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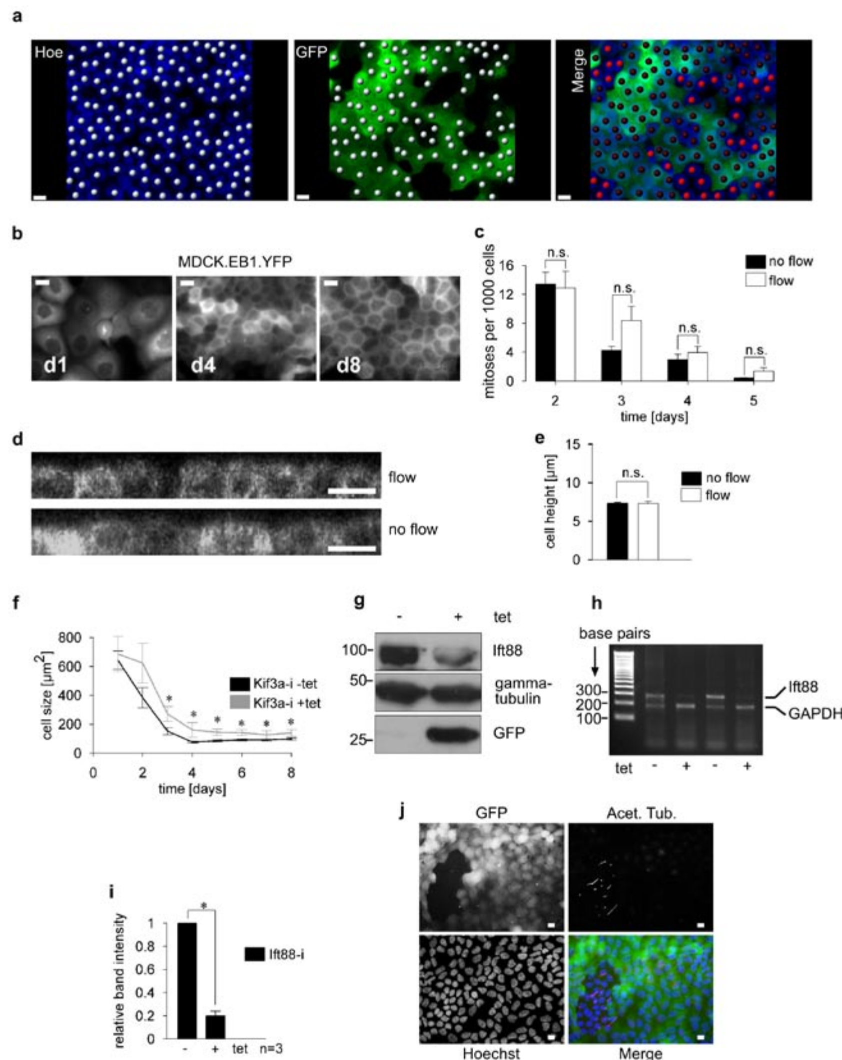

Figure S1 (**a**) Representative field of view of lentivirus transduced, polyclonal Kif3a knock-down cells after tetracycline induction. Shown are the nuclei (Hoechst) and the GFP reporter expression. In the left image each cell is represented by a dot. In the center image only GFP expressing cells are indicated. In the right image GFP negative cells are indicated by a red dot. 85.2% of 2165 cells were GFP-positive (n=3, three fields of view per n). Scale bars: 10 µm. (**b**) Representative images of confluent polyclonal EB1.YFP expressing MDCK cells under flow. EB1.YFP is expressed for better visualization of the cells, the dark areas represent cells expressing little or no EB1.YFP. On day1 (d1) after seeding, the cells are confluent and large. After 4 days of flow the cells have decreased in size (d4) and remain at this size for several days, here day 8 (d8). Scale bars 10 µm. (**c**) EB1-YFP expressing MDCK cells were grown under flow and rest conditions respectively and imaged daily. Mitotic spindles were identified by EB1-YFP and counted as number per 1000 cells (for details see the method section). No significant difference was found in the mitotic index between the flow and no-flow conditions (day 2-4: n=7 (flow), n=3 (no-flow); day 5: n=3; 40 fields of view per n, "n.s.":, not significant). (**d**) The cell height during the plateau phase, measured by confocal microscopy in z-stacks, is similar between cells under flow and cells without flow. Scale bars: 10 µm. (**e**) Quantification of the mean cell height from 3 independent experiments. The cell height is almost identical ("n.s."; not significant) in cells