Supplementary Information

Structures of BCL-2 in complex with venetoclax reveal the molecular basis of resistance mutations

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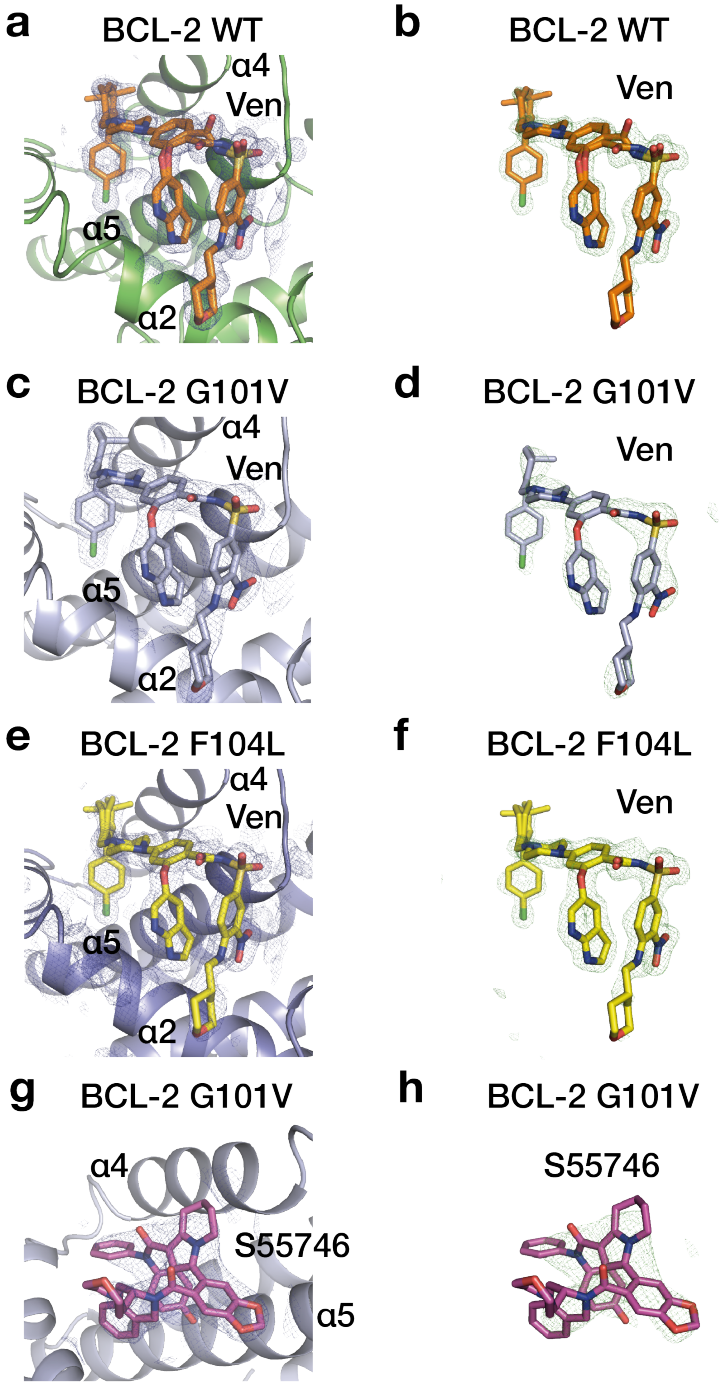
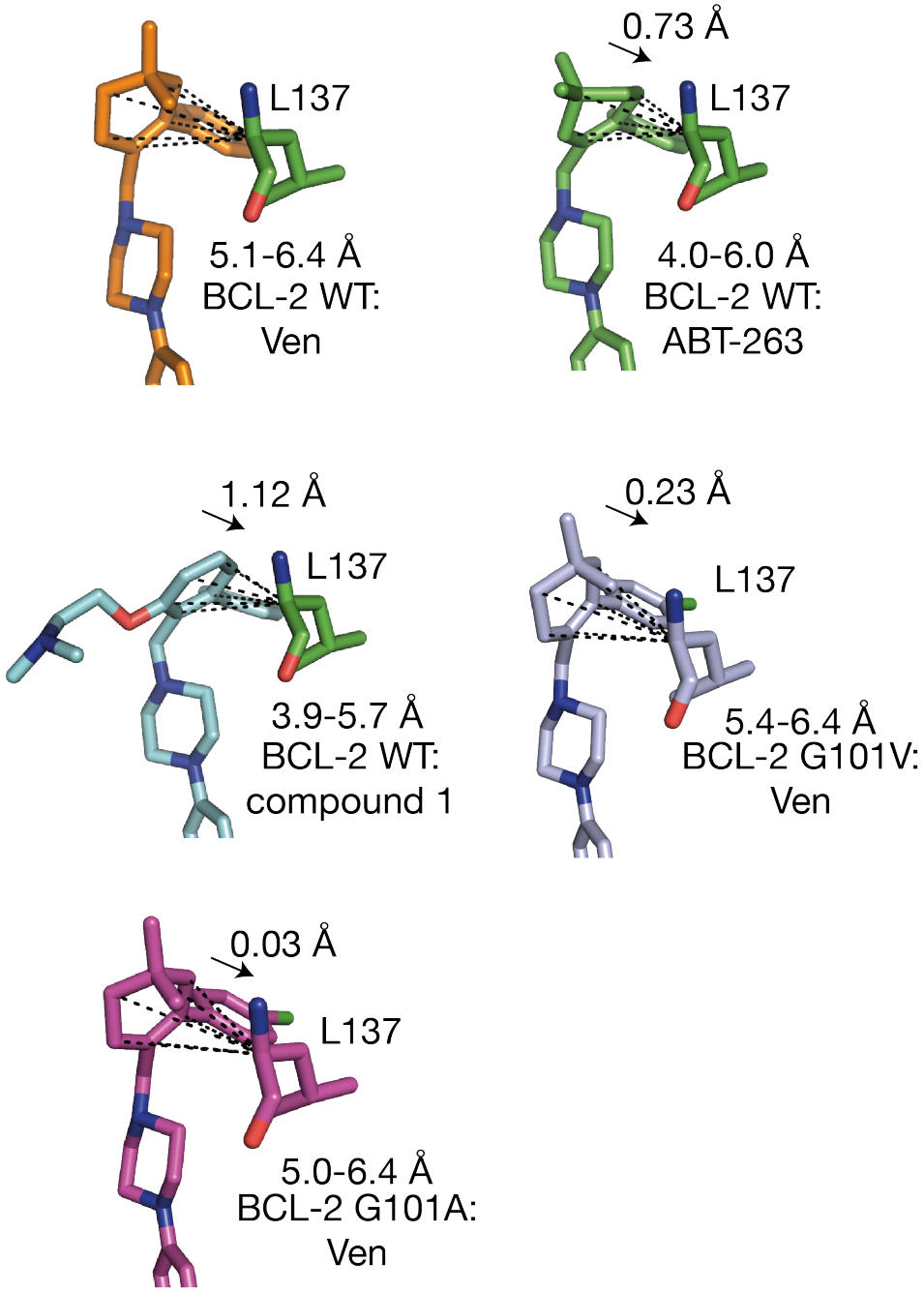
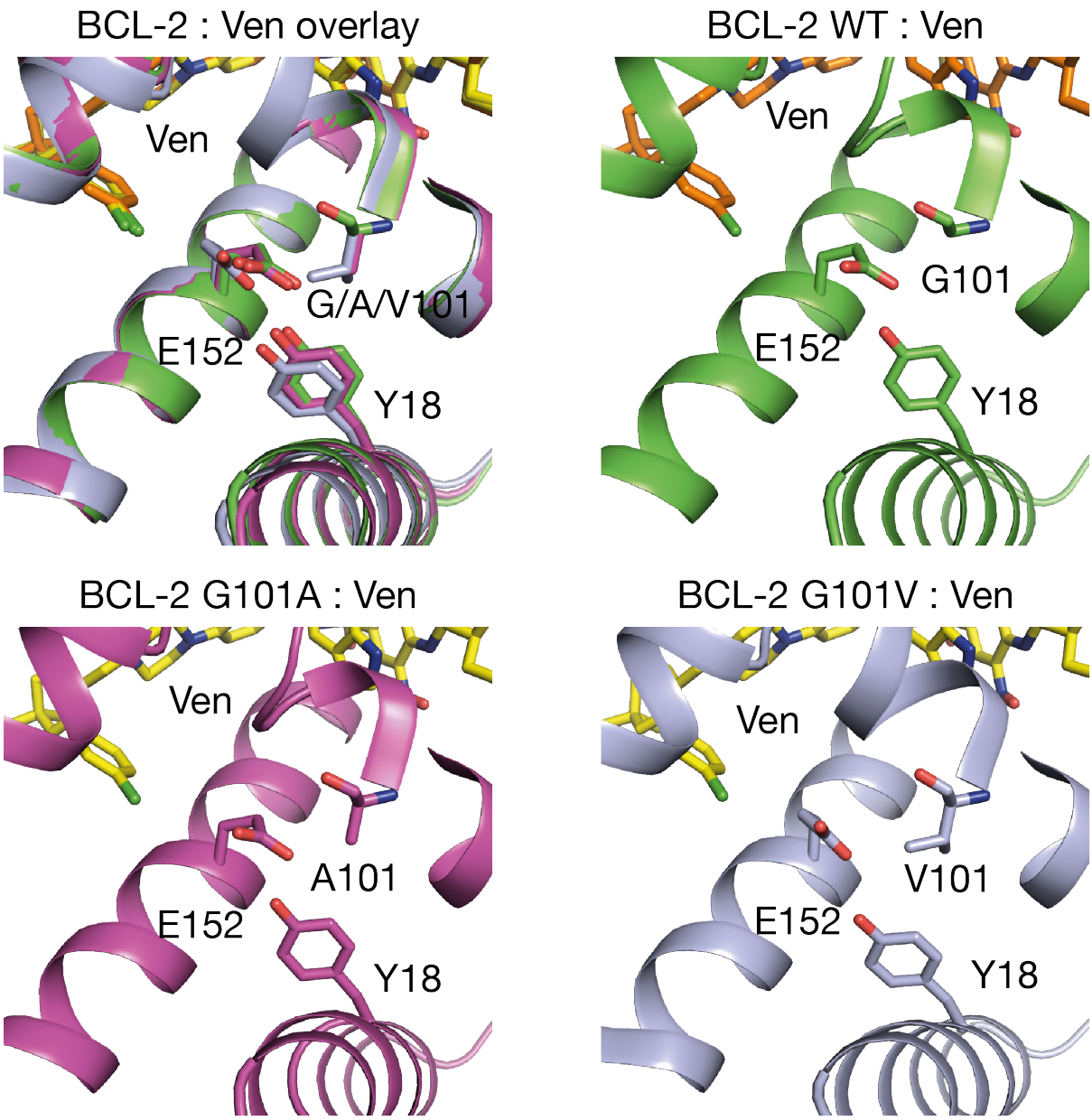


