



Altered GLUT4 trafficking in adipocytes in the absence of the GTPase *Arfrp1*

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ABSTRACT

The GTPase ADP-ribosylation factor related protein 1 (ARFRP1) controls the recruitment of proteins such as golgin-245 to the *trans*-Golgi. ARFRP1 is highly expressed in adipose tissues in which the insulin-sensitive glucose transporter GLUT4 is processed through the Golgi to a specialized endosomal compartment, the insulin-responsive storage compartment from which it is translocated to the plasma membrane in response to a stimulation of cells by insulin. In order to examine the role of ARFRP1 for GLUT4 targeting, subcellular distribution of GLUT4 was investigated in adipose tissue specific *Arfrp1* knockout (*Arfrp1*^{ad-/-}) mice. Immunohistochemical and ultrastructural studies of brown adipocytes demonstrated an abnormal *trans*-Golgi in *Arfrp1*^{ad-/-} adipocytes. In addition, in *Arfrp1*^{ad-/-} adipocytes GLUT4 protein accumulated at the plasma membrane rather than being sequestered in an intracellular compartment. A similar missorting of GLUT4 was produced by siRNA-mediated knockdown of *Arfrp1* in 3T3-L1 adipocytes which was associated with significantly elevated uptake of deoxyglucose under basal conditions. Thus, *Arfrp1* appears to be involved in sorting of GLUT4.

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1. Introduction

The GTPase ARFRP1 (ARF-related protein 1) [1] is a member of the family of ADP-ribosylation factors (ARFs) that operate as GTP-dependent molecular switches in the regulation of intracellular protein traffic and in Golgi function [2,3]. ARFRP1 is ubiquitously expressed with higher levels in white and brown adipose tissue, liver, kidney, and intestine [1] and is associated with *trans*-Golgi membranes [4]. ARFRP1 recruits a second GTPase, ARL1, and its effector the GRIP protein golgin-245 to the *trans*-Golgi network [4–6]. Conventional *Arfrp1*^{-/-} embryos died during early gastrulation between day 6 and 6.5 [7] due to adhesion defects [8]. The adipocyte-specific deletion of *Arfrp1* resulted in a lipodystrophic phenotype due to a defective lipid droplet growth and an elevated lipolysis [9]. We found SNAP23 (synaptosomal-associated protein of 23 kDa) associated with small lipid droplets of control adipocytes as described in the literature [10]. In contrast, SNAP23 was predominantly located in the cytosol and at the cell surface of *Arfrp1*^{ad-/-} adipocytes [9].

SNARE proteins (VAMP2, syntaxin-4, and SNAP23) have been implicated in the insulin-induced translocation of vesicles containing the GLUT4 glucose transporter to the plasma membrane of adi-

pocytes [11–16] indicating that GLUT4 translocation follows typical membrane fusion rules. Because SNAP23 distribution was altered in adipocytes of *Arfrp1*^{ad-/-} mice [9] we reasoned that GLUT4 targeting might be modified in adipocytes lacking *Arfrp1*, thereby allowing further dissection of the different vesicular GLUT4 compartments. Since *Arfrp1*^{ad-/-} mice die early due to the lack of white adipose tissue [9] we studied GLUT4 localization in adipocytes from 18.5 days old *Arfrp1*^{ad-/-} embryos which did not show impaired growth or survival. In addition, we used 3T3-L1 adipocytes in which expression of *Arfrp1* was depleted by siRNA.

2. Materials and methods

2.1. *Arfrp1*^{ad-/-} mice

Generation of *Arfrp1*^{fllox/fllox} mice and the fat cell-specific deletion of *Arfrp1* was described previously [8,9]. Animals were housed in a controlled environment (20 ± 2 °C, 12 h/12 h light/dark cycle) and had free access to water and standard chow diet. All animal experiments were approved by the ethics committee of the Ministry of Agriculture, Nutrition and Forestry (State of Brandenburg, Germany).

2.2. Antibodies

We used the polyclonal antiserum against recombinant GST-ARFRP1 [1,4]. Antiserum against GLUT4 [9] was used for immuno-

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histochemistry and for Western blotting in a dilution of 1:1000, anti-GLUT1 [20] 1:1000. Anti-TGN38 antiserum (Serotec, Oxford, UK) was used in a dilution of 1:100 (immunohistochemistry) or 1:1000 (Western blotting) and anti-GM130 antiserum (BD Transduction Laboratories, NJ, USA) in a 1:100 dilution for immunohistochemistry. For analysis of activation status of Akt and FoxO1 we used the following antibodies for Western blotting in a dilution of 1:1000: anti-Akt (# 9272) and anti phospho-AKT (Ser473; # 9271), anti-FoxO1 (C29H4; # 2880), and anti-phospho-FoxO1 (Thr24)/FoxO3a (Thr32) antibody (#9464, Cell Signaling), Alexa Fluor® 546 F(ab')₂ fragment of goat anti-rabbit IgG (H + L) or Alexa Fluor® 488 F(ab')₂ fragment of goat anti-mouse IgG (H + L) (Molecular Probes, Eugene, Oregon, USA) were used in a dilution of 1:800 as secondary antibodies. Antibody against glyceraldehyde phosphate dehydrogenase (GAPDH, Ambion) was used as loading control.

