

Supplementary Figures

Adipose tissue in health and disease through the lens of its building blocks

Authors: Michael Lenz (ML)^{1,2,3,‡}, Ilja C.W. Arts (IA)^{1,4}, Ralf L.M. Peeters (RP)^{1,5}, Theo M. de Kok (TK)^{1,6} and Gökhan Ertaylan (GE)^{1,7,‡}

Affiliations:

¹Maastricht Centre for Systems Biology (MaCSBio), Maastricht University, the Netherlands

²Institute of Organismic and Molecular Evolution, Johannes Gutenberg University Mainz, Mainz, Germany

³Preventive Cardiology and Preventive Medicine – Center for Cardiology, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

⁴Dept. of Epidemiology, CARIM School of Cardiovascular Diseases, Maastricht University, the Netherlands

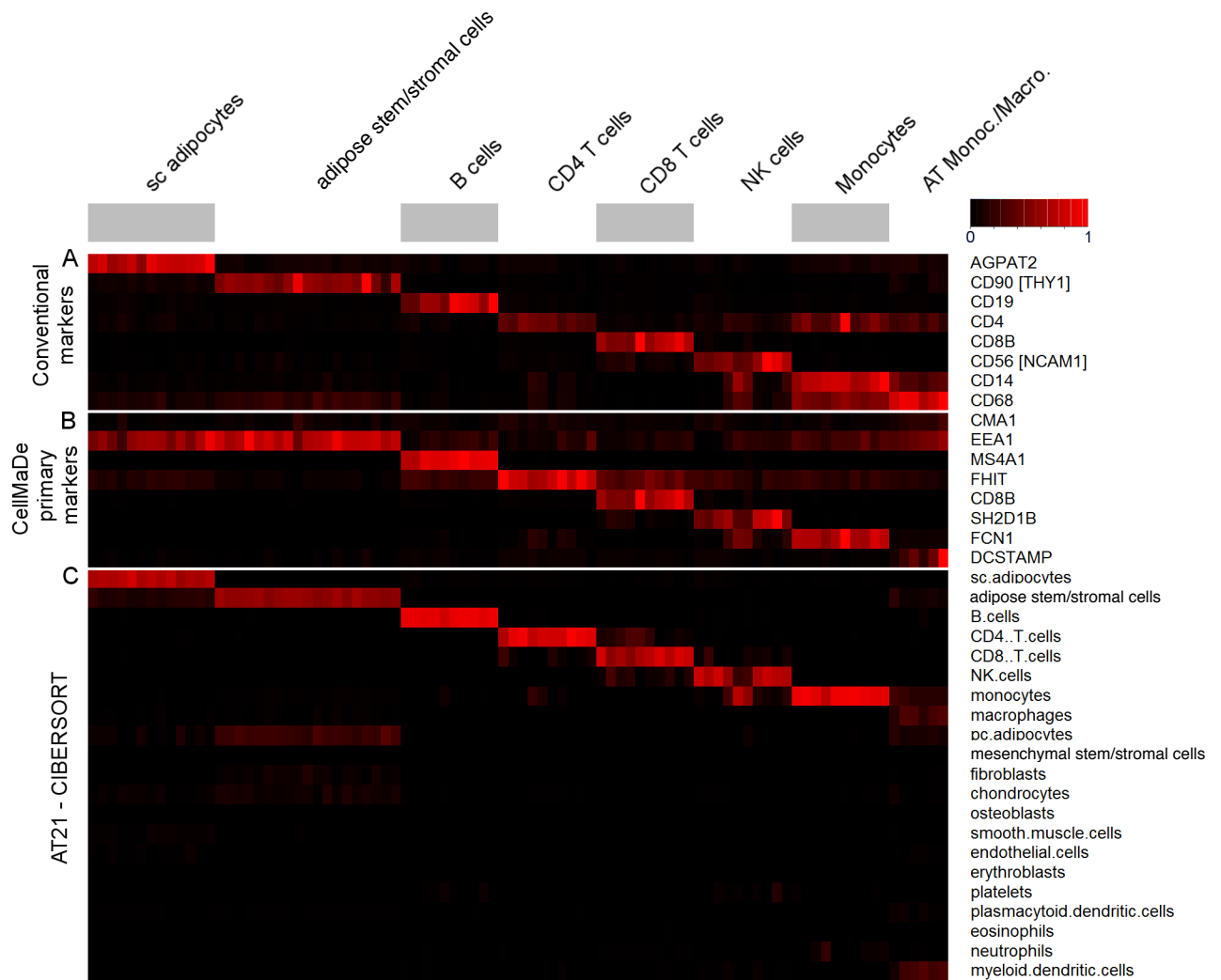
⁵Dept. of Data Science & Knowledge Engineering, Maastricht University, the Netherlands

⁶Dept. of Toxicogenomics, GROW School of Oncology and Developmental Biology, Maastricht University, Maastricht, The Netherlands

⁷Health, Flemish Institute for Technological Research (VITO), Mol, Belgium

‡Corresponding author

Email(s): ML: mlenz@uni-mainz.de;
IA: ilja.arts@maastrichtuniversity.nl;
RP: ralf.peeters@maastrichtuniversity.nl;
TK: t.dekok@maastrichtuniversity.nl;
GE: gokhan.ertaylan@vito.be



Supplementary Figure S1 : Ex-vivo validation of the TissueDecoder framework with an independent dataset. A) Normalized expression of selected conventional markers from literature; B) normalized expression of CellMaDe predicted primary markers; and C) cell type composition prediction from CIBERSORT with the AT21 signature matrix. The common x-axis above the figure denotes the reported cell type of the samples from the validation dataset.

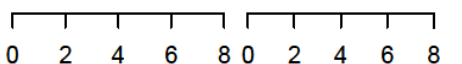
Adipose stem/stromal cells

Primary crit.

Secondary crit.

EEA1 (ME)	COL1A1 (E)
ZBED6 (O)	MFAP5 (E)
Hs.516023 (O)	POSTN (E)
Hs.503911 (O)	COL3A1 (E)
ABCA9 (M)	LUM (E)
SFRP4 (ME)	DCN (E)
U80771 (O)	COL1A2 (E)
Hs.413812 (O)	CDH11 (ME)
Hs.708219 (O)	GREM1 (E)
GUCY1A2 (M)	CCDC80 (E)

CD10 [MME] (ME)	CD10 [MME] (ME)
CD34 (ME)	CD34 (ME)
CD90 [THY1] (ME)	CD90 [THY1] (ME)



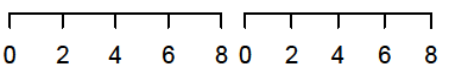
B cells

Primary crit.

Secondary crit.

MS4A1 (O)	MS4A1 (ME)
FCRLA (O)	POU2AF1 (O)
IGKV1D-39 (O)	IGKV1D-39 (O)
VPREB3 (E)	PAX5 (O)
IGKV1-17 (O)	IGHM (ME)
IGHV3-23 (ME)	TCL1A (O)
CD79A (M)	TNFRSF13C (M)
FCRL2 (M)	FCRL3 (ME)
IGHD (ME)	FCRLA (O)
IGHV4-59 (O)	FCRL1 (M)

CD19 (ME)	CD19 (ME)
-----------	-----------



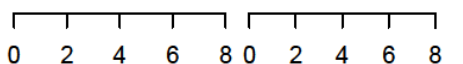
CD4+ T cells

Primary crit.

Secondary crit.

