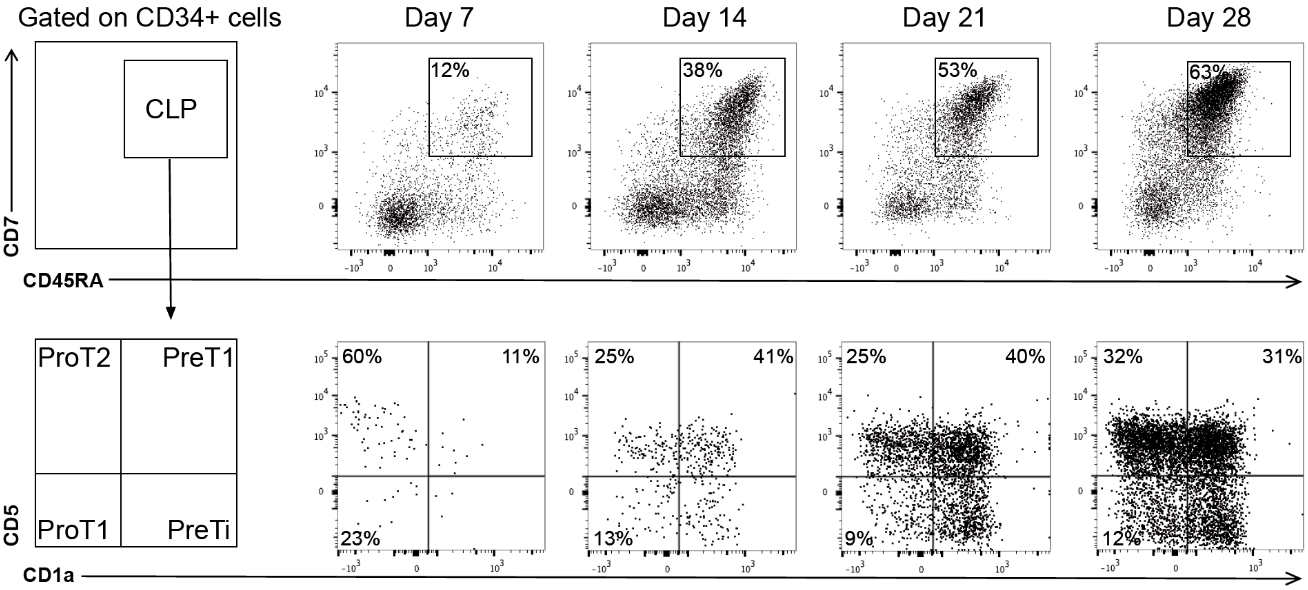
SUPPLEMENTARY FIGURES AND TABLES

Elderly human hematopoietic progenitor cells
express cellular senescence markers and are more susceptible to pyroptosis

