

Supplemental Methods

Mouse embryonic stem (ES) cell generation

To generate inducible ES cell lines (A2lox.Plac8, A2lox.Serpinb2, A2lox.Meis1a, A2lox.Meis2(2a), A2lox.Meis2(4a), and A2lox.HoxA9), individual cDNAs were amplified from embryoid body (EB) RNA using gene-specific primers (supplemental Table 1) and cloned into p2lox targeting vector.¹ Site specific recombination into A2lox ESCs was performed using co-transfected Cre recombinase as described.² An inducible, dual-expression vector was prepared by cloning a cDNA (*Serpinb2*, *HoxA9*, or *Meis2*) into the vector pTet-CMV_{min}pA-zeocin (Dr. Jonathan Green, Washington University School of Medicine), transferring this Tet-CMV_{min} promoter-cDNA-pA cassette into the p2lox targeting vector downstream of the original p2lox pA site, and followed by inserting a second cDNA (*Plac8*, *Meis1*, or *HoxA9*) into the resulting plasmid, allowing simultaneous dox induction of both cDNAs in transfected A2lox ESCs.

Mice

Mesp1-Cre mice were obtained from the Riken BioResource Center as cryo-preserved embryos.³ Heterozygous Mesp1^{Cre/+} mice were bred to homozygous Rosa26-CAG-STOP-eGFP^{+/+} reporter mice. To generate Rosa26-CAG-STOP-eGFP^{+/+} reporter mice, the plasmid pCAG-Cre:GFP (Addgene plasmid 13776)⁴ was digested with *EcoR I* and *Sal I* and the CAG-promoter containing 1718 bp fragment was cloned bluntly into the *Pac I* site of a modified version of pROSA26-1 (Addgene plasmid 11739).⁵ The resulting plasmid was digested using *Pvu I* and the gel purified 13366 bp fragment was electroporated into the 129SvEv ES cell line, EDJ 22 (ATCC number: SCRC-1021). Targeted clones were identified by Southern blot analysis using probes cloned from isogeneic genomic ES cell DNA using oligonucleotides 5'-CTCACTCAGCCCGCTGCCCGAG and 5'-CTCCCGCCAGAGTCCCGATCCCC (probe A), and 5'-GGCTTGTTGGTTCCATACATCTACTGG and 5'-GATCAAGATGAAGGAAGAGACCCTCC (probe B). Mice were generated by injecting

targeted ES cells into C57BL/6J blastocysts and maintained on a 129 background after transmission of the targeted allele through the male germ line.

BM transplantation

BM cells were collected from 8- to 10-week-old *Plac8^{-/-}* or wild type C57BL/6J background littermate control mice (Jackson Laboratory; CD45.2⁺) and transplanted by retro-orbital injection with competitor marrow (B6.SJL-*Ptprc^a/BoyAiTac*; Taconic; CD45.1⁺) at 1:1 ratio into B6.SJL-*Ptprc^a/BoyAiTac* recipients that had received 1,100 rad. Donor-derived cells in BM (CD45.2⁺) were analyzed 4 weeks after transfer by FACS analysis.

References

1. Kyba M, Perlingeiro RC, Hoover RR et al. Enhanced hematopoietic differentiation of embryonic stem cells conditionally expressing Stat5. *Proc Natl Acad Sci U S A*. 2003.
2. Lindsley RC, Gill JG, Murphy TL et al. *Mesp1* coordinately regulates cardiovascular fate restriction and epithelial-mesenchymal transition in differentiating ESCs. *Cell Stem Cell*. 2008;3(1):55-68.
3. Saga Y, Kitajima S, Miyagawa-Tomita S. *Mesp1* Expression Is the Earliest Sign of Cardiovascular Development. *Trends in Cardiovascular Medicine*. 2000;10(8):345-352.
4. Matsuda T, Cepko CL. Controlled expression of transgenes introduced by in vivo electroporation. *Proc Natl Acad Sci U S A*. 2007;104(3):1027-1032.
5. Sasaki Y, Derudder E, Hobeika E et al. Canonical NF-kappaB activity, dispensable for B cell development, replaces BAFF-receptor signals and promotes B cell proliferation upon activation. *Immunity*. 2006;24(6):729-739.
6. Pronk CJ, Rossi DJ, Mansson R et al. Elucidation of the phenotypic, functional, and molecular topography of a myeloerythroid progenitor cell hierarchy. *Cell Stem Cell*. 2007;1(4):428-442.

Supplemental Tables and Figure Legends

Table S1. Oligonucleotide primers used in this study

Cloning Primers	
Name	Sequence
Meis1a_F*	GAAGTAGGAAGGGAGCCAGAGAGG
Meis1a_R*	CACCATTGTAGACAACGCATATTCCC
Meis1b_F*	GAAGTAGGAAGGGAGCCAGAGAGG
Meis1b_R*	CACCATTGTAGACAACGCATATTCCC
Meis2(2a)_F [†]	TACGACACATCCAGGAGTTTATTG
Meis2(2a)_R [†]	GTGTGTTTCCTTCTTCCTTGAGTT
Meis2(2b)_F [†]	TACGACACATCCAGGAGTTTATTG
Meis2(2b)_R [†]	GTGTGTTTCCTTCTTCCTTGAGTT
Meis2(4a)_F [†]	TACGACACATCCAGGAGTTTATTG
Meis2(4a)_R [†]	GTGTGTTTCCTTCTTCCTTGAGTT
Mesp1_F	AATGGTCAGGCCTCCGTTGC
Mesp1_R	TGTCCCCTCCACTCTTCAGGCA
Plac8_F1	TTTTGAGACCTCGCATCGAA
Plac8_R1	TCTTGCCATCCAGCTCCTTA
Serpinb2_F	AGCTGTGTAGAGGATTGAAACAATG
Serpinb2_R	GCTACAAAACCTCATGTGGAAATAG
HoxA9_F	GCGCCGGCAACTTATTAG
HoxA9_R	ACAGAGGGAGACGGACAGTC
Meis1_P_F	CGGGATCCTTTTTCTCTGGAAGTGGGAGC
Meis1_P_R	GAAGATCTGCGTGTGTAAAGTGTGTGTG
Meis2_P_F	CGGGATCCTGTACCGTACTTTCCTGTGG
Meis2_P_R	GAAGATCTCCAAACCAAGGAGACTTCTC
Quantitative RT-PCR Primers	
Name	Sequence
Plac8_F2	ATTTGTAGTAAGACTCAACCCAGAC
Plac8_R2	CAGACAACACTCATTTCATGTCAG
Ptger3_F	GGGATCATGTGTGTGCTGTC
Ptger3_R	AGCAGATAAACCCAGGGATC
Gapdh_F	TGCCCCCATGTTTGTGATG
Gapdh_R	TGTGGTCATGAGCCCTTCC
Mesp1_F	AGAATCGTGGGACCCATCGTTC
Mesp1_R	ATTGTCCCCTCCACTCTTCAGGC

