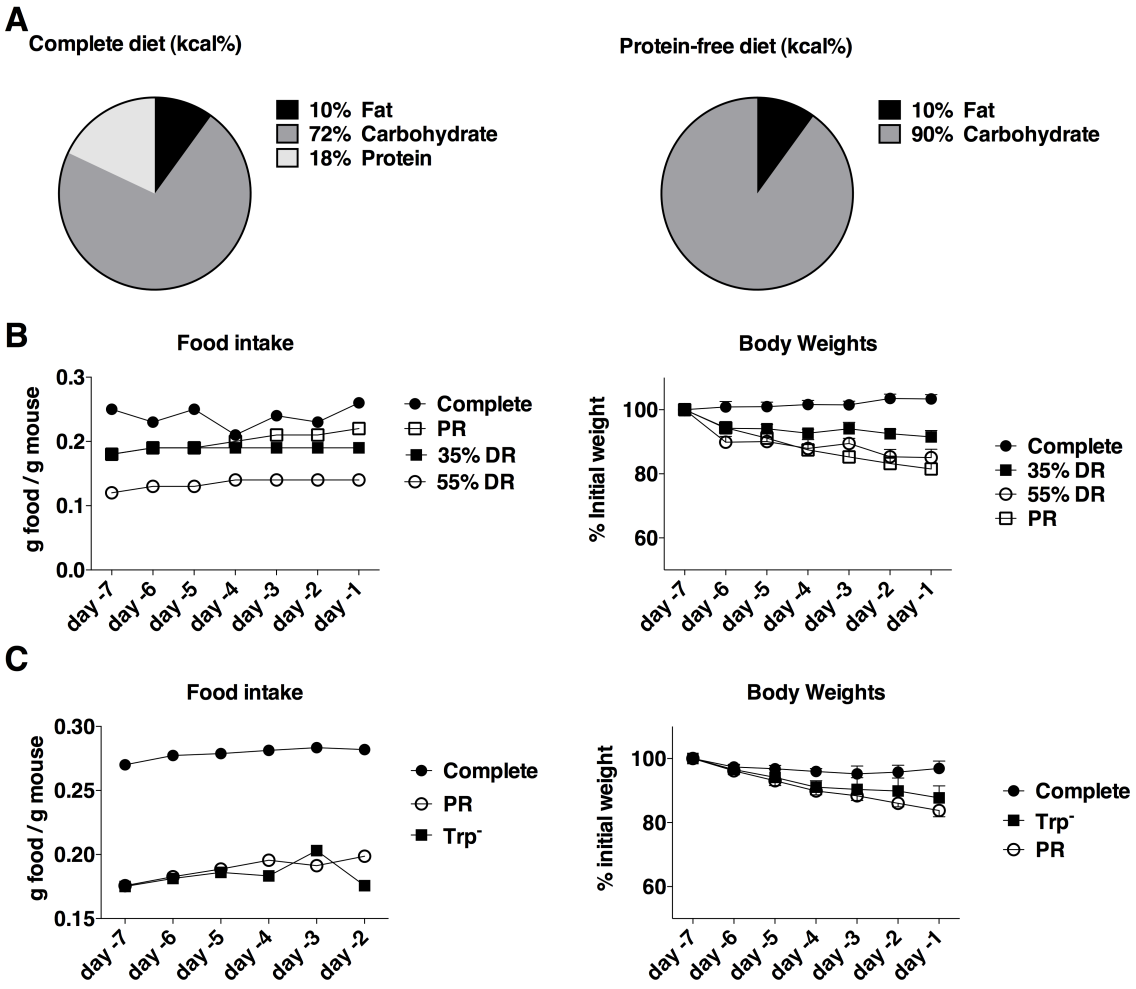
SUPPLEMENTAL INFORMATION

Supplemental information includes supplemental data, with six figures and legends, and supplemental experimental procedures.

SUPPLEMENTAL DATA

Figure S1, related to Figure 1: Dietary protein restriction mediates stress resistance independent of GCN2.