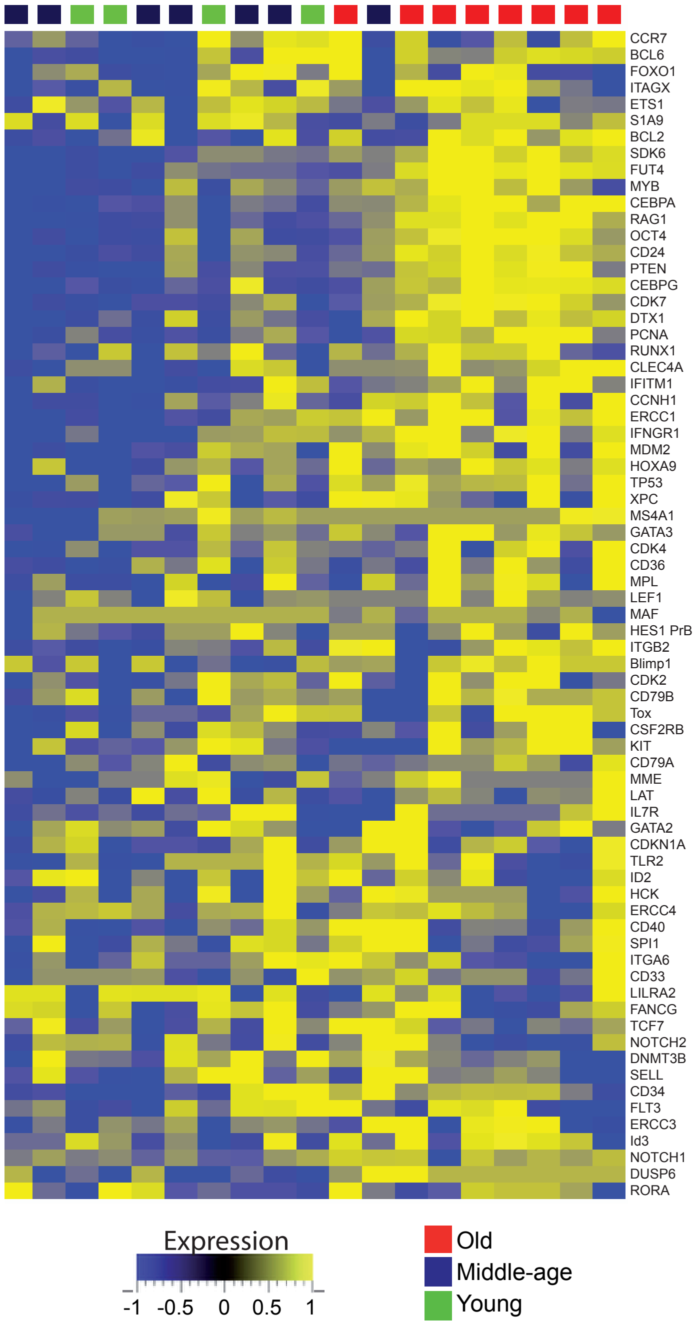
FALI et al.

Fig. S1. Phenotypic characterization of common lymphoid progenitors *in vitro*



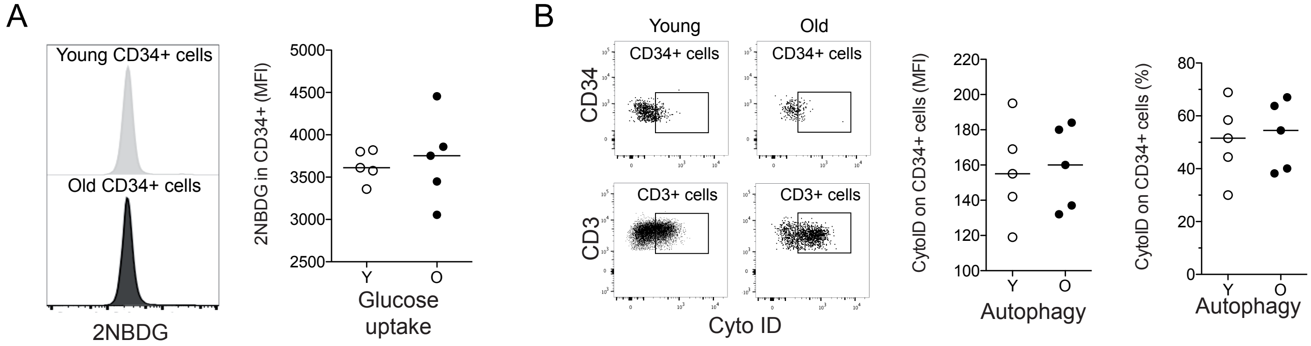
Identification and phenotyping of common lymphoid progenitors (CLP) differentiated *in vitro* from CD34$^+$ cells after 7, 14, 21 and 28 days in the OP9-DL1 culture system. T lymphocyte differentiation subsets were defined as ProT1: CD45RA$^+$ CD7$^+$ CD5$^-$ CD1a$^-$; ProT2: CD45RA$^+$ CD7$^+$ CD5$^+$ CD1a$^-$; PreTimmature: CD45RA$^+$ CD7$^+$ CD5$^-$ CD1a$^+$; and PreT1: CD45RA$^+$ CD7$^+$ CD5$^+$ CD1a$^+$. Numbers indicate percentages of cells within the different quadrants.

Fig. S2. Transcriptional profiling and clustering of CD34$^+$ hematopoietic progenitors from subjects with different age.



