Supporting Information

De Vries-van Leeuwen et al. 10.1073/pnas.1220809110

SI Materials and Methods

Reagents. Fusicoccin was obtained from A. V. Babakov (Institute of Agricultural Biotechnology, Moscow, Russia). β-Estradiol (E2) and 4-hydroxytamoxifen (4OH-TAM) were purchased from Sigma-Aldrich. Carboxyfluorescein labeled SWpTY peptide (FAM-SWpTY, where pT indicates phosphorylated Threonine) peptide was a kind gift from M. Li (Johns Hopkins University, Baltimore, MD) (1).

Peptide Synthesis. All peptides were synthesized via solid phase peptide synthesis using a TentaGel resin with a para-hydroxybenzyl alcohol (Wang) linker [TentaGel R PHB] preloaded with a fluorenylmethyloxycarbonyl (Fmoc)-protected valine (purchased from RAPP polymere GmbH, loading 0.2 μmol/mg). The first amino acid (Fmoc-Thr(PO(OBzl),OH)-OH) was coupled with HATU [O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate]. Most amino acids afterward, except for the Ile/Thr, Ser/Ser, Ala/Thr (IT/SS/AT) fragments, were coupled with HCTU [2-(6-Chloro-1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate] and the last 10 amino acids again with HATU. The IT/SS/AT fragments of the peptides were coupled as Fmoc-Ile-Thr(Ψ-Me,Mepro)-OH, Fmoc-Ser(tBu)-Ser(Ψ-Me,Mepro)-OH and Fmoc-Ala-Thr(Ψ-Me,Mepro)-OH, respectively, with HATU.

The peptide purification was performed by preparative HPLC with MS detection, using a preparative C18 column (Alltima HP) on an LC-MS system (Finnigan) enabling a mass triggered fraction collector. Peptides were separated using a 20%–30% gradient of 0.1% TFA in acetonitrile in 0.1% TFA in H_2O. The analysis of the peptides was performed on a LCQ LC-MS system (water/micromass), using a reverse phase C18 column (Gracesmart) and a mobile phase of A: 0.1% formic acid (FA) in H_2O and B: 0.1% FA in acetonitrile. Linear gradient was run from 2 to 70% of B in A over 10 min.

Antibodies. For Western blotting of estrogen receptor alpha (ERα) we used the rabbit polyclonal HC-20 from Santa Cruz Biotechnology. The antibody raised against the phosphorylated Threonine 594 of ERα (pT^{594}-ERα-antibody), specific for the phosphorylated T^{594} epitope, was prepared by GL Biochem. The T^{594} phosphorylated peptide was conjugated to the carrier protein keyhole limpet hemocyanin (KLH), and this was used to immunize two New Zealand rabbits. The phospho-specific antibody was affinity purified with the antigen.

Cells and Cell Culture. The breast cancer cell line MCF-7, the human osteosarcoma cell line U2OS and the human embryonal kidney 293 (HEK293) cell line were maintained in DMEM (PAA Laboratories) media supplemented with 10% FBS (PAA Laboratories), and standard antibiotics. During experiments, the cells were grown in phenol red-free DMEM (PAA Laboratories) supplemented with 5%–10% charcoal-treated FBS (HyClone) and antibiotics, unless indicated differently. All cell lines were grown in a 37 °C humidified growth chamber containing 5% CO_2.

Cell Proliferation and Apoptosis Assay. MCF-7 cells were seeded at 5,000 cells per well in a 96-well plate. Cells were pretreated 1 h with the indicated concentration of fusicoccin (FC) or methanol (MeOH) as control, before the addition of 1 nM E2. The CellPlayer 96-Well Kinetic Caspase-3/7 Apoptosis assay kit (Essen BioScience) was added to measure apoptosis, according to the manufacturer's instruction. Cell proliferation was measured kinetically every 4 h as an increase in cell confluence. The level of apoptosis was measured every 4 h with fluorescent photography. Cell confluence and apoptosis was determined by analysis of phase-contrast/fluorescent images, using the IncuCyte FLR (Essen BioScience). The algorithm used to analyze images for cell confluence and apoptosis was from Confluence v1.5 in combination with the IncuCyte software, build 1001A Rev2. Two-tailed *t* tests have been performed to analyze the effect of FC treatment.

Gene Expression. MCF-7 cells were seeded at 180,000 cells per well in a six-well plate. Cells were pretreated 1 h with the indicated concentration of fusicoccin (FC) or MeOH as control, before the addition of 10 nM E2 for 5 h. Total RNA was isolated after treatment with TRIZOL reagent (Invitrogen), and cDNA was generated using an oligo(dT) primer and SuperScript-II Reverse Transcriptase (Invitrogen). From this, equal amounts of cDNA were used for expression analysis with SYBR Green (Applied Biosystems) using the MJ Opticon Monitor (BioRad). The data were analyzed with qgene96 (2), using β-actin as reference and pared *t* tests have been performed to analyze the effect of FC treatment.

