Supporting information

Supporting Figure 1



Supporting Figure 1. Comparison of the three-dimensional structures of CD22 and MAG proximal Ig domains. Crystal structures of d7 and d5 proximal Ig-like domains of CD22 and MAG, respectively. C1- and C2-type Ig folding contain different β -strand topology of strands D and C'.



Supporting Figure 2. Variable region of m971 Fab. A) Crystal structure of m971 Fab (grey) in complex with the anti-kappa V_HH domain (magenta). The anti-kappa V_HH domain interacts with the Fab constant region of the light chain (CL). B) Superposition of the crystal structures of the variable domain of m971 Fab unliganded (in blue) and CD22-bound (grey). Except for the HCDR3 loop, no significant changes are observed in the CDRs, indicating that m971 is largely in a predisposed conformation to bind CD22 d7. C) Electrostatic surface representation of the m971 paratope. The d7 domain of CD22 is shown as wheat secondary structure. The calculation of the surface electrostatics was made with the APBS software (1) and prepared using Pymol (2) and displayed on a scale of -5 kT/e (red) to 5 kT/e (blue).



Supporting Figure 3. Thermal stability of WT and mutant m971 Fabs at different **pH's.** A) Melting temperature (T_m) calculated from intrinsic fluorescence. B) Onset aggregation temperature (T_{onset}) calculated from static light scattering.





Supporting Figure 4. Lysis of Raji cells by wild type (wt) and Y^{H52A}R mutant m971 CAR-T cells. WT m971 and Y^{H52A}R m971 scFv were incorporated into the 2nd generation CAR with CD28 transmembrane domain and intracellular 4-1BB and CD3 ζ domain, which were expressed on Jurkat T cells surface by lentiviral-mediated transduction. CAR-T cell killing of Raji cells was detected by the Promega CytoTox-Glo[™] Cytotoxicity Assay kit. Untransduced T cells were used as a negative control.



Supporting Figure 5. Sulphate ions in the CD22_{d6-d7}-m971 Fab co-crystal structure. A) Representative electron density showing the sulphate ion sites I (left), II (middle) and III (right) on the surface of CD22 (in wheat color). The electron density was calculated from a composite omit map and contoured at 1 σ . B) Crystal structure of CD22_{d6-d7} in complex with m971 Fab. Electrostatic surface of the m971 Fab is represented. SO₄ III is located at the interface between CD22 and m971. The calculation of the surface electrostatics was made with the APBS software (1) and prepared using Pymol (2) and displayed on a scale of -5 kT/e (red) to 5 kT/e (blue).

