An invisible ubiquitin conformation is required for efficient phosphorylation by PINK1

Appendix

Christina Gladkova[$], Alexander F. Schubert[$], Jane L. Wagstaff, Jonathan N. Pruneda, Stefan M.V. Freund and David Komander*

Medical Research Council Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, UK.

* Corresponding author: David Komander, dk@mrc-lmb.cam.ac.uk

$ These authors contributed equally to this work

Running title: Ub-CR is a superior PINK1 substrate

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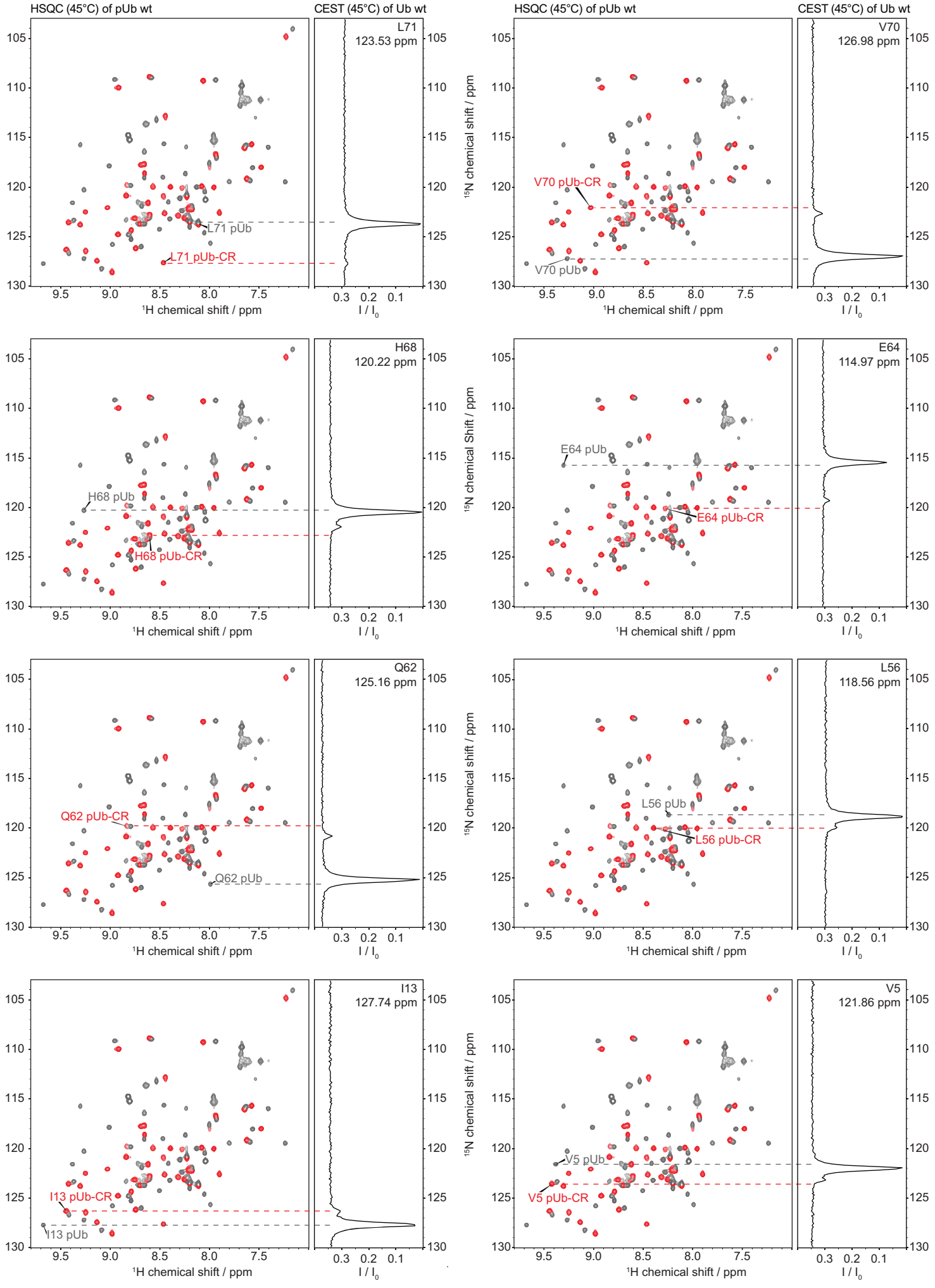
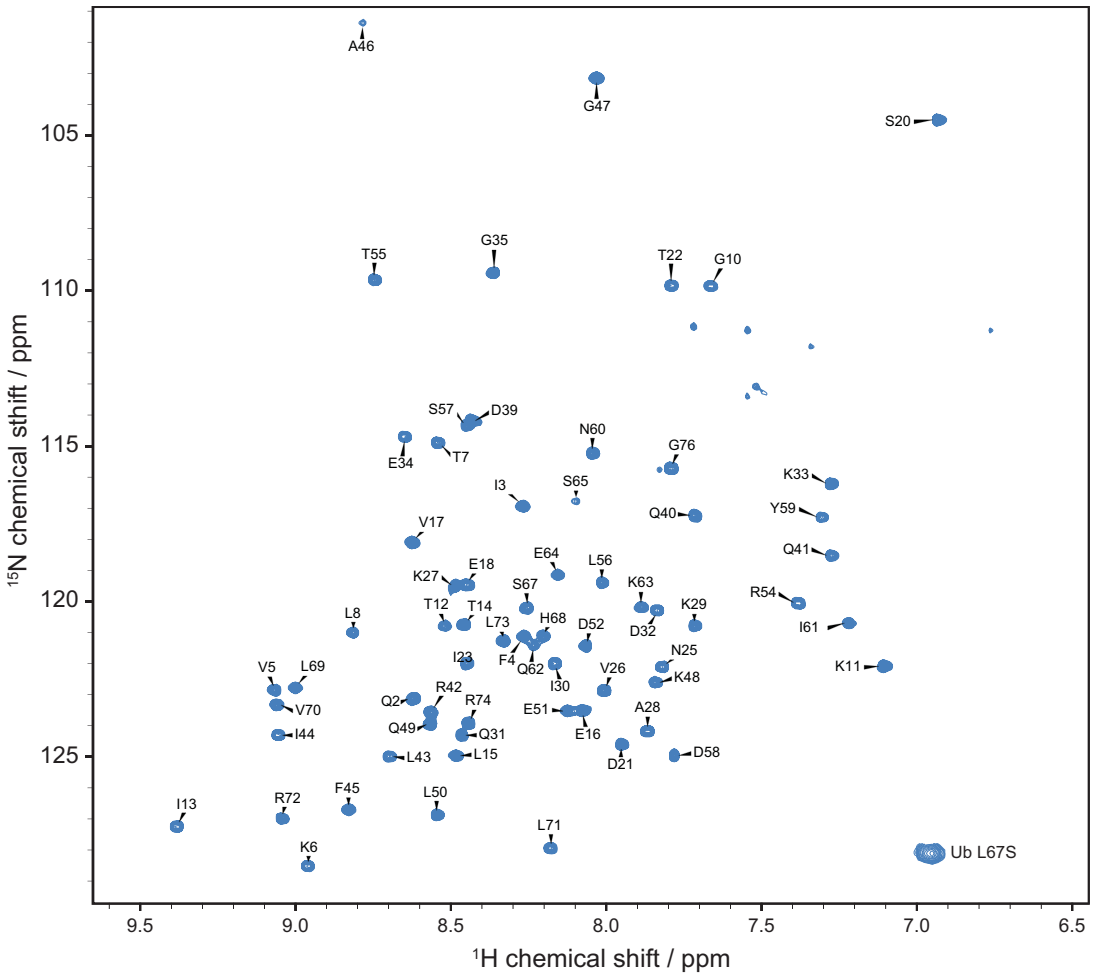
Appendix Figure S1



