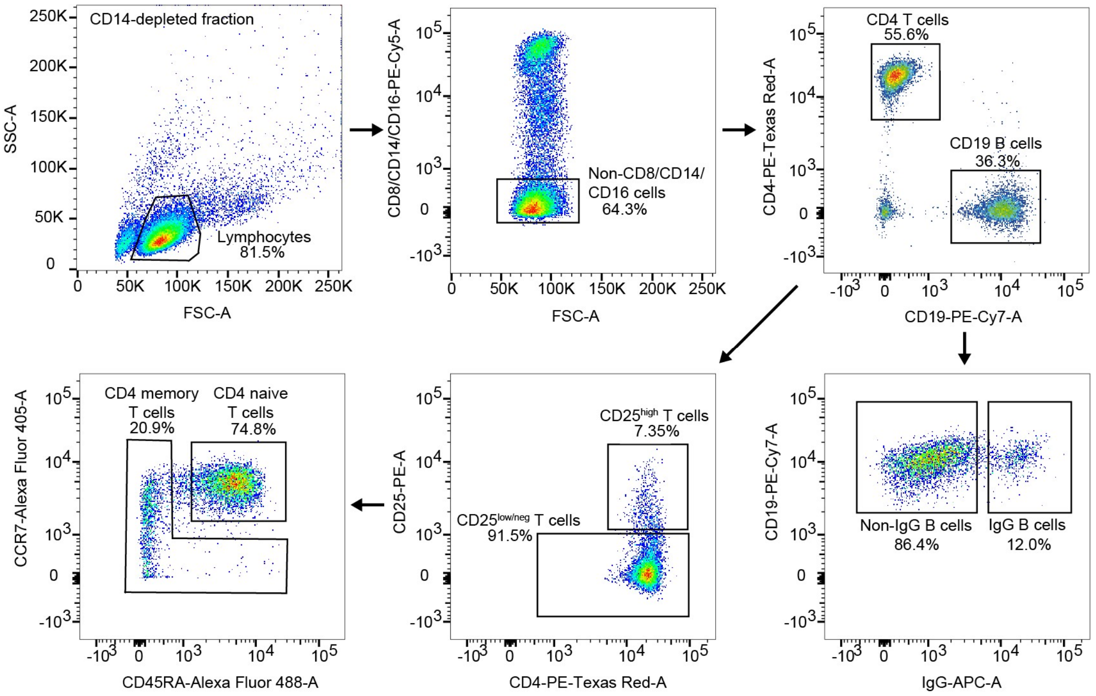

**Supplementary Fig. 1. Sorting strategy of memory IgG$^+$ B cells and memory CD4$^+$ T cells from MS patients.** Representative flow cytometry analysis of CD14 depleted PBMCs stained with fluorochrome-labeled mouse monoclonal antibodies. Cells were FACS-sorted after gating on CD8$^-$, CD14$^-$, CD16$^-$ population. IgG$^+$ memory B cells were sorted from CD19$^+$ B cells. Memory CD4$^+$ T cells were sorted from the CD4$^+$CD25$^{low/neg}$ gate (to exclude CD4$^+$CD25$^{bright}$ T$_{reg}$ cells), after removal of naïve CD4$^+$ T cells (CD45RA$^+$CCR7$^+$).

