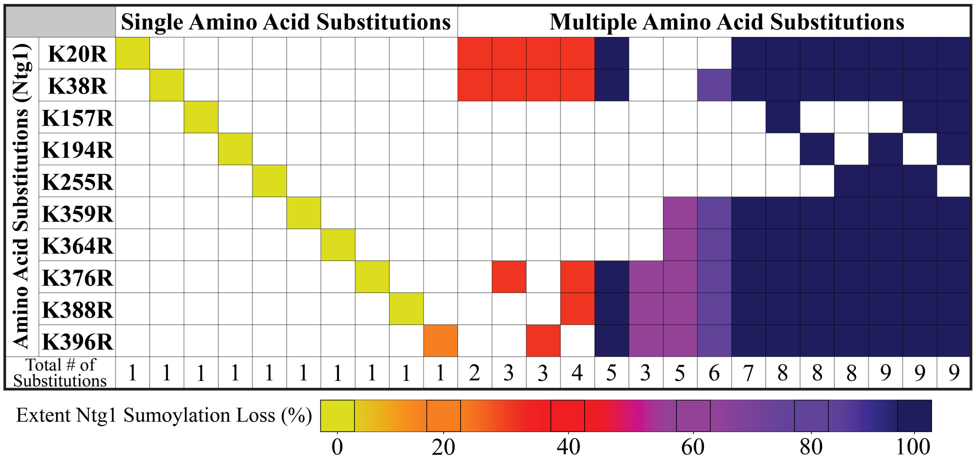
A

Human NTHL1							
Software	**Ψ-K-x-D/E**	**GPS-SUMO**		**PCI-SUMO**		**SUMOplot**	**Combined**
Non-consensus lysine	**Motif**	**Score**	**P-value**	**Confidence**	**Motif?**	**Score**	
56	VK$_{56}$RP	1.795	0.81	0.2403	No	0.82	0.51
60	RK$_{60}$AQ	0.751	0.882	0.1582	No		0.38
75	EK$_{75}$GE	3.818	0.085			0.5	0.75
83	LK$_{83}$VP	1.985	0.411	0.7957	No	0.8	0.52
105	NK$_{105}$KD	2.408	0.306				0.67
126	PK$_{126}$VR	2.07	0.628				0.33
257	TK$_{257}$SP	2.294	0.615				0.33

B

Saccharomyces cerevisiae Ntg1							
Software	**Ψ-K-x-D/E**	**SUMOsp 2.0**		**PCI-SUMO**		**SUMOplot**	**Combined**
Lysine	**Motif**	**Score**	**Confidence**	**Sumo?**	**Confidence**	**Score**	
Consensus							
20	VK$_{20}$TE	3.199	High	Yes	0.8014	0.93	**0.94**
38	IK$_{38}$QE	5.825	High	Yes	0.8771	0.94	**0.96**
376	VK$_{376}$HE	2.711	High	Yes	0.2574	0.93	**0.85**
388	VK$_{388}$LE	2.801	High	Yes	0.3171	0.93	**0.86**
396	VK$_{396}$VE	2.697	High			0.93	**0.97**
Non-consensus							
157	TK$_{157}$DE	2.779	Medium	No	0.2127		**0.53**
194	TK$_{194}$LD	2.75	Medium	Yes	0.6845		**0.75**
255	GK$_{255}$IE			Yes	0.538	0.67	**0.48**
359	VK$_{359}$YL			Yes	0.5946		**0.40**
364	GK$_{364}$RE			No	0.2233	0.67	**0.35**

C



Supplemental Figure 1. NTHL1 and Ntg1 sumoylation scores and Ntg1 variant creation scheme. A. The table shown displays the seven putative non-consensus sumoylation sites and their Motif within NTHL1. The sumoylation scores from GPS-SUMO, PCI-SUMO, and SUMOplot as well as an overall Combined score are shown. B. The table shown displays the five putative consensus sumoylation sites, five putative non-consensus sumoylation sites and their Motif within Ntg1. The sumoylation scores from SUMOsp 2.0, PCI-SUMO, and SUMOplot as well as an overall Combined score are shown. C. A schematic displays the 25 Ntg1 variants where candidate SUMO modification sites were altered from K->R. The specific amino acids altered were selected based on the SUMO scores from (B). Each amino acid is indicated on the left. The filled boxes indicate which specific amino acid(s) was/were altered in each variant. The color of the box provides a quantitative measure of the % sumoylation loss relative to wildtype Ntg1 detected for each of these variants as described in Materials and Methods. Note that all sumoylation was lost when K20, K38, K376, K388, and K396 were simultaneously changed to R. This Ntg1 variant was thus designated ntg1Δ sumo and used for subsequent analyses.

Figure S1
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