Obesity-linked insulin resistance is a major precursor to the development of type 2 diabetes. Previous work has shown that phosphorylation of PPARγ (peroxisome proliferator-activated receptor γ) at serine 273 by cyclin-dependent kinase 5 (Cdk5) stimulates diabetogenic gene expression in adipose tissues. Inhibition of this modification is a key therapeutic mechanism for anti-diabetic drugs that bind PPARγ, such as the thiazolidinediones and PPARγ partial agonists or non-agonists. For a better understanding of the importance of this obesity-linked PPARγ phosphorylation, we created mice that ablated Cdk5 specifically in adipose tissues. These mice have both a paradoxical increase in PPARγ phosphorylation at serine 273 and worsened insulin resistance. Unbiased proteomic studies show that extracellular signal-regulated kinase (ERK) kinases are activated in these knockout animals. Here we show that ERK directly phosphorylates serine 273 of PPARγ in a robust manner and that Cdk5 suppresses ERKs through direct action on a novel site in MAP kinase/ERK kinase (MEK). Importantly, pharmacological inhibition of MEK and ERK markedly improves insulin resistance in both obese wild-type and ob/ob mice, and also completely reverses the deleterious effects of the Cdk5 ablation. These data show that an ERK/Cdk5 axis controls PPARγ function and suggest that MEK/ERK inhibitors may hold promise for the treatment of type 2 diabetes.

Obesity is characterized by dysfunctional adipose tissues in which failure to store excess energy appropriately leads to ectopic lipid deposition, progressive insulin resistance and heightened risk for type 2 diabetes. Disordered secretion of certain fat-derived hormones, called adipokines, also contributes to the metabolic dysfunction in obesity and diabetes. Adipose-tissue-directed insulin-sensitizing drugs, including the thiazolidinediones, potently improve whole-body insulin sensitivity. The thiazolidinedione drugs have two distinct functions as ligands for PPARγ: they promote the differentiation of preadipocytes and they block the phosphorylation of PPARγ at S273 (ref. 1). We recently demonstrated that non-agonist PPARγ ligands capable of blocking the phosphorylation of PPARγ at S273 retain potent anti-diabetic effects despite the inability to promote adipogenesis. These findings strongly suggested that obesity-mediated phosphorylation of PPARγ at S273 may not only correlate positively with the development of insulin resistance but may also be causal to this state.

A variety of protein kinases participate in insulin action and insulin resistance. Insulin signalling activates the Akt/phosphoinositide 3-kinase (PI(3)K) and Grb2/Ras/MEK/ERK kinase cascades. Although much is known about the role of the former in promoting the canonical anabolic actions of insulin, studies in vitro had suggested that the latter cascade downstream of insulin signalling could contribute to insulin resistance, although there is controversy on this point. Obese rodents were shown to have elevated ERK activity, whereas mice lacking ERK1 were shown to be more sensitive to the effects of insulin.

Cdk5 function is both necessary and sufficient in cultured adipocytes to phosphorylate PPARγ at S273 (ref. 1). Mice with global or brain-restricted deletion of Cdk5 show increased perinatal mortality due to a defect in neurogenesis. We therefore set out to test whether modulation of PPARγ phosphorylation at S273 in adipose tissues would lead to altered insulin sensitivity in vivo by creating adipose-selective Cdk5-deficient mice (Cdk5-KO). In contrast to global knockouts, Cdk5-KO mice are grossly normal in appearance, with no apparent differences in body weight or fasting glucose levels when maintained on a standard diet (Extended Data Fig. 1). Deletion of Cdk5 in whole white adipose tissue was confirmed by both western blot analysis (Fig. 1a) and quantitative real-time PCR (Fig. 1b). To determine whether the residual Cdk5 expression in the knockout (KO) mice was emanating from non-adipocytes or from incomplete recombination, tissue fractionation was performed; no detectable Cdk5 protein was observed in the floating adipocyte fraction, whereas residual signal was observed in the stromal vascular fraction (Fig. 1c). On a standard chow diet, KO mice were normal, healthy and indistinguishable from Cdk5-Flox/Flox controls (Extended Data Fig. 1).

