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Supplemental Information

Brain Endothelial Cells Are Exquisite Sensors of Age-Related Circulatory Cues

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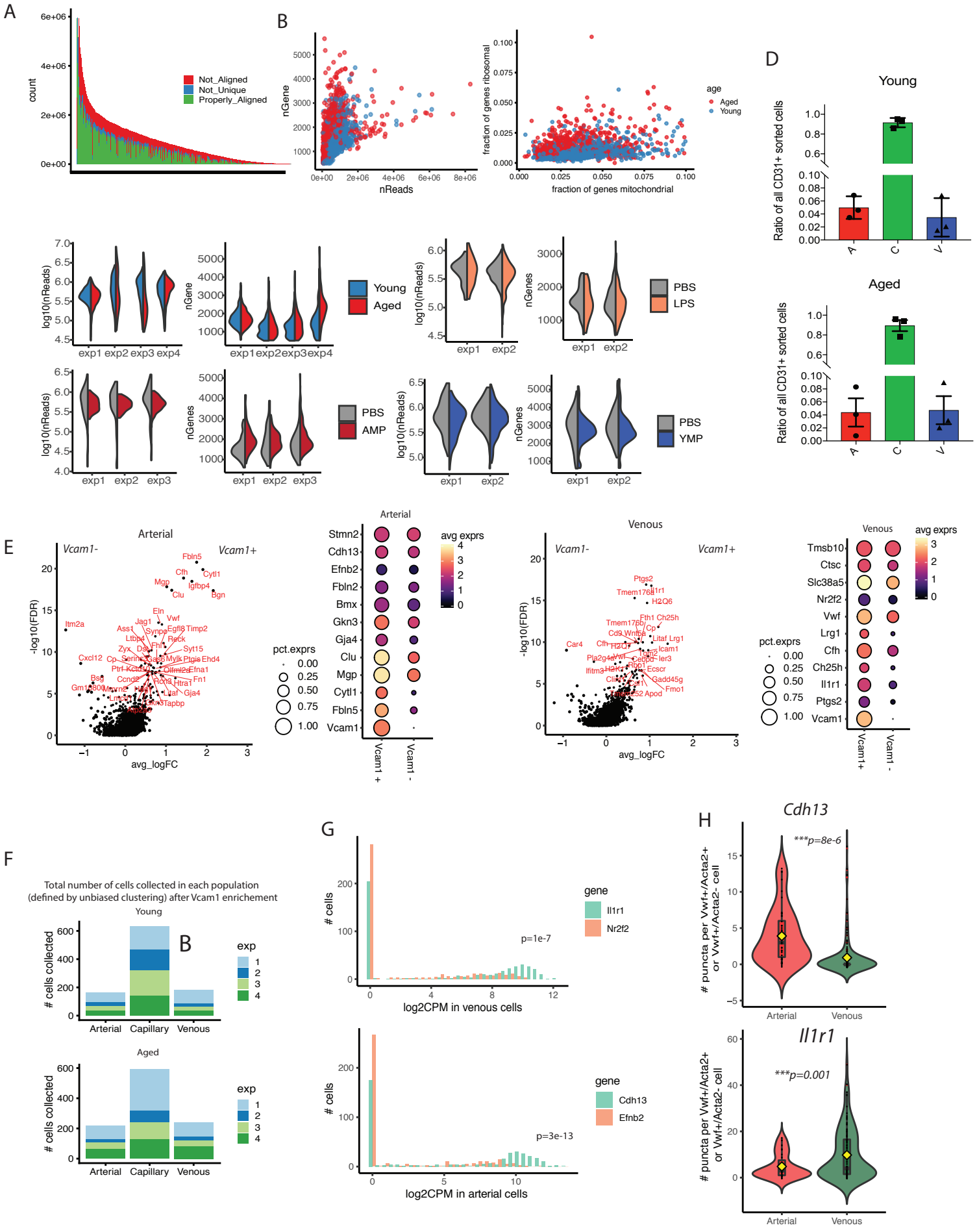

A
count
Not_Aligned
Not_Unique
Properly_Aligned
B
nGene
nReads
fraction of genes ribosomal
fraction of genes mitochondrial
age
Aged
Young
C
log10(nReads)
nGenes
Young
Aged
PBS
LPS
PBS
AMP
PBS
YMP
D
Young
Aged
Ratio of all CD31+ sorted cells
A
C
V
E
Arterial
Vcam1-
Vcam1+
-log10(FDR)
avg_logFC
pct.exprs
avg exprs
Venous
Vcam1+
Vcam1 -
F
Total number of cells collected in each population
(defined by unbiased clustering) after Vcam1 enrichement
Young
Aged
cells collected
exp
Arterial
Capillary
Venous
G
cells
gene
Il1r1
Nr2f2
p=1e-7
log2CPM in venous cells
Cdh13
Efnb2
p=3e-13
log2CPM in arterial cells
H
Cdh13
***p=8e-6
Il1r1
***p=0.001
puncta per Vwf+/Acta2+
or Vwf+/Acta2- cell
Arterial
Venous

Figure S1. Description of data quality metrics and Vcam1 enrichment strategy of arterial and venous cells. Related to Figure 1.

(A) Bar plots of the number of reads for each cell (pre-QC) that is not aligned, not unique and properly aligned, for all 4 replicates of young and aged cells (total 2,034 cells).

(B) (Left) Scatterplot of the number of reads versus the number of genes expressed per cell (pre-QC). Cells are then filtered to retain those expressing at least 500 genes or at least 50000 aligned (mapped) reads. (Right) Relationship between fraction of genes mitochondrial genes and fraction of ribosomal genes, for each cell. Only cells which have <10% mitochondrial genes and <10% ribosomal genes are depicted. Cells are colored by aged (red) or young (blue).

(C) Number of reads and genes detected per biological replicate (see Table S1) for each experimental condition (Aged vs Young, PBS vs LPS, AMP vs PBS, and YMP vs PBS).

(D) Ratio of all sorted cells (based only on $CD31^{+}CD45^{-}$) that identifies as either arterial (A) or venous (V) in segmental identity (based on the expression of at least ⅓ canonical marker genes), for both aged and young mice. Note the break in the axes. Error bars represent stdev.

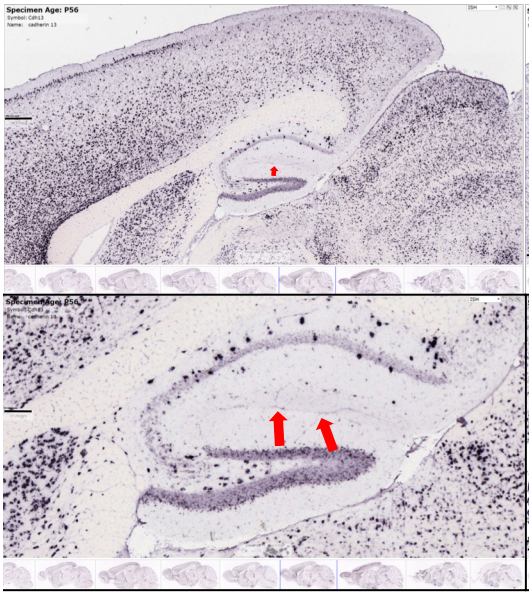
(E) Volcano plots of the top DEGs between *Vcam1*$^{+}$ and *Vcam1*$^{-}$ cells in either arterial or venous populations. Note the transcriptional activation of *Vcam1*$^{+}$ cells in both populations. Dotted heatmaps show the average expression level of key arterial and venous defining markers and the relative differences in percent expression in *Vcam1*$^{+}$ and *Vcam1*$^{-}$ populations. Ideal arterial and venous markers should not be differentially expressed between *Vcam1*$^{+/-}$ populations, in order encompass the range of arterial and venous cells.

(F) The total number of cells collected from each replicate that identifies as A, C or V, based on unbiased transcriptome clustering, after the addition of VCAM1-enriched samples (~20% of all cells).

