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## Supplemental information

# Interplay between FLI-1 and the LDB1 complex in murine erythroleukemia cells and during megakaryopoiesis

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**SUPPLEMENTAL FIGURE LEGEND**

**Figure S1 (Related to Figure 1 and Figure 2) : FLI-1 and the LDB1 complex are co-recruited in MEL cells**

**a** ChIP experiments performed with either the anti-FLI-1 antibody or the control IgG in non induced or induced MEL cells expressing either control shRNA (shSCR) or shRNA against the Fli-1 mRNA (shFli-1) followed by qPCR to amplify the control region in the β-amylase locus (Amy, black bar) or the FLI-1 binding regions identified by ChIP-Seq (see Figure 1d) in the Gp1ba (blue bar) and the Cd41 promoter (red bar) and in the Tgfb1 (green bar), Stat1 (orange bar), Trp53 (purple bar) and Pdcd1lg2 yellow bar) loci. The bars represent the geometric mean of the ratio between the FLI-1 binding regions and the control region of 3 independent experiments. The error bars represent the standard errors. *: $p<0.05$ for the comparison between shSCR and shFli-1 conditions (paired t-test).

**b** Western blot analyses of the FLI-1 (bottom blot) or the VCP (top blot, loading control) protein levels in non-induced (lanes 1 and 2) and induced (lanes 3 and 4) MEL cells expressing the control shRNA (shSCR, lanes 1 and 3) or the shRNA against Fli-1 mRNA (shFli-1, lanes 2 and 4). The blots are representative of 3 independent experiments.

**c** RT-qPCR analyses of the Fli-1 mRNA levels in non-induced and induced MEL cells expressing the control shRNA (shSCR, black bars) or the shRNA against Fli-1 mRNA (shFli-1, blue bars). The bars represent the geometric mean of the ratio of the signals between shFli-1 and shSCR normalized to the signals of the Actb reference gene of 3 independent experiments. The error bars represent the standard errors. *: $p<0.05$ for the comparison between shSCR and shFli-1 conditions (paired t-test).

**d** ChIP experiments performed with either the anti-GATA1 or the anti-LDB1 antibodies or the control IgG in non induced or induced MEL cells followed by qPCR to amplify the control region in the β-amylase locus (Amy, black bar) or the FLI-1 binding regions identified by ChIP-Seq (see Figure 1d) in the Gp1ba (blue bar) and the Cd41 promoter (red bar). The bars represent the geometric mean of the ratio between the FLI-1 binding regions and the control region of 3 independent experiments. The error bars represent the standard error.

**Figure S2 (Related to Figure 3) : FLI-1 and the LDB1 complex directly activate *Fut8* and *Meis1* expression by binding to enhancers in MEL cells**

**a** ChIP-Seq profiles of FLI-1, GATA1, LDB1, TAL1, LMO2, p300 and the Input control in MEL cells (black) and LDB1 and Input control in E14.5 mFL (red) on the Fut8 locus.

**b** ChIP experiments with the anti-FLI-1 (top panel), anti-GATA1 and anti-LDB1 (bottom panel) antibodies and their respective control IgG in MEL cells and E14.5 fetal liver cells (mFL) followed by qPCR experiments to amplify the amylase control region (Amy, black bar), the Meis1 +94 (red bar), the Meis1 +55 (blue bars) or the Meis1 +48 (green bars) regions. The bars represent the geometric mean of the ratio between the FLI-1 binding regions and the control region of 3 independent experiments. The error bars represent the standard error. *: $p<0.05$ for the comparison between MEL cells and fetal liver cells.

**c** ChIP-qPCR experiments performed with either an anti-FLI-1 (top panel), an anti-GATA1 or an anti-LDB1 (bottom panel) antibody and their respective control IgG in MEL cells and E14.5 mFL followed by qPCR amplifying the control Amylase region (black bars) and the Fut8-140 region (blue bar). The bars represent the geometric mean of the ratio between the FLI-1 binding regions and the control region of 3 independent experiments. The error bars represent the standard error. *: $p<0.05$ for the comparison between MEL cells and fetal liver cells.

**d** Quantification of the Meis1 mRNA levels by RT-qPCR experiments in E14.5 fetal liver (red bar) and MEL (blue bar) cells. The bars represent the geometric mean of the ratio between MEL cells and fetal liver cells. The error bars represent the standard error. *: $p<0.05$ for the comparison between MEL cells and fetal liver cells.

**e** Quantification of the Fut8 mRNA levels by RT-qPCR experiments in E14.5 fetal liver (red bar) and MEL (blue bar) cells. The bars represent the geometric mean of the ratio between MEL cells and fetal liver cells. The error bars represent the standard errors. *: $p<0.05$ for the comparison between MEL cells and fetal liver cells.

**f** Left panel: Fut8 mRNA level from non-induced control (shSCR, black bars) or shFli-1 (blue bars) MEL cells. Right panel: Lmo2 and Meis1 mRNA level from control (shSCR, black bar) or shLmo2 transduced (green bar) MEL cells. The values are normalized to the value obtained for the Actb reference gene and those of control cells. Bars represent the geometric mean of 3 independent experiments. The error bars represent the standard errors. *: $p<0.05$ for the comparison between shSCR condition and the others (paired t-test).

**g** ChIP-qPCR experiments using an anti-FLI-1 (top panel), an anti-LDB1 (middle panel) or an anti-

GATA1 (bottom panel) antibody and their respective control IgG in non –induced and induced control (shSCR) or shFli-1 MEL cells followed by qPCR amplifying the control Amylase region (black bars) and the Fut8-140 region (blue bar). The bars represent the average of the ratio between the FLI-1 binding regions and the control region of 3 independent experiments. The error bars represent the standard error. *: $p<0.05$ for the comparison between shSCR and shFli-1 conditions (paired t-test).
**h** Western blot analyses of GATA1 ($2^{nd}$ top panel), LDB1 (bottom panel) VCP (loading control, top panel and $2^{nd}$ bottom panel) protein levels in non-induced (lanes 1 and 2) and induced (lanes 3 and 4) MEL cells expressing the control shSCR (lanes 1 and 3) or shFli-1 (lanes 2 and 4). The pictures are representative of 3 independent experiments.
**i** Left panel : ChIP experiment using an anti-H3K9Ac antibody or its control IgG from MEL cells or E14.5 mouse fetal liver cells followed by qPCR experiments to amplify the *Meis1+94* (red bars), the *Meis1+55* (blue bars) or the *Meis1+48* (green bars) regions. Right panel : ChIP experiment using an anti-H3K9Ac antibody or its control IgG from MEL cells or E14.5 mouse fetal liver cells followed by qPCR experiments to amplify the negative region (Amy, black bars) or the *Fut8-140* (blue bars) region. In the two panels, the bars represent the average of the relative value compared to Input of 3 independent experiments. The error bars represent the standard error. *: $p<0.05$ for the comparison between MEL cells and fetal liver cells.
**j** ChIP-qPCR experiments performed with an anti-H3K9Ac or its respective control IgG in control (shSCR) or shFli-1 non-induced or induced MEL cells followed by qPCR amplifying the control Amylase region (black bars) and the Fut8-140 region (blue bar). The bars represent the average of the relative value compared to Input of 3 independent experiments. The error bars represent the standard error. *: $p<0.05$ for the comparison between control and shFli-1 cells.

