Supplemental Material

Aggressiveness and Metastatic Potential of Breast Cancer Cells Co-Cultured with Preadipocytes and Exposed to an Environmental Pollutant Dioxin: An *in Vitro* and *in Vivo* Zebrafish Study

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Figure S2. Cell death, senescence, proliferation, or autophagy measurements. MCF7 cells were grown with hMADS and/or treated with 25nM TCDD for 48h (Control (vehicle MCF-7 cells, alone), TCDD (MCF-7 cells treated with 25nM TCDD), coculture (MCF-7 co-cultured with hMADS) and coexposure (co-culture with TCDD)). (**A**) Quantification of MCF7 apoptosis using annexin V-FITC (y-axis)/PI staining(x-axis). (a) Flow cytometer results: cells were classified as early apoptotic cells (Annexin V+, PI−), late apoptotic cells (Annexin V+, PI+), and damaged cells (Annexin V−, PI+). (b) Percentage of cells from (a) under each different experimental condition. Graph represent means ± SEM of 5 measurements. (**B**) q-RT-PCR experiments quantifying apoptosis and autophagy biomarkers. Relative levels compared to the control of mRNA expression of ATG5, ATG7 and BAX by real-time qPCR. Graph represent means ± SEM of 5 measurements. (**C**) Quantification of MCF-7 proliferation using the AlamarBlue assay. Mean absorbance is indicated for each condition as a horizontal bar and statistically significant differences were determined by ANOVA and indicated as P values above the comparison, bars, SD. N=3. The numerical information mean +/-SEM and p-values are provided in Table S5, S6 and S7. (Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series, ** $p<0.01$; * $p<0.05$). (**D**) Senescence-associated β-galactosidase activity. The blue-stained cells and the total number of cells were observed.10X magnification (1 field par well). The pictures are representative of at least 3 different experiments.

Table S1. Numerical data mean +/- SEM and p-values for Figure 1C: Cell index and p-values for MCF-7 cells under control, TCDD, coculture, or coexposure conditions using the xCELLigence system.

[a] Data presented are mean of 6 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions compared to control.

Table S2. Numerical data mean +/- SEM and p-values for Figure 2B: ALDH enzymatic analysis for MCF-7 cells under control, TCDD, coculture, or coexposure conditions.

[a] Data presented are mean of 6 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions compared to control.

Table S3. Numerical data mean +/- SEM and p-values for Figure 3: Tumorsphere or spheroid area (µm^2) of MCF-7 cells under control, TCDD, coculture, or coexposure conditions.

[a] Data presented are mean of 14-39 measurements on 3 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions compared to control.

Table S4. Numerical data mean +/- SEM and p-values for Figure 4: Quantitation of cell number and giant cells (very large cells with multiple nuclei) per field (N=3 field per conditions) in MCF-7 cells under control, TCDD, coculture, or coexposure conditions.

[a] Data presented are mean of 3 field in 3 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

Table S5. Numerical data mean +/- SEM and p-values for Figure S2A-a: Percentage of MCF-7 cells that were classified as early apoptotic cells (Annexin V+, PI−), late apoptotic cells (Annexin V+, PI+), and damaged cells (Annexin V−, PI+) using flow cytometry.

[a] Data presented are mean of 5 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

Table S6. Numerical data means +/- SEM and p-values for Figure S2B: mRNA expression of ATG7, ATG5, and Bax in MCF-7 cells under control, TCDD, coculture, or coexposure conditions as determined by q-RT-PCR.

[a] Data presented are mean of 5 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

Table S7. Numerical data means +/- SEM and p-values for Figure S2C: Quantification of proliferation of MCF-7 cells under control, TCDD, coculture, or coexposure conditions as determined using the AlamarBlue assay. Absorbance measured at 540 nm.

[a] Data presented are mean of 3 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

Table S8. Numerical data means +/- SEM and p-values for figure 6B-b.

[a] Data presented are mean of 3 experiments. The total number of fish injected with CM-Dil-labelled MCF-7 was: control (N=32), TCDD (N=21), Coculture (N=36) and Coexposure (N=34). The total number of fish injected with RFP-labelled MDA-MB-231 cells was: control (N=46), TCDD (N=41), Coculture (N=24) and Coexposure (N=42).
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

Table S9. Numerical data means +/- SEM and p-values for Figure S1.

