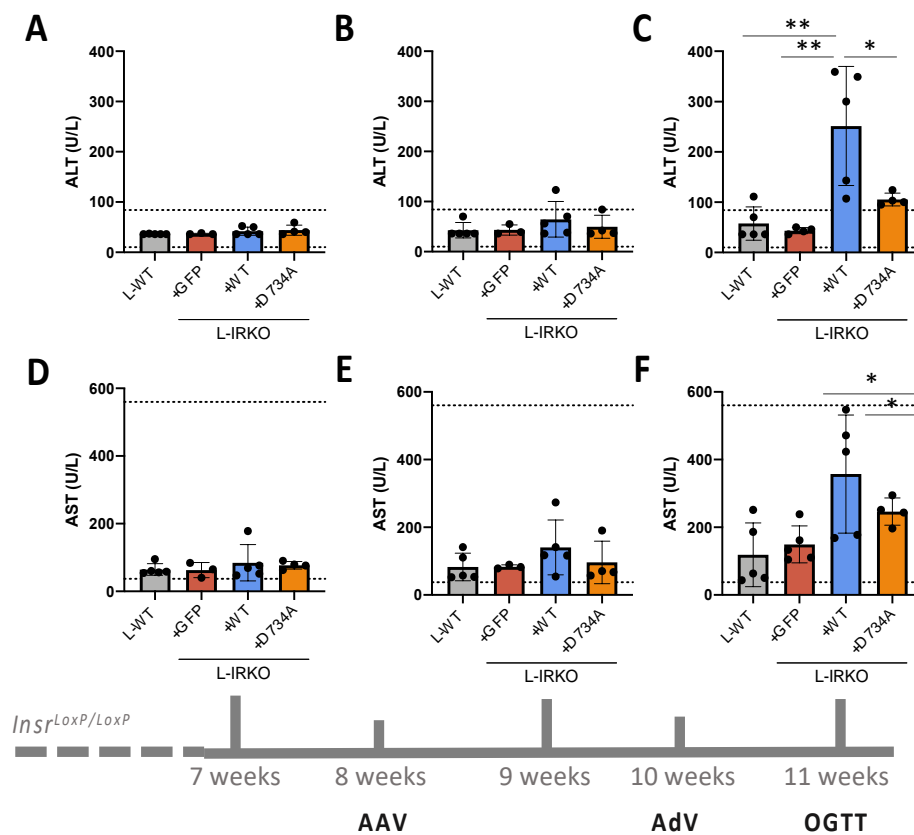# Online Appendix

Anti-Insulin receptor antibodies improve hyperglycemia in a mouse model of human insulin receptoropathy

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Supplementary Figure 1. Plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured before and after virus administration. ALT levels (**A - C**) in the L-IRKO+WT mice at week 11 were 3-fold higher than the reference values for C57BL/6J mice[1] (dotted lines) indicating mild liver inflammation to a degree that would be clinically insignificant. Plasma AST levels were elevated post-AAV and AdV administration but fell within the reference range (dotted lines) for C57BL/6J mice[1] (**D - E**).

Supplementary Reference:

1. *Blood chemistry and hematology in 8 inbred strains of mice.* MPD: Eumorphia1. Mouse Phenome Database web resource (RRID:SCR_003212), The Jackson Laboratory, Bar Harbor, Maine USA. https://phenome.jax.org [Accessed 27/5/20]).
