Supplemental Materials

Molecular Biology of the Cell

Wiegering et al.

Rpgrip1l controls ciliary gating by ensuring the proper amount of Cep290 at the vertebrate transition zone

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Supplemental Materials

This file includes:

4 supplemental figures with legends

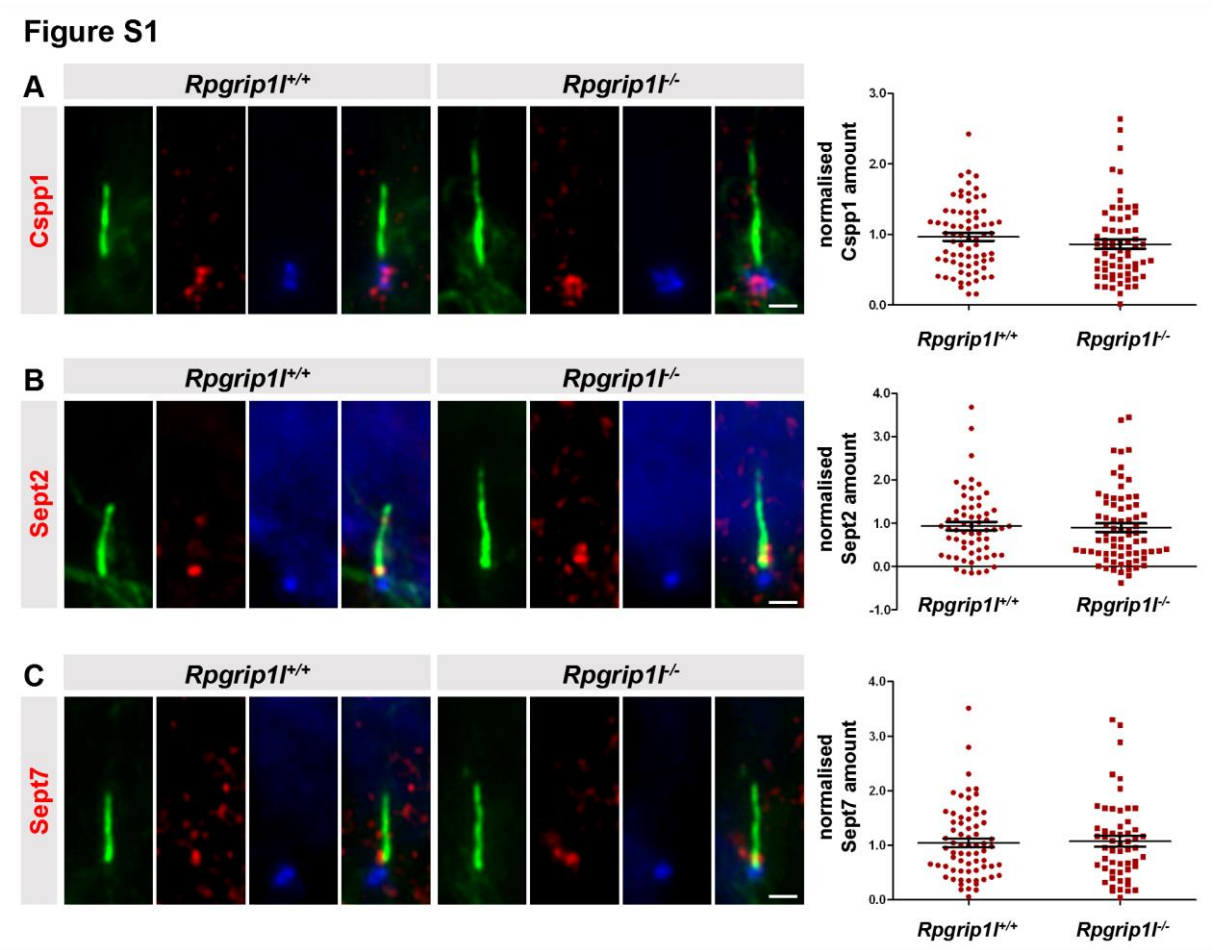

Figure S1: The ciliary amount of Cspp1, Sept2 and Sept7 does not depend on Rpgrip1l.

