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The death effector domain-containing DEDD forms a complex with Akt and Hsp90, and supports their stability

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Abstract

Insulin secretion and glucose transport are the major mechanisms to balance glucose homeostasis. Recently, we found that the death effector domain-containing DEDD inhibits cyclin-dependent kinase 1 (Cdk1) function, thereby preventing Cdk1-dependent inhibitory phosphorylation of S6 kinase 1 (S6K1), downstream of phosphatidylinositol 3-kinase (PI3K), which overall results in maintenance of S6K1 activity. Here we newly show that DEDD forms a complex with Akt and heat-shock protein 90 (Hsp90), and supports the stability of both proteins. Hence, in DEDD$^{-/-}$ mice, Akt protein levels are diminished in skeletal muscles and adipose tissues, which interferes with the translocation of glucose transporter 4 (GLUT4) upon insulin stimulation, leading to inefficient incorporation of glucose in these organs. Interestingly, as for the activation of S6K1, suppression of Cdk1 is involved in the stabilization of Akt protein by DEDD, since diminishment of Cdk1 in DEDD$^{-/-}$ cells via siRNA expression or treatment with a Cdk1-inhibitor, increases both Akt and Hsp90 protein levels. Such multifaceted involvement of DEDD in glucose homeostasis by supporting both insulin secretion (via maintenance of S6K1 activity) and glucose uptake (via stabilizing Akt protein), may suggest an association of DEDD-deficiency with the pathogenesis of type 2 diabetes mellitus.

Keywords

DEDD; Akt; glucose uptake; Cdk1

Introduction

The signalling cascade involving mitogen-related phosphatidylinositol 3-kinase (PI3K), Akt and their downstream TOR (target of rapamycin) is the central pathway that maintains glucose homeostasis in the body [1–4]. In mammals, upon stimulation by growth factors including insulin, the mammalian TOR (mTOR) cooperates with PI3K-dependent effectors to activate p70 ribosomal protein S6 kinase 1 (S6K1), thereby phosphorylating the 40S-ribosomal protein

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S6, and subsequently enhances translation of the 5'-terminal oligopyrimidine (5'-TOP) sequences that encode components of the translational machinery. This reaction increases the number of ribosomes and the efficacy of protein synthesis, thus critically promoting growth of types of cells including insulin-producing β cells within the pancreatic Langerhans islet [5–8]. The insulin mass was diminished in S6K1-deficient ($S6K1^{-/-}$) mice, resulting in ineffective secretion of insulin upon glucose administration [9]. Thus, S6K1 is involved in the machinery controlling glucose tolerance by supporting the size of β cells [10,11]. On the other hand, activation of Akt (in particular Akt2, the primary isoform in insulin-responsive tissues) induces translocation of glucose transporter 4 (GLUT4) to the plasma membrane [12–15]. This response is responsible for glucose transport into cells. Thus, dysfunction of these elements provokes a phenotype similar to type 2 diabetes mellitus, which is a multifactorial disease with a variety of pathological defects in glucose homeostasis [16–18].

Recently, we defined the DEDD molecule as a critical element that maintains the activity of S6K1, thereby supporting the size of β cells and insulin mass in mice [19]. DEDD was initially described as a member of the death effector domain (DED)-containing protein family [20]. We previously found that DEDD is associated with the Cdk1/cyclin B1 complex, and that it decreases the kinase activity of Cdk1 [21]. This response impedes the Cdk1-dependent mitotic program to shut off synthesis of ribosomal RNA (rRNA) and protein, and is consequently useful in gaining sufficient cell growth [21,22]. Interestingly, DEDD also associates with S6K1, and interferes with the Cdk1-dependent inhibitory phosphorylation of S6K1 at several serine/threonine (Ser/Thr) residues, including Ser411 and Ser424 sites within the auto-inhibitory tail [19,23,24]. This response maintains the activity of S6K1 preserving a high level of phosphorylation at Thr389, a hallmark of active S6K1 [19]. Hence in $DEDD^{-/-}$ mice, the activity of S6K1 was reduced in various cell types, and as observed in $S6K1^{-/-}$ mice, the insulin mass within pancreatic islets is reduced, resulting in overt glucose intolerance [19].

Having discovered the functional association of DEDD with S6K1, we here address a possible interaction of DEDD with Akt, and investigate a novel involvement of DEDD in the regulation of the insulin signaling cascade.

Material and methods

Mice

$DEDD^{-/-}$ mice [21] had been backcrossed to C57BL/6 (B6) for 17 generations before used for experiments. Mice are maintained under a SPF condition.

Antibodies

Antibodies used are: anti-total Akt (clone 11E7), anti-Akt phosphorylated at Thr308 (clone 244F9) (all are from Cell Signaling Technology, Beverly, MA); anti-Hsp90 (clone SPA-830) and anti-Cdk1 (clone A17) (from Stressgen, Victoria, BC, Canada, and Zymed laboratories Inc. South San Francisco, CA).

Glucose Incorporation

This assay was performed as described previously [25] with some modifications. Pieces from epididymal white fat pads and the soleus muscles of the mice were used. To determine 2-DG uptake, the muscles and fat pads were transferred to buffer A containing 1mM 2-DG (0.5 μCi/ml 2-deoxy-D-[1-^{14}C]glucose) and 1mM L-glucose (5μCi/ml L-[1-^{3}H]glucose) with or without 10nM insulin and incubated at 30°C for 10 min. After the reaction is terminated, the samples were neutralized with 5N HCl and dissolved in ACSII (Amersham Biosciences). ^{14}C and ^{3}H specific activities were counted by a liquid scintillation counter (Packard Instrument Co.).

