

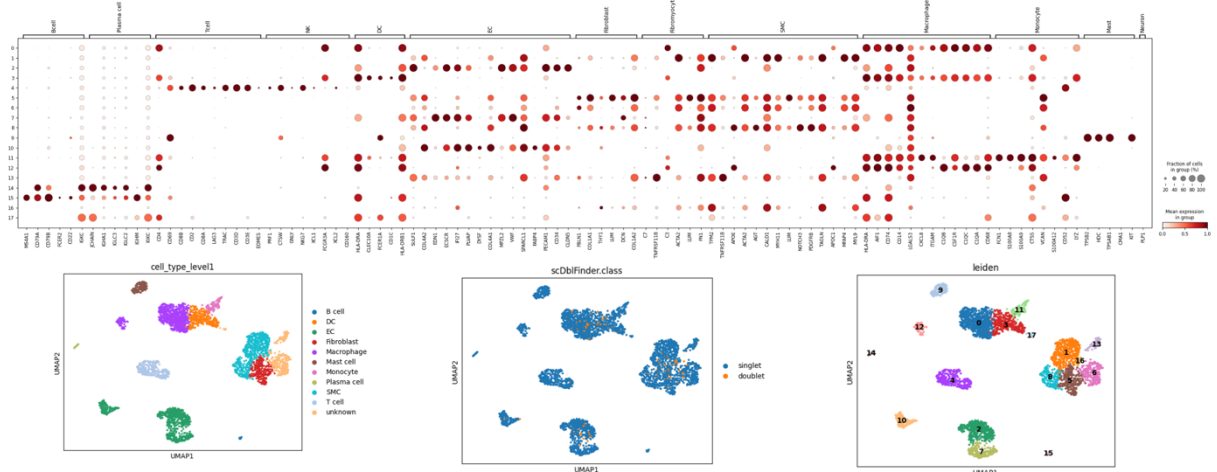
Supplementary Material

Integrated single-cell atlas of human atherosclerotic plaques

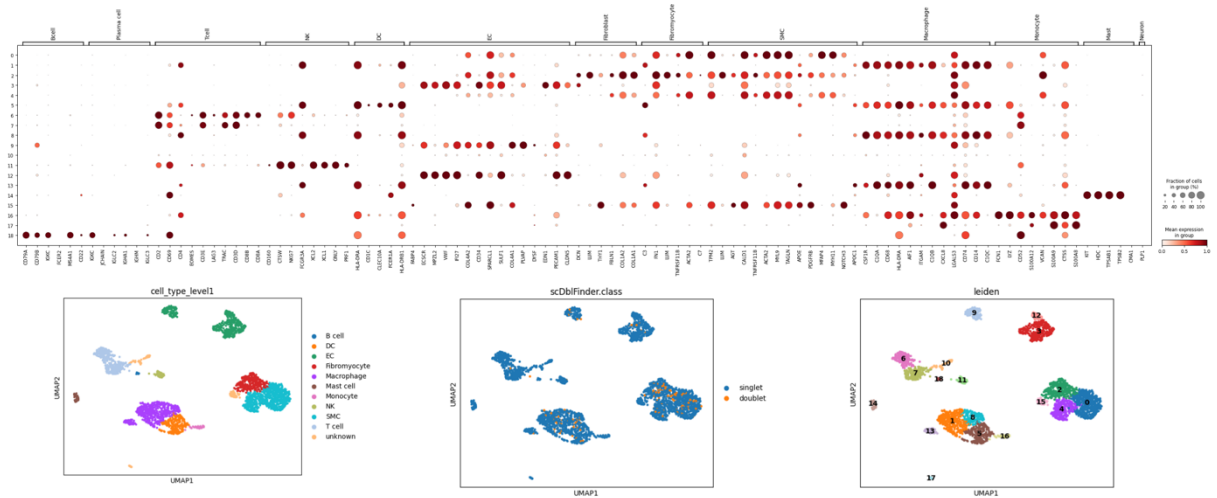
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Supplementary Figures

