



Supplemental figure 6. SIRT1 induces Naged to form mitochondria-dependent vital NETs via opening mPTP channels.

(A and B) NADPH oxidase (Nox) activity analysis **(A)** and expression of the *PAD4* mRNA **(B)** in aged and non-aged neutrophils from the lungs of 2-week tumor bearing mice; total lung neutrophils stimulated with PMA served as the positive control. **(C)** Expression of the Cit-Histone H3 protein in neutrophils from the PB of patients with breast fibroadenoma and the BM of naïve mice treated with SRT1720 or PMA *in vitro*. **(D)** Immunofluorescence staining and quantification for NETs from neutrophils in the BM of naïve mice treated with SRT1720 and PMA in the presence or absence of Apocynin (Nox inhibitor). **(E)** Flow analysis of the survival of neutrophils in PB from patients with breast fibroadenoma and the BM of naïve mice treated with SRT1720 or PMA *in vitro*. **(F)** Representative TEM images showing the morphology of neutrophils from the PB of patients with breast fibroadenoma and the BM of naïve mice treated with SRT1720 or PMA. **(G)** Immunofluorescence staining for NET components produced by neutrophils from the PB of patients with breast fibroadenoma and the BM of naïve mice treated with SRT1720 and PMA. Green, Cit-Histone H3. Red, Sytox Orange. **(H)** Concentration of cfDNA (cell-free DNA) in cultured supernatants from neutrophils in the PB of patients with breast fibroadenoma and the BM of naïve mice treated with SRT1720 and PMA. **(I)** PCR analysis of NET components produced by neutrophils in PB from patients with breast fibroadenoma and the BM of naïve mice treated with SRT1720 in the presence or absence of mPTP inhibitors (TRO19622 or Cyclosporin A). Data are presented as the means \pm SD from one representative experiment. Similar results were obtained from three independent experiments, unless indicated otherwise. Statistical analysis was performed by two-tailed unpaired Student's t test **(D, E)** and one-way ANOVA **(A, B, C and H)**. ns, not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.