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Supporting information for article:

Antibody fragments structurally enable a drug-discovery campaign on the cancer target Mcl-1

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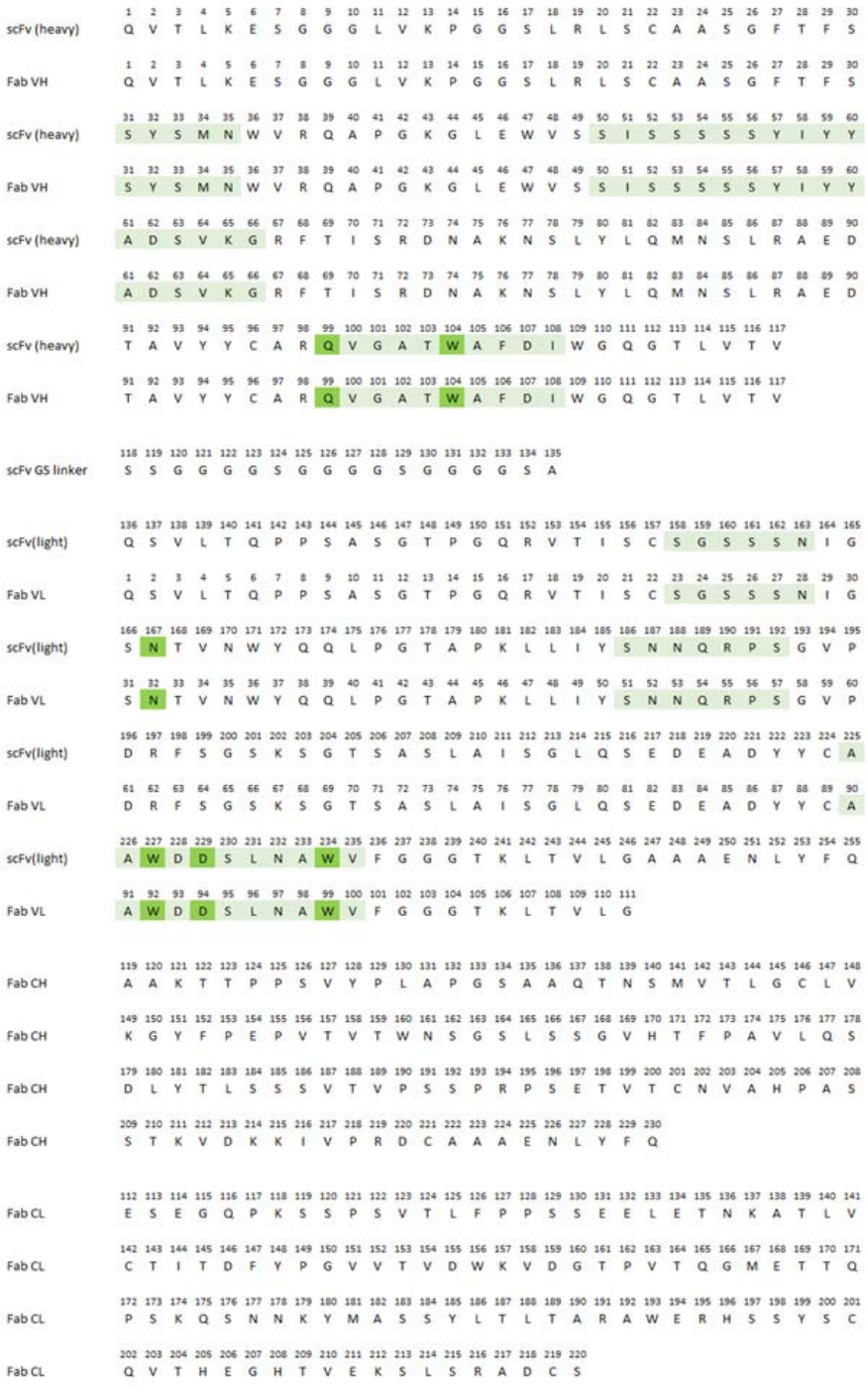
(A)