GLUT4 translocation

Primary MEF cells prepared from DEDD$^{+/+}$ or DEDD$^{-/-}$ embryos were differentiated to adipocytes according to established protocols [26]. GLUT4 translocation assay was performed as previously described [26,27], using a GFP-fused GLUT4 expression vector [27]. Confluent primary MEF cells prepared from DEDD$^{+/+}$ or DEDD$^{-/-}$ mice were induced to differentiate by incubating the cells with DMEM containing 10μg/ml of insulin (Sigma), 1μM of dexamethasone (Sigma), and 500μM of isobutylmethylxanthine (Sigma). After 48 hours, the cells were fed with DMEM containing 10μg/ml of insulin every 2 days prior to use. On 8–10 days after induction of differentiation, cells were transfected with the construction cording GFP-GLUT4-myc [27]. Cells were serum-starved for 4 hours, and then incubated with or without 200nM of insulin for 30 min. GFP-GLUT4 trafficking was followed by a fluorescent microscope. A ratio for GFP-GLUT4 distribution within peri-membrane areas to peri-nuclear areas was measured using NIH Image.

Na_3VO_4 treatment

DEDD$^{+/+}$ or DEDD$^{-/-}$ MEF cells were enriched in metaphase by a treatment with 500nM nocodazole (Sigma) for 24 hours. Cells were further incubated with 10mM Na_3VO_4 for an additional 6 hours, and lysed in SDS sample buffer. The cell extracts were subjected to Western blotting using anti-Akt or anti-Hsp90 antibodies.

Protein degradation and phosphorylation assay

Subconfluent DEDD$^{+/+}$ or DEDD$^{-/-}$ MEF cells were cultured accordingly and harvested at indicated time points after treatment with 100μg/ml cyclohexamide with or without 10μM MG-132 and 0.4mM Chroloquine, lysed with Briji 97 lysis buffer supplemented with 5mM iodoacetamide, 5μg/ml leupeptin, 0.2mM AEBSF, 1mM Na_3VO_4 and 10mM NaF. Lysates were resolved onto SDS-PAGE, and immunoblotted anti Akt antibody.

siRNA transfection

Double strands siRNA targeting DEDD or Cdk1 were purchased from Applied Biosystems or SIGMA, respectively. Wild-type MEF cells at 50% confluency were transfected with 10 μM siRNA using Lipofectamine 2000 (Invitrogen Inc.). Forty-eight hours after the transfection, the cells were harvested and analyzed by Western blotting or RT-PCR.

Sequences of the oligonucleotides were as follows: DEDD siRNA#1: 5’-

GCCCTGATCTTGTAGACAATT-3’, DEDD siRNA#2: 5’-

AAATGGACGTGACTTCTTATT-3’, Cdk1 siRNA#1: 5’-

CTATGATCCTGCCAAACGATT-3’, Cdk1 siRNA#2: 5’-

GTTGTTTACCGTTGGCTCTTT-3’, Cdk1 siRNA#3: 5’-

CAATCAAACTGGCTGATTTTT-3’. For a control, an oligonucleotide targeting GFP sequence (Sigma) was used.

Primers for RT-PCR

Primers used are as follows. for: forward primer; rev: reverse primer.

Hsp90α for : 5’ GCGGCAAAGACAAGAAAAAG 3’; Hsp90α rev: 5’

CAAGTGGTCCTCCCAGTCAT 3’; Hsp90β for: 5’ CTGGGTCAAGCAGAAAGGAG 3’;

Hsp90β rev: 5’ TCTCTGTTGCTTCCCGACTT 3’; Akt1 for: 5’

CCACGCTACTTCCTCCTC 3'; Akt1 rev: 5' TGCCCTTGCCAACAGTCTGAAGCA 3';

Akt2 for: 5' GTCGCCAACAGTCTGAAGCA 3'; Akt2 rev: 5'

GAGAGAGGTGGAAAAACAGC 3'; G3PDH for: 5' ACCACAGTCCATGCCATCAC 3';

G3PDH rev: 5' TCCACCACCCTGTTGCTGTA 3'; β-actin for: 5'

GTGGCTACAGCTTCACCACCACAG 3'; β-actin rev: 5'

GCATCCTGTCAGCAATGCCTGGGT 3'; DEDD for: 5'

GCGGGATCCGCGGGCCTAAAGAGGC 3'; DEDD rev: 5'

GCGTCTAGAGTCTACAAGATCAGGGC 3'

Quantification of Immuno-blots

Quantification of the Immuno-blots was performed using the NIH-Image. Relative phosphorylation levels to those in control (shown as 1.0±S.E.M.) are presented. For all immuno-blotting experiments, at least three independent blotting were performed.

Statistical Analysis

A two-tailed Mann-Whitney test was used to calculate P-values. (**): $P < 0.01$, (*): $P < 0.05$. Error bars: s.e.m.

Results

Lack of DEDD decreases the amount of Akt protein

Having observed reduced activity of S6K1 in the absence of DEDD [19], we wondered whether upstream of S6K1 in the insulin signalling pathway might also be influenced by the lack of DEDD, and thus assessed the situation of Akt protein in $DEDD^{-/-}$ mouse embryonic fibroblast (MEF) cells by Western blotting. To our surprise, the amount of Akt was also greatly decreased in $DEDD^{-/-}$ MEF cells compared with $DEDD^{+/+}$ MEF cells, when tested by using an antibody that detects all isotypes of Akt (Fig. 1A, total Akt). Signals for activated Akt phosphorylated at Thr308 residue were also reduced, along with the diminished amounts of total Akt protein (Fig. 1A, p-Akt). A reduction in the amount of Akt as well as in the Thr308 phosphorylation level, was also clear in $DEDD^{-/-}$ skeletal muscles and adipose tissues, where Akt plays an important role in the regulation of glucose homeostasis [12,13] (Fig. 1B). The effect of an acute loss of DEDD on Akt was also assessed by knocking down DEDD in cells. As presented in Fig. 1C, downregulation of DEDD expression by introducing a double-stranded siRNA for DEDD into wild-type MEF cells significantly reduced the amount of Akt. Consistent with these observations, the activating phosphorylation (at Ser2448) of mTOR, downstream of Akt, was decreased in $DEDD^{-/-}$ compared with $DEDD^{+/+}$ cells (Fig. 1D, left). In contrast, phophorylation levels of 3-phosphoinositide-dependent protein kinase-1 (PDK1), which phosphorylates Akt, were comparable in the presence or absence of DEDD, suggesting that the less phophorylation of Akt in the absence of DEDD is mainly caused by a reduction in the total amount of Akt protein (Fig. 1E). Together, the lack of DEDD decreases the amount of all types of Akt protein both in MEF cells and in tissues from $DEDD^{-/-}$ mice, which is accompanied with lower Akt activity. This result also indicates that in the absence of DEDD, the reduction in Akt activity may partly be responsible for the decreased S6K1 activity, in addition to the increased phophorylation levels at the inhibitory residuesof S6K1 brought about by the hyper activity of Cdk1 [19].

