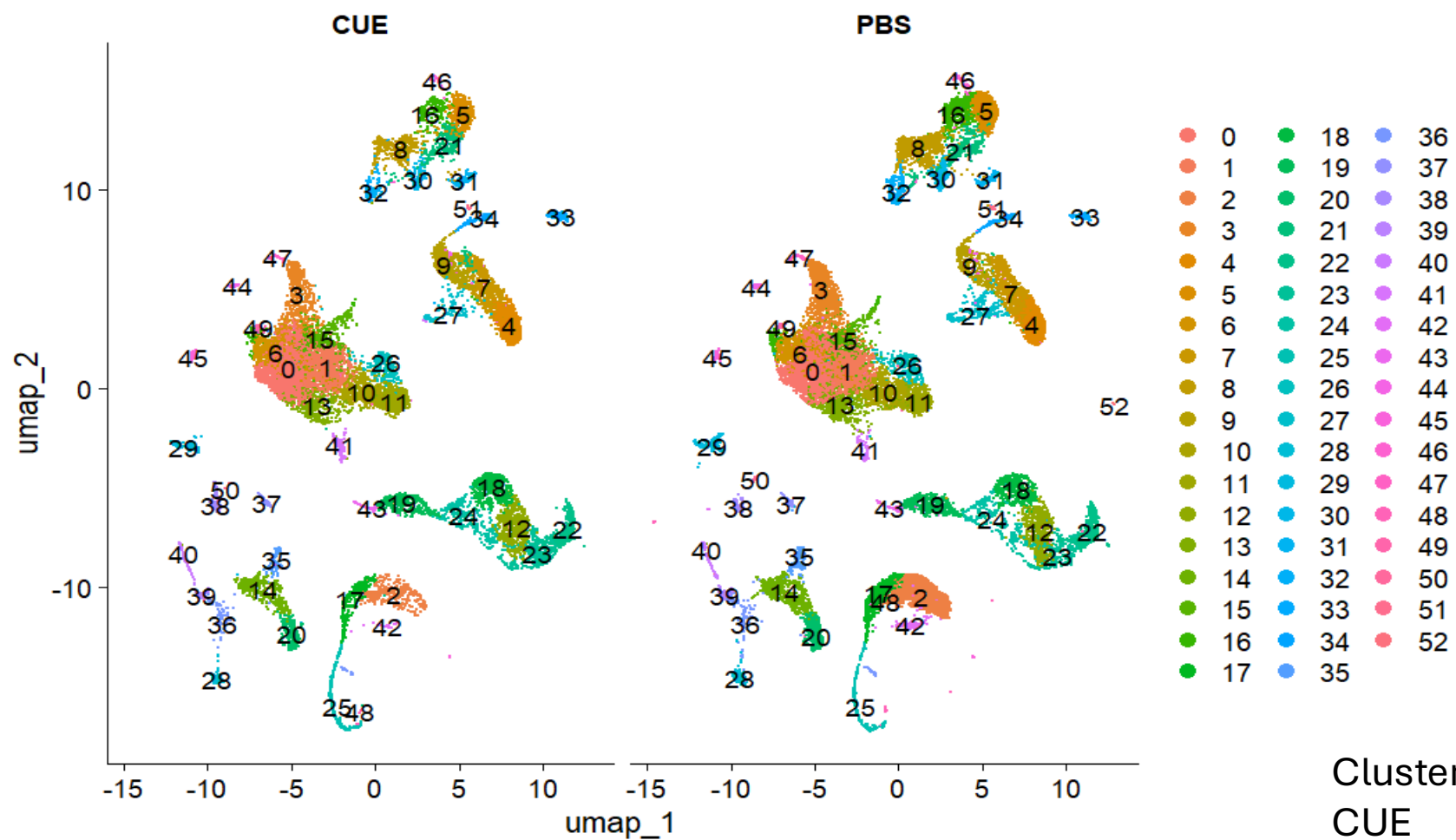
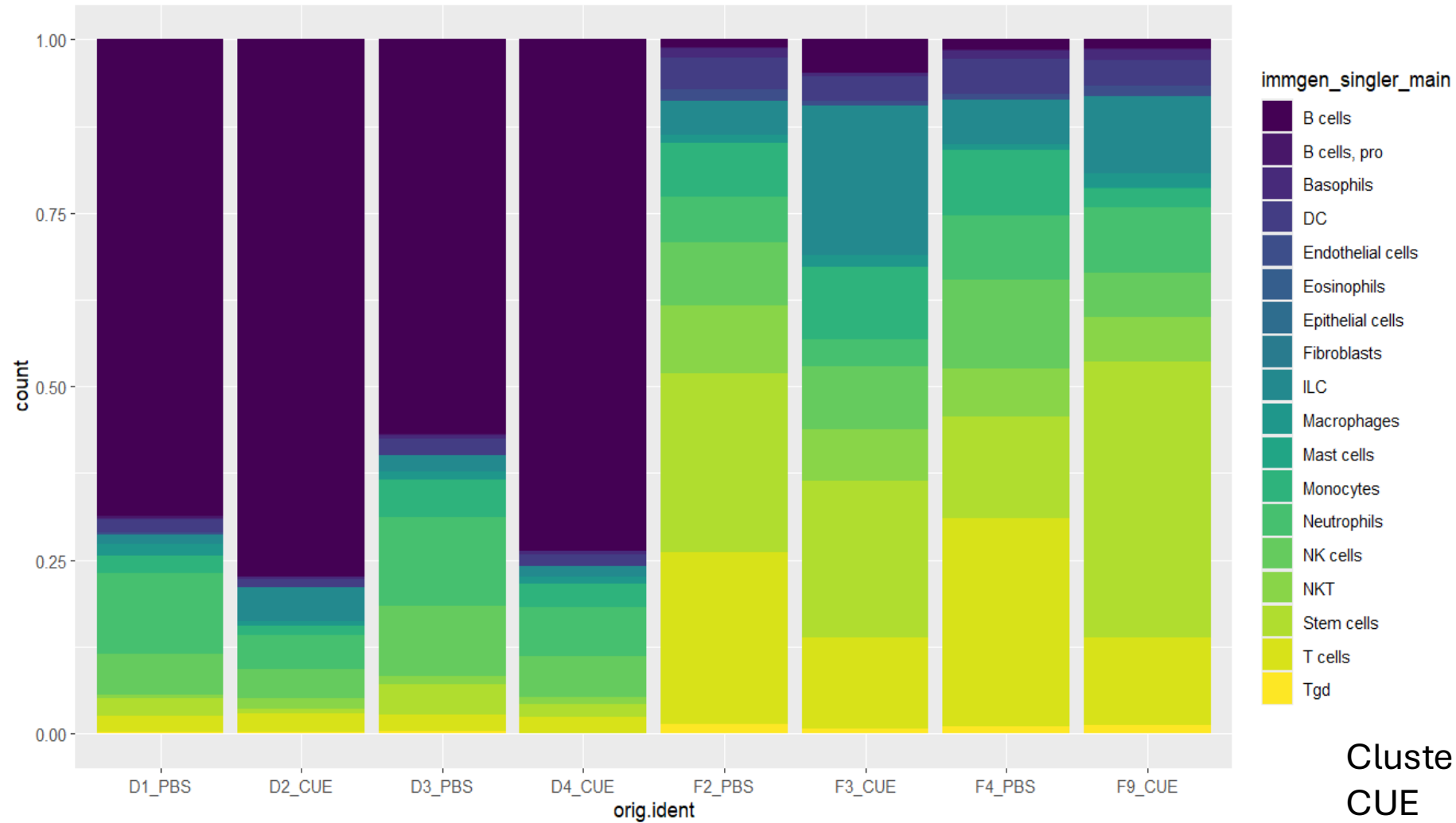


Unannotated clustering

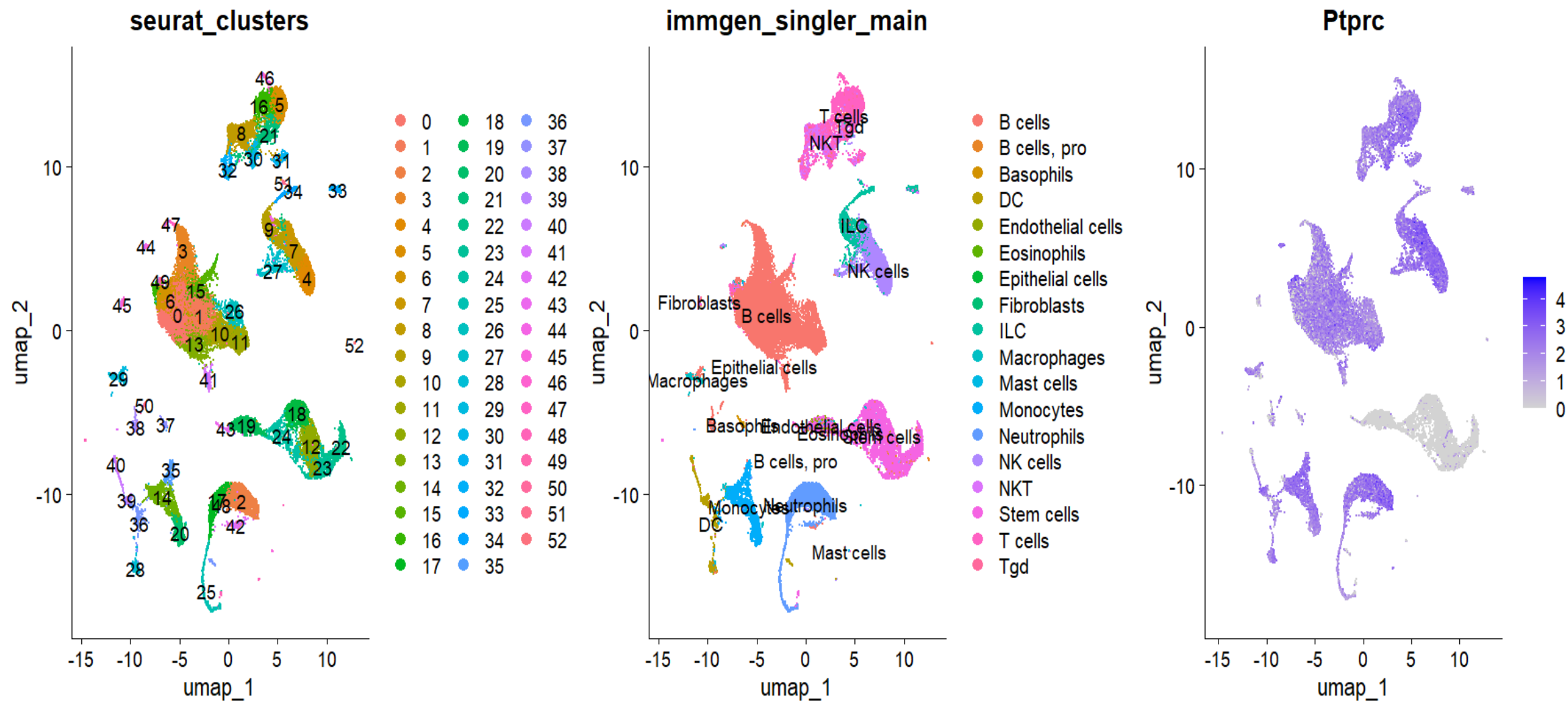


Cell type annotation in each sample using singleR tool – ImmGen dataset

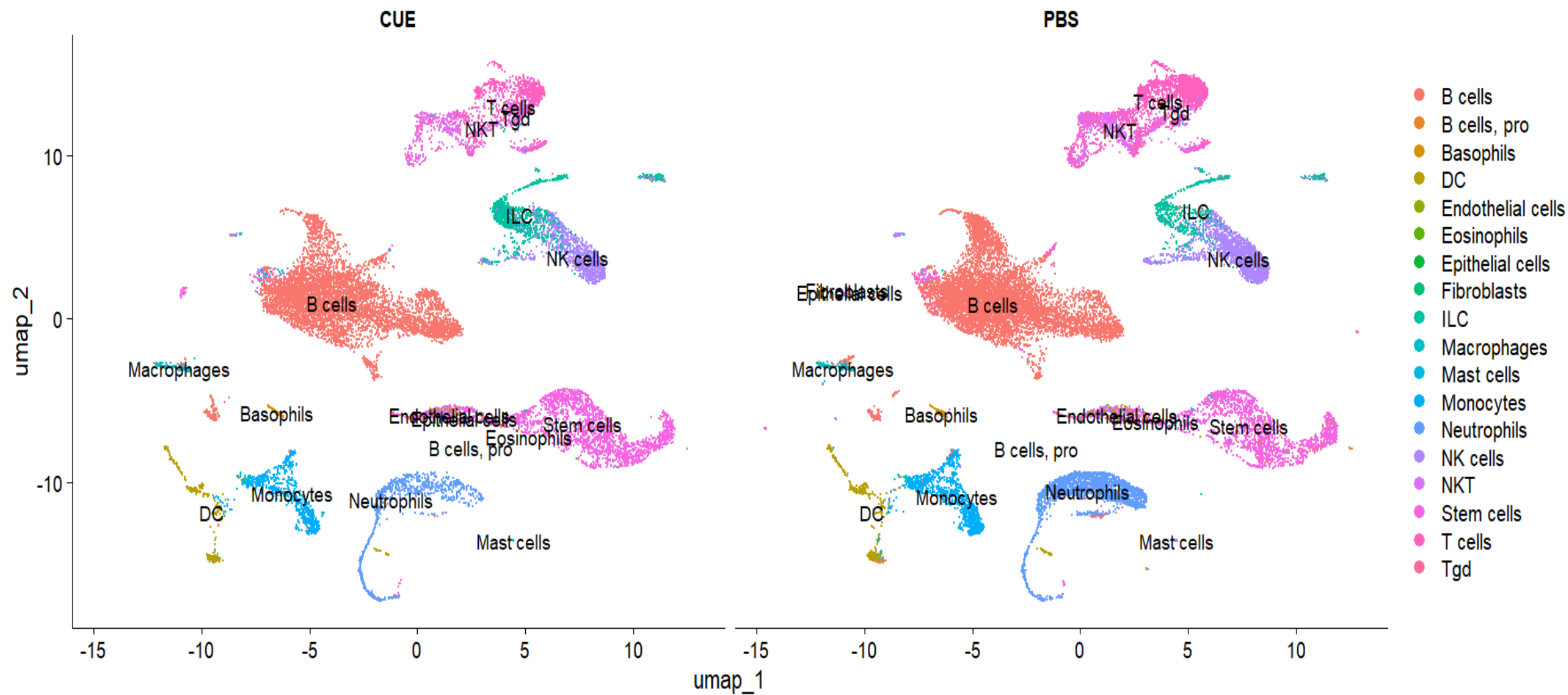


Cluster 52 not found in
CUE

Adding the singleR labels to our dataset



immgen_singler_main

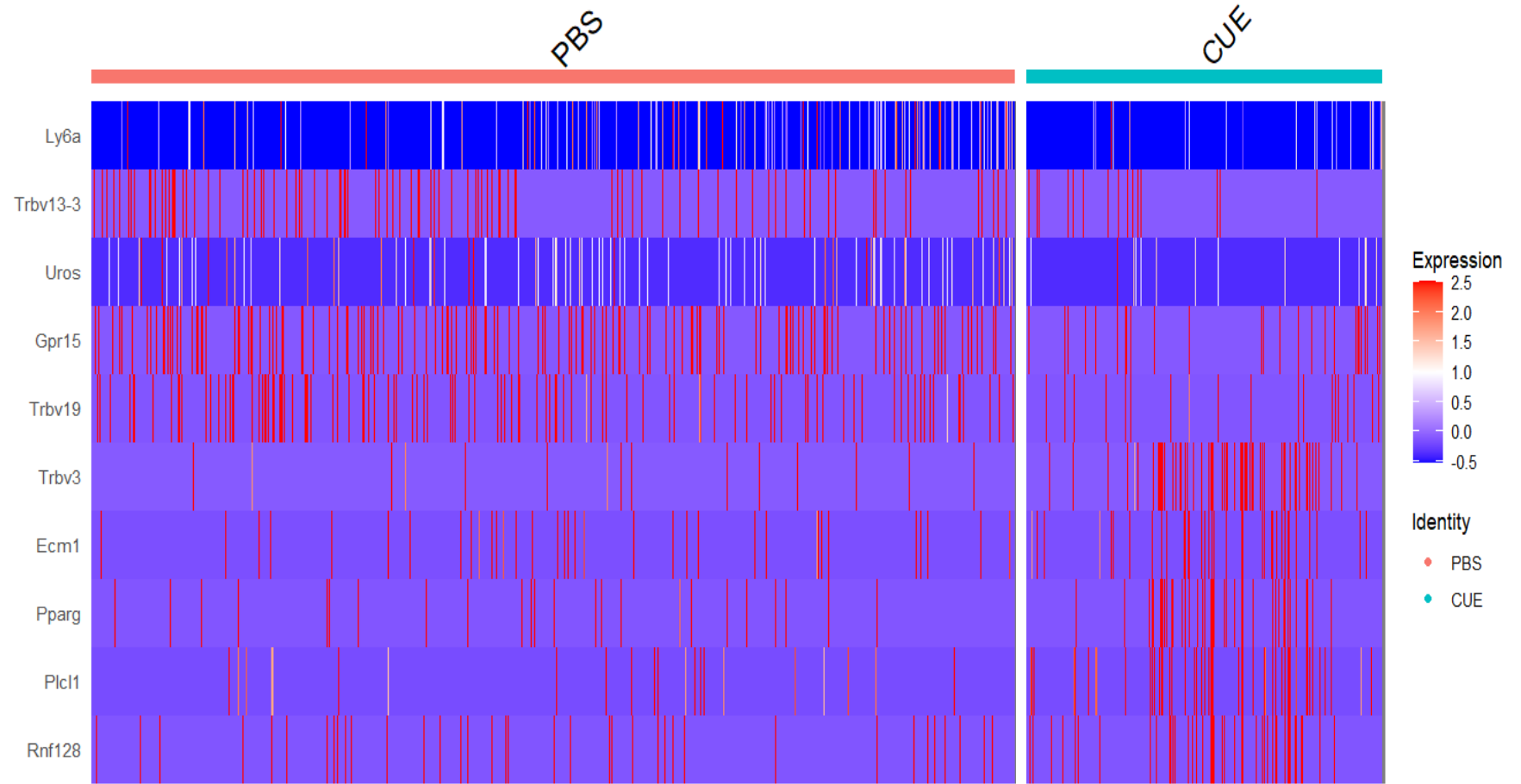


Cluster 8 analysis – CD4

Cluster defining genes

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Cd3e	0.000000e+00	3.160271	0.962	0.122	0.000000e+00
Cd3g	0.000000e+00	3.305363	0.966	0.140	0.000000e+00
Thy1	0.000000e+00	3.159317	0.934	0.170	0.000000e+00
Cd3d	0.000000e+00	2.650256	0.915	0.159	0.000000e+00
Lat	0.000000e+00	3.068581	0.934	0.191	0.000000e+00
Cd5	0.000000e+00	4.073246	0.820	0.084	0.000000e+00
Cd4	0.000000e+00	4.027674	0.760	0.048	0.000000e+00
Skap1	0.000000e+00	2.226144	0.963	0.261	0.000000e+00
Trbc2	0.000000e+00	2.538080	0.869	0.176	0.000000e+00
Themis	0.000000e+00	3.104211	0.763	0.090	0.000000e+00

Differentially expressed genes



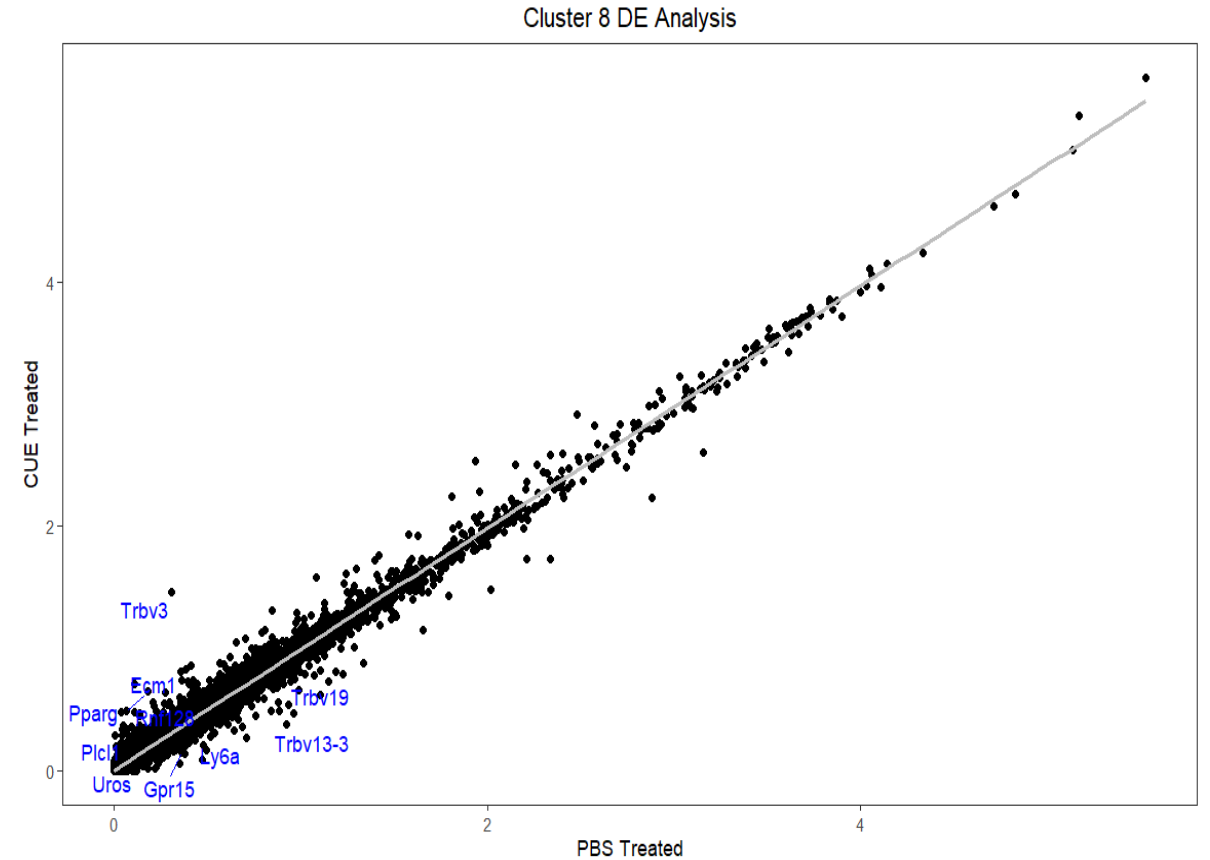
Cluster 8 – DE Analysis

PBS Markers

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ly6a	2.981186e-04	1.7190355	0.137	0.065	1.000000e+00
Trbv13-3	8.928327e-05	1.7171284	0.110	0.041	1.000000e+00
Uros	1.329677e-04	1.6740604	0.104	0.038	1.000000e+00
Gpr15	1.157531e-05	1.4364724	0.165	0.073	2.434055e-01
Trbv19	6.571729e-05	1.4291163	0.134	0.057	1.000000e+00

CUE Markers

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Trbv3	8.904981e-21	-3.2111440	0.020	0.160	1.872539e-16
Ecm1	2.647845e-05	-3.1620310	0.045	0.106	5.567889e-01
Pparg	4.163354e-10	-3.0738031	0.029	0.117	8.754702e-06
Plcl1	4.235490e-14	-2.7070642	0.030	0.144	8.906389e-10
Rnf128	6.559200e-07	-2.2333737	0.046	0.122	1.379269e-02

