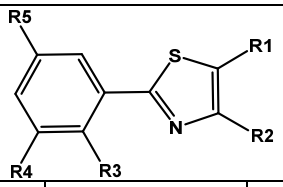
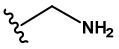
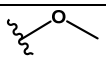
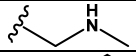
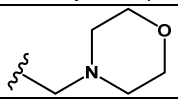
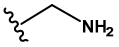
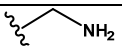
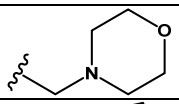
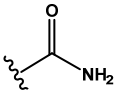
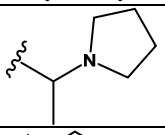
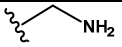
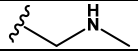
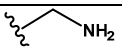
Supporting Information

Supplementary Table S1. Reported fragments tested against hCD44(21-178), grouped according to structural homology in thiazoles, thiophenes and furans.
Rank ordering according to the following criteria: +++, when shifts of HD1 L135, and HD2 L135 peaks are larger than 7 Hz; ++, when shifts of HD1 L135, and HD2 L135 peaks are in between 3 Hz and 7 Hz; when shift of HD1 L135, and HD2 L135 peaks smaller than 3 Hz; -, no shift of the peaks HD1 L135, and HD2 L135. All the shifts refer to the chemical shift difference to the apo for spectrum.

THIAZOLES						
						
ID	**R1**	**R2**	**R3**	**R4**	**R5**	**Ranking by NMR**
131B5		-H	-H		-H	-
131B6	-H		-H	-H	-H	+++
131B7	-H		-H	$-CH_3$	-H	-
131B8		-H		-H	-H	-
131B9	-H		-H	$-CH_3$	-H	-
131B10	$-CH_3$	$-CH_3$	-H	-H		-
131B11	-H		-H	-H		-
131B12	-H	-H	-H	-H		+
131C1	-H	-H	-H		-H	++
131C2	-H		-H	-H	-H	++

Supplementary Table S1. Continued

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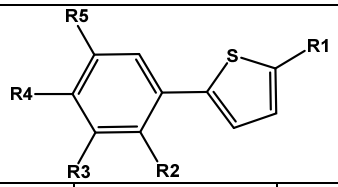						
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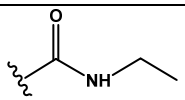
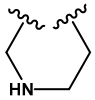
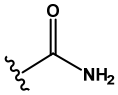
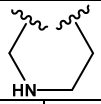
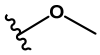
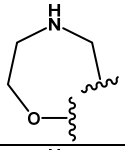
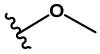
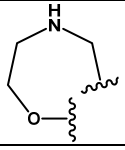
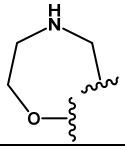
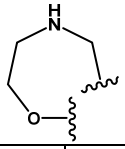
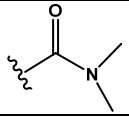
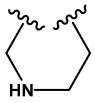
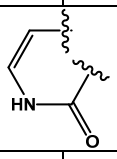
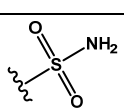
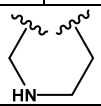
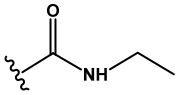
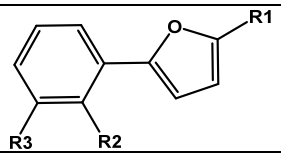
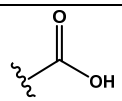
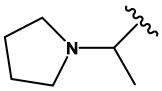
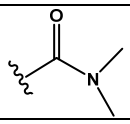
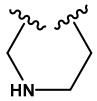
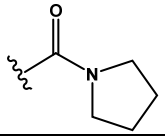
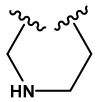
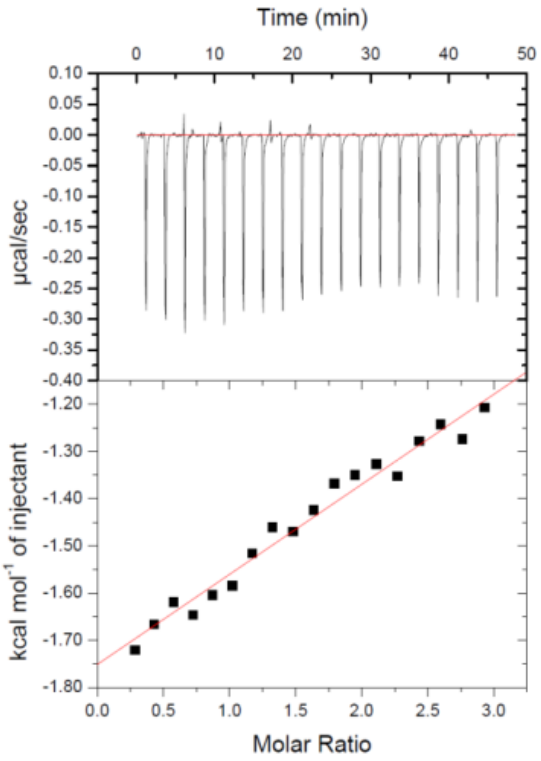
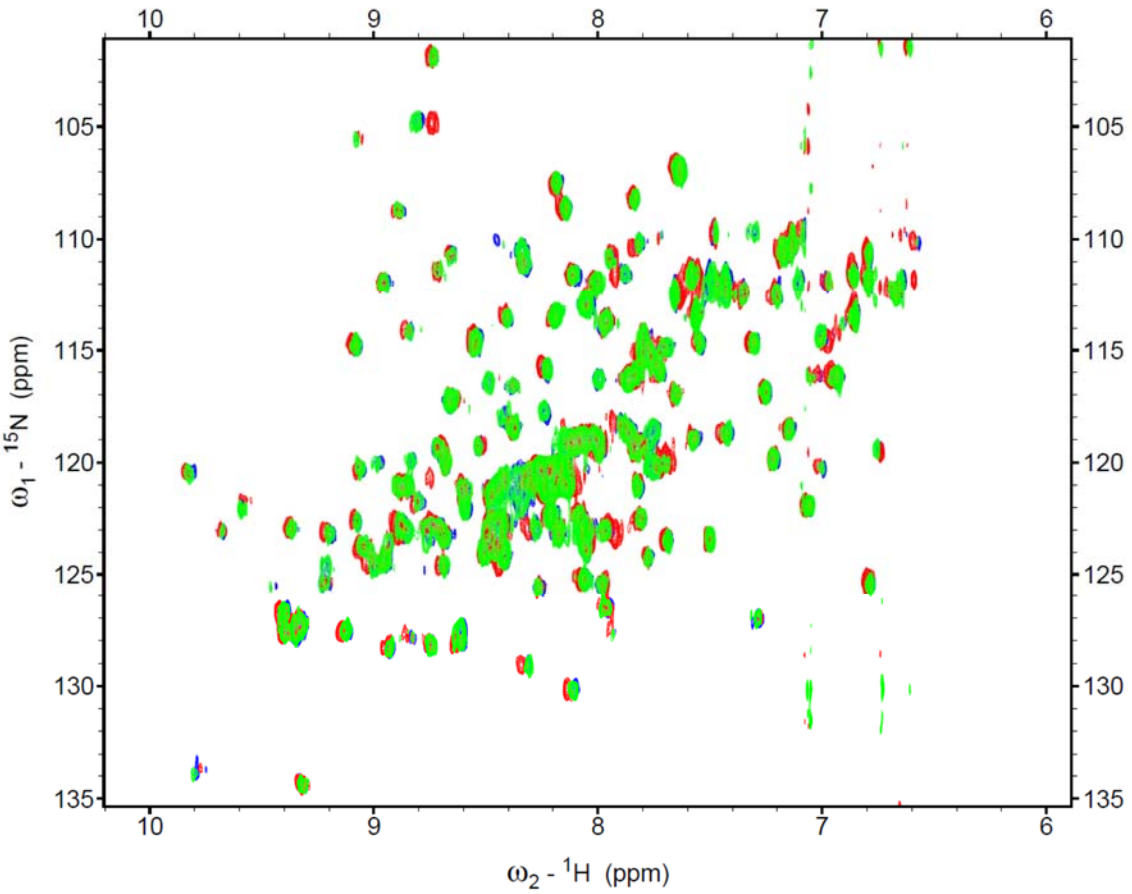
131C3				-H	-H	-
131C4				-H	-H	-
131C5	$-CH_3$	-H				-
131C6	-H	-H				-
131C7	$-CH_3$	-H				+
131C8	-H	-H				++
131C9				-H	-H	-
131C10	-Cl	-H			-H	-
131C11				-H	-H	-
131C12		-H		-H	-H	-