**Figure S3 (Related to Figure 3) : FLI-1 and the LDB1 complex reshape the 3D chromatin landscape of *Meis1* and *Fut8* loci.**
**a** Left panel: Scheme of the Meis1 locus. The promoter and the sense of transcription is indicated by an arrow. The Meis1 +48, Meis1 +55 and the Meis1 +94 regions are indicated by a green, blue and red rectangle respectively. The gRNA used to delete these 3 regions individually are indicated by scissors and the primers couple used to screen for clones having the deletion of the Meis1 +48, Meis1 +55 and the Meis1 +94 regions are indicated by
green, blue and red lanes respectively. Right panel: PCR using the primer couples mentioned above from gDNA of WT, Δ94/Δ94, Δ55/Δ55 and Δ48/Δ48 clones.
**b** T2C profiles on the Meis1 locus from MEL cells expressing either control shRNA (shSCR, middle panel) or shRNA targeting Fli-1 mRNA (shFli-1, top panel) or from mouse fetal liver cells (E14.5 mFL, bottom panel). Below are plotted the LDB1, GATA1, H3K27Ac, CTCF ChIP-Seq profiles and the DNaseI profile. The picture is representative of 2 technical duplicates.
**c** FLI-1 ChIP-Seq and differential contact profiles between Control and shFli-1 MEL cells (blue line) or between MEL cells and E14.5 mFL (orange line). The lines represent the log2 fold change of the aforementioned differences. The *Meis1* promoter is taken as a viewpoint.
**d** FLI-1 ChIP-Seq (black) and T2C plotted as 3C-Seq profile profile view of MEL cells expressing the control shRNA (dark colors) or shRNA against Fli-1 mRNA (medium colors) and E14.5 fetal liver cells (light colors) in the Fut8 locus. The Meis1 +94 enhancer (red profiles), Meis1 +55 (blue profiles) and Meis1 +48 (green profiles) are used as a viewpoint.
**e** FLI-1 ChIP-Seq (black) and 3C-Seq profile view of MEL cells expressing the control shRNA (dark blue) or shRNA against Fli-1 mRNA (light blue) and E14.5 fetal liver cells (red) in the Fut8 locus. The Fut8 promoter is used as a viewpoint.
**f** FLI-1 ChIP-Seq (black) and 3C-Seq profile view of MEL cells expressing the control shRNA (dark blue) or shRNA against Fli-1 mRNA (light blue) and E14.5 fetal liver cells (red) in the Fut8 locus. The Fut8 -140 region is used as a viewpoint.

**Figure S4 (Related to Figure 4) : FLi-1 and the LDB1 complex directly activate the expression of megakaryocytic genes**
**a** ChIP experiments using an anti-GATA-1 or an anti-LDB1 antibody or their respective control IgG performed in non-induced and induced MEL cells expressing a control shRNA (shSCR) or shRNA directed against Fli-1 mRNA (shFli-1) followed by qPCR to amplify the control region in the β-amylase locus (Amy, black bar) or the FLI-1 binding regions identified by ChIP-Seq (see Figure 1d) in the Gp1ba (blue bar) and the Cd41 promoter (red bar). The bars represent the geometric mean of the ratio between the FLI-1 binding regions and the control region of 3 independent experiments. The error bars represent the standard error. *: $p<0.05$ for the comparison between shSCR and shFli-1 MEL cells (paired t-test).

**b, c** (b) ChIP experiments performed with either the anti-FLI-1 antibody (blue bar) or the control IgG (black bar) in L8057 cells followed by qPCR to amplify the control region in the β -amylase locus (Amy) or the FLI-1 binding regions identified by ChIP-Seq (see Figure 1d) in the Gp1ba, Cd41 loci. The bars represent the geometric mean of the ratio between the FLI-1 binding regions and the control region of 3 independent experiments. The error bars represent the standard error.*: $p<0.05$ for the comparison between FLI-1 and control IgG (paired t-test). (c): ChIP experiments with antibodies against GATA-1 (red bars) or LDB1 (orange bars) or the control IgG (black bars) in L8057 cells followed by qPCR to amplify the control amylase region (Negative region), the Gp1ba promoter and the Cd41 promoter. The bars represent the geometric mean of the ratio between the FLI-1 binding regions and the control region (of 3 independent experiments. The error bars represent the standard error. * : p-value <0.05 for the comparison between FLI-1 binding regions and the negative region (paired t-test).
**d** Top panel: Genome view of the RNA-Seq reads of WT (bottom) or the FLI-1 KO MkP (top) displaying the absence of reads in the coding sequence of the Fli-1 exon 9 in the FLI-1 KO MkP. Bottom panel: Fli-1 RPKM value in WT (black bar) and FLI-1 KO (purple bar) MkP.
**e** Scheme of the protocol followed to analyze gene expression in megakaryocytes after Fli-1 and/or Lmo2 repression. cKit+ cells were isolated from E14.5 mouse fetal liver and infected with lentiviruses expressing either a control shRNA (shSCR) or shRNA targeting Fli-1 or Lmo2 or both mRNA. Cells were then cultured for 5 days in presence of mSCF and mTpo followed by 4 extra days in presence of only mTpo. RNA were isolated and RT-qPCR were performed to assess the mRNA levels of megakaryocytic genes (Figure 4f).
**f** Quantification by RT-qPCR analyses of the Fli-1 (left panel) and Lmo2 (right panel) mRNA levels from control mature megakaryocytes (black bars) or mature megakaryocytes knocked-down for Fli-1 (shFli-1, blue bars), or for Lmo2 (shLmo2, red bars) or both (purple bars). The
bars represent the geometric mean of 3 independent experiments. The error bars represent the standard errors. **: $p<0.01$ for the comparison with the shSCR condition (paired t-test).
**g** ChIP-Seq profiles for FLI-1, GATA1 and TAL1 and the Input control at the Gp1ba, Cd41, Selp, Rab27b and vWF loci in megakaryocytes.
**h** Fetal liver cells from E14.5 mouse embryos have been infected by lentiviruses expressing either control shRNA (shSCR, dark coloured bars) or shRNA against *Fli-1* mRNA (light coloured bars) and cultured for 3 days in presence of SCF and Tpo cytokines to prime megakaryopoiesis. Cells were then crosslinked and ChIP experiments were performed using either the anti-FLI-1 (blue bars), the anti-GATA1 (red bars) and the anti-LDB1 (orange bars) antibodies followed by qPCR experiments to amplify the negative region (in the β-amylase locus) and the promoters of *Gp1ba* and *Cd41* gene. The bars represent the fold enrichment relative to the control IgG that have been used in parallel.

