SUPPLEMENTARY DATA

SUPPLEMENTARY METHODS

Early-onset Uncontrolled Diabetic Model. Uncontrolled diabetes was induced with a single intravenous (i.v.) injection of streptozotocin (STZ; Sigma, St. Louis, MO; 60 mg/kg), a potent diabetogenic agent that is cytolytic against pancreatic β–cells, dissolved in saline at the end of the vascular surgery. The glycemic status in diabetic rats was monitored daily using a hand-held glucometer (Accu-Chek Compact Plus, Roche Diagnostics, Laval, QC); only rats that were sufficiently hyperglycemic (blood glucose >15 mM) were included in the diabetic studies. After 54 hr to 60 hr post-STZ injections, the *in vivo* i.h. infusion experiments were carried out and lasted a total of 3.5 hr in rats that were limited to 20 g of standard chow the previous night.

Whole-Body and hypothalamic sustained hyperglycemic models. To induce whole-body hyperglycemia, normal rats received a 24 hr infusion of i.v. glucose. A solution of 37.5% glucose was infused at 35 μL/min for a total of 24 hr. To protect the exteriorized polyethylene catheter from being chewed, the catheter was fed through a stainless steel spring-coil tether (RT-12; Braintree Scientific, Braintree, MA) and was secured to the rodent in a saddle-style Velcro rodent jacket (RJ-M, Braintree Scientific). This infusion apparatus was out of reach to the rodent and still permitted the rodents to be mobile, unrestrained and have free access to food and water. As a control, normal rodents received a 24 hr i.v. infusion of vehicle saline (0.9% w/v NaCl). The next morning, after the 24 hr infusion period ("pre-basal" time point), a blood sample was obtained for plasma glucose and hormone analyses. The i.v. glucose infusion was then terminated at this point, and the plasma glucose levels were allowed to normalize over 1.5 hr prior to the pancreatic clamp. To selectively induce hypothalamic sustained hyperglycemia, normal rats received a 24 hr infusion of i.h. glucose. A solution of 4.5 mM glucose was made and infused at 0.33 μL/hr for a total of 24 hr using a microdialysis pump. The overnight infusion apparatus was setup as described above for the i.v. glucose-infused model. As a control, normal rodents received a 24 hr i.h. infusion of vehicle saline (0.9% w/v NaCl). The i.h. glucose infusion was terminated the next day after 24 hr; to keep the experimental time course consistent with the whole-body hyperglycemic studies, the pancreatic euglycemic clamp was commenced 1.5 hr after this termination.

Phlorizin-treated uncontrolled diabetic model. Once rodents displayed hyperglycemia (~24-30 hr post-STZ injection) STZ-diabetic rodents were administered a continuous infusion of i.v. phlorizin. Phlorizin is a naturally occurring product that produces renal glucosuria and blocks intestinal glucose absorption via the inhibition of the sodium/glucose cotransporters in the proximal renal tubule and the mucosa of the small intestine, respectively (S1). To prepare the infusate, phlorizin dihydrate (Sigma-Aldrich, St. Louis, MO) was dissolved in 20% propylene glycol (PG; Sigma) to a concentration of 5 μg/μL; phlorizin was infused at 2.5 μl/min (12.5 μg/min) i.v. to continuously normalize glucose levels overnight and throughout the pancreatic clamp procedure outlined earlier.

