

Tissue-specific splicing and dietary interaction of a mutant *As160* allele determine muscle metabolic fitness in rodents

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Supplementary Information

Supplementary Table 1 The list of commercial antibodies used in this study

Antibody Name	Company	Cat No.	Species
anti-AS160	Merck Millipore	07-741	Rabbit
anti-pT642-AS160	Invitrogen	441071GCU	Rabbit
anti-GSK3a/b	Cell Signaling Technology	#5676	Rabbit
anti-pSer21/9-GSK3a/b	Cell Signaling Technology	#9331	Rabbit
anti-PKB	Cell Signaling Technology	#9272	Rabbit
anti-pT308-PKB	Cell Signaling Technology	#3038	Rabbit
anti-pS473-PKB	Cell Signaling Technology	#9271	Rabbit
anti-ACSL1(D2H5)	Cell Signaling Technology	#9189	Rabbit
anti-GLUT4 antibody	Abcam	ab654	Rabbit
anti-GLUT1 antibody	Abcam	ab652	Rabbit
anti-CPT1β	Abcam	ab134988	Rabbit
anti-CD36	Abcam	ab133625	Rabbit
anti-PPARδ	Proteintech	10156-2-AP	Rabbit
anti-LPL	Proteintech	16899-1-AP	Rabbit
anti-IRβ	Santa Cruz Biotechnology	sc-711	Rabbit
anti-FATP1	Santa Cruz Biotechnology	sc-25541	Rabbit
anti-GAPDH	Sigma	G8795	Mouse

Supplementary Table 2 Primer information for QPCR analysis of expression of target genes

Gene name	Forward primer	Reverse primer
<i>As160-s</i> (mouse)	5'-AAGGACTTCTCACCAAGGGGA-3'	5'-CTCCCTTCTCCATCACTGCA-3'
<i>As160-m</i> (mouse)	5'-AAGGACTTCTCACCAAGGGGA-3'	5'-AAGCAGTGTCCTGTCTGCATT-3'
<i>As160-l</i> (mouse)	5'-ATGCAGCATTGTCCCCGTCA-3'	5'-TCTCCCTTCTCCATCACTGA-3'
<i>As160-f</i> (mouse)	5'-ATGCAGCATTGTCCCCGTCA-3'	5'-CCATCACTTGACTCTGAAGCAG-3'

