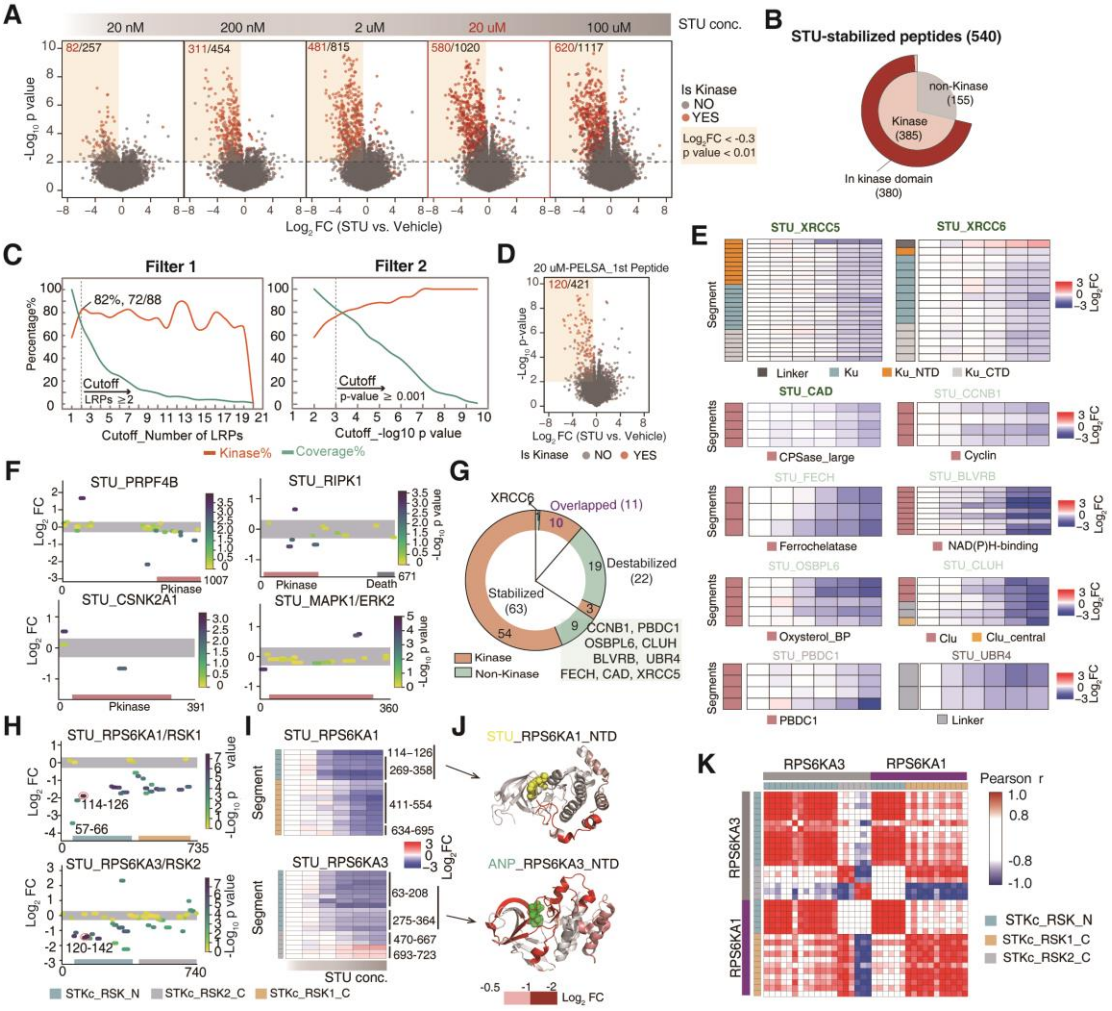
A Two-Dimensional Peptide-Centric Stability Assay Maps Multidimensional Target Landscapes Unveiling 17β-Estradiol (E2)-GPX4 Binding-Induced Ferroptosis

Supplementary Information

SUPPLEMENTARY FIGURES AND FIGURE CAPTION



Supplementary Figure 1. 2D-PELSA strategy for highly reliable determination of ligand-binding proteins, binding regions, and binding affinity.

(A) Volcano plot visualization of all peptides from PELSA analyses of K562 lysates exposed to increasing staurosporine concentrations. Red dots were kinase proteins annotated in uniport or KinHub. log2 fold changes and -log10 p value were plotted as x- and y- axis, respectively. The right boundary and lower boundary of the red shadow denote log2FC of -0.3 and -log10 p value of 2, respectively. The red and block number represent the number of peptides from kinase and total peptides, respectively.

(B) The doughnut chart shows the number of kinase peptides in the STU-stabilized LRPs.

(C) Assessment of the proportion (red) and coverage (green) of kinase proteins among the stabilized proteins by 2D-PELSA under different threshold of LRPs and p-value.

