

Supplemental Figure Legends

Figure S1. The Gal4 DNA-binding domain was fused to the transactivation domain of MTF-1 (Gal4-TAD) then co-expressed with SUMO-1 in the cells. Gal4-TAD protein was immunoprecipitated with anti-His₆ antibodies and the occurrence of sumoylation was analyzed with anti-SUMO-1 antibody. K627R and E629A refer to MTF-1 mutants with Lys627 and Glu629 to arginine (K627R) and alanine (E629A) substitutions, respectively.

Figure S2. HEK293 cells were co-transfected with vector (control), MTF-1 or K627R and SUMO-1 plasmids. Cells were treated with and without 100 μ M Zn for 6 h, then MTIIA mRNA in the cells was quantified. The MTIIA mRNA level of the untreated control was designated as 1. Each value represents a mean \pm standard deviation of three independent experiments. Asterisks (*) denote significant differences ($p < 0.05$) from metal-treated cells transfected with MTF-1.

Figure S3. (A) DBD_{Gal4}, Gal4-TAD, Gal4-K627R or Gal4-E629A mutant was co-transfected with Tk-MH100 \times 4-Luc reporter plasmids into CHO K1 cells. The cells were treated with or without 100 μ M Zn for 6 h, and the luciferase activity was measured. Asterisks (*) denote significant differences ($p < 0.05$) from metal-treated cells transfected with Gal4-TAD gene. (B) DBD_{Gal4}, Gal4-TAD, Gal4-K627R or Gal4-E629A was expressed in CHO K1 cells and the expression level of the proteins was shown. Tubulin was included as loading control. Molecular weight markers are shown because the size of the expressed protein differs markedly.

Figure S4. (A) Gal4-TAD or Gal4-TAD-SUMO gene was co-transfected with Tk-MH100 \times 4-Luc reporter plasmids into CHO K1 cells. Relative luciferase activity was determined after adding 100 μ M Zn to the transfected cells for 6 h. Asterisks (*) denote significant differences ($p < 0.05$) from metal-treated cells transfected with Gal4-TAD gene. (B) The expression level of the proteins was shown. Tubulin was included as loading control. Molecular weight markers are shown because the size of the expressed protein differs markedly.

Figure S5. MTF-1 or K627R was expressed in CHO K1 cells (corresponding to Fig. 2A) and the expression level of the proteins was shown. Tubulin was included as loading control.

Figure S6. MTF-1, MTF-1-SUMO or MTF-1-GFP was expressed in CHO K1 cells (corresponding to Fig. 2C) and the expression level of the proteins was shown. Tubulin was included as loading control.

Figure S7. MTF-1 or MTF-1-SUMO was expressed in CHO K1 cells (corresponding to Fig. 2D)

and the expression level of the proteins was shown. Tubulin was included as loading control.

Figure S8. MTF-1, SIM-2A, SIM-3A or SIM-4A was expressed in CHO K1 cells (corresponding to Fig. 5D) and the expression level of the proteins was shown. Tubulin was included as loading control.

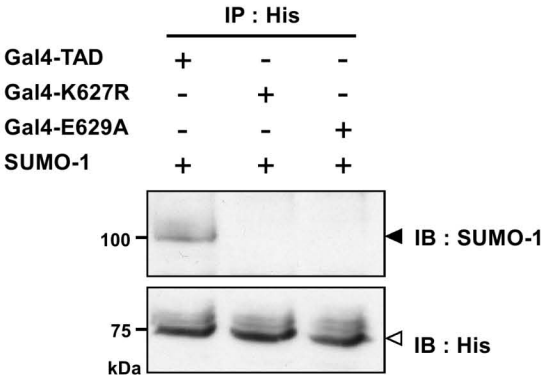
Figure S9. MTF-1 or SIM-3A was expressed in HEK293 cells (corresponding to Fig. 5E) and the expression level of the proteins was shown. Tubulin was included as loading control.

Figure S10. HEK293 cells were co-transfected with vector (control), MTF-1 or SIM-3A and SUMO-1 plasmids. Cells were treated with and without 100 μ M Zn for 6 h, and MTIIA mRNA in the cells was quantified. The MTIIA mRNA level of the untreated control was designated as 1. Each value represents a mean \pm standard deviation of three independent experiments. Asterisks (*) denote significant differences ($p < 0.05$) from metal-treated cells transfected with MTF-1.

