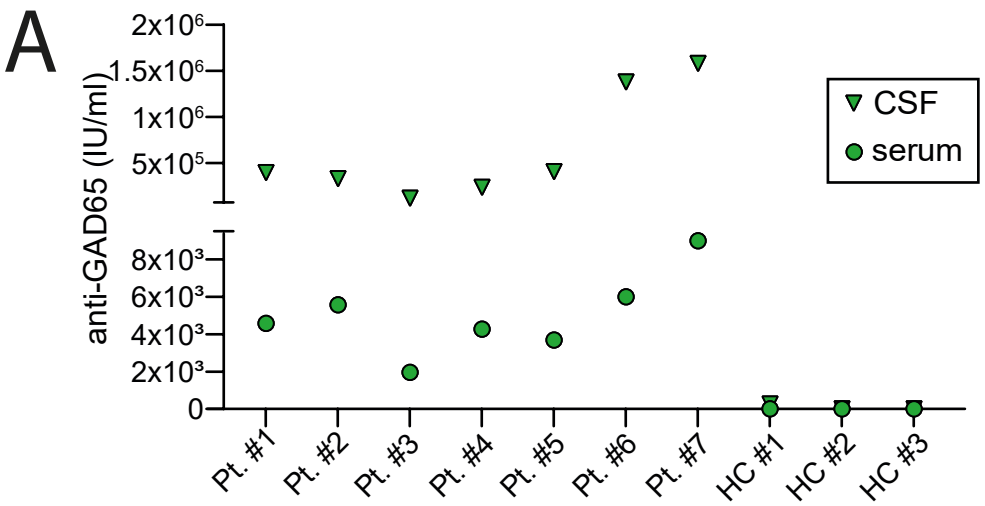
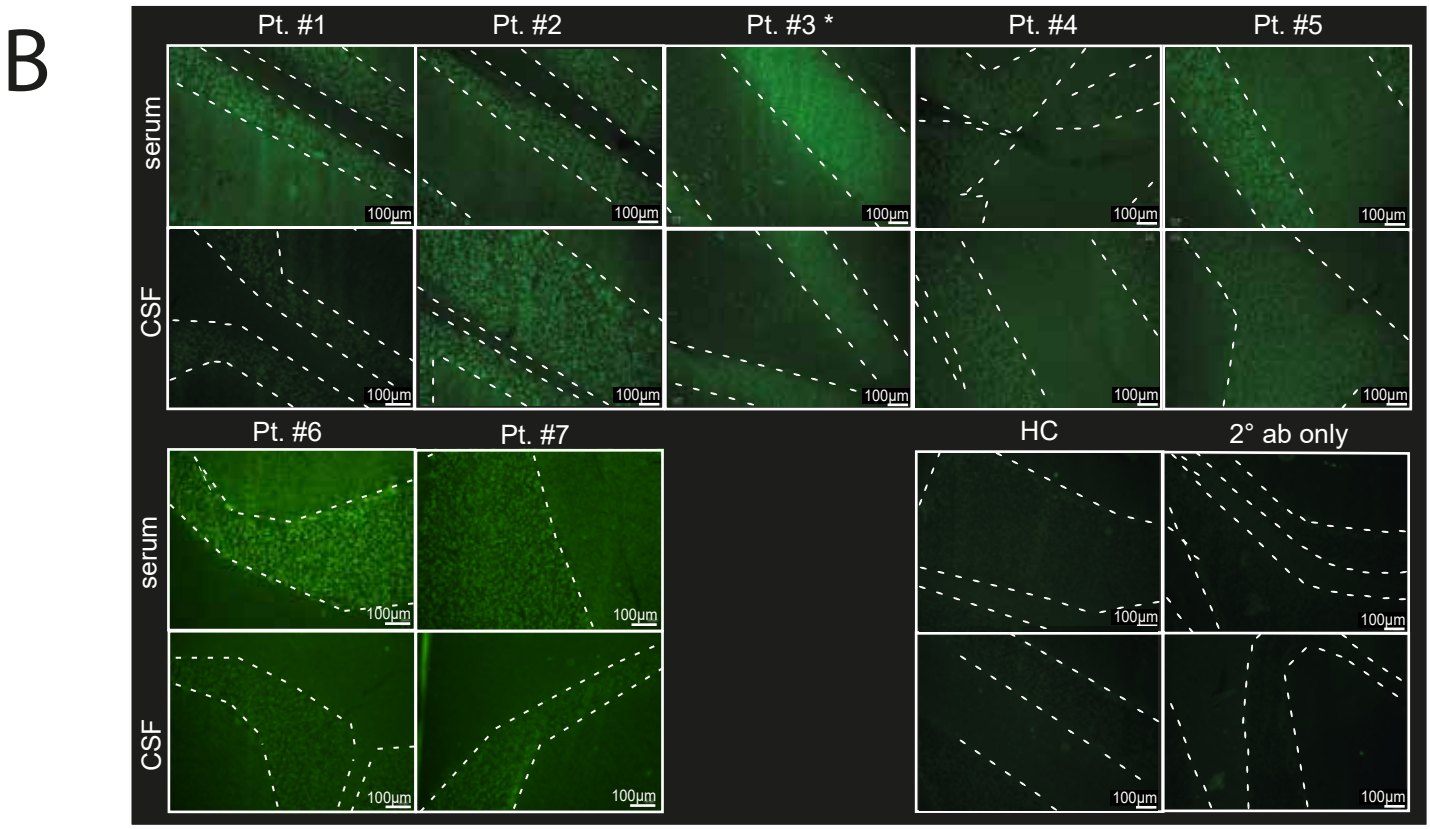
