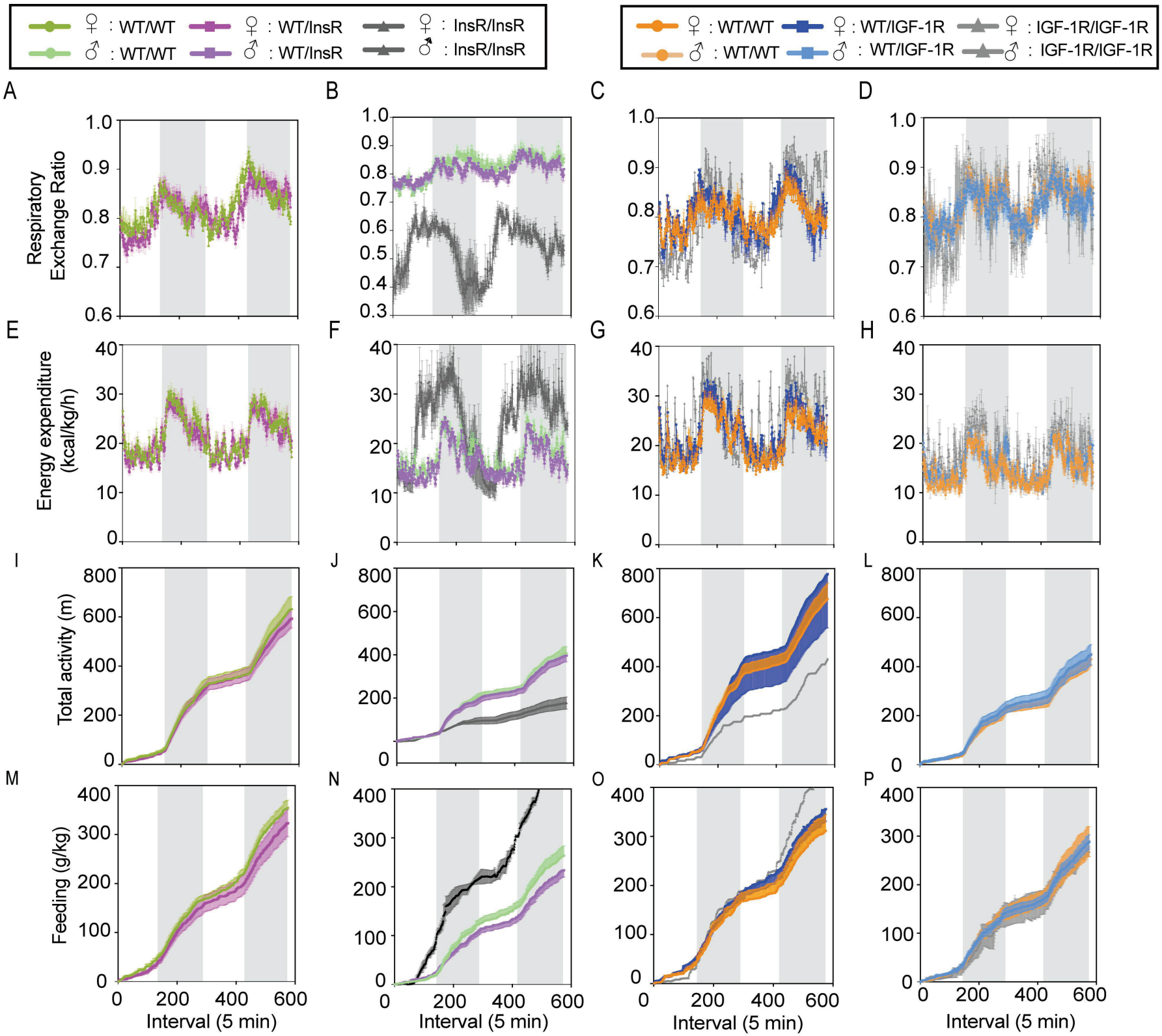
