

miR-193b regulates tumorigenesis in liposarcoma cells via PDGFR, TGF β , and Wnt signaling

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Supplementary data

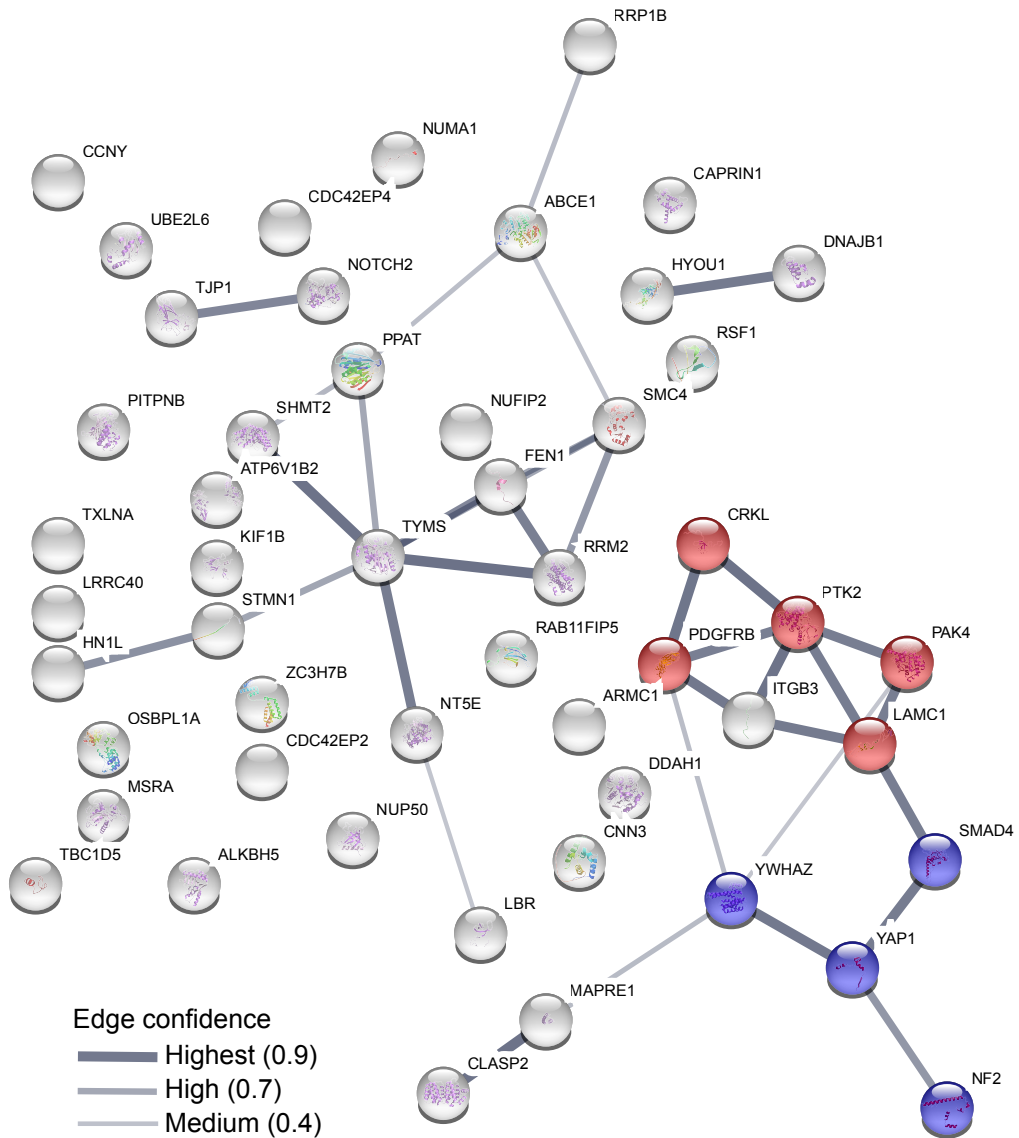


Figure S1. Protein-protein interaction network of putative miR-193b targets. STRING analysis was to construct interaction network among the 50 putative miR-193b targets identified previously¹². STRING interactions with a confidence score of 0.4 or higher are shown and

highlighted in bold. Significantly interacting pathways are listed in the table; colors indicate related protein nodes.

