SUPPLEMENTARY DATA

The inactivation of RabGAP function of AS160 promotes lysosomal degradation of GLUT4 and causes postprandial hyperglycemia and hyperinsulinemia

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

Antibody Name	Company	Cat No.
anti-AS160	Millipore	07-741
anti-pT649-AS160	Life Technologies	441071G
anti-IRAP	Life Technologies	A16604
anti-TfR	Life Technologies	13-6800
anti-PPARγ	Cell Signaling Technology	#2435
anti-perilipin	Cell Signaling Technology	#3467
anti-TBC1D1	Cell Signaling Technology	#4629
anti-PKB	Cell Signaling Technology	#9272
anti-pS473-PKB	Cell Signaling Technology	#9271
anti-LAMP1	Cell Signaling Technology	#9091
anti-IR	Santa Cruz	sc-711
anti-GAPDH	Sigma	G8795

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Supplementary Table 2. Primer information for genotyping

The tissue-specific AS160 knockout mice were genotyped for two loci including the AS160 flox allele and the Cre. The $AS160^{E6/7KO}$ mice were genotyped using three primers to detect WT and KO allele simultaneously.

Mouse Name	Forward primer	Reverse primer
AS160 flox	5′-TGGGCTACAAAATGAGACGATC-3′	5′-AGGAAAGTCAAGGGCTAGCC-3′
Cre	5′-AATGCTTCTGTCCGTTTGC-3′	5′-ACCAGAGTCATCCTTAGCG-3′
$AS160^{E6/7KO}$	5′-AAGGTTTGCTGCACATCACA-3′	5′-TGTTAGTCCCAACCCCTTCC-3′(KO allele) 5′-CTGCTGAGCTACCCCATCAT-3′(WT allele)
$AS160^{R917K}$	5′-AAGAGTAAATCTGGTCCTAG-3′	5′-AACGACCACTCTCTTCCCAC-3′

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Supplementary Table 3. Primer information for QPCR analysis of expression of target genes

Gene name	Accession No.	Forward primer	Reverse primer
AS160	NM_001081278	5′-GAAGGGCCGGCGATTATTTC-3′	5′-TACTTCCAAGCCGACCTCTC-3′
*glut*4	NM_009204	5′-GCCATCGTCATTGGCATTCT-3′	5′-CGCTTTAGACTCTTTCGGGC-3′
*glut*1	NM_011400	5′-ATGAAAGAAGAGGGTCGGCA-3′	5′-TCCAGCTCGCTCTACAACAA-3′
36B4/Rplp0	NM_007475	5′-TAAAGACTGGAGACAAGGTG-3′	5′-GTGTACTCAGTCTCCACAGA-3′
GAPDH	NM_008084	5′-AGGTCGGTGTGAACGGATTTG-3′	5′-TGTAGACCATGTAGTTGAGGTCA-3′

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Supplementary Table 4. Blood chemistry in the AS160E10KO mice upon fasting or refeeding
The male wild-type and AS160^{E10KO} mice (6-month-old) were fasted overnight (16 h) or refed with normal diet for 1 h after overnight fasting. Blood was withdrawn via tail bleeding, and blood chemistry was determined as described in the materials and methods. WT, n = 8; AS160^{E10KO}, n = 5. The data are given as the mean ± SEM.

blood chemistry	fasted		refed	
	WT	AS160^{E10KO}	WT	AS160^{E10KO}
FFA (mEq/L)	1.242 ± 0.090	1.489 ± 0.096	0.398 ±0.045	0.303 ± 0.038
TG (mM)	1.243 ± 0.091	1.516 ± 0.229	0.675 ± 0.094	0.580 ± 0.080
TC (mM)	2.752 ± 0.075	2.750 ± 0.075	2.730 ± 0.111	2.646 ± 0.071
HDL-C (mM)	2.078 ± 0.057	2.032 ± 0.018	2.129 ± 0.097	2.072 ± 0.057
LDL-C (mM)	0.173 ± 0.013	0.194 ± 0.029	0.139 ± 0.017	0.122 ± 0.013

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Supplementary Table 5. Plasma levels of amino acids in the AS160^{E10KO} mice upon fasting or refeeding

