Supplemental Method 1.

Cell treatment Cells were rinsed and incubated with 3 ml of Dulbecco's Phosphate-Buffered Saline (D-PBS) (Invitrogen; Carlsbad, CA) for 1 min followed by a 5 min treatment with 0, 0.0625, 0.125, 0.25, 0.5 and 1 mM peroxynitrite. Medium was then aspirated and cells were washed with 3 ml of D-PBS. Ten ml of complete DMEM medium was added to the cells followed by a 24 hr recovery. In experiments testing the reversal of nitration by N-acetylcysteine (NAC), 10 ml of complete DMEM medium containing 1 mM NAC was added to the cells after removal of peroxynitrite. Then the cells were further incubated at 37°C in a 5 % CO_2 incubator for 24 hr before harvesting. Cells including floating and attached were harvested at end of each treatment and washed twice with D-PBS.

Supplemental Method 2.

Western blotting Proteins separated by the SDS gel were electrotransferred to a nitrocellulose membrane (Amersham Biosciences; Piscataway, NJ) using 45 volts for 3 hr. Proteins separated by the Blue-native gel were electrotransferred to a PVDF membrane (Biorad; Hercules, CA) at 50 volts overnight in cold room. After transfer, the PVDF membrane from the Blue-native gel was incubated with 8% acetic acid for 15 min to fix the proteins. The PVDF Blot with fixed protein and the nitrocellulose membrane from the SDS gel were then blocked with Tris-buffered saline (TBS)/Ody Blocking buffer (LI-COR Biosciences; Lincoln, NE) (1:1 in volume) at 4°C overnight, and incubated with rabbit E1k, E2k or E3 antibody [E1k and E2k antibodies were generated in collaboration with Rockland Immunochemicals Inc. (Gilbertsville, PA); E3 antibody was a generous gift from Gordon Lindsay] and anti-nitrotyrosine antibody (a generous gift from Alvaro G. Estevez) (1:1,000 dilution) at room temperature for 3.5 hr. After washing thoroughly with TBS/0.1% Tween-20 at room temperature, the blots were incubated with Odyssey Goat anti-rabbit IRDye680 antibody and goat anti-mouse IRDye800CW antibody at room temperature for 1 hr (1:5,000; LI-COR Biosciences, Lincoln, NE). After extensive washing, the membranes were scanned with the Odyssey Infrared Imaging System (LI-COR Biosciences; Lincoln, NE) and analyzed with the Odyssey V3.0 software.

Supplemental Method 3.

Isolation of mitochondria from N2a cells Mitochondria were isolated from N2a cells by modification of a previous method (34). Cell pellets were resuspended in 10 ml of ice cold MS buffer (1 mM EGTA, 75 mM Sucrose, 5 mM Hepes, 225 mM D-mannitol and 1 mg/ml BSA). Cell resuspensions were kept on ice for 10 min after addition of 4 μl of 5% digitonin. An additional 10 ml of ice cold MS buffer was added to cell suspension after incubation, and the total suspension was centrifuged at 3,200 g for 10 min. The pellet was resuspended with 7 ml of MS buffer without BSA and homogenized for 7-8 strokes using a Dounce-type homogenizer (pre-chilled) with a glass pestle B. Another 7 ml MS buffer without BSA was added to rinse the homogenizer and was combined with the homogenate followed by centrifugation at 1,000 g for 5 min. The pellet was discarded and the supernatant was further centrifuged at 16,100 g for 10 min. The pellet containing crude mitochondria was washed with 1 ml MS without BSA by centrifugation at 16,100 g for 15 min. The supernatants were discarded and the whitish overheads covering brownish pellets were removed carefully. The mitochondrial pellets (brownish pellets) were resuspended with 700 μl of MS buffer without BSA and centrifuged at 16,100 g for 10 min. The pellets (mitochondria) were saved for mitoplast isolation.

Supplemental Method 4.

