SUPPLEMENTAL MATERIAL

Uysal et al., http://www.jem.org/cgi/content/full/jem.20081862/DC1

SUPPLEMENTAL MATERIALS AND METHODS

DNA sequencing. RNA was extracted from 10^7 hybridoma cells by using the RNeasy total RNA isolation kit (QIAGEN). Total RNA was reverse transcribed into cDNA by using a First-Strand cDNA Synthesis kit (GE Healthcare). DNA fragments that encode heavy and light chains were PCR amplified by using the following primers: κ framework one region, 5′-GACATTGTGATGACACAGTCTCCA-3′ and 5′-GATATTGTGCTAACTCAGTCTCCA-3′; κ constant region, 5′-AAGAGCTTCAACAGGAATGAGTGT-3′; heavy chain framework one region, 5′-CAGATCCAGTTGGTGCAGTCTGGA-3′, 5′-CAGGTCCAACTGCAGCAGCCTGGG-3′, and 5′-GAAGTGAAGCTTGAGGAGTCTGGA-3′; heavy chain constant region, 5′-TGTGGTTGTAAGCCTTGCATATGTA-3′, 5′-GCATGGAGGACAGGGGTTGATTGT-3′, and 5′-TACATATGCAAGGCTTACAACCACA-3′.

PCR fragments were cloned into a pCR-4 TOPO vector (Invitrogen). The DNA sequences of the heavy and light chain, including the variable and constant domains, were determined by automated sequencing using BigDye (Applied Biosystems) and an automated capillary DNA sequencer (ABI 3100; Applied Biosystems).

Heavy Chain

		IMGT			IgBLAST					
Antibody	Isotype	V-Gene	D-Gene	J-Gene	V-Gene	D-Gene	J-Gene	CDR1	CDR2	CDR3
ACC1	IgG2c	IGHV6-6*01	IGHD4-1*01	IGHJ2*01	J606.4.82	DST4.2	JH2	GFTFSDAWMD	IRNKVNNHAT	TGLTFDY
ACC2	IgG2b	IGHV1-64*01	IGHD1-1*01	IGHJ3*01	J558.67.166	DSP2.8	JH3	GYTFTDYWMH	IHPNSGNS	VPSLVGD
ACC3	IgG2b	IGHV1-64*01	IGHD1-1*01	IGHJ3*01	J558.67.166	DSP2.8	JH3	GYTFTDYWMH	IHPNSGNS	VPSLVGD
ACC4	IgG1	IGHV9-2-1*01	IGHD1-2*01	IGHJ3*01	VH9.2	DFL16.2	JH3	GYTFTDYSIH	INTETGEP	ARATTATELAY
GB8	IgG2b	IGHV1-26*01	IGHD5-7*01	IGHJ2*01	J558.26.116	DQ52-C57BL/6	JH2	GYTFTDYYMN	INPINGGT	ARNWDGDY
CIIC1	IgG2a	IGHV1-5*02	IGHD4-1*02	IGHJ3*01	J558.40	DST4.2	JH3	GYTFTSYWMN	IHPSDSET	ARLKPGGTWFAY
UL1	IgG2b	IGHV1-39*01	IGHD2-7*01	IGHJ4*01	J558.39.129	DSP2.2	JH4	GYSFTDYNMN	INPNSGTT	ARLDDYNAMDY
M2139	IgG2b	IGHV1-4*01	IGHD1-1*02	IGHJ2*01	J558.45	DSP2.8	JH2	GYAFISYWMN	INPSDGYT	ARYGGYFDY

Kappa Chain

	IMGT		IgBLAST				
Antibody	V-Gene	J-Gene	V-Gene	J-Gene	CDR1	CDR2	CDR3
ACC1	IGKV3-2*01	IGKJ2*01	21-2	JK2	ESVDNYGISS	AAS	QQSKGVPYT
ACC2	IGKV2-109*01	IGKJ1*01	he24	JK1	KSLLHSNGITY	QMS	AQNLELPWT
ACC3	IGKV2-109*01	IGKJ1*01	he24	JK1	KSLLHSNGITY	QMS	AQNLELPWT
ACC4	IGKV1-135*01	IGKJ5*01	bd2	JK5	QSLLDSDGKTY	LVS	WQGTHFPLT
GB8	IGKV1-133*01	IGKJ1*01	bj2	JK1	QSLLYSNGKTY	LVS	VQGTHFPRT
CIIC1	IGKV3-5*01	IGKJ2*01	21-5	JK2	KSVDSYGNSF	RAS	QQSNEDPYT
UL1	IGKV4-68*01	IGKJ4*01	aq4	JK4	SSVSY	LTS	QQWSSNPFT
M2139	IGKV3-1*01	IGKJ2*01	21-1	JK2	ESVEYFGTSL	AAS	QQSREVPYT