Mesp2_F	CCACTGAACCTGCAGAGCTGACTAAAG
Mesp2_R	AGATAAAGGCACTTCCAAGGC
Meis1a_F	TCCAGCATCTAACACACCCTTAC
Meis1a_R	CATTCCACTCATAGGTCCTGG
Meis1b_F	TCCAGCATCTAACACACCCTTAC
Meis1b_R	GGCATACTTTGCAGCCCTGG
Meis2(2a)_F	AAATCGAGCAGTGAGCCAAG
Meis2(2a)_R	CGAAGGTTACATATAGTGCCACTG
Meis2(2b)_F	AAATCGAGCAGTGAGCCAAG
Meis2(2b)_R	GGCATGCTCTGCAAACCTGCA
Meis1_F	TCCAGCATCTAACACACCCTTAC
Meis1_R	AAAACCTCCCATTGGCTGTC
Meis2_F	ACAACAGCAGTGAGCAAGGC
Meis2_R	GGGTACGGGTGTGTGAGATG
	mir30-based shRNA cassettes
Name	Sequence
Ptger3mir1	TGCTGTTGACAGTGAGCGAGGAGAAGTTTAGCTAAAGACATAGTGA AGCCACAGATGTATGTCTTTAGCTAAACTTCTCCCTGCCTACTGCCTC GGA
Ptger3mir2	TGCTGTTGACAGTGAGCGAGGTTGAGCAATGCAAGACACATAGTGA AGCCACAGATGTATGTGTCTTGCATTGCTCAACCGTGCCTACTGCCT CGGA
Ptger3mir3	TGCTGTTGACAGTGAGCGATGCCAAGAAGGTATACAGTACTAGTGA AGCCACAGATGTAGTACTGTATACCTTCTTGGCACTGCCTACTGCCT CGGA
Ptger3mir4	TGCTGTTGACAGTGAGCGAAGGGAAGGATGACTGAGTATTTAGTGA AGCCACAGATGTAAATACTCAGTCATCCTTCCCTGTGCCTACTGCCT CGGA

* *Meis1a* and *Meis1b* were cloned using the same primers and distinguished by sequencing.

† *Meis2(2a)*, *Meis2(2b)*, and *Meis2(4a)* were cloned using the same primers and distinguished by sequencing.

Table S2. Hematopoietic lineages labeled by Mesp1-Cre

Bone Marrow Progenitors

	HSC	MPP	MkP	GMP	Pre GM	Pre MegE	Pre CFU-E	CFU-E
Mice#1	20.5%	25.5%	26.9%	26.1%	25.5%	28.3%	23.7%	25.1%
Mice#2	31.3%	44.1%	38.1%	43.5%	47.8%	41.1%	35.8%	41.3%
Mice#3	10.8%	9.5%	10.4%	11.2%	11.3%	11.6%	9.7%	11.5%

Spleen

	Lymphoid		Myeloid						Meg	Erythroblast			
	B220 ⁺	CD3 ⁺	Granulocyte	Monocyte	Inflammatory DC	CD4 ⁺ DC	CD8 ⁺ DC	pDC	CD41 ⁺	Pro	Baso	Poly	Ortho
Mice#1	14.4%	20.4%	25.4%	24.3%	19.6%	17.2%	20.0%	25.0%	14.8%	17.4%	9.1%	12.4%	1.3%
Mice#2	26.1%	37.6%	36.4%	38.0%	32.0%	31.8%	32.3%	36.4%	31.4%	33.6%	20.7%	14.0%	1.0%
Mice#3	9.7%	14.4%	10.3%	10.1%	9.3%	9.5%	9.1%	10.5%	10.4%	10.3%	9.7%	1.7%	0.1%

Peripheral Blood

	Lymphocyte			Granulocyte
	B220 ⁺	CD4 ⁺	B220 ⁻ CD4 ⁻	
Mice#4	14.5%	19.3%	17.0%	16.6%
Mice#5	20.5%	21.4%	19.2%	20.2%
Mice#6	97.9%	97.0%	94.0%	99.7%

DC indicates dendritic cell; and pDC, plasmacytoid dendritic cell.

Table S3. Erythroid genes down-regulated by Meis2

Gene Symbol	Fold decrease (+dox/-dox)		
	D7_CD41 ⁺	D8_CD41 ^{int}	D8_CD41 ^{hi}
Hba-a1/2	-3.37	-6.33	-20.15
Hbb-y	-2.22	-5.31	-12.12
Hba-x	-1.85	-3.19	-12.01
Hbb-b1/2	-1.66	-2.75	-4.77
Hbb-bh1	-1.36	-2.36	-3.95
Gypa	-2.45	-5.72	-7.05
Kel	-2.37	-1.40	-1.83
Rhag	-1.80	-2.25	-3.02
Tfrc	-1.68	-1.53	-2.98
Cpox	-2.50	-2.11	-3.00
Spna1	-2.17	-2.22	-4.50
Slc25a37	-1.63	-2.32	-4.26
Nfe2	-2.07	-1.85	-2.14

Figure S1. *In vivo* lineage tracing of myeloerythroid progenitors by *Mesp1*-Cre in mouse bone marrow. Murine bone marrow cells collected from adult *Mesp1*^{Cre/+} *Rosa26-CAG-STOP-eGFP*^{+/+} mice were analyzed by FACS for GFP expression in various myeloerythroid progenitors. (A) Bone marrow cells were stained with antibodies to Sca1, c-kit, B220, CD41, CD105, CD150, and CD16/32. Shown is the gating scheme used to identify progenitor populations LSK, HSC, MPP, MkP, GMP, CFU-E, Pre CFU-E, Pre GM, and Pre MegE.⁶ (B) Shown are two-parameter contours for GFP expression and forward scatter (FSC) for each indicated progenitor population from an individual *Mesp1*^{Cre/+} *Rosa26-CAG-STOP-eGFP*^{+/+} mouse. Numbers indicate the percentage of cells within the indicated gates. (C) Listed hematopoietic subsets were sort-purified from 129S6/SvEV wild-type mice, harvested for RNA, and analyzed for the expression of *Mesp1* by quantitative RT-PCR (n=3). RNA isolated from D4 A2lox.*Mesp1* EBs with or without dox treatment, and from Flk1⁺ Tie2⁺ cells sorted from D5 A2lox.*Mesp1* EBs with or without dox treatment were used as positive controls.

