Supplementary Information for

Cytotoxicity of Activator Expression in CRISPR-based Transcriptional Activation Systems

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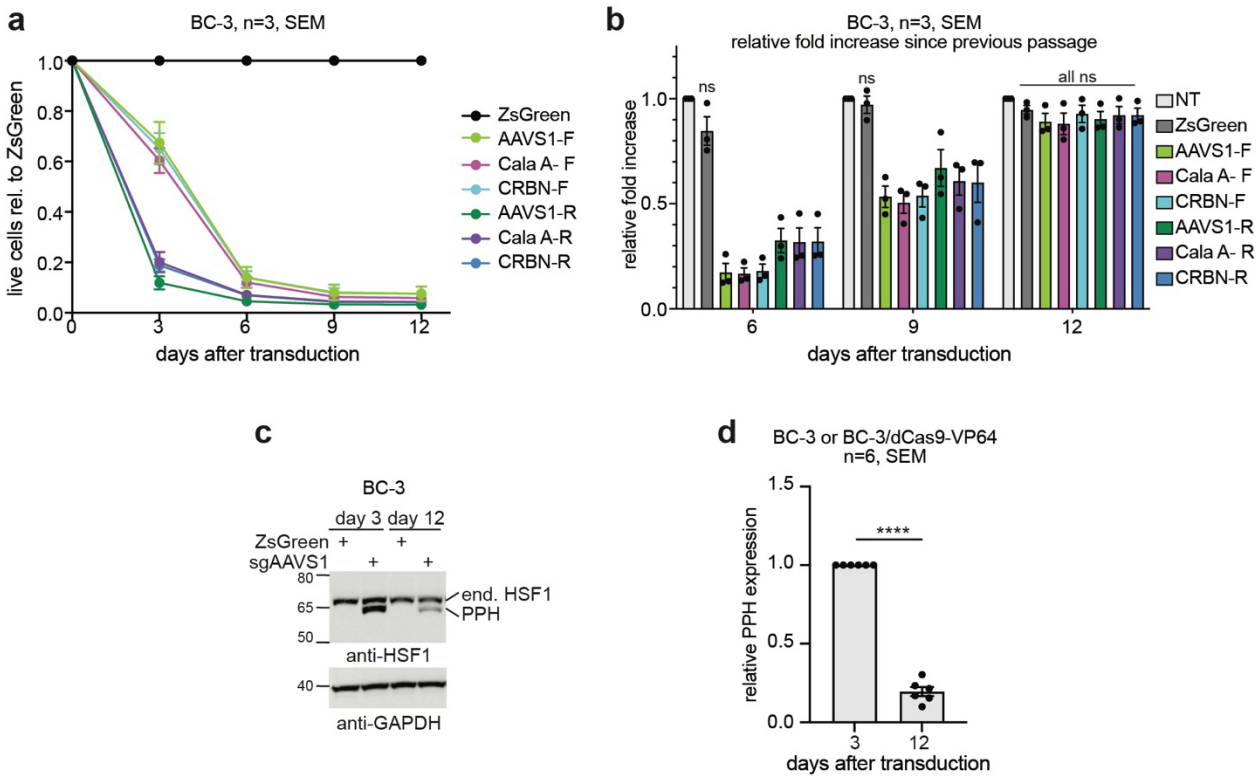
This PDF file includes:
Supplementary Figures 1-10
Uncropped Westerns for Supplementary Figures

Other Supplementary Files for this manuscript include the following:

Supplementary Data 1	Analyzed mRNA-seq results, see Methods for analysis approach.
Supplementary Data 2	Complete GSEA results, see Methods for analysis approach.
Supplementary Data 3	Sequences of oligonucleotide and DNA fragments



Supplementary Figure 1. Extended Data for Fig. 1.

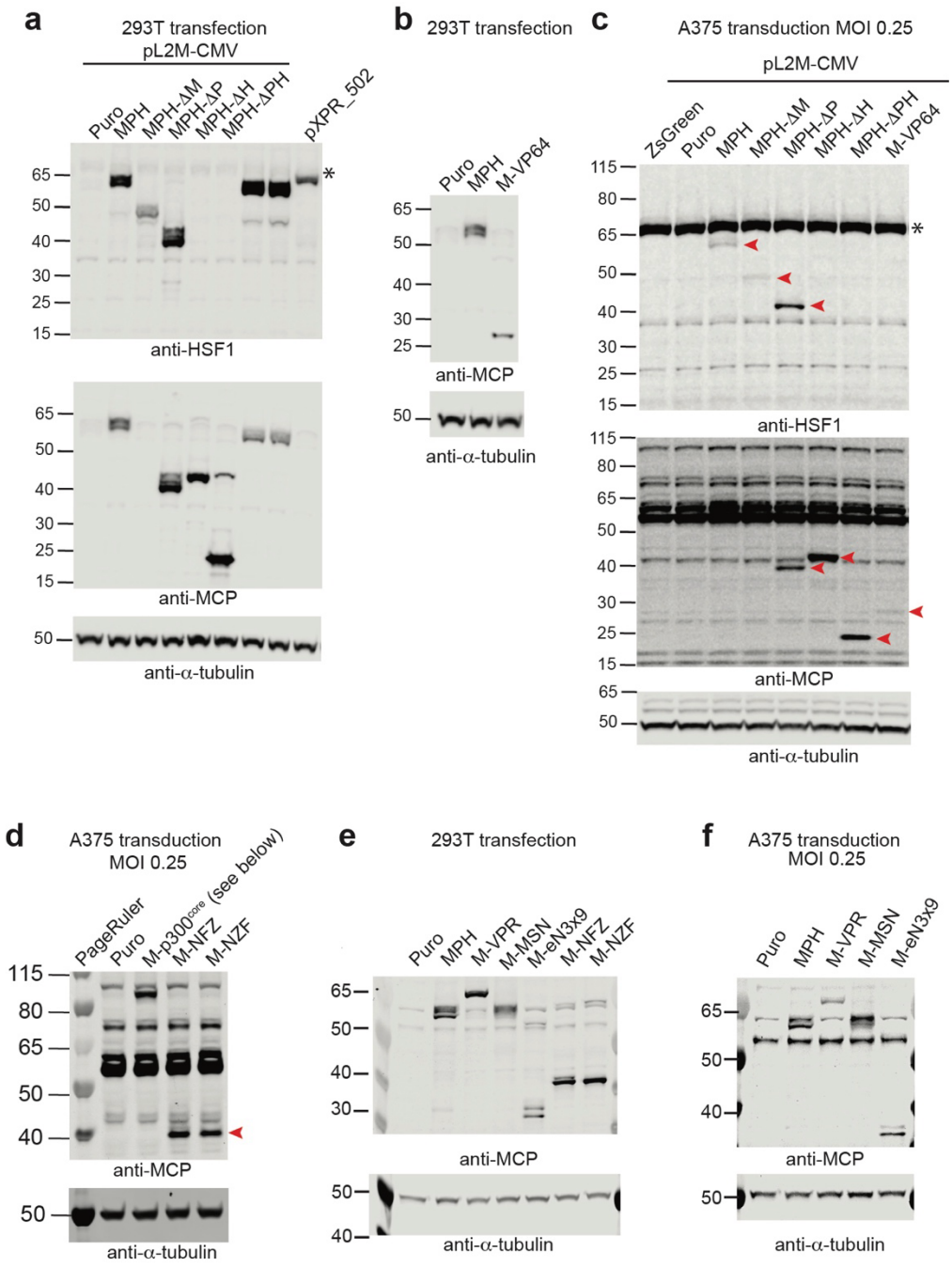
a As in Fig. 1f but using parental BC-3 cells. 3 independent repeats, using the 3 LV preps from Fig. 1 panels d-e. All values differed significantly from the ZsGreen control, determined using One-Way Anova with Tukey's multiple comparison test. For other comparisons, see Source Data file.

b As in Fig. 1g but using parental BC-3 cells, related to the growth curves shown in Supplementary Figure 1a. Values differed significantly from NT at each time point unless specified by ns, determined using One-Way Anova with Tukey's multiple comparison test.

c Western Blot analysis of PPH expression as in Fig. 1h but using parental BC-3, representative result that is quantified over replicates in panel d. Molecular weight marker positions (in kDa) are indicated at left.

d Quantification of PPH expression from results as in Fig. 1h and S1c, combining 2 replicates each for pXPR-sgAAVS1 in BC-3, and Calabrese A or pXPR-sgCRBN-a1 in BC-3/dCas9-VP64. Since these vectors behaved similarly in both cell types (Fig. 1f-g, and Supplementary Figure 1 a-b), we treated these Westerns as six independent replicates for this analysis. Significance in this panel was estimated using an unpaired t test (2-tailed), **** denotes $p<0.0001$.