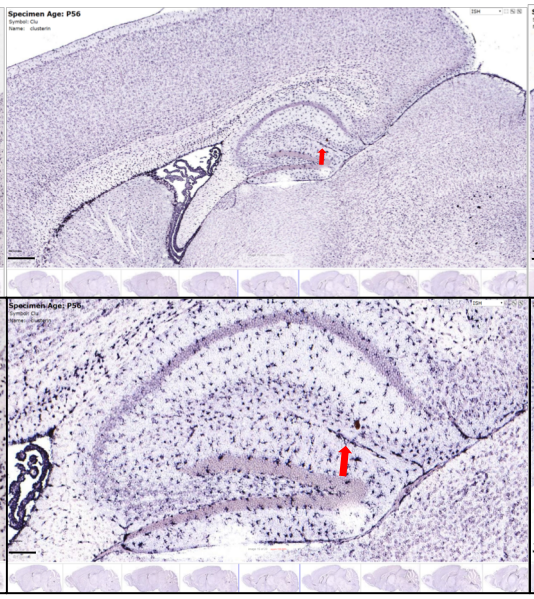
(G) Number of venous cells in this dataset expressing *Il1r1* or *Nr2f2* at various levels. Number of arterial cells in this dataset expressing *Cdh13* or *Efnb2* at various levels.

(H) Quantification of RNA *in situ* hybridization of *Cdh13* and *Il1r1* in arterial (*Vwf*$^{+}$*Acta2*$^{+}$*)* and venous (*Vwf*$^{+}$*Acta2*$^{-}$) cells, respectively (*Cdh13*: n=45 (Arterial) and 89 (Venous); *Il1r1*: n=36 (Arterial) and 92 (Venous)).

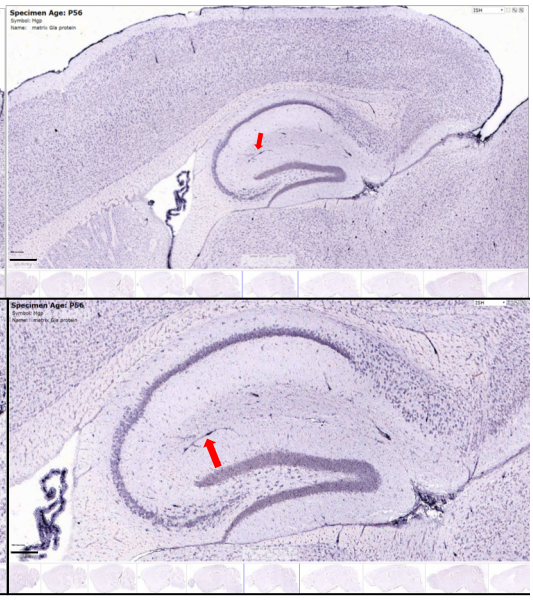
Artery - Cdh13
RP_070205_01_C03



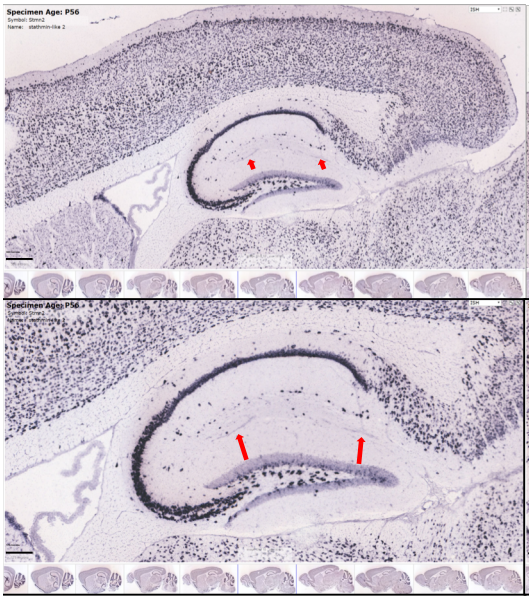
Artery - Clu
RP_070212_01_A02



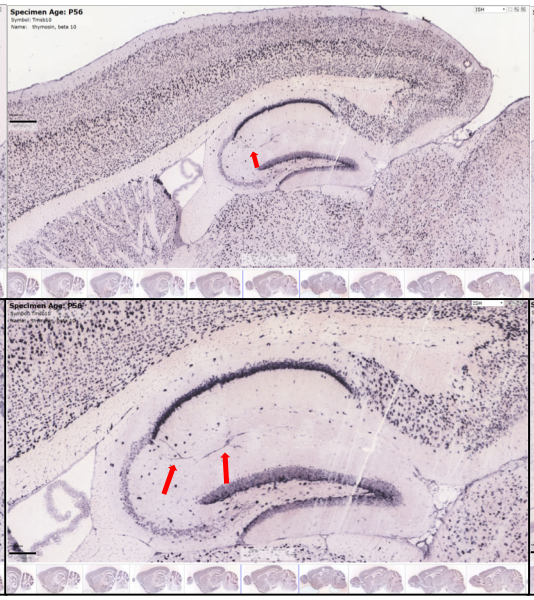
Artery - Mgp
RP_Baylor_102905



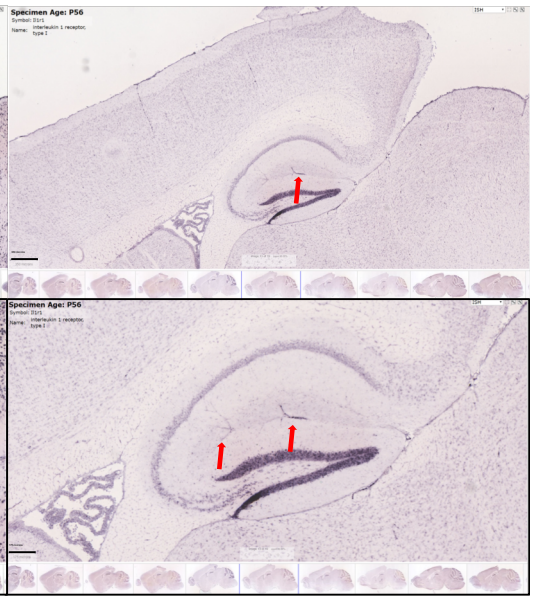
Artery - Stmn2
RP_050329_01_E10



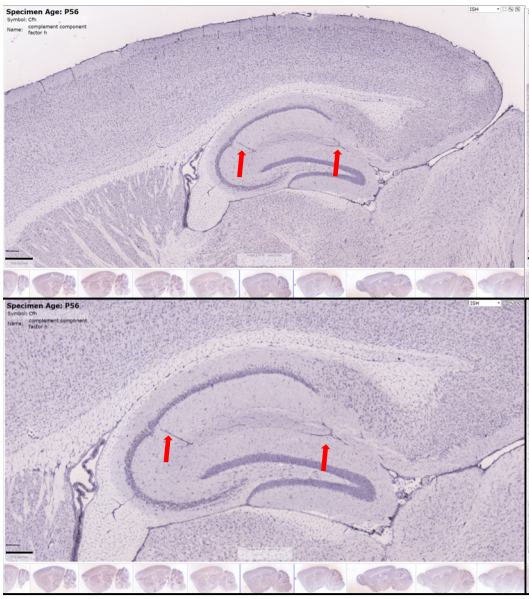
Capillary/Vein - Tmsb10
RP_050510_02_D07