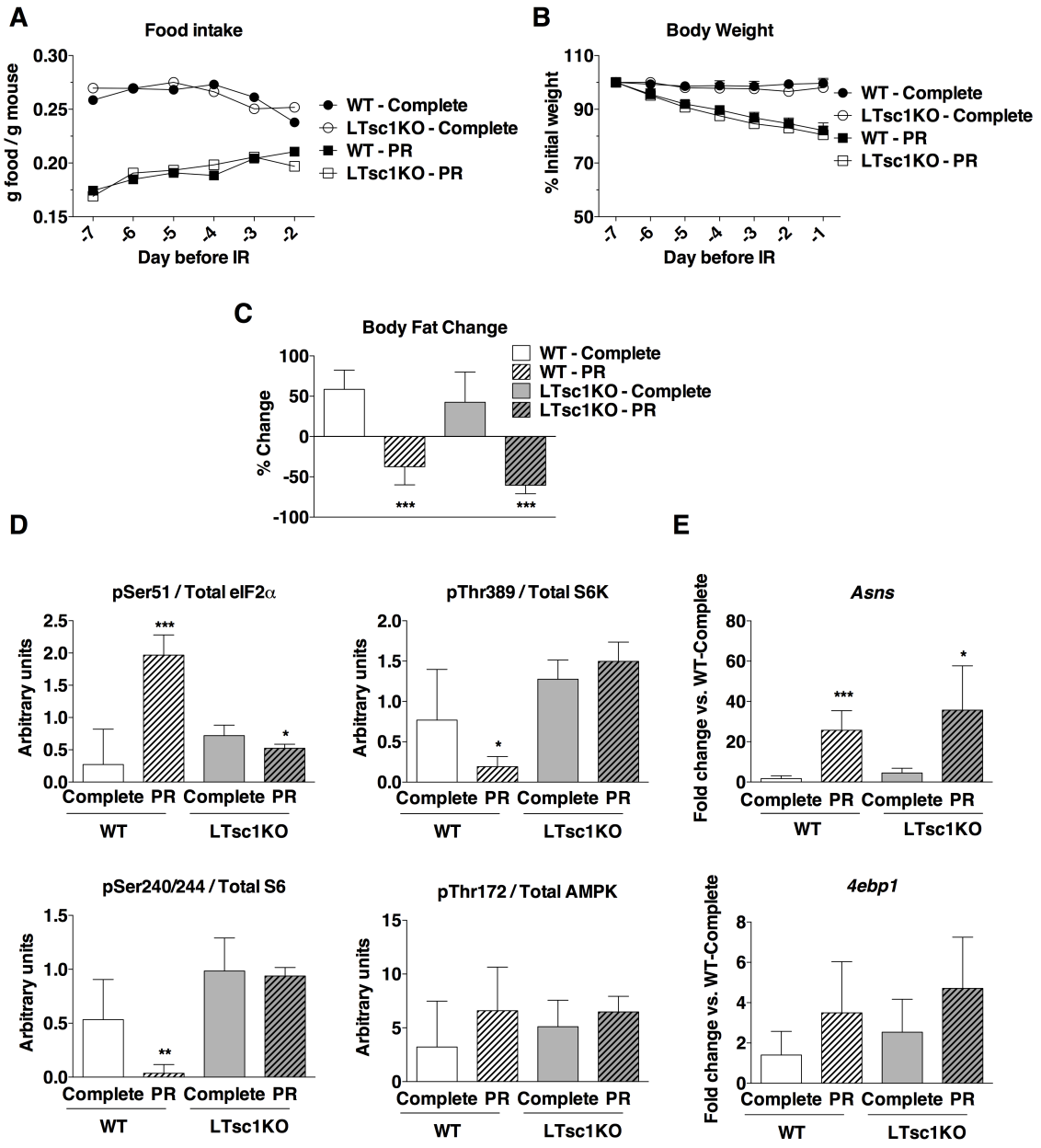
(A) Compositions of the experimental diets, represented as kcal%. Protein was replaced on a per weight basis with isocaloric sucrose in the protein-free diet.

(B) Food intake and body weight curves for the mice in Figure 1A. Hepatic IRI was induced on day 0.

