

Figure S1

Figure S1. IL-10 induces bone marrow T cell senescence *in vivo*. (a-f, i, j) C57BL/6, pMT-10 or pMT10.IL-10Ra^{-/-} were induced with zinc-enriched (+) or control (-) water for 30 days. **(a)** Representative FACS plots of CD4⁺ and CD8⁺ naïve and activated T cell compartments, and SA-β-gal⁺ and γH2AX⁺ cells. **(b)** Numbers of naïve and activated T cell subsets in the CD4⁺ **(b)** and CD8⁺ **(c)** T cell compartments. **(d)** Total numbers of CD3⁺ T cells and **(e)** ratio of CD4/CD8 ratio in the indicated experimental groups. **(f)** Transcriptome directionality in CD4⁺ and CD8⁺ T cells of pMT-10 + Zn vs. pMT-10 – Zn mice. Blue dots represent genes following the same direction of differential expression in CD4⁺ and CD8⁺ T cells; orange dots represent genes following opposite directions. Annotated are the top 25 differentially expressed genes (log₂FC) in CD4⁺ and CD8⁺ T cells of pMT-10 + Zn vs. pMT-10 – Zn mice. **(g, h)** pMT-10 mice were fed with zinc-enriched water and, at the indicated time points, the PD-1⁺ CD38⁺ T cell pool in the bone marrow **(g)** and blood **(h)** analysed by flow cytometry. **(i, j)** DNA methylation clock score of blood samples from induced (pMT-10 + Zn) and control (BL/6 + Zn and pMT-10 – Zn) mice across two different datasets. **(b-e)** Bars represent the mean ± s.e.m. for 5 – 11 mice, from 4 – 5 independent experiments. Each dot represents a single mouse. **(g, h)** Each dot represents the mean ± s.e.m. for 2 – 7 mice, from 2 independent experiments. **(i, j)** Box and whisker plots with individual mice from one experiment represented by single dots. Data were analyzed with one-way ANOVA; *p* values are shown for the indicated comparisons.

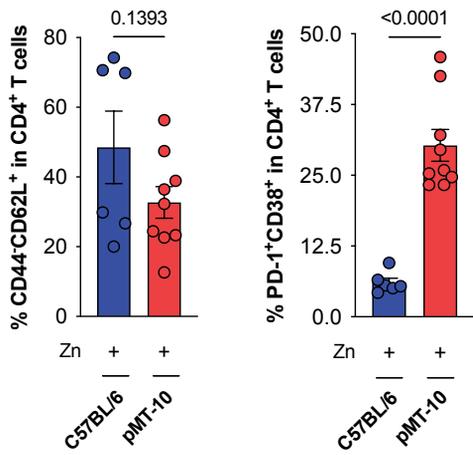
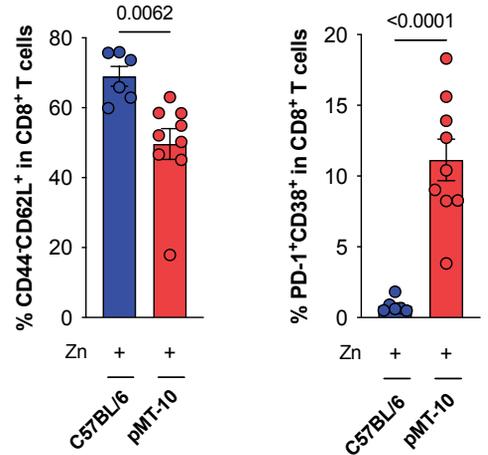
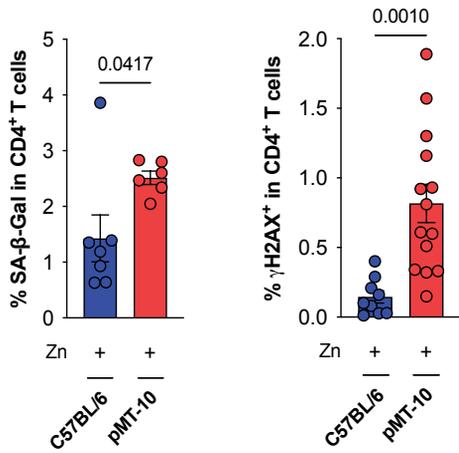
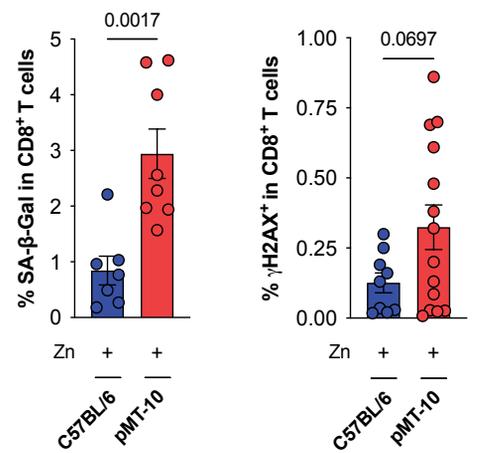
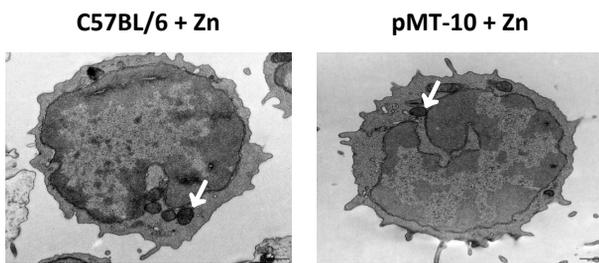
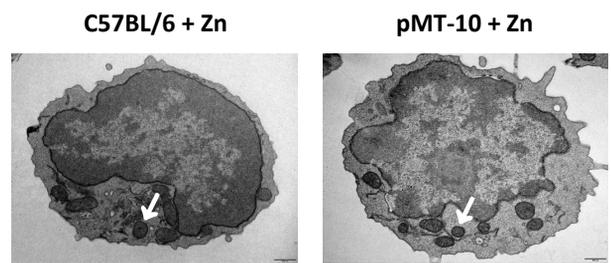
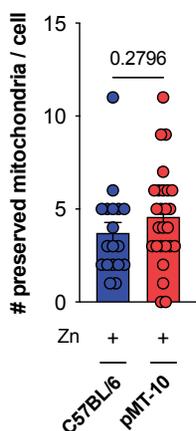
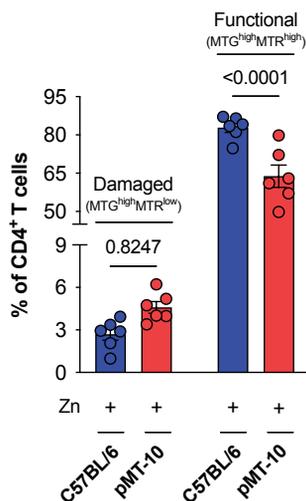
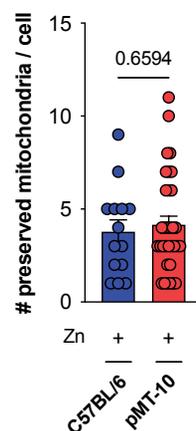
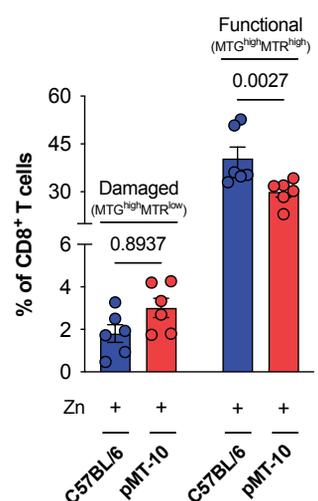
a**CD4⁺ T cells****b****CD8⁺ T cells****c****d****e****CD4⁺ T cells****f****CD8⁺ T cells****g****h****i****j****Figure S2**

