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### **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 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  $\pm$  SEM of 5 measurements. The numerical information mean  $\pm$  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  $\pm$  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  $\pm$  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  $\pm$  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  $\beta$ -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  $\pm$  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  $\pm$  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 ( $\mu\text{m}^2$ ) 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