Expanded View Figures

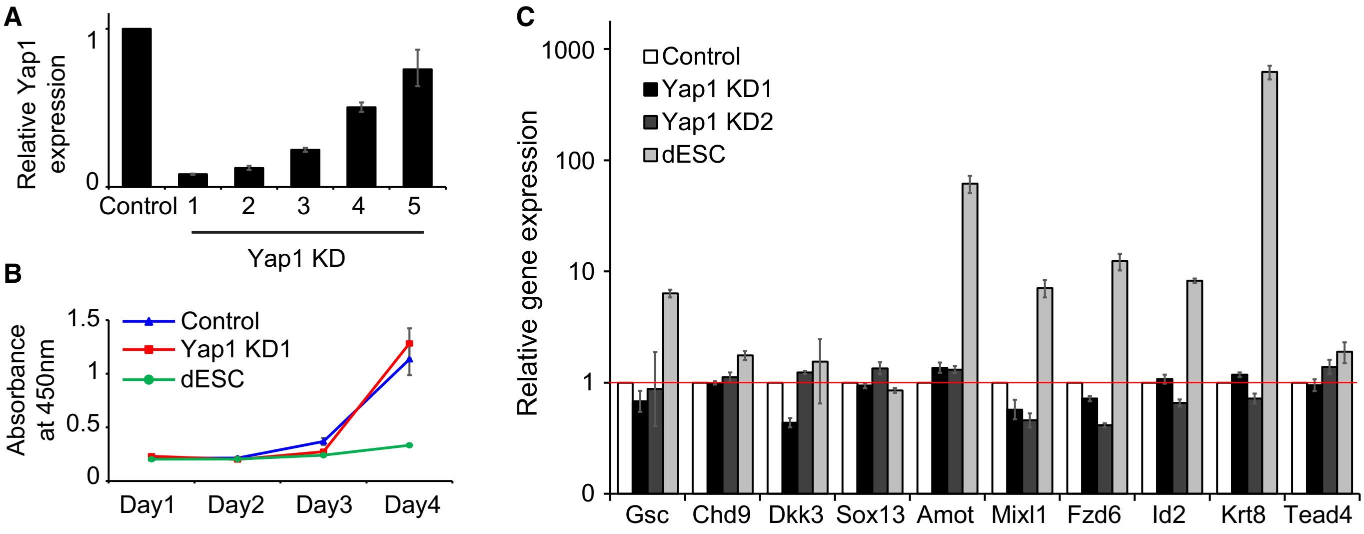


Figure EV1. Yap1 is dispensable for self-renewal of J1 mouse ES cells (related to Fig 1).

A Yap1 mRNA levels measured by RT–qPCR upon shRNA-based KD. Five different shRNA sequences were tested, and shRNAs 1 and 2 (KD1 and KD2) were used for further studies. Data are represented as mean ± SD.

B Cell proliferation rates of Yap1 KD cells and control cells.

C mRNA levels of lineage-specific marker genes upon KD of Yap1. Differentiating ES cells (dESC) were used as control cells. Data are represented as mean ± SD.

Figure EV2. Yap1 is dispensable for self-renewal of E14 and CJ7 mouse ES cells (related to Fig 1).