DEDD forms a complex with Akt and Hsp90, and stabilizes these proteins

Although the amount of Akt protein is markedly reduced, mRNA for both Akt1 and Akt2 were expressed at similar levels in DEDD$^{-/-}$ and DEDD$^{+/+}$ tissues and cells (Fig. 2A). This result suggests that DEDD may be necessary for the maintenance of Akt protein. To test this possibility, we measured the half-life of Akt protein in DEDD$^{-/-}$ and DEDD$^{+/+}$ MEF cells. Importantly, the amount of Akt protein was decreased in 10 hours in DEDD$^{-/-}$ cells, but not in DEDD$^{+/+}$ cells (Fig. 2B). The presence of MG132, a proteasome inhibitor, tempered the reduction observed in DEDD$^{-/-}$ cells (Fig. 2B). Thus, the lack of DEDD results in instability of Akt protein.

Several groups reported that heat shock protein 90 (Hsp90), a chaperone required for the conformational maturation of certain signalling proteins, forms a complex with Akt and is involved in its stabilization [28,29]. Thus, we assessed the protein levels of Hsp90 in DEDD$^{+/+}$ and DEDD$^{-/-}$ MEF and tissues. As depicted in Fig. 2C, the amount of Hsp90 protein also decreased in skeletal muscle, adipose tissue, as well as in MEF cells from DEDD$^{-/-}$ mice compared with those from DEDD$^{+/+}$ mice, whereas the transcripts of *Hsp90* (both α and β) genes were at an equivalent level in both types of mice (Fig. 2D). Furthermore, as depicted in Fig. 2E, immunoprecipitation assays revealed that DEDD associates with Akt (both 1 and 2) and Hsp90. Together, DEDD appears to facilitate a stable complex with Akt and Hsp90, supporting the levels of these proteins.

Suppression of Cdk1 increases Akt protein levels in DEDD$^{-/-}$ cells

As we demonstrated in a previous report, DEDD modulates the activity of S6K1 partly via suppressing Cdk1 activity [19]. To assess whether the inhibitory effect of DEDD on Cdk1 is also involved in stabilizing Akt protein, we knocked down Cdk1 in DEDD$^{-/-}$ MEF cells by introducing double-stranded siRNA for Cdk1, and analyzed the Akt and Hsp90 protein levels. As demonstrated in Fig. 3A, the levels of both proteins increased in DEDD$^{-/-}$ cells when Cdk1 protein was diminished. In addition, treatment of DEDD$^{-/-}$ MEF cells with sodium orthovanadate (VO_4), which is commonly used to inactivate Cdk1 [24], significantly increased the levels of both Akt and Hsp90 (Fig. 3B). These data suggest that in DEDD$^{-/-}$ cells, the increase in Cdk1 activity appeared to be responsible to the instability of Akt protein.

Attenuated glucose incorporation in DEDD$^{-/-}$ skeletal muscles and adipose tissues

One of a variety of functions for Akt is the regulation of incorporation of glucose into cells in response to insulin [30–32]. It is well known that translocation of GLUT4 to the plasma membrane upon insulin stimulation is a key mechanism of glucose transport into cells [12, 13], and that this translocation of GLUT4 is dependent on activation of Akt, in particular Akt2 [33–35]. Therefore, we assessed how the reduction of the amount of Akt caused by the absence of DEDD affects glucose uptake in mice. As shown in Fig. 4A, the uptake of glucose by skeletal muscle (soleus muscles) and adipose tissue in response to insulin was significantly damaged in DEDD$^{-/-}$ mice. We also tested GLUT4 translocation in response to insulin, using DEDD$^{-/-}$ and DEDD$^{+/+}$ adipocytes differentiated from MEF cells. The increase of GLUT4 on the cell membrane after an insulin challenge was significantly less in DEDD$^{-/-}$ compared to DEDD$^{+/+}$ cells (Fig. 4B). Hence, diminished levels of Akt correlated with inefficient induction of GLUT4 translocation, resulting in deficient glucose transport into cells in DEDD$^{-/-}$ skeletal muscle and adipose tissue.

Interestingly, however, Akt levels were almost comparable in the liver in DEDD$^{-/-}$ and DEDD$^{+/+}$ mice, in contrast to levels in skeletal muscle and adipose tissue (Fig. 4C). As the endogenous DEDD expression level was lower in the liver compared with that in the skeletal muscle (Fig. 4D), the loss of DEDD might not strongly influence Akt stability in the liver as it did in the skeletal muscle or adipose tissue.

Discussion

In addition to our previous report in which DEDD maintains the activity of S6K1 supporting the insulin mass within pancreatic islets, our current study has demonstrated that DEDD stabilizes Akt protein, leading to efficient glucose transport into skeletal muscles and adipose tissues. Thus, DEDD is involved in the insulin signalling pathway at diverse levels (summarized in Fig. 4E). As type 2 diabetes mellitus is a multifactorial disease [17], our findings suggest that DEDD deficiency might play a certain role in the pathology of type 2 diabetes mellitus. Indeed, the defect in glucose transport observed in $DEDD^{-/-}$ mice is one of the essential pathogenic features in type 2 diabetes mellitus. Evidence has also shown that a decrease in insulin secretion and reduced β cell mass do contribute to development of the disease [16,18].