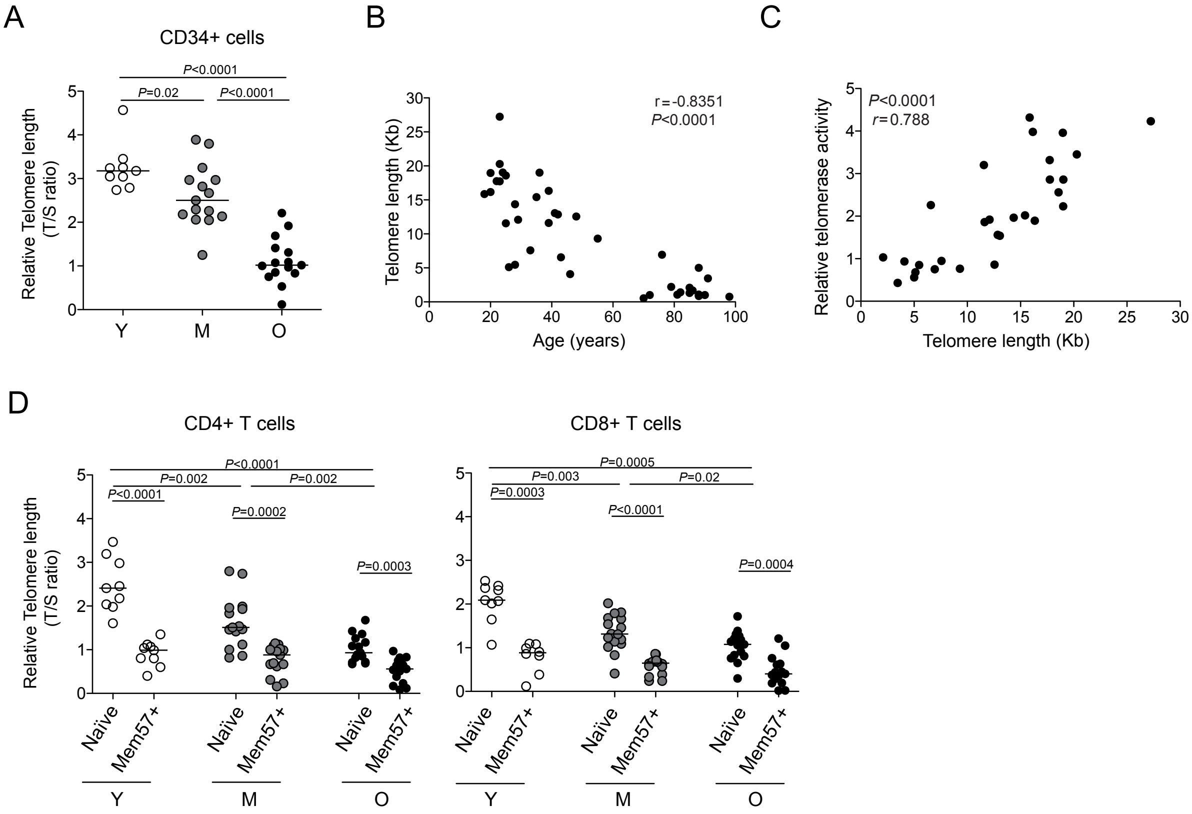
Heat map representation of the transcriptional signature for HPC from young (Y, n=4), middle aged (M, n=7) or old (O, n=8) healthy adults. The expression of 80 selected genes in CD34$^+$ CD45^{low} Lin$^-$ cells, FACS sorted *ex vivo* from donor PBMC, was assessed using Fluidigm microfluidics system.

