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Supplemental Data

E2A Proteins Promote Development of Lymphoid-Primed Multipotent Progenitors

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Figure S1 Dias et al.

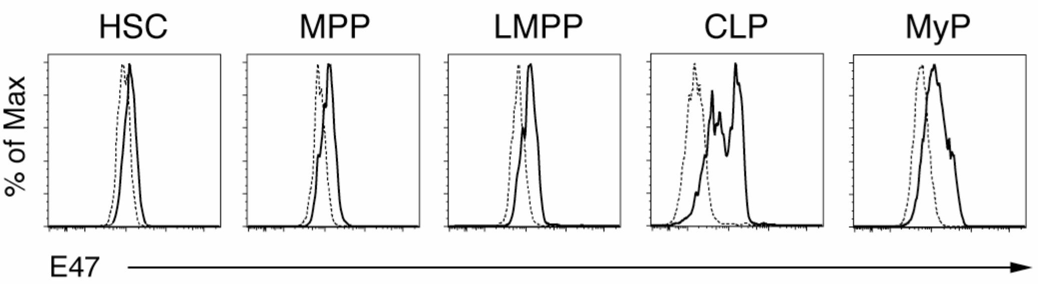


Figure S1. E2A protein in multipotent progenitors

Intracellular staining for the E2A protein E47 in WT HSCs (LSK Flt3^{-}), MPPs (LSK Flt3^{lo}), LMPPs (LSK Flt3^{hi}), CLPs (LSK^{lo}IL-7Rα^{+}Flt3^{+}), and myeloid progenitors (MyP, LS^{-}K expressing FcγRII/III), as indicated by the black line. Secondary antibody only control is shown by the dashed line.

Figure S2 Dias et al.

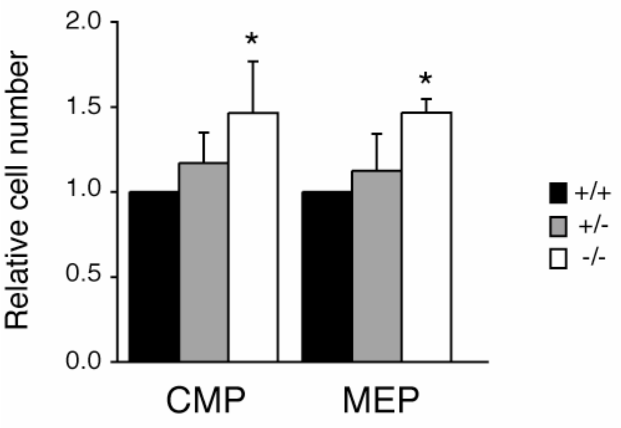
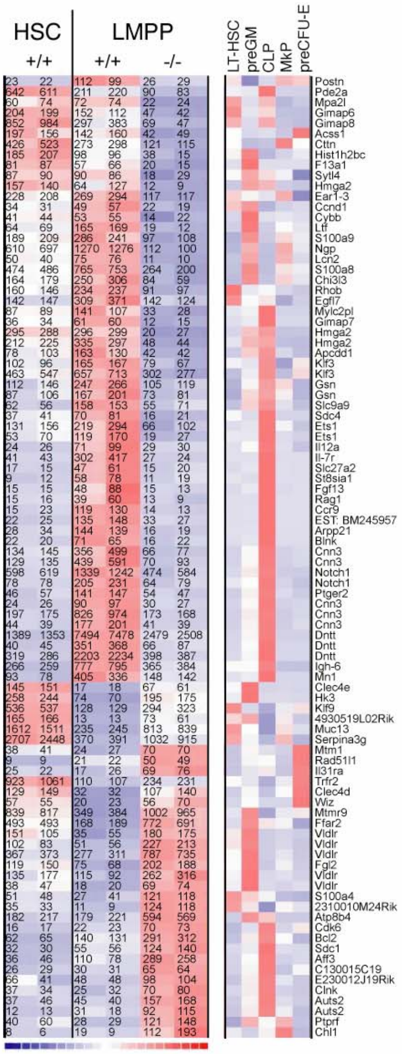


Figure S2. E2A proteins are dispensable for the generation of CMP and MEP.

Number of CMPs ($LS^{-}K$ FcγRII/III^{lo}CD34^{+}) and MEPs ($LS^{-}K$ FcγRII/III^{lo}CD34^{-}) in *Tcfe2a*$^{+/-}$ (grey) and *Tcfe2a* $^{-/-}$ (white) mice relative to WT (black; set to 1). A minimum of 6 mice were analyzed in each group; bars represent the mean ± SD; * $p<0.05$.

Figure S3 Dias et al.



