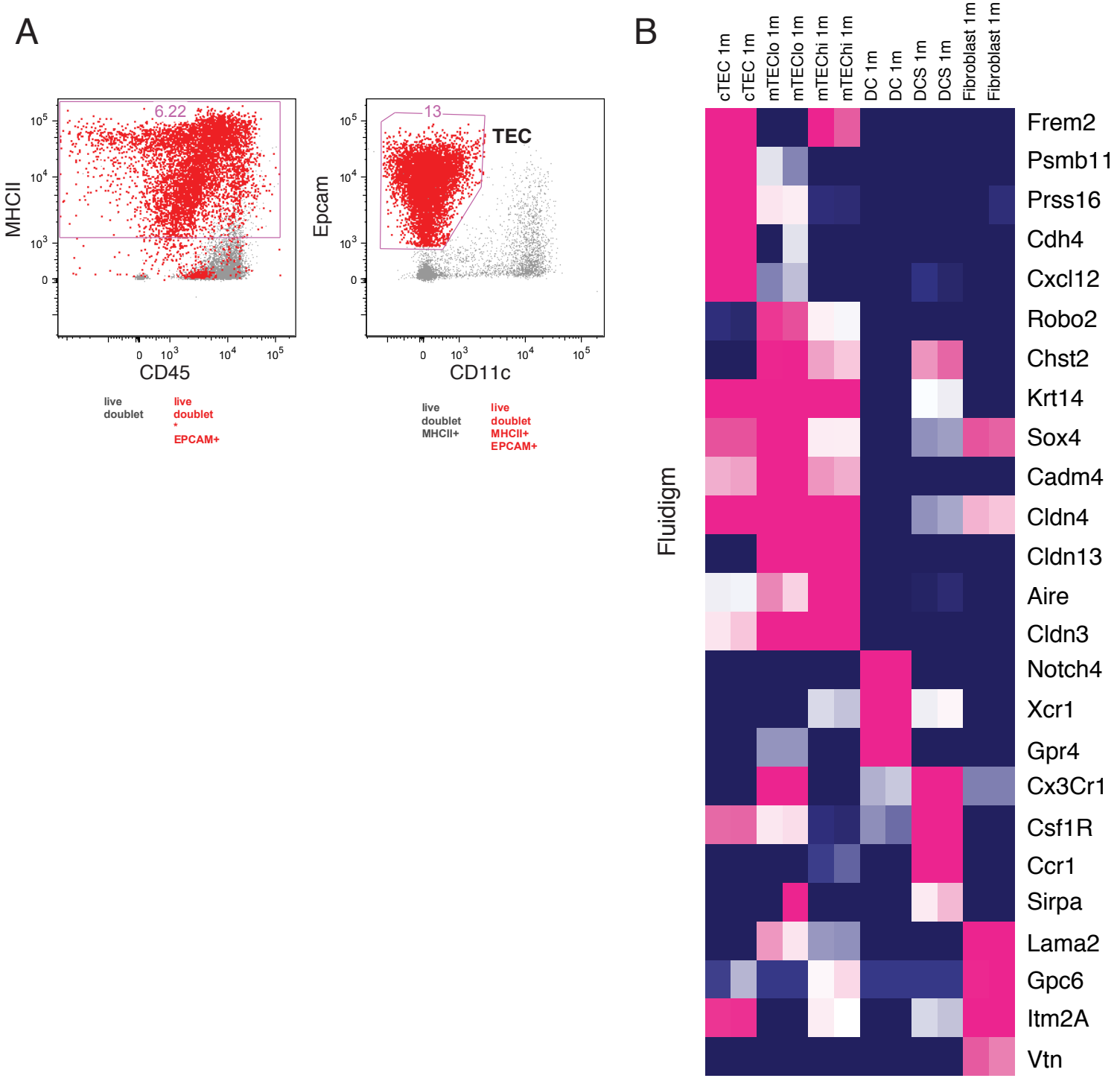
Figure S1


A
6.22
MHCII
CD45
live
doublet
live
doublet
*
EPCAM+
13
TEC
Epcam
CD11c
live
doublet
MHCII+
live
doublet
MHCII+
EPCAM+
B
cTEC 1m
cTEC 1m
mTEClo 1m
mTEClo 1m
mTEChi 1m
mTEChi 1m
DC 1m
DC 1m
DCS 1m
DCS 1m
Fibroblast 1m
Fibroblast 1m
Fluidigm
Frem2
Psmb11
Prss16
Cdh4
Cxcl12
Robo2
Chst2
Krt14
Sox4
Cadm4
Cldn4
Cldn13
Aire
Cldn3
Notch4
Xcr1
Gpr4
Cx3Cr1
Csf1R
Ccr1
Sirpa
Lama2
Gpc6
Itm2A
Vtn
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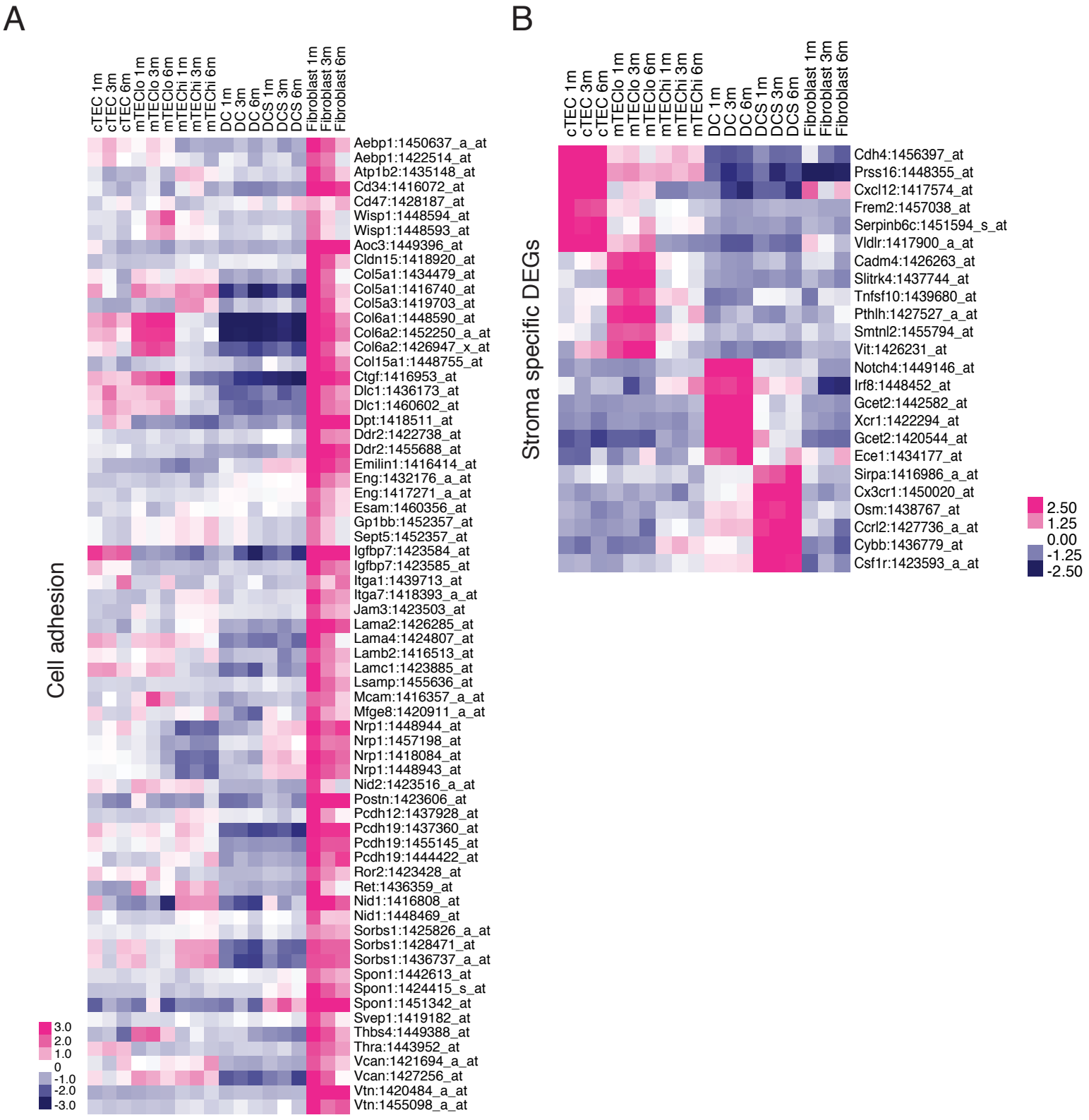
2.0
1.0
0.0
-1.0
-2.0
-3.0

Figure S2


A
cTEC 1m
cTEC 3m
cTEC 6m
mTEClo 1m
mTEClo 3m
mTEClo 6m
mTEChi 1m
mTEChi 3m
mTEChi 6m
DC 1m
DC 3m
DC 6m
DCS 1m
DCS 3m
DCS 6m
Fibroblast 1m
Fibroblast 3m
Fibroblast 6m
Cell adhesion
Aebp1:1450637_a_at
Aebp1:1422514_at
Atp1b2:1435148_at
Cd34:1416072_at
Cd47:1428187_at
Wisp1:1448594_at
Wisp1:1448593_at
Aoc3:1449396_at
Cldn15:1418920_at
Col5a1:1434479_at
Col5a1:1416740_at
Col5a3:1419703_at
Col6a1:1448590_at
Col6a2:1452250_a_at
Col6a2:1426947_x_at
Col15a1:1448755_at
Ctgf:1416953_at
Dlc1:1436173_at
Dlc1:1460602_at
Dpt:1418511_at
Ddr2:1422738_at
Ddr2:1455688_at
Emilin1:1416414_at
Eng:1432176_a_at
Eng:1417271_a_at
Esam:1460356_at
Gp1bb:1452357_at
Sept5:1452357_at
Igfbp7:1423584_at
Igfbp7:1423585_at
Itga1:1439713_at
Itga7:1418393_a_at
Jam3:1423503_at
Lama2:1426285_at
Lama4:1424807_at
