

Stability of Begomoviral pathogenicity determinant β C1 is modulated by mutually antagonistic SUMOylation and SIM interactions

Ashwin Nair^{1, 2}, Kiran Sankar Chatterjee¹, Vikram Jha^{1, 3}, Ranabir Das¹, and P. V. Shivaprasad^{1*}

This file contains following information:

Additional File 1: Fig.S1-19

Fig.S1: SyYVCV β C1 has multiple conserved SUMOylation sites.

Fig.S2: SyYVCV β C1 can interact with *NbSUMO1*.

Fig.S3: SyYVCV β C1 induces various developmental defects in transgenic plants.

Fig.S4: SyYVCV β C1 undergoes SUMOylation *in planta*.

Fig.S5: SyYVCV β C1 weakly interacts with other plant SUMO proteins.

Fig.S6: SUMOylation of β C1 is required for symptom development.

Fig.S7: SUMOylation is required for the stability of β C1 *in planta*.

Fig.S8: SIM sites in SyYVCV β C1 and phylogeny of its potential partner SUMO proteins.

Fig.S9: Expression analysis and purification of plant SUMO proteins.

Fig.S10: Sequence of SIM mutants.

Fig.S11: SIM sites in β C1 C-terminal end interact with *NbSUMO1*.

Fig.S12: SIM sites in SyYVCV β C1 regulate its stability.

Fig.S13: SIM and SUMOylation motifs of β C1 are necessary for its pathogenicity determinant function.

Fig.S14: SUMOylation motif in β C1 is important for its subcellular localization.

Fig.S15: SyYVCV β C1 induces global SUMOylation.

Fig.S16: SIM and SUMOylation motif of β C1 is essential for host defense suppression

Fig.S17: Conservation of SUMOylation sites among viruses producing similar symptoms.

Fig.S18: Uncropped images of blots in main figures 1-7 and replicates of western blots.

Fig.S19: Uncropped images of blots in Supplementary Figures 1-16.

A)

Site	AA	Sequence	Type	P.S.
Ss1	K18	F I V D V K L M Q E D ←	INV. consensus	Low
Ss2	K24	K L M Q E D K I S V Q I ←	INV. consensus	High
Ss3	K83	T I G E F K Q E D M I E →	Consensus	Low

B)

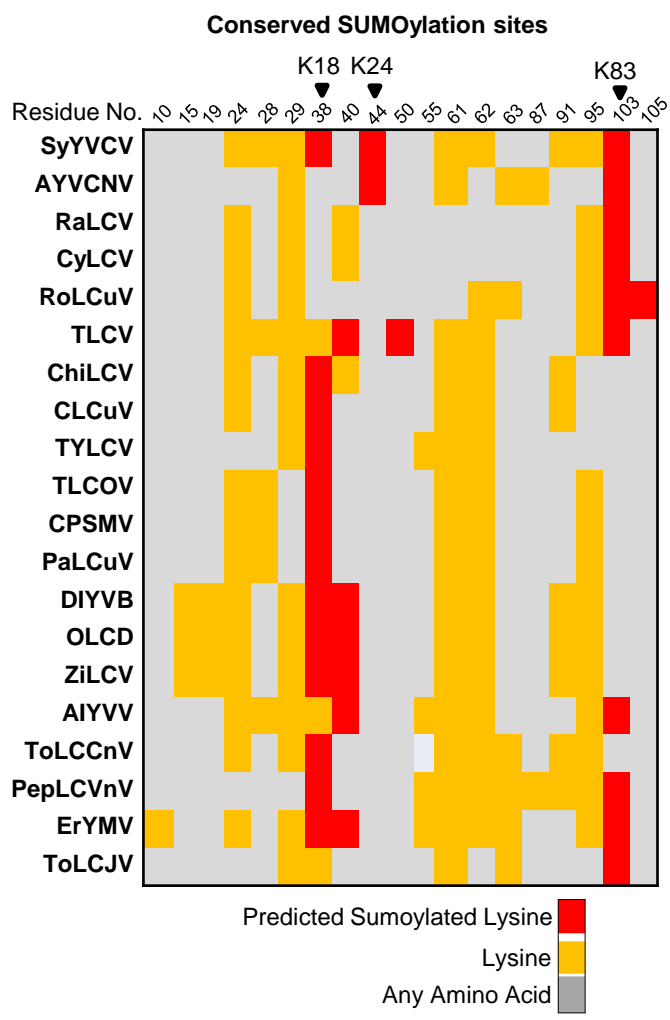


Fig.S1: SyYVCV β C1 has multiple conserved SUMOylation sites.

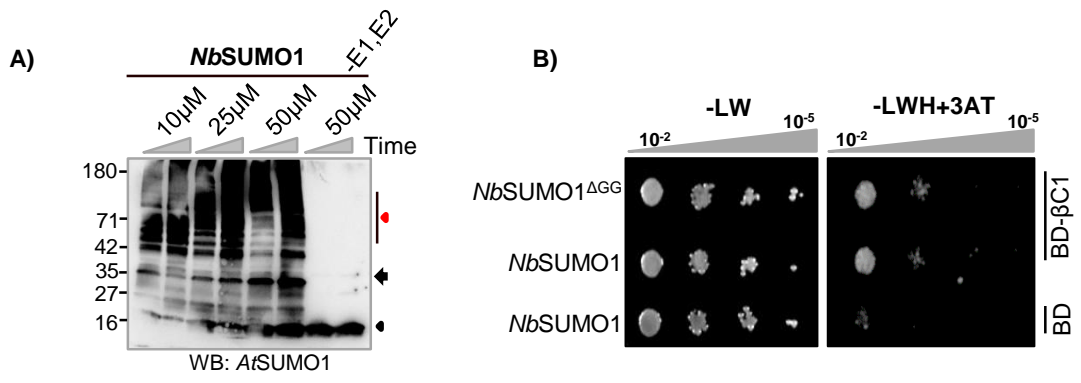


Fig.S2: SyYVCV βC1 can interact with *NbSUMO1*.

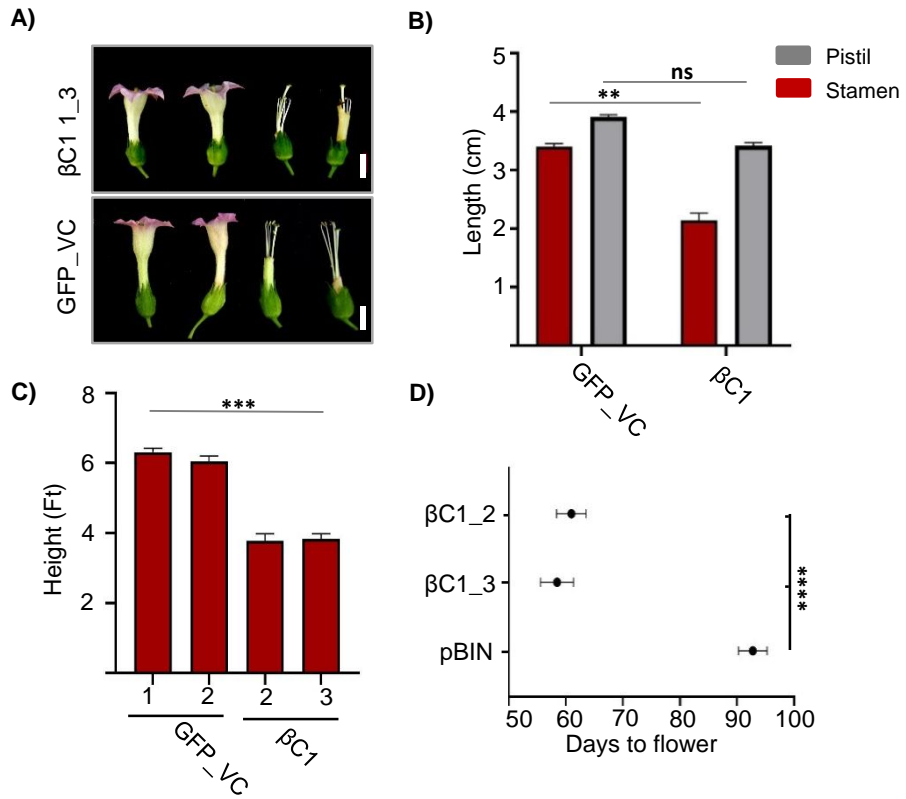


Fig.S3: SyYVCV $\beta C1$ induces various developmental defects in transgenic plants.

