

Fig. S1. Loss of Scrib or Sgt1 increases apoptosis in cysts. (A) Fixed MDCK cysts were stained with actin and cleaved caspase 3 (c-casp 3) as indicated. Nuclei are marked by Hoechst staining and pictured in blue. Localization of Scrib and Sgt1 RNAi cysts have increased cleaved caspase 3 (a marker of apoptosis) positive cells in their luminal space relative to controls. Increased numbers of cleaved caspase 3 positive cells could also be seen for Scrib and Sgt1 shRNA cells grown in 2D culture. (B) Quantification of cleaved caspase 3 staining in 4-5-day-old cysts derived from cells treated with experimental or control shRNAs. Statistically significant: (*)= $P < 0.05$, (**)= $P < 0.01$. Dunnett's multiple comparison test was used.

A

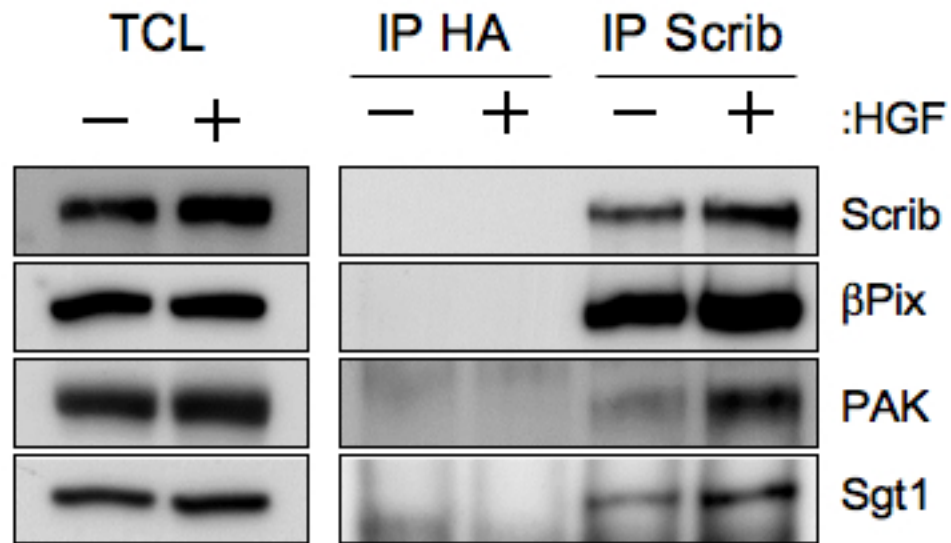
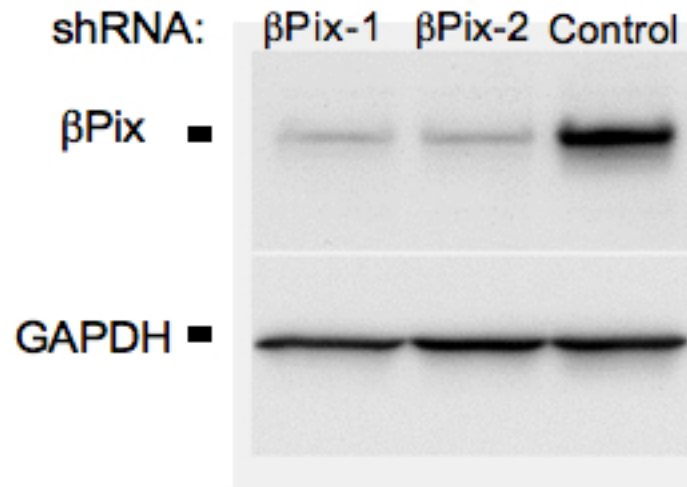


Fig. S2. Scrib forms a complex with β Pix and PAK. (A) MDCK cells were treated with or without HGF and total cell lysates (TCL) were prepared and subject to immunoprecipitation using a Scrib (C-20) or control HA antibody. IPs were isolated and subject to SDS-PAGE and western blot analysis alongside TCLs for the indicated proteins. IP of Scrib demonstrated a specific interaction with β Pix, PAK and Sgt1.

