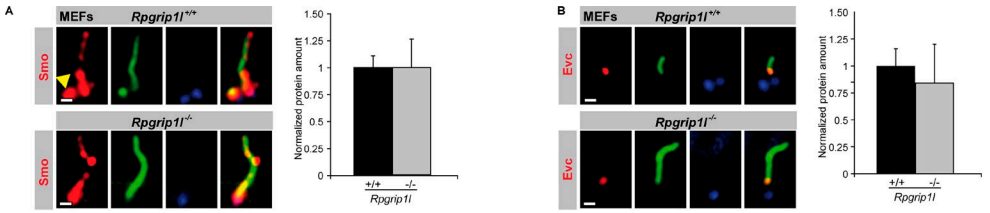
Gerhardt et al., http://www.jcb.org/cgi/content/full/jcb.201408060/DC1



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official symbol	name	accession-no.
Psmd2	proteasome (prosome, macropain) 26S subunit, non-ATPase, 2	NM_134101.2
Hnrpll	heterogeneous nuclear ribonucleoprotein L-like	NM_144802.3
Cspp1	centrosome and spindle pole associated protein 1	NM_026493.3
Srsf2	serine/arginine-rich splicing factor 2	NM_011358.2
Zbtb48	zinc finger and BTB domain containing 48	NM_133879.2
Gtf3a	general transcription factor III A	NM_025652.1

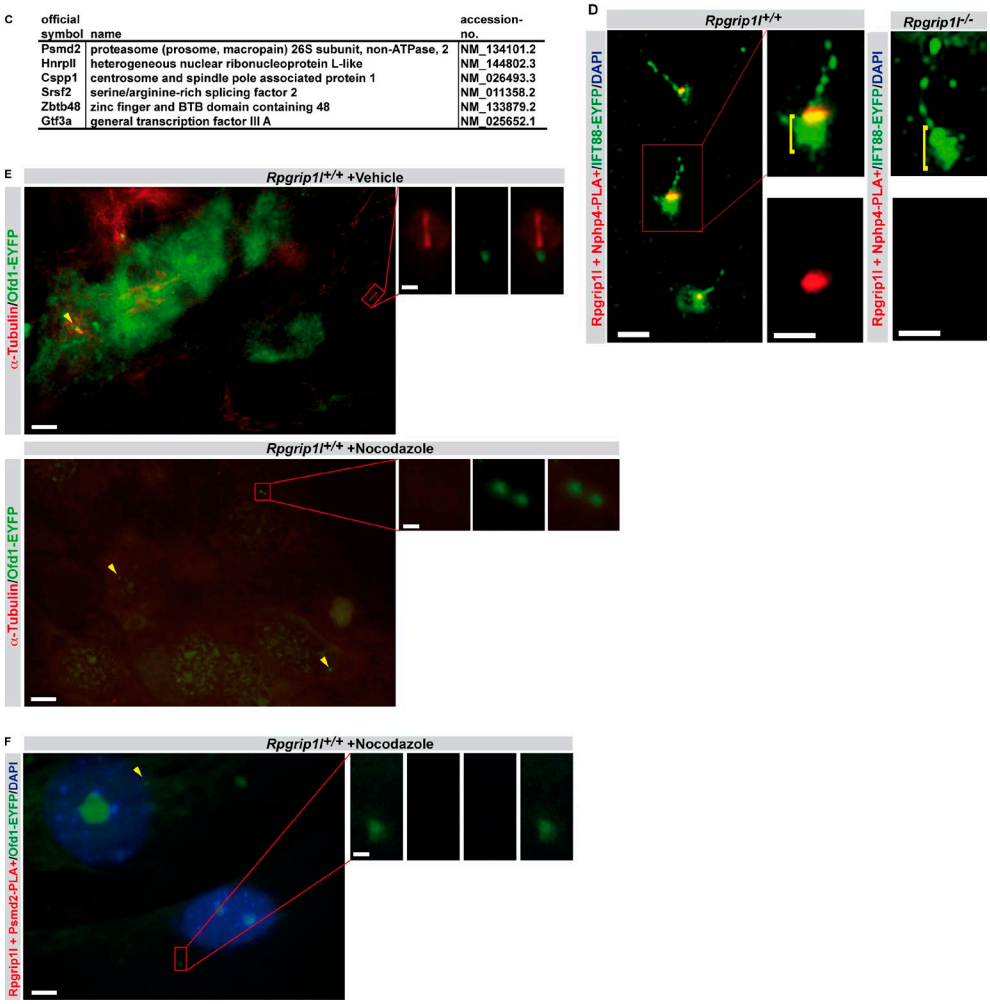

Figure S1. **Ciliary localization of Smo and Evc is not altered by Rpgrip1l deficiency and Rpgrip1l interaction partner experiments.** (A and B) Immunofluorescence on SAG-treated MEFs of E12.5 WT and *Rpgrip1l*$^{-/-}$ embryos (both genotypes: Smo, $n = 3$ embryos; Evc, $n = 3$ embryos). At least 10 cilia per embryo were used for quantification, respectively. The ciliary axoneme is marked by acetylated α-tubulin (green). The BB is marked by γ-tubulin (blue). Quantified proteins are shown in red. Bars: (A) 0.25 µm; (B) 0.5 µm. (A) Yellow arrowhead points at the daughter centriole. Error bars show standard error of the mean. (C) Complete list of Rpgrip1l-interacting proteins identified via yeast two-hybrid screen. Accession numbers were obtained from GenBank. (D) In situ proximity ligation assay (in situ PLA) on MEFs isolated from WT or *Rpgrip1l*$^{-/-}$ E12.5 embryos ($n = 3$, respectively). Cell nuclei are marked by DAPI, and the ciliary axoneme was marked by transiently transfected Ift88-EYFP. Additional accumulation of Ift88-EYFP at the ciliary base is highlighted by yellow brackets. Bars: (overview) 2 µm; (magnifications) 1 µm. (E) Immunofluorescence on MEFs of E12.5 WT embryos (vehicle, $n = 3$ embryos; nocodazole treated, $n = 3$ embryos). MEFs were transfected with an Ofd1-EYFP construct to mark centrosomes and basal bodies. The ciliary axoneme is marked by acetylated α-tubulin (green). Yellow arrowheads point to a cilium with BB (untreated MEFs [vehicle]) or to centrosomes (nocodazole-treated MEFs). Red squares mark a cilium with BB (untreated MEFs [vehicle]) as well as centrosomes (nocodazole-treated MEFs), which are presented magnified. MEFs were treated with 10 µg/ml nocodazole and incubated for 3 h at 4°C to get nonciliated MEFs. After treatment with nocodazole, MEFs do not show any cilia but centrosomes. Bars: (overviews) 5 µm; (magnifications) 1 µm. (F) In situ proximity ligation assay (in situ PLA) on nocodazole-treated MEFs isolated from WT or *Rpgrip1l*$^{-/-}$ E12.5 embryos ($n = 3$, respectively). Cell nuclei are marked by DAPI, and centrosomes are marked by transiently transfected Ofd1-EYFP. A centrosome is highlighted by a yellow arrowhead. Red squares mark another centrosome, which is presented magnified. A PLA signal is not detected at any centrosome. Bars: (overview) 5 µm; (magnification) 0.5 µm.

