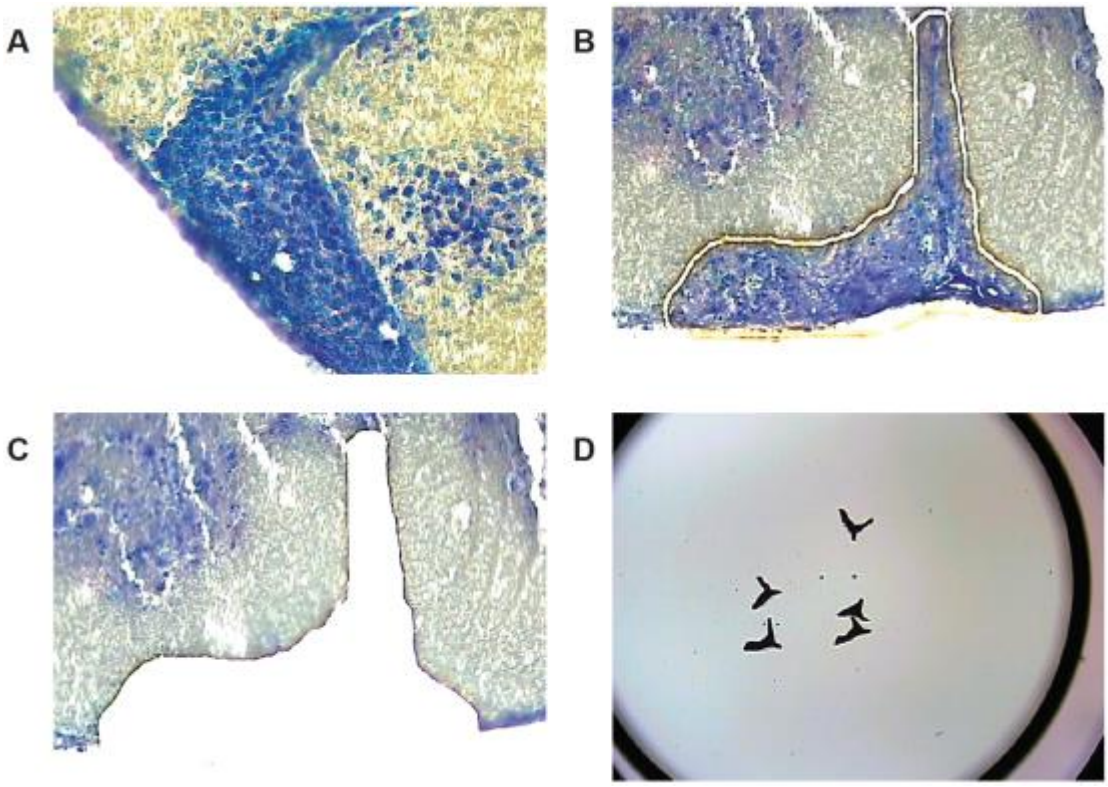
B cell repertoire expansion occurs in meningeal ectopic lymphoid tissue

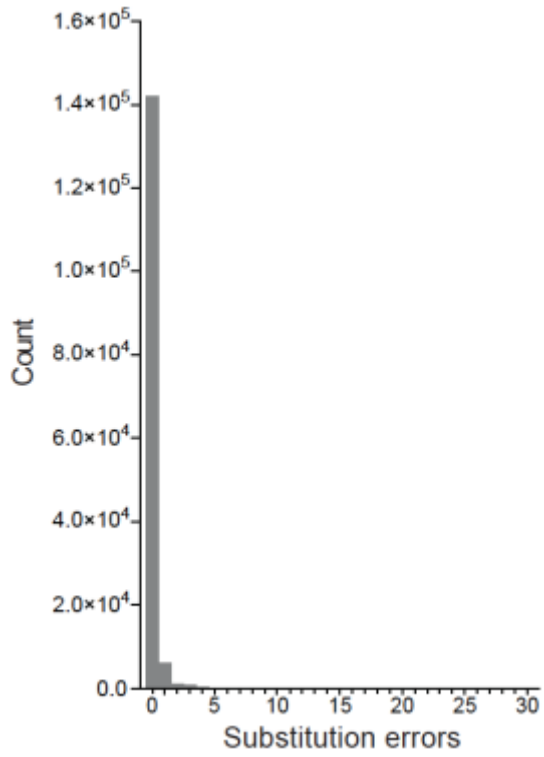
Klaus Lehmann-Horn, Sheng-zhi Wang, Sharon A. Sagan,