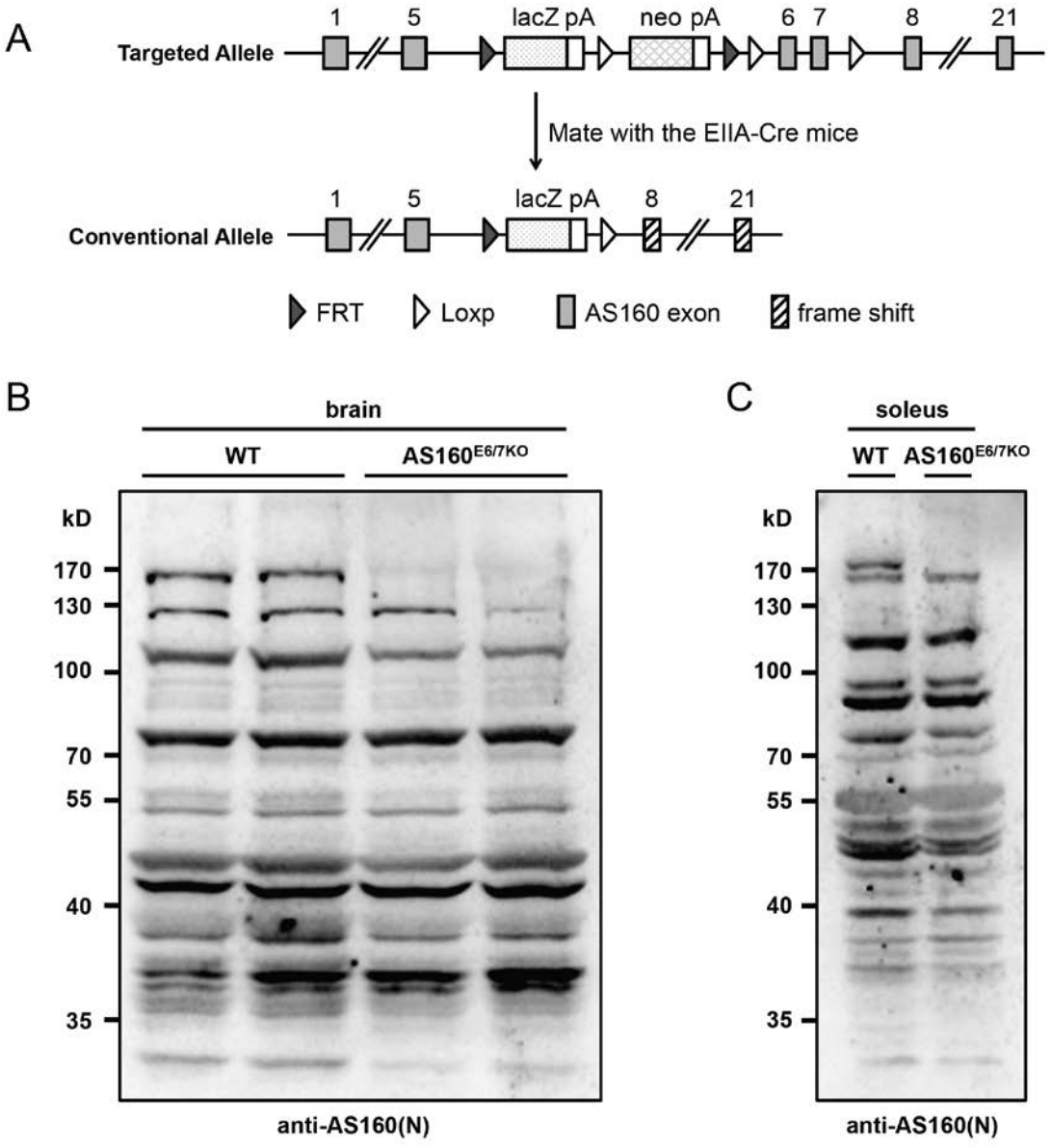
The male wild-type and AS160^{E10KO} mice (2-month-old) were fasted overnight (16 h) or refed with normal diet for 1 h after overnight fasting. Blood was withdrawn via tail bleeding, and amino acids in the plasma were determined as described in the materials and methods. WT fasted, n = 7; AS160^{E10KO} fasted, n = 4; WT refed, n = 6; AS160^{E10KO} refed, n = 6. The data are given as the mean ± SEM. n.d., not detectable. * $p < 0.05$, ** $p < 0.01$.

