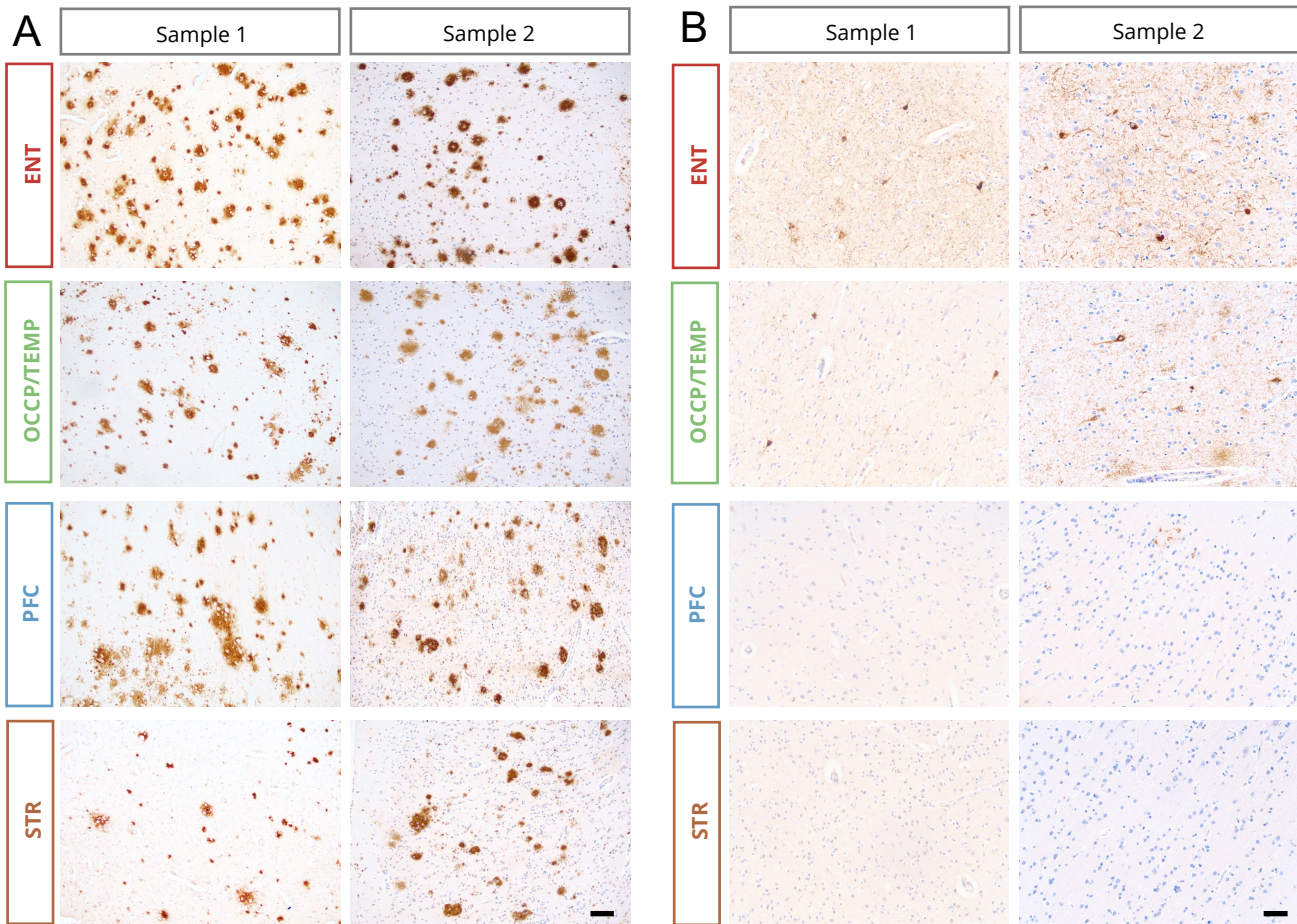
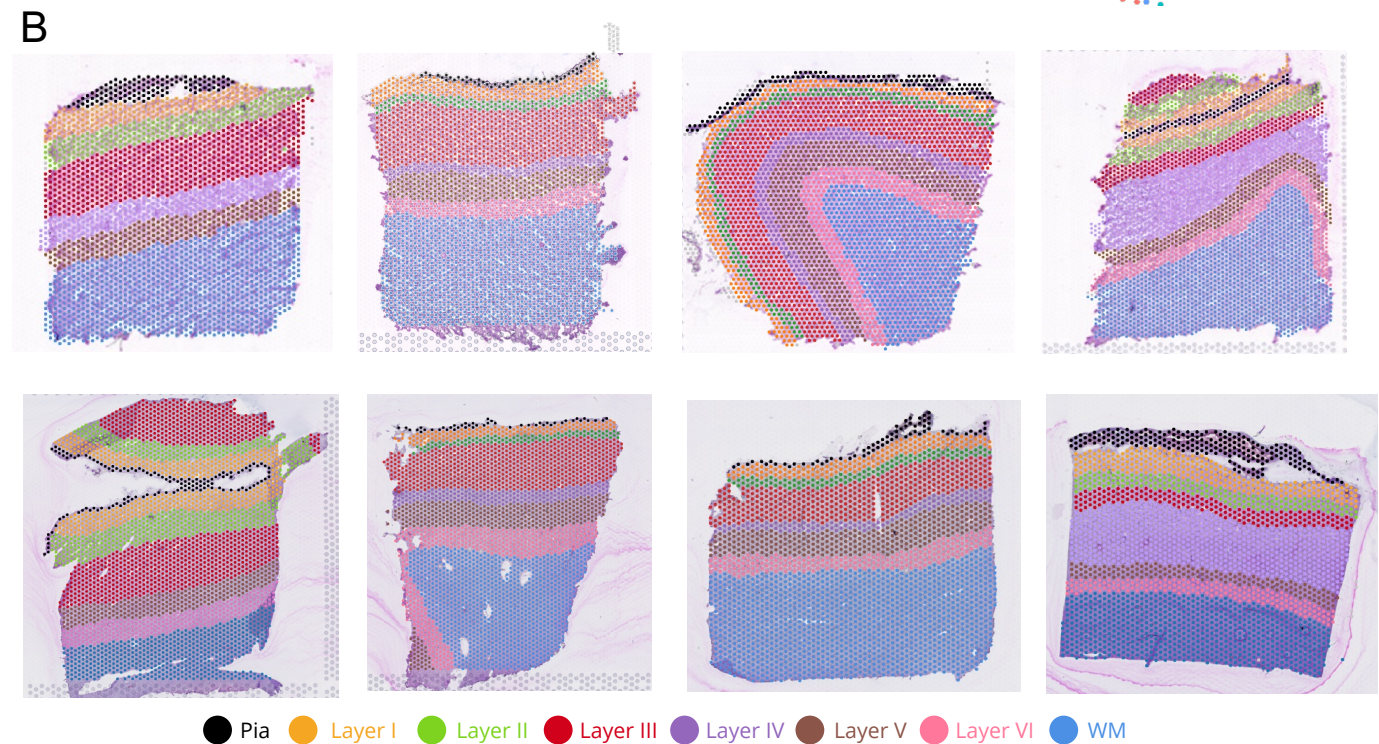
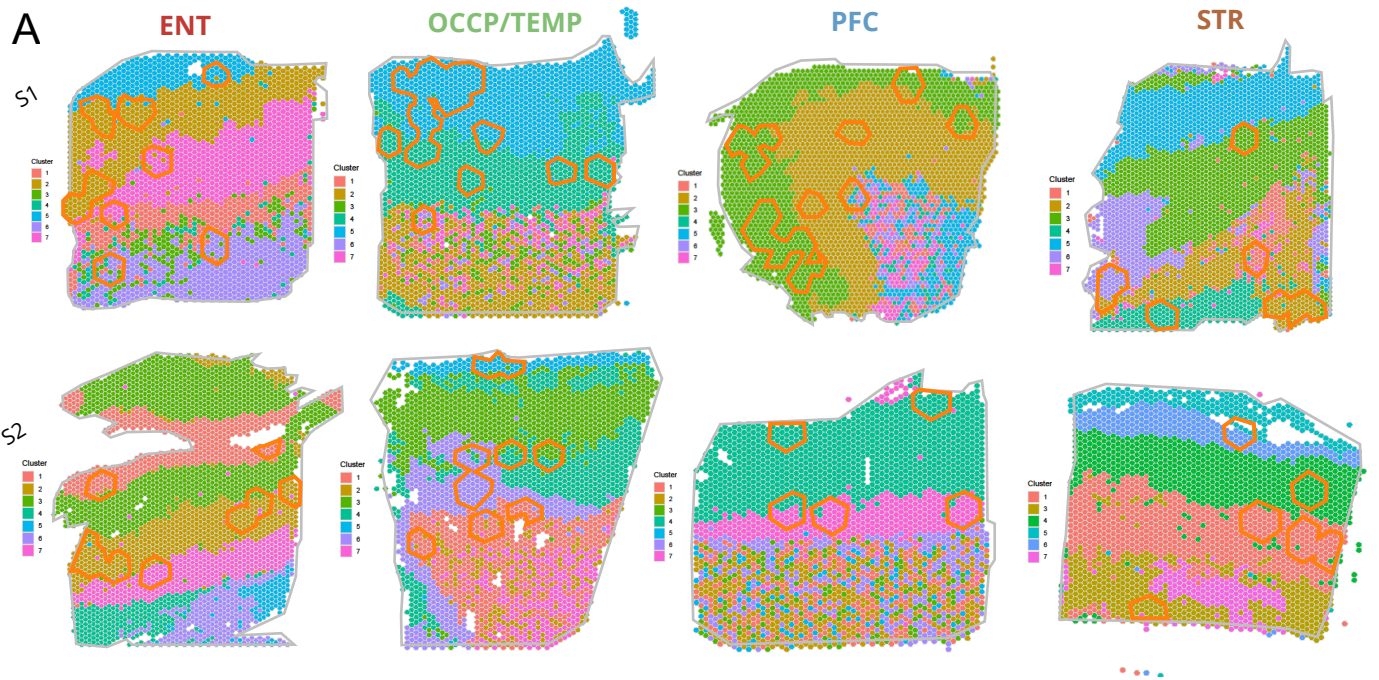
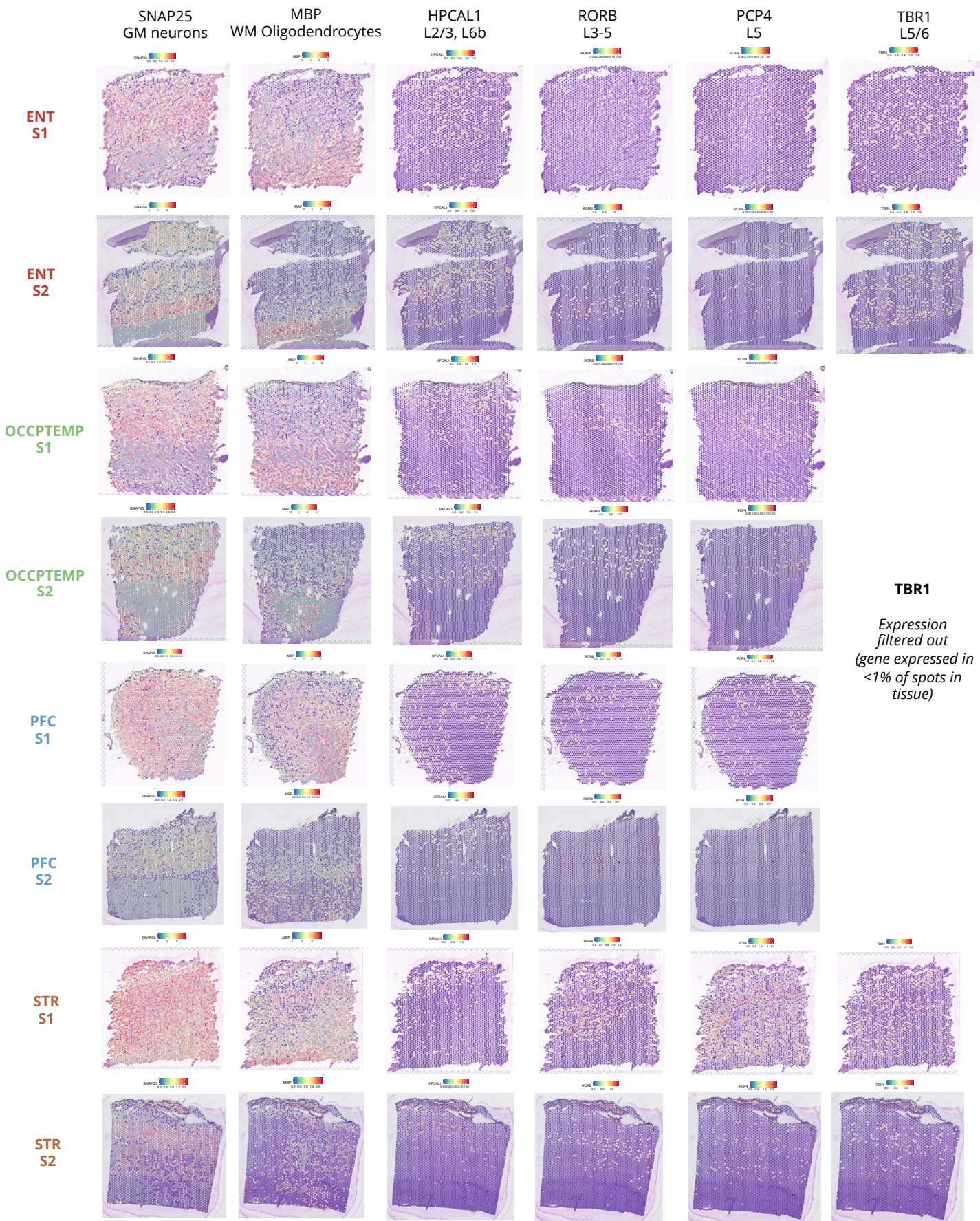