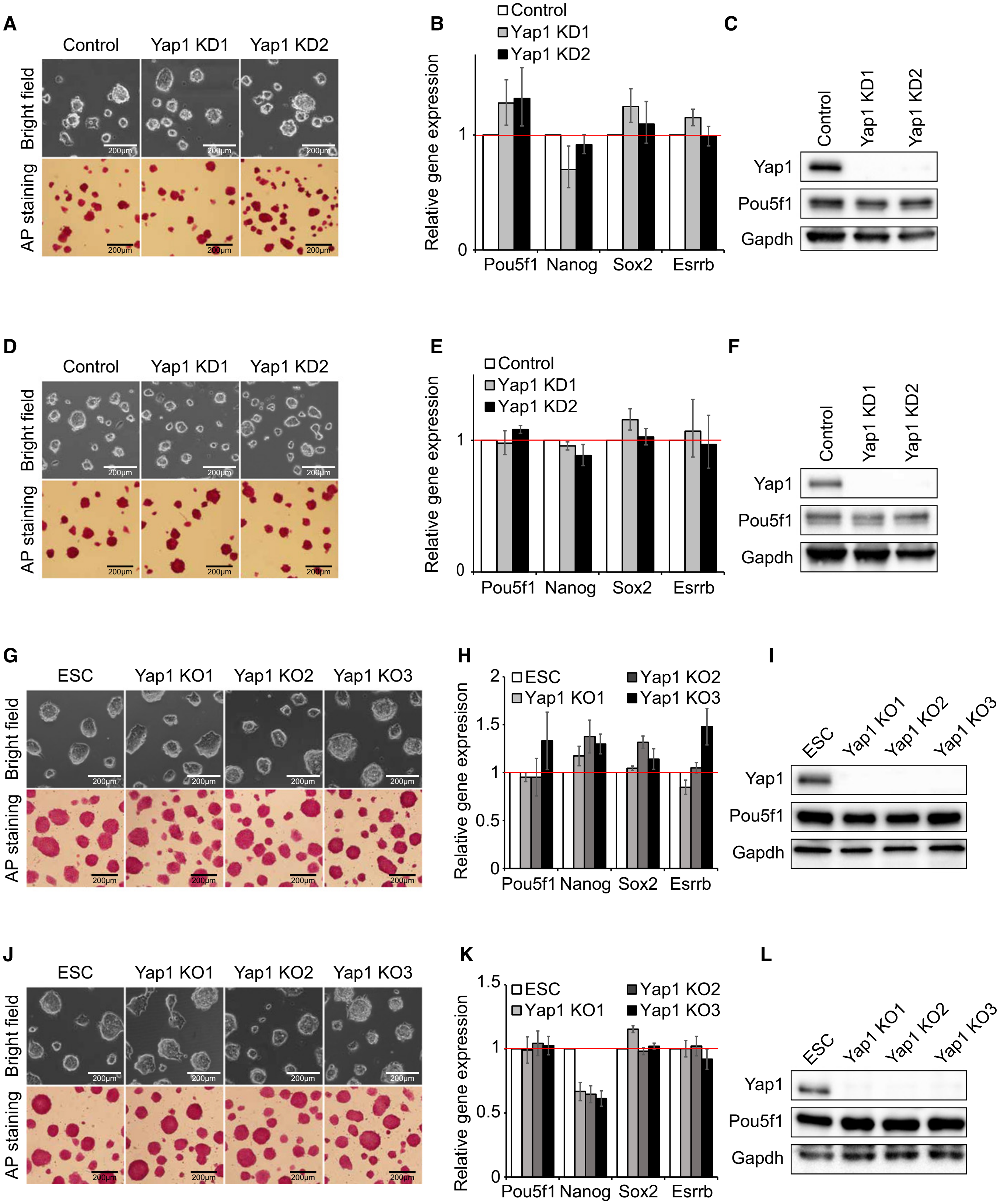
A–C Data from CJ7 ES cells. Colony morphology and AP activity of Yap1 KD cells (A), mRNA levels of Pou5f1, Nanog, Sox2, and Esrrb in Yap1 KD cells (B), and protein expression levels of Yap1, Pou5f1, and Gapdh were measured in Yap1 KD cells (C). mRNA level data are represented as mean ± SD.

D–F Data from E14 ES cells. Colony morphology and AP activity of Yap1 KD cells (D), mRNA levels of Pou5f1, Nanog, Sox2, and Esrrb in Yap1 KD cells (E), and protein expression levels of Yap1, Pou5f1, and Gapdh were measured in Yap1 KD cells (F). mRNA level data are represented as mean ± SD.

G–I Data from CJ7 ES cells (ESC). Colony morphology and AP activity of Yap1 KO clones (G), mRNA levels of Pou5f1, Nanog, Sox2, and Esrrb in Yap1 KO clones (H), and protein expression levels of Yap1, Pou5f1, and Gapdh were measured in Yap1 KO clones (I). mRNA level data are represented as mean ± SD.

