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Supplementary Information for

Bisphenol A replacement chemicals, BPF and BPS, induce pro-tumorigenic changes in human mammary gland organoid morphology and proteome

Juliane Winkler[1,2*], Pengyuan Liu[3,4], Kiet Phong[5], Johanna H. Hinrichs[1,6], Nassim Ataii[1], Katherine Williams[3], Elin Hadler-Olsen[1,7], Susan Samson, Zev J. Gartner[8], Susan Fisher[1,3*], Zena Werb[1†]

[1] Department of Anatomy, University of California, San Francisco, USA;
[2] Department of Cell and Tissue Biology, University of California, San Francisco, USA;
[3] Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, USA;
[4] Department of Chemistry, University of Massachusetts Lowell, USA;
[5] Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, USA;
[6] Institute of Internal Medicine D, Medical Cell Biology, University Hospital Münster, Germany;
[7] Department of Medical Biology, Faculty of Health Sciences, UiT, the Arctic University of Norway, Norway
[8] Department of Pharmaceutical Chemistry, University of California, San Francisco, USA;

*corresponding authors: Juliane Winkler and Susan Fisher

Kiet Phong: kiet.phong@ucsf.edu
Zev Gartner: zev.gartner@ucsf.edu
Nassim Ataii: nataii@berkeley.edu
Elin Hadler-Olsen: elin.hadler-olsen@uit.no
Johanna Hinrichs: johanna.hinrichs@uni-muenster.de
Katherine Williams: Katherine.Williams@ucsf.edu
Pengyuan Liu: Pengyuan_Liu@uml.edu
Juliane Winkler: juliane.winkler@ucsf.edu
Zena Werb: zena.werb@ucsf.edu
Susan Fisher: susan.fisher@ucsf.edu
Susan Samson: ssamson@pacbell.net

This PDF file includes:

