

The long non-coding RNA Meg3 is dispensable for hematopoietic stem cells in mice

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Supplementary Information

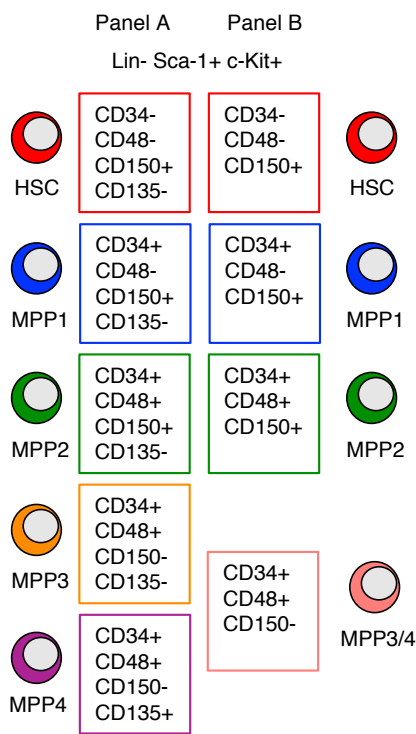
Supplementary Table 1: Flow cytometry stainings

Staining	Surface Marker	Monoclonal Antibody
Differentiated Cells (Peripheral Blood, BM, Spleen)	CD4	GK1.5 (PE-Cy7)
	CD8a	53-6.7 (PE-Cy7)
	CD11b	M1/70 (APC-Cy7)
	Gr1	RB6-8C5 (APC)
	Ter119	Ter-119 (PB)
	CD71	R17217 (PE)
	B220	RA3-6B2 (AF700)
	IgM	II/41 (PE-Cy5)
	Lineage (CD4, CD8a, CD11b, Gr1, Ter119, B220)	Clones see differentiated cells, all PE-Cy7
HSCs (BM)	CD117	2B8 (BV711)
	Sca-1	D7 (APC-Cy7)
	CD48	HM48-1 (PB)
	CD150	TC15-12F12.2 (PE-Cy5)
	CD34	RAM34 (FITC)
	CD16/32	93 (APC)
	CD127	A7R34 (PE)
	Lineage (CD4, CD8a, CD11b, Gr1, Ter119, B220)	Clones see differentiated cells, all AF700
Thymus	CD4	GK1.5 (PE-Cy7)
	CD8a	53-6.7 (PB)
	CD25	PC61 (APC)
	CD44	IM7 (PE-Cy5)
Cell Cycle (BM)	CD117	2B8 (PE)
	Sca-1	D7 (APC-Cy7)
	CD48	HM48-1 (PE-Cy7)
	CD150	TC15-12F12.2 (PE-Cy5)
	CD34	RAM34 (FITC)
	Lineage (CD4, CD8a, CD11b, Gr1, Ter119, B220)	Clones see differentiated cells, all AF700
Differentiated Cells Transplants (Peripheral Blood, BM, Spleen)	CD4	GK1.5 (PE-Cy5)
	CD8a	53-6.7 (PE-Cy5)
	CD11b	M1/70 (APC-Cy7)
	Gr1	RB6-8C5 (APC)
	Ter119	Ter-119 (FITC)
	CD71	R17217 (PE)
	B220	RA3-6B2 (AF700)
	CD45.1	A28 (PE-Cy7)
	CD45.2	104 (PB)
	Lineage (CD4, CD8a, CD11b, Gr1, Ter119, B220)	Clones see differentiated cells, all AF700
HSCs Transplants (BM)	CD117	2B8 (BV711)
	Sca-1	D7 (APC-Cy7)
	CD48	HM48-1 (PE)
	CD150	TC15-12F12.2 (PE-Cy5)
	CD34	RAM34 (FITC)
	CD45.1	A28 (PE-Cy7)
	CD45.2	104 (PB)
	Lineage (CD4, CD8a, CD11b, Gr1, Ter119, B220)	Clones see differentiated cells, all AF700

