# Supplemental data

## The FKBP38 catalytic domain binds to Bcl-2 via a charge-sensitive loop

*Katja Haupt, Günther Jahreis, Miriam Linnert, Mitcheell Maestre-Martínez, Miroslav Malešević, Arndt Pechstein, Frank Edlich, and Christian Lücke*

Table S1: $^{1}H$, $^{13}C$ and $^{15}N$ resonance assignment of the Bcl-$2^{119-131}$ peptide at pH 7.5 and 10 °C.

| residue | N | HN | Hα | Hβ | other H | Cα | Cβ | other C |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
| Leu119 | | | 3.71 | 1.54/1.51 | 1.55γ; 0.91/0.90δ | 55.2 | 44.4 | 26.8γ; 24.8/24.1δ |
| His120 | | | 4.69 | 3.08/3.02 | 6.96δ2; 7.71ε1 | 56.3 | 31.3 | 119.6δ2; 138.8ε1 |
| Leu121 | 125.1 | 8.48 | 4.42 | 1.63/1.54 | 1.55γ; 0.93/0.86δ | 54.9 | 42.5 | 26.8γ; 24.8/23.4δ |
| Thr122 | | | 4.60 | 4.20 | 1.24γ2 | 59.7 | 69.7 | 21.4γ2 |
| Pro123 (*trans*) | | | 4.37 | 2.24/1.76 | 1.95γ; 3.68/3.83δ | 63.4 | 32.1 | |
| Phe124 | 120.7 | 8.42 | 4.61 | 3.10 | 7.25δ; 7.34ε; 7.30ζ | 58.2 | 39.5 | 131.8δ; 131.5 ε; 129.9ζ |
| Thr125 | | 8.01 | 4.24 | 4.14 | 1.18γ2 | 67.7 | 70.1 | 21.6γ2 |
| Ala126 | 126.9 | 8.36 | 4.25 | 1.41 | | 52.6 | 19.1 | |
| Arg127 | 120.3 | 8.36 | 4.31 | 1.87/1.78 | 1.67/1.64γ; 3.19δ | 56.3 | 30.8 | 27.1γ; 43.3δ |
| Gly128 | 110.1 | 8.47 | 3.93 | | | 45.2 | | |
| Arg129 | 120.9 | 8.26 | 4.23 | 1.66 | 1.42γ; 3.11δ | 56.4 | 30.7 | 26.9γ; 43.3δ |
| Phe130 | 121.7 | 8.44 | 4.65 | 3.18/3.04 | 7.27δ; 7.35ε; 7.30ζ | 57.5 | 39.5 | 131.9δ; 131.5 ε; 129.9ζ |
| Ala131 | 127.4 | 8.30 | 4.23 | 1.35 | | 52.4 | 19.4 | |
| $NH_2$ | | 7.08/7.02 | | | | | | |

Figure S1: FKBP38 binding to matrix-bound Bcl-2 peptides. The peptides corresponding to Bcl-2 segments 20-34, 87-102, 167-181, 185-200, 195-210 and 119-131 were loaded onto a streptavidin matrix via biotin labels. The matrix was incubated with *E. coli* lysate containing FKBP38 (50 µg total protein) with CaM/$Ca^{2+}$ (10 µM) in the absence and presence of Bcl-$2^{1-211}$ (5 µM). Input and matrix-bound fractions were subsequently analyzed by Western blotting using polyclonal rabbit anti-FKBP38 antibodies. Specific binding of peptide Bcl-$2^{119-131}$ is shown by the competition between soluble Bcl-2 and matrix-bound peptide.

