Supplemental material

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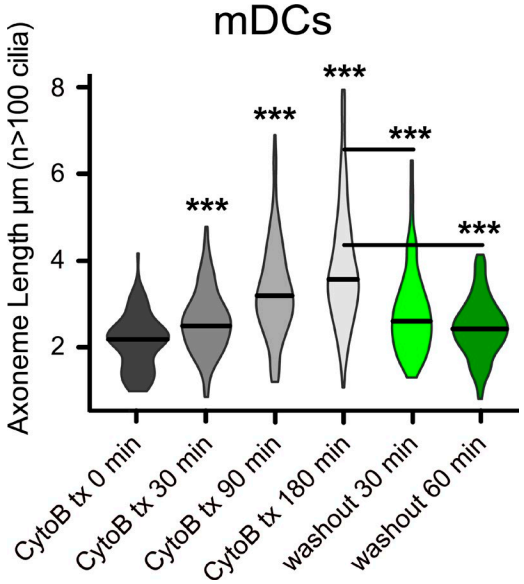

Figure S1. **Cytochalasin B washout restores primary cilium length.** Violin plot of axoneme length in confluent mDCs upon temporal addition and washout of 10 µM cytochalasin B (CytoB) for the indicated time points (n = 3 experiments). tx, treatment. Error bars represent SEM. Significance was determined by unpaired two-tailed t test (***, P < 0.001).