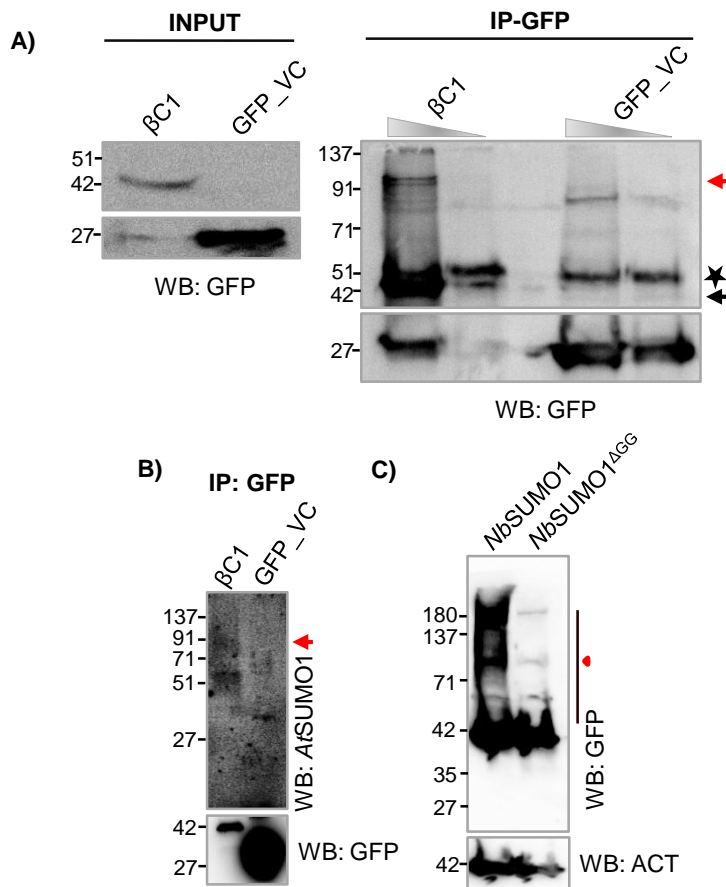


Fig.S4: SyYVCV β C1 undergoes SUMOylation *in planta*.

A)

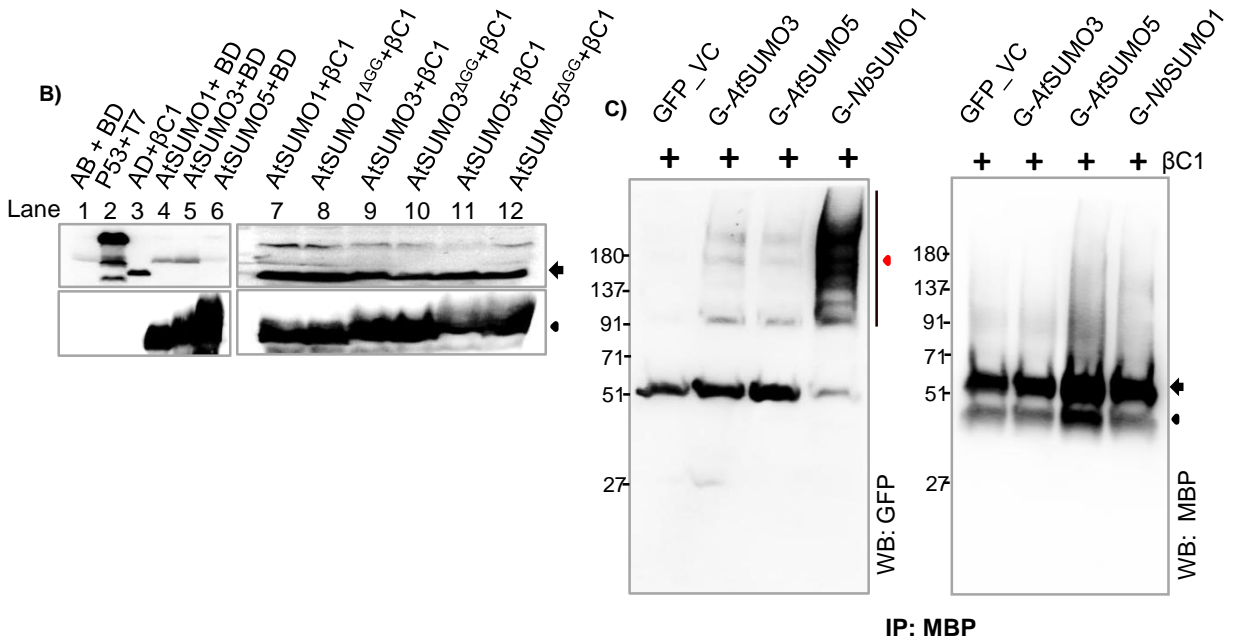
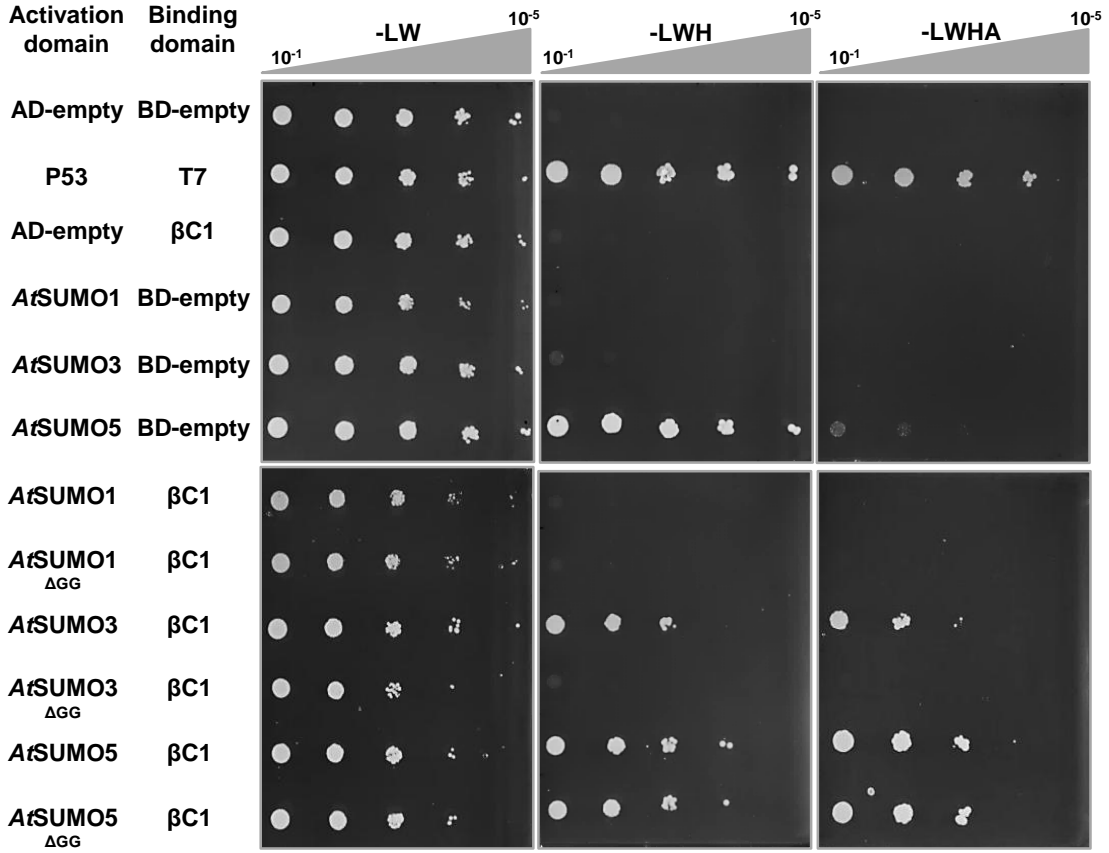


Fig.S5: SyYVCV βC1 weakly interacts with other plant SUMO proteins.