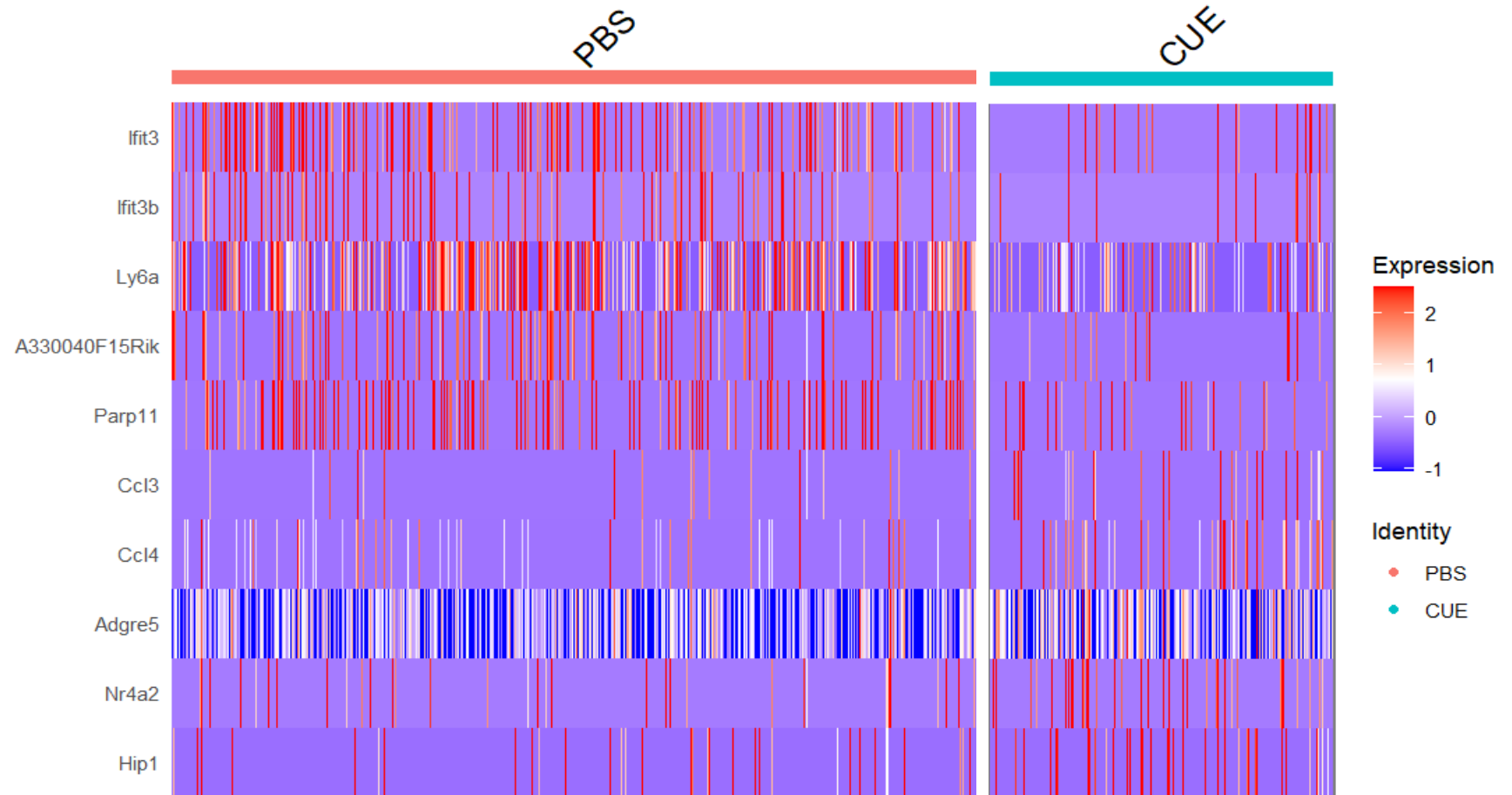


Cluster 16 analysis – CD8

Cluster defining genes

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Cd3e	0.000000e+00	2.4338089	0.857	0.130	0.000000e+00
Cd3d	0.000000e+00	2.6042610	0.846	0.165	0.000000e+00
Cd3g	0.000000e+00	2.1799030	0.828	0.149	0.000000e+00
Skap1	0.000000e+00	2.2863785	0.921	0.267	0.000000e+00
Lat	0.000000e+00	2.2615805	0.829	0.199	0.000000e+00
Ms4a4b	0.000000e+00	1.4524274	0.877	0.270	0.000000e+00
Tcf7	0.000000e+00	2.8252003	0.747	0.141	0.000000e+00
Gm2682	0.000000e+00	1.9989915	0.802	0.211	0.000000e+00
Lef1	0.000000e+00	3.7450245	0.703	0.122	0.000000e+00
Thy1	0.000000e+00	1.9140956	0.745	0.180	0.000000e+00

Differentially expressed genes



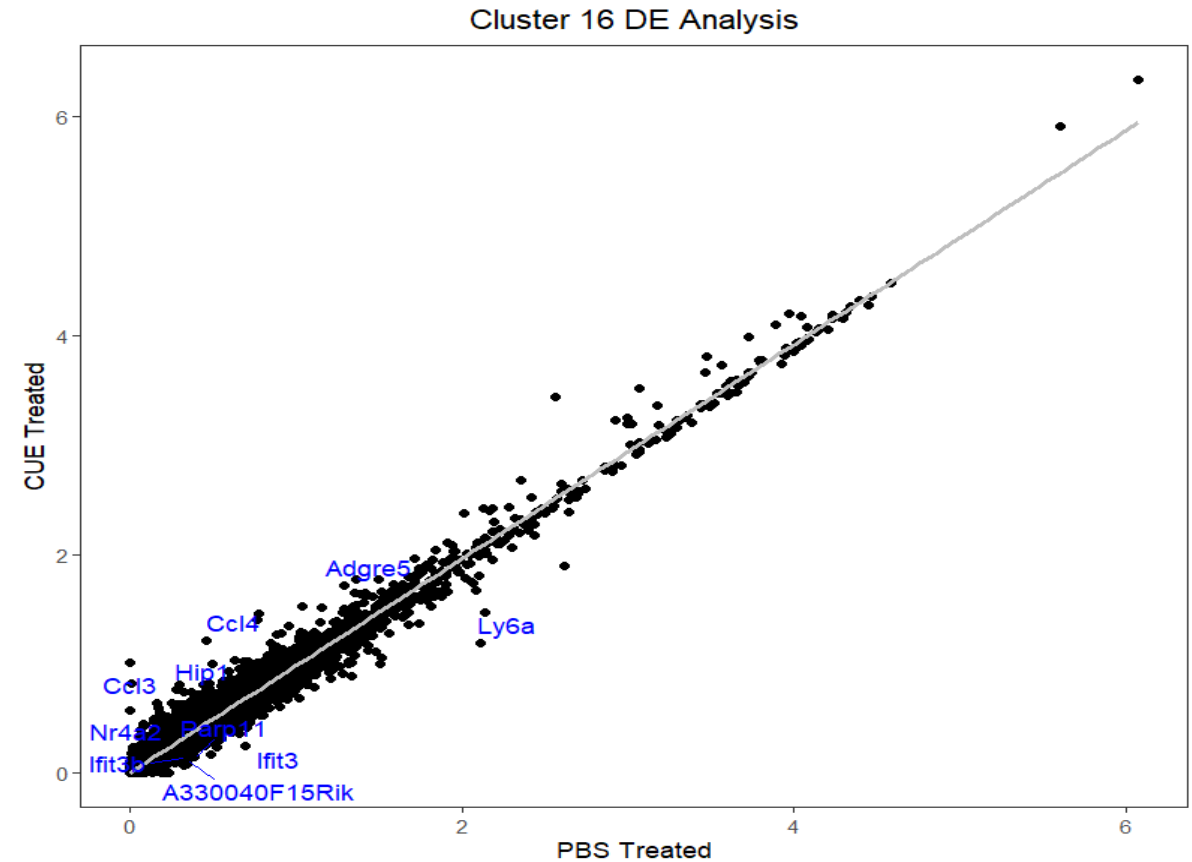
Cluster 16 – DE Analysis

PBS Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ifit3	2.531448e-10	1.8337978	0.260	0.076	5.323129e-06
Ifit3b	4.301648e-06	1.3978491	0.157	0.049	9.045506e-02
Ly6a	7.115525e-13	1.6679535	0.514	0.285	1.496253e-08
A330040F15Rik	9.907569e-08	1.6637100	0.185	0.052	2.083364e-03
Parp11	1.447322e-07	1.5679383	0.214	0.076	3.043428e-03

CUE Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ccl3	6.232912e-07	-2.4132427	0.028	0.104	0.01310657
Ccl4	1.078566e-05	-2.0155427	0.098	0.194	0.22680083
Nr4a2	2.406816e-05	-1.9112002	0.062	0.142	0.50610532
Hip1	9.102858e-06	-1.8512805	0.056	0.139	0.19141490
Adgre5	1.966714e-05	-0.7978877	0.652	0.708	4.135606e-01

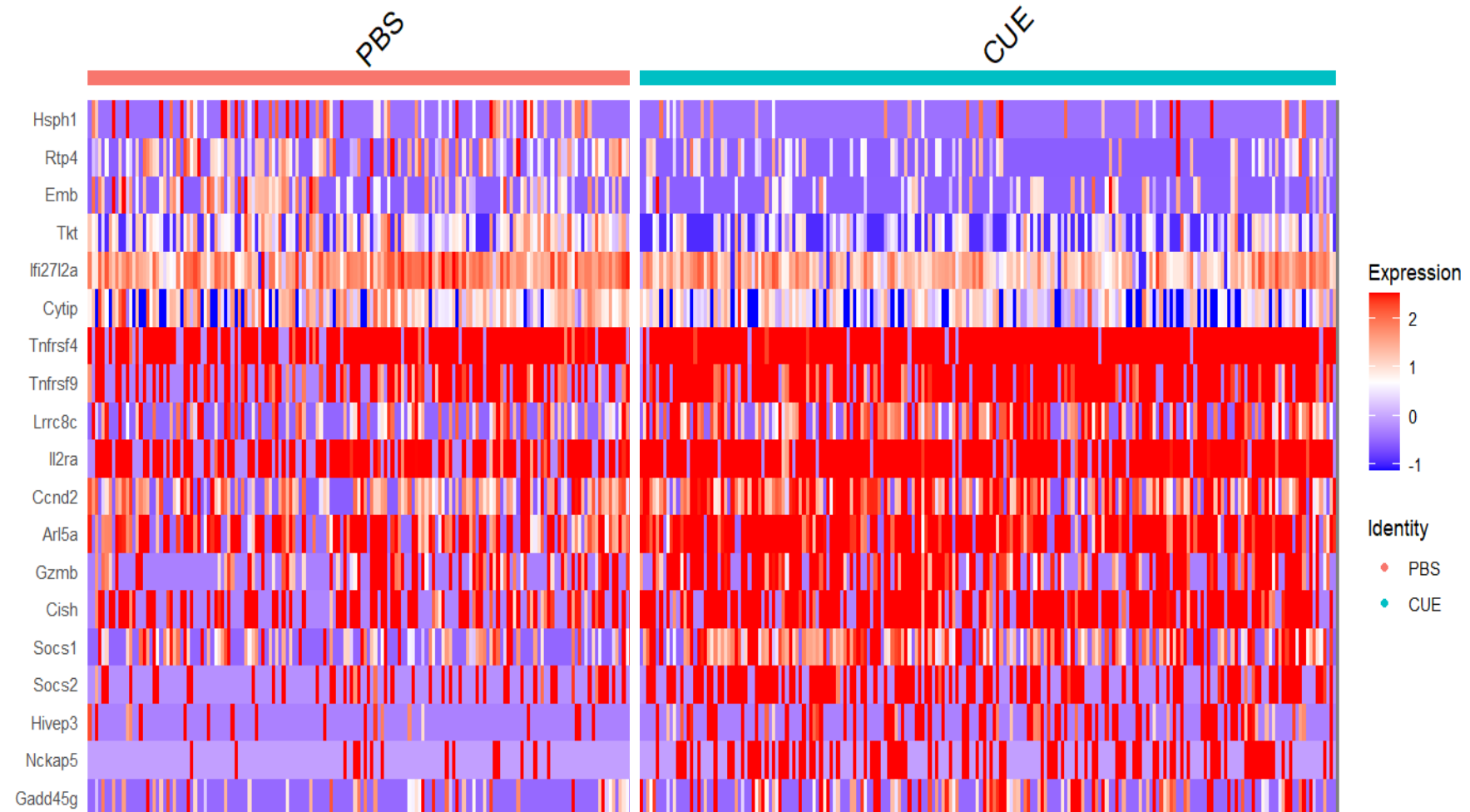


Cluster 31 analysis – Tregs

Cluster defining genes

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ilkzf2	0.000000e+00	5.3858576	0.970	0.078	0.000000e+00
Foxp3	0.000000e+00	8.3273360	0.877	0.006	0.000000e+00
Tnfrsf4	0.000000e+00	6.4250615	0.899	0.031	0.000000e+00
Cd3e	0.000000e+00	3.1895070	0.984	0.140	0.000000e+00
Cd3g	0.000000e+00	2.9501858	0.973	0.159	0.000000e+00
Ctla4	0.000000e+00	5.6641968	0.839	0.033	0.000000e+00
Cd3d	0.000000e+00	2.6279687	0.945	0.175	0.000000e+00
Lat	0.000000e+00	2.6759869	0.964	0.207	0.000000e+00
Il2ra	0.000000e+00	5.3011764	0.806	0.050	0.000000e+00
Tnfrsf18	0.000000e+00	4.1789151	0.956	0.203	0.000000e+00

Differentially expressed genes



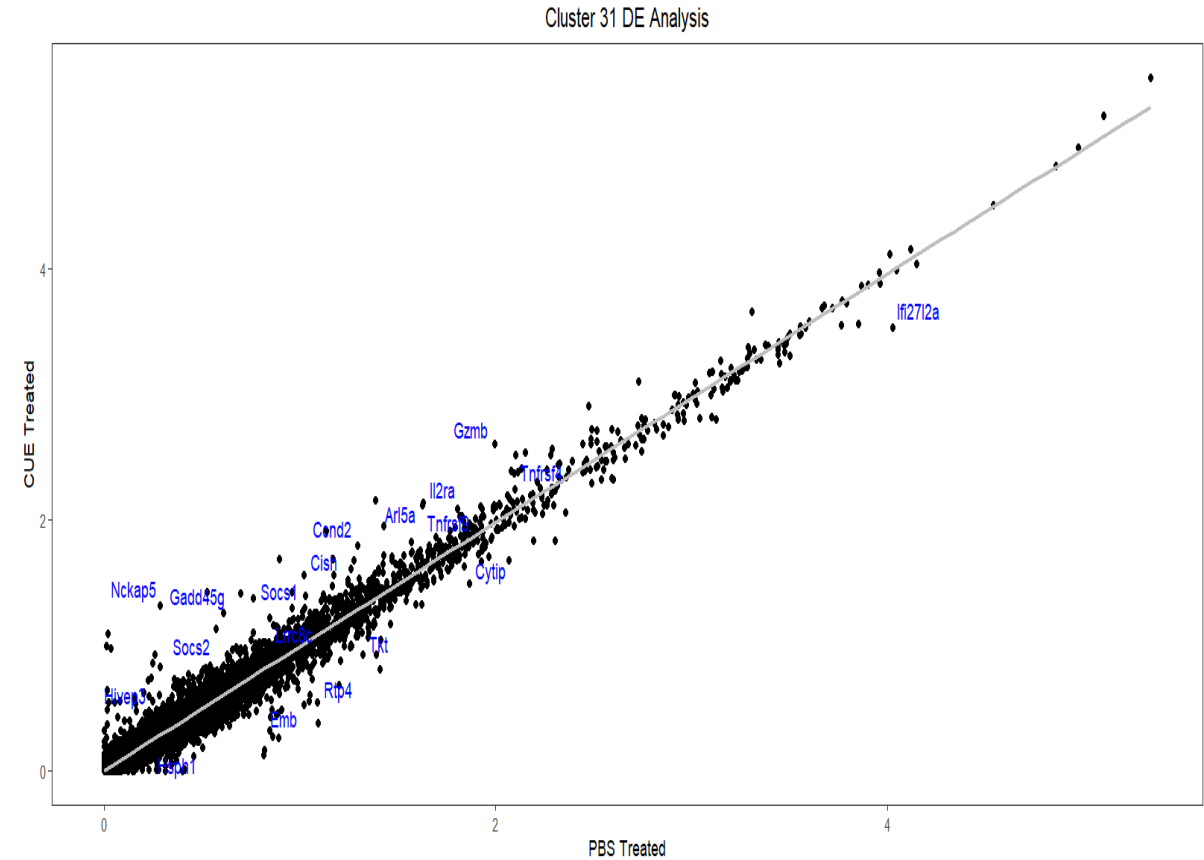
Cluster 31 – DE Analysis

PBS Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Hsph1	1.171040e-06	1.662579	0.344	0.131	0.024625
Rtp4	1.930130e-09	1.434042	0.588	0.296	4.058678e-05
Emb	5.987464e-08	1.353621	0.512	0.257	0.001259
Tkt	1.349497e-07	0.837364	0.775	0.592	0.002838
Ifi27l2a	4.668842e-09	0.72664	0.994	0.966	9.817642e-05
Cytip	1.571513e-06	0.665169	0.85	0.796	0.033046

CUE Markers

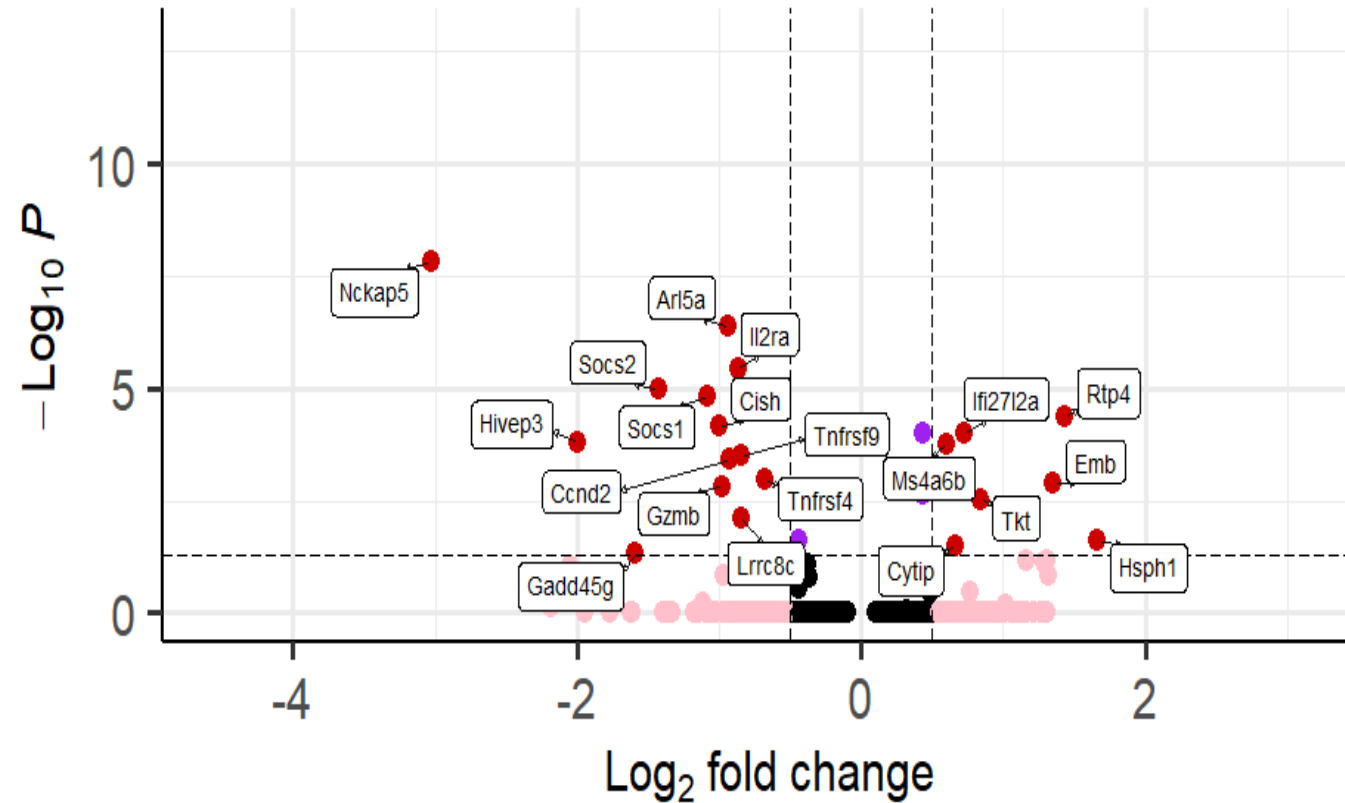
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Nckap5	7.376696520e-13	-3.02995	0.094	0.432	1.551171744e-08
Hivep3	7.1975049300e-09	-2.00026	0.119	0.393	0.000151
Gadd45g	2.1266850686e-06	-1.58799	0.275	0.495	0.04472
Socs2	4.894203637e-10	-1.42981	0.275	0.578	1.0291531409e-05
Socs1	7.26229164e-10	-1.08499	0.494	0.786	1.527114682e-05
Cish	3.3476583239e-09	-1.00202	0.531	0.767	7.03945595e-05
Gzmb	7.03058966562e-08	-0.97852	0.45	0.694	0.001478
Arl5a	1.96424477e-11	-0.93787	0.738	0.893	4.1304139e-07
Ccnd2	1.80916910e-08	-0.92453	0.706	0.854	0.00038
Il2ra	1.682199696e-10	-0.86868	0.688	0.898	3.5373295e-06
Lrrc8c	3.59663909e-07	-0.84863	0.488	0.718	0.007563
Tnfrsf9	1.52411987e-08	-0.84453	0.613	0.835	0.00032
Tnfrsf4	5.0370759e-08	-0.67562	0.838	0.947	0.001059



Volcano Plot of differentially expressed genes

Cluster 31 PBS vs CUE

EnhancedVolcano



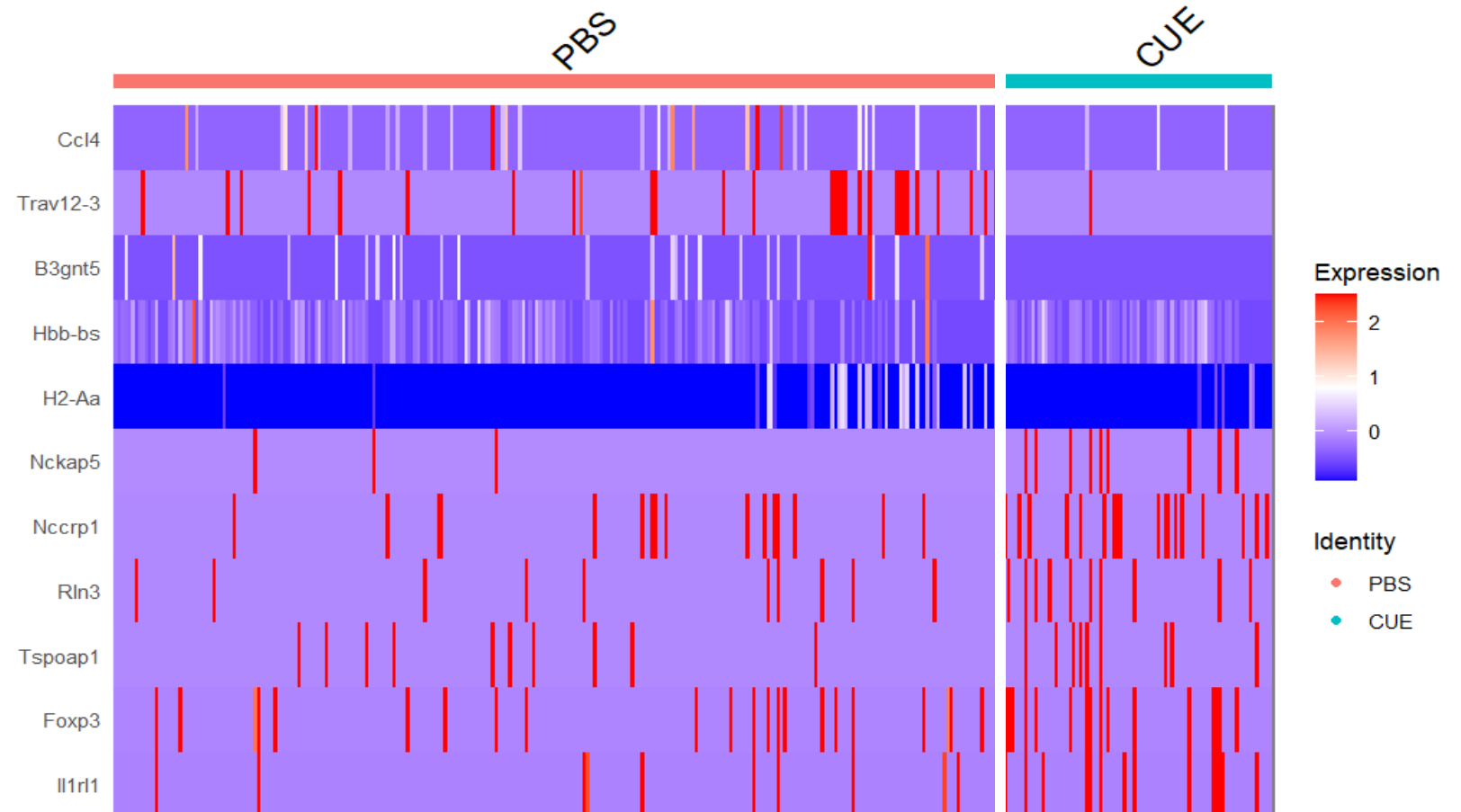
● NS ● \log_2 FC ● p-value ● p-value and \log_2 FC

Cluster 32 analysis – Naïve / Proliferating T cells

Cluster defining genes

	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Pclaf	0.000000e+00	4.222660	0.968	0.112	0.000000e+00
Cd3e	0.000000e+00	2.483317	0.974	0.141	0.000000e+00
Cd3g	0.000000e+00	2.404960	0.971	0.159	0.000000e+00
Stmn1	0.000000e+00	3.359459	0.959	0.149	0.000000e+00
Rrm2	0.000000e+00	2.589541	0.894	0.124	0.000000e+00
Cdk1	0.000000e+00	2.878989	0.874	0.108	0.000000e+00
Lat	0.000000e+00	2.832946	0.971	0.208	0.000000e+00
Birc5	0.000000e+00	2.600172	0.885	0.135	0.000000e+00
Asf1b	0.000000e+00	2.953556	0.865	0.123	0.000000e+00
Top2a	0.000000e+00	2.865339	0.900	0.160	0.000000e+00

