# Supplementary Information

## Identification and Quantification of Glutathionylated Cysteines under Ischemic Stress

Maheeshi Yapa Abeywardana[1], Kusal T. G. Samarasinghe[1] Dhanushka Munkanatta Godage[1], Young-Hoon Ahn[1]*

[1]Department of Chemistry, Wayne State University, Detroit, MI 48202, USA.

Corresponding author: Young-Hoon Ahn, yahn@chem.wayne.edu; (313) 577-1384

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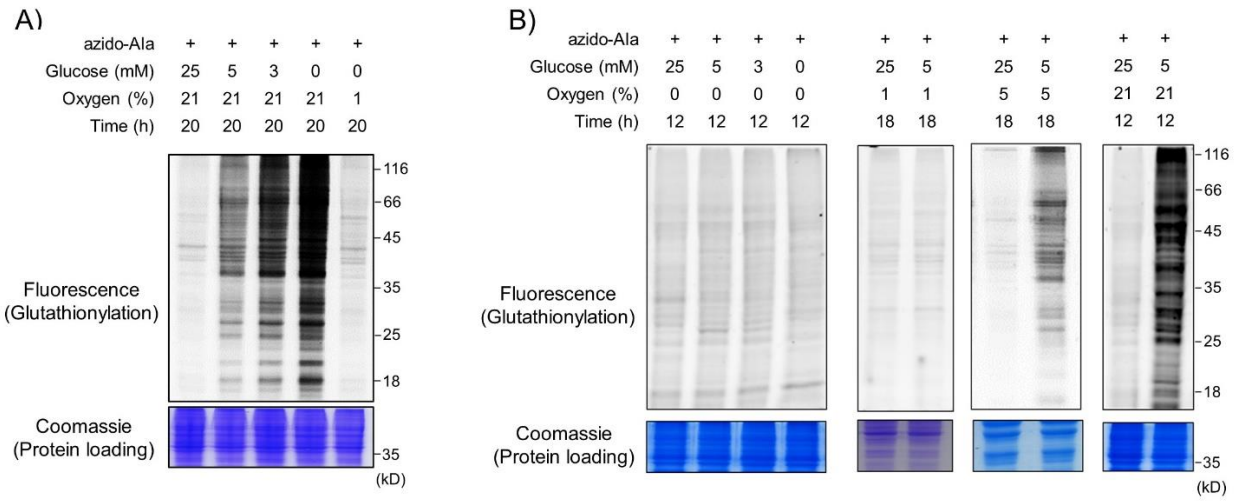


**Figure S-1. Analysis of protein glutathionylation upon glucose deprivation (GD) and hypoxia in HEK293 cell line**. HEK293/GS M4 cells were incubated with azido-Ala and subjected to the following conditions. (A) Different concentrations of glucose in normoxia or hypoxia (1% $O_2$). (B) Glucose deprivation with different percentages of oxygen in a hypoxic chamber. After stimulus, cells were lysed and subjected to click reaction with Cy5-alkyne and analyzed by in-gel fluorescence and Coomassie stains. Data are the representative of at least 3 independent experiments.

