Figures and figure supplements

Gq activity- and β-arrestin-1 scaffolding-mediated ADGRG2/CFTR coupling are required for male fertility

Dao-Lai Zhang et al
Figure 1. The expression of G protein subtypes in the efferent ductules and ADGRG2 promoter-labeled non-ciliated cells. (A) qRT-PCR analysis of mRNA transcription profiles of G proteins in brain tissues and the efferent ductules of WT (n = 3) male mice. Expression levels were normalized to

Zhang et al. eLife 2018;7:e33432. DOI: https://doi.org/10.7554/eLife.33432

Figure 1 continued on next page.
GAPDH levels. *p < 0.05, **p < 0.01, ***p < 0.001, efferent ductules compared with brain tissue. (B) Co-localization analysis of ADGRG2 (red fluorescence) and acetylated-tubulin (green fluorescence) in the efferent ductules of WT mice. Scale bars, 50 μm. (C) Co-localization of ADGRG2 (green fluorescence) and RFP (red fluorescence) in the same cells of male murine efferent ductules infected with the ADGRG2 promoter RFP adenovirus in WT mice. Scale bars, 50 μm. (D) qRT-PCR analysis of mRNA transcription profiles of G protein subtypes in brain tissues and isolated ADGRG2 promoter-labeled non-ciliated cells derived from the efferent ductules of WT (n = 3) male mice. Expression levels were normalized to GAPDH levels. *p < 0.05, **p < 0.01, ***p < 0.001, ADGRG2 promoter-labeled efferent ductule cells compared with brain tissues. n.s., no significant difference. At least three independent biological replicates were performed for Figure 1A and D.

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Figure 1—figure supplement 1. ADGRG2 is specifically expressed in non-ciliated cells. (A) Control experiments: Direct immunofluorescence staining of secondary antibodies used in the manuscript (including donkey anti-sheep, red fluorescence; and donkey anti-rabbit, green fluorescence) in WT male mice efferent ductules. Scale bars, 50 μm. (B) Microscope analysis of efferent ductules and the nucleus in WT male mice, including a light image. Scale bars, 50 μm. (C) Bar graph representation and statistical analyses of co-localization of ADGRG2 and acetylated-tubulin in WT male mice efferent ductules (corresponding to Figure 1B in the main manuscript), n = 3 mice per group; 4–10 random areas were selected from each section, and six sections were randomly selected from each mouse.

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Figure 1—figure supplement 2. The construction of the mouse ADGRG2-promoter-RFP used in the labeling of ADGRG2-expressed cells. (A–B) Schematic representation of the construction of the mouse ADGRG2-promoter-RFP used in the labeling of ADGRG2 expressed cells in the Figure 1—figure supplement 2 continued on next page.
Figure 1—figure supplement 2 continued

epididymal efferent duct epithelium. Sub-cloning strategy of the ADGRG2-promoter (A). Schematic diagram of ADGRG2-promoter-RFP adenovirus vector (B). (C) Isolated epididymal efferent duct epithelium infected with the ADGRG2-promoter RFP adenovirus specifically labeled the ADGRG2-expressing non-ciliated cells. Scale bars, 50 μm.

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Figure 2. Gq activity is required for fluid reabsorption. (A) Images of cultured ligated efferent ductules derived from WT male mice, Adgrg2−/− mice and Gnaq−/− male mice. Ductule segments were selected by examination of the ciliary beat, which is a marker of cell integrity. Ductule pieces from Adgrg2−/−

Figure 2 continued on next page
Figure 2 continued

Y, Gnaq+/− or WT mice were ligated, microdissected and cultured for up to 72 hr. Scale bars, 200 μm. (B–C, E–H) Effects of pharmacological intervention on the diameters of ligated efferent ductules derived from WT or Adgrg2−/− mice. (B) PTX (100 ng/ml), a Gi inhibitory protein. WT (n = 9) or Adgrg2−/− (n = 8); (C) U0126 (10 μM), a MEK inhibitor (ERK pathway blockade). WT (n = 12) or Adgrg2−/− (n = 12). (E) Ro 31–8220 (500 nM), a protein kinase C (PKC) inhibitor, WT (n = 12) or Adgrg2−/− (n = 10); (F) NF449 (1 μM), a Gs inhibitor, WT (n = 9) or Adgrg2−/− (n = 9); (G) PKI14–22 (300 nM), a PKA inhibitor, WT (n = 9) or Adgrg2−/− (n = 9); (H) H89 (500 nM), a non-selective PKA inhibitor, WT (n = 9) or Adgrg2−/− (n = 9). (D) Diameters of the luminal ductules derived from WT (n = 27) mice remained unchanged over 72 hr, whereas the lumens of the ductules derived from Adgrg2−/− (n = 21) mice and Gnaq+/− (n = 16) mice were significantly increased, indicating fluid reabsorption dysfunction. (2B–2H) *p < 0.05, **p < 0.01, ***p < 0.001, Adgrg2−/− mice or Gnaq+/− mice were compared with WT mice. #p < 0.05, ##p < 0.01, ###p < 0.001, treatment with selective inhibitors or stimulators was compared with control vehicles. n.s., no significant difference. At least three independent biological replicates were performed for Figure 2B–H.

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Figure 2—figure supplement 1. The ADGRG2 protein knockout strategy, PCR strategy and western blot results of Adgrg2<sup>+/−</sup> and Gnaq<sup>+/−</sup> mice. (A) Schematic representation of the ADGRG2 knockout strategy for the Adgrg2<sup>+/−</sup> mice. 2 bp nucleotides were removed and 10 bp nucleotides were

Zhang et al. eLife 2018;7:e33432. DOI: https://doi.org/10.7554/eLife.33432
inserted in the first exon of the ADGRG2 gene in the ADGRG2 mutant mice by the CRISP-CAS9 approach. In the ADGRG2-deficient mice, the translation of ADGRG2 was terminated at the 7th amino acid after the signal peptide. (B) Schematic representation for the primers used in the genotyping of the ADGRG2 mutant mice or their wild-type littermates. (C) Schematic description of the PCR strategy and expected results for genotyping. The genotyping of mice was determined by PCR and visualized by bromide staining of agarose Gels. (D) Western blot analysis of ADGRG2 expression in efferent duct tissue of WT and Adgrg2−/− mice. All blots were normalized to GAPDH. (E) Bar graph representation and statistical analyses of (D). At least three independent experiments were carried out. (F) Western blot analysis of Gq expression in efferent duct tissue of WT and Gnaq+/− mice. All blots were normalized to GAPDH. (G) Bar graph representation and statistical analyses of (F). At least three independent experiments were carried out. (E,G) *p<0.05, **p<0.01, ***p<0.001, Adgrg2−/− mice or Gnaq+/− mice were compared with WT mice. n.s., no significant difference. DOI: https://doi.org/10.7554/eLife.33432.006
Figure 2—figure supplement 2. Effects of Forskolin and IBMX on the diameters of ligated efferent ductules derived from WT or Adgrg2−/− mice. (A) Effects of forskolin (10 μM), an adenylyl cyclase (AC) activator, on the diameters of ligated efferent ducts; WT(n = 11) or Adgrg2−/− (n = 10). (B) Effects of IBMX(100 μM), a non-specific inhibitor of cAMP and cGMP phosphodiesterases (PDEs), on the diameters of ligated efferent ducts. WT(n = 9) or Adgrg2−/− (n = 9). (A–B) *p < 0.05, **p < 0.01, ***p < 0.001, Adgrg2−/− mice were compared with WT mice. #p < 0.05, ##p < 0.01, ###p < 0.001, Selective inhibitors or stimulators treated were compared with control vehicles. DOI: https://doi.org/10.7554/eLife.33432.007
Figure 3. Gq expression is required for sperm transportation and male fertility. (A) Representative hematoxylin and eosin staining of WT, Adgrg2^{-/-} or Gnaq^{+/+} mice. Scale bars, 200 μm. (B–D) Corresponding bar graphs demonstrating the accumulation of spermatozoa according to the hematoxylin and eosin staining.
Figure 3 continued

