LETTERS

Deficiency of a β-arrestin-2 signal complex contributes to insulin resistance

Bing Luan[1], Jian Zhao[1], Haiya Wu[3], Baoyu Duan[1], Guangwen Shu[1], Xiaoying Wang[4], Dangsheng Li[2], Weiping Jia[3], Jiuhong Kang[1] & Gang Pei[1,5]

Insulin resistance, a hallmark of type 2 diabetes, is a defect of insulin in stimulating insulin receptor signalling[1,2], which has become one of the most serious public health threats. Upon stimulation by insulin, insulin receptor recruits and phosphorylates insulin receptor substrate proteins[3], leading to activation of the phosphatidylinositol-3-OH kinase (PI(3)K)–Akt pathway. Activated Akt phosphorylates downstream kinases and transcription factors, thus mediating most of the metabolic actions of insulin[4–6]. β-arrestins mediate biological functions of G-protein-coupled receptors by linking activated receptors with distinct sets of accessory and effecter proteins, thereby determining the specificity, efficiency and capacity of signals[7–11]. Here we show that in diabetic mouse models, β-arrestin-2 is severely downregulated. Knockdown of β-arrestin-2 exacerbates insulin resistance, whereas administration of β-arrestin-2 restores insulin sensitivity in mice. Further investigation reveals that insulin stimulates the formation of a new β-arrestin-2 signal complex, in which β-arrestin-2 scaffolds Akt and Src to insulin receptor. Loss or dysfunction of β-arrestin-2 results in deficiency of this signal complex and disturbance of insulin signalling *in vivo*, thereby contributing to the development of insulin resistance and progression of type 2 diabetes. Our findings provide new insight into the molecular pathogenesis of insulin resistance, and implicate new preventive and therapeutic strategies against insulin resistance and type 2 diabetes.

We first investigated the expression pattern of β-arrestins in the *db*/*db* mouse model of type 2 diabetes. There was a decrease in β-arrestin-2 protein and messenger RNA levels in liver and skeletal muscle of *db*/*db* mice (Fig. 1a, b). Consistently, in dietary-induced insulin-resistant model mice (high-fat diet (HFD) mice)[12], expression of β-arrestin-2 also decreased in liver and skeletal muscle (Fig. 1c, d). Expression levels of β-arrestin-2 in adipose tissue (Fig. 1), brain or lung of *db*/*db* or HFD mice did not change (data not shown). β-arrestin-1 was downregulated in liver and skeletal muscle, but to a lesser extent than β-arrestin-2 (data not shown). We also found a similar downregulation of β-arrestins in liver from clinical samples of type 2 diabetes (eight pairs) (Supplementary Fig. 1). Changes in β-arrestin levels in these insulin-resistant mice and clinical samples raise the possibility that β-arrestins might have a role in insulin signalling and that its deficiency might contribute to insulin resistance and type 2 diabetes.

We then assessed the potential roles of β-arrestin-2 in whole-body insulin action and glucose metabolism. β-arrestin-2 knockout (β-arr2-KO) mice are viable and fertile, with normal body weight and food intake (Supplementary Fig. 2a). Their fasted blood glucose and insulin levels were normal, but re-fed blood glucose and insulin levels were much higher than wild-type littermates (Fig. 2a, b), suggesting a potential decrease in systemic insulin sensitivity. In fact, glucose tolerance tests (GTTs) revealed significant deterioration in glucose metabolism in β-arr2-KO mice (Fig. 2c). Insulin secretion in response to glucose load during GTTs was also higher in β-arr2-KO mice (Supplementary Fig. 2c), consistent with insulin tolerance tests (ITTs) which showed significantly decreased insulin sensitivity in β-arr2-KO mice (Fig. 2d). To define the role for β-arrestin-2 in modulating whole-body insulin sensitivity further, we performed hyperinsulinaemic–euglycaemic clamp studies. We found no significant difference in basal hepatic glucose production between β-arr2-KO mice and wild-type littermates, but clamp hepatic glucose production was significantly higher in β-arr2-KO mice (Supplementary Fig. 2d). Whole-body glucose disposal and infusion rates were significantly decreased in β-arr2-KO mice (Supplementary Fig. 2e). These results together demonstrate that β-arr2-KO mice exhibited impaired insulin sensitivity.