(C) Food intake and body weight curves for the mice in Figure 1B. Hepatic IRI was induced on day 0.

Data in all panels are shown as means ± SD.

Figure S2, related to Figure 2: The TSC complex is required for inhibition of mTORC1 upon PR *in vivo*.



(A, B) Daily food intake (A) and body weights (B) of WT and LTsc1KO mice on the indicated diet measured at the same time of the day. Data were pooled from 3 separate experiments; n = 4-5 mice per group for each experiment.

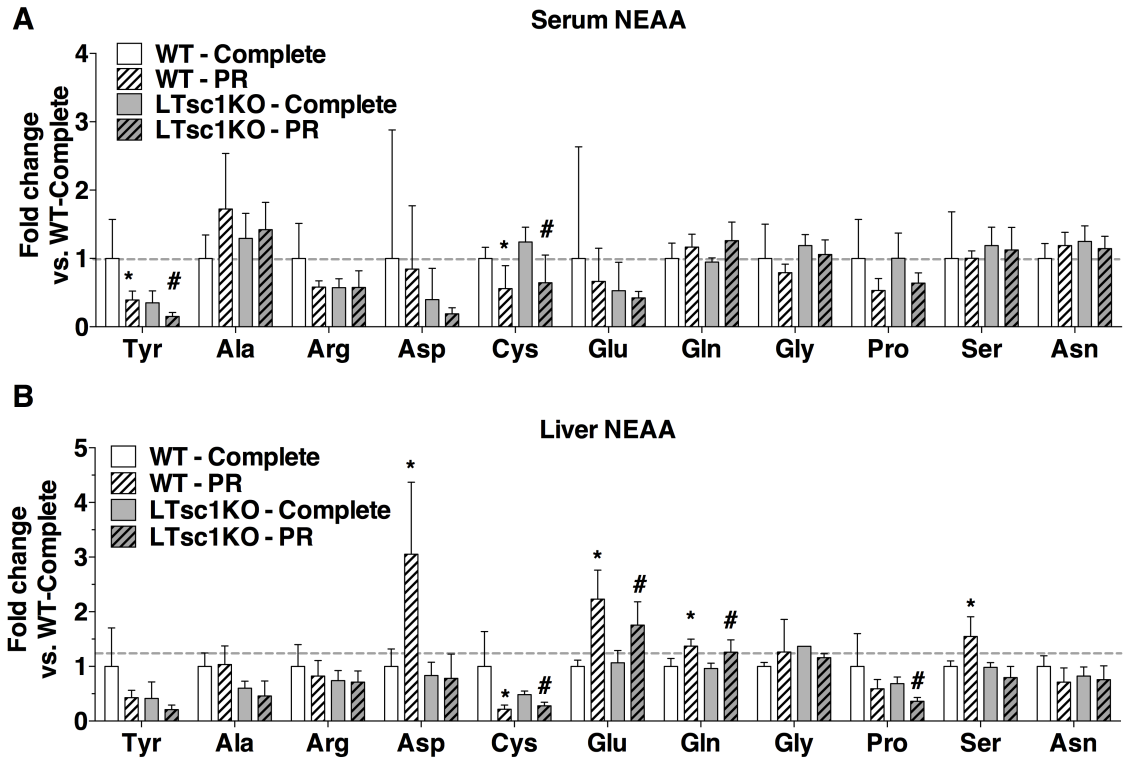
(C) Body fat as measured with Echo-MRI prior to and one week after preconditioning on the indicated diet, expressed as the % change in body fat; n = 5 mice per group. Statistical significance was measured by student's t-test between Complete and PR groups within each genotype; ***$p < 0.0005$.

(D) Quantification of the blots shown in Figure 2B. Statistical significance was measured by student's t-test between Complete and PR groups within each genotype; *$p < 0.05$, **$p < 0.01$, ***$p < 0.0005$.

(E) mRNA levels of ATF4 target genes *Asns* and *4ebp1* in livers of WT and LTsc1KO mice on the indicated diet; n = 4-5 mice per group. Statistical significance was measured by student's t-test between Complete and PR groups within each genotype; *$p < 0.05$, ***$p < 0.001$.