It is interesting that the decrease in Akt protein levels was variable in different $DEDD^{-/-}$ tissues important for glucose homeostasis; *i.e*. it was prominent in the skeletal muscles and adipose tissues, but was not very significant in the liver. This might cause a variable aberrancy in glucose transport in different organs in $DEDD^{-/-}$ mice. Additional experiments such as to test the insulin sensitivity in each tissue, will test this possibility. It may be noteworthy that such a variation in insulin sensitivity in different tissues is often seen in human patients [16–18]. It will also be important to address whether any dysfunction of DEDD is present, either in the whole body or in specific tissues, in a subset of type 2 diabetes patients.

The precise mechanism of how the association of DEDD with Akt and Hsp90 supports the stability of these proteins, is still unclear. The DEDD's effect on Akt stability appears to be achieved through Cdk1. As we previously demonstrated, increased Cdk1 activity in the absence of DEDD accelerates the phophorylation levels at the inhibitory residues of S6K1, resulting in a reduction of S6K1 activity [19]. A similar scenario might also be true for Akt (and/or Hsp90), although so far, inhibitory phosphorylation sites are not known either in Akt or Hsp90. Otherwise, Cdk1 might phosphorylate and activate some ubiquitin ligase(s) that degenerate Akt. As reviewed by Hunter [36], multiple crosstalks between phosphorylation and ubiquitination occur differentially during the protein degradation. Phosphorylation can regulate ubiquitination of a protein in different manners. Firstly, phosphorylation positively or negatively regulates the activity of the E3 ligase responsible for ubiquitine transfer. It is possible that Cdk1 may phosphorylate some E3 ligase(s) responsible for ubiquitination and degradation of Akt. Indeed, some E3 ligases involved in degradation of Akt, including recently identified TTC3 [37], require phophorylation for their activation. Whilst, activity of CHIP, a major E3 ligase responsible for ubiquitination of Akt [38,39], might be modified directly or indirectly by Cdk1-depedent phosphorylative events, though the direct phosphorylation of CHIP has not been reported. On the other hand, it is also known that phosphorylation also promotes recognition of substrates by an E3 ligase [40]. However, phosphorylation events of Akt promoted by Cdk1 have not yet demonstrated. Alternatively, the possibility that formation of DEDD/Akt/Hsp90 might structurally stabilize these participant proteins is not mutually excluded. Thus, the molecular linkage among DEDD, Cdk1 and Akt-degradation still remains to be further addressed..

Conclusions

In summary, we newly demonstrated that DEDD plays an important role in maintenance of the Akt protein level, which in consequence supports the efficient incorporation of glucose into skeletal muscles and adipose tissues. Further investigations might find an unknown relevance of DEDD to the insulin signalling pathway, and thus, with a novel pathogenesis of type 2 diabetes mellitus.

Abbreviations

DEDD	death effector domain-containing DNA binding protein
Cdk1	cyclin-dependent kinase 1
S6K1	S6 kinase 1
PI3K	phosphatidylinositol 3-kinase
rRNA	ribosomal RNA
TOR	target of rapamycin
mTOR	mammalian TOR
5’-TOP	5’-terminal oligopyrimidine
Thr	Threonine
Ser	Serine
MEF	mouse embryonic fibroblast
PDK1	phosphoinositide-dependent protein kinase-1
Hsp90	heat-shock protein 90
GLUT4	glucose-transporter 4

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REFERENCES

1. Jacinto E, Hall MN. Tor signalling in bugs, brain and brawn. Nat. Rev. Mol. Cell Biol 2003;4:117–126. [PubMed: 12563289]
2. Oldham S, Hafen E. Insulin/IGF and target of rapamycin signaling: a TOR de force in growth control. Trends Cell Biol 2003;13:79–85. [PubMed: 12559758]
3. Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. Mol. Pathol 2001;54:133–137. [PubMed: 11376123]
4. Vogt PK. PI 3-kinase, mTOR, protein synthesis and cancer. Trends Mol. Med 2001;7:482–484. [PubMed: 11689313]
5. Fingar DC, Blenis J. Target of rapamycin (TOR): an integrator of nutrient and growth factor signals and coordinator of cell growth and cell cycle progression. Oncogene 2004;23:3151–3171. [PubMed: 15094765]
6. Huang S, Houghton PJ. Targeting mTOR signaling for cancer therapy. Curr. Opin. Pharmacol 2003;3:371–377. [PubMed: 12901945]
7. Shamji F, Nghiem P, Schreiber SL. Integration of growth factor and nutrient signaling: implications for cancer biology. Mol. Cell 2003;12:271–280. [PubMed: 14536067]
8. Sawyers, VivancoCL.; Vivanco; Sawyers, CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat. Rev. Cancer 2002;2:489–501. [PubMed: 12094235]
9. Pende M, Kozma SC, Jaquet M, et al. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. Nature 2000;408:994–997. [PubMed: 11140689]
10. Dann SG, Selvaraj A, Thomas G. mTOR Complex1-S6K1 signaling: at the crossroads of obesity, diabetes and cancer. Trends. Mol. Med 2007;13:252–259. [PubMed: 17452018]

