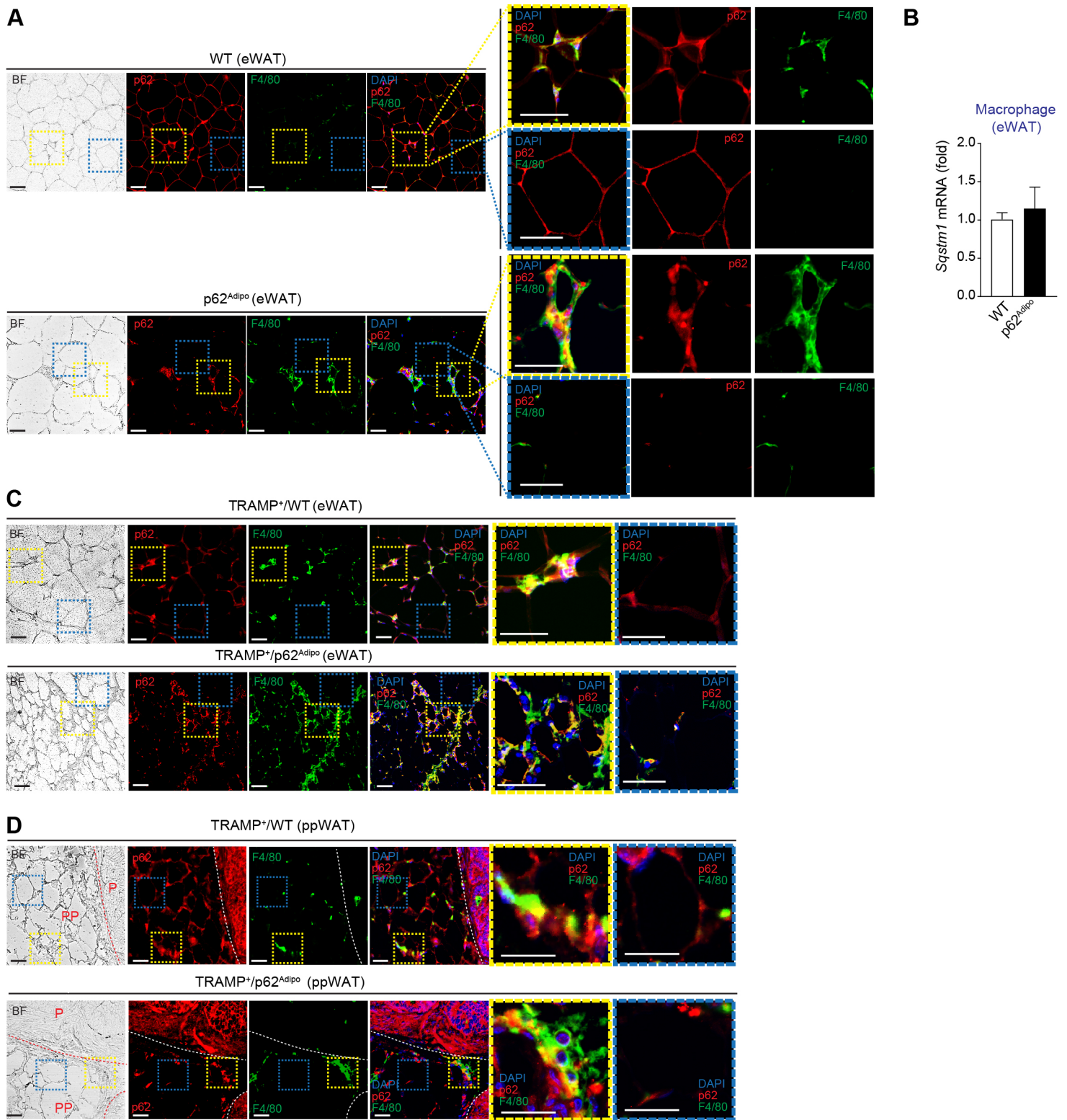


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**Supplemental Information**