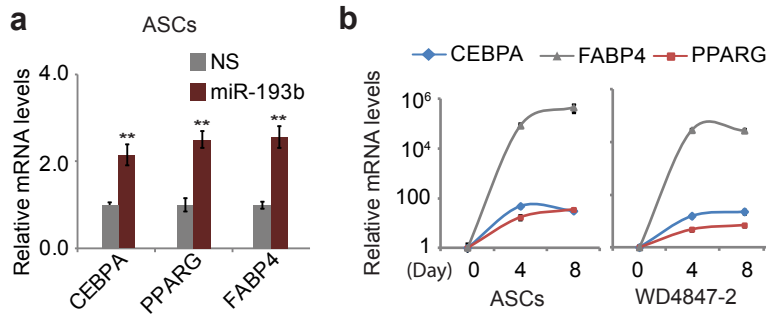


Figure S2. Adipogenic differentiation of ASCs and WD4847-2 cells. **a**, Effects of miR-193b on adipogenic differentiation marker mRNA levels during ASC differentiation. **b**, mRNA levels of differentiation markers during adipogenic differentiation in ASCs and WD4847-2 cells, as detected by qRT-PCR.

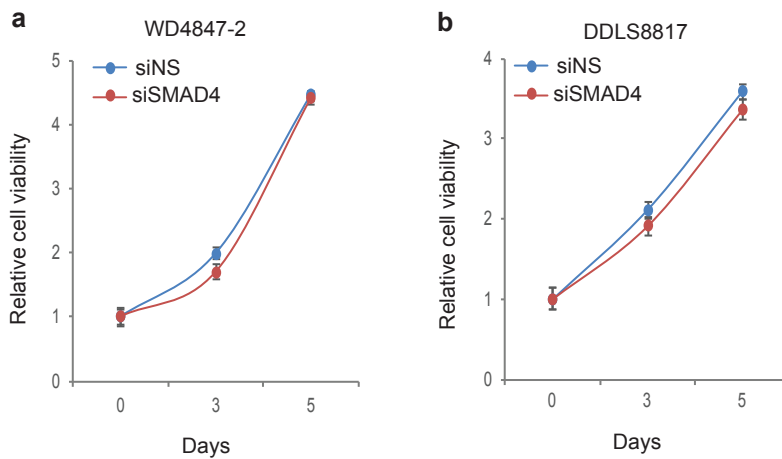


Figure S3. Knockdown of SMAD4 does not affect WDLs and DDLS cell proliferation. Cell proliferation was assessed at day 3 and day 5, after siRNA transfection in WD4847-2 (**a**) and DD8817 (**b**) cells. Values represent the mean \pm SE of 3 independent experiments.