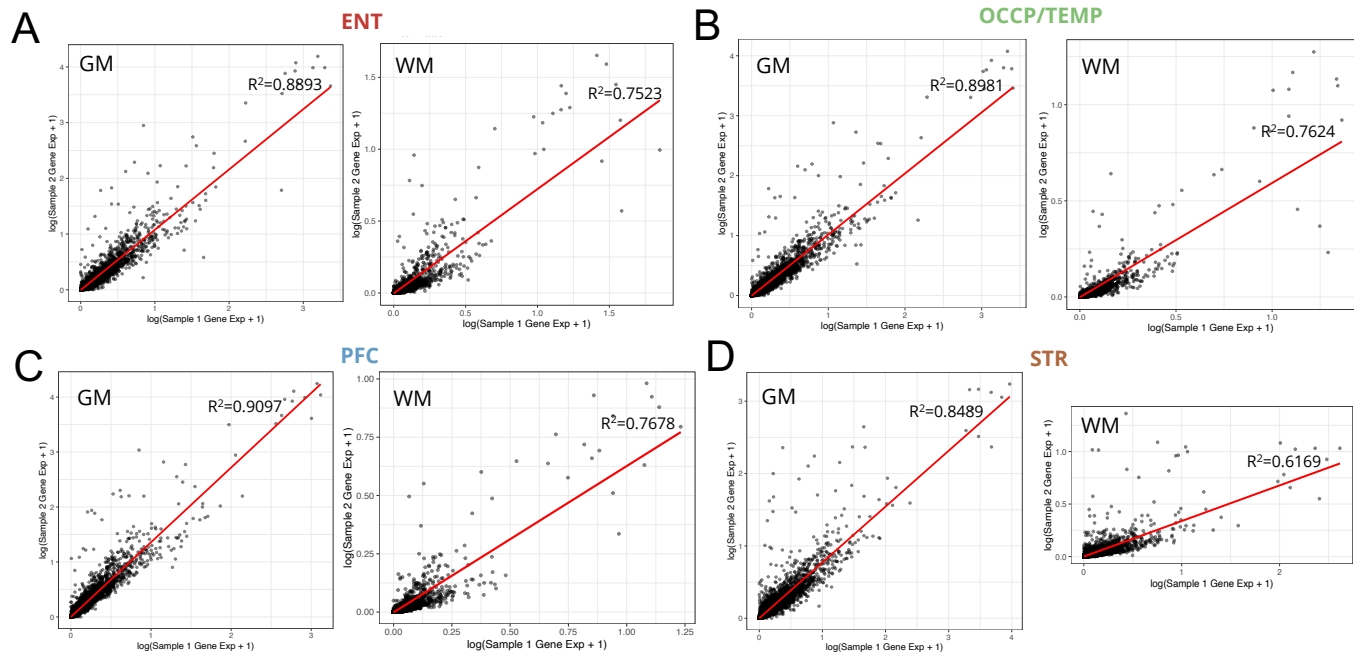
Supplementary Figure 1. Representative images of the entorhinal cortex (ENT), occipito-temporal cortex (OCCP/TEMP), dorsolateral prefrontal cortex (PFC), and striate cortex (STR) from both subjects (S1 and S2) immunostained with **A**) beta-amyloid (A β) and **B**) phosphorylated tau (pTau). Both subjects show widespread amyloid plaques throughout the cortex, and sparse neurofibrillary tangles (NFT) in the ENT and OCCP/TEMP. No NFTs were observed in the PFC or STR of either subject. Scale bar in A = 100 μ m; scale bar in B = 50 μ m.



