

eTable 1. A list of primers for qRT-PCR

Actin beta (ACTB), 5'-CACCATTGGCAATGAGCGGTTC,

3'-AGGTCTTTGCGGATGTCCACGT;

c-MAF, 5'-AGAGACACGTCCTGGAGTCG

3'-TATCAGGGTGGCTAGCTGGA

IL-10, 5'-AGGAGGTGATGCCCCAAGCTGA,

3'-TCGATGACAGCGCCGTAGCC

TNF α , 5'-CTCTTCTGCCTGCTGCACTTTG,

3'-ATGGGCTACAGGCTTGTCACCTC

IFN γ , 5'-GAGTGTGGAGACCATCAAGGAAG,

3'-TGCTTTGCGTTGGACATTCAAGTC

GZMB, 5'-GATGCAGGGGAGATCATCGG,

3'-GGTCGGCTCCTGTTCTTTGA

eTable 2. Clinical Profiles of CSF Samples

	IVMP treatment		Two-sided t-test
	Responders	Nonresponders	
Number	9	8	
Female:male	6:3	5:3	NS
Age (y)	42.1 ± 14.2	34.6 ± 11.3	NS
Disease duration (y)	5.6 ± 10.6	3.8 ± 7.3	NS
Disease activity	1.0 ± 0	1.6 ± 1.1	NS
EDSS score	2.4 ± 1.6	2.6 ± 0.4	NS
CSF			
Cell number (/μL)	2.8 ± 2.3	3.0 ± 2.9	NS
Protein (mg/dL)	45.0 ± 24.3	36.5 ± 6.6	NS
MBP (pg/mL)	16.6 ± 36.6	56.3 ± 91.5	NS
IgG index	0.6 ± 0.1	0.7 ± 0.4	NS

Abbreviations: EDSS = Expanded Disability Status Scale; IgG = immunoglobulin G;

IVMP = intravenous methylprednisolone; MBP = myelin basic protein NS = not significant.

Values are expressed as number or mean ± SD; ns, two-sided unpaired t-test.

1 eTable 3. 170 genes as differentially expressed (DE) between PD-1⁺ and PD-1⁻CD8⁺ cells

Gene Symbol	RefSeq	p-value(PD1+ vs. PD1-)	Fold-Change(PD1+ vs. PD1-)	Fold Change(PD1+ vs. PD1-) 95% lower limit	Fold Change(PD1+ vs. PD1-) 95% upper limit	naïve related genes	memory related genes	exhaustion related genes
HNRNPLL	NM_001142650	0.000169741	5.85039	2.85465	11.9899			
TNFRSF9	NM_001561	4.86E-05	5.16784	2.89101	9.23781		●	
MAF	NM_001031804	0.000142595	3.2704	2.04027	5.2422			●
TOX	NM_014729	0.000479176	3.22833	1.88401	5.53188			
CXCR6	NM_006564	0.000378516	3.19327	1.90154	5.36248			
GZMK	NM_002104	0.000638496	2.9875	1.77387	5.03148			
CCR5	NM_000579	0.000284036	2.87546	1.82322	4.53499			●
HLA-DRA	NM_019111	0.00118303	2.69686	1.61641	4.49953			
HLA-DRB1	NM_001243965	0.000656869	2.68411	1.67429	4.30299			
GALM	NM_138801	0.000510425	2.59941	1.67005	4.04596			
VCAM1	NM_001078	0.000692493	2.40163	1.57543	3.66111			
CD74	NM_001025158	8.70E-05	2.32032	1.68974	3.18621			
CCL5	NM_001278736	0.00161655	2.30695	1.47105	3.61784		●	
MRPL10	NM_145255	4.74E-05	2.16465	1.64857	2.84228			
TIGIT	NM_173799	6.85E-05	2.12025	1.6092	2.79362			●
CST7	NM_003650	0.000572473	2.0748	1.47251	2.92343			
SGPP2	NM_152386	0.00110987	2.05127	1.42033	2.96246			
DOCK2	hsa_circ_0001553	0.00122815	2.02816	1.40557	2.92651			

