

Supplementary Information

An integrated single cell and spatial transcriptomic map of human white adipose tissue

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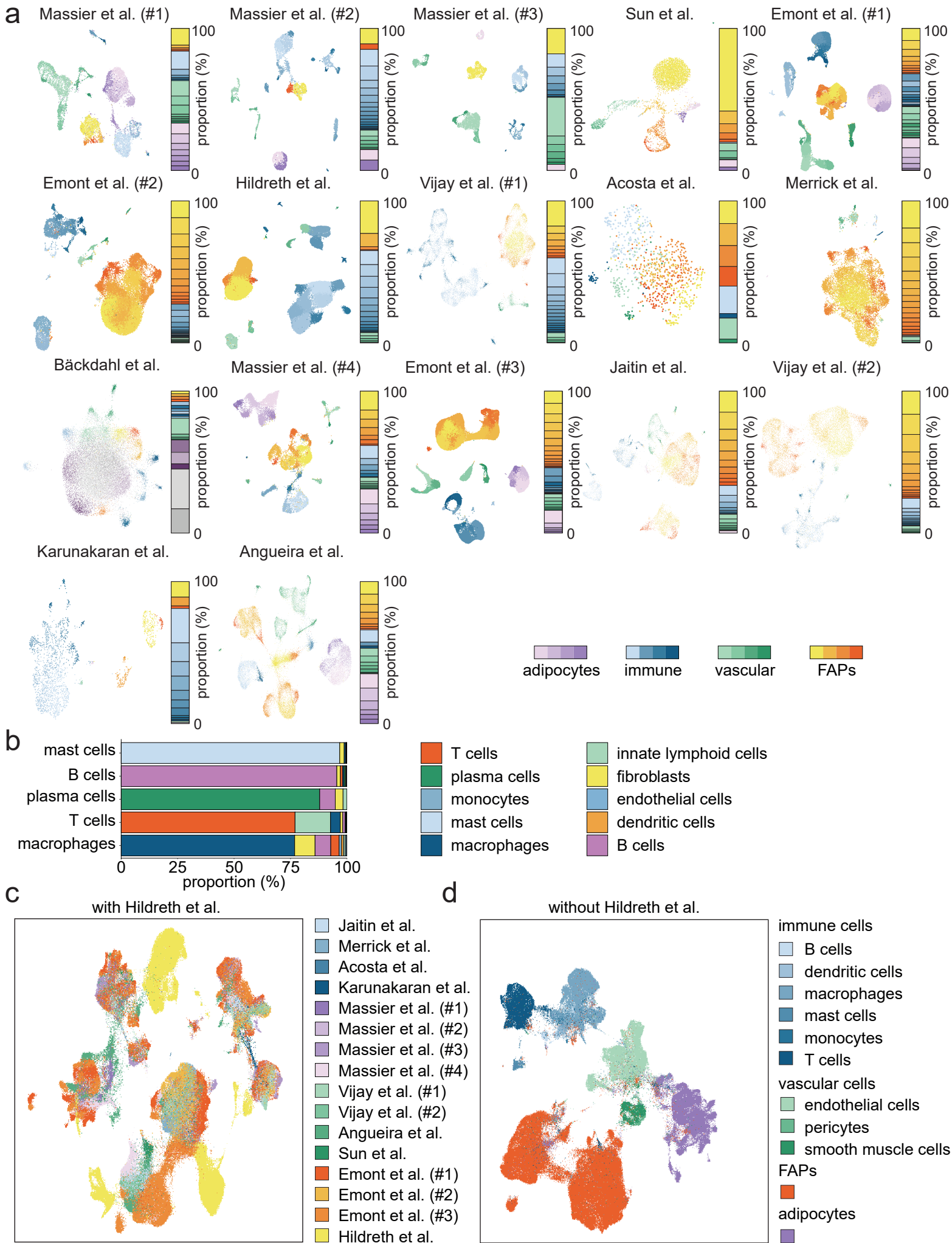
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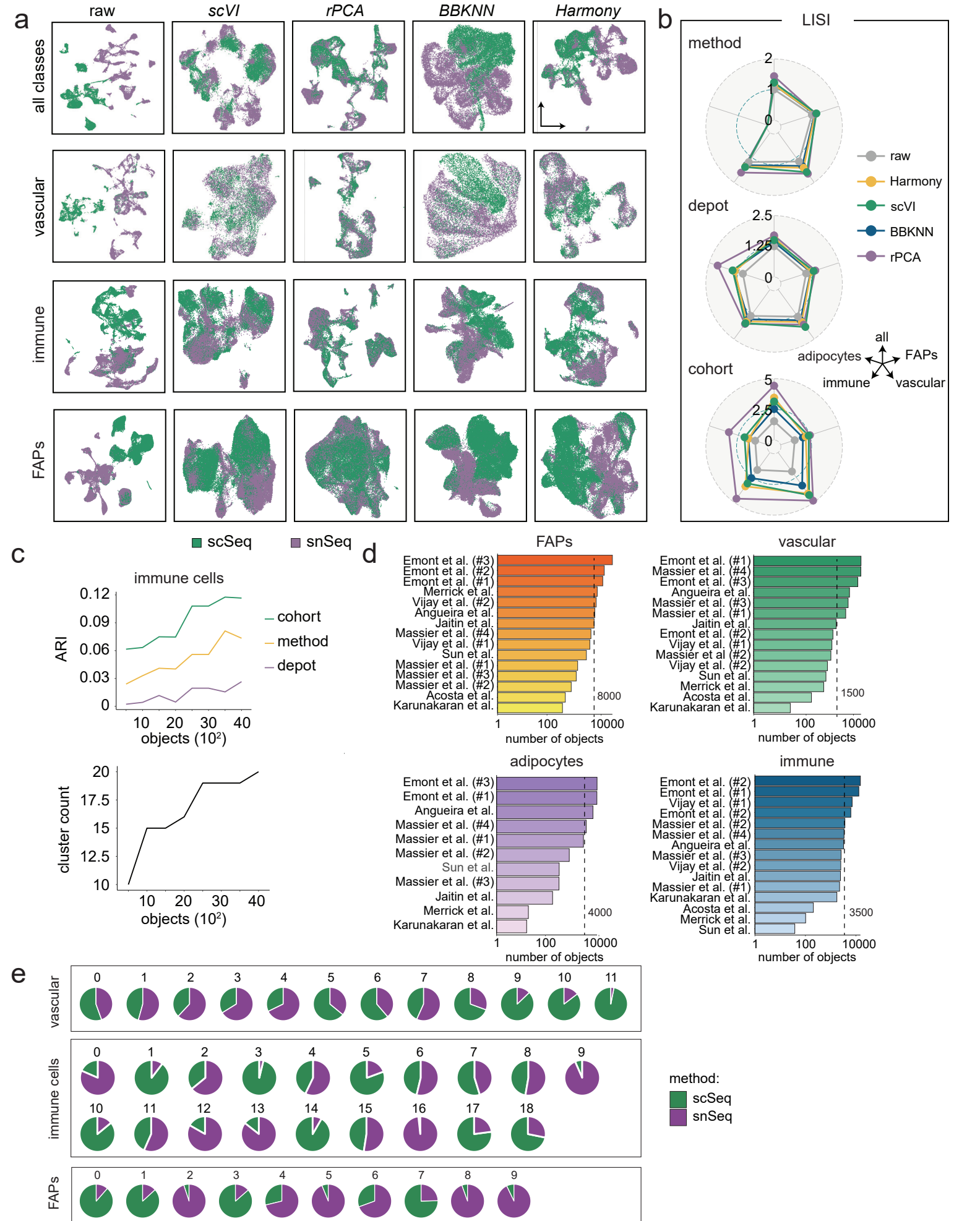


