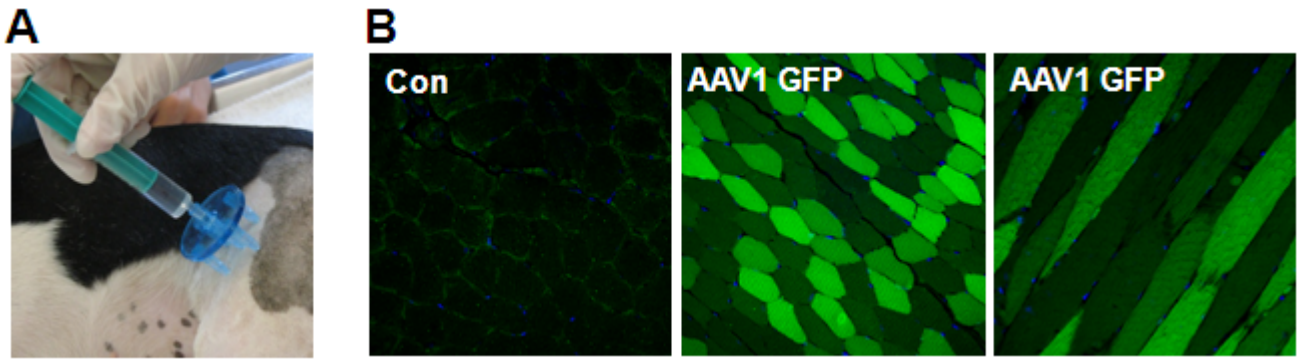
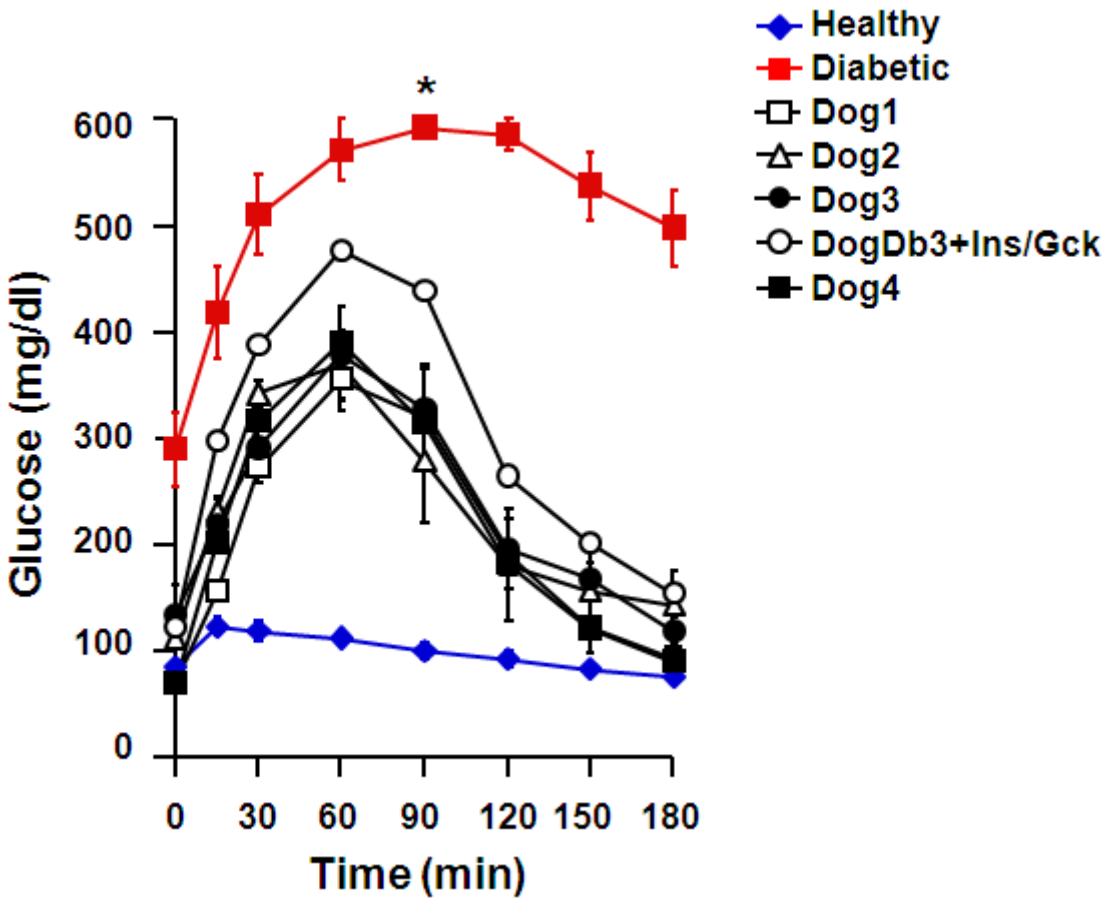
SUPPLEMENTARY DATA

