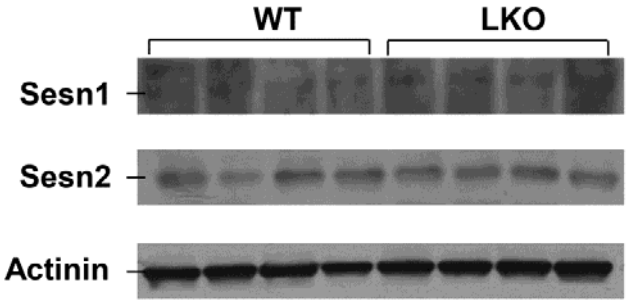
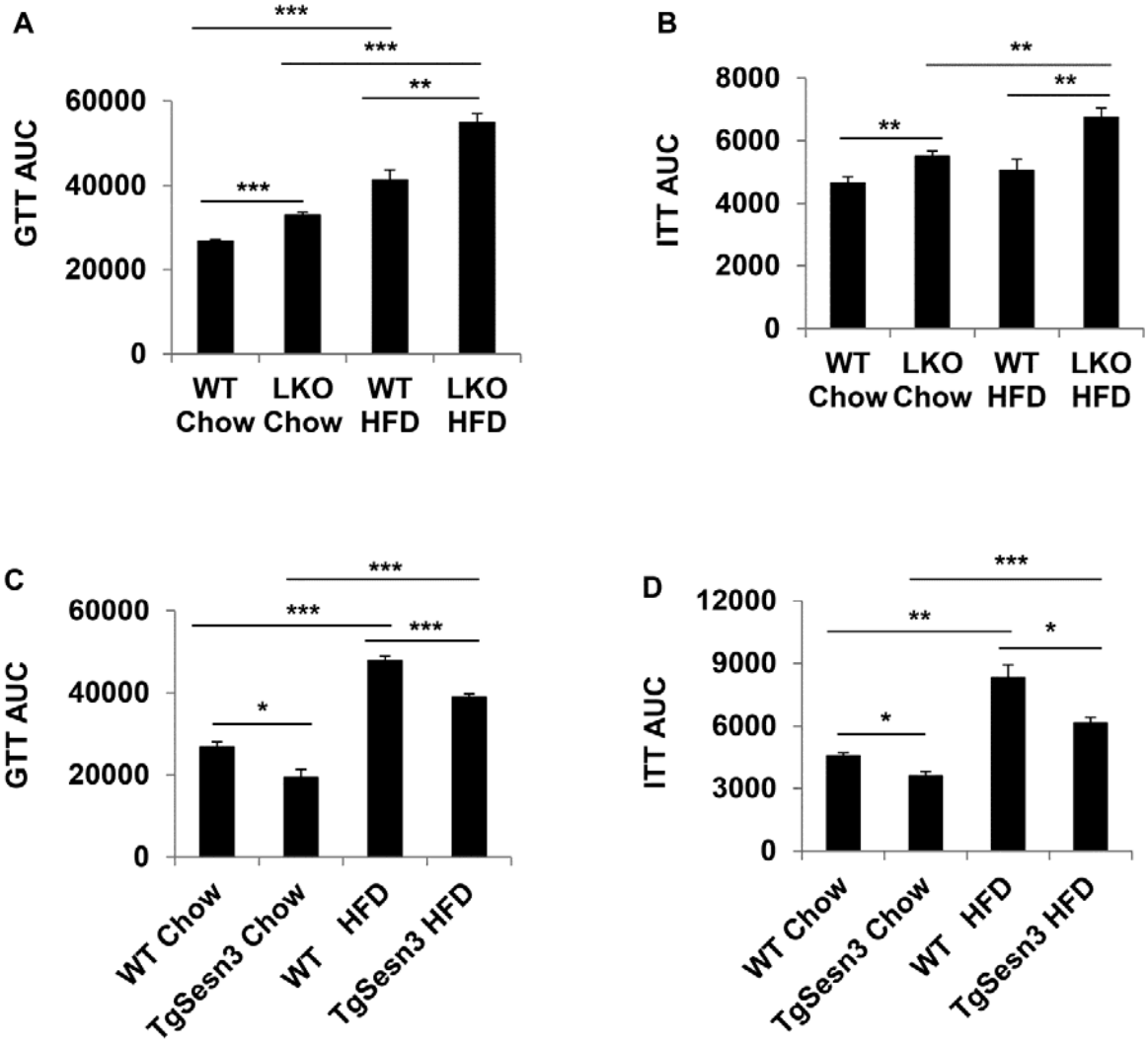
SUPPLEMENTARY DATA

Supplementary Figure 1. Immunoblot analysis of Sesn1 and Sesn2 proteins. Sesn1/2 were analyzed in WT and Sesn3-LKO liver tissues by Western blots.