Appendix Figure S1. CEST experiment full spectra

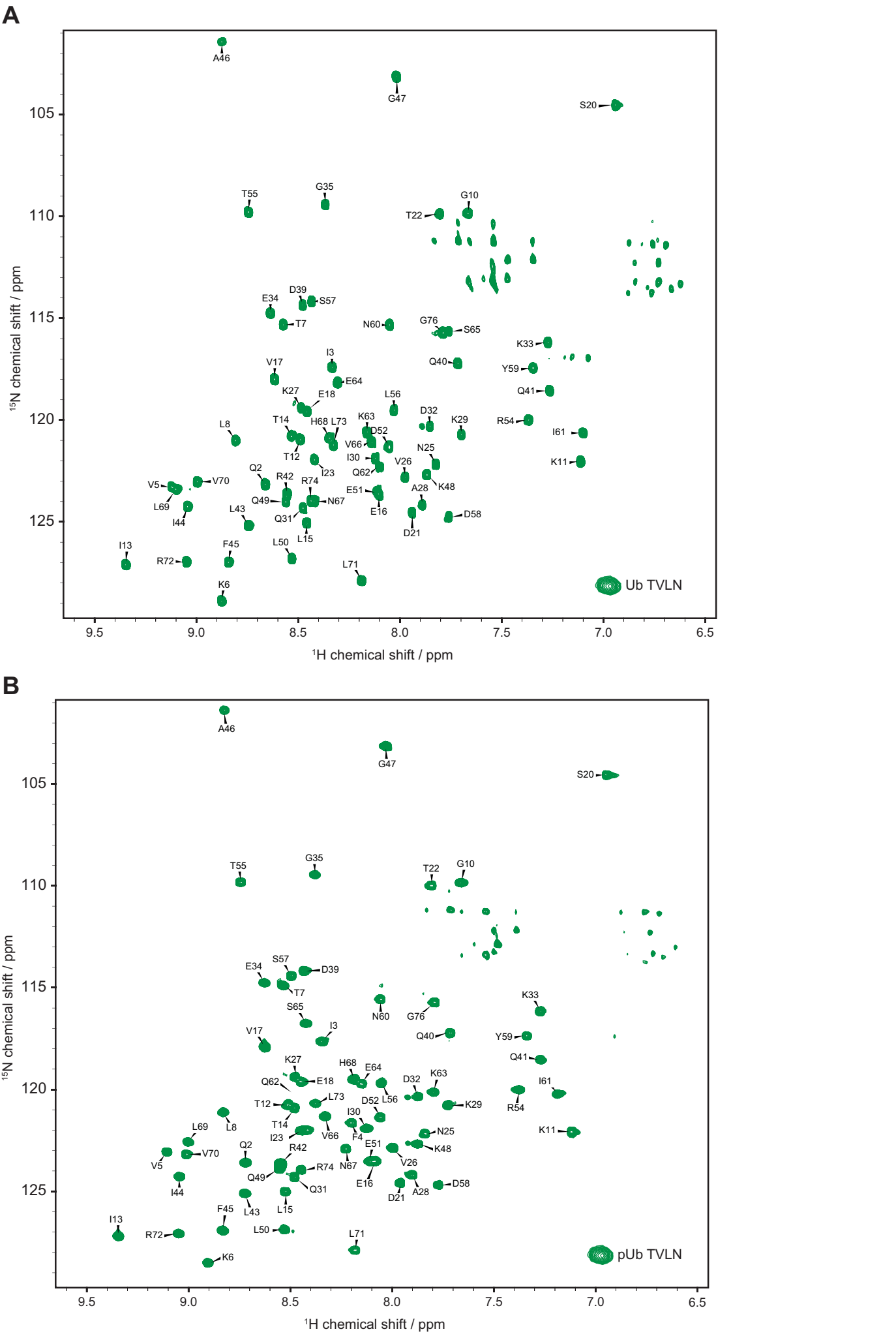
Full CEST ^{15}N profiles are shown alongside phosphoUb HSQC spectra. Residues targeted by the ^{15}N frequency are indicated, and corresponding HSQC positions of the phosphoUb and phosphoUb-CR resonances are indicated.

Appendix Figure S2



Appendix Figure S2. BEST TROSY spectrum of Ub L67S

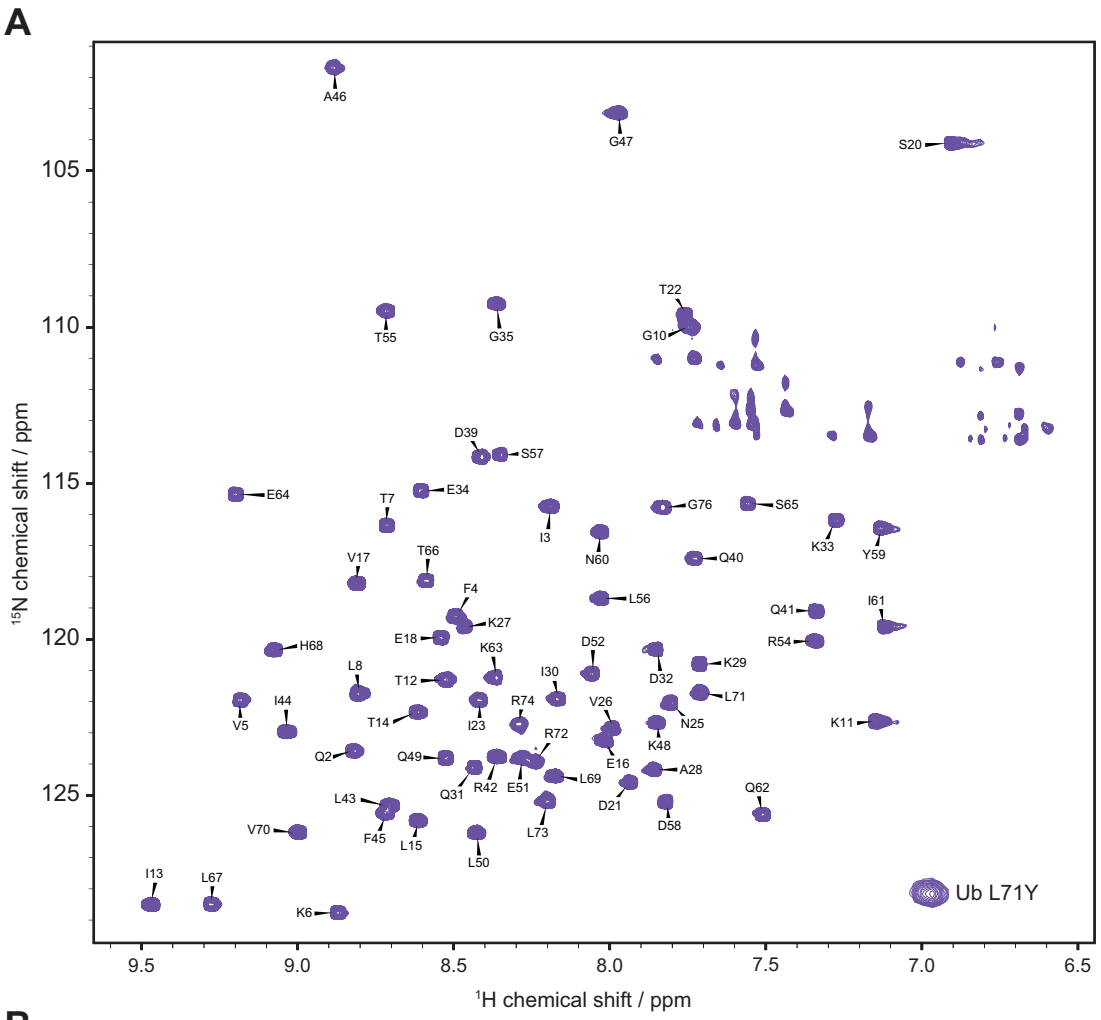
Complete and reassigned BEST TROSY spectrum of ^{13}C, ^{15}N-labelled Ub L67S.

