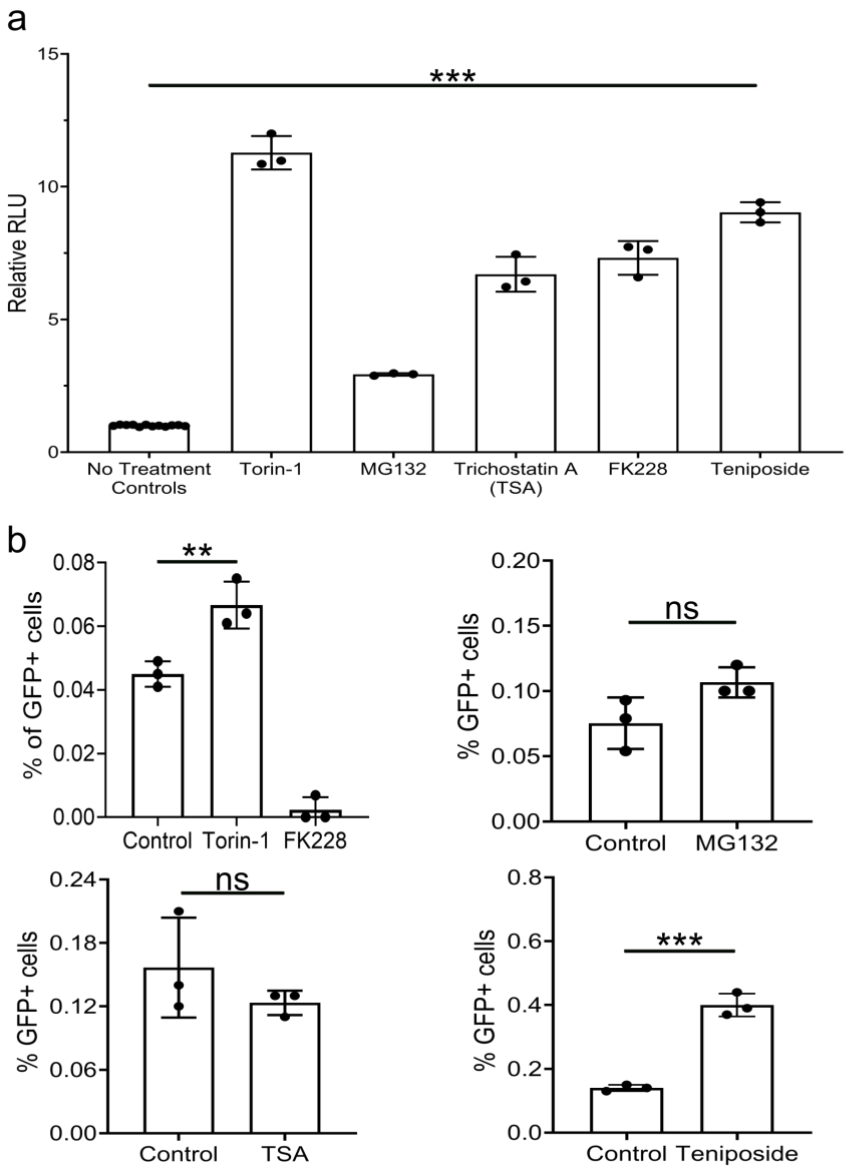
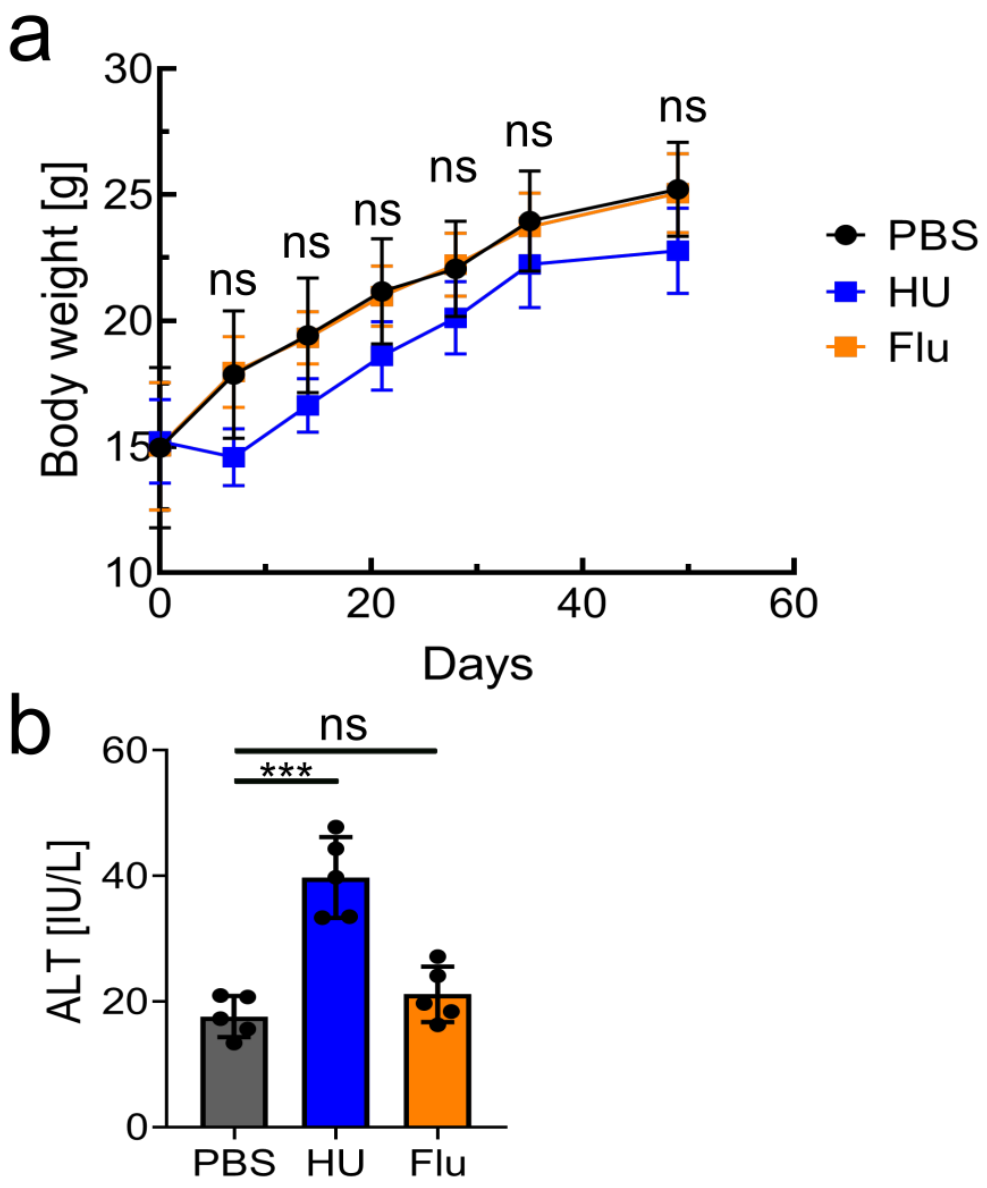


Extended Data Figure 1| Various small molecule compounds enhanced rAAV transduction, but not rAAV-mediated gene targeting efficiency *in vitro*

a, The effect of various compounds on standard rAAV transduction was tested in Huh7 cells. Cells were treated with each compound at the indicated concentration and transduced with an AAVDJ vector expressing Fluc from the CAG promoter. Data is compared to relative luciferase activity of control (DMSO-treated) cells. Error bars represent s.d.; n=3.