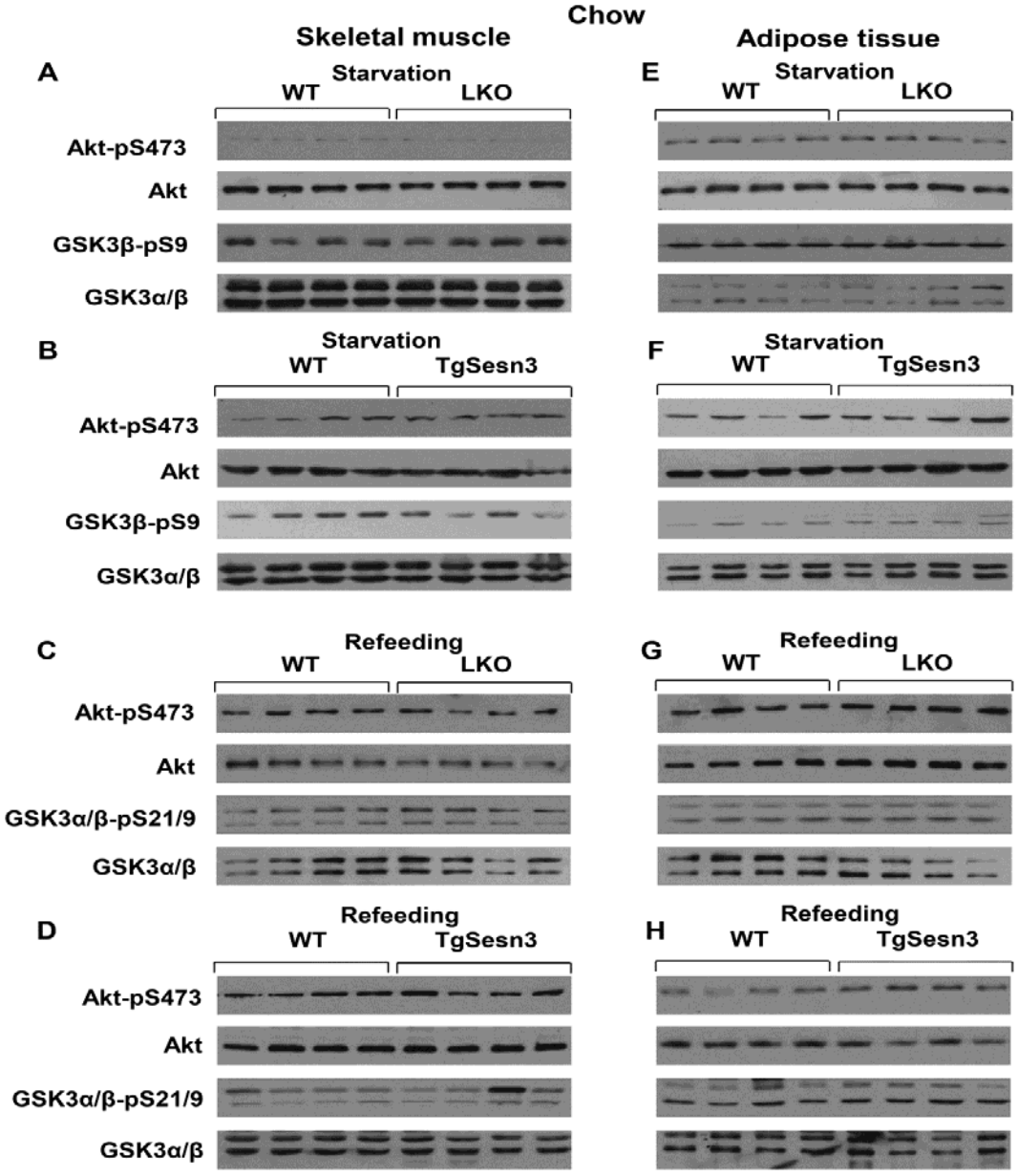
SUPPLEMENTARY DATA

Supplementary Figure 2. The area-under-curve (AUC) analysis of the GTT and ITT data in main Fig. 2. *A, B*: The AUC analysis of GTT and ITT data in chow and HFD fed WT and Sesn3-LKO mice. *C, D*: The AUC analysis of GTT and ITT data in chow and HFD fed WT and TgSesn3 mice. Data are presented as mean ± SEM. **P*<0.05, ***P*<0.0, 1 and ****P*<0.001.