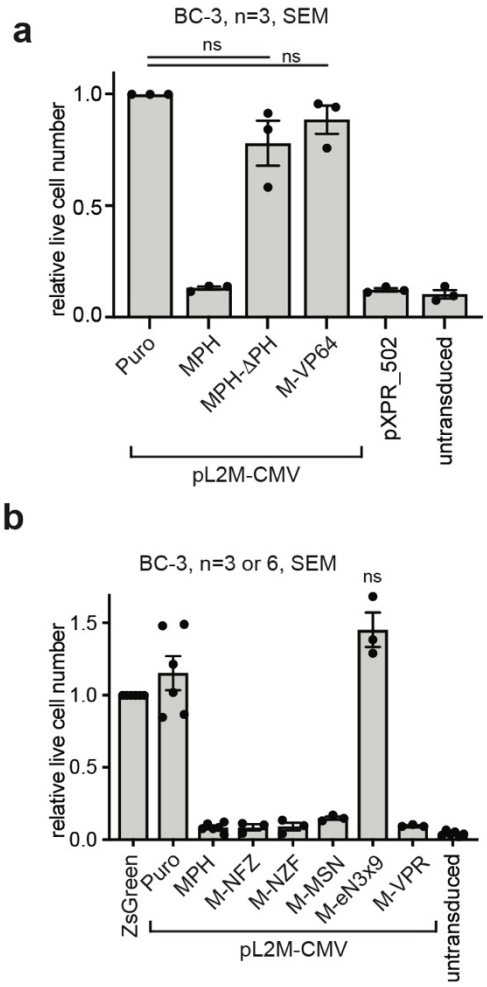
Throughout, error bars represent SEM and ns denotes “not significant”. Source data, adj. p values, or p values are provided in the Source Data file. For uncropped Westerns including molecular weight markers see below in this document.



Supplementary Figure 2. Extended Data for Fig. 2.
a Western Blot analysis of a subset of the LVs from Fig. 2a-b, after expression in 293T. Lysates were probed for the $HSF1^{AD}$ using anti-HSF1 antibodies, for MCP, and for α-tubulin as a loading control. The likely migration of endogenous HSF1 is marked by an asterisk. One repeat in this exact configuration, but each vector was validated similarly at least three times in various configurations. Two unlabeled lanes analyze additional vectors that are not described here.
b Western Blot Analysis of a second subset of the LVs from Fig. 2a-b, after expression in 293T. Lysates were probed for MCP, and α-tubulin served as a loading control. One repeat in this

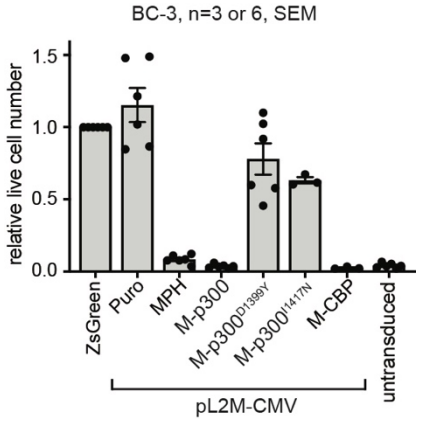
exact configuration, but each vector was validated similarly at least three times in various configurations.
c-d As in Supplementary Figure 2a-b but using lysates from two days after transduction of A375 at MOI 0.25, without puromycin selection. The background is much higher compared to Supplementary Figure a-b, due to low copy transduction and the background of untransduced cells in the absence of puromycin selection. Toxic protein expression is likely weaker in this experiment due to the beginning loss of transduced cells before harvest. Representative of 2 (c) or 3 (d) repeats per vector.
e As in Supplementary Figure 2a-b, after transfection of the indicated LV vectors into 293T. Representative of 2 independent repeats.
f As in Supplementary Figure c-d, again after transduction of A375 at MOI 0.25. Representative of 3 independent repeats.

Throughout, molecular weight marker positions (in kDa) are indicated at left. For uncropped Westerns (including molecular weight markers), see below in this document.

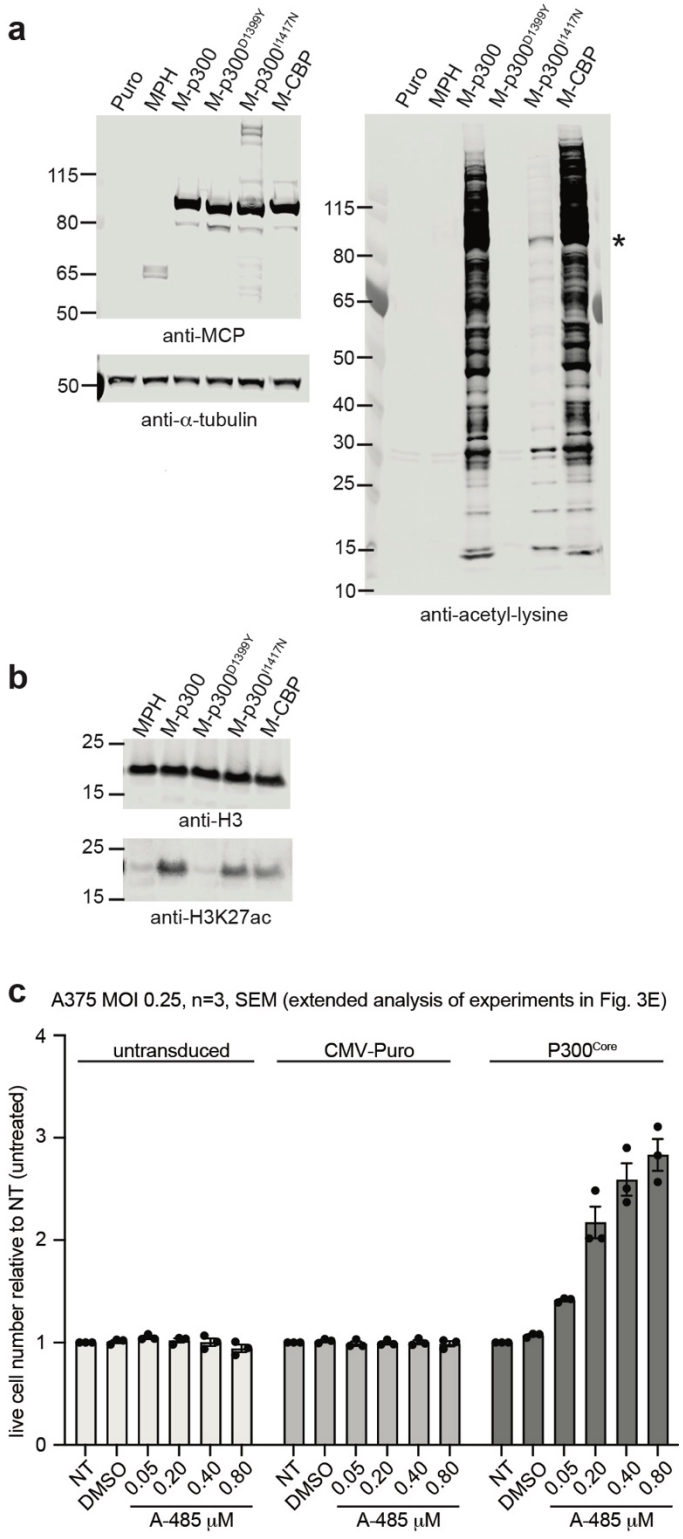


Supplementary Figure 3. Extended Data for Fig. 2.
a Like Fig. 2b, but in BC-3 and including only a subset of the vectors. Relative numbers of BC-3 cells surviving puromycin selection after transduction with the indicated vectors at MOI 0.25 based on LV-gRNA content and the functional titer of the ZsGreen control vector, which was not included in the final experiment. Survival was measured using Cell titer Glo 2.0 on day 3 after transduction, and values were normalized to the CMV-Puro control. Values differed significantly from the CMV-Puro control unless indicated by ns, determined using One-Way Anova with Tukey's multiple comparison test. n=3.
b As in panel a with additional vectors. Values for pL2M-CMV vectors differed significantly from the CMV-Puro control, unless indicated by ns, determined using One-Way Anova with Tukey's multiple comparison test. n=6 (ZsGreen, Puro, MPH, untransduced) or 3 (all others).

