**Molecular Cell, Volume *72***

## Supplemental Information

# An Insulin-Responsive Sensor in the SIRT1 Disordered Region Binds DBC1 and PACS-2 to Control Enzyme Activity

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# Supplemental Information

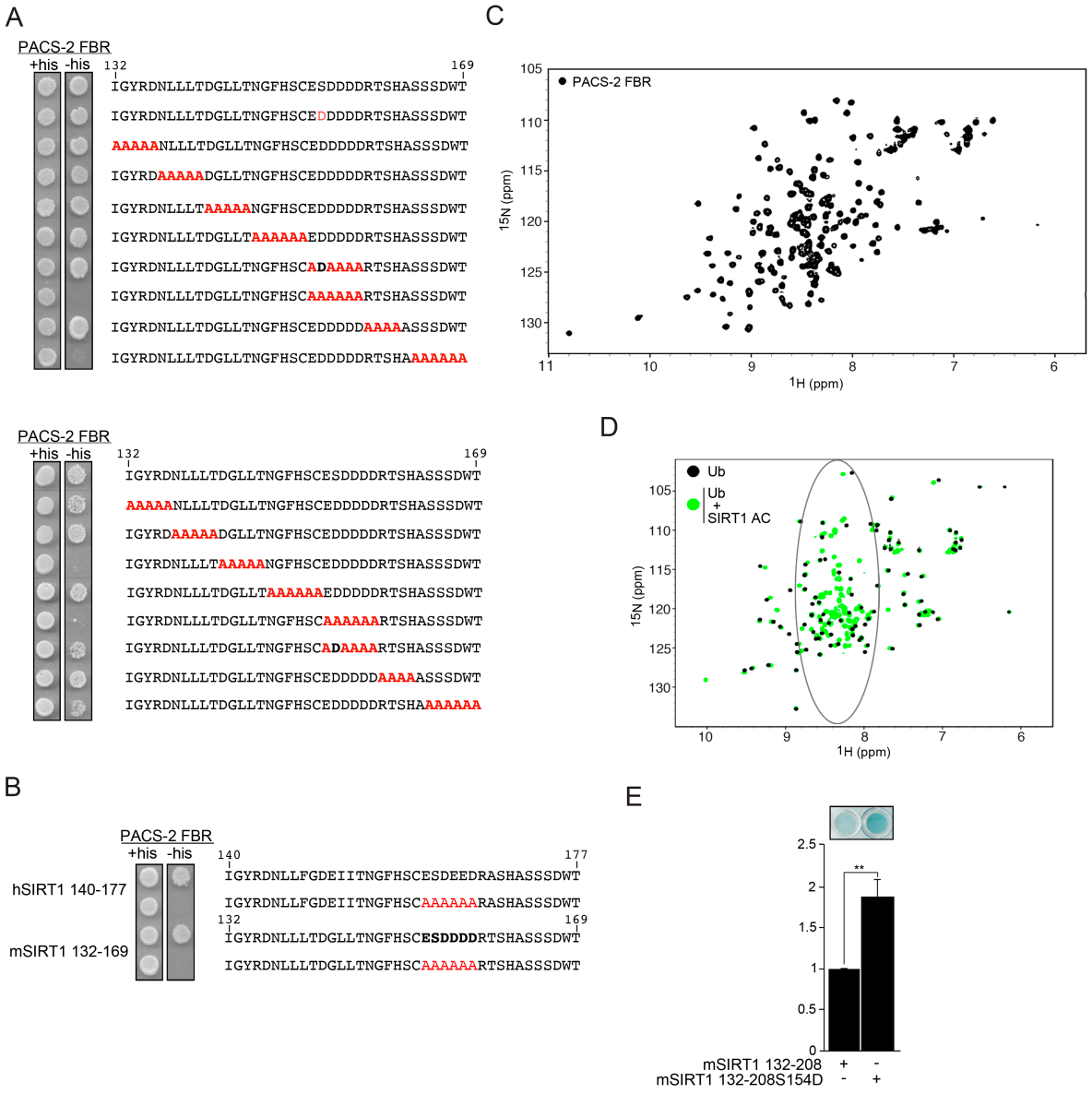


**Figure S1. Related to Figure 1. The SIRT1 acidic cluster serves as a binding site for PACS-2.** **(A)** Y2H screen for binding between PACS-2 FBR and mouse SIRT1 constructs representing a 5-Ala scan from $Ile^{132}$ to $Thr^{169}$ in either $SIRT1^{132\text{-}169}$ (top) or $SIRT1^{132\text{-}208}$ (bottom). **(B)** Y2H screen for binding between PACS-2 FBR and either WT $hSIRT1^{140\text{-}177}$ (corresponding to $mSIRT1^{132\text{-}169}$) or its acidic cluster mutant ($ES^{162}DEED \rightarrow 6A$), WT $mSIRT1^{132\text{-}169}$ or the $mSIRT1^{132\text{-}169}$ acidic cluster mutant ($ES^{154}DDDD \rightarrow 6A$). Lines in panels A and B denote removal of constructs unrelated to this study and alignment of colonies assayed on parallel plates. **(C)** $^{1}H\text{-}^{15}N$ HSQC spectrum of 100 µM PACS-2 FBR **(D)** Superposition of $^{1}H\text{-}^{15}N$ HSQC spectra of 100 µM ubiquitin (black resonances) and of ubiquitin fused to SIRT1 AC ($Ub\text{-}SIRT1^{140\text{-}177}$, green resonances). The ellipse highlights a large number of $SIRT1^{140\text{-}177}$ resonances that are not present in the ubiquitin spectrum. **(E)** Measurement of β-galactosidase activity as a read out for the binding of PACS-2 FBR to $SIRT1^{132\text{-}208}$, containing the native sequence or the $Ser^{154} \rightarrow$ Asp phosphomimic substitution (assay performed in triplicate, error bars = SD).

