Supplementary information

Supplementary Fig 1

(A) shRNA of Prdx6. shRNA of human Prdx6 (GenBank accession number, NM 004905) were predicted by siRNA Target Finder provided by Ambion®. The target sequence of Prdx6 (271~293) was indicated in bold letters. Sense strand and antisense strand as indicated were chemically synthesized and cloned into pSUPER.retro vector according to the manufacturer's instruction (OligoEngine). (B) A control shRNA was predicted from non-coding region of Prdx6. Sense strand and antisense strand as indicated were chemically synthesized and cloned into pSUPER.retro vector according to the manufacturer's instruction (OligoEngine). (C) HEK293 cells were transiently transfected with mock vector (pSUPER. retro), different concentrations of shRNA of Prdx6 as indicated, or different concentrations of control shRNA. At 36 h after transfection, cell lysates were subjected to immunoblotting with anti-Prdx6 and GAPDH antibodies.

Supplementary Table 1

HeLa and HEK293 cells were treated with different concentrations of H_2O_2 as indicated for 24 h. Cells were stained with annexin V–FITC Detection Kit as following manufacturer's protocols. The percentages of Annexin V-positive cells were analyzed by FACSCalibur™ system and determined by CellQuest software. The results are the means ±S.D. for triplicate assays.

Supplementary Fig 2

HeLa cells were treated with different concentrations of H_2O_2 as indicated for 20 min, washed with HBSS, and further incubated for 18 h or 48 h. Apoptotic cells were identified by staining with Annexin V–FITC (PharMingen) according to the manufacturer's protocols. The percentage of Annexin V-positive cells was analyzed with the FACSCalibur™ system and determined with the CellQuest software. The results are expressed as mean ± S.D. for triplicate assays

Supplementary Fig 3

HeLa cells were treated with TNF-α (10 ng/ml) or LPS (5 μg/ml) for different amounts of time as indicated. Cell lysates were subjected to immunoblotting with anti-Prdxs SO_3H that recognizes Prdx1-, Prdx2- and Prdx3-SO_3H, anti-Prdx6 SO_3H, anti-Prdx1, anit-Prdx2. anti-Prdx3, anti-Prdx6, and anti-GAPDH antibodies.

A

1
ATGCCCGGAGGTCTGCTTCTCGGGGACGTGGCTCCCAACTTTGAGGCC
271 293
·····**AAGGGCATGCCTGTGACAGCT**CGTGTGGTGTTTGTTTTTGGTCCTG
ATAAGAAGCTGAAGCTGTCTATCCTCTACCCAGCTACCACTGGCAGGAA
675
····· CAGCCT**TAA**

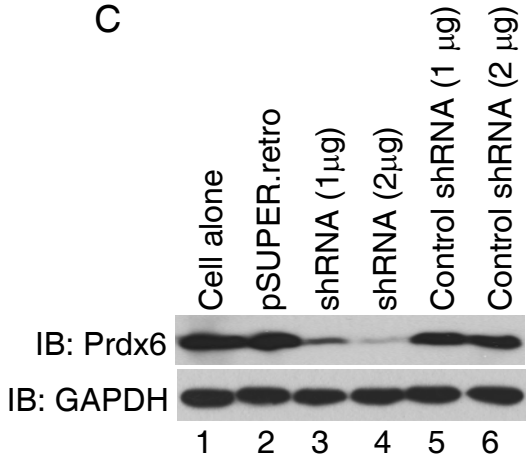
Sense strand shRNA: GGGCAUGCCUGUGACAGCUtt
Antisense strand shRNA: AGCUGUCACAGGCAUGCCCtt

B

AAAAGGCTTCCCTTGGCTCCC

Sense strand siRNA: AAGGCUUCCCUUGGCUCCCtt
Antisense strand siRNA: GGGAGCCAAGGGAAGCCUUtt