Figure S2. Senescent T cells are found in the spleen of IL-10-induced mice. C57BL/6 and pMT-10 were induced with zinc-enriched (+) water for 30 days and, at that time point, their spleens were harvested and T cell populations analysed. Frequency of naïve ($CD44^+ CD62L^+$) and activated ($PD-1^+ CD38^+$) T cells in $CD4^+$ (a) and $CD8^+$ (b) T cells and of SA- β -gal $^+$ and γ H2AX $^+$ $CD4^+$ (c) and $CD8^+$ (d) T cells determined by flow cytometry. Representative TEM images of $CD4^+$ (e) and $CD8^+$ (f) T cells of induced BL/6 and pMT-10 mice. The arrows indicate mitochondria. TEM images were used to quantify the number of preserved mitochondria per cell for $CD4^+$ (g) and $CD8^+$ (i) T cells. In parallel, the frequency of functional and damaged mitochondria in $CD4^+$ (h) and $CD8^+$ (j) T cells was evaluated by flow cytometry using Mitotracker Green/Red levels. (a-d, h, j) Bars represent the mean \pm s.e.m. for 6 – 14 mice, from 1 – 3 independent experiments. Each dot represents a single mouse. (g, i) Bars represent the mean \pm s.e.m. of preserved mitochondria per analysed cell from 3 mice, in one experiment. Each dot represents a single cell. Data were analyzed with Student's t test. *p* values are shown comparing control (C57BL/6) to pMT-10 induced by (+) Zn.

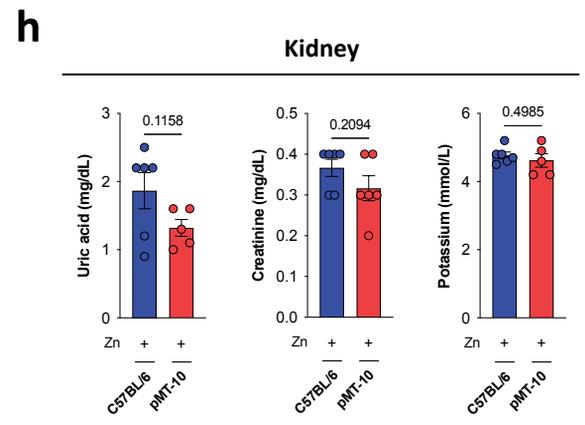
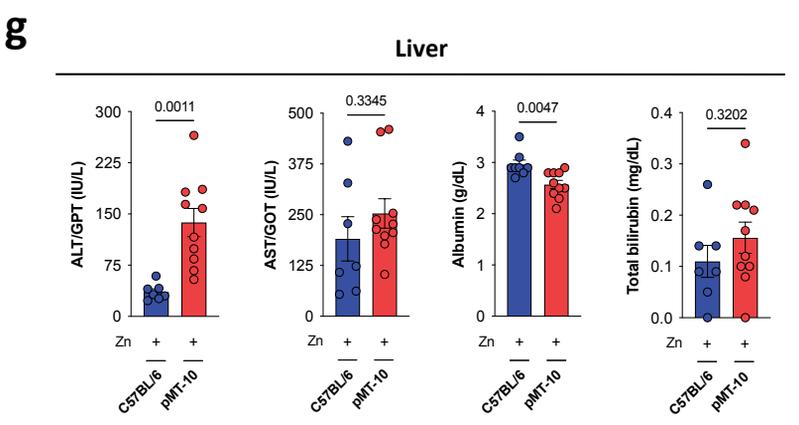
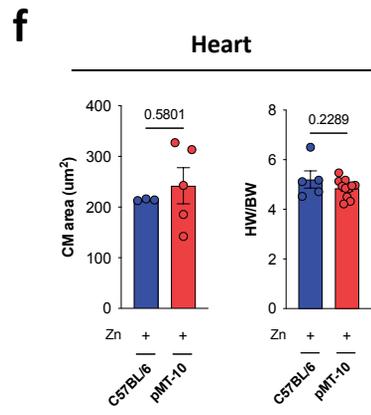
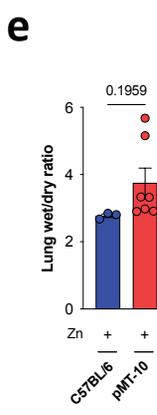
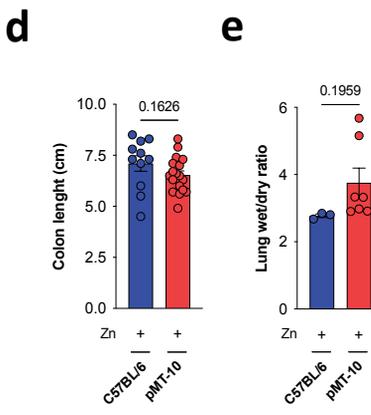
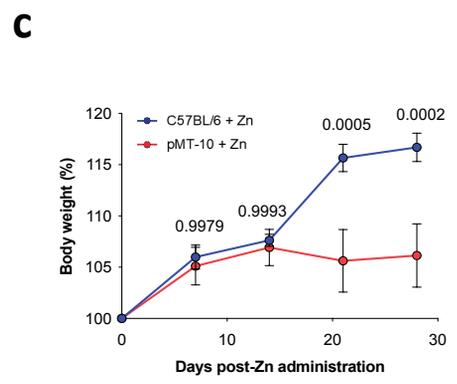
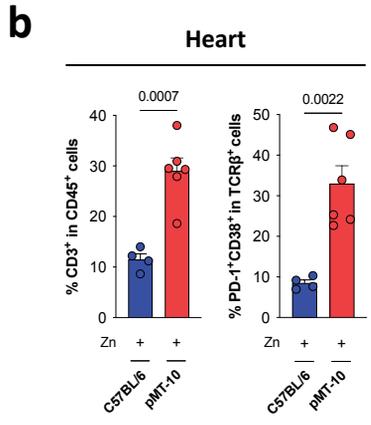
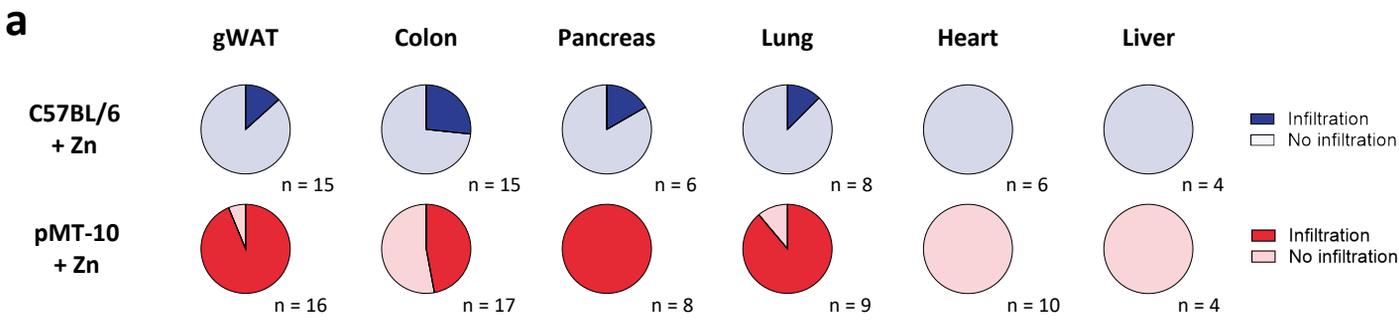