Supplementary Figure 1. Detailed comparison of individual studies (related to main Figure 1).

a. Multiple UMAPs and stacked bar charts from the studies analyzed herein.

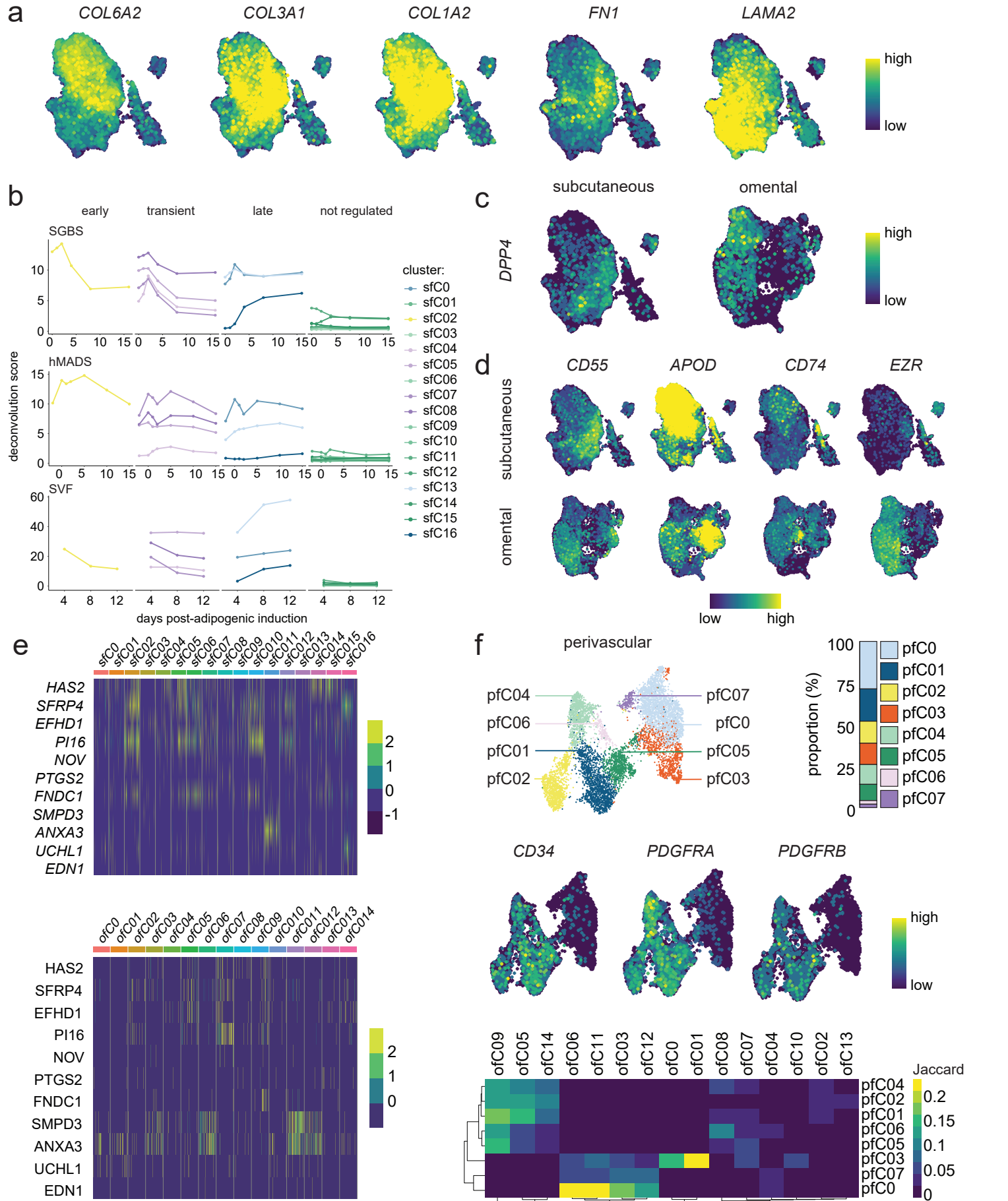
b. Immune models from CellTypist applied to the immune cell class identified herein.

