

Supplemental Table and Figures to

A high-fat diet attenuates AMP-activated protein kinase α 1 in adipocytes to induce exosome shedding and nonalcoholic fatty liver development *in vivo*

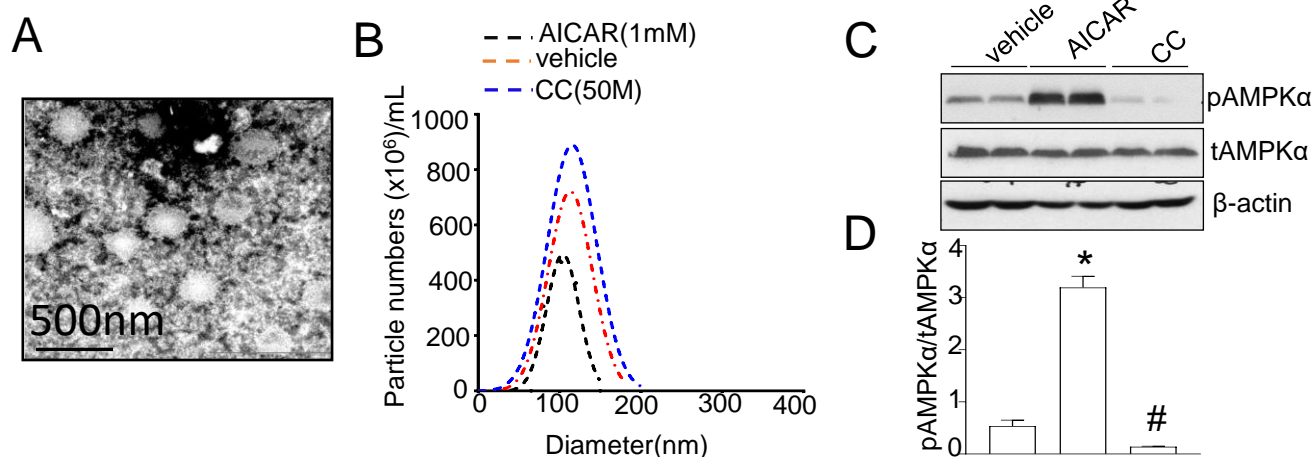
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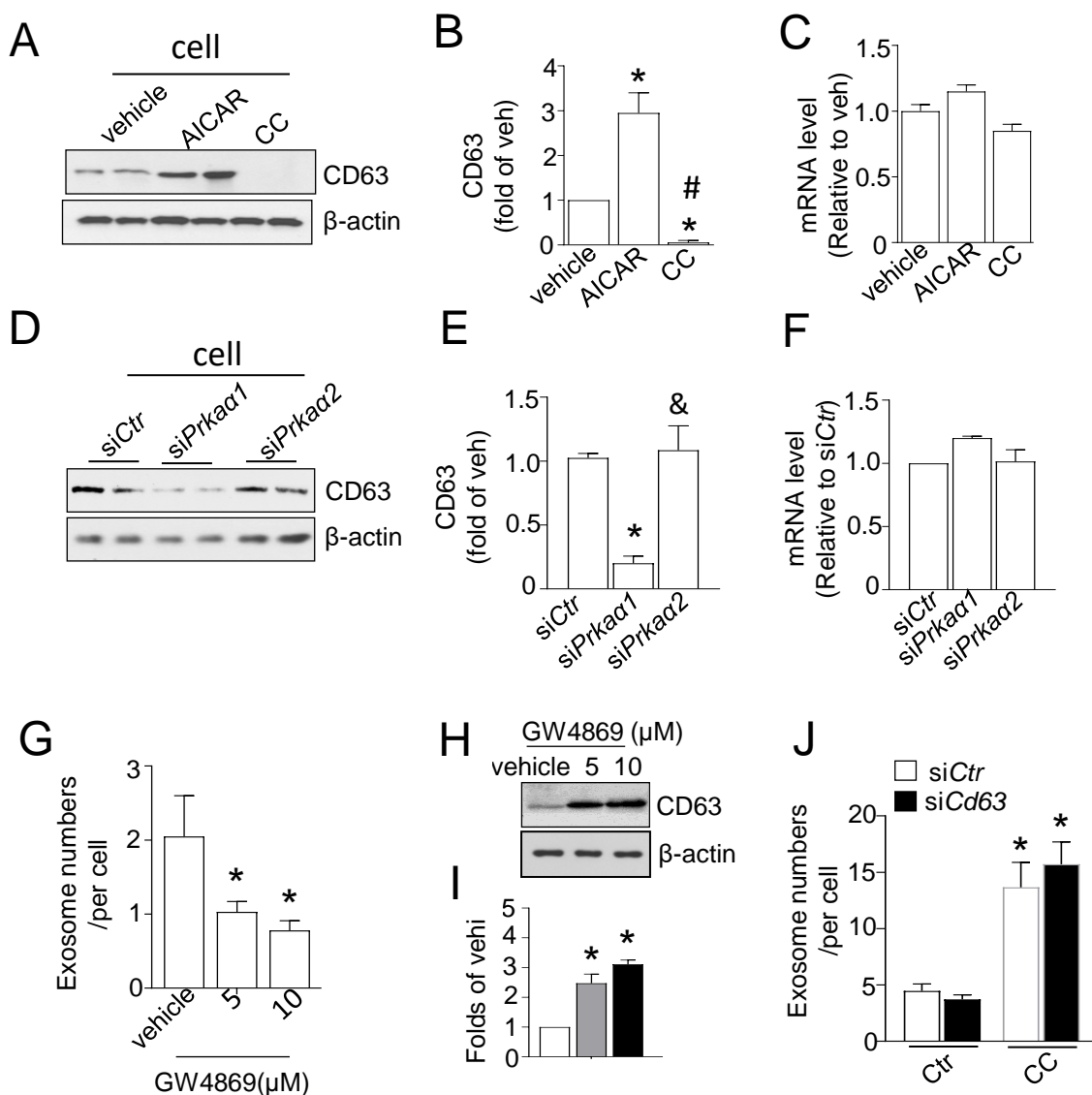
Supplemental Table 1