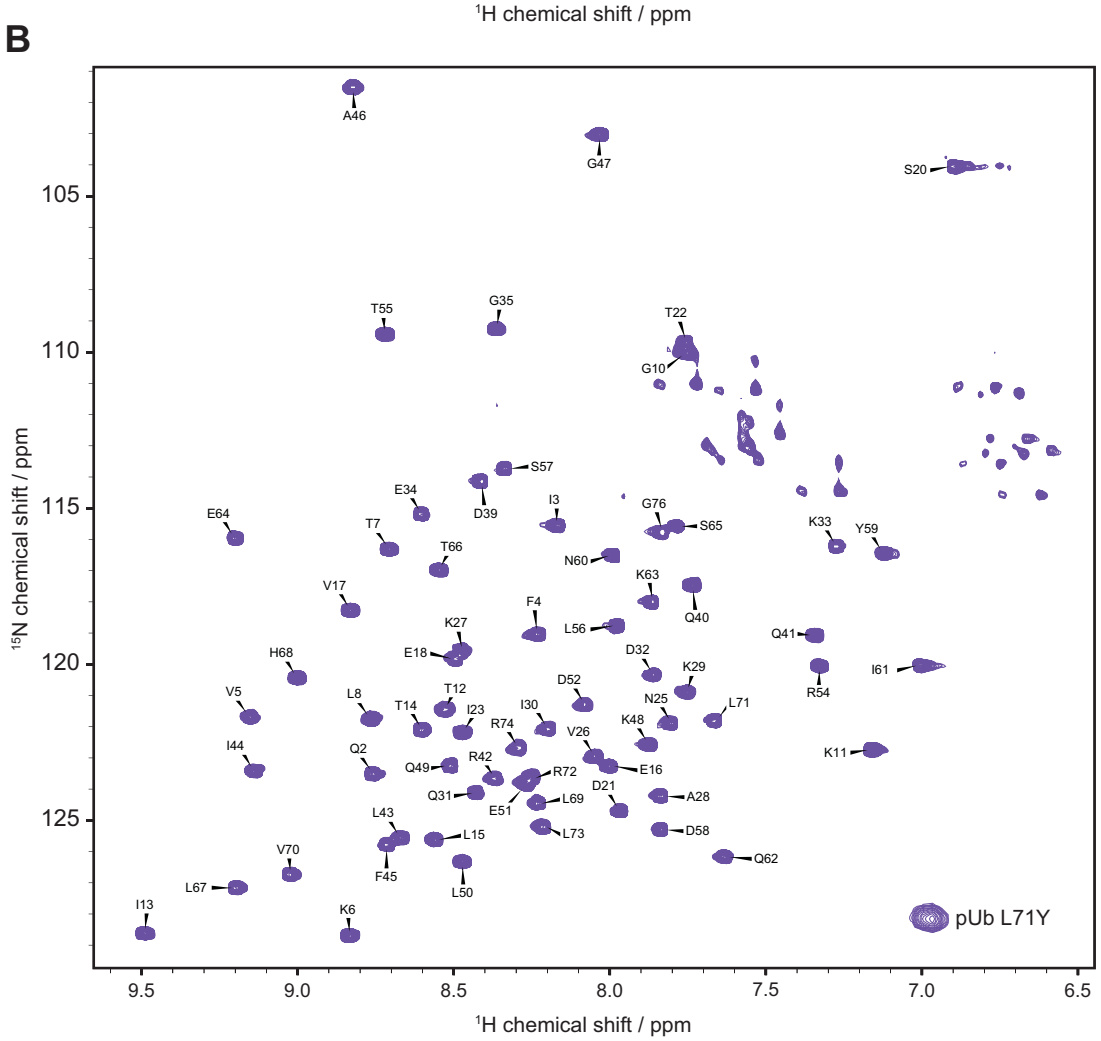

A
B
A46
G47
S20
T55
G35
T22
G10
E34
D39
S57
T7
N60
G76
S65
K33
V17
I3
Q40
Y59
E64
K27
E18
Q41
L56
D32
K29
R54
L8
T14
H68
L73
K63
D52
I61
V66
N25
T12
I30
K11
Q2
I23
Q62
V26
R42
R74
V5
V70
E51
A28
K48
Q49
N67
L69
E16
L43
Q31
D58
I44
D21
L15
I13
F45
L50
R72
L71
K6
Ub TVLN
pUb TVLN
105
110
115
120
125
9.5
9.0
8.5
8.0
7.5
7.0
6.5
15N chemical shift / ppm
1H chemical shift / ppm

Appendix Figure S3. BEST TROSY spectra of Ub TVLN and phosphoUb TVLN

Complete and reassigned BEST TROSY spectra of ^{13}C, ^{15}N-labelled Ub TVLN (A) and phosphoUb TVLN (B).

Appendix Figure S4

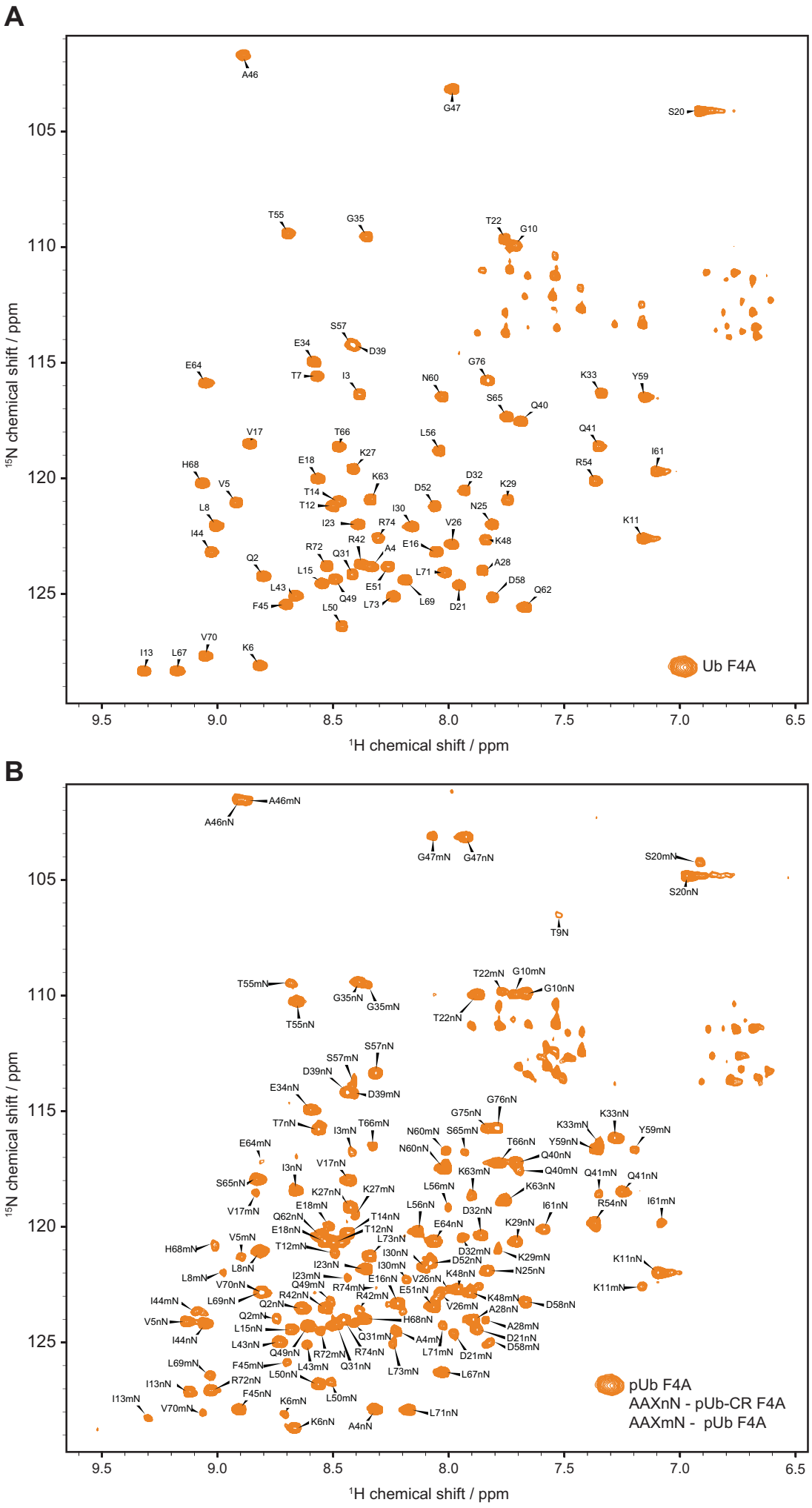




Appendix Figure S4. BEST TROSY spectra of Ub L71Y and phosphoUb L71Y

Complete and reassigned BEST TROSY spectra of ^{13}C, ^{15}N-labelled Ub L71Y (A) and phosphoUb L71Y (B).

