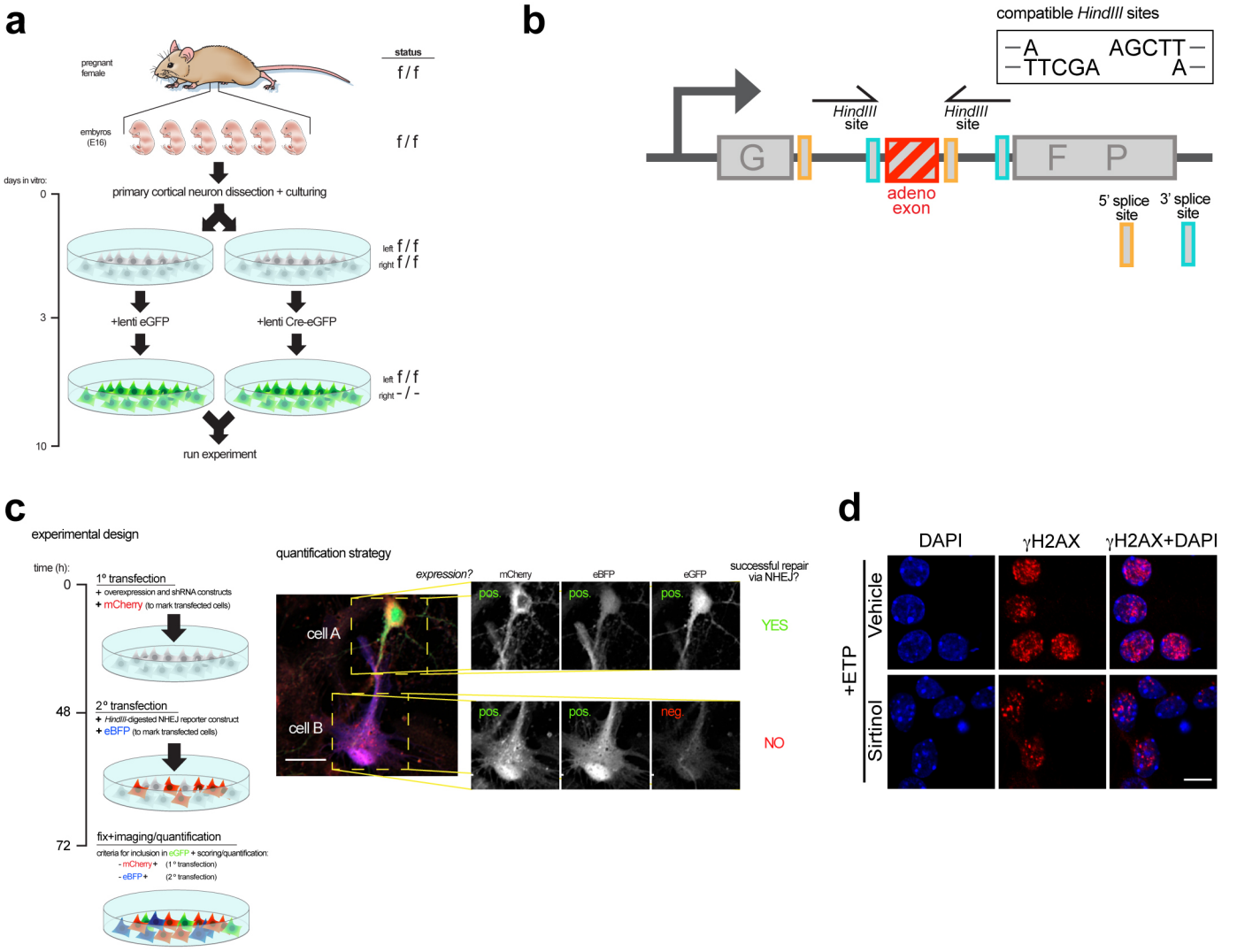