(B)

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Chimera   ------------------------------------------------------------      0
Q07820    MFGLKRNAVIGLNLYCGGAGLGAGSGGATRPGGRLLATEKEASARREIGGGEAGAVIGGS      60

Chimera   ------------------------------------------------------------      0
Q07820    AGASPPSTLTPDSRRVARPPPIGAEVPDVTATPARLLFFAPTRRAAPLEEMEAPAADAIM      120

Chimera   ----------------------------------------------GPLGSEDDLYRQSLE      15
Q07820    SPEEELDGYEPEPLGKRPAVLPLLELVGESGNNTSTDGSLPSTPPPAEEEEDELYRQSLE      180
                                                        .**:*******

Chimera   IISRYLREQATGSKDSKPLGEAGAAGRRALETLRRVGDGVQRNHETAFQGMLRKLDIKNE      75
Q07820    IISRYLREQATGAKDTKPMGRSGATSRKALETLRRVGDGVQRNHETAFQGMLRKLDIKNE      240
          ***********.**:**:*.:**:.*:*******************************

Chimera   DDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAESITDVLVR      135
Q07820    DDVKSLSRVMIHVFSDGVTNWGRIVTLISFGAFVAKHLKTINQESCIEPLAESITDVLVR      300
          ************************************************************

Chimera   TKRDWLVKQRGWDGFVEFFHVEDLEGG---------------------- 162
Q07820    TKRDWLVKQRGWDGFVEFFHVEDLEGGIRNVLLAFAGVAGVGAGLAVLIR 350
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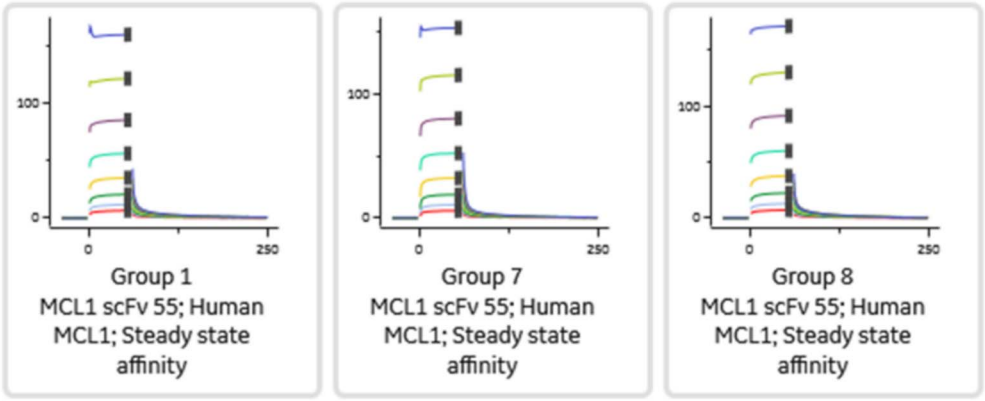
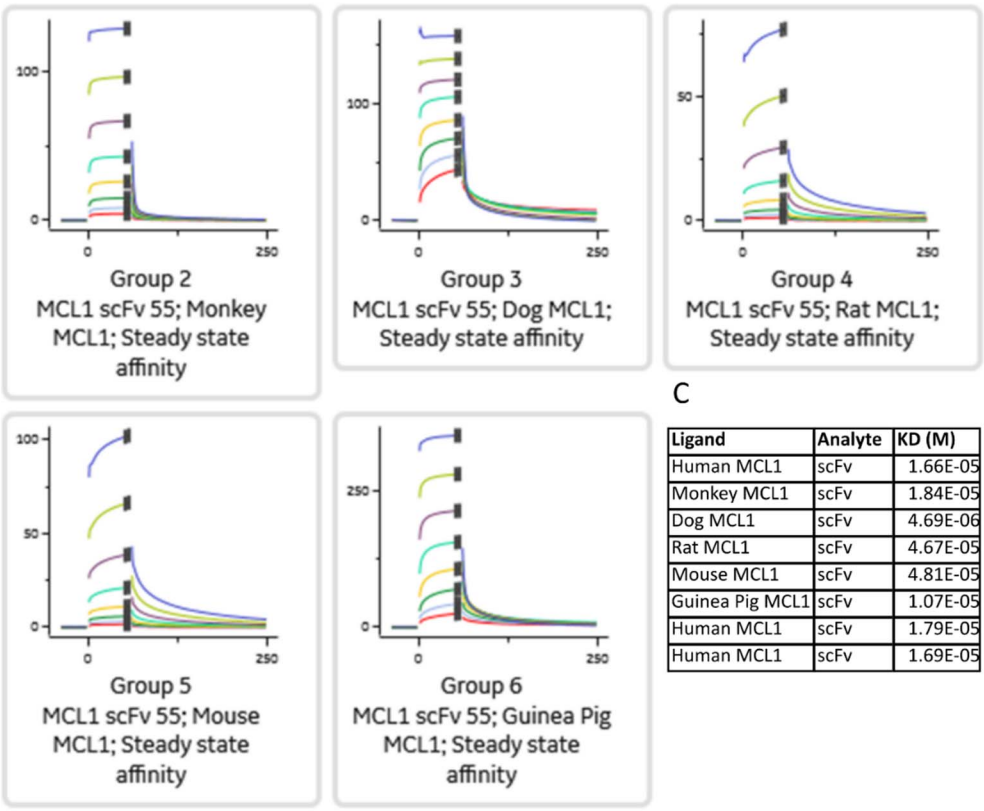
Figure S1 (**A**) Table showing alignment and the amino acid numbering for the scFv and Fab. Light shading shows the range of the CDRs according to MOE antibody modeller software (MOE, 2014). Dark shading highlights specific amino acids involved in direct contact with Mcl-1 BH2. (**B**) Alignment of full length Mcl-1 with the human-mouse chimera used in crystallisation. Blue box indicates the mouse sequence.