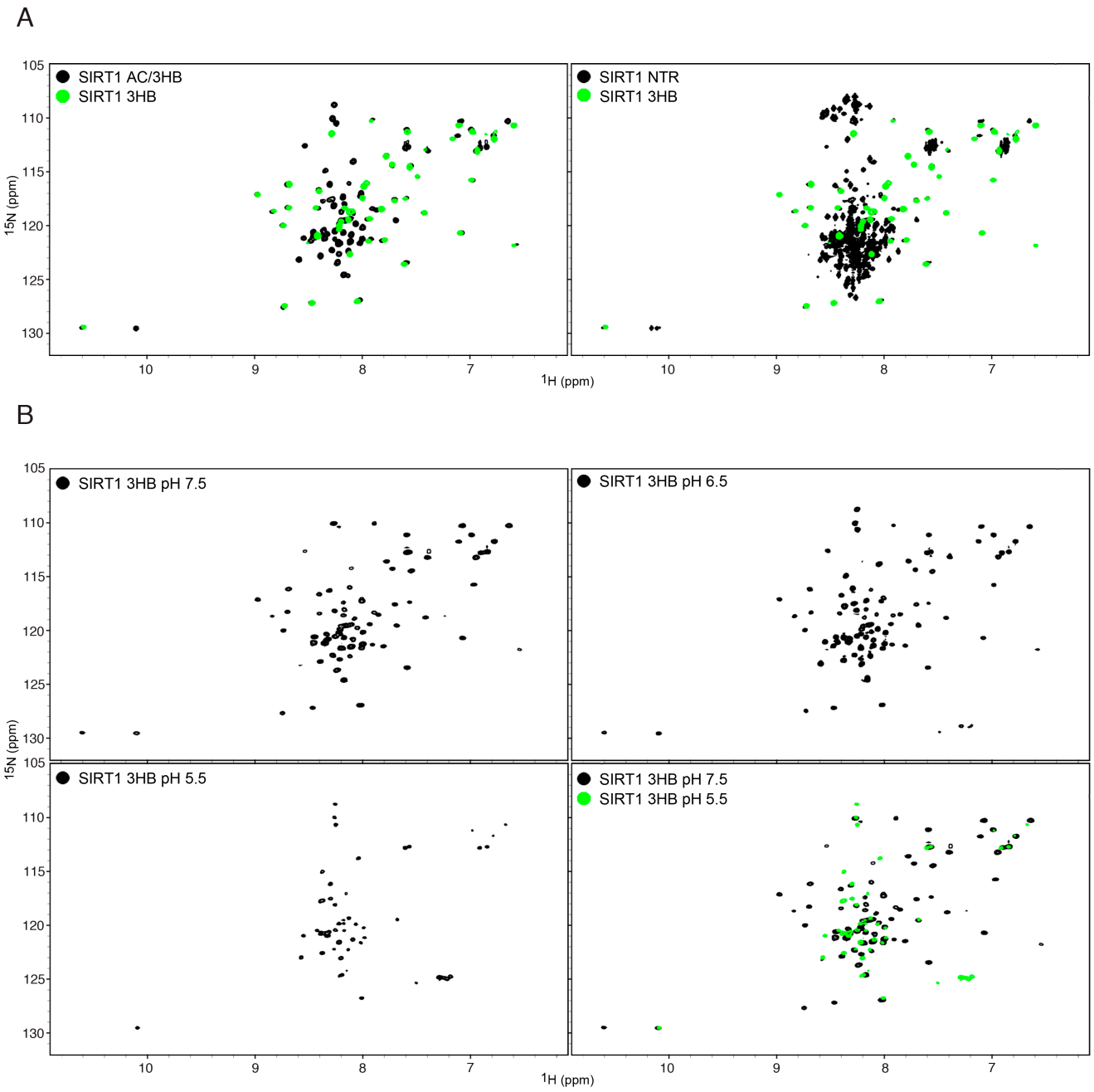


**Figure S2. Related to Figure 2. pH titration of the SIRT1 3HB. (A)** Superposition of $^{1}$H-$^{15}$N HSQC spectra of SIRT1 AC/3HB (SIRT1$^{141-233}$, *left panel*, black resonances) or the SIRT1 NTR (SIRT1$^{1-233}$, *right panel*, black resonances) and SIRT1 3HB (SIRT1$^{183-233}$, green resonances). **(B)** The $^{1}$H-$^{15}$N HSQC spectra of 100 µM SIRT1 3HB (SIRT1$^{183-233}$) in 20mM HEPES pH 7.5 *(top left)*, 20mM HEPES pH 6.5 *(top right)* or 20 mM Na citrate pH 5.5 *(bottom left*). *Bottom right*: Superposition of the $^{1}$H-$^{15}$N HSQC spectra of SIRT1 3HB at pH 5.5 (green resonances) and at pH 7.5 (black resonances).

