Pathway specific effects of ADSL deficiency on neurodevelopment

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Abstract

Adenylosuccinate Lyase (ADSL) functions in *de novo* purine biosynthesis (DNPS) and the purine nucleotide cycle. ADSL deficiency (ADSLD) causes numerous neurodevelopmental pathologies, including microcephaly and autism spectrum disorder. ADSLD patients have normal serum purine nucleotide levels but exhibit accumulation of dephosphorylated ADSL substrates, S-Ado and SAICAr, the latter being implicated in neurotoxic effects through unknown mechanisms. We examined the phenotypic effects of ADSL depletion in human cells and their relation to phenotypic outcomes. Using specific interventions to compensate for reduced purine levels or modulate SAICAr accumulation, we found that diminished AMP levels resulted in increased DNA damage signaling and cell cycle delays, while primary ciliogenesis was impaired specifically by loss of ADSL or administration of SAICAr. ADSL deficient chicken and zebrafish embryos displayed impaired neurogenesis and microcephaly. Neuroprogenitor attrition in zebrafish embryos was rescued by pharmacological inhibition of DNPS, but not increased nucleotide concentration. Zebrafish also displayed phenotypes commonly linked to ciliopathies. Our results suggest that both reduced purine levels and impaired DNPS contribute to neurodevelopmental pathology in ADSLD and that defective ciliogenesis may influence the ADSLD phenotypic spectrum.

Introduction

Adenylosuccinate lyase (ADSL) is a conserved homotetrameric enzyme that catalyzes the eighth reaction in the *de novo* purine synthesis (DNPS) pathway, and also participates in the purine nucleotide cycle (Figure 1-figure supplement 1)(1). Mutations in *ADSL* cause adenylosuccinate lyase deficiency (ADSLD), an autosomal recessive disorder characterized by defects in purine metabolism and heterogeneous neurological phenotypes that include lack of eye-to-eye contact, auto-aggressive behavior, speech impairment, mild psychomotor delay, transient contact defects, autism spectrum disorder, epilepsy and in some cases, microcephaly, encephalopathy, ataxia or coma vigil(2, 3). While the incidence of ADSLD has not been fully established, over 100 patients have been diagnosed to date and subcategorized based on their symptoms that range from premature death to milder developmental and behavioral disorders(3) (www.adenylosuccinatelasedeficiency.com).

ADSLD can be diagnosed by detecting elevated levels of the substrates of ADSL, SAICAR (also referred to as SZMP) and S-AMP, as well as their dephosphorylated riboside forms, SAICAr and S-Ado, in bodily fluids(2). As normal levels of purine nucleotides were detected in serum from ADSLD patients, the accumulation of S-Ado, and particularly SAICAr, were proposed to play a role in the disease pathology(2–4).

In yeast, ADSL (Ade13) loss provokes genomic instability and is lethal(1, 5–7). Lethality in yeast can be rescued by deletion of a number of DNPS enzymes upstream of ADSL, or the transcription factors that regulate the pathway, indicating that the accumulation of metabolic intermediates, rather than impaired DNPS, underlies the toxicity(1, 7). In *C. elegans*, ADSL loss caused delayed growth, infertility, reduced lifespan and locomotion defects. In some studies, growth, lifespan and locomotion could be linked to the accumulation of SAICAR(7–9).

Perfusion of rat brains with SAICAr led to cellular attrition in the hippocampus, leading to the proposition that SAICAr accumulation is neurotoxic, although the potential mechanism remains unknown(4). In glucose deprived cancer cells, SAICAR accumulation was shown to activate PKM2, and a number of other kinases, to promote cancer survival in glucose-limiting conditions, suggesting that purine metabolite accumulation could have distinct signaling outcomes that impact on cell behavior and fate during development(10–12). However, despite extensive enzymology and structural information, the underlying mechanisms by which neuropathology arises in ADSLD remain unknown.

To address the potential roles of ADSL deficiency in neurodevelopment, we systematically examined the consequences of ADSL depletion in diploid human cells and *in vivo*. We found that ADSL depletion in human epithelial cells impaired cell cycle progression, induced DNA damage signaling and impaired primary ciliogenesis. Deletion of p53 or supplementation with nucleosides rescued cell cycle progression and DNA damage signaling, respectively. In contrast, ciliogenesis defects were unaffected by p53 status or nucleoside supplementation and could be reproduced by SAICAr supplementation or attenuated by the inhibition of PAICS, that is required to generate SAICAR/r upstream of ADSL. Depletion of ADSL in chicken or zebrafish embryos impaired neurogenesis and caused developmental defects. Both chicken and zebrafish

embryos exhibited microcephaly, that is observed in some ADSLD patients and increased DNA damage was evident in zebrafish. In addition, fish embryos displayed ciliopathy related phenotypes and interventions to limit SAICAR accumulation rescued impaired neurogenesis. Together our results indicate that ADSL depletion causes context-dependent phenotypes associated with its role in both the purine nucleotide cycle and DNPS, that together impact neurodevelopment.

Results

ADSL depletion causes p53-dependent proliferation defects

To investigate the impact of ADSL on cellular homeostasis, we depleted ADSL with a pool of four siRNAs in hTERT-immortalized human retinal epithelial cells (hTERT-RPE-1, referred to henceforth as RPE-1). Depletion of ADSL was effective, as we observed 80% depletion of the mRNA and a clear reduction in protein levels (Figures 1A-B). This was accompanied by reduced levels of AMP and GMP, as well as accumulation of S-Ado (Figure 1C and Figure 1-figure supplement 2A-B). SAICAr levels were at the limit of detection in RPE-1 cells and differences could not be assessed (Figure 1-figure supplement 2B), but elevated SAICAr could be readily observed following ADSL depletion in HeLa, potentially due to elevated levels of DNPS enzymes that have been observed in cancer cells (Figure 1-figure supplement 2C)(13–16). We deconvolved the siRNA pool and identified an effective single siRNA (#2) to further validate results from the siRNA pool (Figure 1-figure supplement 2D).

As ADSL is critical for DNPS, we examined cell growth following ADSL depletion and found reduced levels of proliferation in ADSL depleted cells compared to controls (Figure 1D). ADSL depleted cells frequently lacked Ki67 expression, indicating that some cells were exiting the cell cycle, had increased levels of p53 and showed accumulation in G1 phase of the cell cycle (Figures 1E, F and Figure 1-figure supplement 2E). Trypan blue and β -galactosidase assays indicated that cell death was not increased (Figure 1-figure supplement 2F) and that the Ki67 negative cells were likely quiescent and not senescent (Figure 1-figure supplement 2G). Deletion of *TP53* rescued proliferation, prevented G1 arrest and restored the number of Ki67-positive cells (Figure 1G, Figure 1-figure supplement 2H-I). The reduction in Ki67-positive cells could be rescued by stable expression of an siRNA resistant allele of *ADSL* (*ADSL**) (Figure 1H, and Figure 1-figure supplement 2J). We also checked whether ADSL depleted RPE-1 cells underwent

differentiation by staining with Vimentin, a marker of undifferentiated cells(17), and Cytokeratin 20 (CK20), a marker of differentiation, upon ADSL depletion. We did not observe any CK20 signal or a reduction in Vimentin-positive cells in the population upon ADSL silencing compared to the controls, arguing against premature differentiation (Figure 1-figure supplement 2K).

To identify the cause of cell cycle exit in ADSL depleted cells, we performed specific treatments. To restore purine levels, we supplemented cells with mixture of nucleosides or with adenosine alone. To reduce potential accumulation of SAICAr that is generated by DNPS, we treated with MRT00252040, a small molecule inhibitor of phosphoribosylaminoimidazole carboxylase (PAICS), the enzyme required to generate SAICAr upstream of ADSL (Figure 1-figure supplement 1) (18). Supplementation of ADSL depleted RPE-1 cells with nucleosides or adenosine did not prevent p53 induction or cell cycle exit (Figures 1I, J). Similarly, treatment with MRT00252040 did not influence p53 induction or loss of Ki67 (Figure 1K, L). This demonstrated that ADSL depletion in non-transformed human epithelial cells leads to a partial p53-dependent cell cycle exit/arrest that is not rescued by complementing the defects of ADSL depletion with nucleoside supplementation or inhibiting PAICS to prevent SAICAR/r production upstream of ADSL.

ADSL depletion causes elevated DNA damage signaling

Reduced levels of purine nucleotides in ADSL-depleted cells may cause replication stress and DNA damage (19–21). To address this, we examined the levels of chromatin bound RPA, a surrogate marker of ssDNA accumulation and indicator of replication stress. In ADSL depleted cells, chromatin bound RPA levels were increased, indicative of replication stress, although the effect was mild (Figure 2A). Additionally, we observed an increased number of cells with more than five 53BP1 foci per cell, indicative of DNA double strand break accumulation (Figures 2B). 53BP1 foci were reduced by treatment with a small molecule inhibitor for ATM (Figure 2C), indicating an active DNA damage response. Supplementation of cells with nucleosides suppressed the appearance of DNA double strand breaks (DSBs) detected by 53BP1 following ADSL depletion or treatment with the ribonucleotide reductase inhibitor Hydroxyurea (HU) that depletes dNTP pools (Figures 2D, E). A similar reduction in 53BP1 foci was also observed with adenosine supplementation of ADSL-depleted cells (Figure 2F). Nucleoside

supplementation also reduced the increase in γ H2AX, the phosphorylated form of the histone variant H2AX, that is also a marker of DSBs (Figures 2G). In contrast to nucleoside supplementation, the PAICS inhibitor MRT00252040 did not rescue the increased levels of DSBs (Figure 2H). These data indicate that ADSL depletion in cultured cells induces mild levels of DNA damage signaling that can be suppressed by nucleoside supplementation, but not by the inhibition of PAICS. This implicates defects in the purine nucleotide cycle, rather than impaired DNPS or specific metabolite accumulation resulting from ADSL depletion, in replication stress and DSB formation. In addition, it indicates that the partial p53-dependent cell cycle exit is not solely a consequence of DNA damage signaling or purine metabolite accumulation, as it was not rescued by either intervention.

ADSL depletion impairs neurogenesis in the developing chicken neural tube

Given the effects of ADSL depletion on cell growth and proliferation, we sought to examine the consequences of its loss *in vivo*. To this end, we used the chicken embryo system to examine the influence of ADSL depletion on nervous system development. We electroporated one side of the neural tube with plasmid expressing *GFP* as a transfection marker in combination with either control or ADSL shRNA vectors. After confirming efficient ADSL depletion (Figure 3A) we evaluated neurogenesis by staining with markers for proliferating neural progenitors (SOX2 positive) and post-mitotic neurons (ELAVL3/4 positive). We found that in the ADSL depleted side, both cell populations were reduced when compared to the non-transfected side (Figure 3B) and that the size of the tissue was smaller, suggesting reduced growth and/or increased cell death. Staining for the apoptotic marker Cleaved-Caspase-3 (CC3) revealed no notable differences, suggesting that this was not due to increased cell death (Figure 3-figure supplement 1A).

We then analyzed SOX2 and ELAVL3/4 staining only within the GFP-positive transfected cells and found that ADSL depletion increased the percentage of SOX2-positive progenitors relative to ELAVL3/4 positive neurons (Figure 3C and Figure 3-figure supplement 1B). This suggested that reduced tissue growth was not due to premature differentiation but possibly due to a proliferation defect in the progenitor population. To study cell cycle progression in neural stem cells we performed FACS analysis of GFP-positive, ELAVL3/4 negative cells following electroporation of control and ADSL shRNA.

We found that there was a slight increase in the G2/M population after ADSL depletion (Figure 3D). Further analysis of stained tissue sections showed that ADSL depletion caused a reduction in the fraction of cells that incorporated EdU and an increase in the fraction of cells positive for the G2/M marker phosphorylated Histone H3-Ser10 (pH3S10) (Figure 3E). We separated the pH3S10 positive cells into two populations; G2 cells, identified by punctate pH3S10 staining, and mitotic cells, displaying broadly distributed pH3S10 staining. This revealed that only the G2 fraction of cells was increased by ADSL depletion, indicating that ADSL depletion caused a specific delay in G2 phase in the SOX2+ population, rather than during mitosis (Figure 3F). Together our *in vitro* data indicate that ADSL depletion leads to a mild induction of DNA damage signaling and impaired cell cycle progression. *In vivo*, this manifests as reduced cellularity in the developing brain, without a clear induction of cell death or senescence.

Ciliogenesis defects following ADSL depletion

As there are non-cycling cells in the brain and ADSL depletion caused cell cycle exit in RPE-1 cells, a condition frequently accompanied by ciliogenesis, we tested the ability of control and ADSL depleted RPE-1 cells to assemble cilia. Following treatment with siRNA, cells were serum-starved for 48 hours and analyzed by immunofluorescence microscopy. Ki67 staining confirmed that most of the cells in both conditions exited the cell cycle (Figure 4A). We next examined ciliogenesis by staining for the ciliary marker ARL13B and the centrosome marker pericentrin (PCNT). Fewer cells treated with the ADSL siRNA pool had cilia and the cilia that were present were shorter when compared to controls (Figure 4B). We also observed shorter cilia upon depletion with single siRNAs for ADSL (Figure 4-figure supplement 1A). To exclude the possibility that ciliogenesis was simply delayed, we quantified the number of ciliated cells 72 hrs after serum starvation and observed a similar defect (Figure 4-figure supplement 1B). Defective ciliogenesis was rescued by expression of an siRNA resistant cDNA (ADSL*), but not by nucleoside supplementation (Figures 4C-D). Inhibition of the DNPS pathway with methotrexate (MTX), that impairs steps in DNPS up and downstream of ADSL (Figure 1-figure supplement 1), had no effect on ciliogenesis in control cells (Figure 4-figure supplement 1C), but rescued both the number of ciliated cells and cilia length in ADSL depleted RPE-1 cells (Figure 4-figure supplement 1D).

Since defective ciliogenesis caused by ADSL depletion was rescued by MTX, but not by nucleoside supplementation, we next examined whether specific inhibition of PAICS was sufficient to induce the phenotype. Following ADSL depletion, we treated cells with the PAICS inhibitor MRT00252040 (18). Similar to MTX, this rescued ciliogenesis, as the number and length of cilia were similar in control and ADSL-depleted cells (Figure 4E). As inhibition of PAICS impairs DNPS upstream of SAICAR production, we treated cells with SAICAr and observed that this recapitulated the ciliogenesis defect observed in ADSL depleted cells (Figures 4F-G). SAICAr treatment did not activate AKT activity, that has recently been shown to inhibit ciliogenesis due to serum starvation (Figure 4-figure supplement 1E)(22). To exclude additional indirect effects as a cause for the reduction in the number of ciliated cells, such as cell cycle progression defects caused by DNA damage, p53 activation or defective cilia removal from interphase cells, we repeated the experiment in p53 KO cells and examined cilia resorption following the addition of serum. While the overall percentage of ciliated cells was slightly lower in p53 KO cells, depletion of ADSL recapitulated the result obtained in RPE-1 wt, a reduction in ciliated cells and cilia length compared to controls (Figure 4-figure supplement 1F). In addition, the kinetics of cilia removal following serum add back was similar in both controls and ADSL depleted cells (Figure 4-figure supplement 1G). We concluded that impairing DNPS specifically at the ADSL-dependent step and/or SAICAr accumulation caused by ADSL depletion impairs the generation of primary cilia.

ADSL depletion and SAICAr accumulation impair CP110 removal

To understand the origin of the ciliogenesis defect, we examined centriole configurations, since mother centrioles, after conversion to basal bodies, template formation of the primary cilium. Centrosomes in ADSL depleted cells had normal levels of PCNT and a normal number of centrioles (Figure 5A and Figure 5-figure supplement 1A). However, we found that the removal of CP110 from the mother centriole, a key step in early ciliogenesis, was impaired in serum-starved, ADSL depleted cells. Compared to controls a larger number of ADSL depleted cells contained centrosomes with 2 CP110 foci (Figure 5B). This could be phenocopied by administration of SAICAr and was rescued by PAICS inhibition (Figure 5C-D). To determine if the retention of CP110 could underlie the phenotype, we co-depleted CP110 with ADSL using three different siRNAs. All three siRNAs silenced CP110, as verified by western blot (Figure 5-figure supplement 1B) and partially depleted CP110 at centrioles (Figure 5-figure supplement 1C). In non-

serum-starved conditions CP110 siRNA treated cells had fewer than the two centriolar CP110 foci typically observed in control cells (Figure 5-figure supplement 1D). Remaining centriolar signal was associated with daughter centrioles (distal to the base of the cilium in ciliated cells; Figure 5-figure supplement 1C). Co-depletion of CP110, using 3 independent siRNAs, with ADSL rescued the ciliogenesis defect (Figure 5E). These data demonstrated that ADSL deficiency or SAICAr administration impair primary ciliogenesis and this can be rescued by CP110 depletion or inhibition of PAICS, but not by supplementation of purine levels.