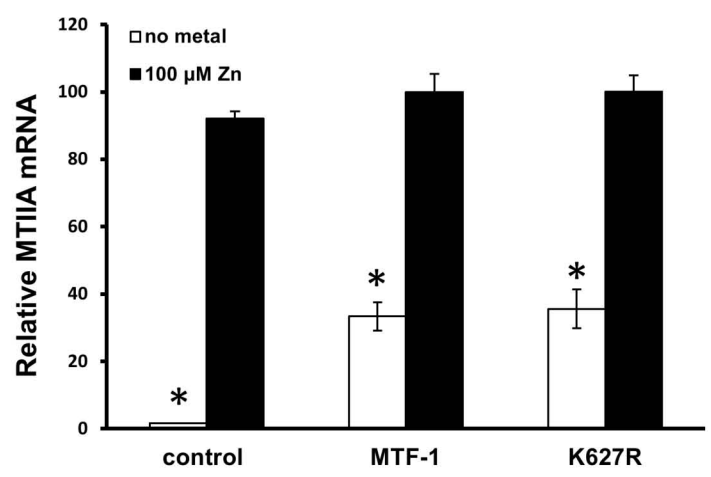
Figure S11. GFP-TAD or GFP-TAD-SKM was expressed in cells with or without 100 μ M Zn treatment for 6 h. Cell extracts were prepared and separated on an analytical Superose 6 HR 10/30 column. Equal volume of each fraction was removed for fluorescence intensity determination (A), or concentrated for immunoblot analysis with anti-GFP antibodies (B).

