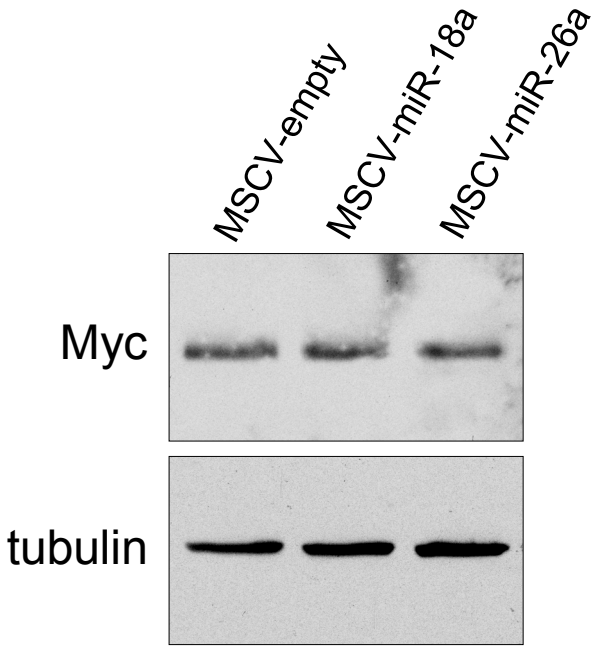

Figure S1. miR-26a does not target Myc
Western blot showing Myc abundance in retrovirally-infected HepG2 cells.

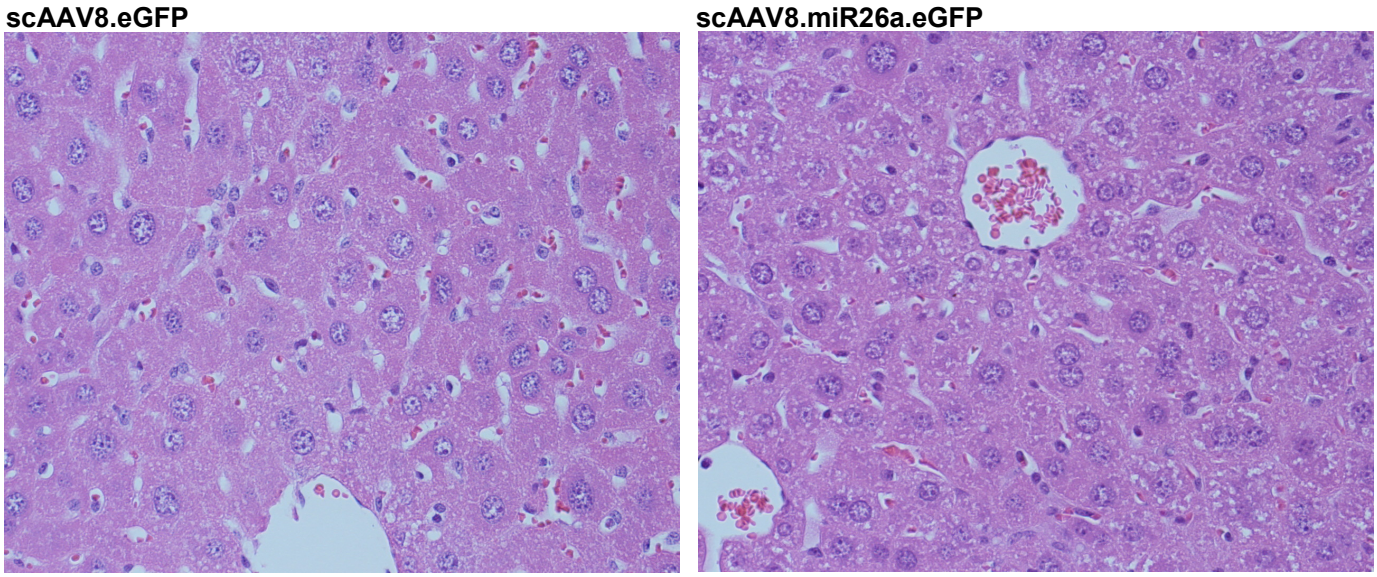

Figure S2. Infection with scAAV8 vectors does not produce overt liver toxicity
Representative H&E-stained sections of liver 21 days following administration of the indicated AAV vectors.

