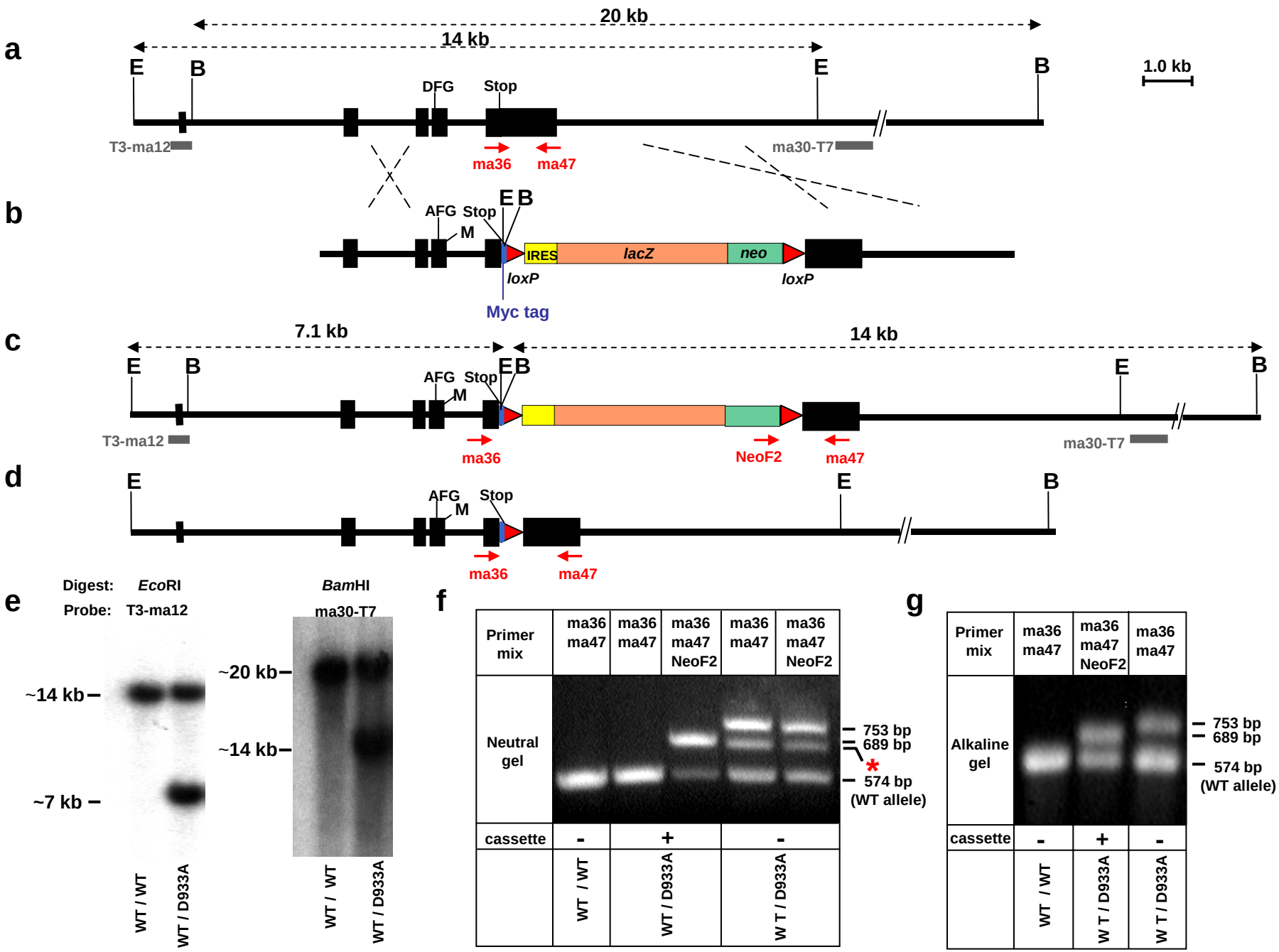
Suppl. Figure 1, Foukas et al.



