Science Advances
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# Supplementary Materials for

## Screening of ETO2-GLIS2–induced Super Enhancers identifies targetable cooperative dependencies in acute megakaryoblastic leukemia

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**The PDF file includes:**



**Other Supplementary Material for this manuscript includes the following:**

**Figure S1**

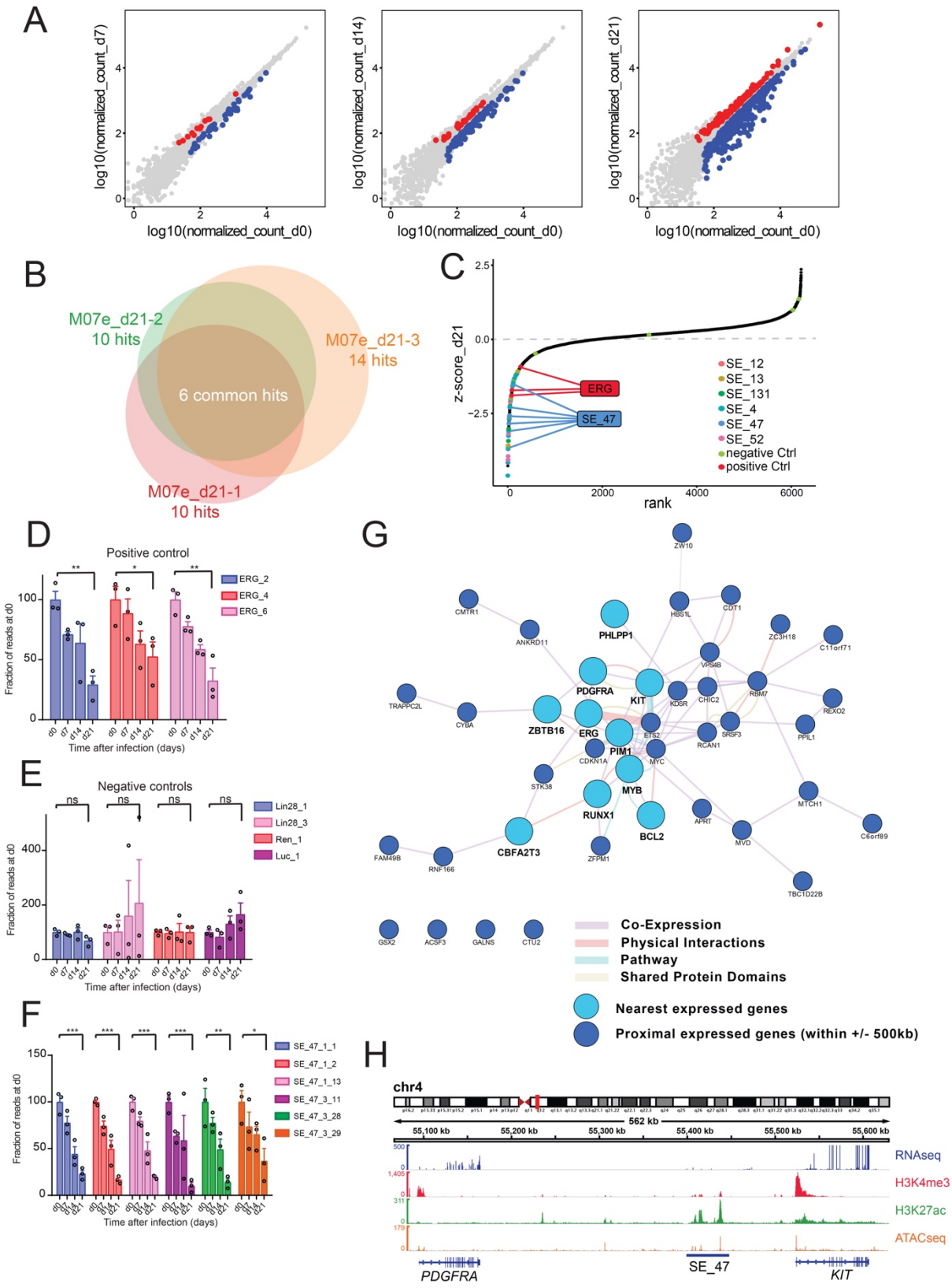
A
log10(normalized_count_d7)
log10(normalized_count_d14)
log10(normalized_count_d21)
log10(normalized_count_d0)
B
M07e_d21-2
10 hits
M07e_d21-3
14 hits
6 common hits
M07e_d21-1
10 hits
C
z-score_d21
rank
ERG
SE_47
SE_12
SE_13
SE_131
SE_4
SE_47
SE_52
negative Ctrl
positive Ctrl
D
Positive control
Fraction of reads at d0
Time after infection (days)
ERG_2
ERG_4
ERG_6
E
Negative controls
ns
Lin28_1
Lin28_3
Ren_1
Luc_1
F
SE_47_1_1
SE_47_1_2
SE_47_1_13
SE_47_3_11
SE_47_3_28
SE_47_3_29
G
ZW10
CMTR1
ANKRD11
PHLPP1
HBS1L
CDT1
ZC3H18
C11orf71
VPS4B
PDGFRA
KIT
KDSR
CHIC2
RBM7
REXO2
TRAPPC2L
CYBA
ZBTB16
ERG
ETS2
RCAN1
SRSF3
PPIL1
CDKN1A
PIM1
MYC
STK38
MYB
RUNX1
BCL2
APRT
MVD
MTCH1
C6orf89
FAM49B
RNF166
CBFA2T3
ZFPM1
TBC1D22B
GSX2
ACSF3
GALNS
CTU2
Co-Expression
Physical Interactions
Pathway
Shared Protein Domains
Nearest expressed genes
Proximal expressed genes (within +/- 500kb)
H
chr4
562 kb
RNAseq
H3K4me3
H3K27ac
ATACseq
PDGFRA
SE_47
KIT

**Figure S1. CRISPRi screening of Super Enhancer in ETO2-GLIS2$^{+}$ AMKL cell line M07e**

**A)** Scatter plots showing significantly depleted (blue) or enriched (red) sgRNA at day 7, 14 and 21 compared to day 0. Only sgRNA with normalized read coverage over 40x at day 0 and with p-value < 0,05 are highlighted. Dots represent average of normalized read counts across the 3 replicates.

**B)** Venn Diagram showing overlap of significant (FDR < 0,25) hits between the 3 replicates of the screen as determined by maximum likelihood of enrichment.

**C)** Dot plot depicting changes in representation of the 6471 sgRNAs covered over 40x during 21 days of culture. sgRNA z-scores were calculated by subtracting to the log2 fold change of average normalized counts at d21 compared to d0 (Log2FC(d21/d0)) the average of all sgRNA Log2FC(d21/d0) and dividing by the standard deviation of all Log2FC(d21/d0) and are plotted in ascending order. Positive control sgRNAs targeting ERG gene are marked in red; negative control sgRNAs are marked in green. sgRNAs against common hits and significantly depleted are colored and sgRNA against SE_47 and ERG are highlighted.

**D)** Bargraph representing variations of normalized counts of sgRNA targeting ERG, positive control of the screen. Data are represented as ratio of counts at day 0. Data are represented as mean± SEM, n=3, statistical significance is determined using Student's *t*-test, * p<0.05, ** p<0.01.

**E)** Bargraph representing variations of normalized counts of sgRNA targeting Luciferase, Renilla and Lin28, negative controls of the screen. Data are represented as ratio of counts at day 0. Data are represented as mean± SEM, n=3, statistical significance is determined using Student's *t*-test, ns=not significant.