<i>As160-s</i> (rat)	5'-GAAGGACTTTCACCAAGGGG-3'	5'-CTCCCTTCTCCATCACTGCA-3'
<i>As160-m</i> (rat)	5'-GAAGGACTTTCACCAAGGGG-3'	5'-AGCCGTGCCTGTCATTG-3'
<i>As160-l</i> (rat)	5'-CAATGCAGTGTTCTCCCGTC-3'	5'-CCTCCCTCTCCATCACTGA-3'
<i>As160-f</i> (rat)	5'-CAATGCAGTGTTCTCCCGTC-3'	5'-GCCGTGCCTGTCATTG-3'
<i>As160-s</i> (human)	5'-ACCCACCTTCAAGCACAAAG-3'	5'-TTCTCCATCACTGCACTGTT-3'
<i>As160-m</i> (human)	5'-ACCCACCTTCAAGCACAAAG-3'	5'-GCAGTGTCTGTCGACTG-3'
<i>As160-l</i> (human)	5'-CAGTGCAGCAATCTTCGTC-3'	5'-CTCCCTCTCCATCACTGATTTC-3'
<i>As160-f</i> (human)	5'-CAGTGCAGCAATCTTCGTC-3'	5'-CCTCTCCATCACTGATTCTGA-3'
<i>Glut4</i>	5'-GCCATCGTCATTGGCATTCT-3'	5'-CGCTTAGACTCTTCGGGC-3'
<i>Glut1</i>	5'-TCCTTATTGCCAGGTGTT-3'	5'-CCTCGAAGCTTTTCAGCAC-3'
<i>Lpl</i>	5'-GAGATTCTCTGTATGGCACA-3'	5'-CTGCAGATGAGAAACTTTCTC-3'
<i>Cd36</i>	5'-CACAGATGCAGCCTCCTTC-3'	5'-AGCACACCACGACGTACA-3'
<i>Fatp1</i>	5'-GGGCATGGATGATCGGCTAT-3'	5'-TGTTCCCTGCTGAGTGGTAG-3'
<i>Fabp3</i>	5'-ACCTGGAAGCTAGTGGACAG-3'	5'-TCACGACCGACTTGACCTTC-3'
<i>Cpt1β</i>	5'-ATCGAACGTGCTGCTTCTT-3'	5'-ATTGCCGTAGAGGCTGAGA-3'
<i>Cpt2</i>	5'-AGGCCCTTAAGTGCTGTC-3'	5'-TCCTGAACCTGGCTGTCATCC-3'
<i>Acadl</i>	5'-AAGAATGGAGAAAGCCGGA-3'	5'-TGACAATATCTGAATGGAGGCTG-3'
<i>Hadha</i>	5'-AGCTCGACTACAAGGGCTTC-3'	5'-CTGGAGTCACGCTTCCACT-3'
<i>Hadhb</i>	5'-TATCTGGGCGGATCACTCT-3'	5'-CATAGCATGACCCCTGCTCC-3'
<i>Acaa2</i>	5'-GCTCCTCAGTTCTGGCTGT-3'	5'-CCACCTCGACGCCCTAACTC-3'
<i>Acsll</i>	5'-AATGGCTGAGTGCATAGGCT-3'	5'-GATGGTCACCCACTCAGGTC-3'
<i>Pparδ</i>	5'-AGCAGCTGCACAGACCTCTC-3'	5'-ACTTGAGCTTCATGCGGATT-3'
<i>Pgc1α</i>	5'-AGCCTTTGCCAGATCTT-3'	5'-GCAATCCGTCTTCATCCACC-3'
<i>Perm1</i>	5'-CGTAAGAAGAGGCGTGGTGT-3'	5'-CTCCTGCTGGCCATCTGTT-3'
<i>Etfb</i>	5'-AGACCATCCGAAC TGCTCTG-3'	5'-ATCAATAGCCTGCTTGGCCA-3'
<i>Etfα</i>	5'-TGCTGGCTTGTCCCAATG-3'	5'-GTCTGCTGTCCTTCATCCC-3'
<i>Etfdh</i>	5'-AGGAAAAGGACATCCGTGTG-3'	5'-ACCTGGAAGAATTGGCACAG-3'
<i>Ndufa9</i>	5'-ATGTCACGTCTGCCATTCT-3'	5'-ACTTGTGACCCATTGTC-3'
<i>Ndufaf4</i>	5'-AAAAGCCATTGAACCACAGC-3'	5'-CAGCAGTCCATGTCCTGGA-3'
<i>Sdhb</i>	5'-AATAAGTGTGGACCGATGGTG-3'	5'-CCAAGGTCTGTCGATCCT-3'
<i>Uqcrq</i>	5'-ACGGCATGTGATCTCCTACA-3'	5'-GATTCTCCGTTGGACTGC-3'
<i>Uqcc1</i>	5'-AATGGACACACCGCACAGTA-3'	5'-CGCGATCTGATCTCCATT-3'
<i>Coa5</i>	5'-CCCCGGTATTATGAGGACAA-3'	5'-TCTTGATCTGGCATCCAACA-3'
<i>Cox10</i>	5'-GGTCAGCACTTGAGCTTCC-3'	5'-CTTCTGGACAGAGGCAGAC-3'
<i>Hccs</i>	5'-AAGGAAAGGCTGCCAGTGA-3'	5'-GATTAGGTGGCTGCAGG-3'
<i>Tfb2m</i>	5'-TTAAGGAAGAACGCGGATGG-3'	5'-ACACCTGCTGACCAAGGAAC-3'
<i>Mrpl14</i>	5'-AGCGCAATCCAGAAGATGAC-3'	5'-CCACGATGAGTGTCTTCTT-3'

