

Fig. S1. Gating strategies used to resolve thymocytes progenitors, HSCs and MPPs from URE Δ/Δ , URE $\Delta/+$ and B6 mice and expression level of *Spi1* in fetal URE Δ/Δ and URE $\Delta/+$ ETPs. (A) Representative FACS plots showing the successive gating steps used to define Lineage negative (Lin⁻) CD117⁺ CD44⁺ CD25⁻ ETPs (which can be further subdivided using anti-CD135 and CD127 antibodies), Lin⁻ CD44⁺ CD25⁺ DN2, Lin⁻ CD44⁻ CD25⁺ DN3 and Lin⁻ CD44⁻ CD25⁺ DN4 thymocytes in 9 wk old B6 thymus. Thymuses from 3 mice were pooled for this particular stain. (B) Representative FACS plots showing the successive gating steps used to define Lineage negative (Lin⁻) CD117⁺ CD150⁺ HSCs and Lin⁻ CD117⁺ CD150⁻ MPPs in the bone marrow of 5 wk old URE $\Delta/+$ mice. Bone marrow cells from 3 mice were pooled for this particular stain. A simplified HSC staining strategy was used to stain/purify HSCs in this study, because URE Δ/Δ bone marrow cells do not express CD135 (30). (C) Expression of *Spi1* relative to *Gapdh* determined by qPCR in HSCs and MPPs isolated from 5wk old URE Δ/Δ and WT mice. qPCR reactions were performed in triplicate using 2 independent samples for each ETP population. Mean \pm SEM and p values are shown.