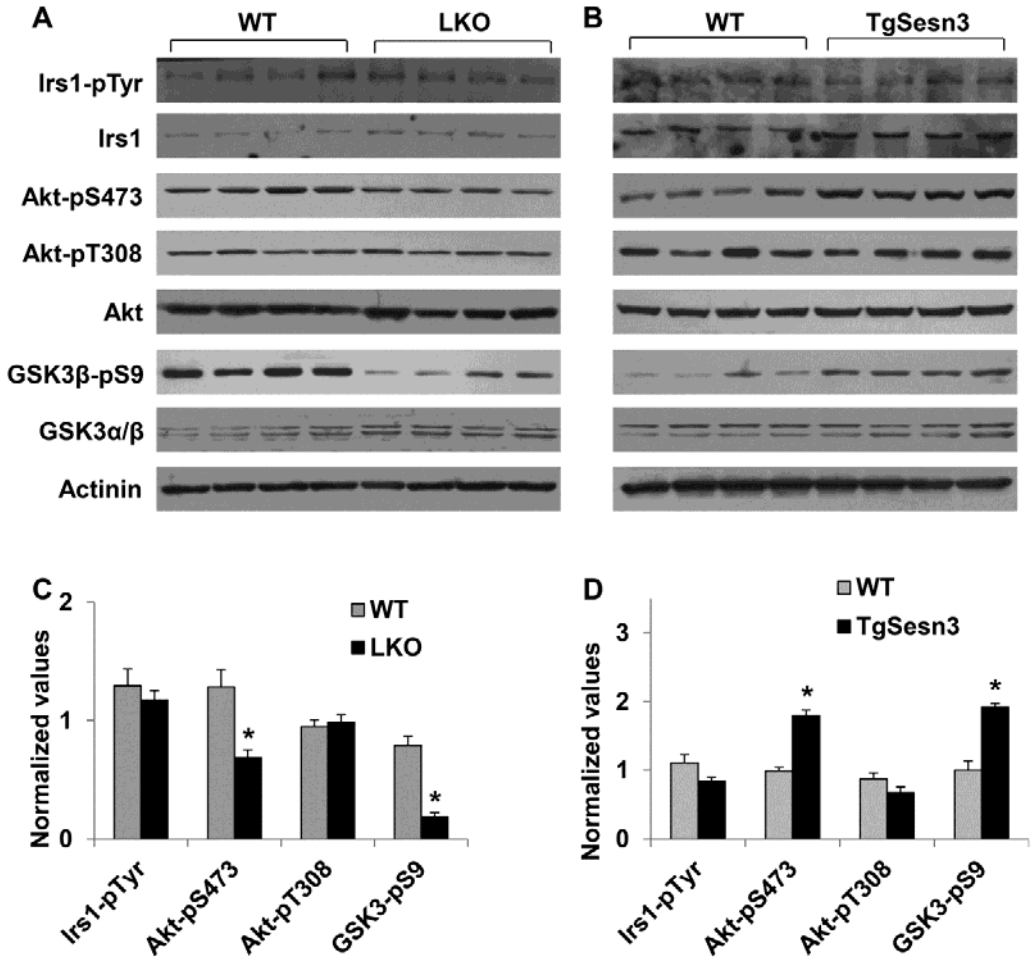
SUPPLEMENTARY DATA

Supplementary Figure 3. Signaling analysis in skeletal muscle and white adipose tissue. *A-H*: Akt and GSK3 phosphorylated and total protein levels were analyzed by Western blots in skeletal muscle and white adipose tissue from chow-fed WT, Sesn3-LKO, and TgSesn3 mice under starvation and refeeding conditions.