**F)** Bargraph representing variations of normalized counts of sgRNA targeting SE_47. Data are represented as ratio of counts at day 0. Data are represented as mean± SEM, statistical significance is determined using Student's *t*-test, * p<0.05, ** p<0.01, *** p<0.001

**G)** Network of nearest expressed genes and proximal expressed genes of top hits Super Enhancer identified through CRISPRi screening. Co-Expression, Physical interactions, common pathway interactions and shared protein domains were included in the network.

**H)** Gene track of SE_47 locus on chromosome 4, including proximal genes *KIT* and *PDGFRA,* showing normalized read density histograms of H3K4me3 ChIP-seq, H3K27ac ChIP-seq and ATAC-seq (bottom panel) in M07e cells. Read densities are shown as unique reads per million.

**Figure S2**

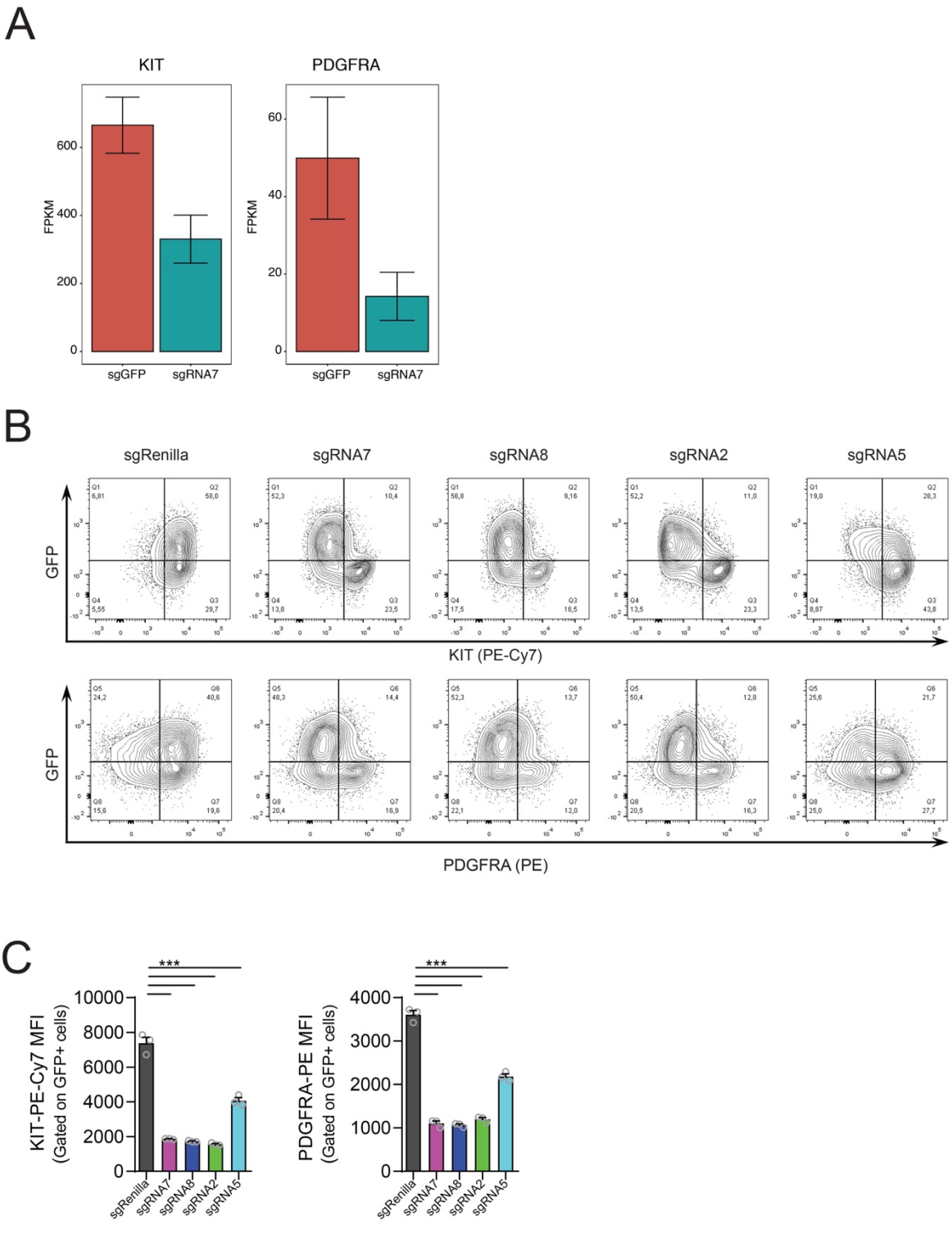
A
KIT
PDGFRA
FPKM
sgGFP
sgRNA7
B
sgRenilla
sgRNA7
sgRNA8
sgRNA2
sgRNA5
GFP
KIT (PE-Cy7)
PDGFRA (PE)
C
KIT-PE-Cy7 MFI (Gated on GFP+ cells)
PDGFRA-PE MFI (Gated on GFP+ cells)
***

**Figure S2. SEKIT inactivation inhibits KIT and PDGFRA protein expression**

**A)** Quantification in Fragments per kilobase per million mapped reads (FPKM) of *KIT* and *PDGFRA* expressions in M07e cells expressing control sgRNA against GFP (brown bar) or sgRNA7 targeting SEKIT (green bar) as assessed by RNA-seq.

**B)** Representative flow cytometry analyses showing KIT (higher panel) and PDGFRA (lower panel) protein expression at cell surface in M07e cells following CRISPRi targeting of SEKIT locus with indicated sgRNAs compared to control sgRenilla.

**C)** Quantification of KIT and PDGFRA protein expression shown as mean fluorescent intensities of PE-Cy7 and PE respectively gated on transduced $GFP^+$ cells. Mean ± SEM, n=3, significance is determined using Student's *t*-test, *** $p<0.001$.

