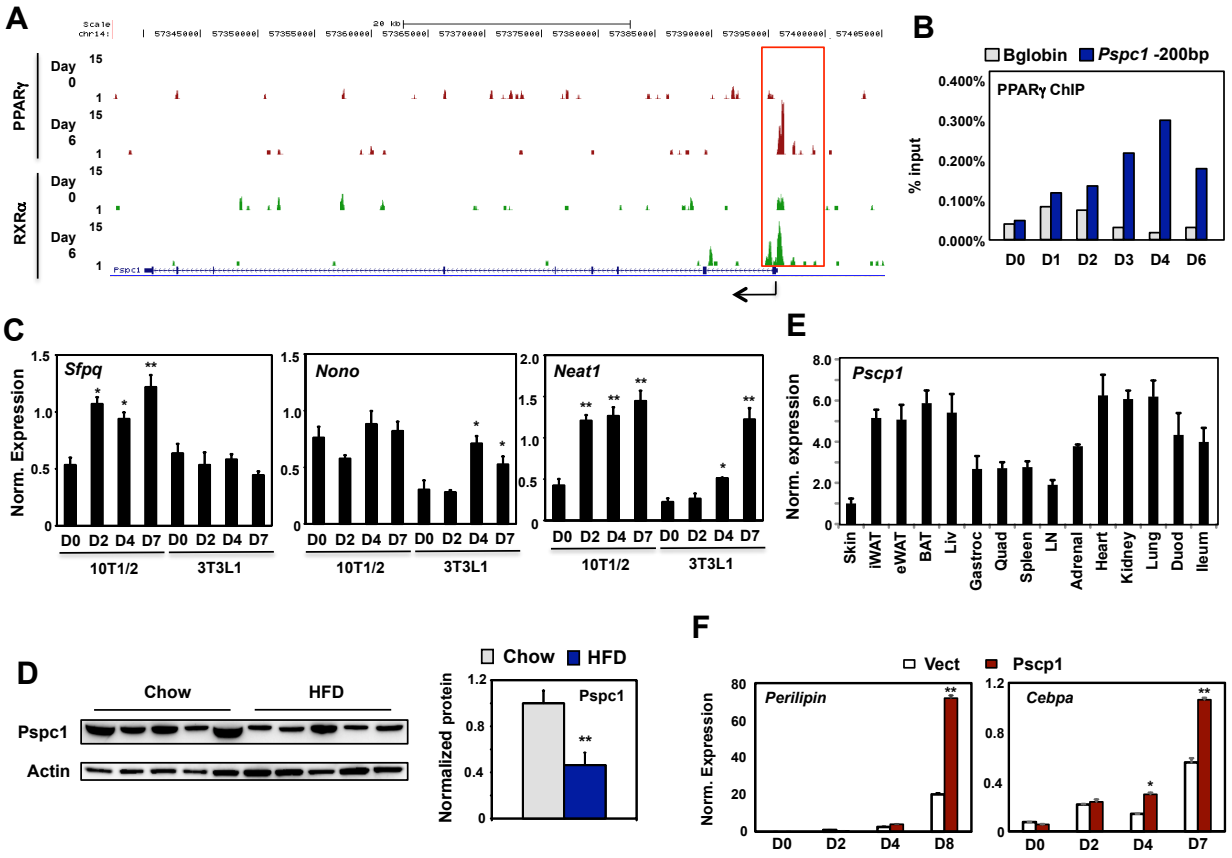



Wang, Fig. S1

**Figure S1. Regulation of Pspc1 expression.** (A) High-resolution ChIP-seq analysis of PPARγ and RXRα binding sites within the mouse *Pspc1* locus from 3T3-L1 cells on differentiation day 0 and day 6. Red square indicates putative PPARγ/RXRα binding site at -200 bp from *Pspc1* transcription start site. Data are from the deep sequencing study of Nielsen et al. (33). (B) Real-time PCR validation of PPARγ occupancy in the –200 bp region of *Pspc1* promoter at the indicated time points during differentiation. A region of the *Bglobin* promoter served as a negative control. (C) mRNA expression of paraspeckle components during adipocyte differentiation. Comparison was made against D0 by one-way ANOVA. Results represent three independent experiments. Unless mentioned otherwise, mRNA expression in this and all subsequent figures were normalized to 36B4 control. (D) Immunoblot analysis of Pspc1 protein levels in epididymal white adipose tissue from chow and high fat diet-fed C57BL/6 mice (Left). Quantification of Pspc1 band intensity normalized to actin (right). $n = 5$ per group. Statistical analysis was performed using Student's t-test compared to the chow group. (E) Real-time PCR analysis of the relative tissue distribution of Pspc1 mRNA. Tissues were harvested from 12-week old C57BL/6 mice fasted for 6 h, $n = 3$. (F) Real-time PCR analysis of adipogenic gene expression in 10T1/2 stable cell lines expressing vector or Pspc1. Cells were stimulated to differentiate with DMI + 20 nM GW. Comparison was made against vector control by Student's t-test. Results represent three independent experiments. Error bars represent mean + SEM. $*p < 0.05$, $**p < 0.01$.