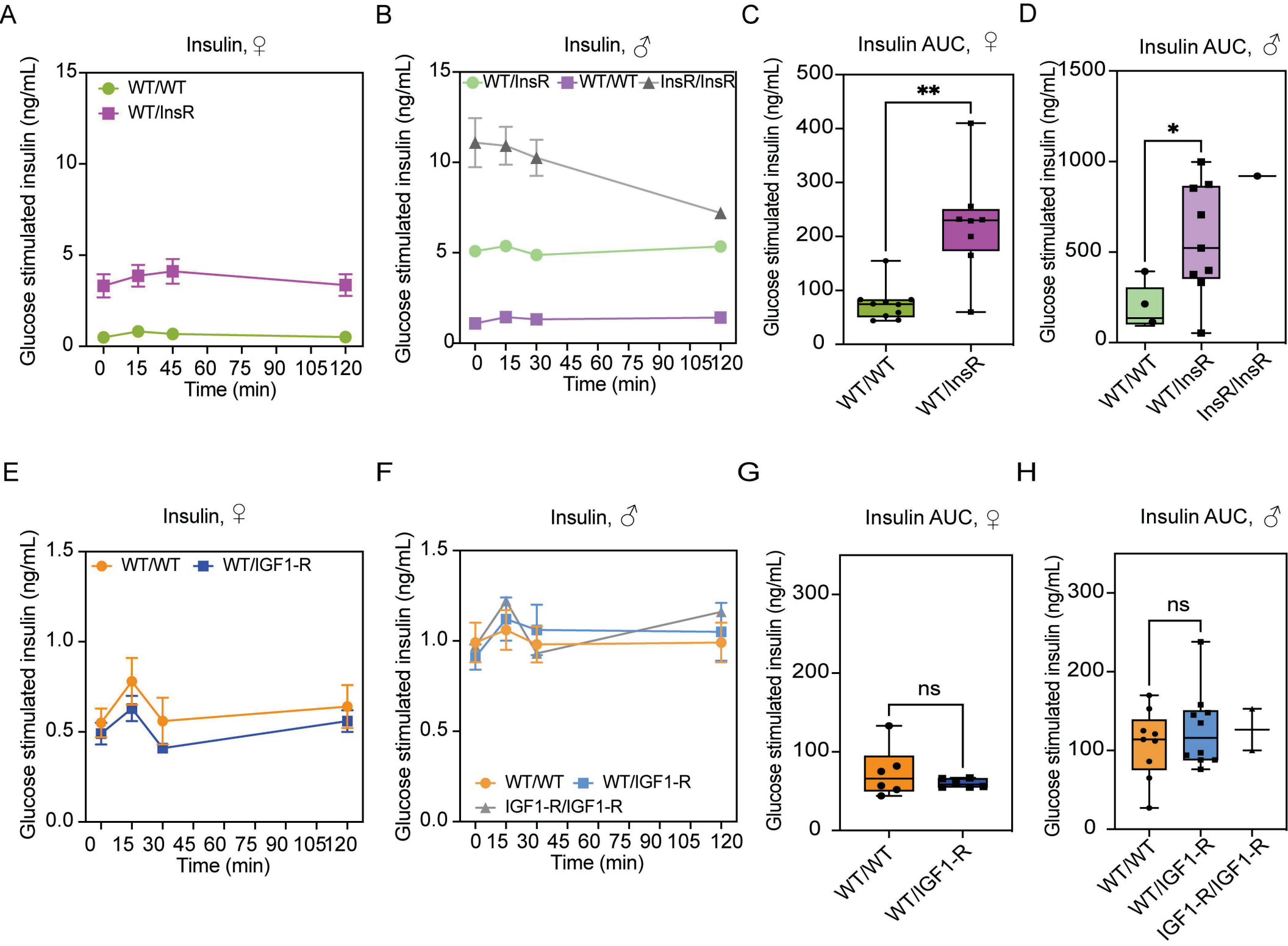

