Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Material

Droplet Microarray: miniaturized platform for rapid formation and high-throughput screening of embryoid bodies

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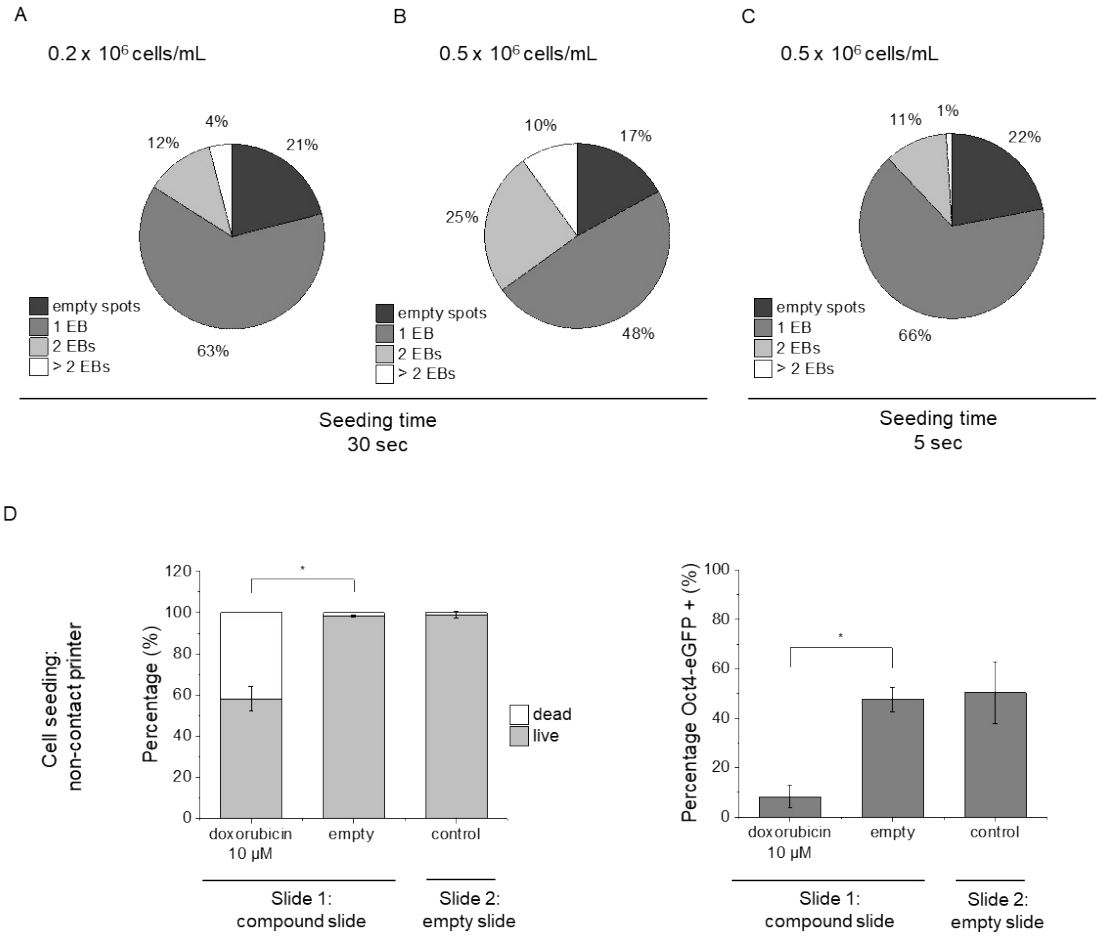

Figure S1. Distribution of EBs on DMA showing percentage of empty spots, single EBs (1EB), 2 EBs or more than 2 EBs (> 2 EBs) per spot using (A) "standing droplet" method with 0.2 x 10^6 cells/mL initial cell concentration and seeding time of 30 sec; (B) "standing droplet" method with 0.5 x 10^6 cells/mL initial cell concentration and seeding time of 30 sec; (C) "standing droplet" method with 0.2 x 10^6 cells/mL initial cell concentration and seeding time of 5 sec. (D) 2D culture of mESC Oct4-eGFP on slides preprinted with doxorubicin using a non-contact cell printer for cell seeding. Left: Percentage of dead (PI positive; hollow bars) and viable cells (PI negative; grey bars) on spots containing doxorubicin (10 µM) and empty spots after 24h incubation. Right: Percentage of GFP positive mESC Oct4-eGFP on doxorubicin and empty spots on the compound slide after 24h incubation. Slide 1: compound slide with doxorubicin (10 µM) printed in checker board format on the DMA; containing doxorubicin containing and empty spots in close proximity. As control serves slide 2: empty slide without compound (compound-free slide). N=3 n > 100; statistical significance: t-test, *indicates p-value ≤0.05.