Supplementary Figure 1. Efficient transduction of canine skeletal muscle with AAV1 vectors. (A) A 5-prong needle syringe was used as vector administration device. (B) Intramuscular delivery of the AAV1 vectors with this device results in efficient transduction of a large number of muscle fibers. AAV1-CMV-GFP vectors were injected at a dose of $5x10^{11}$vg/injection site; representative images of GFP immunostaining are shown. Original magnification 200X.

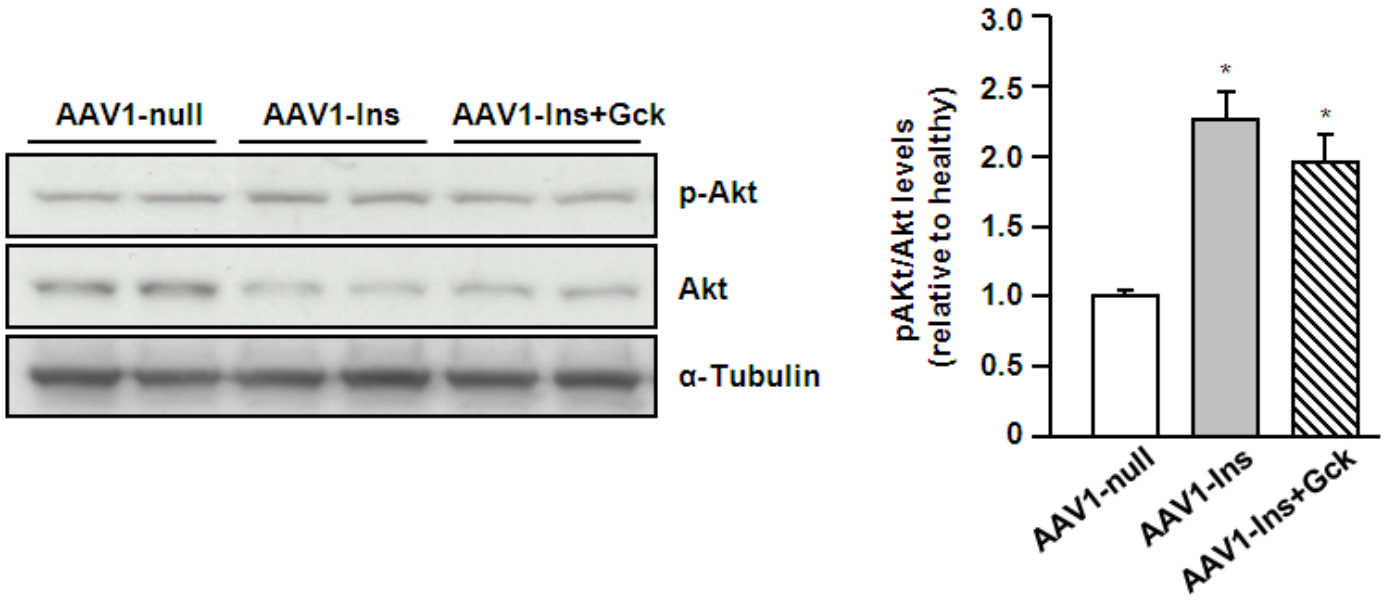


Supplementary Figure 2. OGTT at a dose of glucose of 3g/kg in Ins+Gck-treated dogs showed improved glucose disposal, lower peak glycemia and 2h glycemia dropped below 200 mg/dl. Data are represented as mean ± SEM of 2-3 OGTT performed every year during the study period. * glucose levels >600mg/dl.