Differentially expressed genes



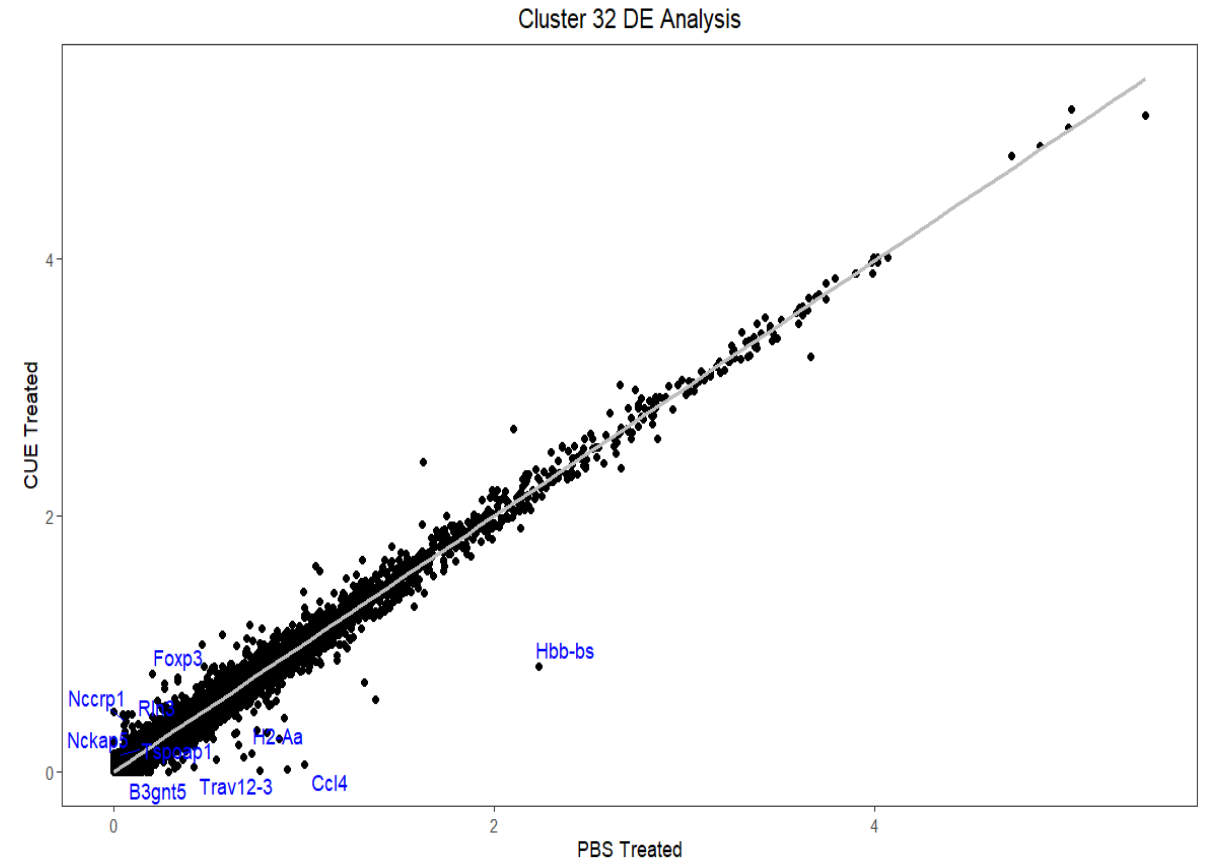
Cluster 32 – DE Analysis

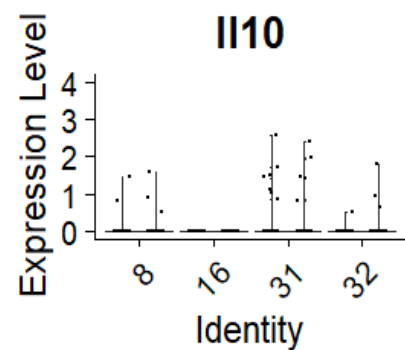
PBS Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ccl4	3.635319e-02	4.5383072	0.119	0.038	1.0000000
Trav12-3	6.757617e-03	3.4187811	0.111	0.013	1.0000000
B3gnt5	2.974806e-03	3.1202108	0.103	0.000	1.0000000
Hbb-bs	2.225673e-01	2.6939815	0.571	0.620	1.0000000
H2-Aa	2.126646e-01	2.6653389	0.107	0.063	1.0000000

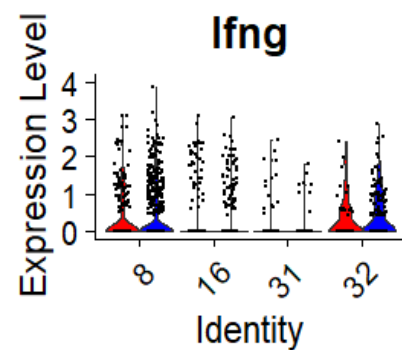
CUE Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Nckap5	1.397895e-05	-3.666536	0.011	0.114	0.2939493
Nccrp1	6.455472e-06	-2.860867	0.061	0.228	0.1357457
Rln3	3.123267e-03	-2.774179	0.038	0.127	1.0000000
Tspoap1	7.478154e-03	-2.364954	0.038	0.114	1.0000000
Foxp3	1.413200e-03	-2.314588	0.084	0.203	1.0000000
l1rl1	2.254006e-05	-2.121949	0.038	0.177	0.473972

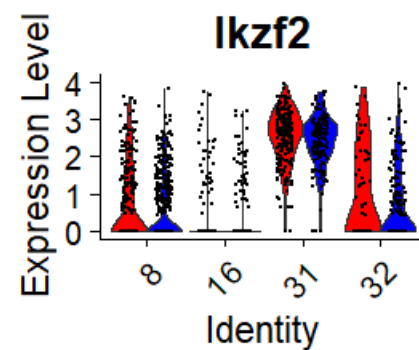




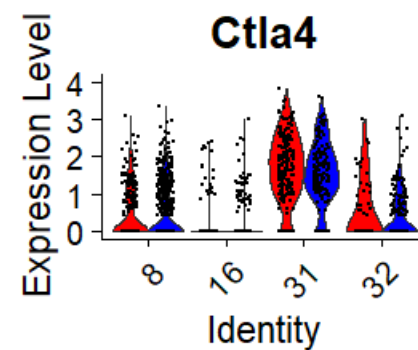
CUE
PBS



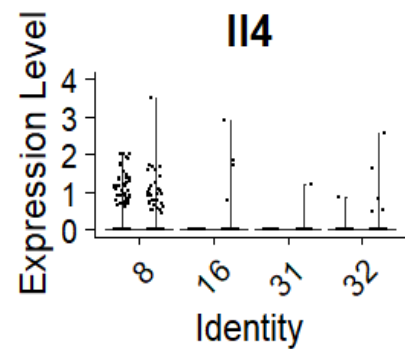
CUE
PBS



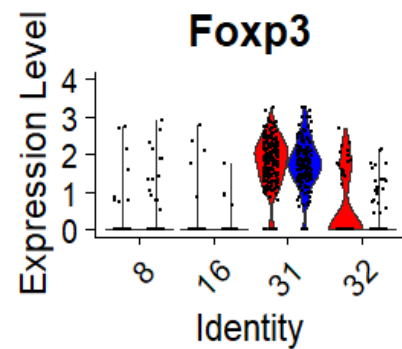
CUE
PBS



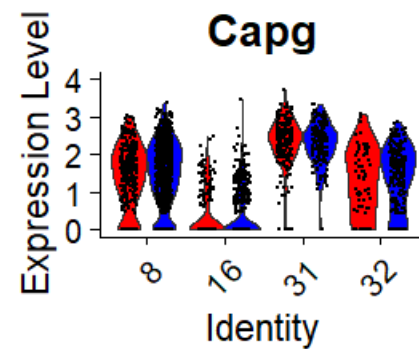
CUE
PBS



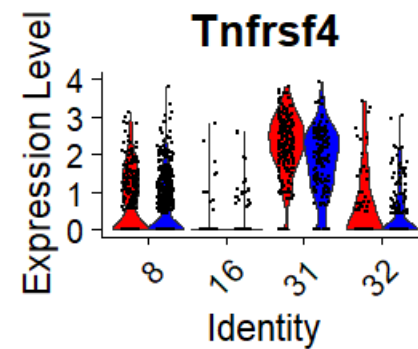
CUE
PBS



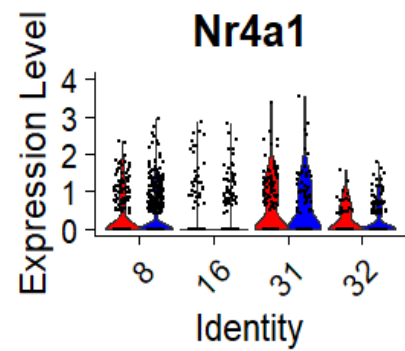
CUE
PBS



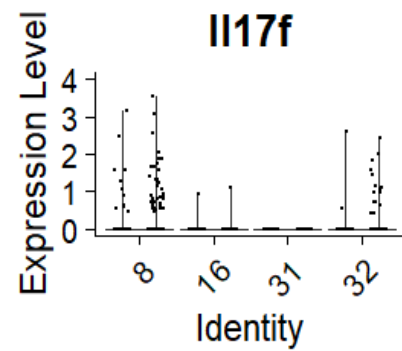
CUE
PBS



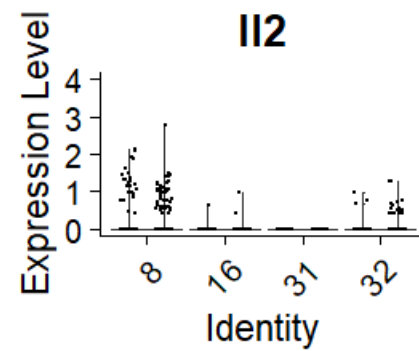
CUE
PBS



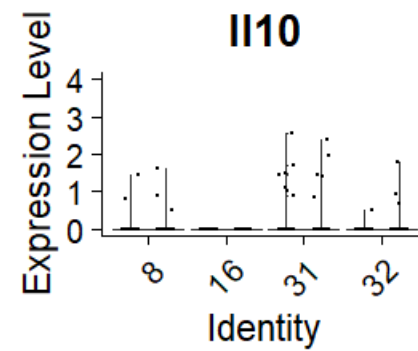
CUE
PBS



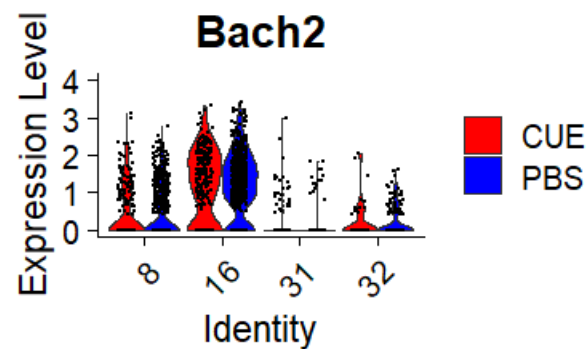
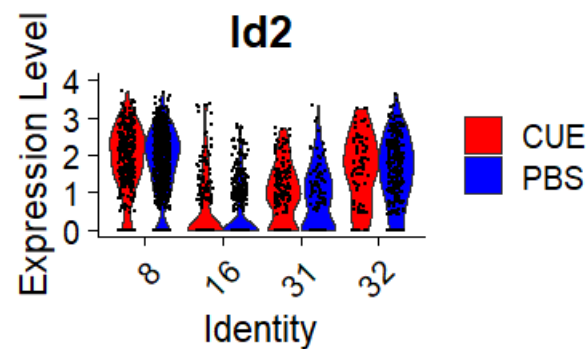
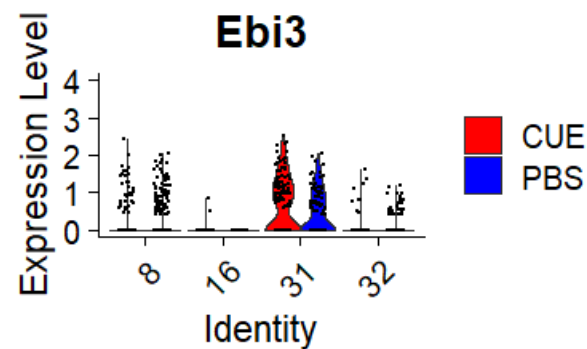
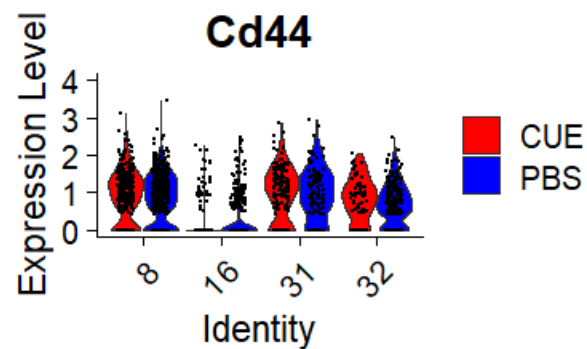
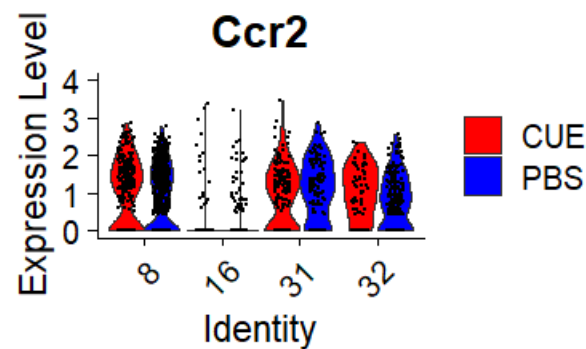
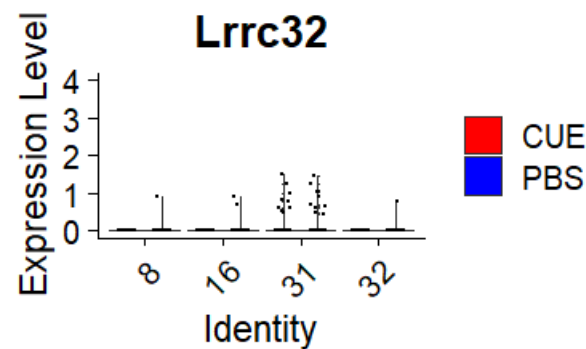
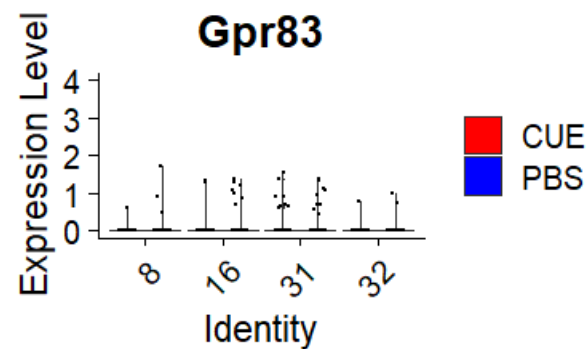
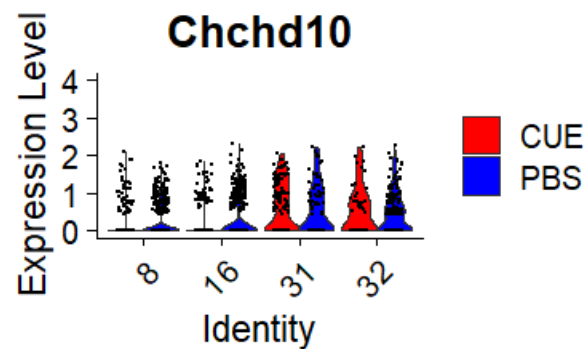
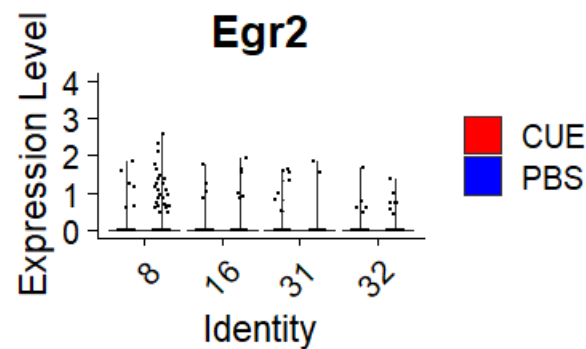
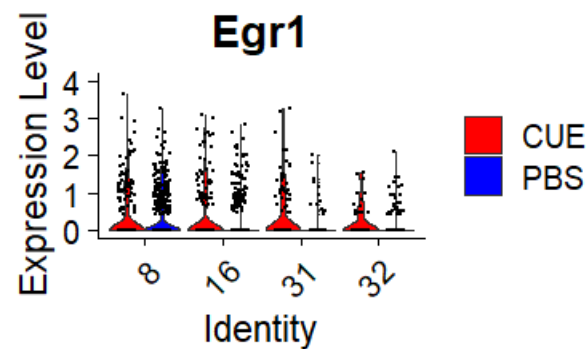
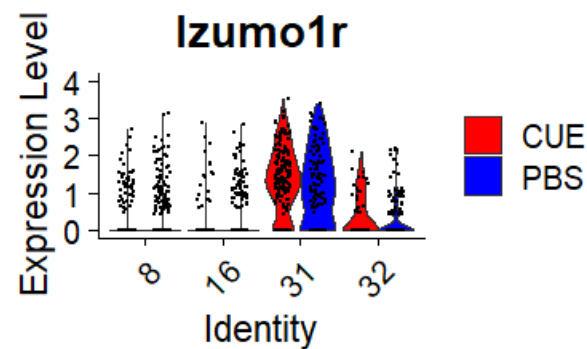
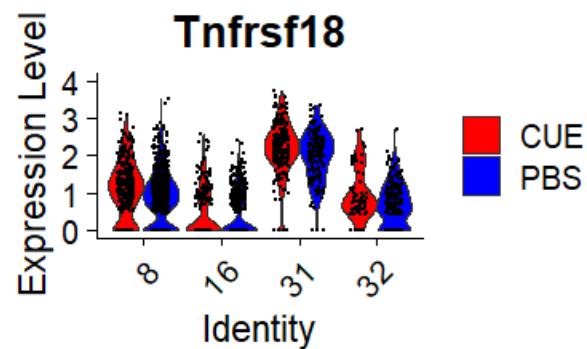
CUE
PBS

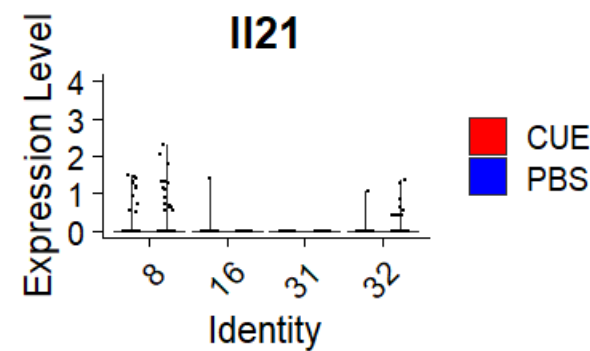
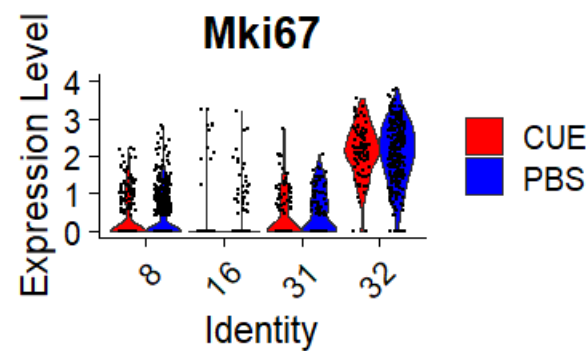
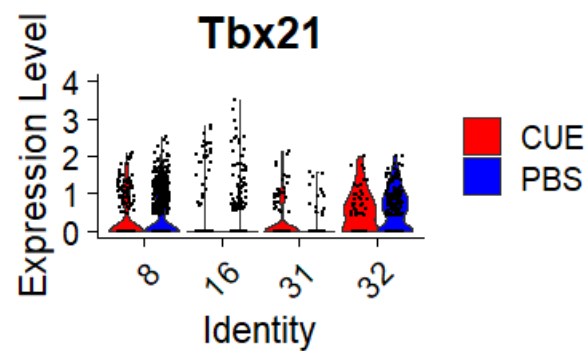
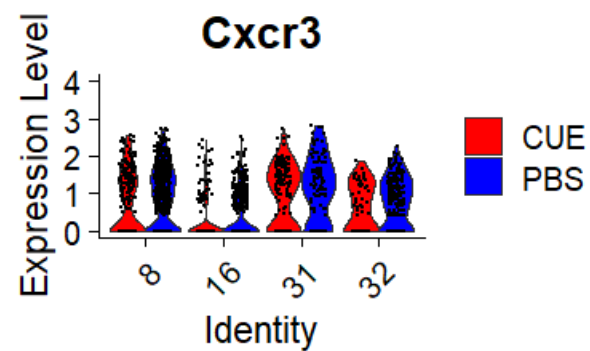
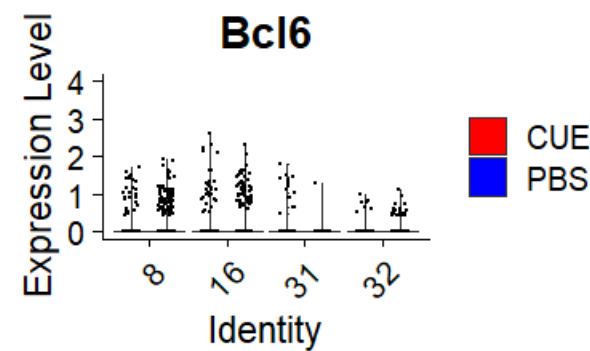
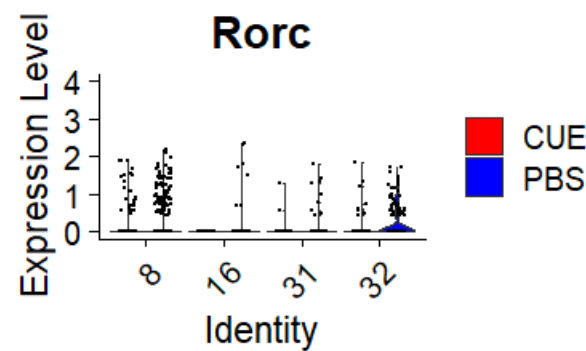
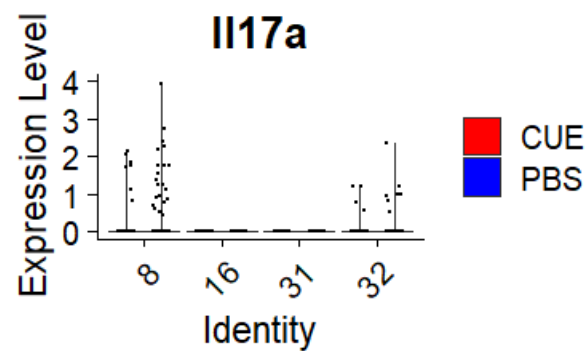
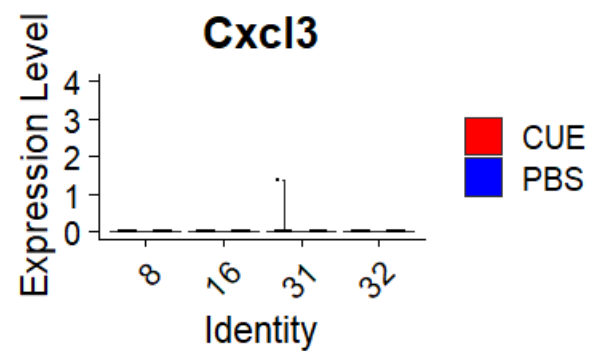
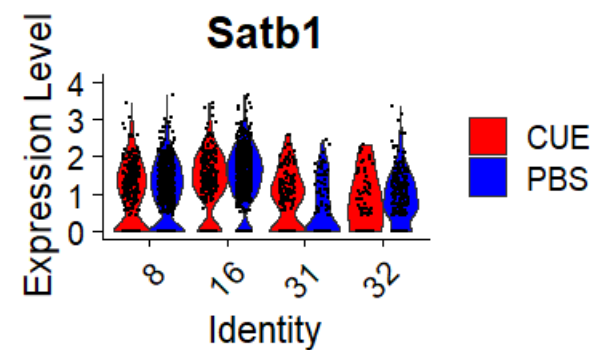
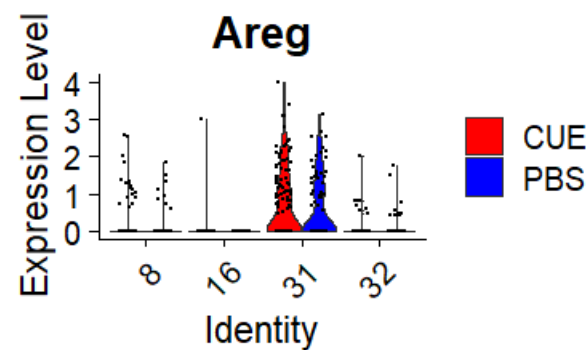
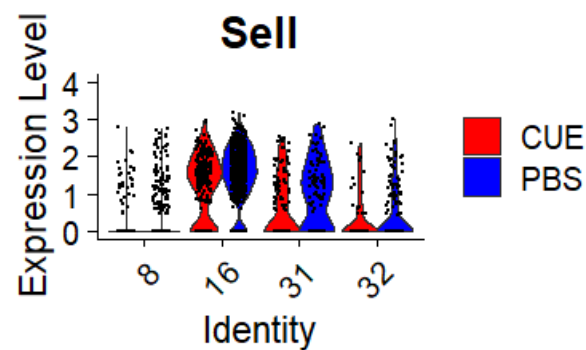
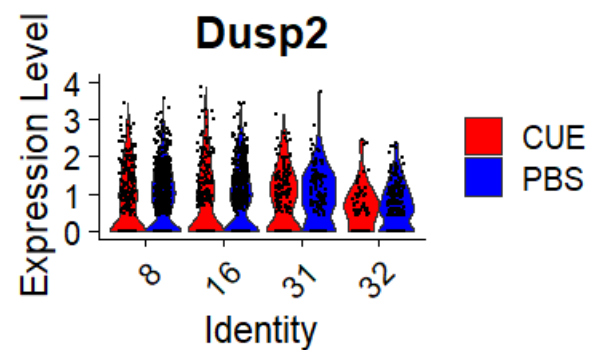


