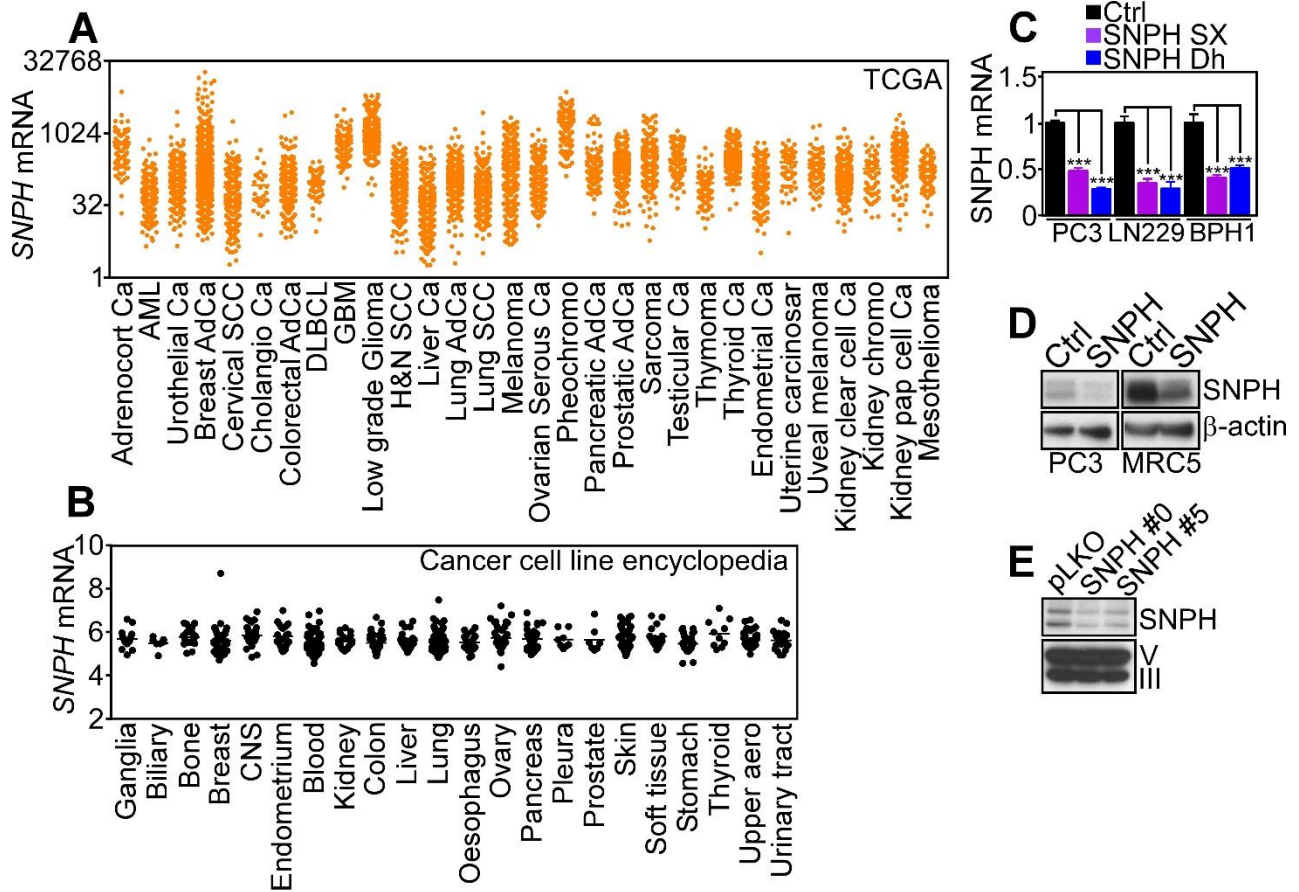


**SYNTAPHILIN CONTROLS A MITOCHONDRIAL *RHEOSTAT* FOR  
PROLIFERATION-MOTILITY DECISIONS IN CANCER**

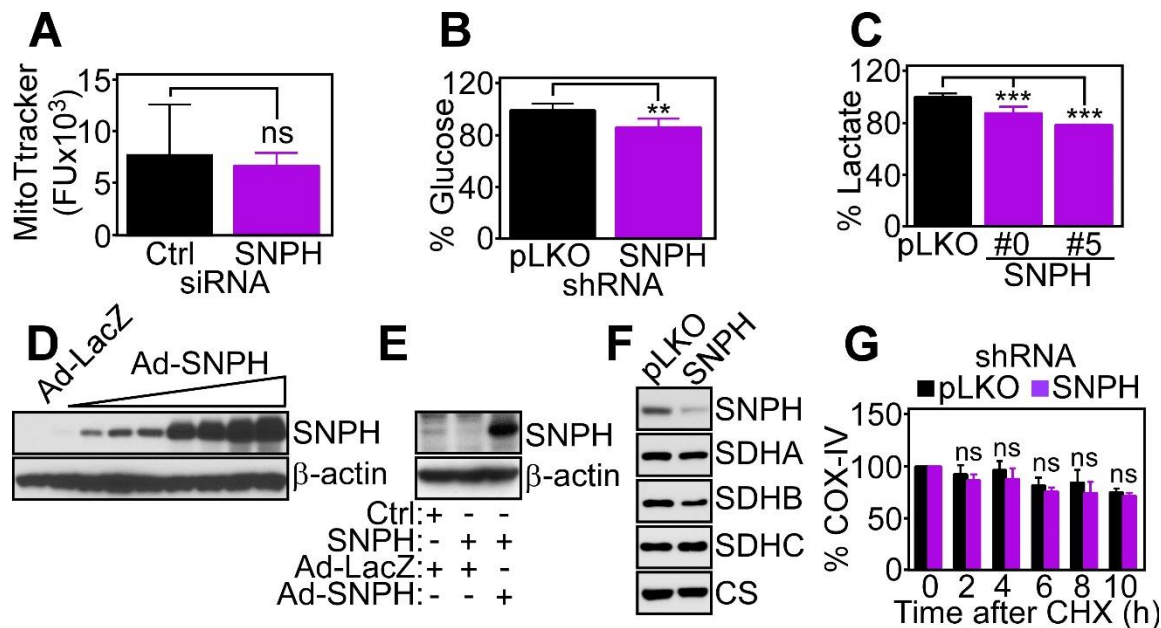
M. Cecilia Caino, Jae Ho Seo, Yuan Wang, Dayana B. Rivadeneira, Dmitry I. Gabrilovich,  
Eui Tae Kim, Ashani T. Weeraratna, Lucia R. Languino, and Dario C.  
Altieri