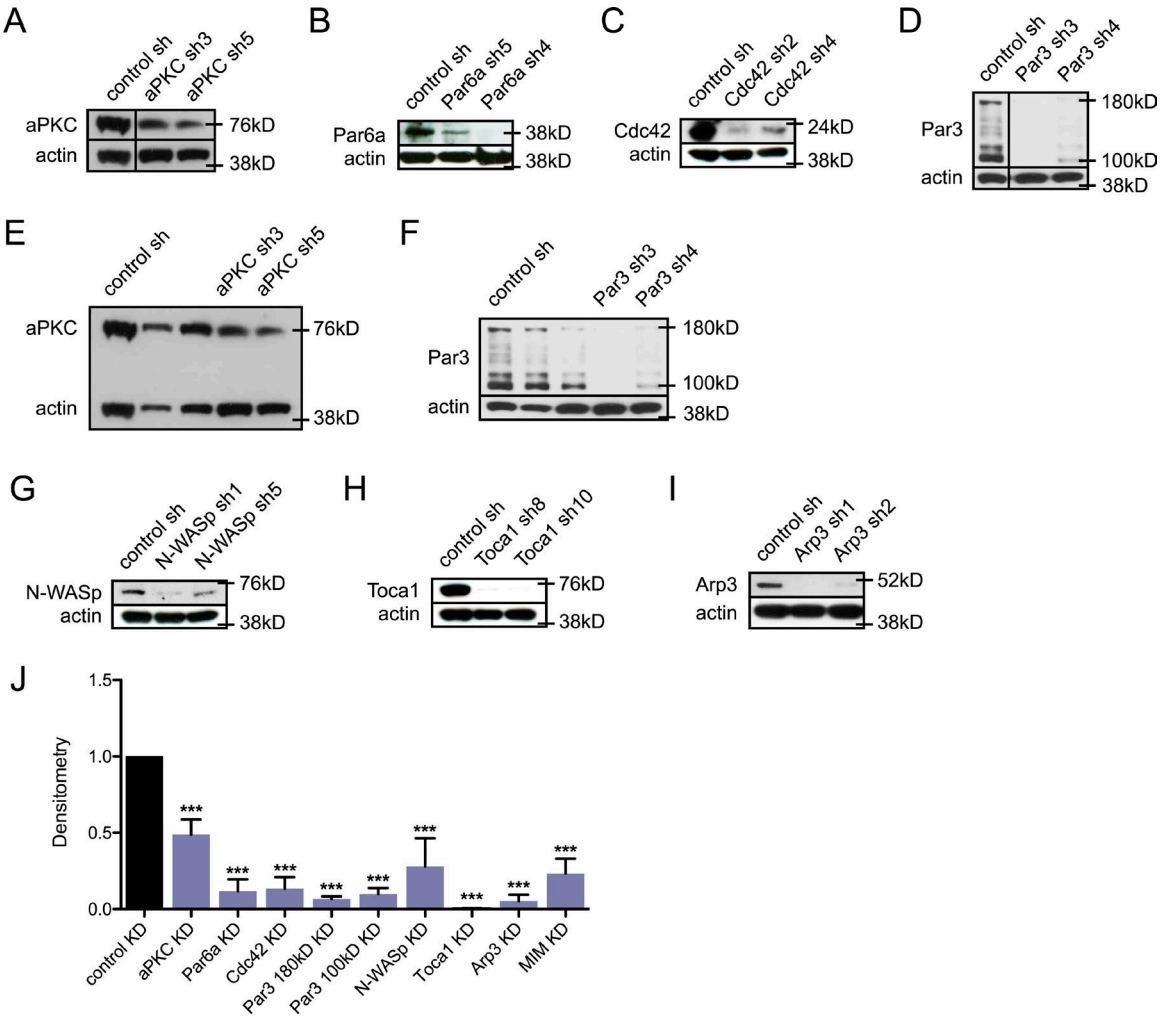

Figure S2. **Western blot analysis of protein knockdown. (A–D)** Western blot of aPKC (A), Par6a (B), Cdc42 (C), or Par3 shRNA-mediated knockdown (KD) mDCs (D). **(E and F)** Whole blot of aPKC (E) or Par3 knockdown mDCs (F). **(G–I)** Western blot of N-WASp (G), Toca1 (H), or Arp3 (I) knockdown mDCs. **(J)** Densitometry of knockdown Western blots. Error bars represent SEM. Significance determined by unpaired two-tailed *t* test (***, P < 0.001).