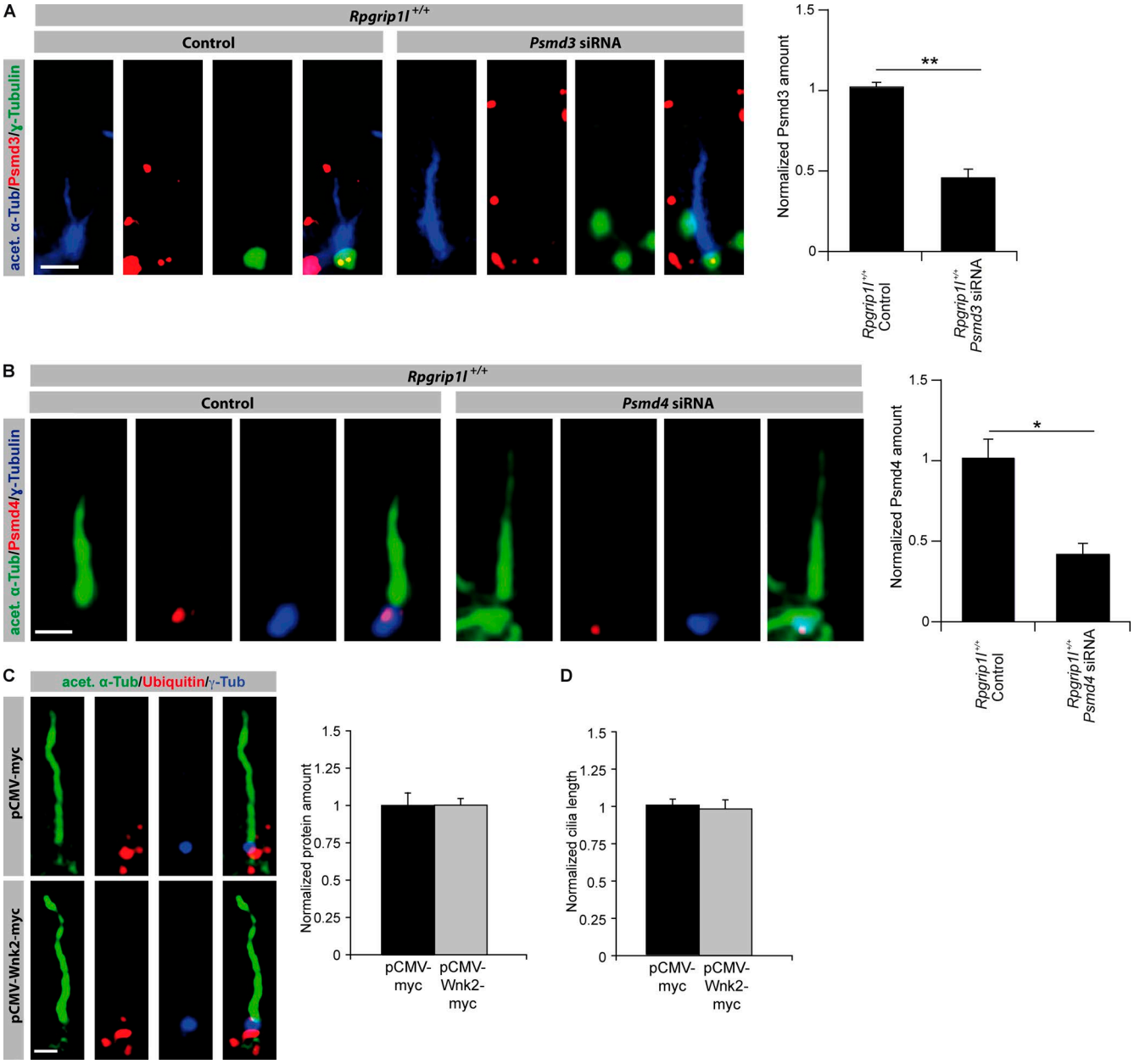

Figure S2. **Transfection of siRNA against *Psmd3* and *Psmd4* significantly reduces Psmd3 and Psmd4 amounts at the ciliary BB and Wnk2 into NIH3T3 cells does not alter the activity of the ciliary proteasome.** (A) Transfection of MEFs isolated at E12.5 with siRNA against *Psmd3*. The ciliary axoneme is marked by acetylated α-tubulin (acet. α-Tub) and the BB is marked by γ-tubulin. (B) Transfection of MEFs isolated at E12.5 with siRNA against *Psmd4*. The ciliary axoneme is marked by acetylated α-tubulin (acet. α-Tub) and the BB is marked by γ-tubulin. (A and B) Psmd3 and Psmd4 amounts were quantified exclusively at cilia, respectively. (C) Immunofluorescence on MEFs of E12.5 WT and *Rpgrip1l*$^{-/-}$ embryos (both genotypes: $n = 3$). The ciliary axoneme is marked by acetylated α-tubulin (acet. α-Tub) and the BB by γ-tubulin. Transfection of Wnk2 in NIH-3T3 cells and quantification of the ciliary Ubiquitin amount. (C and D) Transfection of Wnk2 does not alter the amount of Ubiquitin at the ciliary base or the ciliary length. Error bars show standard error of the mean. *, $P < 0.05$; **, $P < 0.01$. Bars, 1 µm.