Data in all panels are shown as means ± SD.

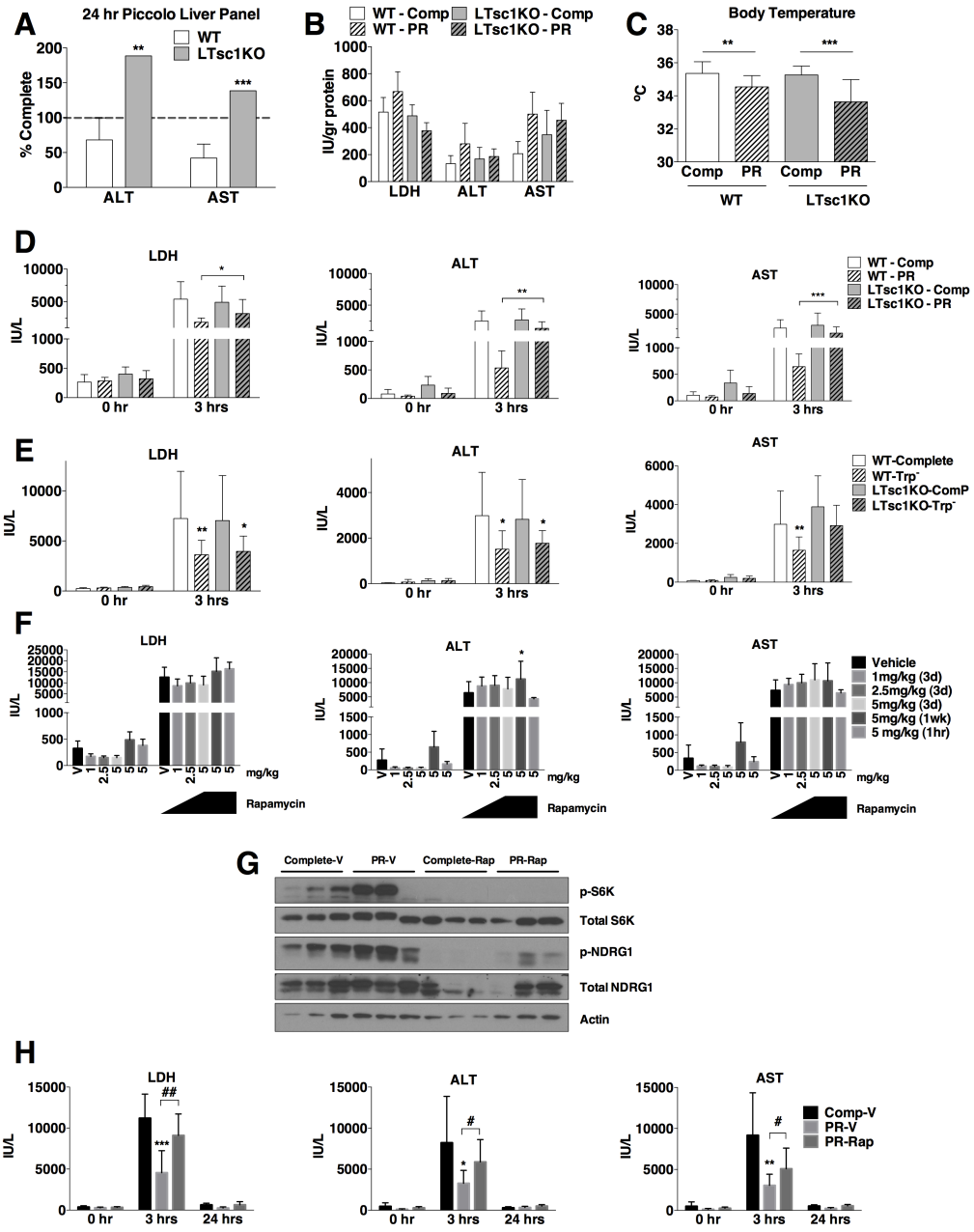
Figure S3, related to Figure 3: Differential reduction of essential amino acids and growth factors *in vivo* upon PR.