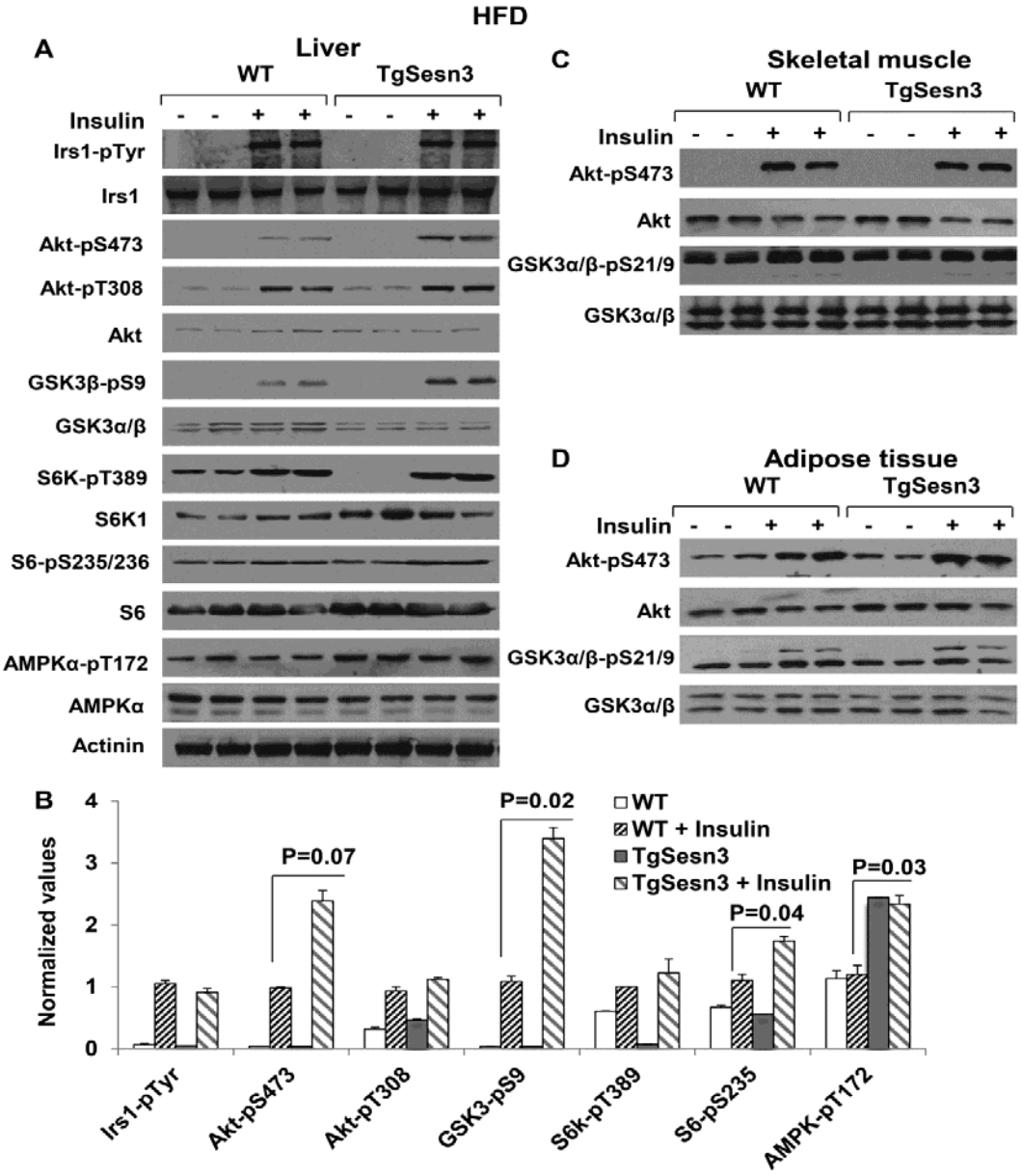
SUPPLEMENTARY DATA

Supplementary Figure 4. Analysis of insulin signaling in chow-fed WT, Sesn3-LKO, and TgSesn3 livers. *A, B*: Western blot analysis of insulin signaling proteins in the liver of WT, Sesn3-LKO and TgSesn3 mice after insulin stimulation, respectively. *C, D*: Quantitative analysis of panels A and B, respectively. Normalization was performed by calculating the ratio of each signaling event in the Sesn3-LKO or TgSesn3 to that in the WT mice. Data are presented as mean ± SEM. **P*<0.05.



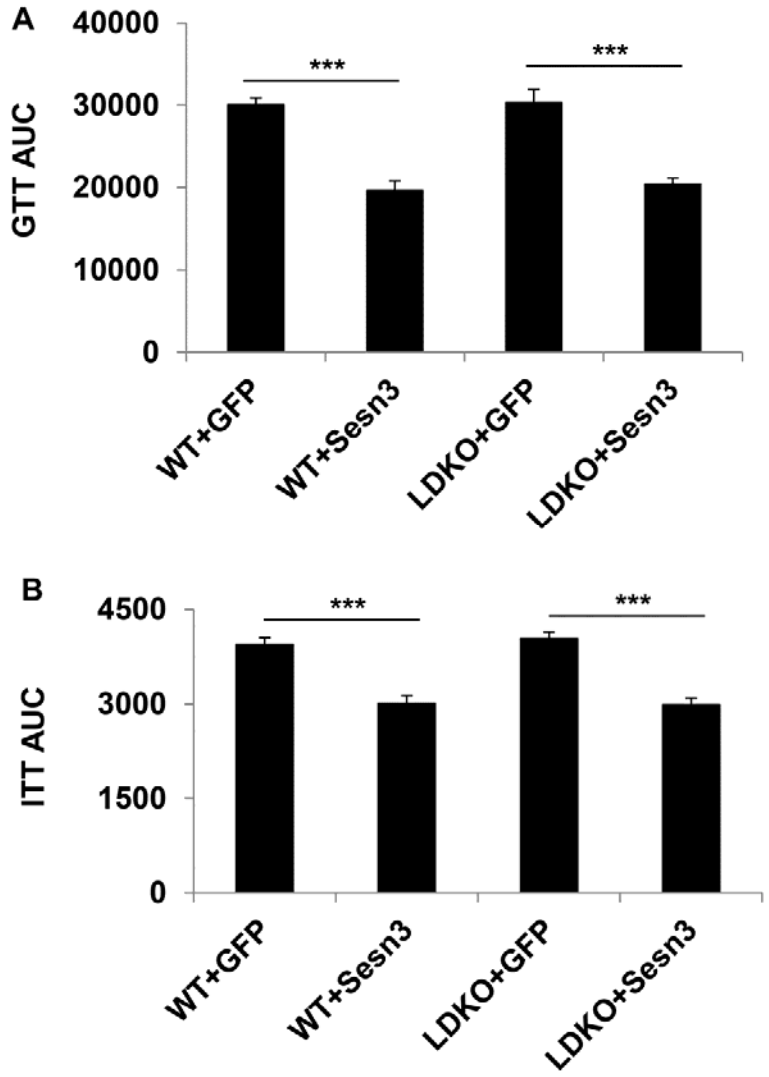
SUPPLEMENTARY DATA

Supplementary Figure 5. Insulin signaling analysis in HFD-fed WT and TgSesn3 mice. *A*: Western blot analysis of insulin signaling in the liver of WT and TgSesn3 mice fed with a HFD. *B*: Quantitative analysis of Panel A. Normalization was performed by calculating the ratio of each signaling event to that in the insulin-stimulated WT mice. *C, D*: Insulin signaling analysis in skeletal muscle and white adipose tissue of HFD-fed WT and TgSesn3 mice by Western blots, respectively. Data are presented as mean ± SEM. **P*<0.05.