11. Um SH, D'Alessio D, Thomas G. Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. Cell Metab. Cell Metab 2006;3:393–402.
12. Hill MM, Clark SF, Tucker DF, et al. A role for protein kinase Bbeta/Akt2 in insulin-stimulated GLUT4 translocation in adipocytes. Mol. Cell Biol 1999;19:7771–7781. [PubMed: 10523666]
13. Huang S, Czech MP. The GLUT4 glucose transporter. Cell Metab 2007;5:237–252. [PubMed: 17403369]
14. Summers SA, Garza LA, Zhou H, Birnbaum MJ. Regulation of insulin-stimulated glucose transporter GLUT4 translocation and Akt kinase activity by ceramide. Mol. Cell Biol 1998;18:5457–5464. [PubMed: 9710629]
15. Whiteman EL, Cho H, Birnbaum MJ. Role of Akt/protein kinase B in metabolism. Trends Endocrinol. Metab 2002;13:444–451. [PubMed: 12431841]
16. Bell GI, Polonsky KS. Diabetes mellitus and genetically programmed defects in beta-cell function. Nature 2001;414:788–791. [PubMed: 11742410]
17. DeFronzo RA. Pathogenesis of type 2 diabetes: metabolic and molecular implications for identifying diabetes genes. Diabetes Rev 1997;5:177.
18. Kahn BB, Rossetti L. Type 2 diabetes--who is conducting the orchestra? Nat. Genet 1998;20:223–225. [PubMed: 9806535]
19. Kurabe N, Arai S, Nishijima A, et al. The death effector domain-containing DEDD supports S6K1 activity via preventing Cdk1-dependent inhibitory phosphorylation. J. Biol. Chem 2009;284:5050–5055. [PubMed: 19106089]
20. Stegh H, Schickling O, Ehret A, et al. DEDD, a novel death effector domain-containing protein, targeted to the nucleolus. EMBO J 1998;17:5974–5986. [PubMed: 9774341]
21. Arai S, Miyake K, Voit R, et al. The death-effector domain containing protein DEDD is a novel mitotic inhibitor requisite for cell growth. Proc. Natl. Acad. Sci. USA 2007;104:2289–2294. [PubMed: 17283331]
22. Miyazaki T, Arai S. Two distinct controls of mitotic Cdk1/cyclin B1 requisite for cell growth prior to cell division. Cell Cycle 2007;6:1419–1425. [PubMed: 17592253]
23. Papst PJ, Sugiyama H, Nagasawa M, et al. Cdc2-cyclin B phosphorylates p70 S6 kinase on Ser411 at mitosis. J. Biol. Chem 1998;273:15077–15084. [PubMed: 9614117]
24. Shah OJ, Ghosh S, Hunter T. J. Biol. Chem 2003;278:16433–16442. [PubMed: 12586835]
25. Kamei N, Tobe K, Suzuki R, et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. J. Biol. Chem 2006;281:26602–26614. [PubMed: 16809344]
26. Bogan S, McKee AE, Lodish HF. Insulin-responsive compartments containing GLUT4 in 3T3-L1 and CHO cells: regulation by amino acid concentrations. Mol. Cell Biol 2001;21:4785–4806. [PubMed: 11416153]
27. Jiang ZY, Chawla A, Bose A, et al. A phosphatidylinositol 3-kinase-independent insulin signaling pathway to N-WASP/Arp2/3/F-actin required for GLUT4 glucose transporter recycling. J. Biol. Chem 2002;277:509–515. [PubMed: 11694514]
28. Basso D, Solit DB, Chiosis G, et al. Akt forms an intracellular complex with heat shock protein 90 (Hsp90) and Cdc37 and is destabilized by inhibitors of Hsp90 function. J. Biol. Chem 2002;277:39858–39866. [PubMed: 12176997]
29. Solit DB, Basso AD, Olshen AB, et al. Inhibition of heat shock protein 90 function down-regulates Akt kinase and sensitizes tumors to Taxol. Cancer Res 2003;63:2139–2144. [PubMed: 12727831]
30. Brazil DP, Yang ZZ, Hemmings BA. Advances in protein kinase B signalling: AKTion on multiple fronts. Trends Biochem. Sci 2004;29:233–242. [PubMed: 15130559]
31. Manning BD, Cantley LC. AKT/PKB Signaling: Navigating Downstream. Cell 2007;129:1261–1274. [PubMed: 17604717]
32. Du K, Tsichlis PN. Regulation of the Akt kinase by interacting proteins. Oncogene 2005;24:7401–7409. [PubMed: 16288287]
33. Cho H, Mu J, Kim JK, Thorvaldsen JL, et al. Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). Science 2001;292:1728–1731. [PubMed: 11387480]

34. Garofalo RS, Orena SJ, Rafidi K, et al. Severe diabetes, age-dependent loss of adipose tissue, and mild growth deficiency in mice lacking Akt2/PKB beta. J. Clin. Invest 2003;112:197–208. [PubMed: 12843127]

35. Ng Y, Ramm G, Lopez JA, James DE. Rapid activation of Akt2 is sufficient to stimulate GLUT4 translocation in 3T3-L1 adipocytes. Cell Metab 2008;7:348–356. [PubMed: 18396141]

36. Hunter T. The age of crosstalk: phosphorylation, ubiquitination, and beyond. Mol. Cell 2007;28:730–738. [PubMed: 18082598]

37. Suizu F, Hiramuki Y, Okumura F, et al. The E3 Ligase TTC3 Facilitates Ubiquitination and Degradation of Phosphorylated Akt. Dev. Cell 2009;17:800–810. [PubMed: 20059950]

38. Dickey CA, Koren J, Zhang Y, et al. Akt and CHIP coregulate tau degradation through coordinated interactions. Proc. Natl. Acad. Sci. USA 2008;105:3622–3627. [PubMed: 18292230]

39. Facchinetti V, Ouyang W, Wei H, et al. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. EMBO J 2008;27:1932–1943. [PubMed: 18566586]

40. Xiang T, Ohashi A, Huang Y, et al. Negative Regulation of AKT Activation by BRCA. Cancer Res 2008;168:10040–10044. [PubMed: 19074868]