A



B



C

Ligand	Analyte	KD (M)
Human MCL1	scFv	1.66E-05
Monkey MCL1	scFv	1.84E-05
Dog MCL1	scFv	4.69E-06
Rat MCL1	scFv	4.67E-05
Mouse MCL1	scFv	4.81E-05
Guinea Pig MCL1	scFv	1.07E-05
Human MCL1	scFv	1.79E-05
Human MCL1	scFv	1.69E-05

Figure S2 Binding sensorgrams of different orthologues of Mcl-1 immobilised on the SPR chip with scFv injected as an analyte. (**A**) shows three replicates of human Mcl-1, while (**B**) shows different species and their binding response to scfv. (**C**) summarises the steady state K_d obtained. The Groups refer to the 8 channels available on the Biacore 8000.

(A) SPR parameters

							A-B-A injection with compound AZD5991	
Ligand (Mcl-1)	**Analyte**	**Kd (µM)**	**RU_{imm}**	**Theoretical R_{max}**	**R_{max}**	**Est. surface activity**	**Kd (µM)**	**Fold Difference**
Human	scFv	16.60	445.6	844	218.4	26%	14.2	1.17
Monkey	scFv	18.40	371.5	704	182.6	26%	16.5	1.11
Dog	scFv	46.90	285.4	541	126.1	23%	3.84	1.22
Rat	scFv	46.70	293.4	556	166.9	30%	39.5	1.18
Mouse	scFv	48.10	352.3	668	224.5	34%	39.3	1.22
Guinea	scFv	10.70	855.9	1622	410.9	25%	12.3	0.87
Human	scFv	17.90	435.8	826	214.3	26%	14.5	1.23
Human	scFv	16.90	476.6	903	234.7	26%	16.8	1.00

