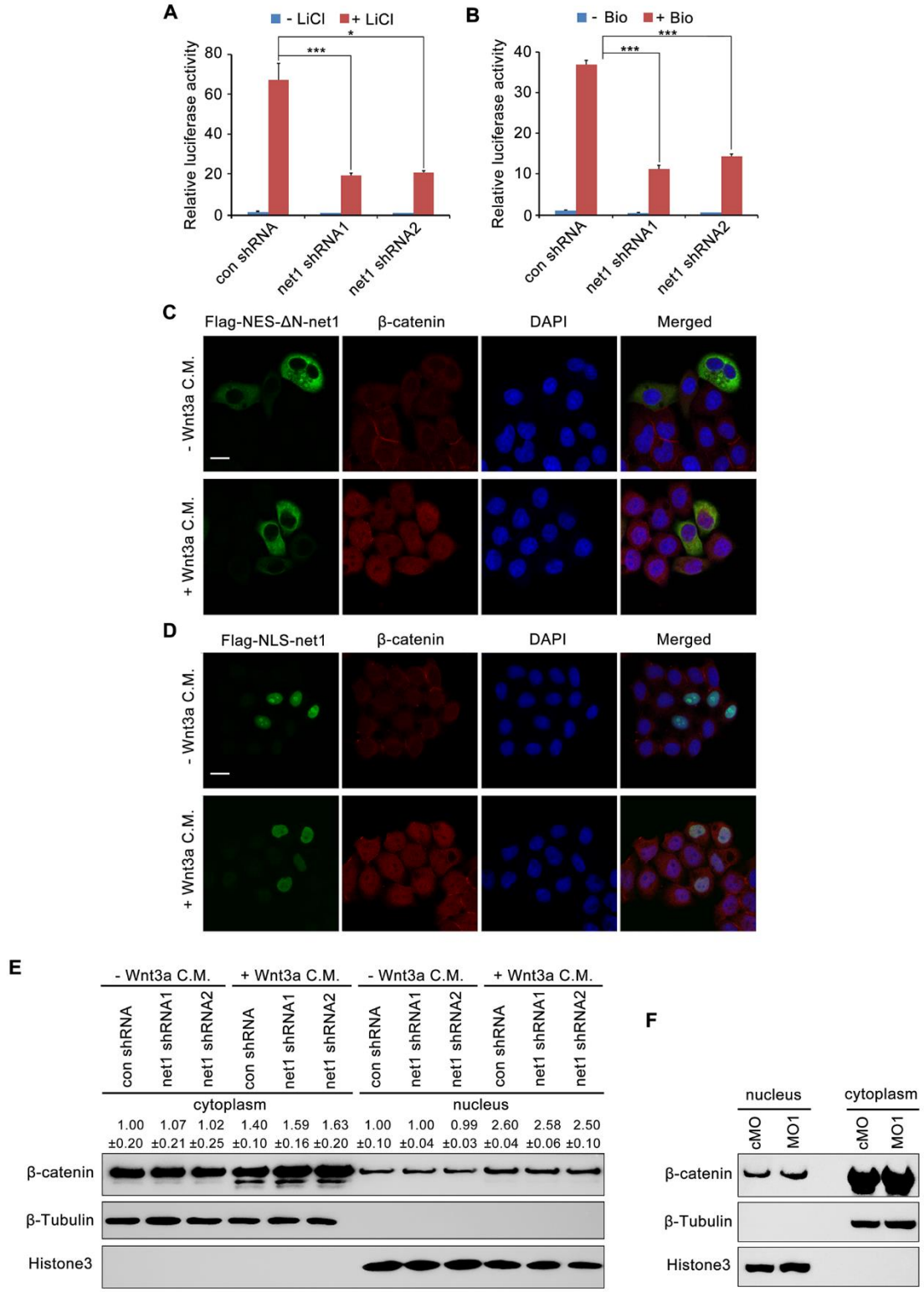

Figure S9 Net1 functions in parallel with or downstream of β-catenin to regulate canonical Wnt signaling. (A and B) net1 knockdown by transfection of shRNAs significantly decreases LiCl and BIO treatment-induced Wnt/β-catenin activity. HEK293T cells were cotransfected with

super-TOPFlash reporter and the indicated shRNA expression plasmids, then treated with 30 mM LiCl (A) and 10 μM BIO (B) for 12 hours before harvesting for luciferase assays. (C and D) Overexpression of net1 does not affect Wnt3a-induced β-catenin nuclear accumulation. Hela cells were transfected with Flag-NES-ΔN-Net1 (C) or Flag-NLS-Net1 (D) plasmids for 24 hours, and then treated with or without Wnt3a C.M. for 5 hours before being subjected to immunostaining with anti-Flag (Green) and anti-β-catenin (red) antibodies. DAPI was used as a counterstain for cell nuclei (blue). Scale bar, 10 μm. (E) Western blots of nuclear or cytosolic fractions from HEK293T cells transfected with the indicated plasmids in the presence or absence of Wnt3a C.M.. Note that the Wnt3a-induced β-catenin nuclear accumulation was not affected in net1-depleted cells. Quantification is the mean relative ratio of cytosolic or nuclear β-catenin signals over β-Tubulin or Histone3 (mean ±SD, three independent biological repeats). (F) Western blots of nuclear or cytosolic fractions of all cells from sphere-stage zebrafish embryos injected with the 4 ng cMO or 4 ng *net1* MO1.