⌘ *Author's Choice*

Sarcoglycan Complex
IMPLICATIONS FOR METABOLIC DEFECTS IN MUSCULAR DYSTROPHIES*⃞S

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The sarcoglycans are known as an integral subcomplex of the dystrophin glycoprotein complex, the function of which is best characterized in skeletal muscle in relation to muscular dystrophies. Here we demonstrate that the white adipocytes, which share a common precursor with the myocytes, express a cell-specific sarcoglycan complex containing β-, δ-, and ε-sarcoglycan. In addition, the adipose sarcoglycan complex associates with sarcospan and laminin binding dystroglycan. Using multiple sarcoglycan null mouse models, we show that loss of α-sarcoglycan has no consequence on the expression of the adipocyte sarcoglycan complex. However, loss of β- or δ-sarcoglycan leads to a concomitant loss of the sarcoglycan complex as well as sarcospan and a dramatic reduction in dystroglycan in adipocytes. We further demonstrate that β-sarcoglycan null mice, which lack the sarcoglycan complex in adipose tissue and skeletal muscle, are glucose-intolerant and exhibit whole body insulin resistance specifically due to impaired insulin-stimulated glucose uptake in skeletal muscles. Thus, our data demonstrate a novel function of the sarcoglycan complex in whole body glucose homeostasis and skeletal muscle metabolism, suggesting that the impairment of the skeletal muscle metabolism influences the pathogenesis of muscular dystrophy.

Muscle fat infiltration is recognized as a hallmark pathological feature in dystrophin glycoprotein complex (DGC)[3]-related muscular dystrophies (1) that include dystrophinopathies (2, 3) and sarcoglycanopathies (LGMD2C-F) (4). In agreement, magnetic resonance imaging measurements of fat infiltration allow accurate assessments of disease severity in Duchenne muscular dystrophy patients (3). Association of adipose tissue development with degenerative/regenerative or atrophic changes in skeletal muscle is also supported by the finding that adipogenesis-competent cells within the skeletal muscle are activated during muscle regeneration (5). However, the molecular mechanism(s) underlying muscle fatty metamorphosis remain unclear.

Ectopic fat deposition in skeletal muscles is primarily described in animals and humans with lipodystrophy and sarcopenia. In these conditions, the accumulation of lipids and adipocytes in skeletal muscle is often accompanied by hyperglycemia and insulin resistance (6–11), both of which are strong indicators of muscle metabolic defects (12, 13) and deregulated adipogenesis (14). Furthermore, both adipose-derived and muscle-derived stem cells differentiate into adipocytes upon exposure to high levels of glucose (15), linking impaired muscle metabolism with muscle fat deposition.

It is long held that the biogenesis of a basement membrane takes place in the earliest steps of adipogenesis and that extensive extracellular matrix (ECM) remodeling occurs throughout adipogenesis (16, 17). The concept that cell surface receptors play a role in the regulation of adipogenesis and thus may underlie metabolic disorders just recently emerged with a study of the integrin complexes (18). Given that the DGC in its capacity as an ECM receptor is critical for muscle integrity (19, 20) and that white adipocytes and skeletal muscle cells originate from the same mesenchymal precursor cells (21, 22), we set out to determine whether components of the skeletal muscle DGC are expressed in white adipocytes. Herein, we describe a unique adipose sarcoglycan (SG) complex that includes β-, δ-, and ε-SG. This complex is tightly associated with sarcospan (Sspn) and dystroglycan (DG). Moreover, we show that DG functions as a novel ECM receptor in white adipocytes. Because adipose tissue and skeletal muscle play critical roles in the maintenance of normal glucose homeostasis and whole body insulin sensitivity (23), we examined the metabolic consequences of the SG complex disruption in both adipose tissue and skeletal muscle. Using *in vivo* approaches, we observed that the β-SG null mouse (24), a mouse model of muscular dystrophy, is glucose-intolerant and exhibits whole body insulin resistance specifically due to impaired insulin-stimulated glucose uptake in skeletal muscle.