AKO22390 (O)	ITK (M)
FHIT (ME)	LEF1 (O)
TRAV23DV6 (O)	BCL11B (O)
RP1-1399P15.1 (O)	GIMAP7 (O)
TRAV9-2 (O)	TRBC1 (M)
INPP4B (O)	CD3G (M)
Hs.499725 (O)	TRAT1 (M)
FAM153A (O)	CCR7 (M)
ADTRP (M)	IL7R (ME)
TNFRSF25 (ME)	TRAC (M)

CD4 (M)	CD4 (M)
---------	---------



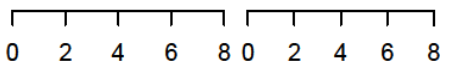
CD8+ T cells

Primary crit.

Secondary crit.

CD8B (ME)	CD8B (ME)
GZMK (E)	ITK (M)
CD8A (ME)	GZMK (E)
REG4 (E)	CD8A (ME)
CXCR6 (M)	CD3G (M)
RF11-103C16.2 (O)	KLRC4-KLRK1 (M)
THEMIS (O)	TRGC2 (M)
CA6 (E)	GZMA (ME)
CRTAM (M)	CCL5 (E)
NELL2 (ME)	TRAC (M)

CD8A (ME)	CD8A (ME)
CD8B (ME)	CD8B (ME)



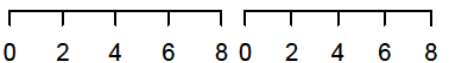
Chondrocytes

Primary crit.

Secondary crit.

SCRG1 (E)	SCRG1 (E)
ANGPTL7 (E)	COL1A1 (E)
OGN (E)	HAPLN1 (E)
CILP2 (E)	COL11A1 (E)
NEBL (E)	COL3A1 (E)
ZNF385B (O)	TNC (ME)
COL2A1 (E)	COL1A2 (E)
TNC (ME)	COL12A1 (E)
OMD (E)	GREM1 (E)
PRELP (E)	DCN (E)

COL1A1 (E)	COL1A1 (E)
COL1A2 (E)	COL1A2 (E)
COL2A1 (E)	COL2A1 (E)



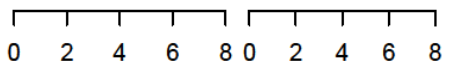
Endothelial cells

Primary crit.

Secondary crit.

CALCRL (M)	MMP1 (E)
IL1RL1 (ME)	CDH5 (M)
HHIP (ME)	HHIP (ME)
CDH5 (M)	SERPINE1 (ME)
EDN1 (E)	CTGF (ME)
BMX (M)	KRT7 (E)
LIPG (E)	EDN1 (E)
MTUS1 (ME)	TFPI2 (E)
APLN (E)	MMRN1 (E)
MMP10 (E)	EFEMP1 (E)

CD31 [PECAM1] (ME)	CD31 [PECAM1] (ME)
CD34 (ME)	CD34 (ME)
VE-Statin (E)	VE-Statin (E)



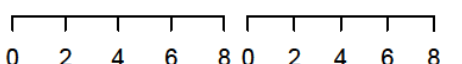
Eosinophils

Primary crit.

Secondary crit.

PRSS33 (E)	CLC (E)
IL5RA (ME)	OLIG1 (O)
OLIG2 (M)	CCR3 (ME)
240131_at (O)	1558048_x_at (O)
CLC (E)	PRSS33 (E)
VSTM1 (ME)	RNASE2 (E)
LOC441666 (O)	VSTM1 (ME)
1563941_at (O)	IL5RA (ME)
RNASE3 (E)	BCL2A1 (M)
SORD (ME)	ADGRE1 (M)

CD66B [CEACAM8] (ME)	CD66B [CEACAM8] (ME)
----------------------	----------------------



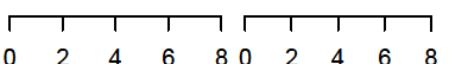
Erythroblasts

Primary crit.

Secondary crit.

SPTA1 (M)	HBD (ME)
RHAG (M)	RHAG (M)
HBBP1 (O)	CA1 (E)
AHSP (O)	HBG2 (E)
CA1 (E)	AHSP (O)
HBD (ME)	SPTA1 (M)
GYPA (ME)	HBBP1 (O)
KLF1 (O)	CA2 (ME)
SLC4A1 (ME)	HEMGN (O)
EPB42 (M)	KLF1 (O)

CD71 [TFRC] (E)	CD71 [TFRC] (E)
-----------------	-----------------



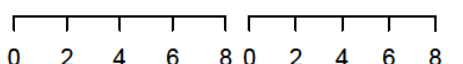
Fibroblasts

Primary crit.

Secondary crit.

HSPB3 (O)	MMP3 (E)
MMP3 (E)	MMP1 (E)
TFAP2C (O)	GREM1 (E)
MKX (O)	PSG5 (E)
PRSS12 (ME)	COL1A1 (E)
NTN1 (E)	C1S (ME)
MAB2L1 (O)	CLDN11 (ME)
LRRC15 (ME)	GPNMB (M)
COLEC12 (ME)	DCN (E)
ACKR4 (M)	COLEC12 (ME)

VIM (ME)	VIM (ME)
FSP1 [ATL1] (M)	FSP1 [ATL1] (M)



Macrophages

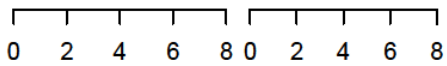
Mesenchymal stem/stromal cells

Monocytes

Primary crit. Secondary crit.

DCSTAMP (M)	MRC1 (M)
CCL22 (E)	SPP1 (ME)
TREM2 (ME)	GPNMB (M)
SPP1 (ME)	PLA2G7 (E)
MSR1 (ME)	MS4A4A (O)
ATP6V0D2 (ME)	ACP5 (ME)
ADAMDEC1 (ME)	APOE (ME)
APOE (ME)	DCSTAMP (M)
ACP5 (ME)	A2M (E)
GM2A (ME)	SUCNR1 (ME)

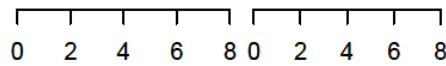
CD68 (M)	CD68 (M)
CD14 (ME)	CD14 (ME)



Primary crit. Secondary crit.

KRTAP1-1 (O)	CHI3L1 (E)
SLC14A1 (M)	CDH11 (ME)
CFI (ME)	GJA1 (ME)
LAMA3 (E)	PENK (ME)
ETV1 (O)	FN1 (ME)
TENM4 (M)	COL1A1 (E)
FAM87A (O)	EDIL3 (E)
PITX2 (O)	CTHRC1 (E)
SUGCT (O)	CLMP (ME)
XYLT1 (ME)	COL1A2 (E)

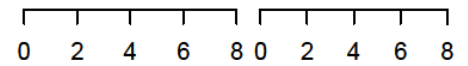
CD105 [ENG] (ME)	CD105 [ENG] (ME)
CD73 [NT5E] (ME)	CD73 [NT5E] (ME)
CD90 [THY1] (ME)	CD90 [THY1] (ME)



Primary crit. Secondary crit.

FCN1 (ME)	S100A12 (ME)
NRG1 (ME)	LYZ (E)
ASGR1 (ME)	CLEC7A (M)
SLOC46A2 (M)	FCN1 (ME)
CLEC5A (M)	SERPINA1 (ME)
CALML4 (O)	CD14 (ME)
MS4A14 (M)	MS4A7 (M)
AATBC (O)	S100A8 (ME)
CYBB (M)	S100A9 (ME)
MTMR11 (E)	TLR8 (M)

CD14 (ME)	CD14 (ME)
-----------	-----------



Myeloid dendritic cells

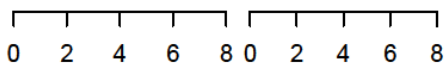
Neutrophils

NK cells

Primary crit. Secondary crit.