DUSP2	NM_004418	0.000330992	1.99738	1.47389	2.7068
MARK4	NM_001199867	0.000616475	1.98456	1.43388	2.74673
CTLA4	NM_001037631	0.00111936	1.95639	1.38732	2.75889
PDLIM1	NM_020992	5.32E-05	1.95231	1.53743	2.47915
ATXN1	NM_000332	0.00144596	1.92996	1.362	2.73476
HEATR9	NM_152781	0.000105461	1.91292	1.4903	2.45538
OPTN	NM_001008211	0.00024635	1.88894	1.4423	2.47389
LRIG1	ENST00000631537	0.000196682	1.8588	1.43878	2.40142
CD84	NM_001184879	0.00103891	1.85311	1.3553	2.53378
S100A5	NM_002962	0.000104007	1.8015	1.43679	2.25879
SEMA4A	NM_001193300	0.000419378	1.79235	1.37675	2.3334
CYB5R3	NM_000398	0.000897394	1.74693	1.32354	2.30576
CXCR5	NM_001716	0.000245989	1.74403	1.37756	2.20797
WSB2	NM_001278557	0.000654737	1.701	1.31969	2.19249
CD80	NM_005191	0.00145636	1.67215	1.27292	2.19661
SURF4	ENST00000626635	0.000237041	1.6353	1.32864	2.01276
SIPA1	NM_006747	0.000231265	1.61413	1.31944	1.97463
C1orf174	NM_207356	0.000365994	1.61284	1.30402	1.9948
EIF3F	ENST00000628952	0.00110251	1.59421	1.25607	2.02337
PHOSPHO1	NM_001143804	3.13E-05	1.54537	1.33428	1.78985
SMS	NM_001258423	0.00105272	1.54349	1.23805	1.92429

CCDC50	NM_174908	0.000369765	1.53582	1.26876	1.8591
CCL3	NM_002983	0.00124722	1.53326	1.2279	1.91454
SPECC1	NM_001033553	2.88E-06	1.5325	1.36837	1.71631
ZBTB49	NM_145291	0.000231986	1.52782	1.27804	1.82643
YPEL1	NM_013313	0.00129551	1.52781	1.22443	1.90637
ARHGAP1	hsa_circ_0000297	0.00167214	1.51846	1.21145	1.90328
RAD51	NM_001164269	0.000432885	1.51032	1.25257	1.82112
SE3A2	NM_007165	0.00104489	1.5061	1.22345	1.85406
MYO1F	hsa_circ_0008990	2.29E-05	1.50152	1.31481	1.71474
KIF15	ENST00000625939	0.00142107	1.48062	1.2031	1.82215
SNX5	NM_001282454	0.00105926	1.45019	1.20046	1.75187
MURC	NM_001018116	0.000355741	1.43497	1.22276	1.68402
HPS1	NM_000195	0.000320757	1.42197	1.21892	1.65884
NDRG1	NM_001135242	0.000865362	1.41358	1.19092	1.67786
LMCD1	NM_001278233	0.00124701	1.40989	1.17941	1.6854
RC3H2	NM_001100588	0.00154494	1.39208	1.16631	1.66155
GPX8	NM_001008397	0.000715785	1.39073	1.18582	1.63104
PDCD1	NM_005018	5.48E-05	1.37247	1.22532	1.53729
ANKRD20A4	NM_001098805	0.000721089	1.33453	1.16066	1.53444
ABHD17C	NM_021214	0.00151104	1.31993	1.13831	1.53053
TP73-AS1	NR_033708	0.000643008	-1.301	-1.47487	-1.14762

GPAT3	NM_001256421	0.00112931	-1.32893	-1.53756	-1.14861
CD2AP	NM_012120	0.000313759	-1.39558	-1.61414	-1.20662
NGRN	NM_001033088	0.000431827	-1.42355	-1.67092	-1.21279
IFIT5	NM_012420	0.00116649	-1.4506	-1.75689	-1.19771
SMARCA2	NM_001289396	0.00167983	-1.51482	-1.89653	-1.20994
NOLC1	NM_001284388	0.00141468	-1.56324	-1.97959	-1.23446
ZNF516	NM_014643	0.00014515	-1.5776	-1.89233	-1.31521
ZNF397	NM_001135178	0.000239817	-1.58762	-1.93031	-1.30577
RALGPS2	NM_001286247	0.00104257	-1.61123	-2.05243	-1.26488
IMPG2	NM_016247	0.000831273	-1.63188	-2.07712	-1.28208
ALPK1	NM_001102406	3.48E-05	-1.63719	-1.9371	-1.38371
MLXIP	ENST00000625214	0.000411748	-1.64598	-2.06085	-1.31463
CDNF	NM_001029954	0.00128499	-1.66078	-2.16397	-1.2746
TMEM204	NM_001256541	0.00159457	-1.67924	-2.21848	-1.27107
IRS1	NM_005544	0.000541707	-1.68924	-2.15739	-1.32268
MLLT3	NM_001286691	0.000309165	-1.69246	-2.1286	-1.34569
ANKRD26	NM_001256053	0.00135012	-1.69862	-2.24359	-1.28603
IMPDH2	NM_000884	0.000779383	-1.70739	-2.21736	-1.3147
TCEAL3	NM_001006933	0.000473735	-1.7176	-2.20153	-1.34004
ZSWIM5	NM_020883	0.000258245	-1.7231	-2.17322	-1.36621
ZDHHC15	NM_001146256	0.00097764	-1.72952	-2.2784	-1.31287

