Supplemental Materials

Molecular Biology of the Cell

Rozycki et al.

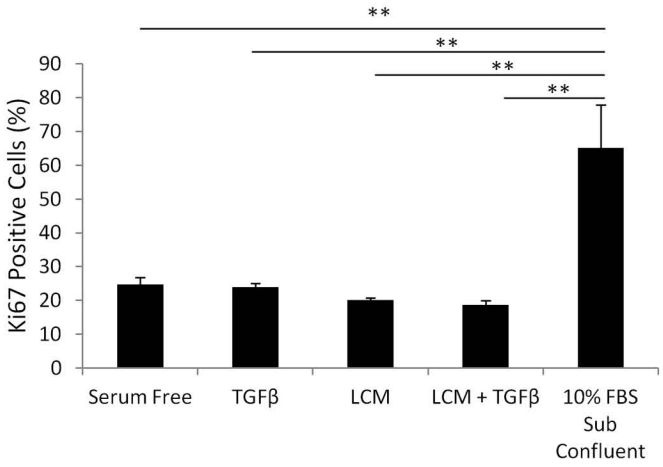

Figure S1. Two hit condition-induced deciliation is not due to re-entry into the cell cycle. LLC-PK1 were grown to confluence and then treated as indicated for 24 h. Subsequently cells were fixed and stained for Ki-67 and the percentage of cells with nuclear Ki-67 was determined (mean ± SEM, n=4, 500 cells/n for each treatment condition).Note that the ratio of Ki-67 positive cells does not change significantly across the four treatment conditions. As a positive control, subconfluent cells were treated with 10% Fetal bovine serum (FBS). These showed a marked increase in the percentage of Ki-67 positive cells.

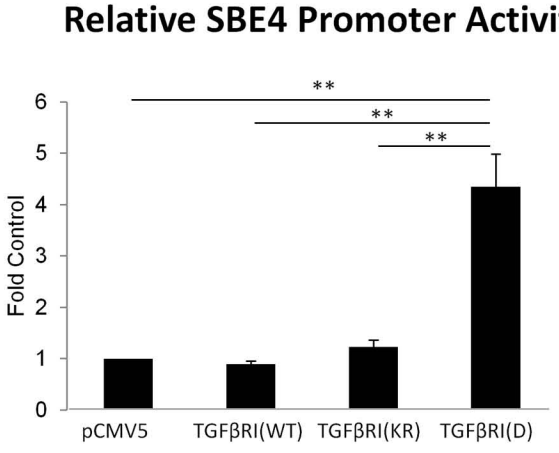

Figure S2. TGFβR1(TD) activates the Smad3-dependent SBE4-Luciferase promoter while the WT or TGFβR1(TD) receptors have no such effect. LLC-PK1 cells were grown to 60% confluence and then transfected with the SBE4-Luciferase promoter and pRL-TK (Renilla) for normalization. Upon reaching confluence cells were serum starved for 24 h and luciferase activity was determined. Data are expressed as fold change over the empty pCMV5-transfected control (mean ± SEM, n=3).