Depletion of *Adsl* in zebrafish results in developmental defects

To test whether ADSL deficiency caused ciliary defects *in vivo*, we employed a zebrafish model. As CRISPR/Cas9-mediated gene knockout did not yield viable mutants, we used two different antisense morpholino oligonucleotides (MO) to deplete *Adsl* in zebrafish embryos. *Adsl* is ubiquitously expressed at early embryonic stages and, by the 18-somite stage, highly expressed in several areas of the developing brain, including the midbrain and mesencephalon (Figure 6-figure supplement 1A-L). Antibody staining demonstrated expression of *Adsl* in neurons, which was abolished upon injection of either MO (Figure 6-figure supplement 2). Examination of embryo morphology 48 hrs post fertilization (hpf) revealed pericardial edema, kinked tail, hydrocephalus and pinhead (microcephaly) phenotypes (Figure 6A-E). Defects in head size, which are consistent with the clinical presentation of ADSLD patients, were further corroborated by staining for skull formation that is coordinated with brain development. Alcian blue staining showed that nearly 50% of the *Adsl* depleted embryos exhibited weak or absent staining (Figure 6F). Defects in skull formation could be largely rescued by zebrafish *Adsl* or human *ADSL* expression but not expression of a human *ADSL* R426H mutant, the most frequently observed ADSLD mutation (Figure 6F)(<https://www.adenylosuccinatelasedeficiency.com/>). Examination of DNA damage signaling in the developing neural tube revealed an increase in γ H2AX positive cells. Similar to what was observed in RPE-1 cells, treatment with nucleosides suppressed DNA damage signaling (Figure 6G). These data demonstrated that *Adsl* depletion strongly impaired normal zebrafish development, leading to DNA damage that could be suppressed with nucleoside supplementation and several phenotypes consistent with ciliary defects.

Adsl depletion impairs ciliogenesis in zebrafish

As the observed phenotypes were potentially indicative of defects in cilium function, we examined heart looping by staining for cardiac myosin light chain 2 (*cmcl2*) mRNA. *Adsl* depleted embryos showed higher frequencies of defects, including inverse looping and to a lesser extent no loops (Figure 7A). Inverse heart looping may be indicative of laterality impairment (*situs inversus*) that can arise due to ciliary defects. To corroborate this possibility, we examined liver placement by staining for angiopoietin-like 3 (*angptl3*). A significant increase in inverse liver placement was observed in *Adsl* depleted embryos compared to controls, supporting a general defect in laterality (Figure 7B). To further investigate the laterality defects, we examined left-right asymmetry at the 20-somite stage, staining for the mRNA of the left lateral plate mesoderm marker *southpaw* (*spaw*). Consistent with the altered distribution of *cmcl2* and *angptl3*, asymmetric *spaw* mRNA localization was changed in about 40% of *Adsl* depleted embryos. Most of these embryos showed symmetric patterning and a smaller fraction no or only weak staining. The correct asymmetric distribution of *spaw* mRNA could be largely restored by expression of mRNA encoding zebrafish *Adsl* (Figure 7C).

To test if impaired laterality may involve ciliary defects, we examined the Kupffer's vesicle (KV, organ of laterality). While KV area and cilia number were not significantly affected by *Adsl* depletion, cilia length was reduced in *Adsl* ATG MO treated embryos, a phenotype that was partially rescued by co-injection of RNA encoding zebrafish *Adsl* (Figure 7D-G). These data, in combination with additional phenotypes, including laterality defects and hydrocephalus, support the role of ADSL in promoting proper cilia formation or function *in vivo*.

MTX treatment rescues neurogenesis in *Adsl* depleted zebrafish

As ciliogenesis defects were linked to impaired DNPS and excess SAICAR in human cells (Figure 4), we examined the effects of inhibiting the DNPS pathway during zebrafish development. We quantified the effects of *Adsl* depletion on differentiating neuronal cells by staining for the marker *Elavl3/4*. Similar to what we observed in the chicken neural tube, depletion of *adsl* caused a significant reduction in *Elavl3/4* positive cells that could be rescued by the co-injection of RNA encoding zebrafish *Adsl* (Figure 8A). We next treated control and *Adsl* depleted embryos with MTX to attenuate the DNPS pathway upstream of ADSL and reduce SAICAR production. Treatment with MTX

completely rescued the reduction in Elavl3/4 positive cells in the neural tube, indicating that this was not a result of impaired DNPS *per se*, but likely a consequence of intermediate metabolite accumulation (Figure 8B). Similar results were observed with a second morpholino targeting *adsl* (Figure 8-figure supplement 1). We next examined the effect of MTX treatment on Sox2 positive neural progenitors in the developing forebrain (Figure 8C, upper right panel). Depletion of *Adsl* reduced the number of Sox2 positive cells and this was rescued by co-treatment with MTX (Figure 8C). In contrast, supplementation with nucleosides did not rescue the reduced number of Sox2 positive progenitors in *Adsl* depleted embryos (Figure 8D). These data indicate a specific role of impaired DNPS, and likely SAICAr accumulation, in the neural progenitor defects associated with *Adsl* depletion.

Discussion

Despite a detailed understanding of the enzymology of DNPS and the purine nucleotide cycle, the specific cell and organismal effects underlying the complex etiology of ADSLD remain unclear. Our results uncovered multiple phenotypes associated with ADSL depletion in human RPE-1 cells, as well as developing chicken and zebrafish embryos (Appendix 1). These can largely be rescued by interventions that suppress the generation of metabolites in the DNPS pathway or restore purine levels, complementing defective DNPS or purine nucleotide pathway activity. DNA damage signaling was suppressed by nucleoside supplementation in human cells and zebrafish, suggesting that this was caused by purine deficiency (Figure 1A). In contrast, defects in primary ciliogenesis resulting from ADSL depletion were rescued by PAICS inhibitor or MTX treatment, but not nucleosides, and phenocopied by SAICAr administration. The inability to rescue these phenotypes with nucleoside supplementation indicated that they most likely resulted specifically from loss of ADSL, and potentially SAICAr accumulation, rather than a general deficiency in purine supply. As SAICAr levels were barely detectable in RPE-1, it could indicate that a very small amount of SAICAr is sufficient to provoke the phenotypes, or that signaling elicited specifically from loss of the ADSL protein is responsible. In ADSL depleted RPE-1 cells, we could also detect p53 activation and defects in cell cycle progression, in the absence of cell death or senescence, and these phenotypes were insensitive to nucleoside supplementation or DNPS modulation, indicating the involvement of additional pathways.

Microcephaly, which is present in a subset of more severe ADSLD patients(24–26), was observed in zebrafish embryos following *Adsl* depletion. Similarly, ADSL depletion in chicken embryos led to a reduction in neural tube size. Together, the results suggest that they are potentially valuable models for understanding the etiology of microcephaly in ADSLD(3). DNA damage, p53 activation and defects in cilia function, that we observed following ADSL depletion, have all been implicated in neurodevelopmental disorders, such as Seckel Syndrome, that is associated with microcephaly(27). In Seckel Syndrome mice expressing hypomorphic, humanized alleles of *Atr*, replication stress and DNA damage preceded neuroprogenitor cell death (28, 29). This was accompanied by extensive p53 activation, but co-deletion of p53 exacerbated the cellular and organismal phenotypes, indicating a protective effect of p53 induction. Mutations in centrosomal proteins, such as CENPJ/SAS4/CPAP or CEP63, were also implicated in Seckel Syndrome(30, 31). In contrast to mice expressing hypomorphic *Atr*, progenitor loss in mice with CEP63 or CENPJ/SAS4 deficiency resulted from mitotic delays and the activation of the USP28-53BP1-dependent mitotic surveillance pathway(32–38). In this case the phenotype was completely rescued by p53 co-deletion, revealing p53-dependent cell death as a main driver of the phenotype. Despite some phenotypic similarities with Seckel Syndrome at the cellular level, including replication stress and p53 activation, we did not detect increased cell death as a result of ADSL depletion. This indicated that the reduced cellularity caused by ADSL depletion occurs in a manner mechanistically distinct from Seckel Syndrome driven by ATR deficiency or centrosome duplication defects.

GMPS (GMP synthase), an essential enzyme in the purine synthesis pathway (Figure 1-figure supplement 1), was identified as a target for p53 repression following p53 activation using Nutlin-3a(39). In addition, GMPS was demonstrated to promote p53 stabilization in response to genotoxic stress in a manner that requires the USP7 ubiquitin protease(40). While we cannot rule out a role for this pathway in p53 stabilization following ADSL depletion, we could not rescue p53 activation with nucleoside supplementation and induction of p53 via the GMPS pathway was demonstrated to induce cell death, which we did not observe clearly in any of the systems we analyzed.

In addition to p53-dependent and independent cell death, defects in cilium removal and premature progenitor differentiation have also been clearly implicated in microcephaly in

Seckel Syndrome and Primary Microcephaly(41, 42). We examined the removal of cilia by adding back serum to serum starved RPE-1 cells depleted for ADSL and observed normal rates of cilia removal (Figure 4-figure supplement 1G), suggesting that impaired cilia resorption was likely not impairing cell cycle exit and indirectly impacting ciliogenesis. Analysis of differentiation in RPE1 cells depleted for ADSL also did not support a premature differentiation phenotype (Figure 1-figure supplement 2K), but we recognize that RPE-1 cells may not respond to purine depletion in the same way as neural progenitors or other cell types *in vivo*.

In RPE-1 and chicken embryos, we observed cell cycle delays following ADSL depletion, as well as an overall reduction in SOX2 positive progenitor cells in both *in vivo* systems. As a result, differentiated ELAVL3/4 positive cell numbers were also reduced in both chicken and fish systems. Cell cycle delay was further supported by the observation that, contrary to overall Sox2 positive cell numbers, the number of transfected, Sox2-positive cells in chicken embryos was increased relative to controls. In agreement with our findings, nutrient restriction was shown to arrest the proliferation of neural progenitors of *Xenopus laevis* and zebrafish reversibly in G2, suggesting that most of the cells were quiescent(43), consistent with the lack of evidence for cell death or senescence in ADSL depleted systems. While arrest in *Xenopus* progenitors did not require mTOR signaling, nutrient dependent cell cycle reentry was mTOR dependent. Purine deficiency inhibits the mTORC1 pathway, which regulates protein synthesis in response to nutrient availability (18) and inactivation of mTORC1 results in microcephaly in mouse models, but this was attributed mainly to increased levels of cell death (44). Thus, while we cannot rule out a role for dysregulated mTOR signaling in the phenotypes associated ADSLD, the origin of microcephaly is likely to be mechanistically distinct to that in mTORC1 deficient mouse models. We therefore propose that the reduction in brain size resulting from ADSL depletion is largely due to the impaired cell cycle progression observed in SOX2 positive progenitors and delayed differentiation.

The molecular targets of SAICAR remain largely unclear, but its accumulation has been linked to activation of PKM2, and other kinases, in the context of glucose deficiency in cancer (10–12). Recent work also implicated ADSL in the activation of MYC, that plays a major role in controlling metabolism and proliferation in cancer cells(13). Considering that ciliogenesis is a highly regulated process and tightly coordinated with the cell cycle,

modulation of central signaling pathways, including AKT and mTOR that control energetics and proliferation, may be one way by which SAICAr could affect cilia(18, 22). In particular, AKT was shown to regulate a key step in ciliary vesicle formation and AKT activation exerts an inhibitory effect on ciliogenesis induced by serum starvation(22). We examined AKT activation following SAICAr treatment and did not observe any detectable increase, indicating that the ability of SAICAr to inhibit ciliogenesis occurs through a distinct mechanism, consistent with the fact that CP110 depletion, that occurs prior to vesicle formation, rescued ciliation in ADSL depleted cells (Figure 5E). ADSL has also been identified as a proximal interactor of numerous centrosomal proteins, including CEP104, CEP128, CEP135, CEP152, CEP89 and Centrobin, suggesting that it may play specific regulatory roles involving direct interactions or its enzymatic activity locally at centrosomes(45). To address this, future work will be needed to determine the cellular interactomes of ADSL and SAICAr, and the signaling they elicit, to identify the disease-relevant targets.

To our knowledge, this is the first demonstration of a specific purine metabolite impairing ciliogenesis. While ciliopathy-like features have not been described for the pathology of ADSLD, we observed a robust rescue of the neural progenitor population in zebrafish following MTX treatment, suggesting that SAICAr and its effects on cilia may be involved. Consistent with effects on cilia *in vivo*, we observed shorter cilia in the KV of zebrafish, as well as several ciliopathy related phenotypes (Figure 6A-F and Figure 7A-G), consistent with impairment of ciliogenesis or cilia function *in vivo*. *Adsl*-depleted zebrafish also presented with defects in skull cartilage formation that is coordinated with brain size, in part through cilia based sonic hedgehog signaling(46). Primary cilia and Hedgehog signaling have well established roles in regulating multiple progenitor populations in the developing brain(47). However, we also note that severe defects in primary cilia function or Hedgehog signaling cause more drastic reductions in progenitor proliferation and numbers than we observed in either system, potentially consistent with the milder ciliogenesis effects observed *in vivo*(48, 49). Moreover, we used ADSL knockdown in our experimental systems and it is currently unclear to what extent the observed ciliary defects would be recapitulated by ADSL mutations in patients and in what tissues and cell types. If ADSL deficiency would be less severe or only a subset of tissues would be affected, patients may not present classic and widespread ciliopathy features. Together, our work provides a cell-level analysis of ADSL deficiency, identifies

specific cellular defects, and ascribes these to defects in specific steps of DNPS or the purine nucleotide cycle. Highlighting the complex etiology of ADSLD, our results add further support to the notion that SAICAr may play a key role in pathological outcomes and establish a framework for deciphering the underlying molecular mechanisms.

Materials and Methods

Human cell culture

Human immortalized hTERT-RPE-1 WT (ATCC), TP53 knock out (kind gift from Brian Tsou), RPE-1 expressing pLenti-EGFP and pLenti-ADSL*EGFP (siRNA resistant mutant) cells were cultured in Dulbecco's modified Eagle Medium-F12 (DMEM-F12; Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum (Millipore Sigma) and 100 U ml⁻¹ penicillin–streptomycin at 37°C and 5% CO₂ in humidified atmosphere. For cilia experiments, silenced RPE-1 cells were serum starved for 48 hrs in OptiMEM (Thermo Fisher Scientific). Hela (ATCC) and AD293 (Agilent) cells were cultured in Dulbecco's modified Eagle Medium with high glucose (DMEM; Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum (Millipore Sigma) and 100 U ml⁻¹ penicillin–streptomycin at 37°C and 5% CO₂ in humidified atmosphere. All cell lines were authenticated by STR testing (ATCC) and routinely tested for mycoplasma and found negative (Universal Mycoplasma Detection Kit; ATCC).

Drugs used and concentrations

1 mg/ml SAICAr (Carbo Synth) was added to the cells for 96 hrs, to mimic ADSL depletion. 60 µM nucleosides (100X Embryomax, Merck Millipore) were added from the first silencing to the end at 1X in the culture medium. MRT00252040 (kindly provided by Simon Osborne, LifeArc, London, UK) dissolved in DMSO was used at 2 µM and MTX (Millipore Sigma) at 4 µM as described in (18). ATM inhibitor (KU-55933; Selleckem) was used at 5 mM for 24 hrs before fixation. Doxorubicin (Millipore Sigma) was used as positive control for senescence at 1 µg/ml for 6 days. Adenosine (Millipore Sigma) was dissolved in water at a concentration of 0.8mg/ml and diluted 100X to reach a final concentration of 80 µg/ml for 96hrs. Hydroxyurea (HU) (from Millipore Sigma) has been freshly dissolved in water and used at a final concentration of 2 mM in cell culture for 6hrs.