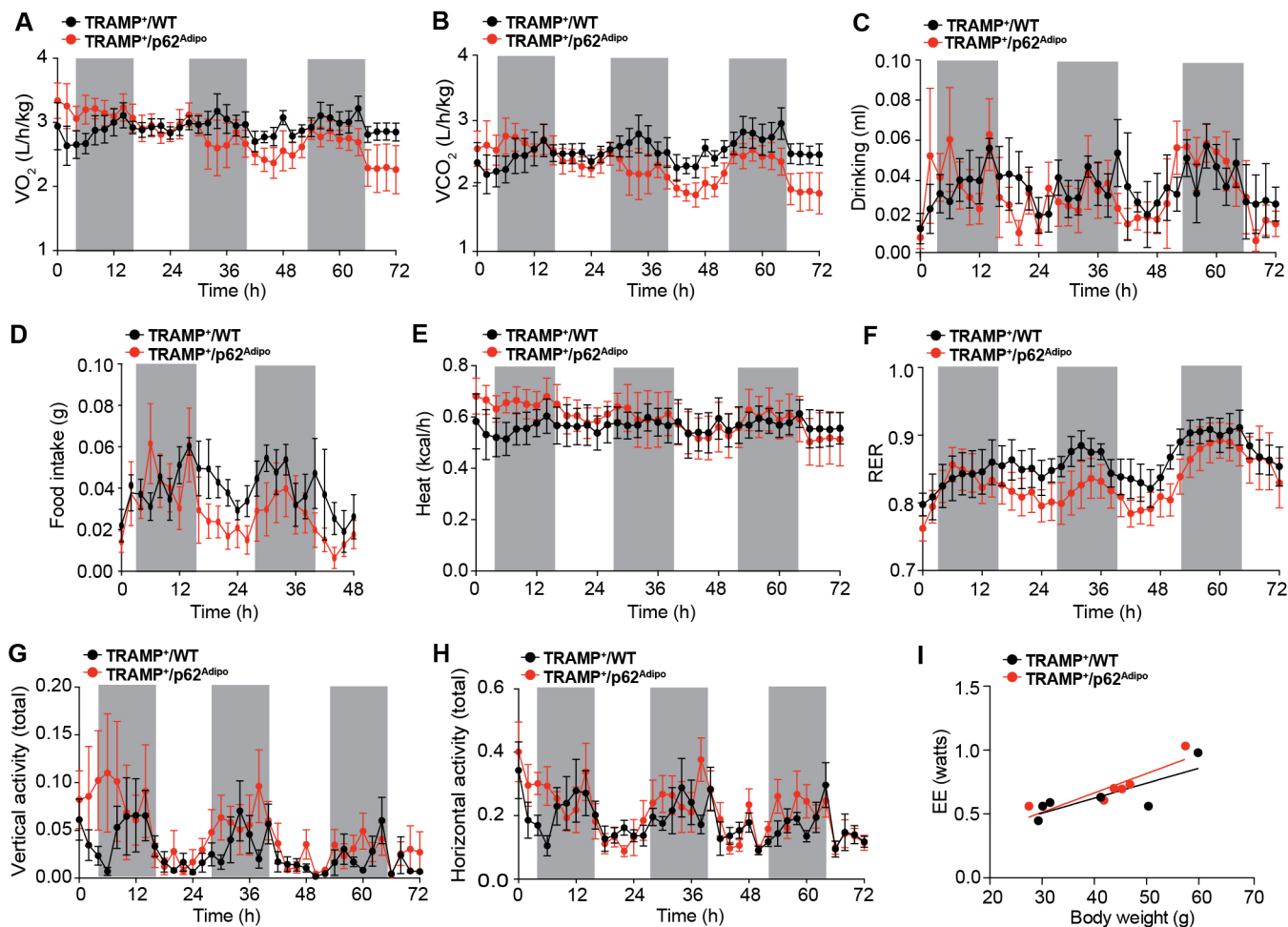
**Adipocyte p62/SQSTM1 Suppresses Tumorigenesis  
through Opposite Regulations of Metabolism  
in Adipose Tissue and Tumor**