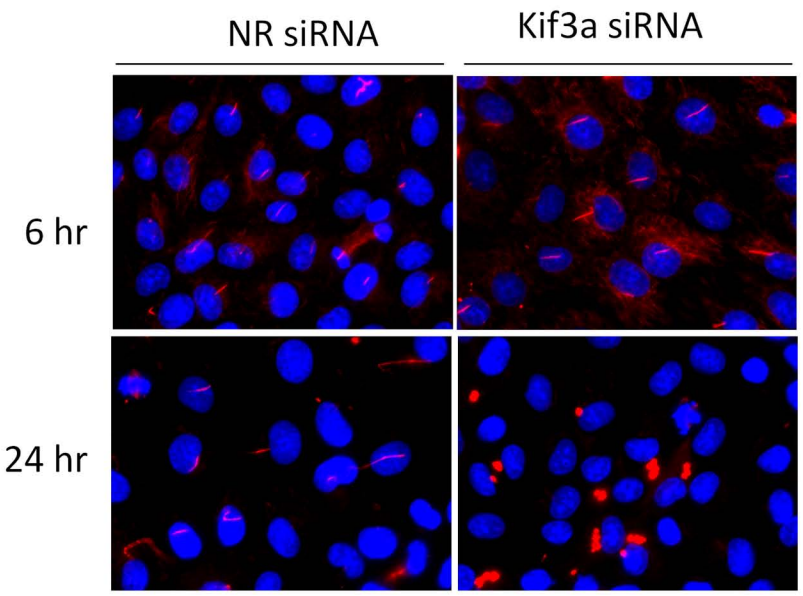

Figure S3. The morphology of the cilium after varying times of Kif3a transfection. LLC-PK1 cells were transfected with 100 nM NR or Kif3a siRNA for 6 or 24 h, as indicated. Subsequently cells were fixed with methanol and stained for the ciliary marker Ac-tub and the nuclear marker Dapi. Note that 6 h after transfection the ciliary structure is still intact, while 24 h after transfection many cells show distorted cilia. Transfection with NR siRNA does not impact cilia at either time.

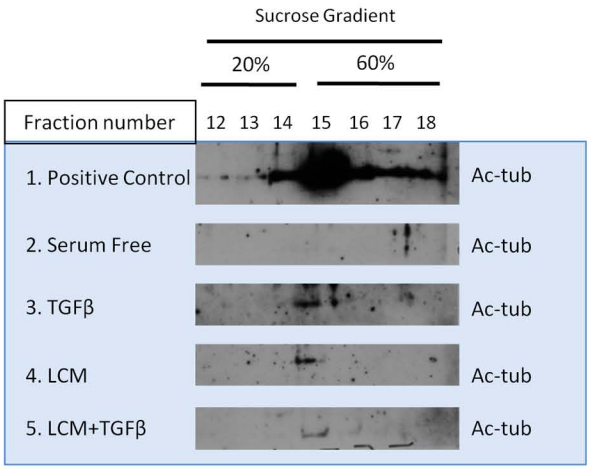

Figure S4. Analysis of primary cilium shedding. To detect cilium shedding, 48 h after treatments the medium bathing the cells was collected and analyzed for the presence of primary cilia by differential centrifugation followed by equilibrium sedimentation on a 20-60% discontinuous sucrose gradient. Shown is the Western blot analysis of the sucrose gradient fractions 12-18. Fractions 14/15 corresponding to the 20-60% sucrose interphase were positive for the cilium marker acetylated tubulin in the positive control in which cilium shedding was achieved by mechanically shaking cells at 360 rpm in high calcium deciliation solution (See Methods). Cells treated with the combined treatment did not show a significant difference compared to LCM or TGFβ alone, suggesting that shedding might not be the key mechanism accounting for deciliation.

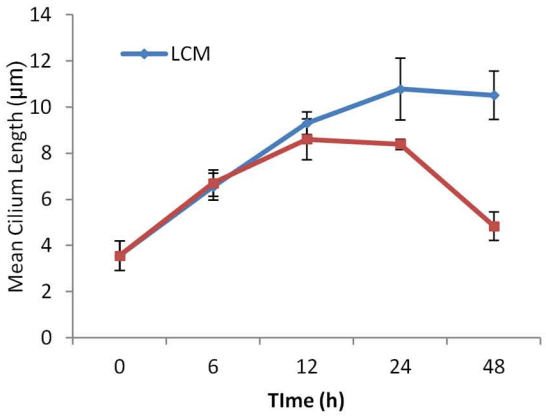

Figure S5. Cilium length undergoes a biphasic change in LCM+TGFβ treated cells. LLC-PK1 cells were grown to confluence and then treated with LCM or LCM+TGFβ for the indicated times. Cells were then fixed and stained for acetylated tubulin and cilium length was measured using morphometry. Data are presented as mean cilium length ± SEM (n=3, 12 frames of ≈30 cells/frame). Note that the vast majority of cilia are lost after 48 h treatment, as indicated in Fig 1. This graph shows that the few remaining cilia are significantly shorter than those present at 12 h under the same treatment conditions or after the same time of LCM treatment. These findings are compatible with a contribution of a resorptive process to deciliation.