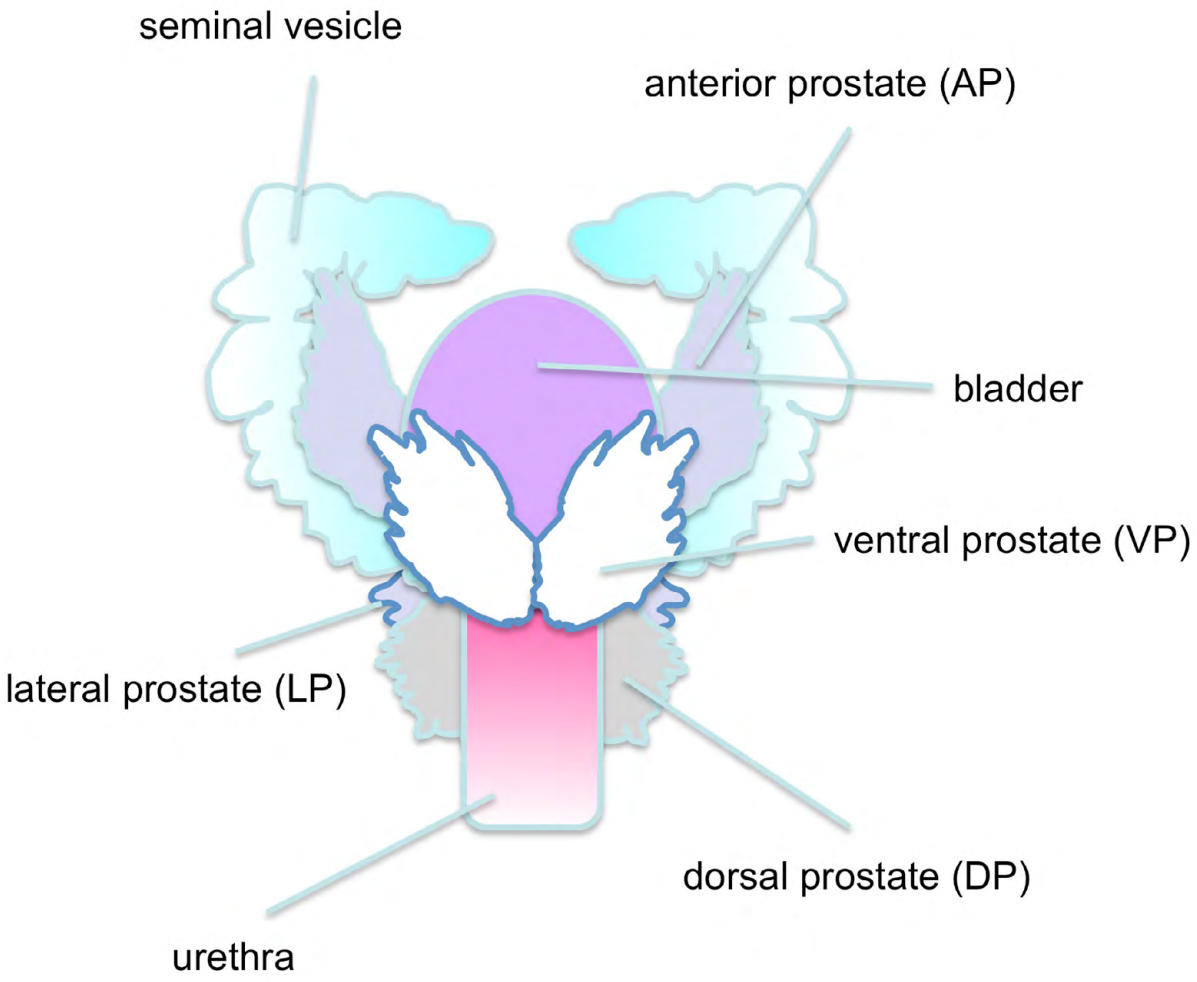

Fig. S1. Cartoon depicting the male urogenital system in adult mice indicating the prostatic lobes.

