Supplementary Materials for

Distinct Inflammatory Profiles of Myelin-Reactive T cells from Patients with Multiple Sclerosis

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References (50-51)

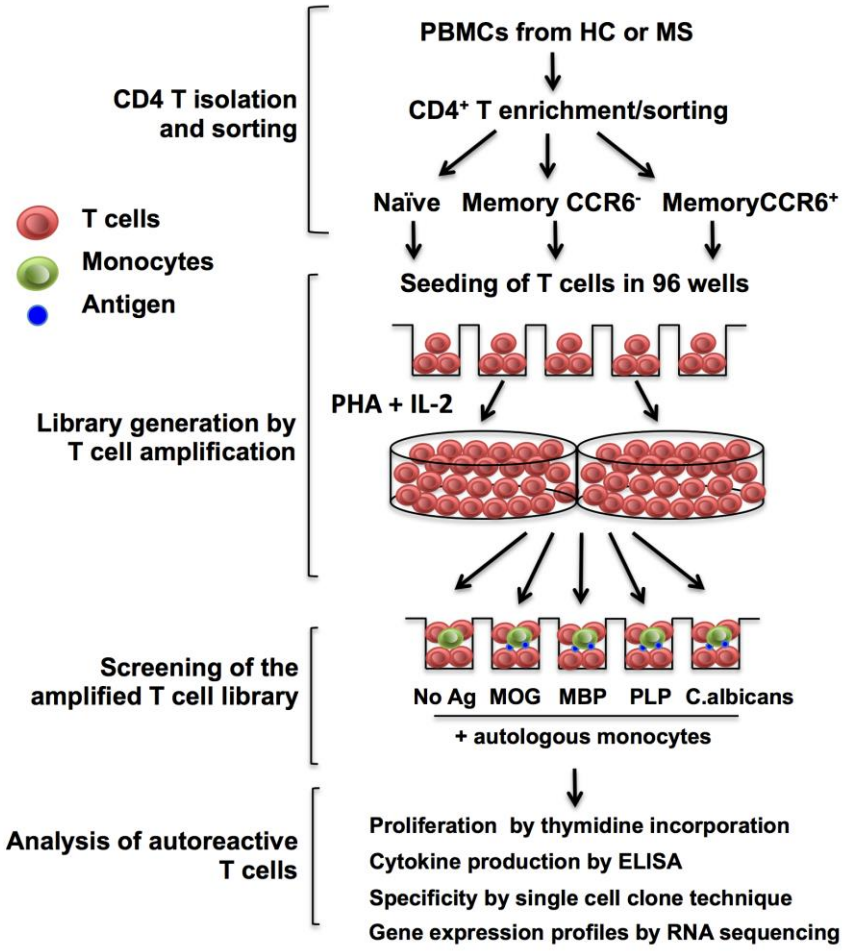

Fig. S1. Schematic representation of amplified T cell library assay. Naïve, CCR6^{-} memory and CCR6^{+} memory CD4^{+} T cells from patients with MS or healthy controls (HC) were sorted and seeded at 2,000 cells per well in 96-well plates, and stimulated with PHA and IL-2 in the presence of irradiated allogeneic feeder cells for 2 weeks. After the stimulation cocktail was washed out, the cells from each well were re-stimulated by autologous monocytes and myelin peptides, *C. albicans*. Myelin-specific CD4^{+} T cell proliferation (day 5) and cytokine production (day 7) were measured by ^{3}H-thymidine incorporation and ELISA assay.

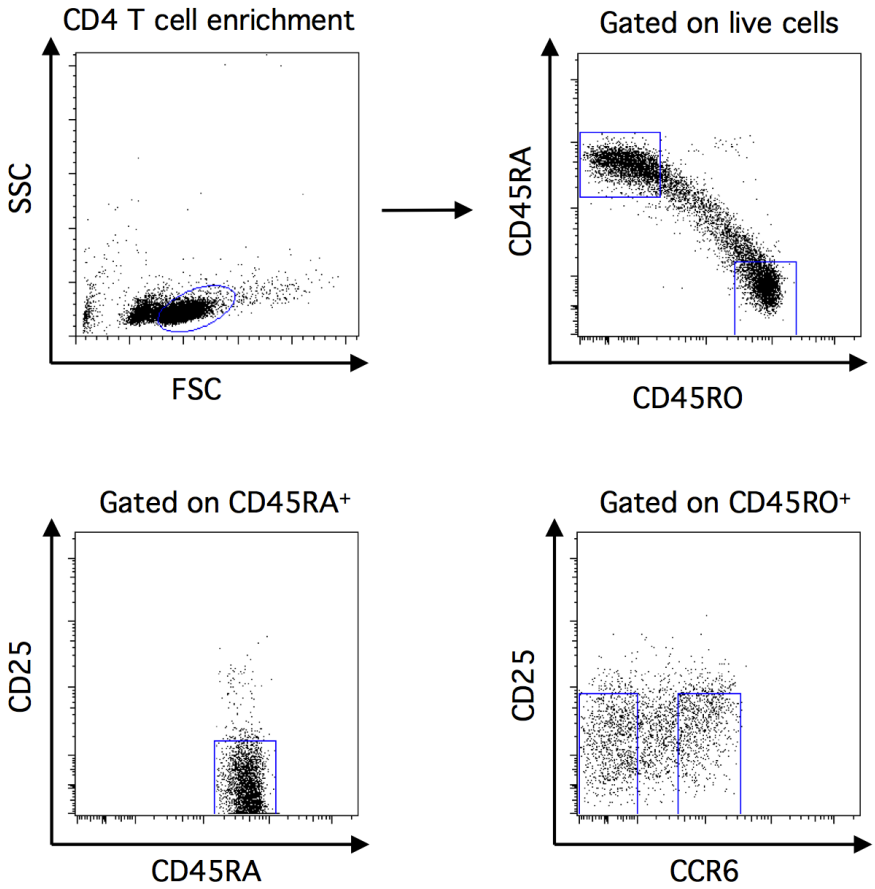

Fig. S2. Sorting strategy of each T cell subpopulation. Total $CD4^+$ T cells were enriched by negative selection from PBMCs. Untouched $CD4^+$ T cells were stained with monoclonal antibodies against CD45RA, CD45RO, CD25 and CCR6 for T cell subpopulation isolation. The following subpopulations were gated and sorted: Naïve ($CD45RA^+CD45RO^-CD25^-$), $CCR6^-$ memory ($CD45RA^-CD45RO^+CD25^-CCR6^-$), and $CCR6^+$ memory ($CD45RA^-CD45RO^+CD25^-CCR6^+$).

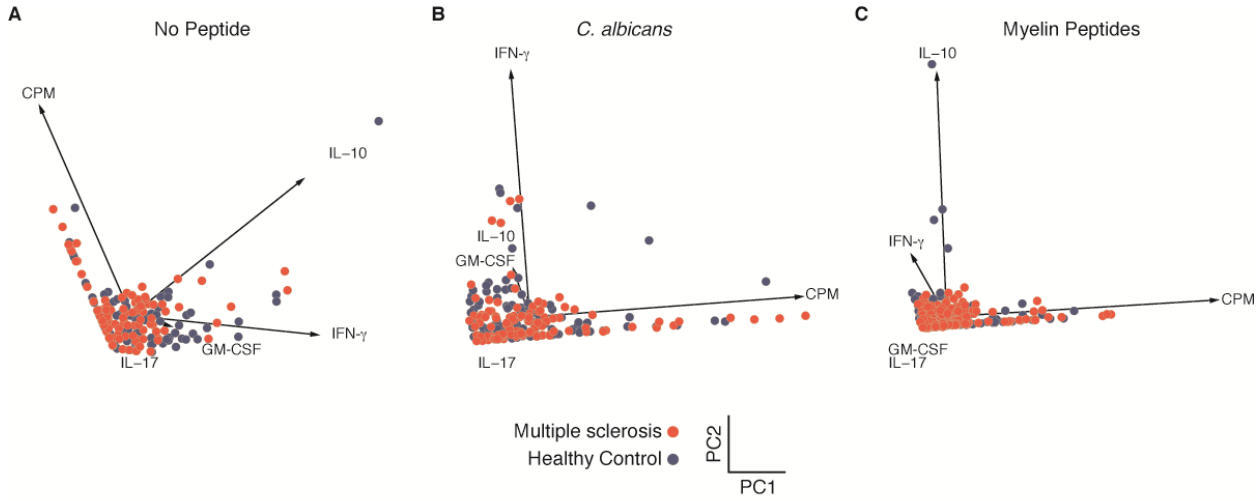

Fig. S3. Principal component analysis of functional phenotypes of myelin-reactive CD4^{+}CCR6^{-} T cells. Scatterplots show measured penta-dimensional responses (proliferation, IFN-γ, IL-17, GM-CSF and IL-10) for individual amplified T cell libraries (each dot) projected onto the first two principal components. Analysis is shown for (**A**) no peptide, (**B**) *C. albicans*, and (**C**) myelin peptides from eight healthy subjects and eight MS patients. Projections of the vectors for each data class are also shown and annotated for reference. Statistically significant p-values are shown below: *C. albicans*-reactive T cells for proliferation ($p = 0.0017$) and GM-CSF ($p < 0.0001$).

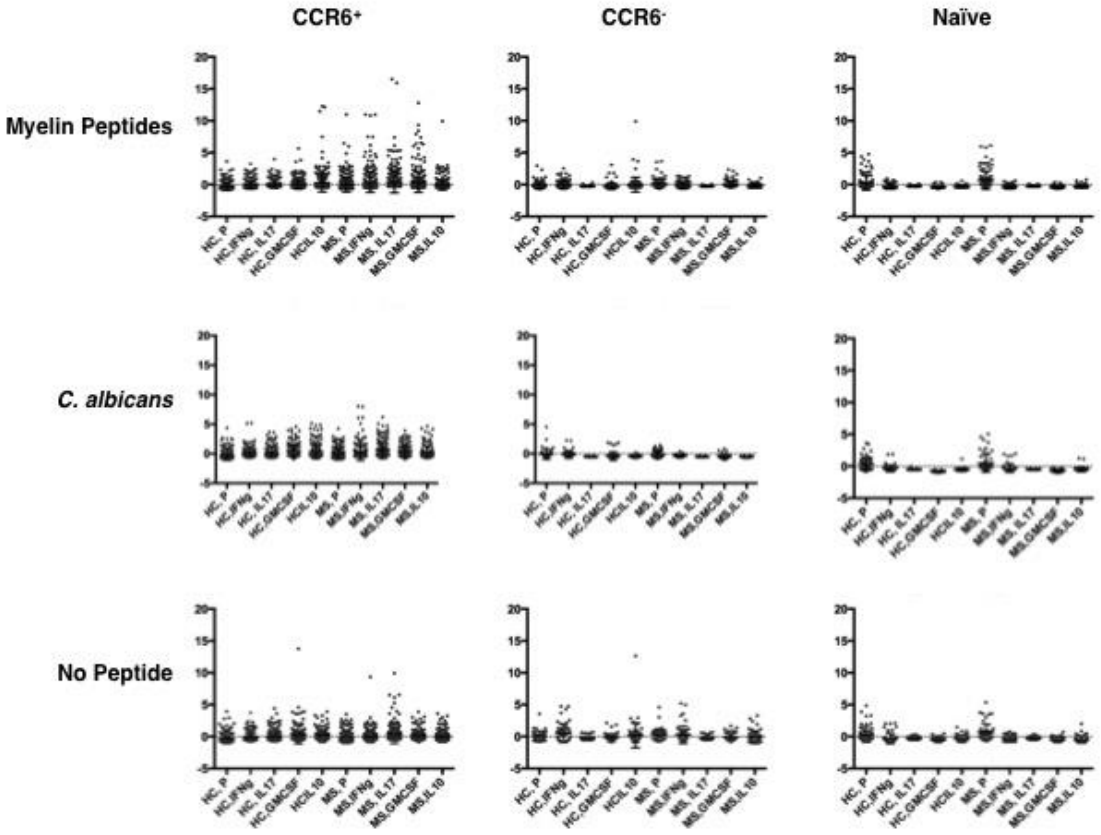

Fig. S4. Functional phenotypes of CCR6^{+} memory, CCR6^{-} memory, and naïve CD4^{+} T cells. Plots show all z –score normalized data for no peptide, *C. albicans*, and myelin peptides corresponding to PCA analysis presented in Figure 2 and Figure S3.