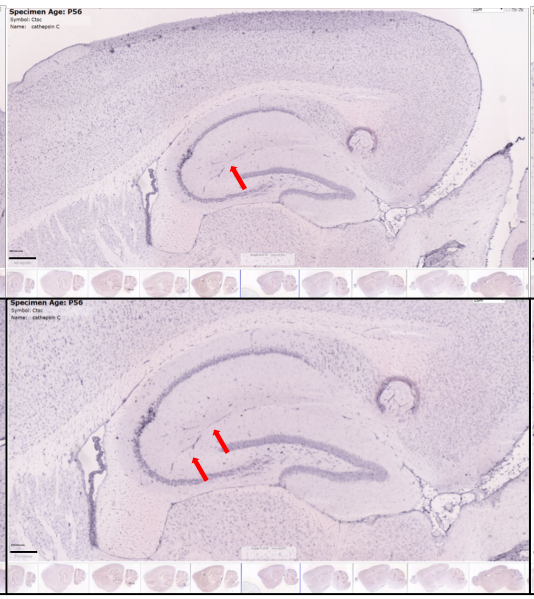
Vein - Il1r1
RP_050503_02_F10



Vein - Cfh
RP_050321_04_B05



Vein - Ctsc
RP_050426_03_G05



Vein - Cldn5
RP_071018_01_F08

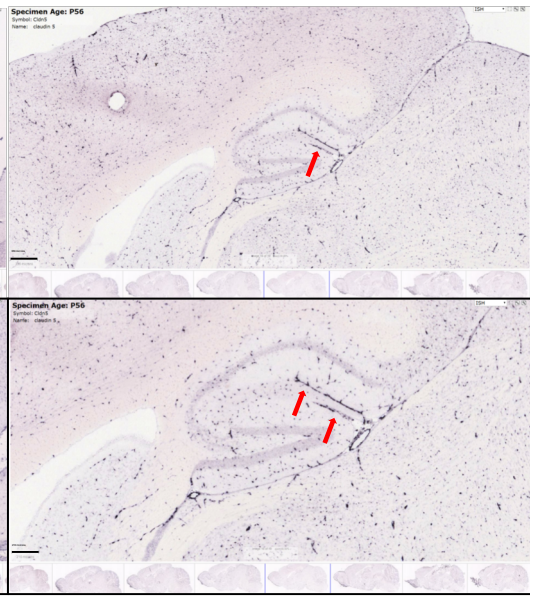


Figure S2. Identification of putative zonation markers in the Allen ISH Brain Atlas.

Related to Figure 1.

Representative images from the Allen ISH Brain Atlas demonstrating the expression of arterial (*Cdh13*, *Clu*, *Mgp*, *Stmn2*) and Venous (*Tmsb10*, *Il1r1*, *Cfh*, *Ctsc*, *Cldn5*) genes in hippocampal vasculature. Scale bars (from Left to Right, Top to bottom = 350 μm, 362 μm, 326 μm, 131 μm, 125 μm, 163 um, 300 μm, 350 μm, 350 μm, 175 μm, 175 μm, 175 um, 350 μm, 300 μm, 350 μm, 210 μm, 210 μm, 210 μm).

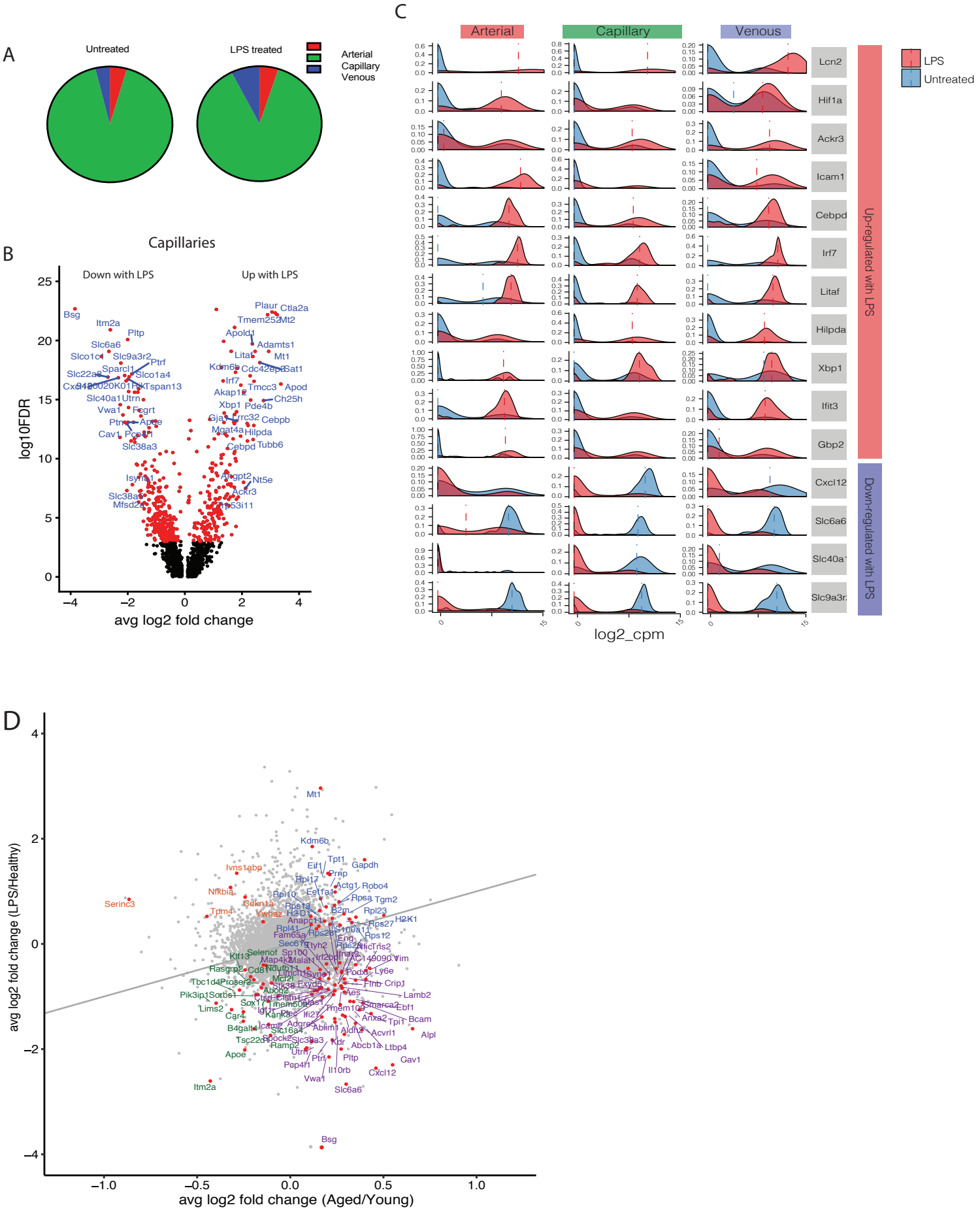

A
Untreated
LPS treated
Arterial
Capillary
Venous
B
Capillaries
Down with LPS
Up with LPS
log10FDR
avg log2 fold change
C
Arterial
Capillary
Venous
LPS
Untreated
Lcn2
Hif1a
Ackr3
Icam1
Cebpd
Irf7
Litaf
Hilpda
Xbp1
Ifit3
Gbp2
Cxcl12
Slc6a6
Slc40a1
Slc9a3r2
Up-regulated with LPS
Down-regulated with LPS
log2_cpm
D
avg log2 fold change (LPS/Healthy)
avg log2 fold change (Aged/Young)

Figure S3. Analysis of DEGs in LPS treated over PBS treated mice. Related to Figure 2.

(A) Ratio of A-C-V cells collected in LPS-treated and untreated mice through unbiased $CD31^{+}CD45^{-}$ sorting remains largely unchanged.

(B) Volcano plot of differentially expressed genes (red: FDR<0.1) when LPS-treated capillaries are compared to PBS-treated capillaries.

(C) Density plots of key genes from showing the single cell distributions of expression levels in A, C and V segments. Dotted lines indicate median of the LPS- or PBS-treated sample distributions. All comparisons shown between LPS- and PBS-treated are significant ($p<0.05$).

(D) Scatterplot showing the genes which are commonly and oppositely differentially expressed (FDR<0.1 in both) between aging capillaries and LPS-treated capillaries. Notes the relatively low number of commonly upregulated DEGs (blue). Commonly downregulated genes (green), differentially upregulated with LPS but downregulated with age (orange), and differentially downregulated with LPS but upregulated with age (purple) are also shown.