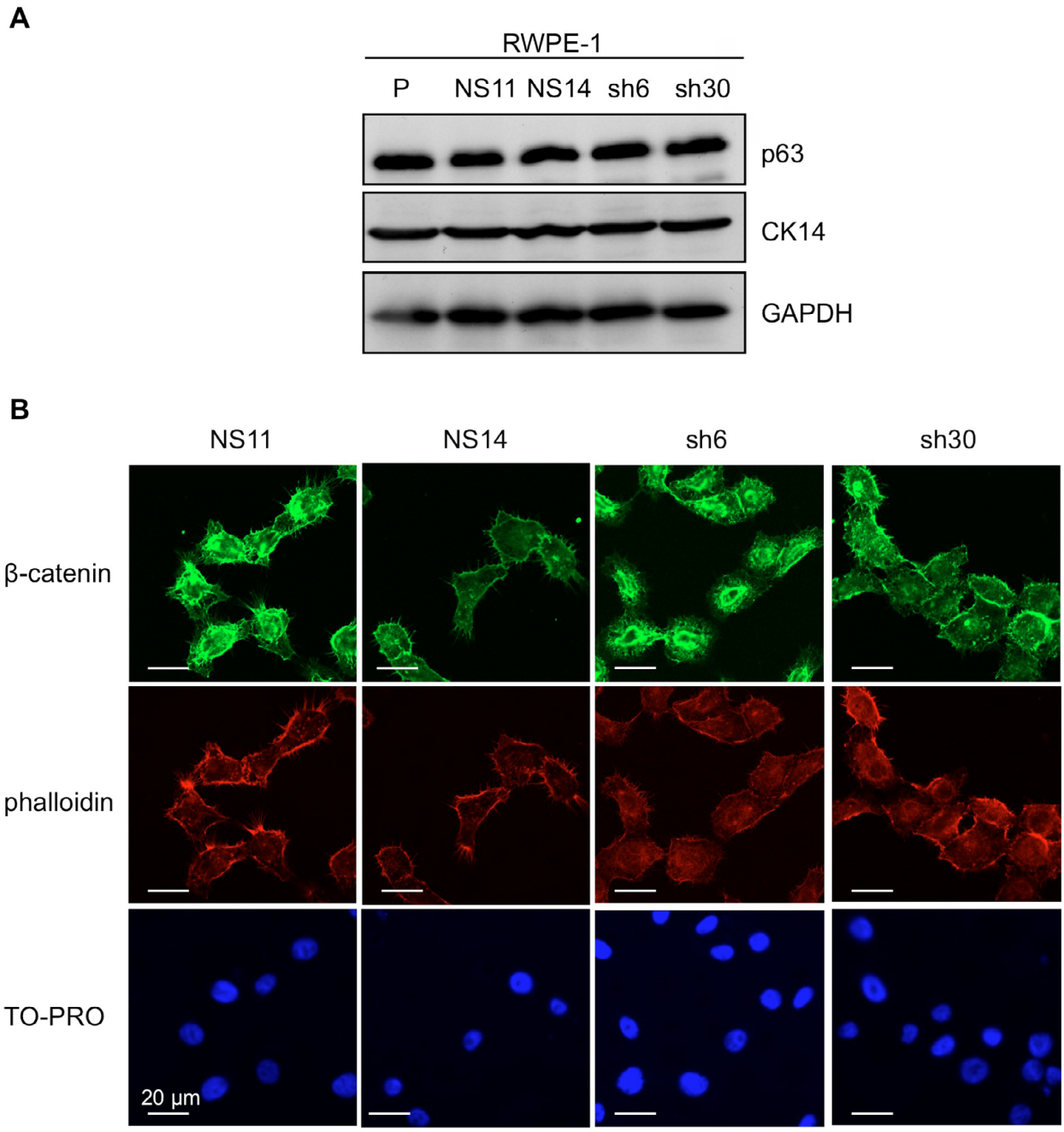

Fig. S2. Phenotypic and morphological characterization of Dkk-3-silenced RWPE-1 sublines. (A) Western blot for p63 and CK14 in RWPE-1 parental cells (P), Dkk-3 depleted clones (sh6 and sh30), and non-silenced control clones (NS11 and NS14). GAPDH was used as a loading control. (B) Immunostaining for β-catenin (green) and F-actin (red, detected using fluorescent phalloidin) in the indicated RWPE-1 sublines; nuclei were stained using TO-PRO-3 (blue).