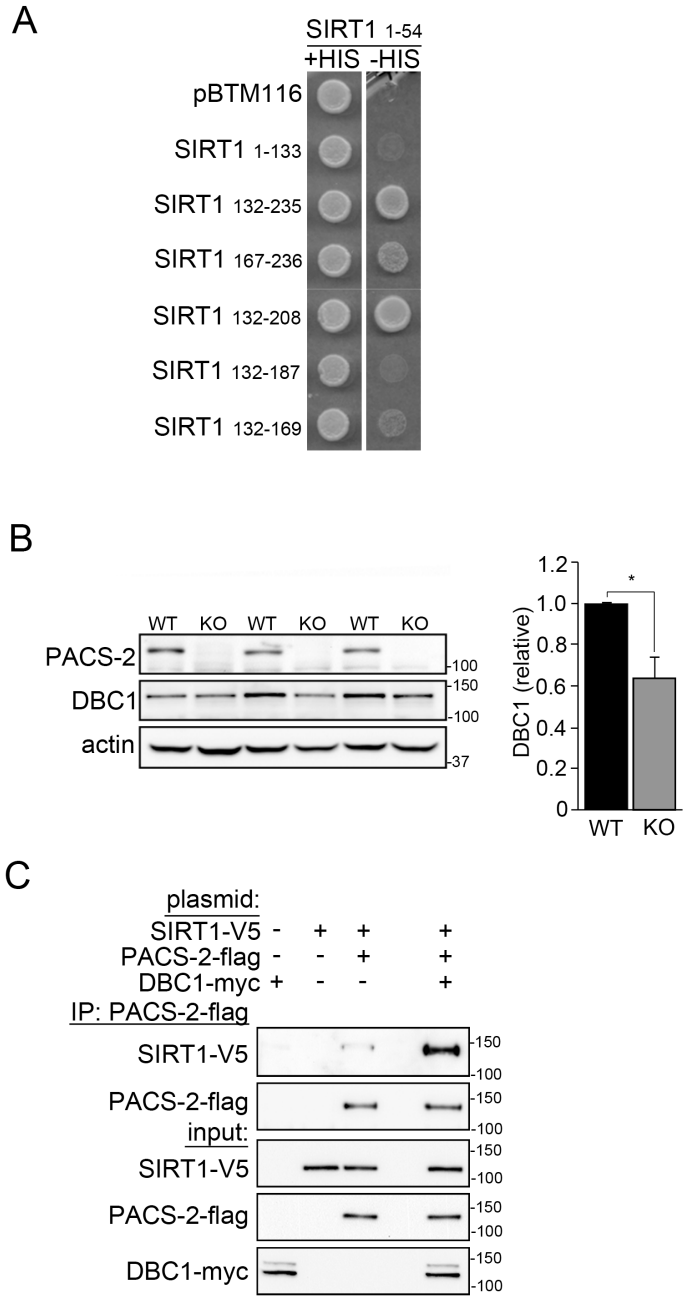


**Figure S3. Related to Figure 3. DBC1 expression in PACS-2$^{-/-}$ liver. (A)** Y2H analysis of the interaction between mouse SIRT1$^{1-54}$ and different SIRT1 NTR mutants (see Figure 1A). Line denotes removal of constructs unrelated to this study and alignment of colonies assayed on parallel plates. **(B)** Livers from WT or PACS-2$^{-/-}$ mice were harvested and the level of endogenous PACS-2, DBC1 and actin were detected by western blot. Mean ± SD, n = 6. **(C)** FLAG-tagged PACS-2 was co-expressed with Myc-tagged DBC1 and V5-tagged human SIRT1 in HCT116 cells as indicated. PACS-2 was immunoprecipitated (FLAG) and co-precipitated human SIRT1 was detected by western blot (V5).