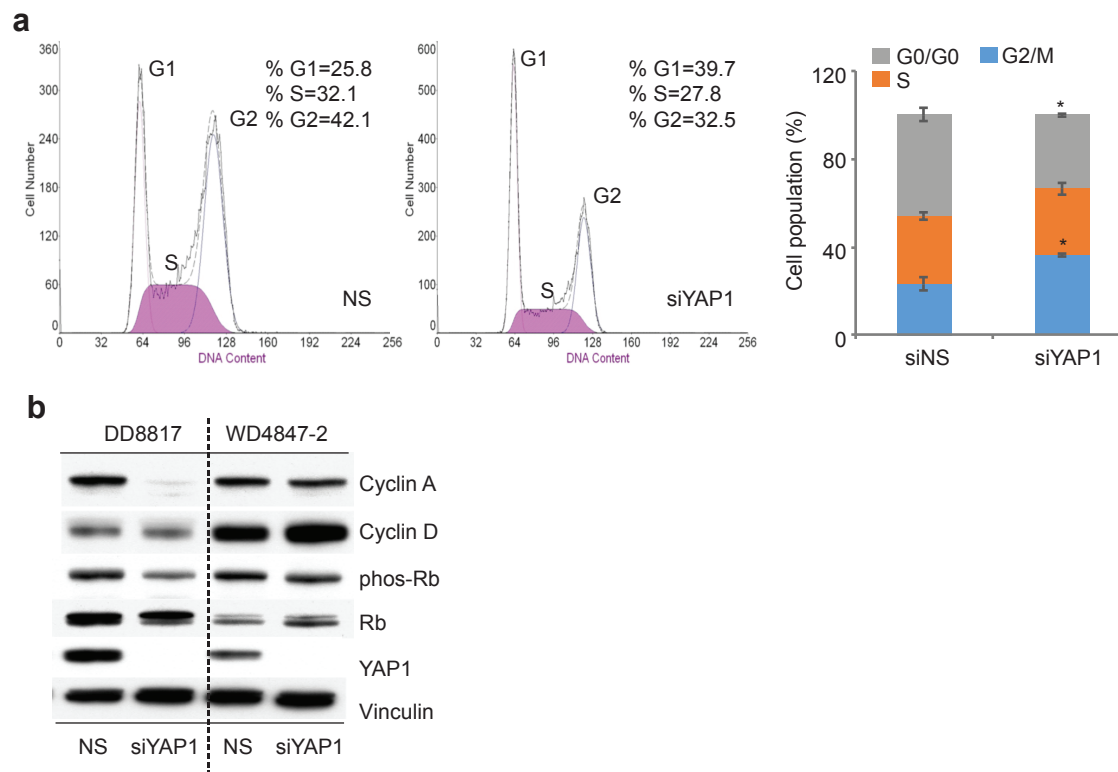


Figure S4. Knockdown of YAP1 by siRNA induces modest cell cycle arrest at G0/G1 in DDLS cells. **a**, Cell cycle FACS analysis was performed after 48h transfection with siRNAs. NS, nonspecific siRNA. Values represent the mean \pm SE of 3 independent experiments. *, $p < 0.05$. **b**, Protein expression of cell cycle markers detected by Western blotting in siRNA-treated DDLS cells (representative results from 2 independent experiments).

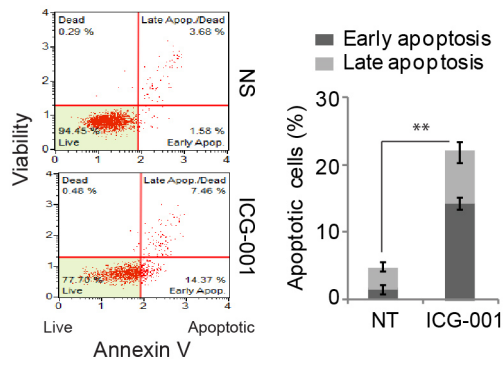


Figure S5. IC-001 induces apoptosis in DDLS cells. Apoptosis was detected in IC-001 (2.5 μ M)-treated DD8817 cells by annexin V assay on day 2. Representative apoptosis profiling panels are shown. Values represent the mean \pm SE of 3 independent experiments. **, $p < 0.01$ compared with vehicle or nonspecific miRNA or siRNA control.