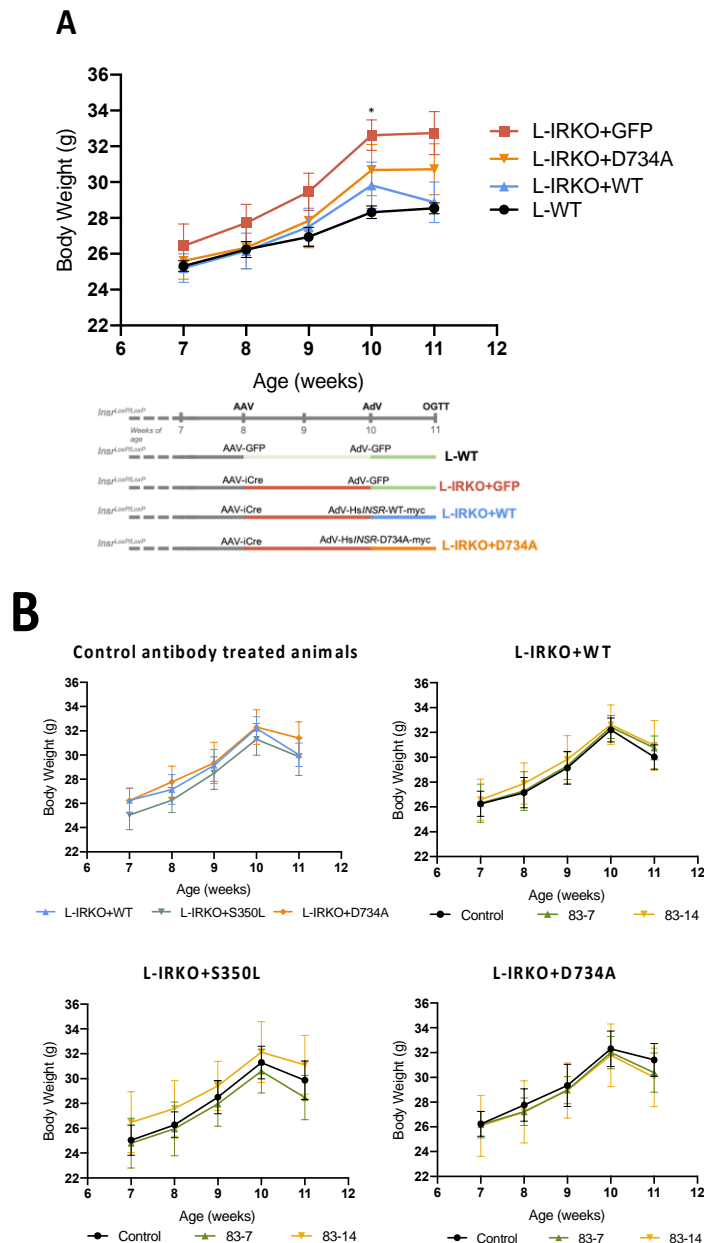
Supplementary Figure 2. Bodyweights of mice were measured throughout the study. (**A**) Body weight data (mean ± SD) for animals reported in Figure 1. Body weight significantly differed (p<0.05) at week 10 between L-IRKO+GFP and L-WT animals only (Two-way ANOVA, Tukey's multiple comparison test). This time point is two weeks post knockout of the *Insr* in the liver of the L-IRKO+GFP animals. Animals were randomly assigned into groups prior to virus administration. Animals exist as L-IRKO mice from 8 weeks of age and become human WT or mutant INSR expressing mice between weeks 10 and 11 of age. No difference in body weight was observed between WT or mutant INSR expressing mice treated with control antibody (**B**). Mice (n = 5-10 per condition, as detailed in the manuscript) all lost some weight over the last week of the protocol, most likely attributable to the increase in procedures over that week (despite extensive handling and habituation).
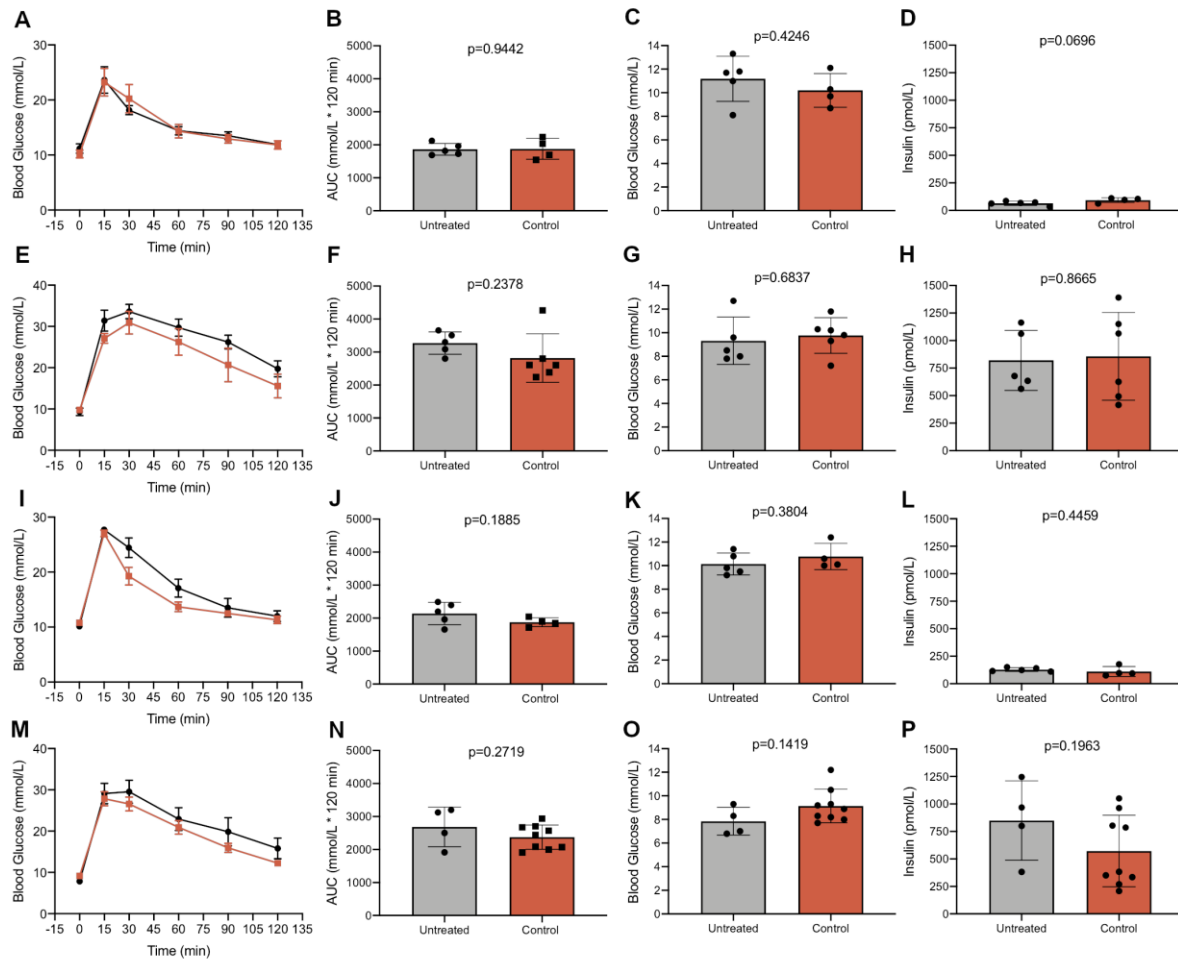