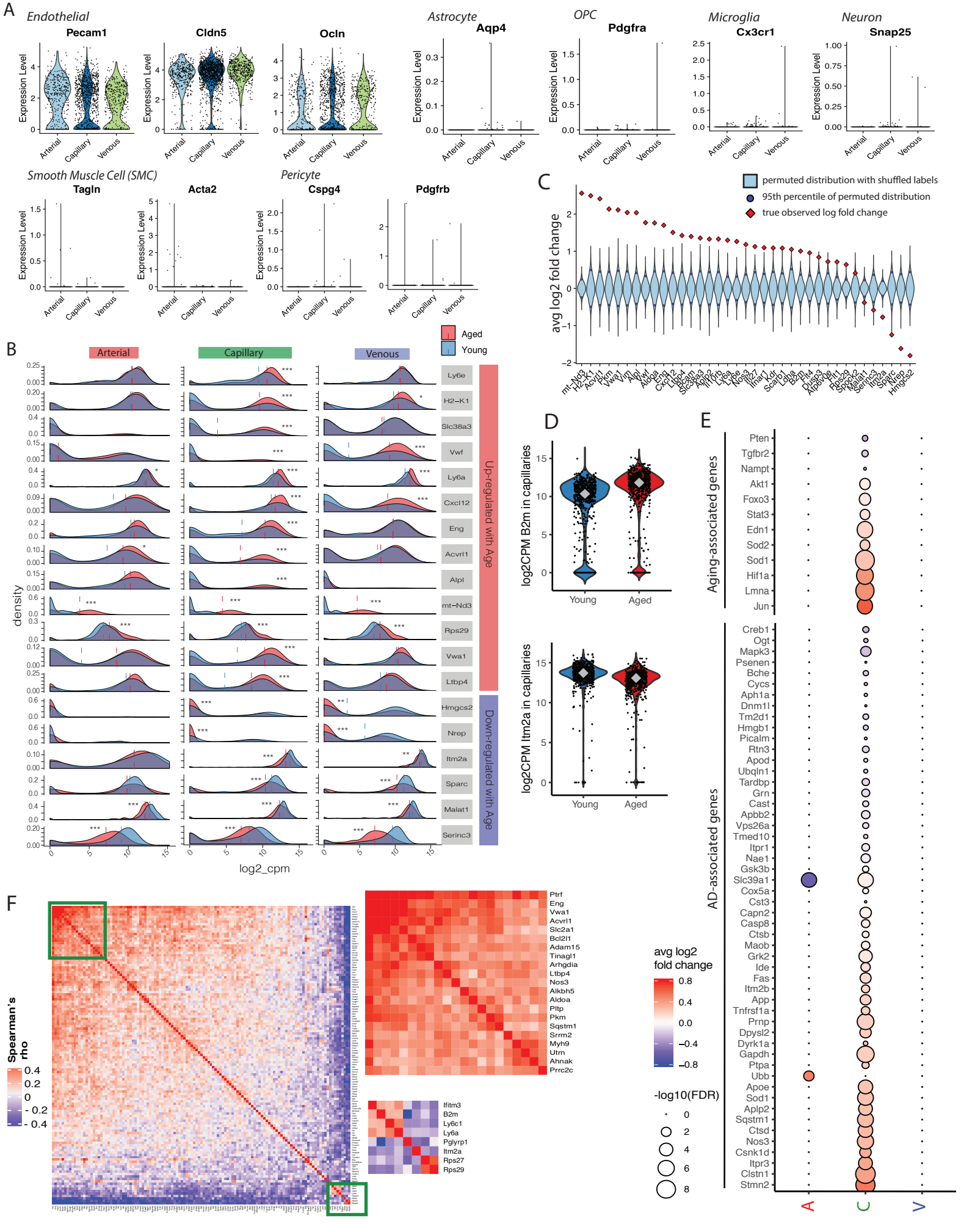

A
Endothelial
Pecam1
Cldn5
Ocln
Astrocyte
Aqp4
OPC
Pdgfra
Microglia
Cx3cr1
Neuron
Snap25
Smooth Muscle Cell (SMC)
Tagln
Acta2
Pericyte
Cspg4
Pdgfrb
Expression Level
Arterial
Capillary
Venous
B
Aged
Young
Arterial
Capillary
Venous
Ly6e
H2−K1
Slc38a3
Vwf
Ly6a
Cxcl12
Eng
Acvrl1
Alpl
mt−Nd3
Rps29
Vwa1
Ltbp4
Hmgcs2
Nrep
Itm2a
Sparc
Malat1
Serinc3
Up−regulated with Age
Down−regulated with Age
density
log2_cpm
C
permuted distribution with shuffled labels
95th percentile of permuted distribution
true observed log fold change
avg log2 fold change
D
log2CPM B2m in capillaries
log2CPM Itm2a in capillaries
Young
Aged
E
Aging−associated genes
Pten
Tgfbr2
Nampt
Akt1
Foxo3
Stat3
Edn1
Sod2
Sod1
Hif1a
Lmna
Jun
AD−associated genes
Creb1
Ogt
Mapk3
Psenen
Bche
Cycs
Aph1a
Dnm1l
Tm2d1
Hmgb1
Picalm
Rtn3
Apod
Ubqln1
Tardbp
Grn
Cast
Apbb2
Vps26a
Tmed10
Itpr1
Nae1
Gsk3b
Slc39a1
Cox5a
Cst3
Capn2
Casp8
Ctsb
Maob
Grk2
Ide
Fas
Itm2b
App
Tnfrsf1a
Prnp
Dpysl2
Dyrk1a
Gapdh
Ptpa
Ubb
Apoe
Sod1
Aplp2
Sqstm1
Ctsd
Nos3
Csnk1d
Itpr3
Clstn1
Stmn2
A
C
V
F
Spearman's rho
Ptrf
Eng
Vwa1
Acvrl1
Slc2a1
Bcl2l1
Adam15
Tinagl1
Arhgdia
Ltbp4
Nos3
Alkbh5
Aldoa
Pltp
Pkm
Sqstm1
Srrm2
Myh9
Utrn
Ahnak
Prrc2c
Ifitm3
B2m
Ly6c1
Ly6a
Pglyrp1
Itm2a
Rps27
Rps29
avg log2 fold change
-log10(FDR)

Figure S4. Analysis of aging-related DEGs. Related to Figure 3.

(A) Violin plots of the expression levels of various endothelial, mural cell and parenchymal cell markers in the dataset.

(B) Density plots of key genes from showing the single cell distributions of expression levels in A, C and V segments. Dotted lines indicate median of the young or aged distribution. *p<0.1, **p<0.01, ***p<0.001.

(C) Violin plot of the permuted distribution (when cell age labels are shuffled) of average log fold changes for several genes of interest. All genes show true observed values well above the 95^{th} percentile of the permuted distribution.

(D) Violin plot of log_2CPM or *B2m* and *Itm2a* in aged vs young capillaries, which were validated via RNAscope.

(E) Dotted heatmap of the average log_2(fold change) and statistical significance of genes associated with Alzheimer's disease and organismal aging as they change with age in arterial, capillary and venous cells.

(F) Correlation heatmap of the expression level of the top 125 aging-associated DEGs in capillaries. Bottom and top inset (of the green boxes) show the higher level of correlation between specific DEGs.

