LIST OF SUPPLEMENTARY TABLES

Table S1: PU.1-interacting molecules in Scid.adh.2c2 cells. Related to Fig. 1.

Table S2: Runx1 consensus Position Weight Matrix (PWM) from DN1 and DN3 cells. Related to Fig. 3.

A. Position weight matrix and occurrence of sequences matching PWM at Group 1 and Group 2 sites

B. List of Runx motifs and Log-odds scores, genome-wide, to default cutoff score

C. Genes with additional Runx1 sites recognized in HOMER, using manually relaxed cutoff score

Table S3: Differentially expressed genes in Scid.Adh.2c2 cells, p.adj<1E-03, sorted from highest to lowest log2 fold change value in response to wildtype PU.1. Related to Figs. 4, 5.

A. Genes showing significant response to transduction of PU.1 into Scid.adh.2c2 cells (p.adj < 1E-03; EdgeR).

B. Genes from A with positive change in response to PU.1; plus effects of sgRunx and sgSatb1 and presence/absence of different site classes.

C. Genes from A with negative change in response to PU.1; effects of sgRunx1 and Satb1 and presence/absence of different site classes.

Table S4: Gene expression effects in Scid.adh.2C2 cells transduced with Runx1DN. Related to Fig. 5.

Table S5: Genes in primary DN cells that are differentially expressed in response to PU.1 deletion: and effects of Satb1 and Runx1 deletion. Related to Fig. 7.

Table S6: List of Oligonucleotides. Related to STAR*Methods and Key Resources Table.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1, related to Figures 1 and 3: PU.1 introduction induces transdifferentiation of Scid.adh.2c2 cells to myeloid phenotype.

(A), Scid.adh.2c2 cells were infected with Myc-Flag-PU.1/hNGFR retrovirus and cultured for two days. Flow cytometric analysis of tagged PU.1 transduced cells (left) shows loss of T-lineage marker CD25 and gain of myeloid marker CD11b (Mac1). Experimental scheme is also indicated (right).

(B), Developmental patterns of expression of PU.1 and candidate interaction partners. The heatmap shows expression profiles for PU.1 (Sfpi1), its binding partners (Runx1, Satb1), an Ets family member with an opposite expression pattern from PU.1, and other lymphoid transcription factors (Gata3, Zfpm1=FOG1, Ikzf1, and Tcf3=E2A), during the multilineage precursor (MLP) to T cell developmental transition. Heatmap is taken from the Immunological Genome web site <www.immgen.org> (Heng *et al.*, 2008; Mingueneau *et al.*, 2013), using the MyGeneSet tool. Color scale indicates z scores with warm colors indicating higher relative levels and cold colors indicating lower relative levels.

(C), Binding patterns of multiple factors associated with Runx1 occupancy sites in Scid.adh.2c2 cells. Tag count distributions for Runx1, Satb1, GATA3, Fog1 and PU.1 ChIP, and ATAC signal around Runx1 peaks using mock- or PU.1-introduced Scid.adh.2c2 cells are shown. All Runx1 sites identified in the presence or absence of exogenous PU.1 were included in the analysis, as in

Fig. 3A for Satb1 peaks. PU.1 ChIP tag count distributions in primary DN1 and DN2a are also indicated to show relation to normal developmental sites of PU.1 binding in primary pro-T cells.

(D), Binding patterns of multiple factors associated with all PU.1 occupancy sites in PU.1-transduced Scid.adh.2c2 cells. Tag count distributions for ChIP and ATAC signal around PU.1 peaks are shown.

(E), Runx1 motif sequence logos from combined non-promoter Runx1 ChIP peak lists of DN1 and DN3 pro-T cells (Fig. 6A, Table S2) (left) and JASPAR reference motif (right) are shown.

(F), Site-quality comparisons between different classes of Runx1 sites occupied in Scid.adh.2c2 cells with and without forced expression of PU.1. Quality scores were established by calculating the preferred position weight matrix for Runx1 occupancy of non-promoter binding sites in DN1 and DN3 primary pro-T cells (E, left), and the occupied sites scored in Scid.adh.2c2 cells were ranked in terms of their log-odds similarities to this PWM. Violin plots show the distribution of motif log-odds similarity scores of Group 4, Group 5 and Group 6 peaks (Fig. S1C) against the PWM for Runx1 motif calculated from occupancy sites in DN1 and DN3 cells (panel E, left). Median, 25% and 75% percentiles are also shown. P-values were determined by Kruskal-Wallis statistical test.

Supplementary Figure 2, related to Figure 3: Satb1 and Runx1 are preferentially recruited to the sites where ATAC opened and directly bound by PU.1.

(A), Tag count distributions are shown for Satb1, Runx1 and PU.1 ChIP, and ATAC signals from mock- or PU.1-introduced Scid.adh.2c2 cells, specifically around the sites that become

ATAC accessible in response to PU.1-transduction. Sites are separated into those that are also bound (upper) and those that are unbound (lower) by PU.1 directly. .

(B), Average tag count distributions are indicated for Satb1, Runx1 and PU.1 ChIP peaks in the sites becoming ATAC accessible by PU.1-transduction, comparing those that are directly bound with those that are unbound by PU.1 in (A).

(C), The figure shows the numbers of Satb1 (left) or Runx1 (right) peaks in mock- or PU.1-introduced Scid.adh.2c2 cells co-occupied with ATAC opened peaks in (A, bound or unbound by PU.1).

Supplementary Figure 3, related to Figure 5: Regulatory impacts of CRISPR/Cas9-mediated knock out of Runx1 and Satb1 in Scid.adh.2c2 cells

(A), Target genes regulated by PU.1 in Satb1- or Runx1-dependent ways in Scid.adh.2c2 cells are also regulated by PU.1 in primary pro-T cells. Heat maps show the acute responses to PU.1 in primary pro-T cells of the same Satb1- or Runx1-responsive genes (sgSatb1 or sgRunx1/sgControl<0.75) identified in Fig. 5C. As described in our related study (Ungerbäck et al., op. cit.), exogenous PU.1 introduced into newly committed DN2b pro-T cells causes some cells to undergo partial reversal of differentiation (PU1WTHA25) and others, expressing higher levels of PU.1, to be driven out of the T-cell program (PU1WTHA44), with stronger overall gene expression differences. Hierarchical clustering analyses of the expression of the Satb1- or Runx1-responsive genes in both subsets of PU.1-introduced primary DN2 cells (Ungerbäck et al., op. cit.) are shown, compared to cells expressing empty vector instead of PU.1 (left). Experimental scheme is also indicated (right).

(B and C), Satb1- or Runx1-responsive genes are significantly enriched among PU.1-dependent (B) or PU.1-repressed (C) genes, as compared to all expressed genes. For comparison with Fig. 5C, the figures show hierarchical clustering analyses of all the expressed genes (RPKM>3 in sgControl/Mock) in the same Scid.adh.2c2 cells used for Fig. 5C (left). The percentages of genes showing a Satb1 or Runx1 response (sgSatb1 or sgRunx1/sgControl<0.75) among the PU.1-dependent (B) or PU.1-repressed (C) genes in Fig. 5C are shown, as compared with percentages among all expressed genes (right). P-value is determined by Fisher's exact test.