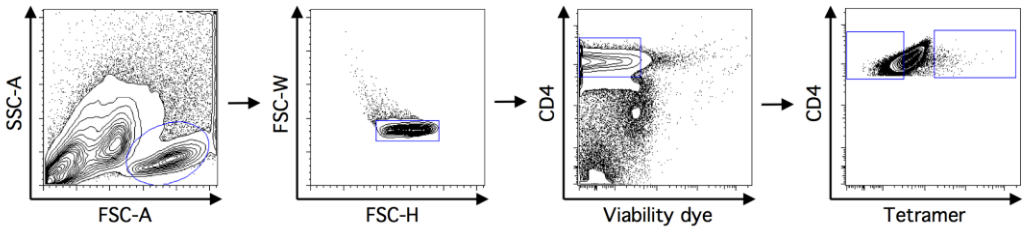

Fig. S5. Representative tetramer staining and sorting strategy of each library from MS patients and healthy controls were chosen for single-cell cloning and RNA sequencing. Two of the highest proliferated wells (red dots shown in fig. S7) were picked and stained by DR4 myelin peptides tetramers (MOG_{97-109} tetramers and $PLP_{180-199}$ tetramers). MOG_{97-109} tetramer-positive and $PLP_{180-199}$ tetramer-positive T cells were sorted for single-cell cloning. Tetramer-positive and tetramer-negative T cells were sorted for RNA sequencing.

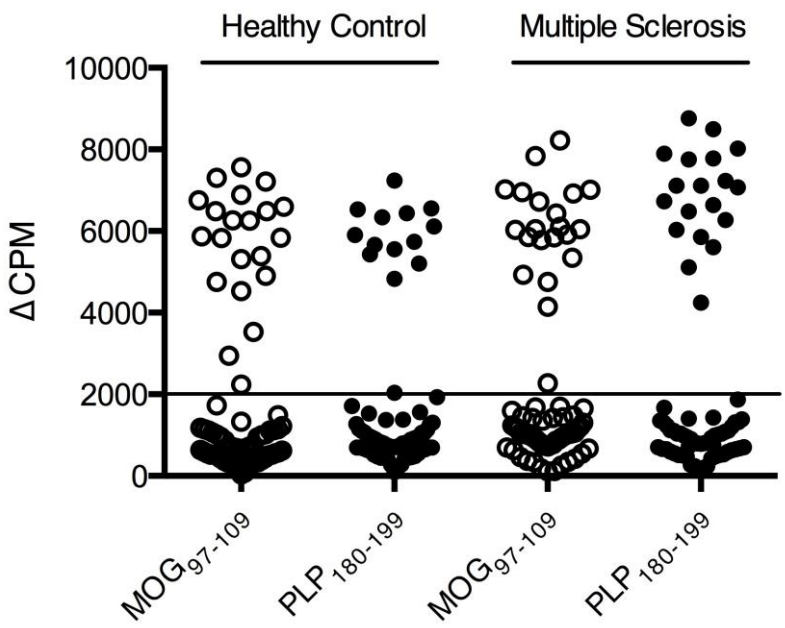

Fig. S6. Specificity of myelin-reactive CD4$^+$ T cells. CCR6$^+$ memory CD4$^+$ T cells from two paired HLA-DR4 healthy subjects and DR4 MS patients were amplified by PHA and IL-2, and scored for proliferation upon re-stimulation with autologous monocytes pulsed with DR4 peptides. The highest sorted wells were used for single-cell sorting using MOG_{97-109}- and $PLP_{180-199}$-tetramers. Single-cell clones were stimulated by monocytes pulsed with no peptide, MOG_{97-109} or $PLP_{180-199}$. Cell proliferation of each clone was measured on day 5. Dot plots show ΔCPM, which was calculated as peptide pulsed culture CPM − no peptide-pulsed culture CPM. Cultures were scored positive when ΔCPM exceeded 2,000 and the stimulation index was > 3.

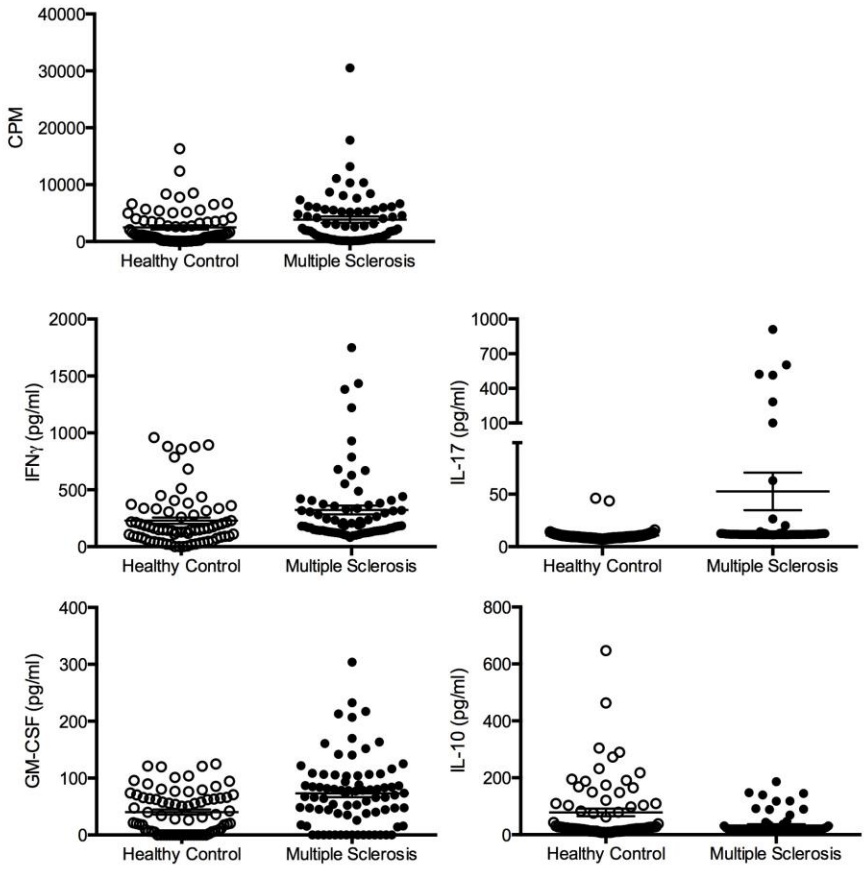

Fig. S7. Phenotypic analysis of myelin-specific single cell clones. Tetramer-sorted single cell clones (n = 144) were stimulated with DR4 myelin peptides (MOG_{97-109} and $PLP_{180-199}$) to verify the specificity. The cell proliferation and cytokine production of these clones were measured on day 5 after stimulation. Data were shown as mean ± SEM. The functional profiles of responsive clones were shown as hierarchical clusters in Figure 3.

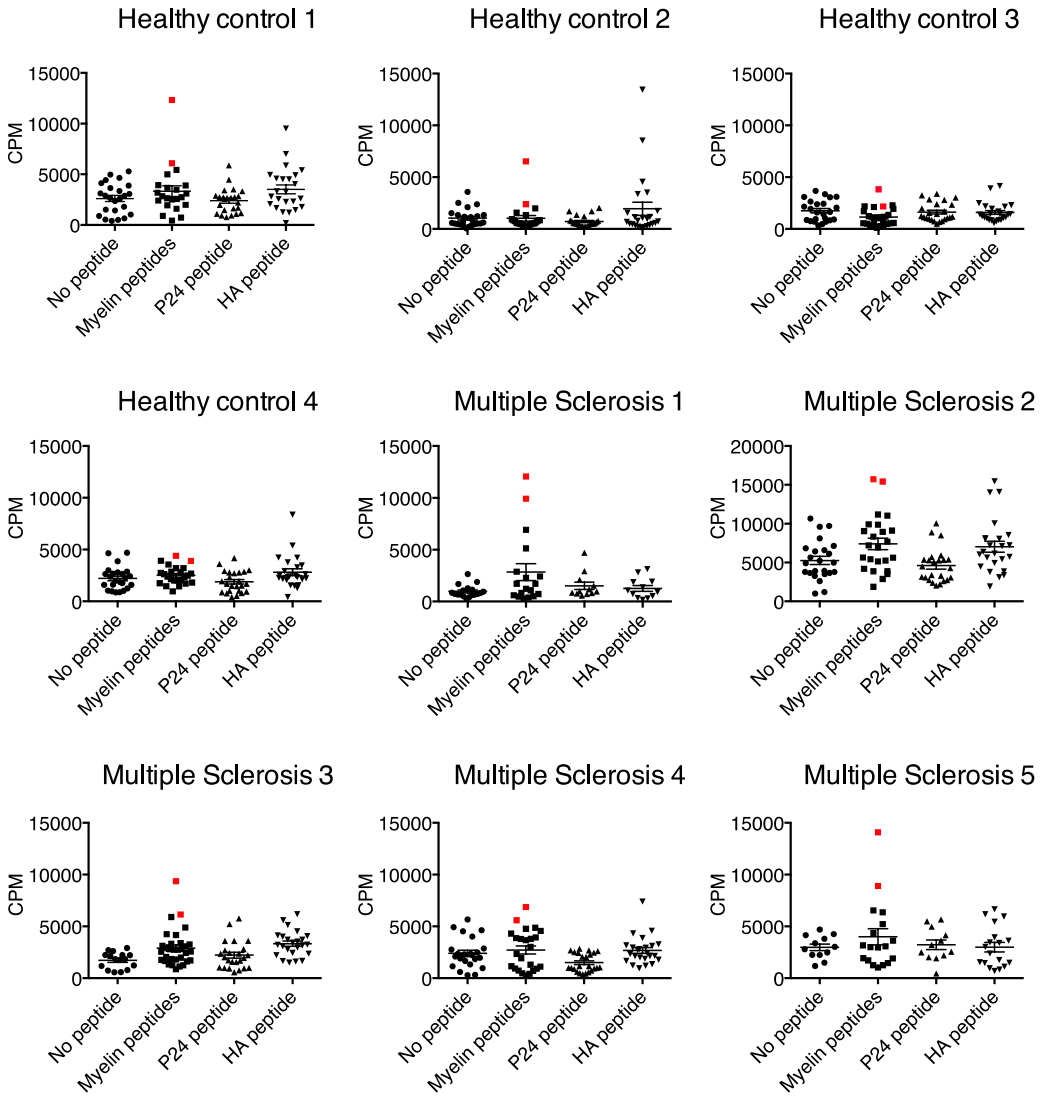

Fig. S8. Cell proliferation of each well from MS patients and healthy controls chosen for RNA sequencing. Four HLA-DR4 healthy controls and five HLA-DR4 MS samples were run. $CCR6^{+}$ memory T cells were amplified by PHA and IL-2 in 96-well plates, and stimulated by irradiated autologous monocytes and DR4 myelin peptides ($MOG_{97\text{-}109}$ and $PLP_{180\text{-}199}$). Two of the highest proliferated wells (red dots) of each sample were picked for RNA sequencing. HIV peptide ($P24_{166\text{-}197}$) and hemagglutinin peptide ($HA_{306\text{-}318}$) used as a negative and positive control.

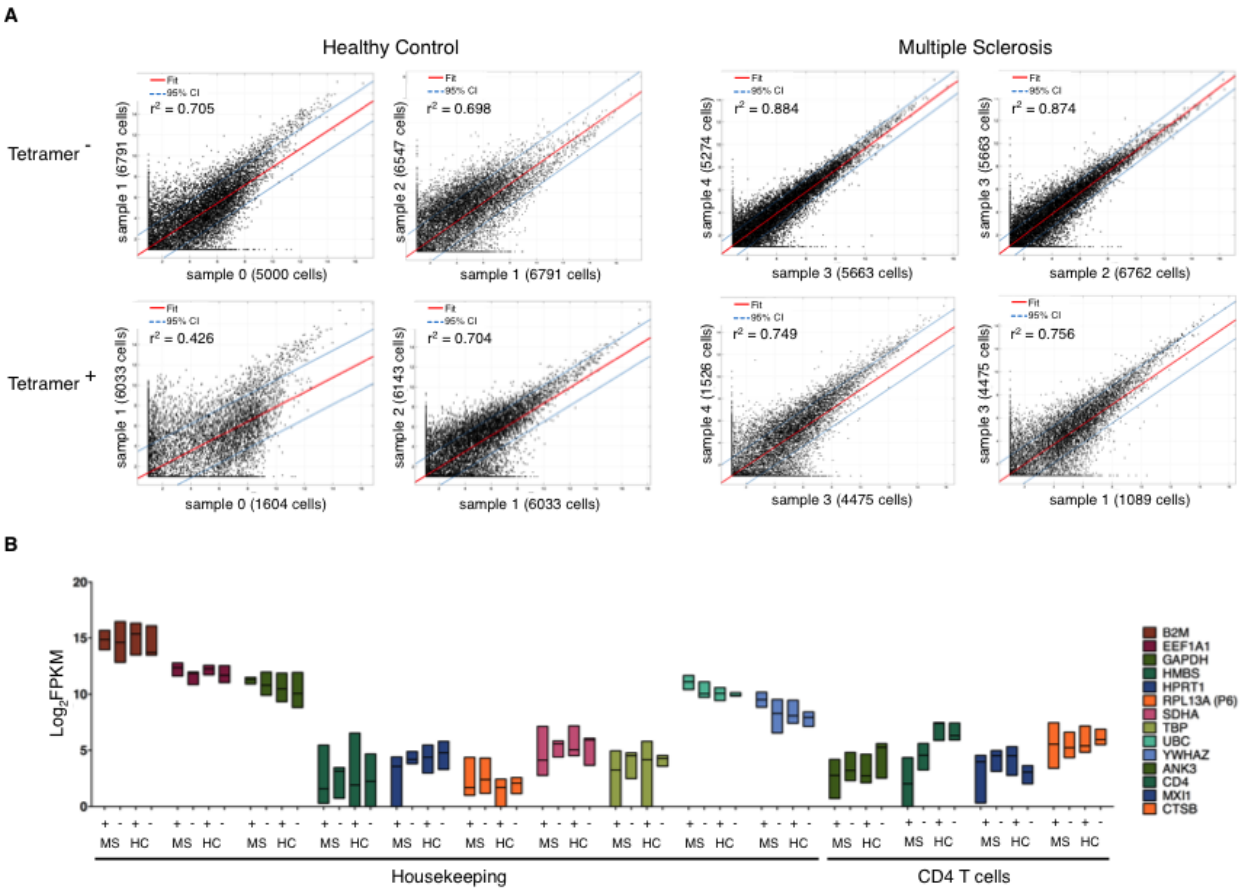

Fig. S9. Correlation of RNA-seq data across biological replicates. (**A**) Correlation scatter plots of log(FPKM+1) values for indicated samples. Linear regression performed in MATLAB using regression toolbox and r^2 values shown. Red lines show the linear fit and blue dotted lines indicate the 95% confidence interval bounds. (**B**) Box-plots are shown for each sample included in down stream analysis (n = 3 HC tetramer$^+$ and tetramer$^-$, n = 5 MS tetramer$^+$ and tetramer$^-$). Line indicates median, boxes extend to the minimum and maximum of replicates included. Housekeeping (50) and CD4 T cell specific (*51*) genes are shown.