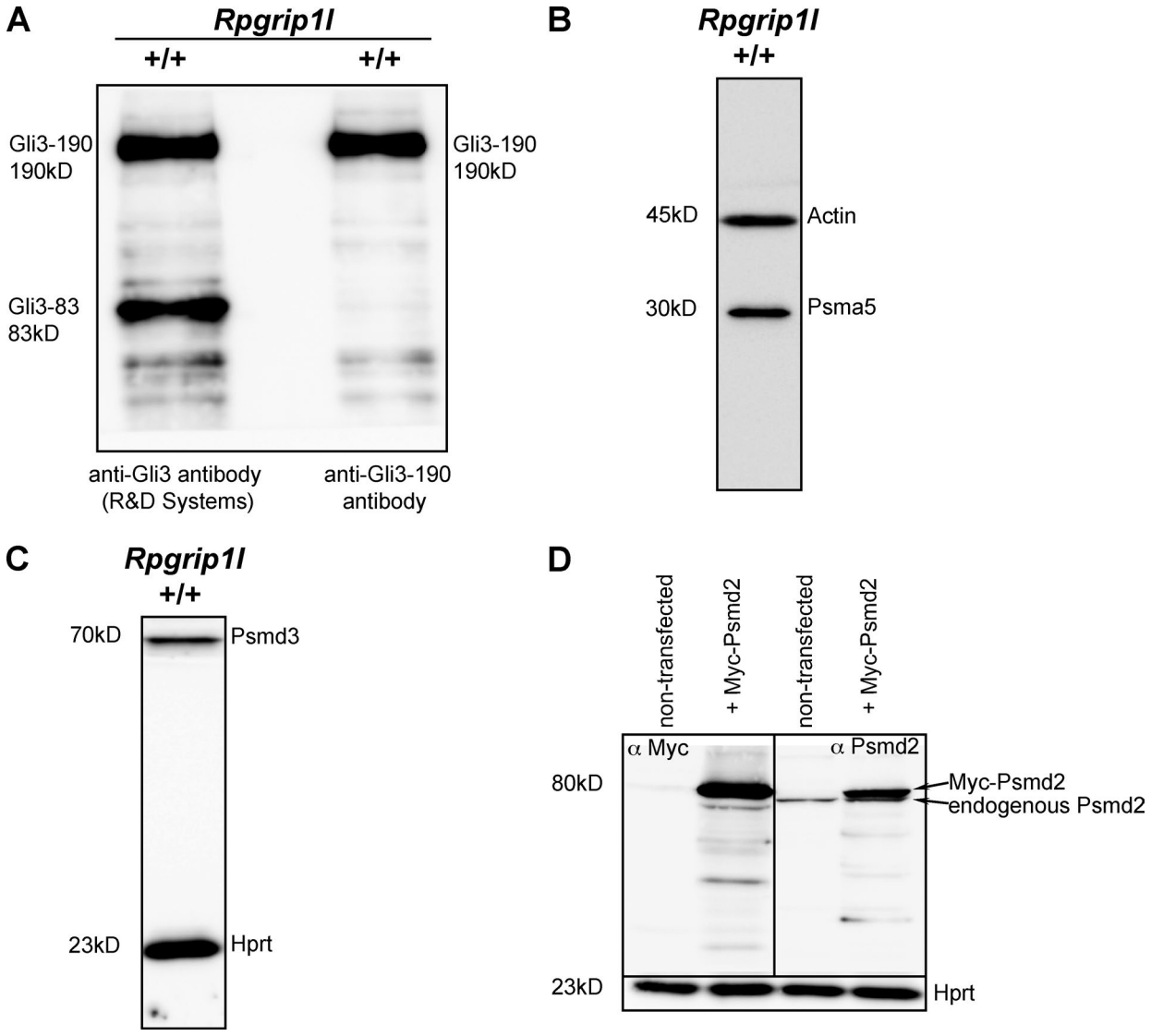

Figure S3. **Illustration of antibody specificity.** (A–D) Western blot analysis on MEFs (obtained from E12.5 WT embryos). Actin (B) and Hprt (C and D) serve as loading controls. (A) Both of our analyses used antibodies to Gli3: anti-Gli3 (R&D Systems) and anti-Gli3-190 antibodies were tested in a Western blot application. The anti-Gli3 antibody (R&D Systems) detects the Gli3 full-length protein (Gli3-190) as well as the processed form (Gli3-83), whereas the anti–Gli3-190 antibody exclusively detects the Gli3 full-length protein (Gli3-190). (B and C) Psma5 as well as Psmd3 were detected at their predicted molecular mass, respectively (Psma5, 30 kD; Psmd3, 70 kD). (D) The anti-Psmd2 antibody detects endogenous as well as overexpressed Psmd2. Black lines indicate that intervening lanes have been spliced out.