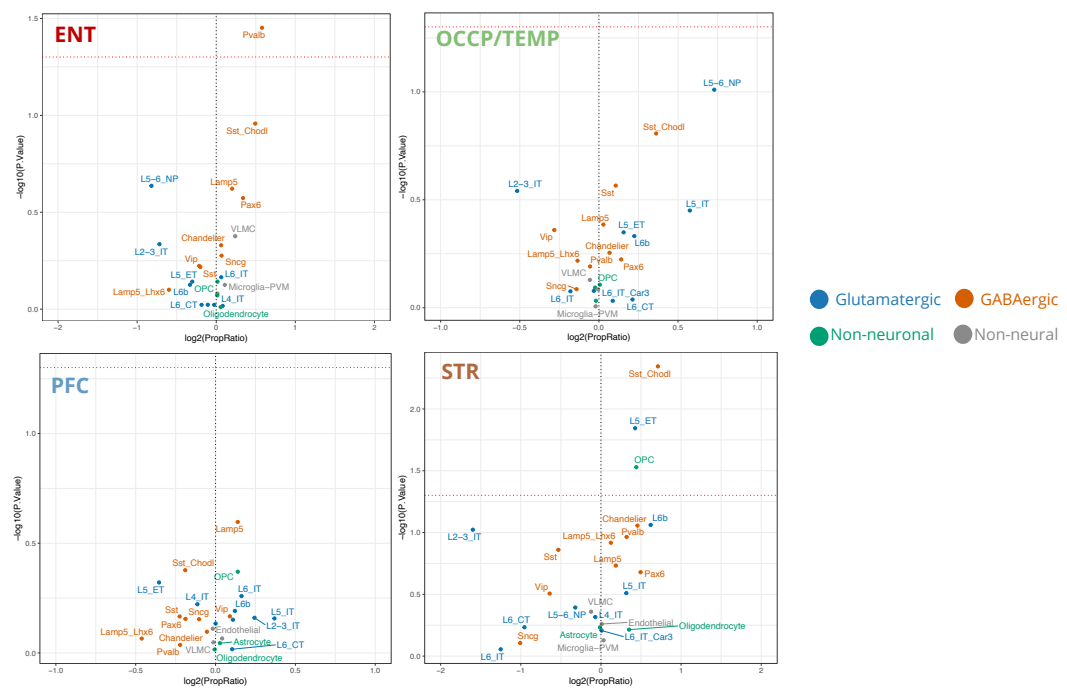
Supplementary Figure 2. Clustering and Annotation of Cortex Laminar Layers **A)** BayesSpace Spatial Clustering of each Sample. Each sample was run separately with the number expected clusters set to 7. **B)** Annotation of Laminar Layers. Laminar layers were annotated referencing nuclei density, number of spots determining the cortical thickness and the expected proportions of each layer and the boundaries of BayesSpace clusters.