(A, B) Free NEAA levels in serum (A) and liver (B) of WT or LTsc1KO mice on the indicated diet expressed as fold change relative to the WT complete diet group; n = 4-5 mice per group. Asterisks/number signs indicate the significance of the difference between diets in WT and LTsc1KO groups, respectively, by student's t-test; $*/^{\#}p < 0.05$.

Data in all panels are shown as means ± SD.

Figure S4, related to Figure 4: The TSC complex is required for benefits of PR against acute hepatic stress.



(A) Liver damage marker analysis using Piccolo Liver Plus Panel discs with serum collected from the indicated groups 24 hours post-reperfusion. Data is

represented as % of complete diet fed group for each genotype; n = 2-5 mice per group; **$p < 0.01$, ***$p < 0.005$.

(B) Total liver enzyme analysis from liver extracts of mice from the indicated groups. Frozen livers were lysed in NP-40 lysis buffer, and the activity of the LDH, ALT and AST enzymes were measured in extracts with kinetic assays. Values were normalized to protein content of each sample; n = 4-5 mice per group.

(C) Core body temperature of mice measured with a rectal probe immediately before induction of ischemia. Results were pooled from 4 separate experiments with n = 4-5 mice per group per experiment; **$p < 0.005$, ***$p < 0.001$ by student's t test between diets within genotype.

(D) Serum levels of LDH, ALT and AST measured before ischemia (0 hr) and 3 hours after reperfusion in male WT or LTsc1KO mice 12-15 weeks of age preconditioned on the indicated diets for 1 week prior to hepatic IRI; pooled results from 3 separate experiments with n = 4-6 mice per group per experiment. Student's t-tests were performed to assess statistically significant differences between WT-PR and LTsc1KO-PR groups; *$p < 0.05$, **$p < 0.01$, ***$p < 0.0005$.

(E) Serum levels of LDH, ALT and AST measured before (0 hr) or 3 hours after reperfusion in WT and LTsc1KO mice preconditioned on the indicated diets for 1 week prior to hepatic IR. Data were pooled from 3 separate experiments with n = 14, 15, 14, 13 mice total for WT-Complete, WT-Trp^-, LTsc1KO-Complete and LTsc1KO-Trp^- groups, respectively. Complete: 65% of ad libitum food intake on a

complete diet; Trp^-: 65% of ad libitum food intake on a tryptophan-free diet. To assess statistical significance, student's t-tests were performed to compare Complete and Trp^- groups within each genotype. *$p < 0.05$, **$p < 0.01$.

(F) Serum levels of LDH, ALT and AST measured before ischemia (left) and 3 hours after reperfusion (right) in WT female C57Bl/6J mice fed a control diet *ad libitum* and subject to the indicated rapamycin or vehicle treatment by intraperitoneal injection. 3 days: daily injection of rapamycin for 3 consecutive days before IRI; 1 week: 3 injections of rapamycin over a week before IRI; 1 hour: a single injection with rapamycin 1 hour prior to IRI. Pooled results from 5 separate experiments are shown, with n = 25 for the vehicle group (V), n = 5 for 3 day treatment groups, and n = 10 for 1 week and 1 hour treatment groups. All groups were compared to the vehicle treated group for statistical analysis using a 1-way ANOVA with Dunnett's test for multiple comparisons. Asterisk indicates multiplicity adjusted p-value below 0.05.