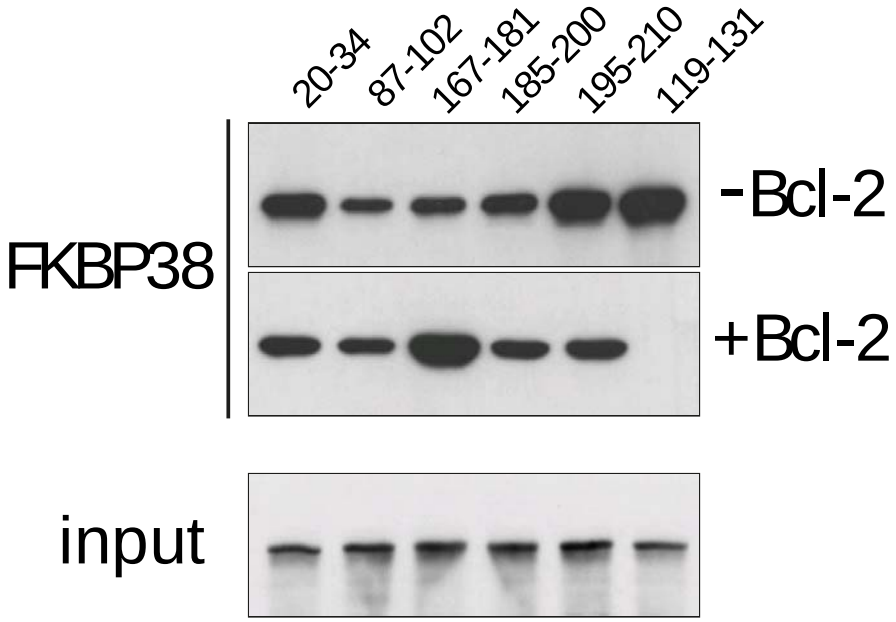

Figure S2: Reverse competition assay. Maltose beads loaded with a maltose binding protein/Bcl-2 fusion protein (MBP/Bcl-2) were incubated with FKBP38 (5 µM) and CaM/$Ca^{2+}$ (10 µM). Competition to the interaction between FKBP38 and Bcl-$2^{1-211}$ was tested by addition of the peptides Bcl-$2^{51-80}$ (i.e., the core region of the long flexible Bcl-2 loop) and Bcl-$2^{119-131}$ (i.e., the specific FKBP38-binding epitope), with subsequent analysis by Western blotting using polyclonal rabbit anti-FKBP38 antibodies. Only the peptide Bcl-$2^{119-131}$ released FKBP38 from the Bcl-2 loaded matrix. (Empty beads served as control.)

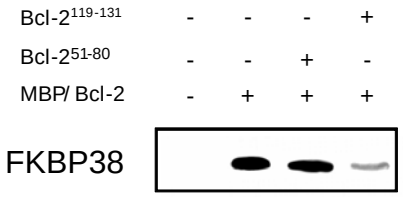

Figure S3: FKBP38 binding to Bcl-$2^{119-131}$. The peptide Bcl-$2^{119-131}$ was loaded onto a streptavidin matrix via a biotin label. The matrix was incubated with FKBP38 (5 µM) in absence and presence of CaM/$Ca^{2+}$ (10 µM) and matrix-bound fractions were subsequently analyzed by Western blotting using polyclonal rabbit anti-FKBP38 antibodies. Specific binding of Bcl-$2^{119-131}$ is shown by the competition between soluble and matrix-bound peptide. On the other hand, the peptide Bcl-$2^{51-80}$, corresponding to the core region of the flexible Bcl-2 loop, did not compete with the matrix-bound peptide Bcl-$2^{119-131}$ for binding of FKBP38. Involvement of the putative FKBP38 active site is indicated by a FK506-induced inhibition of the FKBP38/Bcl-$2^{119-131}$ interaction. (Unloaded matrix served as control.)