Suppl. Figure 1 | p110α gene targeting. The targeting approach to create the p110α^{D933A} allele was similar to the strategy previously used for the creation of the p110δ^{D910A} allele (Okkenhaug et al. (2002), Science 297, 1031). Cloned genomic DNA covering the last 4 exons of the coding region of the p110α gene, *Pik3ca*, was isolated from a 129/Ola library. A point mutation, resulting in the DFG to AFG conversion of the conserved DFG motif in the C-terminal p110α kinase domain, was introduced by site-directed mutagenesis. Three additional features were incorporated into the targeting vector using standard restriction enzyme and PCR-based cloning techniques: (1) a silent mutation introducing a diagnostic *Mls*I restriction site in the third codon of the AFG motif, (2) a sequence encoding a C-terminal Myc-epitope tag introduced immediately preceding the translational stop codon (to allow for recognition of the mutated protein with epitope-specific antibodies), and (3) a reporter/selection cassette flanked by *loxP* sites, inserted into the 3' UTR sequence. The reporter/selection cassette contains an IRES sequence followed by the β-galactosidase *(lacZ)* coding sequence and a neomycin resistance gene expressed from its own promoter *(*MC1*neo*pA*).* Vector-derived *Eco*RI and *Bam*HI sites between the Myc tag and the first *loxP* site were exploited for Southern blot analysis, as detailed below.

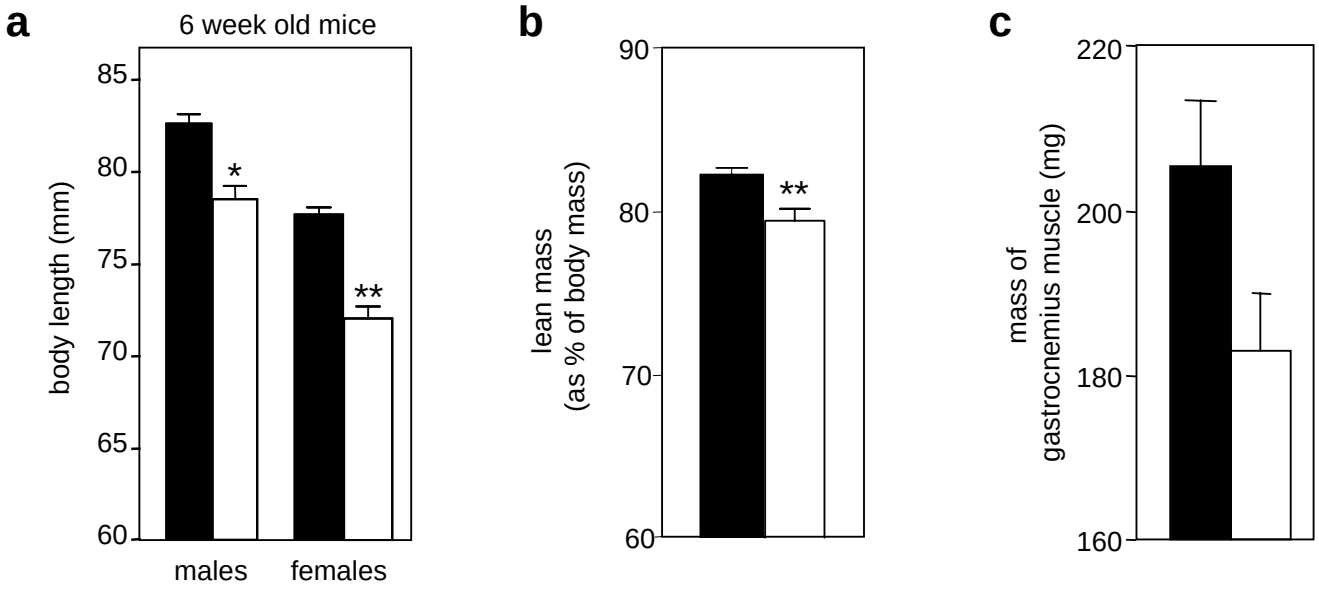
The linearized targeting construct was transfected into E14Tg2a (129/Ola strain) embryonic stem cells. Subsequently, G418-resistant clones with targeted integration of the vector were identified by Southern blot analysis of *Eco*RI- or *Bam*HI-digested genomic DNA using probes (T3-ma12 and ma30-T7, respectively) homologous to genomic sequence flanking and external to the vector homology arms. The presence of the AFG-encoding mutation was confirmed by DNA sequencing. Male chimeras generated from clones with the AFG-encoding mutation were bred with C57BL/6 females and germline transmission confirmed by Southern blot analysis of tail DNA. Deletion of the selection cassette was achieved by intercrossing p110α$^{D933A/WT}$ mice with transgenic mice expressing Cre-recombinase in the germline (Schwenk et al. (1995), Nucleic Acids Res 23, 5080) and verified by PCR analysis.

