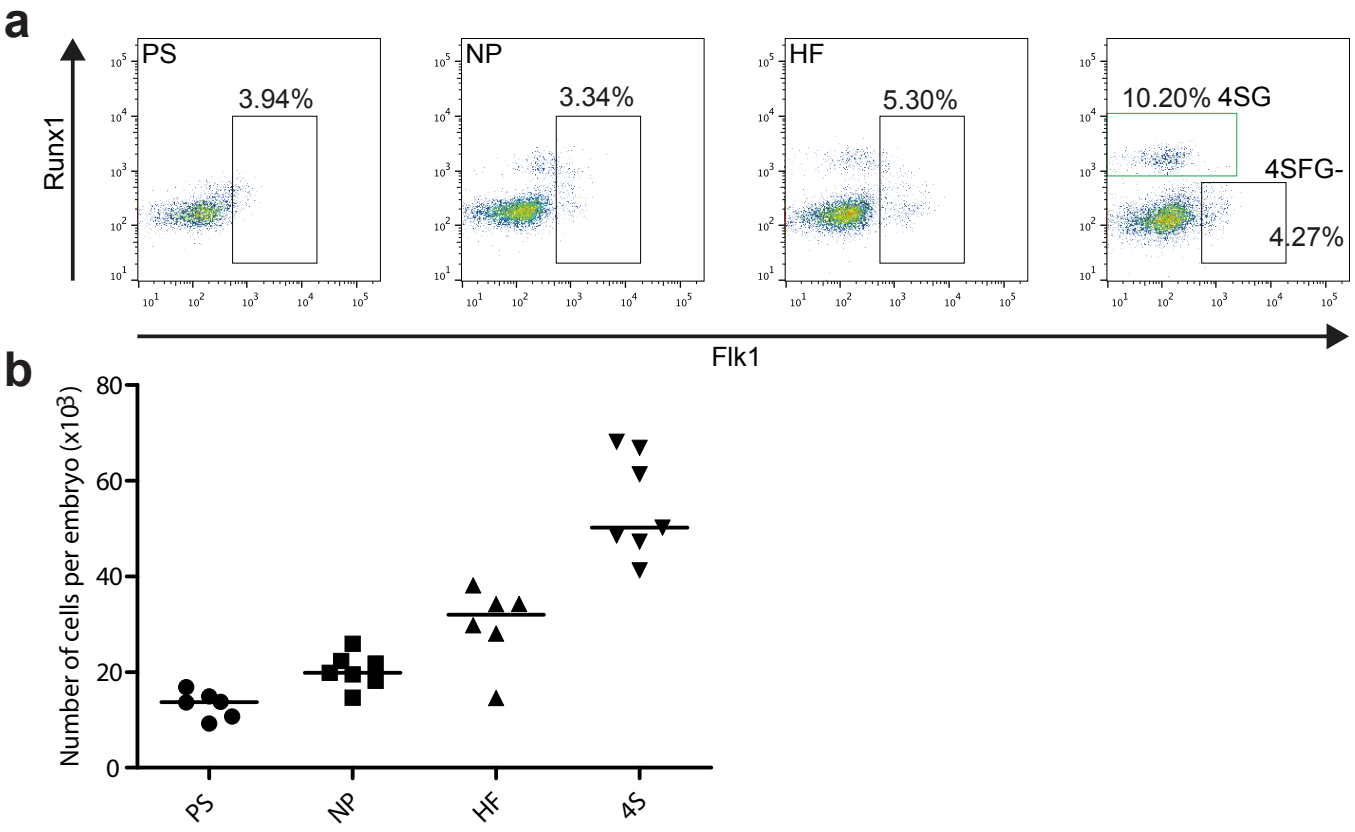
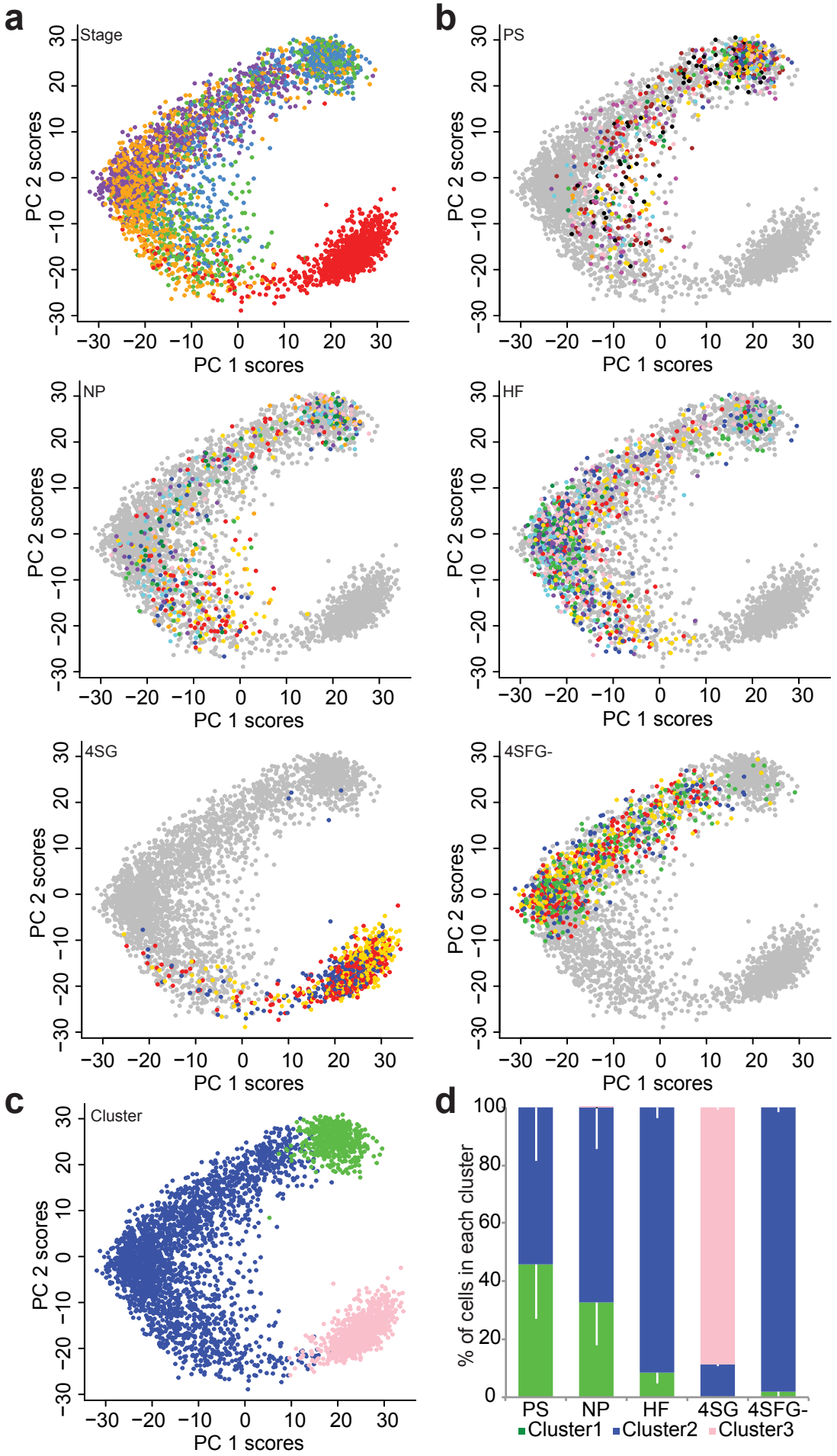
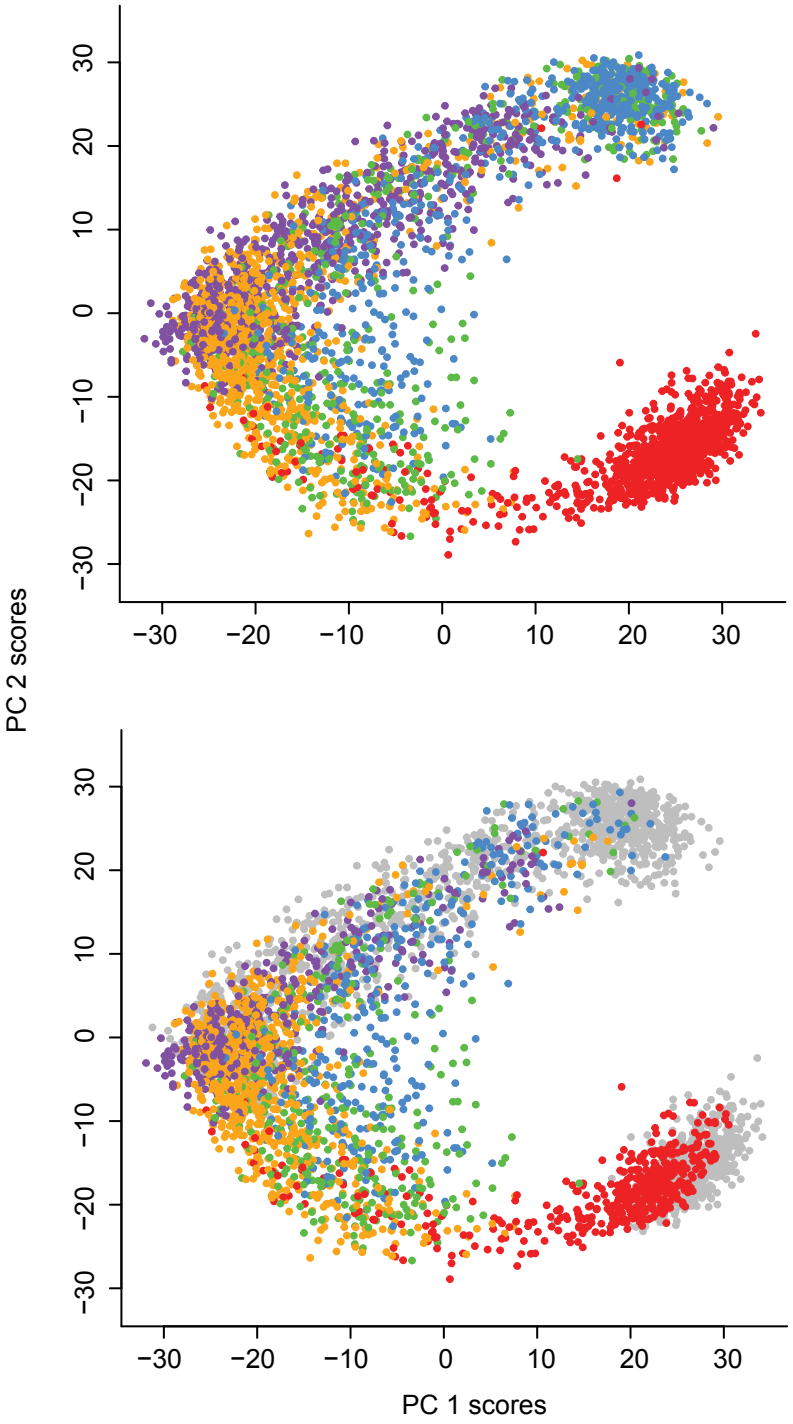


