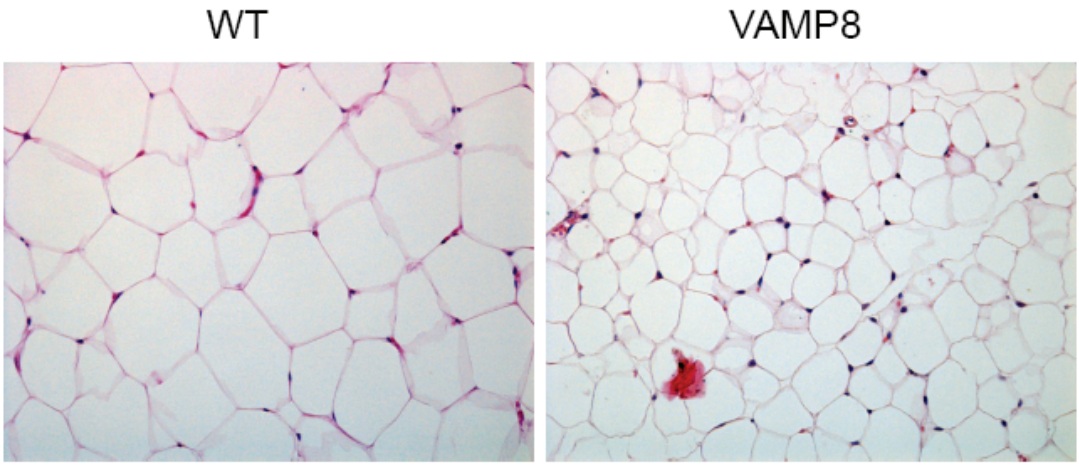
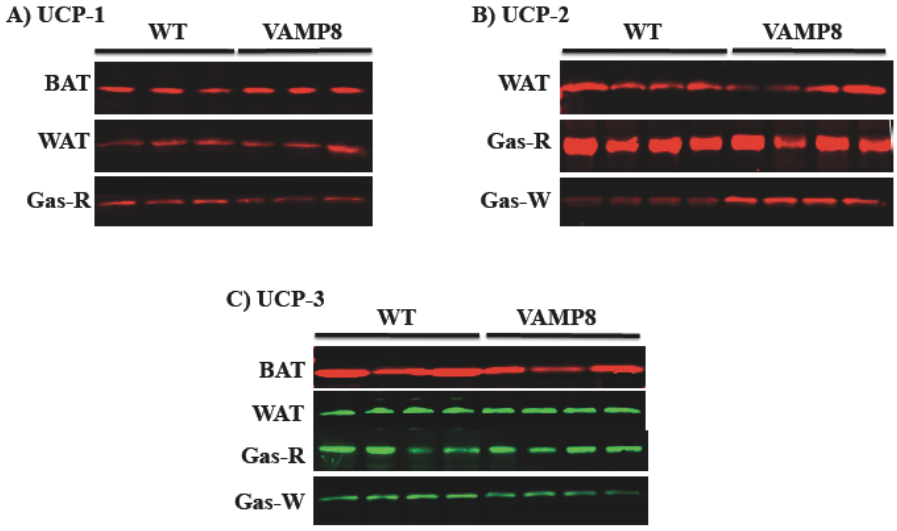
Supplemental Figure 1. VAMP8 null mice display reduced adipocyte cell size. Epididymal adipose tissue was isolated from 12 weeks old wild type (WT) and VAMP8 null (VAMP8) mice fed a normal chow diet. Paraffin sections of isolated epididymal adipose tissue were subjected to H&E staining for morphology of adipocyte. These are representative images taken from 3 WT and VAMP8 null mice.



Supplemental Figure 2. VAMP8 null mice have increased white skeletal muscle expression of UCP-2. Tissue extracts from brown adipose tissue (BAT), epididymal white adipose tisse (WAT), red gastrocnemius skeletal muscle (R-Gas) and white gastrocnemius skeletal muscle (W-Gas) were prepared from wild type (WT) and VAMP8 null (VAMP8) mice. The tissue extracts were immunoblotted for the presence of UCP-1 (A), UCP-2 (B) and UCP-3 (C). These are representative immunoblots performed on extracts isolated from 3-4 independent mice.