Yeast Two-Hybrid and β-Gal Assay. Human ERα [full length and ligand-binding domain (LBD), amino acids 302–595] was cloned into the pGADT7 vector (Clontech), both WT ERα and T^{594}A point mutant. The pBD-GAL4 vector (Stratagene) was used to prepare pBD-14–3-3, pBD-ERα-LBD and pBD-ERα-LBD^{T594A}. Yeast transformations were performed using the lithium acetate (LiAc) protocol (3). Transformed colonies were resuspended in 50 μL Milli-Q water (MQ), of which 2 μL was spotted on selective double dropout (DDO) plates (SD-media without Leu/Trp) or triple dropout (TDO) plates [SD media without Leu/Trp/His containing 7.5 mM 3-Amino-1,2,4-triazole (3-AT)]. These plates were incubated at 30 °C for 1–3 d, to check for colony viability and interaction. To analyze the interaction strength a β-galactosidase (β-gal) assay was performed as described before (3). Colonies were only grown o/n in 3 mL SD-Leu,Trp (SD-LW) media (containing the indicated ligand or solvent as control), and resuspended in 3 mL Z buffer.

Competitive Anisotropy Measurements. Samples containing 100 nM FAM-SWpTY, 1 μM GST-Hs-14-3-3, various concentrations ERα peptide, and when indicated 10 μM FC in a final volume of 200 μL PBS were incubated for 2 h at room temperature (RT) before their anisotropy was measured with the Cary Eclipse fluorescence spectrophotometer (Varian). All anisotropy values are corrected for the background anisotropy of FAM-SWpTY alone.

The ERα peptide consisted of the last 15 (short) or 30 (long) amino acids of ERα, with T^{594} being phosphorylated (pERα). The short pERα peptide was dephosphorylated (dERα) by a 90-min treatment at 37 °C with calf intestinal alkaline phosphatase (CIP) (New England Biolabs), using the recommended buffer. The ERβ peptide consisted of the last 15 aa of ERβ, with S^{552} (penultimate Serine of ERβ) being phosphorylated (pERβ).

Peptide Affinity Determination. L_{tot} = total concentration of fluorescent ligand (FAM-SWpTY) in M, C_{tot} = total concentration of nonfluorescent competitor (ERα peptide) in M, R_{tot} = total 14-3-3 protein concentration in M, $[L]$ = free FAM-SWpTY concentration in M, $[C]$ = free ERα peptide concentration in M, $[R]$ = free 14-3-3 concentration in M, $[RL]$ = concentration of 14-3-3/FAM-SWpTY complex in M, $[RC]$ = concentration of

14-3-3/ERα peptide complex in M, K_d = dissociation equilibrium constant of FAM-SWpTY for the 14-3-3 protein in M, and K_c = dissociation equilibrium constant of ERα peptide for the 14-3-3 protein in M.

When the equilibrium is attained, the total 14-3-3 concentration (R_{tot}) is partitioned into the various complexes according to the following equilibria:

$$R + L \overset{K_d}{\rightleftharpoons} RL \quad \text{and} \quad R + C \overset{K_c}{\rightleftharpoons} RC.$$

Conservation of mass requires

$$R_{tot} = [R] + [RL] + [RC], \qquad \textbf{[S1]}$$

and the concentration of all complexes can be expressed as a function of the equilibrium constants and the free concentration of 14-3-3 (R) and FAM-SWpTY (L) or ERα peptide (C)

$$[RL] = \frac{[R][L]}{K_d} \qquad \textbf{[S2]}$$

and

$$[RC] = \frac{[R][C]}{K_c}. \qquad \textbf{[S3]}$$

By combining Eq. **S2** and Eq. **S3** with the mass-balance equation (Eq. **S1**) the free 14-3-3 concentration [R] can be solved and yields, after rearrangement, Eq. **S4**:

$$[R] = \frac{R_{tot}K_cK_d}{K_cK_d + [L]K_c + [C]K_d}. \qquad \textbf{[S4]}$$

The mass–balance equation for the total FAM-SWpTY concentration L_{tot} partitioned between its free (L) and 14-3-3 complexed (RL) form was derived as

$$L_{tot} = [L] + [RL]. \qquad \textbf{[S5]}$$

Substitution of Eq. **S2** in Eq. **S5** results, after rearrangement, in the free FAM-SWpTY concentration L

$$[L] = \frac{L_{tot}K_d}{K_d + R}. \qquad \textbf{[S6]}$$

In a similar way the free ERα peptide concentration C can be derived

$$[C] = \frac{C_{tot}K_c}{K_c + R}. \qquad \textbf{[S7]}$$

Substitution of Eq. **S6** and Eq. **S7** into Eq. **S4** to express the free 14-3-3 concentration (R) results, after rearrangement and collecting terms, in the cubic Eq. **S8**:

$$R^3 + R^2\left(K_d + K_c + L_{tot} + C_{tot} - R_{tot}\right) + R\left(K_dK_c + K_cL_{tot} + K_dC_{tot} - K_dR_{tot} - K_cR_{tot}\right) - K_cK_dR_{tot} = 0. \qquad \textbf{[S8]}$$

