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# Age-related epithelial defects limit thymic function and regeneration

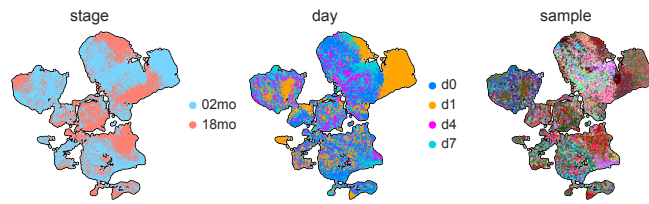
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In the format provided by the authors and unedited

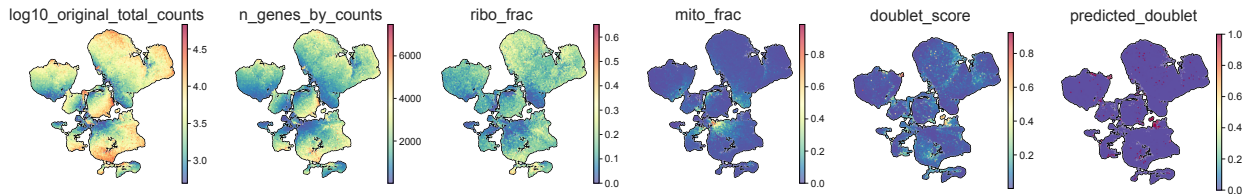
# Supplementary Figure 1

**a**

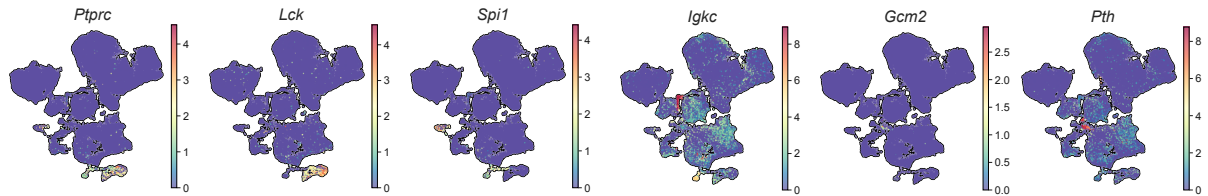
## Metadata info



## QC info

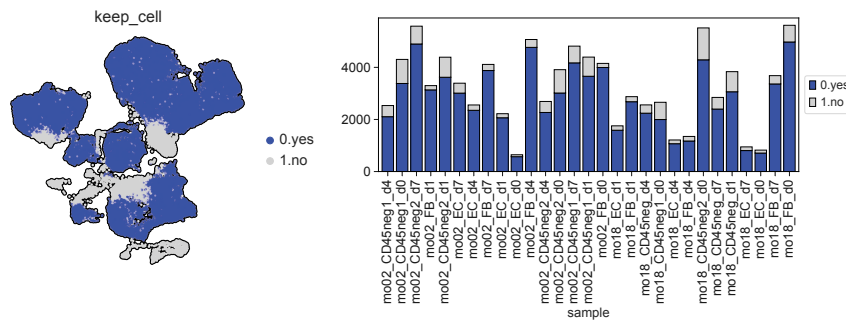


## Contaminants



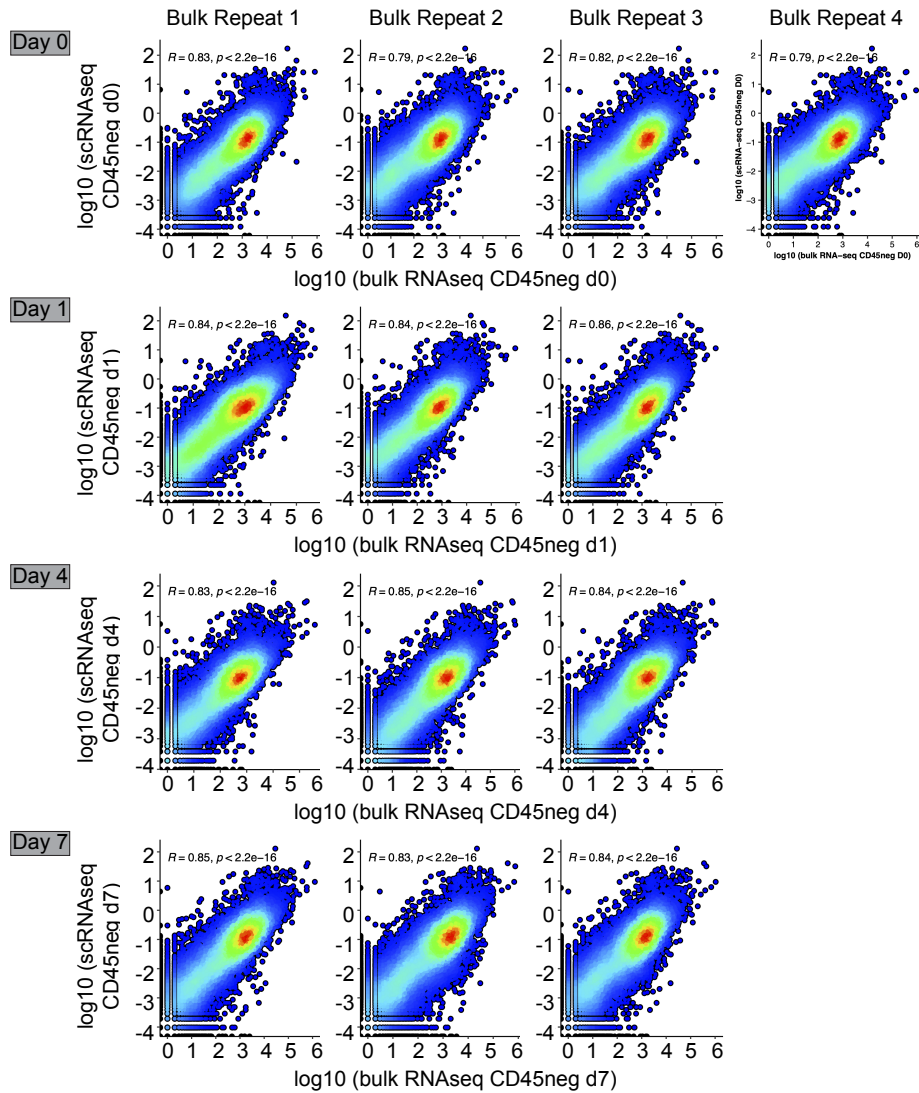
**b**

## Number of cells to discard after QC



**Supplementary Figure 1: a**, UMAPs of 93,738 CD45<sup>-</sup> cells from 2mo or 18mo thymus at baseline (day 0) and at days 1, 4 and 7 after TBI prior to quality control. UMAPs in sequence display age cohort, day after TBI and origin sample (Metadata info); total counts in log10 scale, number of genes, ribosomal fraction, and mitochondrial fraction per cell (QC info); CD45<sup>+</sup> and parathyroid contaminating cells expressing *Ptprc*, *Lck*, *Spi1*, *Igkc* and *Gcm2*, *Pth* genes respectively (Contaminants). **b**, UMAP and associated stacked barplot representation of filtered out cells on a per sample basis. Cells retained for further processing are shown in blue, while cells removed are shown in grey.

## Supplementary Figure 2



**Supplementary Figure 2:** Scatterplots showing correlation (Pearson) between averaged expression profiles of CD45- scRNA-seq averaged technical replicates collected at steady state (row 1), day 1 (row 2), day 4 (row 3) and day 7 (row 4) after TBI versus the expression profiles of CD45- bulk RNA-seq biological replicates (replicate 1; column 1, replicate 2; column 2, replicate 3; column 3, replicate 4; column 4) obtained on the same timeframe.

**Supplementary Table 11: Gating strategy for cell subset analysis by flow cytometry**

Subset	Flow phenotype	Figure
EC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>+</sup> PDGFRa <sup>-</sup>	Ext Fig. 1
Fibroblast	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>-</sup> PDGFRa <sup>+</sup>	
mTEC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>+</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup>	
cTEC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>+</sup> UEA1 <sup>lo</sup> Ly51 <sup>hi</sup>	
mTEC1	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>lo</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup> L1CAM <sup>-</sup> CD104 <sup>+</sup>	Figure 1