**Supplementary Table 1 | Features of the MS patients treated with NZM**

| | Patient A | | Patient B | |
|---|---|---|---|---|
| **Gender** | Female | | Female | |
| **Age at MS diagnosis** | 29 | | 19 | |
| **Age at NZM first infusion** | 40 | | 24 | |
| **Number of NZM infusions** | 3* | | 2* | |
| **Type of hypersensitivity reaction to NZM** | Anaphylactoid-like | | Anaphylactoid-like | |
| ▪ **Symptoms** | Urticaria, flushing, shivering and light dyspnea | | Diffuse urticaria and flushing | |
| ▪ **Post-infusion therapy** | Diphenhydramine and prednisolone | | Chlorpheniramine and methylprednisolone | |
| **ADA serum titers (EC50):** | | | | |
| ▪ **IgG** | 1/700 | | 1/1000 | |
| ▪ **IgM** | nd | | nd | |
| ▪ **IgA** | 1/30 | | 1/170 | |
| **HLA typing:** | | | | |
| ▪ **HLA-DRB1** | 14:01P | 16:01 | 07:01 | 07:01 |
| ▪ **HLA-DQA1** | 01:04 | 01:02 | 02:01 | 02:01 |
| ▪ **HLA-DQB1** | 05:03 | 05:02 | 02:02 | 02:02 |
| ▪ **HLA-DPA1** | 01:03 | 01:03 | 02:01 | 01:03 |
| ▪ **HLA-DPB1** | 04:01P | 04:02P | 01:01P | 04:01P |
| ▪ **HLA-A** | 02:01 | 03:01 | 03:01 | 26:01 |
| ▪ **HLA-B** | 27:02 | 35:03 | 14:01 | 57:02 |
| ▪ **HLA-C** | 02:02 | 12:03 | 04:01 | 08:02 |

nd = not detected; *last infusion was incomplete due to hypersensitivity reaction.

