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Supplementary Data

Short telomere syndromes cause a primary T cell immunodeficiency

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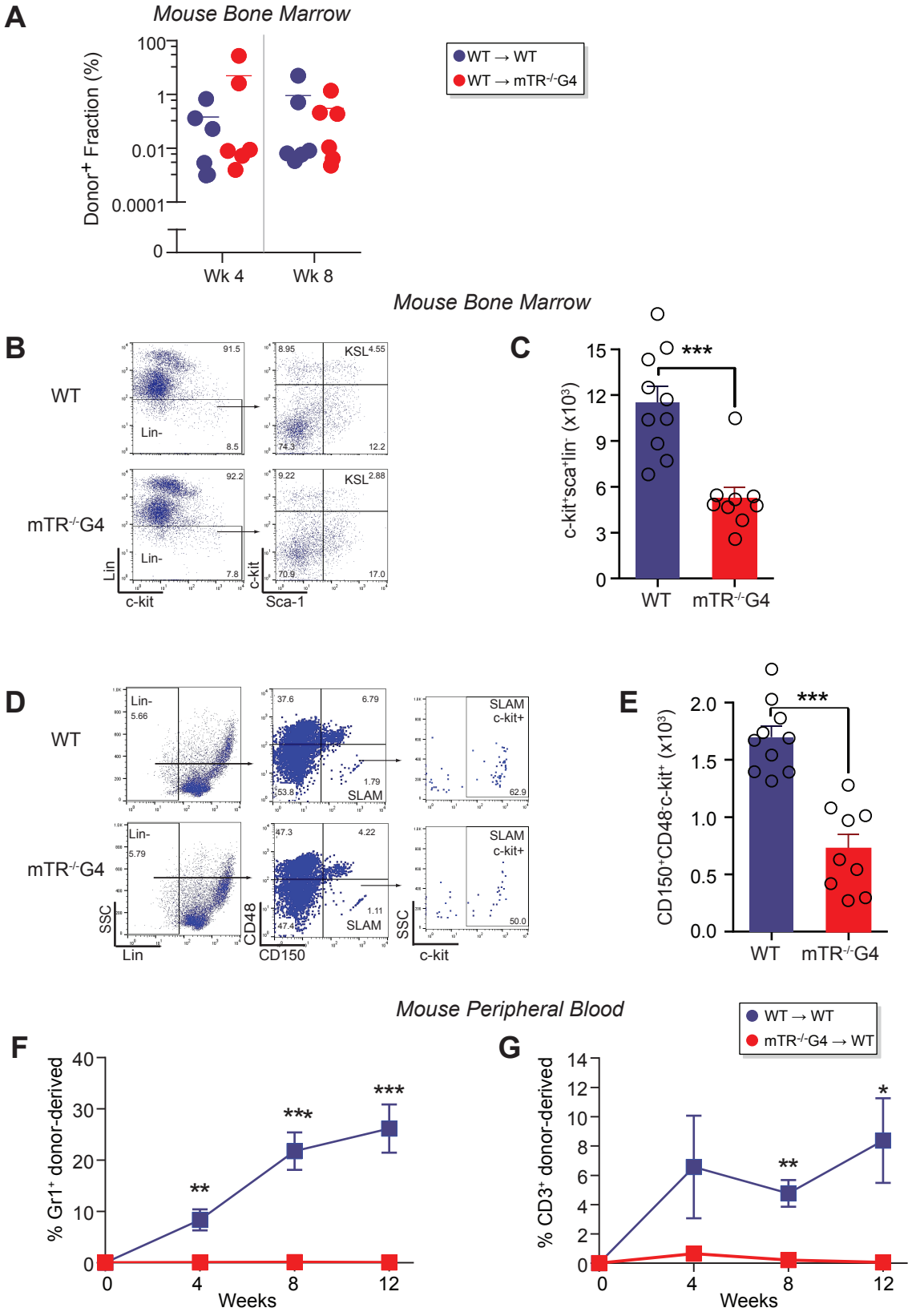
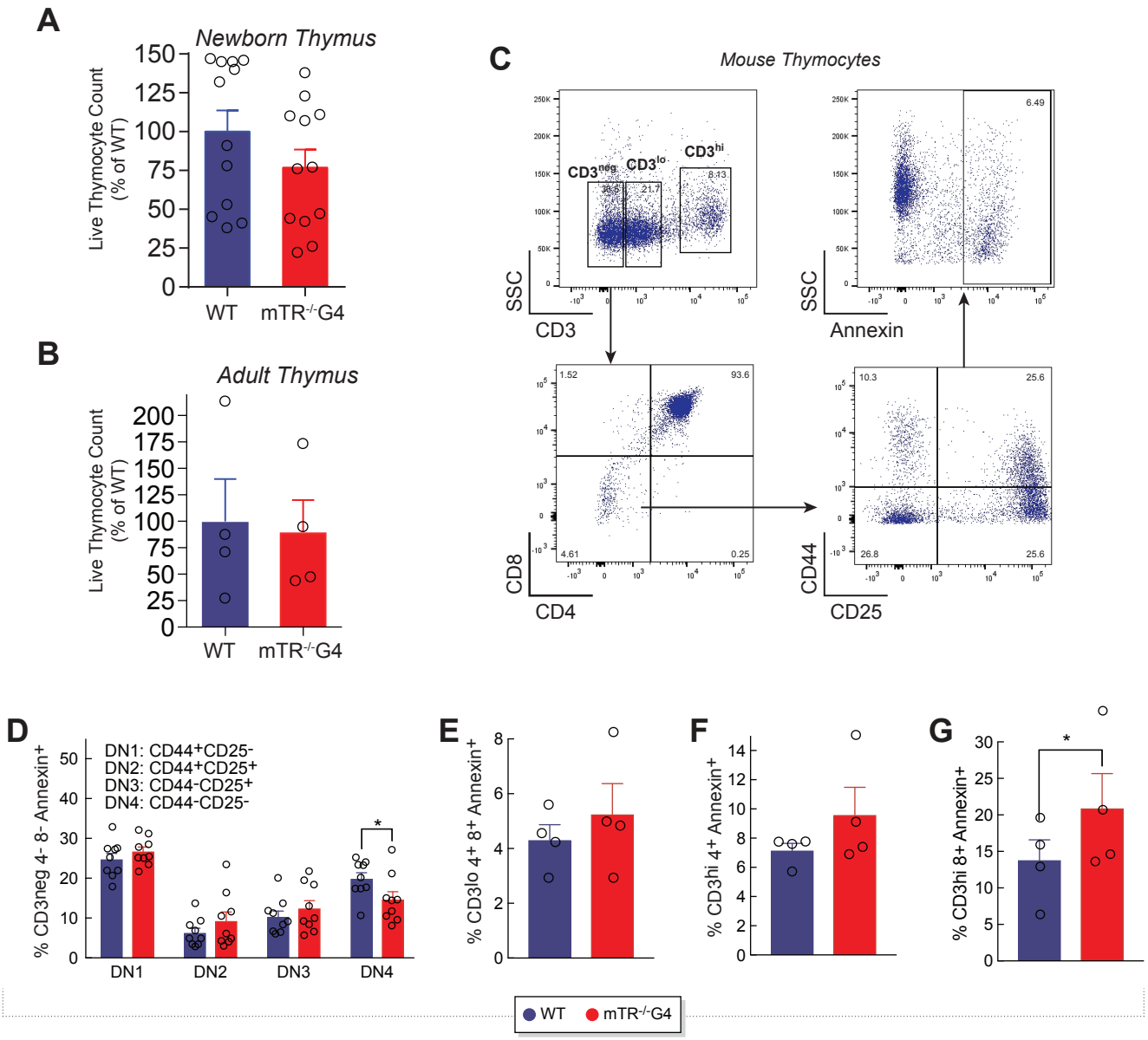
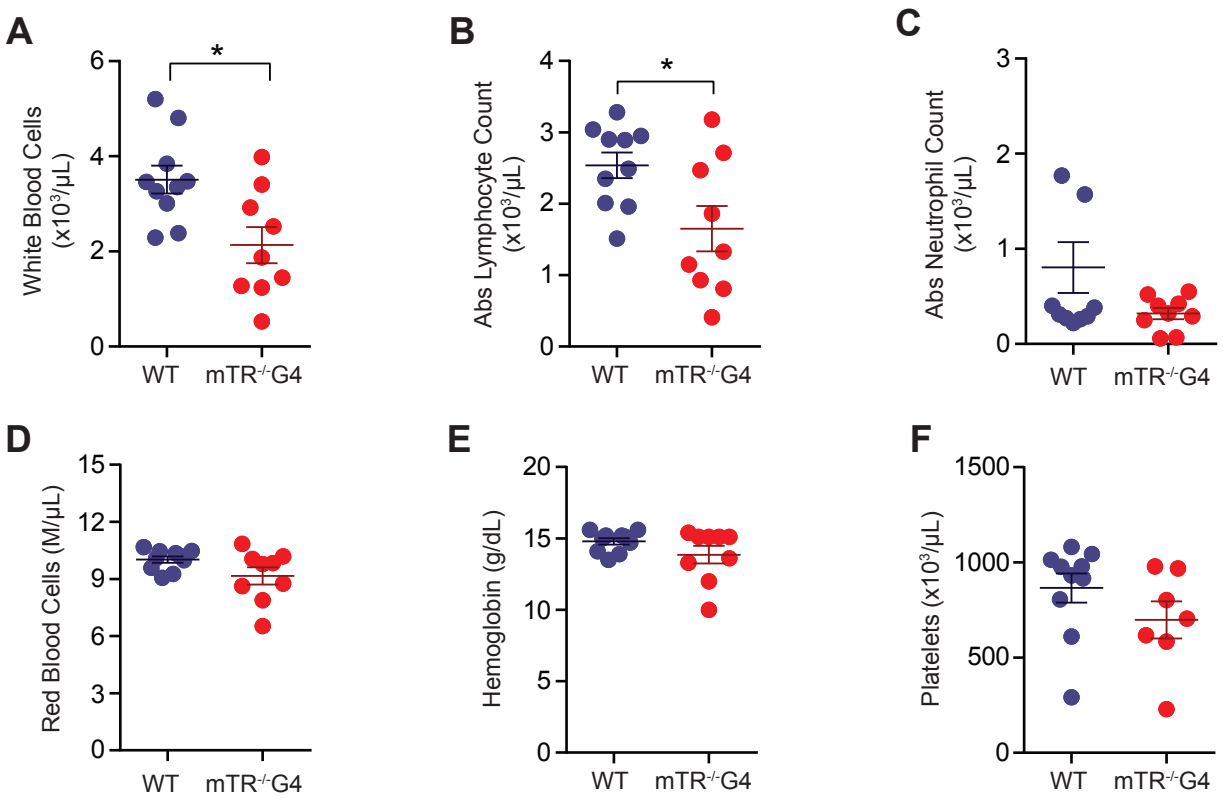
Supplementary Figure 1