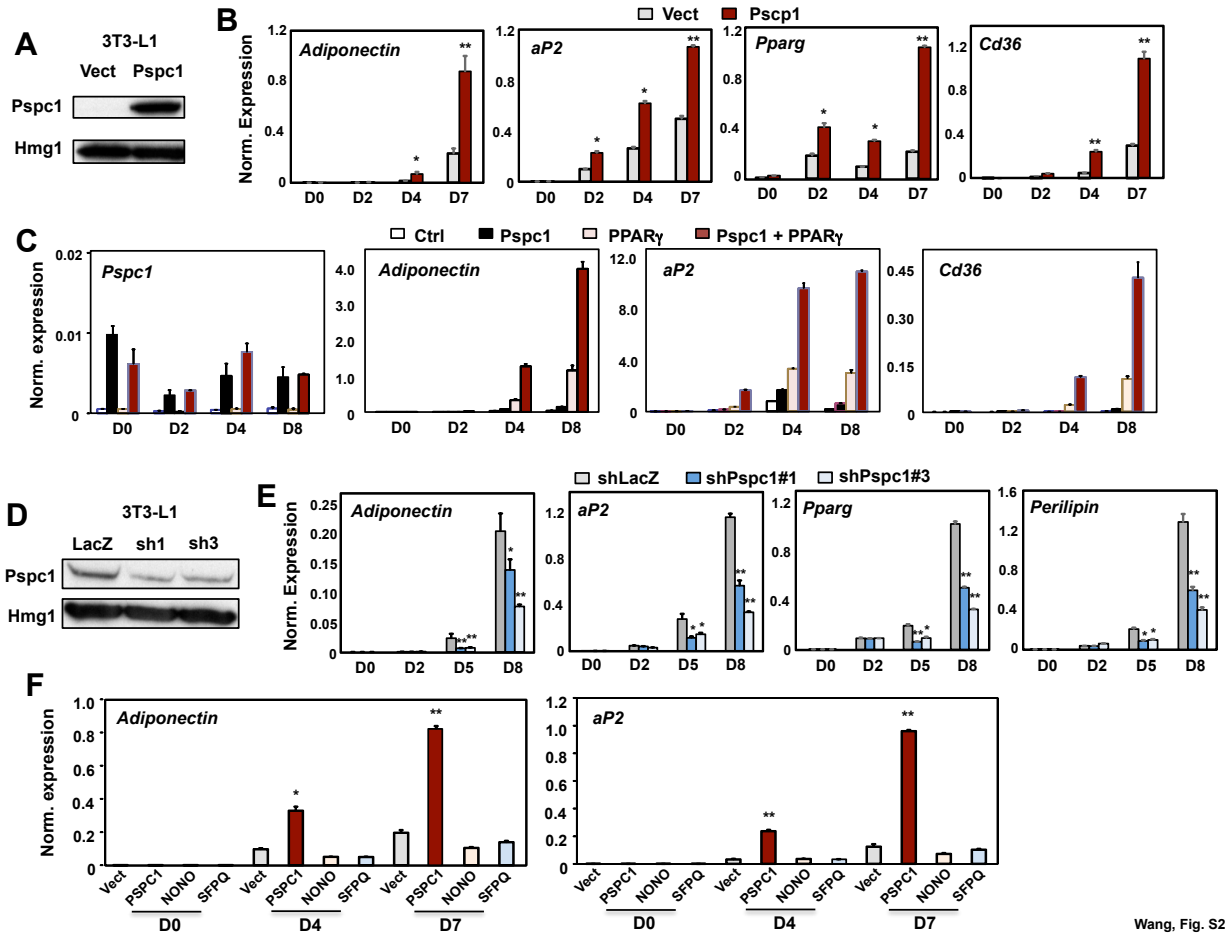



**Figure S2. Effects of Pspc1 on adipocyte differentiation.** (A) Immunoblot analysis of Pspc1 protein expression in retrovirally-derived 3T3-L1 stable cell lines expressing vector or Pspc1. (B) Real-time PCR analysis of adipogenic gene expression in 3T3-L1 stable cells described in (A) on different days during differentiation. Cells were stimulated to differentiate with DMI + 20 nM GW. Comparison was made against vector control by Student's t-test. Results represent three independent experiments. (C) Real-time PCR analysis of adipogenic gene expression in NIH-3T3 cells stably expressing Pspc1, PPARγ, or both. Cells were stimulated to differentiate with dexamethasone (2 μM), insulin (5 μg/ml), and GW1929 (20 nM) for 8 days. Results represent two independent experiments. (D) Immunoblot analysis of Pspc1 protein expression in retrovirally-derived 3T3-L1 stable cell lines expressing Pspc1 shRNAs (sh#1 and sh#3) or lacZ shRNA control. (E) Real-time PCR analysis of adipogenic gene expression in 3T3-L1 stable cells described in (D) on different days during differentiation. Cells were stimulated to differentiate with DMI + 20 nM GW. Comparison was made against shLacZ control by one-way ANOVA. Results represent three independent experiments. (F) Real-time PCR analysis of adipogenic gene expression in 10T1/2 stable cell lines expressing vector, Pspc1, Nono, or Sfpq. Cells were stimulated to differentiate with DMI + 20 nM GW. Comparison was made against vector control by one-way ANOVA. Results represent two independent experiments. Error bars represent mean + SEM. *$p < 0.05$, **$p < 0.01$.