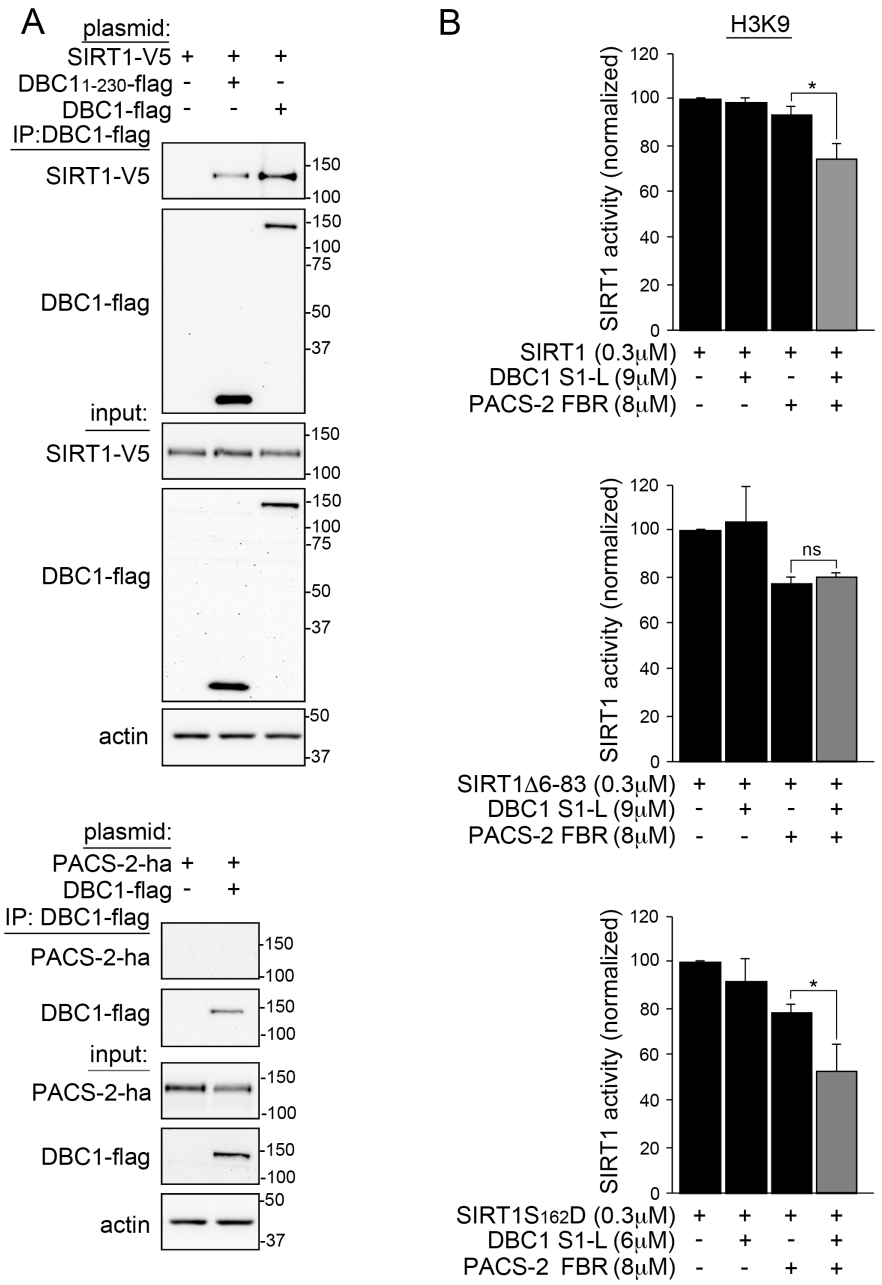


**Figure S4. Related to Figure 4. PACS-2 and DBC1 regulate SIRT1-dependent deacetylation of the H3K9 peptidyl substrate. (A)** *Top*: SIRT1-V5 was expressed alone or together with FLAG-tagged DBC1 or DBC1$^{1\text{-}230}$ in HCT116 cells. DBC1 was immunoprecipitated (FLAG) and co-precipitated SIRT1 was detected by western blot (V5). *Bottom*: HA-tagged PACS-2 was expressed alone or together with FLAG-tagged DBC1. DBC1 was immunoprecipitated (FLAG) and co-precipitated PACS-2 was detected by western blot (HA). **(B)** The Ac-Lys$^{9}$-H3 peptidyl substrate was added to assay tubes containing recombinant human SIRT1, SIRT1$^{84\text{-}747}$ or SIRT1$^{S162D}$ pre-mixed with different combinations of recombinant DBC1$^{52\text{-}120}$ (DBC1 S1-L) and PACS-2$^{22\text{-}180}$ (PACS-2 FBR). Enzyme reactions were initiated by the addition of NAD$^{+}$ or vehicle (background control) and stopped after 1hr. Reaction products were developed with the OPT cocktail and quantified by the fluorescent signal. Error bars represent mean ± SD, n = 4.

