*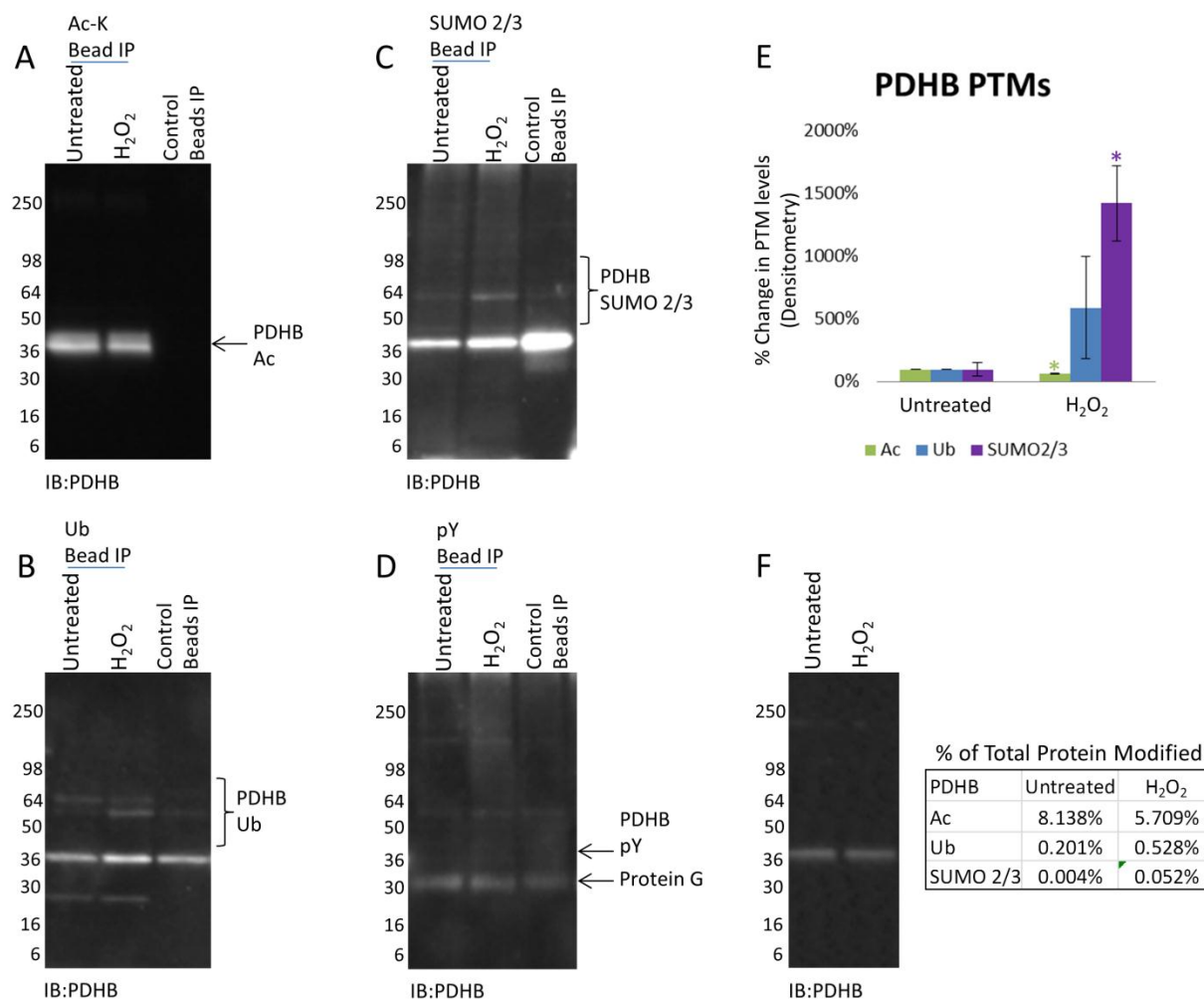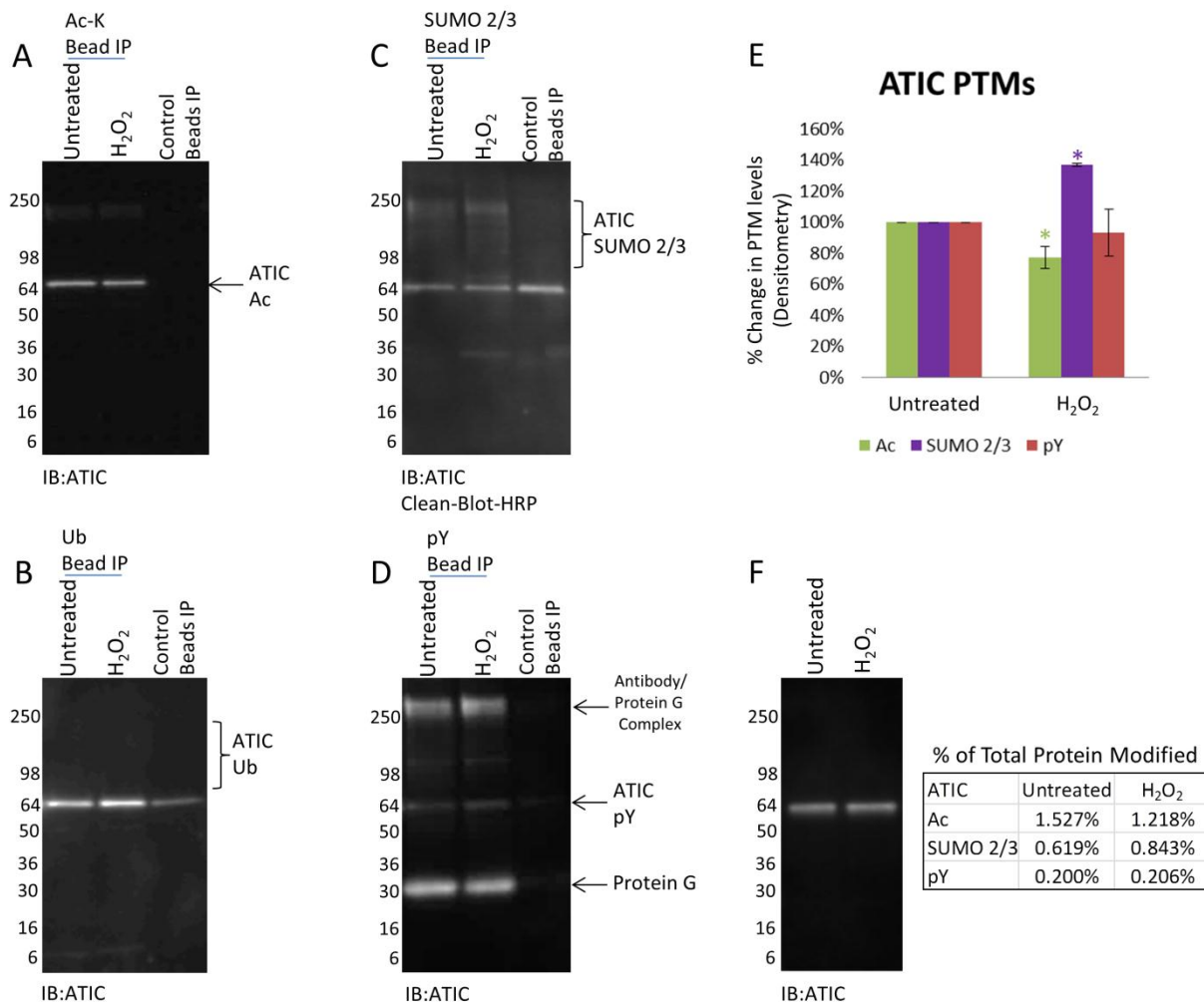**Supplementary Fig 1. Endogenous acetylation of mitochondrial targets.*** A431 cells either untreated or treated with H₂O₂ for 2 hours were lysed with BlastR lysis buffer. IP of acetylated proteins from 800 µg - 1 mg of lysate were performed using Ac-K Affinity beads or Acetyl-lysine IgG control beads. Eluted proteins were resolved in an SDS-PAGE gel and then transferred to a PVDF membrane. Western blots were performed with (A) ATIC, (B) PRDX2, (C) DTYMK, (D) SSBP1, (E) HK2, and (F) DLD antibodies. Shown are representative westerns from N≥3 independent experiments.