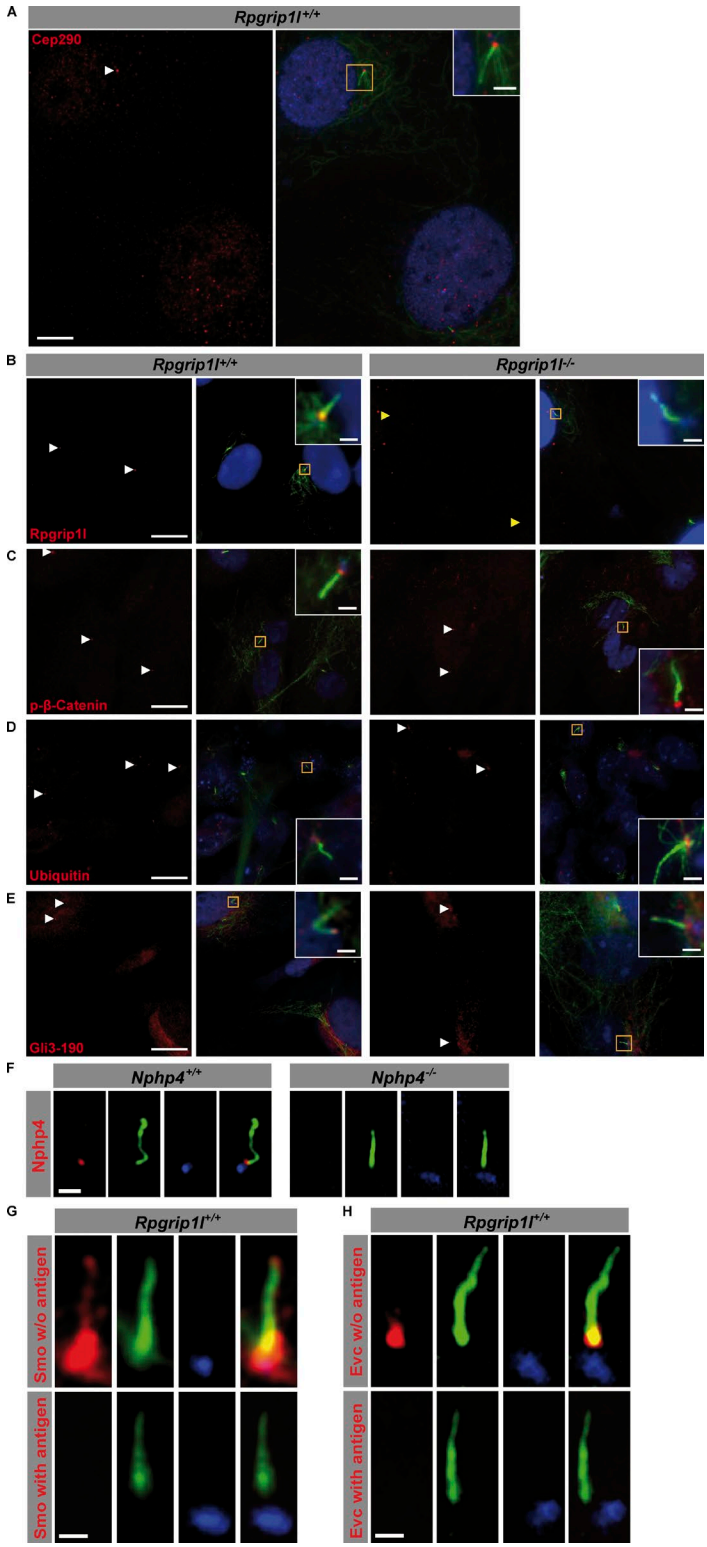

Figure S4. **Wide-field views of immunofluorescence stainings and illustration of antibody specificity.** (A) Immunofluorescence on MEFs isolated from E12.5 WT embryos. Full views of stainings performed by using the anti-Cep290 antibody. The axoneme is marked by acetylated α-tubulin (green), and the BB was marked by γ-tubulin (blue). Cep290 localizes to the TZ. The Cep290 signal is indicated by a white arrowhead. Bars: (overview) 5 µm; (magnification) 1 µm. (B–E) Immunofluorescence on MEFs (isolated from E12.5 WT or *Rpgrip1l*$^{-/-}$ embryos). Ciliary axoneme is marked by acetylated α-tubulin (green). The BB is marked by γ-tubulin (blue). All proteins of interest are marked by their specific antibody. Their ciliary signals are indicated by white arrowheads. Yellow arrowheads point to sites where no ciliary signal is detected by using the anti-Rpgrip1l antibody. Bars: (overview) 10 µm; (magnifications) 1 µm. (F) Immunofluorescence on MEFs isolated from E12.5 WT and *Nphp4*$^{-/-}$ embryos. The axoneme is marked by acetylated α-tubulin (green), and the BB is marked by γ-tubulin (blue). Nphp4 is marked by its specific antibody (red). Bar, 1 µm. (G and H) Immunofluorescence on MEFs isolated from E12.5 WT embryos. The axoneme is marked by acetylated α-tubulin (green), and the BB is marked by γ-tubulin (blue). All proteins of interest are marked by their specific antibody. In combination with the appropriate antigen, anti-Smo and anti-Evc antibodies give no signal. Bars, 1 µm.