EXPERIMENTAL PROCEDURES

Animals—Animal care and procedures were approved and performed in accordance with the standards set forth by the National Institutes of Health and the Animal Care Use and Review Committee at the University of Iowa.

Biochemical Analysis—White adipocytes were isolated from wild-type gonadal white adipose tissue (25). Total RNA extraction using RNA-STAT60™ (IsoTex Diagnostics Inc., Friendswood, TX) and preparation of total membrane extracts (26) were performed from isolated adipocytes. Sucrose gradient

purification of the DGC components (27) was performed from isolated adipocytes and whole WT gonadal adipose tissue.

Physiological Analysis—Glucose tolerance tests (GTTs) were performed on 16-h fasted male mice following intraperitoneal injection of D-glucose (1 g/kg). Blood glucose was measured from tail vein using OneTouch Ultra test strips (LifeScan). Several measurements were done at each time point to ensure reproducibility, and the lowest value was used. Fasting insulin was quantified in serum using enzyme-linked immunosorbent assay (Millipore Corp.). Euglycemic-hyperinsulinemic (EU) clamps were conducted on male mice according to the slightly modified protocol (28). Basal rates of whole body glucose clearance were assessed using a continuous infusion of [^{3}H]glucose for 2 h prior to the start of the clamp. Insulin was infused continuously at 4 milliunits/kg/min. Forty-five min before the end of the clamp, 2-deoxy-D-[1-^{14}C]glucose was administrated as a bolus (10 μCi) to estimate insulin-stimulated glucose uptake in individual tissues.

Statistics—GTT data were analyzed with two-way repeated measures analysis of variance, and all pairwise multiple comparison procedures were done with the Bonferroni *t* test. Other data were analyzed with Student's unpaired *t* test.

RESULTS

To determine whether known components of the DGC are expressed in white adipose tissue while ensuring that our results would not reflect contamination from other tissues, we chose freshly isolated white adipocytes as starting material. RT-PCR data revealed the presence of β-, δ-, and ϵ-SG, Sspn and DG transcripts in adipocytes (Fig. 1*A* and supplemental Table 1s). The identity of these transcripts was confirmed by BLAST analysis. In contrast to skeletal muscle, there was a conspicuous absence of α-SG and γ-SG transcript. The detection of a transcript correlated with the detection of the corresponding protein (Fig. 1*B*). Results were confirmed by immunofluorescence analysis of adipose tissue sections (Fig. 1*C* and data not shown). Of the known DGC proteins, DG is the focus of most research interest due to its widespread expression, association with the basal lamina, and the complex and heterogeneous tissue-specific *O*-glycosylation of its extracellular α-subunit. Post-translational modification of α-DG is critical for its interaction with a broad range of ECM proteins, and loss of this interaction is a common factor in the biogenesis of complex diseases (19). To better characterize the binding properties of α-DG in adipose tissue, we chose to make use of a well characterized mouse model, the myodystrophy mice (Large^{myd} or myd) (29). These mice have a spontaneous loss-of-function mutation in *Large*, which encodes a putative glycosyltransferase involved in α-DG *O*-glycosylation, leading to brain and skeletal muscle α-DG hypoglycosylation and pathology (30). We found adipocyte α-DG reactivity using antibodies directed against either the α-DG core (sheep5) or its sugar moieties (IIIH11), albeit at a lower relative molecular mass (120 kDa) than that in skeletal muscle (supplemental Fig. 1s, *A* and *B, lanes 1* and *3*). The absence of *Large* led to a loss of α-DG glycosylated epitope, with preservation of the α-DG protein core in adipocytes (supplemental Fig. 1s, *A* and *B, lanes 2* and *4*). Agrin and laminin-1 overlay experiments conclusively demonstrated that the adi-
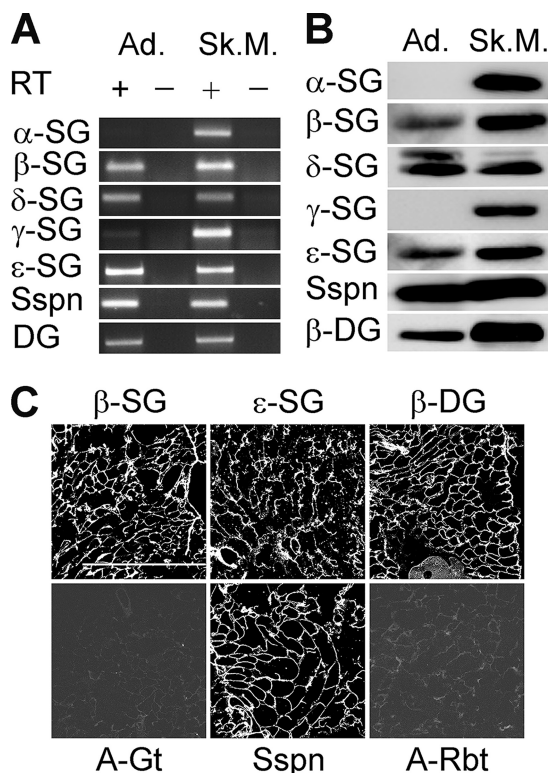

