

Legends for Figure S1-S7, Table S1-S3

Figure S1. A. Post-sort analysis of Ikaros-GFP defined populations used for global gene array analysis. One of three representative samples is shown. A minimum purity of 95% was required for these studies. B. The HSC and MPP composition of the LSK GFP^{neg-lo} sorted population used in our studies is further delineated by expression of Flt3. The LSK GFP^{neg-lo}Flt3^{neg} (defined by a no-stain control for Flt3) consists of LT-HSC and ST-HSC (A) and is ~ 78% of the whole LSK GFP^{neg-lo} population. The LSK GFP^{neg-lo}Flt3^{lo} consists of MPP (B) and represent ~22% of the whole LSK GFP^{neg-lo} population. This is one representative of several independent analyses on the HSC-enriched LSK subset. The LSK GFP⁺ Flt3^{lo-high} is the LMPP population (C) as described in Yoshida et al., NI 2006.

Figure S2. Real-time RT-PCR analysis for lineage-affiliated transcripts used in multiplex single-progenitor studies in Ikaros-GFP defined populations. Genes are indicated on each graph, and colors indicate the lineage affiliation of each gene: lymphoid-blue, myeloid-violet, erythroid-red, stem-orange.

Figure S3. Multiplex single-cell RT-PCR analysis of progenitor populations. (A) Examples of primary data generated from each progenitor type is shown (x: 10 cell sample, ∅ No RT control, * genomic transcript). Lineage-affiliation of transcripts used in this study is shown by the following color designation: lymphoid-blue, myeloid-violet, erythroid- red, stem-yellow. (B) The percent distribution for each lineage-affiliated transcript is shown for each progenitor population. The total number of cells used was;

HSC n=239, LMPP n=211, GMP n=120 and MEP n= 78. Progenitors were isolated from at least two independent sorts and used in 2-4 amplification experiments. Mean +/- SD on percent distribution for the experiments performed per progenitor population is shown.

Figure S4. (A) Comparison between two lineage depletion strategies with (i) or without (ii) anti-Mac-1 in the lineage depletion cocktail. Strategy (ii) leaves 2-4 X more cells in the GMP (LK GFP^{hi}) compartment. (B) IL-7R α expression in various lineage negative (Lin⁻) populations using strategy (i) for lineage depletion. GMP (LK GFP^{hi}) and LSK showed very low level of IL-7R α expression. The significant IL-7R α ⁺ population detected within the Lin⁻c-Kit^{lo}Sca-1^{lo} defines the CLP. Another major IL-7R α ⁺ population was detected within the c-Kit⁻Sca-1^{hi} compartment.

Figure S5. (A) Clonal analysis of LMPP and GMP for combined T cell and myeloid differentiation potential. Single cells were co-cultured with OP9-DL1 for 18 days before FACS analysis for T cell (Thy.1.2, CD44, CD25) and Myeloid (Mac-1 and Gr-1) markers. The percentage of T cell and myeloid developmental potential exhibited by expanding LMPP and GMP clones is shown. Lineage output of two representative GMP clones with T/myeloid differentiation is shown. (B) The differentiation outcome of intrathymic placement of LMPP (n=25) and GMP (n=1000) is shown at 5 and 21 days post-injection.

Figure S6. Hierarchical clustering of up- and down-regulated genes in Ikaros-null HSC (A) and LMPP (B) across WT and KO progenitor populations (HSC, LMPP, GMP and

MEP). The lineage affiliation of deregulated transcripts is shown on the right side of each panel.

Figure S7. Multiplex single-cell gene expression analysis of Ikaros–null HSC. (A) Single progenitor analysis for lineage-affiliated transcripts was performed as described in Figure 2. Mutant HSC (n=181) generated from two independent sorts are shown. (B) The percentage of overall lineage transcript distribution as well as that of individual lineage-specific transcripts is provided for WT and mutant populations. Mean +/- SD on percent distribution for experiments performed per progenitor population is shown.

Table S1. Limiting dilution analysis of LMPP and GMP lymphoid and myeloid developmental potential. Data from four independent experiments is shown noting the stroma used for each assay. T cell potential was scored from OP9-DL1 culture supplemented with 10 ng/ml SCF, 10 ng/ml Flt3L, and 1 ng/ml IL-7. B and myeloid cell potential were simultaneously scored from OP9 stroma supplemented with 10 ng/ml SCF, 10 ng/ml Flt3L, and 10 ng/ml IL-7. Frequency and 95% confidence intervals for each individual sample were calculated using the WEHI limiting dilution analysis web interface. <http://bioinf.wehi.edu.au/software/limdil/>

Table S2. Limiting dilution analysis of wild-type and Ikaros–null HSC and LMPP cells. Data from at least two independent experiments is shown for each sample. Experiments and analysis were performed as in Table S1. Data from wild-type LMPP from

Experiment 2 was also used for Table S1 Experiment 2 as all experiments were performed simultaneously.

Table S3. Limiting dilution analysis of LMPPs transduced with Ikaros-targeted or scrambled shRNA expressing lentiviruses. Data from two independent experiments is shown. LMPPs were harvested and activated with SFEM supplemented with 50 ng/ml SCF, 50 ng/ml Flt3L, 20 ng/ml TPO, and 10 ng/ml IL-7 and cultured on retronectin coated tissue culture plates for 24 hours. Cells were then twice transduced with lentiviral supernatant and polybrene using a 2-hour spin-infection at approximately 2000g followed by a 10-hour culture before being washed and re-cultured in activation conditions for 12 hours. 60 hours after initiation of transduction, GFP positive cells were directly sorted into limiting dilution assays. Subsequent experiments and analysis were performed as with Table S1.

Table S4. The list of primers used for single cell multiplex RT-PCR and qPCR analysis of progenitors.