♀ : WT/WT
♂ : WT/WT
♀ : WT/InsR
♂ : WT/InsR
♀ : InsR/InsR
♂ : InsR/InsR
♀ : WT/WT
♂ : WT/WT
♀ : WT/IGF-1R
♂ : WT/IGF-1R
♀ : IGF-1R/IGF-1R
♂ : IGF-1R/IGF-1R
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Respiratory Exchange Ratio
Energy expenditure (kcal/kg/h)
Total activity (m)
Feeding (g/kg)
Interval (5 min)
Interval (5 min)
Interval (5 min)
Interval (5 min)

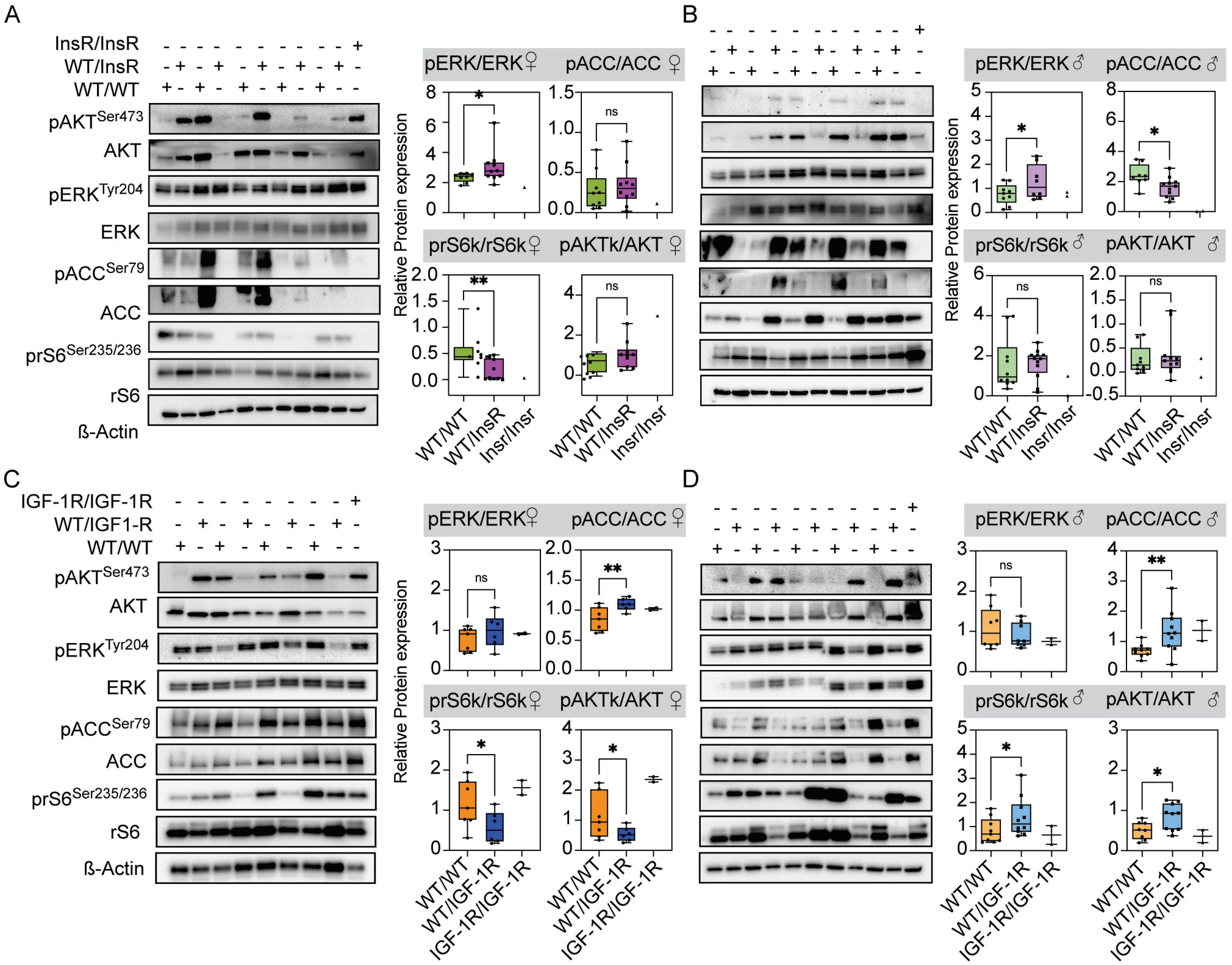
**Supplementary Figure 1. InsR$^{R1109C}$ and IGF-1R$^{R1096C}$ heterozygous mice maintain normal metabolic rates.**

Respiratory exchange ratio (RER) in 5 min intervals over 48 hr. **(A)** female and **(B)** male InsR$^{R1109C}$; **(C)** female and **(D)** male IGF-1R$^{R1096C}$. Energy expenditure (EE) in 5 min intervals over 48 hr (per kg of lean mass): **(E)** female and **(F)** male InsR$^{R1109C}$; **(G)** female and **(H)** male IGF-1R$^{R1096C}$. Total activity in 5 min intervals over 48 hr: **(I)** female and **(J)** male InsR$^{R1109C}$; **(K)** female and **(L)** male IGF-1R$^{R1096C}$. Feeding in 5 min intervals over 48 hr (per kg of lean mass): **(M)** female and **(N)** male InsR$^{R1109C}$; **(O)** female and **(P)** male IGF-1R$^{R1096C}$.