[G] Supplemental Figure 3

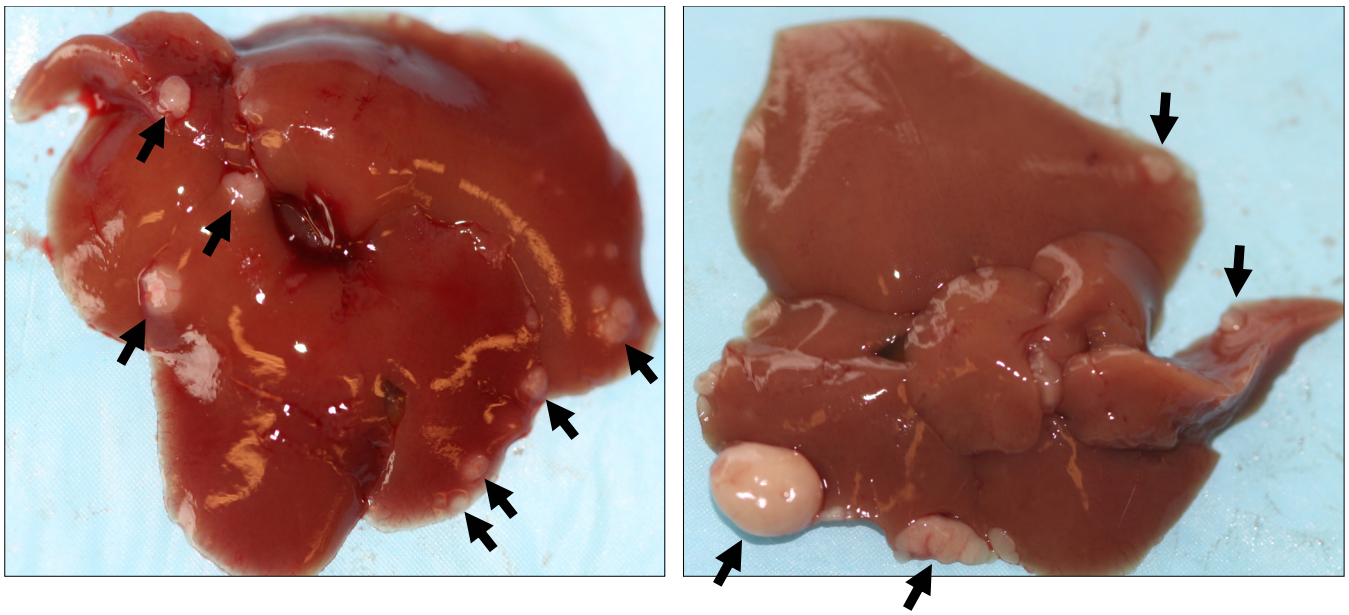

Figure S3. Baseline tumor burden in tet-o-*MYC*; LAP-tTA mice at time of AAV administration

Representative livers 7 weeks following dox removal (dox off at 4 weeks of age, livers harvested at 11 weeks of age).