Supplementary Table S1. Continued

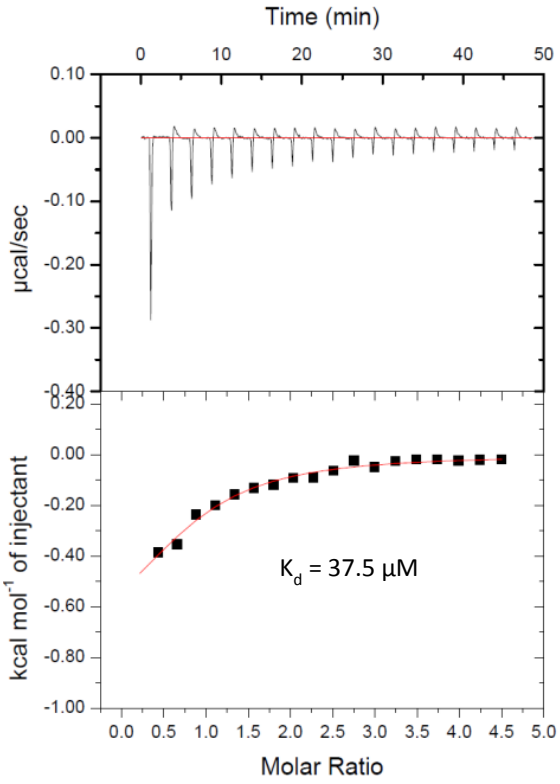
FURANS				
				
ID	**R1**	**R2**	**R3**	**Ranking by NMR**
131D1		-H		-
131D2				-
131D3				-



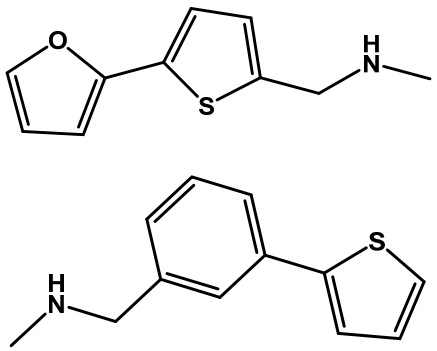
Supplementary Figure S1. Isothermal Titration Calorimetry (ITC) of hCD44(21-178) titrated with A6. There is no evidence of binding in our experimental condition.