EPRS	NM_004446	0.00107502	-1.76656	-2.36063	-1.322	
SOCSS	NM_014011	0.000124489	-1.77126	-2.21636	-1.41555	
FBP1	NM_000507	6.14E-05	-1.80731	-2.24001	-1.45819	
NOG	NM_005450	2.00E-05	-1.8613	-2.27348	-1.52384	●
MAML2	NM_032427	0.000493268	-1.89366	-2.54208	-1.41063	
TSPAN6	NM_001278740	0.00107617	-1.89408	-2.62263	-1.36791	
BCL9	NM_004326	0.000189932	-1.9277	-2.52541	-1.47145	
CHD7	NM_017780	7.91E-05	-1.93694	-2.47836	-1.51379	
NDFIP1	NM_030571	0.000999914	-1.94108	-2.71265	-1.38898	
USP6NL	NM_001080491	0.000809029	-1.95723	-2.72148	-1.40759	
ARRDC4	NM_183376	0.00167083	-2.00438	-2.91922	-1.37624	
FAM153A	ENST00000506011	2.18E-05	-2.01494	-2.52997	-1.60475	
MEST	NM_001253900	0.000963643	-2.02851	-2.89356	-1.42208	●
FCGBP	ENST00000628705	0.000633395	-2.09256	-2.97345	-1.47263	
KIAA0125	NR_026800	0.00160302	-2.09488	-3.11764	-1.40764	
CACHD1	NM_001293274	0.000657866	-2.09604	-2.9858	-1.47143	
SYDE2	NM_032184	2.06E-05	-2.17693	-2.79897	-1.69313	
EPHX2	NM_001256482	0.000361321	-2.19785	-3.11771	-1.54939	●
SPINT2	NM_001166103	0.00135092	-2.22403	-3.38435	-1.46153	
LMO7	NM_001306080	8.76E-06	-2.2398	-2.84392	-1.76401	
HIPK2	NM_001113239	0.000387009	-2.28479	-3.30746	-1.57833	

LRRC16A	NM_001173977	0.000244527	-2.28941	-3.25217	-1.61166
TESPA1	NM_001098815	9.26E-05	-2.29628	-3.14766	-1.67518
THEM4	NM_053055	0.000929643	-2.31205	-3.51502	-1.52077
PDE3B	NM_000922	0.00155035	-2.31287	-3.62274	-1.4766
LINC00282	NR_027047	0.00032275	-2.35218	-3.42112	-1.61723
SELL	NM_000655	0.00103006	-2.35266	-3.62897	-1.52522
SLC16A10	NM_018593	5.00E-06	-2.39293	-3.05539	-1.87409
HAVCR1	NM_001173393	0.000641151	-2.41601	-3.67842	-1.58684
PRKCA	NM_002737	0.000202657	-2.41919	-3.48932	-1.67726
SPG20	NM_001142294	0.00115278	-2.4856	-3.9697	-1.55634
PKIA	NM_006823	0.000368321	-2.53896	-3.84352	-1.67719
FAM216A	NM_013300	0.000207187	-2.56438	-3.793	-1.73373
NPAS2	NM_002518	0.000475165	-2.57811	-3.98213	-1.66913
FAM117B	NM_173511	0.000243449	-2.59435	-3.88509	-1.73243
GTSCR1	NM_001278515	9.52E-06	-2.61568	-3.48559	-1.96288
MAP9	NM_001039580	0.000140238	-2.62353	-3.84919	-1.78814
C1orf162	NM_001300834	0.000592852	-2.64398	-4.18321	-1.67112
ITGA6	NM_000210	0.000174618	-2.67429	-3.99308	-1.79106
UBE2E2	NM_152653	6.09E-05	-2.84162	-4.14875	-1.94632
SLC8B1	NM_024959	0.000806613	-2.86424	-4.80026	-1.70904
ACSM3	NM_005622	0.000993226	-3.0031	-5.2281	-1.72503