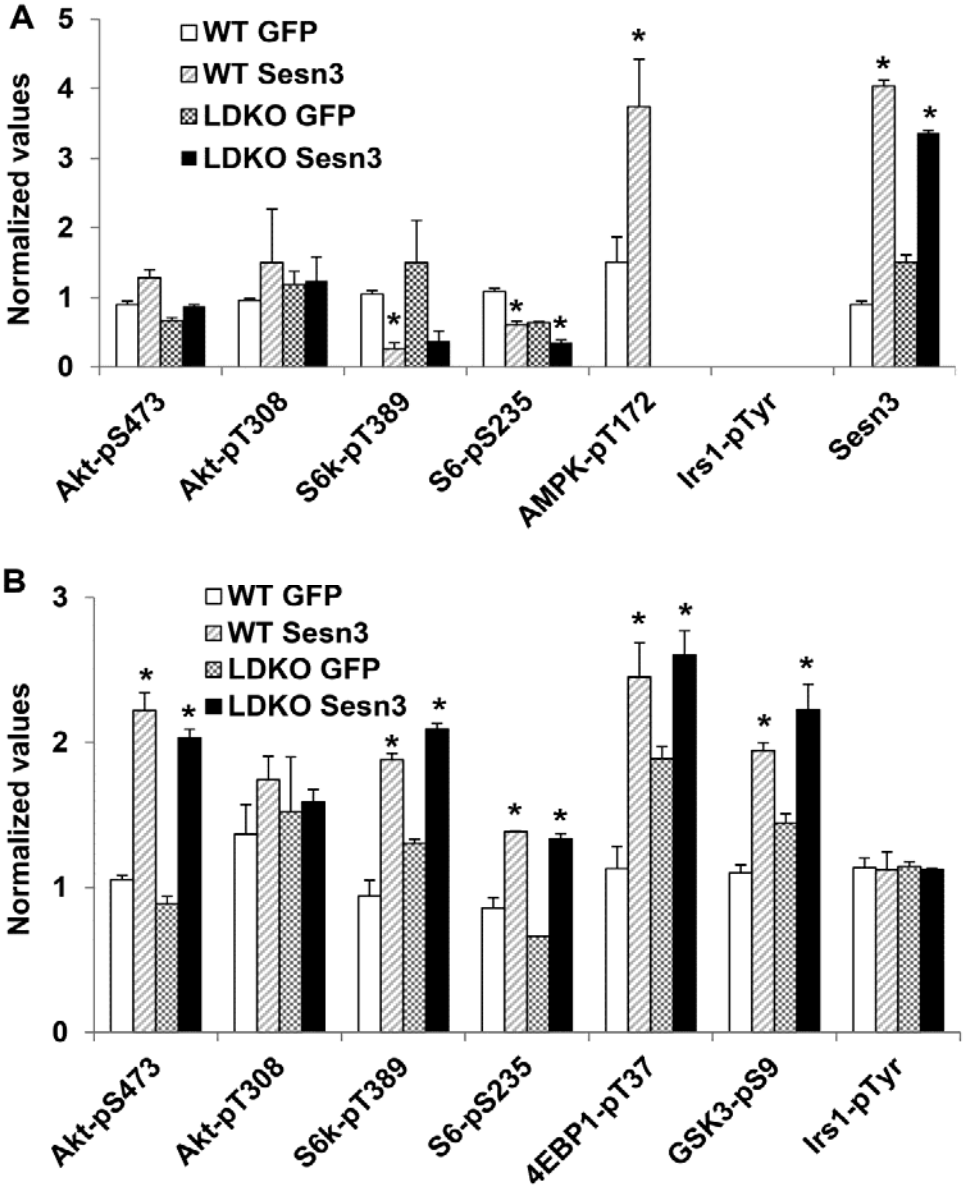
SUPPLEMENTARY DATA

Supplementary Figure 6. The AUC analysis of GTT and ITT data in main Figure 5 (C and D). A, B: The AUC presentation of the GTT and ITT data in WT and AMPK-LDKO mice. Data are presented as mean ± SEM. ***P<0.001.