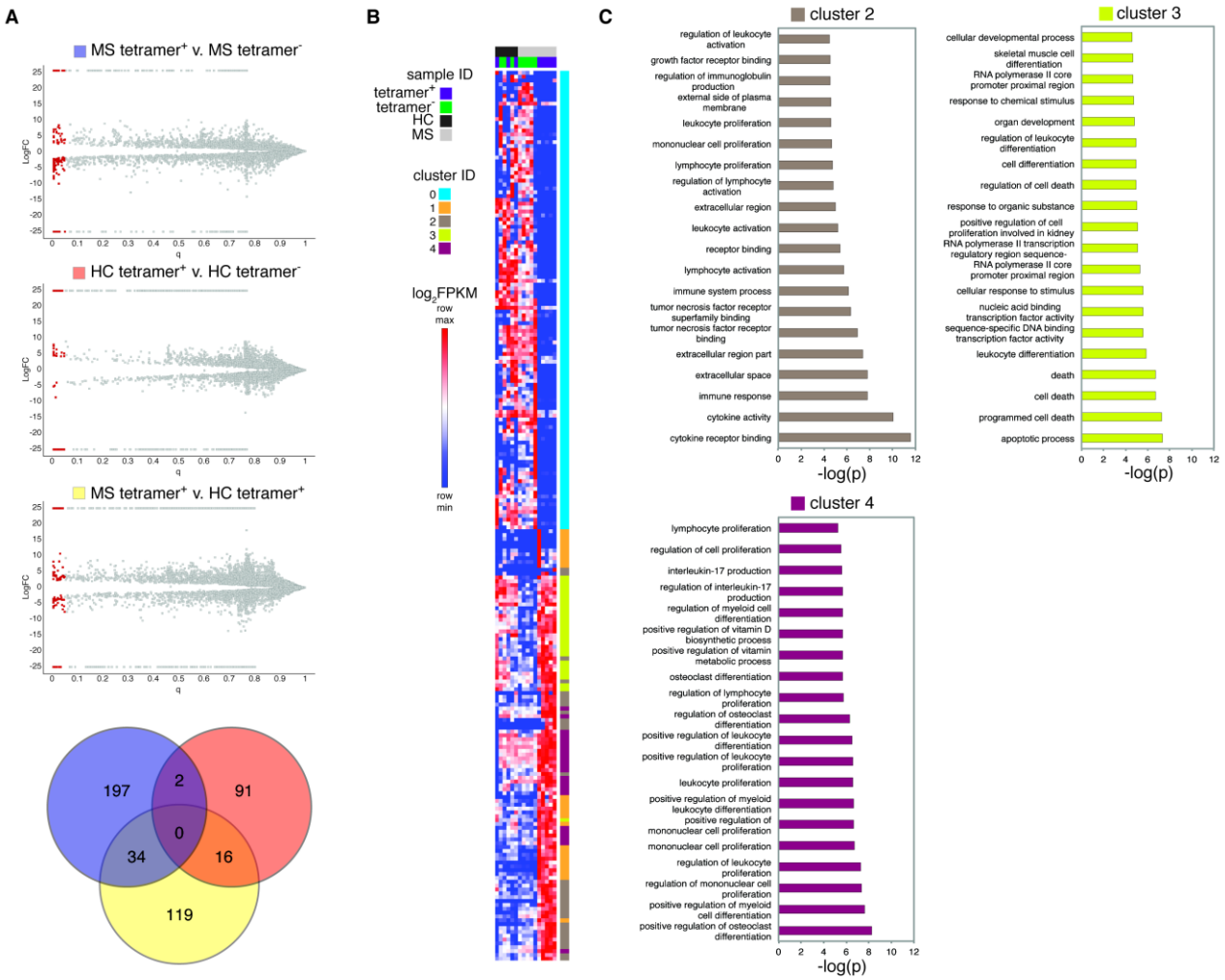

Fig. S10. Differential expression analysis of myelin-reactive T cells in MS and healthy controls. (**A**) Volcano plots show differentially expressed (DE) genes (q<0.05, red), and Venn diagram shows overlap between DE genes from each comparison purple (MS tetramer^{+} vs. MStetramer^{-}), pink (HCtetramer^{+} vs. HCtetramer^{-}, and yellow (MStetramer^{+} vs. HCtetramer^{+}). (**B**) DE genes unique to MStetramer^{+} samples were k means clustered for columns (k = 4) and rows (k = 5) using GeneE. Clusters are shown in colored bars. (**C**) Genes from each cluster were used to perform GO analysis using FuncAssociate to identify biological functions. – logP for each function is plotted for clusters that were found to have enriched functions.

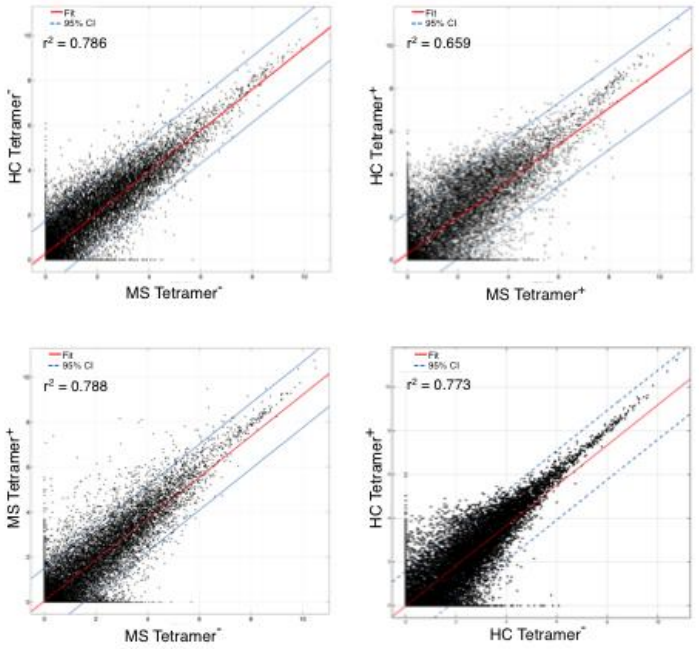

Fig. S11. Correlation scatter plots. Correlation scatter plots of log(averageFPKM+1) values for indicated samples. Genes whose expression was zero for all conditions were removed from the data set. Linear regression performed in MATLAB using regression toolbox and r^2 values shown. Red lines show the linear fit and blue dotted lines indicate the 95% confidence interval bounds.

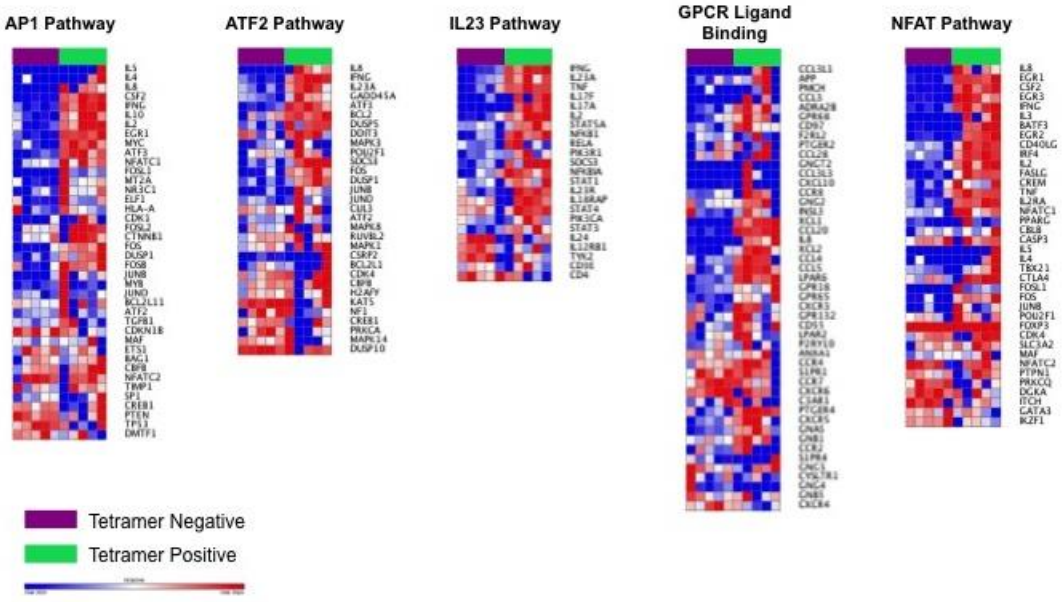

Fig. S12. Heatmaps of selected enriched gene sets identified by GSEA in MS tetramer positive samples. Log_2FPKM values were used to generate each heatmap with GeneE.

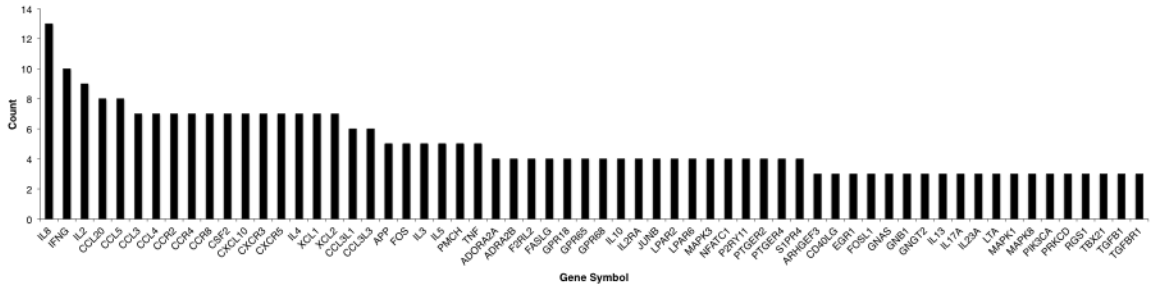

Fig. S13. Histogram showing distribution of the number of times each gene appeared across all gene sets. Of the 224 genes found in the leading edge subset, those appearing at least twice are shown.