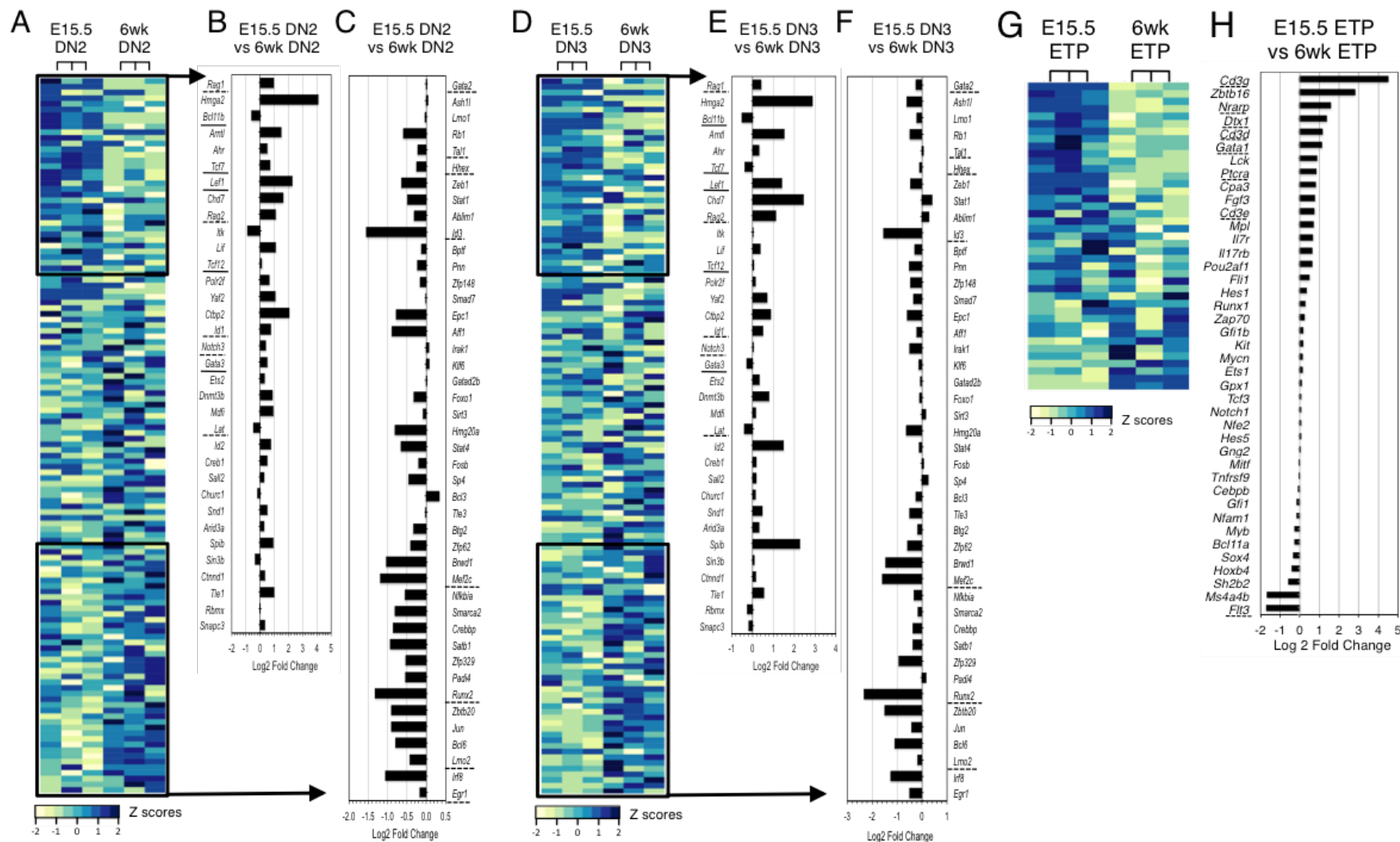


Fig. S2. Expression of key T lineage transcription factors differs between fetal and adult DN2, DN3 and ETP progenitors. (A) Heatmap showing the expression of selected transcription factors in DN2 progenitors isolated from E15.5 and 6 wk old B6 mice. (B) Log₂ fold change of the transcription factors whose expression is higher in fetal than adult DN2 cells (E15.5 versus 6 wk). (C) Log₂ fold change of the transcription factors whose expression is lower in fetal than adult DN2 cells (E15.5 versus 6 wk). (D) Heatmap showing the expression of selected transcription factors in DN3 progenitors isolated from E15.5 and 6 wk old B6 mice. (E) Log₂ fold change of the transcription factors whose expression is higher in fetal than adult DN3 cells (E15.5 versus 6 wk ratios). (F) Log₂ fold change of the transcription factors whose expression is lower in fetal than adult DN3 cells (E15.5 versus 6 wk ratios). (G) Heatmap showing the expression of PU.1 target genes in ETPs isolated from E15.5 and 6 wk old B6 mice. (H) Log₂ fold change of these genes in ETPs isolated from E15.5 embryos versus 6 wk old mice. Log₂ intensities were obtained from microarray data deposited by Belyaev et al (GSE24142) (23). The complete gene list is included in Supplemental Table 2. Dark blue: high expression levels; pale green: low expression levels. All genes were included in the 4,970 probes that passed the $p < 0.05$ filter following ANOVA test as described by Belyaev et al. (23). Z-scores are shown for 3 independent samples for each mouse age.

Supplemental Table 1: List of antibodies used for isolation of hematopoietic cells*.

	<i>Antibody</i>	<i>Clone</i>	<i>Source</i>
<i>Fcγ Blocking</i>	CD16/32	FcγRII-III; 93	Life Technologies
<i>Lineage cocktail</i>			
	CD3α	145-2C11	Life Technologies
	CD8α	53-6.1	Life Technologies
	Gr-1	RB6-8C5	Life Technologies
	IgM	—	Southern Biotechnology
	NK1.1	PK136	Life Technologies
	TCRβ	H57-597	Life Technologies
	TCRγδ	UC7-13D5	Life Technologies
	Ter-119	Ter-119	Life Technologies
	CD11b	M1/70	Life Technologies
<i>HSC / MPP</i>			
	CD117	2B8	Life Technologies
	CD150	TCI5-12F12.2	Biolegend
	Sca-1	D7	Life Technologies
	CD135	A2F10	Biolegend
<i>ETP / DN2 / DN3 / DN4</i>			
	CD117	2B8	Life Technologies
	CD44	IM7	BD Biosciences
	CD25	PC61.5	Life Technologies
	CD135	A2F10	Biolegend
<i>CD4 / CD8</i>			
	CD4	L3T4/GK1.5/RM4-5	Life Technologies
	CD8α	53-6.7	Life Technologies

* Optimal working dilutions of all antibodies were determined before use. *HSC*, Hematopoietic stem cell; *MPP*, Multipotent progenitor; *ETP*, Early Thymic Progenitor.