Supplementary Information

Structure of the VP16 Transactivator Target in ARC/Mediator

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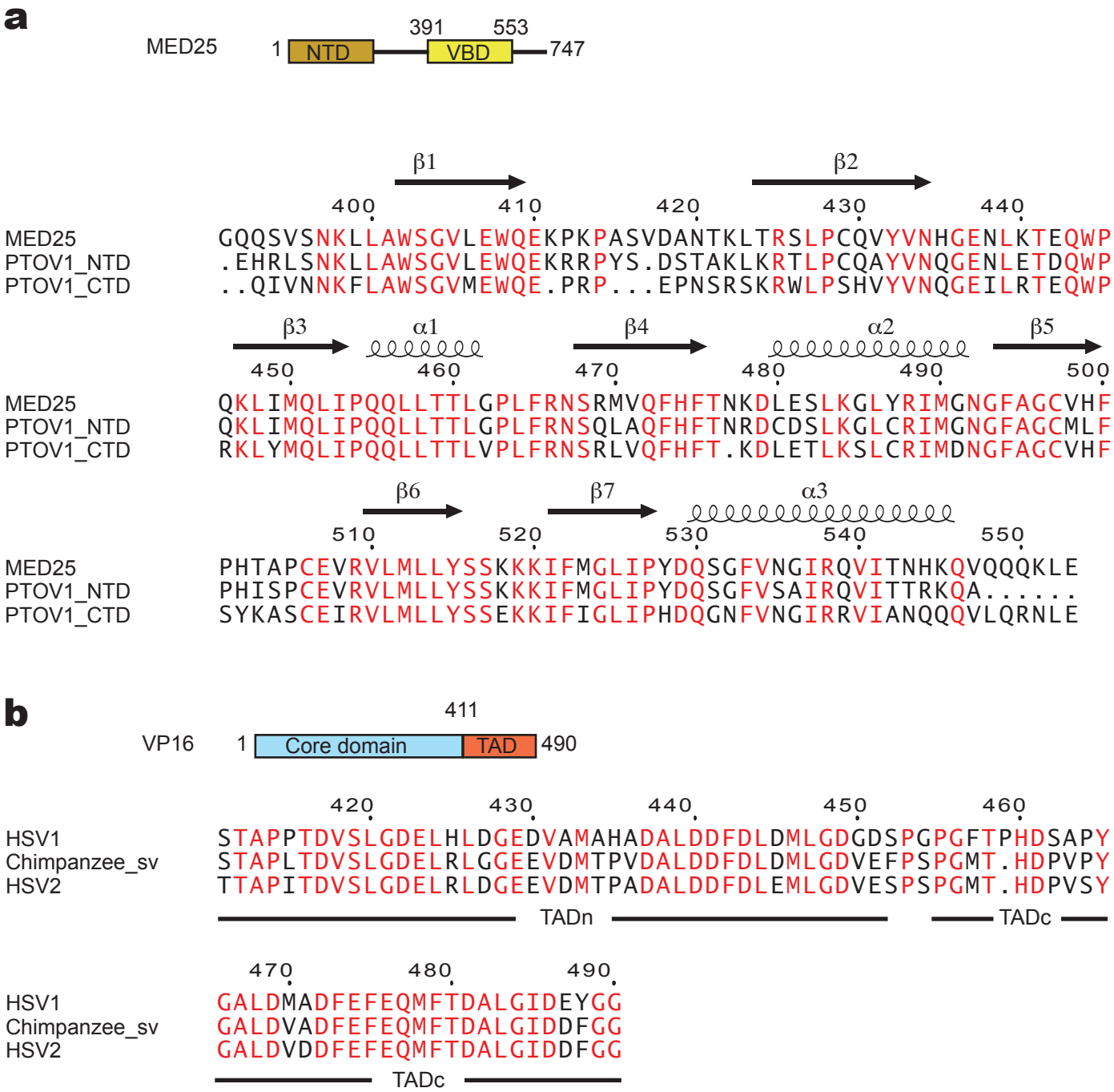
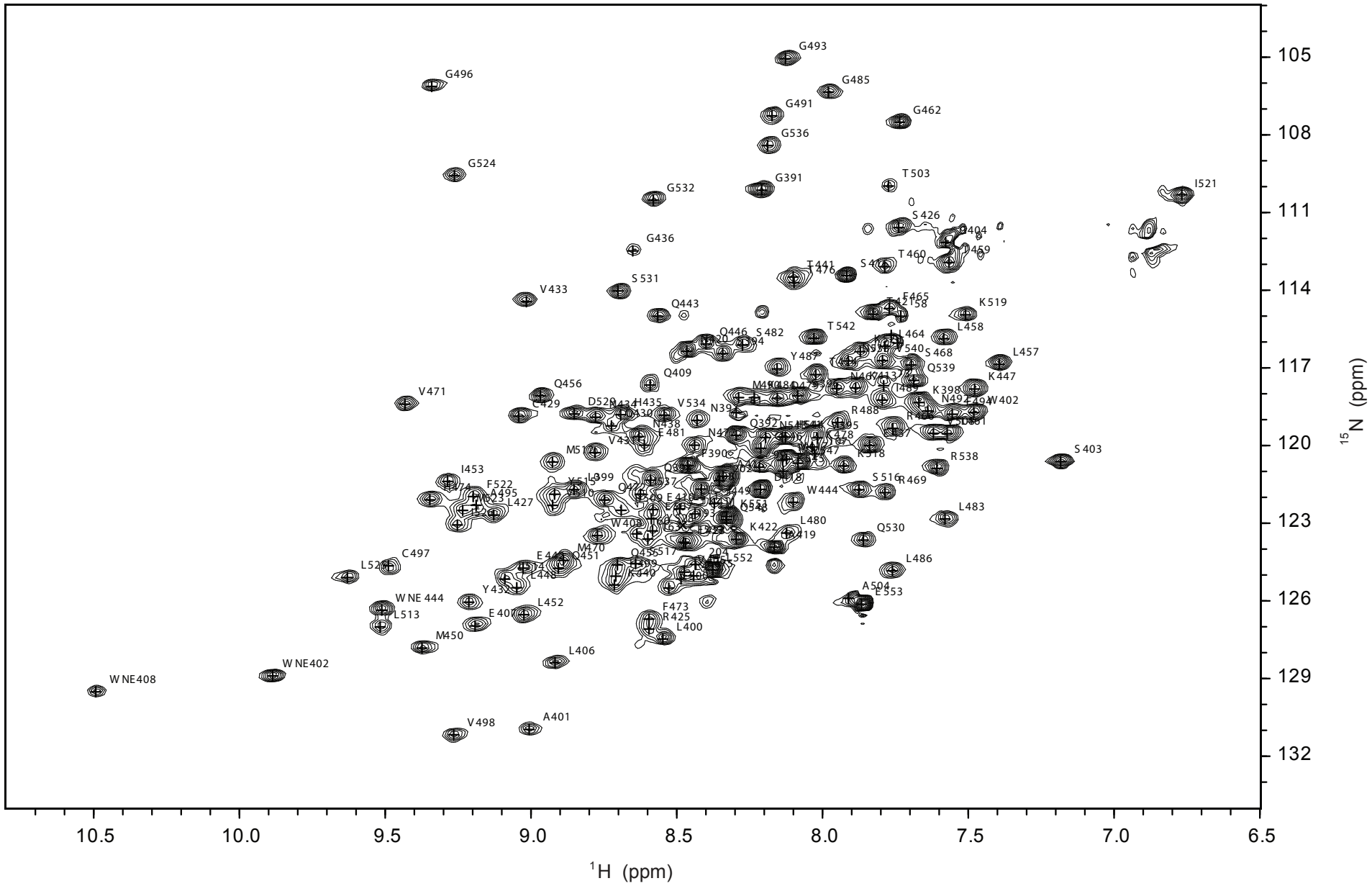
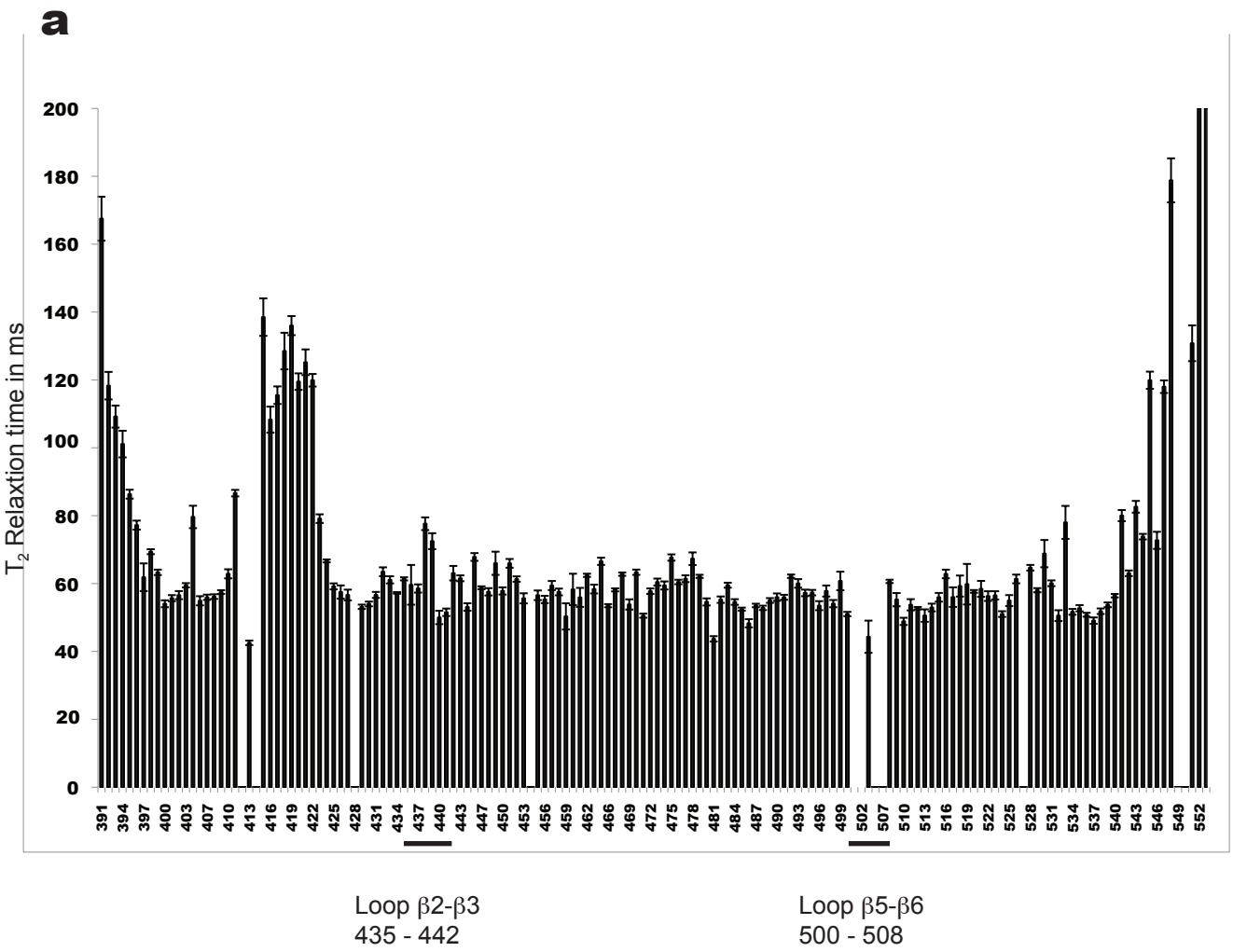


