Mfge8 mediates absorption of dietary fats and uptake of fatty acids by the liver, heart, and white adipose tissue

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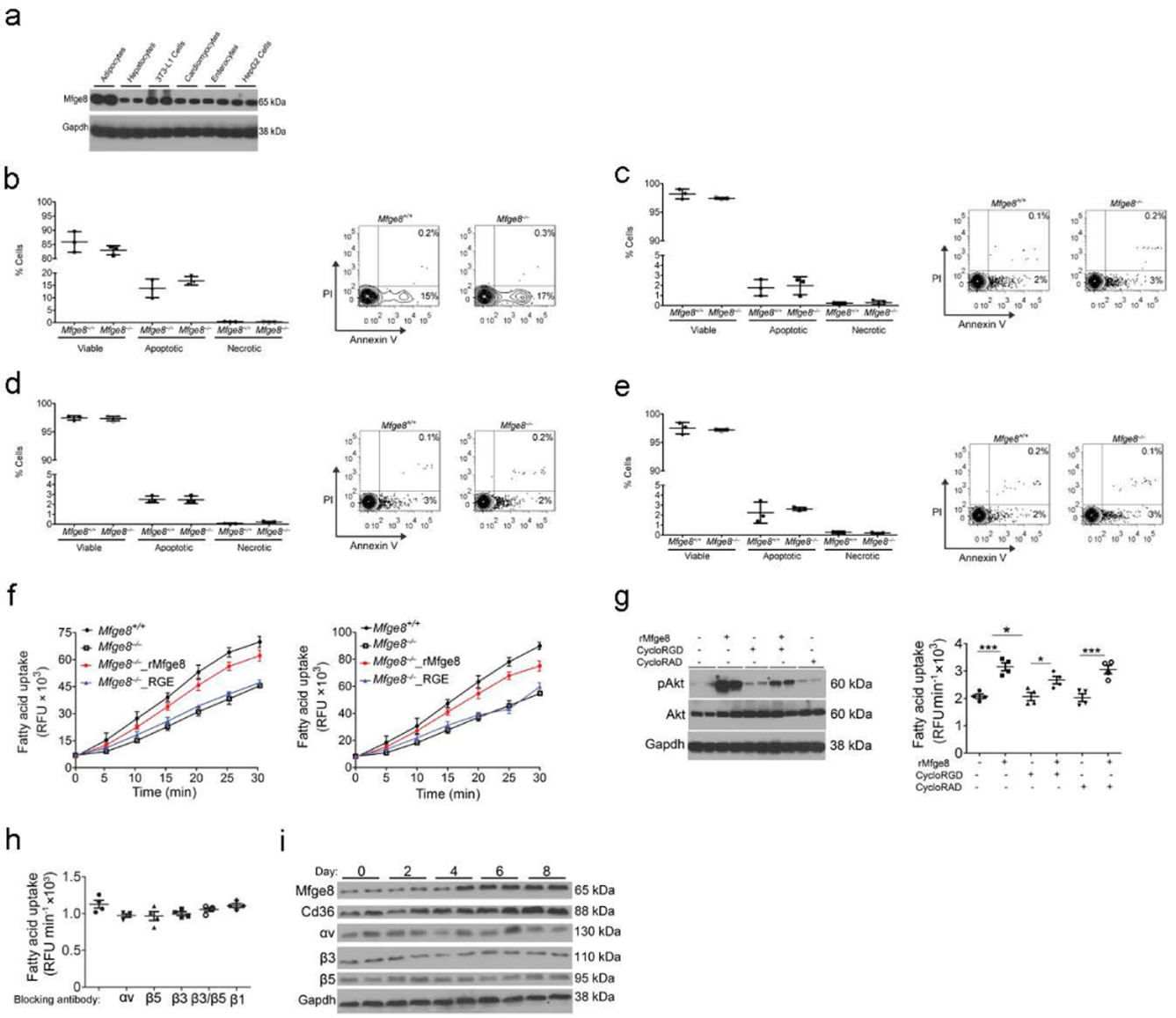
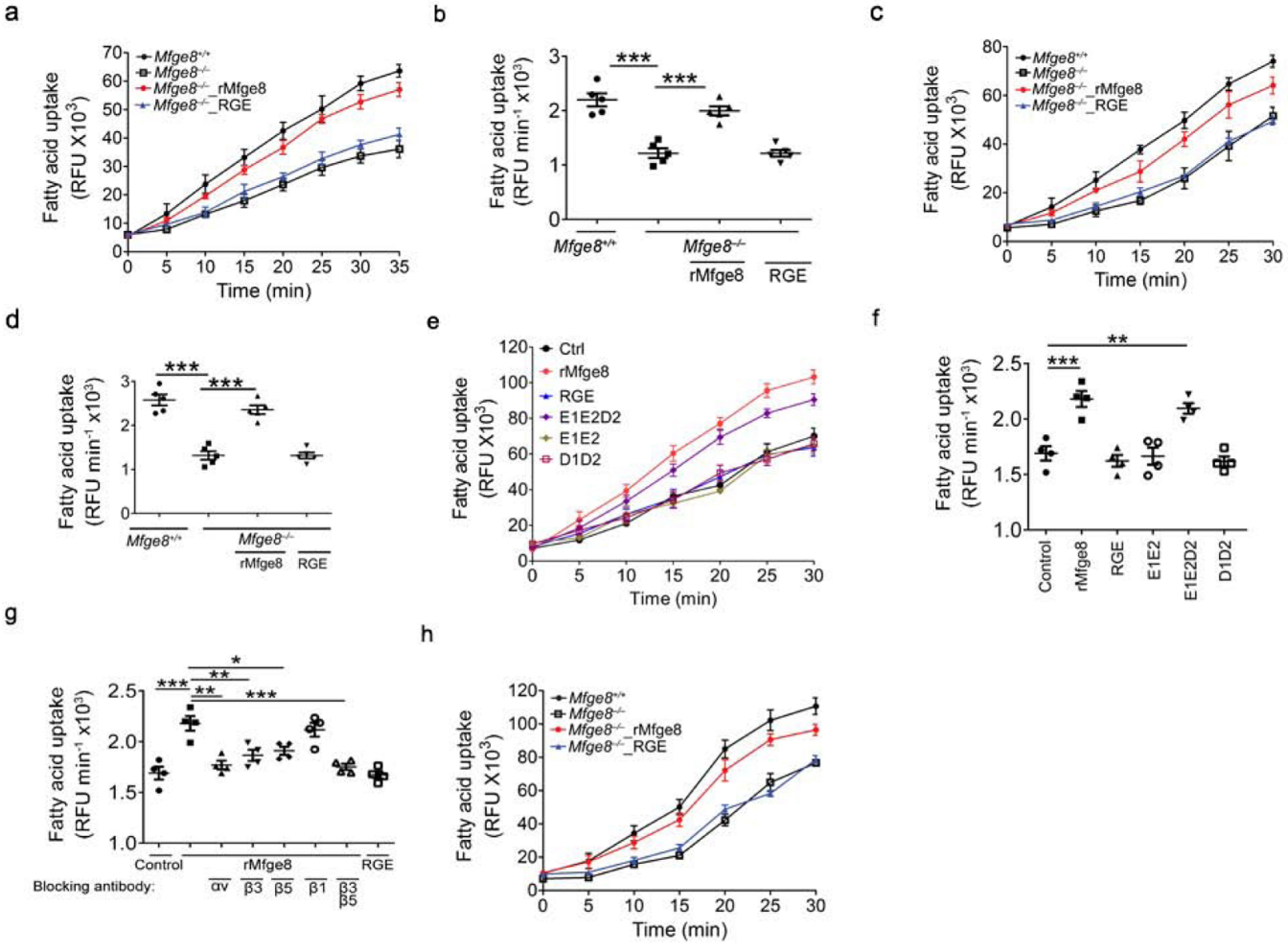
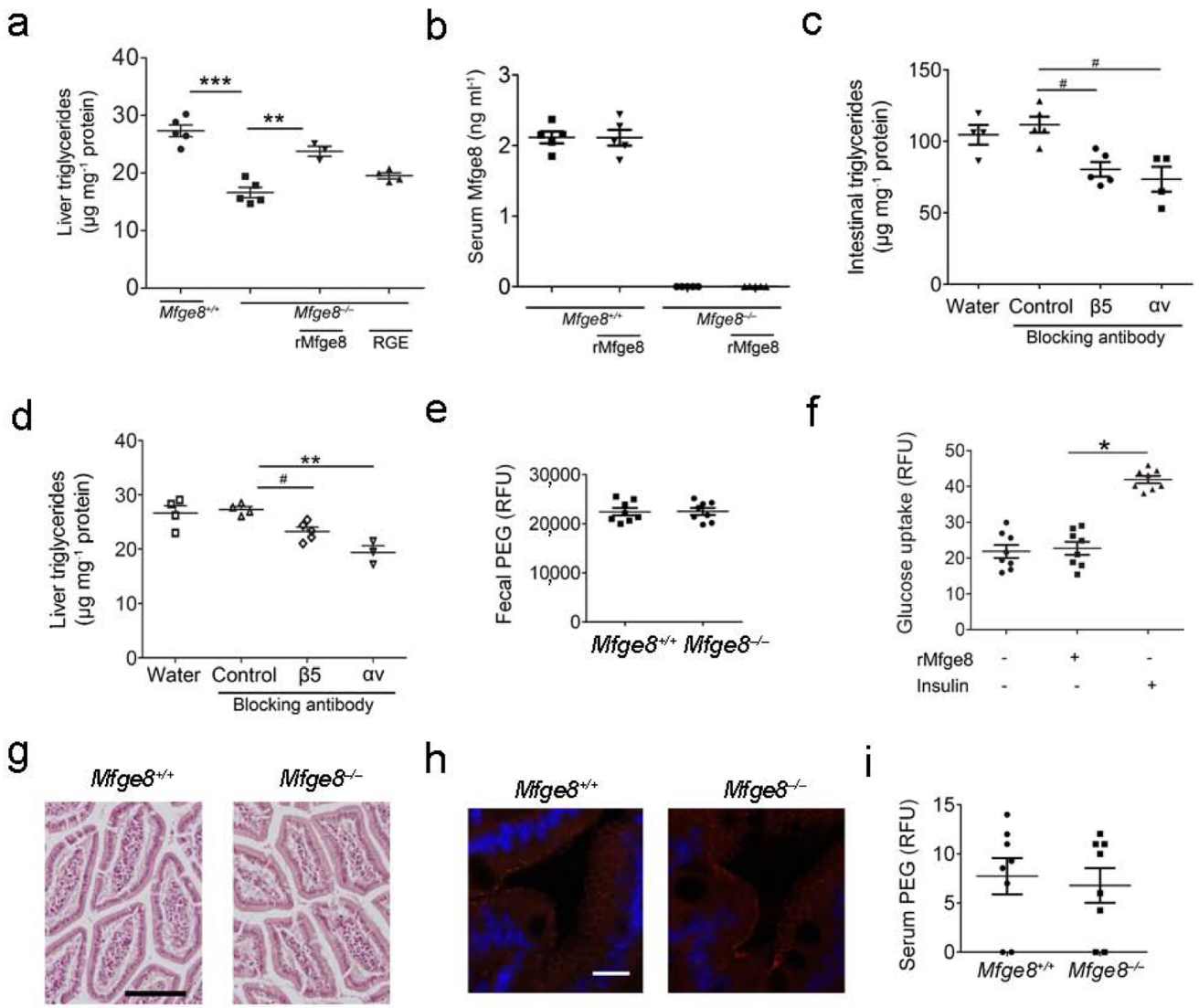


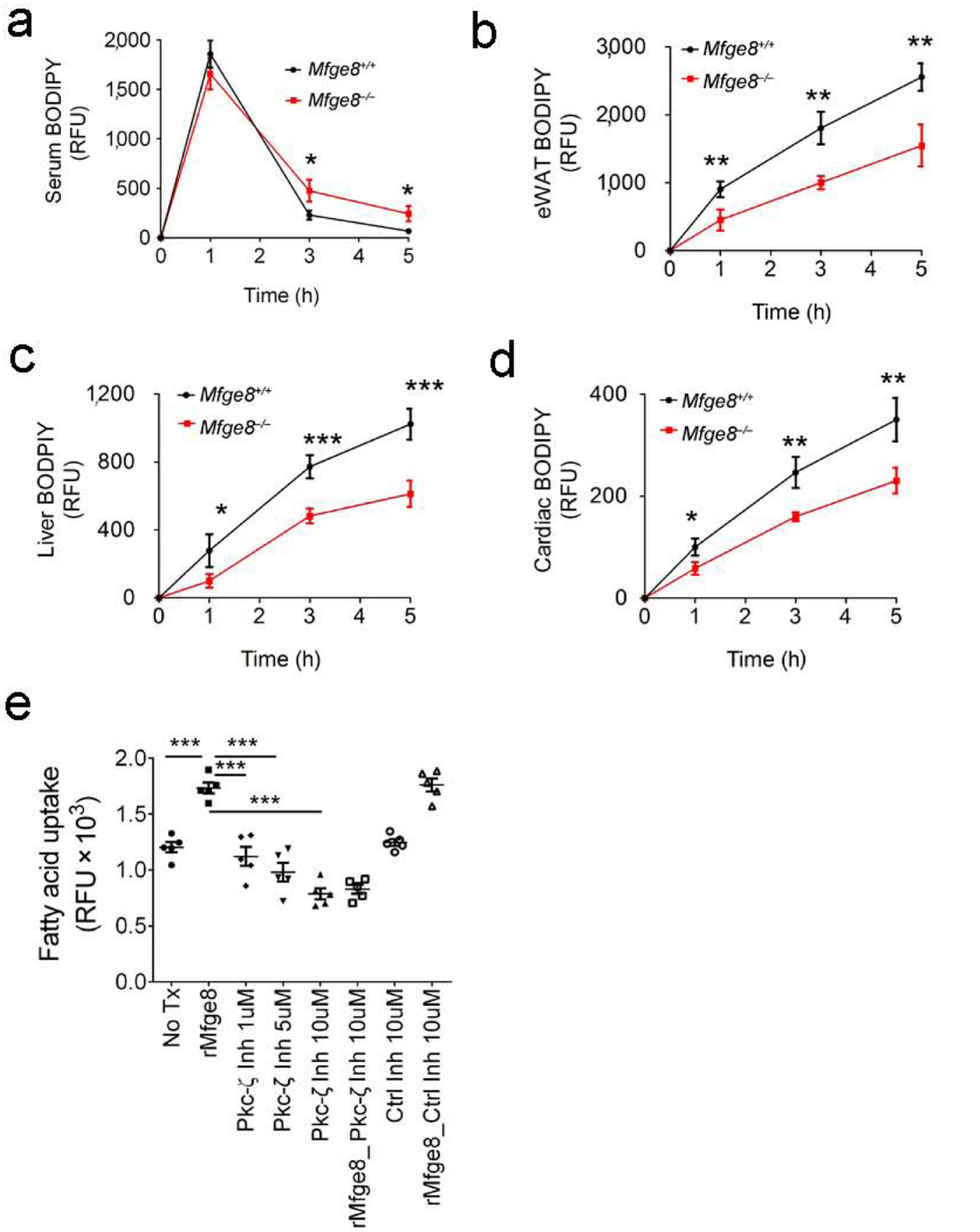
Supplementary Figure 1. Mfge8 mediates fatty acid uptake. Primary cell and cell line Mfge8 expression and cell viability of primary cells. (**a**) Mfge8 expression by Western blot in different cell