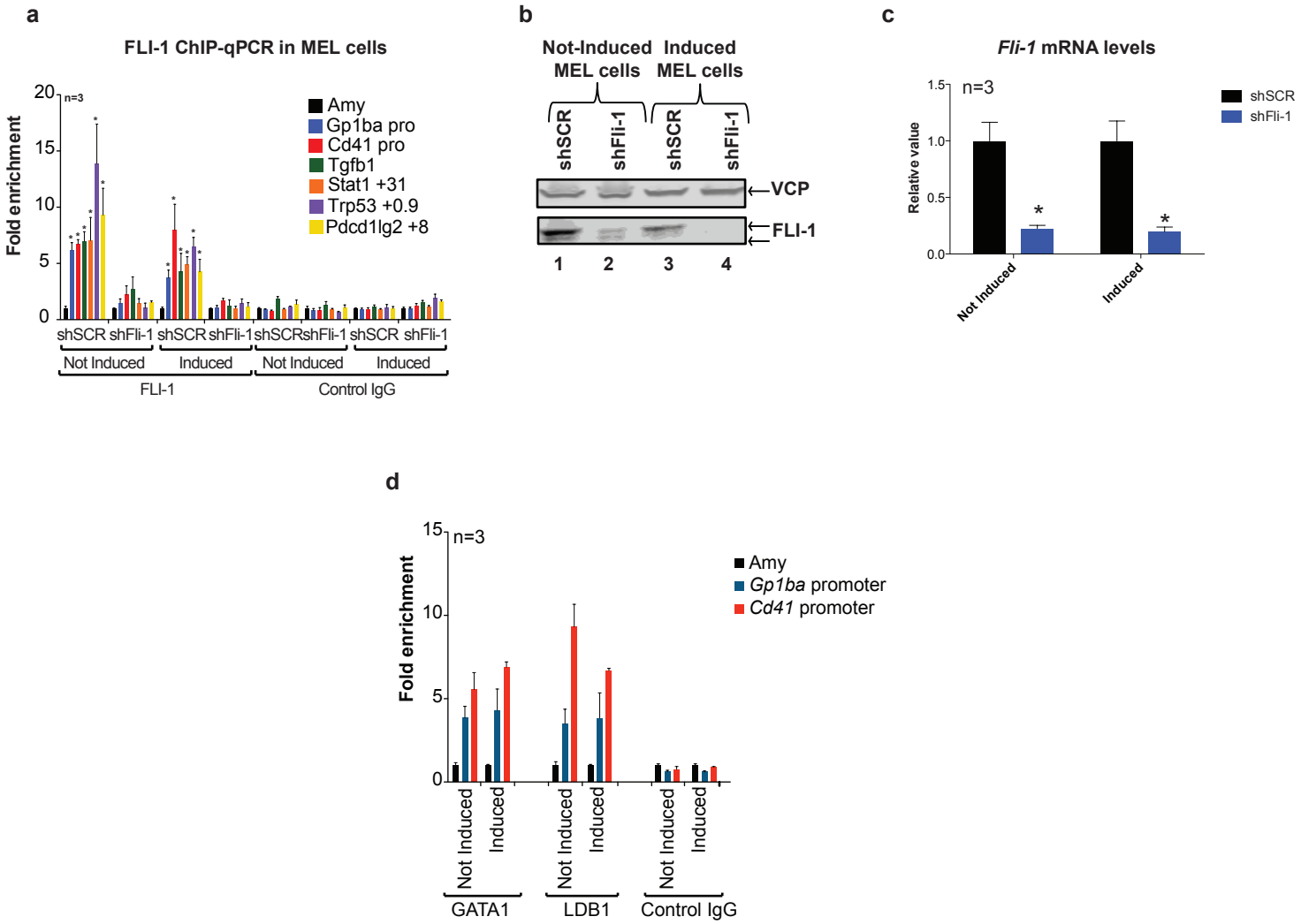
a
FLI-1 ChIP-qPCR in MEL cells
Fold enrichment
n=3
Amy
Gp1ba pro
Cd41 pro
Tgfb1
Stat1 +31
Trp53 +0.9
Pdcd1lg2 +8
shSCR shFli-1 shSCR shFli-1 shSCR shFli-1 shSCR shFli-1
Not Induced
Induced
Not Induced
Induced
FLI-1
Control IgG
b
Not-Induced MEL cells
Induced MEL cells
shSCR
shFli-1
shSCR
shFli-1
VCP
FLI-1
1 2 3 4
c
Fli-1 mRNA levels
n=3
Relative value
shSCR
shFli-1
Not Induced
Induced
d
n=3
Fold enrichment
Amy
Gp1ba promoter
Cd41 promoter
Not Induced
Induced
Not Induced
Induced
Not Induced
Induced
GATA1
LDB1
Control IgG