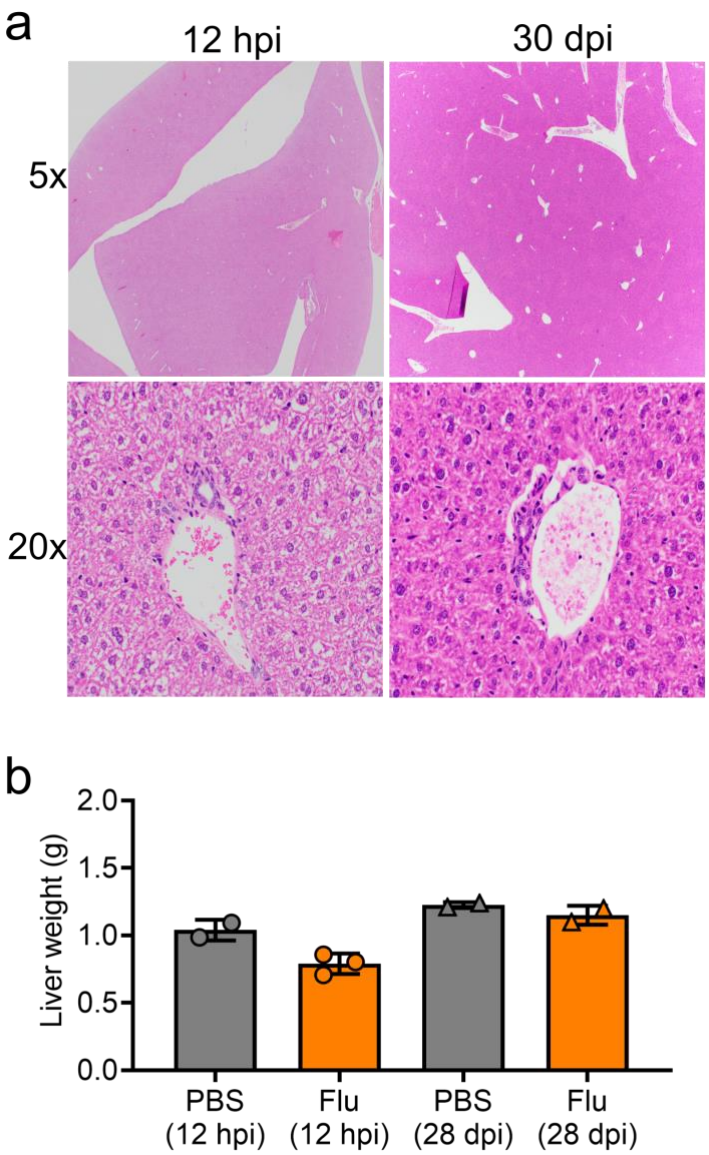
b-f, The effect of each compound on rAAV-mediated gene targeting efficiency was tested in Huh7 cells by treatment with each drug followed by transduction with an AAVDJ gene targeting vector, GAPDH-P2A-GFP. Flow cytometry analysis of GFP positive cells 2 days after treatment is shown. Error bars represent s.d.; n=3. Significance testing was performed by a one-way ANOVA with Dunnett's multiple comparison test and two-tailed t test.



Extended Data Figure 2| Assessment of toxicity from fludarabine or hydroxyurea administration in mice by body weight and transaminase measurements

a, Mice were treated with hydroxyurea (HU) (300 mg/kg/day) or fludarabine (Flu) (375 mg/kg/day) for three days. Body weight was monitored over 6 weeks after drug administration. Error bars represent s.e.; n=5. Significance testing was performed by a two-way ANOVA.

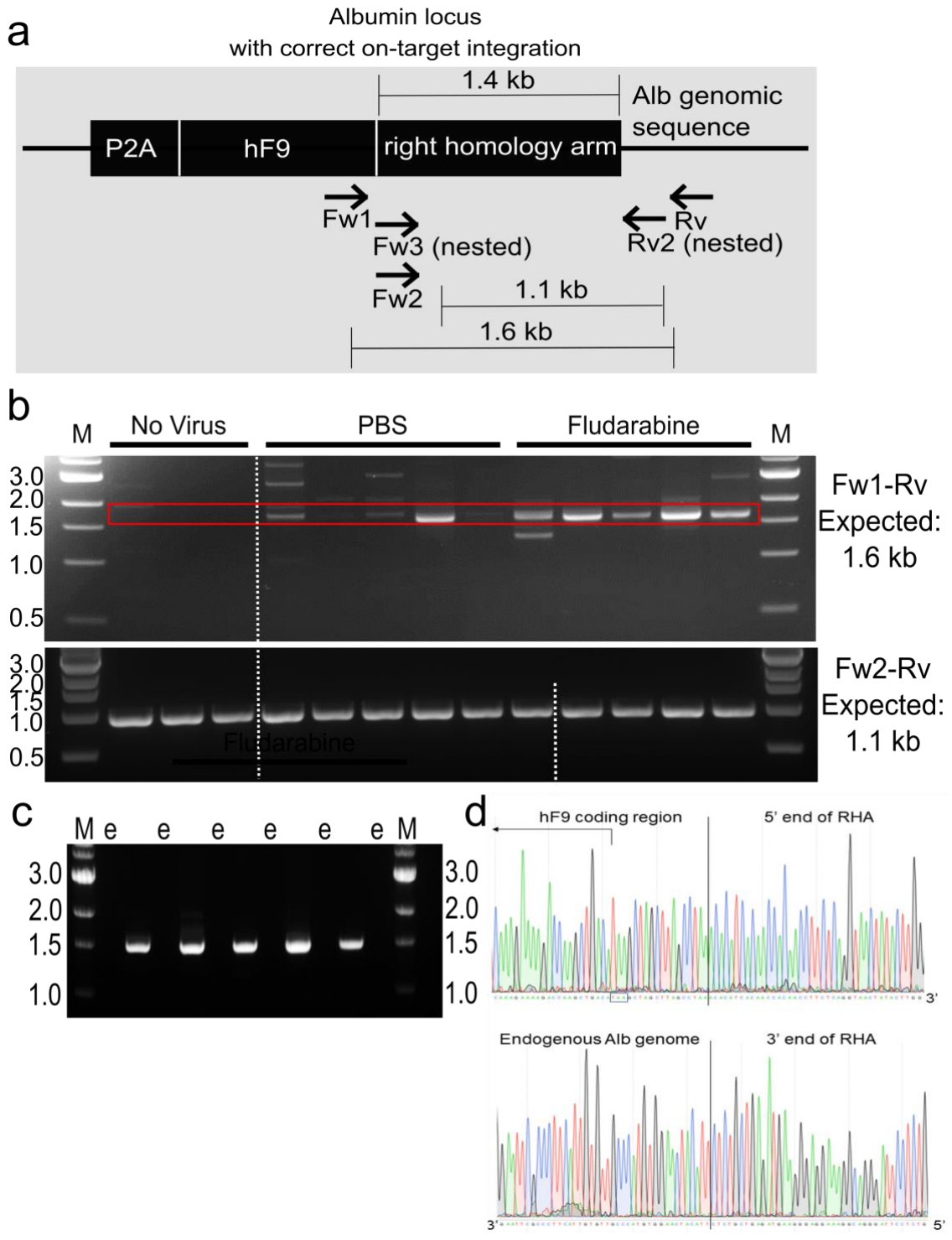
b, Serum alanine transaminase levels (ALT) were evaluated 3 days after the last drug administration. Error bars represent s.e.; n=5. Significance testing was performed by an one-way ANOVA.