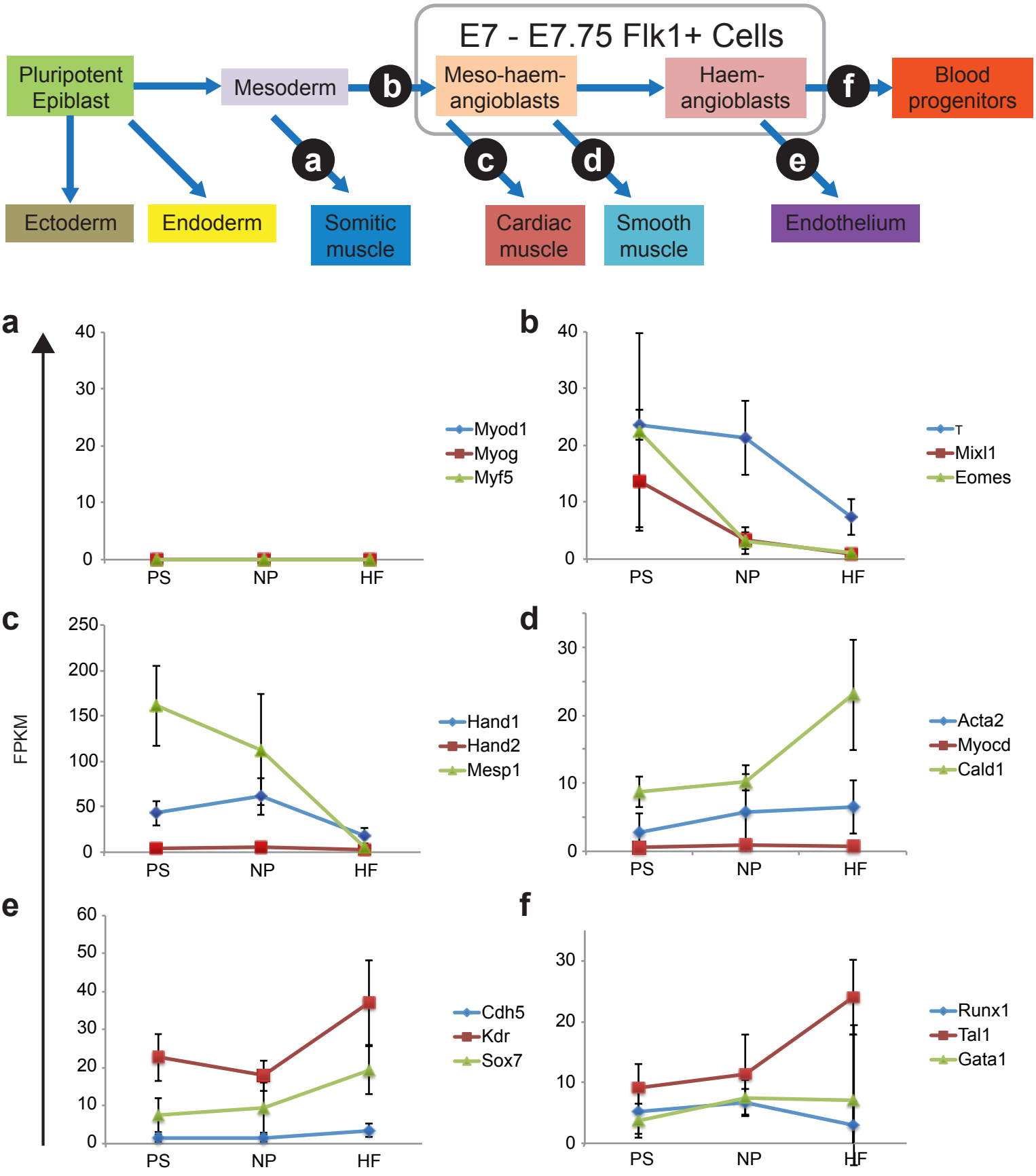
Supplementary Figure 1: Fluorescence activated cell sorting of cells with blood potential. **(a)** Gating strategy for Flk1^{+} cells at PS, NP and HF stages, and Runx1-ires-GFP^{+} cells (4SG) or Flk1^{+}Runx1-ires-GFP^{-} cells (4SFG^{-}) at the 4S stage. **(b)** Total numbers of cells per embryo at each stage, estimated from FACS data. Each point represents one embryo and lines indicate the median.



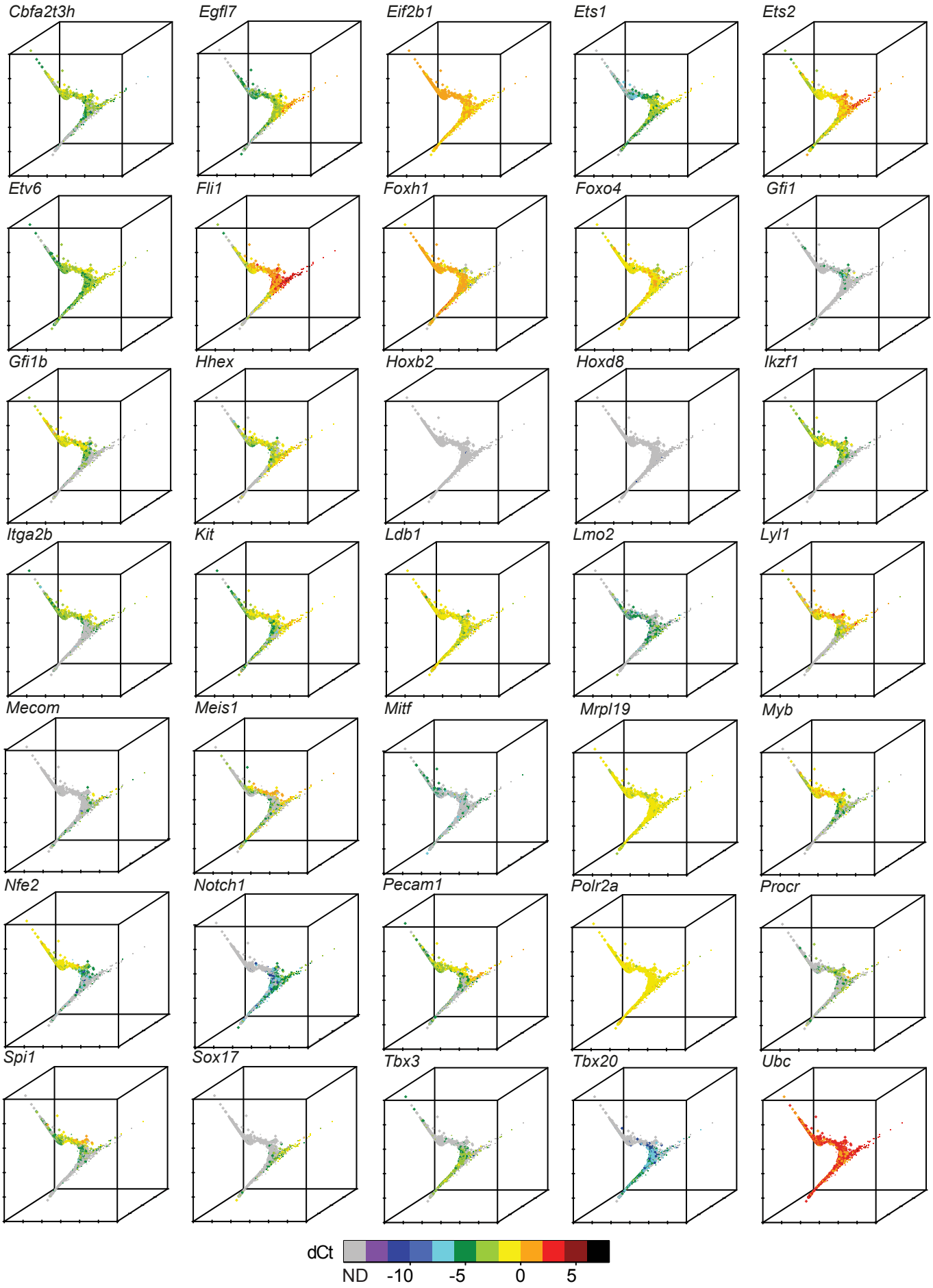
Supplementary Figure 2: Development is asynchronous. **(a)** PCA of the 3,934 cells, coloured retrospectively according to the stage from which they were sorted. Blue, PS; green, NP; orange, HF; red, 4SG; purple, 4SFG^{-}. **(b)** For each stage, the cells from different embryos are shown on the PCA as different colours (cells from other stages shown in grey). **(c)** PCA coloured according to the clusters cells belong to in Figure 1e. Green, I; Blue, II; Pink, III. **(d)** For each embryo, the percentage of cells in clusters I, II and III was calculated. The mean and standard deviation was then calculated for each cluster in each stage. Number of embryos shown in Fig. 1C.



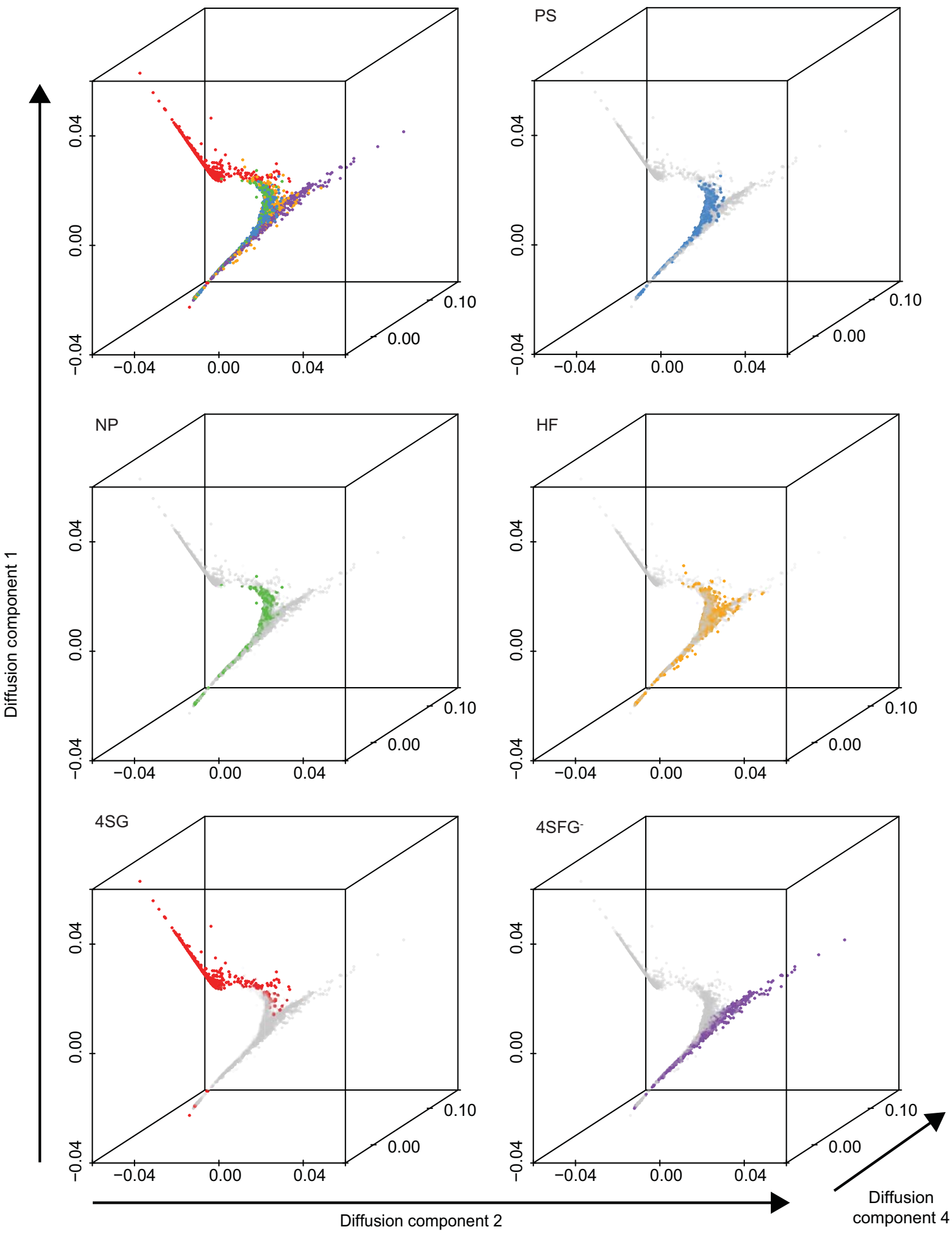
Supplementary Figure 3: Flk1^{+}Runx1^{+} cells are found in all anatomical stages. Principal component analysis coloured according to stage of sorting (top) and whether cells express both Flk1 and Runx1 at the gene expression level (bottom). Cells not expressing both genes shown in grey. Blue, PS; green, NP; orange, HF; red, 4SG; purple, 4SFG^{-}.