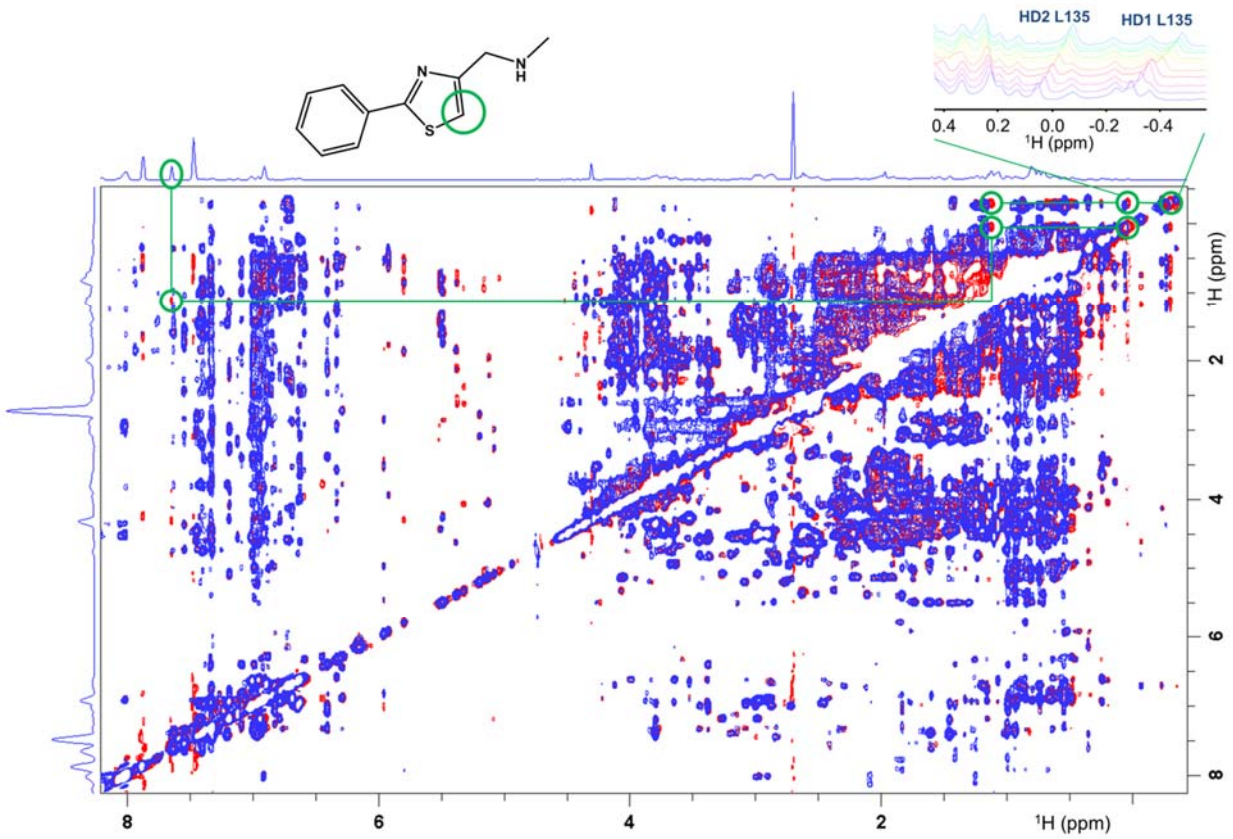
Supplementary Figure S2. 2D [^{1}H,^{15}N]-sofastHMQC NMR spectra of 20 µM ^{15}N-hCD44(21-178) in its apo form (blue), in presence (red) of 20 mM of compound 3 with the pH increase at about 8, and in presence (green) of 20 mM of compound 3 at the correct pH. At the pH of 6.7 (green spectrum) there are no evidences of binding.



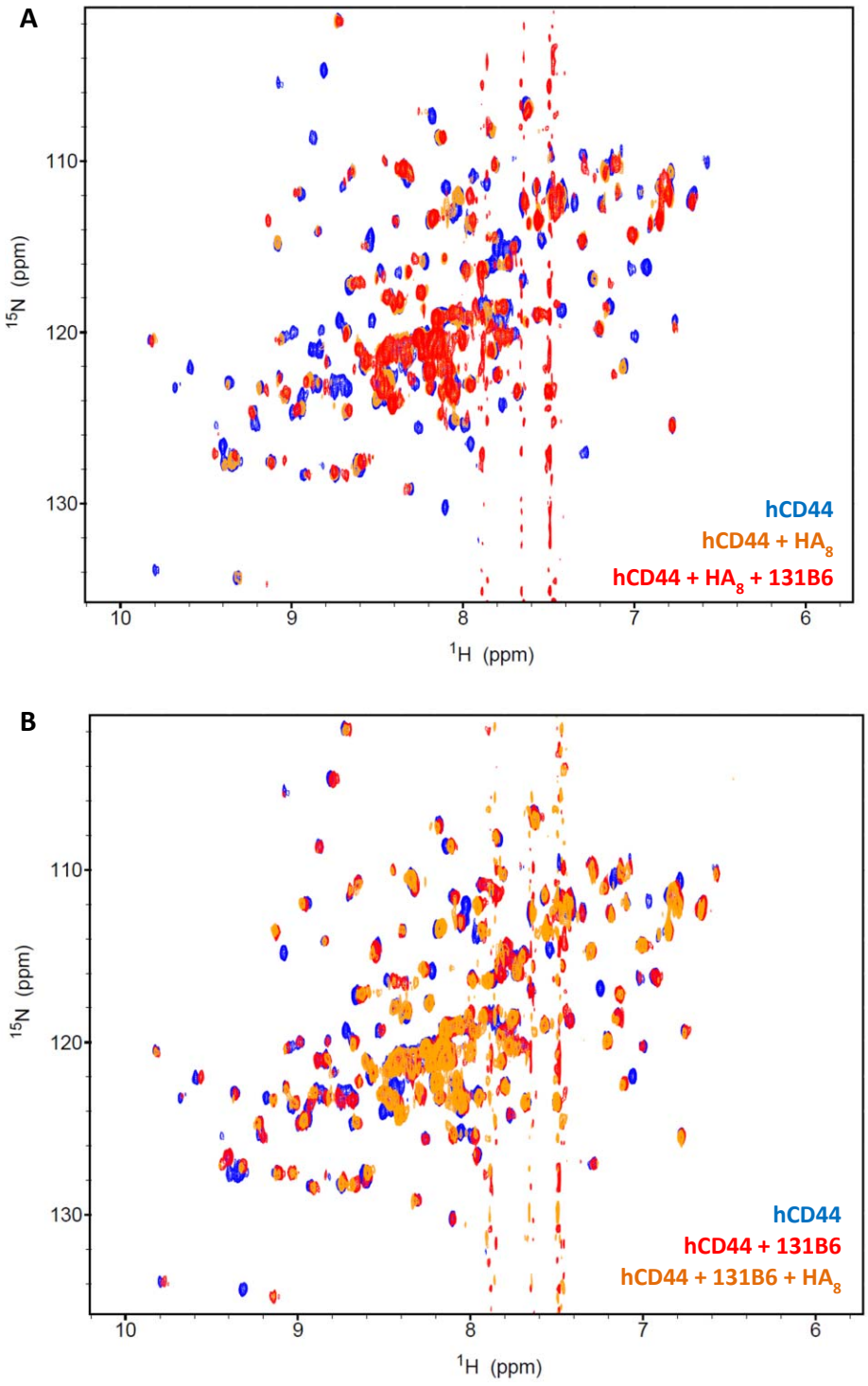
Supplementary Figure S3. Isothermal Titration Calorimetry (ITC) of hCD44(21-178) in presence of 750-fold molar excess of compound 3, titrated with HA_8. The K_d of 37.5 µM is not significantly different than the K_d in absence of the compound 3, suggesting that this fragment not significantly compete with HA_8. The ITC curve we obtained is very similar to the curve reported in S. Banerji, A. J. Wright, M. Noble, D. J. Mahoney, I. D. Campbell, A. J. Day, D. G. Jackson, Nat Struct Mol Biol 2007, 14(3), 234-239.