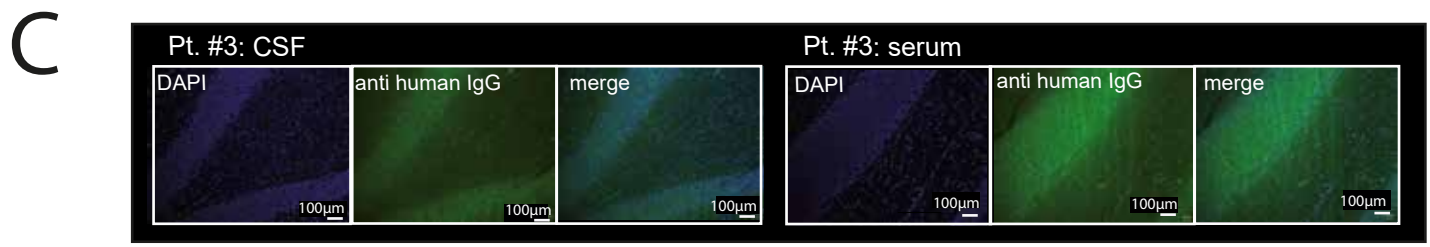
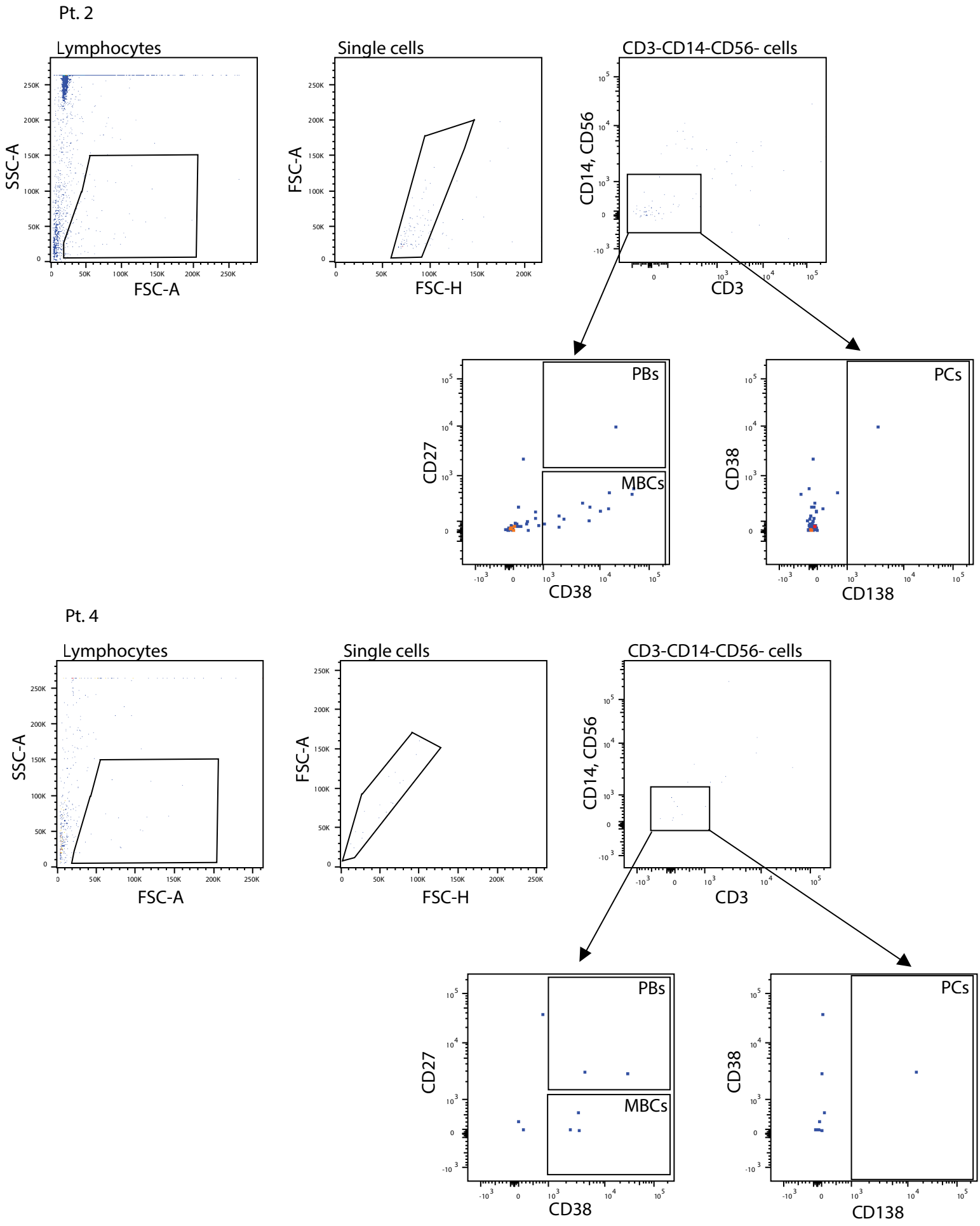