lines and primary cells. (**b-e**) Cell viability assessed by staining for Annexin V and Propridium Iodide (PI) shortly after isolation of (**b**) primary adipocytes, (**c**) hepatocytes, (**d**) cardiac myocytes, and (**e**) enterocytes. *n* = 3. (**f**)Time course of fatty acid uptake in primary *Mfge8*$^{-/-}$ and *Mfge8*$^{+/+}$ adipocytes (**left**, *n* = 7-9) and *Mfge8*$^{-/-}$ and *Mfge8*$^{+/+}$ differentiated adipocyte progenitor cells (**right**, *n* = 3) with and without treatment with rMfge8. (**f left**) Effect of cycloRGD (10 μg/ml) on Akt phosphorylation in 3T3-L1 adipocytes. (**f right**) Fatty acid uptake in 3T3-L1 adipocytes in the presence of cycloRGD. *n* = 4. (**h**) Fatty acid uptake was measured in *Mfge8*$^{-/-}$ adipocytes in the presence of integrin blocking antibodies (20 μg/mL). *n* = 3-4. (**i**) Expression of Mfge8, αvβ3, and αvβ5 integrins in 3T3-L1 adipocytes for 8 days after the addition of differentiation media. Male mice were used for all experiments. Data are expressed as mean ± s.e.m. **P* < 0.05, ****P* < 0.001.