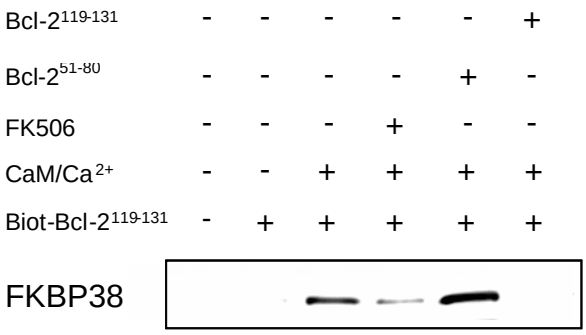

Figure S4: Isothermal titration calorimetry data of FKBP38/CaM/$Ca^{2+}$ titrated with Bcl-$2^{119-131}$. The experiment was performed at 25 °C using a MicroCal VP-ITC microcalorimeter. FKBP38/CaM/$Ca^{2+}$ (35 μM) was dialyzed in 10 mM MES buffer (pH 6.8, 100 mM NaCl, 0.05% $NaN_3$, 2 mM $CaCl_2$) and placed in the calorimeter cell. Bcl-$2^{119-131}$, solubilized in the same MES buffer to 0.20 mM concentration, was loaded into the syringe injector. The titrations were carried out in 510 μl aliquots, with a 240 s delay between each injection. The calorimetric titration data were fitted using the Origin 5.0 program supplied with the instrument. The best fit of the titration curve was obtained with a one-binding-site model, revealing a 1:1 binding stoichiometry (n = 1.014 ± 0.006) and a binding affinity in the lower micromolar range ($K_d$ = 3.17 ± 0.37 μM, ΔH = -34.2 kJ/mol, -TΔS = -24.8 kJ/mol).

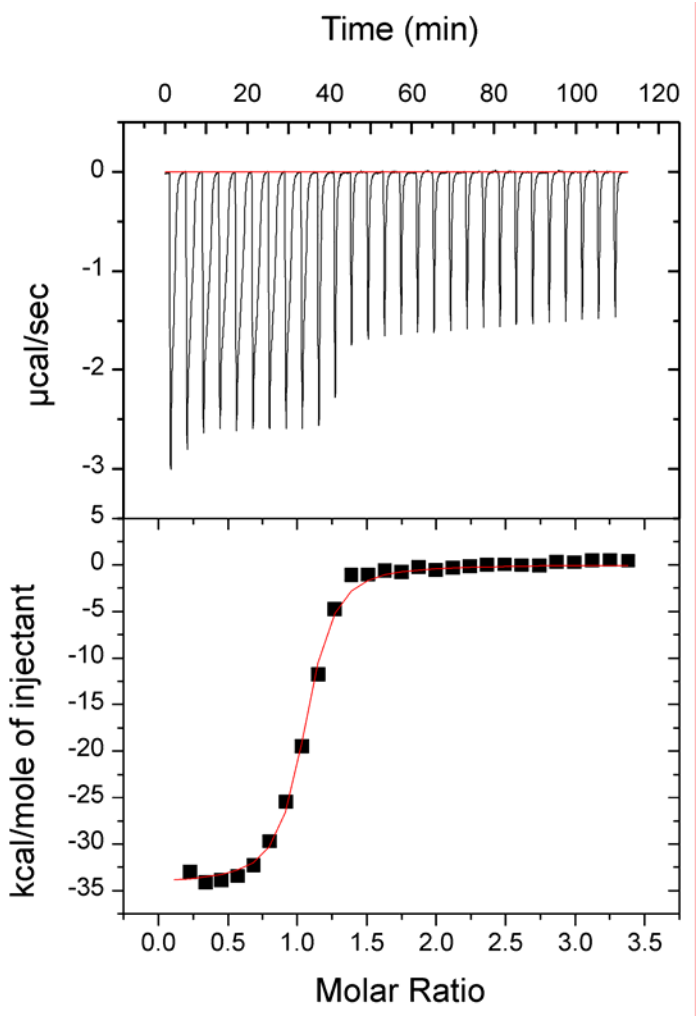

Figure S5: Overlay of $^{1}H/^{15}N$-HSQC spectra of FKBP38$^{35-153}$ in absence (red contours) and presence (blue contours) of the peptide Bcl-2$^{119-131}$. CSP effects are most pronounced in the region of the β5-α1 loop and also around K62, which had been shown previously to be affected by cation binding to the β5-α1 loop (Maestre-Martínez et al. 2011, *J. Mol. Recognit.* **24**, 23-34).