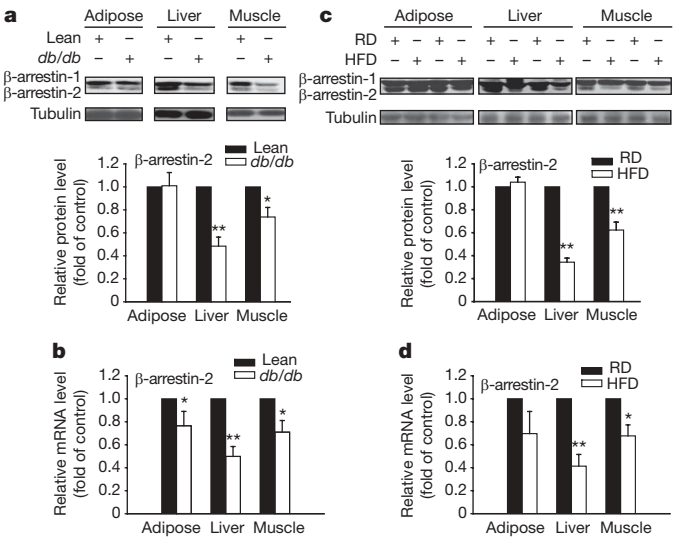

Figure 1 | Downregulation of β-arrestin-2 in diabetic mice. a, b, Immunoblot (**a**) and quantitative RT–PCR (**b**) of β-arrestin expression in adipose tissue, liver and skeletal muscle of lean ($n = 5$) and *db*/*db* mice ($n = 5$). Densitometric analysis is shown. **c, d,** Immunoblot (**c**) and quantitative PCR with reverse transcription (RT–PCR) (**d**) of β-arrestin expression in adipose tissue, liver and skeletal muscle of C57BL/6 mice fed with a regular diet (RD) ($n = 5$) or HFD ($n = 5$). Densitometric analysis is shown. Data are presented as mean and s.e.m. $*P < 0.05$, $**P < 0.005$, versus control.

[1]Laboratory of Molecular Cell Biology, Institute of Biochemistry and Cell Biology, and Graduate School of the Chinese Academy of Sciences, [2]Shanghai Information Center for Life Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 200031, Shanghai, China. [3]Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated Sixth People's Hospital; Shanghai Diabetes Institute; Shanghai Clinical Center of Diabetes, 200233, Shanghai, China. [4]Fudan University Affiliated Zhongshan Hospital, 200032, Shanghai, China. [5]School of Life Science and Technology, Tongji University, 200092, Shanghai, China.