(B) ITC parameters

Syringe	**Sample**	**N (sites)**	**Error +/-**	**K (M^{-1})**	**Error +/-**	**Kd (µM)**	**Error +/-**	**ΔH (cal/mol)**	**Error +/-**	**ΔS (cal/mol/deg)**
scFv	Mcl-1	0.92	0.04	2.87E+05	4.57E+04	3.84	0.66	-1.16E+04	6.86E+02	-1.38E+01
scFv	Mcl-1	1.13	0.03	7.90E+05	1.23E+05	1.27	0.23	-9484	349.5	-4.81
Fab	Mcl-1	1.29	0.03	8.77E+05	1.31E+05	1.14	0.20	-6814	243.3	-4.33

(C) DSF parameters

Method	**Mcl-1 Tm (°C)**	**scFv Tm(°C)**	**scFv Tm(°C) (2 fold excess of Mcl1)**	**scFv Tm(°C) (10 fold excess of Mcl1)**
1st derivative	78.23 ± 0.08	70.53 ± 0.01	71.65 ± 0.01	73.8 ± 0.2

Figure S3 Tables showing biophysical parameters. **(A)** Table lists steady state affinity derived K_d for each of the 8 channels used in the experiment. Ligands denote the immobilised protein, while analyte refers to the injected protein. Ru imm refers to the RU immobilised in each surface, while the theoretical Rmax estimates the extent of response assuming full occupancy of 1:1 binding (Theoretical R_{max}=R_{imm}*($MW_{analyte}$/MW_{ligand}). R_{max} gives the fitted R_{max} which is then used to calculate the estimated surface activity (R_{max}/Theoretical R_{max}).To perform the ABA injection experiment, AZ5991 had been initially injected at a saturating concentration (1 µM) and the scFv had been titrated in a twofold dilution up to 40 µM. Data were evaluated with Biacore 8K Evaluation Software. Last two columns list K_d for scfv binding with AZD5991 pre-bound with an A-B-A injection. **(B)** ITC parameters. **(C)** DSF parameters.