Antibody and Chemical	Company	Catalog number
Anti-CD63	Biosystem	EXOAB-CD63A-1
Anti-CD81	Biosystem	EXOAB-CD81A-1
Anti-AMPKa1	Cell Signaling Technology	#2795
Anti-pAMPKa	Cell Signaling Technology	#2535
Anti-beta-actin	Cell Signaling Technology	#4970
Anti-TSG101	Santa Cruz	sc-7964
Anti-CD36	Santa Cruz	Sc-7309
Anti-cleaved-caspase-3	Cell Signaling Technology	#9579
Anti-CCL2	Cell Signaling Technology	#2027
Anti-IL-6	Cell Signaling Technology	#12153
Anti-beta-tublin	Cell Signaling Technology	#2418
Anti-GAPDH	Cell Signaling Technology	#5174
Anti-AMPKa	Cell Signaling Technology	#2532
Anti-AMPKa2	Cell Signaling Technology	#2757
palmitic acid	Sigma Aldrich	P0500
high fat diet (60% kcal fat)	Research Diets	D12492
AICAR	Sigma Aldrich	06595
Compound C	Sigma Aldrich	C2313
GW4869	Sigma Aldrich	D1692

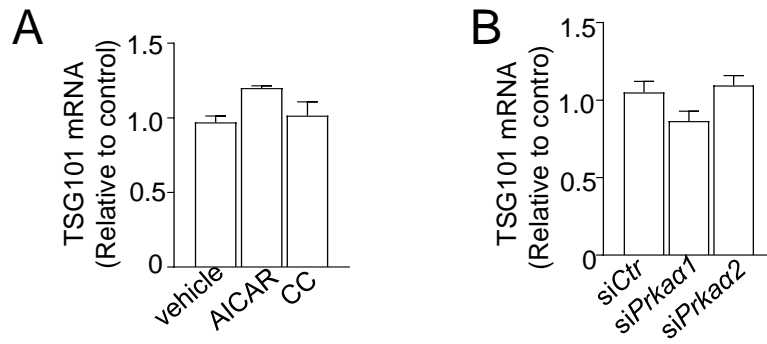


Supplemental Figure 1. Effects of AMPK activation or inhibition on adipocyte-derived exosomes.