Table S1. High-throughput screen (HTS): Primary screen. Table showing results of 12 hit compounds from the primary screen with 774 FDA approved compounds. mESC Oct4-eGFP were cultured in hanging droplets using the DMA in presence of respective compounds for 72h. mESC were stained with Hoechst 333442 (cell nucleus, for automated segmentation of Embryoid bodies (EBs)) and Propidium iodide (PI, dead cells). Evaluated and measured was the formation of EBs (yes/-), EB diameter (in µm), EB roundness, Mean fluorescence intensity (MFI) of Oct4-eGFP of EBs as indication for differentiation and percentage of dead cells (PI positive) of total EB area as indication for toxicity.

Compound	EB formation	Diameter (µm)	Roundness (1-100)	Differentiation (MFI GFP)	Toxicity (% PI)
Control	Yes	85 ± 7	36 ± 3.5	71 ± 11	2
13-cis-retinoic acid	Yes	74 ± 21	23 ± 6	53 ± 23	29
lansoprazole	Yes	168 ± 2	19 ± 0	52 ± 11	0
meclizine dihydrochloride	Yes	41 ± 2	28 ± 15	29 ± 19	0
metaproterenol hemisulfate	Yes	45 ± 4	23 ± 15	23 ± 15	0
propafenone·HCl	Yes	172 ± 1	13 ± 8	77 ± 16	0
dutasteride	Yes	97 ± 21	51 ± 0	166 ± 84	20
eptifibatide	Yes	57 ± 9	40 ± 9	245 ± 84	22
mesna	Yes	74 ± 31	38 ± 18	13 ± 4	19
phentolamine·HCl	Yes	123 ± 34	16 ± 10	161 ± 72	36
busulfan	-	-	-	-	-
digoxin	-	-	-	-	-
mycophenolate mofetil	-	-	-	-	-