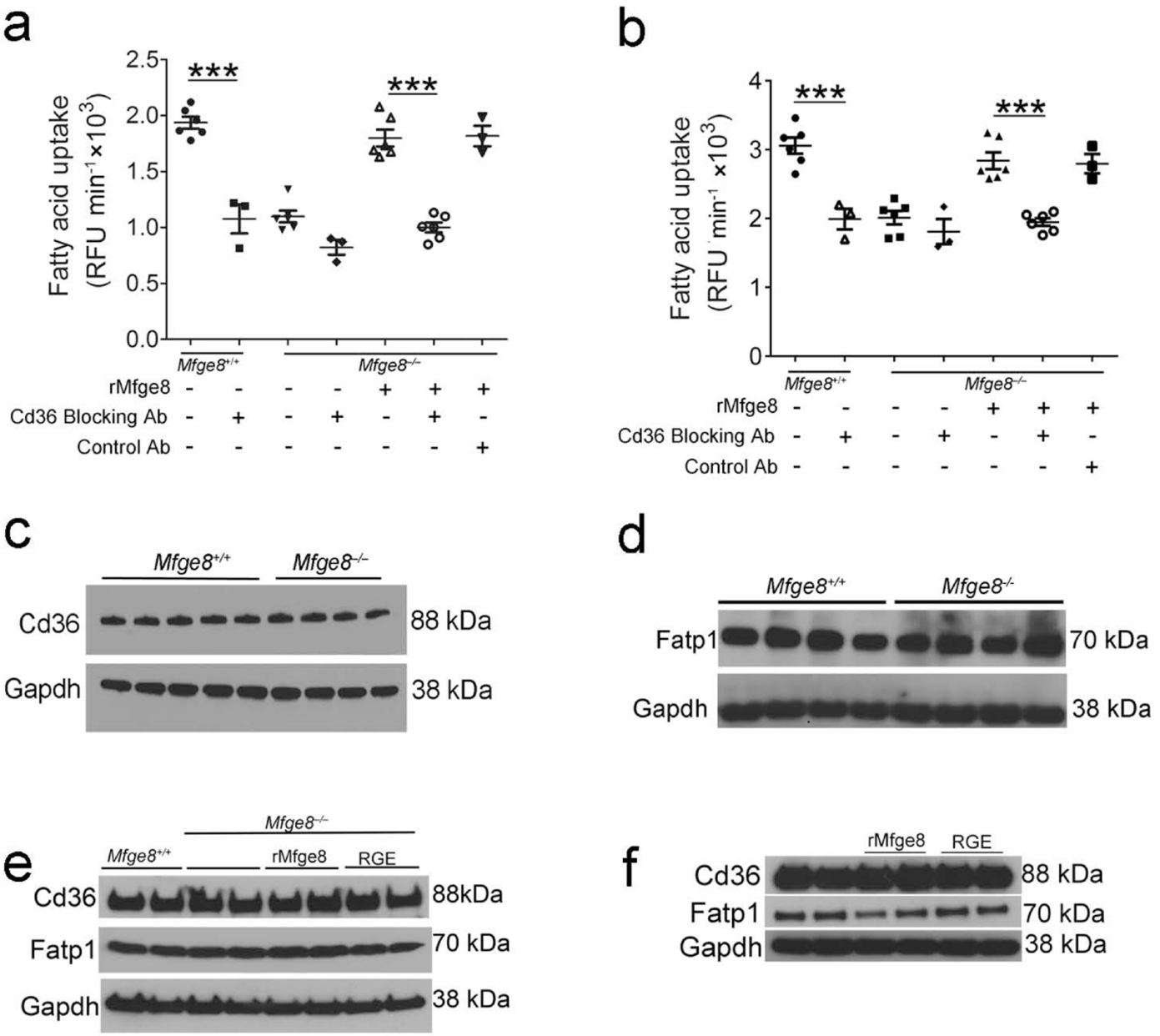
Supplementary Figure 2. Mfge8 mediates fatty acid uptake in hepatocytes and cardiac myocytes. (**a,b**) Time course (**a**) and rate (**b**) of fatty acid uptake in primary *Mfge8*$^{+/+}$ and *Mfge8*$^{-/-}$ hepatocytes, and *Mfge8*$^{-/-}$ hepatocytes treated with rMfge8 or RGE. $n = 5$. (**c,d**) Time course (**c**) and rate (**d**) of fatty acid uptake in primary *Mfge8*$^{+/+}$ and *Mfge8*$^{-/-}$ cardiac myocytes and *Mfge8*$^{-/-}$ cardiac myocytes treated with rMfge8 or RGE. $n = 5$. (**e,f**) Time course (**e**) and rate (**f**) of fatty acid uptake in HepG2 cells treated with mutated Mfge8 constructs. $n = 4$. (G) Fatty acid uptake in HepG2 cells treated with integrin blocking antibodies (20 μg/mL) . $n = 4$. (H) Time course of fatty acid uptake in primary *Mfge8*$^{+/+}$ and *Mfge8*$^{-/-}$ enterocytes, and *Mfge8*$^{-/-}$ enterocytes treated with rMfge8 or RGE. $n = 8$. Male mice were used for these experiments. $^{*}P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$. Data are expressed as mean ± s.e.m. Each replicate represents an independent



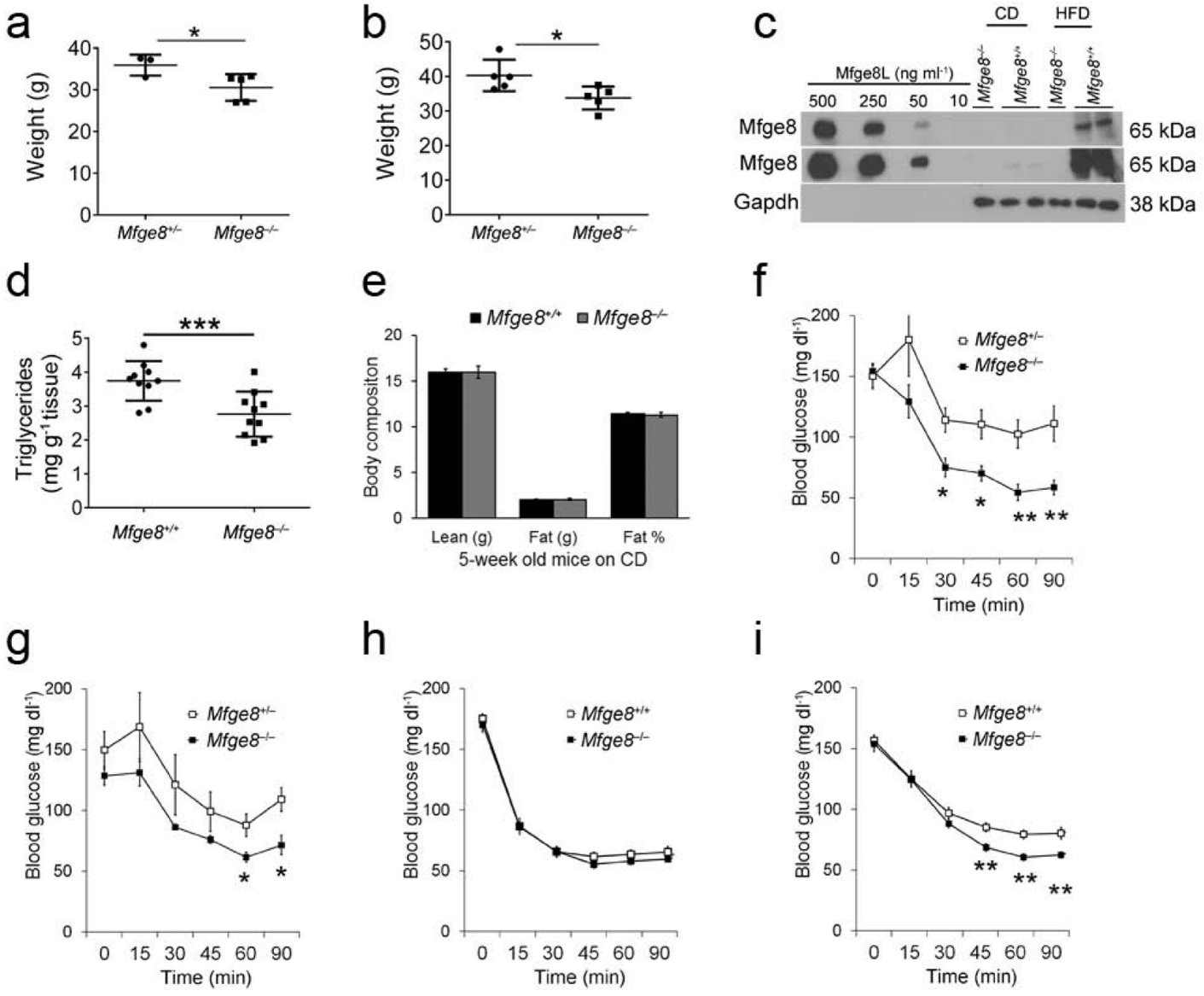