Both PPARγ S273 phosphorylation and insulin resistance are strongly promoted by obesity and inflammatory cytokines. When maintained on a high-fat diet to induce obesity, no differences were observed between wild-type (WT) and KO groups in food intake, energy expenditure or body weight (Fig. 1d and Extended Data Fig. 2). Paradoxically, metabolic analyses of these Cdk5-KO mice demonstrated that their glucose homeostasis was impaired in comparison with control animals. Cdk5-KO mice had elevated fasting insulin levels, as well as impairment in insulin tolerance, with a trend towards impaired glucose tolerance (Fig. 1e–h). We also observed a paradoxical increase in S273 PPARγ phosphorylation in obese Cdk5-KO mice, strongly suggesting compensation from an alternative protein kinase (Fig. 1i, j).

To understand how PPARγ S273 phosphorylation is increased in the absence of Cdk5, unbiased quantitative proteomic kinase profiling was performed on white adipose tissue (Fig. 2a). The most enriched protein kinase-derived peptide in KO mice (VADPDHHTGFLTEpY185VATR) corresponded to the activation loop of MAP kinase, ERK2/Mapk1 (Fig. 2b). We independently confirmed that ERK2 was activated in adipose tissue extracts from the KO mice by examining the phosphorylation of ERK2 at T183 and Y185, using phospho-specific antibodies against ERKs (Fig. 2c). We found no significant differences in the activation of alternative obesity-linked kinases (Extended Data Fig. 3). In addition, elevated T183/Y185 ERK phosphorylation was observed in adipocytes from Cdk5-KO mice that were differentiated for 8 days in vitro, suggesting that this phenomenon is cell-autonomous (Fig. 2d). A small-molecule inhibitor of Cdk5, roscovitine, also promotes ERK phosphorylation in cultured adipocytes, although here the competing inhibition of Cdk5 and activation of ERK had a net effect of leaving PPARγ S273 phosphorylation unchanged (Fig. 2e).

Because even the best available inhibitors of Cdk5, such as roscovitine, are not completely specific for Cdk5 (refs 17, 18), we also used a more precise means of regulating this kinase: an allele of Cdk5 specifically deleted in adipose tissues.
Figure 1 | Insulin resistance after Cdk5 deletion in adipocytes. a, b, Deletion of Cdk5 in epididymal white adipose tissue from control (Ctl) or adipocyte-specific knockout, KO (Cdk5<sup>flx/flx</sup>, adiponectin-Cre) was confirmed by western blotting (a; n = 4) or quantitative real-time PCR (b; n = 5). Ctl, control. c, Fractionated adipose tissue confirmed deletion was confined to the adipocyte fraction of adipose tissue. d, Body weight of control or KO mice when maintained on a high-fat diet. n = 20 Ctl, 25 KO. e, f, Fasting glucose (e) and fasting insulin (f) in mice maintained on a high-fat diet. n = 10 (control) and 12 (KO). g, h, Insulin tolerance test (g) and glucose tolerance tests (h) are consistent with impaired insulin sensitivity. n = 15 (control) and 17 (KO). Histograms show the areas under the curves. i, Western blots of white adipose tissue for pS273 PPAR<sub>γ</sub> in control and KO mice. j, Quantification of I. P < 0.05; **P < 0.01; ***P < 0.001; n.s., not significant. Error bars indicate s.e.m.

Figure 2 | Identification and characterization of ERK as a S273 PPAR<sub>γ</sub> kinase. a, ATP-probe-enriched phosphoproteomic analysis of kinases in control or Cdk5-KO adipose tissue from mice fed on a high-fat diet. Heat map indicating the most highly regulated phosphopeptide between control and Cdk5-KO (n = 3 per group; P = 0.08) corresponding to the peptides containing the activation loop of Mapk1/ERK2. b, Normalized quantification of the abundance of the phosphopeptide (left) and total abundance (right) corresponding to the activation loop of ERK2, determined by mass spectrometry. c, Western blot of ERK1 and ERK2 phosphorylation in brown adipose tissue from mice on a high-fat diet. d, Western blot of phospho-ERK1/2 in primary adipocytes differentiated in vitro for 8 days and serum starved for 18 h. e, Inhibition of Cdk5 by treatment with roscovitine for 6 h in cultured F442A adipocytes at the indicated doses. f, HEK 293 cells expressing WT Cdk5 or an analogue-sensitive (AS) mutant of Cdk5 were treated with the AS-specific inhibitor 1NMPP1 at 0, 0.1, 1.0 or 10 μM for 2 h. g, Cultured adipocytes stably expressing Cdk5-AS treated with the indicated doses of 1NMPP1. h, PPARγ in HEK 293 cells co-transfected with increasing doses of constitutively active ERK2 kinase (ERK-CA) or active Cdk5 (Cdk5 with p35). Western blotting was performed for both pS273 PPARγ and total PPARγ. i, Phosphorylated residues identified by LC-MS/MS after in vitro kinase assay of recombinant Cdk5, ERK2, or MEK2 incubated with full-length recombinant PPARγ. j, In vitro ERK kinase assay incubated with full-length recombinant PPARγ and increasing doses of pioglitazone before western blotting for pS273 and total PPARγ. NT, no treatment. Error bars indicate s.e.m.