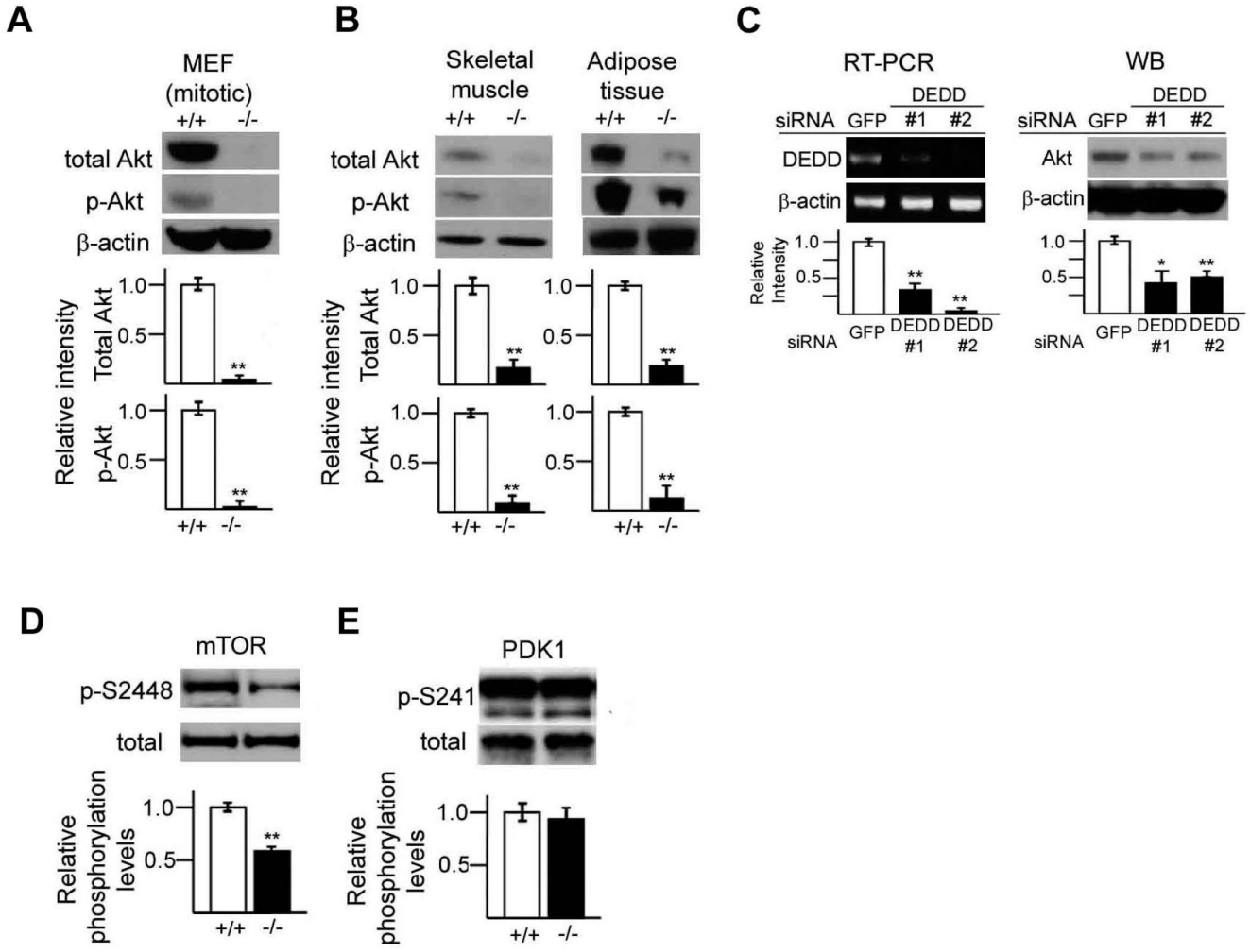

Figure 1. Reduced Akt protein amounts in the absence of DEDD

(A) Total Akt or phosphorylated (activated) Akt analyzed in DEDD$^{+/+}$ and DEDD$^{-/-}$ MEF cells, and (B) in tissues from DEDD$^{+/+}$ and DEDD$^{-/-}$ mice. The representative immuno-blots and the averages of relative intensities (+/+ as 1.0) from all experiments are presented. Error bar: S.E.M. (C) Increase of Akt levels by diminishment of DEDD using siRNA in DEDD$^{-/-}$ MEF cells. DEDD mRNA and Akt protein levels relative to those in control (shown as 1.0) are also presented. (D) Activative phosphorylation of mTOR (at Ser2448 site) and (E) PDK1 (at Ser241 site) in DEDD$^{+/+}$ or DEDD$^{-/-}$ MEF cells was analyzed by Western blotting. For PDK1, two bands appear when this polyclonal antibody (Cell signalling; #3061) is used as described in its data sheet provided from the company. The upper band is the phoshorylated PDK1. Three independent experiments (for both D and E) were performed.