[a] Data presented are mean of 5 experiments.
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

Additional File- Excel Document
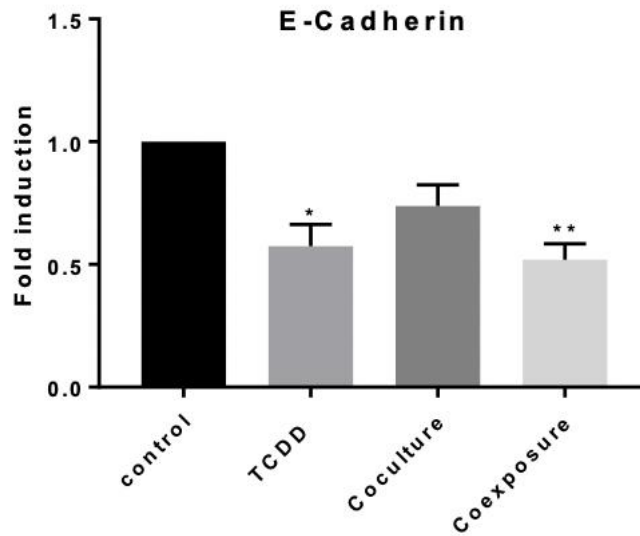
Figure S1: semi-quantitation of E-Cadherin mRNA in MCF-7 cells.
MCF7 cells were grown with hMADS and/or treated with 25nM TCDD for 48h (Control (vehicle MCF-7 cells, alone), TCDD (MCF-7 cells treated with 25nM TCDD), coculture (MCF-7 co-cultured with hMADS) and coexposure (co-culture with TCDD)). Relative levels (compared to the control) of E-Cadherin mRNA were measured by real-time qPCR. Graph represent means ± SEM of 5 measurements. The numerical information mean +/-SEM and p-values are provided in Table S9. (Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), ** $p<0.01$, *$p<0.05$).
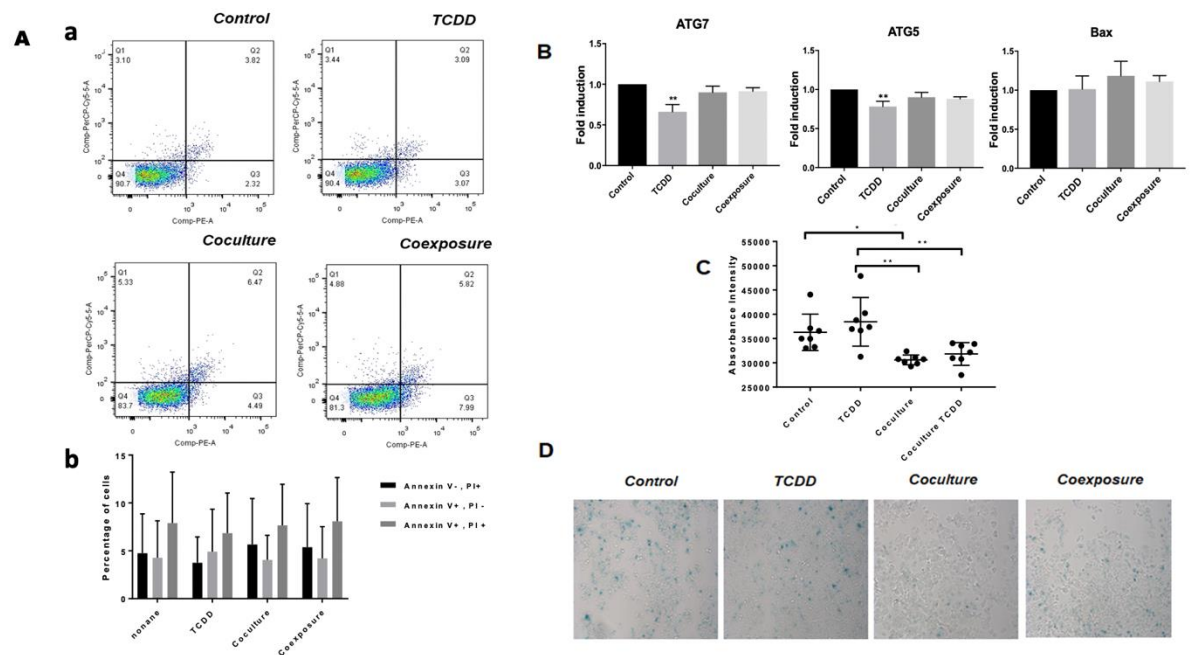
Figure S2: Cell death, senescence, proliferation, or autophagy measurements.
MCF7 cells were grown with hMADS and/or treated with 25nM TCDD for 48h (Control (vehicle MCF-7 cells, alone), TCDD (MCF-7 cells treated with 25nM TCDD), coculture (MCF-7 co-cultured with hMADS) and coexposure (co-culture with TCDD)). **(A)** Quantification of MCF7 apoptosis using annexin V-FITC (y-axis)/PI staining(x-axis). **(a)** Flow cytometer results: cells were classified as early apoptotic cells (Annexin V+, PI−), late apoptotic cells (Annexin V+, PI+), and damaged cells (Annexin V−, PI+). **(b)** Percentage of cells from (a) under each different experimental condition. Graph represent means ± SEM of 5 measurements. **(B)** q-RT-PCR experiments quantifying apoptosis and autophagy biomarkers. Relative levels compared to the control of mRNA expression of ATG5, ATG7 and BAX by real-time qPCR. Graph represent means ± SEM of 5 measurements. **(C)** Quantification of MCF-7 proliferation using the AlamarBlue assay. Mean absorbance is indicated for each condition as a horizontal bar and statistically significant differences were determined by ANOVA and indicated as P values above the comparison, bars, SD. N=3. The numerical information mean +/-SEM and p-values are provided in Table S5, S6 and S7. (Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series, ** $p<0.01$; * $p<0.05$). **(D)** Senescence-associated β-galactosidase activity. The blue-stained cells and the total number of cells were observed.10X magnification (1 field par well). The pictures are representative of at least 3 different experiments.