eosin staining of WT (n = 8), Adgrg2\textsuperscript{−/−} (n = 9) or Gnaq\textsuperscript{+/-} (n = 9) mice. ED: efferent ductules; IS: epididymal initial segment; CA: caput epididymis. (E) Representative photographs of caudal sperm preparation from the caudal epididymis of WT, Adgrg2\textsuperscript{−/−} or Gnaq\textsuperscript{+/-} mice. Scale bars, 50 \textmu m. (F) Bar graph depicting the quantitative analysis of the number of sperm shown in (Figure 1E) of WT (n = 8), Adgrg2\textsuperscript{−/−} (n = 10) or Gnaq\textsuperscript{+/-} (n = 10) mice. (G) Line graph depicting the fertility of Gnaq\textsuperscript{+/-} (n = 6) and WT (n = 6) male mice at various ages, as measured by the median number of embryos. (3B-D and 3 F-G): *p < 0.05, **p < 0.01, ***p < 0.001, Adgrg2\textsuperscript{−/−} mice or Gnaq\textsuperscript{+/-} mice were compared with WT mice. #p < 0.05, ##p < 0.01, ###p < 0.001. Gnaq\textsuperscript{+/-} mice were compared with Adgrg2\textsuperscript{−/−} mice. n.s., no significant difference. At least three independent biological replicates were performed for Figure 3B–D and and F–G.

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Figure 4. Inhibition of CFTR activity in the efferent ductules pheno-copied the activity in Adggr2+/− mice. (A) qRT-PCR analysis of the mRNA transcription profiles of potential osmotic drivers including selective ion channels and transporters in ADGRG2 promoter-labeled cells, non-ADGRG2

Figure 4 continued on next page
promoter-labeled cells and brain tissues of WT (n = 3) male mice. Expression levels were normalized to GAPDH levels. *p < 0.05, **p < 0.01, ***p < 0.001, ADGRG2 promoter-labeled cells were compared with brain tissues. #p < 0.05, ##p < 0.01, ###p < 0.001, non-ADGRG2 promoter-labeled cells were compared with brain tissues. (B–M) Effects of different channel blockers on the diameters of luminal ductules derived from WT or Adgrg2<sup>−/−</sup> mice. (B) Bumetanide (10 μM), an NKCC blocker, WT (n = 9) or Adgrg2<sup>−/−</sup> (n = 10); (C) Ani9 (150 nM), an ANO1 inhibitor, WT (n = 9) or Adgrg2<sup>−/−</sup> (n = 9); (D) NFA (20 μM), a CaCC inhibitor, WT (n = 9) or Adgrg2<sup>−/−</sup> (n = 10), (E) ruthenium red (10 μM), a non-specific TRP channel blocker, WT (n = 12) or Adgrg2<sup>−/−</sup> (n = 12); (F) SKF96365 (10 μM), a TRPC channel inhibitor, WT (n = 12) or Adgrg2<sup>−/−</sup> (n = 9); (G) nifedipine (20 μM), an L-type calcium channel blocker, WT (n = 12) or Adgrg2<sup>−/−</sup> (n = 12); (H) EGTA (5 mM), an extracellular calcium chelator, WT (n = 9) or Adgrg2<sup>−/−</sup> (n = 9); (I) DIDS (20 μM), a chloride-bicarbonate exchanger blocker, WT (n = 9) or Adgrg2<sup>−/−</sup> (n = 10); (J) GlyH-101 (25 μM), a non-specific CFTR inhibitor, WT (n = 17) or Adgrg2<sup>−/−</sup> (n = 15); (K) CFTRinh-172 (10 μM), a specific CFTR inhibitor, WT (n = 12) or Adgrg2<sup>−/−</sup> (n = 10). (L) Effects of angiotensin II (100 nM, an angiotensin receptor agonist) and PD123319 (1 μM, an AT2 receptor antagonist) on the diameters of luminal ductules derived from WT or Adgrg2<sup>−/−</sup> mice (n ≥ 12). (M) Effects of angiotensin II (100 nM) and candesartan (1 μM, an AT1 receptor antagonist) on the diameters of luminal ductules derived from WT or Adgrg2<sup>−/−</sup> mice (n ≥ 12). Application of GlyH-101 and CFTRinh-172 to ligated ductules derived from WT mice recapitulated the phenotype of the ductules derived from Adgrg2<sup>−/−</sup> mice. (4A-M) *p < 0.05, **p < 0.01, ***p < 0.001; Adgrg2<sup>−/−</sup> mice compared with WT mice. #p < 0.05, ##p < 0.01, ###p < 0.001. Treatment with selective inhibitors or stimulators was compared with control vehicles. n.s., no significant difference. At least three independent biological replicates were performed for Figure 4A–M.

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Figure 4—figure supplement 1. Expression and functional analysis of potential osmotic drivers in efferent ductules. (A) Quantitative RT-PCR (qRT-PCR) analysis of mRNA transcription profiles of potential osmotic drivers including selective ion channels and transporters in efferent ductules, brain and liver of wild-type (WT) (N = 3) male mice. Expression levels were normalized with GAPDH levels. *p < 0.05, **p < 0.01, ***p < 0.001, brain were compared with efferent ductules. #p < 0.05, ##p < 0.01, ###p < 0.001, liver were compared with efferent ductules. n.s., no significant difference. (B–C) Effects of different conditions on the width of the ductules. (D) Relative mRNA levels of ADGRG2-pm-labeled (non-ciliated) cells (WT n=3) and ADGRG2-pm-labeled (non-ciliated) cells (Adgrg2-/- n=3).
channel or transporter blockers on the diameters of luminal ductules derived from WT or Adgrg2−/− mice. (B) LaCl3 (100 μM), a non-selective TRPC3/6/7 blocker, WT(n = 12) or Adgrg2−/−(n = 12); (C) Amiloride (1 mM), a sodium/hydrogen antiporter NHE1 inhibitor, WT(n = 10) or Adgrg2−/−(n = 12). (D) Quantitative RT-PCR (qRT-PCR) analysis of the ADGRG2, CFTR, Gαq, Gαq, β-arrestin-1 and β-arrestin-2 expression level in ADGRG2 promoter-labeled efferent ductule cells derived from Adgrg2−/− mice(n = 3) and their WT littersmates(n = 3). Expression levels were normalized with GAPDH levels. (B–D) **p<0.01, ***p<0.001; Adgrg2−/− mice compared with WT mice. ##p<0.01, Selective inhibitors or stimulators treated were compared with control vehicles. n.s., no significant difference.