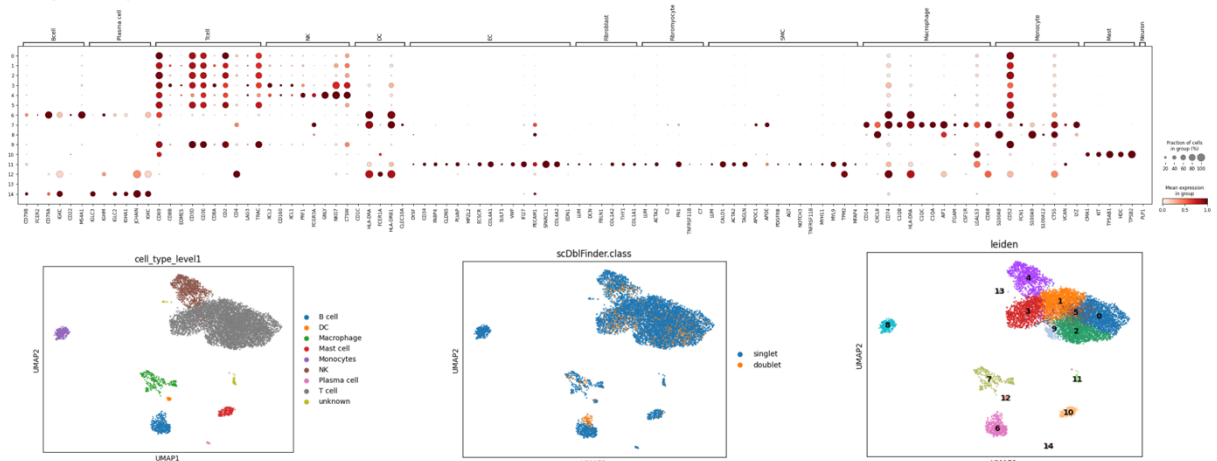
Pan sample 2



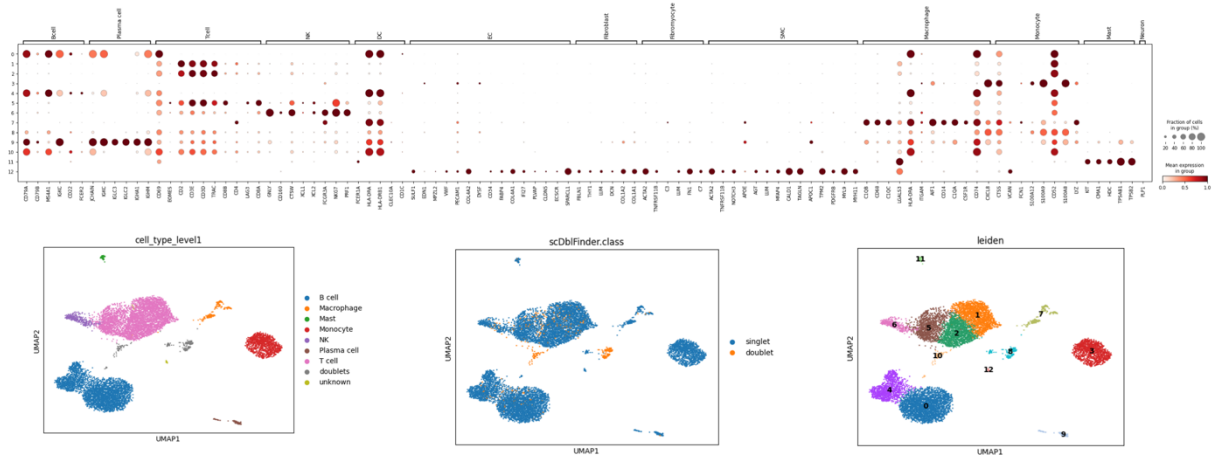
Pan sample 3



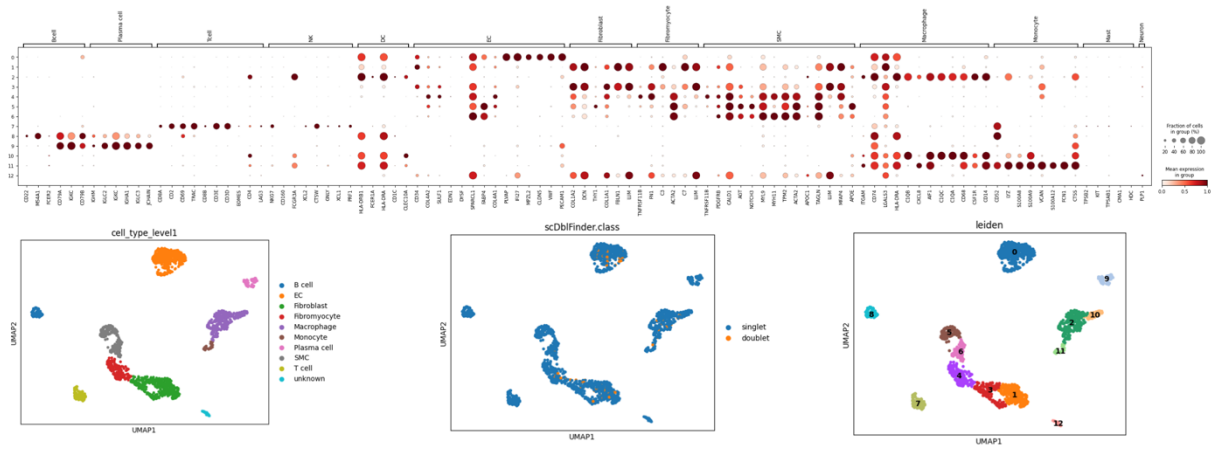
Slysz sample 6



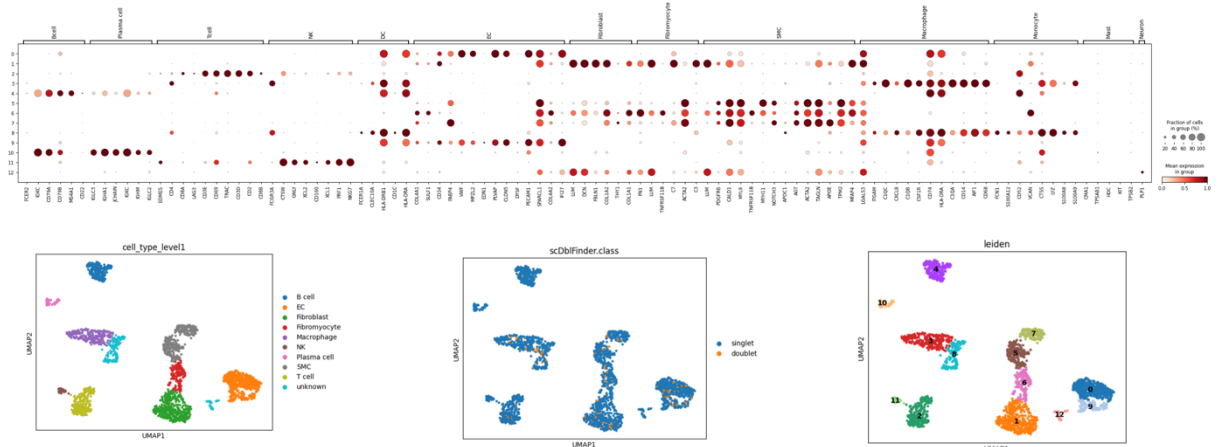
Slysz sample 8



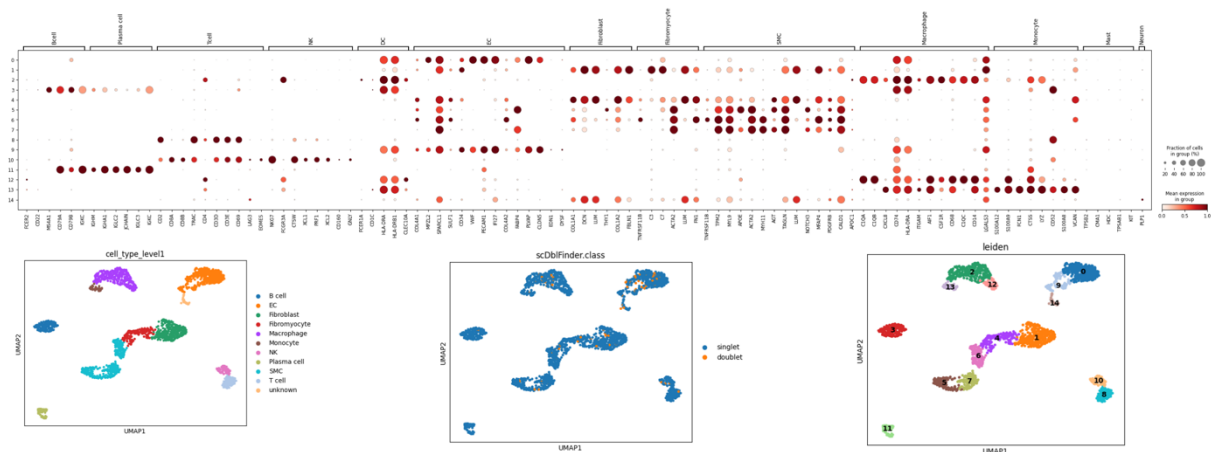
Wirka sample 5



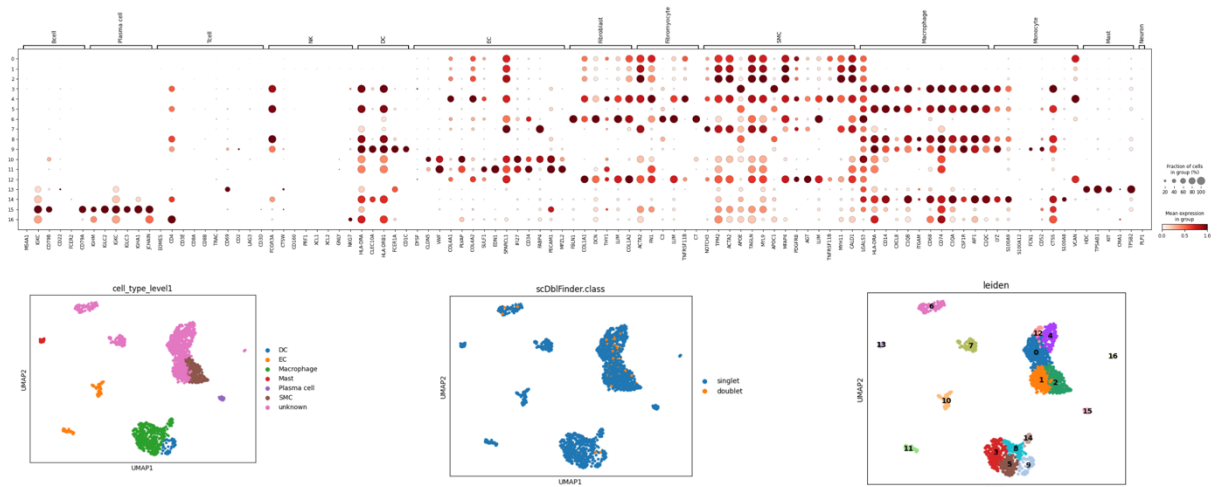
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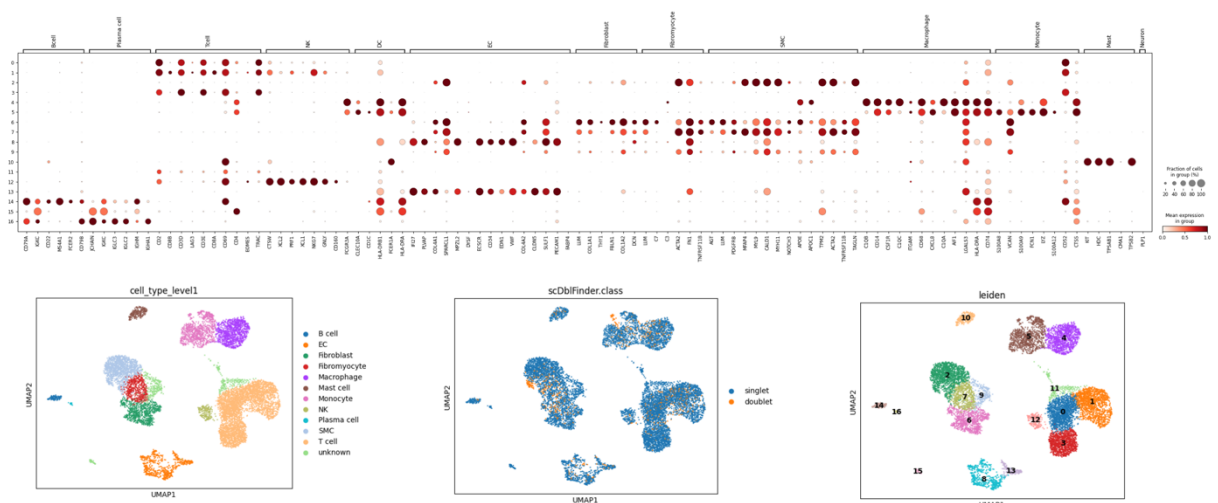
Wirka sample 7