**SUPPLEMENTAL INFORMATION**



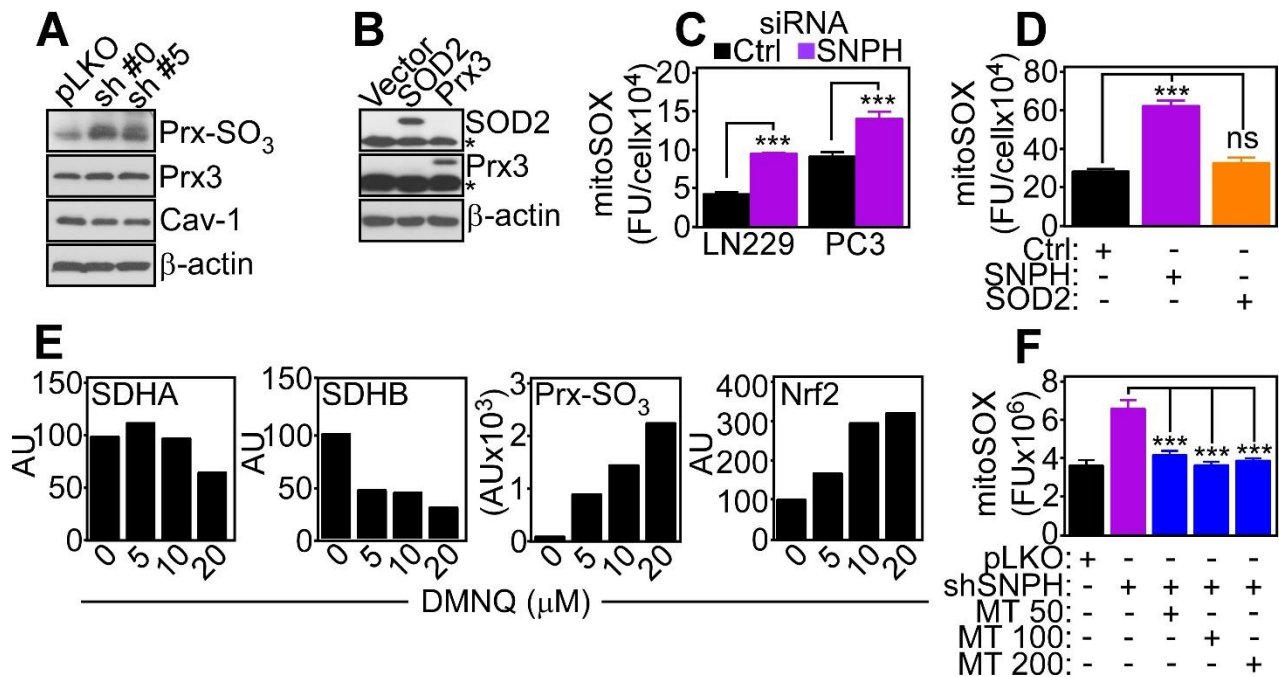
**Supplemental Figure 1. SNPH targeting.** (A) SNPH expression from all available TCGA database studies. Each dot represents an individual patient (RNA seq values for SNPH mRNA). Ca, carcinoma; AdCa, adenocarcinoma; SCC, Squamous Cell Carcinoma; AML, Acute Myeloid Leukemia; DLBCL, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; GBM, Glioblastoma Multiforme; H&N, Head and Neck; Pheochromo, Pheochromocytoma and Paraganglioma; Kidney chromo, Kidney Chromophobe; Kidney pap cell, Kidney Papillary Cell Carcinoma. (B) SNPH expression in the Cancer Cell Line Encyclopedia database. Each symbol represents a cell line (microarray normalized expression values for SNPH mRNA). (C) The indicated cell types were transfected with control non-targeting siRNA (Ctrl) or two independent SNPH-directed siRNAs (SX and Dh) and analyzed by quantitative PCR amplification. Data are expressed as the mean±SEM (n=3). \*\*\*, p<0.001 by ANOVA and Bonferroni's post test. (D) The indicated

tumor (PC3) or normal (MRC5) cell types were transfected with control non-targeting siRNA (Ctrl) or SNPH-directed siRNA and analyzed with a custom-made rabbit polyclonal antibody to SNPH by Western blotting. (E) PC3 cells stably transduced with control pLKO or two independent SNPH- directed shRNA sequences (clones #0 and #5) were analyzed by Western blotting. The levels of individual subunits of mitochondrial oxidative phosphorylation Complex V or Complex III were used as loading control.



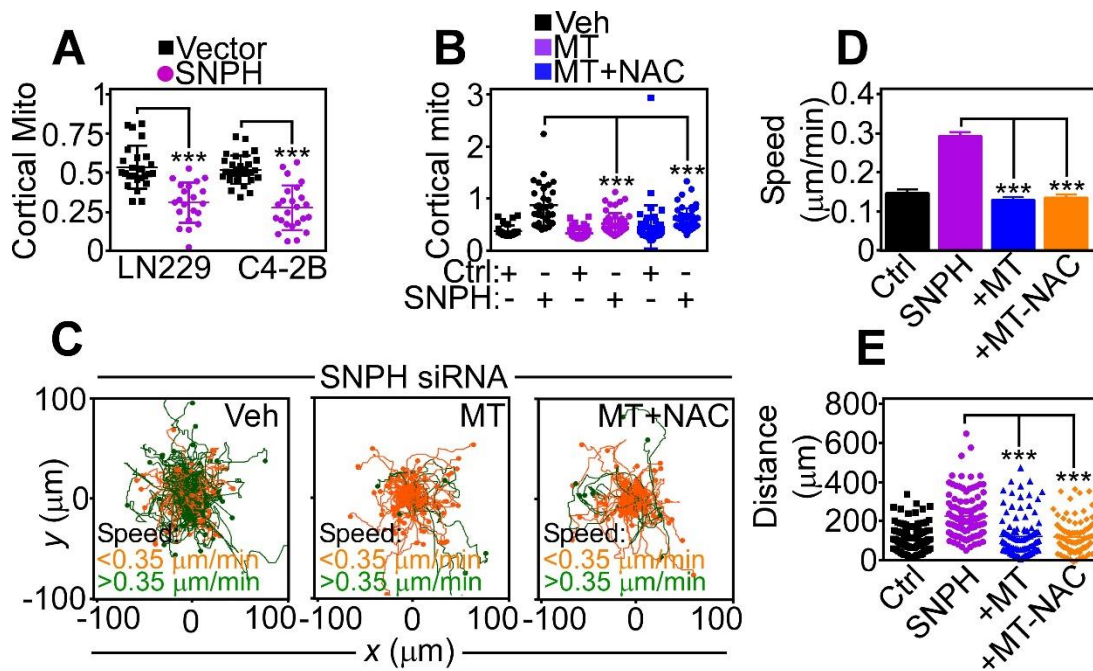
**Supplemental Figure 2. SNPH regulation of bioenergetics.** (A) PC3 cells transfected with control non-targeting siRNA (Ctrl) or SNPH-directed siRNA were analyzed for total mitochondrial mass by MitoTracker Green staining and fluorescence microscopy. Data are expressed as the mean±SD of replicates of a representative experiment (n=6). ns, not significant by 2-tailed Student's *t* test. (B and C) PC3 cells stably transduced with control pLKO or SNPH-directed shRNA were analyzed for glucose consumption (B) or lactate production (C). Data are expressed as the mean±SD of replicates of a representative experiment (n=3). \*\*, p=0.004, by 2-tailed Student's *t* test (B); \*\*\*, p<0.001 by ANOVA and Bonferroni's post test (C). (D) PC3 cells were infected at increasing multiplicity of infection with adenovirus encoding control (Ad-LacZ) or SNPH (Ad-SNPH) and analyzed by Western blotting. (E) PC3 cells transfected with control non-targeting siRNA (Ctrl) or SNPH-directed siRNA were infected with Ad-LacZ or Ad-SNPH and analyzed by Western blotting. (F) PC3 cells transfected with the indicated shRNA were analyzed by Western blotting. (G) PC3 cells transduced as in (F) were treated with cycloheximide (CHX), harvested at the indicated time intervals after release

and COX-IV protein bands were quantified by densitometric scanning. Data are expressed as mean $\pm$ SD (n=4). ns, not significant by 2-tailed Student's *t* test.



**Supplemental Figure 3. Regulation of mitochondrial oxidative stress by SNPH.** (A) PC3 cells transduced with pLKO or SNPH-directed shRNA and analyzed by Western blotting. (B) PC3 cells were transfected with vector, SOD2 or Prx3 cDNA and analyzed by Western blotting. (C) PC3 or LN229 cells transfected with control non-targeting siRNA (Ctrl) or SNPH-directed siRNA were analyzed for mitoSOX reactivity by fluorescence microscopy. Single-cell mitoSOX levels were expressed as the mean±SEM (n>140). \*\*\*, p<0.0001, by 2-tailed Student's *t* test. (D) PC3 cells expressing control non-targeting siRNA (Ctrl) or SNPH-directed siRNA were transfected with SOD2 cDNA and analyzed for mitoSOX reactivity and fluorescence microscopy. Single-cell mitoSOX levels were expressed as the mean±SEM (n>200). \*\*\*, p<0.001 by ANOVA and Bonferroni's post test. ns, not significant. (E) PC3 cells were treated with the indicated increasing concentrations of the oxidative stress stimulus, DMNQ and protein bands representative of SDHA, SDHB, Prx-SO<sub>3</sub> and Nrf2 were quantified by densitometric scanning. Representative experiment. (F) PC3 cells stably transduced with pLKO or SNPH shRNA were incubated with vehicle or the indicated increasing concentrations

of MitoTempo (MT) and analyzed for mitoSOX by fluorescence microscopy. Data are the mean $\pm$ SEM (pLKO, n=15, vehicle, n=51; MT 50, n=31; MT 100, n=6; MT 200, n=49). \*\*\*, p<0.001 by ANOVA and Bonferroni's post test.