Supplementary Figure 3. Spatial Expression of Layer Specific Markers Genes in All 8 Samples. SCTransformed gene expression of SNAP25 (GM neurons), MBP (WM Oligodendrocytes/myelin), HPCAL1 (Layer 2/3 and 6b), RORB (Layer 3-5), PCP4 (L5) and TBR1 (L5/6)

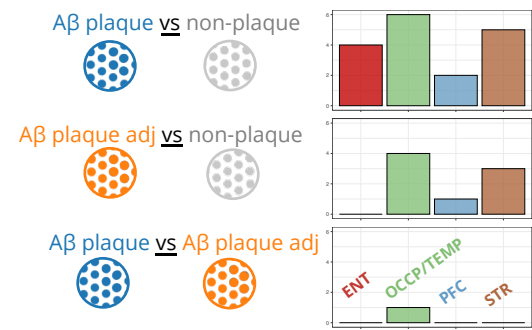


Supplementary Figure 4. Correlation of average gene expression (across all ST spots) of ~30,000 genes in the grey and white matter between Sample 1 and Sample 2 across all four regions of the cortex **A)** ENT **B)** OCCP/TEMP **C)** PFC and **D)** STR. Correlation was measured through Multiple R^2 , representing the proportion of variance of average gene expression in Sample 2 explained by Sample 1 for each gene.