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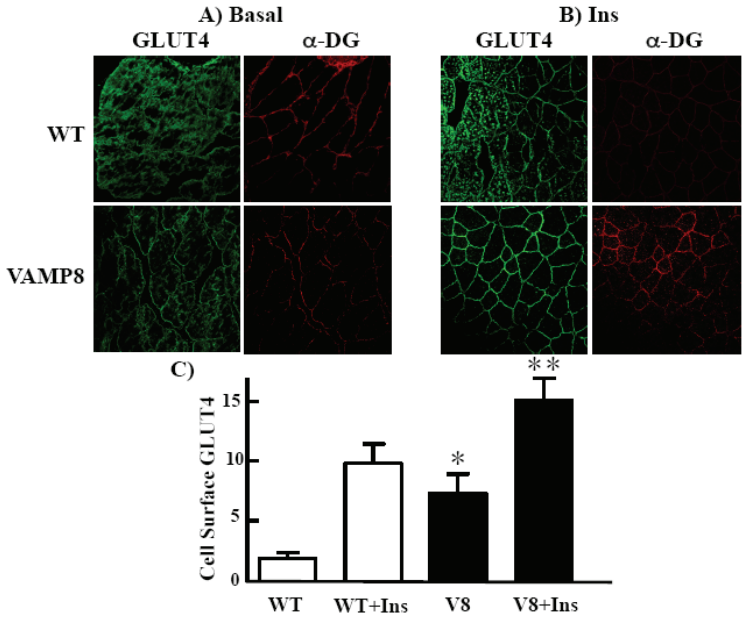
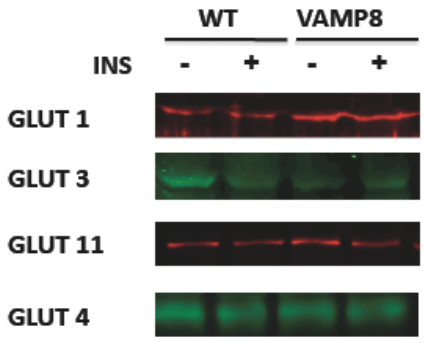
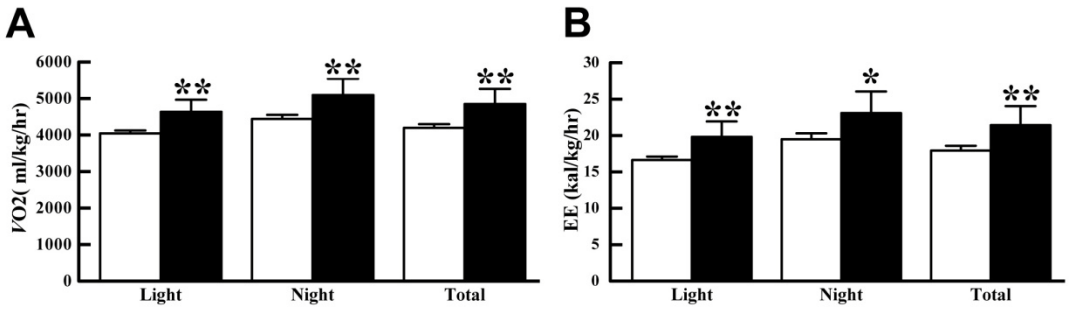
Supplemental Figure 3. VAMP8 null mice have increased GLUT4 protein localized to the sarcolemma membrane in soleus skeletal muscle. Wild type (WT) and VAMP8 null (VAMP8) mice were fasted overnight (12 h) or given an intraperitoneal injection of either (A) isotonic saline or (B) insulin (1 U/kg). Fifteen min later the soleus muscle was isolated, and embedded in OCT as described in Research Design and Methods. The frozen tissue cross-sections (10 μm) were then subjected to confocal immunofluorescence with specific GLUT4 and the α-dystroglycan (αDG) antibody. Representative images are shown and quantification of the relative extent of cell surface distribution of GLUT4 (C) was determined as the mean ± SEM from 5-10 independent mice. *p<0.05, **p<0.003, WT mice (open boxes) and VAMP8 mice (filled boxes).



Supplemental Figure 4. VAMP8 null mice have no change in the total protein levels of the GLUT1, GLUT3, GLUT4 and GLUT11 facilitative glucose transporters expressed in skeletal muscle. Skeletal muscle (gastrocnemius) extracts were isolated from wild type (WT) and VAMP8 null (VAMP8) mice in either the basal or insulin-stimulated state as described in Research Design and Methods. The muscle extracts were immunblotted for the presence of the indicated facilitative glucose transporters. This is a representative immunoblotted independently performed 5 times for each group.



Supplementary Figure 5. Loss of VAMP8 expression results in increased energy expenditure (EE) when corrected for lean mass. The average oxygen consumption in ml/kg/h (A) and energy expenditure in kcal/gm/h (B) determined in Figure 4 was normalized for lean mass from 5 wild type (WT, open boxes) and VAMP8 null (VAMP8, filled boxes) mice. *p<0.05, **p<0.003.