Antibodies

Staining of human cells was performed with the following primary antibodies: α -ADSL (Millipore Sigma, rabbit, 1:100 IF, 1:1000 western), α -ARL13B (Santa Cruz Biotechnology, mouse monoclonal C5, 1:100), PCNT (Novus Biologicals, rabbit, 1:400), α -p53 (Cell Signaling, mouse monoclonal 1C12, 1:100), α -RPA2 (Calbiochem, mouse monoclonal Ab-3, 1:100), α -53BP1 (Novus Biologicals, rabbit, 1:400), α -pSer139-H2A.X (Santa Cruz Biotechnology, rabbit, 1:100), α -AKT (Cell Signaling, rabbit polyclonal, 1:2000), α -pAKT-S473 (Cell Signaling, rabbit polyclonal, 1:2000), α -Vinculin (Cell Signaling, rabbit polyclonal, 1:2000), α -Actin (Millipore Sigma, mouse monoclonal AC-40, 1:1500), α -Vimentin (Abcam, rabbit, 1:100), α -CK20 (DaKo, mouse, 1:200), α -Ki67 (Novocastra, mouse, 1:500), α -Centrobin (a kind gift from Ciaran Morrison, mouse, 1:500 (53)), α -Centrin (EMD Millipore, mouse, 1:1,000), α -CP110 (a kind gift from Andrew Holland, rabbit, 1:1000), α -Mouse-AlexaFluor594 (Molecular Probes, goat, 1:400) and α -Rabbit-AlexaFluor488 (Molecular Probes, goat, 1:400). Staining of chicken tissues was performed with the following primary antibodies: α -ELAVL3/4 (Molecular Probes, mouse, 1:500), α - β -TubulinIII-Tuj1 (Covance, mouse, 1:1000), Pax6 (DSHB, mouse, 1:250), SOX2 (Invitrogen, rabbit, 1:500), pH3S10 (Millipore, rabbit, 1:500), Cleaved-Caspase3 (Millipore, rabbit, 1:500). Staining of zebrafish tissues was performed with the following primary and secondary antibodies: α -ELAVL3/4 (GeneTex, rabbit, 1:1000), α -acetylated-alpha-tubulin (Sant Cruz Biotechnology, mouse monoclonal 6-11B-1, 1:1000), α -SOX2 (Abcam, rabbit, 1:1000), α - γ H2AX (GeneTex, rabbit, 1:400), α -ADSL (Millipore Sigma, rabbit, 1:200), α -PKC ζ (Sant Cruz Biotechnology, rabbit, 1:500), α -Digoxigenin-AP Fab fragments (Roche, sheep, 1:5000 ISH), α -Mouse-AlexaFluor568 (Molecular Probes, donkey, 1:1000) and α -Rabbit-AlexaFluor488 (Molecular Probes, donkey, 1:1000).

siRNA transfections

RPE-1 (hTERT-RPE-1; ATCC) were transfected with 100 nM siRNAs (Millipore Sigma or Dharmacon) with Lipofectamine RNAiMAX (Thermo Fisher Scientific) in Opti-MEM (Gibco) without antibiotics for one or two rounds of 48 hrs, depending on the gene to be silenced. We used siGFP (GGCUACGUCCAGGAGCGCCGCACC) and siGL2 (siC) (CGUACGCGGAUACUUCGA) as negative controls. In this study we used a smart pool

(four siRNAs) against *ADSL* (Dharmacon) or single oligos siADSL#2 5'-CAAGAUUUGCACCGACAUA-3' (Millipore Sigma). The siRNA-resistant mutant was designed to be resistant to siADSL#2. For rescue experiments with siCP110 we used three oligos (#1 5'-GCAAAACCAGAAUACGAGAUU-3', #2 5'-CAAGCGGACUCACUCCAUAATT-3' and #3 5'-TAGACTTATGCAGACAGATAA-3' (Millipore-Sigma) for 24 hrs.

RNA extraction and quantitative real time-PCR

RPE-1 cells (ATCC) were seeded in a 6 well-plate, silenced for 96 hrs, washed twice in PBS and resuspended in 300 µl of Tri-Reagent (Millipore Sigma). RNA was isolated by centrifugation followed by chloroform extraction, isopropanol precipitation, washing twice in 75% ethanol and resuspended in 20 µl DEPC-treated water (Thermo Fisher Scientific). Total RNA was quantified with a Nanodrop 8000 Instrument (Thermo Fisher Scientific). 1 µg of total RNA was used for the reverse transcription reaction performed by High-Capacity RNA-to-cDNA Kit (Applied Biosystems), according to the manufacturer's recommendations, in a 2×RT buffer mix, supplemented with dNTPs, random primers and RT enzyme in a final volume of 20 µl. Quantitative real time PCR (qRT-PCR) was performed using the comparative CT method and a Step-One-Plus Real-Time PCR Instrument (Thermo Fisher). Amplification of the 16 ng of cDNA was done in triplicate with TaqMan Universal PCR Master Mix (Thermo Fisher) for *ADSL* and *GAPDH*.

Plasmid cloning and generation of stable cell line

The siRNA resistant mutant, *ADSL*^{*}, was produced by introducing 5 different silent mutations using the QuikChange mutagenesis kit (Thermo Fisher) with the following primers: forward, 5'-GGTTTGCCAGGAGGCGTAGGTCTTTGCAAATTGTGTGCACTGATGCCCCCA-3'. And reverse 5'-CCAAACGGTCCTCCGCATCCAGAAACGTTTAACACACGTGACTACGGGGGT-3'. Constructs were checked by sequencing (Macrogen) and expression was checked by western blot and immunofluorescence.

into the pLenti-CMV-eGFP-BLAST (659-1) plasmid, a gift from Eric Campeau & Paul Kaufman (Addgene plasmid #17445; <http://n2t.net/addgene:17445>; RRID:Addgene_17445)(50), using primers containing *XhoI* and *EcoRI* overhangs

(ADSL- *Xho*I FW 5'-AAAAC TCGAGCGATGGCGGCTGGAGGCGATCAT-3' and ADSL-
*Eco*RI-RV 5'-TTTTGAATTCCAGACATAATTCTGCTTCA-3'). The siRNA resistant
mutant was produced by introducing 5 different silent mutations using the QuikChange
mutagenesis kit (Thermo Fisher) with the following primers: forward, 5'-
GGTTTGCCAGGAGGCGTAGGTCTTTGCAAATTGTGTGCACTGATGCCCCCA-3'.
And reverse 5'-CCAAACGGTCCTCCGCATCCAGAAACGTTTAACACACGTGACT
ACGGGGGT-3'. Constructs were checked by sequencing (Macrogen) and expression
was checked by western blot and immunofluorescence. For lentivirus preparation: 6×10^6
AD293 cells were plated in 15 cm culture dishes, and transfected with 20 μ g pLenti-
CMV-EGFP empty and pLenti-CMV-ADSL*-EGFP, 2 μ g pRSV-REV, a gift from Didier
Trono (Addgene plasmid # 12253 ; <http://n2t.net/addgene:12253> ;
RRID:Addgene_12253), 6 μ g pMDLg/pRRE, a gift from Didier Trono (Addgene plasmid #
12251 ; <http://n2t.net/addgene:12251> ; RRID:Addgene_12251), and 2 μ g pCMV-VSV-G,
a gift from Bob Weinberg (Addgene plasmid # 8454 ; <http://n2t.net/addgene:8454> ;
RRID:Addgene_8454), plasmids with 160 μ l PEI pH 7.0 (Polyscience Euro) and 150 mM
NaCl(51, 52). After 48 hrs the medium containing the viruses was cleared with a 0.45
mm filter (Millipore) and added to the target cells. Three days after the infection, cells
were selected with blasticidin (Invitrogen) for 7 days.

Immunofluorescence (human cells)

Silenced RPE-1 cells were seeded on 18 mm round coverslips after 96 hrs of silencing
and fixed accordingly with the antibody requirements, with 4% PFA for 10 or 30 min,
followed by permeabilization in 0.1% Triton-PBS for 5 min and stored in 100% EtOH. For
RPA2 and γ H2AX detection, pre-extraction was performed using cold 0.2% Triton X-100
in 1X PBS on ice for 5 minutes, before fixation and permeabilization as described before.
Cells were incubated with the blocking solution of 3% bovine serum albumin (Millipore
Sigma) in PBT for 30 min. Primary antibodies (listed below) were diluted in the same
blocking solution and incubated for 1 hr at RT. After three washes, cells were incubated
with Alexa Fluor-conjugated 594 and 488 secondary antibodies (Thermo Fisher
Scientific) at 1:400 dilution for 1 hr at RT DAPI was used to visualize the DNA. Slides
were imaged using Orca AG camera (Hamamatsu) on a Leica DMI6000B microscope
equipped with 1.4 100X oil immersion objective. AF6000 software (Leica) was used for
image acquisition. Image processing and quantification was performed with ImageJ

software. Intensities were measured in images acquired with the same exposure settings and subtracting the background for each image.

Cell proliferation and cell death

150,000 RPE-1 cells were plated in 6-well plates and silenced with control or siADSL oligos (Millipore Sigma or Dharmacon) for 72 hrs, when they were counted and plated again in the same amount for the second round of silencing. After 3 days cells were counted as second timepoint (144 hrs, 6 days) and seeded for a third timepoint (9 days). Cells were cultured in the presence of serum for all the experiment. The Δ PDL (difference in population doubling levels) was obtained by using the formula: $\log(N1/N0)/\log 2$, where N1 is the number of cells at the timepoint we collected them and N0 is the initial number of cells plated(54). For detecting cell death, cells in suspension were collected in the growth medium and the attached ones were trypsinized and resuspended in complete medium to block trypsin activity. Cells were then mixed in 0.4% trypan blue solution (Gibco). The number of blue-positive cells and total cell number was quantified at the microscope.

Cell cycle profile

10×10^6 cells were collected after 96hrs of siRNA depletion, as previously described, and fixed in cold 100% EtOH dropwise and stored at -20C for 24hrs. Cells were stained with propidium iodide (Millipore Sigma) in PBS and RNase and 10,000 cells were analyzed at the Parc Cientific de Barcelona flow cytometry facility using a Gallios (Beckman Coulter) instrument). The data were collected and analyzed using FlowJo v10.7.2.

Cell extracts and western blotting

RPE-1 cells were seeded in a 6-well plate and after 96 hrs of silencing they were trypsinized, washed once in PBS and resuspended in a 2X SDS lysis buffer (2X SDS lysis buffer contained 4% SDS, 20% glycerol, 120 mM Tris/HCl pH 6.8, 1x protease (Roche) and phosphatase inhibitors (Millipore Sigma)). Protein concentration was quantified using the DC Protein Assay (Bio-Rad), and proteins separated by SDS-PAGE and transferred to 0.2 μ m nitrocellulose membrane (Amersham Protran) or 0.45 μ m PVDF membrane (Millipore Sigma) depending on the molecular weight. Membranes were blocked in 5% milk in PBT (PBS containing 0.2% Tween-20) for 30 min and then incubated with primary antibodies for 1 hr at RT. After three washes in PBS containing

Tween-20 0.02%, membranes were incubated with secondary antibodies conjugated to HRP and protein bands were visualized by ECL-Plus (Millipore Sigma).

Senescence-associated (SA) β -galactosidase assay

RPE-1 were silenced for 96 hrs with siControl and siADSL#2, then fixed in ice-cold X-gal fixative solution (containing 4% formaldehyde, 0.5% gluteraldehyde, 0.1 M sodium phosphate buffer pH 7.2) for 4 minutes. After two washes in PBS, X-gal (Roche) was diluted 1:100 at a final concentration of 1 mg/ml in X-gal solution (containing 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN)_6$, 2 mM $MgCl_2$ in PBS). Incubation was performed at 37°C for 8 hrs in the dark. Two washes in PBS were performed before taking the images. Doxorubicin was used as a positive control.

Targeted liquid chromatography-mass spectrometry (LC-MS) analyses

For Figure 1C and Figure 1-supplement 2A, metabolites were extracted from snap-frozen cell pellets by adding 300uL ACN:MeOH:H₂O (5:4:1, v:v:v) solution and vortexing samples for 30 sec. Samples were immersed in liquid N₂ to disrupt cell membranes followed by 30 sec of ultrasonication. These two steps were repeated three times. Then, samples were incubated at -20°C for 1 hr, centrifuged at 15,200 rpm for 10 min at 4°C, and the supernatant was collected into a LC-MS vial.

For cell media, metabolites were extracted from 200 uL of lyophilized media by adding 300 uL Acetonitrile:MeOH:H₂O (5:4:1, v:v:v) and vortexing samples for 30 sec. Then, samples were incubated at -20°C for 20 min, centrifuged at 15,200 rpm for 10 min at 4°C, and the supernatant was collected into a LC-MS vial. Samples were injected (1 uL of media and 10 uL of cell extracts) in a UHPLC system (1290 Agilent) coupled to a triple quadrupole (QqQ) MS (6490 Agilent Technologies) operated in multiple reaction monitoring (MRM) and positive (POS) or negative (NEG) electrospray ionization (ESI) mode. Source parameters were: Gas Temp (°C) = 270; Gas Flow (L/min) = 15; Nebulizer (psi) = 25; Capillary (V) = 3500 (POS) and 2500 (NEG). Metabolites were separated using an ACQUITY UPLC BEH HILIC 1,7um, 2,1x150mm chromatography (Waters) at flow rate of 0.4 ml/min. The solvent system was: A = 50mM ammonium acetate in water, and B = acetonitrile. The linear gradient elution started at 5% A (time 0–1 min), 50% A (time 1–4 min), 5% A (time 4–4.5 min) and finished at 5% A (time 7 min). MRM transitions are shown in Appendix 2.

681

682 The ribosides SAICAr and SAdo and the internal standards $^{13}\text{C}_4$ -SAICAr and $^{13}\text{C}_4$ -SAdo
683 were synthesized according to previously published procedures(55, 56). SAICAr and S-
684 Ado analysis was performed essentially as previously described (57). For Figure 1-
685 supplement 2B-C,, RPE-1 or HeLa cells were sonicated in 10mM Tris pH 8,2, 2mM
686 EDTA, 10mM KCl, 1mM DTT and 4% glycerol, centrifugated, metabolites extracted from
687 50 μl of lysate diluted to protein concentration 1 mg/ml with 200 μL of extracting solution
688 (ACN:MeOH 1:1, v:v, 0.125M formic acid, 15nM $^{13}\text{C}_4$ -SAICAr and $^{13}\text{C}_4$ -SAdo) and
689 incubated on dry ice for 30min. After centrifugation, the supernatant was deep-freeze
690 dried and pellets were resuspended in 50 μl of water, briefly centrifuged and analyzed by
691 LC-MS/MS.

692

693 The reverse phase column Prontosil 120-3-C18 AQ 150x3mm (Bischoff
694 Chromatography) and Agilent 1290 Infinity LC System coupled with an API 4000 triple
695 quadrupole mass spectrometer with an electron ion source (Agilent Technologies) were
696 used. The mobile phase consisted of 0.1% formic acid in water (mobile phase A) and
697 0.1% formic acid in acetonitrile (mobile phase B). The gradient elution was performed as
698 follows: $t = 0.0$ min, 100% A; $t = 2.5 - 6.5$ min, linear gradient to 20% B; $t = 6.5-7.5$,
699 linear gradient from 20% to 60% B; $t = 7.5-9.0$ min, 90% B and then a regeneration of
700 the column for 12 min. The flow rate was set to 0.3 mL/min for the first step, and
701 increased to 0.4 mL/min. The injection volume was 5 μL . MRM transitions for SAICAr,
702 SAdo, SAICAr, SAMP and their corresponding internal standards are shown in
703 Appendix 2.

704

705 The chromatographic conditions were the same as in the HPLC-HRMSⁿ analysis
706 mentioned above. Detection was performed on an Orbitrap Elite operating in positive
707 ionization mode with the same setting as above. The detection method was divided into
708 four time segments. Full scan analysis within the mass range m/z 70–1000 was
709 performed in the first (0.0–3.0 min) and fourth (12.0–17.0 min) segments. The selected
710 ion monitoring (SIM) method was applied in the second segment (3.0–7.0 min) for the
711 analysis of ribosides (m/z 177–417) and in the third segment (7.0–12.0 min) for the
712 analysis of ribotides (m/z 257–497) to enhance the sensitivity towards these metabolites
713 (except for the measurement of SAdo which had m/z ranges: 379–389). The resolution
714 was set to 60,000 FWHM. The mass error was below 3 ppm. All cell lines were

measured in hexaplicate, and the intensity values are presented as averages. The identities of the accumulated compounds in both cell lysates and media were confirmed by MS² fragmentation analysis. Fragmentation spectra were produced via CID with the fragmentation energy set to 30 units of normalized collision energy.

Statistical analysis (cells)

In vitro data were analyzed with an unpaired two-sided *t*-test when two samples were compared, while one-way ANOVA was used to compare more than two samples in the same graph (GraphPad Prism 6.0, GraphPad Software Inc.). Values of $p < 0.05$ were considered statistically significant (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Two or more independent experiments were performed for each condition and this is indicated in individual figure legends. Statistical analysis for percentages of positive cells: counts of cells belonging to the positive and negative condition were used to fit a generalized linear model with binomial distribution. The R [1] packages "lme4" [2] and "multcomp" [3] were used to fit the model and compute raw and adjusted p-values with the "Shaffer" method.

