Supporting Information

Combination of Metabolomics with Cellular Assays Reveals New Biomarkers and Mechanistic Insights on Xenoestrogenic Exposures in MCF‑7 Cells

Sarah Potratz,[*,†] Patrick Tarnow,[†] Harald Jungnickel,[†] Sven Baumann,[‡,§] Martin von Bergen,[‡,∥,⊥] Tewes Tralau,[†] and Andreas Luch[†]

[†]Department of Chemical and Product Safety, German Federal Institute for Risk Assessment (BfR), Max-Dohrn-Strasse 8-10, 10589 Berlin, Germany

[‡]Department of Molecular Systems Biology, UFZ−Helmholtz-Centre for Environmental Research, Permoserstrasse 15, 04318 Leipzig, Germany

[§]Institute of Pharmacy, Faculty of Biosciences, Pharmacology and Psychology, University of Leipzig, Bru¨derstrasse 34, 04103 Leipzig, Germany

[∥]Institute of Biochemistry, Faculty of Biosciences, Pharmacology and Psychology, University of Leipzig, Bru¨derstrasse 34, 04103 Leipzig, Germany

[⊥]Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Fredrik Bajers Vej 7K, 9220 Aalborg, Denmark

* Correspondence: Sarah.Potratz@bfr.bund.de

Systems Toxicology II

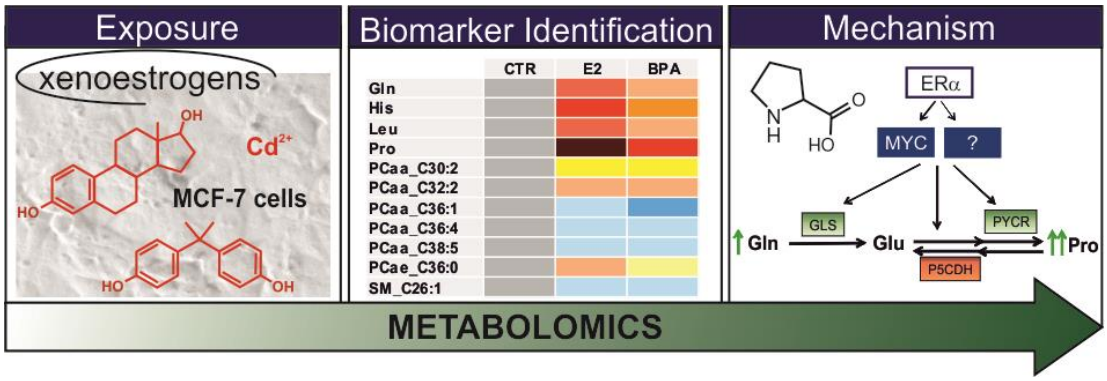
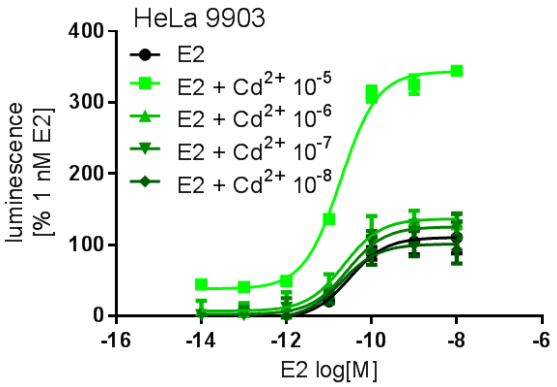
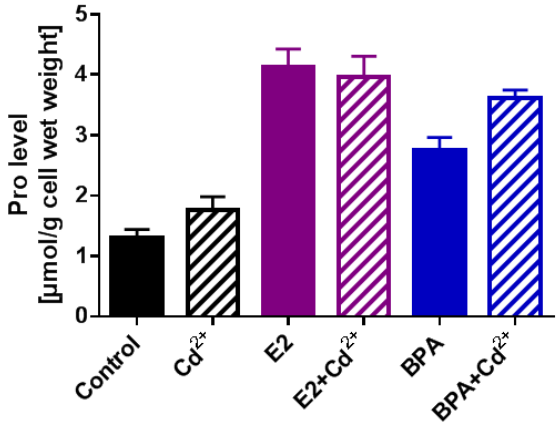


Figure S1.



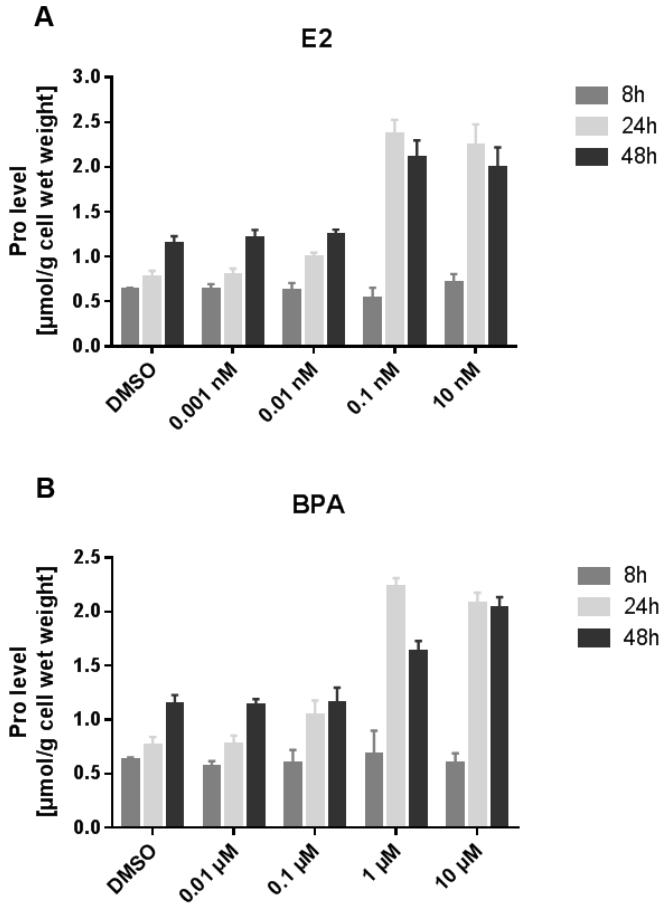
Effect of Cd^{2+} in a luciferase-dependent ER transactivation assay. HeLa9903 cells containing an ER responsive firefly luciferase construct were stimulated with increasing concentrations of E2 in the absence (solvent control) or presence of increasing concentrations of Cd^{2+} (range: 10^{-5} to 10^{-8} µM) for 24 hours as indicated. Signals were corrected for background fluorescence and plotted relative to the signal strength of the positive control (1 nM E2). The data shown represent the mean ± SEM of three independent experiments. Abbreviation used: E2 = 17β-estradiol.

