SUPPLEMENTARY MATERIALS FOR:

PHARMACOLOGICAL ASCORBATE INHIBITS PANCREATIC CANCER METASTASES VIA A PEROXIDE-MEDIATED MECHANISM

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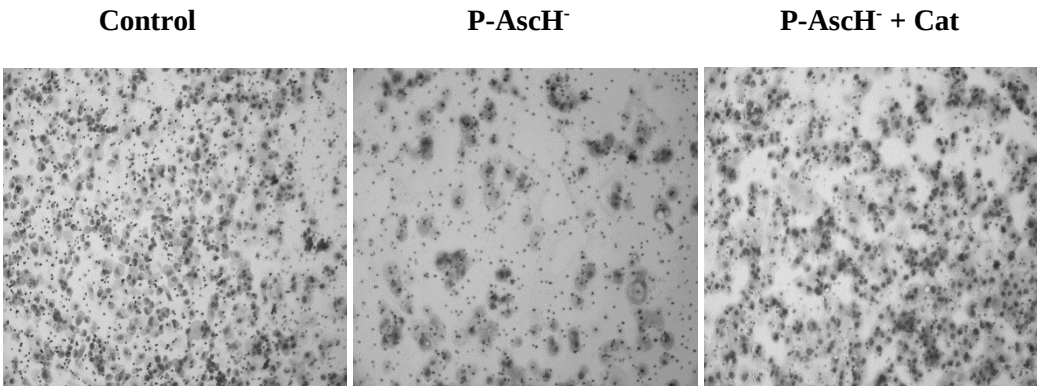
A



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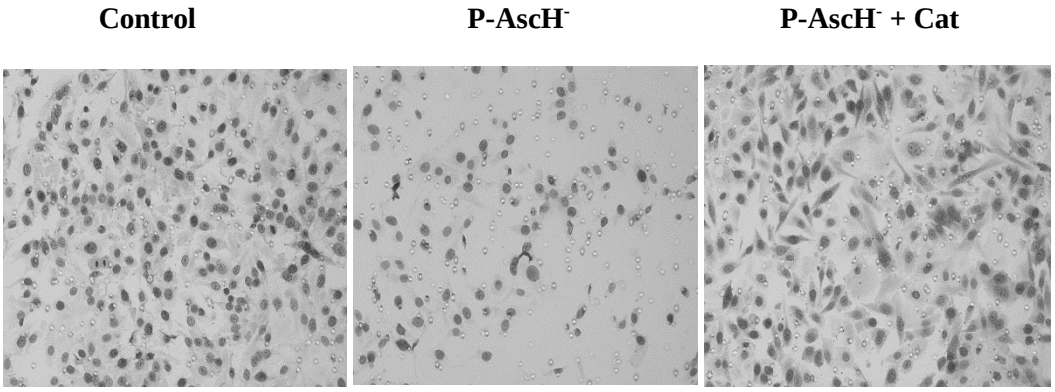
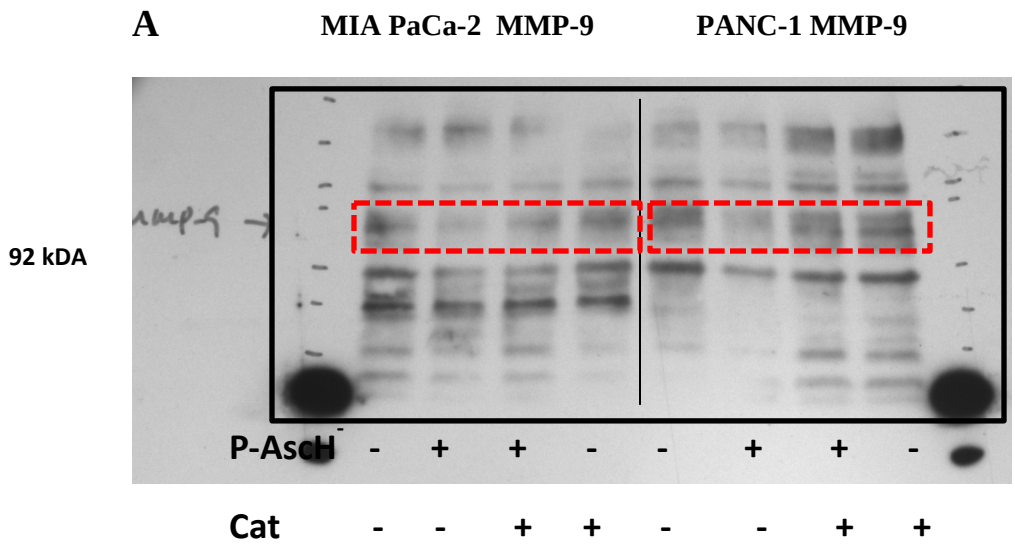
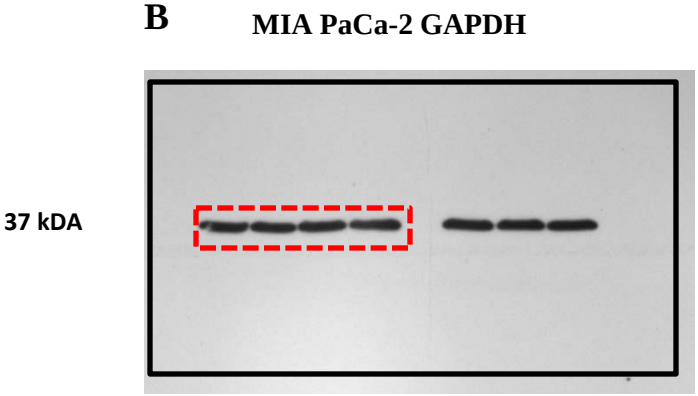
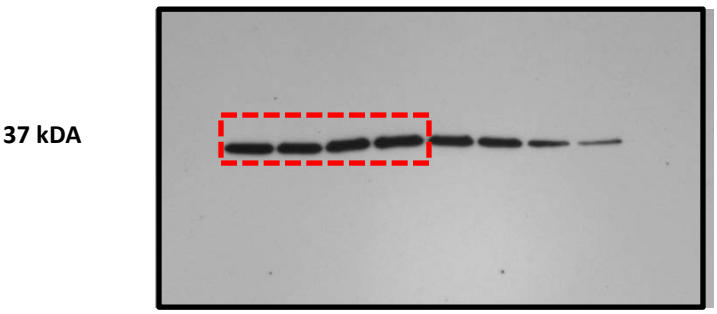