(A-C) Immunofluorescence on MEFs obtained from WT (n = 4) and *Rpgrip1l*$^{-/-}$ (n = 4) embryos at embryonic stage E12.5. The ciliary axoneme is stained in green by acetylated α-tubulin, the basal body is stained in blue by γ-tubulin. The scale bars represent a length of 0.5 µm. The amounts of Cspp1, Sept2 and Sept7 are unaltered in cilia of *Rpgrip1l*$^{-/-}$ MEFs. At least 15 cilia per embryo were used for quantification (Cspp1: Σ(WT) = 72 cilia, Σ(*Rpgrip1l*$^{-/-}$) = 66 cilia; Sept2: Σ(WT) = 63 cilia, Σ(*Rpgrip1l*$^{-/-}$) = 74 cilia; Sept7: Σ(WT) = 69 cilia, Σ(*Rpgrip1l*$^{-/-}$)= 58 cilia). Data are shown as mean ± s.e.m. Asterisks denote statistical significance according to unpaired *t*-tests with Welch's correction (A: t (37) = 1.649, P < 0.1072; B: t (26) = 1.088, P < 0.2867; C: t (39) = 0.926, P < 0.3601).

Figure S2

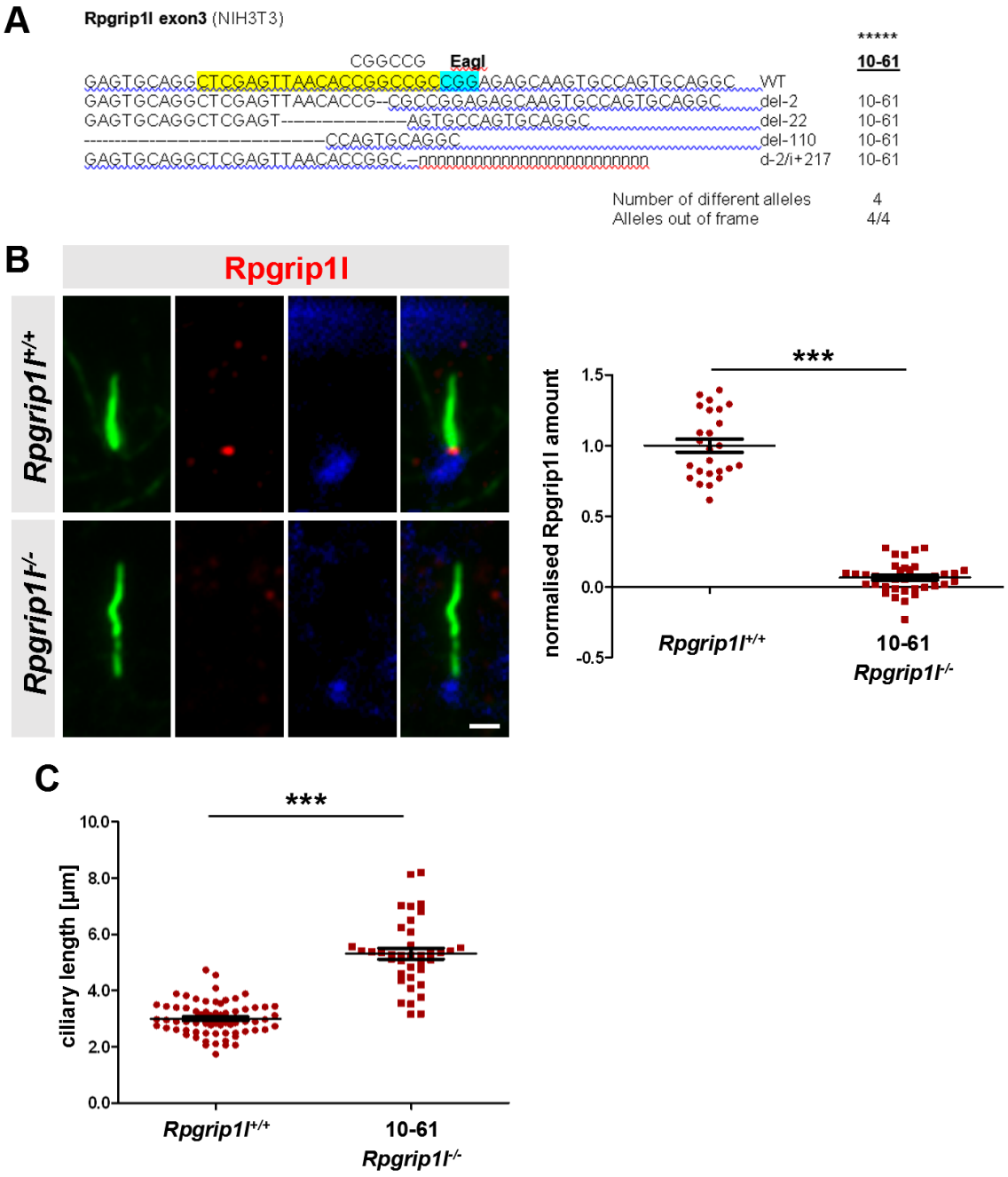

Figure S2: Confirmation of the inactivation of *Rpgrip1l* in *Rpgrip1l*$^{-/-}$ NIH3T3 cells.

(A) Genotype analysis of targeted *Rpgrip1l* on-target alleles. Sequences of CRISPR/Cas9 mutated alleles are compared to the WT sequence (on the left side), corresponding NIH clone 10-61 is depicted on the right side. The sequences of the 20 nt guide and the PAM within the WT sequence are coloured in yellow and cyan, respectively. The recognition sequence of *Eag*I

