

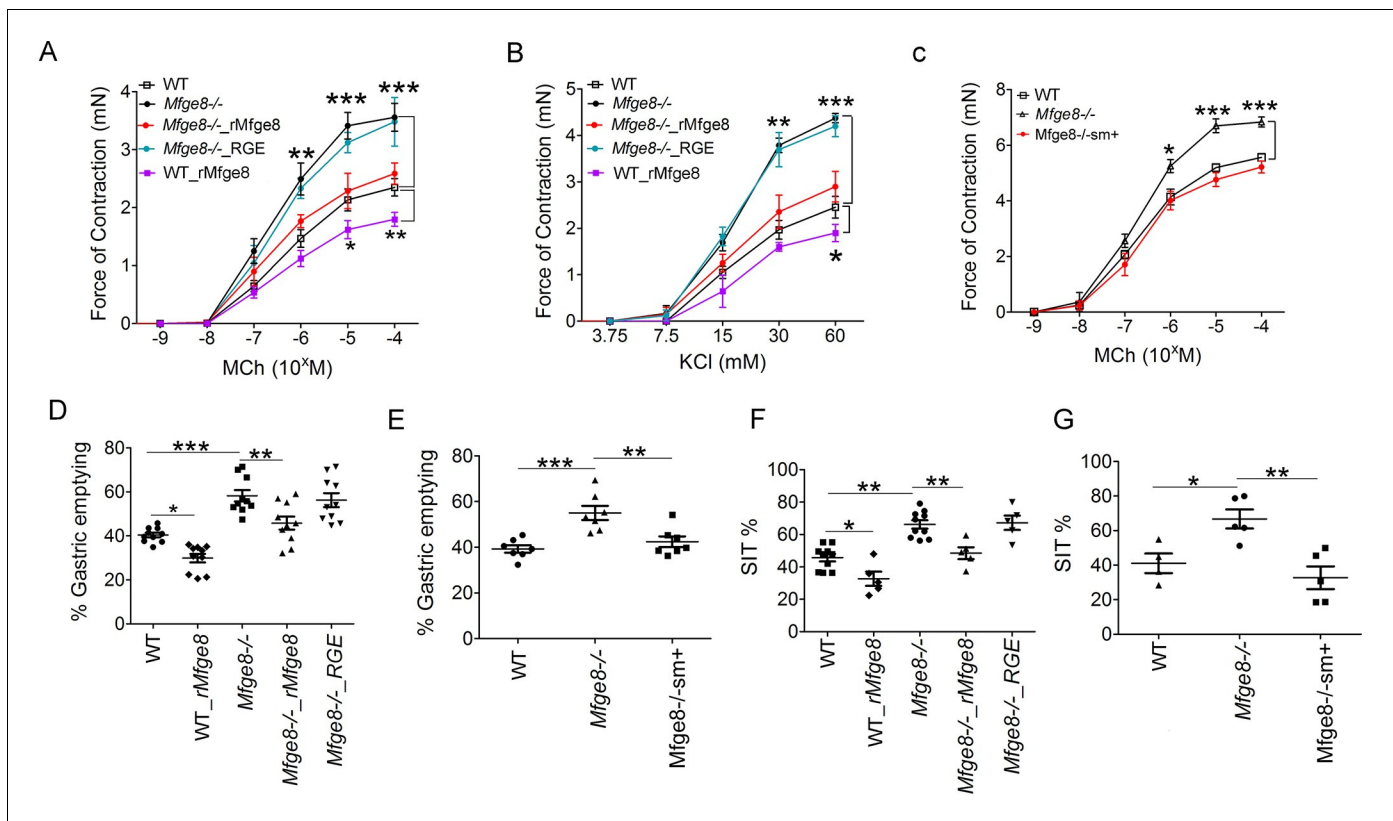


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## Figures and figure supplements

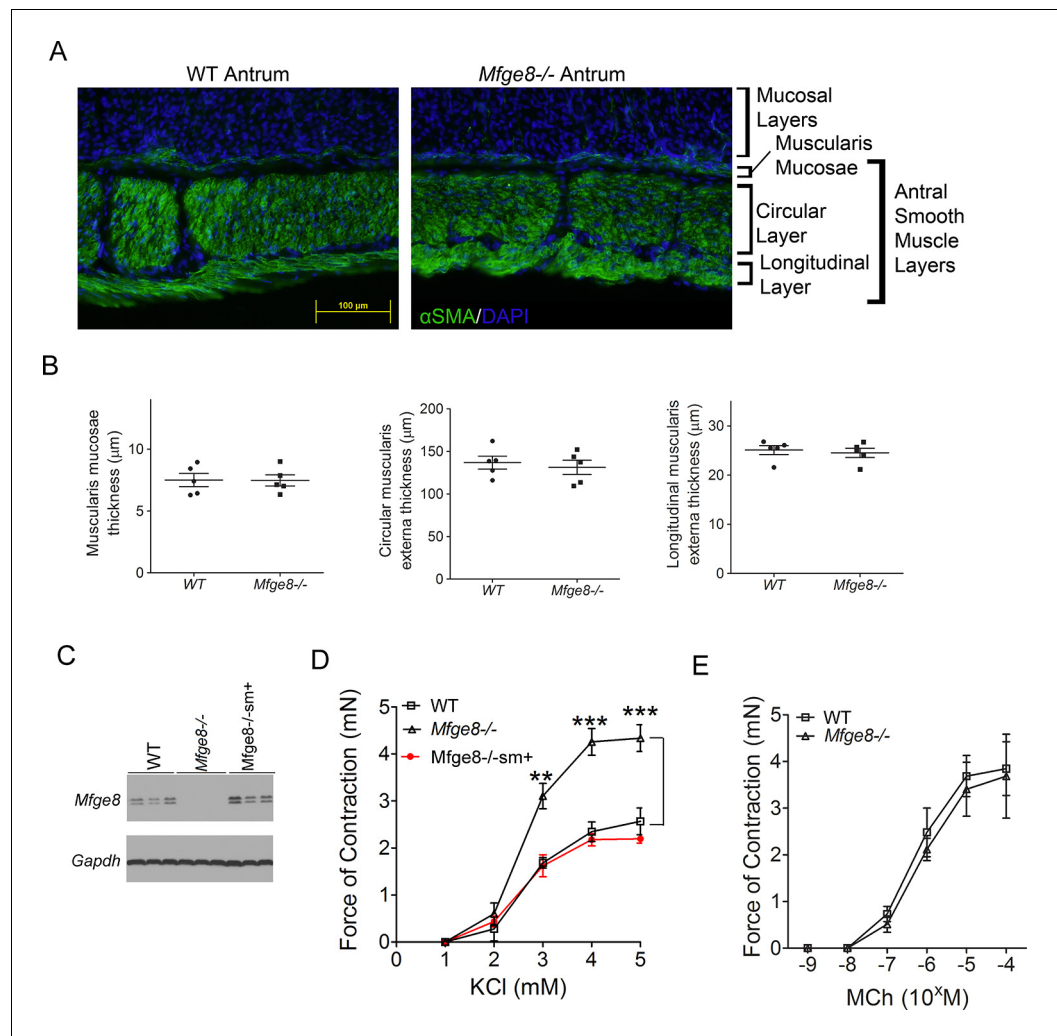
$\alpha 8 \beta 1$  integrin regulates nutrient absorption through an Mfge8-PTEN dependent mechanism

