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

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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±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-Phd1Phd2Phd3f/f 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).
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±SEM (n=8 male mice per group). *p<0.05
Supplementary Figure 5. (a) whole livers of PHD1/2/3fl/ff 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 = 100μm  (d) Time course of fed blood glucose in L-Phd1Phd2Phd3fl/ff treated with either adGFP or adCRE
Supplementary Figure 6. H&E and Oil RedO of indicated genotypes and treatments. Scale bar= 100μm
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, ~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