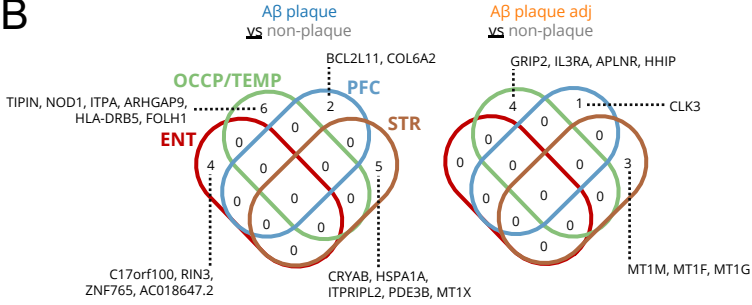


Supplementary Figure 6. Differentially Enriched/Depleted Cell Type Proportions the A β plaque adjacent spots of all regions vs non-plaque. Log2FC>0 indicating cell types with higher proportions in A β plaque adjacent annotated spots, while Log2FC<0 indicate cell types with a lower proportion in A β plaque adjacent spots. Spots are colored by cell type category.

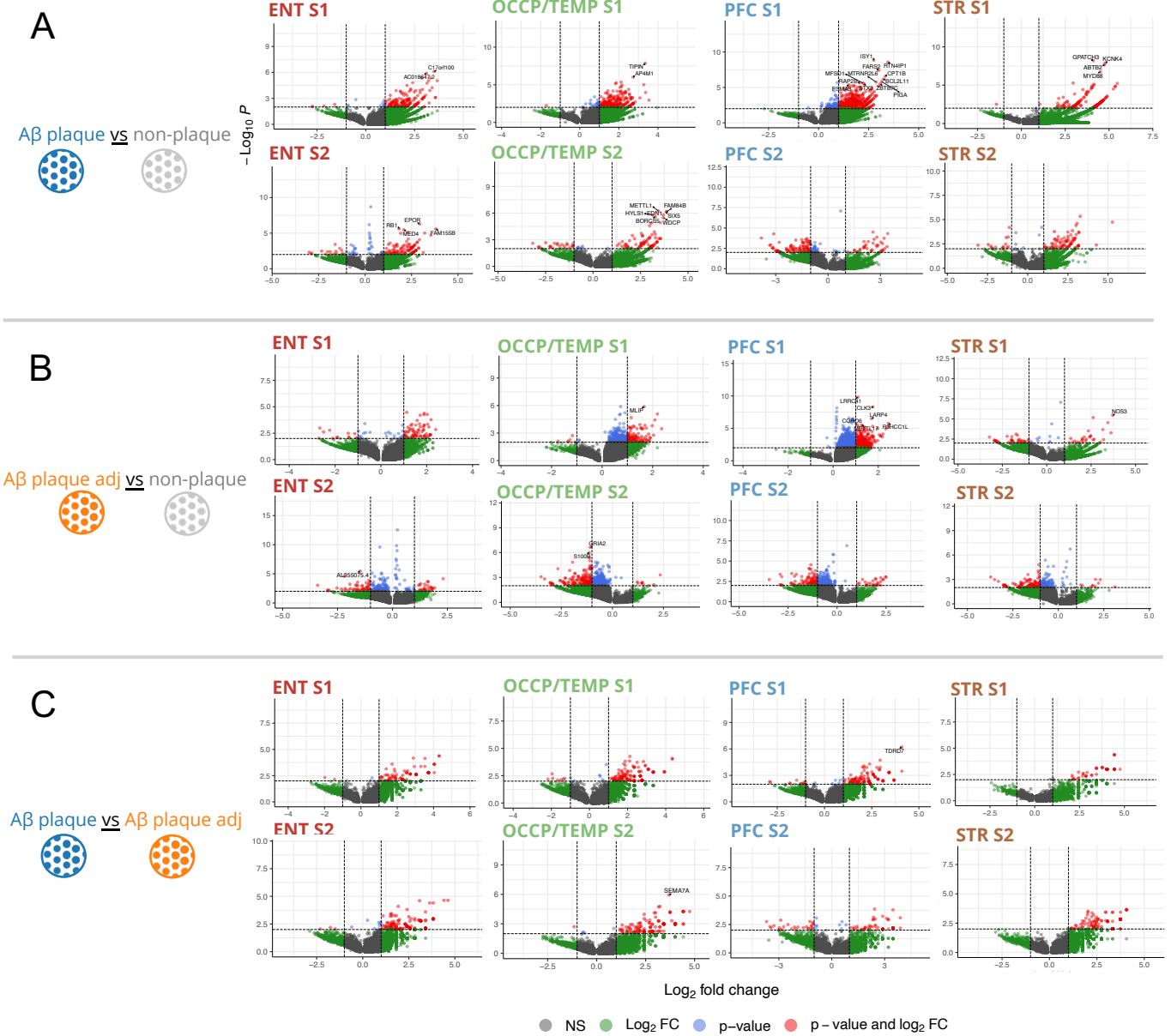
A Count of DEGs (filtered by FDR <0.05 and logFC >1)



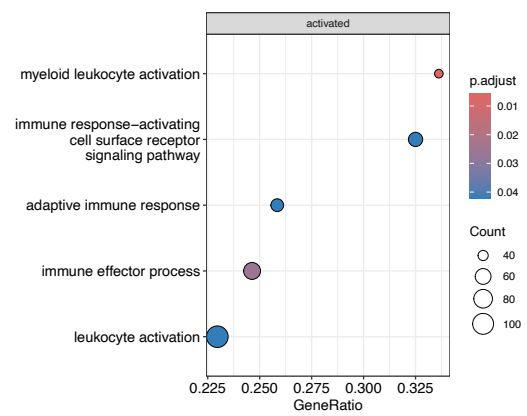
B



Supplementary Figure 7. DEGs in the Aβ plaque Microenvironment with a more stringent filtering approach (FDR < 0.05 and logFC >1) **A)** Number of DEGs for each region for each contrast **B)** Number of Overlapping DEGs between the 4 regions for each contrast