hCD22 mCD22 rCD22	1 1 1	MHLLGPWLLLLVLEYLAFSDSSKWVFEHPETLYAWEGACVWIPCTYRALDGDLESF MRVHYLWLLLILGHAASAQYSSANDWTVDHPQTLFAWEGACIRIPCKYKTPLPKARLDNI MCVPCPWLLLILGHVASFRSATGWSVEHPQTLFAWEGACIQIPCKYKVPKPRSRLDSI * : ****:: : : : : : : : : : : : : : :	56 60 58
hCD22 mCD22 rCD22	57 61 59	ILFHNPEYNKNTSKFDGTRLYESTKDGKVPSEQKRVQFLGDKNKNCTLSIHPVHLN LLFQNYEFDKATKKFKGTVLYNKAEPELYPPKQRRVTFLGNSIDNCTLKIHPIRAN LLFQNYTFDNTTRDFTGKVLYNNTKASIETELYFSQQDRVTFLGNRSNNCTLKIDPIHAN :**:* ::: * .* *. **::: :* ** ***: .****: ****:	112 116 118
hCD22 mCD22 rCD22	113 117 119	DSGQLGLRMESKTEKWMERIHLNVSERPFPPHIQLPPEIQESQEVTLTCLLNFSCYGYPI DSGNLGLRMTAGTERWMEPIHLNVSEKPFQPYIQMPSEIRESQSVTLTCGLNFSCFEYDI DSGKLGLRLISGTDKWMEHIHLNVSERPFQPYIQLPSEIRESQRVSLTCGLNFTCFGYDI ***:****:::::::::::::::::::::::::::::	172 176 178
hCD22 mCD22 rCD22	173 177 179	QLQWLLEGVPMRQAAVTSTSLTIKSVFTRSELKFSPQWSHHGKIVTCQLQDADG LLQWFLEDSKITSVTPSVTSITSSVTSSIKNVYTESKLTFQPKWTDHGKSVKCQVQHSS- SLRWSLDGSEVSPVTSSITSSVENVYTESKLTFQPKWTDHGKSMMCQVWHFS- *:* *:. : . : : : : : : : : : : : : : :	226 235 230
hCD22 mCD22 rCD22	227 236 231	KFLSNDTVQLNVKHTPKLEIKVTPSDAIVREGDSVTMTCEVSSSNPEYTTVSWLKDGT EVLSERTVRLDVKYTPKLEIKVNPTEVEKNNSVTMTCRVNSSNPKLRTVAVSWFKDGR QVLSERTVRLDVKYTPKLQITVNPTEVKEGNSVTMTCQVNSSNPSLSTKKVSWFKDGS :.**: **:*:****************************	284 293 288
hCD22 mCD22 rCD22	285 294 289	SLKKQNTFTLNLREVTKDQSGKYCCQVSNDVGPGRSEEVFLQVQYAPEPSTVQI PLEDQELEQEQQMSKLILHSVTKDMRGKYRCQASNDIGPGESEEVELTVHYAPEPSRVHI FLKEQQEQEELTLHSVTKDMRGKYQCRASNNIGQGQSKEVALTVLYPPEPSKVHI *:.*: * *:.**** *** *:.**::* *.*:** * * *	338 353 343
hCD22 mCD22 rCD22	339 354 344	LHSPAVEGSQVEFLCMSLANPLPTNYTWYHNGKEMQGRTEEKVHIPKILPWHAGTYSCVA YPSPAEEGQSVELICESLASPSATNYTWYHNRKPIPGDTQEKLRIPKVSPWHAGNYSCLA YPSPAEEGQSVELICDSLASPRATNYTWYHNGEMVPGASHEKLQISNVSLWHAGKYSCLA *** ****::* ***.* ******* : : * :.**::* :: ****.***:*	398 413 403
hCD22 mCD22 rCD22	399 414 404	ENILGTGQRGPGAELDVQYPPKKVTTVIQNPMPIREGDTVTLSCNYNSSNPSVTRYEWKP ENRLGHGKIDQEAKLDVHYAPKAVTTVIQSFTPILEGDSVTLVCRYNSSNPDVTSYRWNP ENRLGCGKIEQEAELDVHYVPKAVTTVIQSVTPIREGDSVTLDCRYNSSNPEVTKYEWSP ** ** *: *:***:* ** ****** ** *********	458 473 463
hCD22 mCD22 rCD22	459 474 464	HGAWEEPSLGVLKIQNVGWDNTTIACAACNSWCSWASPVALNVQYAPRDVRVRKIKPLSE QGSGSVLKPGVLRIQKVTWDSMPVSCAACNHKCSWALPVILNVHYAPRDVKVLKVSPASE QGSGSEVTPGVLRIQKATWDSPPIKCAACNYKCSWSFPVSLNVHYAPRAVKILKVSPASE :*:***:**:.**.:***	518 533 523
hCD22 mCD22 rCD22	519 534 524	IHSGNSVSLQCDFSSSHPKEVQFFWEKNGRLLGKESQLNFDSISPEDAGSYSCWVNNSIG IRAGQRVLLQCDFAESNPAEVRFFWKKNGSLVQEGRYLSFGSVSPEDSGNYNCMVNNSIG IHAGQHVLLQCDFSGSFPTEVRFFWKKNGNLVQEGRYLNFSSISPEDSGNYNCMVRNSIG *::*: * *****: * * **:**** *: * *.********	578 593 583
hCD22 mCD22 rCD22	579 594 584	QTASKAWTLEVLYA <mark>PRRLRV</mark> SMSPGDQVMEGKSATLTCESDANPPVSHYTWFDWNNQSLP ETLSQAWNLQVLYA <mark>PRRLRV</mark> SISPGDHVMEGKKATLSCESDANPPISQYTWFDSSQQDLH ETSSQAWSLQVLYA <mark>PRRLRV</mark> SISPGDSVMEGKKATLSCESDAHPPVFKYIWFDANDQDLH :* *:**.**:****************************	638 653 643
hCD22 mCD22 rCD22	639 654 644	YHSQKLRLEPVKVQHSGAYWCQGTNSVGKGRSPLSTLTVYYSPETIGRRVAVGLGSCLAI SSGQKLRLEPLEVQHTGSYRCKGTNGIGTGESPPSTLTVYYSPETIGKRVALGLGFCLTI FSGQKLRLEPLRVQHTGSYRCQGINELGTGKSPPSTLTVYYSPETIGKRVALGLGFCLAI .*******:.***:** *:* *:* *:************	698 713 703
hCD22 mCD22 rCD22	699 714 704	LILAICGLKLQRRWKRTQSQQGLQENSSGQSFFVRNKKVRRAPLSEGPHSLGCYNPMMED CILAIWGMKIQKKWKQNRSQQGLQENSSGQSFFVRNKKARRTPLSEGPQSQGCYNPAMDD CILAIWGMKIQKKWKHNRSQQGPQDNSSGQSFFVRNKKARRTRLTEGPQSQGCYNLAMDD **** *:*::*:**** *:**** *:***********	758 773 763
hCD22 mCD22 rCD22	759 774 764	GISYTTLRFPEMNIPRTGDAESSEMQRPPPDCDDTVTYSALHKRQVGDYENVIPDFPEDE TVSYAILRFPESDMHNAGDAGTPATQAPPPNNSDSVTYSVIQKRPMGDYENVNPSCPEDE TVSYAVLRFPESDTHGAGGARSPATQGPPPNDDDTVTYSVLQKRNMGDYENVSPNCPEDE :**: ***** : :*.* : * ***: .*:****.:** :****** *. ****	818 833 823
hCD22 mCD22 rCD22	819 834 824	GIHYSELIQFGVGERPQAQENVDYVILKH SIHYSELVQFGAGKRPQAKEDVDYVTLKH SIHYSELVQFGAGKRPQAKEDVDYVTLKH ******	847 862 852

