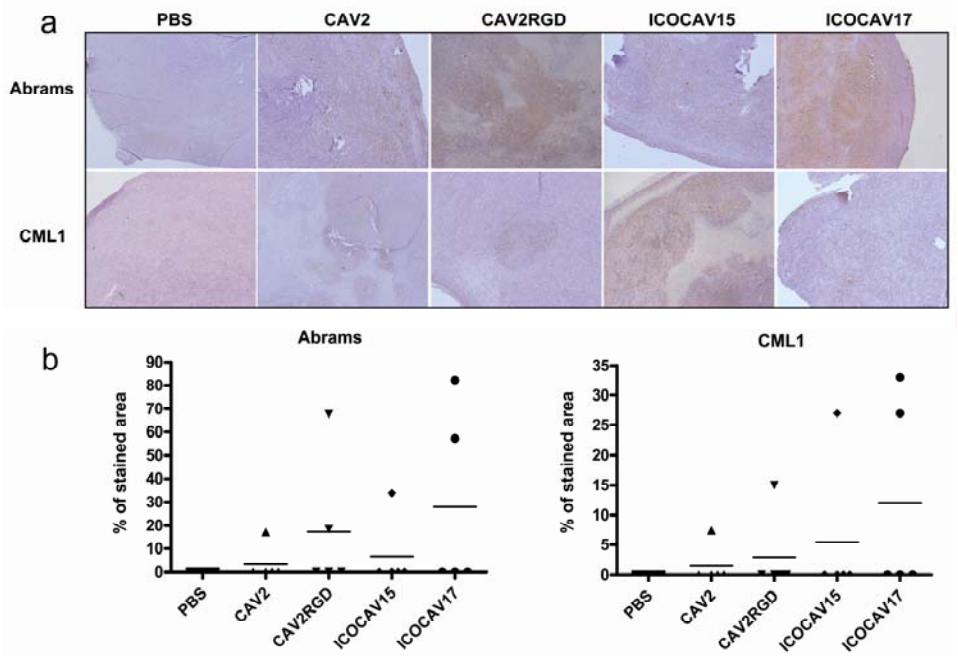

Figure S1. Immunohistochemistry of tumors from in vivo efficacy assays in Abrams and CML1 models.

Tumors were obtained at the end of the experiments. Adenovirus detection was performed in deparaffinized tumor sections with an anti-adenovirus late protein antibody. (a) Representative positive sections of tumors from each treatment group. Upper panel: Abrams model. Lower panel: CML1 model. Original magnification x40. (b) To quantify the stained areas tumor images were captured at original magnification x10 and merged with Photoshop software. The percentage of stained area for each tumor was quantified using the ImageJ software and represented as single dots. Mean value for each group is showed (line). PBS, phosphate-buffered saline.

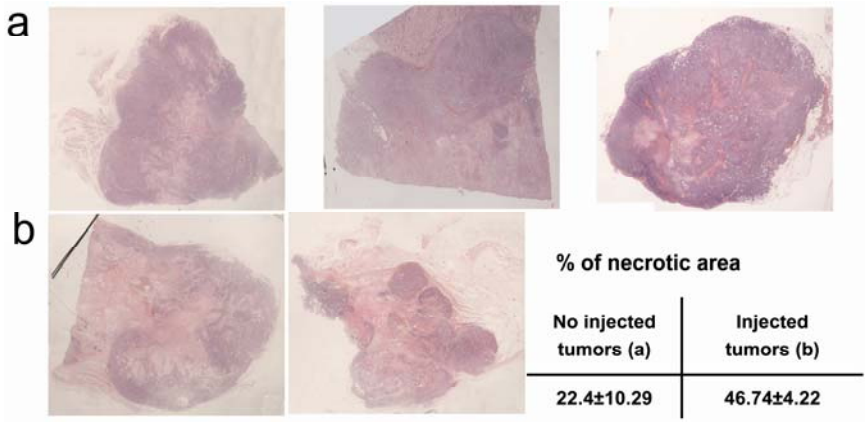

Figure S2. Hematoxilin&Eosin staining of tumors from patient VI.
Patient VI suffered a hepatic neuroendocrine carcinoma with multiple subcutaneous and organ-localized tumors. Two subcutaneous tumors were injected with 5x1011vp of ICOCAV17 each. After 22 days patient died due to progressively aggravated illness. Subcutaneous injected and non-injected tumors and hepatic and renal tumors were collected and stained with hematoxilin and eosin. Complete injected (lower panel) and non-injected (upper panel) tumor sections are shown. To quantify the necrotic areas (eosin staining) tumor images were captured at original magnification x10 and merged with Photoshop software. The percentage of necrotic areas was quantified using the ImageJ software. Percentage of necrotic area ±SD is shown.

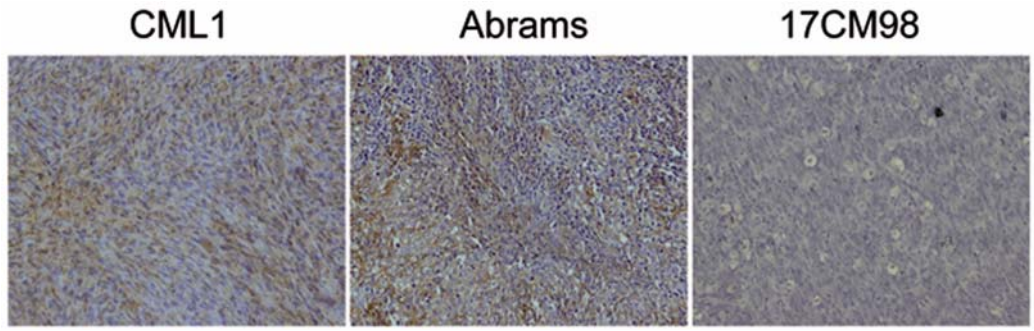

Figure S3. Hyaluronic acid detection within three canine xenografts tumors
Detection of Hyaluronic Acid within CML1, Abrams, and 17CM98 canine xenograft tumors was performed by histochemical analysis using biotinylated hyaluronan-binding protein (HABP-b). Original magnification x200.