C



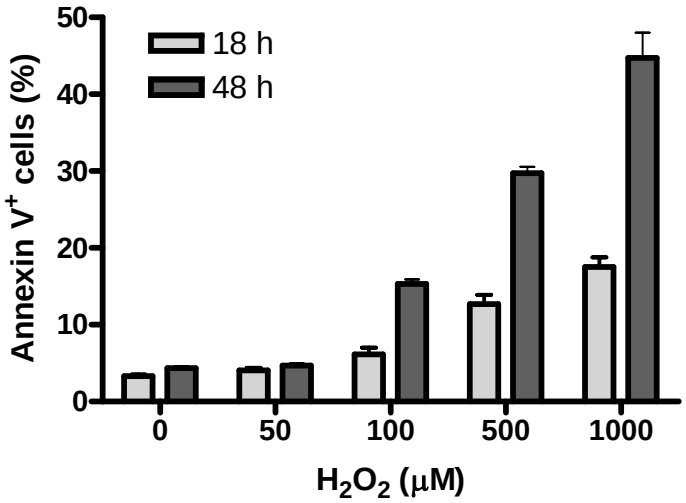
Supplementary Fig 1

Table 1. Relative proportion of apoptotic cells (Annexin V^+ cells) following treatment of H_2O_2

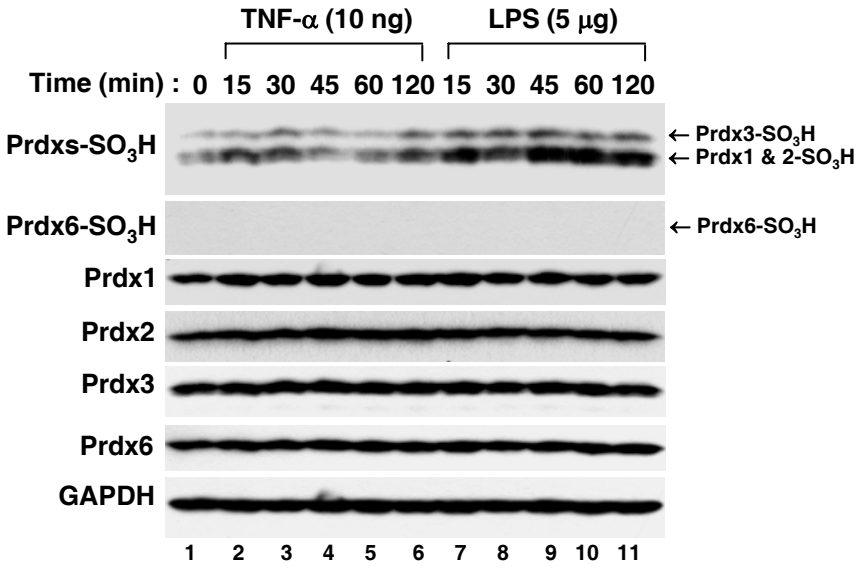
Cell type	H_2O_2 concentration (μM)		
	10 μM	100 μM	500 μM
HEK293 cells			
0 hr	10 ± 1.9	10 ± 1.9	**10 ± 1.9***
4 hr	11 ± 1.7	10 ± 2.1	**29 ± 2.8**
8 hr	10 ± 2.9	11 ± 1.8	**33 ± 3.7**
16 hr	10 ± 2.4	14 ± 3.1	**48 ± 2.9**
24 hr	10 ± 2.3	15 ± 2.2	**55 ± 3.8**
HeLa cells			
0 hr	4 ± 0.9	4 ± 0.9	**4 ± 0.9**
4 hr	4 ± 1.3	4 ± 0.8	**12 ± 1.8**
8 hr	5 ± 1.2	6 ± 0.7	**21 ± 3.1**
16 hr	4 ± 1.0	7 ± 1.1	**25 ± 1.3**
24 hr	5 ± 0.8	6 ± 1.4	**28 ± 2.7**

*: ± SD values from there independent experiments are shown

Supplementary Table 1



Supplementary Fig 2



Supplementary Fig 3