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Supplemental information

Modulation of AAV transduction and integration targeting by topoisomerase poisons

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Figure S1

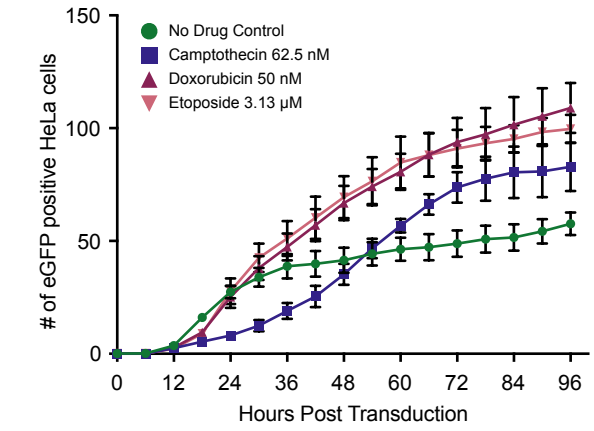


Figure S1. Camptothecin (CPT), Doxorubicin (DOX) and Etoposide (ETO) increase AAV transduction in Hela cells reproducibly. Hela cells were transduced with four biological replicates per drug conditions per experiment across two separate experiments. The second biological replicate followed similar kinetics and at 12 days post transduction onward, cells were quantified by flow cytometry, bringing the count per group to n = 8 biological replicates for Fig. 1C. Data displayed here is the mean value and standard deviation of quadruplicate biological replicates (n = 4) per group. Highlighted timepoints were analyzed using one-way ANOVA with Dunnett's multiple comparison test of each drug against the control cells. Significance is displayed as such (ns = no significance, * = p-value <0.05, ** = p-value <0.01, *** = p-value < 0.001, *** = p-value <0.0001)

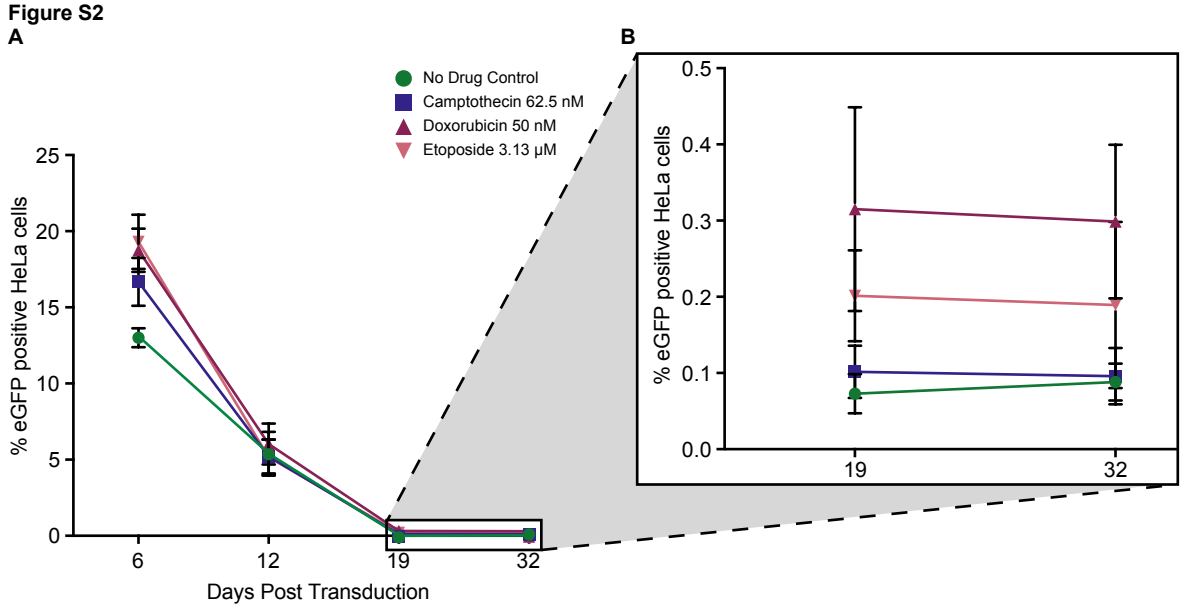


Figure S2. GFP expression from AAV in Hela cells stabilize by 19 days post transduction. Data displayed is (**A**) the mean %GFP positive Hela cells and standard deviation of quadruplicate biological replicates (n=4 for Day 6 and n = 8 for subsequent timepoints) per group from Day 6 to Day 32 post transduction. The cutaway (**B**) depicts Day 19 and Day 32 values, demonstrating relative stabilization of the GFP signal. Control cells are depicted as green circles, cells treated with 62.5 nM of CPT are depicted as blue squares, cells treated with 50nM DOX are depicted as maroon upward triangles and cells treated with 3.13 µM of ETO are depicted as pink downward triangles.