Supplementary Figure 4, related to Figure 5: Runx-dependent gene definition: confirmation with Runx1 dominant negative and correlation with PU.1 effect

Confirmation that genes repressed by introduction of PU.1 into Scid.adh.2c2 cells are frequently also genes that have a dose-dependent requirement for Runx1 activity in the absence of PU.1. (A) Comparison of effects of sgRunx1 and Runx1DN for genes downregulated by sgRunx1. (B) Comparison of effects of sgRunx1 and exogenous PU.1, for same genes as in (B). (C) Same as A, but for genes upregulated by sgRunx1. The genes shown are those that were positively or negatively responsive to exogenous PU.1, and also potential Runx1 regulation candidates based on responses to sgRunx1 and Cas9. Values plotted are from Table S3 and Table S4. Although Cas9-dependent deletion experiments using sgRNA against Runx1 (Fig. 5, Table S3) show detectable but relatively weak effects on these genes (blue bars in panels A-C), the same genes show strong and highly significant responses in the same directions in response to acute introduction of Runx1DN, which binds DNA but neither transactivates nor represses (dark red

bars in panels A & C). This confirms that these genes can be significantly affected by disruption of the Runx input.

Genes chosen for in this analysis were all those that have both (1) substantial responses to PU.1, positive or negative (p.adj < 0.01) and (2) some indication of a response to Runx1 disruption alone, positive or negative. For sgRunx1 responses a very permissive criterion (p.adj <0.5) was used to collect a large number of possible candidates (Table S3). The same genes were then evaluated for their responses to acute transduction with Runx1 DN in Scid.adh.2c2 cells in completely independent experiments (Table S4). The questions addressed were: (1) how often do these permissively chosen potential Runx1 targets actually respond to Runx1DN, and (2) how much do the directions of the genes' responses to Runx1 predict the directions of their responses to PU.1? The genes shown are sorted according to the magnitudes of their responses to sgRunx1, and downregulated genes (Runx1-dependent) are shown in panels (A) and (B) while upregulated genes (Runx1-repressed) are shown in panel (C). Specifically, bars in the figure show $\log_2$ fold changes in expression of the indicated genes for the following perturbations compared to their levels in the corresponding controls: (A)-(C) sgRunx1 (no added PU.1) vs. control guide RNA (no added PU.1)= blue bars; (A) & (C) Runx1DN transduction vs. empty vector transduction for 48 hr = dark red bars; (B) PU1 transduction + control guide RNA vs. mock transduction + control guide RNA = green bars. Dark red bars in A, C are shown for all genes where effect of Runx1DN had p.adj<0.05; most had p.adj <1E-10. All values were calculated (EdgeR) from whole genome transcriptome data in two independent experiments of each type.

Summary of results: Results show, first, that despite the highly permissive criterion used to select this set of sgRunx1-responsive genes (blue bars), the responses to sgRunx1 agree well with

responses to the more potent Runx1DN (dark red bars), whether for activation or repression (panels A, C). Second, this analysis confirms that most of the Runx1-dependent genes that are PU.1 responsive in any direction (blue bars) are repressed by PU.1 (green bars in panel B). Fisher's exact tests for both relationships strongly indicate dependence (both $p < 0.00001$). .

Supplementary Figure 5, related to Figure 6: Correlation of Runx1 peak redirections in transduced Scid.adh.2c2 cells and primary pro-T cells in normal development

(A), Venn diagrams show categorization of Runx1 peaks in primary DN1 and DN3 cells (Fig. 6A) (left). Note shift of Runx1 binding sites between the PU.1-expressing DN1 stage and the PU.1-nonexpressing DN3 stage. Violin plots show the distribution of motif log-odds similarity scores of DN1_specific, Shared and DN3_specific Runx1 peaks (right) against PWM for Runx1 motif in DN1 and DN3 (Fig. S1E). Median, 25% and 75% percentiles are shown with P-values (K-W statistical test). The lower median quality of sites successfully occupied by Runx1 in DN1 stage is notable because Runx1 levels themselves are lower at this stage (Fig. S1B), supporting the need for interaction with PU.1 or some other DN1-stage factor(s) to recruit Runx1 to these sites.

(B), Scatter plots of the Runx1 peaks on promoter (left) or non-promoter (right) sites in primary DN1 and DN3 cells are shown. Dots represent the log2 values of normalized Runx1 ChIP tag count in DN1 (x-axis) and DN3 (y-axis). Runx1 peaks co-occupied with PU.1 in DN1 cells are indicated in yellow.

(C and D), Dynamics of Runx1 site responses to PU.1 in Scid.adh.2c2 cells resemble reversal of natural shifts of Runx1 sites in normal pro-T cell development. Tag count distributions for

Runx1 and PU.1 ChIP, and ATAC signal around peaks in Group 1 (C) and Group 2 (D) in Fig. 3A using mock- or PU.1-introduced Scid.adh.2c2 cells and primary DN1 or DN3 cells are shown.

(E), Average tag count distributions for Runx1 and PU.1 ChIP, and ATAC signals in (C and B) are quantitated: Group 1 sites from (C), Group 2 sites from (D).

(F), Impacts of PU.1 on gene expression tend to be opposite for genes with DN1-unique Runx1 sites as compared to genes with DN3-unique Runx1 sites. Cumulative distributions of expression changes in wild-type PU.1 (PU.1WTHA25)-introduced-transduced DN cells (Ungerbäck et al., op. cit.) for four groups of genes bound by different category of Runx1 peaks in DN1 and DN3 in (A) and differentially expressed in PU.1-introduced cells (FDR<0.1) are shown. The number of genes in each group and p-values (K-S tests for comparisons with 'no peaks') are indicated. Only genes are shown that have sites of the indicated class without other classes. Note that genes with DN1-specific Runx1 sites in normal development tend to be up-regulated in acute response to PU.1 while genes with DN3-specific Runx1 sites tend to be down-regulated by PU.1. Bracket shows comparison between the fractions repressed vs. activated in the different gene groups.

Supplementary Figure 6, related to Figure 7: CRISPR/Cas9-mediated knock out of *Spi1* (PU.1), *Runx1* and *Satb1* in primary DN cells

(A), Phenotype of Cas9, Bcl2-doubly transgenic progenitor cells prior to transduction with sgRNA against *Spi1*, *Runx1*, or *Satb1*. Flow cytometry analysis of BM-derived precursors after 4 days of OP9-DL1 culture (before retrovirus infection) shows that cells are phenotypically Kit+ DN1 cells.

(B), Flow cytometric analysis of sgRNA transduced BM-derived precursors after 8 days of OP9-DL1 culture shows high frequency of transduction in CD45+ Lin^- cells.

(C), Inhibition of early pro-T cell proliferation by Cas9-mediated deletion of *Spi1*. Cell numbers of CD45+ and CFP+ cells in Fig. 7A are shown. P-values were determined by Student *t*-test.