**Supplementary Table 2 | Comparison of neutralizing and binding abilities of anti-natalizumab antibodies**

| Patient | Antibody | Type | IC90 (ng/ml) | EC50 (ng/ml) | EC50$_{diss}$ (ng/ml) | $k_a$ (1/Ms) | $k_d$ (1/s) | KD (M) |
|---|---|---|---|---|---|---|---|---|
| A | NAA104 | BAb | 100,000.0 | 35.1 | 16,704.0 | 1.1E+06 | 1.2E-03 | 1.1E-09 |
| | NAA32 | BAb | 100,000.0 | 68.1 | 18,663.0 | 1.2E+06 | 1.0E-03 | 9.0E-10 |
| | NAA40 | BAb | 100,000.0 | 103.0 | 37,878.0 | 1.3E+06 | 5.1E-03 | 3.9E-09 |
| | NAA94 | BAb | 100,000.0 | 69.8 | 17,478.0 | 1.3E+06 | 6.3E-04 | 4.7E-10 |
| | NAA96 | BAb | 100,000.0 | 32.6 | 29,967.0 | 6.8E+05 | 9.0E-05 | 1.3E-10 |
| | NAA36 | BAb | 29,485.0 | 83.6 | 100,000.0 | 2.6E+05 | 1.0E-06 | 1.0E-12 |
| | NAA2 | BAb | 24,849.0 | 59.2 | 100,000.0 | 4.5E+05 | 1.0E-06 | 1.0E-12 |
| | NAA20 | BAb | 23,763.0 | 99.0 | 4,227.0 | 5.1E+05 | 1.1E-04 | 2.2E-10 |
| | NAA49 | BAb | 7,965.0 | 51.8 | 100,000.0 | 6.6E+05 | 2.2E-05 | 3.4E-11 |
| | NAA110 | BAb | 7,843.0 | 65.9 | 100,000.0 | 6.5E+05 | 2.2E-04 | 3.4E-10 |
| | NAA9 | BAb | 2,859.0 | 78.6 | 100,000.0 | 5.7E+05 | 1.3E-04 | 2.3E-10 |
| | NAA34 | BAb | 1,611.0 | 51.1 | 2,173.0 | 9.6E+04 | 6.5E-04 | 6.8E-09 |
| | NAA59 | NAb | 271.1 | 31.0 | 220.0 | 7.1E+05 | 9.1E-07 | 1.3E-12 |
| | NAA5 | NAb | 222.3 | 51.0 | 392.0 | 5.4E+05 | 1.0E-06 | 1.0E-12 |
| | NAA33 | NAb | 194.6 | 58.3 | 332.0 | 4.7E+05 | 1.0E-06 | 1.0E-12 |
| | NAA7 | NAb | 187.9 | 69.5 | 100,000.0 | 6.2E+05 | 1.0E-06 | 1.0E-12 |
| | NAA62 | NAb | 171.2 | 47.4 | 10,339.0 | 4.6E+05 | 3.6E-05 | 7.8E-11 |
| | NAA116 | NAb | 147.3 | 44.0 | 594.2 | 4.2E+05 | 1.0E-06 | 1.0E-12 |
| | NAA114 | NAb | 133.3 | 48.0 | 32,688.0 | 6.3E+05 | 1.0E-06 | 1.0E-12 |
| | NAA105 | NAb | 88.8 | 50.5 | 889.6 | 6.1E+05 | 4.9E-05 | 8.0E-11 |
| | NAA84 | NAb | 69.1 | 36.4 | 434.7 | 7.6E+05 | 8.4E-06 | 1.1E-11 |
| | NAA92 | NAb | 60.8 | 33.2 | 37.6 | 3.4E+05 | 1.0E-06 | 1.0E-12 |
| | NAA6 | NAb | 58.6 | 51.2 | 87.8 | 4.3E+05 | 1.0E-06 | 1.0E-12 |
| | NAA113 | NAb | 57.7 | 30.2 | 589.8 | 5.6E+05 | 1.0E-06 | 1.0E-12 |
| | NAA45 | NAb | 49.4 | 31.4 | 706.0 | 5.6E+05 | 5.2E-05 | 9.4E-11 |
| | NAA44 | NAb | 48.0 | 43.8 | 141.1 | 8.0E+05 | 1.4E-04 | 1.8E-10 |
| | NAA119 | NAb | 45.5 | 52.7 | 262.7 | 3.6E+05 | 1.0E-06 | 1.0E-12 |
| | NAA80 | NAb | 43.7 | 46.0 | 43.0 | 9.9E+05 | 1.0E-06 | 1.0E-12 |
| | NAA128 | NAb | 41.4 | 39.8 | 64.6 | 5.1E+05 | 1.0E-06 | 1.0E-12 |
| | NAA125 | NAb | 17.1 | 48.4 | 531.3 | 5.2E+05 | 1.0E-06 | 1.0E-12 |
| B | NAE125 | BAb | 100,000.0 | 24.5 | 100,000.0 | 7.8E+05 | 2.3E-03 | 2.9E-09 |
| | NAE194 | BAb | 100,000.0 | 25.6 | 100,000.0 | 1.3E+06 | 2.1E-03 | 1.6E-09 |
| | NAE197 | BAb | 100,000.0 | 823.3 | 100,000.0 | 1.5E+06 | 1.6E-03 | 1.1E-09 |
| | NAE199 | BAb | 100,000.0 | 23.3 | 15,761.0 | 1.8E+06 | 2.6E-03 | 1.5E-09 |
| | NAE205 | BAb | 100,000.0 | 736.3 | 100,000.0 | 2.0E+05 | 5.8E-04 | 2.9E-09 |
| | NAE206 | BAb | 100,000.0 | 710.0 | 100,000.0 | 5.8E+06 | 1.3E-02 | 2.3E-09 |
| | NAE207 | BAb | 100,000.0 | 972.6 | 100,000.0 | 1.4E+06 | 3.1E-03 | 2.2E-09 |
| | NAE210 | BAb | 100,000.0 | 17.5 | 100,000.0 | 2.0E+06 | 8.5E-04 | 4.2E-10 |
| | NAE208 | BAb | 100,000.0 | 6,433.0 | 100,000.0 | 1.9E+05 | 4.4E-03 | 2.3E-08 |
| | NAE203 | BAb | 63,640.0 | 18.5 | 5,924.0 | 2.4E+05 | 9.0E-04 | 3.7E-09 |

IC90 indicates the antibody concentration required for 90% inhibition of NZM binding to T cells.

EC50 indicates half-maximal antibody concentration required to bind to NZM by ELISA.