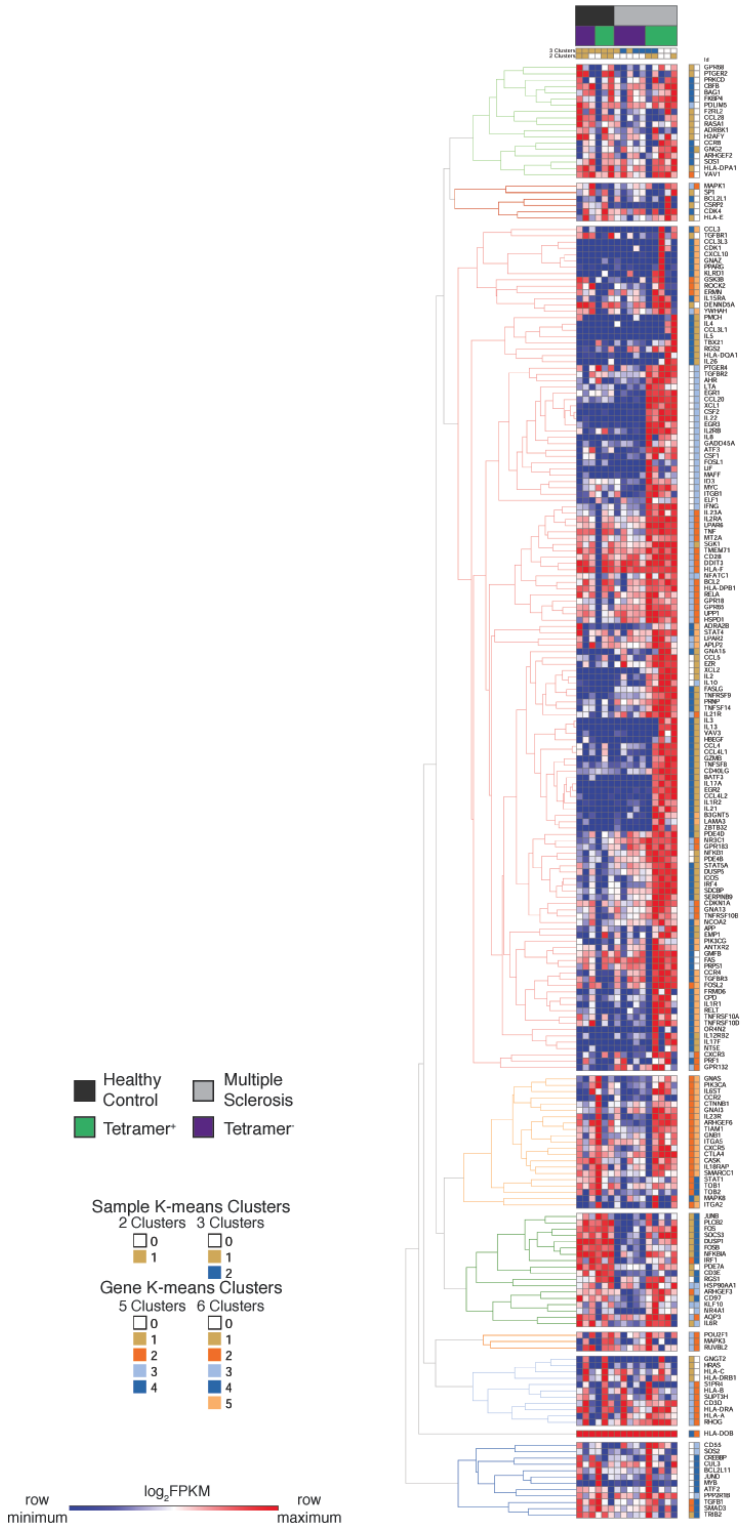

Fig. S14. **Heatmap of log_2FPKM values for the 224-gene leading edge set**. Data are shown for all samples passing quality control filters. Data were clustered hierarchically (dendrogram) row-wise and by k means for columns (k = 2 and k = 3) and rows (k = 5 and k = 6) using GeneE.

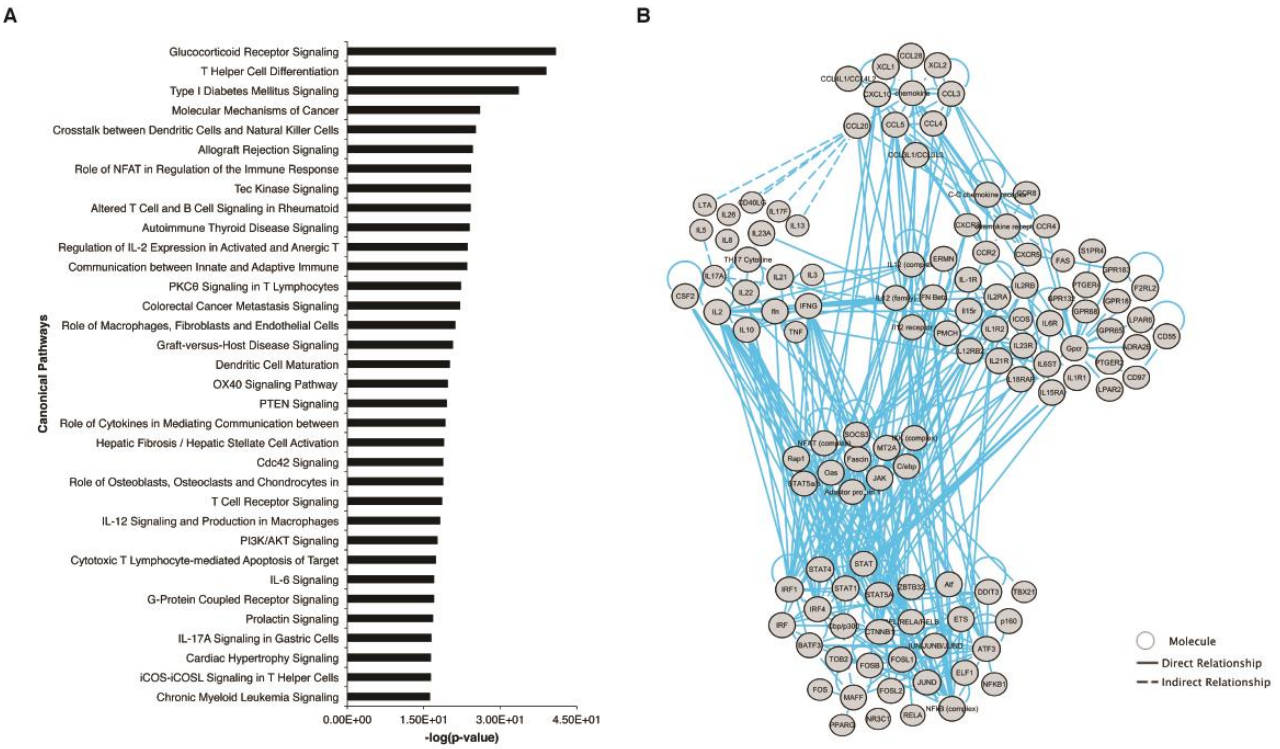

Fig. S15. Enriched canonical pathways and network analysis. (**A**) The top 45 canonical pathways identified as up-regulated in MS tetramer positive samples are plotted as a function of –log(p-value). Canonical pathways were predicted using IPA and the 235-gene signature. (**B**) Fully labeled version of network diagram presented in figure 4C. Molecules are represented by circles and relationships represented by dashed (indirect) or solid (direct) cyan lines.

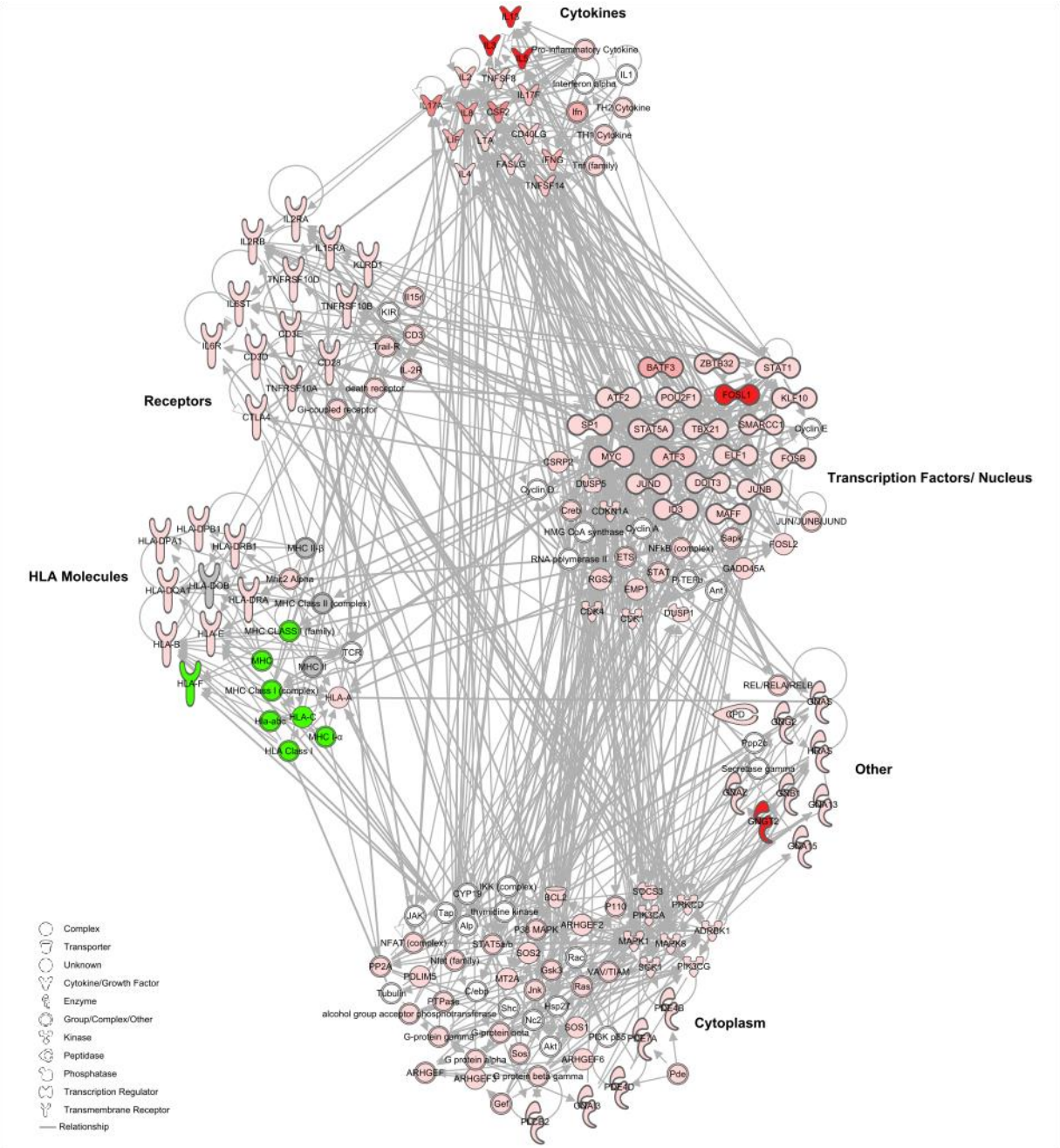

Fig. S16. Additional network analysis. Networks 3-7 from IPA were merged and grouped according to molecular function. Molecules are colored according to logFC(MS^+/MS^-), where red is increased in MS tetramer positive and green in decreased in MS tetramer positive.

Table S1: Patients with MS and paired healthy subjects Information.

Sample ID	Age	Gender	EDSS Score	Date of first Symptoms	Treatment	Experiment used	T cell libraries generated		
							Naïve	CCR6$^-$ memory	CCR6$^+$ memory
MS128	35	F	4.0	2011	Untreated	T cell library assay	192	192	192
MS168	53	F	N.I.	N.I.	Untreated	T cell library assay	96	96	96
MS174	45	F	1.0	2005	Untreated	T cell library assay	96	96	96
MS176	53	F	1.0	N.I.	Untreated	T cell library assay	96	96	96
MS189	61	F	1.0	2007	Untreated	T cell library assay	96	96	96
MS192	33	M	1.0	2011	Untreated	T cell library assay	96	96	96
MS224	25	F	1.0	2011	Untreated	T cell library assay	96	96	96
MS226	57	F	2.0	2011	Untreated	T cell library assay	96	96	96
MS234	20	F	1.0	2011	Untreated	T cell library assay	96	96	96
MS339	29	M	1.0	2011	Untreated	T cell library assay	96	96	96
MS464	37	M	2.0	2014	Untreated	T cell library assay	96	96	192
MS466	51	F	1.0	2000	Untreated	T cell library assay	96	96	192
MS468	33	F	2.0	2012	Untreated	T cell library assay	96	96	192
MS470	47	M	1.0	1995	Untreated	T cell library assay	96	96	192
MS472	32	F	3.0	2014	Untreated	T cell library assay	96	96	192
MS333	29	M	1.0	2012	Untreated	Single cell cloning	66	68	192
MS304	39	M	1.0	2002	Untreated	Single cell cloning	0	0	192
MS361	40	M	1.0	2013	Untreated	Single cell cloning	0	0	192
MS181	64	F	6.5	2000	Untreated	RNA sequencing	0	0	192
MS266	57	F	2.0	1985	Untreated	RNA sequencing	0	0	192
MS386	48	F	6.0	1994	Untreated	RNA sequencing	0	0	192
MS358	50	F	1.0	2013	Untreated	RNA sequencing	0	0	192
MS369	43	M	3.0	2013	Untreated	RNA sequencing	0	0	192

Table S1: Patients with MS and paired healthy subjects Information (continued).

Sample ID	Age	Gender	EDSS Score	Date of first Symptoms	Treatment	Experiment used	T cell libraries generated		
							Naïve	CCR6$^-$ memory	CCR6$^+$ memory
HC292	31	M	N.A.	N.A.	N.A.	T cell library assay	192	192	192
HC304	50	M	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC309	32	M	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC293	25	F	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC326	34	M	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC322	39	M	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC301	27	F	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC310	32	M	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC298	39	F	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC300	54	F	N.A.	N.A.	N.A.	T cell library assay	96	96	96
HC2614	30	M	N.A.	N.A.	N.A.	T cell library assay	96	96	192
HC2623	46	F	N.A.	N.A.	N.A.	T cell library assay	96	96	192
HC2613	22	F	N.A.	N.A.	N.A.	T cell library assay	96	96	192
HC2618	52	M	N.A.	N.A.	N.A.	T cell library assay	96	96	192
HC2622	28	F	N.A.	N.A.	N.A.	T cell library assay	96	96	192
HC226	31	M	N.A.	N.A.	N.A.	Single cell cloning	66	68	192
HC310	32	M	N.A.	N.A.	N.A.	Single cell cloning	0	0	192
HC333	52	M	N.A.	N.A.	N.A.	Single cell cloning	0	0	192
HC391	35	M	N.A.	N.A.	N.A.	RNA sequencing	0	0	192
HC311	32	M	N.A.	N.A.	N.A.	RNA sequencing	0	0	192
HC322	37	M	N.A.	N.A.	N.A.	RNA sequencing	0	0	192
HC330	52	M	N.A.	N.A.	N.A.	RNA sequencing	0	0	192

N.I., no information available;

N.A., not applicable.