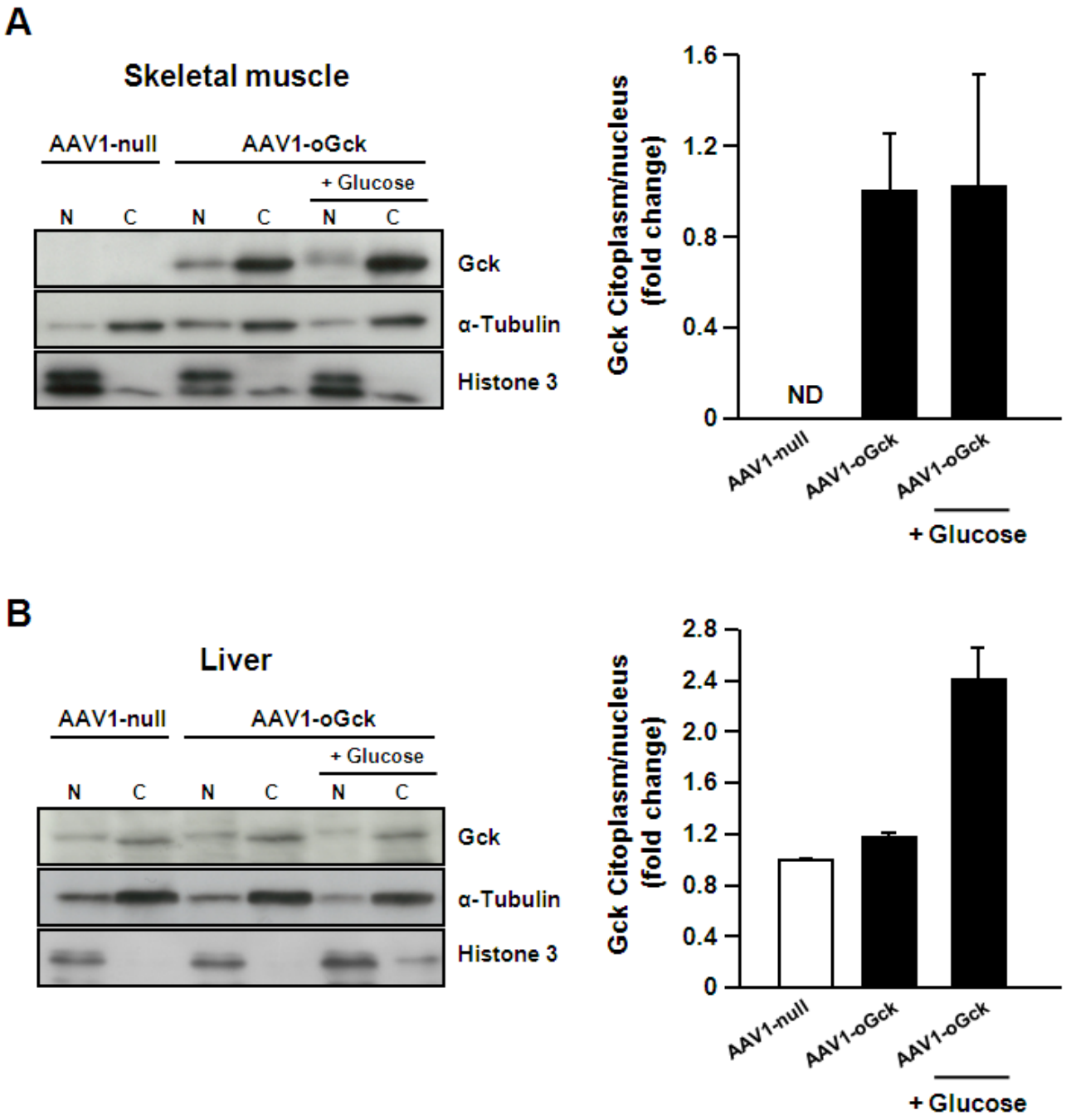
SUPPLEMENTARY DATA

Supplementary Figure 3. Insulin signaling in skeletal muscle of diabetic mice treated with AAV-Ins and AAV-Ins+Gck. Two weeks after diabetes induction mice were injected i.m. with $2x10^{12}$vg/kg of AAV1-null, AAV1-Ins or AAV1-Ins+GcK vectors. Fourteen weeks after vector administration the content of phospho-Akt (p-Akt) and total Akt was measured by Western blot in injected muscle samples. α-Tubulin was used as load control. Representative images of the Western blots are shown in the left panel. The right panel shows the ratio of p-Akt/Akt obtained from densitometric analysis of the western blots (n=4 per group). * $p<0.05$ vs AAV1-null