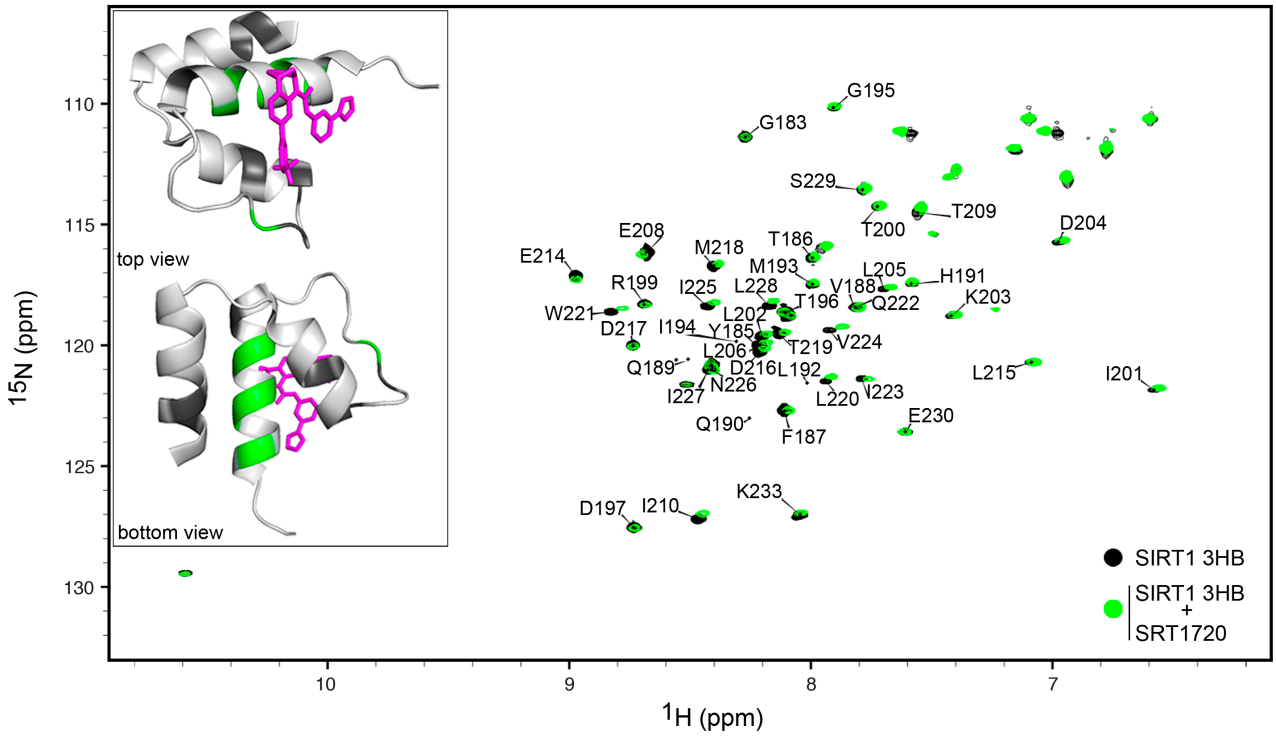


**Figure S5. Related to Figure 5. Complete Resonance Assignments and structural mapping of the SIRT1 3HB/SRT1720 interaction.** Superposition of $^{1}H$-$^{15}N$ HSQC spectra of 100 µM SIRT1 3HB ($SIRT1^{183-233}$) alone (black resonances) and in the presence of 100 µM SRT1720 (green resonances). Resonance assignments are provided by amino acid name and number. *Inset*: top and side views of SRT1720 (magenta) bound to the SIRT1 3HB (PDB:4ZZH). SIRT1 3HB residues that undergo the greatest chemical shift changes (mean + one standard deviation) are highlighted in green and those which exhibit smaller changes are in dark gray. The top view looks down on the hydrophobic core of the 3HB and the bottom view shows the STAC binding interface.

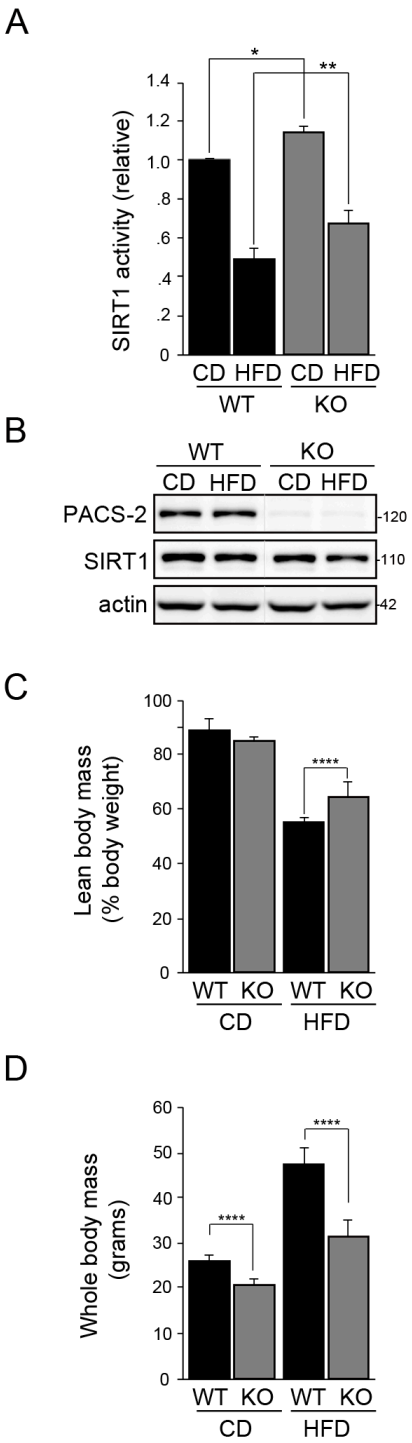


**Figure S6. Related to Figure 6. PACS-2$^{-/-}$ mice are resistant to diet-induced obesity. (A)** Nuclear extracts from WT and PACS-2$^{-/-}$ mice, fed a CD or HFD, were assayed for SIRT1 activity using the Ac-Lys$^{778}$-PGC-1α peptidyl substrate. Data are mean ± SD, n = 3. **(B)** Western blot of PACS-2, SIRT1 and actin from WT and PACS-2$^{-/-}$ mouse liver following 8 weeks of CD or HFD. Line denotes removal of unused samples. **(C and D)** Whole body mass and percent lean body mass of 8 weeks HFD WT and PACS-2$^{-/-}$ mice measured using an EchoMRI 4-in-1 system.