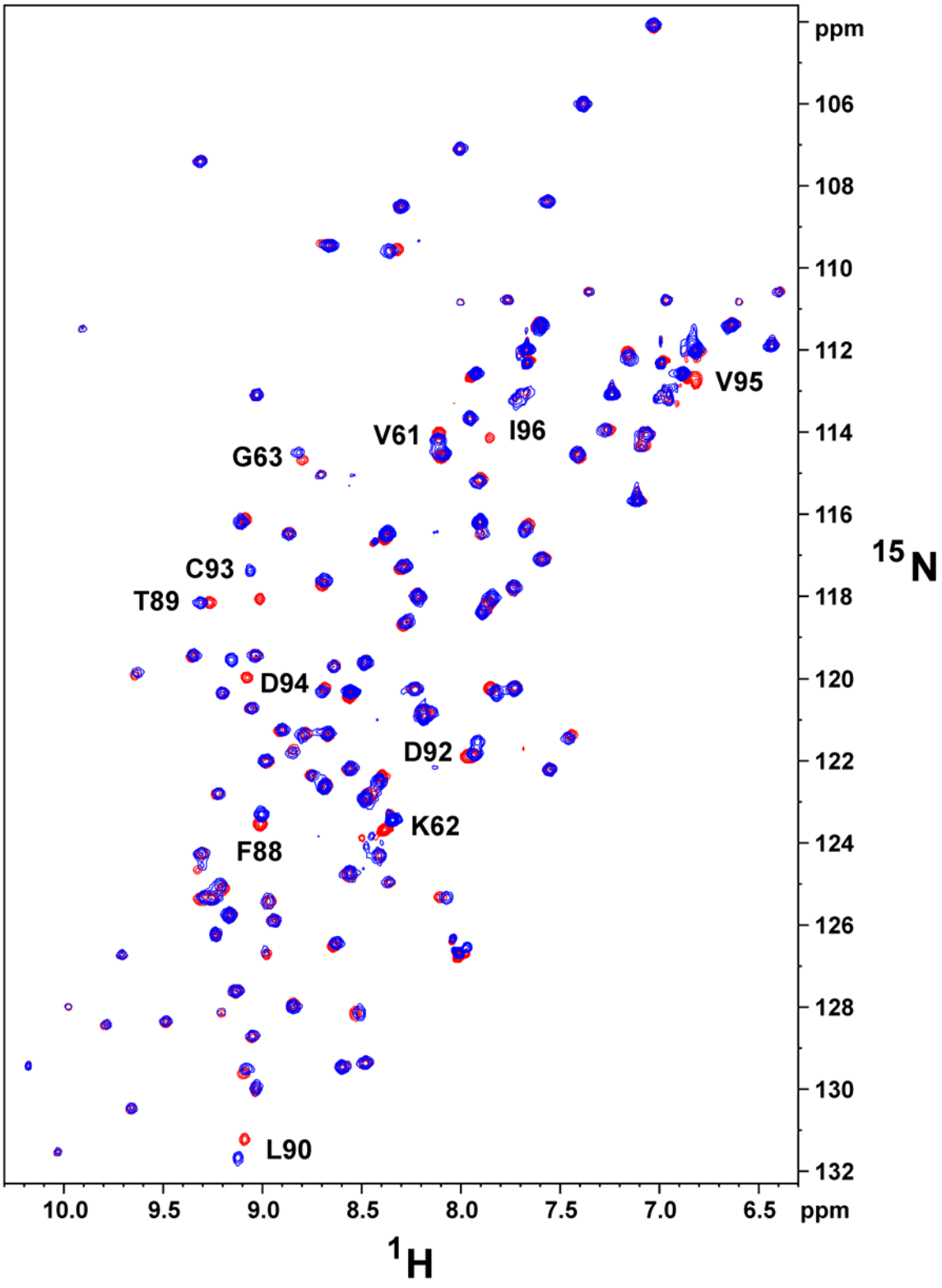

Figure S6: Section of the $^{1}H/^{15}N$-HSQC spectrum which includes the amide signals of residues T89, C93 and D94 in the β5-α1 loop of FKBP38. Superposed are the spectra of FKBP38$^{35-153}$ in the absence of peptide Bcl-2$^{119-131}$ (black contours) as well as in presence of the peptides Bcl-2$^{119-131}$ R127Q/R129Q (blue contours), Bcl-2$^{119-131}$ R127Q (violet contours), Bcl-2$^{119-131}$ R129Q (magenta contours), and Bcl-2$^{119-131}$ (red contours).