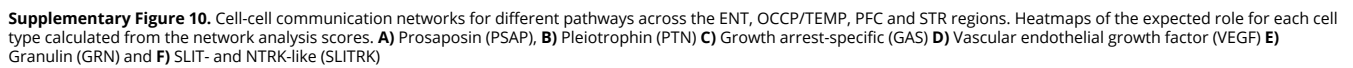
Supplementary Figure 8. Volcano Plot of DEGs in the $\text{A}\beta$ plaque Microenvironment for each sample (FDR < 0.05 and abs logFC ≥ 1 are highlighted) for **A)** $\text{A}\beta$ plaque vs non-plaque **B)** $\text{A}\beta$ plaque adjacent vs non-plaque and **C)** $\text{A}\beta$ plaque vs $\text{A}\beta$ plaque adjacent contrasts



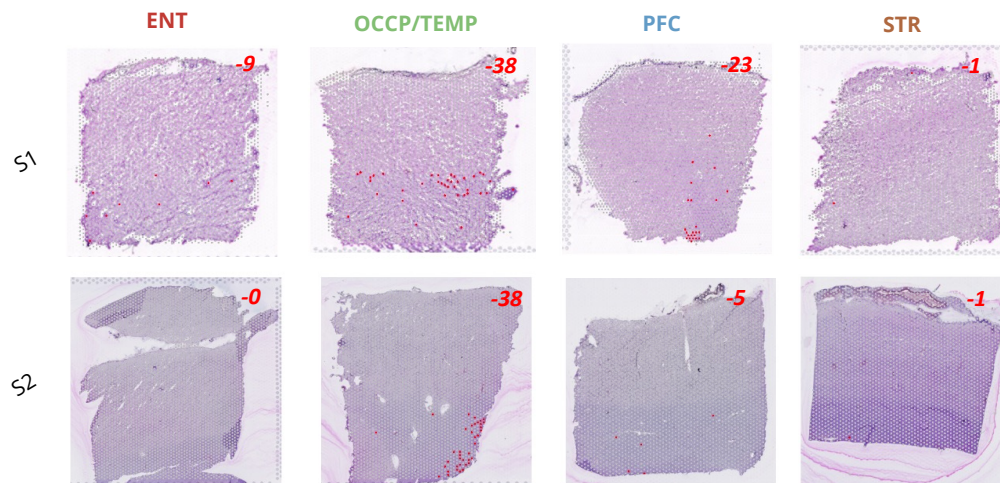
Supplementary Figure 9. Dotplot of enriched pathways in DEGs between ENT plaque vs non-plaque ST spots (adjusted p-value <0.05).

STR

F SLITRK signaling pathway



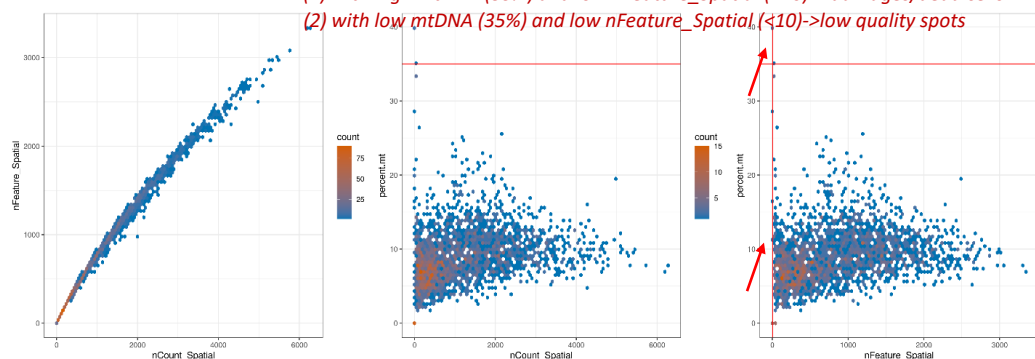
A



B

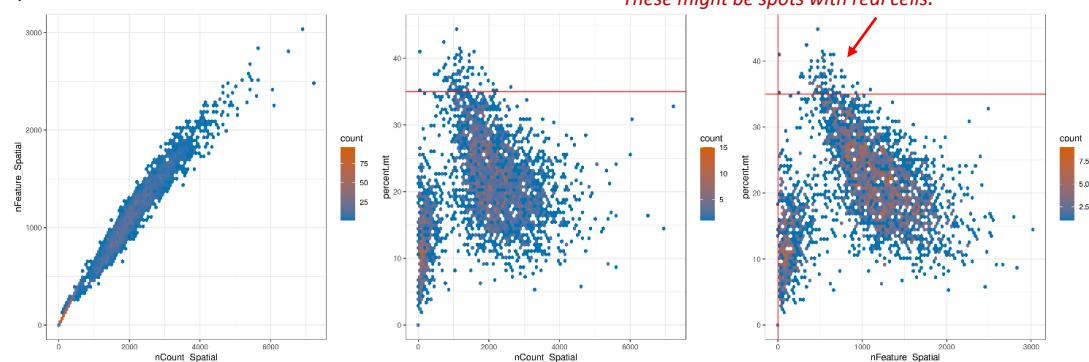
Sample 1 ENT

Only filtering spots in the left quadrants,
 (1) with high mtDNA (35%) and low nFeature_Spatial (<10)-> damages/dead cells
 (2) with low mtDNA (35%) and low nFeature_Spatial (<10)->low quality spots



Sample 2 ENT

High percent.mt but the nFeature_Spatial is ~500.
 These might be spots with real cells.