a, Organization of the p110α (*PIK3CA*) gene locus. DFG represents the conserved amino acid motif in the C-terminal p110α kinase domain. **b,** Linearized p110α targeting vector showing the AFG-encoding mutation. **c,** Targeted p110α allele. **d,** Targeted p110α allele after Cre recombinase-mediated deletion of the reporter/selection cassette. **e**, Southern blot analysis using 5' (T3-ma12) and 3' (ma30-T7) flanking probes of *Eco*RI- or *Bam*HI-digested genomic DNA from tails of WT or p110α$^{D933A/WT}$ mice still containing the reporter/selection cassette. **f, g,** PCR analysis of targeted alleles. *Lane 1,* WT allele: the ma36-ma47 primer set amplifies a 574 bp fragment. *Lanes 2 and 3*, targeted allele before excision of selection cassette: the ma36-ma37 primer set fails to amplify a >12 kb fragment containing the selection cassette (or even if produced, the large size of the amplification product would preclude penetration of the gel). Inclusion of the NeoF2 primer, however, allows amplification of a 689 bp product with the ma47 primer, revealing the presence of the selection cassette. *Lane 4*, targeted allele following Cre-mediated deletion of the selection cassette: the ma36-ma47 primer set amplifies a fragment of 753 bp, which includes DNA sequences encoding the Myc tag and one *loxP* site that was retained following Cre-mediated deletion of the selection cassette. *Lane 5*, inclusion of NeoF2 primer to the ma35-ma47 primer mix shows no difference from lane 4. * designates a PCR product corresponding to heteroduplexes of DNA of the WT and KI allele generated during PCR amplification, as revealed by DNA sequencing (data not shown) and further confirmed by alkaline agarose gel electrophoresis (shown in panel (g)).

Keys: Exon sequences are represented by filled black rectangles, intron sequences by a black line. Restriction sites are E (*Eco*RI), B (*Bam*HI), M (*Mls*I). The relevant restriction fragments are highlighted by a dashed line with double arrows. Probe fragments are represented by gray lines. The Myc tag is shown as a filled blue rectangle, the *loxP* sites as red triangles with the pointed end indicating orientation and the *loxP* flanked IRES/*lacZ*/MC1*neo*pA cassette components as yellow, orange and green filled rectangles, respectively. The positions of the primers used for PCR screening are designated by red arrows. The sequences of the primers are as follows:

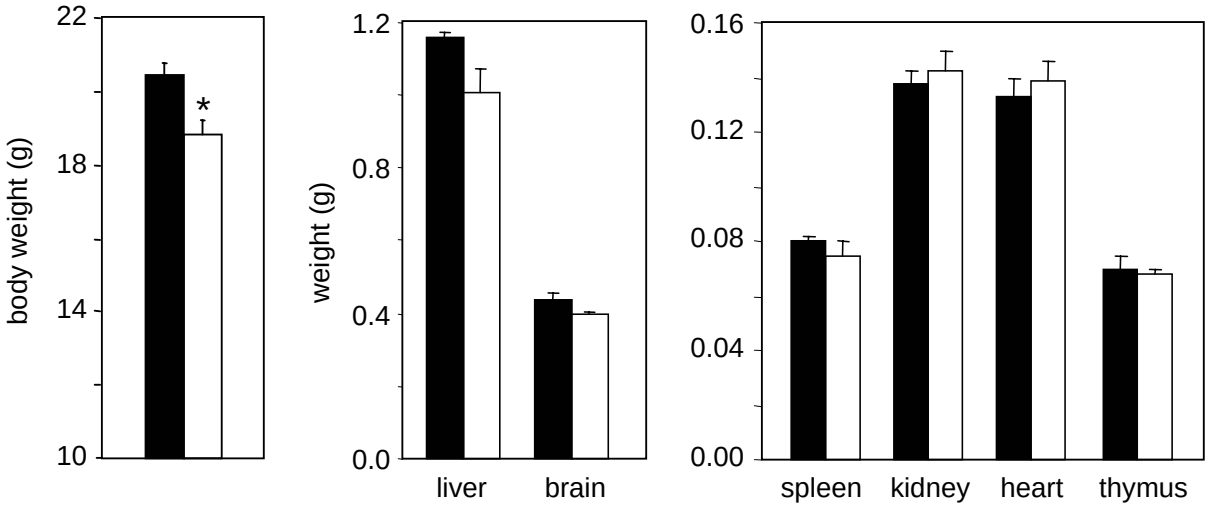
ma36: 5'-CCTAAGCCCTTAAAGCCTTAC-3',
ma47: 5'-ACTGCCATGCAGTGGAGAAGCC-3',
NeoF2: 5'-CTGTCATCTCACCTTGCTCC-3'

Suppl. Figure 2, Foukas et al.