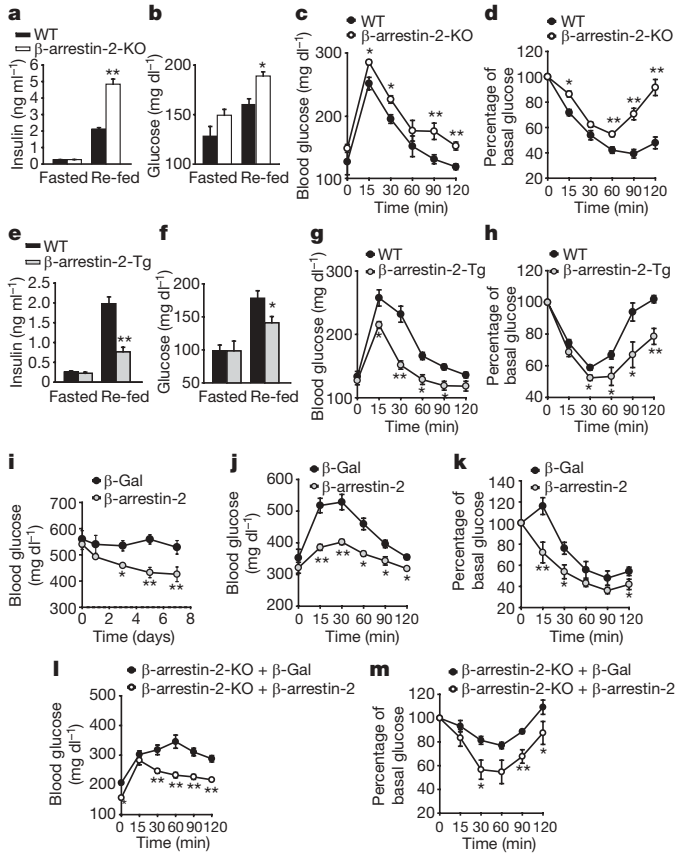

Figure 2 | β-arrestin-2 affects the development of insulin resistance. **a**, **b**, Serum insulin (**a**) and blood glucose (**b**) levels in β-arr2-KO mice ($n = 8$) and wild-type (WT) littermates ($n = 8$) under fasted and re-fed stages. **c**, **d**, Glucose levels during GTTs (1 g kg^{-1}) (**c**) and ITTs (1 U kg^{-1}) (**d**) in β-arr2-KO mice ($n = 10$) and wild-type littermates ($n = 8$). **e**, **f**, Serum insulin (**e**) and blood glucose (**f**) levels in β-arr2-Tg mice ($n = 8$) and wild-type littermates ($n = 8$) under fasted and re-fed stages. **g**, **h**, GTTs (1 g kg^{-1}) (**g**) and ITTs (0.75 U kg^{-1}) (**h**) in β-arr2-Tg mice ($n = 11$) and wild-type littermates ($n = 8$). **i**, Blood glucose concentration of *db*/*db* mice ($n = 8$) injected with indicated adenovirus. **j**, **k**, GTTs (1.5 g kg^{-1}) (**j**) and ITTs (1.5 U kg^{-1}) (**k**) in *db*/*db* mice at 7 days after adenovirus injection. **l**, **m**, GTTs (1.5 g kg^{-1}) (**l**) and ITTs (1.5 U kg^{-1}) (**m**) in β-arr2-KO mice fed on HFD at 7 days after adenovirus injection. Data are presented as mean ± s.e.m. $^{*}P < 0.05$, $^{**}P < 0.005$, versus control.

Transgenic mice expressing human β-arrestin-2 driven by the cytomegalovirus (CMV) promoter (β-arr2-Tg) had an approximate twofold increase in liver β-arrestin-2 expression compared with control mice (Supplementary Fig. 2b). β-arr2-Tg mice develop normally with normal food intake and body weight (Supplementary Fig. 2b). Their serum insulin and blood glucose levels were similar to wild-type mice in fasted stages but much lower in re-fed stages (Fig. 2e, f). GTTs and ITTs showed augmented glucose metabolism and insulin sensitivity in β-arr2-Tg mice (Fig. 2g, h, and Supplementary Fig. 2c). Similar results were obtained using β-arr2-KO, β-arr2-Tg and their wild-type littermates that had been fed on HFD (Supplementary Fig. 3), further suggesting a potential anti-diabetic role of β-arrestin-2.

We next explored the possibility that administration of β-arrestin-2 may have a therapeutic potential against insulin resistance and type 2 diabetes. To this end, we administered β-arrestin-2 in *db*/*db* mice by using adenovirus. Intravenous injection of recombinant adenovirus expressing β-arrestin-2 led to an approximate threefold increase of β-arrestin-2 protein levels in liver, without altering the food intake and body weight (Supplementary Fig. 2f, g). The blood glucose levels under fed conditions were reduced in *db*/*db* mice that received the β-arrestin-2 adenovirus, compared with control mice (Fig. 2i). Injection of β-arrestin-2 adenovirus ameliorated glucose tolerance and insulin sensitivity, as shown in GTTs and ITTs (Fig. 2j, k). Similarly, rescue of β-arrestin-2 expression by adenovirus injection in β-arr2-KO mice fed on HFD amended glucose intolerance and insulin resistance in these mice (Fig. 2l, m). These results indicate that β-arrestin-2 is a positive regulator of insulin sensitivity.

At the molecular level, insulin resistance results from defects in insulin signalling in peripheral tissues[4]. Interestingly, although activation of PI(3)K by insulin was similar in mice of all genotypes (Supplementary Fig. 4a), we observed different activities of Akt in liver of β-arr2-KO, β-arr2-Tg and wild-type mice, as monitored by *in vitro* kinase assay (Supplementary Fig. 4b). Further, insulin-stimulated phosphorylation of Akt, GSK3-β and Foxo1 were dramatically reduced in liver of β-arr2-KO mice but largely increased in β-arr2-Tg mice (Fig. 3a, b, and Supplementary Fig. 4c). Similar results were also observed in skeletal muscle and adipose tissue (Supplementary Fig. 4d–g). These results show that β-arrestin-2 promotes insulin-stimulated activation of Akt but does not affect PI(3)K activity.

Recent studies have shown that phosphorylation of Akt at Tyr 315/326 by Src enhances Akt serine/threonine phosphorylation and is a prerequisite of full Akt activation[13–17]. We have also observed that, in the presence of Src inhibitor PP2, tyrosine phosphorylation and serine/threonine phosphorylation of Akt were remarkably reduced in Hep3B hepatocytes (Supplementary Fig. 5a). Furthermore, Akt activity was reduced when Tyr 315/326 were substituted by Ala (Supplementary Fig. 5b). In mouse liver, we observed that insulin stimulation led to an approximate twofold increase in tyrosine phosphorylation of Akt in wild-type mice (Fig. 3c and Supplementary Fig. 5c), which was reduced by about 40% in β-arr2-KO mice and increased by about 1.5-fold in β-arr2-Tg mice (Fig. 3c and Supplementary Fig. 5c). Interestingly, knockdown of Src by short interfering RNA (siRNA) (Fig. 3d) in primary hepatocytes isolated from β-arr2-KO mice and their wild-type littermates abolished the difference in Akt activities (Fig. 3e) and moderately depressed the differential reduction on g6p/pepck expression (Supplementary Fig. 5d, e). The suppressive effect on hepatocytic glucose production conferred by β-arrestin-2 was also abolished in the presence of Src siRNA (Fig. 3f). Together, these results suggest that β-arrestin-2 promotes Akt activation and glucose metabolism through Src.

