

Supplementary Information for:

Cross-talk between hypoxia and insulin signaling via *Phd3* regulates hepatic glucose and lipid metabolism and ameliorates diabetes

Running Title: A novel Phd3/Hif2/Irs2 axis regulates hepatic metabolism

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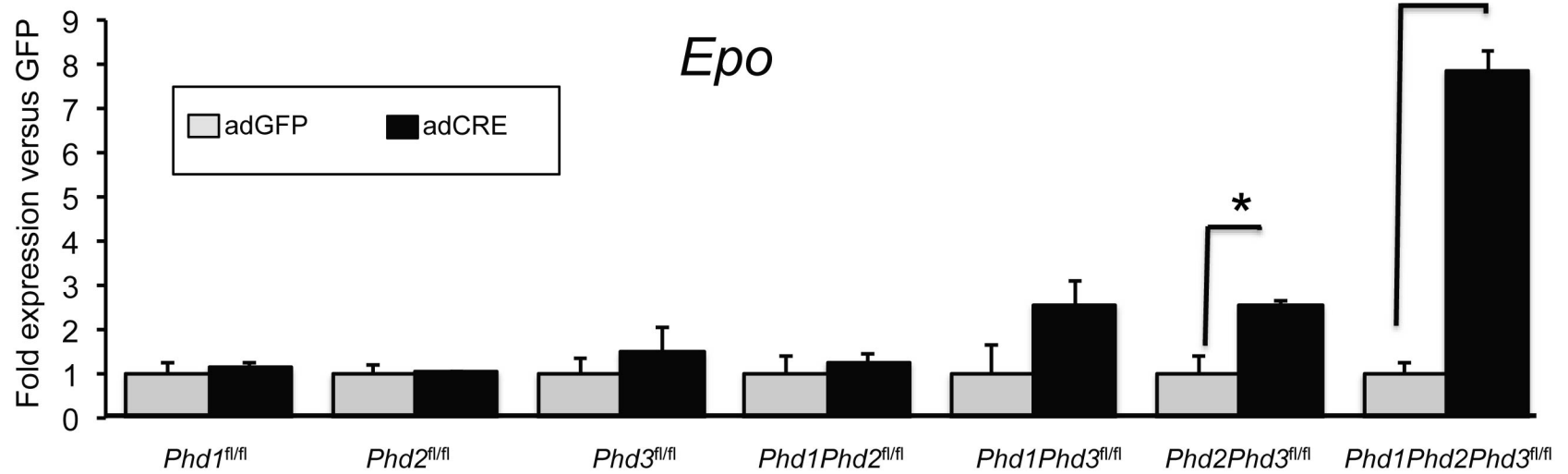
Supplementary Information Titles

Journal: Nature Medicine

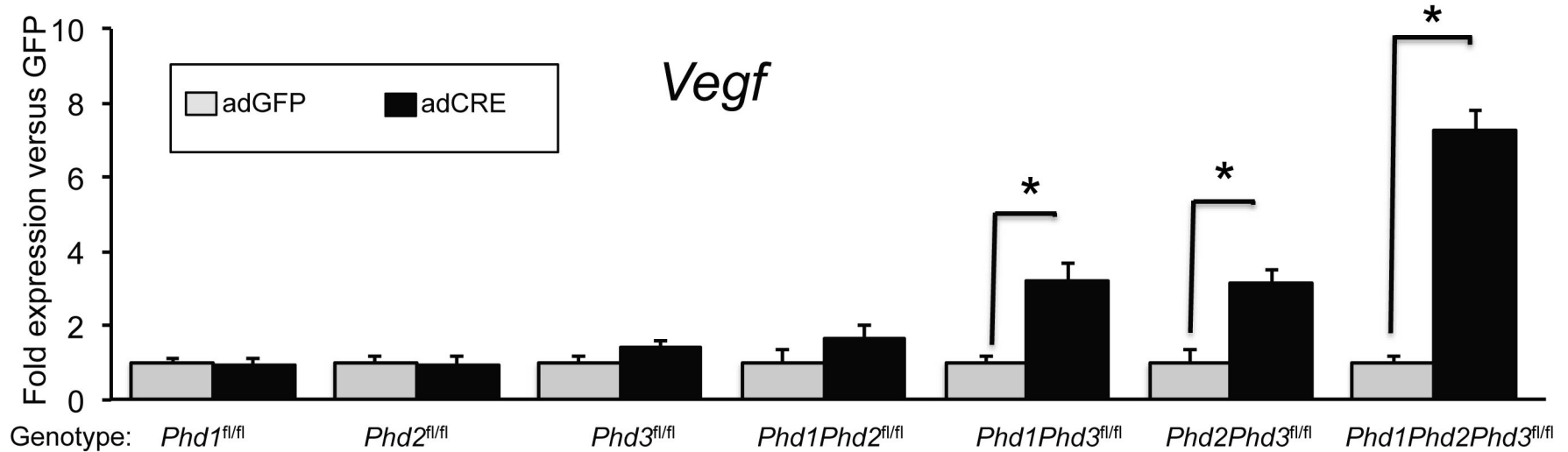
Article Title:	Cross-talk between hypoxia and insulin signaling via <i>Phd3</i> regulates hepatic glucose and lipid metabolism and ameliorates diabetes
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Supplementary Item & Number (add rows as necessary)	Title or Caption
Supplementary Figure 1	<i>Epo</i> and <i>Vegf</i> Expression from <i>Phd</i> knockout livers
Supplementary Figure 2	Chronic blood glucose and AUC measurements from <i>Phd</i> knockout mice
Supplementary Figure 3	Metabolic Measurements from <i>Phd1^{fl/fl}</i> <i>Phd2^{fl/fl}</i> and <i>Phd1Phd2^{fl/fl}</i> mice
Supplementary Figure 4	Gluconeogenic and lipogenic gene expression from <i>Phd1^{fl/fl}</i> <i>Phd2^{fl/fl}</i> and <i>Phd1Phd2^{fl/fl}</i> mice
Supplementary Figure 5	Characterization of toxicity from <i>Phd</i> knockout mice
Supplementary Figure 6	Histology from livers of various <i>Phd</i> knockout animals
Supplementary Figure 7	Human and mouse IRS2 promoters and constructs
Supplementary Figure 8	Body weight, blood glucose and insulin from HFD experiments and multiple AUC calculations.