Lamb2:1416513_at
Lamc1:1423885_at
Lsamp:1455636_at
Mcam:1416357_a_at
Mfge8:1420911_a_at
Nrp1:1448944_at
Nrp1:1457198_at
Nrp1:1418084_at
Nrp1:1448943_at
Nid2:1423516_a_at
Postn:1423606_at
Pcdh12:1437928_at
Pcdh19:1437360_at
Pcdh19:1455145_at
Pcdh19:1444422_at
Ror2:1423428_at
Ret:1436359_at
Nid1:1416808_at
Nid1:1448469_at
Sorbs1:1425826_a_at
Sorbs1:1428471_at
Sorbs1:1436737_a_at
Spon1:1442613_at
Spon1:1424415_s_at
Spon1:1451342_at
Svep1:1419182_at
Thbs4:1449388_at
Thra:1443952_at
Vcan:1421694_a_at
Vcan:1427256_at
Vtn:1420484_a_at
Vtn:1455098_a_at
3.0
2.0
1.0
0
-1.0
-2.0
-3.0
B
cTEC 1m
cTEC 3m
cTEC 6m
mTEClo 1m
mTEClo 3m
mTEClo 6m
mTEChi 1m
mTEChi 3m
mTEChi 6m
DC 1m
DC 3m
DC 6m
DCS 1m
DCS 3m
DCS 6m
Fibroblast 1m
Fibroblast 3m
Fibroblast 6m
Stroma specific DEGs
Cdh4:1456397_at
Prss16:1448355_at
Cxcl12:1417574_at
Frem2:1457038_at
Serpinb6c:1451594_s_at
Vldlr:1417900_a_at
Cadm4:1426263_at
Slitrk4:1437744_at
Tnfsf10:1439680_at
Pthlh:1427527_a_at
Smtnl2:1455794_at
Vit:1426231_at
Notch4:1449146_at
Irf8:1448452_at
Gcet2:1442582_at
Xcr1:1422294_at
Gcet2:1420544_at
Ece1:1434177_at
Sirpa:1416986_a_at
Cx3cr1:1450020_at
Osm:1438767_at
Ccrl2:1427736_a_at
Cybb:1436779_at
Csf1r:1423593_a_at
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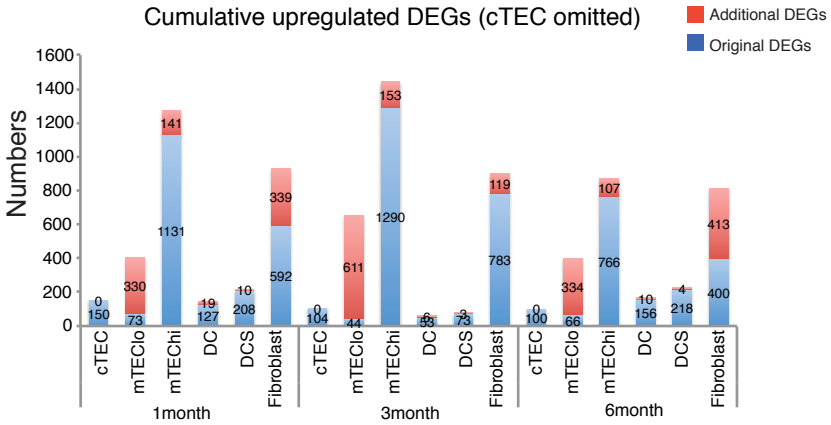
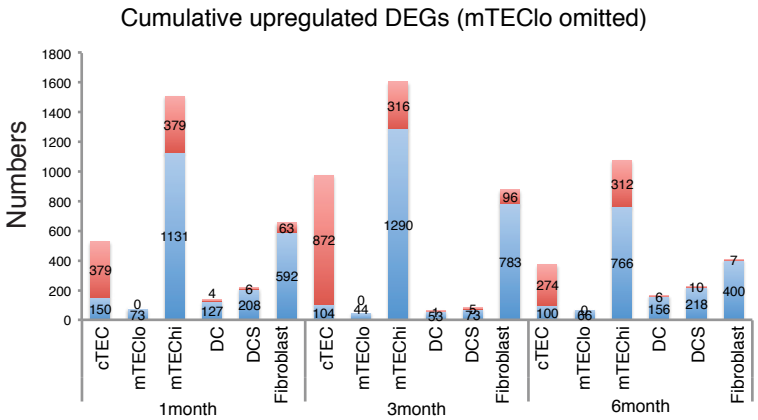
0.00
-1.25
-2.50