**Figure S1**

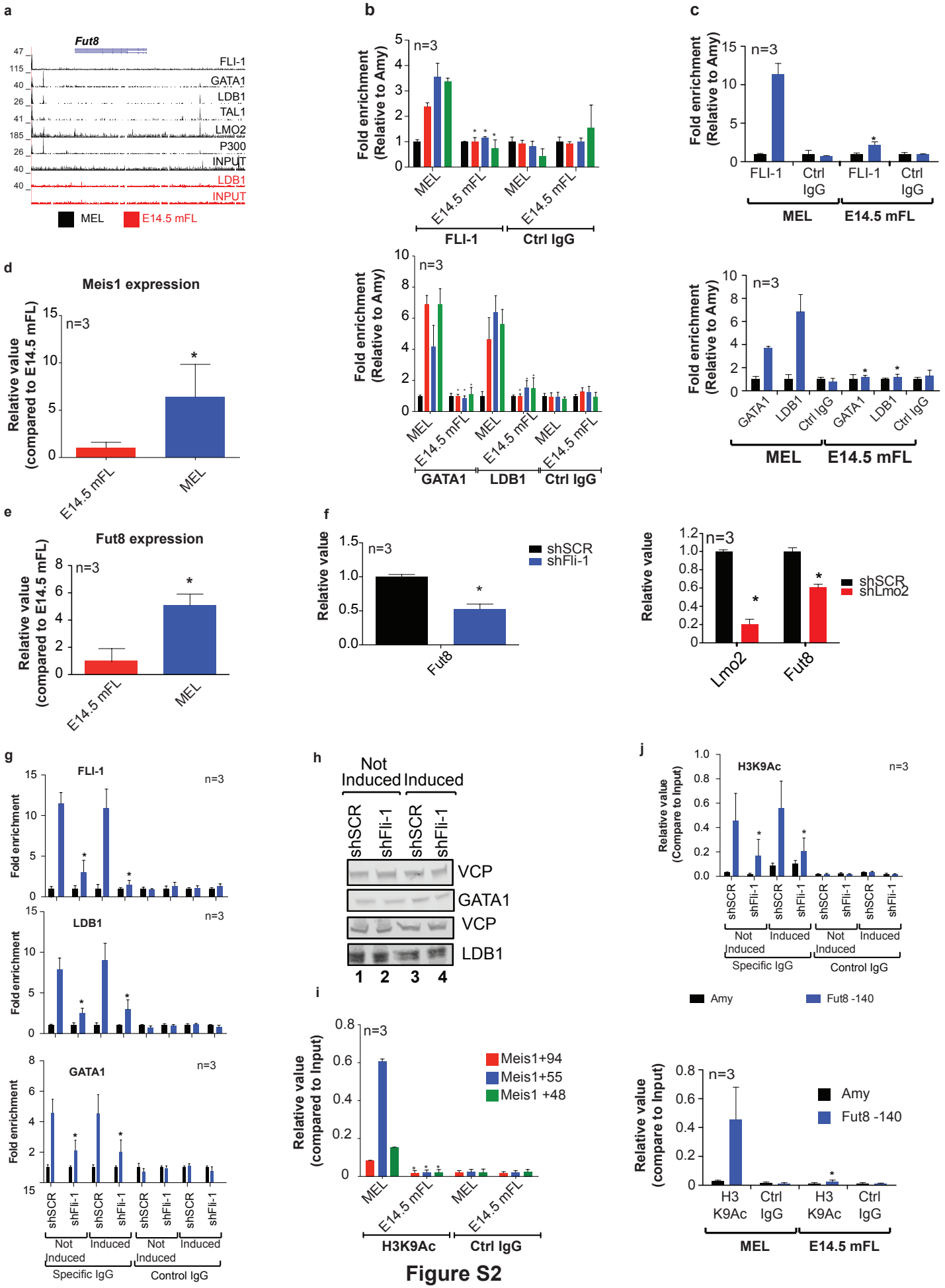
a
Fut8
FLI-1
GATA1
LDB1
TAL1
LMO2
P300
INPUT
LDB1
INPUT
MEL
E14.5 mFL
b
Fold enrichment (Relative to Amy)
n=3
MEL
E14.5 mFL
MEL
E14.5 mFL
FLI-1
Ctrl IgG
GATA1
LDB1
Ctrl IgG
c
Fold enrichment (Relative to Amy)
n=3
FLI-1
Ctrl IgG
FLI-1
Ctrl IgG
MEL
E14.5 mFL
GATA1
LDB1
Ctrl IgG
GATA1
LDB1
Ctrl IgG
MEL
E14.5 mFL
d
Meis1 expression
Relative value (compared to E14.5 mFL)
n=3
E14.5 mFL
MEL
e
Fut8 expression
Relative value (compared to E14.5 mFL)
n=3
E14.5 mFL
MEL
f
Relative value
n=3
shSCR
shFli-1
Fut8
Relative value
n=3
shSCR
shLmo2
Lmo2
Fut8
g
FLI-1
LDB1
GATA1
Fold enrichment
n=3
shSCR
shFli-1
Not Induced
Induced
Specific IgG
Control IgG
Amy
Fut8 -140
h
Not Induced
Induced
shSCR
shFli-1
VCP
GATA1
VCP
LDB1
1 2 3 4
i
Relative value (compared to Input)
n=3
Meis1+94
Meis1+55
Meis1 +48
MEL
E14.5 mFL
H3K9Ac
Ctrl IgG
j
H3K9Ac
Relative value (Compare to Input)
n=3
shSCR
shFli-1
Not Induced
Induced
Specific IgG
Control IgG
Amy
Fut8 -140
Relative value (compare to Input)
n=3
Amy
Fut8 -140
H3 K9Ac
Ctrl IgG
MEL
E14.5 mFL