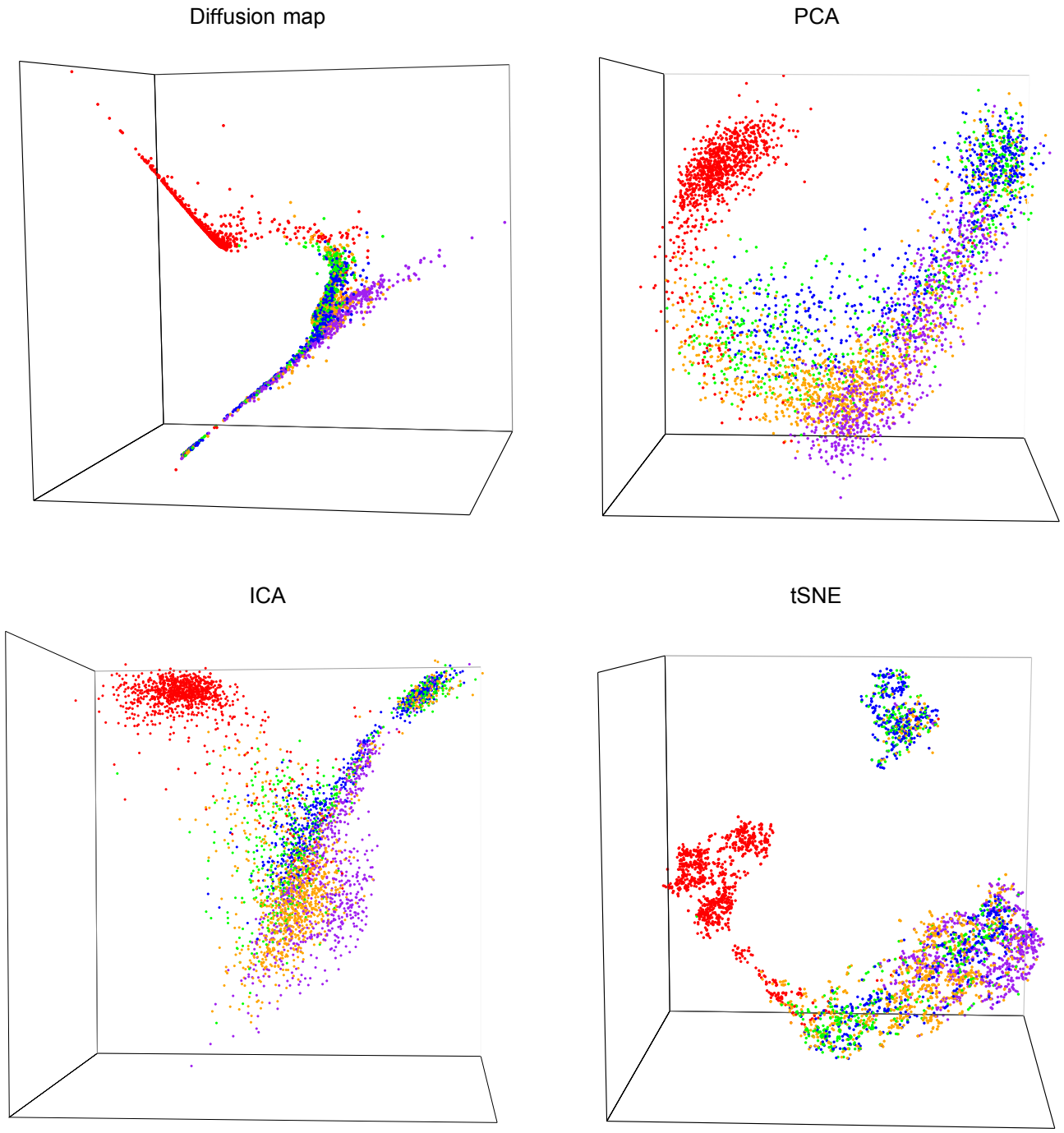
Supplementary Figure 4: RNA sequencing of populations of 50 cells. Schematic of early embryonic development of the mesodermal lineage. The cardiac, smooth muscle, endothelial and blood lineages all diverge from Flk1^{+} mesoderm, while somatic muscle diverges earlier. **(a-f)** Populations of 50 cells were sorted from 5 embryos each at PS, NP and HF stages. FPKM values for three key genes are shown for each of the lineage decisions in the schematic. Points are the mean and s.d. of the 5 replicates.



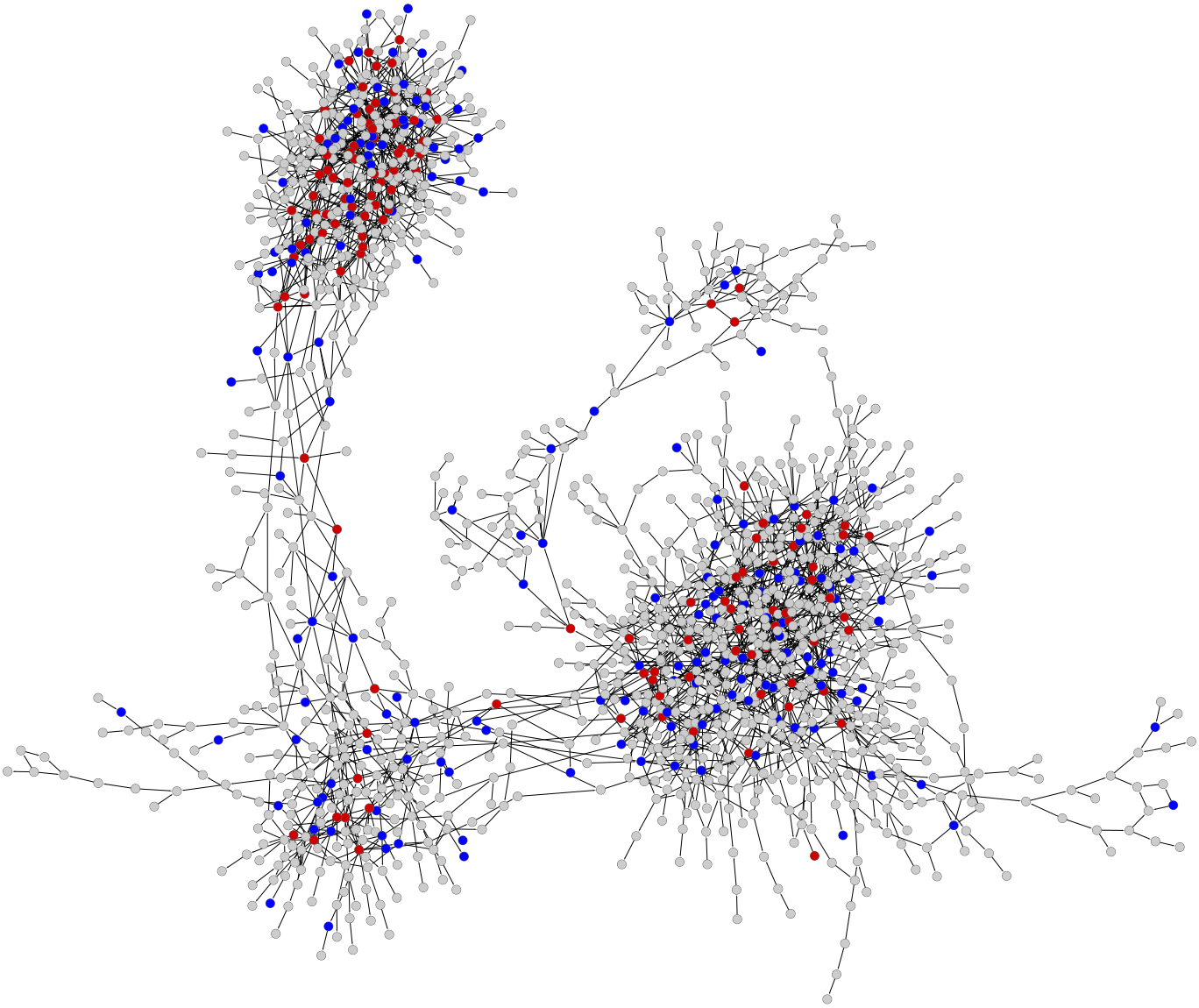
Supplementary Figure 5: Diffusion plots. Diffusion plots showing expression patterns of additional genes not shown in Figure 2.