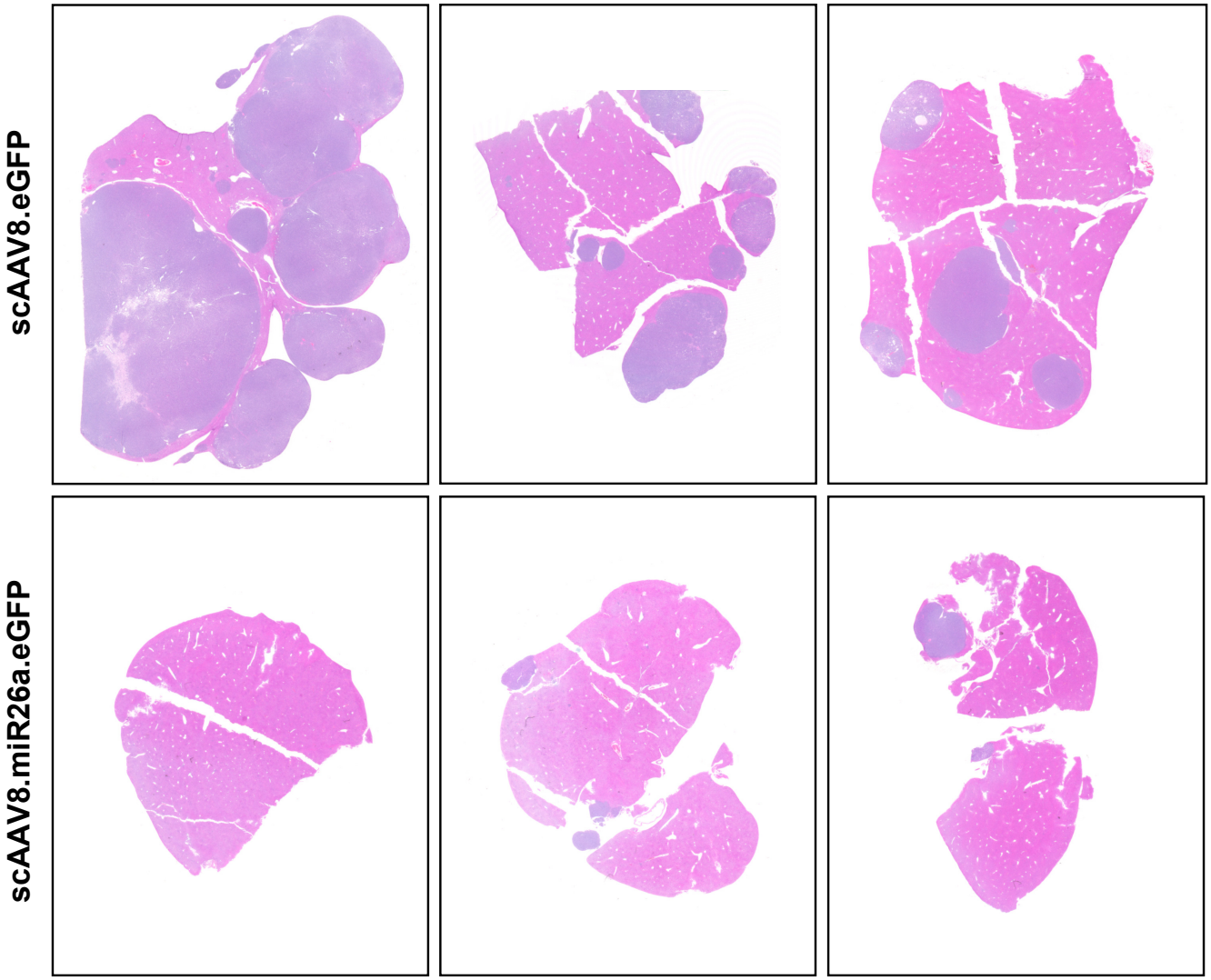

Figure S4. Lobular distribution of tumors in miR-26a-treated and control animals 21 days after AAV administration