Figure S3

Figure S3. IL-10 elevation causes structural and functional alterations in multiple non-lymphoid organs. C57BL/6 or pMT-10 were fed with zinc-enriched water for 30 days and, at that time point, different analyses were performed. **(a)** Hematoxylin and eosin-stained sections of the indicated organs were screened for the presence or absence of immune cell infiltration. *n* indicates number of animals analysed. **(b)** Frequency of CD3⁺ T cells within the CD45⁺ population and of PD1⁺CD38⁺ cells within the TCRβ⁺ population in the heart of the indicated animals was measured by flow cytometry. **(c)** Induced C57BL/6 or pMT-10 mice were weighted every week up to 30 days post-induction. Represented is the body weight variation over time. **(d)** Colon length measured after the removal of fecal matter. **(e)** Weight ratio of wet (immediately after organ harvesting) and dry lung tissue. **(f)** Alterations to the cardiac tissue were assessed by cardiomyocyte (CM) area and heart weight vs. body weight (HW/BW) ratio. Liver **(g)** and kidney **(h)** functions were evaluated by measuring the serum levels of the indicated enzymes. (b, d-h) Bars represent the mean ± s.e.m. for 3 – 18 mice, from 2 – 5 independent experiments. Each dot represents a single mouse. Data were analyzed with Student's t test. (c) Each dot represents the mean ± s.e.m. for 6 mice, from one experiment. Data were analyzed with two-way ANOVA test. *p* values are shown comparing control (C57BL/6) to pMT-10 induced by (+) Zn.

