SUPPLEMENTARY MATERIAL FOR

Delayed mesoderm and erythroid differentiation of murine embryonic stem cells in the absence of the transcriptional regulator FUBP1

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Supplementary Materials and Methods

qPCR primer (designed for use at T_M 60^0C):

mFubp1 fw	5´-*agaagatggagatcagccagatgc*-3´
mFubp1 rev	5´-*ctttgctgctgatgcattggaggt*-3´
mGapdh fw	5´-*tgtgtccgtcgtggatctga*-3´
mGapdh rev	5´-*ttgctgttgaagtcgcaggag*-3´
mc-myc fw	5´-*cctagtgctgcatgaggagac*-3´
mc-myc rev	5´-*cctcatcttcttgctcttcttca*-3´
mp21 fw	5´-*cctgacagatttctatcactcca*-3´
mp21 rev	5´-*caggcagcgtatatcaggag*-3´
mNanog fw	5´-*gaattctgggaacgcctcatc*-3´
mNanog rev	5´-*ccttgtcagcctcaggacttg*-3´
mOct3/4 fw	5´-*ggacatgaaagccctgcagaa*-3´
mOct3/4 rev	5´-*gacagatggtggtctggctgaa*-3´
mBrachyury fw	5´-*catcggaacagctctccaacctat*-3´
mBrachyury rev	5´-*gtgggctggcgttatgactca*-3´
mNestin fw	5´-*gggccagcactcttagctttgata*-3´
mNestin rev	5´-*tgagccttcagggtgatccag*-3´
mSox17 fw	5´-*ggacacgactgcggagtgaa*-3´
mSox17 rev	5´-*ggtcggcaaccgtcaaatg*-3´
mFlk-1 fw	5´-*gggatggtccttgcatcagaa*-3´
mFlk-1 rev	5´-*actggtagccactggtctggttg*-3´
mFoxa2 fw	5´-*tagcggaggcaagaagacc*-3´
mFoxa2 rev	5´-*cttaggccacctcgcttgt*-3´
mSnail fw	5´-*gccggaagcccaactatagcga*-3´
mSnail rev	5´-*ttcagagcgcccaggctgaggtact*-3´
mSnai2/slug fw	5´-*tggtcaagaaacatttcaacgcc*-3´
mSnai2/slug rev	5´-*ggtgaggatctctggttttggta*-3´
mFgfR1 fw	5´-*gcagagcatcaactggctg*-3´
mFgfR1 rev	5´-*ggagaagtaggtggtatcgctg*-3´
mBmp4 fw	5´-*ccgaatgctgatggtcgttt*-3´
mBmp4 rev	5´-*cctgaatctcggcgacttttt*-3´
mß-catenin fw	5´-*tcttcaggacagagccaatgg*-3´
m ß-catenin rev	5´-*accagagtgaaaagaacggtagct*-3´
m Gata4 fw	5´-*caccccaatctcgatatgtttga*-3´
m Gata4 rev	5´-*gcacaggtagtgtcccgtc*-3´

Figure S1: CRISPR/Cas9-induced destruction of the *Fubp1* open reading frame and creation of premature stop codons.

The sequences of the *Fubp1* exon 2 locus for both alleles of two *Fubp1* KO clones revealed a change in the reading frame and the generation of premature stop codons for all 4 alleles. On top of the page, the wildtype *Fubp1* sequence is displayed, and the gRNA 1 target sequence is indicated in bold.

Figure S2: Absence of FUBP1 does not affect ESC pluripotency according to SSEA1/SSEA4 marker expression.

Flow cytometry analysis revealed no difference between FUBP1-deficient and FUBP1-expressing ESC clones in their $SSEA1^{+}/SSEA4^{-}$ status. The results for 2 NTC control and 3 *Fubp1* KO clones are presented, together with the unstained control.

Figure S3: Cell cycle and FUBP1 target gene analysis in undifferentiated *Fubp1* KO and NTC control ESC clones.