c-d. Single-cell annotation using variational inference (scANVI) analysis c) including or d) excluding Hildreth et al.



Supplementary Figure 2. Optimization of integration (related to main Figure 1).

a. Multiple UMAP plots of unintegrated (raw) and integrated data using the indicated approaches (details in Methods). Upper panels show results using data from all cell classes, while the lower panels display results for individual cell classes. Colors indicate method: single-cell (scSeq, green) and single-nucleus (snSeq, purple) sequencing. b. Local inverse Simpson's index (LISI) for raw or integrated data using the indicated methods displayed according to method, depot and cohort. c. A representative example displaying how the adjusted Rand index (ARI, upper panel) and number of immune cell clusters (lower panel) vary depending on the number of included objects. d. Number of objects included from each study per cell class. Maximum cut-off is highlighted by the vertical dashed line. e. Proportion (%) of scSeq or snSeq objects in the indicated cell classes.



Supplementary Figure 4. Depot-specific differences in FAP signatures (related to main Figure 4).

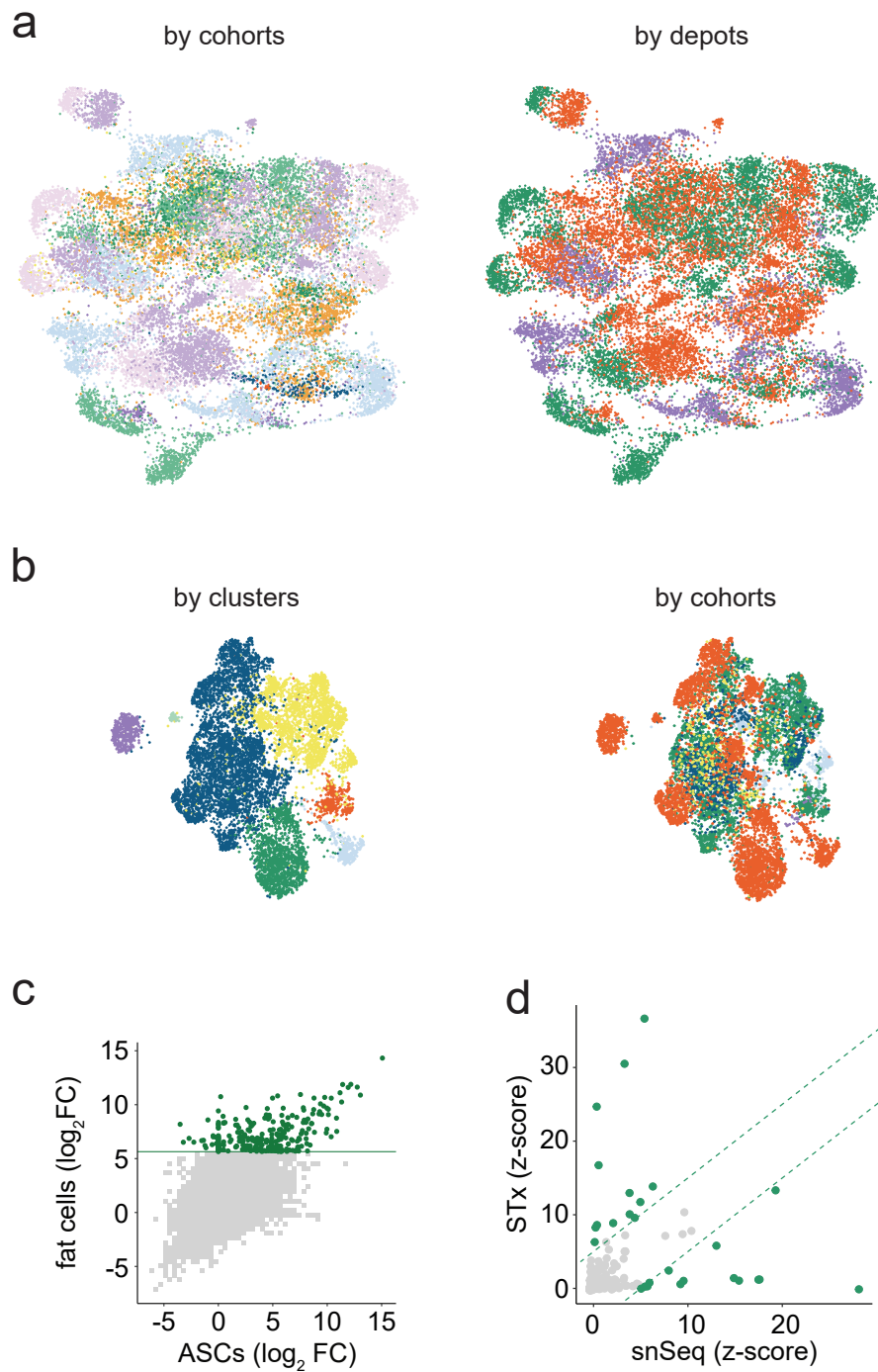
a. Multiple UMAPs showing the expression of selected genes encoding extracellular matrix proteins in subcutaneous FAPs.