In the competitive anisotropy measurements, the ERα peptide is added to a solution containing a fixed amount of FAM-SWpTY (L_{tot}) with known K_d and fixed 14-3-3 concentration. Because the anisotropy originates from the RL complex, the total anisotropy can be determined by

$$A = A_0 + (A_{max} - A_0)\frac{[RL]}{L_{tot}}, \qquad \textbf{[S9]}$$

where A is the observed anisotropy, A_0 the anisotropy for free FAM-SWpTY, and A_{max} the maximum anisotropy of a solution containing FAM-SWpTY and 14-3-3 protein only. The concentration [RL] was calculated by solving Eq. **S8** via a fixed-point iteration algorithm, followed by substitution of the free concentration R into Eq. **S6** and Eq. **S2**. Nonlinear least square minimization of the fluorescent anisotropy data was performed using Eq. **S9** with the Matlab function lsqnonlin, a subspace trust region method based on the interior-reflective Newton method. The adjustable parameters in the nonlinear least square minimization are the ERα peptide dissociation equilibrium constant (K_c) and the maximum anisotropy (A_{max}). To prevent entrapment in a local minimum during optimization, various different initial parameters were defined in the interval $K_c \in [10^{-8}, 10^{-3}]$ and $A_{max} \in [10^{-2}, 10^{0}]$, using a latin hypercube sampling method (Matlab function lhsdesign). The best fit (defined as the fit with the lowest squared 2-norm of residuals) was taken as the final solution for the optimized K_c and A_{max} values. The lower bounds of the 95% confidence intervals and SEs were calculated based on the observed Fisher information matrix using established procedures (4).

Pull-Down Assays and Immunoprecipitation. MCF-7 cells were seeded in 10-cm dishes 3 d before the experiment. Cells were washed 2× with PBS (supplemented with 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$), before lysis with 0.75 mL Nonidet P-40 lysis buffer/plate [50 mM Tris·HCl, 1% Nonidet P-40, 100 mM NaCl, 5 mM EDTA, 2 mM $MgCl_2$, 10% glycerol, 1 mM irreversible serine protease inhibitor (PefaBloc) SC (Fluka), 10 μM cantharidin, 1× protease inhibitor mixture (Roche), pH 7.4]. The isolated protein concentration was determined with a bicinchoninic acid protein assay (BCA assay) (Thermo Scientific). Equal amounts of protein were mixed with GST-ERα-LBD or FC-coated beads (FC was coupled covalently to magnetic hydrazide beads after changing the vinyl group into a reactive aldehyde) and 100 μM noninteracting peptide (NIP) (ERYMGICMRKQYNNFVPVCLRS) or the 14-3-3 interacting peptide R18 (PHCVPRDLS**WLDL**EANMCLP; 14-3-3 interaction motif is in bold) (5), and incubated for 1 h at RT while rotating. Beads were washed with lysis buffer, before a mock elution and R18 elution were performed. Next the beads were resuspended in 100 μL SDS-sample buffer, and the associated endogenous ERα and/or 14-3-3 proteins were visualized by Western blotting.

HEK293 cells were transferred to a six-well plate, 1,000,000 cells per well. Cells were transfected with 1 μg of pMSCV-HA-ERα vector and/or 1 μg pMSCV-GFP-ERα vector, using 24 μg PEI (Polyethyleneimine) (Sigma-Aldrich). Cells were treated for 48 h with 10 μM fusicoccin. After the treatment the cells were lysed with radio-immunoprecipitation assay (RIPA) buffer. The IP for the HA-ERα was performed with monoclonal anti-HA-agarose antibody (Sigma-Aldrich), according to the manufacturer's protocol. HA-ERα was eluted from the anti-HA agarose with 50 mM NaOH (50 μL). Twenty microliters of the elutions was used for Western blotting.

Estrogen Response Element Luciferase Assay. U2OS cells were seeded at 40,000 cells per well in a 24-well plate. Cells were transfected with 15 ng pMSCV-ERα (WT or Δ4), 0.2 μg estrogen response element-tk-luciferase (ERE-tk-Luc) reporter plasmid, and 1 ng Renilla-Luc per well using PEI (Polysciences) for 16 h, before being treated with the indicated concentration of E2 and 10 μM FC or MeOH as control. After 48 h of treatment the ERα activity was determined with a Dual-Luciferase Reporter Assay (Promega), according to the manufacturer's instruction. The

luminescent intensities were recorded on a Berthold Centro XS3-LB-960 (Berthold Technologies). The ERE-luciferase signal was first normalized over the Renilla-luciferase signal; this ratio was subsequently normalized over the ratio of untreated cells. Two-tailed *t* tests were performed to analyze the effect of FC treatment.

ChIP, ChIP-Seq, and Analysis. ChIPs were performed as described previously (6) using the ERα antibody SC-543 (Santa Cruz). Isolated DNA was amplified as described (6). Sequences were generated by the Illumina HisEq. 2000 genome analyzer (using 50-bp reads) and aligned to the Human Reference Genome (assembly hg19, February 2009). Enriched regions of the genome were identified by comparing the ChIP samples to input using the model-bases analysis for ChIP-seq (MACS) peak caller (7) version 1.3.7.1.

ChIP-seq data snapshots were generated using the Integrative Genome Viewer IGV 2.1 (www.broadinstitute.org/igv). Motif analysis were performed through the Cistrome (www.cistrome.org), applying the SeqPos motif tool (8). Heatmaps and quantifications thereof were generated using seqminer (9), with a 5-kb window around the peak region.

Crystallization, Data Collection, and Structure Determination. The complex of 14-3-3σΔC (amino acids 1–231) and the short pERα peptide was preformed in a 1:1.5 molar stoichiometry and a final concentration of 10 mg/mL in 20 mM Hepes, 2 mM $MgCl_2$, 2 mM Tris(2-carboxyethyl)phosphine (TCEP), pH 7.6 for 1 h on ice and then set up in EasyXtal 15-well Tools (Qiagen) using the hanging-drop method by mixing 1 μL protein solution and 1 μL reservoir solution and equilibrating against 1 mL reservoir solution (0.095 M Na Hepes pH 7.5, 25.6% (wt/vol) PEG 400 (polyethylene glycol 400), 0.19 M $CaCl_2$, and 5% glycerol). The obtained crystals grew within a week to dimensions of ∼400 × 100 × 100 μm and could be directly flash cooled in mother liquor using liquid nitrogen. For the soaking, a 10-mM FC stock solution in ethanol was dried on a coverslip and 1- to 3-wk-old crystals were, together with their mother liquid, transferred to the FC dry mass. After 2–5 d of incubation at 4 °C, the crystals were flash cooled for data collection.