β-arrestins have been reported to function as adaptors and to promote the activation of various mitogen-activated protein kinases such as ERK1/2 and JNK3 (ref. 18). A similar scaffolding mechanism might also operate in insulin signalling. We found that in liver of C57BL/6 mice, endogenous β-arrestin-2, Akt and Src were co-purified with each other (Fig. 3g). However, the association between Src and Akt was dramatically reduced in liver samples from β-arr2-KO mice (Fig. 3h and Supplementary Fig. 6a). Conversely, Akt and Src interaction was remarkably enhanced in β-arr2-Tg mice (Fig. 3i and Supplementary Fig. 6b). These results strongly suggest that β-arrestin-2, Akt and Src form a complex *in vivo* and that β-arrestin-2 is essential for mediating the association of Akt with Src. Next, we examined whether the formation of this Akt/β-arrestin-2/Src signalling complex depends on insulin stimulation. Intraperitoneal administration of insulin triggered a marked increase in Akt/Src interaction as well as Akt/β-arrestin-2 association in livers of C57BL/6 mice (Fig. 3j and Supplementary Fig. 6c), implicating the promotion of Akt/β-arrestin-2/Src interaction by insulin stimulation. Furthermore, we found β-arrestin-2 interacts directly with insulin receptor (Supplementary Fig. 6d). β-arrestin-2 as well as Akt and Src associate with insulin receptor in a similar time-dependent manner (Fig. 3k and Supplementary Fig. 6e). Moreover, interaction of Akt and Src with insulin receptor depends on the expression level of β-arrestin-2 (Supplementary Fig. 6f, g), supporting the idea that β-arrestin-2 is essential for the association of Akt/Src with insulin receptor.

We examined a series of truncation mutants of β-arrestin-2 by immunoprecipitation assay. A β-arrestin-2 fragment comprising amino acids 1–185 interacts with Src as efficiently as wild-type β-arrestin-2 but completely loses the interaction with Akt (Fig. 4a).