Figure S2. *Meis1* maintains CD41⁺ hematopoietic progenitors from ES cells co-cultured with OP9 and hematopoietic cytokines. (A) A2lox.*Meis1* ES cells were differentiated as embryoid bodies (EBs) for 6 days. On day 6, EBs were left intact, or dissociated and co-cultured with an OP9-GFP cell monolayer in the absence (OP9) or presence (OP9 + cytokines) of the cytokines stem cell factor (SCF), thrombopoietin (TPO), vascular endothelial growth factor (VEGF), Flt3 ligand (Flt3L), interleukin-3 (IL-3) and IL-6 (IL-6) as described in “Methods”. Doxycycline (dox) was added as indicated on day 6 and replenished every other day until day 12. (B) A2lox.*Meis1* and A2lox.*Meis2* ES cells were differentiated as embryoid bodies (EBs) for 6 days and plated on OP9 cells with cytokines described above in (A). Levels of *Meis1* and *Meis2* were determined by quantitative RT-PCR on day 7 or day 8 as indicated in the presence or absence of doxycycline (dox) administered on day 6. (C) On day 9 and 12, cells from each differentiation condition were analyzed by FACS for expression of CD41 and CD42d. Numbers indicate the percentage of cells in the indicated gates.

Figure S3. Induction of *Meis1* or *Meis2* before day 8 of differentiation is required to maintain CD41⁺ hematopoietic progenitors derived in ES/OP9 co-cultures. (A)

A2lox.*Meis1* or A2lox.*Meis2* ES cells were differentiated as EBs for 6 days before plating on OP9-GFP monolayers and cytokines as described in supplemental Figure 2A. Doxycycline (dox) was added on day 4, 6, 8, or 10 as indicated and replenished every other day until day 12.

(B) A2lox.*Meis1* or A2lox.*Meis2* ES cells from the conditions indicated in panel A were analyzed on day 12 by FACS for CD41 and CD42d expression.

Figure S4. *Plac8* and *Serpinb2* are not sufficient to maintain CD41⁺ hematopoietic progenitors derived in ES/OP9 co-cultures. ES cells with dox-inducible *Plac8* (A2lox.*Plac8*),

Serpinb2 (A2lox.*Serpinb2*), or both (A2lox.*Plac8*tet*Serpinb2*) were differentiated as EBs for 6 days and co-cultured with OP9-GFP monolayers and cytokines as in supplemental Figure 2A.

Doxycycline (dox) was added on day 6 and replenished every other day until day 12. On day 9 and 12, cells were analyzed by FACS for CD41 and CD42d expression.

Figure S5. *Plac8* is dispensable for normal *in vivo* hematopoietic progenitor proliferation.

CD45.2⁺ bone marrow cells isolated from *Plac8*^{-/-} mice (KO) or wild-type (WT) littermate control mice were mixed with CD45.1⁺ competitor bone marrow cells from B6.SJL-

Ptprc^a/BoyAiTac at 1:1 ratio and transplanted into lethally irradiated B6.SJL-*Ptprc*^a/BoyAiTac recipients. Bone marrow from recipients was analyzed by FACS after 4 weeks. (A) Cells were

stained with antibodies against Sca1, c-kit, B220, CD41, CD105, CD150, CD45.1 and CD45.2.

Shown are two-color histograms and gating scheme used to identify the populations indicated in

supplemental Figure 1. (B) Cells from recipients were stained as in panel A. Shown are two-

color histograms for CD45.1 and CD45.2 expression gated on the indicated populations as

shown in panel A. Numbers are the percentage of cells in the indicated gates.

Figure S6. Isoforms of *Meis1* and *Meis2* maintain CD41⁺ hematopoietic progenitors

differentiated from ES cells. (A) Shown is the domain structure of *Meis1* and *Meis2* isoforms.

Meis1a and *Meis1b* differ in the carboxyl-terminal (C-terminal) region due to alternative

splicing. Similarly, the C-terminal regions of *Meis2(2a)* and *Meis2(4a)* differ from *Meis2(2b)*.

(B) ES cells with dox-inducible *Meis1a* (A2lox.Meis1a), *Meis2(2a)* (A2lox.Meis2(2a)), or *Meis2(4a)* (A2lox.Meis2(4a)) were differentiated and analyzed as in supplemental Figure 4.

Figure S7. *HoxA9* is insufficient for maintaining CD41⁺ hematopoietic progenitors differentiated from ES cells either alone or in combination with *Meis1* or *Meis2*.

A2lox.HoxA9 ESCs containing dox-inducible *HoxA9*, A2lox.Meis1tetHoxA9 ESCs containing simultaneously dox-inducible *Meis1* and *HoxA9*, and A2lox.HoxA9tetMeis2 ESCs containing simultaneously dox-inducible *Meis2* and *HoxA9* were differentiated as in supplemental Figure 4. On day 9, cells were analyzed by FACS for CD41 and CD42d expression. On day 12, cells were analyzed by for CD41 and c-kit expression.

Figure S8. Expression pattern of *Ptger3* and *Meis1* in adult hematopoietic subsets.

Expression values are shown for *Ptger3* (A) and *Meis1* (B) for each indicated cell population derived from microarray analysis.⁶ Data are assembled from two to four replicate arrays.

Figure S9. Lack of extinction of *Ptger3* mRNA after miR-based shRNA expression. Four miR30-based shRNA (*Ptger3*mir1-4) cassettes, described in supplemental Table 1, directed against the target gene *Ptger3* were cloned into vector pcDNA3.1 zeo/(+) Vav1 mir-shRNA (from Dr. Andrew Shaw, Washington University School of Medicine, St. Louis, MO) using XhoI and EcoR1, and then subcloned into p2lox.CAGGFP.MCS.pA, a vector expressing GFP, at the NheI and EcoRV sites in the 3' UTR downstream of the GFP coding exon. A2lox ES cell lines with constitutive expression of shRNA targeting *Ptger3* were generated. The extent of knockdown of the *Ptger3* was assessed by quantitative RT-PCR.