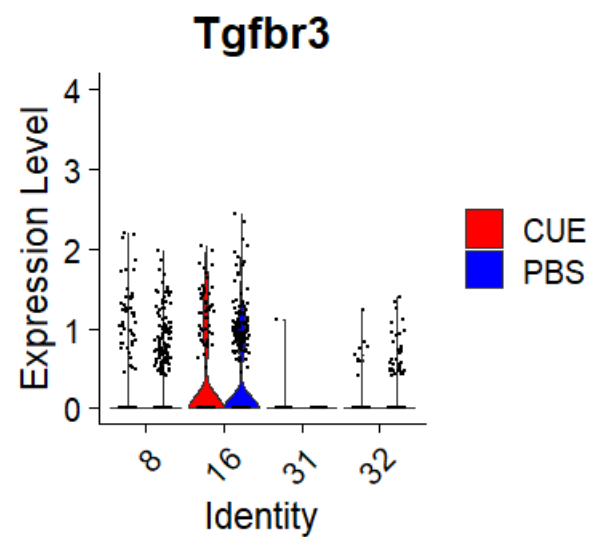
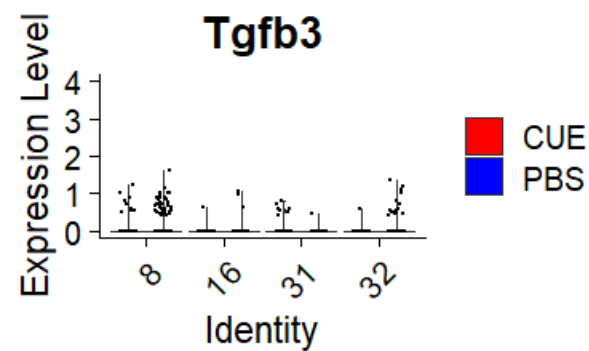
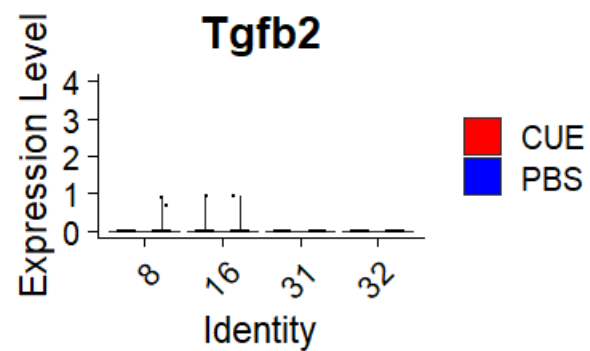
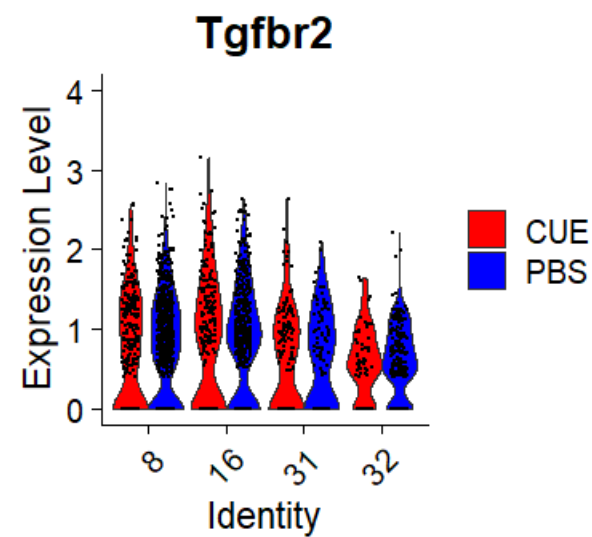
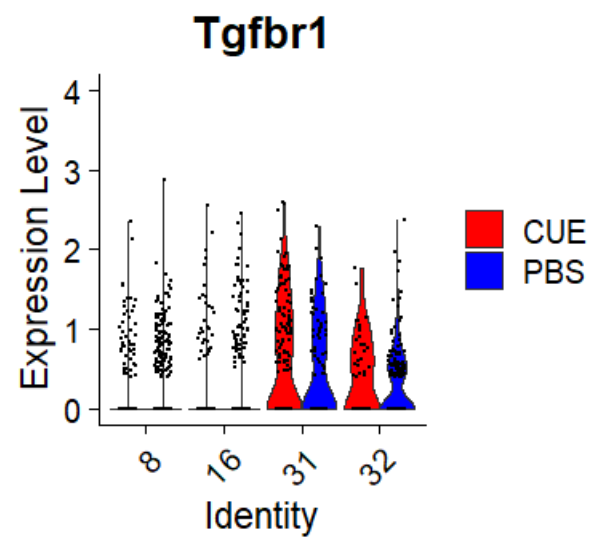
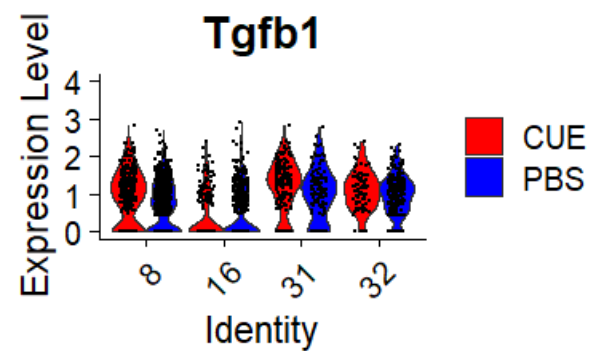
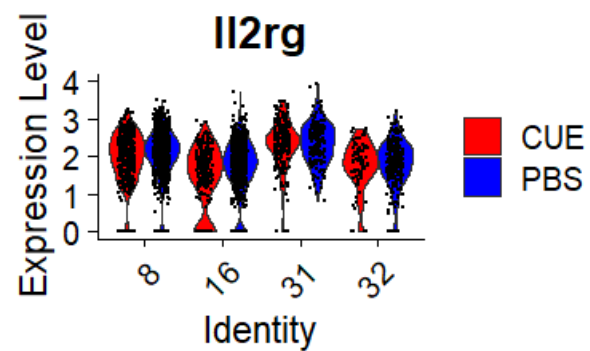
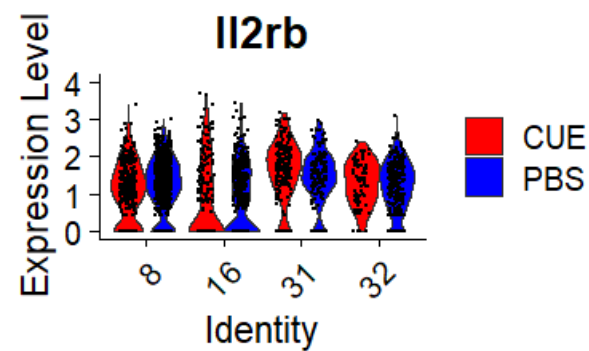
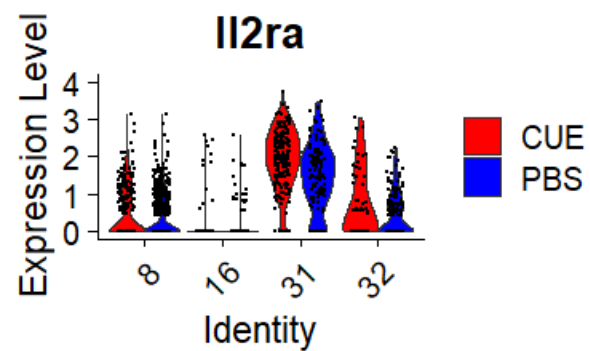
CUE
PBS



CUE
PBS







Average expression of transcripts based on violin plots

Transcript	Avg.exp PBS	Avg.exp CUE
Foxp3 (31)	5.33133	5.798473
Foxp3 (32)	0.228747	1.144332
Il2ra (31)	4.096818	7.487319
Il2ra (32)	0.5956466	1.693708
Tnfrsf18 (31)	7.42269	10.1615
Tnfrsf18 (32)	1.180769	2.055893
Izumo1r (31)	3.060281	3.871478
Izumo1r (32)	0.4034255	0.5442202
Tnfrsf4 (31)	7.164299	11.44851
Tnfrsf4 (32)	1.049225	2.14781
Ctla4 (31)	5.137665	6.631343
Ctla4 (32)	0.7377898	1.15798
Ifng (31)	0.1288564	0.2273403
Ifng (32)	0.6713446	0.4486221

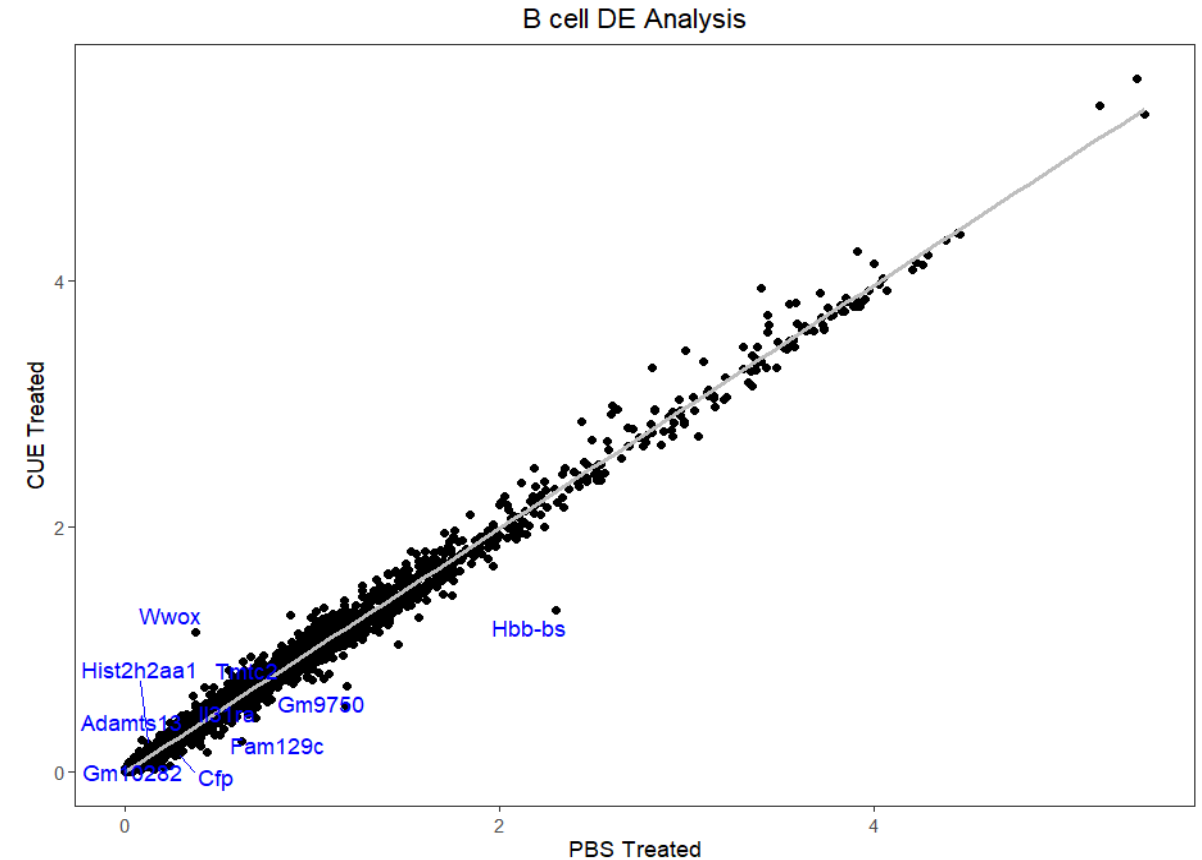
B Cells – DE Analysis

PBS Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Hbb-bs	6.360334e-45	1.7035103	0.221	0.124	1.337451e-40
Gm9750	2.558693e-168	1.1544043	0.578	0.403	5.380420e-164
Cfp	8.132438e-12	1.0819902	0.108	0.073	1.710089e-07
Fam129c	2.444641e-07	0.8916258	0.161	0.134	5.140592e-03
Gm10282	6.094722e-13	0.8762781	0.126	0.087	1.281598e-08

CUE Markers

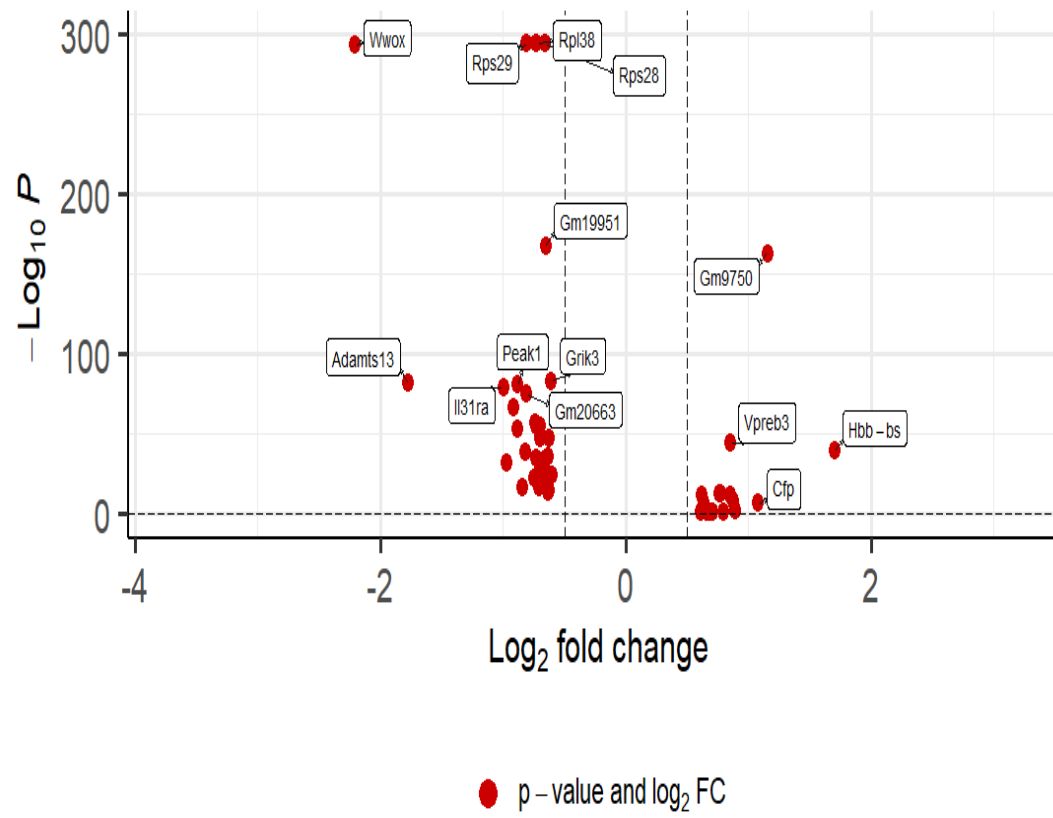
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Wwox	1.656562e-298	-2.2075302	0.160	0.457	3.483419e-294
Adamts13	2.203779e-87	-1.7717733	0.032	0.131	4.634106e-83
Il31ra	3.314820e-84	-0.9958097	0.144	0.299	6.970402e-80
Hist2h2aa1	6.878101e-37	-0.9707538	0.050	0.116	1.446327e-32
Tmtc2	3.483067e-72	-0.9138250	0.177	0.327	7.324193e-68



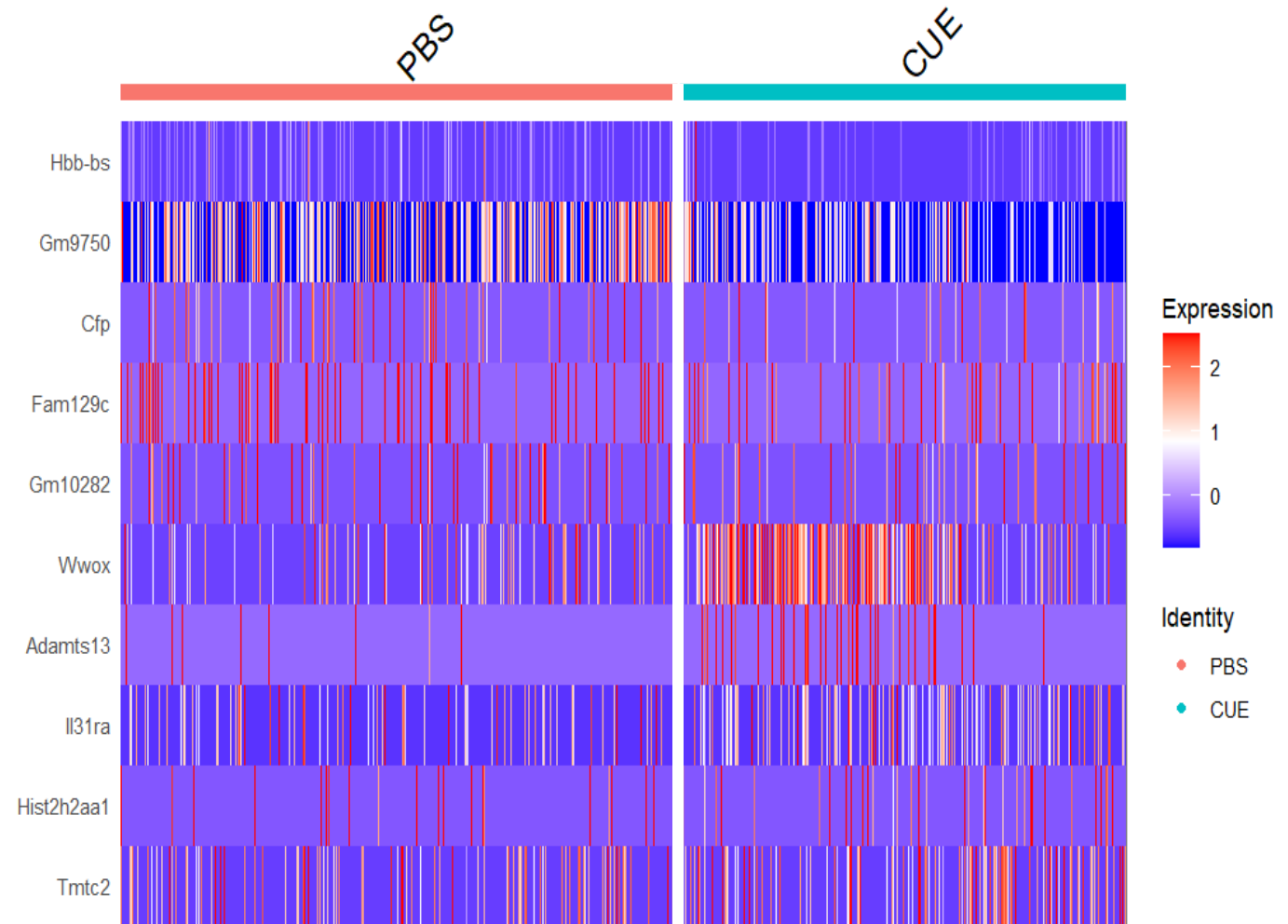
Plots of differentially expressed genes

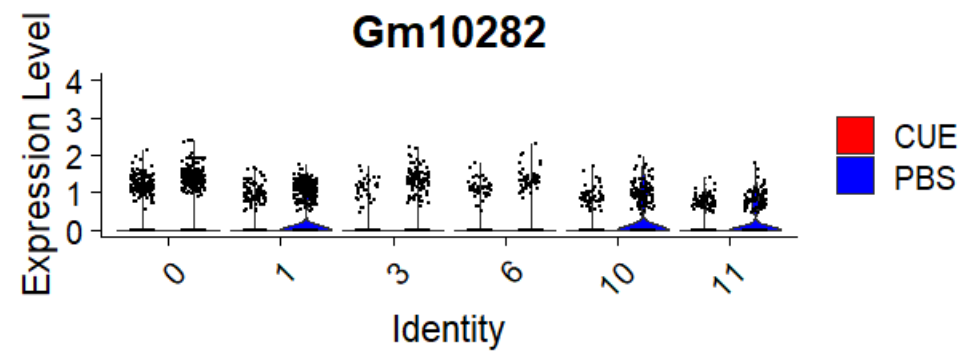
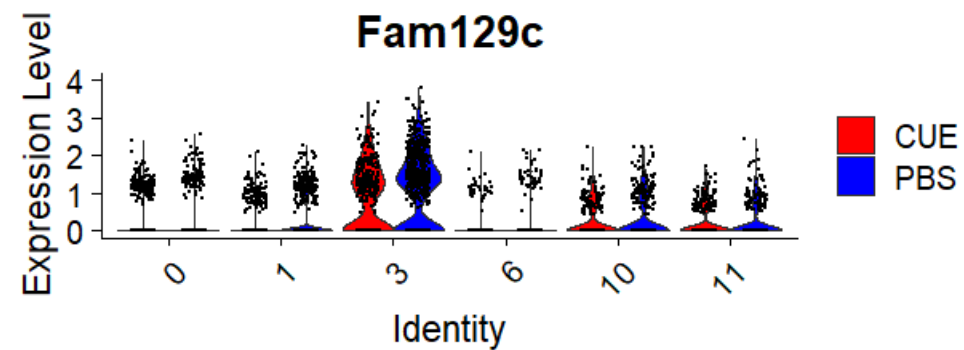
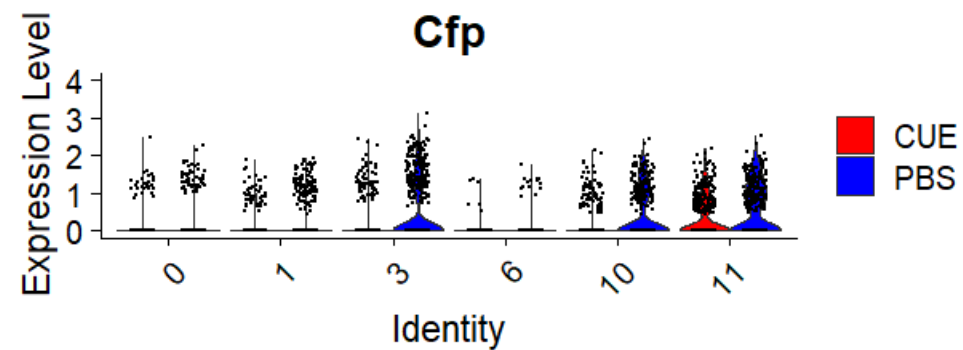
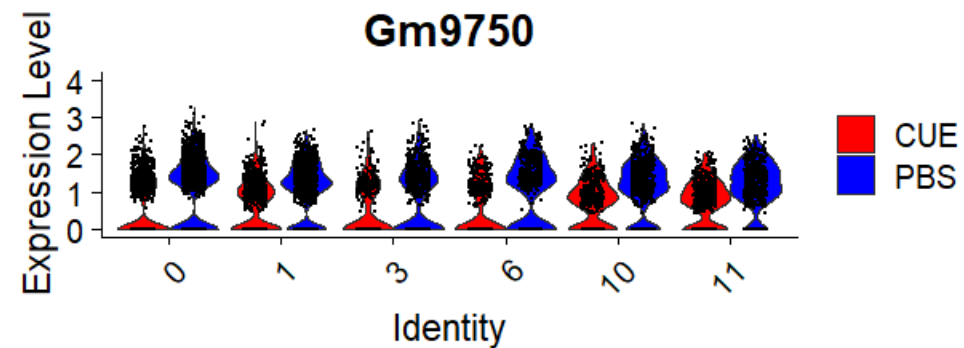
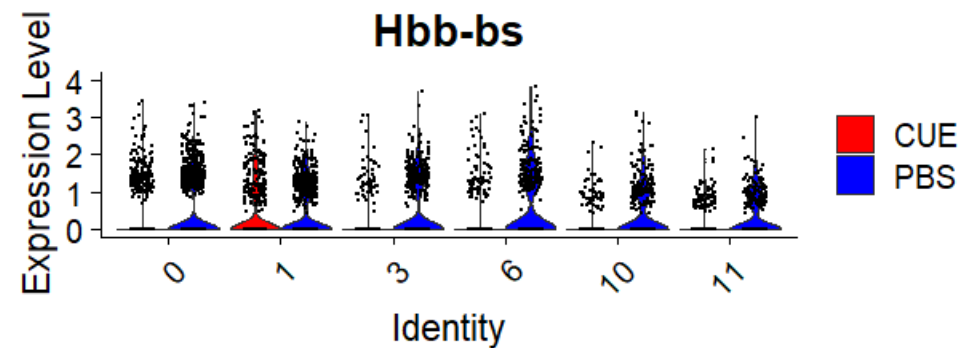
B Cell Differential Expression Analysis

