SUPPLEMENTARY DATA

EXPERIMENTAL PROCEDURES

Purification of recombinant proteins and in vitro binding assays **-** DNA fragments corresponding to full length or deletion mutants of MITF were prepared by PCR, using pEF-BOS-MITF-M (1) (gift from Dr. Funaba (Kyoto University) as template and inserted to pGEX-4T-1 (GE Healthcare) to obtain expression plasmid for GST-fused MITF. GST-MITF and its derivatives were expressed in *Escherichia coli* (BL21) and purified by use of glutathione-agarose beads according to the manufacturer's instructions (Sigma). GST-MITF and its derivatives were incubated with glutathione-agarose beads and purified HSP70 with rotation. The beads were washed four times and proteins bound to beads were extracted by SDS sample buffer. Samples were applied to polyacrylamide SDS gels and subjected to electrophoresis, after which proteins were immunoblotted with each antibody.

Primers **-**The primers used were (name: forward primer, reverse primer): *tyrp1*: 5'-tggaccaatcaggagaaacc-3', 5'-atacacggacctccaagcac-3'; *dct*: 5'-tgtgcaagattgcctgtctc-3', 5'-agtccagtgttccgtctgct-3'; *pme I*: 5'-ggagaattgagctggcaaaa-3', 5'-gccaagagcagcagtttagg-3'.

Figure S1. Effect of UVB irradiation, heat treatment and overexpression of HSP70 on melanin production. A and B, B16 cells were irradiated with indicated doses of UVB and cultured for 72 h. C and D, B16 cells were incubated for 12 h with or without 100 μM IBMX, then for 1.5 h at 42°C (Heat Shock +) or 37°C (Heat Shock -) and finally for 58.5 h at 37°C. E and F, B16 cells were incubated for 1.5 h at 42°C (Heat Shock +) or 37°C (Heat Shock -), then for 6 h at 37°C and finally for 72 h with or without 100 nM α-MSH. G and H, HSP70-overexpressing B16 cells (HSP70 +) and mock transfectant control cells (HSP70 -) were incubated for 72 h with or without 100 nM α-MSH. I and J, B16 cells were pre-incubated for 1.5 h at 42°C (Heat Shock +) or 37°C (Heat Shock -) and further incubated for 6 h at 37°C and/or irradiated with indicated doses of UVB. Cells were further cultured for 72 h with or without 100 μM IBMX. A-J, the amounts of melanin in the conditioned medium (A, C, E, G, I) or cell extract (B, D, F, H, J) were determined as described in the legend of Fig. 1. Values are given as mean ± S.D. (n=3). **P<0.01; *P<0.05.

Figure S2. Effect of heat treatment on IBMX-stimulated activity and expression of tyrosinase and expression of MITF and various genes. A-F, B16 cells were pre-incubated for 1.5 h at 42°C (Heat Shock +) or 37°C (Heat Shock -) and further incubated for 6 h at 37°C. Cells were further incubated for 48 h (A-D) or 3 h (E, F) with or without 100 μM IBMX. A-D, tyrosinase activity, expression of tyrosinase and *tyrosinase* mRNA, and the promoter activity of the *tyrosinase* gene were determined as described in the legend of Fig. 2. E and F, the expression of MITF and *mitf* mRNA were determined as described in the legend of Fig. 3. G-I, HSP70-overexpressing B16 cells (HSP70 +) or mock transfectant control cells (HSP70 -) were incubated for 48 h with or without 100 μM IBMX. The mRNA expression was monitored as described in the legend of Fig. 2. Values are given as mean ± S.D. (n=3). **P<0.01; *P<0.05.

Figure S3. Deletion mapping of GST-MITF, to identify regions which interact with HSP70. Diagram of GST-MITF fragments; numbers refer to amino acid residues (A). Purified HSP70 and GST-MITF and its derivatives were subjected to precipitation with glutathione agarose beads. Samples were analyzed by immunoblotting with an antibody against HSP70 or GST. Arrows show the band of each derivative of GST-MITF (B).