Designed to be inhibited by the ‘bulky’ small molecule 1NMPP1. With the use of this previously validated approach<sup>19,20</sup>, a dose-dependent increase of ERK phosphorylation was observed after Cdk5 inhibition with 1NMPP1, in both fibroblasts and cultured adipocytes (Fig. 2f, g). Thus, we observed elevated levels of an activating ERK phosphorylation as a consequence of Cdk5 loss <i>in vivo</i> or <i>ex vivo</i>. 

We next investigated whether ERK kinase might be capable of directly compensating for Cdk5 deficiency in the phosphorylation of PPARγ. ERK and Cdk5 are structurally similar Ser/Thr kinases with a propensity for phosphorylating sites with proline in the +1 position<sup>21</sup>. In cultured cells, both constitutively active ERK (ERK-CA) and active Cdk5 (Cdk5<sub>γ</sub>) and its activating subunit p35 phosphorylated PPARγ at S273 (Fig. 2h). We confirmed that this was a direct effect by performing <i>in vitro</i> protein kinase assays on recombinant full-length PPARγ. Both ERK2 and Cdk5 resulted in the direct phosphorylation of S273 PPARγ, but a third kinase, MEK2, failed to phosphorylate this site (Fig. 2i). Cdk5 and ERK both phosphorylated the Ser-Pro sites S273 and S112 (Fig. 2i). The action of ERK on S112 has been reported previously by us and others<sup>22</sup>. A novel Cdk5-specific target site at T296 was also identified. In contrast, both ERK and MEK phosphorylated PPARγ at only one common site, S133. Because thiazolidinediones can block the ability of Cdk5 to phosphorylate S273 PPARγ (ref. 1), we sought to determine whether they would similarly block the action of ERKs. ERK phosphorylates both S112 and S273, but increasing concentrations of pioglitazone block phosphorylation only at S273 (Fig. 2j).

To determine how Cdk5 might be regulating ERK, we again turned to ATP probes and quantitative proteomics to identify Cdk5 substrates. Cdk5-deficient adipose tissue extracts were spiked with increasing doses of recombinant active Cdk5 kinase, plus p35 (Fig. 3a). MEK2, the kinase upstream of ERK, was identified as the protein with the single greatest dose-dependent increase in phosphorylation (Fig. 3b). The phosphopeptide identified contained two closely spaced potential phosphothreonine sites at T395 and T397 of mouse MEK2. Although Cdk5 has been reported to regulate MEK1 at T286, this site was not conserved in MEK2.
frequently found MEK2 modifications in the proteomic databases out-
and T397) has not previously been reported, yet they are the most
recombinant ERK protein.

To examine further the efficacy of targeting MEK and ERK pharma-

The most highly regulated peptide includes MEK2 residues T395 and T397.

We next asked a critical question: does inhibition of the MEK/ERK
pathway correct the metabolic defect evident in the Cdk5-KO mice? Mice
of both WT and KO genotypes maintained on a high-fat diet were
-treated with the well-characterized MEK inhibitor PD0325901 for 5 days
before a glucose tolerance test. Inhibition of MEK was able to normalize
glucose tolerance completely in these two groups, consistent with the
role of ERK as a key compensating kinase in Cdk5-deficient adipose
tissue (Fig. 4a–c). This occurred with no effect on adiposity (Extended
Data Fig. 5).

To gain a better understanding of the role of ERK in the pathophys-
ology of diabetes we examined insulin sensitivity by performing insulin
tolerance tests and hyperinsulinaemic–euglycaemic clamp experiments
on diet-induced obese wild-type C57Bl/6 mice. Mice treated with
PD0325901 showed a late divergence in glycaemia 90 min after admin-
istration of insulin, suggesting an exaggerated response to insulin action
(Extended Data Fig. 5c). Similarly, clamp studies revealed a twofold increase in the