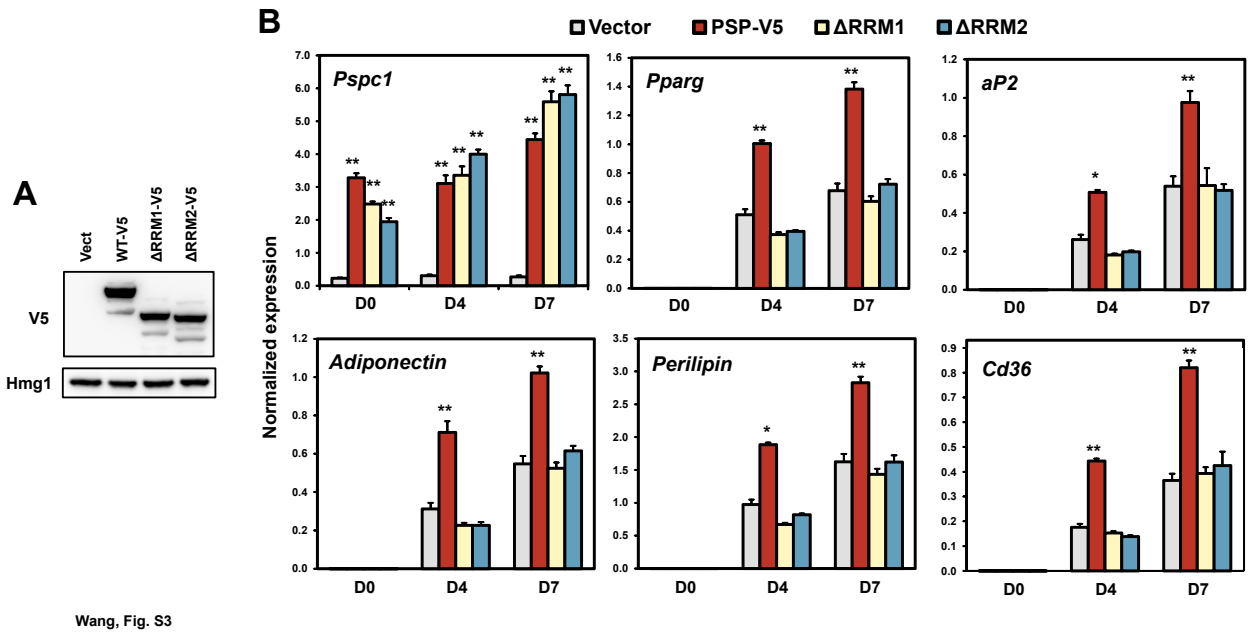



**Figure S3. RRMs of Pspc1 are required for its adipogenic effects.** (A) Immunoblot analysis of V5-Pspc1 in retrovirally-derived 10T1/2 stable cell lines expressing WT Pspc1 or individual RRM deletion mutants. (B) Real-time PCR analysis of gene expression in 10T1/2 stable cells described