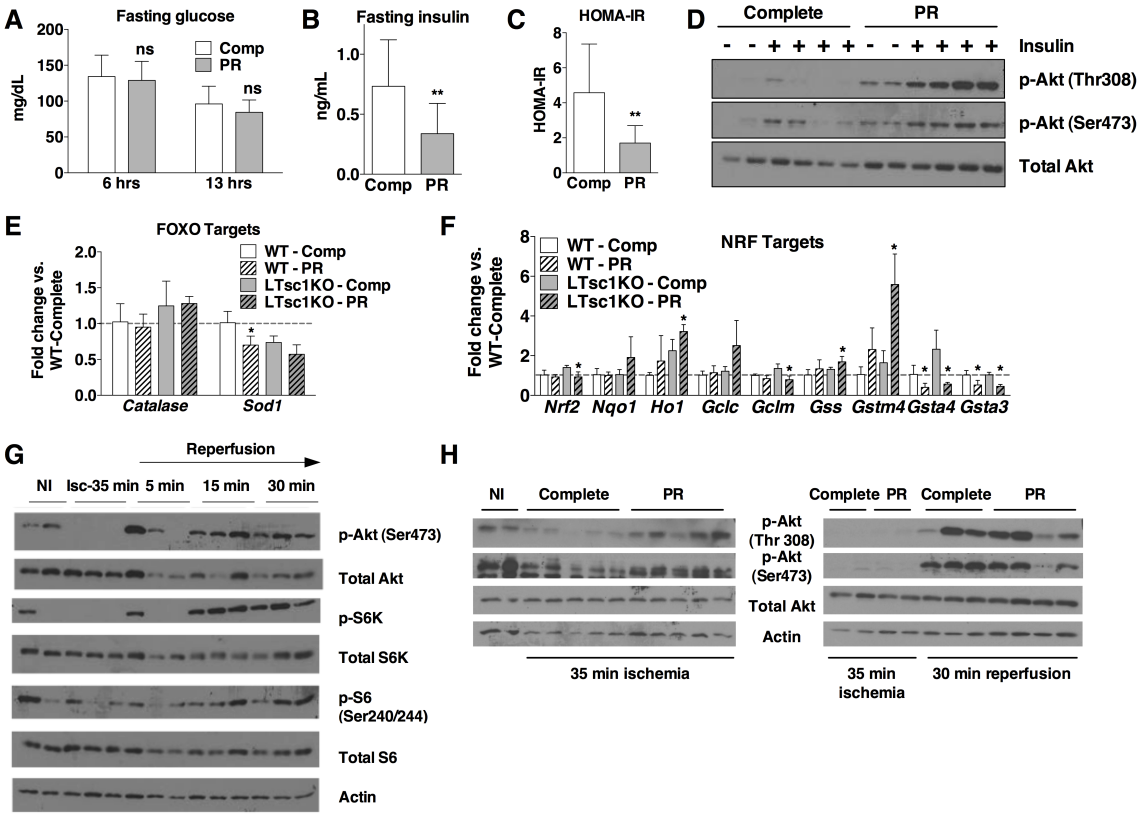
(G) Immunoblots of mTORC1 targets in liver extracts of the indicated groups of WT mice fed a complete diet *ad libitum* or preconditioned with PR and treated with either vehicle or rapamycin (5 mg/kg i.p. 3 times/week) for 1 week. Mice were fasted overnight prior to harvest and stimulated with insulin (0.25 U/kg i.p.) 10 minutes before sacrifice.

(H) Serum levels of LDH, ALT and AST measured before (0 hr), 3 hours and 24 hours post-reperfusion in mice preconditioned with the indicated diets, and

treated with either vehicle or rapamycin (5 mg/kg i.p. 3 times/week) for 1 week prior to hepatic IRI. Results were pooled from 2 separate experiments with n = 4-5 mice per group in each experiment. Complete-V control group data was also used in panel (F). Asterisks indicate statistically significant differences between Complete-V and PR-V groups according to a student's t-test, *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, #$p < 0.05$, ##$p < 0.005$.

Data in all panels are shown as means ± SD.

Figure S5, related to Figure 5: The TSC complex is required for improved hepatic insulin sensitivity upon PR.



(A-C) Glucose homeostasis in WT female C57Bl/6J mice at 11-13 weeks of age (n=12/group) on the indicated diet for up to 10 days. (A) Blood glucose measured after 6 hours (day 10) or 13 hours (day 7) of fasting. (B) Serum insulin levels measured after 13 hours of fasting on day 7. (C) HOMA-IR index was calculated as: [fasting glucose (mg/dl) * fasting insulin (μU/ml)]/405. Asterisks indicate statistically significant differences between diet groups according to a student's t-test; **$p < 0.01$, ns: not significant.