(used in RFLP analyses to screen for mutated clones) is shown on top of the WT sequence. Within the sequences of targeted alleles, deletions are indicated by dashes, insertions of a single base pair are typed bold, and larger insertions are displayed as runs of "n". The karyotype analysis of NIH3T3 revealed, that *Rpgrip1l* is present in 4 copies within this cell line [1]. In accordance with this, we were able to detect four different alleles in clone 10-61 appeared to carry only alleles with out-of-frame mutations. (B) Immunofluorescence on *Rpgrip1l*$^{+/+}$ and *Rpgrip1l*$^{-/-}$ (clone 10.61) NIH3T3 cells. The ciliary axoneme is stained in green by acetylated α-tubulin, the basal body is stained in blue by γ-tubulin. The scale bars represent a length of 1 µm. Rpgrip1l is not detectable in cilia of *Rpgrip1l*$^{-/-}$ NIH3T3 cells (clone 10.61). At least 30 cilia per clone were used for quantification. Data are shown as mean ± s.e.m. Asterisks denote statistical significance according to an unpaired *t*-test with Welch's correction (***$P < 0.001$) (t (30) = 18.72, $P < 0.0001$). (C) Ciliary length measurement. At least 40 cilia per clone were used for quantification. Data are shown as mean ± s.e.m. Asterisks denote statistical significance according to an unpaired *t*-test with Welch´s correction (***$P < 0.001$) (t (47) = 11.06, $P < 0.0001$).