A. The cell cycle analysis of propidium iodide-stained cells by flow cytometry showed no differences in cell cycle distribution and the level of apoptotic cell death ($subG_1$ phase) between *Fubp1* KO and NTC control cells. **B**. The mRNA expression of the direct FUBP1 target genes *p21* and *c-myc* was quantified in *Fubp1* KO and NTC control ESCs. While *p21* expression showed no difference, *c-myc* mRNA was significantly upregulated in *Fubp1* KO clones. Three independent experiments were performed, each with 3 (**A**) and

4 (**B**) NTC and 3 (**A**) and 5 (**B**) *Fubp1* knockout ESC clones. The qPCR data represent the mean values ± SD (**: $p<0.01$).

Figure S4: *Fubp1* mRNA expression in Flk-1$^+$ cells.

After 4 days of EB differentiation, FACS-sorted FLK-1$^+$ showed a slightly higher *Fubp1* mRNA expression compared to FLK-1$^-$ cells. Results were obtained from 3 independent experiments. The data represent the mean values ± SD (**: $p<0.01$).

Figure S5: Differentiation of ESCs into CD45$^+$ hematopoietic cells during OP9 co-culture.

Differentiation of *Fubp1* KO and NTC control ESCs during 12 days of OP9 co-culture plus SCF resulted in the production of CD45$^+$ hematopoietic progenitor cells to a comparable extent. Results were obtained from 3 independent experiments, each with 3 NTC and 5 *Fubp1* knockout ESC clones. The data represent the mean values ± SD.

Fubp1 wildtype sequence

226 bp **gRNA 1 target sequence** 306 bp

-ctgcagagagcgcggcagattgcag**caaaaattgggggtgatgc**tggtacatcattgaattcaaatgactatggttatggg-

-Leu Gln Arg Ala Arg Gln Ile Ala Ala Lys Ile Gly Gly Asp Ala Gly Thr Ser Leu Asn Ser Asn Asp Tyr Gly Tyr Gly -

gRNA 1 #7 (allele 1): deletion of 28 bp

226 bp 278 bp

-ctgcagagagcgcggcag_____gtacatcattgaattcaaatgactatggttatggg-

-Leu Gln Arg Ala Arg Gln _____Val His His STOP

gRNA 1 #7 (allele 2): insertion of 1 bp

226 bp 307 bp

-ctgcagagagcgcggcagattgcag**caaaaattgggggtgattgc**tggtacatcattgaattcaaatgactatggttatggg-

- Leu Gln Arg Ala Arg Gln Ile Ala Ala Lys Ile Gly Gly Asp **Cys Trp Tyr Ile Ile Glu Phe Lys STOP**

gRNA 1 #14 (allele 1): insertion of 1 bp

226 bp 307 bp

-ctgcagagagcgcggcagattgcag**caaaaattgggggtgattgc**tggtacatcattgaattcaaatgactatggttatggg-

- Leu Gln Arg Ala Arg Gln Ile Ala Ala Lys Ile Gly Gly Asp **Cys Trp Tyr Ile Ile Glu Phe Lys STOP**

gRNA 1 #14 (allele 2): insertion of 1 bp

226 bp 307 bp

-ctgcagagagcgcggcagattgcag**caaaaattgggggtgattgc**tggtacatcattgaattcaaatgactatggttatggg-

- Leu Gln Arg Ala Arg Gln Ile Ala Ala Lys Ile Gly Gly Asp **Cys Trp Tyr Ile Ile Glu Phe Lys STOP**

