

# Select Per- and Polyfluoroalkyl Substances (PFAS) Induce Resistance to Carboplatin in Ovarian Cancer Cell Lines

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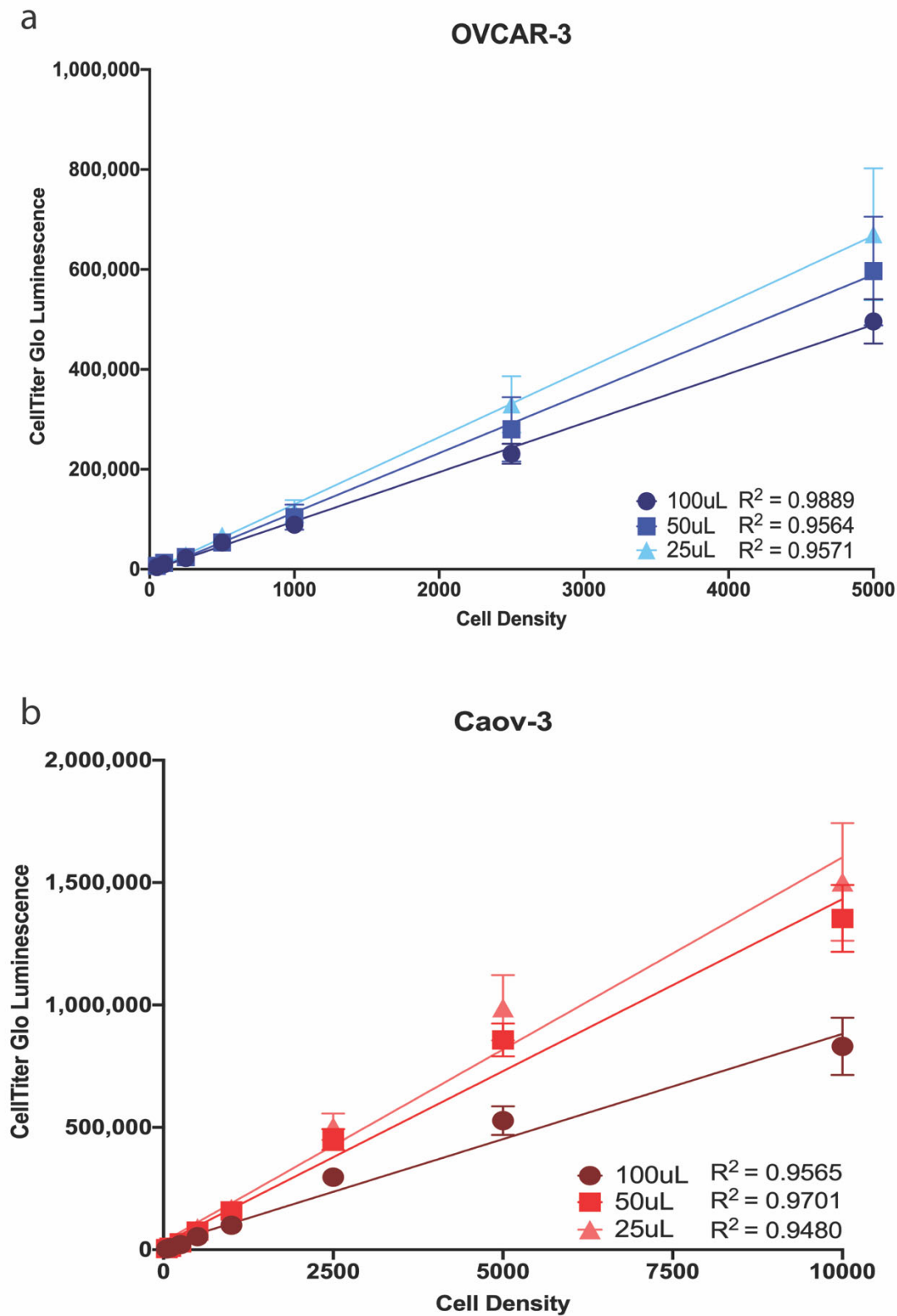
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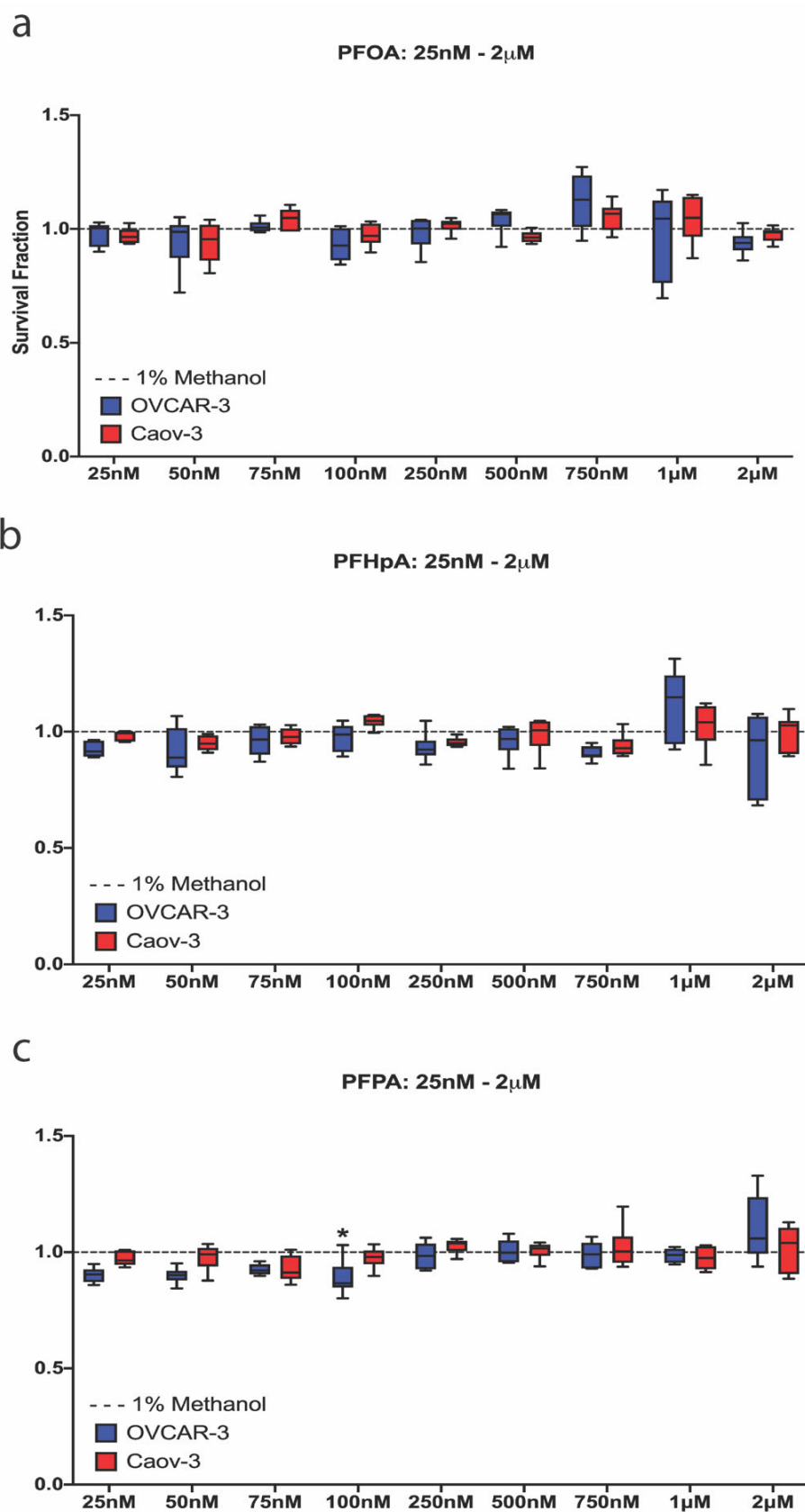