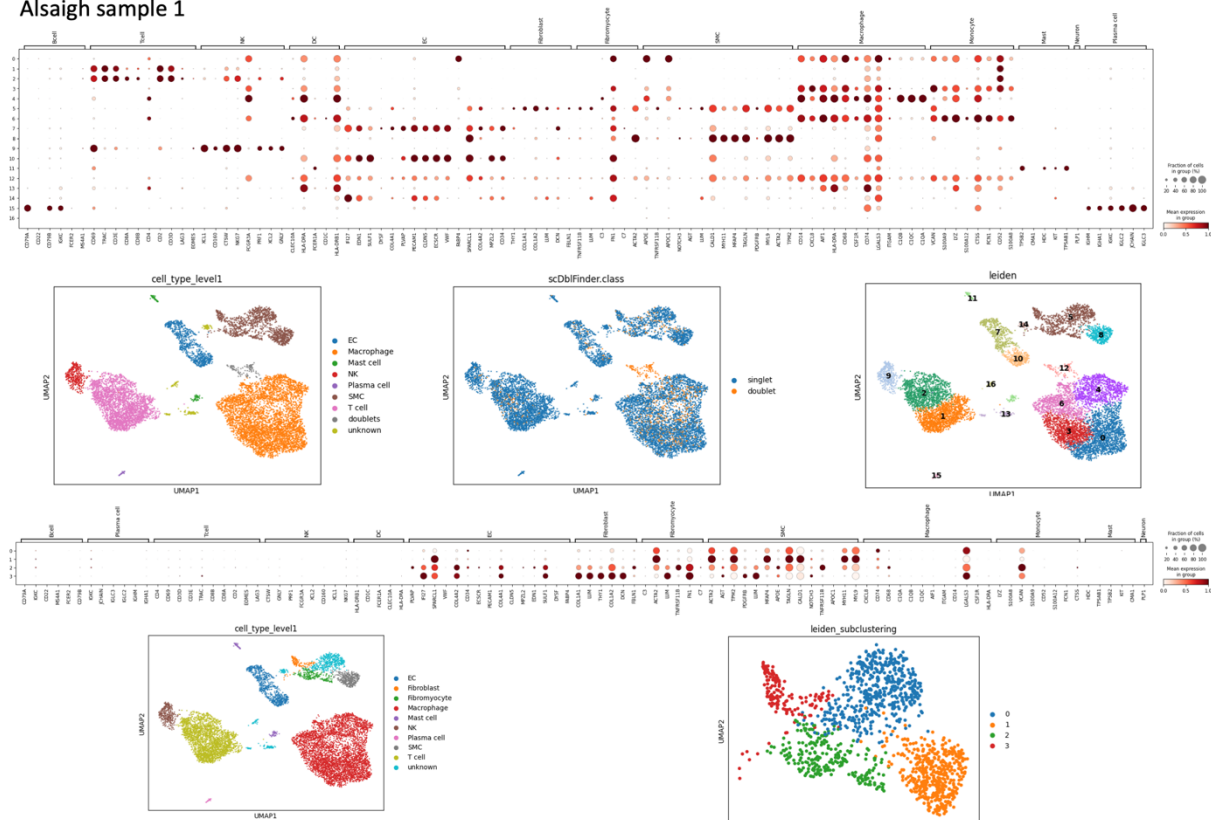
Wirka sample 8



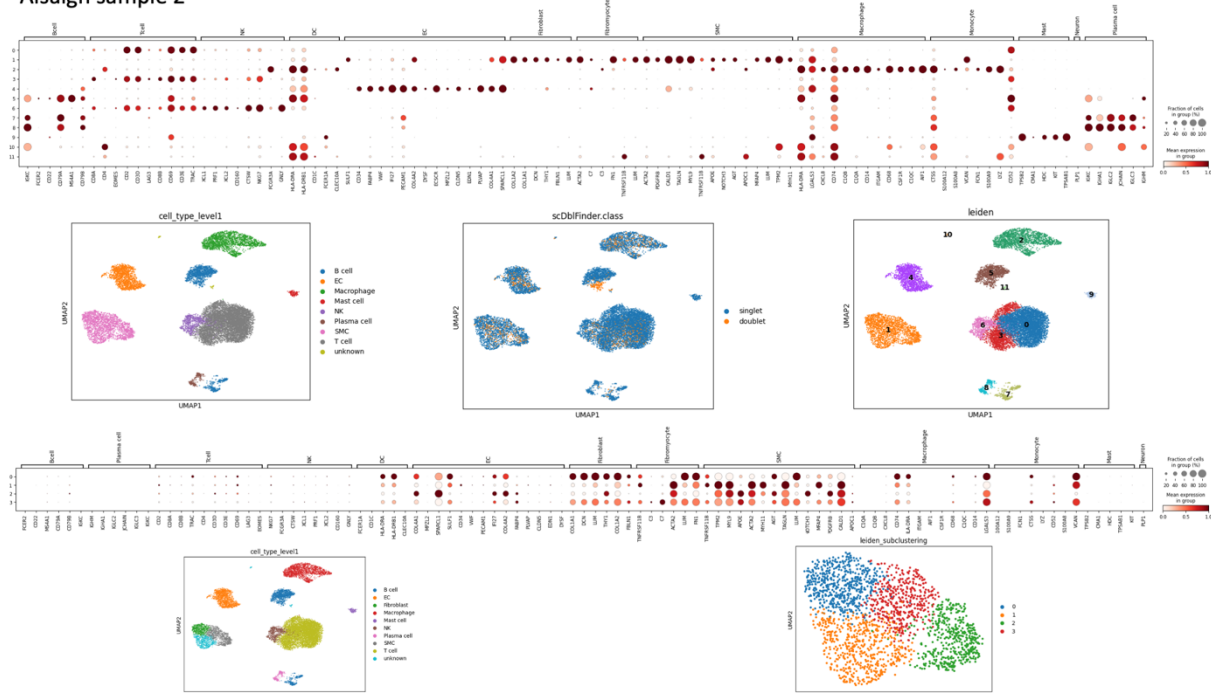
Alsaigh sample 3



Alsaigh sample 1



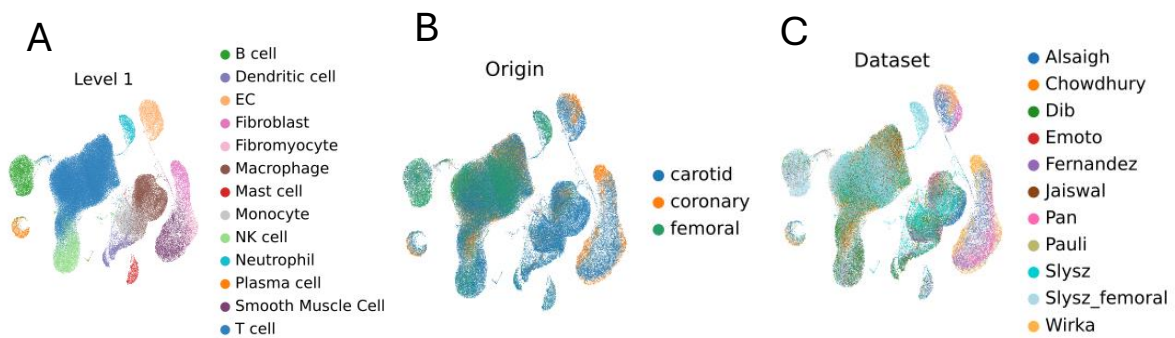
Alsaigh sample 2



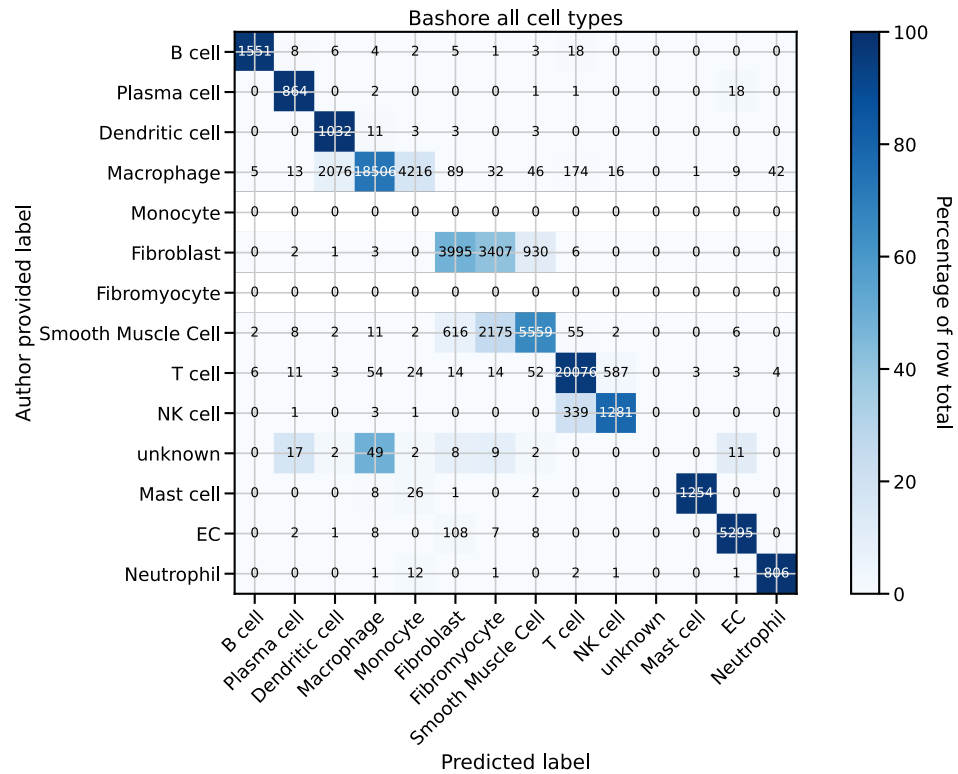
Suppl. Figure 1: Manual annotation of a subset of the samples in the atlas using marker genes. For the manual annotation several samples were preprocessed and annotated with dedicated marker genes. For each sample the dot plot with the Leiden clusters on the y-axis and the marker genes on the x-axis is shown, together with the corresponding UMAPs of the annotated cells with Level 1, the doublet classification from ScDbtFinder, and the Leiden clusters. In some cases, sub-clustering on specific clusters were performed and the corresponding dot plot, cell type UMAP and Leiden UMAP shown.