Fig. S3. Metabolic properties of circulating CD34$^+$ cells



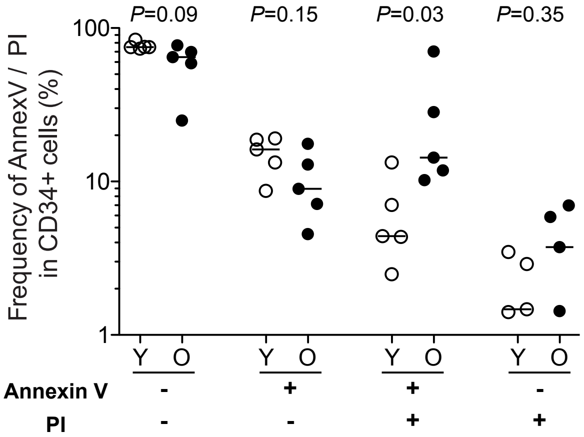
(A) Representative staining and MFI levels for 2NBDG (i.e. glucose fluorescent substitute) uptake in CD34$^+$ cells from young (n=5) or old (n=5) adult PBMC. **(B)** Representative staining in CD34$^+$ or CD3$^+$ cells and MFI levels for Cyto ID (i.e. autophagy marker) in CD34$^+$ cells from young (n=5) or old (n=5) adult PBMC. Bars represent the median.

Fig. S4. Telomere attrition in elderly CD34$^+$ hematopoietic progenitors



Relative telomere length in **(A)** CD34$^+$ HPC from young (Y, n=9), middle aged (M, n=15) or old (O, n=14) healthy adults. Telomere length was measured by qPCR in FACS sorted cells from PBMC, and is expressed as T/S ratio (T: telomere and S: single gene copy). **(B)** Correlation between CLP telomere length and age of donors. **(C)** Correlations between telomere length or telomease activity of CLP. The Spearman's rank test was used to determine correlations. **(D)** Relative telomere length in naïve or senescent CD57+ memory CD4$^+$ or CD8$^+$ T cells from young (Y, n=9), middle aged (M, n=15) or old (O, n=14) healthy adults. Bars indicate the median. The Mann-Whitney test was used for group comparison.

Fig. S5. Assessment of CD34$^+$ cell death using Annexin V and Propidium iodide staining



Staining levels for Annexin V and PI in CD34$^+$ cells from young (open circles) or old (black circles) adult PBMC. Percentages of cells Annexin V -/+ and PI -/+ within CD34$^+$ cells are shown. The Mann-Whitney test was used for comparison. Bars indicate the median.

Table S1. Genes and primers selected for the transcriptional study of CD34^{+} hematopoietic progenitors from old humans