Supplemental Figure 1. P-AscH$^-$ attenuates the invasive phenotype of PDAC *in vitro.*

A & B. Representative invasion images from Panc-1 (A) and 339 (B) PDAC cells in the presence of P-AscH$^-$ (4mM) and/or catalase (200 U/mL). Cells were treated for 1 h then seeded at 1-3 x 10^5 and incubated for 24 (PANC-1) or 48 h (339).



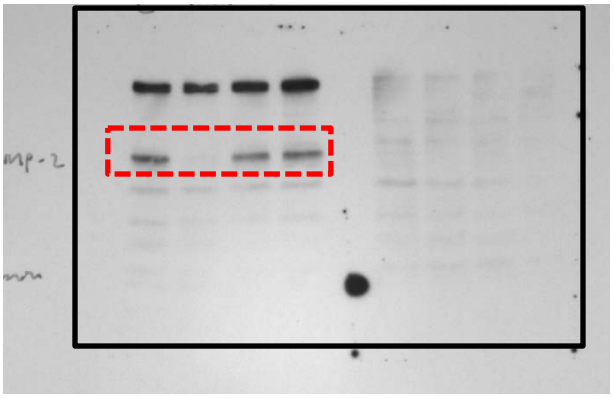




Supplementary Figure 2. Original unprocessed Western blots from main Figure 2C. **A.** MMP-9 expression from MIA PaCa-2 and PANC-1 cells following treatment with ascorbate +/- catalase. **B.** Corresponding GAPDH loading control from MIA PaCa-2 cells. **C.** Corresponding GAPDH loading control from PANC-1 cells.