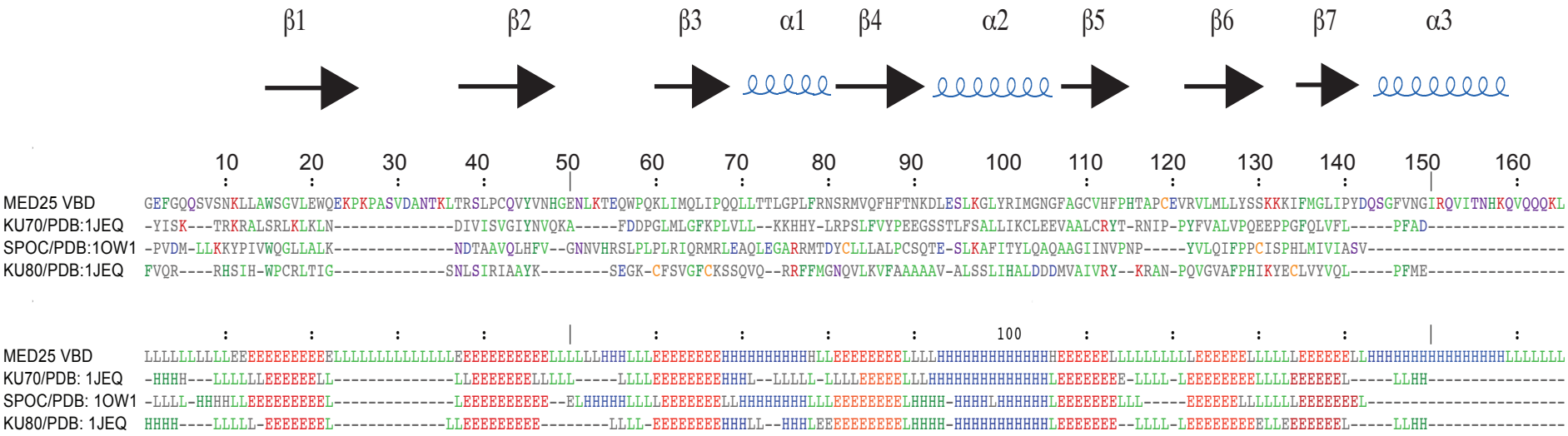
Supplementary Figure 1. Sequence alignments of the MED25 VBD and VP16 TAD. (**a**) Sequence of the human MED25 VBD aligned with the homologous C- and N-terminal domain of human PTOV1. Secondary structure elements are shown above the sequences. (**b**) Sequence of the VP16 TAD from *Herpes simplex virus I* aligned with sequences of TADs from other viruses. Alignments were visualized with ESPript[1]. Conserved residues are highlighted in red.