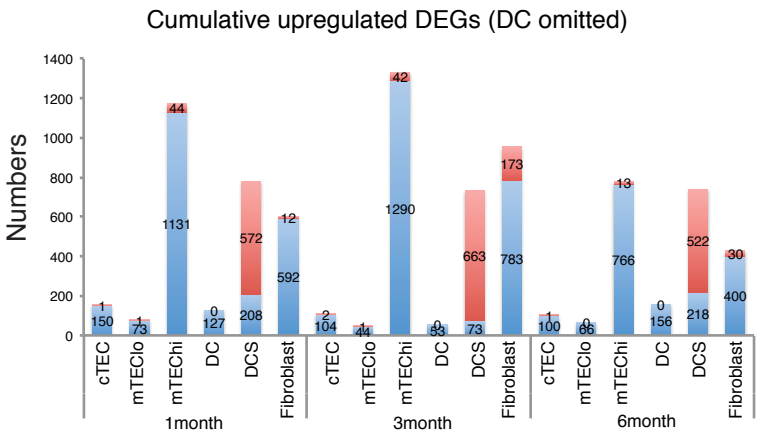
Figure S3

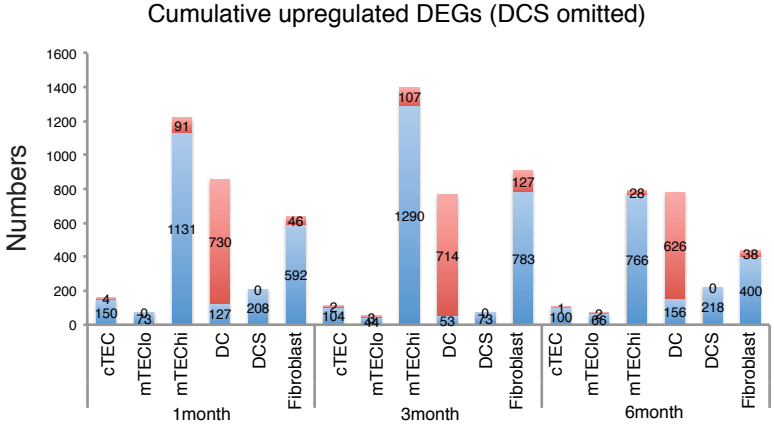
A


Cumulative upregulated DEGs (cTEC omitted)
Additional DEGs
Original DEGs
Numbers
cTEC
mTECIo
mTEChi
DC
DCS
Fibroblast
1month
3month
6month


Cumulative upregulated DEGs (mTECIo omitted)
Numbers
cTEC
mTECIo
mTEChi
DC
DCS
Fibroblast
1month
3month
6month

B


Cumulative upregulated DEGs (DC omitted)
Numbers
cTEC
mTECIo
mTEChi
DC
DCS
Fibroblast
1month
3month
6month


Cumulative upregulated DEGs (DCS omitted)
Numbers
cTEC
mTECIo
mTEChi
DC
DCS
Fibroblast
1month
3month
6month