A, Transmission electron microscope (TEM) image of adipocyte-derived exosomes. **B**, Effects of CC (50 μ M) and AICAR (1mM) on the diameter of the exosomes isolated from 3T3L1-derived adipocytes. **C**, Representative Western blots of AMPK α and pAMPK α in cellular lysates of 3T3L1-derived adipocytes treated with either CC or AICAR; the blots are a representative of three independent experiments; **D**, Quantitative data for AMPK α and pAMPK α . * $p < 0.05$ AICAR-treated verse vehicle-treated; # $p < 0.05$ CC-treated verse vehicle-treated $n=3$.

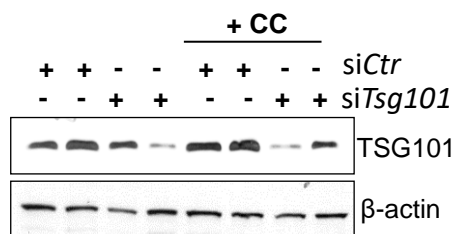


Supplemental Figure 2. AMPK α 1 regulated-exosome release is independent of CD63. **A**, Western blot analysis of CD63 in lysates of adipocytes treated with or without CC (50 nM) or AICAR (1 mM). **B**, Quantitative data for CD63. * $p < 0.05$ vs. Vehicle. $n=3$. **C**, CD63 mRNA in adipocytes treated with or without CC (50 nM) or AICAR (1 mM). **D**, Western blot analysis of CD63 in lysates of adipocytes transfected with siCtrl, siPrkaa1, or siPrkaa2. **E**, Quantitative data for CD63. * $p < 0.05$ vs. siCtrl. **F**, CD63 mRNA in adipocytes transfected with siCtrl, siPrkaa1, or siPrkaa2. **G**, Exosome number in medium from adipocytes treated with GW4869. **H**, Western blot analysis of CD63 in exosomes from 3T3L1-derived adipocytes treated with GW4869. **I**, Quantitative data for CD63. * $p < 0.05$ vs. vehicle. **J**, Exosome number in medium from adipocytes transfected with Cd63 siRNA and treated with CC. * $p < 0.05$ vs. control. # $p < 0.05$ vs. control group treated with AICAR treatment. & $p < 0.05$ vs. control group treated with siPrkaa1^{-/-} or Prkaa1^{-/-}, $n=3$.

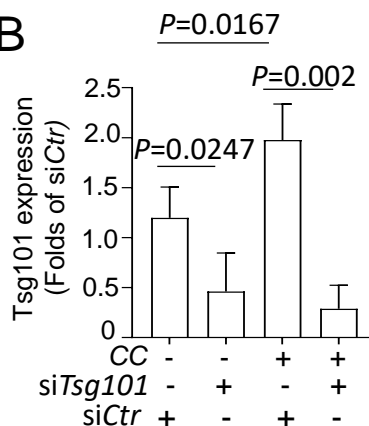


Supplemental Figure 3. Effect of AMPK on TSG101 mRNA expression. **A**, TSG101 mRNA level in adipocytes treated with AICAR (1 mM) or CC (50 nM). **B**. TSG101 mRNA level in adipocytes transfected with *Prkaa1* or *Prkaa2* siRNA, n=6.