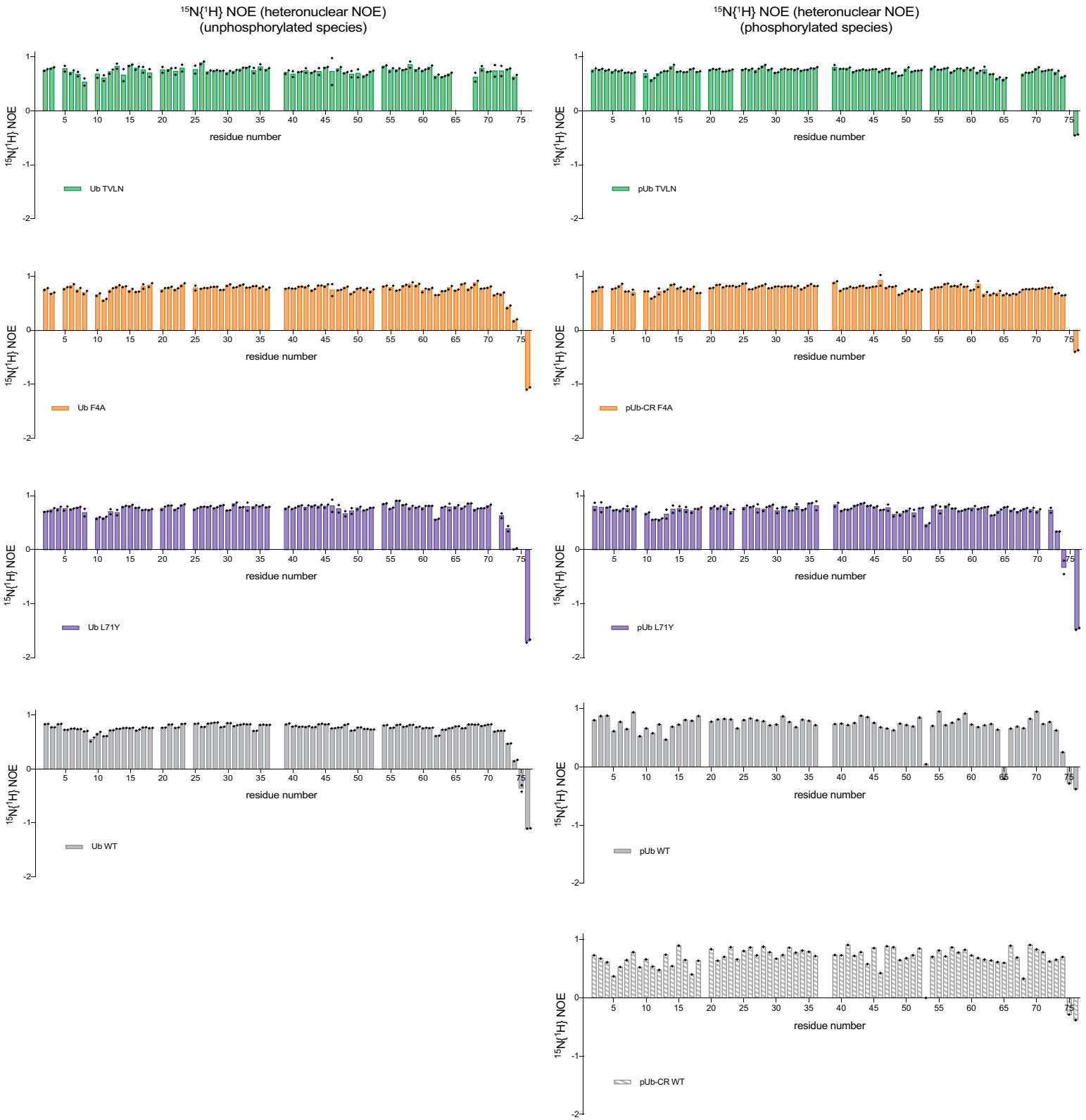
Appendix Figure S5



Appendix Figure S5. BEST TROSY spectra of Ub F4A and phosphoUb F4A

Complete and reassigned BEST TROSY spectra of ^{13}C, ^{15}N-labelled Ub F4A (A) and phosphoUb F4A (B).