Supplementary Figure 1

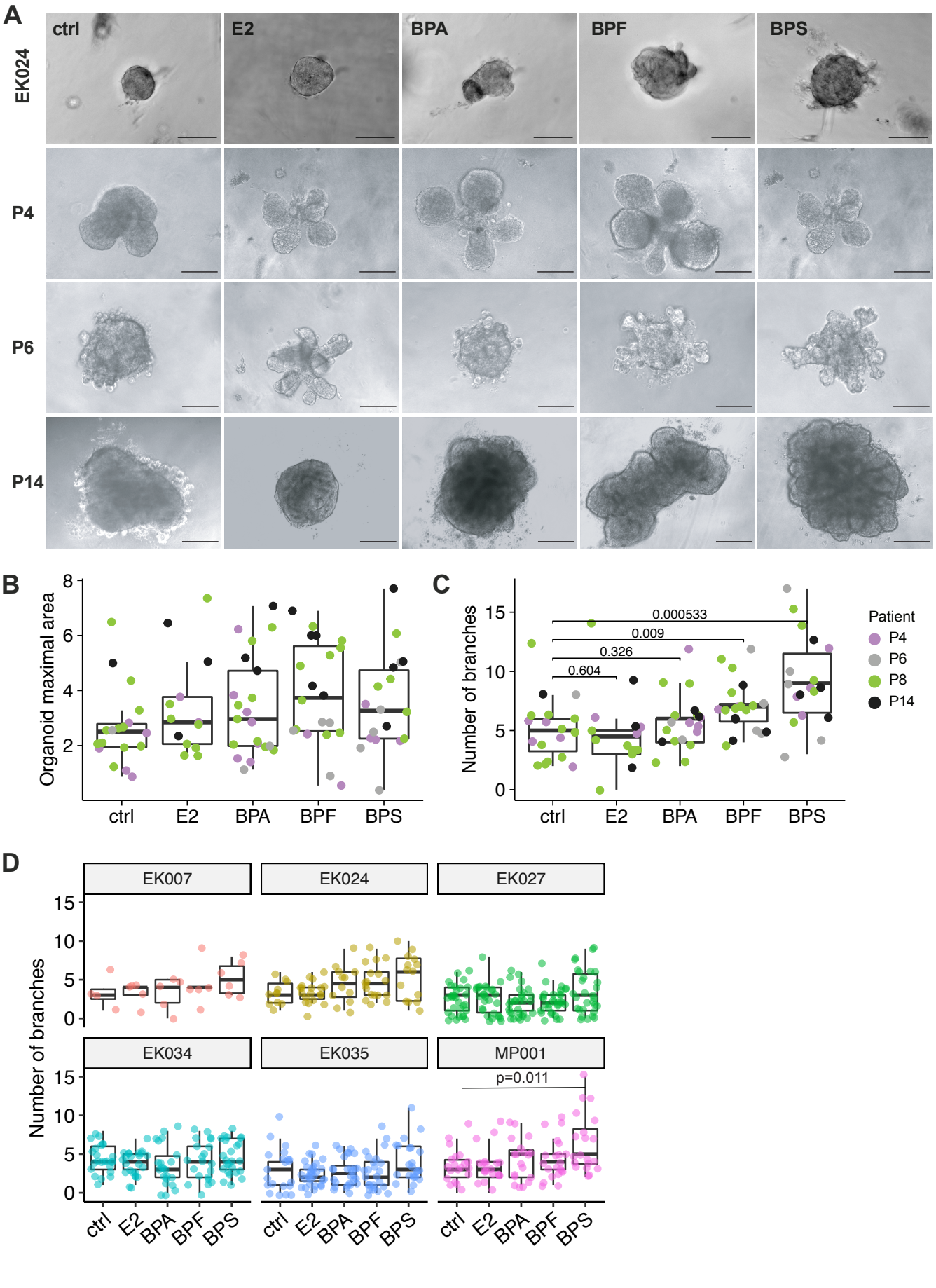


Fig. S1. Response to bisphenols shows interpatient heterogeneity

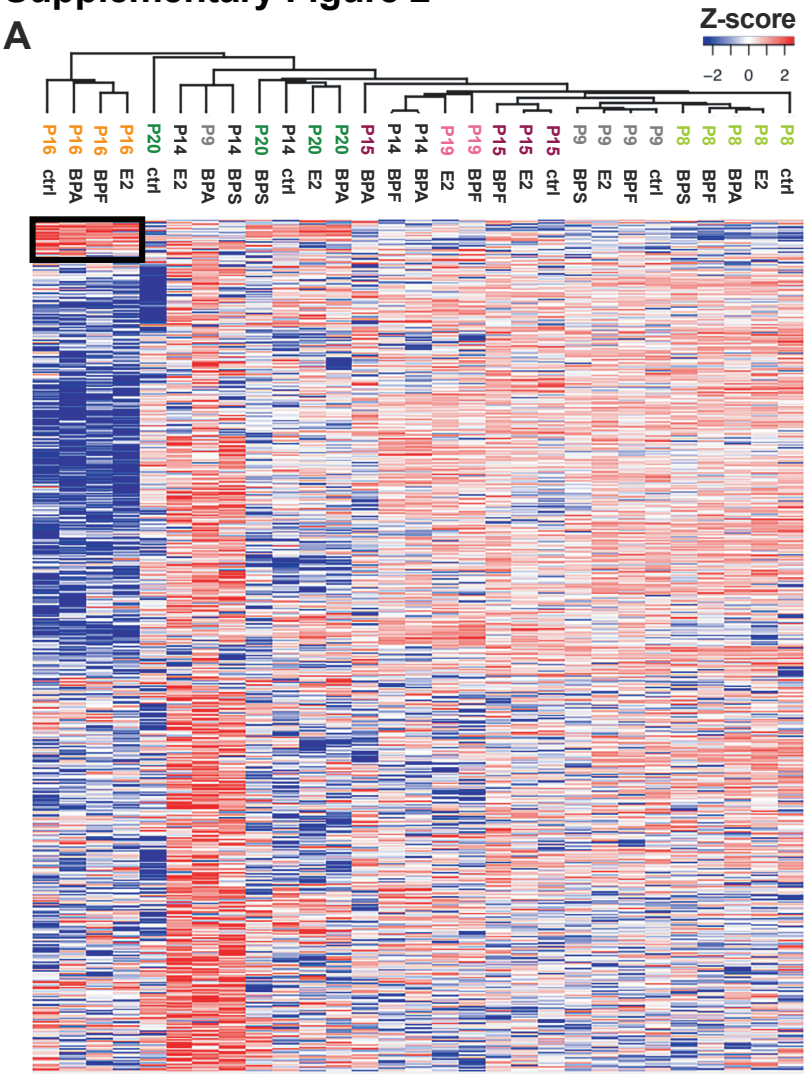
A) Representative brightfield images of human breast organoids from different patients after 6 days of exposure to DMSO as vehicle control (ctrl), estrogen (E2), BPA, BPF or BPS. Scale bars = 100 µm.

