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Supplementary material

TRANSCRIPTIONAL REGULATION OF MOUSE MESENCEPHALIC ASTROCYTE-DERIVED NEUROTROPHIC FACTOR IN Neuro2a CELLS

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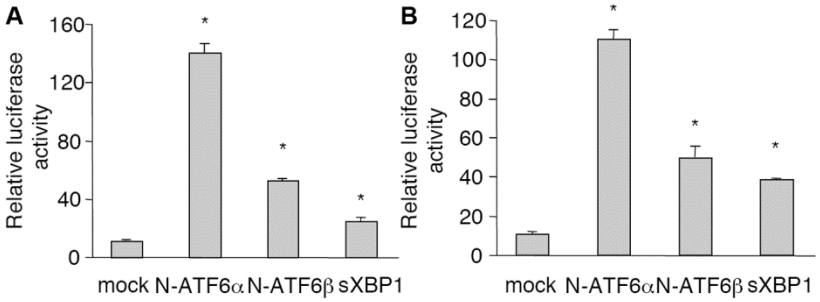
SUPPLEMENTARY MATERIALS AND METHODS

Plasmid construction

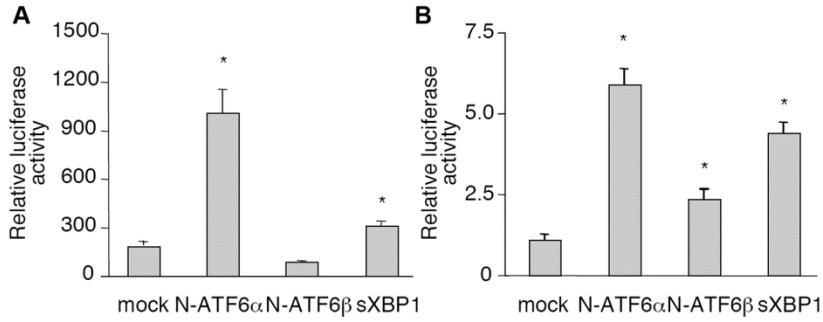
To prepare reporter constructs of human GRP78 and mouse Herp promoters, genomic DNA was extracted from HEK293 and Neuro2a cells, and the human GRP78 (-142/+109) and mouse Herp (-192/+69) promoters were amplified using PCR and then cloned into the pGL3b vector. The promoter regions of human GRP78 and mouse Herp were respectively defined using the NCBI Reference Sequences NM_005347.4 and NM_022331. The 1× UPRE pGL3-promoter contruct was prepared by inserting the 1× UPRE-containing nucleotide sequence gctagcCTCGAGACAGGTGCTGACGTGGCATTCGCTCGagatct into the pGL3-promoter vector at the *Nhe* I and *Bgl* II sites [S1]. The underlined nucleotide sequence is the ATF6/XBP1-recognizing sequence. To prepare untagged ATF6α, ATF6β and sXBP1, the active forms of the N-terminal fragment of the transcription factors N-ATF6α (1-366 aa), ATF6β (1-392 aa) and sXBP1 were amplified via PCR and then cloned into the pcDNA3.1 vector.

Semi-quantitative RT-PCR

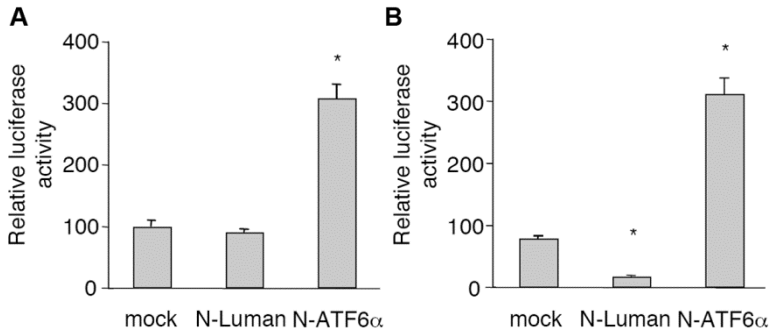
To estimate the expression level of MANF mRMA using semi-quantitative RT-PCR, cDNAs amplified via RT-PCR were separated by electrophoresis on 2.0% agarose gels and visualized using ethidium bromide. The relative expression level of MANF mRNA was normalized by the level of GAPDH in the untreated control cells using NIH imaging.