Supplemental Figure S1

S1

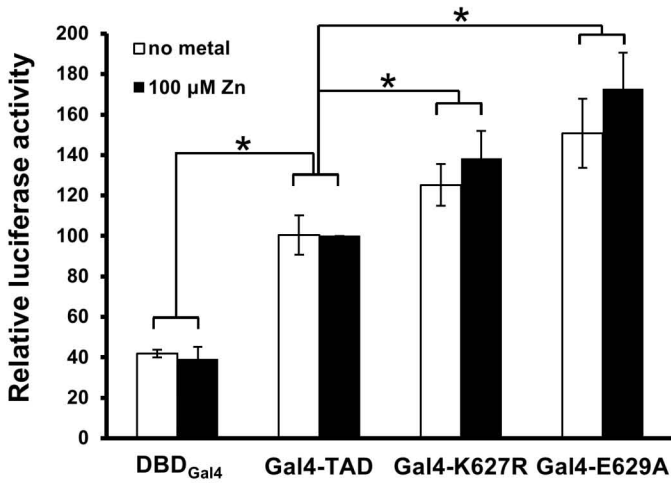


S2

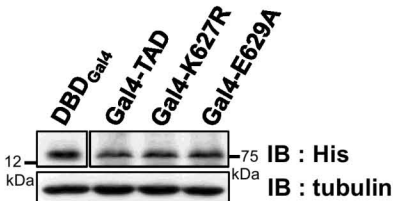


Supplemental Figure S3

S3A

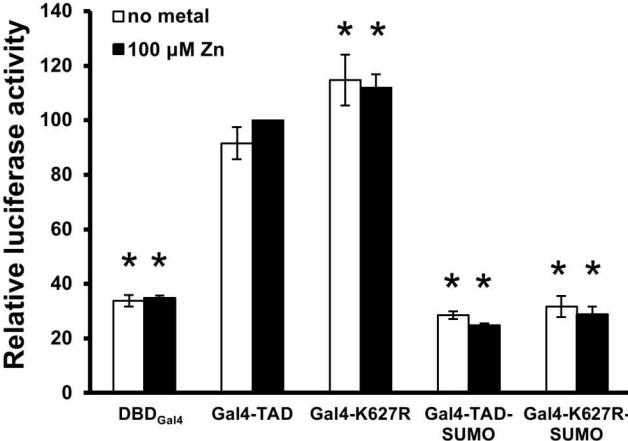


S3B

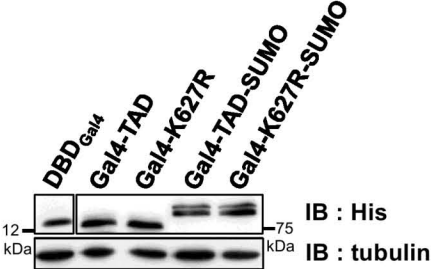


Supplemental Figure S4

S4A

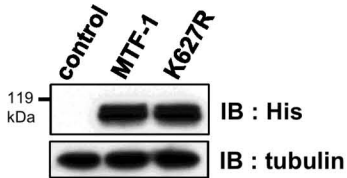


S4B

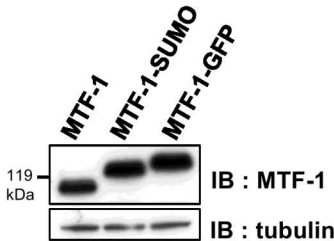


Supplemental Figure S5 to S7

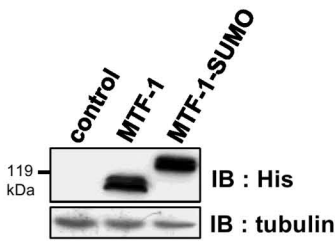
S5



S6

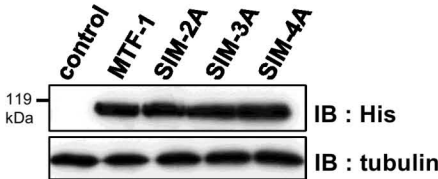


S7

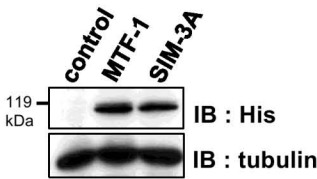


Supplemental Figure S8 and S9

S8

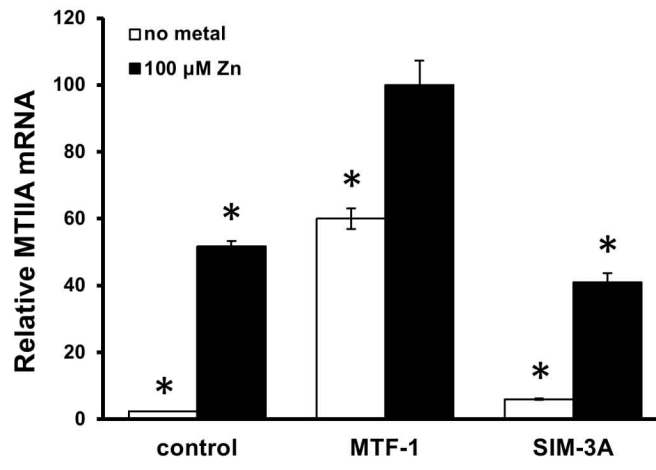


S9



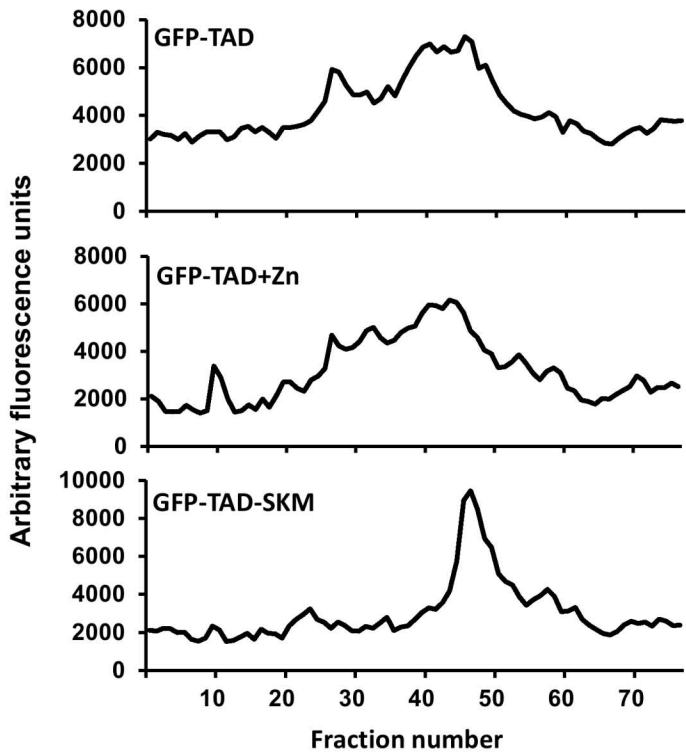
Supplemental Figure S10

S10



Supplemental Figure S11

S11A



S11B