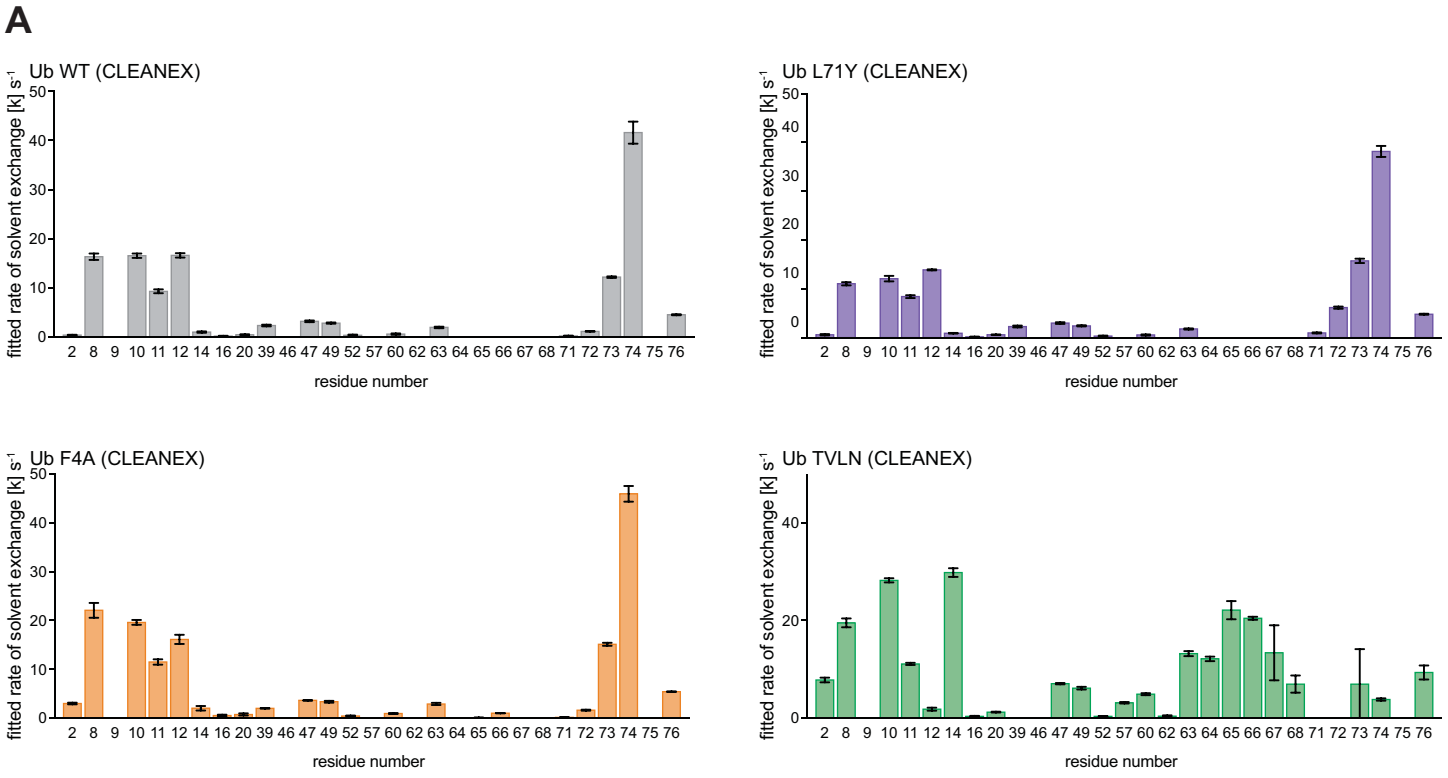
Appendix Figure S6

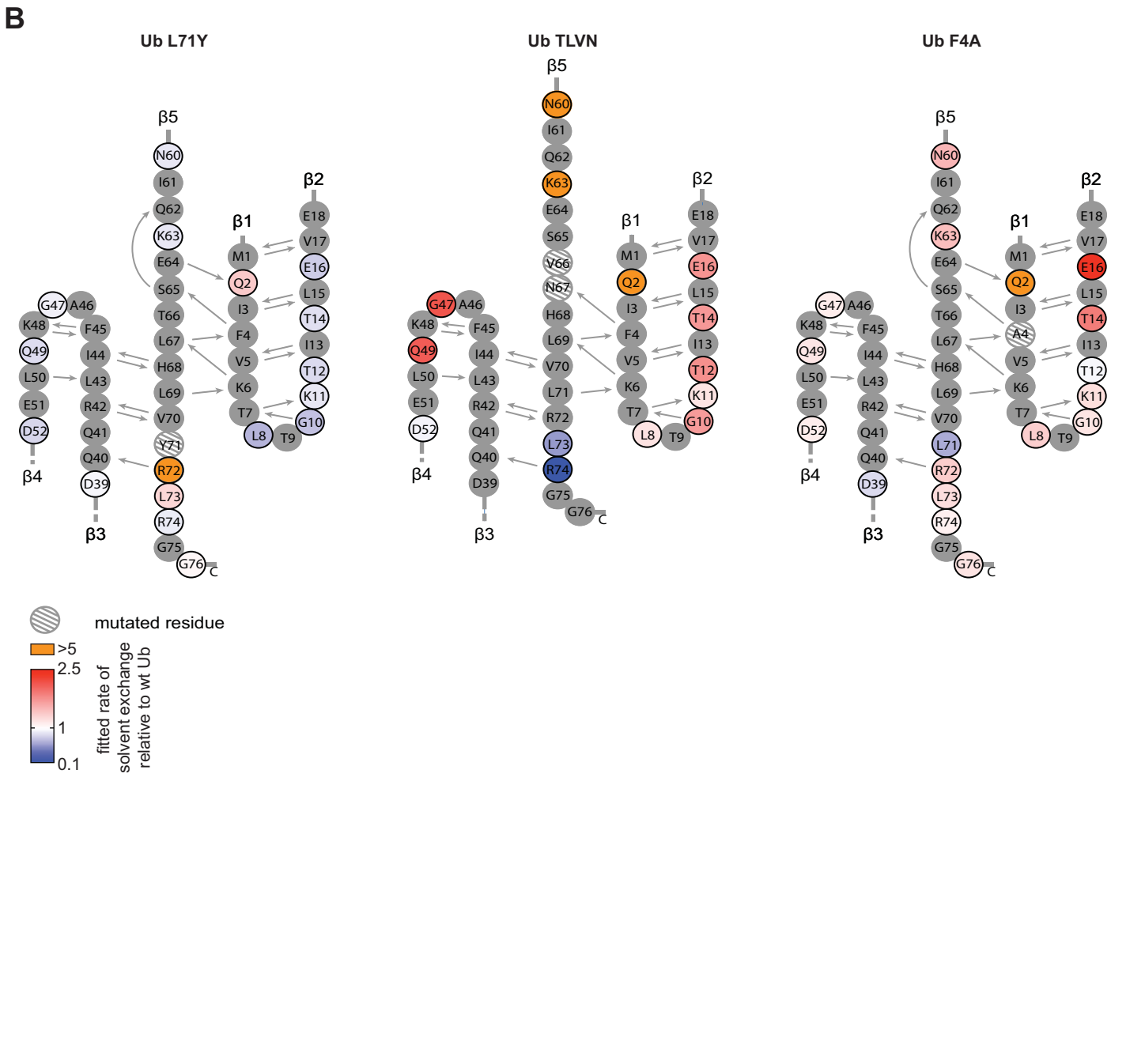


Appendix Figure S6. $^{15}N\{^{1}H\}$ hetNOE experiments on Ub variants

Complete set of $^{15}N\{^{1}H\}$ hetNOE measurements of Ub residues for indicated species. Data for pUb and pUb-CR WT are replotted from (Wauer *et al*, 2015a).

Appendix Figure S7



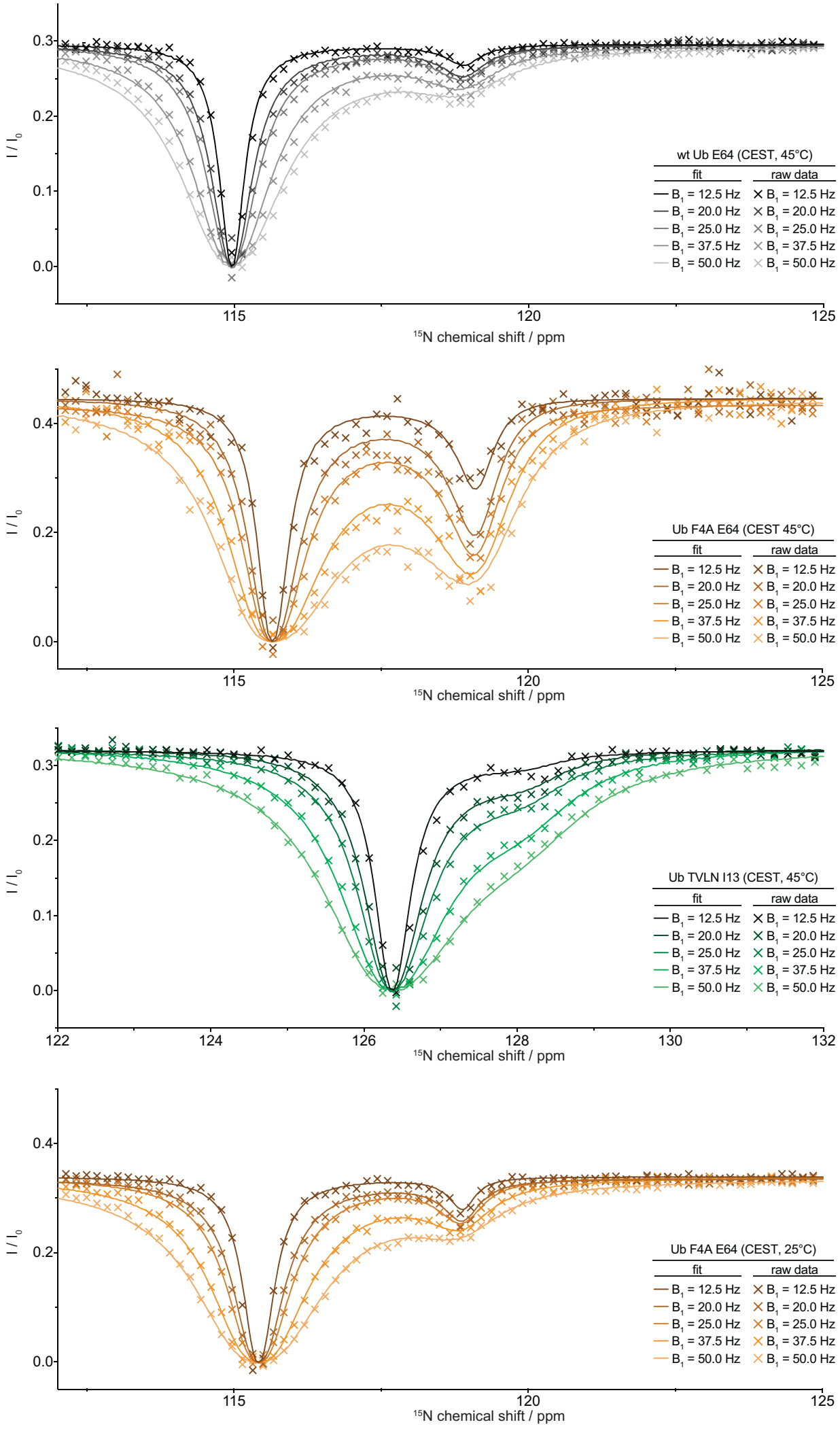


Appendix Figure S7. CLEANEX experiments on Ub variants

A) Bar graph showing the fitted rate of solvent exchange for indicated Ub residues.