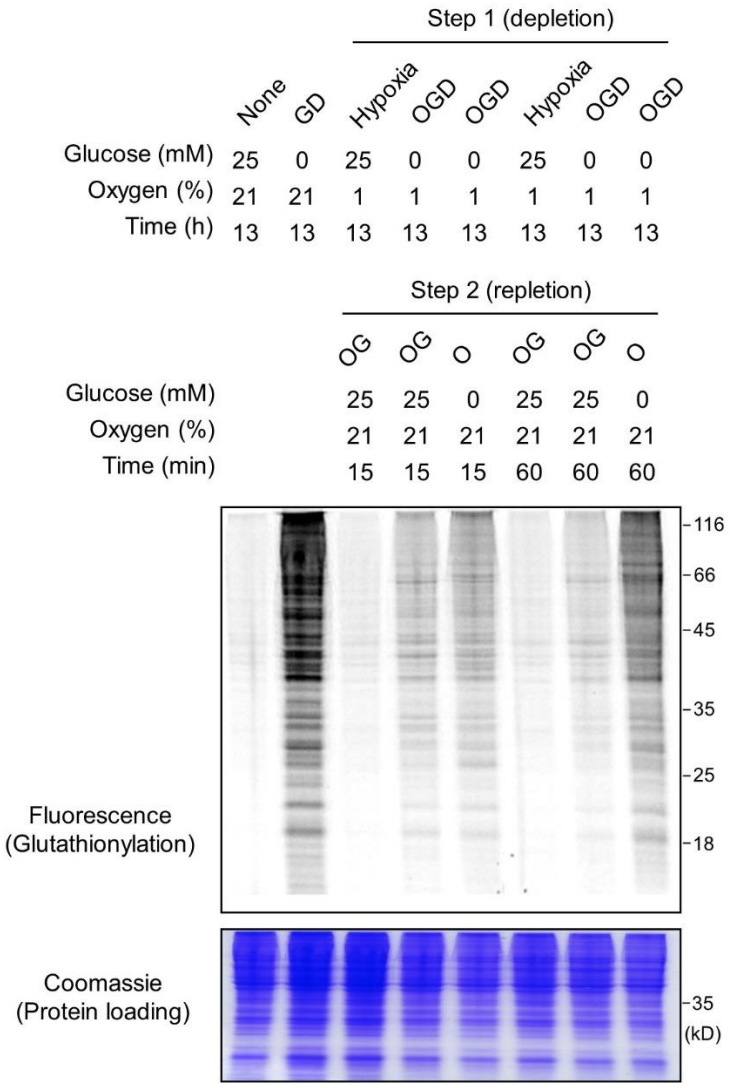


**Figure S-2. Analysis of protein glutathionylation in HEK293 cell line during repletion of glucose, oxygen, and palmitate after OGD (OGD/R).** HEK293/GS M4 stable cells were incubated with azido-Ala and subjected to the indicated conditions in a hypoxic chamber. Cells were then lysed and analyzed by in-gel fluorescence or Coomassie stains after click reaction with Cy5-alkyne. Data are the representative of 3 independent experiments.

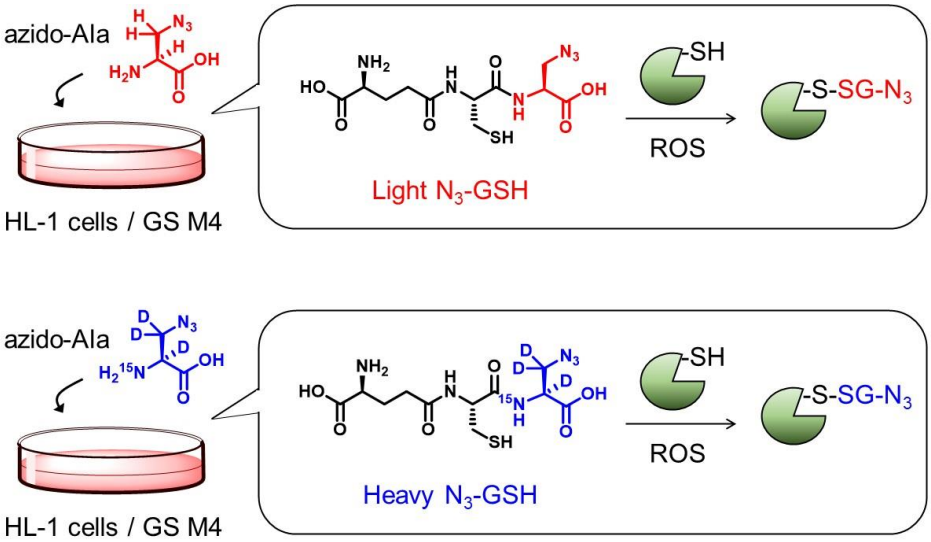


**Figure S-3. Isotopically labelled clickable glutathione.** GS M4 uses light or heavy derivative of azido-Ala to synthesize the corresponding clickable glutathione with +4 Da difference.