in (A). Cells were stimulated to differentiate with DMI + 20 nM GW for 7 days. Comparison was made against vector control by one-way ANOVA. Results represent three independent experiments. Error bars represent mean + SEM. *$p < 0.05$, **$p < 0.01$.

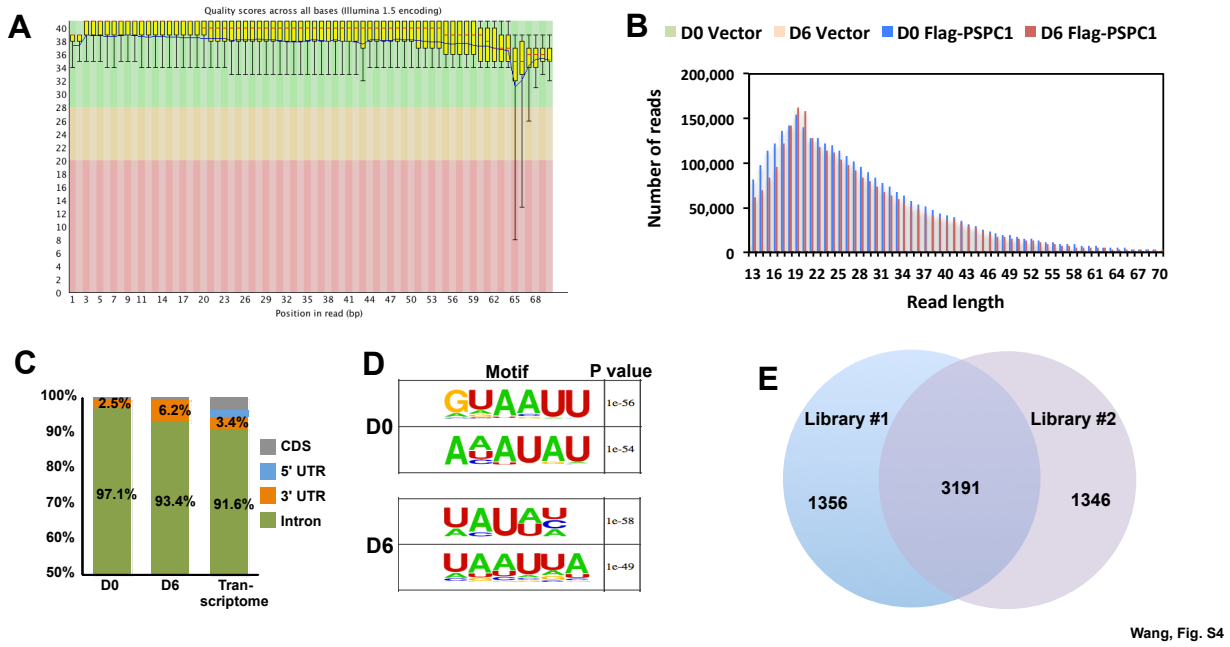
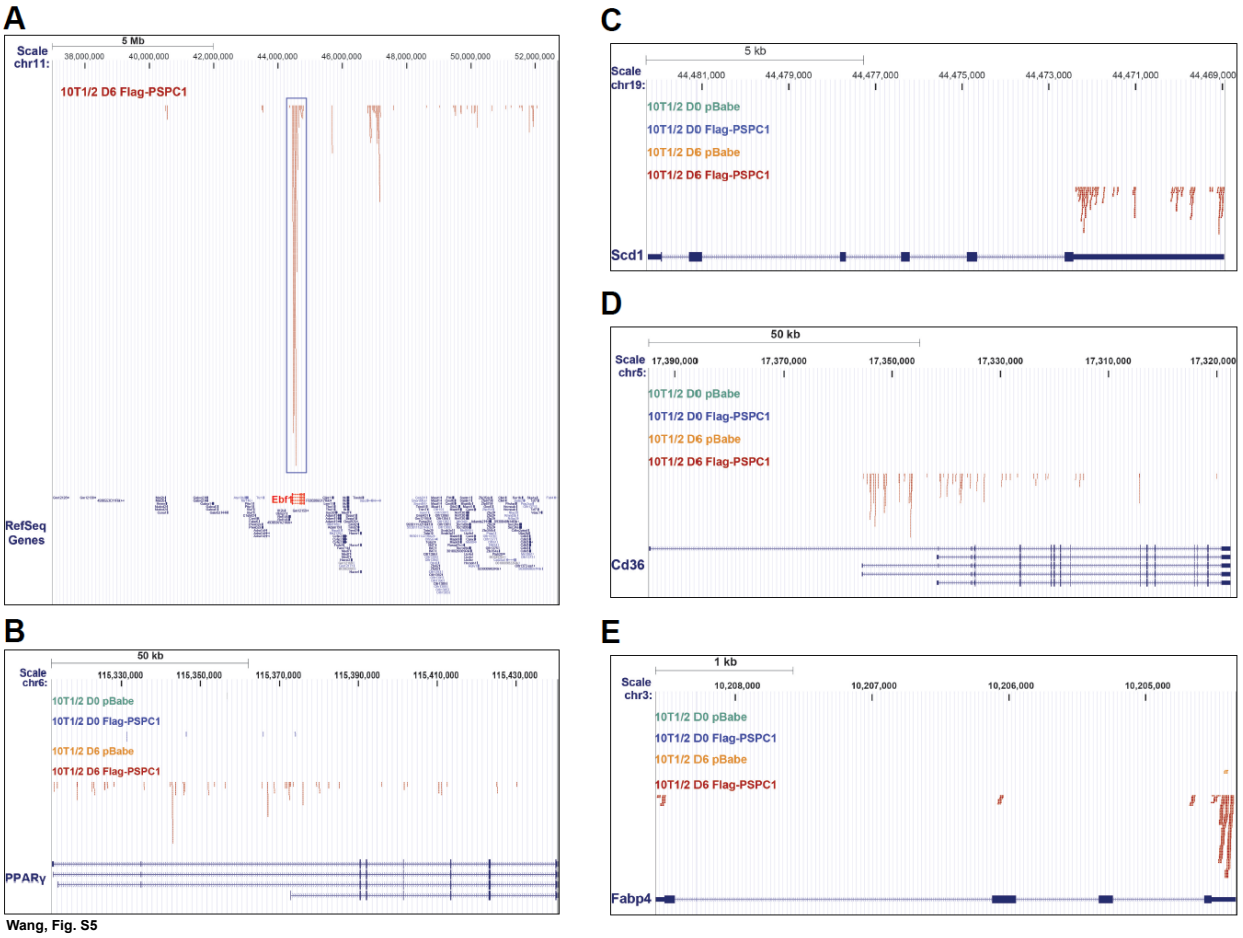



