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Supplemental Information

Anti-adenoviral Artificial MicroRNAs Expressed from AAV9 Vectors Inhibit Human Adenovirus Infection in Immunosuppressed Syrian Hamsters

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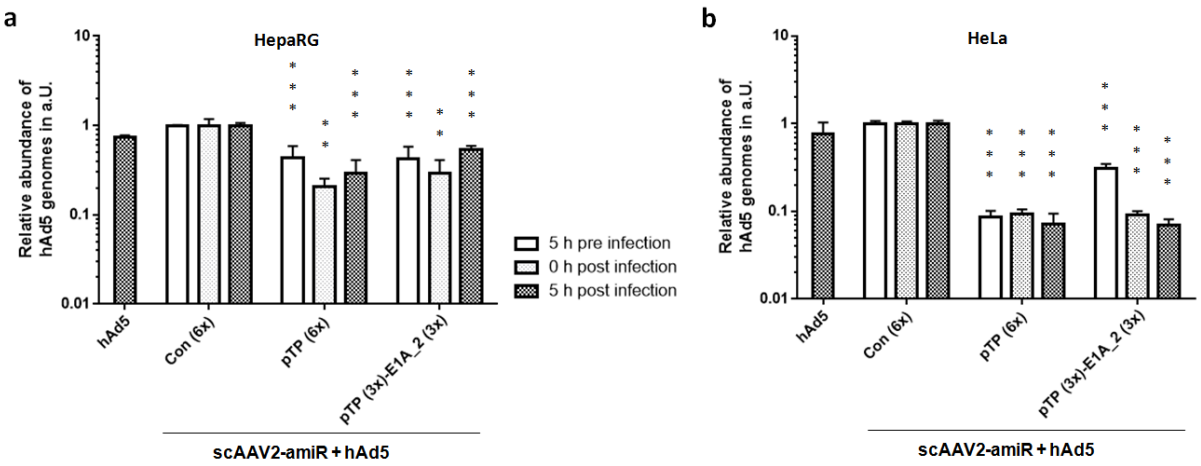
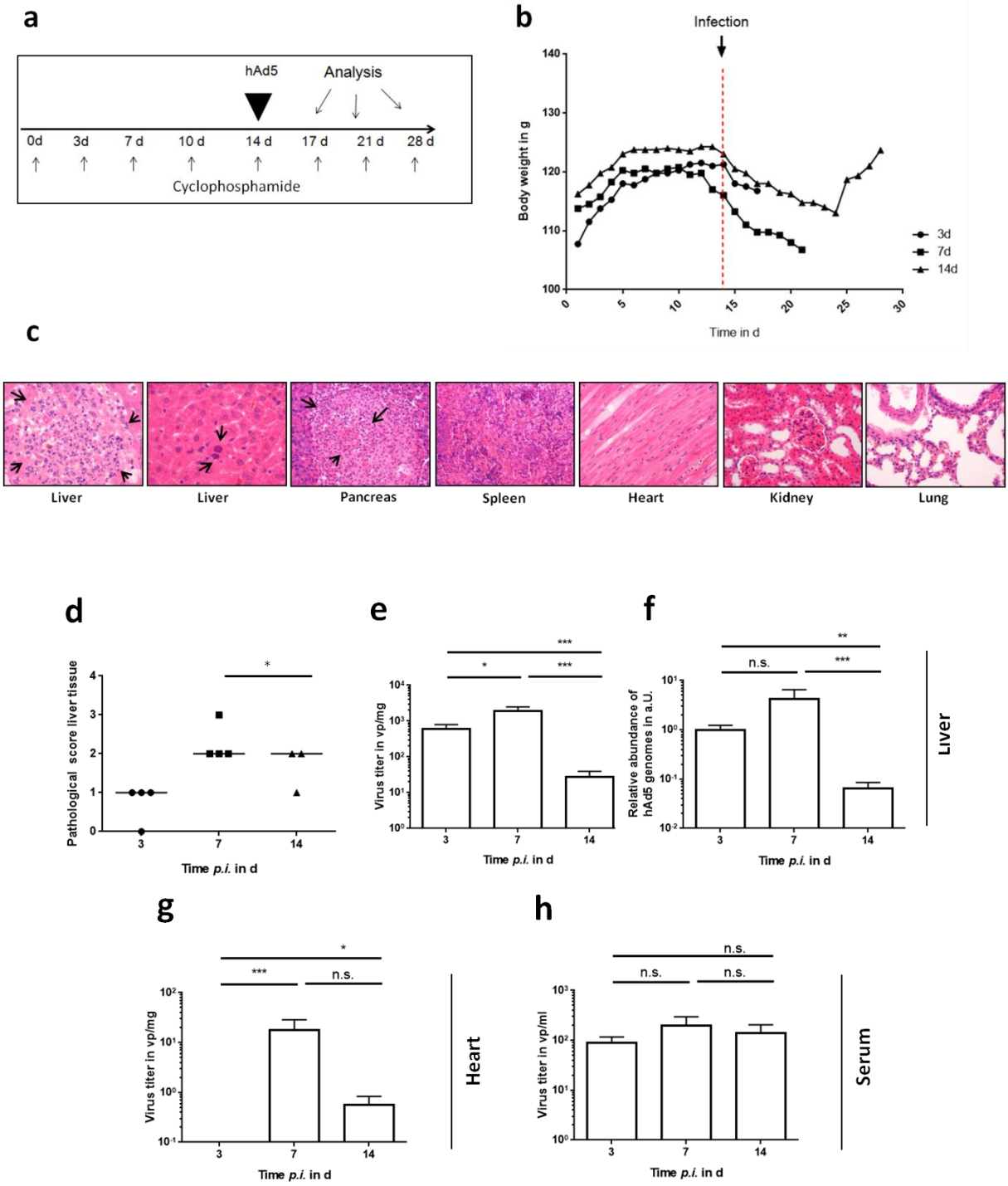
Supplementary material

