SUPPLEMENTARY DATA

Hepatic Expression of Adenovirus 36 E4ORF1 Improves Glycemic Control and Promotes Glucose Metabolism via Akt Activation

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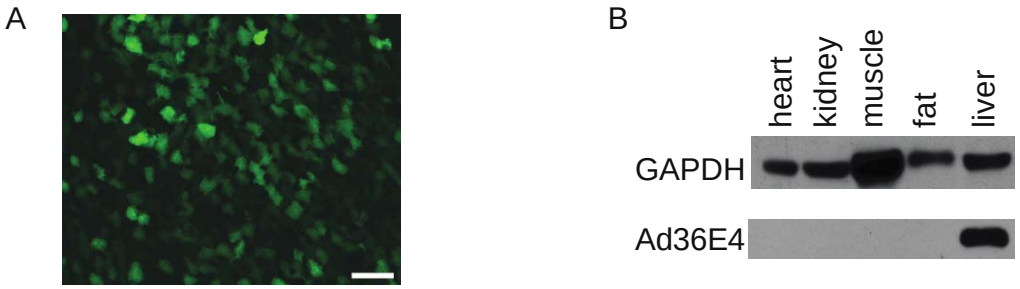
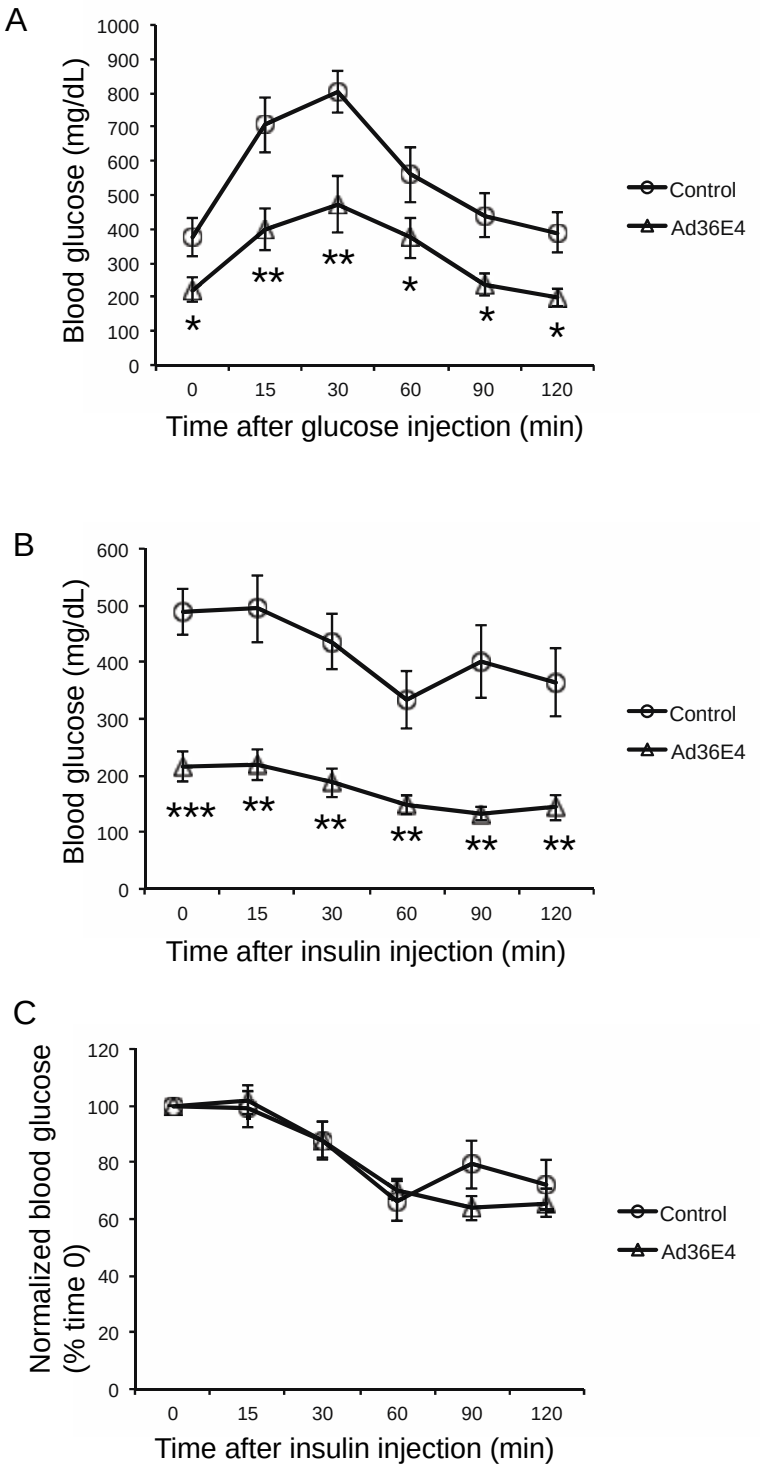
SUPPLEMENTARY DATA