**Amin Khalifeh-Soltani et al**



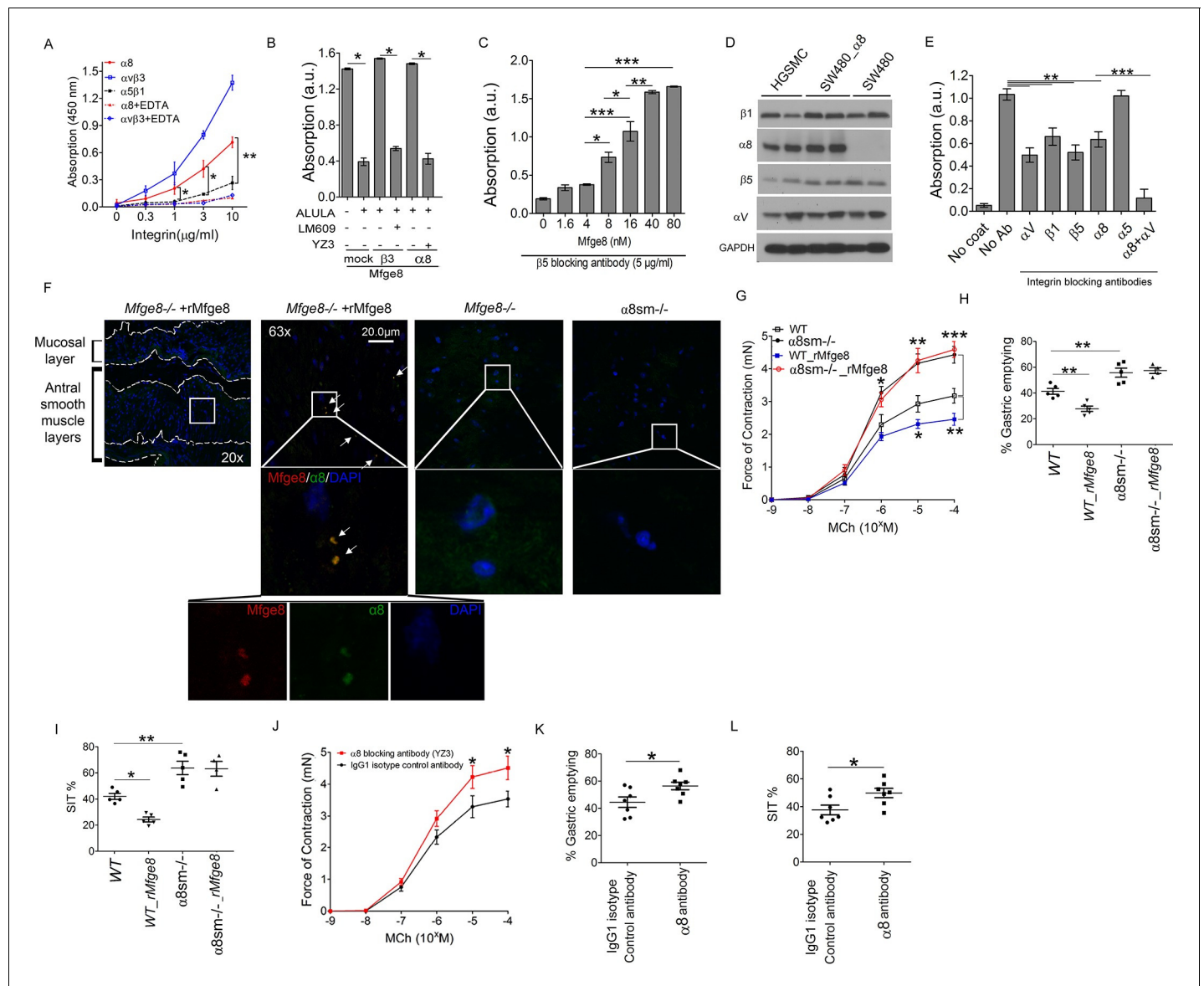
**Figure 1.** Mfge8 regulates gastrointestinal motility. (A–C) Force of antral smooth muscle ring contraction with and without the addition of rMfge8 or RGE construct in *Mfge8*<sup>-/-</sup> and WT in response to MCh (A, N = 4–5) or KCl (B, N = 4–5) or after in vivo induction of smooth muscle Mfge8 expression in *Mfge8*<sup>-/-</sup>\_sm+ mice in response to MCh (C, N = 5). (D, E) The rate of gastric emptying in *Mfge8*<sup>-/-</sup> and WT with and without the addition of rMfge8 or RGE construct (D, N = 10) or after smooth muscle transgenic (*Mfge8*<sup>-/-</sup>\_sm+) expression of Mfge8 (E, N = 7). (F–G) Small intestinal transit time in *Mfge8*<sup>-/-</sup> and WT with and without the addition of rMfge8 or RGE construct (F, N = 5–10) or after smooth muscle transgenic expression of Mfge8 (G, N = 4–5). Female mice were used for all experiments. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Data are expressed as mean ± s.e.m.

