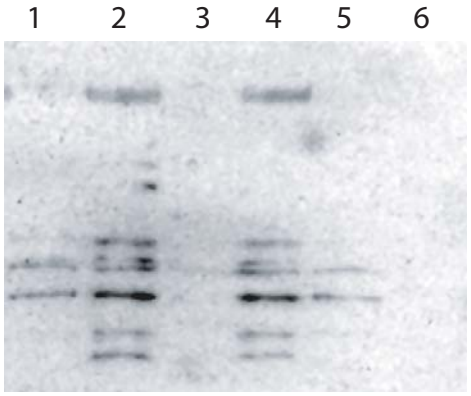
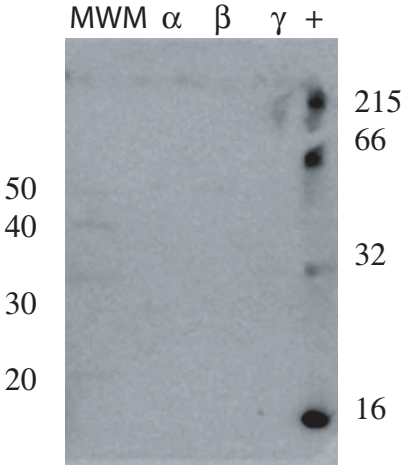
Supplementary Figure 1
At the beginiing of the studies and every time that a new batch of antibody for nitrotyrosine was purchased, the antibody was tested for its specificity towards nitrotyrosine (examples shown below). To this end, the reactivity of the antibody against nitrated mitochondrial proteins and amino-containing mitochondrial proteins was tested (Supplementary Figure 1) without or with previous reduction of the nitro group to amino by using either ascorbic acid (A) or sodium dithionite (not shown). In addition, its reactivity towards nonnitrated, recombinant proteins (B) and nitrated molecular weight markers (+) was also tested.



PANEL A
Western blot of nitrated mitochondrial proteins
One-mg of rat liver mitochondria was pretreated with 0 (lanes 2 & 4), 1 (lanes 1 & 5), 10 (lane 3), and 100 (lane 6) mM ascorbic acid or 0, 1, 5, and 10 mM sodium dithionite (not shown) for 5-6 min on ice.
Then the samples were diluted with PBS, and treated with Laemmli buffer. One microgram of each sample was loaded onto a 12.5% Tris-Gly SDS-PAGE. The gel was electrotransferred to a PVDF membrane and probed with anti-nitroTyr monoclonal antibody (Upstate; cat #5-233, Lot 23305, clone 1A6).

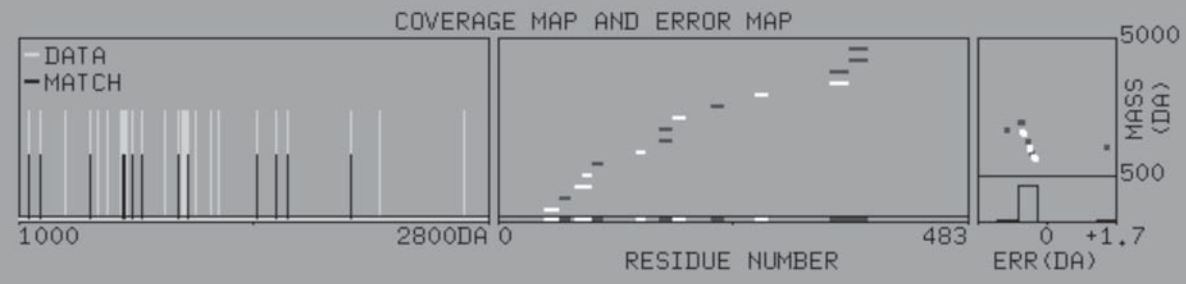


PANEL B
Western blot of ATPase recombinant proteins
One microgram of recombinant alpha, beta and gamma subunits were loaded onto a 12.5% Tris-Gly SDS-PAGE.
The gel was electrotransferred to a PVDF membrane and probed with anti-nitroTyr monoclonal antibody (Upstate; cat #5-233, Lot 23305, clone 1A6).
Abbreviations: MWM, molecular weight markers in kDa.
+: Positive control constituted by nitrated proteins of known MW.

Supplemental Figure 3: Analysis of the peptide masses obtained by MALDI-ToF indicating those peptides that had been modified by the treatment

Name: (unnamed)

Number of Measured Peptides : 30
Number of Matched Peptides : 15
Min. Coverage of Sequence : 34%



Measured Mass	Ave/ Mono	Computed Mass	Error (Da)	Residues Start	Residues End	Missed Cut	Peptide Sequence
1037.302	M	1037.586	-0.284	88	97	0	IPVGPETLGR
1087.322	M	1087.627	-0.305	143	152	0	VVDLLAPYAK
1277.252	M	1277.628	-0.376	64	75	0	TIAMDGTEGLVR (1)+O@M
1400.292	M	1400.696	-0.404	98	109	0	IMNVIGEPIDER (1)+O@M
1405.262	M	1405.673	-0.411	180	193	0	AHGGYSVFAGVGER
1434.332	M	1434.746	-0.414	265	278	0	FTQAGSEVSALLGR
1472.402	M	1472.827	-0.425	167	179	0	TVLIMELINNVAK (1)+O@M
1474.342	M	1472.827	1.515	167	179	0	TVLIMELINNVAK (1)+O@M
1616.332	M	1616.798	-0.466	219	233	0	VALVYGQMNEPPGAR (1)+O@M
1649.462	M	1649.910	-0.448	49	63	0	LVLEVAQHLGESTVR
1917.532	M	1918.088	-0.556	79	97	1	VLDSGAPIKIPVGPETLGR
1986.442	M	1987.026	-0.584	342	360	0	AIAELGIYPAVDPLDSTSR
2031.012	M	2032.011	-0.999	342	360	0	AIAELGIYPAVDPLDSTSR (1)+NO2-H@Y
2271.442	M	2272.090	-0.648	361	380	1	IMDPNIVGSEHYDVARGVQK (1)+NO2-H@Y
2271.482	M	2272.090	-0.608	361	380	1	IMDPNIVGSEHYDVARGVQK (1)+NO2-H@Y