Figure S3

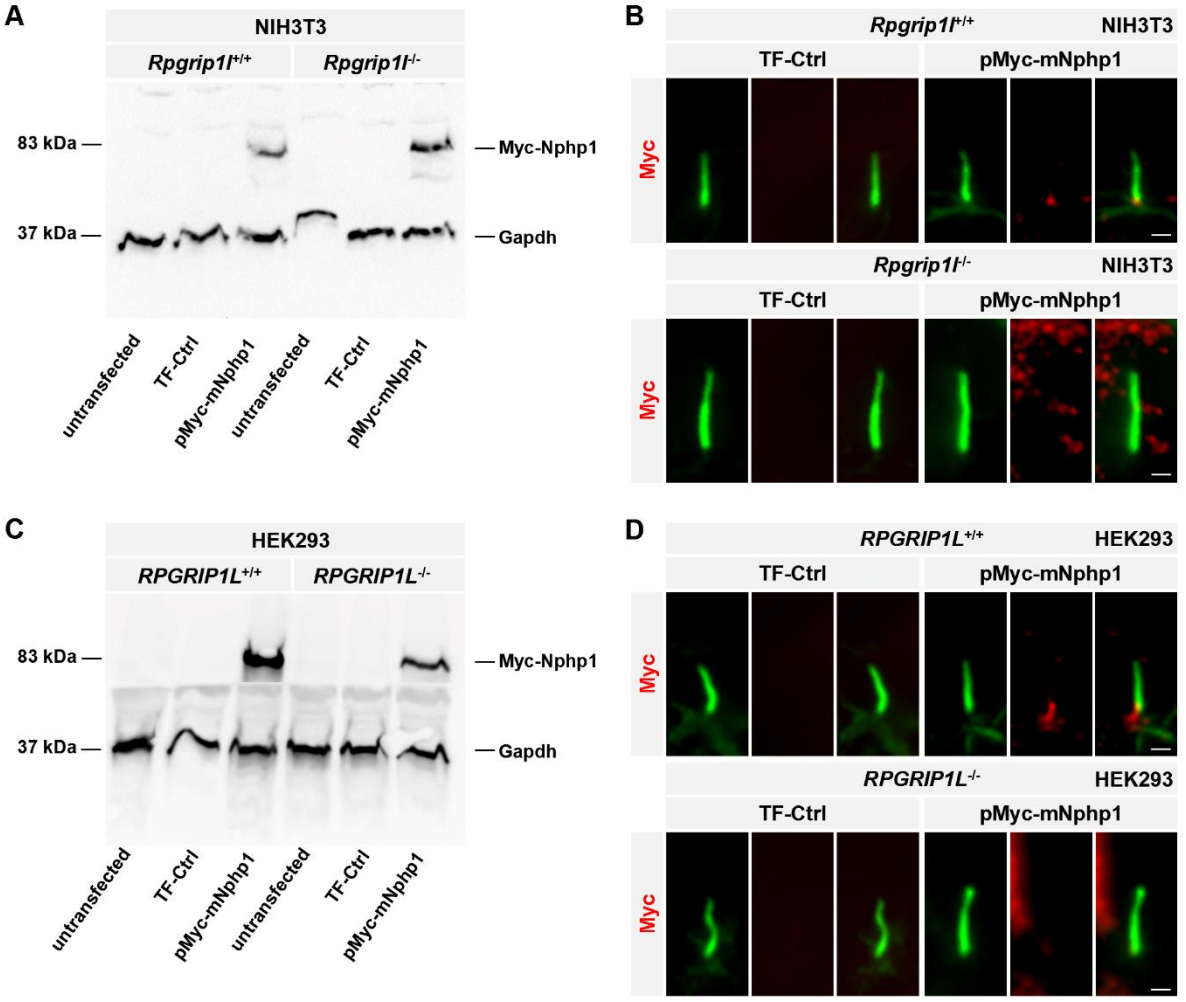

Figure S3: The localisation of Nphp1 at the TZ depends on Rpgrip1l.

(A, C) Western blot analysis with lysates obtained from (A) *Rpgrip1l*$^{+/+}$ and *Rpgrip1l*$^{-/-}$ NIH3T3 cells (clone 10-61) and (C) *RPGRIP1L*$^{+/+}$ and *RPGRIP1L*$^{-/-}$ HEK293 cells (clone 1-7). Gapdh serves as loading control. (B, D) Immunfluorescence on (B) *Rpgrip1l*$^{+/+}$ and *Rpgrip1l*$^{-/-}$ NIH3T3 cells (clone 10-61) and (D) *RPGRIP1L*$^{+/+}$ and *RPGRIP1L*$^{-/-}$ HEK293 cells (clone 1-7). The ciliary axoneme is stained in green by acetylated α-tubulin and Myc is shown in red. The scale bar represents a length of 1 µm. (A-D) Cells were transfected with a plasmid encoding a Nphp1 (full-length)-Myc fusion protein (pMyc-mNPHP1).