Figure S1

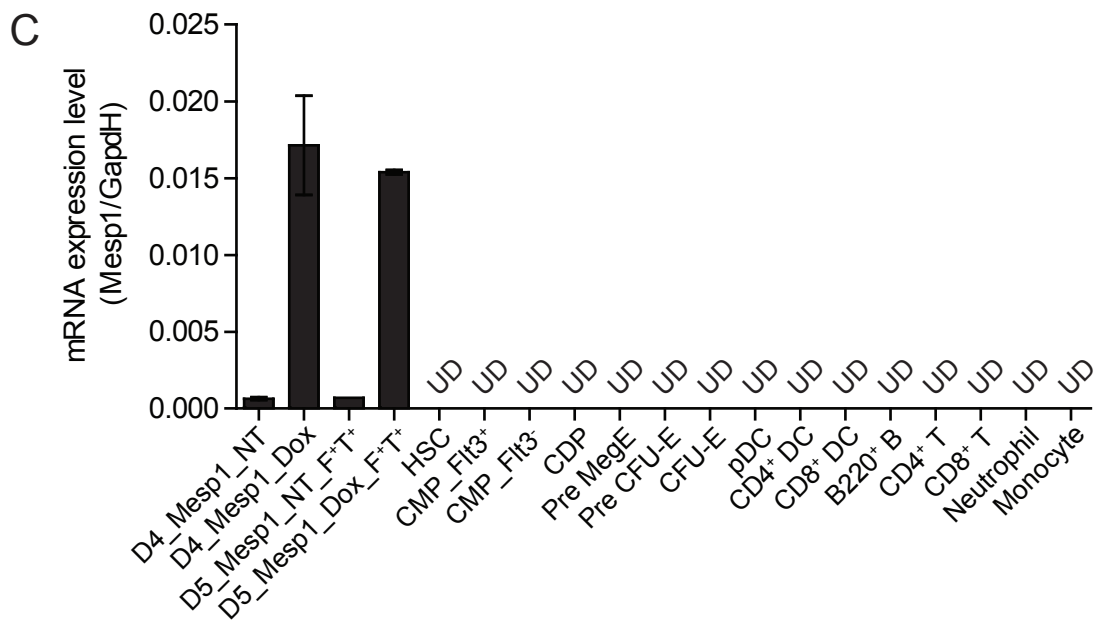
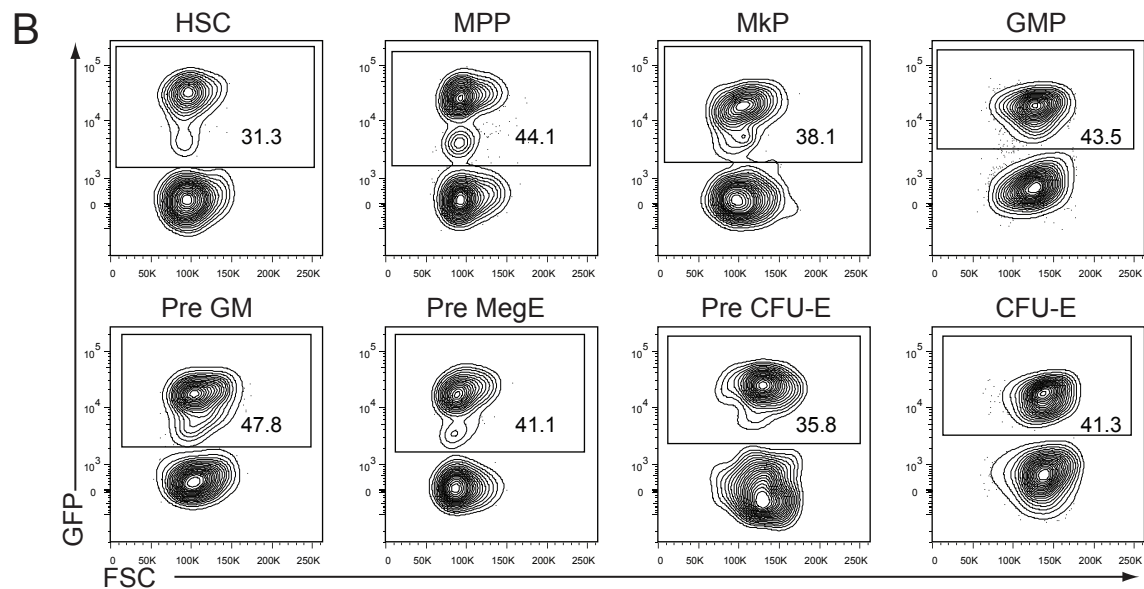
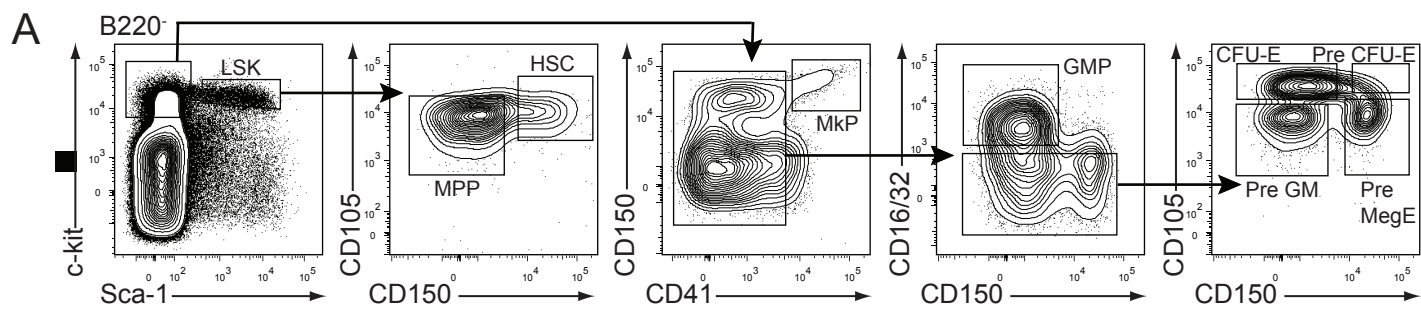