Cloning (fish)

To generate a template for the generation of an antisense *in-situ* probe, a 921 bp fragment of the *Danio rerio* *adsl* open reading frame was cloned into pCRII via TOPO TA cloning (Invitrogen). To have a template for the generation of capped mRNA using the AmpliCap SP6 High Yield Message Maker Kit (Cellscript) the whole open reading frame of zebrafish *adsl* was cloned with a N-terminal Flag-tag into pCS2+ using EcoRI and XhoI.

Immunofluorescence (fish)

Zebrafish embryos were fixed with 4% buffered paraformaldehyde at the indicated stages. Antibody staining was performed as described(58) using the primary antibodies previously described (see also antibodies section above) and detected with Alexa-Fluor-labelled secondary antibodies (1:1000, Molecular Probes).

Statistical analysis (fish)

The number of fertilized eggs per clutch determined the size of experimental groups with clutches having been randomly and equally divided into treatment groups. No additional

statistical methods have been applied to pre-determine sample size. All zebrafish experiments were done at least three times with eggs from different mating tanks or different mating days. Embryo numbers are given in the legends. All statistical analyses were performed with GraphPad Prism 7 and 8, respectively. Data were tested for normality and analyzed accordingly by parametric or non-parametric tests. Graphs display, if not indicated otherwise, individual datapoints and medians in case of non-parametric datasets. An α level of <0.5 was considered significant.

Zebrafish maintenance and manipulation

Zebrafish were maintained in a 14 hrs light and 10 hrs dark cycle in a standardized, water recycling housing system (Tecniplast) with automatic monitoring and adjustments of pH, conductivity and temperature. Fertilized eggs were generated by natural matings of the wild-type strains EK or AB. Eggs were incubated at 28.5 °C and allowed to develop until the desired stages. In order to achieve *Adsl* knockdown, a translation blocking antisense morpholino oligonucleotide (*Adsl* ATG MO) (5'-TCCCTCCATGCCTGCAGCGGTTAAA) was used or a MO which targets the exon-intron boundary at exon 4 of *Adsl* (*Adsl* SplMO) (5'- CCAACTGTGGGAGAGAGCGACTGTA). A standard control MO was also used in all experiments. MOs (GeneTools Inc) were injected at the 1-2 cell stage directly into the yolk. In addition, non-injected wild-type embryos served as internal control for clutch quality. For pharmacological manipulation zebrafish embryos were immersed in embryo water containing 1% DMSO or 1% DMSO and 100 μ M methotrexate (MTX; Cayman Chemicals) from 10 until 24 hrs post fertilization (hpf) or 50 μ M nucleosides. All zebrafish maintenance and procedures have been approved by the Veterinary Care Unit at Ulm University and University of Tübingen, respectively and the animal welfare commissioner of the regional board for scientific animal experiments in Tübingen, Germany. Zebrafish experiments were performed according to the European Union Directive 86/609/EEC for the protection of animals used for experimental and other scientific purposes.

***In situ* hybridization (fish)**

Zebrafish were fixed overnight at 4°C at the indicated stages using 4% buffered paraformaldehyde, dehydrated with a gradual methanol series and stored at -20 °C until further use. For ISH embryos were rehydrated in a methanol series containing PBST (PBS containing 0.1% Tween-20) and processed according to standard protocols(59).

Genes of interest were detected using DIG-labeled *in situ* probes, which were *in vitro* transcribed from linearized plasmids carrying fragments of the gene of interest: *adsl* (Genbank no.199899.2), *angiopoietin-like 3* (*angptl3*, Genbank no. AF379604). The probes against *cardiac myosin light chain 2* (*cmcl2*) and *spaw* have been described before(60).

Analysis of cartilage formation

4 days post fertilization (dpf) old zebrafish embryos were fixed for 2 hours at RT using 4% buffered paraformaldehyde. After rinsing with PBS, embryos were washed for 10 min with 50% EtOH in PBS, before the staining solution (0,02% Alcian blue (Millipore Sigma), 70% EtOH, 50mM MgCl₂) was added and the embryos were incubated o/n at RT. On the next day, embryos were rinsed with H₂O and subsequently bleached for 20 min at RT with opened lid of the reaction tube (bleaching solution: 1,5% H₂O₂ in 1% KOH). A clearing series was performed (30 min 20% glycerol/0,25% KOH, 2h 50% Glycerol/ 0,1% KOH). Stained embryos were stored at 4 °C in 50% Glycerol/ 0,1% KOH.

Measurements of cilia and neural progenitors/differentiated cell populations

To count neural progenitors, anterior views of 24 hpf embryos were taken using a fluorescent whole mount microscope. The number of Sox2 positive cells within the forebrain was determined. To count differentiated neural cells, dorsal views of embryos were captured by fluorescent whole mount microscopy and the number of ELAVL3/4-positive cells per 100 µm were counted. γH2AX positive cells were counted over a distance of 300 µm in the neural tube. Cilia were counted and measured after acquiring confocal z-stacks of flat-mounted tails of 6 somite stage (ss) embryos. The Simple Neurite Tracer in Fiji was used to trace and measure cilia through the whole z-stack. Image J was also used to trace and measure the outline of the KVs.

Microscopy of zebrafish embryos

Live zebrafish embryos and those processed by ISH or for cartilage staining were imaged using a M125 whole-mount microscope equipped with a Leica IC80 HD camera. Zebrafish embryos undergoing immunofluorescence stainings were assessed with a M205 FCA and a DFC 9000 GT sCMOS camera. Confocal z-stacks were acquired on a TCS SP5II with LAS AF software (All microscopes and software: Leica).

Chick embryo *in ovo* electroporation

Eggs from White-Leghorn chickens were incubated at 37.5°C in an atmosphere of 45% humidity and the embryos were staged according to Hamburger and Hamilton(61). Chick embryos were electroporated with column purified plasmid DNA (3 µg/µl for shRNAs) in H₂O containing Fast Green (0.5 µg/µl). Briefly, plasmid DNA was injected into the lumen of HH12 or HH16 neural tubes, electrodes were placed on either side of the neural tube and electroporation was carried out by applying five 50 ms square pulses using an Intracel Dual Pulse (TSS10) electroporator set at 25 V. Transfected embryos were allowed to develop to the specific stages and then dissected under a fluorescence dissection microscope.

DNA constructs

shRNAs were generated using pSHIN plasmid (a GFP expressing evolution of pSUPER): shCONTROL sequence (CCGGTCTCGACGGTCGAGT) and shADSL sequence (GAGCTGGACAGATTAGTGA). The knockdown efficiency of shRNAs was assessed by RT-qPCR in electroporated chicken embryonic fibroblast cultures(62).

Immunostaining and EdU incorporation in chicken embryos

Embryos were fixed overnight at 4°C in 4% paraformaldehyde and immunostaining was performed on vibratome sections (60 µm) following standard procedures. After washing in PBS-0.1% Triton X-100, the sections were incubated overnight with the appropriate primary antibodies diluted in a solution of PBS-0.1% Triton supplemented with 10% bovine serum albumin. After washing in PBS-0.1% Triton, sections were incubated for 2 hr at room temperature with the appropriate Alexa conjugated secondary antibodies diluted in a solution of PBS-0.1% Triton supplemented with 10% bovine serum albumin. After staining, the sections were mounted and examined on a Leica SP5 or a Zeiss Lsm 780 multiphoton microscope. For EdU incorporation, 200 µl of EdU solution (1mM) was added on the vitelline membrane of each embryo 2 h before fixation in 4% paraformaldehyde. EdU was detected in sections using the Click-iT EdU imaging kit (Invitrogen).

Fluorescence Associated Cell Sorting (FACS)

HH-12 chicken embryos were electroporated with shCONTROL or shADSL plasmids and 48 hpe, a single cell suspension was obtained by digestion for 10-15 min with

Trypsin-EDTA (Millipore Sigma) and labeled with Hoechst and α -ELAVL3/4 antibody used with Alexa647-conjugated anti-mouse secondary antibody. Alexa647, Hoechst and GFP fluorescence was determined by FACS Aria Fusion cytometer (BD Biosciences), and the data were analyzed with FlowJo software (Tree Star) and Multicycle software (Phoenix Flow Systems; cell cycle profile analysis).

Quantitative fluorescence image analysis.

Quantification of Cleaved Caspase-3 immunofluorescence intensity was done using ImageJ software. Tuj1+ and Tuj1- areas on the electroporated side and the respective areas on the non-electroporated side were delimited by polygonal selection, and the mean intensity of Cleaved Caspase-3 immunofluorescence was quantified as mean gray values. At least three different images were used to calculate the mean value per embryo. Each mean value was normalized to the mean value obtained for the respective non-electroporated area of the same embryo.

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Conflicts of interest statement

The authors declare that they have no conflict of interest.

Figure Legends

Main Figures

Figure 1. ADSL depletion causes p53-dependent proliferation defects. **A.** Reduced mRNA levels of *ADSL* confirmed by qRT-PCR experiments. hTERT-RPE-1 were silenced with smart pool RNAi for 96 hrs before harvesting. Two independent experiments in triplicate are shown in the panel (n=2 two-tailed *t*-test, *****p*< 0.0001) **B.** Western blot of RPE-1 cell extracts treated as in (A). One experiment is shown as representative of three independent experiments. Actin was used as a loading control. Quantifications of ADSL intensity in four different experiments were performed by ImageJ software and normalized to actin first and then to the relative controls (n=4, two-tailed *t* test, *****p*<0.0001). Ponceau is shown as an additional loading and transfer control. **C.** S-Ado levels in RPE-1 cells silenced with a single control or ADSL siRNA (n=4, two-tailed *t*-test, **p*<0.05). **D.** Cell proliferation rates of RPE-1 cells quantified every three days after treatment with a single control or *ADSL* siRNA in medium with serum (n=3, two-tailed *t* test *****p*<0.0001). Δ PDL represent the difference in population doubling levels quantified through the formula described in materials and methods. **E.** Ki67 positive cells (indicated by arrows) upon 96 hrs of silencing with control or *ADSL* smart pool siRNAs. Scale bar 10 μ m. (n=4, scored 767 cells for siC, 694 cells for siADSL conditions, *****p*<0.0001). **F.** The percentage of p53 positive cells (indicated by arrows) following treatment with control or ADSL smart pool siRNAs were quantified in three independent experiments (n=3, scored 835 cells for siC and 1126 cells for siADSL, **p*<0.05). **G.** Cell proliferation rates in RPE-1 wt and p53 knockout KO cells as in (D) was counted for 6 days. (n=3, two-tailed *t* test, ***p*<0.01, **p*<0.05). **H.** EGFP and ADSL*-EGFP stably expressing RPE-1 were transfected with a single control or ADSL siRNAs for 96 hrs and immunostained with anti-Ki67 antibody. Scale bar=20 μ m. Quantification of Ki67 positive cells (n=3, scored 278 cells for EGFP siC, 264 for EGFP siADSL, 266 for ADSL* siC, 232 cells for ADSL* siADSL conditions, *ns* not significant, *****p*<0.0001). **I.** Quantification of RPE-1 transfected with a single control or ADSL siRNA for 96 hrs in presence or absence of 60 μ M nucleosides. Cells were fixed and immunostained with anti-Ki67 antibody. (n=3, at least 100 cells per conditions were counted in each

experiment, *ns* not significant, **** $p < 0.0001$). **J.** Quantification of RPE-1 in the same conditions of (I) and immunostained with anti-p53 antibody ($n=3$, at least 100 cells were counted for each condition per experiment, *ns* not significant, **** $p < 0.0001$). **K.** Quantification of p53 positive cells in *ADSL* depleted cells in the presence or absence of the PAICS inhibitor MRT00252040. ($n=3$, scored 223 cells for siC, 248 cells for si*ADSL*, 336 cells for siC+MRT00252040, 365 cells for si*ADSL*+MRT00252040, *ns* not significant, **** $p < 0.001$, * $p < 0.05$). **L.** Quantification of Ki67 positive cells in *ADSL*-depleted cells in the presence or absence of MRT00252040 ($n=5$, more than 60 cells were counted in each condition for each experiment, *ns* not significant, *** $p < 0.001$). Positive cells are indicated with arrows in si*ADSL* panels. All graphs depict means $\pm$ SD with individual values shown in circles.

Figure 2. *ADSL* depletion caused elevated DNA damage signaling. **A.** RPE-1 were silenced for 96 hrs with a smart pool of *ADSL* siRNAs, chromatin extracted and immunostained with an anti-RPA2 antibody. Nuclear intensity of cells was measured- Quantification of one representative experiment of four that showed similar results is shown; SD and average is indicated in black. After normalization to the average of the control (siC), two-tailed *t* test was used for statistical analysis of $n=4$ independent experiments: * $p < 0.05$ was observed for si*ADSL* to siC. **B.** RPE-1 were silenced for 96 hrs with a smart pool of *ADSL* siRNAs, fixed and immunostained with anti-53BP1 antibody. Scale bar=10 μ m. Quantification of positive cells that have more than 5 foci per cell ($n=5$ scored 1134 cells for siC, 1144 cells for si*ADSL*, *** $p < 0.001$). **C.** RPE-1 were silenced with a single control or *ADSL* siRNA with or without 5 mM ATM inhibitor (KU5593) ($n=4$, scored 359 cells for siC, 322 cells for si*ADSL*, 307 cells for siC+KU5593, 279 cells for si*ADSL*+KU5593, **** $p < 0.0001$). **D.** Cells were silenced for 96 hrs, treated or not with 60 μ M (1X) nucleosides and stained for 53BP1. Scale bar=10 μ m. ($n=5$, scored 717 cells for siC, 608 cells for si*ADSL*, 576 cells for siC+ nucl, 512 cells for si*ADSL* +nucl, **** $p < 0.0001$). **E.** RPE-1 cells were treated with 2mM HU for 6 hrs and treated or not with 1X nucleosides and stained for 53BP1. Cells with more than 5 foci per nucleus were counted as positive. Three independent experiments were performed ($n=3$, at least 100 cells per experiment per condition were counted, **** $p < 0.0001$). **F.** Cells were silenced for 96 hrs, treated or not with 80 μ g/ml adenosine and stained for 53BP1. ($n=3$, scored 544 cells for siC, 428 cells for si*ADSL*, 485 cells for siC+ adenosine, 411 cells for si*ADSL* +adenosine, **** $p < 0.0001$), **G.** RPE-1 treated as in (A) were fixed and

stained for γ H2AX (H2AX phosphorylated on Ser-139). Scale bar=10 μ m. 5 Gy X-ray irradiation (IR) was used as positive control. Quantification of one representative experiment of two that showed similar results is shown; median is indicated in black. After normalization to the average of the control (siC), one-tailed *t* test was used for statistical analysis of *n*=3 independent experiments: **p*<0.05 was observed for siADSL (to siC), and for siADSL relative to siADSL+nucl. There is no statistical difference between siC and siC+nucl. **H.** RPE-1 were silenced in the presence or absence of 4 μ M MRT00252040, fixed and stained for 53BP1 (*n*=4, scored 367 cells for siC, 313 cells for siADSL, 294 cells for siC + MRT00252040, 241 cells for siADSL+MRT00252040, *ns* not significant, *****p*<0.0001). All bar graphs show means $\pm$ SD with individual values in circles.