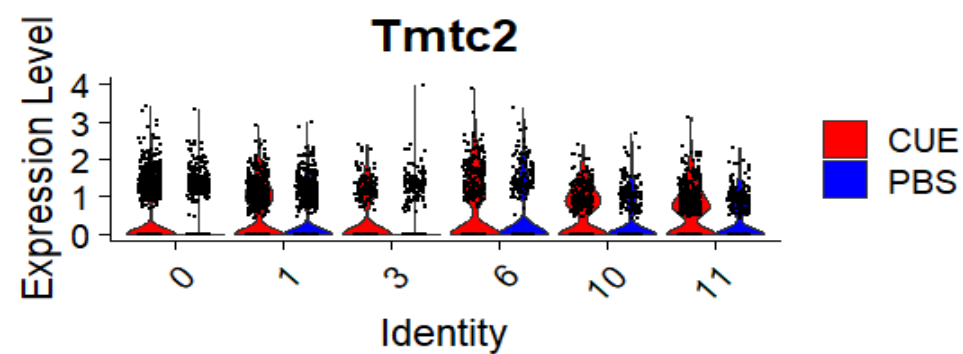
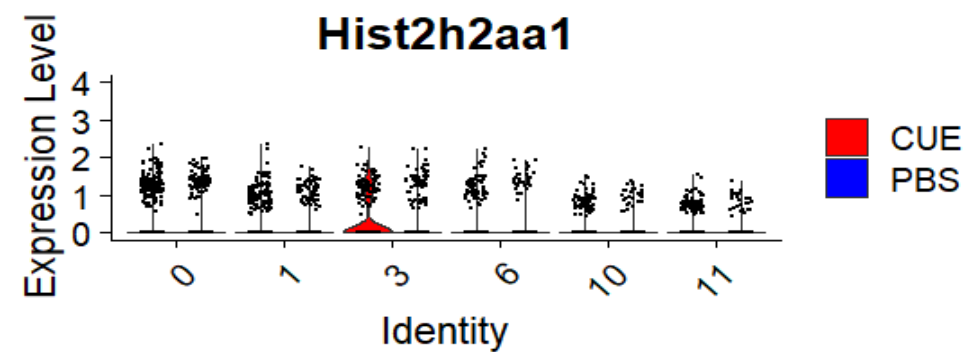
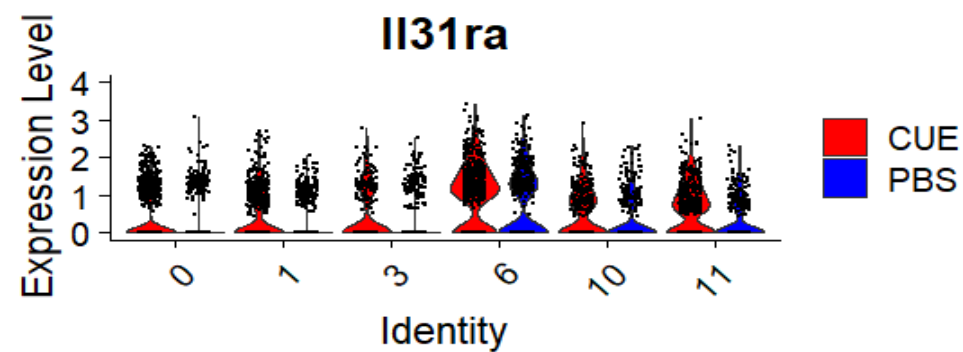
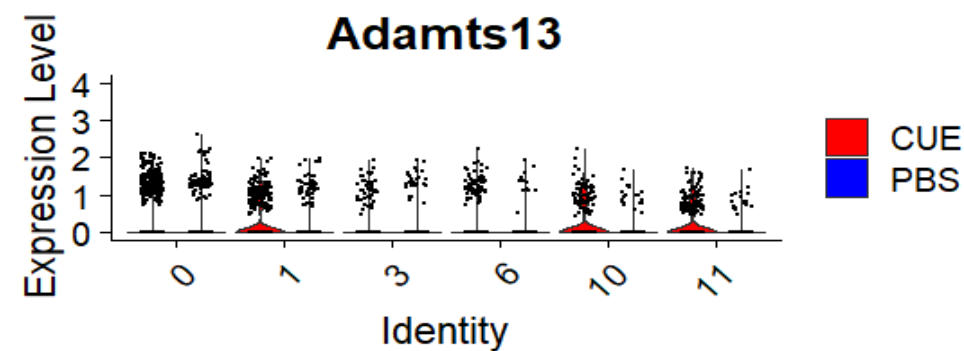
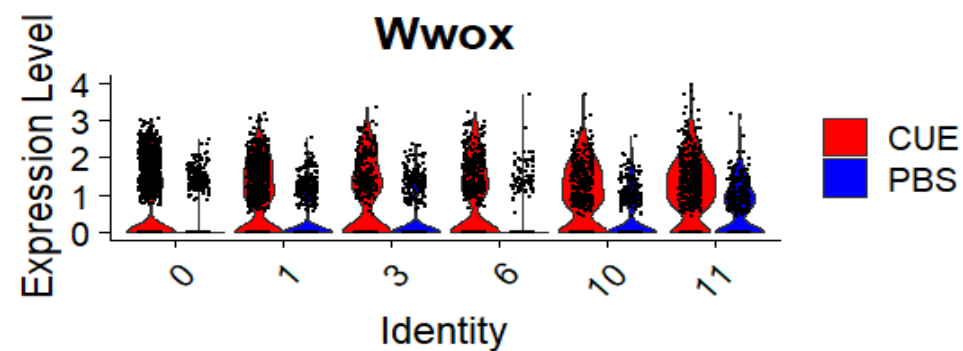
EnhancedVolcano



total = 52 variables







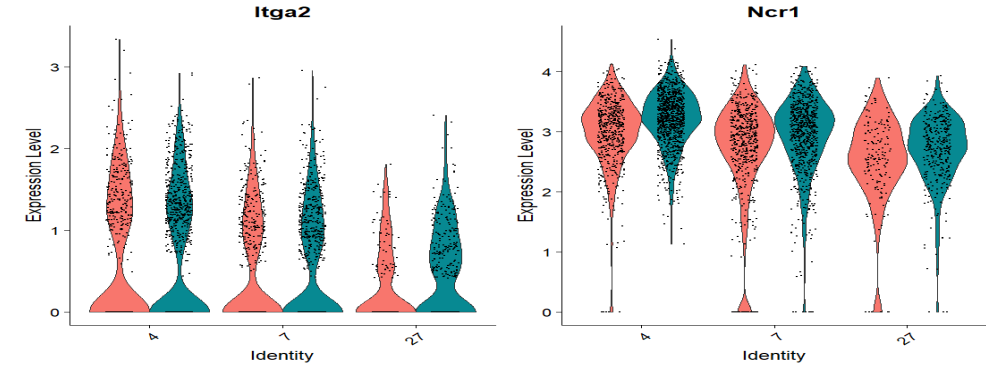
NK Cells – DE Analysis

PBS Markers

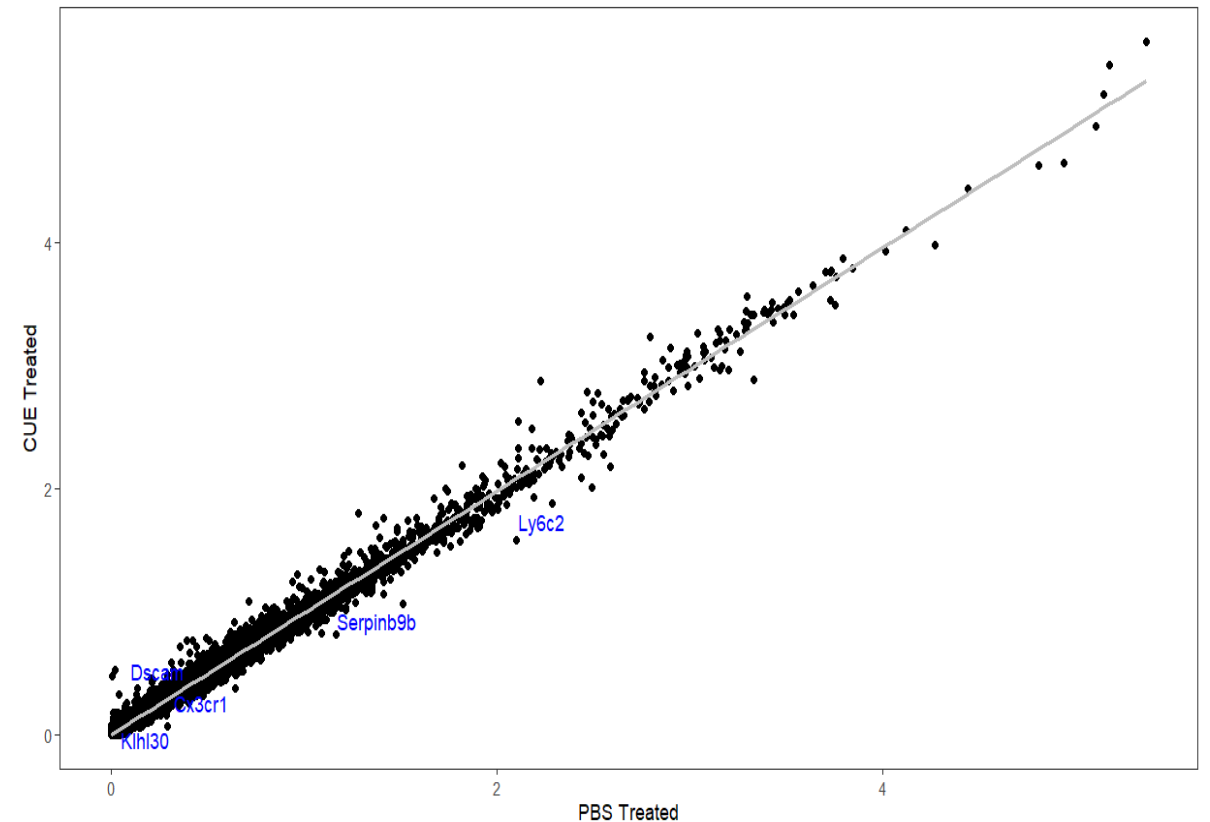
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Cx3cr1	2.215586e-07	0.9297317	0.115	0.060	4.658935e-03
Steap3	1.575695e-08	0.9255460	0.125	0.062	3.313371e-04
Serpina9b	4.336696e-25	0.8914593	0.476	0.289	9.119204e-21
Klhl30	4.361963e-07	0.8852655	0.116	0.061	9.172335e-03
Ly6c2	1.591162e-12	0.8792023	0.350	0.240	3.345895e-08

CUE Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Tcrg-C4	2.112354e-19	-1.8326617	0.042	0.125	4.441857e-15
Serpina3g	1.595519e-15	-1.8156590	0.046	0.120	3.355057e-11
Dtx1	2.539092e-13	-1.7921157	0.047	0.114	5.339202e-09
Dscam	1.292123e-17	-1.6610786	0.051	0.134	2.717076e-13
Podnl1	4.868432e-18	-1.4993302	0.078	0.174	1.023734e-13



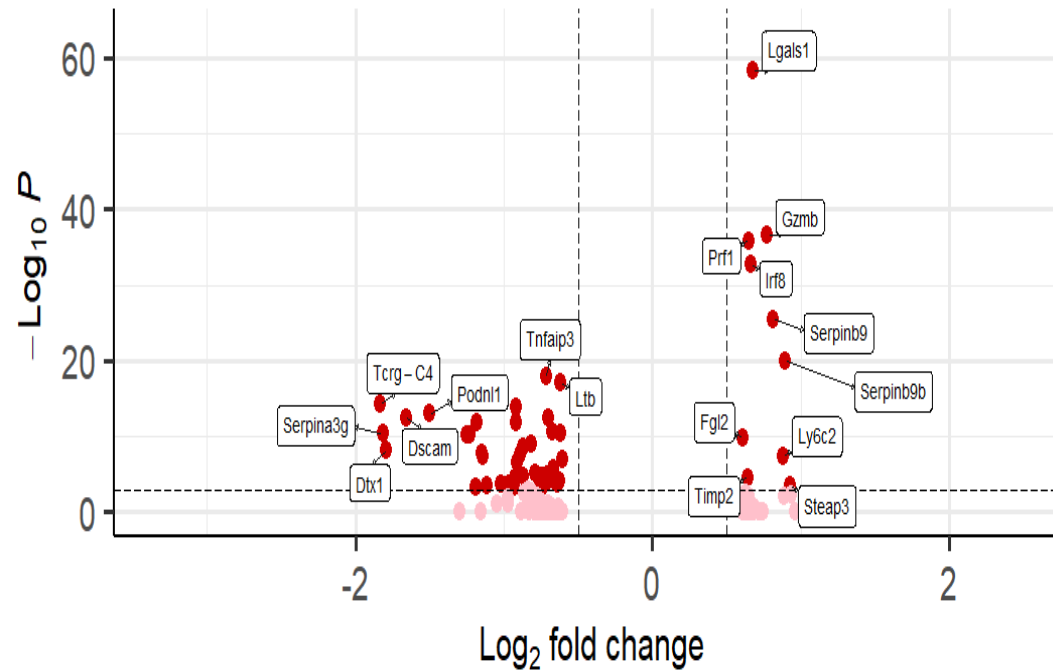
NK cell DE Analysis



Plots of differentially expressed genes

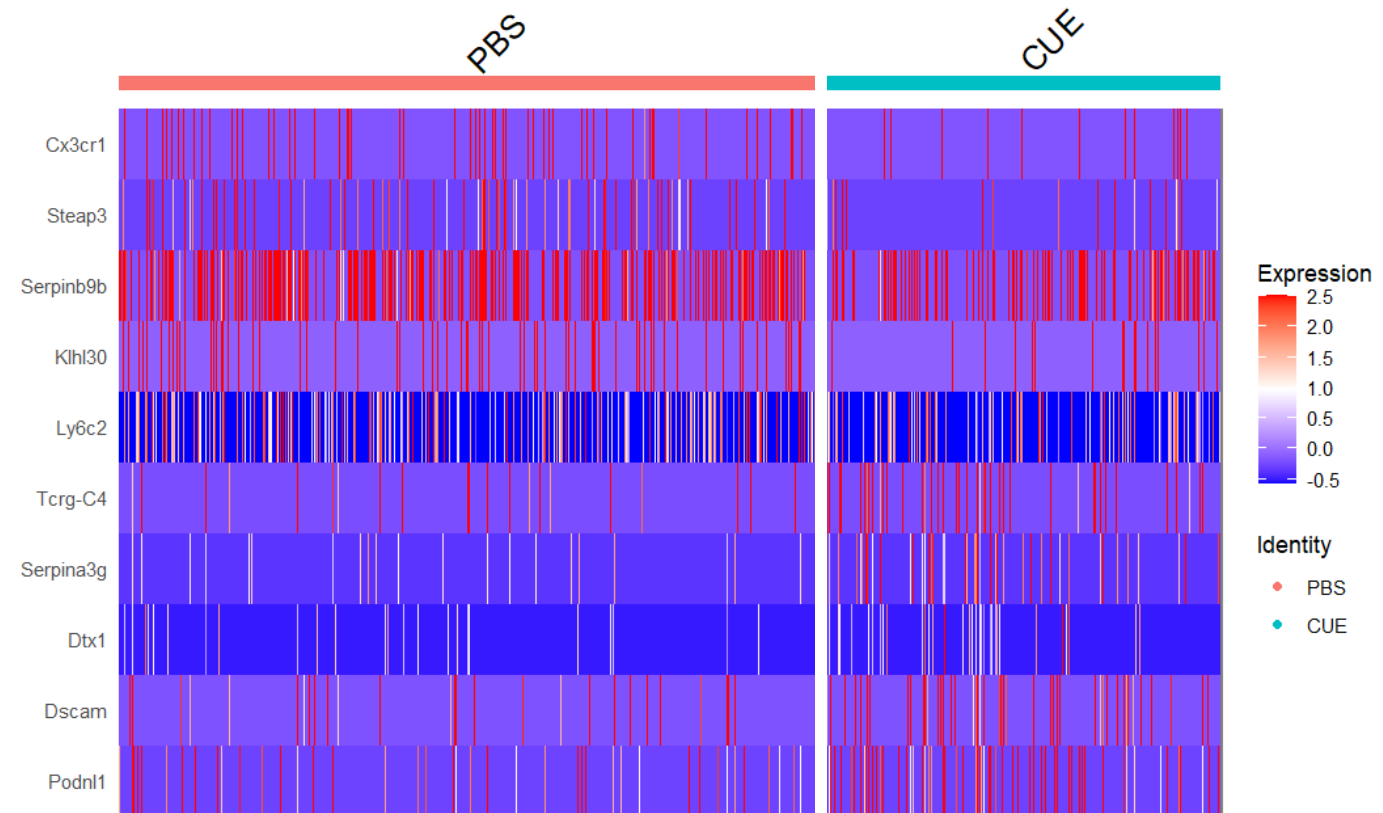
NK Cell Differential Expression Analysis

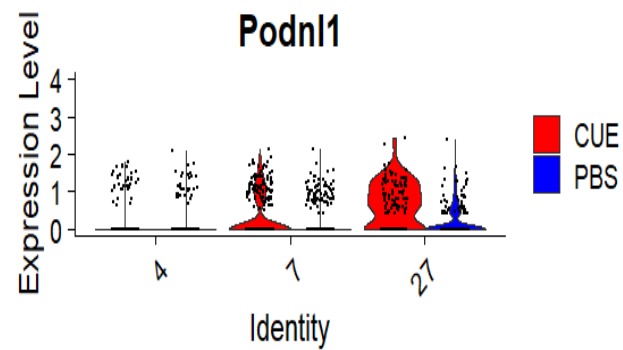
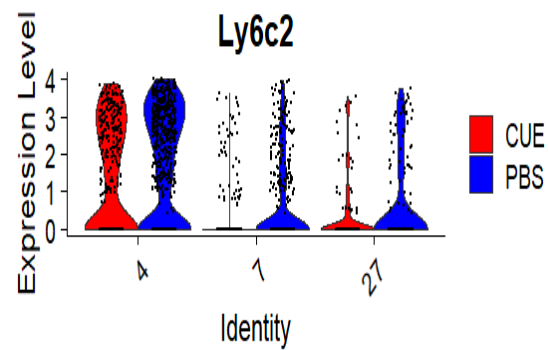
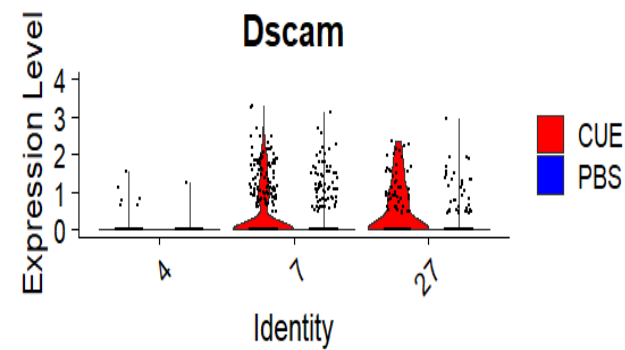
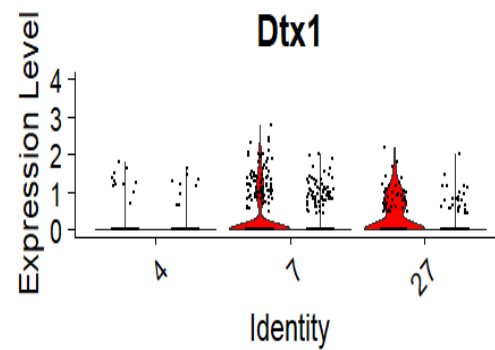
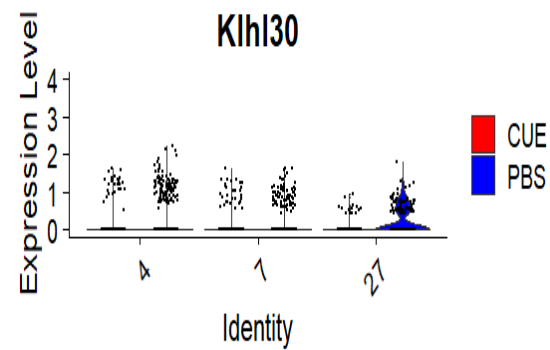
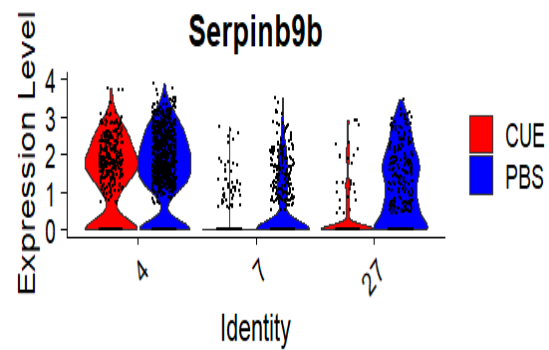
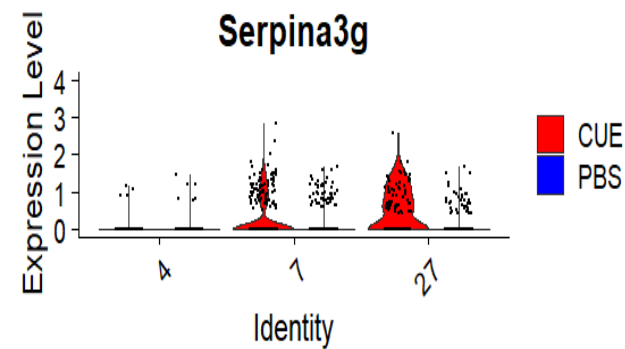
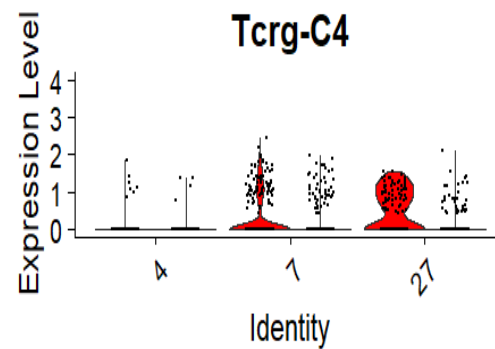
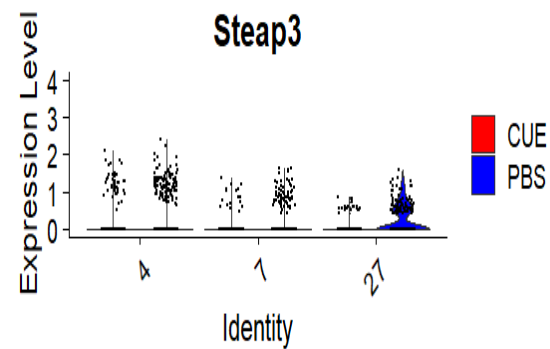
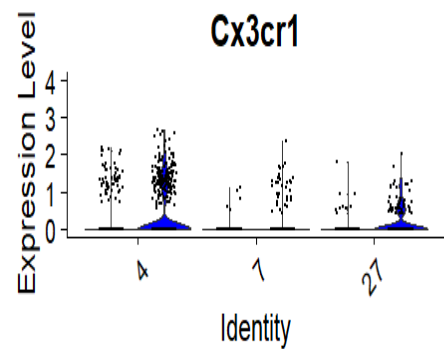
EnhancedVolcano



