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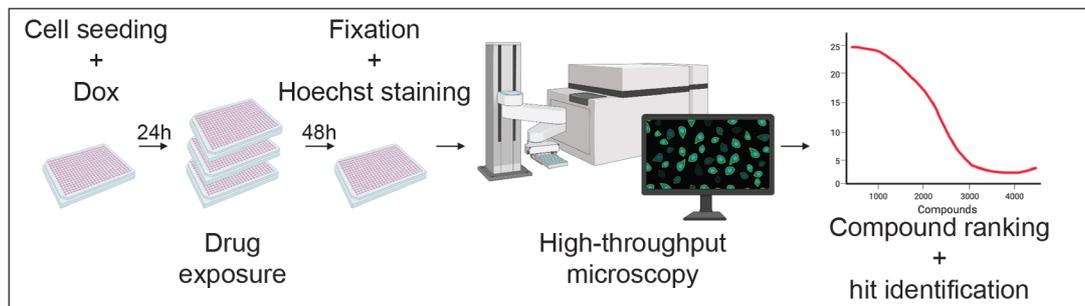


Fig. S1. TDP-43 overexpression reduces cell viability by triggering apoptosis, related to Figure 1.

- A) Viability of U2OS^{T43} cells measured by quantifying nuclei through HTM after 72 hrs of exposure to the indicated Dox concentrations. Data are normalized to untreated cells and represent the mean \pm SEM (n=6 biological replicates). ****p<0.0001 by one-way ANOVA with Dunnett's multiple comparisons test.
- B) TDP-43^{EGFP} levels in U2OS^{T43} cells quantified by HTM using EGFP signal, 72 hrs after being treated with Dox at the indicated concentrations. Data represents the mean \pm SEM of the EGFP integrated intensity measured in 5 independent experiments.
- C) WB illustrating the levels of TDP-43 and TDP-43^{EGFP} in U2OS^{T43} cells after 72 hrs of Dox associated with PARP cleavage as a readout of apoptosis. β -actin levels are shown as a loading control.
- D) Viability of U2OS^{EGFP} cells measured by quantifying nuclei through HTM after 72 hrs of exposure to the indicated Dox concentration. On the right, WB showing endogenous TDP-43 and any cleavage of PARP are reported. β -actin levels are shown as a loading control.
- E) Schematic overview of the screening workflow. U2OS^{T43} cells were seeded together with Dox (10 ng/ml) in triplicate plates. After 24 hrs, compounds from the chemical library were dispensed at a final 1 μ M concentration. 48 hrs after exposure, cells were fixed and stained with Hoechst to enable the quantification of nuclei numbers by HTM.