FIGURE 1. **Expression of DGC components in white adipocytes.** *A*, transcripts of known DGC components were amplified from isolated adipocytes (*Ad.*) and skeletal muscle (*Sk.M.*) by RT-PCR in the presence (+) or absence (−) of reverse transcriptase (RT). *B*, expression of the corresponding proteins in total membrane fraction from white adipocytes (100 μg) and KCl-washed microsomes from skeletal muscle (50 μg), as determined by Western blotting. *C*, expression of DGC proteins in epididymal white adipose tissue, analyzed by confocal microscopy. Negative controls using cyanine 3-labeled anti-goat (*A-Gt*) and Alexa Fluor 555-labeled anti-rabbit (*A-Rbt*) were performed by omitting the primary antibody. *Scale bar*, 500 μm.

pose-specific glycosylation was sufficient for α-DG to function as an ECM ligand receptor (supplemental Fig. 1s, *C* and *D, lanes 1* and *3*). As expected and consistent with the observations made in myd brain and skeletal muscle, laminin and agrin binding to α-DG were abolished in absence of *Large* (supplemental Fig. 1s, *C* and *D, lanes 2* and *4*), with the level of expression of the SGs and Sspn being preserved (data not shown). These data demonstrate that Large-mediated post-translational processing of α-DG takes place in white adipocytes. In support of this conclusion, expression of *Large* and other α-DG post-translational processing enzymes, *i.e. Pomt1*, *Pomt2*, *PomGnt1*, and *Fukutin*, was observed in adipocytes by RT-PCR (supplemental Fig. 2s), confirming a previous gene profiling study performed in white adipocytes (31).

To test whether the DGC components expressed in the white adipocytes associate in a fashion similar to that described for skeletal muscle (19, 20), we analyzed their co-fractionation on sucrose gradient (supplemental Fig. 3s*A*). We found that Sspn migrated in the denser gradient fractions, along with the β-, δ-, and ϵ-SG and β-DG. The tight association of these proteins upon solubilization indicates that these proteins are integral components of a multisubunit complex that via DG can function as a novel ECM receptor in the white adipocytes. Control experiments in differentiated 3T3-L1 cells supported this conclusion as well (data not shown).
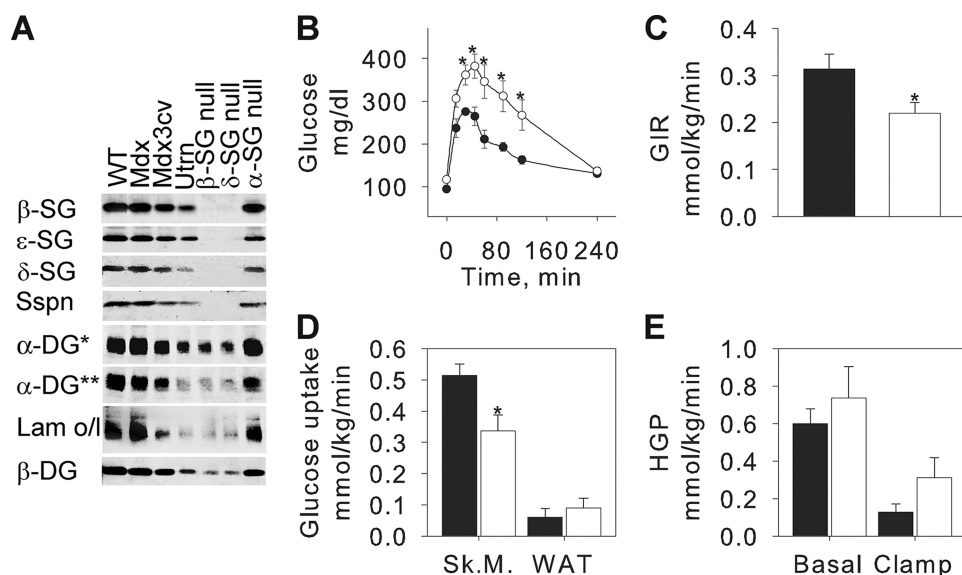
FIGURE 2. **β-SG null mice are glucose-intolerant and insulin-resistant.** *A*, whole adipose tissue from various mouse models was analyzed by Western blotting and laminin-1 overlay (*Lam o/l*) after digitonin solubilization and glycoprotein enrichment. *, anti-core antibody sheep5; **, anti-carbohydrate antibody IIIH11. *B*, intraperitoneal GTTs performed on 3–4-month-old male mice. ●, WT, $n = 14$; ○, β-SG null mice, $n = 7$. Mean ± S.E., *, $p < 0.05$. *C–E*, conscious EU clamps were performed on 3–4-month-old male mice. Whole body glucose infusion rate (*GIR*) (*C*) and glucose uptake in soleus (*Sk.M.*) and white adipose tissue (*WAT*) (*D*) were measured during clamp conditions. Hepatic glucose production was measured in both basal and clamp conditions (*E*). *Filled columns*, WT, $n = 7$; *open columns*, β-SG null mice, $n = 8$. Mean ± S.E., *, $p < 0.05$.

TABLE 1

Organ weights expressed as a percentage of total weight

Organs were carefully dissected and weighed. Results are expressed as mean (S.E.). All experiments were performed in double-blind fashion. IBAT, intrascapular brown adipose tissue. *, $p < 0.001$.

Organ	WT ($n = 15$)	β-SG null mice ($n = 11$)
Quadriceps muscle	1.54 (0.02)	1.79 (0.06)*
Calf muscle	1.09 (0.03)	1.43 (0.03)*
Cardiac muscle	0.56 (0.01)	0.57 (0.03)
Kidney	1.24 (0.02)	1.22 (0.03)
Liver	4.14 (0.09)	5.92 (0.11)*
Epididymal fat	1.57 (0.07)	1.01 (0.06)*
Retroperitoneal fat	0.39 (0.02)	0.22 (0.03)*
Lungs	0.56 (0.02)	0.66 (0.05)
Spleen	0.27 (0.01)	0.39 (0.04)
Pancreas	0.53 (0.02)	0.56 (0.04)
IBAT	0.22 (0.02)	0.27 (0.02)
Testis	0.69 (0.02)	0.67 (0.03)

To determine whether the adipose SG complex functions as a single unit, we tested whether the loss of a single component will influence the expression of the others and impair α-DG function. We took advantage of existing mouse models of muscular dystrophy, each with a systemic genetic ablation of one skeletal DGC component (32). Fig. 2*A* shows the composition of the SG complex and its associated Sspn and DG in mdx and mdx3cv mice, utrophin (utrn), β-SG, δ-SG, and α-SG null mice. As expected, loss of either full-length dystrophin (dys) or α-SG, whose expression are restricted to skeletal muscle, had no consequence on the expression of the SGs, Sspn, and DG at the adipocyte membrane nor on the ability of α-DG to bind laminin. The loss of all dys isoforms in mdx3cv mice or of utrn in the utrn null mice was accompanied by modest decreased expression of all the proteins tested and a reduced laminin binding to α-DG. Therefore, although one cannot rule out that a short dys isoform and utrophin could be expressed in the adipocytes,

their expression does not appear to be critical for proper SG complex expression at the adipocyte plasma membrane. However, loss of either β-SG or δ-SG led to a complete loss of the other SGs as well as Sspn. Moreover, only residual expression of the glycosylated form of α-DG could be detected, which correlated with weak residual laminin binding. Altogether, our data suggest that the adipose SG complex should be considered as a single unit that relies on all its individual components for full functionality.

Because both skeletal muscle and adipose tissue play important roles in glucose homeostasis, we took advantage of the β-SG null mice due to the concomitant loss of expression of the entire adipose and skeletal SG complexes. Upon dissection, we observed that the β-SG null mice had virtually no subcutaneous adipose tissue (data not shown). Furthermore, Table 1 demonstrates that the β-SG null had a significant decrease of their visceral white adipose tissue mass (epididymal and retroperitoneal fat expressed as a percentage of total body mass) as compared with age-matched WT mice. Histological analysis of the white adipose tissue revealed conspicuous changes such as those that characterize lipodystrophy (supplemental Fig. 4s). Specifically, unilocular cells were heterogeneous in size and shape; numerous small multilocular cells were present, and large blood vessels and fibrous strands coursed throughout the adipose tissue.

Functionally, β-SG null mice were glucose-intolerant (Fig. 2*B*) and had a trend to have higher fasting insulin levels than their WT counterparts (data not shown), suggesting an alteration of their whole body insulin sensitivity. To address this last question, we performed conscious EU clamps coupled with [³H]glucose infusion in β-SG null and WT mice (Fig. 2, *C–E*). EU clamps indicated that the glucose clearance was significantly diminished in the β-SG null mice as compared with the WT controls (22% decrease with $p = 0.029$, data not shown), confirming the glucose tolerance test data. The whole body glucose infusion rate was 30% lower ($p = 0.03$) in the β-SG null mice than in the controls (Fig. 2*C*), demonstrating that the null animals were insulin-resistant. Interestingly, insulin resistance was accompanied by decreased insulin sensitivity in skeletal muscle only (35% decrease in insulin-stimulated glucose uptake in skeletal muscle of β-SG null mice as compared with that of WT age-matched controls, $p = 0.016$) (Fig. 2*D*). Insulin sensitivity remained normal in both white adipose tissue (Fig. 2*D*) and liver (Fig. 2*E*).

DISCUSSION

We have demonstrated here that white adipocytes express at their plasma membrane an SG complex unique to this cell type.

It includes β-, δ-, and ϵ-SG but lacks α- and γ-SG as determined by RT-PCR and immunoblotting. Both β-SG and δ-SG proteins can seed the formation of the SG complexes (α-$\beta\delta\gamma$ and ϵ-$\beta\delta\gamma$) found in skeletal and smooth muscle, respectively (33, 34), and a recent model proposed that γ-SG links the β/δ-SG precomplex to α-SG in skeletal muscle (35). As ζ-SG, the latest SG identified is the closest γ-SG homolog (36), it is possible that ζ-SG functionally replaces γ-SG in white adipocytes. We also showed that Sspn co-purifies with the above SG complex and that its expression is gone in the adipose tissue of SG-null mice. It was previously suggested that the assembly of a complete SG complex was a prerequisite for proper expression of Sspn at the sarcolemma (37). Likewise, our data suggest that expression of Sspn at the adipocyte membrane is dependent on proper SG complex expression.

The role played by the ECM receptors (18) and the importance of the cell-cell and cell-matrix interactions in adipose tissue are just emerging concepts (38–40). Here, we found that decreased expression of DG and a loss of extracellular ligand binding to α-DG were associated with a loss of SG complex expression in white adipocytes. This suggests that the SG complex is necessary for functional DG expression as an ECM receptor at the adipocyte plasma membrane. The physiological characterization of β-SG null mice suggests that these mice, primarily known as a genetic model of muscular dystrophy (24, 32), could also be a new genetic model of lipodystrophy (9). It is well established that adipose tissue serves as a crucial integrator of glucose homeostasis (12, 28) and that alterations of its physiology can have a wide range of metabolic consequences (41, 42). Thus, it is tempting to speculate that the alteration of the adipose SG complex could represent a new molecular mechanism underlying phenotypes of altered adiposity, abnormal fat distribution, and metabolic disorders, likely via an alteration of the cell-cell and/or cell-matrix interactions in adipose tissue. However, since the global ablation of β-SG affects both skeletal muscle and adipocytes, future studies using tissue-specific knock-out animals will be necessary to specifically resolve the tissue-specific roles of the SG complex in glucose metabolism.

Nevertheless, these findings raise novel questions that have important implications for clinical studies of muscular dystrophy. DGC-related muscular dystrophies are often accompanied by progressive muscle fat replacement (2–4), and the ECM imparts specific characteristics to each tissue. Thus, we speculate that the SG complex, along with Sspn and DG, plays a role in regulating the adipogenesis/myogenesis cell fate decisions potentially involved in the mechanism of muscle fat replacement. Furthermore, muscular dystrophies are complex diseases likely to involve more than the skeletal muscles (19). However, the efficiency of the therapeutic strategies are so far solely based on the recovery of the skeletal DGC and/or muscle strength (43, 44). Our data indicate that a cell-specific SG complex is expressed in the white adipocytes. Furthermore, we for the first time bring evidence of metabolic defects in the skeletal muscles and whole body of the β-SG null mice. We speculate that the severity of the metabolic symptoms is likely to depend on whether the skeletal SG complex alone (*i.e.* in α-SG null mice) or both adipose and skeletal SG complexes (*i.e.* in β-SG null mice) are disrupted and on the relative functional contribution

of the primary missing protein. Although the association of metabolic defects with the progression of muscular dystrophy is not documented yet in patients, it is possible that the recovery of skeletal muscle function in treated patients might uncover new health problems due to defective SG complexes in non-muscle tissues, such as adipose tissue.

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SUPPLEMENTAL MATERIAL

EXPERIMENTAL PROCEDURES

Animals- Wild type (C57BL/6), Large[myd] (29), mdx and mdx3cv mice (45,46) were bred at the University of Iowa from stocks originally obtained from Jackson Laboratories (Bar Harbor, ME). α-SG null (A575), β-SG null (Q94) and δ-SG null (I74) mice have been described previously (24,47,48) and were maintained on C57BL/6 background (backcross 5).

Antibodies- MAbs against α-SG (Ad1/20A6) and γ-SG (35DAG/21B5), and pAbs against Sspn (R256), α-SG (R98), β-SG (Goat 26), δ–SG (R245), γ-SG (R229), and ε-SG (R232) were described previously (24). pAbs against the core α-DG (sheep 5) and β-DG (R83) were used as previously described (30). MAb IIIH11, which recognizes the α-DG glycoepitope, was obtained from the University of Iowa Hybridoma Facility. MAb 5204 against agrin (Chemicon) was used as described in the company datasheet.

RT-PCR- cDNA was synthesized from 2μg of total RNA, using 10U AMV-RT (Promega), 40U RNAsin (Promega), 20 pmol oligo dT primers, and 20 pmol random nanomers. PCR products were separated on 1.5% agarose gels in the presence of ethidium bromide, gel-purified, sequenced (by The University of Iowa DNA Facility), and compared to the corresponding sequences deposited in the GenBank database.

Immunoblot analysis- Proteins were resolved by SDS-PAGE on 3-15% linear gradient gels and transferred to Immobilon-Fl membranes. Western blot was carried on as previously described (27) and analyzed using the LI-COR Odyssey system. For agrin and laminin overlay, agrin and mouse laminin-1 (Invitrogen) were directly labeled with IRdye® 800CW after extensive dialysis in PBS. Labeling took place for 2 hours at room temperature in the dark (IRDye® 800CW Protein Labeling Kit-High MW, LI-COR Biosciences), and free dye was removed using a Zeba spin desalting column (Pierce).

Confocal immunofluorescence analysis- Seven μm cryosections of epididymal adipose tissue were prepared as described (27) and analyzed by confocal microscopy (Olympus Confocal Laser Scanning Microscope Fluoview FV1000).

H&E staining- Epididymal fat was fixed with 4% paraformaldehyde before to be embedded in paraffin. Five μm paraffin sections were used for H&E.

Glycoprotein enrichment and sucrose gradient fractionation- Glycoprotein enrichment and co-fractionation analysis were performed as previously described (27) with the following modifications: isolated adipocytes (5 ml) were solubilized for 1 hour at 4ºC in a 1% digitonin buffer (50 mM Tris, pH 7.4; 500 mM NaCl; 1 % Digitonin) in the presence of protease inhibitors, after which they were centrifuged at 142,000 *g* for 37 min at 4ºC. Whole adipose tissue was solubilized in the same digitonin-containing buffer at a ratio of 1g of tissue per 2ml of solubilization buffer. After overnight incubation with WGA-Agarose (Vector Laboratories), WGA-bound proteins were eluted with 0.3M N-acetylglucosamine. Eluates were concentrated using Biomax-10K centrifugal concentrators (Millipore Corp., Bedford, MA) before being applied to a 5-30% linear sucrose gradient (Biocomp gradient Master # 105). The gradient was centrifuged for 2 hours at 249,000 g. Eleven fractions of equal volume were collected from the top of the gradient.

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Supplemental table 1

Name	GenBank A.N.	Sequence	Tm °C	Size bp
DG	NM_010017	S: GCTCTTTCGAGTGAGCATTCCC AS: CTAGTTTCCCAGGACAGGAGA	50	561
α-SG	NM_0097161	S: CGACAGCTATGCCCGTTGT AS: AGTCTCGGTCTGTGGCTTCA	62	210
β-SG	NM_011890	S: TTATCCTGGCCGTCATCAAT AS: TGGAAGACAATTGGCTGGTT	58	223
δ-SG	NM_011891	S: TCCGAAGGCTGTGGAAGCAT AS: AACCTTAGTTCCTTGAAGGG	62	195
γ-SG	NM_011892	S: TCACAGGCCGGGTGAAAGT AS: AAATGGGTCAGCTCTGACAA	60	203
ε-SG	NM_011360	S: GGGTGTTTATGTCATGGTTGG AS: AAAATGCCCTCGCCACGAA	60	225
Sspn	NM_010656	S: TGGGATCATTGTCTGCGTGGT AS: AGGGAGGTCTCGCAGGTAAA	60	207
Pomt1	NM_145145.1	S: CACTGAGCCCCCATTCTCAA AS: GTACAAAGCGCACTTCCGAC	60	147
Pomt2	NM_153415.3	S: ATCCCCTTTGCCTACCTCAC AS: GCCGCCATGATGAAGAAC	54	152
Pomgnt1	NM_026651	S: GGGATTGGGACATGTGGAT AS: TGAGCTGGACACCTGGAACT	54	174
Large	NM_010687	S: TTCCTGGCTGCCTCTTTGAC AS: TGGACGTGTGTTTTTCCAGA	61	830
fukutin	NM_139309.4	S: AAGGCCCGGCTTCAGAAT AS: ATCTAGCCGAGGATCCTTGC	62	161
Rpl4	NM_024212.4	S: GCGCAGGAATACCATTCTTC AS: TGTCTGCAGTCCCCTTCTCTG	62	125

<u>Table 1s.</u> The equivalent of 0.5 μg of cDNA was amplified with the above primer pairs. After an initial denaturation step of 2min at 94°C, 35 cycles of 30s at 94°C, 30sec at the specific Tm and 30s at 72°C were performed. Reactions were terminated by a 4min extension at 72°C. S, sense, AS, antisense.
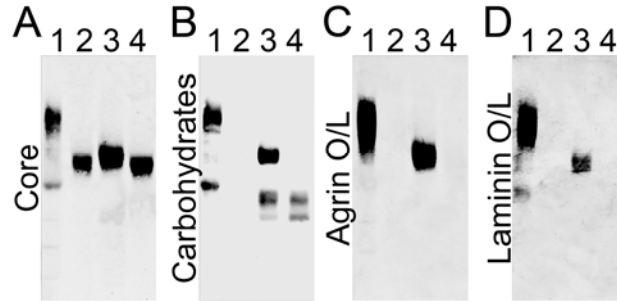
Supplemental figure 1

Fig. 1s. α-DG in white adipocytes is modified by the Large protein. Western blot analysis of WGA-enriched total glycoproteins isolated from WT skeletal muscle (1), Large[myd] skeletal muscle (2), WT fat (3) and Large[myd] fat (4) using anti-core antibody sheep5 (A), anti-carbohydrate antibody IIIH11 (B), agrin overlay (O/L) (C), and laminin-1 overlay (D).
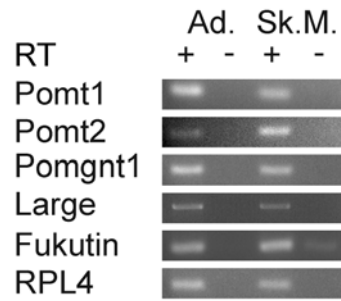
Supplemental figure 2

Fig. 2s.   Expression of the α-DG post-translational processing components in white adipocytes. Transcripts were amplified from isolated adipocytes (Ad.) and skeletal muscle (Sk.M.) by RT-PCR in presence (+) or absence (-) of reverse transcriptase (RT).
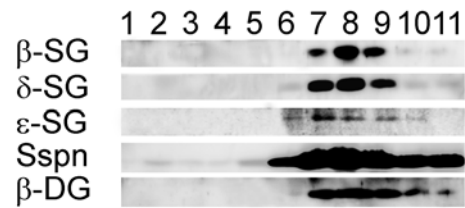
Supplemental figure 3

Fig. 3s. Composition and organization of the adipose DGC. *A*. WT isolated adipocytes were solubilized in digitonin, glycoenriched using WGA and subjected to sucrose gradient fractionation. Each fraction, from the lightest (1) to the heaviest (11), was analyzed by immunoblotting.
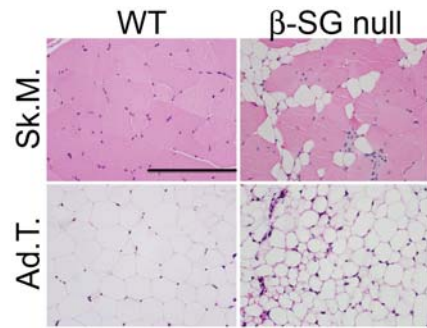
Supplemental figure 4

Fig. 4s. H&E staining of skeletal muscle (Sk.M, top panel) and gonadal adipose tissue (Ad.T., bottom panel) from WT and β-SG null mice. Scale bar, 200μm.