The transcriptional landscape of cTEC and mTEC^{hi}.

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The thymus is the site of positive and negative selection, the processes by which lymphocytes are selected for that are capable, in their T-cell receptors, of adequately recognizing foreign antigen, and selected against in the event that, by their T-cell receptors, they recognize self antigen (1-16). It is anatomically segregated into a medulla and cortex, and epithelial cells in both the medulla and cortex function in the processes of negative and positive selection but there are limited studies assessing in an unbiased, systematic manner their relative basic transcriptional natures (17-26). In this study we compared the transcriptomes of cortical epithelial cells (cTEC) and medullary epithelial cells expressing high levels of the class II major histocompatibility complex (MHC-II) (mTEC^{hi}) (27). We previously described the major transcription factors and epigenetic machinery that uniquely describe cTEC and mTEC^{hi} (28). Here, using a published dataset, we report a series of modules consisting of cell surface receptors, Fbox proteins, proteasomal components, cytochrome P450 components, ATP-related machinery of the lysosome and mitochondria, and matrix metalloproteinases that, by their differential expression, uniquely describe cTEC and mTEC^{hi}. These data will serve as a resource for future efforts towards molecular engineering of the thymus for transplant medicine and targeted treatment of autoimmunity.

Keywords: thymus, medulla, cortex, medullary epithelial cells, cortical epithelial cells, mTEC, cTEC, transcriptome analysis of the immunologically active cells of the thymus, systems analysis of the thymus, positive selection, negative selection.

Introduction

The ability to discern what is self and what is foreign is essential for the immune system to be able to adequately protect the human body from pathogens and at the same time to prevent “friendly fire”, or the recognition of the thousands of self antigens the body possesses as foreign (1-16). The site at which this ability is developed is known as the thymus, an organ in the chest of every human. The thymus possesses a thymus and medulla (3, 4), and epithelial cells in the cortex of the thymus (cTEC) participate in positive selection (1, 2, 8-13, 15), the process by which lymphocytes expressing T-cell receptors that possess an optimal binding affinity for MHC-peptide receptor complexes are selected for, while epithelial cells in the medulla of the thymus (mTEC^{hi}) participate in negative selection, the process by which lymphocytes that express T-cell receptors that have the potential to recognize self-antigen in complex with MHC-II are deleted so as to prevent autoimmune reactions (1, 2, 5, 7, 14).

The general transcriptional behavior of mTEC^{hi} and cTEC have been explored to some extent (17-26), but there are limited unbiased, systematic analyses of their transcriptional landscapes: how they relate to each other, and how they most differ from each other. In this study, we performed global differential gene expression analysis by comparing the transcriptomes of mTEC^{hi} and cTEC isolated from the murine thymus using a published dataset (27). We found a discrete gene expression program consisting of modules that uniquely identified the mTEC^{hi} and cTEC lineages. This program included Fbox genes with opposing expression in either lineage, multiple proteasomal genes, centromere proteins, cell surface receptors, major histocompatibility complex genes, ATP-related genes, matrix metalloproteinases, cell

cycle genes and others. These data will serve as a resource for future efforts aimed at engineering the thymus for novel therapeutics aimed at curbing transplant rejection and autoimmune responses across tissues.

Methods

GSE89965 (27) was utilized for this differential gene expression analysis, performed using GEO2R. This public dataset was generated by Jakub Abramson using microarray (Affymetrix Mouse Gene 1-ST array) analysis of Aire-GFP, EpCAM+Ly51+ cells (cTEC), Aire-GFP Epcam+MHC-II(hi) cells (mTEC^{hi}); $n=4$ from mTEC^{hi}; $n=3$ from cTEC, from C57Bl/6 Adig mice 6 weeks of age (adult). No adjustment was made to p -values, and there was no log-transformation applied to the data. The NCBI generated category of platform annotation was used. An unpaired two-tailed t-test with Welch's correction was utilized to determine the statistical significance of the RNA expression values between each cell type.

Results

We performed global gene expression profiling of medullary and cortical epithelial cells isolated from the murine thymus (27) to systematically compare the transcriptomes of cells participating in negative and positive selection. Using this analysis, we identified the most significant differences in the transcriptional landscapes of the mammalian mTEC^{hi} and cTEC.