Fig. S1

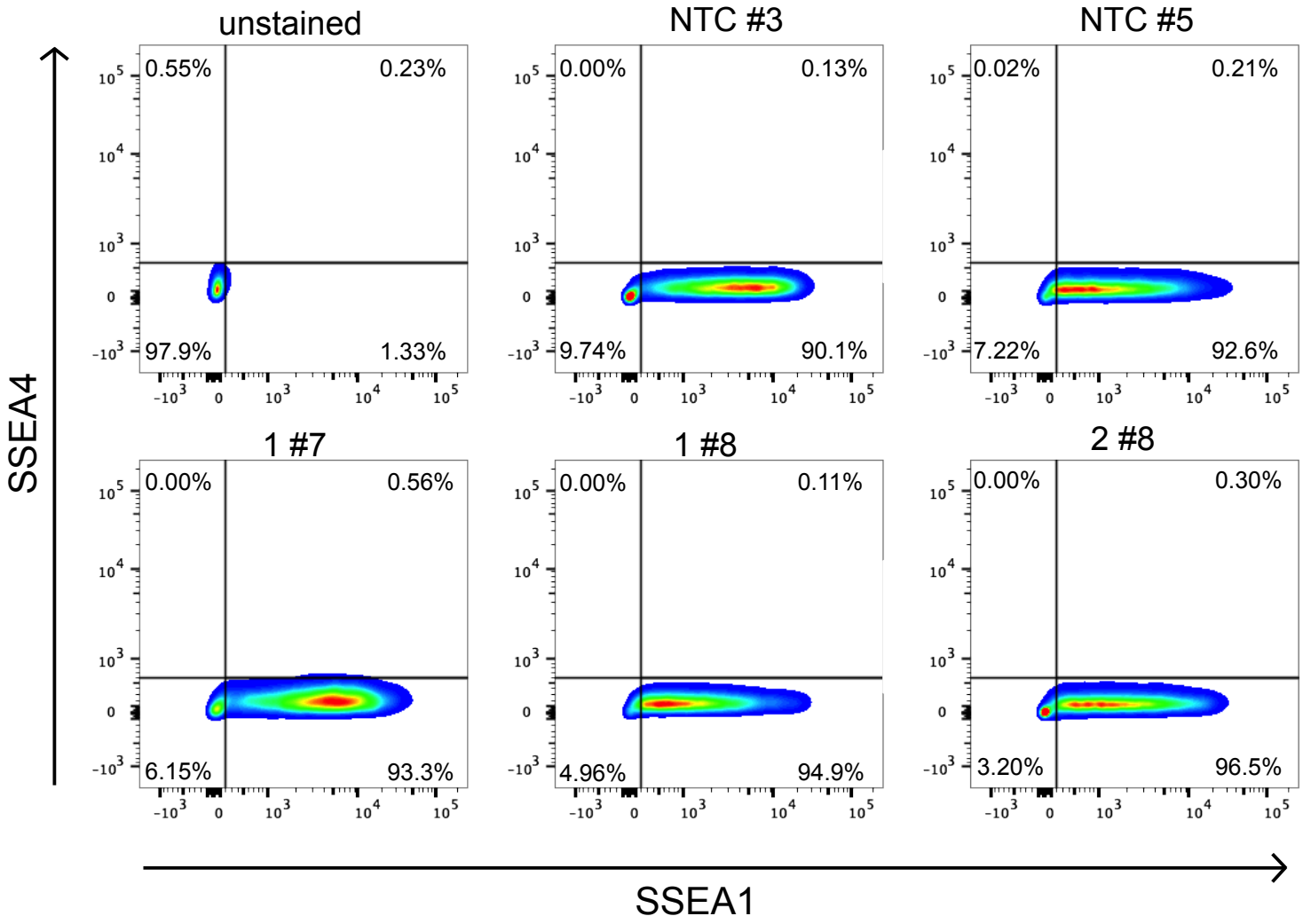
unstained
NTC #3
NTC #5
1 #7
1 #8
2 #8
0.55%
0.23%
97.9%
1.33%
0.00%
0.13%
9.74%
90.1%
0.02%
0.21%
7.22%
92.6%
0.00%
0.56%
6.15%
93.3%
0.00%
0.11%
4.96%
94.9%
0.00%
0.30%
3.20%
96.5%
SSEA4
SSEA1