Figure S3

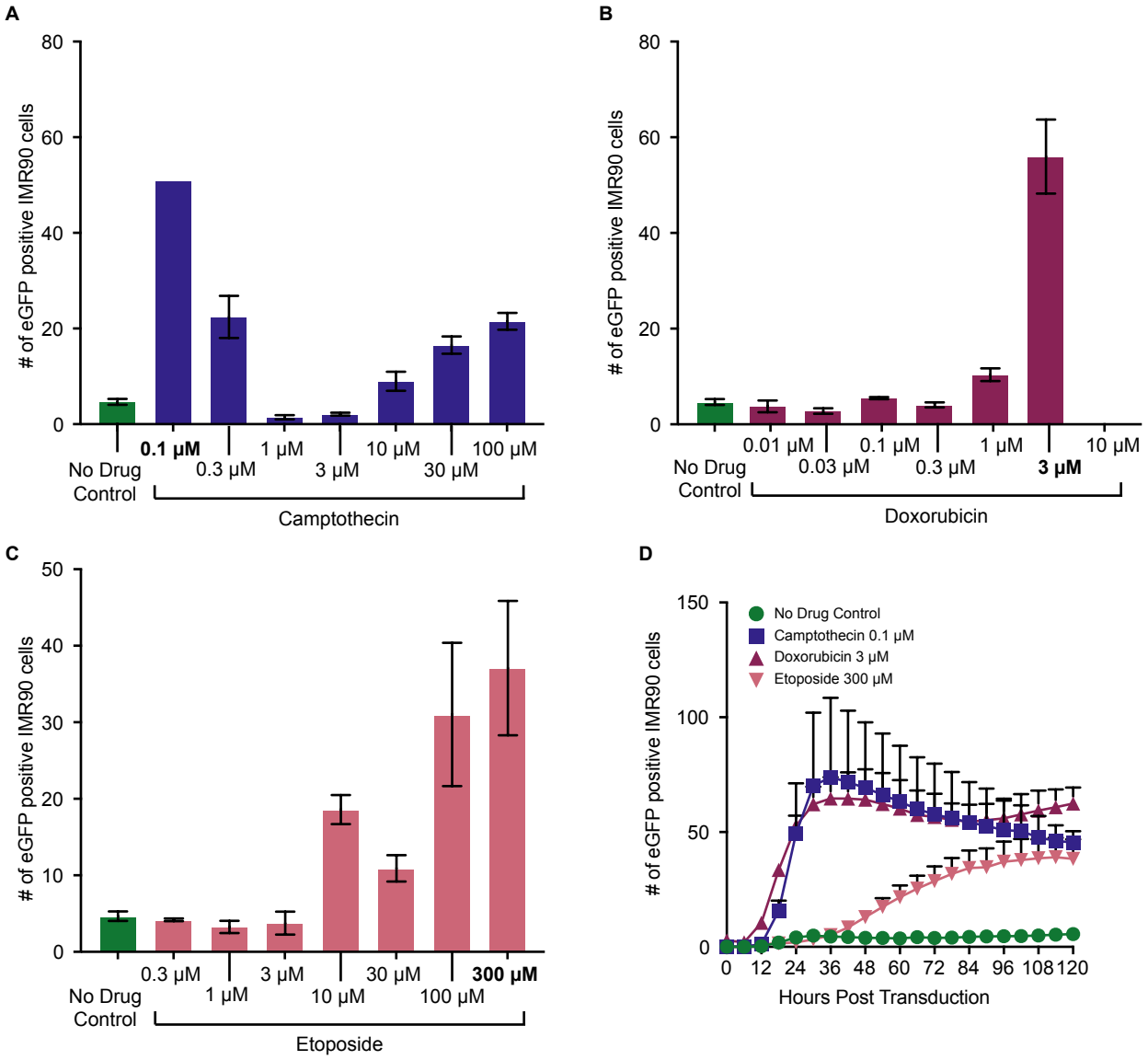