without flow (black bar, 7.30±0.15 µm) and cells under flow (white bar, 7.33±0.18 µm; n=3, 116 cells per n, $P = 0.89$). (**f**) Time course analysis of the average cell-size measured in one single experiment each of induced (+ tet) vs. non-induced (- tet) Kif3a knock-down cells in the flow-chamber. A plateau cell-size is reached at day 4 in both conditions, however the plateau cell-size is larger in unciliated cells after tetracycline induced Kif3a knock-down (+ tet), compared to the ciliated control cells (- tet). Each data point represents 10 visual fields. Asterisks: P < 0.05. Data are mean ± s.d. (**g**) Western Blot analysis of tetracycline induced Ift88 knock-down cells. A GFP reporter is induced after knock-down. (**h**) MDCK cells expressing the inducible shRNA against Ift88 were analyzed by RT-PCR for expression of Ift88 and GAPDH. Tetracycline treatment (+tet) markedly decreases the Ift88 transcript, as shown in 2 independent experiments. (**i**) Quantification of the relative Ift88/GAPDH band intensity of 3 independent experiments. Tetracycline induction results in significant knock-down of Ift88 expression. Asterisk: $P < 0.05$. (**j**) Induction of Ift88 knock-down by tetracycline results in the lack of cilia in GFP positive knock-down cells. Scale bars: 10µm. Data in (**c**), (**e**) and (**i**) are mean ± s.e.m..

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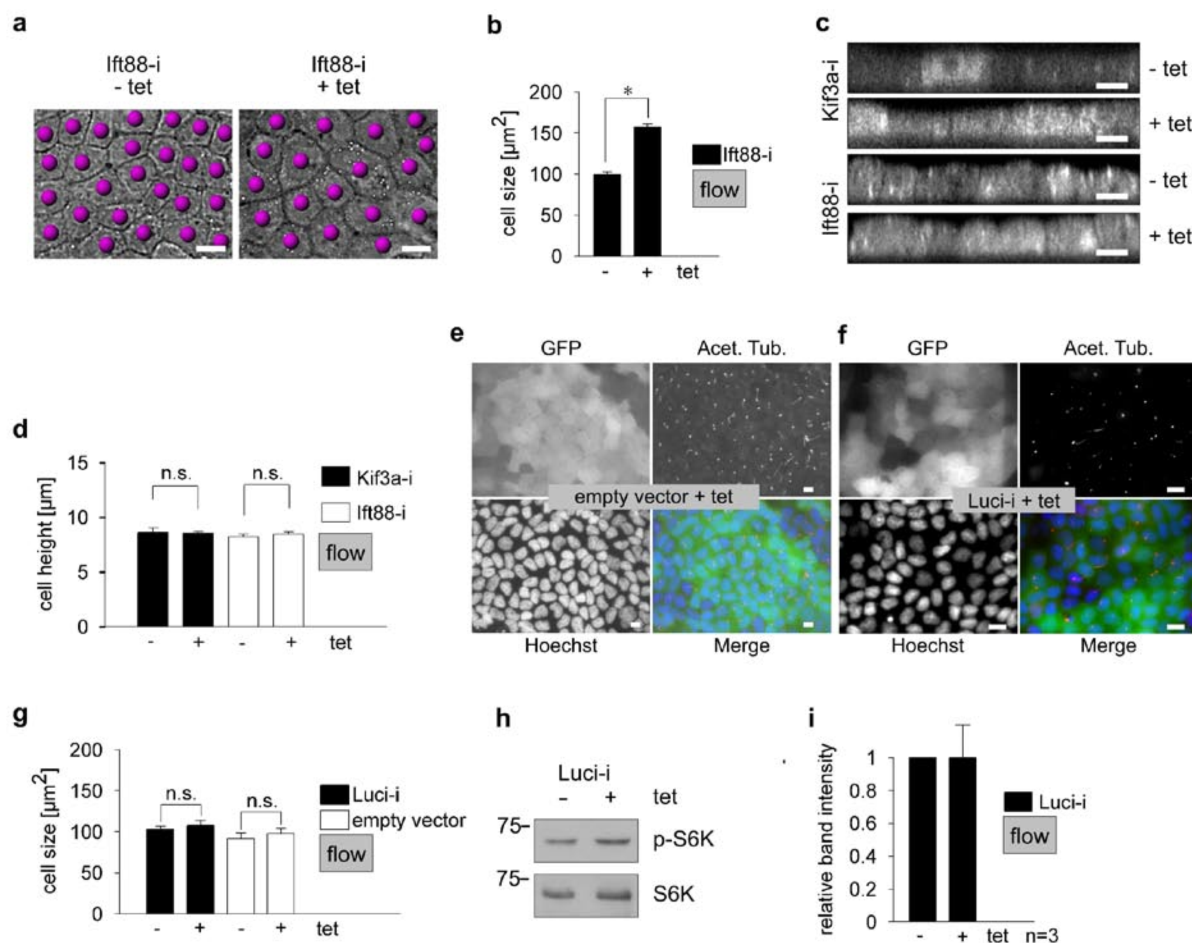

Figure S2 (**a**) Knock-down of Ift88 (+tet) results in larger cells compared to -tet conditions. (**b**) Ift88 knock-down (+tet) results in larger cells after 5 days of flow. 157±4 μm^2 vs. 100±3 μm^2, n=8 and n=11, 10 fields of view per n; $P < 0.0001$. (**c**) Kif3a-i and Ift88-i cells were