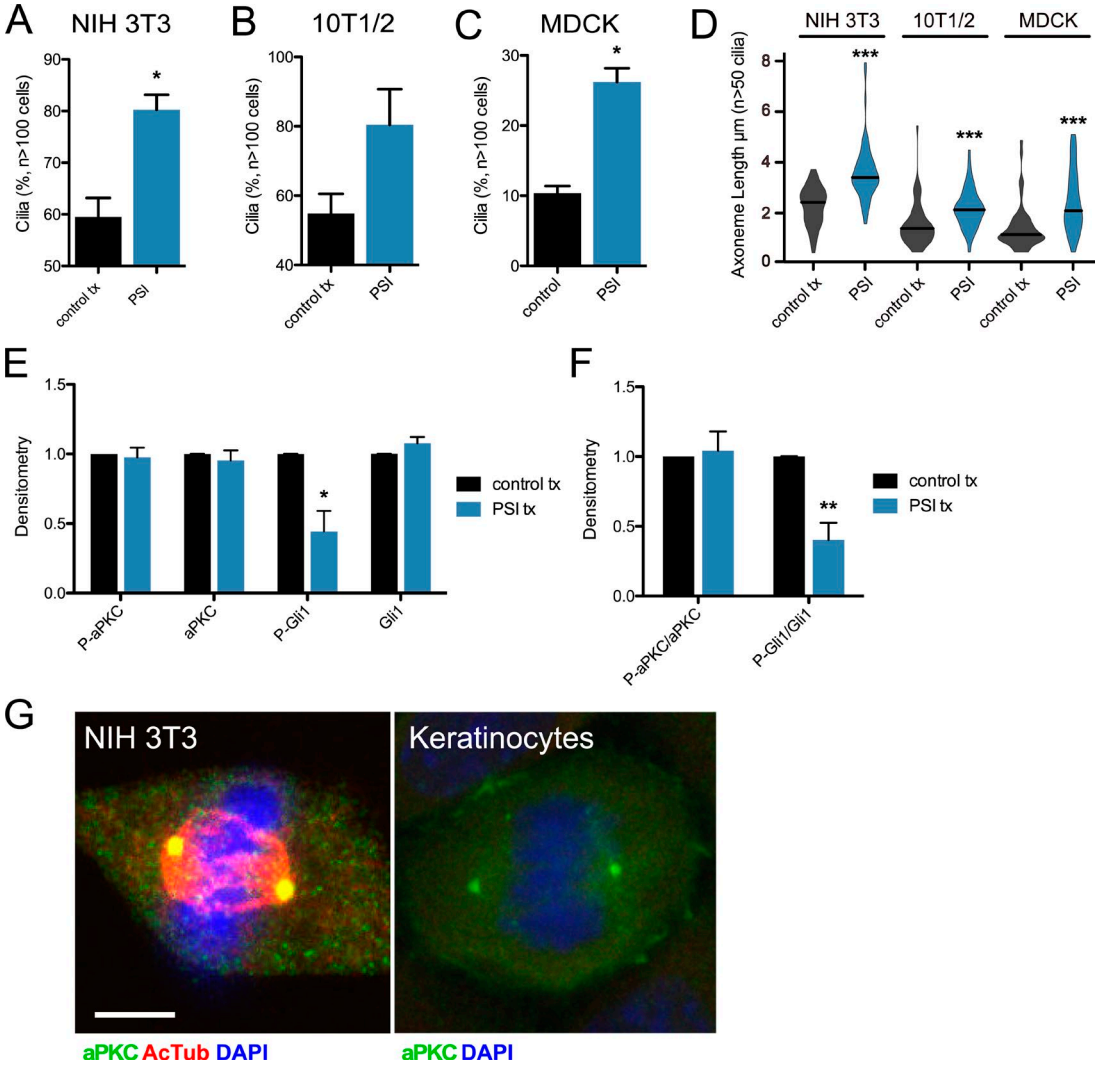

Figure S3. **aPKC controls primary cilia and Hh signaling. (A–C)** Percentage of primary cilia in control or 10 µM PSI–treated (18 h) subconfluent NIH 3T3 (A; *n* = 3 experiments), C3H10T1/2 (G; 10T1/2; *n* = 3 experiments), or MDCK cells (C; *n* = 2 experiments). **(D)** Violin plot of axoneme length. **(E and F)** Densitometry of Western blot bands (E) or ratios (F) from Fig. 4 B. Error bars represent SEM. Significance determined by unpaired two-tailed *t* test (*, P < 0.05; **, P < 0.01; ***, P < 0.001). **(G)** Immunofluorescence of mitotic NIH 3T3 or mouse keratinocytes stained for the indicated proteins. Bar, 5 µm.

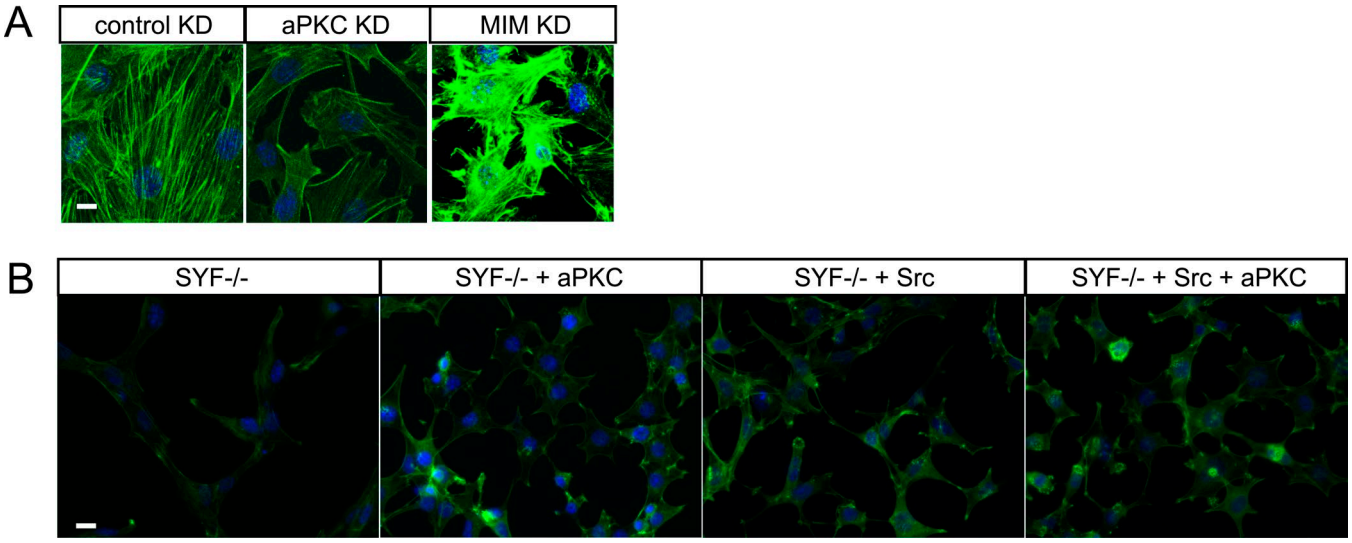

Figure S4. **Phalloidin staining is altered upon disruption of upstream regulators of actin nucleators. (A)** Representative phalloidin staining of subconfluent mDCs after shRNA knockdown of control, aPKC, or MIM. Bar, 10 µm. **(B)** Representative phalloidin staining of sub-confluent *SYF*$^{-/-}$ MEFs with or without stable transfection of aPKC and/or Src (n > 100 cells). Bar, 10 µm.