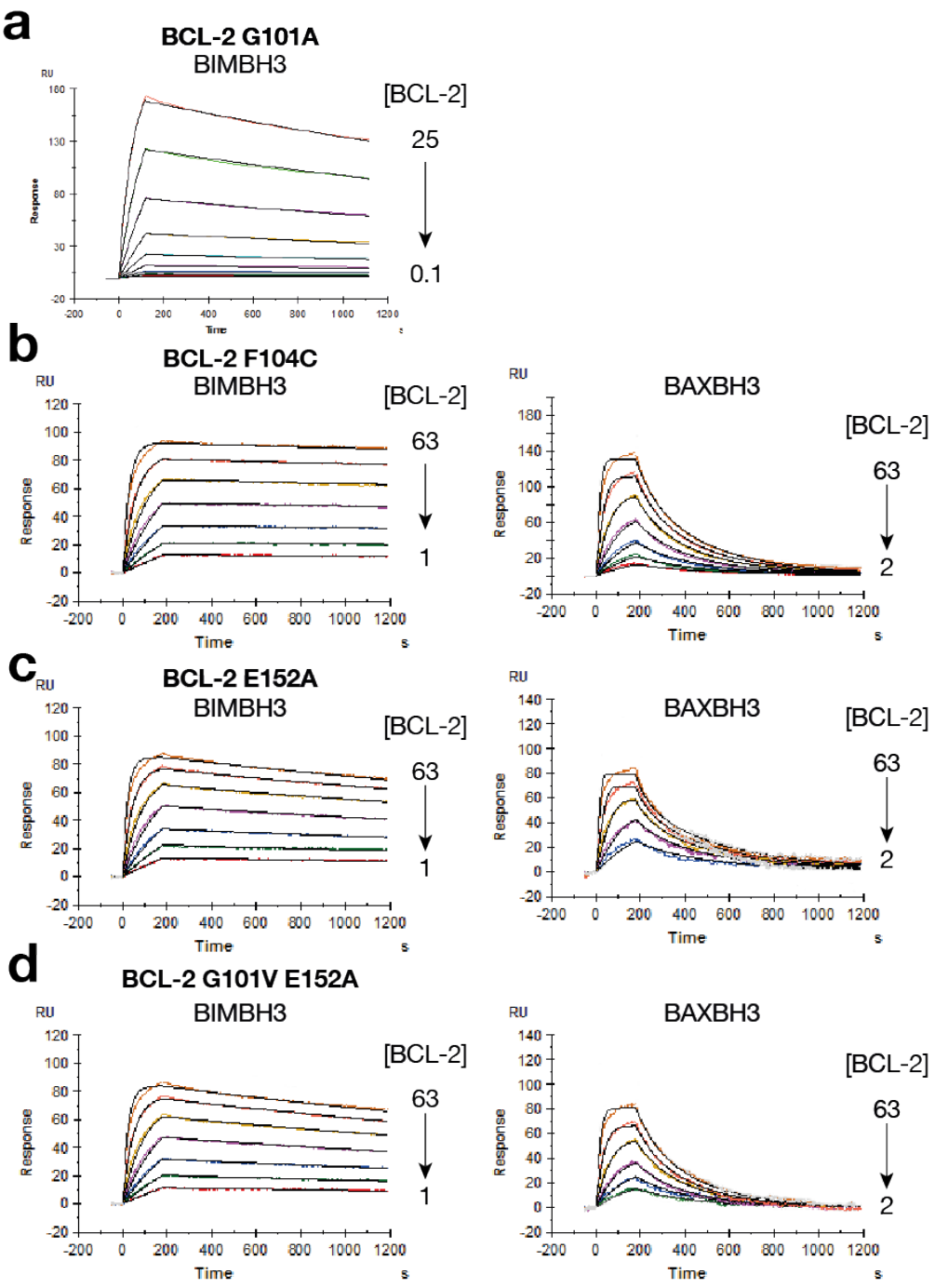
Supplementary Figure 1. BCL-2 mutant with Venetoclax or S55746 electron density maps. Electron density maps for BCL-2 mutants with venetoclax (Ven) or S55746 showing sigma weighted 2Fo-Fc refined density (blue; **a**, **c**, **e**, **g**) or simulated annealing (cartesian) Fo-Fc omit map density (green; **b**, **d**, **e**, **f**) for each crystal structure with BCL-2 mutants: wild-type (WT, green), G101V (light blue) and F104L (darker blue). The 2Fo-Fc density was contoured at 1.0 σ and Fo-Fc density contoured at 3.0 σ. Maps were visualised in pymol using the isomesh function, excluding density 3 Å from either venetoclax or S55746.