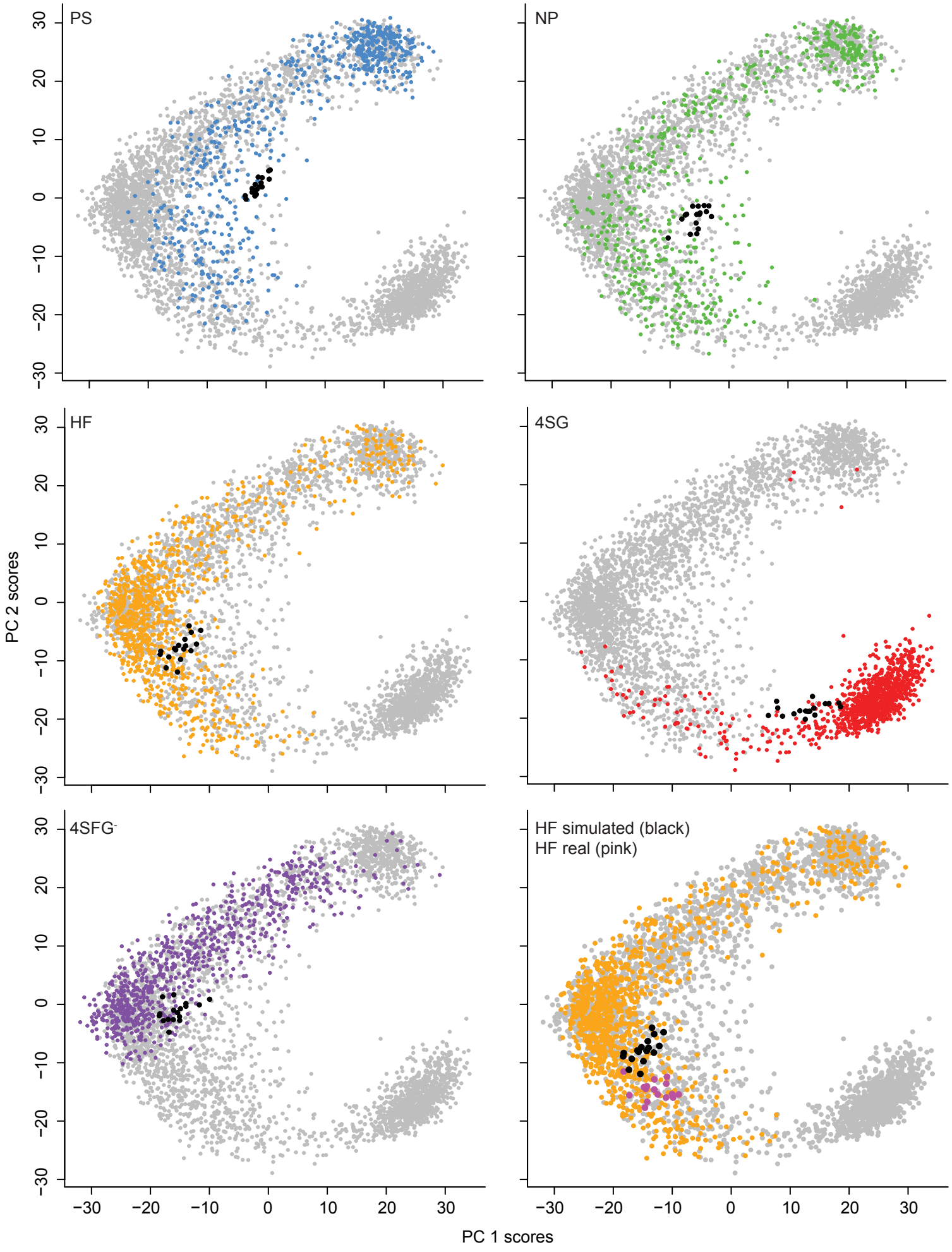
Supplementary Figure 6: Diffusion plots highlighting each stage. Top left plot shows all populations, other plots were coloured according to embryonic stage with cells from other stages shown in grey. Blue, PS; green, NP; orange, HF; red, 4SG; purple, 4SFG^{-}.



Supplementary Figure 7: Comparison of multivariate analysis techniques. The data for all 3,934 cells were plotted using diffusion maps, principal component analysis, independent component analysis and t-SNE. Blue, PS; green, NP; orange, HF; red, 4SG; purple, 4SFG^{-}.



Supplementary Figure 8: Some states occur in multiple cells. State transition graph coloured by the number of times each binary state occurs in the 3,934 expression profiles. Grey, occurs once; blue, occurs twice; red, occurs more than twice.



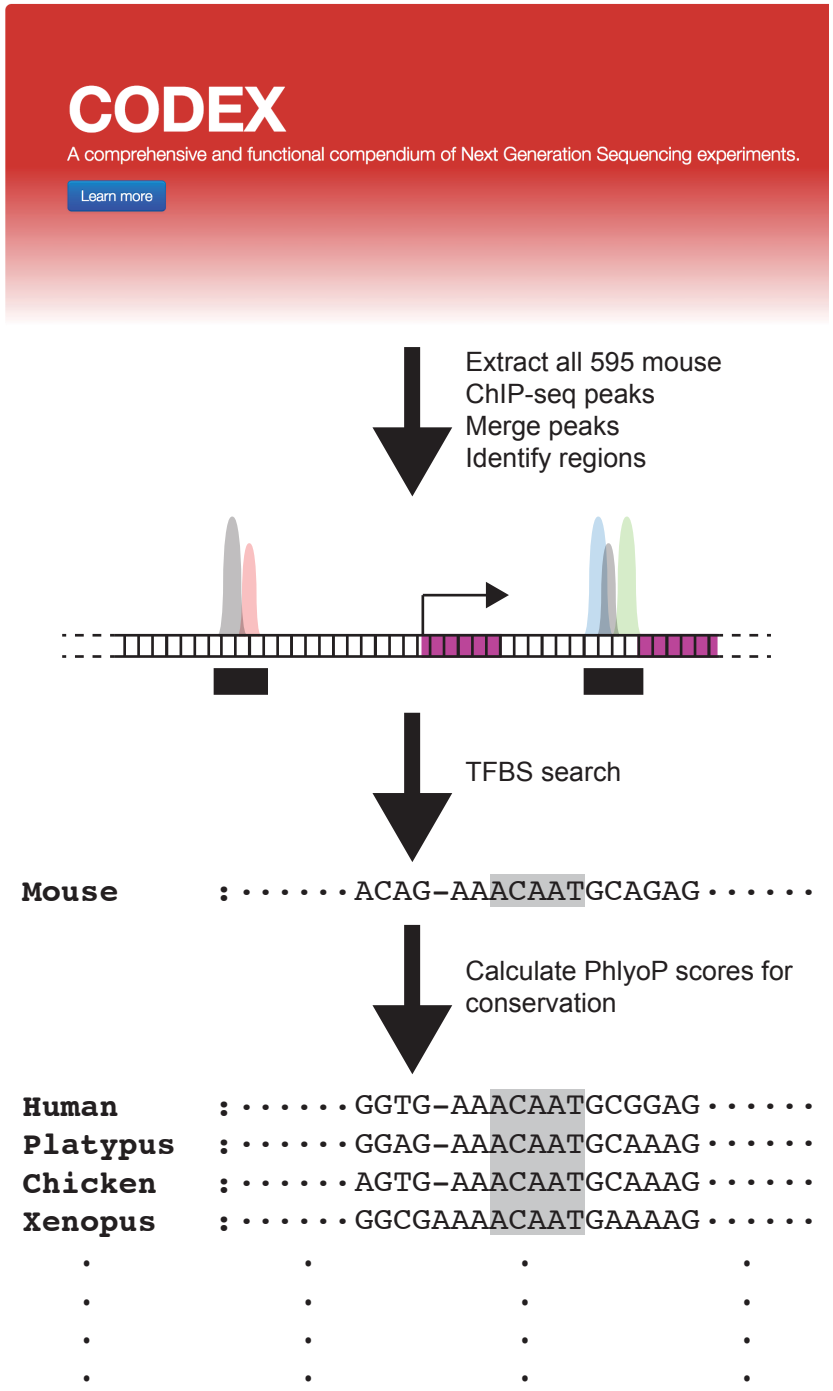
Supplementary Figure 9: Populations mask variation and asynchrony. Pools of 20 cells were simulated from single cell data and projected onto the principal component analysis for all 3,934 cells. Each plot shows one embryonic stage and the simulated pools of 20 cells for that population in black (normalized in the same way as single cells). Bottom right hand plot shows the head fold stage and simulated pools as well as actual pools of 20 cells sorted from embryos (pink).

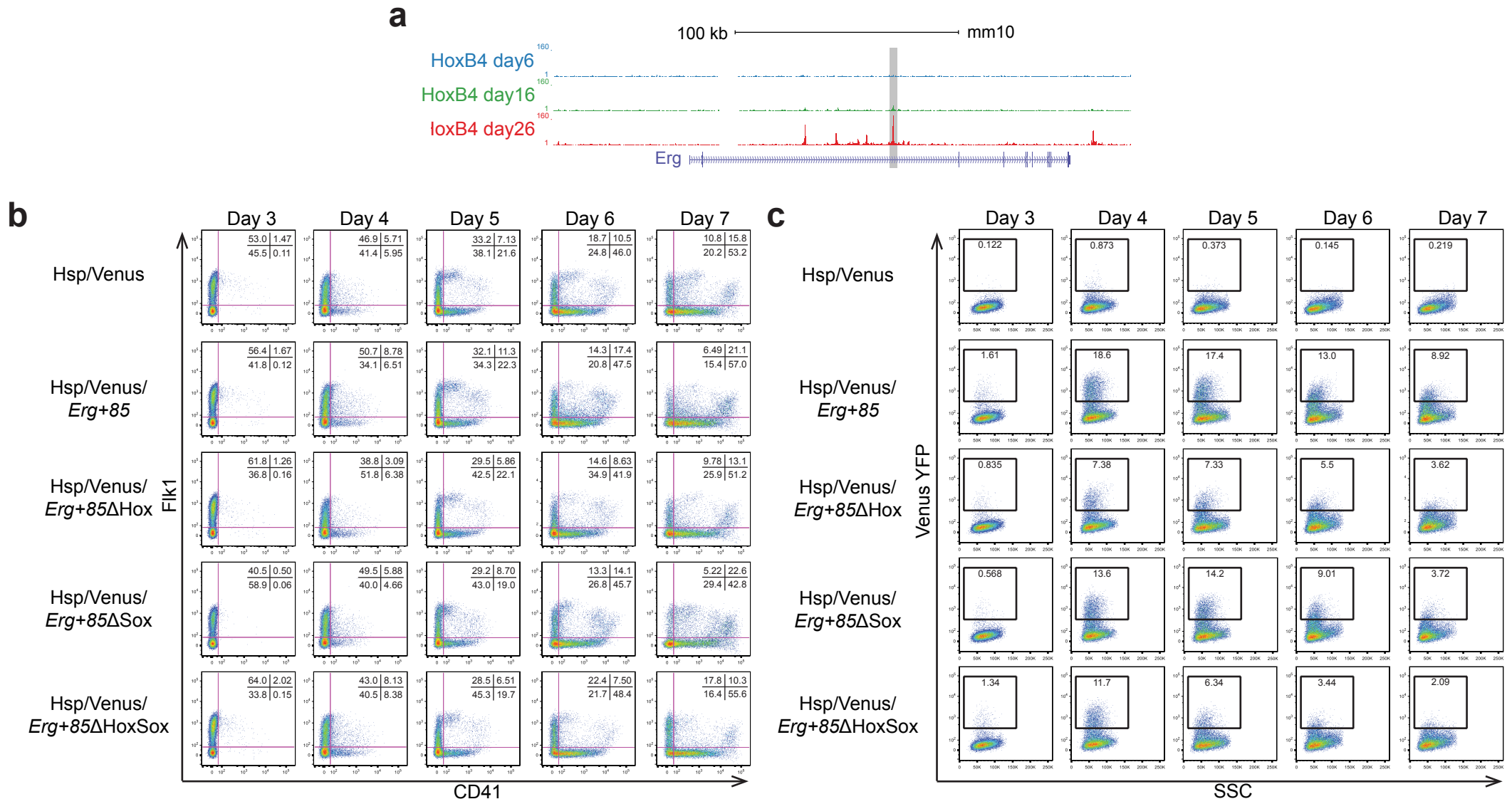
a

Factor type	TFs	Motif
AP1-like	Nfe2	TCAY
Ets	Erg, Ets1, Etv2, Fli1, PU.1	GGAW
Ebox	Scl, Lyl1	CANNTG
Gata	Gata1	GATA
Gfi	Gfi1, Gfi1b	AATC
HMG box	Sox7, Sox17	WWCAAWG
Homeobox	Hhex	YWATTAAR
Homeobbox	Hoxb4	TAAT
Ikaros	Ikaros	HRGGAW
Myb	Myb	YAACNG
Rbpj	Notch1	TTCCCAA