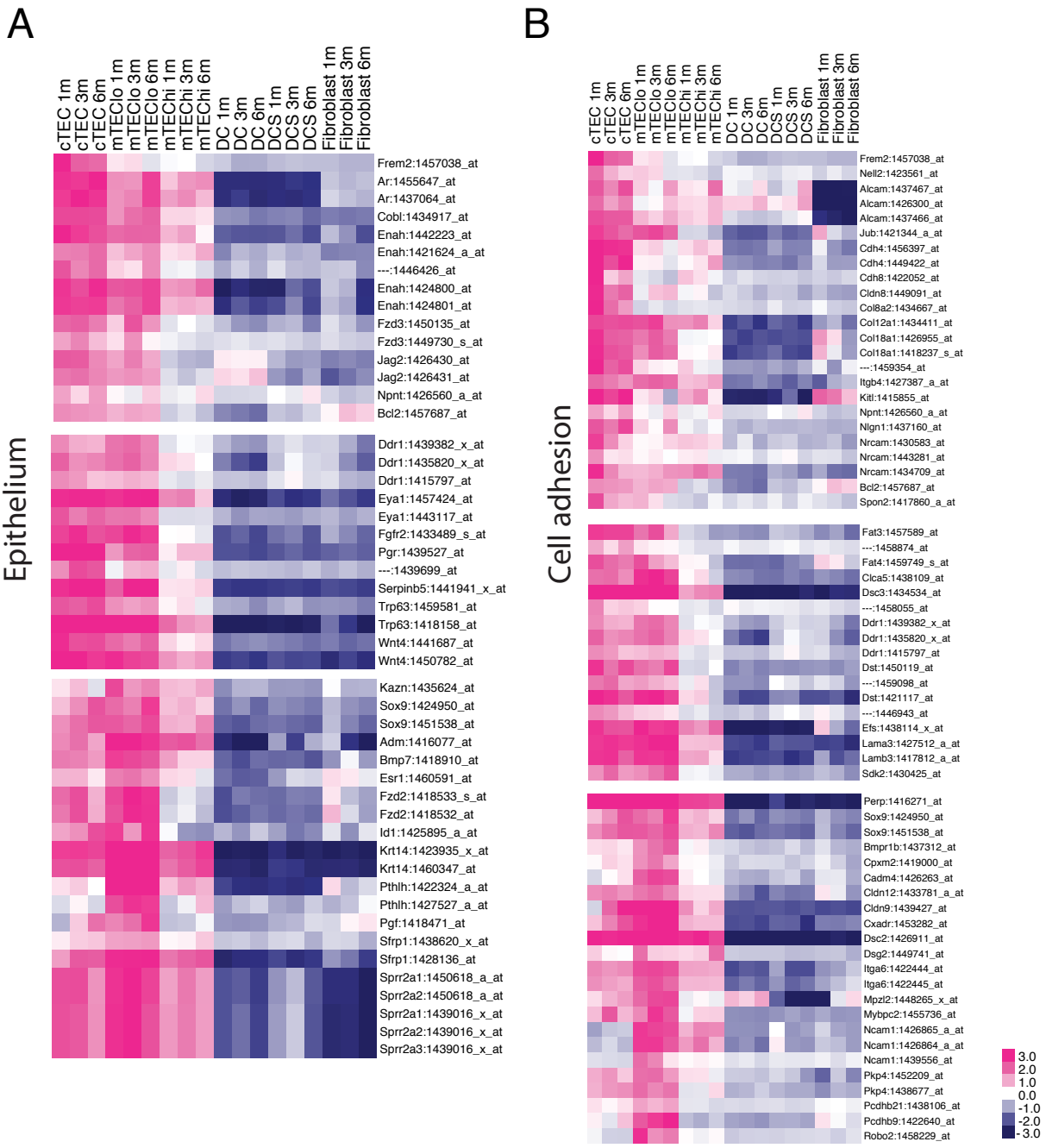
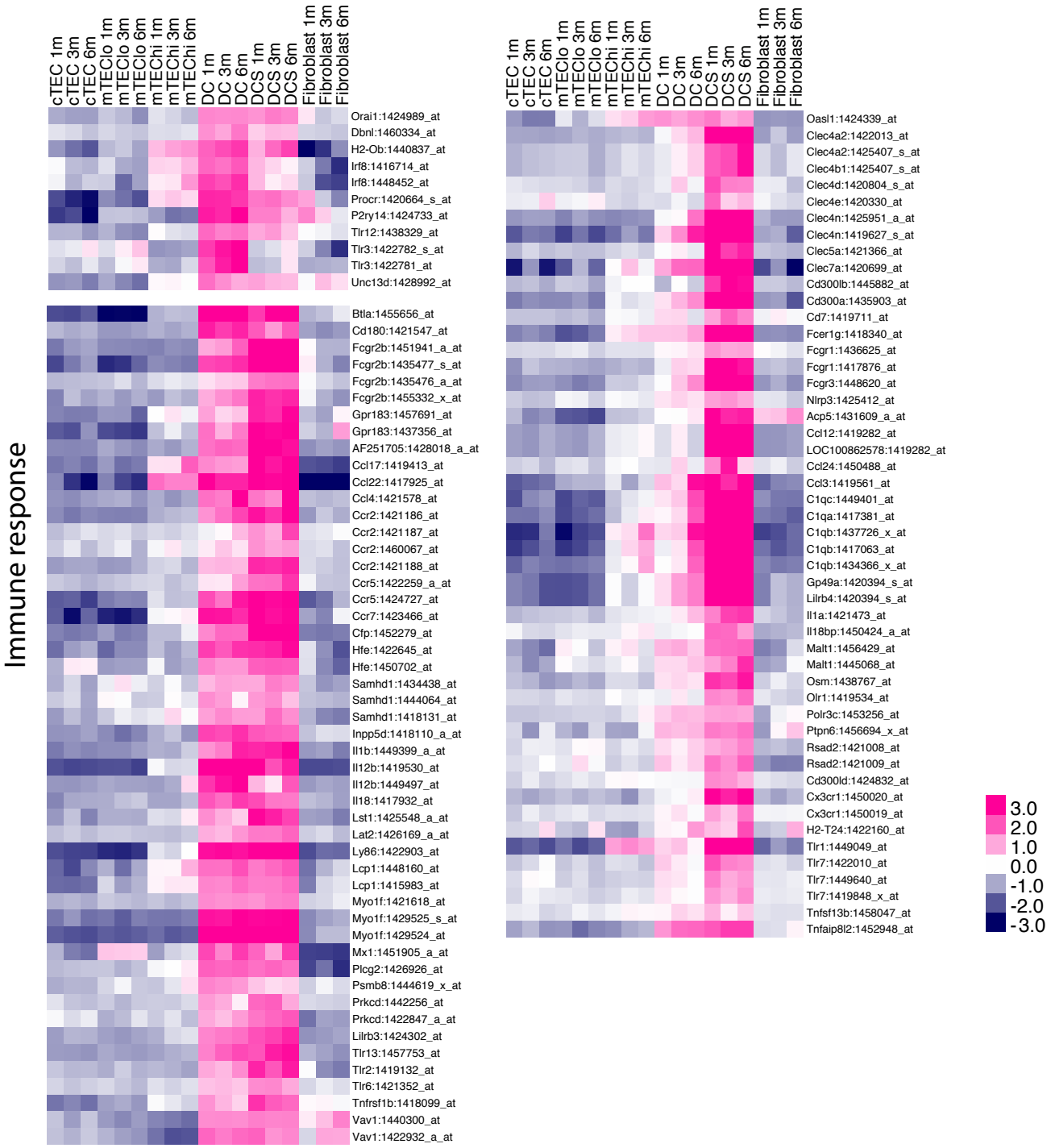