Scott S. Zamvil, H.-Christian von Büdingen

Supplemental data

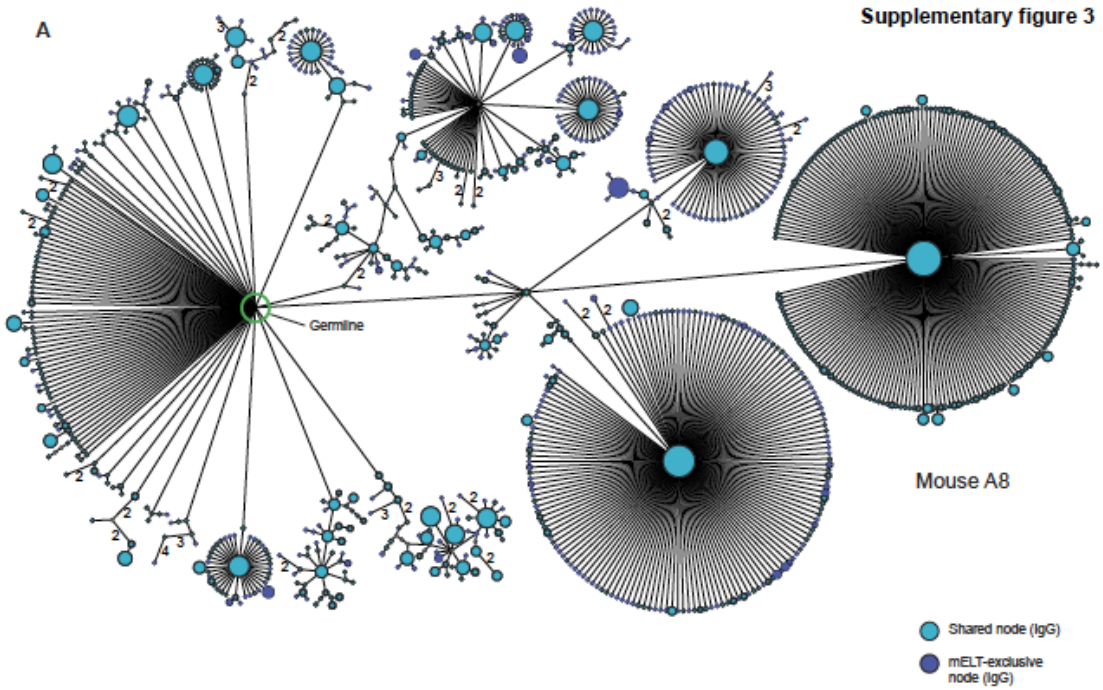


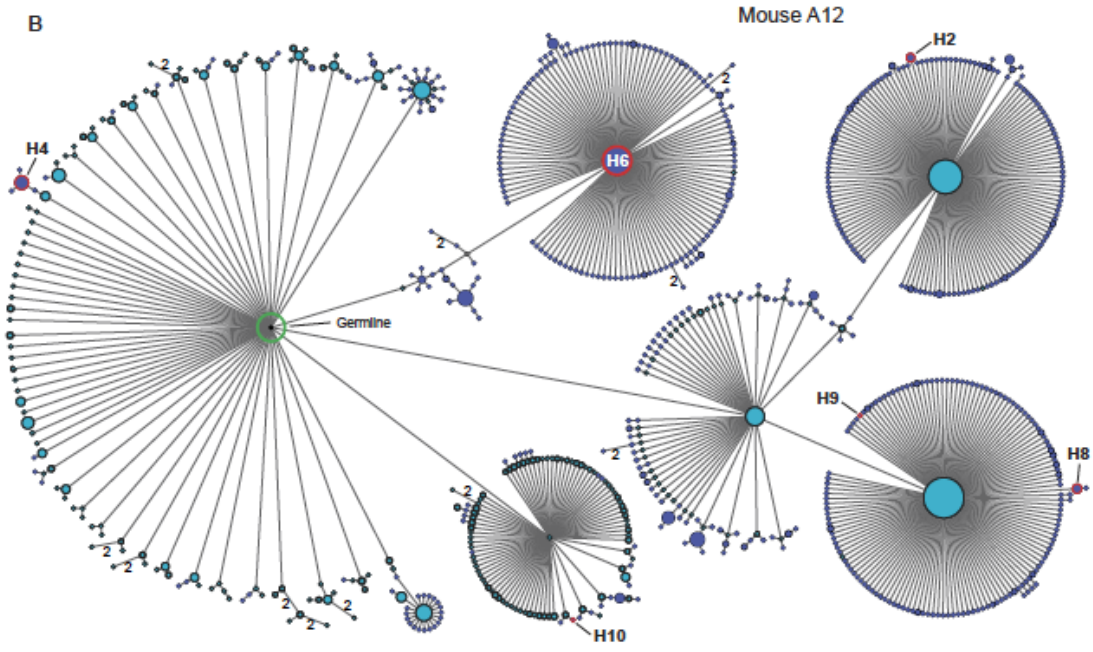
Supplementary figure 1. LCM of spinal cord mELT from a Th×2D2 EAE mouse. **(A)** 40X magnification of mELT. mELT after laser cutting **(B)** and after capture **(C)**; 20X magnification. **(D)** 5 mELT areas from adjacent sections of the spinal cord captured on one cap, including the one from **(B,C)** on the bottom left. 2X magnification. All sections are axial, 20 µm thickness, H&E stain. LCM, laser capture microscopy; mELT, meningeal ectopic lymphoid tissue; EAE, experimental autoimmune encephalomyelitis.