Method	Bio conservation					Batch correction					Aggregate score		
	Isolated labels	KMeans NMI	KMeans ARI	Silhouette label	cLISI	Silhouette batch	iLISI	KBET	Graph connectivity comparison	PCR	Batch correction	Bio conservation	Total
scPoli	0.64	1.00	1.00	1.00	1.00	0.84	0.64	0.54	0.89	0.16	0.61	0.83	0.80
scPoli_nb	0.22	1.00	0.82	0.99	1.00	0.87	0.91	0.99	0.80	0.14	0.74	0.80	0.78
Harmony	1.00	0.79	0.84	0.40	1.00	0.94	0.60	0.33	0.59	0.48	0.59	0.81	0.72
scGen	0.37	0.80	0.52	0.43	1.00	1.00	0.60	0.37	1.00	0.84	0.76	0.62	0.68
scANVI	0.51	0.77	0.91	0.37	1.00	0.63	0.54	0.29	0.82	0.72	0.60	0.71	0.67
PCA	0.87	0.67	0.53	0.24	1.00	0.04	0.00	0.00	0.61	0.00	0.13	0.66	0.45
scVI	0.32	0.00	0.00	0.00	0.65	0.80	0.47	0.11	0.79	0.99	0.65	0.19	0.37
LIGER	0.00	0.29	0.42	0.20	0.00	0.00	1.00	1.00	0.00	1.00	0.60	0.18	0.35

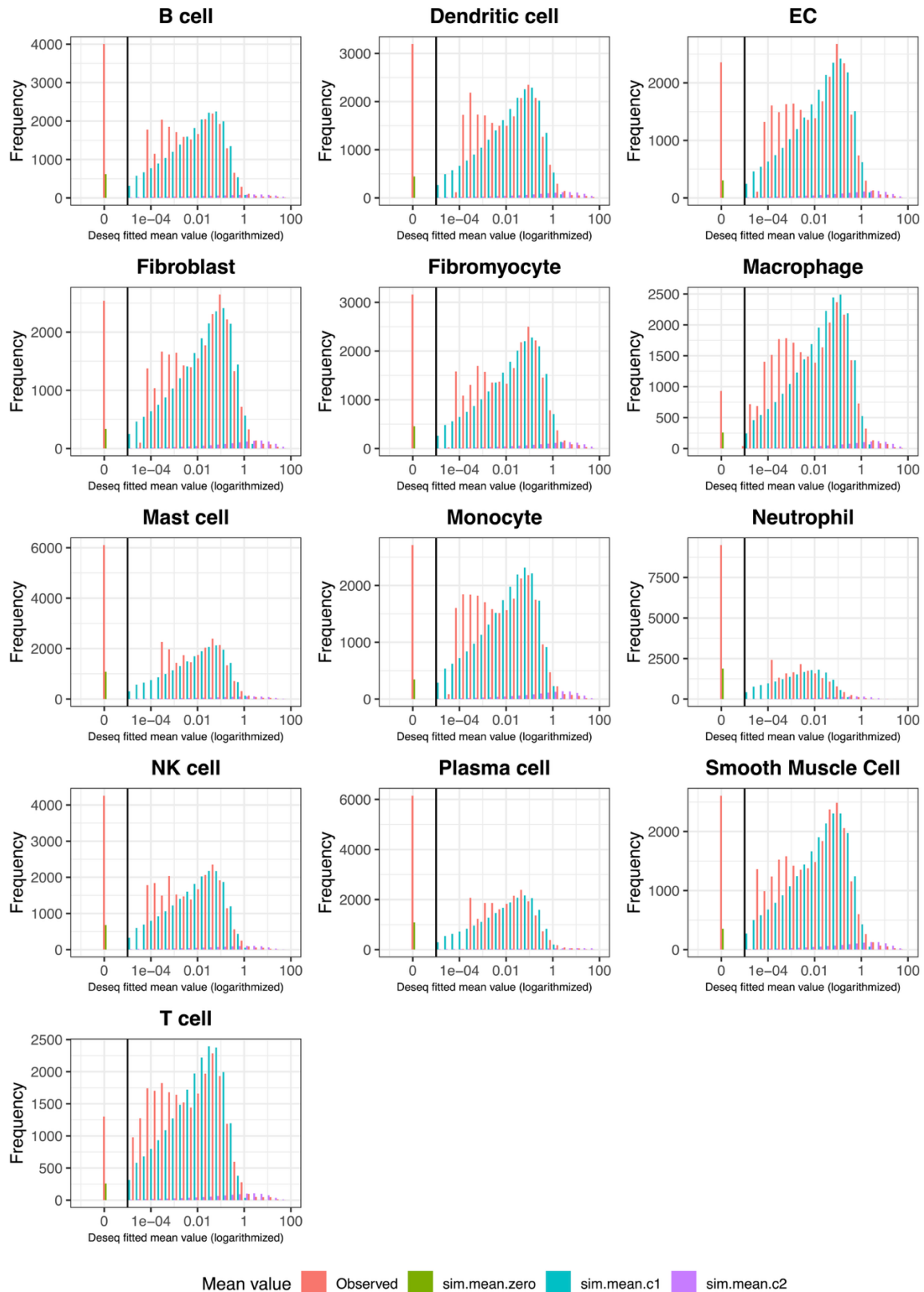
Suppl. Figure 2: Benchmark of integration methods with scib-metrics on a subset of manually annotated samples. For each method, ten metrics are calculated and normalized between 0 and 1 across the methods. Five Bio conservation and five batch correction metrics are aggregated in one total score. scPoli was the best performing method in bio conservation and in total. Source data are provided as a Source Data file.



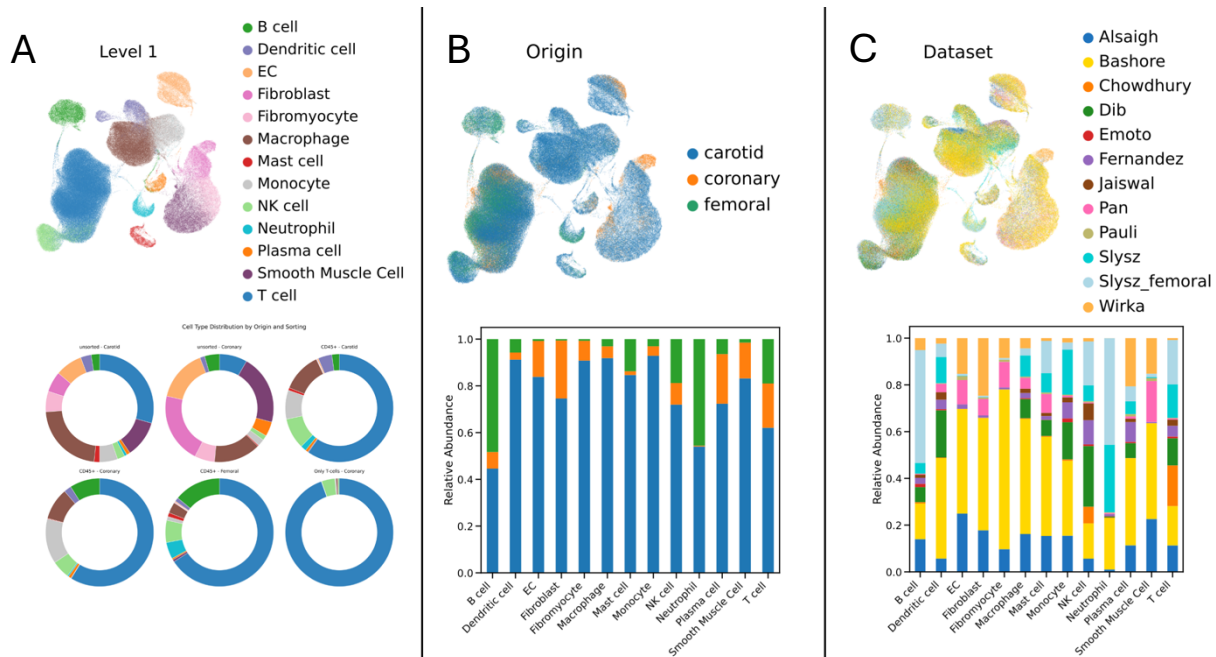
Suppl. Figure 3: Reference atlas after level 1 integration. A) UMAP of the embedding after integration including Level 1 annotations. B) UMAP with the origin site of the cells tissue. C) UMAP with the different datasets used in the reference atlas.



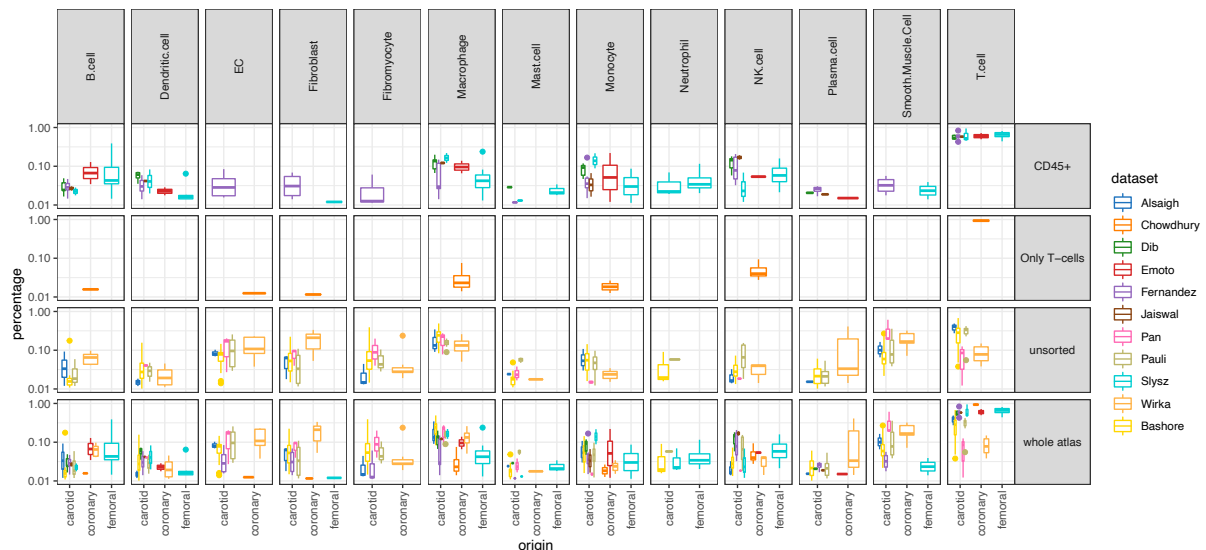
Suppl. Figure 4: Validation of the cell type label transfer. Confusion matrix of the Bashore et al. mappings with all cell types, including the cell types that are not shared between the author provided labels and our predicted labels. To compensate for the differences in cell type abundance the colormap is logarithmized and the overall precision and recall are weighted according to their abundance. Source data are provided as a Source Data file.