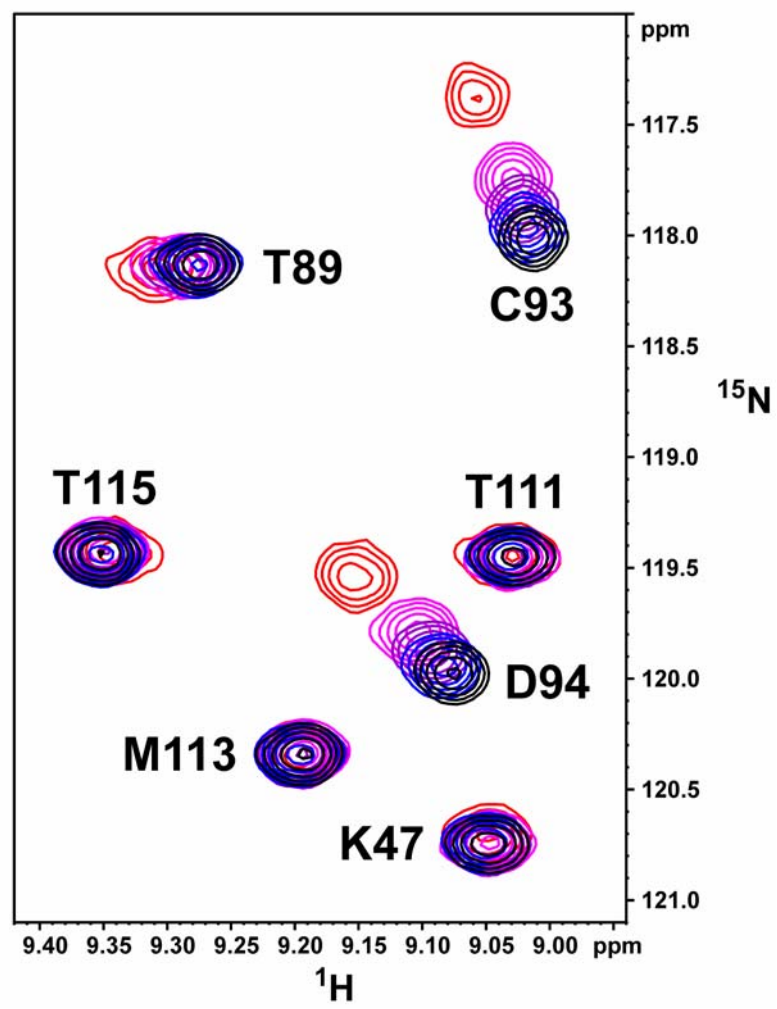

Figure S7: Overlay of $^{1}H/^{15}N$-HSQC spectra of the Bcl-$2^{119-131}$ peptide Npep1 in absence (black contours) and presence (red contours) of FKBP$38^{35-153}$.