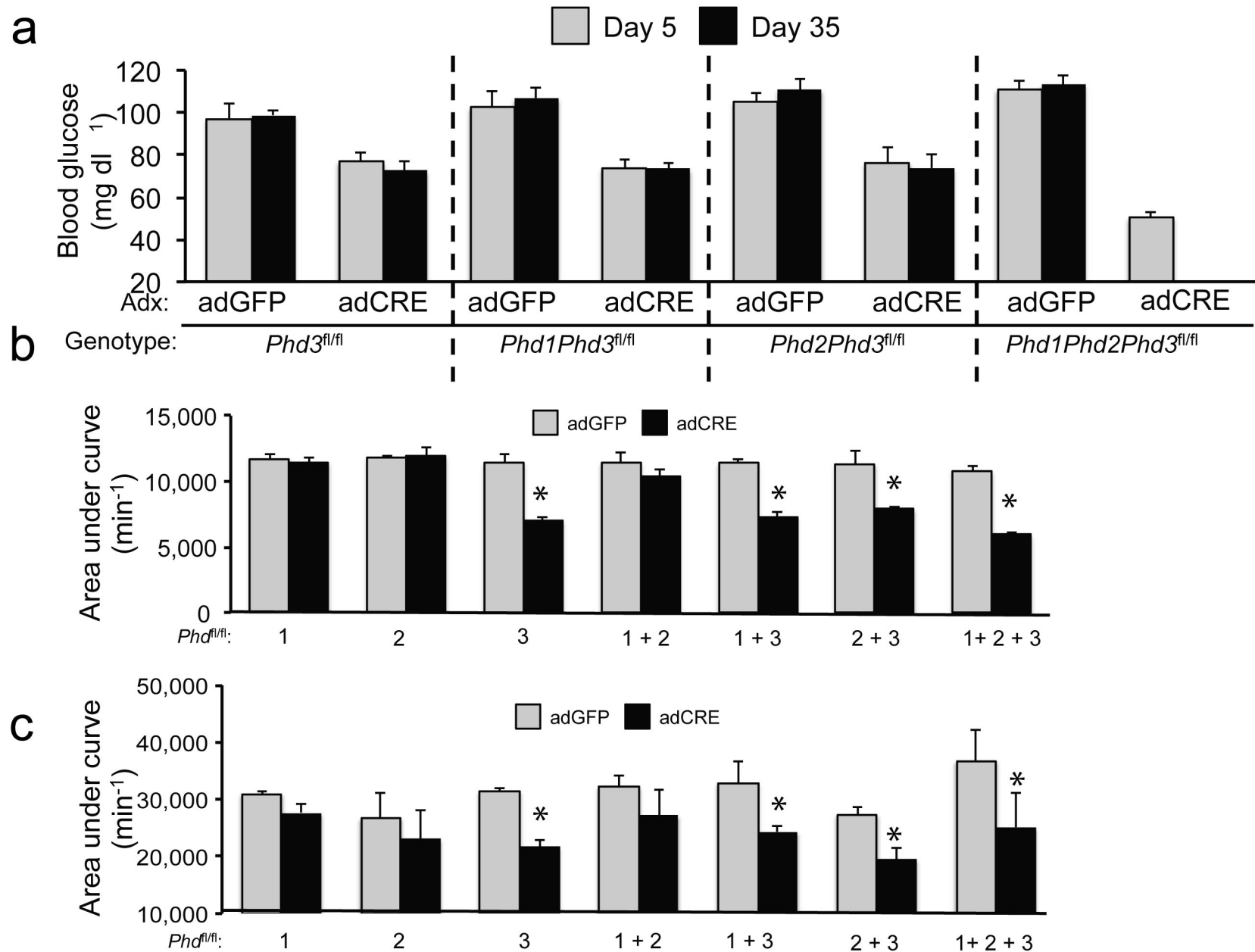
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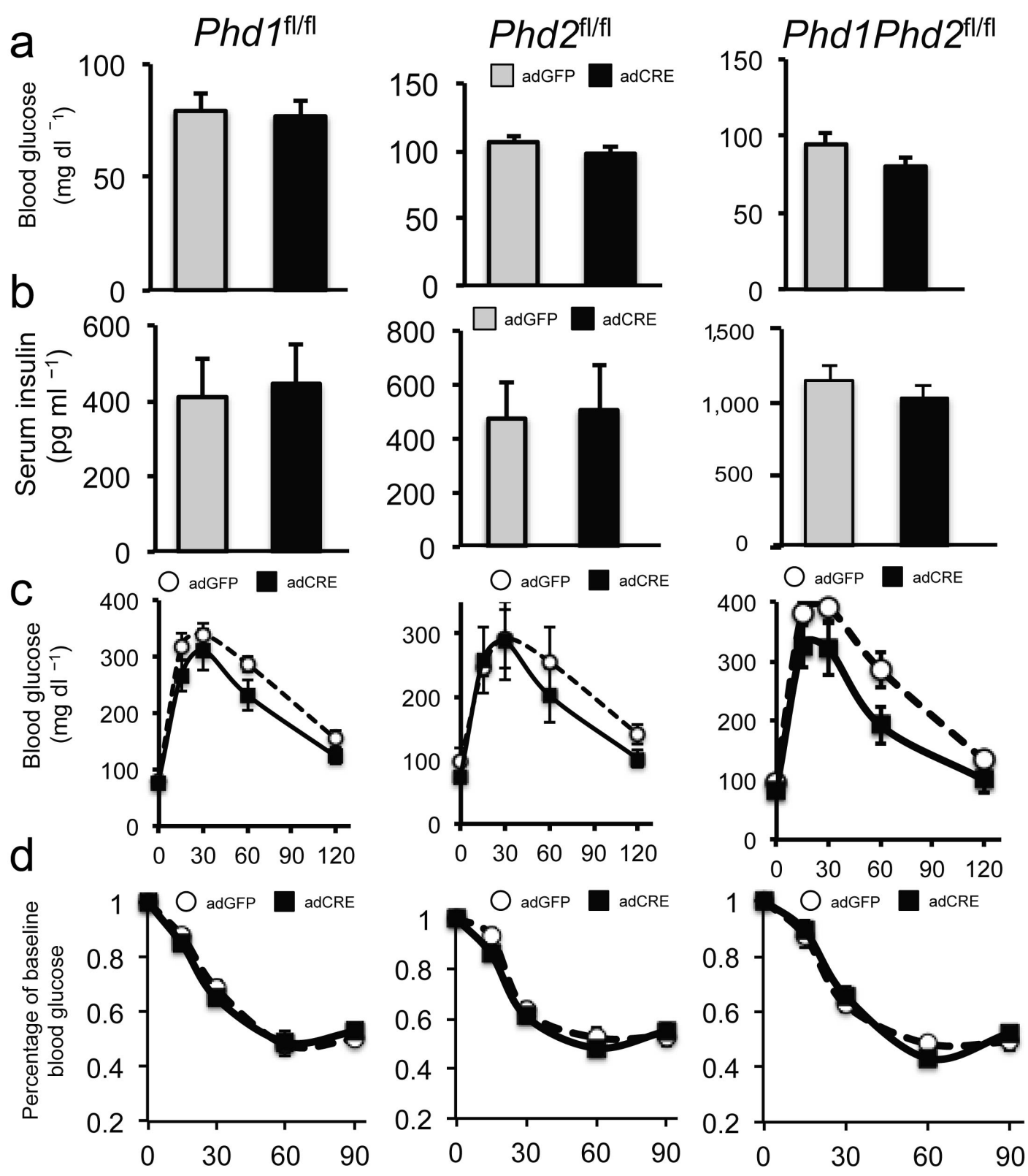
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Supplementary Figure 1. mRNA levels of (a) erythropoietin (*Epo*) and (b) vascular endothelial growth factor (*Vegf*) in total RNA isolated from livers of mice of the following genotypes and viral treatments. Mice are only compared to their littermate controls of the same genotype to account for background effects. Data are expressed as mean \pm SEM (n=8/group). *p<0.05