Throughout, error bars represent SEM and ns “not significant”. Source data and adj. p values are provided in the Source Data file.



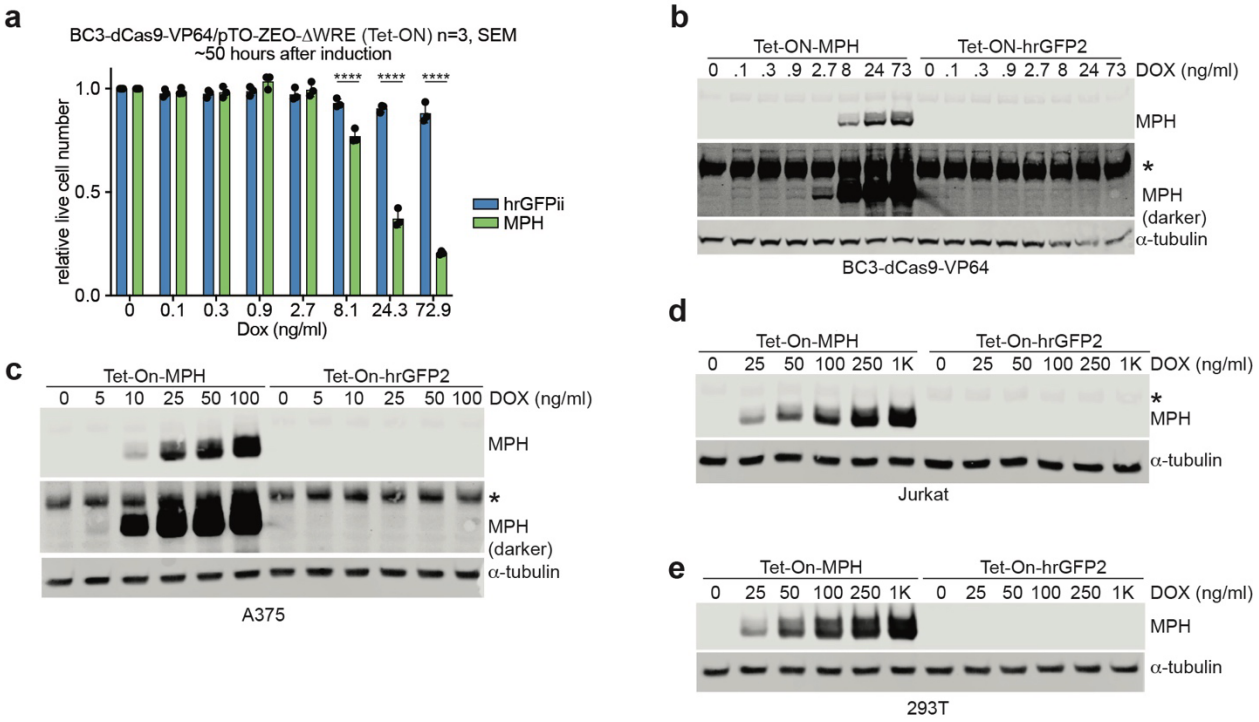
Supplementary Figure 4. Extended Data for Fig. 3c
Like Fig. 3c, but in BC-3. Relative numbers of BC-3 cells surviving puromycin selection after transduction with the indicated vectors at MOI 0.25 based on LV-gRNA content and the functional titer of the ZsGreen control vector. Survival was measured using Cell titer Glo 2.0 on day 3 after transduction, and values were normalized to the CMV-Puro control. Values for all pL2M vectors differed significantly from the CMV-Puro control, determined using One-Way Anova with Tukey's multiple comparison test. n=3 (M-p300^{I1417N}, M-CBP) or 6 (all others). Error bars represent SEM. Source data and adj. p values are provided in the Source Data file.



Supplementary Figure 5. Extended Data for Fig. 3d and 3f.
a Western Blot analyses of a subset of the LVs from Fig. 3d, after expression in 293T. Lysates were probed for MCP, acetylated lysines, and for α-tubulin as a loading control. The likely migration of the autoacetylated MCP-HAT^{Core} fusion proteins is marked by an asterisk.

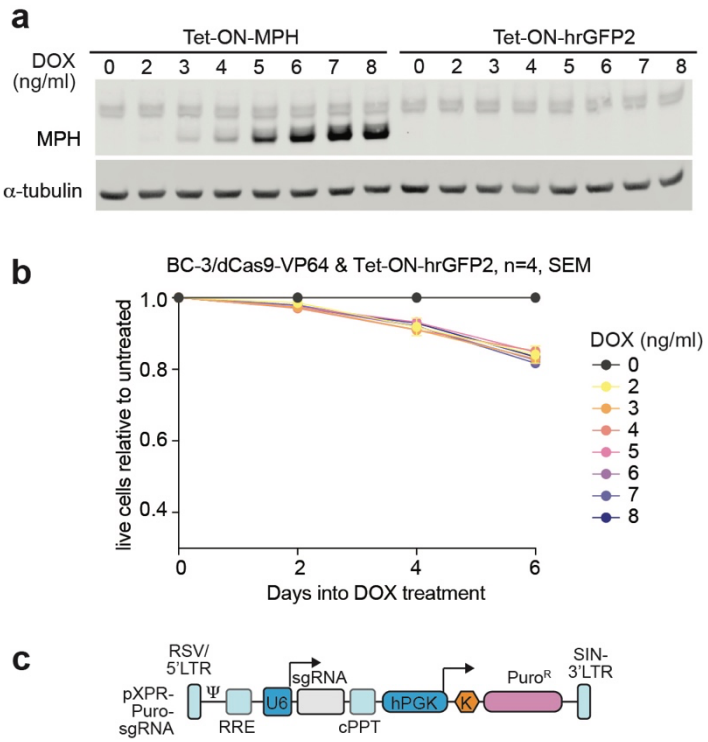
Representative of 2 independent repeats per vector in this exact configuration. Molecular weight marker positions (in kDa) are indicated at left.
b A subset of the lysates analyzed in Supplementary Figure 5a were additionally probed for Histone H3 and Histone H3 with acetylated lysine 27. Representative of 2 independent repeats. Molecular weight marker positions (in kDa) are indicated at left.
c Extended Analysis of all samples included in the experiments also shown in Fig. 3f. For this panel, results were only normalized to the untreated (NT) control, which did not receive either DMSO or A-485. See Source Data File for a complete analysis. 3 independent repeats.

Source data and adj. p values for panel c are provided in the Source Data file, together with data from Fig. 3f. For uncropped Westerns including molecular weight markers see below in this document.