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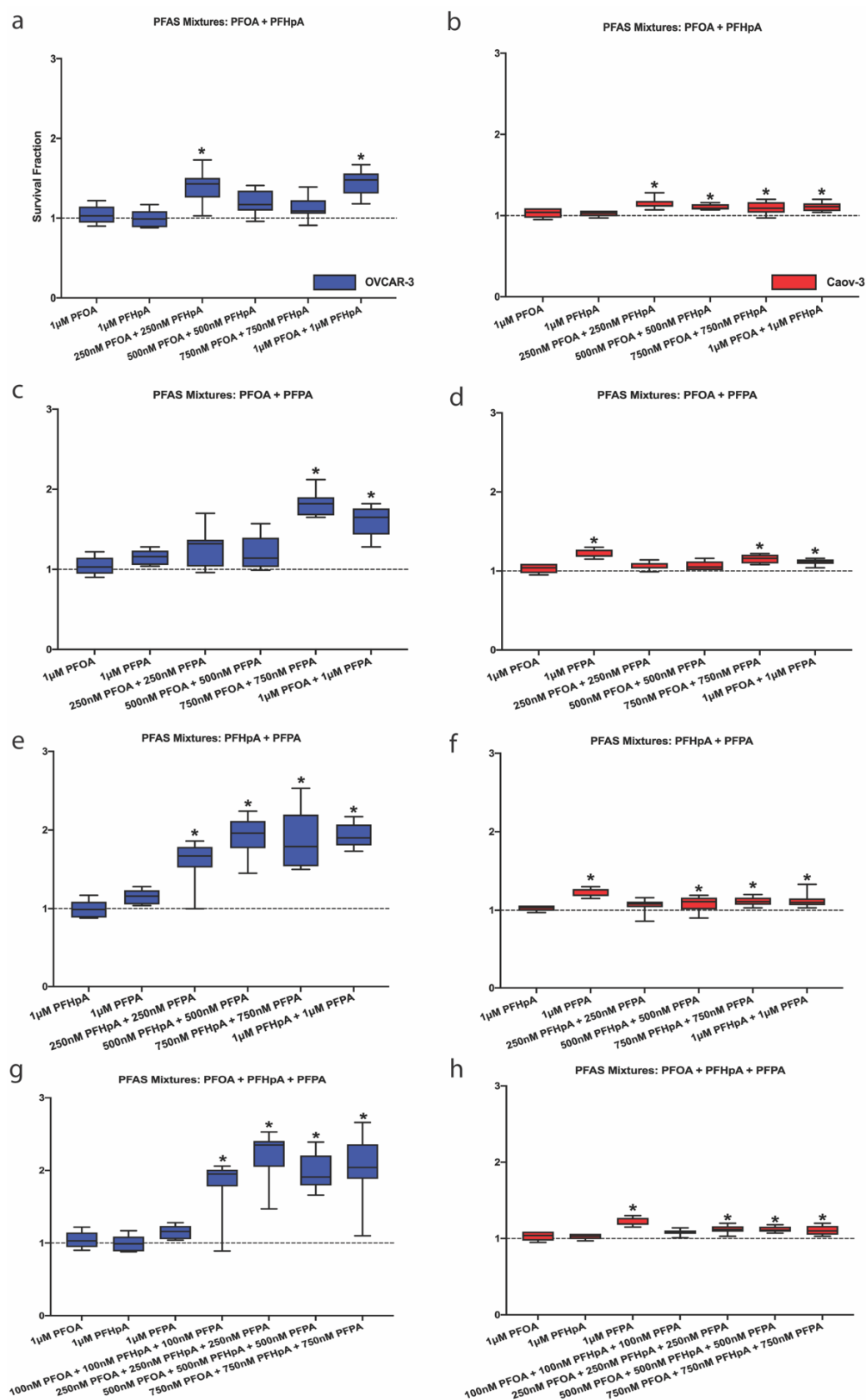
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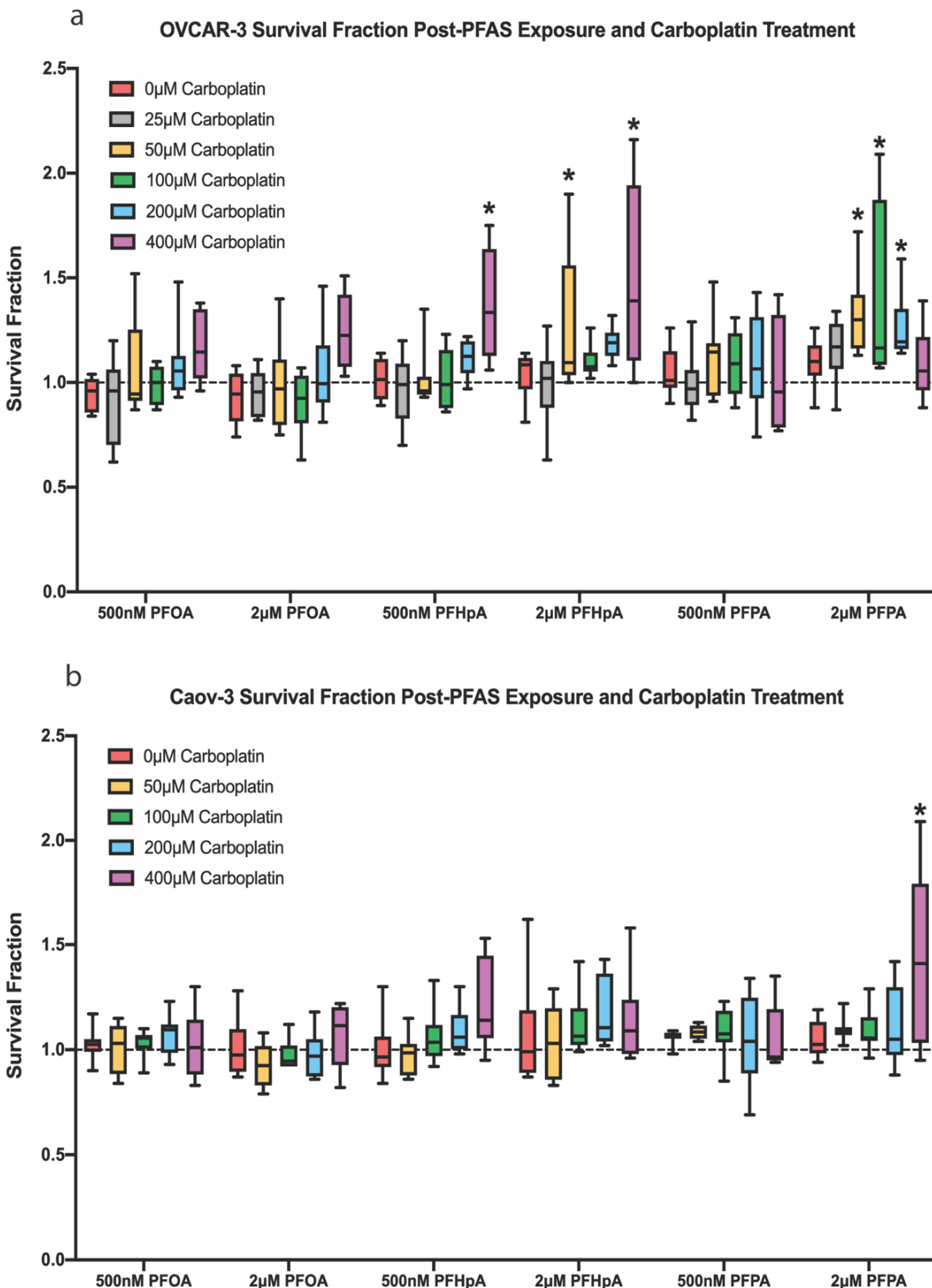
**Figure S1.** OVCAR-3 and Caov-3 optimal seeding densities and reagent volume based on CellTiter Glo linear dynamic range. CellTiter Glo luminescence readouts when **(a)** OVCAR-3 and **(b)** Caov-3 were plated at cell densities ranging from 0 – 5,000 cells/well and 0 – 10,000 cells/well, respectively, with the addition of 25, 50, or 100uL CellTiter Glo reagent [mean  $\pm$  SD;  $n = 3$  independent experiments in duplicate] after 6 days.