**Figure S4. Pspc1 iCLIP-seq analysis.** (A) Quality scores of total trimmed reads at each nucleotide position, using the FastQC tool from Babraham Bioinformatics. Scores within the green area indicate high quality. (B) Length distribution of trimmed reads from vector control samples and Flag-Pspc1 samples at two time points. (C) Genomic distribution of iCLIP-identified Pspc1 binding clusters on differentiation day 0 and day 6, as compared to percentage of nucleotides in the annotated transcriptome. (D) De novo motif analysis using the Homer's findMotifsGenome program. Pentamers and hexamers with top p-value rankings are displayed. (E) Venn diagram indicating overlap of putative Pspc1 interacting RNAs identified in two independent iCLIP analyses.



Wang, Fig. S5

**Figure S5. UCSC genome browser tracks of Pspc1 binding sites on target mRNAs.** Annotations are the same as Figures 2D and 2E.

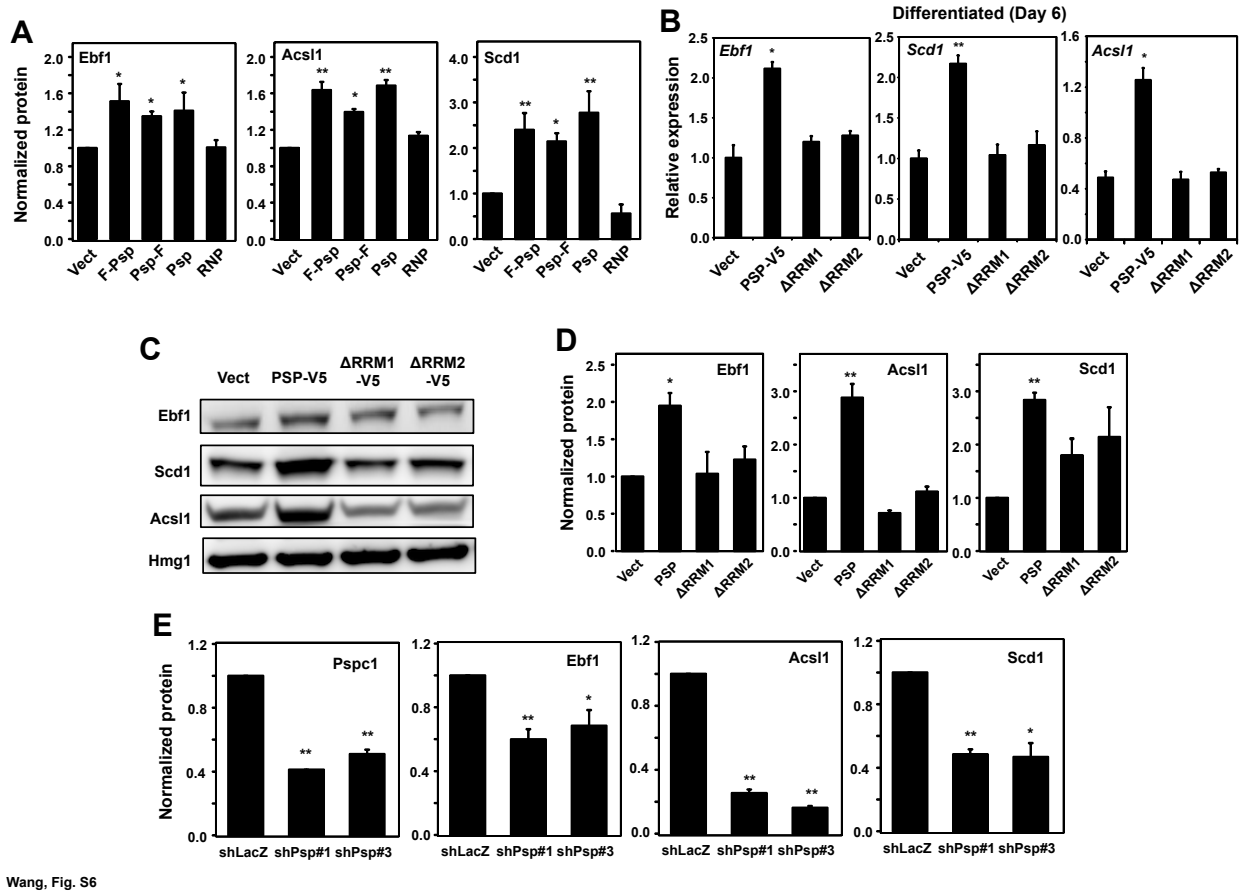



**Figure S6. RNA and protein expression of putative Pspc1 targets.** (A) Quantification of band intensity in Figure 4B. Values represent intensity of target gene bands normalized to intensity of Hmg1 bands. Comparison was made against vector control by one-way ANOVA. Results represent three independent experiments. (B) Real-time PCR analysis of target gene mRNA expression in 10T1/2 cells expressing vector (Vect), V5-tagged WT Pspc1 (Psp), V5-tagged RRM1 deletion mutant (ΔRRM1), or V5-tagged RRM2 deletion mutant (ΔRRM2). Cells were differentiated for 6 days with DMI + 20nM GW. Comparison was made against vector control by one-way ANOVA. Results represent three independent experiments. (C) Immunoblot analysis of target gene protein expression in differentiated 10T1/2 cells described in (B). Results are representative of two independent experiments. (D) Quantification of band intensity in Figure 4D. Values represent intensity of target gene bands normalized to intensity of Hmg1 bands. Comparison was made against vector control by one-way ANOVA. Results represent three independent experiments. (E) Quantification of band intensity in Figure 4F. Values represent intensity of target gene bands normalized to intensity of Hmg1 bands. Comparison was made against shLacZ control by one-way ANOVA. Results represent three independent experiments. Error bars represent mean + SEM. *$p < 0.05$, **$p < 0.01$.