DOI: 10.7554/eLife.13063.003



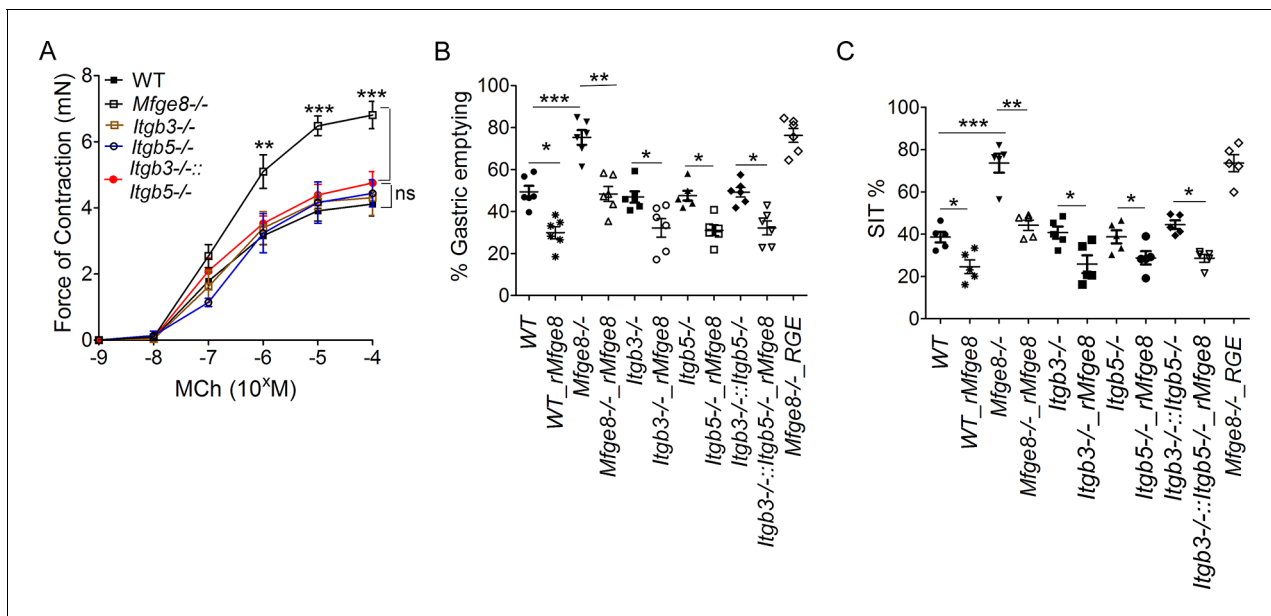
**Figure 1—figure supplement 1.** *Mfge8*<sup>-/-</sup> smooth muscle morphology and smooth muscle expression of Mfge8 in *Mfge8*<sup>-/-</sup>sm<sup>+</sup> mice. (A, B) Morphometric analysis of antrum from WT and *Mfge8*<sup>-/-</sup> mice. (A) Representative image of antral smooth muscle in WT and *Mfge8*<sup>-/-</sup> mice. (B) Thickness quantitation of muscularis mucosae, circular layer, and longitudinal layer of antral smooth muscle. (C) Western blot of antrum lysates probing for Mfge8 after transgenic induction of smooth muscle Mfge8 in *Mfge8*<sup>-/-</sup>sm<sup>+</sup> after doxycycline administration. (D) Force of antral smooth muscle ring contraction after in vivo induction of smooth muscle Mfge8 expression in *Mfge8*<sup>-/-</sup>sm<sup>+</sup> mice in response to KCl (N = 4–5). (E) Force of duodenal smooth muscle ring contraction in response to MCh (N = 4). Both male and female mice were used for these experiments. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Data are expressed as mean ± s.e.m.

DOI: [10.7554/eLife.13063.004](https://doi.org/10.7554/eLife.13063.004)



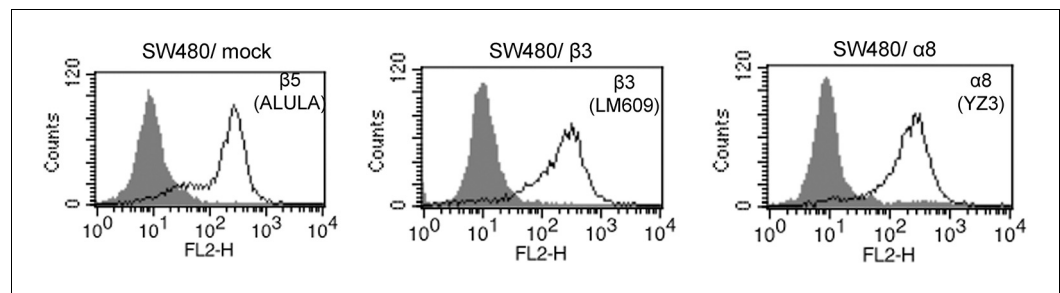
**Figure 2.** Mfge8 binds to  $\alpha 8$  integrin to regulate gastrointestinal motility. (A) Purified  $\alpha 8$ ,  $\alpha v\beta 3$ , or  $\alpha 5\beta 1$  were used for solid-phase binding assays with purified Mfge8 at indicated concentrations in the presence or absence of 10 mM EDTA. (B) Adhesion of SW480 (mock),  $\alpha 8$  transfected SW480 cells ( $\alpha 8$ ) or  $\beta 3$  transfected SW480 cells ( $\beta 3$ ) adhesion to wells coated with rMfge8 (5  $\mu$ g/ml) in the presence or absence of integrin blocking antibodies (5  $\mu$ g/ml) against  $\beta 5$  (ALULA),  $\beta 3$  (LM609) or  $\alpha 8$  (YZ83). (C) Dose-dependent binding of SW480 cells to wells coated with a dose range of rMfge8 in the presence of a  $\beta 5$  blocking antibody. (D) Western blot of integrin expression in human gastric smooth muscle cells (HGSMC), SW480 cells and  $\alpha 8$  transfected SW480 (SW480\_ $\alpha 8$ ) cells. (E) Human gastric smooth muscle cell adhesion to rMfge8-coated wells in the presence of blocking antibodies against the  $\alpha v$ ,  $\beta 1$ ,  $\beta 5$ ,  $\alpha 8$ , or  $\alpha 5$  integrin subunits. (F) Immunofluorescence staining of antral sections from *Mfge8*<sup>-/-</sup> and *α8sm*<sup>-/-</sup> mice with or without rMfge8 gavage proved for  $\alpha 8$  (green), human-FC-Mfge8 recombinant construct (red) and DAPI (blue). Arrows point co-localization of Mfge8 and  $\alpha 8$ . (G) Force of antral contraction in WT and *α8sm*<sup>-/-</sup> mice in response to MCh (N = 3–4). (H) The rate of gastric emptying in *α8sm*<sup>-/-</sup> and WT mice with and without the addition of rMfge8 (N = 4–5). (I) Small intestinal transit time in *α8sm*<sup>-/-</sup> and WT mice with and without the addition of rMfge8 (N = 4–5). (J) Force of antral contraction in WT mice after IP injection of  $\alpha 8$  blocking or control antibody in response to MCh (N = 4–5). (K) The rate of gastric emptying in WT mice after IP injection of  $\alpha 8$  blocking or IgG1 isotype control antibody (N = 7). (L) Small intestinal transit time in WT mice after IP injection of  $\alpha 8$  blocking or IgG1 isotype control antibody (N = 7). Female mice were used in G, H and I and male mice were used for all remaining panels. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Data are expressed as mean  $\pm$  s.e.m.