● Log_2 FC ● p-value and log_2 FC

total = 126 variables





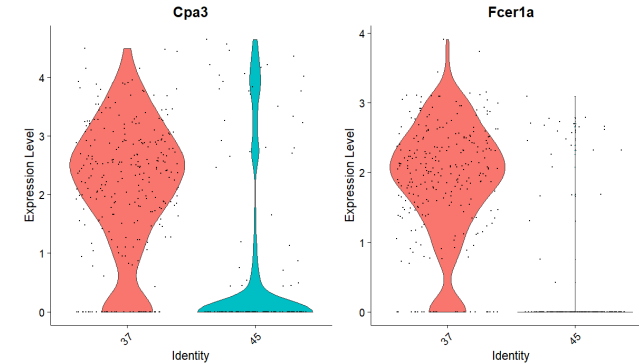
Mast Cells – DE Analysis

PBS Markers

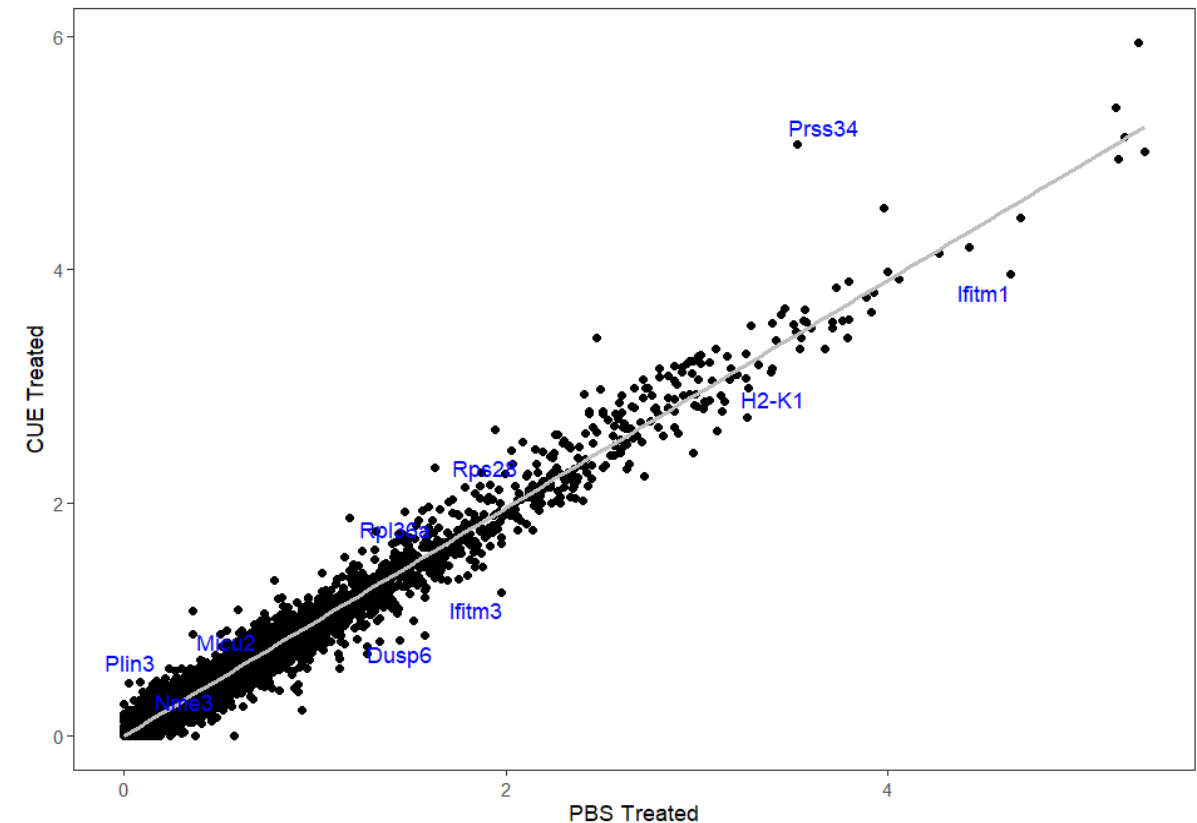
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ifitm1	1.830075e-09	1.0020547	0.964	0.920	3.848282e-05
Dusp6	5.149281e-08	1.4913326	0.744	0.589	1.082791e-03
Ifitm3	1.607946e-07	1.3508286	0.762	0.580	3.381190e-03
H2-K1	1.151582e-06	0.7999928	0.982	1.000	2.421546e-02

CUE Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Prss34	1.463182e-12	-2.2600767	0.304	0.705	3.076778e-08
Rps28	1.106982e-11	-0.6880758	0.899	0.973	2.327763e-07
Plin3	2.106122e-06	-1.7021387	0.131	0.375	4.428753e-02
Micu2	2.642932e-06	-0.9144448	0.232	0.536	5.557557e-02
Rpl36a	2.887380e-06	-0.6535398	0.708	0.929	6.071583e-02
Nme3	4.446521e-06	-1.4630118	0.131	0.375	9.350145e-02



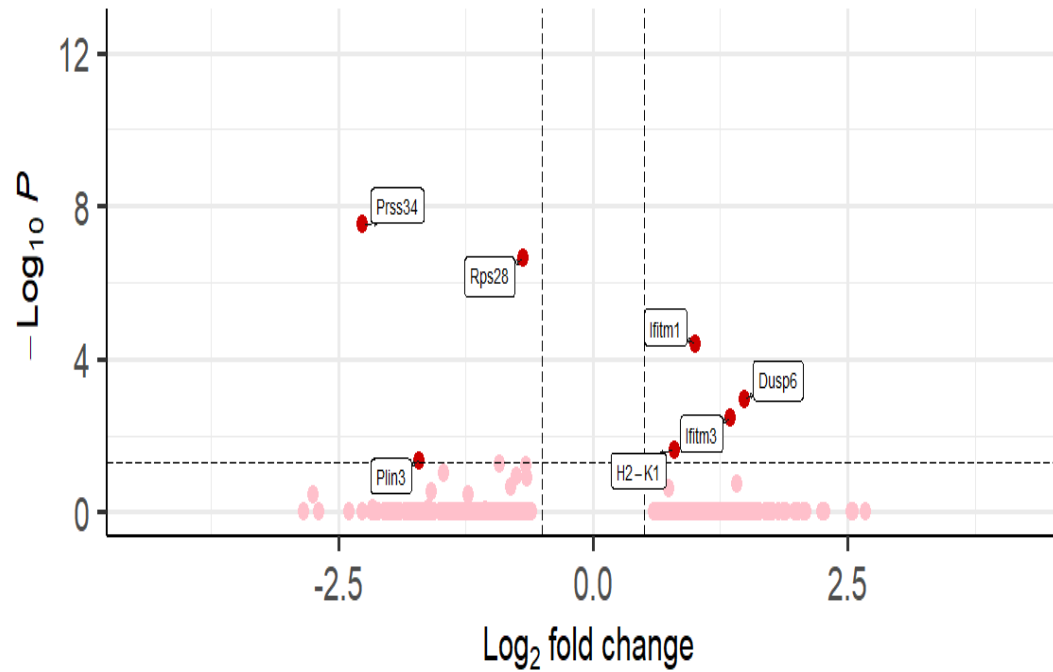
Mast cell DE Analysis



Plots of differentially expressed genes

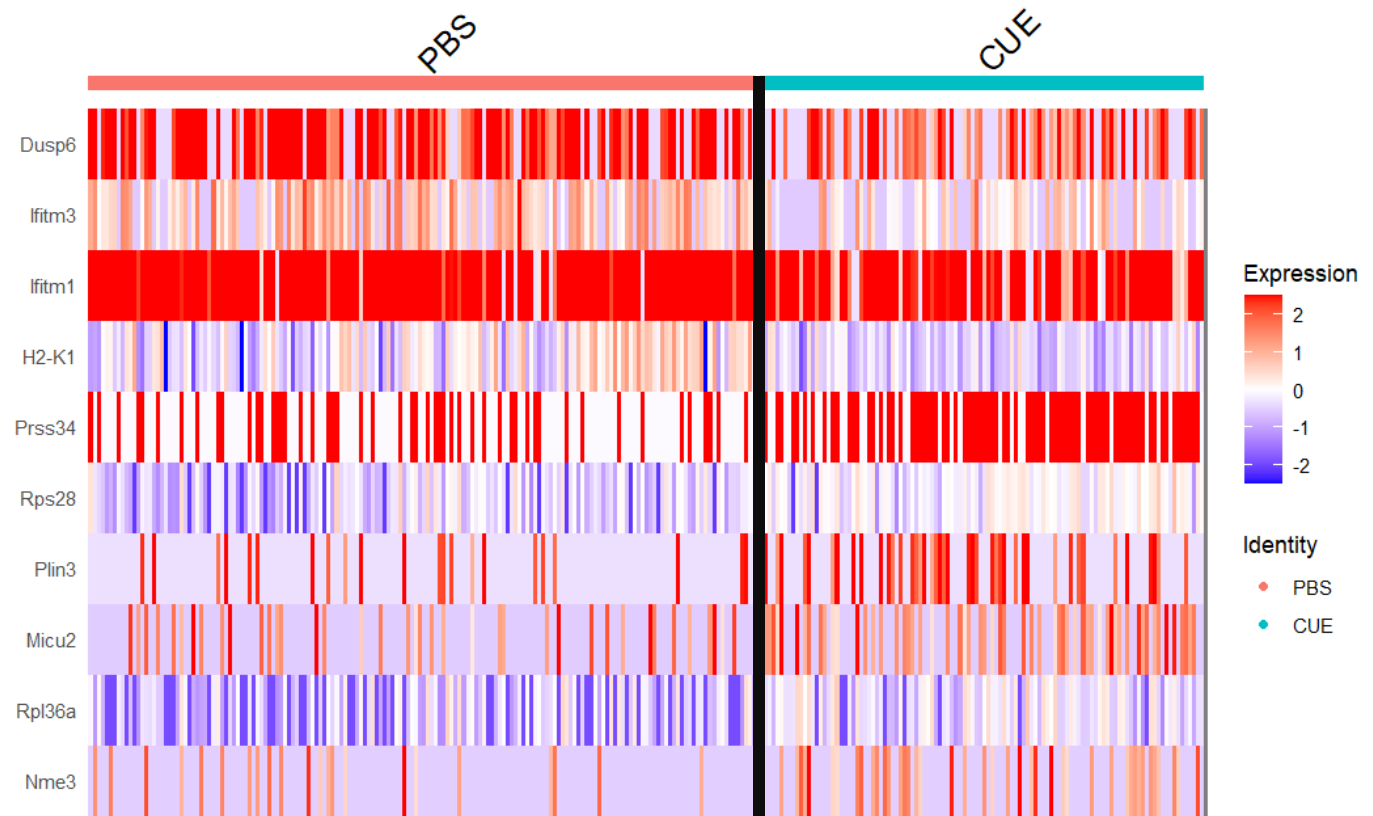
Mast Cell Differential Expression Analysis

EnhancedVolcano

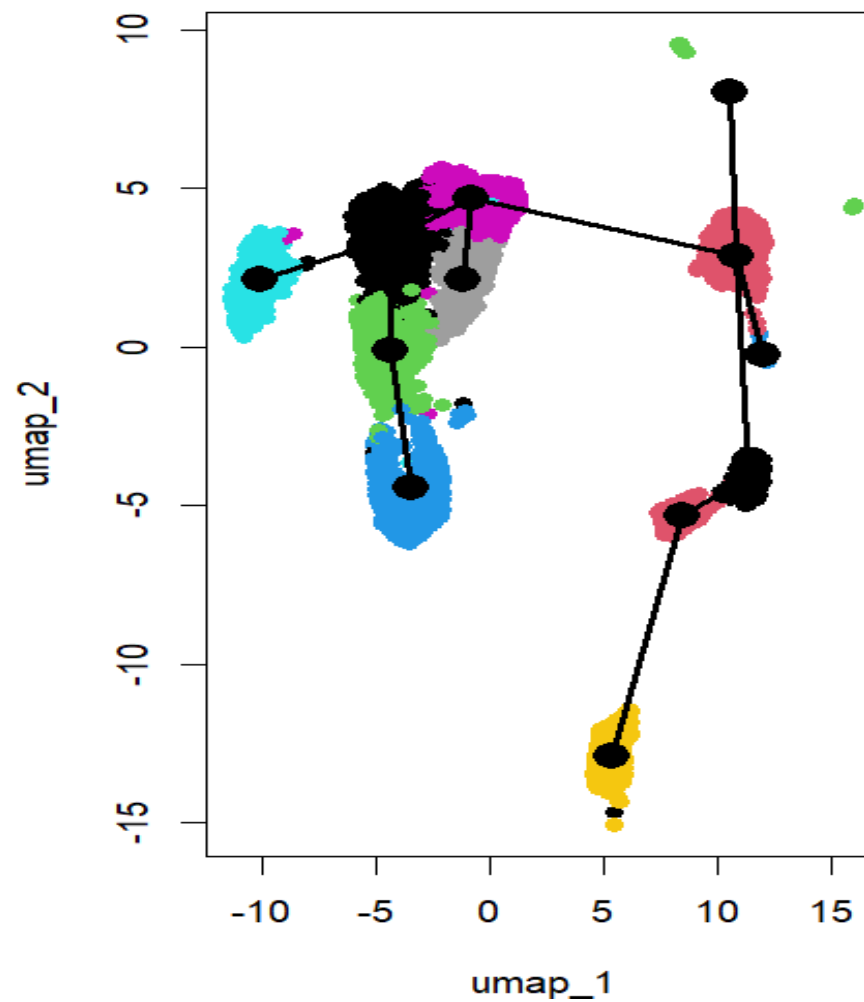
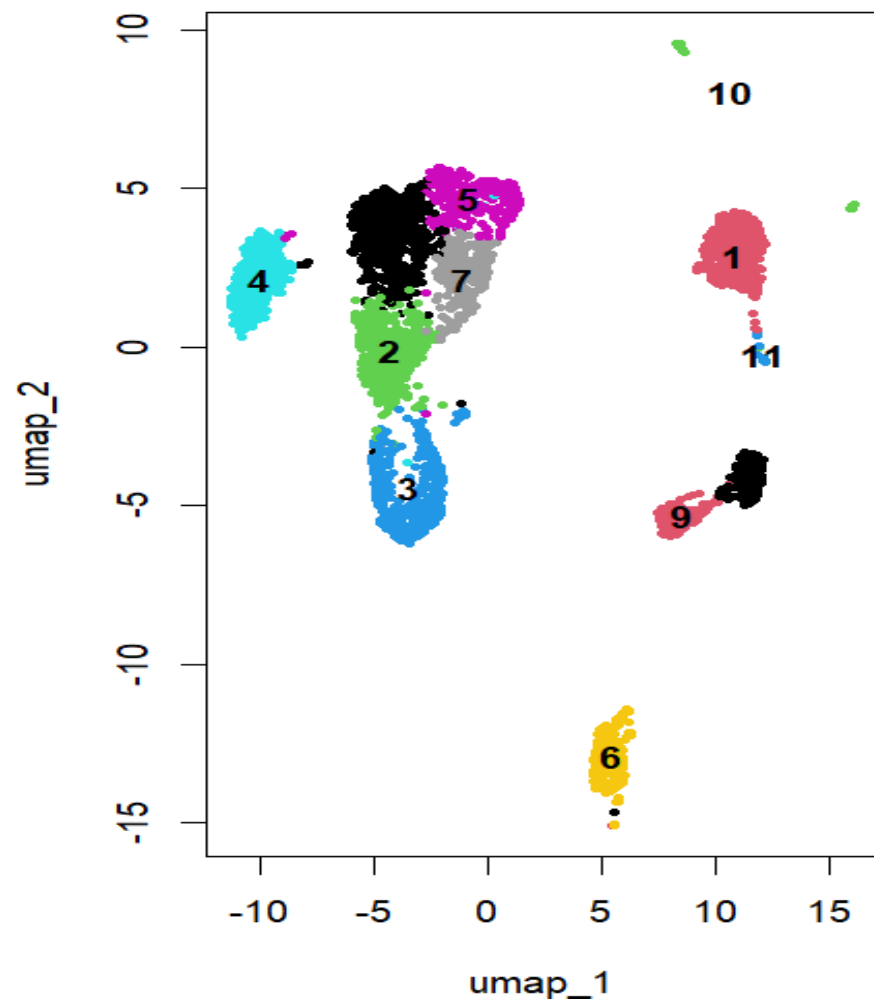


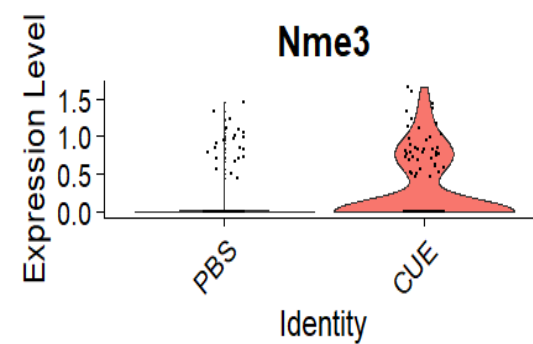
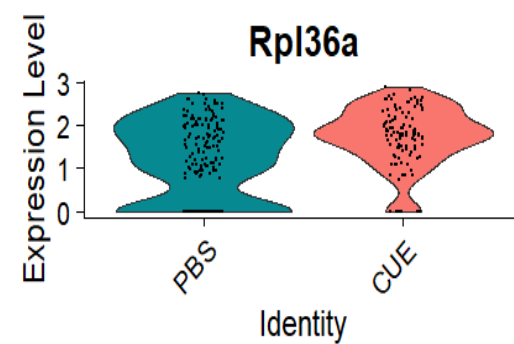
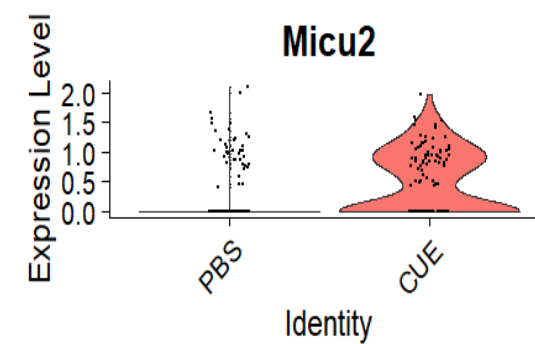
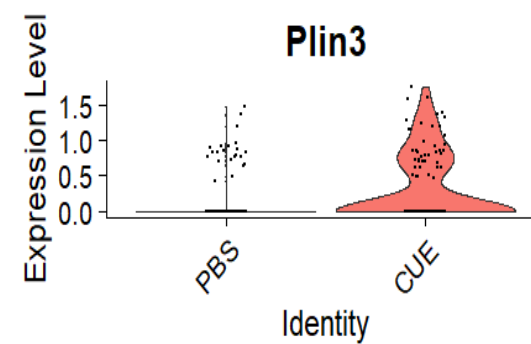
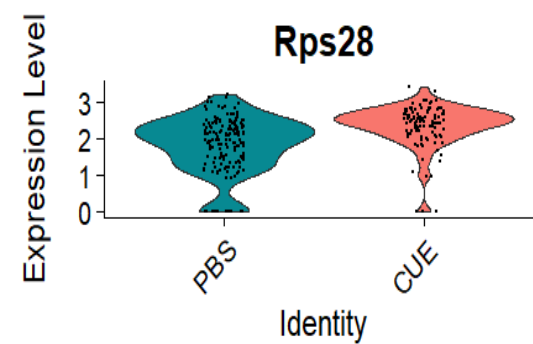
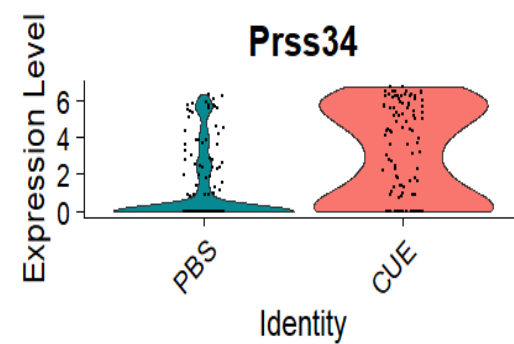
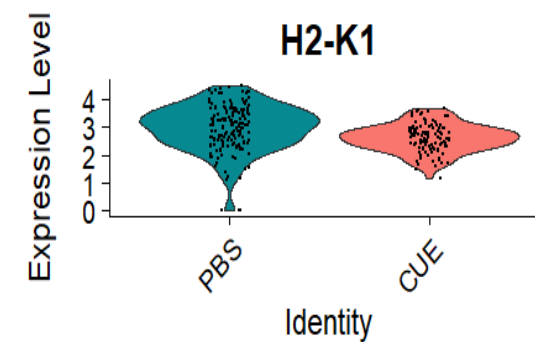
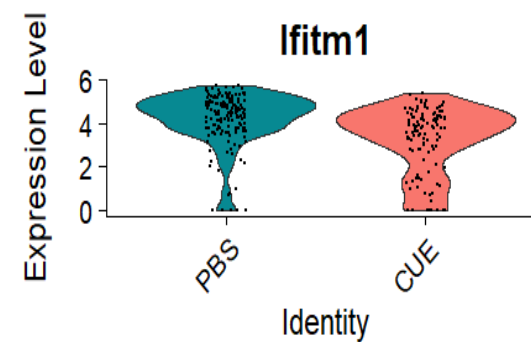
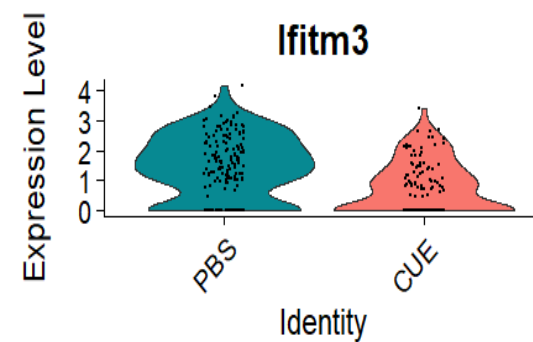
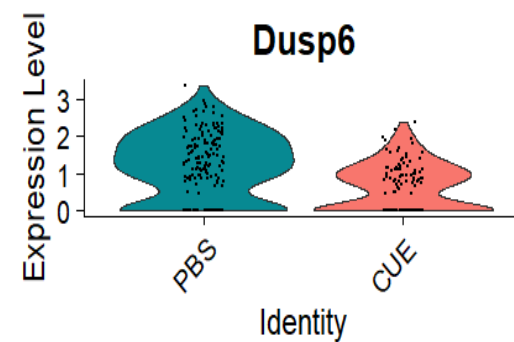
● Log₂ FC ● p-value and log₂ FC

total = 1169 variables



Trajectory inference





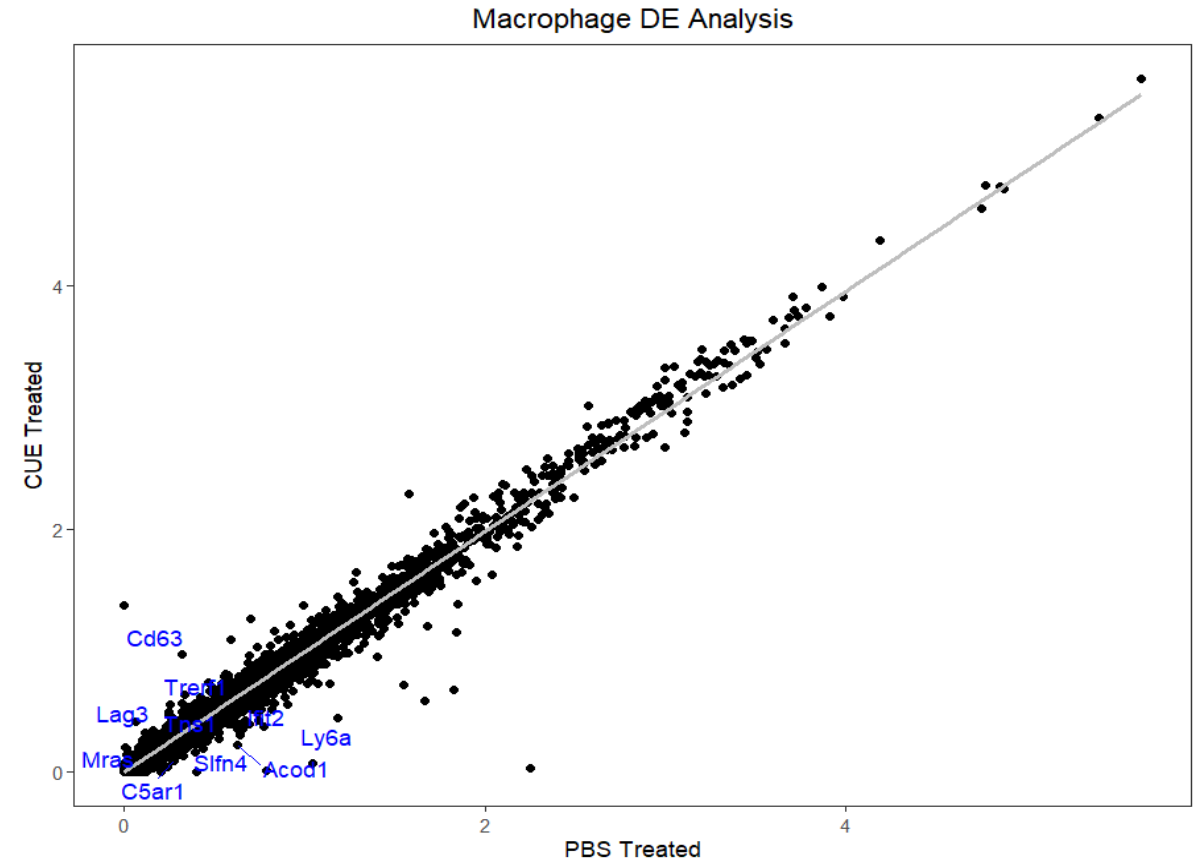
Macrophages – DE Analysis

PBS Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ly6a	1.5255081e-13	2.01538	0.276	0.111	3.2078392e-09
Acod1	1.149032e-06	1.759315	0.175	0.083	0.024162
C5ar1	1.250998e-07	1.64903	0.191	0.091	0.002631
Slfn4	8.970409e-07	1.414479	0.189	0.092	0.018863
Ifit2	1.104227e-10	1.329998	0.35	0.192	2.321970e-06

