Supplementary figure legends

Fig. S1: Further characterization of GAPDH-SIRT1 binding and SIRT1 nitrosylation. (**a**) *In vitro* binding assay showing that GAPDH-C150S does not physically interact with SIRT1. (**b**) SIRT1 is nitrosylated in 293T cells treated with NO donor. Cells were treated with 500 μM GSH or GSNO for 3 hr. (**c**) *In vitro* nitrosylation of SIRT1 by GSNO. Recombinant SIRT1 was treated with the indicated concentrations of GSH or GSNO for 30 min at 37ºC, followed by the biotin switch assay. Reactions were probed with anti-biotin antibody. (**d**) Mutational analysis identifies Cys387 and Cys390 as the sites of SIRT1 nitrosylation. A biotin switch was performed from 293-nNOS cells transfected with the indicated plasmid and treated with A23187 (5 μM) for 2 hr. FH-SIRT1, Flag-HA-tagged SIRT1.

Fig. S2: Quantitation of western blots from Figure 2. (**a**) Quantitation of results from **Fig. 2c**. *P<0.01, n=3, mean±s.e.m., one-way ANOVA. (**b**) Quantitation of results from **Fig. 2d**. *P<0.05, n=4, mean±s.e.m., student's t-test. (**c**) Quantitation of results from **Fig. 2e**. *P<0.05, n=3, mean±s.e.m., one-way ANOVA. (**d**) Quantitation of results from **Fig. 2f**. *P<0.001, n=4, mean±s.e.m., student's t-test. (**e**) Quantitation of results from **Fig. 2g**. *P<0.05, n=3, mean±s.e.m., one-way ANOVA. (**f**) Quantitation of results from **Fig. 2i**. *P<0.01, n=3, mean±s.e.m., student's t-test.

Fig. S3: SNO-GAPDH transnitrosylates SIRT1. (**a**) Peptide-C150, but not a scrambled peptide, prevents SIRT1 transnitrosylation by SNO-GAPDH *in vitro*. (**b**) S-nitrosylated albumin fails to transnitrosylate SIRT1. *In vitro* transnitrosylation assay performed as in **Fig. 2**, with the exception that, after the labeling step, biotinylated proteins were precipitated with NeutrAvidin-agarose before being resolved by SDS-PAGE and probed with

specific antibodies. (**c**) Overexpression of GAPDH augments SIRT1 nitrosylation in 293 cells treated with NO donor. Forty-eight hours after transfection with FH-SIRT1 and either HA-GAPDH or empty HA vector, cells were treated with 500 μM GSH or GSNO for 3 hr and subjected to the biotin switch assay. (**d**) Mutation of GAPDH-T152 has no appreciable effect on its glycolytic activity. n=4, mean±s.e.m. (**e**) Overexpression of GAPDH has no effect on the biotin switch signal for FH-SIRT1-C387/390S. The assay was performed from 293-nNOS cells transfected with the indicated plasmids and treated with A23187 (5 μM) for 2 hr. (**f**) and (**g**) Depletion of GAPDH by RNAi (**f**) and Siah1ΔNLS expression (**g**) in 293-nNOS cells abolishes nitrosylation of exogenous FH-SIRT1. (**h**) The inhibitory effect of SNO-GAPDH on SIRT1 enzymatic activity is lost with mutation of GAPDH-C150. *In vitro* deacetylation assay performed as in **Fig. 3.** (**i**) Overexpression of wild-type GAPDH, but not GAPDH-K225A, increases PGC1α acetylation in 293-nNOS cells. FH-SIRT1, Flag-HA-tagged SIRT1. BSA, purified bovine serum albumin.