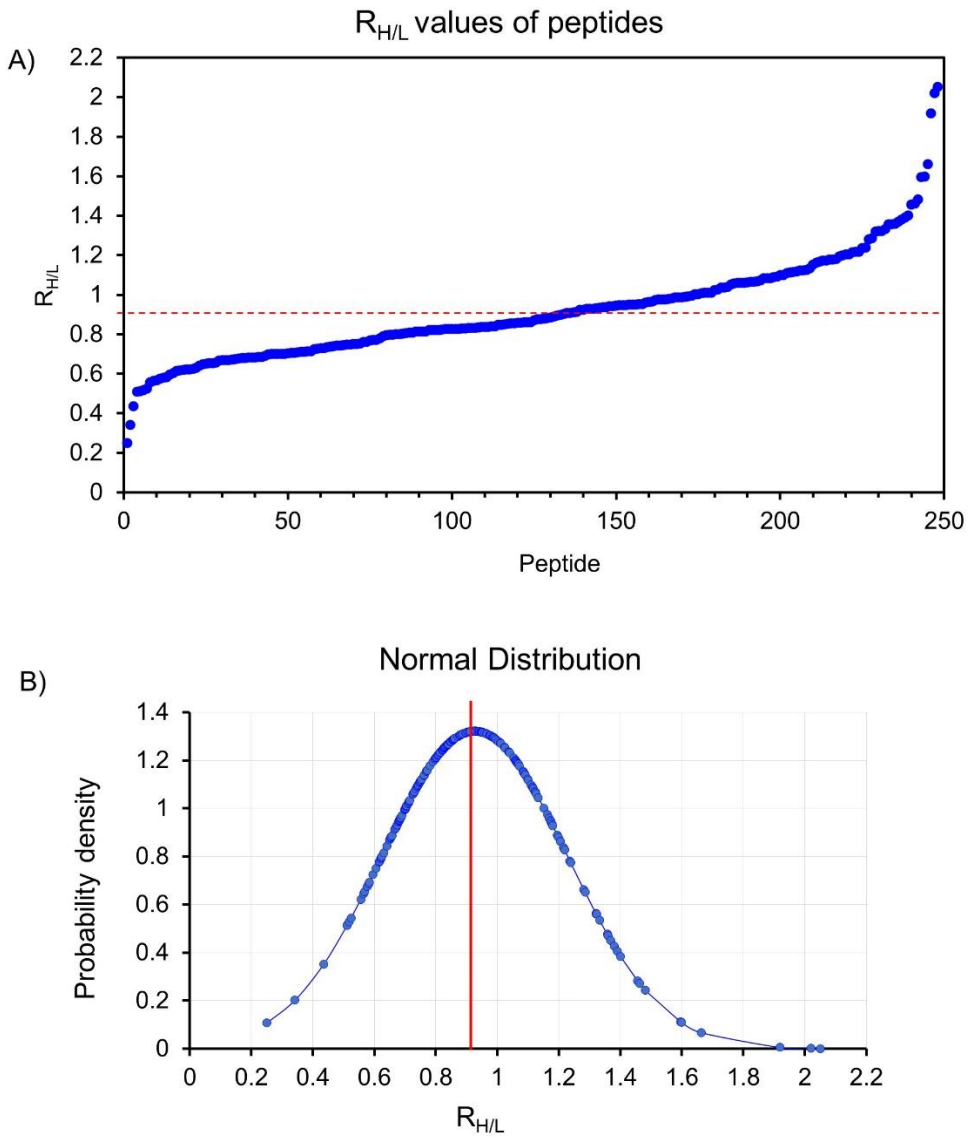


**Figure S-4. $R_{H/L}$ values of identified glutathionylated peptides at E1 experiment.** Two cohorts of cells incubated with heavy or light azido-Ala were subjected to OGD, followed by reoxygenation and repletion of glucose and palmitate (OGD/OGF). The identified glutathionylated peptides were analyzed for $R_{H/L}$ values. (A) A plot of $R_{H/L}$ values of individual peptides. The median $R_{H/L}$ value (0.87) is indicated by a red dot line. (B) A normal distribution plot of $R_{H/L}$ values. The average $R_{H/L}$ value (0.93) is shown by a red line. The 99% of $R_{H/L}$ values are between 1.59 and 0.25.

