Supplemental Figure 2 250 50 0.4 pmol 10 2 250 50 0.4 pmol 10 2 pT276 pep1 -pT276 pep1 pT276 pep2 -pT276 pep2 -Non-pT276 pep -Non-pT276 pep anti-pFIP5-T276 pre-immune B C anti-GFP anti-GFP anti-GFP anti-GFP Lysate IgG IgG IgG GSK-3 Inh. + FIP5-TA-GFP FIP5-GFP kDa kDa -FIP5-TA-GFP <sub>100</sub>--FIP5-GFP 100--FIP5-GFP 75-75--IgG HC -IgG HC -IgG HC 50-50-WB: anti-pFIP5-T276 WB: anti-pFIP5-T276 WB: anti-pFIP5-T276 FIP5-GFP -FIP5-TA-GFP -FIP5-GFP 100-100-75-75--IgG HC 50-IgG HC -IgG HC 50-WB: anti-GFP WB: anti-GFP WB: anti-GFP D (percent of untreated control) 120pFIP5-GFP-T276 levels 100-80-60p<0.01 40-20 GSK-3 Inh. anti-GFP anti-pFIP5-T276

## Fig S2. GSK-3 phosphorylates FIP5-T276.

- (A) Increasing concentrations of either non-phosphorylated FIP5-T276 or FIP5-pT276 peptides were spotted on nitrocellulose membrane and immunoblotted with either pre-immune serum (right) or affinity purified anti-pFIP5-T276 antibody (left).
- (B) MDCK cells expressing either FIP5-GFP or FIP5-T276A-GFP were lysed and FIP5-GFP was immunoprecipitated with anti-GFP antibody. The amounts of the total precipitated FIP5-GFP and pFIP5-T276 were evaluated by immunoblotting with anti-GFP (bottom panels) or anti-pFIP5-T276 (top panels) antibodies. IgG-HC marks anti-GFP immunoglobulin heavy chain.
- (C-D) GSK-3 inhibition significantly decreases FIP5-T276 phosphorylation (p<0.01). MDCK cells expressing FIP5-GFP were incubated for 1 h with DMSO or GSK-3 Inhibitor. Cells were harvested and FIP5-GFP was immunoprecipitated with anti-GFP antibody. The amounts of total FIP5-GFP (bottom) and phosphorylated FIP5-T276 (top) were analyzed using anti-GFP or anti-pFIP5-T276 antibodies. IgG-HC marks anti-GFP immunoglobulin heavy chain. Panel (D) shows quantification of GFP or pFIP5-276 signal. Data shown are means and standard deviations calculated from three independent experiments.