imaged under flow by confocal laser microscopy and analyzed by cross-sectional z-stack analysis (shown stack size is 12μm, 0.5μm z-distance). Visualization of cell height in -tet cells was performed in the RFP channel as a DsRed reporter is expressed with the tet repressor (see the method section). The cell heights are similar with and without tetracycline. (**d**) Quantification of cell heights shows no significant (n.s.) differences after depletion of Kif3a or Ift88. Kif3a-i: 8.6±0.1 μm (+tet) vs. 8.6±0.4 μm (-tet), n=3, 100 cells per n; $P = 0.94$, and Ift88-i: 8.5±0.2 μm (+tet) *vs.* 8.3±0.2 μm (-tet), n=3, 100 cells per n; $P = 0.43$. (**e**) Control cells transduced with lentivirus derived from empty vector express the GFP reporter but no shRNA after tetracycline treatment. Ciliogenesis (acet. tub.) is not impaired. Scale bars 10 μm. (**f**) Control cells expressing shRNA against luciferase (luci-i) show normal cilia formation (Acet. Tub.) after tetracycline treatment. Scale bars 10μm. (**g**) The average size of control cells expressing tetracycline inducible RNA-i against luciferase (luci-i, black bars) or cells transduced with empty vector derived lentivirus (empty vector, white bars) is not significantly different with or without tetracycline (luci-i: 103±4 μm^2 vs. 108±6 μm^2, n=9 and n=11. $P = 0.4$. Empty vector: 92±7 μm^2 vs. 98±6 μm^2, n=3 and n=6, $P = 0.5$; 10 visual fields per n). "n.s": not significant. (**h**) Levels of p-S6K are similar in luci-i cells under flow, irrespective of tetracycline treatment. (**i**) Band quantification of 4 independent experiments shows no significant (n.s.) difference in levels of p-S6K. $P = 0.9$. Data in (**b**), (**d**), (**g**) and (**i**) are mean ± s.e.m..

