APPENDIX

APPENDIX SUPPLEMENTARY METHODS

Generation of stable cell lines

pEF1α-FLBIO vector containing Yap1 full length cDNA was introduced into BirA expressing ES cells (control cells) by electroporation [1]. Positive clones were selected under the selection of puromycin and geneticin. Multiple single colonies were picked 7-10 days later, and then Yap1 overexpressing clones were confirmed by western blot assay using anti-streptavidin-HRP (1:2000 dilution, Sigma-Aldrich Mfr. RPN1231), and anti-Yap1 antibodies (1:1000 dilution, Santa Cruz sc-101199). Yap1 KO, Tead2 KO, and Tead4 KO cell lines were generated using CRISPR-Cas9 system following manufacturer's instructions (Life Technologies) [2,3]. Briefly, GeneArt® CRISPR Nuclease Vector System (Life Technologies Cat. A21175) was used to edit genomic sequence in *Yap1, Tead2,* or *Tead4* gene locus. Six different target sequences are TGCCGTCATGAACCCCAAGA (Yap1), ATTGAAGAGCGCCTCCAAGT (Yap1), GGCTGGACAGGTAGCGAGGA (Tead2), TAGCGAGGAAGGCAGCGAAG (Tead2), GCTCCACTCGTTGGAGGTAA (Tead4), and GGGAGAGCTCCACTCGTTGG (Tead4). J1 ES cells were transfected with the cloned CRISPR-Cas9 nuclease construct using Lipofectamine® 2000 (Life Technologies Cat. 11668027) and incubated for 1 day. Transfected J1 ES cells were enriched by Dynabeads® CD4 Positive Isolation Kit (Life Technologies Cat. 11331D) and incubated for colonization. Each colony was picked, analyzed with Western blotting, and confirmed with sequencing for genomic editing at the target site.

Real time quantitative PCR (RT-qPCR) analysis

Total RNA was isolated using the RNeasy plus mini kit (Qiagen Cat. 74134) and 500ng of RNAs were reverse transcribed using qScript cDNA SuperMix (Quanta Cat. 95048). RT-qPCRs were performed using 10 uL of PerfeCTa® SYBR® Green FastMix®, Low ROX™ (Quanta Cat. 95074), and 1uL of 5 X diluted cDNAs. RT-qPCR primers were designed to amplify the exon junction with amplicon size ~ 100 base pairs. Primer sequences are listed in Table S1. CT values of each primer were normalized against Gapdh using –ΔΔCt method to calculate fold change.

Cell proliferation assay

1×10^3 ES cells were plated on 96-well plate. Every 24 h, 10uL of Cell Counting Kit-8 (Dojindo CK04) was added to the media. Absorbance was measured using Infinite® M1000 PRO microplate reader (Tecan) at 450nm after 2hr incubation.

Quantification of immunofluorescence image

DAPI signal is used to distinguish nuclear portion from cytoplasmic portion of the cells. Total area of the cells is identified by pan-cadherin antibody. Intensity is calculated using ZEN software (ZEISS).

Western blot analysis

Cells were lysed with RIPA buffer (G-BIOSCIENCES Cat. 786-490) with the addition of PhosSTOP (Roche P.N. 04906845001) and 100x Halt™ Protease Inhibitor (Thermo Scientific P.N. 1860932). After three times of sonication with 30 sec intervals, lyses were centrifuged at maximum speed for 10 min at 4°C. Supernatant was transferred to clean tube and Laemmli sample buffer (Biorad Cat. 1610737) was added. Following antibodies were used for Western blot analyses: Yap1 (1:1000, Santa Cruz, SC-101199), p-Yap1 (1:000, Cell signaling 4911S), Pou5f1 (1:1000, Santa Cruz, SC-5279), Nanog (1:1000, Abcam, ab21624), Gapdh (1:2000, Santa Cruz, SC-166545), Actb (1:20000, Abcam, ab20272), Tead1 (1:1000, BD Biosciences, Cat. 610923), Tead3 (1:1000, Abcam, ab75192), and Tead4 (1:1000, Abnova, H00007004-M01).

Gene ontology analysis

For Gene Ontology (GO) analyses, differentially expressed genes were tested for enrichment of functional gene sets using Database for Annotation, Visualization and Integrated Discovery (DAVID) [4]. Enrichment *P*-values were calculated using a modified Fisher Exact test. Similar GO terms are removed except the gene sets with the highest p-value.

Correlation analyses

To generate the gene expression correlation map, $\log_2$ (FPKM) values were used to calculate Pearson correlation coefficients. Clustering analysis and visualization of the data were done by Cluster 3.0 and Java Treeview, respectively [5,6].

APPENDIX REFERENCES

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