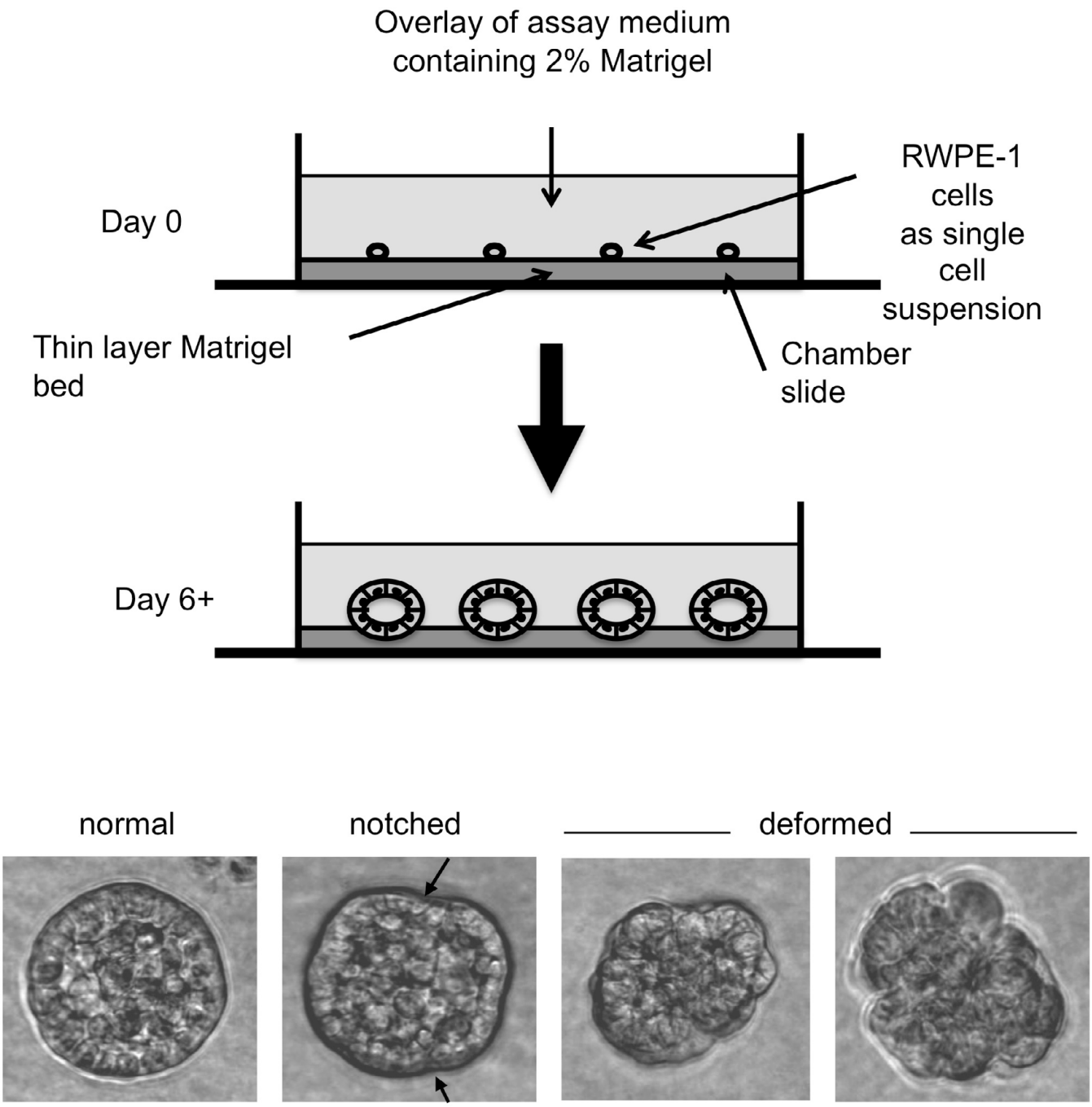

Fig. S3. RWPE-1 acinar morphogenesis assays. Top: schematic diagram of the acinar morphogenesis assay of RWPE-1 cells on Matrigel (adapted from Debnath (Debnath et al., 2003)). RWPE-1 cells are seeded as a single-cell suspension in assay medium containing 2% FCS, 5 ng/ml EGF and 2% Matrigel onto a thin layer bed of solidified Matrigel. The assay medium is replaced every the other day and acinar formation usually becomes apparent by day 6. Bottom: Acinar morphogenesis phenotypes observed in RWPE-1 derived sublines. Representative examples of 'normal', 'notched' (acini with irregular periphery) and 'deformed' (amorphous aggregation without trace of acinar structure) are shown.