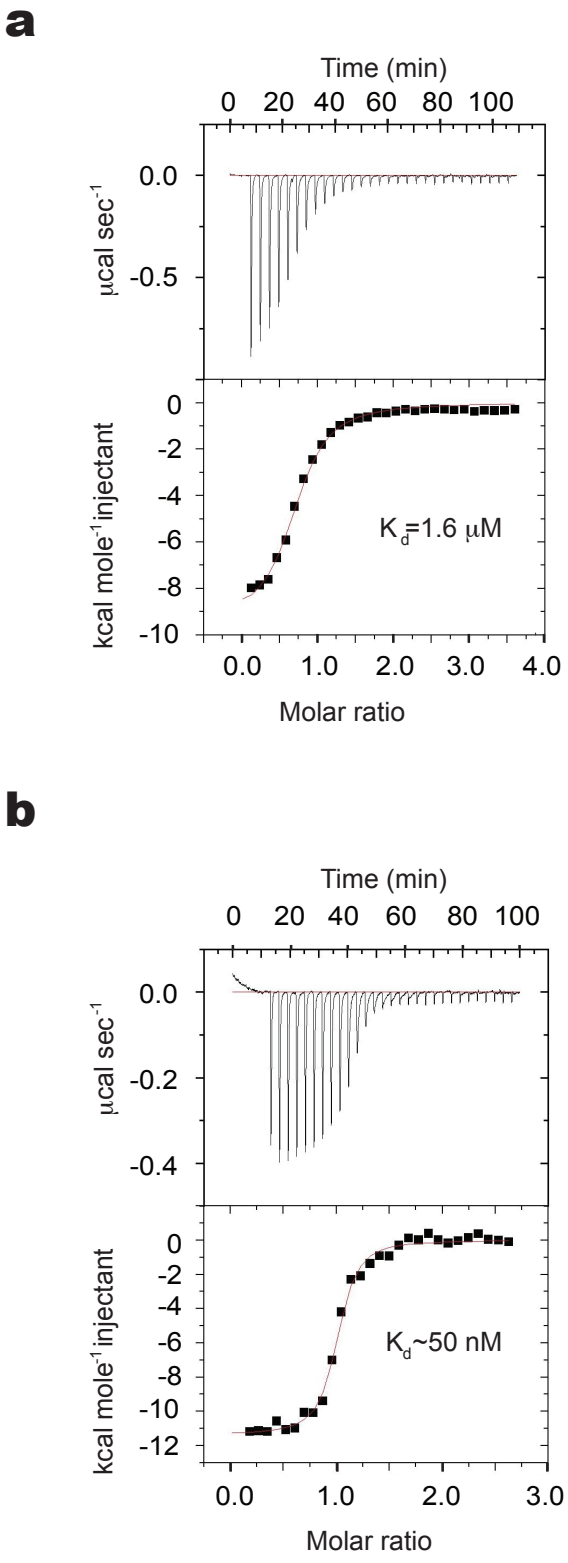
Supplementary Figure 2. ^{1}H-^{15}N-HSQC spectrum of the MED25 VBD with the assigned residue numbers.