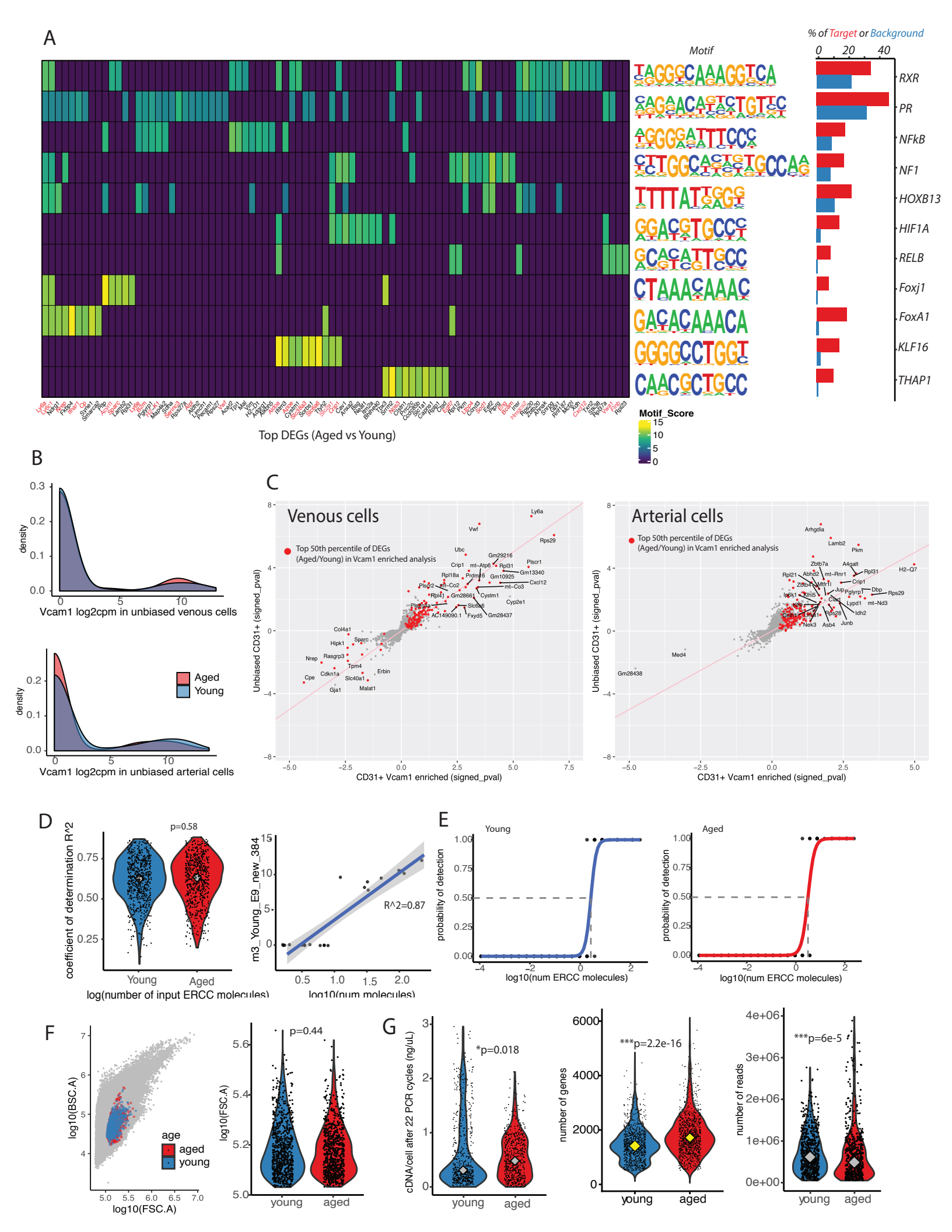

A
Motif
% of Target or Background
0
20
40
RXR
PR
NFkB
NF1
HOXB13
HIF1A
RELB
Foxj1
FoxA1
KLF16
THAP1
Top DEGs (Aged vs Young)
Motif_Score
15
10
5
0
B
density
Vcam1 log2cpm in unbiased venous cells
Aged
Young
Vcam1 log2cpm in unbiased arterial cells
C
Venous cells
Top 50th percentile of DEGs
(Aged/Young) in Vcam1 enriched analysis
Unbiased CD31+ (signed_pval)
CD31+ Vcam1 enriched (signed_pval)
Arterial cells
D
coefficient of determination R^2
p=0.58
Young
Aged
log(number of input ERCC molecules)
m3_Young_E9_new_384
R^2=0.87
log10(num molecules)
E
Young
Aged
probability of detection
log10(num ERCC molecules)
F
log10(BSC.A)
log10(FSC.A)
age
aged
young
p=0.44
G
cDNA/cell after 22 PCR cycles (ng/uL)
*p=0.018
number of genes
***p=2.2e-16
number of reads
***p=6e-5

Figure S5. Analysis of aging-related DEGs, validation of Vcam1 enrichment strategy, analysis of sequencing-associated differences between aged and young BECs. Related to Figure 3.

(A) Regulatory motif enrichment analysis of the top 125 aging-associated DEGs in capillaries was performed using HOMER. For each putative de novo motif, the percentage of target (aging DEGs) vs background genes is depicted. Genes of interest in this study are highlighted in red.

(B) Distribution of *Vcam1* expression in (Left) venous and (Right) arterial cells in aged and young BECs.

(C) Comparison of the signed p-value ($log_2FC^*-log_{10}(FDR)$) of each DEG calculated from using only unbiasedly sorted or VCAM1-enriched (Left) venous or (Right) arterial cells. DEGs in the top 50^{th} percentile (ranked by FDR) derived using the VCAM1-enriched datasets are labeled in red.

(D) Accuracy analysis with ERCCs showing the relationship between the number of input ERCC molecules and the number detected by scRNAseq, for both aged and young cells.

(E) Sensitivity analysis with ERCCs showing the number of ERCC molecules to reach 50% probability of detection, in either aged or young cells.

(F) Forward vs backscatter (from flow cytometry) of aged and young endothelial cells. Violin plot comparison the FSC (representative of size) between aged and young cells.

(G) Violin plots of the concentration of cDNA for each cell after 22 cycles of PCR amplification, the number of unique genes detected, and the sequencing depth, for aged compared to young cells.