Extended Data Figure 3| Assessment of liver toxicity from fludarabine treatment

a, Mice were treated with fludarabine (Flu) (375 mg/kg/day) or PBS for three days. Groups of two PBS mice or three fludarabine-treated mice were submitted 12 hours after the final drug injection (hpi). A second cohort of mice was submitted 28 days after injections (dpi). Livers were collected for H&E staining and subsequent blinded analysis by a trained veterinary pathologist. Representative images of H&E stained liver sections from fludarabine treated mice are shown, at 12 hpi (left column) and 28 dpi (right column).

b, Livers were weighed at the time of collection at either 12 hpi or 28 dpi. Hematological analysis of these mice is available in Table 2.



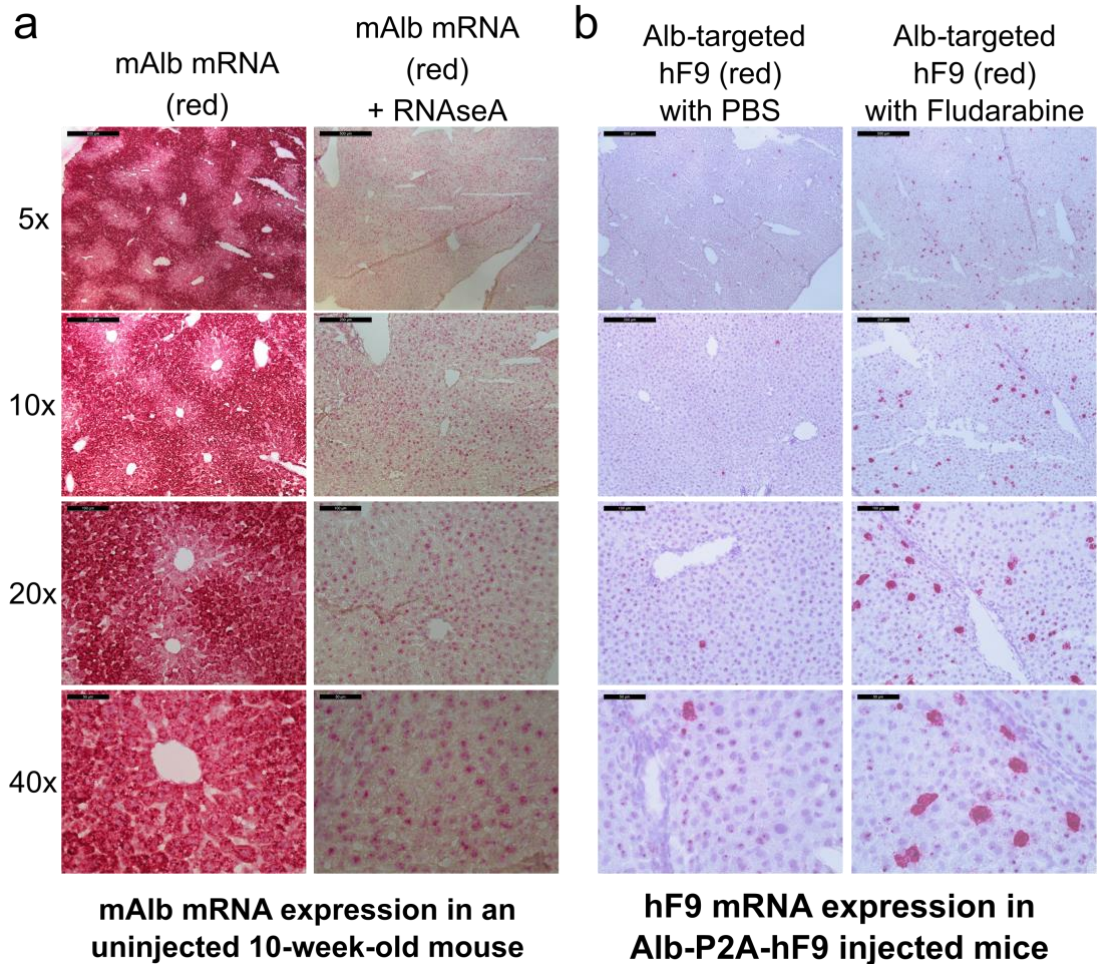
Extended Data Figure 4| Genomic DNA analysis to validate correct integration at the albumin locus in the liver of mice.

a, Schematic of the gene targeting Alb-P2A-hF9 vector integrated at the albumin genomic region. Also shown are positions of semi-quantitative PCR primers (black arrows) used for

analysis on on-target genomic integration. Primers Fw1 (binding in the hF9 transgene) and Rv (binding in the *Alb* genomic locus) are used for initial amplification of integration junctions. **b,** Junction capture PCR samples were run on agarose gels to detect genomic integration in the right homology arm region (above). PCR amplification of endogenous albumin locus was used as a control (below) (primers Fw2 and Rv).

c, Agarose gel image of nested PCR reactions from each fludarabine-treated mouse is shown. Each well represents amplification from an individual mouse, while 'e' represents an empty well.