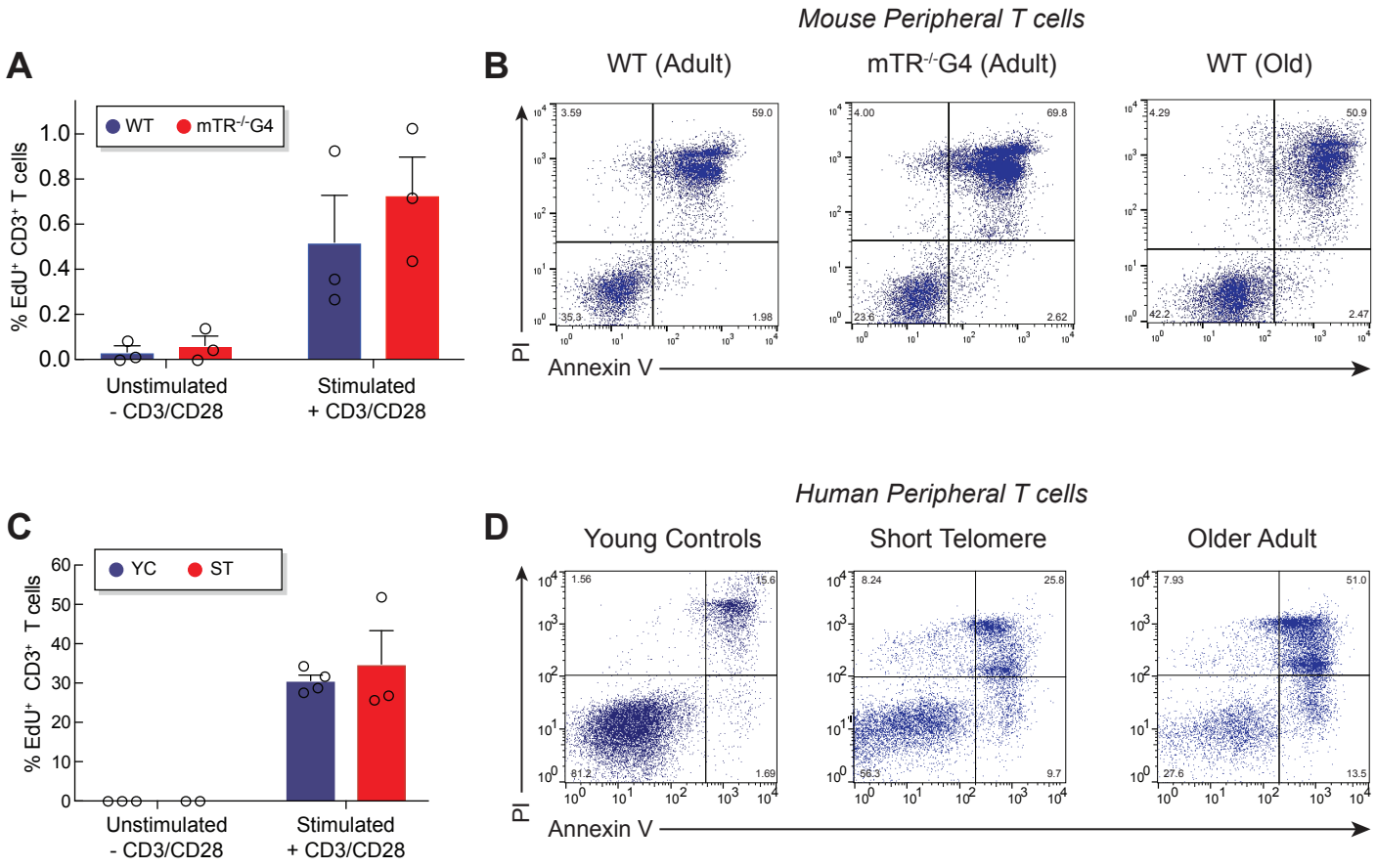
Supplementary Figure 2



Supplemental Figure 3



Supplementary Figure 4



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