Construction of glial-specific adenoviruses. A plasmid containing the promoter for glial fibrillary acidic protein (GFAP), LacZ reporter gene and SV40 poly(A) signal (InvivoGen, San Diego, CA) was digested with SwaI and SpeI. The entire digested cassette was ligated into pShuttle (Stratagene, La Jolla, CA) using the Xba I and EcoR1 sites. Rat GLUT1 cDNA was cloned from prGT3 (Addgene plasmid 15993, from Dr. Morris J. Birnbaum (S2)) with primer pairs 5'-GAACACAAGAATCACATGTGGAGTGTCG and 5'-CAGGAGTGTCCGTGAATTCAGCAGTAAG. The cloned fragment was purified using a PCR gel purification kit (Invitrogen, Carlsbad, CA) and inserted between the BspHI and EcoRI sites of the modified pShuttle vector. The insertion was verified by sequencing. The recombinant adenovirus plasmid was generated with pShuttle-GFAP-GLUT1 or pShuttle-GFAP-LacZ vector using adenoviral E1- and E3-deleted vector (Stratagene) as previously described (S3). Propagation of the virus was performed in HEK 293 cells and purified using a ViraBind Adenovirus Purification Kit (Cell Biolabs, San Diego, CA). The purified virus was aliquotted and stored at -80°C. Purified virus concentration was 2.7 x 10^7 pfu/ml.

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Cell culture and in vitro cell infection. PC12 cells (rat pheochromocytoma), C6 (rat glial cell) and HEK 293 (human embryonic kidney) were purchased from American Type Cell Culture (Manassas, VA). All cell types were cultured according to supplier's recommendations and were maintained at 37°C under a humidified atmosphere of 5% CO2. PC12 cells and C6 cells were seeded at 1 $x10^5$ cells/well in 12-well plates. After one hour of seeding, 1 μM of Datura stramonium agglutinin (DSA) was added to the each well (S4). After 24 hours of initial seeding, the DSA medium was removed and replaced by normal culture medium containing equal number of virus particles for two hours of incubation. Cells were cultured in normal conditions and were ready to be assayed or harvested after 48 hours. To visualize β–galactosidase (LacZ) expression, cells were fixed with 4% PFA and incubated with X-gal staining solution (2 mM $MgCl_2$, 5 mM potassium ferrocyanide, and 5 mM potassium ferricyanide, 1mg/ml X-gal) overnight in 37°C.

Western blot. Cell culture and tissue lysates were obtained via motorized homogenization in 1% Triton X-100, 100 mM KCL, 20 mM HEPES, 2 mM EDTA pH 7.3, 1 mM EDTA and protease inhibitors (Roche). Cell culture and tissue lysates were obtained via motorized homogenization in 10mM Tris-HCL, 5 mM EDTA and protease inhibitors (Roche). Cell culture lysates were centrifuged at 4°C for 20 minutes at 42,000g. The supernatant was collected and protein concentration was measured with protein assay (Bio-rad). 20 μg of protein was subjected to 10% SDS-PAGE and transfer to PVDF membranes. For cell lysate samples the membrane was probed with rabbit anti-GLUT1 (Santa Cruz) overnight (1:500), and for tissue samples the membrane was probed with rabbit polyclonal anti-GLUT1 (GT-11A) from Alpha Diagnostic (1:200). Membranes were incubated with HRP-conjugated secondary anti-rabbit (Cell Signaling) for an hour (1:5000). β–tubulin protein expression was assessed with a rabbit polyclonal β–tubulin antibody from Cell Signalling (1:1000). Chemiluminescence detection was performed with ECL kits (Pierce) according to manufacture instruction. Blots were quantified using densitometry.

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Supplementary Figure 1. Measurements of Plasma Insulin and Glucagon Levels.

Table 1 – Plasma levels of Insulin (ηg/mL) during Pancreatic Clamps

	Start	Basal	Clamp
Normal (n = 20)	0.67 ± 0.14	0.73 ± 0.07	0.66 ± 0.07
STZ-Diabetic (n = 16)	0.18 ± 0.04***	0.13 ± 0.05***	0.73 ± 0.06
24 hr i.v. glucose (n = 14)	2.41 ± 0.05#	1.42 ± 0.22#	0.70 ± 0.06
24 hr i.h. glucose (n = 11)	0.64 ± 0.07	0.74 ± 0.09	0.68 ± 0.10
STZ + Phlorizin (n = 15)	0.19 ± 0.05***	0.19 ± 0.05***	0.73 ± 0.09

Data are means ± SEM.
Start: t=0 min (of clamp). Basal: average of t=60-90 min. Clamp: average of t=180-210 min.
'Normal' pools 1) Normal (n=12), 2) 24h i.v. (n=4) and 3) 24 i.h. (n=4) saline-infused rodents.
'STZ-Diabetic' pools 1) STZ-Diabetic (n=11) and 2) STZ+PG (n=5).
***$P < 0.001$ vs. Normal, #$P < 0.05$ vs. Normal (Student's *t* test).