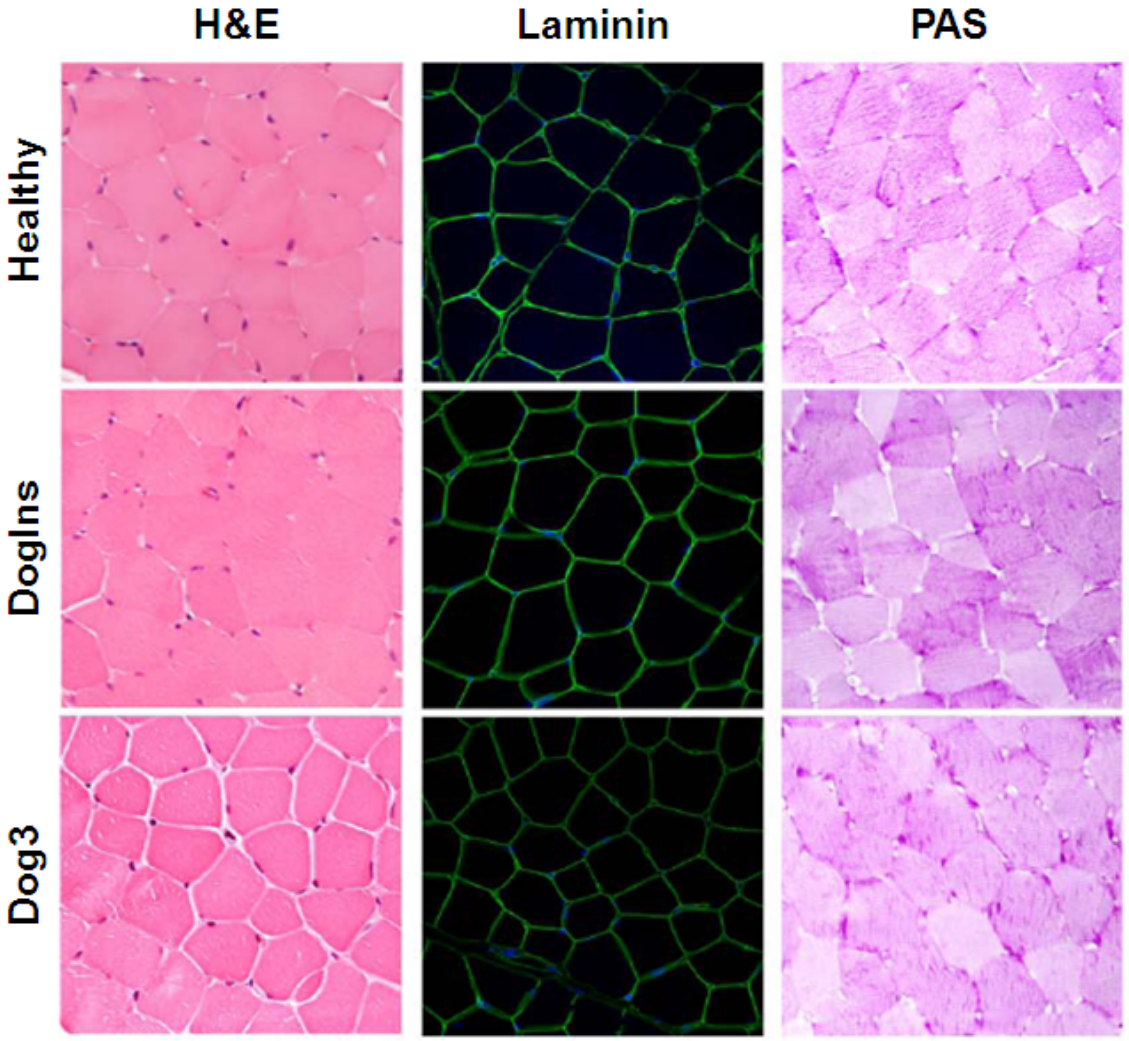
SUPPLEMENTARY DATA

Supplementary Figure 4. Subcellular localization of Gck. Healthy mice were injected i.m. with $4x10^{12}$ vg/kg of AAV1-null or AAV1-oGck. Three weeks after vector delivery One group of AAV1-oGck-treated mice was injected with 3g/kg of glucose and sacrificed 15 min later (shown as + glucose). The rest of the animals were sacrificed in starved conditions. Skeletal muscles (A) and liver (B) were fractioned in nuclear and cytoplasmic portions as indicated in Research Design and Methods. Left panels show representative images of Western blots for glucokinase (Gck), α-tubulin (a cytoplasmic protein) and histone3 (nuclear protein). Left panels show the ratio of Gck protein that was detected in the cytoplasmic fraction (identified as the fraction enriched in α-tubulin) *vs.* Gck that was detected in the nucleic fraction (identified as the fraction enriched in Histone 3. n=2, per group. ND, not detected. N, nucleic fraction. C, cytoplasmic fraction.