IL6ST	NM_001190981	0.000163781	-3.11264	-4.92741	-1.96625	●
MAN1C1	NM_001289010	0.000186547	-3.11481	-4.96655	-1.95347	
CD248	NM_020404	0.000163296	-3.11914	-4.94109	-1.969	●
RCAN3	NM_001251977	0.00103658	-3.14415	-5.62004	-1.75901	
DSEL	NM_032160	0.000700345	-3.24246	-5.71572	-1.83941	
DENND5A	NM_001243254	0.000106627	-3.25721	-5.13429	-2.06639	
TRABD2A	NM_001080824	9.49E-05	-3.27803	-5.14925	-2.08681	
FAM134B	NM_001034850	1.93E-05	-3.30017	-4.8407	-2.24991	
CNKSR2	NM_001168647	7.86E-07	-3.31179	-4.38525	-2.50109	
DSC1	NM_004948	0.000530524	-3.33828	-5.84998	-1.90499	●
NELL2	NM_001145107	0.00113222	-3.34695	-6.21974	-1.80105	●
MYB	NM_001130172	0.00035338	-3.37036	-5.77195	-1.96802	
RBM11	NM_144770	0.000515815	-3.37289	-5.92737	-1.9193	
EFHC2	NM_025184	0.000170727	-3.4234	-5.64546	-2.07594	
CR1	NM_000573	9.78E-05	-3.57397	-5.8114	-2.19797	
CA6	NM_001215	0.00011612	-3.58885	-5.90024	-2.18293	●
BACH2	hsa_circ_0001628	9.69E-05	-3.63399	-5.94362	-2.22186	
CERS6	NM_001256126	0.000999155	-3.63743	-6.97826	-1.89602	
LEF1	NM_001130713	0.00031587	-3.67088	-6.47878	-2.07992	
NGFRAP1	NM_001282674	0.000771656	-3.82601	-7.3634	-1.98799	
ARHGAP5	NM_001030055	0.000209265	-3.85914	-6.76951	-2.2	

VSIG1	NM_001170553	0.000207891	-3.86991	-6.79335	-2.20453
LRRN3	NM_001099658	1.65E-05	-3.89521	-5.98449	-2.53533
FCER1G	NM_004106	0.00119419	-3.91746	-7.93153	-1.93487
RNF130	NM_001280801	0.000152514	-3.95035	-6.85551	-2.27631
CRIL	NM_175710	0.00019683	-3.95276	-6.97493	-2.24007
PLAG1	NM_001114634	2.25E-05	-3.9571	-6.19614	-2.52716
NRCAM	NM_001037132	9.93E-06	-4.01643	-6.0943	-2.64701
CCR7	NM_001301714	7.88E-05	-4.02716	-6.76786	-2.39633
TCEA3	NM_003196	0.000170049	-4.11593	-7.31338	-2.31642
ACTN1	NM_001102	2.59E-05	-4.18484	-6.71939	-2.60632
GCNT4	NM_016591	0.000447873	-4.20603	-8.09454	-2.18551
AK5	NM_012093	0.000156293	-4.22305	-7.53991	-2.3653
FAM153B	OTTHUMT0000037192	0.00126452	-4.30259	-9.19691	-2.01288
	8				
TMEM220	NM_001004313	1.67E-07	-4.31969	-5.81448	-3.20918
PTPRK	NM_001291983	3.66E-05	-4.33509	-7.17036	-2.62093
MAL	NM_002371	6.64E-05	-4.35474	-7.45862	-2.54253
TXK	NM_003328	0.000252903	-4.40076	-8.26676	-2.34272
IFNGR2	NM_005534	6.05E-06	-4.53512	-6.98176	-2.94587
CR2	NM_001006658	3.67E-05	-4.62563	-7.82474	-2.73446
IGF1R	NM_000875	7.76E-05	-4.71948	-8.40658	-2.64953

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KLF7	NM_001270942	6.96E-07	-4.84007	-6.97573	-3.35825	
NT5E	NM_001204813	0.00020992	-5.26757	-10.52	-2.63757	●
S100B	NM_006272	0.00133119	-5.81391	-14.6286	-2.31065	
SLC7A8	NM_001267036	0.000329938	-5.94616	-13.0088	-2.71792	
PCSK5	NM_001190482	7.40E-06	-6.36276	-10.9057	-3.71224	
SCML1	NM_001037535	2.78E-06	-7.45942	-12.6912	-4.38439	

- 1 Filled circles (●) indicate overlap genes with naïve or memory CD8⁺ T cell specific genes
- 2 (<https://www.proteinatlas.org/humanproteome/blood/>) and with co-inhibitory gene-module related to T cell dysfunctions (ref. 4).