ENHO (E)	FCER1A (ME)
CD1E (M)	NAPSB (M)
IDO2 (O)	CD1C (M)
MS4A2 (O)	HLA-DQA1 (M)
XCR1 (M)	CPVL (E)
CLEC10A (M)	HLA-DQA2 (M)
CLNK (O)	RP11-389C8.2 (O)
CD1C (M)	CLEC10A (M)
ELANE (E)	LGALS2 (ME)
HLA-DQA1 (M)	CD1D (M)

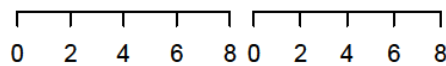
CD11c [ITGAX] (M)	CD11c [ITGAX] (M)
-------------------	-------------------



Primary crit. Secondary crit.

CYP4F3 (M)	FCGR3B (O)
KRT23 (O)	CYP4F3 (M)
PI3 (ME)	PROK2 (E)
216782_at (O)	CMTM2 (ME)
CMTM2 (ME)	CXCL8 (E)
VNN3 (ME)	236495_at (O)
PROK2 (E)	CXCR2 (M)
KCNJ15 (M)	AQP9 (M)
KIAA1324 (ME)	S100A12 (ME)
PTGS2 (M)	TNFRSF10C (M)

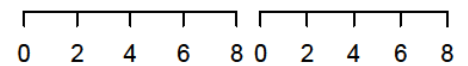
CD16 [FCGR3B] (ME)	CD16 [FCGR3B] (ME)
--------------------	--------------------



Primary crit. Secondary crit.

SH2D1B (O)	GNLY (E)
TRDC (O)	GZMB (M)
S1PR5 (M)	KLRF1 (M)
KIR3DL1 (M)	TRDC (O)
PTGDR (M)	SH2D1B (O)
KLRF1 (M)	FGFBP2 (E)
CD160 (M)	GZMA (ME)
KIR2DS4 (M)	KLRC1 (M)
216050_at (O)	NKG7 (M)
TBX21 (O)	KLRD1 (M)

CD56 [NCAM1] (ME)	CD56 [NCAM1] (ME)
-------------------	-------------------



Osteoblasts

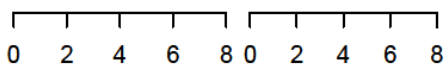
Pc Adipocytes

Plasmacyt. dendritic cells

Primary crit. Secondary crit.

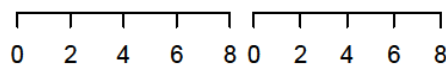
TM4SF20 (M)	COL11A1 (E)
IBSP (ME)	VCAM1 (ME)
EYA4 (O)	ITGBL1 (E)
NRXN3 (ME)	RP11-397O8.7 (O)
TMEM178B (M)	COL1A1 (E)
KRT14 (E)	COL8A1 (E)
PAPPA2 (E)	POSTN (E)
C3orf80 (M)	COL3A1 (E)
VCAM1 (ME)	EDIL3 (E)
TMEM171 (M)	COL1A2 (E)

CBFA1 [RUNX2] (O)	CBFA1 [RUNX2] (O)
CD105 [ENG] (ME)	CD105 [ENG] (ME)
ALPL (ME)	ALPL (ME)



Primary crit. Secondary crit.

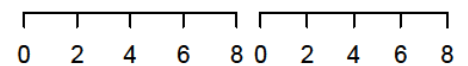
C7 (ME)	ADIPOQ (E)
ADGRG2 (ME)	SPARCL1 (E)
PLN (M)	RBP4 (E)
SLC27A2 (ME)	SORBS1 (M)
SELE (ME)	AOC3 (ME)
AGT (ME)	FABP4 (E)
CNTN4 (ME)	ACACB (M)
SYNPO2 (O)	ADH1B (O)
ADAMTSL3 (E)	LPL (ME)
CCL8 (E)	C11orf96 (O)



Primary crit. Secondary crit.

CUX2 (ME)	GZMB (M)
LINC00996 (O)	LINC00996 (O)
CLEC4C (M)	EPHB1 (ME)
LINC00865 (O)	CUX2 (ME)
PACSIN1 (M)	CLEC4C (M)
PHEX (M)	BLNK (M)
RP11-216L13.19 (O)	TCL1A (O)
COL24A1 (E)	JCHAIN (E)
LILRA4 (M)	NAPSB (M)
THAP2 (O)	C1orf186 (M)

IL3RA (M)	IL3RA (M)
-----------	-----------

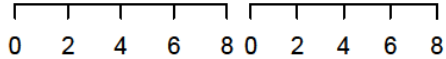


Platelets

Primary crit. Secondary crit.

Primary crit.	Secondary crit.
PF4V1 (E)	TUBB1 (E)
TRBV27 (O)	PF4V1 (E)
HIST1H3H (ME)	CXCL5 (E)
ITGB3 (ME)	TRBV27 (O)
FAM81B (O)	HIST1H3H (ME)
CXCL5 (E)	PPBP (E)
LOC105375547 (O)	MFAP3L (M)
HIST1H2AG (E)	ELOVL7 (M)
CLEC1B (M)	SDPR (M)
BEND2 (O)	SPX (E)

⋮	⋮
CD61 [ITGB3] (ME)	CD61 [ITGB3] (ME)

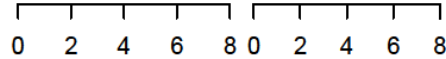


Sc Adipocytes

Primary crit. Secondary crit.

Primary crit.	Secondary crit.
CMA1 (E)	ADIPOQ (E)
TMEM139 (M)	SPARCL1 (E)
IRX1 (O)	RBP4 (E)
PPP1R1B (O)	SORBS1 (M)
ALDOC (E)	ACACB (M)
ANGPTL8 (E)	CXCL14 (E)
CALB2 (O)	LPL (ME)
EGFL6 (ME)	AOC3 (ME)
DEFB132 (E)	FABP4 (E)
PPP2R1B (ME)	LEP (E)

⋮	⋮
CEBPA (O)	CEBPA (O)
CEBPB (O)	CEBPB (O)
AGPAT2 (M)	AGPAT2 (M)

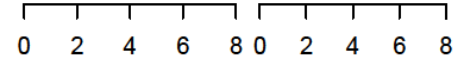


Smooth muscle cells

Primary crit. Secondary crit.

Primary crit.	Secondary crit.
RGS5 (M)	RGS4 (M)
CDH6 (ME)	COL1A1 (E)
SLC1A1 (ME)	GREM1 (E)
FOXS1 (O)	COL3A1 (E)
PLAT (E)	PLAT (E)
TFAP2A (O)	POSTN (E)
BEX1 (O)	COL1A2 (E)
TMEFF2 (ME)	SERPINE1 (ME)
EPHA5 (M)	TFPI2 (E)
LRRC17 (E)	MMP1 (E)

⋮	⋮
DES (ME)	DES (ME)
ACTA1 (E)	ACTA1 (E)
CALD1 (M)	CALD1 (M)



Supplementary Figure S2: Primary and secondary markers for all 21 cell types. Bar plots show the top ten cell type markers identified by the primary criterion and the secondary criterion of CellMaDe. Below each column conventional markers are demonstrated with their associated score. **Bold** font indicates the presence of the gene in the AT21 signature matrix and red colored bars indicate the value of the (primary/secondary) criterion was negative. Letters in brackets (M: membrane, E: extracellular, ME: both membrane and extracellular, or O: other) specify the gene ontology cellular location of the corresponding protein.

Supplementary Figure S3. Tissue expressions of the most significant Primary Marker per cell type identified by CellMaDe analyzed by Genevestigator from 394 human anatomically annotated tissue expression profiles.

