**Supplementary online Materials**

**Figure e-1.** Gating strategy for identifying B cell subsets.

**Figure e-2.** Expression of genes related to cytokines/chemokines, interferon, and inflammatory responses in B cell subsets.

**Figure e-3.** B cell subpopulations in CSF from NMOSD patients.

**Figure e-4.** Frequency of total B cells and subpopulations in CSF and blood in NMOSD patients compared with those in patients with multiple sclerosis and healthy controls.

**Figure e-5.** Geneexpression of IFN-response, inflammation, and exhaustion-related among B cell clusters in NMOSD.

**Figure e-6.** Expression of inflammatory and exhaustion-related genes in B cell clusters across different organs in NMOSD.

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**Table e-11.** Differentially expressed genes in blood ASCs versus CSF ASCs from NMOSD patients.

**Table e-12.** Differentially expressed genes in blood ASCs versus bone marrow ASCs from NMOSD patients.

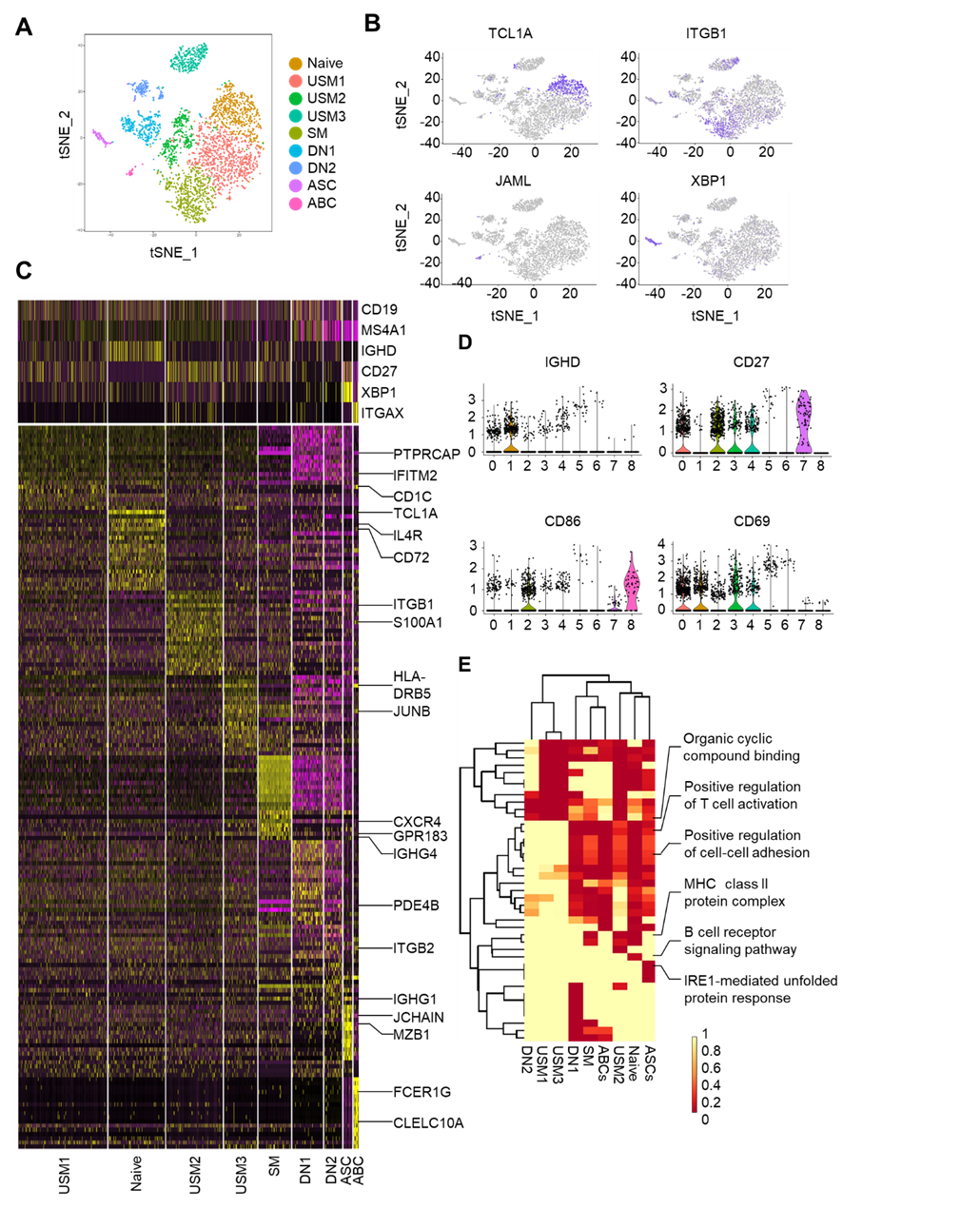
**Figure e-1**

**Figure e-1. Gating strategy for identifying B cell subsets**. Live CD19+ B cells were divided into four canonical subsets based on CD27 and IgD expression: naïve B cells (Q3, IgD+ CD27-), double positive B cells (pre-switch memory B cells) (Q2, IgD+CD27+), memory B cells (switched memory B cells) (Q1, IgD-CD27+) and double negative B cells (Q4, IgD-CD27-). Antibody secreting cells (ASCs) were categorized into two types: CD19+ ASCs (CD19+CD27highCD38high) and CD19- ASCs (CD3-CD14-CD16-CD19-CD56-CD27highCD38high).