Figure S1

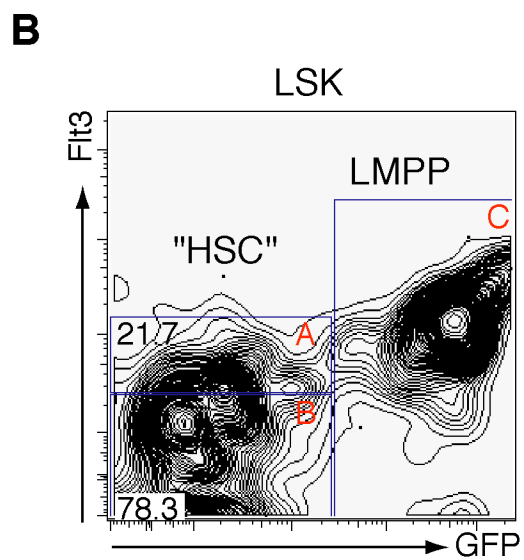
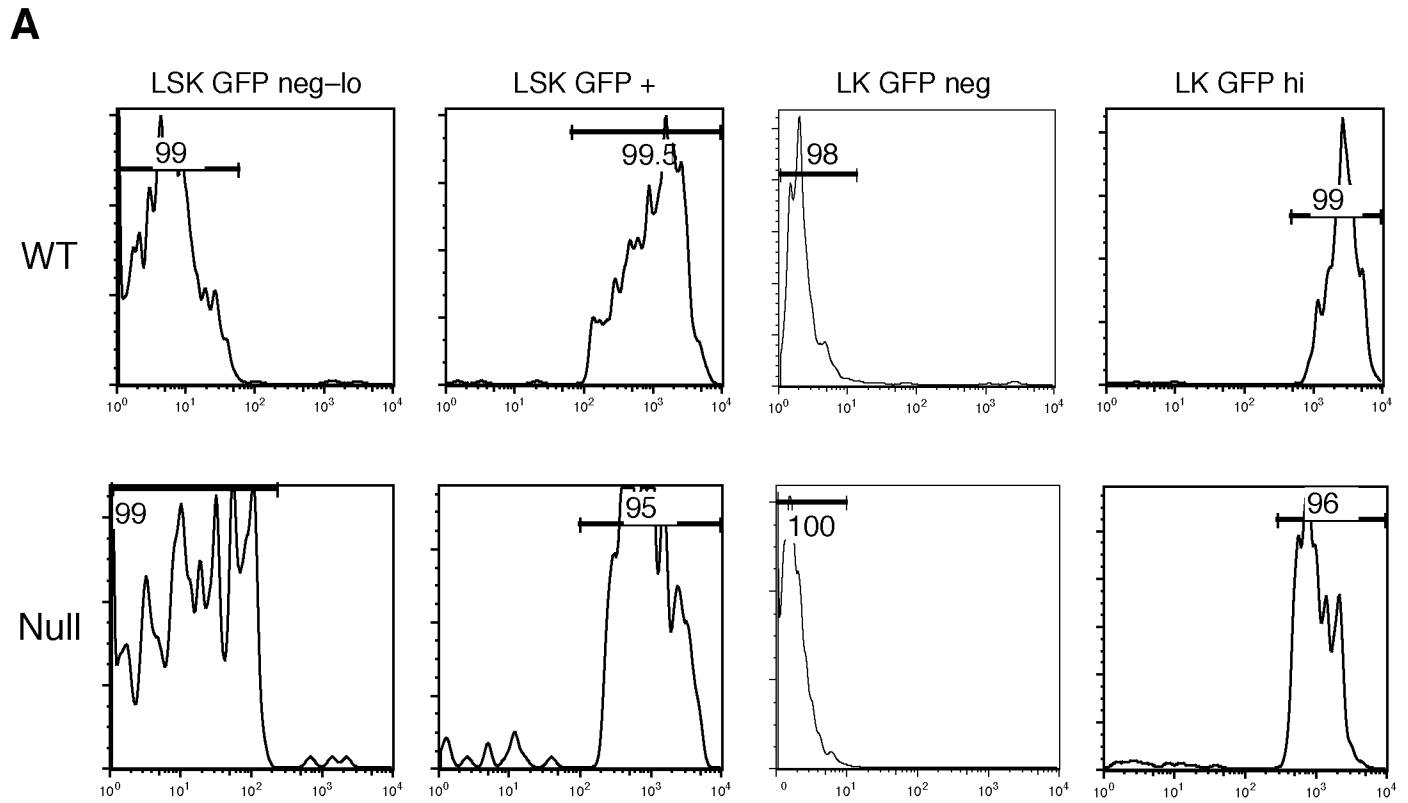


Figure S2

[F] Supplemental Text and Figures

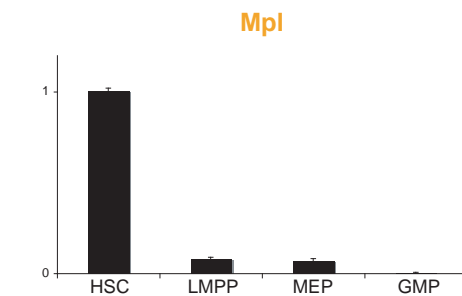
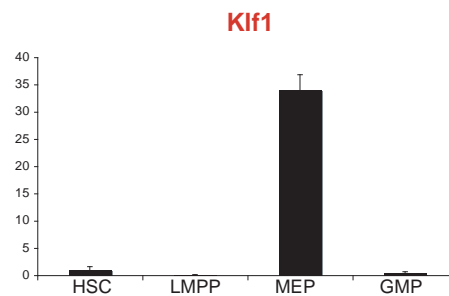
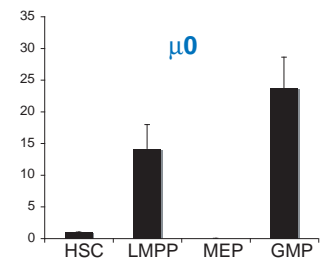
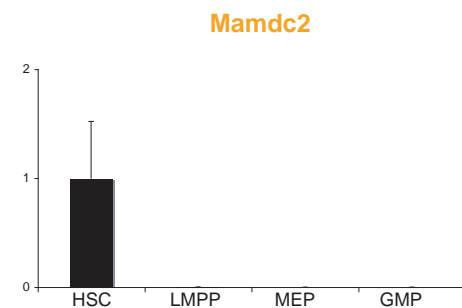
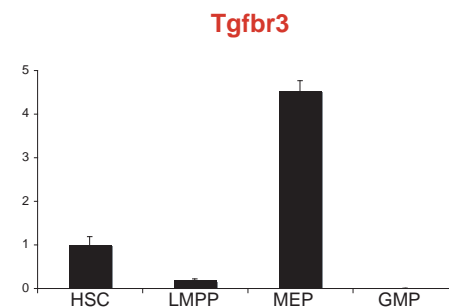
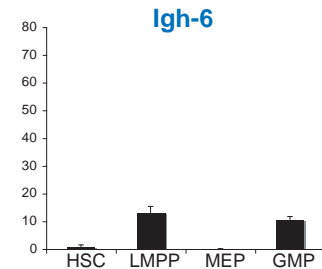
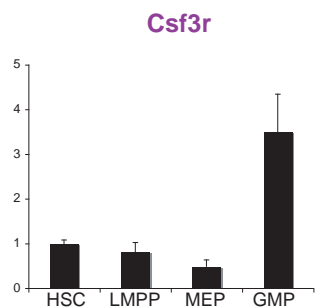
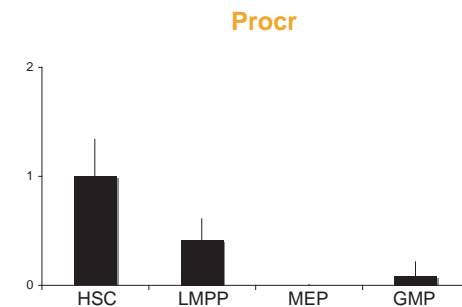
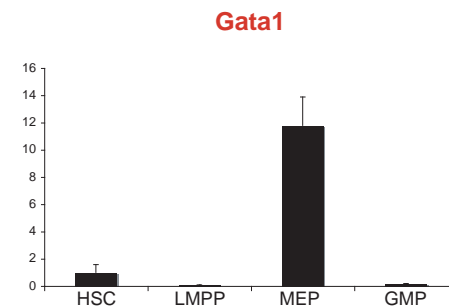
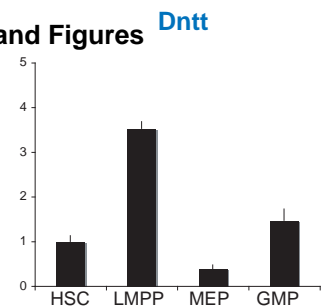
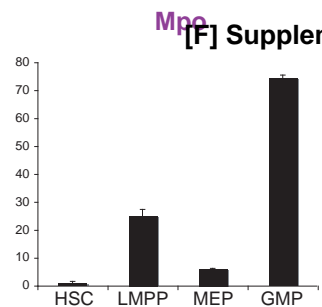