Figure S2

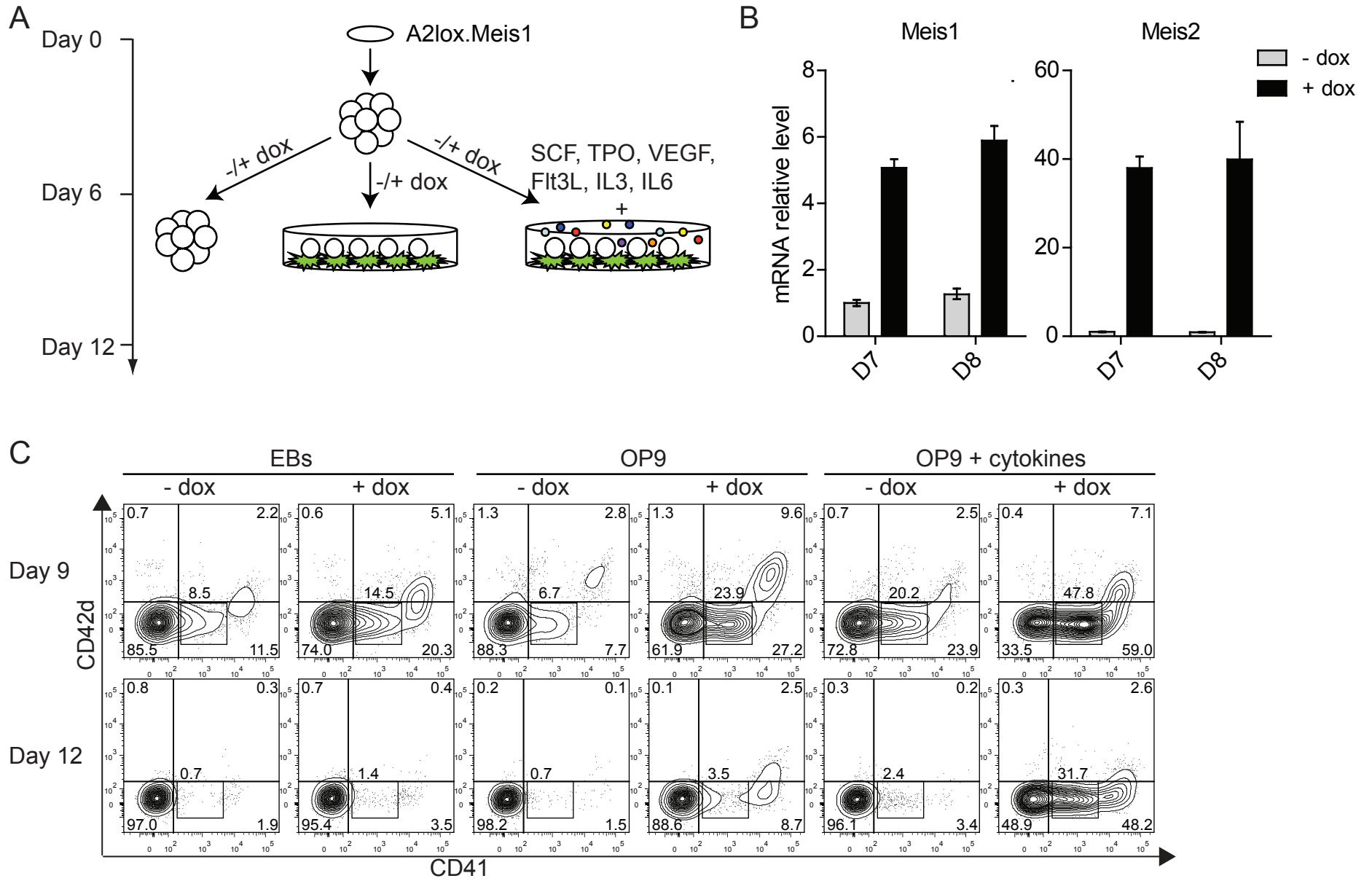


Figure S3

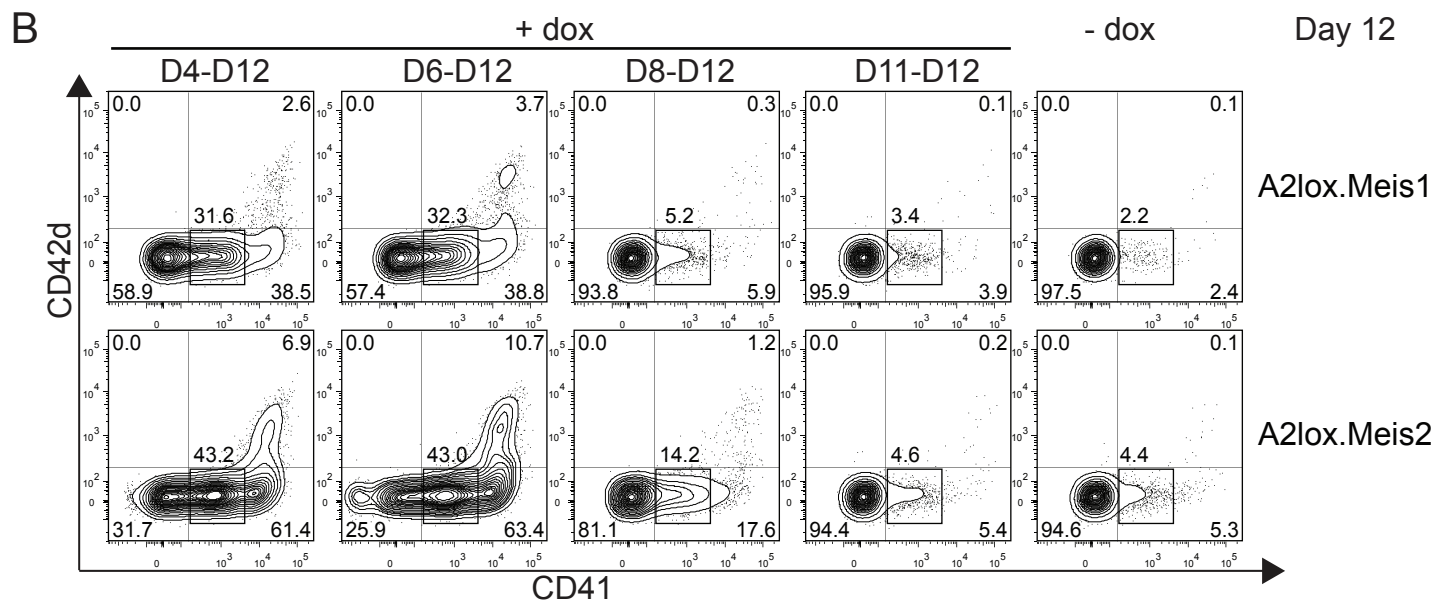
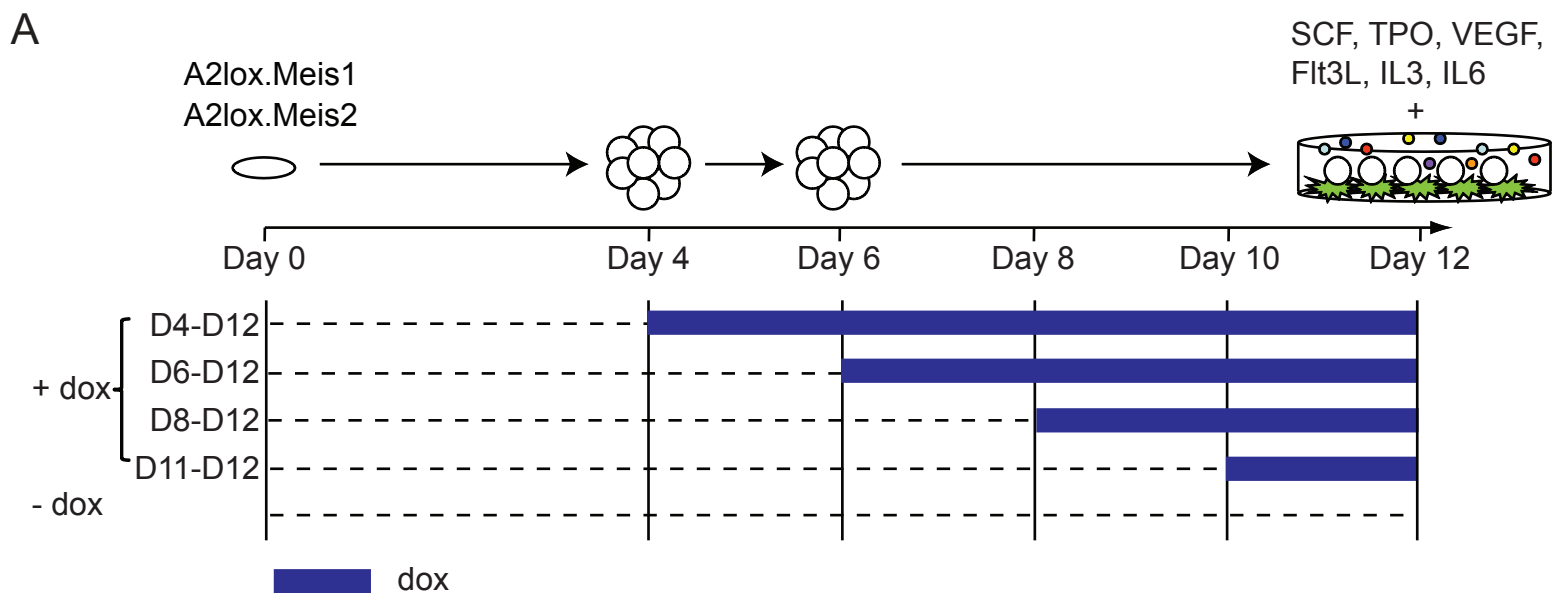