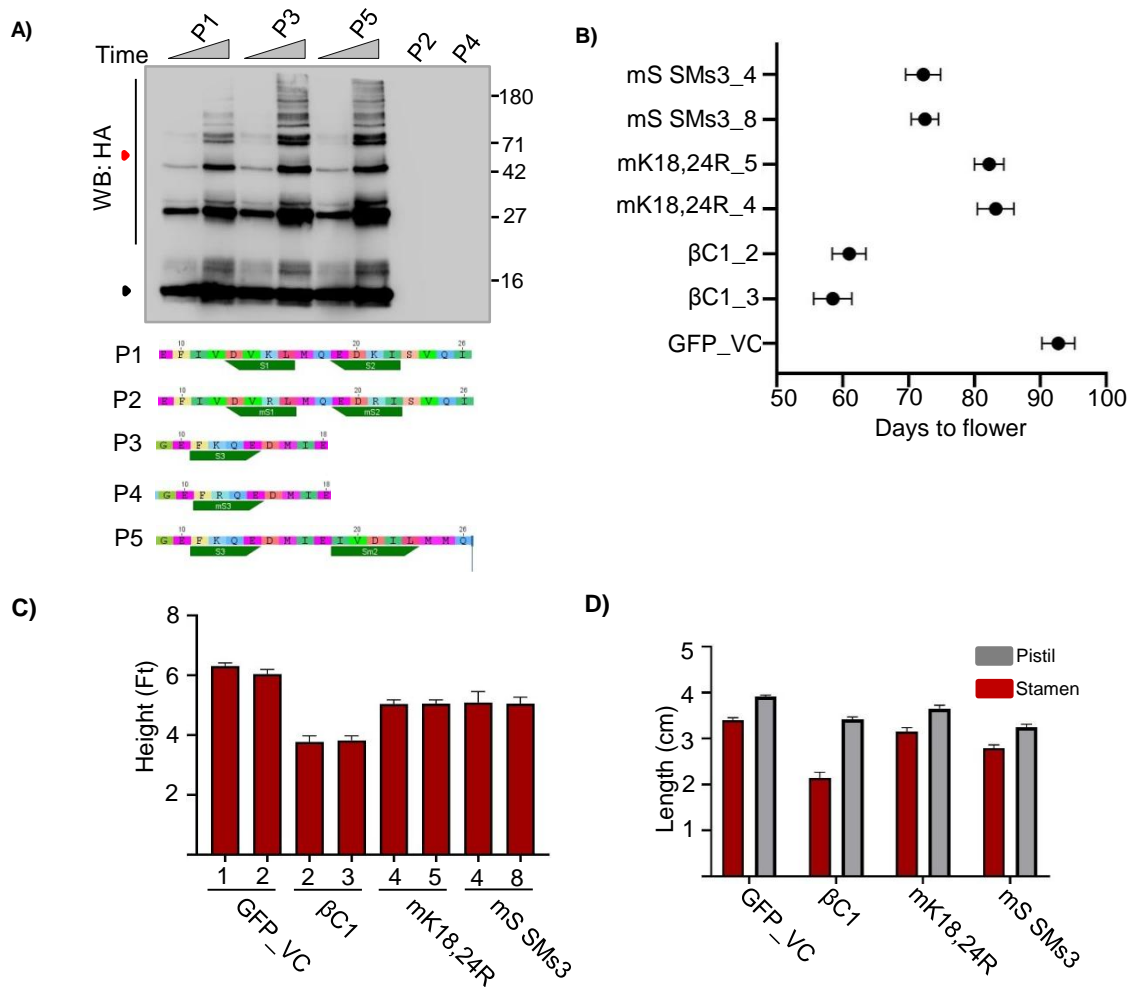


Fig.S6: SUMOylation of β C1 is required for symptom development.

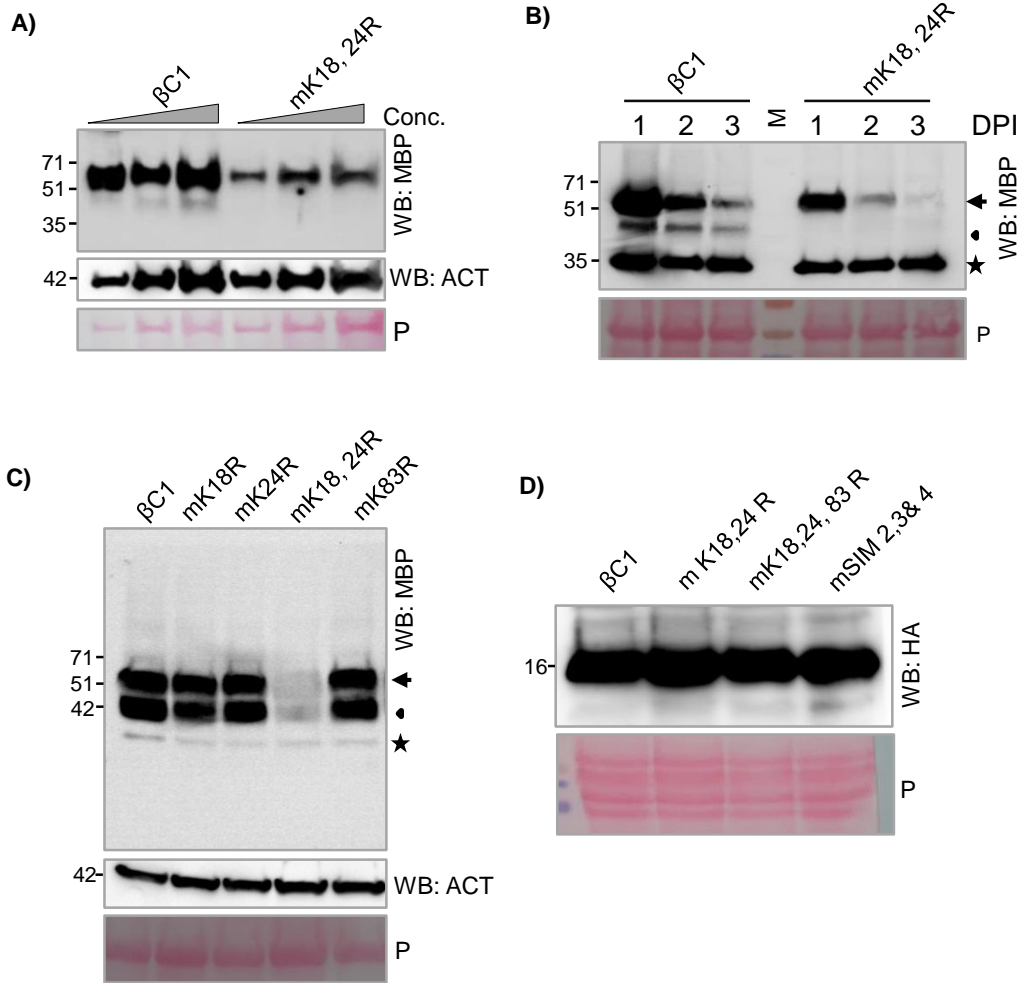


Fig.S7: SUMOylation is required for the stability of $\beta C1$ in planta.

A)

Domain	Sequence	Type	PS	AA No.
SIM s1	G M E F I V D V K L M Q	SIM β	4.191	14-17
SIM s2	D M I E I V D I L M M Q E	SIM β	4.501	90-93
SIM s3	M I E I V D I L M M Q E	SIM 2	0.918	91-94
SIM s4	Q E A P V I D I N V S D E	SIM β	24.07	101-104

B)

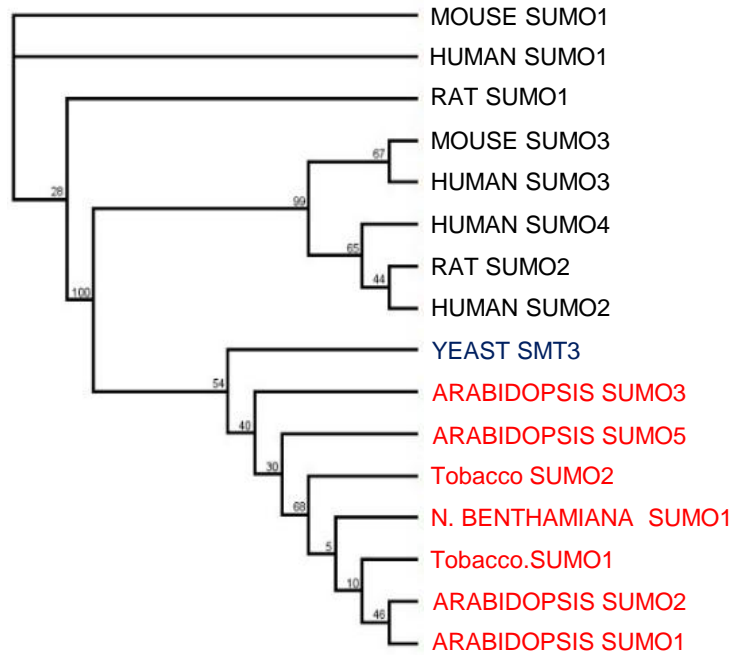


Fig.S8: SIM sites in SyYVCV β C1 and phylogeny of its potential partner SUMO proteins.

