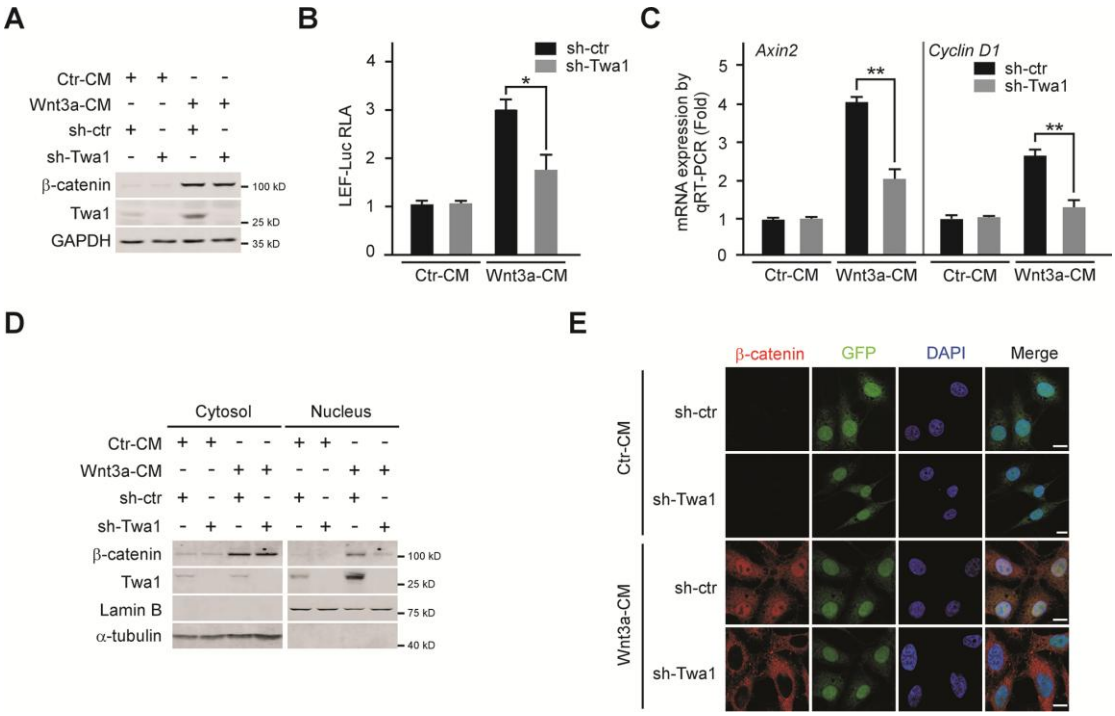
A
Ctr-CM + + - -
Wnt3a-CM - - + +
sh-ctr + - + -
sh-Twa1 - + - +
β-catenin
Twa1
GAPDH
100 kD
25 kD
35 kD
B
LEF-Luc RLA
sh-ctr
sh-Twa1
*
Ctr-CM
Wnt3a-CM
C
Axin2
Cyclin D1
mRNA expression by qRT-PCR (Fold)
sh-ctr
sh-Twa1
**
**
Ctr-CM
Wnt3a-CM
Ctr-CM
Wnt3a-CM
D
Cytosol
Nucleus
Ctr-CM + + - - + + - -
Wnt3a-CM - - + + - - + +
sh-ctr + - + - + - + -
sh-Twa1 - + - + - + - +
β-catenin
Twa1
Lamin B
α-tubulin
100 kD
25 kD
75 kD
40 kD
E
β-catenin
GFP
DAPI
Merge
Ctr-CM
sh-ctr
sh-Twa1
Wnt3a-CM
sh-ctr
sh-Twa1

Supplementary information, Figure S4 Twa1 facilitates β-catenin nuclear accumulation and Wnt target gene expression in RKO cells. RKO cells infected with lentiviruses containing sh-Twa1 or sh-ctr were treated with Wnt3a-CM or Ctr-CM, and then subjected to the following experiments. (**A**) Western analysis of endogenous β-catenin and Twa1 from RKO cell lysates. GAPDH was used as a loading control. (**B**, **C**) Effects of Twa1 depletion on Wnt-induced luciferase reporter activity **(B)** and Wnt target gene expression by qRT-PCR **(C)**. Quantitative data are shown as the mean ± SEM (at least three independent experiments). **P* < 0.05 and ***P* < 0.01, Student's *t* test. (**D**) 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. (**E**) 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.