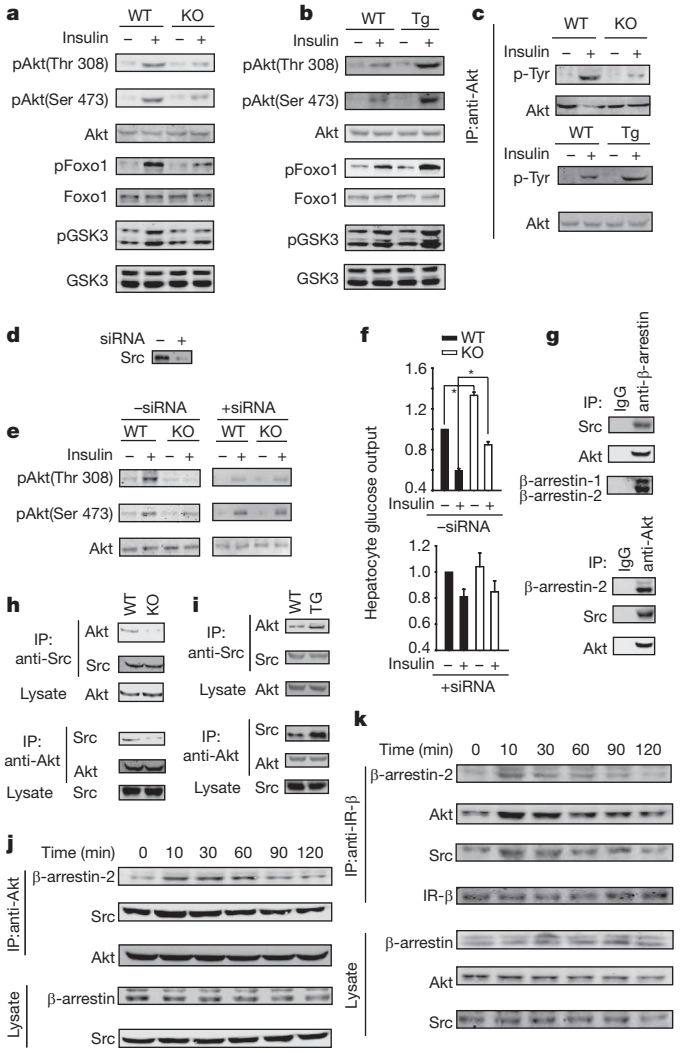

Figure 3 | Insulin stimulated the formation of Receptor/Akt/β-arrestin-2/Src signal complex. **a**, **b**, Activation of Akt in livers of β-arr2-KO mice ($n = 6$) (**a**), β-arr2-Tg mice ($n = 6$) (**b**) and their wild-type littermates ($n = 6$). Mice were injected with either saline or insulin (1 U kg^{-1}) for 10 min. **c**, Tyrosine phosphorylation of Akt immunopurified from livers of β-arr2-KO mice ($n = 6$), β-arr2-Tg mice ($n = 6$), and their wild-type littermates ($n = 6$). **d**, Suppression of Src in primary hepatocytes by Src siRNA. **e**, Akt activities in primary hepatocytes shown in **d** ($n = 3$). **f**, Glucose production treated with or without 10 nM insulin for 3 h in primary hepatocytes shown in **d** ($n = 3$). **g**, Interactions of Akt/β-arrestin-2/Src assayed by immunoprecipitation from livers of C57BL/6 mice ($n = 5$). **h**, **i**, Interaction of Akt and Src in liver extracts of β-arr2-KO mice ($n = 6$) (**h**), β-arr2-Tg mice ($n = 6$) (**i**), and their wild-type littermates ($n = 6$) were assayed by immunoprecipitation. **j**, Insulin induces formation of Akt/β-arrestin-2/Src complex. Immunoprecipitation was conducted in liver extracts of C57BL/6 mice ($n = 6$) injected with 1 U kg^{-1} insulin for the indicated times. **k**, Insulin stimulated interaction of Akt/β-arrestin-2/Src with insulin receptor ($n = 6$). Data are presented as mean and s.e.m. $^{*}P < 0.05$, $^{**}P < 0.005$, versus control.

Overexpression of β-arrestin-2 1–185 in Hep3B hepatocytes inhibited Akt/Src interaction (data not shown) and dramatically reduced insulin-stimulated Akt activation (Fig. 4b), presumably by competing with endogenous β-arrestin-2. Moreover, overexpression of β-arrestin-2 186–409, a truncated mutant that interacts with insulin receptor but not with Akt or Src (Fig. 4c) in Hep3B hepatocytes, dominant-negatively suppressed insulin-stimulated Akt activation (Fig. 4d). These results clearly demonstrate the pivotal role of β-arrestin-2 in scaffolding the active insulin receptor/Akt/β-arrestin-2/Src signalling complex after insulin stimulation.