HSC +/+		LMPP +/+		LMPP -/-		
23	22	112	99	26	29	Postn
642	611	211	220	90	83	Pde2a
60	74	72	74	22	24	Mpa2l
204	199	152	112	47	42	Gimap6
852	984	297	383	69	47	Gimap8
197	156	142	160	42	49	Acss1
426	523	273	298	121	115	Cttn
185	207	98	96	38	15	Hist1h2bc
81	87	57	66	20	15	F13a1
87	90	90	86	18	29	Sytl4
157	140	64	127	12	9	Hmga2
228	208	269	294	117	117	Ear1-3
34	31	49	57	22	19	Ccnd1
41	44	53	55	14	22	Cybb
64	69	165	169	19	12	Ltf
189	209	286	241	97	108	S100a9
610	697	1270	1276	112	100	Ngp
50	40	75	76	11	10	Lcn2
474	486	765	753	264	200	S100a8
164	179	250	306	84	59	Chi3l3
160	146	234	237	91	97	Rhob
142	147	309	371	142	124	Egfl7
87	89	141	107	33	28	Mylc2pl
36	34	61	60	12	15	Gimap7
295	288	296	299	20	27	Hmga2
212	225	335	297	48	44	Hmga2
78	103	163	130	42	42	Apcdd1
102	96	165	167	79	67	Klf3
463	547	657	713	302	277	Klf3
112	146	247	266	105	119	Gsn
87	106	167	201	73	81	Gsn
62	56	158	153	55	71	Slc9a9
37	41	70	81	16	21	Sdc4
131	156	219	294	66	102	Ets1
53	70	119	170	19	27	Ets1
24	26	71	99	29	30	Il12a
41	43	302	417	27	24	Il-7r
17	15	47	61	15	20	Slc27a2
9	12	58	78	11	19	St8sia1
15	15	48	88	15	13	Fgf13
15	16	39	60	13	9	Rag1
15	23	119	130	14	13	Ccr9
22	25	135	148	33	27	EST: BM245957
28	34	144	139	16	19	Arpp21
22	20	71	65	16	22	Blnk
134	145	356	499	66	77	Cnn3
129	135	439	591	70	93	Cnn3
598	619	1339	1242	474	584	Notch1
78	78	205	231	64	79	Notch1
46	57	141	147	54	47	Ptger2
24	26	90	97	30	27	Cnn3
197	175	826	974	173	168	Cnn3
44	39	177	201	41	39	Cnn3
1389	1353	7494	7478	2479	2508	Dntt
40	45	351	368	66	87	Dntt
319	286	2203	2234	398	387	Dntt
266	259	777	795	365	384	Igh-6
93	78	405	336	148	142	Mn1
145	151	17	18	67	61	Clec4e
258	244	74	70	195	175	Hk3
536	537	128	129	294	323	Klf9
165	166	13	13	73	61	4930519L02Rik
1612	1511	235	245	813	839	Muc13
2707	2448	370	391	1032	915	Serpina3g
38	41	24	27	70	70	Mtm1
9	9	21	22	50	49	Rad51l1
25	22	17	26	69	76	Il31ra
923	1061	110	107	234	231	Trfr2
129	149	32	32	107	140	Clec4d
57	55	20	23	56	70	Wiz
839	817	349	384	1002	965	Mtmr9
493	493	168	189	772	691	Ffar2
151	105	35	55	180	175	Vldlr
102	83	51	56	227	213	Vldlr
367	373	277	311	787	735	Vldlr
119	150	75	68	202	188	Fgl2
135	177	115	92	262	316	Vldlr
38	47	18	20	69	74	Vldlr
51	48	27	41	121	118	S100a4
35	33	11	9	124	118	2310010M24Rik
182	217	179	221	594	569	Atp8b4
16	17	22	23	70	73	Cdk6
62	65	140	131	291	312	Bcl2
32	30	55	56	124	140	Sdc1
36	46	110	78	289	258	Aff3
26	29	30	31	65	64	C130015C19
66	41	48	48	98	104	E230012J19Rik
37	34	25	32	70	80	Clnk
37	46	45	40	157	168	Auts2
12	13	31	18	92	115	Auts2
40	60	28	29	121	148	Ptprf
8	6	19	9	112	193	Chl1

Figure S3. Comparative analysis of the *Tcfe2a*$^{+/+}$ and *Tcfe2a*$^{-/-}$ LMPP transcriptome

Clusters show genes that are differentially expressed in replicate samples (one per column) of *Tcfe2a*$^{+/+}$ and *Tcfe2a*$^{-/-}$ LMPPs and expression of these genes in *Tcfe2a*$^{+/+}$ HSCs is shown for comparison. The clustering includes all genes with expression levels >50 in at least one of the four LMPP arrays and differing by 50% (using a lower 90% confidence bound of fold change). Each row corresponds to one unique identifier and numbers are the raw expression values. The right-most clustering shows the lineage association of these differentially expressed genes in LT-HSC, preGM, CLP, MkP and preCFU-E (as defined in (Pronk et al., 2007b)). Red indicates high, blue low, and white intermediate expression levels.