	Slope/Slope control	
	Mean +/-SEM (a)	p-value (b)
TCDD	113.61+/-12.93	0.6215
Coculture	45.83 +/- 7.18	0.0121
Coexposure	37.91 +/- 4.88	0.0035

Table S1. Numerical data mean +/- SEM and p-values for Figure 1C: Cell index and p-values for MCF-7 cells under control, TCDD, coculture, or coexposure conditions using the xCELLigence system

[a] Data presented are mean of 6 experiments
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions compared to control.

	ALDH positive cells (% compared to the control)	
	Mean +/-SEM (a)	p-value (b)
TCDD	407.9+/-136.8	0.0292
Coculture	110.9 +/- 28.44	0.805
Coexposure	606.1 +/- 194	0.0027

Table S2. Numerical data mean +/- SEM and p-values for Figure 2B: ALDH enzymatic analysis for MCF-7 cells under control, TCDD, coculture, or coexposure conditions

[a] Data presented are mean of 6 experiments
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions compared to control.

	Spheroid Area (μm^2)	
	Mean +/-SEM (a)	p-value (b)
Control	9228 +/- 1155	
TCDD	12125 +/-1035	0.3441
Coculture	67205 +/- 13398	<0.0001
Coexposure	46675 +/-5468	<0.0001

Table S3. Numerical data mean +/- SEM and p-values for Figure 3: Tumorsphere or spheroid area (μm^2) of MCF-7 cells under control, TCDD, coculture, or coexposure conditions

[a] Data presented are mean of 14-39 measurements on 3 experiments
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions compared to control.

	Cell number per field	
	Mean +/-SEM (a)	p-value (b)
Control	113+/-10.02	
TCDD	46.33+/-11.02	0.3204
Coculture	42.67 +/- 1.66	>0.9999
Coexposure	31.67 +/- 4.33	0.027

	Gaint Cells per field	
	Mean +/-SEM (a)	p-value (b)
Control	0	
TCDD	1+/-0	0.9485
Coculture	1+/- 0.5774	>0.9999
Coexposure	3.333 +/- 0.33	0.0162

Table S4. Numerical data mean +/- SEM and p-values for Figure 4: Quantitation of cell number and giant cells (very large cells with multiple nuclei) per field (N=3 field per conditions) in MCF-7 cells under control, TCDD, coculture, or coexposure conditions