mTEC2	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>+</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup> L1CAM <sup>-</sup> CD104 <sup>-</sup> Ly6D <sup>-</sup>	
mTEC3	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>+</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup> L1CAM <sup>-</sup> CD104 <sup>-</sup> Ly6D <sup>+</sup>	
TEC <sup>tuft</sup>	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>+</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup> L1CAM <sup>+</sup>	
DN-TEC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>+</sup> UEA1 <sup>-</sup> Ly51 <sup>-</sup>	
aaTEC1	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> PDGFRa <sup>-</sup> CD31 <sup>-</sup> EpCAM <sup>+</sup> MHCII <sup>+</sup> UEA1 <sup>-</sup> Ly51 <sup>-</sup> Claudin-3 <sup>+</sup>	Figure 1j, Ext Data Fig. 4
aaTEC2	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>-</sup> MHCII <sup>+</sup> PDPN <sup>+</sup> PDGFRa <sup>-</sup>	
capFB	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>-</sup> PDGFRa <sup>+</sup> CD26 <sup>+</sup> PDPN <sup>+</sup>	Ext Data Fig. 4
medFB	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>-</sup> PDGFRa <sup>+</sup> CD26 <sup>-</sup> PDPN <sup>+</sup>	
intFB	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>-</sup> PDGFRa <sup>+</sup> PDPN <sup>-</sup>	
aEC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>+</sup> PDGFRa <sup>-</sup> CD309 <sup>-</sup> CD62P <sup>-</sup>	Ext Data Fig. 4
capEC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>+</sup> PDGFRa <sup>-</sup> CD309 <sup>+</sup>	
vEC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>+</sup> PDGFRa <sup>-</sup> CD309 <sup>-</sup> CD62P <sup>+</sup>	
Pericyte	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>-</sup> PDGFRb <sup>+</sup> Ly51 <sup>+</sup> CD9 <sup>+</sup>	Ext Data Fig. 4
vSMC	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>-</sup> PDGFRb <sup>+</sup> Ly51 <sup>-</sup> CD9 <sup>+</sup>	
Mesothelial	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> CD31 <sup>-</sup> PDGFRb <sup>-</sup> CD9 <sup>+</sup> PDPN <sup>+</sup>	
Tuft Cell	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup> GFP <sup>+</sup> DCLK1 <sup>+</sup> Ly6D <sup>lo/-</sup>	Ext Data Fig. 7 (In Foxn1 <sup>nTnG</sup> mice)
Corneocyte	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup> GFP <sup>+</sup> DCLK1 <sup>-</sup> Ly6D <sup>+</sup>	
M cell	LiveTER119 <sup>-</sup> CD45 <sup>-</sup> EpCAM <sup>+</sup> UEA1 <sup>hi</sup> Ly51 <sup>lo</sup> GFP <sup>+</sup> GP2 <sup>+</sup> Ly6D <sup>lo/-</sup>	