Figure S3

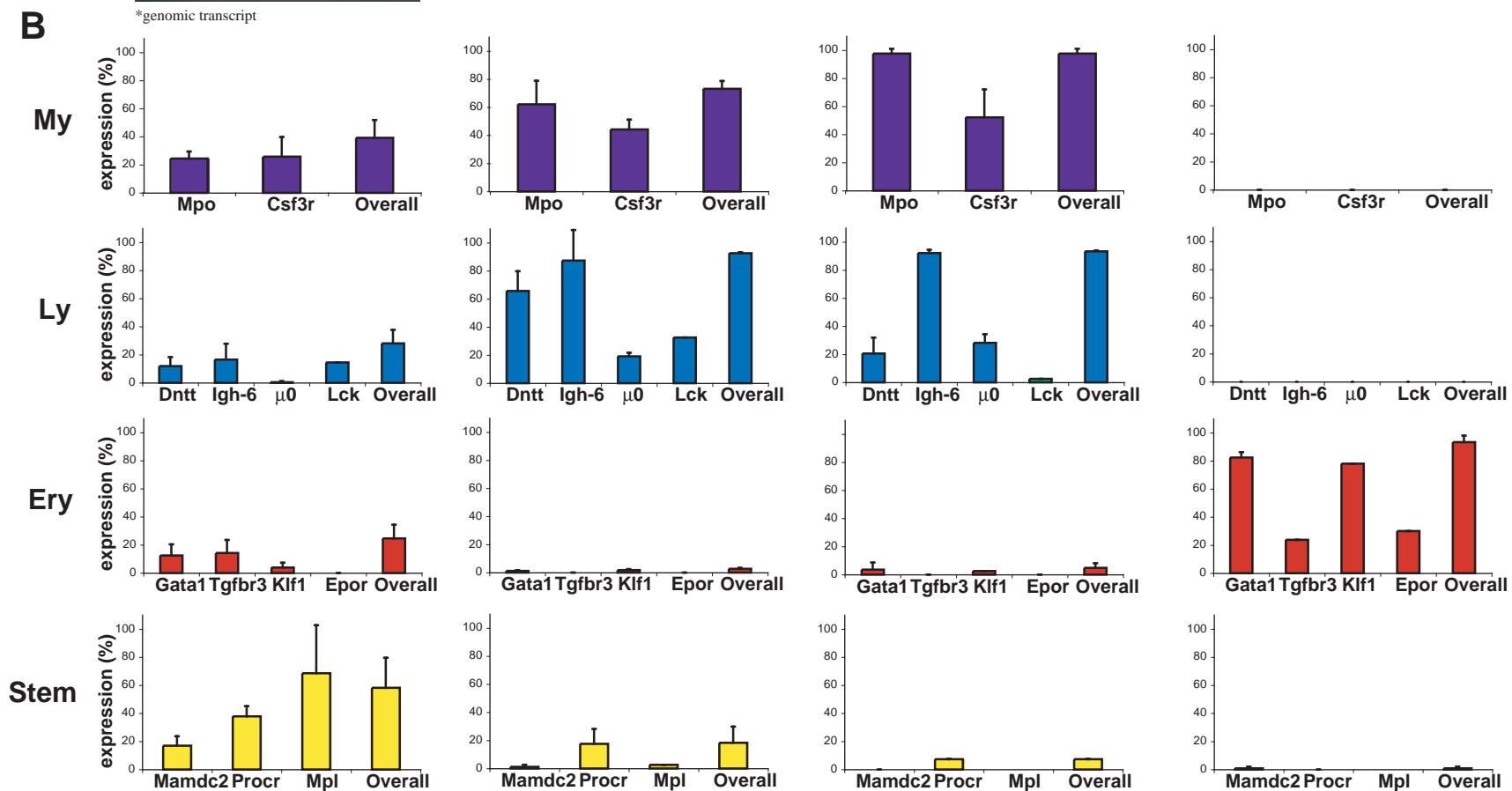
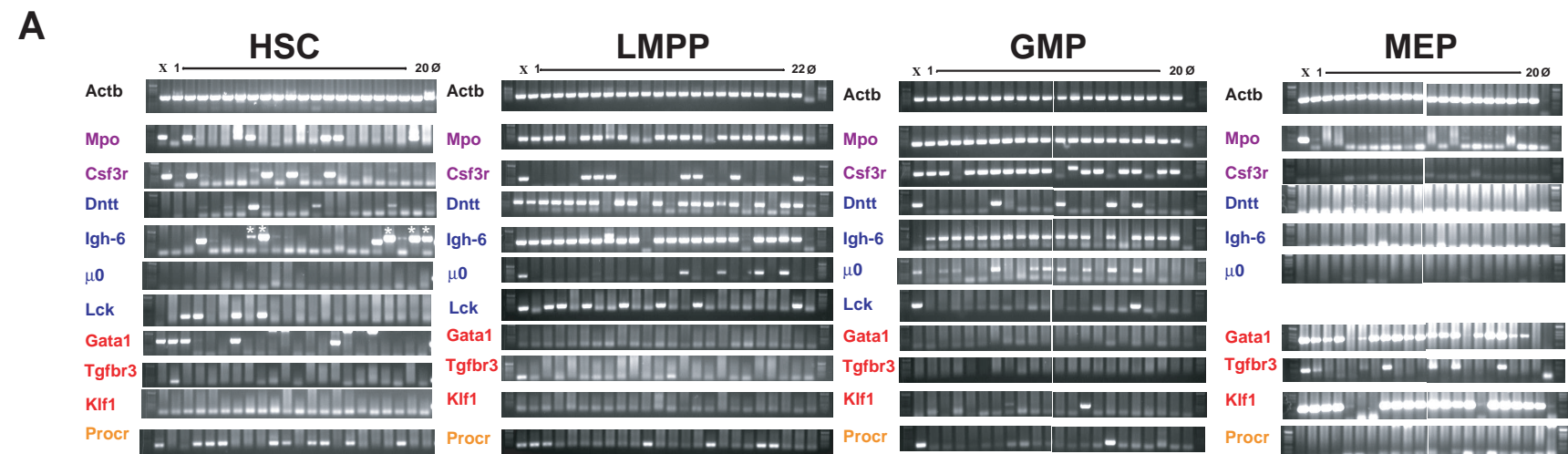
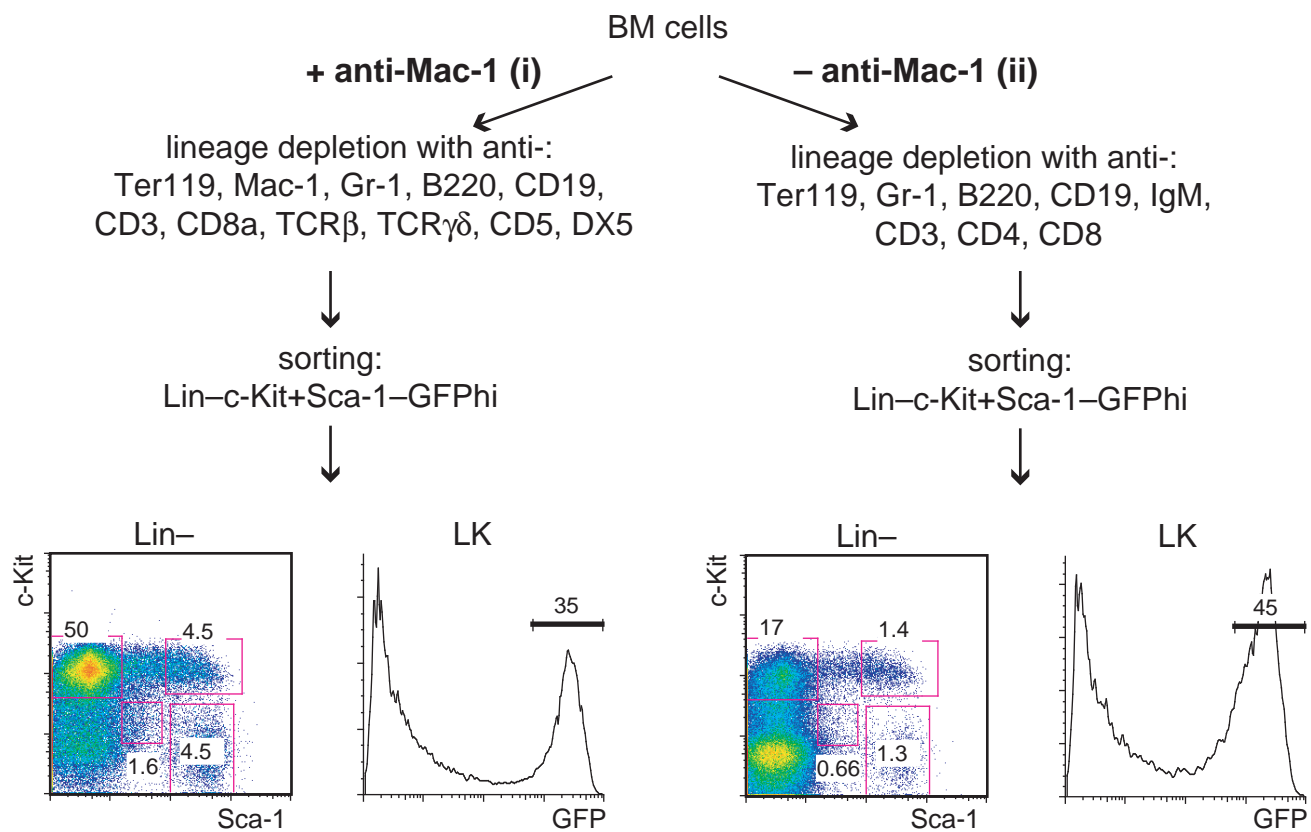