<i>Cluh</i>	5'-CCCTCAGTCATGCTGTTGAA-3'	5'-ATGAGCACCTGGTGGATCTC-3'
<i>Mrm3</i>	5'-GGAAAAGCAAGGGAAAGATCC-3'	5'-GTCAGGTTGGCAAAATCC-3'
<i>Idh2</i>	5'-ACCGTGACCAGACCAATGAC-3'	5'-ATCGGGGGTGTGATTGTAGCAC-3'
<i>Mdh1</i>	5'-AGACCAGCCCATCATTCTG-3'	5'-TCCAGGTCTTGAGGCAAC-3'
<i>Gpam</i>	5'-ACTGGGTTGACTGTGGCTTC-3'	5'-GCAAACCCAGAGATGGGATA-3'
<i>Cact</i>	5'-TGCAGACACAACCACCAAGT-3'	5'-AACAGCTGCGGGTAGGTAA-3'
<i>Slc25a34</i>	5'-TTGTGGGGAGTCCTGCTTAC-3'	5'-CAGCTGAACCCACTGTGACT-3'
<i>Pex11a</i>	5'-CCGACTTTTCAGAGCCACTC-3'	5'-CGGTTCAGGTTGGCTAATGT-3'
<i>Pex12</i>	5'-TGTGGTCAAGGTTCTGCAG-3'	5'-CGCTACAATCCGCTTAACC-3'
<i>Prdx1</i>	5'-CCGGATGGACAATTCAAAGATA-3'	5'-ATCCTCCTGTTCTTGGGTGT-3'
<i>Prdx5</i>	5'-AAAGGAGCAGGTTGGGAGTG-3'	5'-GCAGATGGTCTTGGAACAG-3'
<i>Acox1</i>	5'-CGCCGTCGAGAAATTGAGAAC-3'	5'-AGGCCAACAGGTTCCACAAA-3'
<i>Ech1</i>	5'-TGATCACCGCAATTACGGGA-3'	5'-CTCCGGTCCCAATGACCTT-3'
<i>actin</i>	5'-GAGAGGGAAATCGTGCCTGACA-3'	5'-GTTTCATGGATGCCACAGGAT-3'

Supplementary figure legends

Supplementary Figure 1 Tissue expression of *As160* variants in mouse, rat and human

A-C. Expression of *As160* variants in mouse skeletal muscle (A), heart (B) and WAT (C). n = 6.
D-F. Sequencing of dominant *As160* mRNA variants at the exon boundaries in mouse skeletal muscle (D), heart (E) and WAT (F).

G-I. Expression of *As160* variants in rat skeletal muscle (G, n = 6), heart (H, n = 3) and WAT (I, n = 5).

J-L. Sequencing of dominant *As160* mRNA variants at the exon boundaries in rat skeletal muscle (J), heart (K) and WAT (L).

M-N. Expression of *As160* variants in human skeletal muscle (M) and WAT (N). n = 6.

O-P. Sequencing of dominant *As160* mRNA variants at the exon boundaries in human skeletal muscle (O) and WAT (P).

The data are given as the mean ± SEM. *** indicates p < 0.001.

Supplementary Figure 2 Glucose homeostasis and insulin signalling in the AS160^{R691X} mice

A. Oral glucose tolerance test in the WT and AS160^{R691X} female mice at age of 12 weeks. The values show the glucose area under the curve during glucose tolerance test. n = 7-10.

B. Blood glucose levels in the WT and AS160^{R691X} female mice (8-week-old) in the fasting-refeeding assay. n = 7-10.

C. Expression of *As160*-s in the WAT of WT and AS160^{R691X} male mice. n = 6.

D-E. Expression and phosphorylation of PKB and GSK3 in soleus (D) and EDL (E) muscle of WT and AS160^{R691X} male mice (12-week-old) in response to insulin.

