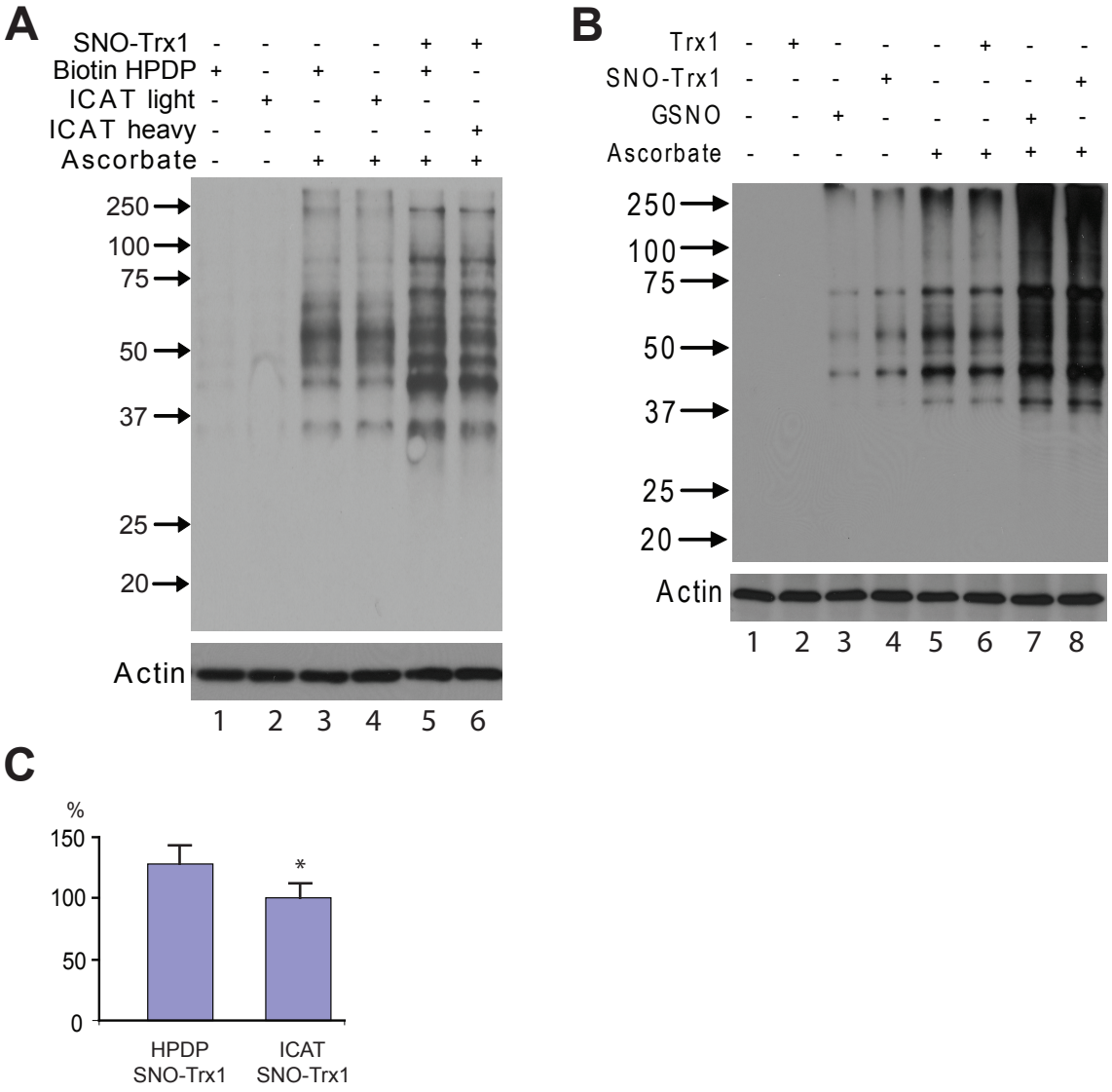

Figure S1. Comparison of Biotin HPDP and ICAT reagent labeled nitrosylated proteins in SH-SY5Y cells using a modified biotin switch method. (**A**) Western blot of biotin-HPDP and ICAT reagent labeled nitrosylated proteins. SH-SY5Y cell lysate was in vitro transnitrosylated by incubation with SNO-Trx1. Resultant SNO-proteins were modified by BST using biotin-HPDP or ICAT-L or -H reagents, resolved by non-reducing SDS-PAGE and detected by Western blotting with an anti-biotin antibody (top panel). Protein levels were normalized to actin (bottom panel). (**B**) Comparison of ICAT reagent specificity in a modified biotin switch assay. SH-SY5Y cell lysate was in vitro transnitrosylated by incubation with SNO-Trx1. GSNO or unmodified Trx1 were used as positive and negative controls, respectively. Resultant SNO-proteins were modified by BST using ICAT-H reagent with or without ascorbate. ICAT-H labeled proteins were detected by reducing SDS-PAGE and Western blotting with an anti-biotin antibody (top panel). Protein levels were normalized to actin (bottom panel). (**C**) Densitometry of lanes 5 and 6 of panel **A**, representing biotin-HPDP or ICAT-H labeled SNO-Trx1 transnitrosylated protein, respectively. Values are the mean ± S.E. for experiments performed in triplicate, *p = 0.03. The faint biotinylated bands detectable in panel **B** in the absence of ascorbate treatment (lanes 3 and 4) are possibly attributable to extremely labile SNO-Cys sites modified during BST work-up.