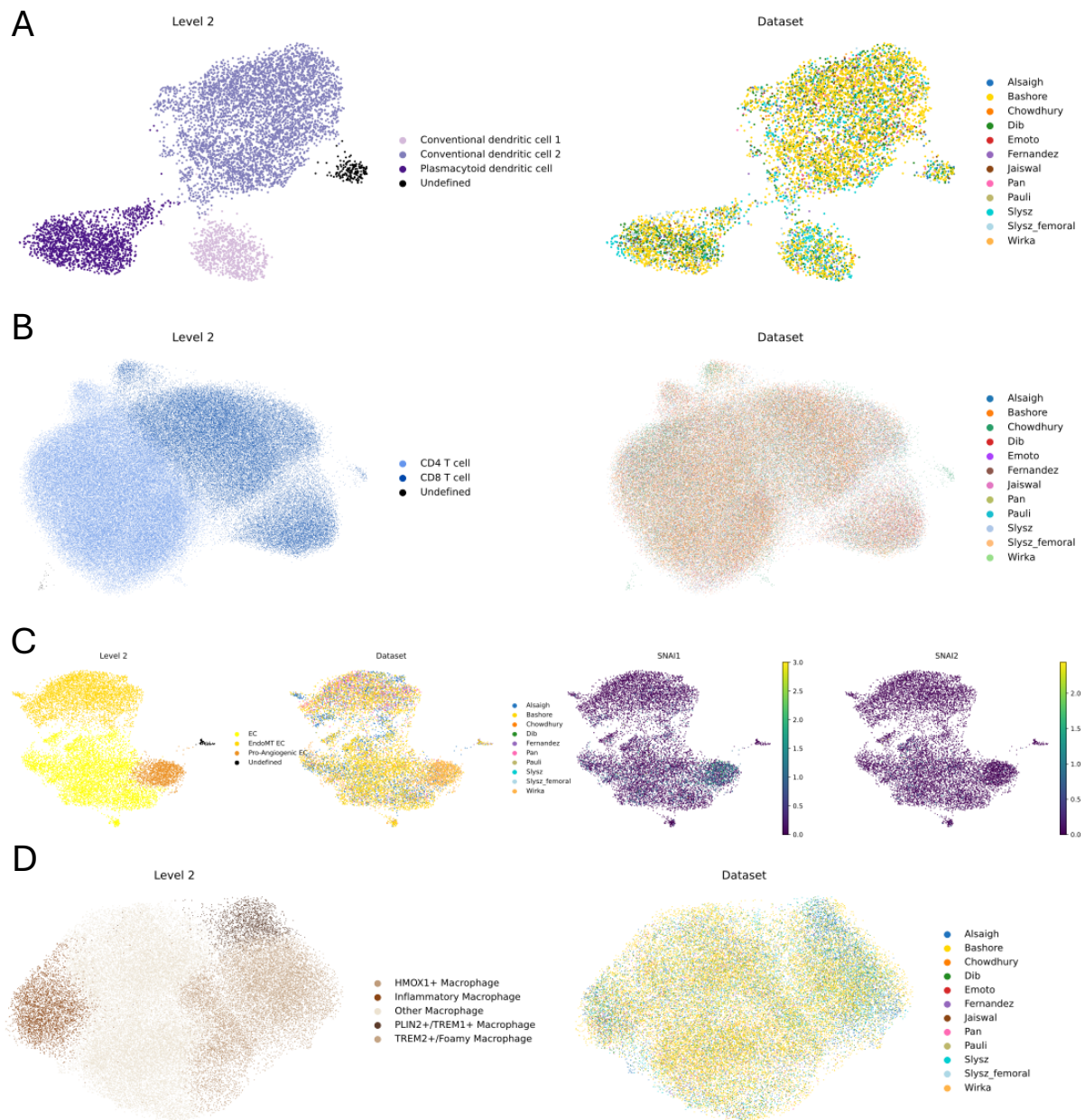
Suppl. Figure 5: Gene expression gamma prior fits used for scPower for each cell type using the atlas as a reference. The panels depict the frequency of the Deseq fitted mean values (logarithmized) stratified in the zero component, two gamma distributions and the observed values per cell type. Source data are provided as a Source Data file.



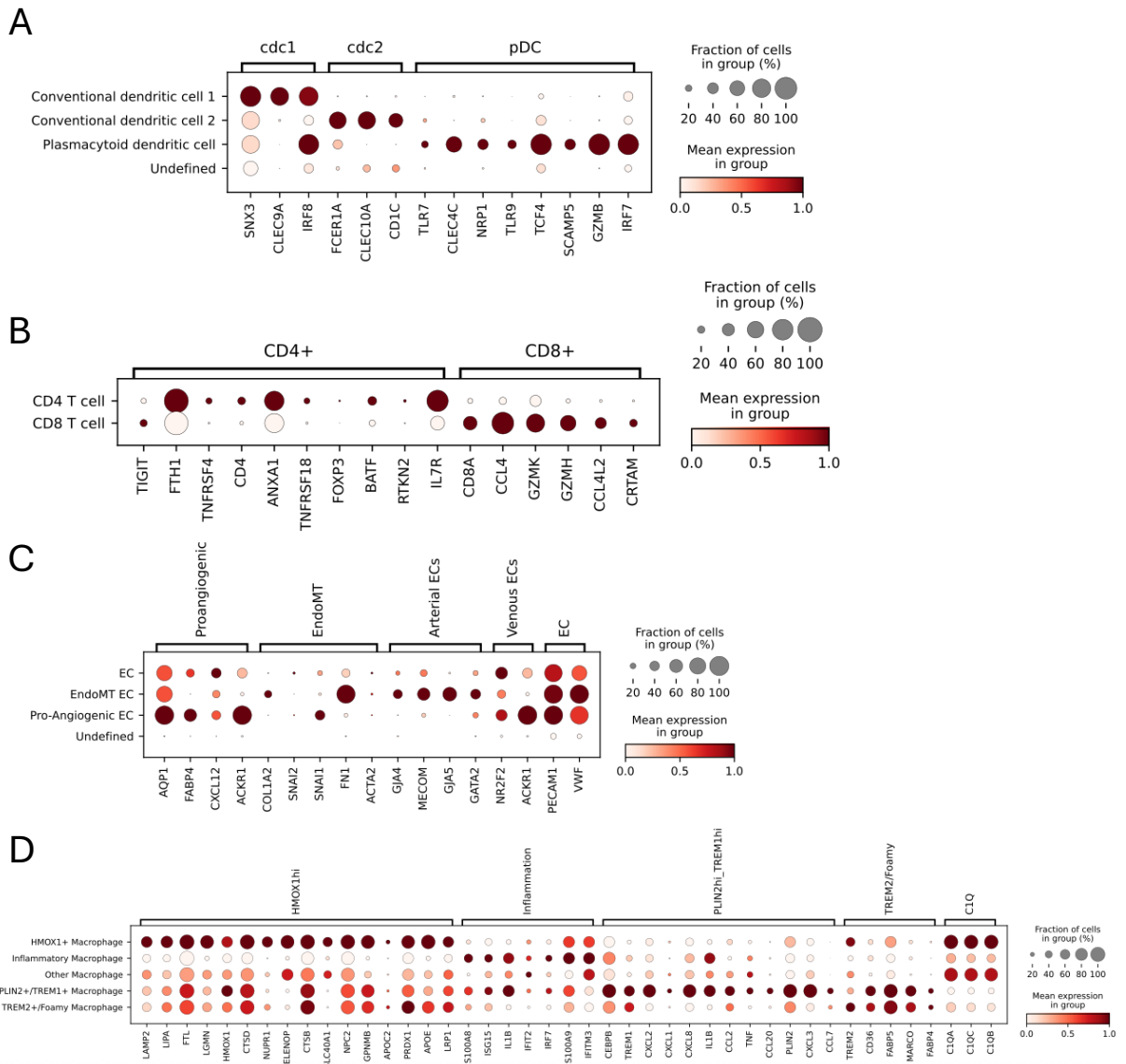
Suppl. Figure 6: Abundances of cells per sorting and per cell type, origin and dataset. A) UMAP and cell type abundances by origin and sorting. B) UMAP and origin abundance of cells by cell type. C) UMAP and dataset abundance per cell type. Source data are provided as a Source Data file.