**Jianfeng Huang, Angeles Duran, Miguel Reina-Campos, Tania Valencia, Elias A. Castilla, Timo D. Müller, Matthias H. Tschöp, Jorge Moscat, and Maria T. Diaz-Meco**



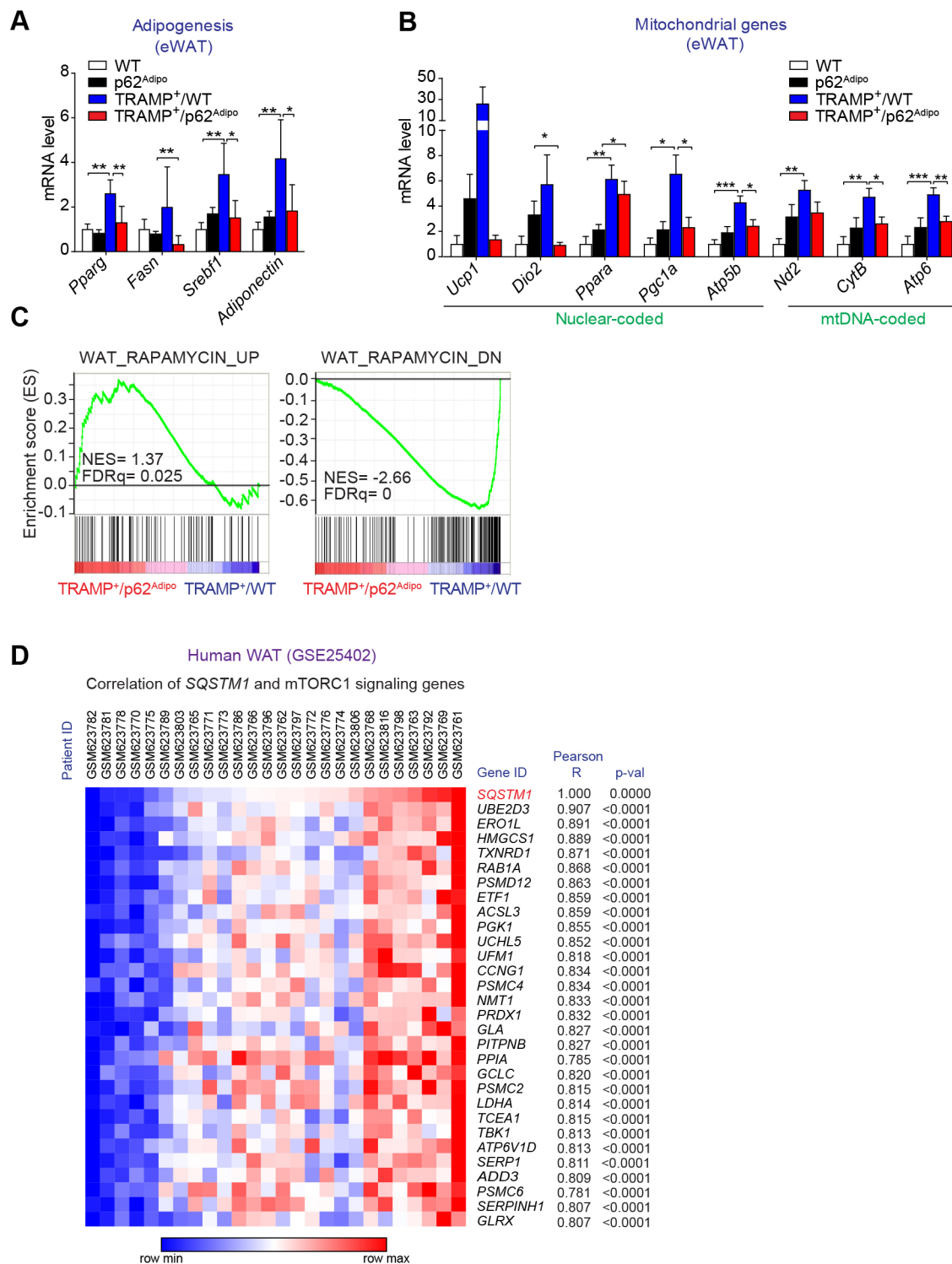
**Figure S1, related to Figure 1. White adipose tissue (WAT) p62 expression pattern in aP2-Cre mice**