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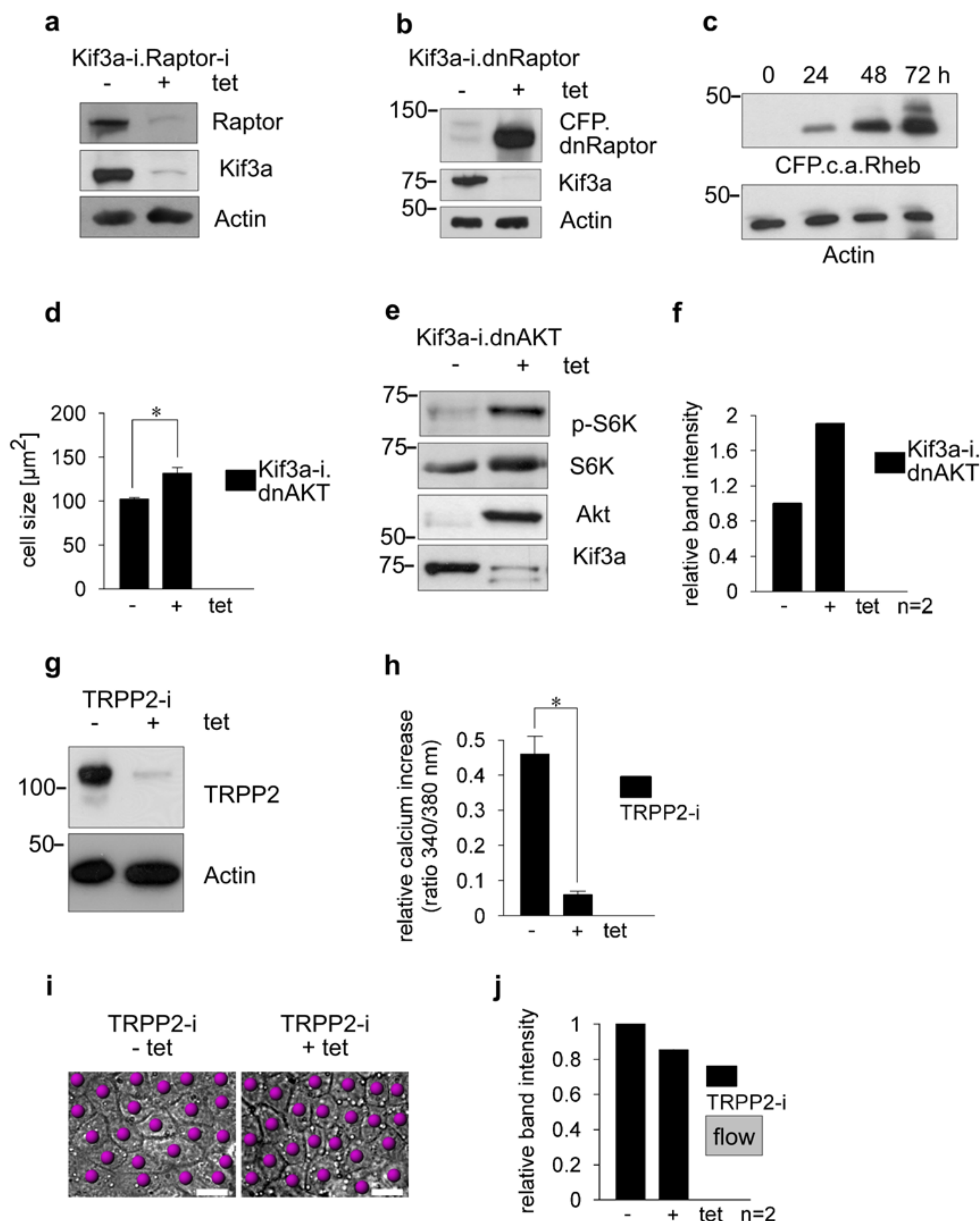

Figure S3 (**a**) Expression control of Kif3a+Raptor knock-down cells. Incubation with tetracycline results in efficient knock-down of Raptor and Kif3 (+tet). (**b**) Expression control of inducible dominant negative (dn) Raptor in Kif3a knock-down cells (+tet). (**c**) Polyclonal (c.a.)Rheb-MDCK cells show strong expression of CFP coupled constitutively active Rheb within 24-78h of induction by tetracycline. (**d**) Expression of dominant negative Akt (dnAkt) does not prevent cell-size deregulation in unciliated Kif3a-i cells after permanent flow for 5 days. Treatment with tetracycline shows a significant increase of cell-size (132±6 μm^2 compared to 102±2 μm^2; both n=8 and, 10 visual fields per n, $P < 0.001$). (**e**) p-S6K under flow is increased in Kif3a-i+dnAKT cells when treated with tetracycline (+tet) compared to non-induced cells (-tet). (**f**) Quantification of the band intensities of phosphorylated over total S6K from two independent experiments shows increased S6K phosphorylation under tetracycline (+tet). (**g**) Analysis of TRPP2-i cell lysates by Western blot demonstrates effective depletion of Polycystin 2 after induction of a specific shRNA by tetracycline. (**h**) Quantification of the mean calcium increase from several independent experiments (-tet: n=5; +tet n=6) reveals a strongly reduced flow response after induction of TRPP2-i (+tet). Asterisk: $P < 0.001$. (**i**) TRPP2-i cells have similar sizes with or without tetracycline. (**j**) Band density quantification of two independent experiments shows no difference in S6K phosphorylation in TRPP2 depleted cells (+tet). "n.s.": not significant. Data in (**d**) and (**h**) are mean ± s.e.m.. The uncropped blot of (**a**) is shown in Supplementary Information, Fig. S6.