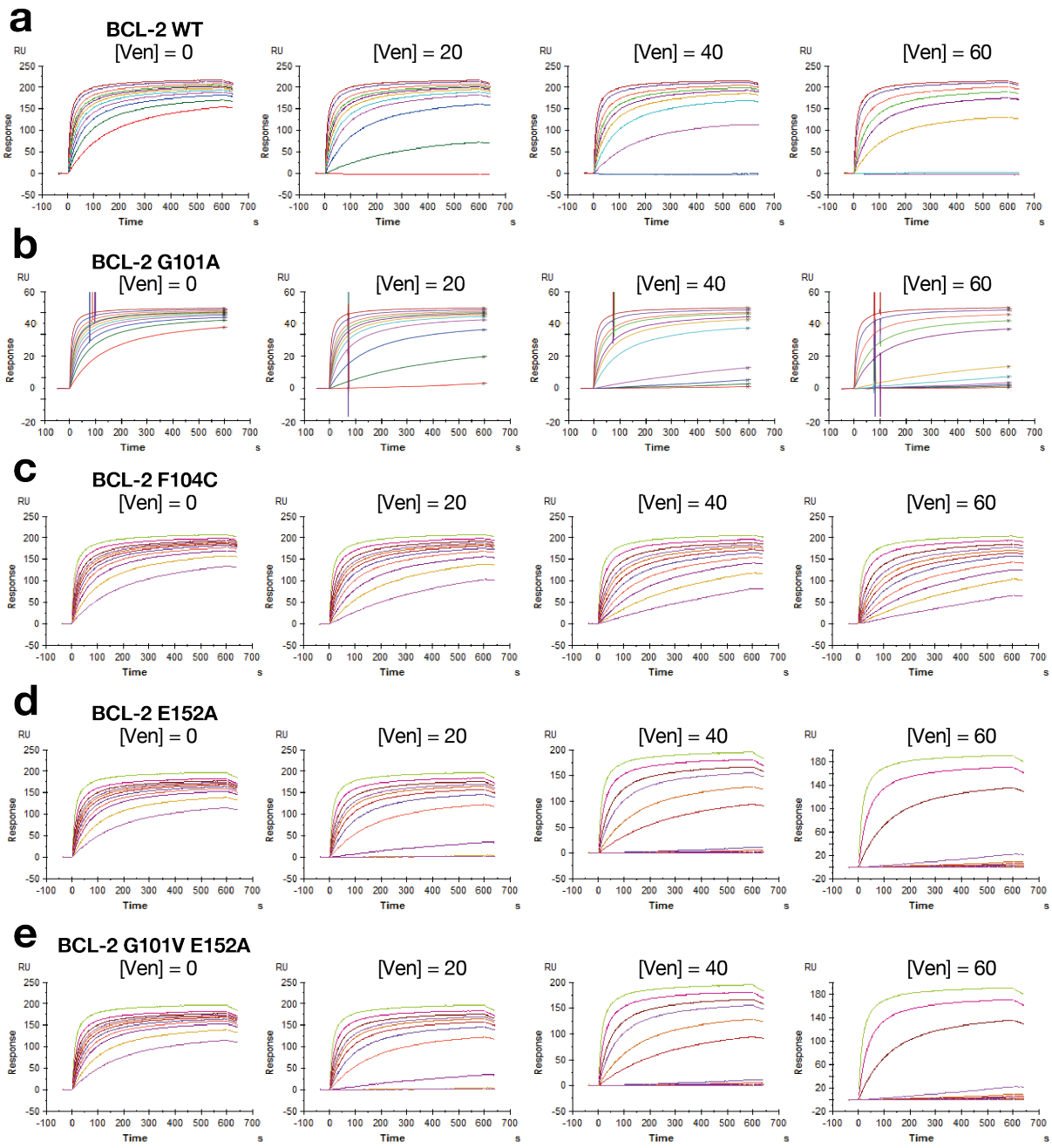
Supplementary Figure 2. Relative distances between compounds and L137. Distances were measured (black dotted lines) between the top 6 membered ring of venetoclax (Ven, orange or light blue), ABT-263 (green) and 4MAN analogue (cyan) to the BCL-2 residue L137 Cα. The range of these distances are shown for each structure. RMSD values relative to the BCL-2 WT:venetoclax (alternate conformer A) are indicated above arrows informing the movement of the ring towards L137.