eFigure 1: Characterization of GAD65-Ab profile of the patient cohort. (A) GAD65-Abs in serum and cerebro-spinal fluid (CSF) of patients (pts) #1-7 as well as three healthy controls (HCs) were determined by ELISA. (**B, C**) Tissue-based assay with indirect immunofluorescence on primate creebellar slices was performed. (**B**) An anti-GAD65 specific staining pattern was identified using serum (1:100) oder CSF (1:1) of patients # 1-7 as well as a healthy control (HC). A condition with only secondary antibody was used as an additional negative ctrl. * indicates an overlaying anti-nuclear antibody -staining. (**C**) An anti-nuclear antibody (ANA)-staining pattern was identified using serum (1:100) or CSF (1:1) of patient #3. Slices were additionally stained with 4´,6-Diamidin-2-phenylindol (DAPI) to viualize cell nuclei and the merge of the stainings is depicted.



eFigure 2: Gating strategy to define B-cell subpopulations. After gating on lymphocytes and single cells, B cells were defined as $CD14^{-}CD56^{-}CD3^{-}$ cells. Memory B cells were defined as $CD3^{-}CD14^{-}CD56^{-}CD27^{+}CD38^{-}$, plasma blasts were defined as $CD3^{-}CD14^{-}CD56^{-}CD27^{+}CD38^{+}$, **and plasma cells as** $CD3^{-}CD14^{-}CD56^{-}CD138^{+}$. Abbreviations. FSC, forward scatter; MBC, memory B cell; PB, plasma blast; PC, plasma cell; Pt, patient; SSC, sideward scatter.