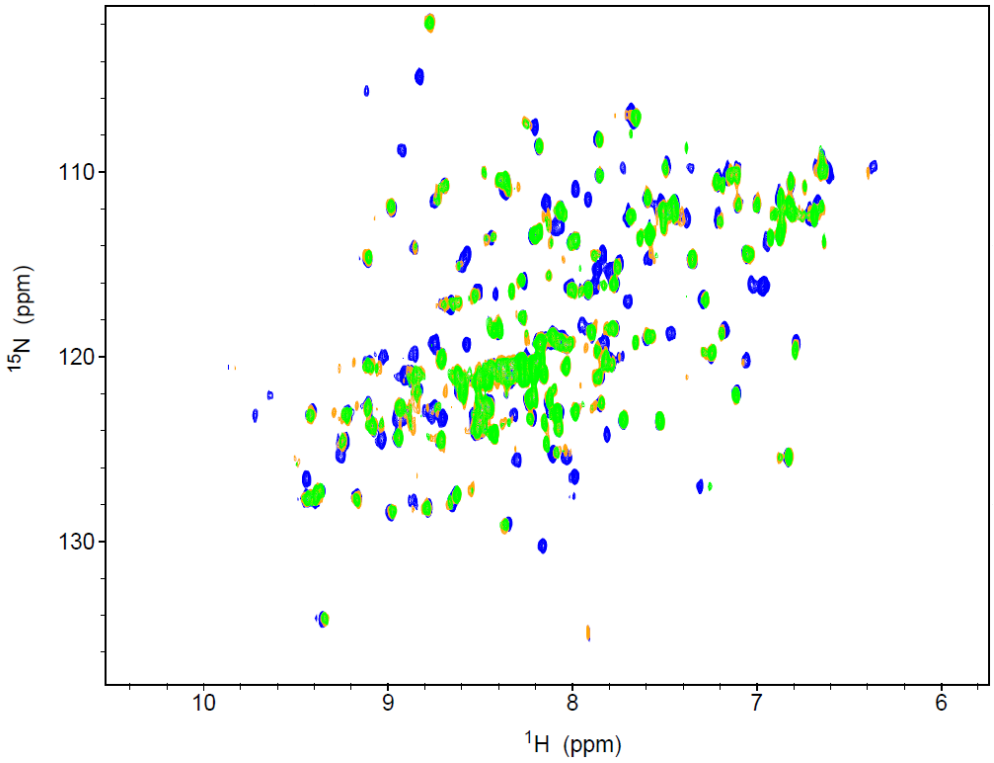
Supplementary Figure S4. The two initial hits came from the 500 fragment screening (Maybridge).



