

Fig S6

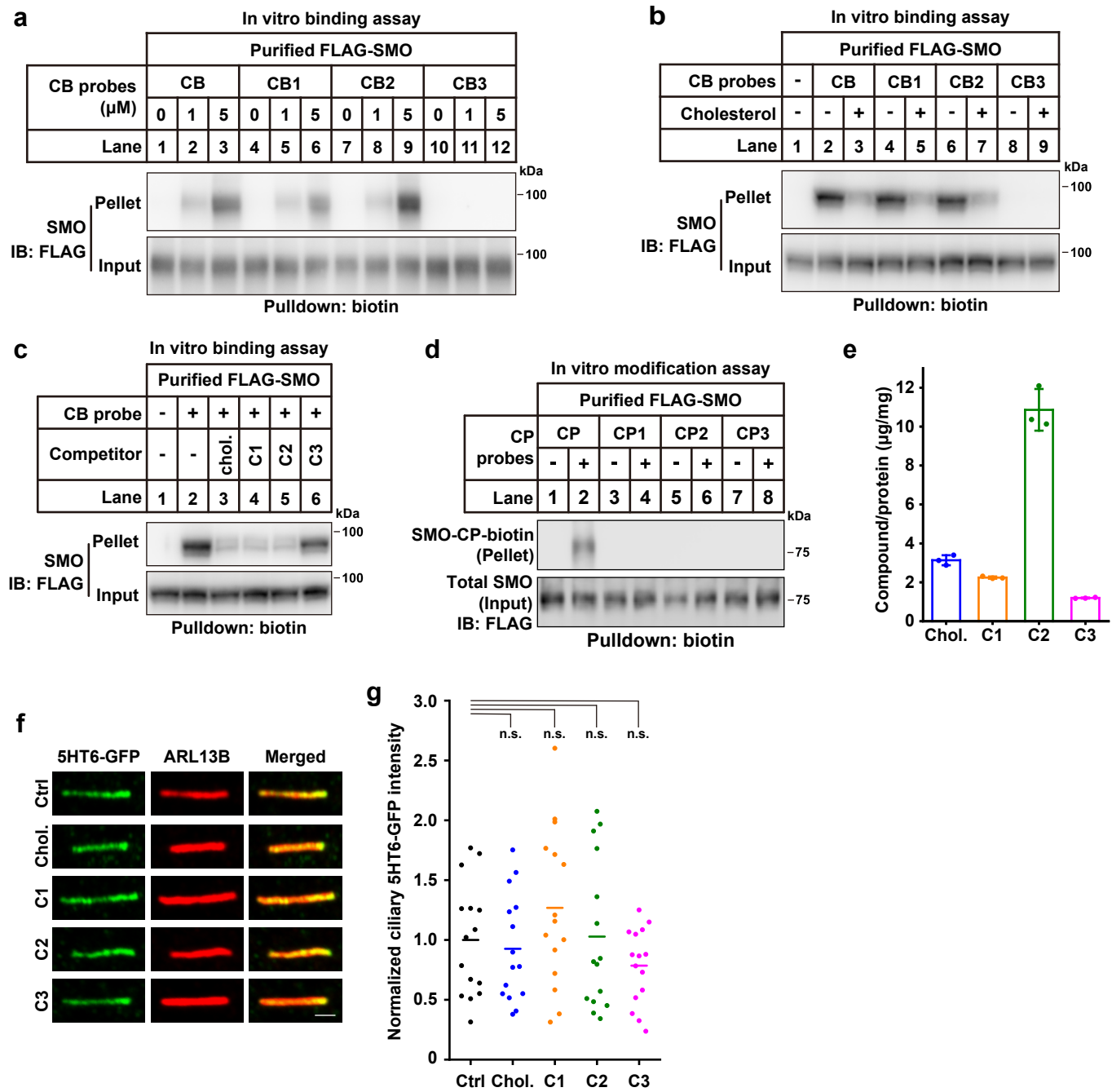


Fig. S6. *In vitro* binding of full-length SMO with CB probes.

- a.** *In vitro* binding of the purified full-length FLAG-tagged SMO protein with CB probes.
- b.** Cholesterol competed off the interactions of SMO with CB probes.
- c.** Free cholesterol, C1 and C2 competed off the interactions of SMO with CB.
- d.** *In vitro* modification of SMO by CP, but not by other azide derivatives of cholesterol analogs.
- e.** Cellular uptake of indicated compounds in complex with M β CD. NIH3T3 cells were treated with 50 μ M of indicated compounds for 4 h. Cellular lipids were extracted and subjected to UPLC-MS/MS analysis. To discriminate exogenous added cholesterol and endogenous cholesterol, hepta-deuterium-labeled cholesterol (cholesterol-d7) was used to treat cells.
- f.** Treatment with cholesterol, or C1-C3 did not affect ciliary trafficking of 5HT6-GFP. NIH3T3 cells expressing 5HT6-GFP were treated with 50 μ M of indicated compounds in complex with M β CD for 2 hours, fixed with paraformaldehyde and stained with ARL13B antibody. Control group was treated with M β CD only. Bar=1 μ m.
- g.** Quantification of ciliary 5HT6-GFP intensity in **f**. Horizontal lines denote mean values. n=15. p=0.9967, 0.7058, 0.9885 and 0.9965 (from left to right). Two-sample T-test.