The data are given as the mean ± SEM.

Supplementary Figure 3 Body weight and whole-body glucose homeostasis in the AS160^{R693X} rats

A. Body weight gain in the AS160^{R693X} male rats. n = 9.

B. Fasting blood glucose in the WT and AS160^{R693X} female rats at age of 8 weeks. n = 5.

C. Blood glucose levels in the WT and AS160^{R693X} female rats (8-week-old) in the fasting-refeeding assay. n = 5.

The data are given as the mean ± SEM. * indicates p < 0.05.

Supplementary Figure 4 Expression of *Glut4* and *Glut1* mRNAs in skeletal muscle of the AS160^{R693X} rats

A-B. Expression of *Glut4* (A) and *Glut1* (B) in soleus muscle of AS160^{R693X} rats. n = 3.

C-D. Expression of *Glut4* (C) and *Glut1* (D) in EDL muscle of AS160^{R693X} rats. n = 4.

E-F. Expression of *Tbc1d1* (E) and *Hk2* (F) in gastrocnemius muscle of AS160^{R693X} rats. n = 5-6.

G-I. Expression of TBC1D1 and HK2 in gastrocnemius muscle of AS160^{R693X} rats. Quantitation of TBC1D1 is shown in 4G, and quantitation of HK2 in 4H. Representative blots are shown in 4I. n = 6.

The data are given as the mean ± SEM.

Supplementary Figure 5 RNA-Seq analyses of mRNA expression in skeletal muscle of the AS160^{R693X} rats

A. Volcano plot of differentially-expressed genes in skeletal muscle of the AS160^{R691X} mice.

B. Volcano plot of differentially-expressed genes in skeletal muscle of the AS160^{R693X} rats.

C. KEGG pathway analysis of differentially-expressed genes in skeletal muscle of the AS160^{R693X} rats.

D. Cellular component analysis of differentially-expressed genes in skeletal muscle of the AS160^{R693X} rats.

Supplementary Figure 6 Effects of down-regulation of AS160 on lipid metabolism in L6 myocytes

A. Protein expression of AS160 in WT control and AS160-knockdown (AS160-KD) L6 myocytes. AS160-KD myocytes were generated by stably-expressing shRNA targeting AS160 (the sequence of shRNA: 5'-TCTTGAAATTCTTCGAGC-3').

B. Fatty acid uptake in L6 myocytes in response to insulin. n = 6.

C-E. Cell surface and total CD36 levels in WT control and AS160-KD myocytes stimulated with or without insulin. C, representative blots. D-E, quantitative results of total (D) and cell surface (E) CD36 levels. n = 3.

The data are given as the mean ± SEM. * indicates $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. n.s., not significant.

Supplementary Figure 7 Body and tissue weights in the AS160^{R693X} rats on the CD and HSD