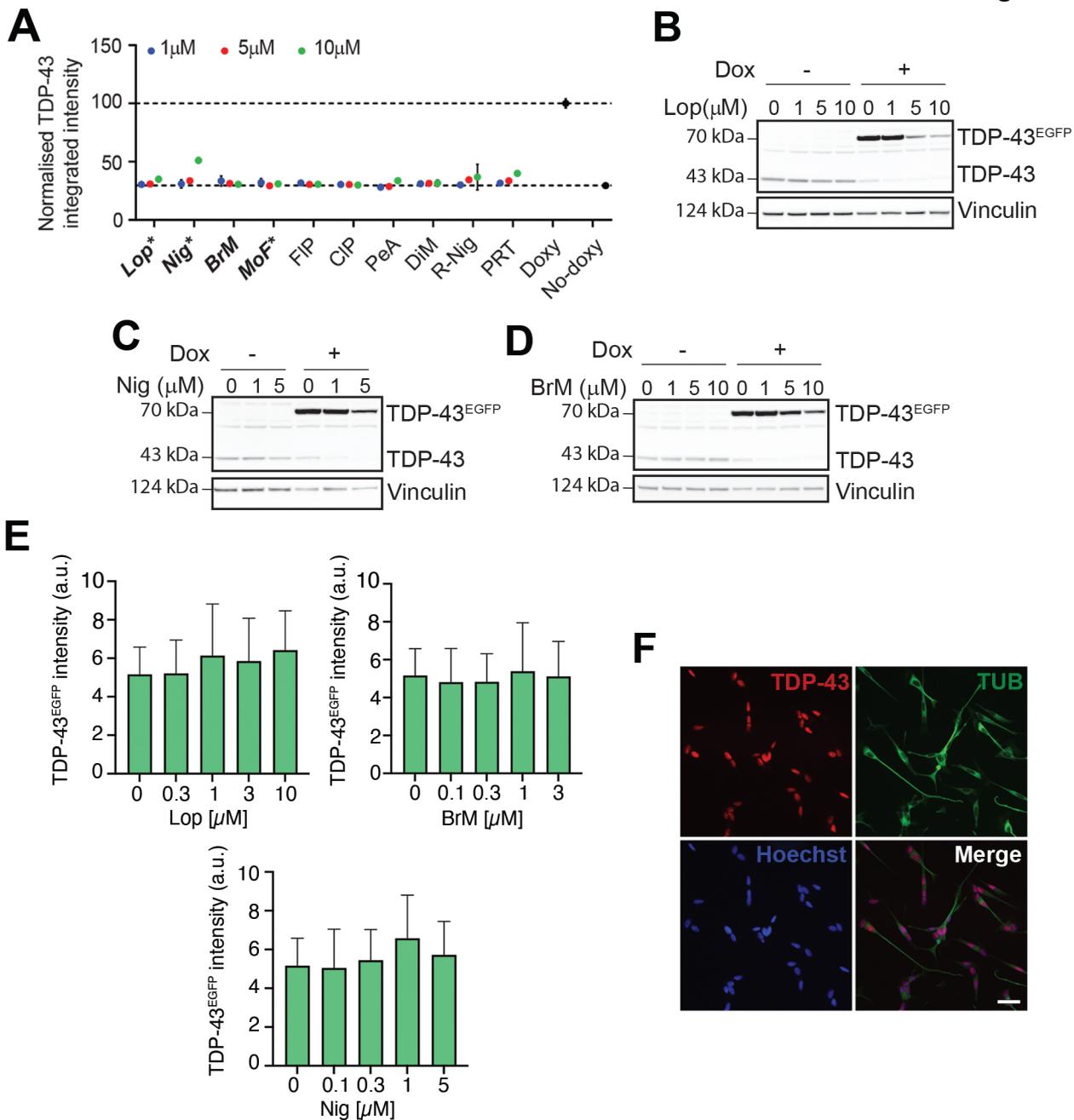


Fig. S2. Effect of the hit compounds on endogenous and Dox-dependent TDP-43^{EGFP} levels, related to Figure 2.

- A) TDP-43 levels in U2OS^{T43} cells quantified by HTM using an antibody against endogenous TDP-43, after 72 hrs after being treated with Dox or after the indicated compounds at three independent concentrations (1, 5 and 10 μ M) without doxycycline. Data are normalized to Dox treated cells (Dox) and represent the mean \pm SEM (n=6 biological replicates).
- B) WB illustrating the levels of TDP-43 and TDP-43^{EGFP} in U2OS^{T43} cells after exposure to Dox for 24 hrs followed by 48 hours of Lop at the indicated concentrations. Vinculin levels are shown as a loading control.
- C) WB illustrating the levels of TDP-43 and TDP-43^{EGFP} in U2OS^{T43} cells after exposure to Dox for 24 hrs followed by 48 hours of Nig at the indicated concentrations. Vinculin levels are shown as a loading control.
- D) WB illustrating the levels of TDP-43 and TDP-43^{EGFP} in U2OS^{T43} cells after exposure to Dox for 24 hrs followed by 48 hours of BrM at the indicated concentrations. Vinculin levels are shown as a loading control.
- E) TDP-43 levels in neurons differentiated from SH-SY5Y cells as quantified by HTM using an antibody against endogenous TDP-43, 48 hrs after being treated with the indicated compounds.
- F) Representative image from the HTM analysis defined in E, revealing the neuronal morphology of the SH-SY5Y cultures stained with antibodies against TDP-43 (red) and β 3-TUBULIN (TUB, green). DNA was stained with Hoechst (blue). Scale bar (white) indicates 10 μ m.