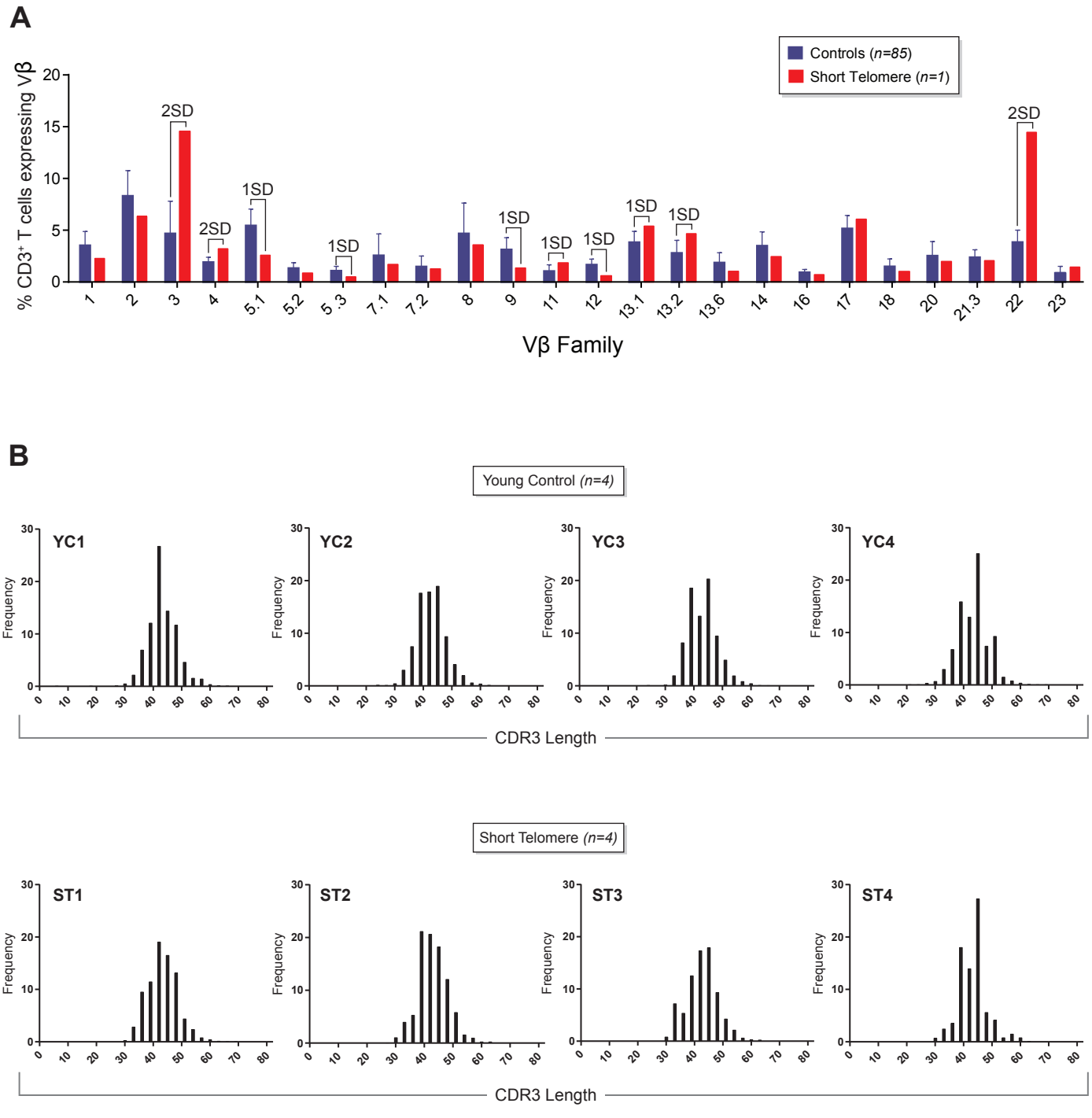
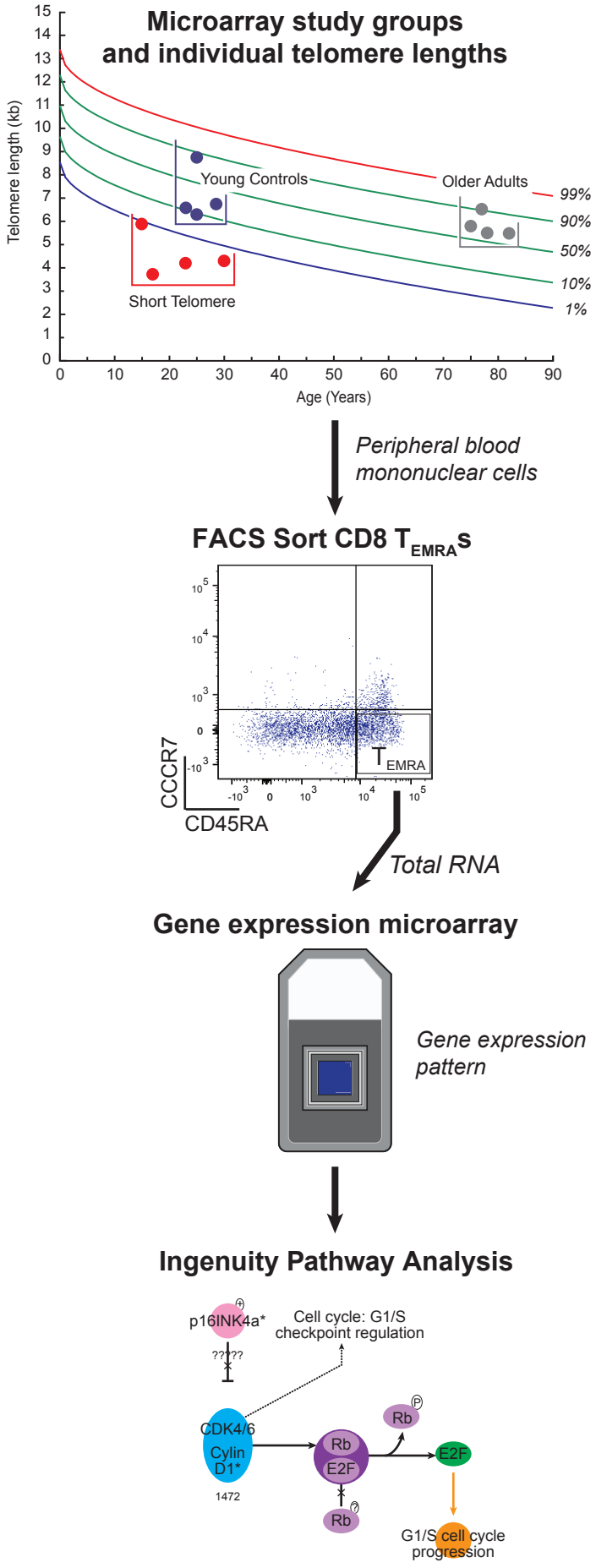
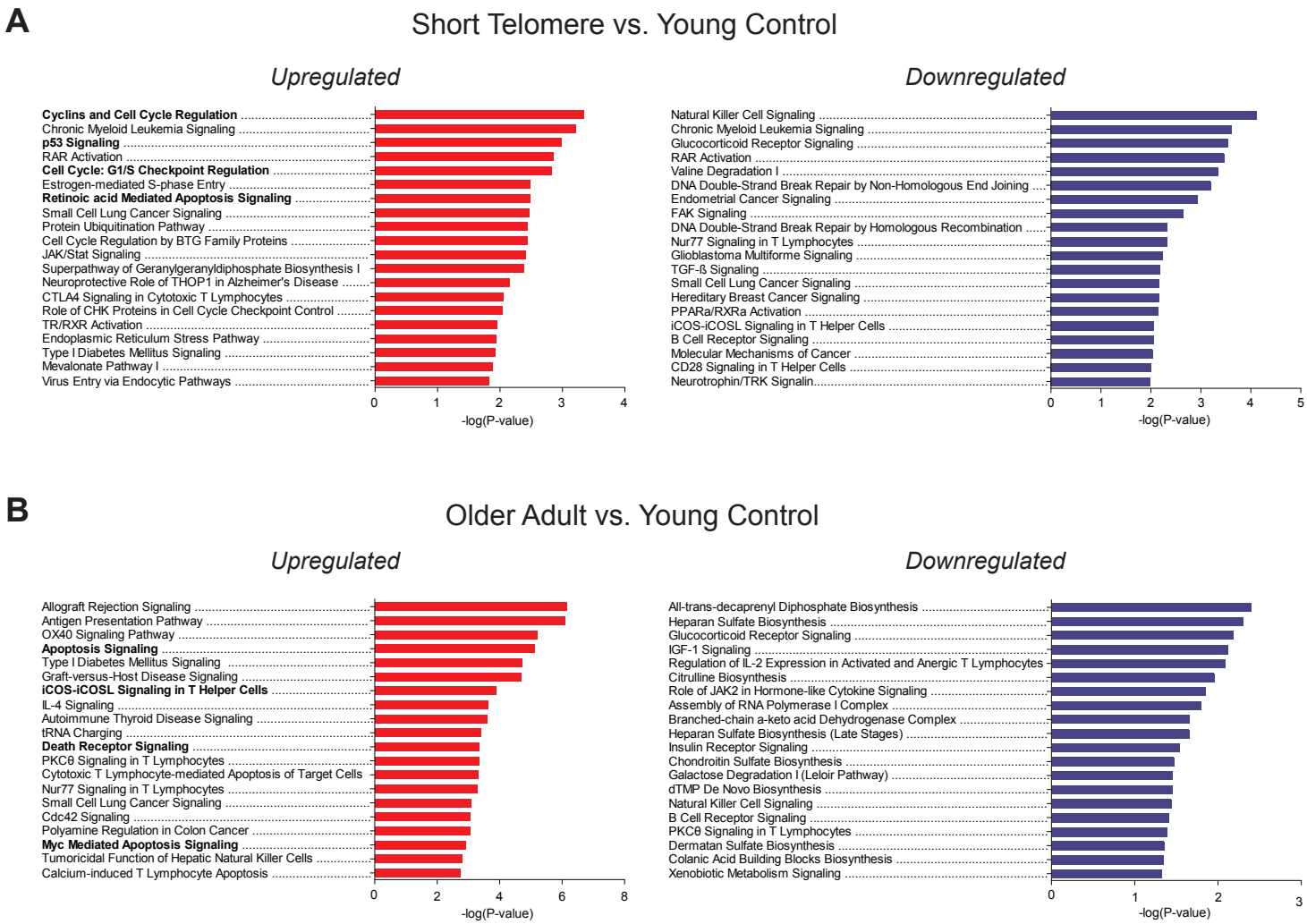