b. Expression of clusters sfC0-16 in Simpson-Golabi-Behmel Syndrome (SGBS), (human multipotent adipose-derived stem (hMADS), and primary cells isolated from human stromal vascular fraction (SVF) during in vitro differentiation.

c-d. Multiple UMAPs displaying the expression of marker genes in subcutaneous (sc) and omental (om).

e. Expression of fibro-inflammatory progenitor marker genes in subcutaneous (upper panel) and omental (lower panel) FAPs.

f. A UMAP and a stacked bar chart displaying the proportion (%) of cell clusters in perivascular WAT (upper panels). Expression of selected genes shown in UMAPs (mid panels). Comparisons of similarities between omental and perivascular FAP marker genes (lower panels).



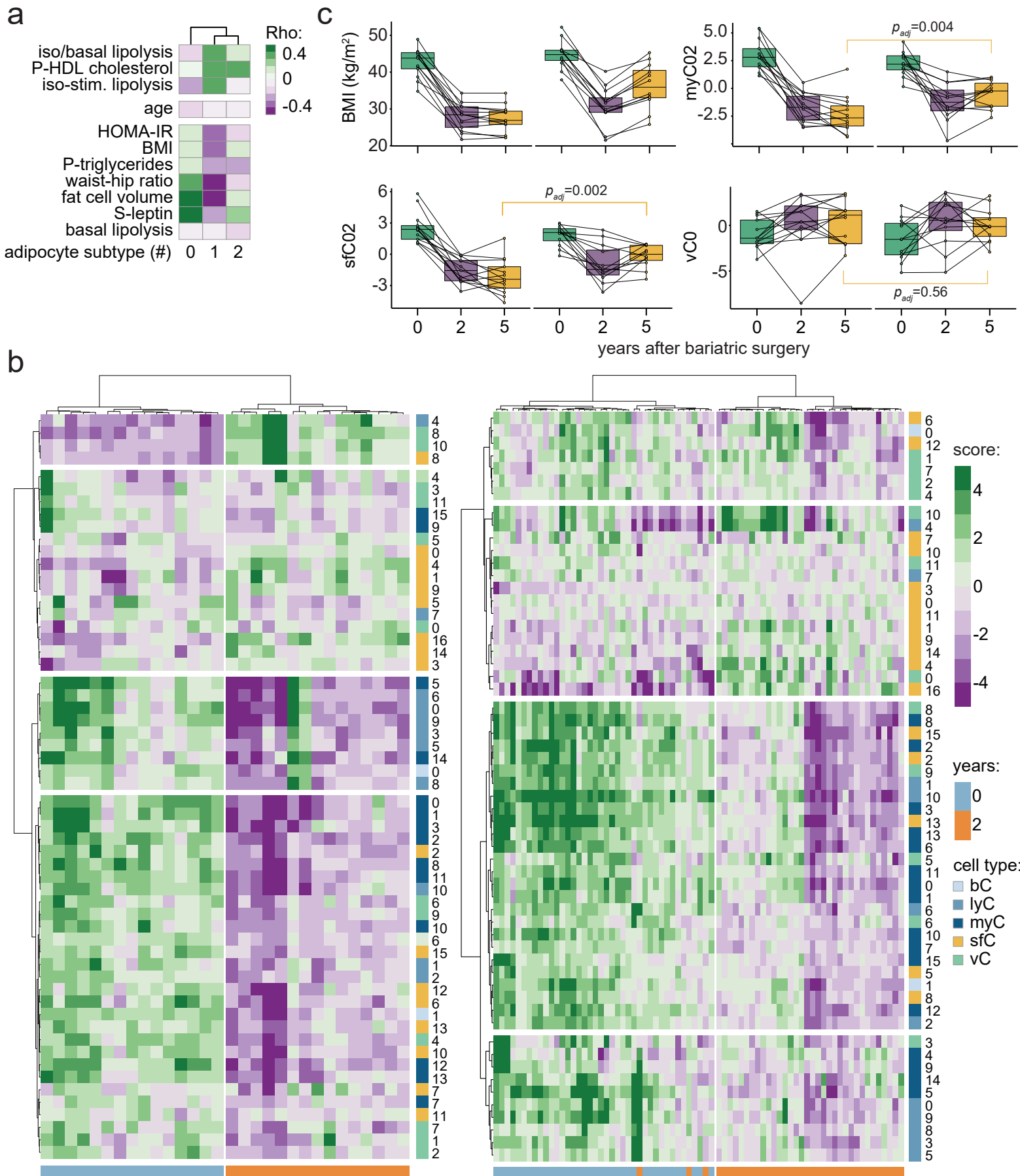
Supplementary Figure 5. Adipocytes separate by cohort and depot after integration (related to main Figure 5).

a. Two UMAPs of adipocyte data from all available studies highlighting cohorts (left panel) and depots (right panel).
b. Two UMAPs of adipocyte data from all available studies highlighting clusters (left panel) and cohorts (right panel) for subcutaneous data only.

c-d. Selection of c) adipocyte-marker genes (vs. adipose-derived stem [ASC] cells) and d) marker genes discordant for spatial transcriptomics (STx) and single-nucleus sequencing (snSeq) data. For details on selection, see Methods.