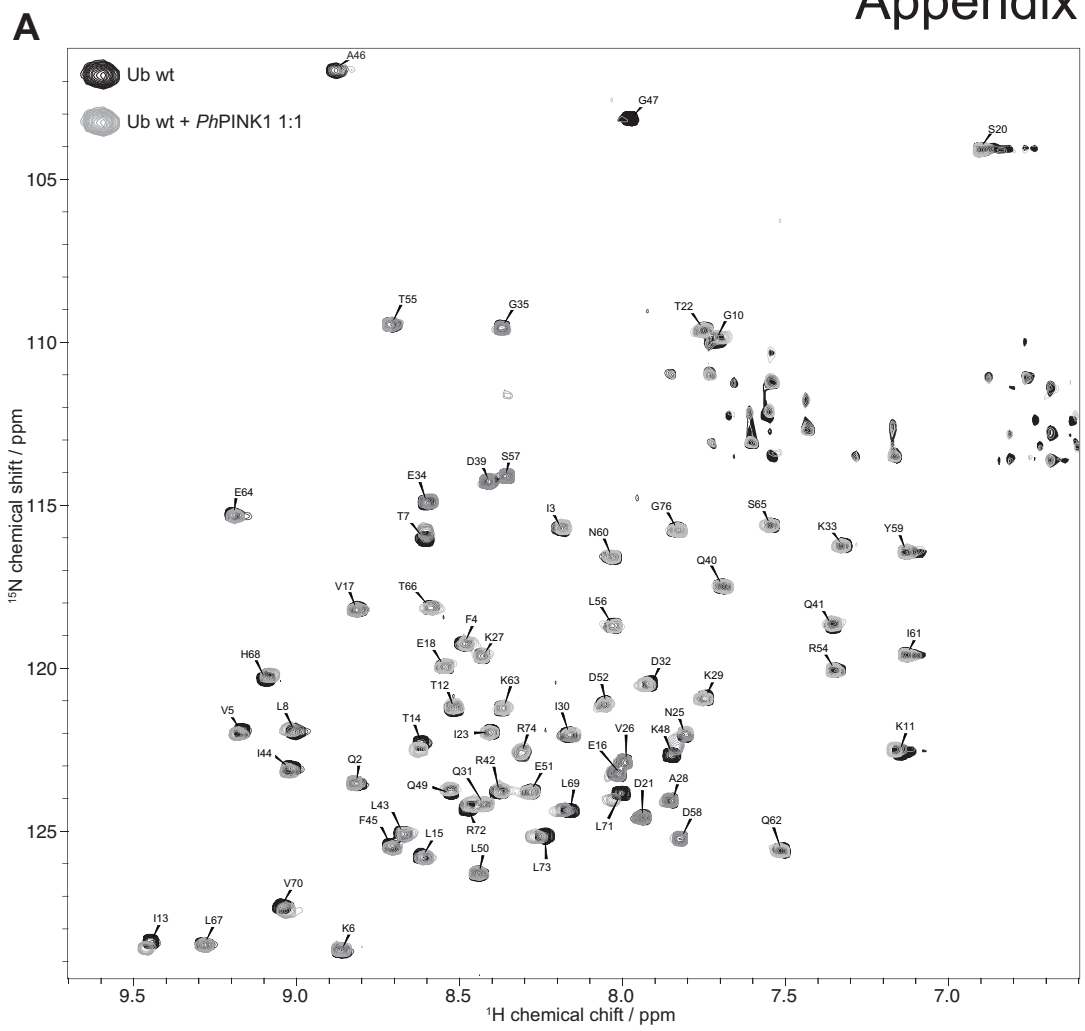
B) Hydrogen bonding pattern of Ub L71Y (in common Ub conformation), Ub TVLN (in Ub-CR conformation) and Ub F4A (in common Ub conformation) colouring residues with increased or decreased rate of solvent exchange as compared to wt Ub.

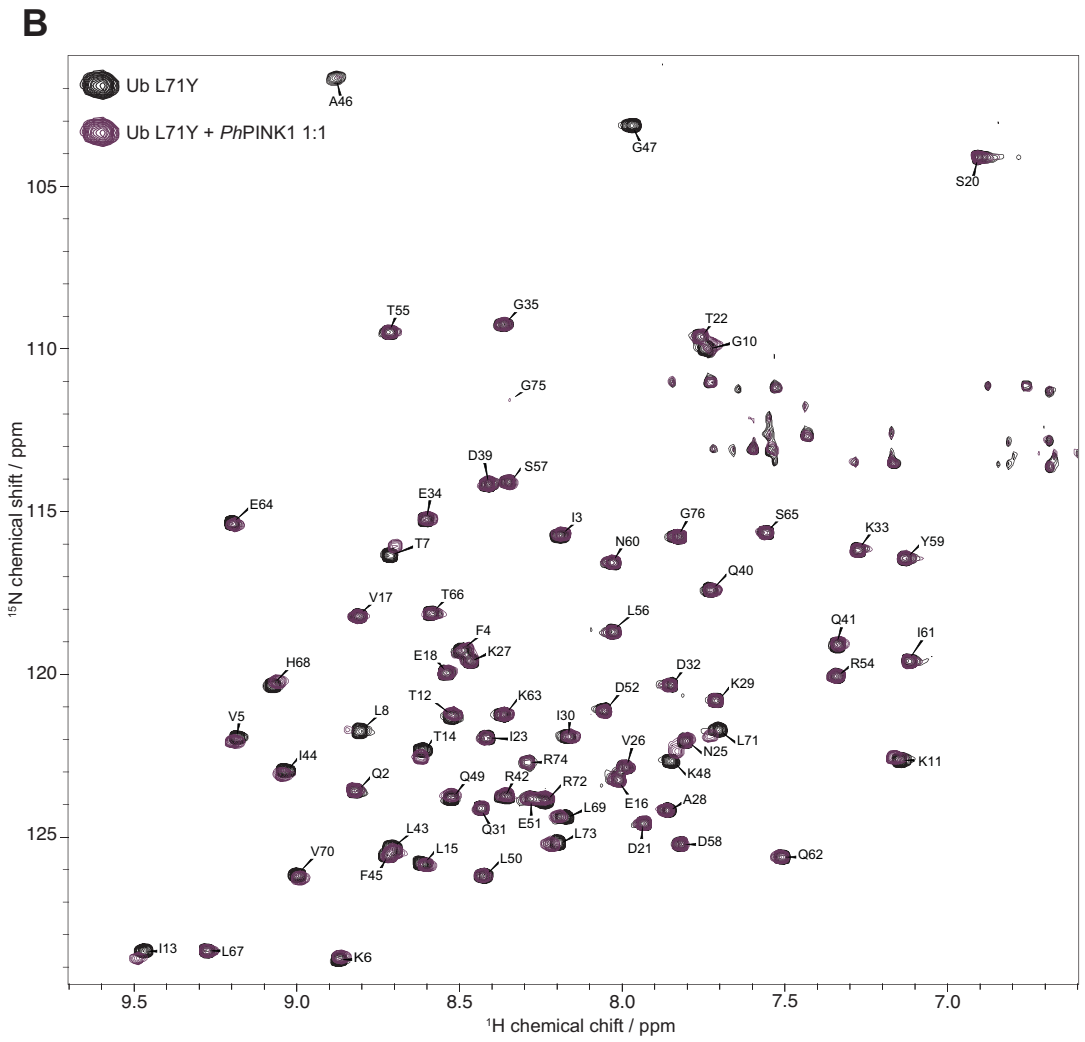
Appendix Figure S8



Appendix Figure S8. Representative CEST fits

Representative peak fits for CEST experiments on wt Ub, Ub TVLN, and Ub F4A at different B_1 fields.