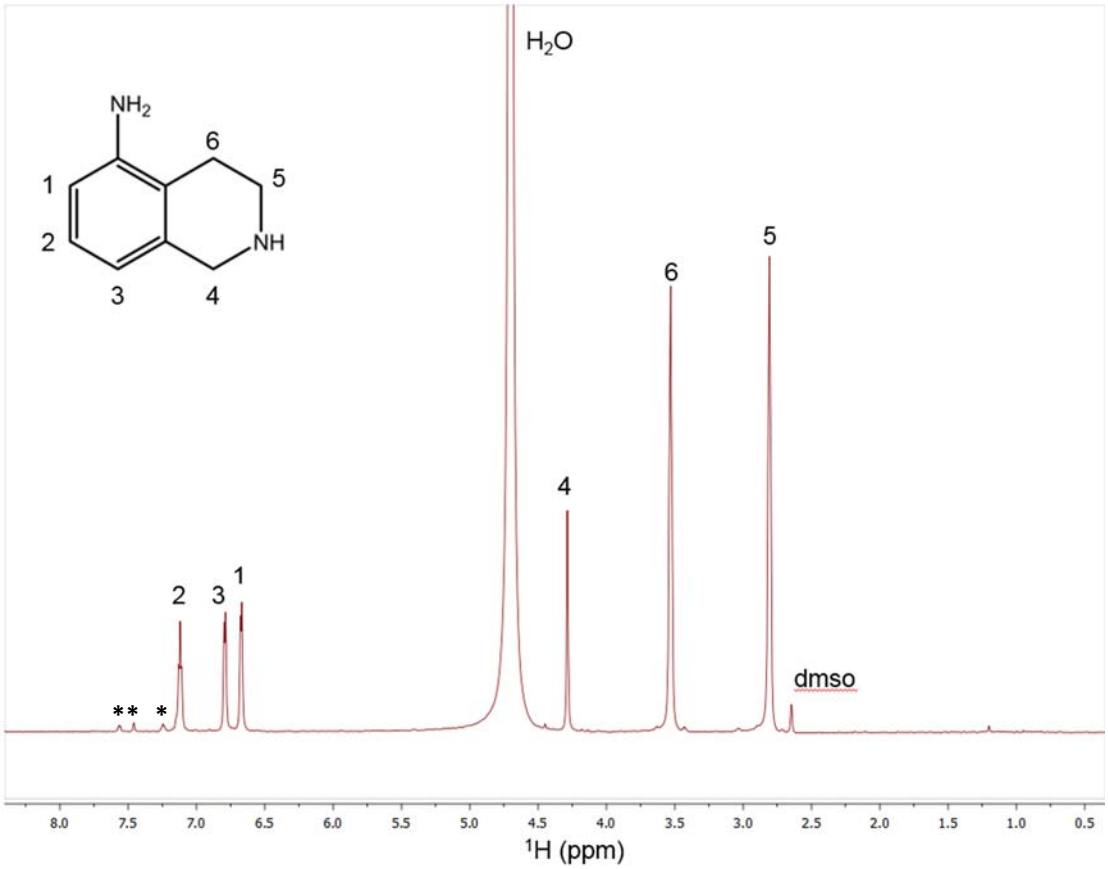
Supplementary Figure S5. 2D [^{1}H,^{1}H]-NOESY NMR spectra of 100 µM hCD44(21-178) in absence (blue) and in presence (red) of 500 µM of compound 131B6. In green is depicted the correlation between the proton of the thiazole (compound 131B6) and probably the β-protons of the leucine 135 of hCD44(21-178).



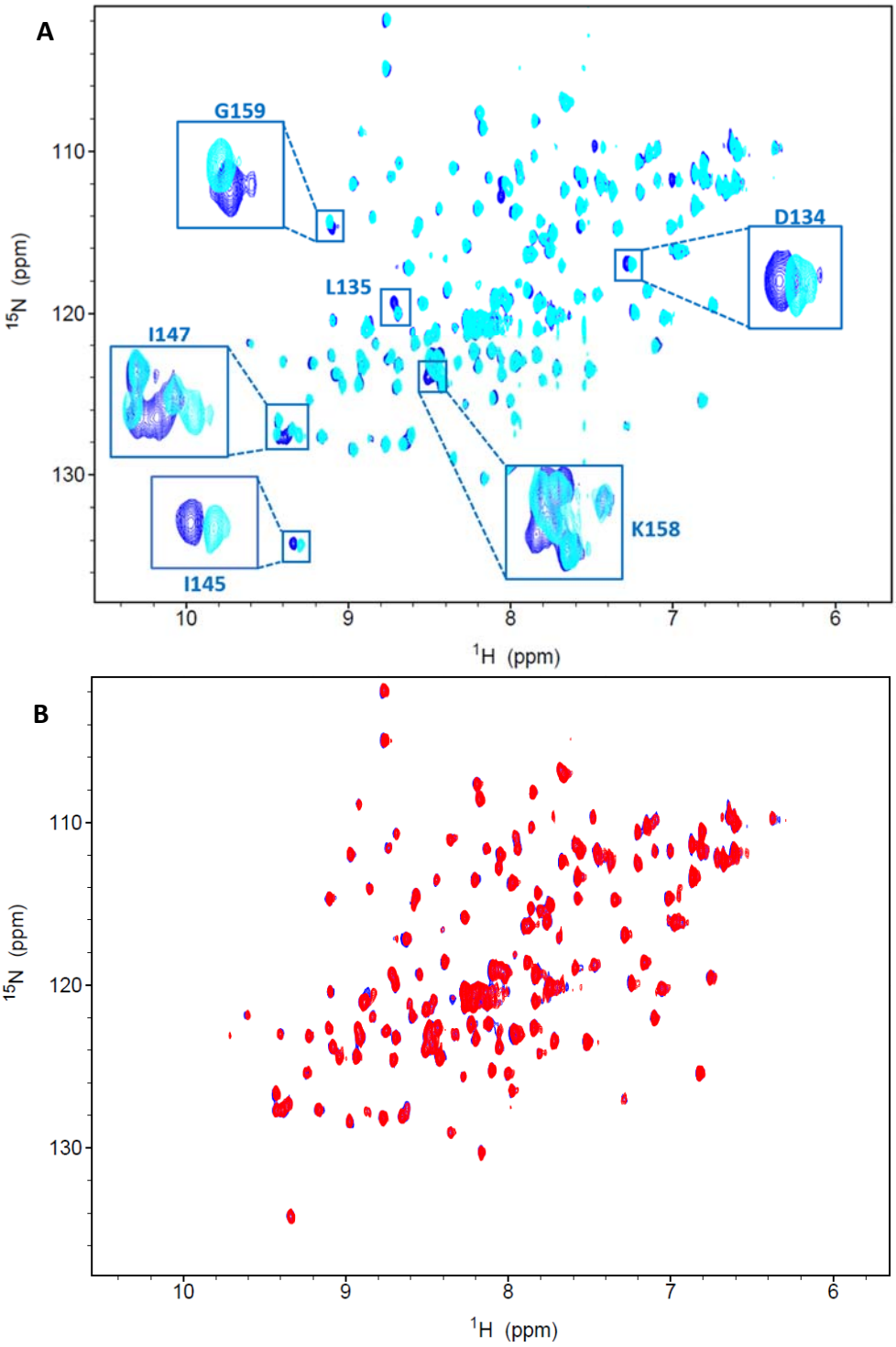
Supplementary Figure S6. (A) ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 µM) recorded in absence (blue) and presence of 80 µM HA$_8$(orange), in presence of 80 µM HA$_8$ and 15 mM of 131B6 (red). (B) ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 µM) recorded in absence (blue) and presence of 15mM 131B6 (red), or in presence of 15 mM 131B6 and 60 µM HA$_8$ (orange).