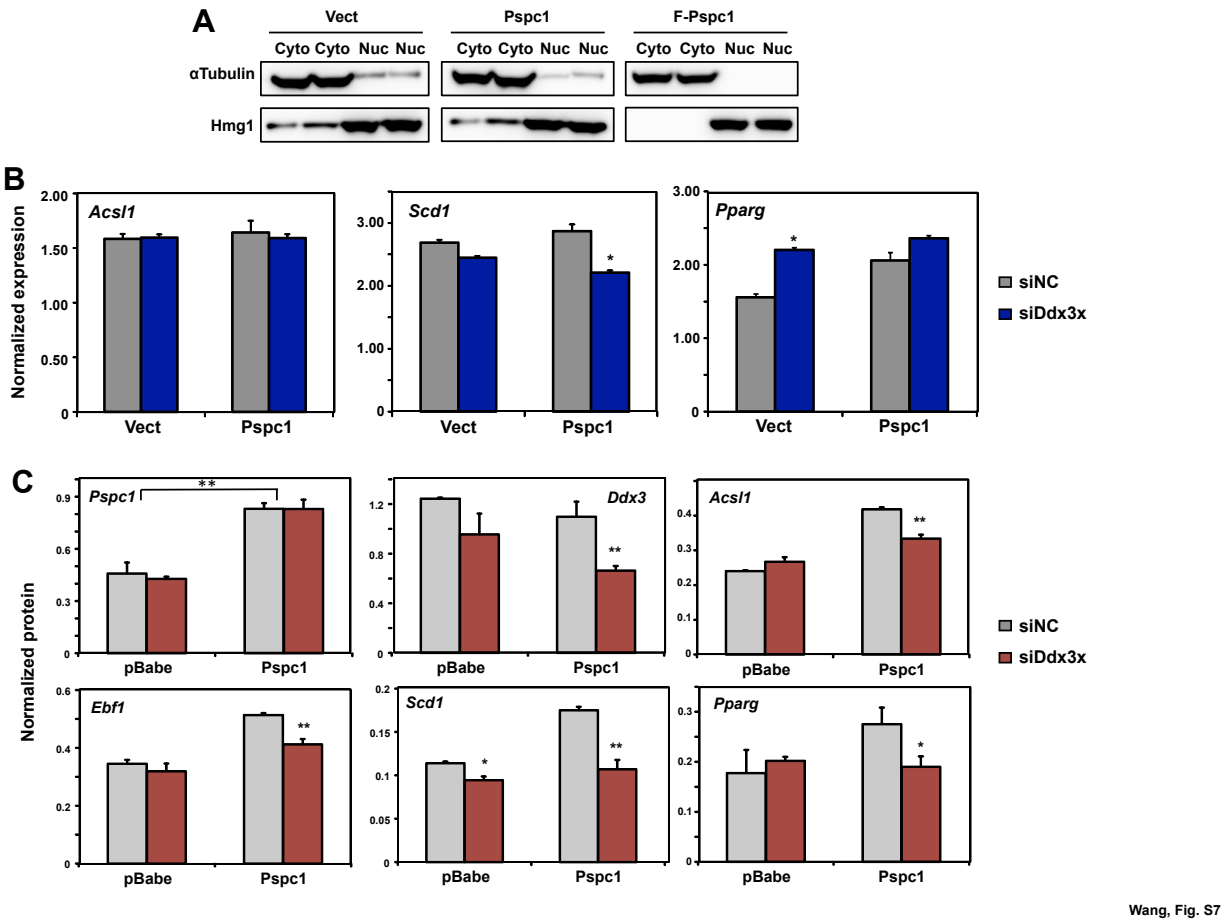



**Figure S7. Knockdown of Ddx3x affects Pspc1 target gene protein but not mRNA levels.** **(**A) Validation of nuclear and cytoplasmic enrichment for the experiment shown in Figure 6C. Immunoblot of nuclear (Nuc) and cytoplasmic (Cyto) fractions from control and Pscp1-expressing cell lines are shown. Hmg1 and αTubulin served as nuclear and cytoplasmic markers, respectively. (B) Real-time PCR analysis of gene expression in control or Pspc1-transduced 10T1/2 cells transfected with non-targeting siRNA control (siNC) or siRNA directed against Ddx3x (siDdx3x). Comparison was made against siNC control by Student's t-test. Results represent two independent experiments. (C) Quantification of band intensity in Figure 6E. Values represent intensity of target gene bands normalized to intensity of Actin bands. Comparison was made against siNC control by Student's t-test. Results represent two independent experiments. Error bars represent mean + SEM. *$p < 0.05$, **$p < 0.01$.

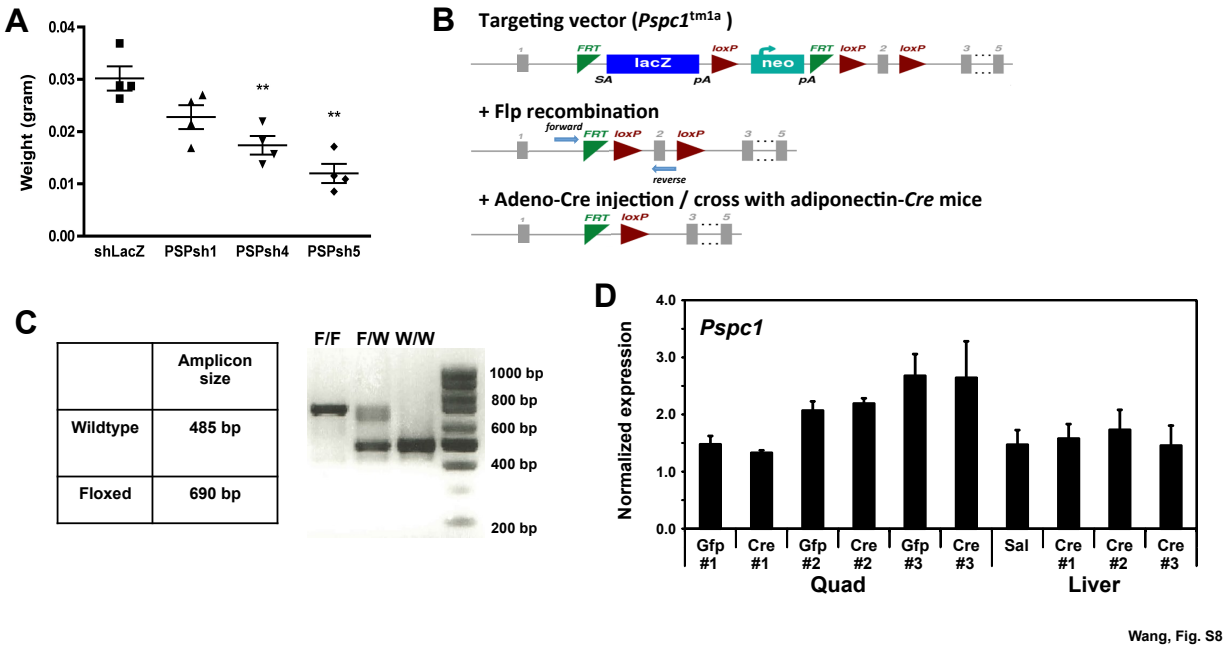



**Figure S8. Effects of loss of Pspc1 in adipose tissue *in vivo*.** (A) Weight of the fat pad formed from subcutaneous transplantation of 3T3-F442A preadipocytes expressing lacZ shRNA or different Pspc1 shRNA constructs. $n = 4$ per group. Comparison was made against shLacZ using One-way ANOVA. (B) Strategy for the generation of *Pspc1*-floxed mice. The targeting vector was electroporated into C57BL/6N ES cells to generate the conditional-potential allele. Validated ES cell clones were subjected to *in vitro* Flp recombination followed by blastocyst injection. Resulting chimeric mice were mated with C57BL/6N mice to generate heterozygous *Pspc1*-floxed mice. forward/reverse, genotyping primer binding sites for detection of the inserted *loxP* site. Homozygous *Pspc1*-floxed mice were used for Adeno-Cre injection or mated with adiponectin-*Cre* mice. (C) PCR genotyping of *Pspc1*-floxed mice. (D) Real-time PCR analysis of Pspc1 mRNA expression in liver and quadriceps muscle of $Pspc1^{F/F}$ mice injected with adenovirus or saline (Sal). Results from 3 representative mice are shown. Error bars represent mean + SEM.

