Figures and figure supplements

Mouse SAS-6 is required for centriole formation in embryos and integrity in embryonic stem cells

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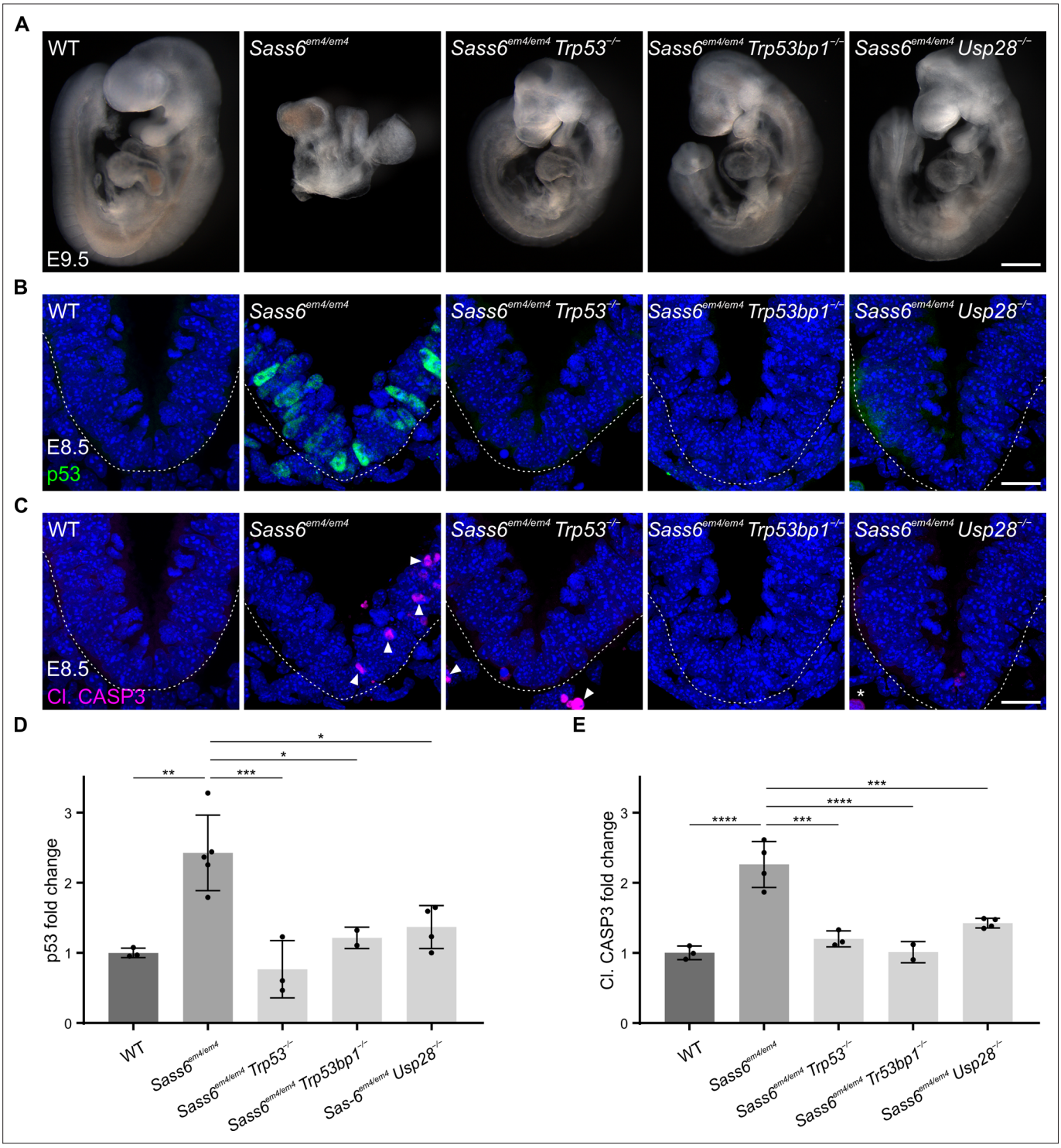

Figure 1. Mutation in mouse *Sass6* activates the 53BP1-USP28-p53 mitotic surveillance pathway. (**A**) Left-side views of wild-type (WT), *Sass6*$^{em4/em4}$, *Sass6*$^{em4/em4}$ *Trp53*$^{-/-}$, *Sass6*$^{em4/em4}$ *Trp53bp1*$^{-/-}$, and *Sass6*$^{em4/em4}$ *Usp28*$^{-/-}$ embryos at E9.5. Anterior is up in all images. At least three embryos per genotype showed similar phenotypes. Scale bar = 500 µm. (**B**) Immunostaining for p53 on transverse sections of WT, *Sass6*$^{em4/em4}$, *Sass6*$^{em4/em4}$ *Trp53*$^{-/-}$, *Sass6*$^{em4/em4}$ *Trp53bp1*$^{-/-}$, and *Sass6*$^{em4/em4}$ *Usp28*$^{-/-}$ embryos at E8.5. The sections shown encompass the neural plate (top) and mesenchyme (bottom), demarcated by the dashed line. Dorsal is up in all images. Scale bars = 25 µm. (**C**) Immunostaining for Cleaved-Caspase3 (Cl. CASP3) as mentioned in (**B**). Arrowheads indicate Cl. CASP3-positive cells, while asterisks mark non-specific staining of blood cells. (**D**) Quantification of the nuclear p53 in (**B**). Values were normalized to WT. Error bars represent mean ± SD WT: 1.00 ± 0.06 (n=2582 cells from three embryos); *Sass6*$^{em4/em4}$: 2.4 ± 0.5 (n=2372 from four embryos); *Sass6*$^{em4/em4}$ *Trp53*$^{-/-}$: 0.8 ± 0.3 (n=2379 from three embryos); *Sass6*$^{em4/em4}$ *Usp28*$^{-/-}$: 1.4 ± 0.3 (n=2775 from four embryos);

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Sass6$^{em4/em4}$ *Trp53bp1*$^{-/-}$: 1.2 ± 0.1 (n=1840 from two embryos). *p<0.05, **p<0.01, ***p<0.001 (one-way ANOVA with Tukey's multiple comparisons). (**E**) Quantification of Cl. CASP3 in (**C**) as mentioned in (**D**). WT: 1.00 ± 0.1 (n=2582 cells from three embryos); *Sass6*$^{em4/em4}$: 2.3 ± 0.3 (n=2372 from four embryos); *Sass6*$^{em4/em4}$ *Trp53*$^{-/-}$: 1.2 ± 0.1 (n=2379 from three embryos); *Sass6*$^{em4/em4}$ *Usp28*$^{-/-}$: 1.4 ± 0.1 (n=2775 from four embryos); *Sass6*$^{em4/em4}$ *Trp53bp1*$^{-/-}$: 1 ± 0.1 (n=1840 from two embryos). ***p<0.001, ****p<0.0001 (one-way ANOVA with Tukey's multiple comparisons).

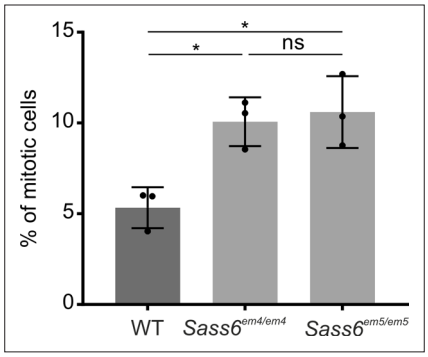

Figure 1—figure supplement 1. Mutations in *Sass6* lead to an increase in the mitotic index in mouse embryos. The percentage of phospho-Histone H3-positive neural and mesenchymal cells of wild-type (WT), *Sass6*$^{em4/em4}$, and *Sass6*$^{em5/em5}$ at E8.5. Three embryos per genotype were used for the quantifications. Error bars represent mean ± SD WT: 5 ± 1% (n=1449 cells); *Sass6*$^{em4/em4}$: 10 ± 1% (n=896); *Sass6*$^{em5/em5}$: 11 ± 2% (n=941). *p<0.05, ns = not significant with p>0.05 (one-way ANOVA with Tukey's multiple comparisons).