DOI: 10.7554/eLife.13063.005



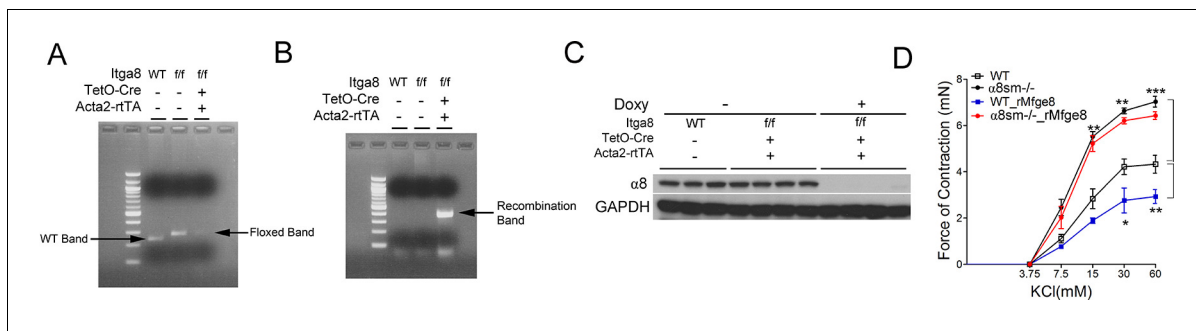
**Figure 2—figure supplement 1.** Normal gastrointestinal motility in  $Itgb3^{-/-}$ ,  $Itgb5^{-/-}$  and  $Itgb3^{-/-}; Itgb5^{-/-}$  mice. (A) Force of antral smooth muscle ring contraction in  $Itgb3^{-/-}$ ,  $Itgb5^{-/-}$ , and  $Itgb3^{-/-}; Itgb5^{-/-}$  mice in response to MCh. (B) The rate of gastric emptying in  $Itgb3^{-/-}$ ,  $Itgb5^{-/-}$  and  $Itgb3^{-/-}; Itgb5^{-/-}$  mice with and without the addition of rMfge8 (N = 5–6). (C) Small intestinal transit time in  $Itgb3^{-/-}$ ,  $Itgb5^{-/-}$  and  $Itgb3^{-/-}; Itgb5^{-/-}$  mice with and without the addition of rMfge8 (N = 5–6). Both male and female mice were used for these experiments. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Data are expressed as mean  $\pm$  s.e.m.

DOI: 10.7554/eLife.13063.006



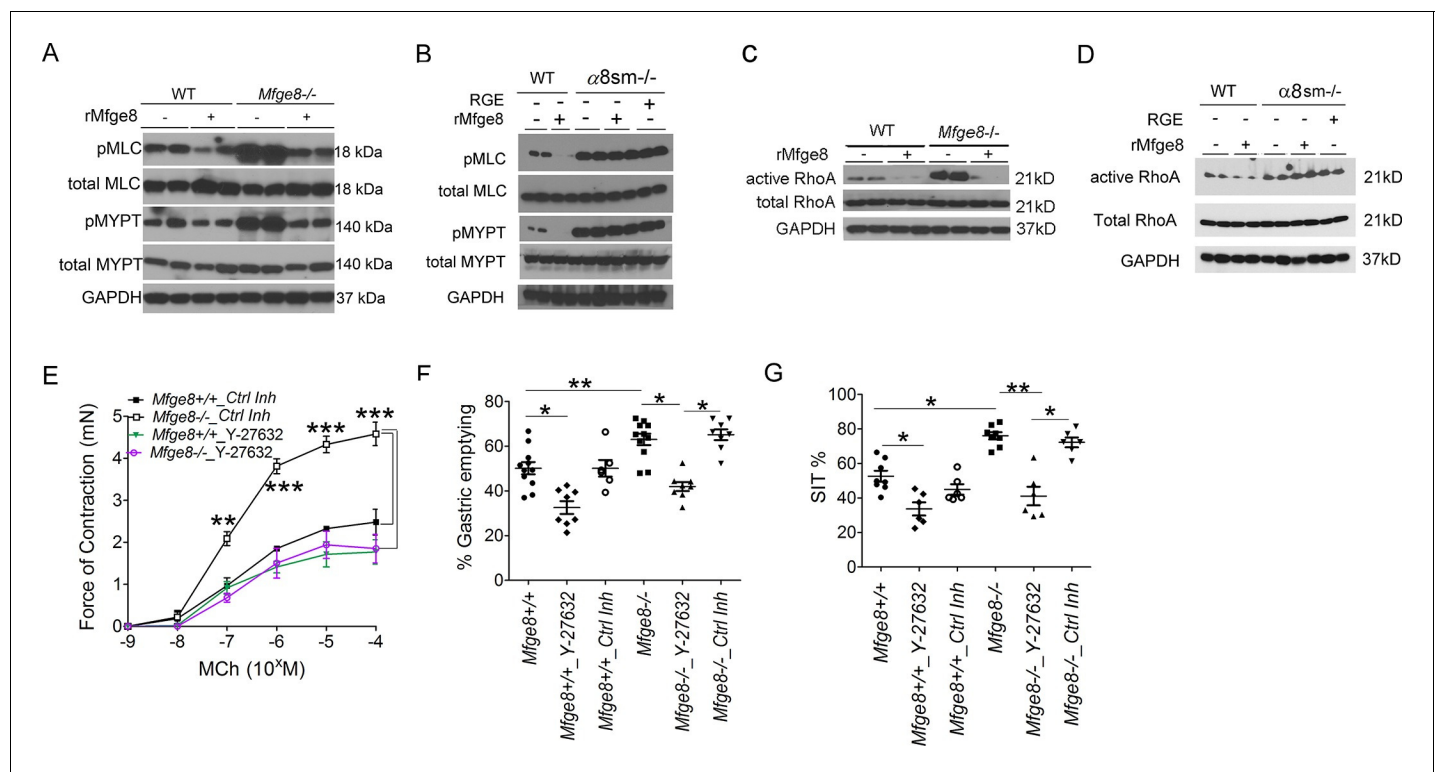
**Figure 2—figure supplement 2.** Integrin expression levels in SW480 cells. Flow cytometric analysis of mock transfected cells and cells transfected with either Itgb3 or Itga8. Gray histogram represents isotype control.