Table S2. Myelin peptides and control peptides used in T cell library assays.

Peptides	Sequences	Tetramers
MBP_{85-99}	NPVVHFFKNIVTPRT	DRB1*1501/$MBP_{\mathbf{85-99}}$
$MOG_{222-241}$	VALIICYNWLHRRLAGQFLE	DRB1*1501/$MOG_{\mathbf{222-241}}$
PLP_{30-49}	ALFCGCGHEALTGTEKLIET	DRB1*1501/$PLP_{\mathbf{30-49}}$
$PLP_{129-148}$	QHQAHSLERVCHCLGKWLGH	DRB1*1501/$PLP_{\mathbf{129-148}}$
MOG_{97-109}	RTELLKDAIGEGK	DRB1*0401/MOG_{97-109}
$PLP_{180-199}$	TWTTCQSIAFPSKTSASIGS	DRB1*0401/$PLP_{180-199}$
$HA_{306-318}$	PRYVKQNTLKLAT	/
$P24_{166-179}$	DRFYKTLRAEQASQ	/

Abbreviations: MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; HA, hemagglutinin.

Table S3. Percent variance explained by each principal component.

	C. albicans		No peptide		Myelin peptides	
Condition	Naïve/ $CCR6^-$	$CCR6^+$	Naïve/ $CCR6^-$	$CCR6^+$	Naïve/ $CCR6^-$	$CCR6^+$
PC1	66.91	30.67	48.26	33.57	58.15	33.16
PC2	16.92	23.54	27.00	26.66	21.58	22.41
PC3	12.37	20.68	17.77	16.99	10.80	21.14
PC4	3.48	15.05	5.52	12.89	8.98	14.64
PC5	0.32	10.06	1.45	9.89	0.49	8.64

Table S4. GSEA results (FDR < 0.25) for comparison of MS tetramer positive to MS tetramer negative.

NAME	NES	NOM p-val	FDR q-val
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	5.255	<0.001	<0.001
REACTOME_3_UTR_MEDIATED_TRANSLATIONAL_REGULATION	4.418	<0.001	<0.001
MIPS_RIBOSOME_CYTOPLASMIC	4.120	<0.001	<0.001
MIPS_NOP56P_ASSOCIATED_PRE_RRNA_COMPLEX	3.941	<0.001	<0.001
KEGG_RIBOSOME	3.848	<0.001	<0.001
REACTOME_PEPTIDE_CHAIN_ELONGATION	3.798	<0.001	<0.001
PID_NFAT_TFPATHWAY	3.797	<0.001	<0.001
REACTOME_NONSENSE_MEDIATED_DECAY_ENHANCED_BY_THE_EXON_JUNCTION_COMPLEX	3.676	<0.001	<0.001
BIOCARTA_CYTOKINE_PATHWAY	3.599	<0.001	<0.001
REACTOME_GPCR_LIGAND_BINDING	3.534	<0.001	<0.001
REACTOME_CLASS_A1_RHODOPSIN_LIKE_RECEPTORS	3.497	<0.001	<0.001
REACTOME_TRANSLATION	3.452	<0.001	<0.001
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	3.351	<0.001	<0.001
PID_AP1_PATHWAY	3.319	<0.001	<0.001
PID_ATF2_PATHWAY	3.213	<0.001	<0.001
BIOCARTA_INFLAM_PATHWAY	3.182	<0.001	<0.001
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	3.177	<0.001	<0.001
MIPS_60S_RIBOSOMAL_SUBUNIT_CYTOPLASMIC	3.158	<0.001	<0.001
REACTOME_FORMATION_OF_THE_TERNARY_COMPLEX_AND_SUBSEQUENTLY_THE_43S_COMPLEX	3.140	<0.001	0.0001
REACTOME_GPCR_DOWNSTREAM_SIGNALING	3.112	<0.001	0.0001
REACTOME_INFLUENZA_LIFE_CYCLE	3.064	<0.001	0.0001
REACTOME_SIGNALING_BY_GPCR	3.047	<0.001	0.0001
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	3.043	<0.001	0.0001
PID_TCRCALCIUMPATHWAY	2.993	<0.001	0.0001
KEGG_CHEMOKINE_SIGNALING_PATHWAY	2.878	<0.001	0.0001
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	2.874	<0.001	0.0001
KEGG_ASTHMA	2.804	<0.001	0.0002
PID_IL23PATHWAY	2.790	<0.001	0.0002
KEGG_ALLOGRAFT_REJECTION	2.772	<0.001	0.0002
PID_REG_GR_PATHWAY	2.769	<0.001	0.0002
KEGG_AUTOIMMUNE_THYROID_DISEASE	2.707	<0.001	0.0005
REACTOME_METABOLISM_OF_PROTEINS	2.691	<0.001	0.0005
KEGG_MAPK_SIGNALING_PATHWAY	2.690	<0.001	0.0005
KEGG_JAK_STAT_SIGNALING_PATHWAY	2.663	<0.001	0.0007
BIOCARTA_NKT_PATHWAY	2.636	<0.001	0.0008
KEGG_TYPE_I_DIABETES_MELLITUS	2.636	<0.001	0.0008
REACTOME_METABOLISM_OF_RNA	2.598	<0.001	0.0013
REACTOME_SIGNALING_BY_ILS	2.583	<0.001	0.0013

KEGG_GRAFT_VERSUS_HOST_DISEASE	2.579	<0.001	0.0013
MIPS_40S_RIBOSOMAL_SUBUNIT_CYTOPLASMIC	2.565	<0.001	0.0014
BIOCARTA_MET_PATHWAY	2.534	<0.001	0.0018
REACTOME_SLC_MEDIATED_TRANSMEMBRANE_TRANSPORT	2.505	<0.001	0.0020
BIOCARTA_TFF_PATHWAY	2.497	<0.001	0.0021
REACTOME_METABOLISM_OF_MRNA	2.493	<0.001	0.0022
BIOCARTA_NTHI_PATHWAY	2.484	<0.001	0.0022
KEGG_HEMATOPOIETIC_CELL_LINEAGE	2.466	<0.001	0.0025
BIOCARTA_DC_PATHWAY	2.462	<0.001	0.0025
PID_MYC_ACTIVPATHWAY	2.447	<0.001	0.0028
PID_IL12_2PATHWAY	2.415	<0.001	0.0038
PID_ECADHERIN_STABILIZATION_PATHWAY	2.396	<0.001	0.0044
PID_IL2_STAT5PATHWAY	2.379	0.0019	0.0051
PID_INTEGRIN1_PATHWAY	2.375	<0.001	0.0051
KEGG_PATHWAYS_IN_CANCER	2.371	<0.001	0.0053
REACTOME_TRANSPORT_OF_INORGANIC_CATIONS_ANIONS_AND_AMINO_ACIDS_OLIGOPEPTIDES	2.365	0.0020	0.0055
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	2.359	<0.001	0.0056
PID_HIF1_TFPATHWAY	2.343	0.0019	0.0062
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	2.332	<0.001	0.0064
KEGG_SMALL_CELL_LUNG_CANCER	2.305	0.0020	0.0077
BIOCARTA_AKT_PATHWAY	2.272	0.0019	0.0095
REACTOME_PLATELET_ACTIVATION_SIGNALING_AND_AGGREGATION	2.270	0.0060	0.0094
MIPS_EIF3_COMPLEX	2.265	<0.001	0.0097
MIPS_CHUK_NFKB2_REL_IKBKG_SPAG9_NFKB1_NFKBIE_COPB2_TNIP1_NFKBIA_RELA_TNIP2_COMPLEX	2.264	<0.001	0.0096
BIOCARTA_GCR_PATHWAY	2.211	0.0020	0.0141
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM	2.209	0.0019	0.0140
PID_AVB3_OPN_PATHWAY	2.207	<0.001	0.0140
KEGG_ADHERENS_JUNCTION	2.191	<0.001	0.0154
KEGG_SPLICEOSOME	2.187	0.0020	0.0156
PID_IL6_7PATHWAY	2.183	<0.001	0.0157
PID_PDGFRBPATHWAY	2.173	0.0020	0.0165
PID_CD8TCRDOWNSTREAMPATHWAY	2.170	0.0058	0.0165
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS	2.166	<0.001	0.0166
PID_AJDISS_2PATHWAY	2.159	0.0020	0.0170
PID_NFKAPPABCANONICALPATHWAY	2.156	0.0062	0.0171
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	2.143	0.0019	0.0185
BIOCARTA_IGF1_PATHWAY	2.139	<0.001	0.0188
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	2.134	<0.001	0.0191
PID_IL12_STAT4PATHWAY	2.130	0.0038	0.0194
MIPS_TRBP_CONTAINING_COMPLEX_1	2.115	0.0019	0.0213
PID_IL2_1PATHWAY	2.114	<0.001	0.0211
MIPS_17S_U2_SNRNP	2.109	0.0020	0.0218

BIOCARTA_IL2RB_PATHWAY	2.104	0.0042	0.0220
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	2.104	<0.001	0.0219
PID_ANGIOPOIETINRECEPTOR_PATHWAY	2.102	0.0020	0.0217
PID_ER_NONGENOMIC_PATHWAY	2.101	<0.001	0.0215
PID_SYNDECAN_2_PATHWAY	2.099	0.0020	0.0215
REACTOME_AMINO_ACID_AND_OLIGOPEPTIDE_SLC_TRANSPORTERS	2.089	0.0021	0.0228
KEGG_ECM_RECEPTOR_INTERACTION	2.089	<0.001	0.0226
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	2.082	0.0039	0.0233
BIOCARTA_TOB1_PATHWAY	2.079	<0.001	0.0234
PID_CD40_PATHWAY	2.075	0.0038	0.0238
KEGG_CHRONIC_MYELOID_LEUKEMIA	2.073	0.0019	0.0239
MIPS_TNF_ALPHA_NF_KAPPA_B_SIGNALING_COMPLEX	2.072	0.0041	0.0238
PID_REELINPATHWAY	2.071	0.0019	0.0236
PID_P53REGULATIONPATHWAY	2.066	<0.001	0.0242
SIG_CD40PATHWAYMAP	2.066	0.0019	0.0240
PID_KITPATHWAY	2.052	0.0040	0.0258
BIOCARTA_INSULIN_PATHWAY	2.050	0.0102	0.0261
PID_EPOPATHWAY	2.049	0.0020	0.0259
REACTOME_GROWTH_HORMONE_RECEPTOR_SIGNALING	2.030	0.0020	0.0292
PID_TELOMERASEPATHWAY	2.027	0.0020	0.0295
REACTOME_HEMOSTASIS	2.026	0.0039	0.0294
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	2.023	0.0020	0.0296
PID_ERBB1_RECEPTOR_PROXIMAL_PATHWAY	2.022	0.0020	0.0295
KEGG_TYPE_II_DIABETES_MELLITUS	2.016	0.0061	0.0306
PID_IL27PATHWAY	2.013	0.0126	0.0310
KEGG_FOCAL_ADHESION	2.011	0.0062	0.0314
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	2.005	0.0063	0.0320
KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY	2.005	0.0059	0.0319
REACTOME_AXON_GUIDANCE	2.003	0.0038	0.0319
PID_SHP2_PATHWAY	2.002	0.0040	0.0318
PID_FRA_PATHWAY	1.998	0.0105	0.0322
PID_AVB3_INTEGRIN_PATHWAY	1.994	0.0058	0.0329
BIOCARTA_NFAT_PATHWAY	1.986	0.0039	0.0339
BIOCARTA_41BB_PATHWAY	1.986	0.0042	0.0336
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	1.966	0.0019	0.0375
KEGG_ARGININE_AND_PROLINE_METABOLISM	1.963	0.0062	0.0378
REACTOME_NFKB_AND_MAP_KINASES_ACTIVATION_MEDIATED_BY_TLR4_SIGNALING_REPERTOIRE	1.958	0.0059	0.0385
REACTOME_IL_3_5_AND_GM_CSF_SIGNALING	1.958	0.0079	0.0383
REACTOME_TRIGLYCERIDE_BIOSYNTHESIS	1.955	0.0104	0.0385
KEGG_LEISHMANIA_INFECTION	1.954	0.0039	0.0385
REACTOME_MYD88_MAL_CASCADE_INITIATED_ON_PLASMA_MEMBRANE	1.945	0.0120	0.0406
REACTOME_TRANSPORT_OF_MATURE_TRANSCRIPT_TO_CYTOPLASM	1.933	0.0108	0.0430