A. Body weight gain in the WT and AS160^{R693X} male rats on the CD and HSD. n = 5-7.

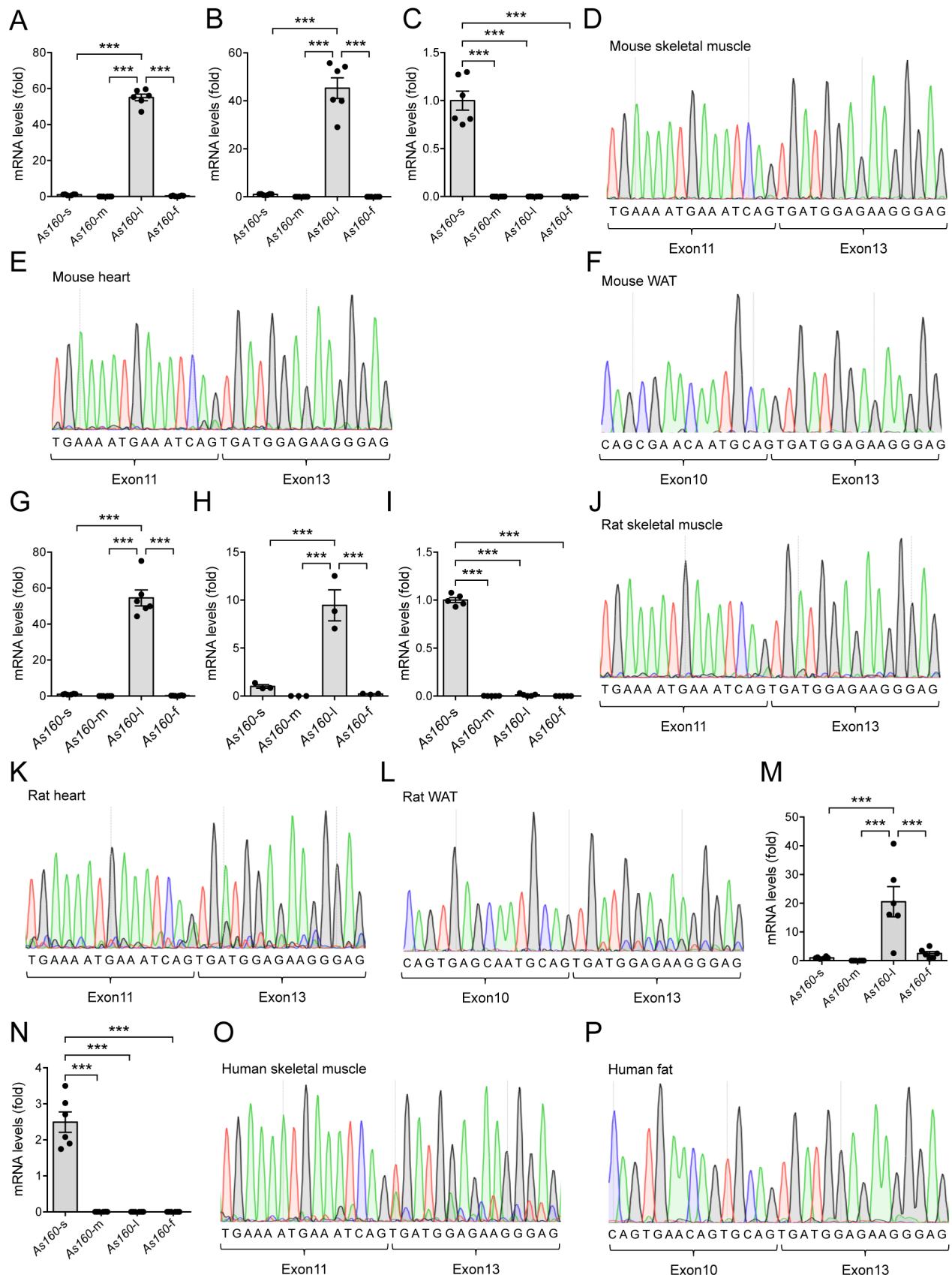
B. Weight of tibialis anterior (TA) muscle in WT and AS160^{R693X} male rats on the CD and HSD. n = 6-7.