Supplementary Figure 3. Mfge8 mediates fat absorption. (**a**) Liver TG levels 8 hours after olive oil gavage mixed with and without rMfge8 or RGE construct . *n* = 3-5. (**b**) Serum Mfge8 levels after olive oil gavage with and without rMfge8. N = 5. (**c,d**) Effect of oral administration of integrin blocking antibodies prior to olive oil gavage on small intestine (**c**) and liver TG content (**d**) in *Mfge8*$^{+/+}$ mice. *n* = 3-5. (**e**) Fecal rhodamine-PEG levels after gavage with a mixture of BODIPY and rhodamine-PEG. *n* = 8. (**f**) Effect of rMfge8 and insulin on glucose uptake in 3T3-L1 adipocytes. *n* = 8. Each replicate represents an independent experiment (**g**) Representative H&E staining of small intestinal histology in *Mfge8*$^{-/-}$ and control mice. Scale bar, 100 µm. *n* = 3. (**h**) Representative section of Tjp1 staining in the small intestines of *Mfge8*$^{-/-}$ and control mice. Scale bar, 30 µm. *n* = 3. (**i**) Serum rhodamine-PEG levels after oral gavage. *n* = 8. Each in vivo experiment was performed one independent time for panels **a-d,f** and 3 independent times for panels **e,i**. Female mice were used for these experiments. #*P* < 0.05, **P* < 0.01, ***P* < 0.001, ****P* < 0.0001. Data are expressed as mean ± s.e.m.