The genes are predicted to be expressed in A) Subcutaneous Adipocytes, B) Pericardial Adipocytes, C) A. Stromal/ Stem Cells, D) Mesenchymal stromal/stem Cells, E) Fibroblasts, F) Chondrocytes, G) Osteoblasts, H) Smooth Muscle Cells, I) Endothelial Cells, J) Erythroblasts, K) Platelets, L) Natural Killer Cells, M) CD4+ T cells, N) CD8+ T cells, O) B Cells, P) Plasmacytoid Dendritic Cells, Q) Eosinophils, R) Neutrophils, S) Myeloid Dendritic Cells, U) Monocytes, V) Macrophages.

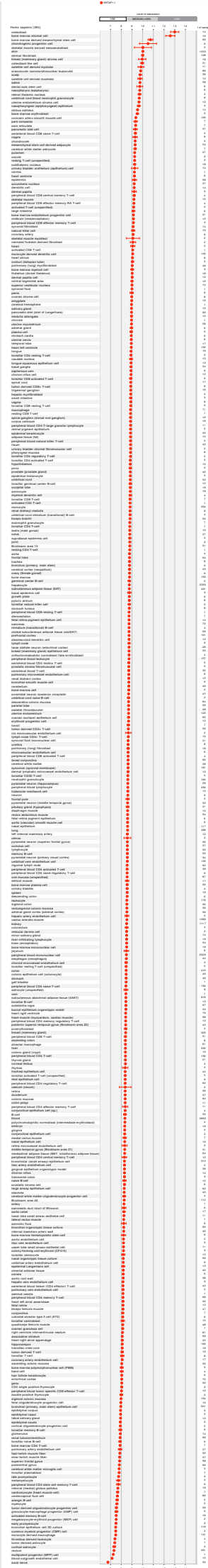
*The data is accessed on 12/08/2017.