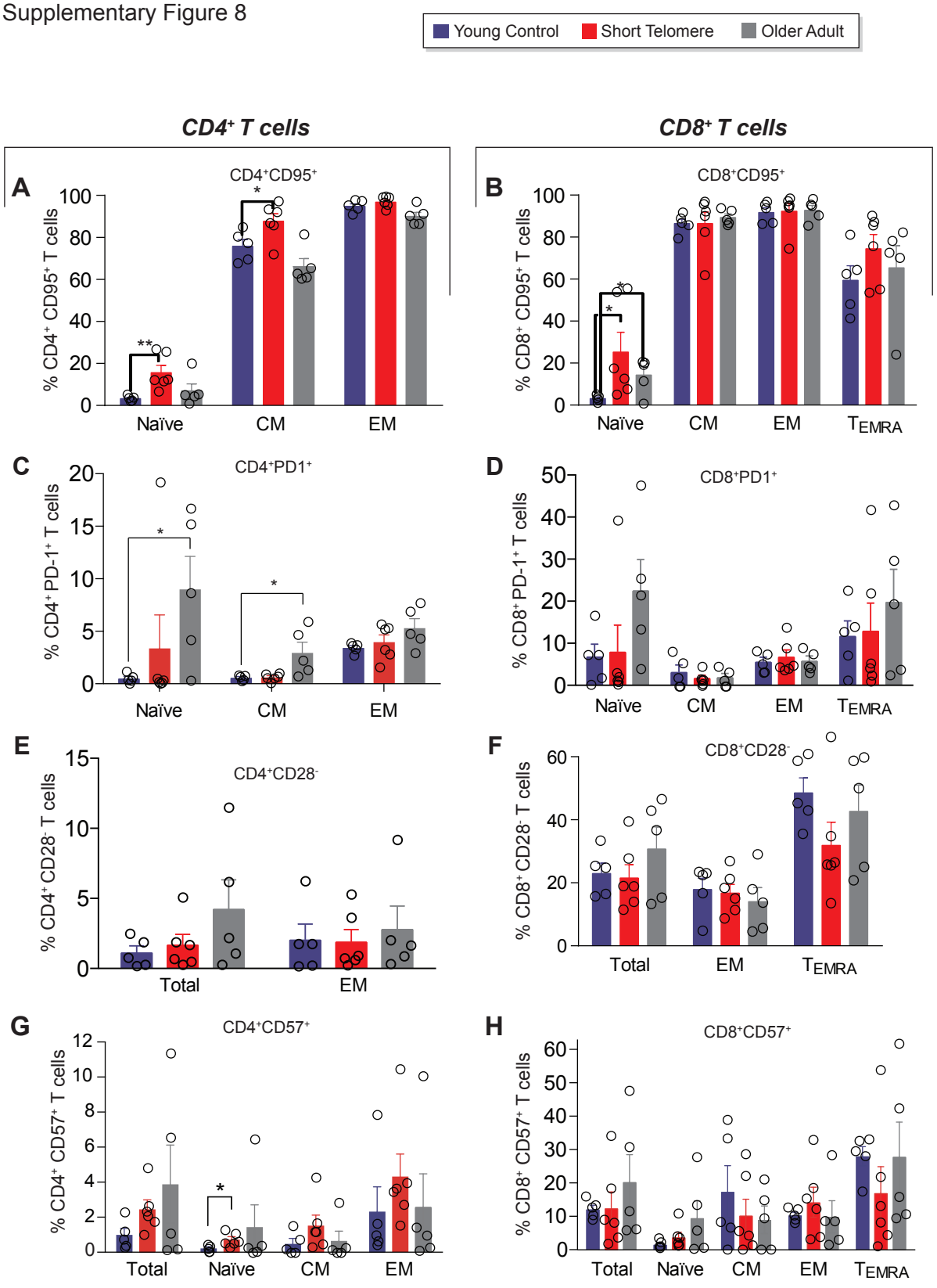
Supplemental Figure 6



Supplementary Figure 7



Supplementary Figure 8



Supplementary Figure Legends

Supplemental Figure 1. Depleted stem cell pools in late generation telomerase RNA null mice, *mTR$^{-/-}$ G4*. **A.** Bone marrow donor-derived engraftment after sublethal irradiation for congenic transplant experimental data shown in Figure 3A-C. **B** and **C**. Representative flow plots and quantification of lineage^{-} c-kit^{+} Sca-1^{+} (KSL) population in *wild-type (WT)* and *mTR$^{-/-}$ G4* mice. **D** and **E**. Representative flow plots and quantification of the bone marrow lineage^{-} c-kit^{+}CD150^{+}CD48^{-} (SLAM) population. For **C** and **E**, *WT* (n=10, 6-16 weeks, 7M/3F) and *mTR$^{-/-}$ G4* mice (n=9, 6-16 weeks, 4M/5F). **F** and **G**. Peripheral blood engraftment after injection of *wild-type (WT)* or *mTR$^{-/-}$ G4* into *wild-type (WT)* mice. Each datapoint represents the mean from 5 individual mice. Donor mice were male and recipients were female. Error bars represent s.e.m and ***P <0.001 (Mann-Whitney test).

Supplemental Figure 2. **A** and **B.** Live thymocyte counts from *wildtype (WT)* and *mTR$^{-/-}$G4* newborn (n=12/genotype, sex undetermined because of early age) and adult mice (all female, n=4/group, 19-20 weeks, representative of 2 experiments), respectively. **C.** Gating strategy for quantifying intra-thymic CD3^{neg}, CD3^{lo} and CD3^{hi} populations. The flowplots shown are representative from a *WT* newborn mouse. The CD3^{neg} CD4^{-}CD8^{-} (DN) population was analyzed with CD25 vs. CD44 gating to determine the relative populations of DN1-4 subsets. **D.** Comparison of thymocyte apoptosis rates in CD3^{neg} CD4^{-} CD8^{-} (double negative, DN) populations 1, 2, 3 and 4 as defined by their cell surface markers shown in the left upper corner (n=9 mice/group, 18-21 weeks, all female). **E.** Apoptotic fraction of CD3^{lo} CD4^{+} CD8^{+} (double positive, DP) thymocytes in adult mice. **F** and **G**. Apoptotic fraction of CD3^{hi} CD4^{+} and CD3^{hi} CD8^{+} thymocytes. For **E-G**, n=4 mice/group, 20-22 weeks, all female and the data are representative of two independent experiments. For panels **D-G**, the apoptotic fraction was quantified as the total Annexin V^{+} population. Error bars represent s.e.m and *P <0.05 (Mann-Whitney test).

Supplemental Figure 3. Lymphopenia in short telomere mice. A-F. Complete blood counts of *wild-type* (*WT*, n=10, 7M/3F, 6-16 weeks) and *mTR$^{-/-}$G4* mice (n=9, 4M/5F, 6-16 weeks) showing abnormally low absolute (Abs) lymphocyte counts. **Fig. 3I** and **J** show the absolute CD4 and CD8 counts, respectively. Error bars represent s.e.m and *P<0.05 (Mann-Whitney test).

Supplemental Figure 4. Mouse and human EdU incorporation with T cell stimulation and representative flow plots of Annexin V staining. A. EdU incorporation in isolated CD3^{+} T cells stimulated with CD3/CD28 ex vivo and quantified at 48h. Unstimulated cells were also included as a negative control. WT and *mTR$^{-/-}$G4* (n=3/genotype). The experiment was replicated twice with similar results and data shown are for one experiment. **B.** Representative flow plots from peripheral mouse splenocyte-derived isolated CD3^{+} T cells, analyzed 48 hours after stimulation with CD3/CD28 antibodies. **C.** EdU incorporation in isolated human CD3^{+} T cells stimulated with CD3/CD28 ex vivo and quantified at 48h. YC represent Young Controls (n=7, 3M/4F) and ST represents short telomere patients (n=5, 1M/4F) studied under both unstimulated

and stimulated conditions. The data are from two independent experiments. **D.** Representative flow plots of CD3+ T cells derived from the 3 groups shown in panel C. They show Annexin V staining at 48 hours after stimulation with CD3/CD28. For **B** and **D**, the total Annexin V^+ PI^{neg} (early) plus Annexin V^+ PI^{lo} (late) apoptotic populations are quantified in **Figures 3K** and **L**, respectively. Error bars represent s.e.m.

Supplemental Figure 5. Raw TCR-Vβ flow cytometry data and complementarity-determining region 3 (CDR3) length by immunoSEQ. A. Example of raw analysis performed to generate the data shown in **Fig. 4A**. The percentage of $CD3^+$ T cells expressing each of 24 Vβ proteins in a patient carrying a *TERT* mutation is plotted relative to data from 85 controls. The error bars in the controls represent +/- 1 standard deviation (SD). This analysis was performed for each of the Young Controls, Short Telomere and Older Adult cases studied in **Fig. 4A. B**. CDR3 length distribution is graphed from data generated by immunoSEQ (Adaptive Technologies, Seattle) on sorted $CD8^+$ T cells from 4 young controls and 4 short telomere subjects (2M, 2F for each).

Supplemental Figure 6. Design and analysis of microarray experiment. At the top, the telogram shows actual lymphocyte telomere length data relative to the age-adjusted nomogram for each of the 12 individuals studied across the three groups. Telomere length was measured by flow cytometry and FISH. $CD8^+$ T_{EMRA} cells were sorted by multi-color flow cytometry using the gating scheme shown. All the short telomere individuals were asymptomatic at the time of lymphocyte procurement. RNA was amplified, reverse-transcribed, and subjected to transcriptional profiling using the Affymetrix GeneChip® PrimeView™ Human Gene Expression Array. The differentially expressed genes were then input into the Ingenuity Pathways Analysis (IPA) platform.

