

Supplementary Table S1. List of primer sequences

Primer	Sequence (5'–3')
ChimHy4-B	ACCCATGGGCCCTTGGTGGAGGCTGAGGAGACTGTGACCGTGGTGCCTGC
Hy4LCDR3b1	GAAGGGATCCTCATTACTTTGCTGRCAGTAATAAGTTGCRAA
Hy4LCDR3b2	GAGGGATCCTCATTACTTTGCTGGCAGTAATAAACCCCRAC
Hy4LCDR3b3	AGGGATCCTCATTACTTTGCTGACAGTAATACACTGCAA
Hy4LCDR3b4	GAAGGGATCCTCATTACTTTGCTGACAGTAATAAACTGCCAC
Hy4LCDR3-F	CAAAGTAATGAGGATCCCTTCACGTTCCGGCCARGGGACCAAG
CK1dX	AGAGAGAGAGCGCCGTCTAGAATTAACACTCTCCCCTGTTGAAGCTCTTTGTGACGGGCGAACTCAG
RSC-F	GAGGAGGAGGAGGAGGAGGCCGGGGCCAGGCCGCGAGCTC

Supplementary Table S2. Antibody (Ab) amino acid content during the humanization of murine mAb 3B4C-4

Amino acid composition is listed as either murine (m) or human (h); h+m indicates the use of h germline genes plus several positions where m residues were allowed as described in Methods. In the CDRs, either m or h residues were used as noted or choice of m or h (m/h) was allowed.

Humanization step*	Ab library, mAb or Fab	Library chain pairing	LC† variable region genes				LC constant κ genes	HC‡ variable region genes				HC CH1§ genes
			Framework	CDR 1	CDR2	CDR3		Framework	CDR1	CDR2	CDR3	
1, 2	Murine mAb 3B4C-4	–	m	m	m	m	m	m	m	m	m	m
3	Humanized LC Library Hy4-11, -14, -43	Chimeric HC	h	h	h	m	h	m	m	m	m	h
4	Humanized HC Library	LC from Hy4-11, -14, or -43	h	h	h	m	h	h+m	m/h	m/h	m	h

5	Hy4-26, -53, -63 Modification of LC Hy4-14	HC from Hy4-26	h+m	m	m	m	h	h+m	m	m	m	h
6, 7	Hy4-26A, -B, -C, -D Humanized Fab Hy4	-	h+m	m	m	m	h	h+m	m	m	m	h

*Humanization steps (see Methods for process details): (1) cDNA cloning of 3B4C-4 Fab genes; (2) generating chimeric 3B4C-4 H chain; (3) humanization of LC by grafting murine CDRs into a library of rearranged human κ LC; (4) humanization of HC; (5) modification of Fab LC clone Hy4-14; (6) Fab conversion to full IgG1; and (7) transient mammalian cell transfection and development of stable cell lines for IgG expression. Fab clones that came from each step in the humanization process are listed under the relevant library.

†LC, Light chain.

‡HC, Heavy chain.

§CH1, Heavy chain constant region 1.

||CDR, Complementary determining region.

Hunt, A. R., Frederickson, S., Hinkel, C., Bowdish, K. S. and Roehrig, J. T. (2006). A humanized murine monoclonal antibody protects mice either before or after challenge with virulent Venezuelan equine encephalomyelitis virus. *J Gen Virol* **87**.