Fbxl15 and Fbxl17 expression uniquely define the mTEC^{hi} and cTEC lineages

Fbxl15, F-box and leucine-rich repeat protein 15, was the 9th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=4.9E-07$), while Fbxl17, F-box and leucine-rich repeat protein 17 was the 62nd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=5.11E-06$).

Fbxl15 and Fbxl17 expression opposed each other, with higher expression of Fbxl15 in cTEC (Figure 1; $p=0.0012$) and higher expression of Fbxl17 in mTEC^{hi} (Figure 1; $p<0.0001$).

Il2rg expression uniquely defines the mTEC^{hi} lineage from the cTEC lineage

The gamma chain of the interleukin 2 receptor, Il2rg, was the 46th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=3.5E-06$). Expression of Il2rg was significantly higher in mTEC^{hi} than in cTEC (Figure 2; $p=0.0003$).

Scarf2, Csf2ra, Tnfrsf11a, Skint7 expression uniquely define the mTEC^{hi} and cTEC lineages.

Scarf2, scavenger receptor class F, member 2, was the 53rd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=4.26E-06$). Expression of Scarf2 was significantly higher in cTEC than in mTEC^{hi} (Figure 3; $p=0.0030$).

Csf2ra, colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage), was the 110th most differentially expressed gene when comparing the

mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.01E-05$). Expression of *Csf2ra* was significantly higher in cTEC than in mTEC^{hi} (Figure 3; $p<0.0001$).

Tnfrsf11a, tumor necrosis factor receptor superfamily, member 11a, NFkB activator, was the 224th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.62E-05$). Expression of *Scarf2* was significantly higher in mTEC^{hi} than in cTEC (Figure 3; $p=0.0002$).

Skint7, selection and upkeep of intraepithelial T cells 7, was the 9th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=4.99E-06$). Expression of *Skint7* was significantly higher in mTEC^{hi} than in cTEC (Figure 3; $p=0.0016$).

Bmp8a and Wnt4 expression uniquely define the cTEC lineage from the mTEC^{hi} lineage.

Bmp8a, bone morphogenetic protein 8a, was the 65th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=5.29E-06$). Expression of *Bmp8a* was significantly higher in cTEC than in mTEC^{hi} (Figure 4; $p<0.0001$).

Wnt4, wingless-type MMTV integration site family, member 4, was the 36th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.6E-06$). Expression of *Wnt4* was significantly higher in cTEC than in mTEC^{hi} (Figure 4; $p=0.0002$).

Psme1, *Psma6* and *Psmd6* expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

Psme1, proteasome (prosome, macropain) activator subunit 1 (PA28 alpha), was the 41st most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=3.21E-06$). Expression of Psme1 was significantly higher in mTEC^{hi} than in cTEC (Figure 5; $p<0.0001$).

Psmd6, proteasome (prosome, macropain) 26S subunit, non-ATPase, 6, was the 155th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.55E-05$). Expression of Psma6 was significantly higher in mTEC^{hi} than in cTEC (Figure 5; $p<0.0001$).

Psma6, proteasome (prosome, macropain) subunit, alpha type 6, was the 187th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2E-05$). Expression of Psmd6 was significantly higher in mTEC^{hi} than in cTEC (Figure 5; $p=0.0051$).

Cep350 and Cep162 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage

Cep350, centrosomal protein 350, was the 98th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=8.91E-06$). Expression of Cep350 was significantly higher in mTEC^{hi} than in cTEC (Figure 6; $p<0.0001$).

Cep162, centrosomal protein 162, was the 48th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=3.63E-06$). Expression of Cep162 was significantly higher in mTEC^{hi} than in cTEC (Figure 6; $p<0.0001$).

A (Nonagouti) expression uniquely defines the cTEC lineage from the mTEC^{hi} lineage.

A (nonagouti), was the 78th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=6.53E-06$). Expression of A (nonagouti) was significantly higher in cTEC than in mTEC^{hi} (Figure 7; $p<0.0001$).

Cyp4f39, Cyp1a2, and Cyp2w1 expression uniquely define the mTEC^{hi} and cTEC lineages.

Cyp1a2, cytochrome P450, family 1, subfamily a, polypeptide 2, was the 45th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=3.49E-06$). Expression of Cyp1a2 was significantly higher in mTEC^{hi} than in cTEC (Figure 8; $p=0.0002$).

Cyp2w1, cytochrome P450, family 2, subfamily w, polypeptide 1, was the 138th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.31E-05$). Expression of Cyp2w1 was significantly higher in cTEC than in mTEC^{hi} (Figure 8; $p<0.0001$).

