

SUPPLEMENTAL MATERIAL

Raposo et al., <http://www.jem.org/cgi/content/full/jem.20130968/DC1>

		CDR1		CDR2	
<i>Ighv1-4</i>	QVQLQQSGAELAKPGTSVKMSCKAS	GYTFTTSYW	MNWKQRPQGLEWIG	AINPSNGYTEYNQKPKD	KAILTADKSSSTAYMQLSSLTSEDSAVYYCAR
M2139	-----	---I---	-----	---D---	---M--R-----L----
M284	-----	---I---	-----	---D---	---M-----
M287	-----	---I---	-----	---DA--D--N---	---R-----L----
<i>Ighv1-4</i>	QVQLQQSGAELAKPGTSVKMSCKAS	GYTFTTSYW	MNWKQRPQGLEWIG	AINPSNGYTEYNQKPKD	KAILTADKSSSTAYMQLSSLTSEDSAVYYCAR
A/J	-----	-----	-----	-----	-----
AKR/J	-----E---R--A-----	---R--	-H-----A-----	--Y-G-SD-S-----G	--K--VT-A-----E-----
BALB/cJ	-----R--A-----	---R--	-H-----	Y---S--D-----	--T-----
C3H/HeJ	-----E---R--A-----	---R--	-H-----A-----	--Y-G-SD-S-----G	--K--VT-A-----E-----
C57BL/6J	-----R--A-----	---T	-H-----	Y---S--K-----	--T-----
C57BL/10.Q	-----R--A-----	---T	-H-----	Y---S--K-----	--T-----
CBA/J	-----E---R--A-----	---R--	-H-----A-----	--Y-G-SD-S-----G	--K--VT-A-----E-----
DBA/2J	-----R--A-----	---T	-H-----	Y---S--K-----	--T-----
LP/J	-----R--A-----	---T	-H-----	Y---S--K-----	--T-----
<i>Ighv1-4</i>	QVQLQQSGAELAKPGTSVKMSCKAS	GYTFTTSYW	MNWKQRPQGLEWIG	AINPSNGYTEYNQKPKD	KAILTADKSSSTAYMQLSSLTSEDSAVYYCAR
M2139 _{ScFv}	-----	---I---	-----	---D---	---M--R-----L----
M2139 _{ScFv-S31R}	-----	---IR---	-----	---D---	---M--R-----L----
M2139 _{ScFv-M33T}	-----	---I--T	-----	---D---	---M--R-----L----
M2139 _{ScFv-S31R-M33T}	-----	---IR-T	-----	---D---	---M--R-----L----
DBA _{ScFv}	-----	---I---	-----	---D---	---M--R-----L----
B6 _{ScFv}	-----R--A-----	---T	-H-----	Y---S--K-----	--T-----

Figure S1. Amino acid sequences of the anti-J1 recognizing V_H segments. M2139, M284, and M287 are all anti-J1 mAb raised in the DBA/1J mouse strain. All sequences are compared with the germline-encoded *Ighv1-4* gene from the DBA/1J mouse strain. All ScFv constructs use an identical V_L sequence, identical to the one expressed by the M2139 mAb.

A
 DIVLTQSPASLAVSLGQRATISCRASESVEYFGTSLMQ
 WYQKPGQPPKLLIYAASNVEGVPARFSGSGGTD
 LNIHPVEDDIAMFYCQQSREVPTFGGSKLEIKRGG
 GSGGGSGGGSGGGGQVQLQQSGAELARPGTSVKM
 SCKASGYTFISYWMNWKQRPQGLEWIGAINPSDGYT
 EYNQKFKDKAIMTADRSSSTAYMQLSSLTSEDSALYYC
 ARYGGYFDYWGGTTLTVSSAASNGLPETGHHHHHH

B
 DIVLTQSPASLAVSLGQRATISCRASESVEYFGTSLMQ
 WYQKPGQPPKLLIYAASNVEGVPARFSGSGGTD
 LNIHPVEDDIAMFYCQQSREVPTFGGSKLEIKRAD
 AAPTVSIFPPSSEQLTSGGASVVCFLNFPYKDI
 NPKVWIDGSRQNGVLSWTDQDSKDYMSSTLTLTKDEY
 ERHNSYTCETHKTSSTPIVKSFNRNEC

C
 QVQLQQSGAELAKPGTSVKMSCKASGYTFISYWMNWK
 QRPQGLEWIGAINPSDGYTEYNQKFKDKAIMTADRSS
 STAYMQLSSLTSEDSALYYCARYGGYFDYWGGTTLTV
 SSAKTTTPSVYPLAPGCGDTTGSVTLGCLVKGYPES
 VTVTWNSGSLSSVHTFPALLQSGLYTMSSSVTPSS
 WPSQVTVCSVAHPASSTIVDKKLEPSGPISTINCP
 PCKECHKCPAPNLEGGPSVFIKPPNPKDVLMI
 SLTPKVTCLVVDVEDDQVQISWVFNVEVHTAQ
 TQTHREDYNSITIRVSTLPIQHQDWMGKFKCKV
 NNKDLPSPIERTISKIKGLVRAPQVYILPPPAE
 QLSRKDVSLTCLVVGFNPDISVEWTSNGHT
 EENYKDTAPVLDSDGSYFIYSKLNMMKTSK
 WEKTDTSFSCNVRHEGLKNYYLKKIISRSPGK

Figure S2. Amino acid sequences of the M2139 antibodies. (A) Sequence of the M2139_{ScFv} expressed in *E. coli*. (B and C) Sequence of the light chain (B) and heavy chain (C) of the M2139_{Ig} expressed in HEK293T cells.