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Figure 5. Functional coupling and co-localization of CFTR and ADGRG2 on the apical membrane in the efferent ductules. (A) Intracellular pH (pHi) of the ligated efferent ductules from WT (n = 9) mice and Adgrg2<sup>−/−</sup> (n = 9) mice were measured by carboxy-SNARF (5 μM), with or without incubation with...
the CFTR inhibitor CFTRinh-172. (B) qRT-PCR analysis of CFTR levels in the efferent ductules of WT (n = 3) or Adgrg2-/-Y (n = 3) mice. (C) Co-localization of ADGRG2 (red fluorescence) and CFTR (sc-8909, Santa Cruz, green fluorescence) in the male efferent ductules of WT mice. Scale bars, 50 μm. (D) Analysis of ADGRG2 and CFTR fluorescence intensities by Pearson’s correlation analysis. The Pearson’s correlation coefficient was 0.76. (E) Immunofluorescence staining of ADGRG2 (red fluorescence) and CFTR (sc-8909, Santa Cruz, green fluorescence) in the efferent ductules of Adgrg2-/-Y mice. Scale bars, 50 μm. (F) Co-localization of ADGRG2 (red fluorescence) and ezrin (green fluorescence) in the male efferent ductules of WT mice. Scale bars, 50 μm. (G) Analysis of ADGRG2 and ezrin fluorescence intensities by Pearson’s correlation analysis. The Pearson’s correlation coefficient was 0.69. (H) ADGRG2 was immunoprecipitated with an anti-ADGRG2 antibody from the male efferent ductules of WT mice or Adgrg2-/-Y mice, and co-precipitated CFTR, Gs, Gq, β-arrestin-1, β-arrestin-2 and Gi-1/2/3 levels were examined by using specific corresponding antibodies (CFTR antibody:20738–1-AP, Proteintech). (5A-5B) *p < 0.05, **p < 0.01, ***p < 0.001, Adgrg2-/-Y mice compared with WT mice. #p < 0.05, ##p < 0.01, ###p < 0.001. Treatment with selective inhibitors or stimulators was compared with control vehicles. n.s., no significant difference. At least three independent biological replicates were performed for Figure 5A–B.

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Figure 5—figure supplement 1. Representative agarose gel for the reverse transcription PCR analysis of CFTR mRNA level in efferent ductules of WT or Adgrg2−/− mice. The upper band (220 bp PCR product) in each lane represents CFTR, whereas the lower band (100 bp product) represents GAPDH (This figure was related to Figure 5B).

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**Figure 5—figure supplement 2.** pH homeostasis in the efferent ductules was impaired in Adgrg2−/− mice. (A) The relationship between R value (fluorescence emission intensity at 635 nm/fluorescence emission intensity at 590 nm) and pH for the indicator 5′(and 6′)-carboxy-10-dimethylamino-3-hydroxy-spiro[7H-benzo[c]xanthene-7,1′(3 hr)-isobenzofuran]-3′-one (carboxy SNARF-1)). (B–D) Intracellular pH (pHi) of the ligated efferent ductules with or without bicarbonate (25 mM) (B), Acetazolamide (500 μM) (C), Amiloride (1 mM) (D) from WT mice and Adgrg2−/− mice. (B–D) *p<0.05, selective inhibitors or stimulators treated were compared with control vehicles.

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Figure S—figure supplement 3. Immunostaining experiments for CFTR location in efferent ductules. (A) Co-Immunofluorescence staining of ADGRG2 (red fluorescence) and ANO1 (green fluorescence) in WT male mice efferent ductules. Scale bars, 50 μm. (B) Immunofluorescent staining of CFTR (sc-8909, Santa Cruz, green fluorescence) and acetylated-tubulin (yellow fluorescence) in WT male mice efferent ductules. Scale bars, 50 μm. A representative image was selected from at least three independent experiments. (C) Bar graph representation and statistical analyses of co-localization cells of CFTR and acetylated-tubulin in WT male mice efferent ductules (related to (B)).

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Figure 5—figure supplement 4. Bar graph representation and statistical analyses of Figure 5H. ***p<0.001, Adgrg2−/− lysates or IP protein were compared with WT lysates or IP protein respectively. n.s., no significant difference.
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Figure 6. The whole-cell Cl$^-$ current recording of ADGRG2 promoter-labeled efferent ductule cells. (A) Time course of whole-cell Cl$^-$ current ($I_{ADGRG2-ED}$) at +100 and –100 mV in ADGRG2 promoter-labeled efferent ductule cells derived from Adgrg2$^{-/-}$ mice or their littermates. An ‘a’ or ‘d’ indicates the
substitution of the Cl\(^-\) bath solution with Gluc\(^-\) (148.5 mM Cl\(^-\) was replaced by 48.5 mM Cl\(^-\) and 100 mM Gluc\(^-\)); and 'b' or 'e' indicates the substitution of the Gluc\(^-\) bath solution with Cl\(^-\) (148.5 mM Cl\(^-\)). 'a', 'b' and 'c' belong to WT mice. 'd', 'e' and 'f' belong to Adgrg2\(^{-/-}\) mice. (B) The current-voltage relationship of I\(_{ADGRG2-ED}\) at specific time points (from 6A) is shown. (C) The whole cell Cl\(^-\) current of I\(_{ADGRG2-ED}\) elicited by voltage steps between –100 mV and +100 mV in a representative ADGRG2-promoter-RFP labeled efferent ductule cells derived from the Adgrg2\(^{-/-}\) mice and their wild type littermates. The outwardly rectifying I\(_{ADGRG2-ED}\) was significantly diminished when bath Cl\(^-\) was substituted for gluconate (Gluc\(^-\)). (D) Representative whole-cell Cl\(^-\) current of ADGRG2 promoter-labeled efferent ductule cells; I\(_{ADGRG2-ED}\) versus voltage (I–V) relationships in response to voltage ramps recorded with a CsCl pipette solution in Adgrg2\(^{-/-}\) (n = 8) or WT mice (n = 8). The outwardly rectifying I\(_{ADGRG2-ED}\) was significantly diminished, and its reversal potential (E\(_{rev}\)) shifted to the positive direction when Cl\(^-\) was substituted for Gluc\(^-\). (E) Average current densities (pA/pF) measured at 100 mV of (C). Inset: average E\(_{rev}\) (± s.e.m., n = 8 for each condition). **p<0.01, I\(_{ADGRG2-ED}\) in Gluc\(^-\) solution was compared with I\(_{ADGRG2-ED}\) in Cl\(^-\) solution. ns, no significant difference. At least three independent biological replicates were performed.

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Figure 7. Cl− currents in the non-ciliated cells of the efferent ductules through CFTR. (A, D and F) Corresponding I-V curves of the whole-cell Cl− IADGRG2-ED currents recorded in Figure 6 and (A, D and F) Corresponding I-V curves of the whole-cell Cl− IADGRG2-ED currents recorded in Figure 7—Figure 7 continued on next page.