plasma amino acids	fasted		refed	
	WT	AS160^{E10KO}	WT	AS160^{E10KO}
Ser (μM)	339.2 ± 9.8	330.6 ± 17.5	176.5 ± 8.9	177.5 ± 9.8
Glu (μM)	n.d.	n.d.	8.2 ± 3.9	14.4 ± 4.6
Gly (μM)	722.1 ± 91.0	648.1 ± 133.3	214.3 ±11.0	343.1 ± 94.9
His (μM)	780.6 ± 27.7	960.6 ± 39.4**	288.8 ± 8.5	281.7 ± 11.8
Arg (μM)	246.2 ± 6.7	253.2 ± 17.5	111.7 ± 7.6	100.5 ± 5.7
Thr (μM)	429.2 ± 20.9	420.4 ± 33.1	224.6 ± 11.5	222.3 ± 9.7
Ala (μM)	402.3 ± 12.3	369.7 ± 39.4	525.6 ± 31.9	498.5 ± 22.8
Pro (μM)	183.7 ±5.0	187.7 ± 9.5	152.6 ± 5.4	139.5 ± 6.6
Tyr (μM)	180.2 ± 3.0	179.2 ± 5.3	83.3 ± 3.9	70.0 ± 1.6**
Val (μM)	414.7 ± 14.7	393.1 ± 33.5	191.0 ± 6.9	164.6 ± 7.1*
Met (μM)	162.2 ± 3.7	156.0 ± 9.1	117.8 ± 2.8	107.6 ± 3.2*
Lys (μM)	458.7 ± 12.6	442.7 ± 27.0	279.5 ± 13.6	247.1 ± 20.2
Ile (μM)	246.0 ± 9.8	244.9 ± 22.6	73.1 ± 2.6	61.2 ± 1.9**
Leu (μM)	385.6 ± 14.6	379.2 ± 35.1	134.4 ± 5.4	113.3 ± 4.5*
Phe (μM)	206.2 ± 4.1	214.2 ± 11.6	86.0 ± 1.5	84.9 ± 2.8

SUPPLEMENTARY DATA

Supplementary Figure 1. Generation and characterization of the AS160$^{f/f}$ and AS160$^{E6/7KO}$ mice
A. Diagram of strategy for generating the AS160$^{f/f}$ and AS160$^{E6/7KO}$ mice. B. AS160 expression in lysates of brain from the WT and AS160$^{E6/7KO}$ mice. The sheep anti-AS160(N) antibody recognizing the N-terminus (1−280 aa) of AS160 was used to detect full-length AS160 and the putative N-terminal fragment. No N-terminal fragment of AS160 could be detected.

C. AS160 expression in lysates of soleus muscle from the WT and AS160$^{E6/7KO}$ mice. The sheep anti-AS160(N) antibody recognizing the N-terminus (1−280 aa) of AS160 was used to detect full-length AS160 and the putative N-terminal fragment. No N-terminal fragment of AS160 could be detected.



