Supplementary Figure Legends

Supplementary Figure 1. **The PI3K pathway is required for branching morphogenesis of NMuMG mammary epithelial cells.** Clusters of NMuMG mouse mammary epithelial cells were embedded in collagen gel and treated with no growth factor (No GF) or EGF with or without PI3K pathway inhibitors and monitored for branching after 24 h. Shown are phase contrast and fluorescence images depicting nuclei (blue) and actin (green). Scale bars, 100 μm.

Supplementary Figure 2. **Treatment with calyculin A enhances pAkt distribution towards the ends of the tubules.** Mammary epithelial tubules were treated with HGF and calyculin A for 2 h. Shown are immunofluorescence images and frequency maps of 50 tubules stained for pAkt and pPTEN. Scale bars, 50 μm.

Supplementary Figure 3. **Loss of SPRY2 leads to unpatterned branching.** A. Immunoblot to confirm the knockdown of SPRY2 in cells transfected with shSPRY2. B. Mammary epithelial tubules were transfected with either scrambled shRNA or SPRY2 shRNA and used to form tubules, which were treated with HGF for 24 h. Shown are images of DAPI staining and frequency maps of 50 tubules stained for nuclei. Scale bars, 50 μm. Color bars indicate frequency.

Supplementary Figure 4. **Loss of SPRY2 leads to elevated levels of pPTEN and pAkt.** A. Mammary epithelial cells were transfected with scrambled shRNA (Sc) or shRNA targeting SPRY2 (shSPRY2). B, C. Mammary epithelial cells were transfected with Sc or shSPRY2 and

used to generate mammary epithelial tubules. Tubules were treated with HGF for 2 h and stained for pAkt (B) or pPTEN (C). Scale bars, 50 μm. Color bars indicate frequency.

Supplementary Figure 5. **Effects of modulating the PI3K pathway on apoptosis.** A. Clusters of EpH4 mammary epithelial cells were embedded in collagen gel and treated with no growth factor (No GF) or HGF with or without LY294002, wortmannin, Akt inhibitor, or NSC27366 and monitored for branching after 24 h. The samples were then fixed and stained for cleaved caspase-3 and nuclei. B. Cells were transfected with pCAGEN control vector or pCAG-Myr-p110-IH plasmid and used to generate clusters, which were treated with HGF and monitored for branching after 24 h. The samples were then fixed and stained for cleaved caspase-3 and nuclei. C. Cells were transfected with Sc or shSPRY2 and used to generate clusters, which were treated with HGF and monitored for branching after 24 h. The samples were then fixed and stained for cleaved caspase-3. Scale bars, 100 μm.

Supplementary Figure 6. **Endogenous mechanical stress alters SPRY2 expression.** A. SPRY2 levels are higher at the ends of the tubules. Shown are immunofluorescence images and frequency maps of 50 tubules stained for SPRY2. Scale bars, 50 μm. Color bars indicate frequency. B. Mammary epithelial cells were treated with either blebbistatin or calyculin A for indicated time periods. SPRY2 expression levels were determined by immunoblot.