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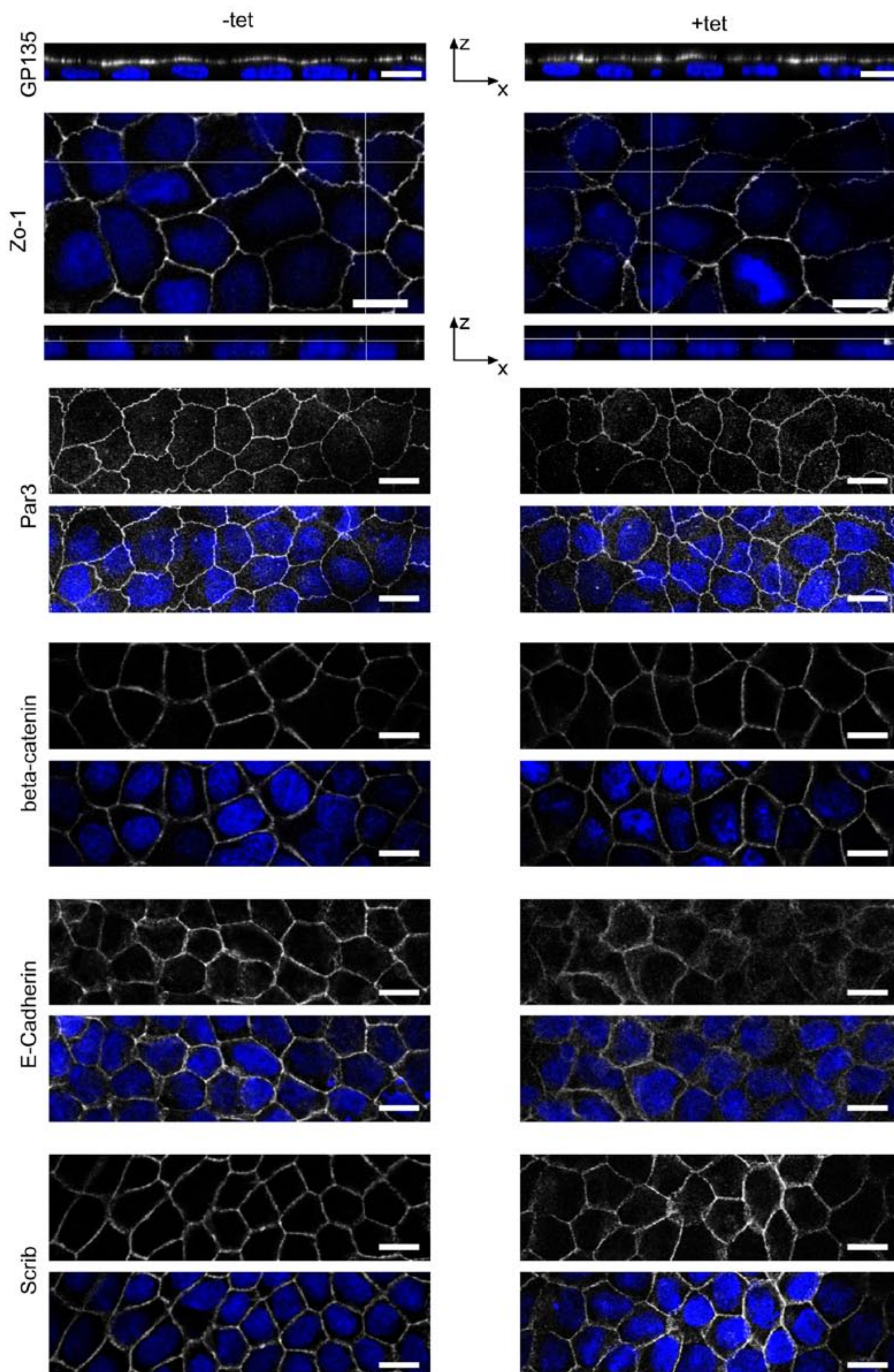

Figure S4 Lkb1 knock-down cells (+tet) and non-induced controls (-tet) were fixed and stained for the apical protein GP135, the tight junction proteins Zo-1 and Par3, and the lateral markers ß-Catenin, E-cadherin and Scrib. Apart from a slight reduction of E-cadherin staining, no differences are observed in Lkb1 depleted cells (+tet). Scale bars 10µm.