(D), Successful removal of PU.1 protein in PU.1-specific sgRNA-transduced pro-T cells from mice with transgenic Cas9. PU.1 protein expression in sgControl and sgPU.1 introduced primary DN cells (in Fig. 7A) was determined by intracellular staining followed by FACS analysis.

(E), Functional collaboration between PU.1 and Runx1 shown by divergent responses in primary pro-T cells to loss of PU.1 by genes with different classes of Runx1 sites. Genes with DN1-specific or DN3-specific Runx1 sites, unique or overlapping with PU.1 sites, are compared for responses to loss of PU.1. Cumulative distributions of expression changes in PU.1-deficient primary DN cells are shown for four groups of genes, bound by DN1-specific (left) and DN3-specific (right) Runx1 peaks depicted in Fig. 6A and differentially expressed in sgPU.1-introduced cells (FDR<0.1). The number of genes in each group and p-values (K-S tests for comparisons with 'PU.1_no_Runx1 peaks') are indicated.

(F), Satb1- or Runx1-responsive genes are significantly enriched in PU.1-dependent or PU.1-repressed genes (Fig. 7B) compare to all expressed genes. For comparison with Fig. 7B, the figures show hierarchical clustering analyses of the all expressed genes (RPKM>3 in sgControl) in the same cells used for Fig. 7B (left). The percentages of Satb1- or Runx1-responsive genes (sgSatb1 or sgRunx1/sgControl<0.75) among the PU.1-dependent and PU.1-repressed genes in Fig. 7B and all expressed genes are shown (right). P-value is determined by Fisher's exact test.

Figure S1

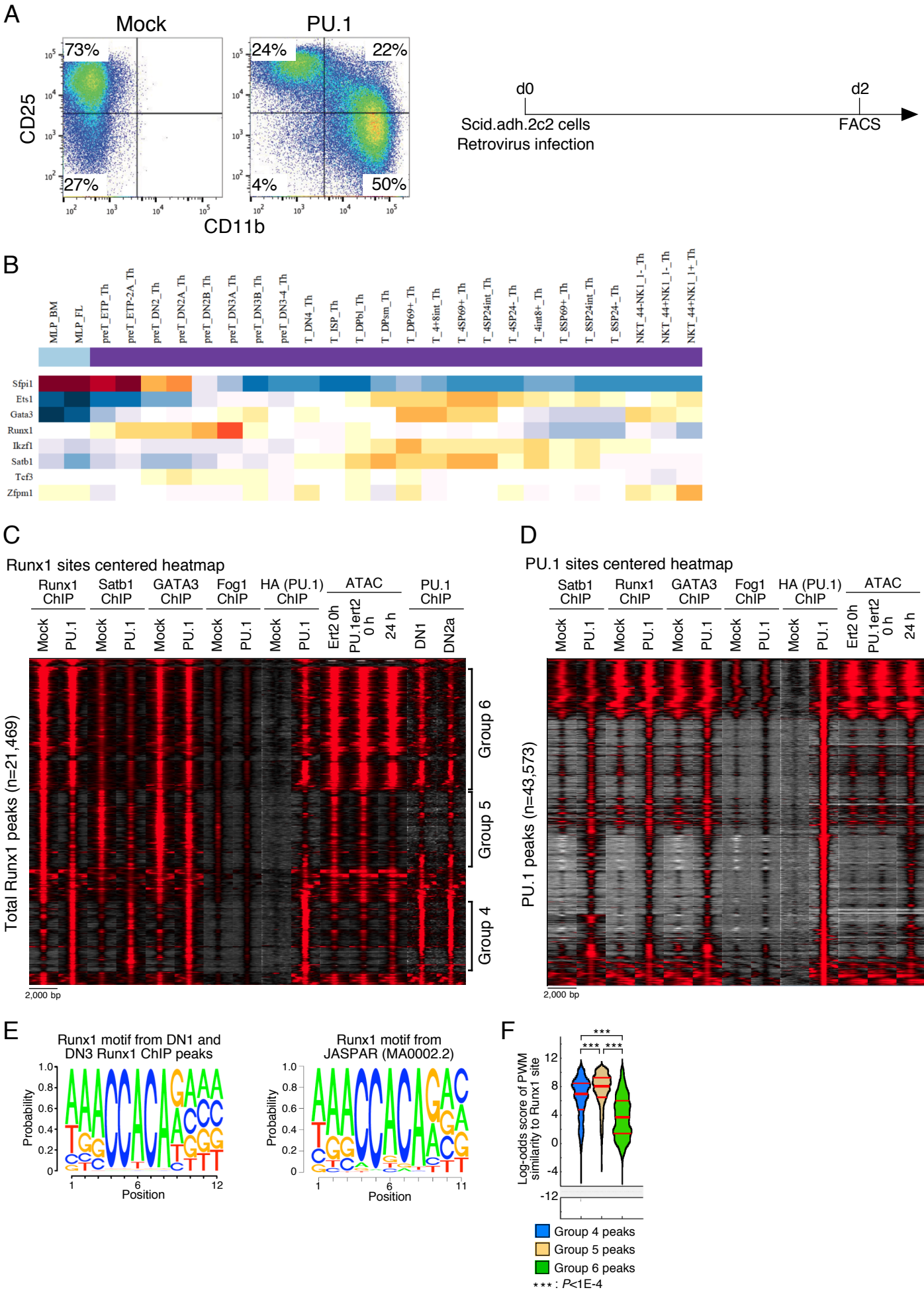

A
Mock
PU.1
73%
24%
22%
27%
4%
50%
CD25
CD11b
d0
d2
Scid.adh.2c2 cells
Retrovirus infection
FACS
B
MLP_BM
MLP_FL
preT_ETP_Th
preT_ETP-2A_Th
preT_DN2_Th
preT_DN2A_Th
preT_DN2B_Th
preT_DN3A_Th
preT_DN3B_Th
preT_DN3-4_Th
T_DN4_Th
T_ISP_Th
T_DPbl_Th
T_DPsm_Th
T_DP69+_Th
T_4+8int_Th
T_4SP69+_Th
T_4SP24int_Th
T_4SP24-_Th
T_4int8+_Th
T_8SP69+_Th
T_8SP24int_Th
T_8SP24-_Th
NKT_44-NK1_1-_Th
NKT_44+NK1_1-_Th
NKT_44+NK1_1+_Th
Sfpi1
Ets1
Gata3
Runx1
Ikzf1
Satb1
Tcf3
Zfpm1
C
Runx1 sites centered heatmap
Runx1 ChIP
Satb1 ChIP
GATA3 ChIP
Fog1 ChIP
HA (PU.1) ChIP
ATAC
PU.1 ChIP
Mock
PU.1
Ert2 0h
PU.1ert2 0 h
24 h
DN1
DN2a
Total Runx1 peaks (n=21,469)
Group 6
Group 5
Group 4
2,000 bp
D
PU.1 sites centered heatmap
Satb1 ChIP
Runx1 ChIP
GATA3 ChIP
Fog1 ChIP
HA (PU.1) ChIP
ATAC
PU.1 ChIP
PU.1 peaks (n=43,573)
2,000 bp
Normalized Tag count density
10
0
E
Runx1 motif from DN1 and DN3 Runx1 ChIP peaks
Runx1 motif from JASPAR (MA0002.2)
Probability
Position
F
Log-odds score of PWM similarity to Runx1 site
***
Group 4 peaks
Group 5 peaks
Group 6 peaks
*** : P<1E-4