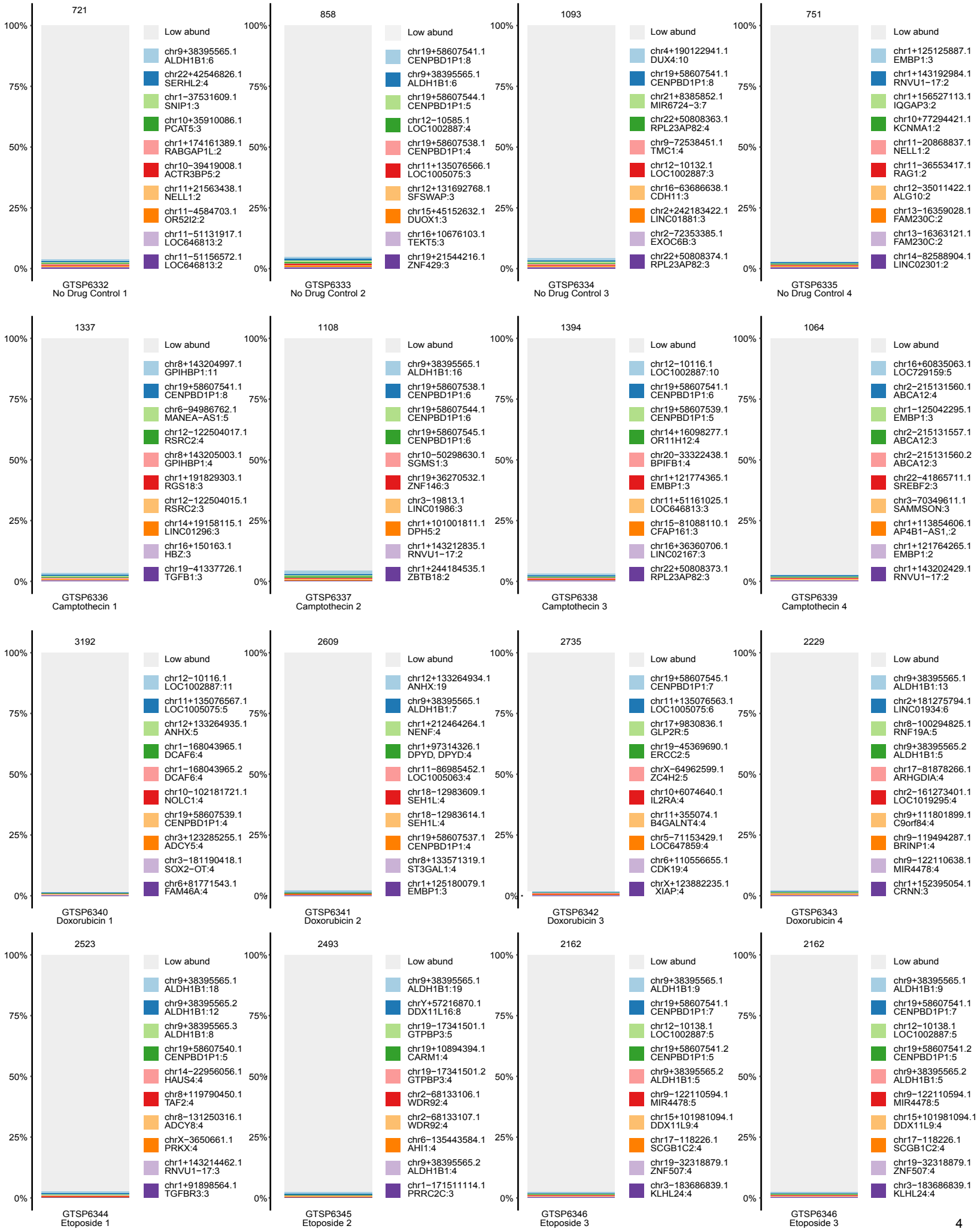