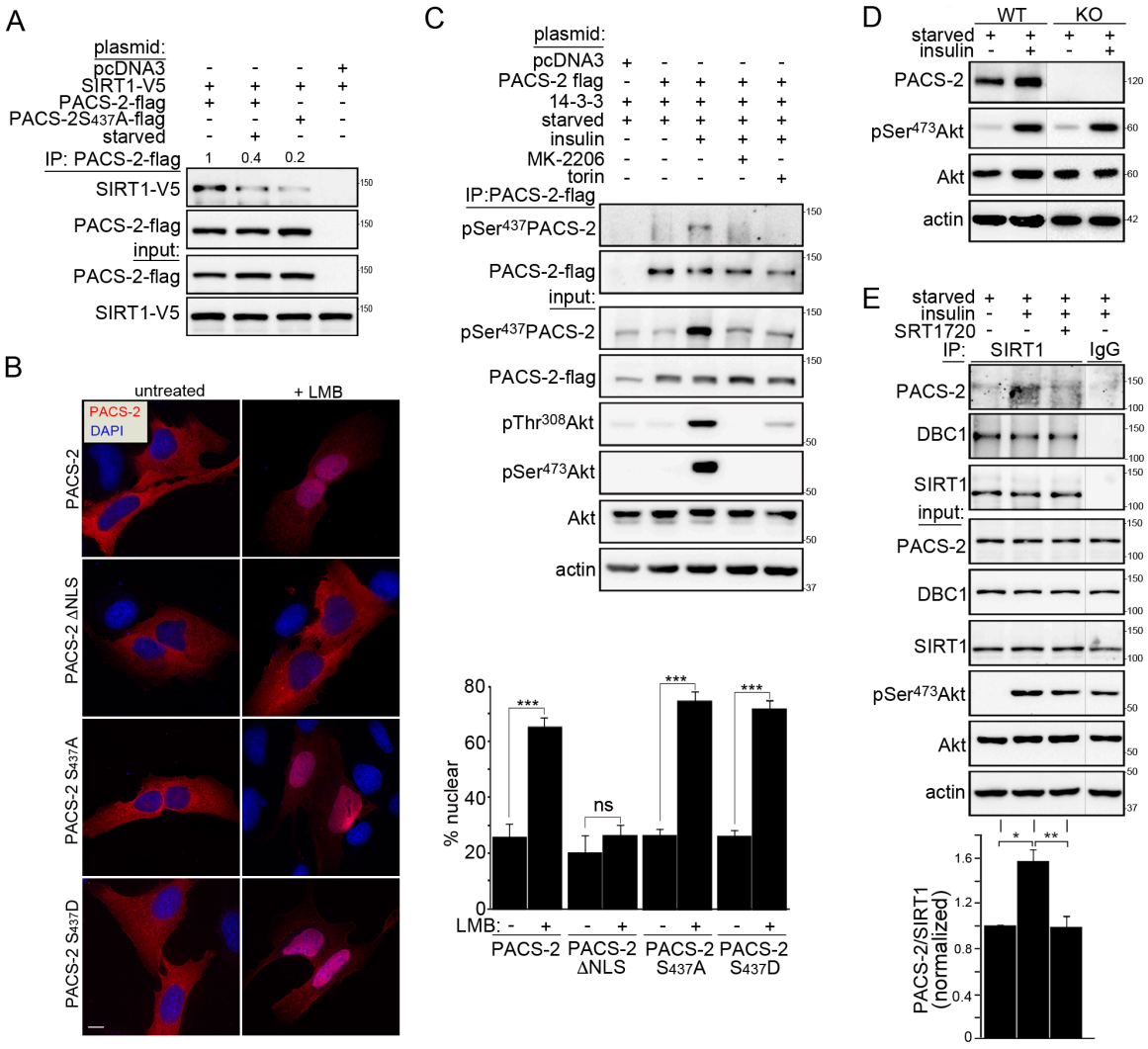


**Figure S7. Related to Figure 7. PACS-2 undergoes nucleocytoplasmic trafficking independent of its Akt phosphorylation status. (A)** SIRT1-V5 was co-expressed with FLAG-tagged PACS-2 or PACS-2$^{S437A}$ in AML12 cells as indicated. The AML12 cells were fed or serum-starved for 16 hrs, PACS-2 proteins were immunoprecipitated (FLAG) and co-precipitating SIRT1 was detected by western blot (V5), n = 5. **(B)** U2OS cells expressing mCherry-tagged PACS-2, PACS-2$^{\Delta NLS}$, PACS-2$^{S437A}$ or PACS-2$^{S437D}$ were treated with 40 nM LMB (3 hrs) and analyzed by deconvolution microscopy. Nuclei were stained with DAPI. The percent total cellular fluorescent protein signal in the nucleus was quantified. Error bars represent mean ± SD from >100 cells in 3 independent experiments. Scale bar, 20 μm. **(C)** Hela cells expressing FLAG-tagged PACS-2 were starved for 14 hrs. The cells were then pre-treated or not with 1 μM Torin 1 or 5 μM MK-2206 and then treated with 100 nM insulin for 30 min. PACS-2 was immunoprecipitated (FLAG) and pSer$^{437}$-PACS-2 was detected by western blot (CST #81E12B5 (Aslan et al., 2009)), n = 3. **(D)** Isolated WT and PACS-2$^{-/-}$ mouse primary hepatocytes were starved overnight and treated for 6 hrs with 10 μM WY-14643, then treated or not with 100 nM insulin treatment for 4 hrs. Cells were harvested and the indicated proteins analyzed by western blot. Line denotes removal of unused samples. **(E)** AML12 cells were starved overnight, pre-treated with 12 μM SRT1720 for one hr and then treated with 10 nM insulin for 30 min. Endogenous SIRT1 was immunoprecipitated and co-precipitated PACS-2 and DBC1 were detected by western blot. Data are mean ± SD, n = 3. Line denotes placement of IgG control on last lane.

**TABLE S1**

TFactS analysis of transcription factors altered in PACS-2$^{-/-}$ mice. False discovery rate = 0.05. Related to Figure 1.