Figure S1. Inhibition of hAd5 ongoing infection in hepatic HepaRG and HeLa cells by anti-adenoviral amiRs



HepaRG cells (**a**) and HeLa cells (**b**) were infected with 0.05 MOI hAd5 and transduced 5 h before, concomitant (0 h) or 5 h after infection of cells with 1,000 vge/cell of scAAV2-amiR vectors containing the indicated amiRs (copy number of each amiR in the vector genome is shown in parentheses). The cells were lysed 48 h later and the amount of viral DNA quantified by qPCR. hAd5 represents cells which were only infected with hAd5 but not transduced. Con represents scAAV2-amiR-control vector containing six copies of amiR-dsRed. Abundance of hAd5 genomes after treatment with anti-adenoviral amiRs expressing scAAV2 vectors was related to Con (= 1). Significance of the anti-adenoviral amiR vectors compared to Con, ** $p < 0.01$, *** $p<0.001$.

Figure S2. hAd5 infection in immunosuppressed Syrian hamsters.



(**a**) **Experimental design**. Syrian hamsters were immunosuppressed with cyclophosphamide for two weeks and then infected with $4x10^{11}$ vp/kg hAd5. Immunosuppression was pursued until days 3 (n = 4), 7 (n = 4) and 14 (n = 3) after infection with hAd5 respectively, on which animals were sacrificed and analyzed.

(**b**) **Development of body weight**. Shown are the mean values of body weight.

(**c**) **Hematoxilin-eosin staining of tissues of hAd5 infected animals at Day 7 post infection**. Liver tissue (left panel) shows local necrotic area. Liver tissue (right) shows inclusion bodies (shown by arrows). Pancreas with necrosis and inflammation of an islet. Spleen shows depletion of immune cells. Heart, lung and kidney were not affected compared to uninfected immunosuppressed animals (not shown). Magnification 400x.

(**d**) **Pathological grading of liver tissue damage**. Liver damage was assessed and given as a pathological score presenting a scale from 0 (no damage) to 3 (severe damage); Score 0 = without necrosis, score 1 = minimal necroses, score 2 = minimal, multifocal necroses, score 3 = moderate, multifocal necroses.