Cyp4f39, cytochrome P450, family 4, subfamily f, polypeptide 39, was the 173rd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.84E-05$). Expression of Cyp4f39 was significantly higher in mTEC^{hi} than in cTEC (Figure 8; $p=0.0005$).

Atp6v1b2, Atp6v01a, and Atp5f1 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

Atp5f1, ATP synthase, H⁺ transporting, mitochondrial F0 complex, subunit B1, was the 135th most differentially expressed gene when comparing the mTEC^{hi} and

cTEC transcriptomes (Table 1; $p=1.27E-05$). Expression of Atp5f1 was significantly higher in mTEC^{hi} than in cTEC (Figure 9; $p<0.0001$).

Atp6v1b2, ATPase, H⁺ transporting, lysosomal V1 subunit B2, was the 167th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.71E-05$). Expression of Atp6v1b2 was significantly higher in mTEC^{hi} than in cTEC (Figure 9; $p=0.0005$).

Atp6v01a, ATPase, H⁺ transporting, lysosomal V0 subunit A1, was the 169th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.76E-05$). Expression of Atp6v01a was significantly higher in mTEC^{hi} than in cTEC (Figure 9; $p<0.0001$).

Dock6 and Dock10 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

Dock10, dedicator of cytokinesis 10, was the 121st most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.11E-05$). Expression of Dock10 was significantly higher in mTEC^{hi} than in cTEC (Figure 10; $p=0.0005$).

Dock6, dedicator of cytokinesis 6, was the 246th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.98E-05$). Expression of Dock6 was significantly higher in mTEC^{hi} than in cTEC (Figure 10; $p=0.0002$).

Vps13a and Vps13d expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

Vps13a, vacuolar protein sorting 13A, was the 52nd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=4.04E-06$). Expression of Vps13a was significantly higher in mTEC^{hi} than in cTEC (Figure 11; $p<0.0001$).

Vps13d, vacuolar protein sorting 13D, was the 232nd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.75E-05$). Expression of Vps13d was significantly higher in mTEC^{hi} than in cTEC (Figure 11; $p=0.0002$).

H2-Ab1, H2-DMb2 , H2-T22 and H2-Aa expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

H2-Aa, histocompatibility 2, class II antigen A, alpha, was the 22nd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.34E-06$). Expression of H2-Aa was significantly higher in mTEC^{hi} than in cTEC (Figure 12; $p=0.0004$).

H2-Ab1, histocompatibility 2, class II antigen A, beta 1, was the 154th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.54E-05$). Expression of H2-Ab1 was significantly higher in mTEC^{hi} than in cTEC (Figure 12; $p=0.0017$).

H2-DMb2, histocompatibility 2, class II, locus Mb2, was the 234rd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.76E-05$). Expression of H2-DMb2 was significantly higher in mTEC^{hi} than in cTEC (Figure 12; $p=0.0001$).

H2-T22, histocompatibility 2, T region locus 22, was the 213th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.51E-05$). Expression of H2-T22 was significantly higher in mTEC^{hi} than in cTEC (Figure 12; $p=0.0004$).

Adam8 and Adam23 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

Adam8, a disintegrin and metallopeptidase domain 8, was the 147th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.39E-05$). Expression of Adam8 was significantly higher in mTEC^{hi} than in cTEC (Figure 13; $p=0.0007$).

Adam23, a disintegrin and metallopeptidase domain 23, was the 199th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=2.25E-05$). Expression of Adam23 was significantly higher in mTEC^{hi} than in cTEC (Figure 13; $p=0.0003$).

Cdca5 and Anapc2 expression uniquely define the mTEC^{hi} lineage and cTEC lineages.

Cdca5, cell division cycle associated 5, was the 183rd most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.98E-05$). Expression of Cdca5 was significantly higher in cTEC than in mTEC^{hi} (Figure 14; $p<0.0001$).

Anapc2, anaphase promoting complex subunit 2, was the 127th most differentially expressed gene when comparing the mTEC^{hi} and cTEC transcriptomes (Table 1; $p=1.18E-05$). Expression of Anapc2 was significantly higher in mTEC^{hi} than in cTEC (Figure 14; $p=0.0005$).