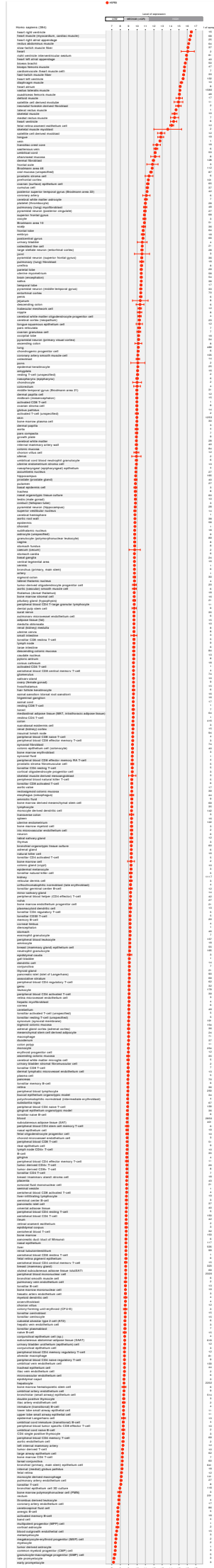


C) Adipose Stem/Stromal Cells



C) Mesenchymal Stem/Stromal Cells



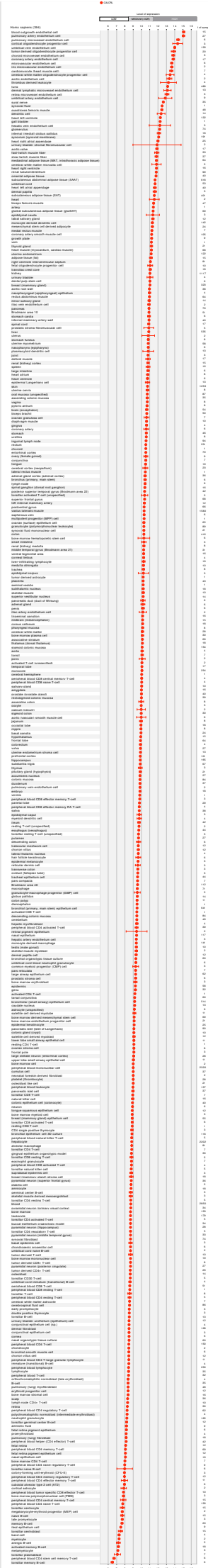


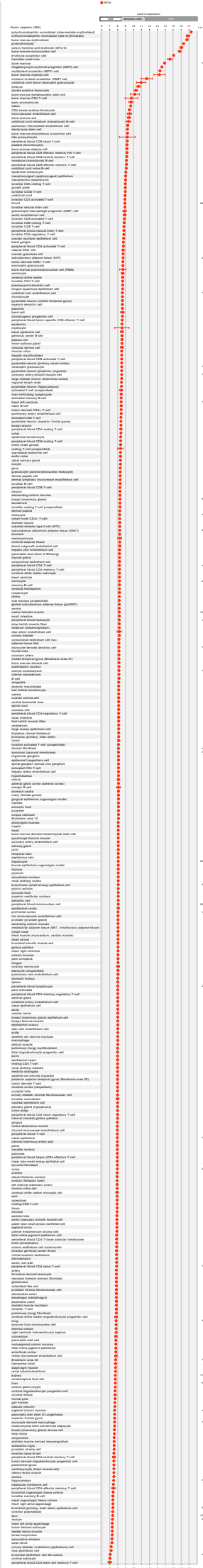
Unit: expression

F) Chondrocytes



I) Endothelial Cells



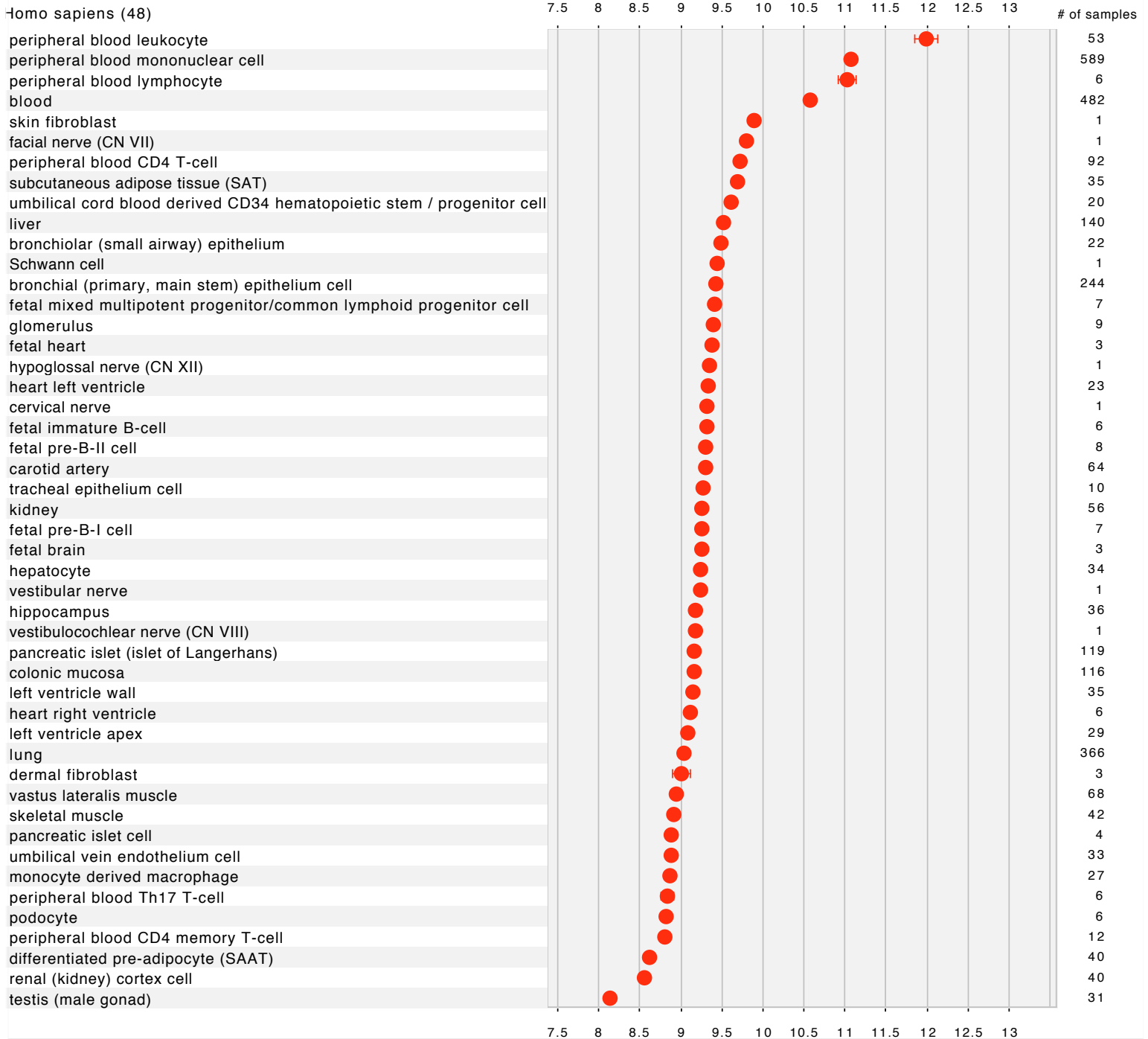


K) Platelets

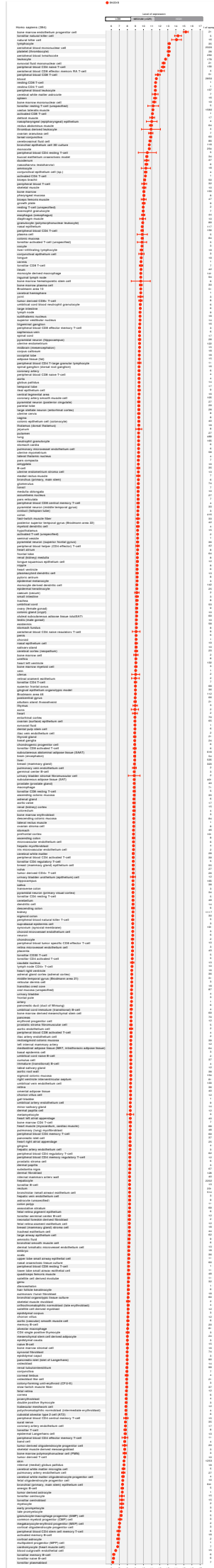
● PF4V1

Level of expression (signal intensity on Affymetrix Human Gene 1.0 ST Array)

