Supplementary Fig. S1. Biological replicate and dye swap experiment for S-FLOS analysis of protein extracts from cells with or without IFNγ/LPS stimulation for 24 h. S-FLOS analysis was performed as in Fig 5B. In contrast, proteins from stimulated cells were labeled with Cy5, whereas proteins from unstimulated control cell were labeled with Cy3 using the S-FLOS assay. The backgrounds and S-FLOS fluorescence increase in proteins from the stimulated cells were similar to Figure 5B

Supplementary Fig. S2. *In situ* staining of *S*-nitrosated proteins using S-FLOS. RAW264.7 cells grown on fibronectin-coated coverslips were treated with IFNγ/LPS (to stimulate NOS2) for 48 h in the presence or absence of a NOS2 inhibitor (1400 W). At 0, 24, and 48 h, cells were fixed, permeabilized, and labeled with Cy3 *in situ* using the S-FLOS assay. There is a time dependent increase in SNO signal. This increase in fluorescence had the same time dependency as NO production in IFNγ/LPS stimulated RAW 264.7 cells, measured using the Griess method (Fig. 5A). Stimulated cells grown in the NOS2 inhibitor 1400 W had a lower fluorescence signal at 48 h (+1400 W 48 h). Pretreatment of cells the 48 h time point with DTT (+DTT 48 h) to remove all nitrosation prior to MMTS blocking or omission of the ascorbate reduction step after blocking free thiols from the S-FLOS assay (-Asc 48 h) lead to complete loss of the fluorescence signal. Merged fluorescence images of DAPI staining for nuclei (blue) and S-FLOS Cy3 labeling (false colored red) at 0 and 48 h, respectively, are shown in bottom panels. The scale bars are 10μm. These results are consistent other fluorescent histochemical staining experiments for *S*-nitrosation in literature [21] and [37].In situ labeling of S-nitrosothiols protocol: All steps were performed in the dark. IFNγ/LP *S*-stimulated and unstimulated RAW264.7 cells grown in the absence or presence of 20 μM NOS2 inhibitor 1400 W (Cayman Chemical, www.caymanchem.com) on coverslips were fixed in 3% paraformaldehyde and permeabilized with 0.05% Triton X-100. After incubating cells with 20 mM MMTS in Wash Buffer (PBS containing 0.5% v/v Tween-20) for 1 h at 50 °C (with or without 5 mM DTT pretreatment), the cells were washed three times in 500 μl Wash Buffer and incubated in 5 mM ascorbate for 1 h at room temperature. The cells were then rinsed three times with 500 μl Wash Buffer and incubated with 10 μM Cy3-maleimide in Labeling Buffer at 37 °C for 30 min. The samples were again washed three times in 500 μl Wash Buffer. Fluorescence images were acquired using a Nikon Eclipse TE200 microscope and an internally-cooled 12-bit CCD camera (CoolSnapHQ, Photometrics, photomet.com). Images were collected using OpenLab software (Improvision, www.improvision.com). Nuclei of cells were labeled with DAPI (Invitrogen) and imaged using fluorescence microscopy and merged corresponding with Cy-dye image