(A) p62 and F4/80 double immunofluorescence (IF) staining in epididymal WAT (eWAT) of p62<sup>Adipo</sup> mice and WT controls (n = 3, per genotype). CLS niche (F4/80 positive) and non-CLS adipocytes (F4/80 negative) were selected and highlighted in dashed boxes. Scale bar = 50  $\mu$ m. (B) *Sqstm1* mRNA in primary macrophages (F4/80 positive cells) isolated from eWAT of p62<sup>Adipo</sup> mice and WT (n = 3, per genotype). Results are presented as mean  $\pm$  SEM. (C and D) p62 and F4/80 double IF staining in eWAT (C) and periprostatic WAT (ppWAT, D) of TRAMP<sup>+</sup>/p62<sup>Adipo</sup> mice and TRAMP<sup>+</sup>/WT controls (n = 3, per genotype). PP: ppWAT; P: prostate gland. CLS niche (F4/80 positive; yellow dashed box) and non-CLS adipocytes (F4/80 negative, blue dashed box) were selected and highlighted. Scale bar, 50  $\mu$ m.

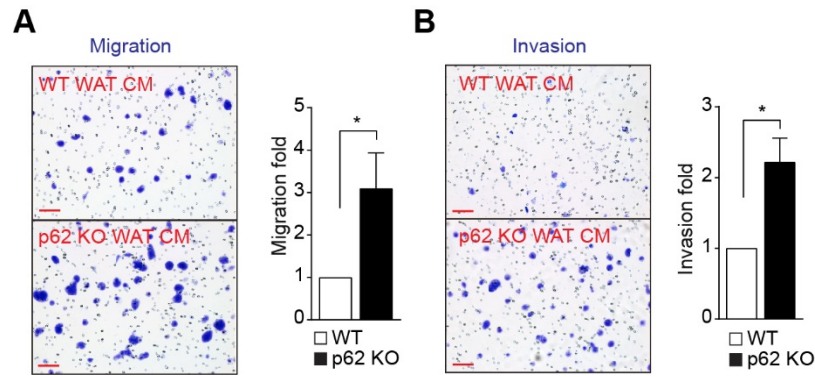


**Figure S2, related to Figure 2. Metabolic analysis of TRAMP<sup>+</sup>/WT and TRAMP<sup>+</sup>/p62<sup>Adipo</sup> mice**

(A-F) 7 month-old TRAMP<sup>+</sup>/p62<sup>Adipo</sup> and TRAMP<sup>+</sup>/WT mice (n = 6, per genotype) were housed in a metabolic chamber and monitored using Comprehensive Lab Animal Monitoring System CLAMS. Oxygen consumption (A), Carbon Dioxide production (B), Drinking volume (C), Food intake (D), Heat production (E), Respiratory Exchange Rate (RER) (F), Locomotor activity (G-H), Energy expenditure in relation to body weight (I) are recorded. Results are presented as mean ± SEM.

