Supplemental Data for

AIRAP: a new human heat shock gene regulated by Heat Shock Factor 1

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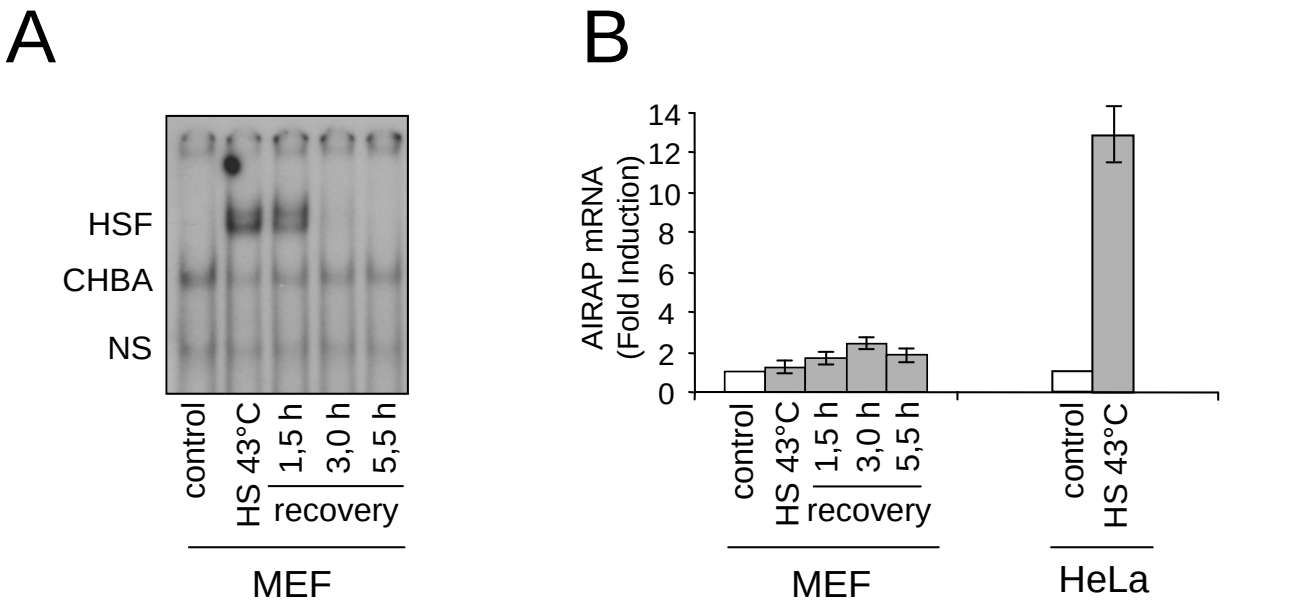
Supplemental Figure Legends
Supplemental Figures S1 and S2

Supplemental Figure legends

Figure S1. Analysis of AIRAP gene expression in heat shocked murine cells. (A) Murine embryonic fibroblasts (MEF) were subjected to heat shock (HS) at 43°C for 40 min. At the end of heat treatment or at different times during the recovery period at 37°C, whole-cell extracts were analyzed for HSF1 DNA-binding activity by EMSA. Positions of the HSF DNA-binding complex (**HSF**), constitutive HSE-binding activity (**CHBA**) and non specific protein-DNA interaction (**NS**) are shown. **(B)** In parallel samples, total RNA was analyzed for murine AIRAP expression by real-time PCR. Total RNA extracted from HeLa-pS cells (HeLa) subjected to heat shock at 43°C for 40 min, and then allowed to recover for 1.5 h at 37°C, was analyzed in parallel for human AIRAP by real-time PCR. Relative quantities of AIRAP RNAs were normalized to β-actin. All reactions were made in duplicates using samples derived from at least three biological repeats. Error bars indicate ± S.D.. Fold induction was calculated by comparing the induction of AIRAP in the treated samples to the relative control, which was arbitrarily set to 1. **(C)** Sequence alignment of the putative HSF1-binding element (mHSE) identified in the mouse promoter region of AIRAP gene is compared to the human HSE2 (hHSE2), described in Fig. 4. Boxes indicate the minimal binding sites detected by TFSEARCH. The nucleotide cores of the adjacent nGAAn monomeric units are indicated in bold.

