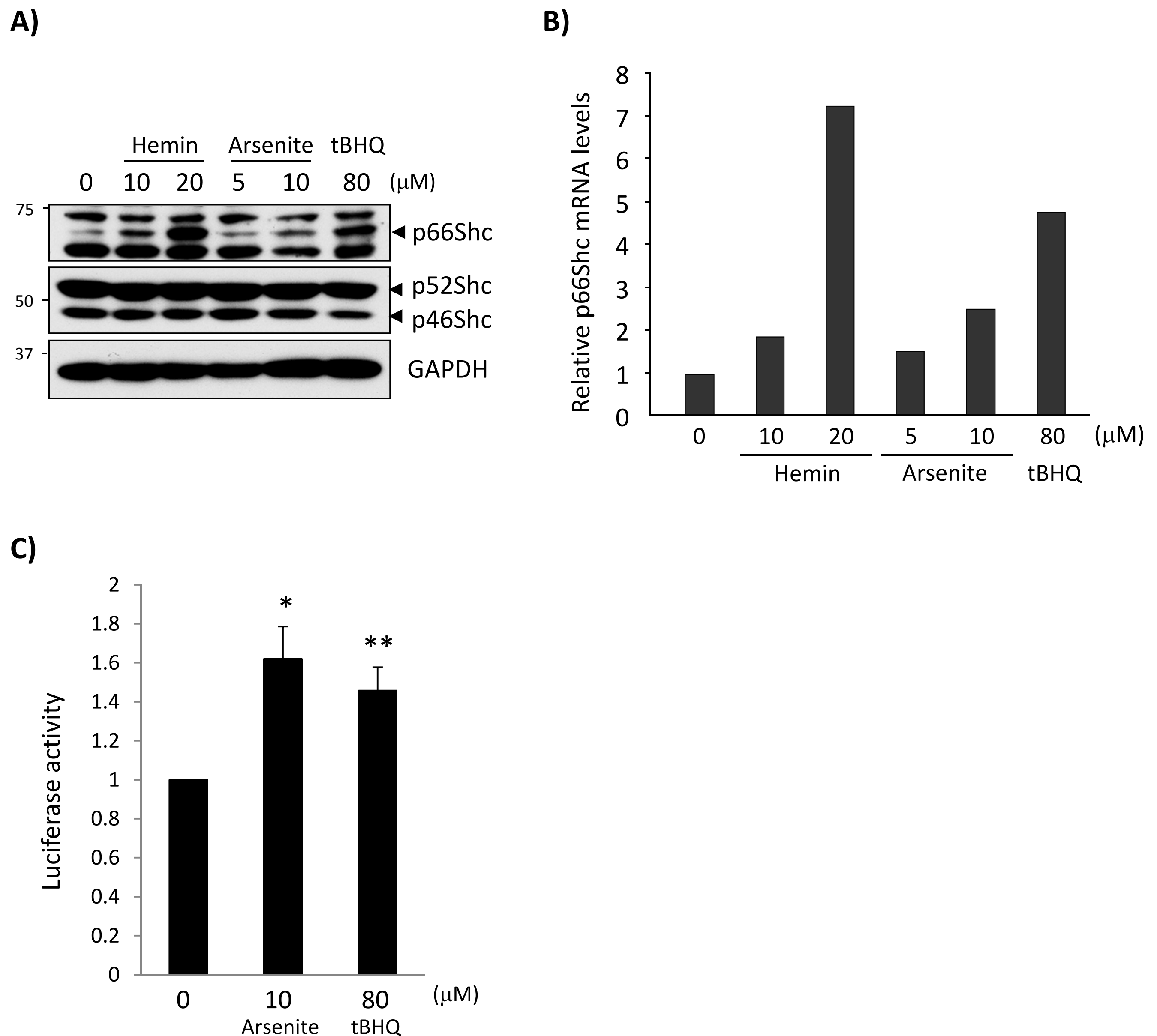
Supplemental Materials

Molecular Biology of the Cell

Miyazawa et al.