Supplementary Figure 3. **Control antibody has no metabolic effect in mice with acute liver insulin receptoropathy** (**A - D**) L-WT mice, (**E – H**) L-IRKO+GFP mice, (**I – L**) L-IRKO+WT mice, (**M – P**) L-IRKO+D734A mice. Results of OGTT (2g/kg glucose) after 5 h fasting (**A, E, I, M**) and OGTT areas under the curve (**B, F, J, N**). (**C, G, K, O**) Blood glucose and (**D, H, L, P**) insulin concentrations in mice after 5 h fasting. L-WT mice = AAV-GFP/AdV-GFP (i.e. GFP control only), L-IRKO+GFP mice = AAV-iCre/AdV-GFP (i.e. liver *Insr* knockout only), L-IRKO + WT = AAV-iCre/AdV-Hs*INSR*-WT-myc (i.e. L-IRKO with WT INSR add back), L-IRKO + D734A = AAV-iCre/AdV-Hs*INSR*-D734A-myc (i.e. L-IRKO with D734A INSR add back). Data in **A, E, I, M** are shown are means ± SEM, with statistical significance of difference from L-IRKO+GFP tested by two-way repeated measures ANOVA with Sidak's multiple comparisons test. All other data are shown as mean ± SD, with statistical significance determined by unpaired two-tailed t-test.