**Figure S3**

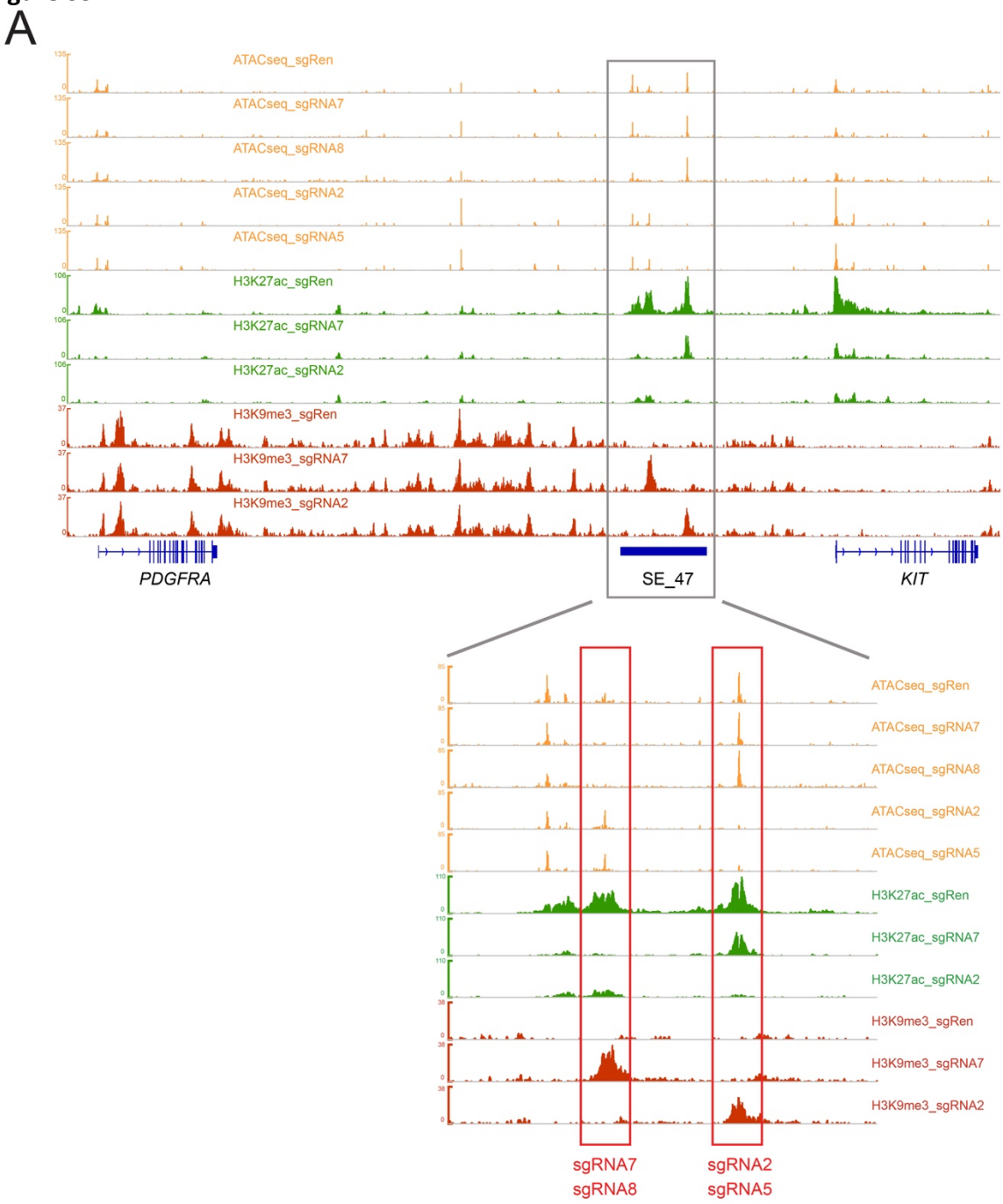
A
ATACseq_sgRen
ATACseq_sgRNA7
ATACseq_sgRNA8
ATACseq_sgRNA2
ATACseq_sgRNA5
H3K27ac_sgRen
H3K27ac_sgRNA7
H3K27ac_sgRNA2
H3K9me3_sgRen
H3K9me3_sgRNA7
H3K9me3_sgRNA2
PDGFRA
SE_47
KIT
sgRNA7
sgRNA8
sgRNA2
sgRNA5
B
% of BFP+ cells
sgGFP
sgRNA2
sgRNA7
ns
Time after infection (days)
C
KIT expression (relative to GAPDH)
ns
sgGFP
sgRNA2
sgRNA7

**Figure S3. CRISPRi targeting of SE is on target**

**A)** Gene track showing normalized read density histograms of ATAC-seq (orange tracks), H3K27ac ChIP-seq (green tracks) and H3K9me3 ChIP-seq (bordeaux tracks) following CRISPRi targeting of SE_47 with indicated sgRNAs compared to control sgRenilla in M07e cells. Lower panel displays a blow up of grey box highlighted SE_47 region. Targeted regions and location of sgRNAs are highlighted by red boxes.

**B)** Percentage of $BFP^+$ HEL 5J20 cells following CRISPRi targeting of SE_47 locus with indicated sgRNAs compared to control sgGFP and normalized to day 4 after infection. Mean ± SEM, n=3, statistical significance is determined using Student's *t*-test, ns=not significant.

**C)** Quantitative PCR showing *KIT* expression in HEL 5J20 cells following CRISPRi targeting of SE_47 locus with indicated sgRNAs compared to control sgGFP. Mean ± SEM, n=3, statistical significance is determined using Student's *t*-test, ns=not significant.

**Figure S4**

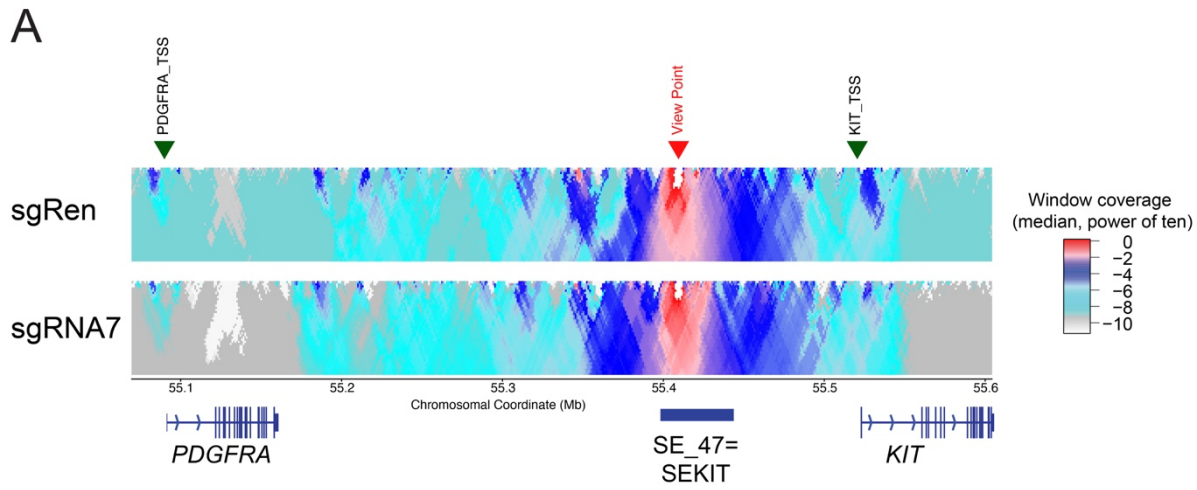
A
PDGFRA_TSS
View Point
KIT_TSS
sgRen
sgRNA7
Window coverage
(median, power of ten)
0
−2
−4
−6
−8
−10
55.1
55.2
55.3
55.4
55.5
55.6
Chromosomal Coordinate (Mb)
PDGFRA
SE_47=
SEKIT
KIT

**Figure S4. SEKIT activity is required for its interaction with *KIT* and *PDGFRA* promoters**
**A)** 4C-seq domainograms with viewpoint on SEKIT (red arrowhead) showing cis-contacts between the SEKIT locus and proximal elements in M07e cells expressing non targeting CRISPRi (sgRen, top panel) or CRISPRi targeting SEKIT (sgRNA7, bottom panel). *PDGFRA* and *KIT* transcription start sites are shown with green arrowheads, and *PDGFRA*, SEKIT and *KIT* locations are shown below.