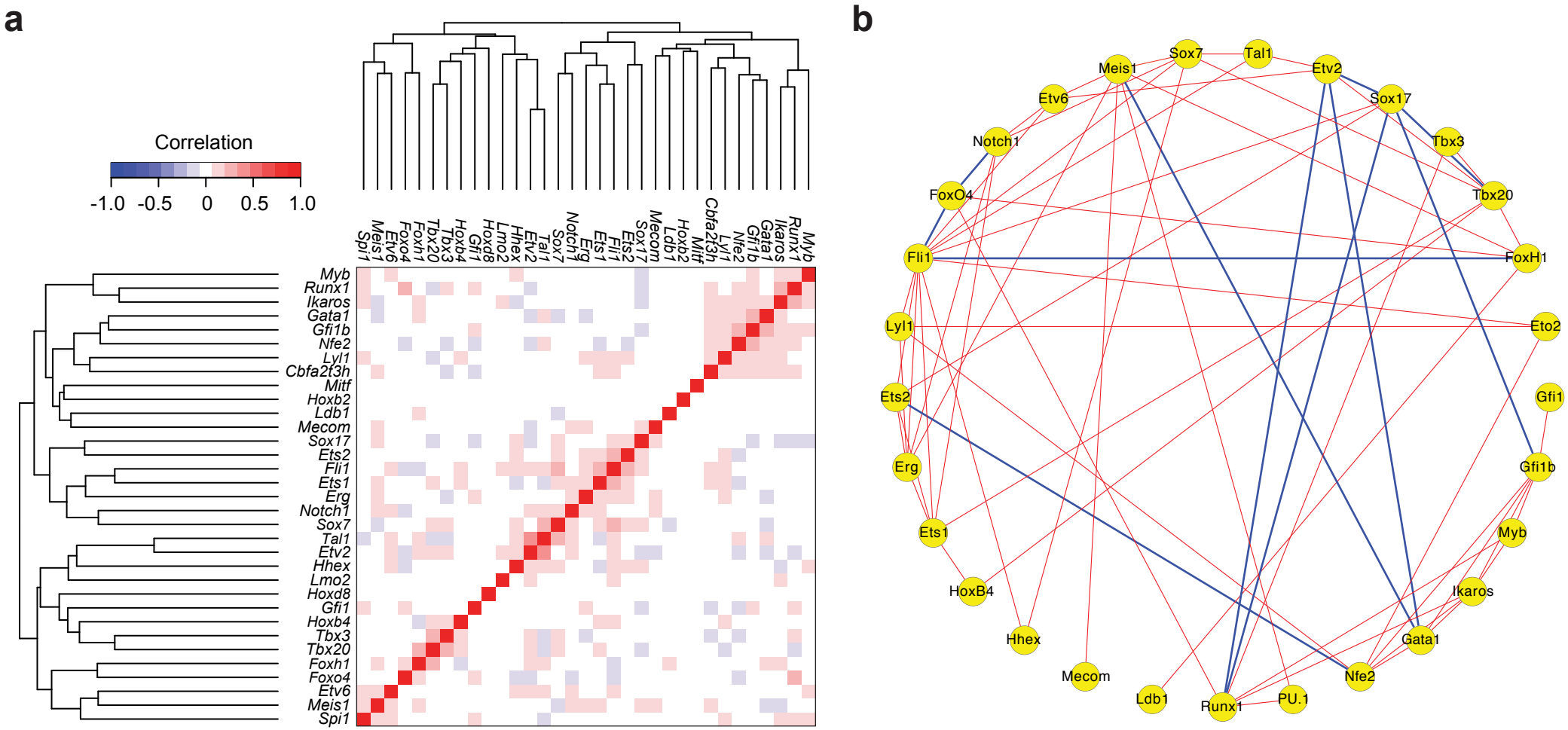
Supplementary Figure 10: Analysis of transcription factor binding sites in network gene loci. **(a)** Motifs for DNA-binding TFs in the core network. Note that Eto2 and Lmo2 do not bind directly to the DNA, and the binding site of the Notch partner Rbpj was used for Notch1 as described previously[1]. **(b)** To search for evidence that predicted regulatory interactions are direct, all haematopoietic TF ChIP-seq data in Codex[2] were searched for peaks within the gene body or 50 kb up- and down-stream of the 20 network genes. Overlapping peaks were merged to identify putative regulatory sequences. Different colours indicate peaks from different experiments. A transcription factor binding site search (TFBS) was performed using TFBSsearch[3] on the mouse genomic sequence of all regions to identify the consensus binding sites of the network TFs. Evolutionary conservation of TF binding motifs is a well-recognised feature of truly functional binding sites[4]. To identify conserved binding sites, PhyloP scores were calculated for each motif across 60 species using the tool provided by the UCSC Genome Browser. Evolutionarily conserved motifs previously validated in functional assays by us and other were characterised by PhyloP scores greater than 1, which was therefore used as the threshold. Results are summarised in Supplementary Table 2.

b





Supplementary Figure 11: The *Erg+85* enhancer is active during haematopoietic development. **(a)** UCSC Genome Browser tracks of re-analysed ChIP-Seq data for HoxB4[5] indicate that HoxB4 binds to the *Erg+85* enhancer (shown in grey) during a 26-day differentiation time course that produces HSCs from ES cells, with HSCs able to contribute to long-term engraftment in recipient mice. Binding is absent at day 6, but becomes apparent by day 16. **(b)** Flow cytometric analysis of Flk1 and CD41 expression across a time-course of EB differentiation from Figure 4B indicates that different clones have similar kinetics of differentiation, with Flk1$^+$ cells giving rise to Flk1$^+$CD41$^+$ and then Flk1$^-$CD41$^+$ cells. **(c)** Flow cytometric analysis of YFP expression in all live cells across a time-course of EB differentiation from Figure 4b illustrates reduction of YFP positive cells in ES cell clones where YFP expression is driven by mutant enhancer constructs. In **(b)** and **(c)**, a representative experiment is shown from three biological repeats of 2-3 clones per construct.



Supplementary Figure 12: Partial correlation analysis. **(a)** Hierarchical clustering of partial correlation coefficients between pairs of transcription factors for all 3,934 expression profiles. Pairwise coefficients were calculated while controlling for the effect of all other transcription factors. *Erg* does not correlate with Sox or Hox factors. **(b)** Network diagram showing putative undirected activating (red) and inhibiting (blue) relationships suggested by significant correlations (p-value < 1e-10).

Supplementary Table 1: List of TaqMan assays used for single cell gene expression analysis.

Gene name	Protein name	Assay ID
Cbfa2t3h	Eto2	Mm00486780_m1
Cdh1	E-cadherin	Mm01247357_m1
Cdh5	VE-cadherin	Mm00486938_m1
Egfl7	Egfl7	Mm00618004_m1
Eif2b1	Eif2b1	Mm01199614_m1
Erg	Erg	Mm01214246_m1
Ets1	Ets1	Mm01175819_m1
Ets2	Ets2	Mm00468977_m1
Etv2	Etv2	Mm00468389_m1
Etv6	Tel	Mm01261325_m1
Fli1	Fli1	Mm00484409_m1
FoxH1	FoxH1	AJD1TLL (custom assay)
FoxO4	FoxO4	AJLJIMX (custom assay)
Gata1	Gata1	Mm00484678_m1
Gata2	Gata2	Mm00492300_m1
Gfi1	Gfi1	Mm00515855_m1
Gfi1b	Gfi1b	Mm00492318_m1
Hbb-bH1	Hbb-bH1	Mm00756487_mH
Hhex	Hhex	Mm00433954_m1
HoxB2	HoxB2	Mm04209931_m1
HoxB3	HoxB3	Mm00650701_m1
HoxB4	HoxB4	Mm00657964_m1
HoxD8	HoxD8	AJFARRT (custom assay)
Ikzf1	Ikaros	Mm01187882_m1
Itga2b	CD41	Mm00439768_m1
Kdr	Flk1	Mm01222421_m1
Kit	c-Kit	Mm00445212_m1
Ldb1	Ldb1	Mm00440156_m1
Lmo2	Lmo2	Mm01281680_m1
Lyl1	Lyl1	Mm01247198_m1
Mecom	MDS1/Evi1	Mm01289155_m1
Meis1	Meis1	Mm00487659_m1
Mitf	Mitf	Mm01182480_m1
Mrpl19	Mrpl19	Mm03048937_m1
Myb	Myb	Mm00501741_m1
Nfe2	Nfe2	Mm00801891_m1
Notch1	Notch1	Mm00435249_m1
Pecam1	CD31	Mm01242584_m1
Polr2a	Polr2a	Mm00839493_m1
Procr	Epcr	Mm00440993_mH
Runx1	Runx1	Mm01213405_m1
Spi1	PU.1	Mm00488142_m1
Sox17	Sox17	Mm04208182_m1
Sox7	Sox7	Mm00776876_m1
Tal1	Scl	Mm01187033_m1
Tbx20	Tbx20	Mm00451517_m1
Tbx3	Tbx3	Mm01195726_m1
Ubc	Ubc	Mm01201237_m1

Supplementary Table 2: Boolean update rules for the network in Figure 3c, synthesized from the state graph in Figure 3b. The final column indicates whether the DNA binding motifs of the predicted regulators are present in the locus of the target gene (see Supplementary Figure 10).