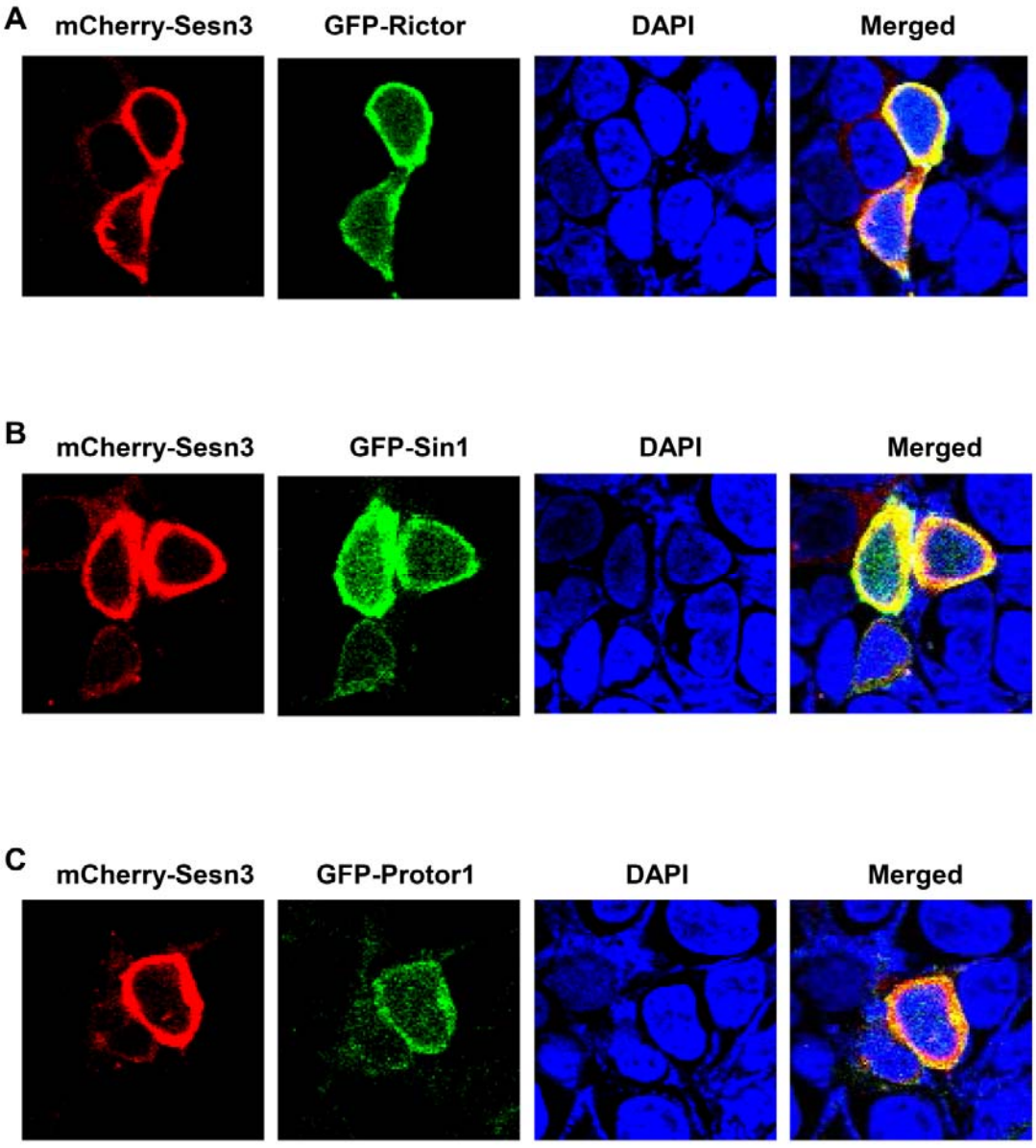
SUPPLEMENTARY DATA

Supplementary Figure 7. Quantitative analysis of insulin signaling in the liver of WT and AMPK-LDKO mice. The quantitative analysis was performed for Panels E and F in main Figure 5. Normalization was performed by calculating the ratio of each signaling event to that in the GFP-transduced WT mice. Data are presented as mean ± SEM. **P*<0.05.



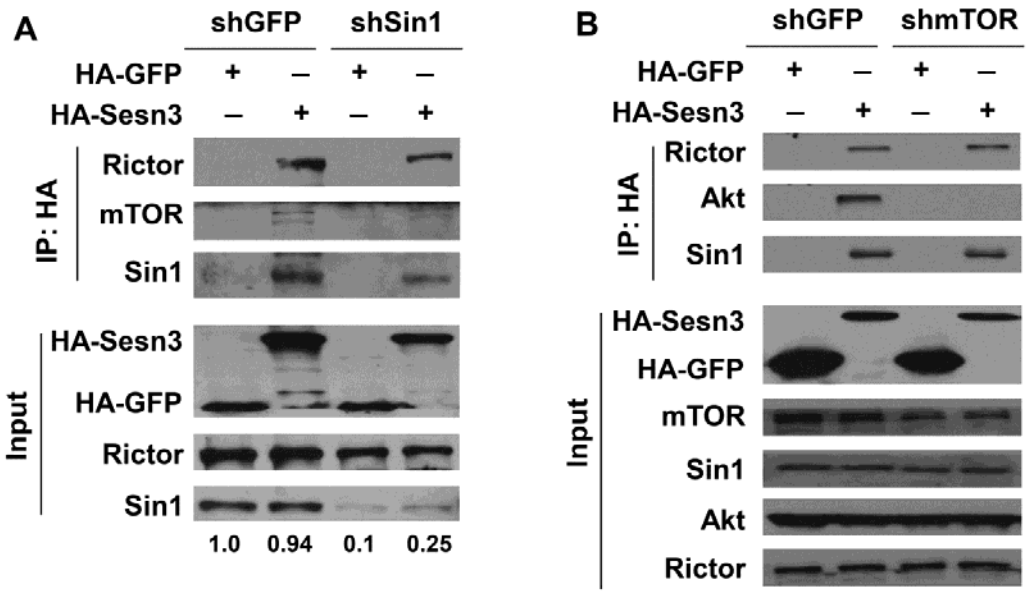
SUPPLEMENTARY DATA

Supplementary Figure 8. Microscopic analysis of colocalization of Sesn3 and mTORC2 complex. (A-C) HEK293T cells were seeded on coated glass cover slips and then transfected with mCherry-Sesn3 and GFP-Rictor, GFP-Sin1 or GFP-Protor1 plasmids. Fluorescence was recorded using a confocal microscope.