Low-power H&E-stained sections of liver lobes from treated and control animals

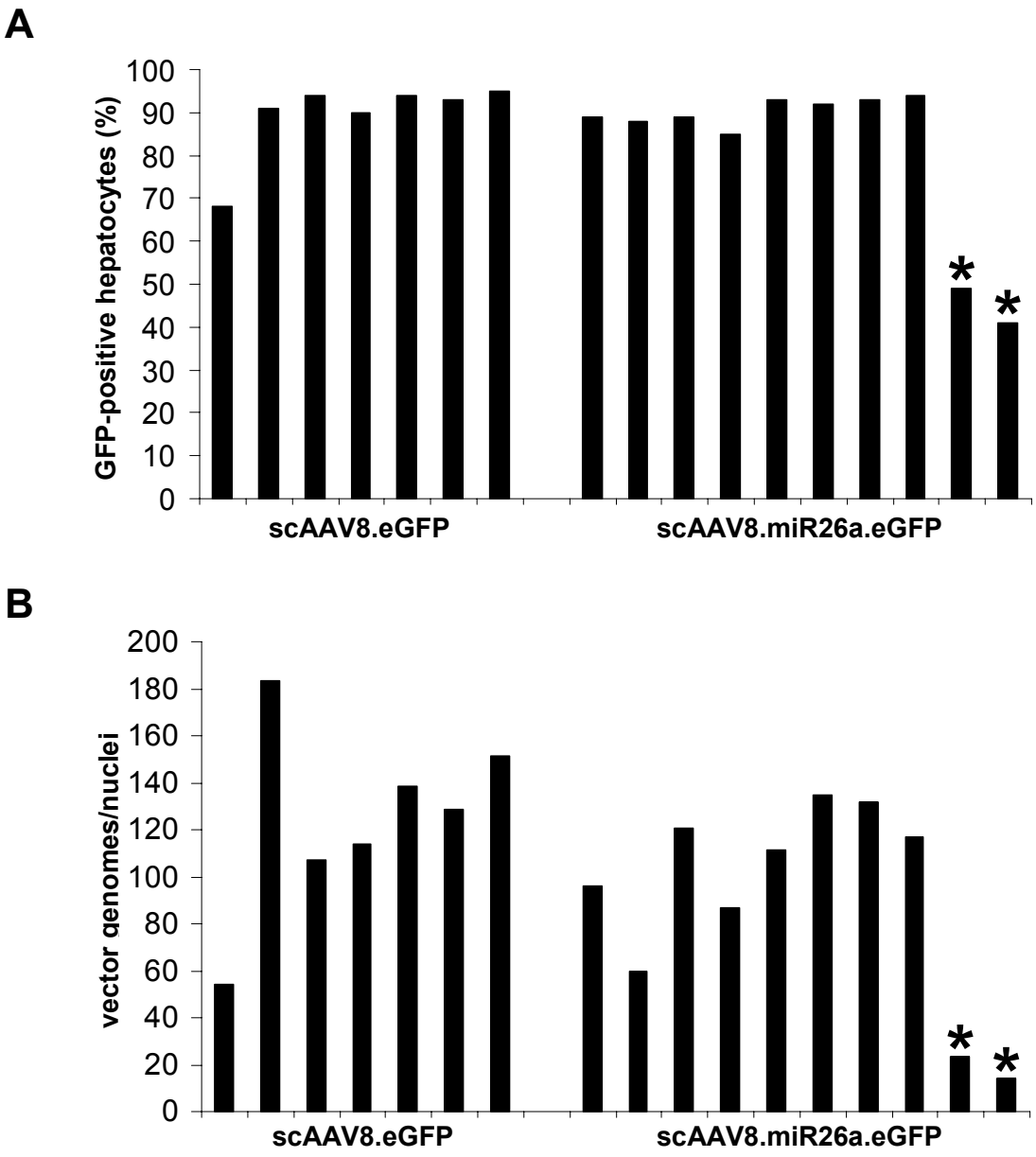

Figure S5. AAV transduction efficiency in miR-26a-treated and control animals

(A) Percentage of GFP-positive hepatocytes in treated animals, as determined by fluorescent microscopy. Asterisks in this panel and in (B) indicate animals which did not demonstrate a therapeutic response to miR-26a delivery (see Fig. 5). (B) Quantification of transduced vector genomes per cell as determined by qPCR.