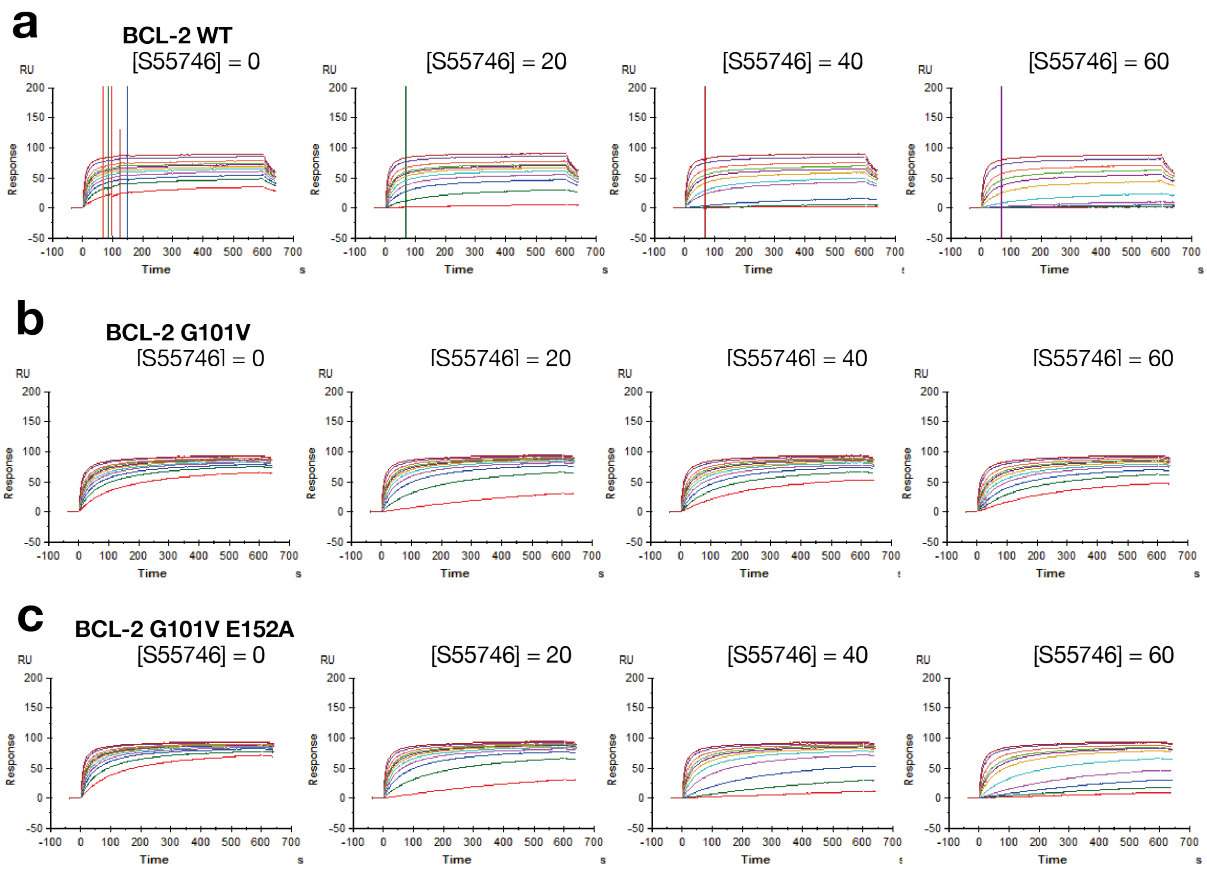
Supplementary Figure 3. Structure of BCL-2 G101A:venetoclax. Structures of BCL-2 WT (green), G101A (magenta) and G101V (light blue) bound to venetoclax (Ven, orange in WT and yellow in mutant structures). Key residues Tyr18, Glu152 and Gly/Ala/Val101 are shown in stick representation.