culture block in vitro, Immunoprecipitation

We performed gene expression analysis to gain a better understand-
ing of the transcriptional basis of the improved glucose homeostasis
after MEK inhibition. We have previously defined a set of 17 genes that
are sensitive to PPARγ S273 phosphorylation in cultured adipose cells
and then further refined that gene set to 10 genes that were also regu-
lated by obesity in mice and responded to treatment with non-agonist
PPARγ ligands1. Although treatment with MEK inhibitors did not affect
the degree of obesity, expression of all of these 10 genes was significantly
regulated by treatment with one or both of the MEK inhibitors used in
this study (Fig. 5f). This included the genes encoding the circulating
insulin sensitivity factors adiponectin and adipisin, previously shown to
be most sensitive to phosphorylation of PPARγ at S273. The direction
of all of these changes was consistent with that predicted by the reversal
of S273 phosphorylation. Conversely, the MEK inhibitors had a min-
imal effect on the expression of genes linked to PPARγ agonism and
adipogenesis, including ap2 and Cebpa (Fig. 5g). We also found increased
expression of genes participating in the induction of thermogenesis and
‘browning’ of white adipose tissue (Fig. 5h). This thermogenic program

(Extended Data Fig. 4)23. The function of these two MEK2 sites (T395
and T397) has not previously been reported, yet they are the most
frequently found MEK2 modifications in the proteomic databases out-
side the canonical activation loop35.

Using a phospho-specific antibody, we were able to show that acute
Cdk5 inhibition abrogates the phosphorylation of MEK2 at T395 (Fig. 3c).
Mutation of both of these neighbouring sites rendered MEK more active
than wild type in a protein kinase assay using recombinant ERK protein
as a substrate (Fig. 3d). Together, these findings strongly suggest that
Cdk5 deletion results in the activation of ERK kinase via derepression
of MEK kinase activity.

We next asked a critical question: does inhibition of the MEK/ERK
pathway correct the metabolic defect evident in the Cdk5-KO mice? Mice
of both WT and KO genotypes maintained on a high-fat diet were
treated with the well-characterized MEK inhibitor PD0325901 for 5 days
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(Extended Data Fig. 5c). Similarly, clamp studies revealed a twofold increase in the
glucose infusion rate (Fig. 4e). This markedly improved sensitivity to insu-
lin infusion was due to increased whole-body glucose utilization and
regulation of free fatty acids.

**P < 0.001. Error bars indicate s.e.m.**

(Fig. 5a). We next treated ob/ob mice with either PD0325901 or a distinct
US Food and Drug Administration-approved MEK inhibitor, Trametinib/
GSK1120212. In comparison with control animals, mice receiving either
GSK1120212 or PD0325901 showed an improvement in glucose tol-
erance (Fig. 5b and Extended Data Fig. 6a). This was accompanied by
decreased insulin levels and increased levels of the insulin-sensitizing
hormone adiponectin (Fig. 5c, d and Extended Data Fig. 6b) without
affecting body weight (Fig. 5e and Extended Data Fig. 6c).

We performed gene expression analysis to gain a better understand-
ing of the transcriptional basis of the improved glucose homeostasis
after MEK inhibition. We have previously defined a set of 17 genes that
are sensitive to PPARγ S273 phosphorylation in cultured adipose cells
and then further refined that gene set to 10 genes that were also regu-
lated by obesity in mice and responded to treatment with non-agonist
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expression of genes participating in the induction of thermogenesis and
‘browning’ of white adipose tissue (Fig. 5h). This thermogenic program

**P < 0.001. Error bars indicate s.e.m.**
does not include induction of the PPARγ coactivators Pgc1a or Prdm16, although post-translational modifications of these proteins cannot be excluded as contributing to this phenotype.8,7,28 Last, we observed decreased expression of the pro-inflammatory cytokine Tnfα messenger RNA and an altered adipose tissue macrophage expression profile (Extended Data Fig. 7). Both MEK inhibitors caused a decrease in PPARγ phosphorylation at S112 and S273, confirming the established role of ERKs in regulating S112 and strongly suggesting a new role in regulating S273 phosphorylation (Extended Data Fig. 8). Taken together, these data identify an insulin-sensitizing role for MEK inhibitors in adipose tissue, which is consistent with the effects of non-agonist PPARγ ligands that specifically block PPARγ phosphorylation at S273 (Extended Data Fig. 8).