Figure S4

A



B

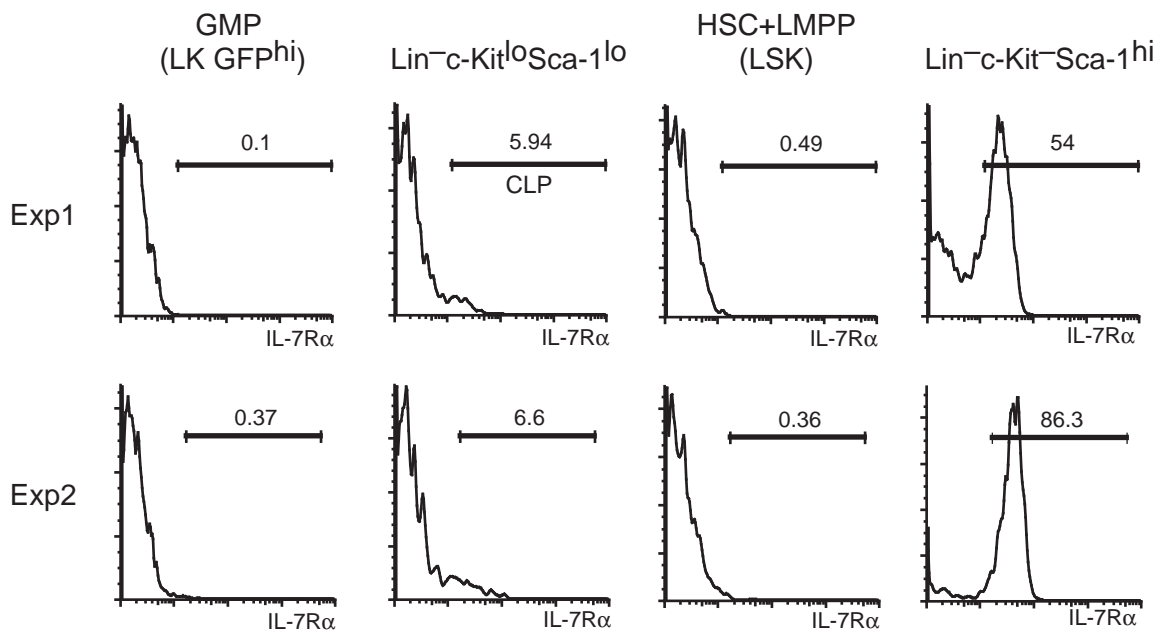
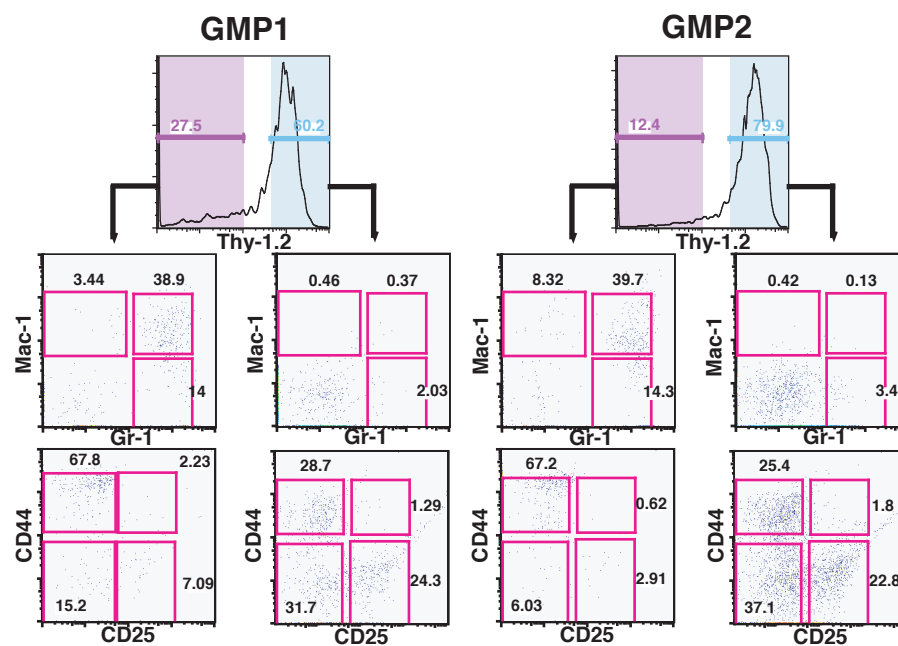
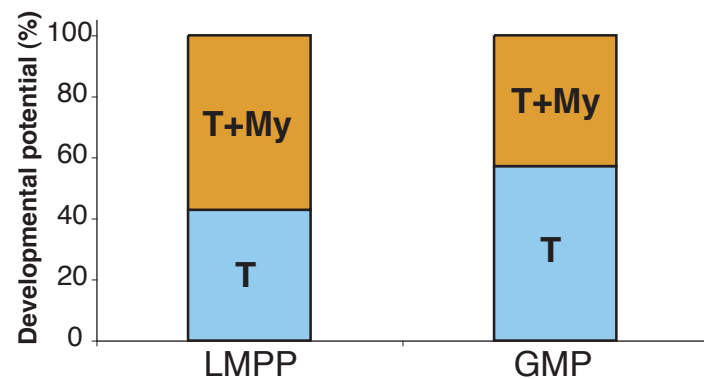