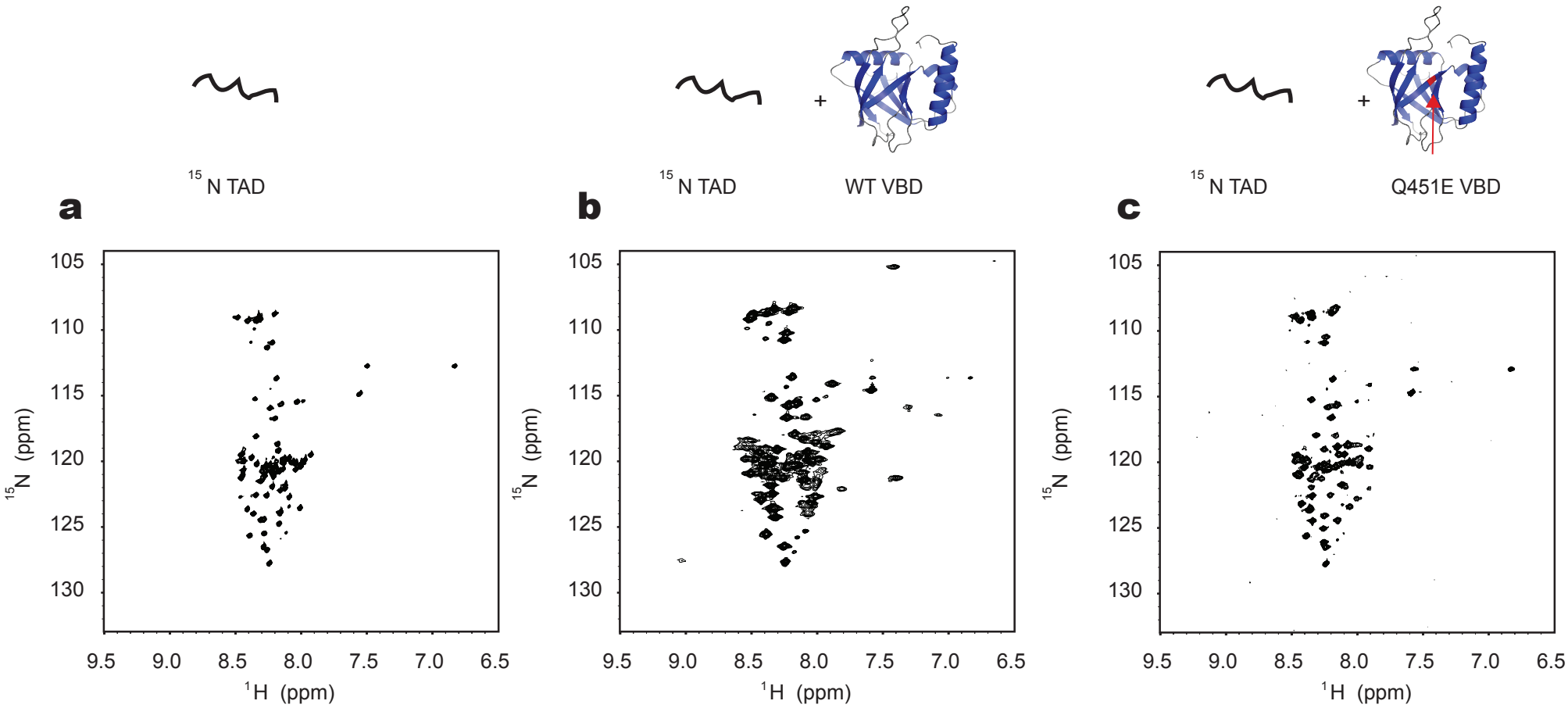
Supplementary Figure 3. ^{15}N T_2 relaxation time measurement of the MED25 VBD. **(a)** ^{15}N T_2 relaxation times of the free MED25 VBD are plotted against the residue number. Error bars represent s.d. The N- and C-terminus and the long loop between β1 and β2 display significantly longer T_2 times, indicating local flexibility. Due to line broadening and peak overlap, T_2 times of the residues forming loop β5/ β6 could only be determined for T503. **(b)** The 25 lowest energy structures are shown overlaid on the secondary structure elements. Loop β2/ β3 and loop β5/ β6 close the barrel from the bottom.