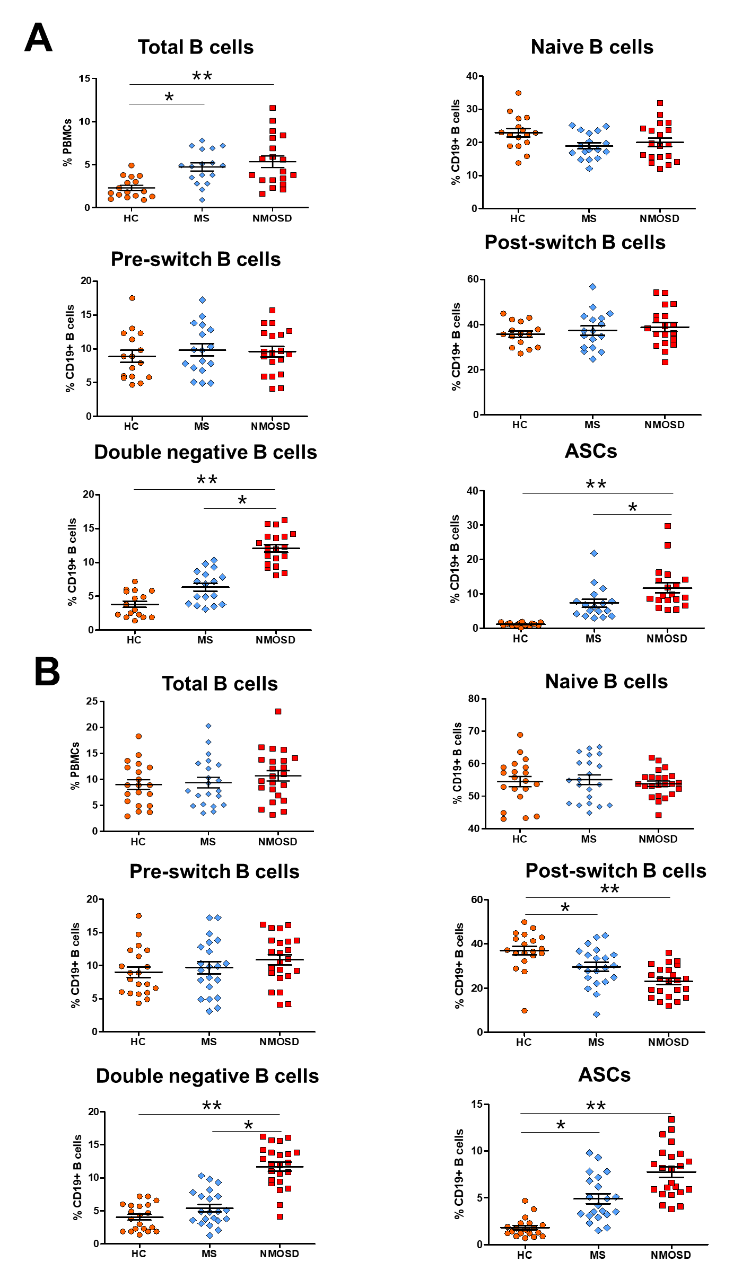
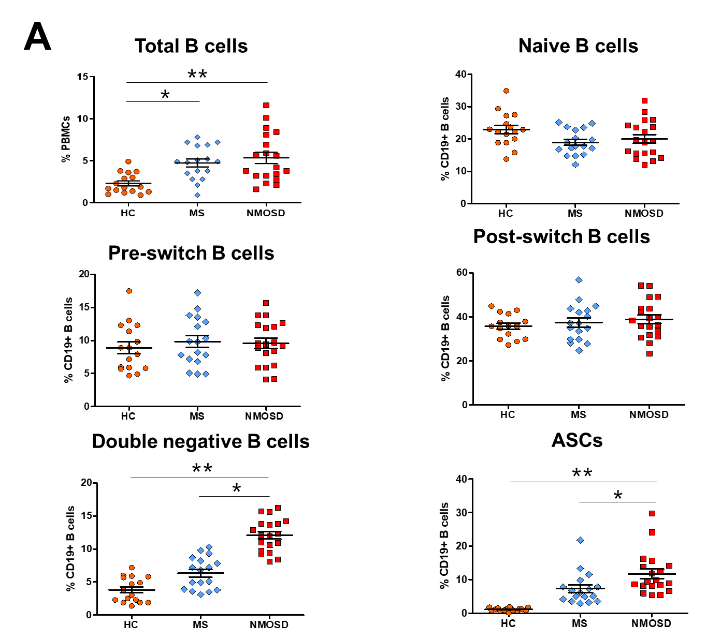
**Figure e-2**

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**Figure e-2**. **Expression of genes related to cytokines/chemokines, interferon, and inflammatory response in B cell subsets.** Cells were pooled from all samples and eight B cell subsets were identified. ASC, antibody secreting cells.