2.3. Immunohistochemistry and immunocytochemistry

Paraffin sections of *Arfrp1^{fllox/fllox}* and *Arfrp1^{ad-/-}* animals at the age of ED18.5 were prepared as described earlier [9]. The indicated primary antibodies were applied overnight at 4 °C in a humid chamber. Subcellular distribution of GLUT4, TGN38, GM130, p115, syntaxin-6 and ARL1 was visualized by fluorescence-conjugated secondary antibodies in a confocal microscope as described [8].

2.4. Quantitative real-time PCR

Quantitative real-time PCR analysis (qRT-PCR) was performed using the Applied Biosystems 7300 Real-time PCR System as described previously [17]. For the determination of the *Arfrp1* mRNA levels and to analyze expression of *aP2* and *GLUT4*, the following TaqMan gene expression assays were used: *Arfrp1* E2_E3 (Mm01220415_g1), *Slc2a4/GLUT4* (Mm00436615_m1), *aP2* (Mm00445880_m1). Data were normalized as described (5) whereas a β -actin expression assay with the probe 5'-TTG AGA CCT TCA ACA CCC CAG CCA-3' and the forward primer 5'-GCC AAC CGT GAA AAG ATG AC-3' and reverse primer 5'-TAC GAC CAG AGG CAT ACA G-3' was used as endogenous control.

2.5. Electron microscopy

Brown adipose tissue of *Arfrp1^{fllox/fllox}* and *Arfrp1^{ad-/-}* embryos (ED 18.5) was sectioned into fragments of approximately 1–2 mm³. These pieces were immersed in a fixative consisting of 7.5% glutaraldehyde and 3% paraformaldehyde (pH 7.4). The specimens were post fixed in 1% OsO₄, dehydrated in ethanol, and embedded in Epon-Araldite. Block staining of specimens was performed with 1% uranyl acetate and 0.5% phosphotungstic acid. Ultra thin sections (50–60 nm) were obtained with an ultramicrotome, stained with lead citrate, and examined with a Zeiss EM10 electron microscope.

2.6. Cell culture and siRNA-mediated knockdown of *Arfrp1* in 3T3-L1 cells

Murine 3T3-L1 fibroblasts were purchased from the American Type Tissue Culture repository. Cells were grown to confluence and differentiated to mature adipocytes for up to 8 days [18] prior to electroporation. For downregulation of *Arfrp1* mature adipocytes (5 × 10⁶ cells/electroporation) were electroporated using the Bio-rad Gene Pulser II with settings of 170 V and 960 microfarads with 200 μ mol *Arfrp1*-specific siRNA (5'-GACUGUACUGUA AGAUU-GUU-3'; also corresponding to si-*Arfrp1*-a). For control experiments cells were electroporated with 200 μ mol scrambled siRNA

(5'-GACUGUACUGAUAGAUUGUU-3'; also corresponding to si-scrambled-a). We used an additional set of an *Arfrp1*-specific siRNA (si-*Arfrp1*-b: 5'-GUAAUUGAUUCCACUGAUGUU-3') and its corresponding scrambled siRNA (si-scrambled-b: 5'-CUAAUCGAUUC-CACUGAAGUU-3'). After electroporation, cells were immediately mixed with fresh medium before being reseeded onto multiple-well plates or plated on glass coverslips for immunocytochemical detection of GLUT4.

2.7. Detection of glucose transport

Seventy-two hours after electroporation of 3T3-L1 adipocytes with siRNA, basal and insulin-stimulated glucose uptake was assayed as described previously [18].

2.8. Preparation of plasma membrane sheets

Preparation of plasma membrane sheets from differentiated 3T3-L1 adipocytes was performed by the method of Olson et al. [19] with minor modifications as described by von der Crone et al. [20].

3. Results

3.1. Specific alteration of the trans-Golgi in brown *Arfrp1^{ad-/-}* adipocytes

Our previous studies have shown that activated, GTP-bound ARFRP1 associates with the trans-Golgi and recruits other proteins such as ARL1 and its effector, golgin-245 to this compartment [4]. In order to test whether the Golgi is also altered *in vivo* in the absence of ARFRP1 we stained brown adipocytes of control and *Arfrp1^{ad-/-}* embryos (ED 18.5) with specific markers for the trans-Golgi (TGN38) and for the cis-Golgi (GM130). Expression of *Arfrp1* in BAT of *Arfrp1^{ad-/-}* embryos was significantly and specifically reduced (Fig. 1A). In contrast to postnatal mice the knockout embryos did not show a growth retardation and a reduced survival [9]; they were therefore the appropriate model to study the influence of *Arfrp1* depletion on Golgi organization and GLUT4 localization because secondary alterations due to growth retardation were avoided. As shown in Fig. 1B (left panel), *Arfrp1^{fllox/fllox}* cells exhibited distinct distribution of TGN38 in a perinuclear region whereas it was nearly undetectable in *Arfrp1^{ad-/-}* cells. In order to test whether a perturbation of TGN38 trafficking might result in its degradation we analyzed TGN38 levels but did not detect differences (Fig. 1B, right panel). Syntaxin-6, an additional TGN protein was still membrane associated in brown adipocytes of *Arfrp1^{ad-/-}* embryos but displaced a broader staining than in control cells (Fig. 1B, lower panel). However, staining of the cis-Golgi with the anti-GM130 antibody was essentially identical in both genotypes, supporting our previous finding that organization of the trans-Golgi is specifically disrupted by *in vitro* knockdown of *Arfrp1*.