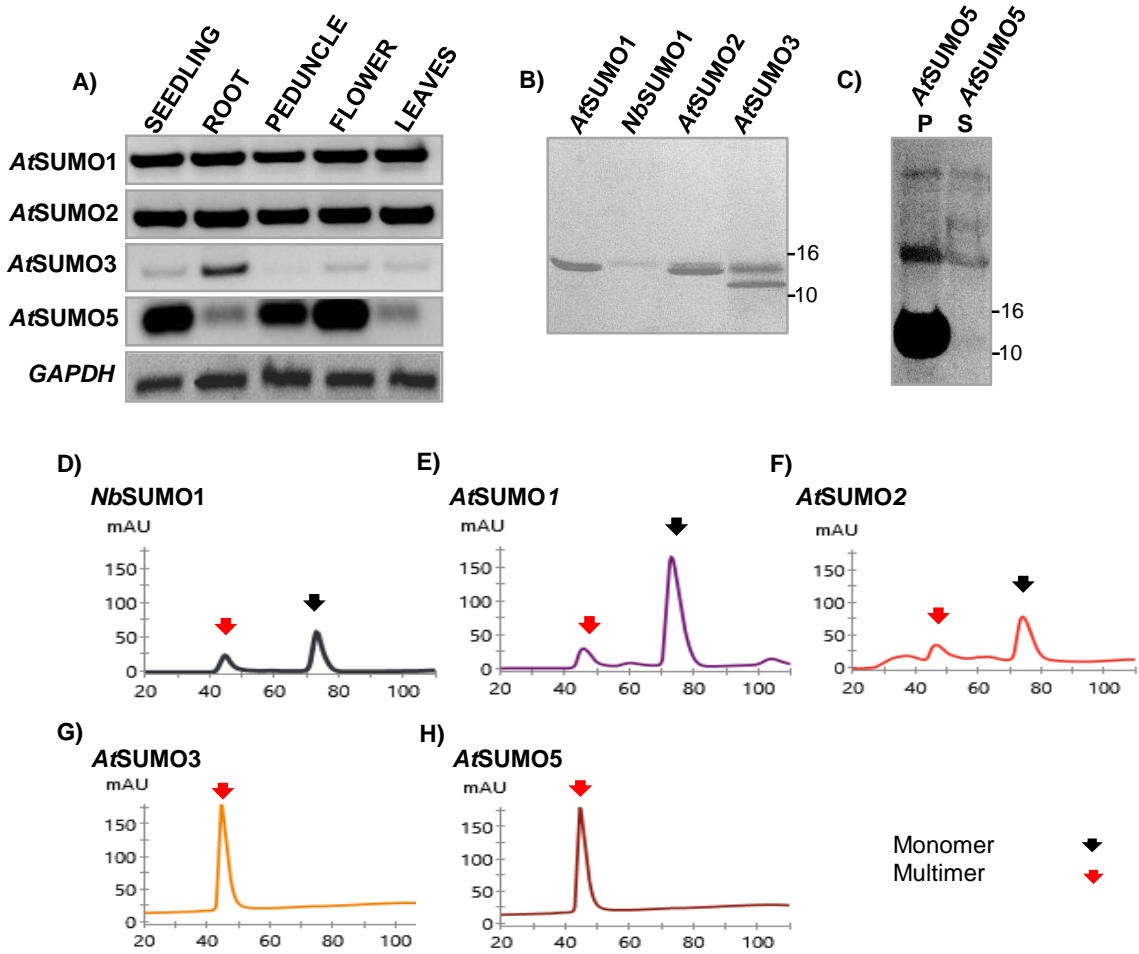


Fig.S9: Expression analysis and purification of plant SUMO proteins.

A)

pSIM1 : KKGMEF**IVDV**KLMQEDKI
pmSIM1 : WKKGMEF**AADA**KLMQEDKI
pSIM 2,3 : KQEDMIE**VDIL**MMQEAPVI
pmSIM2,3 : WKQEDMIEI**ADAA**MMQEAPVI
pSIM4 : ILMMQEAP**VIDI**NVSDEYEV
pmSIM4 : WILMMQEAP**AADA**NVSDEYEV

B)

Residue No.	14	18	90	95	101	105	118
SyYVCV β C1	M- F	I	VDV	K-	I	EI	VDI L- PVI DI N- V
mSIM 1	M- F	AAAA	K	-	I	EI	VDI L- PVI DI N- V
mSIM 2,3	M- F	I	VDV	K-	I	EAAAA	L- PVI DI N- V
mSIM 4	M- F	I	VDV	K-	I	EI	VDI L- PAAAA N- V
mSIM 2,3,4	M- F	I	VDV	K-	I	EAAAA	L- PAAAA N- V
mS SIM 2,3	M- F	I	VDV	K-	I	EI	KAI L- PVI DI N- V
mS SIM 4	M- F	I	VDV	K-	I	EI	VDI L- PVI SGN - V

Fig.S10: Sequence of SIM mutants.