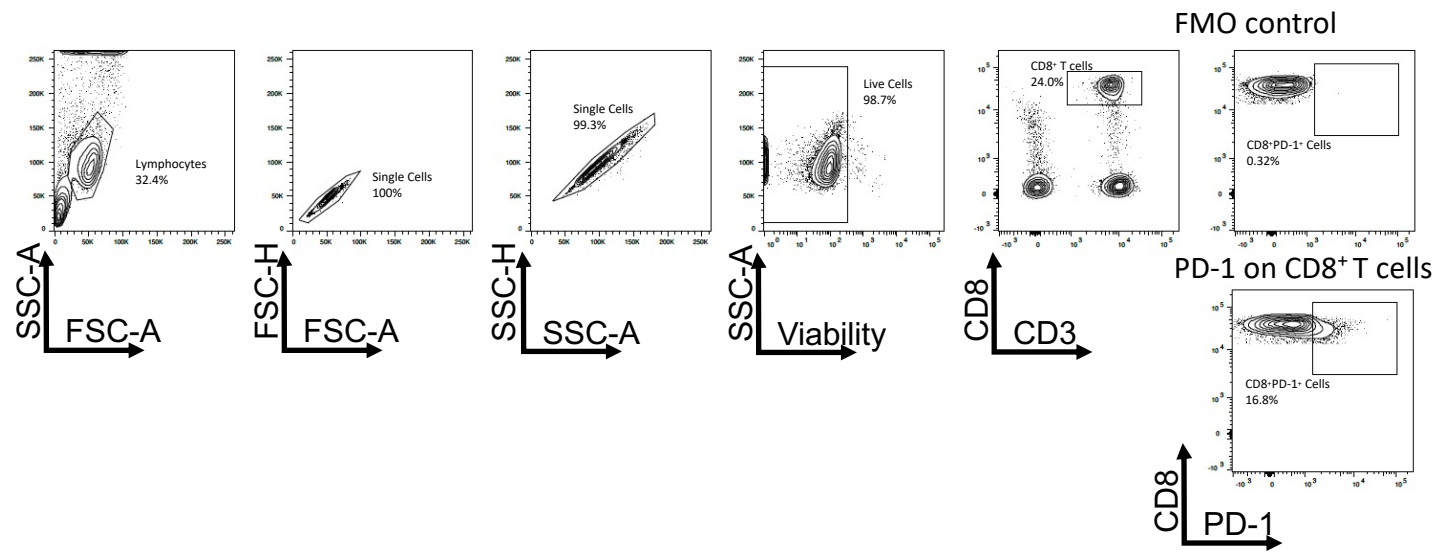
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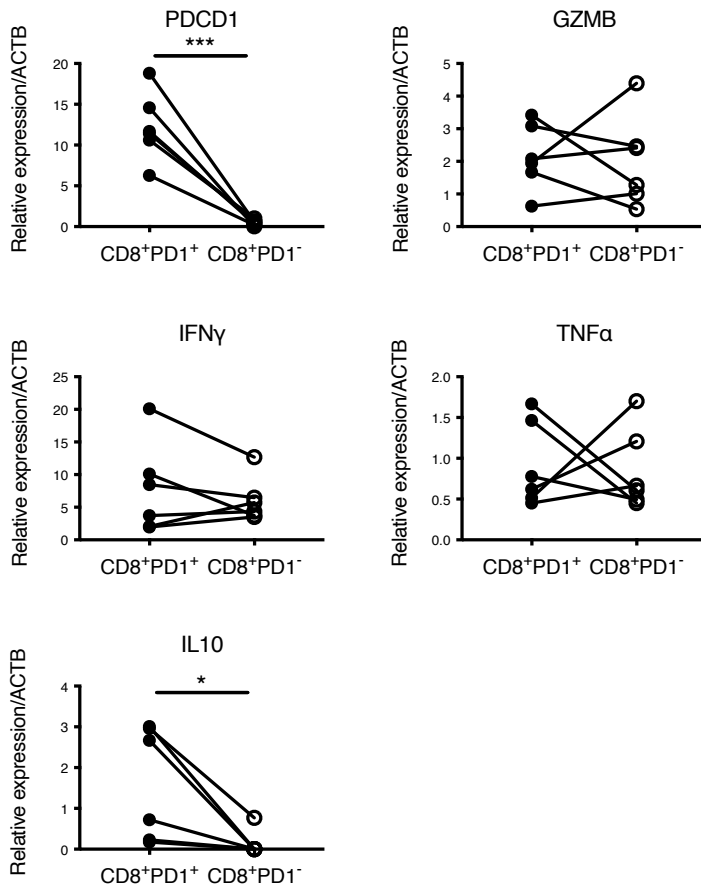
eTable 4. 45 genes as up-regulated in PD-1⁺CD8⁺ cells in MS patients treated with IFN- β