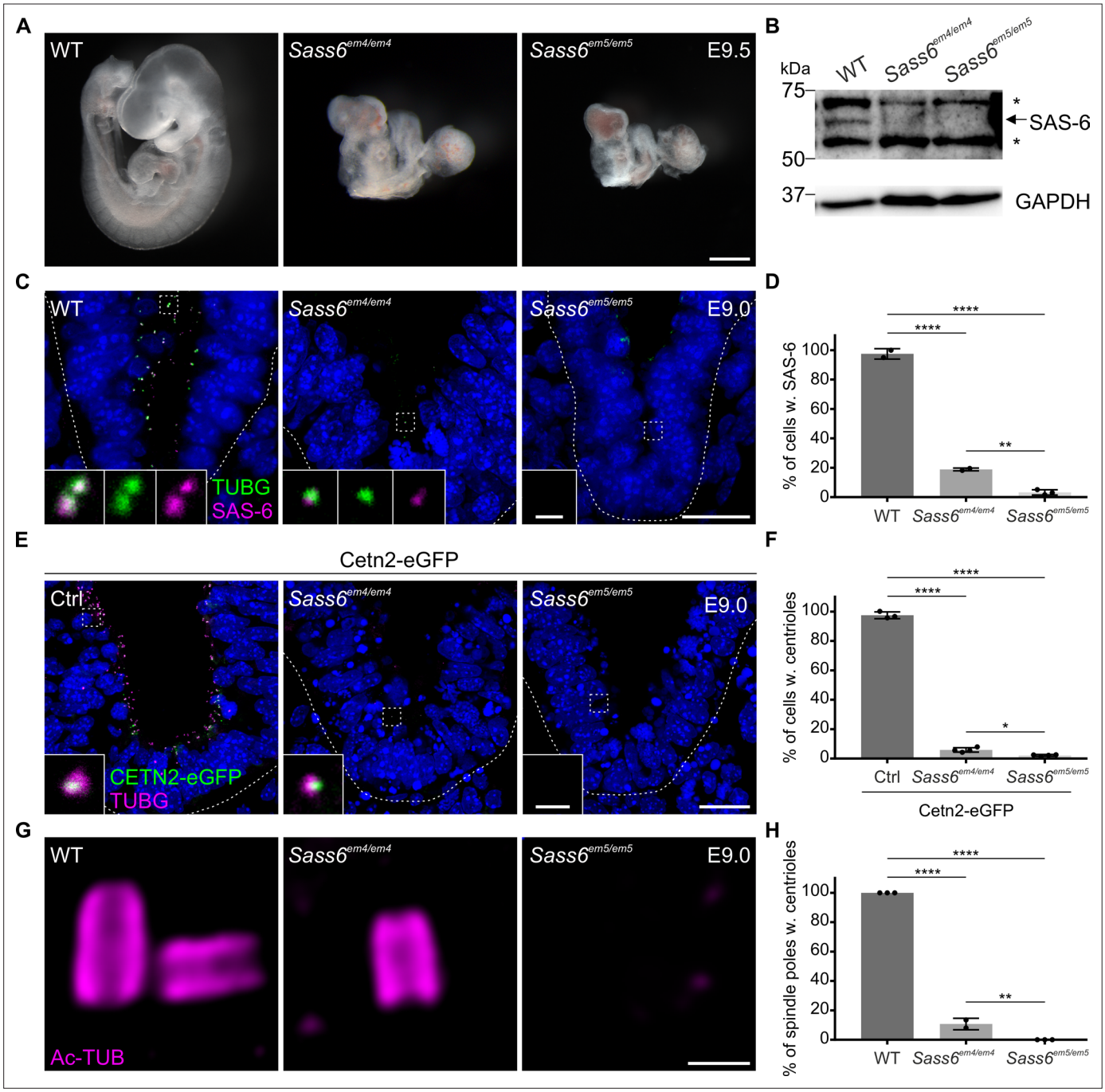

Figure 2. *Sass6*$^{em4/em4}$ are severe hypomorphs while *Sass6*$^{em5/em5}$ embryos lack centrioles. (**A**) Left-side views of wild-type (WT), *Sass6*$^{em4/em4}$, and *Sass6*$^{em5/em5}$ embryos at E9.5. Anterior is up in all images. At least five embryos were analyzed per genotype. Scale bar = 500 µm. (**B**) Western blot analysis using a SAS-6-specific antibody on E9.5 WT, *Sass6*$^{em4/em4}$, and *Sass6* $^{em5/em5}$ embryo extracts. Asterisks mark non-specific bands. GAPDH is used as a loading control. (**C**) Immunostaining for TUBG and SAS-6 on sagittal sections of WT, *Sass6*$^{em4/em4}$, and *Sass6*$^{em5/em5}$ embryos at E9.0. The sections shown encompass the neural plate (top) and mesenchyme (bottom), demarcated by the dashed line. Insets are magnifications of the center of the dashed squares. Dorsal is up in all images. Scale bars = 20 µm and 1 µm (insets). (**D**) Quantification of the percentage of cells with SAS-6 signal co-localization with TUBG in (**C**). Error bars represent mean ± SD WT: 95 ± 3% (n=1929 cells from three embryos); *Sass6*$^{em4/em4}$: 19 ± 1% (n=542 from two embryos); *Sass6*$^{em5/em5}$: 4 ± 2% (n=2458 from four embryos). ****p<0.0001, **p<0.01 (one-way ANOVA with Tukey's multiple comparisons). (**E**) Immunostaining for TUBG on transverse sections of Cetn2-eGFP, *Sass6*$^{em4/em4}$ Cetn2-eGFP, and *Sass6*$^{em5/em5}$ Cetn2-eGFP embryos at E9.0. The sections shown are similar to those described in (**C**). Insets are magnifications of the center of the dashed squares. Scale bars = 20 µm and 1 µm (insets). (**F**) Quantification of the percentage of cells with centrioles (TUBG and Centrin-eGFP) is shown in (**E**). Error bars represent mean ± SD Cetn2-eGFP: 98 ± 2% (n=11,196 cells from three embryos); *Sass6*$^{em4/em4}$ Cetn2-eGFP: 6 ± 1% (n=9752 from four embryos); *Sass6*$^{em5/em5}$ Cetn2-eGFP: 2 ± 0.5% (n=5559 from four embryos).

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****p<0.0001, *p<0.05 (one-way ANOVA with Tukey's multiple comparisons). (**G**) Immunostaining for Ac-TUB on U-ExM sections from E9.0 embryos of the indicated genotypes. Scale bar = 200 nm. (**H**) Quantification of the percentage of mitotic spindle poles with centrioles in (**G**). Error bars represent mean ± SD WT: 100 ± 0% (n=65 spindle poles from three embryos); *Sass6*$^{em4/em4}$: 11 ± 0.03% (n=62 from two embryos); *Sass6*$^{em5/em5}$: 0 ± 0% (n=45 from three embryos). ****p<0.0001, **p<0.01 (one-way ANOVA with Tukey's multiple comparisons).

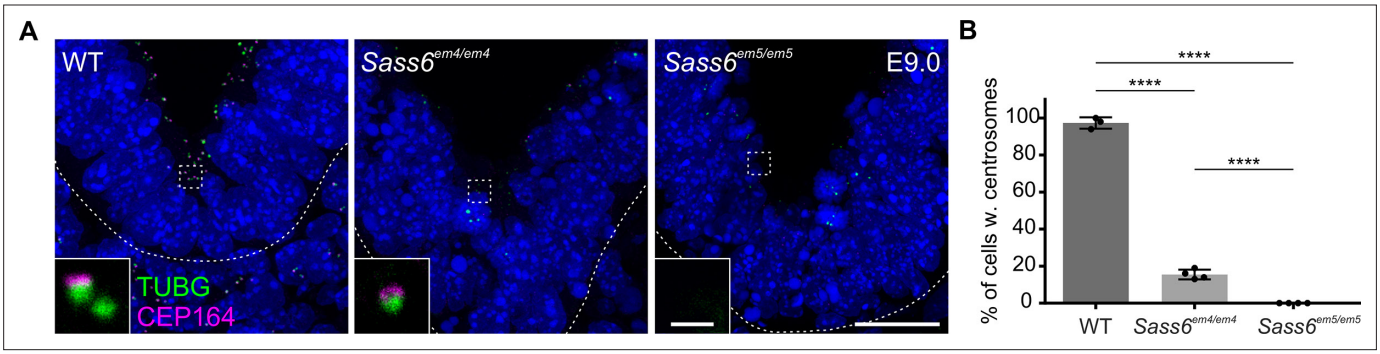

Figure 2—figure supplement 1. *Sass6*$^{em5/em5}$ embryos lack mother centrioles marked with CEP164. (**A**) Immunostaining for TUBG and CEP164 on transverse sections of wild-type (WT), *Sass6*$^{em4/em4}$, and *Sass6*$^{em5/em5}$ embryos at E9.0. The sections shown are similar to those described in ***Figure 1B***. Insets are magnifications of the center of the dashed squares. Scale bars = 20 µm and 1 µm (insets). (**B**) Quantification of the percentage of cells with centrosomes (TUBG and CEP164) in (**B**). Error bars represent mean ± SD WT: 97 ± 0.03% (n=2843 cells from three embryos); *Sass6*$^{em4/em4}$: 16 ± 0.2% (n=2375 from four embryos); *Sass6*$^{em5/em5}$: 0 ± 0% (n=2152 from four embryos). ****p<0.0001 (one-way ANOVA with Tukey's multiple comparisons).