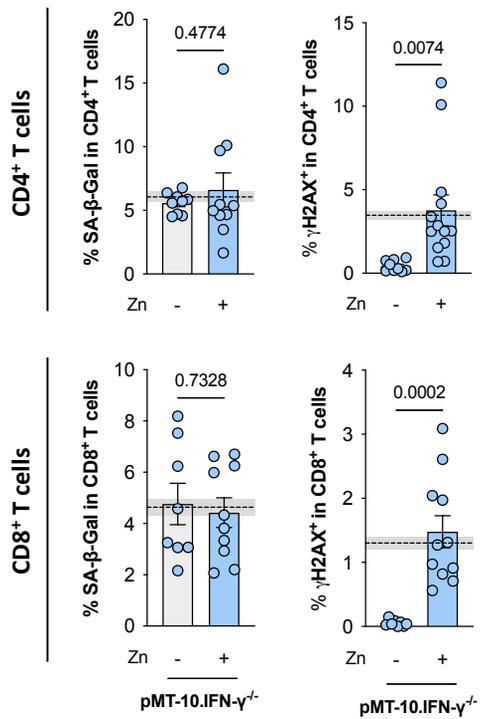
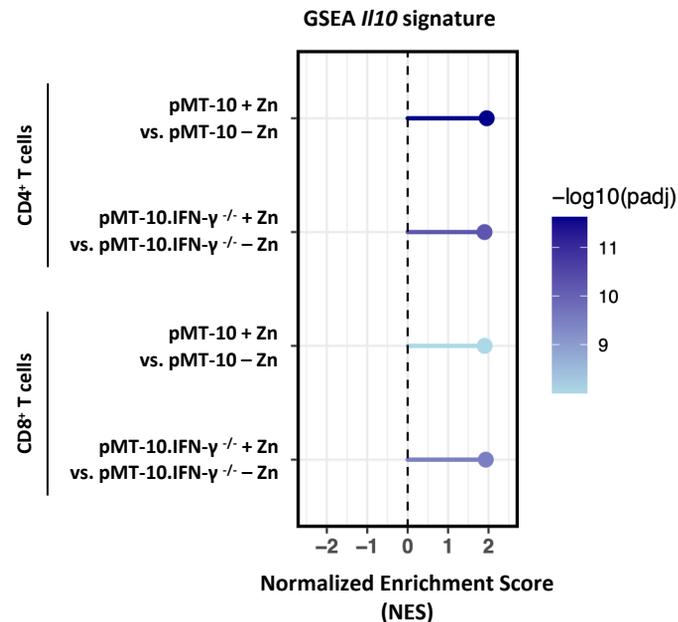
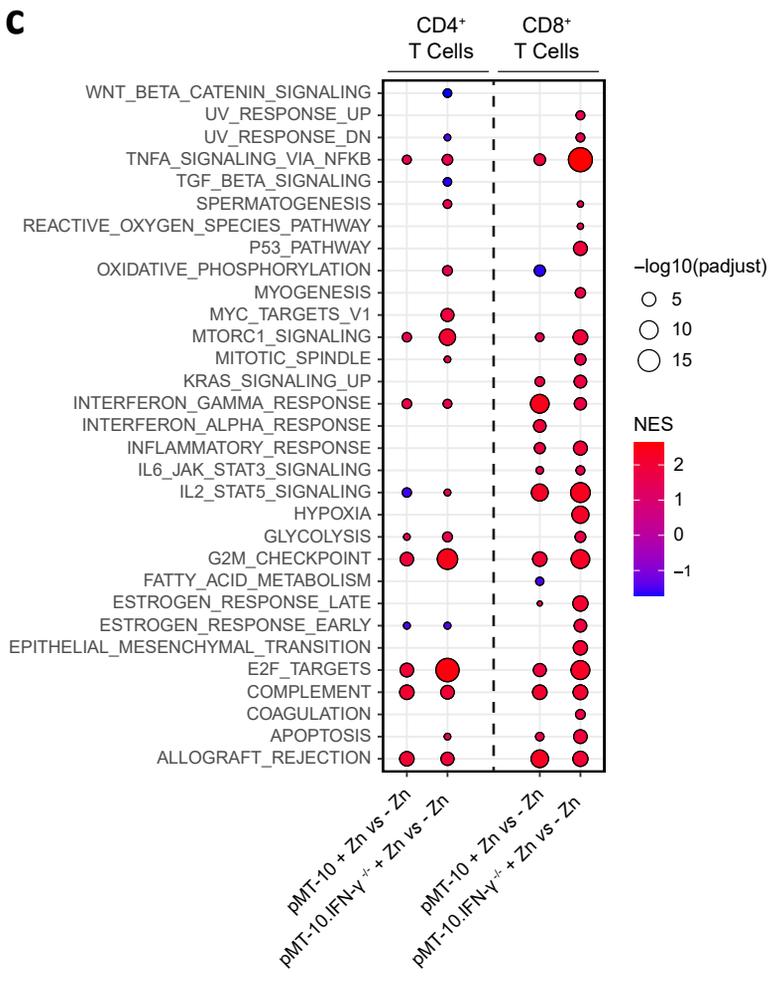
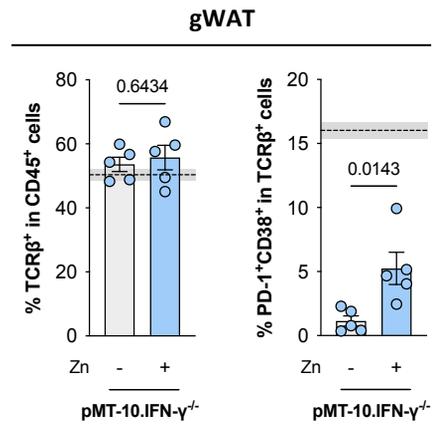
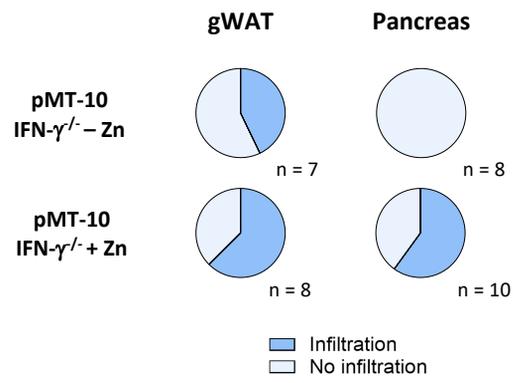
a**b****c****d****e**

Figure S4

Figure S4. Contribution of IFN- γ to the IL-10-induced T cell reprogramming and downstream features in the adipose tissue and pancreas. pMT-10 or pMT-10.IFN $\gamma^{-/-}$ mice were fed for 30 days with control (- Zn) or zinc-enriched (+ Zn) water and, at that time point, different analyses were performed. **(a)** Frequency of SA- β -gal $^{+}$ and γ H2AX $^{+}$ cells within the bone marrow CD4 $^{+}$ and CD8 $^{+}$ T cell subsets. **(b)** GSEA analysis comparing the enrichment of the *Il10* T cell signature genes in CD4 $^{+}$ and CD8 $^{+}$ T cells of pMT10 or pMT-10.IFN $\gamma^{-/-}$ fed with control or zinc-enriched water. **(c)** GSEA analysis of MSigDB Hallmark pathways enriched in CD4 $^{+}$ and CD8 $^{+}$ T cells under the same comparisons: pMT10 plus vs minus Zn and pMT-10.IFN $\gamma^{-/-}$ plus vs minus Zn. **(d)** Frequency of TCR β^{+} and activated (PD-1 $^{+}$ CD38 $^{+}$) T cells in the gonadal white adipose tissue of the indicated experimental groups. **(e)** Prevalence of immune cell infiltrates detected in histological images analyzed for the indicated organs. (a, d) Bars represent the mean \pm s.e.m. for 5 – 13 mice, from 1 – 3 independent experiments. Each dot represents a single mouse. For comparison, dashed lines represent the mean \pm s.e.m. obtained for pMT-10 + Zn mice. Data were analyzed with Student's t test. *p* values are shown comparing pMT-10.IFN $\gamma^{-/-}$ control (-) and induced by (+) Zn.

