Supporting Information

Rivieccio et al. 10.1073/pnas.0907935106

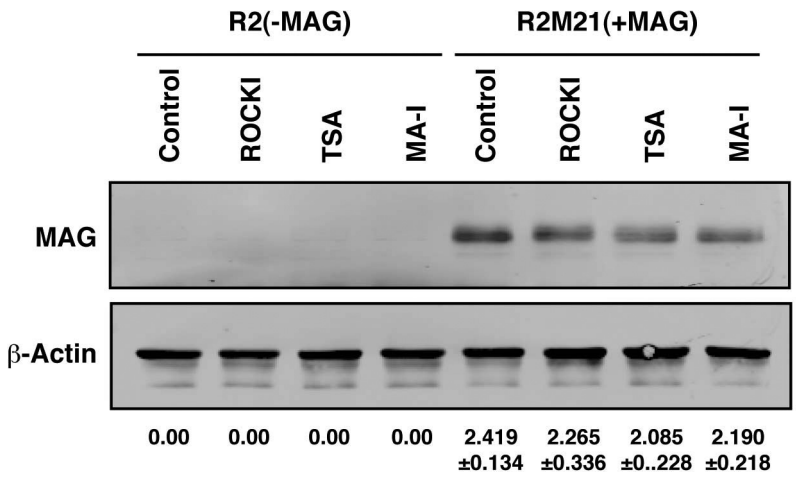


Fig. S1. MAG expression by CHO cells is not affected by HDAC inhibition. Representative Western blot analysis showing relative levels of MAG in total cellular lysates from CHO cells expressing MAG (R2M21+MAG) and control CHO cells (R2) after treatment with TSA (0.66 μM), RhoA kinase Inhibitor (ROCKI), Y27632 (10 μM), or MA-I (10 μM) for 24 h. Relative levels of β-Actin are shown to indicate loadings in Western blots. Quantified levels of MAG (normalized to β-Actin) are shown below Western blot (Mean MAG level $\pm$ Standard Deviation). MAG expression in treated R2M21+MAG CHO cells are not statistically different to nontreated R2M21+MAG CHO controls, by two-way ANOVA followed by Bonferroni posttests.

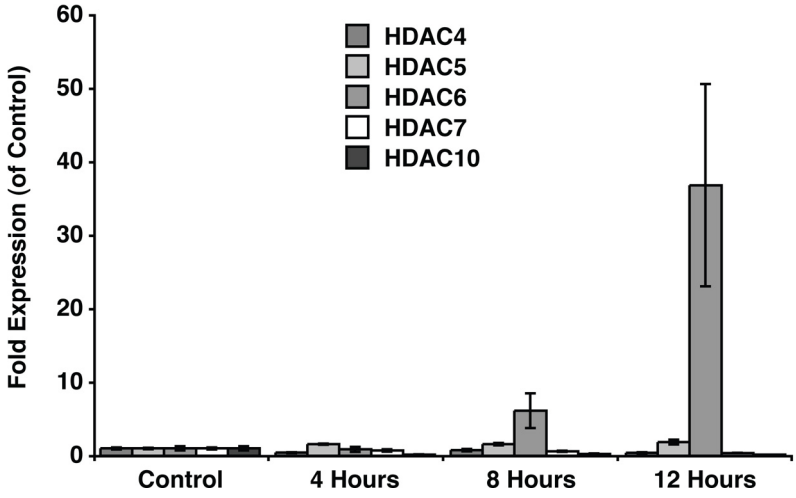
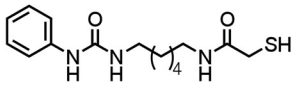


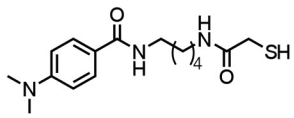
Fig. S2. HDAC6 expression is induced in oxidatively stressed primary neurons. Relative levels of the Class II HDACs as measured by real-time rt-PCR on RNA extracted from rat primary cortical cultures treated for 0, 4, 8, or 12 h with homocysteic acid (HCA; 5 mM). Expression was normalized using rat β-Actin. Data are representative of three independent experiments.

MA-I



2-Mercapto-N-[6-(3-phenylureido)hexyl] acetamide

MA-2



N-{5-[3-(4-Dimethylaminophenyl)ureido] pentyl}-2-mercaptoacetamide

Fig. S3. HDAC6-selective pharmacological inhibitors, MA-I and MA-II. Chemical structures and names of the HDAC6-selective ligands, 2-Mercapto-*N*-[6-(3-phenylureido)hexyl] acetamide (MA-I) and *N*-{5-[3-(4-Dimethylaminophenyl)ureido]pentyl}-2-mercaptoacetamide (MA-II) (38).

