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Supplemental Information

lncRNA SNHG10 Promotes the Proliferation and Invasion of Osteosarcoma via Wnt/β-Catenin Signaling

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Supplementary figures

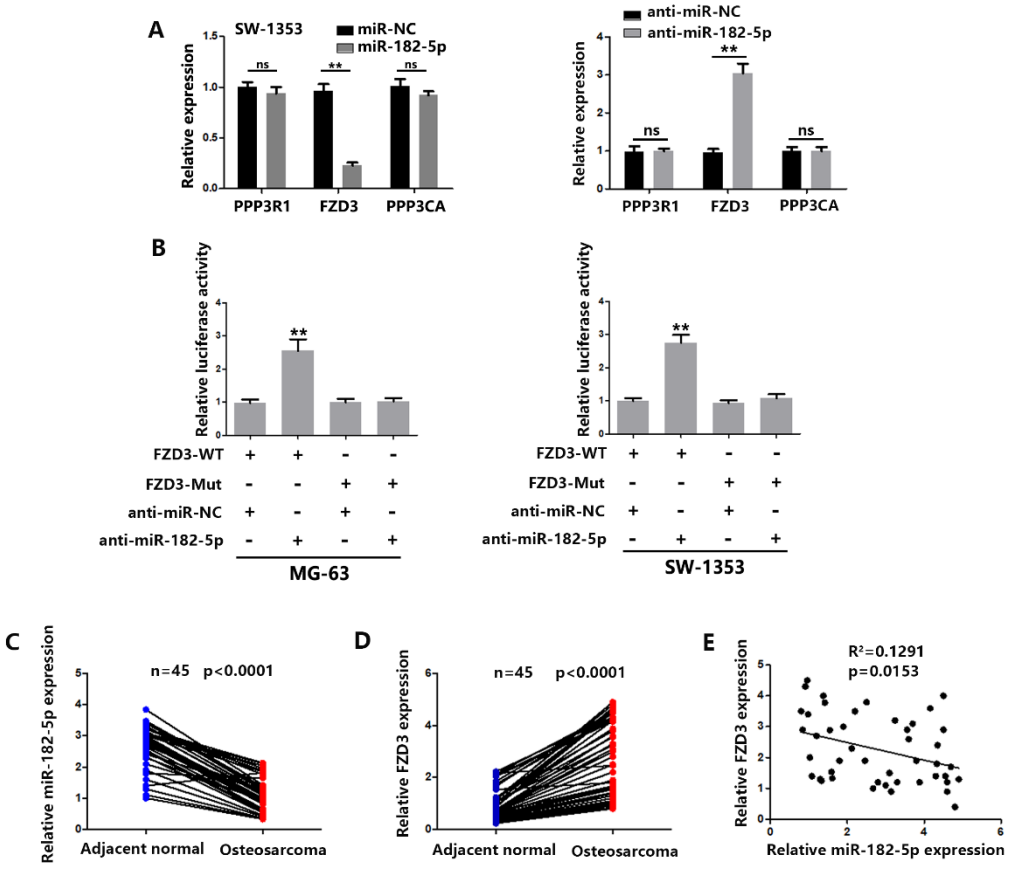

Figure S1 FZD3 is a target of miR-182-5p, Related to Figure 5

(A) mRNA expression of PPP3R1, FZD3 and PPP3CA in OS cells transfected with

miR-182-5p mimic or anti-miR-182-5p.

(B) Relative FZD3 reporter activities in OS cells co-transfected with anti-miR-182-5p and luciferase reporters.

(C) Relative expression of miR-182-5p in OS and adjacent normal samples were quantified by qRT-PCR.

(D) Relative expression of FZD3 in OS and adjacent normal samples were quantified by qRT-PCR.

(E) Spearman correlation analysis of the FZD3 and miR-182-5p expression levels in OS samples.

In all experiments, bars represent mean ± SD from three independent experiments. (*P< .05, **P< .01.)

