ZEB2 and LMO2 drive immature T-cell lymphoblastic leukemia via distinct oncogenic mechanisms

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Supplementary Information

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Supplementary Figures and Legends S1-2

Supplementary Tables S1-2

Supplemental Figure S1

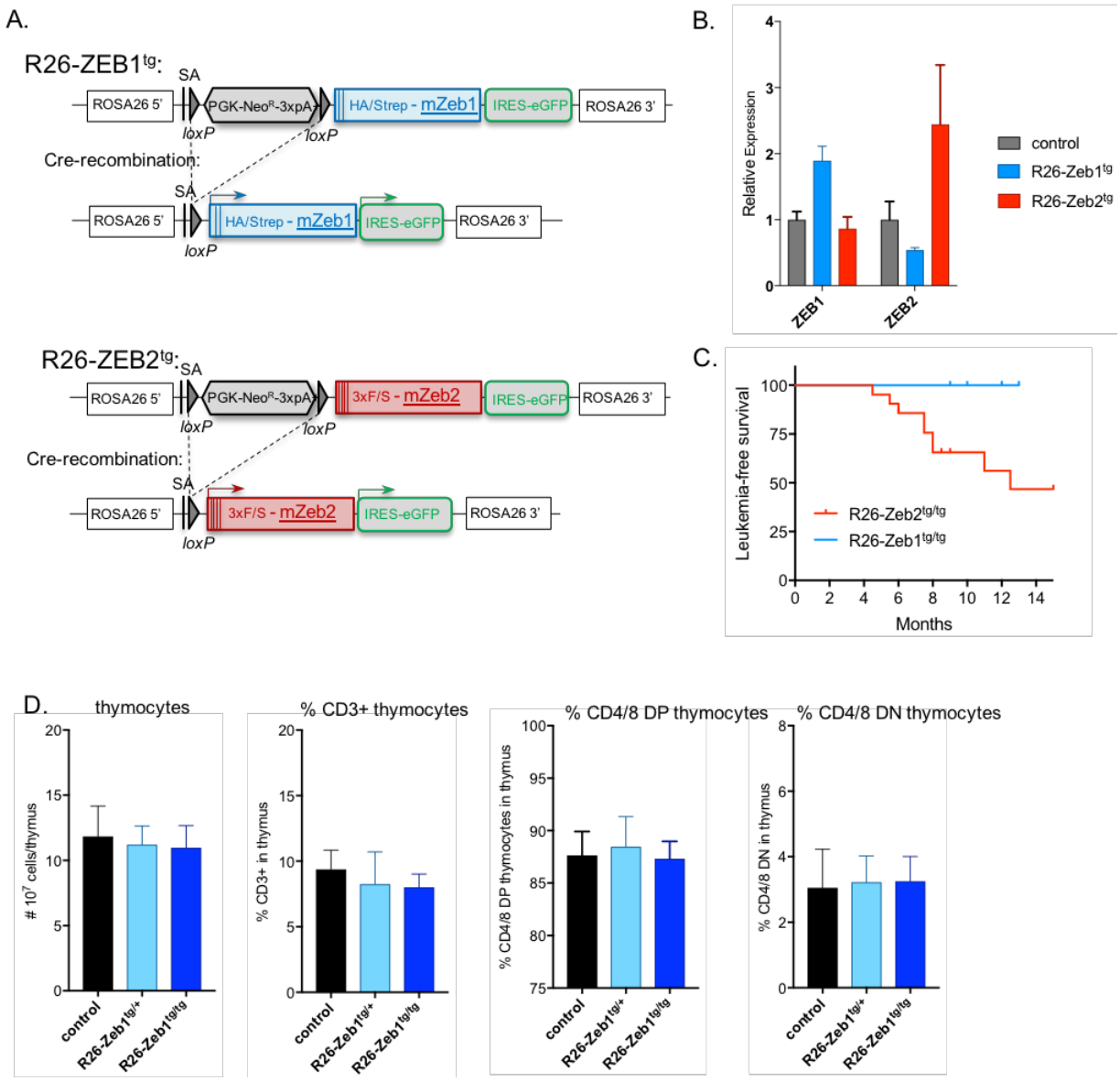

Figure S1: **Zeb1 overexpression does not delay T-cell differentiation and does not lead to T-ALL development** (A) ROSA26 targeting strategies used to generate the conditional *R26-Zeb1*^{tg} and *R26-Zeb2*^{tg} overexpression mice (B) When intercrossed with the Tie2-cre line, this results in a similar moderate (2-3 fold) increase in total Zeb1/2 mRNA levels within the thymus, compared to their littermate Cre-negative controls (n=3/group), as demonstrated by qPCR. (C) Kaplan Meier leukemia-free survival curves comparing *R26-Zeb1*^{tg}(n=15) versus *R26-Zeb2*^{tg} (n=21) mice (D) Flow cytometric analysis of thymus of *Zeb1* overexpressing mice versus control littermates. Absolute numbers of total thymocytes and percentage of CD3, CD4/8 double positive (DP) and CD4/8 double negative cell (DN) populations are shown (n=5/group).