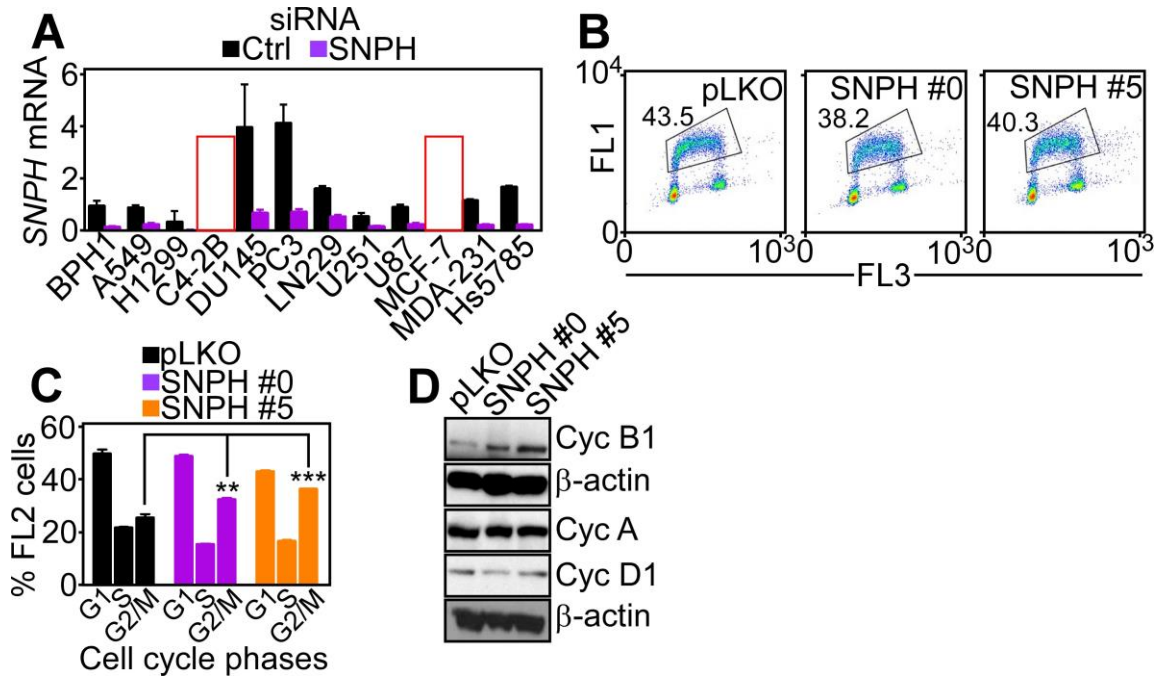


**Supplemental Figure 4. SNPH regulation of mitochondrial oxidative stress controls tumor**

**chemotaxis.** (A) The indicated cell types were transfected with vector or SNPH cDNA and analyzed for mitochondrial repositioning to the cortical cytoskeleton by fluorescence microscopy. Cortical mitochondrial localization was measured as fluorescence units ( $10^6$ ) and normalized to the total mitochondria signal per cell. Each symbol corresponds to an individual cell ( $n>30$ ). \*\*\*,  $p<0.0001$ , by 2-tailed Student's *t* test. (B) LN229 cells transfected with control non-targeting siRNA or SNPH-directed siRNA were incubated with the mitochondrial superoxide scavenger, MitoTempo (MT,  $100 \mu\text{M}$ ) alone or in combination with the antioxidant, N-acetyl-cysteine (NAC,  $10 \mu\text{M}$ ), and quantified for mitochondrial trafficking to the cortical cytoskeleton by fluorescence microscopy. Cortical mitochondrial localization was measured as fluorescence units ( $10^6$ ) and normalized to the total mitochondria signal per cell. Each symbol corresponds to an individual cell ( $n=29-51$ ). \*\*\*,  $p<0.001$  by ANOVA and Bonferroni's post test. (C) LN229 cells were transfected with SNPH-directed siRNA and analyzed in a 2D

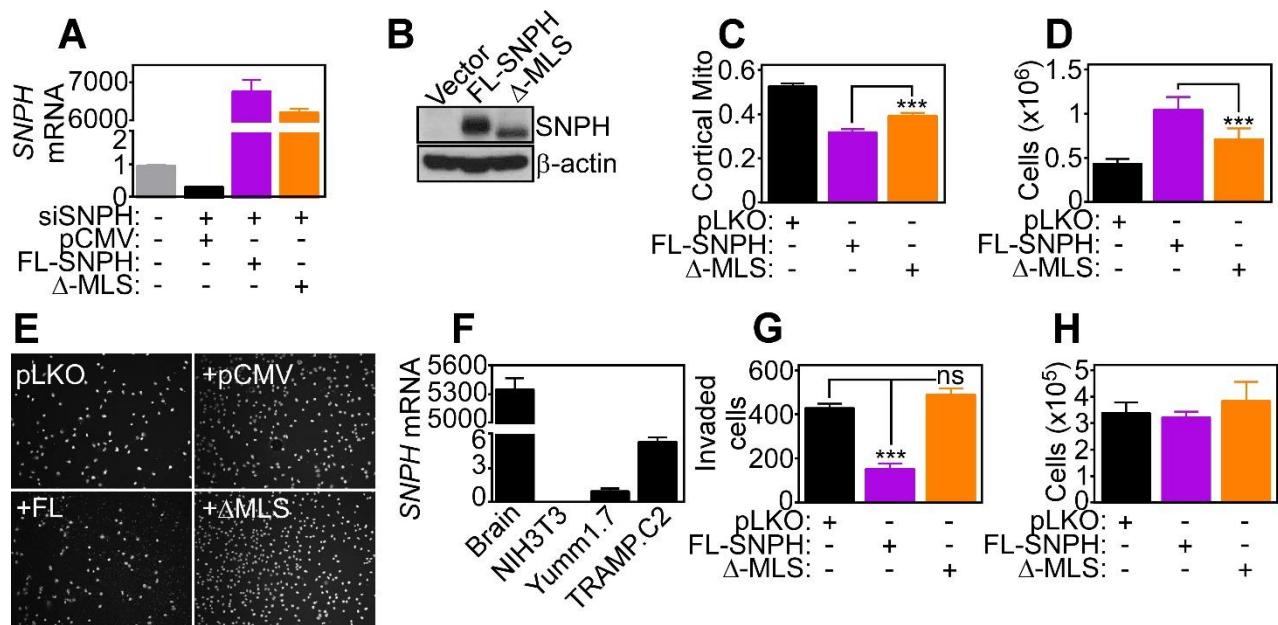


chemotaxis assay in the presence of vehicle (Veh), MitoTempo (MT) alone or the combination with the antioxidant, N-acetyl-cysteine (NAC). Each tracing represents the trajectory of a single cell throughout the analysis. Trajectories are color coded according to speed, as indicated. **(D and E)** The experimental conditions are as in **(C)**, and the speed of cell migration **(D)** or distance traveled by individual cells **(E)** was quantified. Each symbol **(E)** corresponds to an individual cell. Data are expressed as the mean $\pm$ SEM (n=91-112). \*\*\*, p<0.001, by ANOVA and Bonferroni's post test.



