

Supplementary:

Specificity of biotin labeled on the nitrosocysteine residue. **(A)** The time-dependent S-nitrosylation after SNAP treatment is shown. Biotinylated cell lysates (40 µg) were obtained from ECs after cell lysate was treated with 1 mM SNAP for various intervals, followed by biotinylation using the biotin switch method. Control samples of cell lysate were subjected to: no biotinylation, and; de-biotinylation by treating with 1 % (w/v) DTT. Samples were separated by SDS-PAGE, followed by western blot with streptavidin-HRP (1:4000). **(B)** Schematic diagram showing the specificity of the Biotin-HPDP reaction to the reduced thiols of cysteine. **(C)** Following 1 mM SNAP- or NAP-treatment, equal amounts (1 mg) of protein were collected from whole-cell lysate, MMTS-treated lysate, and ascorbate-reduced lysate, and then biotinylated with Biotin-HPDP. Samples were separated using DTT-free 2-DE followed by western blot, with streptavidin-HRP.