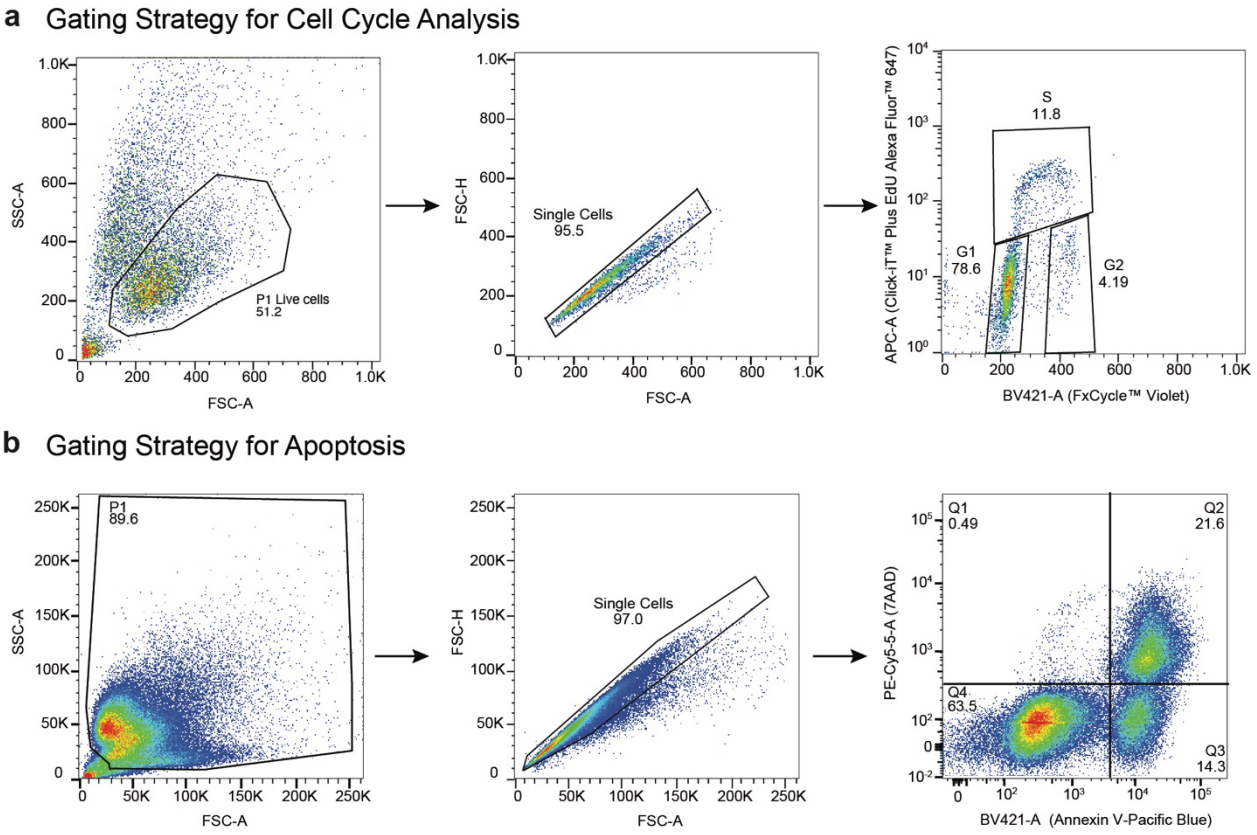
Supplementary Figure 6. Extended Data for Fig. 4.
a Experiment as in Fig. 4b, but in BC-3/dCas9-VP64. 3 independent repeats. Error bars represent SEM and **** denotes adj. p<0.0001, determined using One-Way Anova with Tukey's multiple comparison test.
b Western Blot analysis of MPH expression in samples as in Supplementary Figure 6a. α-tubulin served as a loading control. n=1, since this panel simply confirms MPH expression. For analysis over replicates see Fig. 5b. The panel shows two different displays of the MPH panel to visualize MPH expression over the range of concentrations.
c-e As in panel Supplementary Figure 6b, but in A375, Jurkat, and 293T cells.

The asterisk marks endogenous HSF1 throughout. Source data and adj. p values are provided in the Source Data file. For uncropped Westerns including molecular weight markers, see below in this document.

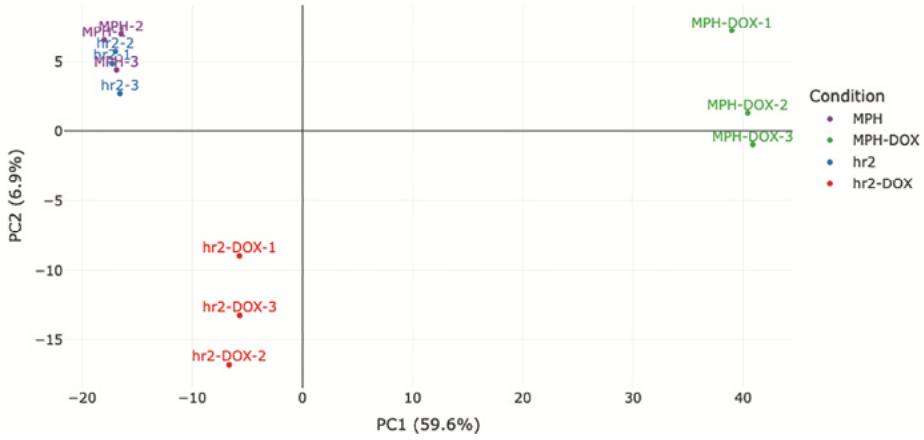


Supplementary Figure 7. Extended Data for Fig. 5
a Full panel showing all samples from the experiment in Fig. 5a. Representative of n=3.
b As in Fig. 5c but showing results from Tet-ON-hrGFP2.
c Schematic of the sgRNA vector used in Fig. 5. Abbreviations are as in Fig. 1b.

Source data and adj. p values are provided in the Source Data file. For uncropped Westerns including molecular weight markers see Source Data File for Fig. 5a.



Supplementary Figure 8. Gating Strategy for FACS Analyses in Fig. 6
a For cell cycle analysis, cells were first gated based on size and granularity (FSC-A and SSC-A, left panel), with live cells selected in gate P1. Singlets were identified using FSC-H vs. FSC-A (middle panels), excluding doublets and aggregates (middle panel). Finally, single cells were analyzed for staining using BV421-A (DNA content based on FxCycle™ Violet) and APC-A (Click-iT™ Plus EdU Alexa Fluor™ 647). The gating strategy is illustrated using the same MPH+DOX sample shown in Fig. 6a.
b For Annexin staining, cells were first gated on FSC-A/SSC-A to exclude debris, but not dead cells (left panel), followed by gating for single cells (middle panel) and analysis for BV421-A (Annexin V-Pacific Blue) vs. PE-Cy5-5-A (7-AAD) (right panel). Quadrant gates were applied to distinguish unstained, single-positive, and double-positive cell populations. The gating strategy is illustrated using the same MPH+DOX sample shown in Fig. 6c.



Supplementary Figure 9. Extended Data for Fig. 6e-f
Principal Component Analysis (PCA) of the RNA-seq data used for Fig. 6e-f.

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GACGTCTGCTTCAAACTTTACTCAGTTCGTGCTCGTGGACAATGGTGGGACAGGGGATGT
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Supplementary Figure 10. MCP-VPR-P2A-Puro coding sequence within pL2M-CMV-MCP-VPR-P2A-Puro.

Components are as follows: NLS from Mus musculus nuclear cap binding protein subunit 1 (Ncbp1) (green), MCP (yellow), VP64 (purple), p65-AD (orange), RTA-AD (blue), P2A-Puro^{R} (brown).