Figure S2

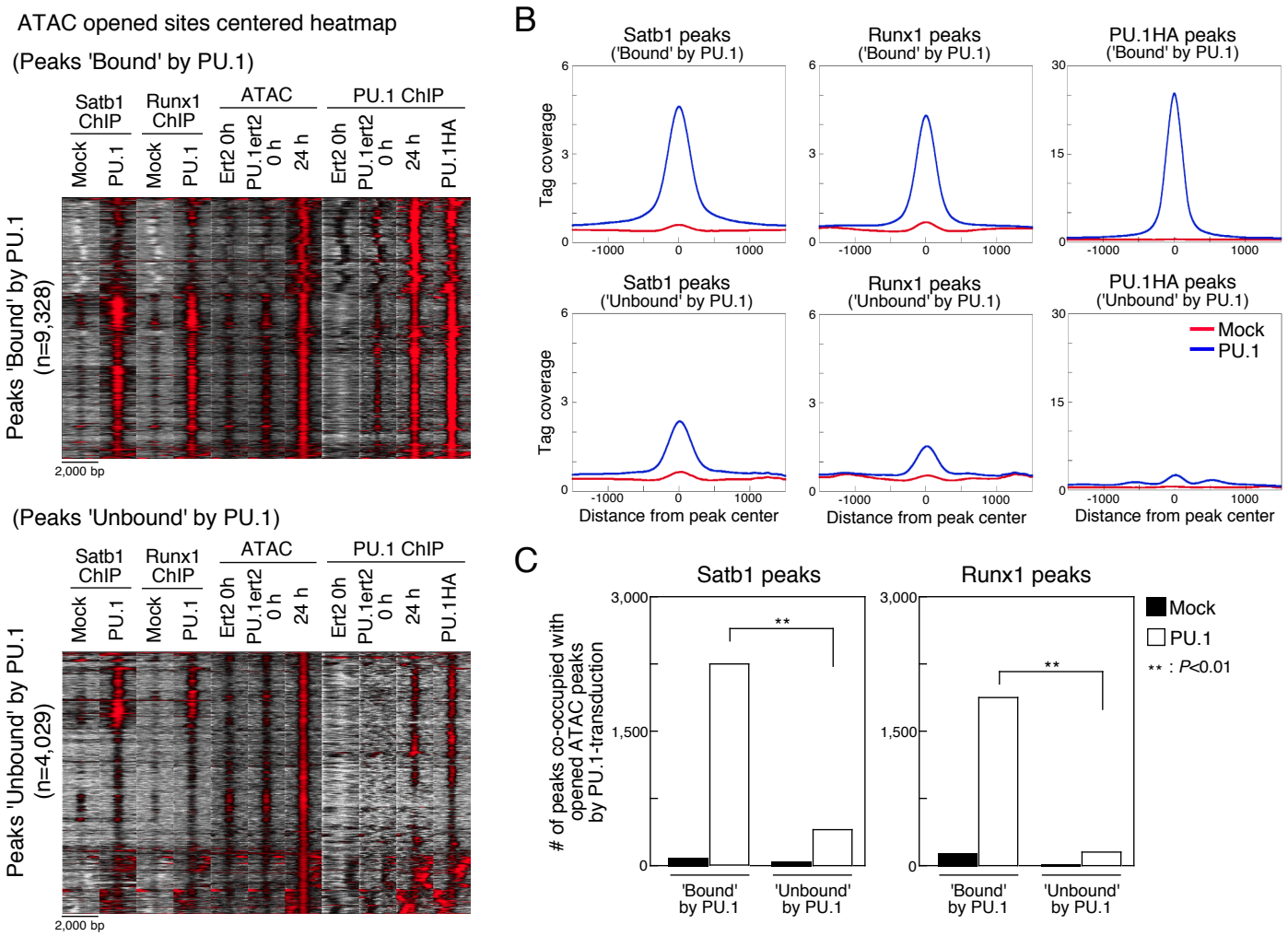
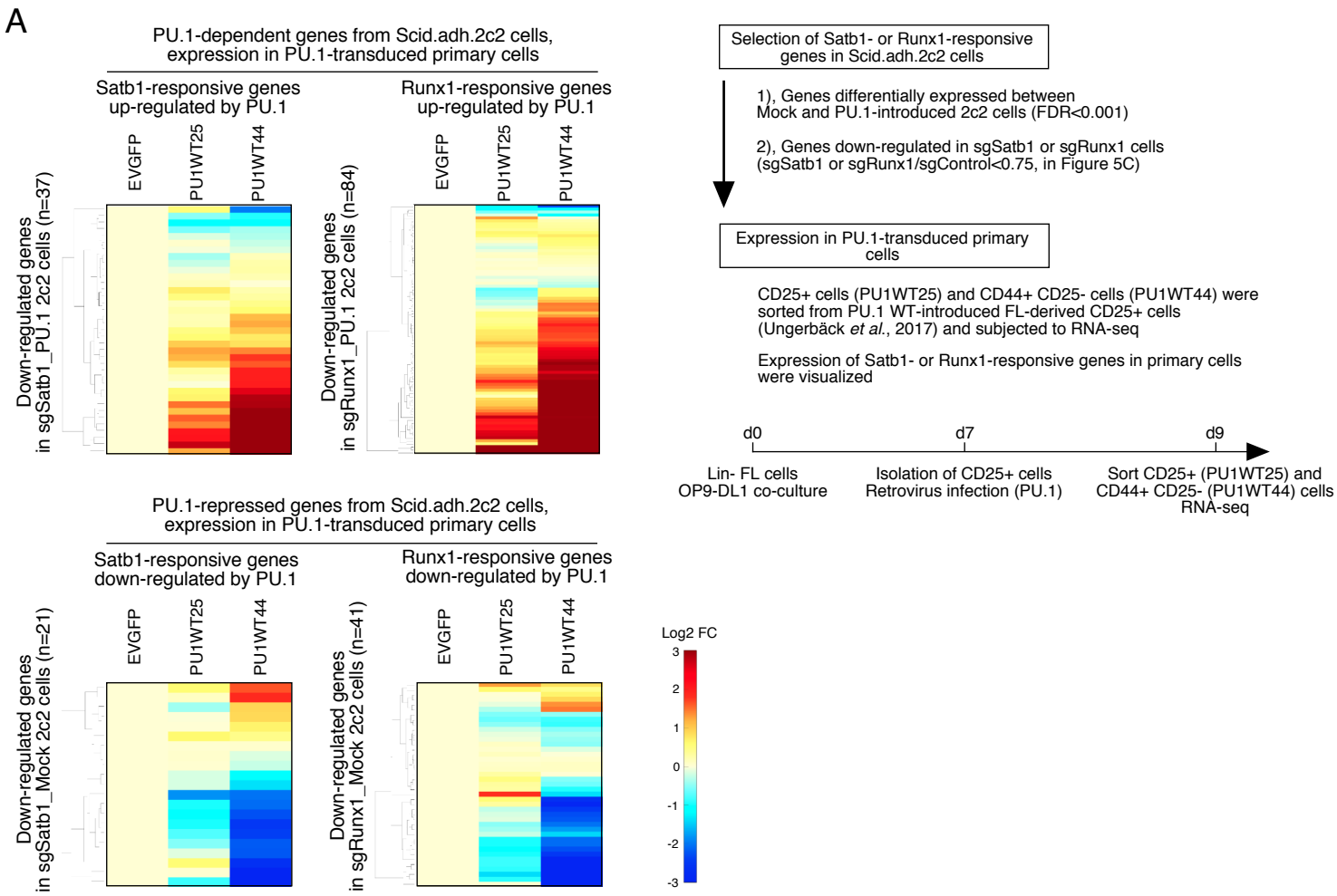
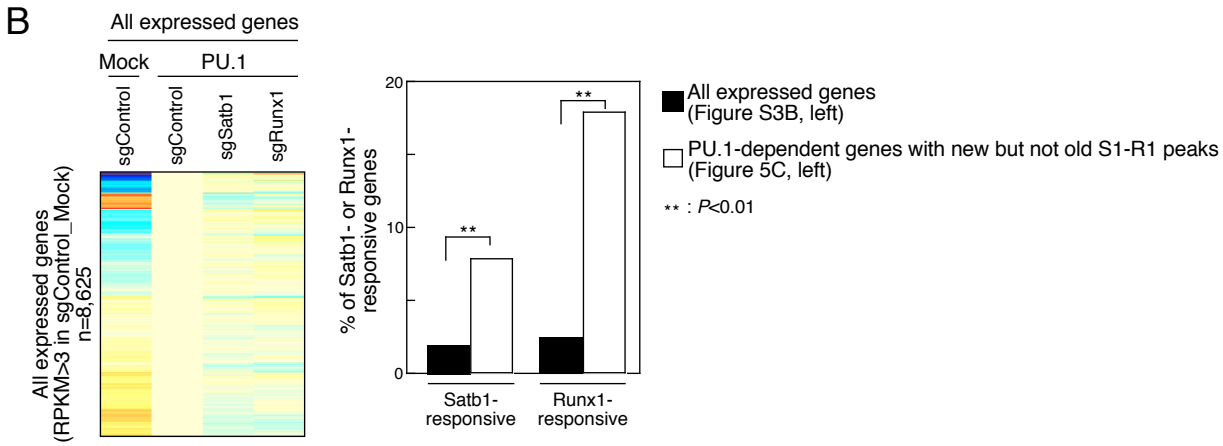