MYO1F
PHOSPHO1
MRPL10
TNFRSF9
PDLIM1
TIGIT
CD74
S100A5
HEATR9
MAF
HNRNPLL
SIPA1
ZBTB49
SURF4
CXCR5
OPTN
HPS1
DUSP2
MURC
C1orf174
SEMA4A
RAD51
TOX
CST7
MARK4
WSB2
HLA-DRB1
GPX8
ANKRD20A4
NDRG1
CYB5R3
SF3A2
SMS
EIF3F
HLA-DRA
DOCK2
LMCD1
CCL3
YPEL1
KIF15
CD80
ABHD17C
RC3H2
CCL5
ARHGAP1

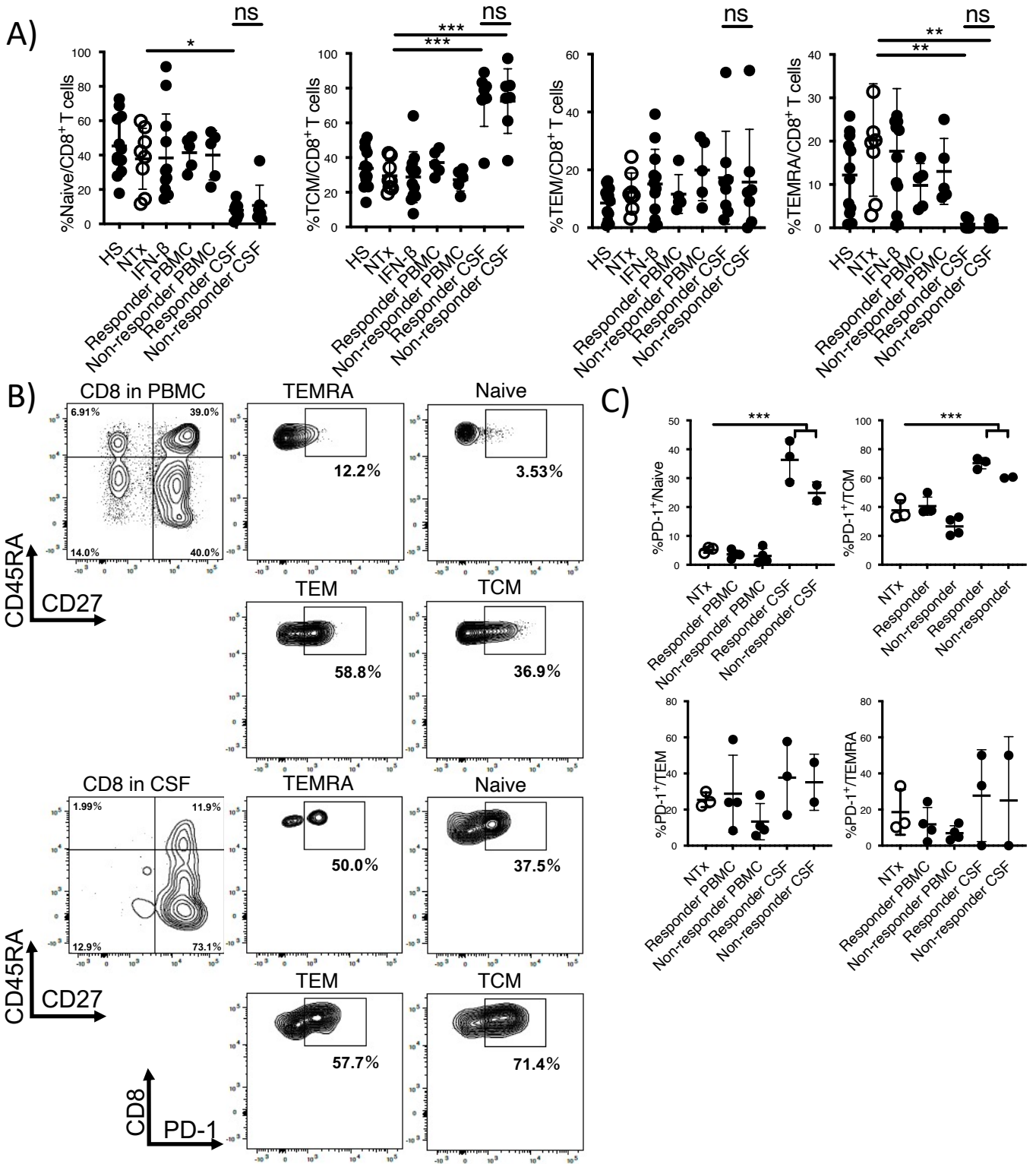
eFigure 1.