DOI: [10.7554/eLife.13063.007](https://doi.org/10.7554/eLife.13063.007)



**Figure 2—figure supplement 3.** Enhanced antral contraction in  $\alpha 8\text{sm}^{-/-}$  mice. (A) Floxed band, (B) recombination band, and (C) western blot of gastric smooth muscle of  $\alpha 8\text{sm}^{-/-}$  after two weeks of doxycycline administration. (D) Force of antral contraction in WT and  $\alpha 8\text{sm}^{-/-}$  mice in response to KCl (N = 3–4). Female mice were used for these experiments. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Data are expressed as mean  $\pm$  s.e.m.

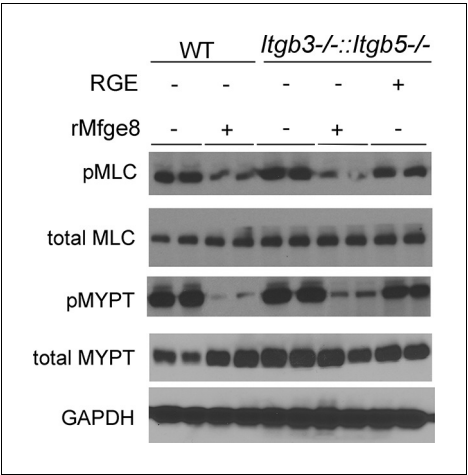
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**Figure 3.**  $\alpha 8$  integrin regulates antrum smooth muscle calcium sensitivity by preventing RhoA activation. (A, B) Western blot of antrum muscle strips obtained from (A) *Mfge8*<sup>-/-</sup> and (B)  $\alpha 8$ sm<sup>-/-</sup> mice and incubated with MCh. (C, D) Western blot of antrum smooth muscle strips obtained from (C) *Mfge8*<sup>-/-</sup> and (D)  $\alpha 8$ sm<sup>-/-</sup> treated with MCh demonstrating active and total RhoA using a GST pull-down assay. (E) Force of antral smooth muscle ring contraction with and without the addition of ROCK inhibitor Y-27632 (N = 3–4). (F) The rate of gastric emptying in *Mfge8*<sup>-/-</sup> and WT with and without the IP injection of ROCK inhibitor (Y-27632) or control inhibitor (N = 5–11). (G) Small intestinal transit times *Mfge8*<sup>-/-</sup> and WT with and without IP injection of ROCK inhibitor (Y-27632) or control inhibitor (N = 6–11). Female mice were used for all experiments. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Data are expressed as mean  $\pm$  s.e.m.