Figure S2. Accumulation of polyubiquitinated proteins in heat-shocked HeLa-pS and HeLa-HSF1i cells. (A) Kinetics analysis of protein ubiquitination. HeLa-pS and HeLa-HSF1i cells were subjected to heat shock (HS) at 43°C for 40 min. At the end of heat treatment, or at 1.5, 3, 5.5 and 8 h during the recovery period at 37°C, whole-cell extracts were analyzed by Western blot for total protein ubiquitination (top panel) and β-actin levels (bottom panel). **(B**) Effect of AIRAP overexpression on protein ubiquitination in heat shocked HeLa-HSF1i cells. HeLa-HSF1i cells were transiently transfected with Flag-AIRAP-pcDNA3 expression vector, or with Flag-pcDNA3 vector and, after 24 h, were subjected to heat shock (HS) at 43°C for 40 min. At the end of heat treatment, or at 3 and 5.5 h during the recovery period at 37°C, whole-cell extracts were analyzed by Western blot for protein ubiquitination (top panel), Flag-AIRAP and β-actin levels (bottom panels).

Figure S1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HSF
CHBA
NS
control
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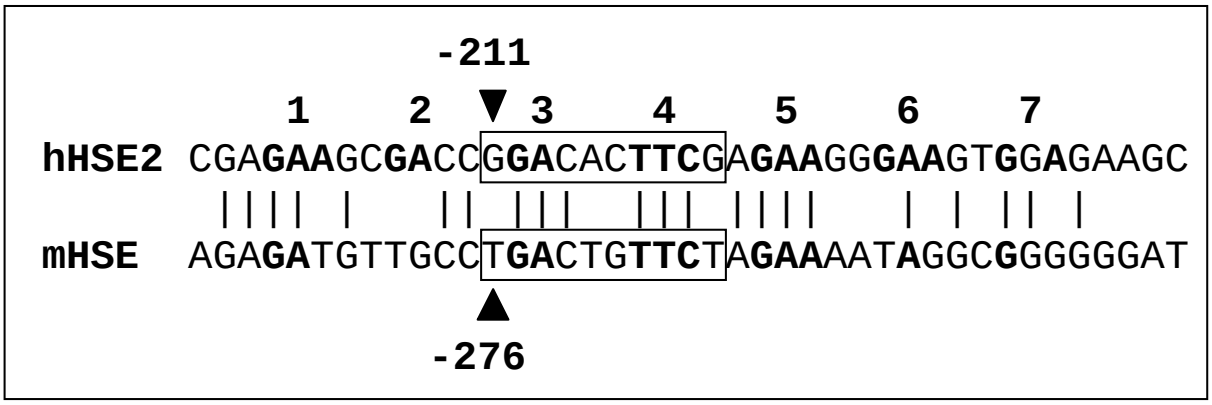
1,5 h
3,0 h
5,5 h
recovery
MEF
B
AIRAP mRNA
(Fold Induction)
14
12
10
8
6
4
2
0
control
HS 43°C
1,5 h
3,0 h
5,5 h
recovery
MEF
control
HS 43°C
HeLa