In addition, the ultrastructure of brown adipocytes was studied by electron microscopy at ED18.5. Fat cells of control embryos showed typical features such as lipid droplets and numerous mitochondria (Fig. 1C, upper panel). In brown fat cells of *Arfrp1^{ad-/-}* embryos lipid droplets were smaller [9] but nuclei, cell-cell contact and structure of mitochondria were normal. The major difference we observed was striking abnormal vacuolization of the Golgi apparatus from *Arfrp1^{ad-/-}* adipocytes (Fig. 1C, middle and lower panel).

3.2. Altered targeting of GLUT4 in adipocytes lacking *Arfrp1*

Since important biosynthesis processing steps of the insulin-regulated glucose transporter GLUT4 are believed to be initiated in the sorting compartments of the TGN [21] where ARFRP1 regu-

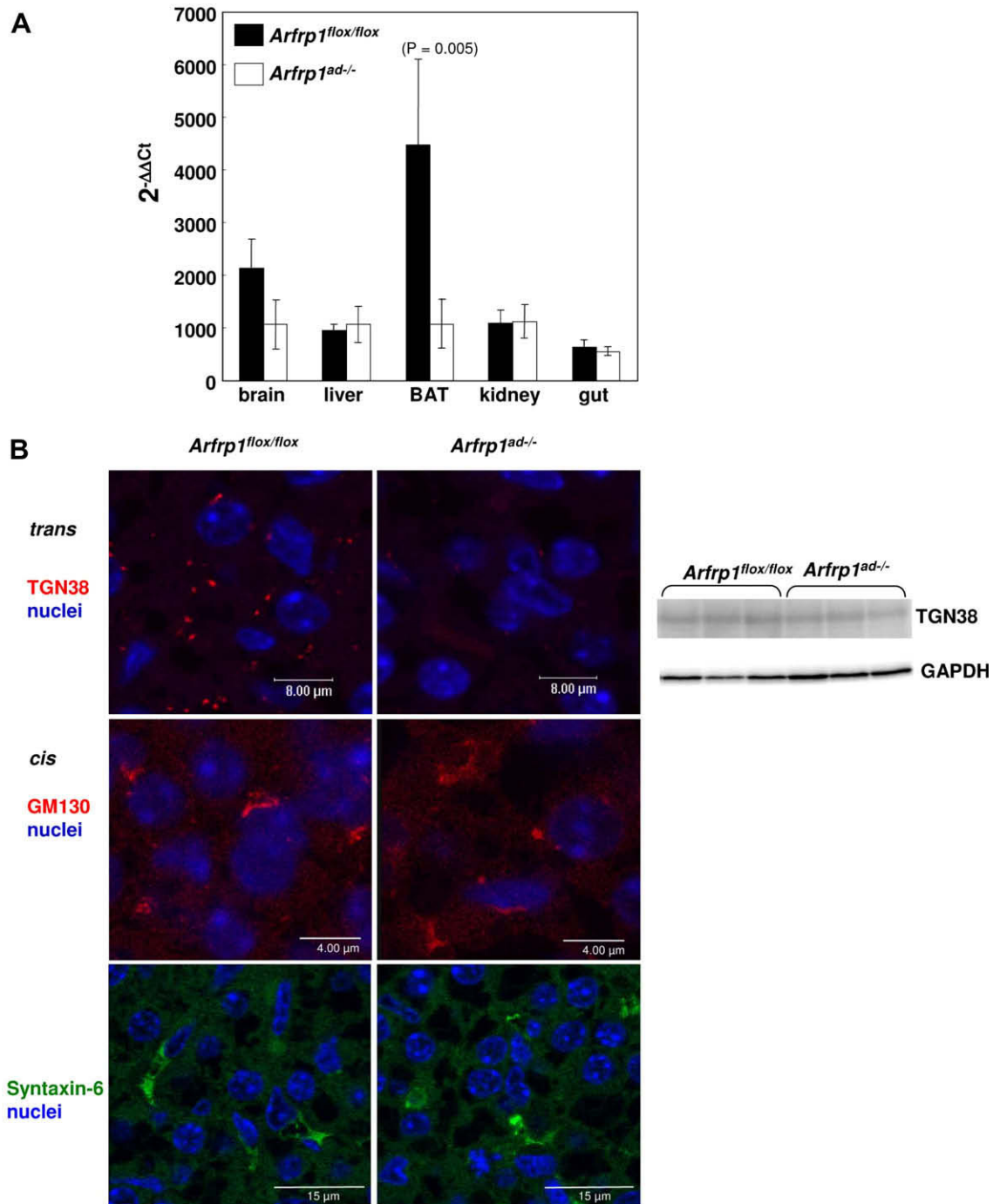


Fig. 1. Altered organization of the *trans*-Golgi in *Arfrp1*^{ad-/-} adipocytes. (A) *Arfrp1* mRNA levels of *Arfrp1*^{flox/flox} and *Arfrp1*^{ad-/-} embryos at ED18.5 in the indicated tissues were detected by quantitative RT-PCR as described in Section 2. (B) Immunohistochemical detection of TGN38 (upper panels), GM130 (middle panels) and syntaxin-6 (lower panels) in brown adipocytes of *Arfrp1*^{flox/flox} and *Arfrp1*^{ad-/-} embryos at day 18.5. Paraffin sections of BAT were stained with the indicated antisera as described in Section 2. Nuclei were detected with TO-PRO³-iodid. Lysates of BAT from two days old *Arfrp1*^{flox/flox} and *Arfrp1*^{ad-/-} mice were analyzed for TGN38 protein levels by Western blotting (right panel). (C) Electron micrographs of BAT of *Arfrp1*^{flox/flox} and *Arfrp1*^{ad-/-} embryos (ED18.5) were obtained as described in Section 2. Adipocytes of *Arfrp1*^{flox/flox} and *Arfrp1*^{ad-/-} embryos at a magnification of 1:2500 (upper panels). Golgi structures of adipocytes from *Arfrp1*^{flox/flox} and *Arfrp1*^{ad-/-} embryos at a magnification of 1:12,500 (middle and lower panels) (ER, endoplasmic reticulum; G, Golgi elements; L, lipid droplet; M, mitochondrium; N, nucleus).

lates recruitment of proteins such as ARL1 we examined the subcellular distribution of GLUT4 in BAT at ED18.5 by immunohistochemistry (Fig. 2, left panel). The subcellular distribution of GLUT4 in brown adipocytes differed between *Arfrp1*^{flox/flox} and *Arfrp1*^{ad-/-} embryos. *Arfrp1*^{flox/flox} adipocytes exhibited a punctate intracellular distribution of GLUT4, whereas *Arfrp1*^{ad-/-} fat cells showed a distinct concentration of GLUT4 at the plasma membrane

(Fig. 2, arrows). As shown in the right panel of Fig. 2, *Slc2a4*/*Glut4* mRNA was not altered in *Arfrp1*^{ad-/-} adipocytes. In addition, no differences in the amount of GLUT4 were visible in Western blot analysis of lysates from BAT of two days old control and *Arfrp1*^{ad-/-} mice (Fig. 2, lower right panel). We therefore can exclude that an increased expression of GLUT4 in *Arfrp1*^{ad-/-} adipocytes might be responsible for a stronger staining of GLUT4 at the cell surface.