Figure S3. IMR90 cells tolerate a range of Camptothecin (CPT), Doxorubicin (DOX) and Etoposide (ETO) doses. (**A-C**) IMR90 cells were treated overnight with either media (No Drug Control) or various doses of (**A**) CPT, (**B**) DOX, or (**C**) ETO before drug washout. Treated cells were transduced with AAV-GFP and cultured in the absence of topoisomerase drugs. The Incucyte analysis software was used to quantify GFP positive foci, with the reported count taking an average across the 25 images. Data displayed is the mean number of GFP foci at 96 hours post transduction for each drug condition (n = 4) with the standard deviation. Doses that increase transduction were highlighted in (**D**). Over the first 120 hours post transduction, phase and green fluorescence live cell images were taken every 6 hours using the Incucyte S3 and 25 images were taken per well. The Incucyte analysis software was used to quantify GFP positive foci, with the reported count taking an average across the 25 images. Data displayed is the mean number of GFP foci for each drug condition (n = 4) with the standard deviation for each timepoint.

Figure S4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chr1+174161389.1 RABGAP1L:2
chr10−39419008.1 ACTR3BP5:2
chr11+21563438.1 NELL1:2
chr11−4584703.1 OR52I2:2
chr11−51131917.1 LOC646813:2
chr11−51156572.1 LOC646813:2
GTSP6332 No Drug Control 1
858
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chr12−10585.1 LOC1002887:4
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chr11−20868837.1 NELL1:2
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chr19+36270532.1 ZNF146:3
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chr1+101001811.1 DPH5:2
chr1+143212835.1 RNVU1−17:2
chr1+244184535.1 ZBTB18:2
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2609
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chr18−12983609.1 SEH1L:4
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GTSP6341 Doxorubicin 2
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chr11+135076563.1 LOC1005075:6
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chr19−45369690.1 ERCC2:5
chrX−64962599.1 ZC4H2:5
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chr11+355074.1 B4GALNT4:4
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chr6+110556655.1 CDK19:4
chrX+123882235.1 XIAP:4
GTSP6342 Doxorubicin 3
2229
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GTSP6346 Etoposide 3
2162
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chr17−118226.1 SCGB1C2:4
chr19−32318879.1 ZNF507:4
chr3−183686839.1 KLHL24:4
GTSP6346 Etoposide 3

Figure S4. IMR90 cells tolerate a range of Camptothecin (CPT), Doxorubicin (DOX) and Etoposide (ETO) doses. (**A-C**) IMR90 cells were treated overnight with either media (No Drug Control) or various doses of (**A**) CPT, (**B**) DOX, or (**C**) ETO before drug washout. Treated cells were transduced with AAV-GFP and cultured in the absence of topoisomerase drugs. The Incucyte analysis software was used to quantify GFP positive foci, with the reported count taking an average across the 25 images. Data displayed is the mean number of GFP foci at 96 hours post transduction for each drug condition (n = 4) with the standard deviation. Doses that increase transduction were highlighted in (**D**). Over the first 120 hours post transduction, phase and green fluorescence live cell images were taken every 6 hours using the Incucyte S3 and 25 images were taken per well. The Incucyte analysis software was used to quantify GFP positive foci, with the reported count taking an average across the 25 images. Data displayed is the mean number of GFP foci for each drug condition (n = 4) with the standard deviation for each timepoint.