**Figure S2.** PFAS are mostly sub-cytotoxic in ovarian cancer cell lines at dose-ranging nanomolar and micromolar concentrations. OVCAR-3 (blue) and Caov-3 (red) survival fraction [mean  $\pm$  SD expressed as a percentage of the vehicle control (dashed line);  $n = 3$  independent experiments in duplicate] after 48 h exposure to 25nM - 2 $\mu$ M (a) PFOA, (b) PFHpA, or (c) PFPA.



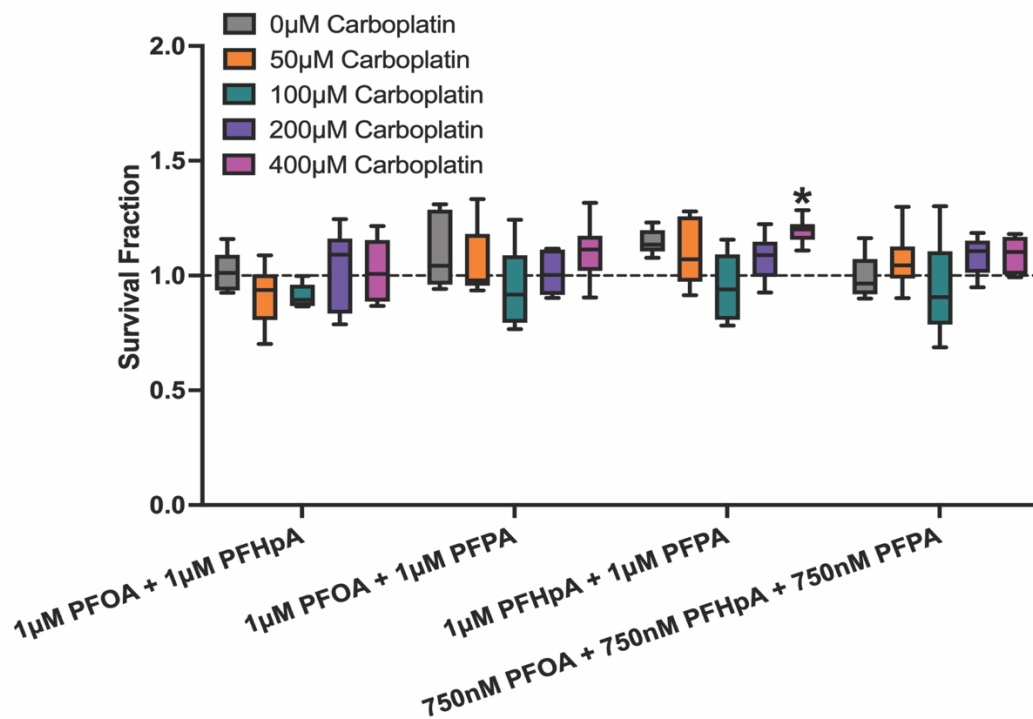
**Figure S3.** PFAS mixtures increase survival fraction in ovarian cancer cells. OVCAR-3 (blue) and Caov-3 (red) survival fraction [mean  $\pm$  SD expressed as a percentage of the vehicle control (dashed line);  $n = 3$  independent experiments in duplicate for individual PFAS,  $n = 3$  independent experiments in triplicate for mixtures] after 48 h exposure to (a,b) PFOA + PFHpA, (c,d) PFOA + PFPA, (e,f) PFHpA + PFPA, or (g,h) PFOA + PFHpA + PFPA.