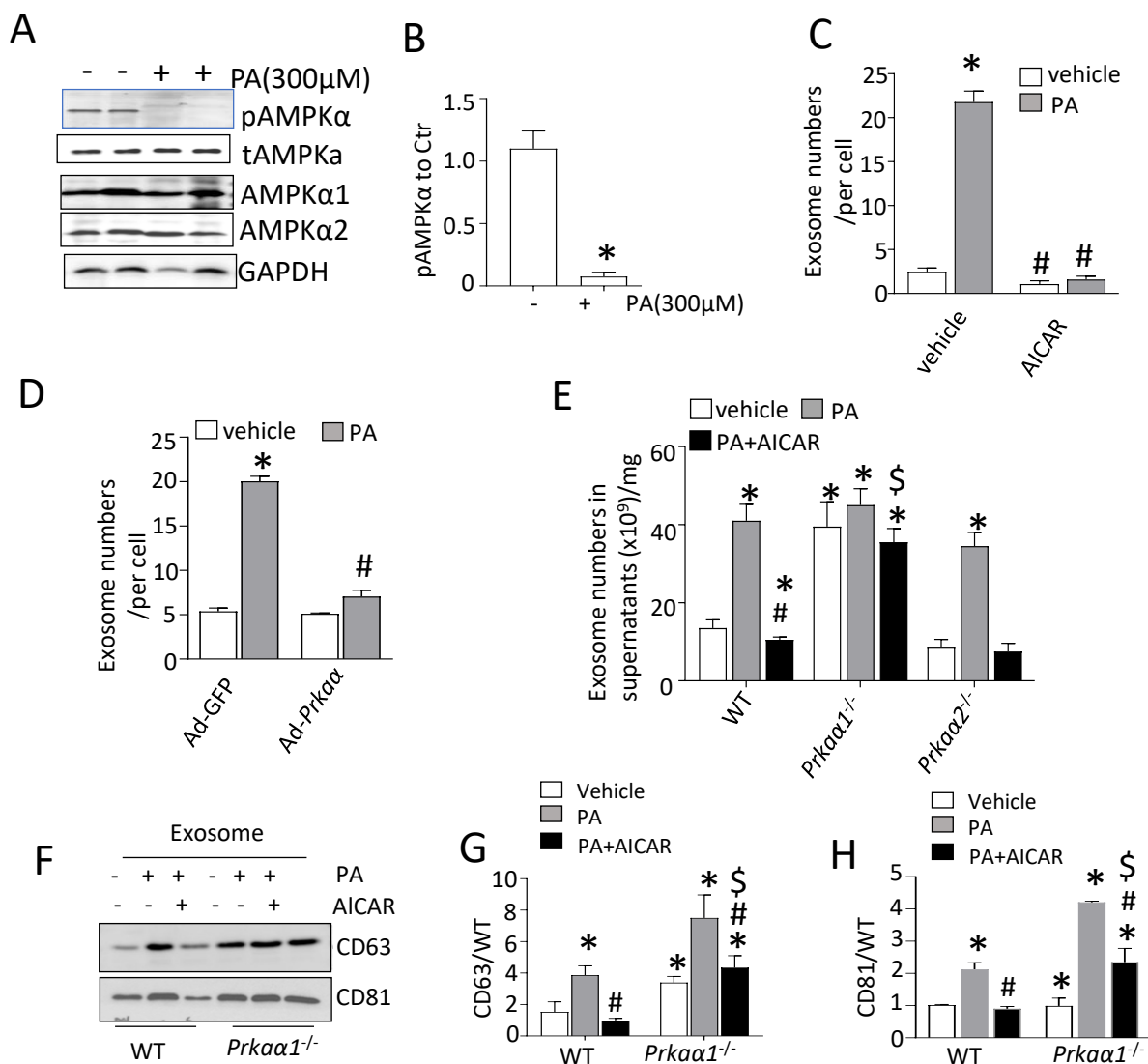
A



B

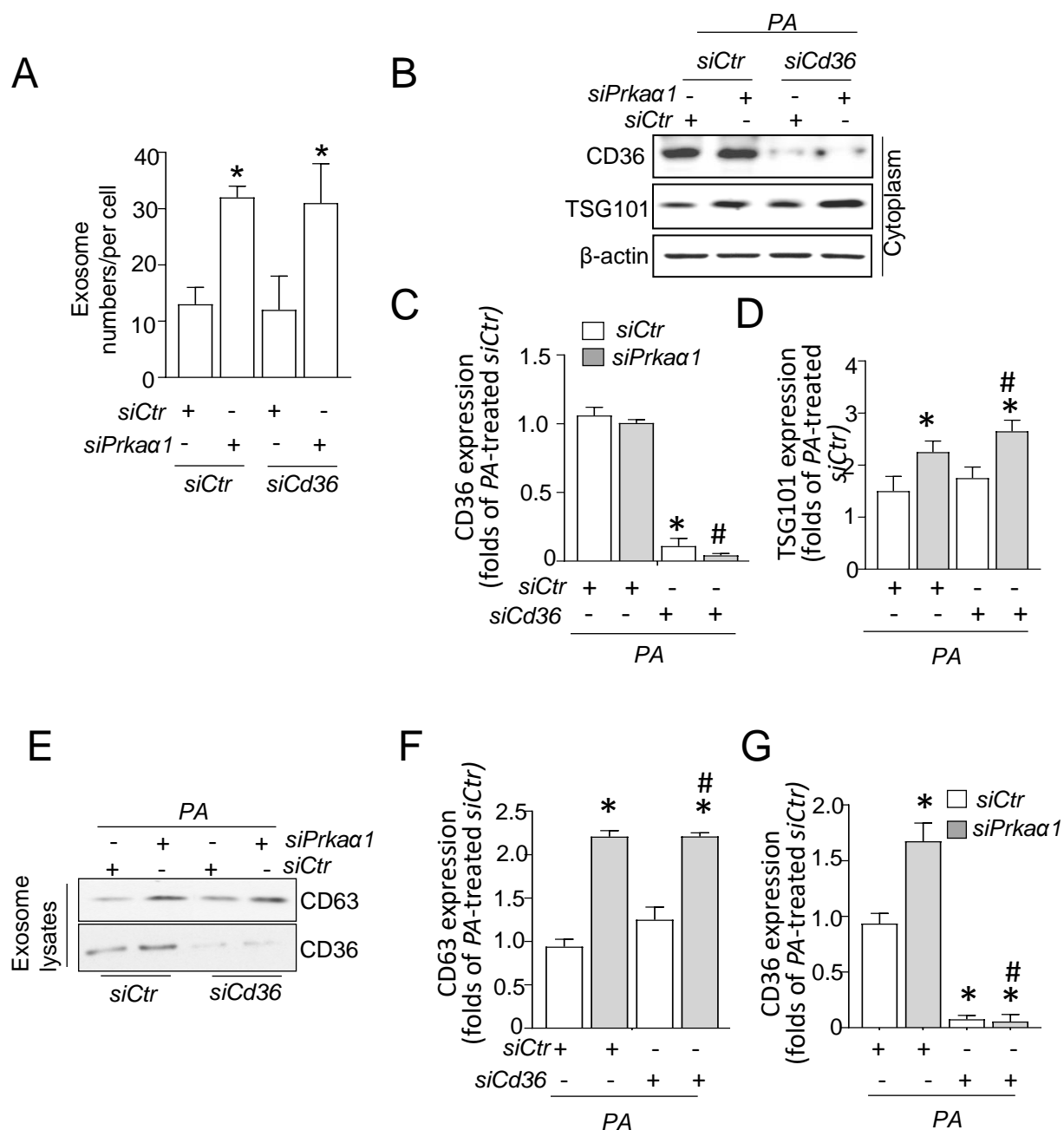


Supplemental Figure 4. Effects of compound C (CC) on TSG101 expression in adipocytes. A, Representative western blots of TSG101 expression in adipocytes treated with either vehicle or without CC was analyzed by western blot. **B,** Densitometric analysis of TSG101 western blots from A. * $p < 0.05$ SiTsg101 siRNA verse control siRNA; # $p < 0.05$ Control siRNA plus verse control siRNA plus CC; \$ $p < 0.05$ control siRNA plus CC verse SiTsg101 plus CC, $n = 3$.