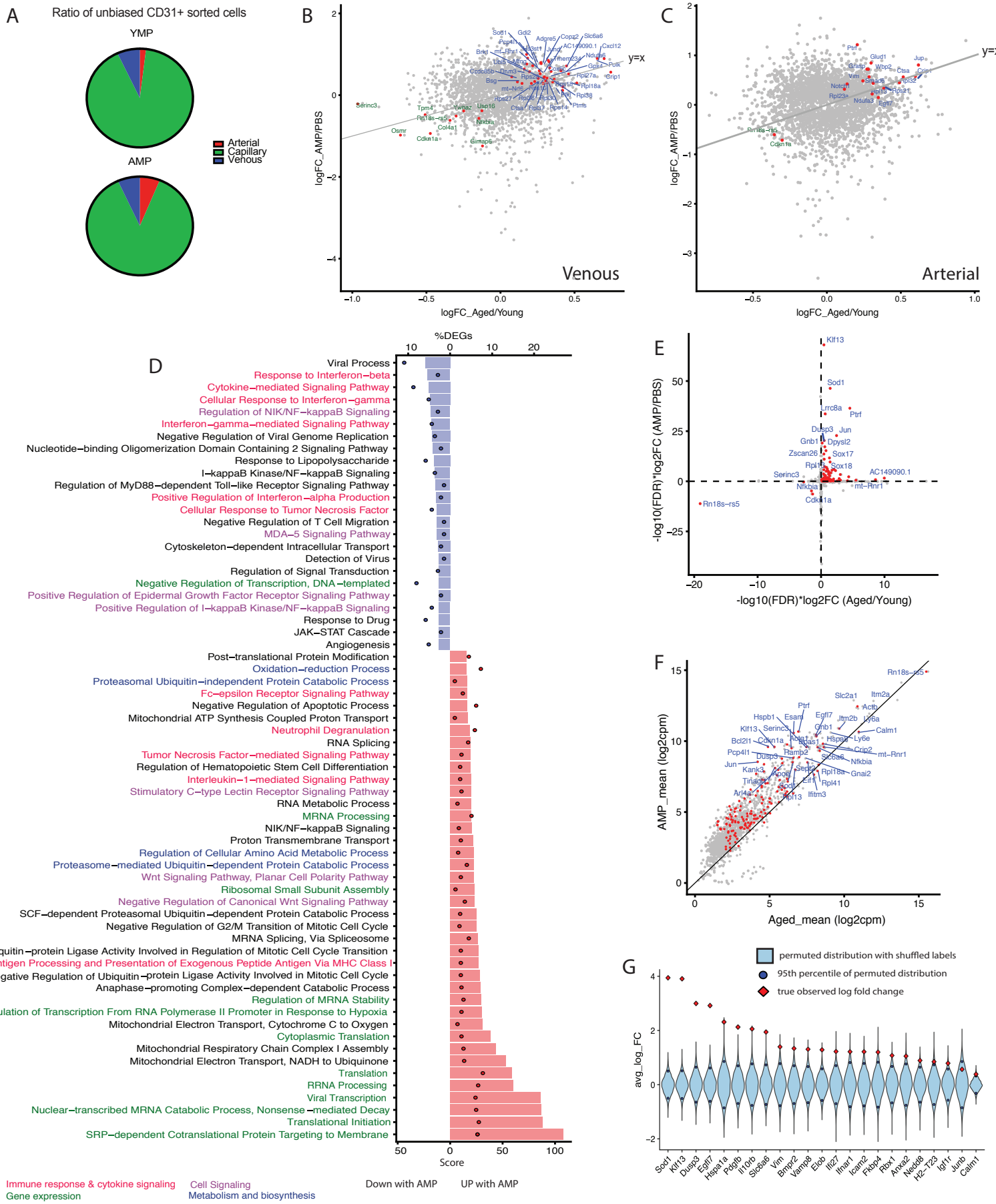

A
Ratio of unbiased CD31+ sorted cells
YMP
AMP
Arterial
Capillary
Venous
B
logFC_AMP/PBS
logFC_Aged/Young
y=x
Venous
C
logFC_AMP/PBS
logFC_Aged/Young
y=x
Arterial
D
%DEGs
Viral Process
Response to Interferon–beta
Cytokine–mediated Signaling Pathway
Cellular Response to Interferon–gamma
Regulation of NIK/NF–kappaB Signaling
Interferon–gamma–mediated Signaling Pathway
Negative Regulation of Viral Genome Replication
Nucleotide–binding Oligomerization Domain Containing 2 Signaling Pathway
Response to Lipopolysaccharide
I–kappaB Kinase/NF–kappaB Signaling
Regulation of MyD88–dependent Toll–like Receptor Signaling Pathway
Positive Regulation of Interferon–alpha Production
Cellular Response to Tumor Necrosis Factor
Negative Regulation of T Cell Migration
MDA–5 Signaling Pathway
Cytoskeleton–dependent Intracellular Transport
Detection of Virus
Regulation of Signal Transduction
Negative Regulation of Transcription, DNA–templated
Positive Regulation of Epidermal Growth Factor Receptor Signaling Pathway
Positive Regulation of I–kappaB Kinase/NF–kappaB Signaling
Response to Drug
JAK–STAT Cascade
Angiogenesis
Post–translational Protein Modification
Oxidation–reduction Process
Proteasomal Ubiquitin–independent Protein Catabolic Process
Fc–epsilon Receptor Signaling Pathway
Negative Regulation of Apoptotic Process
Mitochondrial ATP Synthesis Coupled Proton Transport
Neutrophil Degranulation
RNA Splicing
Tumor Necrosis Factor–mediated Signaling Pathway
Regulation of Hematopoietic Stem Cell Differentiation
Interleukin–1–mediated Signaling Pathway
Stimulatory C–type Lectin Receptor Signaling Pathway
RNA Metabolic Process
MRNA Processing
NIK/NF–kappaB Signaling
Proton Transmembrane Transport
Regulation of Cellular Amino Acid Metabolic Process
Proteasome–mediated Ubiquitin–dependent Protein Catabolic Process
Wnt Signaling Pathway, Planar Cell Polarity Pathway
Ribosomal Small Subunit Assembly
Negative Regulation of Canonical Wnt Signaling Pathway
SCF–dependent Proteasomal Ubiquitin–dependent Protein Catabolic Process
Negative Regulation of G2/M Transition of Mitotic Cell Cycle
MRNA Splicing, Via Spliceosome
Ubiquitin–protein Ligase Activity Involved in Regulation of Mitotic Cell Cycle Transition
Antigen Processing and Presentation of Exogenous Peptide Antigen Via MHC Class I
Negative Regulation of Ubiquitin–protein Ligase Activity Involved in Mitotic Cell Cycle
Anaphase–promoting Complex–dependent Catabolic Process
Regulation of MRNA Stability
Regulation of Transcription From RNA Polymerase II Promoter in Response to Hypoxia
Mitochondrial Electron Transport, Cytochrome C to Oxygen
Cytoplasmic Translation
Mitochondrial Respiratory Chain Complex I Assembly
Mitochondrial Electron Transport, NADH to Ubiquinone
Translation
RRNA Processing
Viral Transcription
Nuclear–transcribed MRNA Catabolic Process, Nonsense–mediated Decay
Translational Initiation
SRP–dependent Cotranslational Protein Targeting to Membrane
Score
Immune response & cytokine signaling
Gene expression
Cell Signaling
Metabolism and biosynthesis
Down with AMP
UP with AMP
E
-log10(FDR)*log2FC (AMP/PBS)
-log10(FDR)*log2FC (Aged/Young)
F
AMP_mean (log2cpm)
Aged_mean (log2cpm)
G
permuted distribution with shuffled labels
95th percentile of permuted distribution
true observed log fold change
avg_log_FC

Figure S6. Analysis of DEGs associated with AMP treatment and overlaps with normal aging. Related to Figure 4.

(A) Ratio of A-C-V cells collected via unbiased $CD31^{+}CD45^{-}CD11b^{-}$ sorting remains largely unchanged in AMP- versus PBS-treated mice.

(B) Scatterplot showing the genes which are oppositely differentially expressed (FDR<0.1 in both) between aging and AMP-treated venous cells. Note the relatively low number of common DEGs (red).

(C) Scatterplot showing the genes which are oppositely differentially expressed (FDR<0.1 in both) between aging and AMP-treated arterial cells. Note the relatively low number of common DEGs (red).

(D) GO analysis of the pathways enriched in the genes upregulated and downregulated with AMP treatment (compared to PBS)

(E) Scatterplot of the signed p-values ($-\log_{10}(FDR)*\log_2 FC$) for each gene. Genes differentially up- and down-regulated are marked in red. Top scoring genes are labeled.

(F) Scatterplot of the mean $\log_2 CPM$ values of all genes in either AMP-treated or aged BECs. Common DEGs between the two conditions (B) are depicted in red. Top expressed genes are labeled. Note that all common DEGs are more highly expressed in AMP treatment than normal, disease-free aging.

(G) Violin plot of the permuted distribution (when cell treatment labels are shuffled) of average log fold changes for several genes of interest in capillary cells, and the relative positions of the true observations and the 95^{th} percentile of the permuted distributions.