Zhang et al. eLife 2018;7:e33432. DOI: https://doi.org/10.7554/eLife.33432

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Biochemistry and Chemical Biology
Figure 7 continued

**Figure supplement 1(A,F) and Figure 7—figure supplement 3(D).** WT (n = 6), Adgrg2−/− (n = 6); WT +CFTRinh-172 (n = 6), Adgrg2−/− +CFTRinh-172 (n = 6); WT +AN9 (n = 6), Adgrg2−/− +AN9 (n = 6); WT +DIDS (n = 6), Adgrg2−/− +DIDS (n = 6); WT +Control RNAi (n = 6), WT +CFTR RNAi (n = 6), Adgrg2−/− +Control RNAi (n = 6), Adgrg2−/− +CFTR RNAi (n = 6); WT +FSK + IBMX (n = 6), Adgrg2−/− +FSK + IBMX (n = 6). (B,E and G) Corresponding bar graph depicting the average current densities (pA/pF) measured at 100 mV in (A), (D) and (F). (C) qRT-PCR analysis of CFTR levels in the efferent ductules treated with CFTR siRNA (n = 3) or control RNAi (n = 3). (B, E and G) *p < 0.05, **p < 0.01, ***p < 0.001, Adgrg2−/− mice compared with WT mice. #p < 0.05, ##p < 0.01, ###p < 0.001. Treatment with selective inhibitors, stimulators or CFTR RNAi was compared with control vehicles or control RNAi. n.s., no significant difference. At least three independent biological replicates were performed for **Figure 7B, E and G.**

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Figure 7—figure supplement 1. Effects of different stimulators or inhibitors of osmotic drivers on $I_{ADGRG2-ED}$ Cl currents of efferent ductule cells derived from Adgrg2$^{−/−}$ mice and their wild type littermates. (A) The whole cell Cl current of $I_{ADGRG2-ED}$ elicited by voltage steps between $−100$ mV.
and +100 mV in a representative ADGREG2-promoter-RFP-labeled efferent ductule cells derived from the Adg22Y mice and their wild-type littermates with or without selective inhibitors or stimulators. (B) Corresponding bar graph of average reversal potential (E_{rev}) (± s.e.m., n = 6 for each condition) in (A) and calculated Nernst potential at according Cl- concentrations. n.s., no significant difference; compared to calculated Nernst potential.

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Figure 7—figure supplement 2. Effects of Cl⁻ concentration change and CFTRinh-172 on the $I_{\text{ADGRG2-ED}}$ Cl⁻ currents. (A) The whole cell Cl⁻ current of $I_{\text{ADGRG2-ED}}$ elicited by voltage steps between −100 mV and +100 mV in a representative ADGRG2-promoter-RFP-labeled efferent ductule cells derived from Zhang et al. eLife 2018;7:e33432. DOI: https://doi.org/10.7554/eLife.33432
Figure 7—figure supplement 2 continued

from the Adgrg2<sup>Y</sup> mice and their wild-type littermates with or without CFTR selective inhibitors CFTRinh-172, and in response to bath Cl<sup>-</sup> concentration change (Cl<sup>-</sup> was substituted for gluconate (Gluc)). (B) Representative whole cell Cl<sup>-</sup> current of I<sub>ADGRG2.ED</sub> versus voltage (I–V) relationships in response to voltage ramps recorded in (A) with a CsCl pipette solution. (C) Corresponding bar graph of average current densities (pA/pF) measured at 100 mV. **p<0.01, ***p<0.001, compared with WT mice in 148.5mM Cl<sup>-</sup> condition. #p<0.05, WT mice in 148.5mM Cl<sup>-</sup> condition treated with CFTRinh-172 was compared with WT mice in 48.5mM Cl<sup>-</sup> condition treated with CFTRinh-172. (D) Corresponding bar graph of average reversal potential (E<sub>rev</sub>) (±s. e.m., n = 8 for each condition) in (A–B).

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Figure 7—figure supplement 3. Effects of CFTR knocked down on the $I_{ADGRG2,ED}$ Cl$^-$ currents. (A) The whole cell Cl$^-$ current of $I_{ADGRG2,ED}$ elicited by voltage steps between −100 mV and +100 mV of primary efferent ductile cells after CFTR-siRNA or Scramble-siRNA treatment. (B) Corresponding bar graph of average reversal potential ($E_{rev}$) (±s.e.m., n = 6 for each condition) in (A). n.s., no significant difference, compared to calculated Nernst potential. DOI: https://doi.org/10.7554/eLife.33432.021
Figure 8. Gq activity regulated Cl⁻ current and pH homeostasis in the efferent ductules. (A) Intracellular pH (pHi) of the ligated efferent ductules from WT (n = 9) mice or Gnaq⁺⁻ (n = 9) mice was measured by carboxy-SNARF. (B) The whole-cell Cl⁻ current of the IADGRG2-ED elicited by voltage steps Figure 8 continued on next page
between −100 mV and +100 mV in representative ADGRG2 promoter-RFP-labeled efferent ductule cells derived from Gnaq+/− mice, their WT littermates, or WT murine cells incubated with the PKC inhibitor Ro 31–8220 (500 nM). The whole-cell Cl− \( I_{\text{ADGRG2-ED}} \) current was recorded with a CsCl pipette solution (101 mM CsCl, 10 mM EGTA, 10 mM Hepes, 20 mM TEACl, 2 mM MgATP, 2 mM MgCl₂, 5.8 mM glucose, pH 7.2, with D-mannitol compensated for osm 290) and a bath solution containing 138 mM NaCl, 4.5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 5 mM glucose, and 10 mM HEPES, pH 7.4 with D-mannitol compensated for osm 310. (C) Corresponding I-V curves of the whole-cell Cl− currents recorded in (B): WT (n = 6), Gnaq+/− (n = 6), WT + Ro 31–8220 (n = 6). (D) Corresponding bar graph of the average current densities (pA/pF) measured at 100 mV according to (C). (E) Co-localization of ADGRG2 (red) and Gq (green) in the male efferent ductules. Scale bars, 50 μm. (F) Co-localization of Gq (red) and acetylated-tubulin (yellow) in the male efferent ductules. Scale bars, 50 μm. (G) IP1 levels in the brain tissues, ligated efferent ductules, and livers of WT (n = 9) or Adgrg2−/− (n = 9) mice in response to ATP (5 mM) or control vehicles, measured by ELISA. (H) cAMP concentrations in the brains, ligated efferent ductules, and livers of WT (n = 9) or Adgrg2−/− (n = 9) mice were measured using ELISA. (8A,8D,8G-H) *p < 0.05, **p < 0.01, ***p < 0.001, Adgrg2−/− mice or Gnaq+/− mice compared with WT mice. #p < 0.05, ##p < 0.01, ###p < 0.001, ATP- or Ro 31–8220-treated cells were compared with control vehicles. n.s., no significant difference. At least three independent biological replicates were performed for Figure 8A,D,G and H.

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Figure 8—figure supplement 1. Effects of G protein signaling on the $I_{\text{ADGRG2-ED}}$ Cl$^-$ currents. (A) The whole cell Cl$^-$ current of $I_{\text{ADGRG2-ED}}$ elicited by voltage steps between $-100$ mV and $+100$ mV in a representative ADGRG2-promoter-RFP-labeled efferent ductule cells derived from the Gnaq$^{+/−}$ mice.