**Figure S5**

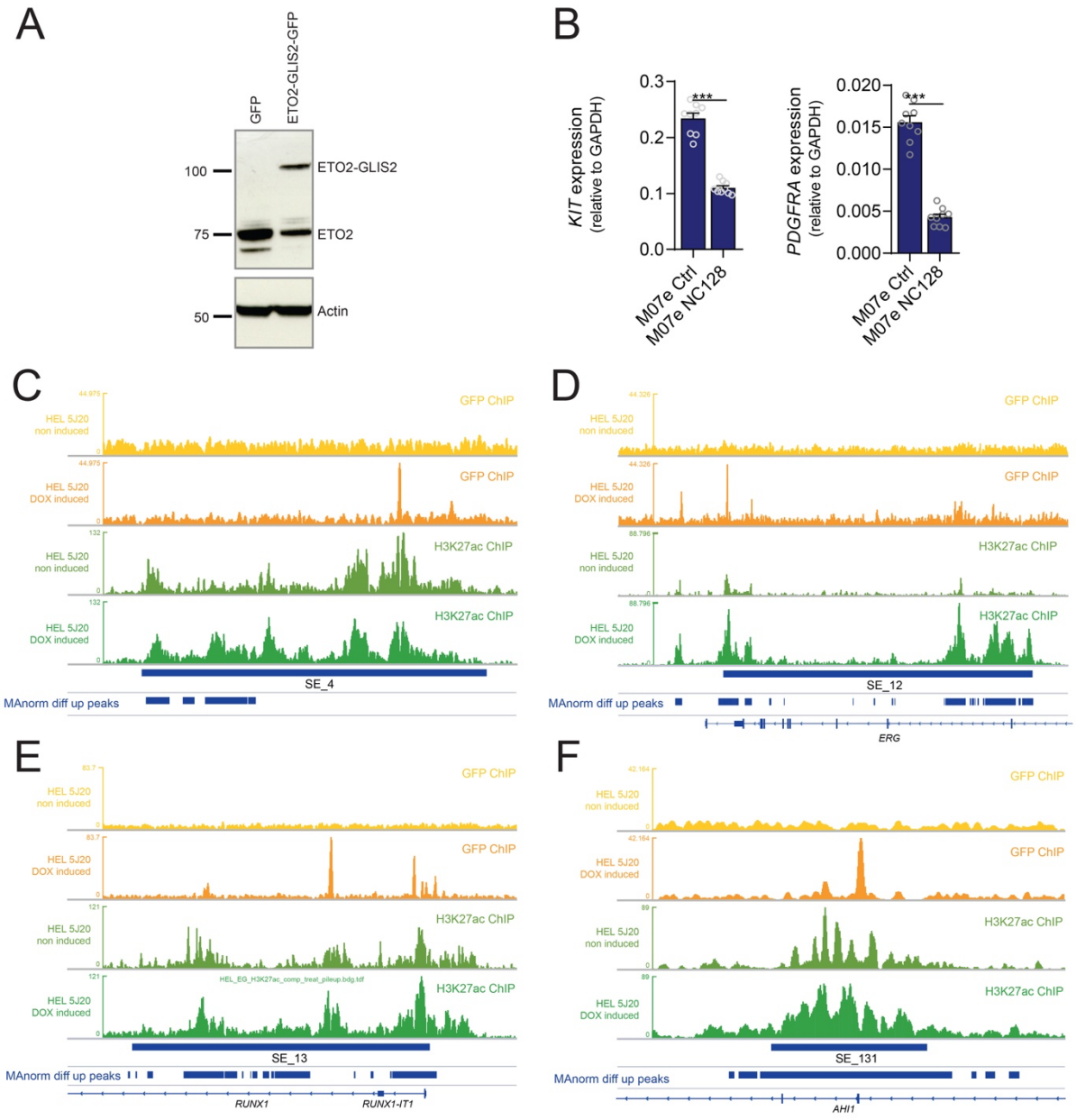
A
GFP
ETO2-GLIS2-GFP
100
75
50
ETO2-GLIS2
ETO2
Actin
B
KIT expression (relative to GAPDH)
PDGFRA expression (relative to GAPDH)
***
***
M07e Ctrl
M07e NC128
C
D
E
F
GFP ChIP
H3K27ac ChIP
HEL 5J20 non induced
HEL 5J20 DOX induced
SE_4
SE_12
SE_13
SE_131
MAnorm diff up peaks
RUNX1
RUNX1-IT1
ERG
AHI1

**Figure S5. SEKIT is induced by ETO2-GLIS2**

**A)** Western blot showing ETO2 and ETO2-GLIS2 expression in an individual clone of HEL 5J20 cells upon doxycycline induction of ETO2-GLIS2 expression compared to empty vector (GFP).

**B)** qPCR showing *KIT* and *PDGFRA* expression in M07e cells expressing NC128 or control peptides. Mean ± SEM, n=3, statistical significance is determined using Student's *t*-test, *** $p<0.001$

**C-F** Gene tracks showing normalized read density histograms of H3K27Ac ChIP-seq analysis at **C)** SE_4, **D)** SE_12, **E)** SE_13 and **F)** SE_131 locci upon ETO2-GLIS2 (EG) expression induction in HEL 5J20 cells compared to non-induced cells (khaki and green tracks respectively) and read densities of GFP ChIP-seq in non-induced versus doxycycline induced HEL 5J20 cells (yellow and orange tracks respectively) showing EG binding in SEKIT locus. Read densities are shown as unique reads per million. Differentially upregulated peaks as determined using MAnorm algorithm are shown at the bottom.

**Figure S6**

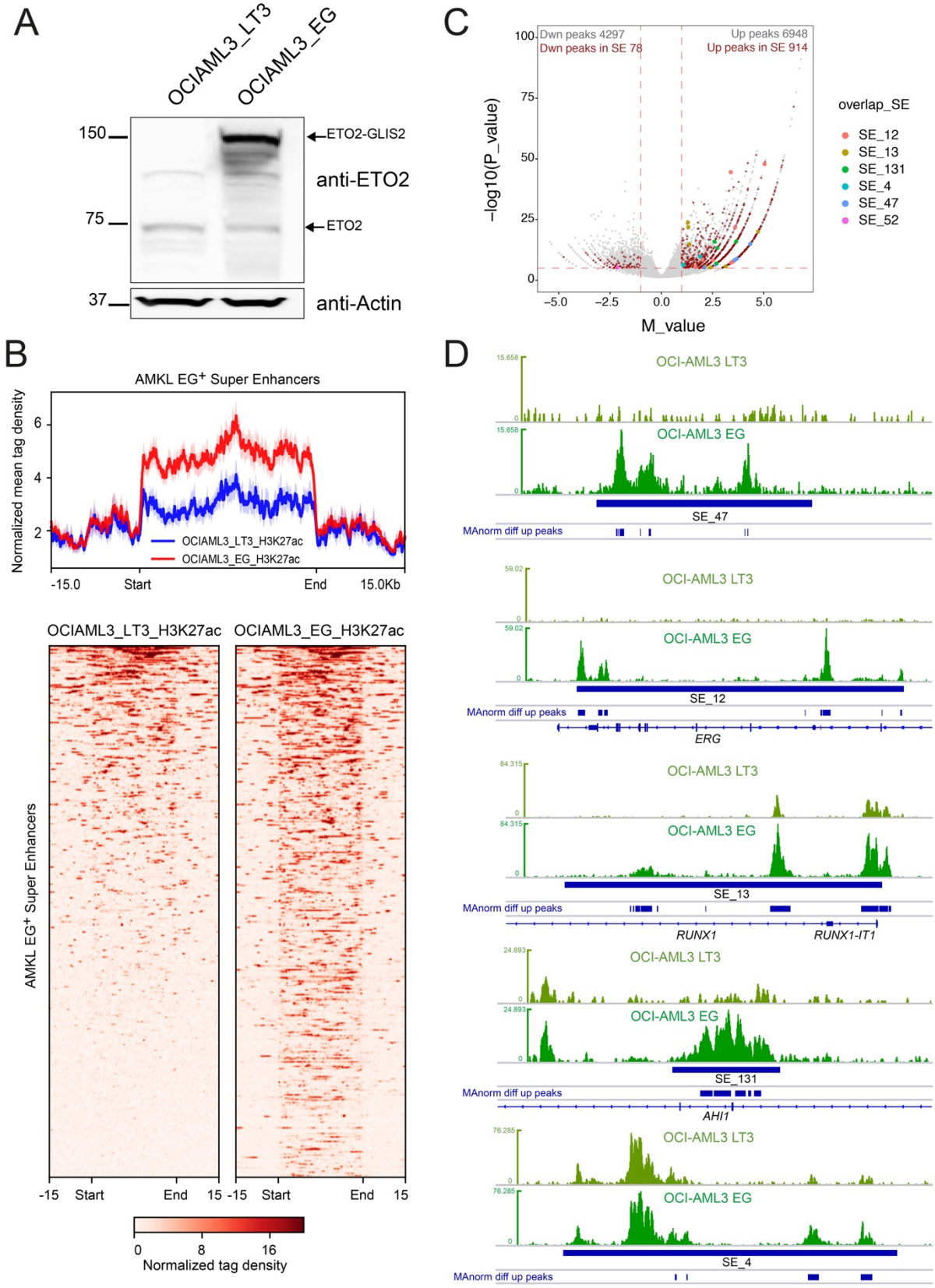
A
OCIAML3_LT3
OCIAML3_EG
150
75
37
ETO2-GLIS2
anti-ETO2
ETO2
anti-Actin
B
AMKL EG+ Super Enhancers
Normalized mean tag density
OCIAML3_LT3_H3K27ac
OCIAML3_EG_H3K27ac
-15.0
Start
End
15.0Kb
OCIAML3_LT3_H3K27ac
OCIAML3_EG_H3K27ac
AMKL EG+ Super Enhancers
-15
Start
End
15
Normalized tag density
C
Dwn peaks 4297
Dwn peaks in SE 78
Up peaks 6948
Up peaks in SE 914
−log10(P_value)
M_value
overlap_SE
SE_12
SE_13
SE_131
SE_4
SE_47
SE_52
D
OCI-AML3 LT3
OCI-AML3 EG
SE_47
MAnorm diff up peaks
SE_12
ERG
SE_13
RUNX1
RUNX1-IT1
SE_131
AHI1
SE_4