A
ATAC opened sites centered heatmap
(Peaks 'Bound' by PU.1)
Satb1 ChIP
Runx1 ChIP
ATAC
PU.1 ChIP
Mock
PU.1
Mock
PU.1
Ert2 0h
PU.1ert2 0 h
24 h
Ert2 0h
PU.1ert2 0 h
24 h
PU.1HA
Peaks 'Bound' by PU.1 (n=9,328)
2,000 bp
(Peaks 'Unbound' by PU.1)
Peaks 'Unbound' by PU.1 (n=4,029)
2,000 bp
B
Satb1 peaks ('Bound' by PU.1)
Runx1 peaks ('Bound' by PU.1)
PU.1HA peaks ('Bound' by PU.1)
Satb1 peaks ('Unbound' by PU.1)
Runx1 peaks ('Unbound' by PU.1)
PU.1HA peaks ('Unbound' by PU.1)
Tag coverage
Mock
PU.1
Distance from peak center
C
Satb1 peaks
Runx1 peaks
# of peaks co-occupied with opened ATAC peaks by PU.1-transduction
3,000
1,500
0
**
'Bound' by PU.1
'Unbound' by PU.1
Mock
PU.1
** : P<0.01

Figure S3


A
PU.1-dependent genes from Scid.adh.2c2 cells, expression in PU.1-transduced primary cells
Satb1-responsive genes up-regulated by PU.1
Runx1-responsive genes up-regulated by PU.1
EVGFP
PU1WT25
PU1WT44
Down-regulated genes in sgSatb1_PU.1 2c2 cells (n=37)
Down-regulated genes in sgRunx1_PU.1 2c2 cells (n=84)
Selection of Satb1- or Runx1-responsive genes in Scid.adh.2c2 cells
1), Genes differentially expressed between Mock and PU.1-introduced 2c2 cells (FDR<0.001)
2), Genes down-regulated in sgSatb1 or sgRunx1 cells (sgSatb1 or sgRunx1/sgControl<0.75, in Figure 5C)
Expression in PU.1-transduced primary cells
CD25+ cells (PU1WT25) and CD44+ CD25- cells (PU1WT44) were sorted from PU.1 WT-introduced FL-derived CD25+ cells (Ungerbäck et al., 2017) and subjected to RNA-seq
Expression of Satb1- or Runx1-responsive genes in primary cells were visualized
d0
d7
d9
Lin- FL cells OP9-DL1 co-culture
Isolation of CD25+ cells Retrovirus infection (PU.1)
Sort CD25+ (PU1WT25) and CD44+ CD25- (PU1WT44) cells RNA-seq
PU.1-repressed genes from Scid.adh.2c2 cells, expression in PU.1-transduced primary cells
Satb1-responsive genes down-regulated by PU.1
Runx1-responsive genes down-regulated by PU.1
Down-regulated genes in sgSatb1_Mock 2c2 cells (n=21)
Down-regulated genes in sgRunx1_Mock 2c2 cells (n=41)
Log2 FC
3
2
1
0
-1
-2
-3


B
All expressed genes
Mock
PU.1
sgControl
sgControl
sgSatb1
sgRunx1
All expressed genes (RPKM>3 in sgControl_Mock) n=8,625
% of Satb1- or Runx1-responsive genes
20
10
0
**
**
Satb1-responsive
Runx1-responsive
All expressed genes (Figure S3B, left)
PU.1-dependent genes with new but not old S1-R1 peaks (Figure 5C, left)
** : P<0.01