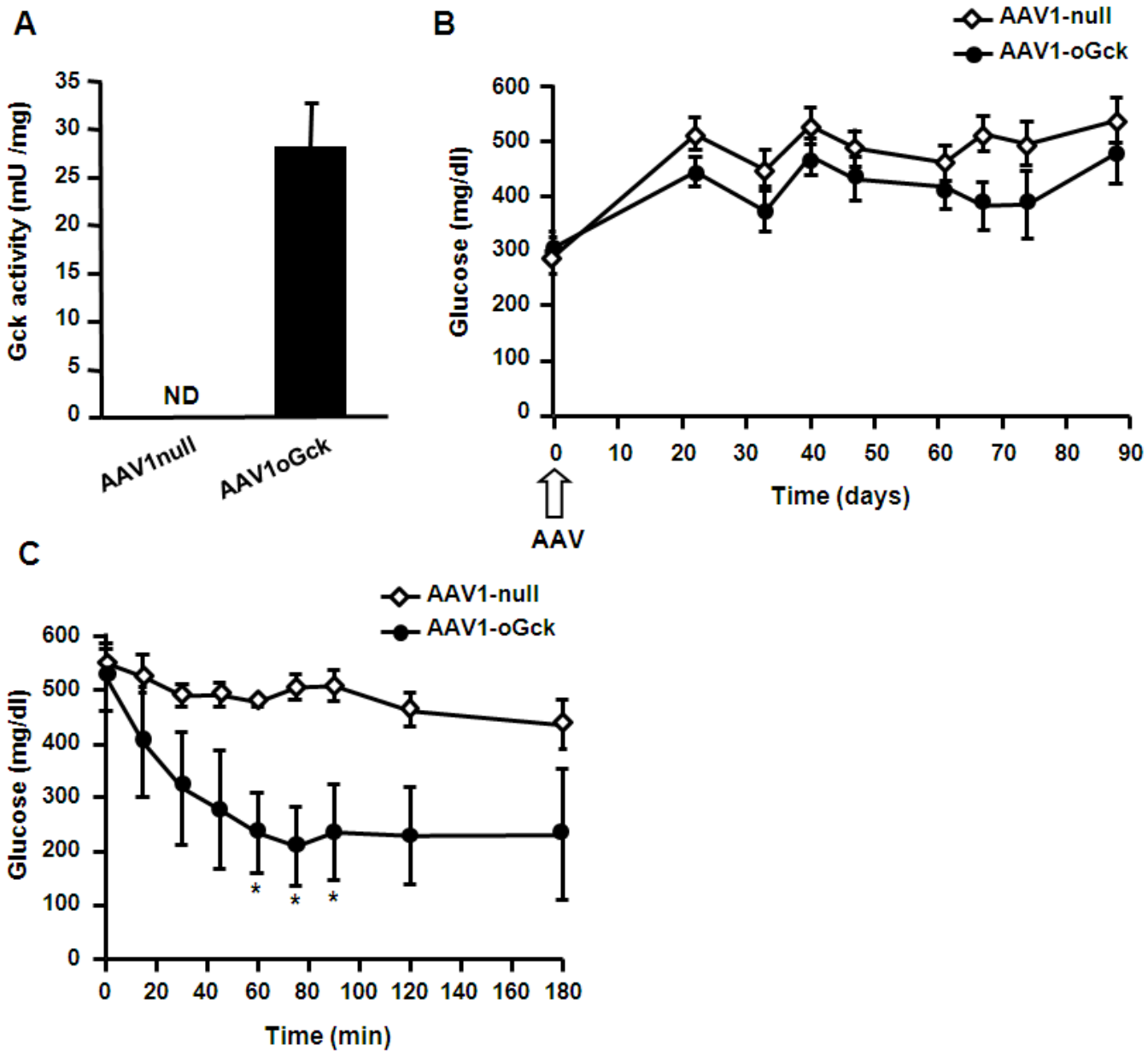
SUPPLEMENTARY DATA

Supplementary Figure 5. Integrity of muscle structure after AAV1-Ins and AAV1-Gck gene transfer. Morphologic analysis performed on skeletal muscle biopsies of DogIns obtained 9 months after AAV1 vector administration and on necropsy samples from Dog3 (2.2 years after treatment). No signs of muscle pathology or inflammation were observed by hematoxylin and eosin (H&E) staining or by laminin immunodetection. Periodic acid Schiff (PAS)-staining showed normal levels of glycogen storage. Original magnification 200X.



SUPPLEMENTARY DATA

Supplementary Figure 6. Expression of Glucokinase in skeletal muscle increases insulin sensitivity. STZ-treated mice were intramuscularly administered with either AAV1- oGck or AAV1-null (non-coding) vectors (4x1012 vg/kg). (A) Glucokinase activity was detected in the skeletal muscle of mice injected with AAV1-oGck 3-months after vector administration. (B) Blood glucose profile of diabetic mice treated with AAV-oGck or AAV-null vectors. Glycemia was determined in fed conditions in the absence of exogenous insulin treatment. (C) Insulin sensitivity was determined in diabetic mice 3-months