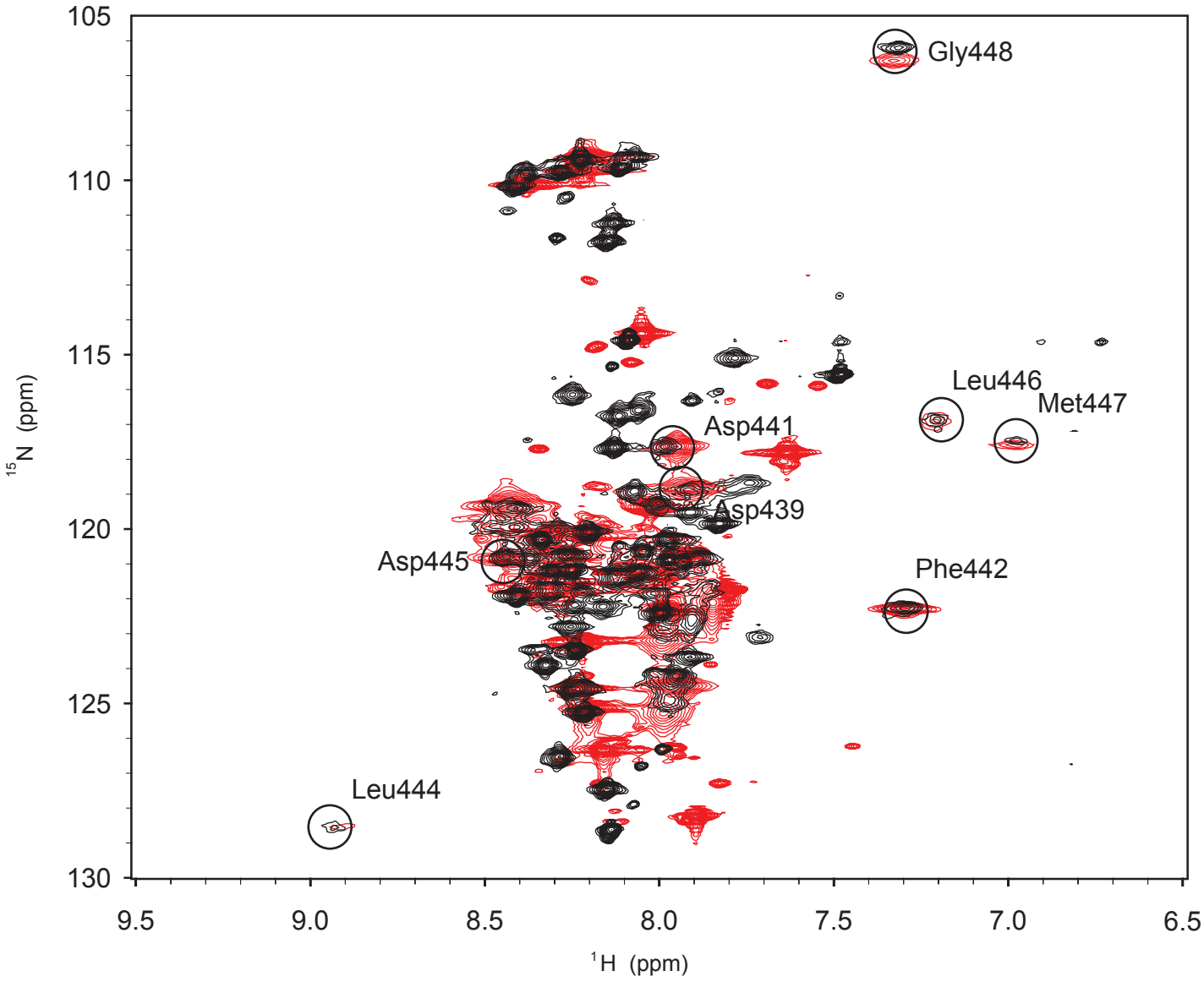
Supplementary Figure 4. Structural homology search by DALI[2]. Alignment of amino acid sequences (upper panel) and structural elements (lower panel) with the three structural homologous proteins of MED25 VBD: KU70/PDB: 1JEQ[3], SPOC/PDB: 1OW1[4], KU80/PDB:1JEQ[3]. Extended regions are highlighted in red and helical portions are shown in blue (lower panel).