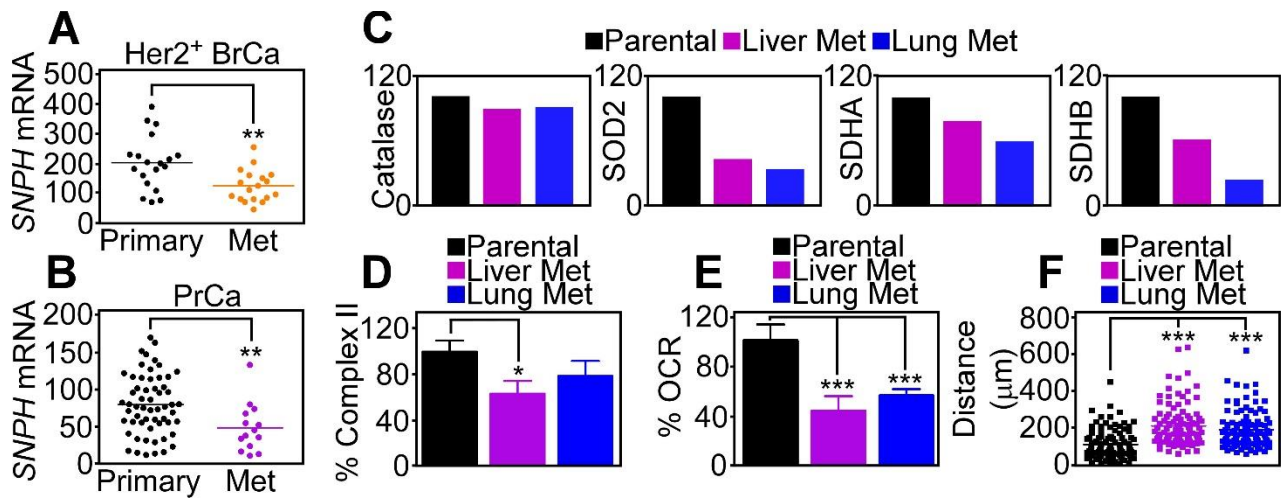
**Supplemental Figure 5. Mitochondrial SNPH regulation of tumor cell proliferation. (A)**

The indicated tumor cell lines transfected with control non-targeting siRNA (Ctrl) or SNPH-directed siRNA were analyzed for *SNPH* mRNA levels by quantitative PCR. Red boxes indicate two cell lines (C4-2B and MCF-7) with undetectable endogenous levels of SNPH. Data are expressed as the mean±SD (n=3). (B) PC3 cells transduced with pLKO or two independent SNPH-directed shRNA sequences (clones #0 and #5) were analyzed for BrdU staining and flow cytometry, and the percentage of cells in S phase (boxes) per condition was quantified. A representative experiment is shown (n=2). (C) The experimental conditions are as in (B) except that transduced PC3 cells were analyzed for DNA content by propidium iodide staining and flow cytometry, and the percentage of cells in each cell cycle phase was quantified. Data are expressed as the mean±SEM (n=3). \*\*, p<0.01; \*\*\*, p<0.001 by ANOVA and Bonferroni's post test. (D) PC3 cells transduced as in (B) were analyzed by Western blotting. A separate loading control is shown directly under each separate gel (one for Cyclin B and one for Cyclin A and Cyclin D1).

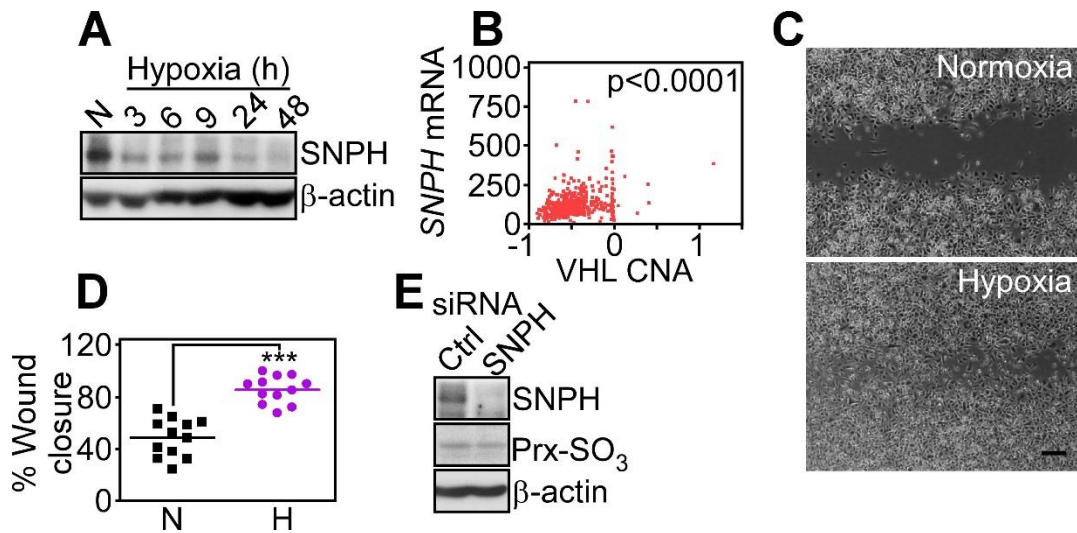


**Supplemental Figure 6. Mitochondrial SNPH regulation of metastasis, in vivo.** (A) PC3 cells stably transduced with pLKO or SNPH shRNA were reconstituted with vector (pCMV6), FL SNPH or a SNPH mutant deleted in the mitochondrial-localization sequence ( $\Delta$ -MLS), and analyzed for expression of *SNPH* mRNA by RT-PCR. Data are the mean $\pm$ SEM (n=3). (B) C42B cells were transfected with pCMV6, FL SNPH or a  $\Delta$ -MLS SNPH mutant, and analyzed by Western blotting. (C and D) LN229 cells were transfected with pCMV6, FL SNPH or  $\Delta$ -MLS SNPH and analyzed for mitochondrial trafficking to the cortical cytoskeleton (C) or cell proliferation (D) by direct cell counting. For (C), data are the mean $\pm$ SEM (pCMV6, n=89; FL SNPH, n=96;  $\Delta$ -MLS SNPH, n=87). For (D), data are the mean $\pm$ SD (n=6). \*\*\*, p<0.001 by ANOVA and Bonferroni's post test. (E) PC3 stably silenced for endogenous SNPH (sh0) were reconstituted with pCMV6, FL SNPH or SNPH  $\Delta$ -MLS as in (A) and analyzed in a Matrigel invasion assay. DAPI-stained nuclei of invaded cells under the various conditions are shown.

Magnification, 10X. **(F)** The indicated murine cell types or tissue extracts of normal mouse brain were amplified for *SNPH* mRNA expression by RT-PCR. Data are the mean $\pm$ SEM (n=3). **(G)** and **(H)** Yumm1.7 cells expressing mCherry were stably transfected with pCMV, FL SNPH or  $\Delta$ -MLS SNPH and analyzed for Matrigel invasion **(G)** or cell proliferation **(H)**. For **(G)**, data are the mean $\pm$ SEM (n=10). \*\*\*, p<0.001 by ANOVA and Bonferroni's post test. ns, not significant. For **(H)**, data are the mean $\pm$ SEM (n=3). All comparisons are not significant (ns) by ANOVA and Bonferroni's post test.

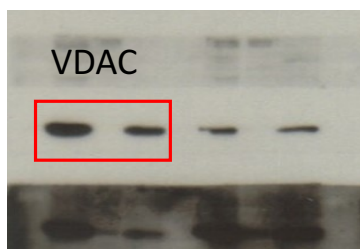
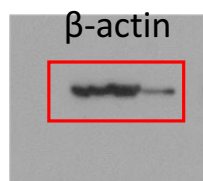
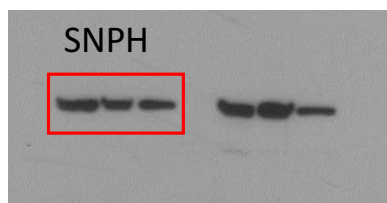


**Supplemental Figure 7. SNPH modulation of cell proliferation-metastasis.** (A and B) *SNPH* expression in primary or metastatic (Met) Her2<sup>+</sup> breast adenocarcinoma (BrCa, A) or prostatic adenocarcinoma (PrCa, B). Each symbol corresponds to an independent case (see methods for sample size). \*\*,  $p=0.008-0.003$ , by 2-tailed Student's *t* test. (C) pLKO-transduced PC3 cells or pLKO-transduced PC3 cells isolated from a lung or liver metastatic site were analyzed by Western blotting, and protein bands for Catalase, SOD2, SDHA and SDHB were quantified by densitometric scanning. Representative experiment. (D and E) The indicated cell types as in (C) were analyzed for mitochondrial oxidative phosphorylation Complex II activity normalized to citrate synthase activity (D), or oxygen consumption rate (OCR, E). Data are expressed as the mean±SD ( $n=3-4$ ). \*,  $p<0.05$ ; \*\*\*,  $p<0.001$  by ANOVA and Bonferroni's post test. ns, not significant. (F) The indicated cell types were analyzed for 2D chemotaxis and the distance traveled by individual cells was calculated. Each symbol corresponds to an individual cell. Data are expressed as the mean±SEM ( $n=52-54$ ). \*\*\*,  $p<0.0001$ , by ANOVA and Bonferroni's post test.