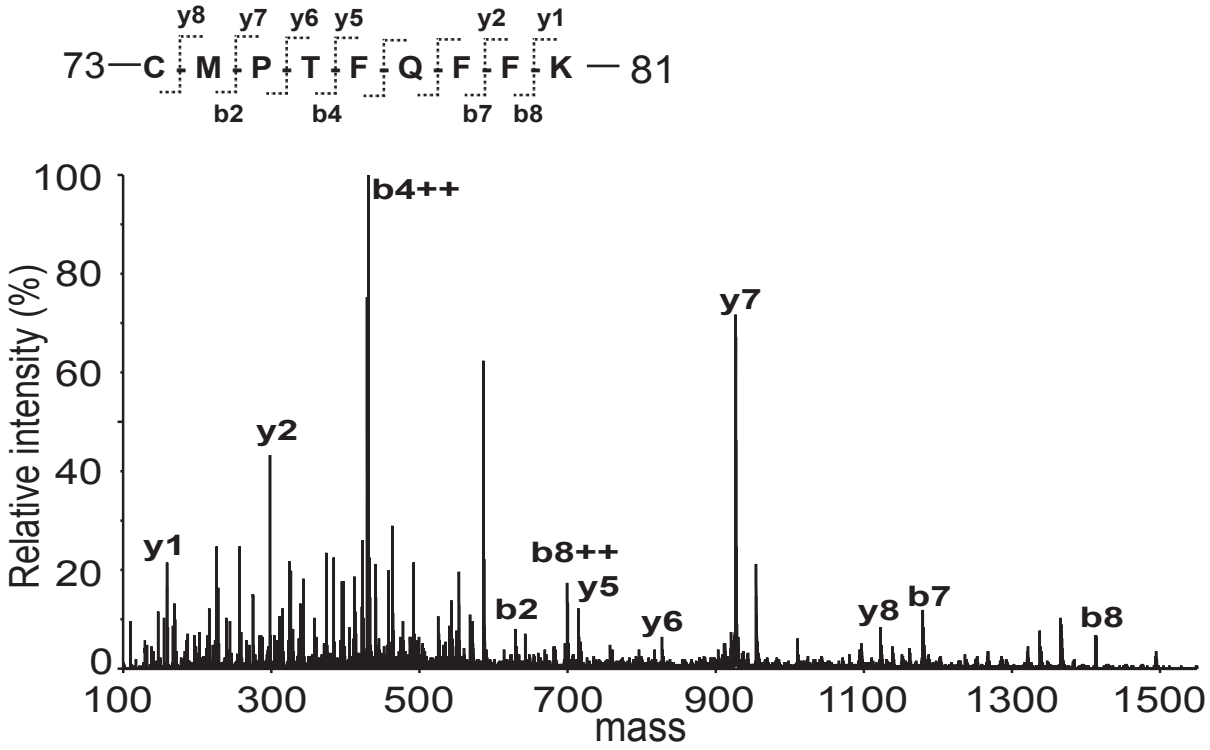

Figure S2. Confirmation of Trx1 Cys73 S-nitrosylation. MS/MS spectrum of ICAT-H labeled Trx1 peptide 73-C*MPTFQFFK-81 (m/z 1383.5). *Cys73 was confirmed to be S-nitrosylated.

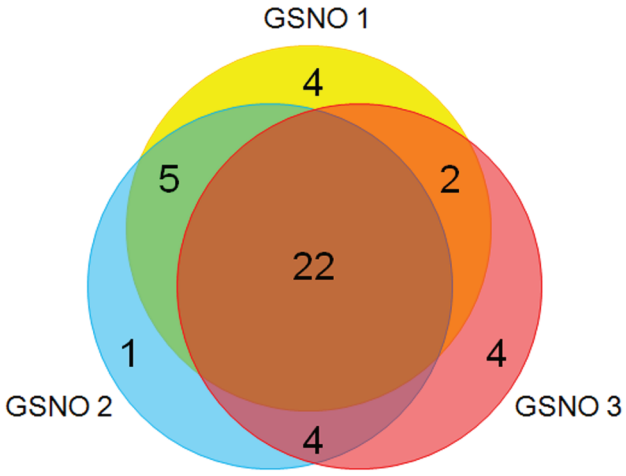

Figure S3. Reproducibility of detecting ICAT reagent labeled peptides. Venn diagram showing the reproducibility of detecting ICAT-H and -L labeled peptide pairs from 3 whole reaction repeats. SH-SY5Y cell lysate was incubated with GSNO, then divided equally and labeled by BST using light or heavy ICAT reagent. After tryptic digest, ICAT samples were combined 1:1 and biotinylated peptides detected by LC/MS/MS. SNO-peptides and location of their SNO-Cys residues were identified using Mascot (v2.2) using ICAT-C (ICAT-L) and ICAT-C:13C(9) (ICAT-H) as variable modifications.