Supplementary Figure S1: Primary cilium and shear stress. (A-D) HK2 cells were subjected to fluid flow from 4h to 4 days (shear 4h, 48h, 96h-4D), or not (static 4h, 48h, 96h-4D). (A) Cells were fixed with methanol, labeled with DAPI and ARL13B antibody to stain the primary cilium. (B) Cilia length was quantified from experiments shown in (A). (C) Levels of the ciliary protein IFT20 were analyzed by western blot and IFT20/actin ratio was quantified (D). Scale bar in (A) = 10μm.
Supplementary Figure S2: FLCN knockdown affects IFT20 protein stability. HK2 cells were transfected with control siRNA (siCTRL) or with siRNA targeting FLCN (siFLCN). 72 h later, they were subjected to fluid flow for 4 days (shear 4D) or not (static 4D). (A) IFT20 and actin levels were analyzed by western blot and IFT20/actin ratio was quantified (B).
Supplementary Figure S3: FLCN and autophagy dynamics. HK2 cells were transfected with control siRNA (siCTRL) or with siRNA targeting FLCN (siFLCN). 72 h later, they were subjected to fluid flow for 4 days (shear 4D) or not (static 4D). Chloroquine (20 μM) was added (+) or not (-) during the 24 last hours of shear stress to block autophagolysosomal maturation. (A) LC3 and actin levels were analyzed by western blot and LC3 II/actin ratio was quantified (B). (C) siCTRL and siFLCN cells were grown under control condition (CTRL) or upon complete starvation condition for 24 h (STV.) and FLCN, LC3 and actin levels were analyzed by western blot.
Supplementary Figure S4: FLCN knockdown affects mTOR signaling pathway in response to fluid flow. HK2 cells were transfected with control siRNA (siCTRL) or with siRNA targeting FLCN (siFLCN). 72 h later, they were subjected to fluid flow for 4 days (shear 4D) or not (static 4D). (A) phospho-TSC2, total TSC2, phospho-S6, total S6 and actin levels were analyzed by western blot and normalized phospho-TSC2/total TSC2 ratio and normalized phospho-S6/total S6 ratio were quantified (B, C).