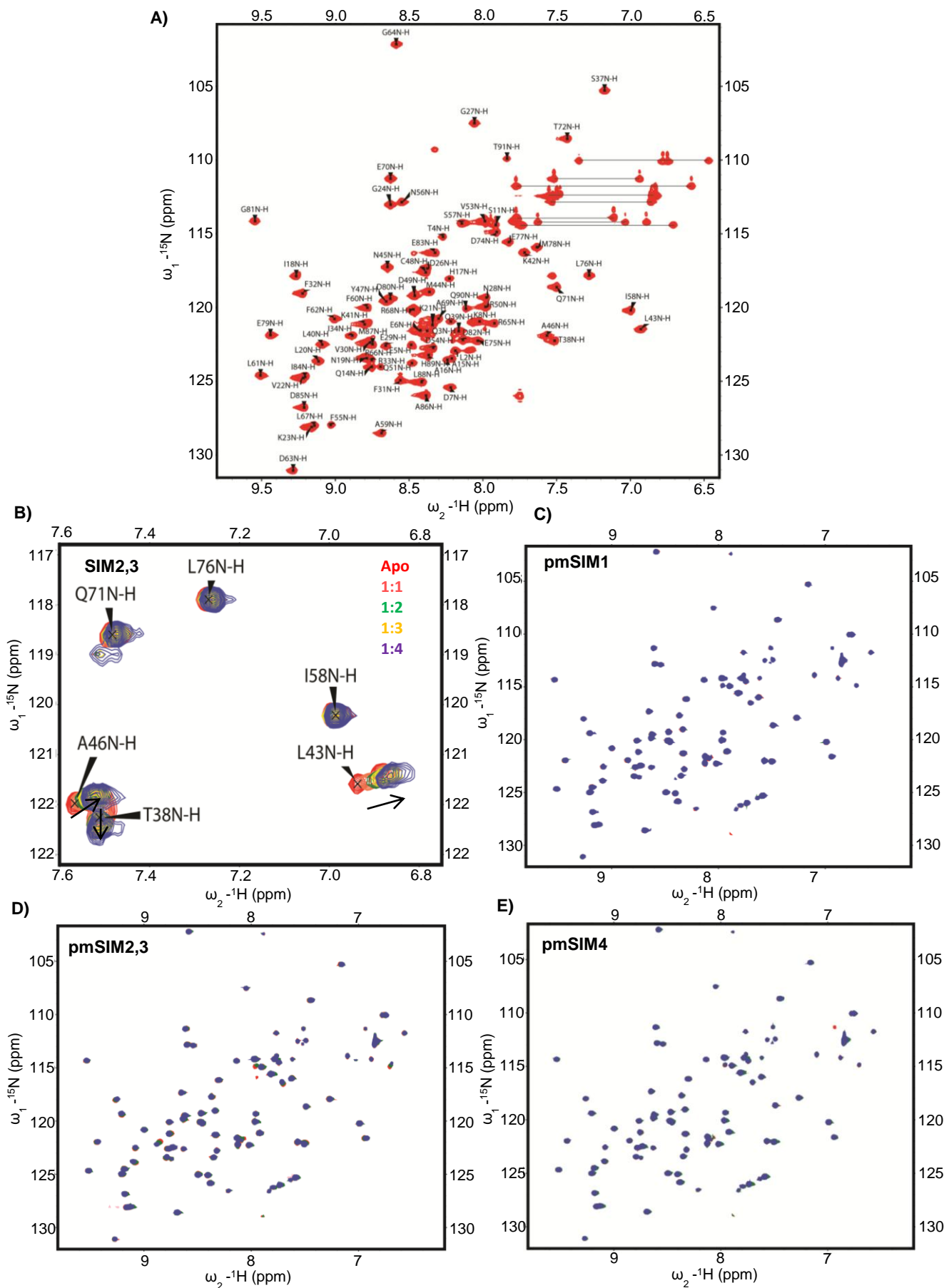


Fig.S11: SIM sites in βC1 C-terminal end interact with *Nb*SUMO1.

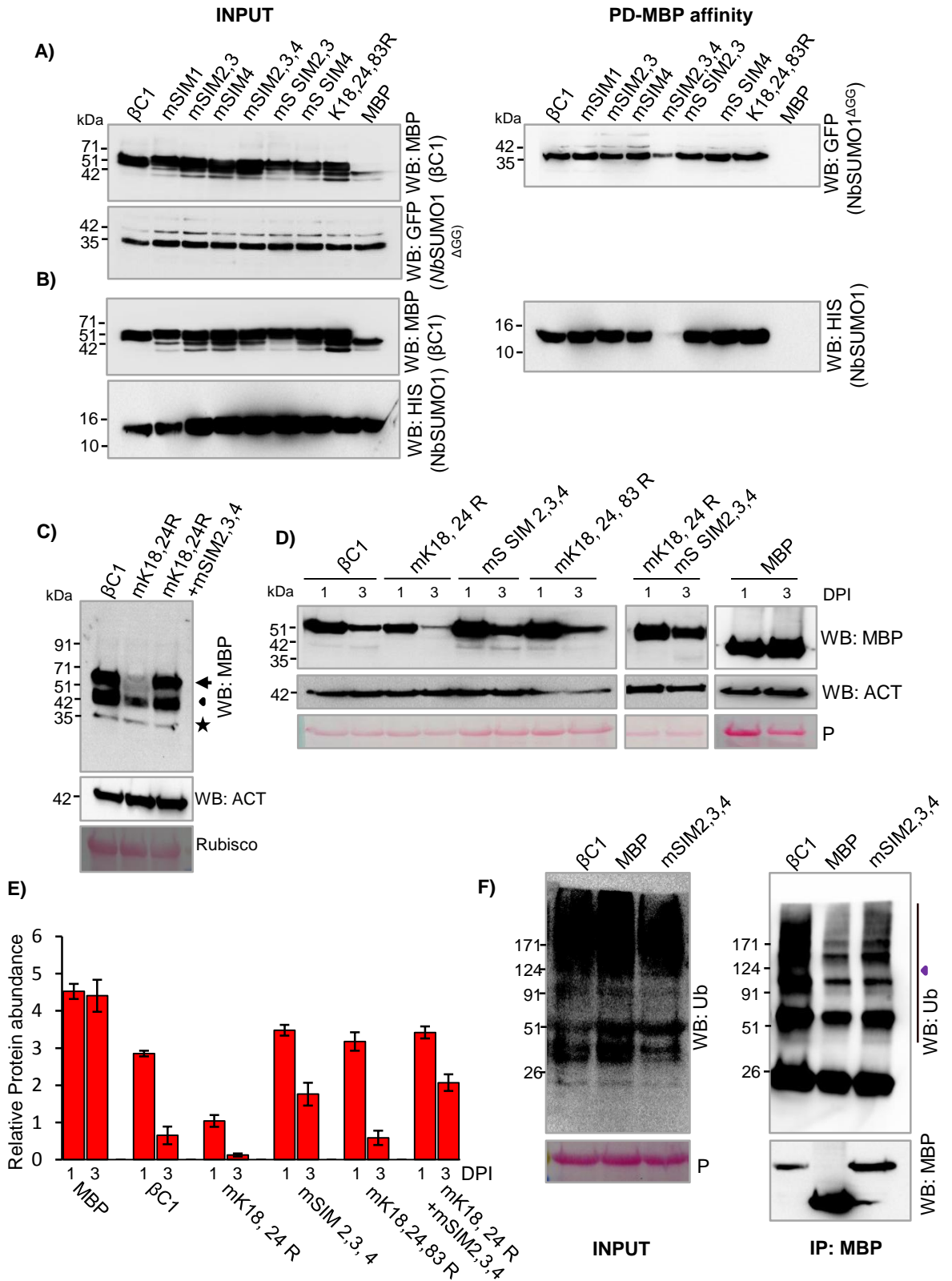


Fig.S12: SIM sites in SyYVVCV βC1 regulate its stability.

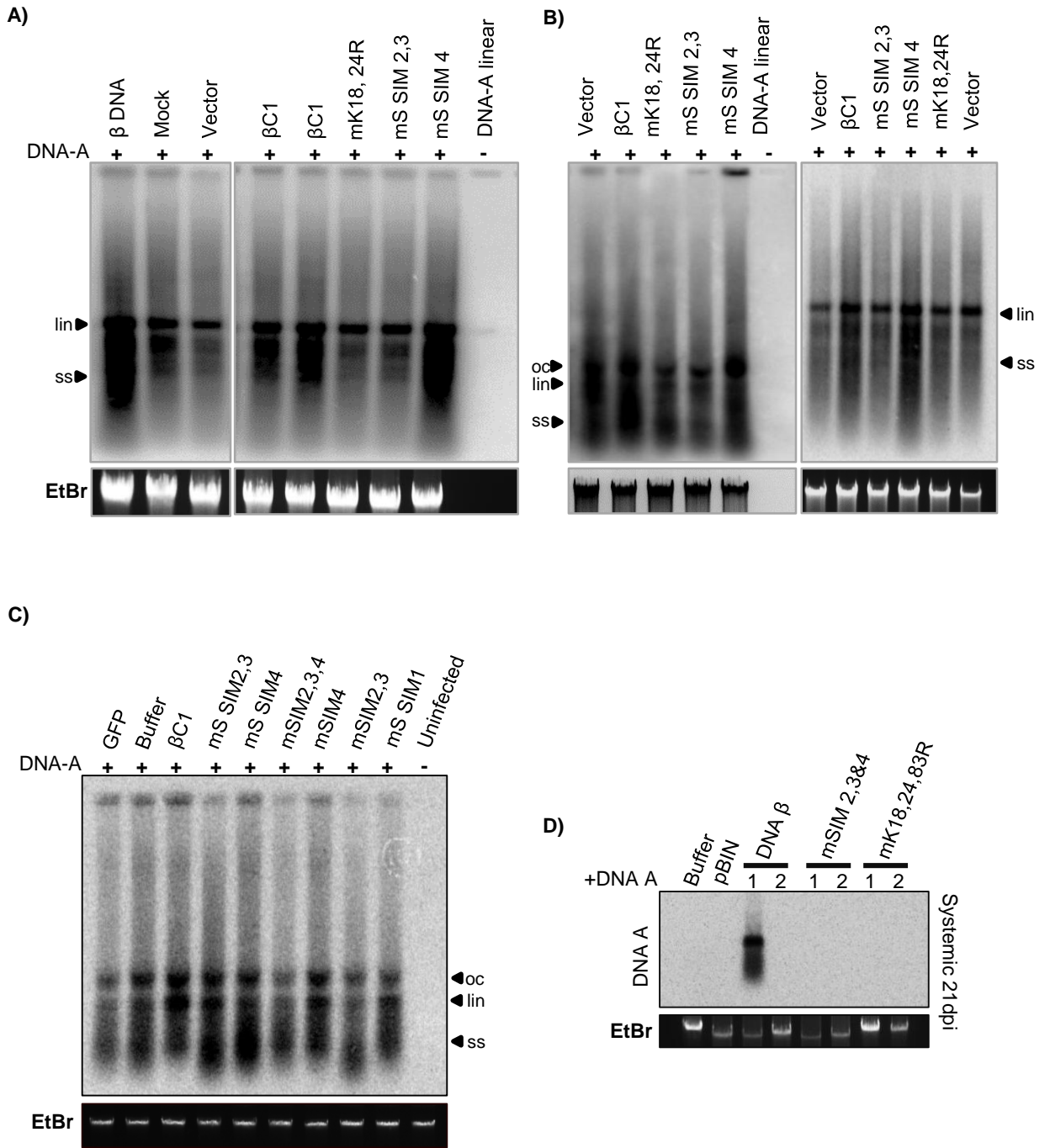


Fig.S13: SIM and SUMOylation motifs of β C1 are necessary for its pathogenicity determinant function.