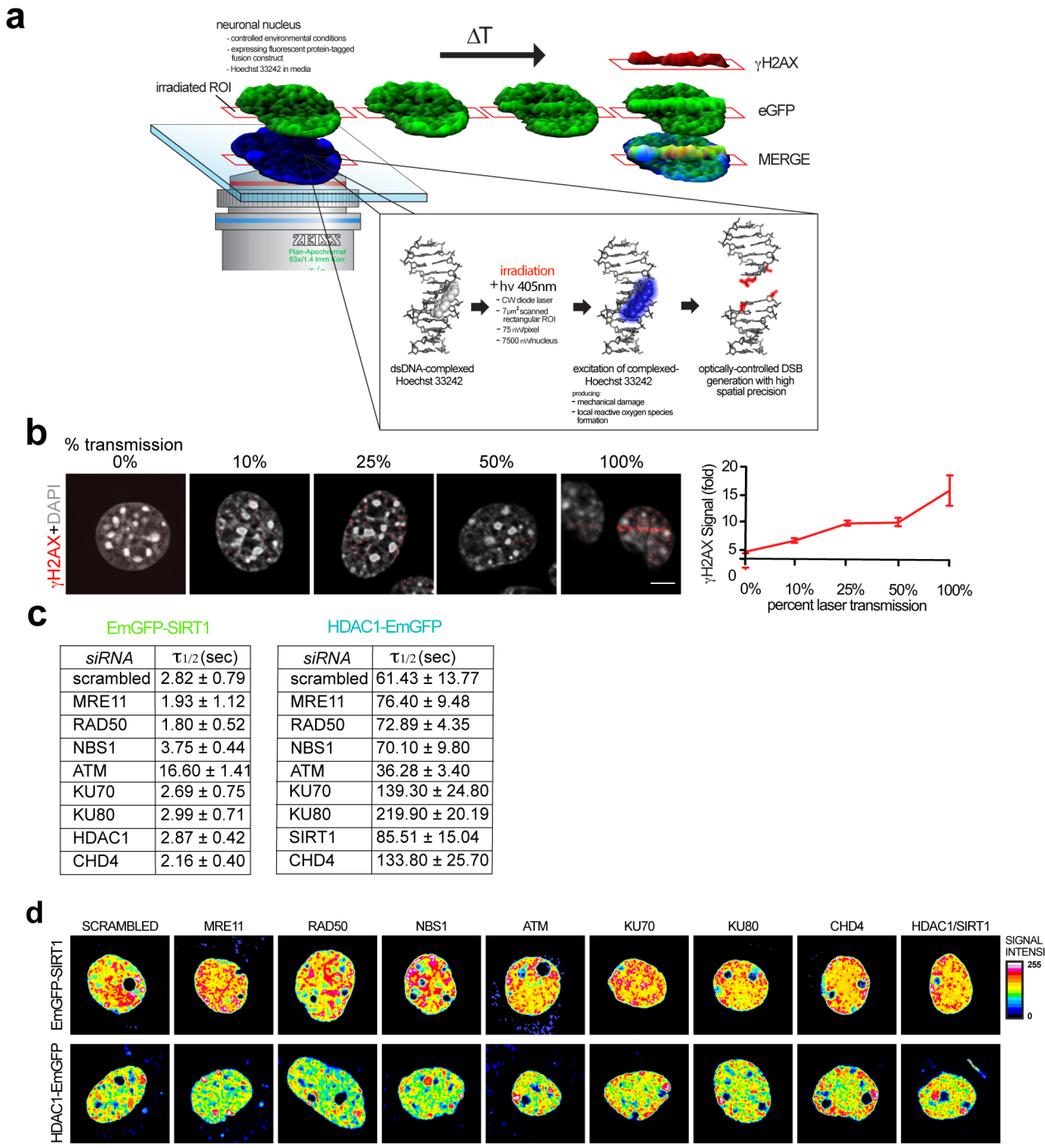
Supplementary Figure 1