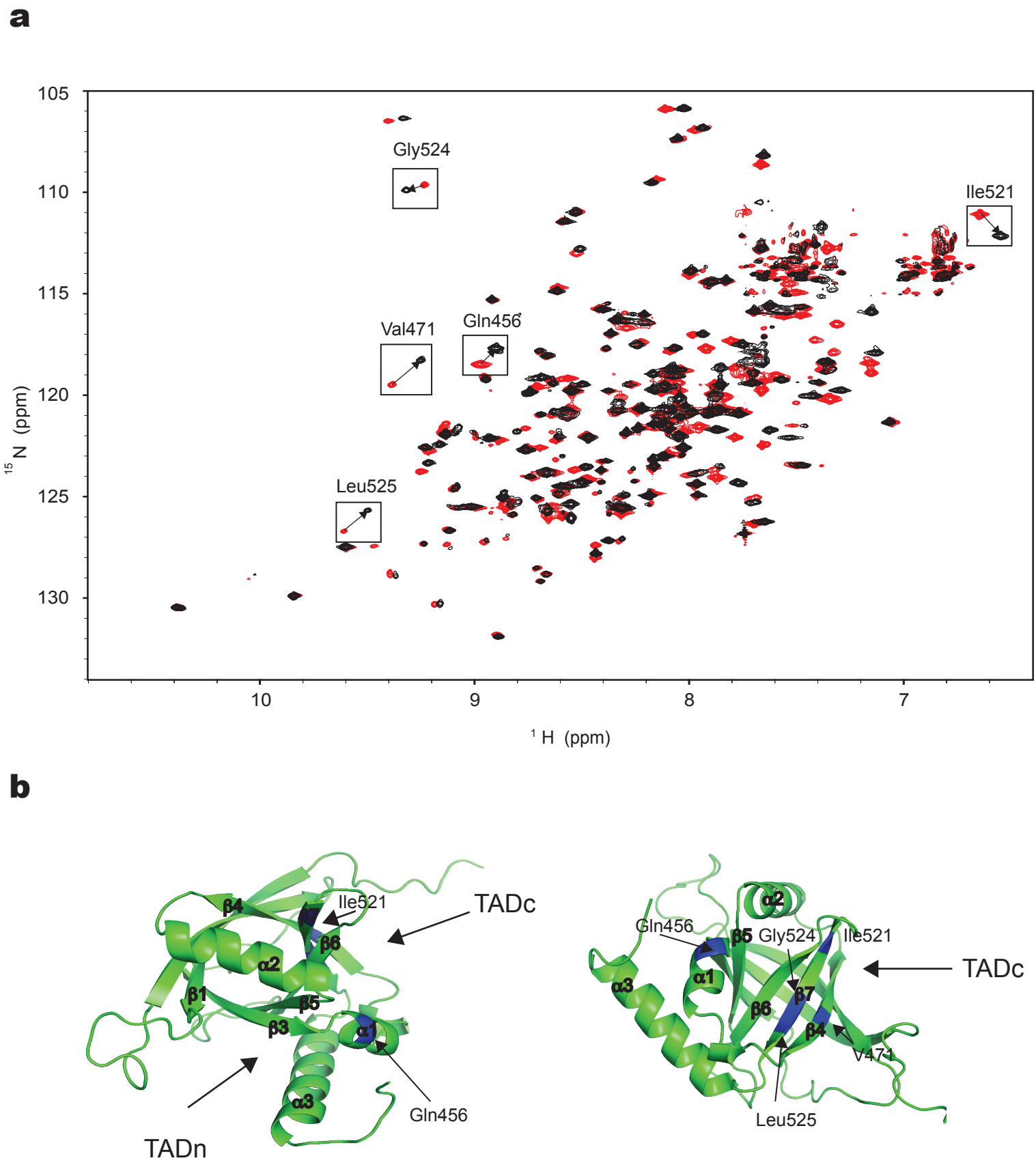
Supplementary Figure 5. Isothermal calorimetry titration. **(a)** Isothermal calorimetry titration of MED25 VBD with VP16 TADn revealed a K_d of 1.6 µM. **(b)** Isothermal calorimetry titration of MED25 VBD with VP16 full-length TAD revealed a K_d of approximately 50 nM. The C-terminal portion of the VP16 TAD contributes to the binding to MED25 VBD and likely accounts for the difference in K_d between TADn and TAD.