**Figure e-3**

**Figure e-3. B cell subpopulations in CSF from NMOSD patients. A.** Single cell analysis revealed CSF B cells subpopulations in NMOSD. **B.** Heterogeneous distribution of B cell subpopulations in NMOSD CSF. **C.** Heatmap showing the expression of canonical markers that identified B cell subpopulations (top) or genes that were differentially upregulated in each subpopulation (bottom). Scaled expression of discriminative gene sets for B cells was shown. Color scheme is based gene expression abundance from -2 (purple) to 2 (yellow). **D.** Expression patterns of *IGHD*, *CD27*, *CD86* and *CD69* amongst the subsets. C0 cluster, unswitched memory B cells type 1 (USM1) had high levels of *CD24* and *PTPRCAP*, key regulators of T- and B-lymphocyte activation. C1 cluster were naïve B cells. C2 cluster were switched memory B cells. Both cluster C3 (unswitched memory B cells type 2 [USM2]) and C4 (unswitched memory B cells type 3 [USM3]) expressed similar levels of *TNFRSF13C (BAFF-R)*, however, cluster C3 had higher levels of *CD69,* but cluster C4 had unique high levels of *IGHG1*, *IGHG4*, *GPR18*3 and *CXCR4*. Both clusters C5 and C6, were double negative memory B cells that expressed very low levels of *CD86* and *CD24*. Cluster C5 had higher levels of *CD69* and *CD83* than cluster. C6 had low expression of *CD19*, similar expression was observed in the C7 cluster. C7 cluster were antibody secreting cells (ASCs). C8 cluster were age-associated B cells (ABCs). **E.** Pathway enrichment analysis showing the expression of the indicated pathways in CSF B cell subsets in NMOSD patients. Scaled expression of signaling pathway by GO analysis for indicated B cell subpopulations was shown. Color scheme is based on relative expression level among B cell subpopulations from 0 (yellow) to 1 (red).

**Figure e-4**

**Figure e-4.** **Frequency of total B cells and subpopulations in CSF and blood of NMOSD patients, compared with those in** **multiple sclerosis patients and healthy controls.** **A.** Counts of total B cells and indicated B cell subpopulations in CSF. **B**. Counts of total B cells and indicated B cell subpopulations in blood. N = 23 in NMOSD cohort, n = 15 in MS cohort, n = 16 in HCs cohort. HCs, healthy controls. MS, multiple sclerosis. Error bars represent S.E.M. \**p* < 0.05, \*\**p* < 0.01.

**Figure e-5**



**Figure e-5. Gene expression of IFN-response, inflammation and exhaustion-related genes among B cell clusters in NMOSD. A.** Plots showing increased expression of IFN-response genes. **B.** Plots showing inflammation-related genes with higher scores. **C.** Plots showing scores of inflammation-related gene in indicated B cell subsets. **D.** Plots showing increased expression of exhaustion-related genes. **E.** Plots showing scores of exhaustion-related genes of indicated B cell subsets. \**p* < 0.05.

**Figure e-6**

**Figure e-6. Expression of inflammatory and exhaustion-related genes in B cell clusters across different organs in NMOSD. A.** Memory B cells and naïve B cells had higher Z-scores of inflammation-related genes in CSF. **B.** Memory B cells and CD27high ASCs had significantly differences in Z-scores of exhaustion genes. \**p* < 0.05.