Supplemental Figure 1

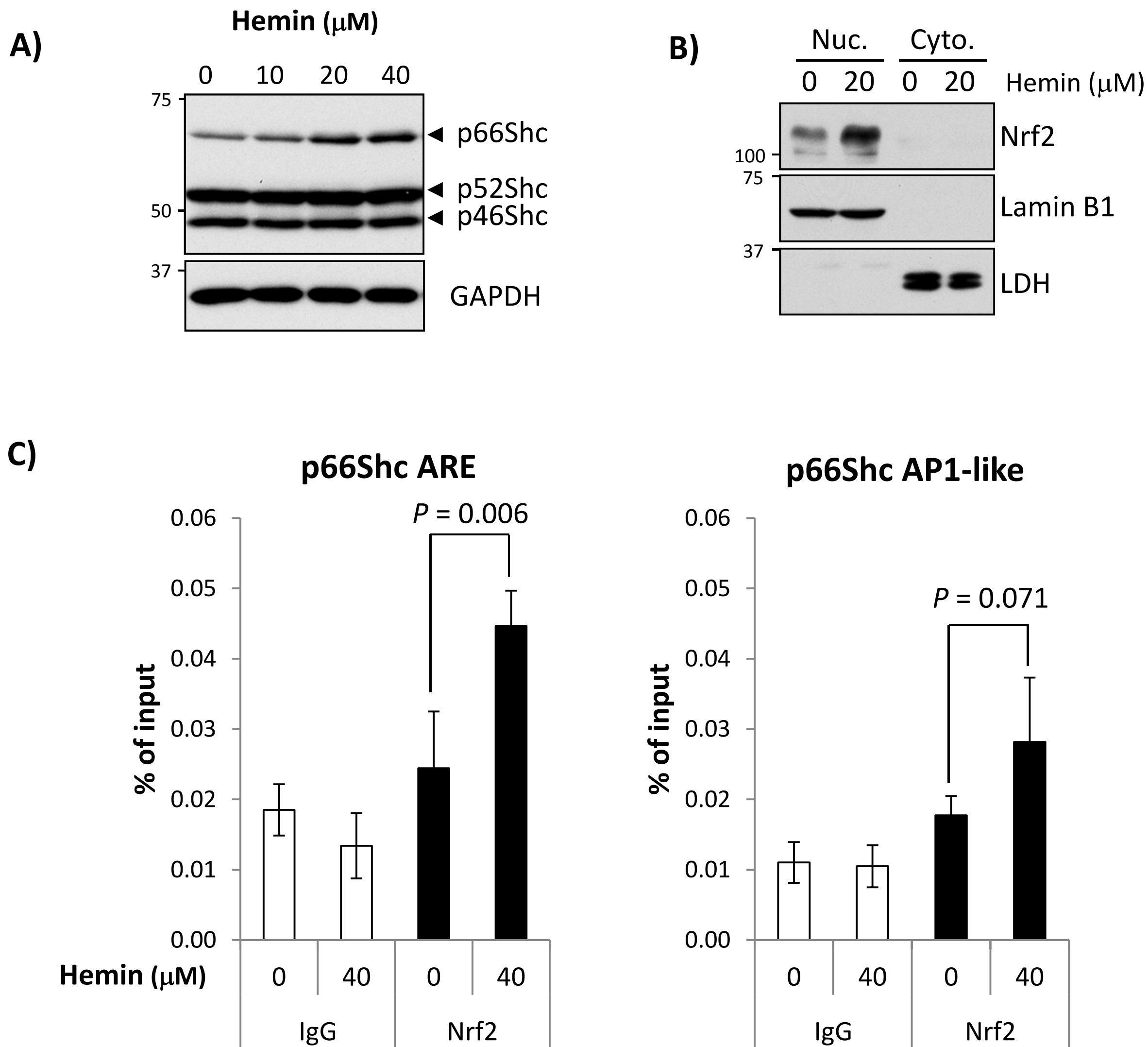
M. Miyazawa and Y. Tsuji



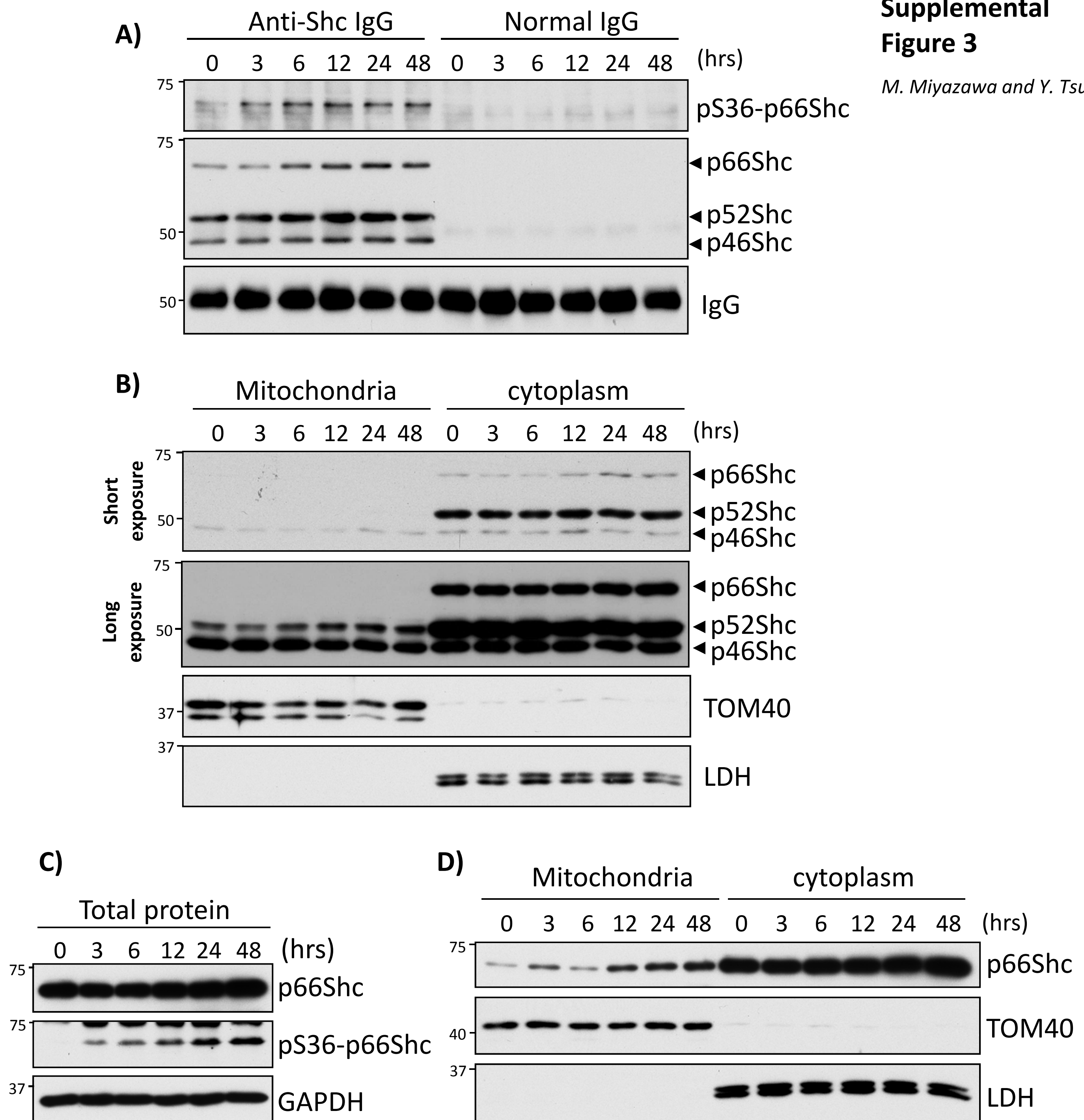
Supplemental Figure 1.
A, B) Jurkat cells were treated with 0, 10, 20 μM hemin, 5, 10 μM sodium arsenite, or 80 μM tBHQ for 24 hrs. A) Whole cell lysates were used for Western blot with Shc and GAPDH antibodies, and B) mRNA was used for real-time PCR to measure p66Shc mRNA levels. Real-time PCR results were presented as relative mRNA expression (0 time point as 1.0), and normalized by β2-microglobulin mRNA levels.
C) K562 cells were transfected with a pGL3 luciferase reporter plasmid containing -450 to +60 5'-regulatry region of the human p66shc gene, and cells were treated with 0, 10 μM arsenite or 80 μM tBHQ for 24 hrs. The firefly luciferase activity was presented as relative activity (0 time point as 1.0). *P* values were calculated using student *t*-test. Asterisk indicates significantly different from non-treatment sample. * $P < 0.001$, ** $P = 0.003$. Error bars represent mean ± s.d. (n = 3).

Supplemental Figure 2

M. Miyazawa and Y. Tsuji



Supplemental Figure 2.
A) TF-1 cells were treated with 0, 10, 20, and 40 μM hemin for 24 hrs and whole cell lysates were used for Western blot with a cross-reactive Shc antibody. The blot is shown along with GAPDH Western as a loading control.
B) TF-1 cells were treated with 20 μM hemin for 24 hrs and nuclear (Nuc.) and cytoplasmic (Cyto.) fractions were used for Western blot with Nrf2, Lamin B1, or lactate dehydrogenese (LDH) antibody. Lamin B1 and LDH are nuclear and cytoplasmic fraction markers, respectively.