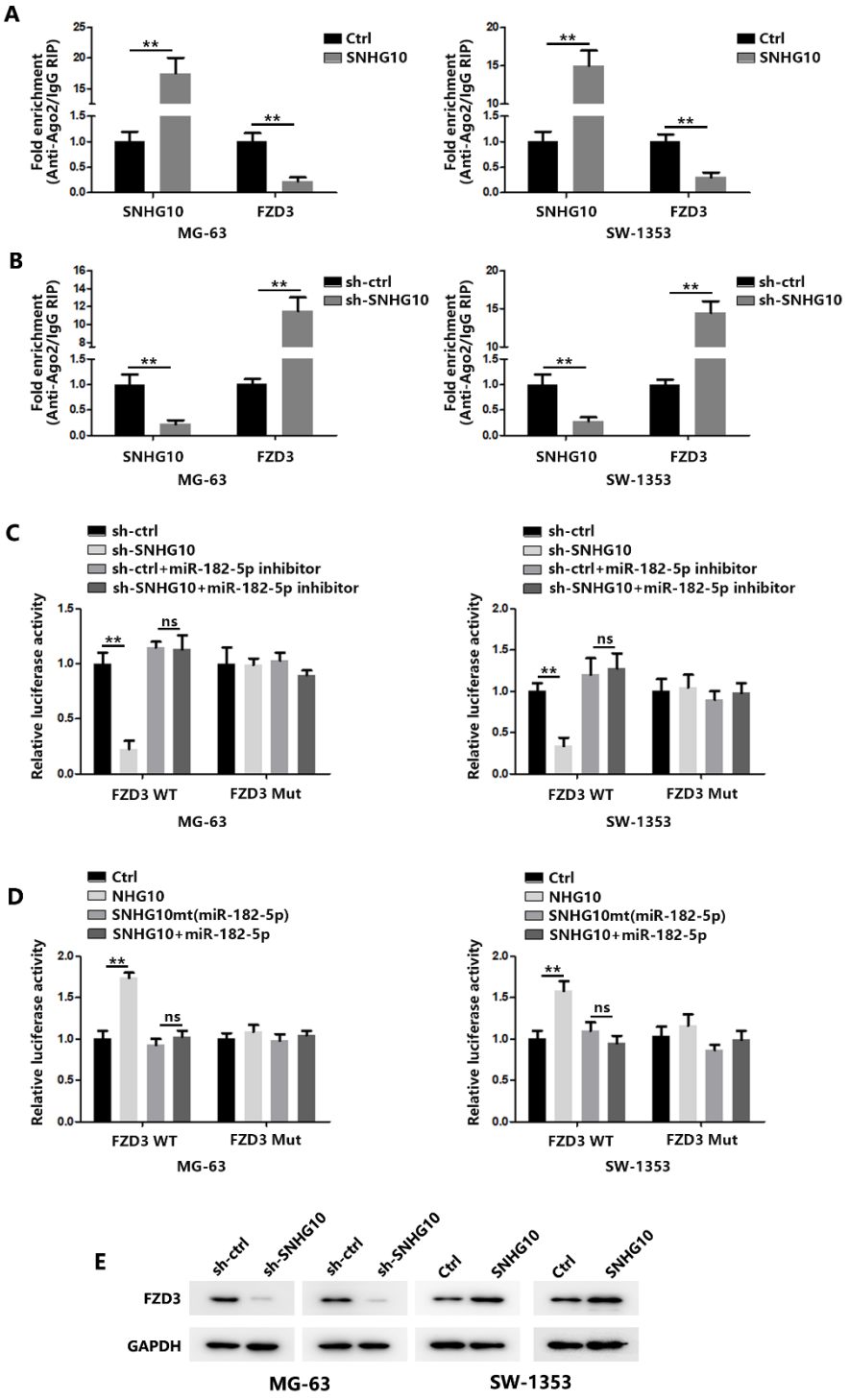

Figure S2 SNHG10 promotes OS progression through FZD3, Related to Figure 6

(A) RIP assay of the enrichment of Ago2 on SNHG10 and FZD3 transcripts relative to IgG in OS cells transfected with SNHG10 or control.