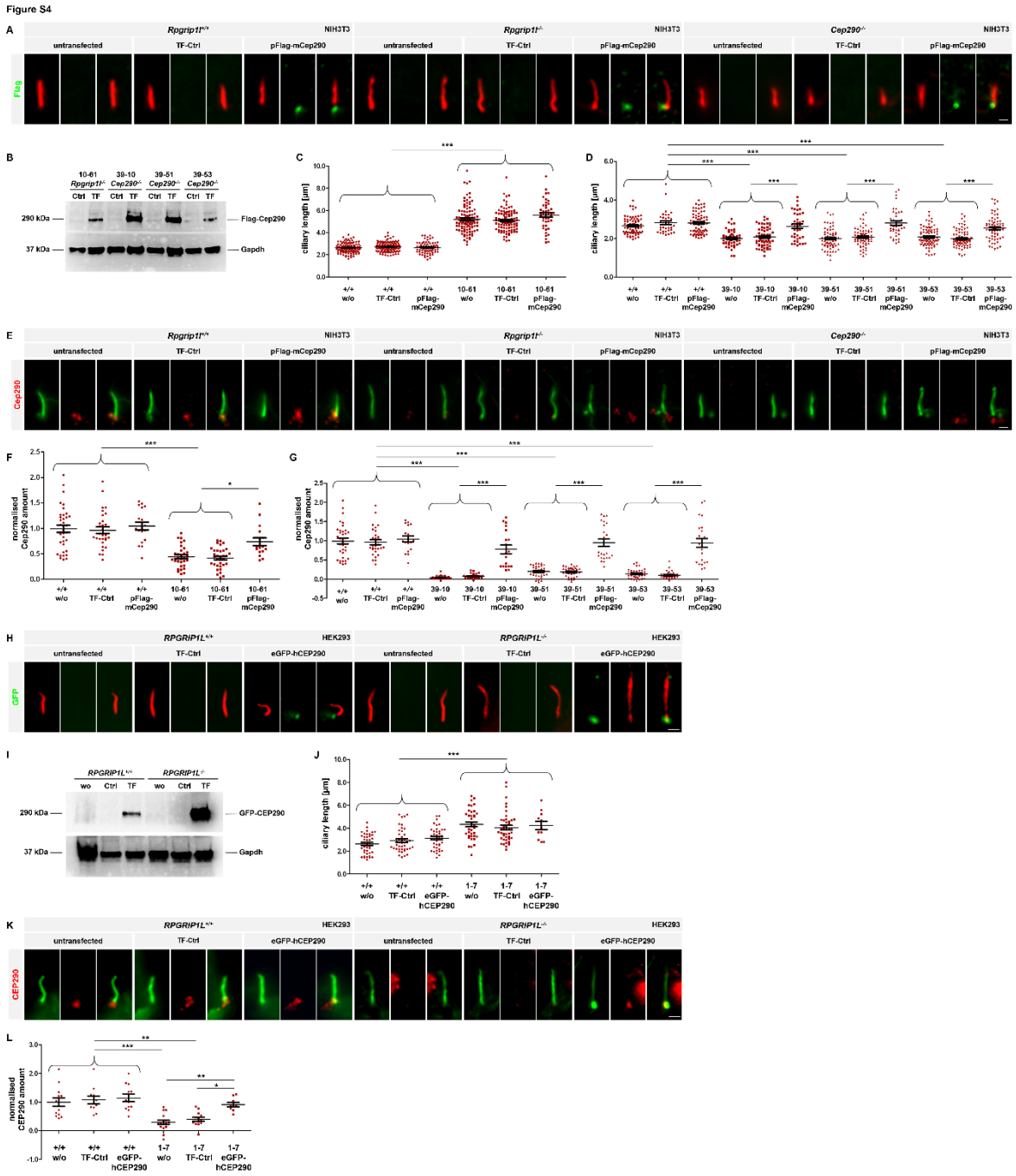

Figure S4: Rescue of the Cep290 amount at the ciliary TZ in NIH3T3 and HEK293 cells.