Supplementary Figure 7. Spot-based deconvolution of spatial transcriptomics data (related to main Figure 7). a-c. Heatmaps for a) the indicated deconvolution tools for the different cell classes, and for the pair-wise within-spot associations between b) cellular subpopulations and c) across all ten subjects for selected cells. In panel b, the area marked in red is magnified in main Figure 7d. In panel c, only correlations with an r-value >0.1 are included.



Supplementary Figure 8. Deconvolution of bulk transcriptomic data in weight loss and regain (related to main Figure 8).

a. Heatmap for the indicated clinical/WAT measures following deconvolution of the bulk transcriptomic data using spatial (STx) marker genes for adipocyte subtype #0 (AdipoLEP), #1 (AdipoPLIN) and #2 (AdipoSAA), respectively. Analyses were performed as in main Figure 8b.

b. Heatmaps detailing cell type deconvolution scores of subcutaneous white adipose tissue before and two years after bariatric surgery in Petrus et al.55 (left panel) and Kerr et al.54 (right panel).

c. Body mass index (upper left panel) and representative examples of cell type deconvolution scores before, two and five years after bariatric surgery in the study from Kerr et al.54 For each panel, data is subdivided according to subjects displaying weight stability (left part) and weight regain (right part) between the two and five year follow-ups. p values were calculated by two-sided Student's t test comparing differences at five years between weight stability (n=13) and weight regain (n=12) and boxplots are presented as interquartile range plus median and Tukey whiskers with individual, paired data points.

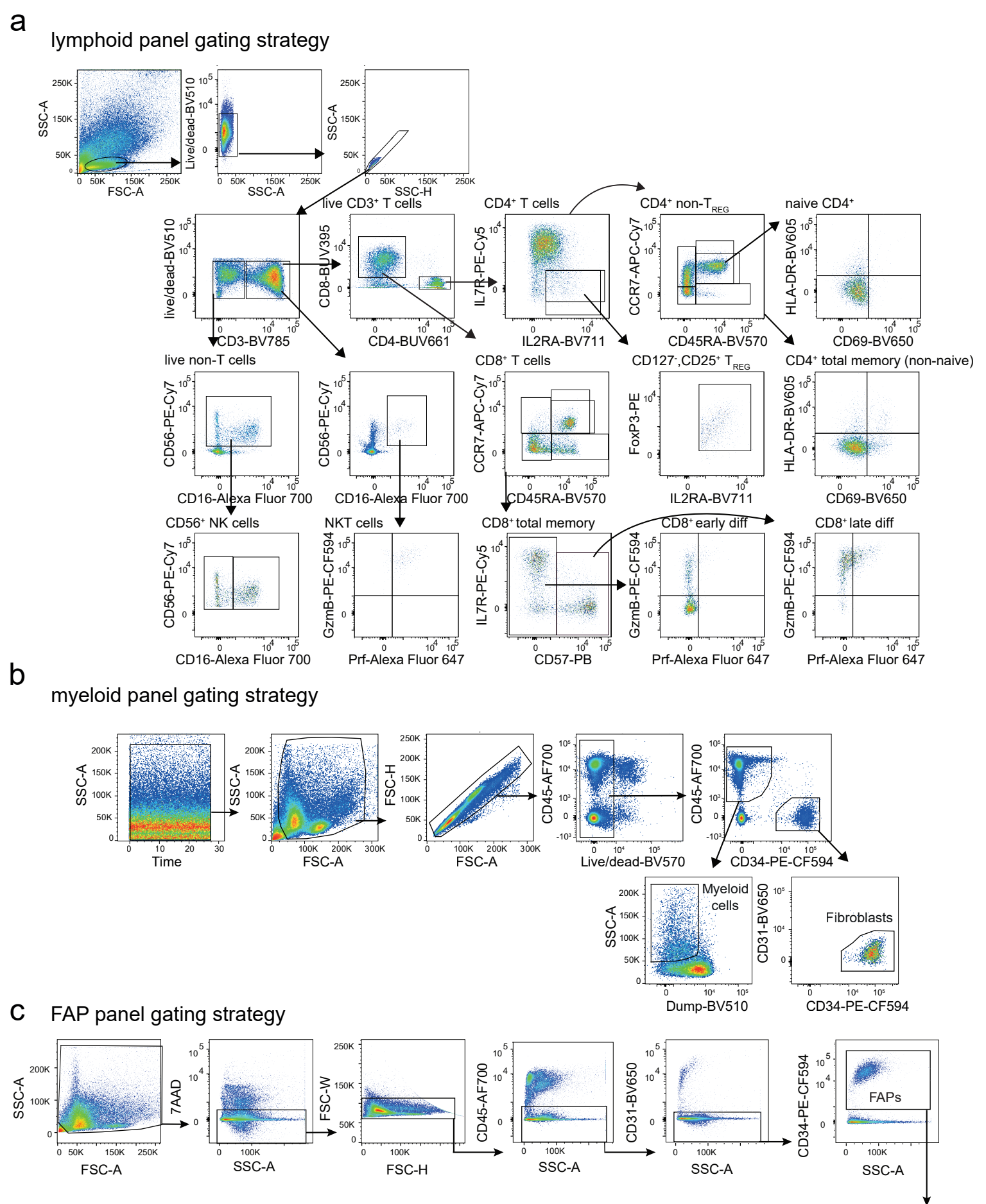


Figure 4K: CD55, CD74, APOD, EZR

Supplementary Figure 9. Gating strategies (related to main Figures 2 and 4).

a-c. Flow-cytometry gating strategies for a) lymphoid, b) myeloid cells and c) fibroblast and adipogenic progenitors (FAPs).