C. Liver weight in WT and AS160^{R693X} male rats on the CD and HSD. n = 6-7.

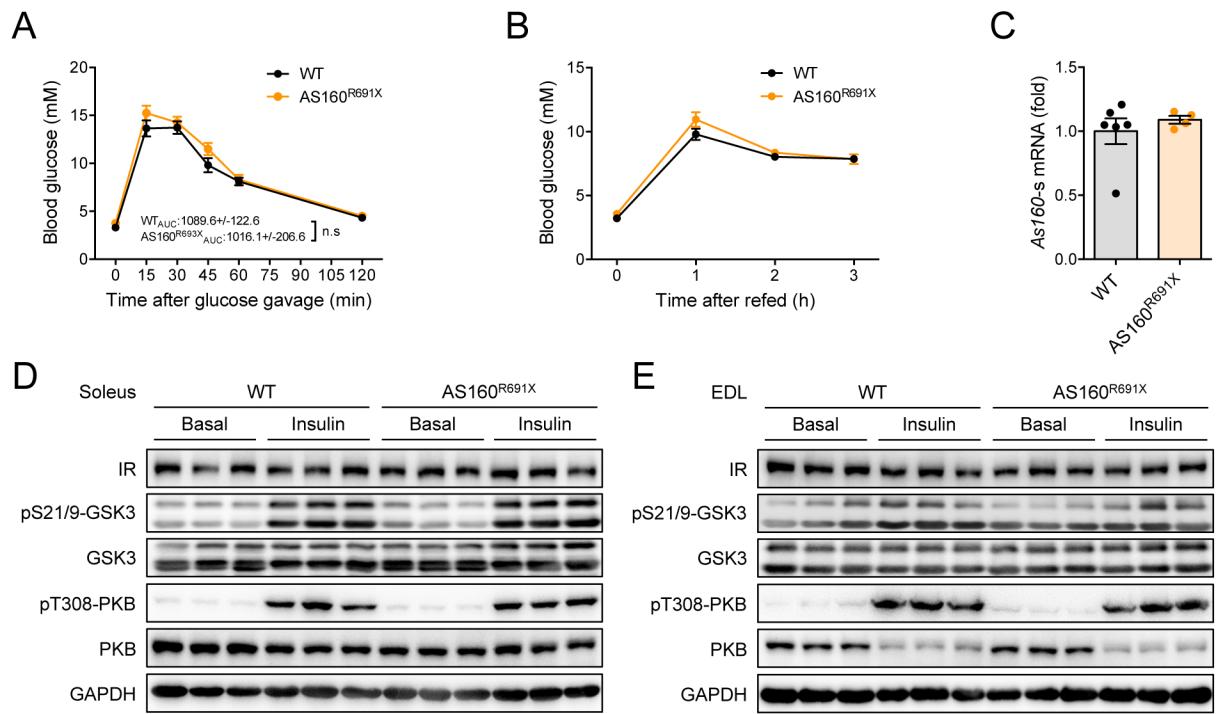
D-F. Expression and phosphorylation of HSL in WAT of CD-fed WT and AS160^{R693X} male rats during fasting and refeeding treatment. Representative blots are shown in 7D. Quantitation of pS563-HSL is shown in 7E, and quantitation of total HSL in 7F. n = 4-5.

The data are given as the mean ± SEM. n.s., not significant.