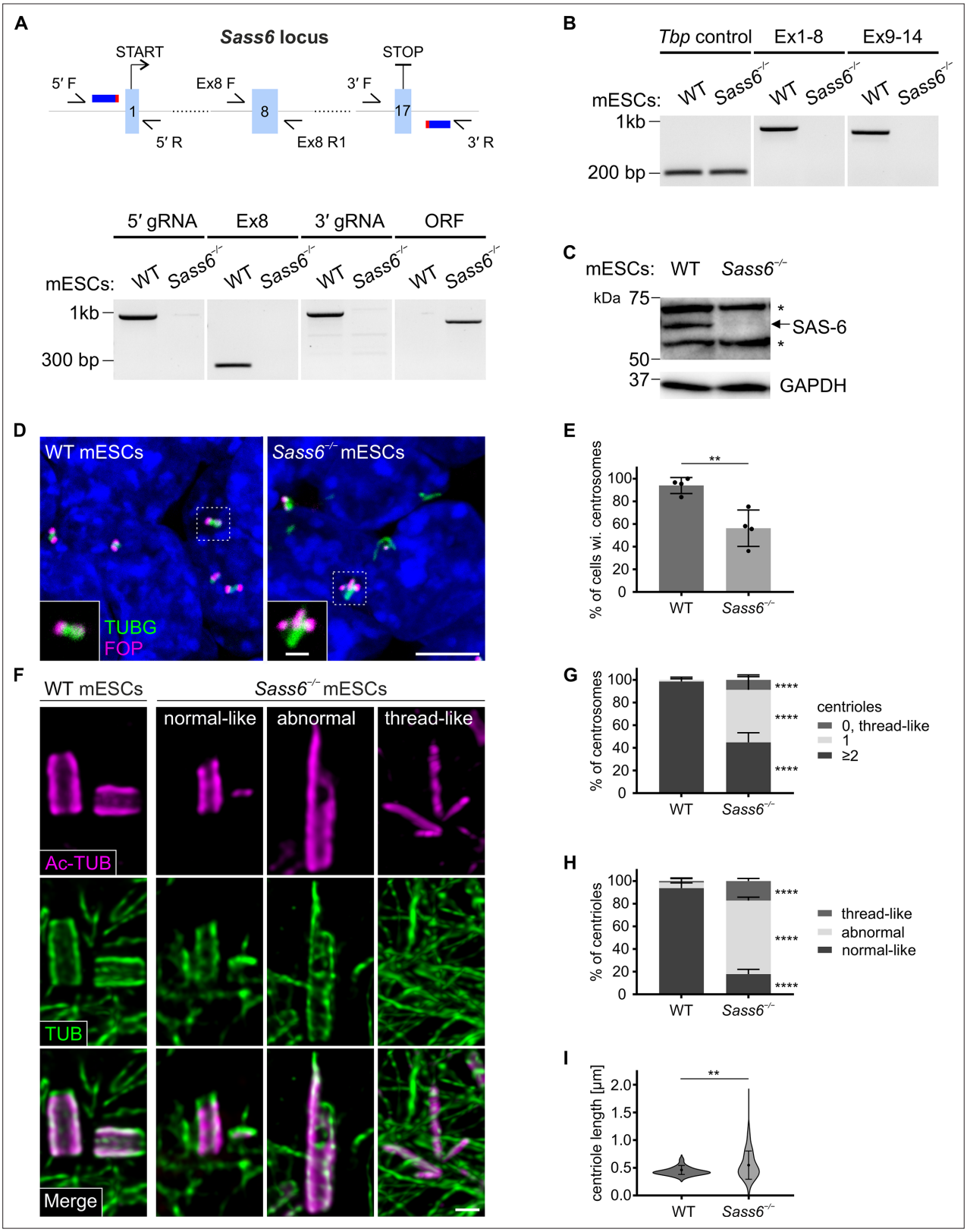

Figure 3. SAS 6 is required for centriole integrity, but not formation, in mouse embryonic stem cells (mESCs). (**A**) (Top) Schematic showing the CRISPR/Cas9 strategy using two gRNAs to delete the entire *Sass6* open reading frame (ORF) in mESCs. Exons (Ex) are represented by light blue boxes, gRNAs by dark blue thick horizontal lines, and PAM sites in red. Half arrows indicate the primers used for PCR analyses (below). (Bottom) Confirmation of the *Sass6* deletion in *Sass6*$^{-/-}$ mESCs by genomic PCR. The picture shows the PCR products using the following primers indicated in the schematic above: 5′

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gRNA (5′ F and 5′ R, band = 977 bp), Ex8 (Ex8 F and Ex8 R1, band = 281 bp), 3′ gRNA (3′ F and 3′ R, band = 992 bp), *Sass6* ORF (5′ F and 3′ R, 825 bp in *Sass6*$^{-/-}$, 34,349 bp in wild-type (WT), product too long to be amplified). (**B**) RT-PCR analyses of *Sass6* transcripts in WT and *Sass6*$^{-/-}$ mESCs. The picture shows the PCR products from RT-PCR using the following primers: from Ex1 to Ex8 (Ex1 F and Ex8 R2, band = 734 bp), from Ex9 to Ex14 (Ex9 F and Ex14 R, band = 617 bp), *Tbp* Ctrl (Tbp F and Tbp R, band = 156 bp). (**C**) Western blot analysis using a SAS-6-specific antibody on WT and *Sass6*$^{-/-}$ mESCs extracts. Asterisks mark non-specific bands. GAPDH is used as a loading control. (**D**) Immunostaining for TUBG and FOP in WT and *Sass6*$^{-/-}$ mESCs. Insets are magnifications of the center of the dashed squares. Scale bars = 5 µm and 1 µm (insets). (**E**) Quantification of the percentage of cells with centrosomes (TUBG and FOP) in (**D**) from four independent experiments. Error bars represent mean ± SD WT: 94 ± 6% (n=2450 cells); *Sass6*$^{-/-}$: 56 ± 14% (n=2766). **p<0.01 (two-tailed Student's t-test). (**F**) Centrioles were visualized using U-ExM and immunostaining for α- and β-tubulin (TUB) and Ac-TUB in WT and *Sass6*$^{-/-}$ mESCs. Scale bar = 200 nm. (**G**) Quantification of the percentage of centrosomes with ≥2, 1, or 0 centrioles in (**F**) in WT and *Sass6*$^{-/-}$ mESCs from five independent experiments. Error bars represent mean ± SD WT (n=156 centrosomes):≥2 centrioles = 99 ± 2%; 1 centriole = 1 ± 2%; *Sass6*$^{-/-}$ (n=254):≥2 centrioles = 45 ± 8%, 1 centriole = 46 ± 10%, 0 centrioles = 9 ± 4%. ****p<0.0001 (two-tailed Student's t-test) (**H**) Quantifications of the percentage of centrioles within each category in (**F**) from five independent experiments. Error bars represent mean ± s.d. WT (n=330 centrioles): normal-like centrioles = 94 ± 4%; abnormal centrioles = 5 ± 3%; thread-like structures = 1 ± 2%; *Sass6*$^{-/-}$ (n=432): normal-like centrioles = 18 ± 4%, abnormal centrioles = 65 ± 3%, thread-like structures = 17 ± 2%. ****p<0.0001, (two-tailed Student's t-test). (**I**) Violin plots of centriole length of normal-like centrioles in (**F**) in WT and *Sass6*$^{-/-}$ mESCs from five independent experiments. Error bars represent mean ± SD WT: 0.46 ± 0.07 µm (n=72 centrioles); *Sass6*$^{-/-}$: 0.55 ± 0.25 µm (n=72). **p<0.01 (two-tailed Student's t-test).