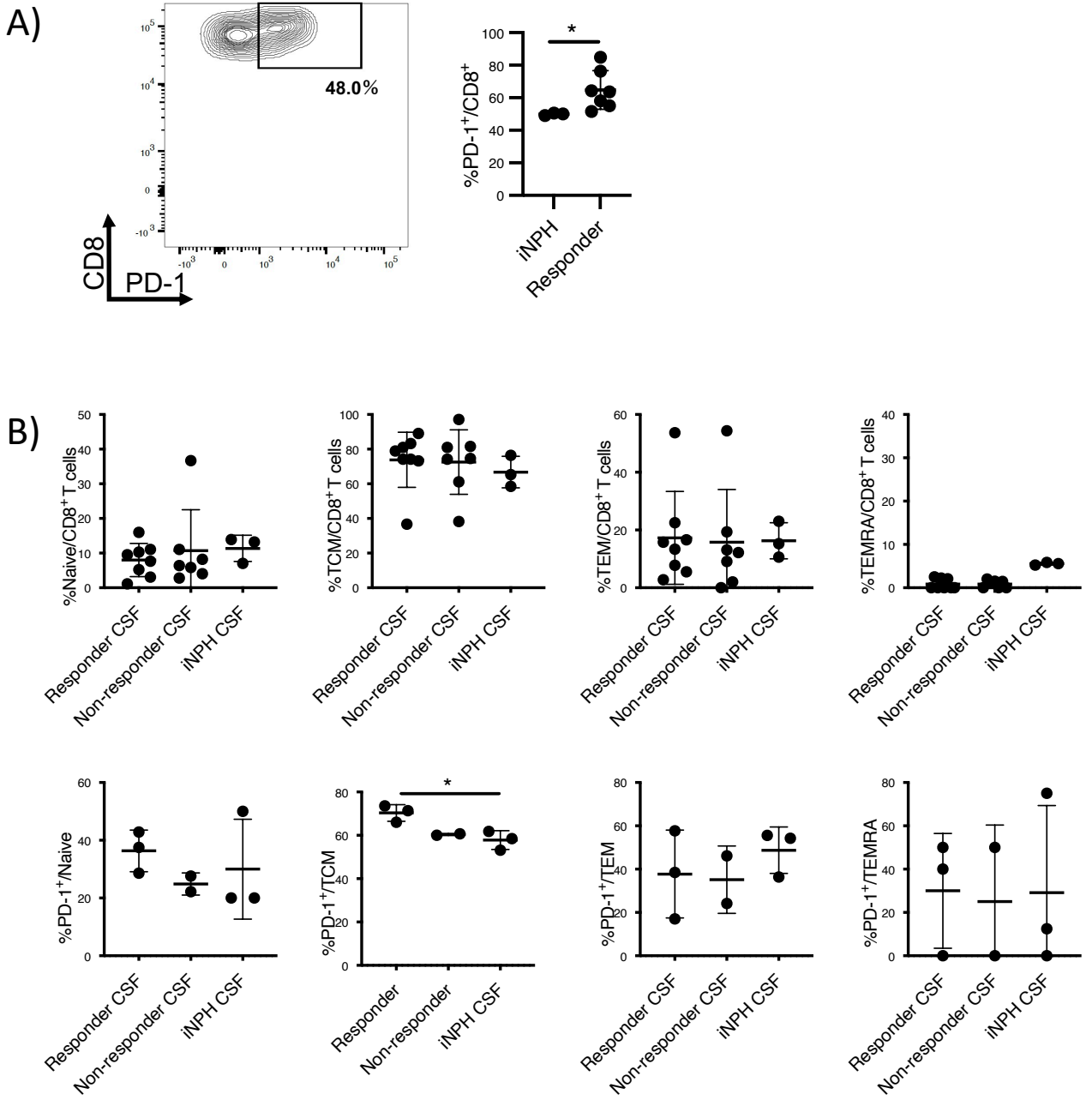
eFigure 2.



