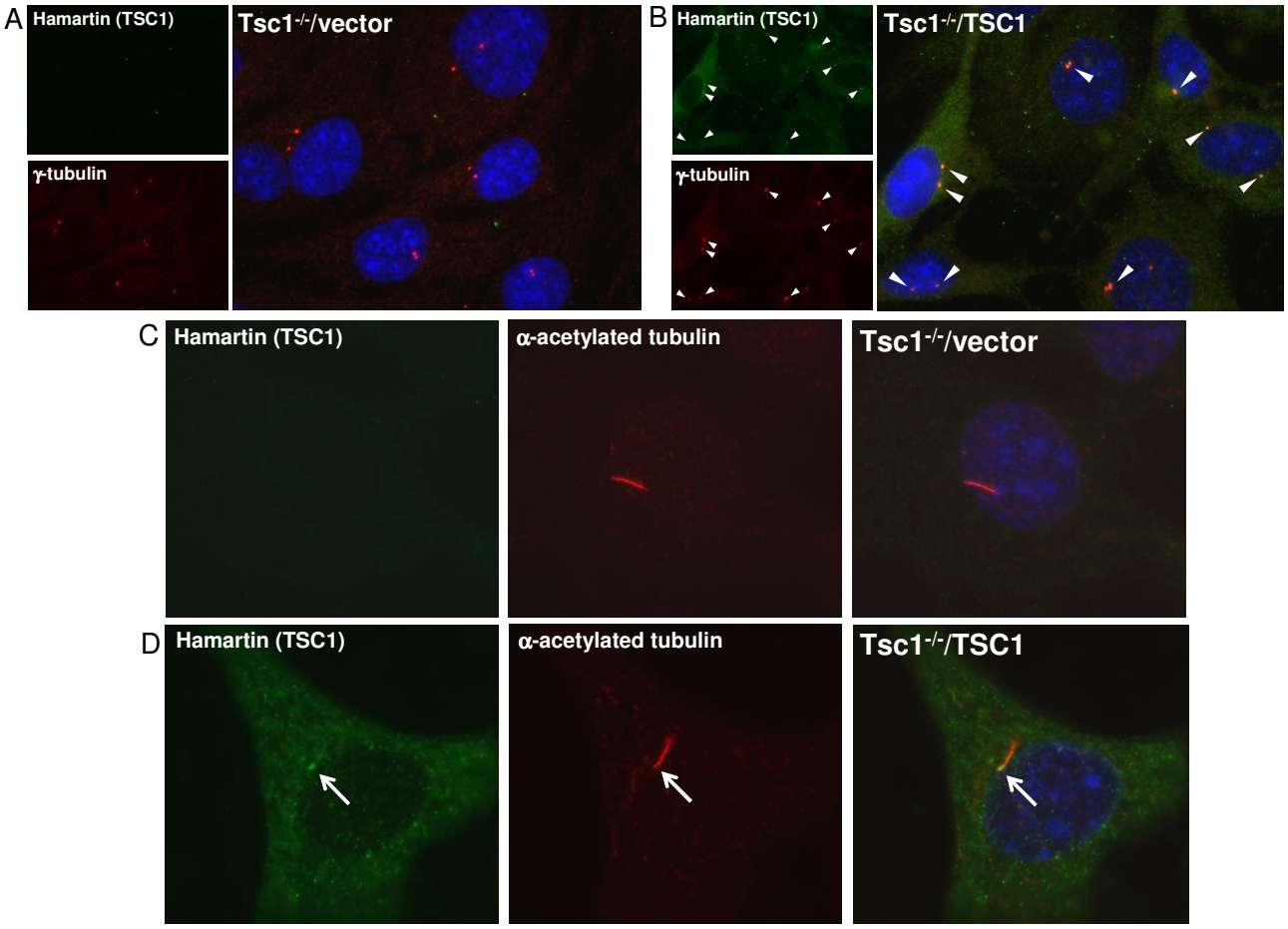
Supplemental Table 1. Percentage of Tsc1 and Tsc2-null and wild type MEFs with 2 or >2 centrosomes per cell