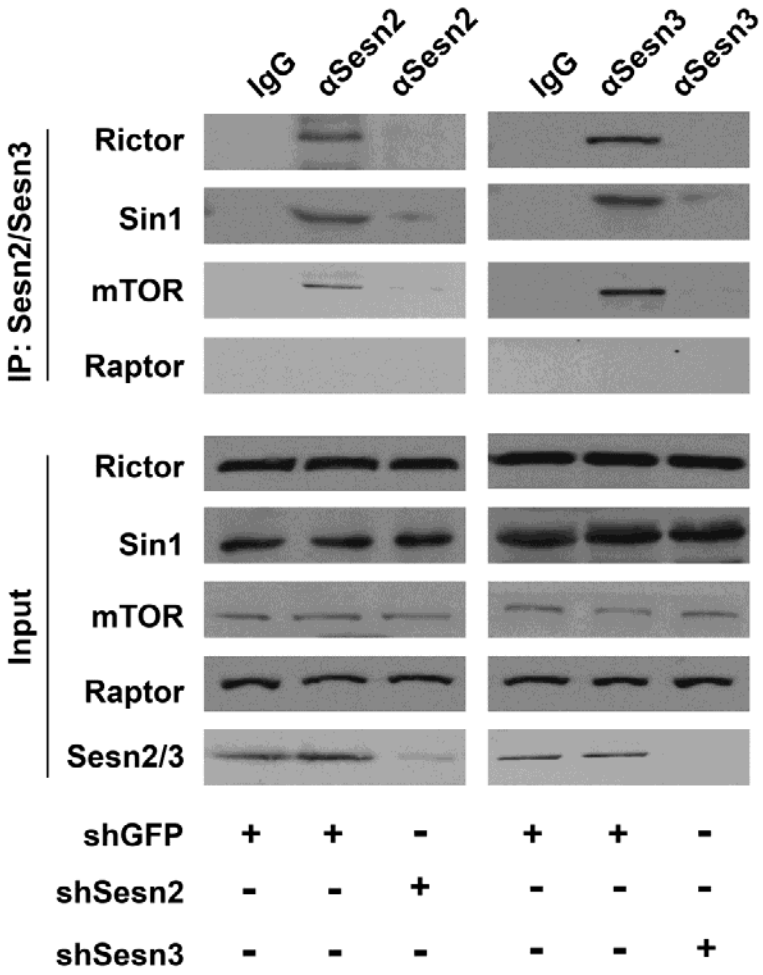
SUPPLEMENTARY DATA

Supplementary Figure 9. Analysis of Sesn3/mTORC2/Akt interactions. *A*: IP analysis of Sesn3-mTORC2 interaction in mouse primary hepatocytes transduced with shGFP (GFP shRNA) or shSin1 (Sin1 shRNA) adenoviruses together with GFP or Sesn3 expressing adenoviruses. *B*: IP analysis of Sesn3-mTORC2 interaction in mouse primary hepatocytes transduced with shGFP or shmTOR (mTOR shRNA) adenoviruses together with GFP or Sesn3 expressing adenoviruses.



SUPPLEMENTARY DATA

Supplementary Figure 10. Confirmation of Sesn2/3-mTORC2 interactions. Specific sestrin-mTORC2 interaction was analyzed in mouse primary hepatocytes transduced with either shGFP, shSesn2, or shSesn3 by IP using specific antibodies against Sesn2 and Sesn3.



SUPPLEMENTARY DATA

Supplementary Table 1.