Fig. S4: An unbiased screen identifying DNA-PK as a target of SNO-GAPDH-dependent S-nitrosylation. Biotin switch assay from 293-nNOS crude nuclear lysates, with or without GAPDH overexpression. After pulldown, proteins were eluted from NeutrAvidin-agarose with β-mercaptoethanol, resolved by SDS-PAGE, and visualized by silver stain (SilverSNAP Stain Kit II, Pierce). The indicated bands were excised from the gel and sent for analysis by mass spectrometry (Taplin Mass Spectrometry Facility, Harvard University). The biotin switch signal from the upper band, which consisted primarily of DNA-PK, was the most consistently and substantially enhanced by GAPDH overexpression. Therefore, DNA-PK was followed up as a potential target of GAPDH-mediated transnitrosylation.

Fig. S5: Full scans of key Western blot data. In most experiments, membranes were cut prior to probing with antibody. In cases in which the separate blots were exposed on the same piece of film, the full scan incorporating all blots is shown.

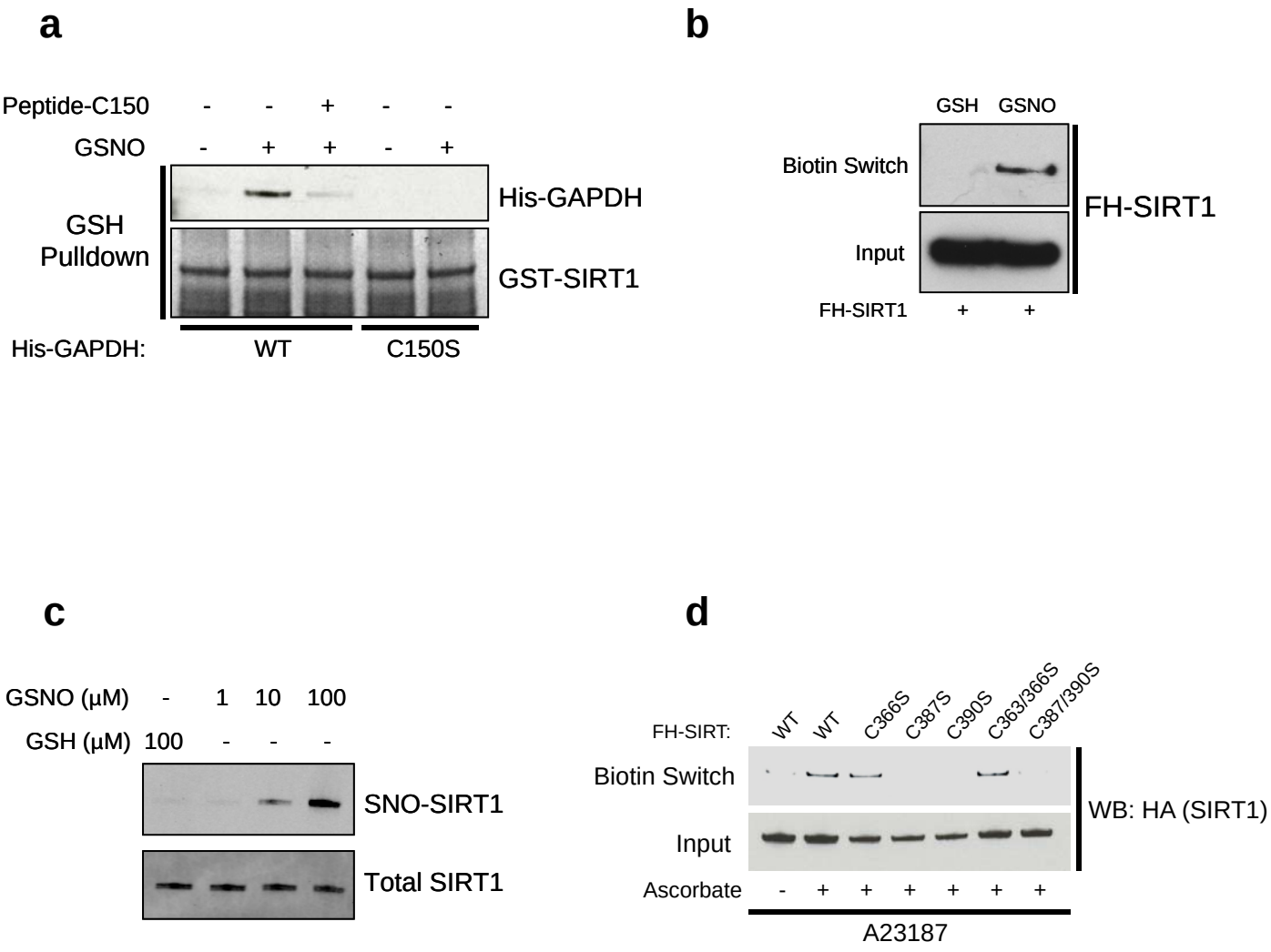
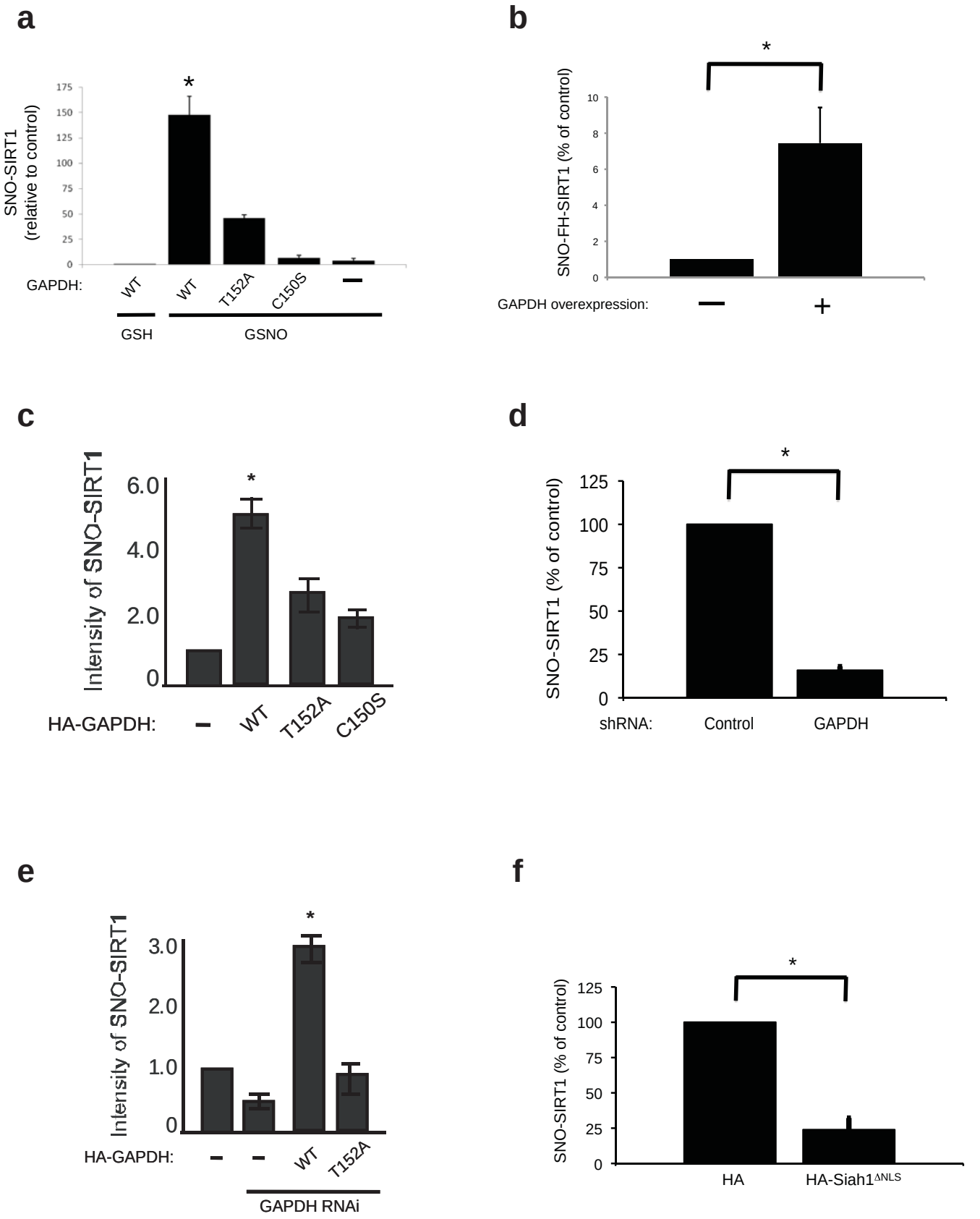