GenBank accession codes and mouse strains

Antibody	Heavy	Kappa	Strain
ACC1	EU159566	EU159567	B10.RIII.Cia5
ACC2	EU159568	EU159569	(B10.QxDBA/1)F1
ACC3	EU159570	EU159571	(B10.QxDBA/1)F1
ACC4	EU159572	EU159573	DBA/1
GB8	EU159574	EU159575	B10.Q
CIIC1	Z72441	Z72442	DBA/1
UL1	EU159576	EU159577	B10.Q
M2139	Z72462	Z72463	DBA/1

Figure S1. Amino acid sequences of antibody CDR regions, corresponding germline genes, and mouse strains that antibodies originated. The most similar germline genes to the antibody light and heavy chain sequences determined by tools provided by the IMGT and GenBank (IgBLAST) databases. CDR regions were determined according to IMGT rules. These sequences are available from GenBank/EMBL/DDBJ under accession nos. EU159566, EU159567, EU159568, EU159569, EU159570, EU159571, EU159572, EU159573, EU159574, EU159575, EU159576, and EU159577.

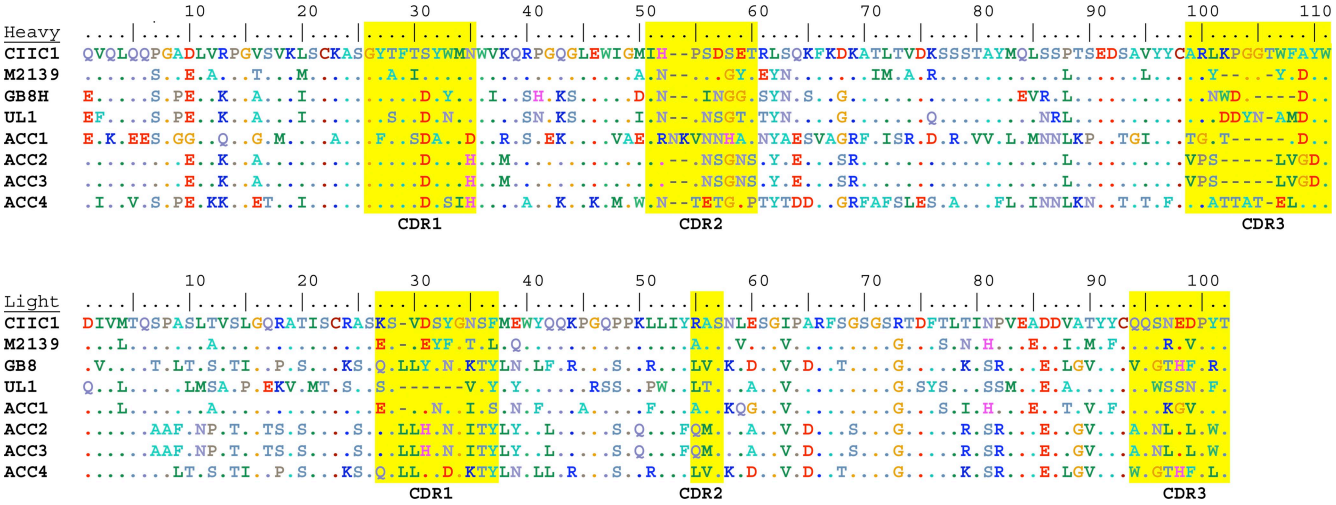

Figure S2. Amino acid sequence alignments. Amino acid sequence alignments of the heavy and light chain variable regions of the sequenced antibodies.