A



B

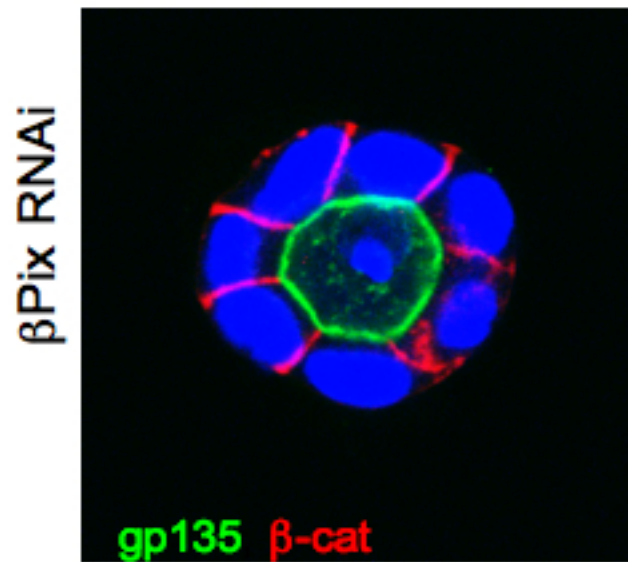


Fig. S3. RNAi of β Pix in MDCK cysts. (A) Western blot image of MDCK total cell lysates treated either with a control shRNA or shRNAs directed against β Pix. GAPDH is included as a loading control for the lysates. (B) Fixed MDCK cysts were stained with the apical domain marker gp135 and the adherens junction marker beta-catenin (β -cat). Nuclei are marked by Hoechst staining and pictured in blue.

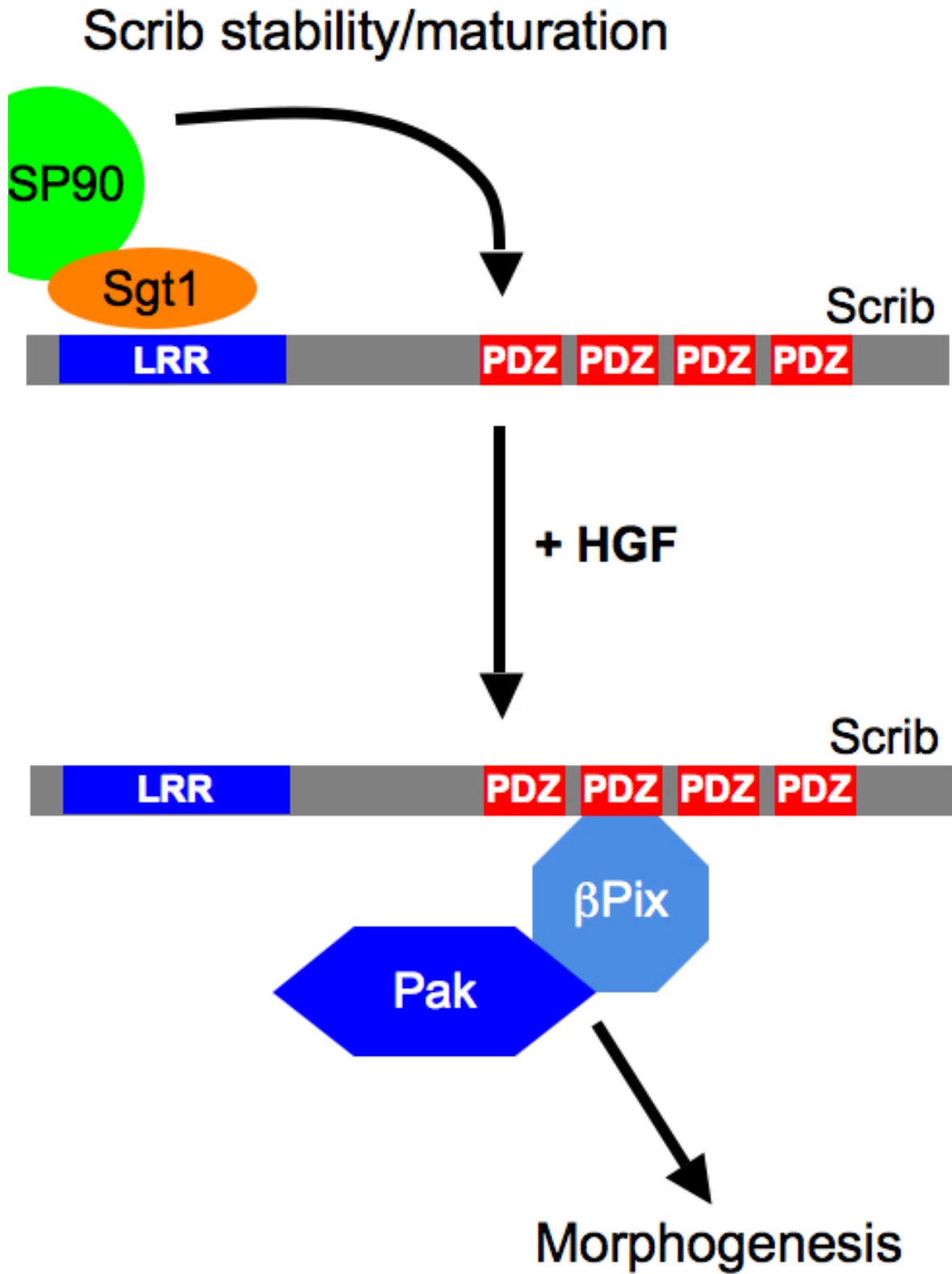


Fig. S4. Model. Model for Scrib complex regulation of epithelial morphogenesis. Sgt1-HSP90 functions to stabilize Scrib via the LRR domain. This enables Scrib to promote HGF-mediated extension formation through β Pix and PAK.