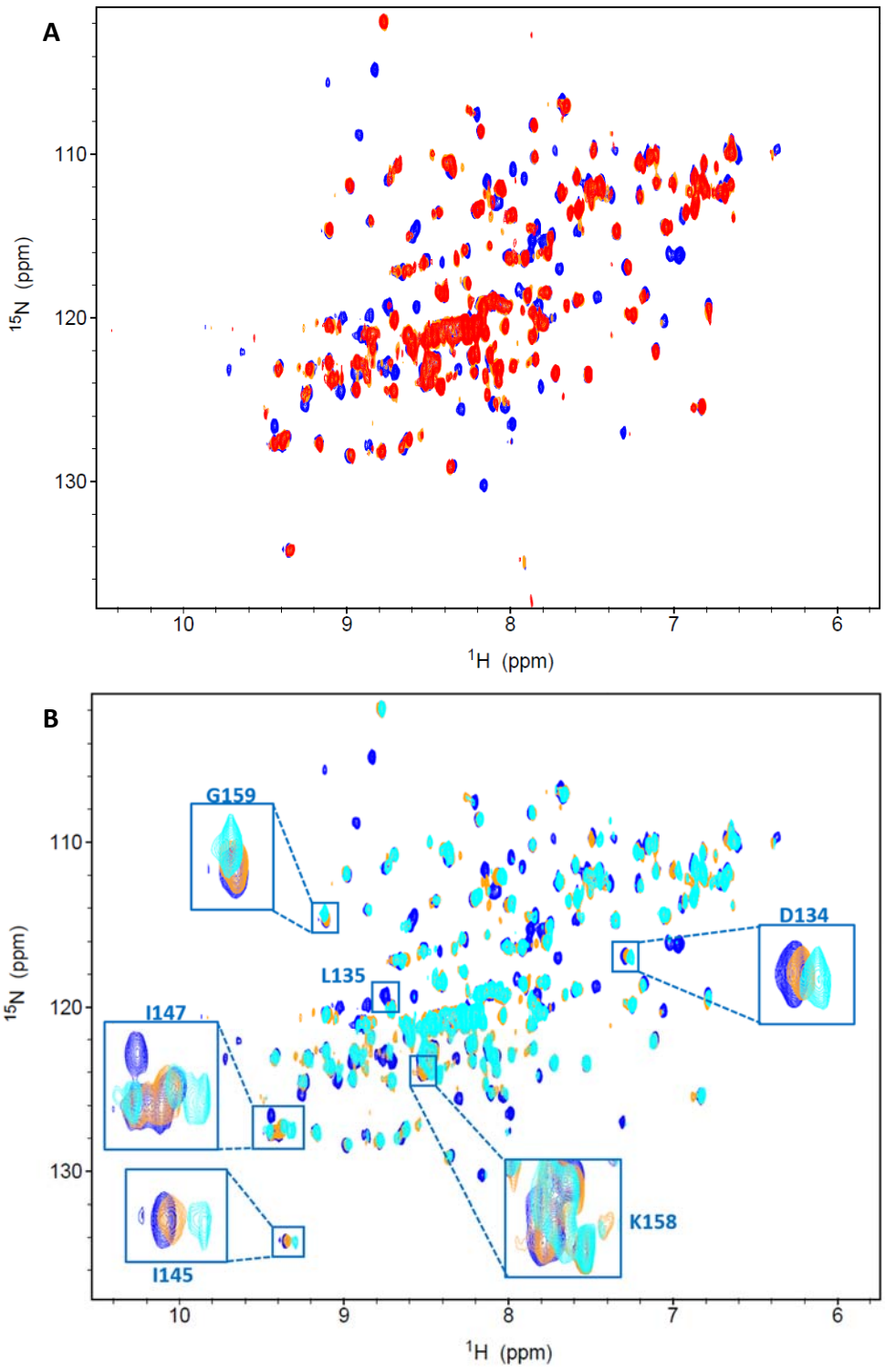
Supplementary Figure S7. (A) ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 μM at pH 6.7) recorded in absence (blue) and presence of 80 μM HA_8(orange), or in presence of 80 μM HA_8 and 200 μM A6 (green).



Supplementary Figure S8. 1D ^{1}H NMR spectrum of 2 mM compound 3 in 50 mM phosphate buffer pH = 7.4 with 150 mM NaCl. The NMR signals due to the compound 3 are assigned. Resonances from impurities are marked with asterisks.