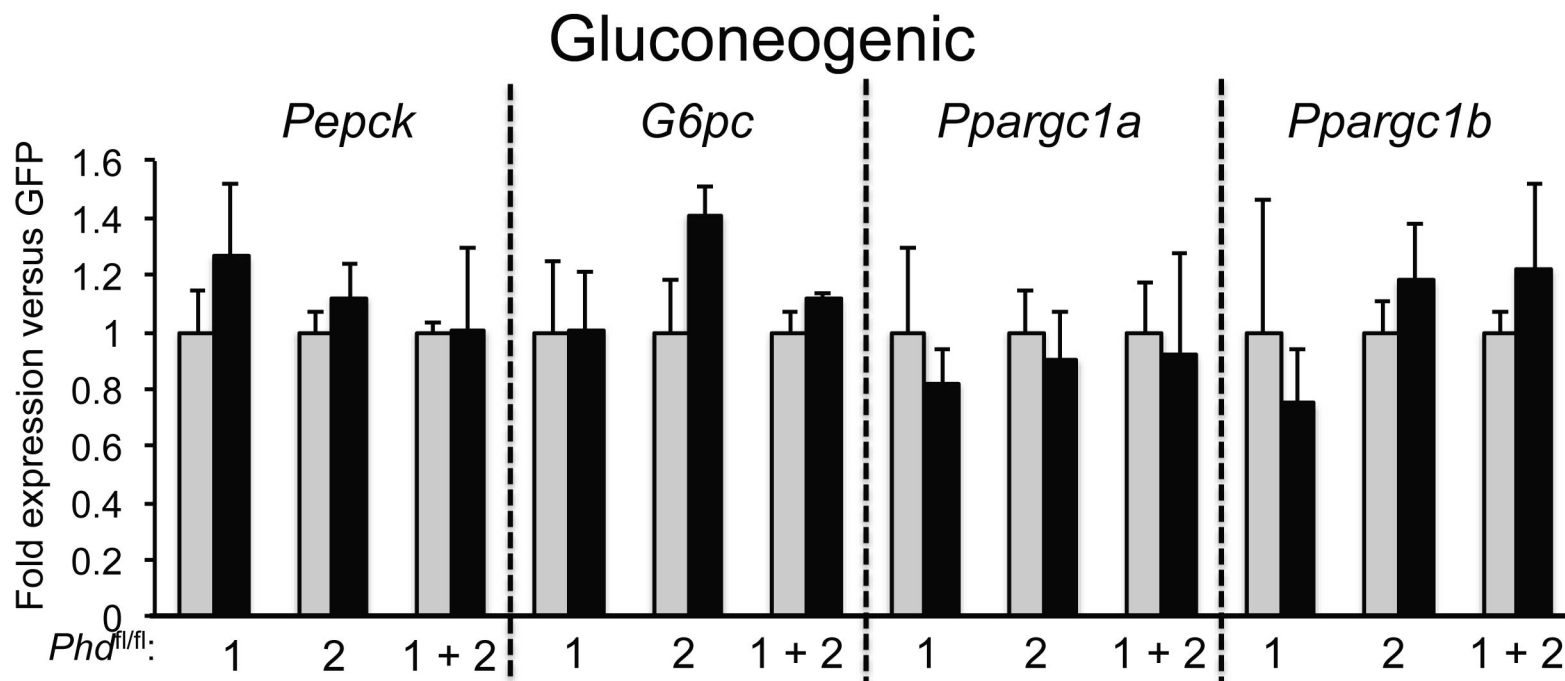


Supplementary Figure 2. (a) Fasting blood glucose at 5 and 35 days after adenoviral injection for each genotype and treatment. Note there is no value for *L-Phd1Phd2Phd3^{fl/fl}* mice after 35 days since they die uniformly within 2 weeks after knockout. Area under the curve (AUC) calculations for (b) glucose tolerance tests and (c) insulin tolerance tests of the indicated genotypes and adenoviral treatments, Data are expressed as mean±SEM (n=6 male mice per group). *p<0.05