GeneName	Gene description	Ref. ID
PTEN	Phosphatase And Tension Homolog	Hs03673482_s1
CEBP	CCAAT/Enhancer Binding Protein Gamma	Hs01922818_s1
CDK7	Cyclin Dependent Kinase 7	Hs00361486_m1
RAG1	Recombination Activating 1	Hs00172121_m1
POU5F1	POU Class 5 Homeobox 1	Hs00999632_g1
CD24	CD24 Molecule	Hs02379687_s1
CEBPA	CCAAT/Enhancer Binding Protein Alpha	Hs00269972_s1
DTX1	Deltex E3 Ubiquitin Ligase 1	Hs01092201_m1
FUT4	Fucocyltransferase 4	Hs00275643_s1
CDK6	Cyclin Dependent Kinase 6	Hs01026371_m1
MYB	MYB Proto-Oncogene, Transcription Factor	Hs00920554_m1
PCNA	Proliferating Cell Nuclear Antigen	Hs00696862_m1
RUNXL	Runt Related Transcritpion Factor 1	Hs00231079_m1
CLEC4A	C-Type Lectin Domain Family 4 Member A	Hs01087625_m1
IFITM1	interferon induced Transmembrane Protein 1	Hs00705137_s1
p53	Tumor Protein P53	Hs01034249_m1
HOXA9	Homeobox A9	Hs00365956_m1
MDM2	MDM2 Proto-Oncogene	Hs01066942_m1
ERCC1	ERCC Excision Repair 1, Endonuclease Non-Catalytic Subunit	Hs00157415_m1
CCNH	Cyclin H	Hs01025418_m1
IFNGR1	Interferon Gamma Receptor 1	Hs00166223_m1
XPC	XPC Complex Subunit, DNA Damage Recognition And Repair Factor	Hs01104206_m1
MS4A1	Membrane Spanning 4-Domains A1	Hs00544818_m1
GATA3	GATA binding Protein 3	Hs00231122_m1
CDK4	Cyclin Dependent Kinase 4	Hs00175935_m1
CD36	CD36 Molecule	Hs00169627_m1
C-MPL	MPL Proto-Oncogene, Thrombopoietin Receptro	Hs00180489_m1
LEF1	Lymphoid Enhacer Binding Factor 1	Hs01547250_m1
MAF	MAF BZIP Transcription Factor	Hs00193519_m1
HES-1	Hes Family BHLH Transcription Factor1	Hs01118948_g1
ITGB2	Integrin subunit Beta 2	Hs00164957_m1
Blimp-1	Beta-Ineterferon Gene Positive regulating Domain I	Hs00153357_m1
CDK2	Cyclin dependent Kinase 2	Hs00608087_g1
CD79B	CD79b Molecule	Hs00236881_m1
Tox	Thymocyte Selection Associated High Mobility Group Box	Hs01049522_m1
CSF2RB	Colony Stimulating Factor 2 Receptor Bata Common Subunit	Hs00166144_m1
KIT	Kit Proto-Ocogene Receptro Tyrosine Kinase	Hs00174029_m1
CD79A	CD79a Molecule	Hs00233566_m1
MME	Memebrane metalloendopeptidase	Hs00153510_m1
LAT	Linker For Activation Of T-Cells	Hs00175561_m1
Foxo1	Forkhead Box O1	Hs01054576_m1
ITGAX	Integrin subunit Alpha X	Hs01015064_m1
CCR7	C-C Motif Chemokine Receptor 7	Hs99999080_m1
BCL6	B-Cell CLL/Lymphoma 6	Hs00277037_m1
ETS1	ETS Proto-Ocogene 1, Transcription Factor	Hs00428293_m1
S1A9	S100 Calcium Binding Protein A9	Hs00610058_m1
BCL2	BCL2, Apoptosis Regulator	Hs99999018_m1
L7R	Interleukin 7 Receptor	Hs00233682_m1
GATA2	GATA Binding Protein 2	Hs00231119_m1
ID2	Inhibitor of DNA Binding 2, HLH Protein	Hs04187239_m1
HCK	HCK Proto-Oncogene, Src Family Tyrosine Kinase	Hs00176654_m1
TLR2	Toll Like Receptor 2	Hs01014511_m1
CDKN1A	Cyclin Dependent Kinase inhibitor 1A	Hs00355782_m1
ERCC4	ERCC Excision Repair 4, Endonuclease Catalytic Subunit	Hs00193342_m1
CD40	Cd40 Molecule	Hs00374176_m1
SPI1	Hematopoietic Transcription Factor PU.1	Hs02786711_m1
ITGA6	Intergrin Subunit Alpha 6	Hs01041011_m1
CD33	CD33 Molecule	Hs01076281_m1
LILRA2	Leukocyte Immunoglobulin Like Receptor A2	Hs00429044_m1
FANCG	Fanconi Anemia Complementation Group G	Hs00922010_g1
TCF7	Transcription Factor 7 (T-Cell Specific, HMG-Box)	Rh02844810_m1
NOTCH2	Neurogenic Locus Notch Homolog Protein 2	Rh02881159_m1
DNMT3B	DNA Methyltransferase 3 Beta	Hs01003402_m1
SELL	Selectin L (Leukocyte Adhesion Molecule)	Hs00174151_m1
CD34	CD34 Molecule	Hs00990732_m1
FLT3	Fms Related Tyrosine Kinase 3	Hs00975664_m1
ERCC3	ERCC Excision Repair 3, TFIIH Core Helicase Subunit	Hs01554457_m1
ID3	Inhibitor of DNA Binding 3, HLH Protein	Hs00954037_g1
NOTCH1	Neurogenic Locus Notch Homolog Protein 1	Hs01062014_m1
DUSP6	Dual Specificity Phosphatase 6	Hs01044001_m1
RORA	RAR Related Orphan Receptor A	Hs00536545_m1

Table S2. Differentially upregulated genes in CD34$^+$ hematopoietic progenitors from old humans