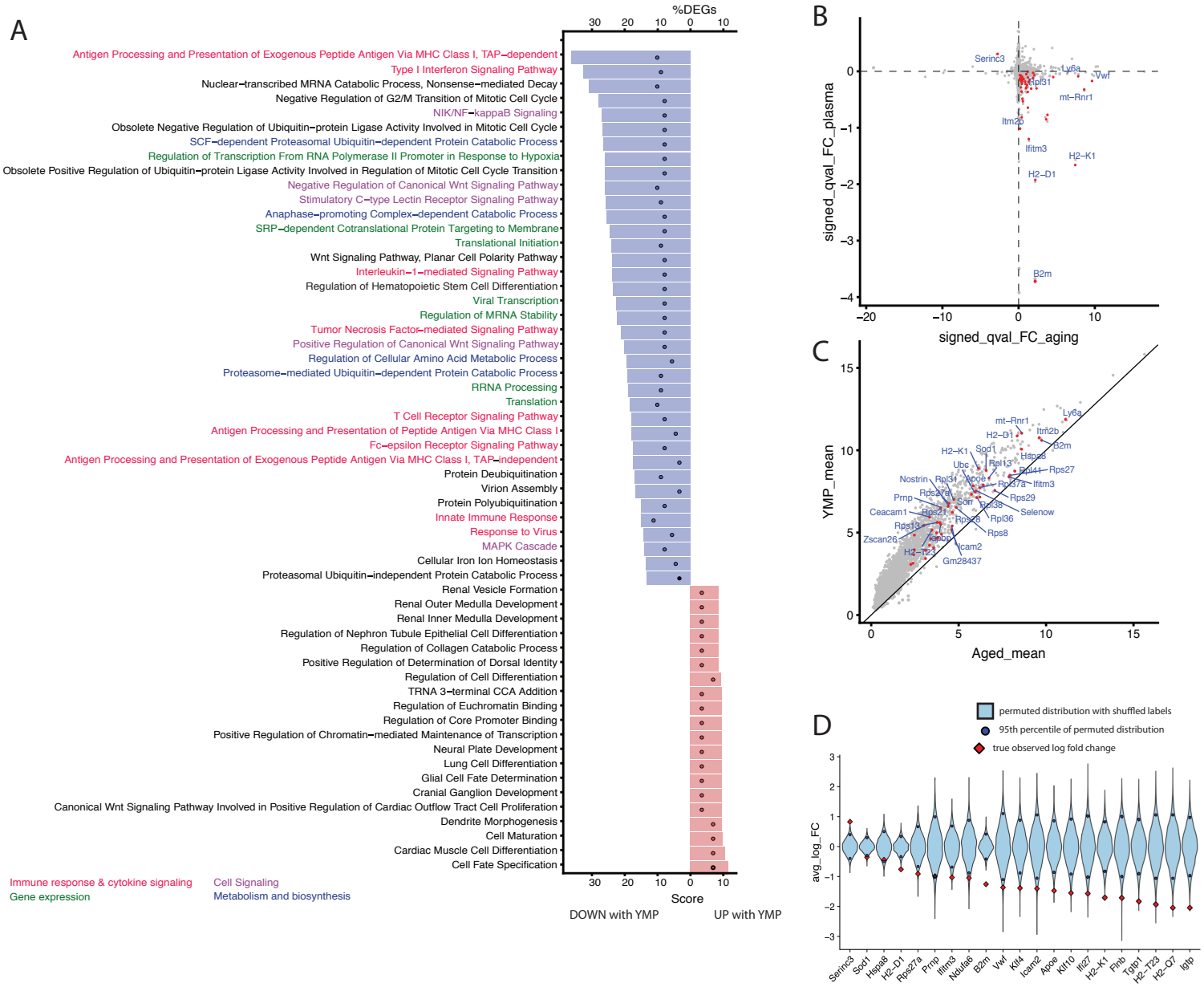

A
%DEGs
30 20 10 0 10
Antigen Processing and Presentation of Exogenous Peptide Antigen Via MHC Class I, TAP−dependent
Type I Interferon Signaling Pathway
Nuclear−transcribed MRNA Catabolic Process, Nonsense−mediated Decay
Negative Regulation of G2/M Transition of Mitotic Cell Cycle
NIK/NF−kappaB Signaling
Obsolete Negative Regulation of Ubiquitin−protein Ligase Activity Involved in Mitotic Cell Cycle
SCF−dependent Proteasomal Ubiquitin−dependent Protein Catabolic Process
Regulation of Transcription From RNA Polymerase II Promoter in Response to Hypoxia
Obsolete Positive Regulation of Ubiquitin−protein Ligase Activity Involved in Regulation of Mitotic Cell Cycle Transition
Negative Regulation of Canonical Wnt Signaling Pathway
Stimulatory C−type Lectin Receptor Signaling Pathway
Anaphase−promoting Complex−dependent Catabolic Process
SRP−dependent Cotranslational Protein Targeting to Membrane
Translational Initiation
Wnt Signaling Pathway, Planar Cell Polarity Pathway
Interleukin−1−mediated Signaling Pathway
Regulation of Hematopoietic Stem Cell Differentiation
Viral Transcription
Regulation of MRNA Stability
Tumor Necrosis Factor−mediated Signaling Pathway
Positive Regulation of Canonical Wnt Signaling Pathway
Regulation of Cellular Amino Acid Metabolic Process
Proteasome−mediated Ubiquitin−dependent Protein Catabolic Process
RRNA Processing
Translation
T Cell Receptor Signaling Pathway
Antigen Processing and Presentation of Peptide Antigen Via MHC Class I
Fc−epsilon Receptor Signaling Pathway
Antigen Processing and Presentation of Exogenous Peptide Antigen Via MHC Class I, TAP−independent
Protein Deubiquitination
Virion Assembly
Protein Polyubiquitination
Innate Immune Response
Response to Virus
MAPK Cascade
Cellular Iron Ion Homeostasis
Proteasomal Ubiquitin−independent Protein Catabolic Process
Renal Vesicle Formation
Renal Outer Medulla Development
Renal Inner Medulla Development
Regulation of Nephron Tubule Epithelial Cell Differentiation
Regulation of Collagen Catabolic Process
Positive Regulation of Determination of Dorsal Identity
Regulation of Cell Differentiation
TRNA 3−terminal CCA Addition
Regulation of Euchromatin Binding
Regulation of Core Promoter Binding
Positive Regulation of Chromatin−mediated Maintenance of Transcription
Neural Plate Development
Lung Cell Differentiation
Glial Cell Fate Determination
Cranial Ganglion Development
Canonical Wnt Signaling Pathway Involved in Positive Regulation of Cardiac Outflow Tract Cell Proliferation
Dendrite Morphogenesis
Cell Maturation
Cardiac Muscle Cell Differentiation
Cell Fate Specification
30 20 10 0 10
Score
DOWN with YMP
UP with YMP
Immune response & cytokine signaling
Cell Signaling
Gene expression
Metabolism and biosynthesis
B
signed_qval_FC_plasma
signed_qval_FC_aging
Serinc3
Ly6a
Vwf
Rtkl1
mt−Rnr1
Itm2b
Ifitm3
H2−K1
H2−D1
B2m
C
YMP_mean
Aged_mean
mt−Rnr1
Ly6a
H2−D1
Itm2b
B2m
H2−K1
Sod1
Hspa8
Ubc
Rpl13
Rps27
Nostrin
Rpl34
Apoe
Rps27a
Ifitm3
Prnp
Rps29
Son
Rpl38
Ceacam1
Rps21
Selenow
Rps13
Rps28
Rpl36
Rps8
Zscan26
Icam2
H2−T23
Gm28437
D
permuted distribution with shuffled labels
95th percentile of permuted distribution
true observed log fold change
avg_log_FC
Serinc3
Sod1
Hspa8
H2−D1
Rps27a
Prnp
Ifitm3
Ndufa6
B2m
Vwf
Klf4
Icam2
Apoe
Klf10
Itl27
H2−K1
Fnb
Tgtp1
H2−T23
H2−Q7
Igfp

Figure S7. Analysis of DEGs associated with YMP treatment and overlaps with normal aging. Related to Figure 5.

(A) GO analysis of the pathways enriched in the genes up- and down-regulated with YMP treatment (compared to PBS).

(B) Scatterplot of the signed p-values ($-\log_{10}(\text{FDR})*\log_2\text{FC}$) for each gene. Genes differentially up- and down-regulated are marked in red. Top scoring genes are labeled.

(C) Scatterplot of the mean $\log_2$CPM values of all genes in either YMP-treated or aged BECs. Common DEGs between the two conditions (B) are depicted in red. Top expressed genes are labeled in blue.

(D) Violin plot of the permuted distribution (when cell treatment labels are shuffled) of average log fold changes for several genes of interest in capillary cells, and the relative positions of the observations and the 95^{th} percentile of the permuted distributions.