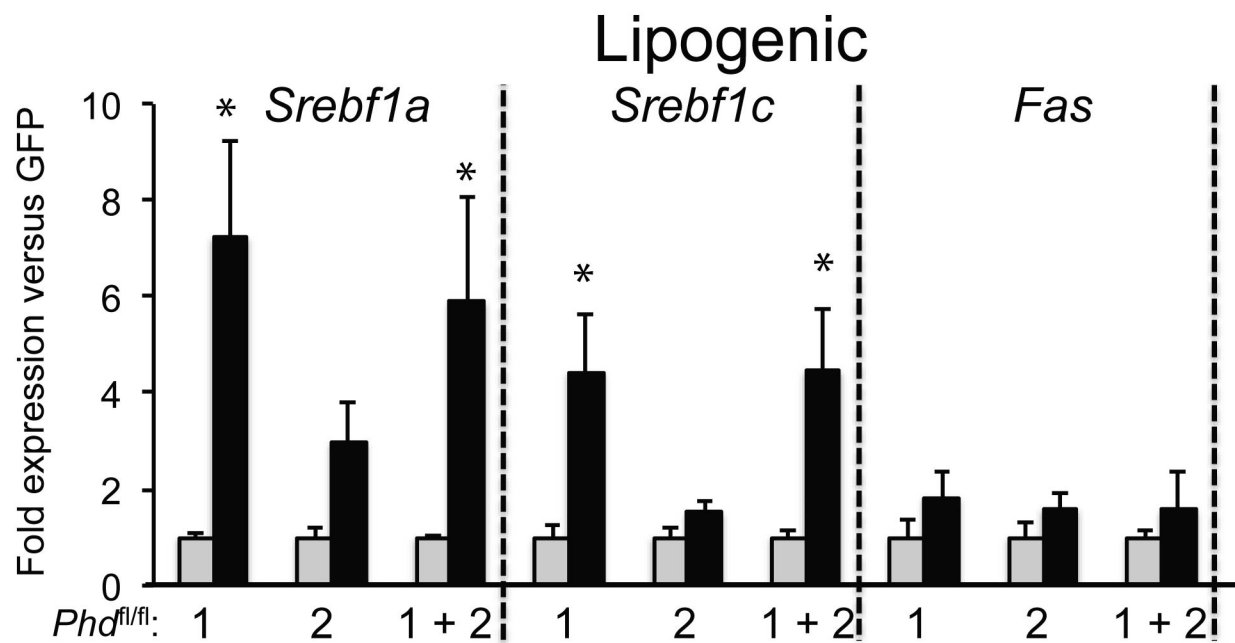


Supplementary Figure 3. (a) Fasting blood glucose **(b)** fasting insulin levels and **(c)** glucose tolerance tests (2 g/kg, intraperitoneally) were performed on *Phd1*^{fl/fl}, *Phd2*^{fl/fl} or *Phd1Phd2*^{fl/fl} mice following a 16h fast five days after adenoviral injection. Blood samples were collected and glucose measured at the times indicated. **(d)** Insulin tolerance tests (ITT). Open circles, dashed black line (○)—adad GFP, closed squares, solid black line (■)—Cre, Data are expressed as mean ± SEM (n=8 male mice per group).