J–L Data from E14 ES cells. Colony morphology and AP activity of Yap1 KO clones (J), mRNA levels of Pou5f1, Nanog, Sox2, and Esrrb in Yap1 KO clones (K), and protein expression levels of Yap1, Pou5f1, and Gapdh were measured in Yap1 KO clones (L). mRNA level data are represented as mean ± SD.

▶


A
Control
Yap1 KD1
Yap1 KD2
Bright field
AP staining
200μm
B
Control
Yap1 KD1
Yap1 KD2
Relative gene expression
0
1
Pou5f1
Nanog
Sox2
Esrrb
C
Control
Yap1 KD1
Yap1 KD2
Yap1
Pou5f1
Gapdh
D
Control
Yap1 KD1
Yap1 KD2
Bright field
AP staining
200μm
E
Control
Yap1 KD1
Yap1 KD2
Relative gene expression
0
1
Pou5f1
Nanog
Sox2
Esrrb
F
Control
Yap1 KD1
Yap1 KD2
Yap1
Pou5f1
Gapdh
G
ESC
Yap1 KO1
Yap1 KO2
Yap1 KO3
Bright field
AP staining
200μm
H
ESC
Yap1 KO1
Yap1 KO2
Yap1 KO3
Relative gene expresion
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2
Pou5f1
Nanog
Sox2
Esrrb
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ESC
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Yap1 KO2
Yap1 KO3
Yap1
Pou5f1
Gapdh
J
ESC
Yap1 KO1
Yap1 KO2
Yap1 KO3
Bright field
AP staining
200μm
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ESC
Yap1 KO1
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Yap1 KO3
Relative gene expression
0
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Pou5f1
Nanog
Sox2
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Yap1 KO2
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Yap1
Pou5f1
Gapdh

Figure EV2.

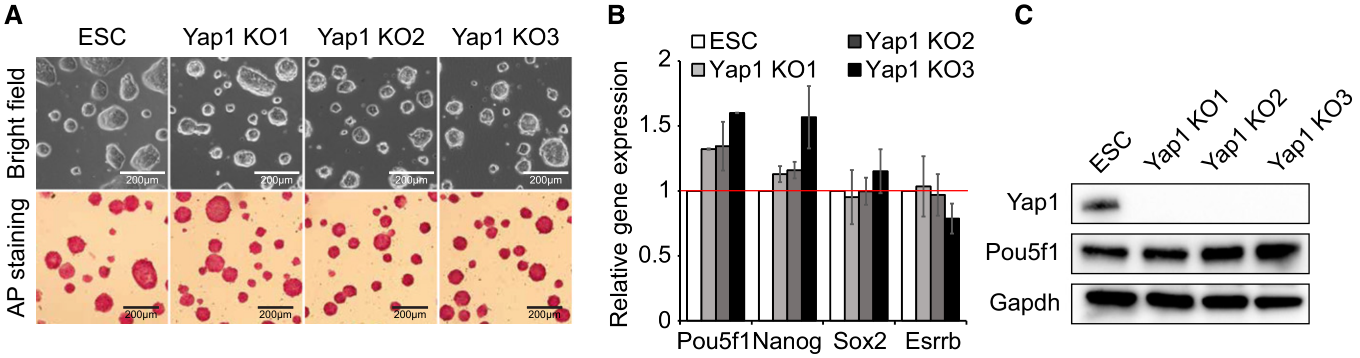

Figure EV3. Yap1-depleted ES cells can maintain self-renewal for more than a month in culture (related to Fig 1).

A Colony morphology and AP activity of Yap1 KO clones cultured for more than a month.

B mRNA levels of Pou5f1, Nanog, Sox2, and Esrrb in Yap1 KO clones shown in (A). Data are represented as mean ± SD.

C Protein expression levels of Yap1, Pou5f1, and Gapdh were measured in Yap1 KO clones shown in (A).