(B) RIP assay of the enrichment of Ago2 on SNHG10 and FZD3 transcripts relative to IgG in OS cells transfected with sh-SNHG10 or sh-control.

(C) Luciferase activity of reporters which contained wild-type or mt FZD3 3'UTR with indicated treatment in OS cells.

In all experiments, bars represent mean ± SD from three independent experiments. (*P< .05, **P< .01.)

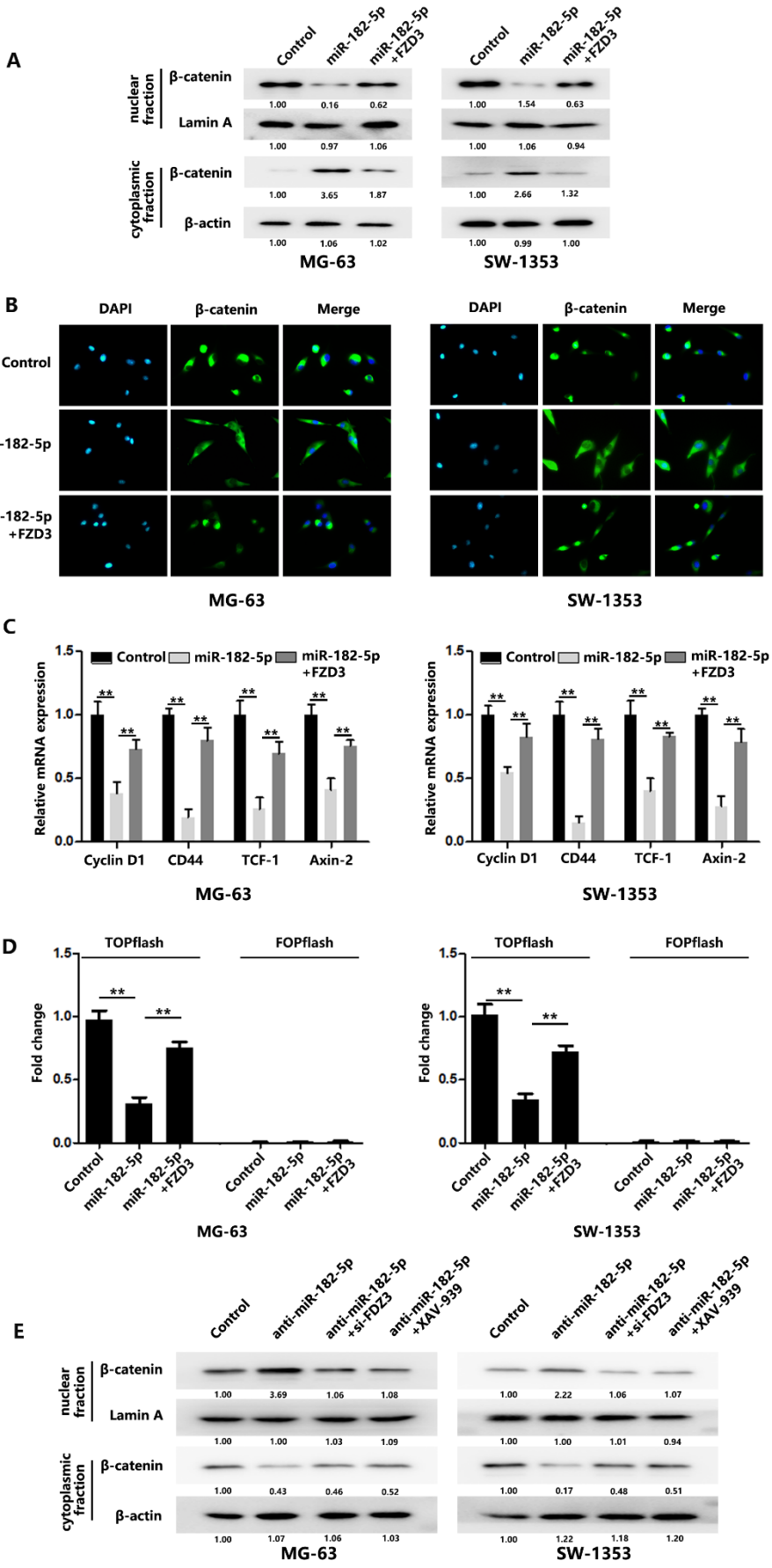
A
Control
miR-182-5p
miR-182-5p +FZD3
nuclear fraction
β-catenin
Lamin A
cytoplasmic fraction
β-catenin
β-actin
1.00 0.16 0.62
1.00 0.97 1.06
1.00 3.65 1.87
1.00 1.06 1.02
1.00 1.54 0.63
1.00 1.06 0.94
1.00 2.66 1.32
1.00 0.99 1.00
MG-63
SW-1353
B
DAPI
β-catenin
Merge
Control
miR-182-5p
miR-182-5p +FZD3
MG-63
SW-1353
C
Control
miR-182-5p
miR-182-5p +FZD3
Relative mRNA expression
1.5
1.0
0.5
0.0
**
Cyclin D1
CD44
TCF-1
Axin-2
MG-63
SW-1353
D
TOPflash
FOPflash
Fold change
1.5
1.0
0.5
0.0
**
Control
miR-182-5p
miR-182-5p +FZD3
MG-63
SW-1353
E
Control
anti-miR-182-5p
anti-miR-182-5p +si-FDZ3
anti-miR-182-5p +XAV-939
nuclear fraction
β-catenin
Lamin A
cytoplasmic fraction
β-catenin
β-actin
1.00 3.69 1.06 1.08
1.00 1.00 1.03 1.09
1.00 0.43 0.46 0.52
1.00 1.07 1.06 1.03
1.00 2.22 1.06 1.07
1.00 1.00 1.01 0.94
1.00 0.17 0.48 0.51
1.00 1.22 1.18 1.20
MG-63
SW-1353