Unmatched Monoisotopic Masses:
1178.252 1306.292 1343.422 1390.342 1395.232 1416.282 1559.392 1627.442 1637.402 1639.422 1681.412 1739.422 1764.222 2382.262 2704.292

Input Summary

Protein

```
   1  *    10   *    20   *    30   *    40   *    50
   1 AAQSSAAPKAGTATGQIVAVIGAVVDVQFDEGLPPILNALEVQGRESRLV
  51 LEVAQHLGESTVRTIAMDGTEGLVRGQKVLDSGAPIKIPVGPETLGRIMN
 101 VIGEPIDERGPIKTKQFAPIHAEAPEFIEMSVEQEILVTGIKVVDLLAPY
 151 AKGGKIGLFGGAGVGKTVLIMELINNVAKAHGGYSVFAGVGERTREGNDL
 201 YHEMIESGVINLKDATSKVALVYGQMNEPPGARARVALTGLTVAEYFRDQ
 251 EGQDVLLFIDNIFRFTQAGSEVSALLGRIPSAVGYQPTLATDMGTMQERI
 301 TTTKKGSITSVQAIYVPADDLTDPAPATTFAHLDATTVLSRAIAELGIYP
 351 AVDPLDSTSRIMDPNIVGSEHYDVARGVQKILQDYKSLQDIIAILGMDEL
 401 SEEDKLTVSRARKIQRFLSQPFQVAEVFTGHMGKLVPLKETIKGFQQILA
 451 GDYDHLPEQAFYMVGPIEEAVAKADKLAEEHGS
```

Enzyme	Name	Site(s)	Terminal	Mod.	Proline	Missed Cut
	Trypsin	KR	C		N	2

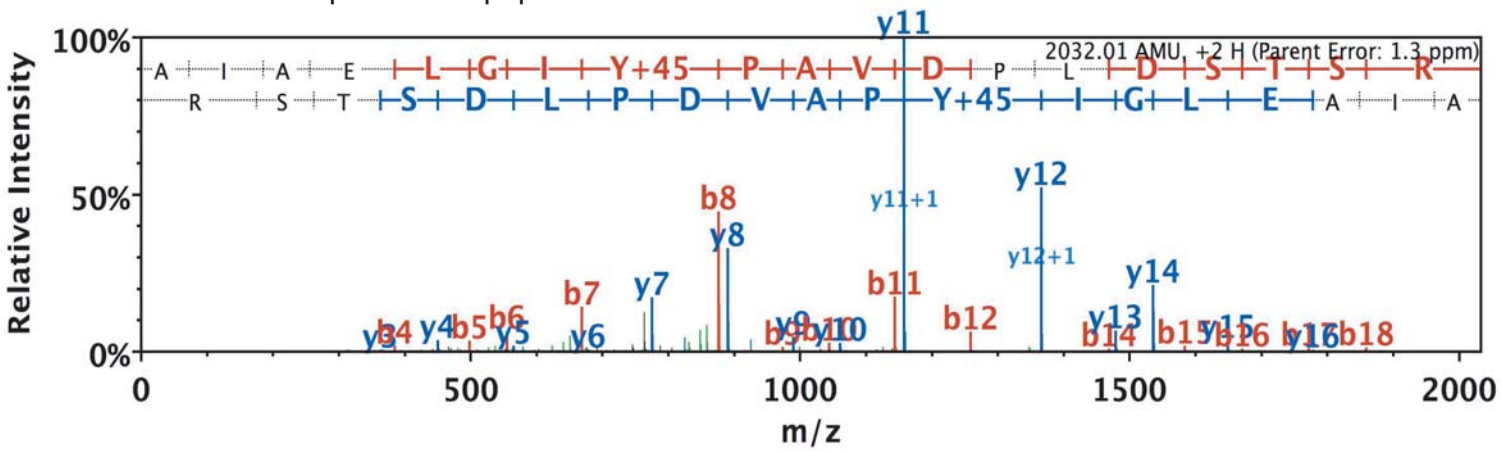
Global Modification	Name	Site(s)	Formula	Min. Occ.	Max. Occ.
	Iodoacetamide (Cys)	C	+C2H3ON	Complete	
	Nitration (Y)	Y	+NO2-H	Partial	
	Oxidation (M)	M	+O	Partial	

Monoisotopic Masses (+/- 2 Da)
1037.302 1087.322 1178.252 1277.252 1306.292 1343.422 1390.342 1395.232 1400.292 1405.262 1416.282 1434.332 1472.402 1474.342 1559.392 1616.332 1627.442 1637.402 1639.422 1649.462 1681.412 1739.422 1764.222 1917.532 1986.442 2031.012 2271.442 2271.482 2382.262 2704.292

Charge State MH+

Supplementary figure 4 Example of one of the MS/MS spectrum obtained with one of the peptides that showed nitration in the MALDI-ToF analysis

A.. MS/MS Spectrum of peptide with mass 2032.01



B. Error spectrum/model of the above spectrum