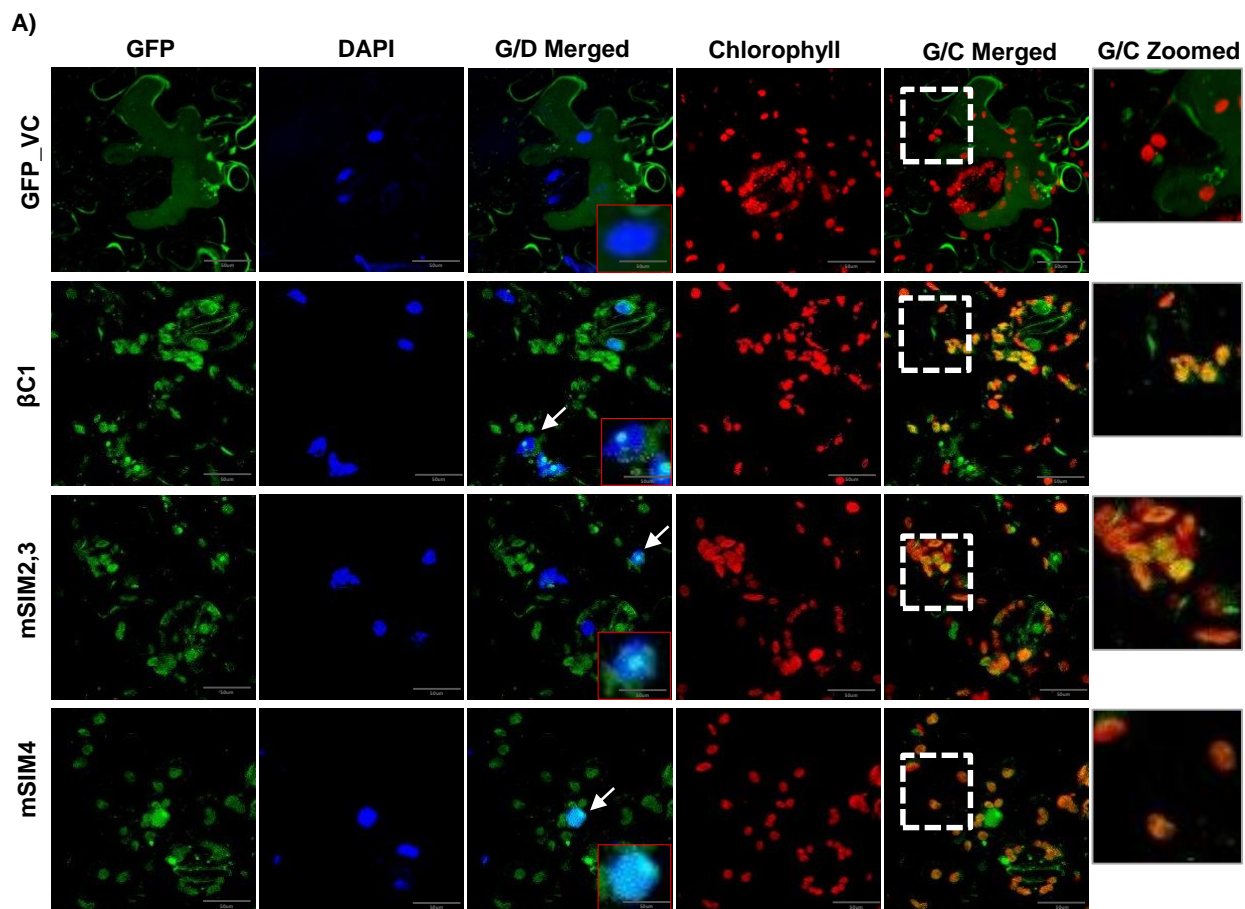


Fig.S14: SUMOylation motif in β C1 is important for its subcellular localization.

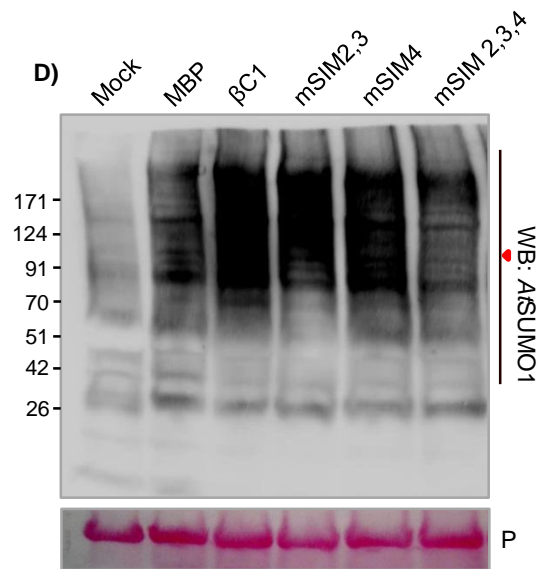
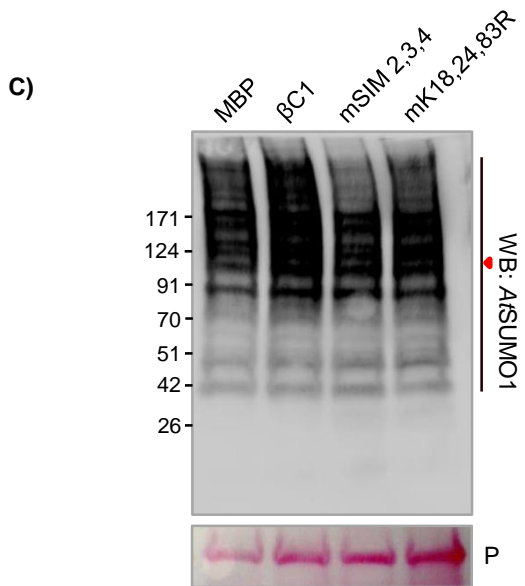
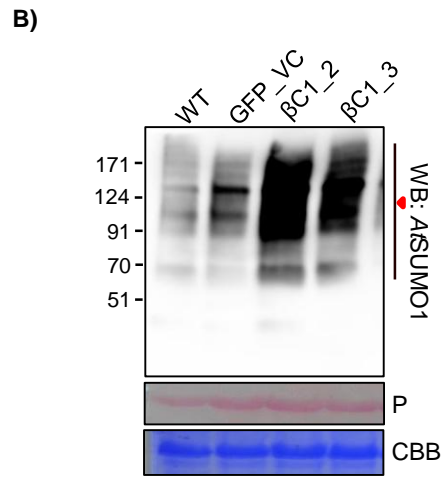
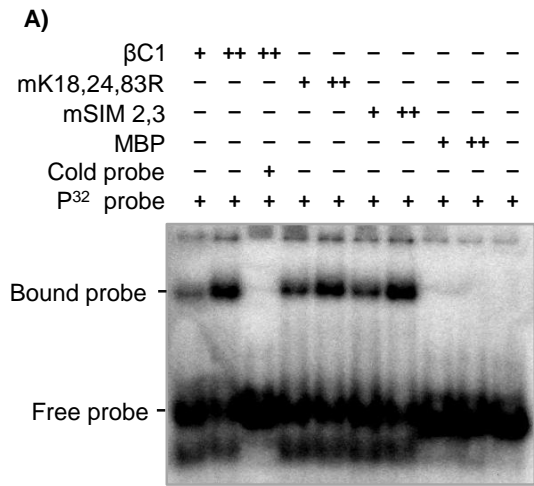


Fig.S15: SyYVCV β C1 induces global SUMOylation.