Supplementary Figure 1. a, Schematic for generating *Sirt1 KO* neurons. Dissociated primary cortical neurons were cultured from E16 *Sirt1 F/F* embryos. DIV3 cultures were infected with a lentiviral vector carrying either a functional Cre recombinase (Cre-eGFP) or a non-functional Cre (eGFP). Neurons were usually used 7 days after infection with Cre-eGFP. A similar strategy was used to generate *Hdac1 KO* neurons. **b,** Construct used to measure efficiency of DNA repair using NHEJ. In this construct, a functional *eGFP* gene is interrupted by an intron. The presence of an adenoviral exon sequence within the intron prevents it from normally being spliced out. Generation of a DSB using HindIII and its subsequent repair using NHEJ disrupts the adenoviral exon sequence. Consequently, the intron is spliced out, allowing for expression of GFP. In this way, GFP+ cells can be used to score the efficiency of NHEJ. **c,** Outline of the experimental scheme used to adapt the NHEJ reporter assay to cultured primary neurons in our study. **d,** Primary cortical neurons (DIV14) were incubated overnight with the SIRT1 inhibitor, sirtinol (20μM final), then treated with 2μM etoposide for 1 h and analyzed as in Fig. 1c. Scale bar = 10μm.

Supplementary Figure 2

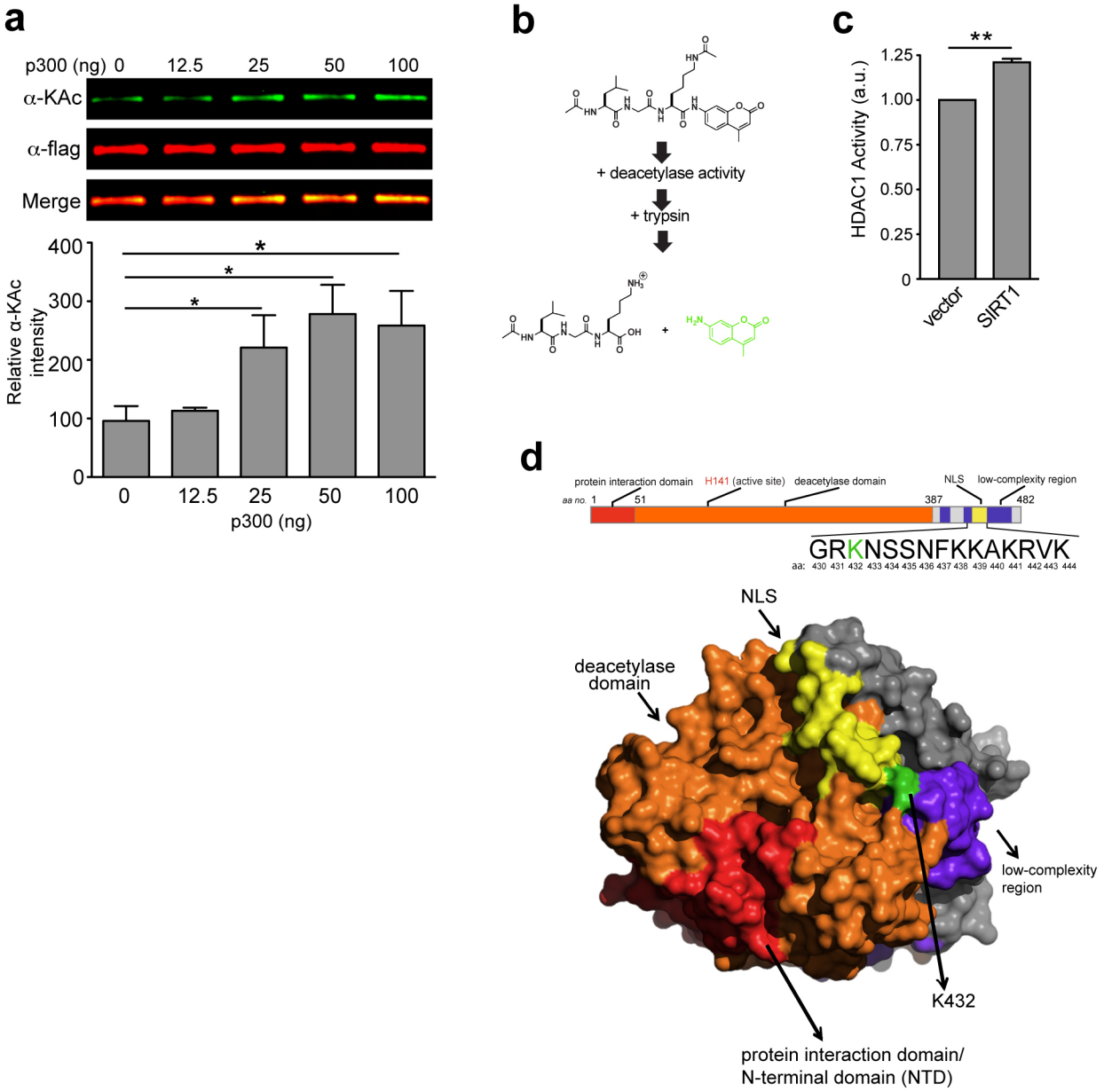


siRNA	$\tau_{1/2}$ (sec)
scrambled	2.82 ± 0.79
MRE11	1.93 ± 1.12
RAD50	1.80 ± 0.52
NBS1	3.75 ± 0.44
ATM	16.60 ± 1.41
KU70	2.69 ± 0.75
KU80	2.99 ± 0.71
HDAC1	2.87 ± 0.42
CHD4	2.16 ± 0.40

siRNA	$\tau_{1/2}$ (sec)
scrambled	61.43 ± 13.77
MRE11	76.40 ± 9.48
RAD50	72.89 ± 4.35
NBS1	70.10 ± 9.80
ATM	36.28 ± 3.40
KU70	139.30 ± 24.80
KU80	219.90 ± 20.19
SIRT1	85.51 ± 15.04
CHD4	133.80 ± 25.70

Supplementary Figure 2. a, Schematic of laser microirradiation. A Zeiss LSM710 inverted laser scanning confocal microscope equipped with a 405nm diode laser was used to irradiate a thin sub-nuclear strip of Hoechst-stained primary neurons. Localization of proteins to sites of laser-induced DNA DSBs can be monitored as increased fluorescence intensity within lesioned regions as visualized either by immunocytochemistry of fixed cells (for