	vp/ng DNA		vp/mg of tissue		vp/organ	
	CAV2	**CAV2RGD**	**CAV2**	**CAV2RGD**	**CAV2**	**CAV2RGD**
CNS	**1,8E+00** ±2,7E-01	**3,0E+00** ±1,9E+00	**3,2E+02** ±4,8E+01	**5,4E+02** ±3,9E+02	**1,4E+05** ±2,3E+04	**2,1E+05** ±1,4E+05
Testis#	**7,6E-01** ±8,9E-02	**7,2E+00** ±3,1E+00	**6,1E+02** ±1,0E+02	**2,4E+03** ±1,2E+03	**2,9E+04** ±6,1E+03	**1,1E+05** ±3,4E+04
Kidney#	**3,3E+01** ±5,6E+00	**7,6E+02** ±3,4E+02	**7,9E+03** ±4,5E+03	**9,6E+04** ±4,5E+04	**4,7E+06** ±3,0E+06	**5,6E+07** ±2,3E+07
Heart#	**4,2E+01** ±9,7E+00	**1,2E+02** ±3,5E+01	**4,4E+03** ±1,1E+03	**9,2E+03** ±3,1E+03	**1,1E+06** ±4,0E+05	**1,8E+06** ±5,5E+05
Lung#	**1,1E+02** ±1,2E+02	**1,3E+03** ±3,8E+02	**3,2E+04** ±3,6E+04	**2,2E+05** ±7,3E+04	**8,0E+06** ±8,5E+06	**5,3E+07** ±1,7E+07
Spleen	**1,3E+02** ±3,5E+01	**4,0E+02** ±3,0E+02	**1,3E+05** ±5,3E+04	**2,0E+05** ±1,4E+05	**2,2E+07** ±9,1E+06	**3,1E+07** ±1,9E+07
Liver	**6,8E+01** ±1,6E+01	**7,2E+01** ±2,1E+01	**7,8E+04** ±2,8E+04	**6,3E+04** ±2,2E+04	**9,8E+07** ±3,5E+07	**7,8E+07** ±2,7E+07
Blood#	**7,82E+00** ±2,78E+00	**8,71E+02** ±3,65E+02	**7,47E+01** ±1,70E+01	**9,70E+03** ±4,09E+03	**7,82E+00** ±2,78E+00	**8,71E+02** ±3,65E+02

Supplementary Table 1. Systemic biodistribution of CAV2 and CAV2RGD in Balb/C mice
Abbreviations: CNS, Central nervous system. Vp, viral particles.
#Statistical significance between CAV2 and CAV2RGD ($p<0.05$) by two-tailed unpaired Student's t-test. Mean values ± SD are shown for the different ratios.

Supplementary Material and Methods

Histochemical staining of hyaluronic acid (HA)

Paraffin-embedded tumors were cut into 4-µm thick sections. Sections were deparaffinized and endogenous peroxidase activity was blocked by incubation for 30 minutes in 0,3 % H2O2 in methanol. After re-hydratation, sections were blocked for 30 minutes with 10% Normal Goat Serum diluted in PBS. For HA staining, the slides were incubated with 5 µg/ml of a bionylated hyaluronic acid binding protein (HABP-b. Seikagaku; Japan) overnight at 4ºC. Some samples were pre-treated with 20 U/ml of bovine testes hyaluronidase (Sigma; St Louis, MO) at 37°C for 1h, prior to the addition of the HABP-b in order to test the specificity of HA staining. After incubation with HABP-b, the sections were washed with PBS and treated with avidin-biotin-peroxidase kit (ABC KIT PK-4000. Vectastain; Burlingame, CA). After washings, sections were developed with DAB (DAKO Laboratories; Glostrup, Denmark), and counterstained with hematoxylin. Images of slices were captured on a Nikon Eclipse 80i microscope (Nikon Instruments), using the software NIS-Element Basic Research 3.2 (Nikon).

Immunohistochemistry and histopathology.

Samples obtained from tumors were fixed in 10% buffered paraformaldehyde for 24 hours and embedded in paraffin. Routine hematoxylin-eosin staining and immunohistochemistry against adenovirus late proteins were performed. Blocks were cut into 4-µm thick sections and were deparaffinized, endogenous peroxidase activity blocked, and antigen retrieval was performed in citrate buffer. After rehydration, sections were blocked for 30 minuteswith 20% normal horse serum diluted in PBS. Samples were incubated with rabbit anti-late adenovirus proteins polyclonal IgG (Ab6982 Abcam; cross reactivity against CAV2) overnight at 4ºC. Slides were washed thrice in PBS 0.2% Triton X-100 and incubated with EnVision (Dako, Hamburg, Germany) according to the manufacturer's instructions. After washings, sections were developed with DAB (Dako Laboratories, Glostrup, Denmark), and counterstained with hematoxylin. Images of sections were captured on a Nikon SMZ800 stereo microscope (Nikon Instruments, Amsterdam, Netherlands), using the software NIS-Element Basic Research 3.2 (Nikon). Tumor images were captured (10x) and merged with Photoshop Software. ImageJ software was used to quantify adenovirus positive areas and percentage of necrotic areas.