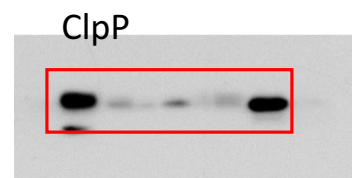
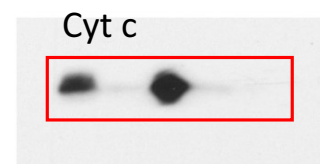
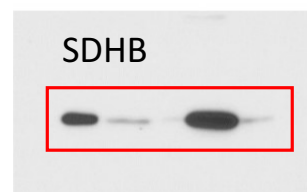
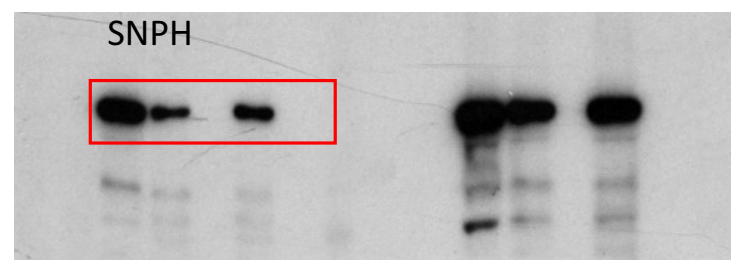


**Supplemental Figure 8. Regulation of SNPH by stress stimuli.** (A) PC3 cells maintained in normoxia (N) or exposed to hypoxia (H, 1% O<sub>2</sub>) were harvested at the indicated time intervals (h=hours) and analyzed by Western blotting. (B) Spearman correlation ( $r=0.30$ ) between *SNPH* mRNA levels and linear VHL copy number alterations (CNA) in cases of renal clear cell carcinoma contained in the TCGA database. Each symbol corresponds to an individual case ( $n=446$ ). (C and D) The experimental conditions are as in (A) except that PC3 cells under the different conditions were examined for cell motility in a wound closure assay (C) and quantified as % of wound closure (D). Each point corresponds to an individual determination. \*\*\*,  $p<0.0001$ , by 2-tailed Student's *t* test. Scale bar, 200  $\mu\text{m}$ . (E) HFF fibroblasts were transiently transfected with control (Ctrl) or SNPH siRNA and analyzed by Western blotting.

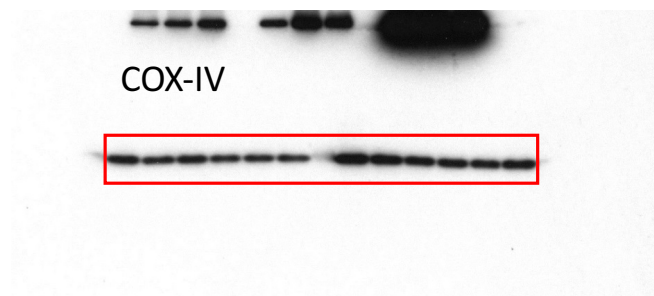
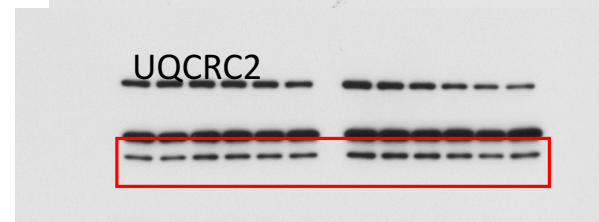
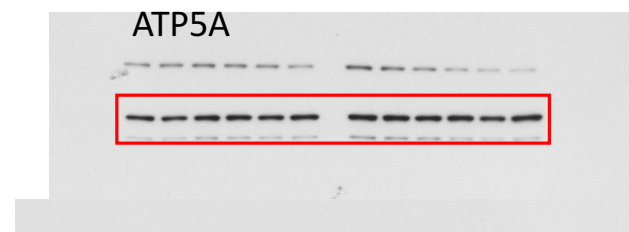
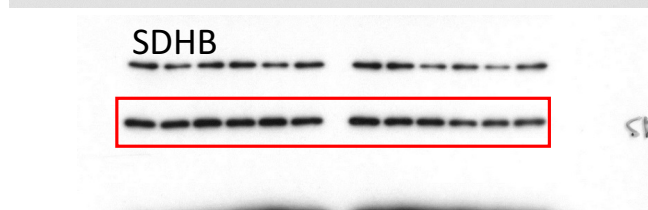
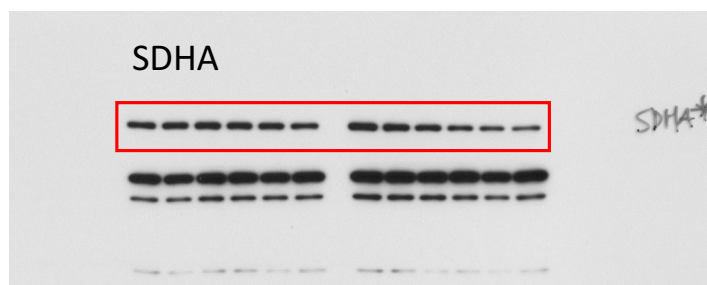
Full uncut blots for Fig. 1E