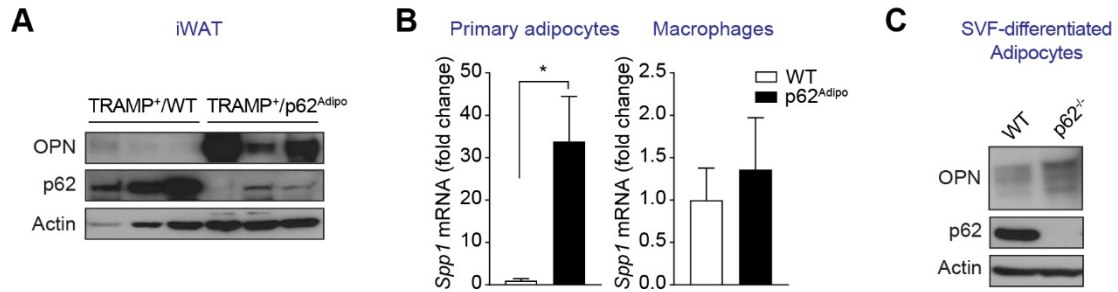


**Figure S3, related to Figure 3. Metabolic alterations in WAT of TRAMP<sup>+/WT</sup> and TRAMP<sup>+/p62<sup>Adipo</sup></sup> mice** (A and B) qPCR analysis of adipogenesis-related genes (A) and mitochondrial genes (B) in eWAT (n = 5-6, per genotypes). Results are presented as mean ± SEM. Student's T test (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001). (C) GSEA plot of enrichment in “WAT\_RAPAMYCIN\_UP” signature in eWAT of TRAMP<sup>+/p62<sup>Adipo</sup></sup> mice and “WAT\_RAPAMYCIN\_DN” signature in TRAMP<sup>+/WT</sup> controls. (D) Heatmap showing the Pearson correlation between the expression of *SQSTM1* and leading-edge genes of the “mTORC1 signaling” gene set.