(A-G) *Rpgrip1l*$^{+/+}$, *Rpgrip1l*$^{-/-}$ and *Cep290*$^{-/-}$ NIH3T3 cells were transfected with a plasmid encoding a murine Cep290 (full-length)-Flag fusion protein (pFlag-mCep290). (A) Immunofluorescence on *Rpgrip1l*$^{+/+}$, *Rpgrip1l*$^{-/-}$ (clone 10-61) and *Cep290*$^{-/-}$ (clones 39-10, 39-51, 39-53) NIH3T3 cells. The ciliary axoneme is stained in red by acetylated α-tubulin and Flag is shown in green. The scale bar represents a length of 1 µm. (B) Western blot analysis for Flag

with lysates obtained from *Rpgrip1l*$^{-/-}$ (clone 10-61) and *Cep290*$^{-/-}$ (clones 39-10, 39-51, 39-53) NIH3T3 cells. Gapdh serves as loading control. (C, D) Ciliary length measurements. At least 40 cilia per clone were used for quantification. Data are shown as mean ± s.e.m. Asterisks denote statistical significance according to one-way ANOVA and Tukey HSD tests (***P < 0.001) (C: F (5, 548) = 246.1, P < 0.0001; D: F (11, 708) = 27.23, P < 0.0001). (E) Immunofluorescence on *Rpgrip1l*$^{+/+}$, *Rpgrip1l*$^{-/-}$ (clone 10-61) and *Cep290*$^{-/-}$ (clones 39-10, 39-51, 39-53) NIH3T3 cells. The ciliary axoneme is stained in green by acetylated α-tubulin and Cep290 is shown in red. The scale bar represents a length of 1 µm. (F, G) Normalised ciliary Cep290 amount in (F) *Rpgrip1l*$^{-/-}$ (clone 10-61) and (G) *Cep290*$^{-/-}$ (clones 39-10, 39-51, 39-53) NIH3T3 cells. At least 20 cilia per clone were used for quantification. The same quantification of WT serves as comparison to Rpgrip1l-negative and Cep290-negative cells. Data are shown as mean ± s.e.m. Asterisks denote statistical significance according to one-way ANOVA and Tukey HSD tests (* P < 0.05; ***P < 0.001) (F: F (5, 159) = 23.05, P < 0.0001; G: F (11, 330) = 59.74, P < 0.0001). (H-L) *RPGRIP1L*$^{+/+}$ and *RPGRIP1L*$^{-/-}$ HEK293 cells were transfected with a plasmid encoding a human CEP290 (full-length)-GFP fusion protein (eGFP-hCEP290). (H) Immunofluorescence on *RPGRIP1L*$^{+/+}$ and *RPGRIP1L*$^{-/-}$ (clone 1-7) HEK293 cells. The ciliary axoneme is stained in red by acetylated α-tubulin and GFP is shown in green. The scale bar represents a length of 1 µm. (I) Western blot analysis for GFP with lysates obtained from *RPGRIP1L*$^{+/+}$ and *RPGRIP1L*$^{-/-}$ (clone 1-7) HEK293 cells. Gapdh serves as loading control. (J) Ciliary length measurements. At least 20 cilia per clone were used for quantification. Data are shown as mean ± s.e.m. Asterisks denote statistical significance according to one-way ANOVA and Tukey HSD tests (***P < 0.001) (F (5, 218) = 20.21, P < 0.0001). (K) Immunofluorescence on *RPGRIP1L*$^{+/+}$ and *RPGRIP1L*$^{-/-}$ (clone 1-7) HEK293 cells. The ciliary axoneme is stained in green by acetylated α-tubulin and CEP290 is shown in red. The scale bar represents a length of 1 µm. (L) Normalised ciliary CEP290 amount. At least 15 cilia per clone were used for quantification. Data are shown as mean ± s.e.m. Asterisks denote statistical significance

according to one-way ANOVA and Tukey HSD tests (**P < 0.01; ***P < 0.001) (F (5, 106) = 22.64, P < 0.0001).

References

1. Leibiger, C., Kosyakova, N., Mkrtchyan, H., Glei, M., Trifonov, V., and Liehr, T. (2013). First molecular cytogenetic high resolution characterization of the NIH 3T3 cell line by murine multicolor banding. J. Histochem. Cytochem. *61*, 306-312.