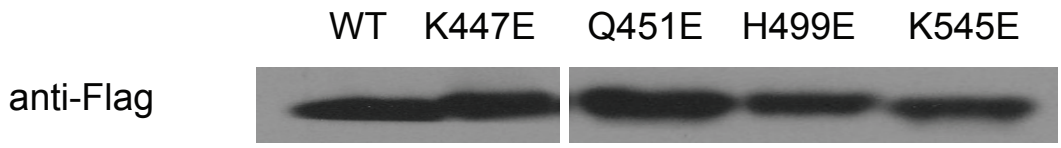
Supplementary Figure 6. The MED25 VBD Q451E mutation on β3 adjacent to the hydrophobic pocket also disrupts binding of full-length VP16 TAD to MED25 VBD. ^{1}H-^{15}N-HSQC spectra of free VP16 TAD **(a)**, at 1:1 ratio of wild-type MED25 VBD **(b)** and with 1:1.5 excess of Q451E MED25 VBD **(c)** show that VP16 TAD only loosely binds, as seen by minor chemical shift changes, to mutant MED25 VBD without adopting a folded conformation. Far-shifted and broadened signals caused by the addition of wild-type MED25 VBD **(b)**, are missing when the mutant MED25 VBD is added to ^{15}N-labeled VP16 TAD **(c)**.



Supplementary Figure 7. ^{1}H-^{15}N-HSQC spectra of the VP16 TAD (black signals) and the VP16 TADn (red signals) in the presence of 1.3 equivalents MED25 VBD. Far-shifted resonances overlap for both peptides and are indicated by circles and assignment.



Supplementary Figure 8. Mapping the TADc binding site on MED25 VBD. **(a)** Overlay of ^{1}H-^{15}N-TROSY-HSQC[5] spectra of the MED25 VBD bound to the VP16 TADn (red) and the MED25 VBD bound to the VP16 TAD (black). **(b)** A cartoon representation shows the clustering of the residues experiencing distinct chemical shifts upon interaction with VP16 TADc on β4 and β7. The TADn and TADc binding sites are located on different sides of the MED25 VBD barrel.



Supplementary Figure 9. Wild-type and mutant MED25 VBD displayed comparable level of expression in transfected HEK293T cells when immunoblotted with anti-Flag antibody.

Supplementary Table 1. Ramachandran Plot Summary of the MED25 VBD structure from PROCHECK[6].

Ramachandran Plot Summary from PROCHECK	
Most favoured regions	89.1%
Additionally allowed regions	10.4%
Generously allowed regions	0.5%
Disallowed regions	0.0%

Supplementary References

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