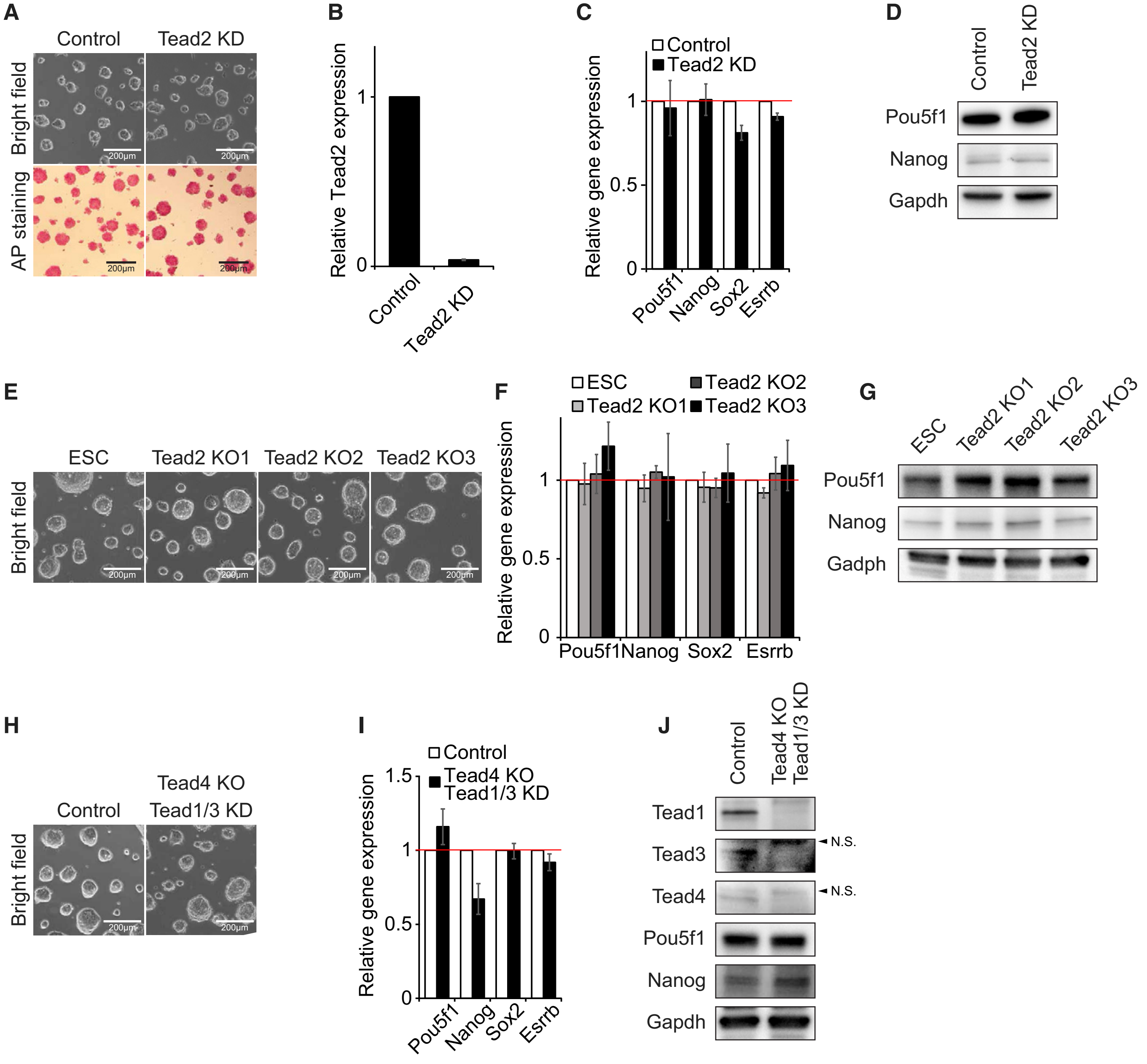

Figure EV4. Tead family proteins are not required for the self-renewal of ES cells (related Fig 2).