Supplementary Table 2: Primer Sequences

Primer	Sequence
<i>Cdk6</i> fwd	cgagtgcagaccagtgagg
<i>Cdk6</i> rev	tgtgcacacatcaaacaacct
<i>c-Myc</i> fwd	caccagcagcgactctga
<i>c-Myc</i> rev	ggggtttgcctcttctcc
<i>Egr1</i> fwd	agttccaacgcctctgac
<i>Egr1</i> rev	acaggaaccgaaccaca
<i>Hoxa5</i> fwd	agctgcacattagtcacgaca
<i>Hoxa5</i> rev	gcggttgaagtgaattctt
<i>Hoxb4</i> fwd	ctggatgcgcaaagttcac
<i>Hoxb4</i> rev	gtgaaactccttctccaactcc
<i>Hoxb5</i> fwd	gcttcacatcagccacgata
<i>Hoxb5</i> rev	caggtagcgattgaagtggaat
<i>Meis1</i> fwd	gacgctttaaagagagataaagatgc
<i>Meis1</i> rev	catttctcaaaaatcagtgctaaga
<i>Meg3</i> fwd	cgaggacttcacgcacaa
<i>Meg3</i> rev	attccagatgatggctttg
<i>Oaz1</i> fwd	tttcagctagcatcctgtactcc
<i>Oaz1</i> rev	gaccctggcttctgtcgttaga
<i>Tgm2</i> fwd	cctgaccctggatccctact
<i>Tgm2</i> rev	cacccgctgtacttctcgtag

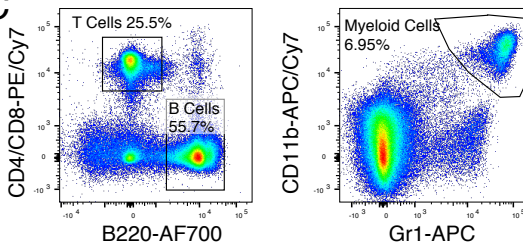
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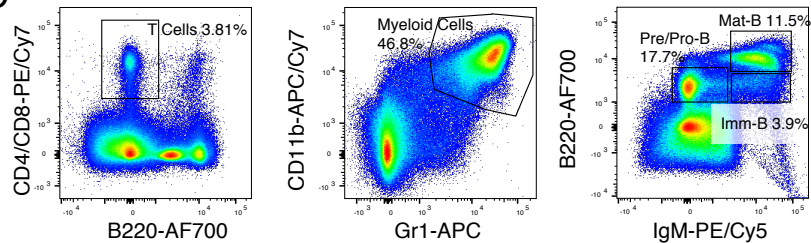
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Symbol	log2FC	p-value adjusted	Direction in <i>Meg3</i> KO	Chr	<i>Dlk1-Meg3</i> Locus
mmu-miR-379-5p	-6,3902	0,0038	down	12	Yes
mmu-miR-300-3p	-6,102	0,0311	down	12	Yes
mmu-miR-541-5p	-5,9404	0,0311	down	12	Yes
mmu-miR-376b-5p	-5,9643	0,0311	down	12	Yes
mmu-miR-326-3p	-2,5344	0,0311	down	7	No
mmu-miR-136-3p	-5,3738	0,0375	down	12	Yes
mmu-miR-382-5p	-5,2985	0,0389	down	12	Yes
mmu-miR-376c-5p	-5,6075	0,0553	down	12	Yes
mmu-miR-411-5p	-5,7243	0,0553	down	12	Yes
mmu-miR-134-5p	-5,7421	0,0553	down	12	Yes
mmu-miR-712-5p	-3,2345	0,0553	down	unknown	unknown
mmu-miR-136-5p	-5,6663	0,0588	down	12	Yes