Table 2 – Plasma levels of Glucagon (ρg/mL) during Pancreatic Clamps

	Start	Basal	Clamp
Normal (n = 20)	69.58 ± 7.70	66.60 ± 9.69	37.11 ± 5.95
STZ-Diabetic (n = 16)	68.19 ± 9.74	61.89 ± 5.33	42.13 ± 1.68
24 hr i.v. glucose (n = 14)	53.37 ± 6.30	56.35 ± 2.23	39.75 ± 1.53
24 hr i.h. glucose (n = 11)	60.09 ± 4.46	56.32 ± 2.53	41.75 ± 2.46
STZ + Phlorizin (n = 15)	68.41 ± 10.46	66.59 ± 9.69	36.73 ± 7.09

Data are means ± SEM.
Start: t=0 min (of clamp). Basal: average of t=60-90 min. Clamp: average of t=180-210 min.
'Normal' pools 1) Normal (n=12), 2) 24h i.v. (n=4) and 3) 24 i.h. (n=4) saline-infused rodents.
'STZ-Diabetic' pools 1) STZ-Diabetic (n=11) and 2) STZ+PG (n=5).
Start: t=0 min. Basal: average of t=60-90 min. Clamp: average of t=180-210 min.

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Supplementary Figure 2. Additional Measurements of Glucose Fluxes during Pancreatic Clamps. Shown are the average rates of whole-body glucose disposal (rate of disappearance, Rd) and the glucose infusion rate (GIR) required to maintain euglycemia during the final 30 minutes of the pancreatic clamp procedure. **P<0.01 vs. i.h. saline infused rats. *P<0.001 vs. Ad-GFAP-LacZ injected rats that have received either i.h. saline or 2 mM glucose infusion during the clamp.

	i.h. Infusion	Clamp Rd	GIR
Normal	Saline	12.6 ± 0.6	1.6 ± 0.4
	Glucose	11.0 ± 0.8	5.0 ± 1.0**
STZ -Diabetic	Saline	19.2 ± 2.5	0.4 ± 0.4
	Glucose	17.9 ± 2.9	0.6 ± 0.3
24 hr i.v. glucose	Saline	11.0 ± 0.9	1.1 ± 0.5
	Glucose	11.1 ± 0.8	1.8 ± 1.3
24 hr i.h. glucose	Saline	14.9 ± 1.2	1.2 ± 0.6
	Glucose	16.3 ± 1.2	2.2 ±1.0
STZ + Phlorizin	Saline	14.4 ± 1.1	4.5 ± 0.9
	Glucose	14.3 ± 1.1	8.3 ± 1.2**
24 hr i.h. glucose + Ad -GFAP -LacZ	Saline	11.6 ± 0.7	1.3 ± 0.3
	Glucose	10.9 ± 0.5	0.7 ± 0.2
24 hr i.h. glucose + Ad -GFAP -GLUT1	Glucose	11.1 ± 1.1	5.2 ± 0.2*