CUE Markers

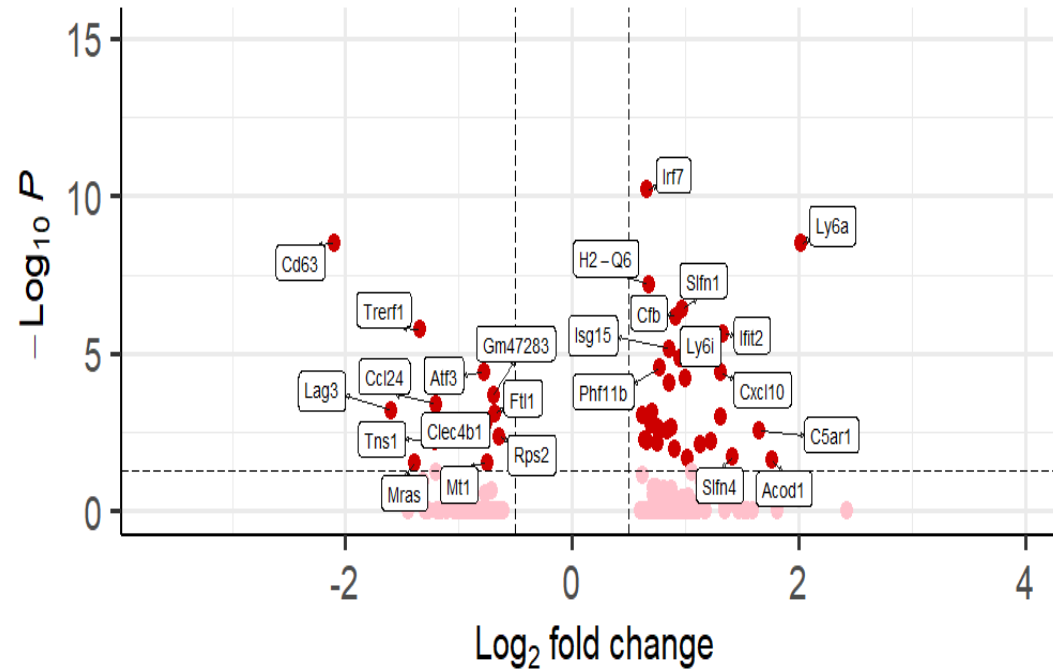
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Tns1	3.00411459e-07	-1.21298	0.12	0.215	0.006317
Trerf1	8.30161938e-11	-1.34054	0.182	0.323	1.74566452e-06
Mras	1.37117861e-06	-1.39138	0.063	0.138	0.028833
Lag3	3.037773e-08	-1.59791	0.058	0.143	0.000639
Cd63	1.47943521e-13	-2.09819	0.098	0.234	3.11095637e-09



Plots of differentially expressed genes

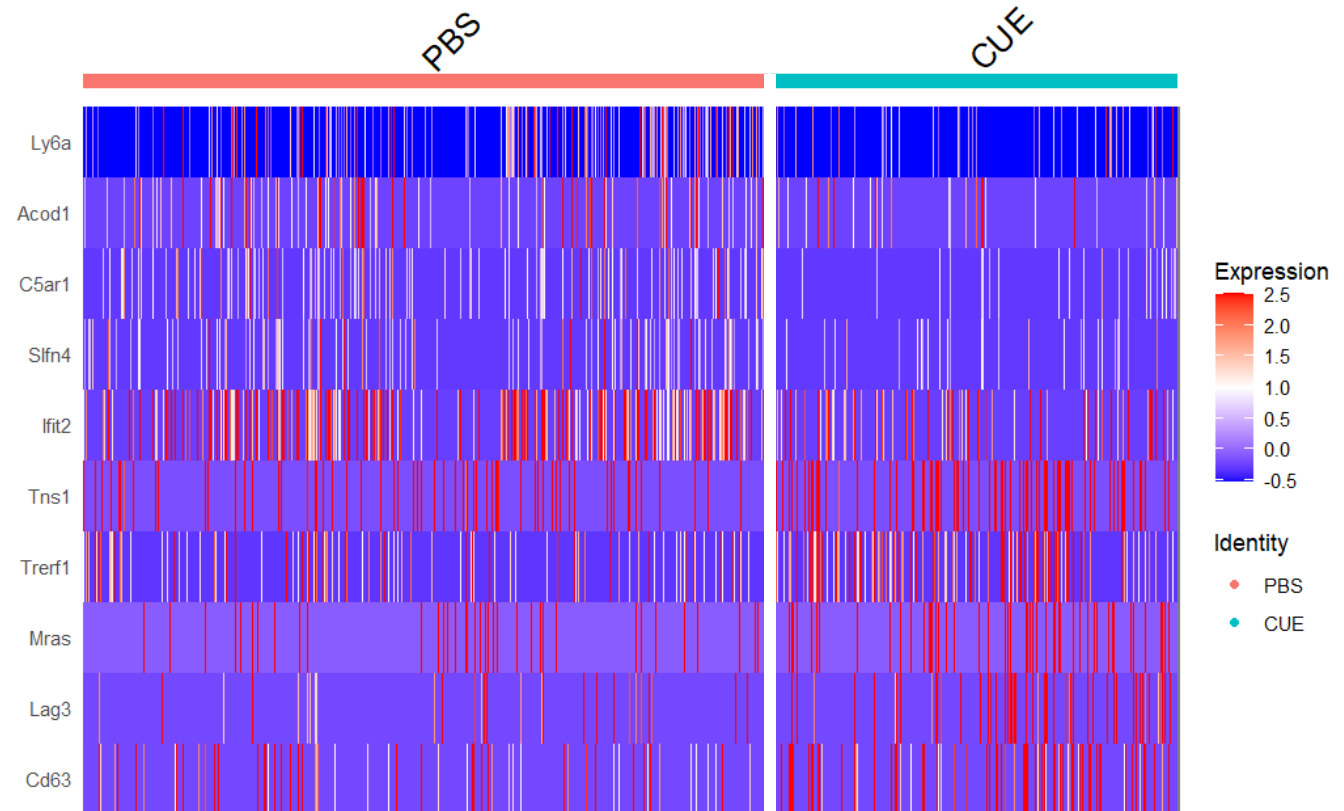
Macrophage Differential Expression Analysis

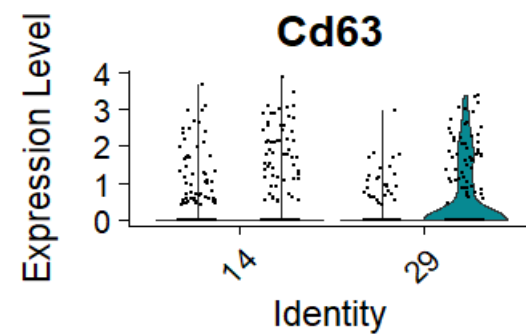
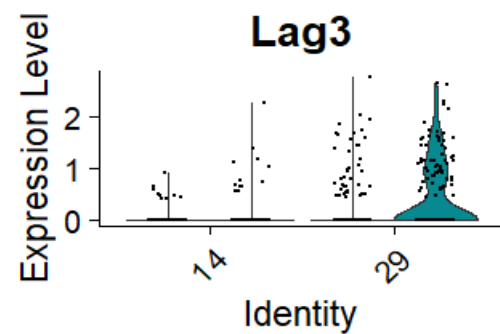
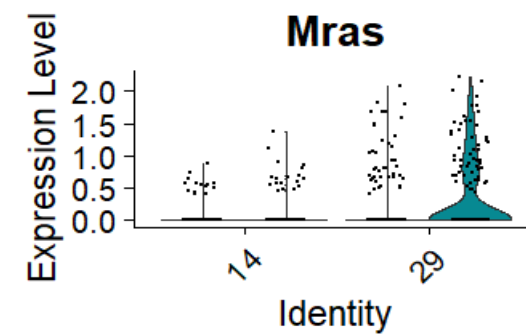
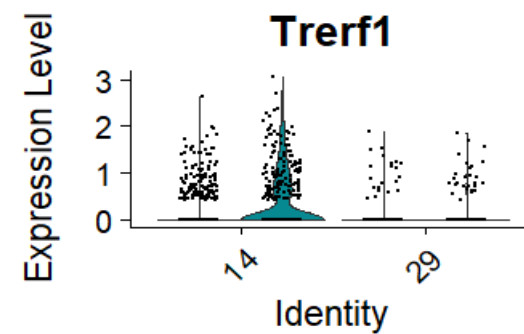
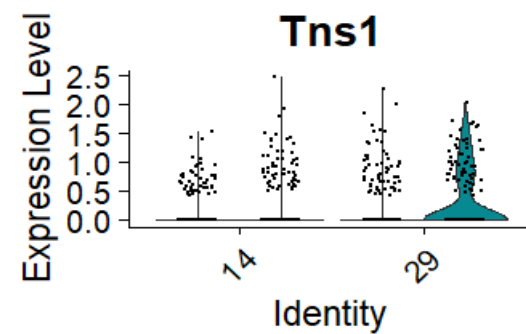
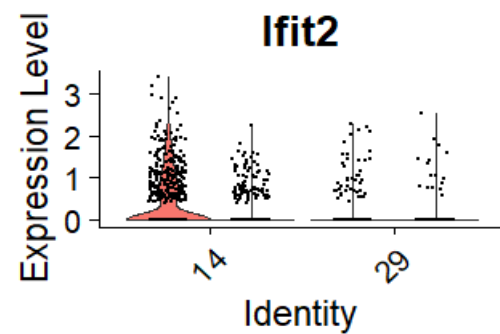
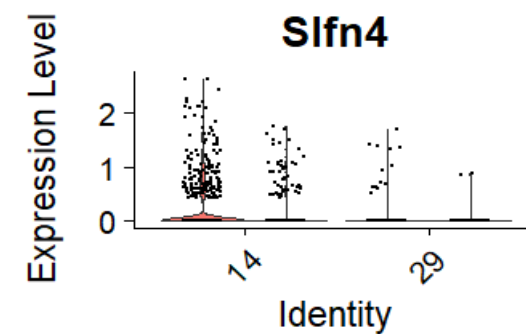
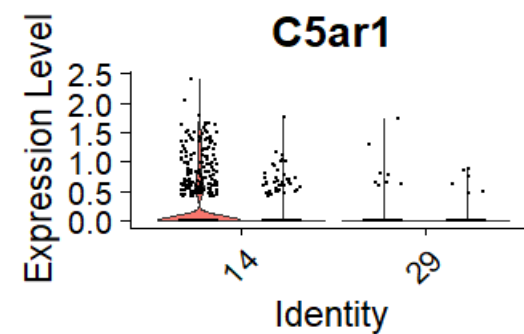
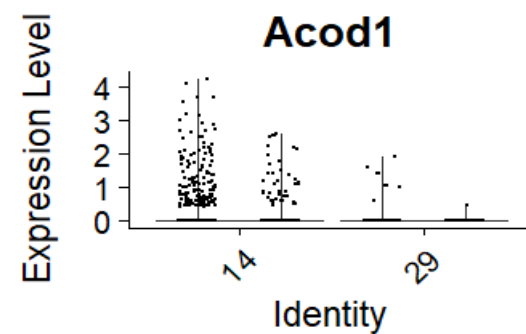
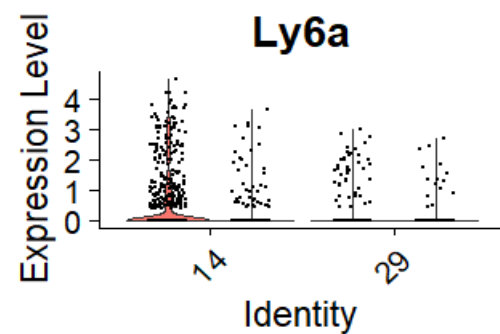
EnhancedVolcano



● Log_2 FC ● p-value and log_2 FC

total = 338 variables





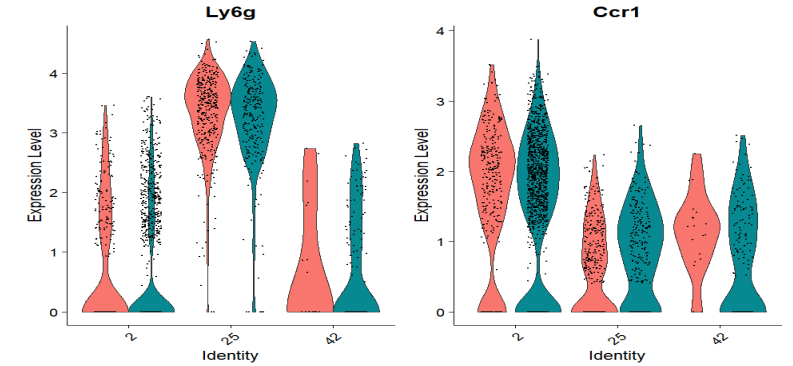
Neutrophils – DE Analysis

PBS Markers

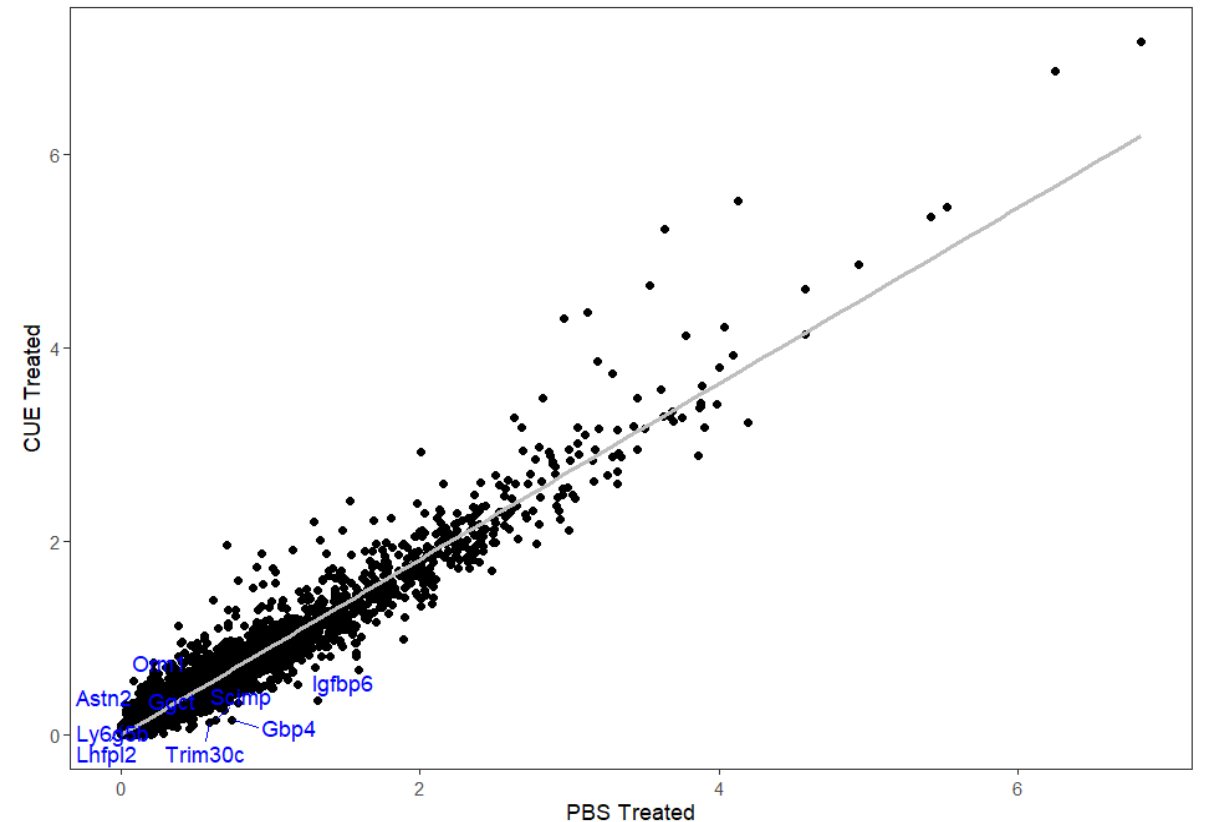
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Gbp4	2.519343e-12	2.661560	0.150	0.048	5.297674e-08
Igfbp6	1.476391e-36	2.632104	0.345	0.091	3.104555e-32
Trim30c	4.930909e-16	2.537589	0.156	0.036	1.036872e-11
Scimp	2.730560e-14	2.368014	0.157	0.043	5.741822e-10
Ggct	1.258806e-07	2.062208	0.101	0.036	2.647016e-03

CUE Markers

Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Orm1	3.716955e-47	-3.095475	0.047	0.241	7.816013e-43
Tmem45a2	4.432013e-37	-3.029938	0.029	0.175	9.319637e-33
Astn2	2.217602e-29	-2.822520	0.016	0.123	4.663173e-25
Lhfp12	1.449393e-20	-2.614300	0.020	0.106	3.047783e-16
Ly6g5b	2.542623e-24	-2.612564	0.024	0.127	5.346628e-20



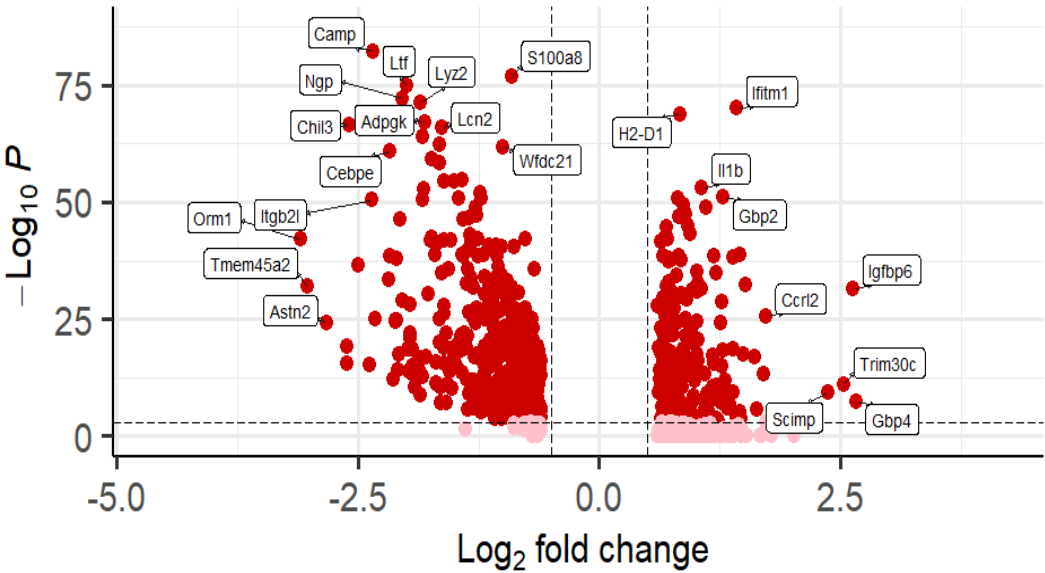
Neutrophil DE Analysis



Plots of differentially expressed genes

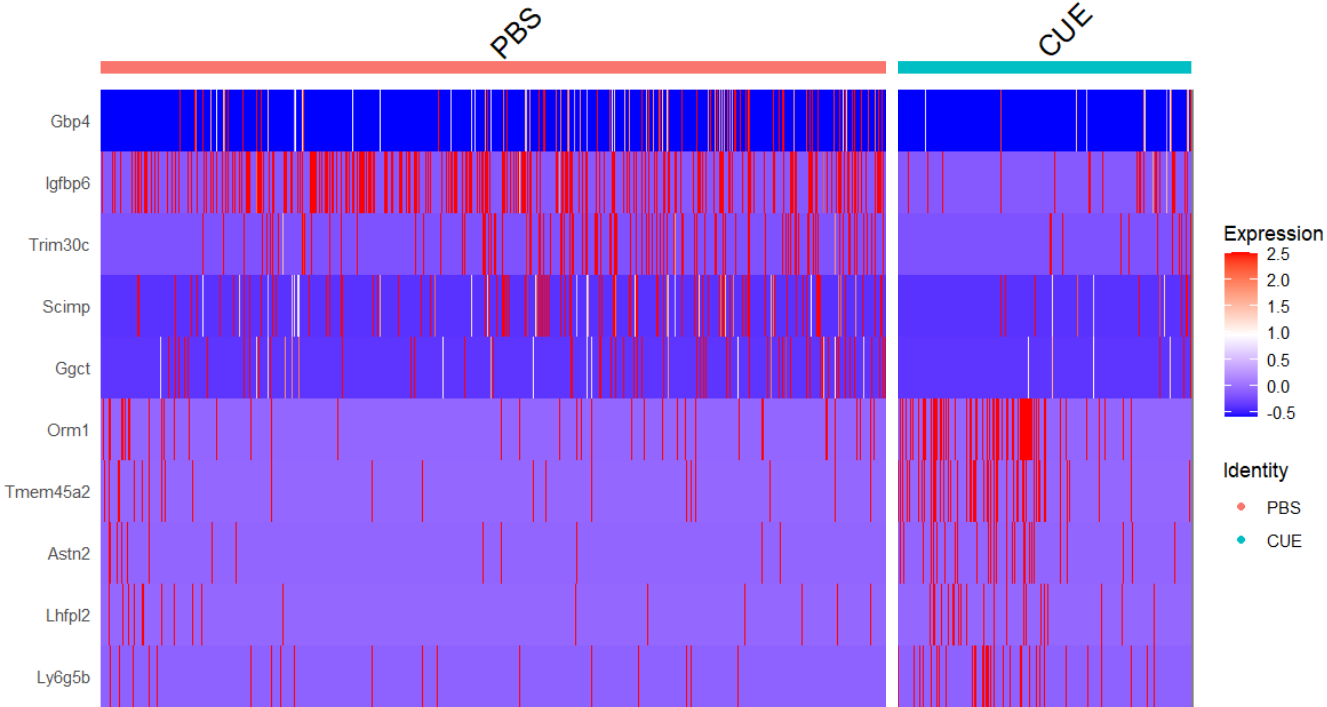
Neutrophil Differential Expression Analysis

