



**Supplementary information, Figure S6** Knockout of *Twa1* suppresses  $\beta$ -catenin nuclear accumulation and Wnt activation. **(A)** Diagram of the sgRNA target site and the sequence of indels in the *Twa1* locus in HEK-293 cells generated by the Cas9/sgRNA system. **(B)** Agarose gel electrophoresis of the PCR products from the genomic DNA of wild-type (WT) and *Twa1* knockout HEK-293 cells (KO-1 and KO-2). **(C)** Western analysis of endogenous *Twa1* in wild-type and *Twa1* knockout HEK-293 cells. **(D-H)** Wild-type and *Twa1* knockout cells were transfected with the indicated constructs or not, treated with Wnt3a-CM or Ctr-CM, and then subjected to luciferase reporter activity **(D)**, Wnt target gene expression **(E)**, western blotting **(F)**, confocal microscopy **(G)** and co-IP analyses **(H)**. DNA was visualized with DAPI (blue). Scale bars, 10  $\mu$ m. Quantitative data are shown as the mean  $\pm$  SEM (at least three independent experiments). \*\* $P < 0.01$ , Student's *t* test.