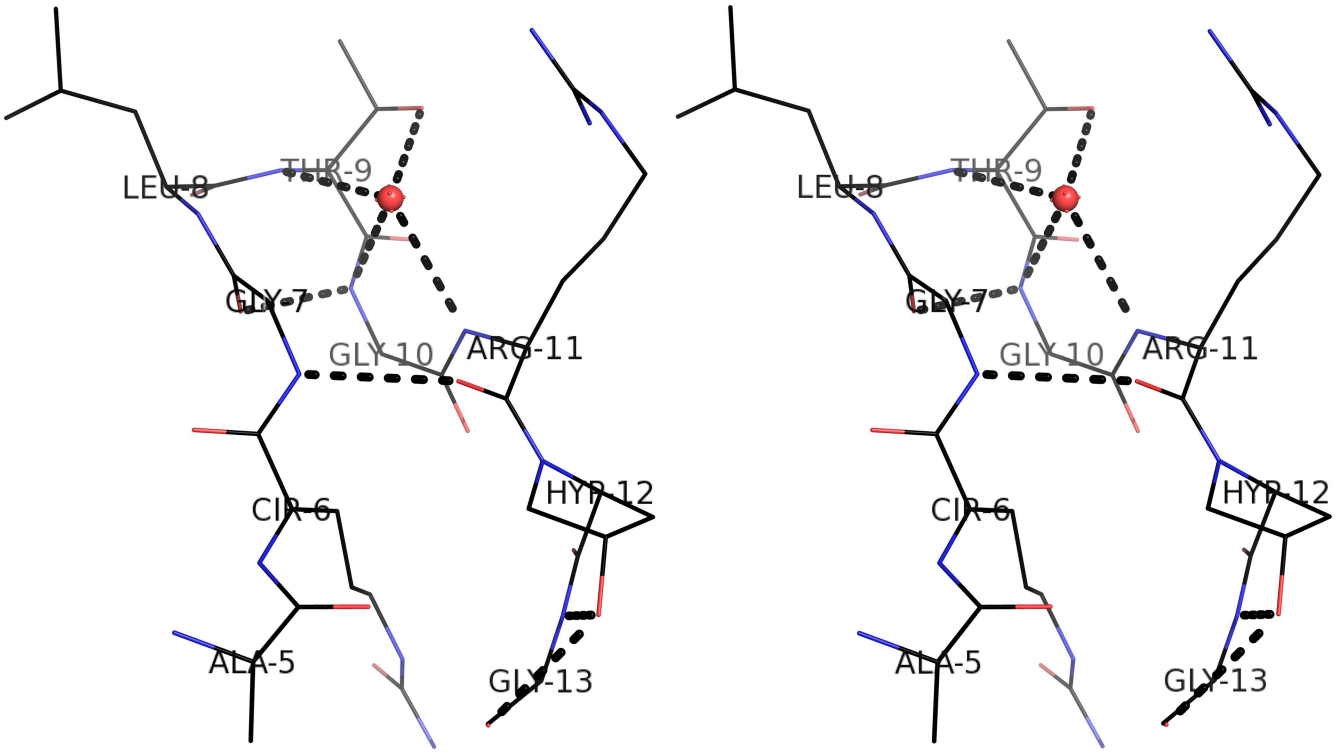

Figure S3. Stereoscopic view of the pCII-Cit1 peptide. Hydrogen bonds are represented by gray dashes. The water molecule is shown as a red ball.

Table S1. Somatic mutation statistics in light and heavy chains of antibodies

	Antibody	FR1	CDR1	FR2	CDR2	FR3	CDR3	Nonsilent	Silent
Heavy chain mutations	ACC1	1	0	0	1	8	1	9	2
	ACC2	0	2	1	2	5	0	9	1
	ACC3	0	2	1	2	5	0	9	1
	ACC4	0	0	1	0	3	0	4	0
	GB8	1	0	3	1	3	0	6	2
	CIIC1	3	1	2	12	0	2	15	5
	UL1	0	0	1	1	2	0	4	0
	M2139	1	4	4	2	5	0	16	4
Light chain mutations	ACC1	0	1	2	0	7	1	9	2
	ACC2	2	1	1	0	1	0	3	2
	ACC3	2	1	1	0	1	0	3	2
	ACC4	0	0	0	0	0	0	0	0
	GB8	1	1	1	0	0	0	1	2
	CIIC1	1	3	1	0	1	1	4	3
	UL1	0	0	0	0	0	3	2	1
	M2139	0	2	0	0	0	1	2	1

The mutations were determined by the tools provided by the IMGT database.