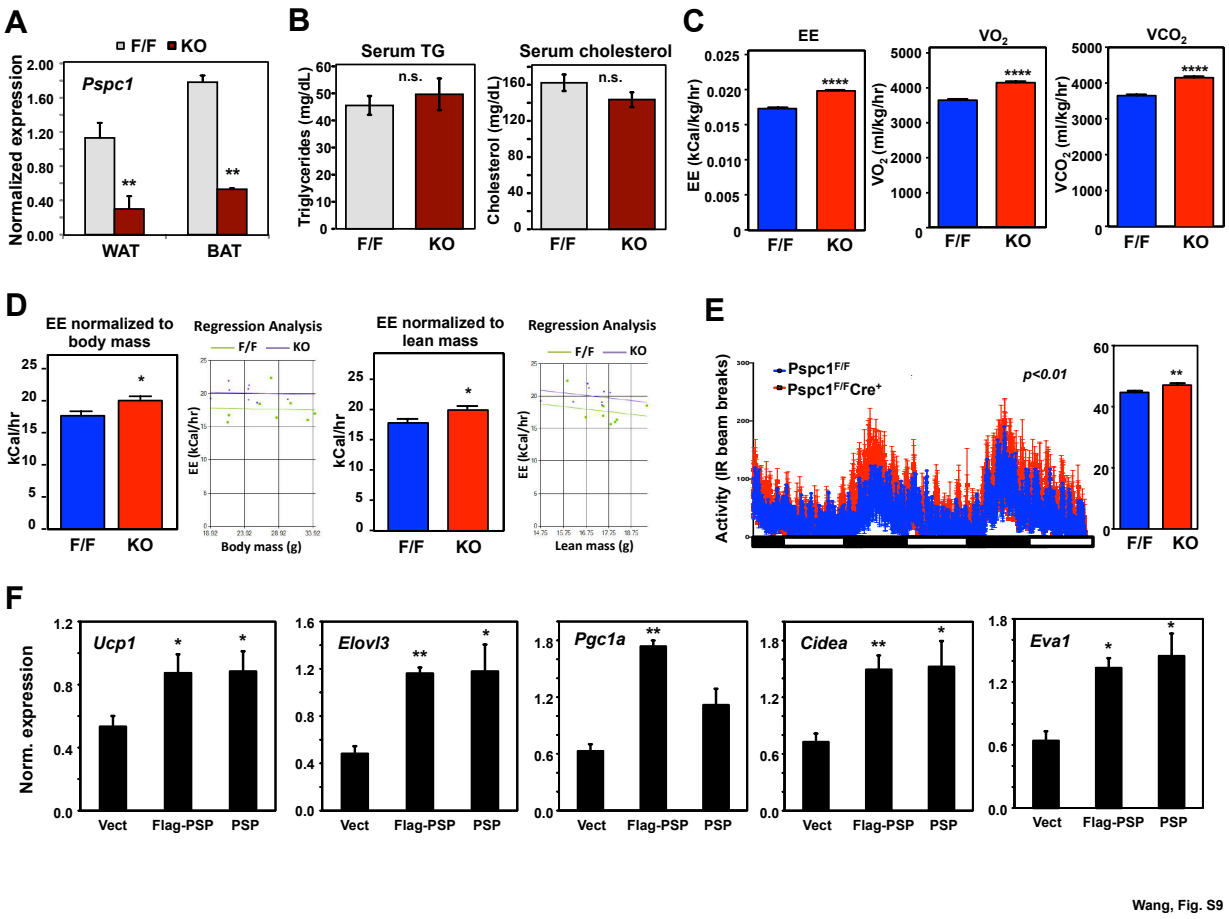



**Figure S9. Energy expenditure of adipose-specific *Pspc1* KO mice.** (A) Expression of Pspc1 mRNA in WAT and BAT from *Pspc*1$^{F/F}$ (F/F) and *Pscp1*$^{F/F}$ *Cre*+ (KO) mice. $n = 8$ per group, female mice. Comparison was made against F/F control mice by Student's t-test. (B) Serum triglyceride and serum cholesterol in F/F and KO mice after 10 weeks on HFD. $n = 8$ per group, female mice. Comparison was made against F/F control mice by Student's t-test. (C) Energy expenditure rate (kCal/kg/hr), $VO_2$ (ml/kg/hr), and $VCO_2$ (ml/kg/hr) of F/F and KO mice in metabolic chamber after 4 weeks on HFD. Values were normalized to whole body weight. 12 hr light/dark cycles, 72 hr total duration. $n = 8$ per group, female mice. $^{****}p < 0.0001$ by two-way ANOVA. (D) ANCOVA analysis on energy expenditure (EE) data in (C) with body weight and lean mass covariates. (E) Activity of F/F and KO mice in metabolic chamber after 4 weeks on HFD.12 hr light/dark cycles, 72 hr total duration, each light/dark bar represents 12 hr duration. $n = 8$ per group, female mice. $^{**}p < 0.01$ by two-way ANOVA. (F) Gene expression in differentiated brown preadipocytes stably expressing vector, native, tagged Pspc1 determined by real-time PCR. Comparison was made against vector control by one-way ANOVA. Results represent two independent experiments. Error bars represent mean + SEM. $^{*}p < 0.05$, $^{**}p < 0.01$.

| 10T1/2 | Total reads | Unique mapped reads | Significant clusters |
|---|---|---|---|
| **D0 Vect** | 178,928 | 8,822 | 776 |
| **D0 Fl-PSPC1** | 3,568,750 | 1,024,730 | 25,743 |
| **D6 Vect** | 173,921 | 7,279 | 361 |
| **D6 Fl-PSPC1** | 2,915,249 | 894,301 | 38,084 |