B) Quantification of the organoid maximum cross-sectional area colored by patient (cohort B). Each dot represents one organoid. The median and the interquartile range are shown. The values were not statistically different among the groups.

C) Quantification of the total number of branches per organoid colored by patient (cohort B). Each dot represents one organoid. The median and interquartile range are shown. Results that reached statistical significance using two-sample Wilcoxon test are noted.

D) Quantification of the total number of branches per organoid grouped and colored by patient (cohort A). Each dot represents one organoid. The median and interquartile range are shown. Results that reached statistical significance using two-sample Wilcoxon test are noted.

Supplementary Figure 2



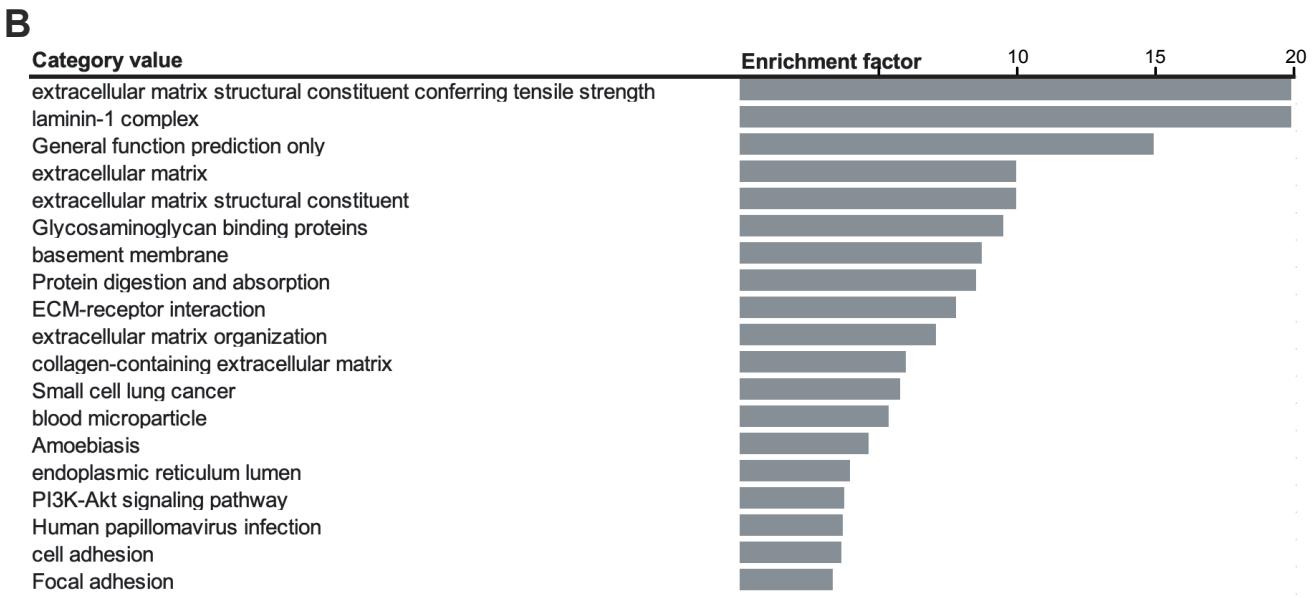


Fig. S2.
A) Heatmap shows unsupervised clustering of proteins that were detected in organoid samples treated as described in Fig1A from 7 individuals. Z-scores of protein abundance with low in blue and high in red. P16 shows strikingly different protein expression compared to all other patients. The box highlights an upregulated distinct cluster.
B) Annotation enrichment analysis of the upregulated cluster in P16 highlighted by the box in (A).