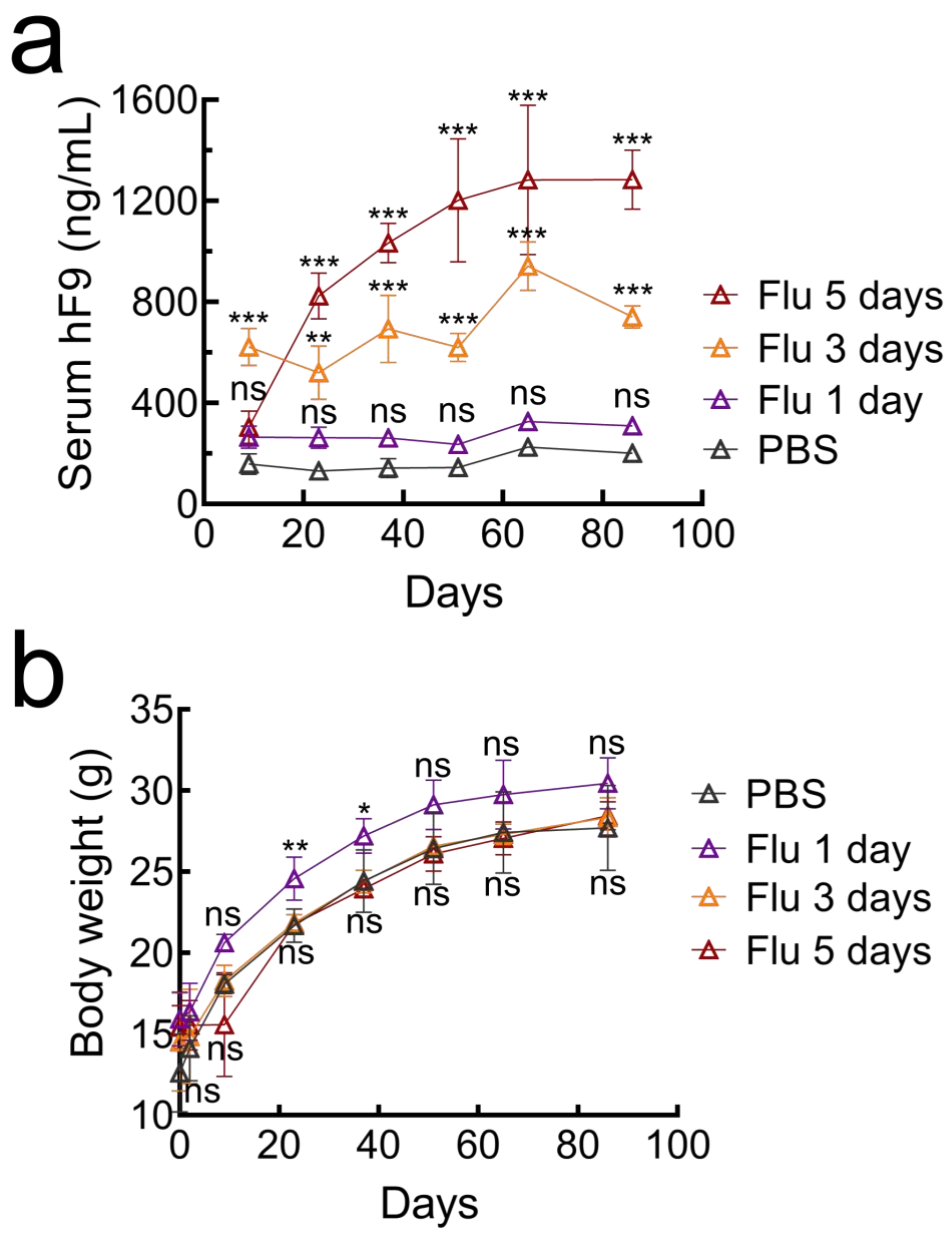
d, Nested PCR amplicons, using primers Fw3 and Rv2 (expected product size of 1.5kb), from fludarabine-treated mice samples were Sanger sequenced. DNA was extracted from the gel and cloned into a plasmid for sequencing analysis using TOPO cloning. Sanger sequencing confirmed on-target genomic integration of the P2A-hF9 cassette without indels or mutations.



Extended Data Figure 5| RNAScope *in situ* hybridization of mAlb and hF9 in mouse liver

a, Representative images of RNAScope *in situ* hybridization are provided. In the first two columns, *mAlb* mRNA was stained in a non-injected normal mouse to determine the *albumin* locus expression characteristics. Specificity for RNA was confirmed by digestion of tissue with RNAseA.

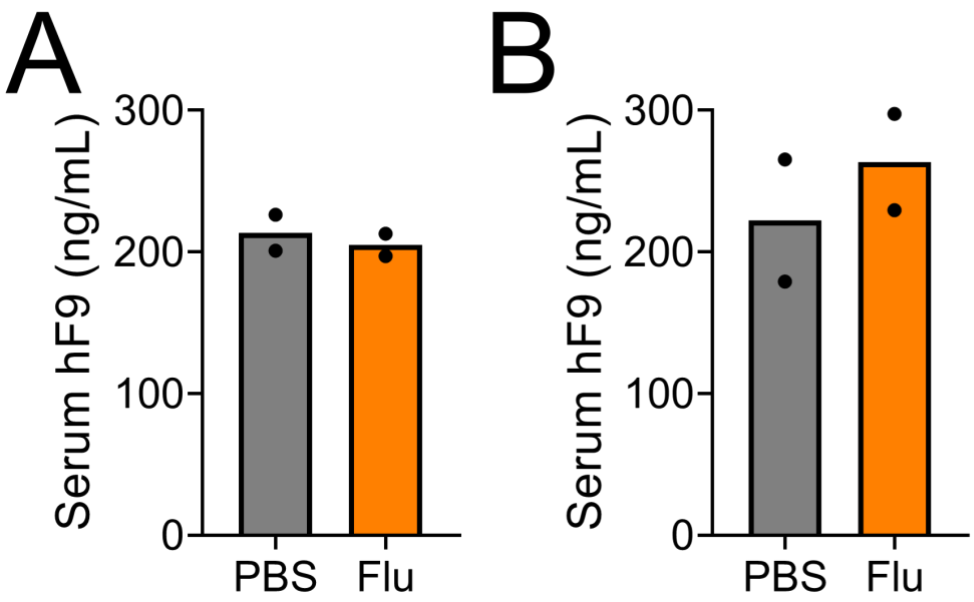
b, Representative images of *hF9* mRNA staining in mice injected with Alb-P2A-hF9 vector, with or without fludarabine treatment.