**Figure e-7**



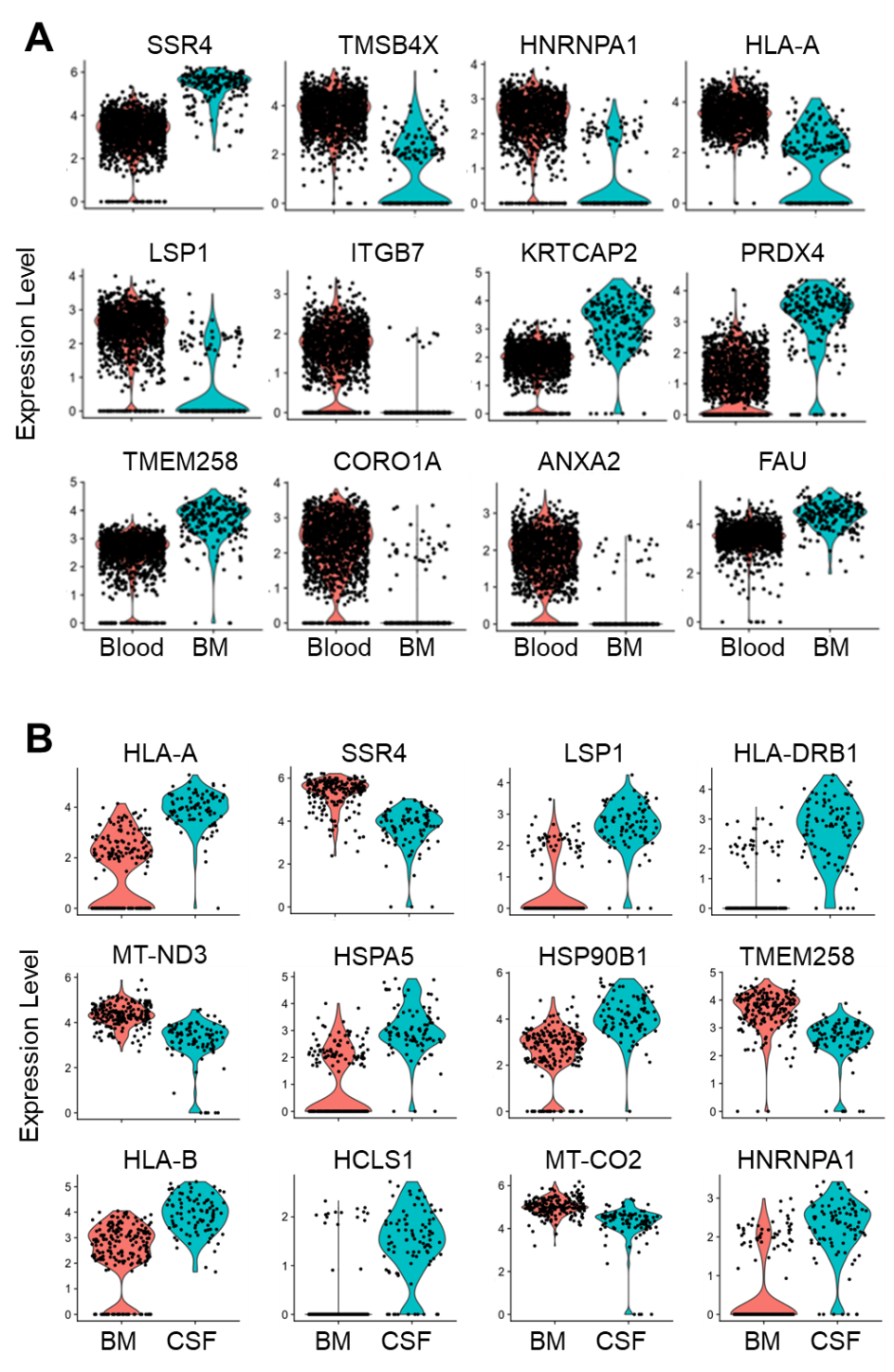
**Figure e-7. Expression of type I interferon-related genes in patients with NMOSD, MS and HCs.** RT-PCR analysis revealed expression of type I interferon-related genes (*IRF4*, *IFI44*, *IFI44L*, *MX1* and *IFI6*) of blood B cells were significantly higher in NMOSD patients (n = 27) compared with MS patients (n = 15) and healthy controls (n = 16). Relative mRNA expression was normalized to house housekeeping gene *GAPDH* and calculated by the 2\_ΔΔCt method. The data were presented as fold change relative to control samples. MS, multiple sclerosis. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**Figure e-8**

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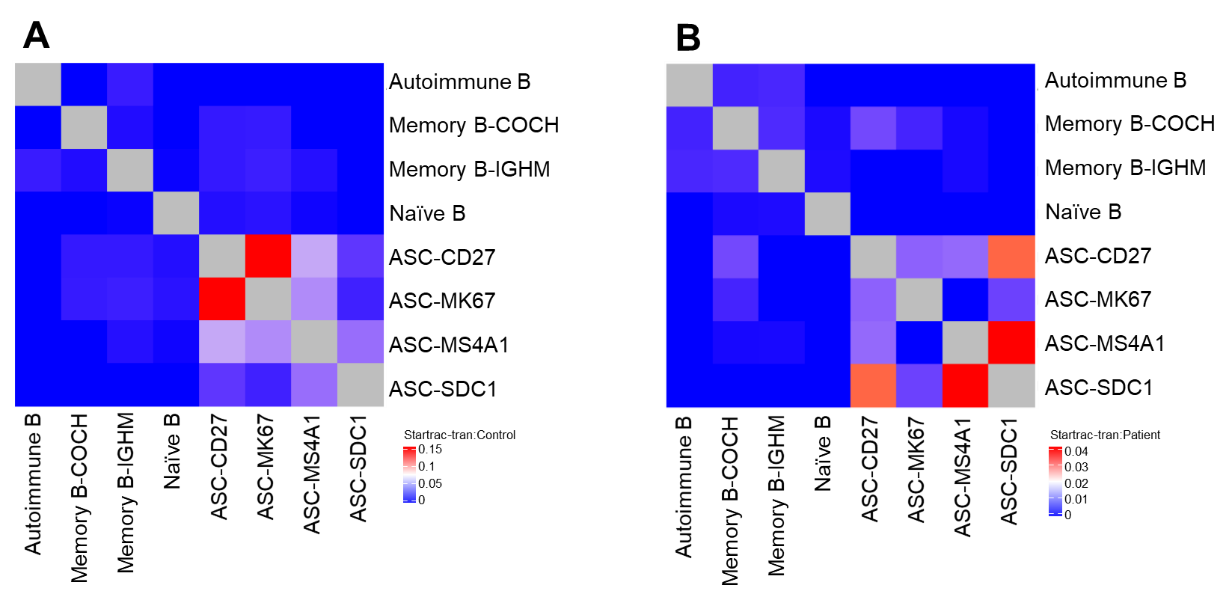
**Figure e-8. Effects of IFN-α on cell viability and apoptosis of transitional B cells.** Purified B cells from NMOSD patients were cultured with indicated concentrations of IFN-α for 24 h, followed by flow cytometry analysis of Annexin V and 7-AAD. **A.** Representative flow cytometry graph showing IFN-α promoted the survival of transitional B cells, **B.** Plot graph showing the effects of IFN-α in the cell viability and apoptosis of transitional B cells. n = 6 per group. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

