SUPPLEMENTARY METHODS

***Generation of Doc2b Transgenic mice* -** The pUC-Combi^{CMV} plasmid used for generation of tetracycline-repressible Doc2b Tg mice contains the tetO minimal promoter to drive the Doc2b gene in one direction, and a CMV promoter to drive the transactivator gene in the other direction. The full-length cDNA for *DOC2B* carrying an N-terminal Myc tag was inserted into the vector at the PmeI site. The construct was linearized by digestion with NotI and microinjected into the nucleus of pre-implantation embryos. These embryos were then transferred into the oviduct of pseudo-pregnant C57BL/6J female mice by the Indiana University, School of Medicine Transgenic Animal Facility. Thirty eight pups were screened for the presence of the transgene using PCR of genomic DNA, with four founders resulting. Of the four lines, F5170, the line with the highest expression levels in pancreas, fat and skeletal muscle (~2-3.5-fold) was selected for full phenotypic characterization. Genotypes determined by PCR on DNA from tail-biopsy specimens (primers for Tg: 5' to 3': #3-ggcagaggacaagtccctgg; #9-agaggattgagcgttggcac; and #10-acgacgaggcttgcaggatcataa. Primers for Wt: 5' to 3': O-ggaaagaaggcgaatggaag and F-tcactccagggttttcatcc), whereby the PCR product of the wild-type (Wt) allele was 500 bp and that of the Tg allele was 681 and 277 bp.

Intraperitoneal Glucose Tolerance Test (IPGTT) and Insulin Tolerance Test (ITT) –Female C57BL/6J Doc2b Tg and Wt mice (4-6 months old) were fasted for 6 h (08:00-14:00) before the IPGTT. Following sample collection of fasted blood, animals were given glucose (2 g/kg body weight) by intraperitoneal injection, and blood glucose readings were taken at 30, 60, 90 and 120 min for IPGTT. For the ITT, female mice (4-6 months old) were fasted for 6 hrs, sampled for fasted blood and then injected intraperitoneally with Humulin R (0.75 U/kg body weight) and

blood glucose readings taken at 15, 30, 60 and 90 min after injection. Blood was collected from the tail vein and measurement of blood glucose was performed using the Hemocue glucometer (Mission Viejo, CA, U.S.A.).

Isolation, Culture and Perifusion of Mouse Islets – Pancreatic mouse islets were isolated from pancreata of 10-14 week old female mice. After isolation, islets were cultured overnight in CMRL-1066 medium, and hand-picked into groups of 40 onto cytodex bead columns. The islets were pre-incubated in Krebs-Ringer bicarbonate buffer (10 mmol/l HEPES pH 7.4, 134 mmol/l NaCl, 5 mmol/l $NaHCO_3$, 4.8 mmol/l KCl, 1 mmol/l $CaCl_2$, 1.2 mmol/l $MgSO_4$, 1.2 mmol/l KH_2PO_4) containing 2.8 mmol/l glucose and 0.1% BSA. Islets were perifused for 10 min at a rate of 0.3 ml/min, followed by stimulation with 16.7 mmol/l glucose for 35 min. Fractions were collected every 1-3 min and insulin secreted into fractions and the corresponding islet lysate insulin content was quantified by radioimmunoassay (RIA).

Skeletal Muscle Subcellular Fractionation – Hind limb skeletal muscle was sub fractionated into sarcolemmal/t-tubule plasma membrane and intracellular membrane components. Doc2b Tg and Wt littermate female mice (4-6 months old) were fasted for 16 h (18:00-10:00), injected intraperitoneally with 21 U/kg body weight of Humulin or saline, and sacrificed within 40 min for removal of the hindquarter muscles into homogenization buffer (20 mmol/l Hepes pH 7.4, 250 mmol/l Sucrose, 1 mmol/l EDTA, 5 mmol/l benzamidine, 10 μg/ml aprotinin, 5 μg/ml leupeptin, 1 μg/ml pepstatin, 1 mmol/l PMSF) for Polytron homogenization. Homogenates were centrifuged at 2000 x g for 5 min at 4 °C, and supernatant then centrifuged at 9000 x g for 20 min at 4 °C. That supernatant was subsequently centrifuged at 180,000 x g for 90 min. Pellets

containing t-tubule and sarcolemmal membrane fractions were resuspended in 1% NP-40 lysis buffer (25 mmol/l Tris, pH 7.4, 1% NP40, 10% glycerol, 50 mmol/l sodium fluoride, 10 mmol/l sodium pyrophosphate, 137 mmol/l sodium chloride, 1 mmol/l sodium vanadate, 1 mmol/l PMSF, 10 µg/ml aprotinin, 1 µg /ml pepstatin and 5 µg /ml leupeptin) and proteins resolved by 10% SDS-PAGE for subsequent immunoblotting for GLUT4, Syn4, Munc18c and Doc2b.

L6 Muscle Cell Culture - Rat L6 muscle cells stably expressing GLUT4 with an exofacial myc-epitope were obtained from Dr. Amira Klip (Department of Biochemistry, University of Toronto, Canada). Myoblasts were maintained in α-MEM containing 5.5 mmol/l glucose (Life Technologies, Gaithersburg, MD, U.S.A) and 10% fetal bovine serum (Fisher Scientific, Pittsburgh, PA, U.S.A.), and 1% (v/v) antimycotic antibiotic solution (Life Technologies, Gaithersburg, MD, U.S.A.). Myoblasts at 80-90% confluence were electroporated (0.20 kV and 960 µFarad) with 150 µg of GFP vector or GFP-*DOC2B* plasmid DNA per 10 cm^2 dish. After electroporation, cells were allowed to adhere to plates for 48 hours. Cells were pre-incubated in serum-free media for 2 h followed by stimulation for 5 min with 100 nmol/l insulin. Cells were harvested in 1% NP-40 lysis buffer and detergent lysates used for co-immunoprecipitation or GST-VAMP2 interaction assays.

CHO-K1 Cell Culture - CHO-K1 cells were cultured in Ham's F-12 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/ml streptomycin, and 292 µg/ml L-glutamine. At 80-90% confluence, cells were electroporated with 40 µg of plasmid DNAs: pN2-GFP-*DOC2B* or pN2-GFP-*DOC2A*, per 10 cm^2 dish. After 48 h of incubation, cells were harvested in 1% NP-40 lysis buffer and detergent lysates were prepared and proteins resolved by

10% SDS-PAGE for immunoblotting for Doc2b and GFP. GFP-Doc2a and -Doc2b fusion proteins migrate at ~73 and 75 kDa, respectively (pN2-eGFP encodes GFP of 29 kDa, Doc2b migrates at ~46 kDa, Doc2a at~44 kDa).

Co-immunoprecipitation – Cleared detergent lysates from L6 myoblasts (2.5 mg) were combined with rabbit anti-Syn4 antibody for 2 h at 4°C, followed by a second incubation with protein G Plus-agarose for 2 h. The resultant immunoprecipitates were subjected to 10-12% SDS-PAGE followed by transfer to PVDF membranes for immunoblotting.

***Recombinant Proteins and Interaction Assays* -** The GST-VAMP2 protein was generated in E. *coli* and purified by glutathione-agarose affinity chromatography for use in the Syntaxin 4 accessibility assay. GST-VAMP2 protein linked to Sepharose beads was combined with 2 mg of L6 myoblast detergent cell lysate for 2 h at 4 °C in 1% NP-40 lysis buffer, followed by three stringent washes with the lysis buffer. The associated proteins were resolved on 10% SDS-PAGE followed by their transfer to PVDF membranes for immunoblotting for Syn4.