Cell Reports, Volume *25*

Supplemental Information

Dynamic Phosphorylation of the C Terminus of Hsp70 Regulates the Mitochondrial Import of SOD2 and Redox Balance

Sara Zemanovic, Maxim V. Ivanov, Lena V. Ivanova, Amogh Bhatnagar, Teresa Michalkiewicz, Ru-Jeng Teng, Suresh Kumar, Rajendra Rathore, Kirkwood A. Pritchard Jr., Girija G. Konduri, and Adeleye J. Afolayan

Supplemental materials

Dynamic Phosphorylation of The C-terminal Domain of Hsp70 Regulates Mitochondrial Import of SOD2 and Redox Balance.

Sara Zemanovic, BSc,[1,2,3] Maxim V. Ivanov, Ph.D.,[6] Lena V. Ivanova, Ph.D.,[6] Amogh Bhatnagar,[6] Teresa Michalkiewicz MSc,[1,2,3] Ru-Jeng Teng, MD,[1,2,3] Suresh Kumar, Ph.D.,[4,2,3] *Rajendra Rathore, Ph.D.,[6] Kirkwood A. Pritchard Jr. Ph.D.,[5,2,3], Girija G. Konduri, MD,[1,2,3] **Adeleye J. Afolayan, MD[1,2]

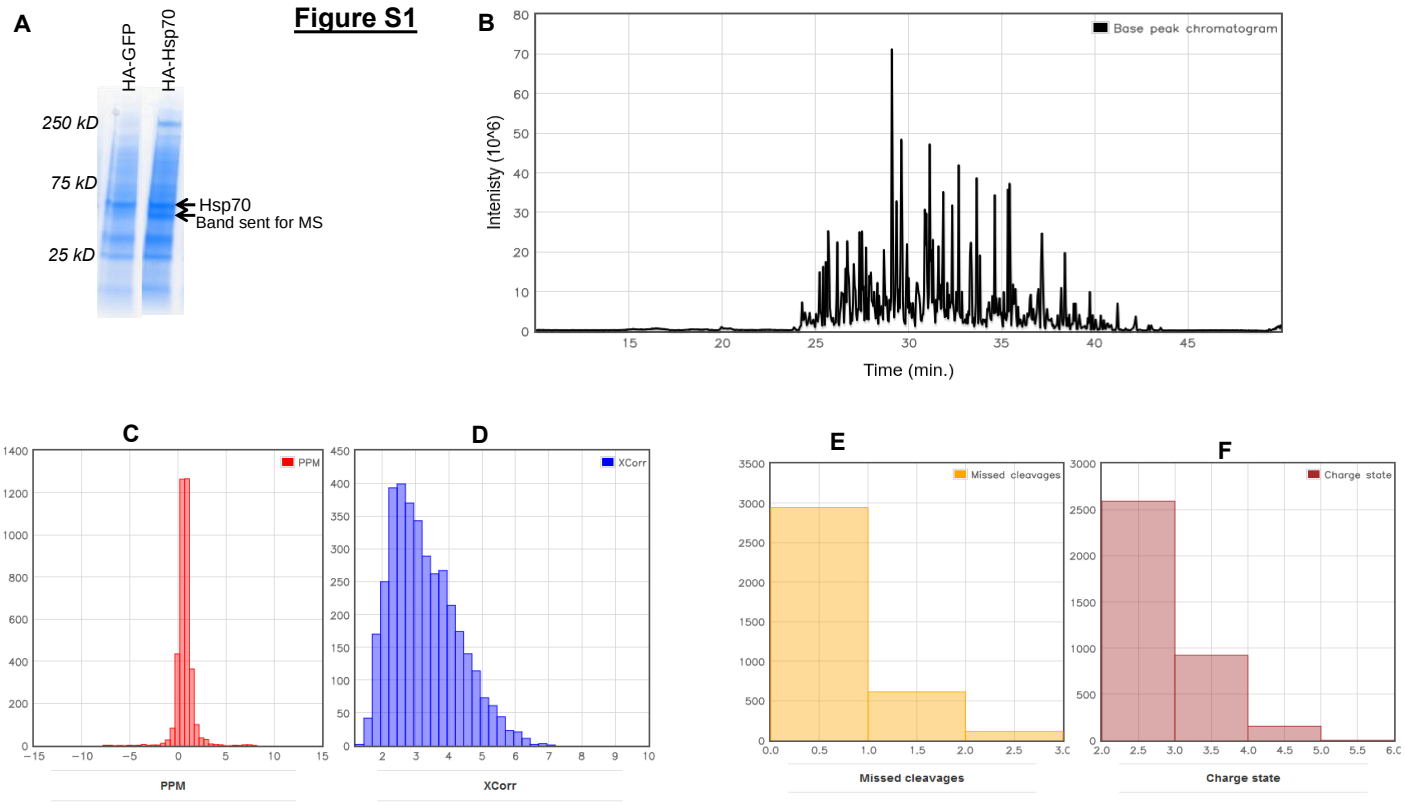

Figure S1 (Related to Fig. 1). Hsp70 interactors. HEK-293T cells were transfected with HA-tagged Hsp70, and the cells were then subjected to Coomassie blue staining after acetylcholine stimulation. (**A**) Differential band sent for MALDI-mass spectrometry. (**B**) MS spectra tracing of Hsp70. (**C**) Mass measurement error in parts per million, ppm. (**D**) Plots showing the Xcorr score distribution for candidate peptides from searches against a shuffled human protein sequence database by SEQUEST. (**E**) The number of cleavage sites in a peptide sequence that a cleavage reagent (enzyme) did not cleave excluding cases where an amino acid (e.g., Pro) inhibits the cleaving enzyme (trypsin). (**F**) The charge state of the peptide, z (usually >1 for the MS analysis).