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and their wild-type littermates, in control bath solution or in response to substitution of Cl\(^-\) with the gluconate (Gluc\(^-\)). (B) Representative whole cell Cl\(^-\) current of ADGRG2-promoter-labeled efferent ductule cells \(I_{\text{ADGRG2-ED}}\) versus voltage (I–V) relationships in response to voltage ramps recorded in (A) with a CsCl pipette solution. (C) Corresponding bar graph of average current densities (pA/pF) measured at 100 mV. Inset: Average \(E_{\text{rev}}\) (±s.e.m., \(n = 8\) for each condition). (D) The whole cell Cl\(^-\) current of \(I_{\text{ADGRG2-ED}}\) elicited by voltage steps between \(-100\) mV and \(+100\) mV in a representative ADGRG2-promoter-RFP-labeled efferent ductule cells derived from the Adgrg2\(^{-/-}\) mice and their wild type littermates, with or without specific G protein signaling pathway inhibitors (including the Gs-PKA inhibitor PKI14-22 or the Gq-PKC inhibitor Ro 31–8220). (E) Corresponding I-V curves of the whole-cell Cl\(^-\) currents \(I_{\text{ADGRG2-ED}}\) recorded in (D). (F) Corresponding bar graph of average current densities (pA/pF) measured at 100 mV in (D–E). (G) Corresponding bar graph of average reversal potential (\(E_{\text{rev}}\)) and calculated Nernst potential (±s.e.m., \(n = 6\) for each condition) in (D–E). n.s., no significant difference; the \(E_{\text{rev}}\) were compared with calculated Nernst potential. (C,F) *\(p<0.05\), **\(p<0.01\), Gnaq\(^{+/–}\) or Adgrg2\(^{-/-}\) mice were compared with their wild-type littermates. #\(p<0.05\), substitution of Cl\(^-\) with the gluconate (Gluc\(^-\)) or cells treated with different inhibitors were compared with control solution.

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Figure 8—figure supplement 2. Gq is localized in the ADGRG2 expressed cells, but not the acetylated-tubulin-labeled cells in efferent ductules. (A) Bar graph representation and statistical analyses of co-localization of Gq and ADGRG2 in WT male mice efferent ductules (corresponding to Figure 8E), n = 3 mice per group; 4–10 random areas were selected from each section, and six sections were randomly selected from each mouse. (B) Bar graph representation and statistical analyses of co-localization of Gq and acetylated-tubulin in WT male mice efferent ductules (corresponding to Figure 8F), n = 3 mice per group; 4–10 random areas were selected from each section, and six sections were randomly selected from each mouse.

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Figure 8—figure supplement 3. The expression of ADGRG2, CFTR, Gs, Gq, β-arrestin-1, β-arrestin-2 in efferent ductules, brain and liver tissue of WT and Adgrg2^{−/−} mice. (A) Western blot analysis of ADGRG2, CFTR, Gs, Gq, β-arrestin-1, β-arrestin-2 expression in efferent ductules, brain and liver tissue.
Figure 8—figure supplement 3 continued

of WT and Adgrg2−/− mice. A representative western blot from at least three independent experiments was shown. (CFTR antibody:20738–1-AP, Proteintech). (B) Bar graph representation and statistical analyses of (A). All blots were normalized to GAPDH. n.s., no significant difference; Adgrg2−/− mice compared with WT mice in the same tissue.

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Figure 9. β-arrestin-1 is required for fluid reabsorption in the efferent ductules via scaffolding ADGRG2/CFTR complex formation. (A) Diameters of the luminal ductules derived from WT (n = 12), Adgrg2−/− (n = 12) or Arrb1−/− (n = 15) mice. (B) Diameters of the luminal ductules derived from WT (n = 12), Adgrg2−/− (n = 12) or Arrb2−/− (n = 15) mice. Figure 9 continued on next page.
Adgrg2^−/− (n = 12) or Arrb2^−/− (n = 15) mice. (C) Intracellular pH (pHi) of the ligated efferent ductules derived from WT (n = 9), Arrb1^−/− (n = 9) or Arrb2^−/− (n = 9) mice were measured by carboxy-SNARF. (D) Co-localization of ADGRG2 (red fluorescence) and CFTR (sc-8909, Santa Cruz, green fluorescence) in the male efferent ductules of Arrb2^−/− mice. (E) Analysis of ADGRG2 and CFTR fluorescence intensities in Arrb2^−/− mice by Pearson’s correlation analysis. The Pearson’s correlation coefficient was 0.62. (F) Localization of ADGRG2 (red fluorescence) and CFTR (sc-8909, Santa Cruz, green fluorescence) in the male efferent ductules of Arrb1^−/− mice. (G) Analysis of ADGRG2 and CFTR fluorescence intensities in Arrb1^−/− mice by Pearson’s correlation analysis. The Pearson’s correlation coefficient was −0.15. (H) Co-localization of ezrin (red fluorescence) and CFTR (sc-8909, Santa Cruz, green fluorescence) in the male efferent ductules of Arrb2^−/− mice. (I) Analysis of ezrin and CFTR fluorescence intensities in Arrb2^−/− mice by Pearson’s correlation analysis. The Pearson’s correlation coefficient was 0.66. (J) Co-localization of ezrin (red fluorescence) and CFTR (sc-8909, Santa Cruz, green fluorescence) in the male efferent ductules of Arrb1^−/− mice. (K) Analysis of ezrin and CFTR fluorescence intensities in Arrb1^−/− mice by Pearson’s correlation analysis. The Pearson’s correlation coefficient was −0.15. (L) ADGRG2 was immunoprecipitated by an anti-ADGRG2 antibody in the male efferent ductules of Arrb1^−/− mice or Arrb2^−/− mice, and co-precipitates with CFTR, β-arrestin-1, and β-arrestin-2 were examined by using specific corresponding antibodies (CFTR antibody:20738–1-AP, Proteintech). (9A-C) *p<0.05, **p<0.01, ***p<0.001, Adgrg2^−/− mice compared with WT mice. #p<0.05, ##p<0.01, ###p<0.001, Arrb1^−/− mice or Arrb2^−/− mice compared with WT mice. ns, no significant difference. At least three independent biological replicates were performed for Figure 9A–C and L.

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Figure 9—figure supplement 1. Western blot analysis of β-arrestin1/2 expression in the efferent duct tissue. (A,C) Western blot analysis of β-arrestin1/2 expression in the efferent duct tissue of WT and Arrb2−/− (A) or Arrb1−/− (C) mice. A representative western blot from at least three independent experiments was shown. (B,D) Bar graph representation and statistical analyses of (A,C). All blots were normalized to GAPDH. ***p<0.001, Arrb2−/− mice or Arrb1−/− mice were compared with WT mice, respectively. n.s., no significant difference.