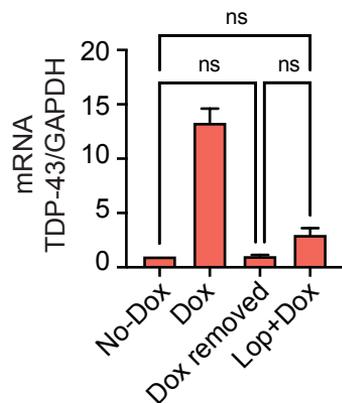
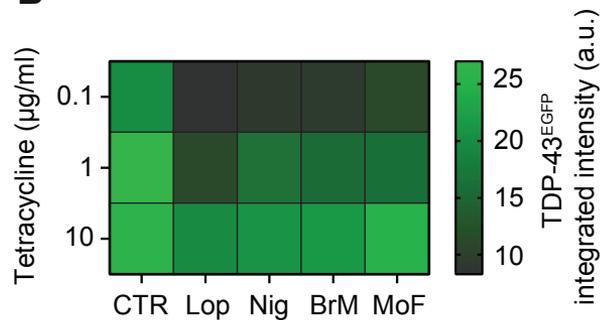
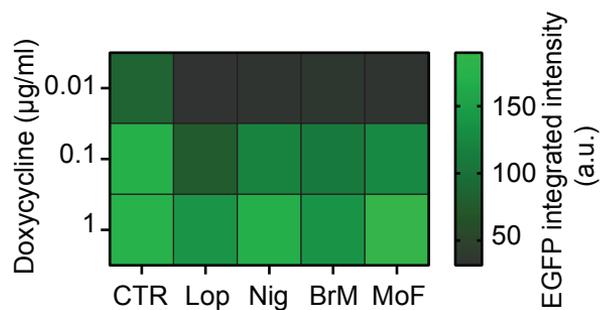
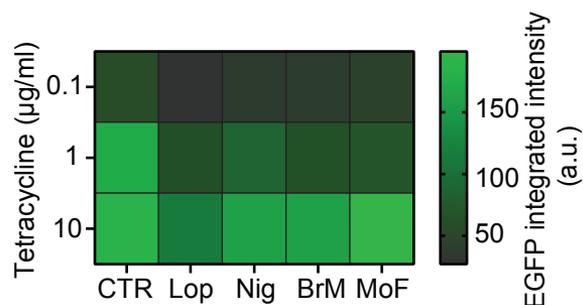
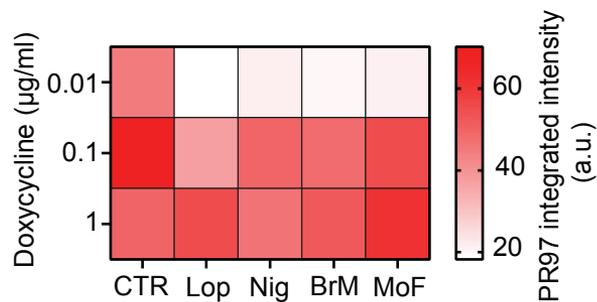
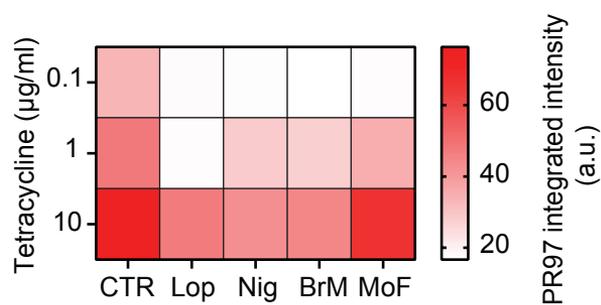
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Fig. S3. A dose-dependent antagonistic effect of hit compounds on Dox- and tet-dependent induction of gene expression, related to Figure 2 and 3.

- A) TDP-43 mRNA levels quantified by RT-qPCR in U2OS^{T43} cells treated as in Fig.2C. GAPDH levels were used to normalize expression levels to that of a housekeeping gene. Data represents the mean \pm SEM (n=2 biological replicates) of TDP-43 expression normalized on the untreated control (No-Dox). Statistical analysis has been performed by one-way ANOVA with Tukey's multiple comparison test.
- B) Heatmap of TDP-43^{EGFP} expression levels quantified by HTM in U2OS^{T43} cells exposed to increasing concentrations of tet with or without the indicated compounds. Data represents the mean \pm SEM (n=3 biological replicates).
- C) Heatmap of EGFP expression levels quantified by HTM in U2OS^{EGFP} cells exposed to increasing concentrations of Dox with or without the indicated compounds. Data represents the mean \pm SEM (n=3 biological replicates).
- D) Heatmap of EGFP expression levels quantified by HTM in U2OS^{EGFP} cells exposed to increasing concentrations of tet with or without the indicated compounds. Data represents the mean \pm SEM (n=3 biological replicates).
- E) Heatmap of PR97 expression levels quantified by HTM in U2OS^{PR97} cells exposed to increasing concentrations of Dox with or without the indicated compounds. Data represents the mean \pm SEM (n=3 biological replicates).
- F) Heatmap of PR97 expression levels quantified by HTM in U2OS^{PR97} cells exposed to increasing concentrations of tet with or without the indicated compounds. Data represents the mean \pm SEM (n=3 biological replicates).