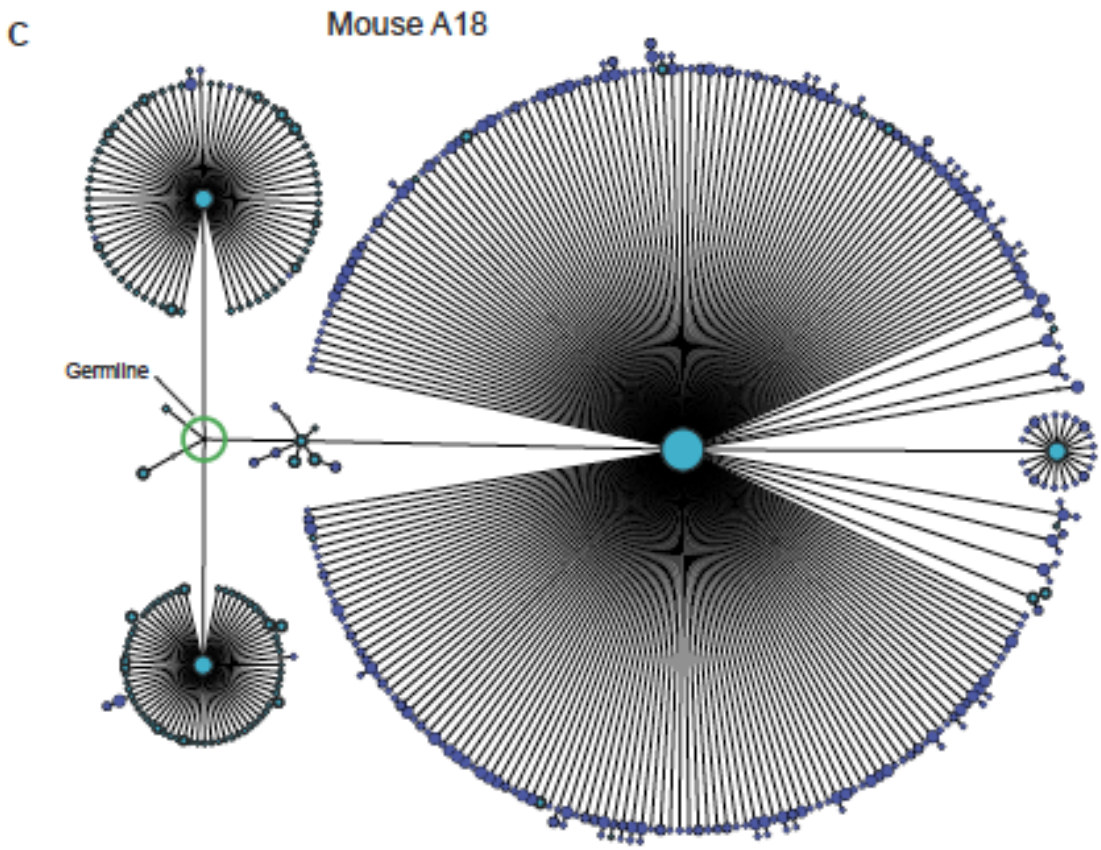


Supplementary figure 2. Repertoire sequencing of an IgG-VH plasmid reveals a low rate of substitution sequencing errors. A plasmid containing a human IgG-VH with known sequence (45C-1G3-H; von Büdingen et al., unpublished) was amplified using a suitable (human IGHV3-30) specific forward primer and a human IGHC specific reverse primer. PCR conditions were 1) 95°C – 60 s; 2) 95°C – 30 s; 66.6°C – 30 s; 72°C – 60 s (33 cycles); 3) 72°C – 7 min. The count of redundant IgG heavy chain sequences according to their number of substitution errors in a 290-nucleotide portion of the VDJ-region of the Ig-VH plasmid 45C-1G3-H is shown. VH, heavy chain variable region.

Supplementary figure 3







Supplementary figure 3. IgG lineage trees evidence intrinsic affinity maturation in mELT. IgG lineage trees from mELT from Th×2D2 EAE mouse A8 **(A)**, A12 **(B)**, and A18 **(C)**. Each node represents all sequencing reads with an identical CDR1-CDR3 region. To reduce the risk that rare sequencing errors result in 'false nodes' and to reduce the size of the trees, only those nodes are shown with ≥ 5 identical sequences (A8 and A12) or ≥ 20 identical sequences (A18). Dark blue nodes are IgG sequences found exclusively in mELT. Turquoise nodes are IgG sequences found in mELT and one or more peripheral compartments (blood, spleen, LN; = shared nodes). Black nodes highlighted with a green circle represent the 'germline' sequence ('knock-in' Ig-VH). Grey nodes represent sequences that were not found in the sequencing data but were computed to complete the tree (internal nodes). The size of each node correlates with the number of identical sequencing reads. Two nodes connected by a line differ from each other by one particular nucleotide mutation (if not indicated differently by a number) in a specific position of the entire