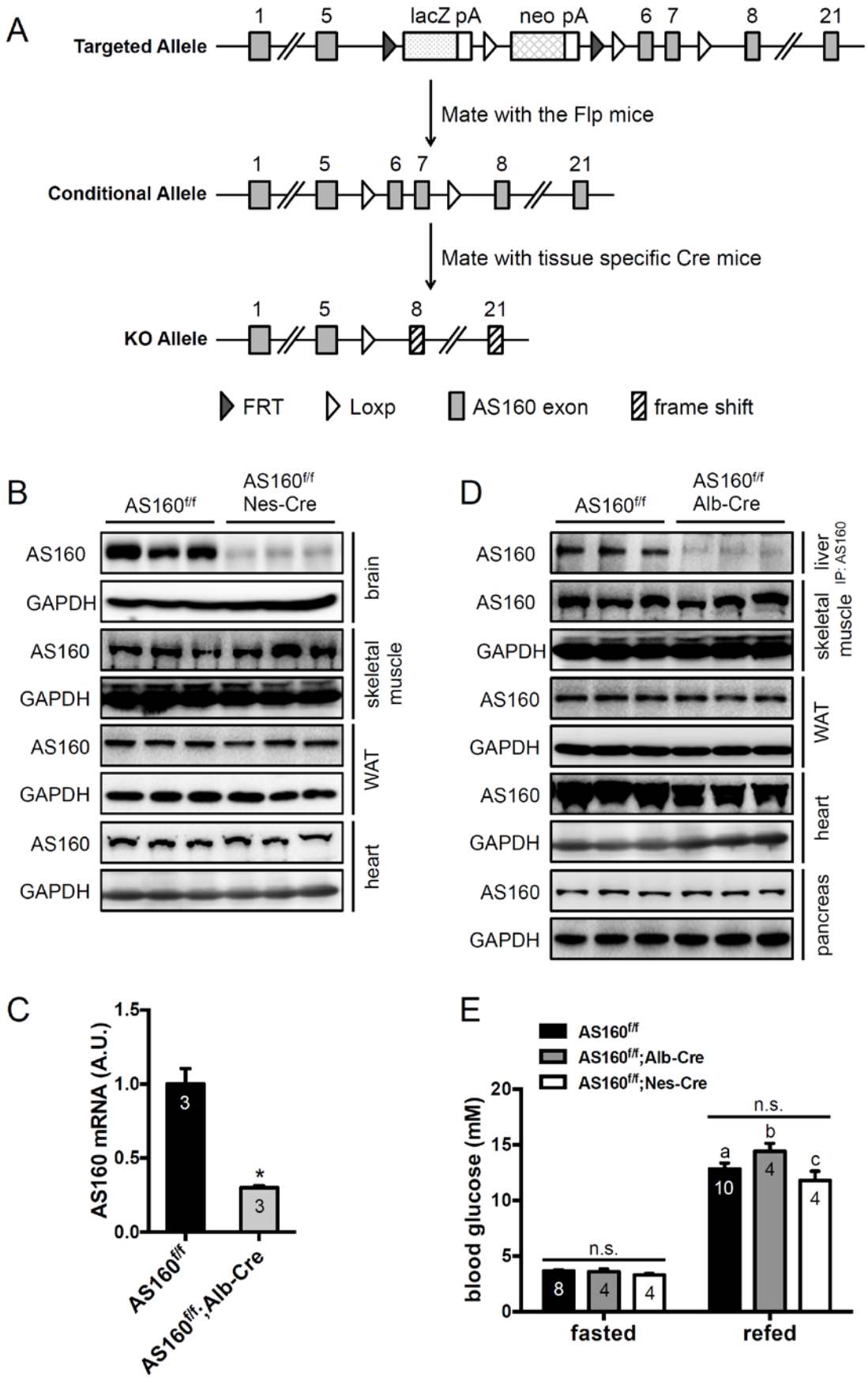
SUPPLEMENTARY DATA

Supplementary Figure 2. Generation and characterization of the tissue-specific AS160 knockout mice

A. Diagram of strategy for generating the tissue-specific AS160 knockout mice.
B. AS160 protein levels in various tissues of the $AS160^{f/f}$;Nes-Cre mice.
C. AS160 mRNA levels in the liver of the $AS160^{f/f}$;Alb-Cre mice.
D. AS160 protein levels in various tissues of the $AS160^{f/f}$;Alb-Cre mice.
E. Blood glucose levels before and after refeeding for 60 min with normal diet after an overnight fast in male AS160 liver- or neuron-specific knockout mice at 6 weeks of age. a indicates $p < 0.001$ ($AS160^{f/f}$ refed vs $AS160^{f/f}$ fasted), b indicates $p < 0.001$ (AS160f/f;Alb-Cre refed vs $AS160^{f/f}$;Alb-Cre fasted), and c indicates $p < 0.001$ (AS160f/f;Nes-Cre refed vs $AS160^{f/f}$;Nes-Cre fasted).

Data are given as means ± SEM. Statistical analyses were carried out via t-test for C, or via two-way ANOVA for E. n.s., not significant. * $p < 0.05$. The numbers shown on the columns indicate the number of samples used in the experiments.