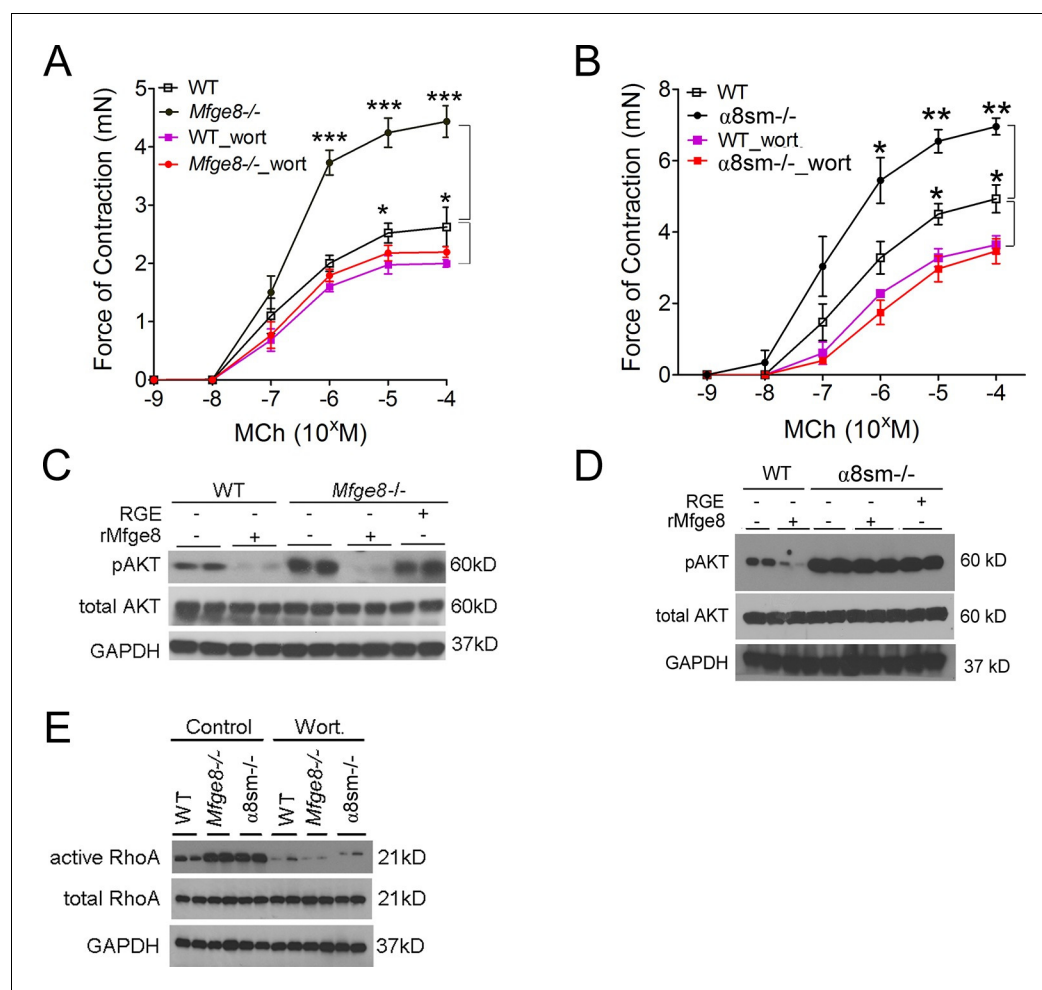
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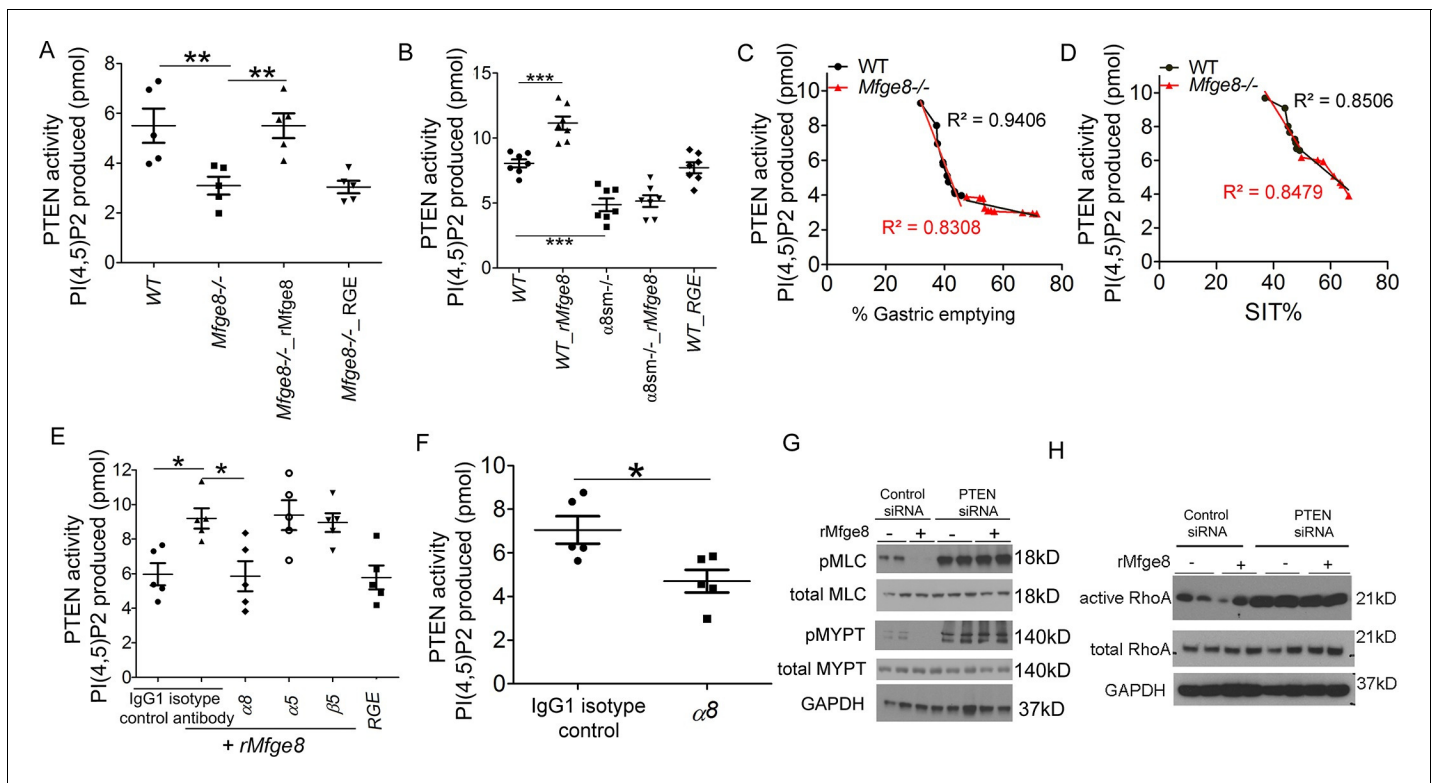
**Figure 3—figure supplement 1.** Normal calcium sensitivity in *Itgb3<sup>-/-</sup>::Itgb5<sup>-/-</sup>* antrum smooth muscle. Western blot of antrum muscle strips obtained from WT and *Itgb3<sup>-/-</sup>::Itgb5<sup>-/-</sup>* mice incubated with MCh. N = 3. Both male and female mice were used for these experiments.