Discussion

In this study we compared the transcriptomes of epithelial cells from the cortex (cTEC) and medulla of the thymus (27); specifically, medullary epithelial cells expressing high levels of the peptide antigen receptor MHC-II (mTEC^{hi}) to understand in an unbiased, systematic fashion the major transcriptional differences between these two cell types that participate in the critical immunological processes of positive and negative selection. We found a discrete gene expression program organized into modules consisting of Fbox genes, proteasome components, ATP-related proteins of the lysosome and mitochondria, matrix metalloproteinases, major histocompatibility genes, genes involved in trafficking of cellular cargoes including vacuolar sorting proteins and Dock proteins, cytochrome oxidases, centromere proteins as well as cell surface receptors whose differential expression uniquely defined the mTEC^{hi} and cTEC lineages.

We previously described a set of epigenetic proteins and transcription factors whose expression uniquely define the mTEC^{hi} and cTEC lineages (28). We are the first to report that opposite expression of Fbox genes Fbxl15 and Fbxl17 mark the cTEC and mTEC^{hi} lineages, respectively. The function of these genes in cTEC and mTEC^{hi} remains to be discerned but analysis of their function in either cell type *in vivo* requires the development of more specific Cre-lox drivers than Foxn1 and $\beta 5t$ as either of these would mark both cTEC and mTEC^{hi}.

One study assessed the level of Psma6 in mTEC^{hi} but did not distinguish between cTEC and mTEC^{hi} in that study and instead measured Psma6 expression by

flow cytometry in the mTEC^{hi} of mice that were doubly deficient in immunoproteasome components Psmb8 and Psmb10 (29).

Unique expression of centromere proteins Cep162 and Cep350 has not previously been reported.

One study has previously reported that Bmp8a could only be detected in spleen, testis, and brain (30). We found here that expression of Bmp8a not only could be detected in the thymus but its differential expression marked cells of the cortex of the thymus. Another study that analyzed expression of Bmp family genes in the thymus of Aire knockout mice found that, unlike other Bmp family genes, the expression of Bmp8a was not different between Aire^{-/-} and Aire wild-type mice but did not assess the expression of Bmp8a in cTEC and mTEC^{hi} (31).

Wnt4 expression in the thymus has been widely reported (32, 33) and it is thought to regulate cellularity in the thymus (34) as well as, in a more artificial setting, protect cells of the thymus against dexamethasone-induced senescence (35). Wnt4 expression in the thymus is reported to decrease upon aging (36). Transduction of fetal thymocytes *in vitro* with Wnt4 results in enhanced survival (32). Deletion of Wnt4 in mice results in a 20-30% decrease in thymocytes in the fetal thymus at E15-16 (34). We also previously reported that Wnt4 expression is higher in mTEC^{lo} than in mTEC^{hi} (37), thus establishing a Wnt4 expression gradient in the thymus, of highest Wnt4 expression in mTEC^{lo}, then in mTEC^{hi}, and lowest Wnt4 expression in cTEC.

Nude mice are a type of immunodeficient mutant mouse that are athymic due to a mutation in the gamma chain of the IL-2 receptor, Il2rg (38). We found that while mTEC^{hi} express relatively high levels of Il2rg, cTEC expression virtually no Il2rg. Thus,

while cTEC essentially lack expression of Il2rg, Il2rg is essential for the development of cells of the thymus including cTEC.

Skint7 is poorly characterized. Skint1 is a specific marker for V γ 5V δ 1+ dendritic epidermal T-cells (39). Skint7 shares 83% homology with Skint8 which was recently shown to negatively regulate T-cell responses *in vitro* and share sequence homology to the B7 family of co-stimulatory molecules (40). We found that differential Skint7 expression marks the mTEC^{hi} lineage.

Tnfrsf11a, or RANK (receptor activator of NF κ B signaling) expression in the thymus has previously been reported (41).

Together with previous findings (37), we demonstrate a graded expression of Scarf2 in the thymus, with highest expression in the cortex, then lower expression in mTEC^{lo}, and the lowest relative expression in mTEC^{hi}.

A (non agouti) expression has not been previously demonstrated in the thymus.

Cyp4f39 expression in the thymus has been reported but not specifically in mTEC or cTEC (42). There has been no functional assignment for this cytochrome protein in cells of the thymus.

We previously reported that Cyp1a2 expression is also relatively very low in the mTEC^{lo} (37). Thus, Cyp1a2 expression uniquely marks mTEC^{hi} from mTEC^{lo} and cTEC.