Figure S2

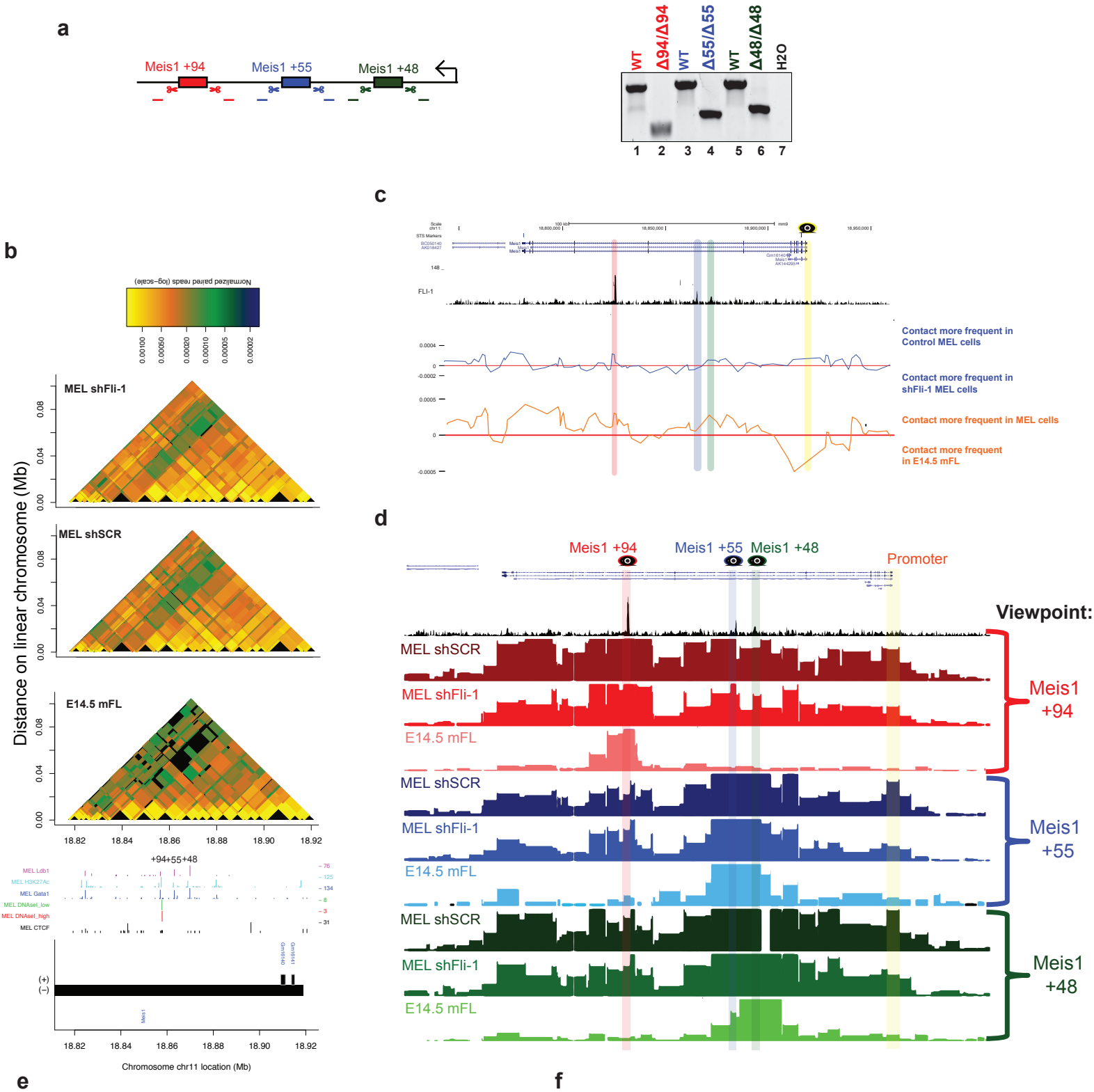
a
Meis1 +94
Meis1 +55
Meis1 +48
WT
Δ94/Δ94
WT
Δ55/Δ55
WT
Δ48/Δ48
H2O
1 2 3 4 5 6 7
b
Normalized paired reads (log-scale)
0.00100
0.00050
0.00020
0.00010
0.00005
0.00002
MEL shFli-1
MEL shSCR
E14.5 mFL
Distance on linear chromosome (Mb)
18.82 18.84 18.86 18.88 18.90 18.92
+94+55+48
MEL Ldb1
MEL H3K27Ac
MEL Gata1
MEL DNaseI_low
MEL DNaseI_high
MEL CTCF
Chromosome chr11 location (Mb)
c
FLI-1
Contact more frequent in Control MEL cells
Contact more frequent in shFli-1 MEL cells
Contact more frequent in MEL cells
Contact more frequent in E14.5 mFL
d
Meis1 +94
Meis1 +55
Meis1 +48
Promoter
Viewpoint:
MEL shSCR
MEL shFli-1
E14.5 mFL
Meis1 +94
Meis1 +55
Meis1 +48
e
Fut8
FLI-1 ChIP-Seq (MEL)
shSCR
shFli-1
E14.5 mFL
f
Fut8
FLI-1 ChIP-Seq (MEL)
MEL shSCR
MEL shFli-1
E14.5 mFL

**Figure S3**

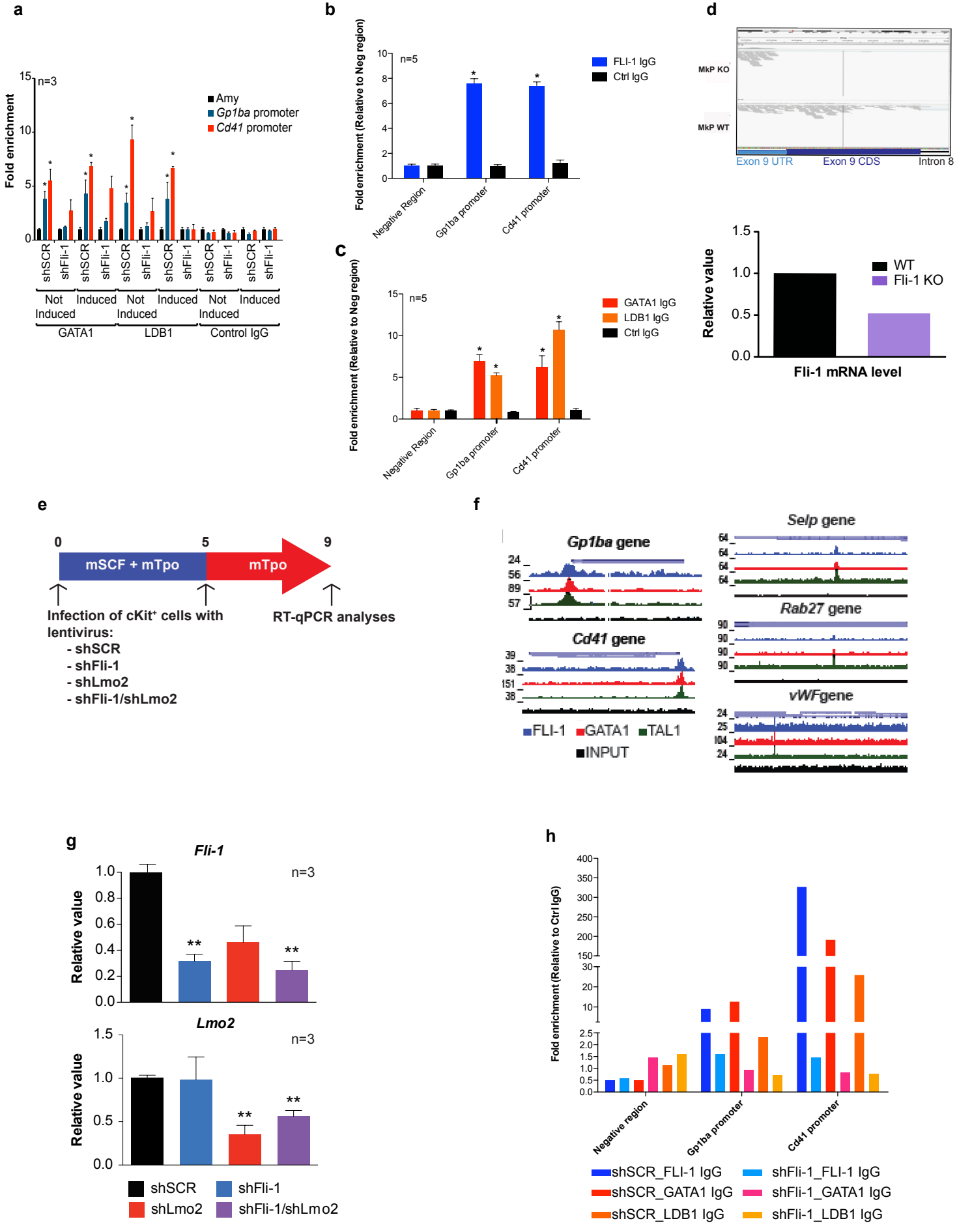
a
Fold enrichment
n=3
Amy
Gp1ba promoter
Cd41 promoter
shSCR
shFli-1
Not Induced
Induced
GATA1
LDB1
Control IgG
b
Fold enrichment (Relative to Neg region)
n=5
FLI-1 IgG
Ctrl IgG
Negative Region
Gp1ba promoter
Cd41 promoter
c
Fold enrichment (Relative to Neg region)
n=5
GATA1 IgG
LDB1 IgG
Ctrl IgG
d
MkP KO
MkP WT
Exon 9 UTR
Exon 9 CDS
Intron 8
Relative value
WT
Fli-1 KO
Fli-1 mRNA level
e
0
5
9
mSCF + mTpo
mTpo
Infection of cKit+ cells with lentivirus:
- shSCR
- shFli-1
- shLmo2
- shFli-1/shLmo2
RT-qPCR analyses
f
Gp1ba gene
Cd41 gene
Selp gene
Rab27 gene
vWFgene
FLI-1
GATA1
TAL1
INPUT
g
Fli-1
Lmo2
Relative value
n=3
shSCR
shFli-1
shLmo2
shFli-1/shLmo2
h
Fold enrichment (Relative to Ctrl IgG)
Negative region
Gp1ba promoter
Cd41 promoter
shSCR_FLI-1 IgG
shFli-1_FLI-1 IgG
shSCR_GATA1 IgG
shFli-1_GATA1 IgG
shSCR_LDB1 IgG
shFli-1_LDB1 IgG