Supplementary Figure 3

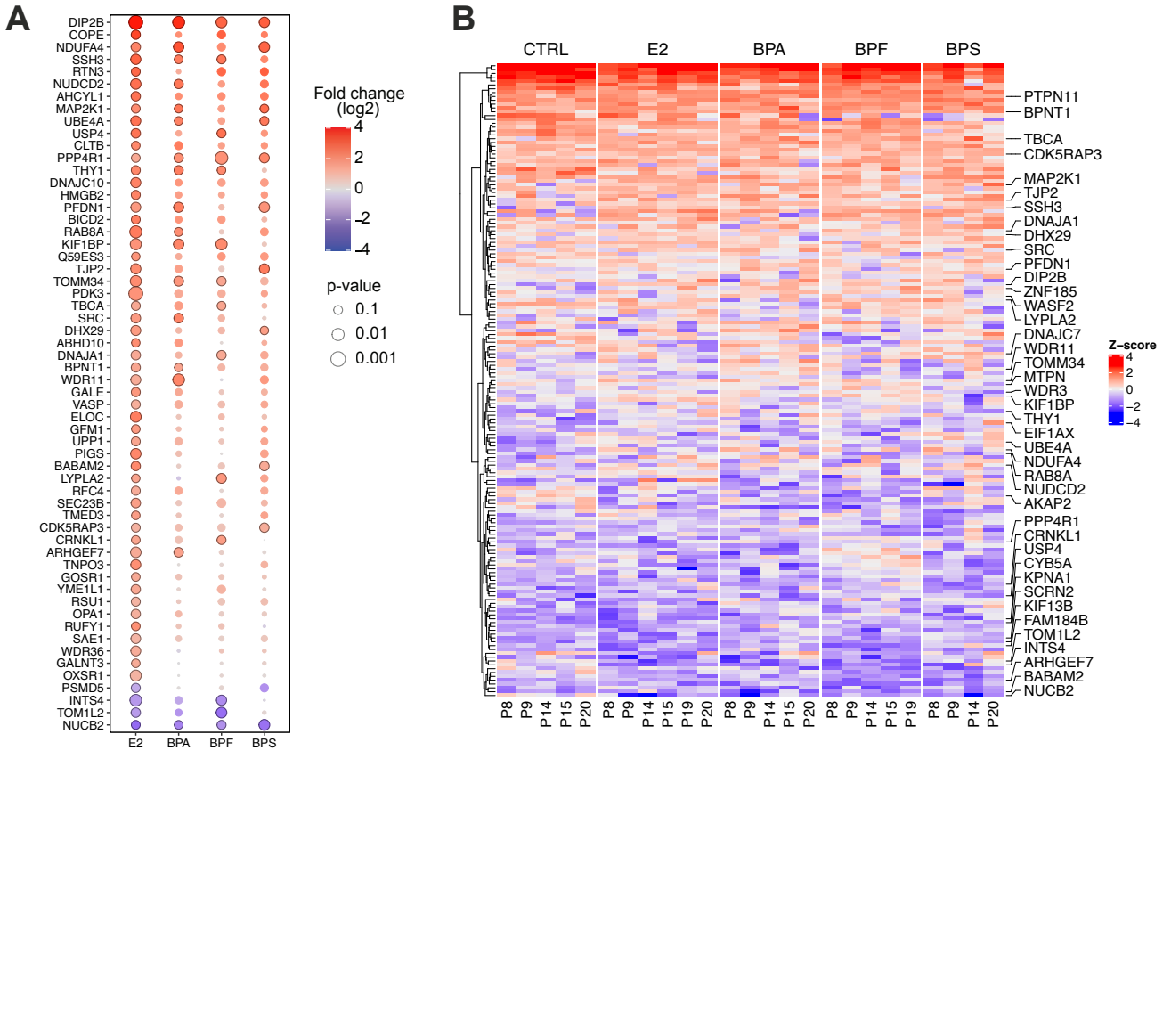


Fig. S3.
A) Bubble plot shows proteins significantly modulated by E2 compared to control conditions. Color represents log2 fold change (downregulation in blue and upregulation in red) and dot size p-value. Circles indicate significant p-value ($p \leq 0.05$).
B) Heatmap shows the abundance of all 166 DE proteins with shared proteins highlighted on the right. Z-score of protein abundance with low in blue and high in red.

Table S1. Corresponding patient data of tissue used for this study

Feature/ Patient	Cohort	Age	Race	BMI	Smoking history	Alcohol history	Children	Reduction mammoplasty
P4	B	25	W	>30	NA	NA	NA	0
P6	B	43	W	27.6	1	0	0	2
P8	B	37	B	45.8	0	0	3	0
P9	B	46	W	25.6	1	1	NA	0
P14	B	20	B	37.3	0	0	NA	0
P15	B	21	W	41.2	0	0	NA	0
P16	B	44	B	33.7	0	0	NA	1
P19	B	24	W	30.5	0	0	NA	0
P20	B	42	W	34.1	0	0	0	0
UCSF011	B	44	W	43.2	NA	NA	NA	0
UCSF013	B	41	B	44.6	NA	NA	NA	0
UCSF016	B	19	B	37.6	NA	NA	NA	0
EK007	A	26	W	31.0	1	1	0	NA
MP001	A	21		NA	NA	NA	0	NA
EK035	A	24	W	28.8	0	0	0	NA
EK024	A	19	H	23.8	0	0	0	NA
EK027	A	25	H	36.1	0	0	0	NA