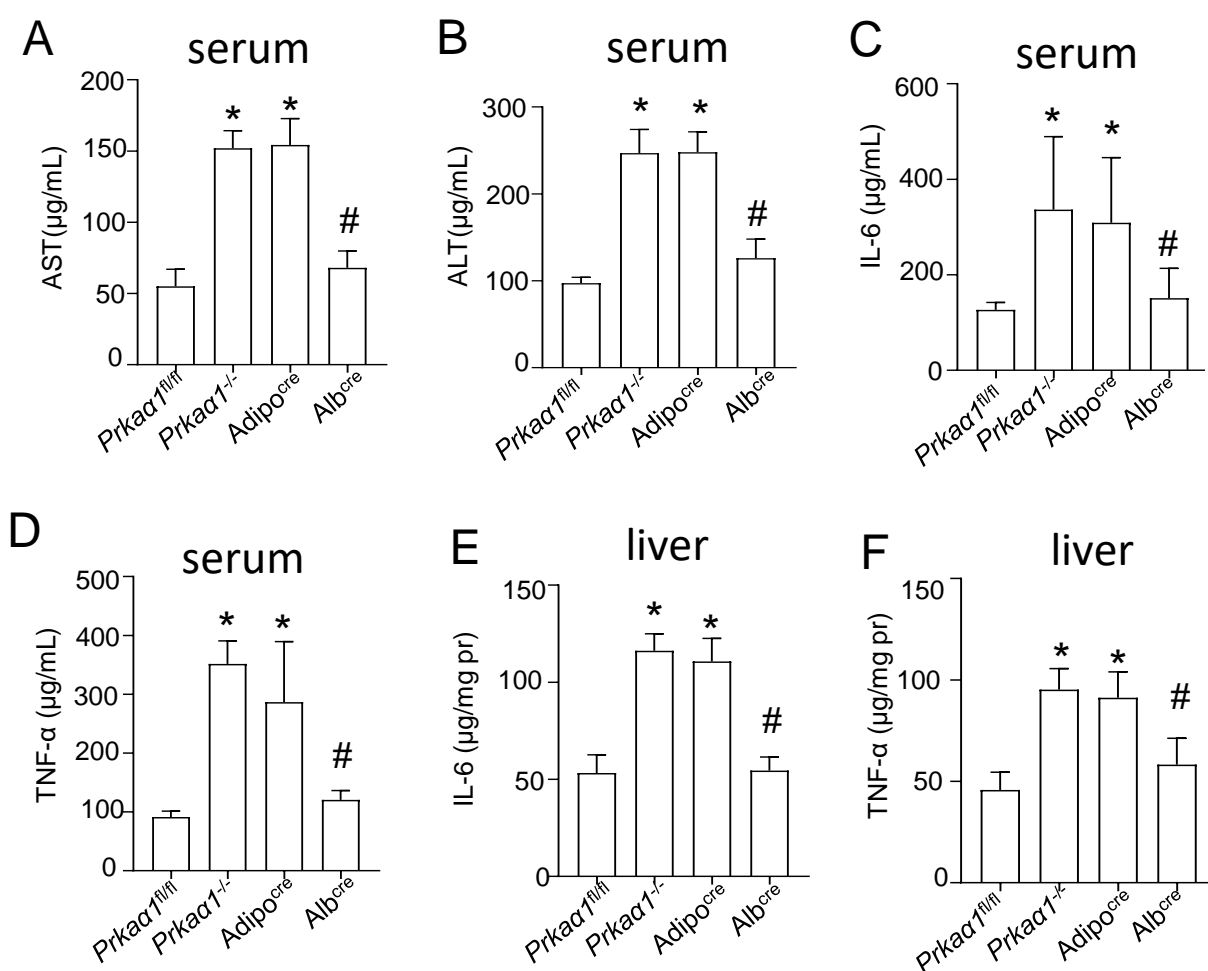


Supplemental Figure 5. Palmitic acid (PA, 300 μM) increases exosome release by inhibiting AMPKα1.