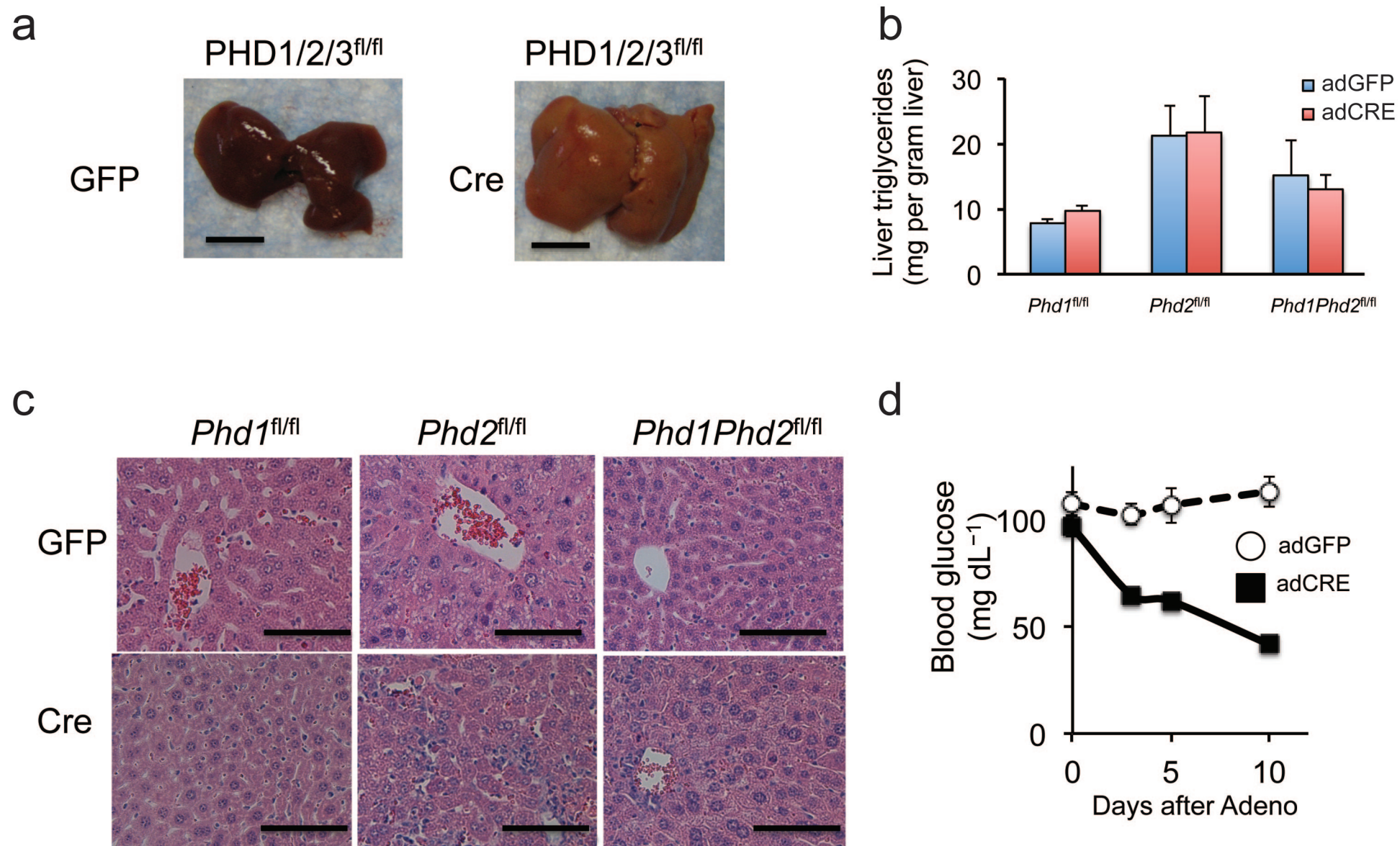
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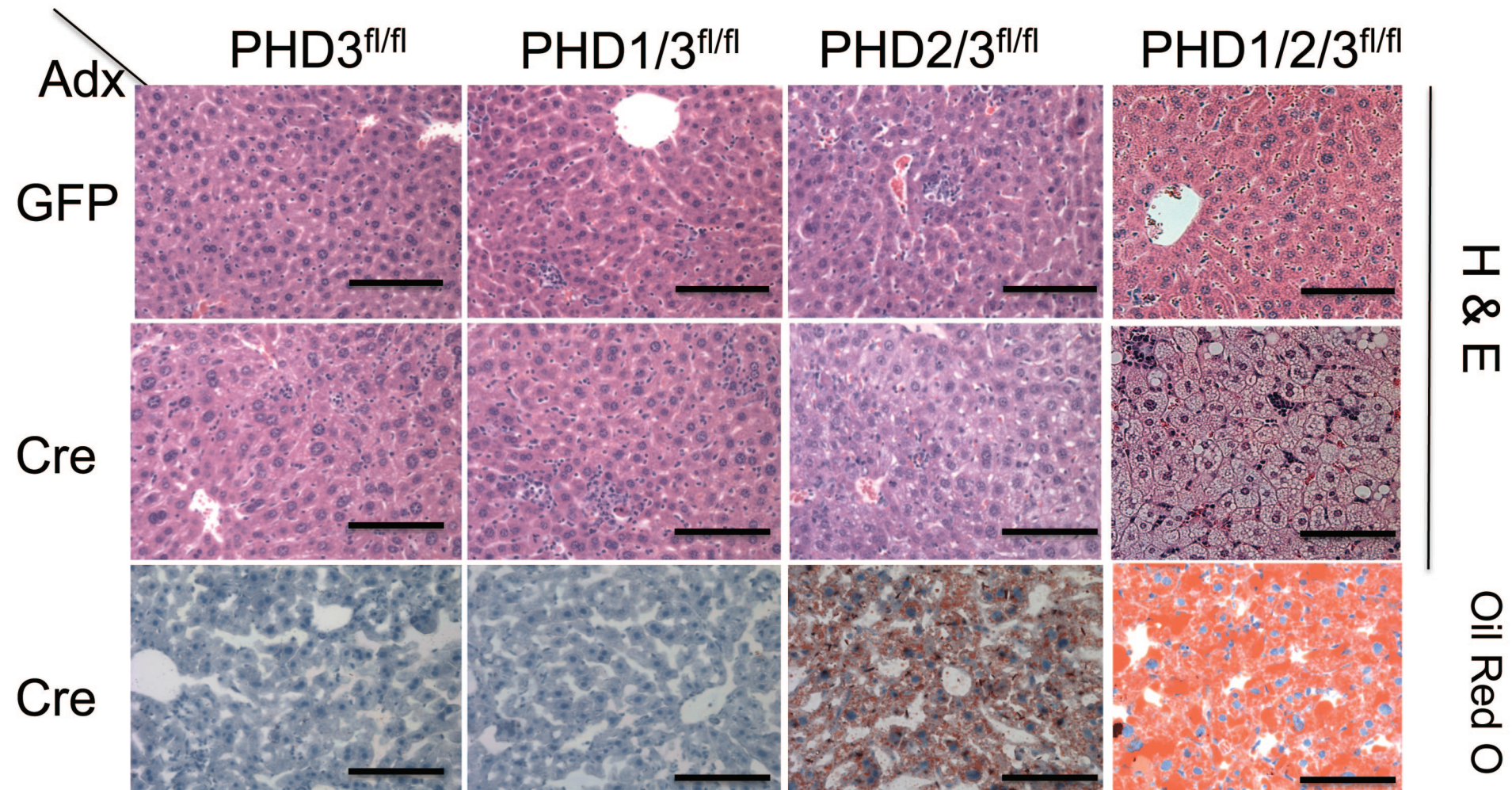
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Supplementary Figure 4 (a) gluconeogenic and (b) lipogenic gene expression of indicated genes in the livers of mice of the indicated genotypes. Data are expressed as mean \pm SEM (n=8 male mice per group). *p<0.05