Table S1. M2139_{Fab}-J1 crystal data collection and refinement statistics

Parameters	M2139 _{Fab} -J1
Data collection	
Space group	P3 ₁ 21
Cell dimensions a, b, c (Å)	95.2, 95.2, 190.4
Resolution (Å)	82.5–3.25 (3.43–3.25) ^a
R_{merge}	0.082 (0.643)
Mean I (σI)	13.5 (2.0)
Completeness (%)	99.7 (99.4)
Redundancy	4.7 (4.0)
Unique reflections	16,327 (2,325)
Refinement	
Resolution (Å)	50.0–3.25
Number of reflections	15,467
R_{work}/R_{free} (%)	22.2/26.3
Number of atoms/B-factors (Å ²)	
Protein (M2139 _{Fab})	3,291/115.3
J1 peptide	479/147.6
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angles (°)	1.56
Ramachandran plot, number of residues	
Favored region	440 (94.8%)
Allowed	22 (4.7%)
Outliers	2 (0.4%)

^aValues given in parenthesis refer to the highest resolution shell.

Table S2. CDR contributions to the surface area buried upon interaction of M2139_{Fab} and J1 peptide

CDR	Residue	Δ BSA	Sum Δ BSA
		\AA^2 (% of ASA)	
L1	E27	41.2 (44)	95
	S28	2.1 (3)	
	Y31	42.5 (37)	
	L36	9.5 (21)	
L2	-	-	-
L3	S95	8.1 (61)	235
	R96	80.7 (74)	
	E97	52.8 (92)	
	V98	65.4 (84)	
	P99	0.7 (4)	
	Y100	27.3 (97)	
	H1	Y27	
H1	T28	48.0 (59)	
	I30	38.7 (53)	
	S31	73.3 (100)	
	Y32	47.3 (69)	
	W33	91.3 (99)	
H2	N35	1.4 (100)	117
	A50	6.9 (100)	
	N52	28.3 (98)	
	S54	13.6 (30)	
	D55	14.7 (19)	
	Y57	15.8 (10)	
	E59	38.2 (36)	
H3	Y99	31.8 (75)	48
	G100	11.2 (30)	
	G101	4.9 (16)	
Other M2139_{Fab} residues			
H	W47	2.5 (34)	
L	D1	8.3 (7)	
L	I2	1.2 (55)	

Δ BSA = change in accessible surface area (ASA) as calculated by the PISA server at EBI.

Table S3. Peptide chain contributions to the surface area buried upon interaction of M2139_{Fab} and J1 peptide

Chain	Residue	Δ BSA	Sum Δ BSA in \AA^2
		\AA^2 (% of ASA)	
E	E20	33.5 (24)	301
	R21	81.2 (52)	
	G22	7.1 (64)	
	A23	34.9 (56)	
	A24	63.5 (100)	
	G25	9.4 (100)	
	I26	33.9 (33)	
	A27	33.1 (58)	
F	G28	3.9 (36)	194
	R21	11.7 (10)	
	I26	89.7 (70)	
	A27	14.9 (29)	
	G28	1.9 (46)	
G	P29	73.9 (78)	378
	K30	3.2 (3)	
	G22	0.2 (4)	
	A23	54.5 (82)	
	A24	24.4 (35)	
	G25	9.4 (65)	
	I26	131.5 (99)	
	A27	54.1 (92)	
	G28	13.4 (96)	
	P29	32.6 (40)	
K30	62.3 (55)		

Δ BSA = change in accessible surface area (ASA) as calculated by the PISA server at EBI.

Table S4. Hydrogen bonds between J1 peptide and M2139_{Fab} observed in the M2139_{Fab}-J1 complex

Number	Fab chain:residue:atom	Distance	Peptide chain:residue:atom
		\AA	
1	H:I30:O	3.2	E:A24:N
2	H:S31:O	2.9	E:A23:N
3	H:S31:O γ	2.7	E:G22:O
4	H:Y32:O η	3.0	E:R21:N η 1
5	H:W33:N ϵ 1	2.7	E:G25:O
6	H:N52:N δ 2	3.0	E:A24:O
7	L:E27:O ϵ 2	2.6 (SB)	G:K30:N ζ
8	L:R96:N η 1	2.9	F:A27:O
9	L:E97:O ϵ 1	2.8 (SB)	G:K30:N ζ
10	L:V98:N	3.3	G:G28:O
11	L:Y100:O η	3.0	G:A27:N

SB, salt bridge.