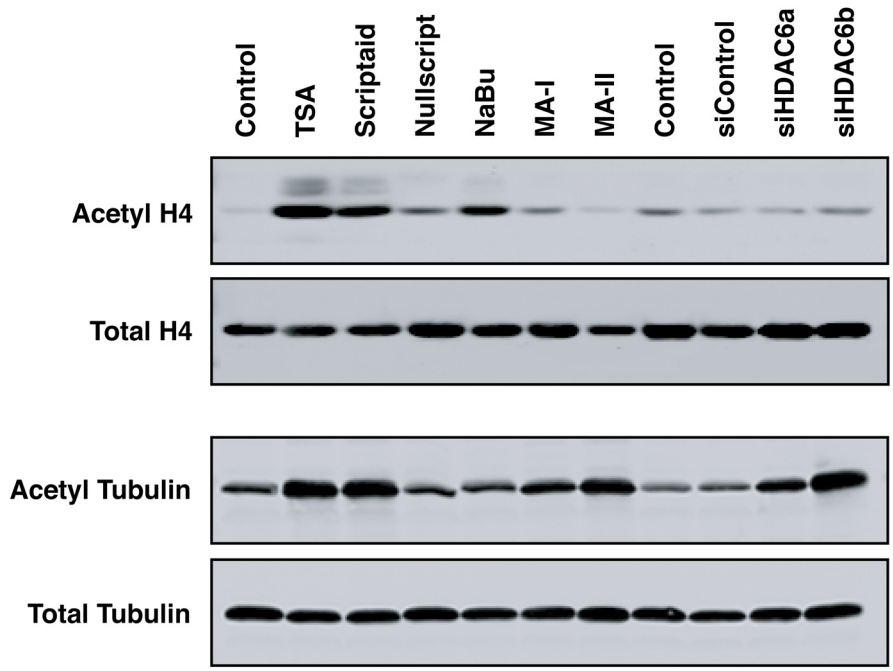


Fig. S4. HDAC6-selective inhibition or knockdown increases α-tubulin acetylation but not histone H4 acetylation. Western blot analysis to detect relative levels of acetylated histone H4 (Acetyl H4) and acetylated tubulin (Acetyl Tubulin) in total cellular lysates from rat primary cortical neurons treated with the pan-HDAC inhibitors TSA (0.66 μM) or Scriptaid (6.13 μM); the structural analog of Scriptaid that lacks HDAC inhibitory activity, Nullscript (6.13 μM); the HDAC Class I-selective inhibitor, NaBu (1 mM); the HDAC6-selective inhibitors MA-I (10 μM) or MA-II (10 μM); the penetratin-1-linked nonspecific 21-nt duplex (siControl; 200 nM); the penetratin-1-linked HDAC6 specific siRNAs (siHDAC6a or siHDAC6b; 200 nM) for 24 h. Relative levels of total histone H4 and total tubulin are shown to indicate loadings in Western blots.

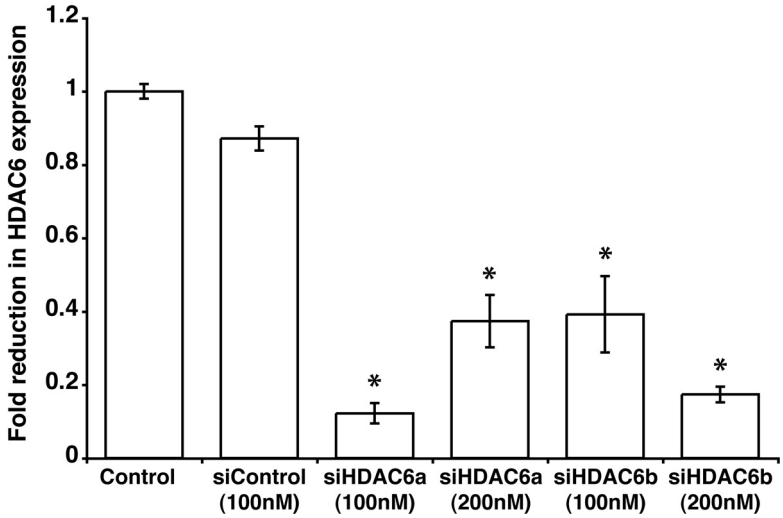
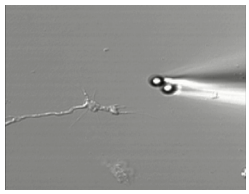
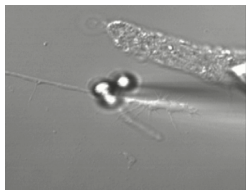


Fig. S5. Penetratin-1-linked HDAC6-specific siRNAs result in efficient HDAC6 knockdown in primary cortical neuron cultures. Graph showing relative levels of HDAC6 message as measured by real-time PCR on cDNAs prepared from total RNA extracted from rat primary neuronal cultures treated with penetratin-1-linked nonspecific 21-nt duplex (siControl; 200 nM), or penetratin-1-linked HDAC6 specific siRNAs (siHDAC6a and siHDAC6b; 200 nM) for 24 h. Expression was normalized using rat β-Actin. Data are representative of at least three independent experiments. *, Significant decrease in HDAC6 expression relative to nontreated control, $P < 0.001$, by one-way ANOVA followed by Turkey's Multiple Comparison Test.



Movie S1. MAG-coated microspheres (4.5 μm) presented to a DRG growth cone treated with vehicle control (DMSO). Growth was monitored by DIC with confocal microscopy, and cells were imaged for a total of 60 min, with image acquisition every 3 min during this period. The resultant .avi file was converted to .mov using QuickTime media player (Apple Computer) and Sorenson compression (Sorenson Media). The video shows three frames per s.

Movie S1 (MOV)



Movie S2. MAG-coated (4.5 μm) microspheres presented to a DRG growth cone treated with the HDAC6-selective inhibitor MA-I. Growth was monitored by DIC with confocal microscopy, and cells were imaged for a total of 60 min, with image acquisition every 3 min during this period. The resultant .avi file was converted to .mov using QuickTime media player (Apple Computer) and Sorenson compression (Sorenson Media). The video shows three frames per s.

Movie S2 (MOV)