

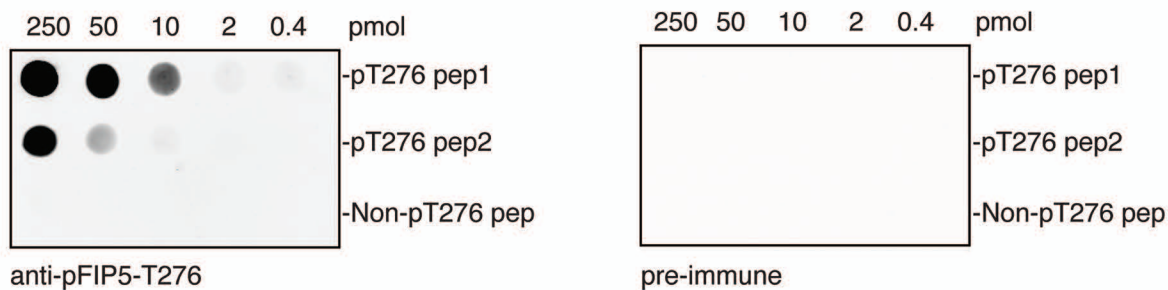
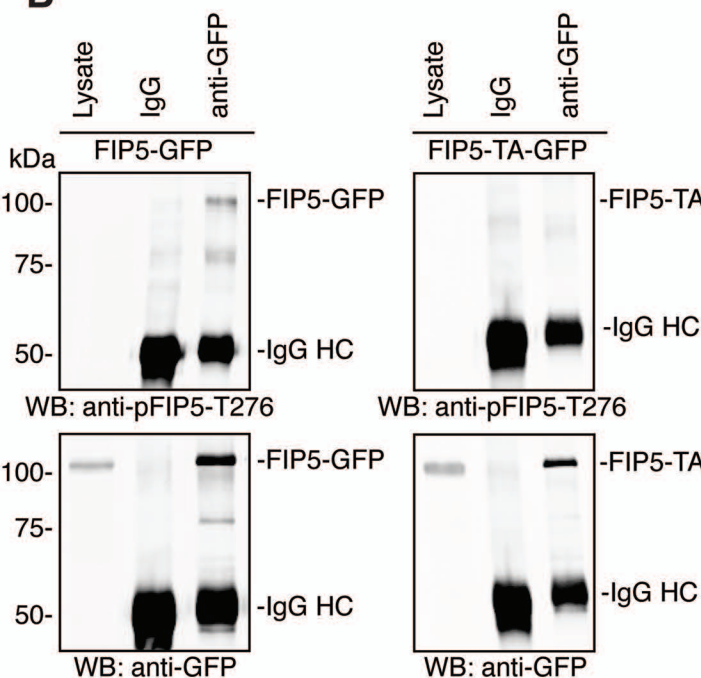
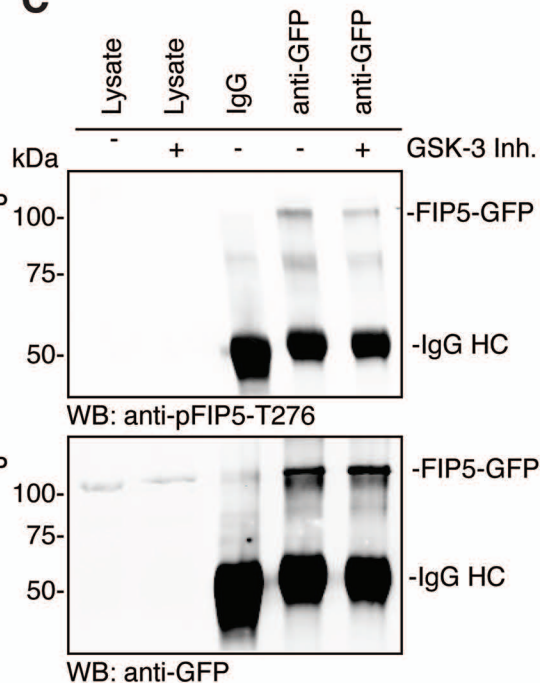
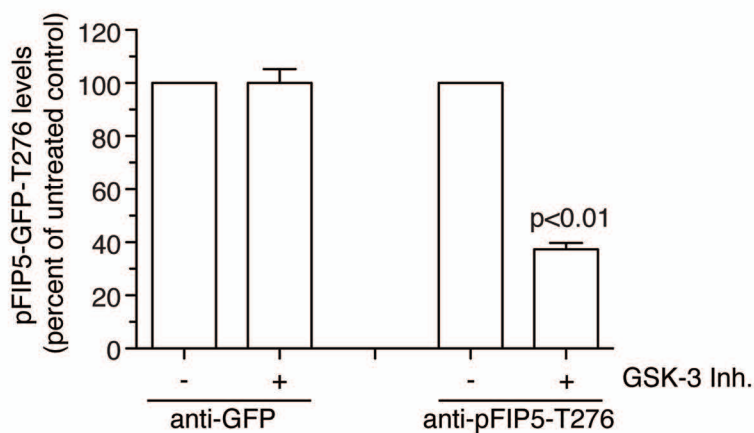
A**B****C****D**

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.