Figure S5

A



B

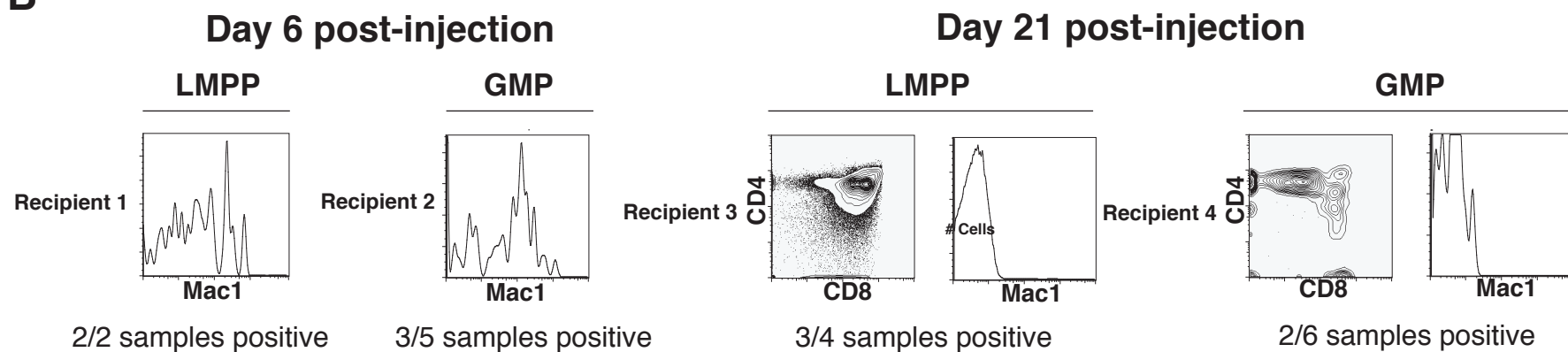


Figure S6

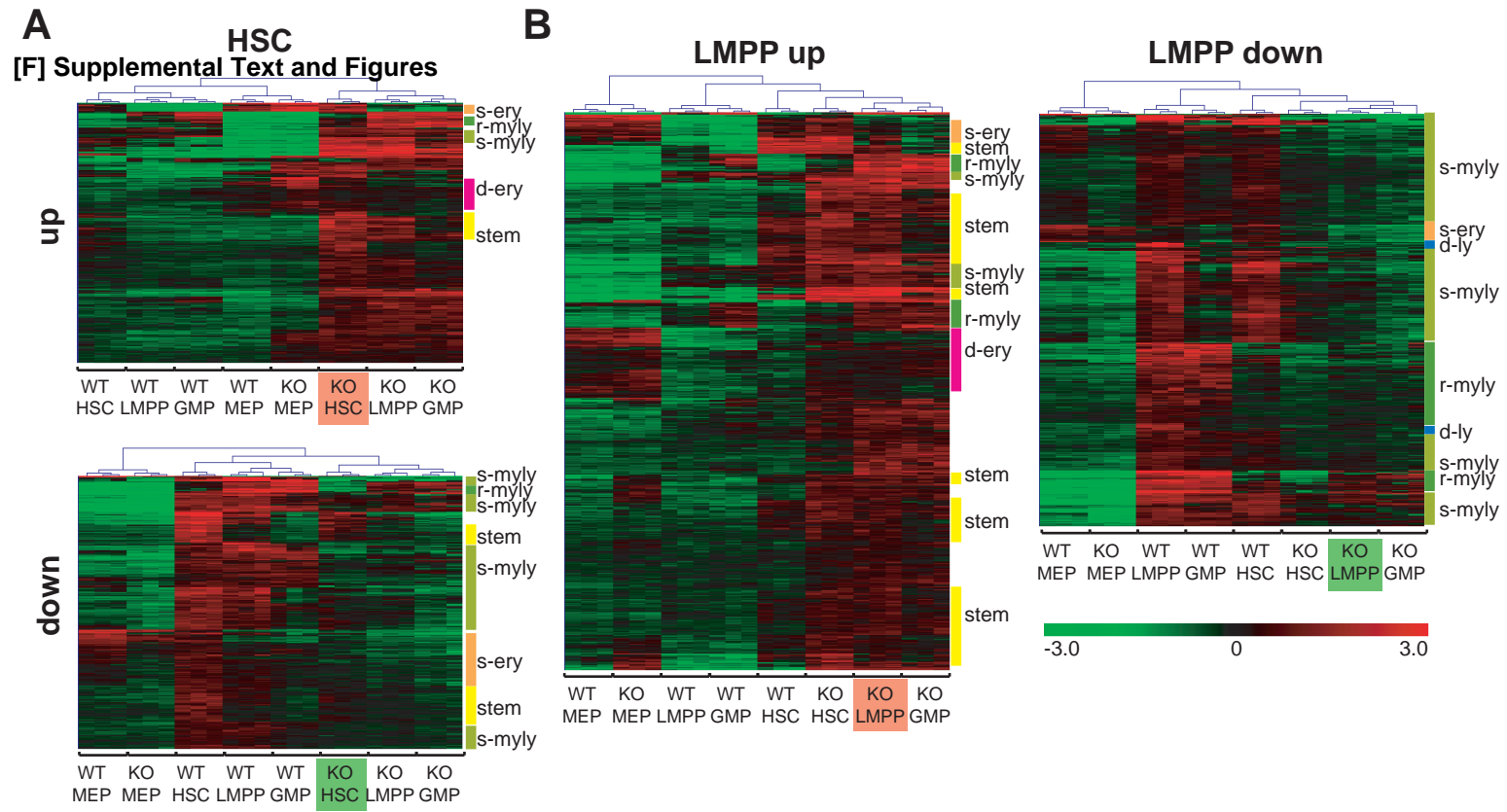
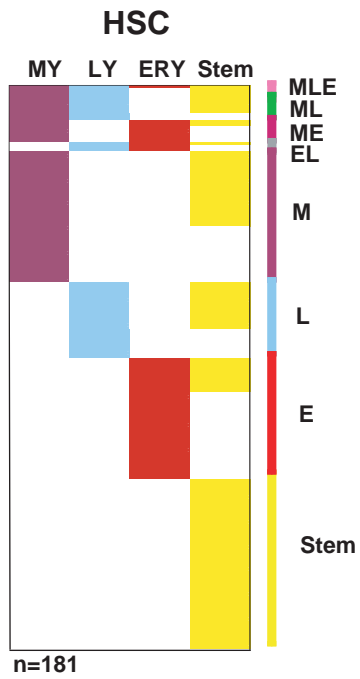


Figure S7

A



B

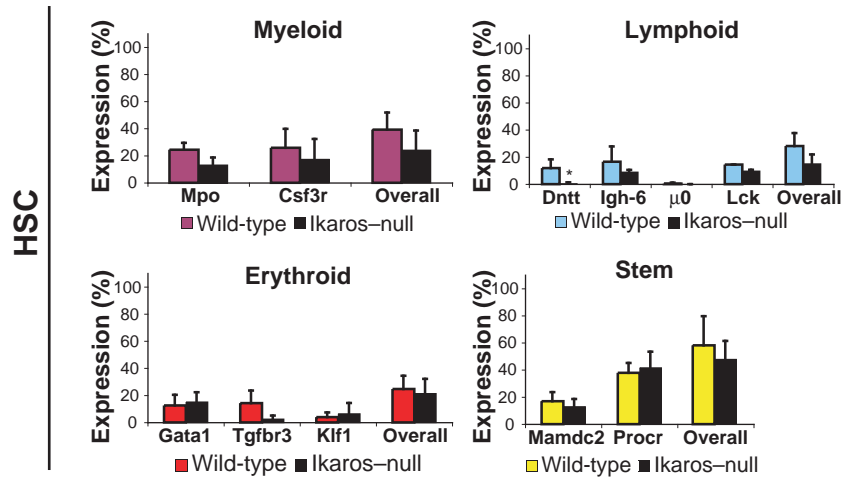


Table S1

Experiment 1

OP9-DL1

95 percent Confidence Intervals

LMPP	Cells/well	Responses	Lower	Estimate	Upper
	25	10/10	1 in 4.25	1 in 2.91	1 in 2.07
	5	18/20			
	1	6/20			
GMP	Cells/well	Responses	Lower	Estimate	Upper
	100	10/10	1 in 25.10	1 in 14.81	1 in 8.82
	20	7/10			
	4	6/20			
	1	1/20			

OP9-B

95 percent Confidence Intervals

LMPP	Cells/well	Responses	Lower	Estimate	Upper
	25	10/10	1 in 4.77	1 in 2.99	1 in 1.98
	5	8/10			
	1	1/20			
GMP	Cells/well	Responses	Lower	Estimate	Upper
	500	8/10	1 in 642.55	1 in 347.64	1 in 188.19
	100	3/10			
	20	0/20			
	4	0/20			

OP9-Myeloid

95 percent Confidence Intervals

LMPP	Cells/well	Responses	Lower	Estimate	Upper
	25	10/10	1 in 7.04	1 in 4.20	1 in 2.60
	5	6/10			
	1	7/20			
GMP	Cells/well	Responses	Lower	Estimate	Upper
	500	10/10	1 in 59.74	1 in 36.52	1 in 22.41
	100	10/10			
	20	8/20			
	4	1/20			

Experiment 2

OP9-DL1

95 percent Confidence Intervals

LMPP	Cells/well	Responses	Lower	Estimate	Upper
	25	10/10	1 in 12.47	1 in 7.17	1 in 4.23
	5	6/10			
	1	1/20			
GMP	Cells/well	Responses	Lower	Estimate	Upper
	100	10/10	1 in 36.53	1 in 21.00	1 in 12.16
	20	7/10			
	4	3/20			
	1	0/20			