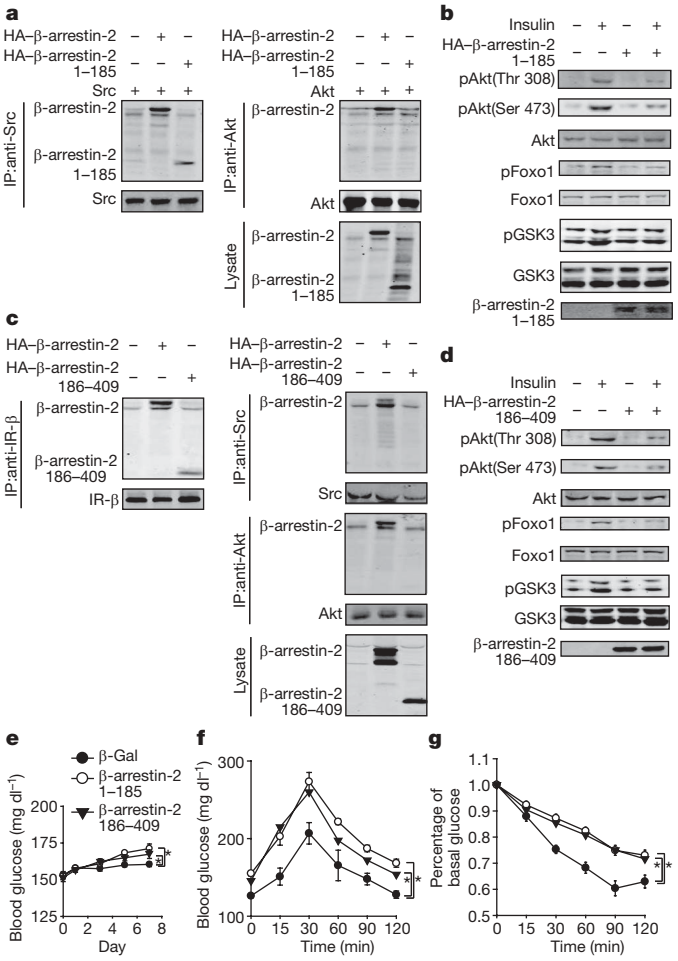

Figure 4 | Mutation of β-arrestin-2 contributes to insulin resistance *in vivo*. **a**, β-arrestin-2 1–185 interacts with Src but not Akt. Immunoprecipitation was conducted from Hep3B hepatocytes transfected with indicated plasmids ($n = 3$). **b**, β-arrestin-2 1–185 inhibits activation of Akt in Hep3B hepatocytes ($n = 3$). **c**, β-arrestin-2 186–409 interacts with insulin receptor-β but not Akt or Src in Hep3B hepatocytes ($n = 3$). **d**, β-arrestin-2 186–409 inhibits activation of Akt in Hep3B hepatocytes ($n = 3$). **e**, Blood glucose concentration of C57BL/6 mice ($n = 8$) injected with indicated adenovirus. **f**, **g**, GTTs (1 g kg^{-1}) (**f**) and ITTs (1 U kg^{-1}) (**g**) in C57BL/6 mice at 7 days after adenovirus injection. Data are presented as mean ± s.e.m. $^{*}P < 0.05$, $^{**}P < 0.005$, versus control.

We further tested whether expression of these β-arrestin-2 mutants would contribute to insulin resistance *in vivo*. Adenoviruses encoding β-Gal, β-arrestin-2 1–185 or β-arrestin-2 186–409 were injected intravenously into C57BL/6 or *db*/*db* diabetic mice. C57BL/6 mice infected with adenoviruses expressing β-arrestin-2 1–185 and β-arrestin-2 186–409 showed higher blood glucose levels than those of control mice (Fig. 4e). β-arrestin-2 1–185 and 186–409 adenovirus injection also deteriorated glucose tolerance and insulin sensitivity, as shown in GTTs and ITTs (Fig. 4f, g). Similar results were obtained in *db*/*db* diabetic mice (Supplementary Fig. 7).

Taken together, we have shown that assignment of Akt with Src and activated insulin receptor by β-arrestin-2 is essential for proper insulin signalling and whole-body insulin action. In contrast to the classical known pathway, β-arrestin-2 mediates activation of Akt through Src, which does not affect PI(3)K. However, this β-arrestin-2-Src pathway and PI(3)K pathway are not necessarily separated. One possibility could be that insulin receptor substrates and PI(3)K, as well as PDKs, may be involved in the signal complex, integrating these two pathways into one insulin-signalling network for efficient signal transduction (Supplementary Fig. 7d). Current and future investigations of the function and mechanism of this

novel signal complex will provide new insight into the understanding of insulin resistance and type 2 diabetes, and uncover potential molecular targets for treating metabolic diseases.

METHODS SUMMARY

Immunoprecipitation and immunoblotting. Mouse tissues were quickly excised and frozen in liquid nitrogen. Tissue lysate was prepared and used for immunoprecipitation and immunoblotting as described[19,20]. Blots were incubated with IRDyeTM800CW-conjugated secondary antibody. The image was captured and analysed by the Odyssey infrared imaging system and Scion Image (Li-Cor Bioscience).

mRNA analysis. We analysed β-arrestin mRNA levels by real-time PCR after reverse transcription as described[21]. Hypoxanthine–guanine phosphoribosyltransferase (HPRT) mRNA levels were used for normalization between samples.

GTTs and ITTs. For GTTs, mice were injected intraperitoneally with glucose after starvation for 6 h. Blood glucose was measured at indicated time points. We injected insulin intraperitoneally to mice under fed conditions for ITTs. We collected blood and determined the glycaemia using a glucometer (Roche Accu-chek). Serum insulin levels were measured using a rat/mouse enzyme-linked immunosorbent assay (ELISA) kit.

Statistical analysis. All data are shown as mean ± s.e.m. Measurements at single time points were analysed by analysis of variance (ANOVA) or, if appropriate, by Student's *t*-test. Time courses were analysed by repeated-measurements (mixed-model) ANOVA with Bonferroni post-tests.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 25 January; accepted 7 November 2008.
Published online 4 January 2009; corrected 26 February 2009 (details online).