C) TF-1 cells treated with 0 and 40 μM hemin for 12 hrs were subjected to ChIP assays with control IgG or Nrf2 antibody, followed by real-time PCR using p66Shc primers spanning the ARE or AP1-like element. Data were normalized by input DNA and shown as mean ± s.d. (n = 4).



Supplemental Figure 3.

A) Phospho-Ser36 p66Shc protein levels were measured in TF-1 cells treated with 20 μM hemin for 0, 3, 6, 12, 24 and 48 hrs. Whole cell lysates were used for IP/Western (IP with normal IgG or anti-Shc antibdy/Western with anti-Phospho-Ser36 p66Shc antibody. Equal protein loading of IP samples was assessed by probing the blot with anti-rabbit IgG.

B) Mitochondrial and cytoplasmic p66Shc protein levels were measured in TF-1 cells treated with 20 μM hemin for 0, 3, 6, 12, 24 and 48 hrs. TOM40 and LDH are markers of mitochondrial and cytoplasmic fractions, respectively.

C) p66Shc and Phospho-Ser36 p66Shc protein levels were measured in SH-SY5Y cells treated with 20 μM hemin for 0, 3, 6, 12, 24 and 48 hrs. Whole cell lysates were used for Western blot with a cross-reactive anti-Shc, anti-Phospho-Ser36 p66Shc, and anti-GAPDH antibody.

D) p66Shc protein levels were measured in SH-SY5Y cells treated with 20 μM hemin for 0, 3, 6, 12, 24 and 48 hrs. Mitochondrial and cytoplasmic fractions were used. TOM40 and LDH are markers of mitochondrial and cytoplasmic fractions, respectively.