**Figure S6. Inducible ETO2-GLIS2 expression in OCI-AML3 cells induces AMKL specific SE activation**

**A)** Western blot showing ETO2-GLIS2 expression in OCI-AML3 cells transduced with empty vector (OCI-AML3_LT3) or doxycycline-inducible ETO2-GLIS2 expressing vector (OCI-AML3_EG) upon doxycycline induction.

**B)** Profile plot of normalized mean tag density (top panel) and Heatmap of normalized tag density (bottom panel) of H3K27ac ChIP-seq in OCI-AML3 cells stably transduced with empty vector (OCI-AML3_LT3) or doxycycline-inducible ETO2-GLIS2 expressing vector (OCI-AML3_EG) after doxycycline treatment at 448 Super Enhancer regions defined in ETO2-GLIS2 AMKL cells.

**C)** Volcano plot showing M value against -log10(P-value) of H3K27ac peaks upon differential analysis using MAnorm. Peaks overlapping Super Enhancer regions defined in ETO2-GLIS2 AMKL cells are highlighted in claret and peaks overlapping top hit SE from CRISPRi screen are highlighted in colors.

**D)** Gene tracks showing normalized read density histograms of H3K27ac ChIP-seq analysis at top hit SE from CRISPRi screen loci upon doxycycline treatment of OCI-AML3 cells stably transduced with empty vector (OCI-AML3_LT3) or doxycycline-inducible ETO2-GLIS2 expressing vector (OCI-AML3_EG) (khaki and green tracks respectively). Read densities are shown as unique reads per million. Differentially upregulated peaks as determined using MAnorm algorithm are shown at the bottom.

**Figure S7**

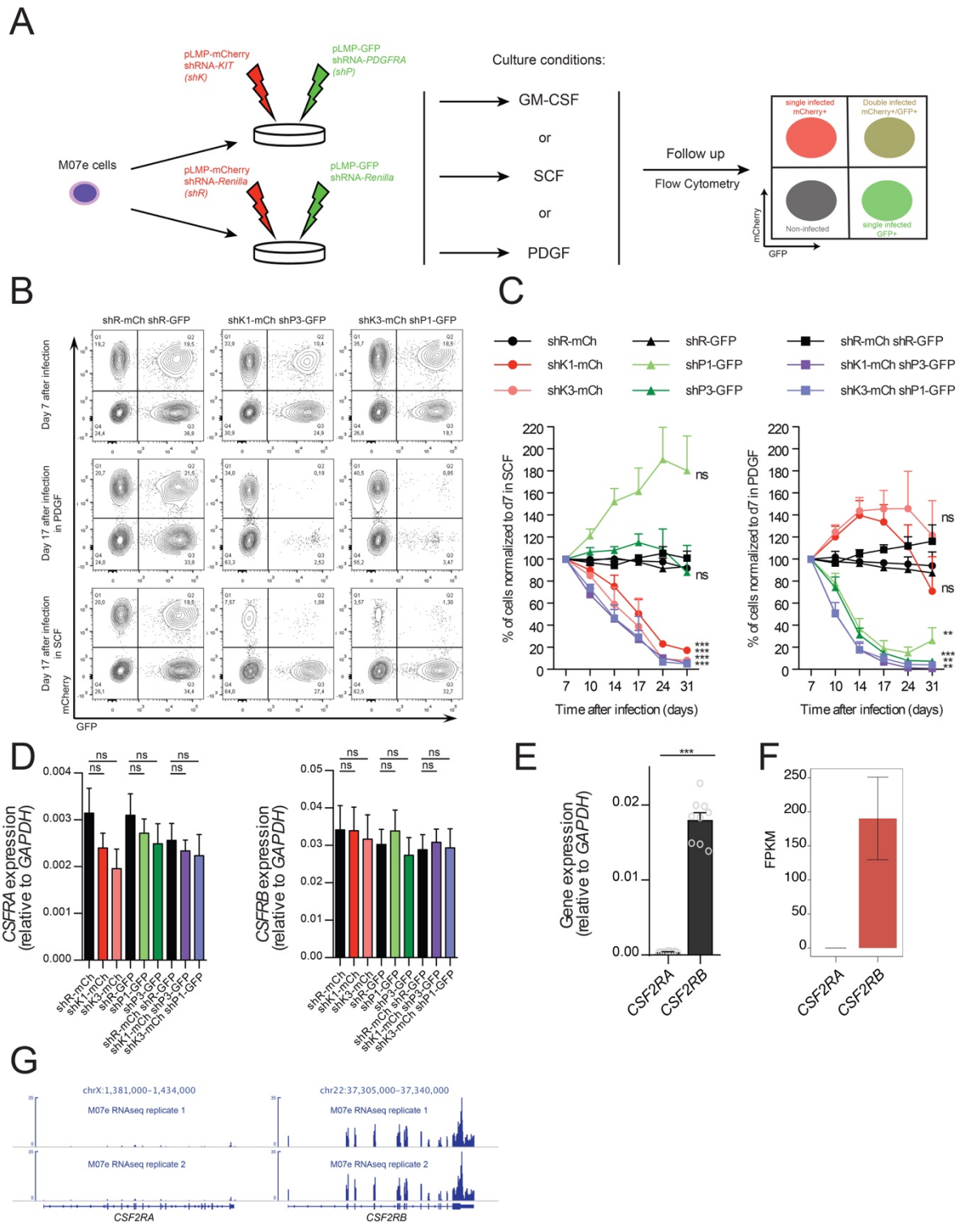
A
pLMP-mCherry shRNA-KIT (shK)
pLMP-GFP shRNA-PDGFRA (shP)
M07e cells
pLMP-mCherry shRNA-Renilla (shR)
pLMP-GFP shRNA-Renilla
Culture conditions:
GM-CSF
or
SCF
or
PDGF
Follow up
Flow Cytometry
single infected mCherry+
Double infected mCherry+/GFP+
Non-infected
single infected GFP+
mCherry
GFP
B
shR-mCh shR-GFP
shK1-mCh shP3-GFP
shK3-mCh shP1-GFP
Day 7 after infection
Day 17 after infection in PDGF
Day 17 after infection in SCF
mCherry
GFP
C
shR-mCh
shK1-mCh
shK3-mCh
shR-GFP
shP1-GFP
shP3-GFP
shR-mCh shR-GFP
shK1-mCh shP3-GFP
shK3-mCh shP1-GFP
% of cells normalized to d7 in SCF
% of cells normalized to d7 in PDGF
Time after infection (days)
ns
***
**
D
CSFRA expression (relative to GAPDH)
CSFRB expression (relative to GAPDH)
E
Gene expression (relative to GAPDH)
CSF2RA
CSF2RB
F
FPKM
G
chrX:1,381,000-1,434,000
chr22:37,305,000-37,340,000
M07e RNAseq replicate 1
M07e RNAseq replicate 2
CSF2RA
CSF2RB

**Figure S7. shRNA targeting of *KIT* and *PDGFRA* is on target**

**A-C,** Two independent shRNAs targeting either *KIT* (shK) or *PDGFRA* (shP) were expressed in M07e cells separately or in combination. shK are expressed with mCherry (mCh) in cells while shP are expressed with GFP. Corresponding shRNAs targeting Renilla (shR) were used as control. 7 days after transduction, cells were washed and maintained in the presence of PDGF-AA or SCF as indicated.