1. Matthaei, S., Stumvoll, M., Kellerer, M. & Haring, H. U. Pathophysiology and pharmacological treatment of insulin resistance. *Endocr. Rev.* **21,** 585–618 (2000).
2. Taniguchi, C. M., Emanuelli, B. & Kahn, C. R. Critical nodes in signalling pathways: insights into insulin action. *Nature Rev. Mol. Cell Biol.* **7,** 85–96 (2006).
3. Sun, X. J. *et al.* Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. *Nature* **352,** 73–77 (1991).
4. Biddinger, S. B. & Kahn, C. R. From mice to men: insights into the insulin resistance syndromes. *Annu. Rev. Physiol.* **68,** 123–158 (2006).
5. Franke, T. F. *et al.* The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. *Cell* **81,** 727–736 (1995).
6. Burgering, B. M. & Coffer, P. J. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature* **376,** 599–602 (1995).
7. McDonald, P. H. *et al.* β-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science* **290,** 1574–1577 (2000).
8. Luttrell, L. M. *et al.* Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proc. Natl Acad. Sci. USA* **98,** 2449–2454 (2001).
9. Luttrell, L. M. *et al.* β-arrestin-dependent formation of β2 adrenergic receptor-Src protein kinase complexes. *Science* **283,** 655–661 (1999).
10. Beaulieu, J. M. *et al.* An Akt/β-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell* **122,** 261–273 (2005).
11. Beaulieu, J. M. *et al.* A β-arrestin 2 signaling complex mediates lithium action on behavior. *Cell* **132,** 125–136 (2008).
12. Yang, Q. *et al.* Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature* **436,** 356–362 (2005).
13. Chen, R. *et al.* Regulation of Akt/PKB activation by tyrosine phosphorylation. *J. Biol. Chem.* **276,** 31858–31862 (2001).
14. Jiang, T. & Qiu, Y. Interaction between Src and a C-terminal proline-rich motif of Akt is required for Akt activation. *J. Biol. Chem.* **278,** 15789–15793 (2003).
15. Craxton, A., Jiang, A., Kurosaki, T. & Clark, E. A. Syk and Bruton's tyrosine kinase are required for B cell antigen receptor-mediated activation of the kinase Akt. *J. Biol. Chem.* **274,** 30644–30650 (1999).
16. Wong, B. R. *et al.* TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. *Mol. Cell* **4,** 1041–1049 (1999).
17. Datta, K., Bellacosa, A., Chan, T. O. & Tsichlis, P. N. Akt is a direct target of the phosphatidylinositol 3-kinase. Activation by growth factors, v-src and v-Ha-ras, in Sf9 and mammalian cells. *J. Biol. Chem.* **271,** 30835–30839 (1996).
18. DeWire, S. M., Ahn, S., Lefkowitz, R. J. & Shenoy, S. K. β-arrestins and cell signaling. *Annu. Rev. Physiol.* **69,** 483–510 (2007).
19. Gao, H. *et al.* Identification of β-arrestin2 as a G protein-coupled receptor-stimulated regulator of NF-κB pathways. *Mol. Cell* **14,** 303–317 (2004).
20. Luan, B., Zhang, Z., Wu, Y., Kang, J. & Pei, G. β-arrestin2 functions as a phosphorylation-regulated suppressor of UV-induced NF-κB activation. *EMBO J.* **24,** 4237–4246 (2005).
21. Kang, J. *et al.* A nuclear function of β-arrestin1 in GPCR signaling: regulation of histone acetylation and gene transcription. *Cell* **123,** 833–847 (2005).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We are grateful to R. J. Lefkowitz for providing us with β-arr2-KO mice. We thank J.-L. Guan for discussions and comments on the manuscripts. We thank all members of the laboratory for sharing reagents and advice. This research was supported by the Ministry of Science and Technology (2005CB522406, 2006CB943900, 2007CB947904, 2007CB947100, 2007CB948000 and 2009CB941100), National Natural Science Foundation of China (30621091, 30625014, 30623003, 30871285 and 90713047), Shanghai Municipal Commission for Science and Technology (07PJ14099 and 06DZ22032), Chinese Academy of Sciences (KSCX2-YW-R-56 and 2007KIP204).

Author Contributions This study was designed by B.L., J.Z. and G.P. The experiments were performed by B.L., B.D. and G.S. H.W. and W.J. contributed to the hyperinsulinaemic–euglycaemic clamp experiments. X.W. provided type 2 diabetes clinic samples. G.P. supervised the project. B.L. and J.Z. contributed to the writing of the paper. D.L. helped with the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to G.P. (gpei@sibs.ac.cn).

