Figure S1 The CaaX motif of FBXL2 is necessary to induce the degradation of p85β. (a) FBXL2 does not bind p110α and p110β. U2OS cells were stably transfected with either an empty vector (EV) or the indicated FLAG-tagged proteins. Cells were treated with MG132 for three hours prior to harvesting for immunoprecipitations and immunoblotting as indicated. (WCL, whole cell lysate). (b) HeLa cells were transfected with either GFP-tagged FBXL2 or GFP-tagged FBXL2(CaaX/SaaX) constructs. Twenty-four hours after transfection, cells were fixed and incubated with an anti-FLAG antibody (green) and anti-p85β antibody (red). In merged images, yellow shows colocalization of FBXL2(CaaX/SaaX) and p85β. Arrows in the middle panels point to the position of GFP-positive cells shown in the top panels. The graphs show the comparison of mean fluorescence between FBXL2-positive and FBXL2-negative cells (top), FBXL2(CaaX/SaaX)-positive and FBXL2(CaaX/SaaX)-negative cells (middle), and FBXL2-positive and FBXL2(CaaX/SaaX)-positive cells (bottom). Fluorescence intensity was measured by a Nikon Eclipse TE2000-E fluorescence microscope using Metamorph software.
Figure S2  p85β is targeted by FBXL2 for degradation. (a) FBXL2 mutants impaired in their binding to SKP1 form more stable complexes with p85β. HEK-293T cells were transfected with either an empty vector (EV) or the indicated FLAG-tagged FBXL2 constructs (wild type and mutants). Twenty-four hours post-transfection, cells were treated with either MG132 or solvent for three hours prior to harvesting for immunoprecipitations and immunoblotting as indicated. (b) During a 72-hour serum starvation, NHFs were transfected with either an siRNA targeting FBXL2 (#2) or a non-silencing siRNA (NS). Cells were subsequently stimulated with media containing serum and harvested at the indicated time points for immunoblotting. (AS, asynchronous growing cells; SR, serum re-addition.)
**Figure S3** Mapping of the FBXL2 binding domain on p85β. (a–e) HEK-293T cells were transfected with GFP-tagged FBXL2 and the indicated FLAG-tagged p85β mutants. Whole cell extracts (WCL) were immunoprecipitated (IP) with either anti-FLAG resin or anti-GFP resin as indicated, and immunocomplexes were probed with antibodies to the indicated proteins.
**Figure S4** Identification of p85β degron. (a) HEK-293T cells were transfected with GFP-tagged FBXL2 and the indicated FLAG-tagged p85β mutants. Whole cell lysates (WCL) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins. (b) Alignment of the amino acid regions containing the FBXL2 binding motif in p85β orthologs. (c) Alignment of the amino acid regions containing the FBXL2 binding motif in human p85β with corresponding region in p85α and p85γ from different species.
**Figure S5** Tyr655 inhibits p85β binding to FBXL2. (a-b) HEK-293T cells were transfected with GFP-tagged FBXL2 and the indicated FLAG-tagged p85β mutants. Whole cell lysates (WCL) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins. (c) Schematic representation of p85β mutants used in (a-b). Binding of p85β to FBXL2 is indicated with symbol (+).
Figure S6 The binding domain on p85β for PTPL1 and FBXL2 overlap. (a) HEK-293T cells were transfected with HA-tagged PTPL1 in combination with an empty vector (EV) or FLAG-tagged p85β. Whole cell lysates (WCL) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins. (b) The experiment was performed as in (a), except that the IP was performed with an anti-HA antibody. (c) HEK-293T cells were transfected with an empty vector (EV), FLAG-tagged p85β, or FLAG-tagged p85β(ΔSH2C). Whole cell lysates (WCL) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins. (d) HEK-293T cells were transfected with GFP-tagged FBXL2 in combination with an empty vector (EV), HA-tagged PTPL1, or HA-tagged p85β. Whole cell lysates (WCL) were immunoprecipitated (IP) with anti-HA antibody, and immunocomplexes were probed with antibodies to the indicated proteins. (e) HEK-293T cells were transfected with GFP-tagged FBXL2 together with either wild type p85β or the indicated FLAG-tagged p85β mutants. Whole cell lysates (WCL) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins. (f) HEK-293T cells were transfected with wild type p85β or the indicated FLAG-tagged p85β mutants. Whole cell lysates (WCL) were immunoprecipitated (IP) with anti-FLAG resin, and immunocomplexes were probed with antibodies to the indicated proteins. (g) Table summarizing the results presented in (a-f) and Fig. 4a. (NT, not tested).
Figure S7  FBXL2, p85β and PTPL1 form a trimeric complex. (a) HEK-293T cells were transfected with different combinations of HA-tagged PTPL1, FLAG-tagged p85β, and GFP-tagged FBXL2 constructs. Whole cell lysates (WCL) were immunoprecipitated (IP) with an anti-FLAG resin, and immunoblotting was performed as indicated. The first lane shows cells transfected with an empty vector (EV). (c) During a 72-hour serum starvation, NHFs were transfected twice with either an siRNA targeting PTPL1 or a non-silencing siRNA (NS). Cells were subsequently re-stimulated with media containing serum for 30 minutes and harvested for immunoblotting. (SR, serum re-addition). (d) During a 72-hour serum starvation, NHFs were transfected with either siRNAs targeting FBXL2, both FBXL2 and p85β, or a non-silencing siRNA (NS). Cells were subsequently stimulated with media containing serum and harvested at the indicated time points for immunoblotting. The graph in the bottom shows FBXL2 mRNA levels in the different samples analyzed using real-time PCR in triplicate measurements. The values represent the ratios between FBXL2 and GAPDH mRNAs. (SR, serum re-addition). (e) Cells were treated as in (d), except that cells were stimulated with media containing only insulin. (f) Cells (different lines as indicated) were serum starved for 48 hours, then, one hour after serum re-addition, cells were lysed, immunoprecipitated with an anti-IRS1 antibody, and immunoblotted as indicated.
Figure S8 Full scans of immunoblots used in the main figures.
Figure S8 continued
Figure S8 continued
Figure S8 continued
Supplementary Table Legend

Table S1  MudPIT analysis of FBXL2 immunopurification. Two biological replicate FLAG-affinity purifications were analyzed for both FBXL2 and FBX01 (control). Distributed normalized spectral abundance factors (dNSAF) were averaged, while unique peptides and total spectral counts were merged from the replicate analyses.