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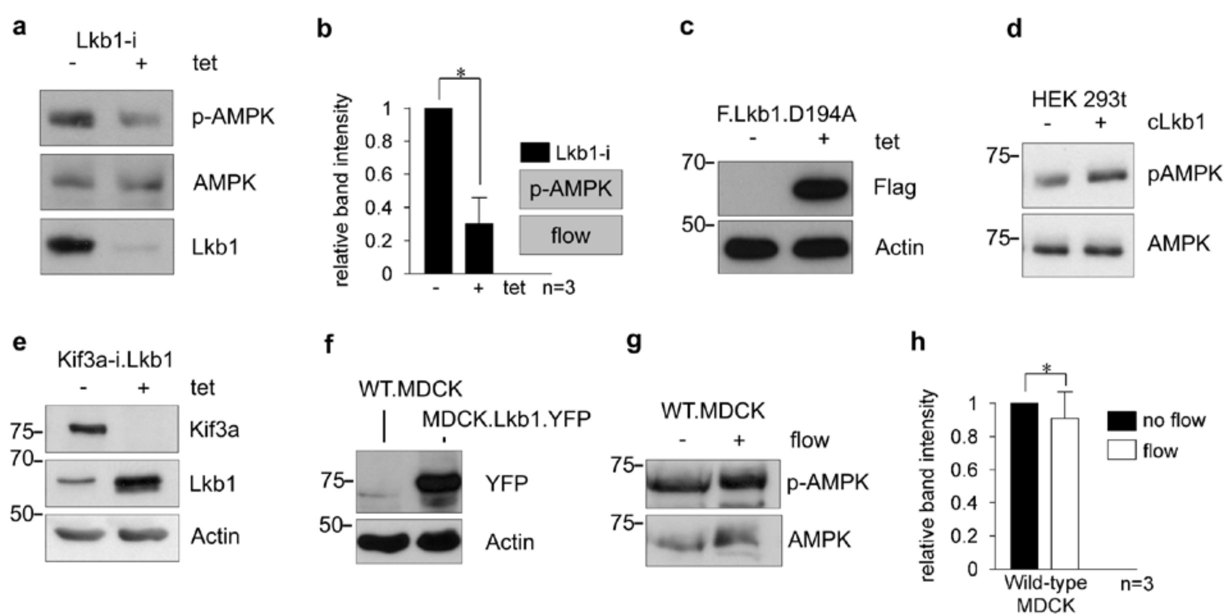

Figure S5 (**a**) Phosphorylation of AMPK is decreased in lysates of Lkb1 depleted cells (+tet) compared to controls after 5 days of flow. (**b**) Band quantification of phosphorylated AMPK (p-AMPK) of three independent experiments demonstrates significantly less p-AMPK in Lkb1 depleted cells (+tet) compared to non-induced cells (-tet) under flow. Asterisk: $P < 0.05$. (**c**) Expression control of inducible flag tagged dominant negative Lkb1 (Lkb1.D194A) in MDCK cells. Robust expression is seen after incubation with tetracycline. (**d**) Overexpression of canine Lkb1 (cLkb1) in HEK 293t cells results in increased phosphorylation of AMPK (representative of three independent experiments) (**e**) Expression control of inducible Lkb1 in Kif3a knockdown cells. Under tetracycline treatment (+tet) Kif3a is depleted and Lkb1 expression is induced, resulting in a stronger band. (**f**) Expression control of Lkb1.YFP expressing MDCK cells (right lane) compared to wild type controls (left lane). (**g**) p-AMPK is similar in lysates of MDCK cells after 5 days of flow compared to no flow. (**h**) Band quantification of phosphorylated AMPK (p-AMPK) from 3 independent experiments demonstrates no significant difference (n.s.) in relative levels of p-AMPK between flow and no flow conditions. Data in (**b**) and (**h**) are mean ± s.e.m.. The uncropped blot of (**a**) is shown in Supplementary Information, Fig. S6.