Extended Data Figure 6| The effect of different Flu dosing regimens on gene targeting efficiency

a, Mice were treated with various Flu doses, differing in the length of administration, as described in Fig.2i, as well as injected i.v. with the gene targeting Alb-P2A-hF9 vector on Day 1 of Flu treatment. Plasma hF9 protein levels from the same mice in Fig.2i were determined via ELISA for a 90-day time course. Error bars represent s.e.; n=4. Significance testing was performed by a two-way ANOVA with Dunnett's multiple comparison test.

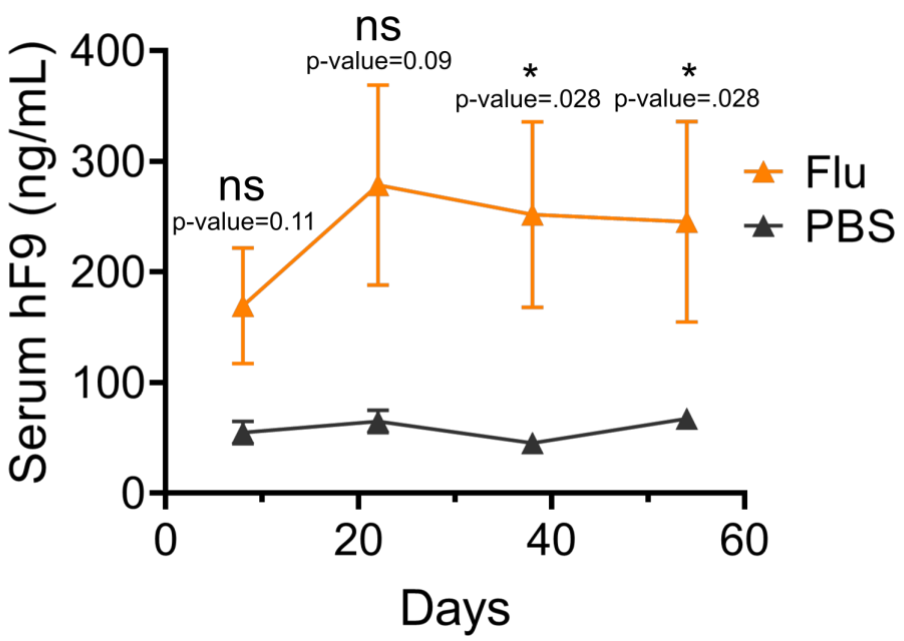
b, Body weight of mice in each treated group were monitored throughout the time course. Error bars represent s.e.; n=4. Statistics were performed by two-way ANOVA.



Extended Data Figure 7| Delayed dosing of fludarabine failed to increase the efficiency of gene targeting *in vivo*

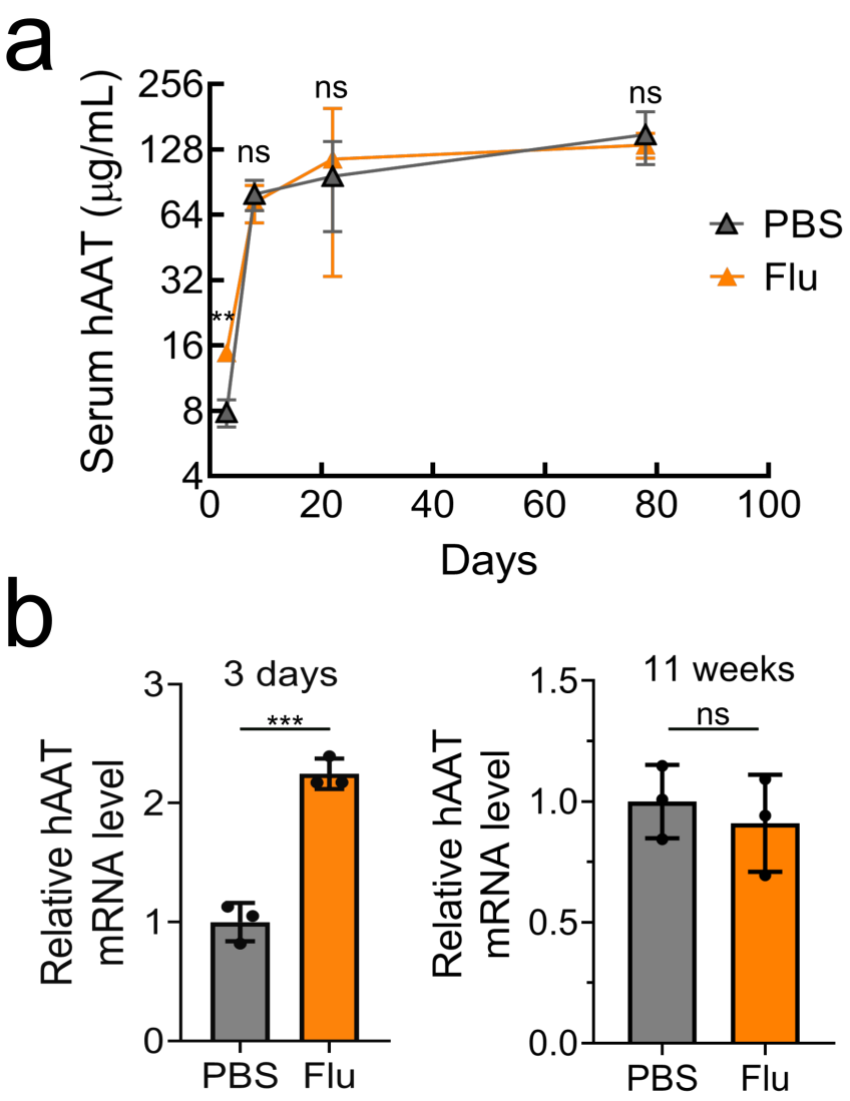
a, Mice were i.v. injected with rAAV8 Alb-P2A-hF9 targeting vector (1.0E11 vg/mouse). Plasma samples were collected at Day 22 and analyzed for hF9 levels via ELISA. Animals were then grouped for equal expression between a control and treatment group. n=2 per group.

b, Next, PBS (control) or fludarabine (125 mg/kg) was administered i.p. three times per day for three days (days 28-30 post vector-injection). hF9 protein levels were then determined 24 days later



Extended Data Figure 9| Fludarabine administration increased the efficiency of gene targeting at the *ApoE* locus *in vivo*

Mice were treated with Flu or PBS, as described before, and injected with 1.0E11 vg of a gene targeting vector, rAAV8-ApoE-P2A-hF9, targeting the murine ApoE locus. Serum was collected at various times across a nearly 60-day time course and hF9 protein levels were determined. Error bars represent s.e.; n=4. Significance was determined using a two-tailed t test or Mann-Whitney U test at each individual time point.