**Figure e-9**



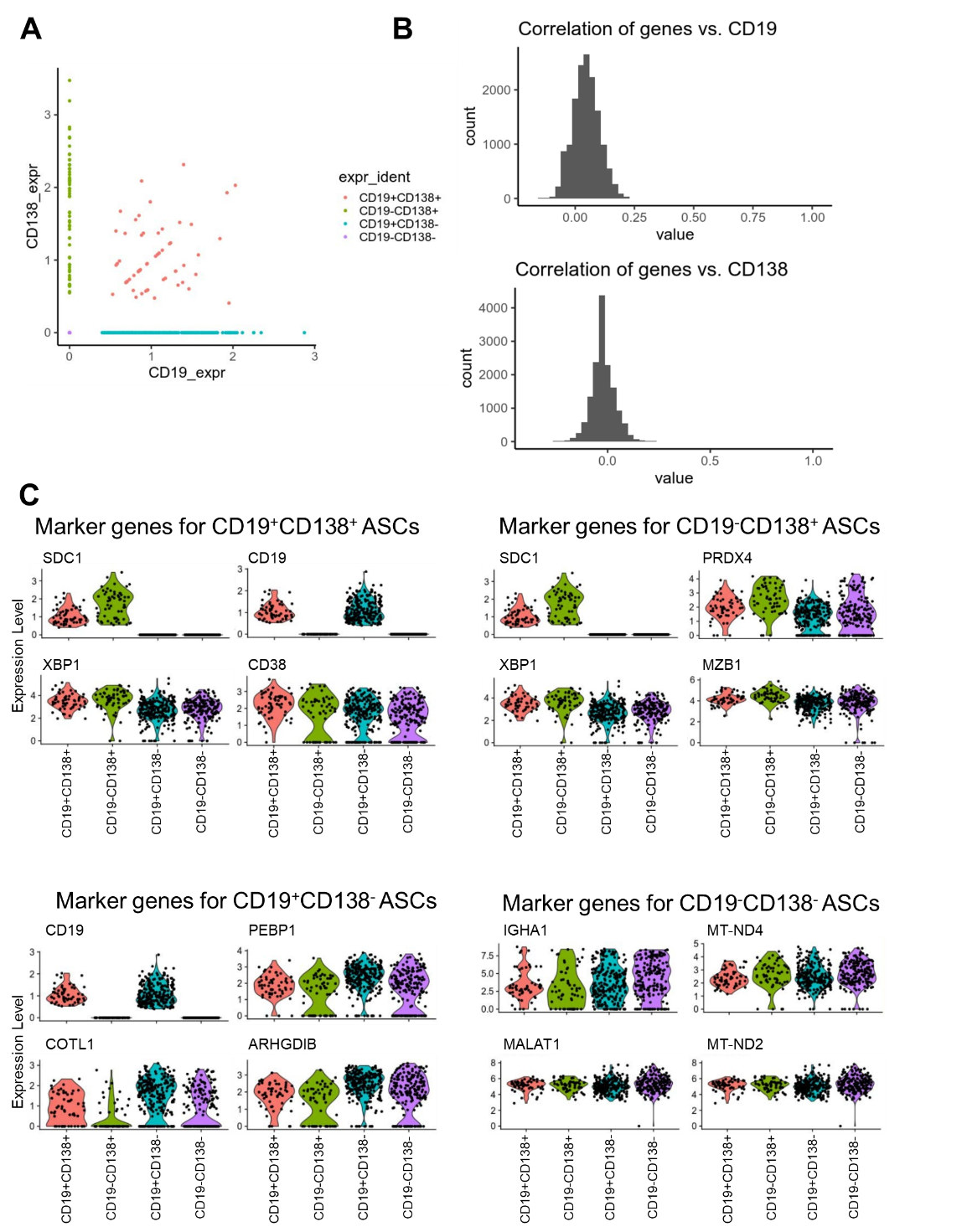
**Figure e-9. Comparative analysis of genetic expression between different tissues for ASCs.** **A.** Plots showing higher expression of *ANXA2* and *ITGB7* in blood ASCs than bone marrow ASCs. **B.** Plots showing higher expression of *HLA-A, HLA-DRB1* and *HLA-B* in bone marrow ASCs than CSF ASCs.