(D) Volcano plot visualization of all proteins from a PELSA analysis of HeLa lysates exposed to 20 μM E2. The peptides with the lowest p value among all quantified peptides of the same protein was selected to represent its corresponding protein. The right boundary and lower boundary of the red shadow denote log2FC of -0.3 and -log10 p value of 2, respectively. The red and block number represent the number of kinases and total proteins, respectively.

(E) Heat map representation of log2 peptide fold changes for 10 non-kinase targets with increasing E2 concentrations (0 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM).

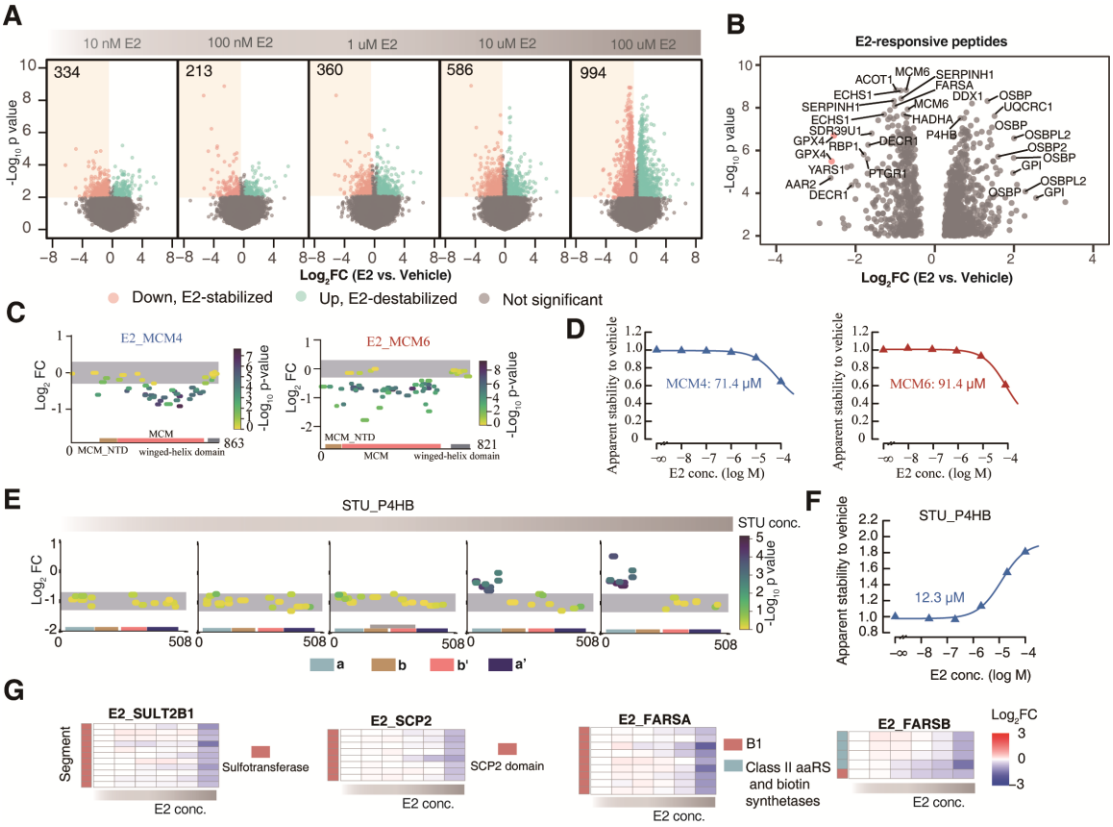
(F) 2D local stability profiles of multiple proteins that destabilized peptide has the lowest p value as measured by 2D-PELSA.

(G) Overlap analysis of STU-stabilized segments and STU-destabilized segments as measured by 2D-PELSA.

(*H-I*) 2D and 3D local stability profiles of RPS6KA1 and RPS6KA3 as measured by 2D-PELSA.

(*J*) Structure of N-terminal RPS6KA1 (PDB: 2Z7R) and RPS6KA3 (PDB:3G51). The quantified LRPs with log2FC < -0.3 are colored in red.

(*K*) Pearson correlation analysis of the dose-dependent curves of E2-LRPs from RPS6KA1 and RPS6KA3.



Supplementary Figure 2. 2D-PELSA allows proteomes scale investigation of E2-binding spectrum in BT474 cell lysates.

(*A*) Volcano plot visualization of all peptides from PELSA analyses of BT474 lysates exposed to increasing E2 concentrations. The right boundary and lower boundary of the red shadow denote log2FC of -0.3 and -$\log_{10}$ p value of 2, respectively.

(*B*) Volcano plot visualization of E2-responsive peptides (LRPs) of BT474 cell lysates by 2D-PELSA analysis.