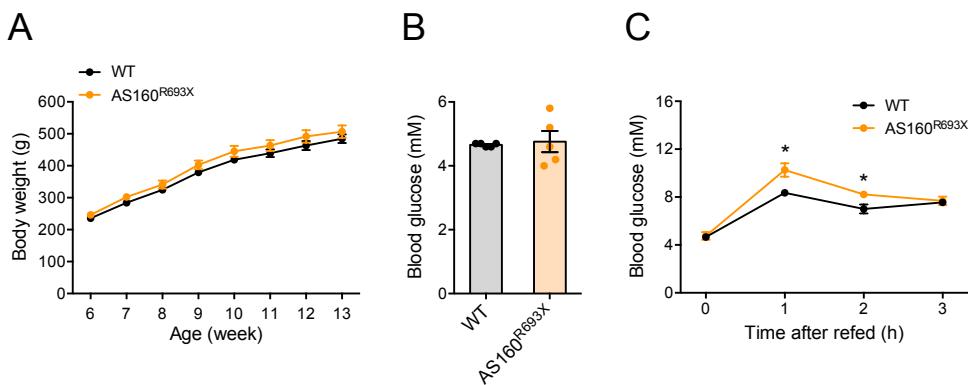
Suppl Figure 1



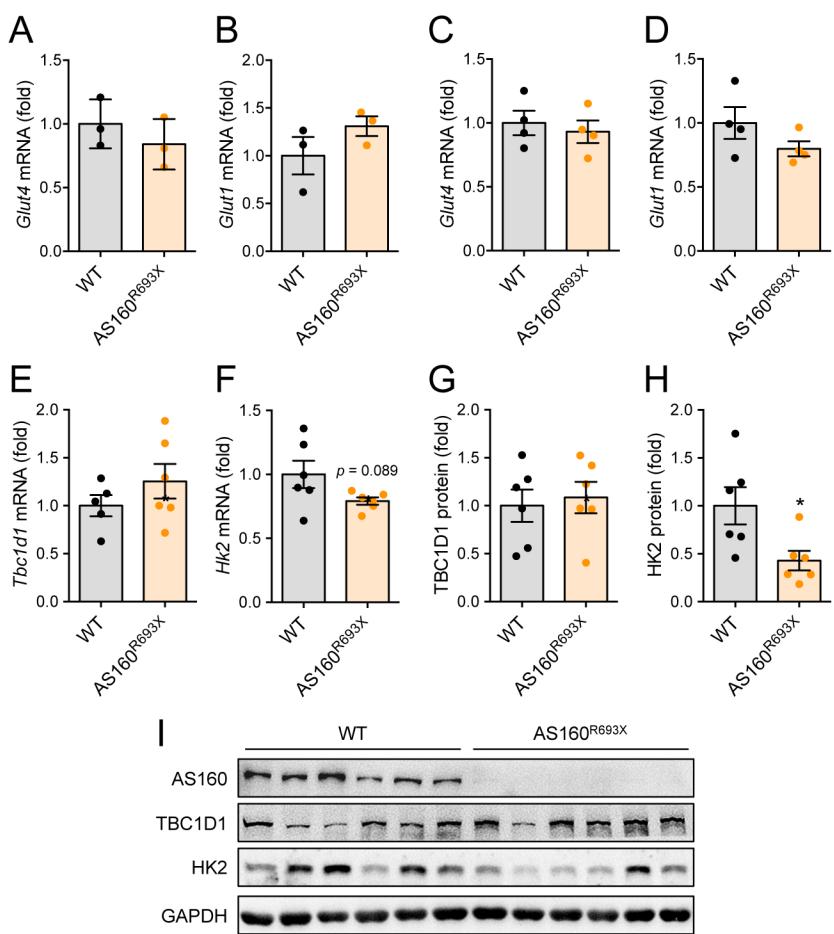
Suppl Figure 2



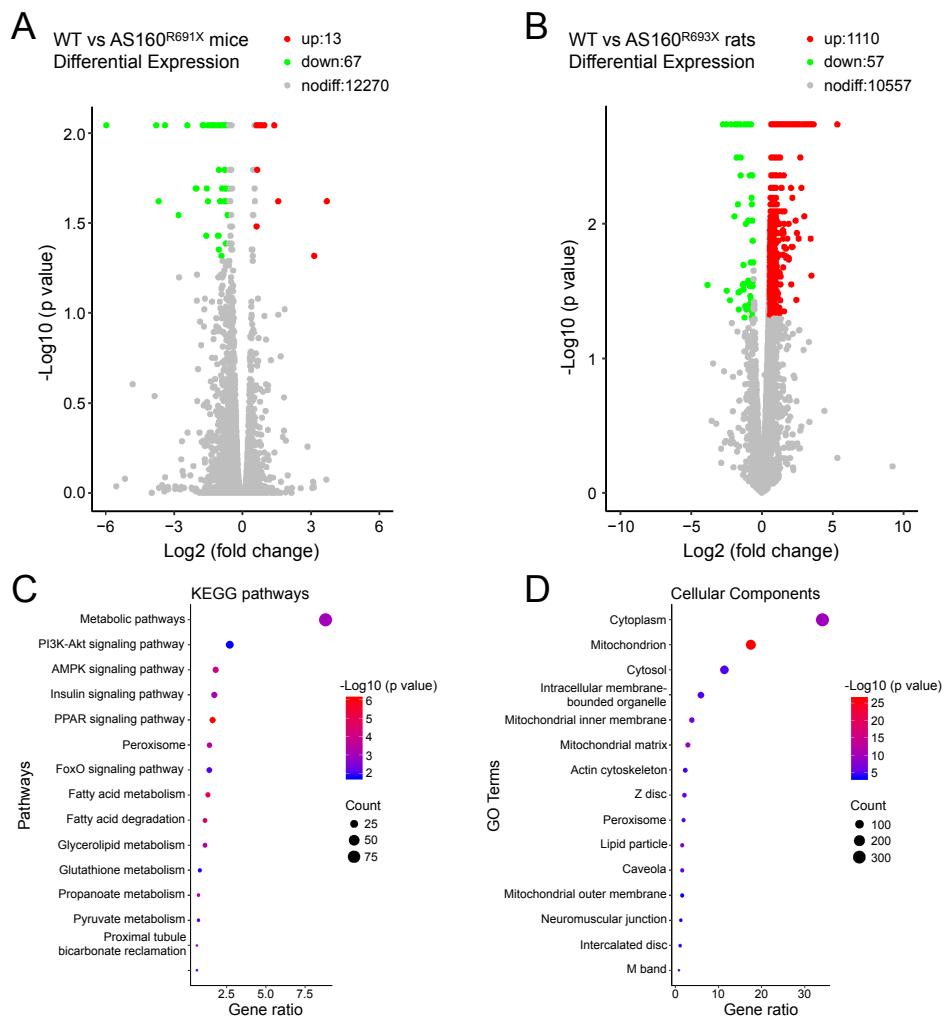