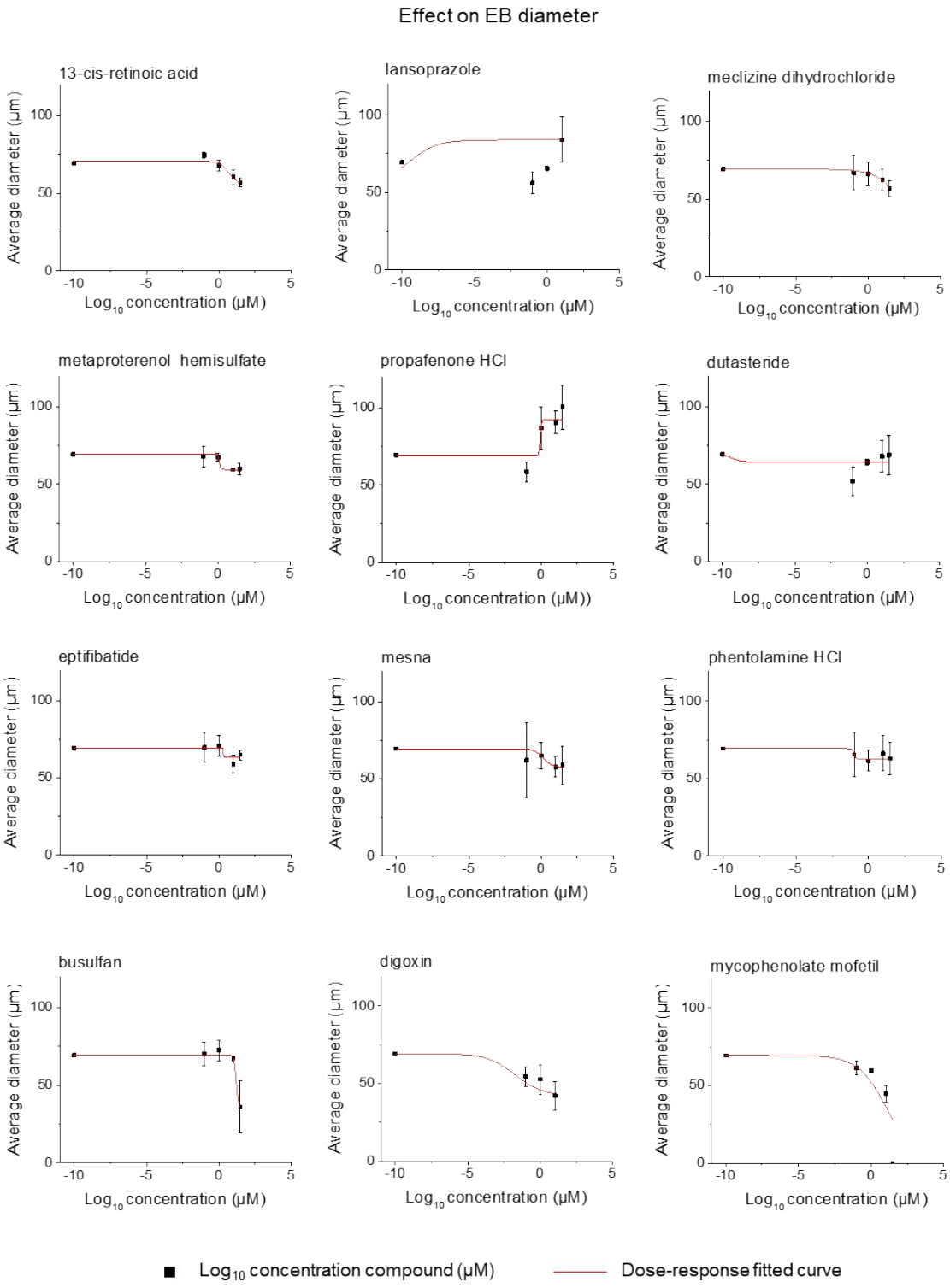

Figure S2. Secondary screen: dose-response curve of 12 hit compounds. mESC Oct4-eGFP were cultured in presence of respective compounds in concentrations from 0.1 µM to 30 µM in hanging droplet using the DMA for 72h. mESC were stained with Hoechst 333442 (cell nucleus, for automated segmentation of Embryoid bodies (EBs)) and Propidium iodide (PI, dead cells). Graphs show measured EB diameter (µm) for respective compounds and concentration (black line). EB diameter of vehicle control is depicted as red line. n=4, N=3.