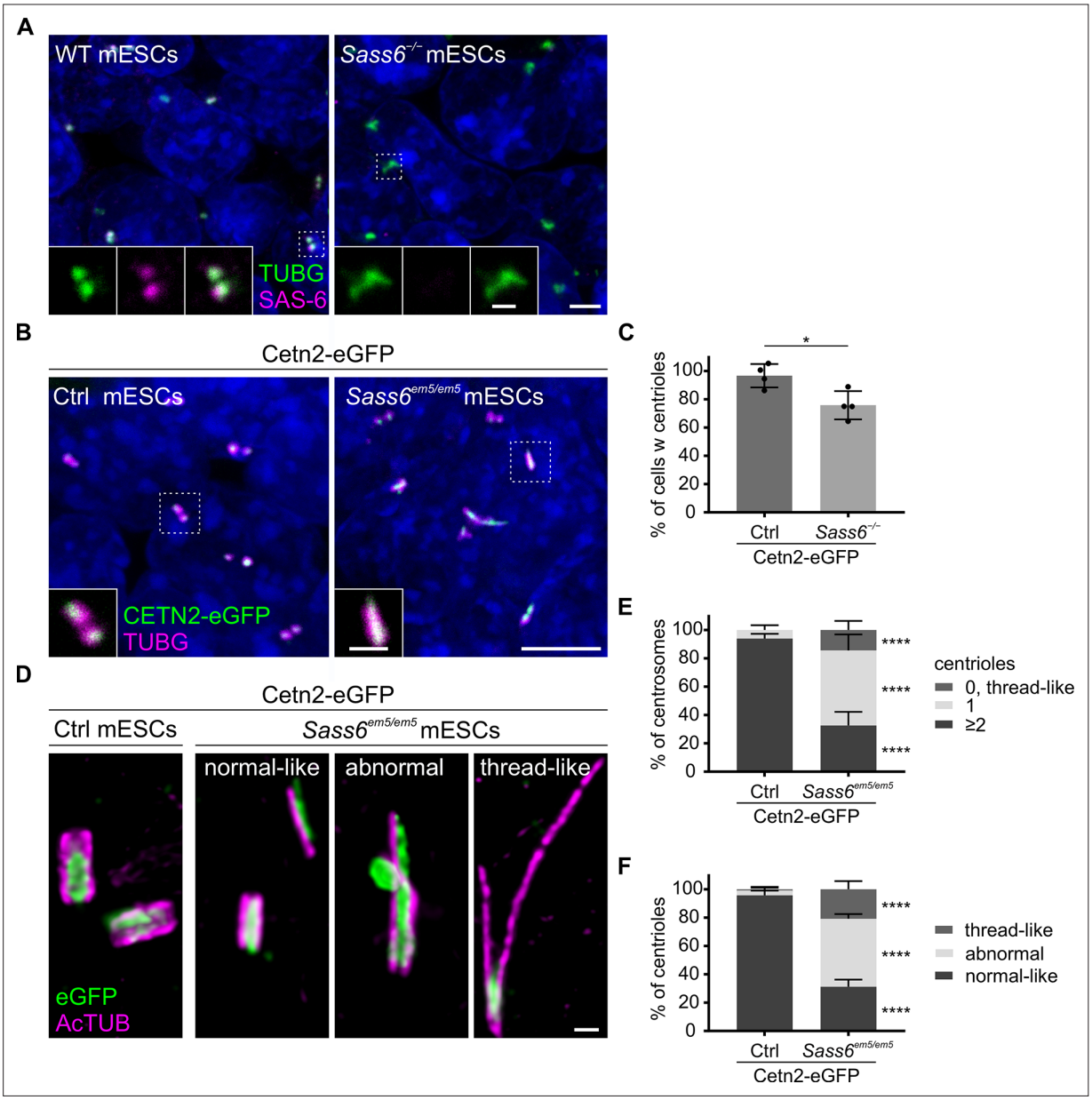

Figure 3—figure supplement 1. SAS-6 is not essential for centriole formation in mouse embryonic stem cells (mESCs). (**A**) Immunostaining for TUBG and SAS-6 in wild-type (WT) and *Sass6*$^{-/-}$ mESCs. Insets are magnifications of the center of the dashed squares. Scale bars = 5 µm and 1 µm (insets). (**B**) Immunostaining for TUBG in control Cetn2-eGFP and *Sass6*$^{em5/em5}$ Cetn2-eGFP mESCs. Insets are magnifications of the center of the dashed squares. Scale bars = 5 µm and 1 µm (insets). (**C**) Quantification of the percentage of cells with centrosomes (TUBG and CETN2-eGFP) in (**B**) from four independent experiments. Error bars represent mean ± SD WT: 97 ± 7% (n=10,858 cells); *Sass6*$^{-/-}$: 76 ± 9% (n=6436). *p<0.05 (two-tailed Student's t-test). (**D**) Immunostaining for Ac-TUB and eGFP of U-ExM of centrioles from control Cetn2-eGFP and *Sass6*$^{em5/em5}$ Cetn2-eGFP mESCs divided into categories: normal-like centrioles, abnormal centrioles, thread-like structures. Scale bar = 200 nm. (**E**) Quantification of the percentage of centrosomes with ≥2, 1, or 0 centrioles in (**D**) in Cetn2-eGFP and *Sass6*$^{em5/em5}$ Cetn2-eGFP mESCs. Compare to ***Figure 3G***. Error bars represent mean ± s.d. Cetn2-eGFP (n=121 centrosomes from four independent experiments):≥2 centrioles = 94 ± 3%; 1 centriole = 6 ± 3%; *Sass6*$^{-/-}$ (n=152 from five independent experiments):≥2 centrioles = 33 ± 9%, 1 centriole = 53 ± 10%, 0 centrioles = 14 ± 6%. ****p<0.0001 (two-tailed Student's t-test) (**F**) Quantifications of the percentage of centrioles within each category in (**D**). Compare to ***Figure 3H***. Error bars represent mean ± SD WT (n=249 centrioles from four independent experiments): normal-like centrioles = 96 ± 3%; abnormal centrioles = 3 ± 2%; thread-like structures = 1 ± 1%; *Sass6*$^{-/-}$ (n=231 from five independent experiments): normal-like centrioles = 31 ± 4%, abnormal centrioles = 48 ± 3%, thread-like structures = 21 ± 5%. ****p<0.0001, (two-tailed Student's t-test).