95 percent Confidence Intervals

LMPP	Cells/well	Responses	Lower	Estimate	Upper
	25	10/10	1 in 18.62	1 in 10.63	1 in 6.17
	5	2/10			
	1	2/20			
GMP	Cells/well	Responses	Lower	Estimate	Upper
	500	10/10	1 in 292.88	1 in 165.87	1 in 94.03
	100	4/10			
	20	1/20			
	4	1/20			

95 percent Confidence Intervals

LMPP	Cells/well	Responses	Lower	Estimate	Upper
	25	10/10	1 in 4.39	1 in 2.79	1 in 1.87
	5	9/10			
	1	7/20			
GMP	Cells/well	Responses	Lower	Estimate	Upper
	500	10/10	1 in 59.74	1 in 36.52	1 in 22.41
	100	10/10			
	20	8/20			
	4	1/20			

Experiment 3

OP9-DL1

95 percent Confidence Intervals

LMPP	Cells/well	Responses	Lower	Estimate	Upper
	25	5/5	1 in 3.32	1 in 2.19	1 in 1.55
	5	10/10			
	1	8/20			
GMP	Cells/well	Responses	Lower	Estimate	Upper
	100	9/10	1 in 71.05	1 in 40.41	1 in 23.08
	20	3/10			
	4	4/20			
	1	0/20			

Experiment 4

95 percent Confidence Intervals

GMP	Cells/well	Responses	Lower	Estimate	Higher
	200	8/20	1 in 628.63	1 in 353.6	1 in 198.90
	50	3/20			
	10	1/20			

95 percent Confidence Intervals

GMP	Cells/well	Responses	Lower	Estimate	Higher
	200	19/20	1 in 84.78	1 in 55.65	1 in 36.53
	50	11/20			
	10	6/20			

Table S2

Experiment 1

		OP9-DL1			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
HSC	25	10/10	1 in 17.36	1 in 10.69	1 in 6.67
	5	5/20			
	1	3/20			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
LMPP	25	10/10	1 in 2.24	1 in 1.6	1 in 1.25
	5	20/20			
	1	12/20			

		OP9-DL1			
Ikaros-null	Cells/well	Responses	Lower	Estimate	Higher
HSC	25	5/10	1 in 48.5	1 in 26.34	1 in 14.41
	5	5/20			
	1	1/20			
Ikaros-null	Cells/well	Responses	Lower	Estimate	Higher
LMPP	25	8/10	1 in 28.85	1 in 16.92	1 in 10.01
	5	6/20			
	1	0/20			

Experiment 2

		OP9-DL1			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
LMPP	25	10/10	1 in 4.25	1 in 2.91	1 in 2.07
	5	18/20			
	1	6/20			

		OP9-DL1			
Ikaros-null	Cells/well	Responses	Lower	Estimate	Higher
HSC	25	4/10	1 in 79.21	1 in 39.07	1 in 19.40
	5	2/20			
	1	2/20			
Ikaros-null	Cells/well	Responses	Lower	Estimate	Higher
LMPP	25	6/10	1 in 40.47	1 in 22.64	1 in 12.77
	5	6/20			
	1	0/20			

Experiment 3

		OP9-DL1			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
HSC	30	7/10	1 in 42.71	1 in 23.85	1 in 13.42
	10	4/10			
	3	1/10			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
LMPP	30	10/10	1 in 4.18	1 in 2.35	1 in 1.48
	10	10/10			
	3	8/10			

Experiment 4

		OP9-DL1			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
HSC	10	2/10	1 in 111.48	1 in 42.01	1 in 16.03
	5	1/10			
	2	1/10			
	1	0/10			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
LMPP	10	10/10	1 in 7.58	1 in 4.95	1 in 3.31
	5	7/10			
	2	3/10			
	1	1/10			

Experiment 5

		OP9-DL1			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
HSC	25	9/10	1 in 21.92	1 in 12.62	1 in 7.37
	5	4/10			
	2	1/10			
	1	0/10			
Wild Type	Cells/well	Responses	Lower	Estimate	Higher
LMPP	25	10/10	1 in 2.69	1 in 1.86	1 in 1.38
	5	10/10			
	2	7/10			
	1	6/10			

Table S3

Experiment 1

OP9 B cell

Ikaros shRNA 1	Cells/well	Responses	Lower	Estimate	Higher
	50	5/20	1 in 115.78	1 in 67.66	1 in 39.54
	10	1/20			
	2	1/20			

Ikaros shRNA 2	Cells/well	Responses	Lower	Estimate	Higher
	50	13/20	1 in 20.17	1 in 12.94	1 in 8.30
	10	9/20			
	2	6/20			

Control shRNA	Cells/well	Responses	Lower	Estimate	Higher
	50	19/20	1 in 7.90	1 in 5.05	1 in 3.22
	10	12/20			
	2	6/20			

OP9 Myeloid

Ikaros shRNA 1	Cells/well	Responses	Lower	Estimate	Higher
	50	20/20	1 in 4.05	1 in 2.5	1 in 1.54
	10	20/20			
	2	10/10			

Ikaros shRNA 2	Cells/well	Responses	Lower	Estimate	Higher
	50	20/20	1 in 6.5	1 in 4.14	1 in 2.64
	10	20/20			
	2	7/20			

Control shRNA	Cells/well	Responses	Lower	Estimate	Higher
	50	4/20	1 in 115.81	1 in 67.67	1 in 39.54
	10	8/20			
	2	4/20			

OP9-DL1

Ikaros shRNA 1	Cells/well	Responses	Lower	Estimate	Higher
	50	20/20	1 in 14.46	1 in 9.35	1 in 6.12
	10	12/20			
	2	6/20			

Ikaros shRNA 2	Cells/well	Responses	Lower	Estimate	Higher
	50	19/19	1 in 16.38	1 in 10.57	1 in 6.90
	10	14/20			
	2	1/20			

Control shRNA	Cells/well	Responses	Lower	Estimate	Higher
	50	20/20	1 in 5.81	1 in 3.87	1 in 2.65
	10	19/20			
	2	10/20			

Experiment 2

OP9 B cell

Ikaros shRNA 1	Cells/well	Responses	Lower	Estimate	Higher
	25	3/10	1 in 80.16	1 in 39.22	1 in 19.32
	5	5/20			
	1	0/20			

Ikaros shRNA 2	Cells/well	Responses	Lower	Estimate	Higher
	25	6/10	1 in 30.7	1 in 17.86	1 in 10.49
	5	6/20			
	1	3/20			

Control shRNA	Cells/well	Responses	Lower	Estimate	Higher
	25	10/10	1 in 10.86	1 in 6.89	1 in 4.45
	5	11/20			
	1	2/20			