Supporting Figure 6. Sequence alignment of CD22. Amino acid sequence alignment of CD22 from human (hCD22), mouse (mCD22) and rat (rCD22). The red box indicates the putative heparan sulfate binding site, with XBBXBX motif (X, aliphatic residues and B, amino acids R, K or H). Sequence alignment was done using the Clustal Omega software (3).

Supporting Table 1. Residues involved in the m971-CD22 interaction. S= salt bridge; H= hydrogen bond.

Contact	CD22 residue	BSA (Ų)
type		
	P623	12
	V624	10
	S625	56
S	H626	71
	Y627	8
Н	T628	18
Н	D631	16
	W632	35
Н	N633	11
Н	Q635	62
	S636	28
	L637	32
Н	P638	109
Н	Y639	70
HS	H640	136
Н	S641	52
	Q642	3
	R645	11
	L646	22
HS	E647	65
	P648	43
	K650	15
	Q652	36
Н	H653	57
Н	Y657	12

	Total	990
	m971 HC residue	BSA (Ų)
Н	N32	32
	A35	1
Н	R50	32
Н	Y52	38
	R52b	78
Н	S53	64
	K54	65
Н	W55	25
Н	Y56	99
	N57	37
Н	D58	33
	Y59	28
HS	K64	30
	T97	20
	G98	12
HS	D99	74
	L100	26
HS	D100b	28
	Total	722
	m971 LC residue	BSA (Ų)
	W30	71
Н	Y32	60
	S91	11
Н	Y92	78
	S93	15

194	41
Total	276

References

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