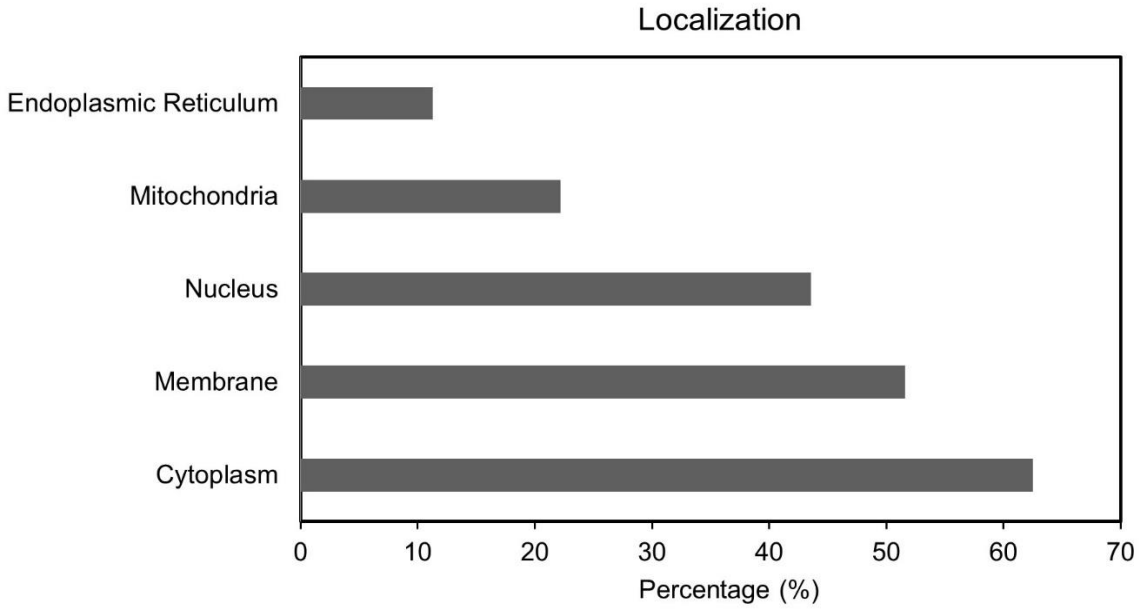


**Figure S-5. Localization of glutathionylated proteins.** All identified proteins (n = 248) were subjected to DAVID GO analysis to identify their cellular localizations.

