Fig. S1

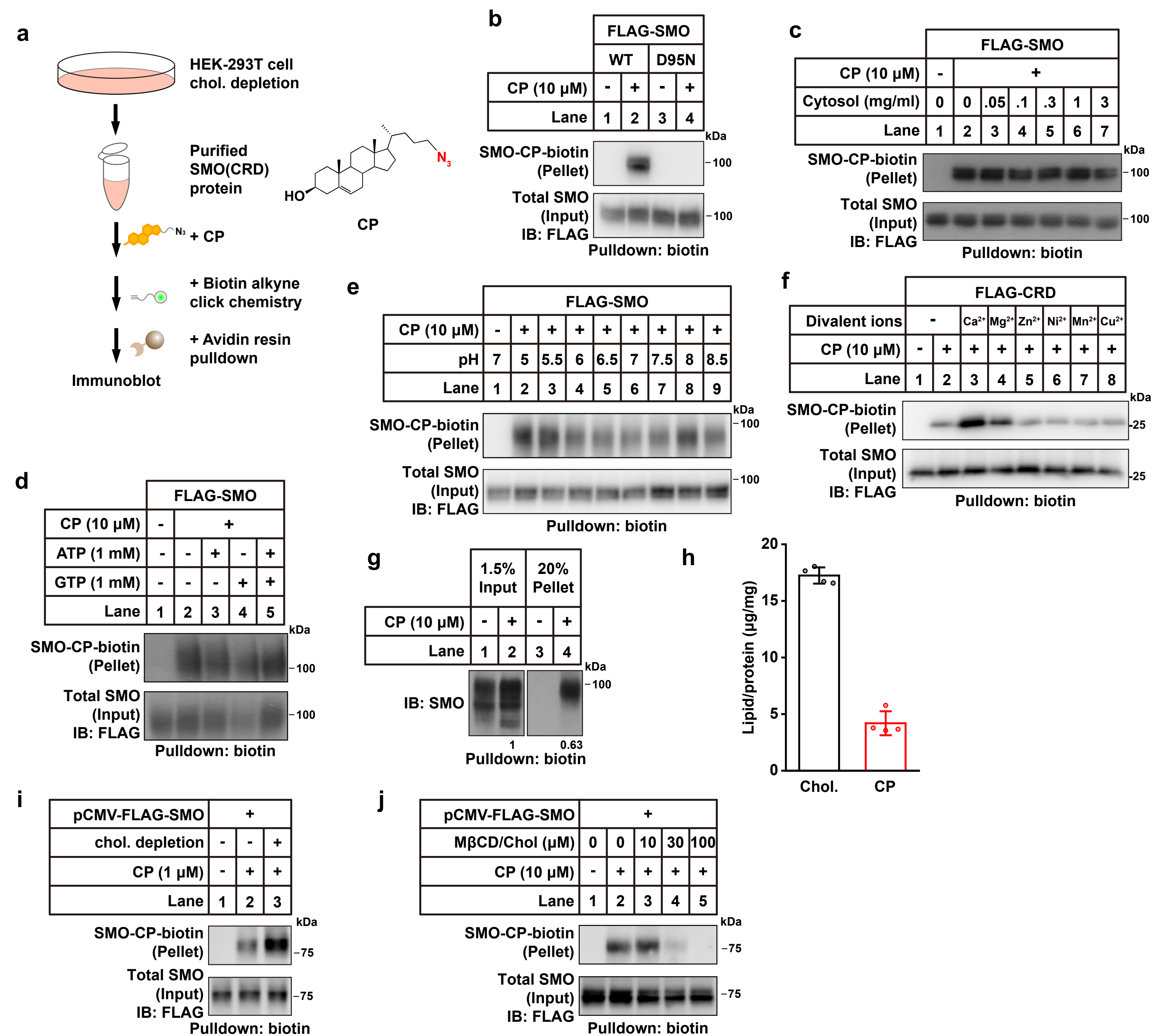

Fig. S1. Analysis of SMO cholesterylation *in vitro*.

a. Illustration of *in vitro* cholesterylation assay and the structure of CP probe.

b. SMO(D95N) mutant is not modified *in vitro*.

c-e. Effects of cytosol extract (**c**), ATP/GTP (**d**), or pH (**e**) on the *in vitro* cholesterylation of SMO.

f. The effect of divalent ions (2 mM each) on the cholesterol modification of SMO-CRD *in vitro*. Ca^{2+}, Mg^{2+}, Zn^{2+} and Mn^{2+} were suppled as chloride salts, while Ni^{2+} and Cu^{2+} were suppled as sulfate salts.

g. The percentage of CP-modified SMO in cultured cells. These four lanes were on the same blot. The relative band intensities were quantified and labeled under the blot.

h. Quantification of CP and endogenous cholesterol in HEK-293T cells by UPLC-MS/MS. Cells received 16 h of 10 μM CP treatment before lipid extraction.

i. Depletion of endogenous cholesterol increased SMO modification by CP probe. HEK-293T cells expressing FLAG-SMO were treated with 1.5% MβCD dissolved in DMEM for 30 min at 37 ºC and rinsed with PBS. Cells were then incubated in DMEM plus 5% lipoprotein-deficient serum, 1 μM lovastatin and 1 μM CP for 16 h and harvested for cholesterylation assay.

j. Exogenous cholesterol competed CP for SMO cholesterylation. HEK-293T cells expressing FLAG-SMO were incubated in complete medium plus 10 μM CP in the presence of MβCD-solubilized cholesterol as competitor for 16 h and harvested for cholesterylation assay.