Figure S3 SNHG10 activates Wnt/β-catenin pathway to promote OS progression, Related to Figure 7

(A) β-catenin protein redistribution in different cellular compartments of transfected OS cells was quantified using western blot.

(B) β-catenin protein redistribution in different cellular compartments of transfected OS cells was quantified using immunofluorescence staining. Scale bar, 100 μm.

(C) Relative expression levels of Wnt/β-catenin pathway downstream target genes were quantified using RT-PCR.

(D) Relative TCF/LEF reporter activity was measured after transfection with miR-182-5p or co-transfection with FZD3.

(E) β-catenin protein redistribution in different cellular compartments of transfected or XAV-939 treated OS cells was quantified using western blot.

In all experiments, bars represent mean ± SD from three independent experiments. (*P< .05, **P< .01.)

Supplementary Material and Methods

Primers used in this study

	Forward	Reverse
SNHG10	CCAGCTTAGATTCATTGATTCC	TTAAGTGCACCAGATGCTG
FZD3	GGATTGTTCTCGGGATTTCC	AGTGTGACACGTCCATATTCC
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	GAACGGGAAGCTCACTGG	GCCTGCTTCACCACCTTCT

Antibodies used in this study

Name	Company	Catalog
Cyclin D1	Cell Signaling Technology	55506
Cyclin E1	Cell Signaling Technology	20808
N-cadherin	Cell Signaling Technology	13116
E-cadherin	Cell Signaling Technology	14472
Vimentin	Cell Signaling Technology	5741
FZD3	Abcam	ab217032
GAPDH	Cell Signaling Technology	5174
β-actin	Cell Signaling Technology	3700