A
B
cTEC 1m
cTEC 3m
cTEC 6m
mTEClo 1m
mTEClo 3m
mTEClo 6m
mTEChi 1m
mTEChi 3m
mTEChi 6m
DC 1m
DC 3m
DC 6m
DCS 1m
DCS 3m
DCS 6m
Fibroblast 1m
Fibroblast 3m
Fibroblast 6m
Epithelium
Frem2:1457038_at
Ar:1455647_at
Ar:1437064_at
Cobl:1434917_at
Enah:1442223_at
Enah:1421624_a_at
---:1446426_at
Enah:1424800_at
Enah:1424801_at
Fzd3:1450135_at
Fzd3:1449730_s_at
Jag2:1426430_at
Jag2:1426431_at
Npnt:1426560_a_at
Bcl2:1457687_at
Ddr1:1439382_x_at
Ddr1:1435820_x_at
Ddr1:1415797_at
Eya1:1457424_at
Eya1:1443117_at
Fgfr2:1433489_s_at
Pgr:1439527_at
---:1439699_at
Serpinb5:1441941_x_at
Trp63:1459581_at
Trp63:1418158_at
Wnt4:1441687_at
Wnt4:1450782_at
Kazn:1435624_at
Sox9:1424950_at
Sox9:1451538_at
Adm:1416077_at
Bmp7:1418910_at
Esr1:1460591_at
Fzd2:1418533_s_at
Fzd2:1418532_at
Id1:1425895_a_at
Krt14:1423935_x_at
Krt14:1460347_at
Pthlh:1422324_a_at
Pthlh:1427527_a_at
Pgf:1418471_at
Sfrp1:1438620_x_at
Sfrp1:1428136_at
Sprr2a1:1450618_a_at
Sprr2a2:1450618_a_at
Sprr2a1:1439016_x_at
Sprr2a2:1439016_x_at
Sprr2a3:1439016_x_at
Cell adhesion
Frem2:1457038_at
Nell2:1423561_at
Alcam:1437467_at
Alcam:1426300_at
Alcam:1437466_at
Jub:1421344_a_at
Cdh4:1456397_at
Cdh4:1449422_at
Cdh8:1422052_at
Cldn8:1449091_at
Col8a2:1434667_at
Col12a1:1434411_at
Col18a1:1426955_at
Col18a1:1418237_s_at
---:1459354_at
Itgb4:1427387_a_at
Kitl:1415855_at
Npnt:1426560_a_at
Nlgn1:1437160_at
Nrcam:1430583_at
Nrcam:1443281_at
Nrcam:1434709_at
Bcl2:1457687_at
Spon2:1417860_a_at
Fat3:1457589_at
---:1458874_at
Fat4:1459749_s_at
Clca5:1438109_at
Dsc3:1434534_at
---:1458055_at
Ddr1:1439382_x_at
Ddr1:1435820_x_at
Ddr1:1415797_at
Dst:1450119_at
---:1459098_at
Dst:1421117_at
---:1446943_at
Efs:1438114_x_at
Lama3:1427512_a_at
Lamb3:1417812_a_at
Sdk2:1430425_at
Perp:1416271_at
Sox9:1424950_at
Sox9:1451538_at
Bmpr1b:1437312_at
Cpxm2:1419000_at
Cadm4:1426263_at
Cldn12:1433781_a_at
Cldn9:1439427_at
Cxadr:1453282_at
Dsc2:1426911_at
Dsg2:1449741_at
Itga6:1422444_at
Itga6:1422445_at
Mpzl2:1448265_x_at
Mybpc2:1455736_at
Ncam1:1426865_a_at
Ncam1:1426864_a_at
Ncam1:1439556_at
Pkp4:1452209_at
Pkp4:1438677_at
Pcdhb21:1438106_at
Pcdhb9:1422640_at
Robo2:1458229_at
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0