Figure S1 (Snyder)



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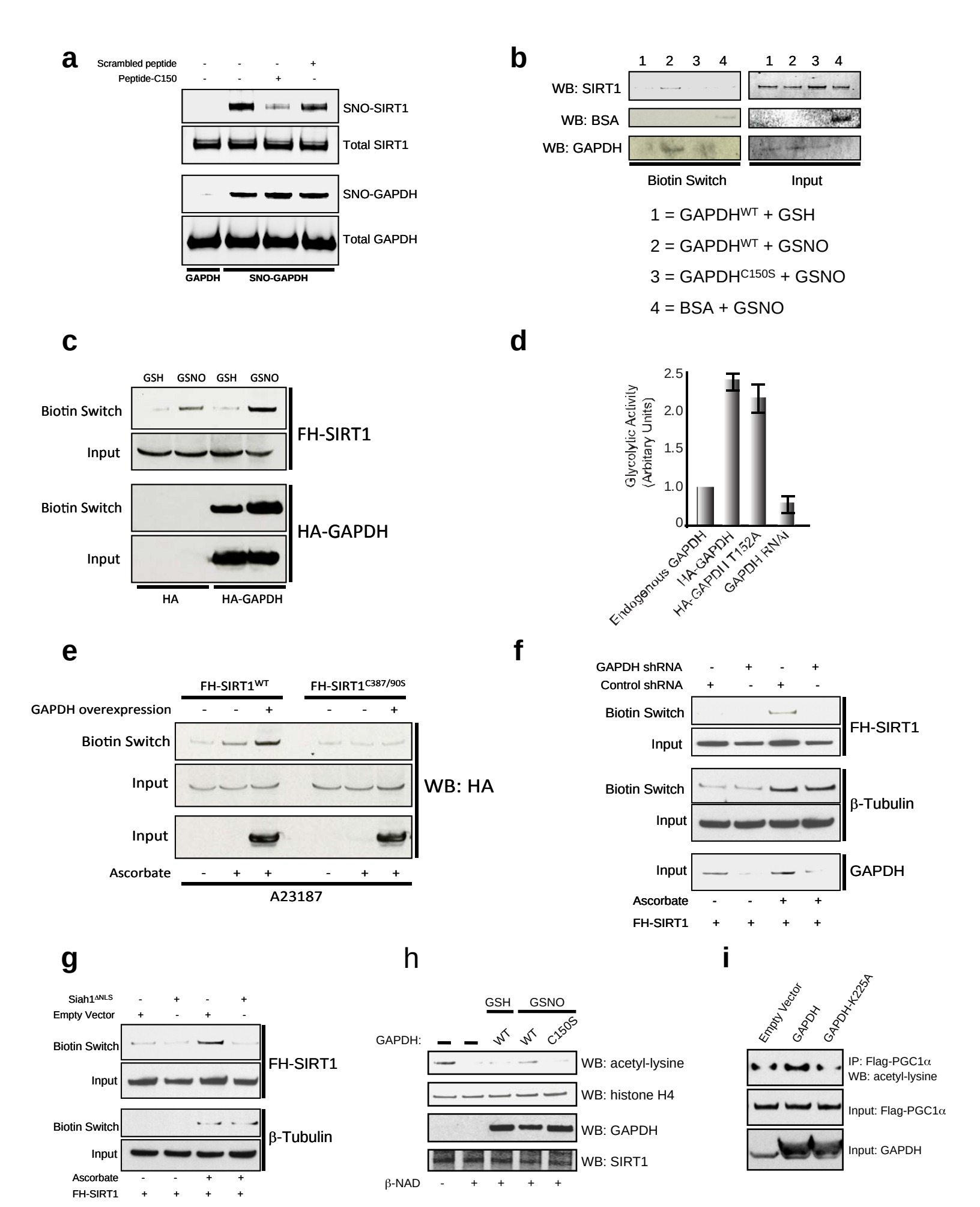