Supplemental Figure S2

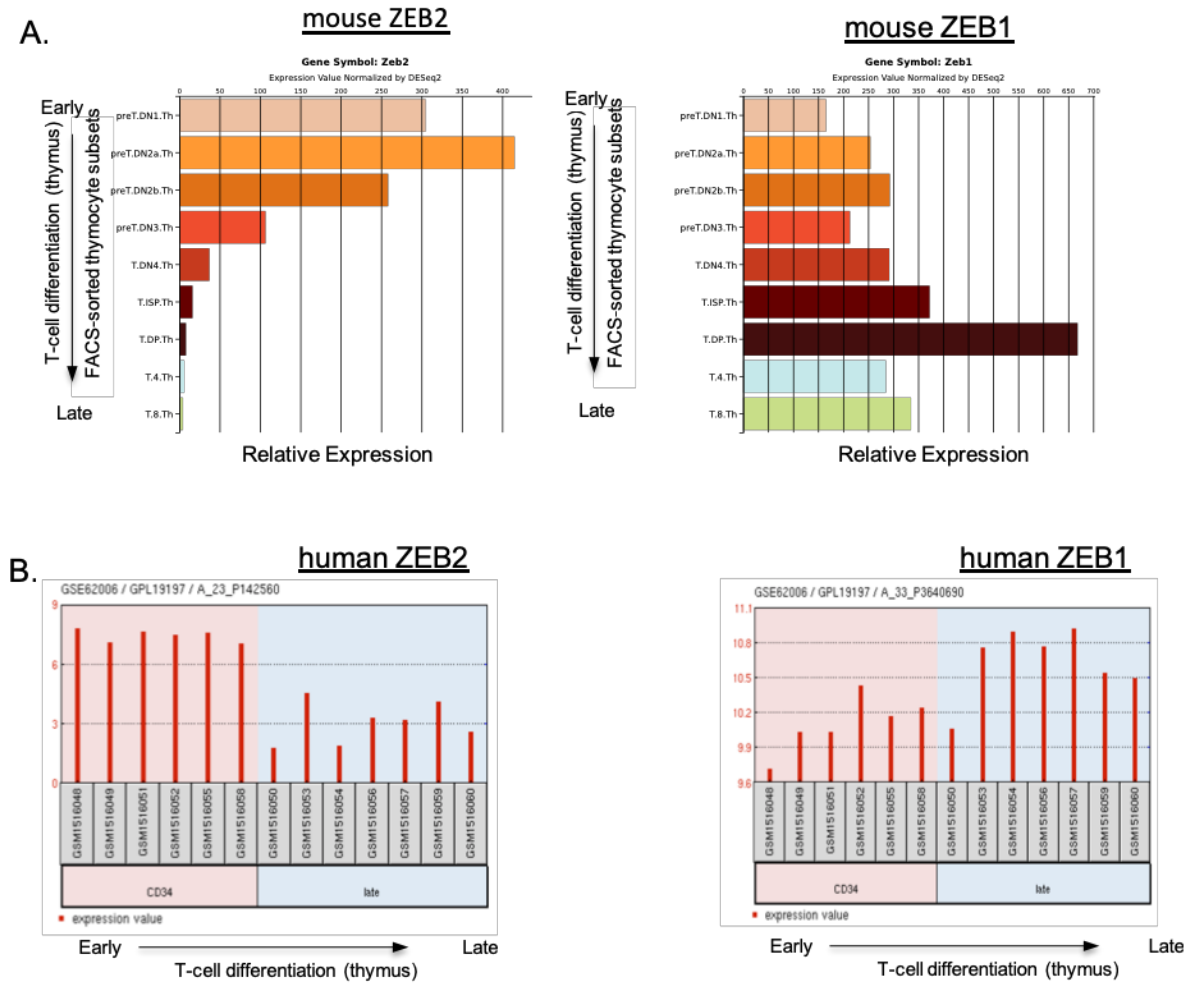

Figure S2: Zeb1 and Zeb2 are differentially expressed during early T-cell differentiation. (A) Publicly available mRNA expression data for *Zeb1* and *Zeb2* in FACS sorted T-cell differentiation subsets from normal mouse thymus. http://rstats.immgen.org/Skyline/skyline.html (B) Publicly available mRNA expression data for *ZEB1* and *ZEB2* in FACS sorted T-cell differentiation cell subsets from normal human thymus (Ref: Durinck, K., et al., The Notch driven long non-coding RNA repertoire in T-cell acute lymphoblastic leukemia. Haematologica, 2014, **99**(12). p.1808-16)

Supplementary Table 1. Antibodies used for FACS and flow cytometry

Antigen	Conjugated	dilution	Company	Experiment
Lineage: Gr-1	Biotin	2μl:10^6 cells	eBioscience	FACS sort
Lineage: CD3e	Biotin	2μl:10^6 cells	eBioscience	FACS sort
Lineage: B220	Biotin	2μl:10^6 cells	eBioscience	FACS sort
Lineage: Ter119	Biotin	2μl:10^6 cells	eBioscience	FACS sort
Streptavidin	PE	1:500	BD Bioscience	FACS sort
cKit/CD117	APC	1:200	Immunoscource	FACS sort + flow cytometry
cKit/CD117	APC.H7	1:100	BD Bioscience	Flow cytometry
CD4	Biotin	1:200	BD Bioscience	Flow cytometry
CD4	AlexaFluor700	1:100	eBioscience	Flow cytometry