A
Insulin, ♀
Glucose stimulated insulin (ng/mL)
WT/WT
WT/InsR
Time (min)
B
Insulin, ♂
WT/InsR
WT/WT
InsR/InsR
C
Insulin AUC, ♀
**
D
Insulin AUC, ♂
*
E
Insulin, ♀
WT/IGF1-R
F
Insulin, ♂
IGF1-R/IGF1-R
G
Insulin AUC, ♀
ns
H
Insulin AUC, ♂

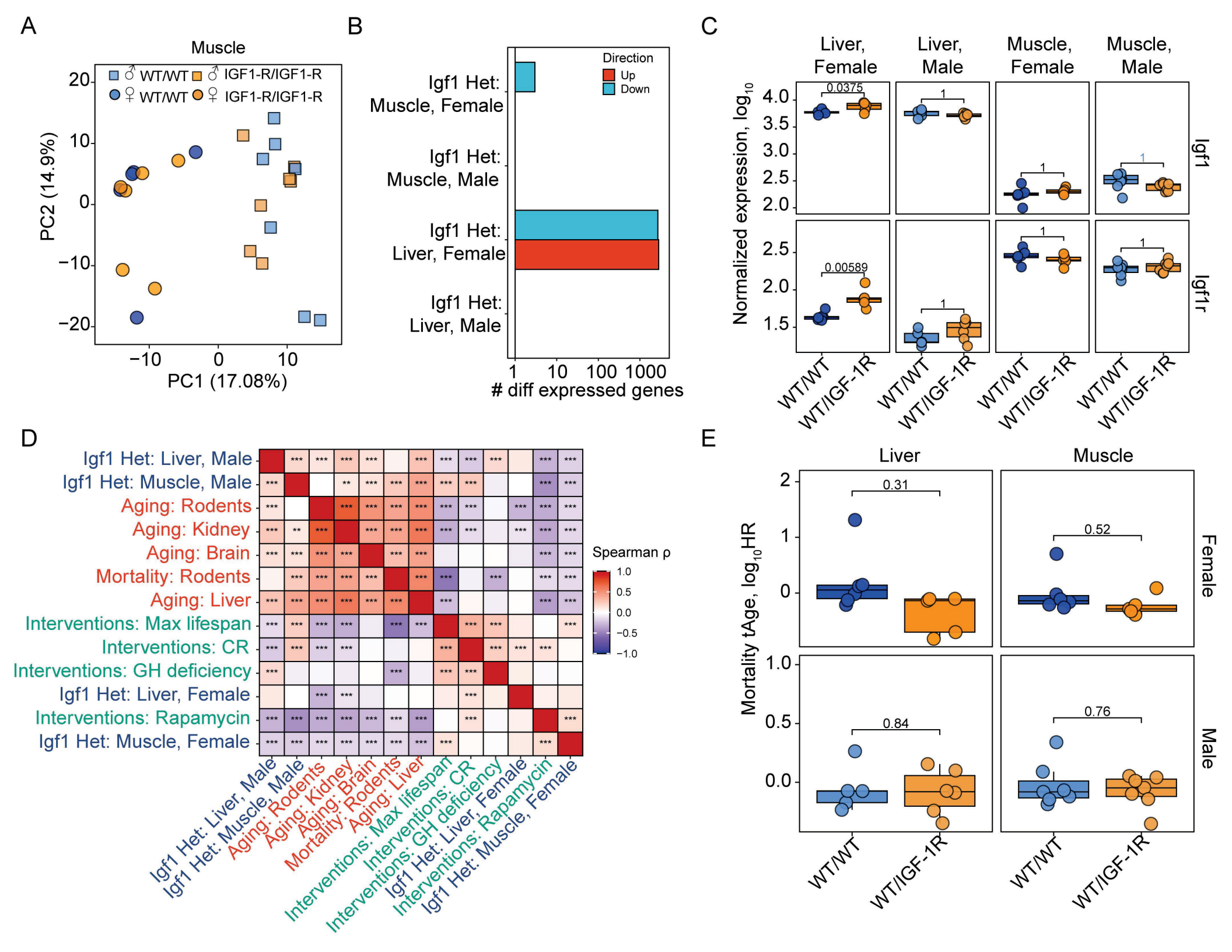
**Supplementary Figure 2. InsR$^{R1109C}$ and IGF-1R$^{R1096C}$ heterozygous mice maintain normal glycated hemoglobin.**