**Figure S4.** PFAS increase survival fraction post-carboplatin treatment in ovarian cancer cells. Select PFAS induce carboplatin resistance, indicated by an increase in survival fraction post-PFAS exposure and carboplatin treatment compared to the vehicle control (dashed line), in **(a)** OVCAR-3 and **(b)** Caov-3 cells. OVCAR-3 cell viability [mean  $\pm$  SD expressed as a percentage of the vehicle control for each carboplatin group;  $n = 4$  independent experiments in duplicate (0, 25, 50, 100, and 200  $\mu$ M carboplatin) or  $n = 3$  independent experiments in duplicate (400  $\mu$ M carboplatin)] after 48 h exposure to PFOA, PFHpA, or PFPA followed by 48 h treatment with carboplatin. Caov-3 viability [mean  $\pm$  SD expressed as a percentage of the vehicle control for each carboplatin group;  $n = 4$  independent experiments in duplicate] after 48 h exposure to PFOA, PFHpA, or PFPA followed by 48 h treatment with carboplatin.

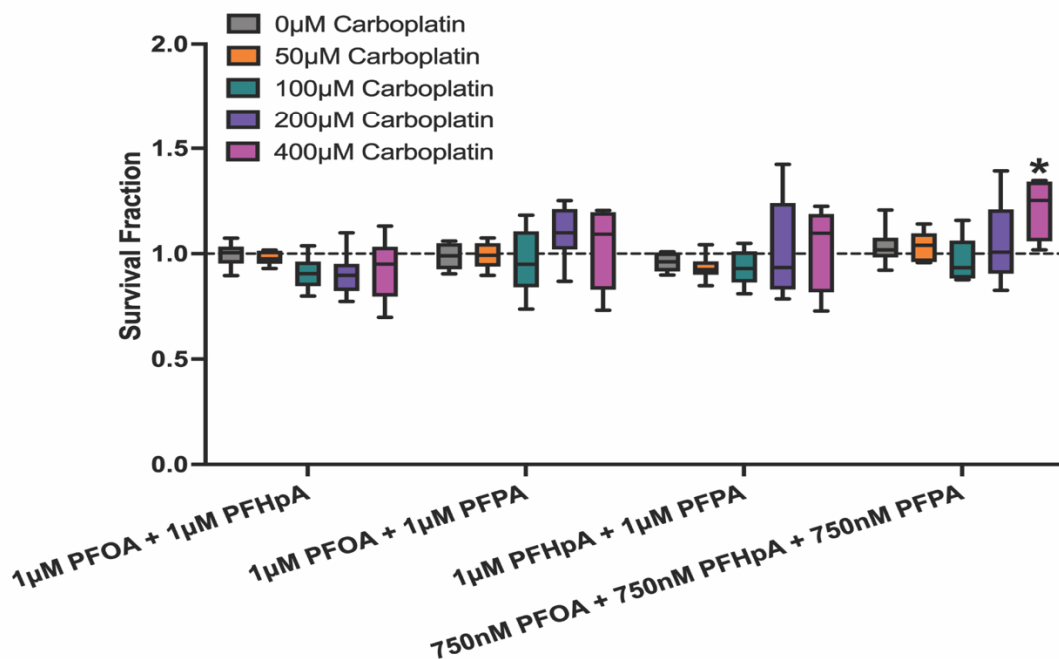