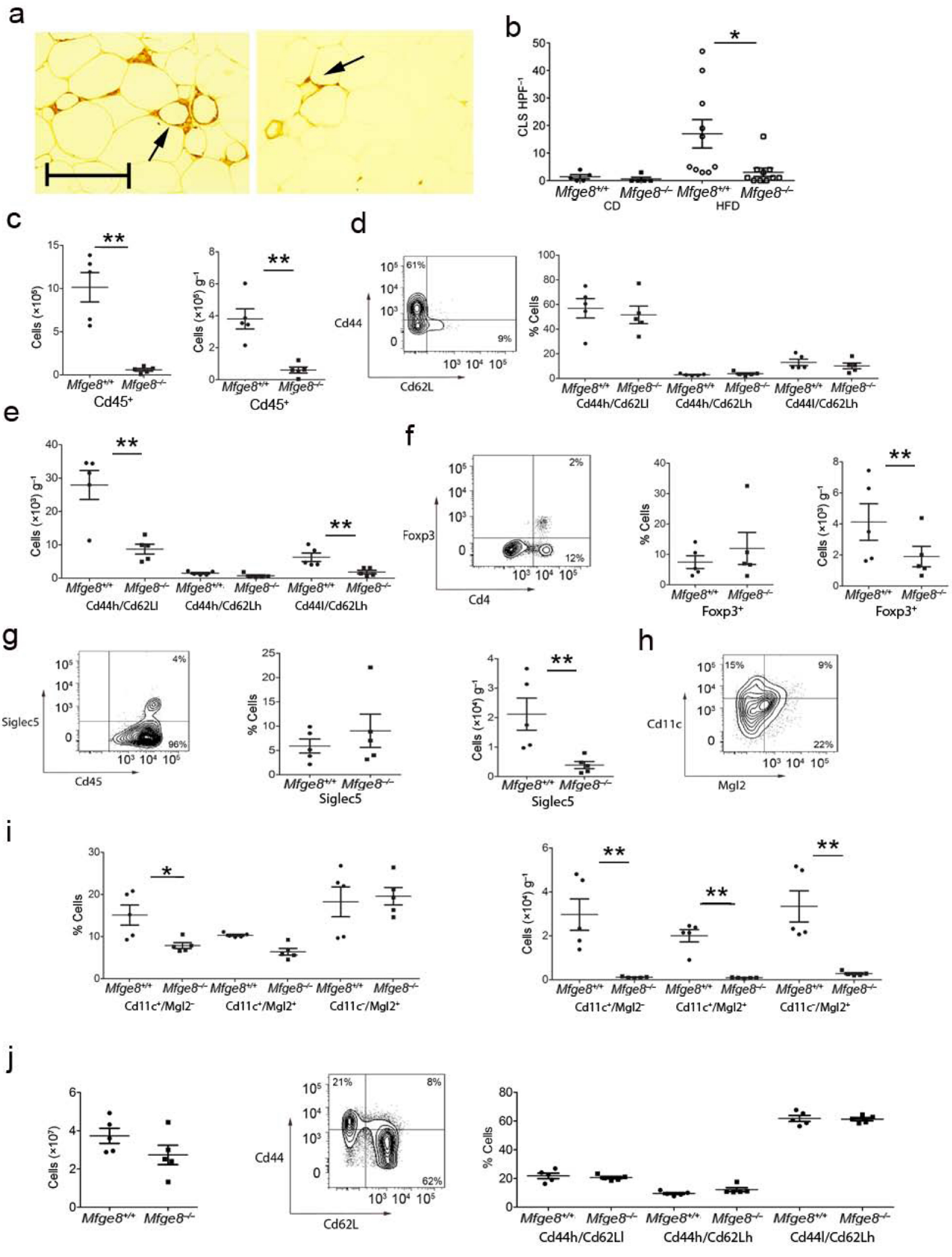
Supplementary Figure 4. Mfge8 mediates serum clearance of fatty acids. (**a-d**) BODIPY levels measured in (**a**) serum, (**b**) eWAT, (**c**) liver, and (**d**) cardiac tissue at indicated time points after IP administration. n = 5, results represent one experiment. (**e**) 3T3-L1 adipocytes were incubated with Pkc-ζ or control inhibitor with and without rMfge8. n = 4 independent experiments, *P < 0.01, **P < 0.001, ***P < 0.0001. Male mice were used for all experiments. Data are expressed as mean ± s.e.m.