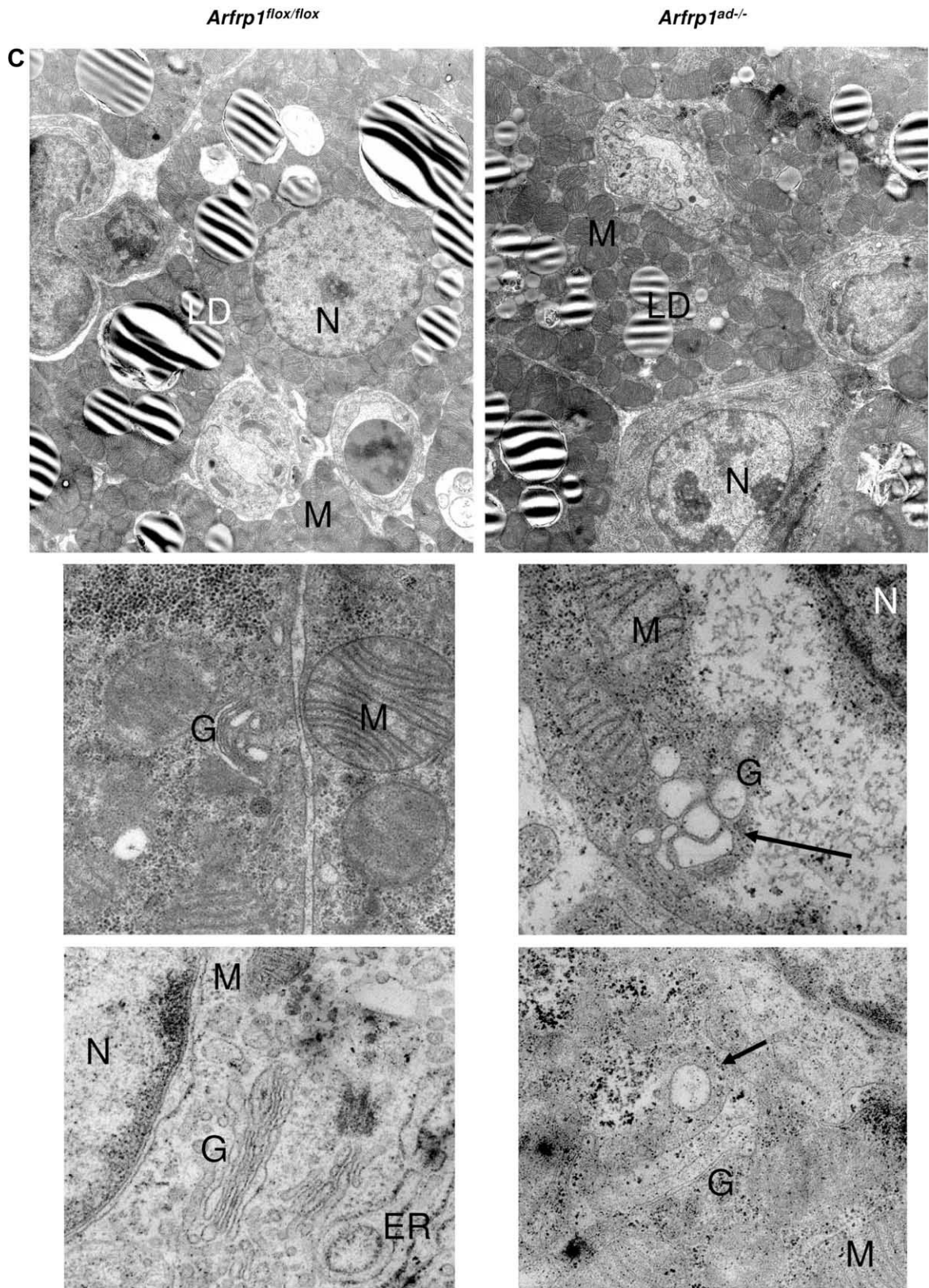


Fig. 1 (continued)

To examine the GLUT4 distribution in adipocytes in the absence of ARFRP1 in cultured adipose cells we suppressed *Arfrp1* expression in 3T3-L1 adipocytes by siRNA (Fig. 3A) and analyzed GLUT4 distribution under basal and insulin-stimulated conditions. In the

absence of insulin, GLUT4 was detected in intracellular membranes in control 3T3-L1 adipocytes which were electroporated with scrambled siRNA, whereas GLUT4 appeared at the cell surface in the presence of insulin (Fig. 3B). In contrast, knockdown of *Arfrp1*

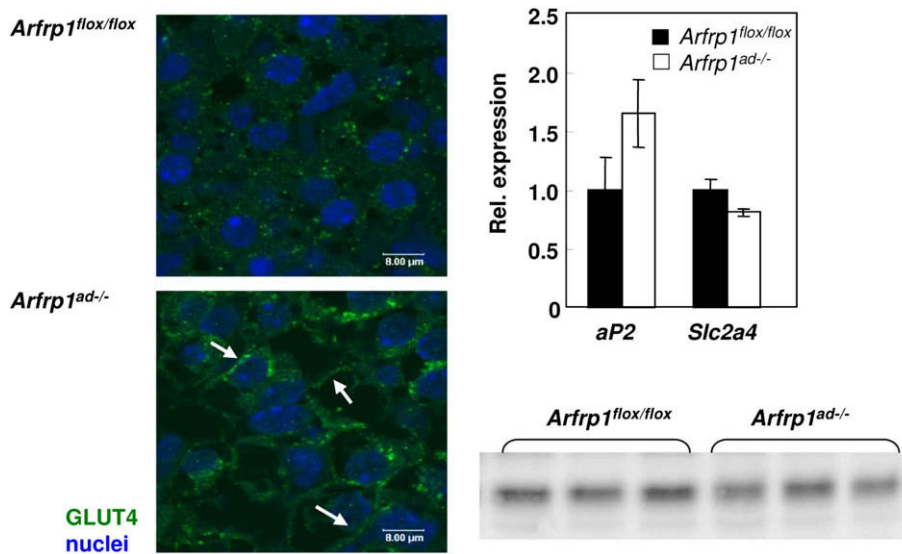


Fig. 2. Altered targeting of GLUT4 in *Arfrp1^{ad-/-}* adipocytes. Immunohistochemical detection of GLUT4 in BAT of *Arfrp1^{lox/lox}* and *Arfrp1^{ad-/-}* embryos at day 18.5 (left panels). Paraffin sections were stained with a polyclonal anti-GLUT4 antiserum in combination with an Alexa488-conjugated secondary antibody. mRNA expression of *aP2* and *Slc2a4* (GLUT4) in BAT of embryos at day 18.5 detected by qRT-PCR analysis (upper right panel). Western blot of GLUT4 in lysates of BAT from two days old *Arfrp1^{lox/lox}* and *Arfrp1^{ad-/-}* mice (lower right panel).

resulted in plasma membrane localization of GLUT4 already under basal conditions (Fig. 3B, arrows), confirming the observation obtained in brown adipocytes of *Arfrp1^{ad-/-}* embryos (Fig. 2, left panel). This finding was verified by a second *Arfrp1*-specific siRNA (*siArfrp1-b*) and the corresponding scrambled control (*si-scrambled-b*). Both sets of *Arfrp1*-specific siRNAs result in a cell surface localization of GLUT4 in the basal state (Supplementary Fig. 1).