LOW	MEDIUM (=IQR)	HIGH
-----	---------------	------



L) Natural Killer Cells



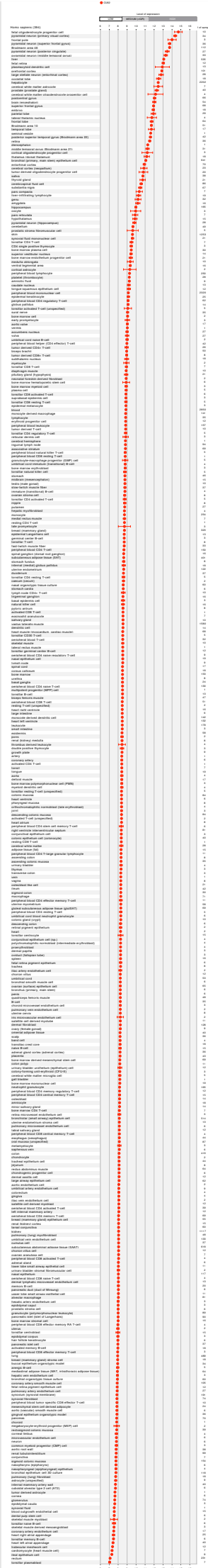
N) CD8+ T cells



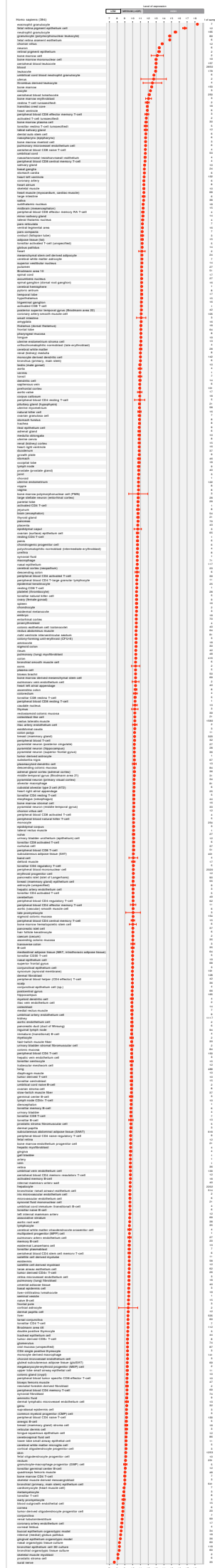
O) B Cells



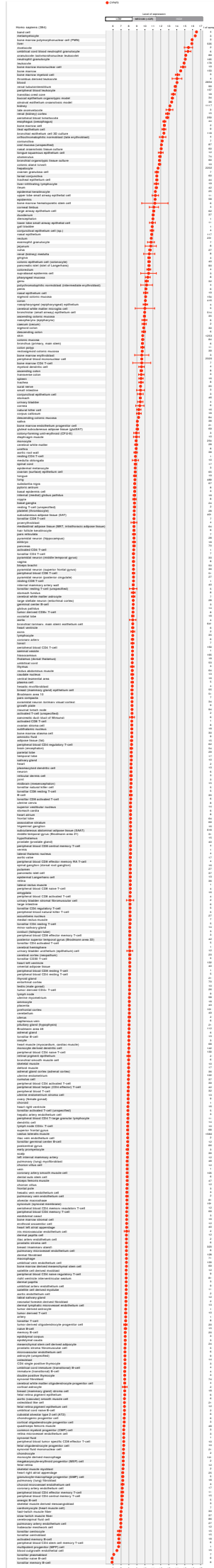
P) Plasmacytoid Dendritic Cells



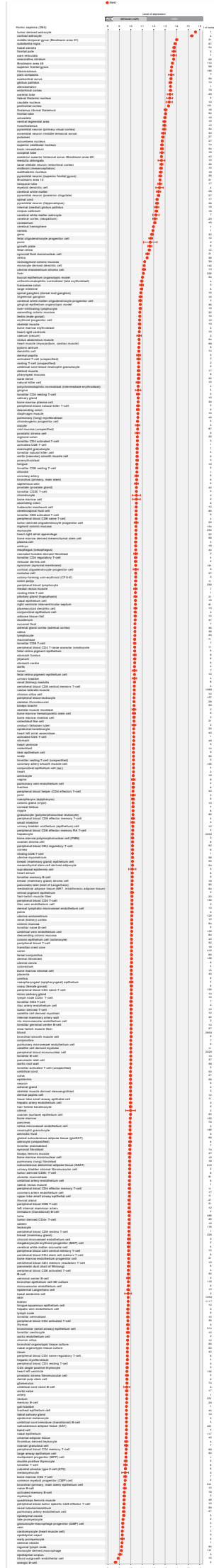
Q) Eosinophils

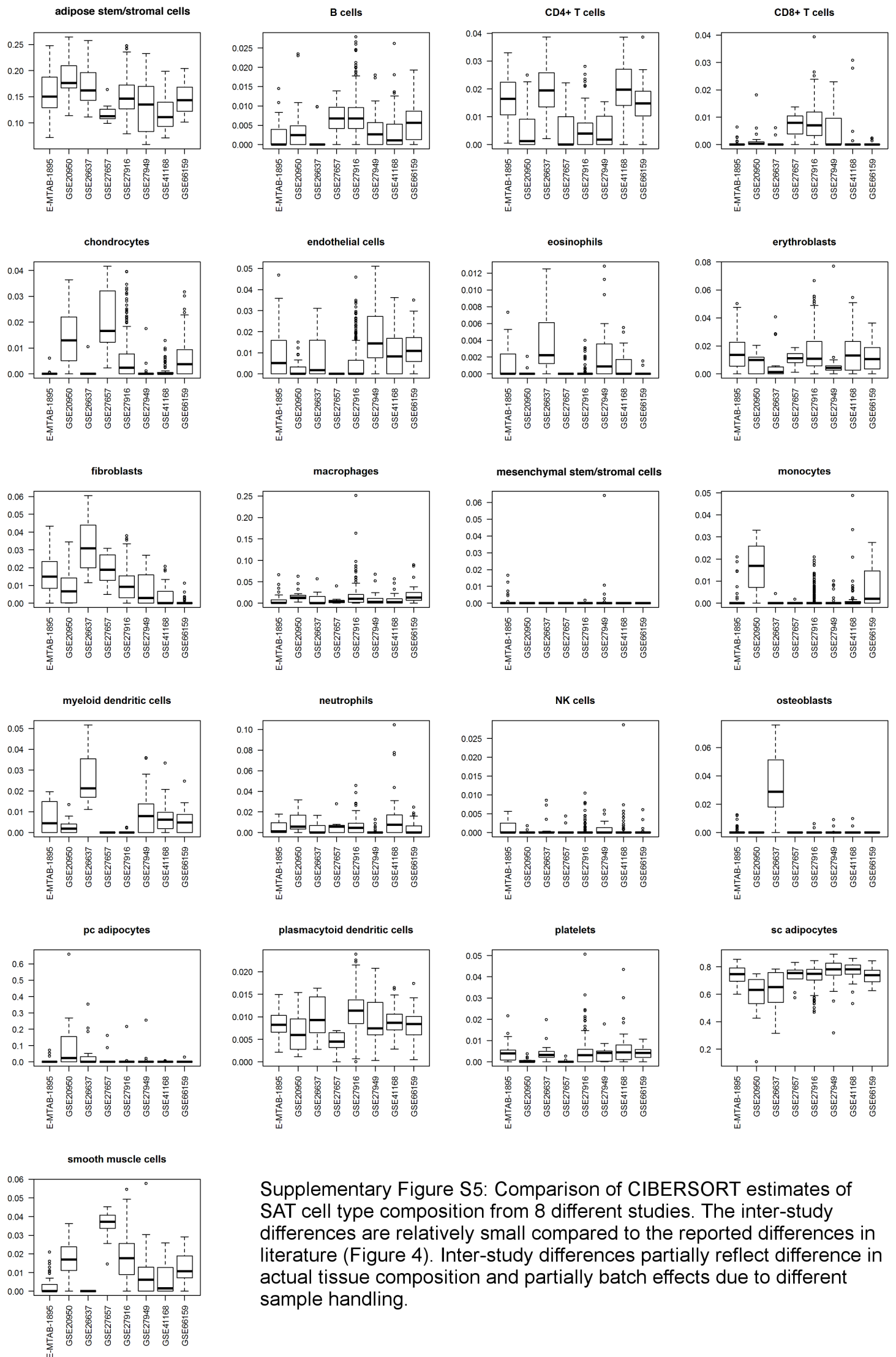


R) Neutrophils

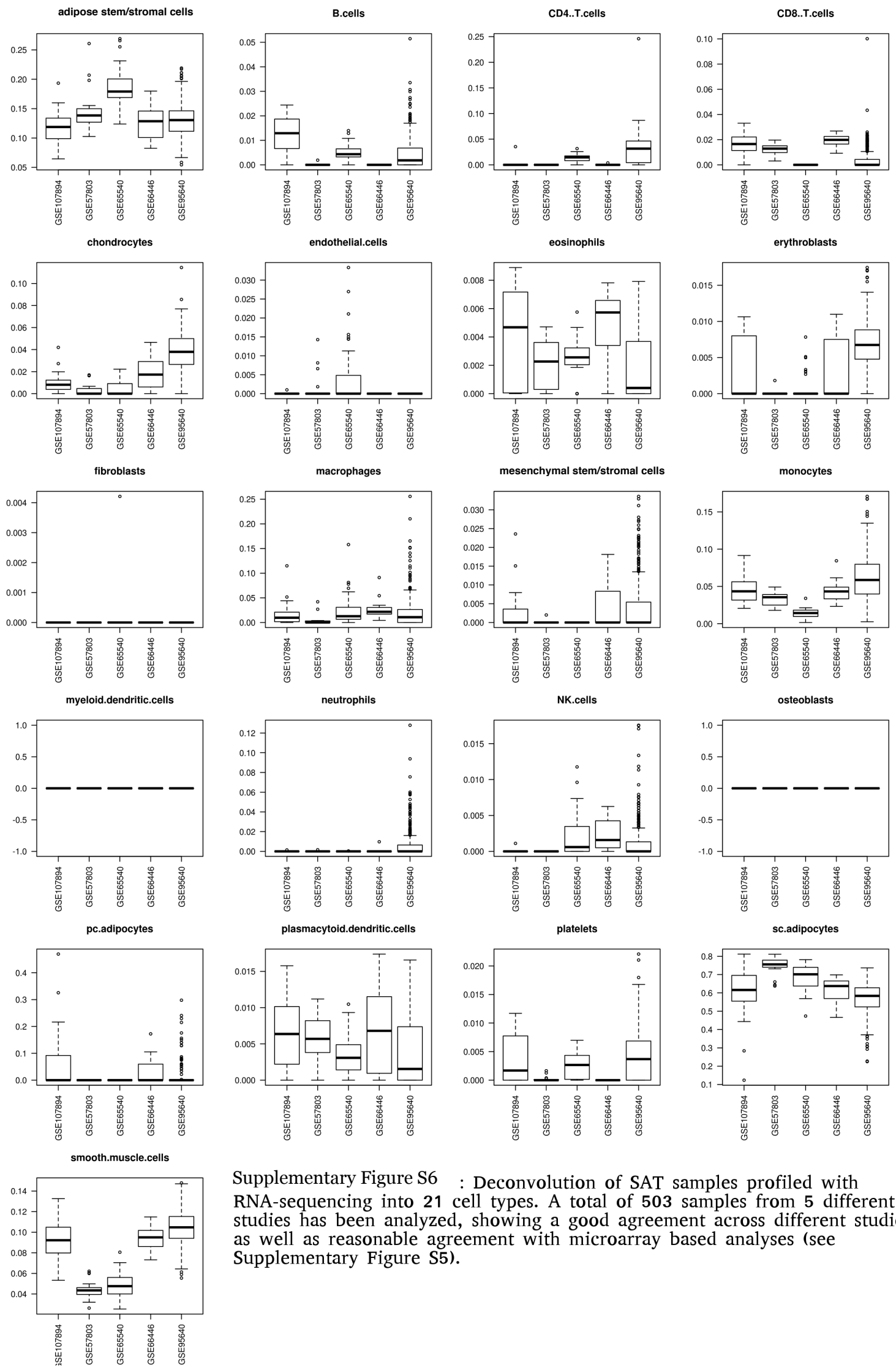


S) Myeloid Dendritic Cells