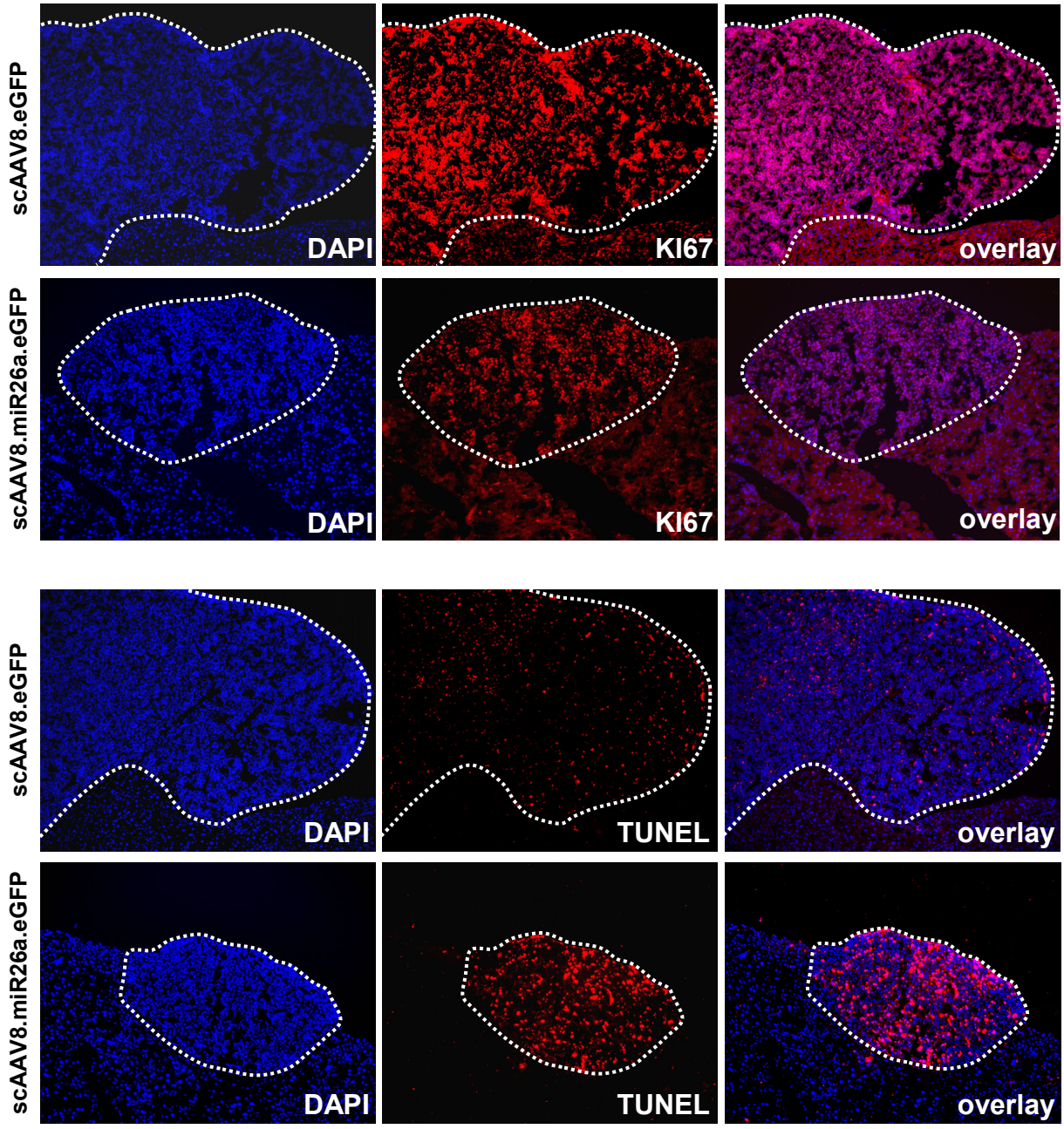

Figure S6. Cellular proliferation and apoptosis in tumors 10 days after AAV administration

Representative DAPI, Ki67, and TUNEL-stained sections from miR-26a-treated and control tet-o-*MYC*; LAP-tTA animals showing tumors (outlined with dotted line) and adjacent normal-appearing liver.