Gene	**Synthesised update functions**	**% Non-observed transitions disallowed (N_i)**	**Motifs present**
Scl	*Fli1*	98	Yes
Etv2	*Notch1*	96	Yes
Fli1	*Etv2*	96	Yes
	Sox7	97	Yes
Lyl1	*Sox7*	92	Yes
Sox7	*Sox17* $\vee$ *HoxB4*	82	No (Sox missing)
Erg	(*HoxB4* $\wedge$ *Lyl1*) $\vee$ *Sox17*	84	Yes
	(*HoxB4* $\wedge$ *Tal1*) $\vee$ *Sox17*	83	Yes
Notch1	*Sox7*	94	Yes
Gata1	*Gfi1b* $\wedge$ *Lmo2*	86	Yes
	Gfi1b $\wedge$ *Hhex*	84	No (Hhex missing)
	Gfi1b $\wedge$ *Ets1*	84	Yes
HoxB4	(*Lyl1* $\wedge$ *Ets1*) $\wedge$ $\neg$*Gata1*	65	Yes
	(*Lyl1* $\vee$ *Nfe2*) $\wedge$ $\neg$*Gata1*	65	Yes
	(*Lyl1* $\vee$ *Ikaros*) $\wedge$ $\neg$*Gata1*	65	No (Ikaros missing)
Sox17	*Lyl1* $\wedge$ $\neg$*Gfi1b*	77	No (Gfi missing)
	(*Eto2* $\wedge$ *Sox7*) $\wedge$ $\neg$*Gfi1b*	76	No (Gfi missing)
	(*Eto2* $\wedge$ *Tal1*) $\wedge$ $\neg$*Gfi1b*	75	No (Gfi missing)
Ets1	*Notch1*	96	Yes
Gfi1	*Gata1* $\wedge$ $\neg$*Sox17*	88	Yes
	Nfe2 $\wedge$ $\neg$*Sox17*	88	Yes
Gfi1b	*Nfe2* $\wedge$ *Myb*	87	Yes
	Pu.1 $\wedge$ *Ikaros*	86	No (Ikaros missing)
	Pu.1 $\wedge$ *Nfe2*	86	Yes
	Pu.1 $\wedge$ *Myb*	86	Yes
Eto2	*Sox7*	93	No (Sox missing)
	Hhex	92	No (Hhex missing)
	Ets1 $\wedge$ *Fli1*	94	No (Ets missing)
Hhex	*Sox7*	97	No (Sox missing)
	Notch1	93	No (Rbpj missing)
Ikaros	*Nfe2* $\vee$ *Gfi1b*	84	Yes
	Nfe2 $\vee$ *Gata1*	83	Yes
	Nfe2 $\vee$ *Gfi1*	82	Yes
Lmo2	*Sox7* $\vee$ *Gfi1*	79	Yes
	Sox7 $\vee$ *Erg*	79	Yes
	Sox7 $\vee$ *HoxB4*	77	Yes
Nfe2	*Ikaros*	78	Yes
Pu.1	*Gfi1* $\vee$ *Erg*	67	Yes
Myb	*HoxB4*	64	Yes

Supplementary Table 3: Boolean update rules after multiple rounds of bootstrapping **(a-e)**. In general, the number of possible solutions for a gene's update function grows as the amount of data used is decreased, and including the full data set narrows these possibilities.

Supplementary Table 3a

Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
Scl	*Hhex*	96
	Sox7	98
	Etv2	98
	Fli1	99
	Ets1	100
	Scl	100
Etv2	No solution	
Fli1	***Etv2***	98
	Sox7	98
	Notch1	98
Lyl1	*Etv2*	90
	Notch1	91
	Sox7	92
Sox7	***Sox17*** $\vee$ ***HoxB4***	85
Erg	*Sox7* $\vee$ *HoxB4*	90
	Sox7 $\vee$ *Notch1*	89
	Sox7	89
	Notch1	89
	Sox7 $\wedge$ *Notch1*	88
	Sox17 $\vee$ *HoxB4*	84
Notch1	No solution	
Gata1	*Gfi1*	89
HoxB4	*Lyl1*	76
Sox17	***Lyl1*** $\wedge \neg$ ***Gfi1b***	78
Ets1	*Sox7*	98
	Notch1	99
Gfi1	*Gfi1b* $\wedge \neg$ *Sox17*	88
	Nfe2 $\wedge \neg$ *Sox17*	90
	Gata1	90
	Gata1 $\wedge \neg$ ***Sox17***	91
Gfi1b	*Gata1*	87
	Nfe2 $\wedge$ ***Myb***	87
	Nfe2 $\wedge$ *Ikaros*	86
	Pu.1 $\wedge$ ***Nfe2***	86
	Sox7 $\wedge$ *Gata1*	86
	Pu.1 $\wedge$ *Myb*	85
Eto2	*Ets1*	95
	Sox7	94
	Hhex	93
	Notch1	93
	Etv2	93
Hhex	***Sox7***	98
	Notch1	98
Ikaros	***Nfe2*** $\vee$ ***Gfi1b***	81
Lmo2	*Notch1*	79
	Sox7	79
Nfe2	***Ikaros***	75
	Gfi1	81

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Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
	Gfi1b	83
	Gata1	84
Pu.1	*Erg*	71
	Sox7 $\wedge$ *Eto2*	71
	Lyl1 $\wedge$ *Erg*	71
	Notch1 $\wedge$ *Eto2*	71
	Gfi1 $\vee$ ***Erg***	72
	HoxB4 $\vee$ *Erg*	72
	Erg $\wedge$ *Eto2*	73
	Ikaros $\wedge$ *Erg*	73
	Notch1 $\wedge$ *Ikaros*	75
	Sox7 $\wedge$ *Ikaros*	75
Myb	***HoxB4***	60
	Gfi1	67

Supplementary Table 3b

Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
Scl	*Hhex*	96
	Notch1	96
	Etv2	96
	Ets1	97
	Sox7	98
	Fli1	98
Etv2	No solution	
Fli1	*Notch1*	96
	Etv2	96
	Sox7	98
Lyl1	*Erg*	84
	Notch1	87
	Etv2	87
	Sox7	91
Sox7	*Sox17* $\vee$ *Erg*	90
	HoxB4 $\vee$ *Erg*	88
	Gfi1 $\vee$ *Erg*	85
	Sox17 $\vee$ ***HoxB4***	84
	Scl $\wedge$ *Erg*	82
	Fli1 $\wedge$ *Erg*	82
	Erg	82
Erg	*Notch1* $\wedge$ *Etv2*	81
	Sox17 $\vee$ ***HoxB4***	83
	Sox7 $\wedge$ *Notch1*	85
Notch1	No solution	
Gata1	*Gfi1*	84
	Gfi1b $\wedge$ *Ets1*	85
	Lmo2 $\wedge$ ***Gfi1b***	87
HoxB4	*Lyl1*	75
Sox17	***Lyl1*** $\wedge \neg$ ***Gfi1b***	78
Ets1	*Sox7*	95
	Notch1	97
Gfi1	*Gata1*	86
	Nfe2 $\wedge \neg$ ***Sox17***	88
	Gfi1b $\wedge \neg$ *Sox17*	88
	Gata1 $\wedge \neg$ ***Sox17***	88
	Gfi1b $\wedge \neg$ *Erg*	87
	Gata1 $\wedge \neg$ *Erg*	86
	Nfe2 $\wedge \neg$ *Hhex*	86
	Gata1 $\wedge \neg$ *Hhex*	86
	Ikaros $\wedge \neg$ *Hhex*	86
	Nfe2 $\wedge \neg$ *Erg*	86
Gfi1b	*Gfi1*	81
	Pu.1 $\wedge$ ***Nfe2***	88
	Nfe2 $\wedge$ ***Myb***	89
Eto2	***Sox7***	94
	Hhex	93
	Ets1	92
	Etv2	91
	Notch1	91
Hhex	***Notch1***	94

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Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
	Sox7	97
Ikaros	*Myb* $\wedge$ *Eto2*	80
	Nfe2 $\vee$ ***Gata1***	81
	Nfe2 $\vee$ ***Gfi1***	82
	Nfe2 $\vee$ ***Gfi1b***	83
Lmo2	*Sox7*	78
Nfe2	***Ikaros***	77
	Gfi1	80
	Gata1	83
	Gfi1b	86
Pu.1	*Nfe2* $\wedge$ *Erg*	67
	HoxB4 $\wedge$ *Gfi1b*	67
	Myb $\wedge$ *Erg*	67
	Sox7 $\wedge$ *Myb*	67
	Erg $\wedge$ *Eto2*	68
	Gfi1 $\vee$ ***Erg***	68
	Lyl1 $\wedge$ *Erg*	68
	Notch1 $\wedge$ *Nfe2*	68
	Tbx20 $\wedge$ *Gfi1b*	68
	Sox7 $\wedge$ *Nfe2*	69
	Gfi1b $\wedge$ *Erg*	72
	Ikaros $\wedge$ *Erg*	73
	Notch1 $\wedge$ *Gfi1b*	74
	Sox7 $\wedge$ *Gfi1b*	75
	Notch1 $\wedge$ *Ikaros*	75
	Sox7 $\wedge$ *Ikaros*	75
Myb	***HoxB4***	65
	Gfi1	66

Supplementary Table 3c

Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
Scl	*Hhex*	97
	Notch1	97
	Sox7	98
	Etv2	98
	Fli1	99
	Ets1	100
	Scl	100
Etv2	No solution	
Fli1	*Notch1*	97
	Etv2	97
	Sox7	98
Lyl1	*Notch1*	90
	Sox7	92
Sox7	***Sox17*** $\vee$ ***HoxB4***	85
Erg	*Sox7*	88
	Notch1	89
	Sox17 $\vee$ *HoxB4*	85
Notch1	No solution	
HoxB4	*Lyl1*	76
Sox17	***Lyl1*** $\wedge \neg$ ***Gfi1b***	75
	Erg $\wedge \neg$*Gfi1b*	74
	Fli1 $\wedge \neg$*Gfi1b*	74
	Eto2 $\wedge \neg$*Gfi1b*	74
	Sox7 $\wedge \neg$*Gfi1b*	74
	Lyl1 $\wedge \neg$*Gata1*	73
Ets1	***Notch1***	98
	Sox7	98
Gfi1	*Gfi1b* $\wedge \neg$*Sox17*	86
	Gata1	87
	Nfe2 $\wedge \neg$ ***Sox17***	87
	Gata1 $\wedge \neg$ ***Sox17***	89
Gfi1b	*Gfi1* $\wedge$ *Eto2*	80
	Ikaros $\wedge$ *Gfi1*	80
	Nfe2 $\wedge$ *Ets1*	82
	Notch1 $\wedge$ *Gata1*	82
	Myb $\wedge$ *Ikaros*	83
	Gata1 $\wedge$ *Etv2*	83
	Pu.1 $\wedge$ ***Nfe2***	83
	Sox7 $\wedge$ *Gata1*	83
	Pu.1 $\wedge$ *Gata1*	84
	Pu.1 $\wedge$ ***Ikaros***	84
	Pu.1 $\wedge$ ***Myb***	84
	Gata1 $\wedge$ *Ets1*	85
	Myb $\wedge$ *Gata1*	85
	Nfe2 $\wedge$ ***Myb***	85
Eto2	*Ets1*	96
	Sox7	95
	Hhex	94
	Etv2	94
	Notch1	93
Hhex	***Notch1***	96