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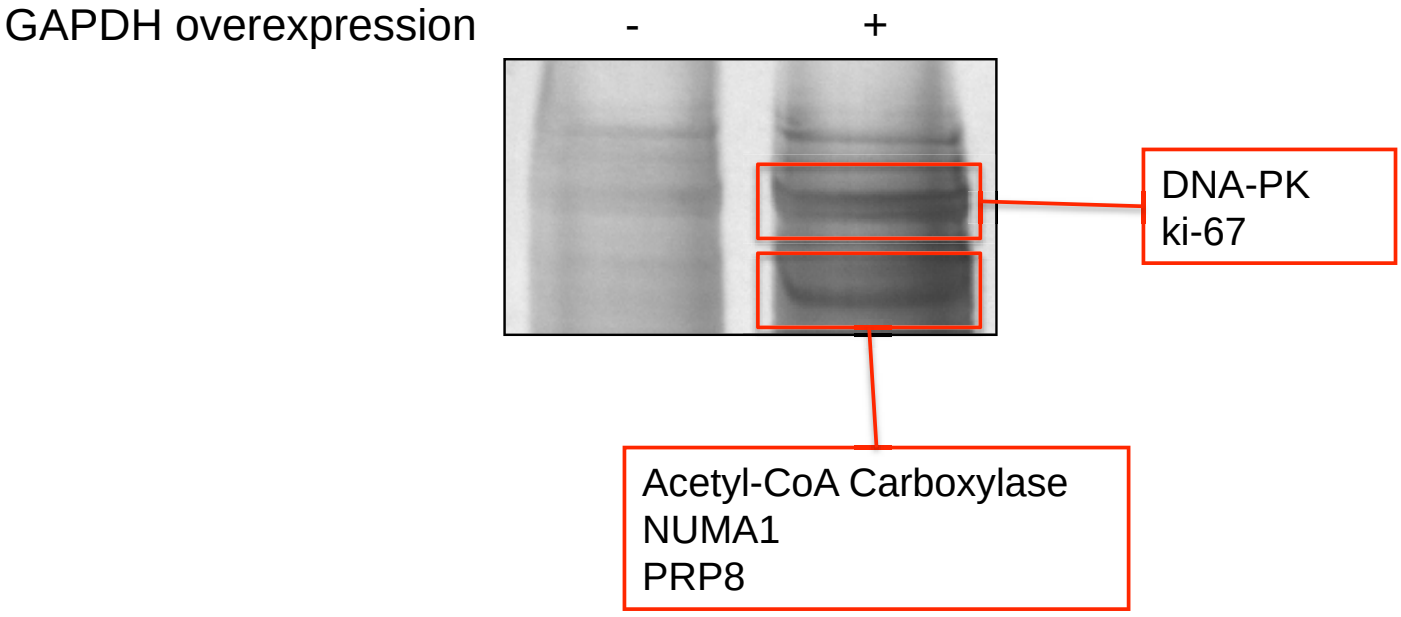


Figure S4 (Snyder)