C


-211
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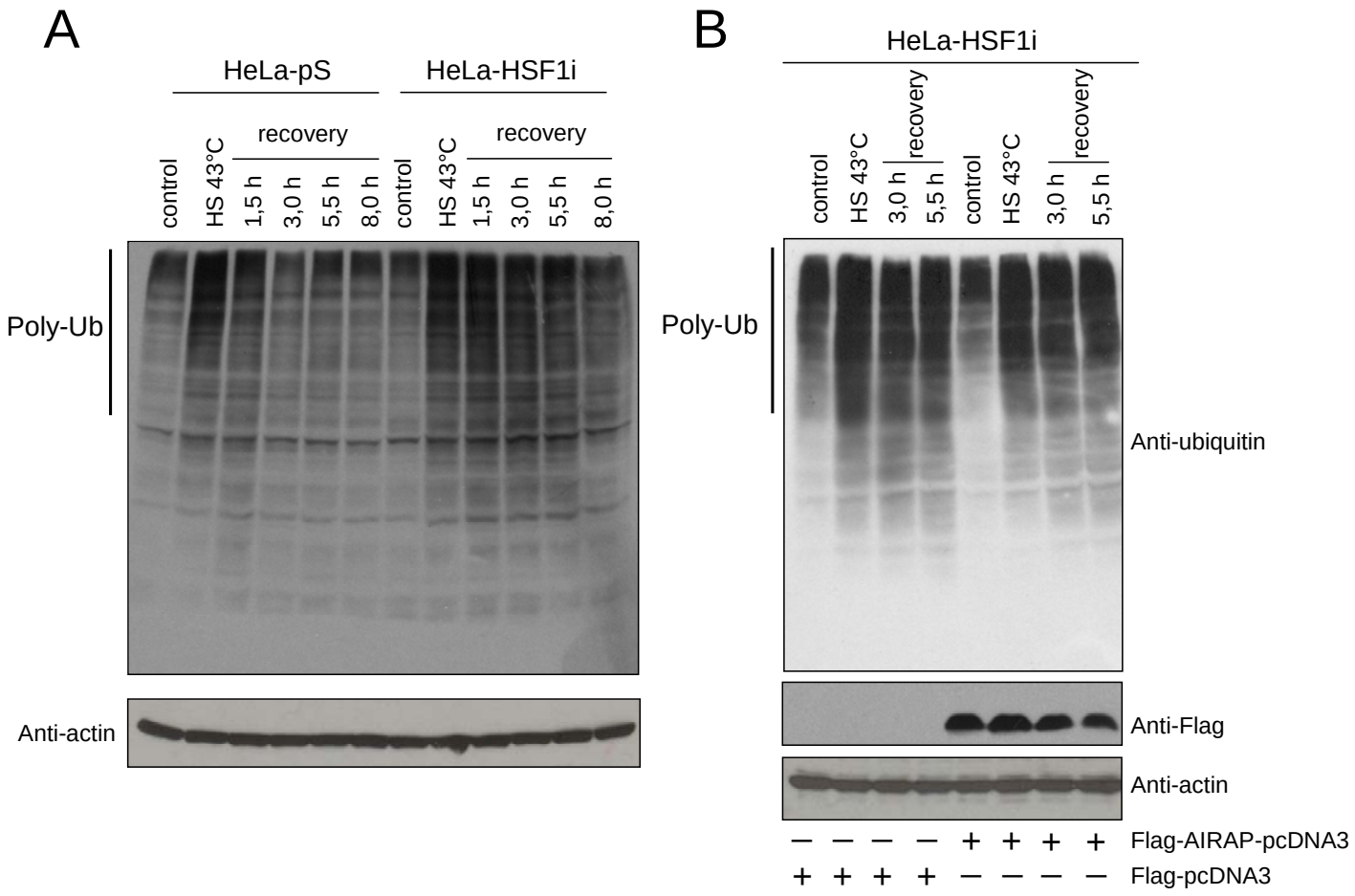
mHSE AGAGATGTTGCCTGACTGTTCTAGAAAATAGGCGGGGGGAT
-276

Figure S2


A
HeLa-pS
HeLa-HSF1i
control
HS 43°C
recovery
1,5 h
3,0 h
5,5 h
8,0 h
Poly-Ub
Anti-actin
B
HeLa-HSF1i
control
HS 43°C
3,0 h
5,5 h
recovery
Poly-Ub
Anti-ubiquitin
Anti-Flag
Anti-actin
Flag-AIRAP-pcDNA3
Flag-pcDNA3