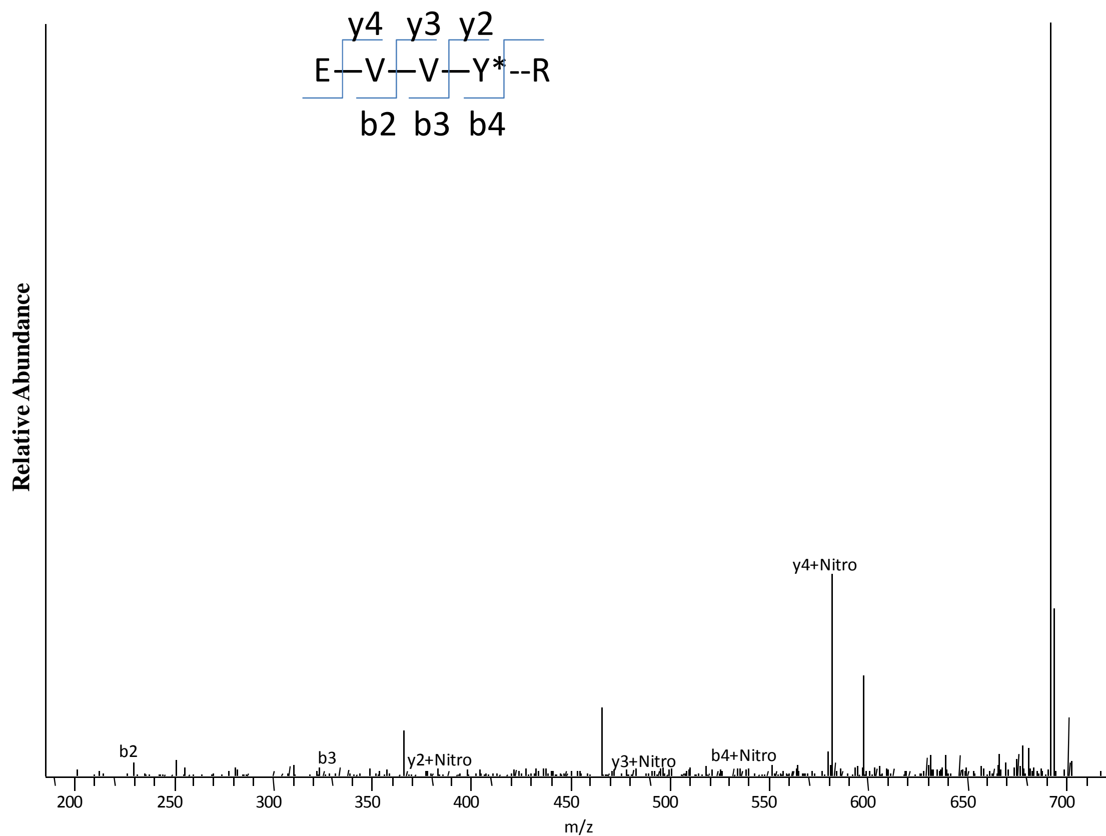
Mitoplast preparation and KGDHC purification Mitoplasts were isolated by modification of a published method (35). The mitochondria were resuspended with 0.5 ml of ice cold H_2O and incubated on ice for 20 min. At the end of incubation, the resuspension was pelleted by centrifugation at 4,000 g for 10 min. The pellets containing mitoplasts were used for KGDHC purification. Mitoplasts were suspended in 200 µl of 1x native sample buffer (50 mM Tris, 50 mM NaCl, 10 % w/v Glycerol, 0.001 % Ponceau S and 10 % n-dodecyl β-D-maltoside, pH7.2), and the suspension was homogenized using a Kontes* Pellet Pestle* Cordless Motor (Fisher Scientific) for 30 sec. The samples were then centrifuged at 100,000 g for 60 min using an Optima™ TL Ultracentrifuge (BECKMAN Coulter; Brea, CA). The pellets were re-suspended with 600 µl 1x native sample buffer and homogenized using the Kontes* Pellet Pestle* Cordless Motor for 30 sec. The homogenates were centrifuged with the optima Ultracentrifuge at 100,000 g (rotor TLA 100.1) for 30 min at 4°C. The supernatants (500 µl) were subjected to KGDHC purification using the same method with that of KGDHC from Sigma KGDHC stock (see the first section in Experimental Procedures).