(**e**) **hAd5 titers in the liver.**

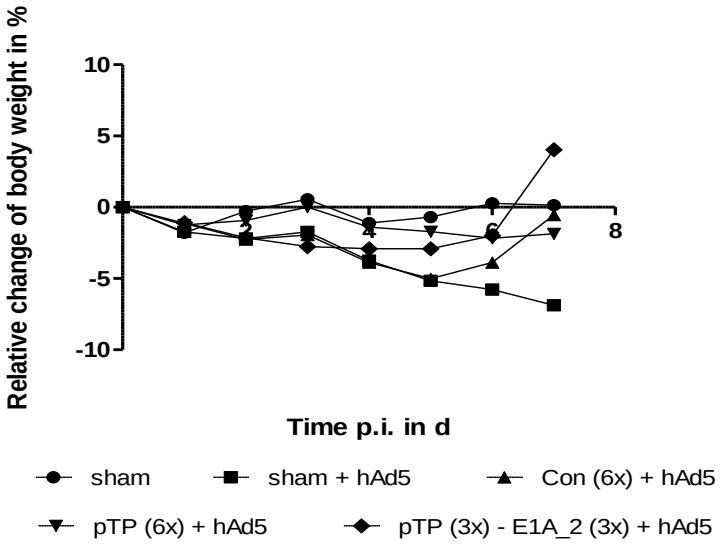
(**f**) **Relative abundance of hAd5 genomes copies in the liver.**

(**g**) **hAd5 titers in the heart.**

(**h**) **hAd5 titers in the serum.**

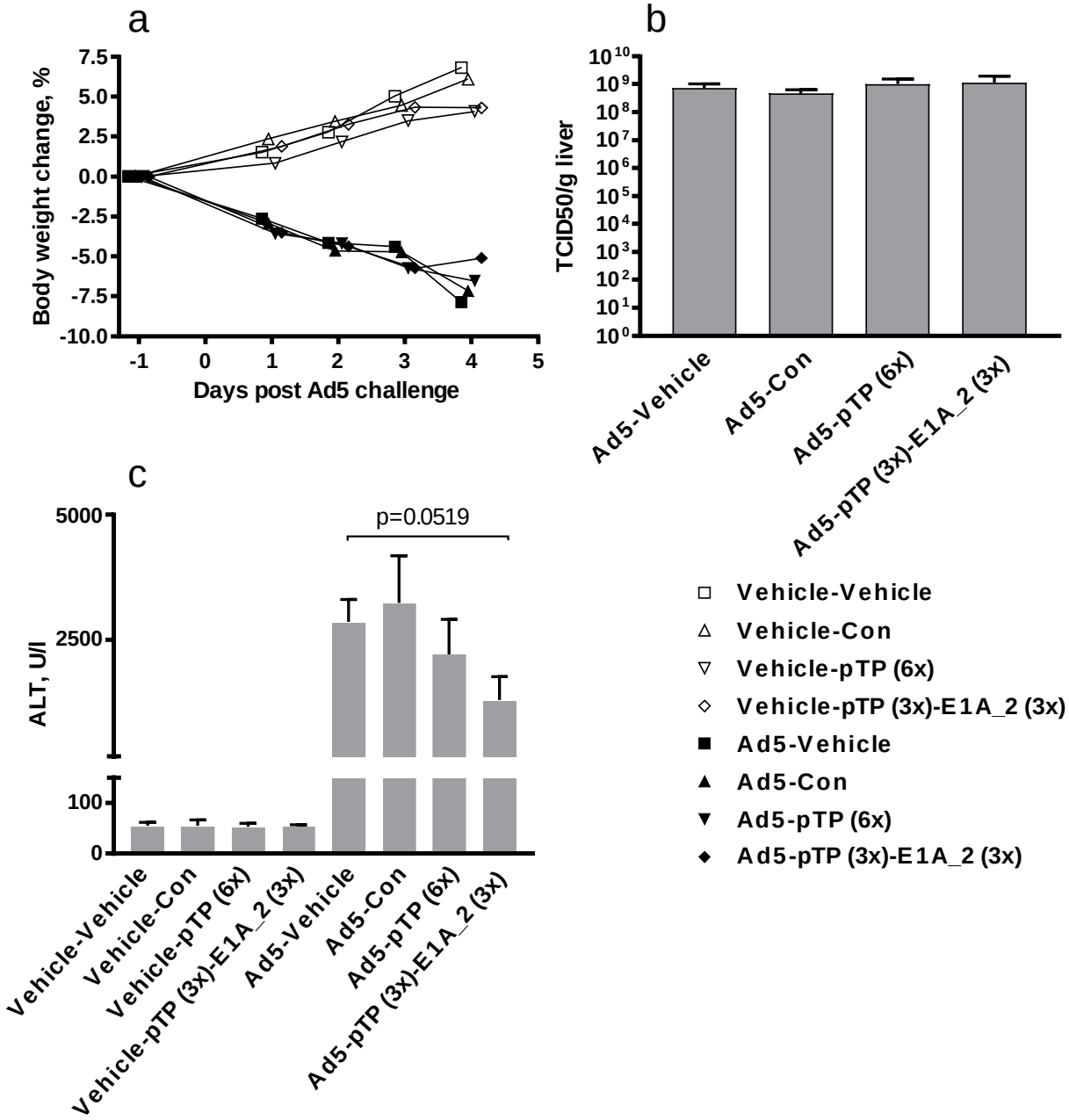
Number of animals, n = 3 to 4; * $p>0.05$, ** $p<0.01$, *** $p<0.001$; n.s., not significant.