The MEK inhibitory compounds that we have used here are now safe and effective enough to be used in patients with metastatic melanoma and are tolerated well enough to permit studies of metabolism in rodents and perhaps in humans. We find anti-diabetic effects when fasting insulin values (c), plasma adiponectin levels (d) and body weights (e) (n = 8). f–h, Gene expression in ob/ob epididymal white adipose tissue after treatment with vehicle or either of two MEK inhibitors, PD0325901 or GSK1120212 (n = 7, 7 and 8, respectively). i, Genes responsive to PPARγ agonism. b. Genes controlling ‘browning’ of white adipose tissue and thermogenesis. j, Phosphorylation of PPARγ in epididymal white adipose tissue in ob/ob mice after treatment with MEK inhibitors. Rates under the curve and gene expression were analysed by analysis of variance. *P < 0.05; **P < 0.01; ***P < 0.001; n.s., not significant. Error bars indicate s.e.m.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.
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Author Contributions A.B., B.M.S., F.E., S.G., J.P.C., M.J. and G.S. designed the experiments. A.B., D.B., F.E., J.C.P. and P.Z. performed the experiments. A.B., B.M.S. and F.E. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to A.B. (abanks@research.bwh.harvard.edu) or B.M.S. (bruce_spiegelman@dfci.harvard.edu).
**METHODS**

**Animal experiments.** Animal experiments were performed with approval from the Institutional Animal Care and Use Committees of both Beth Israel Deaconess Medical Center and The Harvard Center for Comparative Medicine. Glucose and insulin tolerance tests were performed as described previously by researchers blinded to the genotype and treatment group to which each mouse belonged41. Glucose doses used were as follows: for mice maintained on a standard-diet, 2 kg kg\(^{-1}\); high-fat diet, 1.5 kg kg\(^{-1}\); ob/ob mice, 1 kg kg\(^{-1}\). Male C57Bl/6 ob/ob mice were purchased from Jackson Labs at 5–6 weeks of age and allowed to acclimate for 1–2 weeks before treatment. Cdk5\(^{Ko}\) mice were provided by P. Greengard (Rockefeller University). Cdk5\(^{Ko}\), control, adiponectin-Cre and the Cdk5\(^{Ko}\)-Cre mice were healthy and viable, in contrast to mice with whole-body deletion of Cdk5. Adiponectin-Cre mice were provided by E. Rosen (Beth Israel Deaconess Medical Center). Both strains were previously backcrossed to a body deletion of CDK5. Adiponectin-Cre mice were provided by E. Rosen (Beth Israel Deaconess Medical Center). Both strains were previously backcrossed to a

**Indirect calorimetry.** Mass spectrometry.

**PCR with expression normalized to levels of TATA-binding protein (TBP)2.** Cell lines described: protein and RNA preparation, western blotting, quantitative real-time

**C57Bl/6 background.** For diet-induced obesity, male animals were fed on a high-fat

**(60%) diet (Research Diets, catalogue no. D12492i).** For insulin tolerance tests were performed as described previously by researchers blinded to the Institutional Animal Care and Use Committees of both Beth Israel Deaconess Animal experiments. **METHODS**

**Cell culture.** Cell culture of HEK 293 and F442A pre-adipocytes was performed as described: protein and RNA preparation, western blotting, quantitative real-time PCR with expression normalized to levels of TATA-binding protein (TBP)2. Cell lines were found free of mycoplasma before initiation of studies. Inhibition of analogue-sensitive CDK5 kinase (F80G) was performed by treating cells with 1NMP1 (1,1-dimethylethyl)-3-(1-naphthalenylmethyl)-1-pyrazolo[3,4-d]pyrimidine-4-amine (Cayman Chemical) at the indicated doses for 2 h.

**Plasmids.** The constitutively active ERK kinase, ERK-CA, is the product of a fusion amine) (Cayman Chemical) at the indicated doses for 2 h.

**Antibodies.** Antibodies were obtained from Cell Signaling Technology (anti-phospho-ERK1/2 (catalogue no. 9101), anti-ERK (catalogue no. 4695), anti-phospho p38 (catalogue no. 4511), anti-p38 (catalogue no. 9212), anti-phospho JNK (cata-logue no. 4671), anti-JNK (catalogue no. 9252), anti-phospho AKT (catalogue no. 13038) and total AKT (catalogue no. 9272)), Millipore (anti-phospho-S112 PPARγ (catalogue no. 04-816) and Santa Cruz Biotechnology (anti-PPARγ (catalogue no. sc-7273) and anti-phospho-394-PPARγ (catalogue no. sc-101734)). Antibodies against anti-phospho-S273 PPARγ were generated as described previously.