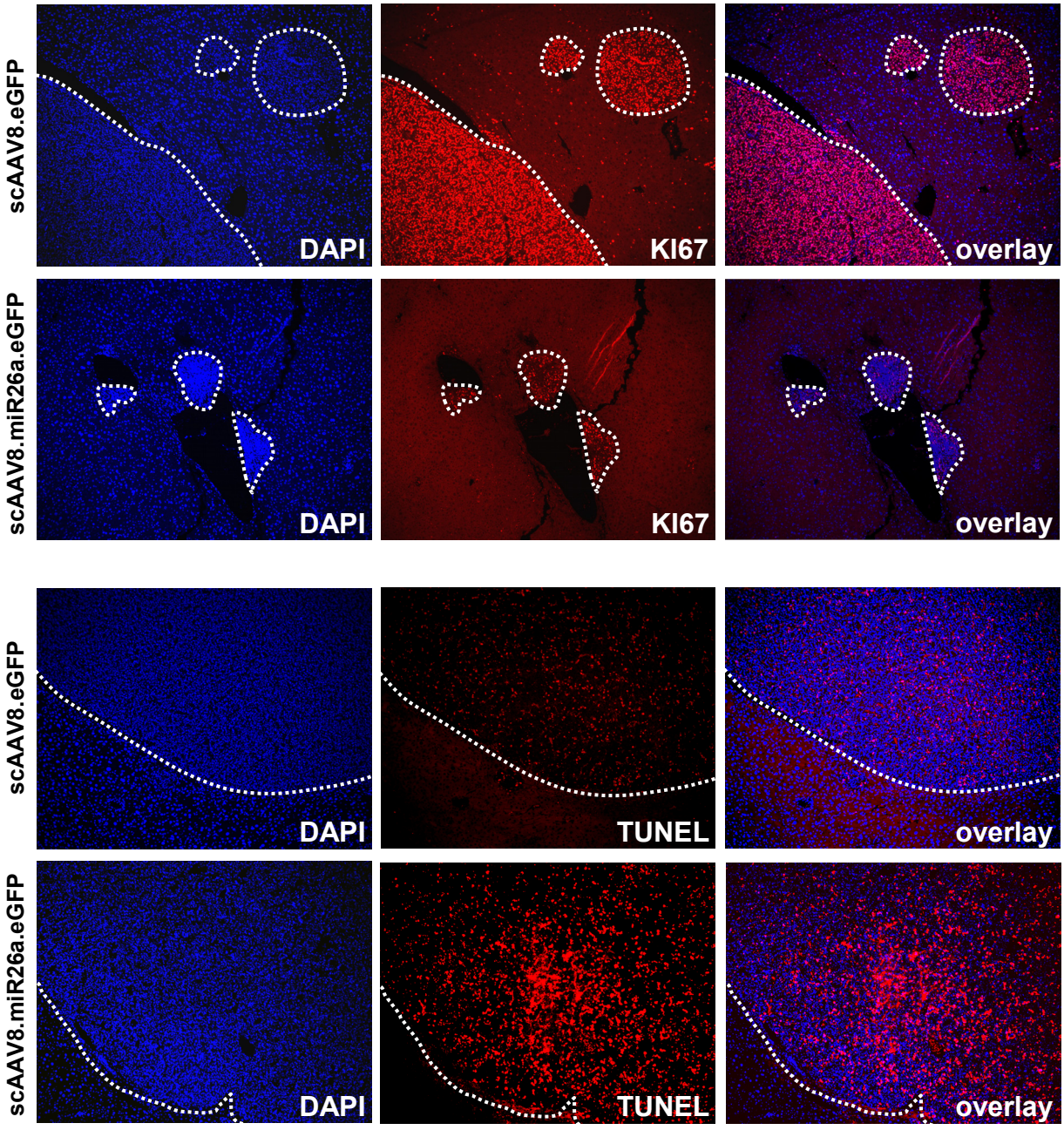

Figure S7. Cellular proliferation and apoptosis in tumors 21 days after AAV administration

Representative DAPI, Ki67, and TUNEL-stained sections from miR-26a-treated and control tet-o-*MYC*; LAP-tTA animals showing tumors (outlined with dotted line) and adjacent normal-appearing liver.