Supplementary Figure S1. Pilot study of intravenous injection of rAAV to wild type mice. (A) GFP fluorescence in the liver of mice receiving rAAV-GFP. Scale bar: 100 μm. (B) Western blots of Ad36E4ORF1 in various tissues of a mouse receiving rAAV-Ad36E4ORF1.



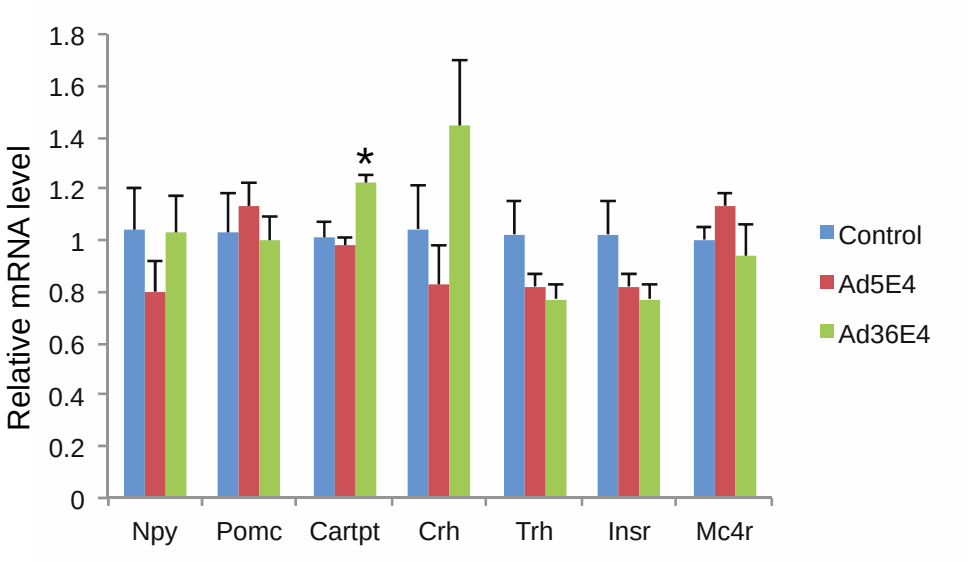
SUPPLEMENTARY DATA

Supplementary Figure S2. Ad36E4ORF1 gene transfer in *db*/*db*. (A) Glucose tolerance test. (B, C) Insulin tolerance test. n=8 per group. Data are means ± SEM. * P<0.05, ** P<0.01, *** P<0.001.