GeneName	Gene descritption	Y Avg FC	M Avg FC	O Avg FC	p-values
CEBPa	CCAAT/Enhancer Binding Protein Alpha	-2,4196	-1,3885	0,2285	0,0002
RAG1	Recombination Activating 1	-1,1240	-0,6129	1,1154	0,0003
CDK6	Cyclin Dependent Kinase 6	2,0593	1,9882	2,9737	0,0008
CDK7	Cyclin Dependent Kinase 7	-0,6813	-0,1469	0,6877	0,0010
CD15 (FUT4)	Fucocyltransferase 4	-2,1527	-2,2744	-0,3035	0,0014
Oct4 (POU5F1)	POU Class 5 Homeobox 1	0,5381	1,1793	2,4558	0,0014
PTEN	Phosphatase And Tension Homolog	2,0877	2,2631	3,3953	0,0018
CD24	CD24 Molecule	-3,0528	-2,1376	-0,5343	0,0028
C/EBP (Cebpg)	CCAAT/Enhancer Binding Protein Gamma	1,5319	1,5435	2,5918	0,0089
MDM2	MDM2 Proto-Oncogene	0,6185	0,8767	1,4578	0,0153
CD11c (ITGAX)	Integrin subunit Alpha X	-4,2033	-7,6045	-3,7764	0,0184
PCNA	Proliferating Cell Nuclear Antigen	-2,8843	-3,1070	-1,8585	0,0189
CLEC4A	C-Type Lectin Domain Family 4 Member A	-5,4330	-4,6949	-3,6966	0,0199
IFNGR1	Interferon Gamma Receptor 1	-1,5483	-1,8931	-0,9983	0,0204
p53	Tumor Protein P53	1,4414	1,1994	1,7966	0,0211
BCL2	BCL2, Apoptosis Regulator	-4,8703	-4,8892	-3,6456	0,0218
C-MYB (v-myb)	MYB Proto-Oncogene, Transcription Factor	1,9033	1,9980	2,8030	0,0225
HOXA9	Homeobox A9	-1,9430	-1,8006	-1,2293	0,0290
DUSP6	Dual Specificity Phosphatase 6	-3,9821	-3,4279	-2,2305	0,0291
ERCC1	ERCC Excision Repair 1, Endonuclease Non-Catalytic Subunit	-0,2441	-0,2287	0,4640	0,0302
Deltex-1 (DTX1)	Deltex E3 Ubiquitin Ligase 1	-6,3014	-6,1170	-4,4440	0,0405

Statistically significant upregulation of genes in CD34+ HPC from elderly people compared to young and middle age individuals. Multigroup comparison was performed using the Annova test of the Qlucore Omics explorer software.

Table S3. Primers and probes used in RTqPCR

Gene Name	Primer Forward (5'→ 3')	Primer reverse (5'→ 3')
TCF7	AGCCAGAAGCAAGTTCACAGG	GCCTCCTTCTCTGCCTTGGAC
LEF1	GATCACACCCGTCACACATC	ACCCGGAGACAAGGGATAAA
FZD3	CCTTGAGGATGTGCCAAGAT	GAAATCCCGAGAACAATCCA
mTOR	TTGAGGTTGCTATGACCAGAGAGAA	TTACCAGAAAGGACACCAGCCAATG
P70-S6K	GGAGCCTGGGAGCCCTGATGTA	GAAGCCCTCTTTGATGCTGTCC
CLPP	GGCCAAGCCACAGACATT	AGAGCTGCTTCTTGAGCTTCAT
CLPX	GGTGCTTTCAATGGTTTAGACAG	GATGGTGTTCCAAATCCAAGA
LON	TCATGGTGGAGGTAGAGAACG	TCATGGTGGAGGTAGAGAACG
HSP60	GTGGAAAAAGGAATCATTGACC	GTAGTTAACAGAGAGGCCACACC
HSP70	CCCTCTTCGCCATCTCCT	GCCCTCATCAACACAGAGAAG
P2X7	ACACCTTCCCTTTGCAGGGGAACT	TCGATGGGGCACCAGGCAGA
TP53	ATGGCGACTGTCCAGCTTTG	GACGCTAGGATCTGACTGCG
p15 (CDKN2A)	GCGTTCACTCCAATGTCTGCTG	TCCACTTTGTCCTCAGTCTTCAGG
p21 (CDKN1A)	CGAAGTCAGTTCCTTGTGGAG	CATGGGTTCTGACGGACAT