Figure 3. ADSL depletion causes neurodevelopmental delay in the chicken neural tube. **A.** mRNA levels of *ADSL* and *GAPDH* were measured by qRT-PCR in chicken embryonic fibroblasts (CEFs) transfected for 24h with shCONTROL and shADSL to confirm knockdown efficiency (*n*=3, two-tailed *t*-test, ***p*<0.01). **B.** Transverse sections of HH12 chicken neural tubes 48 hrs post electroporation (hpe) with shCONTROL and shADSL plasmids and stained with antibodies against SOX2 (green) and ELAVL3/4 (magenta). Transfection was detected by GFP (blue). Scale bar=50 μ m. Average ratio of neural stem cells (NSCs, SOX2+) 48 hpe with shCONTROL and shADSL obtained by comparing the mean number of SOX2+ cells on the electroporated and non electroporated side (*n*=7 embryos, two-tailed *t*-test, ***p*<0.01). Average ratio of cells differentiated into neurons (ELAVL3/4) at 48 hpe with shCONTROL and shADSL obtained by comparing the mean number of ELAVL3/4 positive cells on the electroporated and the non electroporated side (*n*=7 embryos, two-tailed *t*-test, ****p*<0.001). **C.** Percentage of electroporated cells indentified as NSCs (SOX2) or neurons (ELAVL3/4) 48hpe with shCONTROL and shADSL (*n*=11 embryos, two-tailed *t*-test, *****p*<0.0001). **D.** The cell cycle profiles of NSCs (GFP+/ELAVL3/4-) obtained by FACS 48 hpe with shCONTROL and shADSL into HH12 chicken neural tubes. The mean of two independent experiments is shown in the left panel. 6-8 embryos per condition were used for each experiment. Two-tailed *t*-test was used for statistical analysis of *n*=2 independent experiments, *ns* not significant, **p*<0.05 Cell cycle profiles of a representative experiment are shown in the right panels. **E.** Transverse sections of HH12 chicken neural tubes 48 hpe with shCONTROL and shADSL plasmids, and

stained with EdU (green) and an antibody against pH3S10 (magenta). Transfection was detected by GFP (blue). Scale bar=50 μ m. Areas indicated in yellow are amplified in the right panels showing separated channels in black. Green circles in pH3S10 amplification show punctate pH3S10 positive cells. Percentage of transfected cells identified as EdU 48 hpe with shCONTROL and shADSL (n=12 embryos (shCONTROL) and 14 embryos (shADSL), two-tailed t-test, ****p<0.0001). Percentage of pH3S10 among the GFP+ cell population 48 hpe with shCONTROL and shADSL (n=14 embryos, two-tailed t-test, *ns* not significant, **p<0.01, ****p<0.0001). Average ratio of EdU and pH3S10 positive cells 48 hpe of shCONTROL and shADSL plasmids, obtained by comparing the mean number of EdU cells on the electroporated and the non electroporated side (EdU: n=11 embryos (shCONTROL), 15 embryos (shADSL), two-tailed t-test, ***p<0.001; pH3S10: n=18 embryos (shCONTROL), 15 embryos (shADSL), two-tailed t-test, *ns* not significant). **F.** Percentage of punctate pH3S10 (G2 phase) and mitotic pH3S10 (M phase) among the GFP+ cell population 48 hpe of shCONTROL and shADSL plasmids (n=14 embryos, two-tailed t-test, *ns* not significant, ****p<0.0001). Bar graphs show means $\pm$ SD.

Figure 4. SAICAr-dependent ciliogenesis defects following ADSL depletion. A. RPE-1 were transfected with control and ADSL smart pool siRNAs. After 96 hrs cells were serum starved for 48 hrs to induce ciliogenesis followed by staining against Ki67 and negative cells quantified (n=2, two-tailed t-test, *ns* not significant). **B.** Ciliated cells silenced as in **(A)** were stained for ARL13B (magenta) and PCNT (green). Scale bar=10 μ m. Magenta squares show enlargements of the areas. Graphs show quantification of ciliated cells and cilia length (line indicates median) (n=3, scored 108 cells for siC, 82 cells for siADSL, ****p<0.0001). **C.** EGFP and ADSL*-EGFP stably expressing RPE-1 were silenced for 96 hrs with control and a single ADSL siRNA, serum starved for 48 hrs, fixed and stained for ARL13B (red). Scale bar=5 μ m. Graphs summarizes three experiments (one-way ANOVA, *ns* not significant, ***p<0.001, **p<0.01, *p<0.05). **D.** RPE-1 silenced with a single ADSL siRNA (siADSL#2) for 96 hrs in the absence or presence of 1X nucleosides. Cilia frequency and cilia length were quantified; mean $\pm$ SD of n=3 siC (scored 557cells) and siADSL (scored 499cells), n=5 for siC (588 cells) and siADSL (scored 297cells) with nucleosides, *ns* not significant, ***p<0.001.). **E.** RPE-1 cells were ADSL-depleted, treated or not with MRT00252040 and serum starved, and then immunostained for ARL13B (magenta) and PCNT (green). Cilia frequency and cilia length were quantified (n=3, scored 261 cells for siC, 166 cells for siADSL, 287 cells for

siC+MRT0025204, 170 cells for siADSL+MRT00252040, ** $p<0.01$, **** $p<0.0001$). Scale bar=10 μm . **F.** Quantification of the cilia frequency in control and SAICAR-treated cells ($n=4$, scored 589 cells for control, 456 cells for SAICAR-treated, **** $p<0.0001$). **G.** Cilia length measurement of cells treated as in **(F)** ($n=5$, two-tailed t test, * $p<0.05$).

Figure 5. ADSL depletion and SAICAr impair CP110 removal. **A.** Quantification of the number of Centrin foci present in mitotic RPE-1 cells transfected with control or ADSL smart pool siRNAs for 96 hrs ($n=2$, two-tailed t test, *ns* not significant). **B.** ADSL silenced cells and controls were stained for Centrin (magenta) and CP110 (green). Nuclei are shown by DAPI (blue). Graph depicts the number of ciliated cells with two CP110 foci per centrosome. ($n=3$, scored 342 cells for siC, 221 cells for siADSL, * $p<0.05$). **C.** Cells mock or treated with SAICAr were processed and analyzed as described in panel **(B)** ($n=3$, scored 288 cells for control and 253 cells for SAICAr, *** $p<0.001$). **D.** RPE-1 depleted with ADSL and control siRNAs were treated with vehicle or MRT00252040 and stained as in **(B,C)**. Graph depicts the percentage of cells presenting 2 CP110 foci per centrosome ($n=2$; scored 177 cells for siC+DMSO, 180 cells for siADSL+DMSO, 75 cells for siC+MRT00252040, 78 cells for siADSL+MRT00252040, * $p<0.05$). **E.** RPE-1 depleted with ADSL and/or CP110 (silenced for 24 hrs with three different siRNAs) were serum starved for 48 hrs, fixed and stained for ARL13B (magenta) and PCNT (green). Graphs show number of ciliated cells ($n=3$ for siC, siADSL, siCP110#1, siADSL+siCP110#1; $n=2$ for siCP110#2, siCP110#3, siADSL+siCP110#2 and siADSL+siCP110#3, scored 461 cells for siC, 301 cells for siADSL, 277 cells for siCP110#1, 289 cells for siADSL+siCP110#1, 119 cells for siCP110#2, 90 cells for siADSL+siCP110#2, 141 cells for siCP110#3, 98 cells for siADSL+siCP110#3, **** $p<0.0001$, *** $p<0.001$) and cilia length ($n=3$, one-way ANOVA *** $p<0.001$, ** $p<0.01$, * $p<0.05$). All graphs show means $\pm$ SD with individual values shown in circles.

Figure 6. Depletion of Adsl in zebrafish causes developmental phenotypes and DNA damage signaling. **A.** Live images of 48 hpf zebrafish embryos showing pericardial edema (green arrow), kinked tail (yellow arrow), hydrocephalus (red arrow) and pinhead (blue arrow). NI (uninjected controls), CTRL MO (embryos injected with a standard control MO), Adsl ATG MO (injected with a translation blocking MO against Adsl). Scale bar=500 μm . **B-E.** Quantification of the percentage of embryos developing the indicated phenotypes. For **(B-E)** Each circle indicates one experiment. Data from 8

experiments with 311 embryos (NI), 275 (CTRL MO), 227 (Adsl ATG MO) is shown. Kruskal-Wallis test with Dunn's multiple comparison. Dashes show median. **p=0.0042 (pericardial edema), **p=0.0032 (kinked tail), **p=0.0011 (pinhead), ***p=0.0005 (hydrocephalus). **F.** Adsl depleted zebrafish display skull formation defects. Cartilage staining of zebrafish embryos (4 days post fertilization, dpf) with Alcian blue. Embryos were classified according to the severity of their phenotype in normal staining, weak staining or absent cartilage. Lateral and ventral view. Cartilage formation could be rescued by co-injection of capped mRNA encoding zebrafish Adsl. 6-8 experiments with a total of 178 embryos (NI), 133 (CTRL MO), 169 (Adsl ATG MO), 123 (Rescue). Injection of mRNA encoding human wt ADSL, but not the R426H ADSLD variant, restores cartilage formation in embryos. 4 experiments with a total of 116 embryos (NI), 81 (CTRL MO), 80 (Adsl ATG MO), 91 (+ *hADSL wt*) and 89 (+ *hADSL R426H*). Two-tailed Fisher's exact test; **** p<0.0001. Scale bar=200μm. **G.** Immunofluorescence staining of the neural tube (dorsal view) of control and Adsl depleted embryos 24 hpf for γH2AX (green) and Acetylated-tubulin (Ac-tub: red). Treatment with 60mM nucleosides was carried out in indicated samples. Experiments with 45 embryos per treatment are shown, dashes indicate median. Data were analyzed by using Kruskal-Wallis test with Dunn's correction. *p<0.05, ****p<0.0001. Scale bar=300μm. Unless indicated, comparisons are not significant.

Figure 7. Impaired LR asymmetry and cilium formation in the organ of laterality.

A. At 48 hpf ventricle (V) of the two-chambered zebrafish heart is placed left and above the atrium (A). Adsl depleted embryos more frequently develop inversely looped hearts or developed unlooped hearts (no loop) (as scored by whole mount *in situ* hybridization for *cardiac myosin light chain 2 (cmlc2)*). N=6 experiments with a total of 266 embryos (NI), 176 embryos (CTRL MO), 188 embryos (Adsl ATG MO). Scale bar=100μm. **B.** Whole mount *in situ* hybridization for *angiopoietin-like 3 (angptl3)* to assess liver placement in 48 hpf embryos. Dorsal view. Scale bar=200μm. 185 NI, 121 CTRL MO and 99 Adsl ATG MO embryos. (A, B) Two-tailed Fisher's exact test; **p<0.0015, ****p<0.0001. **C.** Whole mount *in situ* hybridization for the left lateral plate mesoderm marker *southpaw (spaw)* at 20 somite stage (ss). *Spaw* is normally expressed in the left lateral plate mesoderm. When LR asymmetry is disturbed, *spaw* can be detected on the right side or on both sides. Aberrant expression of *spaw* in Adsl morphants. Co-injection of RNA encoding zebrafish Adsl restores proper *spaw* expression. Two-tailed Fisher's

exact test; * $p=0.0451$, ** $p=0.0016$, **** $p<0.0001$. Results from 5 experiments with 121 embryos (NI), 142 (CTRL MO), 128 (Adsl ATG MO) and 105 (Rescue) are shown. Scale bar=200 μ m. **D.** Confocal z-stacks of the Kupffer's vesicle (KV) of 6 somite stage (ss) embryos. Cilia are stained red (acetylated tubulin), while apical cell borders were stained for PKC ζ (green). Scale bar=10 μ m. **E.** No significant changes in the size of the KV upon Adsl depletion. $n=25$ (CTRL MO), 25 (Adsl ATG MO) and 18 embryos (rescue with zebrafish *ads/* RNA). Each circle is one embryo, line indicates median. Kruskal-Wallis test with Dunn's correction. p -values: CTRL MO vs. Adsl ATG MO: 0.2582, CTRL MO vs. Rescue: >0.9999, Adsl ATG MO vs. Rescue: 0.1684. **F.** No significant changes in the number of cilia per KV. $n=32$ (CTRL MO), 30 (Adsl ATG MO) and 20 embryos (rescue with zebrafish *ads/* RNA). Each circle is one embryo, lines show means. One-way ANOVA with Sidak's multiple comparison test. $p=0.5538$ (CTRL MO vs. Adsl ATG MO), 0.2844 (CTRL MO vs. Rescue), 0.9225 (Adsl ATG MO vs. Rescue). **G.** Shorter cilia in Adsl morphants can be partially elongated by coinjection of RNA encoding zebrafish Adsl. $n=960$ cilia (CTRL MO), 798 (Adsl ATG MO), 540 (Rescue). Kruskal-Wallis test with Dunn's correction, lines indicate medians; *** $p=0.0008$.

Figure 8. Adsl depletion reduces neuronal lineage cell numbers that can be rescued by Methotrexate (MTX) treatment. **A.** Immunofluorescence whole-mount microscopy of neural tubes of 24 hpf zebrafish embryos (dorsal view) stained for acetylated tubulin (axons, red) and Elavl3/4 (green). Fewer Elavl3/4 positive cells in Adsl depleted embryos, that can be rescued by co-injection with RNA encoding zebrafish Adsl (Rescue). Graph shows Elavl3/4 counts of individual embryos, line indicates median. 3 experiments with 45 embryos (NI), 45 (CTRL MO), 45 (Adsl ATG MO), 45 (Rescue). Kruskal-Wallis test with Dunn's correction. Not significant = n.s. $p>0.9999$, **** $p<0.0001$. Scale bar=100 μ m. **B.** Methotrexate (MTX) treatment rescues Elavl3/4 positive cell numbers. Staining of the neural tube (dorsal view) of 24 hpf zebrafish embryos for acetylated tubulin (red) and or Elavl3/4 (green). Adsl morphants show fewer Elavl3/4 positive cells, which could be rescued by treatment with 100 μ M MTX. 5 experiments with 69 (CTRL MO), 75 (CTRL MO + MTX), 63 (Adsl ATG MO) and 58 (Adsl ATG MO + MTX) embryos. One-way ANOVA with sidak's multiple comparison. ns $p>0.9999$, **** $p<0.0001$. Scale bar=100 μ m. **C.** Forebrains of 24 hpf zebrafish embryos (left panels) stained for acetylated tubulin (red) and Sox2 positive neural progenitors (green), anterior view. Scale bar=200 μ m. Schematic of the developing brain of zebrafish embryos

adapted from (23), top right panel. The forebrain (red) is composed of the telencephalon with the olfactory bulb (1), the pallium (2), the optic recess region (3) and the diencephalon with the hypothalamus (4). Quantification of phenotypes (bottom right panel). *Adsl* morphants show fewer neural progenitor cells in the forebrain, that can partially be rescued with 100 μ M MTX from tailbud stage on. Data were analyzed using one-way ANOVA with Sidak's multiple comparison. Dashes show medians. Experiments with 45 embryos (CTRL MO + DMSO), 45 embryos (CTRL MO + MTX), 45 embryos (*Adsl* ATG MO + DMSO), 47 embryos (*Adsl* ATG MO + MTX). If not shown in the graph all other comparisons are not significant. D. Forebrains of 24 hpf zebrafish embryos (left panels) stained for acetylated tubulin (red) and Sox2 positive neural progenitors (green), anterior view. Scale bar=100 μ m. Quantification of phenotypes (right panel). *Adsl* morphants show similar neural progenitor cells in the forebrain regardless of supplementation with 1X nucleosides. Data were analyzed using one-way ANOVA with Sidak's multiple comparison, n.s. = $p > 0.9999$. Dashes show medians. Experiments with 45 embryos (CTRL MO + DMSO), 45 embryos (CTRL MO + MTX), 45 embryos (*Adsl* ATG MO + DMSO), 47 embryos (*Adsl* ATG MO + MTX).

Figure Supplements

Figure 1-figure supplement 1. The role of ADSL in DNPS and the purine nucleotide cycle. Schematic of the de novo purine synthesis pathway (DNPS), purine nucleotide cycle, purine salvage pathway and tetrahydrofolate cycle. Enzymes are indicated in purple and metabolites in blue. ADSL is shown in orange and the small molecule inhibitors used in red. Circles in corresponding colors indicate the reactions affected by ADSL or inhibitors, respectively. Metabolites that accumulate following ADSL depletion are shown in green.

Figure 1-figure supplement 2. ADSL depletion does not cause senescence or promote differentiation. **A.** Analysis of AMP and GMP levels following ADSL depletion in RPE-1 cells (see also Figure 1C). **B.** Analysis of S-Ado and SAICAr levels following ADSL depletion in RPE-1 cells treated or not with with 4 μ M MRT00252040 (PAICS inhibitor). Box cox transformation was applied using $\lambda=0$ for SAICAr and $\lambda=-0.5$ for S-Ado values. **C.** Analysis of S-Ado and SAICAr levels following ADSL depletion

in HeLa cells treated or not with with 4 uM MRT00252040 (PAICS inhibitor). Box cox transformation was applied using $\lambda=0$ for SAICAr and $\lambda=-0.5$ for S-Ado values. **D.** qRT-PCR confirmed ADSL depletion with a single siRNA against *ADSL* (siRNA#2; n=4 in triplicate, two-tailed *t* test **** $p<0.0001$). **E.** Flow cytometry analysis of cell cycle using propidium iodide staining in RPE-1 cells treated with control (Ci) siRNA or siRNA against ADSL. Representative data is shown (left) and data from n=3 independent cultures is graphed (right). Student *t*-test was applied (n=3 scored more than 10000 cells for each condition in each experiment, * $p<0.05$). **F.** Quantification of cell viability by Trypan blue in RPE-1 cells transfected with single control or ADSL siRNAs. (n=5, scored 807 cells for siC, 682 cells for siADSL, *ns* not significant). **G.** β -galactosidase assay in RPE-1 cells upon ADSL depletion treated as in panel F. Doxorubicin (doxo) was used as a positive control to induce senescence. **H.** Flow cytometry analysis of cell cycle using propidium iodide staining in RPE-1 p53 KO cells treated with control (Ci) siRNA or siRNA against ADSL. Representative data is shown (left) and data from n=3 independent cultures is graphed (right). Student *t* test was applied (n=3 scored more than 10000 cells for each condition in each experiment, *ns* not significant). **I.** Ki67 staining in RPE-1 p53 KO upon silencing with a single control or ADSL siRNA (siRNA#2; n=2, at least 200 cells for each condition per experiment were counted, *ns* not significant). Scale bar=20 μ m. All graphs depict means $\pm$ SD with individual values shown in circles. **J.** Control of ADSL depletion and siRNA resistant mutant expression 96 hrs post ADSL depletion. Scale bar=20 μ m. **K.** Cells were transfected with a single control or ADSL siRNA (siADSL#2) for 96 hrs, fixed and stained against Vimentin and Cytokeratin-20 (CK20). Serum starvation (SS) for 144 hrs was used as positive control for differentiation and CK20 staining. Quantification of the percentage of cells positive for Vimentin is shown. No CK20 positive cells were observed in ADSL depleted cells. Scale bar=20 μ m.