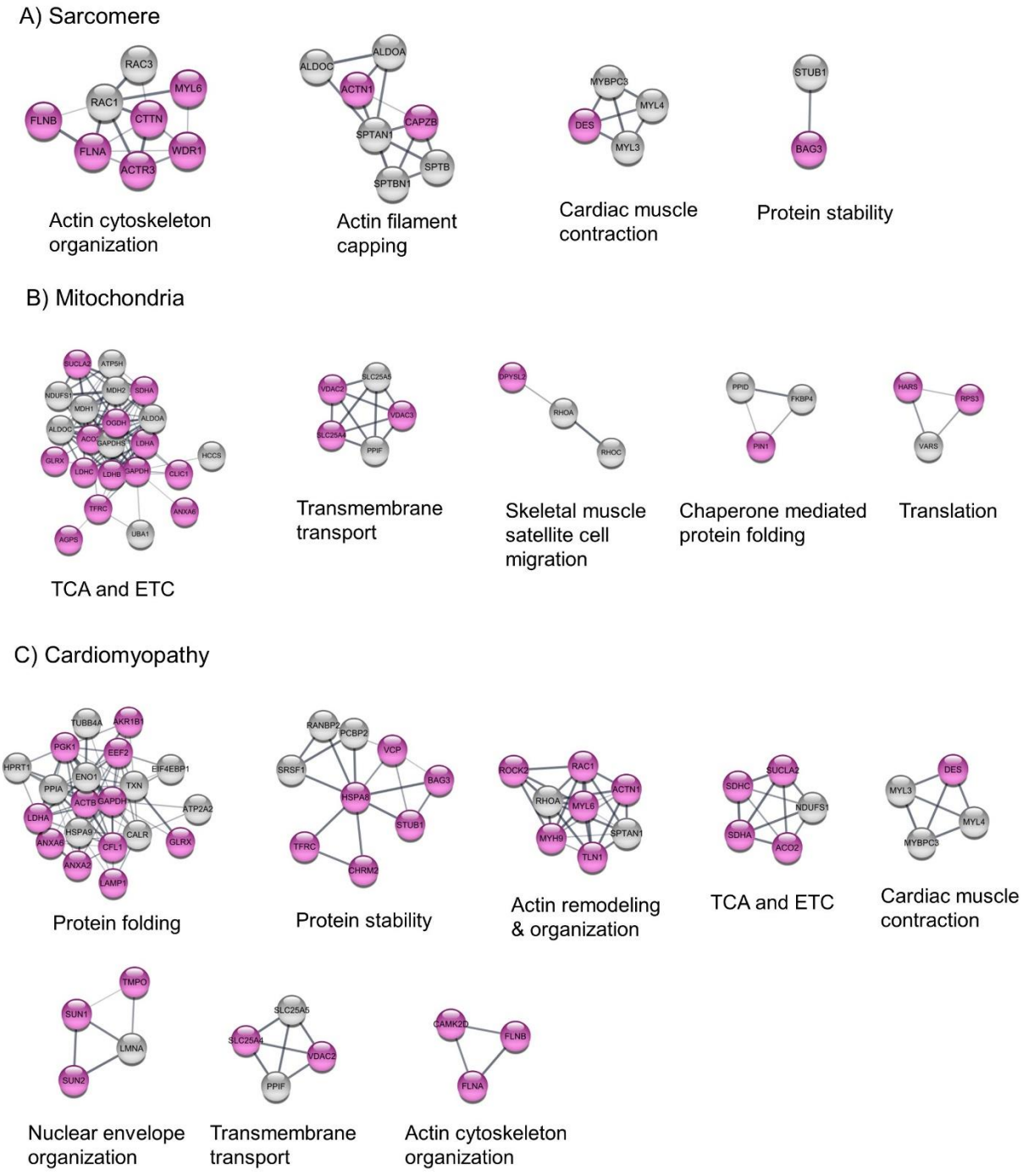


**Figure S-6. Analysis of glutathionylated proteins in association with sarcomere, mitochondria, and cardiomyopathy.** Glutathionylated proteins having $R_{H/L}$ value above 2 (n = 221) were analyzed to classify them into sarcomere associated (A), mitochondrial (B), or cardiomyopathy associated (C). Sarcomere and mitochondrial proteins were identified by

submitting proteins to DAVID GO analysis. Cardiomyopathy associated proteins are identified by comparing cardiomyopathy genes in the STRING disease query. The identified proteins were analyzed by STRING program and clustered using MCL clustering. The name of clusters was assigned after analyzing proteins in individual clusters by DAVID GO analysis.

Figure 6A


Desmin


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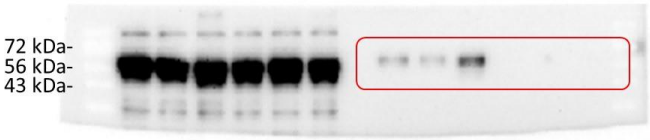


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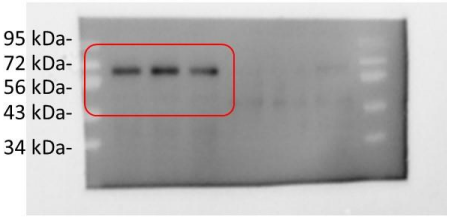
Figure 6B

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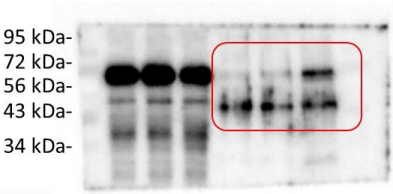


BAG3 After Strep-Beads


FLAG BAG3

**Figure S-7. Original western blots used in Figure 6.**