Molecular Medicine

Supplemental Data

Cysteine Oxidation Targets Peroxiredoxins 1 and 2 for Exosomal Release through a Novel Mechanism of Redox-Dependent Secretion

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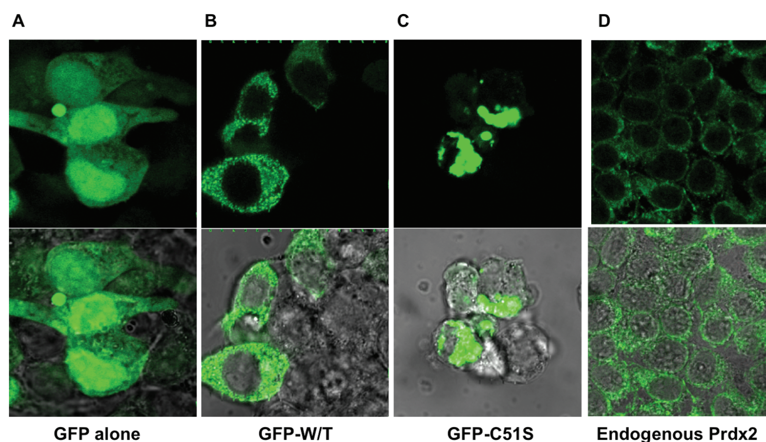
MATERIALS AND METHODS

Preparation of Exosomes from HEK293T Supernatants

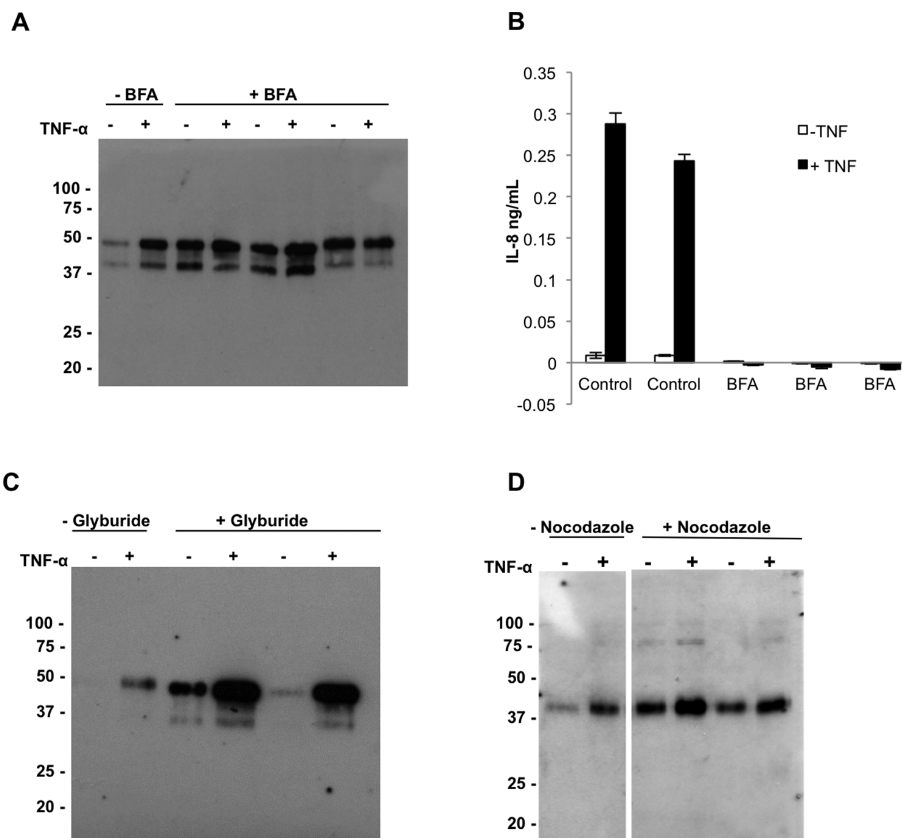
To confirm that the pellets collected after ultracentrifugation contained the exosomes, a second method of exosome preparation was used. Exosomes were isolated from cell culture supernatants from 293T cells using the Exo-spin exosome purification kit according to the manufacturer's instructions (Cell Guidance Systems, Cambridge, UK).