**Figure S4, related to Figure 5. Role of p62 in the adipose tissue-PCa cell interaction in vitro**

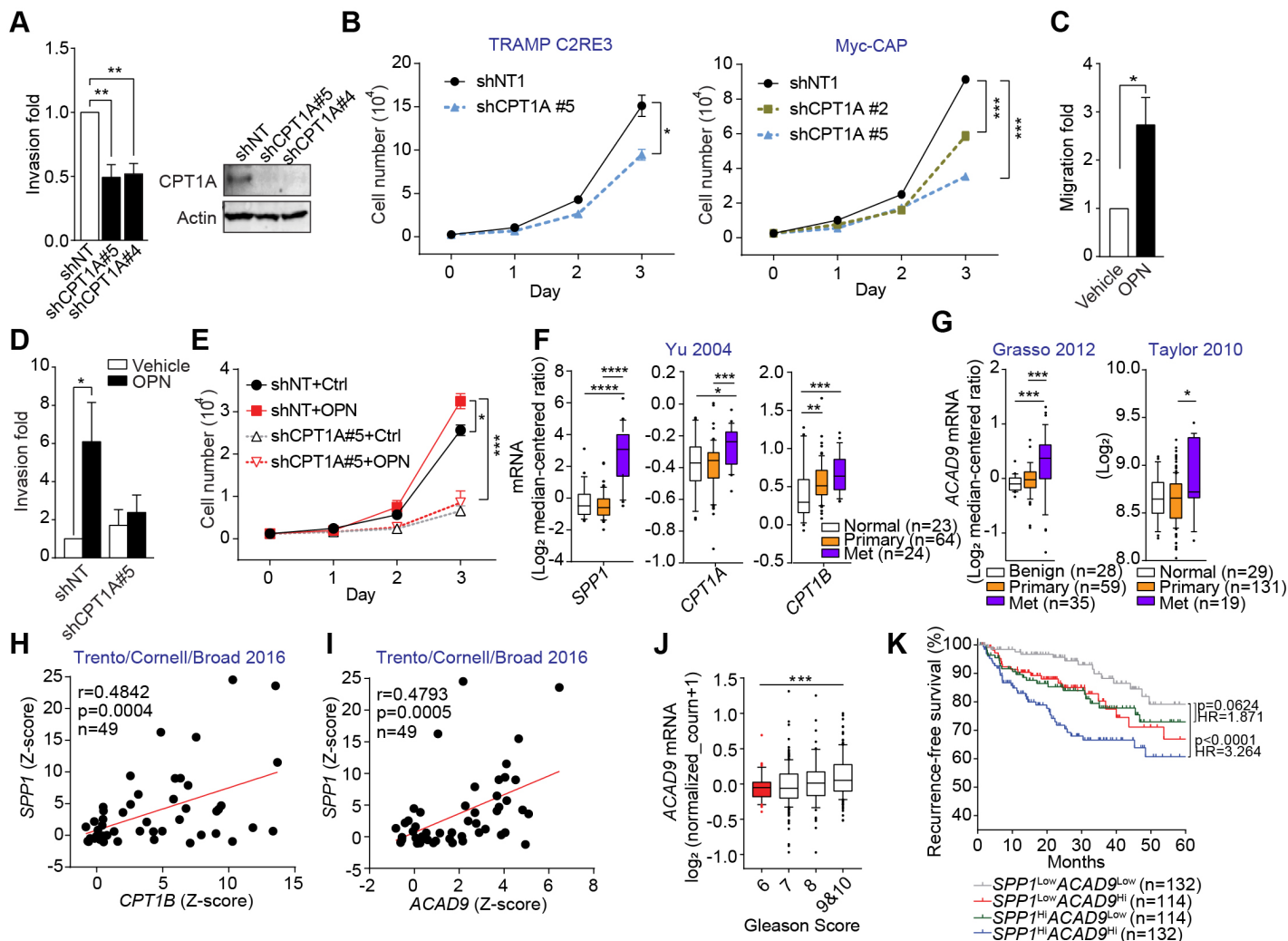
(A) Migration of Myc-CAP in response to CM of p62 KO WAT or WT control for 23 hr. Representative filters and quantification of three independent experiments (n = 4). (B) Invasion of Myc-CAP in response to CM as described in (A) for 28 hr. Representative images and quantification of three independent experiments (n = 3). Scale bar, 100  $\mu$ m. Results are presented as mean  $\pm$  SEM. Student's T-test (\*p<0.05).



**Figure S5, related to Figure 6. OPN expression in p62-deficient adipose tissue**

(A) Immunoblot analysis of OPN and p62 in iWAT of the indicated genotypes. (B) qPCR analysis of *Spp1* (encoding the OPN protein) mRNA expression in primary mature adipocytes (left) and macrophages (right) isolated from eWAT of indicated genotypes (n = 3). Results are presented as mean  $\pm$  SEM. Student t-test. \*, p<0.05. (C) Immunoblot analysis of OPN and p62 in WT and p62 KO primary adipocytes differentiated from SVF. Representative result of two independent experiments.