Supplementary Figure 5. (a) whole livers of PHD1/2/3^{fl/fl} mice treated with indicated adenovirus. Scale bars= 10mm (b) Liver triglycerides of the indicated PHD mice treated with adGFP or AdCre (c) H&E analysis of the indicated genotypes. Scale bar = 100 μ m (d) Time course of fed blood glucose in L-Phd1Phd2Phd3^{fl/fl} treated with either adGFP or adCRE



Supplementary Figure 6. H&E and Oil RedO of indicated genotypes and treatments. Scale bar= 100 μ m

a

Consensus HRE: [a/g]cgtg **HRE**

WT SEQ: -906 GCGCGCCGTGGCCCCCGCCGCCCC
 -900 HRE MUTANT -906 GCGCGCGCTCGCCCCCGCCGCCCC

HRE

WT SEQ: -136 GGGCCCCGAGGGACGCGTGATCGGGCGGGCG
 -123 HRE MUTANT -136 GGGCCCCGAGGGACCGCTCATCGGGCGGGCG

SRE Ebox IRE

WT SEQ: -606 TGC GTAACGCCGAGT CACATGTTGTTTTGC
 E BOX MUTANT: -606 TGC GTAACGCCGAGT CGCATGTTGTTTTGC

SRE Ebox IRE

WT SEQ: -606 TGC GTAACGCCGAGT CACATGTTGTTTTGC
 IRE MUTANT: -606 TGC GTAACGCCGAGT CACATGTAGATCTGC

b

mm_IRS2 -CCCTTGAAGTCTGCTGCTTTGAATTTCTCTATCTACGTGGCCTTTGTGACTTTCAAAGTCG
 hs_IRS2 -CCCTTGAGAACTGCTTCGAATTCATGTATCTACTGGGCTTTTGTGACTTTCAAAGCCT
 rn_IRS2 GCTAGCGCGCCCCTGCCTGGGCCCCCCCGCGTCCGCGGG----CGGGGACGGCCCCGAACCT
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mHRE1