**Indirect calorimetry.** Energy expenditure, O\(_2\) consumption, CO\(_2\) production, respiratory exchange ratio, total locomotor activity and food intake measurements were made with a 16-cage Columbus Instruments Oxymax Comprehensive Lab Animal Monitoring System at ambient room temperature (21–23°C). Whole-body composition was assessed with an EchoMRI 3-1 in-on conscious mice both before and after calorimetry. Because body weight and body composition were unchanged between WT and Cdk5-KO mice, data were analysed by analysis of variance (ANOVA).

**Mass spectrometry.** Enrichment using ActivX ATP probes (Thermo) combined with phosphopeptide enrichment were used to profile kinases46. In brief, tissue extracts were incubated in the presence of non-hydrolysable ATP analogues coupled to a desthiobiotinylated tag. These small-molecule probes are designed to covalently attach to ATPases, including protein kinases. Peptides were then labelled with TMT isotopic tags and the resulting mass spectra were analysed quantitatively. The method was recently adapted to include an additional phosphopeptide enrichment step, thereby improving the identification and quantification of protein kinase activities47. The methods are similar to those described in ref. 37. Mouse tissue homogenate from three WT and three Cdk5-KO mice was subjected to gel filtration in spin columns (Zeba; Pierce) in accordance with the manufacturer’s instructions to remove endogenous ATP, ADP and small molecules and then diluted with reaction buffer (25 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P40, 5% glycerol) to a final protein concentration of 2 mg m\(^{-1}\). Protease inhibitors (1 × ‘complete’; Roche 04693132001) and phosphatase inhibitors (final concentration 2 mM imidazole, 1 mM sodium fluoride, 1.15 mM sodium molybdate, 4 mM sodium tartrate dehydrate, 1 mM β-glycerophosphate, 50 μM phenyl-

**larsine) were added along with MnCl\(_2\) to a final concentration of 10 μM. Lysates were incubated with ActivX ATP probes for 10 min at room temperature at a concentration of 20 μM. The reaction was quenched with 8 M urea, reduced with dithiothreitol (5 mM final concentration) and then alkylated with iodoacetamide (15 mM final concentration). The solution was then subjected again to gel filtration (Zeba; Pierce). Streptavidin was then added to the lysate to capture the undigested, desthiobiotinylated proteins and kinases. After extensive washing (lysis buffer containing 6 M urea (five times with 100 μl), then 50 mM HEPES (five times with 100 μl)), the captured proteins were subjected to on-bead digestion with trypsin (5 ng μl\(^{-1}\) for 4 h at 37°C in a tandem-mass-tag-compatible buffer (50 mM HEPES pH 7.4, 0.5 M guanidinium chloride). After digestion, the resulting peptides were extracted and the beads were washed with 50 mM HEPES (twice with 50 μl) and these washes were added to the peptide mixture. Acetonitrile was added to the peptide mixture to a final concentration of 30%, and the peptides were subjected to Tandem mass tags (TMT; Thermo Scientific) labelling. For labelling, 0.8 μg of each TMT reagent (126, 127, 128, 130 and 131) was resuspended in 40 μl of anhydrous acetonitrile. Peptides were resuspended in 17.5 μl of 50 mM HEPES pH 8.5 and 5 μl of acetonitrile, to which 2.5 μl TAMT reagent was added. The TMT labelling reaction was performed at room temperature for 1 h, and individual labelling reactions were stopped by the addition of 3 μl of 5% hydroxylamine. The six samples were then combined and desalted with StageTip

**For phosphopeptide enrichment, peptides were resuspended in 100 μl of binding buffer (2 M lactic acid, 50% acetonitrile). Phosphopeptides were enriched with TIO2 as described.** TIO2 resin (600 μg; GL Sciences) was prepared by washing twice with 200 μl of binding buffer and was then added to the peptides in binding buffer and incubated for 1 h at room temperature. After incubation, beads were recovered by centrifugation (4 min at 200 g) and washed with binding buffer (five times with 200 μl). Bound phosphopeptides were then eluted with 50 mM K\(_2\)HPO\(_4\) pH 10 (three times with 20 μl) and further purified with StageTip. The purified phosphopeptides were resuspended in 8 μl of 5% formic acid, and 4 μl was injected and analysed by LC-MS.

In the Cdk5 experiment, tissue homogenate was pretreated with Cdk5/p35 (Millipore) for 10 min with the indicated amounts before being processed with the above protocol. ActivX ATP probes, high-capacity binding streptavidin and TMT were obtained from Thermo Scientific; modified trypsin was obtained from Promega; and TIO2 beads were obtained from GL Sciences. SepPak C18 solid-phase extraction cartridges were purchased from Waters Corporation.