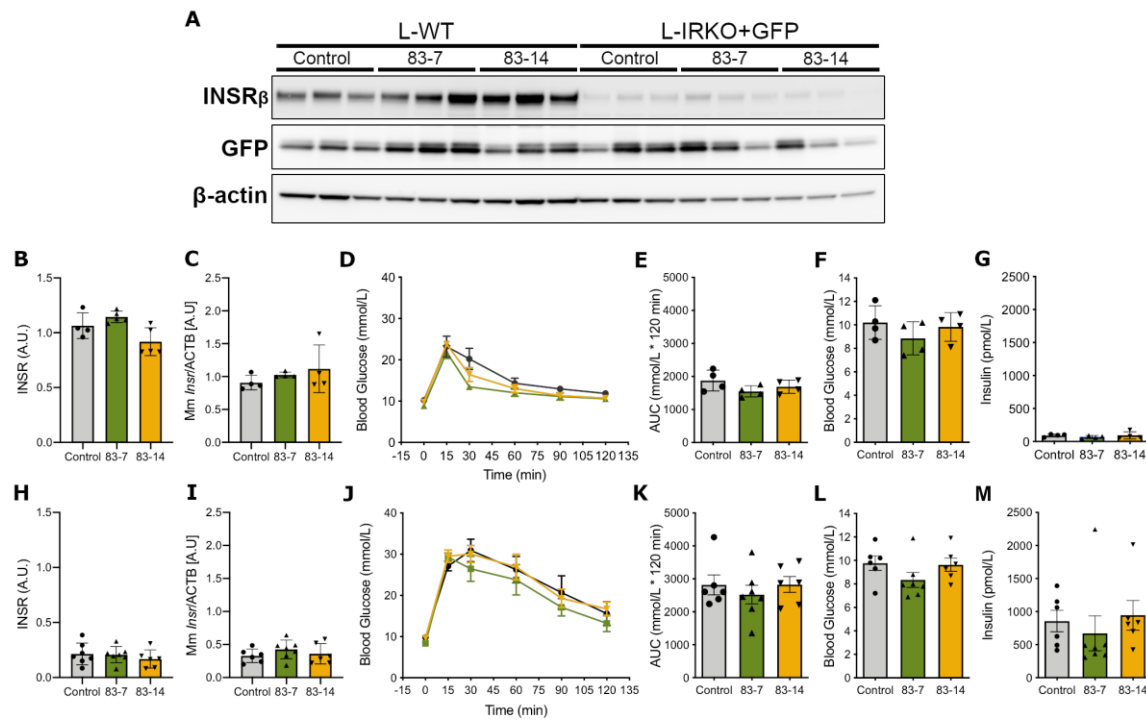
Supplementary Figure 4. Antibody treatment has no effect in mice not expressing human INSR. L-WT (**B** - **G**) and L-IRKO+GFP (**H**- **M**) mice were treated twice over the course of a week with 10mg/kg control or anti-INSR antibodies 83-7 or 83-14 as indicated. (**A**) Representative Western blot of lysates from livers harvested from L-WT and L-IRKO+GFP mice at the completion of oral glucose tolerance test (OGTT) and probed for specific proteins as indicated. INSR protein expression (**B**, **H**) and *Insr* gene expression (**C**, **I**) in antibody treated L-WT and L-IRKO+GFP mice, respectively. Glucose tolerance test 2g/kg administered by oral gavage after 5 h fast, in antibody treated L-WT (**D**) and L-IRKO+GFP (**J**). Data are mean ± SEM. Circles = control antibody. Upward triangles = 83-7 antibody. Downward triangles = 83-14 antibody. Lack of statistical significance determined by two-way repeated measures ANOVA with Tukey's multiple comparisons test. Cumulative measurement of blood glucose during 120 min OGTT in antibody treated L-WT (**E**) and L-IRKO+GFP (**K**) mice. Blood glucose concentrations in antibody treated L-WT (**F**) and L-IRKO+GFP (**L**) mice after 5 h fast. Insulin concentrations in antibody treated L-WT (**G**) and L-IRKO+GFP (**M**) mice after 5 h fast. All data (except D and J) are mean ± SD, lack of statistical significance was determined by one-way ANOVA with Tukey's multiple comparison test. L-WT n = 4 per group. L-IRKO+GFP n = 6 per group control and 83-7 treated animals and n = 7 for the 83-14 treated group.