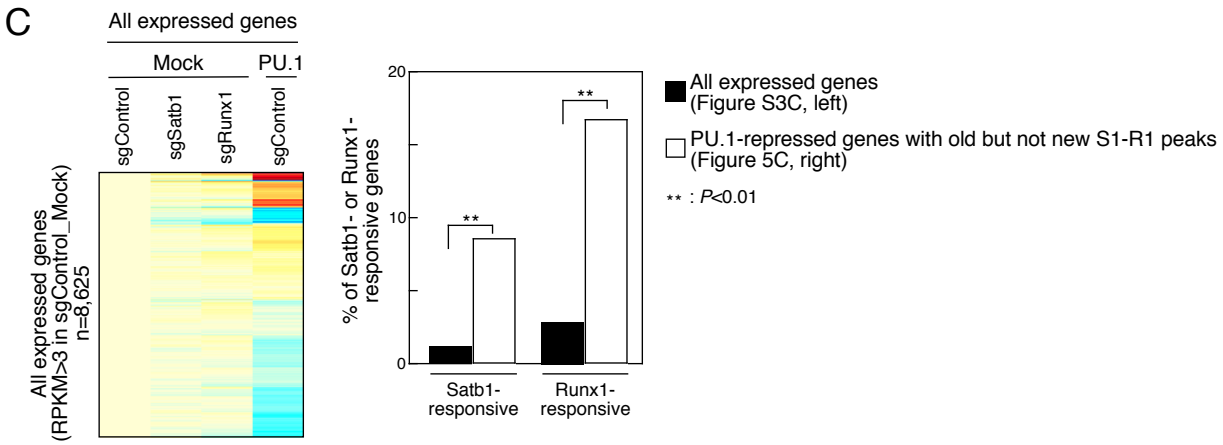

C
All expressed genes
Mock
PU.1
sgControl
sgSatb1
sgRunx1
sgControl
All expressed genes (RPKM>3 in sgControl_Mock) n=8,625
% of Satb1- or Runx1-responsive genes
20
10
0
**
**
Satb1-responsive
Runx1-responsive
All expressed genes (Figure S3C, left)
PU.1-repressed genes with old but not new S1-R1 peaks (Figure 5C, right)
** : P<0.01