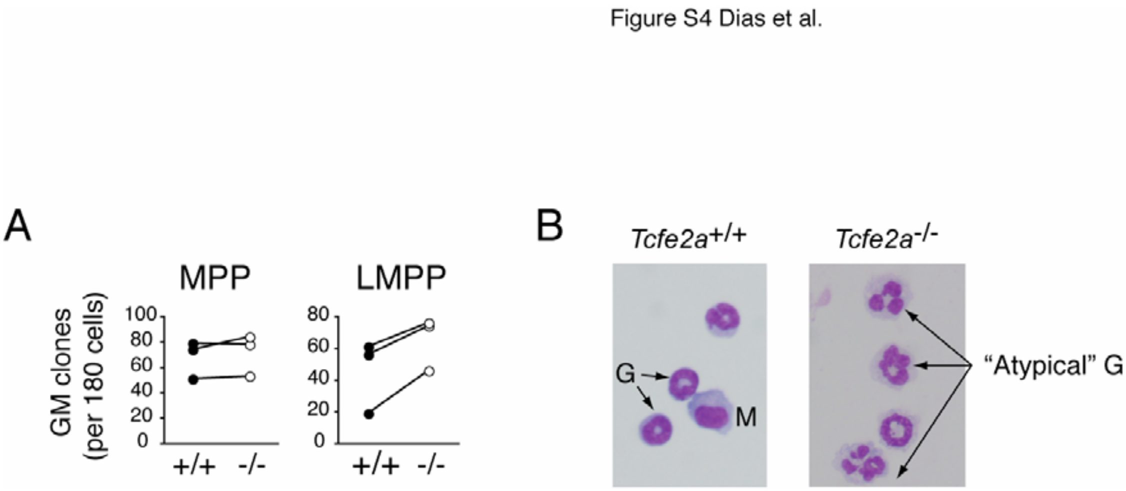


Figure S4. *Tcfe2a*$^{-/-}$ LMPPs have a higher GM clonogenic potential than *Tcfe2a*$^{+/+}$ LMPPs.

(A) Total number of GM colonies generated per 180 single MPPs or LMPP plated from mice of the indicated *Tcfe2a* genotype. Data from 3 independent experiments is shown. (B) Wright-Giemsa staining of a GM *Tcfe2a*$^{+/+}$ (left) and *Tcfe2a*$^{-/-}$ (right) clone. G = granulocyte; M = macrophage. *Tcfe2a*$^{-/-}$ derived G frequently have an atypical morphology consistent with apoptosis.

Table S1. Surface markers for the progenitor populations studied.

	Population	Markers	Lineage
Thymus	ETP	**Lin^{-}*c-kit*^{high}CD25^{-}**	**CD3ε, CD8α, TCRβ, TCRγδ, NK1.1, CD11c, Ter119, CD11b, Ly-6G, B220, CD19**
	DN2	**Lin^{-}*c-kit*^{high}CD25^{+}**	
	DN3	**Lin^{-}*c-kit*$^{-}$CD25^{+}**	
Bone Marrow	HSC	**LSK Flt3^{-}**	**CD3ε, CD4, CD8α, NK1.1, Ter119, CD11b, Ly-6G, B220**
	MPP	**LSK Flt3^{low}**	
	LMPP	**LSK Flt3^{high}**	
	CLP	**LS^{low}K^{low} IL-7Rα^{+}Flt3^{+}**	
	CMP	**LS^{-}K FcgRII/III^{low}CD34^{+}**	**CD3ε, CD4, CD8α, NK1.1, Ter119, Ly-6G, B220, CD19, IgM, IL-7Rα**
	MEP	**LS^{-}K FcgRII/III^{low}CD34^{-}**	
Fetal Liver	MPP	**Lin^{-}*c-kit*^{high}CD27^{+}**	**Ter119, Ly-6G**

Antibody clones: CD3ε, CD4 (GK1.5), CD8α (53-6.7), TCRβ (H57-597), TCRγδ (UC7-13D5), NK1.1 (PK136), CD11c (HL3), Ter-119, FcγIII/IIR (2.4G2), CD11b (M1/70), Ly-6G (RB6-8C5), B220 (RA3-6B2), CD19 (1D3), IgM (R6-60.2), IL-7Rα (A7R34), CD25 (PC61.5), CD27 (LG. 3A10), c-*kit* (2B8), Sca-1 (D7), Flt3 (A2F10), CD34 (RAM34).

Table S2. QPCR primers.

mGene	**Sequence**
Hprt	**For 5’ ACCTCTCGAAGTGTTGGATA** **Rev 5’ CAACAACAAACTTGTCTGGA**
Ikzf1	**For 5’ CAATGTCGCCAAACGTAAGA** **Rev 5’ GTTGATGGCATTGTTGATGG**
Sfpi1	**For 5’ GCTTCCCTTATCAAACCTTGTCCC** **Rev 5’ GGCGAATCTTTTTCTTGCTGC**
Gfi1	**For 5’ TCCGAGGGTCCAAACATCG** **Rev 5’ TTGAAAGGCAGCGTGTAGGG**
Notch1	**For 5’ GCATTGATGATGTCGCTGGATAC** **Rev 5’ GCATACCCCGCTGTTTTT**
Ccr9	**For 5’ CAATCTGGGATGAGCCTAAACAAC** **Rev 5’ ACCAAAAACCAACTGCTGCG**