**Liquid chromatography (LC) and mass spectrometry (MS) analysis.** LC–MS/MS analysis was performed on an LTQ Orbitrap Velos or an LTQ Orbitrap Elite mass spectrometer (Thermo-Fisher Scientific) linked to an Accela 600 quaternary LC pump (Thermo) and a Famos autosampler (LC Packings). Flow rates of 300 nl min\(^{-1}\) over the column were achieved by using a flow-split method. A hand-pulled fused silica capillary column (125 μm × 18 cm) was used for peptide separation. The column was first packed with about 0.5 cm of Magic C4 resin (particle size 5 μm, pore size 100 Å; Michrom Bioroseses) and then with 18 cm of Macel C3 AQ resin (particle size 3 μm, pore size 200 Å; Nest Group). The total LC–MS run length for each sample was 180 min and consisted of a 150-min gradient from 3% to 33% acetonitrile in 0.125% formic acid. A recently developed MS3 method was used to overcome the interference problem in the acquisition of TMT data40. In brief, a high-resolution MS3 scan in the Orbitrap (300–1500 m/z; 60k resolution; automatic gain control (AGC) 10\(^{6}\)) was collected from which the top ten precursors were selected for MS2 analysis followed by MS3 analysis. The MS2 scan was performed in the quadrupole ion trap (collision-induced dissociation, AGC 2 × 10\(^{6}\), isolation width 15% of AGC, isolation energy 35 eV, collision energy 100 eV) and the MS3 scan was analysed in the Orbitrap (HCD, 30k resolution, maximum AGC 1.5 × 10\(^{6}\), maximum injection time 250 ms, normalized collision energy 50). Multiple fragment ions from each MS2 spectrum were selected for MS3 analysis using isolation windows with multiple frequency notches41.

**Data analysis.** Statistical data analysis was performed with GraphPad Prism and Microsoft Excel, including Daniel’s XL Toolbox Add-In. Unless otherwise specified, analyses were by one-tailed Student’s t-test. Sequence alignment was performed with a modified Clustal W algorithm using Vector NTI AlignX. Mass spectrometry data were processed using an in-house software pipeline48. Raw files were converted to mzXML files and searched using the Sequest algorithm49 against a composite database containing sequences from the mouse uniprot database in forward and reverse orientations as well as the sequences of common contaminating proteins (for example trypsin). Database searching matched MS/MS spectra with fully tryptic peptides from this composite database with a 20 pp.m. precursor ion and a product ion tolerance of 1 Da. Carbamidomethylation of cysteine residues (+57.02146 Da) and TMT tags on peptide amino termini and lysines (+229.162932 Da) were set as
static modifications. Variable modifications of oxidation of methionine residues (+15.99492 Da) and phosphorylation (+79.966330 Da) on serine, threonine and tyrosine residues were used. The data were filtered to a false discovery rate of less than 1% based on the target-decoy database approach at both the peptide and protein levels.\textsuperscript{41} Linear discriminant analysis was performed to generate a classifier to distinguish between correct and incorrect MS2 spectra assignments based on the following parameters: XCorr, $\Delta Cn$, peptide ion mass accuracy, charge state and peptide length, as described\textsuperscript{42}. Peptides were then assembled into proteins that were scored probabilistically and further filtered to a protein-level false discovery rate of about 1%. Peptide quantification using TMT reporter ion intensity was performed using in-house software, as described\textsuperscript{42}. In brief, a 0.06$m/z$ window around the theoretical $m/z$ value of each reporter ion was scanned for ions, and the intensity of the signal nearest to the theoretical $m/z$ value was recorded. The intensities of the reporter ions were adjusted to account for isotopic impurities in each TMT variant (as provided by the manufacturer). For comparisons, the whole data sets were filtered to a 1% false discovery rate, and proteins and phosphoites were then quantified by summing reporter ion counts for all the peptide-spectral matches. Filtering was performed to remove poor-quality MS3 spectra in a manner similar to that described previously\textsuperscript{41}. Protein quantification values were exported for further analysis in Excel or Matlab. Hierarchical clustering was performed using Matlab.

