SUPPLEMENTARY MATERIALS

SUMO modification of NZFP mediates transcriptional repression through TBP binding

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Table S1. Primers used for this experiments

Name		Sequence (5′→3′)
(for SUMO modification site mutant construction)		
K123R-F	forward	GAAGGGATAAGAGAGGAGCC
K123R-R	reverse	GGCTCCTCTCTATCCCTTC
K187R-F	forward	CAATGGGATTAGAGAGGAAG
K187R-R	reverse	CTTTCCTCTCTAATCCCATTG
K233R-F	forward	GAATTATATAAGAGAGGAGGTT
K233R-R	reverse	AACCTCCTCTCTTATATAATTC
(for expression assay constructs)		
Gal4-F	forward	GGATCCAAGCTTGCCACCATGAAGCTACTGTCTTCTAT
Gal4-R	reverse	AAGCTTGGATCCCGGCGGGAATTCCGGCGATACAG
VP16-F	forward	GGTACCGGATCCACCATGAAAGCGCCCCCCCGACCGATGT
VP16-R(*XhoI*)	reverse	GGTACCCTCGAGCCCACCGTACTCGTCAAT
VP16-R(*BamHI*)	reverse	GGTACCGGATCCCCCACCGTACTCGTCAAT
(for immunoprecipitation assay constructs)		
HA-TBP-F	forward	GGATAGGTACCATGATGTACCCATACGATGTTCCAGATTACGCTATGGATC
HA-TBP-R	reverse	GACTCTAGATCTTTACGTCGTCTTCC

Supplementary Fig. S1. NZFP interacts with the ubiquitin-like E2 conjugating enzyme, Ubc9.

(A) Identification of a clone encoding a protein that interacts with NZFP in yeast. Clone #3 which had been screened on a His deficient medium was streaked on minimal medium and β-galactosidase activity was detected through X-gal staining. Yeast cells expressing SNF1/SNF4 were used as a positive control and cells containing pGAD10 and pBAH-NZFP were used as a negative control. (B) *In vitro* interaction of *Xenopus* Ubc9 with NZFP. The *in vitro* pull-down assay was performed by using a GST-Ubc9 fusion protein and in vitro translated [^{35}S]-labeled NZFP. Input designates the in vitro translated NZFP used for assay. Bound NZFP is indicated by an arrow.

Supplementary Fig. S2. SENP1/2 mediates desumoylation of NZFP.

(A) Samples from 293T cells transfected with the expression vectors for NZFP-Flag, EGFP-SUMO1 and Myc-SENP, separately or together as described (+/-) were immunoprecipitated with anti-Flag antibody and the bound proteins were analyzed by western blotting using anti-GFP antibody as the primary antibody. SENP (desumoylating proteins) expressing vectors were added in lanes 4 to 6. (1, 2, and 2m designate SENP1, SENP2, and SENP2 mutant, respectively). Samples from above lysates were also tested for the expression of NZFP, SENP, and β-Actin by western blotting using anti-Flag, anti-Myc, and anti- β-Actin antibodies, respectively. (B) Experiments were performed as the same as given in (A) using the expression vectors for NZFP-Flag, Myc-SENPs, Flag-Ubc9, and His-SUMO1.