Data were collected at 1.548 Å on a rotating anode Nonius/Bruker AXS MICRO Star and a MARdtb image plate detector. The crystal-to-detector distance was 150 mm and 100 images were obtained with an oscillation of 1.00° per image. X-ray diffraction data were processed and scaled using XDS (10). The structure was solved using Protein Data Bank (PDB) ID 3P1N as a template. The obtained model was subjected to iterative rounds of model building and refinement using Coot (11) and REFMAC (12). Figures were created using PyMOL. The structures were deposited in the PDB under ID codes PDB 4JC3 and PDB 4JDD.

Mass-Spectrometry Analysis of Immunopurified ERα C-Terminal Phosphopeptide. ***Phosphopeptide enrichment.*** Cell lysate (11.2 mg) from MCF-7 cells grown for 5 h with 5 μM MG-132 and 30 μM FC was digested with trypsin and the resulting peptides were desalted on a Seppak C18 cartridge (300 mg) (Waters). Peptides were bound and washed in 0.1% TFA and eluted in 40% (vol/vol) ACN+0.1% TFA. After elution, peptides were frozen in liquid nitrogen and freeze dried. Peptides were dissolved in 700 μL PBS, incubated with 20 μg pT^{594} rabbit polyclonal antibody for 2 h at 4 °C. Protein G agarose beads (200 μL) (Santa Cruz) were added to the lysate and incubated for 2 h to bind the antibody-peptide complex. After washing with 4 × 1 mL PBS the beads were resuspended in 0.2 mL MilliQ water and transferred to a Vivacon 500 spinfilter (10 K cutoff) (Sartorius), allowed to settle, and residual water was removed by aspiration. Peptides were eluted in 50 μL 0.15% TFA and centrifuged at 14,000 × *g* for 5 min. Eluted peptides were desalted on a 20 μL C18 STAGE tip (ThermoScientific), dried in a vacuum centrifuge, and redissolved in loading solvent [4% (vol/vol) ACN+0.5% TFA].
LC-MS/MS. Peptides were trapped on a 100-μm ID × 2 cm fused silica column packed with 5 μm ReproSil Pur C18 aqua (A. Maisch, Ammerbuch-Entringen, Germany) at 6 μL/min in 0.5% HAc. Peptides were separated in a 120-min gradient of 4% (vol/vol) ACN to 32% (vol/vol) acetonitrile (ACN) in 0.5% HAc at 300 nL/min on a 75-μm ID fused silica column packed with 3 μm ReproSil Pur C18 aqua. NanoLC separation was performed on a Dionex U3000 nanoLC (Dionex). Eluting peptides were ionized on-line at 2.2 kV and sprayed into a Q Exactive mass spectrometer (ThermoScientific). MS spectra were acquired from *m/z* 350–1400 in data dependent mode [top-10 higher energy collisional dissociation (HCD)] at an automatic gain control (ACG) target of 3E6 at a resolution of 70.000 (at *m/z* 200). MS/MS spectra were acquired in the HCD cell at 25% normalized collision energy, an AGC target of 2E5, and a resolution of 12.500 (at *m/z* 200). MS/MS isolation width was set to 4 Da.

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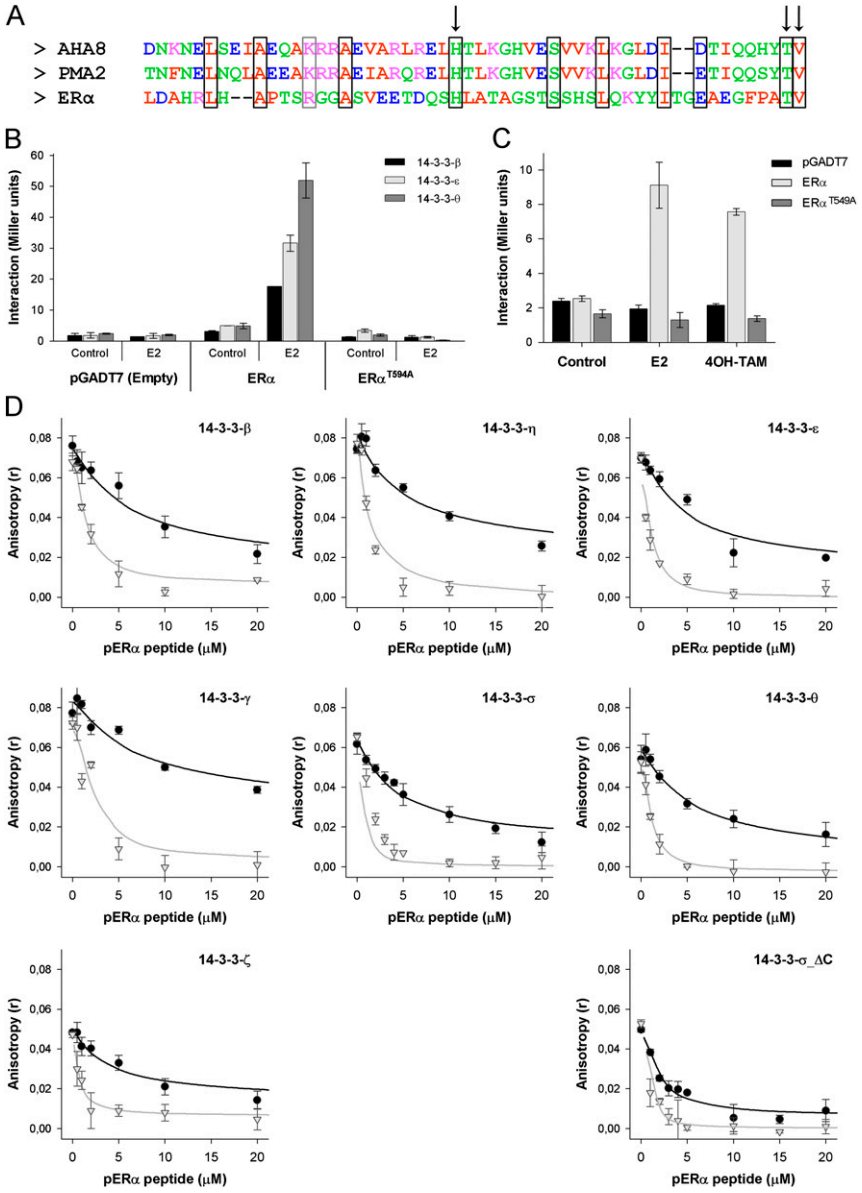