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Figure 9—figure supplement 2. β-arrestin-1 is an essential component in a signaling complex encompassing the ADGRG2 and CFTR in efferent ductules. (A) Co-localization of Ezrin (red fluorescence) and CFTR (sc-8909, Santa Cruz, green fluorescence) in male efferent ductules of the WT mice and Adgrg2-/- mice. Scale bars, 50 μm. Analysis of Ezrin and CFTR fluorescence intensities by Pearson’s correlation. The pearson’s correlation coefficient is 0.6 for WT mice and 0.65 for Adgrg2-/- mice. (B) Bar graph representation and statistical analyses of Figure 9L. ***p<0.001, Arrb2-/- lysates or IP protein were compared with Arrb1-/- lysates or IP protein, respectively. n.s., no significant difference.
DOI: https://doi.org/10.7554/eLife.33432.028
Figure 9—figure supplement 3. The complex formation between ADGRG2, β-arrestin-1 and CFTR in HEK293 cells. (A) HEK293 cells were transfected with equal amount plasmids encoding ADGRG2, CFTR, β-arrestin-1 or β-arrestin-2 plasmids. The Flag- ADGRG2 were pulled down by M2-Flag beads and the associated CFTR, β-arrestins were detected by western blot. Representative images from at least three independent experiments are shown (CFTR antibody:20738–1-AP, Proteintech). (B) Bar graph representation and statistical analyses of (A). ***p<0.001, IP protein of ADGRG2 overexpressed cells were compared with control plasmids transfected cells respectively. n.s., no significant difference.

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ADGRG2 upregulates CFTR Cl⁻ currents through G protein signaling. (A) Whole-cell Cl⁻ currents recorded with a CsCl pipette solution in HEK293 cells transfected with plasmids encoding ADGRG2 or/and CFTR, with or without CFTR inhibitor CFTRinh-172 (10 μM) or its activator (FSK (10 μM)).

B

Figure 10 continued on next page

Zhang et al. eLife 2018;7:e33432. DOI: https://doi.org/10.7554/eLife.33432

Figure 10. ADGRG2 upregulates CFTR Cl⁻ currents through G protein signaling. (A) Whole-cell Cl⁻ currents recorded with a CsCl pipette solution in HEK293 cells transfected with plasmids encoding ADGRG2 or/and CFTR, with or without CFTR inhibitor CFTRinh-172 (10 μM) or its activator (FSK (10 μM)).
mM+IBMX (100 μM)). (B) Corresponding I-V curves of the whole-cell Cl− currents recorded in (C). ADGRG2 (n = 6), CFTR (n = 6), CFTR + CFTRinh-172 (n = 6), CFTR + FSK + IBMX (n = 6), CFTR + ADGRG2 (n = 6), CFTR + ADGRG2+CFTRinh-172 (n = 6), CFTR + ADGRG2+FSK + IBMX (n = 6). (C and D) Bar graph representation of average current densities (pA/pF) measured at 100 mV according to (B) and Figure 9; (C and D) Bar graph representation of average current densities (pA/pF) measured at 100 mV according to (B) and Figure 10-figure supplement 5C. (10C-10D) *p<0.05, **p<0.01, ***p<0.001, HEK293 cells transfected with CFTR compared with cells transfected with pCDNA3.1. #p<0.05, ##p<0.01, ###p<0.001, HEK293 cells transfected with ADGRG2 compared with non-ADGRG2 transfected cells. $p<0.05, $$, p<0.01, $$$, p<0.001, CFTRinh-172, FSK, NF449, U73122 or Ro 31–8220 compared with control vehicle. n.s., no significant difference. At least three independent biological replicates were performed for Figure 10C–D.

DOI: https://doi.org/10.7554/eLife.33432.030
Figure 10—figure supplement 1. Co-localization analysis of ADGRG2 and CFTR in HEK293 cells. (A) Co-localization of ADGRG2 (red fluorescence) and CFTR (green fluorescence) in HEK293 cells. Scale bars, 20 μm. A representative figure from at least three independent experiments was shown. (B) Analysis of ADGRG2 and CFTR fluorescence intensities by Pearson’s correlation. The pearson’s correlation coefficient is 0.56.

DOI: https://doi.org/10.7554/eLife.33432.031
Figure 10—figure supplement 2. Construction and expression of ADGRG2-full length (ADGRG2FL) and a truncated form ADGRG2β. (A–C) Construction and expression of ADGRG2-full length (ADGRG2FL) and a truncated form ADGRG2β. (A) Schematic illustration of the structure of the ADGRG2FL and the ADGRG2β used in the current study. The full length ADGRG2 encompasses a GPS site, which was cleaved after its transportation to the plasma membrane through an auto-hydrolysis mechanism. Therefore, we made a truncation version of the ADGRG2β, which starts at the auto-cleaved site, T584. A signal peptide and a flag-tag were added at the N-terminal for both ADGRG2FL and ADGRG2β. (B) Confocal images showed that the ADGRG2FL and ADGRG2β were primarily localized at the plasma membrane. Scale bars, 10 μm. (C) The expression of ADGRG2FL and ADGRG2β were detected by Western blot. The observed 180 kDa band of the ADGRG2FL is the N-terminal part of the ADGRG2 protein due to the auto-hydrolysis. The apparent 200 kDa band of the ADGRG2β is due to the glycosylation. Representative western blots from at least three independent experiments were shown.

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Overexpression of ADGRG2FL and ADGRG2β lead to constitutive increased cellular cAMP levels. (A–C) Overexpression of ADGRG2FL leads to constitutively increased intracellular cAMP levels. HEK293 cells were co-transfected with the GloSensor plasmid.

Figure 10—figure supplement 3 continued on next page.
and the control pcDNA3.1 vector or the ADGRG2FL expression vector at the indicated concentrations. (A) Representative curve of the intracellular cAMP level measured by the GloSensor assay. (B) A dose-dependent cAMP increase by overexpression of ADGRG2FL was detected. (C) The corresponding ADGRG2FL protein expression level was examined by western blot. **p<0.01, ***p<0.001, the ADGRG2FL-transfected cells were compared to pcDNA3.1-transfected cells. (D–F) Overexpression of ADGRG2β leads to constitutively increased intracellular cAMP levels. HEK293 cells were transfected with the GloSensor plasmid and the control pcDNA3.1 vector or the ADGRG2β expression vector at the indicated concentrations. (D) Representative curve of the intracellular cAMP level measured by the GloSensor assay. (E) A dose-dependent cAMP increase by overexpression of ADGRG2β was detected. (F) The corresponding ADGRG2β protein expression level was examined by western blot. ***p<0.001, the ADGRG2β transfected cells were compared to pcDNA3.1-transfected cells. (G–H) ADGRG2FL overexpressed cells showed higher cAMP levels than the control cells. (G) The constitutive cAMP accumulation in HEK293 cells transfected with ADGRG2FL was further enhanced by the overexpression of Gs in a dose-dependent manner. (H) The corresponding ADGRG2FL and Gs protein expression levels were examined by western blot. ***p<0.001, the Gs and ADGRG2 transfected cells were compared to only ADGRG2 transfected cells. #p<0.05, ##p<0.01, ###p<0.001, the Gs and pcDNA3.1 transfected cells were compared to only pcDNA3.1 transfected cells. $$, p<0.01, $$$, p<0.001, The ADGRG2FL overexpressed cells were compared to the corresponding vector pcDNA3.1 transfected cells with equal Gs protein amount. (I–J) ADGRG2β overexpressed cells showed higher cAMP levels than the control cells. (I) The constitutive cAMP accumulation in HEK293 cells transfected with ADGRG2β was further enhanced by the overexpression of Gs in a dose-dependent manner. (J) The corresponding ADGRG2β and Gs protein expression levels were examined by a western blot. ***p<0.001, the Gs and ADGRG2β transfected cells were compared to only ADGRG2β transfected cells. #p<0.05, ##p<0.01, ###p<0.001, the Gs and pcDNA3.1 transfected cells were compared to only pcDNA3.1 transfected cells. $$, p<0.01, $$$, p<0.001, The ADGRG2β overexpressed cells were compared to the corresponding vector pcDNA3.1 transfected cells with equal Gs protein amounts. (K–M) Comparison of The cAMP levels in the cells with similar expression levels of ADGRG2FL and ADGRG2β. (K–L) The ADGRG2β exhibits higher constitutive cAMP activity compared to ADGRG2FL. (M) The corresponding ADGRG2FL and ADGRG2β protein expression levels were examined by the ELISA assay. ***p<0.001, ADGRG2FL or ADGRG2β transfected cells were compared to control vector transfected cells ###p<0.001, ADGRG2β transfected cells were compared to ADGRG2FL transfected cells.