The expression of Cyp2w1 expression in cTEC has not previously been reported. Interestingly, Cyp2w1 is cited as a biomarker for cancers and its expression is thought to be highest in adrenal glands (43-45).

Atp6v1b2 is a high confidence Foxn1 target gene, supporting our finding that it is differentially expressed in cells of the thymus (46). Recurrent *de novo* misense

mutations of Atp6v1b2 have been found in Zimmerman-Laband Syndrome, a rare genetic disorder featuring craniofacial abnormalities and children with extremely large gums (47).

There is essentially no literature describing any role for Atp6v01a. Atp5f1 expression in the thymus has also not been previously reported.

Dock6 expression has been reported in the epithelium of the thymus in the context of interaction with the Mob1 protein (48). Dock10 has also been reported to be expressed in the thymus (51, 52).

Vps13a has been reported broadly in the thymus but not specifically in mTEC or cTEC (49). Vps13d has been reported as a peptide that eluted from MHC-I complexes and belonging to set of genes possessing a “high thymic Z score” and having expression almost exclusively in hematolymphoid organs (50). A specific function for this cellular transport module in mTEC^{hi} has not been established.

H2-Ab1 is a class II MHC molecule and its expression has been reported in the medullary epithelial cells of the thymus; H2-Aa expression has also been reported in mTEC (5). H2-Dmb2 expression has been reported in the thymus, specifically in mTECs, and its expression is dependent on the Class II transcriptional activator CIITA (5). A study of gamma delta T-cells ablated the entire H2-T locus and found no deficits in T-cell development in the thymus (54).

Adam8 is expressed in the thymic stroma and deficiency in Adam8 leads to hypercellularity in the thymus (55). Adam23 has been reported to possess highest expression in the brain (56), but we could not find locate any literature describing differential expression of Adam23 in mTEC and cTEC.

With respect to the cell cycle module identified here, *Cdca5* expression has been reported in an mTEC cell line but its expression in the medulla or cortex of the mammalian thymus has not specifically been described (57). *Anapc2* expression has been reported in the thymus but not differentially in mTEC^{hi} and cTEC (58).

In conclusion, we demonstrate the existence of several key modules that differentially mark the mTEC^{hi} and cTEC lineages by their relative expression, consisting of transcripts produced by Fbox genes, proteasome genes, genes encoding multiple cell surface receptors, centromere proteins, Adam-family matrix metalloproteinases, ATP-related machinery of the lysosome and mitochondria, cellular transport proteins of the Dock and VPS families, as well as cell cycle proteins. The molecular descriptions provided in this study, by comparing the transcriptomes of mTEC^{hi} and cTEC, will serve as a resource for the specific and targeted engineering of the epithelial cells of the thymus for the treatment of transplant rejection and for the re-establishment of tolerance in autoimmune maladies across tissues.

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Figure 1: Fbxl15 and Fbxl17 expression uniquely define the mTEC^{hi} and cTEC lineages.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 2: Expression of the gamma chain of the IL-2 receptor uniquely defines the mTEC^{hi} lineage from the cTEC lineage.

The expression of Il2rg is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 3: Scarf2, Csf2ra, Tnfrsf11a, Skint7 expression uniquely define the mTEC^{hi} and cTEC lineages.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 4: Bmp8a and Wnt4 expression uniquely define the cTEC lineage from the mTEC^{hi} lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 5: Psme1, Psma6 and Psmd6 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 6: Cep350 and Cep162 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

A (nonagouti)

Figure 7: A (Nonagouti) expression uniquely defines the cTEC lineage from the mTEC^{hi} lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 8: Cyp4f39, Cyp1a2, and Cyp2w1 expression uniquely define the mTEC^{hi} and cTEC lineages.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 9: Atp6v1b2, Atp6v01a, and Atp5f1 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 10: Dock6 and Dock10 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 11: Vps13a and Vps13d expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 12: H2-Ab1, H2-DMb2 and H2-Aa expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 13: Adam8 and Adam23 expression uniquely define the mTEC^{hi} lineage from the cTEC lineage.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Figure 14: *Cdca5* and *Anapc2* expression uniquely define the mTEC^{hi} lineage and cTEC lineages.

The expression of each gene is graphically represented by dots denoting mRNA expression level in each cell sample (mTEC^{hi}: $n=4$; cTEC: $n=3$) with the horizontal black bar representing the mean expression level in each cell type. A statistical test was performed to evaluate the significance of difference between cell type and the result of this test, a p -value, is shown on each graph.