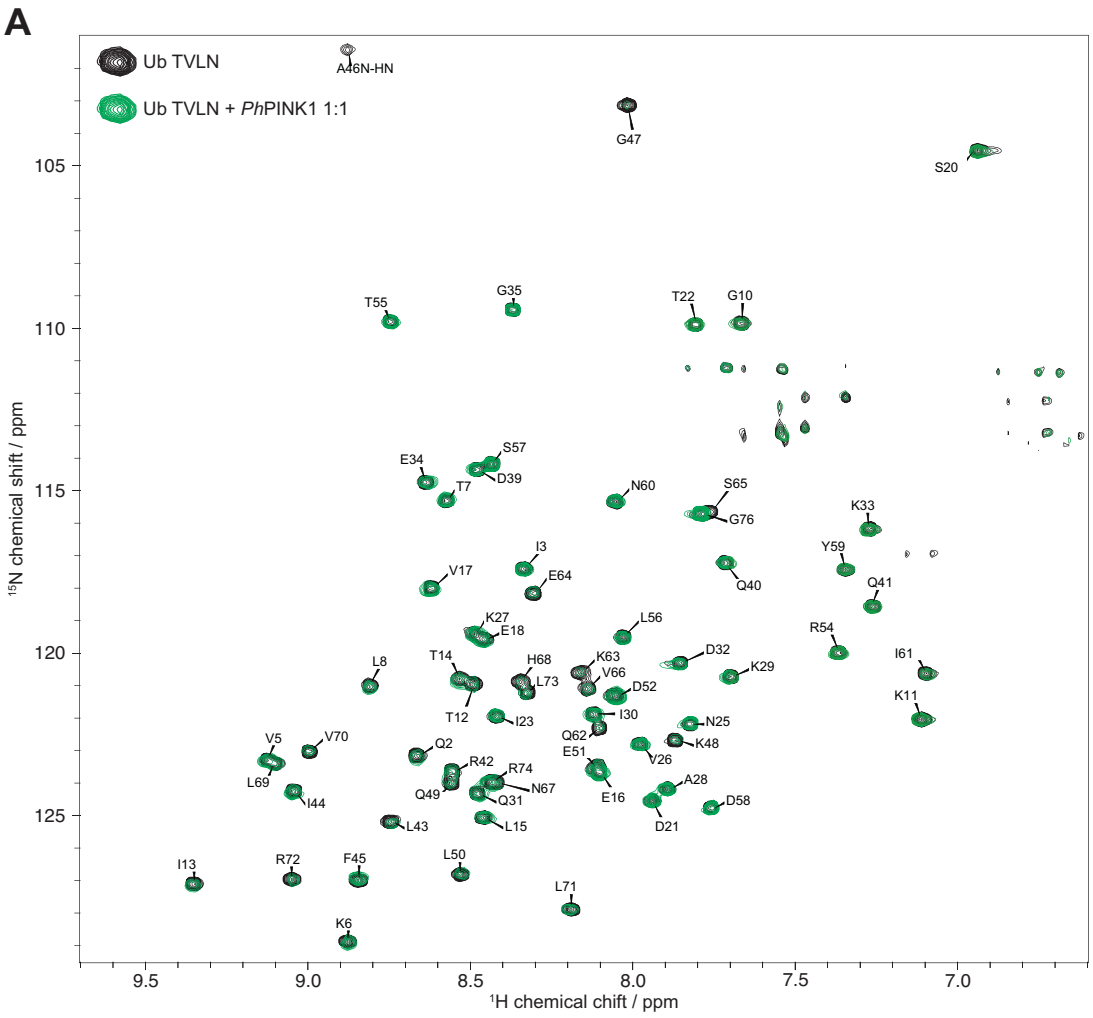

A
Ub wt
Ub wt + PhPINK1 1:1
15N chemical shift / ppm
1H chemical chift / ppm
105
110
115
120
125
9.5
9.0
8.5
8.0
7.5
7.0
A46
G47
S20
T55
G35
T22
G10
D39
S57
E34
E64
I3
G76
S65
T7
N60
K33
Y59
Q40
V17
T66
L56
Q41
F4
I61
E18
K27
H68
R54
D32
K29
T12
K63
D52
V5
L8
I30
N25
T14
I23
R74
V26
K48
K11
I44
Q2
E16
Q31
R42
E51
A28
Q49
L69
D21
L43
F45
L15
R72
L71
D58
Q62
L50
L73
V70
I13
L67
K6


B
Ub L71Y
Ub L71Y + PhPINK1 1:1
15N chemical shift / ppm
1H chemical chift / ppm
105
110
115
120
125
9.5
9.0
8.5
8.0
7.5
7.0
A46
G47
S20
T55
G35
T22
G10
G75
D39
S57
E34
E64
I3
G76
S65
K33
Y59
T7
N60
Q40
V17
T66
L56
Q41
F4
I61
E18
K27
H68
R54
D32
K29
T12
K63
D52
V5
L8
I30
L71
T14
I23
V26
N25
K11
I44
R74
K48
Q2
Q49
R42
R72
E16
A28
L69
E51
Q31
L43
L73
D21
D58
L15
V70
Q62
F45
L50
I13
L67
K6

Appendix Figure S9. PINK1 interaction analysis

Complete BEST TROSY spectral overlay in the absence and presence of equimolar *Ph*PINK1 for wt Ub (A) and Ub L71Y (B).

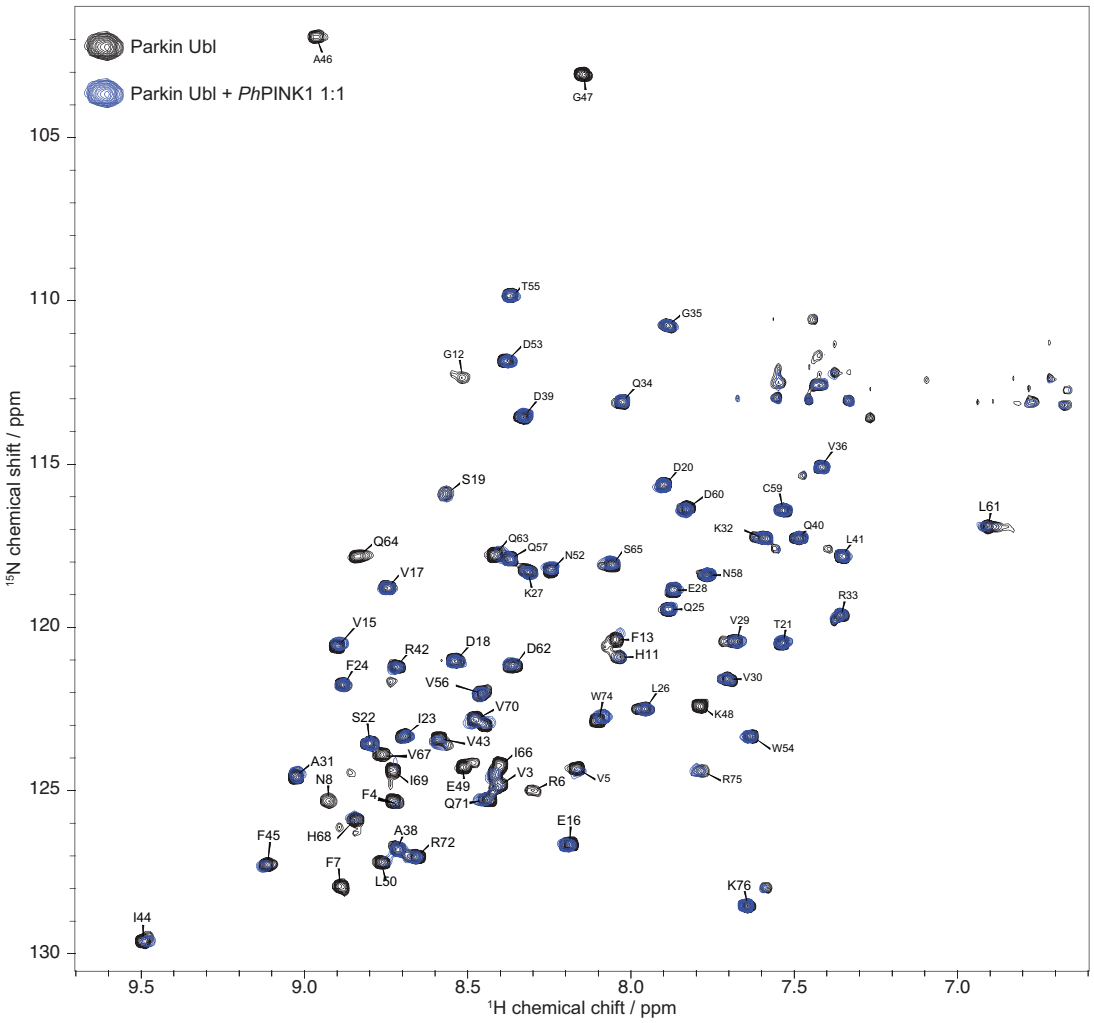
Appendix Figure S10



Appendix Figure S10. PINK1 interaction analysis

Complete BEST TROSY spectral overlay in the absence and presence of equimolar *Ph*PINK1 for Ub TVLN (A) and Ub F4A (B).