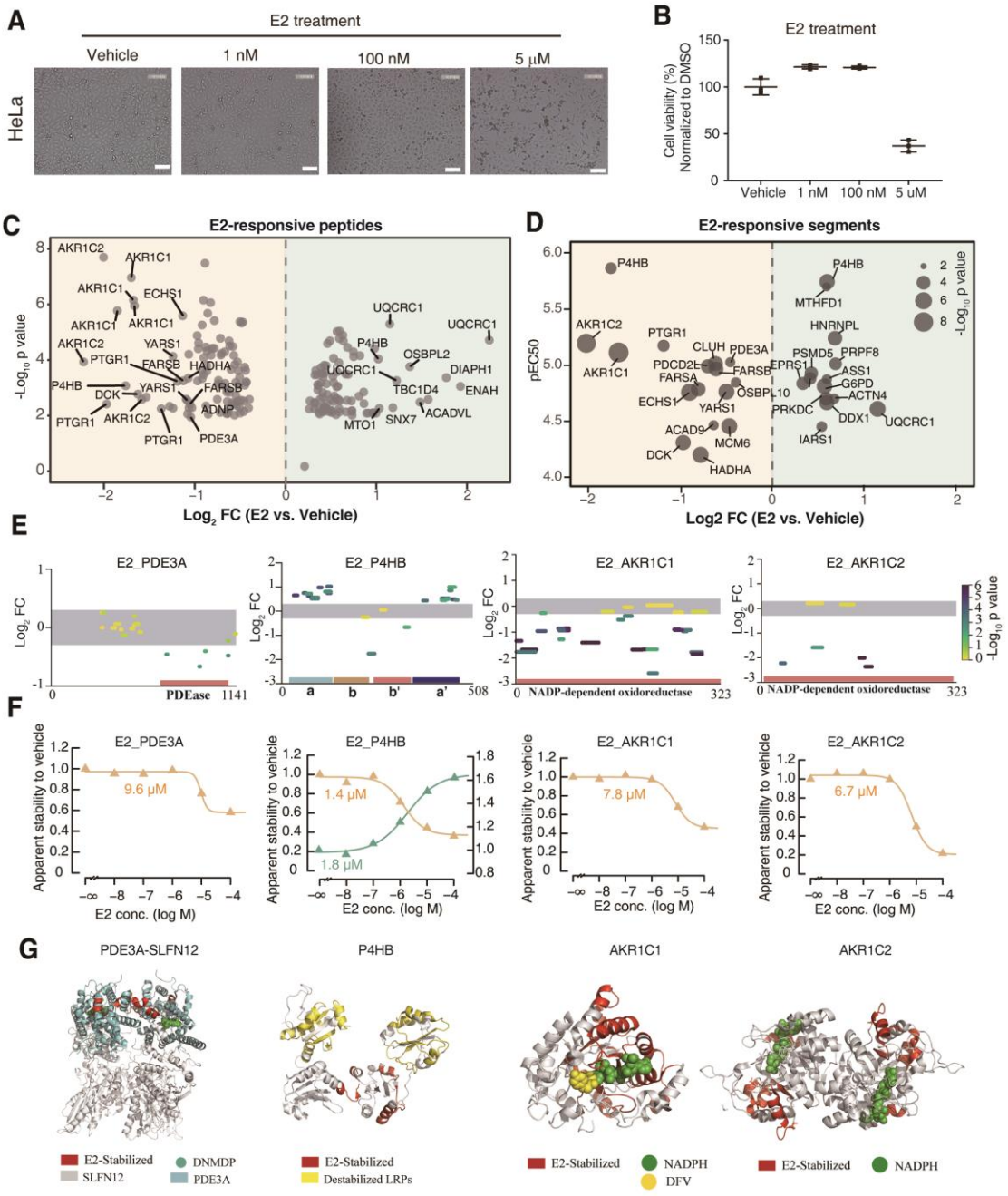
(*C*) 2D local stability profiles of MCM4 and MCM6 as measured by 2D-PELSA.

(*D*) Dose-response profiles of MCM4 and MCM6 as measured by 2D-PELSA.

(*E*) 2D local stability profiles of P4HB demonstrating stability changes exposed to increasing STU concentrations as measured by 2D-PELSA in K562 cell lysates.

(*F*) Dose-response profile of P4HB as measured by 2D-PELSA.

(*G*) 3D local stability profiles of SULT2B1, SCP2, FARSA and FARSB as measured by 2D-PELSA. Heat map representation of log2 peptide fold changes of LRPs with increasing E2 concentrations (0 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM).



Supplementary Figure 3. 2D-PELSA allows proteomes scale investigation of E2-binding spectrum in HeLa cell lysates.

(*A***)** Microscope imaging of HeLa cells in the presence of increasing concentrations of E2 for 2d; scale bars: 100 μm.