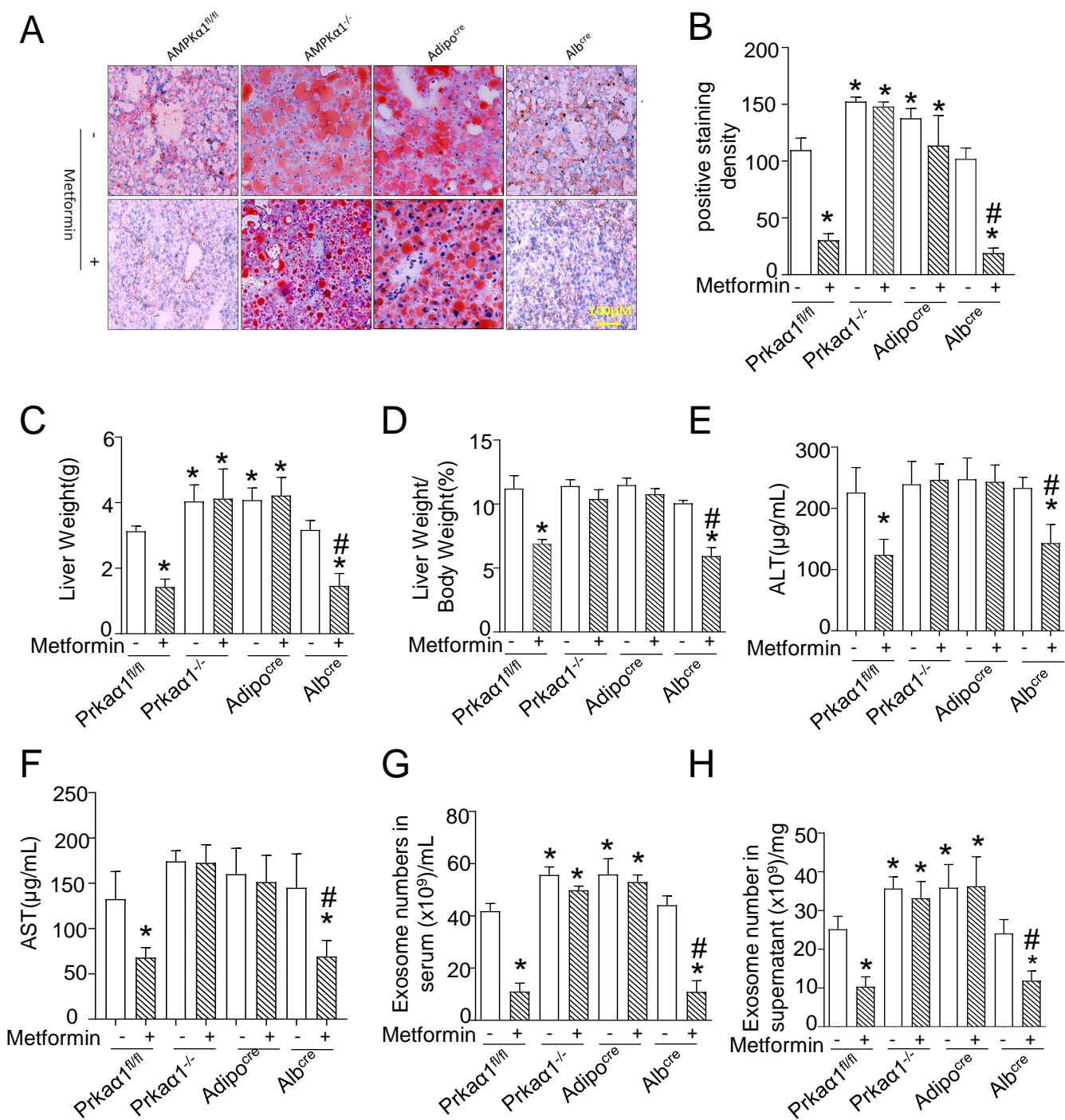
A, PA inhibits AMPK in adipocytes. Representative western blot analysis of pAMPKα, total AMPKα1(tAMPKα), AMPKα1, and AMPKα2 in 3T3L1-derived adipocytes with or without PA treatment. **B**, Quantitative analysis for pAMPKα in PA-treated adipocytes. * $p < 0.05$ PA versus vehicle control, $n = 3$; **C**, AICAR treatment suppresses PA-enhanced exosome release from adipocytes. * $p < 0.05$ PA versus vehicle control group; # $p < 0.05$ PA versus PA plus AICAR(1mM) for 24 hours, $n = 3$. **D**, Effects of overexpression of adenovirus-encoding *Prkaa* or GFP on exosome numbers in PA-treated adipocytes. * $p < 0.05$ PA versus vehicle control group; # $p < 0.05$ PA versus PA plus AICAR(1mM) for 24 hours, $n = 3$. **E**, Effects of AICAR in white adipose tissues (WAT) isolated from AICAR-treated WT, *Prkaa1*^{-/-} and *Prkaa2*^{-/-}. * $p < 0.05$ PA versus vehicle control group; # $p < 0.05$ PA versus PA plus AICAR(1mM), The \$ $p < 0.05$ WT versus PA plus AICAR, $n = 5$. **F**, Representative Western blot of CD63 and CD81 in exosomes derived from PA- and AICAR-treated WT and *Prkaa1*^{-/-} WAT. **G-H**, Quantitative data for CD63 and CD81. * $p < 0.05$ PA versus vehicle control group; # $p < 0.05$ PA versus PA plus AICAR(1mM), \$ $p < 0.05$ WT versus PA plus AICAR, $n = 3$.