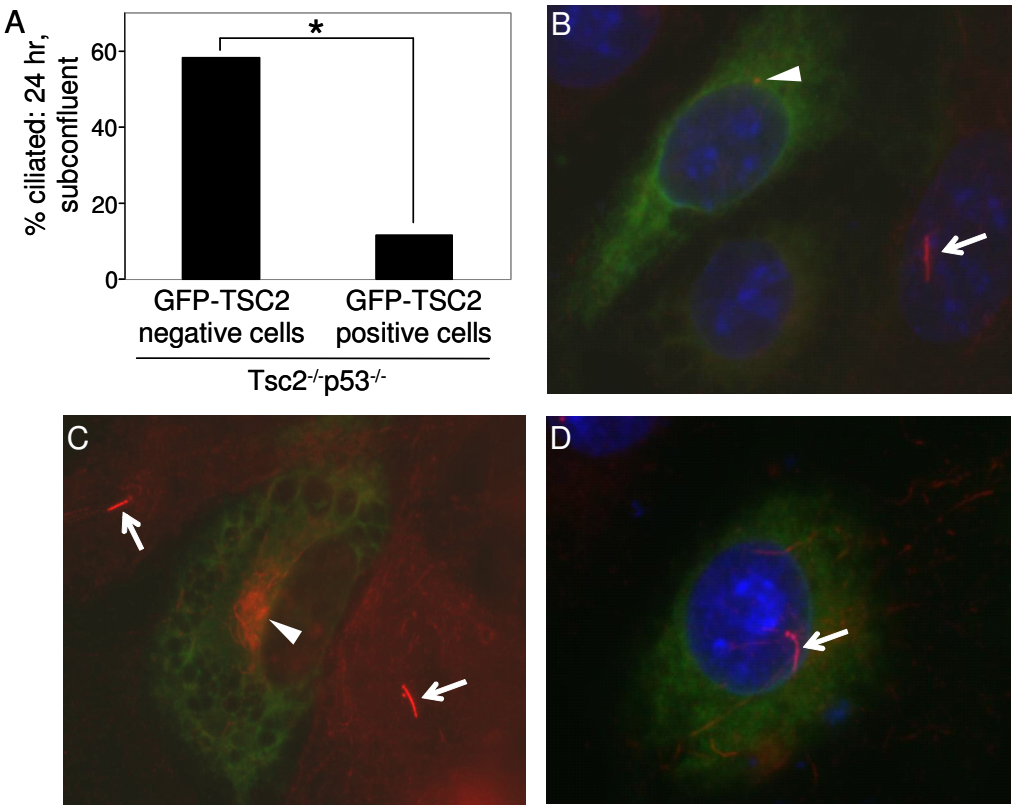
Cell Type	Serum Condition	Percentage of Cells with 2 Centrosomes	Percentage of Cells with >2 Centrosomes
Tsc1$^{-/-}$/vector	24 hr FBS subconfluent	97.6	2.4
	48 hr FBS confluency	98.9	1.1
	48 hr serum deprivation	95.1	4.9
	48 hr FBS, DMSO	98.0	2.0
	48 hr FBS, rapamycin	96.4	3.6
Tsc1$^{-/-}$/TSC1	24 hr FBS subconfluent	98.8	1.2
	48 hr FBS confluency	98.1	1.9
	48 hr serum deprivation	96.0	4.0
	48 hr FBS, DMSO	97.8	2.2
	48 hr FBS, rapamycin	97.1	2.9
Tsc2$^{-/-}$p53$^{-/-}$	24 hour FBS subconfluent	97.6	2.4
	48 hour FBS confluency	98.7	1.3
	48 hour serum deprivation	97.8	2.2
	48 hr FBS, DMSO	98.3	1.7
	48 hr FBS, rapamycin	97.8	2.2
	72 hr no serum, DMSO	97.0	3.0
	72 hr no serum, rapamycin	97.1	2.9
Tsc2$^{+/+}$p53$^{-/-}$	24 hr FBS subconfluent	98.0	2.0
	48 hr FBS confluency	98.2	1.8
	48 hr serum deprivation	96.5	3.5
	48 hr FBS, DMSO	98.1	1.9
	48 hr FBS, rapamycin	98.7	1.3
	72 hr no serum, DMSO	96.5	3.5
	72 hr no serum, rapamycin	97.1	2.9

There are no significant differences in the number of centrosomes per cell for any of the cell lines in any culture condition tested, two-tailed Fisher's exact test.