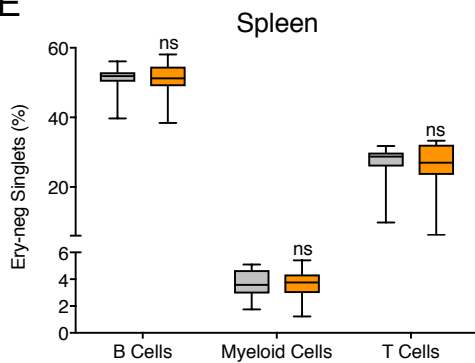
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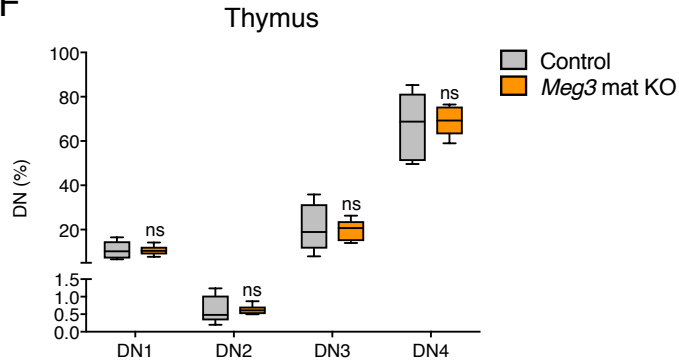
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