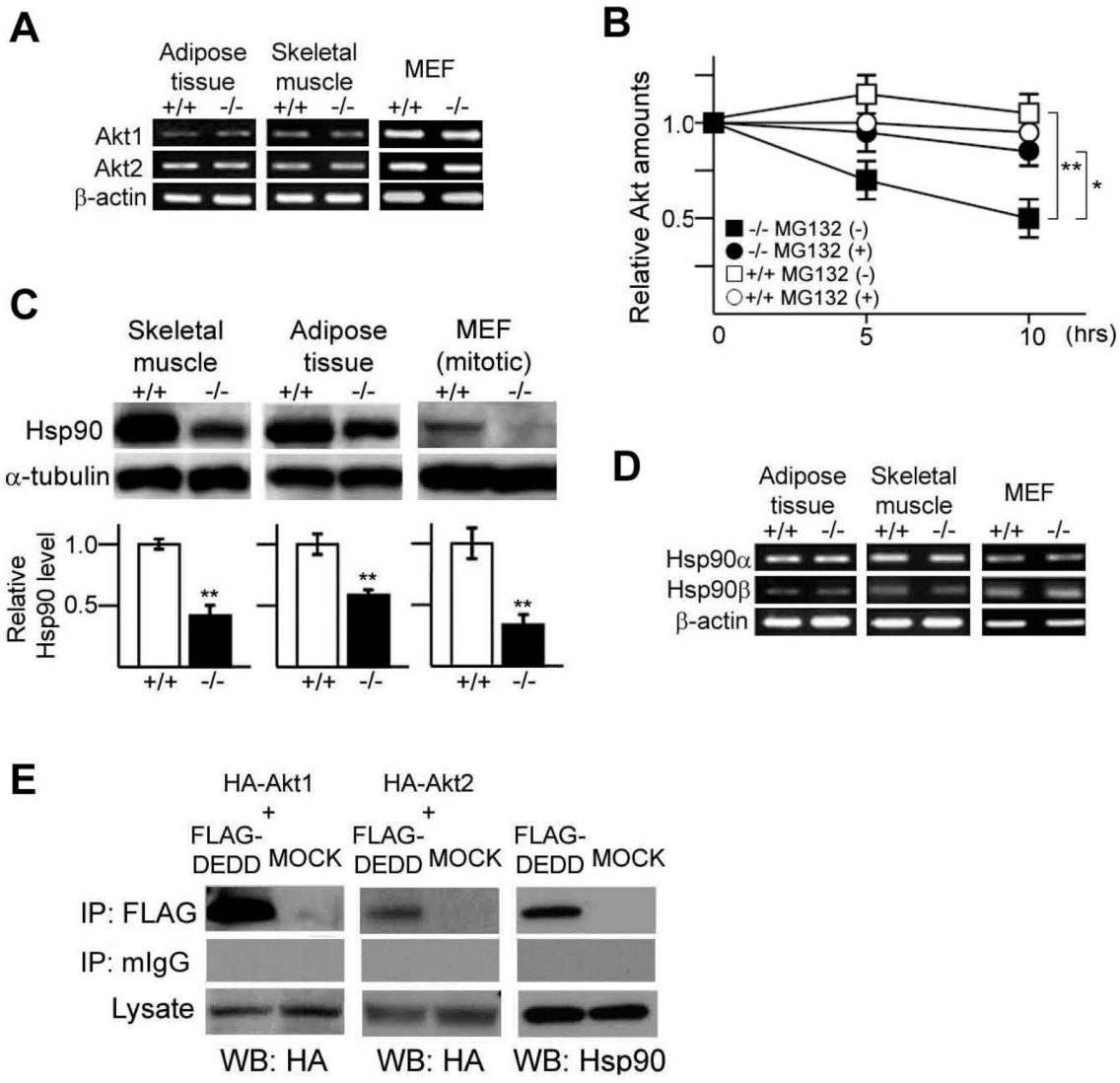

Figure 2. DEDD forms a complex with Akt and Hsp90 and supports the stability of these proteins

(A) RT-PCR for Akt1, Akt2, and β-actin as a control (performed within the linear range). (B) Protein degradation assay for Akt. Representative data out of comparable results obtained by two independent experiments is presented. (C) Hsp90 protein levels in DEDD$^{+/+}$ or DEDD$^{-/-}$ skeletal muscle, adipose tissue or mitotic MEF cells. (D) RT-PCR assay for Hsp90 mRNA. (E) Association of DEDD with Akt1, Akt2 and Hsp90. DEDD was precipitated from 293T cells expressing HA-tagged AKt1 or Akt2 with or without FLAG-DEDD, and the precipitates were analyzed for HA-Akt1 or 2 using an anti-HA antibody (left and middle). Likewise, endogenous Hsp90 was co-precipitated with FLAG-DEDD from 293T cells expressing FLAG-DEDD (right). Results from the control IP using a mouse-IgG (mIgG) are also demonstrated.