Supplemental Figure 7. Top 20 up- and down-regulated pathways derived from Ingenuity Pathways Analysis (IPA) from differentially expressed genes in sorted $CD8^+$ terminally differentiated effector memory $CD45RA^+(T_{EMRA})$ cells. A. Comparison of short telomere patients with young controls. Up- and down-regulated pathways are shown respectively sorted by the –log(P-value). **B**. The top 20 up- and down-regulated pathways of differentially expressed genes in older adults compared to young controls similarly sorted by P-values. For these analyses, the most highly differentially expressed genes, defined as a minimum of 2 standard deviations, increased or decreased in each of short telomere and older adult subjects relative to the mean for the young control group. P-values were calculated using the Fisher's exact test (one-sided). There was no overlap across the comparisons in the top 20 up-regulated pathways. Three down-regulated pathways were shared: natural killer cell signaling, glucocorticoid receptor signaling and B cell receptor signaling. The list of the differentially expressed genes for each pathway is included in Supplementary Tables 3 and 4, for the short telomere vs. young control and older adult vs. young control comparisons, respectively.

Supplemental Figure 8. Immunophenotype of peripheral T cells from telomerase mutation carriers (ST) compared to age-matched young controls (YC) and older adults (OA). A and **B.** CD95 expression in $CD4^+$ and $CD8^+$ T cell subsets, respectively. **C** and **D.** PD-1 expression on $CD4^+$ and $CD8^+$ T cell subsets, respectively. **E** and **F**.

Percentage of total and T cells subsets without CD28 expression on $CD4^+$ and $CD8^+$ T cells, respectively. Per convention, CD28 loss occurs in the terminally differentiated populations of $CD4^+$ and $CD8^+$ T cells, respectively, and these were the populations studied. Total refers to all $CD4^+$ and $CD8^+$ T cell sub-populations, including the naïve populations, respectively. **G** and **H**. CD57 expression on total and $CD4^+$ and $CD8^+$ T cell subsets, respectively. For **A-H**, YC (n=5-6, 2M/3-4F), ST (n=6-7, 1-2M/5F), and OA (n=5, 3M/2F). Error bars represent s.e.m, *P<0.05 (Student's t-test, two sided). CM, Central Memory; EM, Effector Memory; T_{EMRA}, Effector Memory, $CD45RA^+$.

Supplementary Table 1. Demographics of subjects studied*

	Young Controls **(n=22)**	***Short Telomere*** **(n=16)**	***Older Adults*** **(n=12)**
Mean Age (y) Range	25.8 (14-33)	20.8 (1-39)	73.3 (61-82)
Sex			
Male	12	8	7
Female	10	8	5
Mean deltaT (kb)** Range	-0.40 (-2.0, 1.1)	-3.63 (-4.51, -2.36)	-0.37 (-2.26, 1.36)

*These demographics correspond to the subjects for whom telomere length is shown in Fig. 2a,b. Notably, thte Young Controls listed here include 4 additional healthy individuals for whom telomere length data were not available.

**deltaT refers to the difference of lymphocyte telomere length from the age-adjusted median as measured by flowFISH.

Supplementary Table 2. Mutations of short telomere syndrome subjects studied (n=16)

Age (y)	M/F	First Clinical phenotype	Mutation	Mode of Inheritance	Prior reports[1-7]
0.7	M	Enterocolitis	DKC1 Ala308Gly	*de novo*	Alder et al. 2018
6	M	Aplastic anemia	TR del375-377	AD	Alder et al. 2011
8	F	Aplastic anemia	TERT Phe71Leu	AD	Alder et al. 2018
10	M	Enteropathy	DKC1 IVS1+592C>G	X-linked	Knight et al. 2001
15	M	Sinopulmonary infections	Classic DC	X-linked	n/a
17	F	Asymptomatic*	TERT Lys902Asn	AD	Armanios et al. 2005
20	M	Asymptomatic	TERT Gly135Glu	AD	Gorgy et al. 2015
20	F	CMV pneumonitis *P. jiroveci*	TR U80A	AD	Alder et al. 2018
22	M	Aplastic anemia	Classic DC	AD	n/a
23	M	Aplastic anemia	TERT Gly135Glu	AD	Gorgy et al. 2015
23	F	Asymptomatic	TERT Gly135Glu	AD	Gorgy et al. 2015
30	F	Asymptomatic*	TR del375-377	AD	Alder et al. 2011
32	F	Asymptomatic	TR del375-377	AD	Alder et al. 2011
34	F	Asymptomatic	TR C204G	AD	Fogarty et al. 2003
34	M	Aplastic anemia	DKC1 Leu317Phe	*de novo*	Marrone et al. 2013
39	F	Aplastic anemia	TR C204G	AD	Fogarty et al. 2003

Abbreviations AD, autosomal dominant; CMV, cytomegalovirus; DC, dyskeratosis congenita

*These two individuals were asymptomatic at the time of procurement of lymphocytes but subsequently developed herpes zoster infection

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7. Knight, S.W., Vulliamy, T.J., Morgan, B., Devriendt, K., Mason, P.J., and Dokal, I. 2001. Identification of novel DKC1 mutations in patients with dyskeratosis congenita: implications for pathophysiology and diagnosis. *Hum Genet* 108:299-303.

Supplementary Table 3A. Canonical pathways for up-regulated genes in sorted CD8$^+$ T_{EMRA} cells from individuals with short telomeres compared to young controls

Ingenuity Canonical Pathway	*-log(P-value)***	*R****	*Molecules*
Cyclins and Cell Cycle Regulation	3.34E00	1.43E-01	E2F6,E2F4,HDAC5,SUV39H1,CCNE1,CDKN2A,PPP2R5D,CCNA2,RB1,HDAC3,PPP2CA
Chronic Myeloid Leukemia Signaling	3.22E00	1.3E-01	E2F6,E2F4,HDAC5,NFKB1,GAB2,PIK3C2A,SUV39H1,CDKN2A,RB1,HDAC3,STAT5B,AKT2
p53 Signaling	2.98E00	1.22E-01	CSNK1D,PIK3C2A,MED1,HIF1A,JMY,CDKN2A,RB1,TP53INP1,THBS1,AKT2,PMAIP1,TNFRSF10B
RAR Activation	2.86E00	9.83E-02	REL,ADCY4,NFKB1,RARA,PRKAR2B,CSNK2B,MED1,AKT2,CSNK2A1,SMAD7,SNW1,RELB,RXRA,TNIP1,STAT5B,RAC1,GTF2H1
Cell Cycle: G1/S Checkpoint Regulation	2.83E00	1.43E-01	E2F6,E2F4,HDAC5,SUV39H1,CCNE1,CDKN2A,RB1,HDAC3,MAX
Estrogen-mediated S-phase Entry	2.49E00	2.08E-01	E2F6,E2F4,CCNE1,CCNA2,RB1
Retinoic acid Mediated Apoptosis Signaling	2.48E00	1.38E-01	IRF1,RARA,PARP11,ZC3HAV1,TNKS2,RXRA,CASP3,TNFRSF10B
Small Cell Lung Cancer Signaling	2.47E00	1.27E-01	PIAS3,NFKB1,PIK3C2A,SUV39H1,CCNE1,RXRA,RB1,MAX,AKT2
Protein Ubiquitination Pathway	2.45E00	8.27E-02	DNAJC6,UBE2L3,USP22,DNAJB7,USP14,UBE2V1,DNAJB12,UBE2N,BIRC3,USP36,USP46,PSMC2,HLA-A,USP12,HSPA5,PSMA2,HLA-C,DNAJB4,HSPH1,PSMB2,PSMD2
Cell Cycle Regulation by BTG Family Proteins	2.45E00	1.71E-01	E2F6,E2F4,CCNE1,PPP2R5D,RB1,PPP2CA
JAK/Stat Signaling	2.42E00	1.25E-01	PIAS3,GNAQ,NFKB1,SOCS5,PIK3C2A,SOCS4,SOCS6,STAT5B,AKT2
Superpathway of Geranylgeranyldiphosphate Biosynthesis I (via Mevalonate)	2.38E00	2.5E-01	HMGCS1,HMGCR,FNTB,HADHB
Neuroprotective Role of THOP1 in Alzheimer's Disease	2.15E00	1.5E-01	HLA-A,MME,PRKAR2B,HLA-C,PNOC,GNRH1
CTLA4 Signaling in Cytotoxic T Lymphocytes	2.05E00	1.1E-01	HLA-A,PIK3C2A,CLTA,PPP2R5D,HLA-C,CLTB,AKT2,CTLA4,PPP2CA