| Transcription Factor | P.value | E.value | Q.value | FDR control (B-H) | Intersection | Target genes | Random Control(%) |
|---|---|---|---|---|---|---|---|
| NR1H3 | 3.000e-5 | 6.300e-4 | 1.075e-4 | 2.381e-3 | 2 | 17 | 0 |
| NR1H2 | 3.000e-5 | 6.300e-4 | 1.075e-4 | 4.762e-3 | 2 | 17 | 0 |
| PPARG | 2.100e-4 | 4.410e-3 | 5.018e-4 | 7.143e-3 | 2 | 41 | 0 |
| HNF1A | 3.700e-4 | 7.770e-3 | 6.630e-4 | 9.524e-3 | 2 | 54 | 0 |
| CREBBP | 3.510e-3 | 7.371e-2 | 4.898e-3 | 1.190e-2 | 1 | 6 | 0 |
| FOXM1 | 4.100e-3 | 8.610e-2 | 4.898e-3 | 1.429e-2 | 1 | 7 | 0 |
| NR2F2 | 7.600e-3 | 1.596e-1 | 7.782e-3 | 1.667e-2 | 1 | 13 | 0 |
| FOXA2 | 1.226e-2 | 2.575e-1 | 1.023e-2 | 1.905e-2 | 1 | 21 | 0 |
| PPARA | 1.284e-2 | 2.696e-1 | 1.023e-2 | 2.143e-2 | 1 | 22 | 0 |
| ETS2 | 1.748e-2 | 3.671e-1 | 1.253e-2 | 2.381e-2 | 1 | 30 | 0 |
| SP1 | 2.164e-2 | 4.544e-1 | 1.410e-2 | 2.619e-2 | 2 | 428 | 0 |
| SREBF1 | 2.957e-2 | 6.210e-1 | 1.659e-2 | 2.857e-2 | 1 | 51 | 0 |
| STAT5B | 3.186e-2 | 6.691e-1 | 1.659e-2 | 3.095e-2 | 1 | 55 | 0 |
| STAT5A | 3.244e-2 | 6.812e-1 | 1.659e-2 | 3.333e-2 | 1 | 56 | 0 |
| RARA | 3.472e-2 | 7.291e-1 | 1.659e-2 | 3.571e-2 | 1 | 60 | 0 |
| CEBPB | 4.099e-2 | 8.608e-1 | 1.836e-2 | 3.810e-2 | 1 | 71 | 0 |
| FOXO3 | 4.496e-2 | 9.442e-1 | 1.880e-2 | 4.048e-2 | 1 | 78 | 0 |
| HNF4A | 4.722e-2 | 9.916e-1 | 1.880e-2 | 4.286e-2 | 1 | 82 | 0 |
| CEBPA | 6.797e-2 | 1.427e+0 | 2.564e-2 | 4.524e-2 | 1 | 119 | 1 |
| SP3 | 7.518e-2 | 1.579e+0 | 2.694e-2 | 4.762e-2 | 1 | 132 | 2 |
| FOXO1 | 9.112e-2 | 1.914e+0 | 3.110e-2 | 5.000e-2 | 1 | 161 | 0 |

**TABLE S2**

qRT-PCR primers used in this study. Related to Figure 1.