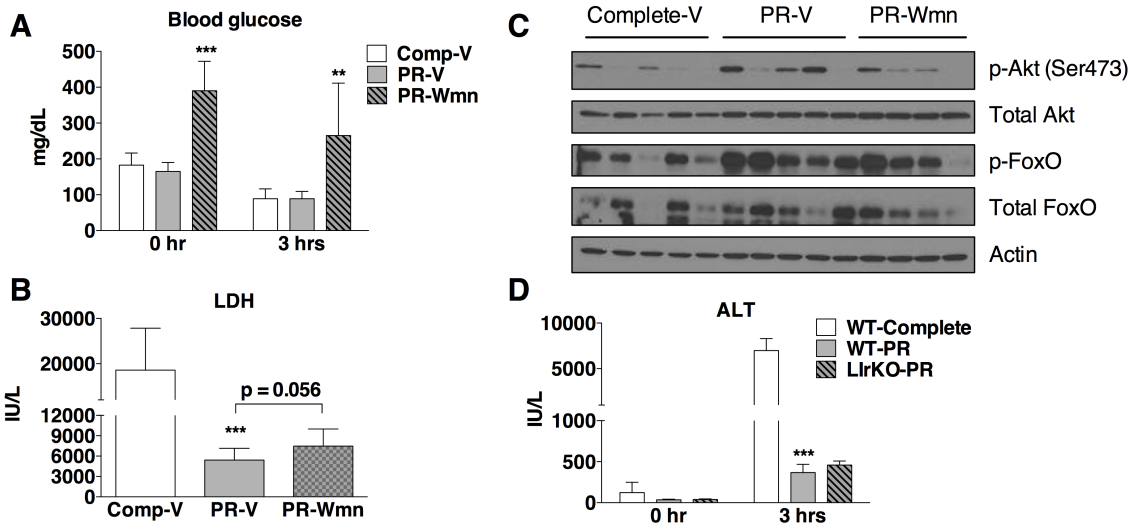
(D) Hepatic insulin sensitivity as determined by immunoblotting for markers of Akt pathway activation in liver extracts from mice fasted overnight and then stimulated with insulin (0.25 U/kg i.p.) 10 minutes prior to harvest.

(E, F) Gene expression analysis of FOXO (E) or NRF2 (F) targets by qRT-PCR from livers of WT and LTsc1KO mice (n = 4-5 mice per group) on the indicated diet. For each gene, data are represented as fold changes with respect to the WT-AL group. Asterisks indicate statistically significant differences between diet groups within genotype according to a student's t-test; *$p < 0.05$.

(G) Hepatic insulin signaling as determined by immunoblotting for markers of Akt and mTORC1 pathways in liver extracts prepared from WT mice fed *ad libitum* and harvested either without surgery (NI: non-ischemic) or at various time points during surgery. Isc-35 min: at the end of the 35 minutes ischemic period; 5, 15 and 30 minutes: Time after reperfusion. Each well represents an individual animal.

(H) Hepatic insulin signaling as determined by immunoblotting for markers of Akt activation in extracts of livers from WT mice on the indicated diet for 1 week. Livers were harvested either without surgeries (NI: non-ischemic), at the end of the ischemic period (35 min ischemia) or 30 minutes into the reperfusion period. Data in all panels are shown as means ± SD.

Figure S6, related to Figure 6: Increased prosurvival signaling and reduced apoptosis contribute to PR-mediated protection



(A) Blood glucose values of mice measured before (0 hr) and 3 hours after reperfusion for the experiment described in Figure 6F. Statistical significance was assessed by student's t-test between PR-V and PR-Wmn groups; $^{**}p < 0.005$, $^{***}p < 0.000001$.

(B) Serum LDH values for the experiment described in Figure 6F. Statistical significance was assessed by student's t-test between Complete-V and PR-V groups; $^{***}p < 0.0005$.

(C) Immunoblots of liver extracts showing markers of Akt signaling; mice that were harvested 3 hours post-reperfusion, from the experiment described in Figure 6F.

(D) Serum ALT values for the experiment described in Figure 6H. Statistical significance was assessed by student's t-test, ***$p < 0.0005$ between WT-Complete and WT-PR groups.

