Supporting Information

Sen and Snyder 10.1073/pnas.1117820108

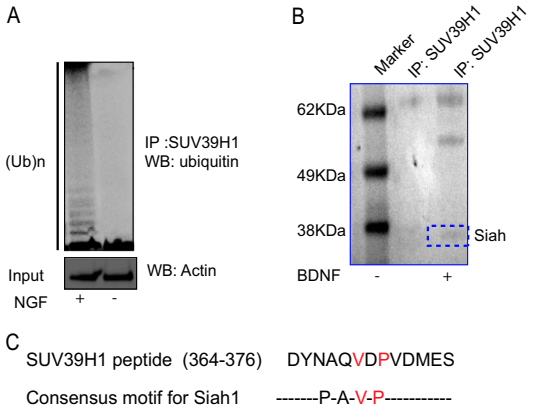

Fig. S1. (*A*) Degradation of suppressor of variegation 3–9 homolog 1 (SUV39H1) by seven in absentia homolog (Siah). SUV39H1 is polyubiquitinated endogenously in primary neurons upon treatment with nerve-growth factor (NGF). IP, immunoprecipitation; (Ub)n, polyubiquitin chains. (*B*) Identification of Siah as one of the interacting proteins with SUV39H1 in brain-derived neurotrophic factor (BDNF)-treated primary cortical neurons. (*C*) Amino acid sequence of the SUV39H1 peptide sequence, which contains the Siah-binding motif.

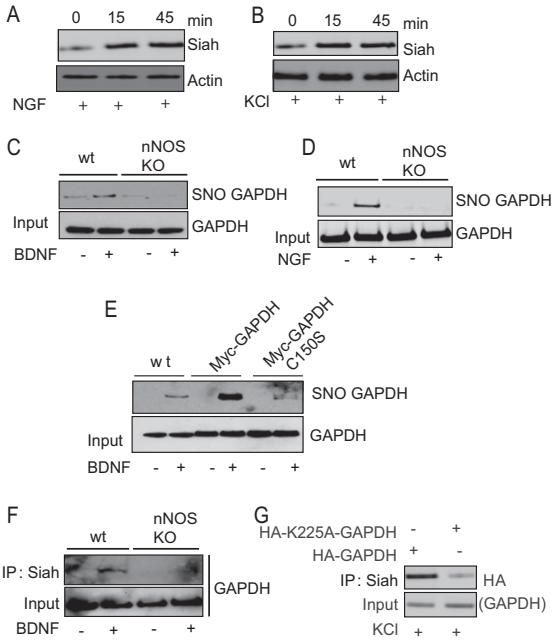

Fig. S2. BDNF induces S-nitrosylation of GAPDH and binding with Siah. (*A* and *B*) Immunoblot analysis of Siah protein in primary cortical neuronal cells treated with NGF and in PC-12 cells treated with KCl. (*C* and *D*) Biotin-switch assay of BDNF- or NGF-treated cortical neurons derived from wild-type or neuronal NOS (nNOS)-null mouse embryos. Neurons were stimulated with BDNF (*C*) or NGF (*D*). Precipitations were performed using streptavidin followed by Western blotting for GAPDH. SNO-GAPDH, nitrosylated GAPDH. (*E*) Biotin-switch assay of PC-12 cells transfected with myelocytomatosis viral oncogene homolog (Myc)-GAPDH or Myc-GAPDH-C150S and treated with BDNF. Streptavidin precipitation was followed by Western blotting for GAPDH. (*F*) BDNF-stimulated binding between GAPDH and Siah is abolished in nNOS-deleted primary neurons. Cell lysates were immunoprecipitated with anti-Siah1 antibody. (*G*) Overexpression of GAPDH-K225A prevents GAPDH–Siah interaction upon treatment with KCl in PC-12 cells.