after treatment with AAV-oGck or AAV-null vectors. Fed mice were given an intraperitoneal injection of insulin (0.75IU/kg, Humulin regular, Eli Lilly) and glycemia was followed for 180 min. In contrast to diabetic null mice that remained highly hyperglycemic, Gck-expressing diabetic mice showed marker reduction of glycemia. Results are expressed as the mean ± S.E.M. of n=12 mice per group. *, indicates significant difference with $p<0.05$.



SUPPLEMENTARY DATA

Supplementary Table 1. Summary of the experimental groups and the time of follow-up of each dog.

Dog identification	Treatment	Dose of AAV vector	Follow up (months)
DogDb1	Once or twice daily subcutaneous injection of insulin (Lantus)	N.A.	8.3
DogDb2			8.3
DogDb3			4.6
DogDb4			2.6
DogIns	AAV1-Ins	$1x10^{12}$vg/kg	53.3
Dog1	AAV1-Ins +AAV1-Gck	$1x10^{12}$vg/kg (each vector)	53.3
Dog2			53.3
Dog3		$2x10^{12}$vg/kg (each vector)	26.6
DogDb3+Ins/Gck			4.3
Dog4	AAV1-oIns +AAV1-oGck	$1x10^{12}$vg/kg (each vector)	4.6
DogGck1	AAV1-oGck + insulin	$2x10^{12}$vg/kg	10
DogGck2			10

N.A., not applicable.

SUPPLEMENTARY DATA

Supplementary Table 2. Evaluation of secondary complications in AAV-treated and untreated diabetic dogs.