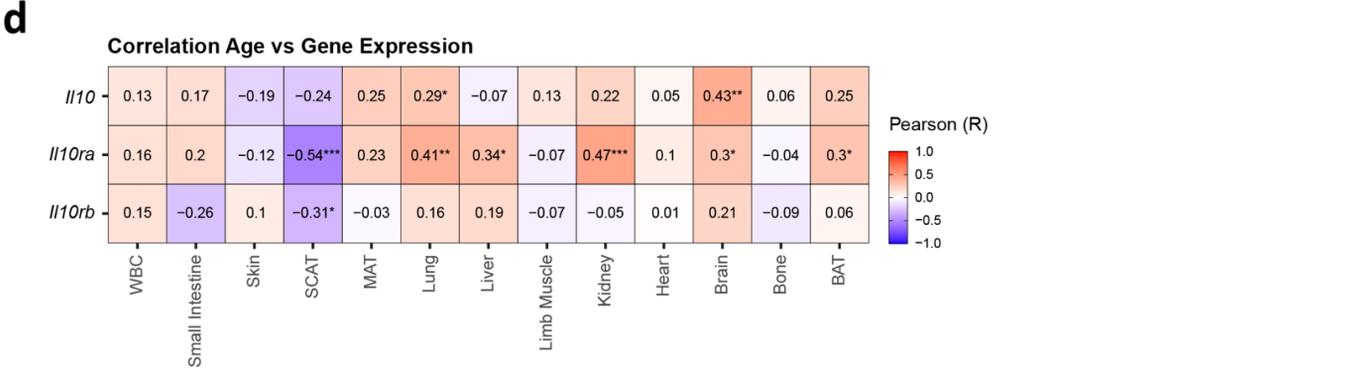
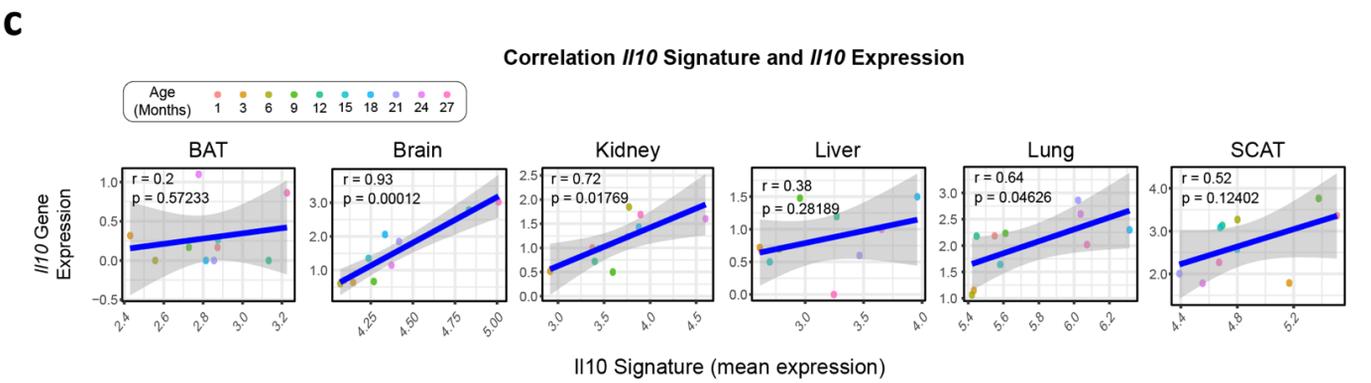
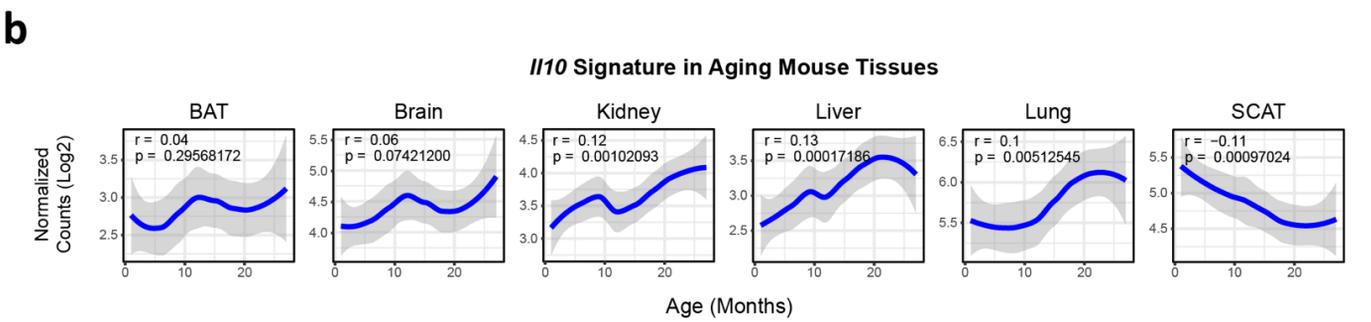
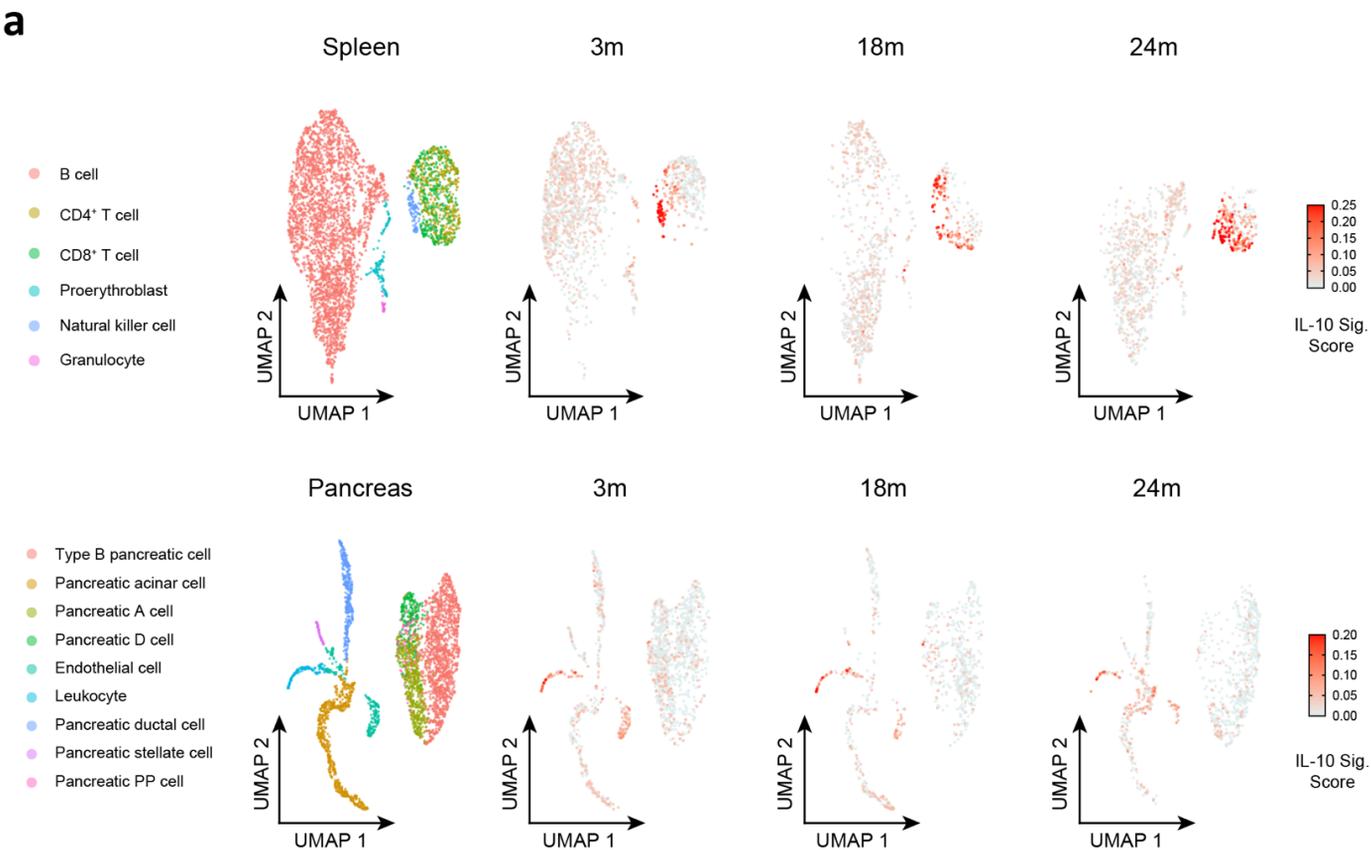


