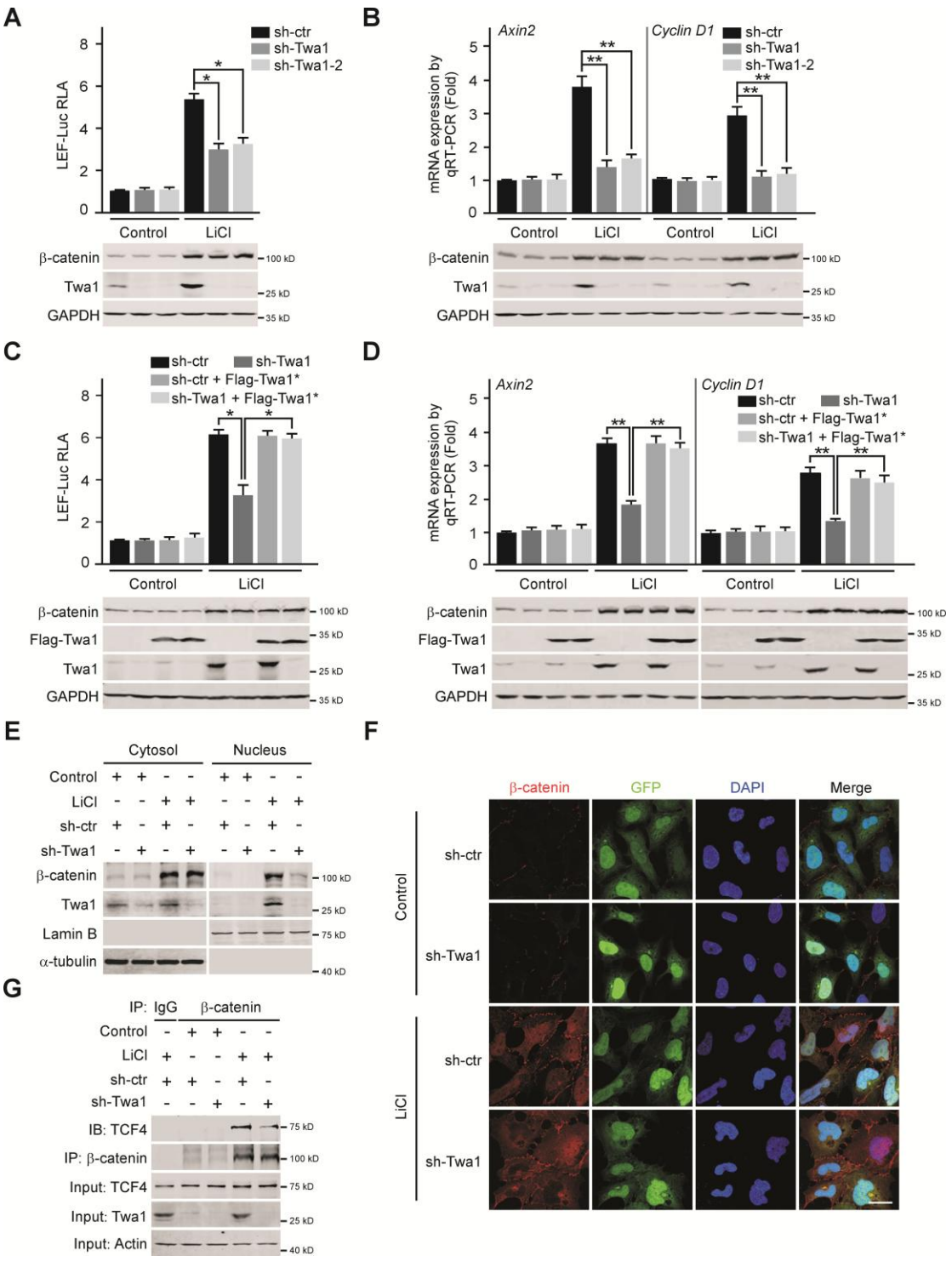
A
LEF-Luc RLA
sh-ctr
sh-Twa1
sh-Twa1-2
Control
LiCl
β-catenin
Twa1
GAPDH
100 kD
25 kD
35 kD
B
Axin2
Cyclin D1
mRNA expression by qRT-PCR (Fold)
C
sh-ctr + Flag-Twa1*
sh-Twa1 + Flag-Twa1*
Flag-Twa1
D
E
Cytosol
Nucleus
Lamin B
α-tubulin
75 kD
40 kD
F
β-catenin
GFP
DAPI
Merge
G
IP: IgG
IP: β-catenin
IB: TCF4
Input: TCF4
Input: Twa1
Input: Actin

Supplementary information, Figure S5 Twa1 promotes β-catenin nuclear accumulation and Wnt target gene expression in response to LiCl treatment. HEK-293 cells infected with lentiviruses containing sh-Twa1, sh-Twa1-2 or sh-ctr were transfected with an RNAi-resistant human Twa1 (Twa1*) construct or not. The cells were then treated with LiCl (40 mM) or not, and subjected to the following experiments. (**A**-**D**) Effect of Twa1 depletion on LiCl-induced luciferase reporter activity (**A**, **C**) and Wnt target gene expression by qRT-PCR (**B**, **D**). Quantitative data are shown as the mean ± SEM (at least three independent experiments). *P < 0.05 and **P < 0.01, Student's t test. (**E**) Western blotting of endogenous β-catenin and Twa1 from cytosolic and nuclear fractions of HEK-293 cells. Lamin B and α-tubulin were used as loading controls for nuclear and cytoplasmic fractions, respectively. (**F**) Confocal microscopy showing the nuclear localization of β-catenin. Green signals indicate the cells infected with lentiviruses. DNA was stained with DAPI (blue). Bars, 10 μm. (**G**) Western analysis of the indicated proteins co-immunoprecipitated by anti-β-catenin antibody in HEK-293 cells. IB, immunoblotting; IP, immunoprecipitation.