| Gene Name | Forward primer (5'-3') | Reverse primer (5'-3') | Accession number |
|---|---|---|---|
| mPpar γ | GATGGAAGACCACTCGCATT | AACCATTGGGTCAGCTCTTG | NM_001127330 |
| mPparδ | CCATCGTCAACAAAGACGGG | ACTTGGGCTCAATGATGTCAC | NM_011145 |
| mPparα | TCGGCGAACTATTCGGCTG | GCACTTGTGAAAACGGCAGT | NM_011144 |
| mNrf1 | CTGCAGGTCCTGTGGGAATG | ACTCGCGTCGTGTACTCATC | NM_001164226 |
| mCpt1α | GCAGTCGACTCACCTTTCCT | ATTTCTCAAAGTCAAACAGTTCCA | NM_013495 |
| mCpt2 | CCAAAGAAGCAGCGATGG | TAGAGCTCAGGCAGGGTGA | NM_009949 |
| mAcadvl | GGTGGTTTGGGCCTCTCTA | GGGTAACGCTAACACCAAGG | NM_017366 |
| mMcad | AGTACCCTGTGGAGAAGCTGAT | TCAATGTGCTCACGAGCTATG | NM_007382 |
| mAcad4 (Lcad) | CCCATGGCATTAGCCTCTT | AGAATAGTTCTGCTGTGTCCTGAG | NM_007381 |
| mG6pase | CCTCCTCAGCCTATGTCTGC | AACATCGGAGTGACCTTTGG | NM_008061 |
| mPepck | GTCAACACCGACCTCCCTTA | ATTTGCCCCTAGCCTGTTCT | NM_011044 |
| mPgc-1α | ATGTGTCGCCTTCTTGCTCT | ATCTACTGCCTGGGGACCTT | NM_008904 |
| mCyt-c | GAGTTTTGGGCTGATGGGTA | ATCCCGCTGTAACACCAGTC | NM_007808 |
| mLxrα | CTGAAGCGGCAAGAAGAGGA | CTGTGGCAGGACTTGAGGAG | NM_013839 |
| mFasn | CCCTTGATGAAGAGGGATCA | ACTCCACAGGTGGGAACAAG | NM_007988 |
| mGlut4 | ACTCTTGCCACACAGGCTCT | CCTTGCCCTGTCAGGTATGT | NM_009204 |
| mSrebp-1c | GTGAGCCTGACAAGCAATCA | GGTGCCTACAGAGCAAGAGG | NM_011480 |
| mTnfa | AGCCCCCAGTCTGTATCCTT | GAGTTGGACCCTGAGCCATA | NM_013693 |
| mSerpin1 | ATCCCAGACCTCGGACTCTT | TGAGACCTTTGTGGGGTAGG | NM_008871 |
| mHmgcr | CGTAACCCAAAGGGTCAAGA | GACCCAAGGAAACCTTAGCC | NM_008255 |
| mCyclophilin B | GGAGATGGCACAGGAGGAA | GCCCGTAGTGCTTCAGCTT | NM_011149 |
| mCyp7a1 | CCGTCTACGCATGTTTCTCA | GAAGGTTGCAGGAATGGTGT | NM_007824 |
| mCyp8b1 | AAGCACCAGGATGCCATGAA | AGACTCTCCTCCATCACGCT | NM_010012 |
| mCyp27a1 | CAAGGACTTTGCCCACATGC | ATCCGGGAGTTTGTGGGAAC | NM_024264 |
| mShp | CATGGCCTCTACCCTCAAGA | TGATAGGGCGGAAGAAGAGA | NM_011850 |
| mBsep | GGACAATGATGTGCTTGTGG | CACACAAAGCCCCTACCAGT | NM_021022 |
| mMrp3 | ACAACAGGGTCCTGGTCTTG | CTTGGCCATCCCATAGAAGA | NM_029600 |
| mHnf4 | TACCTTCCTCCGCCATCTGA | TCCTACCCTCTGCCTTACCC | NM_008261 |
| mFxr | AGGGGATGAGCTGTGTGTTG | CGGAAGAAACCTTTGCAGCC | NM_001163700 |
| mScd1 | GCGATACACTCTGGTGCTCA | CCCAGGGAAACCAGGATATT | NM_009127 |
| mAcc | GCCTCTTCCTGACAAACGAG | TGATCTGTGCTGTCCTGGAG | NM_133360 |
| mCD36 | GCTTGCAACTGTCAGCACAT | GCCTTGCTGTAGCCAAGAAC | NM_007643 |
| mLpl | CCTACTTCAGCTGGCCTGAC | CTTTCACTCGGATCCTCTCG | NM_008509 |
| mFabp4 | TCACCTGGAAGACAGCTCCT | AAGCCCACTCCCACTTCTTT | NM_024406 |
| mLdlr | GAACTCAGGGCCTCTGTCTG | AGCAGGCTGGATGTCTCTGT | NM_010700 |
| mSrebp2 | ATGAACCGGACTCTCCTCCT | CCAGGAAGGTGAGGACACAT | NM_033218 |
| mIl-6 | AGTTGCCTTCTTGGGACTGA | GTCTCCTCTCCGGACTTGTG | NM_031168 |
| mInfg | ACTGGCAAAAGGATGGTGAC | GCTGATGGCCTGATTGTCTT | NM_008337 |
| mAbca1 | CCAGACAGTTGTGGATGTGG | CCTGTGTGAACGGGATTCTT | NM_013454 |
| mFgf21 | CCTCTAGGTTTCTTTGCCAACAG | AAGCTGCAGGCCTCAGGAT | NM_020013 |
| mPcsk9 | CATTGCAGACACCATTTTGG | CTGGAGGTGTGCAGAACTGA | NM_153565 |