EK034	A	25	H	20.4	0	0	0	NA

W: White; B: Black; H: Hispanic or Latino; NA: no data available;

Table S2: Primer sequences used for the qPCR analysis in this study

Target	Forward/ Reverse	Sequence	Reference
AREG	F	GAGCCGACTATGACTACTCAGA	PrimerBank
AREG	R	TCACTTTCCGTCTTGTTTTGGG	PrimerBank
HPRT	F	GACCAGTCAACAGGGGACAT	(1)
HPRT	R	CCTGACCAAGGAAAGCAAAG	(1)
KRT18	F	CACAGTCTGCTGAGGTTGGA	(1)
KRT18	R	GAGCTGCTCCATCTGTAGGG	(1)
PRa/b	F	AGCATGTCGCCTTAGAAAGTGC	(2)
PRa/b	R	TAGGGCTTGGCTTTCATTTG	(2)
TFF1	F	GGAGCAGAGAGGAGGCAAT	(3)
TFF1	R	GGCGCAGATCACCTTGTT	(3)
GREB1	F	GACCTGCCAAATGGAAGAAG	(3)
GREB1	R	AAAGCCATGTCCTTCCACAC	(3)

1. T. Tanos, *et al.*, Progesterone/RANKL is a major regulatory axis in the human breast. *Sci. Transl. Med.* **5** (2013).
2. C. M. Luetjens, *et al.*, Tissue expression of the nuclear progesterone receptor in male non-human primates and men. *J. Endocrinol.* **189**, 529–539 (2006).
3. N. Hah, *et al.*, A Rapid, Extensive, and Transient Transcriptional Response to Estrogen Signaling in Breast Cancer Cells. *Cell* **145**, 622–634 (2011).

Dataset S1 (separate file). Differential expressed proteins