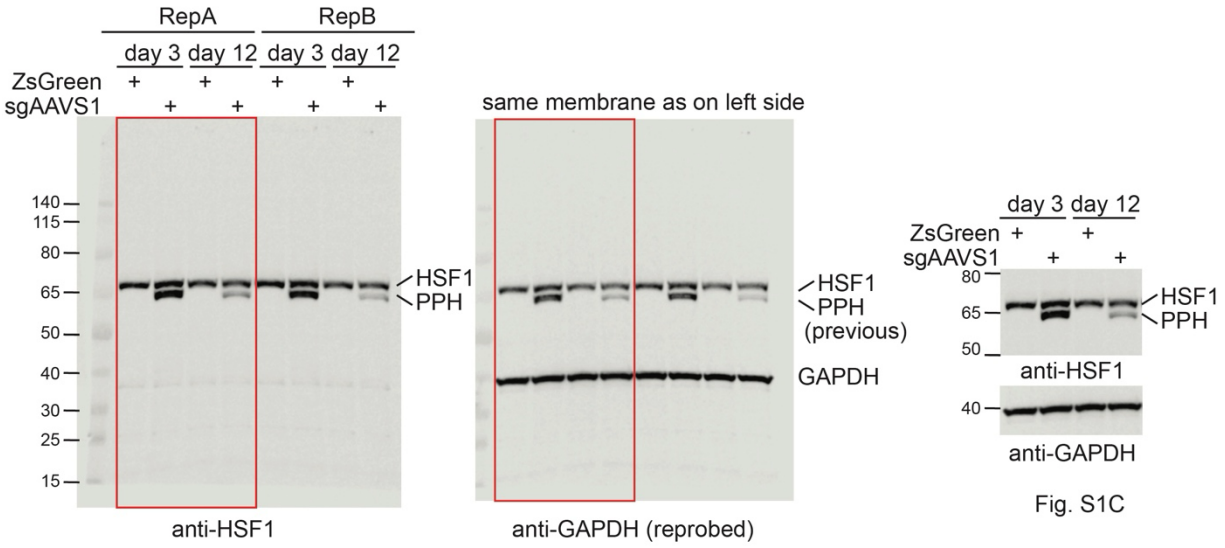
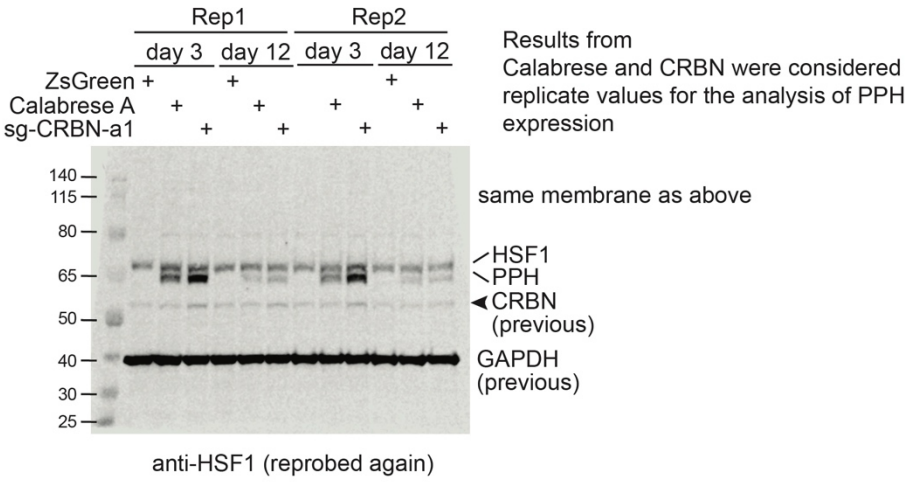
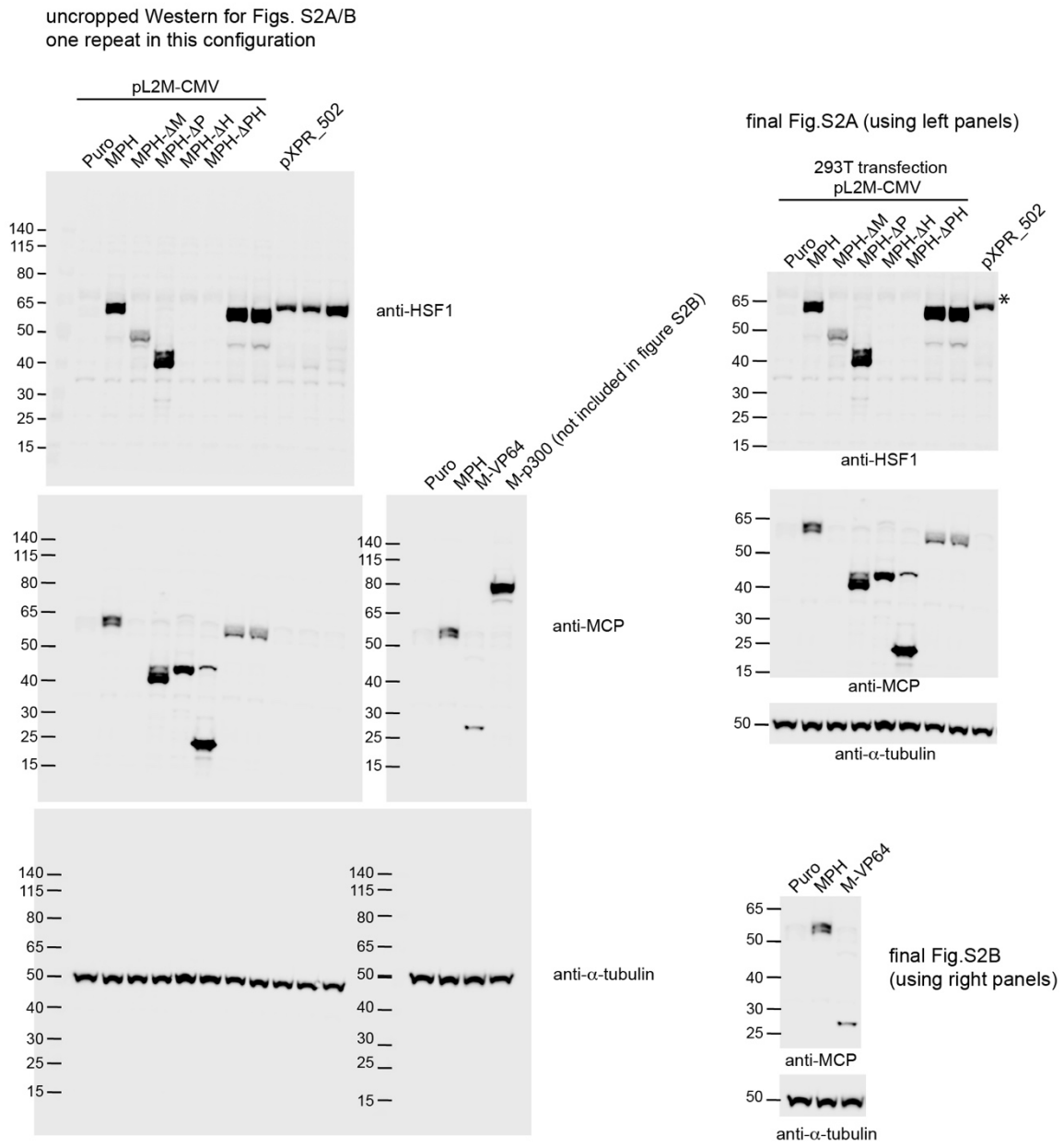


Fig. S1C

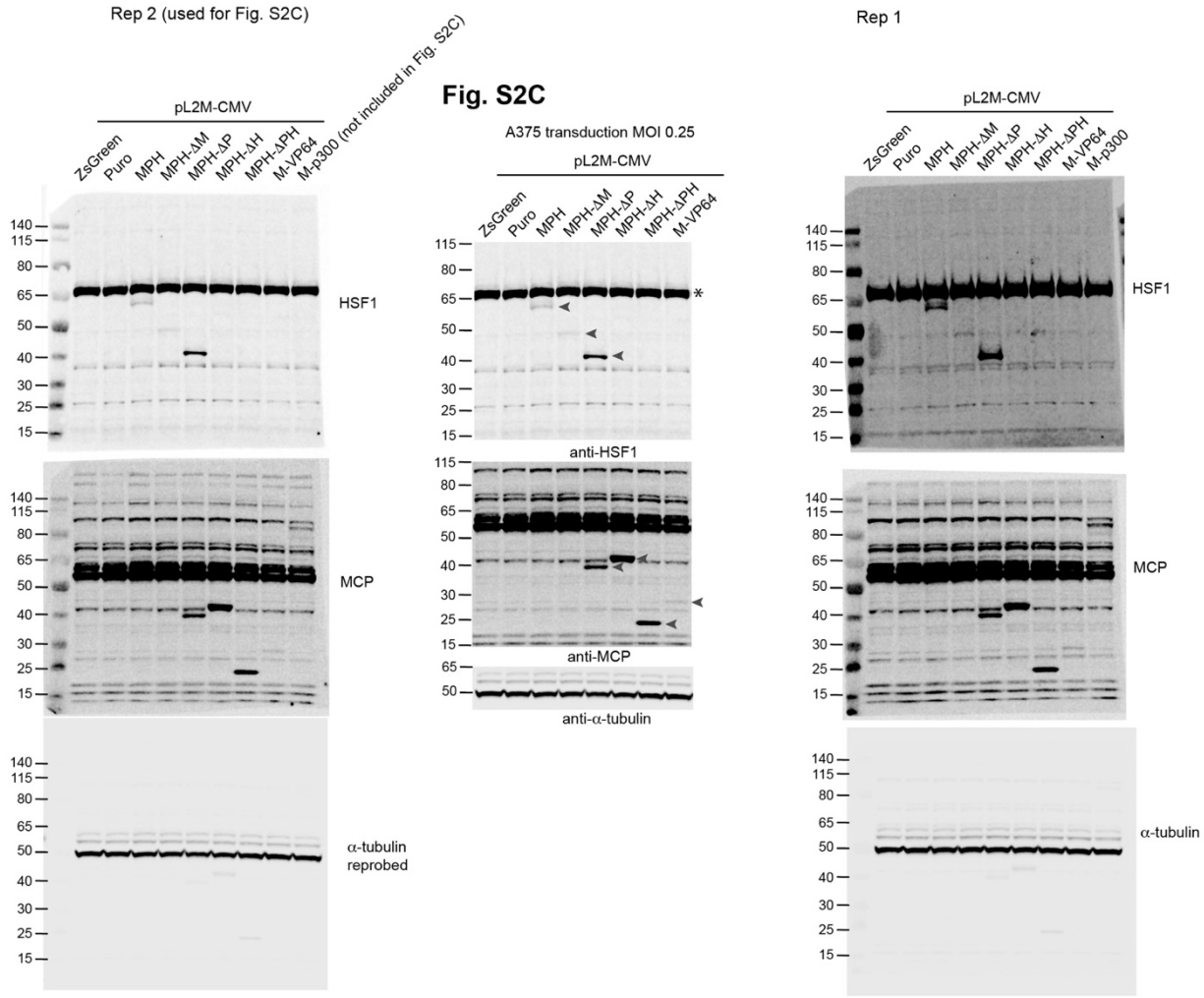
Membrane 1: Reps 1 and 2 - also part of Fig. 1I



