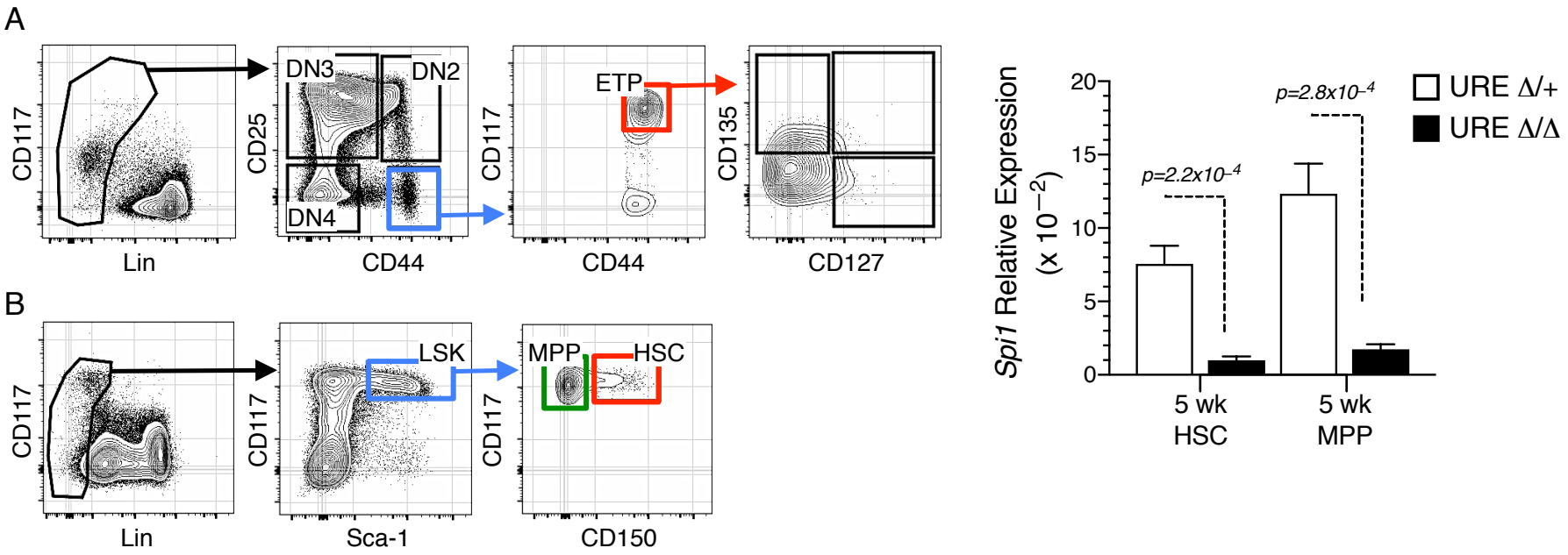

Fig. S1. Gating strategies used to resolve thymocytes progenitors, HSCs and MPPs from UREΔ/Δ, UREΔ/+ and B6 mice and expression level of *Spi1* in fetal UREΔ/Δ and UREΔ/+ ETPs. (**A**) Representative FACS plots showing the successive gating steps used to define Lineage negative (Lin^-) $CD117^+$ $CD44^+$ $CD25^-$ 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 UREΔ/+ 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 UREΔ/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 UREΔ/Δ and WT mice. qPCR reactions were performed in triplicate using 2 independent samples for each ETP population. Mean ± SEM and p values are shownare shown.