To further verify the necessary role of ARFRP1 function in the intracellular retention of endogenous GLUT4, we examined the effect of *Arfrp1* knockdown on glucose transport by measuring uptake of labeled deoxyglucose. Scrambled siRNA-transfected adipocytes displayed a 5.6-fold insulin-stimulated increase in the rate of glucose uptake. In comparison, knockdown of *Arfrp1* resulted in a higher basal transport rate (3-fold higher than basal scrambled siRNA-transfected cells) with only an approximate 1.6-fold stimulation by insulin (Fig. 3C). These data strongly support a requirement for ARFRP1 in the sorting of proteins such as GLUT4. However, the total insulin-stimulated deoxyglucose transport did not differ between scrambled and *siArfrp1* transfected cells indicating that a recruitment of GLUT4 occurs from a similar intracellular compartment. In order to test whether elevated GLUT4 levels at the plasma membrane are the result of a higher GLUT4 expression or insulin-like effects in *Arfrp1* knockdown cells we analyzed lysates of transfected 3T3-L1 adipocytes for GLUT4 expression and levels of phosphorylated Akt and FoxO1, two prominent downstream signals of the insulin receptor. Expression of GLUT4, Akt and FoxO1 as well as the levels of phosphorylated Akt and FoxO1 were not affected in two sets of experiments (Fig. 3D).

In order to characterize the role of ARFRP1 for the Golgi structure we stained some Golgi proteins in 3T3-L1 adipocytes after transfection with scrambled and *Arfrp1*-specific siRNA. In control cells (*si-scrambled*), the *trans*-Golgi marker TGN38 was colocalized with GLUT4 in the perinuclear region. In contrast, TGN38 was nearly not detectable after downregulation of *Arfrp1* when GLUT4 was located at the cell surface (Supplementary Fig. 2A). In addition, ARL1 (ARF-like 1) which has been described to associate with the *trans*-Golgi only in the presence of *Arfrp1* [4] was detected in the perinuclear compartment in control cells, whereas it was rarely located at the Golgi apparatus after downregulation of *Arfrp1* (Sup-

plementary Fig. 2B, upper panel). In contrast, distribution of the *cis*-Golgi marker p115 was not affected in 3T3-L1 adipocytes transfected with *Arfrp1*-specific siRNA (Supplementary Fig. 2B, lower panel).

In order to confirm that GLUT4 is recruited from its intracellular compartment in the absence of ARFRP1 we employed the 'sheet assay' [19] which allows the isolation of very pure plasma membranes that are not contaminated with intracellular membranes and organelles. The high degree of purity is demonstrated by the very low immunoreactivity of GLUT4 in membranes obtained from unstimulated 3T3-L1 cells while stimulation with insulin results in an increase of the GLUT4 signal as shown for untransfected 3T3-L1 cells (Fig. 4A). In order to elucidate the effect of *Arfrp1* depletion for plasma membrane localization of GLUT4 we performed the sheet assay with scrambled siRNA and *Arfrp1*-specific siRNA-transfected 3T3-L1 cells under basal conditions. Fig. 4B (upper panel) shows the results of two from in total four experiments demonstrating that the GLUT4 content in plasma membranes is higher after suppression of *Arfrp1* expression than in cells transfected with control siRNA. The specificity of the effect on GLUT4 localization is demonstrated by detection of GLUT1 (Fig. 4B, middle panel). The loading control as shown for GAPDH signals indicates that equal amounts of plasma membrane lawn were used in the Western blots (Fig. 4B, lower panel). The results clearly demonstrate that specifically GLUT4 but not GLUT1 is sorted in a different way when ARFRP1 is absent. However, in comparison to untransfected cells (Fig. 4A, first lane) the amount of GLUT4 in the plasma membrane is higher when cells were transfected with scrambled siRNA.

4. Discussion

The present data demonstrate that deletion of the ARF-like GTPase *Arfrp1* in adipose cells resulted in marked alterations of *trans*-Golgi structures and a mistargeting of GLUT4. *In-vitro* studies performed in 3T3-L1 adipocytes confirmed that sequestration of GLUT4 in an intracellular compartment was altered in the absence of *Arfrp1*.