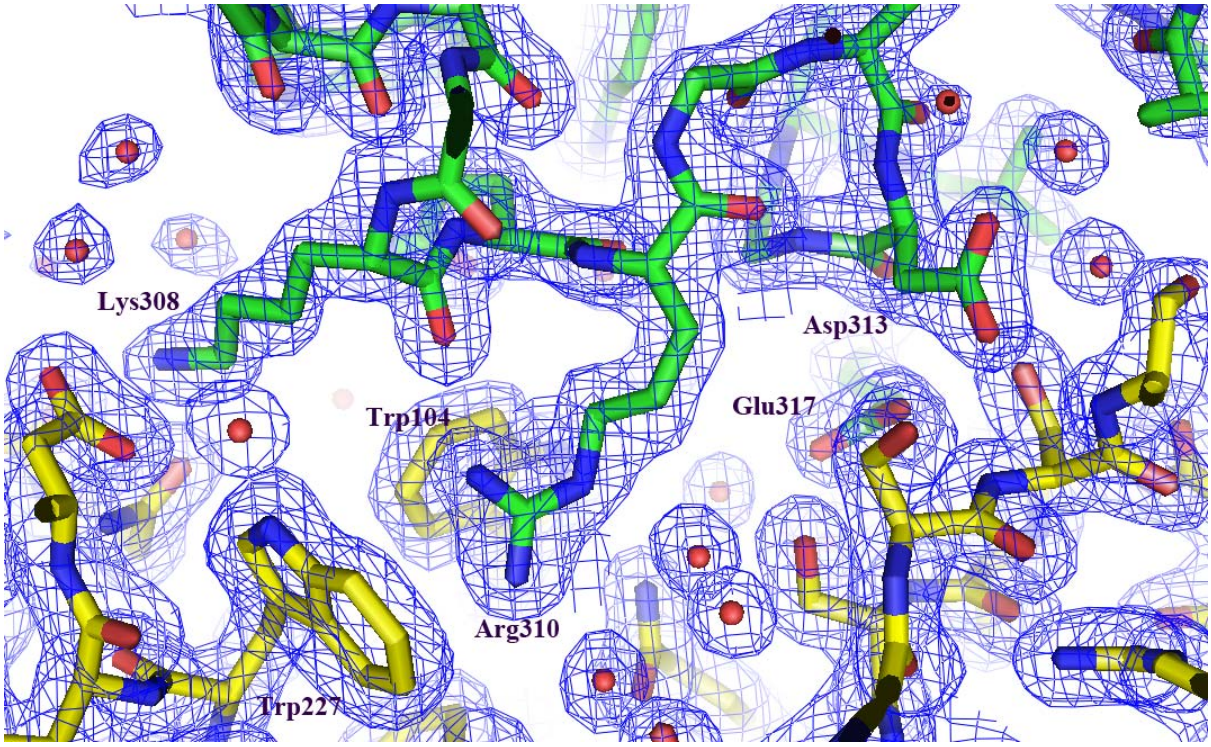


Figure S4 Figure showing an example of the 2Fo-Fc electron density for epitope-paratope interaction between Mcl-1 and the scFv (deposition 6QB3) at 1.9Å. The buried Arginine 310 side chain (green) is shown in the CDR pocket (yellow). Waters are shown as red spheres. Key residues referred to in the main text are labelled.

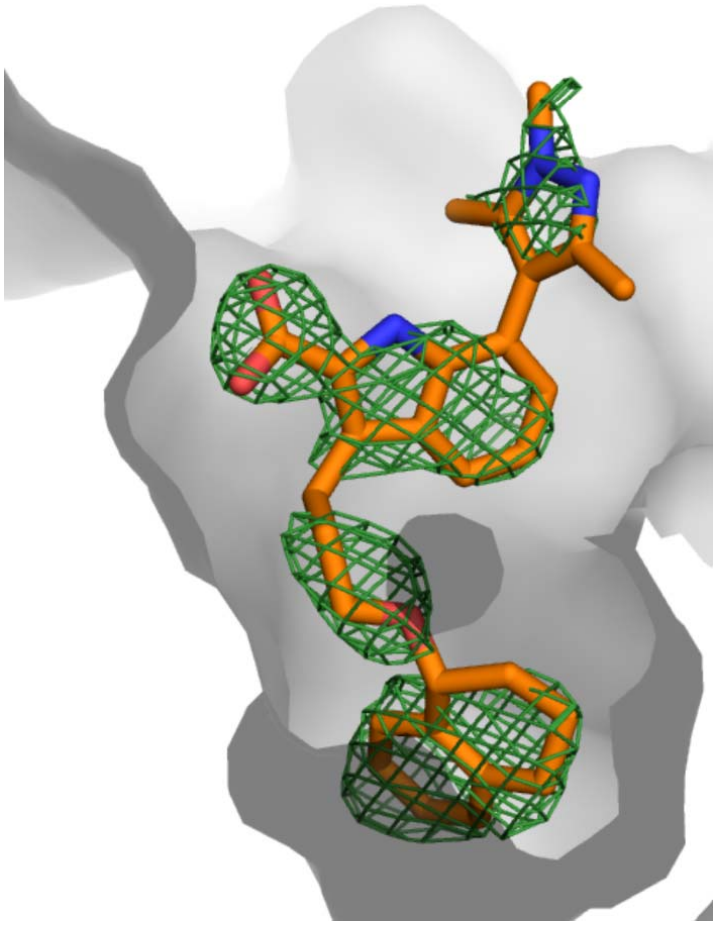


Figure S5 Figure showing a simple omit map of the ligand binding site. The Fo-Fc density was calculated by removing the ligand from the model and re-refining the phases using Buster. The map is contoured at 3σ.