Figure S5

Figure S5. Dynamics of IL-10 and IL-10-derived signature in normative aging. (a) UMAP showing the IL-10-derived T cell signature scores in individual cells of the indicated populations detected in the spleen and pancreas of 3, 18 or 24 months-old mice. Data were extracted from a publicly available single-cell RNA-seq dataset ⁴⁰. (b) Temporal, tissue-specific trajectories of the IL-10-induced signature expression across multiple time points of the mouse life-span, for the indicated tissues. (c) Pearson correlation plots illustrating the relationship between the expression of *Il10* and *Il10* signature across the indicated tissues and the mouse life-span indicated in months. In B,C plots, Pearson correlations (r) and corresponding p values are indicated for each tissue. (d) Pearson correlation between the expression of the *Il10*, *Il10ra* and *Il10rb* genes and the mouse age across the indicated tissues. Statistically significant correlations are indicated: *p<0.05; **p<0.01; ***p<0.001. Data were extracted from the *Tabula Muris Senis* dataset of 17 different aging tissues ⁵¹.

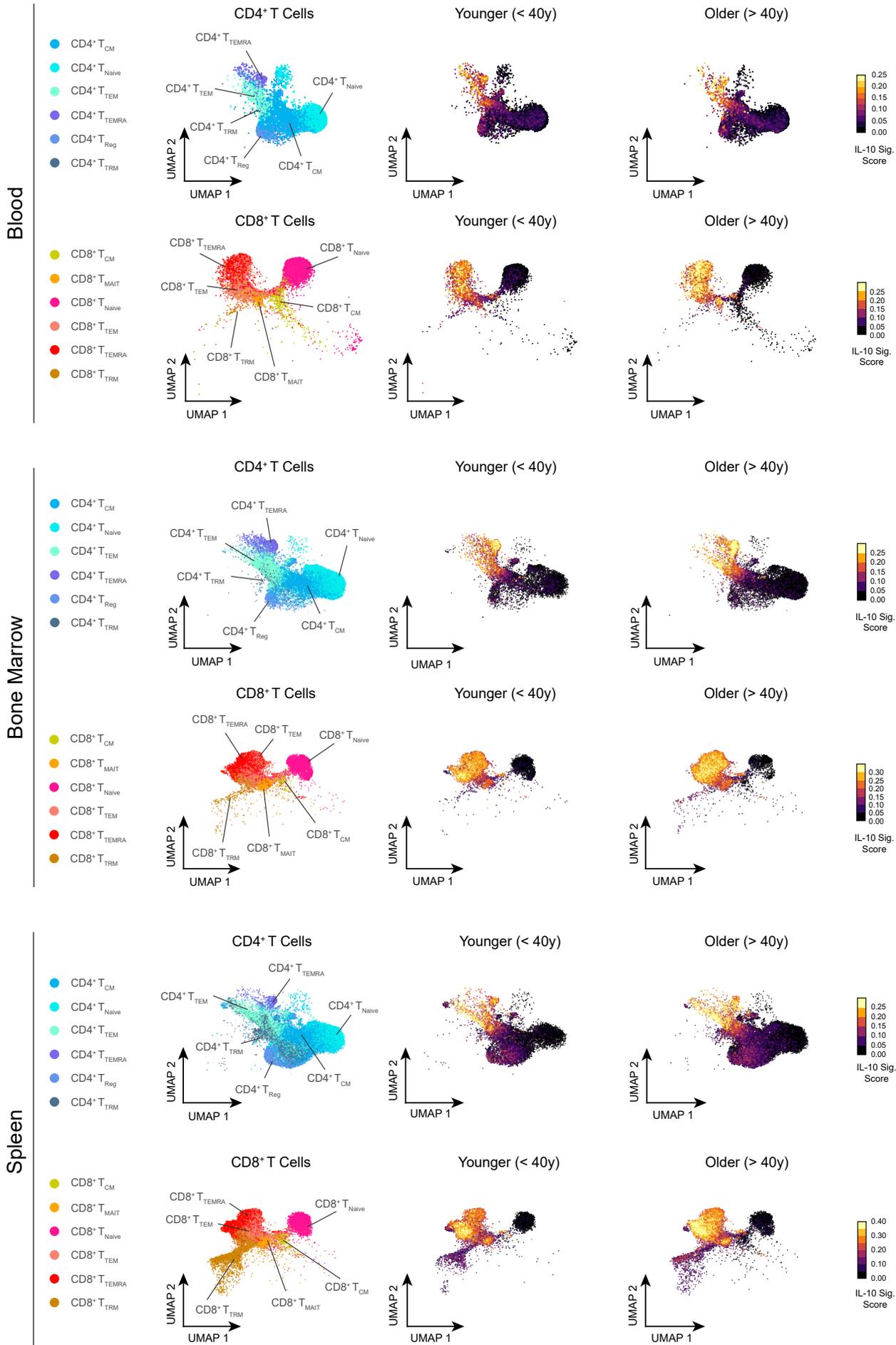
a**Figure S6**

Figure S6. Detection of IL-10-induced T cell-derived transcriptional signature in human CD4 and CD8 T cell subsets during aging. UMAP plots of the IL-10-induced transcriptional signature expression in human CD4⁺ and CD8⁺ T cells derived from the blood, bone marrow and spleen of younger (<40 years old) and older donors (>40 years old). Gene expression scores were obtained using the UCell package. T_{CM}, central memory T cells; T_{TEM}, effector memory T cells; T_{TEMRA}, effector memory re-expressing CD45RA T cells; T_{REG}, regulatory T cells; T_{TRM}, tissue-resident memory T cells; T_{MAIT}, mucosal-associated invariant T cells.

Supplementary Table 1. Log2FC and pAdj values for the genes selected as encoding SASP or exhaustion/senescence markers and analysed in CD4 T cells of the indicated mice.

gene_name	CD4 ⁺ T cells					
	pMT10 + vs - Zn		pMT10 + vs C57/BL6 + Zn		pMT-10.IL-10R $\alpha^{-/-}$ + vs - Zn	
	log2FC	pAdj	log2FC	pAdj	log2FC	pAdj
<i>Ccl3</i>	5,298	0	4,607	1,03E-39	0,087	0,712
<i>Ccl5</i>	4,659	1,27E-60	3,930	1,53E-09	0,163	0,743
<i>Ifng</i>	3,697	4,85E-46	3,361	5,06E-19	1,392	2,59E-24
<i>Ccl4</i>	2,421	7,55E-104	2,291	1,59E-68	0,026	1,000
<i>Il10</i>	1,235	1,31E-15	0,931	9,79E-07	0,303	0,718