We and others have previously shown that ARFRP1 is required for correct targeting of some proteins. In the absence of ARFRP1 the GTPase ARL1 was dislocated from *trans*-Golgi membranes to the

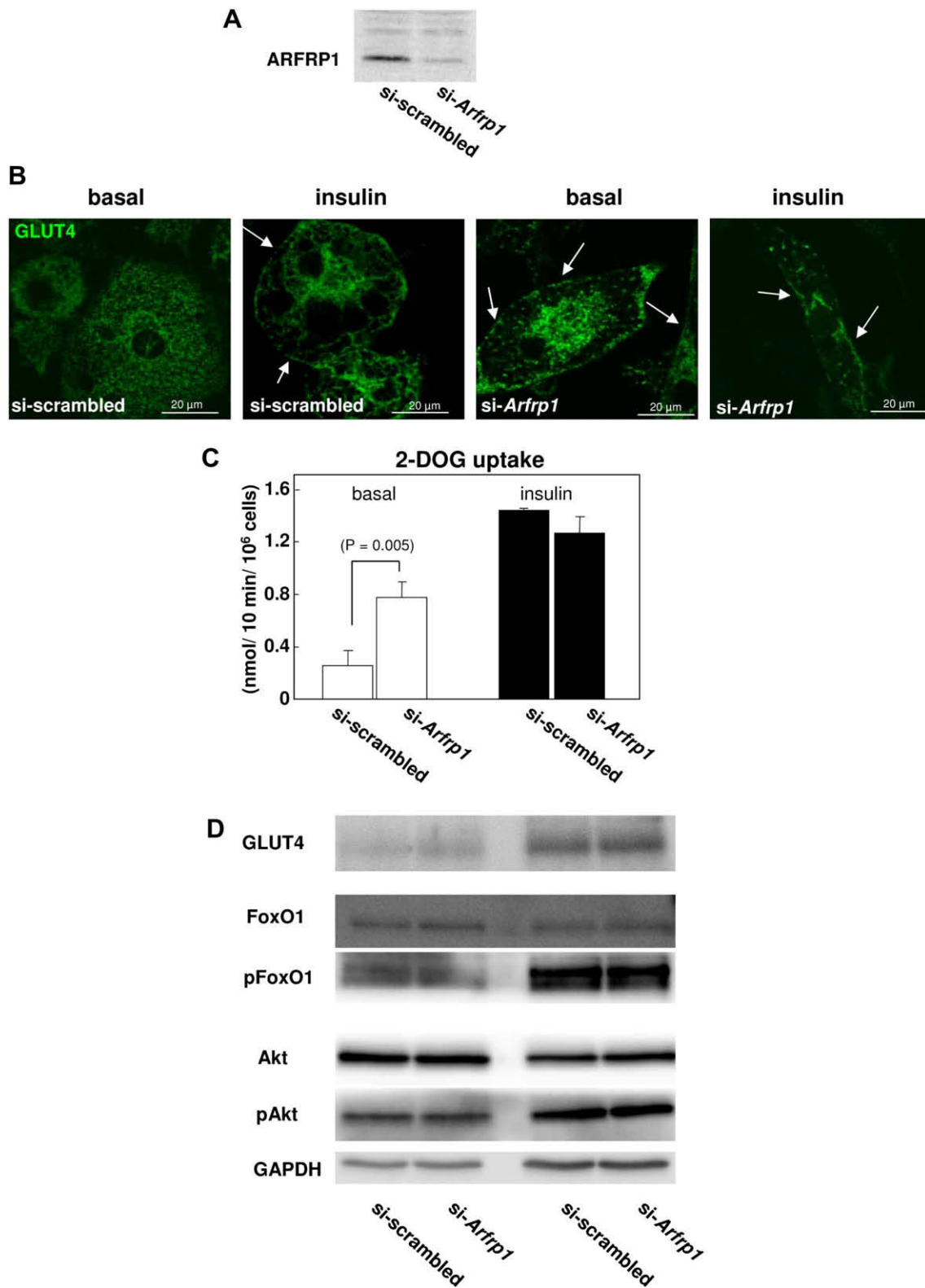


Fig. 3. Suppression of *Arfrp1* expression in 3T3-L1 adipocytes by siRNA alters GLUT4 distribution and glucose transport. 3T3-L1 adipocytes were electroporated with scrambled or *Arfrp1*-specific siRNA and 72 h later, (A) ARFRP1 expression was analyzed by Western blotting and (B) subcellular distribution of GLUT4 was examined immunohistochemically by confocal laser scanning microscopy and (C) glucose transport was detected under basal and insulin-stimulated conditions by assessment of labeled deoxyglucose uptake, respectively. (D) Lysates of transfected 3T3-L1 adipocytes were analyzed by Western blotting with the indicated antibodies.

cytosol [4–6]. Mice lacking *Arfrp1* died during early gastrulation [7,8] due to defective E-cadherin targeting to the plasma membrane which resulted in adhesion defects. Here we demonstrated

that in adipocytes targeting of the insulin-sensitive glucose transporter GLUT4 was affected in the absence of ARFRP1. At early stages after deletion of *Arfrp1* in adipocytes (*Arfrp1*^{ad-/-} embryos