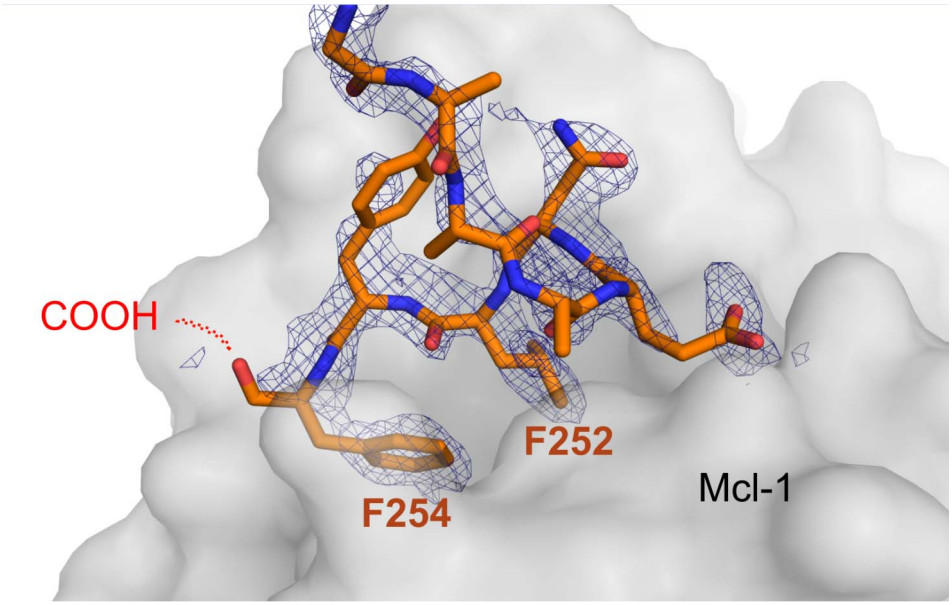


Figure S6 The 2Fo-Fc density was calculated by removing the C-terminal from the model and re-refining the phases using Buster. The map is contoured at 1σ. The carboxy terminal is marked.

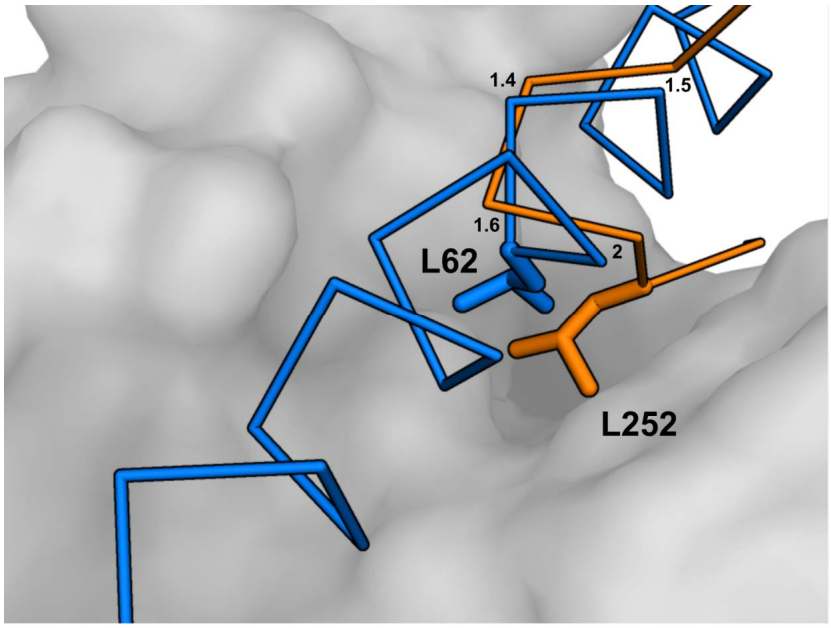


Figure S7 Figure showing and Cα overlay between the C-terminal region of the scFv bound in the BH3 binding site (orange) and the BimBH3 peptide from 2NL9 (blue). Mcl-1 is shown as a surface. Cα distances are shown. The close overlay of L252 and L62 side chains are shown.

References

Molecular Operating Environment (MOE) software. Chemical Computing Group Inc. 2014. http://www.chemcomp.com