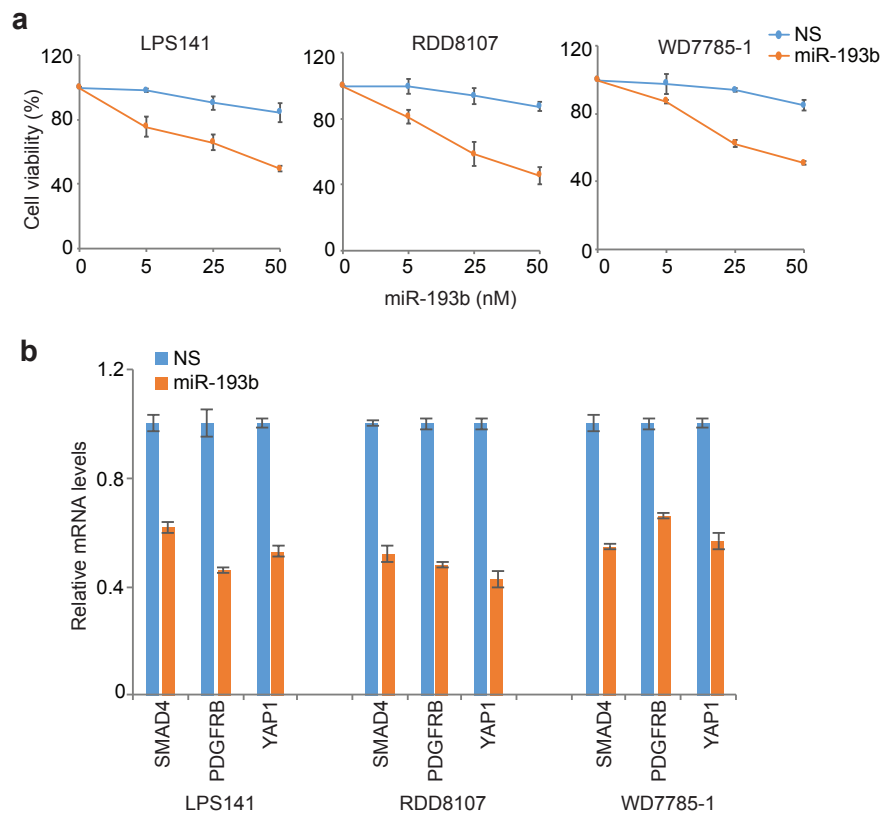
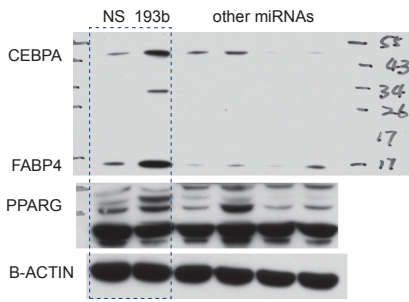


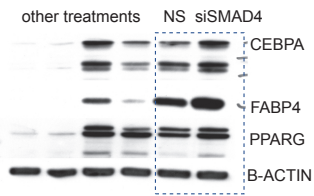
Figure S6. miR-193b slows growth of cells that express intermediate levels of miR-193b.

The selected cell lines were previously shown to express miR-193b at levels between those of ASCs and the cell lines used in preceding experiments¹². **a**, Proliferation of liposarcoma cells on day 5 after transfection with varying doses of miR-193b. **b**, Expression of miR-193b targets was evaluated by qRT-PCR after 3 days of transfection with miR-193b. Values represent the mean \pm SE of 3 independent experiments.

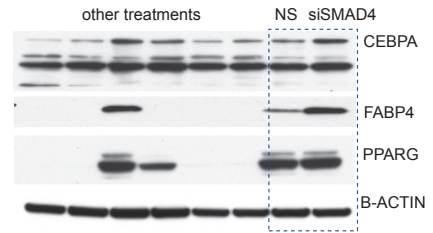
a Full blots for Fig. 4F



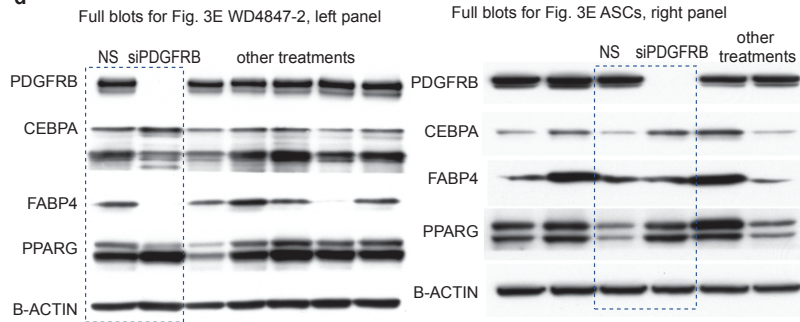
b Full blots for Fig. 4G



c Full blots for Fig. 4I



d



f Full blots for Fig. 5C

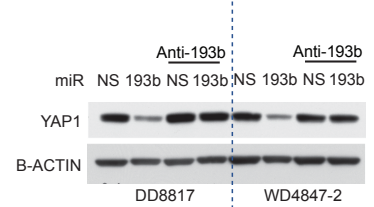


Figure S7. Full western blots for all figures.