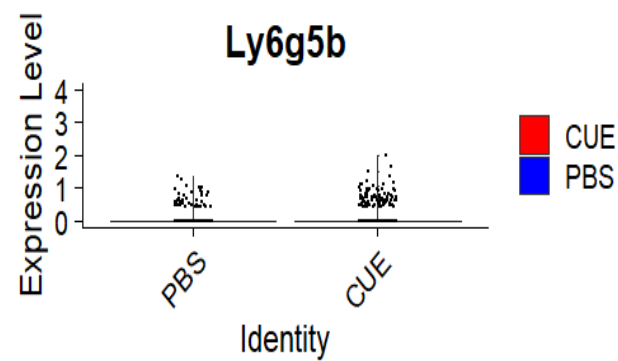
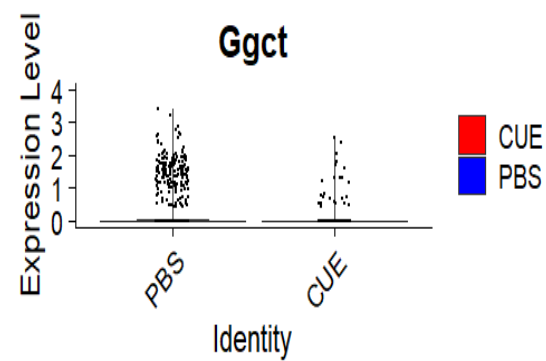
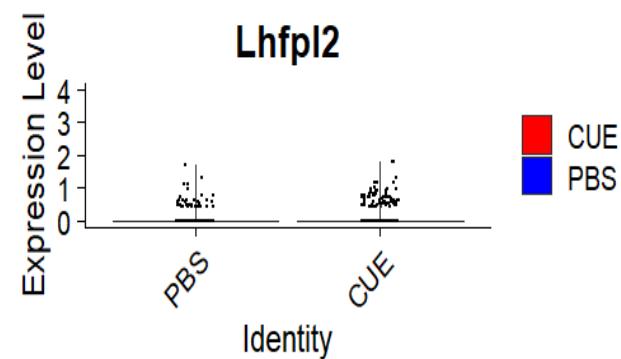
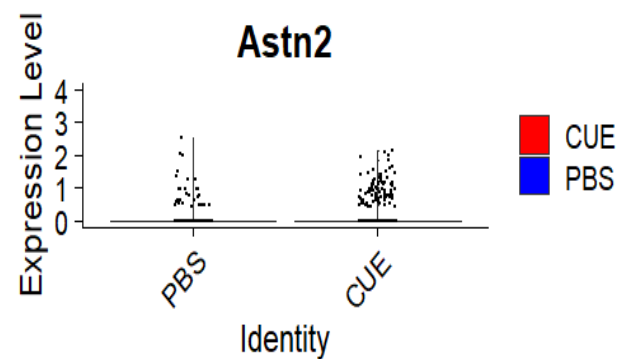
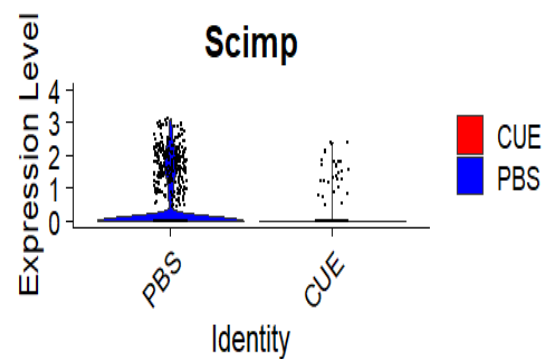
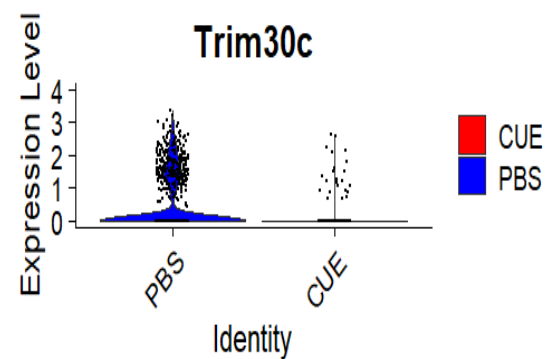
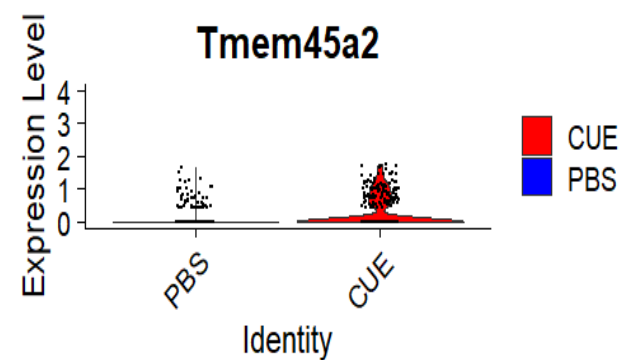
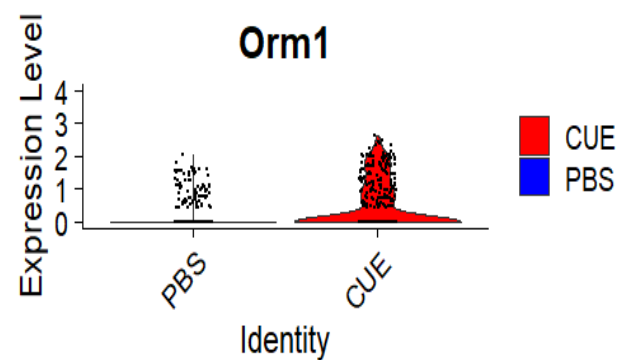
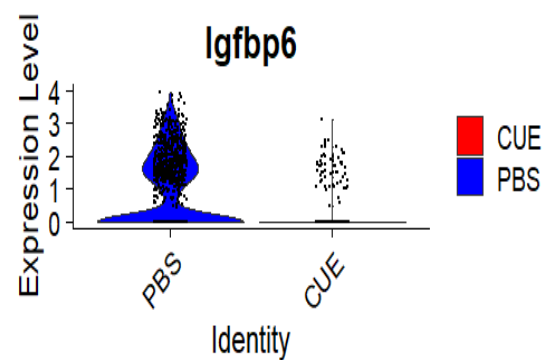
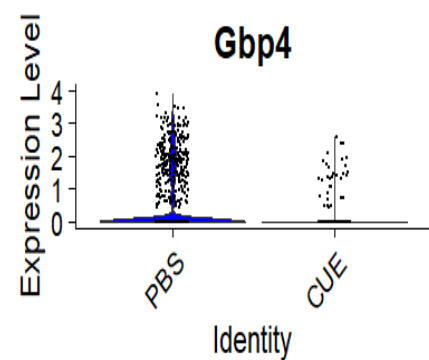
EnhancedVolcano



● Log_2 FC ● p-value and Log_2 FC

total = 1094 variables





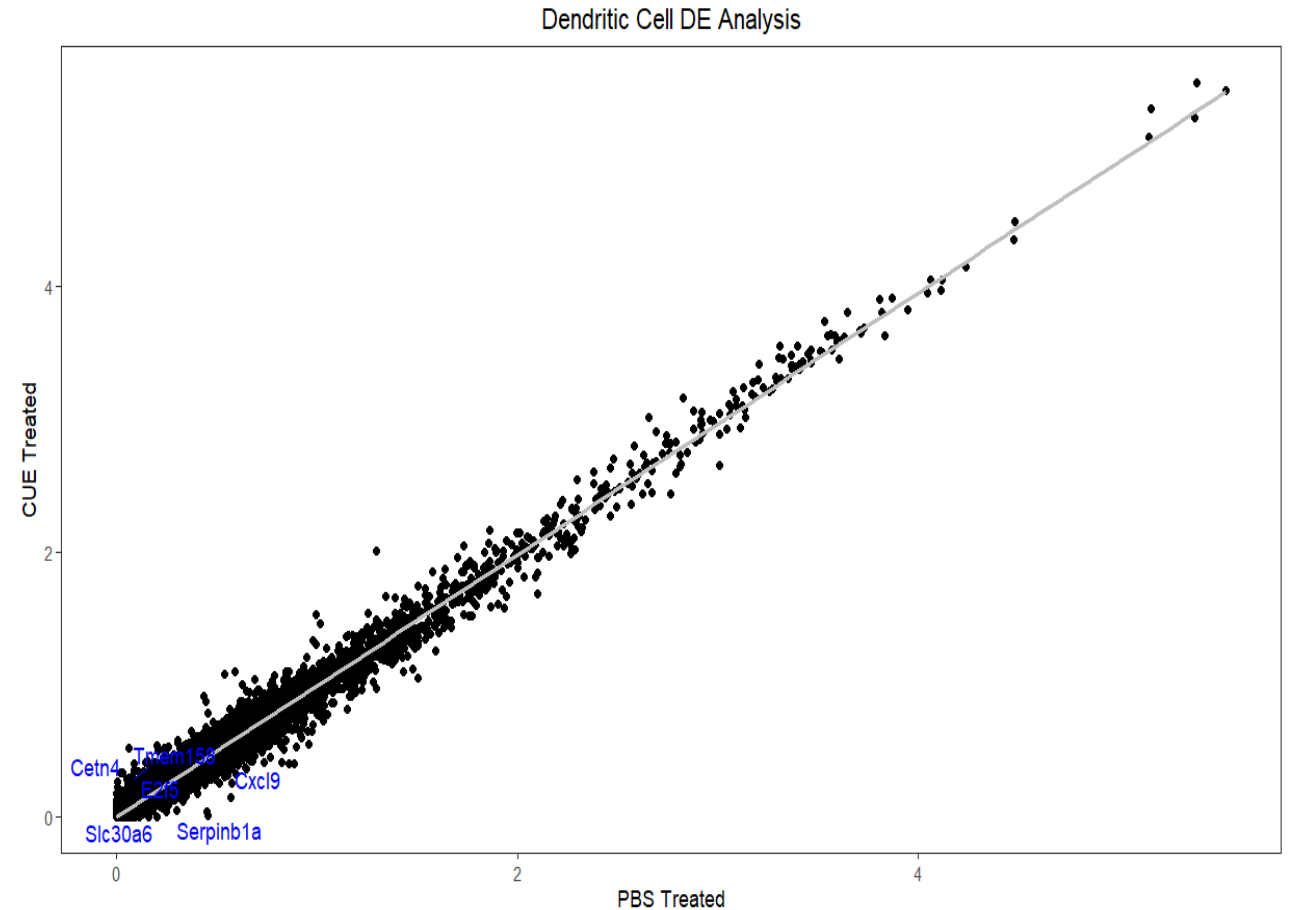
Dendritic Cells – DE Analysis

PBS Markers

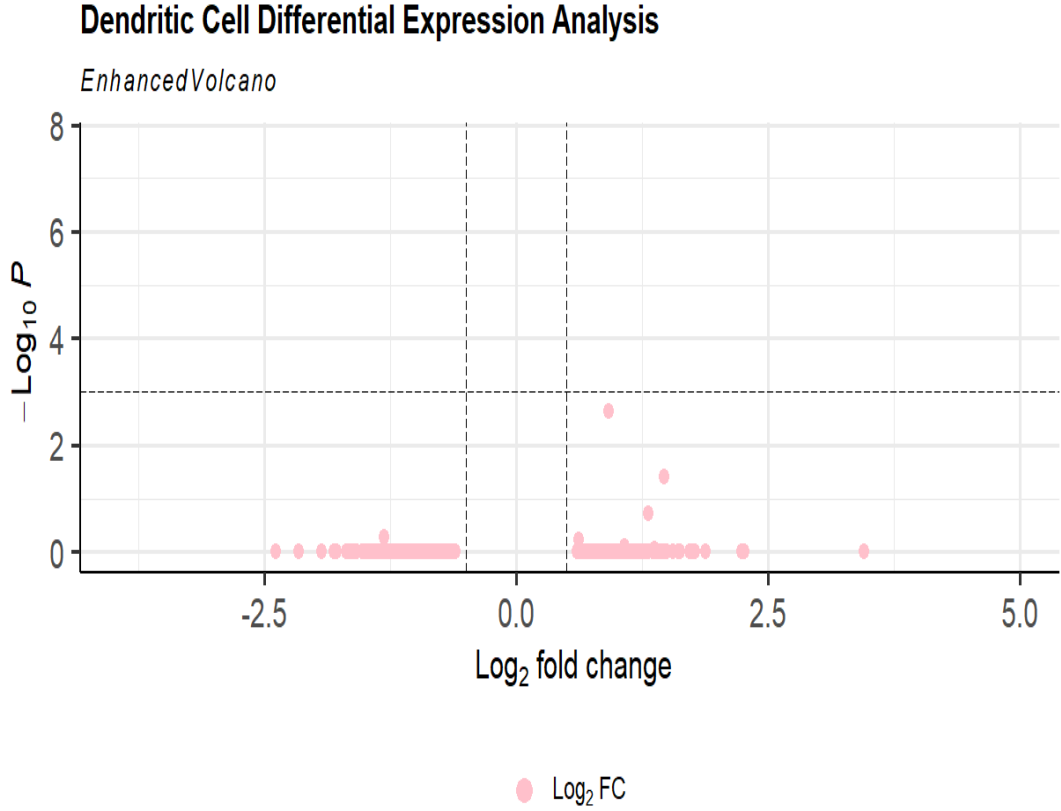
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Serpinb1a	3.286064e-03	3.4503364	0.112	0.029	1.00000000
Slc30a6	1.140005e-03	2.2681447	0.126	0.029	1.00000000
Cxcl9	3.504167e-03	2.2386678	0.147	0.050	1.00000000
Nup188	3.662870e-03	1.8806996	0.133	0.043	1.00000000
Ifit2	4.744546e-02	1.7716046	0.133	0.071	1.00000000

CUE Markers

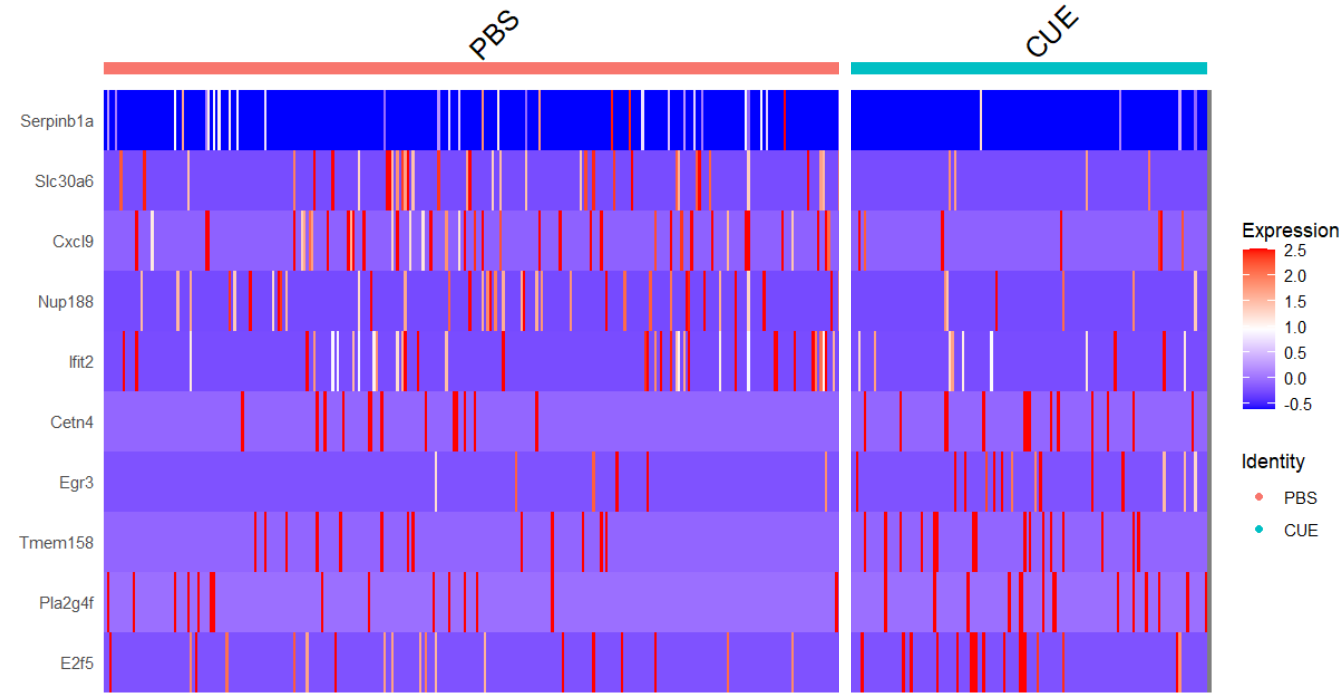
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Egr3	0.0002852447	-2.3852650	0.021	0.100	1.00000000
Cetn4	0.0288757349	-2.1636228	0.046	0.100	1.00000000
Tmem158	0.0071811975	-1.9356980	0.046	0.114	1.00000000
Pla2g4f	0.0324197921	-1.8070181	0.053	0.107	1.00000000
E2f5	0.0401445145	-1.7809758	0.067	0.121	1.00000000



Plots of differentially expressed genes



total = 1021 variables



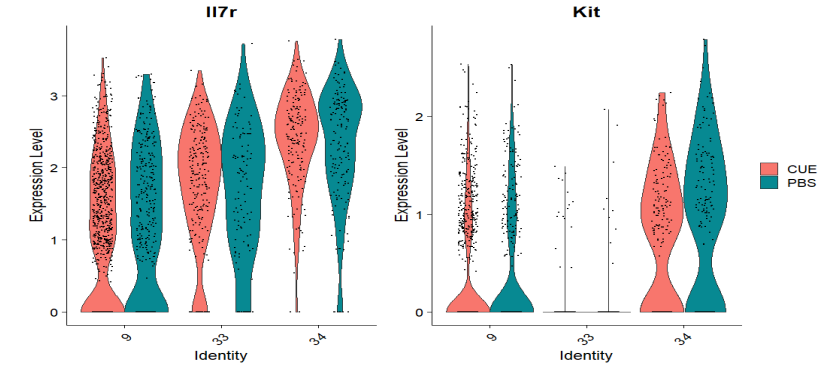
ILC – DE Analysis

PBS Markers

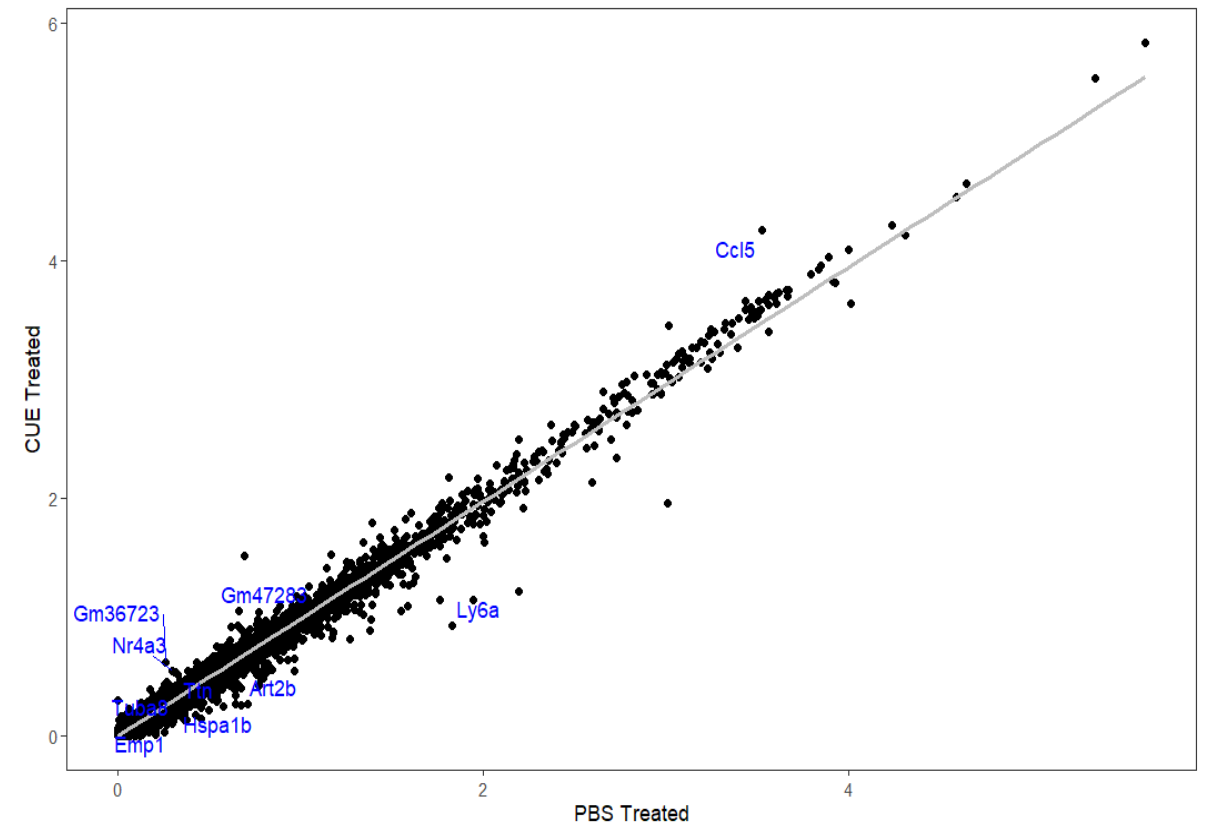
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Ly6a	1.662244e-10	1.7906676	0.225	0.119	3.495368e-06
Art2b	1.270850e-14	1.7788018	0.170	0.060	2.672343e-10
Hspa1b	2.616422e-16	1.7325270	0.147	0.042	5.501811e-12
Emp1	1.474503e-06	1.7168288	0.112	0.053	3.100586e-02
Tuba8	2.551521e-08	1.6624243	0.125	0.054	5.365338e-04

CUE Markers

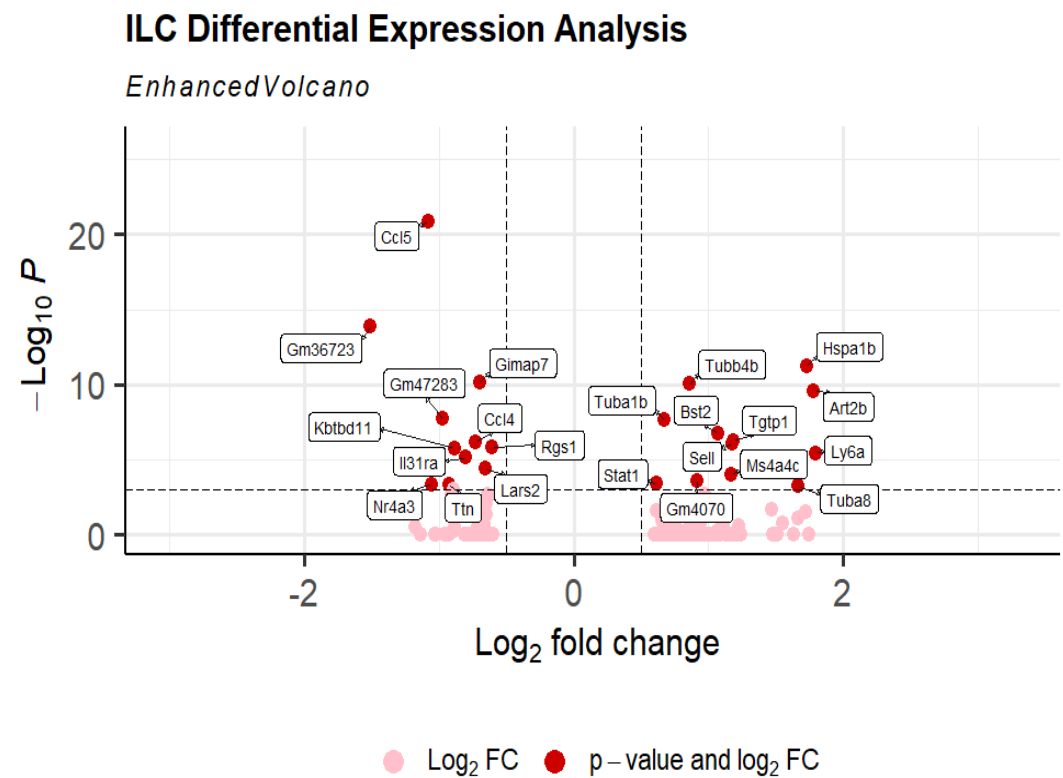
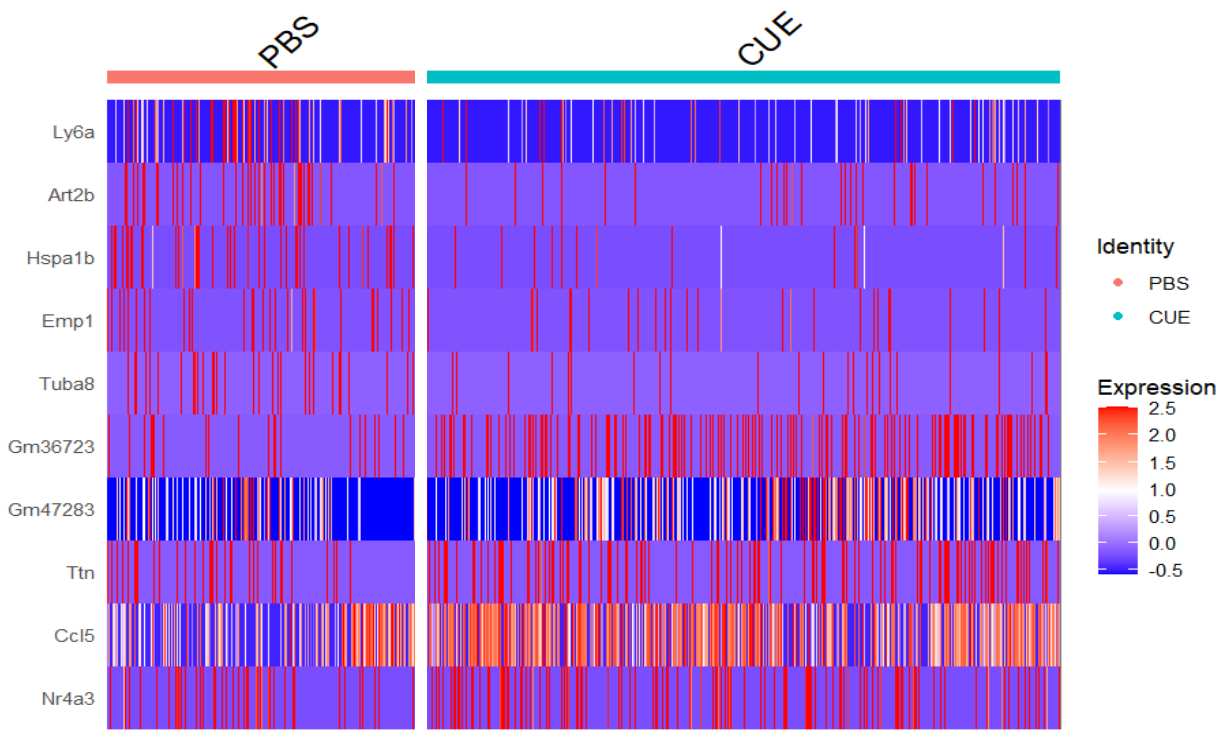
Gene	p_val	avg_log2FC	pct.1	pct.2	p_val_adj
Gm36723	5.564324e-19	-1.5076817	0.117	0.302	1.170066e-14
Ccl5	6.611076e-26	-1.0812870	0.568	0.718	1.390177e-21
Nr4a3	1.937568e-08	-1.0548174	0.163	0.279	4.074317e-04
Gm47283	8.151858e-13	-0.9729894	0.301	0.463	1.714173e-08
Ttn	2.061210e-08	-0.9253745	0.147	0.264	4.334312e-04



ILC DE Analysis



Plots of differentially expressed genes



total = 262 variables