Figure 3-figure supplement 1. Lack of cell death or increased differentiation in developing ADSL-depleted chicken neural tubes. **A.** Representative transverse neural tube sections and quantification of mean Cleaved-Caspase-3 (CC3; green) immunofluorescence intensity obtained by comparing mean CC3 intensity on TUJ1- or TUJ1+ area (magenta) on the electroporated side (GFP area, blue) with the respective area on the non electropated side after 48 hpe with shCONTROL and shADSL (n=7 embryos (shCONTROL) and 6 embryos (shADSL), two-tailed *t*-test, *ns* not significant).

Scale bar=50 μ m. **B.** Rate of differentiation was analyzed by FACS after 48 hpe into HH12 neural tubes with shCONTROL and shADSL. 6-8 embryos per condition were used for each experiment. The mean of two independent experiments are shown in the left panel. Dot plots in right panels representing ELAVL3/4 intensity versus GFP intensity of a representative experiment.

Figure 4-figure supplement 1. ADSL-depletion impairs ciliogenesis that can be rescued by MTX treatment. **A.** Quantification of cilia length in RPE-1 cells transfected with a single control or ADSL siRNA (n=3, two-tailed *t*-test, **p<0.01). **B.** Quantification of cilia frequency in RPE-1 silenced as in (A) and serum starved for 72 hrs. (n=3, two-tailed *t*-test **p<0.01). **C.** Quantification of cilia length in RPE-1 treated for 24 hrs with 5 μ M MTX (DHFR inhibitor) (n=3, two-tailed *t*-test, *ns* not significant). **D.** Quantification of cilia frequency and cilia length in RPE-1 transfected with single control and ADSL siRNAs treated or not with 5 μ M MTX for 24 hrs before serum starvation (n=3, scored 324 cells for siC, 294 cells for siADSL, 253 cells for siC+MTX, 271 cells for siADSL+MTX, **p<0.01, *p<0.05). **E.** Western blotting for total AKT or active AKT (pS473) following 24 hrs SS, SS with SAICAr (48 hr pre-treatment, 24 hr SS) or 24 hrs SS followed by 20% serum for 30 min. **F.** Quantification of the number of ciliated cells and cilia length in RPE-1 p53KO upon ADSL depletion (n=3, scored 338 cells for siC, 321 cells for siADSL, **p<0.01, *p<0.05). **G.** Quantification of the number of ciliated cells and cilia length in RPE-1 upon ADSL depletion for 96hrs, serum starved for 48 hrs (t=0) and serum add back at different timepoints (t=3, t=6 hrs) (n=2, scored 95 cells for siC, 89 cells for siADSL).

Figure 5-figure supplement 1. Analysis of Pericentrin accumulation and CP110 depletion. **A.** Quantification of PCNT intensity upon ADSL depletion with a single siRNA#2 (n=2, two-tailed *t* test applied, *ns* not significant; median is shown). **B.** Western blot to confirm CP110 depletion after 24 hrs of silencing with three different siRNAs. **C.** CP110 presence in both centrioles (stained by anti-Centrin antibody, in magenta) upon 24 hrs of CP110 depletion in normal serum (10%) conditions. Quantification of centrioles presenting two foci per centrosome in control and in CP110 depleted cells with three different siRNAs (n=2, scored 82 cells for siC, 85 cells for siCP110#1, 77 cells for siCP110#2, 79 cells for siCP110#3, **p<0.01, *p<0.05). Scale bar=5 μ m. **D.** CP110 foci (in green) colocalizing with Centrobilin (marker of daughter centriole, in red) upon CP110

depletion for 24 hrs in normal serum (10%). Three different siRNAs were used. Scale bar=5µm.

Figure 6-supplement 1. *Adsl* expression in zebrafish development. Whole mount *in situ* hybridization for detection of *adsl* expression during zebrafish development. All scale bars=100 µm. **A-D.** *adsl* is ubiquitously expressed. **E-F.** *adsl* is expressed in the anterior part of the embryo including the optic primordium (arrow in F). **G-H.** At 18 somite stage (ss) *adsl* is expressed in the developing midbrain and hindbrain (arrows). **I-J.** *adsl* is expressed in several areas of the brain including the mesencephalon (arrows). **K-L.** *adsl* is expressed in several areas of the brain including the midbrain hindbrain boundary (arrows). **D, F, H, J, L.** dorsal views.

Figure 6-supplement 2. Test of knockdown efficiency. Whole mount antibody staining for acetylated tubulin (red) to visualize neurons and *Adsl* (green) in 24 hpf zebrafish embryos. Injection of ATG morpholino resulted in very weak expression of *Adsl* along axons. Images show anterior views of zebrafish heads. Scale bar=200 µm.

Figure 8-figure supplement 1. Dorsal view of part of the neural tube of 24 hpf zebrafish embryos (left panels). Acetylated tubulin (Ac-tub) is shown in red. Neural cells are stained for Elavl3+4 (green). *Adsl* morphants (*spIMO*) show fewer neuronal cells, which can be partially rescued by treatment with 100 µM Methotrexate from tailbud stage on. Data were analyzed by using one-way ANOVA with Sidak's multiple comparison. Dashes show median. Experiments with 45 embryos (CTRL MO + DMSO), 45 embryos (CTRL MO + MTX), 45 embryos (*Adsl* *spIMO* + DMSO), 45 embryos (*Adsl* *spIMO* + MTX). If not shown in graph, all other comparisons are not significant. Scale bar=100µm

Appendix 1. Summary of phenotypes identified in the systems used. Corresponding figures are indicated, as well as interventions that rescue or recapitulate the phenotype.

Appendix 2. Multiple reaction monitoring transitions. See details in materials and methods.

Source Data (provided in a single zip file Dutto source data v2.zip)

1260 **Figure 1-source data 1**
1261 **Related to Figure 1A, C-L**
1262 **Graphpad Prism file**
1263
1264 **Figure 1-source data 2**
1265 **Related to Figure 1B**
1266 **Jpg file**
1267
1268 **Figure 1-source data 3**
1269 **Related to Figure 1-figure supplement 2**
1270 **Graphpad Prism file**
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1272 **Figure 2-source data 1**
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1276 **Figure 3-source data 1**
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1280 **Figure 4-source data 1**
1281 **Related to Figure 4**
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1292 **Figure 5-source data 2**
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1300 **Figure 6-source data 1**
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1304 **Figure 6-source data 2**
1305 **Related to Figure 6F**
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1308 **Figure 6-source data 3**
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1314 **Graphpad Prism file**
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1316 **Figure 7-source data 1**
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1324 **Figure 7-source data 3**
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1328 **Figure 7-source data 4**
1329 **Related to Figure 7F**
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1332 **Figure 7-source data 5**
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1352 **Figure 8-source data 5**

1353 **Related to Figure 8-figure supplement 1**

1354 **Graphpad Prism file**

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1357 **Source raw microscopy data have been published in FigsharePlus**

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1359 Travis Stracker, Jens Luders, Melanie Philipp, Sebastian Pons, Ilaria Dutto and Antonio
1360 Herrera

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1364 Pathway specific effects of ADSL deficiency on neurodevelopment

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1368 <https://doi.org/10.25452/figshare.plus.c.5793614>

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Bibliography

1. B. Daignan-Fornier, B. Pinson, Yeast to study human purine metabolism diseases. *Cells*. **8** (2019), doi:10.3390/cells8010067.
2. J. Jaeken, G. Van den Berghe, An infantile autistic syndrome characterised by the presence of succinylpurines in body fluids. *Lancet*. **2**, 1058–1061 (1984).
3. A. Jurecka, M. Zikanova, S. Kmoch, A. Tylki-Szymańska, Adenylosuccinate lyase deficiency. *J. Inherit. Metab. Dis.* **38**, 231–242 (2015).
4. T. W. Stone, L. A. Roberts, B. J. Morris, P. A. Jones, H. A. Ogilvy, W. M. Behan, J. A. Duley, H. A. Simmonds, M. F. Vincent, G. van den Berghe, Succinylpurines induce neuronal damage in the rat brain. *Adv. Exp. Med. Biol.* **431**, 185–189 (1998).
5. G. Giaever, A. M. Chu, L. Ni, C. Connelly, L. Riles, S. Véronneau, S. Dow, A. Lucau-Danila, K. Anderson, B. André, A. P. Arkin, A. Astromoff, M. El-Bakkoury, R. Bangham, R. Benito, S. Brachat, S. Campanaro, M. Curtiss, K. Davis, A. Deutschbauer, K.-D. Entian, P. Flaherty, F. Foury, D. J. Garfinkel, M. Gerstein, D. Gotte, U. Güldener, J. H. Hegemann, S. Hempel, Z. Herman, D. F. Jaramillo, D. E. Kelly, S. L. Kelly, P. Kötter, D. LaBonte, D. C. Lamb, N. Lan, H. Liang, H. Liao, L. Liu, C. Luo, M. Lussier, R. Mao, P. Menard, S. L. Ooi, J. L. Revuelta, C. J. Roberts, M. Rose, P. Ross-Macdonald, B. Scherens, G. Schimmack, B. Shafer, D. D. Shoemaker, S. Sookhai-Mahadeo, R. K. Storms, J. N. Strathern, G. Valle, M. Voet, G. Volckaert, C. Wang, T. R. Ward, J. Wilhelmy, E. A. Winzeler, Y. Yang, G. Yen, E. Youngman, K. Yu, H. Bussey, J. D. Boeke, M. Snyder, P. Philippsen, R. W. Davis, M. Johnston, Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature*. **418**, 387–391 (2002).
6. B. Pinson, S. Vaur, I. Sagot, F. Coulpier, S. Lemoine, B. Daignan-Fornier, Metabolic intermediates selectively stimulate transcription factor interaction and modulate phosphate and purine pathways. *Genes Dev.* **23**, 1399–1407 (2009).
7. P. Chen, D. Wang, H. Chen, Z. Zhou, X. He, The nonessentiality of essential genes in yeast provides therapeutic insights into a human disease. *Genome Res.* **26**, 1355–1362 (2016).
8. R. Marsac, B. Pinson, C. Saint-Marc, M. Olmedo, M. Artal-Sanz, B. Daignan-Fornier, J.-E. Gomes, Purine Homeostasis Is Necessary for Developmental Timing, Germline Maintenance and Muscle Integrity in *Caenorhabditis elegans*. *Genetics*. **211**, 1297–1313 (2019).
9. A. R. Fenton, H. N. Janowitz, M. R. McReynolds, W. Wang, W. Hanna-Rose, A *Caenorhabditis elegans* model of adenylosuccinate lyase deficiency reveals neuromuscular and reproductive phenotypes of distinct etiology. *BioRxiv* (2017), doi:10.1101/181719.
10. K. E. Keller, I. S. Tan, Y.-S. Lee, SAICAR stimulates pyruvate kinase isoform M2 and promotes cancer cell survival in glucose-limited conditions. *Science*. **338**, 1069–1072 (2012).
11. K. E. Keller, Z. M. Doctor, Z. W. Dwyer, Y.-S. Lee, SAICAR induces protein kinase activity of PKM2 that is necessary for sustained proliferative signaling of cancer cells. *Mol. Cell*. **53**, 700–709 (2014).
12. M. Yan, S. Chakravarthy, J. M. Tokuda, L. Pollack, G. D. Bowman, Y.-S. Lee, Succinyl-5-aminoimidazole-4-carboxamide-1-ribose 5'-Phosphate (SAICAR) Activates Pyruvate Kinase Isoform M2 (PKM2) in Its Dimeric Form. *Biochemistry*. **55**, 4731–4736 (2016).
13. G. Zurlo, X. Liu, M. Takada, C. Fan, J. M. Simon, T. S. Ptacek, J. Rodriguez, A. von Kriegsheim, J. Liu, J. W. Locasale, A. Robinson, J. Zhang, J. M. Holler, B.

- Kim, M. Zikánová, J. Bierau, L. Xie, X. Chen, M. Li, C. M. Perou, Q. Zhang, Prolyl hydroxylase substrate adenylosuccinate lyase is an oncogenic driver in triple negative breast cancer. *Nat. Commun.* **10**, 5177 (2019).
14. S. Agarwal, B. V. S. K. Chakravarthi, M. Behring, H.-G. Kim, D. S. Chandrashekar, N. Gupta, P. Bajpai, A. Elkholy, S. A. H. Balasubramanya, C. Hardy, S. A. Diffalha, S. Varambally, U. Manne, PAICS, a purine nucleotide metabolic enzyme, is involved in tumor growth and the metastasis of colorectal cancer. *Cancers (Basel)*. **12** (2020), doi:10.3390/cancers12040772.
15. T. Yamauchi, K. Miyawaki, Y. Semba, M. Takahashi, Y. Izumi, J. Nogami, F. Nakao, T. Sugio, K. Sasaki, L. Pinello, D. E. Bauer, T. Bamba, K. Akashi, T. Maeda, Targeting leukemia-specific dependence on the de novo purine synthesis pathway. *Leukemia* (2021), doi:10.1038/s41375-021-01369-0.
16. S. Taha-Mehlitz, G. Bianco, M. Coto-Llerena, V. Kancherla, G. R. Bantug, J. Gallon, C. Ercan, F. Panebianco, S. Eppenberger-Castori, M. von Strauss, S. Staubli, M. Bolli, R. Peterli, M. S. Matter, L. M. Terracciano, M. von Flüe, C. K. Y. Ng, S. D. Soysal, O. Kollmar, S. Piscuoglio, Adenylosuccinate lyase is oncogenic in colorectal cancer by causing mitochondrial dysfunction and independent activation of NRF2 and mTOR-MYC-axis. *Theranostics*. **11**, 4011–4029 (2021).
17. S. Tamiya, L. Liu, H. J. Kaplan, Epithelial-mesenchymal transition and proliferation of retinal pigment epithelial cells initiated upon loss of cell-cell contact. *Invest. Ophthalmol. Vis. Sci.* **51**, 2755–2763 (2010).
18. G. Hoxhaj, J. Hughes-Hallett, R. C. Timson, E. Ilagan, M. Yuan, J. M. Asara, I. Ben-Sahra, B. D. Manning, The mTORC1 Signaling Network Senses Changes in Cellular Purine Nucleotide Levels. *Cell Rep.* **21**, 1331–1346 (2017).
19. M. P. Kim, Y. Zhang, G. Lozano, Mutant p53: Multiple Mechanisms Define Biologic Activity in Cancer. *Front. Oncol.* **5**, 249 (2015).
20. B. Benedict, T. van Harn, M. Dekker, S. Hermsen, A. Kucukosmanoglu, W. Pieters, E. Delzenne-Goette, J. C. Dorsman, E. Petermann, F. Foijer, H. Te Riele, Loss of p53 suppresses replication-stress-induced DNA breakage in G1/S checkpoint deficient cells. *Elife*. **7** (2018), doi:10.7554/eLife.37868.
21. V. Gottifredi, S. Shieh, Y. Taya, C. Prives, p53 accumulates but is functionally impaired when DNA synthesis is blocked. *Proc. Natl. Acad. Sci. USA*. **98**, 1036–1041 (2001).
22. V. Walia, A. Cuenca, M. Vetter, C. Insinna, S. Perera, Q. Lu, D. A. Ritt, E. Semler, S. Specht, J. Stauffer, D. K. Morrison, E. Lorentzen, C. J. Westlake, Akt Regulates a Rab11-Effector Switch Required for Ciliogenesis. *Dev. Cell*. **50**, 229–246.e7 (2019).
23. R. Vaz, W. Hofmeister, A. Lindstrand, Zebrafish models of neurodevelopmental disorders: limitations and benefits of current tools and techniques. *Int. J. Mol. Sci.* **20** (2019), doi:10.3390/ijms20061296.
24. A. Jurecka, M. Zikanova, A. Tylki-Szymanska, J. Krijt, A. Bogdanska, W. Gradowska, K. Mullerova, J. Sykut-Cegielska, S. Kmoch, E. Pronicka, Clinical, biochemical and molecular findings in seven Polish patients with adenylosuccinate lyase deficiency. *Mol. Genet. Metab.* **94**, 435–442 (2008).
25. A. Jurecka, E. Jurkiewicz, A. Tylki-Szymanska, Magnetic resonance imaging of the brain in adenylosuccinate lyase deficiency: a report of seven cases and a review of the literature. *Eur. J. Pediatr.* **171**, 131–138 (2012).
26. K. Mouchegh, M. Zikánová, G. F. Hoffmann, B. Kretzschmar, T. Kühn, E. Mildenerberger, G. Stoltenburg-Didinger, J. Krijt, L. Dvoráková, T. Honzík, J. Zeman, S. Kmoch, R. Rossi, Lethal fetal and early neonatal presentation of adenylosuccinate lyase deficiency: observation of 6 patients in 4 families. *J.*