**Figure S4**

**SUPPLEMENTAL TABLE**

**Table S1 : List of antibodies used (Related to Figure 1, 2 3 and 4)**

| Antibody against | Techniques | Conjugated with | Brand | Reference |
|---|---|---|---|---|
| CD117 | FACS | PE-Cy7 | BioLegend | 105813 |
| | | APC | BioLegend | 105812 |
| CD41 | FACS | FITC | BioLegend | 133903 |
| CD61 | FACS | PE | BioLegend | 104307 |
| TER119 | FACS | Biotin | BioLegend | 116203 |
| SCA1 | FACS | Biotin | BioLegend | 108103 |
| CD3 | FACS | Biotin | BioLegend | 100243 |
| CD127 | FACS | Biotin | BioLegend | 135005 |
| CD19 | FACS | Biotin | BioLegend | 101504 |
| Streptavidin | FACS | APC | BioLegend | 405207 |
| CD150 | FACS | PerCp-Cy5.5 | BioLegend | 115921 |
| FLI-1 | Western blot/ChIP | NA | Abcam | ab-15289 |
| GATA1 | Western blot/ChIP | NA | Santa Cruz Biotechnologies | sc-1234 |
| LDB1 | Western blot/ChIP | NA | Santa Cruz Biotechnologies | sc-11198 |
| VCP | Western blot | NA | Abcam | ab-11433 |
| Goat anti-mouse | Western blot | IRDye800CW | LiCOR | 926-32210 |
| Donkey anti-goat | Western blot | IRDye800CW | LiCOR | 926-32214 |
| Goat anti-rabbit | Western blot | IRDye800CW | LiCOR | 926-32211 |
| FLI-1 | ChIP-Seq | NA | Santa Cruz Biotechnologies | sc-356 |
| Rabbit IgG | ChIP-qPCR | NA | Santa Cruz Biotechnologies | sc-2027 |
| Goat IgG | ChIP-qPCR | NA | Santa Cruz Biotechnologies | sc-2028 |

**Table S2 : List of primers (Related to Figure 1, 2, 3 and 4)**

| **Primers for RT-qPCR** | | |
|---|---|---|
| **Genes** | **Forward** | **Reverse** |
| **Actb** | AGCACAGCTTCTTTGCAG | GATGGAGGGGAATACAGC |
| **Fli-1** | GTCAATGTCAAGCGGGAGTA | ATGACTCTCCGTTCGTTGGT |
| **Lmo2** | GAAAGGAAGAGCCTGGAC | ATGGCTTTCAGGAAGTAGC |
| **Gp1ba** | TAGAGAGAAGGACCGAGTCA | GCTGGTCACTTTGGAGATAC |
| **Cd41** | CTGCTGACCCTGCTAGTTT | CCCTCTGCTGTCACTCTTC |
| **Thbs1** | CTCTGCTTTCACAATGGAGT | TTTTGCAGATGGTAACCGA |
| **Selp** | TGGAATGATGAACCCTGTTT | CAATGGTCTCGATGCACT |
| **Rab27b** | ATCCCAAATTCATCACCACA | ACCTTAAACGCTTTTCCTGA |
| **vWF** | TGCAGTTATCTCCTGGCT | AAACTCCCCAAGATACACAG |
| **Meis1** | ACGCTTTTTGTGACGCTTTT | TCCACTCGTTCAGGAGGAAC |
| **Trp53** | TGTCATCTTTTGTCCCTTCT | CTTATTGAGGGGAGGAGAGT |
| **Pdcd1lg2** | GGCCATAGTGATAATCCAGA | GAAGTCTCTTGAGGGTTTCC |
| **Stat1** | ACAGAAGGAGCTGGACAGTA | AAAAACATCTTGTGGAGCAG |
| **Tgfb1** | CGAAGCGGACTACTATGCTA | CGAATGTCTGACGTATTGAA |
| **Primers for ChIP-qPCR** | | |
| **Region** | **Forward** | **Reverse** |
| **β-Amylase** | CTCCTTGTACGGGTTGGT | AATGATGTGCACAGCTGAA |
| **Gp1ba pro** | TCACAGGAGCTGATTATCAG | AGACAGACAGTCCTTTGGAG |
| **Cd41 pro** | CTCTTGAATGCTGTGATGTG | AGGAAGTGGGTAAATGTCCT |
| **Tgfb1** | GGTGTCAGTAGCTTCTCCAG | GAAATGGGGTGACATAGAGA |