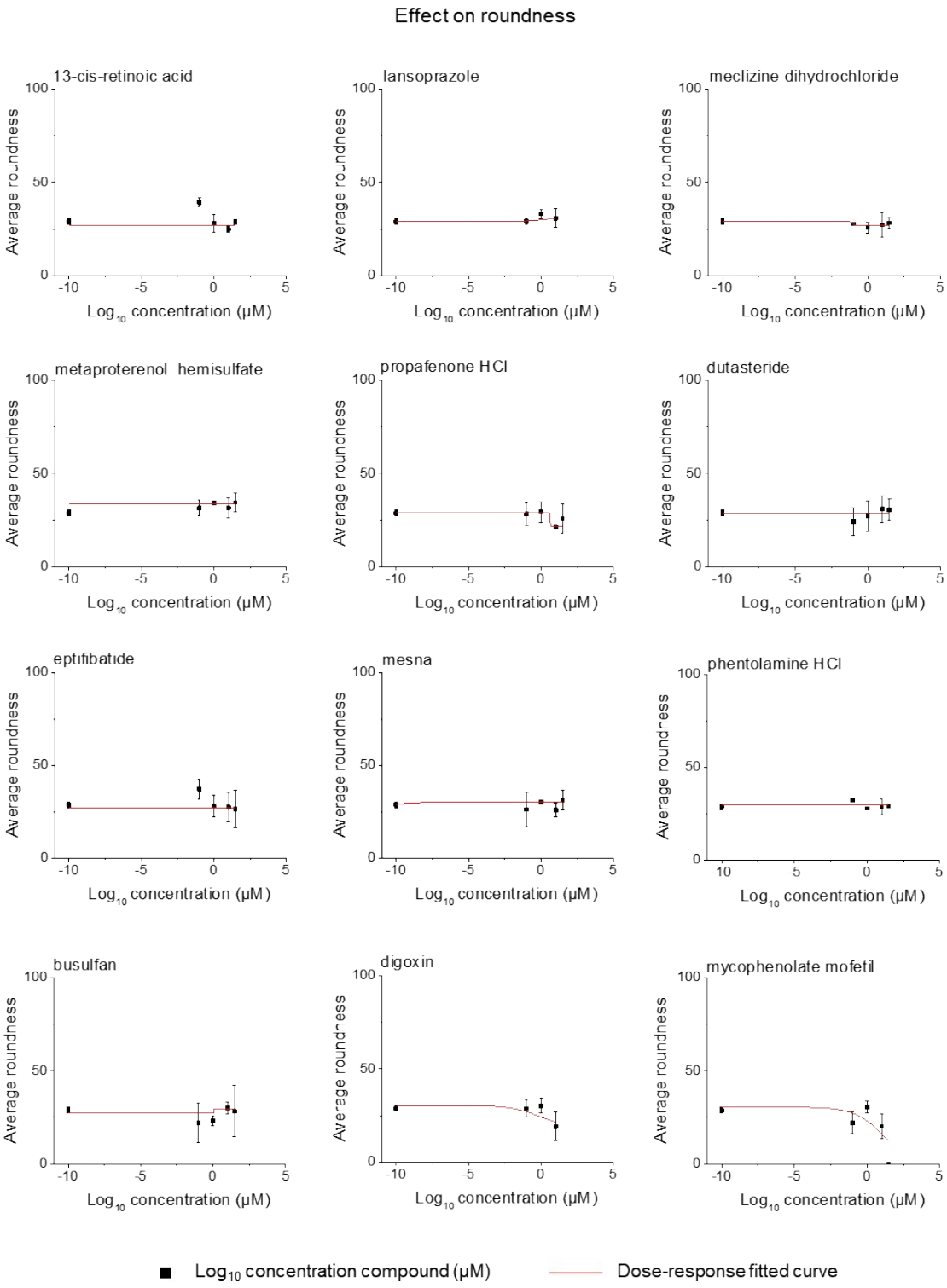

Figure S3. Secondary screen: dose-response curve of 12 hit compounds. mESC Oct4-eGFP were culture in presence of respective compounds in concentrations from 0.1 µM to 30 µM in hanging droplet using the DMA for 72h. mESC were stained with Hoechst 333442 (cell nucleus, for automated segmentation of Embryoid bodies (EBs)) and Propidium iodide (PI, dead cells). Graphs show measured EB roundness (0-100) for respective compounds and concentration (black line). EB roundness of vehicle control is depicted as red line. n=4, N=3.

Regarding the assessment of cytotoxic effect of the respective compounds, in some cases we observed high standard deviations between the replicates (Figure S4). This could be due to the way of measurement and the 3 dimensionality of the EBs. Due to time limitation during live cell imaging and in order to ensure data assessment of all compounds in a timely manner, the imaging of EBs had to be reduced to a single plane. This could result in imaging of planes in different z-positions between replicates, which could in turn exhibit different percentages of viable and dead cells depending on the position within the EB. Another possible factor influencing the measurement of viable and dead cells could be, that during imaging of a 3D object the fluorescent signal emitted by stained cells lying underneath the current plane could also be detected and measured during imaging.