\textbf{In vitro, MS-based kinase activity assay.} PPAR$\gamma$ (1 µg; Active Motif) was incubated with 50 ng of recombinant kinases MEK2, ERK2 (SignalChem) and Cdks/p35 (Millipore) and kinase reaction buffer containing 25 mM Tris-HCl pH 7.5, 5 mM ATP, 7.5 mM MgCl$_2$, 0.2 mM EGTA, 7.5 mM $\beta$-glycerophosphate, 0.1 mM Na$_2$VO$_4$ and 0.1 mM dithiothreitol in a final reaction volume of 50 µl. All proteins corresponded to the human sequences. After incubation for 45 min at room temperature, the reaction mixture was subjected to both LysC and trypsin digestion (individual reactions). After purification, the samples were then analysed by LC-MS/MS in a similar manner to that described above but without the additional MS3 dimension.

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Extended Data Figure 1 | Metabolic profiling of adipose-specific Cdk5-KO mice on a standard chow diet. a–e, Fasting plasma levels of glucose (a), insulin (b), total triacylglycerols (c), free fatty acids (FFA) (d) and total cholesterol (e) (n = 16 (control) and 17 (KO)). f, g, Body weights (f) and intraperitoneal glucose tolerance test (g). Mice were 12 weeks of age (n = 14 (control) and 11 (KO)). No significant differences were observed. Error bars indicate s.e.m.
Extended Data Figure 2 | Energy homeostasis of adipose-specific Cdk5-KO mice maintained on a high-fat diet. a–f. After a 48-h acclimatization period, singly housed mice were monitored for oxygen consumption ($V_{O2}$) (a), carbon dioxide production ($V_{CO2}$) (b), respiratory exchange ratio (RER) (c), ambulatory locomotor activity (d), cumulative food intake (e) and body weights (f) ($n = 8$ per group). Shaded areas signify the dark phase of the light cycle. No significant differences were observed. Error bars indicate s.e.m.
Extended Data Figure 3 | Activity of alternative kinases in adipose tissue from Cdk5-KO mice. Brown adipose tissue protein lysates from mice maintained on a high-fat diet for 12 weeks. Blotting for phospho-p38, phospho-JNK and phospho-S473 and pT308 AKT was performed before loading for total protein amounts.
Extended Data Figure 4 | Conservation of the sites on MEK2 phosphorylated by Cdk5. Mouse MEK2 T395/T397 corresponds to human MEK2 T394/T396. These sites share identity with MEK1 T386/T388 in both humans and mouse. Cdk5 has previously been shown to phosphorylate MEK1 at T286, a site not shared with MEK2. ERK has been shown to phosphorylate MEK1 T386 and contribute to regulation of kinase activity. Homo, Homo sapiens; trog, Pan troglodytes; mus, Mus musculus; rat, Rattus norvegicus; bos, Bos taurus; canis, Canis lupus familiaris.
Extended Data Figure 5 | Body weight of control and of adipose-specific Cdk5-KO mice maintained on a high-fat diet after treatment with PD0325901. Treatment similar to that in Fig. 4a–c. The body weights are not significantly different by ANOVA. Error bars indicate s.e.m.
Extended Data Figure 6 | Effects of PD0325901 treatment on ob/ob mice. a–c, Glucose tolerance test (a), adiponectin levels (b) and body weights (c) of ob/ob mice treated with PD0325901 (n = 7 (vehicle) and 8 (PD)). *P ≤ 0.05 by Student’s t-test. Error bars indicate s.e.m.
Extended Data Figure 7 | Inflammatory markers in epididymal white adipose tissue from ob/ob mice treated with MEK inhibitors. Gene expression analysis was performed on M1 macrophage markers Nos2 and tumour necrosis factor-α (TNF-α); M2 macrophage markers Arg1, Chil3, Il10, Itgax, Clec10a/Mgl1 and Mgl2; chemotactic ligand Ccl2 and receptor Ccr2; and macrophage surface markers Emr1, Cd68 and Csf1r (n = 7 or 8 mice per group as in Fig. 5f, h). Gene expression was analysed by ANOVA. Error bars indicate s.e.m. *P < 0.05; **P < 0.01; ***P < 0.001.
Extended Data Figure 8 | Schematic model of PPARγ regulation at S273.

a. In the lean state, PPARγ is not phosphorylated. b. In the obese state, S273 phosphorylation is driven by both Cdk5 and ERK with CDK5 repressing MEK and ERK activity. c. Cdk5-KO results in derepression of MEK and ERK kinases and increased phosphorylation of S273 PPARγ. d. MEK inhibition markedly decreases S273 PPARγ phosphorylation. e. PPARγ ligands, including the thiazolidinediones, block the accessibility of S273 PPARγ by either ERK or CDK5 kinases.