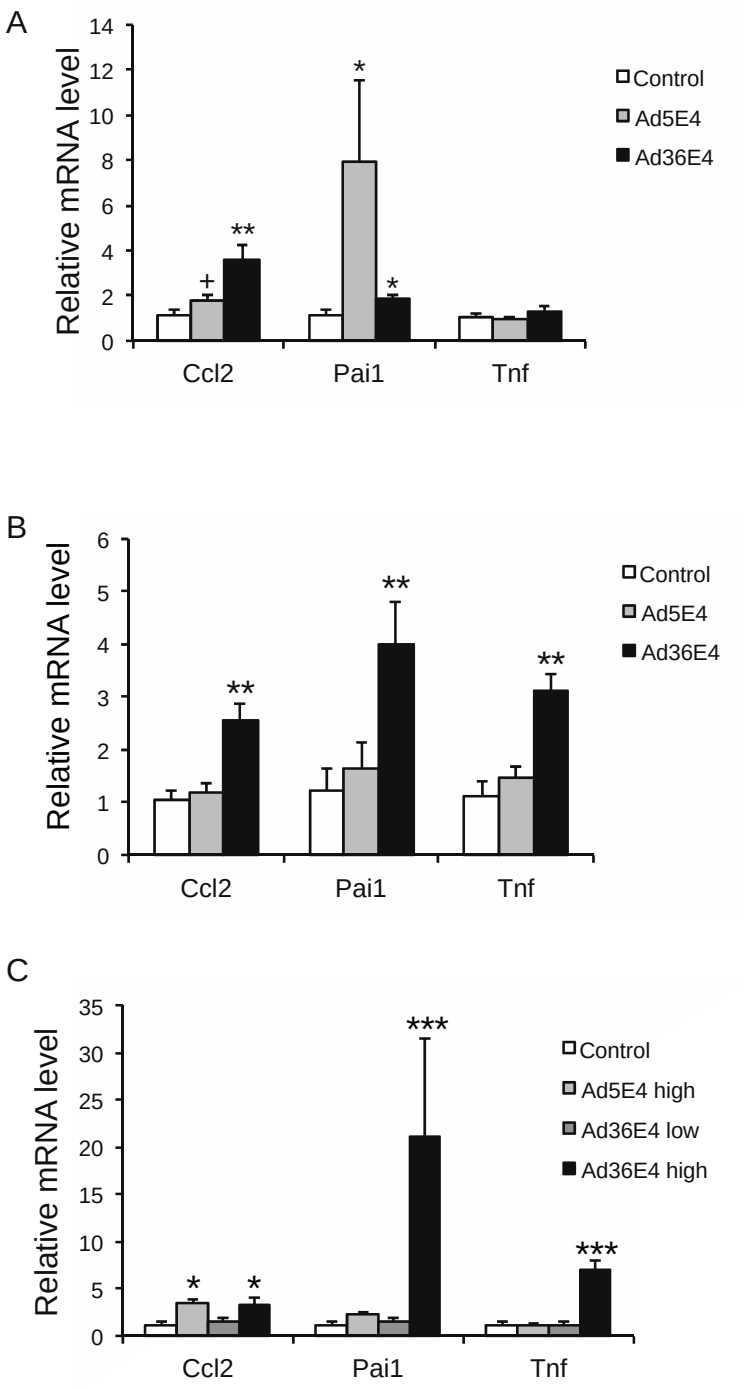
SUPPLEMENTARY DATA

Supplementary Figure S3. Gene expression of hypothalamus in Ad36E4ORF1 and Ad5E4ORF1 treated *db*/*db* mice. n=4 per group. * *P*<0.05 compared to YFP control. Npy, neuropeptide Y; Pomc, proopiomelanocortin; Cartpt, cocaine-amphetamine-regulated transcript; Crh, corticotropin-releasing hormone; Trh, thyrotropin-releasing hormone; Insr, insulin receptor; Mc4r, melanocortin-4 receptor



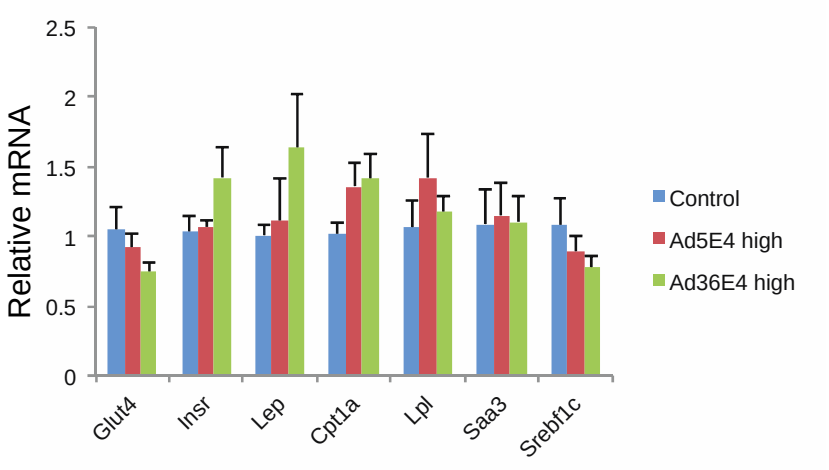
SUPPLEMENTARY DATA

Supplementary Figure S4. Inflammatory gene expression of liver in Ad36E4ORF1 and Ad5E4ORF1 treated *db*/*db*, DIO, and normal wild type mice. (A) *db*/*db* mice. n=8 Control, n=5 Ad5E4ORF1, n=5 Ad36E4ORF1. (B) DIO mice. n=5 per group. (C) Normal wild type mice. n=5 per group. Low: $2x10^9$, high: $2x10^{10}$ vg per mouse. Data are means ± SEM. * P<0.05, ** P<0.01, *** P<0.001 compared to YFP control.