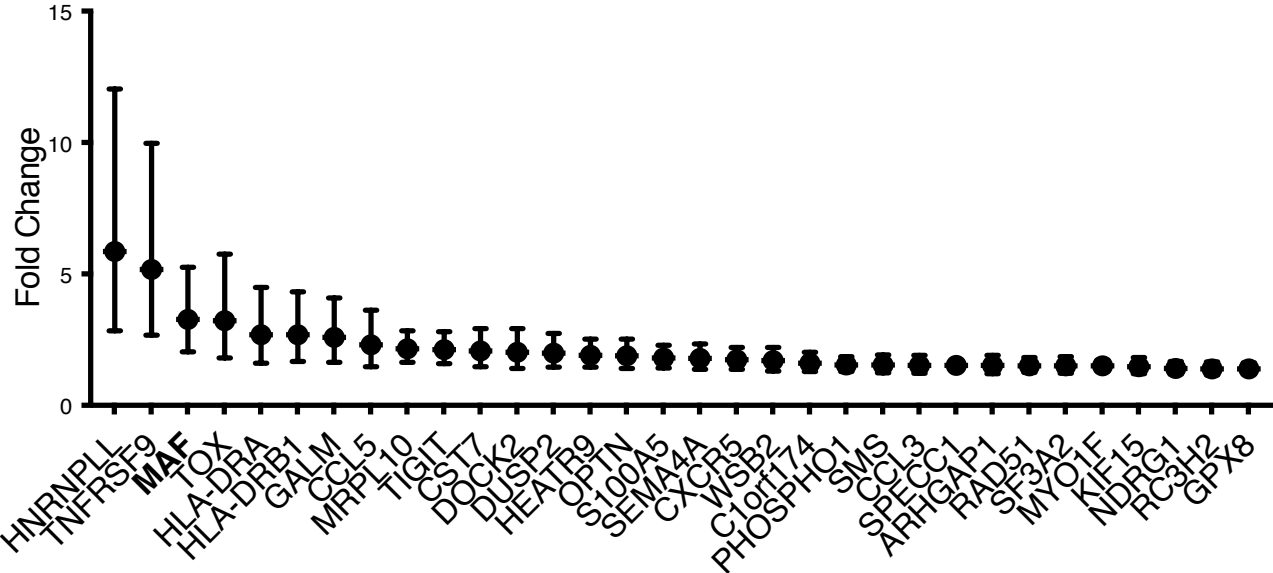
eFigure 3.



eFigure 4.



eFigure 5.



eFigure 1. A representative flow cytometric scheme to identify PD1⁺CD8⁺ T cells

A representative flow cytometric scheme to identify PD1⁺CD8⁺ T cells is shown. Lymphocytes identified by FSC/SSC gating were excluded doublets for “Single Cells” and dead cells for “Live Cells”. CD8⁺ T cells were gated out from the live cells based on their expression of CD3 (CD3-PerCP/Cy5.5 antibody, BD Biosciences) and CD8. CD8⁺ T cells are identifiable based on the high levels of CD8 expression. CD8⁺ T cells were further analyzed for their PD-1 expression using FMO control.

eFigure 2. Differences in gene expression of the indicated genes between PD1⁺CD8⁺ T cells and PD1⁻CD8⁺ T cells

Gene expression was analyzed by quantitative reverse transcription PCR, normalized to the expression of the house-keeping gene *ACTB* expression. * $p < 0.05$, *** $p < 0.001$, two-sided paired t-test.

eFigure 3. Distribution of differentiation subsets of CD8⁺ T cells in PBMC and CSF

A) The proportions of CD8⁺ T-cell subsets are shown. The data were obtained from healthy subjects (HS, $n = 12$), patients with MS in remission receiving no treatment (NTx, $n = 8$), patients with MS treated with IFN- β (IFN- β , $n = 12$), patients with relapsed MS or CIS (PBMCs, $n = 10$; CSF, $n = 15$). Values are expressed as mean \pm SD; ns = not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, one-way analysis of variance followed by Tukey’s multiple comparison test for NTx vs CSF. B) Representative flow cytometry data for PD-1 expression on each CD8⁺ T cell subset. PBMC of MS patients receiving no treatment in remission and CSF of responder MS patient in disease flare. C) Summary plots of PD-1 expression on each CD8⁺ T cell subset from

multiple donors; MS patients in remission receiving no treatment (N = 3), relapsed MS or CIS patients (PBMCs, N = 8; CSF, N = 5). Values are expressed as means \pm s.d.; ***p < 0.001, one-way analysis of variance followed by Tukey's multiple comparison test for NTx vs CSF.

eFigure 4. Proportion of PD-1 expressing cells on CD8⁺ T cells in the CSF of non-inflammatory neurological disease (idiopathic normal pressure hydrocephalus, iNPH). A)

The data are representative of three different patients and summary plots. Values are expressed as means \pm s.d.; *p < 0.05, two-sided Mann–Whitney U test (compared with the treatment responders shown in Figure 2B). B) The proportions of CD8⁺ T-cell subsets and PD-1 expression on each CD8⁺ T cell subset including iNPH samples were shown. Values are expressed as means \pm s.d. *p < 0.05, one-way analysis of variance followed by Tukey's multiple comparison test.

eFigure 5. c-Maf regulating genes are differentially upregulated in PD-1⁺CD8⁺ T cells

Fold changes in gene expression in microarray analysis of PD-1⁺CD8⁺ T cells compared with PD-1⁻CD8⁺ T cells are shown. Values are expressed as mean \pm 95% confidence interval.