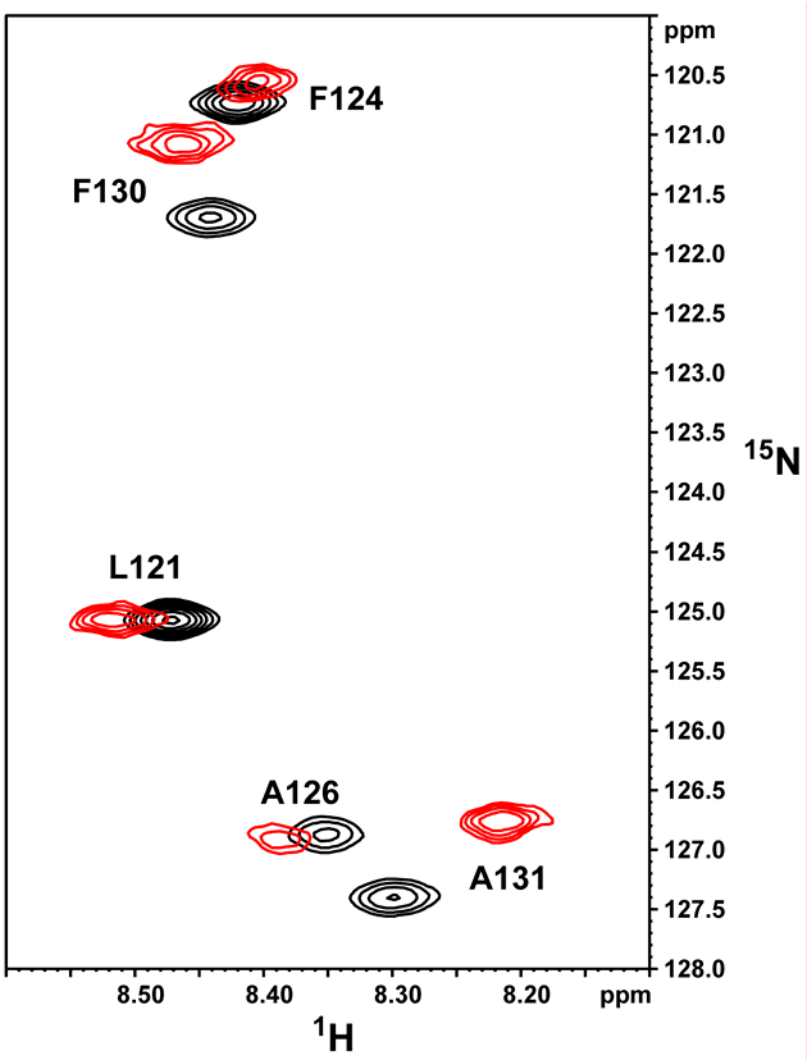

Figure S8: $^{1}H/^{15}N$-HSQC of Npep2 in absence (blue contours) and presence (red contours) of non-labelled $FKBP38^{35-153}$. The side-chain $N_{\varepsilon}H$ signals of R127 and R129, which were not detected in absence of $FKBP38^{35-153}$, became observable in the complex.