Data in all panels are shown as means ± SD.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Dietary and Pharmacological Treatments of Mice

Experimental control diets were based on a modified version of Research Diets D12450B prepared with 18% calories from protein (individual crystalline amino acids (Ajinomoto) based on the proportions present in casein), 10% from fat (lard, soybean oil) and 72% from carbohydrate (sucrose, maltodextrin, corn starch). For protein free and individual amino acid free diets, missing amino acids were replaced with isocaloric sucrose. All diets were prepared in a final 1% agar mixture. Mice on experimental diets were fed and weighed daily, at the same time of the day. When mice were subject daily to reduced food availability (dietary restriction, DR), they tended to eat their daily allotment quickly, resulting in periods of extended fasting for the remainder of the 24-hour period. However, when presented with incomplete diets lacking protein or essential amino acids, mice self-restricted food intake by up to 30-35% (Peng et al., 2012). As a result, even when availability of protein or amino acid deficient diets was restricted daily by 35%, animals did not eat the available food quickly (as with DR) and thus didn't experience extended periods of fasting. Procedures including surgery and tissue harvesting were performed in the morning without prior fasting unless otherwise indicated. Rapamycin for *in vivo* use was purchased from LC Laboratories, dissolved in 100% EtOH and diluted in sterile 5% Tween-80/5% PEG-400 solution for i.p. injections. Wortmannin was purchased from

Selleckchem, dissolved in DMSO and diluted in sterile 30%PEG/1%Tween-80 solution for i.p. injections.

RNA isolation and quantitative PCR

Total RNA was isolated from frozen tissues by RNA Bee reagent (Qiagen) and cDNAs were synthesized by using random hexamer primers and Verso cDNA synthesis kit (Thermo Scientific) according to the manufacturer's instructions. SYBR green dye was used for quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) and fold changes were calculated by $\Delta\Delta C_t$ method using *Hprt* housekeeping gene. Each sample was tested in duplicates. Primer sequences for each gene are listed in the table.

qRT-PCR Primer sequences

Gene	**Forward primer (5' → 3')**	**Reverse primer (5' → 3')**
Asns	GTCAAGAACTCCTGGTTCAAG	GATCTGACGGTAGAAGTAGC
4ebp1	ACTAGCCCTACCAGCGATGAG	CGCAGACATAGAAGCATCATTGCG
Nrf2	AGGACATGGAGCAAGTTTGG	TCTGTCAGTGTGGCTTCTGG
Nqo1	AGGATGGGAGGTACTCGAATC	AGGCGTCCTTCCTTATATGCTA
Ho1	AAGCCGAGAATGCTGAGTTCA	GCCGTGTAGATATGGTACAAGGA
Gclc	GGGGTGACGAGGTGGAGTA	GTTGGGGTTTGTCCTCTCCC
Gclm	AGGAGCTTCGGGACTGTATCC	GGGACATGGTGCATTCCAAAA
Gss	CAAAGCAGGCCATAGACAGGG	AAAAGCGTGAATGGGGCATAC

Gstm4	AGCTCACGCTATTCGGCTG	GCTCCAAGTATTCCACCTTCAGT
Gsta4	TGATTGCCGTGGCTCCATTTA	CAACGAGAAAAGCCTCTCCGT
Gsta3	AAGAATGGAGCCTATCCGGTG	CCATCACTTCGTAACCTTGCC
Catalase	TCCCTGCTGTCTCACGTTCC	CGGGTCTCCTATTGGGTTCCCG
Sod1	GGGACAATACACAAGGCTGT	GCCAATGATGGAATGCTCTC
Hprt	TTTCCCTGGTTAAGCAGTACAGCCC	TGGCCTGTATCCAACACTTCGAGA

Serum damage measurements using Piccolo Liver Plus Panel discs

Piccolo Liver Plus Panel discs (Abaxis) were used as a second platform for confirming ALT and AST levels according to the manufacturer's instructions. This platform can measure serum ALT and AST levels up to 2000 IU/L. For the mice which had damage values greater than 2000, we estimated these values to be 2000 IU/L and performed calculations accordingly.