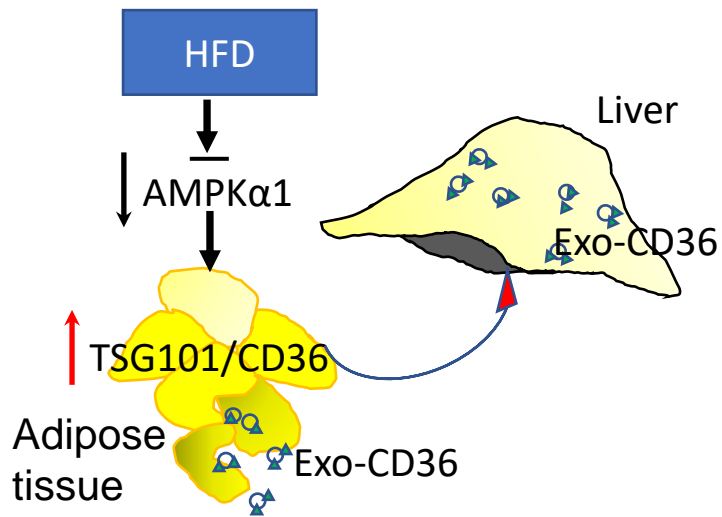
Supplemental Figure 6. Effects of AMPK α 1 and CD36 on PA-induced exosome secretion. PA-treated adipocytes were transfected with siCtrl, siPrkaa1, siCd36, and siPrkaa1+ siCd36. **A**, Exosome numbers in the media. * $p < 0.05$ vs. siCtrl. **B**, Western blot analysis of CD36 and TSG101 in cell lysates. **C** and **D**, Quantitative data for CD36(**C**) and TSG101(**D**). * $p < 0.05$ vs. siCtrl. # $p < 0.05$ vs. PA treated siPrkaa1 group. **E**, Western blot analysis of CD63 and CD36 in exosome lysates. **F** and **G**, Quantitative analysis of expression CD63(**F**) and CD36(**G**) in exosome from adipocytes. siCtrl: Control siRNA. * $p < 0.05$ vs. siCtrl. # $p < 0.05$ vs. PA treated siPrkaa1 group.



Supplemental Figure 7. AMPKα1 deficiency exacerbates liver impairment and inflammation in the serum and liver. *Prkaa1^{-/-}*, *Prkaa1^{fl/fl}*, *Adipo^{Cre}* and *Alb^{Cre}* mice were treated with HFD for 3 months. **A to D**, Quantification of serum AST, ALT, IL-6 and TNF-α in *Prkaa1^{-/-}*, *Prkaa1^{fl/fl}*, *Adipo^{Cre}* and *Alb^{Cre}* mice fed a HFD for 3 months (n=4 for each group). **E, F**, hepatic IL-6 and TNF-α levels. **p* < 0.05 vs. *Prkaa1^{-/-}* mice. #*p* < 0.05 vs. *Adipo^{Cre}* mice



Supplemental Figure 8. Activation of AMPK α 1 by metformin reduces exosome release and mitigates fatty liver only in *Prkaa1^{fl/fl}* and *Prkaa1^{fl/fl}:Alb^{Cre}* mice. *Prkaa1^{-/-}*, *Prkaa1^{fl/fl}*, *Adipo^{Cre}* and *Alb^{Cre}* mice were treated with HFD and metformin (5mg/kg/day) for 3 months. **A**, Representative images of Oil-red O staining. **B**, Quantitative data for Oil-red O staining. **C**, Liver weight. **D**, Ratio of Liver weight to Body weight. **E**, ALT. **F**, AST. **G**, Exosome number in serum. **H**, Exosome number in supernatant. (n=6). ***Adipo^{Cre}***: *Prkaa1^{fl/fl}:Adipo^{Cre}* mice; ***Alb^{Cre}***: *Prkaa1^{fl/fl}:Alb^{Cre}* mice. **p* < 0.05 vs. *Prkaa1^{fl/fl}* mice. #*p* < 0.05 vs. same group mice treated with vehicle.



Supplemental Figure 9: Schematic illustration of HFD-inhibited AMPKα1 inducing fatty liver by instigating exosome release from adipose tissue in mice.