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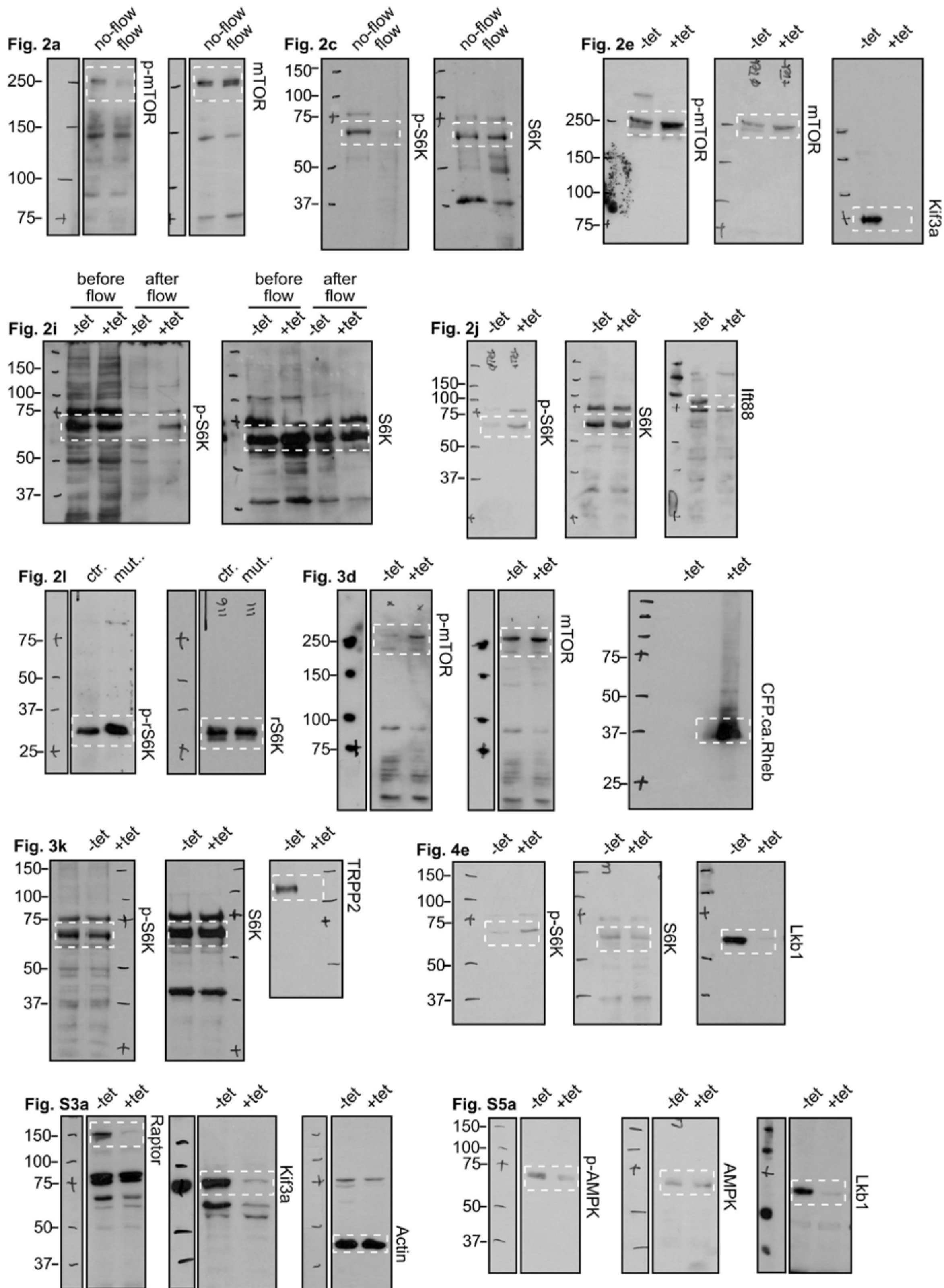

Figure S6 Uncropped key blots.

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