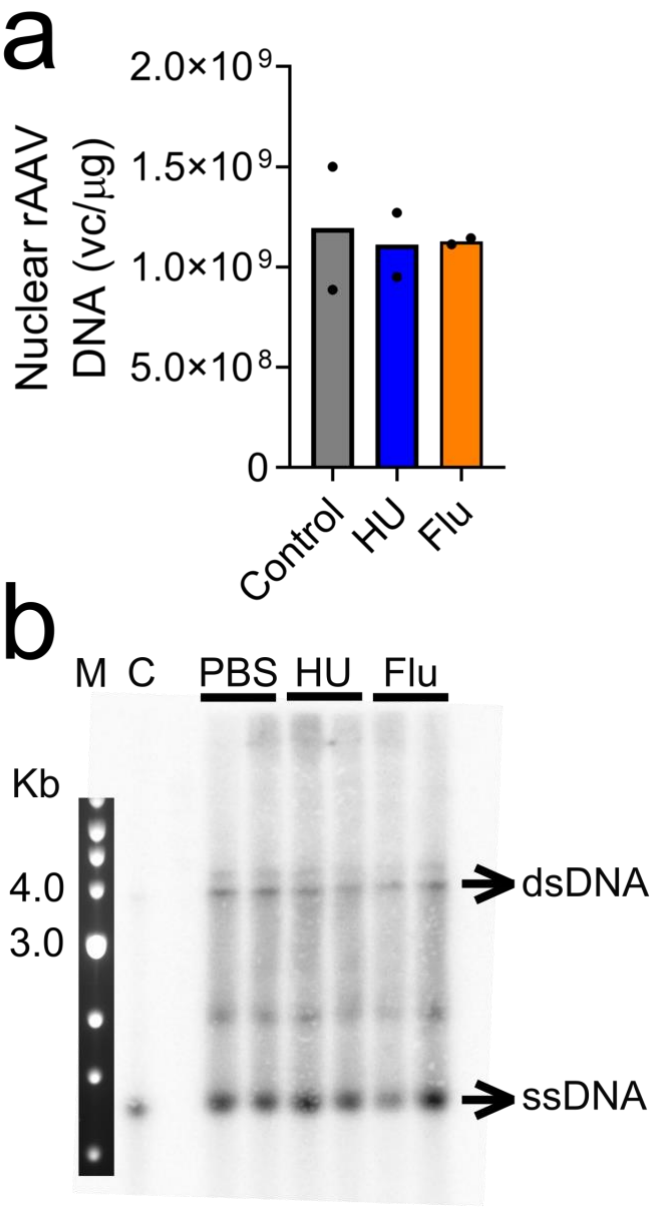
Extended Data Figure 10| The effect of fludarabine administration on promoter-driven rAAV transduction

Flu (125 mg/kg) was administered i.p three times per day for three days. Mice were also injected i.v. with a rAAV8 vector expressing hAAT from the HLP promoter (3.0E10 vg/mouse) on the first day of drug treatment. Blood was collected at indicated time points and liver tissues were extracted at the end of the time course.

a, Serum hAAT protein levels were determined via ELISA for an 80-day time course. Error bars represent s.e.; n=3. Significance was determined using one-way ANOVA with Sidak's multiple comparisons test.

b, Total RNA was extracted from liver tissues at three days and 11 weeks, then qPCR was performed to quantify hAAT mRNA expression. Actb mRNA was used for normalization and

data shown as relative expression to the PBS-treated group. Error bars represent s.e.; n=3. Significance was determined using an unpaired two-tailed t test.



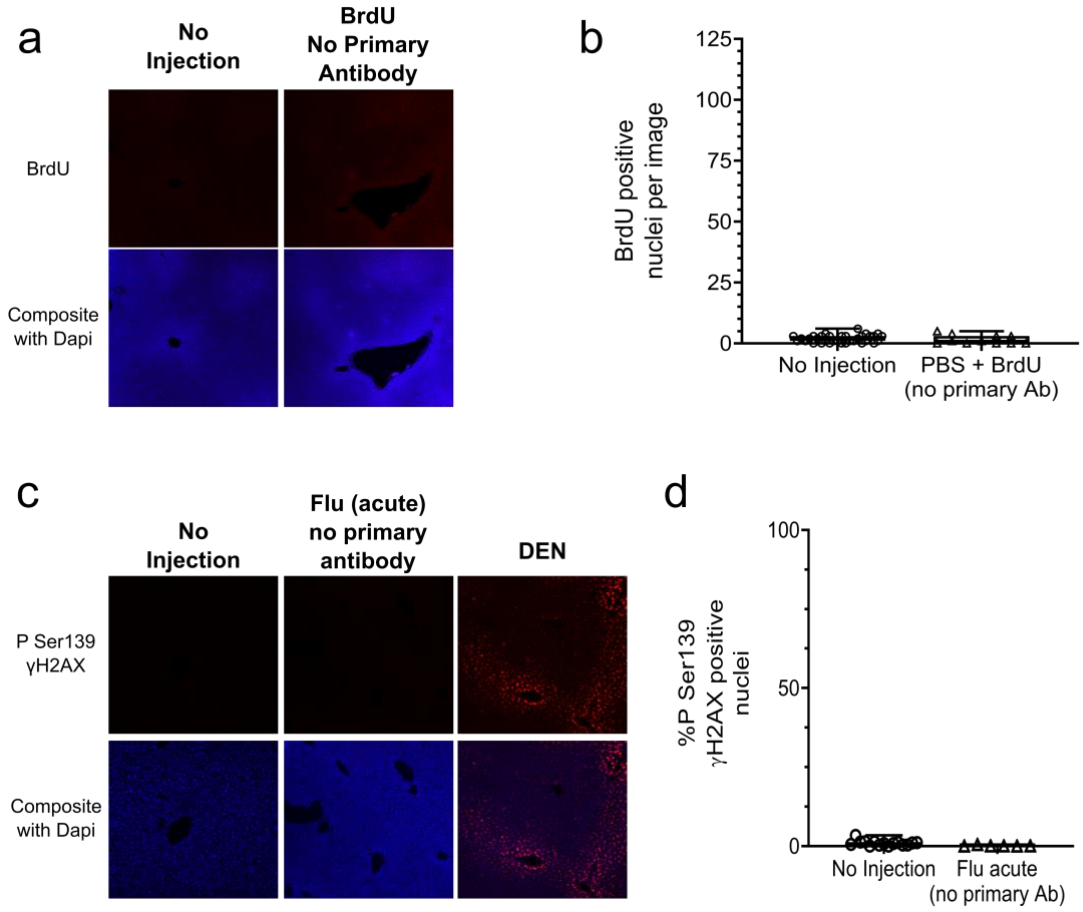
Extended Data Figure 11| Treatment with RNR inhibitors did not alter the processing of rAAV genomes from ssDNA to dsDNA in cell culture