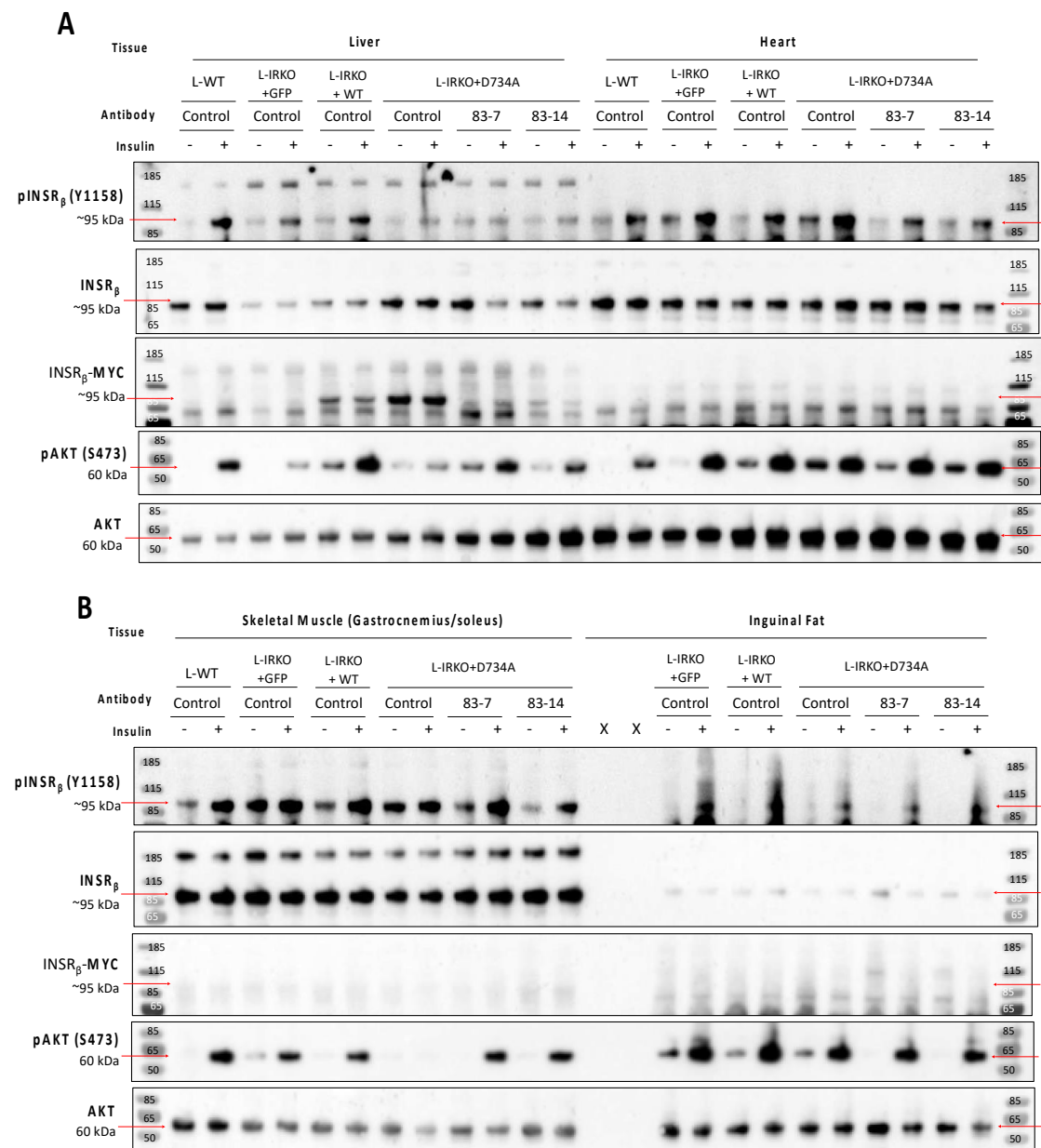
Supplementary Figure 5. Insulin receptor expression and insulin signalling is unaffected in tissues not targeted by AAV/AdV or antibody treatment. Western blot of lysates from liver, heart (**A**), skeletal muscle (gastrocnemius/soleus) and inguinal fat (**B**) demonstrate selective *Insr* knockout in the liver followed by selective hepatic add back of human INSR was achieved using liver-specific promoters and AAV/AdV serotypes that exhibit efficient liver transduction. The non-targeted tissues still express wild type murine *Insr* and the treatment antibodies are selective for the human INSR (added back in the liver). Insulin signalling in non-hepatic tissues and organs is intact and unaffected by AAV/AdV or antibody treatment, and reflects the prevailing plasma insulin concentrations.