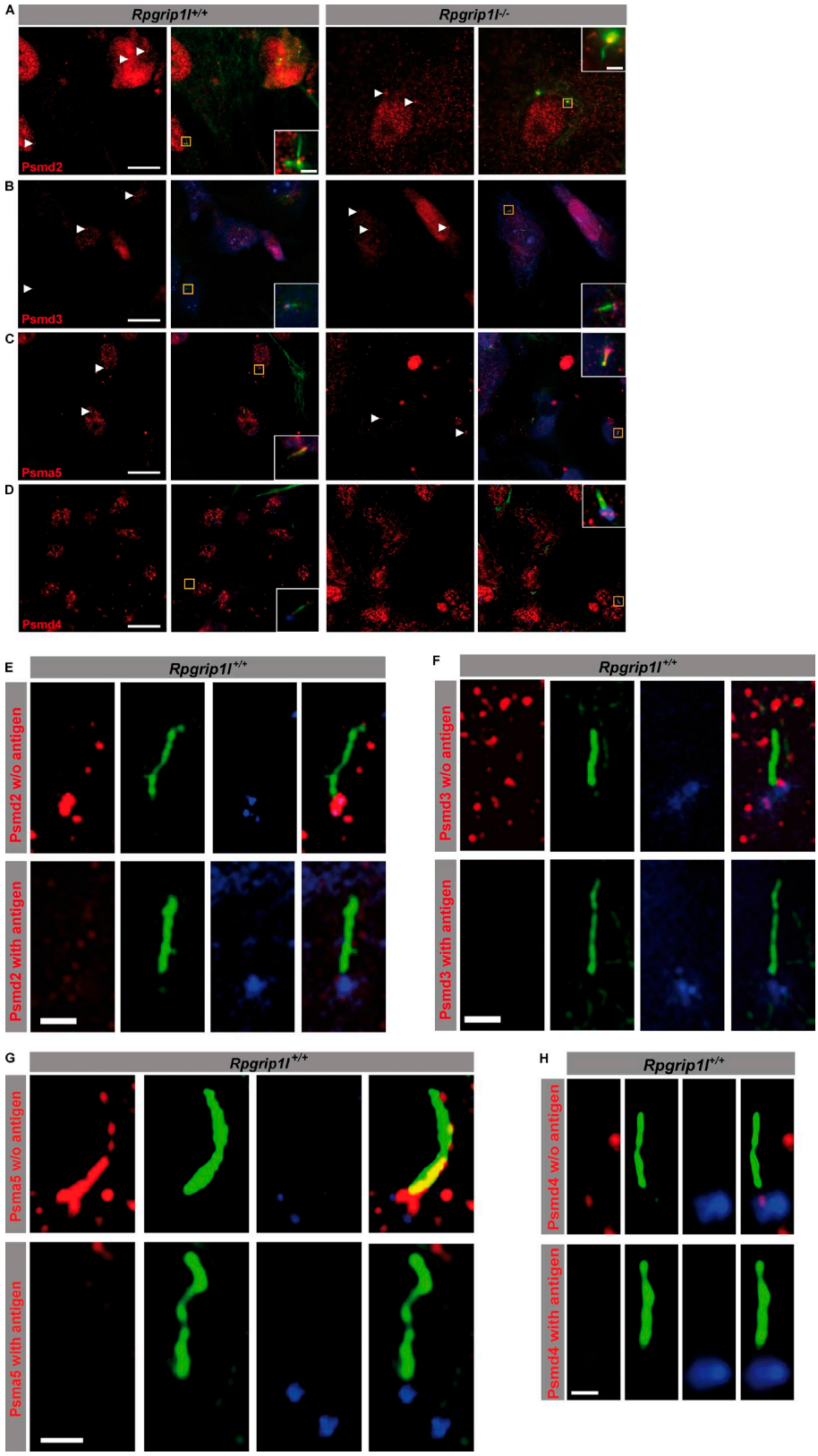

Figure S5. **Additional examples of immunofluorescence stainings and illustration of antibody specificity.** (A–D) Immunofluorescence on MEFs isolated from E12.5 WT and *Rpgrip1l*$^{-/-}$ embryos. (E–H) Immunofluorescence on MEFs isolated from E12.5 WT embryos. Bars: (A–D, overviews) 10 µm; (A–D, magnifications; and E–H) 1 µm. (A) The axoneme and the BB are marked in green (by acetylated α-tubulin and by Ptcn, respectively). (B–H) The axoneme is marked by acetylated α-tubulin (green), and the BB is marked by γ-tubulin (blue). All proteins of interest are marked by their specific antibody. Their ciliary signals are indicated by white arrowheads. (E–H) In combination with the appropriate antigen, anti-Psmd2, anti-Psmd3, anti-Psma5, and anti-Psmd4 antibodies give no signal.