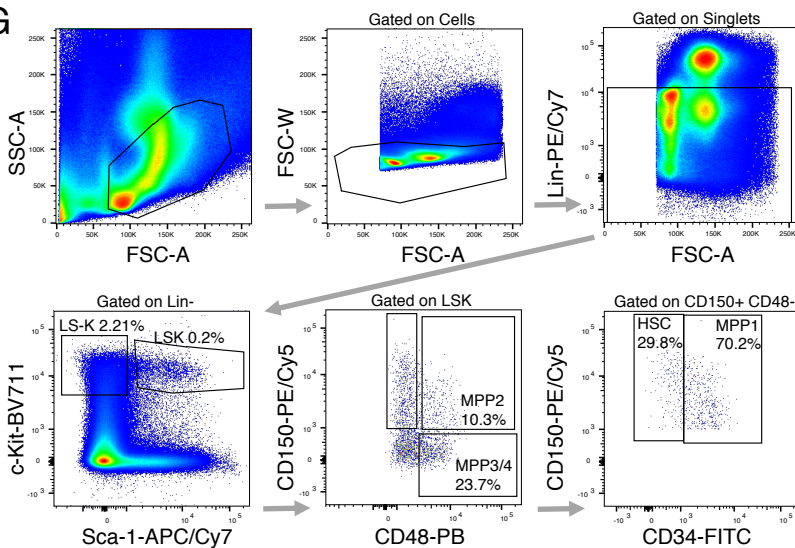
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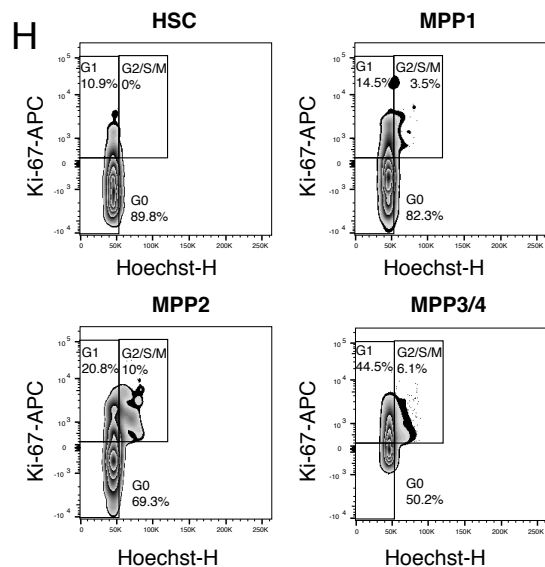