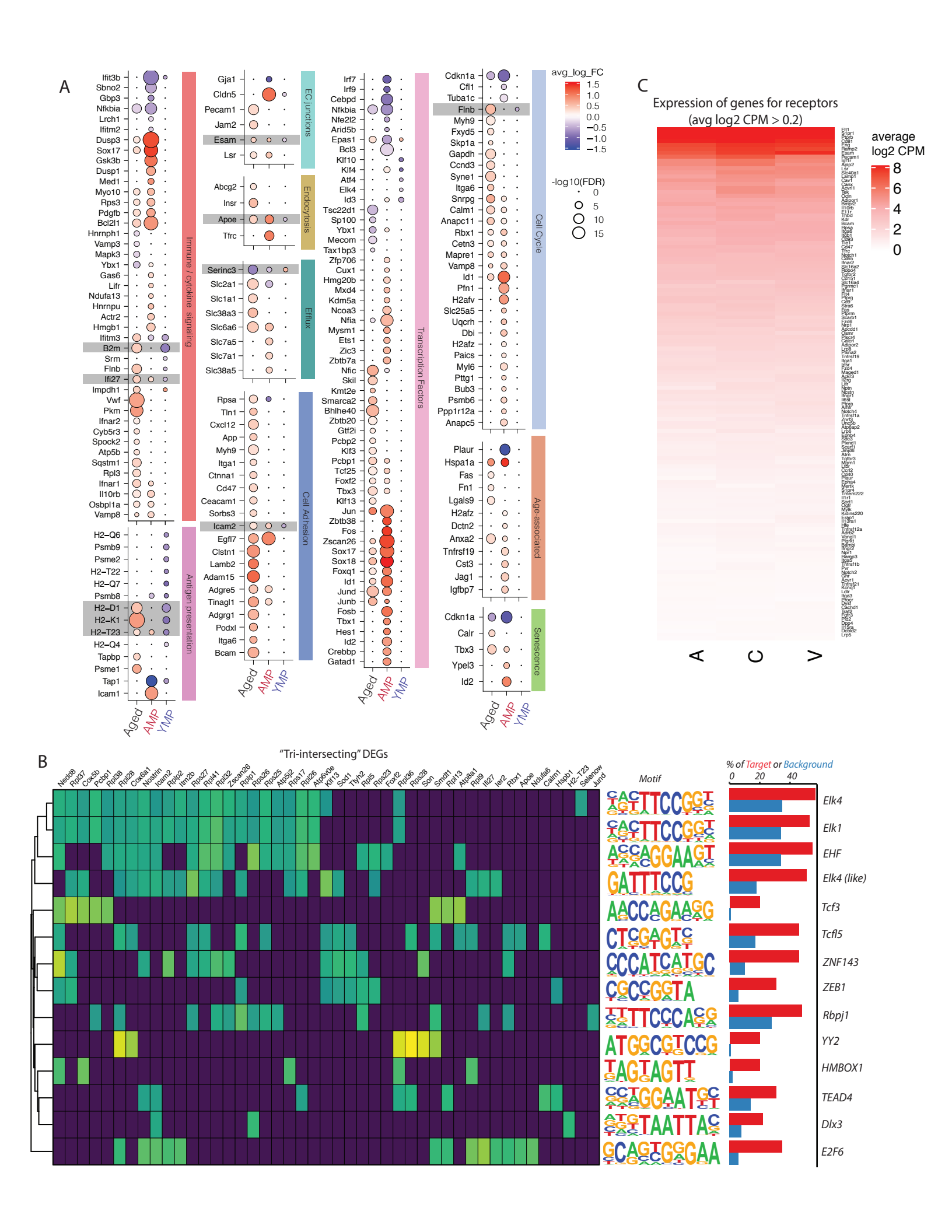

A
Ifit3b
Sbno2
Gbp3
Nfkbia
Lrch1
Ifitm2
Dusp3
Sox17
Gsk3b
Dusp1
Med1
Myo10
Rps3
Pdgfb
Bcl2l1
Hnrnph1
Vamp3
Mapk3
Ybx1
Gas6
Lifr
Ndufa13
Hnrnpu
Actr2
Hmgb1
Ifitm3
B2m
Srm
Flnb
Ifi27
Impdh1
Vwf
Pkm
Ifnar2
Cyb5r3
Spock2
Atp5b
Sqstm1
Rpl3
Ifnar1
Il10rb
Osbpl1a
Vamp8
Immune / cytokine signaling
H2−Q6
Psmb9
Psme2
H2−T22
H2−Q7
Psmb8
H2−D1
H2−K1
H2−T23
H2−Q4
Tapbp
Psme1
Tap1
Icam1
Antigen presentation
Aged
AMP
YMP
Gja1
Cldn5
Pecam1
Jam2
Esam
Lsr
EC junctions
Abcg2
Insr
Apoe
Tfrc
Endocytosis
Serinc3
Slc2a1
Slc1a1
Slc38a3
Slc6a6
Slc7a5
Slc7a1
Slc38a5
Efflux
Rpsa
Tln1
Cxcl12
App
Myh9
Itga1
Ctnna1
Cd47
Ceacam1
Sorbs3
Icam2
Egfl7
Clstn1
Lamb2
Adam15
Adgre5
Tinagl1
Adgrg1
Podxl
Itga6
Bcam
Cell Adhesion
Irf7
Irf9
Cebpd
Nfkbia
Nfe2l2
Arid5b
Epas1
Bcl3
Klf10
Klf4
Atf4
Elk4
Id3
Tsc22d1
Sp100
Ybx1
Mecom
Tax1bp3
Zfp706
Cux1
Hmg20b
Mxd4
Kdm5a
Ncoa3
Nfia
Mysm1
Ets1
Zic3
Zbtb7a
Nfic
Skil
Kmt2e
Smarca2
Bhlhe40
Zbtb20
Gtf2i
Pcbp2
Klf3
Pcbp1
Tcf25
Foxf2
Tbx3
Klf13
Jun
Zbtb38
Fos
Zscan26
Sox17
Sox18
Foxq1
Id1
Jund
Junb
Fosb
Tbx1
Hes1
Id2
Crebbp
Gatad1
Transcription Factors
Cdkn1a
Cfl1
Tuba1c
Flnb
Myh9
Fxyd5
Skp1a
Gapdh
Ccnd3
Syne1
Itga6
Snrpg
Calm1
Anapc11
Rbx1
Cetn3
Mapre1
Vamp8
Id1
Pfn1
H2afv
Slc25a5
Uqcrh
Dbi
H2afz
Paics
Myl6
Pttg1
Bub3
Psmb6
Ppp1r12a
Anapc5
Cell Cycle
Plaur
Hspa1a
Fas
Fn1
Lgals9
H2afz
Dctn2
Anxa2
Tnfrsf19
Cst3
Jag1
Igfbp7
Age-associated
Cdkn1a
Calr
Tbx3
Ypel3
Id2
Senescence
avg_log_FC
1.5
1.0
0.5
0.0
−0.5
−1.0
−1.5
-log10(FDR)
0
5
10
15
C
Expression of genes for receptors
(avg log2 CPM > 0.2)
average
log2 CPM
8
6
4
2
0
A
C
V
B
"Tri-intersecting" DEGs
Motif
% of Target or Background
0
20
40
Elk4
Elk1
EHF
Elk4 (like)
Tcf3
Tcf15
ZNF143
ZEB1
Rbpj1
YY2
HMBOX1
TEAD4
Dlx3
E2F6

Figure S8. Analysis of DEGs intersecting with normal aging, AMP treatment and YMP treatment. Related to Figure 6.

(A) Bubble plots of key genes grouped by function, and their log fold change values in each condition (Aged/Young, YMP/PBS, AMP/PBS). Gene of interest are highlighted in grey.

(B) Regulatory motif enrichment analysis of the 42 “tri-intersecting” DEGs (upregulated with aged, AMP and downregulated with YMP) in capillaries was performed using HOMER. For each putative de novo motif, the percentage of target (DEGs) versus background genes is shown. Genes of interest in this study are highlighted in red.

(C) Heatmap of the expression level of expressed genes encoding surface receptors in arterial, venous or capillary cells.

Table S1. Distribution of numbers of mice, cells and independent sequencing runs. Related to Figure 1.

Condition	# mice	# cells Past (QC)	# sequencing runs spread over		# mice per run								
					Run1	Run2	Run3	Run4	Run5	Run6	Run7	Run8	Exps
Healthy Young	6	981	4		3	1	1	1					**4**
Healthy Aged	6	1053	4		3	1	1	1					**4**
LPS	2	156	2			1	1						**2**
PBS	2	276	2			1	1						**2**
Young + AMP	4	333	3					1	1	2			**3**
Young + PBS	4	205	3					1	1	2			**3**
Aged +YMP	4	256	2								2	2	**2**
Aged + PBS	4	121	2								2	2	**2**
Total	**32**	**3381**											
				Aging	Exp1	Exp2	Exp3	Exp4					
				LPS		Exp1	Exp2						
				AMP				Exp1	Exp2	Exp3			
				YMP							Exp1	Exp2	

SUPPLEMENTARY TABLES

Table S1

Table outlining the experimental layout (number of mice, number of sequencing runs spread out over) for each treatment condition.