[a] Data presented are mean of 3 field in 3 experiments
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

	Annexin V- . PI+		Annexin V+ . PI -		Annexin V+ . PI +	
	Mean +/-SEM (a)	p-value (b)	Mean +/-SEM (a)	p-value (b)	Mean +/-SEM (a)	p-value (b)
Control	4.742+/-1.839		4.264+/-1.729		7.89+/-2.381	
TCDD	3.744+/-1.207	0.8307	4.902+/-1.987	0.6492	6.846+/-1.869	0.7083
Coculture	5.652+/-2.146	0.6305	4.048+/-1.151	0.8725	7.662+/-1.916	>0.9999
Coexposure	5.382+/-2.033	0.6305	4.212+/-1.476	0.8097	8.07+/-2.044	0.9574

Table S5 : Numerical data mean +/- SEM and p-values for Figure S2A-a: Percentage of MCF-7 cells that were classified as early apoptotic cells (Annexin V+, PI–), late apoptotic cells (Annexin V+, PI+), and damaged cells (Annexin V–, PI+) using flow cytometry.
[a] Data presented are mean of 5 experiments
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control

	ATG7 Expression (compared to the control)		ATG5 Expression (compared to the control)		Bax Expression (compared to the control)	
	Mean +/-SEM (a)	p-value (b)	Mean +/-SEM (a)	p-value (b)	Mean +/-SEM (a)	p-value (b)
Control	1		1		1	
TCDD	0.662+/-0.088	0.0015	0.782+/-0.067	0.009	1.014+/-0.1687	0.8084
Coculture	0.9 +/- 0.076	0.1459	0.902 +/- 0.061	0.1613	1.184 +/- 0.1872	0.5005
Coexposure	0.914 +/- 0.044	0.1961	0.88 +/- 0.026	0.0558	1.112 +/- 0.074	0.2357

Table S6 : Numerical data means +/- SEM and p-values for Figure S2B: mRNA expression of ATG7, ATG5, and Bax in MCF-7 cells under control, TCDD, coculture, or coexposure conditions as determined by q-RT-PCR

[a] Data presented are mean of 5 experiments

[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control

	Absorbance intensity	
	Mean +/-SEM (a)	p-value (b)
Control	36283+/-1420	
TCDD	38451+/-1893	0.4747
Coculture	30615 +/- 377.8	0.0025
Coexposure	31833 +/- 833	0.0552

Table S7 : Numerical data means +/- SEM and p-values for Figure S2C: Quantification of proliferation of MCF-7 cells under control, TCDD, coculture, or coexposure conditions as determined using the AlamarBlue assay. Absorbance measured at 540 nm

[a] Data presented are mean of 3 experiments

[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control.

	Metastasis/Fish	
	Mean +/-SEM (a)	p-value (b)
Control	12.7+/-2.044	
TCDD	6.167+/-1.447	0.1101
Coculture	15.06+/-2.167	0.5133
Coexposure	21.72+/-2.286	0.0257
	Metastasis/Fish	
	Mean +/-SEM (a)	p-value (b)
Control	10.21+/-1.178	
TCDD	8.417+/-1.323	0.2547
Coculture	8.556+/-0.9734	0.4811
Coexposure	15.95+/-2.136	0.0147

Table S8: Numerical data means +/- SEM and p-values for figure 6B-b

[a] Data presented are mean of 3 experiments. The total number of fish injected with CM-Dil-labelled MCF-7 was: control (N=32), TCDD (N=21), Coculture (N=36) and Coexposure (N=34). The total number of fish injected with RFP-labelled MDA-MB-231 cells was: control (N=46), TCDD (N=41), Coculture (N=24) and Coexposure (N=42).

[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control

	E-Cadherin Expression (compared to the control)	
	Mean +/-SEM (a)	p-value (b)
Control	1	
TCDD	0.574+/-0.088	0.0105
Coculture	0.738 +/- 0.086	0.1457
Coexposure	0.52 +/- 0.064	0.0028

Table S9: Numerical data means +/- SEM and p-values for Figure S1

[a] Data presented are mean of 5 experiments
[b] p-values calculated via Kruskal–Wallis's H test (nonparametric comparison of k independent series) followed by a 1-factor ANOVA test (parametric comparison of k independent series), All conditions were compared to control