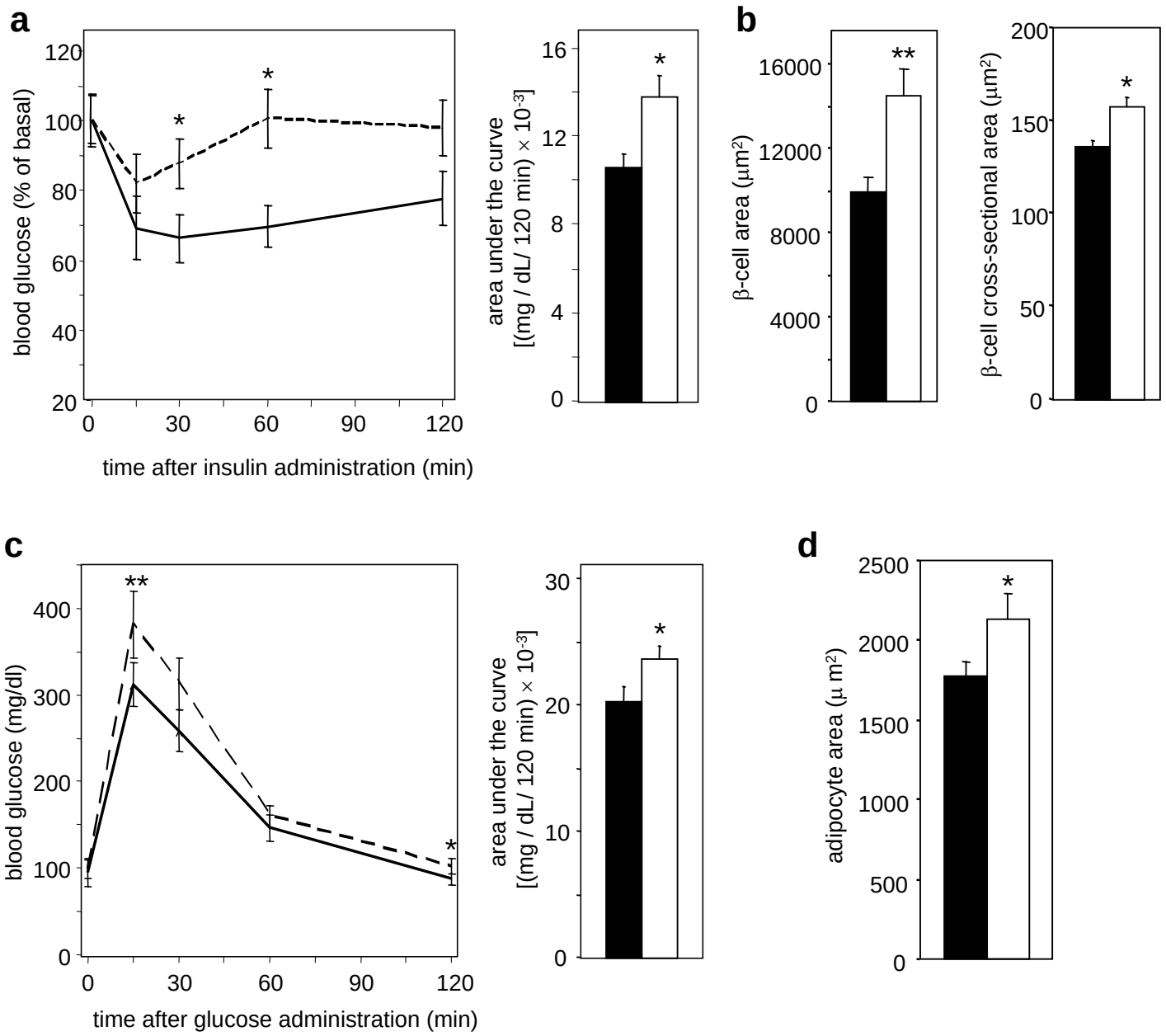
Suppl. Figure 2 | a, Mouth-to-anus length of 6-week old p110α$^{D933A/WT}$ mice and WT littermates. **b**, Lean mass of p110α$^{D933A/WT}$ mice (n=11) and WT littermates (n=11) was determined by DEXA analysis and expressed as % of total body mass. **c,** Gastrocnemius muscle weights from 12 week old male mice (n=6 per genotype). Filled bars, WT mice; open bars, p110α$^{D933A/WT}$ mice.