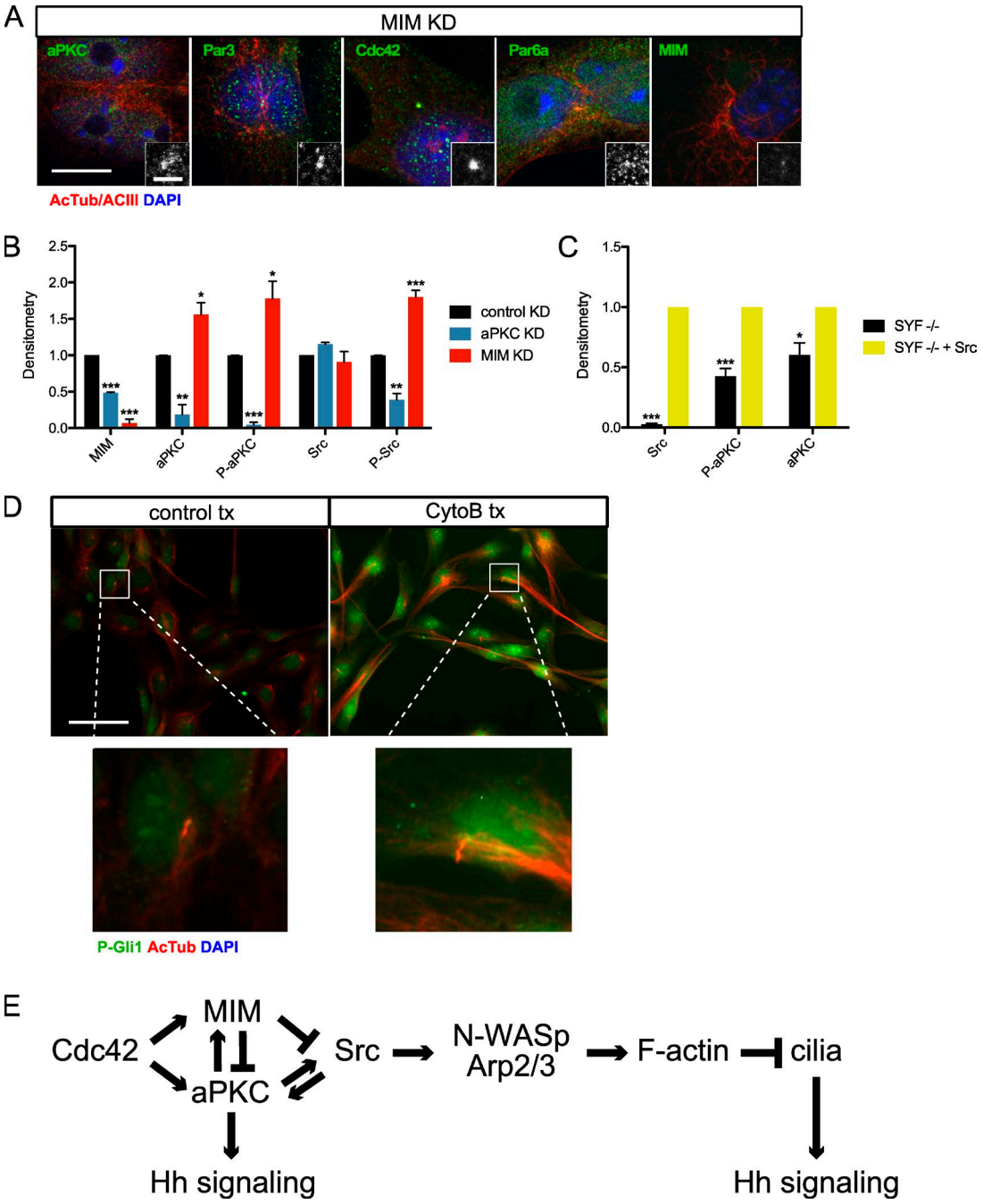

Figure S5. **MIM and phospho-Gli1 staining. (A)** Immunofluorescence of MIM knockdown mDCs stained for indicated proteins and acetylated-tubulin (AcTub)/adenyl cyclase III (ACIII) positive primary cilia. Immunoreactivity around the basal body is highlighted in the lower right of each panel. Bar: 10 µm; (inset) 2 µm. **(B and C)** Densitometry of Western blots from Fig. 6 A (B) or Fig. 6 I (C). Error bars represent SEM. Significance determined by unpaired two-tailed *t* test (*, P < 0.05; **, P < 0.01; ***, P < 0.001). **(D)** Immunofluorescence of NIH 3T3 cells treated with or without 2.5 µM cytochalasin B (CytoB) for 3 h and stained for the indicated proteins, as in Fig. 6 K. Bar, 50 µm. **(E)** Model showing Cdc42 recruits MIM and aPKC to antagonize Src activity and control actin polymerization, primary cilia, and HH signaling.

Provided online are three supplemental tables in Excel. Table S1 shows protein–protein interaction hub proteins. Table S2 shows kinase enrichment analysis proteins. Table S3 shows human endogenous complexome proteins.