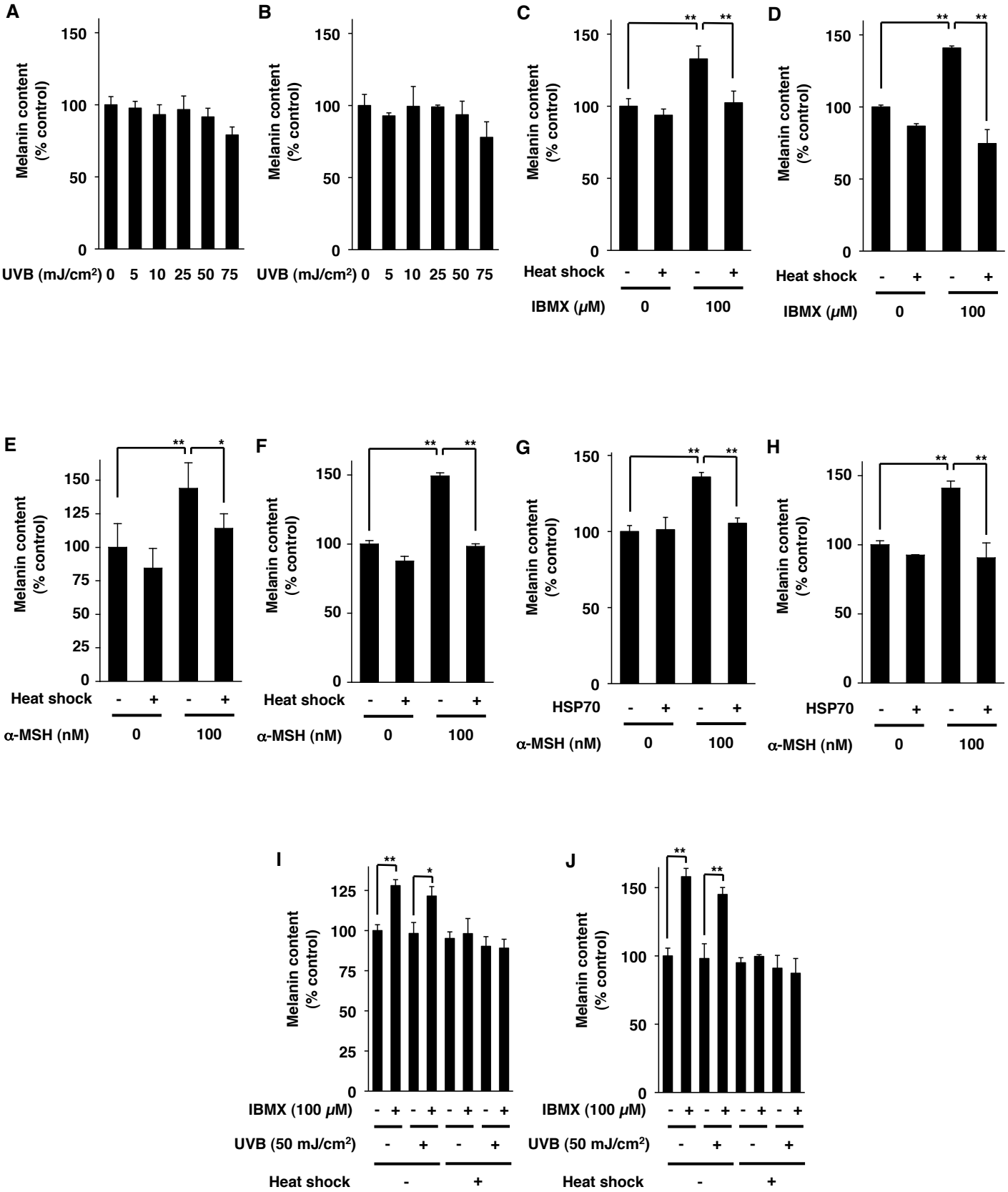
Figure S4. Effect of heat treatment on UVB-induced melanin production in the skin *in*

***vivo*.** Wild-type mice were treated at 42°C (Heat shock) or R.T. (Control) for 20 min (A-C). The tail skin samples were prepared after 6 h and subjected to immunoblotting analysis (A). Mice were irradiated with 180 mJ/cm^2 UVB once per 2 days for 8 days (totally 4 times) (B, C). Sections were prepared from the tail skin and subjected to Fontana–Masson staining (B). The amount of melanin in the tail skin was measured as described in the legend of Fig. 8. Values are given as mean ± S.E.M. (n=8-9). **P<0.01 (C).