Figure S3. Development of body weight of immunosuppressed Syrian hamsters after treatment with anti-adenoviral amiR-expressing scAAV9 vectors and hAd5.



Shown are relative changes of body weight beginning at day 14 after scAAV9 vector transduction of immunosuppressed Syrian hamsters.

Figure S4. Treatment of hAd5 infection with anti-adenoviral amiRs in a high hAd5 doses infection model of immunosuppressed Syrian hamsters



Syrian hamsters were immunosuppressed with cyclophosphamide. Concomitant with the first CP injection, three groups of animals were injected i.v. with one of the three scAAV9-amiR vectors at a dose of $5x10^{13}$ vge/kg, and a fourth group received vehicle (PBS). At 14 days after vector administration, half of the animals in all groups were injected i.v. with hAd5 at a dose of $5.58x10^{12}$ vp/kg, while the remaining animals received vehicle (PBS) injections. Animals were sacrificed seven days later. Number of animals per group, n = 6.

a) Body weight changes. The symbols represent the group mean.

(**b**) **Infectious hAd5 burden in the liver.** There was no significant reduction of virus titers after treatment of hAd5 infected animals with scAAV9-amiR-pTP (6x) or scAAV9-amiR-pTP

(3x)-E1A_2 (3x). For this graph and the one shown in panel **c**, the columns represent the group mean, and the error bars show the standard error of the mean.

(**c**) **Alanine transaminase (ALT) levels in the serum.** There was no significant reduction of ALT levels after treatment of hAd5 infected animals with scAAV9-amiR-pTP (6x) or scAAV9-amiR-pTP (3x)-E1A_2 (3x), but a clear tendency for an protective effect of scAAV9-amiR-pTP(3x)-E1A_2 (3x), p = 0.0519.

Table S1. siRNA sequences of anti-adenoviral amiRs

amiR	antisense / sense strand 5' → 3'	Target gene	Target sequence in hAd5 Gene bank: AC_000008.1
E1A_1	UUU ACA GCU CAA GUC CAA AGG CCU UUG GAU GAG CUG UAA A	E1A	1510-1530
E1A_2	UAU UGC AUU CUC UAG ACA CAG CUG UGU AGA GAA UGC AAU A	E1A	1334-1354
E1A_3	UCG GUA AUA ACA CCU CCG UGG CCA CGG AUG UUA UUA CCG A	E1A	577-597
E1A_4	AAA AUC UGC GAA ACC GCC UCC GGA GGC GGU CGC AGA UUU U	E1A	736-756
E1A_5	AGU GAG UAA GUC AAU CCC UUC GAA GGG AUA CUU ACU CAC U	E1A	785-805
Hex	UUU CCA CUU GAC UUU CUA GCU AGC UAG AAU CAA GUG GAA A	Hexon	19611-19631
IVa2	AUU UCU GGG AUC ACU AAC GUC GAC GUU AGA UCC CAG AAA U	IVa2	4649-4669
pTP	AAG AGA GUU CGA CAG AAU CAA UUG AUU CUC GAA CUC UCU U	pTP	8789-8809
Con_1	GUA UAG UCU UCG UUG UGG CUU AAG CCA CAG AAG ACU AUA C	drFP383	
Con_2	UUU UAU AGU CUG GUA UGU CGG CCG ACA UAA GAC UAU AAA A	drFP383	
Con_3	UUC UAU UUC AAA CUC GUG CCC GGG CAC GAU UGA AAU AGA A	drFP383	