[G] Supplemental Figure 8

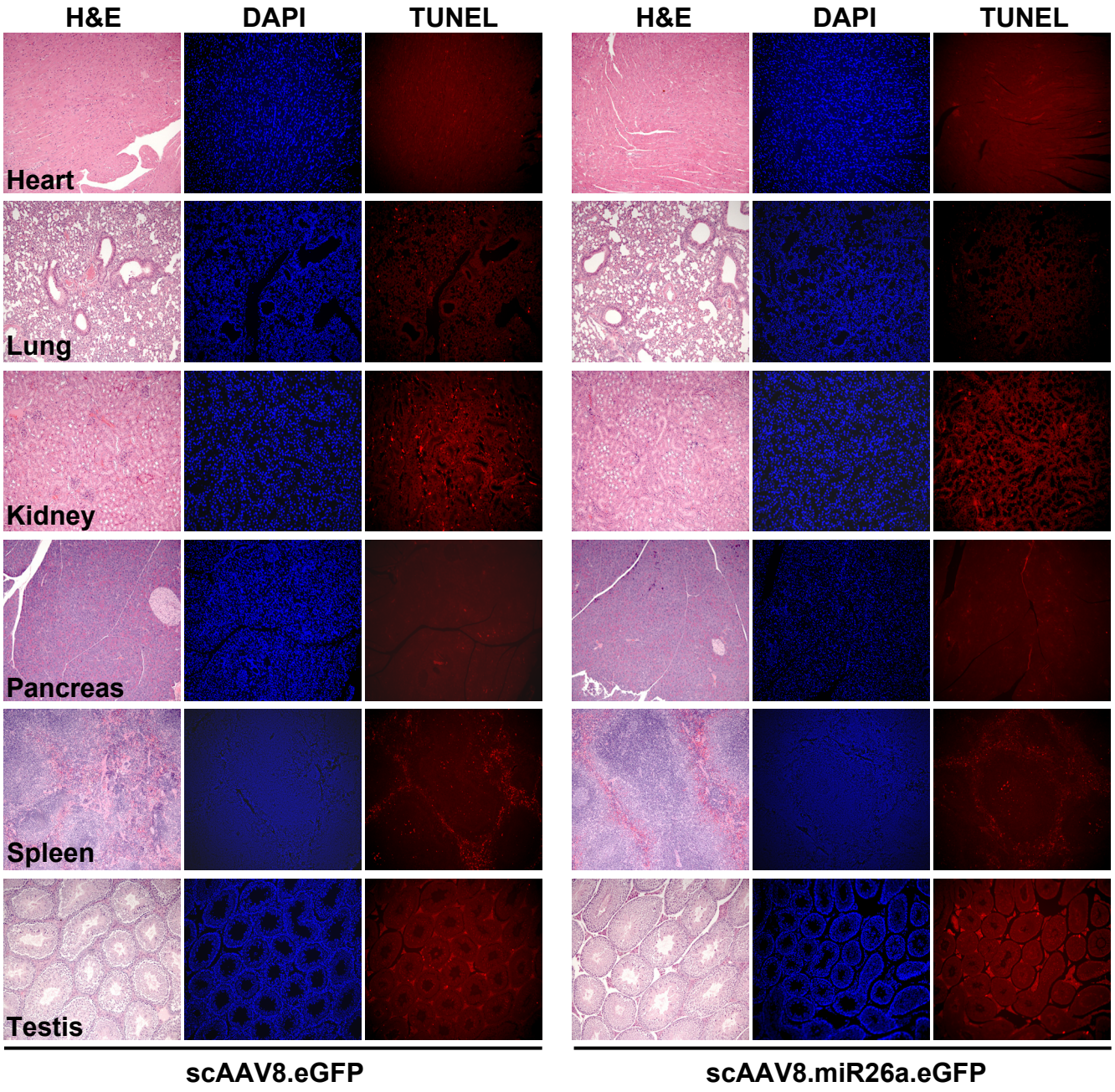

Figure S8. Analysis of apoptosis in various tissues following AAV8-mediated delivery of miR-26a

Tissues were collected 21 days after AAV administration and stained with H&E, DAPI, or TUNEL.

Table S1 : Liver Function Tests in C57/BL10 mice Pre- and Post-scAAV8.miR-26a.eGFP Administration

	Baseline	3 wks Post Injection
ALT (normal 24-140 U/L)	70.6 ± 10.2	89.6 ± 18.6
AST (normal 72-288 U/L)	112.8 ± 12.7	127.4 ± 32.1
ALP (normal 45-222 U/L)	76.4 ± 4.3	147.4 ± 21.9
Bilirubin, Total (normal 0.0-0.9 mg/dL)	0.24 ± 0.03	0.3 ± 0.03
Bilirubin, Direct (normal 0.0- 0.2 mg/dL)	0 ± 0.00	0.02 ± 0.02

Mean and Standard Error of the Mean shown (n=5)
ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase

[G] Supplemental Tables 2-4

Table S2: Primers used in luciferase reporter assays (Fig. 3)

	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
CCND2.XbaI amplification	ATCGATTCTAGAGGTGCAAAGATAGATGGCTGA	ATCGATTCTAGAAACAAATAGCTTCATTACCTGTCAA
CCNE2.XbaI amplification	ATCGATTCTAGATGACTAGTGCAATTTGGTTCTTG	ATCGATTCTAGAAGAGCACAGGTTGAACACCA
CCND2 mutagenesis	GCAAAGTTGTATTCAGCGAAGTAGTATTTTTCTTCCTCTC CACTTCTTAGAGGC	GCCTCTAAGAAGTGGAGAGGAAGAAAAATACTACTTCGC TGAATACAACTTTGC
CCNE2 mutagenesis	CTAATTTATCTATAGCTGCTATAGCAAGCTATTATAAAAG TAGTATTTCTACAAATGGTGAAATTTAATGTTT	AAACATTAAATTTCACCATTTGTAGAAATACTACTTTTATA ATAGCTTGCTATAGCAGCTATAGATAAATTAG

Table S3: Primers used in constructing scAAV vectors and pcDNA-miR-122a (Fig. 4)

	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
miR-26a-2.NheI/NotI amplification	CCGCCGGCTAGCCGGCAGGGTGTCTGTCTAGT	AAGGAAAAAAGCGGCCGCCAGGCTTCCAATGGATCAGT
miR-26a-2.FseI amplification	ATCGATCGGGCCGGCCCCCTGGTGCAATTCATTACC	ATCGATCGGGCCGGCCATCAGTGGTCCCAACCTCAA
miR-122a.XhoI amplification	ATACCGCTCGAGTTGCAAACAGAGTTCCTGTCCA	ATACCGCTCGAGAGCATGTGAGAGGCAGGGTTC

Table S4: Primers and probes used in Taqman assays for viral genome quantitation (Fig. S5)

	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Taqman probe sequence (5'-3')
EF1α Taqman amplicon	GGTGAGTCACCCACACAAAGG	GGTACTCCGTGGAGTCACATGAA	AAAGGGCCTTTCCGTCCTCAGCC
eGFP Taqman amplicon	CACTACCTGAGCACCCAGTC	TCCAGCAGGACC ATGTGATC	TGAGCAAAGACCCCAACGAGAAGCG

Supplemental Methods

Tumor Tissue Procurement

After obtaining appropriate Institutional Review Board approval, fresh tissues from hepatitis C-positive patients were collected at the time of surgery from primary liver neoplasms and adjacent non-neoplastic liver tissues, snap frozen in liquid nitrogen, and stored at -80C. The tissue diagnoses were confirmed in all cases by routine light microscopy.