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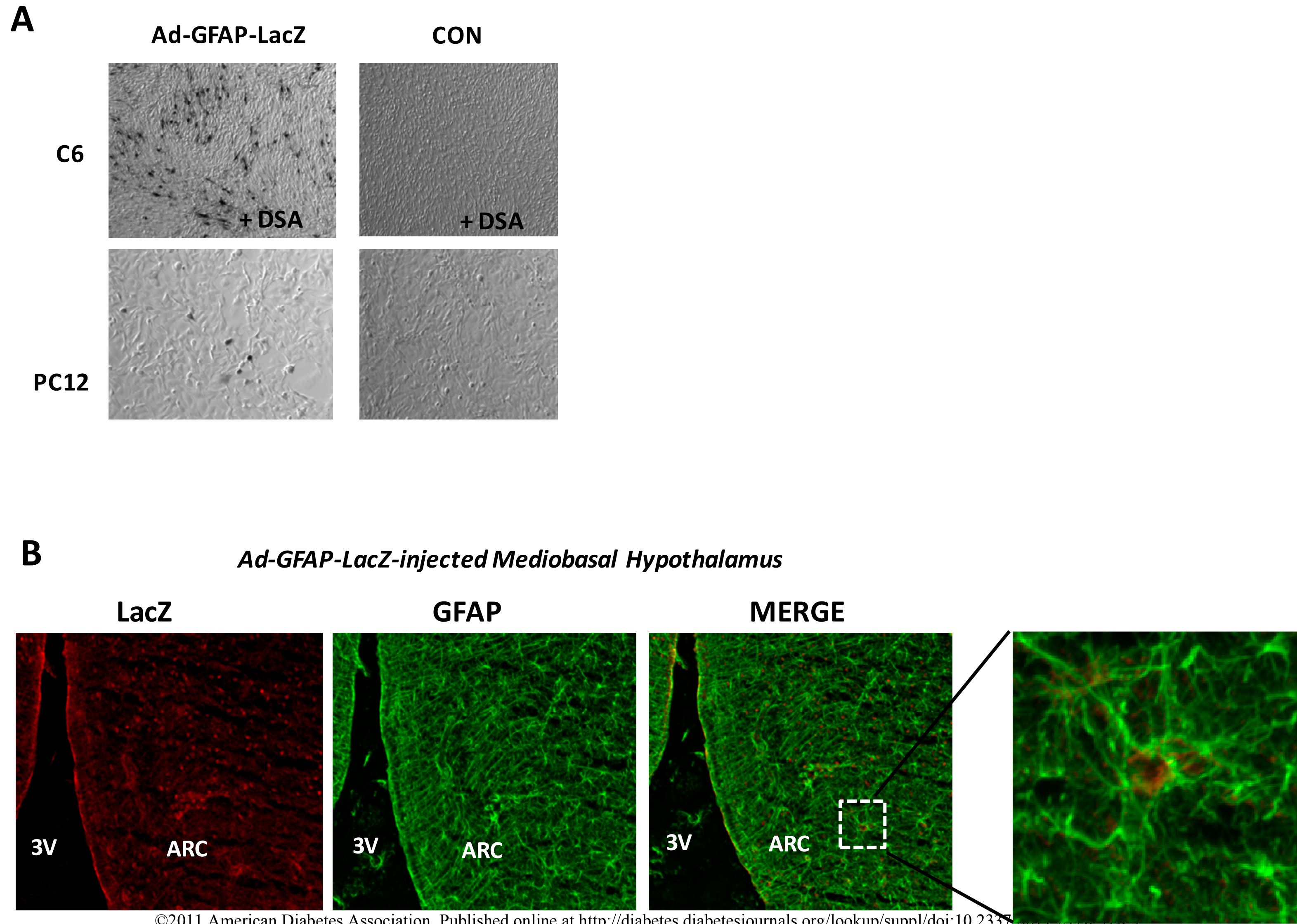
Supplementary Figure 3. The Specificity of the Glial Fibrillary Acidic Protein (GFAP) Promoter. (**A**) C6 and PC12 cells were transduced with adenovirus expressing LacZ driven by a GFAP promoter (Ad-GFAP-LacZ). In vitro β-galactosidase staining 48 hours post transduction showed that high level of LacZ activity was observed in C6 cells, whereas minimal activity was detected in PC12 cells. No adenovirus expression was detectable in the control (CON) cells. (**B**) Ad-GFAP-LacZ was injected into the mediobasal hypothalamus of the rats for 4 days. Co-immunostaining of LacZ with GFAP confirmed the selective distribution of the adenovirus in the arcuate nucleus (ARC) and the promoter specificity. Datura stramonium agglutinin (DSA).



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