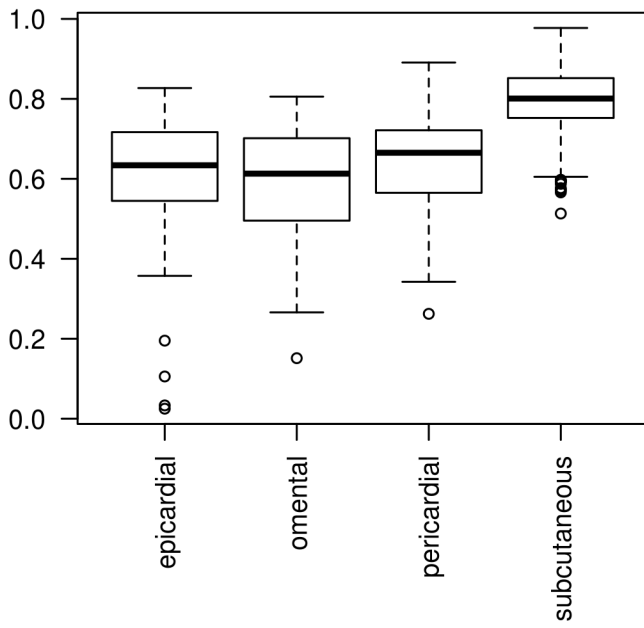




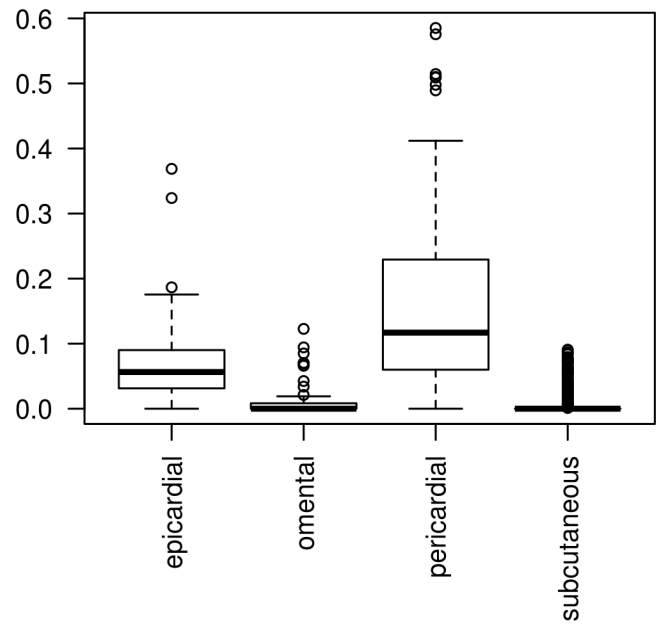
Supplementary Figure S5: Comparison of CIBERSORT estimates of SAT cell type composition from 8 different studies. The inter-study differences are relatively small compared to the reported differences in literature (Figure 4). Inter-study differences partially reflect difference in actual tissue composition and partially batch effects due to different sample handling.