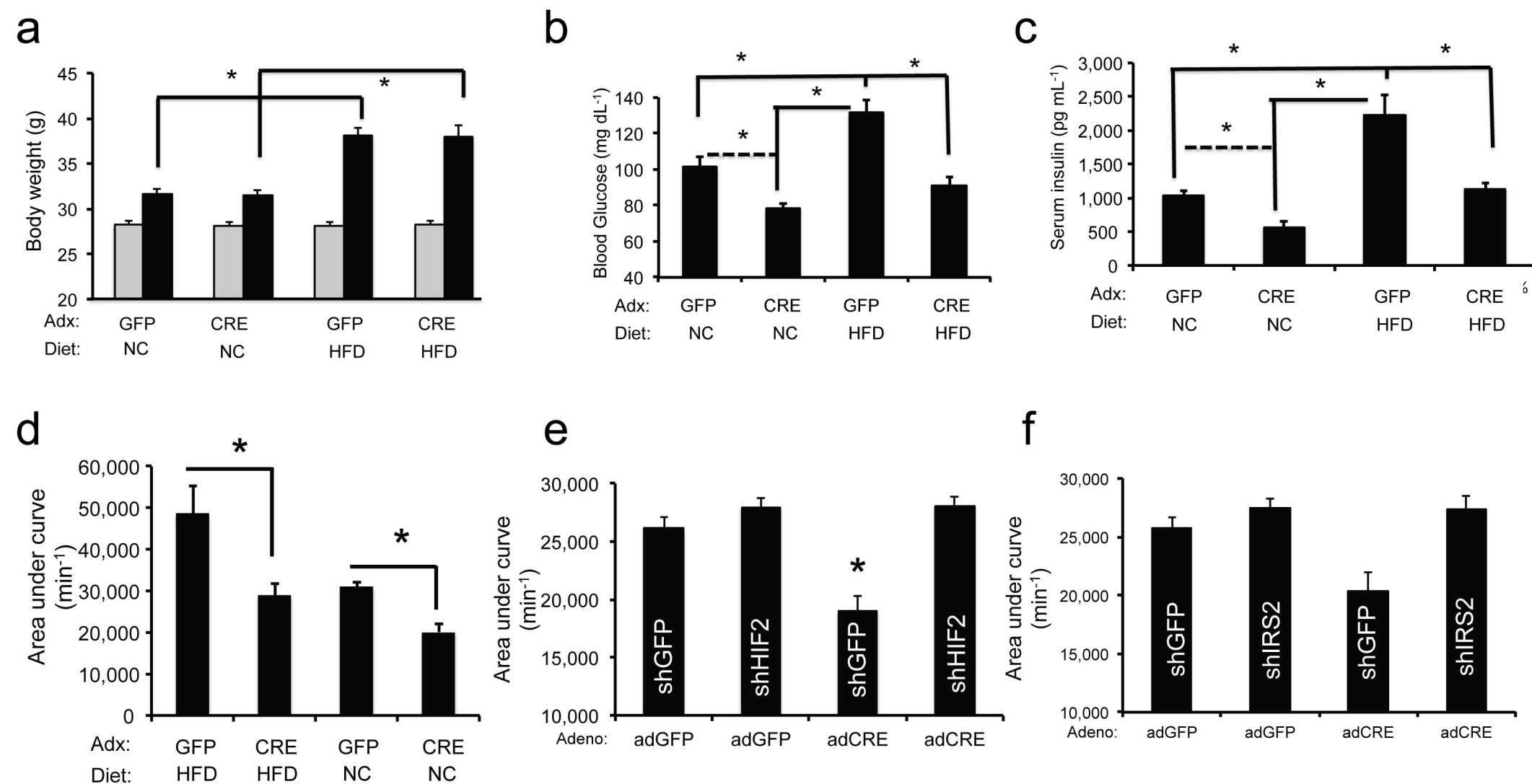
mm_IRS2 TCCCCGACGGACAGCGAGACGGACCAATGAGCGTGAGGCACGGGGGGGGGGGGAGGCGG
 hs_IRS2 CCCCCGACGGACGGCGCGCTCGGCCAATCGGCGCGGCGCTCGCGGGGGCGGGCCGCGCGC
 rn_IRS2 TCCGCGGCGGA-AGCCTG--GGCCAGTTAG--TAGGGCGCGAGGCGGGAGCACAAAC--
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mHRE2

E-Box

mm_IRS2 GAGTAACACATCGCGCACCGAGTGACTGAACTAAGA---GCAAACAACACGTTGACTCTG
 hs_IRS2 GAGTAACACATCGCGCACCGAGTGACTGAACTAAGAAGAGCAAACAACATGTGACTCGG
 rn_IRS2 GGGCACCGC--CTGCGCCGCGCGCCC-ACCCAGA--TGC-----TGGGTCCGA
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Supplementary Figure 7. a) Human IRS2 promoter mutants used for this study b) HRE1 and HRE2 are not conserved between humans and mice, but E-box sequence is.



Supplementary Figure 8. (a) Body weight at 0 weeks (grey bars, at ~10wks of age) compared to after 6 weeks of a of HF or NC diet (black bars, ~16wks of age). (b) fasting blood glucose and (c) serum insulin levels of the following genotypes and viral treatments while on normal chow (NC) or high-fat diet (HFD). Data are expressed as mean±SEM (n=8 male mice per group). *p<0.05 (d) Area under the curve calculations for glucose tolerance tests of the indicated HFD and NC diets and adenoviral treatments (as in Figure 3e). (e,f) Area under the curve calculations for glucose tolerance tests of the indicated adenovirus treatments. Data are expressed as mean±SEM (n=8 male mice per group). *p<0.05