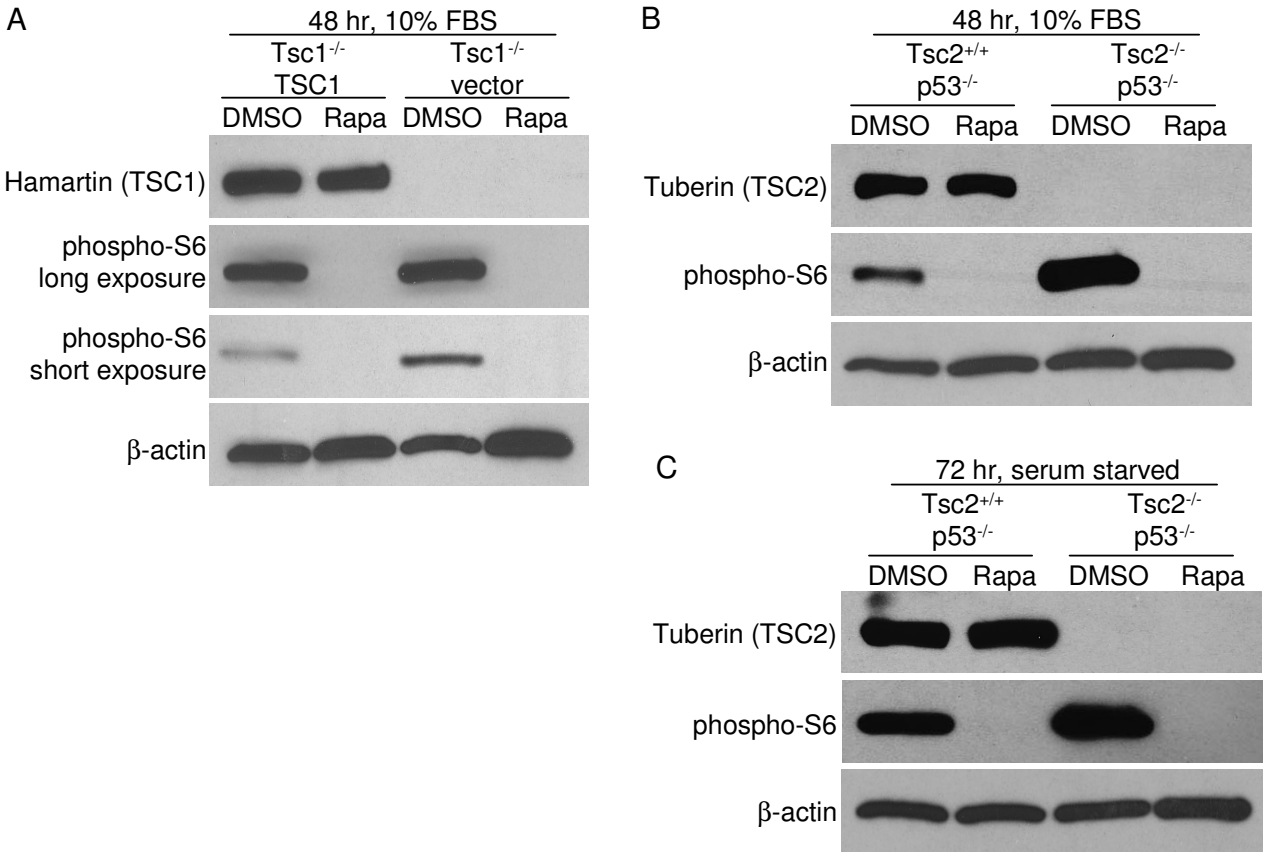


Supplemental Figure S1. Hamartin localizes to the centrosome and basal body of *Tsc1*$^{-/-}$ */TSC1* MEFs. *Tsc1*$^{-/-}$*/vector* (Tsc1-null) MEFs and *Tsc1*$^{-/-}$*/TSC1* (TSC1 re-expressing) MEFs were grown in 10% FBS for 24 hours. **A**) *Tsc1*$^{-/-}$*/vector* MEFs were fixed and immunostained with anti-gamma-tubulin antibody (red) to identify centrosomes, and anti-hamartin antibody (TSC1, green). Hamartin was undetectable in the Tsc1-null cells. **B**) *Tsc1*$^{-/-}$*/TSC1* MEFs were fixed and immunostained with anti-gamma-tubulin antibody (red) to identify centrosomes, and anti-hamartin antibody (TSC1, green). Arrowheads indicate hamartin colocalization with the centrosome. **C**) *Tsc1*$^{-/-}$*/vector* MEFs were fixed and immunostained with anti-alpha-acetylated-tubulin antibody (red) to identify cilia, and anti-hamartin antibody (TSC1, green). Hamartin was undetectable in the Tsc1-null cells. **B**) *Tsc1*$^{-/-}$*/TSC1* MEFs were fixed and immunostained with anti-alpha-acetylated-tubulin antibody (red) to identify cilia, and anti-hamartin antibody (TSC1,

green). Arrows indicate hamartin localization at the basal body. Re-expressed hamartin staining was also observed in the cytoplasm. Images for hamartin, alpha-acetylated tubulin, and gamma-tubulin were captured with identical settings and exposure times for *Tsc1*$^{-/-}$*/vector* and *Tsc1*$^{-/-}$*/TSC1* MEFs. Nuclei were stained with DAPI. All images shown at 100X magnification.