Figure S2.



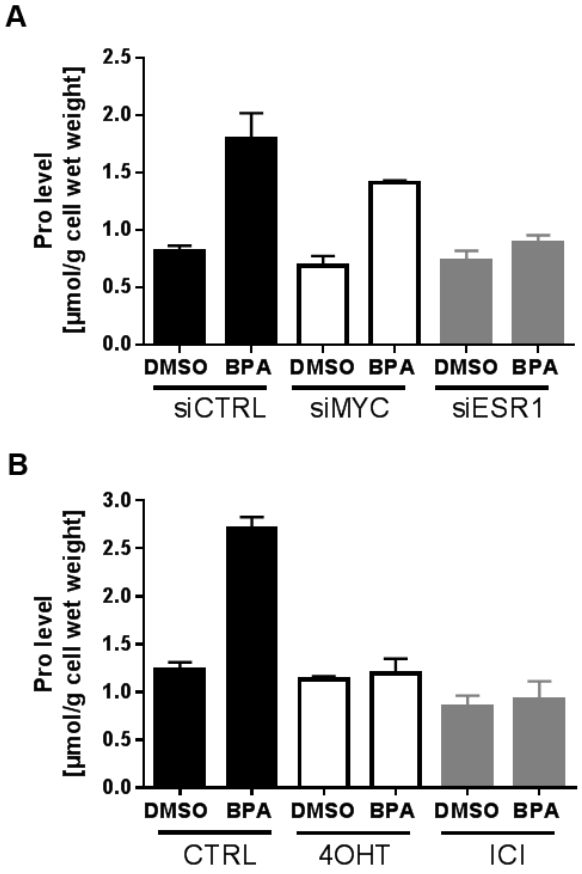
Metabolomics results for Pro. (A) Mass spectrometric results of Pro levels after treatment with Cd^{2+} (0.1 nM), E2 (10 nM) and BPA (10 μM) as indicated. Data represent the mean ± SEM of six replicates.

Figure S3.



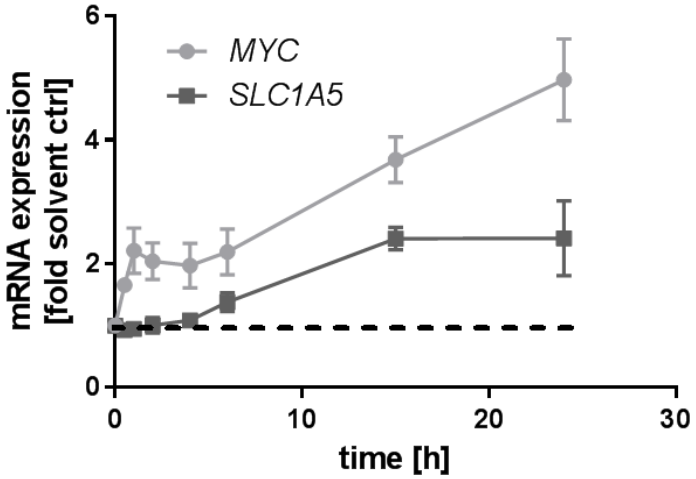
Pro levels after E2 and BPA treatments over time. MCF-7 cells were stimulated with different concentrations of (A) E2 and (B) BPA and Pro levels were determined after 8, 24 and 48 hours. The data represent mean ± SEM from three independent experiments. Abbreviations used: BPA = bisphenol A, E2 = 17β-estradiol, Pro = proline.

Figure S4.



Influence of *ESR1* or *MYC* knockdown and ER inhibitors on Pro levels upon BPA treatment. (A) MCF-7 cells were transfected with control siRNA (siCTRL) or siRNA against *ESR1* (siESR1) or *MYC* (siMYC) and treated with 10 µM BPA or solvent only for 24 h. (B) MCF-7 cells were stimulated with BPA (10 µM) or with BPA plus ER inhibitors 4-hydroxytamoxifen (4OHT, 1 µM) or ICI-182,780 (ICI, 0.1 µM) for 24 h. The data represent mean ± SEM from three independent experiments. Abbreviations used: BPA = bisphenol A, *ESR1* = estrogen receptor 1 (ERα gene), ICI = ICI-182,780, 4OHT = 4-hydroxytamoxifen, Pro = proline.

Figure S5.



mRNA expression of *MYC* and the Gln transporter (*SLC1A5*) upon E2 stimulation over time. MCF-7 cells were treated with E2 (1 nM) or solvent only and *MYC* and *SLC1A5* mRNA levels were assessed subsequently over 24 h. Gene expression levels shown are normalized to endogenous transcription of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The data represent mean ± SEM from four independent experiments.