Supplementary Table 1. Details on datasets included in the meta-analysis.					
Publication	Method	Illumina platform	Collection	Data source	Reference
Sun et al.	snSeq	NovaSeq 6000	Switzerland	E-MTAB-9199	Nature 587, 98-102 (2020)
Hildreth et al.	scSeq	NovaSeq 6000S2	USA	GSE155960, GSE156110	Nat Immunol 22, 639-653 (2021)
Vijay et al.	scSeq	NovaSeq 6000	Canada	GSE136230	Nat Metab 2, 97-109 (2020)
Acosta et al.	scSeq	HiSeq 2000	Sweden	Communication with author	Stem Cell Res Ther 8, 250 (2017)
Merrick et al.	scSeq	HiSeq 2500	USA	GSE128889	Science 364(2019)
Karunakaran et al.	scSeq	NovaSeq S2	Canada	GSE151889	Nat Metab 2, 1113-1125 (2020)
Emont et al.	snSeq, scSeq	NextSeq 500	USA	E-MTAB-6677, GSE135134, GSE128889	Nature 603, 926-933 (2022)
Jaitin et al.	scSeq	NextSeq 500	USA	GSE128518	Cell 178, 686-698 e614 (2019)
Angueira et al.	snSeq	NovaSeq 6000	USA	GSE164528	Nat Metab 3, 469-484 (2021)
Bäckdahl et al.	STx	NovaSeq 6000	Sweden	https://doi.org/10.17632/3bs5f8mvbs.1	Cell Metabolism 33, 1–14 (2021)

Supplementary Table 2: Confidence score and references for cluster assignment.				
Cluster	Cell Annotation	Marker	PMID	Confidence
Lymphoid cells				
lyC0	CD4 Th1	<i>CD4</i>	34193602	high
		<i>STAT4</i>	30714682	
		<i>HLA-DR</i>	22474485	
lyC01	CD4 TRM	<i>CD4</i>	34671115	high
		<i>CD69</i>		
		<i>CD44</i>	29262342	
		<i>SELL (CD62L)-</i> <i>and CCR7-</i>	29221731	
lyC02	Early-diff CD8	<i>CD8</i>	35677061	high
		<i>IFNG</i>	35477960	
lyC03	Late-diff CD8	<i>CD8</i>	12766758	
		<i>GZMB</i>	12001998	
		<i>PRF1</i>		
		<i>GNLY</i>		
lyC04	Naive/early-diff CD4	<i>CD4</i>	30559746	medium
		<i>CCR7</i>	22990666	
		<i>SELL</i>	22474485	
		<i>STAT4</i>	35092032	
		<i>LEF1</i>		
lyC05	NKT	<i>NCAM1</i>	11359797	high
		<i>CD56</i>	15238416	
		<i>GZMB</i>		
lyC06	CD16+ NK cells	<i>FCG3RA /CD56</i>	11698225	high
		<i>KLRF1</i>	10671213	
		<i>NKG7</i>	32839609	
		<i>CD3- (NK cells</i> <i>lack CD3)</i>	11698225	
			15368309	
lyC07	Unknown	<i>N/A</i>		
lyC08	Naive /Treg CD4	<i>CD25 (IL2RA)</i>	26122357	medium
		<i>CD127 (IL7R)</i>	21635972	
		<i>CD4</i>		
		<i>LEF1</i>	35092032	
lyC09	CD56+CD16- NK-cells	<i>CD56</i>	11698225	high
		<i>CYRIB</i>		
lyC10	CD4 T cells	<i>CD4</i>	28280496	high (phenotype low)
		<i>HLA-DR</i>	28167943	
		<i>LAMP1</i> <i>(CD107)</i>		
		<i>CD69</i>		
Myeloid cells				
myC0	M2-like macrophage	<i>MRC1</i>	35480482	high
		<i>F13A1</i>	33221826	
		<i>SLC9A9</i>		
		<i>RBPJ</i>	26874522	

myC01	M2-like macrophage	<i>MRC1</i>	35480482	high
		<i>RBPJ</i>	26874522	
		<i>SLC9A9</i>		
		<i>F13A1</i>	33221826	
myC02	Lipid associated macrophage (LAM)	<i>TREM2</i>	31257031	high
		<i>CD9</i>	29760084	
		<i>FABP4</i>		
		<i>CD68</i>	34794433	
myC03	Dendritic cell type 2 (cDC2)	<i>CD1c</i>	33101275	high
		<i>CD74</i>		
		<i>HLA-DR</i>	31474513	
		<i>CLEC10A</i>	31668803	
		<i>TIMP1</i>		
myC04	M2-like macrophage	<i>LYVE1</i>	18227385, 17272806	high
		<i>FOS</i>	31178859	
		<i>LGMN</i>	25205715	
Comments: LYVE1high macrophages indicated role in tissue support and angiogenesis.				
myC05	Non-classical monocyte	<i>FCGR3A=CD16</i>	21653326	high
		<i>LST1</i>		
		<i>TCF7L2</i>	34331874	
		<i>LYST</i>		
myC06	M1-/M2-like macrophage	<i>ITGAX (CD11c)</i>	25035950	high
		<i>CTSB</i>	17200717	
		<i>ITGAM (CD11b)</i>	20357360	
myC07	M2-like macrophage	<i>FN1</i>	35202577	high
		<i>MARCO</i>	25035950	
		<i>TIMD4</i>	34381461	
		<i>FCGR3A</i>	18227385	
Comment: Expressing genes related to vasculature-associated macrophages with high scavenging capacity.				
myC08	M2-like macrophage	<i>TIMP1</i>	7593617	low
		<i>C3</i>	22194611	
		<i>IFITM3</i>	33364194	
		<i>S100A10</i>	31615030	
		<i>MT1E</i>	17322100	
Comment: Gene expression profile indicative of cell-matrix interaction and ECM remodeling				
myC09	M2-like macrophage	<i>MRC1</i>	35480482	high
		<i>TGFBI</i>	25242226	
		<i>ABCA1</i>		
		<i>PPARG</i>	18522830	