a

### OVCAR-3 Survival Fraction Post-PFAS Mixture Exposure and Carboplatin Treatment



b

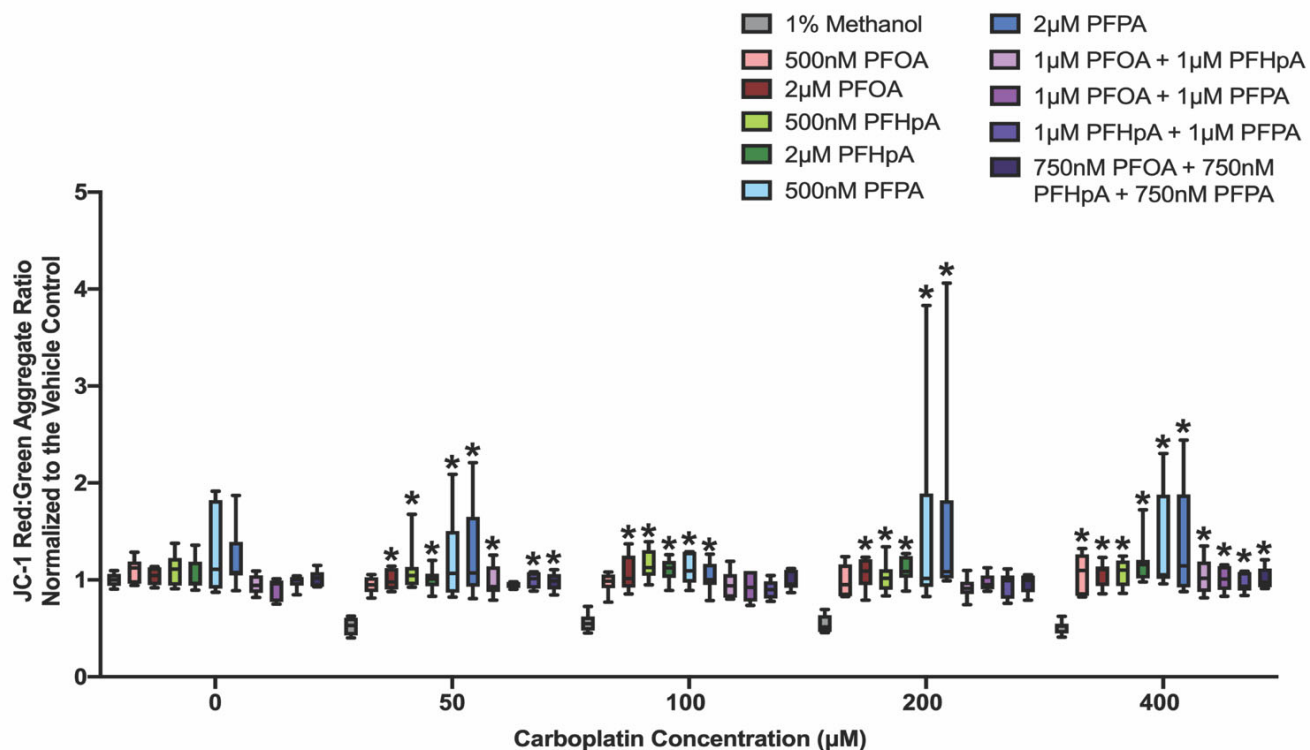
### Caov-3 Survival Fraction Post-PFAS Mixture Exposure and Carboplatin Treatment



**Figure S5.** Select PFAS mixtures increase survival fraction post-carboplatin treatment in ovarian cancer cells. **(a)** OVCAR-3 cell survival fraction increases post-1µM PFHpA + 1µM PFPA and 400µM carboplatin treatment. **(b)** Caov-3 cell survival fraction increases post-750nM PFOA + 750nM PFHpA + 750nM PFPA and 400µM carboplatin treatment. OVCAR-3 and Caov-3 cell viability [mean ± SD expressed as a percentage of the vehicle control (dashed line) for each carboplatin group;  $n = 3$  independent experiments in duplicate] after 48 h exposure to PFOA + PFHpA, PFOA + PFPA, PFHpA + PFPA or PFOA + PFHpA + PFPA followed by 48 h treatment with carboplatin.