CDR1-CDR3 region. In **(B)**, sequences that were selected for subsequent cloning and expression are labeled (e.g. “H2”) and highlighted with a red circle. mELT, meningeal ectopic lymphoid tissue; EAE, experimental autoimmune encephalomyelitis; CDR, complementarity determining region; LN, lymph node; VH, heavy chain variable region.

Mouse	Sex	Clinical score (at EAE onset)	Clinical score (when tissue was obtained)	Age at EAE onset [days]	Age when tissue was obtained [days]	Duration of EAE [days]
A8	female	3.5	3.5	25	61	37
A12	female	3.5	3.5	36	50	15
A18	female	3.5	3.5	71	114	44
A20	female	3.0	3.5	40	86	47
A22	female	3.0	3.5	40	86	47

Supplementary table 1. Clinical features of Th×2D2 mice with spontaneous EAE, which were included in the RepSeq analysis. These mice were randomly chosen from our colony of Th×2D2 EAE mice. EAE, experimental autoimmune encephalomyelitis.

Number of nucleotide substitutions in one single Ig-VH sequencing read	Probability of the occurrence of the event
1	0.079800
2	0.003500
3	0.000100
4	2.19E-06
5	3.81E-08

Supplementary table 2. Estimation of the probability of sequencing errors potentially confounding RepSeq results based on sequencing data from a 290-nucleotide portion of the Ig-VH plasmid 45C-1G3-H. Calculation of the probability of sequencing errors resulting in 1, 2, 3, 4, or 5 nucleotide substitutions in a single Ig-VH sequencing read.