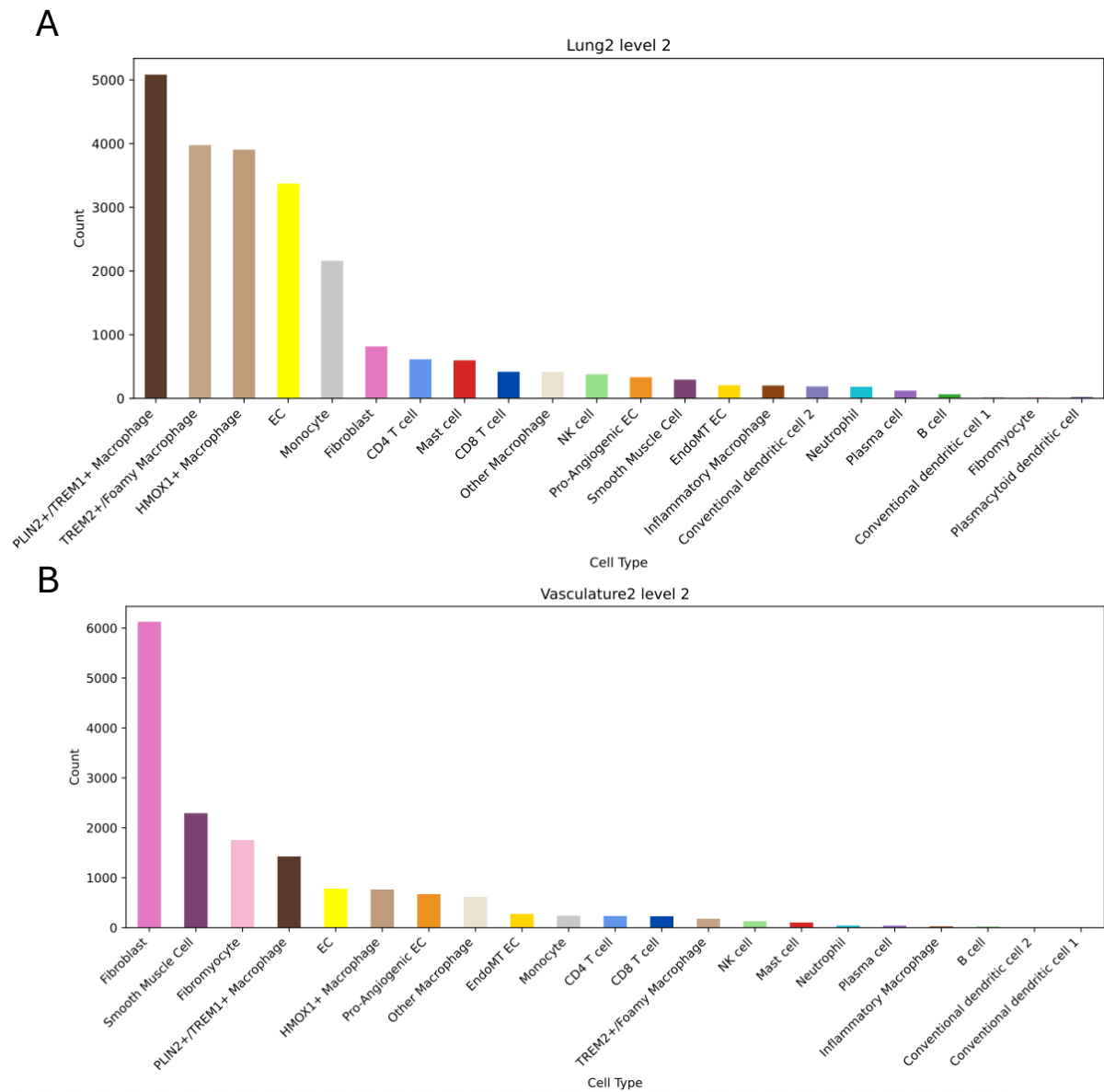
Suppl. Figure 7: Abundance comparisons by origin and sorting in the atlas. Absolute cell type abundances per sample were converted into percentages, with values below 1% excluded. The y-axis was log-transformed to highlight differences in smaller abundances. Comparisons were stratified by cell type, origin, and sorting method. The final row summarizes abundances across all sorting algorithms. In the legend, the *Slysz_femoral* and *Slysz* datasets are pooled, as they are differentiated by origin in the plot. Source data are provided as a Source Data file.



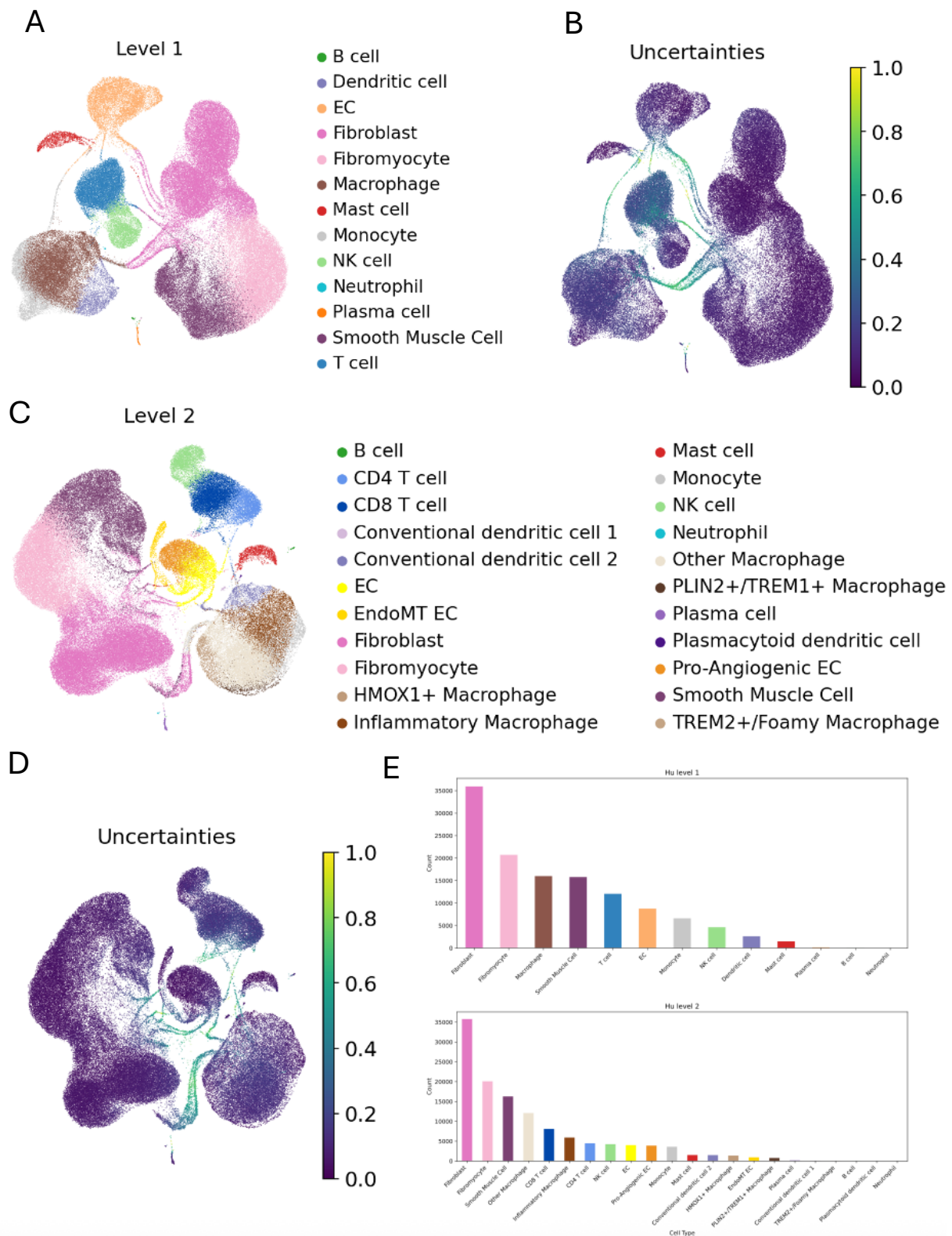
Suppl. Figure 8: *Level 2 annotation of subclusters.* UMAPs for the level 2 sub clustering for A) Dendritic cells, B) T cells, C) Endothelial cells, D) Macrophages. Cells with no clear signature were termed Undefined.