PID_LYSOPHOSPHOLIPID_PATHWAY	1.932	0.0084	0.0427
BIOCARTA_PPARA_PATHWAY	1.928	0.0141	0.0435
REACTOME_TAK1_ACTIVATES_NFKB_BY_PHOSPHORYLATION_AND_ACTIVATION_OF_IKKS_COMPLEX	1.927	0.0173	0.0435
BIOCARTA_EIF_PATHWAY	1.925	0.0078	0.0436
PID_RHOA_PATHWAY	1.923	0.0060	0.0436
BIOCARTA_IL1R_PATHWAY	1.920	0.0058	0.0441
PID_BCR_5PATHWAY	1.904	0.0122	0.0485
MIPS_LARGE_DROSHA_COMPLEX	1.901	0.0079	0.0489
PID_SYNDECAN_4_PATHWAY	1.899	0.0082	0.0490
REACTOME_TRANSMEMBRANE_TRANSPORT_OF_SMALL_MOLECULES	1.892	0.0081	0.0507
BIOCARTA_BAD_PATHWAY	1.891	0.0134	0.0505
PID_ERBB1_DOWNSTREAM_PATHWAY	1.889	0.0081	0.0506
REACTOME_MAP_KINASE_ACTIVATION_IN_TLR_CASCADE	1.888	0.0064	0.0507
REACTOME_G_ALPHA_Q_SIGNALLING_EVENTS	1.884	0.0065	0.0513
PID_AR_TF_PATHWAY	1.880	0.0156	0.0519
REACTOME_MRNA_PROCESSING	1.878	0.0099	0.0523
PID_IGF1_PATHWAY	1.877	0.0141	0.0521
SIG_INSULIN_RECEPTOR_PATHWAY_IN_CARDIAC_MYOCYTES	1.876	0.0134	0.0522
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	1.870	0.0039	0.0535
REACTOME_INNATE_IMMUNE_SYSTEM	1.865	0.0098	0.0547
KEGG_PROSTATE_CANCER	1.863	0.0100	0.0551
PID_FAK_PATHWAY	1.862	0.0143	0.0548
REACTOME_RESPONSE_TO_ELEVATED_PLATELET_CYTOSOLIC_CA2_	1.854	0.0121	0.0571
REACTOME_ACTIVATED_TLR4_SIGNALLING	1.848	0.0106	0.0588
BIOCARTA_TH1TH2_PATHWAY	1.848	0.0103	0.0584
BIOCARTA_ASBCELL_PATHWAY	1.843	0.0039	0.0597
REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA	1.843	0.0078	0.0594
PID_IL4_2PATHWAY	1.841	0.0099	0.0598
REACTOME_TRAF6_MEDIATED_INDUCTION_OF_NFKB_AND_MAP_KINASES_UPON_TLR7_8_OR_9_ACTIVATION	1.839	0.0243	0.0599
PID_TCPTP_PATHWAY	1.833	0.0138	0.0613
REACTOME_IL_RECEPTOR_SHC_SIGNALING	1.831	0.0121	0.0618
KEGG_VIRAL_MYOCARDITIS	1.816	0.0158	0.0664
REACTOME_CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	1.808	0.0183	0.0692
ST_INTEGRIN_SIGNALING_PATHWAY	1.808	0.0104	0.0688
REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION	1.806	0.0178	0.0688
BIOCARTA_GSK3_PATHWAY	1.799	0.0247	0.0711
PID_IL2_PI3KPATHWAY	1.790	0.0117	0.0743
REACTOME_GASTRIN_CREB_SIGNALLING_PATHWAY_VIA_PKC_AND_MAPK	1.788	0.0144	0.0749
REACTOME_NUCLEOTIDE_BINDING_DOMAIN_LEUCINE_RICH_REPEAT_CONTAINING_RECEPTOR_NLR_SIGNALING_PATHWAYS	1.786	0.0177	0.0753
REACTOME_CLEAVAGE_OF_GROWING_TRANSCRIPT_IN_THE_TERMINATION_REGION_	1.785	0.0253	0.0752
REACTOME_G_ALPHA1213_SIGNALLING_EVENTS	1.785	0.0139	0.0748
MIPS_SPLICEOSOME	1.785	0.0208	0.0744

KEGG_BLADDER_CANCER	1.779	0.0118	0.0764
PID_CDC42_PATHWAY	1.778	0.0223	0.0762
REACTOME_TRIF_MEDIATED_TLR3_SIGNALING	1.777	0.0255	0.0760
PID_TRKRPATHWAY	1.759	0.0241	0.0835
BIOCARTA_CTLA4_PATHWAY	1.757	0.0137	0.0840
BIOCARTA_HER2_PATHWAY	1.751	0.0230	0.0862
BIOCARTA_GATA3_PATHWAY	1.746	0.0249	0.0880
BIOCARTA_ATM_PATHWAY	1.745	0.0224	0.0879
REACTOME_BASIGIN_INTERACTIONS	1.743	0.0158	0.0881
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	1.742	0.0230	0.0880
KEGG_P53_SIGNALING_PATHWAY	1.738	0.0173	0.0895
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	1.737	0.0182	0.0893
BIOCARTA_CD40_PATHWAY	1.737	0.0209	0.0892
REACTOME_JNK_C_JUN_KINASES_PHOSPHORYLATION_AND_ACTIVATION_MEDIATED_BY_ACTIVATED_HUMAN_TAK1	1.734	0.0177	0.0897
REACTOME_TOLL_RECEPTOR_CASCADES	1.732	0.0207	0.0905
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	1.729	0.0195	0.0912
BIOCARTA_P53HYPOXIA_PATHWAY	1.724	0.0280	0.0927
PID_CIRCADIANPATHWAY	1.721	0.0181	0.0940
BIOCARTA_PTDINS_PATHWAY	1.719	0.0321	0.0941
MIPS_TNF_ALPHA_NF_KAPPA_B_SIGNALING_COMPLEX_3	1.705	0.0327	0.1007
PID_GMCSF_PATHWAY	1.705	0.0290	0.1002
ST_PHOSPHOINOSITIDE_3_KINASE_PATHWAY	1.692	0.0195	0.1060
REACTOME_FORMATION_OF_TUBULIN_FOLDING_INTERMEDIATES_BY_CCT_TRIC	1.688	0.0298	0.1076
PID_CXCR4_PATHWAY	1.687	0.0261	0.1076
BIOCARTA_RANKL_PATHWAY	1.679	0.0342	0.1114
PID_THROMBIN_PAR1_PATHWAY	1.675	0.0408	0.1130
BIOCARTA_IL6_PATHWAY	1.673	0.0351	0.1133
REACTOME_PLATELET_AGGREGATION_PLUG_FORMATION	1.673	0.0192	0.1131
REACTOME_MRNA_SPLICING	1.671	0.0395	0.1133
ST_T_CELL_SIGNAL_TRANSDUCTION	1.671	0.0329	0.1130
KEGG_PANCREATIC_CANCER	1.671	0.0370	0.1125
BIOCARTA_TALL1_PATHWAY	1.667	0.0348	0.1136
PID_RET_PATHWAY	1.659	0.0235	0.1173
PID_VEGFR1_2_PATHWAY	1.652	0.0439	0.1209
REACTOME_NCAM_SIGNALING_FOR_NEURITE_OUT_GROWTH	1.652	0.0308	0.1206
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	1.651	0.0381	0.1204
REACTOME_IL_2_SIGNALING	1.651	0.0416	0.1200
PID_AR_NONGENOMIC_PATHWAY	1.649	0.0375	0.1201
ST_B_CELL_ANTIGEN_RECEPTOR	1.647	0.0371	0.1210
BIOCARTA_RACCYCD_PATHWAY	1.642	0.0304	0.1237
REACTOME_CLASS_B_2_SECRETIN_FAMILY_RECEPTORS	1.633	0.0359	0.1285
REACTOME_PERK_REGULATED_GENE_EXPRESSION	1.624	0.0475	0.1336

PID_INSULIN_GLUCOSE_PATHWAY	1.624	0.0372	0.1331
PID_CERAMIDE_PATHWAY	1.621	0.0498	0.1343
PID_NCADHERINPATHWAY	1.620	0.0428	0.1346
SIG_REGULATION_OF_THE_ACTIN_CYTOSKELETON_BY_RHO_GTPASES	1.617	0.0493	0.1358
REACTOME_DEVELOPMENTAL_BIOLOGY	1.615	0.0432	0.1361
MIPS_DGCR8_MULTIPROTEIN_COMPLEX	1.608	0.0505	0.1402
REACTOME_ACTIVATED_TAK1_MEDIATES_P38_MAPK_ACTIVATION	1.603	0.0589	0.1431
BIOCARTA_IL3_PATHWAY	1.599	0.0503	0.1447
ST_ERK1_ERK2_MAPK_PATHWAY	1.599	0.0397	0.1441
PID_IL8CXCR1_PATHWAY	1.599	0.0443	0.1437
BIOCARTA_HSP27_PATHWAY	1.598	0.0367	0.1436
PID_ARF6DOWNSTREAMPATHWAY	1.593	0.0521	0.1465
BIOCARTA_TNFR2_PATHWAY	1.588	0.0306	0.1493
PID_THROMBIN_PAR4_PATHWAY	1.587	0.0614	0.1488
PID_LYMPHANGIOGENESIS_PATHWAY	1.583	0.0581	0.1511
KEGG_GAP_JUNCTION	1.582	0.0619	0.1513
REACTOME_ACTIVATION_OF_CHAPERONES_BY_ATF6_ALPHA	1.581	0.0472	0.1515
BIOCARTA_CDMAC_PATHWAY	1.576	0.0535	0.1542
BIOCARTA_GLEEVEC_PATHWAY	1.576	0.0339	0.1539
REACTOME_IMMUNE_SYSTEM	1.564	0.0469	0.1621
BIOCARTA_CREB_PATHWAY	1.563	0.0477	0.1625
ST_GA13_PATHWAY	1.560	0.0581	0.1636
REACTOME_NOD1_2_SIGNALING_PATHWAY	1.558	0.0531	0.1649
REACTOME_MRNA_3_END_PROCESSING	1.557	0.0373	0.1643
BIOCARTA_AGR_PATHWAY	1.557	0.0639	0.1638
BIOCARTA_MAPK_PATHWAY	1.556	0.0593	0.1641
REACTOME_TRANSPORT_TO_THE_GOLGI_AND_SUBSEQUENT_MODIFICATION	1.550	0.0536	0.1679
PID_EPHA2_FWDPATHWAY	1.550	0.0435	0.1674
REACTOME_G_ALPHA_S_SIGNALLING_EVENTS	1.549	0.0509	0.1670
PID_FGF_PATHWAY	1.548	0.0663	0.1672
BIOCARTA_ALK_PATHWAY	1.543	0.0631	0.1704
REACTOME_RIG_I_MDA5_MEDIATED_INDUCTION_OF_IFN_ALPHA_BETA_PATHWAYS	1.540	0.0635	0.1724
MIPS_KINASE_MATURATION_COMPLEX_1	1.534	0.0781	0.1759
PID_INTEGRIN_A4B1_PATHWAY	1.533	0.0507	0.1766
REACTOME_GLUCOSE_TRANSPORT	1.529	0.0586	0.1788
MIPS_CDC5L_COMPLEX	1.524	0.0744	0.1819
BIOCARTA_TPO_PATHWAY	1.517	0.0774	0.1871
KEGG_RENAL_CELL_CARCINOMA	1.513	0.0830	0.1898
BIOCARTA_ECM_PATHWAY	1.509	0.0597	0.1923
BIOCARTA_ARF_PATHWAY	1.508	0.0758	0.1920
PID_CD8TCRPATHWAY	1.508	0.0669	0.1913
PID_MAPKTRKPATHWAY	1.507	0.0553	0.1912
BIOCARTA_RNA_PATHWAY	1.507	0.0646	0.1906