Uncropped Westerns for Supplementary Figure 1c. Membrane 1 was also used for Fig. 1I (see separate tab)**.** Panels show all results that were used for quantification in Supplementary Figure 1d. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.

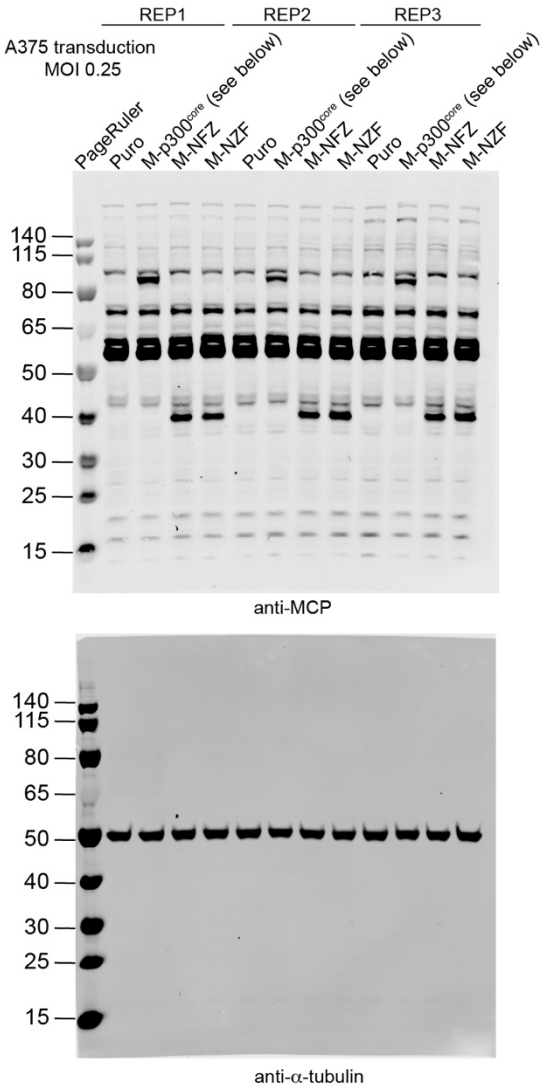


Uncropped Westerns for Supplementary Figure 2a-b. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.

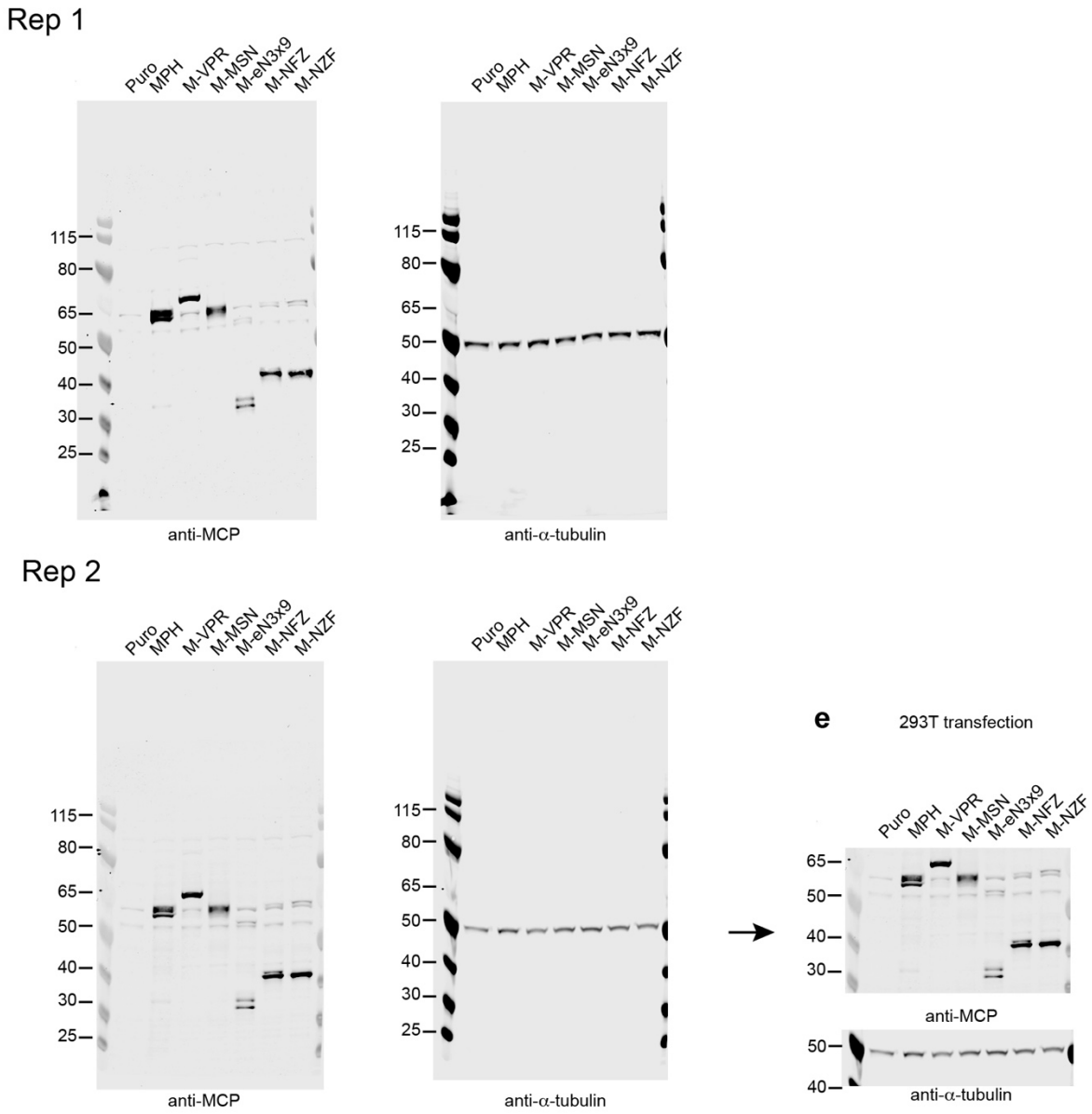


Uncropped Westerns for Supplementary Figure 2c.