Suppl. Figure 3, Foukas et al.



Suppl. Figure 3 | Weight of visceral organs in WT and p110α$^{D933A/WT}$ mice. A cohort of female p110α$^{D933A/WT}$ mice and WT littermates (n=5 per genotype) were weighed *(left panel)*, then sacrificed and the indicated organs isolated and weighed *(middle and right panel)*. Filled bars, WT mice; open bars, p110α$^{D933A/WT}$ mice. Values are mean ± sem.

Suppl. Figure 4, Foukas et al.



Suppl. Figure 4 | a, Insulin tolerance test in male p110α$^{D933A/WT}$ (n=10) and WT (n=11) littermate mice. Calculated areas under the curves are shown. **b,** Morphometric analysis of pancreatic islets from WT and p110α$^{D933A/WT}$ mice. Pancreata were isolated from 11-12 week old male mice (n=6 per genotype). The tissues were fixed, processed for histology and analyzed as described in Methods. **c**, Glucose tolerance test in male p110α$^{D933A/WT}$ (n=18) and WT (n=19) littermate mice. Calculated areas under the curves are shown. **d,** Morphometric analysis of white adipose tissue from WT and p110α$^{D933A/WT}$ mice. Epididymal fat pads were isolated from 11-12 week old male mice (n=6 per genotype). The tissues were fixed, processed for histology and analyzed as described in Methods. Solid lines/filled bars, WT mice; dashed lines/open bars, p110α$^{D933A/WT}$ mice. Values are mean ± sem.

Supplementary Methods

Protein extraction, immunoprecipitation and immunoblotting. Tissues were removed, snap-frozen in liquid nitrogen and homogenized in a mortar with a pestle in lysis buffer (50 mM Tris.HCl pH 7.4, 5 mM EDTA, 150 mM NaCl, 50 mM NaF and 1% Triton X-100 supplemented with 2 mg/ml aprotinin, 1 mM pepstatin, 1 ng/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride and 1 mM sodium orthovanadate). Homogenates were clarified by centrifugation in a microcentrifuge. Supernatants were either analyzed directly or immunoprecipitated for 1 h with the indicated antibodies. Immune complexes were collected with 20 µl of 50% slurry of protein A- or protein G-Sepharose, washed with lysis buffer, resolved on 8% SDS-PAGE, and transferred onto PVDF membranes. The blots were probed with the indicated antibodies. Detection was performed by enhanced chemiluminescence (ECL, Amersham Biosciences).

PI3K assay. Lipid kinase assays were performed essentially as described before (Vanhaesebroeck et al. (1997), Proc. Natl. Acad. Sci. USA 94, 4330). Briefly, p110α, p110β were immunoprecipitated using either an affinity matrix of the phosphopeptide YpVPMLG corresponding to Tyr 751 of the human PDGF receptor β coupled to Actigel (Sterogene, CA), p110 isoform-specific antibodies or non-immune control antibodies, and subjected to an *in vitro* kinase reaction using PtdIns(4,5)P2 as a substrate. The lipids were resolved by thin-layer chromatography and quantified on a Molecular Imager FX (Bio-Rad).

Supplementary Table 1: List of organs and tissues subjected to histological examination.

adrenal gland	ovary
blood vessels (aorta, vena cava, organ vessels)	oviduct
bones	pancreas
brain	parotid gland
brainstem	penis
cerebellum	peripheral nerve (sciatic nerve)
coagulating gland	pituitary gland
clitoral gland	prostate gland
dorsal root ganglia	seminal gland
ear	skeletal muscle (gastrocnemius, soleus)
epididymis	skin
esophagus	small intestine (duodenum, jejunum, ileum)
eye	spinal cord
fat tissue	spleen
gallbladder	stomach
harderian gland	sublingual gland
heart	submandibular gland
kidney	testicle
lachrymal gland (exorbital)	thymus
large bowel (cecum, colon, rectum)	thyroid gland
larynx	tongue
liver	trachea
lung	urinary bladder
lymph nodes	uterus
mammary glands	vagina
nose	vas deferens