- A Colony morphology and AP activity of Tead2 KD ES cells.
- B mRNA levels of Tead2 measured by RT–qPCR upon shRNA-based KD.
- C mRNA expression levels of Pou5f1, Nanog, Sox2, and Esrrb upon KD of Tead2. Data are represented as mean ± SD.
- D Protein levels of Pou5f1 and Nanog upon KD of Tead2.
- E Colony morphology of three Tead2 KO clones (KO1-KO3) and control ES cells.
- F mRNA levels of Pou5f1, Nanog, Sox2, and Esrrb upon KO of Tead2. Data are represented as mean ± SD.
- G Protein levels of Pou5f1 and Nanog in Tead2 KO clones.
- H Colony morphology of ES cells upon KO of Tead4 and KD of Tead1/3.
- I mRNA levels of Pou5f1, Nanog, Sox2, and Esrrb upon KO of Tead4 and KD of Tead1/3. Data are represented as mean ± SD.
- J Protein levels of Pou5f1 and Nanog in Tead4 KO and Tead1/3 KD ES cells. N.S., non-specific bands.

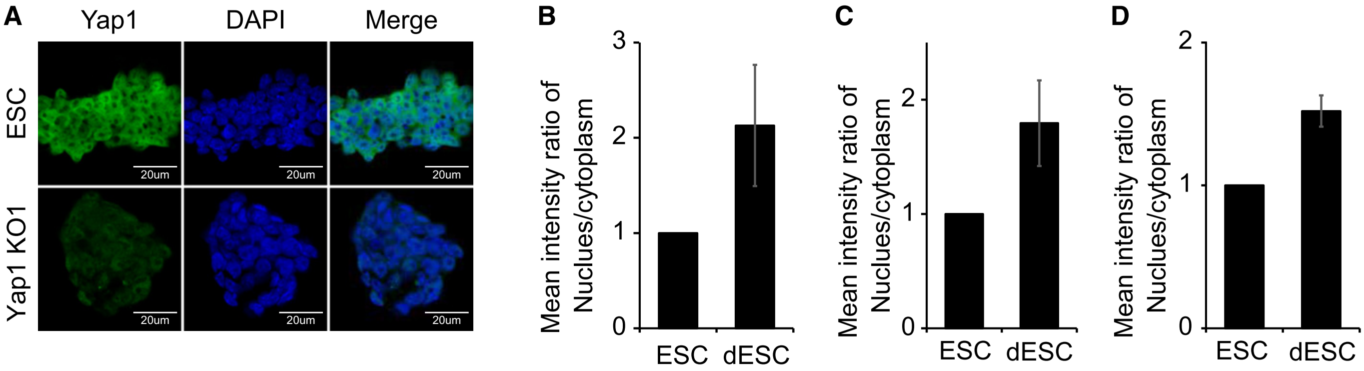

Figure EV5. Yap1 is translocated into the nucleus upon differentiation of ES cells (related to Fig 3).

A Immunofluorescence (IF) images depicting Yap1 signals in J1 ES cells (ESC) and Yap1 KO clone.

B–D Quantification of relative Yap1 localization between ESC and differentiating ES cells (dESC) from three different cell lines: J1 (B), CJ7 (C), and E14 (D). See Appendix Supplementary Methods for detailed quantification method. Data are represented as mean ± SD.