Fig. 1a

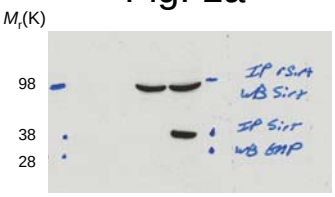


Fig. 1d

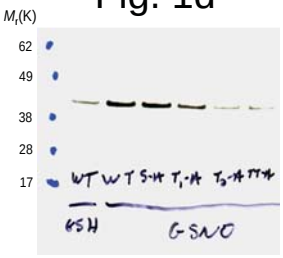


Fig. 2c

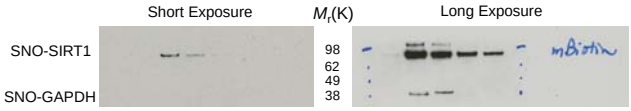


Fig. 2d

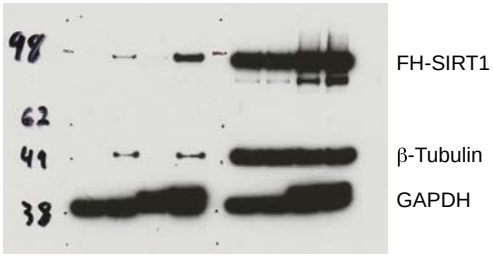


Fig. 2f

Fig. 2i

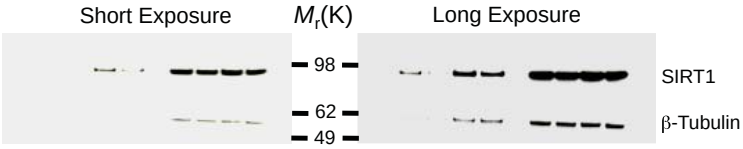


Fig. 3b

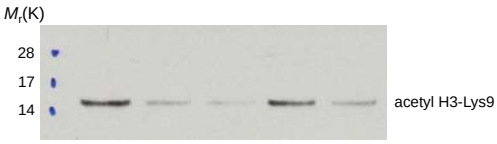


Fig. 3c

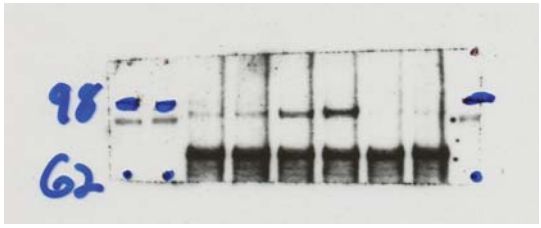


Fig. 3d

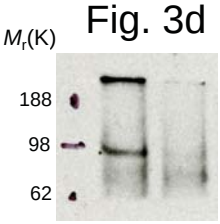
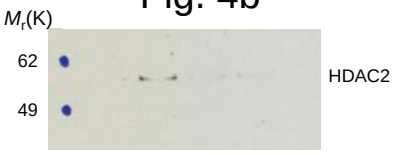


Fig. 4b



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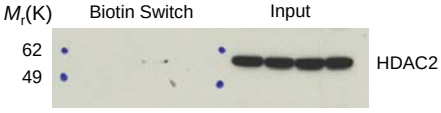

Fig. 4d

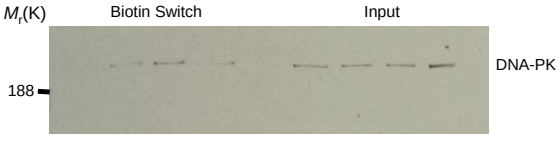

Fig. 4e

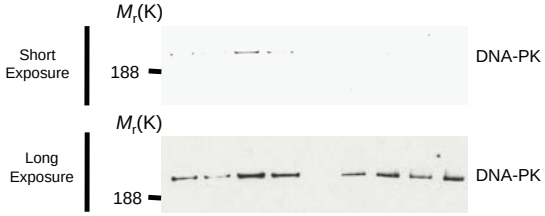

Figure S5