Figure S4

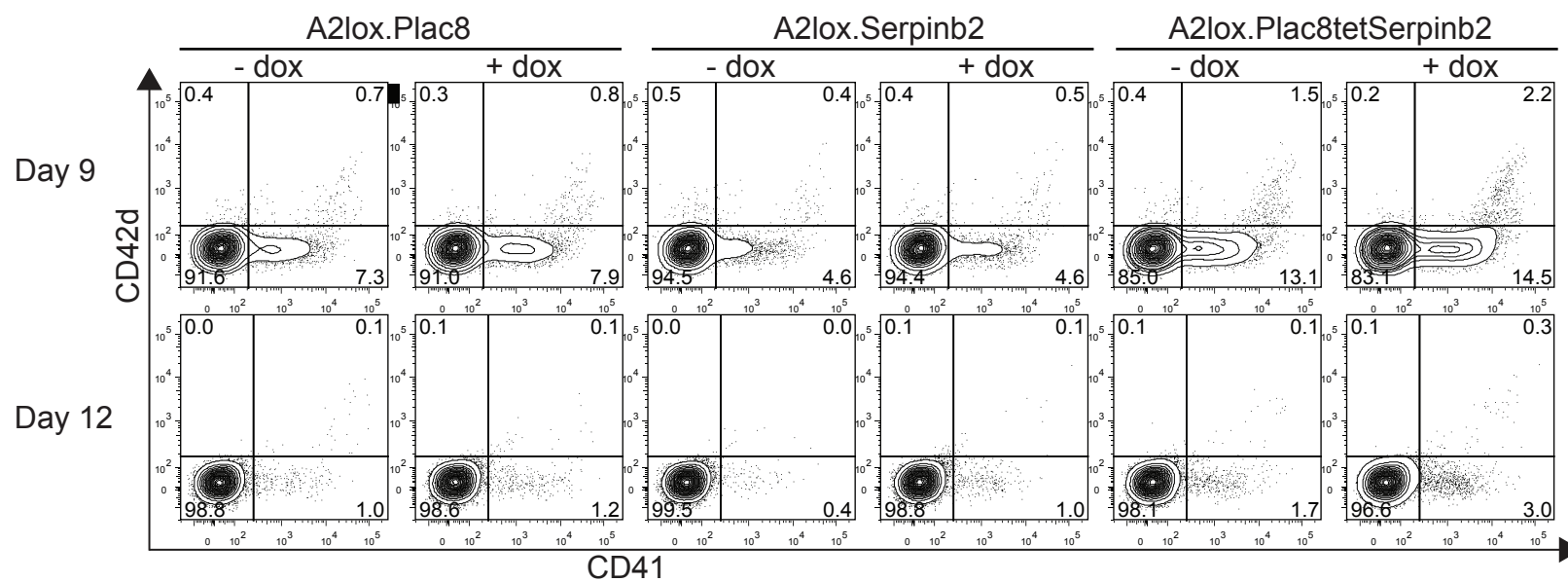


Figure S5

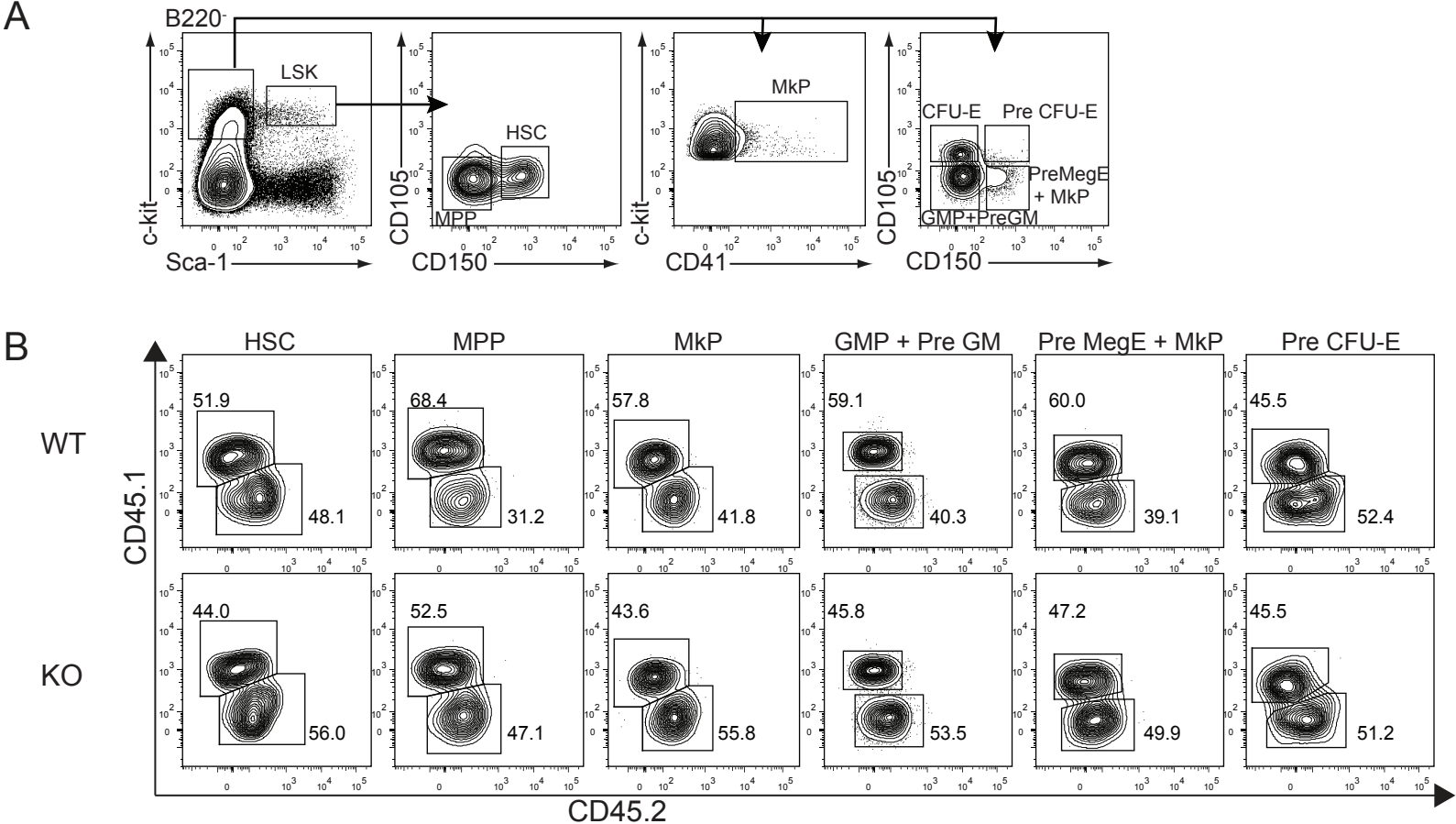