**Figure S6, related to Figure 7. Cpt1a Is Required for OPN-stimulated Proliferation and Invasion in PCa Tumor**

(A) Cell invasion of TRAMP C2RE3 deficient in *Cpt1a* (shCPT1A) or control (shNT) in response to 5% FBS DMEM in Transwell system for 20 hr. Three independent experiments (n = 3). Right: immunoblot of CPT1A in TRAMP C2RE3 cells. (B) Growth curve of Myc-CAP deficient in *Cpt1a* (shCPT1A) or control (shNT) in media containing 10% FBS (n = 3). (C) Cell migration of TRAMP C2RE3 in response to recombinant OPN (5 µg/ml) in Transwell system for 20 hr (n = 4). (D) Cell invasion of TRAMP C2RE3 (shCPT1A and shNT) in response to recombinant OPN (5 µg/ml) in Transwell system for 22 hr (n = 5). (E) Growth curve of Myc-CAP deficient in CPT1A (shCPT1A) or control (shNT) in response to recombinant OPN (5 µg/ml) (n = 4). (F) Box-and-whisker plots of *CPT1A* and *IB* transcript levels in prostate samples from different lesion sites in human prostate cancer dataset (Yu 2004) extracted from Oncomine. Mann-Whitney test. (G) Box-and-whisker plots of *ACAD9* transcript levels in prostate samples from different lesion sites in two different human prostate cancer datasets (Grossa 2012 and Taylor 2010) extracted from Oncomine or GEO (GSE21034), respectively. Mann-Whitney test. (H and I) Scatter plots of expression of *SPP1* related to *CPT1B* (H) or to *ACAD9* (I) in patient samples (n = 49) from castration-resistant prostate cancer (CRPC) dataset (Trento 2016) extracted from cBioportal. Pearson's correlation analysis. (J) Box-and-whisker plots of *ACAD9* transcript levels in patients with different Gleason Scores from TCGA PRAD dataset. Gleason 6 (n = 45), 7 (n = 247), 8 (n = 64), 9 plus 10 (n = 142). Mann-Whitney test. (K) Kaplan-Meier curve of five-year freedom from biochemical



recurrence in patients from TCGA PRAD cohort (n = 493) according to *SPP1* and *ACAD9* co-expression. Median as expression cutoff. Log-Rank test. HR: Hazard ratio. Results are presented as mean  $\pm$  SEM expect (A - E). \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001. Student's T-test unless indicated elsewhere.

## Supplemental Tables

**Table S1, related to Figure 4. Human PPARalpha Target Gene List**

Lipid metabolism-related	Glucose metabolism-related
ADIPOR2, CD36, LEPR, SLC27A, SLC27A2, SLC27A4, ACOT1, ACOT7, ACOT12, ACSL1, ACSL3, ACSL4, ACSL5, ACSM3, ACSS2, FABP1, FABP2, FABP3, FABP4, FABP5, ACAA2, ACADL, ACADM, ACADS, ACADVL, ACAD8, ACAD9, ACAD10, ACOT2, ACOT9, CPT1A, CPT1B, CPT2, CRAT, DECR1, ETFA, ETFB, ETFDH, HADHA, HADHB, HADH, HSD17B10, HIBCH, SLC25A20, SLC22A5, TXNIP, UCP2, UCP3, ACAT1, BDH1, BDH2, FGF21, HMGCL, HMGCS2, ABCD2, ABCD3, ACAA1, ACAA1B, ACOT1, ACOT4, ACOT5, ACOT8, ACOX1, CROT, DECR2, ECH1, EHHADH, HACL1, HSD17B4, ECI2, PEX11A, ALDH3A1, ALDH3A2, ALDH9A1, CYP4A11, CYP4A22, CYP4A14, CYP4F8, CYP4X1, ACACA, ACACB, AGPAT2, AGPAT3, AGPAT5, AGPAT6, DGAT1, ELOVL5, ELOVL6, ELOVL7, FADS2, FADS1, FASN, GPAM, HSD17B12, LPIN2, MLYCD, MOGAT1, SCD, SCD2, SLC25A10, SREBF1, PLIN2, CES1, CES3, CIDEA, CIDEA, G0S2, LIPA, LIPE, MGLL, PLIN5, PLIN1, PNPLA2, PLIN4, ANGPTL4, APOA1, APOA2, APOA5, APOC3, LIPC, LIPG, LPL, LRP4, PCTP, PLTP, MTTP, VLDLR, ABCA1, ABCB4, ABCB11, ABCG5, ABCG8, CAV1, CYP7A1, CYP8B1, CYP27A1, FXR1, NR1H3, NPC1, RAB9A, SCARB2, SLC10A1, SLC10A2	AQP3, AQP7, AQP9, FBP2, G6PC, GPD1, GPD2, GYK, GYS2, LDHA, PC, PCK1, PDK1, PDK4