Supplementary Figure 4. SPR sensograms of BCL-2 mutants binding to BIMBH3 and BAXBH3 peptides. Direct binding experiments to determine affinity of BCL-2 mutants G101A (**a**), F104C (**b**), E152A (**c**), G101V E152A double mutant (**d**) for BIMBH3 and BAXBH3 peptides. Raw data (coloured curves) were fitted (black curves) to a 1 site specific kinetic model to determine on and off rates and the equilibrium binding constant K_D. Data are representative of at least 2 independent experiments. The BCL-2 mutant proteins were diluted in a 2-fold dilution series, concentration ranges for curves are indicated. All experiments shown were performed on a BIAcore 4000, with the exception of (**a**) which was performed on a BIAcore S200.

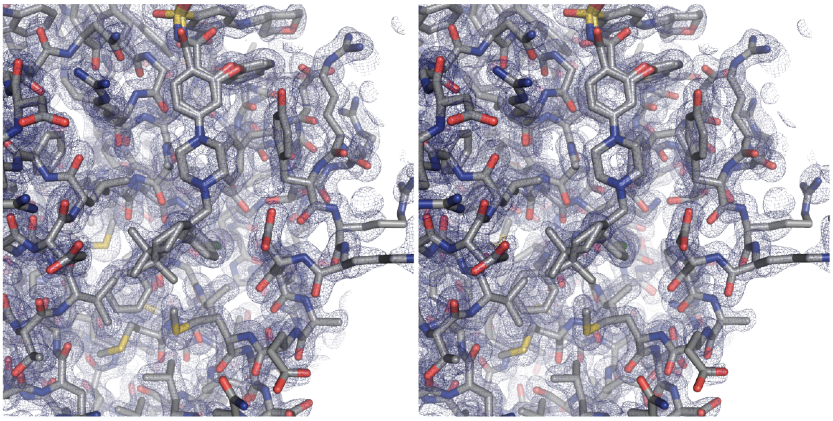


Supplementary Figure 5. BCL-2 mutants with venetoclax steady-state SPR sensograms. Double referenced sensorgrams for BCL-2 wild-type (WT, **a**), G101A (**b**), F104C (**c**), E152A (**d**) and G101V E152A (**e**) double mutant (**d**) binding to a BIMBH3 peptide immobilised chip in the presence of various venetoclax (Ven) concentrations (0-60 nM). Average response at the end of injection ~600 sec, were plotted as a function of BCL-2 and venetoclax concentration in figure 3a-d. Data are representative of 3 independent experiments.