<i>tnf</i>	-0,146	0,24	0,148	0,259	0,109	0,681
<i>Gzmk</i>	5,042	1,23E-44	3,966	7,45E-10	0,439	0,456
<i>Gzmb</i>	4,832	2,97E-134	3,687	5,56E-18	0,245	0,756
<i>Cd38</i>	2,356	3,71E-50	2,433	5,07E-43	1,156	1,20E-07
<i>Eomes</i>	3,778	7,78E-83	3,360	4,77E-48	0,032	0,999
<i>Lag3</i>	4,468	1,05E-24	3,592	2,63E-10	-0,775	0,371
<i>Tox2</i>	2,622	9,60E-69	1,589	1,12E-05	-0,461	0,229
<i>Ctla4</i>	-1,308	3,61E-38	-1,198	6,32E-09	0,194	0,634
<i>Pdcd1</i>	1,560	3,91E-19	1,216	2,30E-05	-0,419	0,706
<i>Tigit</i>	1,284	9,25E-34	0,760	1,38E-02	-0,210	0,789
<i>Prfl</i>	2,374	7,78E-33	1,985	1,85E-15	-0,050	0,990
<i>Runx1</i>	0,706	4,61E-15	0,446	0,0025	0,053	0,911
<i>Cish</i>	-0,006	0,734	0,047	0,843	0,140	0,439
<i>Cdkn1a</i>	1,818	2,65E-24	1,575	1,13E-15	0,249	0,223
<i>Cdkn2a</i>	1,174	2,97E-11	1,199	3,55E-11	0,040	0,969
<i>Ccna2</i>	1,288	1,59E-10	0,984	2,06E-04	-0,127	0,907

*red indicates non-significant variations

Supplementary Table 2. Log2FC and pAdj values for the genes selected as encoding SASP or exhaustion/senescence markers and analysed in CD8 T cells of the indicated mice.

gene_name	CD8 ⁺ T cells					
	pMT10 + vs - Zn		pMT10 + vs C57/BL6 + Zn		pMT-10.IL-10R α ^{-/-} + vs - Zn	
	log2FC	pAdj	log2FC	pAdj	log2FC	pAdj
<i>Ccl3</i>	3,817	9,84E-25	3,834	4,01E-28	0,5231	0,066
<i>Ccl5</i>	2,947	1,17E-21	2,917	1,73E-26	0,2605	0,414
<i>Ifng</i>	3,598	1,21E-21	3,447	2,12E-17	1,2167	0,003
<i>Ccl4</i>	1,369	8,93E-15	1,331	6,89E-14	0,5742	2,64E-06
<i>Il10</i>	1,049	9,43E-03	1,048	8,60E-03	-0,0102	0,999
<i>tnf</i>	-0,050	0,932	-0,132	0,506	0,2098	0,116
<i>Gzmk</i>	3,252	1,10E-12	3,324	5,67E-14	0,6881	0,268
<i>Gzmb</i>	6,173	1,57E-52	5,732	8,16E-29	0,8049	0,393
<i>Cd38</i>	1,899	1,17E-08	1,908	1,22E-08	1,0284	0,0002
<i>Eomes</i>	0,732	9,02E-03	0,611	0,0715	0,3566	0,134
<i>Lag3</i>	3,862	2,06E-20	3,782	2,41E-21	-0,0034	0,999
<i>Tox2</i>	1,541	1,01E-04	1,517	1,21E-04	-0,0257	0,999
<i>Ctla4</i>	0,640	3,67E-03	0,640	8,19E-03	0,3220	0,008
<i>Pdcd1</i>	3,341	3,41E-14	3,412	1,11E-12	-0,3852	0,602
<i>Tigit</i>	1,883	3,42E-09	1,792	2,09E-08	0,0619	0,950
<i>Prfl</i>	0,873	0,128	0,602	0,400	0,1507	0,779
<i>Runx1</i>	0,462	0,229	0,374	0,092	-0,0620	0,908
<i>Cish</i>	0,026	0,997	0,013	0,996	0,0541	0,926
<i>Cdkn1a</i>	0,147	0,458	0,098	0,699	0,0328	0,992
<i>Cdkn2a</i>	0,248	0,090	0,247	0,0886	0,0552	0,922
<i>Ccna2</i>	1,191	8,07E-05	1,040	1,89E-03	0,0309	0,999