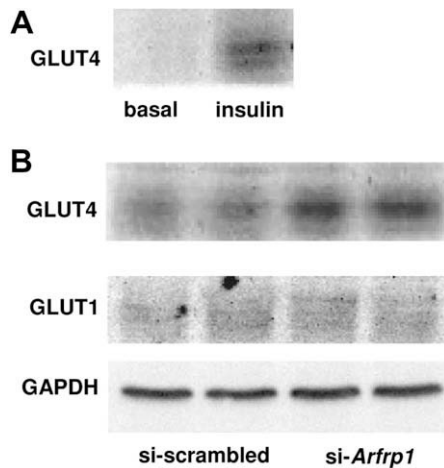


Fig. 4. Effect of downregulation of *Arfrp1* on GLUT4 protein in plasma membranes in 3T3-L1 adipocytes. 3T3-L1 adipocytes were electroporated with scrambled or *Arfrp1*-specific siRNA and 72 h later, cells were incubated with serum-free medium for 2 h and stimulated with or without 250 nM insulin for 20 min at 37 °C. Untransfected (upper panel) or with siRNA electroporated (lower panel) 3T3-L1 adipocytes were stimulated with and without (basal) insulin and plasma membrane sheets were prepared and analyzed by Western blotting with the anti-GLUT4 antibody. The experiment was repeated four times.

at ED 18.5), GLUT4 protein specifically accumulated at the plasma membrane rather than being sequestered in an intracellular compartment (Fig. 2). A similar altered distribution of GLUT4 was produced by siRNA-mediated knockdown of *Arfrp1* in 3T3-L1 adipocytes (Fig. 3B). A plasma membrane localization of GLUT4 in *Arfrp1* knockdown 3T3-L1 adipocytes was also evident by measuring elevated basal glucose transport rates (Fig. 3C) and an increased immunoreactivity obtained in isolated plasma membranes (Fig. 4). The cell surface localization of GLUT4 could be the result of (i) an insulin stimulation which induces the translocation of GLUT4 to the plasma membrane, or (ii) defects in GLUT4 trafficking. An increase in insulin levels as the trigger of GLUT4 translocation is unlikely, as embryonic BAT was exposed to the maternal environment of normoglycemic and normoinsulinemic mothers. Since inhibition of *Arfrp1* expression in fully differentiated 3T3-L1 adipocytes also resulted in plasma membrane localization of GLUT4 (Fig. 3B), increased glucose uptake under basal conditions (Fig. 3C), and elevated GLUT4 levels in purified plasma membranes (Fig. 4) we conclude that cell surface localization of GLUT4 is a consequence of defects in Golgi structure as detected in *Arfrp1*^{ad-/-} adipocytes.

The Golgi and *trans*-Golgi network (TGN) are major sorting compartments for proteins and membrane traffic [21]. Important biosynthesis processing steps of the insulin-regulated glucose transporter GLUT4 are believed to be initiated in the sorting compartments of the TGN [22]. Peripheral Golgi proteins such as golgin-160 [23] and golgin-p115 [24] have been described to be required for correct intracellular tethering of GLUT4. Williams et al. [23] who downregulated golgin-160 in differentiated 3T3-L1 adipocytes observed a similar mistargeting of GLUT4 as detected in cells lacking ARFRP1. Thus it was assumed that in the absence of golgin-160, GLUT4 was targeted to the plasma membrane by default, bypassing the sorting to the insulin-responsive compartment [23]. These data are consistent with a scenario in which ARFRP1 is a crucial component of golgin targeting. Mistargeting of GLUT4 has to be a consequence of specific Golgi alterations. Treatment of adipocytes with the fungal metabolite brefeldin A (BFA) for instance did not affect the intracellular sequestration of GLUT4 or its insulin-dependent translocation to the plasma membrane [25] indicating that not alterations of Golgi structures *per se* result in a cell surface location of GLUT4.

The finding that Golgi structures of *Arfrp1*^{ad-/-} adipocytes exhibited pronounced alterations of the *trans*-Golgi (Fig. 1B) is consistent with previous conclusions derived from *in vitro* studies with heterologous expression systems. We and others have shown previously that downregulation of ARFRP1 in HeLa cells by shRNA disrupted the recruitment of the GTPase ARL1 and of its effector golgin-245 to Golgi membranes [4–6]. Golgin proteins are coiled-coil proteins which are required for the structure of the Golgi apparatus in eukaryotic cells. A number of recent studies reveal a common theme for the targeting of golgins containing the ARL-binding GRIP domain [26]. Similarly, other golgins such as the vesicle tethering factor p115 are targeted by the Rab GTPases, Rab1 and Rab6, respectively. Together, golgins and their regulatory GTPases form a complex network, commonly known as the Golgi matrix, which organizes Golgi membranes and regulates membrane trafficking [27]. The present data support this concept, and provide definitive proof for the essential role of ARFRP1 in Golgi function.

In conclusion, the present data demonstrate that deletion of the GTPase *Arfrp1* severely alters GLUT4 sorting as well as Golgi structures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2010.03.059.

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