METHODS

Mice. β-arr2-KO mice were provided by R. J. Lefkowitz (Duke University Medical Center). β-arr2-Tg mice were generated as described[22]. All other mice were from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. Mice were fed with a regular diet (Formulab 5008, Labdiet 5053) or high-fat diet (55% fat calories) (Harlan-Teklad 93075) and had free access to water and diet. We injected adenovirus (7×10^9 viral particles per 100 μl saline) into the tail vein of *db*/*db* and C57BL/6 mive specifically to target the liver. Body weight and food intake were measured as described[23]. Lean mice were 8 weeks old and fed with a regular diet (body weight 20.3 ± 1.2 g, food intake 3.5 ± 0.5 g), *db*/*db* mice were 8 weeks old and fed with a regular diet (body weight 38.5 ± 1.4 g, food intake 4.8 ± 0.7 g). Six-week-old C57BL/6 mice were fed on regular diet (body weight 22.6 ± 2.1 g, food intake 3.6 ± 0.3 g) or HFD (body weight 36.7 ± 1.9 g, food intake 4.1 ± 0.2 g) for an additional 8 weeks. All mice except for HFD feeding were 8 weeks old when experiments were conducted; *db*/*db* mice were 8 weeks old when they received adenovirus injection. GTTs and ITTs were conducted at 7 days after adenovirus injection.

Cell transfection and plasmids. Human hepatocyte Hep3B cells were transfected by LipofectAMINE (Invitrogen). For all transfection experiments, CMV-β-Gal was used to compensate the total DNA input. Full lengths of Akt and Src were cloned into modified pcDNA3 vector in-frame with HA or Flag at the amino (N) terminus. Akt Tyr315/326Ala was also cloned into modified pcDNA3 vector in-frame with Flag at the N terminus. Plasmids containing complementary DNA (cDNA) encoding β-arrestin-2 and its truncation mutants were generated as described[19]. The authenticity of the DNA sequences was confirmed by sequencing.

Materials and reagents. Anti-β-arrestin rabbit polyclonal antibody (A1CT and A2CT) was a gift from R. J. Lefkowitz (Duke University Medical Center). Antibodies directed against Akt (total), Akt (phosphorylated Thr308), Akt (phosphorylated Ser473), GSK3-α/β (phosphorylated Ser21/9), Foxo1 (total), Foxo1 (phosphorylated Ser256), Src, phosphorylated Tyr and insulin receptor were obtained from Cell Signalling. GSK3-α/β antibody was from Santa Cruz. PP2 and wortmannin were from Sigma. The Rat/Mouse Insulin ELISA Kit was from Linco Research. The PI3-Kinase ELISA Kit was from Echelon Biosciences. The Rat/Mouse Glucagon ELISA Kit, NEFA, triglycerides, and cholesterol detection kit were from WAKO Chemicals USA. The Mouse Epinephrine ELISA Kit was from USCN Life Science. The Akt Kinase Assay Kit was from Cell Signalling.

Adenovirus preparation and injection. We generated adenoviruses encoding Gal, β-arrestin-2, β-arrestin-2 1–185 and β-arrestin-2 186–409 using the adEasy system according to the manufacturer's instructions (Stratagene).

Quantitative PCR coupled with RT–PCR. The primer pairs used were: mouse β-arrestin-1 sense, 5′- AAGGGACACGAGTGTTCAAGA-3′; antisense, 5′-CCC GCT TTC CCA GGT AGA C-3′; mouse β-arrestin-2 sense, 5′-GGC AAG CGC GAC TTT GTA G-3′; antisense, 5′- GTG AGG GTC ACG AAC ACT TTC-3′; mouse HPRT sense, 5′-CCT GCT GGA TTA CAT TAA AGC ACT G-3′; antisense, 5′-TTC AAC ACT TCG AGA GGT CCT-3′.

Primary hepatocyte culture and glucose production. Primary hepatocytes were isolated and cultured after perfusion and collagenase digestion of the liver[24]. Glucose production in primary hepatocytes was measured as described[12], and a Glucose (GO) Assay Kit from Sigma was used to detect glucose concentration.

Hyperinsulinaemic–euglycaemic clamps. Hyperinsulinaemic–euglycaemic clamps were performed in 8-week-old mice as described[25,26]. Insulin was infused at 2.5 mU kg^{-1} min^{-1}.

22. Shi, Y. *et al.* Critical regulation of $CD4^+$ T cell survival and autoimmunity by β-arrestin 1. *Nature Immunol.* **8**, 817–824 (2007).
23. Netea, M. G. *et al.* Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nature Med.* **12**, 650–656 (2006).
24. Arkan, M. C. *et al.* IKK-β links inflammation to obesity-induced insulin resistance. *Nature Med.* **11**, 191–198 (2005).
25. Shen, H. Q., Zhu, J. S. & Baron, A. D. Dose-response relationship of insulin to glucose fluxes in the awake and unrestrained mouse. *Metabolism* **48**, 965–970 (1999).
26. Haluzik, M. M. *et al.* Improvement of insulin sensitivity after peroxisome proliferator-activated receptor-alpha agonist treatment is accompanied by paradoxical increase of circulating resistin levels. *Endocrinology* **147**, 4517–4524 (2006).