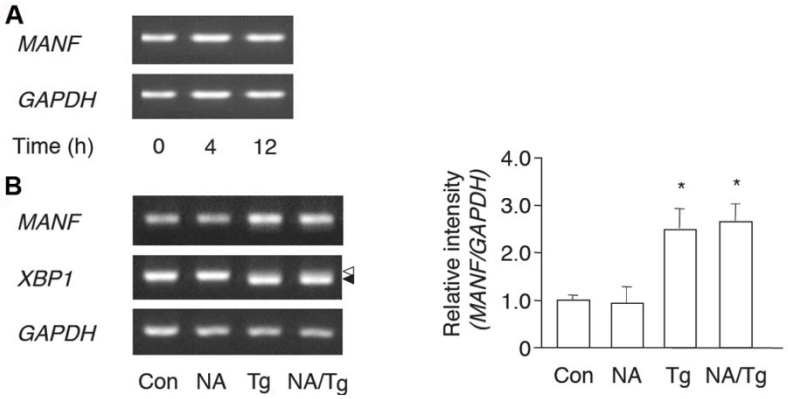
Suppl. Fig. 1. Overexpression of untagged ATF6α, ATF6β and sXBP1 upregulated the mouse MANF promoter activity in Neuro2a cells. Untagged ATF6α, ATF6β and sXBP1 constructs at low (A, 0.0025 μg/well) and high (B, 0.0125 μg/well) concentrations or an empty vector together with the mouse MANF promoter construct (-129/+34) and pGL4.70 were transfected into Neuro2a cells. 36 h after transfection, the cells were lysed, and the activity of each luciferase was measured as described in the Materials and Methods section. Values represent the means ± SD from 3 independent cultures and are expressed relative to the luciferase activity of the cells co-transfected with the pGL3-Basic vector and the indicated constructs of transcription factors. Data were analyzed using one-way ANOVA followed by Scheffé's method to evaluate the effects of co-expression of the indicated transcription factors on the MANF promoter activity. Values marked with an asterisk are significantly different from the MANF promoter activity (-129/+34) without transfection of each transcription factor ($p < 0.05$).



Suppl. Fig. 2. Overexpression of Flag-tagged N-ATF6α, N-ATF6β and sXBP1 upregulated the human GRP78 promoter and UPRE-dependent transcription activity in Neuro2a cells. Flag-tagged ATF6α, ATF6β or sXBP1 construct or an empty vector together with human GRP78 promoter (A) or 1×UPRE pGL3-promoter (B) and pGL4.70 were transfected into Neuro2a cells. 36 h after transfection, the cells were lysed, and the activity of each luciferase was measured as described in the Materials and Methods section. Values represent the means ± SD from 6 independent cultures and are expressed relative to the luciferase activity of the cells co-transfected with the pGL3-Basic vector and the indicated constructs of transcription factors. Data were analyzed using one-way ANOVA followed by Scheffé's method to evaluate the effects of co-expression of the indicated transcription factors on the activity of each luciferase. Values marked with an asterisk are significantly different from those of the human GRP78 promoter (A) and 1×UPRE pGL3-promoter (B) activities without transfection of each transcription factor ($p < 0.05$).



Suppl. Fig. 3. Effects of overexpression of Flag-tagged N-Luman and N-ATF6α on the mouse Herp promoter activity in Neuro2a cells. Flag-tagged N-Luman and N-ATF6α constructs at low (A, 0.0005 μg/well) and high (B, 0.005 μg/well) concentrations or an empty vector together with the mouse Herp promoter and pGL4.70 were transfected into Neuro2a cells. 36 h after transfection, the cells were lysed, and the activity of each luciferase was measured as described in the Materials and Methods section. Values represent the means ± SD from 6-7 independent cultures and are expressed relative to the luciferase activity of the cells co-transfected with the pGL3-Basic vector and the indicated constructs of transcription factors. Data were analyzed using one-way ANOVA followed by Scheffé's method to evaluate the effects of co-expression of the indicated transcription factors on the activity of each luciferase. Values marked with an asterisk are significantly different from the mouse Herp promoter activity without transfection of each transcription factor ($p < 0.05$).



Suppl. Fig. 4. Nicotinamide failed to induce MANF mRNA in Neuro2a cells. A – Neuro2a cells were treated with nicotinamide (NA, 10 mM) for the indicated time. B – Neuro2a cells were treated with NA (10 mM) in the presence or absence of Tg (0.1 μM) for 12 h. Expression of each mRNA was evaluated via RT-PCR as described in the Suppl. Materials and Methods section. Open and closed arrow-heads respectively indicate uXBP1 and sXBP1. The relative expression level of MANF mRNA was normalized by the level of GAPDH in the untreated control cells using NIH imaging. Values represent the means ± SD from 4 independent cultures. Data were analyzed using one-way ANOVA followed by Scheffé's method to evaluate the effects of NA and Tg treatment on the expression of MANF mRNA. Values marked with an asterisk are significantly different from those for the untreated control cells ($p < 0.05$).

SUPPLEMENTARY REFERENCE

1. Wang, Y., Shen, J., Arenzana, N., Tirasophon, W., Kaufman, R.J. and Prywes, R. Activation of ATF6 and an ATF6 DNA-binding site by the endoplasmic reticulum stress response. **J. Biol. Chem.** 275 (2000) 27013-27020.