Table S1. Oligonucleotide sequences for 3'UTR reporters, RT-PCR primers, and miRNAs.

Cloning primers for 3'UTR reporters

Reporter	Sequence
YAP1-WT	AAACTAGCGGCCGCAGCTACTTGGAAGGAGTGCCTATAATTTGCCAGTAGCCACAGAGCTACTTGGAAGGAGTGCCTATAATTTGCCAGTAGCCACA GT
YAP1-WT as	CTAGACTGTGGCTACTGGCAAATTATAGGCACTCCTTCCAAGTAGCTCTGTGGCTACTGGCAAATTATAGGCACTCCTTCCAAGTAGCTGCGGCCGC TAGTTT
YAP1-Mut	AAACTAGCGGCCGCAGCTACTTGGAAGGAGTGCCTATAATTTGCATATAGCCACAGAGCTACTTGGAAGGAGTGCCTATAATTTGCATATAGCCACA GT
YAP1-Mut as	CTAGACTGTGGCTATATGCAAATTATAGGCACTCCTTCCAAGTAGCTCTGTGGCTATATGCAAATTATAGGCACTCCTTCCAAGTAGCTGCGGCCGCT AGTTT
SMAD4-WT	AAACTAGCGGCCGCCCACTCGTAGCTTCTGCTTTGGGGACAACCTGGTCAGTTGAAACCACTCGTAGCTTCTGCTTTGGGGACAACCTGGTCAGTTGAA AT
SMAD4-WT as	CTAGATTTCAACTGACCAGTTGTCCCAAAGCAGAAGCTACGAGTGGTTTCAACTGACCAGTTGTCCCAAAGCAGAAGCTACGAGTGGGCGGCCG CTAGTTT
SMAD4-Mut	AAACTAGCGGCCGCCCACTCGTAGCTTCTGCTTTTCTAACAACCTCATGCATTGAAACCACTCGTAGCTTCTGCTTTTCTAACAACCTCATGCATTGAAAT
SMAD4-Mut as	CTAGATTTCAATGCATGAGTTGTTAGGAAAGCAGAAGCTACGAGTGGTTTCAATGCATGAGTTGTTAGGAAAGCAGAAGCTACGAGTGGGCGGCCGC TAGTTT
PDGFRB-WT	AAACTAGCGGCCGCATGTGCCAGTGTGGAGTGGCCACGTGTGTGTGCCAGTATATGATGTGCCAGTGTGGAGTGGCCACGTGTGTGTGCCAGTATA TGT
PDGFRB-WT-as	CTAGCTAGACATATACTGGCACACACACGTGGCCACTCCACACTGGCACATCATATACTGGCACACACACGTGGCCACTCCACACTGGCACATGCG GCCGCTAGTTT
PDGFRB-WT	AAACTAGCGGCCGCATGTGCCAGTGTGGAGTGGCCACGTGTGTGTGCATGGCATATGATGTGCCAGTGTGGAGTGGCCACGTGTGTGTGCATGGCATA TGT
PDGFRB-WT-as	CTAGACATATGCCATGACACACACGTGGCCACTCCACACTGGCACATCATATGCCATGACACACACGTGGCCACTCCACACTGGCACATGCGGCCG CTAGTTT

Taqman primers

Primer	Assay ID (from Thermo Fisher Scientific)
SMAD4	Hs00929647_m1
PDGFRB	Hs01019589_m1
YAP1	Hs00902712_g1
BIRC5	Hs04194392_s1
PTTG	Hs00747713_sH
CEBPA	Hs00269972_s1
PPARG	Hs00234592_m1
FABP4	Hs00609791_m1
GAPDH	Hs99999905_m1

miRNA primers:

Primer	Sequence
miR-193b-3p	AACTGGCCCTCAAAGTCCCGCT