Supplementary Figure 11. Filtering of ST spots in dataset **A**) Spatial distribution and Count of ST spots removed for nFeature_Spatial (total genes per spot) <10 **B**) Scatterplots of QC metrics (nFeature_Spatial, nCount_Spatial and percent.mt) for ENT region of Samples 1 and 2

Suppl Table 1. Sample Information and Visium Spatial Transcriptomics Raw Quality Control Metrics

<i>Sample ID</i>	<i>Age</i>	<i>Sex</i>	<i>APOE Status</i>	<i>Thal Phase</i>	<i>Braak Stage</i>	<i>CERAD score</i>	<i>Sections</i>	<i>Number of spots under tissue</i>	<i>Fraction Reads in Spots Under Tissue</i>	<i>Number of reads (million)</i>	<i>Total Genes Detected</i>	<i>Mean reads per spot</i>	<i>Median genes per spot</i>	<i>Median UMI counts per spot</i>
S1	72	M	APOE3/4	4	III	2	ENT	2,543	62.90%	228	18,261	89,699	755	986
							OCCPTEMP	2,819	76.50%	242	18,072	85,770	709	913
							PFC	3,102	83.30%	229	18,062	73,711	800	1,052
							STR	3,314	73.70%	180	18,446	54,439	977	1,381
S2	89	F	APOE3/4	4	III	1	ENT	3,221	95.80%	201	18,936	62,600	1,042	1,927
							OCCPTEMP	2,907	95.20%	210	18,201	72,082	728	1,185
							PFC	2,578	90.40%	186	18,081	71,967	447	632
							STR	2,421	85.90%	217	17,942	89,611	514	664

Suppl Table 2. Mean RCTD weights of 24 cell types for each plaque category (Aβ plaque, Aβ plaque-adjacent, non-plaque)