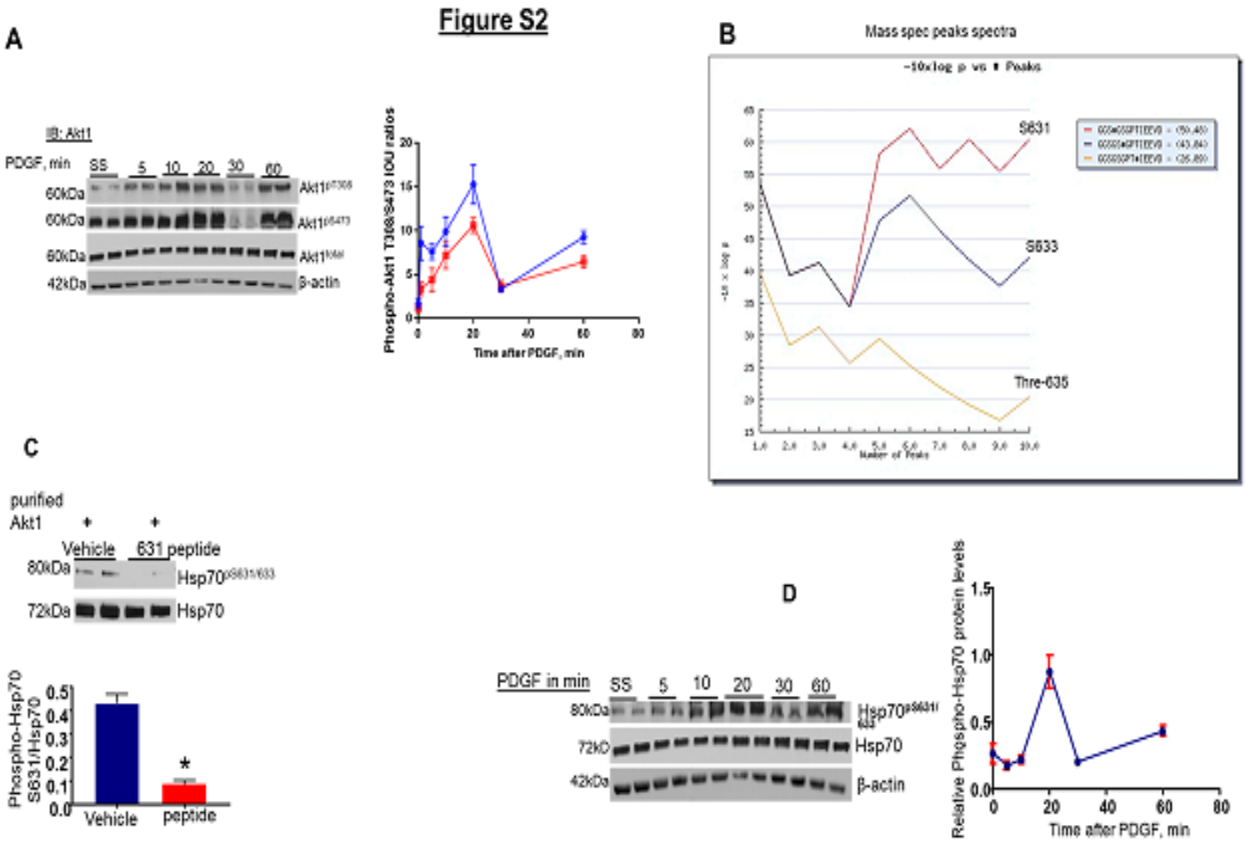

Figure S2 (Related to Fig.2). Akt1 phosphorylates the C-terminus of Hsp70 on Ser631. (**A**) Detection of phosphorylated Akt1 on T308 /S473 at indicated time points for 1-hr after PDGF treatment of serum-starved cells. The bar graph shows densitometric quantification analysis. (**B**) MS peak spectra of phosphorylated residues in the C-terminus of Hsp70. (**C**) Generated antiserum against phospho-Ser631/633 is specific. Purified Hsp70 proteins were incubated with peptide antigen and measured phosphorylated Ser631/633 by IB (n=4, * $p<0.05$ from vehicle-treated proteins). (**D**) Detection of Ser631/633 phosphorylation of Hsp70 after PDGF treatment of serum-starved PAECs. Densitometric quantification