Role of CHK Proteins in Cell Cycle Checkpoint Control	2.04E00	1.27E-01	E2F6,E2F4,RFC1,TLK2,PPP2R5D,HUS1, PPP2CA
TR/RXR Activation	1.95E00	1.06E-01	LDLR,PIK3C2A,MED1,HIF1A,RXRA,BCL3, HDAC3,AKT2,THRB
Endoplasmic Reticulum Stress Pathway	1.94E00	1.9E-01	HSPA5,CASP3,MBTPS1,EIF2AK3
Type I Diabetes Mellitus Signaling	1.92E00	9.9E-02	IRF1,NFKB1,HLA-A,SOCS5,HLA-DMA,SOCS4,CASP3,HLA-C,SOCS6,RIPK1
Mevalonate Pathway I	1.88E00	2.5E-01	HMGCS1,HMGCR,HADHB
Virus Entry via Endocytic Pathways	1.83E00	1.01E-01	HLA-A,PIK3C2A,CLTA,DNM2,HLA-C,CLTB,RAC1,TFRC,ITGA5

Supplementary Table 3B. Canonical pathways for down-regulated genes in CD8$^+$ T_{EMRA} cells from individuals with short telomeres compared to young controls

Ingenuity Canonical Pathway	*-log(P-value)***	*R****	*Molecules*
Natural Killer Cell Signaling	4.11E00	2.08E-01	PAK2,PIK3R3,AKT3,RRAS2,CD244,LILRB1,KIR3DL1,KLRB1,KLRC3,PIK3C2B,FCER1G,KLRC1,SOS1,FCGR2A,NCR3,PIK3CA,CD300A,KLRC2,SH3BP2,PRKCB,PTPN6,FCGR3A/FCGR3B
Chronic Myeloid Leukemia Signaling	3.6E00	2.07E-01	CTBP1,TGFB3,HDAC2,CDK4,PIK3R3,AKT3,RRAS2,RBL2,HDAC8,PIK3C2B,IKBKB,SOS1,MYC,PIK3CA,CTBP2,TGFBR1,MDM2,HDAC4,CDK6
Glucocorticoid Receptor Signaling	3.53E00	1.52E-01	HSPA1A/HSPA1B,MAPK14,AKT3,CREB1,PRKAA1,SMARCA2,NR3C2,PRKACB,NCOA3,FOS,A2M,POLR2L,FKBP5,PIK3CA,DUSP1,CD3D,CREBBP,TGFBR1,NFAT5,GTF2E1,TAF15,TGFB3,PIK3R3,RRAS2,NCOA1,SMAD2,PPP3CB,IKBKB,PIK3C2B,HLTF,POLR2C,PPP3CA,SOS1,BCL2,IL5,GTF2H5,FOXO3,PRKAB2,GTF2H1
RAR Activation	3.46E00	1.68E-01	MAPK14,PML,PTEN,AKT3,SMARCA2,NSD1,PRKACB,FOS,PIK3CA,DUSP1,PRKCB,CREBBP,PNPLA4,TGFB3,PIK3R3,RDH11,NCOA1,CSNK2B,SMAD2,RXRB,HLTF,PRMT1,PARP1,PDPK1,SMAD5,PRMT2,GTF2H5,ADCY9,GTF2H1
Valine Degradation I	3.34E00	3.89E-01	HIBCH,HIBADH,AUH,ALDH6A1,DBT,BCKDHB,HADHA
DNA Double-Strand Break Repair by Non-Homologous End Joining	3.19E00	4.29E-01	PARP1,RAD50,XRCC1,PRKDC,XRCC4,MRE11A
Endometrial Cancer Signaling	2.92E00	2.31E-01	CTNNA1,MYC,PIK3R3,PDPK1,AKT3,PTEN,PIK3CA,RRAS2,LEF1,PIK3C2B,FOXO3,SOS1
FAK Signaling	2.63E00	1.86E-01	ASAP1,PAK2,PTEN,PIK3R3,AKT3,TLN1,RRAS2,VCL,PIK3C2B,GIT2,ACTA2,SOS1,PDPK1,CAPN5,PIK3CA,ARHGAP26
DNA Double-Strand Break Repair by Homologous Recombination	2.32E00	3.57E-01	POLA1,RAD52,RAD50,ATRX,MRE11A
Nur77 Signaling in T Lymphocytes	2.32E00	2.17E-01	MAP2K5,HDAC2,CD28,APAF1,HLA-DQA1,CD3D,BCL2,PPP3CB,FCER1G,PPP3CA
Glioblastoma Multiforme Signaling	2.23E00	1.52E-01	PLCB1,CDC42,CDK4,PTEN,PIK3R3,AKT3,PDGFD,RRAS2,LEF1,PIK3C2B,RHOQ,S

			OS1,APC,FZD4,MYC,PIK3CA,MDM2,PLCL2,WNT4,CDK6,TSC1,NF1
TGF-β Signaling	2.18E00	1.72E-01	TGFB3,MAPK14,CDC42,RRAS2,SMAD2,SMURF2,ACVR2A,SOS1,FOS,SMAD5,BCL2,CREBBP,TGFBR1,ACVR1,RUNX2
Small Cell Lung Cancer Signaling	2.17E00	1.83E-01	CDK4,PTEN,PIK3R3,AKT3,APAF1,PIK3C2B,IKBKB,RXRB,MYC,PIK3CA,SKP2,BCL2,CDK6
Hereditary Breast Cancer Signaling	2.17E00	1.61E-01	HDAC2,CDK4,PTEN,PIK3R3,AKT3,RAD50,RRAS2,HDAC8,SMARCA2,PIK3C2B,HLTF,POLR2C,POLR2L,PIK3CA,CREBBP,HDAC4,CDK6,MRE11A
PPARα/RXRα Activation	2.15E00	1.45E-01	PLCB1,TGFB3,MAPK14,RRAS2,SMAD2,PRKAA1,IKBKB,ACVR2A,MED24,PRKACB,CLOCK,SOS1,NCOA3,ACOX1,IL18RAP,AP2A2,PRKCB,CREBBP,TGFBR1,PLCL2,ACVR1,ADCY9,PRKAB2,GPD2
iCOS-iCOSL Signaling in T Helper Cells	2.05E00	1.63E-01	PTEN,PIK3R3,AKT3,HLA-DQA1,PPP3CB,PIK3C2B,IKBKB,FCER1G,PPP3CA,PDPK1,CD28,PIK3CA,TRAT1,CD3D,CAMK2G,NFAT5
B Cell Receptor Signaling	2.05E00	1.43E-01	MAPK14,CDC42,PAG1,PTEN,PIK3R3,AKT3,RRAS2,CREB1,PPP3CB,PIK3C2B,IKBKB,EGR1,GAB1,PPP3CA,SOS1,FCGR2A,PDPK1,PIK3CA,PIK3AP1,CAMK2G,PRKCB,CREBBP,PTPN6,NFAT5
Molecular Mechanisms of Cancer	2.02E00	1.23E-01	MAPK14,CDC42,AKT3,APAF1,PTCH1,RHOQ,GAB1,PRKACB,APC,FOS,HHAT,PIK3CA,PRKCB,CREBBP,XIAP,TGFBR1,CDK6,PRKDC,NF1,CTNNA1,PLCB1,TGFB3,PAK2,CDK4,PIK3R3,RRAS2,SMAD2,LEF1,PIK3C2B,NLK,SOS1,FZD4,MYC,RALBP1,SMAD5,BCL2,CAMK2G,RALA,RASGRF2,WNT4,MDM2,HIPK2,ADCY9,CDC25B
CD28 Signaling in T Helper Cells	1.99E00	1.57E-01	CDC42,PIK3R3,AKT3,HLA-DQA1,PPP3CB,PIK3C2B,IKBKB,FCER1G,PPP3CA,FOS,ARPC5,PDPK1,CD28,PIK3CA,CD3D,PTPN6,NFAT5
Neurotrophin/TRK Signaling	1.97E00	1.79E-01	FOS,MAP2K5,CDC42,PIK3R3,PDPK1,PIK3CA,RRAS2,CREB1,PIK3C2B,CREBBP,GAB1,SOS1

Supplementary Table 4A. Canonical pathways for up-regulated genes in sorted CD8$^+$ T_{EMRA} cells from older adults with normal telomere length compared to young controls