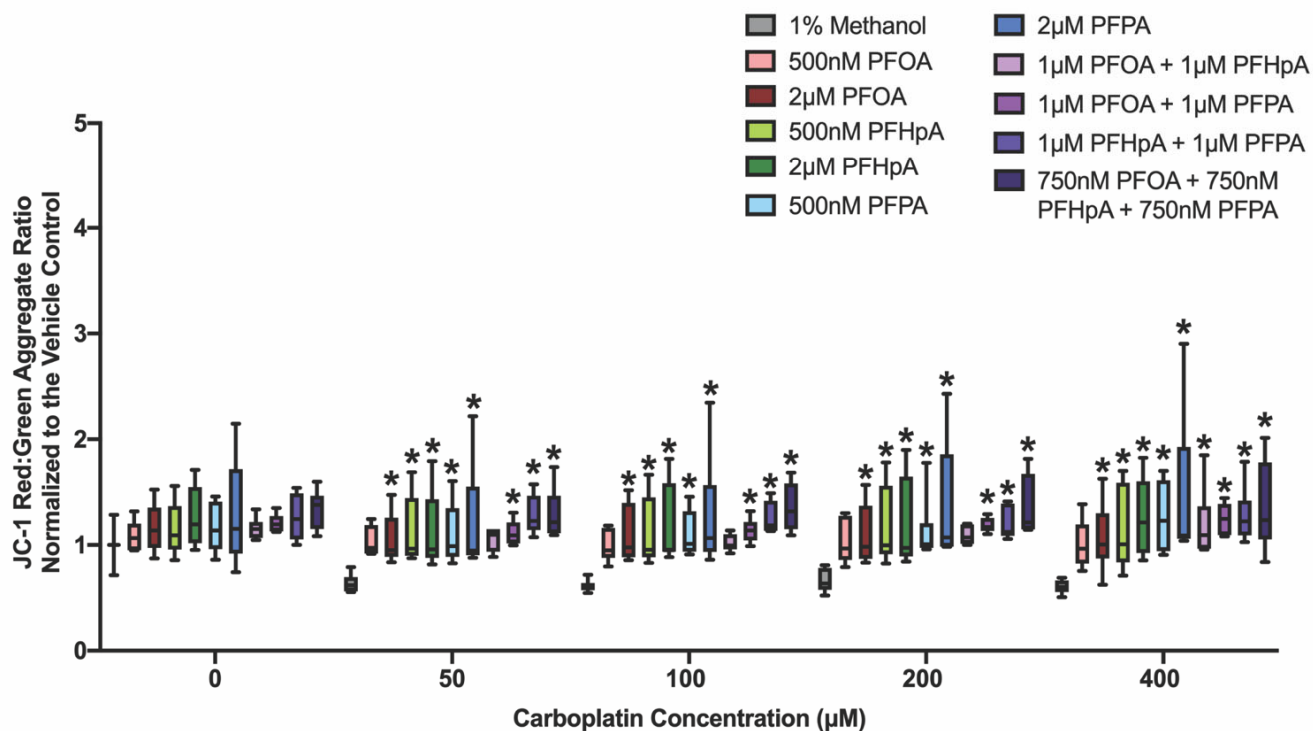
a

### OVCAR-3 JC-1 Aggregate Ratio Post-PFAS Exposure and Carboplatin Treatment

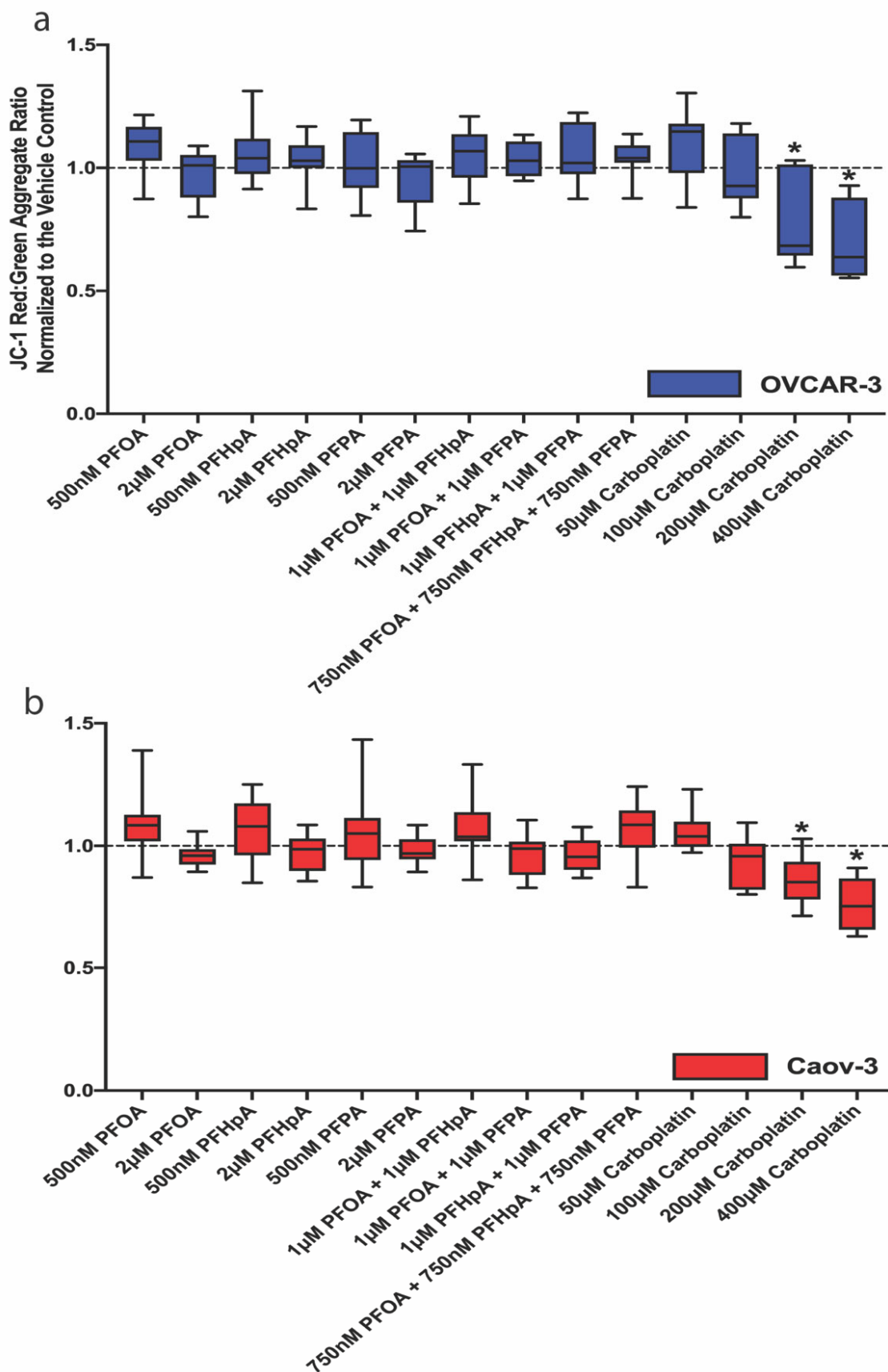


b

### Caov-3 JC-1 Aggregate Ratio Post-PFAS Exposure and Carboplatin Treatment



**Figure S6.**  $\Delta\Psi_m$  increases post-PFAS exposure and carboplatin treatment in OVCAR-3 and Caov-3 cells. In (a) OVCAR-3 and (b) Caov-3 cells,  $\Delta\Psi_m$  increases in every PFAS exposure group and at least one carboplatin concentration [mean  $\pm$  SD expressed as a percentage of the vehicle control for each carboplatin group;  $n = 3$  independent experiments in triplicate].



**Figure S7.** Carboplatin treatment, but not PFAS exposure, decreases  $\Delta\Psi_m$  in ovarian cancer cells on experimental day 6.  $\Delta\Psi_m$  decreases in (a) OVCAR-3 and (b) Caov-3 cells following 48-hour treatment with 200µM and 400µM carboplatin, but is unaffected by 48-hour PFAS or PFAS mixture exposure followed by a 48-hour incubation with fresh medium [mean  $\pm$  SD expressed as a percentage of the vehicle control (dashed line);  $n = 4$  independent experiments in triplicate].

Exposure Group by Cell Line (OVCAR-3 = blue-shaded region, Caov-3 = red-shaded region)	IC <sub>50</sub> (μM carboplatin)