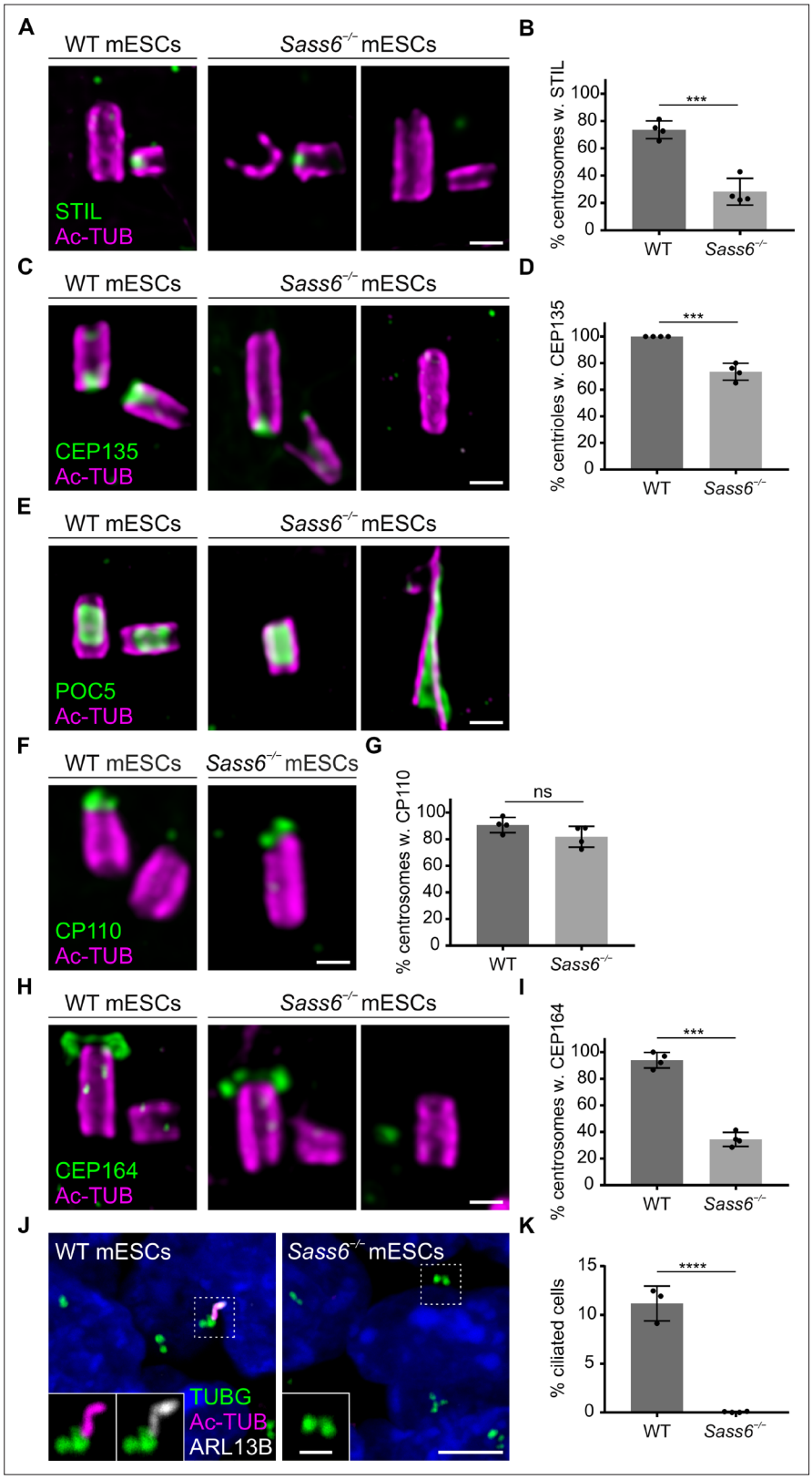

Figure 4. Centrioles in *Sass6*$^{-/-}$ mouse embryonic stem cells (mESCs) exhibit proximal and distal defects. (**A**) Immunostaining for Ac-TUB and STIL of U-ExM of centrioles from wild-type (WT) and *Sass6*$^{-/-}$ mESCs. Examples of centrioles with or without STIL are shown. Scale bar = 200 nm. (**B**) Quantification of the percentage of centrosomes with (w.) STIL in (**A**) from four independent experiments. Error bars represent mean ± SD WT: 74 ± 6%

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(n=72 centrosomes); *Sass6*$^{-/-}$: 29 ± 8% (n=94). ***p<0.001, (two-tailed Student's t-test). (**C**) Immunostaining for Ac-TUB and cartwheel protein (CEP135) of U-ExM of centrioles from WT and *Sass6*$^{-/-}$ mESCs. Examples of centrioles with or without CEP135 are shown. Scale bar = 200 nm. (**D**) Quantifications of the percentage of centrioles with CEP135 in (**C**) from four independent experiments. Error bars represent mean ± SD WT: 100 ± 0% (n=160 centrioles); *Sass6*$^{-/-}$: 73 ± 6% (n=98). ***p<0.001, (two-tailed Student's t-test). (**E**) Immunostaining for Ac-TUB and the inner scaffold protein POC5 of U-ExM of centrioles from WT and *Sass6*$^{-/-}$ mESCs. Examples of normal-like or abnormal centrioles with POC5 are shown. Scale bar = 200 nm. (**F**) Immunostaining for Ac-TUB and the distal-end capping protein CP110 of U-ExM of centrioles from WT and *Sass6*$^{-/-}$ mESCs. Scale bar = 200 nm. (**G**) Quantification of the percentage of centrosomes with CP110 in (**F**) from four independent experiments. Error bars represent mean ± SD WT: 91 ± 5% (n=116 centrosomes); *Sass6*$^{-/-}$: 82 ± 7% (n=106). ns = not significant with p>0.05 (two-tailed Student's t-test). (**H**) Immunostaining for Ac-TUB and CEP164 of U-ExM of centrioles from WT and *Sass6*$^{-/-}$ mESCs. Examples of centrioles with or without CEP164 are shown. Scale bar = 200 nm. (**I**) Quantification of the percentage of centrosomes with mother centrioles (Ac-TUB) with the distal appendage marker (CEP164) in (**H**). Error bars represent mean ± SD WT: 94 ± 5% (n=104 centrosomes from four independent experiments); *Sass6*$^{-/-}$: 28 ± 14% (n=140 from five experiments). ***p<0.001 (two-tailed Student's t-test). (**J**) Immunostaining of the cilia markers ARL13B and Ac-TUB, and basal bodies marked with TUBG, on WT and *Sass6*$^{-/-}$ mESCs. The insets show separate channels for the magnifications of the center of the dashed squares. Scale bars = 5 µm and 1 µm (insets). (**K**) Quantification of the percentage of ciliated cells in (**J**). Error bars represent mean ± SD WT: 11 ± 1% (n=2602 cells from three experiments); *Sass6*$^{-/-}$: 0 ± 0% (n=4602 from four experiments). ****p<0.0001 (two-tailed Student's t-test).