- 1473 *Pediatr.* **150**, 57–61.e2 (2007).
- 1474 27. T. H. Stracker, C. G. Morrison, F. Gergely, Molecular causes of primary
1475 microcephaly and related diseases: a report from the UNIA Workshop.
1476 *Chromosoma*. **129**, 115–120 (2020).
- 1477 28. M. O'Driscoll, V. L. Ruiz-Perez, C. G. Woods, P. A. Jeggo, J. A. Goodship, A
1478 splicing mutation affecting expression of ataxia-telangiectasia and Rad3-related
1479 protein (ATR) results in Seckel syndrome. *Nat. Genet.* **33**, 497–501 (2003).
- 1480 29. M. Murga, S. Bunting, M. F. Montaña, R. Soria, F. Mulero, M. Cañamero, Y. Lee,
1481 P. J. McKinnon, A. Nussenzweig, O. Fernandez-Capetillo, A mouse model of ATR-
1482 Seckel shows embryonic replicative stress and accelerated aging. *Nat. Genet.* **41**,
1483 891–898 (2009).
- 1484 30. M. S. Al-Dosari, R. Shaheen, D. Colak, F. S. Alkuraya, Novel CENPJ mutation
1485 causes Seckel syndrome. *J. Med. Genet.* **47**, 411–414 (2010).
- 1486 31. J.-H. Sir, A. R. Barr, A. K. Nicholas, O. P. Carvalho, M. Khurshid, A. Sossick, S.
1487 Reichelt, C. D'Santos, C. G. Woods, F. Gergely, A primary microcephaly protein
1488 complex forms a ring around parental centrioles. *Nat. Genet.* **43**, 1147–1153
1489 (2011).
- 1490 32. H. Bazzi, K. V. Anderson, Acentriolar mitosis activates a p53-dependent apoptosis
1491 pathway in the mouse embryo. *Proc. Natl. Acad. Sci. USA*. **111**, E1491-500
1492 (2014).
- 1493 33. R. Insolera, H. Bazzi, W. Shao, K. V. Anderson, S.-H. Shi, Cortical neurogenesis in
1494 the absence of centrioles. *Nat. Neurosci.* **17**, 1528–1535 (2014).
- 1495 34. M. Marjanović, C. Sánchez-Huertas, B. Terré, R. Gómez, J. F. Scheel, S.
1496 Pacheco, P. A. Knobel, A. Martínez-Marchal, S. Aivio, L. Palenzuela, U. Wolfrum,
1497 P. J. McKinnon, J. A. Suja, I. Roig, V. Costanzo, J. Lüders, T. H. Stracker, CEP63
1498 deficiency promotes p53-dependent microcephaly and reveals a role for the
1499 centrosome in meiotic recombination. *Nat. Commun.* **6**, 7676 (2015).
- 1500 35. R. E. McIntyre, P. Lakshminarasimhan Chavali, O. Ismail, D. M. Carragher, G.
1501 Sanchez-Andrade, J. V. Forment, B. Fu, M. Del Castillo Velasco-Herrera, A.
1502 Edwards, L. van der Weyden, F. Yang, Sanger Mouse Genetics Project, R.
1503 Ramirez-Solis, J. Estabel, F. A. Gallagher, D. W. Logan, M. J. Arends, S. H.
1504 Tsang, V. B. Mahajan, C. L. Scudamore, J. K. White, S. P. Jackson, F. Gergely, D.
1505 J. Adams, Disruption of mouse Cenpj, a regulator of centriole biogenesis,
1506 phenocopies Seckel syndrome. *PLoS Genet.* **8**, e1003022 (2012).
- 1507 36. Y.-N. Lin, Y.-S. Lee, S.-K. Li, T. K. Tang, Loss of CPAP in developing mouse brain
1508 and its functional implication for human primary microcephaly. *J. Cell Sci.* **133**
1509 (2020), doi:10.1242/jcs.243592.
- 1510 37. C. S. Fong, G. Mazo, T. Das, J. Goodman, M. Kim, B. P. O'Rourke, D. Izquierdo,
1511 M.-F. B. Tsou, 53BP1 and USP28 mediate p53-dependent cell cycle arrest in
1512 response to centrosome loss and prolonged mitosis. *Elife*. **5** (2016),
1513 doi:10.7554/eLife.16270.
- 1514 38. B. G. Lambrus, V. Daggubati, Y. Uetake, P. M. Scott, K. M. Clutario, G. Sluder, A.
1515 J. Holland, A USP28-53BP1-p53-p21 signaling axis arrests growth after
1516 centrosome loss or prolonged mitosis. *J. Cell Biol.* **214**, 143–153 (2016).
- 1517 39. K. Holzer, E. Drucker, S. Roessler, D. Dauch, F. Heinzmann, N. Waldburger, E.-M.
1518 Eiteneuer, E. Herpel, K. Breuhahn, L. Zender, P. Schirmacher, A. Ori, S. Singer,
1519 Proteomic analysis reveals GMP synthetase as p53 repression target in liver
1520 cancer. *Am. J. Pathol.* **187**, 228–235 (2017).
- 1521 40. B. A. Reddy, J. A. van der Knaap, A. G. M. Bot, A. Mohd-Sarip, D. H. W. Dekkers,
1522 M. A. Timmermans, J. W. M. Martens, J. A. A. Demmers, C. P. Verrijzer,
1523 Nucleotide biosynthetic enzyme GMP synthase is a TRIM21-controlled relay of p53

- stabilization. *Mol. Cell.* **53**, 458–470 (2014).
41. E. Gabriel, A. Wason, A. Ramani, L. M. Gooi, P. Keller, A. Pozniakovsky, I. Poser, F. Noack, N. S. Telugu, F. Calegari, T. Šarić, J. Hescheler, A. A. Hyman, M. Gottardo, G. Callaini, F. S. Alkuraya, J. Gopalakrishnan, CPAP promotes timely cilium disassembly to maintain neural progenitor pool. *EMBO J.* **35**, 803–819 (2016).
42. M. Farooq, L. Lindbæk, N. Krogh, C. Doganli, C. Keller, M. Mönnich, A. B. Gonçalves, S. Sakthivel, Y. Mang, A. Fatima, V. S. Andersen, M. S. Hussain, H. Eiberg, L. Hansen, K. W. Kjaer, J. Gopalakrishnan, L. B. Pedersen, K. Møllgård, H. Nielsen, S. M. Baig, N. Tommerup, S. T. Christensen, L. A. Larsen, RRP7A links primary microcephaly to dysfunction of ribosome biogenesis, resorption of primary cilia, and neurogenesis. *Nat. Commun.* **11**, 5816 (2020).
43. C. R. McKeown, H. T. Cline, Nutrient restriction causes reversible G2 arrest in *Xenopus* neural progenitors. *Development.* **146** (2019), doi:10.1242/dev.178871.
44. D. Cloëtta, V. Thomanetz, C. Baranek, R. M. Lustenberger, S. Lin, F. Oliveri, S. Atanasoski, M. A. Rüegg, Inactivation of mTORC1 in the developing brain causes microcephaly and affects gliogenesis. *J. Neurosci.* **33**, 7799–7810 (2013).
45. G. D. Gupta, É. Coyaoud, J. Gonçalves, B. A. Mojarad, Y. Liu, Q. Wu, L. Gheiratmand, D. Comartin, J. M. Tkach, S. W. T. Cheung, M. Bashkurov, M. Hasegan, J. D. Knight, Z.-Y. Lin, M. Schueler, F. Hildebrandt, J. Moffat, A.-C. Gingras, B. Raught, L. Pelletier, A Dynamic Protein Interaction Landscape of the Human Centrosome-Cilium Interface. *Cell.* **163**, 1484–1499 (2015).
46. G. M. Xavier, M. Seppala, W. Barrell, A. A. Birjandi, F. Geoghegan, M. T. Cobourne, Hedgehog receptor function during craniofacial development. *Dev. Biol.* **415**, 198–215 (2016).
47. F. Bangs, K. V. Anderson, Primary cilia and mammalian hedgehog signaling. *Cold Spring Harb. Perspect. Biol.* **9** (2017), doi:10.1101/cshperspect.a028175.
48. J. J. Breunig, M. R. Sarkisian, J. I. Arellano, Y. M. Morozov, A. E. Ayoub, S. Sojitra, B. Wang, R. A. Flavell, P. Rakic, T. Town, Primary cilia regulate hippocampal neurogenesis by mediating sonic hedgehog signaling. *Proc. Natl. Acad. Sci. USA.* **105**, 13127–13132 (2008).
49. Y.-G. Han, N. Spassky, M. Romaguera-Ros, J.-M. Garcia-Verdugo, A. Aguilar, S. Schneider-Maunoury, A. Alvarez-Buylla, Hedgehog signaling and primary cilia are required for the formation of adult neural stem cells. *Nat. Neurosci.* **11**, 277–284 (2008).
50. E. Campeau, V. E. Ruhl, F. Rodier, C. L. Smith, B. L. Rahmberg, J. O. Fuss, J. Campisi, P. Yaswen, P. K. Cooper, P. D. Kaufman, A versatile viral system for expression and depletion of proteins in mammalian cells. *PLoS One.* **4**, e6529 (2009).
51. T. Dull, R. Zufferey, M. Kelly, R. J. Mandel, M. Nguyen, D. Trono, L. Naldini, A third-generation lentivirus vector with a conditional packaging system. *J. Virol.* **72**, 8463–8471 (1998).
52. S. A. Stewart, D. M. Dykxhoorn, D. Palliser, H. Mizuno, E. Y. Yu, D. S. An, D. M. Sabatini, I. S. Y. Chen, W. C. Hahn, P. A. Sharp, R. A. Weinberg, C. D. Novina, Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA.* **9**, 493–501 (2003).
53. Y. A. Ogungbenro, T. C. Tena, D. Gaboriau, P. Lalor, P. Dockery, M. Philipp, C. G. Morrison, Centrobins controls primary ciliogenesis in vertebrates. *J. Cell Biol.* **217**, 1205–1215 (2018).
54. C. Pantoja, M. Serrano, Murine fibroblasts lacking p21 undergo senescence and are resistant to transformation by oncogenic Ras. *Oncogene.* **18**, 4974–4982

- 1575 (1999).
- 1576 55. V. Baresova, M. Krijt, V. Skopova, O. Souckova, S. Kmoch, M. Zikanova, CRISPR-
- 1577 Cas9 induced mutations along de novo purine synthesis in HeLa cells result in
- 1578 accumulation of individual enzyme substrates and affect purinosome formation.
- 1579 *Mol. Genet. Metab.* **119**, 270–277 (2016).
- 1580 56. M. Zikánová, J. Krijt, H. Hartmannová, S. Kmoch, Preparation of 5-amino-4-
- 1581 imidazole-N-succinocarboxamide ribotide, 5-amino-4-imidazole-N-
- 1582 succinocarboxamide riboside and succinyladenosine, compounds usable in
- 1583 diagnosis and research of adenylosuccinate lyase deficiency. *J. Inherit. Metab.*
- 1584 *Dis.* **28**, 493–499 (2005).
- 1585 57. L. Mádrová, M. Krijt, V. Barešová, J. Václavík, D. Friedecký, D. Dobešová, O.
- 1586 Součková, V. Škopová, T. Adam, M. Zikánová, Mass spectrometric analysis of
- 1587 purine de novo biosynthesis intermediates. *PLoS One.* **13**, e0208947 (2018).
- 1588 58. K. M. Jaffe, S. Y. Thiberge, M. E. Bisher, R. D. Burdine, Imaging cilia in zebrafish.
- 1589 *Methods Cell Biol.* **97**, 415–435 (2010).
- 1590 59. B. Thisse, C. Thisse, In situ hybridization on whole-mount zebrafish embryos and
- 1591 young larvae. *Methods Mol. Biol.* **1211**, 53–67 (2014).
- 1592 60. M. D. Burkhalter, G. B. Fralish, R. T. Premont, M. G. Caron, M. Philipp, Grk5l
- 1593 controls heart development by limiting mTOR signaling during symmetry breaking.
- 1594 *Cell Rep.* **4**, 625–632 (2013).
- 1595 61. V. Hamburger, H. L. Hamilton, A series of normal stages in the development of the
- 1596 chick embryo. 1951. *Dev. Dyn.* **195**, 231–272 (1992).
- 1597 62. A. Herrera, M. Saade, A. Menendez, E. Marti, S. Pons, Sustained Wnt/ β -catenin
- 1598 signalling causes neuroepithelial aberrations through the accumulation of aPKC at
- 1599 the apical pole. *Nat. Commun.* **5**, 4168 (2014).
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Appendix 1

<i>In vitro (RPE-1)</i>				
Phenotype	Defined by	Complemented with	Recapitulated by	Figures
Cell cycle exit	Ki67 positivity	ADSL expression, p53-KO	-	1E, 1H, 1I, 1L, 1-FS2I
Cell cycle arrest (G1)	Increased P53 levels, flow cytometry, cumulative growth	P53 deletion	-	1D, 1F, 1G, 1J, 1K, 1-FS2E, 1-FS2H
Elevated DNA damage signaling	γ H2AX, 53BP1 foci	ATM inhibitor (53BP1), nucleosides, adenosine	Hydroxyurea, ionizing radiation	2B-H
Elevated replication stress	Chromatin bound RPA	Nucleosides, adenosine	-	2A
Ciliogenesis defect	IF analysis of ARL13B, centriole markers, CP110	PAICS inhibition, MTX treatment, CP110 depletion	SAICAr addition	4B-G, 4-FS1C-E, 5A-E
<i>In vivo (chicken)</i>				
Phenotype	Defined by	Complemented with	Recapitulated by	Figure
Microcephaly	Reduced neural tube size	-	-	3B
Cell cycle arrest (G2)	Edu/H3S10 IF, Flow cytometry	-	-	3C-F
Reduced Sox2+ progenitors	SOX2 IF, flow cytometry	-	-	3B, 3C
Reduced differentiated cells	ELAVL3/4 IF	-	-	3B, 3C, 3-FS1B
<i>In vivo (fish)</i>				
Phenotype	Defined by	Complemented with	Recapitulated by	Figure
Microcephaly	Pinhead, hydrocephalus, pericardial edema, kinked tail, defective skull formation	Fish or human ADSL expression	-	6A-F

Reduced Sox2+ progenitors	SOX2 IF	MTX treatment	-	8B, 8C
Reduced differentiated cells	ELAVL3/4 IF	MTX treatment, ADSL expression	-	8A-B, 8-FS1
Ciliogenesis defect	Left-right patterning defect, reduced cilia length	ADSL expression	-	6A-F, 7A-G
Elevated DNA damage signaling	γH2AX IF	Nucleosides	-	6G

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Appendix 2

Multiple reaction monitoring transitions			
Metabolite	1 st transition	2 nd transition	Polarity
GMP	364→152	364→135	Positive
AMP	348→136	348→119	Positive
SAICAr	375→243	375→110	Positive
SAdo	384→252	384→234	Positive
¹³ C ₄ -SAICAr	379→247		Positive
¹³ C ₄ -SAdo	388→256		Positive