Fig. S1. C-terminal ERα F domain tip interacts with 14-3-3 proteins. (*A*) Alignment of the last 52 amino acids of human ERα and the H^+-ATPase of *Nicotiana plumbaginifolia* (PMA2) and *Arabidopsis thaliana* (AHA2). The three amino acids most important for the FC/14-3-3/receptor complex formation are indicated (↓). (*B*) Yeast two-hybrid assay; ligands stimulate the interaction of various 14-3-3 isoforms with WT ERα-LBD, but not ERα-LBD^{T594A}. (*C*) Quantification of the interaction of 14-3-3θ with full-length WT ERα and $ER\alpha^{T594A}$, in the presence of ERα ligands. (*D*) Analysis of the interaction between the short (15 aa) phosphorylated C-terminal ERα peptide (pERa) and all human 14-3-3 isoforms and 14-3-3σ-ΔC. Curves are fitted as described in *SI Material and Methods*.

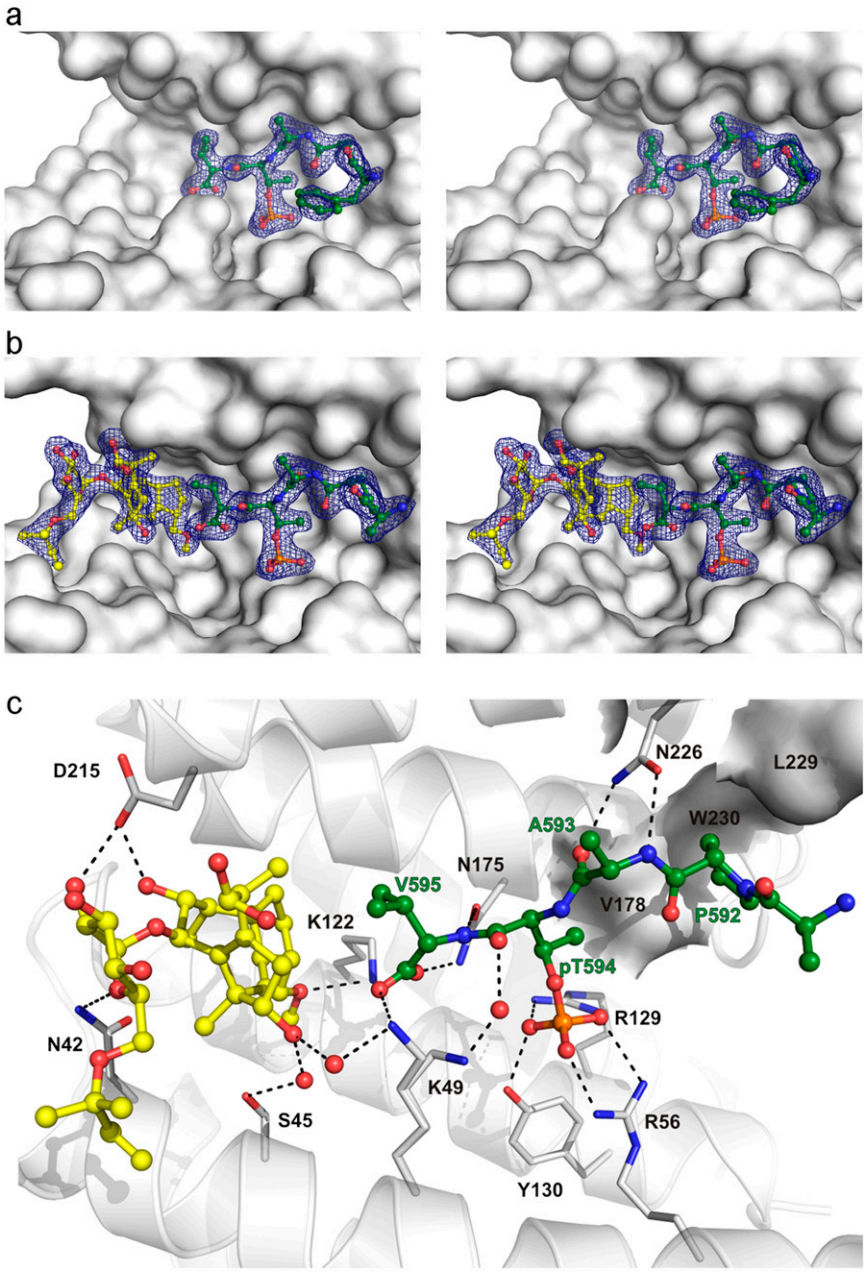

Fig. S2. Cocrystallization of 14-3-3 and the pERα peptide and soaked fusicoccin. (*A*) Stereoview of the pERα peptide (green) in the 2Fo-Fc density map (contoured at 1σ) occupying half of the amphipathic 14-3-3 binding groove (white surface). (*B*) Stereoview of the pERα peptide (green) and fusicoccin (yellow) in the 2Fo-Fc density map (contoured at 1σ) occupying the amphipathic full binding groove of a 14-3-3σ monomer (white). (*C*) Detailed view of the interaction of ERα (green) and fusicoccin (yellow) with 14-3-3σ (white). Polar interactions are indicated by dashed lines and 14-3-3 residues implicated in these interactions are labeled in black. Hydrophobic 14-3-3 interaction surface is depicted as white solid surface. Red spheres are water molecules conferring polar interactions.

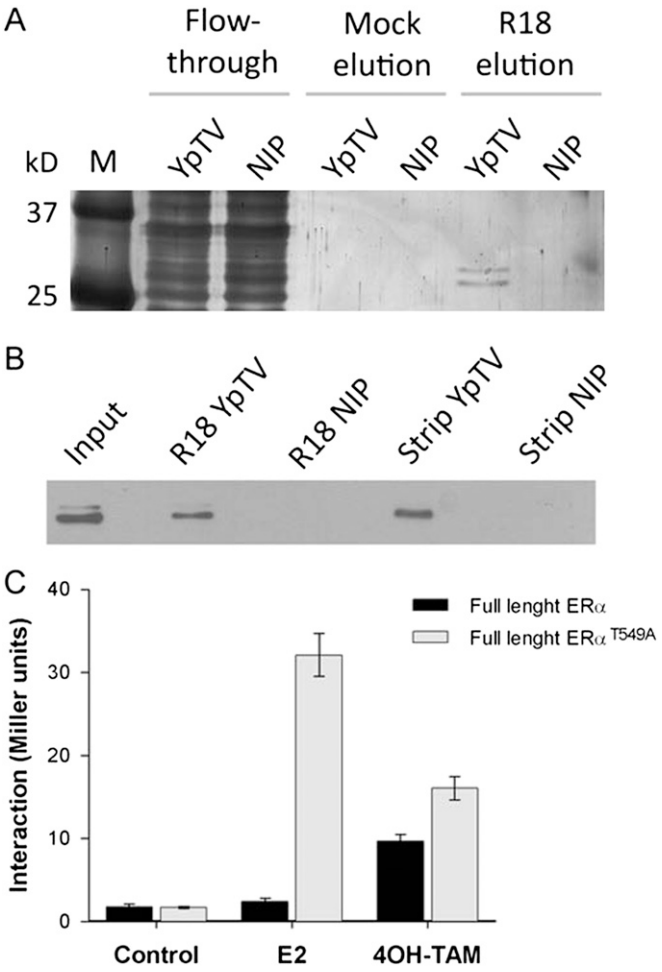

Fig. S3. Functional test on FC beads and 14-3-3 interaction with full-length ERα affects dimerization. (*A*) Specifically bound endogenous 14-3-3 proteins can only be eluted from FC-coupled beads (FC beads) with the 14-3-3 interacting R18 peptide, when bound in the presence of a C-terminal peptide derived from the plant H^+-ATPase (YpTV) and not with a noninteracting peptide (NIP). (*B*) 14-3-3 Western blot on the pull-down samples of *A* confirms the specificity of the 14-3-3 interaction to the FC-coupled beads. (*C*) Yeast two-hybrid assay for ERα dimerization: The full-length-ERα/ERα-LBD and full length-ERα^{T594A}/ERα-LBD^{T594A} dimerize in the presence of ERα ligands.