| | | |
|---|---|---|
| **Meis1 +94** | CTGGTGGCAAGAGTTACTTC | CCACTTGACTTTCTCCACA |
| **Meis1 +55** | GTTGAGGTTTAGGCACTCTG | AGAGGTTCTCACAGGAACAA |
| **Meis1 +48** | CACTGAGATAGGAACCTGGA | GCATTTCTTGTCACTTCCTC |
| **Stat1 +31** | CACACCCACTAGGACAAGTT | GTGTTATCACTCAGGCAGGT |
| **Trp53 +0.9** | GATTCTAGGCTGGTTCTGTG | TAAAACCGGATACTCGGTAA |
| **E2f2 +5.7** | AGAGTGTTTGCACTGTTTCC | GTGAGAGGCAAATAATCAGG |
| **Dusp1 +5.5** | AAAGGGAAACTCCTCAGTGT | GTCCGTGGTCTCAACTTAAC |
| **Pdcd1lg2 +8** | GATCAGGCTTTATTGCTCAC | TCTAGGCTCTTCAGTCTCCA |
| **Primers for screening Meis1 CRISPR clones** | | |
| **Viewpoint** | **Forward** | **Reverse** |
| ***Meis1 ∆94*** | ATGTCTCAAAACAAACAAAAGC | TGGAGTCACCTTGGGATT |
| ***Meis1 ∆55*** | TCTGACTCACAATTGAAAAAGG | GCACAGAGCGTGTAGACG |
| ***Meis1 ∆48*** | CCCATCATGTAGCATTGTG | AGAGCAATGGAGATATTTGC |
| **gRNA for deleting Meis1 enhancer** | | |
| | Sense | Antisense |
| ***5' Meis1 ∆94*** | GGTCTACAATAGCTCCTTCC | CGGAAGGAGCTATTGTAGAC |
| ***3' Meis1 ∆94*** | TGAGGGGTTAATGTTAGAGG | CCTCTAACATTAACCCCTCA |
| ***5' Meis1 ∆55*** | GCTATCTCACCCGACCCCCC | GGGGGGTCGGGTGAGATAGA |
| ***3' Meis1 ∆55*** | GCTGAGGAGGGTCGAGCCCT | AGGGCTCGACCCTCCTCAGC |
| ***5' Meis1 ∆48*** | ACTCAGGGCTGGCCGTTGGC | GCCAACGGCCAGCCCTGAGT |
| ***3' Meis1 ∆48*** | ACCTGCTGCTTTAGGTATTC | GAATACCTAAAGCAGCAGGT |

## TRANSPARENT METHODS

### Mice
Ethical approval was obtained from the Committee on the Ethics of Animal Experiments (DEC) of the Erasmus MC as well as from the was obtained from the Ministère Délégué de la Recherche et des Nouvelles Technologies, agreement no. 4936; Direction des Services Vétérinaires, agreement n°69266317 and 7462.
All animal experiments were carried out according to institutional and national and EU guidelines.

### Megakaryocyte differentiation, staining and transduction
Fetal liver cells were isolated from E14.5 embryos and homogenized to obtain a single-cell suspension. cKit $(CD117)^+$ cells were isolated by incubating the cells with biotin-conjugated CD117 antibody for 30 minutes followed by 30 minutes incubation with magnetic streptavidin microbeads (Miltenyi biotech) in a buffer containing PBS, 2 mM EDTA and 0.5 % biotin-free bovine serum albumin (Thermo Fisher). Cells were passed in a LS column (Miltenyi biotech) attached to a magnet, washed 3 times with a buffer containing PBS and 2 mM EDTA and the $cKit^+$ cells were collected by adding the plunger to the column. The positive population was then transduced with 20 µL of lentivirus in presence of 8 µg/mL polybrene and cultured for 5 days in IMDM (Life technologies) supplemented with 10 % FCS, 50 ng/mL mTPO, 10 ng/mL mSCF. Cells were then washed 5 times with IMDM, transduced with lentiviruses as above and cultured for 4 additional days in IMDM supplemented with 10 % FCS, 50 ng/mL mTPO.

### Constructs
gRNAs targeting the *Meis1* enhancers (sequences in Table S1) were cloned in the BbsI site of the pX459 plasmid (gift from Feng Zhang Addgene plasmid # 62988) (Ran et al., 2013).

### Isolation of MkP from WT and FLI-1 KO mice
MkP were isolated from the bone marrow of WT and FLI-1 KO mice following the protocol described by Pronk *et al.* and using a FACS Aria III (Pronk et al., 2007; Starck et al., 2010). The antibodies used are listed in Table S2.

### Western blot
Western blot experiments were performed as described previously (Giraud et al., 2014). The primary and secondary antibodies used are listed in Table S1.

### Chromatin immunoprecipitation (ChIP)
ChIP experiments were performed as described previously(Giraud et al., 2014). The antibodies used are listed in Table S1.

### cDNA synthesis
500 ng of RNA isolated using the TRI Reagent (Sigma-Aldrich) was retro-transcribed into cDNA by using the SuperScript II First Strand Synthesis System (Life technologies) and oligo-dT primers (Life technologies) according to manufacturer's instruction.

### Real-time PCR
Real-time PCR was performed using the platinum Taq Polymerase (Life technologies), SYBR green (Biorad) and the primers listed in Table S2 on a Biorad CFX96 apparatus. The specificity and the linear efficiency of each primer have been tested using different amount of mouse genomic DNA or mouse cDNA. Melting curves were checked to confirm the amplification of only the expected amplicon. A negative control was performed in parallel with the real experiment. For gene expression, qPCR reactions were performed in technical duplicates from 5 ng of cDNA and relative DNA levels were calculated using the $\Delta\Delta$Ct method and were normalized to the expression of the *Actb* RNA level. For ChIP, qPCR reactions were performed in technical duplicates from 1/25 of ChIP DNA solution and 1/67 of Input DNA solution. Relative DNA levels were calculated using the $\Delta\Delta$Ct method and were normalized to the Input level.

### T2C
T2C experiments were performed as described(Kolovos et al., 2014, 2018) using HindIII as a first enzyme and DpnII as second enzyme. The visualization of the interactome of specific DNA fragments (viewpoints) was performed as described before(Kolovos et al., 2014, 2018).

**3C-Seq**
3C-Seq experiments were performed as described by Stadhouders *et al.* by using HindIII as first enzyme and NlaIII (*Fut8* enhancer as a viewpoint) or DpnII (*Fut8* promoter as a viewpoint) as second enzyme (Stadhouders et al., 2013). The primers used are listed in Table S1. The samples were sequenced in Illumina Hiseq2500. Raw data were mapped to the reference genome (mm9) and analyzed using the r3Cseq (Thongjuea et al., 2013).