Figure S1

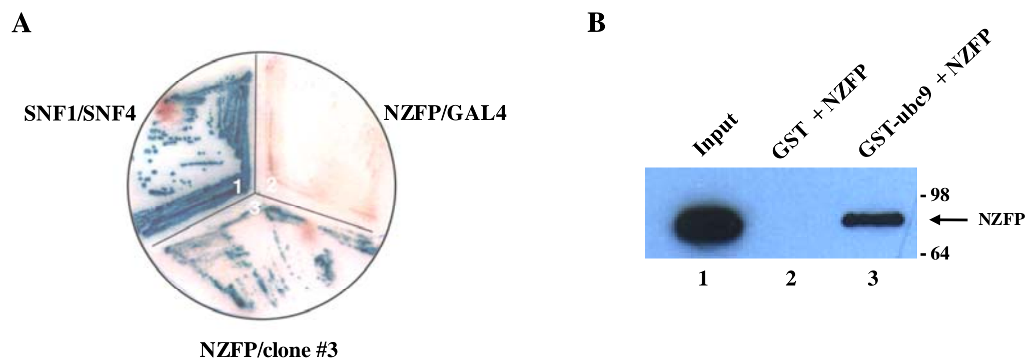
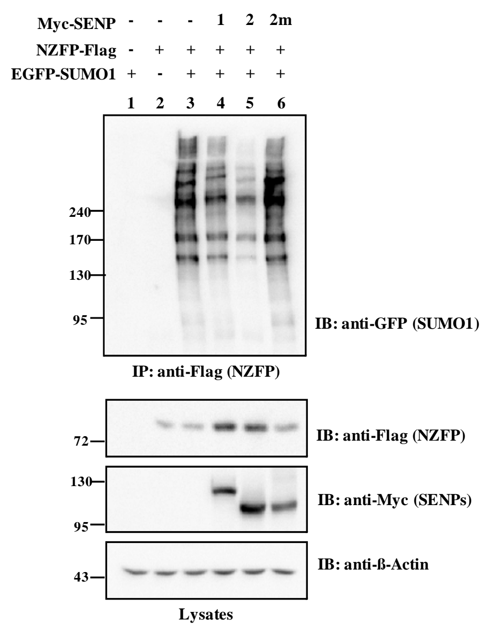
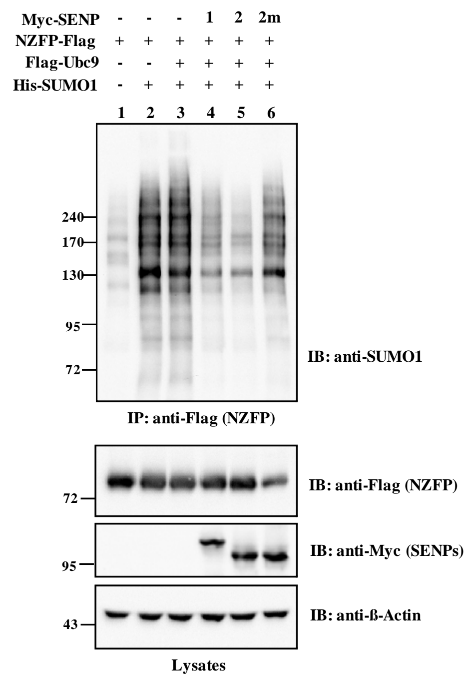


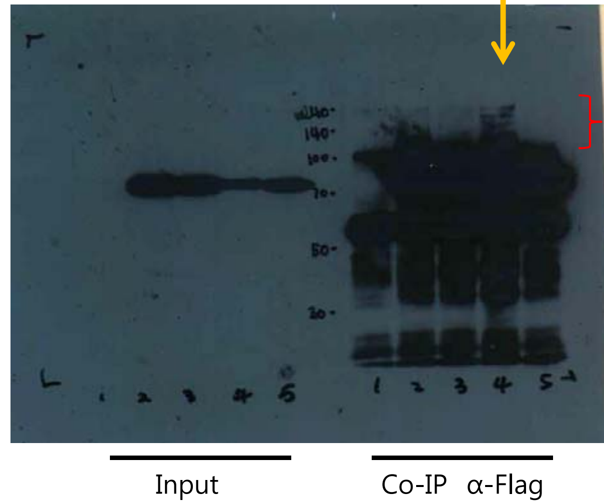
Figure S2





Review only

western : α-Flag



Exposure time : 1 minute

1 : HA-TBP single transfection
2 : Flag-NZFP single transfection
3 : Flag-3P single transfection
4 : Flag-NZFP + HA-TBP Co-T
5 : Flag-3P + HA-TBP Co-T