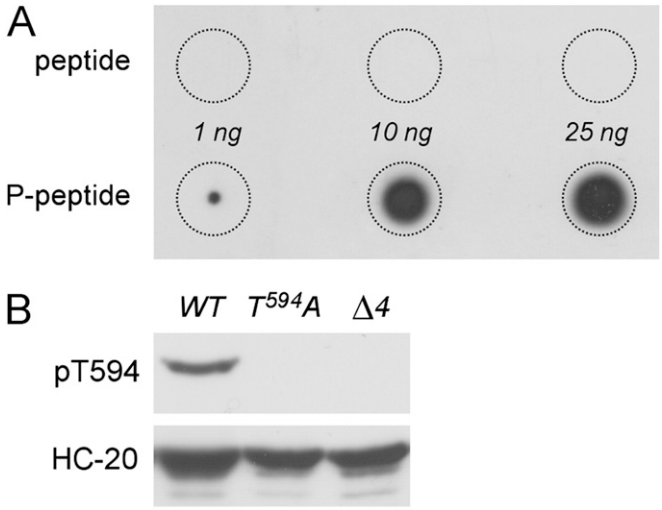

Fig. S4. The pT^{594}-antibody is specific for the T^{594}-phosphorylated ERα C-terminal epitope. (*A*) Dot blot with different amounts of nonphosphorylated (peptide: KYYITGEAEGFPATV) and phosphorylated peptide (P-peptide: KYYITGEAEGFPAT^{P}V) spotted. The blot was probed with the pT^{594}-ERα antibody. (*B*) Western blot with HC-20 antibody and pT^{594}-ERα antibody on cell lysate of HEK293 cells expressing wild-type ERα, ERα-T^{594}A, and ERα-Δ4; cells were grown with FC (10 μM) for 24 h. Whereas the HC-20 antibody shows that ERα is expressed in all three transfected cells, the pT^{594}-ERα antibody only recognizes a band in wild-type ERα transfected cells. This demonstrates that the pT^{594}-ERα antibody is specific for the T^{594} phosphorylated ERα protein.

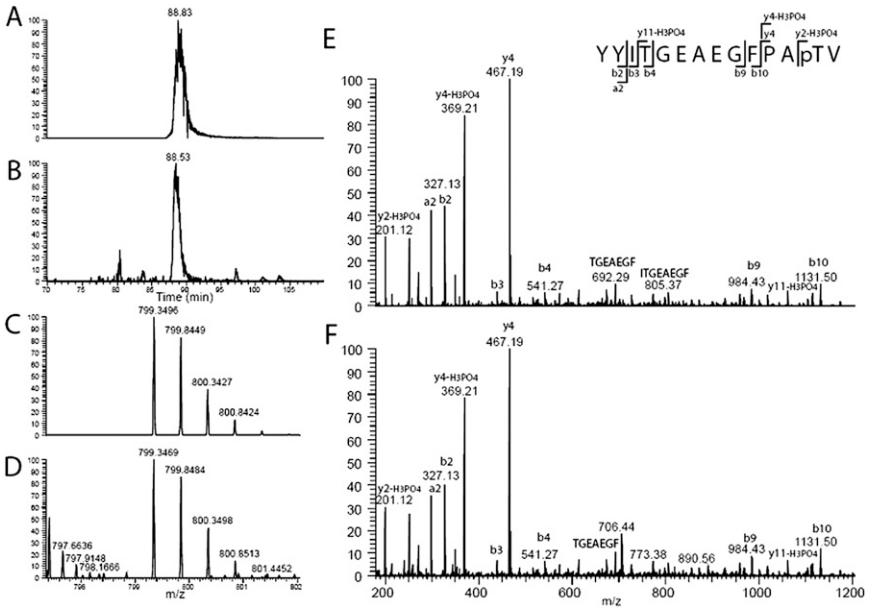

Fig. S5. Mass-spectrometry analysis of the C-terminal ERα peptide shows phosphorylation of the penultimate threonine, T^{594}. (*A*) Extracted ion chromatogram of synthetic ERα C-terminal tryptic phosphopeptide YYITGEAEGFPApTV phosphorylated at T^{594}. The extraction window is *m/z* 799.31–38. (*B*) The corresponding peptide from MCF-7 digest, after IP is shown. In *A* and *B* coelution at 89–90 min is observed. (*C* and *D*) Accurate intact mass of the peptide, corresponding to the $[M+2H]^{2+}$ monoisotopic peak at *m/z* 799.3447, for the synthetic and MCF7-derived phosphopeptide, respectively. (*E*) MS/MS spectrum of synthetic YYIT-GEAEGFPApTV. The absence of basic residues (protein C terminus) and presence of acidic residues, as well as a phosphate group, result in a poor MS/MS spectrum. However, the y2-H_3PO_4 (*m/z* 201.1234), y4 (*m/z* 467.1901), and y4-H_3PO_4 (*m/z* 369.2132) ions conclusively localize the phosphate group to T^{594}. (*F*) Corresponding MS/MS spectrum for the MCF-7–derived phosphopeptide. The fragment ions b2, a2, y2-H_3PO_4, y4-H_3PO_4, and y4 ions are detected in similar intensity ratios as observed for the synthetic phosphopeptide identifying the MCF-7–derived phosphopeptide and localizing the site of phosphorylation to T^{594}.