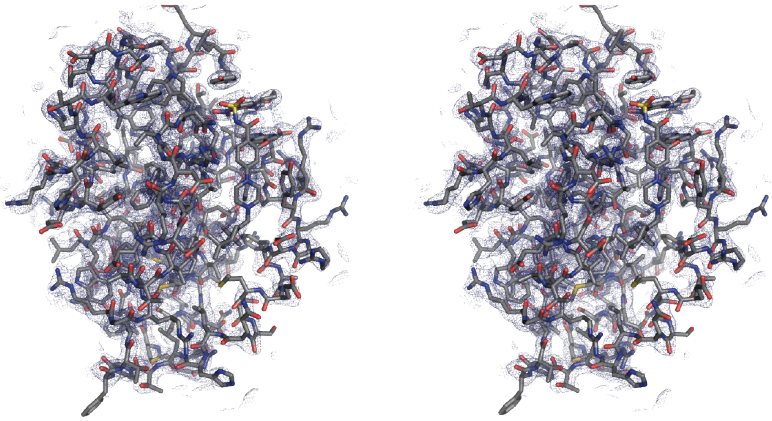


Supplementary Figure 6. BCL-2 mutants with S55746 steady-state SPR sensograms. Double referenced sensorgrams for BCL-2 wild-type (WT, **a**), G101V (**b**) and G101V E152A double mutant (**c**) binding to a BIMBH3 peptide immobilised chip in the presence of various S55746 concentrations (0-60 nM). Average response at the end of injection ~600 sec, were plotted as a function of BCL-2 and S55746 concentration in figure 3e-f. Data are representative of at least 2 independent experiments.

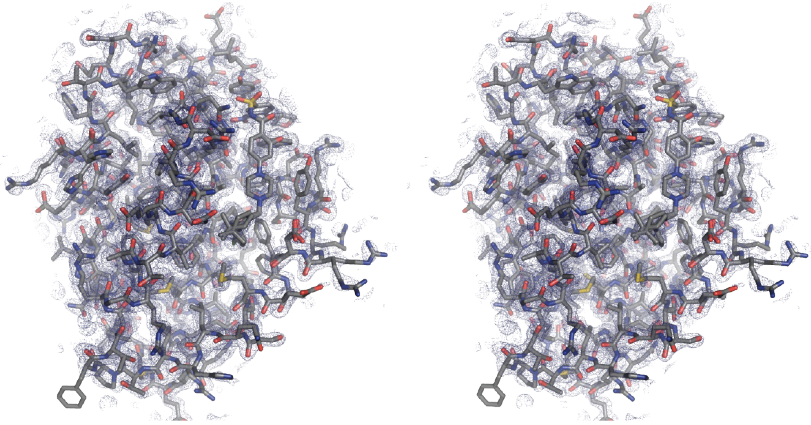
a BCL-2 WT:Venetoclax

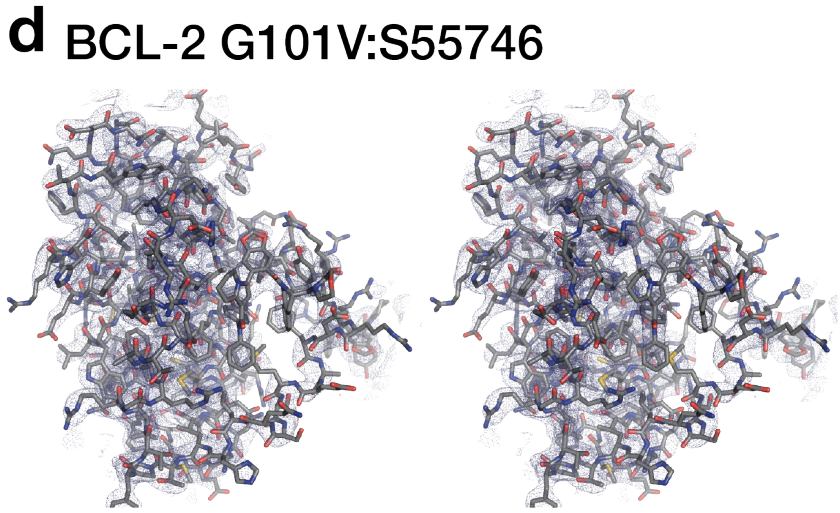


b BCL-2 G101V:Venetoclax



c BCL-2 F104L:Venetoclax





Supplementary Figure 7. Stereo images of 2Fo-Fc electron density maps contoured at 1 σ for the BCL2 mutant small molecule structures reported.