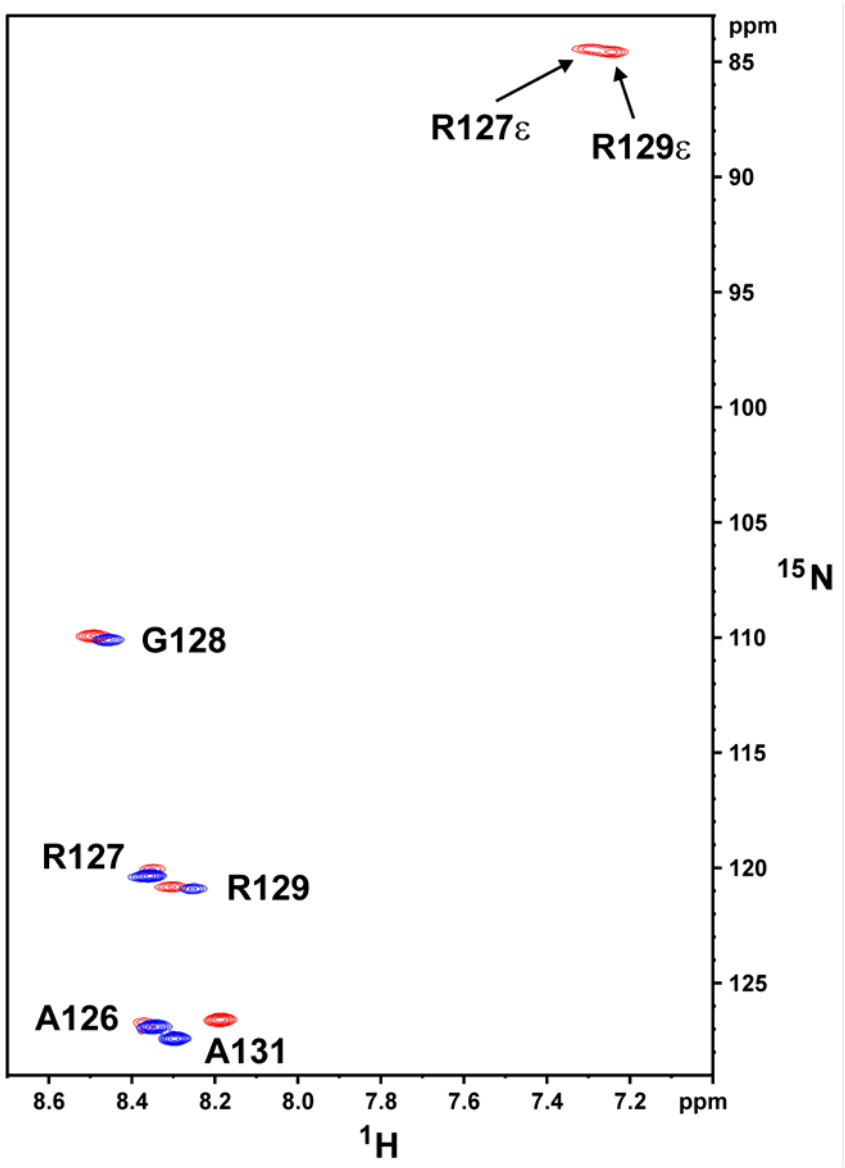

Figure S9: Overview of the chemical shift changes (Δδ) observed in $^{15}$N-labelled FKBP38$^{35\text{-}153}$ upon addition of a 3-fold molar excess of various Bcl2$^{119\text{-}131}$ peptide variants. CSP effects in the β5-α1 loop region were observed with all peptide variants except Bcl2$^{119\text{-}131}$ R127Q/R129Q, corroborating the electrostatic character of the FKBP38$^{35\text{-}153}$/Bcl2$^{119\text{-}131}$ interaction.