Key Resources Table				
Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
gene (<i>Homo sapiens</i>)	<i>ADSL isoform1</i>	NCBI Gene	CCDS14001.1	
gene (<i>Gallus gallus</i>)	<i>Adsl</i>	Genbank	M37901.1	
gene (<i>Danio rerio</i>)	<i>adsl</i>	Genbank	NM_199899.2	
gene (<i>Danio rerio</i>)	<i>angptl3</i>	Genbank	AF379604	
gene (<i>Danio rerio</i>)	<i>spaw</i>	Genbank	NM_180967	
gene (<i>Danio rerio</i>)	<i>cmlc2</i>	Genbank	PMID: 10491254	
strain, strain background (<i>Gallus gallus</i> , eggs)	White Leghorn fertilized eggs	Granja Gilbert S.A.		
strain, strain background (<i>Danio rerio</i> , male and female)	AB	ZFIN	ZFIN ID: ZDB-GENO-960809-7	
strain, strain background (<i>Danio rerio</i> , male and female)	EK	ZFIN	ZFIN ID: ZDB-GENO-990520-2	
cell line (human)	hTERT-RPE-1	ATCC	Cat. #: CRL-4000 RRID:CVCL_4388	Authenticated by STR testing, routinely tested for mycoplasma and found negative

cell line (human)	HeLa	ATCC	Cat. #: CCL-2 RRID: CVCL_0030	Authenticated by STR testing, routinely tested for mycoplasma and found negative
cell line (human)	hTERT-RPE-1 p53KO	Kind gift from Bryan Tsou, Sloan-Kettering Institute	PMID: 27371829	P53 genotype confirmed by western blotting, routinely tested for mycoplasma and found negative.
cell line (human)	AD-293	Agilent	Cat. #: 240085 RRID:CVCL_9804	Authenticated by STR testing, routinely tested for mycoplasma and found negative.
transfected construct (human)	pLenti-CMV-GFP- BLAST (659-1)	Addgene	Cat.#: 17445 RRID:Addgene_1 7445	pLenti CMV GFP Blast (659-1) was a gift from Eric Campeau & Paul Kaufman (Addgene plasmid # 17445 ; http://n2t.net/addgene:17445 ; RRID:Addgene _17445)
transfected construct (human)	pMDLg/pRRE	Addgene	Cat.#:12251 RRID:Addgene_1 2251	pMDLg/pRRE was a gift from Didier Trono (Addgene plasmid # 12251 ; http://n2t.net/addgene:12251 ; RRID:Addgene _12251)
transfected construct (human)	pCMV-VSV-G	Addgene	Cat.#:8454 RRID:Addgene_8 454	pCMV-VSV-G was a gift from Bob Weinberg (Addgene plasmid # 8454 ;

				http://n2t.net/addgene:8454 ; RRID:Addgene_8454)
transfected construct (human)	pRSV-REV	Addgene	Cat.#:12253 RRID:Addgene_12253	pRSV-Rev was a gift from Didier Trono (Addgene plasmid # 12253 ; http://n2t.net/addgene:12253 ; RRID:Addgene_12253)
antibody	anti-acetylated α Tubulin (6-11B-1) (Mouse monoclonal)	Santa Cruz Biotechnology, INC.	Cat. #: sc-23950 RRID:AB_628409	IF (1:1000 human cells)
antibody	anti-Elav3/4 (Rabbit polyclonal)	GeneTex	Cat. #: GTX128365 RRID:AB_2885764	IF (1:1000 human cells)
antibody	anti-SOX2 (Rabbit polyclonal)	Abcam	Cat. #: ab97959 RRID:AB_2341193	IF (1:1000 human cells)
antibody	anti- γ H2AX (Rabbit polyclonal)	GeneTex	Cat. #: GTX127342 RRID:AB_2833105	IF (1:400 human cells)
antibody	anti-AKT (pan) (Rabbit polyclonal)	Cell Signaling	Cat. #: 2920 RRID:AB_1147620	Western (1:2000 human cells)
antibody	anti-AKT p-S473 (Rabbit polyclonal)	Cell Signaling	Cat. #: 4060 RRID:AB_2315049	Western (1:2000 human cells)
antibody	anti-Vinculin (Rabbit polyclonal)	Cell Signaling	Cat. #: 13901 RRID:AB_2728768	Western (1:2000 human cells)
antibody	anti-ADSL (Rabbit polyclonal)	Millipore Sigma	Cat. #: HPA000525 RRID:AB_1078106	IF (1:200 fish, 1:100 human cells)
antibody	anti-PKC ζ (C-20) (Rabbit polyclonal)	Santa Cruz Biotechnology, INC.	Cat. #: sc-216 RRID:AB_2300359	IF (1:500 fish)

antibody	Alexa Fluor® 568 donkey anti-mouse IgG (Donkey polyclonal)	Molecular Probes	Cat. #: A10037 RRID:AB_253401 3	IF (1:1000 fish)
antibody	Alexa Fluor® 488 donkey anti-rabbit IgG (Donkey polyclonal)	Molecular Probes	Cat. #: A32790 RRID:AB_276283 3	IF (1:1000 fish)
antibody	Alexa Fluor® 594 goat anti-mouse IgG cross-adsorbed (goat polyclonal)	Invitrogen/ ThermoFisher Scientific	Cat. #: A32742 RRID:AB_276282 5	IF (1:400 human cells)
antibody	Alexa Fluor® 488 goat anti-rabbit IgG cross-adsorbed (goat polyclonal)	Invitrogen/ ThermoFisher Scientific	Cat. #: A11034 RRID:AB_257621 7	IF (1:400 human cells)
antibody	Anti-Digoxigenin-AP Fab fragments (Sheep polyclonal)	Roche	Cat. #: 11093274910 RRID:AB_514497	ISH (1:5000 fish)
antibody	Anti-Actin (AC-40)	Millipore Sigma	Cat. #: A4700 RRID:AB_476730	Western (1:1500 human cells)
antibody	Anti-Ki67 (Mouse monoclonal)	Novocastra	Cat. #: NCL-Ki-67p RRID:AB_442102	IF (1:500 human cells)
antibody	Anti-p53 (1C12)	Cell Signaling	Cat. #: 2524 RRID:AB_331743	IF (1:100 human cells)
antibody	Anti-Vimentin (rabbit polyclonal)	Abcam	Cat. #: ab45939 RRID: AB_2257290	IF (1:100 human cells)
antibody	Anti-CK20 (Ks20.8) (mouse monoclonal)	Dako/Agilent	Cat. #: GA77761-2	IF (1:200 human cells)
antibody	Anti-53BP1 (rabbit polyclonal)	Novus Biologicals	Cat. #: NB100-304 RRID: AB_10003037	IF (1:400 human cells)

antibody	Anti- γ H2AX-pS139 (rabbit polyclonal)	Santa Cruz Biotechnology, INC.	Cat. #: sc-517336 RRID:AB_2133718	IF (1:100 human cells)
antibody	Anti-RPA32	Millipore Sigma	Cat. #: NA19L RRID:AB_565123	IF (1:100 human cells)
antibody	Anti-SOX2 (rabbit polyclonal)	Invitrogen/ ThermoFisher Scientific	Cat. #: 48-1400 RRID:AB_2533841	IF (1:500 chicken)
antibody	Anti-ELAVL3/4 (HuC/HuD) 16A11)	Molecular Probes/ ThermoFisher Scientific	Cat. #: A21271 RRID:AB_221448	IF (1:500 chicken)
antibody	Anti-pH3S10 (rabbit polyclonal)	Millipore Sigma	Cat. #: 06-570 RRID:AB_310177	IF (1:500 chicken)
antibody	Anti-Cleaved Caspase 3 (CC3)	Millipore Sigma	Cat. #: AB3623 RRID:AB_91556	IF (1:500 chicken)
antibody	Anti-TUJ1	Covance	Cat. #: MMS-435P RRID:AB_2313773	IF (1:1000 chicken)
antibody	PAX6 (mouse monoclonal)	DSHB	Cat.#: AB_528427 RRID:AB_528427	IF (1:250 chicken)
antibody	Anti-ARL13B (C5) (mouse monoclonal)	Santa Cruz Biotechnology	Cat. #: sc-515784 RRID:AB_2890034	IF (1:100)
antibody	Anti-Pericentrin (PCNT) (rabbit polyclonal)	Novus Biologicals	Cat. #: NBP1-87772 RRID:AB_11018354	IF (1:400)
antibody	Anti-CP110 (rabbit polyclonal)	Kind gift from Andrew Holland	Unpublished reagent	IF (1:1000)

antibody	Anti-centrin (20H5) (mouse monoclonal)	Millipore Sigma	Cat. #: 04-1624 RRID:AB_AB_10563501	IF (1:1000)
antibody	Anti-Centrobin (mouse monoclonal)	Kind gift from Ciaran Morrison	PMID: 29440264	IF (1:500)
recombinant DNA reagent	pLenti-CMV-ADSL*-EGFP siRNA resistant	This paper.		
recombinant DNA reagent	pCR2.1-TOPO	Invitrogen/ ThermoFisher Scientific	Cat. #: 450640 RRID:Addgene_26778	
recombinant DNA reagent	pCRII-zfAdsl	This paper		Template for generation of antisense <i>in-situ</i> probe (zebrafish)
recombinant DNA reagent	pCS2+Flag-zfAdsl	This paper		Template for generation of capped mRNA (zebrafish)
recombinant DNA reagent	pCS2+Flag-zfAdsl MO mut	This paper		Template for generation of capped mRNA (insensitive to ATG MO) (zebrafish)
recombinant DNA reagent	pCS2+hAdsl	This paper		Template for generation of capped mRNA (zebrafish)
recombinant DNA reagent	pCS2+hAdsl R426H	This paper		Template for generation of capped mRNA (zebrafish)
recombinant DNA reagent	pSHIN	Kind gift of Dr. Kojima	PMID: 24741441	
sequence-based reagent	ADSL Smartpool siRNA	Dharmacon	Cat. #: M-010986-01-0005	
sequence-based reagent	ADSL siRNA#2	Millipore Sigma	Custom	CAAGAUUUG CACCGACAU A

sequence-based reagent	CP110 siRNA#1	Millipore Sigma	Custom	GCAAAACCA GAUACGAG AUU
sequence-based reagent	CP110 siRNA#2	Millipore Sigma	Custom	CAAGCGGAC UCACUCCAU ATT
sequence-based reagent	CP110 siRNA#3	Millipore Sigma	Custom	TAGACTTAT GCAGACAGA TAA
sequence-based reagent	EGFP siRNA	Millipore Sigma	Custom	GGCUACGU CCAGGAGC GCCGCACC
sequence-based reagent	GL2 siRNA (siC, targets luciferase)	Millipore Sigma	Custom, published in PMID: 11373684	CGUACGCG GAUACUUC GA
sequence-based reagent	ADSL-BsiWI-F	Millipore Sigma	Custom	5'AAAACGTA CGATGG CGGCTGGA GGCGATCAT 3'
sequence-based reagent	ADSL- <i>EcoR1-R</i>	Millipore Sigma	Custom	5'TTTTGAATT CCAGACA TAATTCTGC TTTCA3'
sequence-based reagent	shCONTROL	Millipore Sigma	Custom, for control in chicken embryo	5'- CCGGTCTCG A CGGTCGAGT -3'
sequence-based reagent	shADSL	Millipore Sigma	Custom, for ADSL depletion in chicken embryo	5'- GAGCTGGAC A GATTAGTGA- 3'
sequence-based reagent	Adsl ATG MO	GeneTools	Custom, for Adsl depletion in fish	5'- TCCCTCCAT GC CTGCAGC GGTTAAA
sequence-based reagent	Adsl splMO	GeneTools	Custom, for Adsl depletion in fish	5'- CCAACTGTG GG AGAGAGC GACTGTA

sequence-based reagent	Std. CTRL MO	GeneTools	Custom, for control in fish	5'- CCTCTTACC TCA GTTACAATTT ATA
commercial assay or kit	Click-iT EdU imaging kit	Invitrogen/ ThermoFisher Scientific	Cat.#: C10340	
commercial assay or kit	TOPO TA cloning	Invitrogen/ ThermoFisher Scientific	Cat. #: 450640	
commercial assay or kit	AmpliCap™ SP6 High Yield Message Maker Kit	Cellscript	Cat. #: C-AC0706	
commercial assay or kit	QuikChange Lightning mutagenesis kit	ThermoFisher Scientific	Cat. #: 210518	
commercial assay or kit	DC protein assay	Biorad	Cat. #: 500-0111	
commercial assay or kit	Immobilion ECL Ultra	Millipore Sigma	Cat. #:WBULS0100	
chemical compound, drug	Formaldehyde, 37%	Millipore Sigma	Cat. #:47608-500ML-F	Used at 4% for cell fixation.
commercial assay or kit	Senescence β -Galactosidase Staining Kit	Cell Signaling	Cat. #: 9860	
commercial assay or kit	High Capacity RNA-to-cDNA Kit	Applied Biosystems	Cat. #: 4387406	
commercial assay or kit	TaqMan Universal PCR Master Mix	Thermo Fisher Scientific	Cat. #: 4324018	
commercial assay or kit	Quick ligation kit	NEB	Cat. #: 4324018	

chemical compound, drug	Lipofectamine RNAiMAX	ThermoFisher Scientific	Cat. #: 13778150	
chemical compound, drug	Opti-MEM	Gibco	Cat. #: 31985070	
chemical compound, drug	DMEM-F12	Gibco	Cat.#: 21331046	
chemical compound, drug	Tri-reagent	Millipore Sigma	Cat. #: T2494	
chemical compound, drug	KOD Hot start DNA polymerase	Millipore Sigma	Cat. #: 71086-3	
chemical compound, drug	Polyethylenimine (PEI), linear (MW 25,000)	Polyscience Euro	Cat. #: 23966-2	
chemical compound, drug	Bovine serum albumin	Millipore Sigma	Cat. #: F7524	10% in culture media
chemical compound, drug	Trypan Blue	Gibco/ ThermoFisher Scientific	Cat. #: 15250061	0.4% solution
chemical compound, drug	Triton X-100 (TX-100)	Millipore Sigma	Cat.#: T8787	
chemical compound, drug	Phosphatase inhibitor cocktail 2	Millipore Sigma	Cat.#: P5726-5ML	1X in lysis buffer
chemical compound, drug	Phosphatase inhibitor cocktail 3	Millipore Sigma	Cat.#: P0044-5ML	1X in lysis buffer
chemical compound, drug	cOmplete, EDTA free Protease inhibitors	Roche/Millipore Sigma	Cat.#: 4693132001	1X in lysis buffer

chemical compound, drug	Alcian Blue Solution	Sigma	Cat. #: B8438	
chemical compound, drug	MTX (Methotrexate)	Cayman Chemicals	Cat. #: 13960	100 μ M in DMSO
chemical compound, drug	EmbryoMax® Nucleosides 100x	Merck	Cat. #: ES-008-D	1X in media or saline
chemical compound, drug	TWEEN® 20	Sigma	Cat. #: P2287	
chemical compound, drug	MRT00252040	kindly provided by Simon Osborne, LifeArc, London, UK		PAICS inhibitor. Stock solution 2mM in DMSO, used at final concentration of 2 μ M in DMSO
chemical compound, drug	Methotrexate (MTX)	Millipore Sigma	Cat. #: M8407-100MG	DHFR inhibitor. Stock solution of 100 μ M and used at final concentration of 4 μ M in DMSO.
chemical compound, drug	KU-55933	Selleckchem	Cat. #: 118500-2MG	ATM inhibitor. Used at final concentration of 5 μ M in DMSO in cell culture media.
chemical compound, drug	penicillin–streptomycin (10K U/ml)	ThermoFisher Scientific	Cat. #: 15140122	Used 1% in cell culture media.
chemical compound, drug	SAICAr	CarboSynth	Cat. #: NS16860	Stock solution 20mg/ml in water and used as final concentration at 1 mg/ in cell culture media

chemical compound, drug	Doxorubicin	Millipore Sigma	Cat. #: D1515	Used at 1 ug/ml in cell culture media.
chemical compound, drug	Ascl	NEB	Cat. #: R0558S	
chemical compound, drug	NotI-HF	NEB	Cat. #: R3189S	
chemical compound, drug	Carbenicillin	Millipore Sigma	Cat. #: C9231-1G	
chemical compound, drug	Blasticidin	Invitrogen/ ThermoFisher Scientific	Cat. #: A1113902	
chemical compound, drug	Paraformaldehyde (PFA)	Panreac AppliChem	Cat. #: 14145.1211	Used at 2% in cells
software, algorithm	Graphpad Prism 7-9	Graphpad Prism	RRID:SCR_002798	Version: 7.0e, 8.4.3, 9.3.0
software, algorithm	Adobe Photoshop	Adobe Photoshop	RRID:SCR_014199	Version: 22.4.2
software, algorithm	Adobe Illustrator	Adobe Illustrator	RRID:SCR_010279	Version: 24.3, 25.2.3
software, algorithm	Fiji	Fiji	RRID:SCR_002285	Version: 2.0.0-rc-69/1.52p
software, algorithm	LAS AF	Leica	RRID:SCR_013673	Version: 3.7.3.23245

A **B**

A **B** **C** **D**

A **B** **C** **D** **E** **F** **G**