Glucose (1.5g/kg) stimulated insulin in female **(A)** and male **(B)** InsR$^{R1109C}$. Area under the curve (AUC) for glucose-stimulated insulin in female **(C)** and male **(D)** InsR$^{R1109C}$. Glucose (1.5g/kg) stimulated insulin in female **(E)** and male **(F)** IGF-1R$^{R1096C}$. Area under the curve (AUC) for glucose-stimulated insulin in female **(G)** male **(H)** IGF-1R$^{R1096C}$.


A
InsR/InsR
WT/InsR
WT/WT
pAKT Ser473
AKT
pERK Tyr204
ERK
pACC Ser79
ACC
prS6 Ser235/236
rS6
ß-Actin
pERK/ERK♀
pACC/ACC ♀
prS6k/rS6k♀
pAKTk/AKT ♀
Relative Protein expression
WT/WT
WT/InsR
Insr/Insr
B
pERK/ERK♂
pACC/ACC ♂
prS6k/rS6k ♂
pAKT/AKT ♂
C
IGF-1R/IGF-1R
WT/IGF1-R
WT/WT
pAKT Ser473
AKT
pERK Tyr204
ERK
pACC Ser79
ACC
prS6 Ser235/236
rS6
ß-Actin
pERK/ERK♀
pACC/ACC ♀
prS6k/rS6k♀
pAKTk/AKT ♀
Relative Protein expression
WT/WT
WT/IGF-1R
IGF-1R/IGF-1R
D
pERK/ERK♂
pACC/ACC ♂
prS6k/rS6k ♂
pAKT/AKT ♂

**Supplementary Figure 3. Liver AMPK and mTORC1 activity in InsR$^{R1109C}$ and IGF-1R$^{R1096C}$ heterozygous mice.**

Representative blot showing five wildtype, five heterozygotes and one homozygous mouse after 6 hr morning fast: InsR$^{R1109C}$ **(A)** Females, **(B)** Males; IGF-1R$^{R1096C}$ **(C)** Females and **(D)** Males. Relative pERK$^{Tyr204}$/ERK, pACC$^{Ser79}$/ACC, PAKT$^{Ser473}$/AKT and prS6$^{Ser235/236}$/rS6 respectively for InsR$^{R1109C}$ females and males; and IGF-1R$^{R1096C}$ females and males. Ratios of phosphorylated protein to total protein. Box plot for minimum and maximum with median. T-test sample size: Females WT/WT n= 9 and WT/InsR n= 10; Males WT/WT n= 8-10 and WT/InsR n =8-12; Females WT/WT n= 7 and WT/IGF-1R n= 6; Males WT/WT n= 10 and WT/IGF-1R n= 9-10). * $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$: ns, no significant differences.


A
Muscle
♂ WT/WT ♂ IGF1-R/IGF1-R
♀ WT/WT ♀ IGF1-R/IGF1-R
PC2 (14.9%)
PC1 (17.08%)
B
Direction
Up
Down
Igf1 Het: Muscle, Female
Igf1 Het: Muscle, Male
Igf1 Het: Liver, Female
Igf1 Het: Liver, Male
# diff expressed genes
C
Liver, Female
Liver, Male
Muscle, Female
Muscle, Male
Normalized expression, log10
0.0375
0.00589
Igf1
Igf1r
WT/WT
WT/IGF-1R
D
Igf1 Het: Liver, Male
Igf1 Het: Muscle, Male
Aging: Rodents
Aging: Kidney
Aging: Brain
Mortality: Rodents
Aging: Liver
Interventions: Max lifespan
Interventions: CR
Interventions: GH deficiency
Igf1 Het: Liver, Female
Interventions: Rapamycin
Igf1 Het: Muscle, Female
Spearman ρ
1.0
0.5
0.0
−0.5
−1.0
E
Liver
Muscle
Mortality tAge, log10HR
0.31
0.52
0.84
0.76
Female
Male
WT/WT
WT/IGF-1R