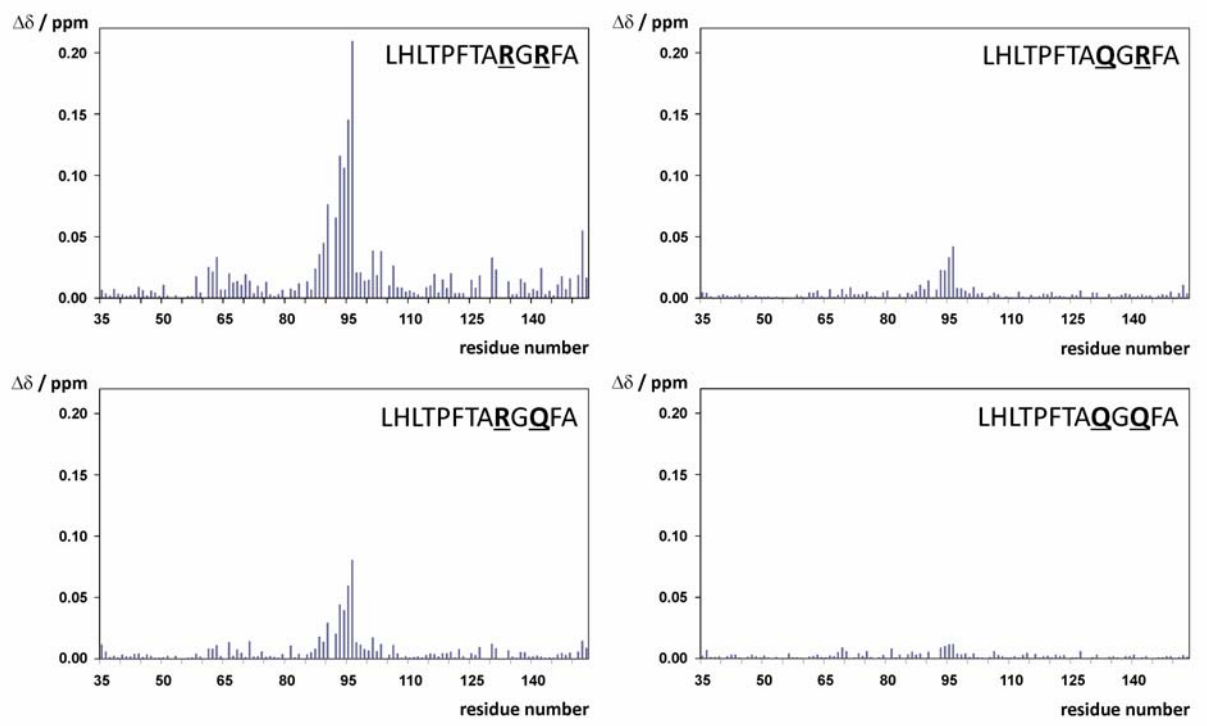

Figure S10: Section of the $^{1}H/^{15}N$-HSQC spectrum which includes the amide signals of residues T89, C93 and D94 in the β5-α1 loop of FKBP38. Superposed are the spectra of FKBP38$^{35-153}$ in the absence (black contours) and presence (red contours) of Bcl-2$^{1-211}$.

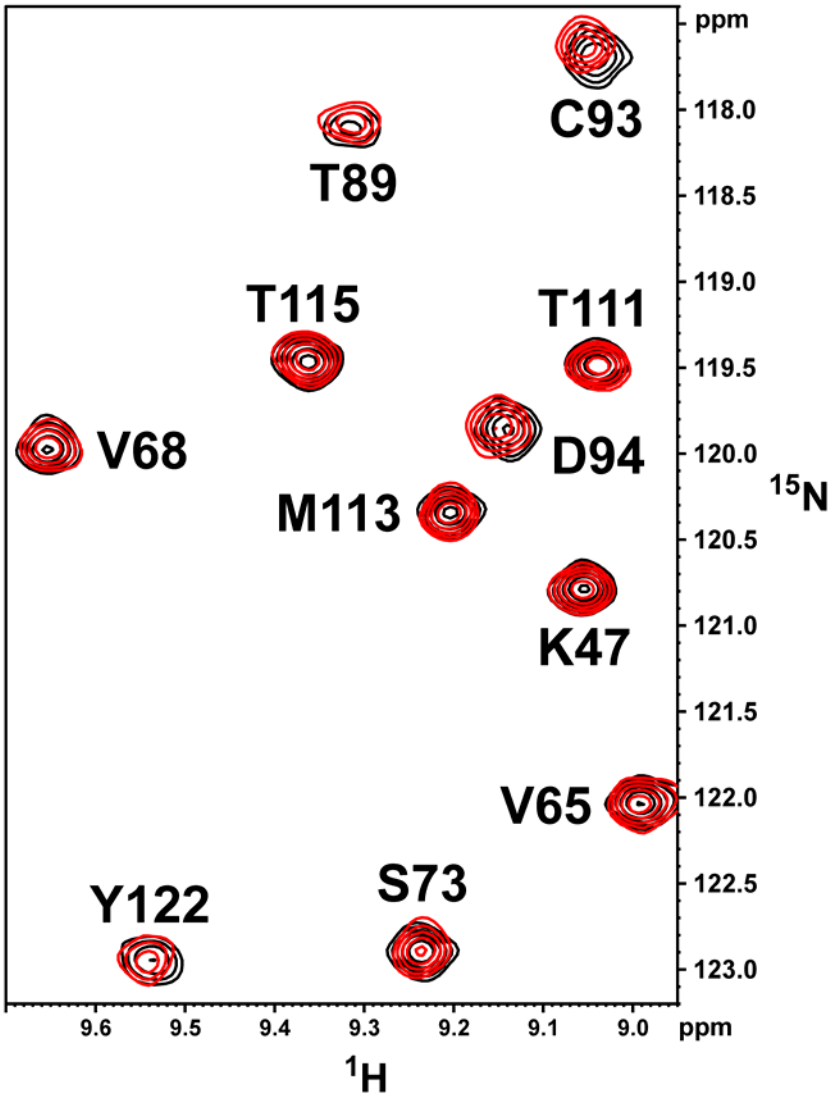

Figure S11: Graphic representation of all arginine residues (yellow sticks) in the Bcl-2 molecule (PDB ID code 1GJH). The arrow on the left marks the long flexible loop between the BH4 and BH3 domains, which is truncated in this structure (i.e. residues S51-P91 are missing).