**Supplementary Table 12: Flow cytometry and cell sorting antibodies:**

Antigen	Clone	Fluorochrome	Vendor	Cat#	Lot#	Dilution
CD45	30-F11	PercP-Cy5.5	Biolegend	103132	B363634	1/100
		PE-CF594	BD	562420	0013784	1/300
		BUV615	BD	751170		1/300
CD31	390	PercP-Cy5.5	Biolegend	102420	B247171	1/1300
TER119	TER-119	PercP-Cy5.5	Biolegend	116228	B356105	1/100
		BUV563	BD	741257	2265032	1/500
		PE-Cy5.5	Invitrogen	35-5921-82		1/650
MHCII	M5/114.15.2	BV421	Biolegend	107631	B360795	1/300
		BV711	Biolegend	107631	B377900	1/400
		APC-e780	Invitrogen	47-5321-82		1/2000
EpCAM	G8.8	APC-Cy7	Biolegend	118218	B393333	1/100
		Percp-e710	Invitrogen	46-5791-82	4278076	1/400
Ly51	6C3	PE-Cy7	Biolegend	108313	B215053	1/100
		PE	Invitrogen	12-5891-83	4300066	1/1000
		Alexa 647	Biolegend	108312	B222435	1/400
PDGFRa	APA5	BUV737	BD	741789	3228775	1/200
		Alexa594	Invitrogen	14-1401-82		1/100
		BV750	BD	747220		1/100
PDGFRb	APB5	SB600	Invitrogen	63-1402-80		1/100
CD104	346-11A	PE-Cy7	Biolegend	123615	B343426	1/200
		BV711	BD	743082		1/150
L1CAM	555	PE	RD system	AAYQ0223061	FAB5674P	1/100
Ly6D	49-H4	eFluor450	eBio	48-597480	2020836	1/200
PDPN	8.1.1	APC-Fire750	Biolegend	127425	B313124	1/200
		BUV805	BD (custom)	624287		1/650
		Purified	Biolegend	127402	B219285	1/100
	Alexa 647	Anti-IgG	Invitrogen	A-21451	2527971	1/100

CD26	H194-112	BUV737	BD	741729		1/20
CD62P	RB40.34	BV480	BD	746249		1/300
CD309	Avas12a	BUV661	BD	741571		1/50
CD9	KMC8	BUV563	BD	741270		1/50
Claudin-3	Polyclonal	Purified	Invitrogen	34-1700	Y1376567	1/100
Anti-Rabbit	Secondary	APC	Invitrogen	A31573	1786284	1/400
UEA1	Lectin	Fitc	VectorLabs	FL-1061	ZC0426	1/1000
		Biotin	VectorLabs	B1065	ZD0810	1/200
		Strep-BV605	Biolegend	405229	B387251	1/1000
pAKT	Polyclonal	Pac Blue	Cell Signaling	68035S	1	1/100
FOXN1	2/41	Alexa 647	Gift	N/A	N/A	
DCLK1	aa690-720	BV421	Lsbio	LSC100746	134142	1/1000
GP2	2F11-C3	Alexa 594	MBL	D278-3	010	1/100
GFP	GFP-1020	Dylight 488	Aves	GFP-1020	GFP3717982	1/1000

**Supplementary Table 13: Antibodies for immunofluorescence.**

	<b>Antigen</b>	<b>Format</b>	<b>Vendor</b>	<b>Catalog#</b>
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Primaries	pan-Cytokeratin	Purified	Dako	Z0622
	Keratin 5	Purified	Biolegend	poly19055
	anti-mouse K8/18	Purified	Developmental Studies Hybridoma Bank	TROMA-1
	Keratin 14	Purified	Abcam	EPR17350
	AIRE	Purified	WEHI	Clone# 5H12
	DCLK1	Purified	LSBio	LS-C100746
	UEA1 lectin	Purified	Vector labs	B-1065

Secondaries	Donkey anti-rabbit IgG	Alexa Fluor® 647	Invitrogen	A31573
	Goat anti-rat IgG	Alexa Fluor® 647	Invitrogen	A-21247
	Streptavidin conjugate	Alexa Fluor® 647	Invitrogen	S21374