Figure S5


Immune response
cTEC 1m
cTEC 3m
cTEC 6m
mTEClo 1m
mTEClo 3m
mTEClo 6m
mTEChi 1m
mTEChi 3m
mTEChi 6m
DC 1m
DC 3m
DC 6m
DCS 1m
DCS 3m
DCS 6m
Fibroblast 1m
Fibroblast 3m
Fibroblast 6m
Orai1:1424989_at
Dbnl:1460334_at
H2-Ob:1440837_at
Irf8:1416714_at
Irf8:1448452_at
Procr:1420664_s_at
P2ry14:1424733_at
Tlr12:1438329_at
Tlr3:1422782_s_at
Tlr3:1422781_at
Unc13d:1428992_at
Btla:1455656_at
Cd180:1421547_at
Fcgr2b:1451941_a_at
Fcgr2b:1435477_s_at
Fcgr2b:1435476_a_at
Fcgr2b:1455332_x_at
Gpr183:1457691_at
Gpr183:1437356_at
AF251705:1428018_a_at
Ccl17:1419413_at
Ccl22:1417925_at
Ccl4:1421578_at
Ccr2:1421186_at
Ccr2:1421187_at
Ccr2:1460067_at
Ccr2:1421188_at
Ccr5:1422259_a_at
Ccr5:1424727_at
Ccr7:1423466_at
Cfp:1452279_at
Hfe:1422645_at
Hfe:1450702_at
Samhd1:1434438_at
Samhd1:1444064_at
Samhd1:1418131_at
Inpp5d:1418110_a_at
Il1b:1449399_a_at
Il12b:1419530_at
Il12b:1449497_at
Il18:1417932_at
Lst1:1425548_a_at
Lat2:1426169_a_at
Ly86:1422903_at
Lcp1:1448160_at
Lcp1:1415983_at
Myo1f:1421618_at
Myo1f:1429525_s_at
Myo1f:1429524_at
Mx1:1451905_a_at
Plcg2:1426926_at
Psmb8:1444619_x_at
Prkcd:1442256_at
Prkcd:1422847_a_at
Lilrb3:1424302_at
Tlr13:1457753_at
Tlr2:1419132_at
Tlr6:1421352_at
Tnfrsf1b:1418099_at
Vav1:1440300_at
Vav1:1422932_a_at
Oasl1:1424339_at
Clec4a2:1422013_at
Clec4a2:1425407_s_at
Clec4b1:1425407_s_at
Clec4d:1420804_s_at
Clec4e:1420330_at
Clec4n:1425951_a_at
Clec4n:1419627_s_at
Clec5a:1421366_at
Clec7a:1420699_at
Cd300lb:1445882_at
Cd300a:1435903_at
Cd7:1419711_at
Fcer1g:1418340_at
Fcgr1:1436625_at
Fcgr1:1417876_at
Fcgr3:1448620_at
Nlrp3:1425412_at
Acp5:1431609_a_at
Ccl12:1419282_at
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Ccl3:1419561_at
C1qc:1449401_at
C1qa:1417381_at
C1qb:1437726_x_at
C1qb:1417063_at
C1qb:1434366_x_at
Gp49a:1420394_s_at
Lilrb4:1420394_s_at
Il1a:1421473_at
Il18bp:1450424_a_at
Malt1:1456429_at
Malt1:1445068_at
Osm:1438767_at
Olr1:1419534_at
Polr3c:1453256_at
Ptpn6:1456694_x_at
Rsad2:1421008_at
Rsad2:1421009_at
Cd300ld:1424832_at
Cx3cr1:1450020_at
Cx3cr1:1450019_at
H2-T24:1422160_at
Tlr1:1449049_at
Tlr7:1422010_at
Tlr7:1449640_at
Tlr7:1419848_x_at
Tnfsf13b:1458047_at
Tnfaip8l2:1452948_at
3.0
2.0
1.0
0.0
-1.0
-2.0
-3.0

Figure S6


DC 1m
DC 3m
DC 6m
LPS induced genes
Ier3
Hes1
Btg2
Dusp1
Il1b
Zc3h12a
Stard4
Cd83
Sav1
Sod2
Ccl4
Nedd4l
Nfkbiz
Rgs1
Twistnb
Dusp5
Selk
Cxcl2
Taok3
Usp12
Dnajb4
Tpd52
Il1a
Socs3
Ddx5
Ptp4a1
Peli1
Rel
Pnrc2
Pts
Egr1
Nupl1
Plk3
Rybp
Arl5b
Nfkbia
Adamdec1
Map3k4
Yrdc
Pim3
Ptger4
Maff
Areg
Id2
Ptgs2
Il12b
Edn1
Serpinb8
Bsnd
Il18
Kcnj2
Acsl1
Plek
Rtp3
Wtap
Map3k8
Eif1b
Plekhf2
Icam1
Xbp1
Ptx3
Dusp2
Rpgr
Il10
Pdss1
Rnd1
Tnfaip3
Rap2c
DC 1m
DC 3m
DC 6m
Ppp1r15b
Gng2
Ccrl2
Marcks
E2f7
Tnf
Il6
Phlda2
F8
G0s2
Rin2
Ell2
Ets2
Pld1
Fosl1
Tmem88
Ube2d1
Gch1
Il23a
Ffar2
Fjx1
Hs3st3b1
Pfkfb3
Csf3
Gpr84
Sgpp2
Rhcg
Birc3
Tdh
Dcun1d3
Psmd5
Osr2
Rhoh
Plaur
Gadd45b
Hecw2
Mmp7
Tifa
Clcf1
Ripk2
Cxcl1
Cd274
Gbp1
Rab33a
Siah2
Efna1
Gbp2
Ddit4
B3gnt2
Dyrk3
Cflar
Gadd45a
Rab21
Hivep2
Phf1
Synpo2
Inhba
Rbbp8
Lama3
Tnfaip6
Ccr7
Tfrc
Adm
Ccrn4l
Aqp9
Rbm17
Rapgef2
Piga
Cdkn1a
Stat5a
C1qtnf1
DCS 1m
DCS 3m
DCS 6m
LPS induced genes
Twistnb
Rel
Nedd4l
Ptgs2
Cd83
Nfkbiz
Dusp5
Yrdc
Tmem88
Maff
Acsl1
Dusp1
Hivep2
Rgs1
Pim3
Hes1
Ccrl2
Tnf
Id2
Dnajb4
Arl5b
Eif1b
Egr1
Ccl4
Pts
Stard4
Pfkfb3
Cflar
Sav1
Birc3
Ptp4a1
Nupl1
Il1b
Ptger4
Dusp2
Usp12
Cxcl2
Il1a
Tpd52
Btg2
Plk3
Wtap
Map3k8
Rab21
Areg
Selk
Rap2c
Il10
Cd274
Socs3
Xbp1
Ier3
Zc3h12a
Edn1
Ddx5
Taok3
Tnfaip3
Plek
Pnrc2
Ppp1r15b
Rin2
Il6
Rybp
Nfkbia
Ets2
Gch1
Tnfaip6
Gadd45b
DCS 1m
DCS 3m
DCS 6m
Peli1
Piga
Cxcl1
Map3k4
E2f7
Plekhf2
Ffar2
Lama3
Pdss1
Hecw2
Hs3st3b1
Adamdec1
Mmp7
C1qtnf1
Rpgr
Ell2
Rab33a
Bsnd
Gbp2
Icam1
Gbp1
Ube2d1
Dyrk3
Clcf1
Ddit4
Sod2
F8
Osr2
Tifa
Gpr84
Ccr7
Serpinb8
Marcks
Rnd1
Tfrc
Adm
Il12b
Cdkn1a
Rapgef2
Psmd5
Phf1
Phlda2
Rhoh
Aqp9
Tdh
Efna1
Ptx3
Pld1
Kcnj2
Dcun1d3
Plaur
Synpo2
Fosl1
Gng2
Ripk2
Siah2
B3gnt2
Rtp3
Rhcg
Il23a
Rbbp8
Il18
Inhba
Csf3
Fjx1
G0s2
Ccrn4l
Stat5a
Sgpp2
Gadd45a
Rbm17
0.50
0.25
0.00
-0.25
-0.50