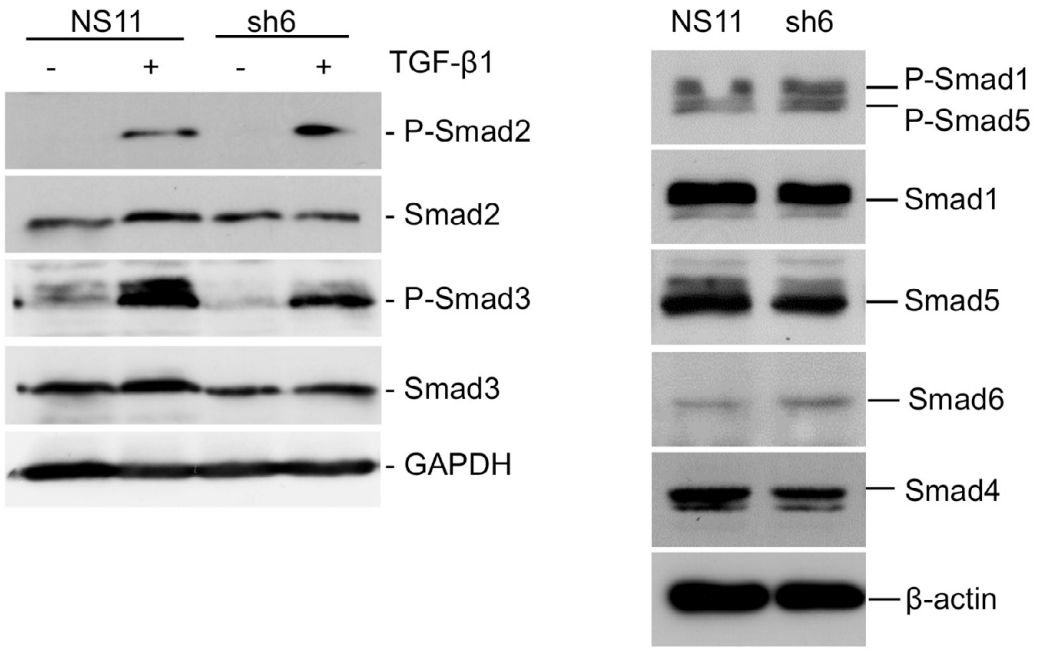

Fig. S4. Expression and phosphorylation of Smad proteins in Dkk-3-silenced cells. Extracts from the RWPE-1 NS11 and sh6 cells (left panels treated for 1 h with vehicle (PBS) or 1 ng/ml TGF-β1) were probed by western blotting for the indicated proteins.