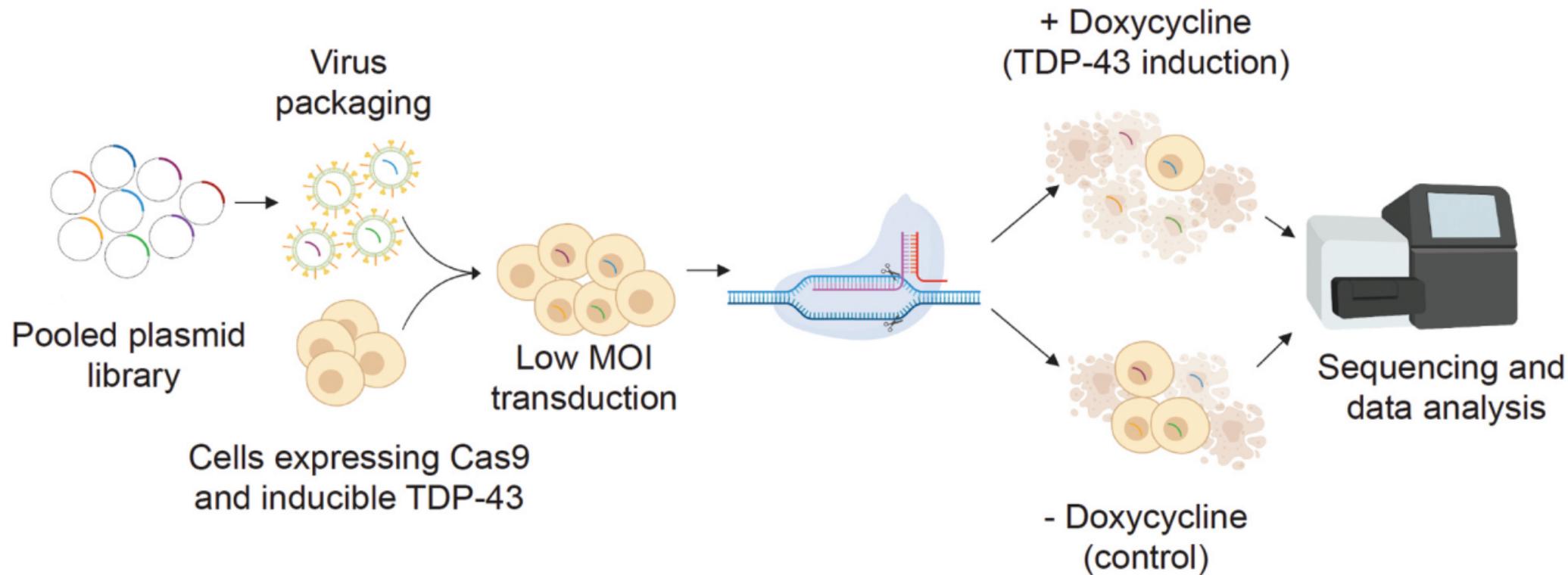


Fig. S4. Workflow of the CRISPR screen performed in U2OS^{T43} cells, *related to Figure 4.*

Schematic overview of the forward genome-wide CRISPR screen conducted in this study. Briefly, U2OS^{T43} cells stably expressing Cas9 were transduced with a pooled sgRNAs library at a low MOI. Transduced cells were then grown in the absence or presence of Dox for 10 days. At this point, the abundance of each sgRNA in the remaining cell populations was calculated by next generation sequencing of PCR-amplified sgRNA sequences. The experiment was performed in 2 independent biological replicates and enrichment scores calculated independently from each of them.