PID_LKB1_PATHWAY	1.507	0.0593	0.1903
PID_ECADHERIN_NASCENTAJ_PATHWAY	1.505	0.0762	0.1910
BIOCARTA_MPR_PATHWAY	1.503	0.0752	0.1920
REACTOME_INTEGRIN_ALPHAIIB_BETA3_SIGNALING	1.498	0.0679	0.1952
PID_MET_PATHWAY	1.494	0.0827	0.1985
MIPS_HCF_1_COMPLEX	1.493	0.0676	0.1984
PID_A6B1_A6B4_INTEGRIN_PATHWAY	1.492	0.0777	0.1989
PID_NFAT_3PATHWAY	1.488	0.0955	0.2018
BIOCARTA_IGF1R_PATHWAY	1.478	0.0738	0.2093
BIOCARTA_ETS_PATHWAY	1.475	0.0774	0.2116
PID_NETRIN_PATHWAY	1.472	0.0864	0.2138
REACTOME_TRNA_AMINOACYLATION	1.471	0.0823	0.2130
BIOCARTA_ERK_PATHWAY	1.469	0.0929	0.2144
REACTOME_L1CAM_INTERACTIONS	1.469	0.0720	0.2140
REACTOME_PROLONGED_ERK_ACTIVATION_EVENTS	1.466	0.1004	0.2154
BIOCARTA_PDGF_PATHWAY	1.465	0.0857	0.2152
PID_PI3KPLCTRKPATHWAY	1.465	0.0630	0.2147
REACTOME_AMYLOIDS	1.464	0.0918	0.2144
PID_P53DOWNSTREAMPATHWAY	1.459	0.0829	0.2191
KEGG_DILATED_CARDIOMYOPATHY	1.455	0.0984	0.2218
REACTOME_GLYCOLYSIS	1.454	0.0761	0.2224
REACTOME_RNA_POL_I_PROMOTER_OPENING	1.454	0.0923	0.2217
PID_IL1PATHWAY	1.452	0.0812	0.2231
PID_ECADHERIN_KERATINOCYTE_PATHWAY	1.451	0.1143	0.2224
REACTOME_MAPK_TARGETS_NUCLEAR_EVENTS_MEDIATED_BY_MAP_KINASES	1.451	0.0822	0.2216
REACTOME_ACTIVATION_OF_GENES_BY_ATF4	1.451	0.0873	0.2213
PID_IL8CXCR2_PATHWAY	1.450	0.1075	0.2216
BIOCARTA_IL2_PATHWAY	1.445	0.1091	0.2252
REACTOME_NRAGE_SIGNALS_DEATH_THROUGH_JNK	1.443	0.0870	0.2261
REACTOME_THROMBIN_SIGNALLING_THROUGH_PROTEINASE_ACTIVATED_RECEPTORS_PAR S	1.442	0.0780	0.2268
REACTOME_IL1_SIGNALING	1.438	0.1025	0.2295
REACTOME_TRANSPORT_OF_MATURE_MRNA_DERIVED_FROM_AN_INTRONLESS_TRANSCRIP T	1.437	0.1020	0.2302
BIOCARTA_INTEGRIN_PATHWAY	1.431	0.0976	0.2350
MIPS_TNF_ALPHA_NF_KAPPA_B_SIGNALING_COMPLEX_6	1.429	0.0958	0.2356
REACTOME_DOWNSTREAM_TCR_SIGNALING	1.427	0.1029	0.2369
ST_P38_MAPK_PATHWAY	1.426	0.1010	0.2364
PID_IFNGPATHWAY	1.426	0.0911	0.2362
BIOCARTA_PAR1_PATHWAY	1.425	0.1036	0.2357
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	1.423	0.1018	0.2370
KEGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTION	1.420	0.0937	0.2395
ST_DIFFERENTIATION_PATHWAY_IN_PC12_CELLS	1.419	0.1086	0.2394
PID_SMAD2_3NUCLEARPATHWAY	1.417	0.1033	0.2400

BIOCARTA_TCR_PATHWAY	1.414	0.0961	0.2424
BIOCARTA_KERATINOCYTE_PATHWAY	1.414	0.0984	0.2416
BIOCARTA_NKCELLS_PATHWAY	1.413	0.1130	0.2413
REACTOME_ANTIGEN_PRESENTATION_FOLDING_ASSEMBLY_AND_PEPTIDE_LOADING_OF_CLASS_I_MHC	1.412	0.1083	0.2419
PID_S1P_S1P4_PATHWAY	1.410	0.1022	0.2431
REACTOME_INTERFERON_GAMMA_SIGNALING	1.405	0.1040	0.2473
MIPS_TNF_ALPHA_NF_KAPPA_B_SIGNALING_COMPLEX_5	1.405	0.0935	0.2466
REACTOME_RNA_POL_II_TRANSCRIPTION	1.404	0.1300	0.2469
BIOCARTA_HCMV_PATHWAY	1.404	0.0943	0.2462
REACTOME_NUCLEAR_RECEPTOR_TRANSCRIPTION_PATHWAY	1.402	0.1098	0.2469
PID_PRLSIGNALINGEVENTSPATHWAY	1.400	0.1138	0.2480
BIOCARTA_BIOPEPTIDES_PATHWAY	1.400	0.1133	0.2477
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	1.398	0.1063	0.2487

Table S5. GSEA results (FDR < 0.25) for comparison of healthy control tetramer positive to healthy control tetramer negative.

NAME	NES	NOM p-val	FDR q-val
REACTOME_SRP_DEPENDENT_COTRANSLATIONAL_PROTEIN_TARGETING_TO_MEMBRANE	4.5784	<0.001	<0.001
REACTOME_PEPTIDE_CHAIN_ELONGATION	4.4660	<0.001	<0.001
REACTOME_TRANSLATION	4.4433	<0.001	<0.001
MIPS_RIBOSOME_CYTOPLASMIC	4.4039	<0.001	<0.001
KEGG_RIBOSOME	4.3425	<0.001	<0.001
REACTOME_METABOLISM_OF_PROTEINS	4.3121	<0.001	<0.001
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	4.2863	<0.001	<0.001
REACTOME_3_UTR_MEDIATED_TRANSLATIONAL_REGULATION	4.2010	<0.001	<0.001
REACTOME_INFLUENZA_LIFE_CYCLE	3.9434	<0.001	<0.001
REACTOME_METABOLISM_OF_MRNA	3.9010	<0.001	<0.001
REACTOME_METABOLISM_OF_RNA	3.6973	<0.001	<0.001
REACTOME_NONSENSE_MEDIATED_DECAY_ENHANCED_BY_THE_EXON_JUNCTION_COMPLEX	3.6825	<0.001	<0.001
MIPS_NOP56P_ASSOCIATED_PRE_RRNA_COMPLEX	3.2647	<0.001	<0.001
MIPS_60S_RIBOSOMAL_SUBUNIT_CYTOPLASMIC	3.1934	<0.001	<0.001
REACTOME_ACTIVATION_OF_THE_MRNA_UPON_BINDING_OF_THE_CAP_BINDING_COMPLEX_AND_EIFS_AND_SUBSEQUENT_BINDING_TO_43S	2.9852	<0.001	0.000 2
REACTOME_FORMATION_OF_THE_TERNARY_COMPLEX_AND_SUBSEQUENTLY_THE_43S_COMPLEX	2.9110	<0.001	0.000 2
MIPS_40S_RIBOSOMAL_SUBUNIT_CYTOPLASMIC	2.8496	<0.001	0.000 5
REACTOME_IMMUNE_SYSTEM	2.6550	<0.001	0.001 4
REACTOME_ADAPTIVE_IMMUNE_SYSTEM	2.5564	<0.001	0.003 3
MIPS_TRBP_CONTAINING_COMPLEX_1	2.5334	<0.001	0.003 8
REACTOME_ANTIGEN_PROCESSING_CROSS_PRESENTATION	2.4612	<0.001	0.007 2
PID_CXCR4_PATHWAY	2.4525	<0.001	0.007 4
KEGG_PARKINSONS_DISEASE	2.4241	<0.001	0.008 3
REACTOME_SIGNALING_BY_WNT	2.3765	0.0020	0.011 1
REACTOME_CTLA4_INHIBITORY_SIGNALING	2.3647	<0.001	0.011 7
REACTOME_MRNA_SPLICING	2.3517	0.0019	0.012 6
BIOCARTA_ERK_PATHWAY	2.3293	<0.001	0.014 0
REACTOME_INTERFERON_SIGNALING	2.3281	<0.001	0.013 6
REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1_APC_C	2.3079	<0.001	0.015 2
REACTOME_CLASS_I_MHC_MEDIATED_ANTIGEN_PROCESSING_PRESENTATION	2.3024	<0.001	0.015 3
REACTOME_ER_PHAGOSOME_PATHWAY	2.3011	0.0020	0.015 0
REACTOME_APC_C_CDC20_MEDIATED_DEGRADATION_OF_MITOTIC_PROTEINS	2.2783	<0.001	0.016 6

REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA	2.2775	<0.001	0.016 2
REACTOME_MRNA_PROCESSING	2.2308	0.0020	0.021 7
MIPS_55S_RIBOSOME_MITOCHONDRIAL	2.1984	<0.001	0.027 0
REACTOME_CELL_CYCLE_MITOTIC	2.1896	0.0043	0.028 0
KEGG_OXIDATIVE_PHOSPHORYLATION	2.1375	0.0020	0.038 4
PID_TELOMERASEPATHWAY	2.1303	0.0021	0.039 4
REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS	2.1297	0.0040	0.038 5
REACTOME_COSTIMULATION_BY_THE_CD28_FAMILY	2.0994	0.0020	0.045 0
REACTOME_MITOTIC_PROMETAPHASE	2.0919	<0.001	0.046 0
KEGG_SPLICEOSOME	2.0764	0.0020	0.049 6
REACTOME_MEMBRANE_TRAFFICKING	2.0732	0.0084	0.049 8
MIPS_PA28_20S_PROTEASOME	2.0694	0.0039	0.049 6
REACTOME_P53_INDEPENDENT_G1_S_DNA_DAMAGE_CHECKPOINT	2.0686	0.0041	0.048 6
REACTOME_APOPTOSIS	2.0643	0.0036	0.048 7
REACTOME_DESTABILIZATION_OF_MRNA_BY_AUF1_HNRNP_D0	2.0556	<0.001	0.050 8
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT_ATP_SYNTHESIS_BY_CHEMIOSMOTIC_COUPLING_AND_HEAT_PRODUCTION_BY_UNCOUPLING_PROTEINS_	2.0501	0.0061	0.051 2
KEGG_PRIMARY_IMMUNODEFICIENCY	2.0356	0.0042	0.055 4
REACTOME_HIV_INFECTION	2.0339	0.0021	0.055 0
REACTOME_REGULATION_OF_MITOTIC_CELL_CYCLE	2.0319	0.0041	0.054 6
REACTOME_REGULATION_OF_MRNA_STABILITY_BY_PROTEINS_THAT_BIND_AU_RICH_ELEMENTS	2.0100	0.0039	0.061 2
MIPS_F1F0_ATP_SYNTHASE_MITOCHONDRIAL	1.9992	0.0019	0.063 8
MIPS_C_COMPLEX_SPLICEOSOME	1.9984	<0.001	0.062 8
REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1	1.9931	0.0021	0.064 0
REACTOME_MRNA_SPLICING_MINOR_PATHWAY	1.9904	0.0040	0.063 7
REACTOME_SYNTHESIS_OF_SUBSTRATES_IN_N_GLYCAN_BIOSYTHESIS	1.9857	0.0058	0.064 4
MIPS_26S_PROTEASOME	1.9680	0.0057	0.070 1
REACTOME_REGULATION_OF_APOPTOSIS	1.9583	0.0083	0.073 3
REACTOME_CROSS_PRESENTATION_OF_SOLUBLE_EXOGENOUS_ANTIGENS_ENDOSOMES	1.9569	0.0041	0.072 7
REACTOME_CLEAVAGE_OF_GROWING_TRANSCRIPT_IN_THE_TERMINATION_REGION_	1.9561	0.0097	0.071 9
REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G	1.9543	0.0059	0.071 6
REACTOME_INTERFERON_GAMMA_SIGNALING	1.9356	0.0083	0.078 6
REACTOME_CELL_CYCLE_CHECKPOINTS	1.9337	0.0122	0.078 2
REACTOME_APC_C_CDH1_MEDIATED_DEGRADATION_OF_CDC20_AND_OTHER_APC_C_CDH1_TARGETED_PROTEINS_IN_LATE_MITOSIS_EARLY_G1	1.9316	0.0061	0.077 8
MIPS_20S_PROTEASOME	1.9260	0.0080	0.079 1
REACTOME_DEADENYLATION_OF_MRNA	1.9129	0.0061	0.084 4