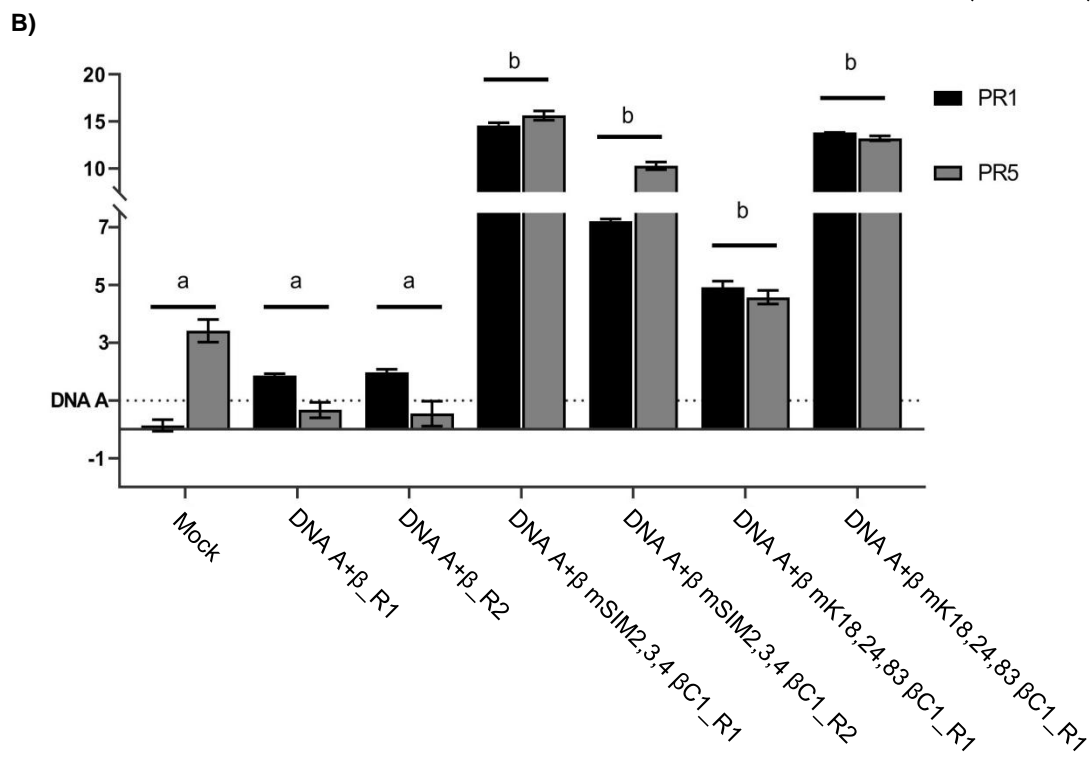
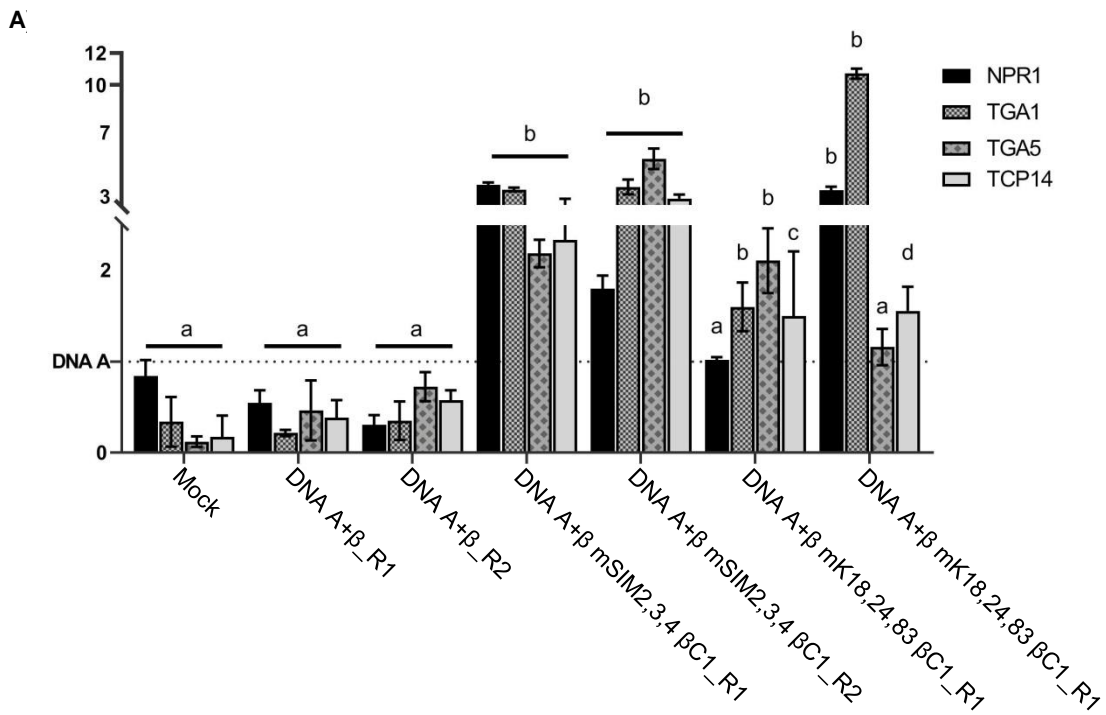
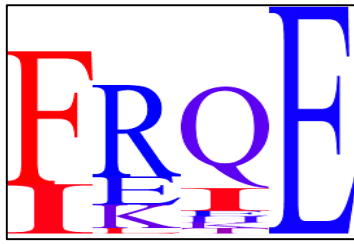


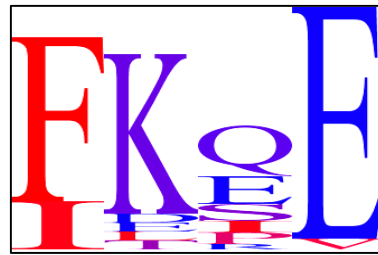
Fig.S16: SIM and SUMOylation motifs of β C1 is essential for host defense suppression.

A)



F	R	Q	E	Ageratum leaf curl virus
F	R	Q	E	Chilli leaf curl virus
F	R	Q	E	Chilli leaf curl virus pakistan
I	R	T	E	Cotton leaf curl Gezira virus
I	E	I	E	Cotton leaf curl Multan virus
I	E	I	E	Cotton leaf curl virus
I	E	K	E	Cotton leaf curl virus
F	R	Q	E	Cowpea severe leaf
I	E	I	E	Kenaf leaf curl virus pakistan
I	E	I	E	Ludwigia leaf curl distortion virus
F	R	H	E	Malvastrum leaf curl virus Guangdong
I	L	E	E	Okra leaf curl virus
F	R	Q	E	Okra leaf curl virus
F	K	Q	E	Papaya leaf curl virus NBRI
F	R	Q	E	Pepper leaf curl virus
F	K	Q	E	Potato apical leaf curl virus
F	R	Q	E	Sida leaf curl virus
F	K	Q	E	Tobacco leaf curl virus S2202
F	K	Q	E	Tomato leaf curl China virus
F	R	Q	E	Tomato leaf curl virus
F	R	Q	E	Tomato leaf curl virus
F	R	Q	E	Tomato leaf curl virus
F	R	Q	E	Tomato leaf curl virus Jeddah
F	R	Q	E	Tomato yellow leaf curl China virus SC65
F	R	Q	E	Tomato yellow leaf curl China virus Y10
F	R	Q	E	Tomato yellow leaf curl China virus_Y278

B)



F	K	Q	E	Synedrella yellow vein clearing virus
F	K	Q	E	Ageratum yellow vein China virus
F	K	Q	E	Ageratum yellow vein virus
F	K	Q	E	Alternanthera yellow vein virus
F	K	Q	E	Andrographis yellow vein virus
I	L	Q	E	Bhendi yellow vein mosaic virus
F	K	Q	E	Corchorus yellow vein mosaic virus
F	K	Q	E	Croton yellow vein mosaic virus
F	K	Q	E	Digera arvensis yellow vein virus
I	E	Q	E	Eclipta yellow vein virus
F	K	Q	E	Emilia yellow vein virus
F	K	Q	E	Eupatorium yellow vein virus
F	K	Q	E	Honeysuckle yellow vein mosaic virus
F	K	Q	E	Kadam yellow mosaic virus
F	K	Q	E	Leucas zeylanica yellow vein virus
F	K	Q	E	Lindernia anagallis yellow vein virus
F	K	Q	E	Lindernia anagallis yellow vein virus
I	D	Q	V	Malvastrum yellow vein virus
F	K	Q	E	Radish leaf curl virus
I	T	Q	E	Siegesbeckia yellow vein Guangxi virus
F	K	Q	E	Spinach yellow vein virus
F	K	Q	E	Vernonia yellow vein Fujian virus

Fig.S17: Conservation of SUMOylation sites among viruses producing similar symptoms..