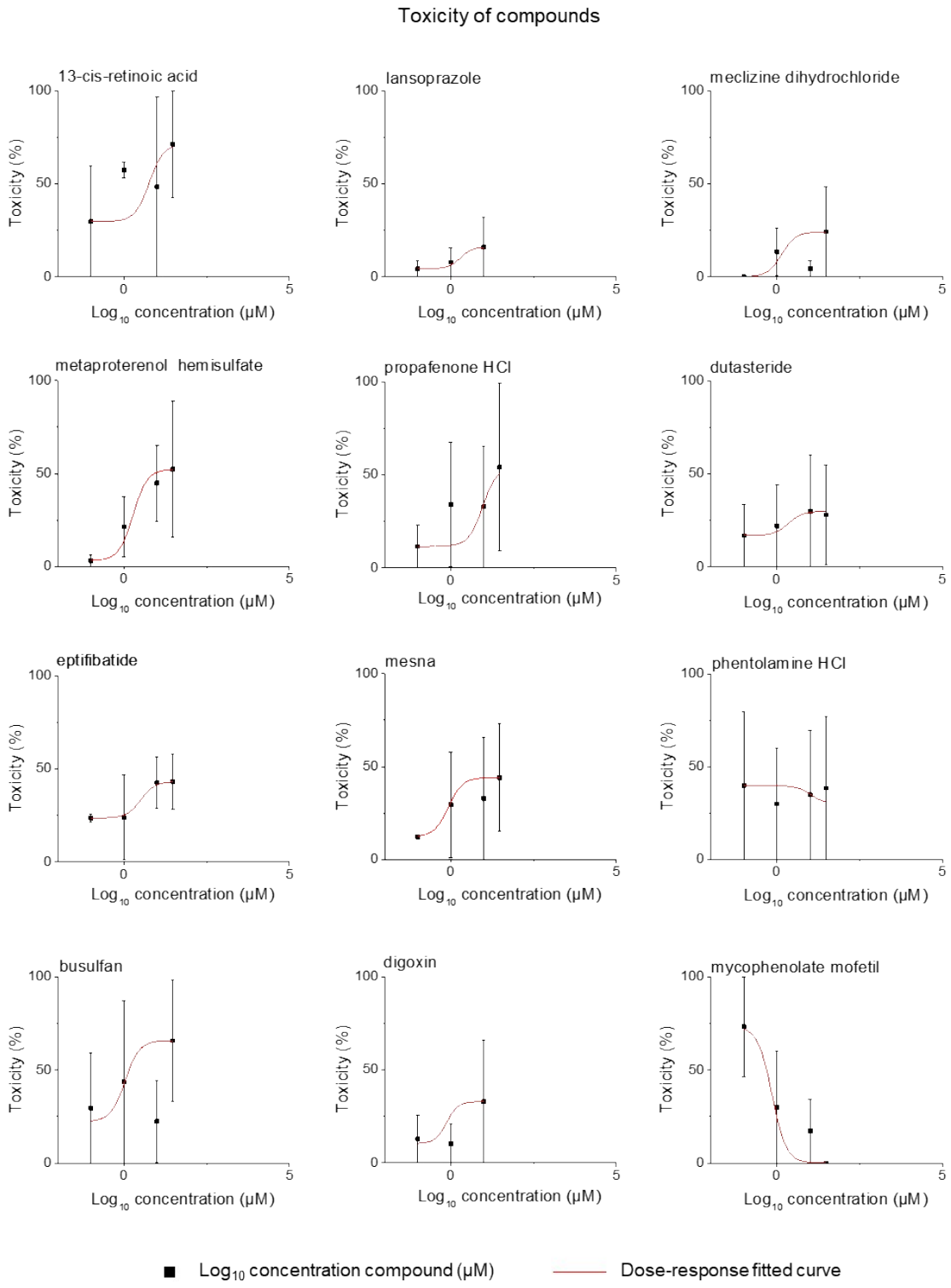

Figure S4. Secondary screen: dose-response curve of 12 hit compounds. mESC Oct4-eGFP were cultured in presence of respective compounds in concentrations from 0.1 µM to 30 µM in hanging droplet using the DMA for 72h. mESC were stained with Hoechst 333442 (cell nucleus, for automated segmentation of Embryoid bodies (EBs)) and Propidium iodide (PI, dead cells). Graphs show toxicity of respective compounds in different concentrations (area fraction of PI positive cells from total EB area; black line). n=4, N=3