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Figure 10—figure supplement 4. Overexpression of ADGRG2FL and ADGRG2β have constitutive Gq-NFAT signaling activities. (A–B) Dose-dependent effect of ADGRG2FL (A) or ADGRG2β (B) overexpression on the luciferase activity of the NFAT-DLR. *p<0.05, **p<0.01, ***p<0.001, the Figure 10—figure supplement 4 continued on next page
ADGRG2 transfected cells were compared to non-transfected cells. (C–D) ADGRG2FL overexpressed cells showed higher NFAT signaling activity than the control cells. (C) The luciferase activity of the NFAT-DLR in HEK293 cells transfected with ADGRG2FL was further enhanced by the overexpression of Gq in a dose-dependent manner. (D) The corresponding ADGRG2FL and Gq protein expression levels were examined by western blot. ***p<0.001, the Gq and ADGRG2FL transfected cells were compared to only ADGRG2FL transfected cells. #p<0.05, ##p<0.01, ###p<0.001, the Gq and pcDNA3.1 transfected cells were compared to only pcDNA3.1 transfected cells. $$, p<0.01,$$$, p<0.001, The ADGRG2FL overexpressed cells were compared to the corresponding vector pcDNA3.1 transfected cells with equal Gq protein amounts. (E–F) ADGRG2β overexpressed cells showed higher NFAT signaling activity than the control cells. (E) The luciferase activity of the NFAT-DLR in HEK293 cells transfected with ADGRG2β was further enhanced by the overexpression of Gq in a dose-dependent manner. (F) The corresponding ADGRG2β and Gq protein expression levels were examined by western blot. *p<0.05, **p<0.01, ***p<0.001, the Gq and ADGRG2β transfected cells were compared to only ADGRG2β transfected cells. #p<0.05, ##p<0.01, ###p<0.001, the Gq and pcDNA3.1 transfected cells were compared to only pcDNA3.1 transfected cells. $$, p<0.01,$$$, p<0.001, The ADGRG2β overexpressed cells were compared to the corresponding vector pcDNA3.1 transfected cells with equal Gq protein amounts. (G) Comparison of the luciferase activity of the NFAT-DLR in the cells with similar expression levels of ADGRG2FL and ADGRG2β. The ADGRG2β exhibits higher constitutive activity in NFAT luciferase assay when compared to ADGRG2FL. ***p<0.001, ADGRG2FL or ADGRG2β transfected cells were compared to control vector transfected cells. ###p<0.001, ADGRG2β transfected cells was compared to ADGRG2FL transfected cells.

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Figure 10—figure supplement 5. ADGRG2 upregulates CFTR Cl\(^{-}\) currents and Cl\(^{-}\) efflux through G protein signaling. (A) Corresponding bar graph of average reversal potential (E\(_{rev}\)) (±s.e.m., n = 6 for each condition) in HEK293 cells transfected with plasmids encoding ADGRG2 or/and CFTR, with or without CFTRinh-172.

Zhang et al. eLife 2018;7:e33432. DOI: https://doi.org/10.7554/eLife.33432

Figure 10—figure supplement 5 continued on next page
without CFTR inhibitor CFTRinh-172 (10 μM) or its activator (FSK (10 μM) + IBMX (100 μM)) and calculated Nernst potential. n.s., no significant difference; the $E_{\text{rev}}$ were compared with calculated Nernst potential. (B) Whole-cell Cl$^-$ currents recorded with a CsCl pipette solution in HEK293 cells transfected with plasmids encoding ADGRG2 or/and CFTR, with or without the PKA inhibitor PKI14-22 or the PKC inhibitor Ro 31–8220. (C) Corresponding I–V curves of the whole-cell Cl$^-$ currents (n = 6 for each condition) recorded in (B). (D) Corresponding bar graph of average reversal potential ($E_{\text{rev}}$) (± s.e.m., n = 6 for each condition) recorded in (B–C) and calculated Nernst potential. n.s., no significant difference; the $E_{\text{rev}}$ were compared with calculated Nernst potential.

DOI: https://doi.org/10.7554/eLife.33432.035
Figure 11. Key mutations of ADGRG2 downregulates CFTR Cl⁻ currents through G protein signaling. (A) Schematic representation of the location of the selected ADGRG2 mutants in intracellular loop 2 and loop 3 of ADGRG2. (B) Effects of the overexpression of ADGRG2 (n = 6) and its mutations
(n = 6) on cAMP levels. (C) Effects of the overexpression of ADGRG2 (n = 6) and its mutations (n = 6) on NFAT-DLR activation. (D) Whole-cell Cl− currents recorded with a CsCl pipette solution in HEK293 cells overexpressing CFTR, CFTR and ADGRG2-WT, CFTR and ADGRG2-HM696AA, CFTR and ADGRG2-Y698A, CFTR and ADGRG2-F705A, CFTR and ADGRG2-Y708A or CFTR and ADGRG2-RK803EE. (E) Corresponding I-V curves for the whole-cell Cl− currents recorded in (D). (F) Bar graph representation of average current densities (pA/pF) measured at 100 mV according to (E). (11B-11C and 11F) *p<0.05, **p<0.01, ***p<0.001, cells transfected with ADGRG2-WT or mutants compared with the control plasmid (pCDNA3.1). #p<0.05, ##p<0.01, ###p<0.001, cells overexpressing ADGRG2 mutants compared with ADGRG2-WT. n.s., no significant difference. At least three independent biological replicates were performed for Figure 11B–C,F.