Complication **(Frequency in diabetic companion dogs)**		**Dog**
Ocular complications	*Cataract* (Common)	Detected in DogDb1-3, DogGck1 and DogGck2 soon after diabetes induction with worsening over follow up period. Not detected in DogDb4, DogIns and Dog1-Dog4.
	Uveitis (Common, secondary to cataract)	Not detected
	Retinopathy (Uncommon)	Not detected
Urinary tract infection	(Common)	Not detected
Nephropathy	*Azotemia* (Uncommon)	Not detected in DogDb1-4, DogIns, Dog1, Dog2, Dog4, DogDb3+Ins/Gck, DogGck1 and DogGck2. Drug-induced moderate azotemia in Dog3*.
	Proteinuria (Uncommon)	Not detected
Clinical Peripheral Neuropathy	*Weakness, knuckling, abnormal gait, muscle atrophy, depressed limb reflexes, deficits in postural reaction testing* (Uncommon)	Not detected

Dogs were periodically monitored at the Veterinary Clinical Hospital at UAB. This Table summarizes the secondary complications described in companion diabetic dogs and the evaluation of these complications in AAV-treated and untreated diabetic dogs throughout the study over a period of 4 years. *Dog3 showed moderate azotemia immediately after STZ and Alloxan administration, compatible with a toxic effect of these drugs. No proteinuria or urinary tract infections were detected in Dog3 for the duration of the study.

SUPPLEMENTARY DATA

Supplementary Table 3. AAV1 vector genome biodistribution in Dog3.

Tissue	vg/diploid genome
Right Triceps brachii (untreated muscle)	0.21
Right Triceps brachii (untreated muscle)	0.11
Left Biceps femoris	0.05
Left Biceps femoris	0.03
Left Biceps femoris	5.68
Left Quadriceps femoris-Vastus lateralis	6.88
Left Semitendinosus	3.16
Left Tibialis anterior	1.57
Left Tibialis anterior	8.36
Left Tibialis anterior	15.14
Right Biceps femoris	0.18
Right Biceps femoris	0.02
Right Biceps femoris	0.75
Left Quadriceps femoris-Vastus lateralis	1.21
Right Semitendinosus	6.14
Right Extensor digitorum longus	1.35
Right Extensor digitorum longus	15.31
Right Extensor digitorum longus	2.38
Right Tibialis anterior	7.42
Kidney (cortex)	0.22
Kidney (medulla)	0.38
Lung (right middle lobe)	0.67
Lung (right caudal lobe)	0.61
Heart (left ventricle)	0.19
Heart (left atrium)	0.22
Gonads (testis)	0.04
Liver (left lateral lobe)	1.15
Liver (quadrate lobe)	1.12
Liver (right medial lobe)	0.40
Liver (right lateral lobe)	0.88
Liver (caudate process)	0.55
Liver (papillary process)	0.81
Pancreas (left lobe)	0.15
Pancreas (right lobe)	0.03
Spleen	0.57

Systemic biodistribution of vector genomes in Dog3 2.2 years after the administration of $2.0x10^{12}$ vg/kg of AAV1-Ins and AAV1-Gck vectors. Most of the detectable vector was found in injected muscles. Low vector gene copy numbers were detected in most peripheral tissues. Expression of insulin in the liver was undetectable (data not shown) despite the detection of vector genomes. This is likely due to silencing of the CMV promoter in liver.

SUPPLEMENTARY DATA

Supplementary Table 4. Glycogen content in skeletal muscle biopsies. Glycogen content in skeletal muscle was determined in necropsy samples from an uninjected control (Con) dog and from Dog3 (2.2 years post-treatment), and in muscle biopsies from DogIns and from Dog1 obtained 9 months after AAV1 administration. Results are mean±SEM of 3 samples for dog Con and Dog3 samples obtained from different muscle areas. Results for DogIns and Dog1 correspond to a single biopsy sample.

Dog	Glycogen (mg/g)
Con	3.31 ± 0.55
DogIns	0.910
Dog1	2.711
Dog3	2.76 ± 0.47

Supplementary Video 1. Dog exercise test. Fasted dogs (24 h) were subjected to 37- minute exercise under increasing speed and slope in a variable speed belt treadmill.

Supplementary Video 2. Dog2 before and after combined AAV1-Ins and AAV1-Gck treatment. One month after development of hyperglycemia and without exogenous insulin treatment, Dog2 showed apathetic behavior and significant cachexia. Seven months after treatment with AAV1-Ins and AAV1-Gck, Dog2 restored normoglycemia and recovered weight. Accordingly, the dog behaved more actively, like healthy control dogs.