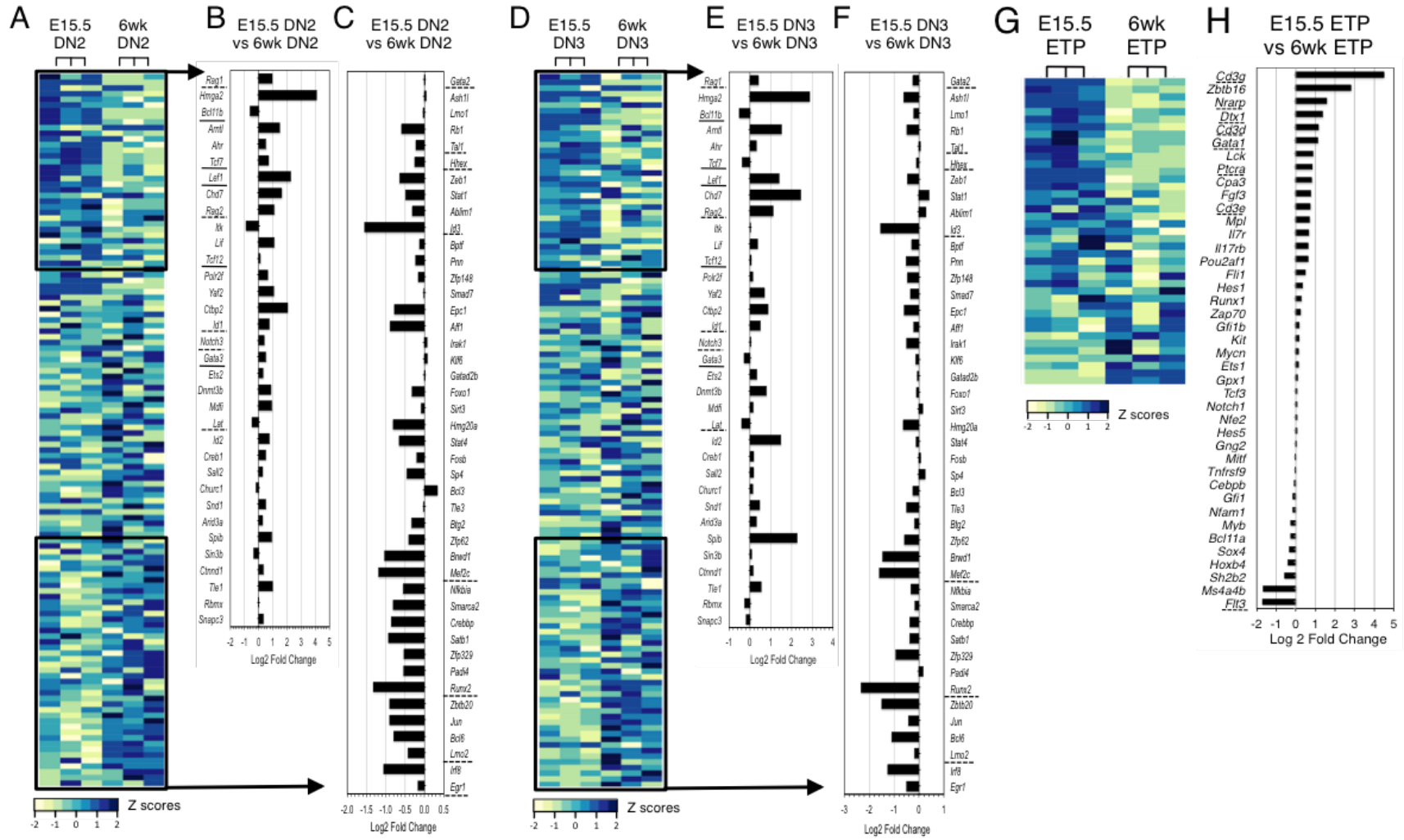

Fig. S2. **Expression of key T lineage transcription factors differs between fetal and adult DN2, DN3 and ETPprogenitors.** (**A**) Heatmap showing the expression of selected transcription factors in DN2 progenitors isolated from E15.5 and 6 wk old B6 mice. (**B**) Log_2 fold change of the transcription factors whose expression is higher in fetal than adult DN2 cells (E15.5 versus 6 wk). (**C**) Log_2 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**) Log_2 fold change of the transcription factors whose expression is higher in fetal than adult DN3 cells (E15.5 versus 6 wk ratios). (**F**) Log_2 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**) Log_2 fold change of these genes in ETPs isolated from E15.5 embryos versus 6 wk old mice. Log_2 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*.**

	Antibody	*Clone*	*Source*
Fcγ Blocking	CD16/32	FcγRII-III; 93	Life Technologies
Lineage cocktail			
	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
HSC / MPP			
	CD117	2B8	Life Technologies
	CD150	TCI5-12F12.2	Biolegend
	Sca-1	D7	Life Technologies
	CD135	A2F10	Biolegend
ETP / DN2 / DN3 / DN4			
	CD117	2B8	Life Technologies
	CD44	IM7	BD Biosciences
	CD25	PC61.5	Life Technologies
	CD135	A2F10	Biolegend
CD4 / CD8			
	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.