REACTOME_AUTODEGRADATION_OF_THE_E3_UBIQUITIN_LIGASE_COP1	1.9110	0.0122	0.084 2
REACTOME_FORMATION_OF_ATP_BY_CHEMIOSMOTIC_COUPLING	1.9053	0.0038	0.085 7
REACTOME_ASSEMBLY_OF_THE_PRE_REPLICATIVE_COMPLEX	1.8950	0.0140	0.089 6
REACTOME_PREFOLDIN_MEDIATED_TRANSFER_OF_SUBSTRATE_TO_CCT_TRIC	1.8841	0.0109	0.094 4
REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE_ODC	1.8813	0.0196	0.094 5
REACTOME_ANTIGEN_PRESENTATION_FOLDING_ASSEMBLY_AND_PEPTIDE_LOADING_OF_CLASS_I_MHC	1.8812	0.0101	0.093 3
REACTOME_PROTEIN_FOLDING	1.8799	0.0118	0.092 7
MIPS_SPLICEOSOME	1.8724	0.0096	0.095 5
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	1.8644	0.0102	0.098 0
REACTOME_FORMATION_OF_TUBULIN_FOLDING_INTERMEDIATES_BY_CCT_TRIC	1.8609	0.0061	0.098 4
REACTOME_CELL_CYCLE	1.8472	0.0180	0.104 2
REACTOME_APC_CDC20_MEDIATED_DEGRADATION_OF_NEK2A	1.8331	0.0220	0.111 3
REACTOME_CDK_MEDIATED_PHOSPHORYLATION_AND_REMOVAL_OF_CDC6	1.8210	0.0104	0.117 7
REACTOME_ANTIGEN_PROCESSING_UBIQUITINATION_PROTEASOME_DEGRADATION	1.8157	0.0142	0.119 9
KEGG_PROTEASOME	1.8124	0.0101	0.120 7
REACTOME_P53_DEPENDENT_G1_DNA_DAMAGE_RESPONSE	1.8077	0.0161	0.121 9
REACTOME_LOSS_OF_NLP_FROM_MITOTIC_CENTROSOMES	1.7845	0.0136	0.136 8
REACTOME_MRNA_3_END_PROCESSING	1.7772	0.0044	0.140 4
MIPS_PA700_20S_PA28_COMPLEX	1.7735	0.0123	0.141 2
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION_	1.7688	0.0288	0.143 2
REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT	1.7638	0.0200	0.145 5
KEGG_NITROGEN_METABOLISM	1.7591	0.0133	0.147 1
KEGG_BLADDER_CANCER	1.7570	0.0232	0.147 2
BIOCARTA_CTLA4_PATHWAY	1.7537	0.0100	0.148 2
REACTOME_RESPIRATORY_ELECTRON_TRANSPORT	1.7530	0.0292	0.147 0
REACTOME_METABOLISM_OF_AMINO_ACIDS_AND_DERIVATIVES	1.7374	0.0218	0.157 8
REACTOME_TRANSPORT_TO_THE_GOLGI_AND_SUBSEQUENT_MODIFICATION	1.7357	0.0343	0.157 3
REACTOME_CYTOKINE_SIGNALING_IN_IMMUNE_SYSTEM	1.7297	0.0196	0.160 1
MIPS_CDC5L_COMPLEX	1.7279	0.0242	0.159 9
KEGG_PROTEIN_EXPORT	1.7275	0.0211	0.158 6
REACTOME_POST_TRANSLATIONAL_PROTEIN_MODIFICATION	1.7270	0.0240	0.157 5
REACTOME_ASPARAGINE_N_LINKED_GLYCOSYLATION	1.7135	0.0252	0.167 4
REACTOME_CHOLESTEROL_BIOSYNTHESIS	1.6956	0.0222	0.181 3
PID_IL3_PATHWAY	1.6934	0.0287	0.181 6
REACTOME_CDT1_ASSOCIATION_WITH_THE_CDC6_ORC_ORIGIN_COMPLEX	1.6927	0.0462	0.180 4

REACTOME_GOLGI_ASSOCIATED_VESICLE_BIOGENESIS	1.6876	0.0205	0.183 8
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	1.6875	0.0342	0.182 1
REACTOME_GENERATION_OF_SECOND_MESSENGER_MOLECULES	1.6854	0.0205	0.182 4
REACTOME_ANTIVIRAL_MECHANISM_BY_IFN_STIMULATED_GENES	1.6847	0.0297	0.181 3
MIPS_39S_RIBOSOMAL_SUBUNIT_MITOCHONDRIAL	1.6812	0.0324	0.183 0
KEGG_CHEMOKINE_SIGNALING_PATHWAY	1.6772	0.0367	0.185 2
REACTOME_IMMUNOREGULATORY_INTERACTIONS_BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	1.6688	0.0400	0.191 3
BIOCARTA_IL2RB_PATHWAY	1.6623	0.0347	0.195 5
REACTOME_MITOTIC_G1_G1_S_PHASES	1.6586	0.0348	0.197 4
MIPS_TNF_ALPHA_NF_KAPPA_B_SIGNALING_COMPLEX_6	1.6576	0.0218	0.196 8
MIPS_28S_RIBOSOMAL_SUBUNIT_MITOCHONDRIAL	1.6572	0.0315	0.195 6
REACTOME_APC_C_CDC20_MEDIATED_DEGRADATION_OF_CYCLIN_B	1.6556	0.0345	0.195 5
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	1.6472	0.0386	0.201 8
REACTOME_INHIBITION_OF_THE_PROTEOLYTIC_ACTIVITY_OF_APC_C_REQUIRED_FOR_THE_ONSET_OF_ANAPHASE_BY_MITOTIC_SPINDLE_CHECKPOINT_COMPONENTS	1.6381	0.0270	0.209 5
BIOCARTA_BCELLSURVIVAL_PATHWAY	1.6252	0.0414	0.221 2
BIOCARTA_PROTEASOME_PATHWAY	1.6242	0.0227	0.220 5
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	1.6228	0.0425	0.220 0
PID_IL8CXCR2_PATHWAY	1.6211	0.0454	0.219 9
REACTOME_CTNNB1_PHOSPHORYLATION_CASCADE	1.6209	0.0387	0.218 2
REACTOME_RNA_POL_II_TRANSCRIPTION	1.6194	0.0417	0.218 1
BIOCARTA_EIF_PATHWAY	1.6178	0.0476	0.218 1
REACTOME_POST_CHAPERONIN_TUBULIN_FOLDING_PATHWAY	1.6122	0.0528	0.222 0
REACTOME_SCFSKP2_MEDIATED_DEGRADATION_OF_P27_P21	1.6112	0.0454	0.221 5
PID_TCR_PATHWAY	1.6094	0.0431	0.221 6
REACTOME_MITOTIC_G2_G2_M_PHASES	1.6030	0.0413	0.226 6
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	1.5905	0.0626	0.238 9
PID_IL6_7PATHWAY	1.5888	0.0422	0.239 0
REACTOME_CONVERSION_FROM_APC_C_CDC20_TO_APC_C_CDH1_IN_LATE_ANAPHASE	1.5860	0.0398	0.240 5
BIOCARTA_MTOR_PATHWAY	1.5772	0.0530	0.248 4
REACTOME_PHOSPHORYLATION_OF_THE_APC_C	1.5754	0.0494	0.248 5
PID_GMCSF_PATHWAY	1.5735	0.0575	0.248 7
KEGG_PROGESTERONE_MEDIATED_OOCYTE_MATURATION	1.5725	0.0447	0.247 8

Table S6: Curated gene signatures corresponding to Figure 4C.

Gene Set	Description	Reference	Gene Symbols
MOG induced EAE[+]	Gene signature comprised of differentially expressed genes in MOG induced EAE in rats.	(*37*)	See Supplementary Table 4.
Th1 signature[+]	Th1-specific gene signature derived from C57BL/6 mice.	(*38*)	See Supplementary Table 4.
Th2 signature[+]	Th2-specific gene signature derived from C57BL/6 mice.	(*38*)	See Supplementary Table 4.
Th17 signature[+]	Th17-specific gene signature derived from C57BL/6 mice.	(*38*)	See Supplementary Table 4.
Pathogenic Th17[+]	Pathogenic signature of Th17 cells derived by Lee et. al.	(*16*)	See Figure 4D for 23-list gene set.
Th17 differential expression[+]	Differentially expressed Th17 genes derived from comparison of Th17 cells induced with TGFb1 and IL-6 versus TGFb3 and IL-6.	(*16*)	See Supplementary Table 1.
Th17 cytokines	Cytokines up-regulated in mouse Th17 cells relative to Th0 cells.	(*39*) Figure 3B	IL23R, IL9, IL17R, IL17A, IL24, IL12RB1, IL17F, LIF, IL10, TNFRSF8, IL21, IL1R1
Th17 combinatorial core	Combinatorial core of transcription factor targets involved in Th17 cell specification.	(*39*) Figure 6D	STAT3, IRF4, RORC, MAF, BATF, FOSL2, CYSLTR2, CCL5, HIF1A, IL18RAP, IL17A, IL17F, CCL20, CCR8, IL2, CXCR3, TBX21, CCL4, CCL3, CCR6, IL1R1, GPR15, IL18R1, IL21RB1, IL7R, IL2RA, LTB4R1, IL12RB2, LTB, IL10RA, SMAD3, IL23A, CCR7, IL24, IL4RA, IL21, CXCL10, IL6RA, IL16
PLP induced EAE[+]	Gene signature comprised of differentially expressed genes in PLP induced EAE (SJL/J mice).	(*35*)	See Supplementary Table 5.
Th17.1 EM selective	Th17.1 effector memory selective genes identified using FACs-sorted and *ex-vivo* stimulated human $CD4^{+}CD25^{-}CRTH2^{-}CCR7^{lo}CCR6^{+}CCR4^{lo}CXCR3^{hi}$ cells.	(40) Table S1	ME1, ENPP1, ADAM23, COLQ, SLC4A10, CA2, SCRN1, ABCB1, CLEC2B, CCR2, DPP4, KLRB1
Th17 EM core	Th17 effector memory core genes identified using FACs-sorted and *ex-vivo* stimulated human $CD4^{+}CD25^{-}CRTH2^{-}CCR7^{lo}CCR6^{+}CCR4^{lo}CXCR3^{hi}$ cells.	(*40*) Table S1	IL17RE, PIK3R6, HLF, CCR6, RORC, CTSH, LRP12, CHN1, HPGD, ADAM12, PTPN13
MDR1 Th17.1 EM in Crohn's patients	*Ex vivo* transcriptional signature of $MDR1^{+}$Th17.1 effector memory cells isolated from Crohn's disease patient lesions.	(*40*) Table S2	ABCB1, ND6, GNLY, CDR1, CCL5, IL2, ADAM23, MALAT1, CA2, LRRN3, IL22, GPR15, DSE, IL18RAP, CCL20, GAB3, PELO, ARHGEF4, CSF2, PDE4DIP, SLC4A10, TUBB2A, MIR221, CDC42EP5, MYBL1, SYTL2, FERMT2, B3GALT2, RPL41, PRF1, PLCB1, TLE1, AUTS2, ERN1, CXCR6, IL23R, LGALS3
Th17 EM selective	Th17.1 EM specific genes of FACs-sorted and *ex-vivo* stimulated human $CD4^{+}CD25^{-}CRTH2^{-}CCR7^{lo}CCR6^{+}CCR4^{lo}CXCR3^{hi}$ cells.	(*40*) Table S1	SHF, IL17A, IL17F, ATP1B1, S100A4, NTRK2, PLD1, PPARG, ITGAE, PAK3, TMOD1, IL26, TIMP4, HRH4, TIMP1

[+]For gene sets derived from rat or mice, gene symbols were mapped using HGNC gene checker.

Table S7. Gene sets used for leading edge analysis to define pathogenic gene signature.

NAME	SIZE	NES	FDR q-val
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	87	5.2552	<0.001
PID_NFAT_TFPATHWAY	39	3.7971	<0.001
BIOCARTA_CYTOKINE_PATHWAY	12	3.5986	<0.001
REACTOME_GPCR_LIGAND_BINDING	53	3.5342	<0.001
REACTOME_CLASS_A1_RHODOPSIN_LIKE_RECEPTORS	43	3.4971	<0.001
PID_AP1_PATHWAY	44	3.3188	<0.001
PID_ATF2_PATHWAY	32	3.2134	<0.001
REACTOME_GPCR_DOWNSTREAM_SIGNALING	106	3.1118	0.0001
REACTOME_SIGNALING_BY_GPCR	139	3.0468	0.0001
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	21	3.0431	0.0001
PID_TCRCALCIUMPATHWAY	20	2.9931	0.0001
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	27	2.8743	0.0001
PID_IL23PATHWAY	24	2.7896	0.0002
PID_REG_GR_PATHWAY	53	2.7694	0.0002
KEGG_TYPE_I_DIABETES_MELLITUS	22	2.6357	0.0008
PID_SYNDECAN_2_PATHWAY	22	2.0995	0.0215
BIOCARTA_TOB1_PATHWAY	16	2.0789	0.0234

Table S8. Summary of networks constructed by IPA from pathogenic signature. Networks 1,2 and 8 were merged for subsequent analysis in figure 4D and networks 3-7 were merged for presentation in figure S15.

Network ID	Score	Number Focus Molecules	Top Diseases and Functions
1	56	29	Cell Signaling, Molecular Transport, Vitamin and Mineral Metabolism
2	41	23	Cellular Development, Hematological System Development and Function, Hematopoiesis
3	38	22	Cellular Development, Cellular Growth and Proliferation, Hematological System Development and Function
4	31	19	Cell Signaling, Molecular Transport, Vitamin and Mineral Metabolism
5	31	19	Endocrine System Disorders, Gastrointestinal Disease, Metabolic Disease
6	31	19	Cell Cycle, Amino Acid Metabolism, Post-Translational Modification
7	31	21	Cell Death and Survival, Cellular Development, Hematopoiesis
8	26	17	Immunological Disease, Inflammatory Disease, Inflammatory Response
9	20	15	Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking
10	17	12	Cell Death and Survival, Hematological System Development and Function, Immune Cell Trafficking
11	16	12	Cell Death and Survival, Cellular Compromise, Cellular Assembly and Organization
12	15	11	Cell Cycle, Gastrointestinal Disease, Organismal Injury and Abnormalities
13	2	1	Cell Death and Survival, Connective Tissue Development and Function, Gene Expression
14	1	1	Cancer, Reproductive System Disease, Lymphoid Tissue Structure and Development