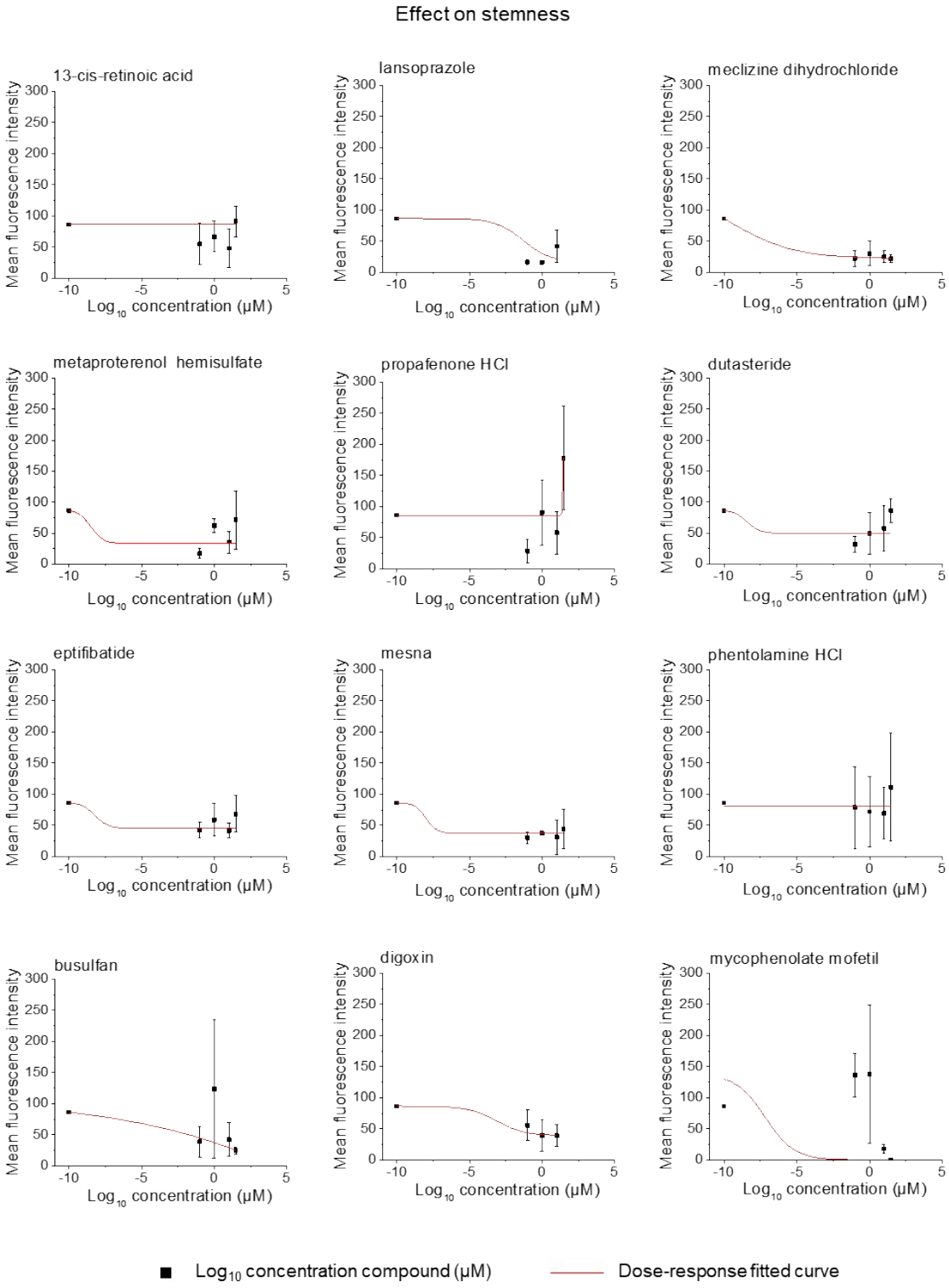

Figure S5. Secondary screen: dose-response curve of 12 hit compounds. mESC Oct4-eGFP were cultured in presence of respective compounds in concentrations from 0.1 µM to 30 µM in hanging droplet using the DMA for 72h. mESC were stained with Hoechst 333442 (cell nucleus, for automated segmentation of Embryoid bodies (EBs)) and Propidium iodide (PI, dead cells). Graphs show mean fluorescence intensity of Oct4-eGFP signal for respective compounds and concentrations as indication for differentiation (black line). Mean fluorescence intensity of vehicle control is depicted as red line. n=4, N=3.