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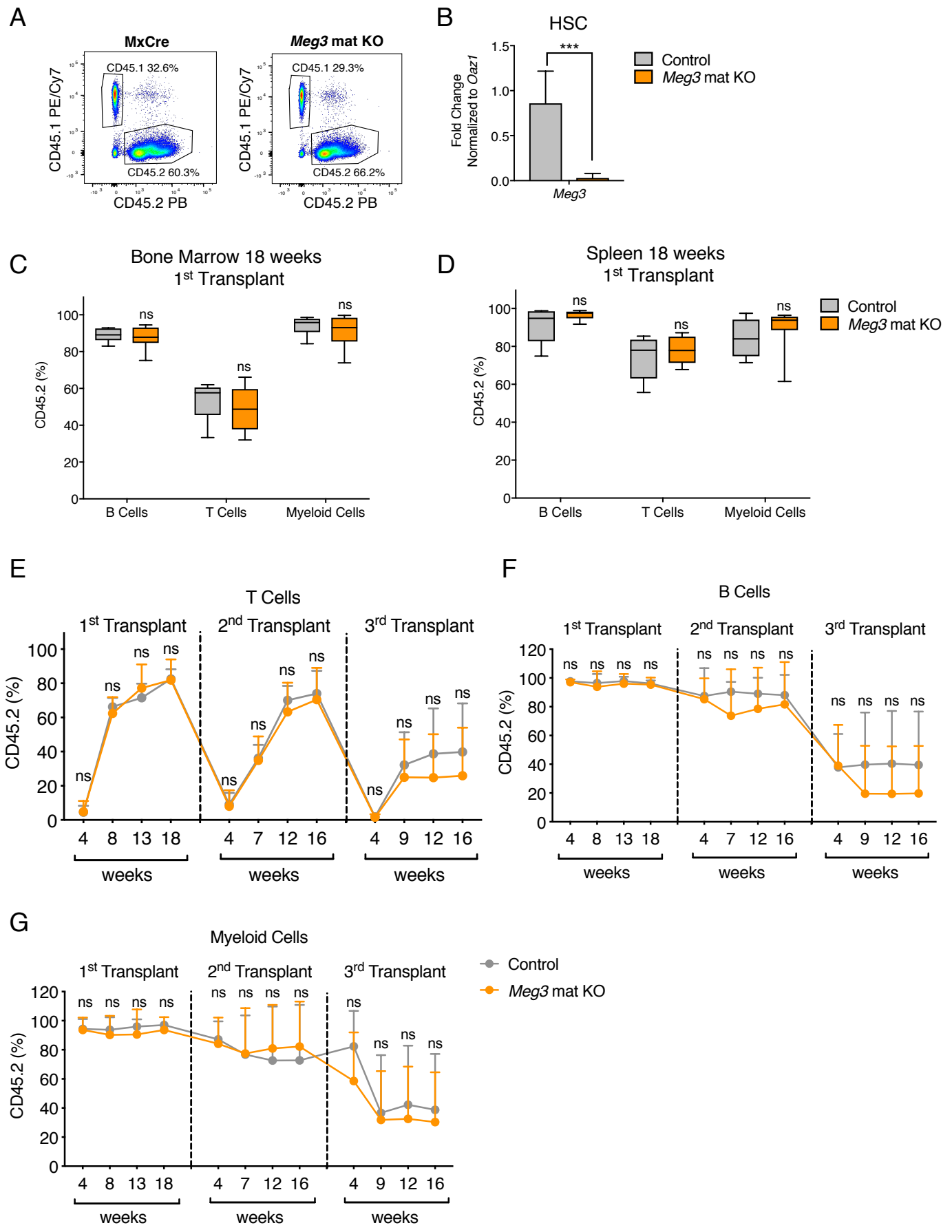
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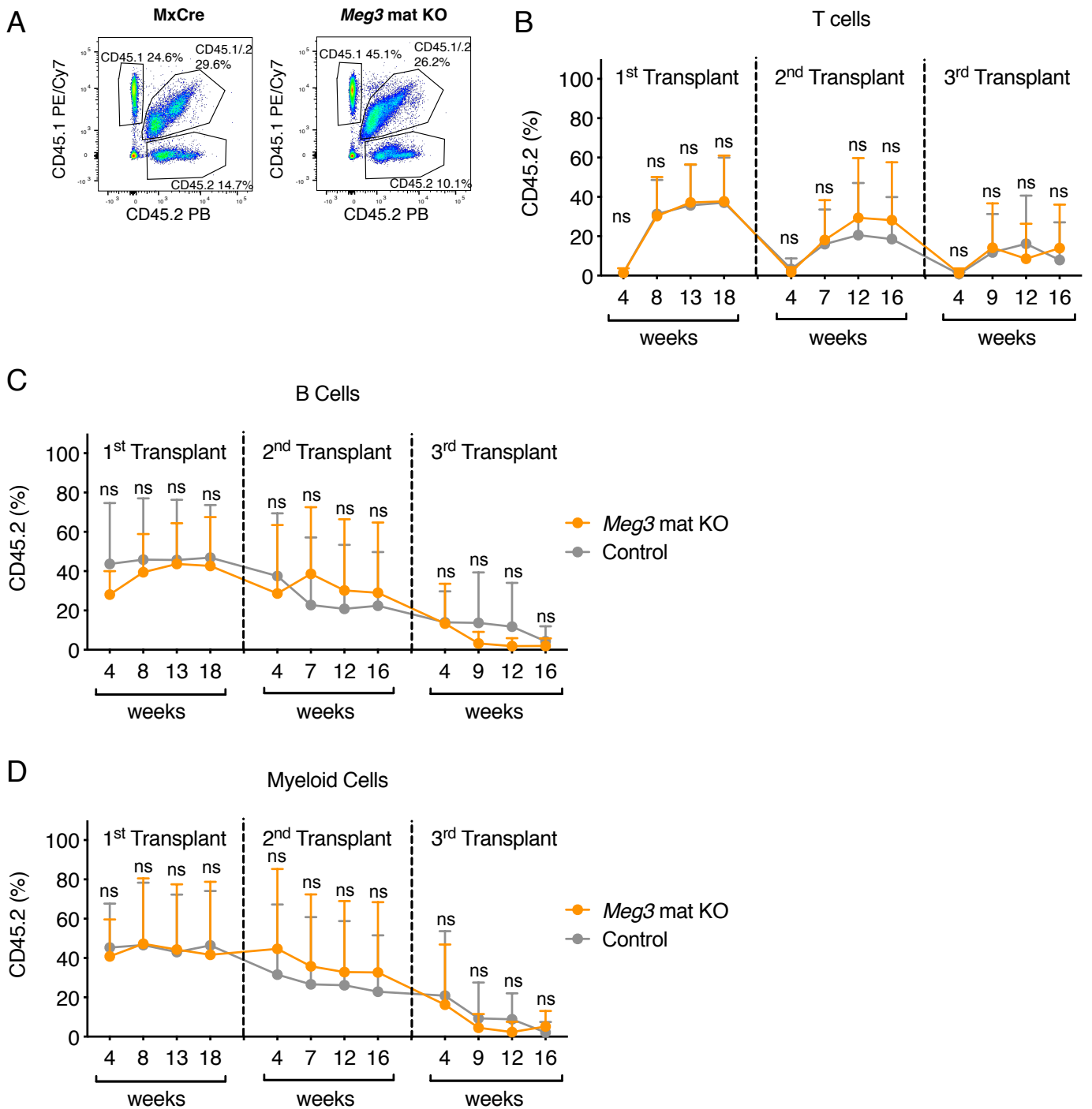
H