Rank	Symbol	Gene name	p-value	t	B	ID
9	Fbxl15	F-box and leucine-rich repeat protein 15	4.9E-07	23.8775	-3.32	10463625
22	H2-Aa	histocompatibility 2, class II antigen A, alpha	1.34E-06	-20.0652	-3.33	10450154
36	Wnt4	wingless-type MMTV integration site family, member 4	2.6E-06	17.8656	-3.34	10509267
41	Psme1	proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)	3.21E-06	-17.2298	-3.34	10415282
45	Cyp1a2	cytochrome P450, family 1, subfamily a, polypeptide 2	3.49E-06	-16.9802	-3.34	10593981
46	Il2rg	interleukin 2 receptor, gamma chain	3.5E-06	-16.9718	-3.34	10606016
48	Cep162	centrosomal protein 162	3.63E-06	-16.8583	-3.34	10595529
52	Vps13a	vacuolar protein sorting 13A	4.04E-06	-16.5509	-3.34	10466441
53	Scarf2	scavenger receptor class F, member 2	4.26E-06	16.3977	-3.34	10434105
60	Skint7	selection and upkeep of intraepithelial T cells 7	4.99E-06	-15.9489	-3.34	10507059
62	Fbxl17	F-box and leucine-rich repeat protein 17	5.11E-06	-15.8827	-3.34	10452430
65	Bmp8a	bone morphogenetic protein 8a	5.29E-06	15.7872	-3.34	10516093
78	A	nonagouti	6.53E-06	15.2121	-3.35	10477595
98	Cep350	centrosomal protein 350	8.91E-06	-14.4065	-3.35	10359050
110	Csf2ra	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	1.01E-05	14.0961	-3.35	10468893
121	Dock10	dedicator of cytokinesis 10	1.11E-05	-13.8628	-3.36	10356020
127	Anapc2	anaphase promoting complex subunit 2	1.18E-05	-13.7073	-3.36	10469965
135	Atp5f1	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit B1	1.27E-05	-13.5253	-3.36	10386005
138	Cyp2w1	cytochrome P450, family 2, subfamily w, polypeptide 1	1.31E-05	13.4683	-3.36	10526931
147	Adam8	a disintegrin and metallopeptidase domain 8	1.39E-05	-13.3183	-3.36	10568873
154	H2-Ab1	histocompatibility 2, class II antigen A, beta 1	1.54E-05	-13.0748	-3.36	10444291
155	Psmd6	proteasome (prosome, macropain) 26S subunit, non-ATPase, 6	1.55E-05	-13.0659	-3.36	10417689
167	Atp6v1b2	ATPase, H+ transporting, lysosomal V1 subunit B2	1.71E-05	-12.8356	-3.37	10572146
169	Atp6v0a1	ATPase, H+ transporting, lysosomal V0 subunit A1	1.76E-05	-12.7746	-3.37	10381187
173	Cyp4f39	cytochrome P450, family 4, subfamily f, polypeptide 39	1.84E-05	-12.6695	-3.37	10443854
183	Cdca5	cell division cycle associated 5	1.98E-05	12.5166	-3.37	10460738
187	Psma6	proteasome (prosome, macropain) subunit, alpha type 6	2E-05	-12.4892	-3.37	10395816
199	Adam23	a disintegrin and metallopeptidase domain 23	2.25E-05	-12.2359	-3.37	10346882
213	H2-T22	histocompatibility 2, T region locus 22	2.51E-05	-11.9966	-3.37	10450694
224	Tnfrsf11a	tumor necrosis factor receptor superfamily, member 11a, NFkB activator	2.62E-05	-11.9075	-3.38	10349051
232	Vps13d	vacuolar protein sorting 13D	2.75E-05	-11.8045	-3.38	10518228
234	H2-DMb2	histocompatibility 2, class II, locus Mb2	2.76E-05	-11.7949	-3.38	10444236
246	Dock6	dedicator of cytokinesis 6	2.98E-05	-11.6361	-3.38	10591630

Table 1: Comparative transcriptome analysis defines the mTEC^{hi} and cTEC lineages.

The rank of differential expression, gene symbol and name, p-value, t statistic, B, the log-odds of differential expression, and the transcript ID (all provided by GEO2R) are shown.