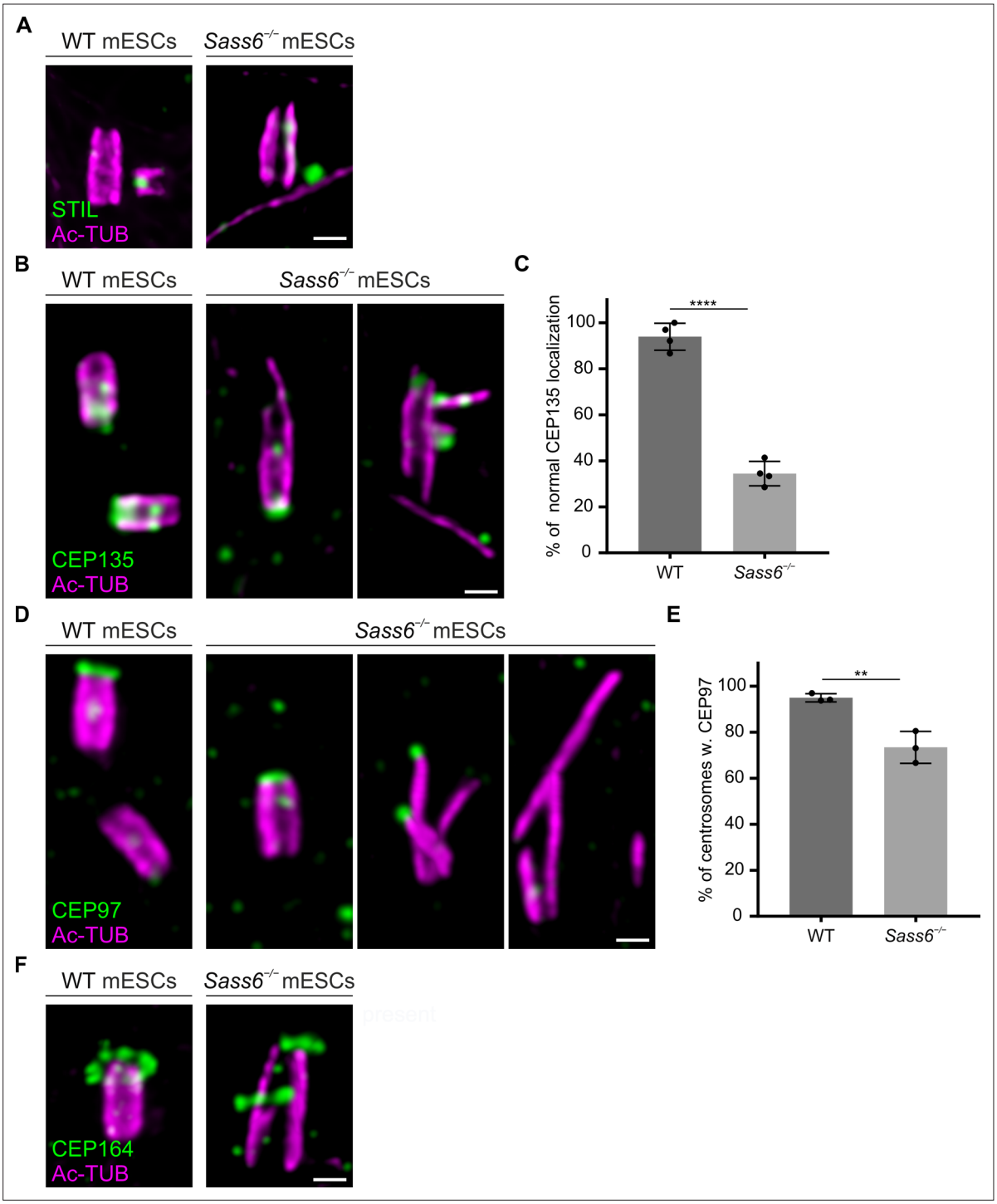

Figure 4—figure supplement 1. Centrioles in *Sass6*$^{-/-}$ mouse embryonic stem cells (mESCs) exhibit structural abnormalities. (**A**) Immunostaining for Ac-TUB and STIL of U-ExM of centrioles from wild-type (WT) and *Sass6*$^{-/-}$ mESCs. Examples of centrioles with STIL are shown. Scale bar = 200 nm. (**B**) Immunostaining for Ac-TUB and the cartwheel protein CEP135 of U-ExM of centrioles from WT and *Sass6*$^{-/-}$ mESCs. Examples of centrioles with normal or abnormal localization of CEP135 are shown. Scale bar = 200 nm. (**C**) Quantifications of normal localization of CEP135 in CEP135-positive centrosomes in (**B**) from four independent experiments. Error bars represent mean ± SD WT: 96 ± 3% (n=160 centrioles); *Sass6*$^{-/-}$: 12 ± 8% (n=98). ****p<0.0001, (two-tailed Student's t-test). (**D**) Immunostaining for Ac-TUB and the distal-end capping protein CEP97 of U-ExM of centrioles from WT and *Sass6*$^{-/-}$ mESCs. Examples of centrioles with or without CEP97 are shown. Scale bar = 1 µm. (**E**) Quantification of the percentage of centrosomes with CEP97 in (**D**) from four independent experiments. Error bars represent mean ± SD WT: 95 ± 1% (n=116 centrosomes); *Sass6*$^{-/-}$: 73 ± 6% (n=106). **p<0.01, (two-tailed Student's t-test). (**F**) Immunostaining for Ac-TUB and CEP164 in U-ExM of centrioles from WT and *Sass6*$^{-/-}$ mESCs. Examples of centrioles with CEP164 are shown. Scale bar = 200 nm.

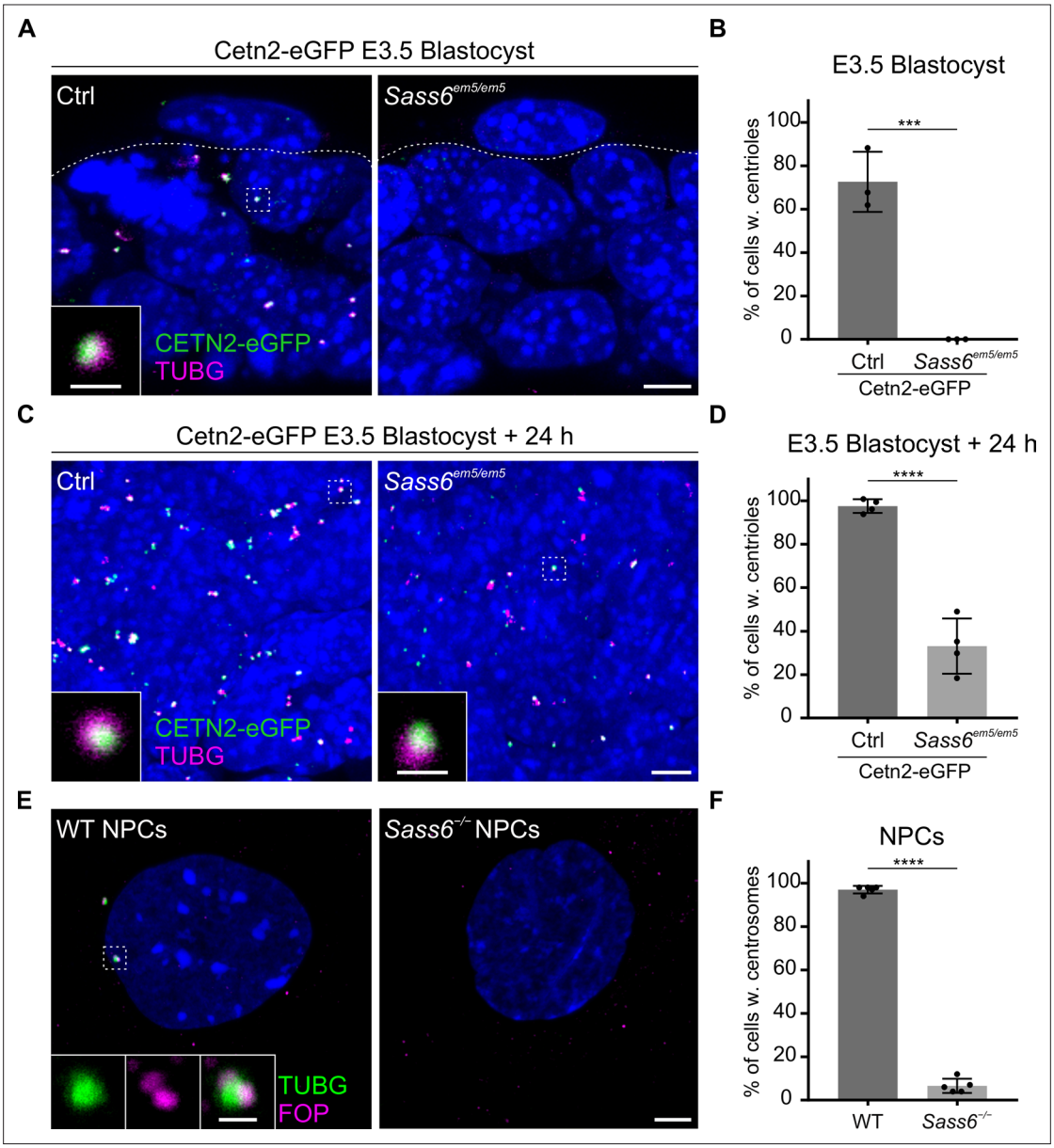

Figure 5. Centrioles in *Sass6* $^{em5/em5}$ mouse embryonic stem cells (mESCs) are formed *de novo* during derivation from blastocysts and are lost upon differentiation. (**A**) Whole-mount immunostaining for TUBG on Cetn2-eGFP and *Sass6*$^{em5/em5}$ Cetn2-eGFP blastocysts at E3.5. Trophoblasts (top) and inner cell mass cells (bottom) are demarcated by the dashed line. The Inset is a magnification of the dashed square. Scale bars = 5 µm and 1 µm (inset). (**B**) Quantification of the percentage of cells with centrioles (TUBG and Centrin-eGFP) from E3.5 blastocysts in (**A**). Three blastocysts per genotype were used for the quantifications. Error bars represent mean ± SD WT: 73 ± 11% (n=200 cells); *Sass6*$^{em5/em5}$: 0 ± 0% (n=175). ***p<0.001, (two-tailed Student's t-test). (**C**) Whole-mount immunostaining as mentioned in (**A**) on blastocysts after 24 hr in culture. (**D**) Quantification from (**C**) as mentioned in (**B**). Four blastocysts per genotype were used for the quantifications. WT: 98 ± 30% (n=630 cells); *Sass6*$^{em5/em5}$: 33 ± 11% (n=690). ****p<0.0001. (**E**) Immunostaining for TUBG and FOP in WT and *Sass6*$^{-/-}$ cells after *in vitro* neural differentiation (NPCs). Insets are magnifications of the center of the dashed squares. Scale bars = 5 µm and 1 µm (insets). (**F**) Quantification of the percentage of cells with centrosomes (TUBG and FOP) in (**E**) from five independent experiments. Error bars represent mean ± SD WT: 97 ± 0% (n=1388 cells); *Sass6*$^{-/-}$: 6 ± 0% (n=1068). ****p<0.0001, (two-tailed Student's t-test).