Ingenuity Canonical Pathway	*-log(P-value)**	*R***	*Molecules*
Allograft Rejection Signaling	6.15E00	3.5E-01	HLA-DPB1,HLA-DOA,HLA-DQA1,HLA-DQB1,HLA-DRB4,HLA-DRA,HLA-A,HLA-DRB1,HLA-DMA,TNF,HLA-DMB,HLA-DPA1,HLA-C,FAS
Antigen Presentation Pathway	6.09E00	3.71E-01	HLA-DPB1,HLA-DOA,CD74,HLA-DQA1,HLA-DRB4,HLA-DRA,HLA-A,HLA-DRB1,HLA-DMA,HLA-DMB,HLA-DPA1,HLA-C,CIITA
OX40 Signaling Pathway	5.2E00	2.98E-01	HLA-DPB1,CD3E,HLA-DOA,HLA-DQA1,HLA-DQB1,HLA-DRB4,HLA-DRA,HLA-A,HLA-DRB1,MAP2K4,HLA-DMA,HLA-DMB,HLA-DPA1,HLA-C
Apoptosis Signaling	5.13E00	2.27E-01	MAP4K4,BCL2L11,CAPN1,MAP3K14,IKBKB,BAD,BIRC3,PARP1,CAPN3,MAP2K4,BCL2A1,TNF,RPS6KA1,CASP3,DFFB,FAS,BID,CASP8,MAPK3,SPTAN1
Type I Diabetes Mellitus Signaling	4.72E00	2.08E-01	IRF1,CD3E,HLA-DOA,MYD88,HLA-DQA1,HLA-DQB1,MAP3K14,HSPD1,IKBKB,HLA-DRA,HLA-A,HLA-DRB1,MAP2K4,HLA-DMA,TNF,HLA-DMB,CASP3,HLA-C,FAS,BID,CASP8
Graft-versus-Host Disease Signaling	4.7E00	3.08E-01	HLA-DRB1,HLA-A,HLA-DOA,HLA-DMA,HLA-DQA1,HLA-DQB1,TNF,KIR2DL1/KIR2DL3,HLA-DMB,HLA-C,FAS,HLA-DRA
iCOS-iCOSL Signaling in T Helper Cells	3.89E00	1.94E-01	CD3E,HLA-DOA,PIK3C2A,HLA-DQA1,HLA-DQB1,CHP1,IKBKB,NFATC1,AKT2,BAD,HLA-DRA,HLA-DRB1,PIK3CD,ITPR3,HLA-DMA,CAMK2G,HLA-DMB,RAC1,SHC1
IL-4 Signaling	3.62E00	2.11E-01	RPS6KB2,HLA-DOA,PIK3C2A,HLA-DQA1,HLA-DQB1,JAK3,NFATC1,AKT2,HLA-DRA,HLA-DRB1,PIK3CD,HLA-DMA,HLA-DMB,SYNJ2,SHC1
Autoimmune Thyroid Disease Signaling	3.6E00	2.78E-01	HLA-DRB1,HLA-A,HLA-DOA,HLA-DMA,HLA-DQA1,HLA-DQB1,HLA-DMB,HLA-C,FAS,HLA-DRA

tRNA Charging	3.39E00	2.63E-01	WARS2,CARS2,MARS,HARS,CARS,YARS2,FARSB,AARS2,WARS,QARS
Death Receptor Signaling	3.35E00	1.87E-01	MAP4K4,MAP3K14,IKBKB,ACTA2,BIRC3,TNFRSF10B,PARP1,PARP11,MAP2K4,ZC3HAV1,TNF,CASP3,DFFB,FAS,BID,CASP8,SPTAN1
PKCθ Signaling in T Lymphocytes	3.33E00	1.76E-01	CD3E,HLA-DOA,PIK3C2A,HLA-DQA1,HLA-DQB1,MAP3K14,CHP1,IKBKB,NFATC1,MAP3K13,HLA-DRA,HLA-DRB1,PIK3CD,MAP2K4,HLA-DMA,CAMK2G,HLA-DMB,RAC1,MAPK3
Cytotoxic T Lymphocyte-mediated Apoptosis of Target Cells	3.31E00	3.08E-01	CD3E,HLA-A,CASP3,HLA-C,DFFB,FAS,BID,CASP8
Nur77 Signaling in T Lymphocytes	3.28E00	2.39E-01	CD3E,HLA-DRB1,HLA-DOA,HLA-DMA,HLA-DQA1,HLA-DQB1,CHP1,HLA-DMB,CASP3,NFATC1,HLA-DRA
Small Cell Lung Cancer Signaling	3.1E00	1.97E-01	CCNE2,PIAS3,CDK4,PIK3C2A,CDK2,IKBKB,MAX,AKT2,PIK3CD,SUV39H1,SKP2,TRAF1,RB1,BID
Cdc42 Signaling	3.07E00	1.64E-01	HLA-DPB1,CD3E,HLA-DOA,HLA-DQA1,HLA-DQB1,MYL6B,HLA-DRB4,WASL,APC,CFL2,HLA-DRA,HLA-A,HLA-DRB1,MAP2K4,HLA-DMA,ITGB1,HLA-DMB,HLA-DPA1,HLA-C,EXOC7
Polyamine Regulation in Colon Cancer	3.05E00	3.18E-01	OAZ1,OAZ2,PSMF1,SAT2,MAX,AZIN1,APC
Myc Mediated Apoptosis Signaling	2.92E00	2.07E-01	PIK3CD,PIK3C2A,MAP2K4,CDKN2A,CASP3,FAS,YWHAE,BID,AKT2,SHC1,BAD,CASP8
Tumoricidal Function of Hepatic Natural Killer Cells	2.8E00	2.92E-01	SERPINB9,CASP3,DFFB,FAS,BID,M6PR,CASP8
Calcium-induced T Lymphocyte Apoptosis	2.74E00	2.08E-01	CD3E,PRKCD,HLA-DRB1,HLA-DOA,ITPR3,HLA-DMA,HLA-DQA1,HLA-DQB1,CHP1,HLA-DMB,HLA-DRA
Allograft Rejection Signaling	6.15E00	3.5E-01	HLA-DPB1,HLA-DOA,HLA-DQA1,HLA-DQB1,HLA-DRB4,HLA-DRA,HLA-A,HLA-DRB1,HLA-DMA,TNF,HLA-DMB,HLA-DPA1,HLA-C,FAS

Supplementary Table 4B. Canonical pathways for down-regulated genes in CD8$^+$ T_{EMRA} cells from older adults with normal telomere length compared to young controls

Ingenuity Canonical Pathway	*-log(P-value)***	*R****	*Molecules*
All-trans-decaprenyl Diphosphate Biosynthesis	2.39E00	1E00	PDSS2,PDSS1
Heparan Sulfate Biosynthesis	2.3E00	1.73E-01	CHST2,HS3ST3B1,HS2ST1,GLCE,NDST1,B3GALT6,UST,B3GAT2,EXTL2
Glucocorticoid Receptor Signaling	2.18E00	1.05E-01	HSPA1A/HSPA1B,CREB1,TAF4B,NR3C2,PRKAG2,POLR2L,GTF2F1,CDKN1C,NFAT5,MAPK8,NRIP1,MAP3K7,TGFB3,CHUK,PIK3R3,RRAS2,SMAD2,HLTF,POLR2C,PPP3CA,HSPA4,PPP3R1,BCL2,IL5,PRKAG1,ICAM1,PRKAB2
IGF-1 Signaling	2.12E00	1.35E-01	PIK3R3,IRS2,SOCS5,RRAS2,PRKAG2,CSNK2A1,IGF1R,NEDD4,SOCS7,IGFBP7,MAPK8,PRKAG1,FOXO1
Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes	2.08E00	1.45E-01	MALT1,TGFB3,CD28,CHUK,RRAS2,PPP3R1,SMAD2,NFAT5,MAPK8,PPP3CA,VAV3
Citrulline Biosynthesis	1.95E00	3.75E-01	ALDH18A1,ARG1,GLS
Role of JAK2 in Hormone-like Cytokine Signaling	1.85E00	1.88E-01	SH2B3,SOCS7,IRS2,SOCS5,HLTF,EPOR
Assembly of RNA Polymerase I Complex	1.79E00	3.33E-01	POLR1B,TAF1A,POLR1C
Branched-chain α-keto acid Dehydrogenase Complex	1.65E00	5E-01	DLD,DBT
Heparan Sulfate Biosynthesis (Late Stages)	1.65E00	1.56E-01	CHST2,HS3ST3B1,HS2ST1,GLCE,NDST1,UST,EXTL2
Insulin Receptor Signaling	1.54E00	1.11E-01	PIK3R3,IRS2,RRAS2,PDE3B,PRKAG2,GAB1,GYS2,PPP1R3D,STXBP4,MAPK8,CRK,PRKAG1,SYNJ1,FOXO1
Chondroitin Sulfate Biosynthesis	1.47E00	1.43E-01	CHST2,HS3ST3B1,HS2ST1,NDST1,B3GALT6,UST,B3GAT2
Galactose Degradation I (Leloir Pathway)	1.45E00	4E-01	GALE,GALK2
dTMP De Novo Biosynthesis	1.45E00	4E-01	DHFR,TYMS
Natural Killer Cell Signaling	1.44E00	1.13E-01	PIK3R3,KIR2DL2,RRAS2,KLRC2,PRKD3,KIR3DL1,PAK1,KLRB1,KLRC3,KLRC1,SYNJ1,VAV3
B Cell Receptor Signaling	1.41E00	1.01E-01	MAP3K7,PIK3R3,CHUK,RRAS2,CREB1,EGR1,GAB1,PPP3CA,MALT1,PPP3R1,PIK