Fig. S2

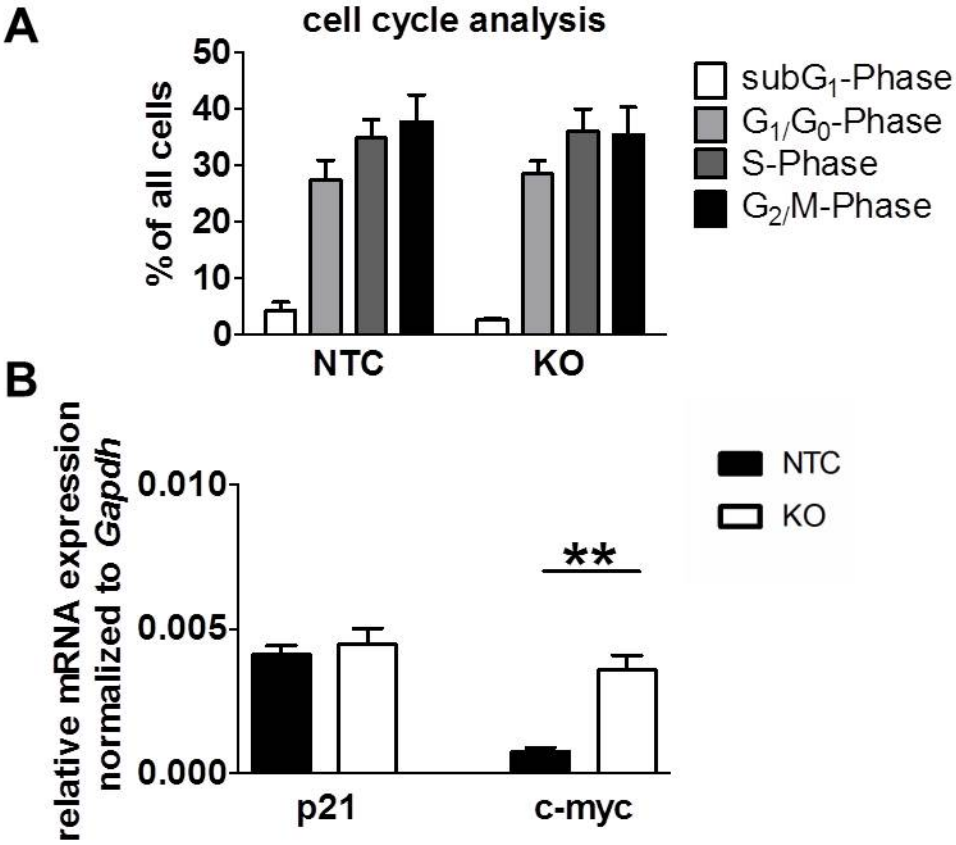
A
cell cycle analysis
% of all cells
50
40
30
20
10
0
NTC
KO
subG1-Phase
G1/G0-Phase
S-Phase
G2/M-Phase
B
relative mRNA expression
normalized to Gapdh
0.010
0.005
0.000
**
p21
c-myc
NTC
KO

Fig. S3

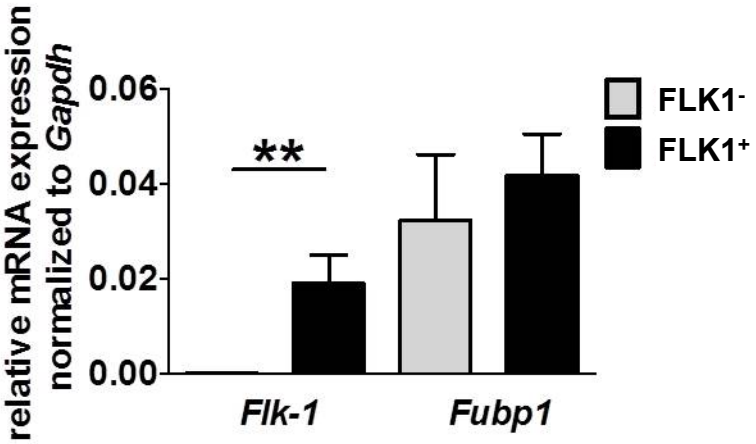
relative mRNA expression
normalized to Gapdh
0.06
0.04
0.02
0.00
**
Flk-1
Fubp1
FLK1-
FLK1+