eMethods

CSF single cell isolation

For fluorescence-activated cell sorting (FACS) CSF B cells were pre-enriched by negative selection using the following kits: human CD3 positive selection kit II (Stem Cell Technologies, #17851), human CD14 positive selection kit II (Stem Cell Technologies, #17858), human CD56 positive selection kit II (Stem Cell Technologies, #17855). Enriched B cells were stained on ice using the following abs: CD14-FITC (Biolegend, clone: 63D3, 1:40), CD56-FITC (Biolegend, clone: HCD56, 1:40), CD19-APC Fire (Biolegend, clone: HIB19, 1:40), CD27-BV605 (Biolegend, clone: O323, 1:40), CD20-PerCP (Biolegend, clone: 2H7, 1:40), CD24-APC (Biolegend, clone: ML5, 1:50), CD138-PE (Stem Cell Technologies, clone: MI15, 1:40), CD38-ef450 (Invitrogen, clone: HB7, 1:40), CD3-AF700 (Invitrogen, clone: OKT3, 1:40), Fc receptor blocking (Miltenyi Biotec, 1:50). Cells were sorted into 96-well plates using the FACS BD FACSAria Fusion Cell Sorter with wells containing 5 µl of RNA lysis buffer (0.4 Unit RNaseOUT™ Recombinant Ribonuclease Inhibitor (Invitrogen), 0.038% Triton-X-100, 1 µM Oligo dT primer, 1 mM dNTP mix (Invitrogen)).

Immunoglobulin sequence analysis

Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed by adding 5 µl RT master mix to the lysed cells containing 50 Units SuperScript™ II Reverse Transcriptase (Invitrogen), 1x superscript II first strand buffer (5x) (Invitrogen), 5 mM DTT (Invitrogen), 0.92 M Betaine, 6 mM $MgCl_2$, 10 Units RNase OUT (Invitrogen), and 1 µM TSO no. 1 (eTable 1). The following PCR conditions were used: 42°C for 90 minutes/ 10 cycles: 50°C for 2 minutes and 42°C for 2 minutes/ 70°C for 15 minutes. cDNA amplification was performed by adding 15 µl containing 0.2 µM TSO no. 2 (eTable 1), 1x KAPA Hifi Mix (2x) (Roche Applied Science). The following PCR conditions were used: 98°C for 3 minutes/ 25 cycles: 98°C for 20 seconds, 67°C for 15 seconds, 72°C for 6 minutes/ 72°C for 5 minutes. For the gene

amplification of the variable immunoglobulin heavy chain (IGH) and the possible lambda (IGL) and kappa (IGK) light chains a nested PCR-strategy was applied as described previously [14]. In brief, PCRs were performed in 25 µl containing 0.4 µM gene specific primers: TSO no. 3, HG_Out, Kappa_Out, and Lambda_Out with all PCR primers together in one master mix for the outer PCR, and TSO_no. 3, HG_IN, Kappa_IN, and Lambda-IN in separate reactions for heavy chain (HC), kappa chain (KC), and lambda chain (LC) for the inner PCR. 1x Phusion buffer (5x) (Roche Applied Science), 0.2 µM dNTPs (Invitrogen), 1 µl DNA template, 0.5 µl Phusion® HF DNA Polymerase (2000 U/mL) (New England biolabs) were added to the respective primers. The following PCR conditions were used: 98°C for 30 seconds/ 30 cycles: 98°C for 20 seconds, 60°C for 20 seconds, 72°C for 50 seconds/ 72°C for 10 minutes. All products from the second PCRs were applied to agarose gel electrophoresis and bands at the correct height with matching heavy and light chains were isolated by gel purification (Qiagen Minelute kit). Purified PCR-products were sequenced with inner primers for IGH, IGK, and IGL. All primers used in the single-cell analysis are listed in eTable 1. Sequences were compared with variable (diversity) joining (V(D)J) segments in the IMGT database. The number of somatic hypermutations (SHMs) in the complementary determining regions (CDR) as well as in the framework regions (FR) were analysed and the length of the CDR3 was determined. Furthermore, IgG subclasses were determined as described previously [15].

Generation of mAbs

Immunoglobulin cloning was performed by introducing restriction sites into heavy and light chains (SalI-HF and SacII for IGH, BssHII and KasI for IGK and IGL) using individual immunoglobulin gene-specific primers (eTable 1). PCR conditions were as described above for the nested PCR approach. Digested and purified PCR-products were cloned into the pTT5 expression vector which adds a C-terminal HIS6-tag to the Abs and contains the IgG1 constant domain. Ig vector pairs were transiently transfected into HEKEBNA cells cultured in Freestyle Medium 293 supplemented with 1% Pluronic F68 (Gibco) and 25 µg/ml Geneticin (Gibco)

using Polyethylenimine (Polysciences). Supernatants were harvested after 4 days of culture. Ab purification was performed by immobilized metal affinity chromatography as described previously [16].