DOI: 10.7554/eLife.13063.010



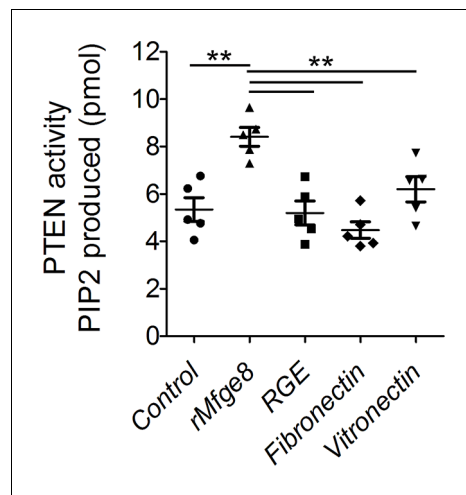
**Figure 4.** Mfge8 ligation of  $\alpha 8 \beta 1$  integrin inhibits PI3 kinase activity. (A–B) Force of antral smooth muscle ring contraction with and without the addition of PI3K inhibitor wortmannin (wort 100 ng/ml) in response to MCh in WT and Mfge8<sup>-/-</sup> (A, N = 4–5) or WT and  $\alpha 8 \text{sm}^{-/-}$  (B, N = 4–5). (C–D) Western blot of antrum muscle strips obtained from WT and Mfge8<sup>-/-</sup> (C) and WT and  $\alpha 8 \text{sm}^{-/-}$  mice (D) incubated with MCh. (E) Western blot of antrum from WT, Mfge8<sup>-/-</sup> and  $\alpha 8 \text{sm}^{-/-}$  treated with wortmannin (100 ng/ml) and MCh demonstrating active and total RhoA using a GST pull-down assay. Male mice were used in panel A and B. The remaining panels include both male and female mice. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Data are expressed as mean  $\pm$  s.e.m.

DOI: 10.7554/eLife.13063.011

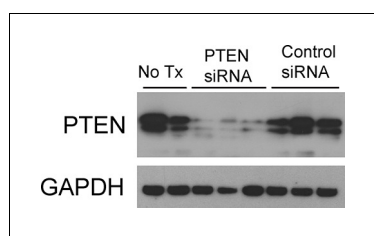


**Figure 5.** Mfge8 modulates PTEN activity. (A–B) PTEN activity in antral smooth muscle of WT and *Mfge8*<sup>-/-</sup> (A, N = 5) and WT and *α8sm*<sup>-/-</sup> (B, N = 7) with and without the addition of rMfge8 and RGE construct. (C–D) Correlation between the PTEN activity and the rate of gastric emptying (C, N = 11) and the small intestinal transit time in WT mice (D, N = 13). (E) PTEN activity in antral smooth muscle strips with addition of rMfge8 in presence of blocking antibody against *α8*, *α5* and *β5*. (F) PTEN activity in antral smooth muscle strips of WT mice after IP injection of *α8* blocking or IgG1 isotype control antibody. (N = 5). (G) Western blot in human gastric smooth muscle cells (HGSMC) treated with siRNA targeting PTEN with or without rMfge8 and then treated with 5-HT (100 μM). (H) Western blot of human gastric smooth muscle cells (HGSMC) treated with PTEN siRNA and with 5-HT demonstrating active and total RhoA using a GST pull-down assay. Both male and female mice were used for these experiments. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Data are expressed as mean ± s.e.m.