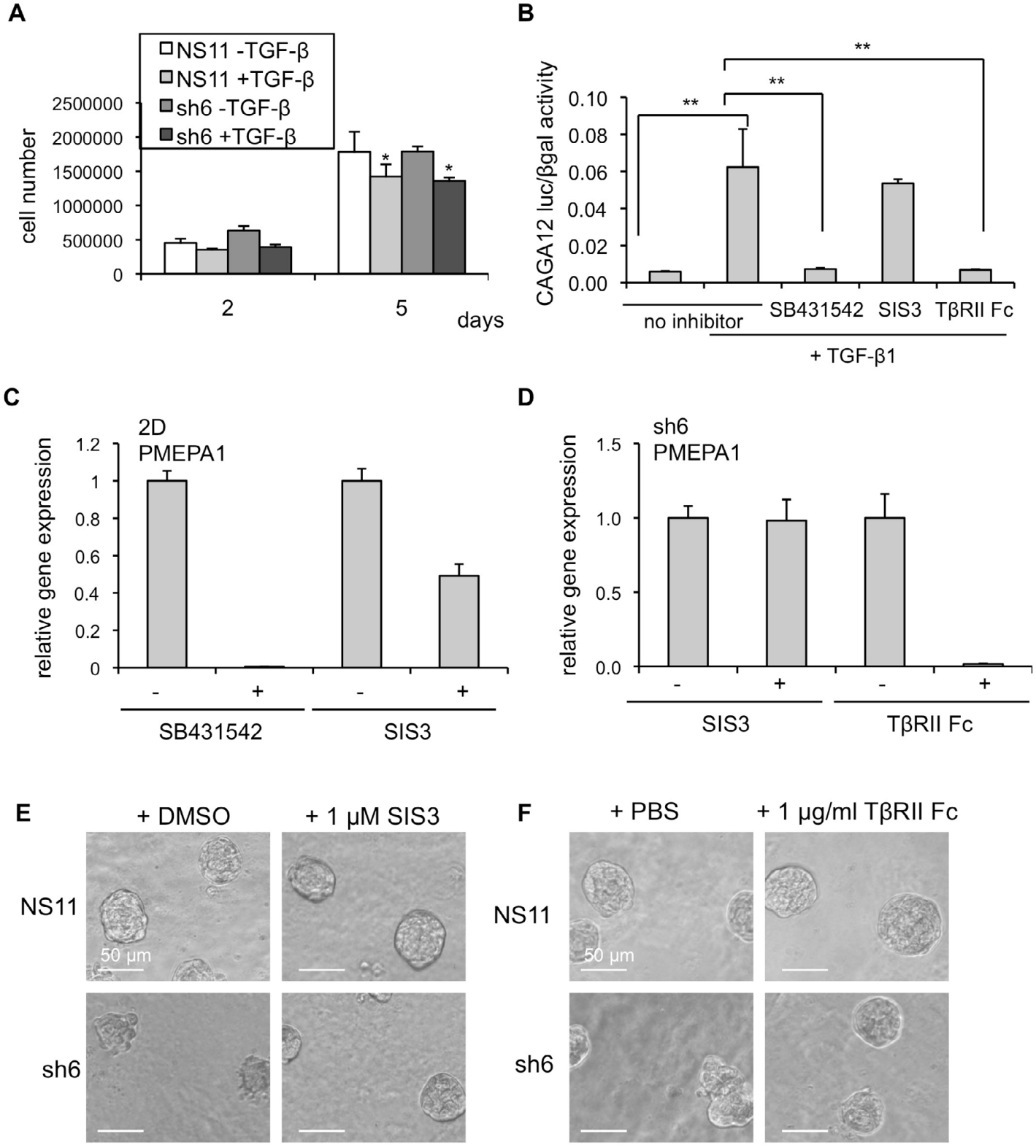

Fig. S5. Effects of TGF-β and TGF-β signalling inhibitors on RWPE-1 cells. (**A**) Cell proliferation assays for RWPE-1 sublines cultured as monolayers. 2.5×10^5 cells were plated in triplicate for each time point and cells were counted after 2 and 5 days. The average and s.d. of cell number are shown from a representative experiment (n=3). TGF-β treatment reduces cell number in sh6 and NS11 cells at 5 days; **P*<0.05 Student's t test, *P*=0.12 (NS11) and 0.12 (sh6) at 2 days. (**B**) Effects of TGF-β signalling inhibitors on CAGA-luciferase activity. RWPE-1 sh6 cells transfected with pGL3-CAGA12-luciferase and pDM-β-gal, untreated or treated with TGF-β1 (1 ng/ml) and either SB431542 (1 μM), SIS3 (1 μM), TGFβRII-Fc (1 μg/ml) or an equivalent volume of vehicle (DMSO or PBS, in the case of TGFβRII-Fc) for 21 h were assayed for gene reporter activity; ***P*<0.001 vs TGF-β1, Student's t test, n=3. (**C**) Effects of TGF-β signalling inhibitors on PMEPA1 expression. Graph shows relative expression of PMEPA1 in RWPE-1 NS11 cells growing as a monolayer (2D), as determined by q-PCR. (**D**) Effects of TGF-β signalling inhibitors on PMEPA1 expression. Graph shows relative expression of PMEPA1 in RWPE-1 sh6 cells growing in 3D, as determined by q-PCR. (**E**) Representative images of acini from the experiment shown in Fig. 6D. (**F**) Representative images of acini from the experiment shown in Fig. 6E.