Supplemental Figure S2. Re-expression of TSC2 reduces the number of ciliated *Tsc2*$^{-/-}$*p53*$^{-/-}$ MEFs. *Tsc2*$^{-/-}$*p53*$^{-/-}$ MEFs were transfected with GFP-TSC2 plasmid DNA and grown in 10% FBS at subconfluency for 24 hours. **A**) Percent of cells containing a primary cilium is shown for nontransfected *Tsc2*$^{-/-}$*p53*$^{-/-}$ MEFs and GFP-TSC2 transfected *Tsc2*$^{-/-}$*p53*$^{-/-}$ MEFs from the same coverslip, asterisk indicates $p<0.005$. **B-C**) Cells were fixed and immunostained with anti-alpha-acetylated-tubulin antibody (cilia, red) and anti-gamma-tubulin antibody (centrosomes/basal body, red). (B) Two GFP-TSC2 positive cells (green) lacking a cilium are shown next to a GFP-TSC2 negative cell with a cilium. Arrow indicates the primary cilium, and arrowheads indicate a centrosome in a nonciliated cell. The centrosome of the lower GFP-TSC2 positive cell is out of the plane of this image. (C) A GFP-TSC2 positive cell (green) lacking a cilium is shown next to two GFP-TSC2 negative cells with cilia. (D) A GFP-TSC2 positive cell (green) with a cilium is shown, indicating that the presence of over-expressed GFP-TSC2 does not block all ciliogenesis. Nuclei were stained with DAPI (B and D). 100X magnification.



Supplemental Figure S3. The mTOR pathway is sensitive to rapamycin in cilia inducing conditions. MEFs were treated with either DMSO vehicle or 20 nmol rapamycin. Immunoblots were probed with phospho-S6 to determine the level of mTOR activation. β-actin was used as a loading control. **A)** Western immunoblot of *Tsc1*$^{-/-}$ MEFs with re-introduced TSC1 (*Tsc1*$^{-/-}$*/TSC1*) or Tsc1-null MEFs (*Tsc1*$^{-/-}$*/vector*) treated with DMSO or rapamycin for 48 hours in 10% FBS. **B**) Western immunoblot of *Tsc2*$^{+/+}$*p53*$^{-/-}$ or *Tsc2*$^{-/-}$*p53*$^{-/-}$ MEFs treated with DMSO or rapamycin for 48 hours in 10% FBS. **C**) Western immunoblot of *Tsc2*$^{+/+}$*p53*$^{-/-}$ or *Tsc2*$^{-/-}$*p53*$^{-/-}$ MEFs treated with DMSO or rapamycin for 72 hours in serum starvation conditions.