Figure S4

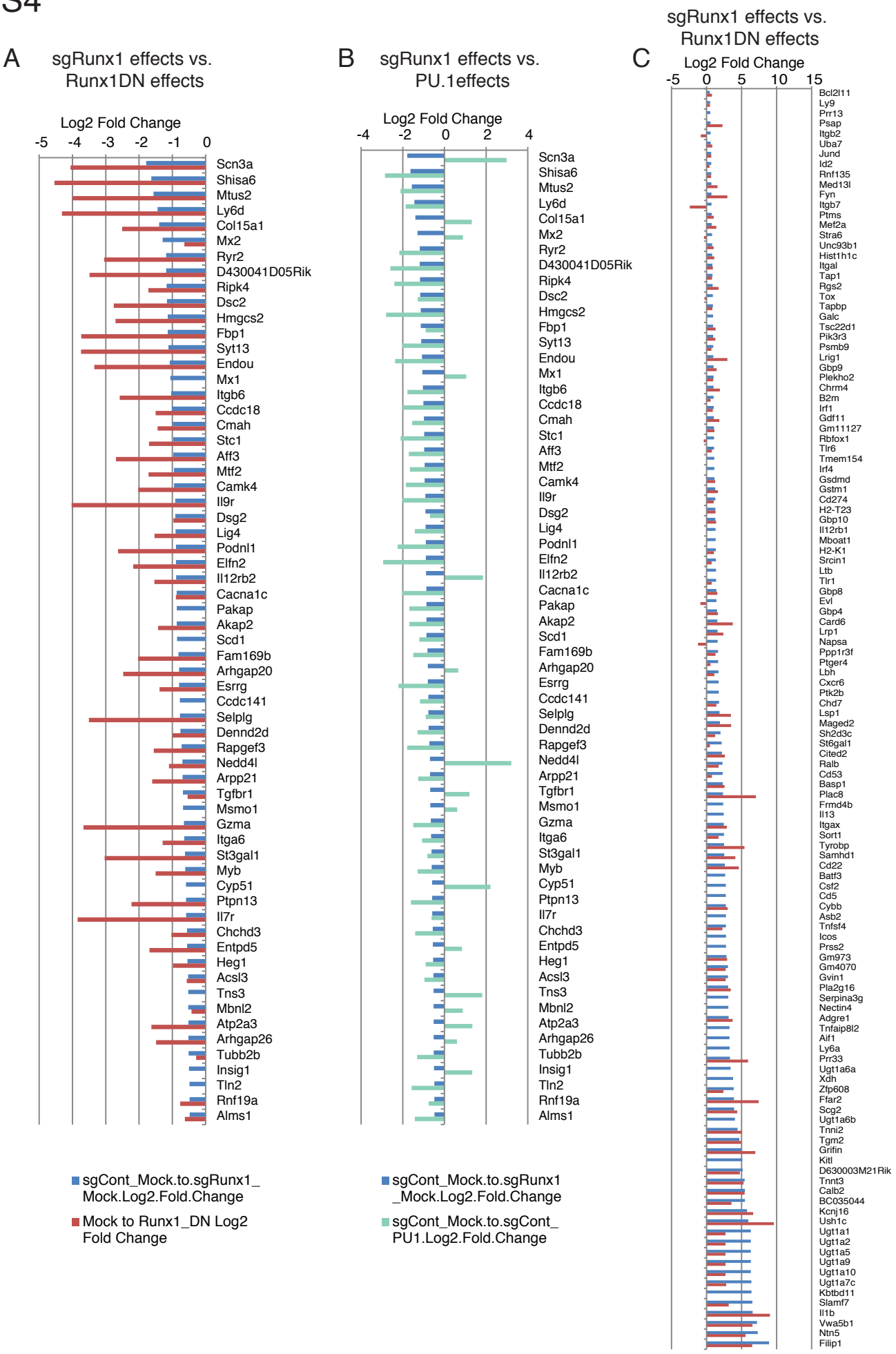

A
sgRunx1 effects vs. Runx1DN effects
Log2 Fold Change
-5 -4 -3 -2 -1 0
Scn3a
Shisa6
Mtus2
Ly6d
Col15a1
Mx2
Ryr2
D430041D05Rik
Ripk4
Dsc2
Hmgcs2
Fbp1
Syt13
Endou
Mx1
Itgb6
Ccdc18
Cmah
Stc1
Aff3
Mtf2
Camk4
Il9r
Dsg2
Lig4
Podnl1
Elfn2
Il12rb2
Cacna1c
Pakap
Akap2
Scd1
Fam169b
Arhgap20
Esrrg
Ccdc141
Selplg
Dennd2d
Rapgef3
Nedd4l
Arpp21
Tgfbr1
Msmo1
Gzma
Itga6
St3gal1
Myb
Cyp51
Ptpn13
Il7r
Chchd3
Entpd5
Heg1
Acsl3
Tns3
Mbnl2
Atp2a3
Arhgap26
Tubb2b
Insig1
Tln2
Rnf19a
Alms1
sgCont_Mock.to.sgRunx1_Mock.Log2.Fold.Change
Mock to Runx1_DN Log2 Fold Change
B
sgRunx1 effects vs. PU.1effects
Log2 Fold Change
-4 -2 0 2 4
Scn3a
Shisa6
Mtus2
Ly6d
Col15a1
Mx2
Ryr2
D430041D05Rik
Ripk4
Dsc2
Hmgcs2
Fbp1
Syt13
Endou
Mx1
Itgb6
Ccdc18
Cmah
Stc1
Aff3
Mtf2
Camk4
Il9r
Dsg2
Lig4
Podnl1
Elfn2
Il12rb2
Cacna1c
Pakap
Akap2
Scd1
Fam169b
Arhgap20
Esrrg
Ccdc141
Selplg
Dennd2d
Rapgef3
Nedd4l
Arpp21
Tgfbr1
Msmo1
Gzma
Itga6
St3gal1
Myb
Cyp51
Ptpn13
Il7r
Chchd3
Entpd5
Heg1
Acsl3
Tns3
Mbnl2
Atp2a3
Arhgap26
Tubb2b
Insig1
Tln2
Rnf19a
Alms1
sgCont_Mock.to.sgRunx1_Mock.Log2.Fold.Change
sgCont_Mock.to.sgCont_PU1.Log2.Fold.Change
C
sgRunx1 effects vs. Runx1DN effects
Log2 Fold Change
-5 0 5 10 15
Bcl2l11
Ly9
Prr13
Psap
Itgb2
Uba7
Jund
Id2
Rnf135
Med13l
Fyn
Itgb7
Ptms
Mef2a
Stra6
Unc93b1
Hist1h1c
Itgal
Tap1
Rgs2
Tox
Tapbp
Galc
Tsc22d1
Pik3r3
Psmb9
Lrig1
Gbp9
Plekho2
Chrm4
B2m
Irf1
Gdf11
Gm11127
Rbfox1
Tlr6
Tmem154
Irf4
Gsdmd
Gstm1
Cd274
H2-T23
Gbp10
Il12rb1
Mboat1
H2-K1
Srcin1
Ltb
Tlr1
Gbp8
Evl
Gbp4
Card6
Lrp1
Napsa
Ppp1r3f
Ptger4
Lbh
Cxcr6
Ptk2b
Chd7
Lsp1
Maged2
Sh2d3c
St6gal1
Cited2
Ralb
Cd53
Basp1
Plac8
Frmd4b
Il13
Itgax
Sort1
Tyrobp
Samhd1
Cd22
Batf3
Csf2
Cd5
Cybb
Asb2
Tnfsf4
Icos
Prss2
Gm973
Gm4070
Gvin1
Pla2g16
Serpina3g
Nectin4
Adgre1
Tnfaip8l2
Aif1
Ly6a
Prr33
Ugt1a6a
Xdh
Zfp608
Ffar2
Scg2
Ugt1a6b
Tnni2
Tgm2
Grifin
Kitl
D630003M21Rik
Tnnt3
Calb2
BC035044
Kcnj16
Ush1c
Ugt1a1
Ugt1a2
Ugt1a5
Ugt1a9
Ugt1a10
Ugt1a7c
Kbtbd11
Slamf7
Il1b
Vwa5b1
Ntn5
Filip1

Figure S5

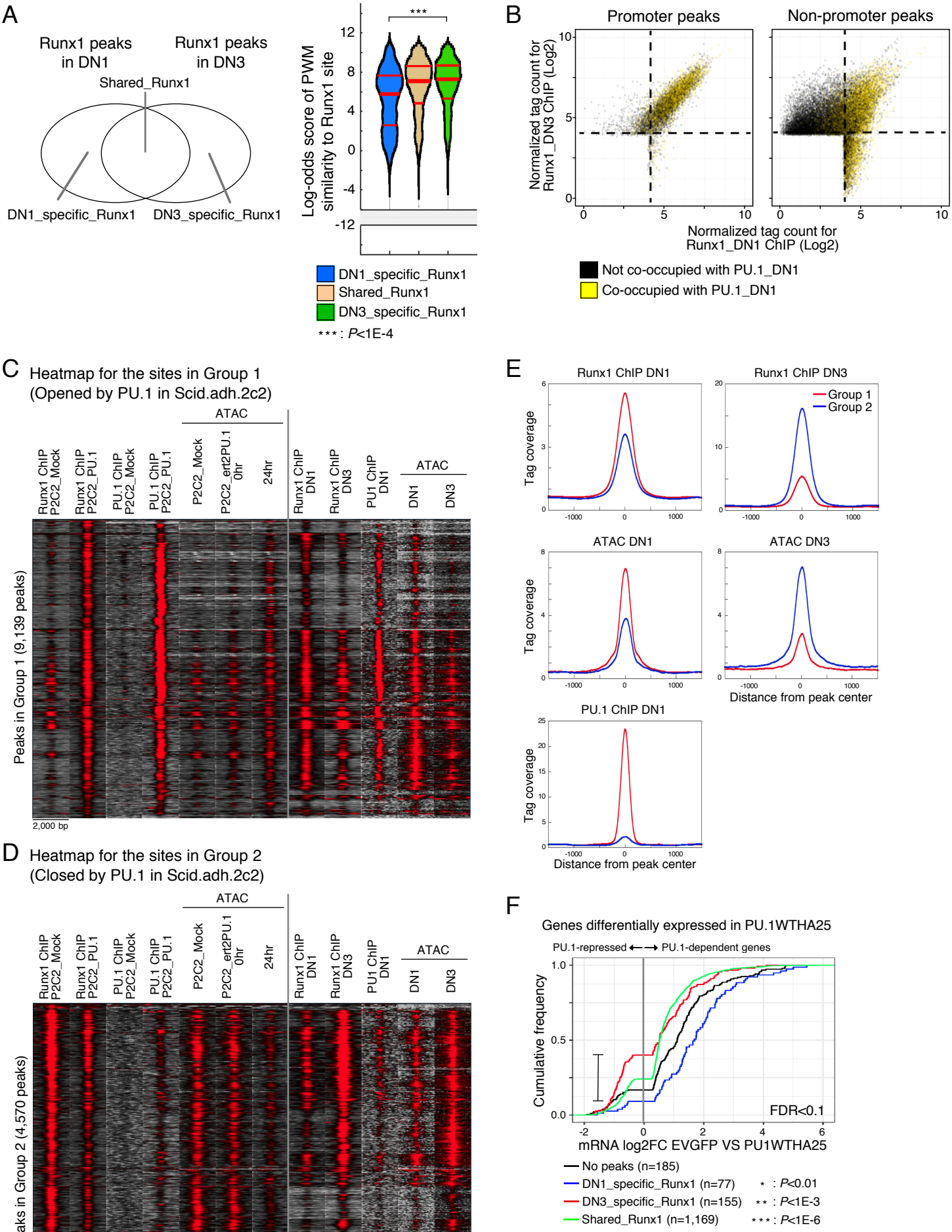
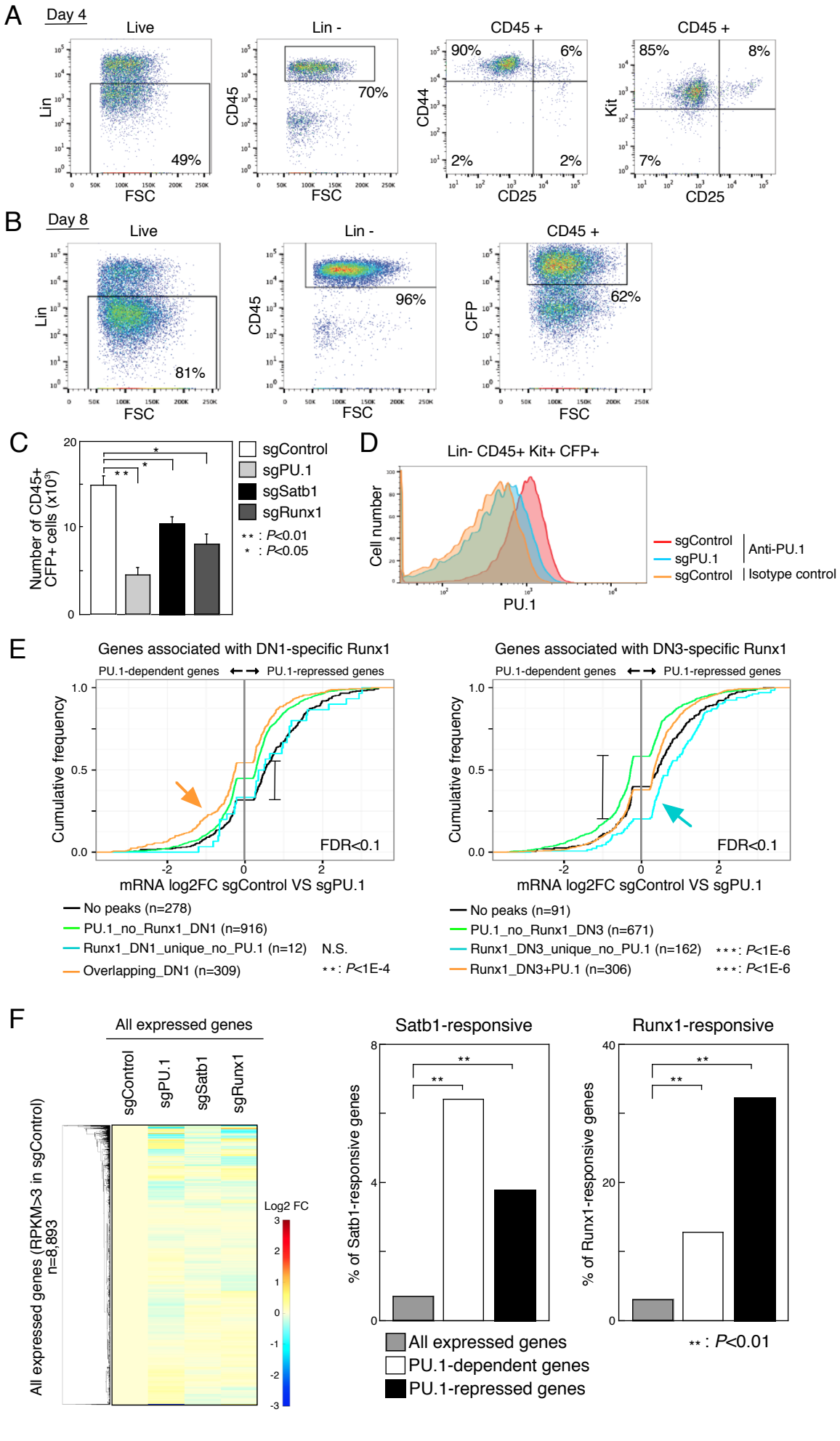