EC50$_{diss}$ values were calculated by NZM ELISA in presence of high-pH dissociation buffer (maximum value 100,000 for no binding).

Kinetics parameters measured by SPR: $k_a$, association constant, $k_d$, dissociation constant and KD, equilibrium dissociation constant (when no dissociation is measured, $k_d$ and KD are set at minimum values of $10^{-6}$ and $10^{-12}$, respectively).

NAb, neutralizing antibody (IC90 < 1,000 ng/ml). BAb, binding (non-neutralizing) antibody (IC90 > 1,000 ng/ml, maximum 100,000).

Values are shown with a two-color gradation scale from minimum (green) to maximum (red).

### Supplementary Table 3 | Data collection and refinement statistics (molecular replacement)

| | NZM-NAA32 | NZM-NAA84 |
|---|---|---|
| **Data collection** | | |
| Space group | P4212 | P21 |
| Cell dimensions | | |
| *a*, *b*, *c* (Å) | 189.1, 189.1, 87.26 | 105.4, 66.6, 199.5 |
| $\alpha$, $\beta$, $\gamma$ (°) | 90, 90, 90 | 90, 98.9, 90 |
| Resolution (Å) | 1.96 (2.22-1.96) | 2.74 (3.14-2.79) |
| $R_{\mathrm{merge}}$ | 0.132 (1.529) | 0.143 (0.583) |
| $I / \sigma I$ | 11.9 (1.8) | 6.3 (1.8) |
| Completeness (%) | 99.8 (100) | 98.6 (99.2) |
| Redundancy | 8 (12.5) | 3 (3.1) |
| | | |
| **Refinement** | | |
| Resolution (Å) | 2.0 | 2.8 |
| No. reflections | 63528 | 40000 |
| $R_{\mathrm{work}}$ / $R_{\mathrm{free}}$ | 0.266 / 0.285 | 0.238 / 0.263 |
| No. atoms | | |
| Protein | 6714 | 13096 |
| Ligand/ion | 18 | 0 |
| Water | 253 | 0 |
| *B*-factors | | |
| Protein | 39.3 | 56.8 |
| Ligand/ion | 48.5 | na |
| Water | 33.5 | na |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.008 | 0.007 |
| Bond angles (°) | 1.08 | 1.03 |

One crystal for each complex was used to solve the structures.

Values in parentheses are for highest-resolution shell. na, not applicable.

### Supplementary Table 4 | TCR Vβ sequence analysis and MHC restriction of NZM-reactive T cell clones

| Patient | Nr. of clones | Vβ gene | Jβ gene | Vβ CDR3 | Specificity | Epitope | Start | End | MHC restriction |
|---|---|---|---|---|---|---|---|---|---|
| A | 6 | V2 | J2-3 | CASGFTDTQYF | NZM LC | GKAPRLLIHYTSALQPGI | 41 | 58 | HLA-DR |
| | 1 | V7-2 | J2-3 | CASSLYSGANTDTQYF | NZM LC | GKAPRLLIHYTSALQPGI | 41 | 58 | HLA-DR |
| | 3 | V7-3 | J1-2 | CASSSQRDSPYTF | NZM LC | GKAPRLLIHYTSALQ | 41 | 55 | nd |
| | 1 | V11-2 | J1-5 | CASSLDNGAGYNQPHHF | NZM LC | KYMAWYQQTPGKAPR | 31 | 45 | nd |
| | 1 | V20-1 | J1-5 | CSASTRQGVGRNQPQHF | NZM LC | GKAPRLLIHYTSALQPGIPS | 41 | 60 | HLA-DR |
| B | 3 | V28 | J1-5 | CASSPAGAYSNQPQHF | NZM HC | PGQRLEWMGRIDPAN | 41 | 55 | nd |
| | 1 | V3-1 | J1-4 | CASSPPGVANEKLFF | NZM LC | KYMAWYQQTPGKAPR | 31 | 45 | HLA-DR |
| | 11 | V12-3 | J2-1 | CASSPTGTGLNEQFF | NZM LC | GKAPRLLIHYTSALQPGI | 41 | 58 | HLA-DR |
| | 2 | V12-3 | J2-7 | CASSPTGTGLTEQYF | NZM LC | GKAPRLLIHYTSALQPGI | 41 | 58 | HLA-DR |
| | 2 | V12-4 | J1-3 | CASSXPPGRVGGNTIYF* | NZM LC | GKAPRLLIHYTSALQPGI | 41 | 58 | nd |
| | 1 | V5-1 | J1-5 | CASSYLPDSNQPQHF | NZM LC | GKAPRLLIHYTSALQPGI | 41 | 58 | HLA-DR |
| | 7 | V29-1 | J2-7 | CSVAQGGGSYEQYF | NZM LC | PRLLIHYTSALQPGI | 44 | 58 | HLA-DR |