instance, γH2AX) or through live imaging of cells carrying fluorescently tagged repair proteins. **b,** Serial attenuation of transmitted 405 nm wavelength light emitted from a continuous-wave diode laser yields a dose-dependency in γH2AX signal intensity within lesion ROIs. ROI area (2μm2), laser power (100%), and scan iterations were held constant for Hoechst33242 pre-sensitized neuronal nuclei, while percent transmission was varied as indicated. Neurons were then fixed and stained with γH2AX. Scale bar = 7μM. **c,** Table indicating the time taken by EmGFP-SIRT1 (left) and HDAC1-EmGFP (right) to attain half-maximal fluorescence intensity in the lesioned region following the knockdown of the DSB components in Fig. 3d. **d,** Representative images of EmGFP-SIRT1 (top) and HDAC1-EmGFP (bottom) expressing neurons that were transfected with the indicated siRNAs to show that the various siRNAs did not affect expression of the two proteins.

Supplementary Figure 3



Supplementary Figure 3. a, Increasing amounts of p300 were incubated with a fixed amount of HDAC1 and the effect of p300 on the acetylation of HDAC1 was assessed using quantitative western blotting (* $p < 0.05$, one-way ANOVA). **b,** Schematic of a fluorescence-based reporter assay used to measure HDAC1 enzymatic activity. Deacetylation of the substrate sensitizes it to cleavage by trypsin, which results in the release of a fluorescent moiety (green). Fluorescence intensity is thus used as an indicator of deacetylase activity. **c,** HEK293T cells were transfected with either an empty vector or a vector carrying SIRT1. HDAC1 was then immunoprecipitated, and its activity was measured as described in b (*$p < 0.01$, unpaired t-test). **d,** Sequence and structural information from an already crystallized HDAC1 ancestor from the hyperthermophillic bacterium Aquifex aeolicus (PMID: 10490031) was used to generate a computational model predicting the tertiary structure of HDAC1. Identical domain color scheme was utilized in domain illustration and predicted structure rendering. Green indicates the position of the lysine residue, K432.

Supplementary Figure 4