CD8a	PE.Cy7	1:100	eBioscience	Flow cytometry
CD8a	PerCP.Cy5.5	1:250	BD Bioscience	Flow cytometry
CD3e	V500	1:100	BD Bioscience	Flow cytometry
CD3e	PE.Cy7	1:100	eBioscience	Flow cytometry
CD25	PerCP.Cy5.5	1:100	BD Bioscience	Flow cytometry
CD25	PE.Cy7	1:250	BD Bioscience	Flow cytometry
CD44	APC	1:100	eBioscience	Flow cytometry
CD44	APC.Cy7	1:250	BD Bioscience	Flow cytometry
CD28	APC	1:100	Biolegend	Flow cytometry
Thy1/CD90.2	FITC	1:100	BD Bioscience	Flow cytometry
Thy1/CD90.2	V500	1:250	BD Bioscience	Flow cytometry
Streptavidin	V500	1:200	BD Bioscience	Flow cytometry
Streptavidin	eFluor780	1:200	eBioscience	Flow cytometry
Streptavidin	PE-Texas Red	1:500	BD Bioscience	Flow cytometry
CD45.1	APC.Cy7	1:250	Biolegend	Flow cytometry
CD45.2	PE	1:250	Biolegend	Flow cytometry

Supplementary Table 2. Primers sequences used for qPCR

GENE	forward primer	reverse primer
beta-actin	5'-AGTGTGACGTTGACATCCGTA-3’	5’-GCCAGAGCAGTAATCTCCTTCT-3’
Gapdh	5’-AGGTTGTCTCCTGCGACTTCA-3’	5’-GGTGGTCCAGGGTTTCTTACTC-3’
Rpl13	5’-CCTGCTGCTCTCAAGGTTGTT-3’	5’-TGGTTGTCACTGCCTGGTACTT-3’
Tbp	5’-TCTACCGTGAATCTTGGCTGTAAA-3’	5’-TTCTCATGATGACTGCAGCAAA-3’
Zeb2	5’-AGCGACACGGCCATTATTTAC-3’	5’-GTTGGGCAAAAGCATCTGGAG-3’
Zeb1	5’-TTGCGTGTCAGGCATGGAT-3’	5’-GAAAACGGCTGTGAACCAAAA-3’