Full uncut blots for Fig. 1G

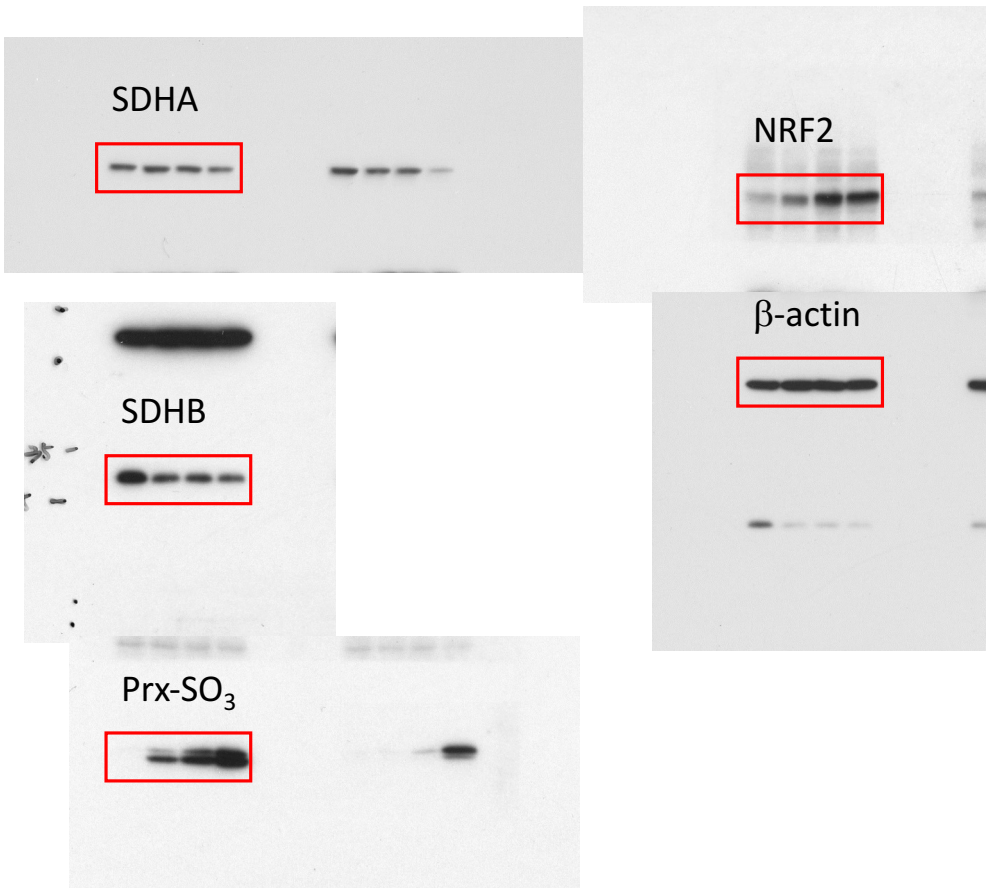


Full uncut blots for Fig. 2I

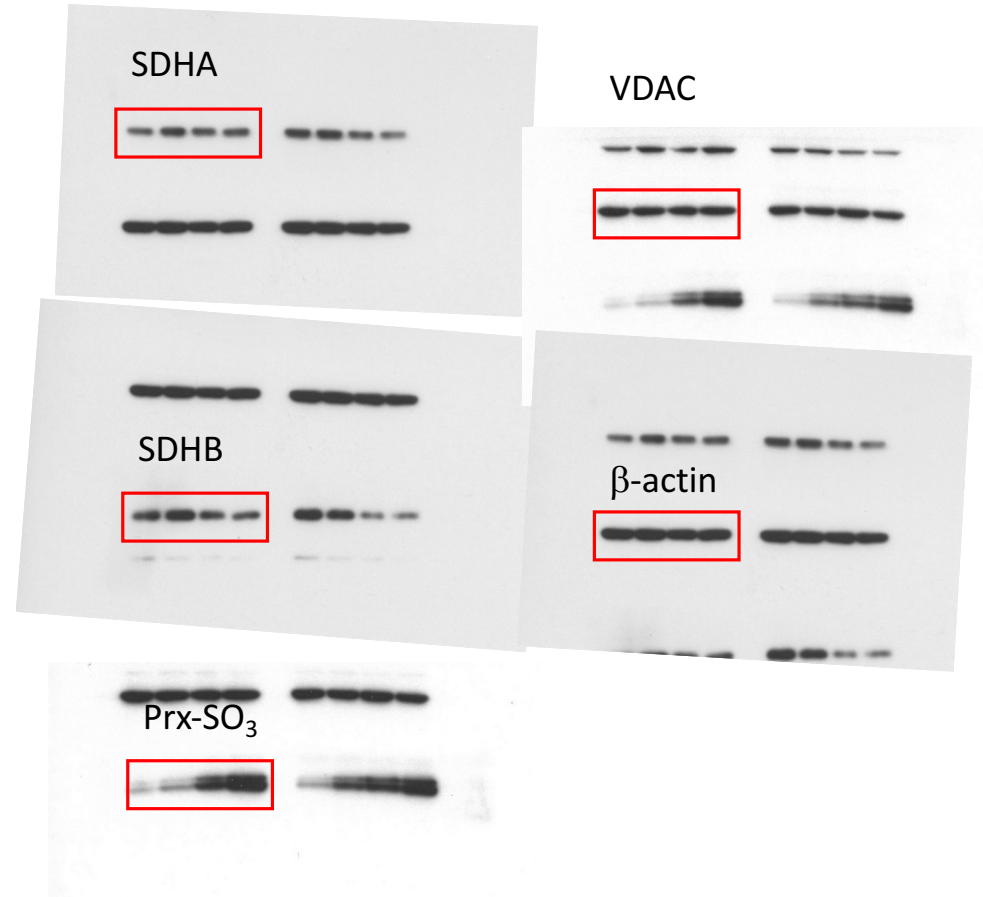




Full uncut blots for Fig. 3D



Full uncut blots for Fig. 3E



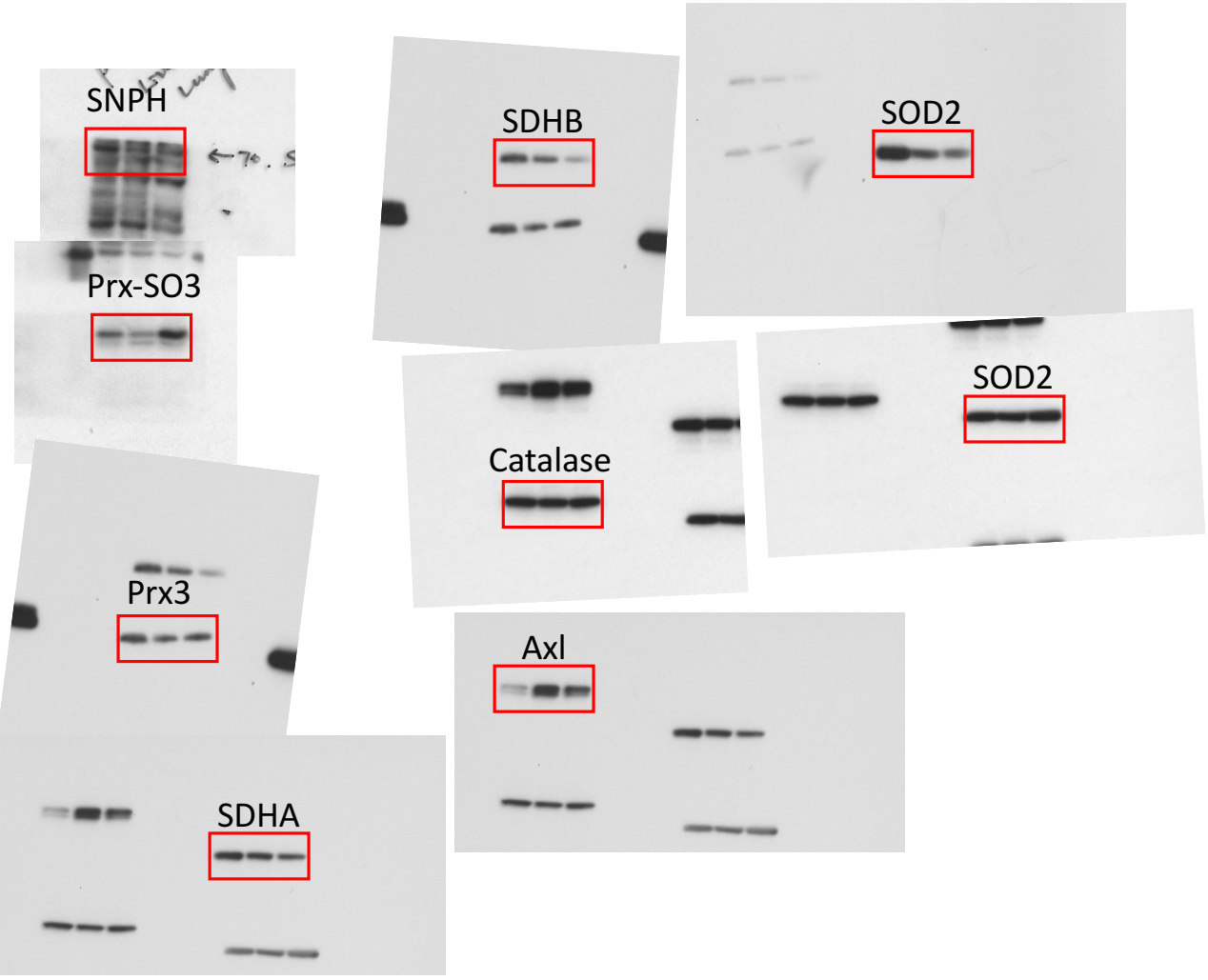
Full uncut blots for Fig. 3F



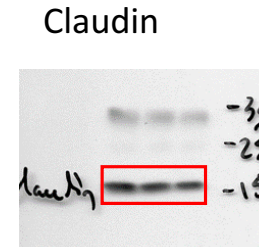
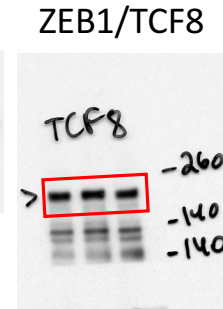
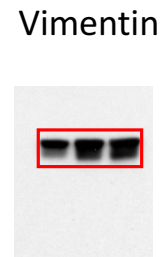
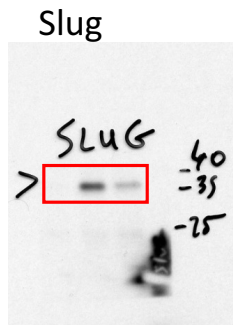
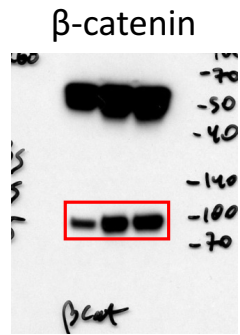
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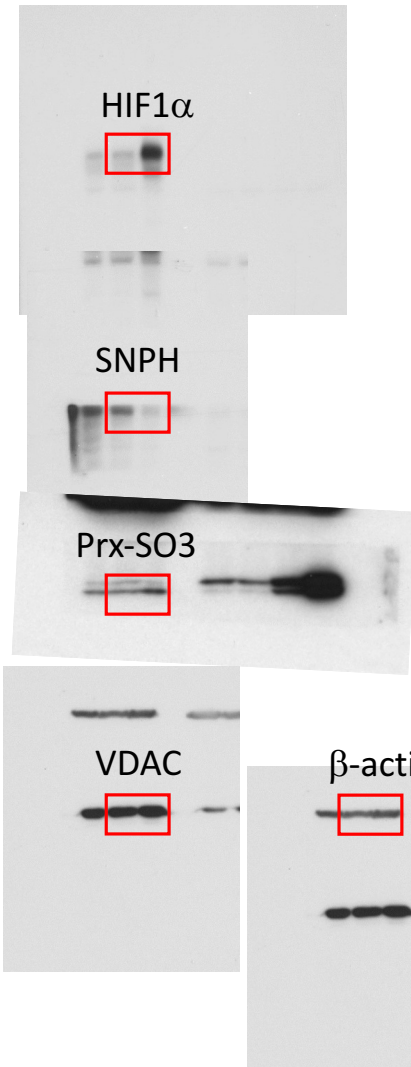