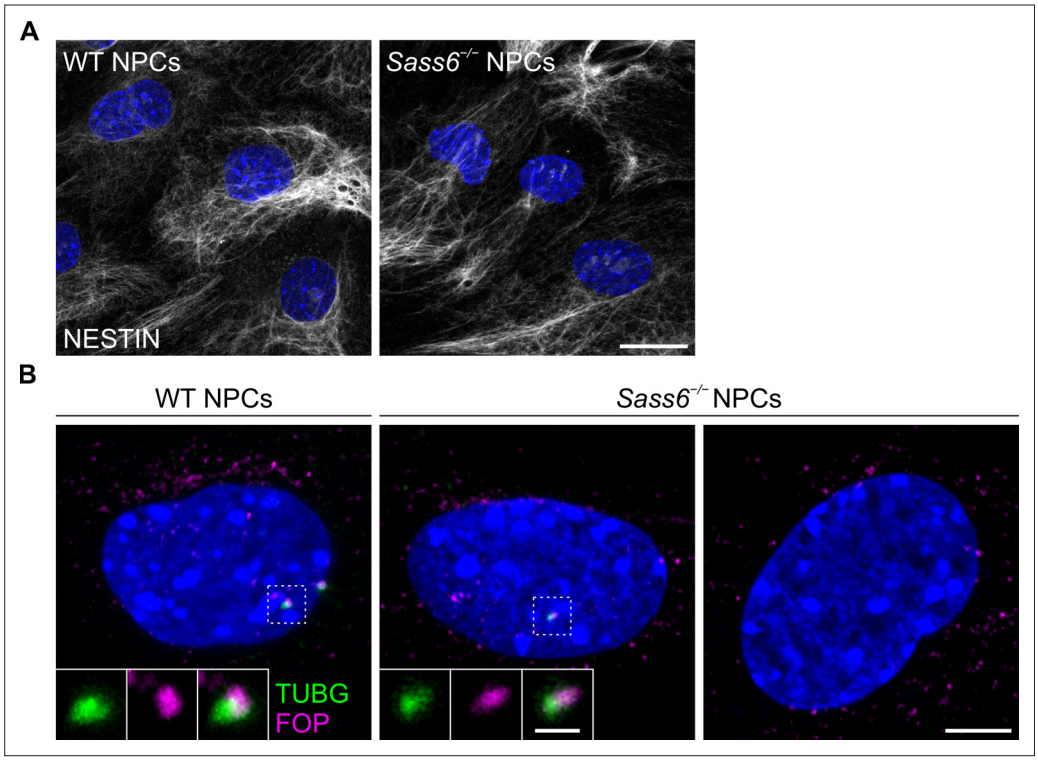

Figure 5—figure supplement 1. Wild-type (WT) and *Sass6*$^{-/-}$ mouse embryonic stem cells (mESCs) differentiated into neural progenitor cells (NPCs). (**A**) Immunostaining for NESTIN on WT and *Sass6*$^{-/-}$ cells after *in vitro* neural differentiation of mESCs into NPCs. Scale bar = 20 µm. (**B**) Immunostaining for TUBG and FOP in WT and *Sass6*$^{-/-}$ cells after *in vitro* neural differentiation (NPCs). Examples of cells with or without (in *Sass6*$^{-/-}$) centrosomes are shown. Insets are magnifications of the center of the dashed squares. Scale bars = 5 µm and 1 µm (insets).

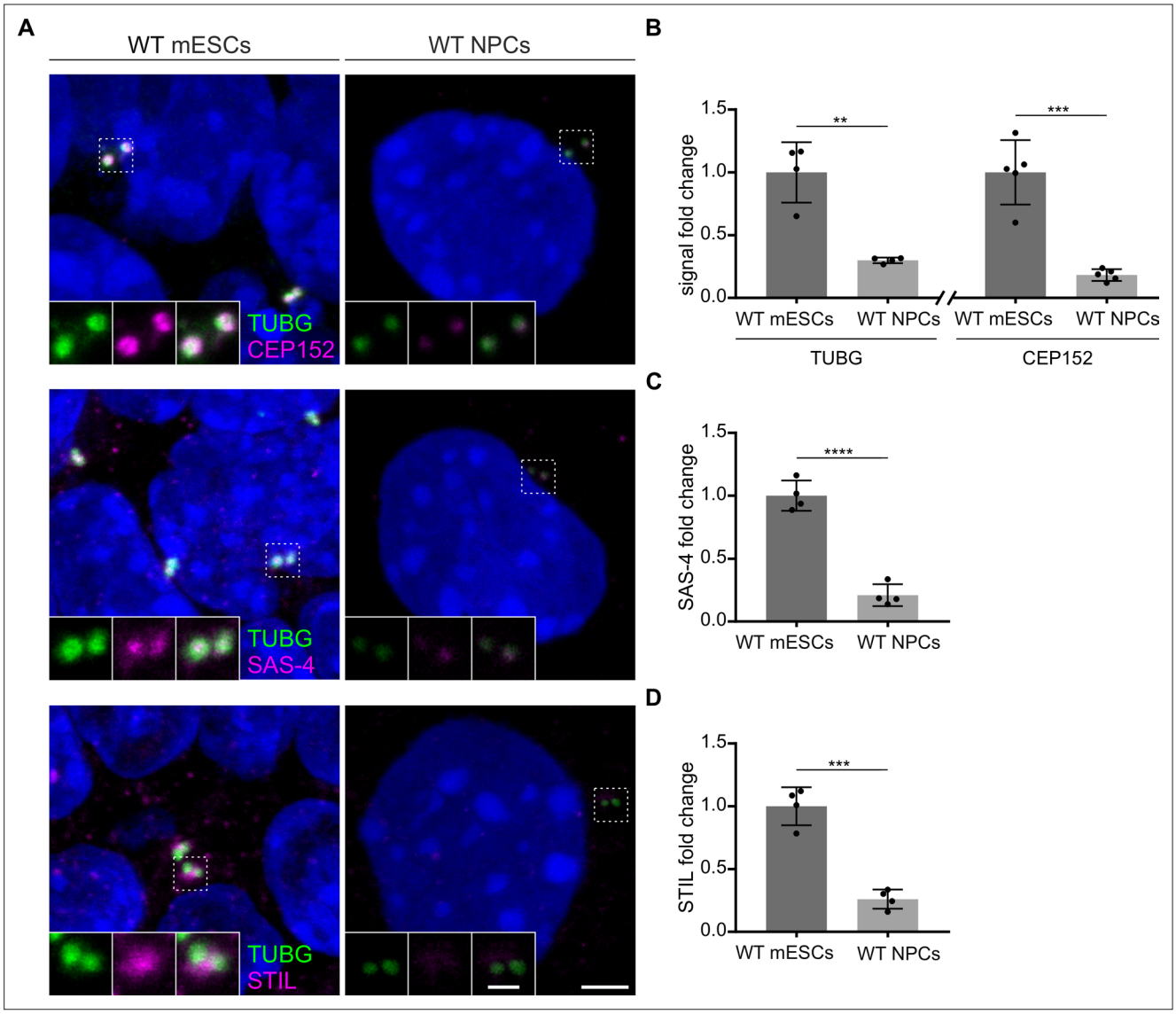

Figure 6. Levels of centrosomal components are reduced upon neural differentiation. (**A**) Immunostaining for TUBG and CEP152, TUBG and SAS-4, or TUBG and STIL in wild-type (WT) mouse embryonic stem cells (mESCs) and *in vitro* differentiated (neural progenitor cells, NPCs). Insets are magnifications of the center of the dashed squares. Scale bars = 3 µm and 1 µm (insets). (**B**) Quantification of the centrosomal TUBG and CEP152 signal from (**A**). Values were normalized to mESCs. Error bars represent mean ± s.d. Quantification of TUBG, mESCs: 1.00 ± 0.2 (n=1325 centrosomes from four independent experiments); NPCs: 0.03 ± 0.02% (n=789 from 4fourindependent experiments). Quantification of CEP152, mESCs: 1.00 ± 0.2 (n=1006 cells from five independent experiments); NPCs: 0.2 ± 0.04% (n=973 from five independent experiments). **p<0.01, ***p<0.001 (two-tailed Student's t-test). (**C**) Quantification of the centrosomal SAS-4 signal from (**A**) from four independent experiments. Values were normalized to mESCs. Error bars represent mean ± SD mESCs: 1.00 ± 0.1 (n=1297 centrosomes); NPCs: 0.2 ± 0.08% (n=790). ****p<0.0001, (two-tailed Student's t-test). (**D**) Quantification of the centrosomal STIL signal from (**A**) from four independent experiments. Values were normalized to mESCs. Error bars represent mean ± SD mESCs: 1.00 ± 0.13 (n=1132 centrosomes from four independent experiments); NPCs: 0.3 ± 0.07% (n=798). ***p<0.001, (two-tailed Student's t-test).