SUPPLEMENTARY DATA

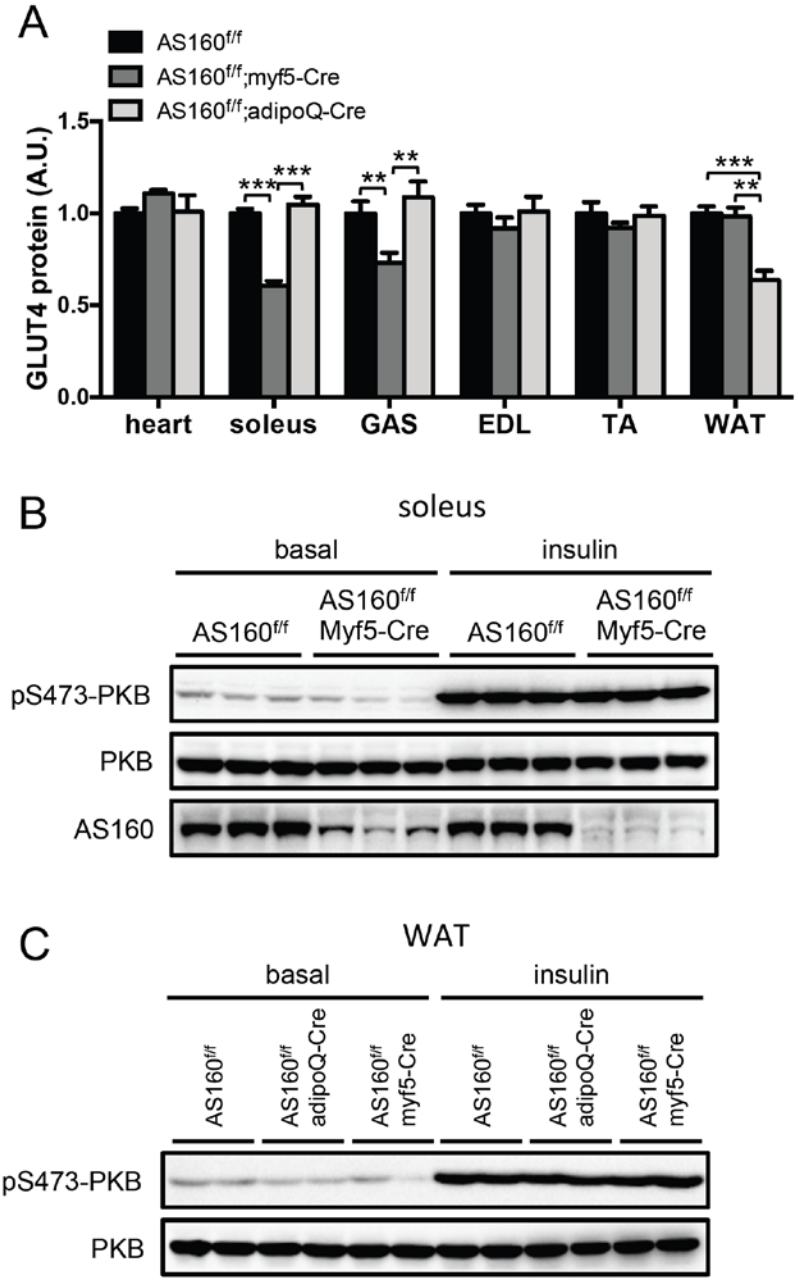


SUPPLEMENTARY DATA

Supplementary Figure 3. GLUT4 quantitation and PKB phosphorylation in the muscle- and fat-specific AS160 knockout mice

A. Quantitation of GLUT4 in various tissues of the muscle- and fat-specific AS160 knockout mice. Representative blots were shown in Fig. 3E. At least four samples per tissue were used for quantitation. Statistical analyses were carried out via t-test. ** $p < 0.01$, *** $p < 0.001$.

B. PKB phosphorylation in soleus muscle of the skeletal muscle-specific AS160 knockout mice in response to insulin. C. PKB phosphorylation in WAT of the skeletal muscle- or adipose-specific AS160 knockout mice in response to insulin.!



SUPPLEMENTARY DATA

Supplementary Figure 4. Generation and characterization of the AS160^{R917K} knockin mice
A. Diagram of strategy for generating the AS160^{R917K} mice. The Arg^{917} (the surrounding sequence is LVDLG**r**TFP, Arg^{917} shown in lower case bold, and numbering is according to NP_001074747.2) on AS160 was changed to lysine by point mutagenesis. The selection marker, Neo gene, which was flanked by *loxP* sites was removed via *in vivo* Cre-mediated cleavage in the AS160^{R917K} knockin mouse.
B. Genomic DNA sequences of the WT and knockin AS160 allele in the heterozygous AS160^{R917K} mice.
C. Quantitation of GLUT4 in various tissues of the AS160^{R917K} knockin mice. The blots shown in Fig. 5B were used for quantitation. n = 3. Statistical analyses were carried out via t-test. ** $p < 0.01$, *** $p < 0.001$.