Plasmid construction

MSCV.PIG retroviral vectors expressing miR-18a, miR-26a, and miR-34a were previously described (Chang et al., 2008). For luciferase reporter constructs, the predicted miR-26a binding site and approximately 225 bp of flanking 3' UTR sequence was amplified from human genomic DNA and cloned into the XbaI site of pGL3-control (Promega). Mutagenesis was performed with the QuickChange XL site-directed mutagenesis kit (Stratagene). Primer sequences are provided in **Table S2**.

The self-complementary vector plasmid scAAV.eGFP includes a deletion in the terminal resolution site in a single inverted terminal repeat. As a result, this construct produces only dimeric inverted repeat genomes during replication and encapsidation (McCarty et al., 2003). The vector consists of the human EF1α promoter [EF1α promoter core domain (592 bp), Exon 1 (33 bp), Intron 1, (593 bp) and Exon 2 leader sequence (9 bp)], an enhanced green fluorescent protein (GFP) open reading frame, and a SV40 polyadenylation signal. To construct scAAV.miR26a, eGFP sequences were removed by NheI/NotI digestion and replaced with miR-26a-2 and approximately 200 bp of flanking genomic sequence amplified from human genomic DNA. To produce scAAV.miR26a.eGFP, miR-26a-2 was amplified and cloned into the FseI site within the EF1α intron in scAAV.eGFP. pcDNA-miR-122a was constructed by amplifying miR-122a from human genomic DNA and cloning into the XhoI site of pcDNA3.1(+) (Invitrogen). Primer sequences are provided in **Table S3**.

Retroviral infection of HepG2 cells

$8x10^6$ HEK293T cells were co-transfected with MSCV.PIG constructs, gag-pol, and VSV-G helper plasmids using the FuGene6 reagent (Roche). Following transfection, the retroviral supernatant was collected, filtered, and supplemented with 12μg/mL polybrene. $1x10^6$ HepG2 cells were infected twice for 8 hours each and, 48 hours after the second infection, plated into medium containing 1.25μg/mL puromycin and selected for 48 hours. Cells were then plated for cell-cycle profiling and RNA/protein collection.

Transient transfection of HeLa cells

$1.5x10^6$ HeLa cells were plated and transfected 16 hours later using the *Trans*IT-HeLaMONSTER reagent (Mirus) with 4μg each of the indicated scAAV construct and pcDNA-miR-122a. 24 hours after transfection, cells were imaged by fluorescence microscopy and harvested for RNA isolation.

AAV vector production

Recombinant AAV vectors were produced by a standard triple transfection calcium phosphate precipitation method using HEK293 cells. The production plasmids were: (i) scAAV.eGFP or scAAV.miR26a.eGFP, (ii) rep2-cap8 modified AAV helper plasmid encoding the cap serotype 8-like isolate rh.74, and (iii) an adenovirus type 5 helper plasmid (pAdhelper) expressing adenovirus E2A, E4 ORF6, and VA I/II RNA genes. Purification was accomplished from clarified 293 cell lysates by sequential iodixanol gradient purification and ion exchange column chromatography using a linear NaCl salt gradient for particle elution. Vector genome (vg) titers

were determined by quantitative polymerase chain reaction (qPCR) as described (Clark et al., 1999). Primer and probe sequences are provided in **Table S4**.

AAV transduction efficiency

Transduction efficiency was determined by counting the number of GFP positive and negative hepatocytes using four random 20x GFP and DAPI overlap images. To further quantify transduction, eGFP transgene qPCR was performed on total DNA isolated from liver tissue. Total tissue DNA was isolated using the Gentra Puregene kit (Qiagen) according to the manufacturer's instructions. 60 ng of DNA (10,000 cell equivalents) was used as PCR template in triplicate reactions and vg number was extrapolated from a linearized plasmid standard. Vector genome/cell calculations assume 6 pg of total DNA per cell. Primer and probe sequences are provided in **Table S4**.

Supplemental References

Chang, T. C., Yu, D., Lee, Y. S., Wentzel, E. A., Arking, D. E., West, K. M., Dang, C. V., Thomas-Tikhonenko, A., and Mendell, J. T. (2008). Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet *40*, 43-50.

Clark, K. R., Liu, X., McGrath, J. P., and Johnson, P. R. (1999). Highly purified recombinant adeno-associated virus vectors are biologically active and free of detectable helper and wild-type viruses. Hum Gene Ther *10*, 1031-1039.

McCarty, D. M., Fu, H., Monahan, P. E., Toulson, C. E., Naik, P., and Samulski, R. J. (2003). Adeno-associated virus terminal repeat (TR) mutant generates self-complementary vectors to overcome the rate-limiting step to transduction in vivo. Gene Ther *10*, 2112-2118.