Huh7 cells were treated with control DMSO or RNR inhibitors, hydroxyurea (HU) or fludarabine (Flu), for 16 hours and transduced with an AAVDJ CAG-promoter expressing Fluc vector after a media change to washout drugs. 48 hours after transduction, nuclear fractions were isolated and genomic DNA was purified

a, The copy number of rAAV genomes in the nuclear fraction was measured by qPCR. Data shown is with 2 individual replicates along with the average.

b, Southern blot analysis using luciferase probes was performed using the same amount of

genomic DNA to distinguish the double- or single-stranded forms of rAAV genomes. Non-denaturing agarose gel (1%) electrophoresis was used to separate single- or double-stranded form of rAAV genomes. DNA ladder (dsDNA) and purified single-stranded AAV genomes from rAAV preps were used as size controls.



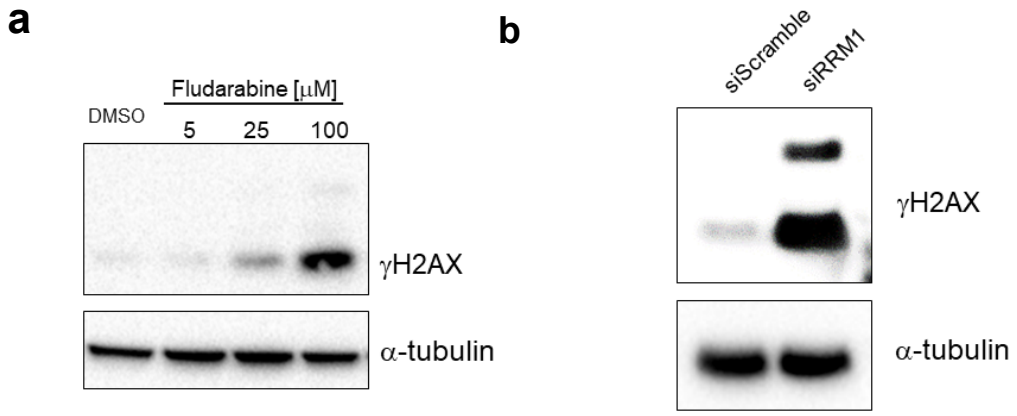
Extended Data Figure 12|- Positive or negative control staining of BrdU or γH2AX in mouse liver (in Figures 3 and 4)

a, Representative 20x confocal images from IHC staining of BrdU in control no injection mouse liver is provided. Also shown is staining of mouse liver from a BrdU and Flu treated animal, stained without the primary anti-BrdU antibody.

b, Images from control stained livers, taken at identical settings to Figure 3b images, were quantified. Each dot represents the data from a single image. n=1 animal for no injection and n=2 animals for no primary antibody control stains.

c, Representative 20x confocal images from IHC staining of γH2AX in control no injection or DEN-injected mouse livers are provided. Also shown is staining of mouse liver from a Flu treated acute-phase animal, stained without the primary anti-γH2AX antibody.

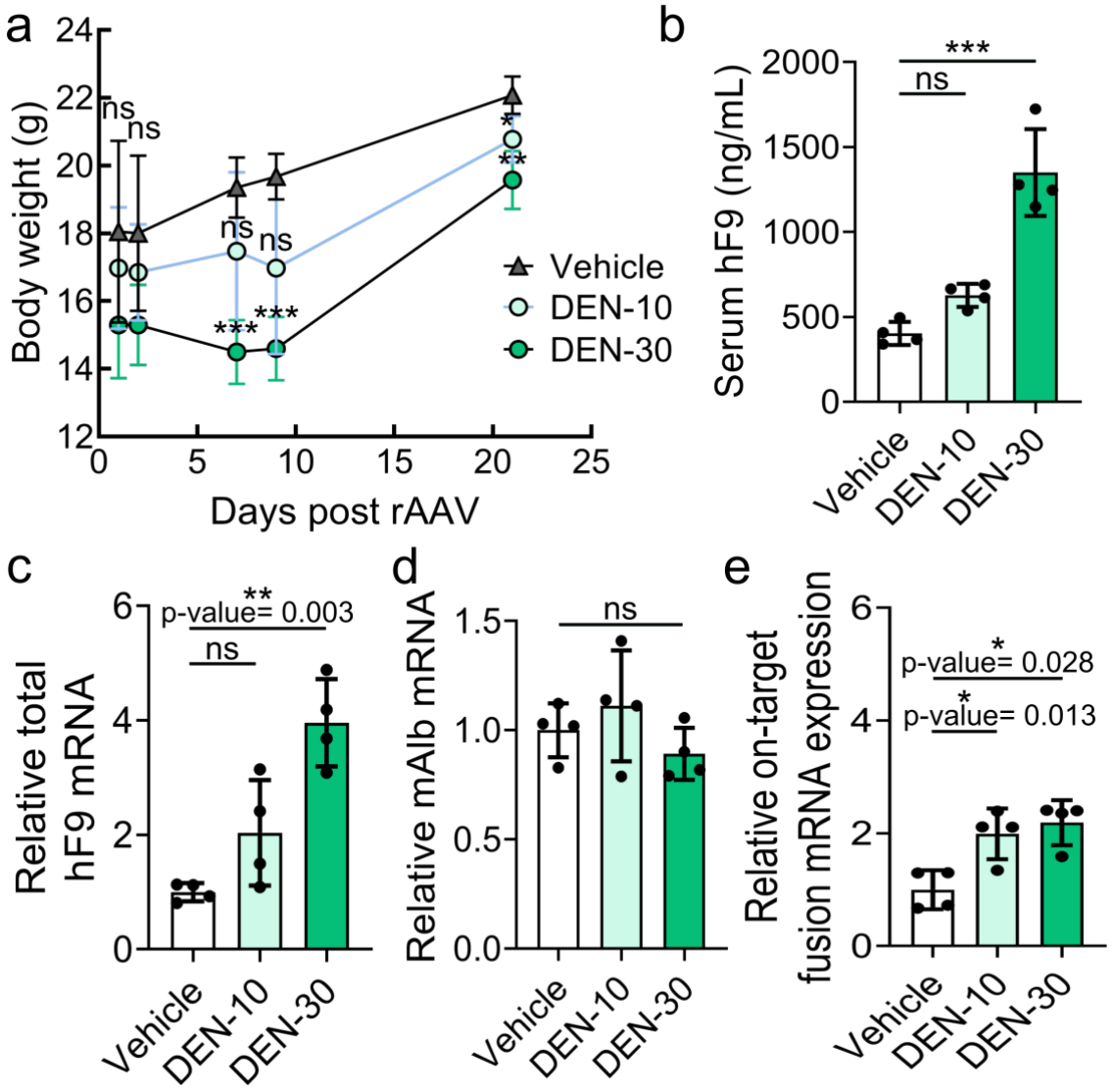
d, Images from control stained livers, taken at identical settings to Figure 4a images, were quantified. Each dot represents the data from a single image. n=1 animal for each control.