Supplemental Figure Legends

Figure S1, Related to Figure 1. (A) Backgating analysis of the EPCAM^{+}CD11c^{-} TEC population gated in the panel on the right demonstrates that the majority of TECs are within the MHCII^{+} gate in the left panel. Comparable backgating was used to adjust the MHCII+ gate during the initial sort setup to ensure MHCII^{lo} TECs were not excluded during cell sorting. Red events on the left panel represent cells gated as EPCAM+CD11c- in the absence of the MHCII+ gate displayed in the left panel. (B) To confirm the microarray expression profiling data, 3 - 4 genes identified as DEGs in each subset were chosen for validation by high throughput qRT-PCR (Fluidigm) on an additional biological replicate for each stromal subset.

Figure S2, Related to Figure 2. (A) The heat map displays the relative expression of all genes in the 'cell adhesion' GO term that overlapped with up-regulated DEGs in 1-month Fibroblasts. (B) The heat map displays the relative expression of select genes identified as unique DEGs in cTEC, mTEClo, DC and DCS subsets.

Figure S3, Related to Figure 3. (A) The graph depicts the number of cTEC and mTEClo DEGs identified before and after excluding each other during the analysis. The blue bars show the original number of DEGs when all subsets were compared in pairwise comparisons, while the red bar shows the number of additional DEGs newly identified after omitting the indicated subtype from the analysis. (B) The graph depicts the number of DC and DCS DEGs identified before and after excluding each other during the analysis, as in A.

Figure S4, Related to Figure 3. The heat maps display the relative expression of all genes in the (A) 'epithelium' and 'epithelium development' GO terms and (B) 'cell adhesion' GO term that overlapped with up-regulated DEGs identified in 1-month cTEC and mTEClo cells.

Figure S5, Related to Figure 3. The heat maps display the relative expression of all genes in the 'immune response' GO term that overlapped with up-regulated DEGs identified in 1-month DC and DCS subsets.

Figure S6, Related to Figure 5. The heat maps display the relative expression of all LPS induced genes in DC and DCS subsets. Some genes are up-regulated, while others are down-regulated in aging dendritic cells. The heat map is ordered from genes most up-regulated to most down-regulated with age in dendritic cell subsets, according to GSEA (Figure 5A).

Table S1, Related to Figure 2. Combined list of subset-specific DEGs that are up- or down-regulated in each cell type based on pairwise comparisons with other stromal subsets.

Table S2, Related to Figure 4. Combined list of age-associated DEGs that are up- or down- regulated in mTEClo, DC, and DCS based on pairwise comparisons of 1-month versus 3-month, 3-month versus 6-month, and 1-month versus 6-month expression values within each subset.

Table S3, Related to Experimental Procedures. Primers used for qRT-PCR detection of gene expression on the Fluidigm platform.

Table S3

	Forward (5'-…-3')	Reverse (5'-…-3')
Frem2	5'-GTGCAGCACTTCAGTCCCT-3'	5'-CAGCACCAAGTCACGCAAC-3'
Psbm11	ACTCCCGACACTCCCAGAC	CCGTGACGAAAGCGAAAAGC
Prss16	CCTGAGGGAACACATTCAGAAG	GTTCTAACCACCCTTGTTTGGG
Cdh4	CAGGCCACTGACATGGAAGG	ATGATTCGGTAGACGGCGTTC
Cxcl12	TGCATCAGTGACGGTAAACCA	TTCTTCAGCCGTGCAACAATC
Robo2	TGATGGATCTCGTCTTCGTCA	GTCGGCCCTCTGCTTTACAG
Chst2	CCGCTCGGGATGAAGGTATTT	CCACTTGTAGTCCAAGAGGTTGA
Krt14	AGCGGCAAGAGTGAGATTTCT	CCTCCAGGTTATTCTCCAGGG
Sox4	GACAGCGACAAGATTCCGTTC	GTTGCCCGACTTCACCTTC
Cadm4	ACAGGAAGTACAGACCGAGAAT	GGTGCCATTGAAAAAGAGGGT
Cldn4	GTCCTGGGAATCTCCTTGGC	TCTGTGCCGTGACGATGTTG
Cldn13	ATGGTCGTCAGCAAACAAGAG	CATCATCTGGAAAGGTCACCC
Aire	AGGTCAGCTTCAGAGAAAACCA	TCATTCCCAGCACTCAGTAGA
Cldn3	ACCAACTGCGTACAAGACGAG	CAGAGCCGCCAACAGGAAA
Notch4	CTCTTGCCACTCAATTTCCCT	TTGCAGAGTTGGGTATCCCTG
Xcr1	CTCAGCCTTGTGGGTAACAGC	ACAGGCAGTAGACAGGAGAAC
Gpr4	GCTGGGCGTCTACCTGATG	AGGCGATGCTGATATAGATGTTG
Cx3cr1	GAGTATGACGATTCTGCTGAGG	CAGACCGAACGTGAAGACGAG
Csf1r	TGTCATCGAGCCTAGTGGC	CGGGAGATTCAGGGTCCAAG
Ccr1	CTCATGCAGCATAGGAGGCTT	ACATGGCATCACCAAAAATCCA
Sirp**α**	CACGGGGACAGAAGTGAAGG	TGCAGTTGAGAATGGTCGAATC
Lama2	TCCCAAGCGCATCAACAGAG	CAGTACATCTCGGGTCCTTTTTC
Gpc6	CCAATCAGGCAGATTTGGACA	GGGCCGAAAACGGGTGTTA
Itm2a	AGGCGCGGCAAGATATAGAG	GTCCTGCCAAGATGAATGAGAG
Vtn	CCCCTGAGGCCCTTTTTCATA	CAAAGCTCGTCACACTGACA