OP9 Myeloid

Ikaros shRNA 1	Cells/well	Responses	Lower	Estimate	Higher
	25	10/10	1 in 7.15	1 in 4.63	1 in 3.08
	5	14/20			
	1	3/20			

Ikaros shRNA 2	Cells/well	Responses	Lower	Estimate	Higher
	25	10/10	1 in 9.51	1 in 6.08	1 in 3.97
	5	11/20			
	1	4/20			

Control shRNA	Cells/well	Responses	Lower	Estimate	Higher
	25	0/10	1 in 251.28	1 in 90.98	1 in 33.14
	5	3/20			
	1	1/20			

OP9-DL1

Ikaros shRNA 1	Cells/well	Responses	Lower	Estimate	Higher
	25	9/9	1 in 9.67	1 in 6	1 in 3.81
	5	10/17			
	1	3/19			

Ikaros shRNA 2	Cells/well	Responses	Lower	Estimate	Higher
	25	8/10	1 in 29.18	1 in 17.09	1 in 10.10
	5	5/20			
	1	1/20			

Control shRNA	Cells/well	Responses	Lower	Estimate	Higher
	25	10/10	1 in 5.15	1 in 3.45	1 in 2.40
	5	16/19			
	1	5/20			

SC-PCR primers

Actb Flanking S
 Actb Flanking A
 Actb Nested S
 Actb Nested A

Lymphoid

Igh-6 Flanking S
 Igh-6 Flanking A
 Igh-6 Nested S
 Igh-6 Nested A
 Lck Flanking S
 Lck Flanking A
 Lck Nested S
 Lck Nested A
 Dntt Flanking S
 Dntt Flanking A
 Dntt Nested S
 Dntt Nested A
 Sterile IgH (μ 0)-1
 Sterile IgH (μ 0)-2
 Sterile IgH (μ 0)-3
 Sterile IgH (μ 0)-4

Myeloid

Mpo Flanking S
 Mpo Flanking A
 Mpo Nested S
 Mpo Nested A
 Csf3r-1
 Csf3r-2
 Csf3r-3
 Csf3r-4

Erythroid

Tgfr3 Flanking S
 Tgfr3 Flanking A
 Tgfr3 Nested S
 Tgfr3 Nested A
 Klf1 Flanking S
 Klf1 Flanking A
 Klf1 Nested S
 Klf1 Nested A
 Gata1-1
 Gata1-2
 Gata1-3
 Gata1-4

Stem

Mamdc2 Flanking S
 Mamdc2 Flanking A
 Mamdc2 Nested S
 Mamdc2 Nested A
 Procr Flanking S
 Procr Flanking A
 Procr Nested S

Sequence

GCTACAGCTTCACCACCACA
 CTTCTGCATCCTGTCAGCAA
 AATCGTGCCTGACATCAAAGAG
 ACGGATGTCAACGTCACACTTC

CGTCCAAGAATTGGATGTGA
 AGGGACACGAGCTCATTGAG
 GTTCAGATGCCAGCCTCACA
 TGGGGTGGGAAGGTGTTCA
 TGCAAGATTGCAGACTTTGG
 CCACACAGATATGGCACAGG
 CAAGTCAGACGTGTGGTCCTTC
 CACAGCATCATGAGGTGGTACA
 CTGTGTTTGGTGTGGGACTG
 CTGGGCTGCTTGAACCTTTTC
 AGCTGTGTGAACAGGCCAGAAG
 CCCTGCTGCTTCCAAAAGTCTG
 GCTAGCCTGAAAGATTACC
 TGAGTTTCTGAGGCTTGG
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 GCGAATGGTGTATGTTGTTCCCTG
 ACAGGAGTGTGAACCTTCGCT
 TACCAGCCACAGCTCAAAGG
 ACGTGTCCAGTCTGATGGTG
 TTGCTTCTTCTGACACCAGC

CCCCAGATGGTGTGGTTTAC
 TCGGACAGATGTTCTCGATG
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 CAAATCCCAGATCTTGGTCAGC
 ATTGTCTGGGACCTGGGACT
 ACGCTTCATGTGCAGAGCTA
 GGCGAACTTTGGCACCTAAGAG
 GTCCAGTGTGCTTCCGGTAGTG
 CCAGTTTGTGGATTCTGC
 GTCCTCACCATCAGATTCC
 AACGTGTTGTTGCTCTTCC
 AAGAATAGGCTGCTGAGC

GCAGTACTGTGTGCGCTTTC
 CTCCCTTGGGTCCTGTGTAG
 GCAAGCGGAAATCAGTTTCAAG
 ATTCTCCAGGTGGAGGTGGAAG
 AAAACGTCAACATCCTCCAG
 TGGTTCTCCAGGAACTCCAC
 GCCTGGTGAAGCTGGTATATCG

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 Mansson **Immunity** 26:407-

Procr Nested A

Mpl-1

Mpl-2

Mpl-3

Mpl-4

TCGAAGAAGACATGGGGTTCAG

CGGGTGCTGTTTGTGGATAG

TGCTCTCCACAGAAACCTGC

AGTCACAGGGAAGGACCAGG

GCGGTTCCCTCCTTCACAT

Mansson **Immunity** 26:407-

Mansson **Immunity** 26:407-

Mansson **Immunity** 26:407-

Mansson **Immunity** 26:407-

Realttime Primers

Actb S

Actb A

Ikaros S

Ikaros A

Dntt S

Dntt A

Igh-6 S

Igh-6 A

μ0 (Sterile IgH)-S

μ0 (Sterile IgH)-A

Mpo S

Mpo A

Klf1 S

Klf1 A

Mamdc2 S

Mamdc2 A

Mpl S

Mpl A

Taqman Primer Sets

Gata1

Procr

Tgfbr3

CGTGAAAAGATGACCCAGATC

CACAGCCTGGATGGCTACGT

CAATGTGCCAAACGTAAGA

GTTGATGGCATTGTTGATGG

GAGGCGGTTGTGACATTTCT

GCTGCTGCTCTTCATCCTCT

TCTGCCTTCACCACAGAAGA

ACACAGCAGGTGGATGTTTG

CCTAAGGCAGGATGTGGAGA

AGACGAGGGGGAAGACATTT

CCTTCTTCACTGGCCTCAAC

GGTTCTTGATTTCGAGGGTCA

CAAGAGCTCGCACCTCAAG

GAAGGGACGATGTCCAGTGT

TACAGCTGCAGGAGGAGACA

TCCTGATGACTGTTTCAGTTGTATCT

CAGACCTACACCGGGTCCTA

GCTGGGTTCCACTTCTTCAC

Mm00484678_m1

Mm00440992_m1

Mm03024063_m1

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