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Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
	Sox7	97
Ikaros	*Myb*	80
	Myb $\wedge$ *Ets1*	80
	Myb $\wedge$ *Fli1*	80
	Scl $\wedge$ *Myb*	80
	Myb $\wedge$ *Lyl1*	80
	Myb $\vee$ *Gfi1*	80
	Myb $\vee$ *Gata1*	81
	Myb $\vee$ *Gfi1b*	81
	Nfe2 $\vee$ *Myb*	81
	Myb $\wedge$ *Eto2*	82
	Nfe2 $\vee$ ***Gata1***	82
	Nfe2 $\vee$ ***Gfi1***	82
	Nfe2 $\vee$ ***Gfi1b***	83
Lmo2	*Sox7*	79
	Notch1	78
Nfe2	***Ikaros***	77
	Gfi1	79
	Gfi1b	83
	Gata1	84
Pu.1	*Erg*	67
	Scl $\wedge$ *Erg*	67
	Ets1 $\wedge$ *Erg*	67
	Fli1 $\wedge$ *Erg*	67
	Notch1 $\wedge$ *Eto2*	68
	HoxB4 $\vee$ *Erg*	68
	Sox7 $\wedge$ *Eto2*	68
	Erg $\wedge$ *Eto2*	69
	Gfi1b $\wedge$ *Erg*	69
	Notch1 $\wedge$ *Lyl1*	69
	Gfi1 $\vee$ ***Erg***	70
	Ikaros $\wedge$ *Erg*	70
	Lyl1 $\wedge$ *Erg*	70
	Notch1 $\wedge$ *Gfi1b*	70
	Sox7 $\wedge$ *Lyl1*	70
	Sox7 $\wedge$ *Gfi1b*	71
	Notch1 $\wedge$ *Ikaros*	72
	Sox7 $\wedge$ *Ikaros*	73
Myb	*Gfi1*	64
	HoxB4	62
	Erg	62

Supplementary Table 3d

Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
Scl	*Etv2*	96
	Notch1	96
	Ets1	96
	Hhex	96
	Fli1	97
	Sox7	97
Etv2	No solution	
Fli1	*Notch1*	96
	Etv2	96
	Sox7	98
Lyl1	*Notch1*	86
	Erg	88
	Sox7	91
Sox7	*Lmo2* $\vee$ *HoxB4*	89
	Sox17 $\vee$ *Erg*	89
	HoxB4 $\vee$ *Erg*	87
	Gfi1 $\vee$ *Erg*	85
	Sox17 $\vee$ *Lmo2*	85
	Scl $\wedge$ *Erg*	83
	Fli1 $\wedge$ *Erg*	84
	Erg	83
	Sox17 $\vee$ ***HoxB4***	83
Erg	*Sox7*	86
	Notch1	82
	Sox7 $\vee$ *Gfi1*	86
	Sox17 $\vee$ *HoxB4*	87
	Sox7 $\wedge$ *Fli1*	87
	Notch1 $\wedge$ *Eto2*	88
	Sox7 $\wedge$ *Eto2*	89
	Notch1 $\wedge$ *Lyl1*	90
	Sox7 $\wedge$ *Lyl1*	90
Notch1	No solution	
Gata1	*Gfi1b*	85
	Gfi1	88
HoxB4	*Lyl1* $\wedge \neg$*Gfi1*	64
Sox17	***Lyl1*** $\wedge \neg$***Gfi1b***	78
	Erg $\wedge \neg$*Gfi1b*	76
	Lyl1 $\wedge \neg$*Gata1*	75
	Erg $\wedge \neg$*Gata1*	74
	Eto2 $\wedge \neg$*Gfi1b*	73
	Lyl1 $\wedge \neg$*Myb*	72
	Hhex $\wedge \neg$*Gfi1b*	72
	Sox7 $\wedge \neg$*Gfi1b*	72
	Erg $\wedge \neg$*Gfi1*	71
	Lyl1 $\wedge \neg$*Gfi1*	71
Ets1	*Sox7*	94
	Notch1	97
Gfi1	*Gata1*	88
	Gata1 $\wedge \neg$***Sox17***	89
Gfi1b	*Gata1*	85
	Nfe2	84

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Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
	Nfe2 $\vee$ *Gfi1*	85
	Nfe2 $\vee$ *Gata1*	86
	Pu.1 $\wedge$ ***Nfe2***	86
	Myb $\wedge$ *Gata1*	86
	Pu.1 $\wedge$ ***Ikaros***	86
	Nfe2 $\wedge$ *Ikaros*	87
	Pu.1 $\wedge$ ***Myb***	87
	Nfe2 $\wedge$ ***Myb***	88
	Gfi1 $\vee$ *Gata1*	88
Eto2	*Fli1*	93
	Sox7	92
	Scl	92
	Hhex	92
	Lyl1	92
	Ets1	90
Hhex	***Notch1***	94
	Sox7	97
Ikaros	*Myb* $\vee$ *Gfi1b*	80
	Myb $\wedge$ *Lyl1*	81
	Nfe2 $\vee$ ***Gata1***	81
	Myb $\wedge$ *Eto2*	82
	Nfe2 $\vee$ ***Gfi1b***	83
Lmo2	*Sox7*	76
	Erg	72
Nfe2	*Myb*	70
	Ikaros	76
	Gfi1	81
	Gata1	85
	Gfi1b	86
Pu.1	*Erg*	69
	Gfi1b $\wedge$ *Erg*	74
	Notch1 $\wedge$ *Gfi1b*	75
	Sox7 $\wedge$ *Gfi1b*	75
Myb	*Erg*	62
	Gfi1	64
	HoxB4	66

Supplementary Table 3e

Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
Scl	*Lyl1*	96
	Hhex	97
	Eto2	97
	Notch1	97
	Etv2	98
	Sox7	98
	Ets1	100
	Fli1	100
	Scl	100
Etv2	***Notch1***	96
	Sox7	98
Fli1	*Meis1*	90
	Notch1	97
	Etv2	98
	Sox7	98
Lyl1	*Hhex*	95
	Notch1	95
	Sox7	97
Sox7	***Sox17*** $\vee$ ***HoxB4***	88
Erg	*Notch1*	91
	Sox7	90
Notch1	No solution	
Gata1	*Gfi1*	85
HoxB4	*Sox17* $\vee$ *Lmo2*	67
	Eto2 $\wedge \neg$ *Gfi1*	59
	Lyl1 $\wedge \neg$ *Gfi1*	59
	Fli1 $\wedge \neg$ *Gfi1*	59
	Scl $\wedge \neg$ *Gfi1*	59
	Lmo2	57
Sox17	*Fli1* $\wedge \neg$ *Gfi1b*	79
	Sox7 $\wedge \neg$ *Gfi1b*	79
	Scl $\wedge \neg$ *Gfi1b*	79
	Ets1 $\wedge \neg$ *Gfi1b*	79
	Etv2 $\wedge \neg$ *Gfi1b*	79
	Notch1 $\wedge \neg$ *Gfi1b*	79
	Hhex $\wedge \neg$ *Gfi1b*	78
	Eto2 $\wedge \neg$ *Gfi1b*	77
	Lyl1 $\wedge \neg$ ***Gfi1b***	75
Ets1	***Notch1***	97
	Sox7	98
Gfi1	*Gata1*	87
	Nfe2 $\wedge \neg$ ***Sox17***	87
	Gata1 $\wedge \neg$ ***Sox17***	88
Gfi1b	*Notch1* $\wedge$ *Gata1*	81
	Nfe2 $\wedge$ *Etv2*	81
	Sox7 $\wedge$ *Nfe2*	81
	Gata1 $\wedge$ *Etv2*	82
	Sox7 $\wedge$ *Gata1*	82
	Pu.1 $\wedge$ *Gata1*	82
	Nfe2 $\wedge$ *Ets1*	83
	Gata1 $\wedge$ *Ets1*	84

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Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
	Myb ∧ *Ikaros*	84
	Myb ∧ *Gata1*	84
	Pu.1 ∧ ***Ikaros***	84
	Pu.1 ∧ ***Myb***	85
	Pu.1 ∧ ***Nfe2***	85
	Nfe2 ∧ ***Myb***	86
Eto2	*Ets1*	99
	Sox7	97
	Etv2	97
	Notch1	96
	Hhex	96
Hhex	***Notch1***	96
	Sox7	98
Ikaros	*Myb* ∧ *Lyl1*	80
	Myb ∨ *Gata1*	80
	Myb ∨ *Gfi1*	80
	Myb ∨ *Mitf*	80
	Myb ∨ *Gfi1b*	81
	Nfe2 ∨ *Myb*	81
	Nfe2 ∨ ***Gfi1b***	82
Lmo2	*Sox7*	78
	Notch1	78
	Notch1 ∨ *Erg*	79
	Sox7 ∨ ***Gfi1***	79
	Sox7 ∨ ***HoxB4***	79
	Sox7 ∨ ***Erg***	79
	Sox7 ∨ *Notch1*	79
Nfe2	*Myb*	71
	Ikaros	74
	Gfi1	80
	Gata1	82
	Gfi1b	84
Pu.1	*Erg*	68
	Sox7	67
Myb	*Erg*	62
	Gfi1	63
	HoxB4	65

Supplementary Table 4: Boolean update rules synthesised from data filtered according to a more stringent limit of detection (Ct 23).

Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
Scl	*Scl*	100
	Fli1	99
	Sox7	99
Etv2	*Sox7*	98
	Notch1	94
Fli1	*Scl*	99
	Ets1	99
	Sox7	98
	Etv2	98
	Hhex	97
Lyl1	*Fli1*	93
	Eto2	93
	Scl	93
	Ets1	93
	Sox7	91
	Hhex	91
	Etv2	91
Sox7	*Notch1*	95
	Sox17 $\vee$ ***HoxB4***	96
Erg	*Sox7*	87
	Notch1	84
	HoxB4 $\vee$ *Sox17*	85
Notch1	***Sox7***	96
	Scl	96
	Ets1	96
	Fli1	96
	Etv2	96
Gata1	*Gfi1b*	86
	Gfi1	87
HoxB4	*Lyl1*	74
	Erg	74
Sox17	***Lyl1*** $\wedge \neg$ ***Gfi1b***	75
Ets1	*Sox7*	99
	Notch1	94
Gfi1	*Gata1*	87
	Gata1 $\wedge \neg$ ***Sox17***	87
	Nfe2 $\wedge \neg$ ***Sox17***	87
	HoxB4 $\wedge \neg$*Notch1*	87
	Gfi1b $\wedge \neg$*Sox17*	86
Gfi1b	*Gata1*	86
	Nfe2	82
	Gfi1	81
Eto2	*Fli1*	97
	Tal1	96
	Ets1	96
	Sox7	94
	Hhex	94
	Etv2	94
Hhex	*Scl*	99
	Fli1	99
	Ets1	99

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Gene	Synthesised update functions	% Non-observed transitions disallowed (N_i)
	Sox7	98
	Etv2	97
Ikaros	***Nfe2*** $\vee$ ***Gfi1b***	83
	Gata1 $\vee$ *Gfi1b*	80
	Myb $\vee$ *Gfi1b*	80
	Gfi1 $\vee$ *Gfi1b*	80
	Myb $\wedge$ *Eto2*	80
	Myb $\vee$ *Lyl1*	81
Lmo2	*Sox7*	72
Nfe2	*Gata1*	84
	Gfi1b	84
	Gfi1	80
	Ikaros	73
Pu.1	*Ikaros*	75
	Gfi1b	74
Myb	*Ikaros*	79
	Gfi1b	75

Supplementary Table 6: Statistical analysis of *Erg+85* enhancer assays. Results of statistical analysis for Figure 4B, comparing each construct to wild type on each day. P values were generated for each differentiation with t-tests, and differentiations were combined by the Fisher's method to calculate overall significance. Where replicates differed in whether the percentage of YFP+ cells was higher or lower than wild type, Stouffer's Z trend was used rather than Fisher's method. *, p <0.05; **, p<0.01; ***, p<0.001; ns, not significant (p >0.05).