Ac-K Bead IP
SUMO 2/3 Bead IP
Ub Bead IP
pY Bead IP

PDHB PTMs

% of Total Protein Modified

PDHB	Untreated	H₂O₂
Ac	8.138%	5.709%
Ub	0.201%	0.528%
SUMO 2/3	0.004%	0.052%

Supplementary Fig 2. H₂O₂ induced Ac, Ub, SUMO 2/3, and pY modifications of PDHB. A431 cells either untreated or treated with H₂O₂ for 2 hours were lysed with BlastR lysis buffer. Untreated and treated A431 lysates were incubated with (A) Ac-K beads to IP acetylated proteins and analyzed for acetylated PDHB, (B) Ub beads to IP ubiquitinated proteins and analyzed for ubiquitinated PDHB, (C) SUMO 2/3 beads to IP SUMOylated 2/3 proteins and analyzed for SUMO 2/3 modified PDHB, (D) and pY beads to IP tyrosine phosphorylated proteins and analyzed for tyrosine phosphorylated PDHB. All IPs were performed with appropriate control beads to detect non-specific detection. Shown are representative westerns from N≥3 independent experiments. (E) Quantification of background subtracted densitometric analysis of PDHB PTMs. Error bars represent s.e.m. T-test statistical analysis was performed. *P<0.05. (F) WCL was analyzed for PDHB levels. The percentage of PTM modified PDHB relative to the total PDHB levels for each modification are shown.

Supplementary Fig 3. H₂O₂ induced Ac, Ub, SUMO 2/3, and pY modifications of ATIC. A431 cells either untreated or treated with H₂O₂ for 2 hours were lysed with BlastR lysis buffer. Untreated and treated A431 lysates were incubated with (A) Ac-K beads to IP acetylated proteins and analyzed for acetylated ATIC, (B) Ub beads to IP ubiquitinated proteins and analyzed for ubiquitinated ATIC, (C) SUMO 2/3 beads to IP SUMOylated 2/3 proteins and analyzed for SUMO 2/3 modified ATIC, (D) and pY beads to IP tyrosine phosphorylated proteins and analyzed for tyrosine phosphorylated ATIC. All IPs were performed with appropriate control beads to detect non-specific detection. Shown are representative westerns from N≥3 independent experiments. (E) Quantification of background subtracted densitometric analysis of ATIC PTMs. Error bars represent s.e.m. T-test statistical analysis was performed. *P<0.05. (F) WCL was analyzed for ATIC levels. The percentage of PTM modified ATIC relative to the total ATIC levels for each modification are shown.