**Supplementary Figure 4. Estimated biological age in heterozygous IGF-1R$^{R1096C}$ females**

**(A)** Principal component analysis (PCA) of skeletal muscle gene expression profiles from WT and IGF-1R$^{R1096C}$ heterozygous mice. **(B)** Number of differentially expressed genes (Benjamini-Hochberg adjusted p-value < 0.05) between WT and IGF-1R$^{R1096C}$ heterozygous mice identified by tissue and sex. **(C)** *Igf1* and *Igf1r* expression in muscle and liver of WT and IGF-1R$^{R1096C}$ heterozygous mice. Differential expression assessed with ANOVA model using edgeR; p-values adjusted for multiple comparisons with Benjamini-Hochberg. **(D)** Spearman correlation of enriched pathways within organs of IGF-1R$^{R1096C}$ heterozygous mice (blue) that are associated with signatures of aging, with mortality (red), and with lifespan-extending interventions (green). Pairwise Spearman correlations were calculated based on NES values determined for each signature via GSEA. * p.adjusted < 0.05, ** p.adjusted < 0.01, *** p.adjusted < 0.001. **(E)** Mortality transcriptomic age (tAge) of tissue from WT and IGF-1R$^{R1096C}$ heterozygous female mice by organ and sex, assessed with the rodent multi-tissue Elastic Net (EN) clock. tAges between the groups were compared with ANOVA, and corresponding BH-adjusted p-values.

**Supplementary Table 1**. Differentially expressed genes in livers and muscles of heterozygous IGF-1R$^{R1096C}$ mice.

**Supplementary Table 2**. Gene set enrichment analysis (GSEA) from IGF-1R$^{R1096C}$ liver samples.

**Supplementary Table 3**. The corresponding numbers for mouse and human IR and IGF-1R with and without signal peptide.

**Supplementary Table 4.** Sequence details of reagents for *Igf1r* and *Insr* gene replacement.

**Supplementary Table 5. KEY RESOURCES TABLE.**

**Supplementary Table 6. Extended Materials and Methods**.

**Supplementary Table 3**. The corresponding numbers for mouse and human IR and IGF-1R with and without signal peptide.

| Protein | With signal peptide (precursor) | Without signal peptide (mature) |
|---|---|---|
| Human IR-A | R1107 | R1080 |
| Human IR-B | R1119 | R1092 |
| Mouse IR-A | R1109 | R1082 |
| Mouse IR-B | R1121 | R1094 |
| Human IGF1R | R1095 | R1065 |
| Mouse IGF1R | R1096 | R1066 |

**Supplementary Table 4.** Sequence details of reagents for *Igf1r* and *Insr* gene replacement.

| **Mouse model** | ***Igf1r*-R1096C (AGG >tGc)** |
|---|---|
| Alt-R™ CRISPR-Cas9 crRNA (PAM) | AAAGTTATCTCCGGTCTCTG (AGG) |
| Ultramer™ DNA oligos (reverse strand) | GCCAGCTCCTGAAAAAAGTGGACCAAGCAAGATAAC ACAGCGAGGAACAAAACTGACCTCCACTTCTGGgCa CAGAGACCGGAGATAACTTTTGAGATCACCGCGTGT CATTAGTTCCATGATGACCAGGGTGGGCTGGCCTTG GGATACCACACCCAG |
| Genotyping primer, forward | GGGTAGTTTCCCCGTTGCAT |
| Genotyping primer, reverse | CCATGACACGTGGTAGAGCA |
| **Mouse model** | ***Insr*-R1109C (AGG>tGc)** |
| Alt-R™ CRISPR-Cas9 crRNA (PAM) | AAAGTCACCTCCGTTCTCTG (AGG) |
| Ultramer™ DNA oligos (reverse strand) | GCATTTAGTGAGGTTGGTATACGTAAGATCAGGTACC CTGTTATGGGTCTTACCTAGAGGCAGCTTACCTCAG CATCTGGgCaCAGAGAACGGAGGTGACTTTTCAGGT CTCCATGAGCCATCAATTCCATCACTACCAGCGTTGG CTGTCCTTTG |
| Genotyping primer, forward | GGCAAGTGAGATTTGCTTGGG |
| Genotyping primer, reverse | ACAGGGGTTGCAATTAGCACT |