Extended Data Figure 13| Inhibition of RNR induced Ser139 phosphorylation of H2AX (γH2AX) *in vitro*

a, Huh7 cells were treated with fludarabine for 16 hours at the indicated concentrations and then total cell lysates were prepared, followed by western blotting analysis of γH2AX. α-tubulin was used as a loading control.

b, Huh7 cells were transfected with RRM1 siRNA and total cell lysates were prepared, followed by western blotting for γH2AX and α-tubulin 2 days after transfection.

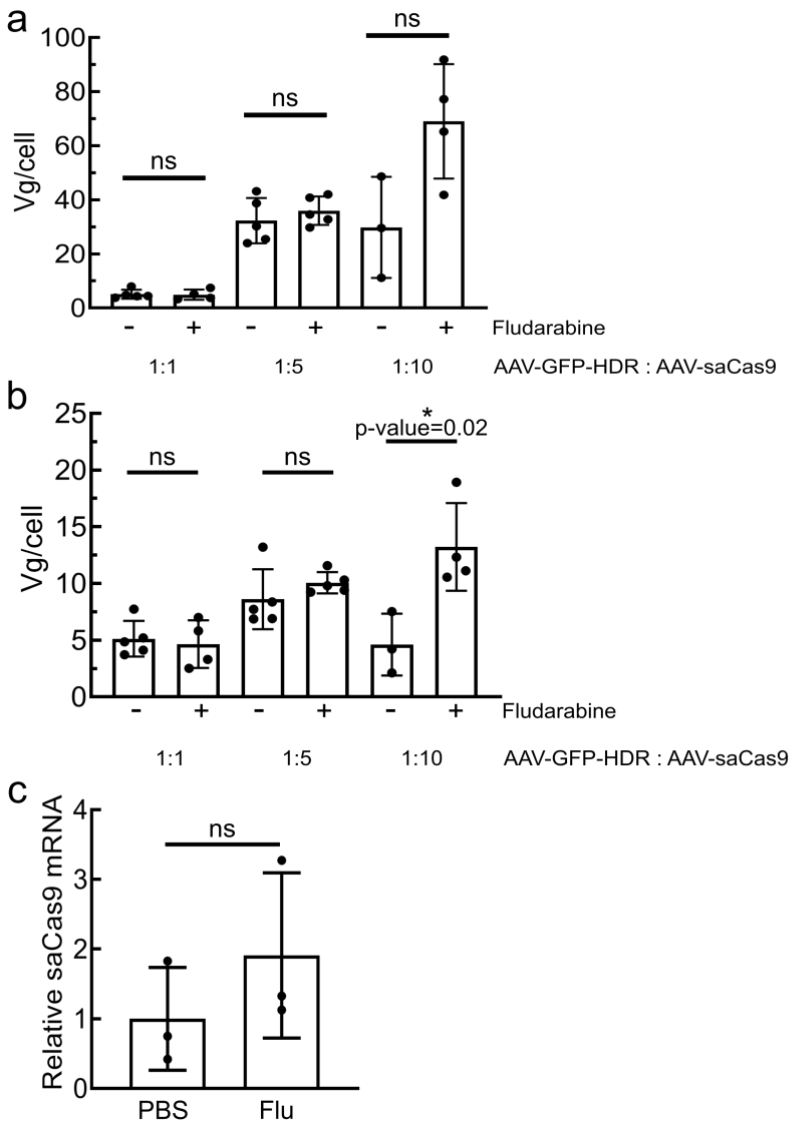


Extended Data Figure 14| The effect of DEN administration on the efficiency of gene targeting in mice liver

a, DEN (10 or 30 mg/kg) was administered through a single i.p. injection per day for three days. Mice were also injected i.v. with rAAV8 packaged Alb-P2A-hF9 gene targeting vector (1.0E11 vg/mouse) on Day 1. Body weight was measured at the indicated time points. Error bars represent s.e.; n=4. Significance testing was performed by two-way ANOVA of multiple comparisons with Dunnett's multiple comparison test.

b, Plasma hF9 protein levels in each treatment group was determined by ELISA. Error bars represent s.e.; n=4. Significance testing was performed by one-way ANOVA with Dunnett's multiple comparison test.

c-e, Total RNA was extracted from liver tissues and qPCR was performed to quantify the expression levels of (c) total hF9 mRNA, (d) endogenous albumin mRNA and (e) on-target integration derived Alb-P2A-hF9 fusion mRNA . Actb mRNA was used for normalization and each data is shown as relative expression to the vehicle (saline)-injected control group. Error bars represent s.e.; n=4. Significance was determined using two-tailed unpaired t tests for normally distributed data with equal variance or Welch's correction for data with significantly different variance. Testing of non-normally distributed data was performed with non-parametric Mann-Whitney testing.



Extended Data Figure 15| Fludarabine does not increase transduction of CRISPR/Cas9 encoded rAAV vectors or saCas9 mRNA.

a-b, At the conclusion of the two-week experiment in Fig.5, DNA was extracted from livers of rAAV-injected animals. Viral genomes were quantified by qPCR and normalized to cellular genomic DNA. The donor rAAV repair template AAV-GFP-HDR is presented on the left (a), while AAV-Cas9 is displayed in the middle panel (b). n=3 to 5 animals per group. Significance testing was performed using two-tailed t tests or Mann-Whitney U test if data was non-normally distributed.

c, Mice were injected with SaCas9 (6.0E12 vg/kg) following fludarabine or PBS treatment as in Fig.5f-g. Two weeks later, total RNA was extracted and saCas9 mRNA levels quantified by

qPCR. n=3 per group. Significance was determined using two-tailed t test.