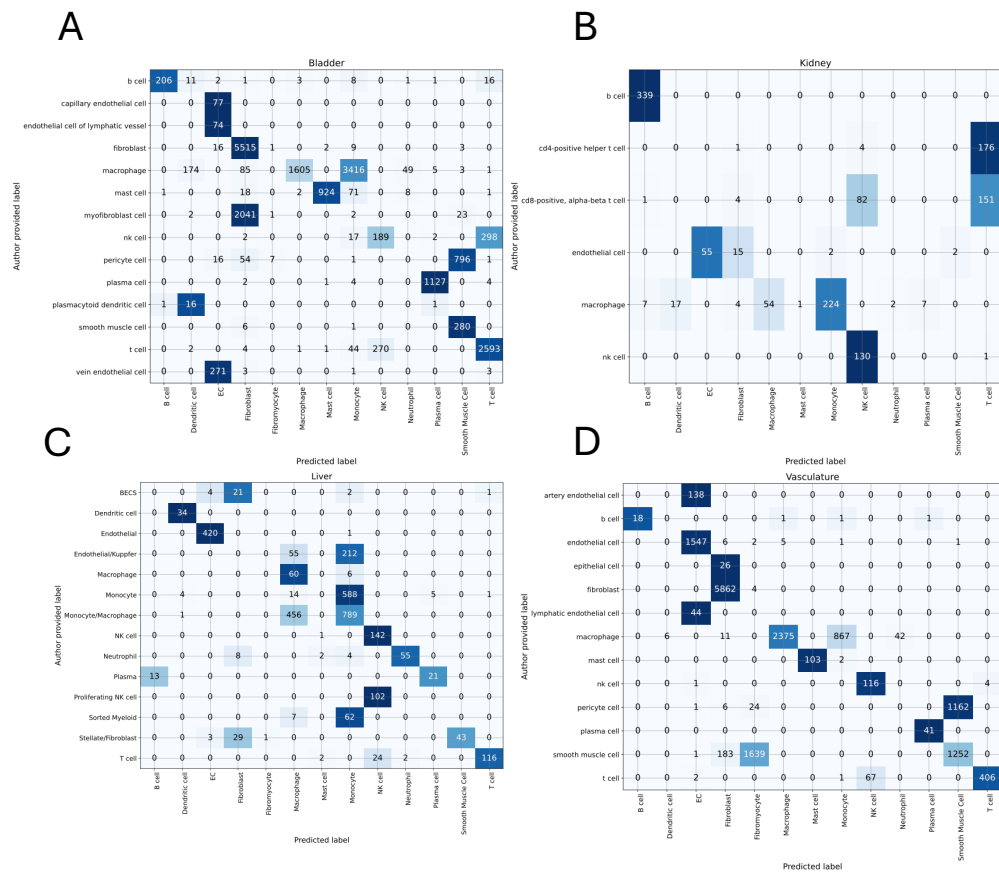
Suppl. Figure 9: Level 2 annotation of subclusters. Dot plots for the level 2 subclustering for A) Dendritic cells, B) T cells, C) Endothelial cells, D) Macrophages.



Suppl. Figure 10: *Abundance of the predicted level 2 cell types in Tabula Sapiens.* Level 2 cell type abundance of the Tabula Sapiens subsets after mapping the Lung (A) and Vasculature (B) dataset to the plaque atlas. Source data are provided as a Source Data file.



Suppl. Figure 11: Reference mapping of healthy arteries of the Hu et al. dataset on the plaque atlas. UMAPs of the mapped Hu et al. cells to the plaque atlas and their uncertainties depicted with Level 1 (A+B), Level 2 (C+D) and the level 1 and level 2 cell type abundance after excluding cells with a higher uncertainty then 0.7 (E). Source data are provided as a Source Data file.



Suppl. Figure 12: Confusion matrices for the *Tabula sapiens* mappings. Panels A-D show the confusion matrices for different data sets (data set name given in the panel headers) providing the number of cells with specific combinations of expert annotations (“Author provided label” on the y-axis) and labels predicted by the atlas (“Predicted Label” on the x-axis). Organ specific cell types and cells with a higher uncertainty than 0.7 are removed. The colormap is normalized for each row to compensate for differences in cell type abundance. Source data are provided as a Source Data file.

Supplementary Tables

Level 1	Level 2	Marker genes	Role in disease progression
B cell	B cell	CD79A ¹⁻⁵ , CD79B ³⁻⁵ , MS4A1 ¹ , IGKC ¹ , CD22 ^{4,6} , FCER2 ⁴	Plaque formation regulation through production of antibodies and cytokines ⁷
Mast cell	Mast cell	TPSAB1 ^{8,9} , TPSB2 ^{2,4,8} , KIT ^{4,10,11} , HDC ^{4,11} , CMA1 ^{4,11}	Can influence plaque stability under stress in mouse model ¹²
T cells	T cell	CD2 ¹ , TRAC ¹ , CD69 ¹ , CD3E ¹³ , CD3D ² , CD4 ² , CD8A ² , CD8B ² , EOMES ¹⁴ , LAG3 ¹⁴	T cells are the predominant lymphoid cells in atherosclerotic plaques, where they are more activated, differentiated, and exhausted compared to their blood counterparts, indicating a significant role in disease progression ^{14,15}
T cell	CD4	IL7R ¹⁵ , ANXA1 ¹⁵ , FTH1 ¹⁵ , CD4 ^{14,15} , BATF ¹⁵ , TNFRSF4 ¹⁵ , TNFRSF18 ¹⁵	An expansion of activated effector memory CD4+ T cells, particularly in symptomatic patients, suggests their involvement in promoting inflammation and contributing to atherosclerosis progression following recent cardiovascular events ^{14,15}
T cell	CD8	CCL4L2 ¹⁵ , CRTAM ¹⁵ , GZMK ¹⁵ , CCL4 ¹⁵ , CD8A ¹⁵ , GZMH ¹⁵	Cytotoxic CD8+ T cells are more abundant in carotid plaques compared to femoral plaques; they

			exhibit activation, differentiation, and exhaustion signatures, indicating their significant role in promoting inflammation and cytotoxicity in atherosclerosis ^{14,15}
Fibroblast	Fibroblast	LUM ^{3,8} , DCN ^{3,8} , COL1A1 ⁸ , COL1A2 ⁸ , FBLN1 ⁸ , THY1 ² , C3 ³ , C7 ³	Fibroblasts play a role in all stages of atherosclerosis including initial phase, fibrous cap formation and plaque formation. Functional and phenotypic changes yield a high diversity and plasticity in their role in the disease ¹⁶
Natural killer cell	Natural killer cell	NKG7 ¹ , XCL1 ^{1,17} , CTSW ¹ , XCL2 ¹⁷ , CD160 ¹⁷ , FCGR3A ¹⁷ , PRF1 ¹⁷ , GNLY ¹⁷	In human atherosclerosis, their role is still not completely known. One study suggests a role in plaque stability ¹⁸
Endothelial cell	Endothelial cell	PECAM1 ^{1,3,4,8} , VWF ^{1,2,4,8} , FABP4 ⁸ , CLDN5 ^{3,8} , IFI27 ⁸ , ECSCR ¹ , DYSF ² , CD34 ⁴ , COL4A1 ⁴ , COL4A2 ⁴ , SPARCL1 ⁴ , PLVAP ⁴ , MPZL2 ⁴ , SULF1 ⁴ , EDN1 ⁴	Endothelial cells play a central role in the development of atherosclerosis by becoming dysfunctional and inflamed in response to risk factors, especially in areas of disturbed blood flow, which leads to monocyte recruitment, LDL oxidation, foam cell formation, and a cycle of chronic inflammation ¹⁹
Endothelial cell	Proangiogenic	ACKR1 ³ , AQP1 ^{3,20} ,	Proangiogenic

		FABP4 ^{3,21} , CXCL12	phenotype which promotes angiogenesis, vascular permeability and leukocyte recruitment ²²
Endothelial cell	EndoMT	COL1A2 ³ , FN1 ³	ECs that acquire a myofibroblast-like phenotype and lose their EC characteristics. This process is important during arterial and cardiovascular remodeling, which may affect plaque stability ²³
Smooth muscle cell	Smooth muscle cell	ACTA2 ^{4,8} , MYH11 ^{2,4,8,24} , MYL9 ^{1,8} , TPM2 ⁸ , CALD1 ¹ , TAGLN ^{1,4} , TNFRSF11B ² , LUM ² , APOE ³ , APOC1 ³ , AGT ³ , NOTCH3 ^{4,25} , PDGFRB ^{4,25} , MFAP4 ^{4,26}	Smooth muscle cells play dual roles in atherosclerosis by contributing to lesion expansion and forming a stabilizing fibrous cap, though they also undergo phenotypic switching to macrophage-like, foam cell-like, and other forms that influence plaque growth and stability, potentially increasing plaque vulnerability ²⁷
Fibromyocyte	Fibromyocyte	FN1 ^{8,28} , LUM ²⁸ , TNFRSF11B ²⁸ , ACTA2 ³ , TCF21 ²⁸	Modulated SMCs that transform into fibroblast-like cells, that establish a protective role in coronary artery disease ²⁸
Dendritic cell	Dendritic cell	CLEC10A ^{3,4,9,10} , FCER1A ^{4,9,10} , CD1C ^{3,4,10,29} , HLA-DRA ¹⁰ , HLA-DRB1 ¹⁰	They act as antigen-presenting cells in atherosclerosis, driving T-cell responses and

			inflammation that advance disease progression ³⁰
Dendritic cell	cDC1	CLEC9A ¹⁷ , IRF8 ¹⁷ , SNX3 ¹⁷	Conventional dendritic cell subtype 1 (cDC1) may help stabilize plaques by promoting atheroprotective Treg cells ³⁰
Dendritic cell	cDC2	CD1C ¹⁷ , CLEC10A ¹⁷ , FCER1A ¹⁷	Conventional dendritic cell subtype 2 (cDC2) supports inflammation by recruiting pro-atherogenic T helper cells ³⁰
Dendritic cell	Plasmacytoid dendritic cell	TCF4, GZMB, TLR7, TLR9, NRP1, SCAMP5, CLEC4C, IRF7. Marker genes are taken from Ghanem et al ³¹ .	Plasmacytoid dendritic cells (pDCs) contribute to vascular inflammation and atherogenesis by producing type I interferons (IFN-I) and chemokines, which recruit inflammatory cells to sites of inflammation ³²
Neutrophil	Neutrophil	NAMPT ³³ , IFITM2 ³⁴ , G0S2 ³⁵ , CXCL8 ³⁶ , NEAT1 ³⁵ , SRGN ³⁷ , AQP9 ³⁸ , SOD2, FCGR3B, IVNS1ABP	Neutrophils are rarely found in atherosclerotic lesions but can promote LDL vascular deposition and oxidation during early stages of atherosclerosis ³⁹
Plasma cell	Plasma cell	IGKC ⁸ , IGHM ^{3,8} , IGHA1 ⁸ , IGLC2 ^{3,8} , IGLC3 ⁸ , JCHAIN ³	Produce antibodies and can regulate plaque formation ⁷
Monocyte	Monocyte	FCN1 ⁴ , S100A8 ^{3,4,10} , S100A9 ^{3,4,10} , S100A12 ^{4,10} , VCAN ⁴ ,	Monocytes infiltrate the intima and differentiate into