**Figure e-10**



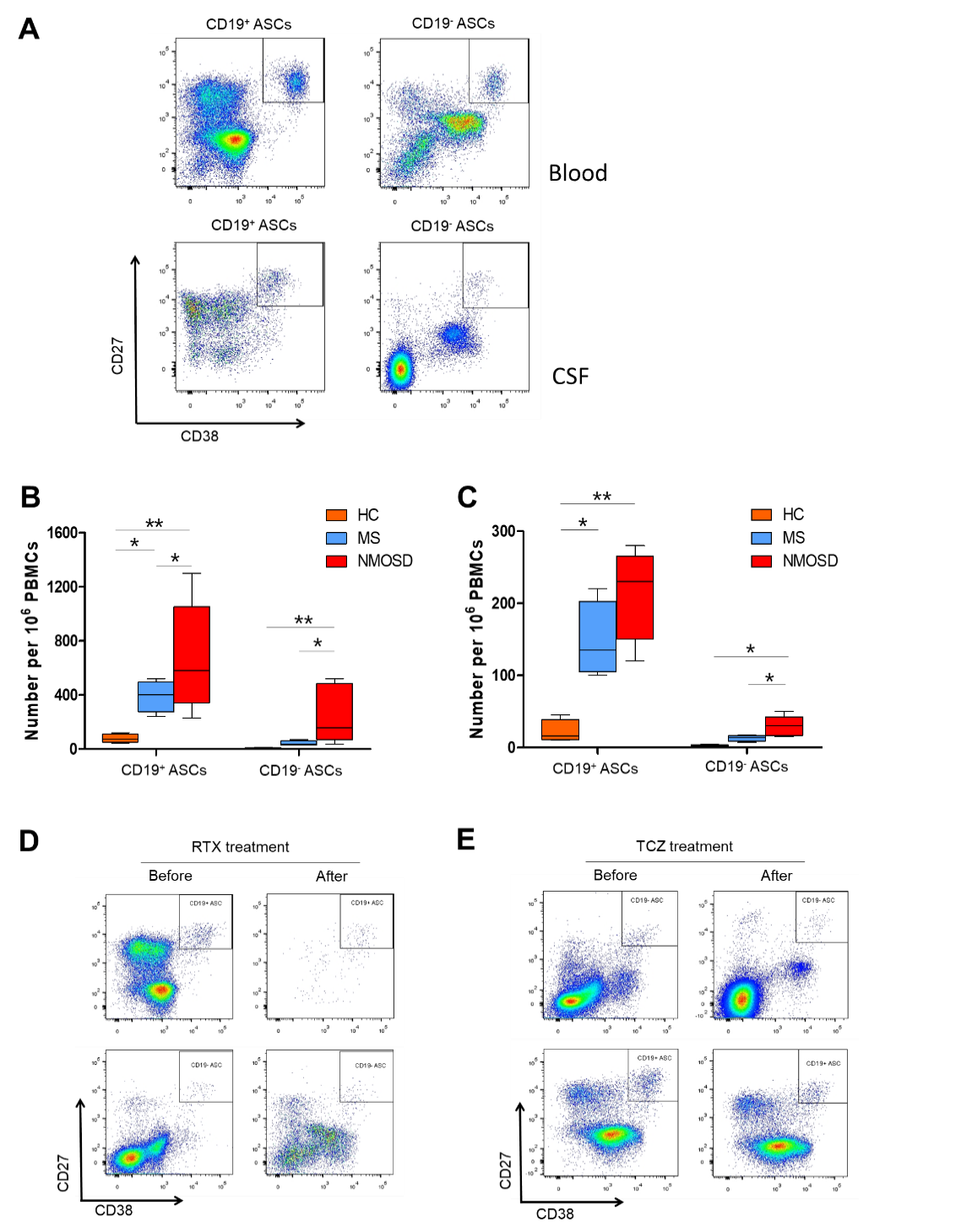
**Figure e-10. Clonal expansion of B cells in patients with NMOSD. A-B.** Distinct Startrac-tran pattern found between healthy controls (**A**) and the patients with NMOSD (**B**). ASC-SDC1 plasma cells have the highest Startrac-expa index across all clusters. n = 3 in healthy control group; n = 11 in NMOSD group.

**Figure e-11**



**Figure e-11. Differential expression of CD19 in NMOSD ASCs. A.** Distribution of NMOSD ASCs *in silico* FACS based on original expression. **B.** Correlation analysis of ASC genes versus *CD19* or *CD138* in NMOSD ASCs. **C.** Four subsets of ASCs based on the expression of *CD19* and *CD138,* and related marker genes. ASCs, antibody secreting cells.