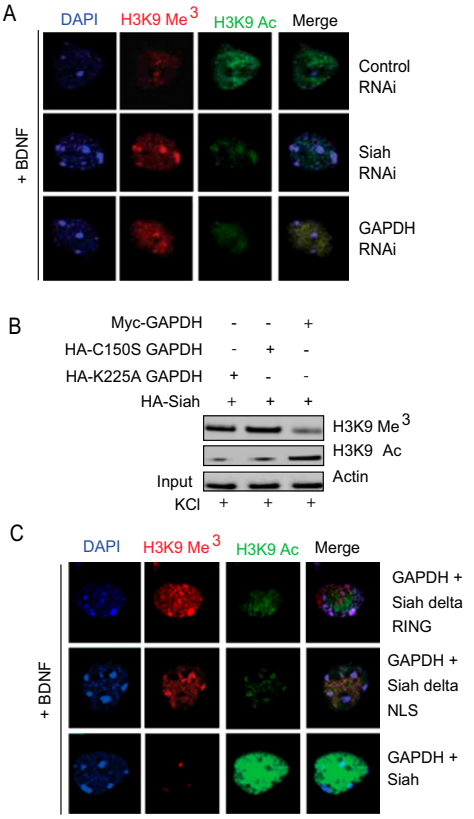

Fig. S3. GAPDH–Siah complex regulates H3K9 methylation (*A*) Confocal microscopic analysis of methylated and acetylated H3K9 and actin after depletion of GAPDH or Siah. Cells were stained with immunofluorescent anti-trimethylated histone H3 at lysine 9 ($H3K9Me^3$) (red) and anti-histone 3 on lysine 9 acetylated (H3K9Ac) antibodies (green). (*B*) Level of methylated and acetylated H3K9 and actin upon treatment with BDNF. (*C*) Confocal microscopic analysis of methylated and acetylated H3K9 and actin after overexpression of Siah lacking the nuclear-localization signal (NLS) or really interesting new gene (RING) domain. Cells were stained with immunofluorescent anti-$H3K9Me^3$ (red) and anti-H3K9Ac (green) antibodies.

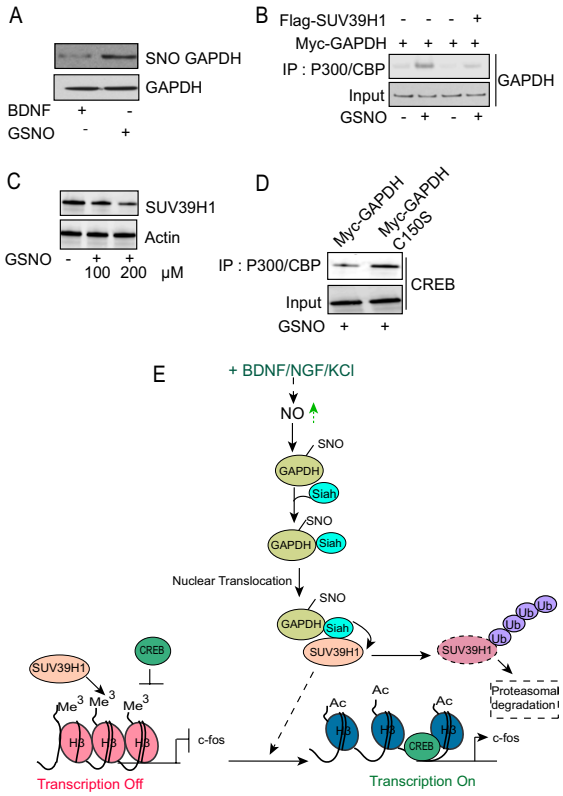

Fig. S4. (*A*) SNO-GAPDH in BDNF- or nitrosoglutathione (GSNO)-treated cells. S-nitrosylation was detected by biotin-switch assay. (*B*) Overexpression of SUV39H1 decreases p300/cAMP response element-binding (CREB)-binding protein (CBP) binding of GAPDH. (*C*) Treatment with GSNO (100 μM or 200 μM overnight) causes degradation of SUV39H1. (*D*) Effect of overexpression of GAPDH and GAPDH-C150S mutant on binding between p300/CBP and CREB. (*E*) Schematic diagram of BDNF signaling mediated through degradation of histone methyltransferase SUV39H1. NO triggers S-nitrosylation of GAPDH and augments its binding to Siah, translocating the GAPDH–Siah protein complex to the nucleus. In the nucleus Siah binds with SUV39H1, which is degraded via ubiquitination. This binding leads to augmentation of histone acetylation and CREB-mediated transcription for neuronal outgrowth.