		CD52 ⁴ , LYZ ^{3,10} , CTSS ¹⁰	macrophages that influence plaque stability ⁴⁰
Macrophage	Macrophage	C1QA ⁸ , C1QB ⁸ , C1QC ⁸ , CD74 ^{8,14,41} , CXCL8 ⁸ , AIF1 ¹ , CD14 ^{1,4,10} , CD68 ^{1,4,10} , ITGAM ¹⁰ , CSF1R ¹⁰ , HLA-DRA ¹⁴ , LGALS3 ⁴	Macrophages play several roles in atherosclerosis depending on the subset ⁴⁰
Macrophage	Foamy	TREM2 ^{4,42-45} , MARCO ¹⁷ , FABP4/5 ¹⁷ , CD36 ¹⁷ .	Foam cells are the hallmark of atherosclerosis. They take up modified LDL cholesterol and form the plaque ⁴⁰
Macrophage	Inflammatory	S100A8 ¹⁷ , IL1B ¹⁷ , S100A9 ¹⁷ , IRF7 ⁴⁶ , IFITM3 ⁴⁶ , ISG15 ⁴⁶ , IFIT2 ⁴⁶	These macrophages promote inflammation in plaques and hence participate substantially in plaque destabilization and rupture ⁴⁰ . However this sub cell types role is under active discussion depending on the definition.
Macrophage	Other	No distinct markers within macrophages	Macrophages with no indicative signature.
Macrophage	PLIN2/TREM1	PLIN2, TREM1, CXCL1/2/3/8, CCL2/7/20, IL1B, TNF, CEBPB. All marker genes are taken from Dib et al ¹⁷ .	They are introduced by Dib et al ¹⁷ and their signature is correlated with vascular events. However, our results indicate no active role with respect to abundance of this cell type.
Macrophage	HMOX1+	HMOX1, APOC2, GPNMB, LIPA, NPC2, PRDX1, SLC40A1, NUPR1,	This sub cell types role is still not fully defined.

		APOE, LAMP2, CTSB, SELENOP, LGMN, LRP1, CTSD, FTL. All marker genes are taken from Dib et al ¹⁷ .	
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Suppl. Table 1: *Summary of cell types included in the atlas.* The first column presents the Level 1 annotation, while the second column provides the corresponding finer-grained Level 2 annotation. The third column lists marker genes along with their associated references. The final column outlines the role of each cell type in disease progression.

cell_type	t_stat	df	p_value	p_adj	ci_lower	ci_upper	label
NK cell	-5.432763738	108.23 34228	0.000000344732 2452	0.00000224075 9594	-3.779801166	-1.75899173 7	t(108.2) = -5.433, p = 3.4e-07
Plasma cell	-5.106035325	112.19 92516	0.000001357275 007	0.00000588152 5032	-4.062727499	-1.79118837 7	t(112.2) = -5.106, p = 1.4e-06
T cell	-4.181707942	117.82 30903	0.000055909300 92	0.00012113681 86	-3.231860513	-1.15459604 8	t(117.8) = -4.182, p = 5.6e-05
Mast cell	-6.256553144	71.520 04444	0.000000025660 42161	0.00000033358 5481	-3.850528417	-1.98954901 6	t(71.5) = -6.257, p = 2.6e-08
Monocyte	-4.244020051	123.29 51268	0.000042769034 39	0.00011119948 94	-3.795024825	-1.38096619 4	t(123.3) = -4.244, p = 4.3e-05
Dendritic cell	0.217456046 8	111.29 11931	0.8282511355	0.8282511355	-1.009458401	1.258331605	ns
Neutrophil	-1.428966512	99.567 97923	0.1561436692	0.1714371415	-2.006490374	0.326343260 1	ns
B cell	-3.061233516	98.683 70664	0.002839856312	0.00369181320 6	-3.817726317	-0.81487074 96	t(98.7) = -3.061, p = 2.8e-03
Smooth Muscle Cell	3.764370466	101.15 18905	0.000280372339 5	0.00045560505 17	1.230701808	3.972736265	t(101.2) = 3.764, p = 2.8e-04
EC	-1.42875124	60.134 91828	0.1582496691	0.1714371415	-0.753483416	0.125572106 4	ns
Fibroblast	-4.470313289	68.736 99793	0.000030002792 26	0.00009750907 484	-4.54371826	-1.73953109 1	t(68.7) = -4.470, p = 3.0e-05
Macrophage	-3.952417962	61.847 82884	0.000201343902 1	0.00037392438 95	-1.550020635	-0.50873512 52	t(61.8) = -3.952, p = 2.0e-04
Fibromyocyte	3.155359873	184.88 84231	0.001871588996	0.00270340632 8	0.244994364	1.06250178	t(184.9) = 3.155, p = 1.9e-03

cell_type	t_stat	df	p_value	p_adj	ci_lower	ci_upper	label
Conventional dendritic cell 2	0.2585641 005	96.5123 014	0.7965222091	0.8344518 381	-0.74411099 03	0.967017758 4	ns

CD8 T cell	-2.3225434 9	124.108 5059	0.02183208483	0.0343075 6187	-1.67092314 7	-0.13333821 48	t(124.1) = -2.32254, p = 2.2e-02
NK cell	-6.2336325 34	112.975 1211	0.000000008089 978863	0.0000000 889897674 9	-4.02739509 3	-2.08480416 8	t(113) = -6.23363, p = 8.1e-09
Plasmacytoid dendritic cell	-0.3245483 386	111.098 0121	0.7461332236	0.8344518 381	-1.13276463	0.813925427	ns
Plasma cell	-4.1846336 09	113.332 0527	0.000056620160 91	0.0001557 054425	-3.52806199 8	-1.26085875 2	t(113.3) = -4.18463, p = 5.7e-05
Monocyte	-4.9213255 94	146.385 1864	0.000002287318 527	0.0000125 802519	-3.27768993 7	-1.39944831	t(146.4) = -4.92133, p = 2.3e-06
Inflammatory Macrophage	-0.0016918 44058	108.990 1371	0.9986531972	0.9986531 972	-1.03872506 7	1.036953231	ns
Neutrophil	-3.4007195 83	99.0334 7275	0.000970291327 6	0.0019405 82655	-3.02428883 1	-0.79554229 66	t(99) = -3.40072, p = 9.7e-04
Mast cell	-6.0097638 24	70.4234 4898	0.000000074032 04001	0.0000005 429016268	-3.59587482 9	-1.80402150 7	t(70.4) = -6.00976, p = 7.4e-08
Conventional dendritic cell 1	-2.1236726 24	105.292 2537	0.0360397572	0.0528583 1056	-2.85677548 2	-0.09803690 661	ns
CD4 T cell	0.2957487 534	104.701 222	0.7680077136	0.8344518 381	-0.90268480 67	1.21916014	ns
B cell	-3.6858879 21	96.9596 0016	0.000375607787 9	0.0008263 371335	-4.23974627 1	-1.27189982 6	t(97) = -3.68589, p = 3.8e-04
EndoMT EC	6.8611693 39	119.698 5861	0.000000000321 6009964	0.0000000 070752219 21	2.768341814	5.014214963	t(119.7) = 6.86117, p = 3.2e-10
Pro-Angiogenic EC	-2.9135911 4	107.593 2988	0.004346393285	0.0073554 34791	-3.91149578 2	-0.74408010 33	t(107.6) = -2.91359, p = 4.3e-03
PLIN2+/TREM1+ Macrophage	1.9412114 9	97.8905 4178	0.05510841945	0.0757740 7675	-0.03530326 997	3.201904829	ns
Other Macrophage	1.1887181 52	121.916 7921	0.2368608604	0.2894966 072	-0.53589875 41	2.1468139	ns
EC	-1.9100211 19	96.0312 1307	0.05911452365	0.0765011 4826	-2.75957152 4	0.053104933 93	ns
TREM2+/Foamy Macrophage	-4.8008437 86	80.5190 4317	0.000007175243 419	0.0000315 7107105	-5.34688767	-2.21331482 6	t(80.5) = -4.80084, p = 7.2e-06
Smooth Muscle Cell	4.2744475 03	102.294 7466	0.000043096944 74	0.0001354 475406	1.569993989	4.288404826	t(102.3) = 4.27445, p = 4.3e-05
Fibroblast	-4.4903319 58	69.4102 0305	0.000027592369 02	0.0001011 720197	-4.50586131 2	-1.73395482 3	t(69.4) = -4.49033, p = 2.8e-05
HMOX1+ Macrophage	-4.0281718 79	70.0968 2403	0.000140642076 9	0.0003437 917434	-3.87000644 7	-1.30687836 5	t(70.1) = -4.02817, p = 1.4e-04
Fibrocyte	3.0800286 36	191.159 9506	0.002374835622	0.0043538 65307	0.215264741	0.981981979 4	t(191.2) = 3.08003, p = 2.4e-03

Suppl. Table 2: Summary test statistics. Test statistics from level 1 and level 2 cell type deconvolution used in abundance comparisons of the bulk data.

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