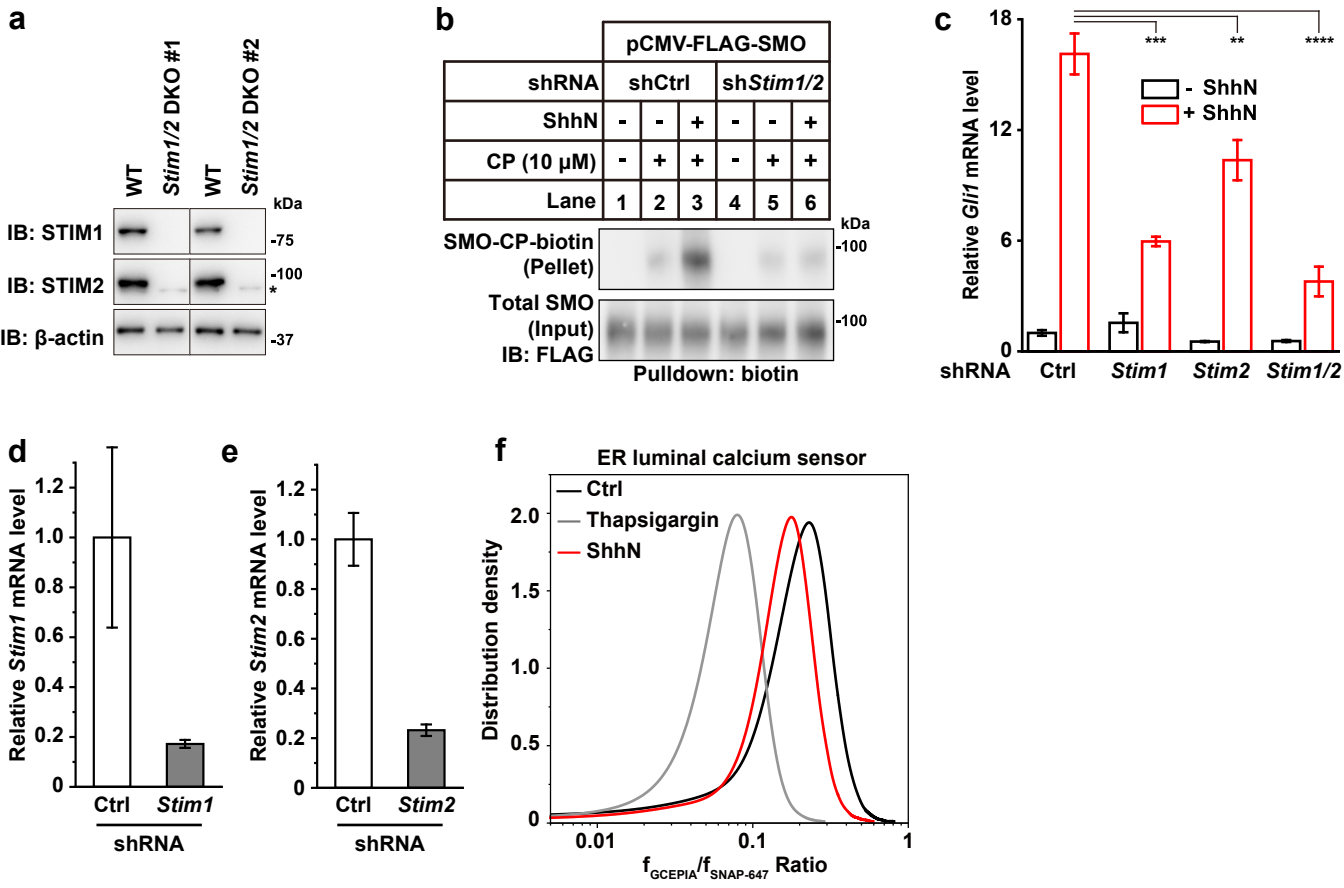


**Fig. S5**



**Fig. S5. Ablation of STIM proteins inhibits Hh signaling and SMO cholesterylation.**

- a.** Validation of two monoclonal *Stim1/2* double knockout cells confirmed by immunoblotting.
- b.** Short-hairpin RNA (shRNA)-mediated knockdown of *Stim1/2* decreased SMO cholesterylation.
- c.** Knockdown of *Stim1*, *Stim2* or double knockdown decreased *Gli1* expression upon ShhN treatment.  $p=1.020E-4$ ,  $0.003$  and  $9.886E-5$  (from left to right). Two sample T-test.
- d-e.** Knockdown efficiencies of *Stim1* and *Stim2* shRNAs validated by qPCR.
- f.** ShhN decreased  $Ca^{2+}$  concentration in the ER. NIH3T3 cells stably expressing the ER  $Ca^{2+}$  sensor GCEPIA-SNAP-ER were seeded in 60-mm dishes.  $10^4$  cells were analyzed in each condition. In **c**, **d** and **e**, data were presented as means  $\pm$  SD.