Vehicle Control (Figure S3A Internal)	58.42
500nM PFOA	61.72
2μM PFOA	58.26
500nM PFHpA	62.83
2μM PFHpA	71.49
500nM PFPA	46.96
2μM PFPA	63.47
Vehicle Control (Figure S4A Internal)	42.50
1μM PFOA + 1μM PFHpA	40.72
1μM PFOA + 1μM PFPA	44.28
1μM PFHpA + 1μM PFPA	41.62
1μM PFOA + 1μM PFHpA + 1μM PFPA	46.02
Vehicle Control (Figure S3B Internal)	164.5
500nM PFOA	179.3
2μM PFOA	169.2
500nM PFHpA	189.1
2μM PFHpA	193.9
500nM PFPA	150.9
2μM PFPA	163.1
Vehicle Control (Figure S4B Internal)	116.0
1μM PFOA + 1μM PFHpA	119.6
1μM PFOA + 1μM PFPA	135.9
1μM PFHpA + 1μM PFPA	130.9
1μM PFOA + 1μM PFHpA + 1μM PFPA	128.8

**Table S1.** OVCAR-3 and Caov-3 IC<sub>50</sub> values post-PFAS or PFAS mixture exposure and carboplatin treatment. IC<sub>50</sub> values for OVCAR-3 cells (blue-shaded region) and Caov-3 cells (red-shaded region) post-PFAS or PFAS mixture exposure and carboplatin treatment determined using nonlinear regression analysis. Each PFAS exposure group was internally normalized to its own vehicle control which is appropriately labeled for accurate comparison.