SUPPLEMENTARY DATA

Supplementary Figure S5. Gene expression of abdominal fat depot in Ad36E4ORF1 and Ad5E4ORF1 treated wild type mice. n=5 per group. Data are means ± SEM. high: $2x10^{10}$ vg per mouse. Glut4, glucose transporter type 4; Insr, insulin receptor; Lep, leptin; Cpt1a, carnitine palmitoyltransferase 1A; Lpl, lipoprotein lipase; Saa3, serum amyloid A3; Srebf1c, sterol regulatory element-binding protein.



SUPPLEMENTARY DATA

Supplementary Table S1. Liver function panel of *db*/*db* mice

	ALB (g/dL)	ALP (U/L)	ALT (U/L)	AST (U/L)	DBILI (mg/dL)	GGT (U/L)	GLOB (U/L)	TBILI (mg/dL)	TPROT (g/dL)
Control	3.45±0.09	193.86±10.26	205.88±41.20	230.25±23.66	0.26±0.04	3.13±0.44	2.09±0.06	0.29±0.04	5.54±0.11
Ad5E4	3.35±0.10	238.38±13.03*	244.75±49.44	383.75±87.35	0.24±0.02	4.5±0.65	2.25±0.06	0.24±0.02	5.6±0.10
Ad36E4	3.56±0.06	326.88±13.20*	289.50±23.51	295.25±22.12	0.28±0.05	3.75±0.53	2.48±0.08*	0.31±0.04	6.04±0.13*

Data are means ± SEM. n=8 per groups, * $P<0.05$ compared to Control.

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Supplementary Table S2. Quantification of western blot of *db*/*db* mice

	pAKT S473	pAKT T308	Ras	rictor	Myc	pFoxo1	pIR	pIRS2	Chrebp	Srebp1
Control	1±0.08	1±0.26	1±0.04	1±0.21	1±0.01	1±0.11	1±0.19	1±0.10	1±0.13	1±0.32
Ad5E4	0.74±0.08	0.97±0.60	1.34±0.09*	0.77±0.05	0.99±0.09	0.68±0.09	0.92±0.13	1.24±0.62	0.53±0.04	0.33±0.10
Ad36E4	1.51±0.05**	3.38±0.81*	1.16±0.14	0.71±0.20	0.95±0.03	0.66±0.17	0.69±0.08	0.80±0.08	0.60±0.04	0.35±0.12

Data are means ± SEM. n=3 per groups, * $P<0.05$, ** $P<0.01$ compared to Control. Phospho-proteins are calibrated to the respective total protein.

SUPPLEMENTARY DATA

Supplementary Table S3. Quantification of western blot of DIO mice

	pAKT S473	pAKT T308	Ras	rictor	pFoxo1	pIR	Chrebp	Srebp1
Control	1±0.38	1±0.30	1±0.28	1±0.13	1±0.09	1±0.30	1±0.19	1±0.11
Ad5E4	0.42±0.23	0.40±0.19	1.17±0.23	0.74±0.19	1.16±0.12	1.38±0.12	0.89±0.11	0.58±0.07*
Ad36E4	1.59±0.13*	2.88±0.45*	0.64±0.06	0.54±0.06*	0.75±0.14	1.05±0.21	0.52±0.03	0.70±0.11

Data are means ± SEM. n=3 per groups, * $P<0.05$, ** $P<0.01$ compared to Control. Phospho-proteins are calibrated to the respective total protein. Quantification by Image J or Image Studio. Some blots had high background and were unable to be processed by the software.

SUPPLEMENTARY DATA

Supplementary Table S4. Quantification of western blot of normal WT mice

	pAKT S473	pAKT T308	Ras	rictor	pFoxo1	Chrebp	Srebp1
Control	1±0.12	1±0.33	1±0.10	1±0.15	1±0.15	1±0.14	1±0.10
Ad5E4	1.99±0.75	3.05±0.92	0.74±0.10	1.54±0.06*	1.27±0.11*	1.06±0.21	1.21±0.34
Ad36E4	2.48±0.14***	5.72±1.15*	0.85±0.16	1.11±0.08	2.28±0.26*	1.49±0.09*	1.47±0.08*

Data are means ± SEM. n=3-5 per groups, * $P<0.05$, *** $P<0.001$ compared to Control. Phospho-proteins are calibrated to the respective total protein. Quantification by Image J or Image Studio.