**A)** Schematic depicting experimental design.

**B)** Representative flow cytometry analyses of shRNA expressing cells at day 7 and day 17 after infection.

**C)** Percentage of M07e cells expressing indicated shRNAs normalized to day 7 after infection. Mean ± SEM, n=3, significance is determined using Student's *t*-test, statistical significance is determined using Student's *t*-test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns=not significant.

**D)** Quantitative PCR showing *CSF2RA* and *CSF2RB* expression at day 4 after infection. Mean ± SEM, n=3, significance is determined using Student's *t*-test, statistical significance is determined using Student's *t*-test, ns=not significant.

**E)** Quantitative PCR comparing *CSF2RA* and *CSF2RB* expression in M07e cells. Mean ± SEM, n=3, significance is determined using Student's *t*-test, ***$p < 0.001$

**F)** Quantification in Fragments per kilobase per million mapped reads (FPKM) of *CSFR2A* and *CSFR2B* expressions in M07e cells as assessed by RNA-seq.

**G)** Gene tracks showing normalized read density histograms at *CSF2RA* and *CSF2RB* genes in two replicates of M07e cells RNA-seq.

**Figure S8**

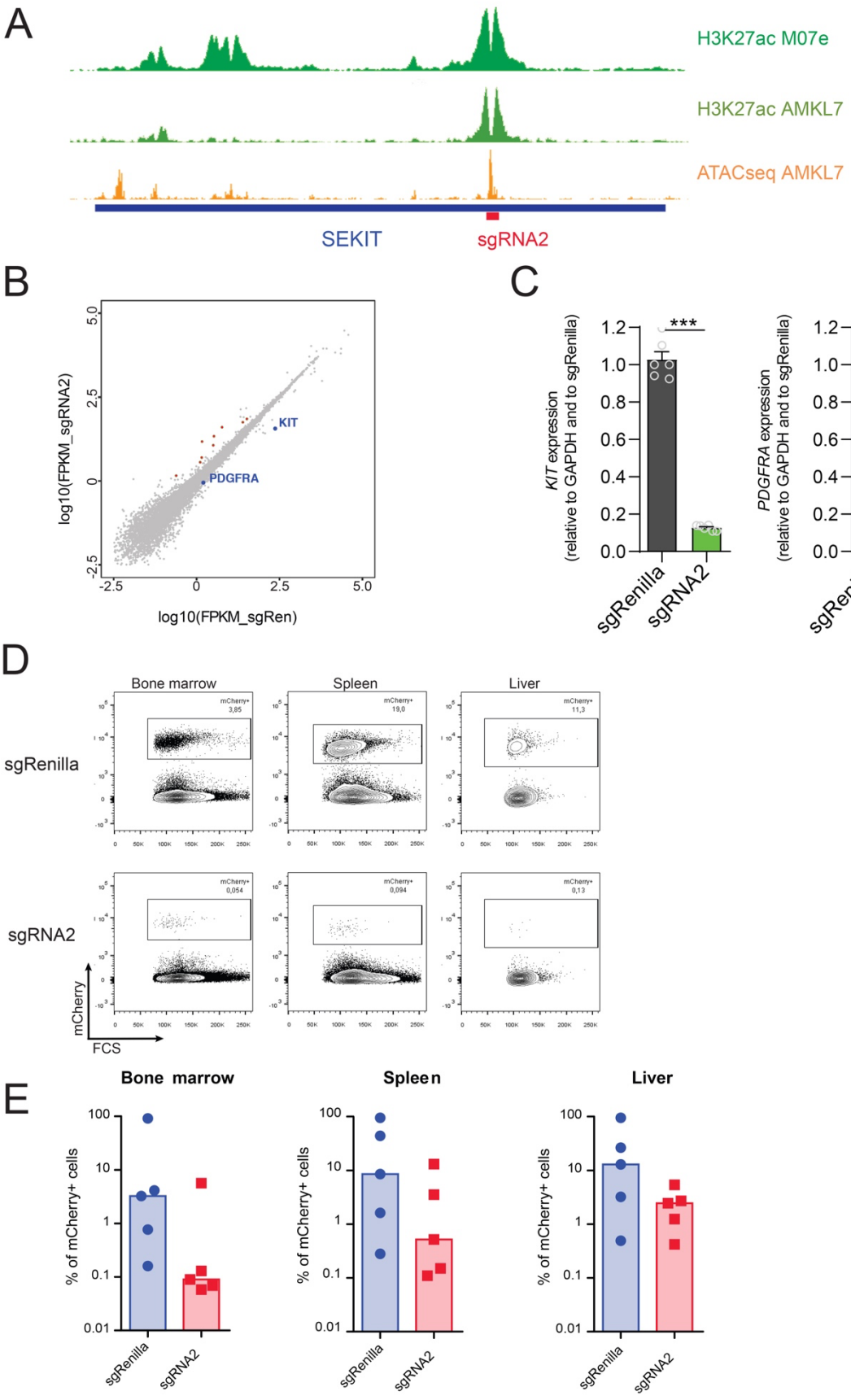
A
H3K27ac M07e
H3K27ac AMKL7
ATACseq AMKL7
SEKIT
sgRNA2
B
log10(FPKM_sgRNA2)
log10(FPKM_sgRen)
KIT
PDGFRA
C
KIT expression (relative to GAPDH and to sgRenilla)
PDGFRA expression (relative to GAPDH and to sgRenilla)
***
sgRenilla
sgRNA2
D
Bone marrow
Spleen
Liver
sgRenilla
sgRNA2
mCherry
FCS
E
Bone marrow
Spleen
Liver
% of mCherry+ cells
sgRenilla
sgRNA2

**Figure S8. Inhibition of SEKIT impairs AMKL progression *in vivo***

**A)** Gene track showing normalized read density histograms of H3K27ac ChIP-seq and ATAC-seq (bottom panel) in AMKL7 patient cells (kaki and orange respectively) and H3K27ac ChIP-seq in M07e cells (green) at SEKIT locus. Read densities are shown as unique reads per million. Location of sgRNA2 is shown in red.

**B)** Scatter plot showing genes whose expression is significantly altered following CRISPRi targeting of SEKIT with sgRNA2 compared to control sgRenilla in AMKL7 patient cells. Dots represent average of $\log_{10}$FPKM across the 2 replicates. Green dots $p<0.05$, red dots $p<0.05$ and >2-fold change. Statistical significance is determined using Student's *t*-test. *KIT* and *PDGFRA* genes are highlighted.

**C)** qPCR showing *KIT* and *PDGFRA* expression following CRISPRi targeting of SE_KIT with sgRNA2 compared to control sgRenilla in AMKL7 patient cells. Mean ± SEM, n=6, Student's *t*-test, ***$p < 0.001$.

**D)** Representative flow cytometry analyses of sgRNA2- or sgRenilla-transduced AMKL7$^{luc}$mCherry$^{+}$ cells in the bone marrow, spleen and liver of xenografted mice.