Population	Mutant	Day3	Day4	Day5	Day6	Day7
Flk1+CD41-	**Hox**	*	ns	ns	ns	ns
	Sox	ns	ns	ns	ns	ns
	HoxSox	ns	*	*	*	ns
Flk1+CD41+	**Hox**	ns	**	ns	**	*
	Sox	*	ns	ns	ns	ns
	HoxSox	ns	*	*	**	*
Flk1-CD41+	**Hox**	ns	***	**	ns	ns
	Sox	ns	ns	ns	ns	ns
	HoxSox	ns	**	*	ns	ns

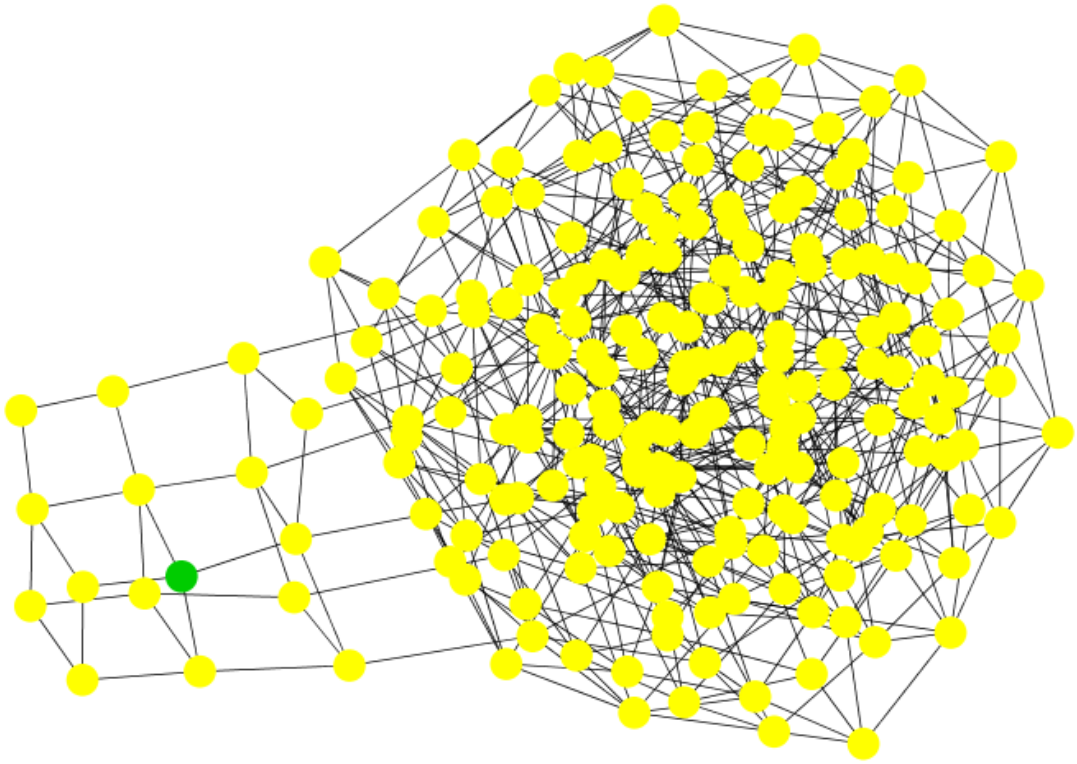
Supplementary Note: Reconstructing an existing Boolean network

To assess the ability of the synthesis method to reconstruct existing asynchrnonous Boolean networks from their state spaces, we applied the method to a Boolean network model of the core regulatory network active in common myeloid progenitor cells[9]. We took the existing Boolean network, constructed its associated state space (shown in the figure below), and then used this state space as input to our synthesis method in order to try to reconstruct the Boolean network that we started with.

Table 1: Original Boolean rules.

Gene	Update function
Gata2	$Gata2 \wedge \neg(Pu.1 \vee (Gata1 \wedge Fog1))$
Gata1	$(Gata1 \vee Gata2 \vee Fli1) \wedge \neg Pu.1$
Fog1	$Gata1$
EKLF	$Gata1 \wedge \neg Fli1$
Fli1	$Gata1 \wedge \neg EKLF$
Scl	$Gata1 \wedge \neg Pu.1$
Cebpa	$Cebpa \wedge \neg(Scl \vee (Fog1 \wedge Gata1))$
Pu.1	$(Cebpa \vee Pu.1) \wedge \neg(Gata1 \vee Gata2)$
cJun	$Pu.1 \wedge \neg Gfi1$
EgrNab	$(Pu.1 \wedge cJun) \wedge \neg Gfi1$
Gfi1	$Cebpa \wedge \neg EgrNab$

Figure 1: Network state space



The results of applying our synthesis method are shown in the table below. The method successfully reconstructs the Boolean update function for all but one gene (*EgrNab*), in some cases uniquely identifying the correct function. We note that when multiple solutions are found for an update function, these solutions, while not exact, all provide useful regulatory information that could be verified in an experimental scenario. For example, both solutions for *Scl* successfully predict *Scl*'s activation by *Gata1*, although one of the two solutions omits its repression by *Pu.1*. We found that these results were robust when performing bootstrapping, randomly discarding 10% of the state space data and rerunning the analysis.

Table 2: Synthesised Boolean functions

Gene	Synthesised update functions	Comments
Gata2	$Gata2 \wedge \neg(Fog1 \vee Pu.1)$ $Gata2 \wedge \neg(Fog1 \vee (Pu.1 \wedge Cebpa))$ $Gata2 \wedge \neg(Fog1 \vee (Pu.1 \wedge Gata2))$ $Gata2 \wedge \neg(Gata2 \wedge (Pu.1 \vee Fog1)$ $\mathbf{Gata2} \wedge \neg(\mathbf{Pu.1} \vee (\mathbf{Gata1} \wedge \mathbf{Fog1}))$ $Gata2 \wedge \neg(Pu.1 \vee (Gata2 \wedge Fog1))$	
Gata1	$(Gata1 \vee Cebpa) \wedge \neg Pu.1$ $(Gata2 \vee Fog1) \wedge \neg Pu.1$ $(Gata1 \vee Gata2) \wedge \neg Pu.1$ $(\mathbf{Gata1} \vee \mathbf{Gata2} \vee \mathbf{Fli1}) \wedge \neg \mathbf{Pu.1}$ Other functions of the form $(X \vee Y \vee Z) \wedge \neg Pu.1$	
Fog1	$\mathbf{Gata1}$	Unique
EKLF	$\mathbf{Gata1} \wedge \neg \mathbf{Fli1}$	Unique
Fli1	$\mathbf{Gata1} \wedge \neg \mathbf{EKLF}$	Unique
Scl	$Gata1$ $\mathbf{Gata1} \wedge \neg \mathbf{Pu.1}$	
Cebpa	$Cebpa \wedge \neg(Fog1 \vee Scl)$ $Cebpa \wedge \neg(Cebpa \wedge (Scl \vee Fog1))$ $Cebpa \wedge \neg(Fog1 \wedge (Scl \vee Cebpa))$ $Cebpa \wedge \neg(Fog1 \vee (Scl \wedge Gata1))$ $Cebpa \wedge \neg(Fog1 \vee (Scl \wedge Gata2))$ $Cebpa \wedge \neg(Gata1 \wedge (Fog1 \vee Scl)$ $Cebpa \wedge \neg(Scl \vee (Fog1 \wedge Cebpa)$ $\mathbf{Cebpa} \wedge \neg(\mathbf{Scl} \vee (\mathbf{Fog1} \wedge \mathbf{Gata1})$	
Pu.1	$Pu.1 \wedge \neg Gata2$ $(Pu.1 \wedge Cebpa) \wedge \neg Gata2$ $Pu.1 \wedge \neg(Gata1 \vee Gata2)$ Other functions of the form $Pu.1 \wedge \neg(Gata2 \vee X)$ $Pu.1 \wedge \neg(Gata2 \wedge Cepba)$ $Pu.1 \wedge \neg(Gata2 \wedge Pu.1)$ $Cebpa \wedge \neg(Gata1 \vee Gata2)$ $Cebpa \wedge \neg(Gata2 \vee Fog1)$ $(\mathbf{Cebpa} \vee \mathbf{Pu.1}) \wedge \neg(\mathbf{Gata1} \vee \mathbf{Gata2})$ $(Cebpa \wedge Pu.1) \wedge \neg(Gata1 \vee Gata2)$ Other functions of the form $(Cebpa \vee X) \wedge \neg(Gata2 \vee Y)$ Other functions of the form $(Pu.1 \vee X) \wedge \neg(Gata2 \vee Y)$ Other functions of the form $(Cebpa \wedge Pu.1) \wedge \neg(Gata2 \vee X)$	
cJun	$\mathbf{Pu.1} \wedge \neg \mathbf{Gfi1}$	Unique
EgrNab	$(cJun \vee Gata1) \wedge \neg Gfi1$	Incorrect
Gfi1	$\mathbf{Cebpa} \wedge \neg \mathbf{EgrNab}$	Unique

REFERENCES

1. Sacilotto, N. *et al.* Analysis of Dll4 regulation reveals a combinatorial role for Sox and Notch in arterial development. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 11893–8 (2013).

2. Sánchez-Castillo, M. *et al.* CODEX: a next-generation sequencing experiment database for the haematopoietic and embryonic stem cell communities. *Nucleic Acids Res.* (2014). doi:10.1093/nar/gku895

3. Chapman, M. A. *et al.* Analysis of multiple genomic sequence alignments: a web resource, online tools, and lessons learned from analysis of mammalian SCL loci. *Genome Res.* **14,** 313–8 (2004).

4. Göttgens, B. *et al.* Analysis of vertebrate SCL loci identifies conserved enhancers. *Nat. Biotechnol.* **18,** 181–186 (2000).

5. Fan, R. *et al.* Dynamic HoxB4-regulatory network during embryonic stem cell differentiation to hematopoietic cells. *Blood* **119,** e139–47 (2012).

6. Warren, A. J. *et al.* The Oncogenic Cysteine-rich LIM domain protein Rbtn2 is essential for erythroid development. *Cell* **78,** 45–57 (1994).

7. Hart, A. *et al.* Fli-1 is required for murine vascular and megakaryocytic development and is hemizygously deleted in patients with thrombocytopenia. *Immunity* **13,** 167–77 (2000).

8. Spyropoulos, D. D. *et al.* Hemorrhage, impaired hematopoiesis, and lethality in mouse embryos carrying a targeted disruption of the Fli1 transcription factor. *Mol. Cell. Biol.* **20,** 5643–52 (2000).

9. Krumsiek, J., Marr, C., Schroeder, T. & Theis, F. J. Hierarchical differentiation of myeloid progenitors is encoded in the transcription factor network. *PLoS One* **6,** e22649 (2011).