ring position	WT Ven*	WT ABT-263	WT 4MAN	G101V Ven	G101A Ven
C1	5.06	4.91	3.88	5.39	5.01
C2	6.08	5.43	4.09	5.75	6.03
C3	6.22	6.01	5.04	6.17	6.17
C4	6.43	5.85	5.70	6.41	6.38
C5	6.22	5.05	5.52	6.20	6.17
C6	5.61	3.98	4.67	5.52	5.56
RMSD	**-**	**0.73**	**1.12**	**0.23**	**0.03**

Supplementary Table 1. Distance between compounds and BCL-2 residue L137 on helix 4. Distances are reported in ångström (Å) from each carbon in the 4-4-dimethylcyclohex-1-ene (venetoclax), 5-5-dimethylcyclohex-1-ene (ABT-263), 4′-biphenyl (compound 1) relative to the L137 Cα. *venetoclax showed two conformations for the cyclohex-1-ene ring the A conformer was used as a reference as it matched the conformers from the ABT-263 and G101V:venetoclax structures. See structural analyses for further details.

BCL2 variant	Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	R_{max} (RU)	χ^2 (RU^2)
G101A	BIMBH3	1.07E+06	2.87E-04	2.67E-10	33	0.08
G101A	BIMBH3	1.02E+06	2.22E-04	2.17E-10	35	0.22
G101A	BIMBH3	4.47E+05	8.57E-05	1.92E-10	395	75
F104C	BIMBH3	5.50E+05	4.86E-05	8.84E-11	102	1.1
F104C	BIMBH3	2.85E+05	4.17E-05	1.46E-10	248	11
F104C	BIMBH3	2.96E+05	8.38E-05	2.83E-10	230	12
F104C	BAXBH3	1.67E+06	7.09E-03	4.25E-09	123	4.0
F104C	BAXBH3	2.00E+06	7.91E-03	3.96E-09	90	3.8
F104C	BAXBH3	2.06E+06	7.90E-03	3.84E-09	86	3.9
E152A	BIMBH3	9.84E+05	4.34E-04	4.41E-10	340	17
E152A	BIMBH3	7.23E+05	2.04E-04	2.82E-10	89	1.1
E152A	BAXBH3	2.36E+10	9.86E+01	4.18E-09	29	0.8
E152A	BAXBH3	4.19E+06	1.23E-02	2.93E-09	71	5.0
G101V, E152A	BIMBH3	6.89E+05	6.24E-04	9.05E-10	204	15
G101V, E152A	BIMBH3	6.66E+05	2.38E-04	3.57E-10	87	1.1
G101V, E152A	BAXBH3	4.56E+09	2.60E+01	5.70E-09	29	0.7
G101V, E152A	BAXBH3	1.19E+06	6.34E-03	5.31E-09	82	2.3

Supplementary Table 2. SPR direct binding fit parameters. The kinetic parameters for direct binding between BCL-2 variants and either BIMBH3 or BAXBH3 described in Table 2 and Supplementary Figure 4. Data are shown for each independent experiment used in the analysis.

Primer purpose	Primer sequence (5' to 3')	
	Forward	*Reverse*
Bacterial expression plasmids		
pGEX sequencing	GGGCTGGCAAGCCACGTTTGGTG	
pGEX sequencing		GAAACGCGCGAGGCAGATCG
G101V mutation	CCCTGCGCCAGGCGGTCGATGACTTTAGCCG	CGGCTAAAGTCATCGACCGCCTGGCGCAGGG
G101A mutation	CCCTGCGCCAGGCGGCCGATGACTTTAGCCG	CGGCTAAAGTCATCGGCCGCCTGGCGCAGGG
F104L mutation	CAGGCGGGCGATGACTTAAGCCGTCGCTATCGTCGC	ACGATAGCGACGGCTTAAGTCATCGCCCGCCTGG
F104C mutation	CAGGCGGGCGATGACTGTAGCCGTCGCTATCGTCGC	ACGATAGCGACGGCTACAGTCATCGCCCGCCTGG
E152A mutation	GCATTGTCGCATTTTTCGCATTTGGCGGTGTTATGTGCGTC	GACGCACATAACACCGCCAAATGCGAAAAATGCGACAATGC
mamalian expression plasmids		
G101V mutation reaction 1	GAATTAGATCTTTCGAAATCACCA	CCTGGCGGAGGGTCAGGTGGA
G101V mutation reaction 2	TGGTCCACCTGACCCTCCGCCAGGCAGTTGACGACTTCTCCCG CCGCTACCGCCGCG	TTATCCTGGATCCAGGTGTGCAGGT

Supplementary Table 3. Sequences of oligonucleotide primers used.