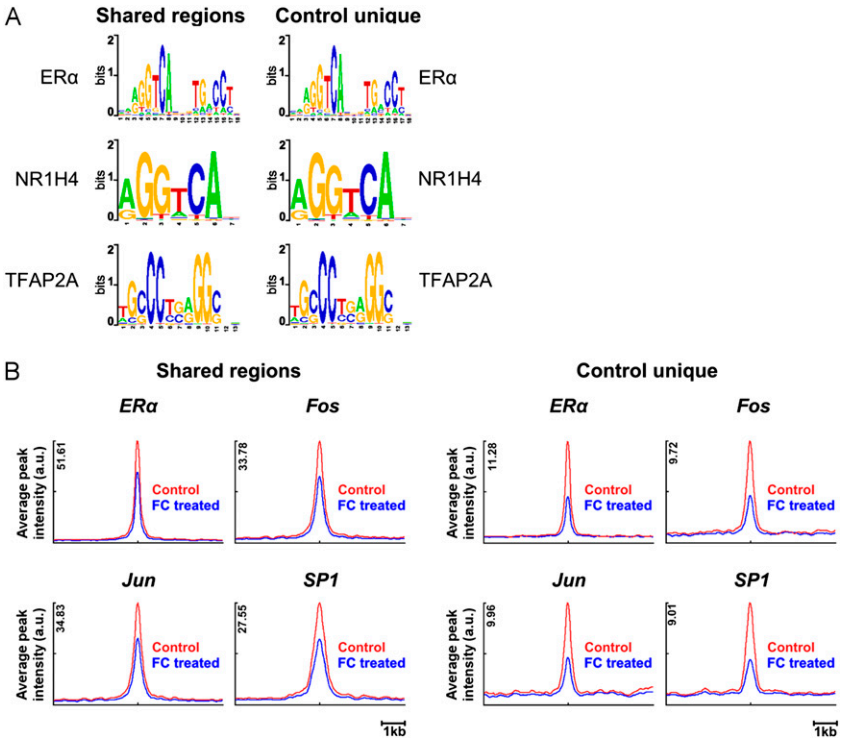

Fig. S6. ERα/DNA binding mode is not selectively altered by FC treatment. (*A*) Top three motifs for the shared (*Left*) and control unique (*Right*) ERα binding events. (*B*) Average peak intensity of shared (*Left*) and control unique (*Right*) ERα binding events mediated directly by ERα or through specificity protein 1 (SP1) and complexes of the transcription factors Fos and Jun (Fos/Jun) as identified through motif scanning of ERα-binding sites, in the absence (red) or presence (blue) of FC.

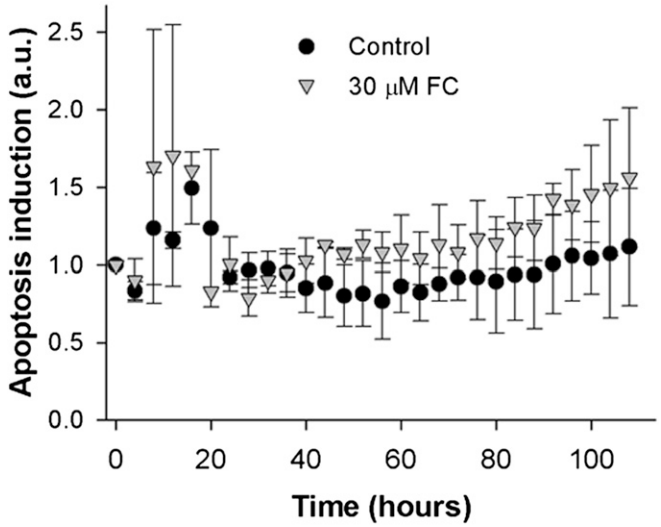

Fig. S7. Fusicoccin does not induce apoptosis in E2-treated MCF-7 cells. E2-induced MCF-7 apoptosis was assessed over time in the presence of various FC concentrations (30 μM FC shown here), by analyzing the CellPlayer 96-Well Kinetic Caspase-3/7 Apoptosis assay kit. For all FC concentrations, no significant apoptosis induction was observed.

Table S1. FC enhances the affinity of the short pERα peptide for all 14-3-3 isoforms

K_d, μM, ±SEM	Control	10 μM FC	Affinity increase
β	0.99 (0.29)	0.11 (0.03)	9.1
η	0.94 (0.32)	0.19 (0.06)	4.9
ε	0.69 (0.21)	0.07 (0.04)	10.4
γ	1.09 (0.36)	0.15 (0.11)	7.1
σ	1.61 (0.32)	0.10 (0.03)	16.4
θ	0.90 (0.20)	0.06 (0.01)	15.1
ζ	0.57 (0.18)	0.05 (0.02)	10.9
σ-ΔC	0.33 (0.07)	0.07 (0.05)	5

Interaction affinity (K_d) of the short pERα peptide for all 14-3-3 isoforms, as calculated from the curves in Fig. S1*D*. Values in parenthesis are +/– SEM.

Table S2. Data collection and refinement statistics for 4JC3 and 4JDD

	4JC3	4JDD
Data collection		
Space group	$C222_1$	$C222_1$
Cell dimensions		
A, b, c, Å	82.2, 112.49, 62.45	82.49, 111.43, 62.24
A, β, γ, °	90, 90, 90	90, 90, 90
Resolution, Å	19.52–2.05 (2.1–2.05)	19.80–2.1 (2.15–2.0)
R_{meas}, %	8.4 (34.9)	12.0 (51.0)
I/σI	24.16 (6.85)	17.79 (4.77)
Completeness, %	99.3	100.0
Redundancy	14.94 (14.7)	15.1 (14.9)
Refinement		
Resolution, Å	19.5–2.05 (2.1–2.05)	19.80–2.1 (2.15–2.0)
No. reflections	141777	135424
Unique reflections	18518	17090
R_{work}/R_{free}	0.1778/0.235	0.1924/0.2316
No. atoms		
Protein	1850	1847
Ligand	42	42 (FUSICOCCIN 48)
Solvent	177	101
B factors, $Å_2$		
Protein	14.3	19.70
Ligand	23.7	26.78 (FUSICOCCIN 23.14)
Water	19.01	21.0
R.m.s. deviations		
Bond lengths, Å	0.019	0.020
Bond angles, °	1.767	1.992
Ramachandran plot		
Favored, %	98.7	97.9
Allowed, %	1.7	2.1
Generously allowed, %	0	0
Disallowed, %	0	0

Values in parenthesis are for the highest resolution shell.