



Fig. S1. Development of human CD45+ cells within a grafted human fetal RFTOC. (A-C') Thymic primordia were microdissected free of connective tissue from two week 8 human fetuses, dissociated to single cell suspensions, and then reaggregated together with mouse primary embryonic fibroblasts as described in the methods. This cellular reaggregate was grafted under the kidney capsule of a NOD/SCID mouse. The graft was recovered after 2 weeks and analyzed by immunohistochemistry with the antibodies shown. (A) Pancytokeratin (panK) and MHC Class II (Class II); (B) Pancytokeratin (panK) and UEA1; (C) Pancytokeratin (panK) and human CD45. C' shows higher magnification images showing detail from C. (D) Positive and negative control staining with anti-human CD45. Left panels, adult mouse thymus; right panels, 17 week human fetal thymus. (E) Positive and negative control staining with anti-human MHC Class II (HLA-DR). Left panel, adult mouse thymus, right panel, 15 week human fetal thymus. PanK, pancytokeratin.

Primer name	Primer sequence
FOXN1F	5' GAAACCTGTGGGAACAGTTG3'
FOXN1R	5' ACTTCCAGACCAGGCAAACG 3'
HOXA3F	5' CTGGAATGAAAGAGTCGCCACC 3'
HOXA3R	5' CCAGCGAATGCATAGAGTTC 3'
PAX1F	5' CCAACGTGGTCAAGCACATC 3'
PAX1R	5' CGAAGGCAGGTTTCTCTAGC 3'
PAX9F	5' CCAGCAGGGTCAGGACGACT 3'
PAX9R	5' ATGCTGGATGCTGACACAAA 3'
BMP4F	5' GAAGCTAGGTGAGTGTGGC 3'
BMP4R	5' AGACCAGTGCTGTGGATCTG 3'
BMP2F	5' GTTGTGTGTCAGCACTTGGC 3'
BMP2R	5' TCGGATACAGGTCTAGCATG 3'
TBX1F	5' TGCAGCTAGAGATGAAGGCG 3'
TBX1R	5' CAATCTTGAGCTGCGTGATA 3'
CLDN4 F	5' CCCCAGCGCTTGGAAATCC 3'
CLDN4R	5'TGGATGATGTTGTGGGCCG3'
P63F	5'AGGAAGGCGGATGAAGATAGC3'
P63R	5'AGGAATGGTTGTAGGAGTGAG3'

Table S1. Primer sequences used to amplify *in situ* hybridization probes. All probes were made to 5' UTR regions. F, forward. R, Reverse.

Affymetrix identifier	Gene symbol	CB094		CB106		CB388	
		mas5 flag	RMA expression rank	mas5 flag	RMA expression rank	mas5 flag	RMA expression rank
205051_s_at	CD117 (c Kit)	P	14420	A	17793	P	10630
205798_at	IL7R	A	31037	M	22399	P	9679
212588_at	PTPRC (CD45)	P	25795	P	10471	P	1658
206591_at	RAG1	A	32543	A	31822	P	16451
215492_x_at	PTCRA	A	17682	A	17021	P	17525
213539_at	CD3D	M	21878	P	11721	P	3121
213193_x_at	TRBC1	P	27612	P	19363	P	6372
215806_x_at	TARP TRGC2	A	31092	P	24829	P	11009
201839_s_at	EPCAM	P	1187	P	3308	P	2468

Table S2. Expression of genes associated with early human thymocyte development in mid-week 8 human thymic lobes. RNA was prepared from three independent mid-week 8 thymic lobes microdissected free of any other cells or tissues (CB094, CS20; CB388, CS21/22; CB106, CS22) and was processed for microarray analysis as described in Materials and methods. Note, the name of each sample includes both an identifier (e.g. CB094) and the Carnegie stage of that sample (e.g. CS20). The microarray data were normalized independently using RMA and MAS5, and the probe sets were then ranked based on the resulting expression values, with the probe set having the highest expression given rank 1. The data presented show ranks for selected probe sets along with the corresponding MAS5 PMA flag [present (P), marginal (M) and absent (A)]. For a given probe set the lower the rank, the higher the probability of the gene being present in the sample, whereas MAS5 predicts the presence or absence of a gene using different statistics. Data are presented for selected genes involved in early human thymocyte development and for EPCAM, which marks all thymic epithelial cells in the thymic primordium. CD117, c-KIT; IL7R, interleukin 7 receptor; PTPRC, protein tyrosine phosphatase receptor type C (CD45); RAG1, recombination activating gene 1; PTCRA, pre-T cell antigen receptor alpha; CD3D, CD3 delta chain; TRBC1, T cell receptor beta constant region 1; TARP/TRGC2, TCR gamma alternate reading frame protein/T cell receptor gamma constant region 2; EPCAM, epithelial cell adhesion molecule. CS, Carnegie stage. Note that pre-Ta and RAG1 expression is detected only in the oldest of the three thymic primordia.

	Human	Mouse	Human	Mouse	Human	Mouse
	Wk 6	E10-E11	Wk7	E11-E12	Wk8	E12-E13
Genes	Pharyngeal endoderm and thymus organogenesis					
HOXA3	+	+	NT	+	NT	+
TBX1	+	+	NT	+	NT	+
PAX1	+	+	NT	+	NT	+
PAX9	+	+	NT	+	NT	+
FOXP1	+	+	NT	+	+	+
BMP4	+	+	NT	+	NT	+
BMP2	+	+	NT	+	NT	+
P63	+	+	NT	+	+	+
PLET1	-	+	-	+	-	+
RHOX4	-	+	-	+	-	+
	Thymic epithelial cells					
EPCAM	NT	+	+	+	+	+
CLDN4	NT	+	+	+	+	+
UEA1	NT	-	+	-	+	-
MHC Class II	NT	-	-	-	+	+
Keratin 5	NT	+	+	+	+	+
Keratin 8	NT	+	+	+	+	+
CDR2	NT	NT	-	HS	+	HS
CD205	NT	NT	-	-	+	+
	Chemokine expression by thymic epithelial cells					
CCL21			+	+	+	+
CCL25			+	+	+	+
	Hematopoietic cells					
CD45	-	-	+	+	+	+
CD34	-		-		+	
CD7	-		-		+	
MAC1	-	-	NT	+	+	+
	Mesenchymal and endothelial cells					
TE7			+	HS	+	HS
CD34			+		+	
CD31			+	+	+	+
VE Cadherin			+	+	+	+

Table S3. Summary of comparative analysis of human and mouse thymus development. The table summarizes the data shown in Figs 1-7. The time of onset of TEC differentiation is week 8, defined by the first point at which markers restricted to prospective cortical and medullary TEC are evident. 1, time of onset of expression; 2, time at which expression restricted to medullary-fated TECs; 3 time at which expression restricted to cortical-fated TECs; 4, time of initial colonization. *Rare intrathymic CD45+ cells present at week 7. **E11.25-E11.5. Shading of adjacent columns indicates equivalent stages of thymus development. HS, human specific; NT, not tested.