**E)** Quantification of mCherry$^{+}$ cells as analyzed in panel **a**. Mean ± SEM, n=5.

**Figure S9**

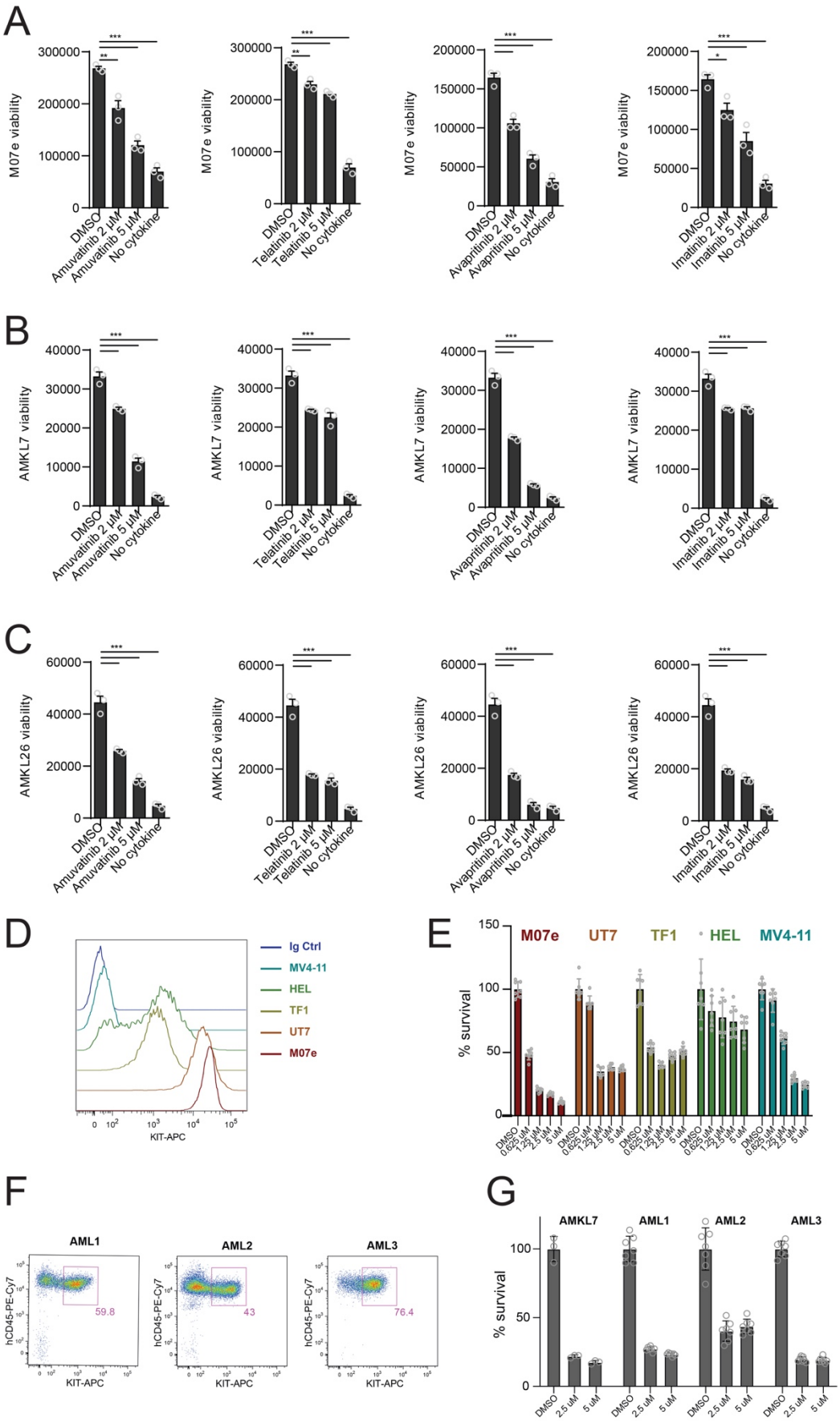
A
M07e viability
DMSO
Amuvatinib 2 μM
Amuvatinib 5 μM
No cytokine
Telatinib 2 μM
Telatinib 5 μM
Avapritinib 2 μM
Avapritinib 5 μM
Imatinib 2 μM
Imatinib 5 μM
B
AMKL7 viability
C
AMKL26 viability
D
Ig Ctrl
MV4-11
HEL
TF1
UT7
M07e
KIT-APC
E
M07e
UT7
TF1
HEL
MV4-11
% survival
F
AML1
AML2
AML3
hCD45-PE-Cy7
KIT-APC
59.8
43
76.4
G
AMKL7
AML1
AML2
AML3
% survival

**Figure S9. Tyrosine kinase inhibitors affect AMKL cell growth**

**A-C,** Viability of M07e cells **(A)**, AMKL7 **(B)** and AMKL26 **(C)** patient cells after 96h treatment with indicated kinase inhibitors or vehicle (DMSO). Negative control of culture without cytokines (No cytokine) is shown. Mean ± SEM, n=3, ***p<0.001, *p<0.05, **p<0.01, and ***p<0.001.

**D)** Histogram of flow cytometry analysis showing KIT expression in depicted AML cell lines.

**E)** Viability of AML cell lines after 96h treatment with indicated concentrations of Axitinib or vehicle (DMSO). Mean ± SEM, n=7.

**F)** Flow cytometry analysis for KIT and CD45 expression on AML blasts isolated from PDX mice.

**G)** Viability of AMKL7 and three AML patient cells derived from PDX mice after 96h treatment with indicated concentrations of Axitinib or vehicle (DMSO). Mean ± SEM, n=3 for AMKL7 and n=7 for AML1-3.

## Supplementary Tables

### Table S1: sgRNA composition and normalized counts of CRISPRi screen replicates

Related to Figure 1

See excel file “Table_S1.xlsx”

### Table S2: Cell counts of FISH experiments

Related to Figure 2

| Cell type: | *KIT*-SEKIT < *KIT*-control number | % | *KIT*-SEKIT > *KIT*-control number | % | *KIT*-SEKIT = *KIT*-control number | % | Sample size (chromosomes) |
|---|---|---|---|---|---|---|---|
| M07e | 139 | 69.5 | 27 | 13.5 | 34 | 17 | 200 |
| HEL | 65 | 43.3 | 46 | 30.7 | 39 | 26 | 150 |

**Chi2 test**

| | | | | | | | Chi2 value: | p-value: |
|---|---|---|---|---|---|---|---|---|
| **M07e vs expected:** | | | | | | | | |
| Observed | 139 | 83.7 | 27 | 16.3 | | 166 | 75.57 | <0.00001*** |
| Expected | 83 | 50 | 83 | 50 | | 166 | | |
| **HEL vs expected:** | | | | | | | | |
| Observed | 65 | 58.6 | 46 | 41.4 | | 111 | 3.25 | 0.71 ns |
| Expected | 55.5 | 50 | 55.5 | 50 | | 111 | | |
| **M07e vs HEL:** | | | | | | | | |
| M07e | 139 | 83.7 | 27 | 16.3 | | 166 | 21.72 | <0.00001*** |
| HEL | 65 | 58.6 | 46 | 41.4 | | 111 | | |