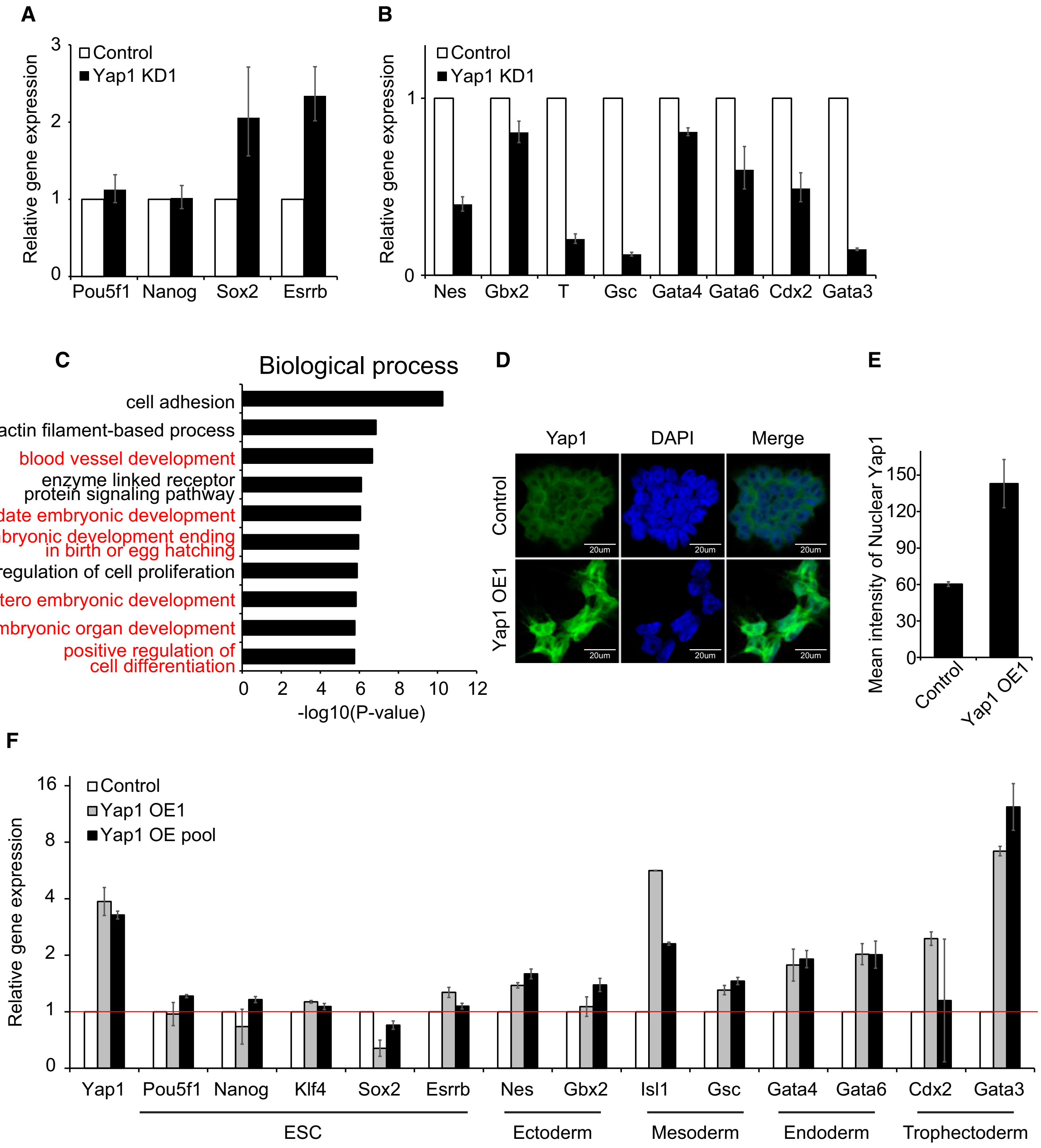

Figure EV6. Alteration of Yap1 affects differentiation of ES cells (related to Fig 4).

A, B mRNA levels of ES cell core factors (A) and lineage-specific markers (B) in Yap1 KD1 cells upon 4 days of differentiation. White bars indicate the levels of genes tested in control virus-infected ES cells (Control) upon differentiation. Data are represented as mean ± SD.

C Bar graphs showing significantly enriched gene ontology (GO) terms (biological functions). GO analysis of genes down-regulated in Yap1 KD cells upon differentiation was performed using David 6.7 tools. Developmental process-related terms are highlighted in red.

D IF images showing localization of Yap1 in control and Yap1 OE cells.

E Quantification of nuclear Yap1 in control and Yap1 OE cells. Data are represented as mean ± SD.

F mRNA levels of ES cell (ESC) core factors and lineage-specific marker genes in Yap1 OE cells and Yap1 OE pool. Data are represented as mean ± SD.