LC, light chain; HC, heavy chain. Start and end position of the epitope residues in the NZM chains are indicated. nd, not determined. *X can be either S or Y.

### Supplementary Table 5 | NZM peptides identified by mass spectrometry-based peptidomics

| Protein | Peptide sequence | Start | End | Length | Identification (patient-replicate) | | | | Uniquely present In NZM |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | A-rep1 | A-rep2 | B-rep1 | B-rep2 | |
| NZM heavy chain | DTYIHWVRQAPGQR | 31 | 44 | 14 | | | + | | * |
| NZM heavy chain | DTSASTAYMELSSLRSEDT | 73 | 91 | 19 | + | | | | |
| NZM heavy chain | ASTAYMELSSLRSEDT | 76 | 91 | 16 | + | + | | | |
| NZM heavy chain | ASTAYMELSSLRSEDTA | 76 | 92 | 17 | + | + | | | |
| NZM heavy chain | STAYMELSSLRSED | 77 | 90 | 14 | + | + | | | |
| NZM heavy chain | STAYMELSSLRSEDT | 77 | 91 | 15 | + | + | | | |
| NZM heavy chain | STAYMELSSLRSEDTA | 77 | 92 | 16 | + | + | | | |
| NZM heavy chain | TAYMELSSLRSEDT | 78 | 91 | 14 | + | | | | |
| NZM heavy chain | GQGTLVTVSSASTKGP | 114 | 129 | 16 | + | | | | * |
| NZM heavy chain | GQGTLVTVSSASTKGPS | 114 | 130 | 17 | + | | | | * |
| NZM heavy chain | QGTLVTVSSASTKGP | 115 | 129 | 15 | + | + | | | * |
| NZM heavy chain | QGTLVTVSSASTKGPS | 115 | 130 | 16 | + | + | | | * |
| NZM light chain | TPGKAPRLLIHYTSALQP | 39 | 56 | 18 | + | | | | * |
| NZM light chain | TPGKAPRLLIHYTSALQPG | 39 | 57 | 19 | + | | + | + | * |
| NZM light chain | TPGKAPRLLIHYTSALQPGIPS | 39 | 60 | 22 | + | | | | * |
| NZM light chain | TPGKAPRLLIHYTSALQPGIPSR | 39 | 61 | 23 | + | + | | | * |
| NZM light chain | KAPRLLIHYTSALQPG | 42 | 57 | 16 | | + | | | * |
| NZM light chain | APRLLIHYTSALQP | 43 | 56 | 14 | + | + | | | * |
| NZM light chain | APRLLIHYTSALQPG | 43 | 57 | 15 | + | + | | | * |
| NZM light chain | APRLLIHYTSALQPGIPS | 43 | 60 | 18 | + | | | | * |
| NZM light chain | TFTISSLQPEDIAT | 72 | 85 | 14 | + | | | | * |

Start/end position and length of the peptide residues in the NZM chains are indicated. + indicates identification of a peptide in an experiment replicate.
* indicates a peptide belonging uniquely to NZM and not to the B cell receptors of the antigen-presenting cells.