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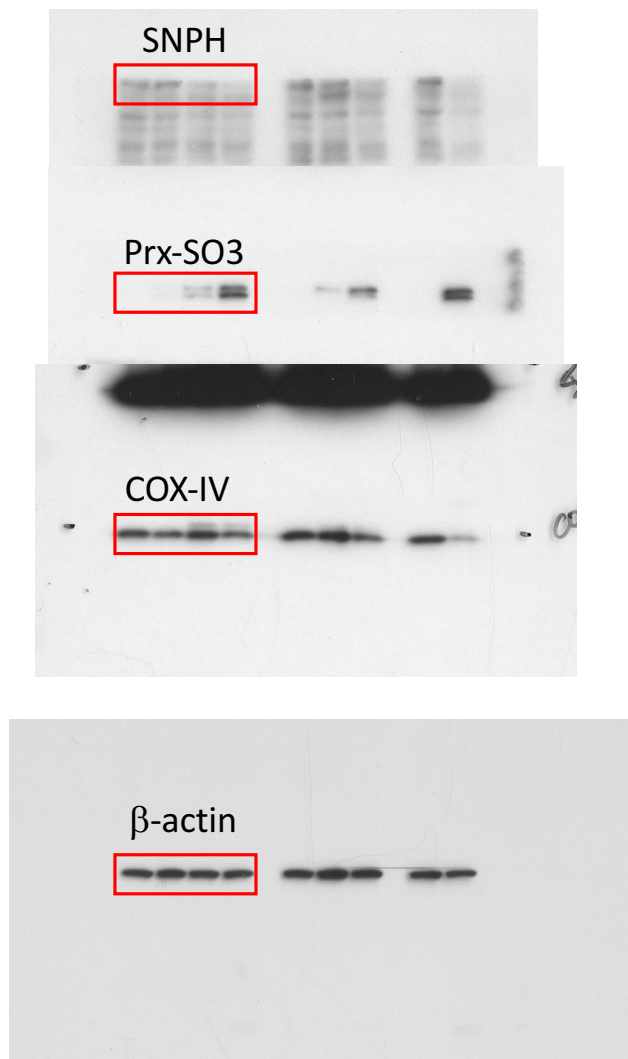
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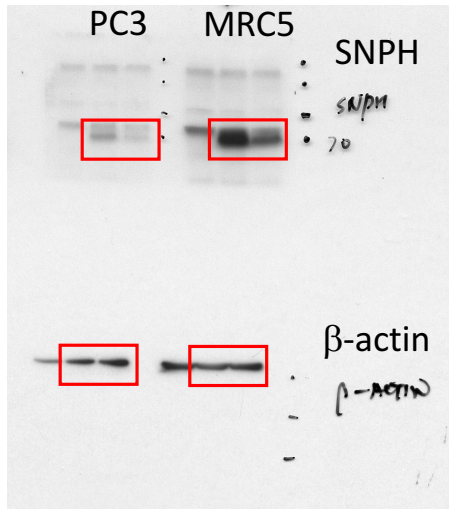
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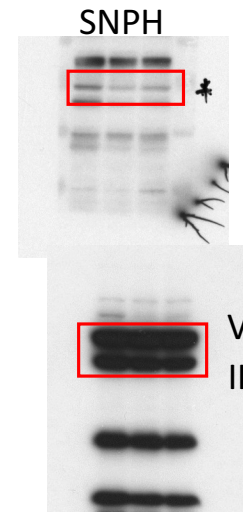
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Full uncut blots for Fig. S1D

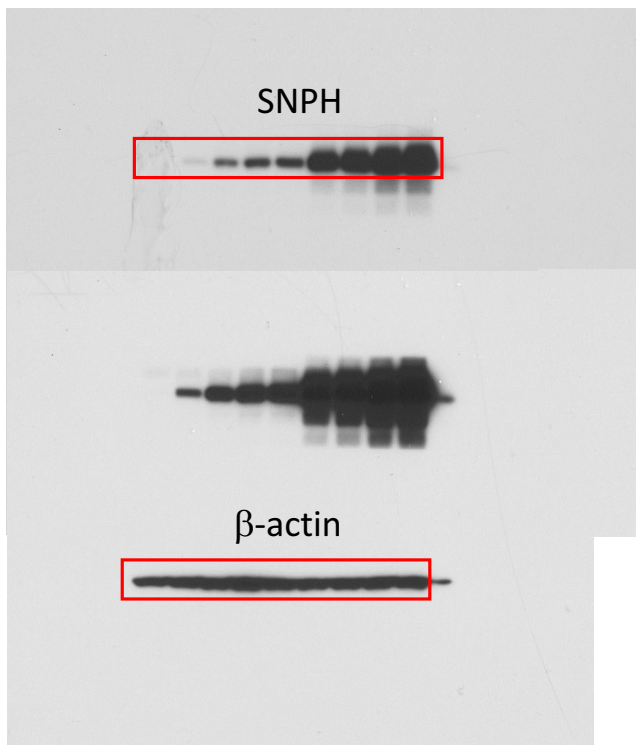


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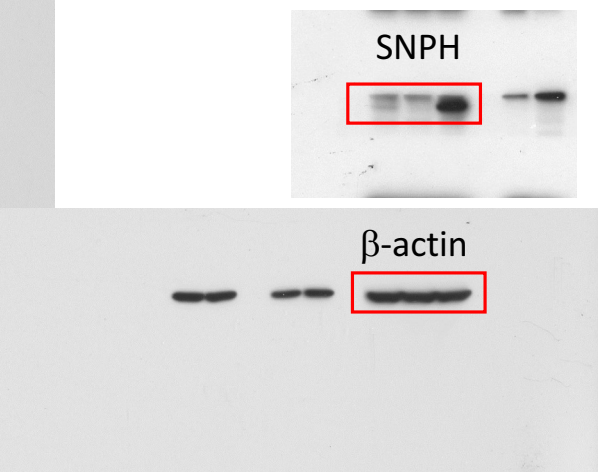