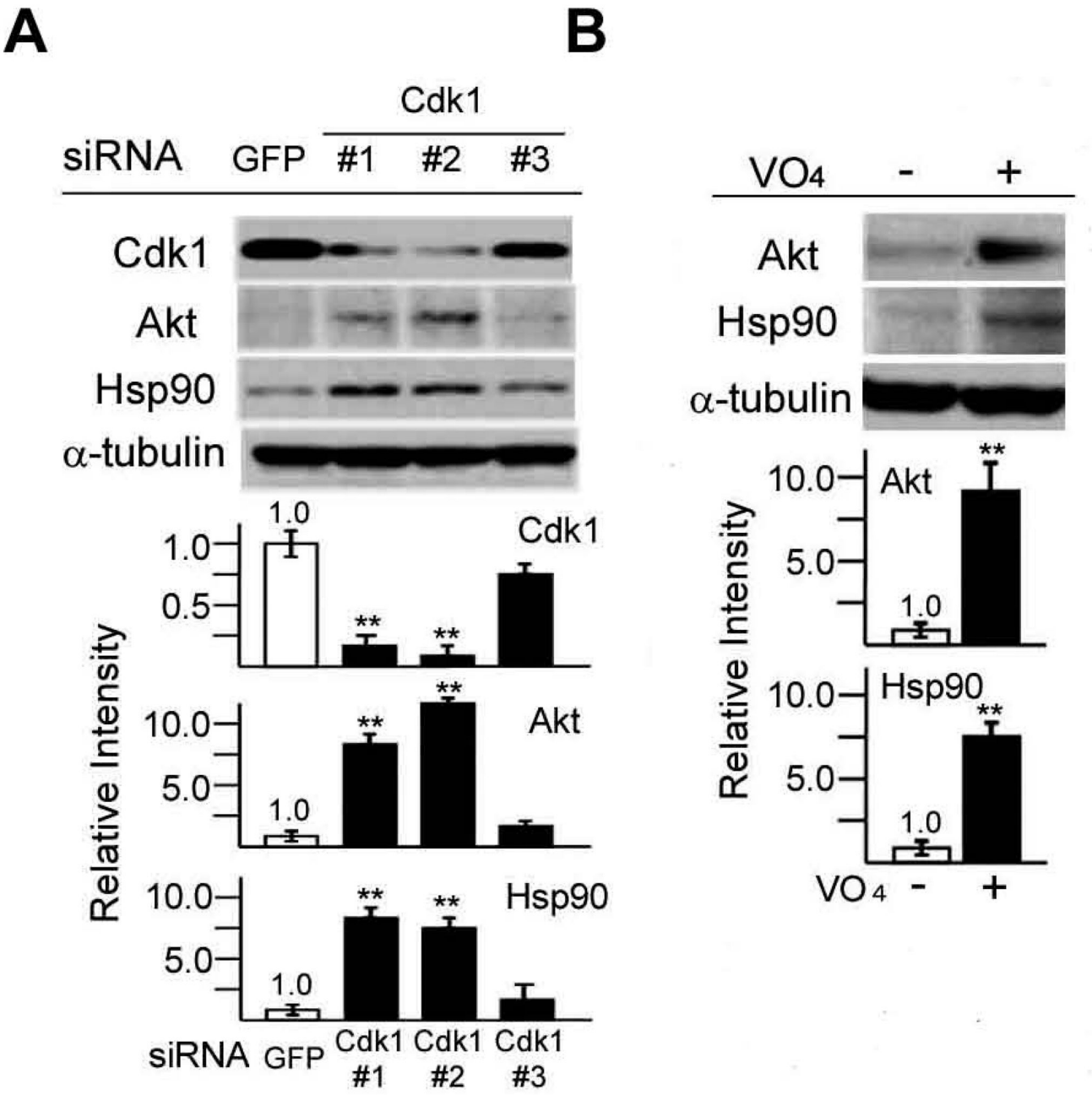

Figure 3. Involvement of Cdk1 in stabilization of Akt by DEDD

(A) Akt and Hsp90 levels in mitotic DEDD$^{-/-}$ MEF cells after treatment with siRNA targeting Cdk1 (three distinct sequences), or (B) with Na_3VO_4 (VO_4) for 6 hours. The levels of Akt and Hsp90 relative to those in control (shown as 1.0) are also presented.

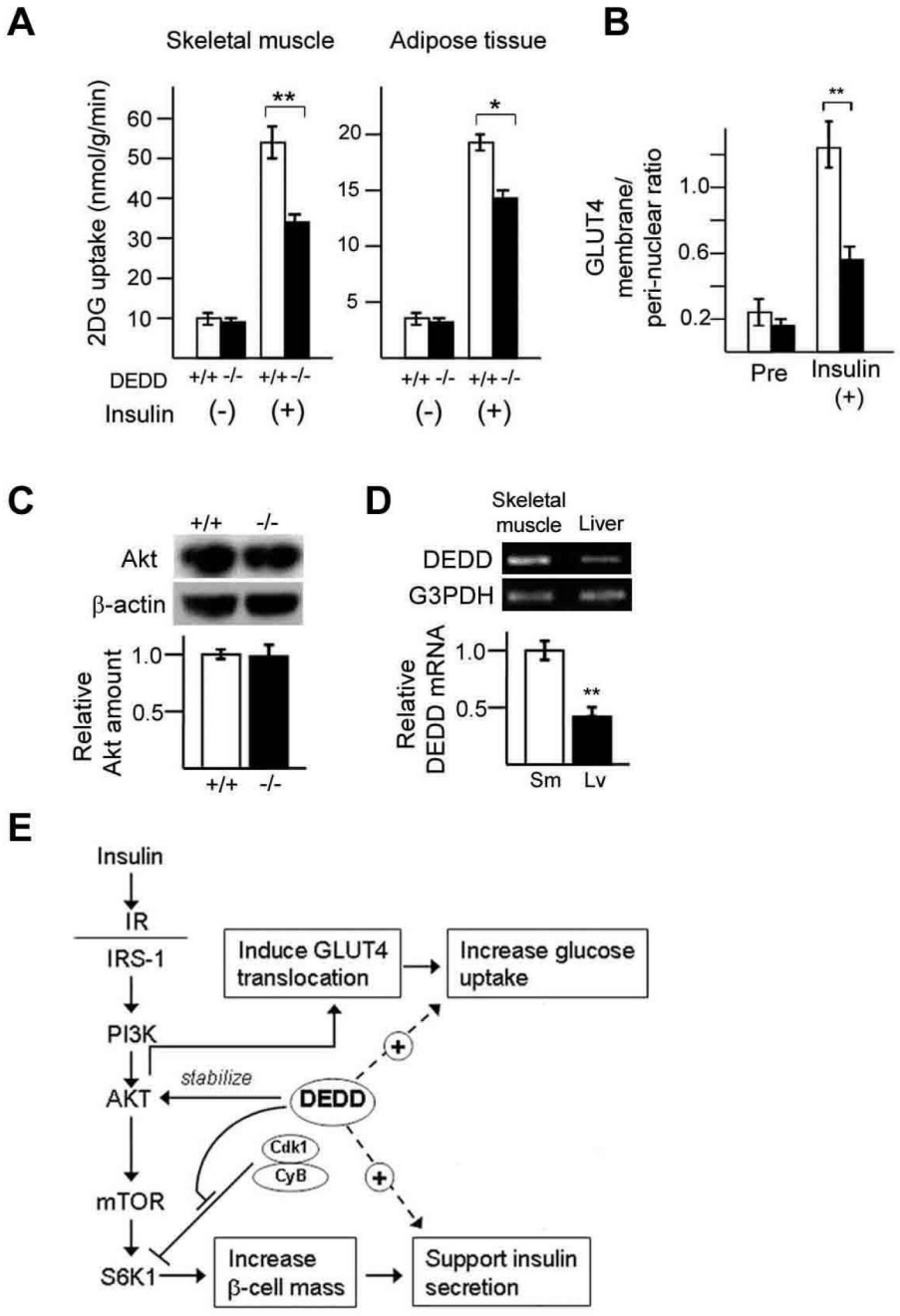

Figure 4. Defects in glucose incorporation in DEDD$^{-/-}$ tissues

(A) Glucose incorporation by skeletal muscle (left) or adipose tissue (right). (n=5 each). (B) Translocation of GLUT4 in DEDD$^{+/+}$ (white bars) or DEDD$^{-/-}$ (black bars) adipocytes. Data represent the relative amount of GFP-GLUT4 at the membrane locus to that at the peri-nuclear area. Data are the averages of results from 30 cells. (C) Akt protein levels in DEDD$^{-/-}$ and DEDD$^{+/+}$ liver. (D) DEDD mRNA level expressed in the liver (Lv) relative to that in the skeletal muscle (Sm) is presented (lower panel). (E) A scheme for the plausible involvement of DEDD in glucose homeostasis. IR: insulin receptor; CyB: cyclin B1. The + with a dashed line (that starts from DEDD) means positive effect.