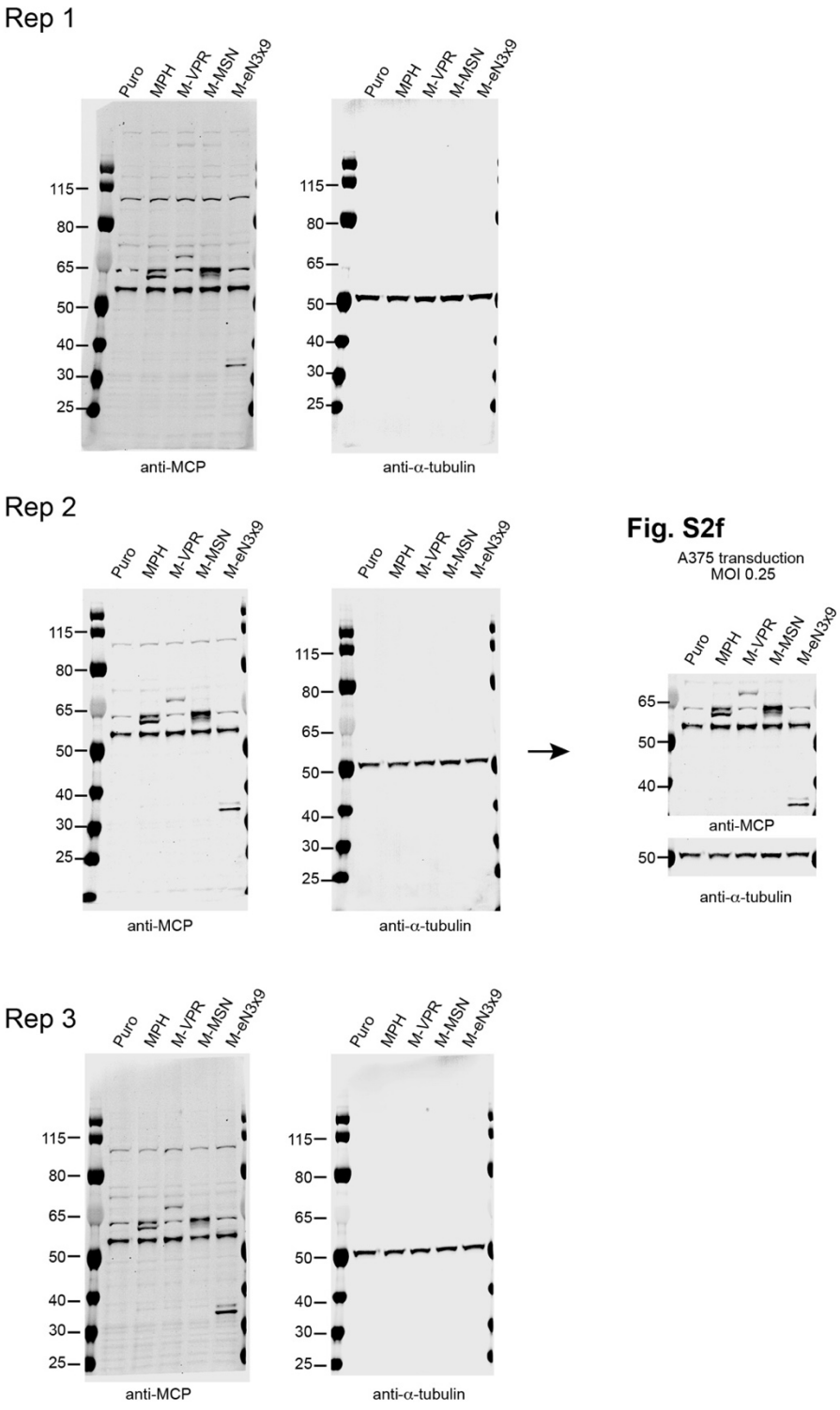
Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



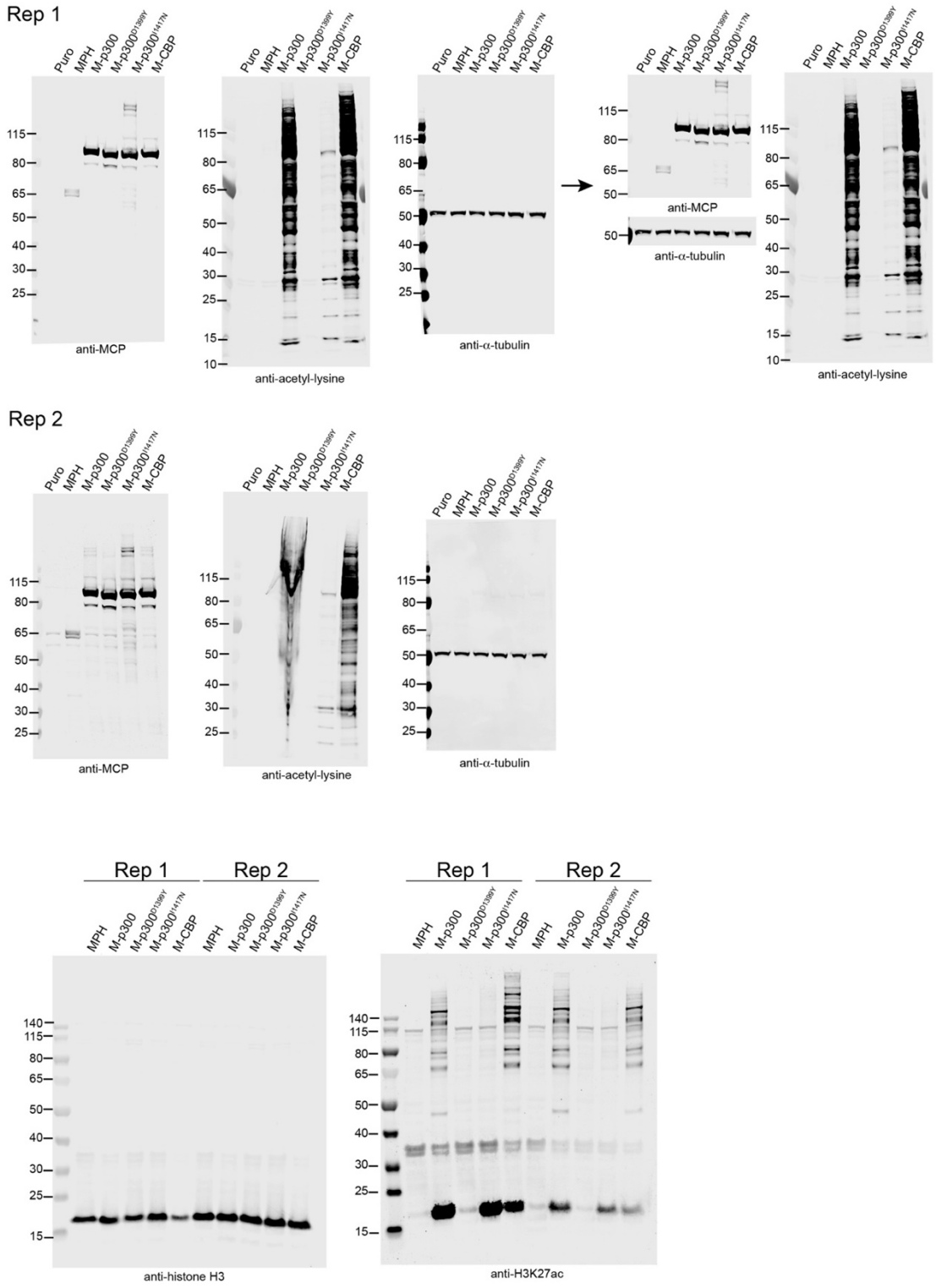
Uncropped Westerns for Supplementary Figure 2d. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



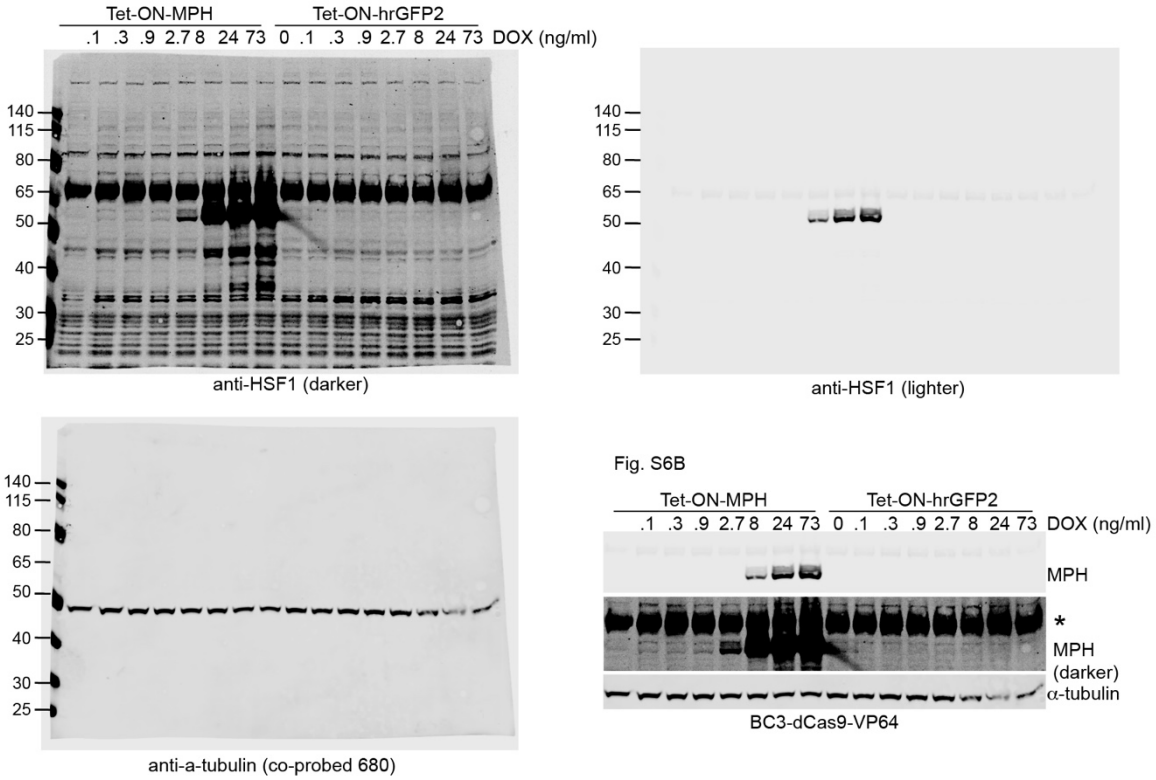
Uncropped Westerns for Supplementary Figure 2e. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