**Table S2, related to STAR METHODS**

<b>Gene Symbol</b>	<b>Forward</b>	<b>Reverse</b>
<i>Pparg</i>	GAACGTGAAGCCCATCGAGGAC	CTGGAGCACCTTGGCGAACA
<i>Fasn</i>	CTTCAACCTGGCCATGGTTTT	GTTGGCGAAGCCGTAGTTAGTT
<i>Srebf1</i>	TATGGAGGGCATGAAACCCGAAGT	TTGACCTGGCTATCCTCAAAGGCT
<i>Adiponectin</i>	GCTTATGTGTATCGCTCAG	TGTGGTAAGAGAAGTAGTAGAG
<i>Ucp1</i>	TCTTCTCAGCCGGAGTTTCAGCTT	ACCTTGGATCTGAAGGCGGACTTT
<i>Dio2</i>	AAGGCTGCCGAATGTCAACGAATG	TGCTGGTTCAGACTCACCTTGGA
<i>Ppara</i>	GACGCTTGTGGCCAAGAT	GTGATAAAGCCATTGCCGT
<i>Pgc1a</i>	AGCTGTGTTTGACGACAAATC	CGACACGGAGAGTTAAAGGAAG
<i>Atp5b</i>	ACCCATCCTAAATGCCCTGGAAGT	ACTTTCTGGCCTCTAACCAAGCCT
<i>Cox2</i>	ACCTGGTGAACCTACGACTGCT	CCTAGGGAGGGGACTGCTCA
<i>Nd2</i>	GCCTGGAATTCAGCCTACTAGC	GGCTGTTGCTTGTGTGACGA
<i>CytB</i>	CCTTCATGTCTGGACGAGGCTT	TGCTGTGGCTATGACTGCGAA
<i>Atp6</i>	TGGCATTAGCAGTCCGGCTT	ATGGTAGCTGTTGGTGGGCT
<i>Cpt1a</i>	GCTCGCACATTACAAGGACAT	TGGACACCACATAGAGGCAG
<i>Acadl</i>	GGGAATGAAAGCTCAGGACA	AGAATCCGCATTAGCTGCAT
<i>Acadm</i>	AGGTTTCAAGATCGCAATGG	CATTGTCCAAAAGCCAAACC
<i>Acads</i>	TGGCGACGGTTACACACTG	GTAGGCCAGGTAATCCAAGCC
<i>Acot1/2</i>	GACAAGAAGAGCTTCATTCCCGTG	CATCAGCATAGAACTCGCTCTTCC
<i>Txnip</i>	CCCACCTACACTGAGGTGGAT	GAGGCAGAAAGAAATGCGCT
<i>Syp</i>	CCTCGGTGGTGTTCGGCTTC	AGCCTGTCTCCTTGAACACG
<i>Eno2</i>	AGCCCTCATCAGCTCAGGTA	TCTCAGTCCCATCCAGTTCC
<i>Mcp1</i>	AGAGCCAGACGGGAGGAAG	CCAGCCTACTCATTGGGATC
<i>Spp1</i>	GATGATGATGACGATGGAGACC	CGACTGTAGGGACGATTGGAG
<i>Tnfa</i>	TGTCTACTCCCAGGTTCTCT	GGGGCAGGGGCTCTTGAC
<i>Il6</i>	ATCCAGTTGCCTTCTTGGGACTGA	TAAGCCTCCGACTTGTGAAGTGGT
<i>Arg1</i>	AGTCTGGCAGTTGGAAGCAT	AGGGGAGTGTTGATGTCAGT
<i>Mgl2</i>	AGGCAGCTGCTATTGGTTCTCTGA	AGTTGACCACCACCAGGTGAGAAT
<i>Tgfb1</i>	CTTCCCGAATGTCTGACGTA	GACCGCAACAACGCCATCT
<i>18s</i>	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG