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

Table 1s. 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

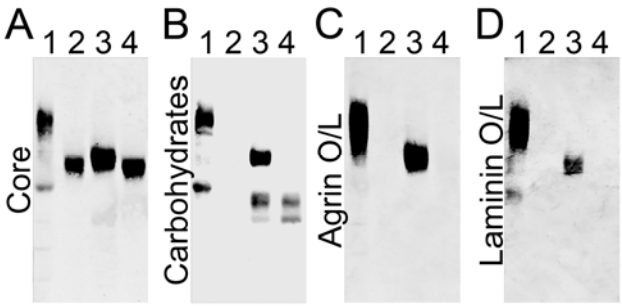

Fig. 1s. α-DG in white adipocytes is modified by the Large protein. Western blot analysis of WGA-enriched total glycoproteins isolated from WT skeletal muscle (1), Large^{myd} skeletal muscle (2), WT fat (3) and Large^{myd} fat (4) using anti-core antibody sheep5 (A), anti-carbohydrate antibody IIIH11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

Supplemental figure 2

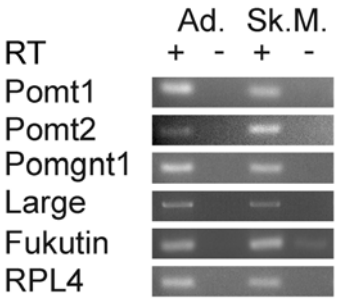

Fig. 2s. Expression of the α-DG post-translational processing components in white adipocytes. Transcripts were amplified from isolated adipocytes (Ad.) and skeletal muscle (Sk.M.) by RT-PCR in presence (+) or absence (-) of reverse transcriptase (RT).

Supplemental figure 3

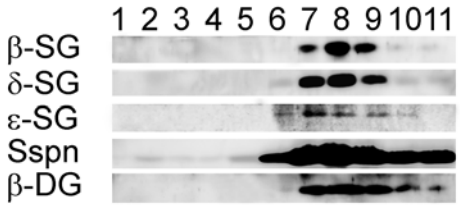

Fig. 3s. Composition and organization of the adipose DGC. *A.* WT isolated adipocytes were solubilized in digitonin, glycoenriched using WGA and subjected to sucrose gradient fractionation. Each fraction, from the lightest (1) to the heaviest (11), was analyzed by immunoblotting.

Supplemental figure 4

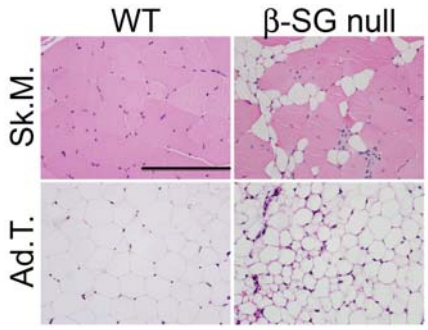

Fig. 4s. H&E staining of skeletal muscle (Sk.M, top panel) and gonadal adipose tissue (Ad.T., bottom panel) from WT and β-SG null mice. Scale bar, 200μm.