DOI: 10.7554/eLife.13063.012

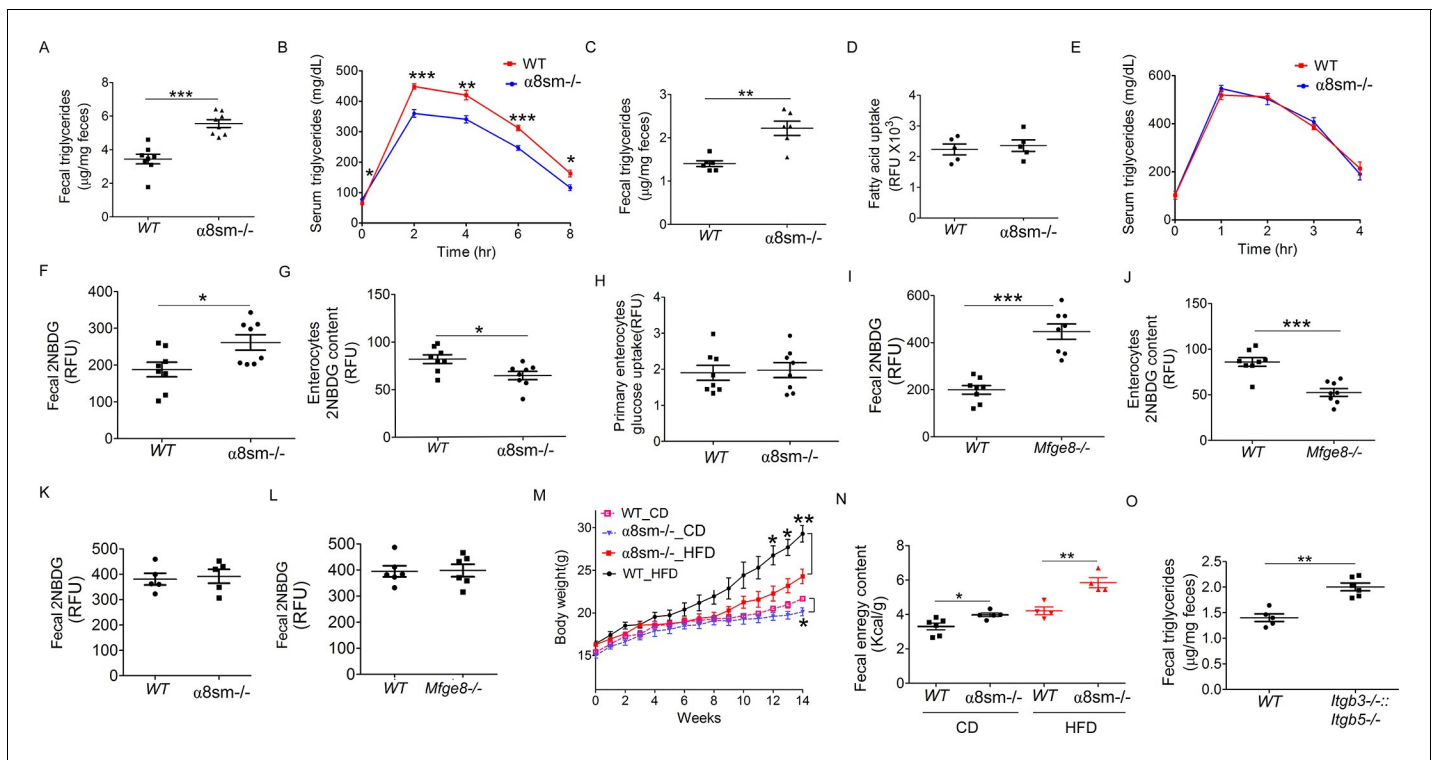


**Figure 5—figure supplement 1.** Mfge8 increases PTEN activity. PTEN activity assay in Human Gastric Smooth Muscle Cells after treatment with rMfge8, RGE construct, fibronectin or vitronectin (N = 5). \*\*p<0.01, \*\*\*p<0.001. Data are expressed as mean  $\pm$  s.e.m.  
[DOI: 10.7554/eLife.13063.013](https://doi.org/10.7554/eLife.13063.013)



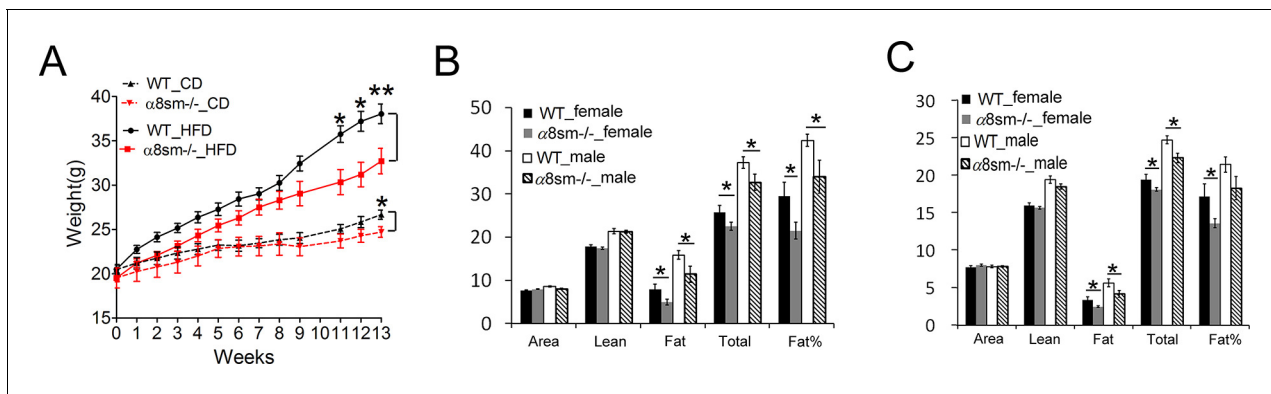
**Figure 5—figure supplement 2.** siRNA knockdown of PTEN. Western blot showing efficiency of PTEN-targeting siRNA and control siRNA in Human Gastric Smooth Muscle Cells.

DOI: [10.7554/eLife.13063.014](https://doi.org/10.7554/eLife.13063.014)



**Figure 6.**  $\alpha 8sm^{-/-}$  mice are protected from diet-induced obesity. (A) Fecal triglycerides in WT and  $\alpha 8sm^{-/-}$  mice after an olive oil gavage ( $N = 8$ ). (B) Serum triglycerides levels in WT and  $\alpha 8sm^{-/-}$  mice after an olive oil gavage ( $N = 5$ ). (C) Fecal triglycerides in WT and  $\alpha 8sm^{-/-}$  mice on a normal chow control diet ( $N = 6$ ). (D) Primary enterocyte fatty acid uptake in the isolated enterocytes from WT and  $\alpha 8sm^{-/-}$  mice ( $N = 5$ ). (E) Serum triglycerides levels after IP administration of olive oil in WT and  $\alpha 8sm^{-/-}$  mice ( $N = 5$ ). (F, G) Fecal (F,  $N = 8$ ) and enterocytes (G,  $N = 8$ ) 2NBDG content in WT and  $\alpha 8sm^{-/-}$  mice after gavage with a 2NBDG-methylcellulose mixture. (H) Glucose uptake assay in isolated primary enterocytes from WT and  $\alpha 8sm^{-/-}$  mice ( $N = 8$ ). (I, J) Fecal (I,  $N = 8$ ) and enterocytes (J,  $N = 8$ ) 2NBDG content in WT and  $Mfge8^{-/-}$  mice after gavage with a 2NBDG-methylcellulose mixture. (K, L) Fecal 2NBDG content in WT and  $\alpha 8sm^{-/-}$  (K,  $N = 5$ ) and  $Mfge8^{-/-}$  (L,  $N = 6$ ) after gavage with a 2NBDG in PBS. (M) Weight gain in female WT and  $\alpha 8sm^{-/-}$  mice on a normal chow diet (CD) ( $N = 6-8$ ) or HFD ( $N = 8-12$ ). (N) Fecal energy content in WT and  $\alpha 8sm^{-/-}$  mice on a normal chow diet (CD) ( $N = 5-6$ ) or HFD ( $N = 4-5$ ). Each sample represents stool combined from 3 mice. Female mice were used for all experiments. (O) Fecal triglycerides in WT and  $Itgb3^{-/-}:Itgb5^{-/-}$  integrin-deficient mice with normal chow control diet ( $N = 5-6$ ). For all in vivo experiments, each group of 5 mice represents 1 independent experiment. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . Data are expressed as mean  $\pm$  s.e.m.

DOI: 10.7554/eLife.13063.015



**Figure 6—figure supplement 1.** Protection from weight gain in  $\alpha 8sm^{-/-}$  mice on a HFD. (A) Weight gain in WT and  $\alpha 8sm^{-/-}$  male mice on a CD (N = 6–8) or HFD (N = 8–10). (B–C) Body composition of WT and  $\alpha 8sm^{-/-}$  mice aged 14 weeks on a HFD (B, N = 8–12) or on a CD (C, N = 6–8). \* $p < 0.05$ , \*\* $p < 0.01$ . Data are expressed as mean  $\pm$  s.e.m.

DOI: [10.7554/eLife.13063.016](https://doi.org/10.7554/eLife.13063.016)