Primer Name	Sequence (5' to 3')
pShuttle-mSesn3HA-F1	ATCAGCTAGCCACCATGAACCGCGGTGGCAG
pShuttle-mSesn3HA-R1	ATCACTCGAGGGTCAGATGCCGAGTTATGGC
pcDNA-mSesn3HA-from-Shuttle-F1	ATCTGCGGCCGCAGTA
pcDNA-mSesn3HA-from-Shuttle-R1	ATCTGCGGCCGCTATTAAGCGTAGTCAGGTACATC
pcDNA-FLAG-mSesn3-F1	ATCAGCTAGCAACCGCGGTGGCAGCAG
pcDNA-FLAG-mSesn3-R1	ATCACTCGAGTCAGGTCAGATGCCGAGTTATGGCTC
pShuttle-FLAG-mSesn3-F1	TCTATGGATATCGCCGCCACCATGGATTATAAAG
pShuttle-FLAG-mSesn3-R1	CCCTCTAGATGCATGCTCGAG
pcDNA-FLAG-mAkt1-F1	ATCGGCTAGCAACGACGTAGCCATTGTGAAG
pcDNA-FLAG-mAkt1-F1	ATCGGCTAGCTCAGGCTGTGCCACTGG
pcDNA-FLAG-mAkt2-F1	ATCGGCTAGCAATGAGGTATCTGTCATCAAAGAAG
pcDNA-FLAG-mAkt2-R1	ATCGGCTAGCTCACTCTCGGATGCTGG
pcDNA-FLAG-mPdpk1-F1	ATCAGCTAGCATGGCCAGGACCACCAGC
pcDNA-FLAG-mPdpk1-R1	ATCACTCGAGTCACTGCACAGCAGCATCTG
pcDNA-FLAG-mPras40-F1	ATCAGCTAGCATGGCGTCTGGGCGGCCA
pcDNA-FLAG-mPras40-R1	ATCACTCGAGTTAATATTTCCGCTTCAGCTTCTGGAAGTCG
pcDNA-mProtor1-FLAG-F1	TAGCGGCCGCCATGAGGACTCTCCGCAGGTTGA
pcDNA-mProtor1-FLAG-R1	AATTGCTAGCCACAACACTTGGCCGGCCT
pcDNA3-NSF-mSin1-F1-NheI	ATC AGC TAG CAT GGC CTT CTT GGA CAA TCC AAC
pcDNA3-NSF-mSin1-R1-XhoI	ATC ACT CGA GTC ACT GCT GCC CTG ATT TCT TC
pShuttle-mSin1-F1	TCTATGGCTAGCGCCGCCACCATGGATTATAAAG
pShuttle-mSin1-R1	CCCTCTAGATGCATGCTCGAG
mSesn3-shRNA2-top	CACCGGAGAAGAACATTTGCCAACATTCAAGAGATGTTGGCAAATGTTCTTCTCC
mSesn3-shRNA2-bottom	AAAAGGAGAAGAACATTTGCCAACATCTCTTGAATGTTGGCAAATGTTCTTCTCC
mSin1-shRNA1-top	CACCGCCGAAGCTCAATGACAATGTTTCAAGAGAACATTGTCATTGAGCTTCGGC
mSin1-shRNA1-bottom	AAAAGCCGAAGCTCAATGACAATGTTCTCTTGAAACATTGTCATTGAGCTTCGGC
mMtor-shRNA2-top	CACCGCATGACAAGTACTGCAAAGATTCAAGAGATCTTTGCAGTACTTGTCATGC
mMtor-shRNA2-bottom	AAAAGCATGACAAGTACTGCAAAGATCTCTTGAATCTTTGCAGTACTTGTCATGC
mRictor-shRNA2-top	CACCCAGGCCAGACCTCATGGACAATTCAAGAGATTGTCCATGAGGTCTGGCCTG
mRictor-shRNA2-bottom	AAAACAGGCCAGACCTCATGGACAATCTCTTGAATTGTCCATGAGGTCTGGCCTG
pET24-mProtor1-F1	GCTGGAATTCGCCGCCA
pET24-mProtor1-R1	TCTCAAGCTTTTTATCATCATCATCTTTATAATCCTCTCCG
pET24-RICTOR-C900-F1	TATAGGATCCATGGATTATAAAGATGATGATGATAAACTCTCCATTCCAAAAGGATTTTC
pET24-RICTOR-C900-R1	TATACTCGAGGGATTCAGCAGATGTATCAACTATA
pET24-mSin1-F1	GCTGGAATTCGCCGCCA
pET24-mSin1-R1	ATTACTCGAGCTGCTGCCCTGATTTCTTCTCC
pET24-mSesn3-F1	ATCAGCTAGCAACCGCGGTGGCAGCAG
pET24-mSesn3-R1	ATCACTCGAGGGTCAGATGCCGAGTTATGGC

SUPPLEMENTARY DATA

pEGFP-mSin1-F1	TATACTCGAGGCCTTCTTGGACAATCCAACTAT
pEGFP-mSin1-F1	TATCGGTACCTAACTGCACATCCGTCGTG
pEGFP-mProtor1-F1	GCTGGAATTCGCCGCCA
pEGFP-mProtor1-R1	TCTCAAGCTTTTTATCATCATCATCTTTATAATCCTCTCCG
pLP-mCherry-mSesn3-F1	ATGGCGCGCCAACCGCGGTGGCAGCA
pLP-mCherry-mSesn3-R1	GCTTAATTAATCAGGTCAGATGCCGAGTTATGG
mSesn3-qPCR-F2	GGATGTTGACACGACCACAC
mSesn3-qPCR-B2	TAAACCTTCAGGCTCCGTTC
mSesn3-Loxp-PCR-F1	CAGAAACCTGCAGTTGTG
mSesn3-Loxp-PCR-R1	CCATAATGCAACACTAAGTCA
mSesn3-Tg-PCR-F1	AAGGGAGCTGCAGTGGAGTA
mSesn3-Tg-PCR-R1	CTTTAAGCCTGCCCAGAAGA
mSesn3-Tg-PCR-R2	GGAAAGTCCCTATTGGCGTTA
AMPKa1-loxp-PCR-F1	CCCACCATCACTCCATCTCT
AMPKa1-loxp-PCR-F1	AGCCTGCTTGGCACACTTAT
AMPKa2-loxp-PCR-F1	GCAGGCGAATTTCTGAGTTC
AMPKa2-loxp-PCR-F1	TCCCCTTGAACAAGCATACC
Cre-PCR-Primer 42	CTAGGCCACAGAATTGAAAGATCT
Cre-PCR-Primer 43	GTAGGTGGAAATTCTAGCATCATCC
Cre-PCR-Primer 567	ACCAGCCAGCTATCAACTCG
Cre-PCR-Primer 568	TTACATTGGTCCAGCCACC