Supplementary information, Materials and Methods

Reagents and antibodies.

Recombinant TGF-β1, Wnt3a, and BMP4 were obtained from R&D Systems, recombinant LIF from Chemicon, and N2 and B27 from Invitrogen. Antibodies were purchased from Santa Cruz (α-GST tag, α-β-catenin and α-Tubulin), Millipore (α-active β-catenin) and GE Healthcare (ECL HRP linked anti-mouse lgG and ECL HRP linked anti-rabbit lgG). Wnt3a CM was generated in our lab [1]and α-Axin2 antibody was generated by SC Lin [2].

Peptide synthesis.

All peptides were synthesized manually using standard 9-fluorenylmethoxycarbonyl (Fmoc)-peptide chemistry on Rink Amind AM resign, and cross-linked using Grubbs-I catalyct. Peptides were synthesized as previously described [3].

Determination of α-helicity.

Peptides were dissolved in PBS buffer to concentration 50 μM. We obtained the CD spectra on a Jasco J-715 spectropolarimeter at 25℃. We collected the spectra using 0.2 cm pathlength quartz with the following parameters: wavelength, 190-255 nm; step resolution, 0.5 nm; speed, 20 nm/min; accumulations, 3. We calculated the helical content of each peptide as reported previously [4].

In vitro pulldown assay.

GST-β-catenin (133-665) fusion protein was expressed in E. coli and purified with glutathione-sepharose (Amersham Pharmacia Biotech). The streptavidin beads

(Thermo) binding biotin-SAHPAs were washed extensively with binding buffer (50mM Tris-HCl pH 8.0, 250mM NaCl) and were incubated with purified GST-β-catenin (133-665), respectively, for 6 hour at 4°C. Bound proteins were extracted with loading buffer and analyzed by immunoblotting..

Isothermal titration calorimetry.

β-catenin (133-665) was obtained after on-beads-cleavage of GST-β-catenin (133-665) fusion protein with Thrombin (Sigma). ITC was performed as previously described [5].

Cell cultures and transfection.

Hela, HEK293T and HEK293 were maintained in DMEM medium supplemented with 10% FBS (Hyclone) in a 37°C humidified incubator containing 5% CO2. R1 cells were maintained as described previously [6]. For serum- and feeder-free culture, cells were grown on gelatinized tissue culture plates in N2B27 medium [7] with indicated (in the figure legends) concentrations of Wnt3a (R&D Systems), SAHPA1, LIF (Chemicon), and/or BMP4 (R&D Systems). Transfection was performed with VigoFect (Vigorous) following the manufacturer's recommendations.

Reporter assay, immunoblotting, immunoprecipitation and alkaline phosphatase (AP) staining.

These assays were performed as previously described [8].

Quantitative RT-PCR (qRT-PCR).

Total RNA was extracted with Trizol reagent (Invitrogen) and cDNA was synthesized

with Revertra Ace (Toyobo). A Mx3000p Quantitative PCR system (Stratagene) was employed to perform qRT-PCR using EvaGreen dye (Biotium). The primers were: human c-myc (5'-TCTCCTTGCAGCTGCTTAG-3' and 5'-GTCGTAGTCGAGGTCATAG-3'), human axin2 (5'-AGTGTGAGGTCCACGGAAAC-3' and 5'-CTTCACACTGCGATGCATTT-3'), human cyclin D-1 (5'-CTGGCCATGAACTACCTGGA-3' and 5'-CTCCGCCTCTGGCATTTTGG-3'), human GAPDH (5'-GAAGGTGAAGGTCGGAGTC-3' and 5'-GAAGATGGTGATGGGATTTC-3'), mouse Oct4 (5'-CACGAGTGGAAAGCAACTCA-3' and 5'-AGATGGTGGTCTGGCTGAAC-3'), mouse Nanog (5'-ACCTGAGCTATAAGCAGGTTAAGAC-3' and 5'-GTGCTGAGCCCTTCTGAATCAGAC-3'), mouse Rex1 (5'-GATTCACATCCTAACCCACGCA-3' and 5'-TATCCCCAGTGCCTCTGTCATT-3'), mouse Sox1 (5'-TTACTTCCCGCCAGCTCTTC-3' and 5'-TGATGCATTTTGGGGGTATCTCTC-3'), mouse nestin (5'-CTGCAGGCCACTGAAAAGT-3' and 5'-TTCCAGGATCTGAGCGATCT-3') and mouse GAPDH (5'-CATGGCCTTCCGTGTTCCTA-3' and 5'-CCTGCTTCACCACCTTCTTGAT-3').

Statistic analysis.

All the values were shown as mean ± SEM (n=3). The significance between groups was determined by Student's T test.

References

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