A
Runx1 peaks in DN1
Runx1 peaks in DN3
Shared_Runx1
DN1_specific_Runx1
DN3_specific_Runx1
Log-odds score of PWM similarity to Runx1 site
***
DN1_specific_Runx1
Shared_Runx1
DN3_specific_Runx1
***: P<1E-4
B
Promoter peaks
Non-promoter peaks
Normalized tag count for Runx1_DN3 ChIP (Log2)
Normalized tag count for Runx1_DN1 ChIP (Log2)
Not co-occupied with PU.1_DN1
Co-occupied with PU.1_DN1
C
Heatmap for the sites in Group 1
(Opened by PU.1 in Scid.adh.2c2)
ATAC
Runx1 ChIP P2C2_Mock
Runx1 ChIP P2C2_PU.1
PU.1 ChIP P2C2_Mock
PU.1 ChIP P2C2_PU.1
P2C2_Mock
P2C2_ert2PU.1 0hr
24hr
Runx1 ChIP DN1
Runx1 ChIP DN3
PU1 ChIP DN1
ATAC
DN1
DN3
Peaks in Group 1 (9,139 peaks)
2,000 bp
D
Heatmap for the sites in Group 2
(Closed by PU.1 in Scid.adh.2c2)
Peaks in Group 2 (4,570 peaks)
2,000 bp
E
Runx1 ChIP DN1
Runx1 ChIP DN3
Group 1
Group 2
Tag coverage
ATAC DN1
ATAC DN3
Distance from peak center
PU.1 ChIP DN1
F
Genes differentially expressed in PU.1WTHA25
PU.1-repressed ←→ PU.1-dependent genes
Cumulative frequency
FDR<0.1
mRNA log2FC EVGFP VS PU1WTHA25
No peaks (n=185)
DN1_specific_Runx1 (n=77)
DN3_specific_Runx1 (n=155)
Shared_Runx1 (n=1,169)
*: P<0.01
**: P<1E-3
***: P<1E-6

Figure S6


A
Day 4
Live
Lin -
CD45 +
CD45 +
Lin
FSC
CD45
70%
49%
CD44
CD25
90%
6%
2%
2%
Kit
85%
8%
7%
B
Day 8
Live
Lin -
CD45 +
96%
62%
81%
CFP
C
Number of CD45+ CFP+ cells (x10^3)
sgControl
sgPU.1
sgSatb1
sgRunx1
** : P<0.01
* : P<0.05
D
Lin- CD45+ Kit+ CFP+
Cell number
PU.1
sgControl
sgPU.1
sgControl
Anti-PU.1
Isotype control
E
Genes associated with DN1-specific Runx1
Genes associated with DN3-specific Runx1
PU.1-dependent genes
PU.1-repressed genes
Cumulative frequency
FDR<0.1
mRNA log2FC sgControl VS sgPU.1
No peaks (n=278)
PU.1_no_Runx1_DN1 (n=916)
Runx1_DN1_unique_no_PU.1 (n=12)
N.S.
Overlapping_DN1 (n=309)
** : P<1E-4
No peaks (n=91)
PU.1_no_Runx1_DN3 (n=671)
Runx1_DN3_unique_no_PU.1 (n=162)
*** : P<1E-6
Runx1_DN3+PU.1 (n=306)
*** : P<1E-6
F
All expressed genes
sgControl
sgPU.1
sgSatb1
sgRunx1
All expressed genes (RPKM>3 in sgControl) n=8,893
Log2 FC
Satb1-responsive
% of Satb1-responsive genes
Runx1-responsive
% of Runx1-responsive genes
** : P<0.01
All expressed genes
PU.1-dependent genes
PU.1-repressed genes