**Figure e-12**



**Figure e-12. Flow cytometry analysis of CD19+ ASCs and CD19- ASCs. A.** Flow cytometry plots showing the gating of CD19+ and CD19- ASCs in NMOSD blood and CSF. **B.** Bar graph showing the counts of CD19+ and CD19- ASCs in peripheral blood from patients with NMOSD, MS, and healthy controls. **C.** Bar graph showing the counts of CD19+ and CD19- ASCs in CSF from patients with NMOSD, MS and healthy controls. n = 10 per group. **D.** Representative flow cytometry plots showing changes of ASCs in NMOSD patients before and after rituximab treatment. RTX, rituximab. **E.** Representative flow cytometry plots showing changes of ASCs in NMOSD patients before and after tocilizumab treatment. TCZ, tocilizumab. \**p* < 0.05, \*\**p* < 0.01.

**Table e-1. Demographic information of patients with NMOSD and healthy controls enrolled for single cell RNA sequencing.**

|  |  |  |
| --- | --- | --- |
|  | **NMOSD Patients (n = 11)** | **Controls (n = 3)** |
| **Age, mean (SD)⁎** | 44.2 ± 7.3 | 45.3 ± 6.2 |
| **Female, n (%)** | 10 (91) | 3 (100) |
| **Mean years of disease duration (SD)** | 5.5 ± 1.2 | - |
| **AQP4-ab positive, n (%)** | 11 (100) | - |
| **EDSS at attack⁎** | 5.5 ± 1.4 | - |
| **Preventive medications before attack** |  | |
| Prednisone | 6 (75) | - |
| Azathioprine | 2 (67) | - |
| Prednisone+Azathioprine | 2 (13) | - |
| Prednisone+Mycophenolate mofetil | 1 (4) | - |

**⁎**EDSS are shown as average ± standard deviation.

**Table e-2. Interferon stimulator genes (ISGs)⁎**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ABCA1 | GALM | IL1RN | P38/MAPK | TIMM10 |
| ACTA2 | GBP1 | IL28RA | PARP10 | TLR3/4 |
| ADAR | GBP2 | IRF3 | PARP12 | TMEM140 |
| AIM2 | GBP3 | IRF4 | PARP14 | TNFAIP6 |
| APOL6 | GBP4 | IRF5 | PARP9 | TNFSF10 |
| ASPRV1 | GBP5 | IRF6 | PHF11 | TNFSF13B |
| BATF2 | GBP6 | IRF7 | PISK | TRAFD1 |
| BCL2L13 | HELZ2 | IRF8 | PSMB8 | TRANK1 |
| BST2 | HERC5 | IRF9 | PSMB9 | TRIM21 |
| BTN3A1 | HERC6 | ISG15 | RBCK1 | TRIM22 |
| CARD16 | HES4 | ISG20 | REC8 | TRIM38 |
| CARD17 | Histone h3 | ISGF3 | RHBDF2 | TRIM5 |
| CCL8 | HSH2D | LAP3 | RNASEL | TRIM56 |
| CCR1 | IDO1 | LDL | RNF213 | TRIM6 |
| CEACAM1 | IFI16 | LGALS9 | RSAD2 | TRIM69 |
| CHMP5 | IFI27 | LY6E | RTP4 | TYMP |
| CMPK2 | IFI27L2 | MOV10 | SARM1 | UBA7 |
| CPT1B | IFI35 | MT1A | SERPING1 | UBE2L6 |
| CXCL10 | IFI44 | MX | SOCS1 | UNC93B1 |
| DDX58 | IFI44L | MX1 | SP100 | WARS |
| DDX60 | IFI44L | MX2 | SP110 | XAF1 |
| DHRS9 | IFIH1 | NBN | SP140 | ZBP1 |
| DHX58 | IFIT1 | NCOA7 | SPATS2L | ZC3HAV1 |
| DYNLT1 | IFIT1L | NMI | SRBD1 | ZNF684 |
| EIF2AK2 | IFIT2 | NT5C3A | STAT1 | ZNFX1 |
| ERK | IFIT3 | OAS1 | STAT2 |  |
| FBXO6 | IFIT5 | OAS2 | STING |  |
| FCGR1C | IFITM1 | OAS3 | TAP1 |  |
| FSH | IFITM3 | OASL | TAP2 |  |
| GADD45B | IL12 | OTOF | TCN2 |  |

|  |
| --- |
| **⁎**Adapted from Arazi et al., Nat Immunol 2019, 20 (7), 902-914. |

**Table e-3. Inflammation-related gene markers****⁎**

|  |  |  |
| --- | --- | --- |
| CHUK | MYD88 | TNFRSF1B |
| FADD | NFKB1 | TRADD |
| IKBKB | NFKBIA | TRAF6 |
| IKBKG | RELA |  |
| IL1A | RIPK1 |  |
| IL1R1 | TAB1 |  |
| IRAK1 | TLR4 |  |
| MAP3K1 | TNF |  |
| MAP3K14 | TNFAIP3 |  |
| MAP3K7 | TNFRSF1A |  |