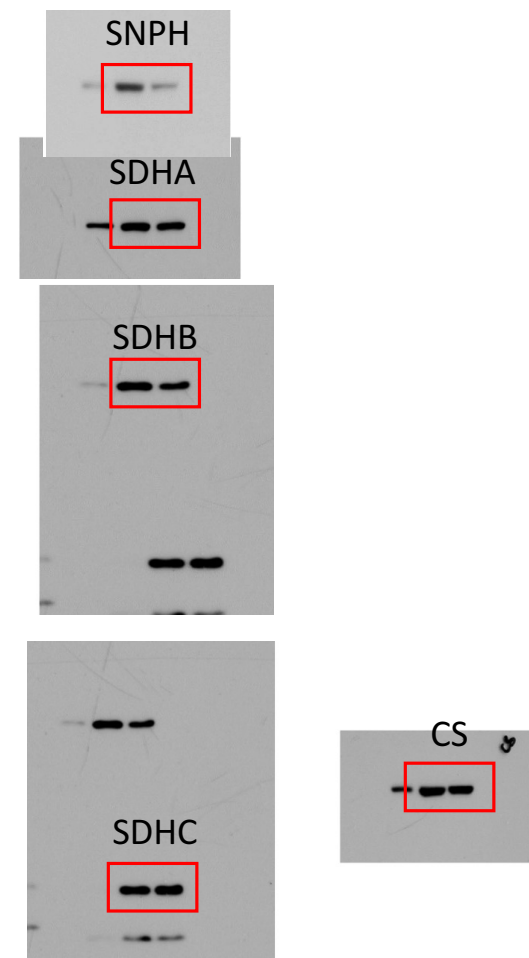
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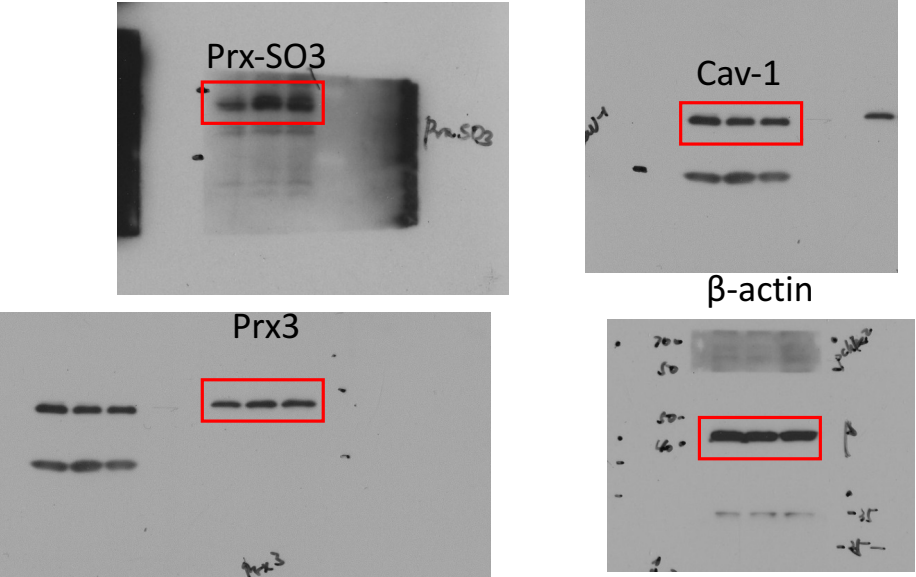
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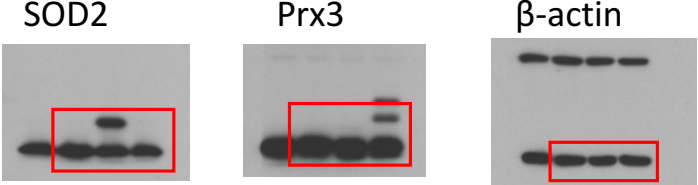
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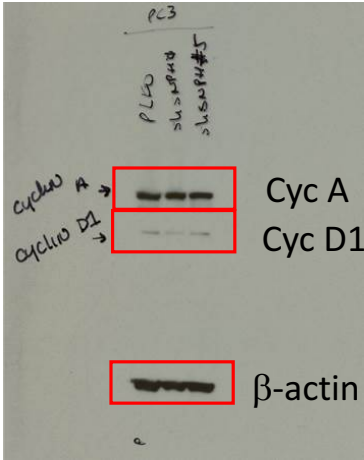
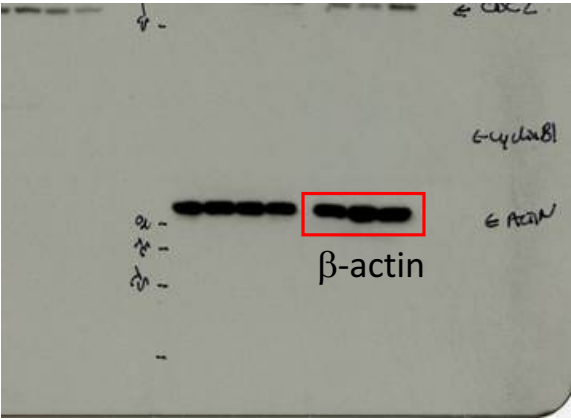
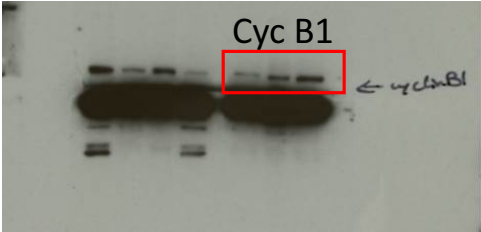


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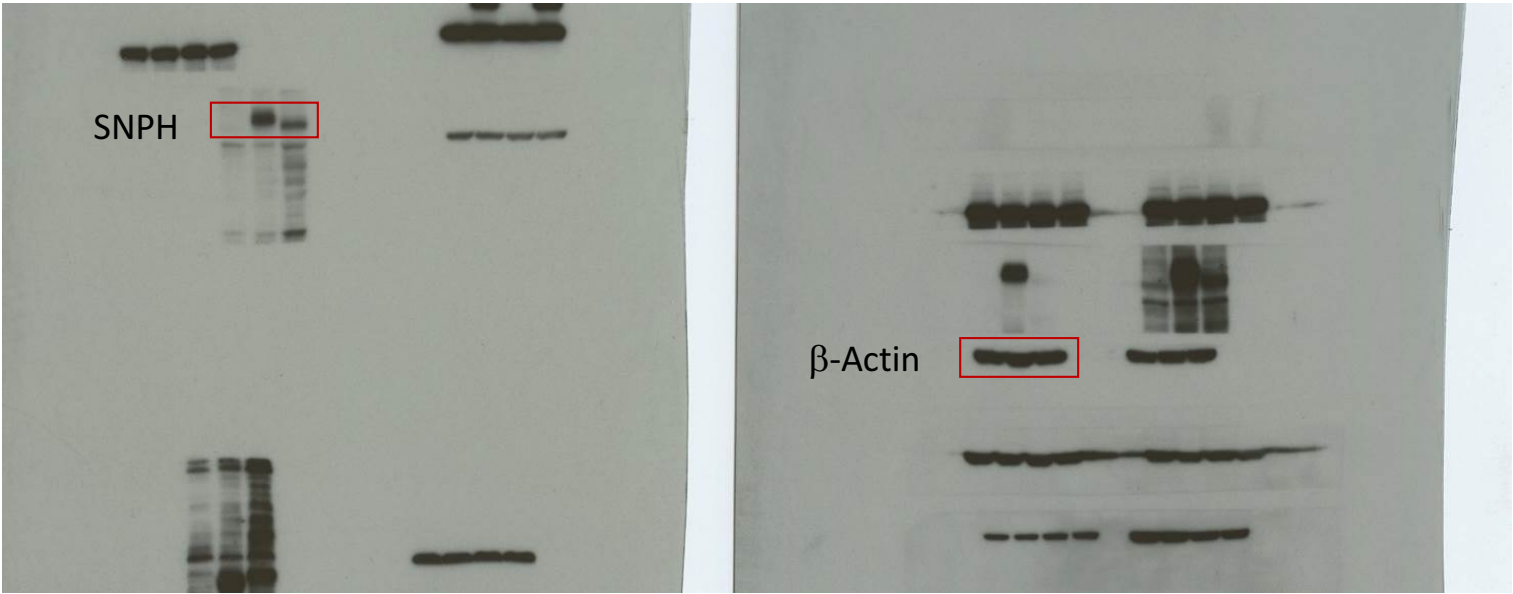




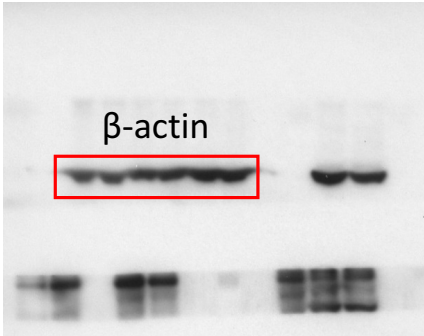
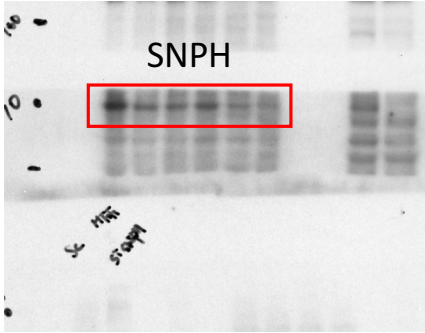
Full uncut blots for Fig. S5D



Full uncut blots for Fig. S6B



Full uncut blots for Fig. S8A



Full uncut blots for Fig. S8E