**Table S1. Statistics of iCLIP-seq analysis.**

| Function/Type (number) | Protein Name |
|---|---|
| **Protein synthesis (5)** | Eif2ak3, ribosomal protein L9 (RPL9), RPL13, RPL12, RPL4 |
| **RNA transport (2)** | Ddx3x, Purb |
| **preRNA processing (2)** | Ptbp1, hnRNPh2 |
| **Other RNA associated (6)** | SFPQ, NONO, Pabpc4, Ybx1, Igf2bp1, Igf2bp2 |

**Table S3. Candidate Pspc1-interacting proteins.**

**Table S4. qPCR primers used.**

| | | |
|---|---|---|
| Pspc1 | F | ATGCAGAAAACTCAACAGTACCAT |
| | R | AATGTCCCAGGTTGAGCAAA |
| aP2 | F | CACCGCAGACGACAGGAAG |
| | R | GCACCTGCACCAGGGC |
| Adiponectin | F | CCGGAACCCCTGGCAG |
| | R | CTGAACGCTGAGCGATACACA |
| CD36 | F | TTGAAAAGTCTCGGACATTGAG |
| | R | TCAGATCCCAACACAGCGTA |
| PPARγ | F | AACTCTGGGAGATTCTCCTGTTGA |
| | R | TGGTAATTTCTTGTGAAGTGCTCATA |
| Perilipin | F | CCATCTCTACCCGCCTTCG |
| | R | CTTGTCAGAGGTGCTTGCAATG |
| C/EBPα | F | GCGGGCAAAGCCAAGAA |
| | R | GCGTTCCCGCCGTACC |
| Nono | F | CCCCACCAATACCTGCAA |
| | R | TTCAGGTCAATAGTCAAGCCTTC |
| Sfpq | F | TGGTGGCATAGGTTATGAAGC |
| | R | CCAAAGCGCTCAGTACGC |
| Neat1 | F | ACCCTTTTTCATGGGGGTAG |

| | | |
|---|---|---|
| | R | GCTGGATGGAGGCTTGTTTA |
| Acsl1 | F | CCACCATCTTCCCTGTGG |
| | R | GGAAGTGTTTGCTTGTCCAAA |
| Scd1 | F | TTCCCTCCTGCAAGCTCTAC |
| | R | CAGAGCGCTGGTCATGTAGT |
| Ebf1 | F | GGGATGATGGGTGTGAACTC |
| | R | GAGTTGCGGGTGAAACCTT |
| Ddx3x | F | GGAAGTGGATCAAGGGGAAG |
| | R | CAAATTTGCCAAAGCCACTT |
| Lpl | F | TTTGTGAAATGCCATGACAAG |
| | R | CAGATGCTTTCTTCTCTTGTTTGT |
| Glut4 | F | ATGAGAAACGGAAGTTGGAGAGA |
| | R | GTGGGTGCGGCTGCC |
| Fasn | F | TGCTCCCAGCTGCAGGC |
| | R | GCCCGGTAGCTCTGGGTGTA |
| Ucp1 | F | GGCCTCTACGACTCAGTCCA |
| | R | TAAGCCGGCTGAGATCTTGT |
| Pgc1α | F | GAAAAGGCCAAACAGAGAGA |
| | R | GTAAATCACACGGCGCTCTT |

| | | |
|---|---|---|
| Cidea | F | AGCCACCAACATCACCAAT |
| | R | CGACTTGACCCGACCTTG |
| Elovl3 | F | TCCGCGTTCTCATGTAGGTCT |
| | R | GGACCTGATGCAACCCTATGA |
| Cox8b | F | GAACCATGAAGCCAACGACT |
| | R | GCGAAGTTCACAGTGGTTCC |
| CD137 | F | CGTGCAGAACTCCTGTGATAAC |
| | R | GTCCACCTATGCTGGAGAAGG |
| CD40 | F | TTGTTGACAGCGGTCCATCTA |
| | R | CCATCGTGGAGGTACTGTTTG |
| Sp100 | F | TGATGGAGGGAACCCAAACTC |
| | R | CTTCCTTGAGAATAGCTGGCAC |
| Tmem26 | F | ACCCTGTCATCCCACAGAG |
| | R | TGTTTGGTGGAGTCCTAAGGTC |
| Tbx1 | F | TTTGTGCCCGTAGATGACAA |
| | R | CTCGGCCAGGTGTAGCAG |
| MyoD1 | F | AGCACTACAGTGGCGACTCA |
| | R | GGCCGCTGTAATCCATCAT |
| PPARα | F | CCGAGGGCTCTGTCATCA |
| | R | GGGCAGCTGACTGAGGAA |

| | | |
|---|---|---|
| AOX | F | TGGCCAAGGCGACCTG |
| | R | AAGCCTTCAGCCCAGCTGT |
| Ucp2 | F | CCCTAATGGCTGCCTACCAA |
| | R | GGGTCCAGGTCAGCATGG |
| Nrf1 | F | GCAGCACCTTTGGAGAATGTG |
| | R | GCCAGAGCAGACTCGAGGTC |
| LXRα | F | TGTGCGCTCAGCTCTTGT |
| | R | CCCTGGACATTACCAAGACAC |
| LXRβ | F | CAGGAGATTGTGGACTTTGC |
| | R | TTGTAGCGTCTGGCTGTTTC |
| 36B4 | F | AGATGCAGCAGATCCGCAT |
| | R | GTTCTTGCCCATCAGCACC |
| Cyclophilin | F | TGGAGAGCACCAAGACAGACA |
| | R | TGCCGGAGTCGACAATGAT |