Suppl Figure 3



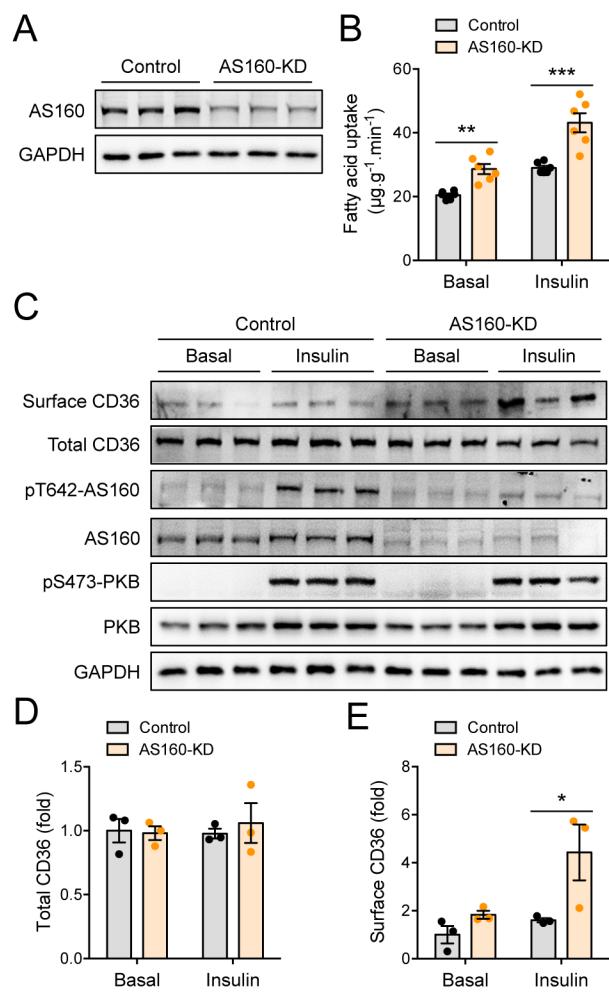
Suppl Figure 4



Suppl Figure 5



Suppl Figure 6



Suppl Figure 7