myC10	Metabolic-regulated macrophage (Mme)	<i>MSR1</i>	32066456	high
		<i>CD36</i>	25242226	
		<i>PPARG</i>		
		<i>ITGAX (CD11c)</i>		
		<i>PLIN2</i>		
myC11	M2-like macrophage	<i>C1QA</i>	34331874, 35288093	medium
		<i>C1QB</i>		
		<i>C1QC</i>		
		<i>SEPP1</i>	33033253	
		<i>RNASE1</i>		
Comment: Resembles C1Qhi macrophage with M2-like gene expression.				
myC12	M2-like macrophage	<i>C3</i>	22194611	low
		<i>STAT3</i>	30015355	
		<i>FKBP5</i>	29241532	
		<i>PTPRG</i>	16896153	
myC13	M2-like macrophage	<i>MRC1</i>	35480482	high
		<i>MERTK</i>	23023392	
		<i>SQSTM1</i>	18083104	
		<i>MARCH-proteins</i>	32973799	
Comment: M2 macrophages with high levels of E3 ligases indicating increased autophagy and inhibition of inflammasome signaling.				
myC14	Classical monocyte	<i>S100A8</i>	21653326	high
		<i>S100A9</i>		
		<i>CD14</i>		
		<i>LYZ</i>		
		<i>VCAN</i>	34331874	
		<i>CCL3</i>	31543877	
myC15	Redox-regulatory metabolic macrophages (MOX)	<i>HMOX</i>	25600948	high
		<i>FTH1</i>	25600948	
		<i>IL1B</i>	29891687	
		<i>TNF</i>		
		<i>TXNRD1</i>	30229891	
		<i>NFE2L2 (NRF2)</i>	20651288	
		<i>HSPA5</i>	20514049	
		<i>CCL2</i>	19404946	
Comment: Both Ox-phospholipid induced proinflammatory and antioxidant phenotype; also expressing iron handling-related genes.				
Vascular cells				
vC0	BEC - Capillary	<i>CD36, BTNL9</i>	34030460	high
vC01	BEC - Vein	<i>EPHB4, ACKR1</i>	30154011, 28526034	high

vC02	BEC - Artery	<i>EFNB2</i>	11161569	high
vC03	BEC - Capillary/venous	<i>CA4</i>	34030460	high
vC04	Pericyte	<i>PDGFRB, CSPG4, RGS5</i>	21839917	high
vC05	BEC	<i>CFD, PDGFRA</i>	33846639	high, (subtype low)
vC06	LEC	<i>PROX1, LYVE1</i>	10499794	high
vC07	VSMC	<i>ACTA2, MYH11, TAGLN</i>	31340668	high
vC08	BEC	<i>TIMP1</i>	7517405	high, (subtype low)
vC09	BEC	<i>FTL</i>		high, (subtype low)
vC010	Unknown	<i>N/A</i>		low
vC011	BEC - Vein	<i>ACKR1</i>	28526034	medium

Comparing enriched marker genes in the respective cluster to a manually curated list of specific endothelial cell subtype markers from the publications PMID: 34030460 and 32059779

Cluster	Capillary (n=35)	Artery (n=28)	Vein (n=32)	
vC0	14	2	5	
vC01	1	2	18	
vC02	4	19	1	
vC03	8	2	9	
vC05	5	5	2	
vC08	2	1	1	
vC09	2	0	0	
vC011	3	1	8	

List of included genes

Capillary: *MFSD2A, AQP7, KDR, RGCC, CA4, SPARC, SGK1, PRX, CYB5A, ACVRL1, ADGRF5, ADGRL2, F2RL3, INFR1, VIPR1, ADRB1, ARHGAP6, IFI27, PREX1, PRKCE, SH2D3C, SORBS1, EMP2, ITGA1, SLC9A3R2, AFF3, MEIS1, CD36, GPIHBP1, FCN3, BTNL9, BTNL8, CD14, IL7R, IL18R1*

Artery: *CYTL1, GKN3, EFNB2, SOX17, BMX, SEMA3G, HEY1, LTBP4, GJA5, GJA4, CLDN10, FBLIM1, FBLN2, FBLN5, MGP, FN1, LTBP1, SERPINE2, CPAMD8, CXCL12, EFBN2, VEGFA, NOS1, DKK2, DLL4, SOX5, HES4, PRDM16*

Vein: *LCN2, SLC38A5, BGN, PTGS1, CH25H, BST2, CAR4, VCAM1, SELP, SELE, ACKR1, NR2F2, ADAMTS9, IGFBP7, HDAC9, RORA, ACTN1, LDLRAD3, LRRC1, EBF1, EBF3, MEOX1, MEOX2, ZNF385D, TACR1, ROBO1, CYSLTR1, CPXM2, MMP16, PDE7B, PDE2A, SPRY1*