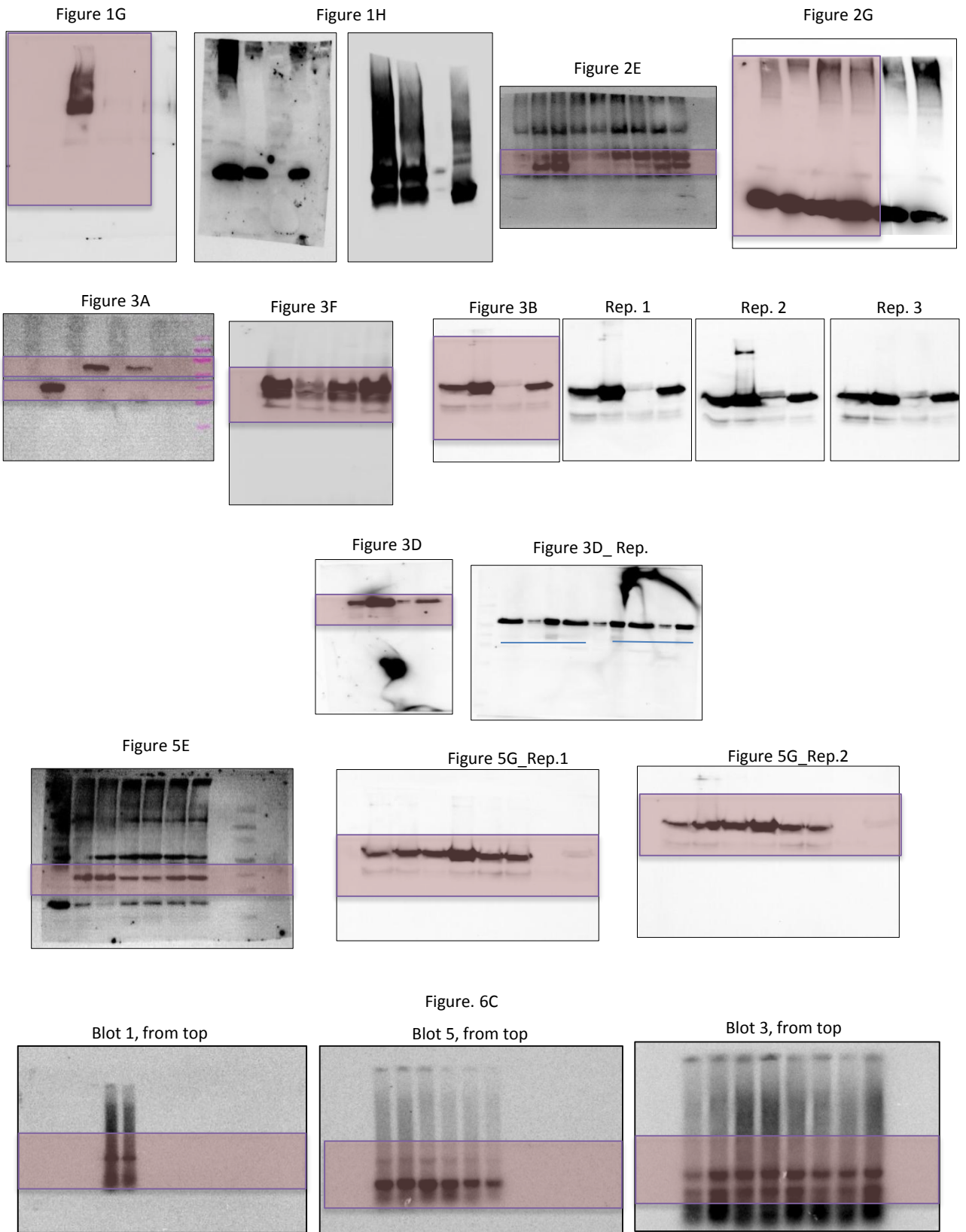
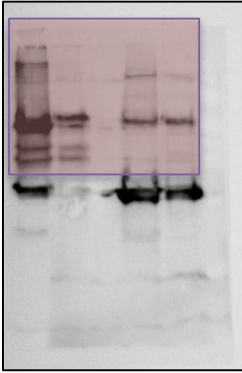


Fig.S18: Uncropped images of blots in main figures 1-7 and replicates of western blots.

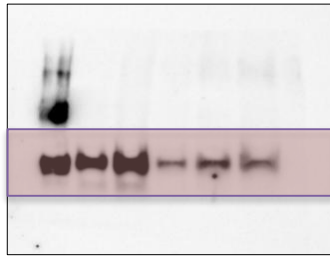
S. Figure 4A



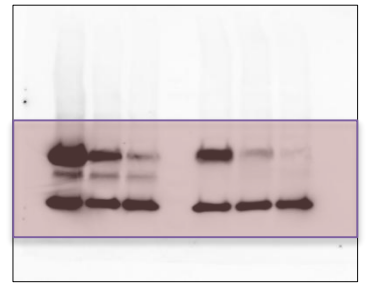
S. Figure 4B



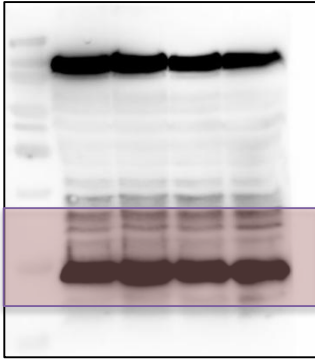
S. Figure 7A



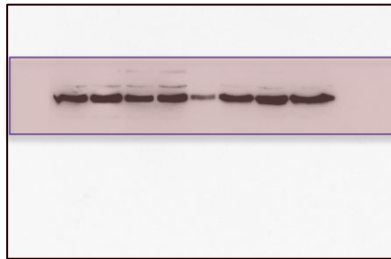
S. Figure 7B



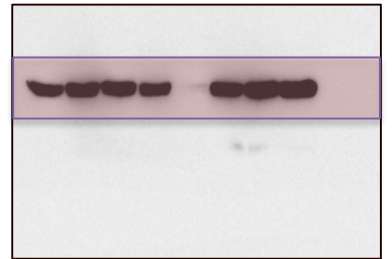
S. Figure 7D



S. Figure 12A



S. Figure 12B



S. Figure 12D

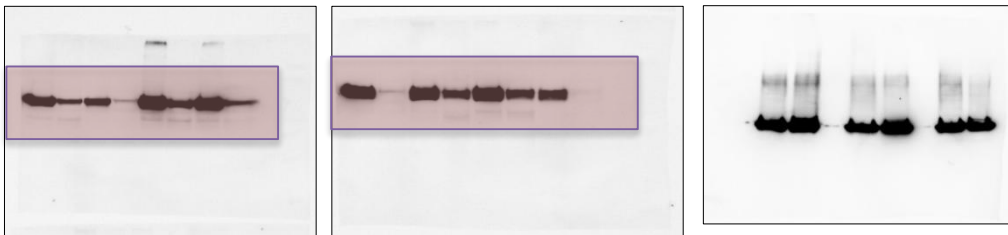


Fig.S19: Uncropped images of blots in Supplemental Figure 1-16.