(*B*) CCK8 assay of HeLa cells in the presence of increasing concentrations of E2 for 2 days. n = 3.

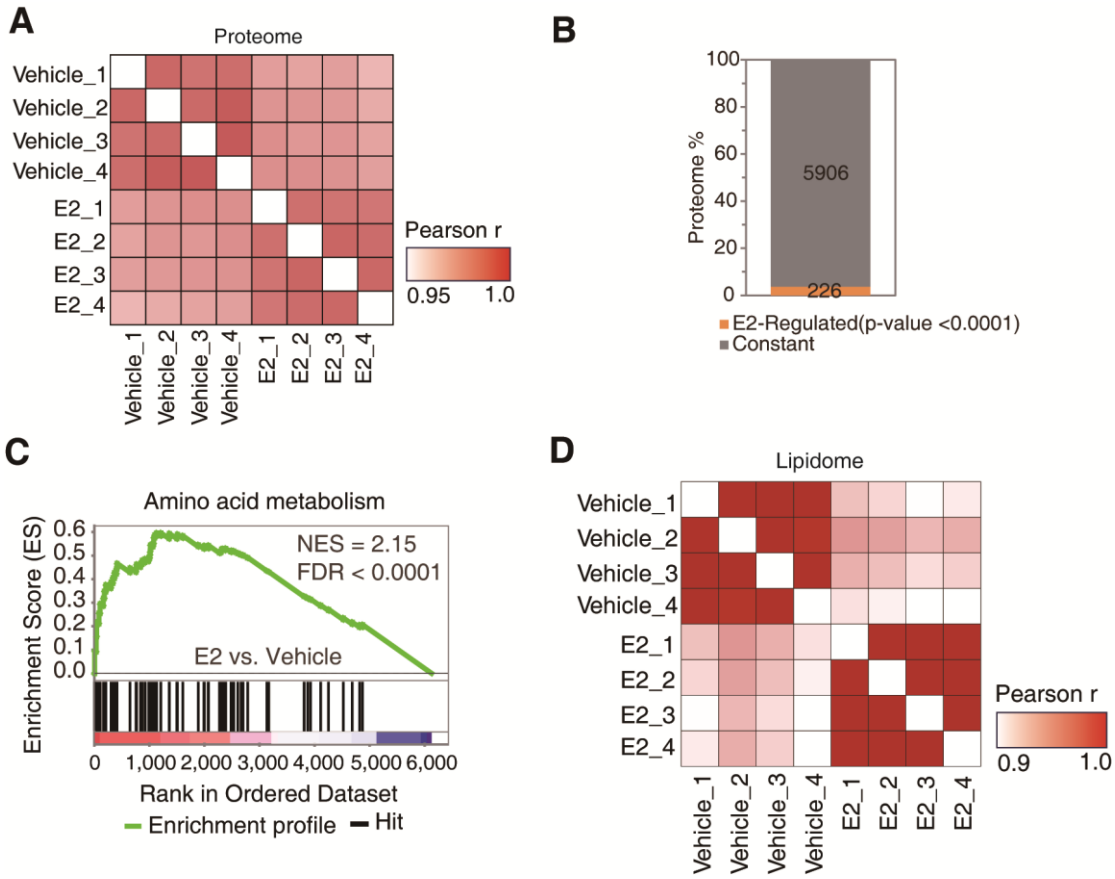
(*C*) Volcano plot visualization of E2- LRPs of HeLa cell lysates by 2D-PELSA analysis.

(*D*) Volcano plot visualization of E2-responsive segments as measured by 2D-PELSA.

(*E*) 2D local stability profiles of multiple known E2-binding proteins as measured by 2D-PELSA.

(*F*) Dose-dependent profiles of multiple known E2-binding proteins as measured by 2D-PELSA.

(*G*) Structure of PDE3A (PDB: 7EG1), P4HB (PDB: 4ekz), AKR1C1 (PDB: 8JP2), and AKR1C2 (PDB:2HDJ). The quantified LRPs with log2FC < -0.3 are colored in blue and these with log2FC >0.3 are colored in red.



Supplementary Figure 4. Quantitative proteome and lipidome profiling of E2-regulated proteins in BT474 cells.

(*A*) Pearson correlation analysis of protein intensities between samples in the E2-treated and DMSO-treated cell samples using Prism.

(*B*) Bar chart showing the proportion of regulated proteins per experiment by (uM) E2 (empirical Bayes moderated t-test, p value < 0.0001).

(*C*) GSEA of the differentially expressed genes in E2 versus Vehicle treated BT474 cells showing significant enrichment of amino acid metabolism in the proteomics.

(D) Pearson correlation analysis of lipid intensities between samples in the E2-treated and DMSO-treated cell samples using Prism.