Supplementary Figure 5. Mfge8 does not induce expression of Cd36 or Fatp1. (**a,b**) Effect of Cd36 blocking antibody or control antibody (ab) on the ability of rMfge8 to increase fatty acid uptake in primary *Mfge8*$^{-/-}$ and *Mfge8*$^{+/+}$ hepatocytes (**a**) and enterocytes (**b**). *n* = 3-4 for experiments with antibodies and 6 for experiments with and without rMfge8. (**c,d**) Expression by Western blot in eWAT of mice on a HFD of Cd36 (**c**) and Fatp1 (**d**). (**e**) Cd36 and Fatp1 expression by Western blot in primary *Mfge8*$^{+/+}$ adipocytes and primary *Mfge8*$^{-/-}$ adipocytes treated with and without treatment with rMfge8 or RGE construct. (**f**) Cd36 and Fatp1 expression by Western blot in 3T3-L1 adipocytes with and without treatment with rMfge8 or RGE construct. Male mice were used for these experiments. ***$P < 0.0001$, Data are expressed as mean ± s.e.m.



Supplementary Figure 6. Mfge8$^{-/-}$ mice are protected from diet-induced obesity. (**a**) Body weight of female Mfge8$^{-/-}$ and Mfge8$^{+/-}$ mice after 12 weeks on a HFD. n = 3-5. (**b**) Body weight of male Mfge8$^{-/-}$ and Mfge8$^{+/-}$ mice after 12 weeks on a HFD. n = 5. (**c**) Western blot showing 100-fold increase in Mfge8 expression in eWAT of male mice after 12 weeks on a CD or HFD with a standard of recombinant Mfge8. (**d**) Heart triglyceride content in mice on a CD is reduced in male Mfge8$^{-/-}$ mice. n = 10. (**e**) Body composition of 5-week-old Mfge8$^{+/+}$ and Mfge8$^{-/-}$ male mice. n = 14-15. (**f**) Insulin tolerance test in 20-week old female Mfge8$^{-/-}$ and Mfge8$^{+/-}$ controls after 12 weeks on a HFD. n = 3-5. (**g**) Insulin tolerance tests in 20-week old male Mfge8$^{-/-}$ and Mfge8$^{+/-}$ control mice after 12 weeks on a HFD. n = 5. (**h,i**) Insulin tolerance tests in 5- (**h**, n = 20) and 10-week old (**i**, n = 22) Mfge8$^{-/-}$ and Mfge8$^{+/+}$ control mice on a CD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data are expressed as mean ± s.e.m.



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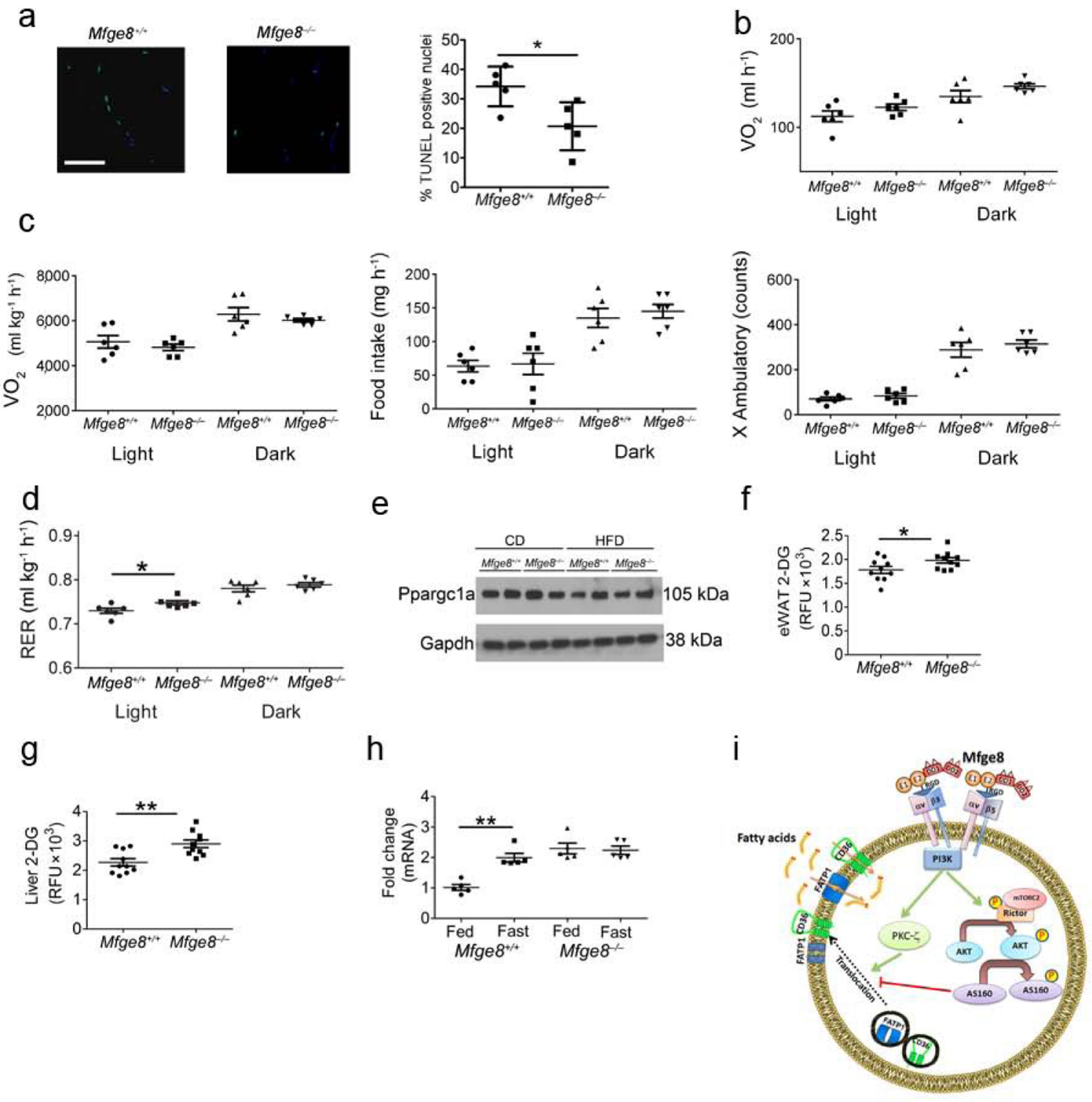
Supplementary Figure 7. *Mfge8*$^{-/-}$ mice are protected from adipose tissue inflammation and lymphocyte activation. (**a-b**) The number of crown like structures (arrows, CLS) per high-power field (HPF, 100x) in male 20-week-old *Mfge8*$^{+/+}$ (**a left**) and *Mfge8*$^{-/-}$ (**a right**) mice on a HFD was quantified (**b**) after staining tissue sections with an antibody against Lgals3. (Scale bar, 100 μm, n = 5 for CD and 10 for HFD). (**c-i**) Flow cytometry from cells of the eWAT of male 20-week-old *Mfge8*$^{-/-}$ and *Mfge8*$^{+/+}$ mice on a HFD. *Mfge8*$^{-/-}$ mice had fewer Cd45^{+} cells (**c left**, n = 5 for all subsequent experiments), and Cd45^{+} cells per gram eWAT (**c right**, all subsequent data normalized for gram weight). (**d-e**) The proportion of Cd4^{+} T cells expressing Cd44 high (Cd44h) and Cd62L low (Cd62Ll) was evaluated to assess T cell activation. (**d**) *Mfge8*$^{-/-}$ eWAT had similar proportions (**d right**) and fewer numbers of activated T cells (**e**). (**f**) *Mfge8*$^{-/-}$ eWAT had similar proportion (**f middle**) and fewer total (**f right**) regulatory T cells (Foxp3^{+}). (**g**) *Mfge8*$^{-/-}$ eWAT had similar proportion (**g middle**) and fewer total (**g right**) eosinophils (Siglec5^{+}). (**h-i**) M1 and M2 macrophage populations were evaluated by first gating on F4/80+ cells and then quantifying cells positive for Cd11c (officially known as Itgax) and Mgl2 (**h**). *Mfge8*$^{-/-}$ eWAT had decreased proportions of M1 macrophages (**i left**) and decreased total numbers of both M1 (Cd11c^{+}/Mgl2^{-}) and M2 macrophages (Cd11c^{-}/Mgl2^{+}) (**i right**). (**j**) Single cell populations of splenocytes obtained from male 20-week-old *Mfge8*$^{+/+}$ and *Mfge8*$^{-/-}$ mice on a HFD. The proportion of Cd4^{+} splenocytes expressing Cd44 high (Cd44h) and Cd62L low (Cd62Ll) was evaluated to assess T cell activation. *P < 0.02, **P < 0.001.Data are expressed as mean ± s.e.m.



Supplementary Figure 8. Mfge8 regulation of fatty acid uptake is independent of impaired apoptotic cell clearance or increased energy expenditure. (**a**)TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) staining in the eWAT of Mfge8$^{+/+}$ and Mfge8$^{-/-}$ on a HFD for 12 weeks and quantification of the percentage of nuclei that are TUNEL positive. $n = 5$. Scale bar, 50 μm (**b-d**) Energy expenditure in 12-week-old Mfge8$^{-/-}$ and Mfge8$^{+/+}$ mice 10 days after being placed on a HFD. (**b**) Unadjusted oxygen consumption (VO_2). (**c left**) Oxygen consumption adjusted for lean body mass. (**c middle**) Food intake. (**c right**) Activity measured by movement in the X axis. (**d**) Respiratory exchange ratio (RER). $n = 6$ for panels b-d. (**e**) Pparg1a protein expression in eWAT of mice on a HFD or CD. eWAT (**f**) and liver (**g**) 2-Deoxy-D-Glucose (2-DG) levels 45 minutes after IP injection of 2-DG. $n = 10$, results represent 2 independent experiments. (**h**) Pdk4 mRNA expression in skeletal muscle of mice on a HFD or CD. $n = 5$. $^{*}P < 0.05$, $^{**}P < 0.01$. (**i**) Model by which Mfge8 increases fatty acid uptake by inducing translocation of Cd36 and Fatp1 to the cell surface. Male mice were used for these experiments. Data are expressed as mean ± s.e.m.