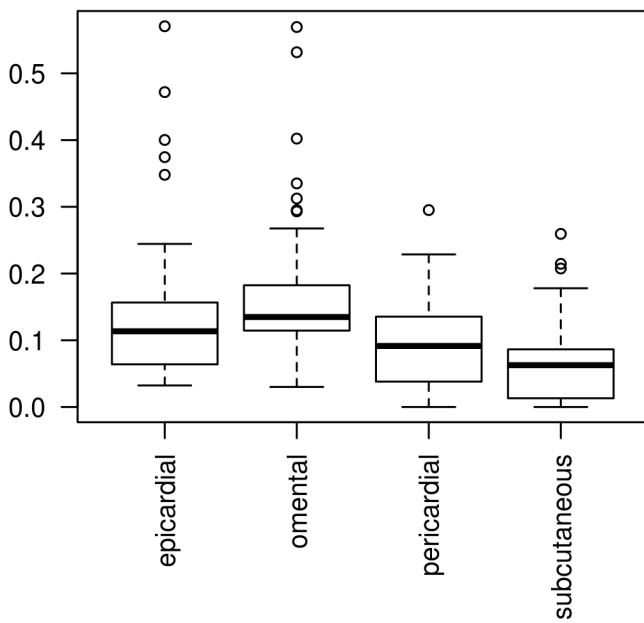
Adipocyte



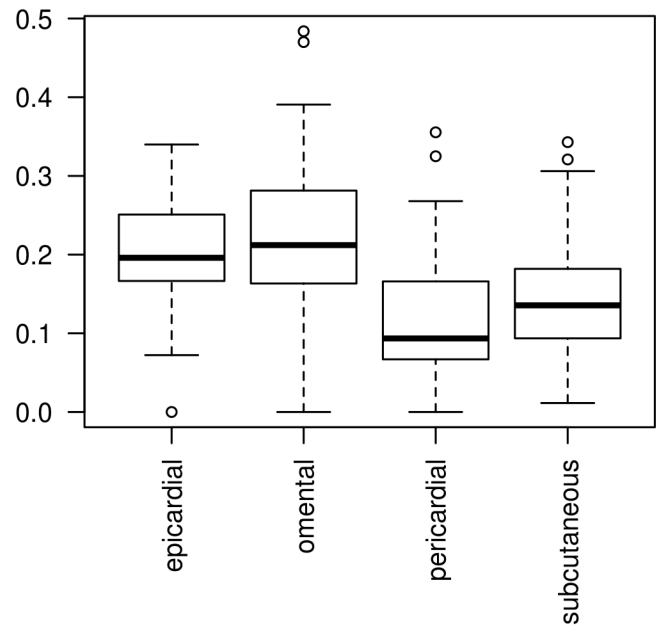
**CD14 - (negative)
Leukocyte**



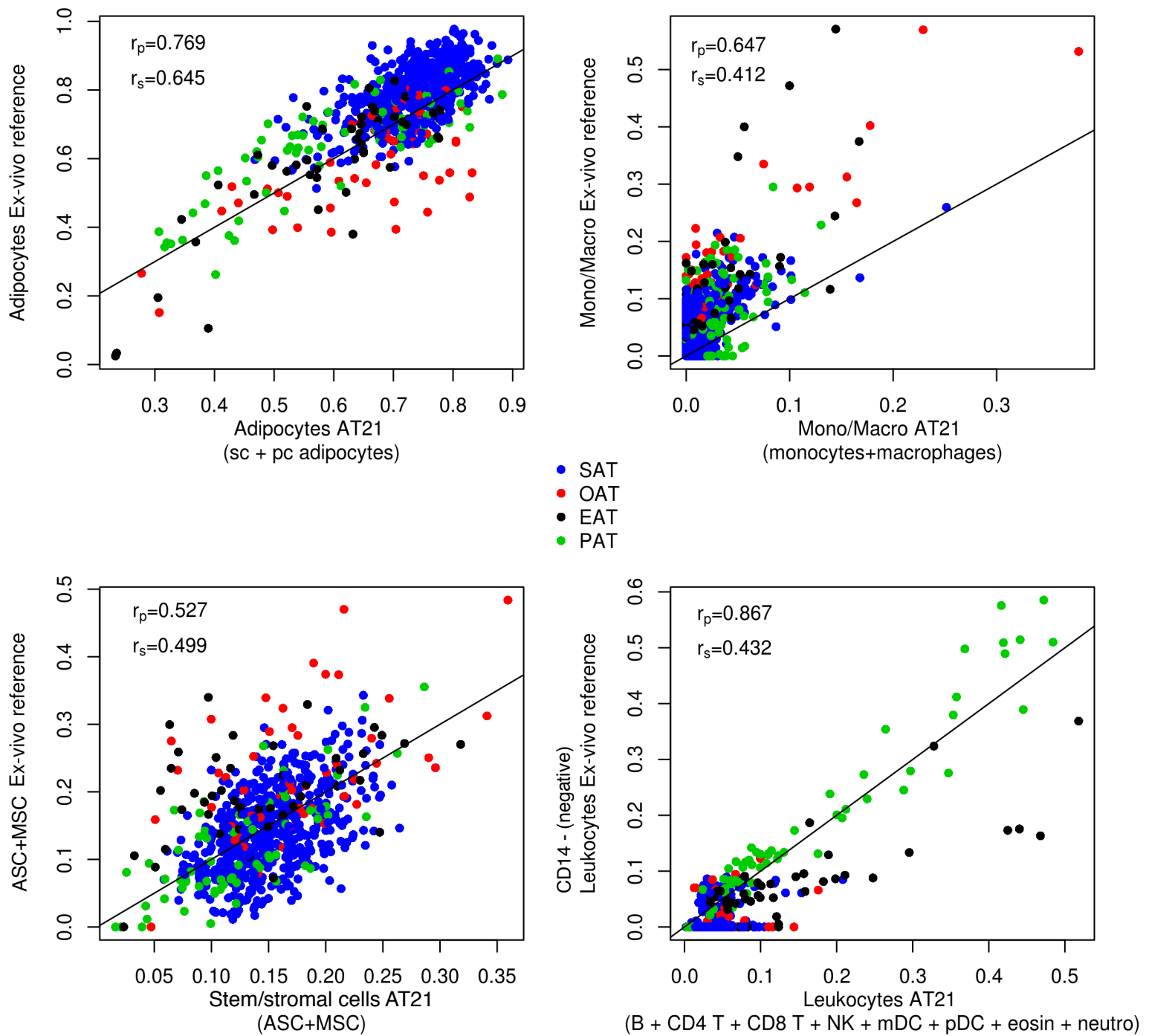
Monocyte.Macrophage



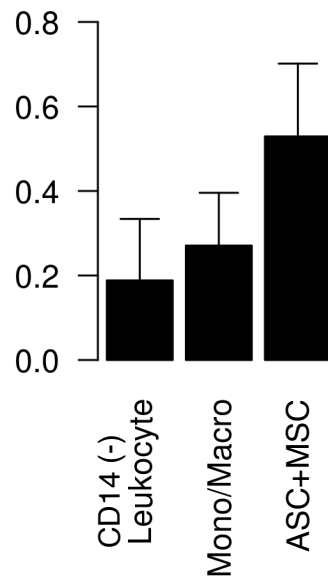
ASC + MSC



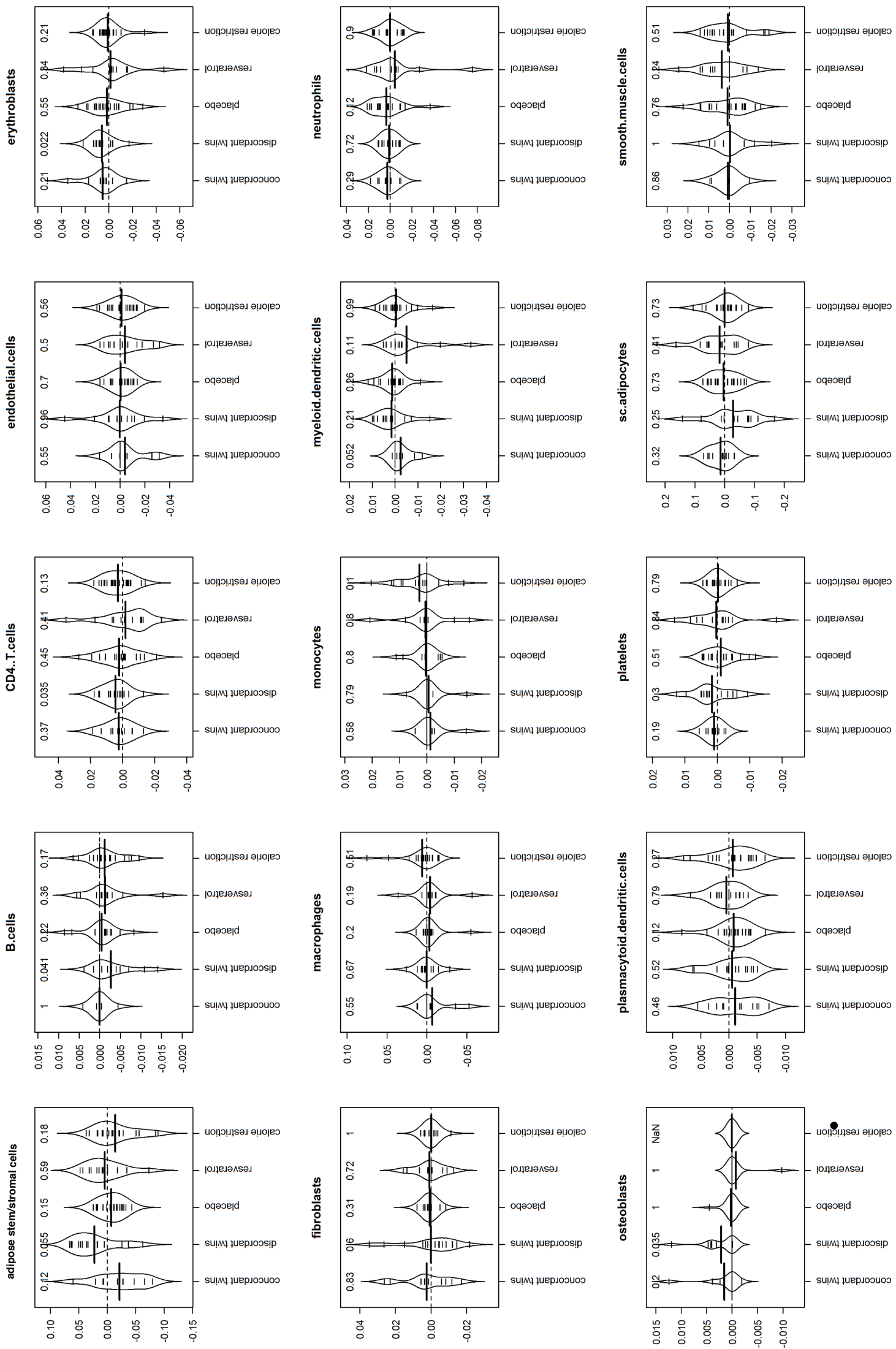
Supplementary Figure S7 : Estimated cell type fractions per adipose depot showing boxplots for the four cell fractions from the ex-vivo reference dataset.



Supplementary Figure S8 : Scatterplot comparing the cell type estimates based on the AT21 signature matrix to the estimates based on the ex-vivo reference dataset for 779 microarray adipose tissue samples from four different adipose tissue depots. For the AT21 estimates, cell fractions have been added up to resemble the less detailed cell fractions represented in the ex-vivo reference dataset.



Supplementary Figure S9 : Barplot of estimated cell fractions (mean and standard deviation) from adipose tissue stromal vascular fraction (SVF) deconvoluted using the ex-vivo reference dataset.



Supplementary Figure S10 : Bean plots showing the population distribution of the cell type percentages for the heavier vs leaner twin, discordant study. The y axis describes differences in estimated cell fractions between the heavier and leaner twin within a pair. P-values are provided above each bean.