Figure S6

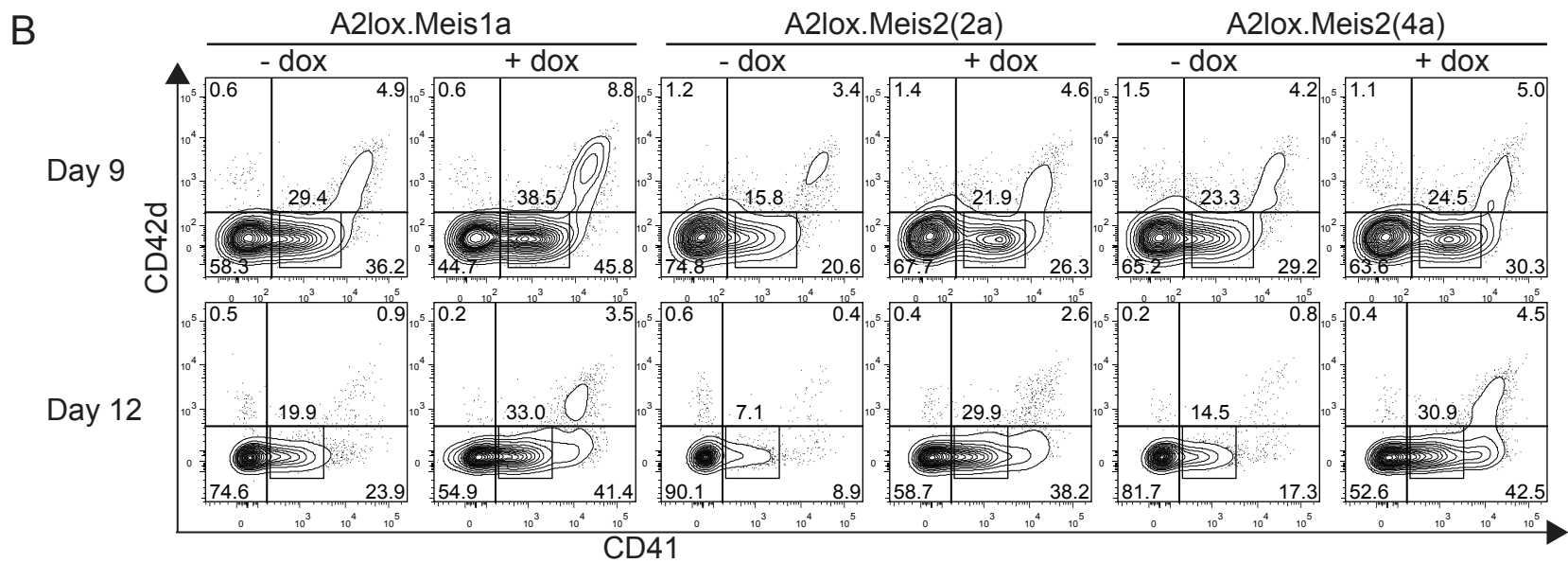
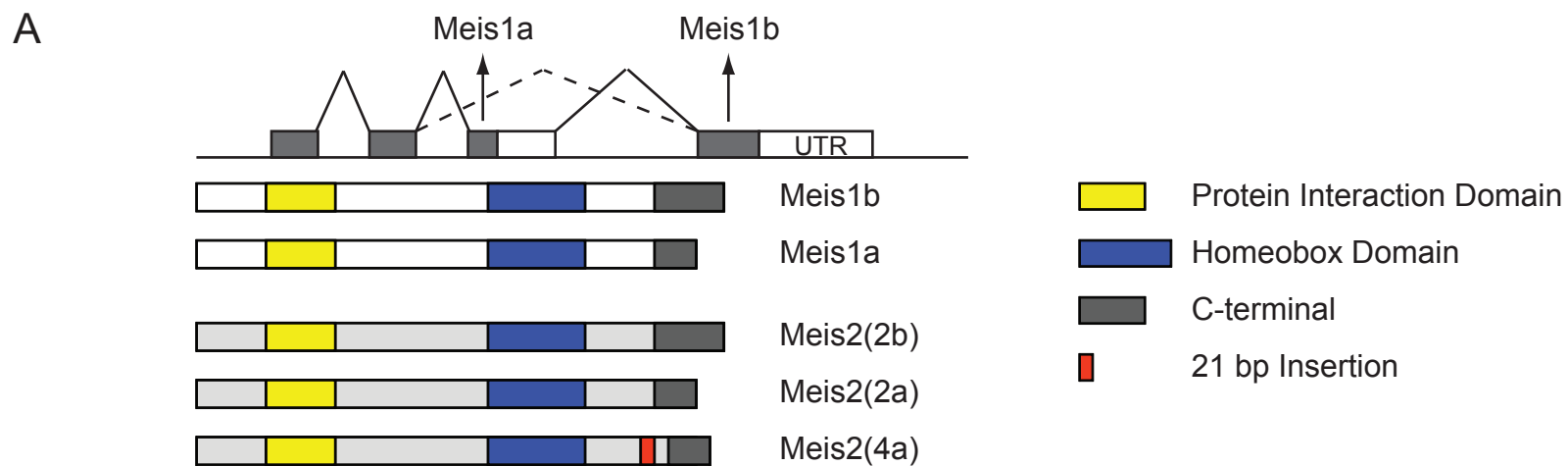