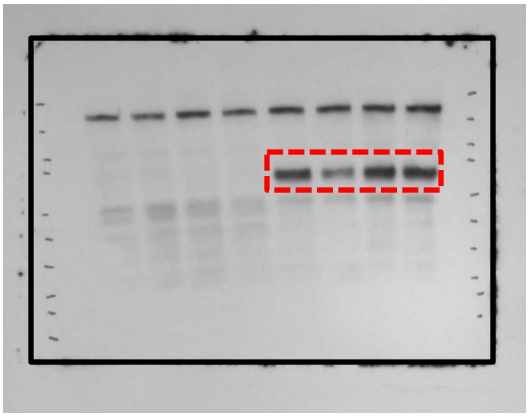
D

339 MMP-2



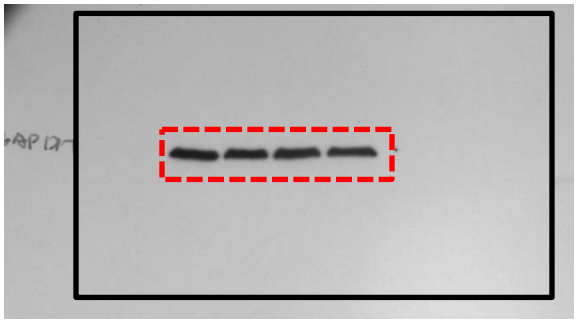
E

PANC-1 MMP-2



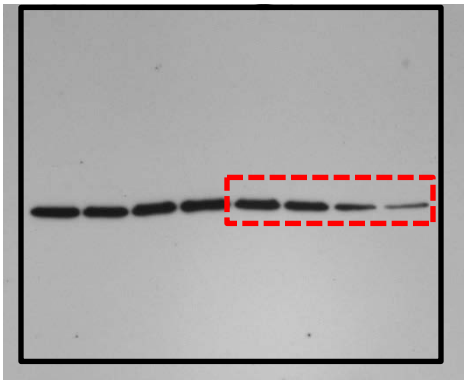
F

339 GAPDH

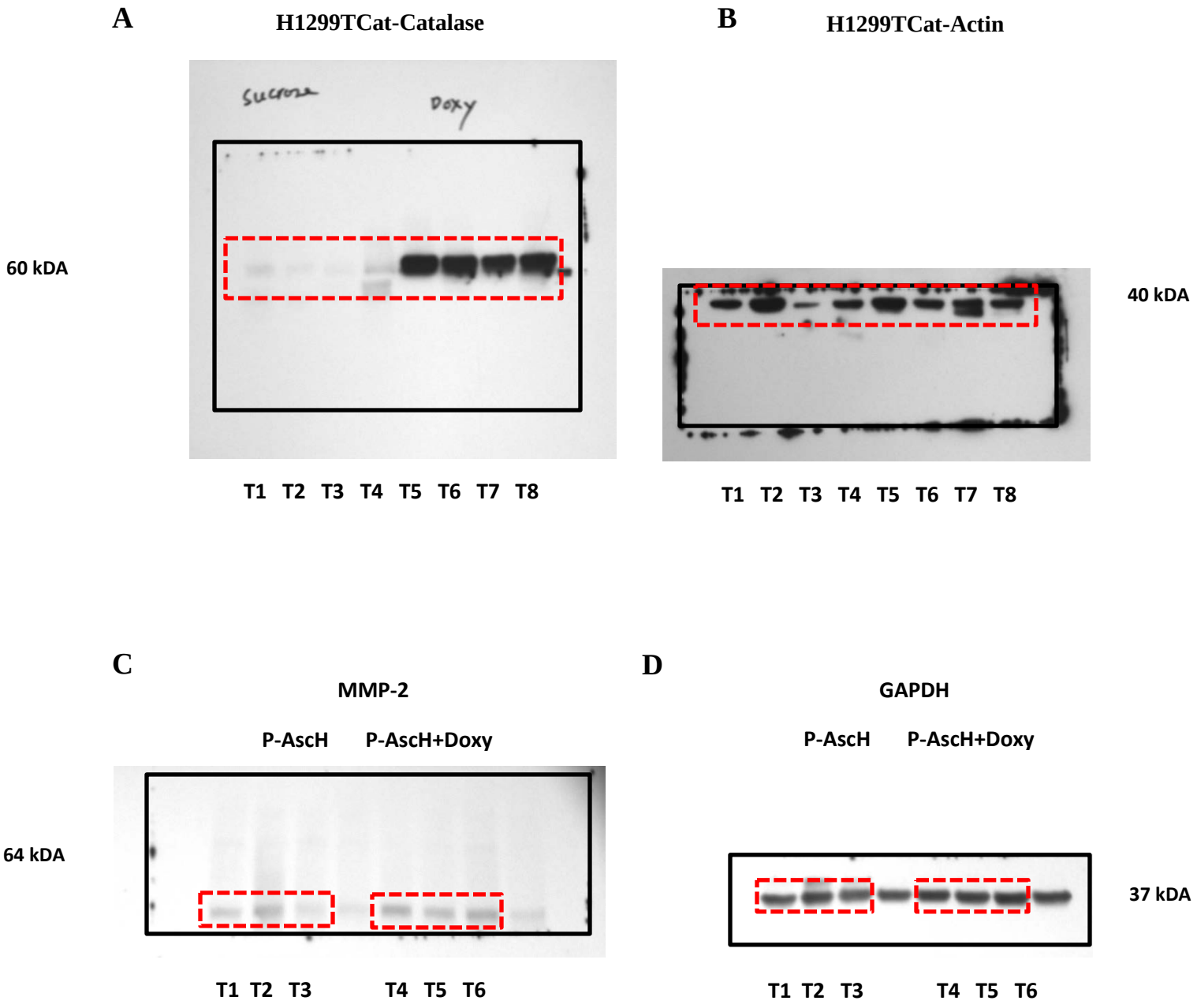


G

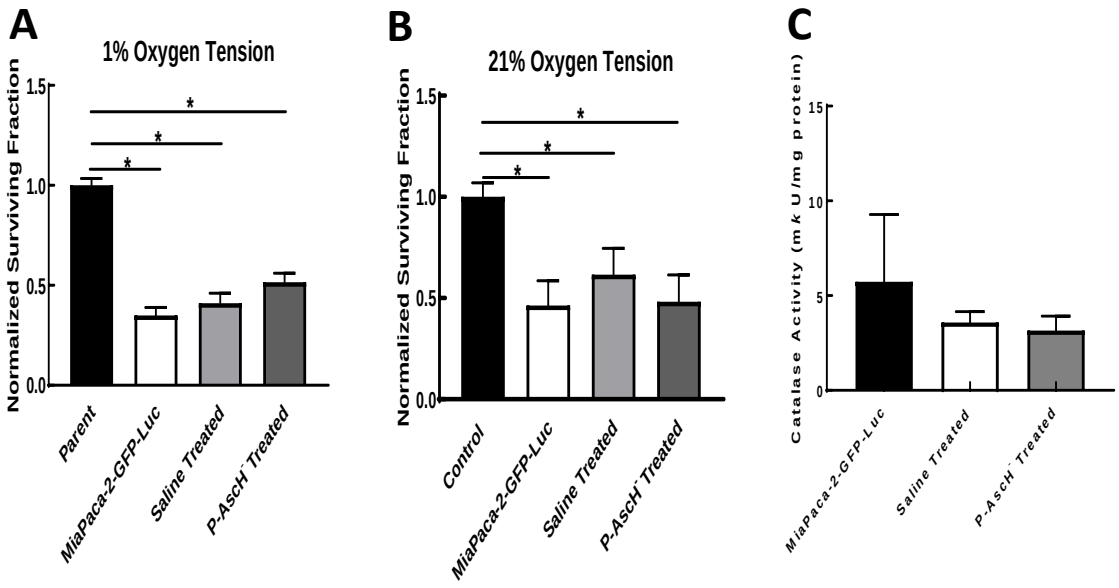
PANC-1 GAPDH



Supplementary Figure 2 Continued. Original unprocessed Western blots from main Figure 2D. **D & E.** MMP-2 expression from 339 and PANC-1 cells following treatment with ascorbate +/- catalase. **F.** Corresponding GAPDH loading control from 339 cells. **G.** Corresponding GAPDH loading control from PANC-1 cells.



Supplementary Figure 3. Original unprocessed Western blots from main Figures 4H&I. **A.** Catalase immunoreactive protein is increased in tumors from mice treated with P-AscH$^-$ + doxycycline. **B.** Corresponding Actin loading control from mouse tumors. **C.** MMP-2 immunoreactive protein is decreased in mice treated with P-AscH$^-$ compared to mice treated with P-AscH$^-$ + doxycycline. **D.** Corresponding GAPDH loading control from mouse tumors.



Supplemental Figure 4. No changes in P-AscH$^-$ toxicity or catalase activity in metastatic disease.