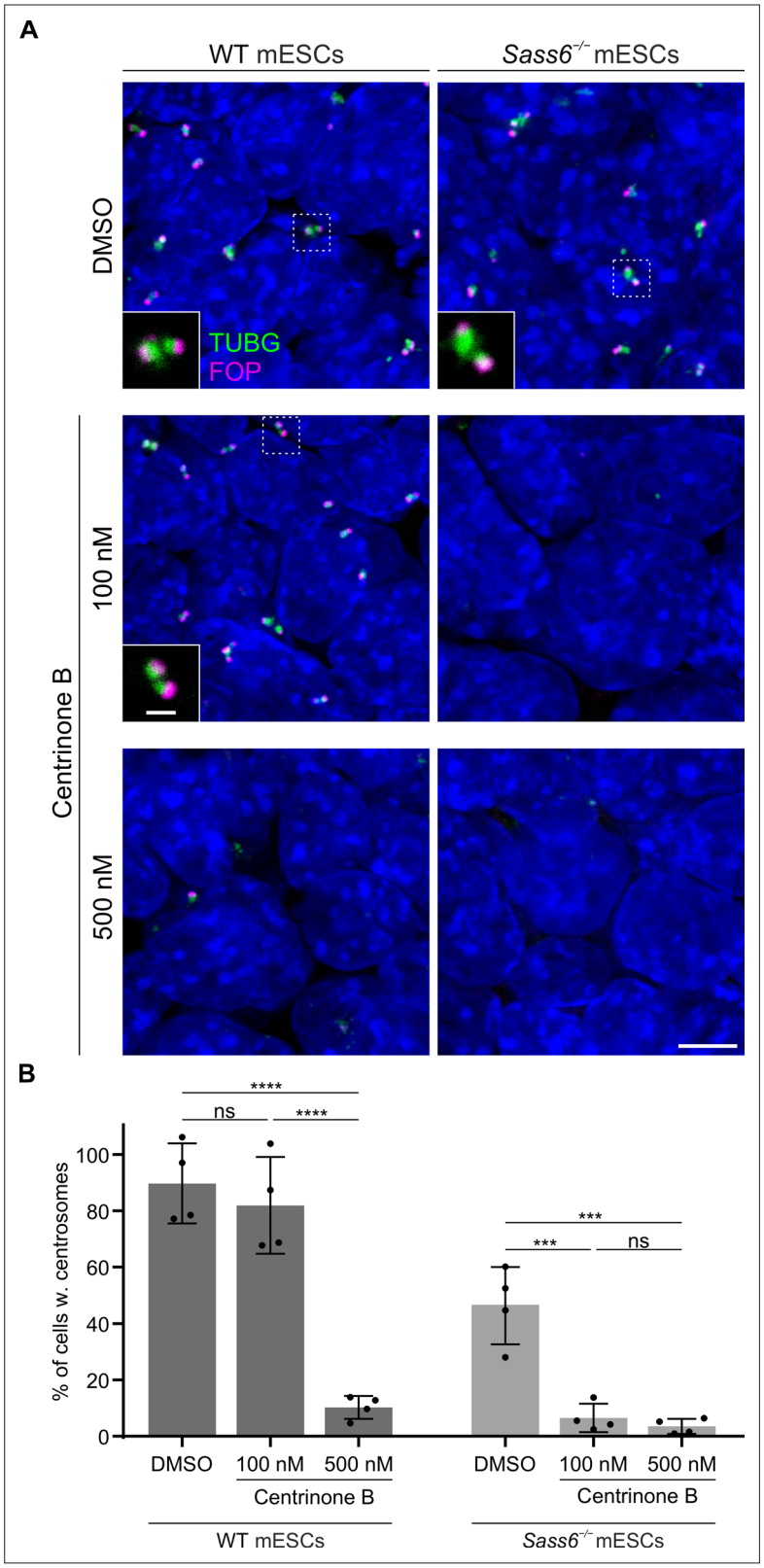

Figure 7. SAS-6-independent centriole formation in mouse embryonic stem cells (mESCs) depends on a threshold Polo-Like Kinase 4 (PLK4) activity. (**A**) Immunostaining for TUBG and FOP in wild-type (WT) and *Sass6*$^{-/-}$ mESCs treated for 4 days with DMSO, 100 nM or 500 nM centrinone B. Insets are magnifications of the center of the dashed squares and show the representative image of the majority of population. Scale bars = 5 µm and 1 µm

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(insets). (**B**) Quantification of the percentage of cells with centrosomes (TUBG and FOP) from (**A**) from four independent experiments. Error bars represent mean ± SD WT, DMSO: 90 ± 12% (n=5280 cells), 100 nM: 82 ± 15% (n=6083 cells), 500 nM: 10 ± 4% (n=4809 cells); *Sass6*$^{-/-}$, DMSO: 46 ± 12% (n=5786 cells), 100 nM: 6 ± 4% (n=7502 cells), 500 nM: 4 ± 2% (n=6220 cells). ***p<0.001, ****p<0.0001, ns = not significant with p>0.05 (one-way ANOVA with Tukey's multiple comparisons).

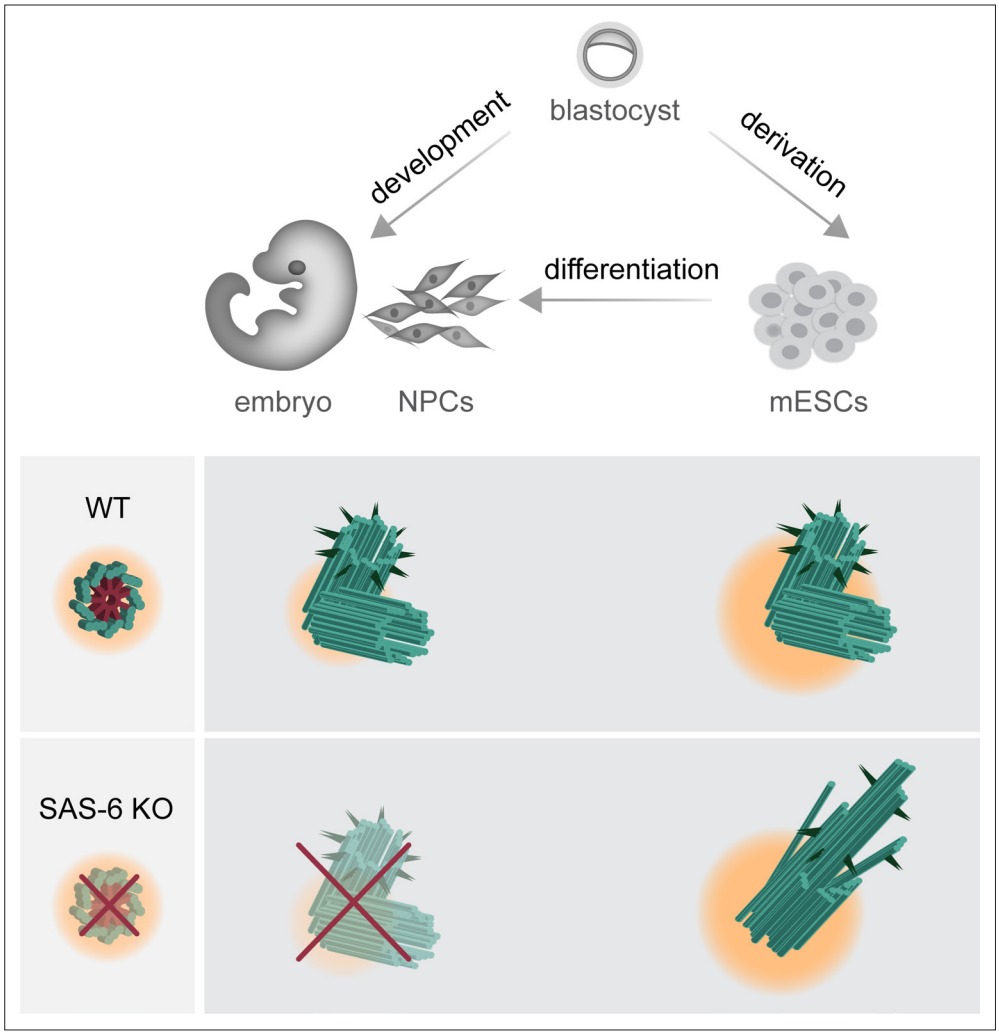

Figure 8. Graphical model depicting the consequences of SAS-6 loss in mouse embryos, mouse embryonic stem cells (mESCs), and neural progenitor cells (NPCs). Compared to mouse embryos and *in vitro* differentiated NPCs, mESCs exhibit a higher concentration of centrosomal components and a robust Polo-Like Kinase 4 (PLK4) activity, as indicated by changes in pericentriolar material color and size. This difference permits the formation of abnormal centrioles in *Sass6*$^{-/-}$ mESCs, while it results in the loss of centrioles in developing mouse embryos and NPCs.