Fig. S4

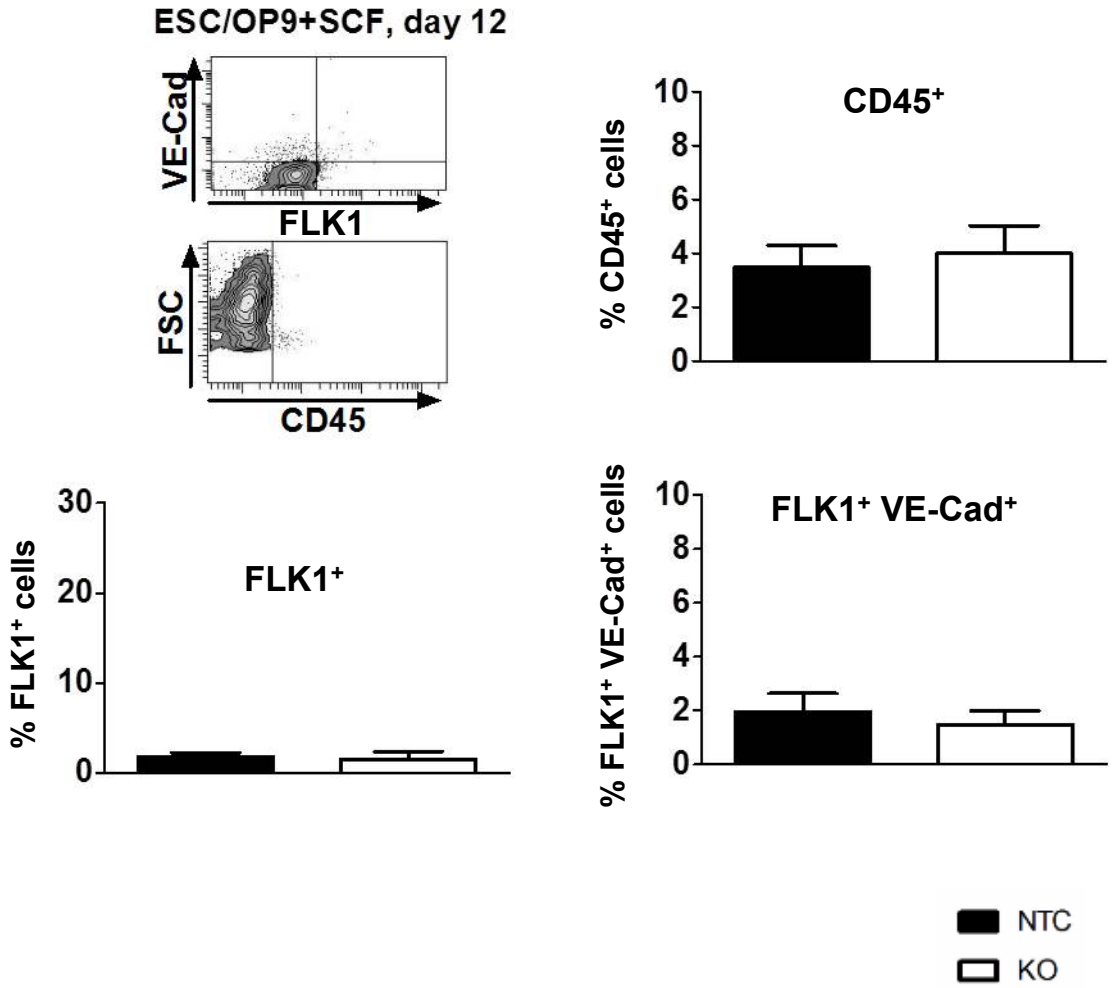
ESC/OP9+SCF, day 12
VE-Cad
FLK1
FSC
CD45
CD45+
% CD45+ cells
10
8
6
4
2
0
FLK1+
% FLK1+ cells
30
20
10
0
FLK1+ VE-Cad+
% FLK1+ VE-Cad+ cells
NTC
KO

Fig. S5