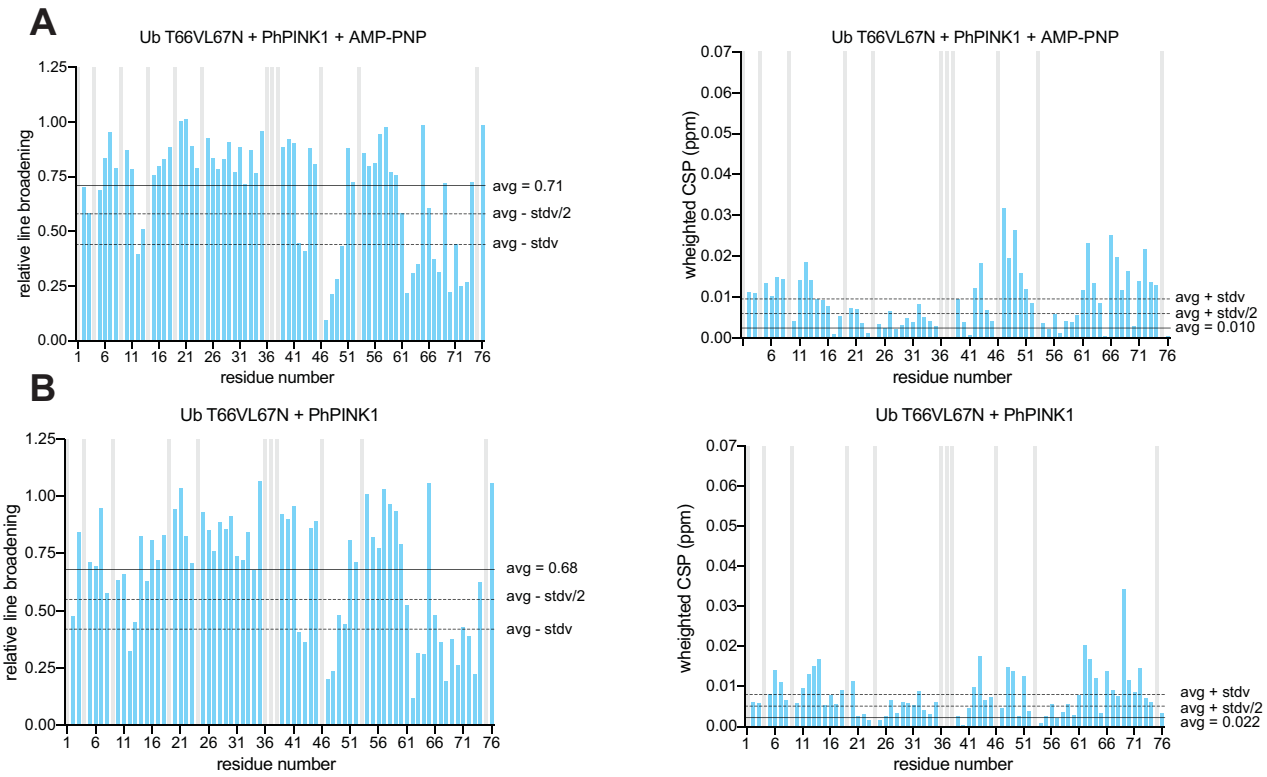
Appendix Figure S11



Appendix Figure S11. PINK1 interaction analysis

Complete BEST TROSY spectral overlay in the absence and presence of equimolar *Ph*PINK1 for Parkin Ubl.

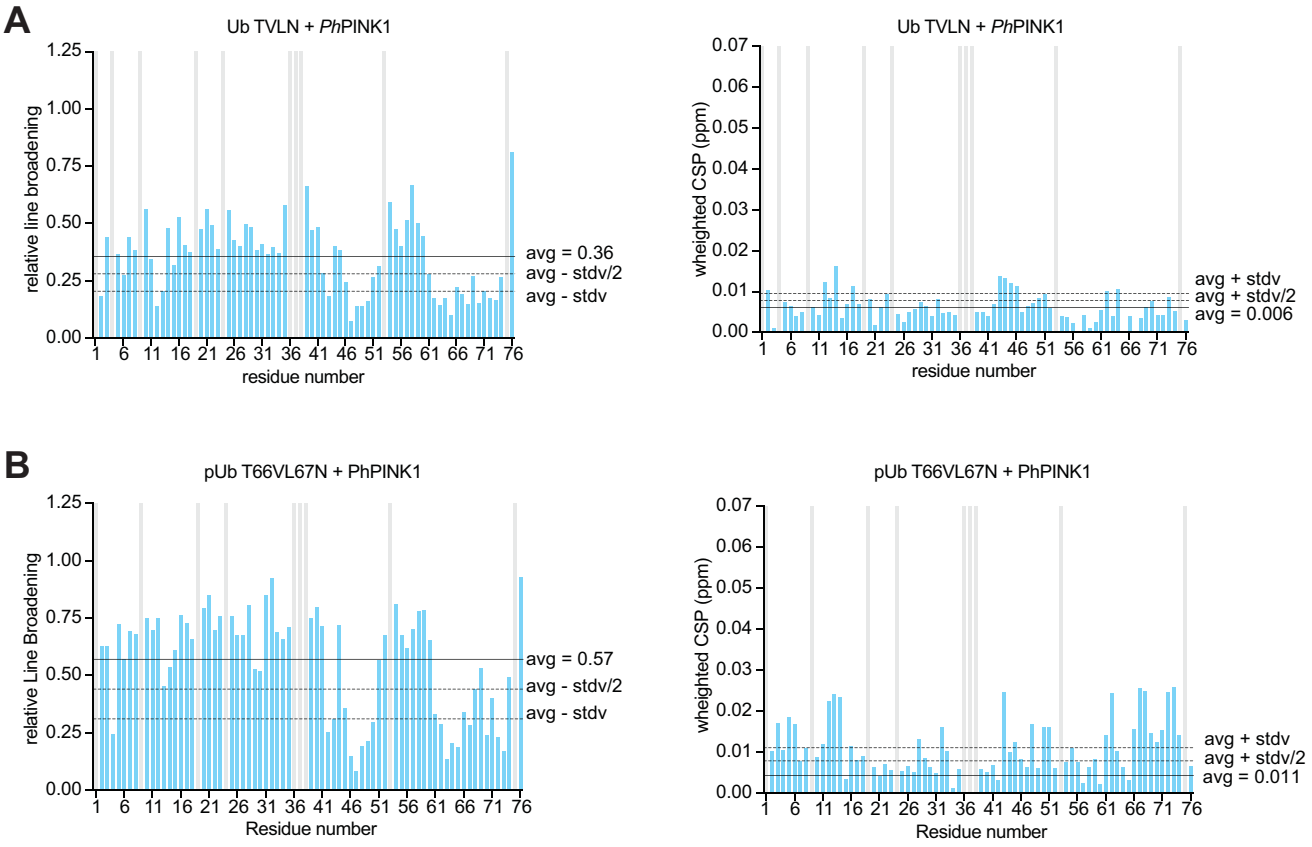
Appendix Figure S12



Appendix Figure S12. PINK1 interaction analysis in the presence of MgAMP-PNP

Line broadening and chemical shift perturbation analyses of the Ub TVLN interaction with *Ph*PINK1 in the presence (A) and absence (B) of MgAMP-PNP.

Appendix Figure S13



Appendix Figure S13. PINK1 interaction analysis with phosphoUb

Line broadening and chemical shift perturbation analyses of the Ub TVLN (A) and phosphoUb TVLN (B) interaction with *Ph*PINK1.