A and **B**. Liver specimens with visible metastatic lesions were used to isolate single tumor cells from both saline treated and P-AscH$^-$ treated mice. Cells were cultured at both 1% and 21% oxygen tension following confirmation of both GFP and luciferase expression after isolation. The stably expressing cell line (MIA PaCa-2-GFP-Luc) and isolated tumor cells from both saline treated and P-AscH$^-$ treated mice were incubated with 1 mM for 1 h and assessed for clonogenic cell survival. Colony formation was determined after 7-10 days. No differences were detected in clonogenic survival between the stably expressing cell line, saline treated, and P-AscH$^-$ treated isolated cells compared to the parental cell line. Surviving fraction was decreased with the addition of P-AscH$^-$ in the stably expressing cell line and isolated tumor cell lines compared to controls. Data represent normalized surviving fractions compared to controls ± SE ($n = 3$, * $p < 0.05$; one-way ANOVA with Bonferroni's multiple comparisons).

C. Isolated tumor cells from saline treated and P-AscH$^-$ treated mice where tested for catalase activity and compared to MIA PaCa-2-GFP-Luc cells. No differences were detected in catalase activity between stable expressing cells and saline or P-AscH$^-$ treated tumor cells. Data represent catalase activity in mK U\mg protein ± SE ($n = 3$, $p > 0.05$; one-way ANOVA with Bonferroni's multiple comparisons).