Confocal Microscopy

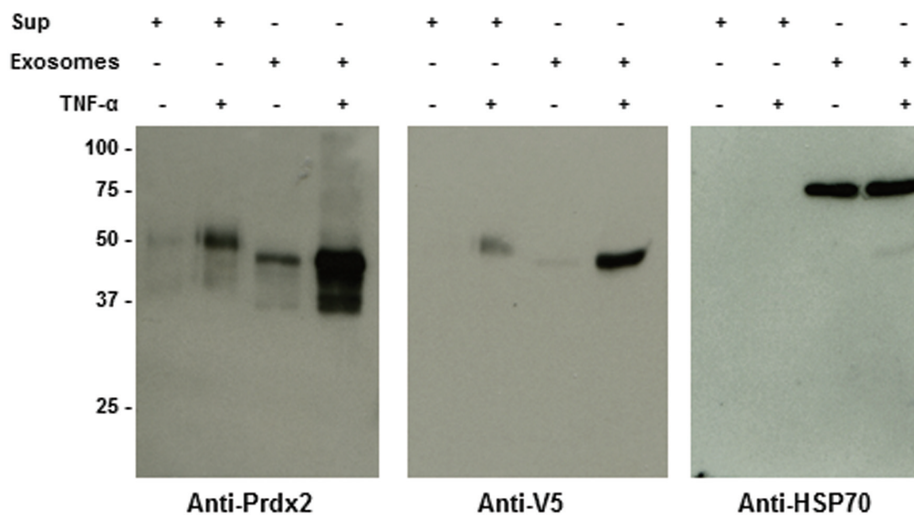
HEK293T cells were plated on poly-l-lysine coated glass-bottomed 35mm dishes (Mattek) and allowed to adhere for 24 h. Cells were then treated with 50 ng/mL TNF-α for 24 h. Culture medium was removed and cells were washed with PBS, before the addition of 4% paraformaldehyde for 10 min at room temperature. After washing with PBS, cells were treated with PBS containing 0.1% Triton x100 and incubated on ice for 20 min. Blocking was done with 5% BSA in PBST for 1 h at RT. Anti-Prdx2 (Sigma) was used at a dilution of 1:1000 in blocking buffer overnight at 4° C. Secondary antibody was anti-mouse conjugated with Alexa Fluor 488 (Santa Cruz Biotechnology). A Zeiss LSM510 META Axiovert 200M microscope supported by LSM software was used for fluorescent imaging by laser scanning confocal microscopy. An oil 40× objective lens was used with an argon ion laser emitting at 488 nm.



Supplementary Figure S1. Aggregation of GFP-Prdx2 C51S fusion protein in HEK 293T cells. Wild-type and C51S Prdx2 were expressed as GFP-fusion proteins for analysis by confocal microscopy. Upper panels – fluorescent signal images only; lower panels – fluorescence and phase contrast images. (A) Wild-type GFP-Prdx2 was visible as punctate cytoplasmic staining, in contrast to (B) the GFP control, which showed diffuse staining throughout the cytoplasm and nucleus. (C) C51S mutant GFP-fusion was present in much larger aggregates within the cytoplasm. (D) Staining of endogenous Prdx2 using anti-Prdx2 antibody followed by immunoflourescent labelling confirmed that the punctate cytoplasmic staining observed for the wild-type recombinant Prdx2 accurately reflected the localisation of the endogenous protein.



Supplementary Figure S2. Release of Prdx2 does not occur via the classical pathway. (A) Western blot of Prdx2 released by HEK 293T cells expressing recombinant wild-type Prdx2 in response to treatment with TNF-α in the presence and absence of BFA. (B) Release of IL-1 from HEK 293T cells in measured by ELISA. (C and D) Western blot of Prdx2 released by HEK 293T cells expressing recombinant wild-type Prdx2 in response to treatment with TNF-α in the presence and absence of glyburide (C) or nocadazole (D).



Supplementary Figure S3. Prdx2 is present in exosomes isolated via Exo-spin exosome purification kit. Western blot analysis of Prdx2 in cell supernatants and exosomes from HEK293T cells transiently transfected with DNA coding for Prdx2. Blots were probed with anti-Prdx2 antibody (left panel) or anti-V5 tag antibody (middle panel). The presence of exosomes was confirmed by detection of HSP70 in supernatants and exosomes (right panel).