Abbreviations: BEC; Blood endothelial cell, LEC; Lymphatic endothelial cell, VSMC; vascular smooth muscle cell

Supplementary Data Table 3. Datasets used for clinical correlations in Figure 8.					
Publication	Method	Platform	Collection	Data source	Reference
Arner, E. et al.	Microarray	GeneChip Human Gene 1.0 ST Array	Sweden	GSE25402	Diabetes 61, 1986-1993 (2012)
Arner, P. et al.	Microarray	Clariom D array	Sweden	GSE113080	Cell Metabolism 28, 45-54 (2018)
Kerr, A. et al.	Microarray	Clariom D array	Sweden	GSE199063	J Intern Med 288, 219-233 (2020)
Arner, P. et al.	Microarray	GeneChip Human Transcriptome Array 2.0	Sweden	GSE76399	Diabetologia 59, 2393-2405 (2016)
Petrus, P. et al.	Microarray	Affymetrix Human Gene 1.1 ST array	Sweden	GSE59034	Cell Rep 25, 551-560 (2018).
Krieg, L et al.	Microarray	HumanHT - 12	Germany	Suppl. Table 7	Gut 71, 2179-2193 (2022).
Imbert, A. et al.	RNASeq	HiSeq 2500	Netherlands, Denmark, United Kingdom, Greece, Bulgaria, Germany, Spain, and Czech Republic	GSE141221	J Clin Endocrinol Metab 107, e130-e142 (2022).
Armenise, C. et al.	RNASeq	HiSeq 2000	Netherlands, Denmark, United Kingdom, Greece, Bulgaria, Germany, Spain, and Czech Republic	GSE95640	Am J Clin Nutr 106, 736-746 (2017).

Supplementary Table 4. Fluorochrome conjugated antibodies used in the flow cytometric analysis.

Myeloid panel

Fluorochrome	Marker	Clone	Supplier	Cat no.	Titration
BUV395	CD8	RPA-T8	BD Biosciences	563795	1 in 250
BUV661	CD4	SK3	BD Biosciences	612962	1 in 50
BUV737	PD-1	EH12.1	BD Biosciences	612791	1 in 50
Aqua (BV510)	Live/Dead		Invitrogen	L34957	1 in 1000
BV570	CD45RA	HI100	Biolegend	304132	1 in 200
BV605	HLA-DR	G46-6	BD Biosciences	562845	1 in 250
BV650	CD69	FN50	Biolegend	310934	1 in 50
BV711	CD25	2A3	BD Biosciences	563159	1 in 50
BV785	CD3	OKT3	Biolegend	317330	1 in 300
Pacific Blue	CD57	HCD57	Biolegend	322316	1 in 600
PE	FoxP3	236A/E7	BD Biosciences	560852	1 in 50
PE-CF594	Granzyme B	GB11	BD Biosciences	562462	1 in 500
PE-Cy5	CD127	A019D5	Biolegend	351324	1 in 100
PE-Cy7	CD56	NCAM16.2	BD Biosciences	335826	1 in 300
AlexaFluor647	Perforin	dG9	Biolegend	308110	1 in 180
AlexaFluor 700	CD16	3G8	BD Biolegend	557920	1 in 300
APC-Cy7	CCR7 (CD197)	G043H7	Biolegend	353212	0.5µl directly to cell pellet

Lymphoid panel

Fluorochrome	Marker	Clone	Supplier	Cat no.	Titration
BUV395	CD14	MφP9	BD	563561	1 in 100
BUV615	CCR2	LS132.1D9	BD	751045	1 in 100
BUV661	HLA-DR	G46-6	BD	612981	1 in 50
BUV737	CD16	3G8	BD	612786	1 in 50
BV421	CD163	GHI/61	Biolegend	333612	1 in 50
BV510	CD3	OKT3	Biolegend	317332	1 in 50
BV510	CD19	HIB19	Biolegend	302242	1 in 50
BV510	CD56	5.1H11	Biolegend	362534	1 in 50
BV510	CD304				1 in 50
BV570	live/dead yellow		Invitrogen	L34968	1 in 1000
BV650	CD31	WM59	BD	740571	1 in 100
BV711	CD141	1A4	BD	563155	1 in 25

BV785	CD55	IA10	BD	742681	1 in 40
BB515	CD206	19.2	BD	564668	1 in 25
BB700	CD1c	F10/21A3	BD	746095	1 in 50
PE-CF594	CD34	563	BD	562449	1 in 200
PECy5	CD5	-	BD	555354	1 in 100
PE-Cy7	CD11c	3.9	Biolegend	301608	1 in 25
AlexaFluor647	CD9	HI9a	Biolegend	312107	1 in 50
AlexaFluor 700	CD45	HI30	BD	560566	1 in 50
APC-Cy7	CD36	GP11b	Biolegend	312108	1 in 50
FAP panel					
Fluorochrome	Marker	Clone	Supplier	Cat no.	Titration
BV650	CD31	WM59	BD	740571	1 in 100
BV786	CD55	IA10	BD	742681	1 in 40
7-AAD	live/dead		BD	559925	5 µl per sample
PE	CD74	LN2	Biolegend	326807	1 in 20
PE-CF594	CD34	563	BD	562449	1 in 100
AlexaFluor647	EZR		NovusBiologicals	NBP2-52977AF647	1 in 20
AlexaFluor700	CD45	HI30	BD	560566	1 in 100