Supplementary Figure 1: (A) Surface marker profiles of HSCs and MPPs as used in flow cytometry analysis and sorting of BM cells. CD135 was only included for analysis in Fig. 1 A and 1 C (Panel A), for all other experiments Panel B was used. (B) Differential mature miRNA expression detected by small RNA-Seq in sorted LSK CD150+ CD48- cells (LSK Slam). Adjusted p-value <0.1 is shown. (C) Representative flow cytometry dot plots showing PB analysis. (D) Representative flow cytometry dot plots showing analysis of BM differentiated cells. (E-F) Flow cytometry analysis of differentiated Spleen (E) and double negative (DN) Thymus cells (F). n=9-18, unpaired student's t-test. ns: not significant. (G) Representative flow cytometry dot plots showing analysis of BM HSC/MPP populations. (H) Representative flow cytometry zebra plots (including outliers) showing cell cycle analysis of BM HSC/MPP populations. For all experiments: *p <0.05; **p <0.01; ***p <0.001., ns: not significant. n indicates number of biological replicates; E-F,H: 3 independent experiments.

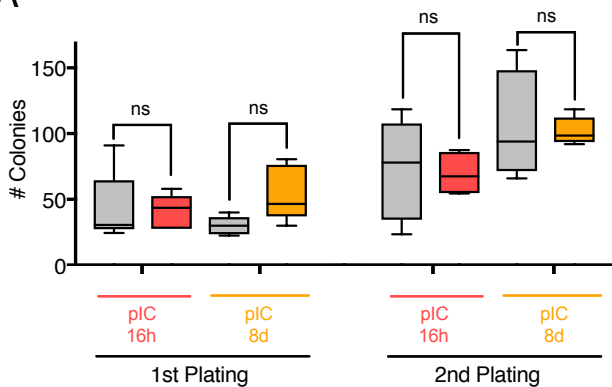


Supplementary Figure 2: (A) Representative flow cytometry dot plots showing PB analysis of full chimeras. (B) qPCR analysis of *Meg3* in sorted CD45.2⁺ HSCs of primary recipients 18 weeks after transplantation. n=5-6, mean +SD, unpaired student's t-test. (C-D) Flow cytometry analysis of differentiated BM (C) and Spleen cells (D) in primary recipients. n=8-9, unpaired student's t-test. (E-G) Flow cytometry analysis of serial transplantation experiments. The data for CD45.2% positive cells in the chimeras is shown for T cells (E), B cells (F) and myeloid cells (G). n=7-9, mean +SD, two-way ANOVA. For all experiments: *p <0.05; **p <0.01; ***p <0.001., ns: not significant. n indicates number of biological replicates; B: 3 independent experiments, C-G: 1 independent experiment.

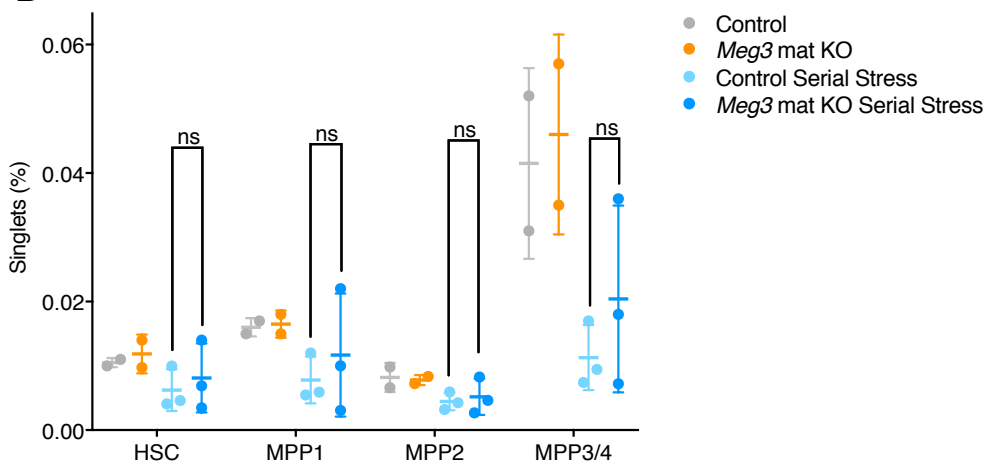


Supplementary Figure 3: (A) Representative flow cytometry dot plots showing PB analysis of competitive chimeras. **(B-D)** Flow cytometry analysis of serial transplantation experiments. The data for CD45.2% positive cells in the chimeras is shown for T cells **(B)**, B cells **(C)** and myeloid cells **(D)**. $n=7-9$, mean +SD, two-way ANOVA. For all experiments: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$., ns: not significant. n indicates number of biological replicates; B-D: 1 independent experiment.

A



B



Supplementary Figure 4: (A) Functional analysis by serial CFU assays. $n=5$, one-way ANOVA. ns: not significant. n indicates number of biological replicates; 2 independent experiments. **(B)** HSCs, MPP1, MPP2, and MPP3/4 frequencies determined by flow cytometry analysis. Percentage of BM HSC-MPPs are represented. $n=2-3$, unpaired student's t-test. ns: not significant. n indicates number of biological replicates; 1 independent experiment.