	<i>ENT_S1_plaque</i>	<i>ENT_S1_plaque_adjacent</i>	<i>ENT_S1_non_plaque</i>	<i>ENT_S2_plaque</i>	<i>ENT_S2_plaque_adjacent</i>	<i>ENT_S2_non_plaque</i>	<i>PFC_S1_plaque</i>	<i>PFC_S1_plaque_adjacent</i>	<i>PFC_S1_non_plaque</i>	<i>PFC_S2_plaque</i>	<i>PFC_S2_plaque_adjacent</i>	<i>PFC_S2_non_plaque</i>	<i>OCCPTEMP_S1_plaque</i>	<i>OCCPTEMP_S1_plaque_adjacent</i>	<i>OCCPTEMP_S1_non_plaque</i>	<i>OCCPTEMP_S2_plaque</i>	<i>OCCPTEMP_S2_plaque_adjacent</i>	<i>OCCPTEMP_S2_non_plaque</i>	<i>STR_S1_plaque</i>	<i>STR_S1_plaque_adjacent</i>	<i>STR_S1_non_plaque</i>	<i>STR_S2_plaque</i>	<i>STR_S2_plaque_adjacent</i>	<i>STR_S2_non_plaque</i>
<i>Astrocyte</i>	0.196081	0.190835	0.198314	0.226229	0.215702	0.204191	0.14651	0.148698	0.156143	0.20376	0.184602	0.170911	0.171814	0.16972	0.167829	0.178942	0.160523	0.168058	0.100071	0.076487	0.089238	0.172054	0.180937	0.170291
<i>Chandelier</i>	0.022078	0.015963	0.015532	0.015154	0.014884	0.014019	0.016053	0.018113	0.020454	0.021476	0.016102	0.015064	0.013382	0.016281	0.016342	0.011455	0.01761	0.015999	0.007874	0.031776	0.030324	0.040163	0.045534	0.026037
<i>Endothelial</i>	0.135015	0.135226	0.137546	0.173724	0.156475	0.151113	0.116663	0.116248	0.114444	0.136256	0.138715	0.143554	0.127297	0.125014	0.126043	0.119826	0.130763	0.130615	0.110422	0.120791	0.114321	0.152121	0.147232	0.151647
<i>L2-3_IT</i>	0.037724	0.031717	0.026622	0.050001	0.049612	0.107099	0.067247	0.057329	0.041529	0.033384	0.054699	0.053056	0.055808	0.061921	0.058434	0.009044	0.023819	0.064196	0.003889	0.009811	0.028993	0.019511	0.013002	0.040108
<i>L4_IT</i>	0.087971	0.082338	0.087864	0.132817	0.10711	0.091157	0.242942	0.234894	0.201656	0.073518	0.121724	0.184155	0.194505	0.181831	0.157124	0.112062	0.142381	0.174367	0.270904	0.339213	0.367743	0.140908	0.177145	0.174283
<i>L5_ET</i>	0.005437	0.011777	0.013748	0.011488	0.014093	0.01817	0.009023	0.010449	0.015358	0.011895	0.014636	0.016698	0.014188	0.015976	0.015915	0.036408	0.020847	0.017127	0.025158	0.014551	0.008778	0.028164	0.022088	0.018476
<i>L5_IT</i>	0.005469	0.014723	0.021725	0.033652	0.033023	0.026872	0.013806	0.018207	0.027585	0.041067	0.057316	0.03089	0.018335	0.027529	0.034429	0.047865	0.076946	0.035736	0.005674	0.026753	0.020094	0.030293	0.026068	0.022299
<i>L5-6_NP</i>	0.003808	0.009413	0.014811	0.003113	0.007989	0.015889	0.011755	0.010419	0.014074	0.028722	0.019498	0.013666	0.009825	0.015361	0.011596	0.020392	0.027713	0.014395	0.029495	0.006533	0.008227	0.015819	0.014967	0.018613
<i>L6_CT</i>	0.0061	0.016497	0.016352	0.004908	0.007792	0.011256	0.004196	0.0042	0.010088	0.00815	0.019247	0.011728	0.006211	0.005248	0.009842	0.028253	0.023319	0.014812	0.005614	0.002977	0.004878	0.002398	0.006071	0.012654
<i>L6_IT</i>	0.011686	0.019544	0.01529	0.004372	0.01801	0.02064	0.005814	0.014089	0.014397	0.019378	0.01768	0.013983	0.012476	0.015408	0.018811	0.027898	0.018616	0.019725	0.007057	0.004023	0.008108	0.011014	0.005476	0.014498
<i>L6_IT_Car3</i>	0.023421	0.027699	0.023317	0.021552	0.026503	0.035044	0.015063	0.01621	0.029838	0.064212	0.04938	0.035725	0.013485	0.020092	0.027293	0.08978	0.061052	0.049081	0.006909	0.015481	0.017146	0.016404	0.025703	0.023808
<i>L6b</i>	0.007324	0.015355	0.017203	0.008269	0.006835	0.01072	0.01341	0.00811	0.010748	0.002762	0.014792	0.010307	0.012163	0.010991	0.009943	0.008827	0.013585	0.011098	0.021535	0.018933	0.005357	0.010869	0.005325	0.010419
<i>Lamp5</i>	0.011668	0.017318	0.013666	0.007217	0.007307	0.007747	0.014846	0.014781	0.01293	0.008121	0.009122	0.008775	0.016399	0.012654	0.011683	0.002632	0.007668	0.00824	0.033857	0.023188	0.01818	0.008449	0.010276	0.011236
<i>Lamp5_Lhx6</i>	0.01157	0.005333	0.009696	0.004082	0.005766	0.007102	0.008701	0.009099	0.011164	0.006289	0.003896	0.006739	0.008602	0.009388	0.010703	0.010476	0.006041	0.006235	0.028376	0.012855	0.010936	0.005099	0.009055	0.009177
<i>Microglia-PVM</i>	0.050517	0.049925	0.045353	0.034483	0.038303	0.03646	0.034612	0.032244	0.030802	0.041664	0.037603	0.03707	0.033323	0.035372	0.039212	0.038943	0.036527	0.033718	0.016152	0.018418	0.025741	0.049026	0.045255	0.036493
<i>Oligodendrocyte</i>	0.143163	0.129647	0.124893	0.053617	0.051543	0.049319	0.100936	0.100485	0.112755	0.08714	0.082837	0.071269	0.095525	0.096789	0.099572	0.054392	0.057961	0.057179	0.074999	0.089695	0.074155	0.105756	0.099685	0.074368
<i>OPC</i>	0.021841	0.022305	0.020764	0.015936	0.022351	0.02338	0.01746	0.017997	0.019262	0.010066	0.022008	0.01706	0.023005	0.020743	0.02138	0.013687	0.021573	0.020762	0.030632	0.025073	0.01441	0.024952	0.021515	0.0199
<i>Pax6</i>	0.057771	0.054315	0.050135	0.046401	0.052162	0.03407	0.027668	0.025508	0.02252	0.025715	0.01698	0.025884	0.036482	0.028312	0.02606	0.026405	0.031904	0.028549	0.044394	0.048779	0.027494	0.020901	0.019738	0.021114
<i>Pvalb</i>	0.02203	0.01614	0.010397	0.00872	0.013489	0.009435	0.010764	0.011989	0.011172	0.007651	0.0052	0.008874	0.008696	0.010372	0.010823	0.007826	0.009935	0.010285	0.030537	0.023433	0.020881	0.011367	0.01985	0.01378
<i>Sncg</i>	0.011206	0.013906	0.013601	0.009634	0.012772	0.011857	0.006846	0.009927	0.012052	0.025911	0.009382	0.008686	0.014462	0.010415	0.012716	0.021517	0.009498	0.009242	5.52E-05	0.002724	0.011407	0.009582	0.007527	0.009198
<i>Sst</i>	0.006958	0.006513	0.00967	0.003619	0.008082	0.0071	0.003125	0.007467	0.009763	0.003961	0.006405	0.006447	0.010349	0.010156	0.010791	0.008381	0.008041	0.006113	0.02866	0.000649	0.007718	0.015907	0.010307	0.008115
<i>Sst_Chodl</i>	0.010474	0.012849	0.009156	0.004178	0.005476	0.003856	0.004553	0.004555	0.005305	0.00772	0.003534	0.00392	0.005588	0.005996	0.00516	0.008811	0.00627	0.004386	5.52E-05	0.00656	0.003108	0.005767	0.006665	0.00497
<i>VLMC</i>	0.09082	0.072974	0.07539	0.096295	0.098158	0.069467	0.0689	0.071377	0.062008	0.10803	0.075217	0.085895	0.064816	0.059795	0.058295	0.095848	0.069379	0.076079	0.105221	0.064926	0.056497	0.079631	0.064984	0.084975
<i>Vip</i>	0.019869	0.027689	0.028954	0.030538	0.026564	0.034034	0.039109	0.037605	0.033954	0.02315	0.019426	0.019644	0.033265	0.034636	0.040005	0.020328	0.018027	0.024001	0.01246	0.016371	0.026266	0.023847	0.015594	0.023543