**Supplementary Table 5. KEY RESOURCES TABLE**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| **Mouse strains** | | |
| C57BL/6J wildtype (WT) mice | The Jackson Laboratory | #:**000664** |
| IGF1R-R1096C | In-house made, Brown Tg Core | NA |
| InsR-R1109C | In-house made, Brown Tg Core | NA |
| Oligo 22007-F1: GGCAAGTGAGATTTGCTTGGG | IDT | NA |
| Oligo 22007-R1: ACAGGGGTTGCAATTAGCACT | IDT | NA |
| 22006-F1: GGGTAGTTTCCCCGTTGCAT | IDT | NA |
| 22006-R1: CCATGACACGTGGTAGAGCA | IDT | NA |
| **Plasmids** | | |
| pCS2-hInsR | (1) | NA |
| pCS2-hInsR-R1107C-Myc (labeled as R1109C) | This paper | NA |
| pCS2-hIGF1R | (2) | NA |
| pCS2-hIGF1R-R1095C (labeled as R1096C) | This paper | NA |
| hInsR R1109C-F | CCGTTCTCTGtgcCCAGAGGCTG | NA |
| hInsR R1109C-R | AGGTAGCTCTTCAGGTCTC | NA |
| hIGF1R-R1095C-F | CCGGTCTCTGtgcCCAGAAATGG | NA |
| hIGF1R-R1095C-R | AGATAACTTTTGAGATCGC | NA |

| | | |
|---|---|---|
| **Hot Start Taq BLUE** Master Mix | **Apex Bioresearch** | Cat #: 42-144 |
| Q5 site-directed mutagenesis | NEB | E0554S |
| SnapGene 7.2 | From Dotmatics; available at snapgene.com) | University of Pittsburgh license |
| **DNeasy Blue & Tissue kit** | **QiaGen** | 69506 |
| RIPA Buffer (10X) | Cell signaling | 9806 |
| Pierce™ Protease Inhibitor Mini Tablets, EDTA-free | Thermo Fisher | A32955 |
| PhosSTOP Easy pack | Roche | 4906845001 |
| SYBR™ Safe DNA Gel Stain | Thermo Fisher | S33102 |
| BenchMark™ Pre-stained Protein Ladder | Thermo Fisher | 0748010 |
| Trans-Blot Turbo RTA Mini 0.2 µm PVDF Transfer Kit | Bio-RAD | 1704272 |
| ProSignal Femto | Prometheus | 20302 |
| Mouse Hemoglobin A1c kit | Crystal Chem | 80310 |
| Mini-Protean TGX gels | Bio-Rad | 4561095 |
| Zirconium Oxide Beads 1.0 mm | Next Advance | ZROB10 |
| 2x Laemmli Sample Buffer | Bio-rad | 1610737EDU |
| Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) | Cell Signaling | 4370 |
| p44/42 MAPK (Erk1/2) | Cell Signaling | 4695 |

| | | |
|---|---|---|
| Phospho-Acetyl-CoA Carboxylase (Ser79) | Cell Signaling | 3661 |
| pY 1150/1151 IR (pY1135/1136 IGF1R) | Cell signaling | 3024 |
| IR (CT-3) | Santa Cruz | sc-57342 |
| IGF1R (Zl001) | Invitrogen | 39-6700 |
| pERK1/2 (197G2) | Cell signaling | 4377 |
| ERK1/2 (L34F12) | Cell signaling | 4696 |
| pAKT (D9E) | Cell signaling | 4060 |
| AKT (40D4) | Cell signaling | 2920 |
| Acetyl-CoA Carboxylase | Cell Signaling | 3676 |
| Phospho-S6 Ribosomal Protein | Cell Signaling | 2211 |
| S6 Ribosomal Protein | Cell Signaling | 2317 |
| Sheep Anti-Mouse IgG, HRP | Prometheus Protein Biology Products | 84-848 |
| anti-rabbit IgG (H + L) (Dylight 800 conjugates) | Cell Signaling | 5151 |
| anti-mouse IgG (H + L) (Dylight 680 conjugates) | Cell Signaling | 5470 |


1. Choi E, et al. Mitotic checkpoint regulators control insulin signaling and metabolic homeostasis. Cell. 2016;166(3):567–581.

2. Li J, et al. Structural basis of the activation of type 1 insulin-like growth factor receptor. Nat Commun. 2019;10(1):4567.