Supplemental Method 5.

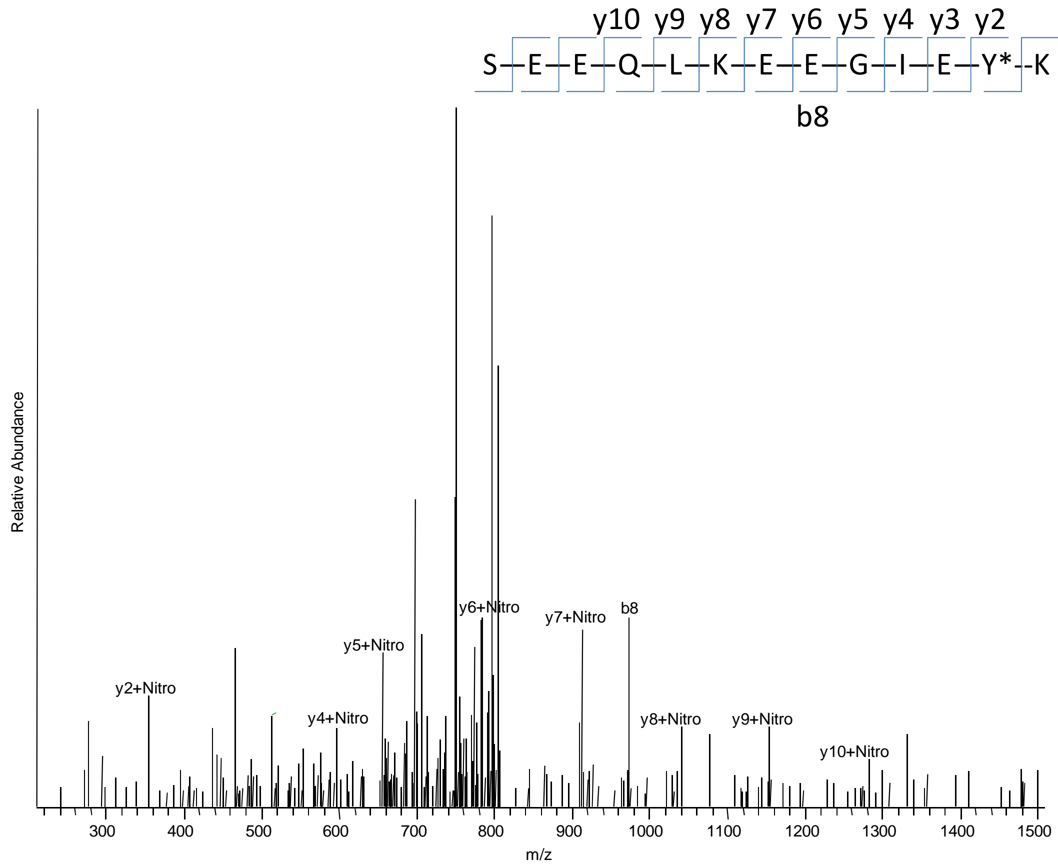
Real-time PCR Each amplification mixture (20 µl) contained 9 µl of cDNA template, 10 µl of TaqMan® Fast Universal PCR Master Mix, 1 µl of a FAM™ dye labeled TaqMan® MGB probe and two PCR primers. Thermal cycler conditions were 95°C for 20 sec, and 40 cycles of 95°C for 3 sec and 60°C for 30 sec. All samples were normalized for beta-2-microglobulin (b2m) expression. A comparative *Ct* (the threshold cycle of PCR at which amplified product was first detected) method was used to compare the mRNA levels in samples from treated to that of the control.

Supplemental Figure 1.

A.



B.



Legend for Supplemental Figure 1.

The selected MS/MS spectra of a nitrated peptide EVVY*R of E2k at *m/z* 710.36 **(A)** and a nitrated peptide SEEQLKEEGIEY*K of E3 at *m/z* 813.89 **(B)**. The fragment ions match most of the y and b ions of the peptides, in which the tyrosine (Y) residue was nitrated with a mass difference of 45Da.