*red indicates non-significant variations

Supplementary Table 3. Log2FC and pAdj values for the genes selected as encoding SASP or exhaustion/senescence markers and analysed in CD4 T cells of the indicated mice.

gene_name	CD4 ⁺ T cells			
	pMT10 + vs pMT-10.IFN- $\gamma^{-/-}$ + Zn		pMT-10.IFN- $\gamma^{-/-}$ + vs - Zn	
	log2FC	pAdj	log2FC	pAdj
<i>Ccl3</i>	-0,653	3,42E-05	5,924	9,01E-244
<i>Ccl5</i>	-0,220	0,530	5,453	3,02E-185
<i>Ifng</i>	5,717	6,58E-100	–	–
<i>Ccl4</i>	-1,004	2,33E-26	3,405	1,76E-203
<i>Il10</i>	-1,125	4,61E-06	2,346	1,52E-29
<i>tnf</i>	-0,126	0,299	0,217	0,169
<i>Gzmk</i>	-0,712	6,56E-03	6,541	1,57E-193
<i>Gzmb</i>	0,559	2,31E-03	4,306	1,94E-117
<i>Cd38</i>	0,074	0,886	2,547	3,35E-32
<i>Eomes</i>	-0,324	0,099	4,426	4,17E-174
<i>Lag3</i>	-0,265	0,682	5,154	7,31E-78
<i>Tox2</i>	-0,391	0,015	2,819	1,26E-90
<i>Ctla4</i>	-0,800	0,036	-0,126	0,826
<i>Pdcd1</i>	-0,092	0,773	2,362	1,21E-71
<i>Tigit</i>	-0,058	0,756	2,400	1,18E-66
<i>Prfl</i>	-0,907	4,39E-05	2,676	5,11E-74
<i>Runx1</i>	0,511	2,15E-06	0,273	0,021
<i>Cish</i>	0,129	0,342	0,031	0,888
<i>Cdkn1a</i>	-1,282	1,75E-14	2,984	4,10E-117
<i>Cdkn2a</i>	0,070	0,896	1,147	1,35E-13
<i>Ccna2</i>	-1,676	1,56E-08	1,647	2,78E-11

*red indicates non-significant variations

Supplementary Table 4. Log2FC and pAdj values for the genes selected as encoding SASP or exhaustion/senescence markers and analysed in CD8 T cells of the indicated mice.

gene_name	CD8 ⁺ T cells			
	pMT10 + vs pMT-10.IFN- $\gamma^{-/-}$ + Zn		pMT-10.IFN- $\gamma^{-/-}$ + vs - Zn	
	log2FC	pAdj	log2FC	pAdj
<i>Ccl3</i>	-1,124	7,53E-04	4,941	5,16E-123
<i>Ccl5</i>	-0,167	0,616	3,114	4,38E-42
<i>Ifng</i>	4,440	8,48E-37	–	–
<i>Ccl4</i>	-1,539	1,07E-17	2,908	1,15E-84
<i>Il10</i>	-0,658	0,084	1,707	4,69E-27
<i>tnf</i>	-0,168	0,159	0,119	0,359
<i>Gzmk</i>	-1,158	9,57E-03	4,410	1,40E-73
<i>Gzmb</i>	-0,260	0,629	6,433	3,07E-176
<i>Cd38</i>	-0,423	0,323	2,322	3,97E-25
<i>Eomes</i>	-0,682	1,12E-02	1,414	6,60E-16
<i>Lag3</i>	-0,952	2,62E-02	4,814	4,29E-87
<i>Tox2</i>	-0,962	8,14E-03	2,503	3,49E-79
<i>Ctla4</i>	-1,661	4,15E-05	2,300	3,86E-10
<i>Pdcd1</i>	-0,835	0,051	4,176	4,08E-118
<i>Tigit</i>	-0,836	5,79E-03	2,719	4,53E-114
<i>Prfl</i>	-1,098	9,87E-03	1,971	3,43E-29
<i>Runx1</i>	-0,112	0,798	0,574	2,81E-05
<i>Cish</i>	0,042	0,845	-0,016	0,963
<i>Cdkn1a</i>	-1,361	4,47E-08	1,509	1,33E-10
<i>Cdkn2a</i>	-0,556	2,52E-03	0,803	2,11E-07
<i>Ccna2</i>	-1,502	4,20E-04	2,693	8,69E-15

*red indicates non-significant variations

Supplementary Table 5. Antibodies used for flow cytometry analysis.

Anti-mouse Antibody	Fluorochrome	Clone	Source	Identifier
CD3	BV510	145-2C11	BioLegend	Cat: 100353 Lot: B390775
	V450	500A2	BD Horizon	Cat: 560801 Lot: 6238596
CD45	PerCP-Cy5.5	I3/2.3	BioLegend	Cat:147706 Lot: B391648
CD4	BV785	GK1.5	BioLegend	Cat: 100453 Lot: B422958
	APC	GK1.5	BD Pharmingen	Cat: 569845 Lot: 3131914
CD8a	BV650	53-6.7	BioLegend	Cat: 100741 Lot: B417847
	PE-Cy7	53-6.7	BioLegend	Cat: 100722 Lot: B329645
	APC	53-6.7	BD Biosciences	Cat: 553035 Lot: 3183954
TCR β	PE-Cy7	H57-597	BioLegend	Cat: 109221 Lot: B436062
	APC-C7	H57-597	BioLegend	Cat: 109219 Lot: B425945
	BUV737	H57-597	BD Biosciences	Cat: 612821 Lot: 4292059
CD44	APC-Cy7	IM7	BioLegend	Cat: 103027 Lot: B399688
CD62L	PE	MEL-14	BioLegend	Cat: 104408 Lot: B127594
CD279 (PD-1)	BV421	29F.1A12	BioLegend	Cat: 135221 Lot: B431857
CD38	FITC	90	BioLegend	Cat: 102705 Lot: B416720
	APC	90	BioLegend	Cat: 170381
CD11b	APC	M1/70	BioLegend	Cat: 101212 Lot: B386638
Ly-6G/Ly-6C (GR-1)	BV510	RB6-8C5	BioLegend	Cat: 108437 Lot: B401758
CD19	PE	6D5	BioLegend	Cat: 115508 Lot: B120253
yH2AX	Alexa Fluor 647	N1-431	BD Pharmingen	Cat: 560447 Lot: 3247924