**Data availability**
The accession number for the FLI-1 ChIP-Seq, the RNA-Seq and the T2C experiments reported in this paper is SRA: SRP158024.

**Cell culture and treatment**
MEL and HEK cells were cultured in DMEM medium (Lonza) supplemented with 10 % fetal calf serum and penicillin/streptomycin. Cells were induced with 2 % DMSO for 2 days. Expression of control shRNA or shRNAs against Fli-1 mRNA was induced by treating the cells with 100 ng/mL doxycyclin 2 days prior to DMSO treatment. L8057 cells were cultured in vol/vol IMDM/RPMI1640 (Life technologies) supplemented with10 % fetal calf serum and penicillin/streptomycin.

**Lentivirus production**
20 µg of pLKO.1 (containing the shRNA), 15 µg PAX2 and 5 µg VSV-G plasmids were incubated for 20 minutes with 100 µg PEI and applied to HEK cells. Cells were cultured for 4 hours in DMEM supplemented with 1 % FCS and then for 3 days in DMEM supplemented with 10 % FCS and penicillin/streptomycin. Each day, the medium containing the lentiviral particles was collected and stored at 4 °C. The collected medium was then centrifuged 5 minutes at 1500 rpm, passed through a 0.45 µm filter and ultra-centrifuged 2:15 hours at 20000 rpm at 4 °C. Supernatant was discarded and the lentivirus pellet was resuspended in 100 µL cold PBS. 20 µL were used to transduce cells in presence of 8 µg/mL polybrene.

**ChIP in transduced fetal liver cells**
Fetal liver cells were isolated from E14.5 embryos and homogenized to obtain a single-cell suspension. Cells were then transduced with 20 µL of lentivirus in presence of 8 µg/mL polybrene and cultured for 3 days in IMDM (Life technologies) supplemented with 10 % FCS, 50 ng/mL mTPO, 10 ng/mL mSCF. Cells were then crosslinked using 1 % formaldehyde and quenched with 0.125 M Glycine. Cells were lysed in a freshly made TE/NP40 buffer (10 mM Tris pH 8, 1 mM EDTA, 0.5 % NP-40, 1 X PIC (Roche Diagnostics)) for 10 minutes in ice. After a centrifugation of 5 minutes at 4 ˚C and 2500 g, the nuclei pellet was resuspend in the NEB buffer (50 mM Tris pH 8, 1 % SDS, 10 mM EDTA, 1 X PIC) and incubated for 10 minutes in ice. The chromatin was then sheared in the Bioruptor (Diagenode) during 30 minutes with the cycle 30 sec ON 30 sec OFF at high intensity. The sonicated chromatin was then precleared using Protein A/G coated agarose beads in the RIPA-150 buffer (50 mM Tris pH8, 0.15 M NaCl, 1 mM EDTA, 0.1 % SDS, 1 % Triton-X100, 0.1 % Na DOC) supplemented with 1 X PIC and 1 % BSA for 3 h at 4 ˚C. Beads were then discarded, new Protein A/G agarose beads attached to 5 µg of specific IgG were added to the pre-cleared chromatin and the mixture was incubated overnight at 4 ˚C. Beads were then washed once with RIPA-150 buffer, twice with RIPA-500 buffer (50 mM Tris pH8, 0.5 M NaCl, 1 mM EDTA, 0.1 % SDS, 1 % Triton-X100, 0.1 % Na DOC), twice with RIPA-$LiCl_2$ buffer (50 mM Tris pH 8, 1 mM EDTA, 1 % NP-40, 0.7 % Na DOC, 0.5 M $LiCl_2$) and twice with TE buffer (10 mM Tris pH 8, 1 mM EDTA). Chromatin was then eluted in Elution Buffer (1% SDS, 0.1 M $NaHCO_3$) and decrosslinked overnight at 65 ˚C. Proteins were then degraded by 20 µg Proteinase K for 1 h at 56 ˚C and chromatin was purified using Phenol:Chloroform, precipitated with 1 volume of isopropanol and washed once with 70 % EtOH.

**ChIP-Seq**
The samples were sequenced at Illumina Hiseq2500. Raw read files were aligned to the mouse genome (NCBI build 37; mm9) using Bowtie (Langmead et al., 2009) and discarding reads mapping to multiple genomic locations. Data processing and analysis were mainly performed as previously described (Soler et al., 2011 ; Stadhouders et al., 2015 ; Kolovos et al., 2016). The ShortRead package (Morgan et al., 2009) was used to convert these into genome-wide coverage files. Using negative binomial distributions, we assigned P-values and false discovery rates (FDR) to each binding region (Rozowsky et al., 2009), and the final peak lists were compiled according with the following criteria: ≥20 read counts at each peak summit and FDR ≤0.001 (using non-specific, IgG, ChIP-seq data as background). Overlaps between binding peaks in the different datasets were identified using

the “findOverlaps” function from the GenomicRanges suite, while the iRanges package (Lawrence et al., 2013) was used in custom R scripts for annotating the genomic location of peaks and defining the closest gene to each. For motif analysis, +/- 100 bp from the centre of the peaks were selected using the rGADEM Bioconductor package (Droit et al., 2010), which then compares discovered sequences to the Jaspar database (Khan et al., 2018) based on similarity score with TOMTOM (Gupta et al., 2007).
FLI-1, GATA1 and TAL1 ChIP-seq in megakaryocytes were acquired from the ENCODE database (Pimkin et al., 2014; Yue et al., 2014) and analyzed as previously described.The coordinates of the FLI-1 total binding regions and common binding regions in MEL cells and in Megakaryocytes are listed in **Table S3**.

**RNA-Seq**
RNA were isolated with either the miRNeasy Mini Kit (QIAGEN) (for MEL cells) or miRNeasy MicroKit (QIAGEN) (for MkP) according to manufacturer’s instructions. The samples were sequenced at Illumina Hiseq2500. Reads were aligned to the mouse genome (NCBI build 37; mm9) using Tophat (Trapnell et al., 2009) and default parameters (“no-coverage-search”, “segment-length 18” as input options) while reads that did not map uniquely were discarded. Uniquely mapped reads were counted per RefSeq gene exon using HTseq (Anders et al., 2015) and statistical analysis of differentially-expressed genes was performed via DESeq (Anders and Huber, 2010). In all cases up- and downregulated genes were selected to have at least ±0.6 log2 fold-change in RNA levels. GO analysis was performed by PANTHER (Mi et al., 2019). The list of mis-regulated genes in MEL cells and in MkP after FLI-1 repression are listed in **Table S4**.

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