Supplemental Experimental Procedures

FACS isolation of thymic stromal subsets

Thymi from 1, 3, and 6-month-old male C57Bl/6J mice were digested with Collagenase D (Roche, Switzerland), followed by Collagenase/Dispase (Roche) in the presence of DNase I, as described previously (Gray et al., 2008). Cells were immunostained with FITC-conjugated *Ulex europaeus* agglutinin I (UEA-1) (Vector Laboratories) and the following fluorochrome-conjugated antibodies (eBioscience or BioLegend, unless indicated): EpCAM (G8.8), TER-119 (TER- 119), CD11c (N418), CD31 (390), Sirpα (P84), B220 (RA3-6B2), I-A/I-E (M5/114.15.2), CD80 (16-10A1), CD45 (30-F11, BD Biosciences), and biotinyated Ly-51 (6C3). Stromal subsets were FACS purified by double sorting to >95% purity on a FACSAria (BD Biosciences); data were analyzed with FlowJo (Treestar).

Sample preparation and transcriptional profiling

Stromal subsets were collected directly in 1ml of TRIzol (Life Technologies), and RNA was purified. 1 μg of high-quality total RNA was amplified, converted to cDNA using the two-cycle cDNA synthesis protocol, labeled, and hybridized onto Mouse Genome 430 2.0 arrays (Affymetrix) at the Stanford PAN facility core according to Affymetrix's specifications. CEL files were uploaded to GExC (Seita et al., 2012), where the data were normalized using the RMA algorithm (McCall et al., 2010) for normalization. An in-silico model of gene expression data from thymic stromal subsets

and previous thymocyte subsets (GSE34723) (Seita et al., 2012) is available (https://gexc.stanford.edu/model/detail/475). Thymic stromal expression data have been deposited in GEO (GSE56928).

Bioinformatics analysis

R (version 2.15.2) was used for hierarchical clustering (function "hclust") and DEG identification (package "limma" version 3.14.4) (Smyth, 2004). p-values were adjusted using the Benjamini–Hochberg procedure to correct for multiple comparisons, and an adjusted p-value of 0.01 and a fold change of 2 were used as cut-offs for DEG. Subtype-specific DEGs were defined as genes differentially expressed in all pairwise comparisons with other subsets of the same age. Age-associated DEGs were defined as the union of differentially expressed genes from pairwise comparisons of single stromal subsets compared at different ages. To identify Aire-regulated genes in mTEChi cells, we analyzed Aire deficient and wild-type mTEChi expression data from the following datasets using R (package "affy" version 1.36.1): GSM49731, GSM49732, GSM49735, GSM49736 (Derbinski, 2005; Gautier et al., 2004). We defined the top 400 DEGs, ranked according to fold change, as Aire-regulated genes. Probe IDs were annotated using version 33 of these array platforms, and gene symbols were then compared to our mTEChi DEG list to identify the extent of overlap. TRAs identified on the basis of unique tissue expression (Griffith et al., 2012) were also compared to our mTEChi DEG list for overlap.

PCA was performed with the "princomp" function in MATLAB R2012b, and Cluster 3.0 was used for K-means clustering. For Gene Ontology analysis, DAVID

version 6.7 was used with probe IDs (Huang et al., 2009). GSEA was carried out by searching Molecular Signature Database (MSigDB) version 4.0 provided by the Broad Institute (http://www.broad.mit.edu/gsea/) (Subramanian et al., 2005). When multiple probe IDs mapped to a gene symbol, the maximum probe value was used for GSEA. For identification of aging associate gene sets, 1:3:6 was used for phenotype labels of ages 1, 3, and 6 months to calculate Pearson correlations, and then the immunologic signatures (C7) and the curated gene sets (C2) of the MSigDB gene sets were queried. A nominal (NOM) p-value cut-off was applied to the results from C7. Boxplots were generated after converting human genes into mouse orthologs using the mouse annotation from Mouse Genome Informatics (MGI) released April 4, 2012. Statistical significance was determined using paired Student's t-tests.

To generate heat maps, log2-transformed expression intensities were centered on the mean (defined as zero) for each probe to compare relative expression across subtypes and ages.

RT-PCR on the Fluidigm platform

For each FACS sorted stromal subset, cDNA was synthesized from 300ng RNA using qScriptTM cDNA SuperMix (Quanta). cDNAs were diluted and preamplified with 2X Taqman preamp Master Mix (Applied BioSystems), using a pool of custom primers for transcripts of interest. qPCRs were carried out on cDNA distributed on 48.48 Dynamic Arrays, using primers (Table S3) for genes of interest on a BioMark System according to manufacturer’s instructions (Fluidigm).

Supplemental Reference

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