**Table e-4. Exhaustion gene markers⁎**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| BUB1 | CXCL10 | JAK3 | SERPINA3 | TRIM25 |
| CAPZB | CXorf56 | LAG3 | SH2D2A |  |
| CCRL2 | ENTPD1 | LMAN2 | SHKBP1 |  |
| CD160 | EOMES | NR4A2 | SNRPB2 |  |
| CD244 | ETF1 | PDCD1 | SPP1 |  |
| CIT | GPD2 | PON2 | TANK |  |
| CPSF2 | GPR65 | PTGER2 | TCEA2 |  |
| CPT2 | IER5 | PTGER4 | TNFRSF9 |  |
| CRYGB | ISG20 | RNF11 | TOR3A |  |
| CTLA4 | ITGAV | S100A13 | TRGV11 |  |

|  |  |
| --- | --- |
| **⁎**This list is based on the genes upregulated in exhaustion, according to Wherry et al, Immunity 2007;27(4):670-84. |  |

**Table e-5. Characteristics of NMOSD patients, MS patients, and healthy controls for B cell subsets analysis by flow cytometry and specific expression of type I interferon related genes**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **NMOSD (n = 27)** | | **MS (n = 15)** | | **Controls (n = 16)** |
| **Age, mean (SD)⁎** | 44.3 ± 14.5 | | 38.8 ± 11.4 | | 42.5 ± 12.3 |
| **Female, n (%)** | 25 (92.6) | | 9 (60) | | 14 (87.5) |
| **Mean years of disease duration (SD)** | 4.3±1.6 | | 5.8±2.8 | | - |
| **AQP4-ab positive, n (%)** | 20 (70) | | 0 (0) | | - |
| **EDSS at attack⁎** | 4.5±1.7 | | 2.5±1.6 | | - |
| **Preventive medications before attack** |  |  | |  | |
| Prednisone | 12 (44.4) | | 5 (33.3) | | - |
| Tocilizumab | 5 (18.5) | | 0 (0) | | - |
| Rituximab | 1 (3.7) | | 2 (13.3) | | - |
| Azathiophrine | 2 (7.4) | | 0 (0) | | - |
| Teriflunomide | 0 (0) | | 4 (26.7) | | - |

**⁎**EDSS are shown as average ± standard deviation. MS, multiple sclerosis. NMOSD, neuromyelitis optica spectrum disorder.

Inclusion criteria for NMOSD patients were the same as previously described. Inclusion criteria for MS patients were: 1) older than 18 years of age; 2) diagnosed in accordance with 2017 McDonald diagnostic criteria; 3) experiencing acute MS attacks without receiving high dose intravenous methylprednisolone. Exclusion criteria were those with concomitant autoimmune diseases, acute myocardial infarction, heart failure, liver diseases, tumor or hematological system diseases, and concomitant use of B cell depleting drugs.

**Table e-6. RT-PCR primer sequence for IFN-related genes.**

|  |  |  |
| --- | --- | --- |
| Gene | Forward Primer (5’-3’) | Reverse Primer (5’-3’) |
| IRF4 | GAGCAATGACTTTGAGGAACTG | CATCATGTAGTTGTGAACCTGC |
| IFI6 | CATGCGGCAGAAGGCGGTATC | TCCGACGGCCATGAAGGTCAG |
| MX1 | CAGGACATTTGAGACAATCGTG | TCGAAACATCTGTGAAAGCAAG |
| IFI44 | TTTTCGATGCGAAGATTCACTG | AAGTTCTCAAGGCAGACAGTAA |
| IFI44L | CTTTCCTAGAGTCTCTGAAGCCA | GCAGCTTGCGCAGATGATTT |
| GAPDH | GCACCGTCAAGGCTGAGAAC | TGGTGAAGACGCCAGTGGA |

**Table e-7. Demographic information of patients with NMOSD receiving rituximab or tocilizumab**

|  |  |  |
| --- | --- | --- |
|  | **Rituximab (n = 10)** | **Tocilizumab (n = 10)** |
| **Age, mean (SD)** | 45.1 ± 13.5 | 43.8 ± 12.7 |
| **Female, n (%)** | 10 (100) | 10 (100) |
| **Mean years of disease duration (SD)** | 5.3±2.6 | 4.9±2.3 |
| **AQP4-ab positive, n (%)** | 10 (100) | 10 (100) |
| **EDSS at initiation of treatment⁎** | 4.2±1.7 | 4.6±1.8 |
| **EDSS at 6 months after treatment** | 3.1±1.0 | 2.7±0.9 |

**⁎**EDSS are shown as average ± standard deviation. Rituximab was administered at 100 mg/d for three consecutive days to reduce the frequency of CD19+ B cells to less than 1% of peripheral blood mononuclear cells, as quantified by flow cytometry. Tocilizumab was administered to patient at the dose of 8 mg/kg/months. NMOSD, neuromyelitis optica spectrum disorder.

**Table e-8.** Gene expression profiles of blood B cells from NMOSD patients versus heathy controls.

**Table e-9.** Gene expression profiles of bone marrow B cells versus blood B cells in NMOSD.

**Table e-10.** Gene expression profiles of blood B cells versus CSF B cells in NMOSD.

**Table e-11.** Differentially expressed genes in blood B cells versus CSF B cells from NMOSD patients.

**Table e-12.** Differentially expressed genes in blood B cells versus bone marrow B cells from NMOSD patients.

**Table e-8-12** are provided in separate Excel spreadsheets.