**Supplementary Table 6. Extended Materials and Methods**.

## Growth and weight

Mice were weighed weekly to the nearest 0.1g from age 2 weeks up to 4 months of age. To calculate the growth rates of mice, we fitted a curve to the relationship between weight (g) and age (weeks) using a logistic 3 -parameter model. Differences among genotypes were determined by comparing the growth rate, inflection point, and asymptote of the curve using equivalence tests.

## Body Composition

Body composition (percent fat mass and percent lean mass) was assessed once at 4 months of age by NMR (EchoMRI, Echo Medical System). Non-anesthetized mice were placed in a restraint cylinder and scanned for approximately 2 min. Data expressed as percent of body mass.

## Metabolic cage analysis

The Sable Systems Promethion Multi-plexed Metabolic Cage system was used to evaluate feeding, activity, energy expenditure (EE), and respiratory exchange ratio (RER) by indirect calorimetry. Mice were individually housed during 72 h, with the first 24 h for acclimatation and the subsequent 48hrs data to calculate values across light and dark cycle for each metabolic parameter.

## In vivo glucose homeostasis

Glucose tolerance tests were conducted in the morning following a 6 hr fast. Mice received an intraperitoneal injection of glucose at a dose of 1.5 g/kg, and blood samples were collected from the tail at specific time points to measure plasma insulin and glucose.

**Rotarod**

Mice were tested on a rotarod (Ugo-Basile, model 47600) started at 4 RPM and accelerated to 40 RPM over a 300-second period. Each mouse underwent three consecutive trials, with approximately a 1-minute interval between. Latency to fall was recorded. Mice that fell immediately upon placement, before the rod began to accelerate, were given a score of 0 seconds for that trial.

**Open Field**

Mice acclimated to the testing room were placed in a Versamax Open Field Arena (40 cm × 40 cm × 40 cm; Omnitech Electronics, OH, United States). Infrared beams tracked their movements, recording distance traveled (in cm), vertical activity, and time spent in the arena perimeter and center. Data were collected in 5-minute intervals over a total period of 60 minutes.

**Home Cage Wheel Running**

Subjects were individually housed with a wireless running wheel affixed with a revolution sensor (Lafayette Instruments, Actimetrics Wireless Low-profile running wheel) and left undisturbed for three consecutive nights except for daily welfare checks.

Data were analyzed using ClockLab Data Software (Lafayette Instruments) in 1 min epochs and calculated as the cumulative time spent running and the average total distance traveled over 30 min periods.

**Homogenization and Protein Extraction for Western blotting**

Mice were sacrificed in small groups over four consecutive days at the same time of day. Sacrifices were conducted within two hours. Liver tissue (20-30mg) was homogenized in RIPA protein extraction reagent (Cell signaling); 1 mL per 100 mg of tissue weight) supplemented with protease and phosphatase inhibitors cocktail (Sigma-Aldrich) by using Zirconium Oxide Beads (Next Advance). Total protein concentration was measured using bicinchoninic acid (BCA; Pierce). Lysates were aliquoted and stored at -80C.

For analysis, protein lysates were boiled with loading buffer (Laemmli buffer and ßME) in a 1:1 ratio for 10 min at 95ºC. Proteins (20µg or 100µg) were separated on 4-20% mini-Protean TGX gels (Bio-Rad) SDS-PAGE, transferred to PVDF membranes for Trans-Turbo transfer (Bio-Rad), and incubated with specific primary antibodies against anti-p44/42 MAPK (Erk1/2), anti-Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204, anti-phospho-ACC, anti-ACC, anti-Phospho-S6 ribosomal, anti-S6 Ribosomal, anti-phospho-Akt (Thr308), anti-Akt (all Cell signaling). Membranes were washed three times with PBS-Tween and incubated with anti-Mouse HRP secondary antibody (Prometheus Protein Biology Products) for 1 h. After three consecutive washes, the blots were developed using a commercial chemiluminescence reagent ProSignal Femto (Prometheus). The proportion of these proteins was quantified by densitometric analysis using ImageJ (1.51v).

**RNA isolation**

Total RNA from liver and gastrocnemius muscle tissues of female WT and IGF1R heterozygous mice was isolated with 1 mL TRIzol Reagent (Invitrogen, CA, USA) and PureLink RNA Mini Kit (Invitrogen, CA, USA). Phase separation was performed with chloroform, and the aqueous phase recovered with 70% ethanol. DNase treatment was performed directly on the column. Eluted RNA quality was within the 260/280 of 1.9 and 2.0.