### Table S3: Genesets negatively correlated with CRISPRi inhibition of SEKIT

Related to Figure 5

| GENESET_NAME | SIZE | ES | NES | NOM_p-val | FDR_q-val |
|---|---|---|---|---|---|
| KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION | 80 | -0.5101066 | -1.858465 | 0.006024096 | 0.15128882 |
| GO_CYTOKINE_BINDING | 45 | -0.5284298 | -1.7387868 | 0.019543974 | 0.18277322 |
| AP1_Q4 | 156 | -0.4322358 | -1.7331688 | 0.002853067 | 0.18397884 |
| GO_PLATELET_DERIVED_GROWTH_FACTOR_RECEPTOR_BINDING | 9 | -0.7660931 | -1.6965766 | 0.02097902 | 0.18942948 |
| REACTOME_SIGNALING_BY_PDGF | 81 | -0.46986297 | -1.6964734 | 0.016897082 | 0.18859418 |
| AP1_Q6 | 139 | -0.43164703 | -1.6909294 | 0.007587254 | 0.18844871 |
| GO_CYTOKINE_ACTIVITY | 51 | -0.48939356 | -1.6587768 | 0.02724359 | 0.18603332 |
| TGANTCA_AP1_C | 554 | -0.35058564 | -1.5819211 | 0.0001 | 0.20607616 |
| REACTOME_SIGNALING_BY_SCF_KIT | 68 | -0.4470207 | -1.5715805 | 0.042944785 | 0.20909369 |
| PDGF_UP.V1_DN | 44 | -0.47405526 | -1.5650235 | 0.04715447 | 0.21040736 |
| AP1_01 | 138 | -0.4016879 | -1.5597069 | 0.017595308 | 0.21429409 |
| GO_CYTOKINE_RECEPTOR_BINDING | 114 | -0.4026892 | -1.5483524 | 0.016467066 | 0.21501972 |
| REACTOME_PIP3_ACTIVATES_AKT_SIGNALING | 25 | -0.53417414 | -1.5436361 | 0.042016808 | 0.21708472 |
| REACTOME_PI3K_AKT_ACTIVATION | 32 | -0.502016 | -1.5235479 | 0.0461285 | 0.22794746 |
| AP1_Q2 | 143 | -0.3835286 | -1.5055175 | 0.023988007 | 0.2345746 |
| AP1_Q2_01 | 143 | -0.3823649 | -1.502686 | 0.021770682 | 0.23683275 |
| AP1_Q6_01 | 141 | -0.3752396 | -1.4760147 | 0.023809524 | 0.25127 |
| GO_CYTOKINE_RECEPTOR_ACTIVITY | 36 | -0.45751223 | -1.4679023 | 0.044701986 | 0.25599676 |
| AP1_C | 138 | -0.3734915 | -1.4593756 | 0.038402457 | 0.26026753 |
| REACTOME_REGULATION_OF_KIT_SIGNALING | 13 | -0.56535697 | -1.4172556 | 0.10175438 | 0.2858632 |
| PID_KIT_PATHWAY | 45 | -0.41782665 | -1.3744195 | 0.080314964 | 0.31436348 |

## Table S4: sequences of sgRNA used in single CRISPRi experiments

Related to Figures 1, 2 and 7

| sgRNA ID | 20nt-sgRNA guide | 73mer sgRNA PCR oligos for cloning in pU6-sgRNA-EF1Alpha-puro-T2A-BFP using XhoI and BstXI | 24mer annealing oligos for cloning in pLV-hU6-sgRNA-hUbC-dCas9-KRAB-T2a-GFP using BsmBI | |
|---|---|---|---|---|
| sgRNA2 | CAGATAATAGACTGGTCATG | GGAGAACCACCTTGTTGGCAGATAATAGACTGGTCATGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC | Forward | CACCCAGATAATAGACTGGTCATG |
| | | | Reverse | AAACCATGACCAGTCTATTATCTG |
| sgRNA5 | TCCGTATAGCAAGTGTACCC | GGAGAACCACCTTGTTGGTCCGTATAGCAAGTGTACCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC | Forward | CACCTCCGTATAGCAAGTGTACCC |
| | | | Reverse | AAACGGGTACACTTGCTATACGGA |
| sgRNA7 | AAGGGTCTTATCTAACTACT | GGAGAACCACCTTGTTGGAAGGGTCTTATCTAACTACTGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC | Forward | CACCAAGGGTCTTATCTAACTACT |
| | | | Reverse | AAACAGTAGTTAGATAAGACCCTT |
| sgRNA8 | AGGGCCTTCCGCTAAAGGCA | GGAGAACCACCTTGTTGGAGGGCCTTCCGCTAAAGGCAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC | Forward | CACCAGGGCCTTCCGCTAAAGGCA |
| | | | Reverse | AAACTGCCTTTAGCGGAAGGCCCT |
| sgRenilla | GGAACACGGCCGTATTAGGG | GGAGAACCACCTTGTTGGGGAACACGGCCGTATTAGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC | Forward | CACCGGAACACGGCCGTATTAGGG |
| | | | Reverse | AAACCCCTAATACGGCCGTGTTCC |
| sgRNA3 | AGACCCTTGCCTTTAGCGGA | GGAGAACCACCTTGTTGGAGACCCTTGCCTTTAGCGGAGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC | | |
| sgGFP | GACCAGGATGGGCACCACCC | GGAGAACCACCTTGTTGGGACCAGGATGGGCACCACCCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC | | |

## Table S5: sequences of shRNA used in gene knock-down experiments

Related to Figures 6 and S5

| Gene Symbol | shRNA ID | 22nt-shRNA guide | mRNA target site | 97mer oligo |
|---|---|---|---|---|
| *KIT* | shKIT1 | TTCTGCTCAGACATCGTCGTGC | GCACGACGATGTCTGAGCAGAA | TGCTGTTGACAGTGAGCGACACGACGATGTCTGAGCAGAATAGTGAAGCCACAGATGTATTCTGCTCAGACATCGTCGTGCTGCCTACTGCCTCGGA |
| *KIT* | shKIT3 | TTATCTACTACTTCCAAGGTTG | CAACCTTGGAAGTAGTAGATAA | TGCTGTTGACAGTGAGCGAAACCTTGGAAGTAGTAGATAATAGTGAAGCCACAGATGTATTATCTACTACTTCCAAGGTTGTGCCTACTGCCTCGGA |
| *PDGFRA* | shPDGFRA1 | TTAAACATCAACCAAGTCCTCC | GGAGGACTTGGTTGATGTTTAA | TGCTGTTGACAGTGAGCGAGAGGACTTGGTTGATGTTTAATAGTGAAGCCACAGATGTATTAAACATCAACCAAGTCCTCCTGCCTACTGCCTCGGA |
| *PDGFRA* | shPDGFRA3 | ATACTGTGTAGTATCAGCCTGC | GCAGGCTGATACTACACAGTAT | TGCTGTTGACAGTGAGCGACAGGCTGATACTACACAGTATTAGTGAAGCCACAGATGTAATACTGTGTAGTATCAGCCTGCTGCCTACTGCCTCGGA |

## Table S6: sequences of primers used in quantitative PCR experiments

Related to Figures 2, 3, 6, S3, S4, S5 and S6

| Gene Symbol | Primer ID | Primer sequence |
|---|---|---|
| *KIT* | hKIT fwd | CAATTCTGACGTCAATGCTGCCA |
| | hKIT rev | GAGCATGCCATTCACGAGCC |
| *PDGFRA* | hPDGFRA fwd | CCTGTCCCTGAGGAGGAGGAC |
| | hPDGFRA rev | GAAGGTGGAACTGCTGGAACCC |
| *CSF2RA* | hCSF2RA fwd | TCCCGCCAGTTCCACAGATC |
| | hCSF2RA rev | GACCTCTTCGCGGTAGCCTT |
| *CSF2RB* | hCSF2RB fwd | AACGGGATCTGGAGCGAGTG |
| | hCSF2RB rev | AGATCACGATGAGGGCCAGC |
| *GAPDH* | hGAPDH fwd | CTTTTGCGTCGCCAGCCGAG |
| | hGAPDH rev | CCAGGCGCCCAATACGACCA |