Figure S5

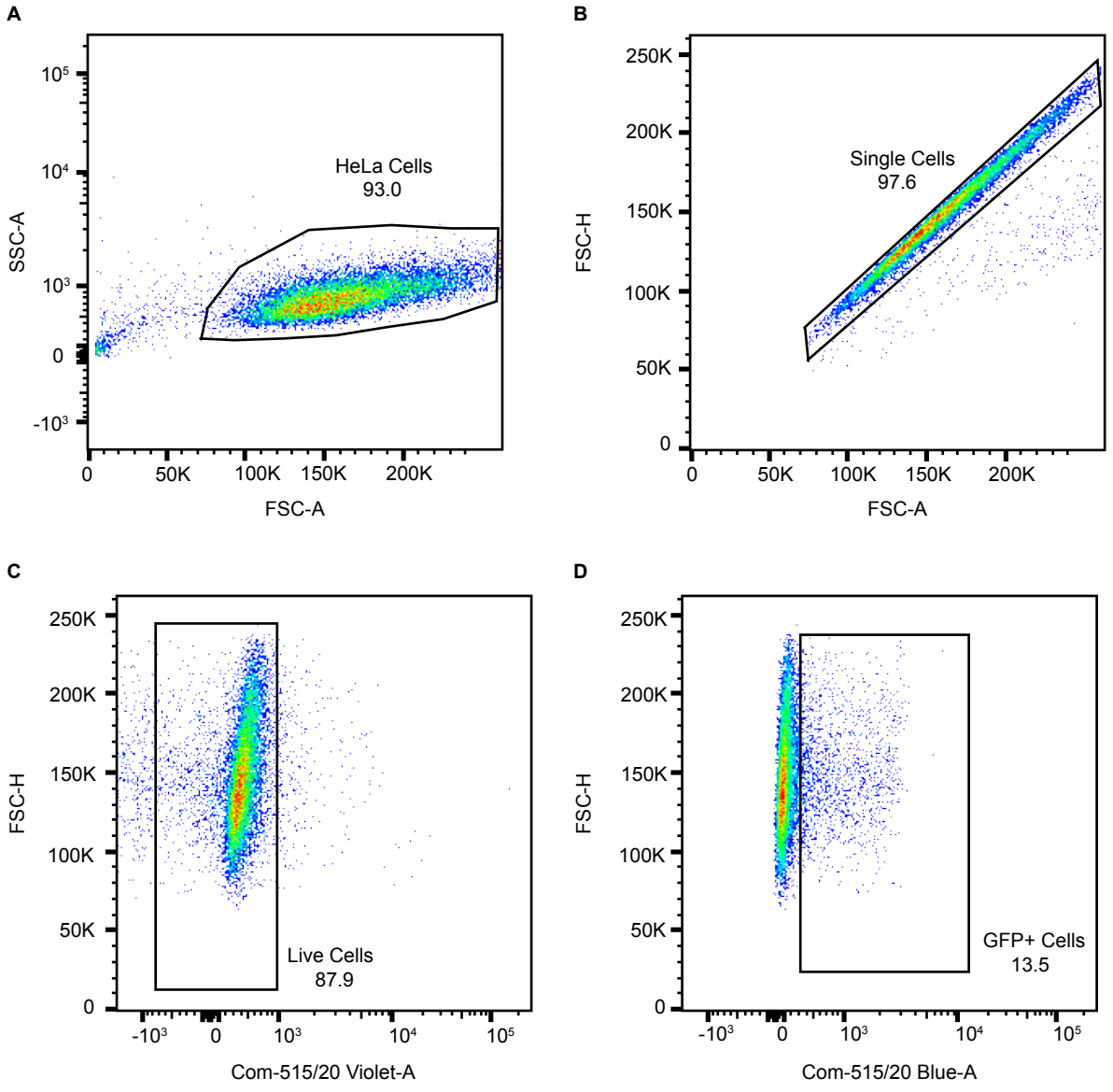

Figure S5. Flow cytometry gating strategy. Cell samples were measured on an BD LSR II (BD BioSciences) and analyzed using FlowJo v10.9.0 software. GFP BrightComp beads and ArC Amine reactive beads (Thermo Fisher) with and without Live/Dead Aqua staining were used to calculate compensation. (**A**) An FSC-A/SSC-A plot was used to gate on epithelial cells and an (**B**) FSC-A/FSC-H plot was used to gate on single cells. (**C**) The cell viability stain LIVE/DEAD Aqua was used to distinguish live vs. dead cells using a FSC-H/ Violet 515/20-A plot.(**D**) A FSC-H/Blue 515/20-A plot was used for GFP fluorescence and untransduced Hela cells were used to draw the GFP+ gate boundaries. Data displayed is from No Drug Control replicate 1, Day 6.

Table S1. Summary of Integration Site Samples

SRA ID: PRJNA1131384

GTSP	Cell Type	Timepoint	Drug	# of Reads	# of Sites	Chao1	# of Breaks	% Reads w/Breaks
6332	HeLa	Day 6	N/A	59027	303	1741.5	184	23.86
6333	HeLa	Day 6	N/A	88861	333	2757	231	28.97
6334	HeLa	Day 6	N/A	80150	428	2568.324	208	20.35
6335	HeLa	Day 6	N/A	77676	344	3305.167	109	14.05
6336	HeLa	Day 6	Camptothecin 62.5 nM	96414	576	2488.924	229	17.95
6337	HeLa	Day 6	Camptothecin 62.5 nM	80234	423	2476.333	366	34.87
6338	HeLa	Day 6	Camptothecin 62.5 nM	123775	594	2925.356	234	17.69
6339	HeLa	Day 6	Camptothecin 62.5 nM	92778	494	3445	149	14.5
6340	HeLa	Day 6	Doxorubicin 50 nM	91085	1198	5093	565	18.61
6341	HeLa	Day 6	Doxorubicin 50 nM	90619	990	5717.317	445	17.96
6342	HeLa	Day 6	Doxorubicin 50 nM	72197	1056	4805.875	512	18.56
6343	HeLa	Day 6	Doxorubicin 50 nM	79027	816	3981.366	485	23.2
6344	HeLa	Day 6	Etoposide 3.13 μM	139483	881	3793.143	808	32.88
6345	HeLa	Day 6	Etoposide 3.13 μM	84096	862	4588.013	580	24.84
6346	HeLa	Day 6	Etoposide 3.13 μM	152823	858	4005.483	575	26.48

Table S2. List of Key Reagents
Table S3. List of DNA Primers Used in This Study.