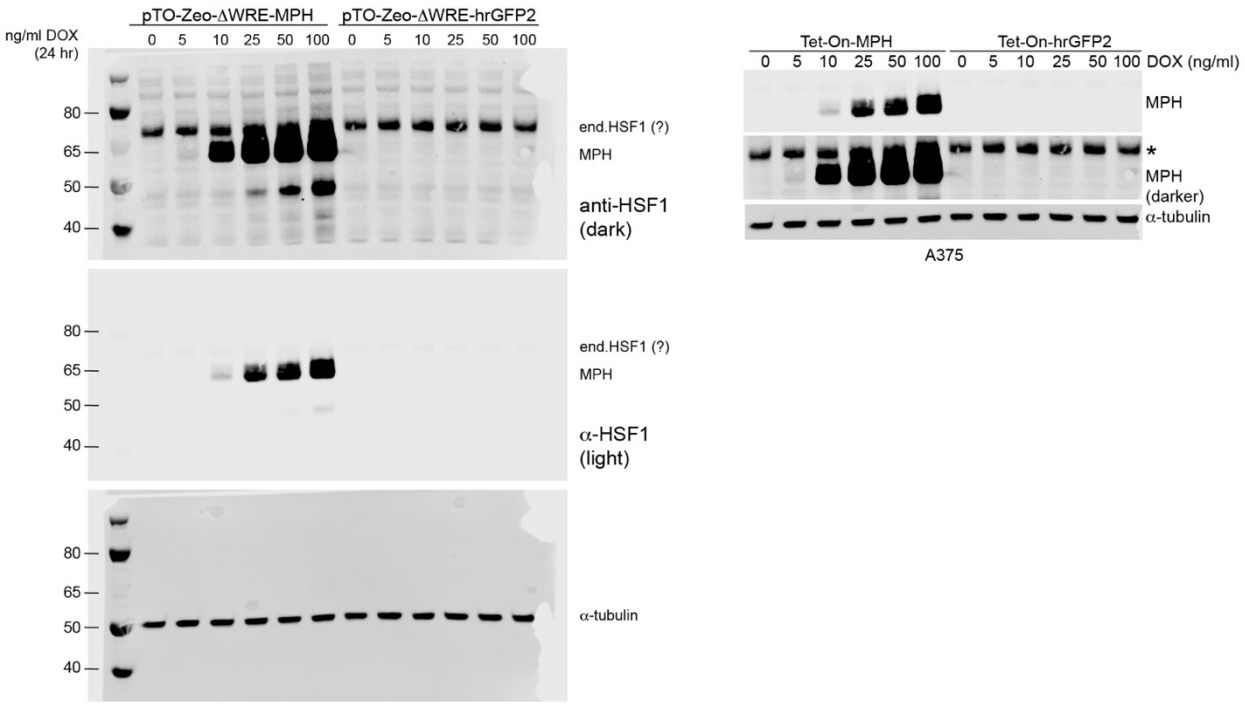
Uncropped Westerns for Supplementary Figure 2f. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



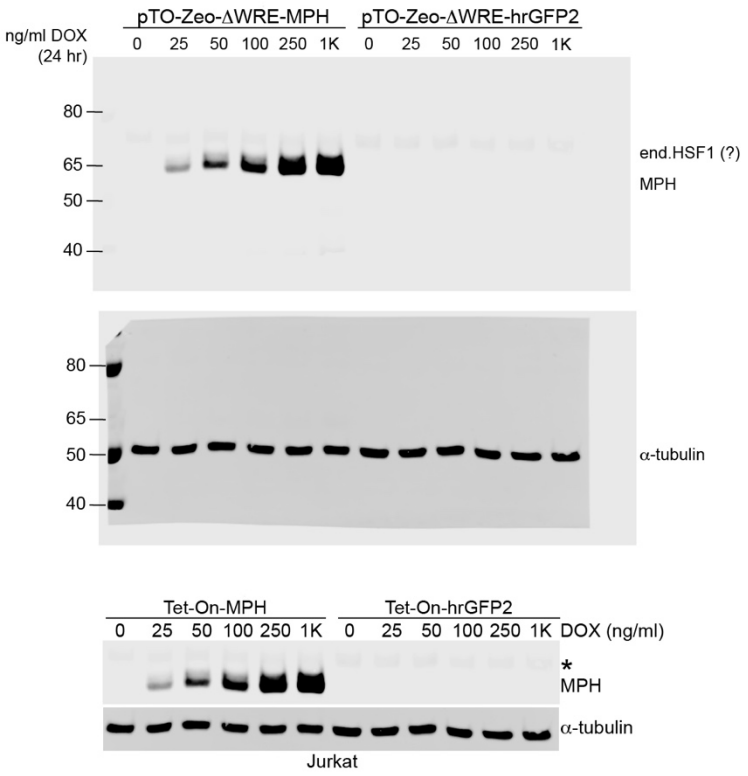
Uncropped Westerns for Supplementary Figure 5a-b. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



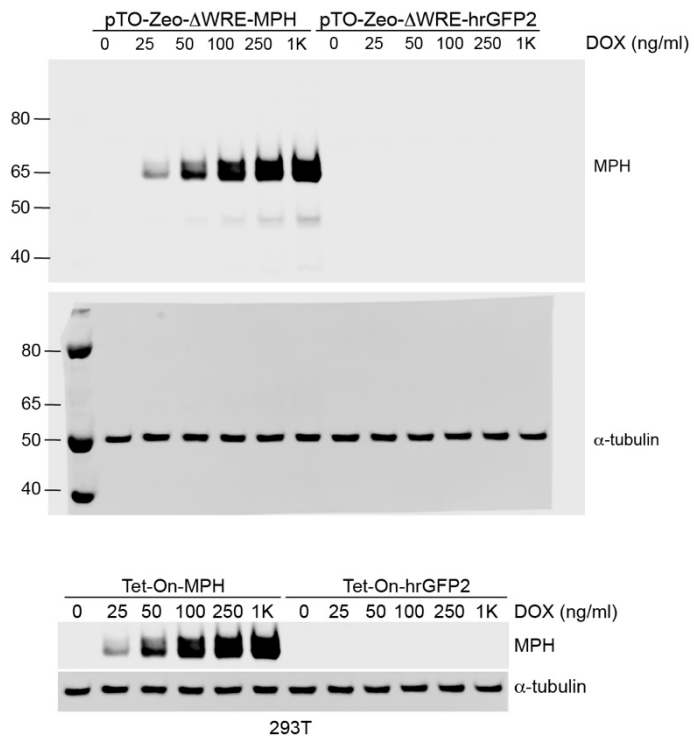
Uncropped Westerns for Supplementary Figure 6b. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



Uncropped Westerns for Supplementary Figure 6c. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



Uncropped Westerns for Supplementary Figure 6d. Pageruler Protein ladder was loaded in the left lanes and molecular weights (in kDa) are indicated at left.



Uncropped Westerns for Supplementary Figure 6e. Pageruler Protein ladder was loaded in the left lane and molecular weights (in kDa) are indicated at left.