DOI: https://doi.org/10.7554/eLife.33432.036
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Figure 11—figure supplement 1. Sequence alignment of the transmembrane domains of ADGRG2 (Homo sapiens, Mus musculus, Rattus norvegicus), β2AR (H. sapiens, M. musculus, and R. norvegicus), and GPR126 (H. sapiens). The ADGRG2 mutation sites studied in the current work were highlighted. The green color indicated that the mutants caused both Gs and Gq defects of ADGRG2 coupling; the yellow color indicated that the mutants induced only Gs defects of ADGRG2 coupling, and the blue color indicated that the mutant induced only Gq defects of ADGRG2 coupling. DOI: https://doi.org/10.7554/eLife.33432.037
Figure 11—figure supplement 2. Western blot and ELISA analysis of the expression of these mutants in the cell membrane. (A) Western blot of ADGRG2 WT and its mutations (H696A, H696A, M697A, Y698A, K703A, V704A, F705A and Y708A in intracellular loop 2; and QL798AA and RK803EE in intracellular loop 3). Representative western blot from at least three independent experiments. (B) ELISA of the expression level of ADGRG2-WT and its mutations in the plasma membrane. ***p<0.001, the protein level of ADGRG2-WT and its mutations in the plasma membrane were compared to control.
DOI: https://doi.org/10.7554/eLife.33432.038
Figure 11—figure supplement 3. Corresponding bar graph of average reversal potential ($E_{\text{rev}}$) (±s.e.m., n = 6 for each condition) recorded in Figure 11D–11E and calculated Nernst potential. ns., no significant difference; the $E_{\text{rev}}$ were compared with calculated Nernst potential.

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Figure 12. Conditional expression of ADGRG2 wild-type or its selective G-subtype signaling mutants in the efferent ductules in Adgrg2^{−/−} mice and their effects on the morphology, sperm maturation of efferent ductules. (A) Schematic representation of the mouse ADGRG2 promoters used in the study.
rescue experiment. (B) Representative hematoxylin-eosin staining of the WT mice, Adgrg2+/Y mice or Adgrg2−/− mice infected with lentivirus encoding ADGRG2-WT or different G-subtype mutants at the efferent ductules, initial segment or caput of the epididymis. Scale bars, 200 μm. (C) Bar graph representing the quantitative analysis of the number of sperm shown in Figure 12B from at least four independent experiments. (D–E) The corresponding bar graph of the accumulation of spermatozoa according to the hematoxyline-eosin staining of the WT, Adgrg2+/Y mice or Adgrg2−/− mice infected with lentivirus encoding GRP64-WT or different G subtype mutants at the efferent ductules (D) or initial segment (E) of epididymis. (C–E) *p<0.05, **p<0.01, ***p<0.001; Adgrg2−/− mice compared with WT mice. #p<0.05, ##p<0.01, ###p<0.001; Adgrg2−/− mice infected with the lentivirus encoding different ADGRG2 constructs compared with Adgrg2−/− mice infected with the control lentivirus. $, p<0.05, $$$, p<0.001; Adgrg2−/− mice infected with the lentivirus encoding different ADGRG2 constructs compared with Adgrg2−/− mice infected with the ADGRG2-WT lentivirus. n.s., no significant difference. At least three independent biological replicates were performed for Figure 12C–E. DOI: https://doi.org/10.7554/eLife.33432.040
Figure 12—figure supplement 1. Effect of the conditional expression of ADGRG2-WT or its selective G-subtype signaling mutants on the rescue of reproductive defects in Adgrg2⁻/⁻ mice. (A) The enlarged images of the ADGRG2 expression in the epididymal initial segment at 3 weeks after the...
injection of the lentivirus of ADGRG2-WT or mutants. Scale bars, 100 μm. (B) Photographs of caudal sperm preparations from the caudal epididymis of the WT mice, Adgrg2-/mice or Adgrg2-/mice infected with a lentivirus encoding ADGRG2 or its different mutants. Scale bars, 50 μm. (C) The corresponding bar graph of the accumulation of spermatozoa according to the hematoxyline-eosin staining of the WT, Adgrg2-/mice or Adgrg2-/mice infected with lentivirus encoding ADGRG2-WT or different G subtype mutants at the caput of epididymis. *p<0.05, ***p<0.001, Adgrg2-/mice and Adgrg2-/mice infected with lentivirus were compared to WT mice. ###p<0.001, Adgrg2-/mice infected with lentivirus were compared to Adgrg2-/mice. $, p<0.05, $$, p<0.01; ADGRG2 mutant virus compared with ADGRG2-WT virus. n.s., no significant difference.

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Figure 13. Effects of conditional expression of ADGRG2 wild-type or its selective G-subtype signaling mutants in Adgrg2^+/− mice on the fluid reabsorption of efferent ductules. (A) Effects of the expression of the ADGRG2-WT adenovirus on the diameter of the ligated efferent ductules derived from WT+Ad-empty mice. (B) Effects of the expression of the ADGRG2-GM adenovirus on the diameter of the ligated efferent ductules derived from WT+Ad-empty mice. (C) Effects of the expression of the ADGRG2-Y698A adenovirus on the diameter of the ligated efferent ductules derived from WT+Ad-empty mice. (D) Effects of the expression of the ADGRG2-F705A adenovirus on the diameter of the ligated efferent ductules derived from WT+Ad-empty mice. (E) Effects of the expression of the ADGRG2-Y708A adenovirus on the diameter of the ligated efferent ductules derived from WT+Ad-empty mice. (F) Effects of the expression of the ADGRG2-RK803E adenovirus on the diameter of the ligated efferent ductules derived from WT+Ad-empty mice.
(B–F) Effects of the expression of adenovirus encoding different ADGRG2 mutants on the diameter of the ligated efferent ductules derived from the WT (n = 12) or Adgrg2−/− (n = 12) mice. (A–F) *p < 0.05, **p < 0.01, ***p < 0.001; Adgrg2−/− mice infected with the empty adenovirus compared with WT mice infected with the empty adenovirus. #p < 0.05, ##p < 0.01, ###p < 0.001; Adgrg2−/− mice infected with the adenovirus encoding different ADGRG2 constructs compared with Adgrg2−/− mice infected with the control adenovirus. n.s., no significant difference.

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Figure 14. Schematic diagram depicting the GPCR signaling pathway in the regulation fluid reabsorption in the efferent ductules. The ADGRG2 and CFTR localized at cell plasma membrane, whereas Gs and Gq localize at the inner surface of non-ciliated cells. Deficiency of ADGRG2 in Adgrg2−/− mice, reducing the Gq protein level by half in Gnaq+/− mice or PKC inhibitor Ro 31–8220 significantly destroyed the coupling of ADGRG2 to CFTR, thus impaired Cl− and H+ homeostasis and fluid reabsorption of efferent ductules. Structurally, residues in intracellular loops 2 and 3 of ADGRG2 are required for the specific interactions between ADGRG2 and Gq, which are required for CFTR and ADGRG2 coupling and fluid reabsorption. In addition Figure 14 continued on next page.
to G protein signaling, \( \beta \)-arrestin-1 is also required for fluid reabsorption in efferent ductules by scaffolding the ADGRG2 and CFTR coupling and complex formation. Therefore, a signaling complex including ADGRG2, Gq, \( \beta \)-arrestin-1 and CFTR that specifically localizes in non-ciliated cells is responsible for the regulation of Cl\(^-\) and H\(^+\) homeostasis and fluid reabsorption in the efferent ductules; thus, these functions are important for male fertility. Moreover, activation of the AGTR2 could rescue the H\(^+\) metabolic disorder caused by ADGRG2 deficiency, which restored the ability of fluid reabsorption in efferent ductules, providing a potential therapeutic strategy in treatment of male infertility caused by dysfunction of GPCR-CFTR signaling in non-ciliated cells.

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