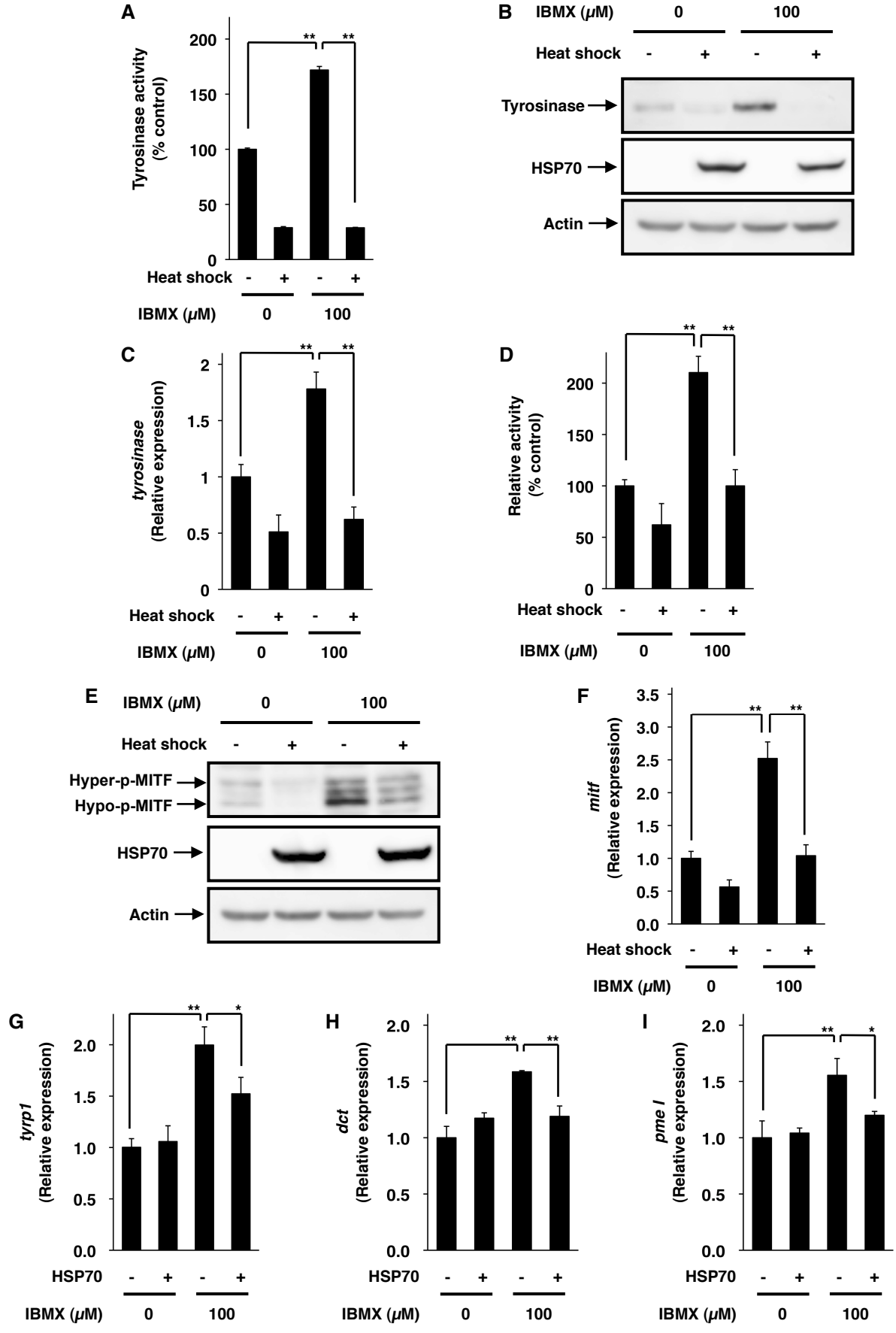
References

1. Murakami, M., Iwata, Y., and Funaba, M. (2007) *Mol Cell Biochem* 303, 251-257

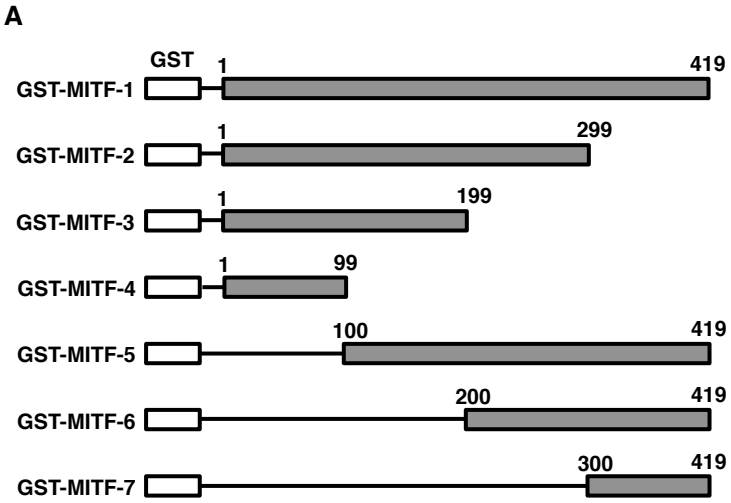
Hoshino *et al*., Supplemental Figure S1

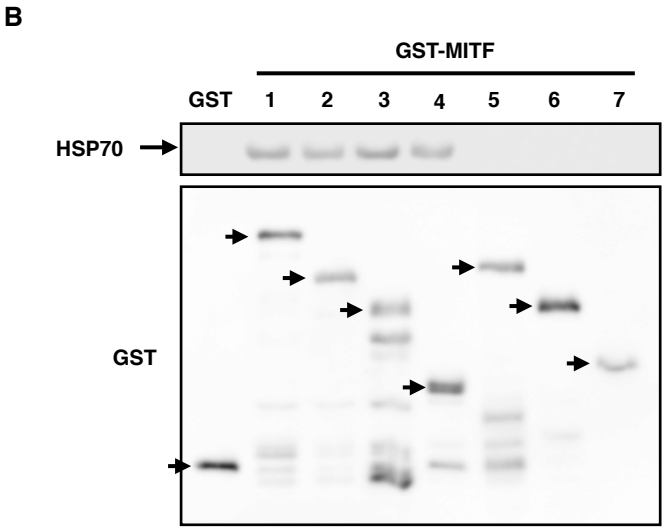


Hoshino *et al*., Supplemental Figure S2



Hoshino *et al*., Supplemental Figure S3





Hoshino *et al*., Supplemental Figure S4