			3AP1,MAPK8,NFAT5,MAP3K4,SYNJ1,FOXO1,VAV3
PKCθ Signaling in T Lymphocytes	1.39E00	1.11E-01	MALT1,MAP3K7,PIK3R3,CD28,CHUK,RRAS2,PPP3R1,NFAT5,MAPK8,MAP3K4,PPP3CA,VAV3
Dermatan Sulfate Biosynthesis	1.35E00	1.35E-01	CHST2,HS3ST3B1,HS2ST1,NDST1,B3GALT6,UST,B3GAT2
Colanic Acid Building Blocks Biosynthesis	1.34E00	2.31E-01	MPI,GALE,GALK2
Xenobiotic Metabolism Signaling	1.32E00	9.2E-02	ABCB1,MAP2K5,CHST2,HS3ST3B1,MAP3K7,GSTO2,PIK3R3,CYP3A7,HS2ST1,RRAS2,PRKD3,GSTM3,ALDH18A1,NQO1,UST,ALDH3A2,PPP2R2B,MAPK8,MAP3K4,NDST1,ALDH8A1,CUL3,NRIP1
All-trans-decaprenyl Diphosphate Biosynthesis	2.39E00	1E00	PDSS2,PDSS1

* P-value was calculated by Fisher's exact test (right-tailed).
**R refers to the ratio of the number of genes in the indicated pathway divided by the total number of genes that make up that pathway.

Supplementary Table 5. Human Flow Cytometry Antibodies Utilized (n=26)

Marker	***Fluorochrome***	***Clone***	***Manufacturer***
CD3	AF700	UCHT1	Biolegend
CD3	APC	UCHT1	BD Biosciences
CD3	FITC	UCHT1	Biolegend
CD4	AF488/FITC	RPA-T4	BD Biosciences
CD4	BV605	OKT-4	BioLegend
CD4	PE-CY5	RPA-T4	Biolegend
CD4	PerCP/Cy5.5	RPA-T4	eBioscience
CD8	AF700	OKT-8	eBioscience
CD8	PE-CF594	RPA-T8	BD Biosciences
CD8	V450	RPA-T8	BD Biosciences
CD8a	FITC	RPA-T8	Biolegend
CD8a	PE	RPA-T8	eBioscience
CD19	BV421	HIB19	Biolegend
CD19	BV650	HIB19	Biolegend
CD28	PE-CY7	CD28.2	BD Biosciences
CD31	FITC	WM59	eBioscience
CD31	PE	WM59	Biolegend
CD45RA	APC/CY7	HI100	Biolegend
CD56	PE-CF594	B159	BD Biosciences
CD56	PE-CY7	HCD56	Biolegend
CD57	FITC	HCD57	Biolegend
CD95	PE-CF594	DX2	BD Biosciences
CD197 (CCR7)	BV421	G043H7	Biolegend
CD197 (CCR7)	PE	3D12	eBioscience
CD197 (CCR7)	V450	150503	BD Biosciences
CD279 (PD-1)	APC	ebioJ105	eBioscience

Supplementary Table 6. Mouse Flow Cytometry Antibodies Utilized (n=18)

Marker	***Fluorochrome***	***Clone #***	***Manufacturer***
CD3	AF700	17A2	Biolegend
CD3e	PE	145-2C11	eBioscience
CD4	FITC	RM4-5	Biolegend
CD8a	APC	53-6.7	Biolegend
CD8a	BV605	53-6.7	Biolegend
CD19	PECF594	1D3	BD Biosciences
CD19	PECY7	6D5	Biolegend
CD25	BV421	BC61	Biolegend
CD44	PECY7	IM7	eBioscience
CD45.1	AF700	A20	Biolegend
CD45.1	PE	A20	eBioscience
CD45.2	PECY7	104	BD Biosciences
CD45.2	PerCP-Cy5.5	104	Biolegend
CD48	PECY7	HM48-1	BD Biosciences
CD117 (c-kit)	APC	2B8	eBioscience
CD150 (SLAM)	PE	TC15-12F12.2	Biolegend
Lineage panel: CD3/GR1/CD11b/ CD45R (B220)/Ter-119	FITC	n/a	Biolegend
Ly-6a/e (sca-1)	PE	E13-161.7	Biolegend

mouse T cells
IR total splenocytes

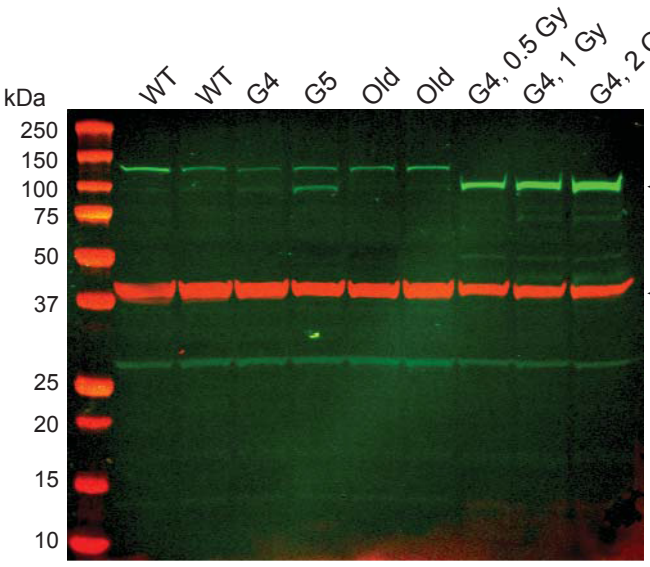
1:2000 Actin
1:500 **pKap1**

Full unedited gel for Figure 5G

The same gel was stripped and re-probed for Kap1.

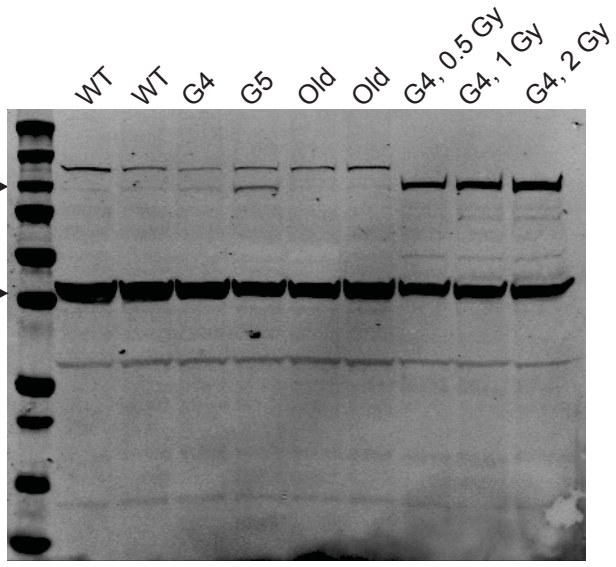
Two colors

8 lanes used for manuscript



Black and white

8 lanes used for manuscript

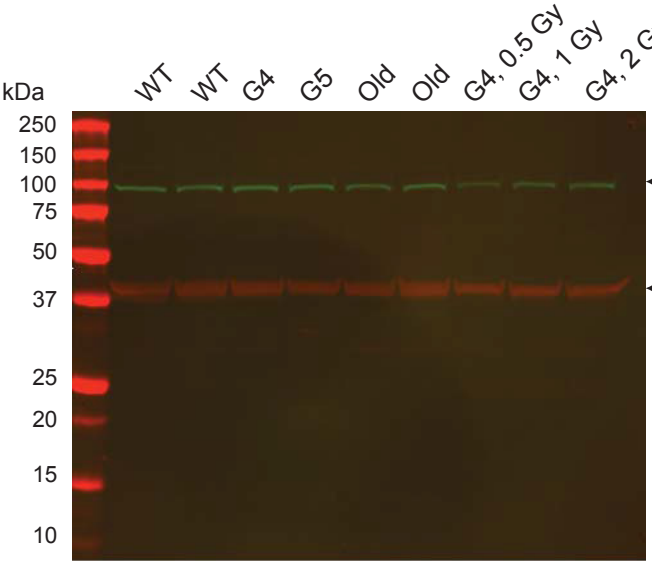


mouse T cells
IR total splenocytes

1:2000 Actin
1:1000 **Kap1**

Two colors

8 lanes used for manuscript



Black and white

8 lanes used for manuscript