Figure S7

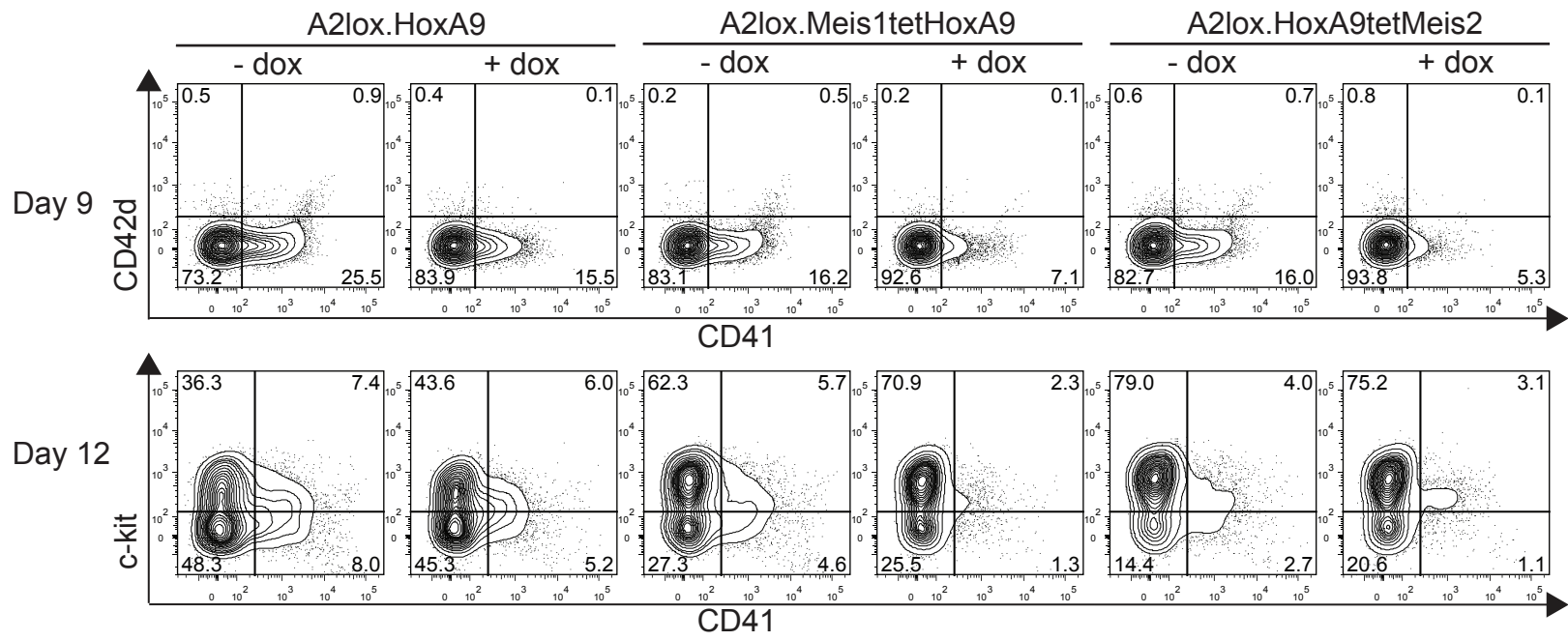


Figure S8

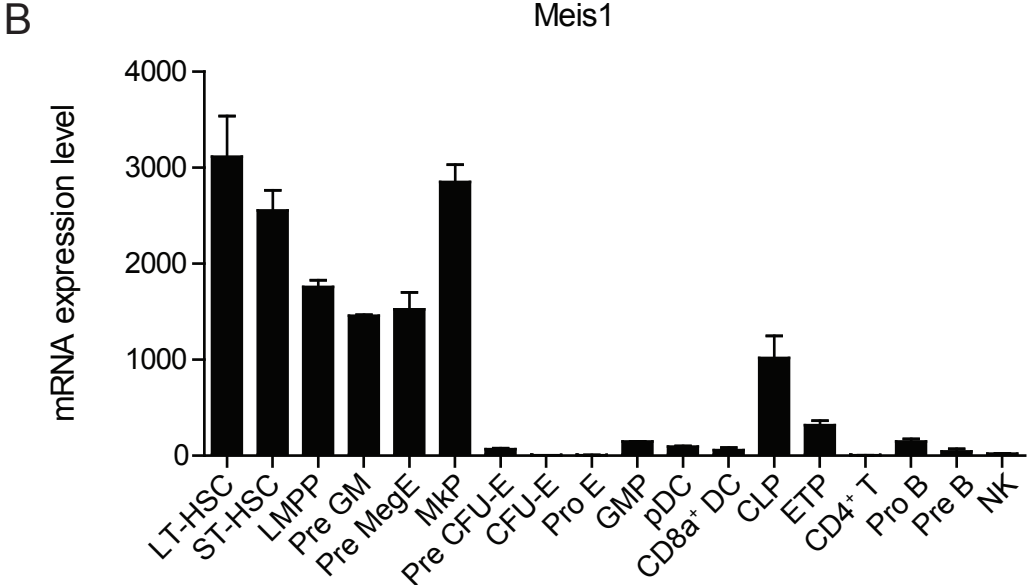
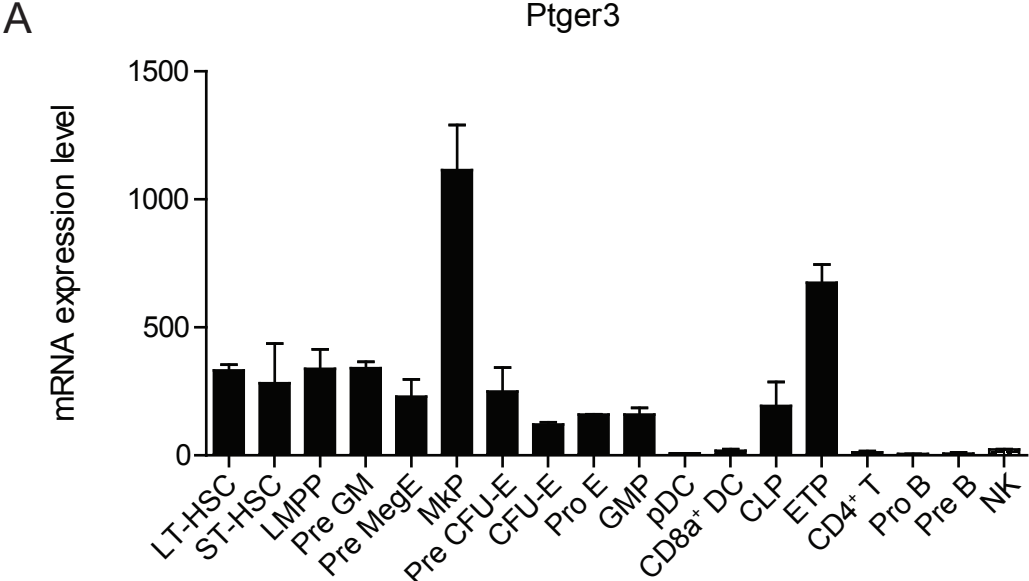


Figure S9