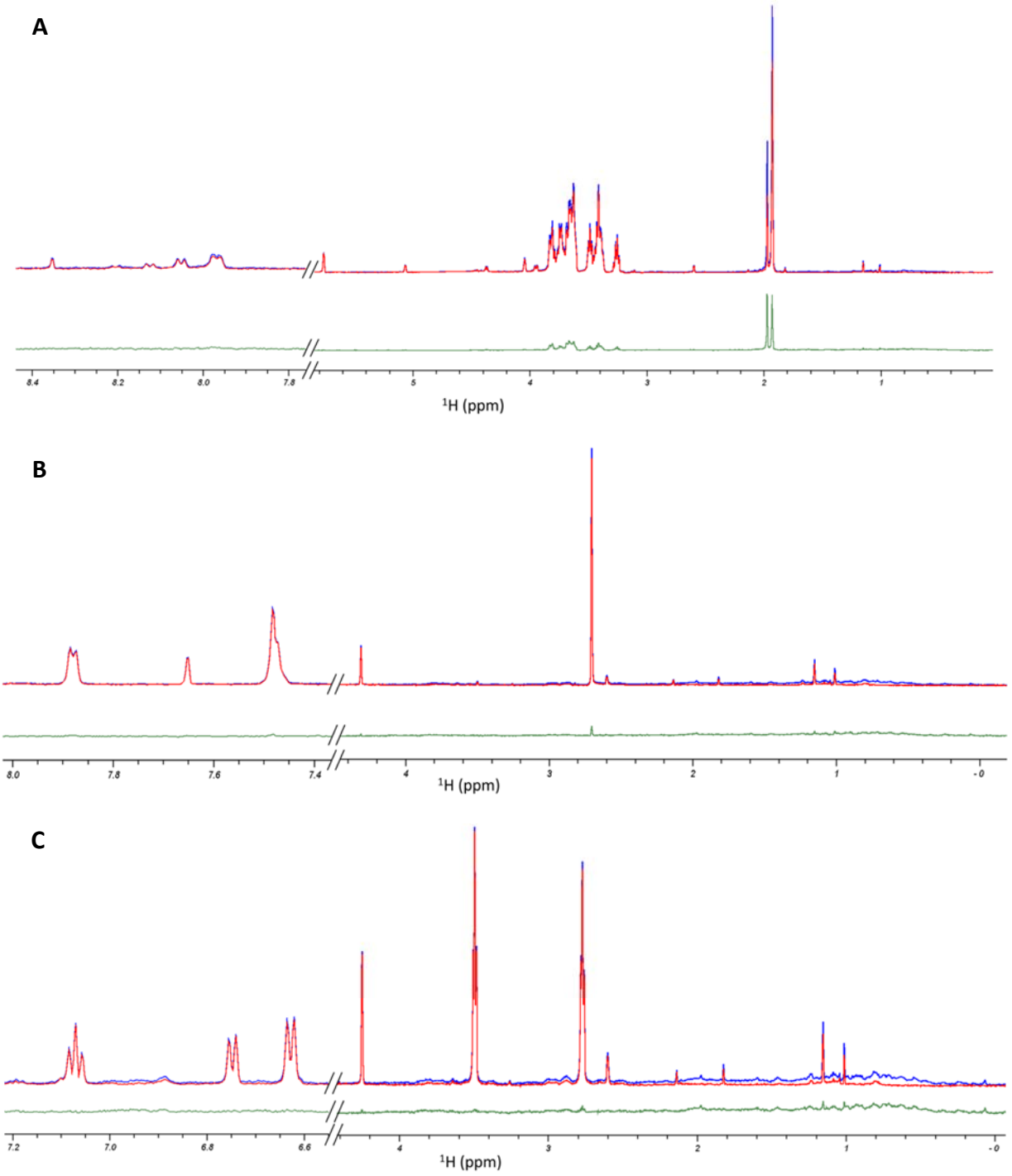


Supplementary Figure S9. (A) ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 µM) at pH=7.4, recorded in absence (blue) and presence of 2 mM 131B6 (cyan). (B) ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 µM) recorded in absence (blue) and presence of 2mM compound 3 (red). Residues that are significantly perturbed are labeled.