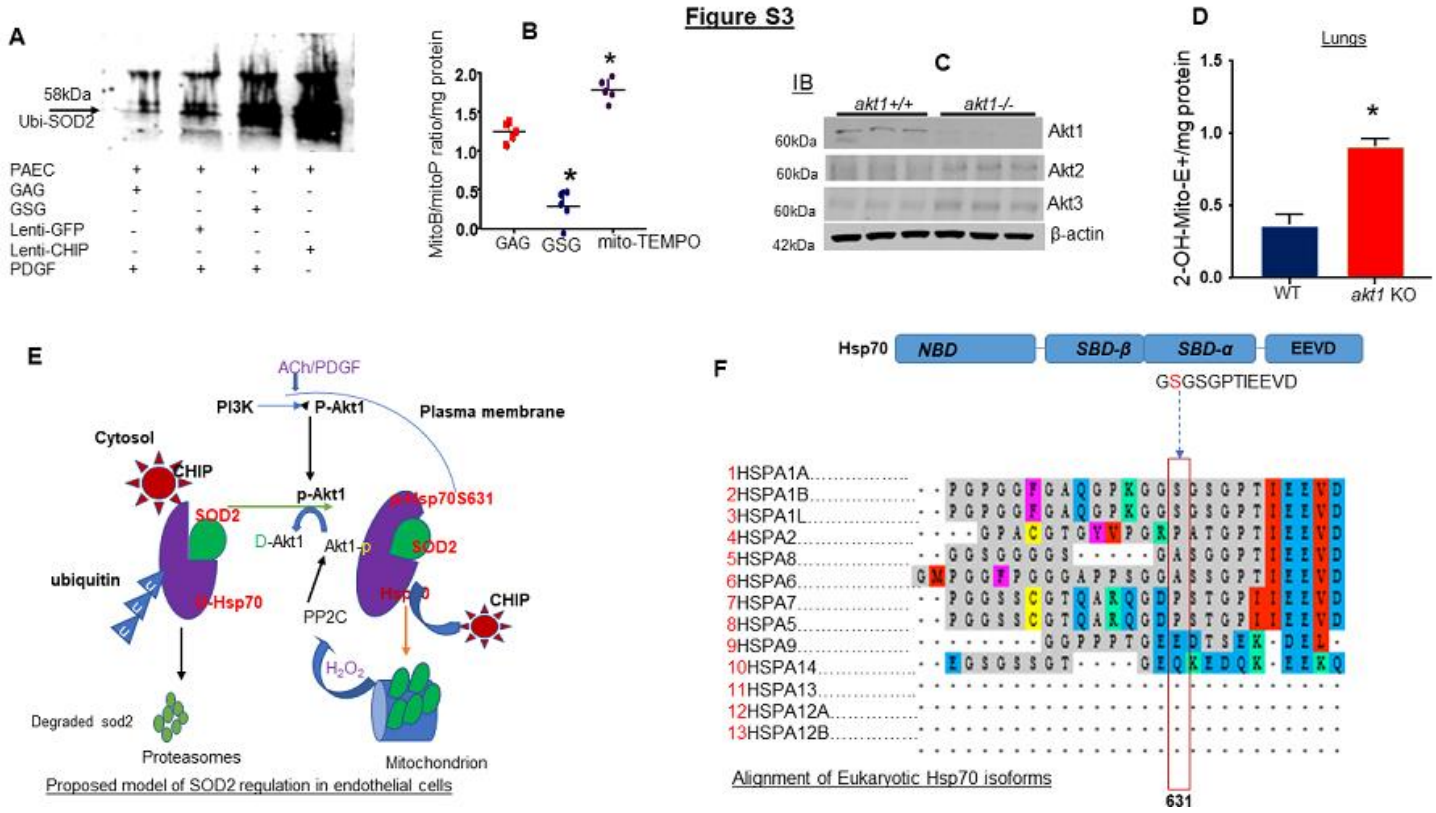

Figure S3 (Fig. S3A is related to Fig.4, Fig. S3C is related to Fig. 6, and Figs. S3E &F are related to discussion). The functional significance of Ser631 phosphorylation on SOD2 activity. The effect of Hsp70 phosphorylation on the stability of SOD2. (**A**) PAECs were treated with GSG, Lentivirus carrying human akt1 and chip genes and measured the ubiquitylated SOD2 protein levels using anti-ubiquitin antibody. (**B**) Bar graph showing LC-MS quantification of MitoP/MitoB ratio in GSG vs GAG treated lungs. (n=5, *p<0.05 from GAG controls). (**C**) Global akt1 null mice were generated, and their lungs were harvest at birth and examined for the levels of Akt1, Akt2 and Akt3 protein levels by IB. Loss of Akt1 activity increases mitochondrial oxidative stress. (**D**) LC-MS quantification of 2-OH-Mito-E+ in the lungs of akt1 null and WT mice (n=6, *p<0.05 from WT). Alignment of sequences in Eukaryotic Hsp70 isoforms. (**E**) Only inducible Hsp70 contains a serine at sixth position from its C-terminus. (**F**) Schematic representation of the proposed new model of SOD2 regulation.