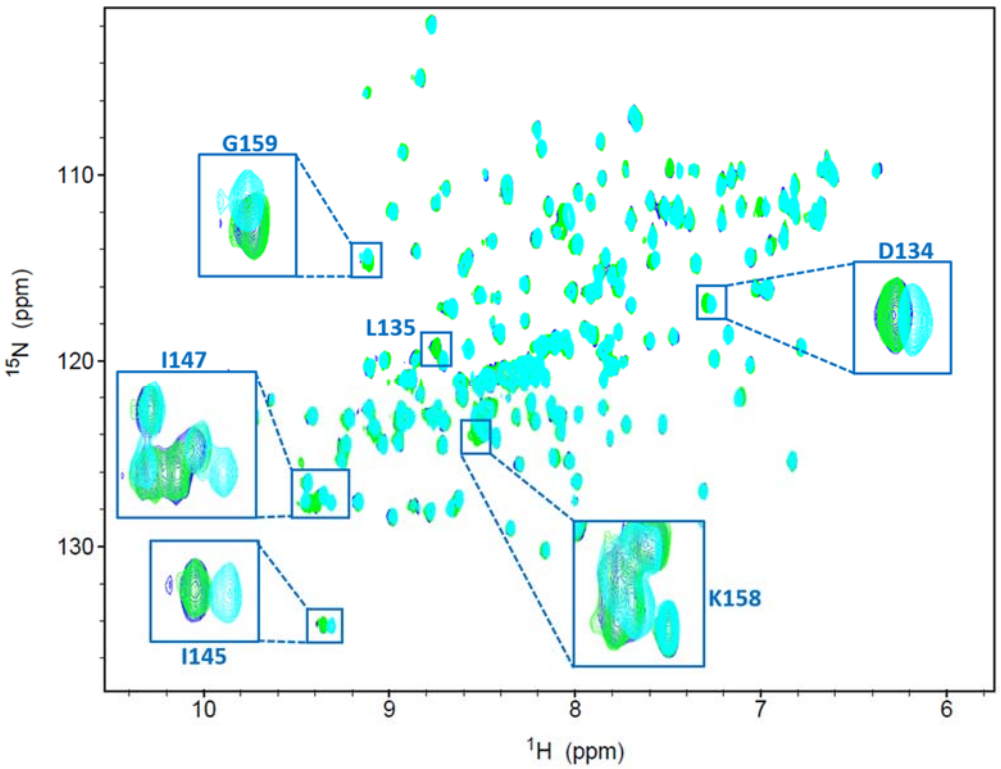


Supplementary Figure S10. (A) ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 µM at pH 6.7) recorded in absence (blue) and presence of 80 µM HA_8(orange), or in presence of 80 µM HA_8 and 2 mM compound 3 (red). (B) ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 µM at pH

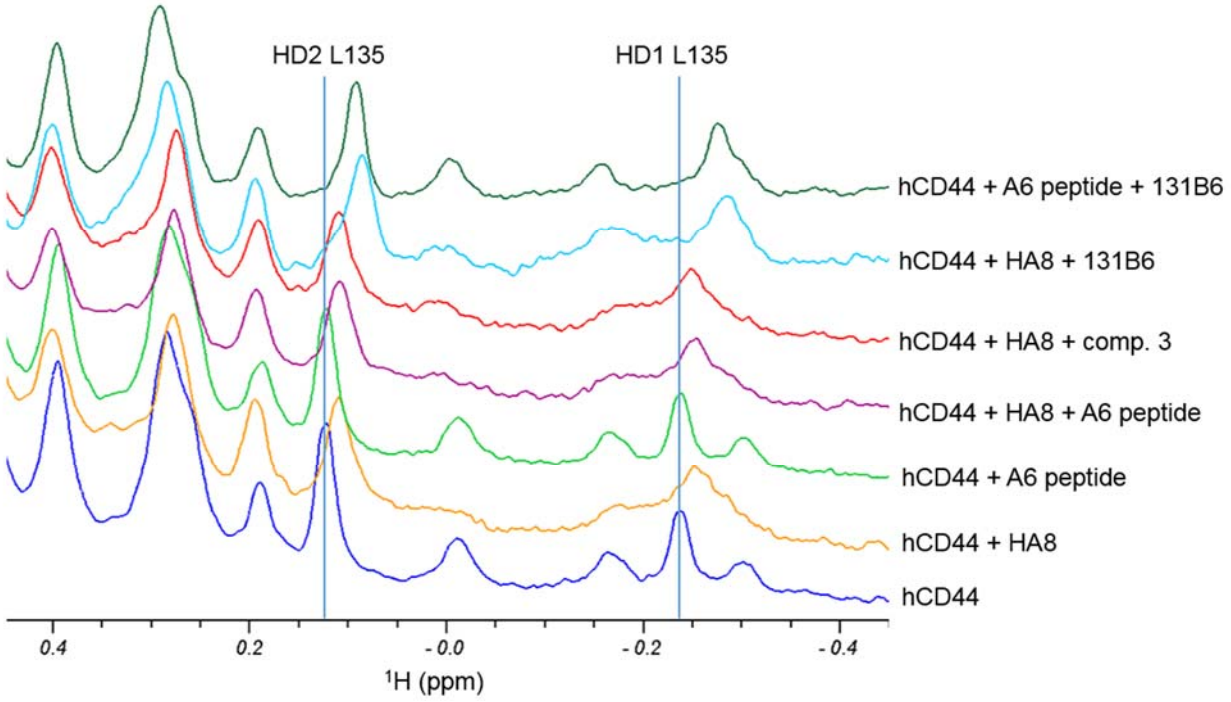
6.7) recorded in absence (blue) and presence of 80 µM HA_8 (orange), or in presence of 80 µM HA_8 and 2 mM 131B6 (cyan). Residues that are significantly perturbed are labeled.



Supplementary Figure S11. Saturation Transfer Difference (STD) experiments of 5 µM hCD44(21-178) in presence of 400 µM HA_8 (A), 400 µM compound 131B6 (B) and, 400 µM compound 3 (C). In all the spectra in blue is represented the off-resonance spectrum, in red the on-resonance and, in green the STD.



Supplementary Figure S12. ^{15}N-hCD44(21-178) spectra of hCD44(21-178) (20 µM at pH 6.7) recorded in absence (blue) and presence of 200 µM A6 (green), or in presence of 200 µM A6 and 2 mM 131B6 (cyan). Residues that are significantly perturbed are labeled.



Supplementary Figure S13. Superimposition of hCD44(21-178) 1D ^{1}H-aliphatic NMR spectra: 20 µM hCD44(21-178), blue; 20 µM hCD44(21-178) + 80 µM HA8, orange; 20 µM hCD44(21-178) + 200 µM A6 peptide, light green; 20 µM hCD44(21-178) + 80 µM HA8 + 200 µM A6 peptide, purple; 20 µM hCD44(21-178) + 80 µM HA8 + 2 mM compound 3, red; 20 µM hCD44(21-178) + 80 µM HA8 + 2 mM 131B6, cyan; 20 µM hCD44(21-178) + 200 µM A6 + 2 mM 131B6, green. The resonances of the methyl groups of L135 are identified.