

SUPPLEMENTARY INFORMATION

Effects of electrochemotherapy on immunologically important modifications in tumor cells

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Supplementary materials

Table S1: Cell densities for clonogenic assay with CDDP and OXA.

		CDDP and OXA									
Chemotherapeutic concentration (μM)		0	2.5	5	10	15	20	30	40	60	80
No. of cells/well	without EP	75	75	75	75	75	75	100 (75 for B16-F10 with OXA)	150 (75 for B16-F10 with OXA)	75	75
	with EP	100	100	150	150	200	200	300	400	400	400

Table S2: Cell densities for clonogenic assay with BLM.

		BLM						
Chemotherapeutic concentration (nM)		0	0.01	0.1	0.5	1.0	1.3	1.8
No. of cells/well	without EP	50	75	75	75	75	100	100
	with EP	100	100	150	150	150	200	250

Table S3: List of antibodies used.

Antibodies	Sources	Cat#	Volume per sample (μL)
MHC class I (H-2Kb) Monoclonal Antibody (AF6-88.5.5.3), APC	Thermo Fisher Scientific	17-5958-82	0.5
MHC class I (H-2Kd) Monoclonal Antibody (SF1-1.1.1), APC	Thermo Fisher Scientific	17-5957-82	0.5
MHC class II (I-A/I-E) Monoclonal Antibody (M5/114.15.2), Super Bright 780	Thermo Fisher Scientific	78-5321-82	0.5
Anti-Mouse CD274 (B7-H1), PE 100 μg (PD-L1)	Thermo Fisher Scientific	12-5982-82	0.5
Calreticulin Antibody (DyLight 405), 0.1 mL	Novus Biologicals	NV-NB600-101V	1
CD40 Monoclonal Antibody (1C10), Super Bright 600	Thermo Fisher Scientific	63-0401-82	1.5

Table S4: Determined IC values for all chemotherapeutics.

the

A				B			
CDDP (μM)	IC30	IC50	IC70	OXA (μM)	IC30	IC50	IC70
B16-F10	6.86 ± 0.73	12.90 ± 1.46	17.87 ± 1.93	B16-F10	8.89 ± 2.42	24.46 ± 2.57	39.29 ± 3.58
4T1	5.23 ± 1.65	13.80 ± 1.91	22.24 ± 2.67	4T1	5.65 ± 0.76	13.95 ± 1.45	18.92 ± 1.10
CT26	4.54 ± 0.60	11.70 ± 0.71	17.69 ± 0.78	CT26	2.68 ± 0.37	14.62 ± 1.38	23.58 ± 2.07

C			
BLM (nM)	IC30	IC50	IC70
B16-F10	0.15 ± 0.05	0.34 ± 0.05	0.63 ± 0.04
4T1	0.16 ± 0.04	0.35 ± 0.06	0.50 ± 0.10
CT26	0.06 ± 0.02	0.27 ± 0.05	0.56 ± 0.07

Supplementary figures

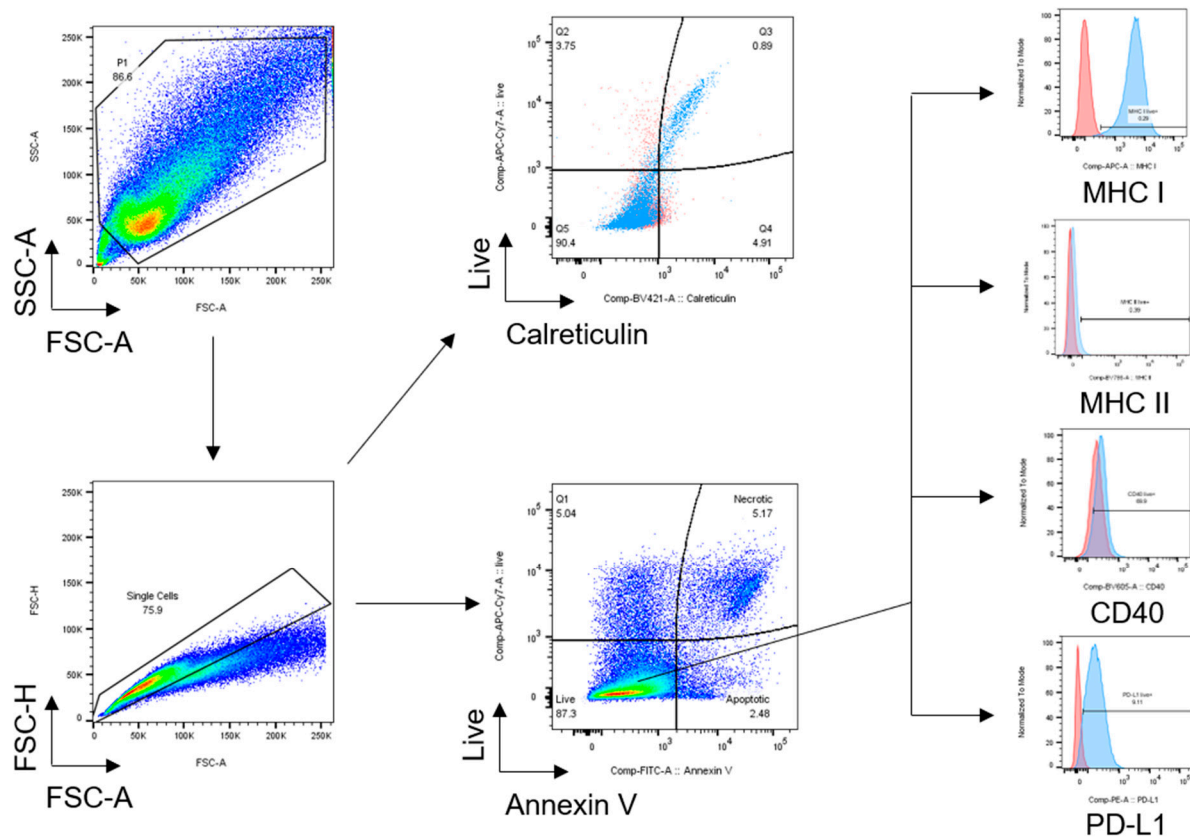


Figure S1: Gating strategy. After ECT experiments were performed, single cells were isolated from debris (SSC-A vs FSC-A) and doublets (FSC-H vs FSC-A). After gating of live and dead cells (efluor 780; APC-Cy7-A vs Annexin V), cells that express MHC I, MHC II, CD40 and PD-L1 were identified. After gating of single cells, cells that express Calreticulin (efluor 780; APC-Cy7-A vs BV421-A) were identified. Percentages shown are derived from the proportion of cells positive for each marker. A total of 100,000 events were collected. SSC-A, side-scatter area; FSC-A, forward-scatter area; FSC-H, forward-scatter height.

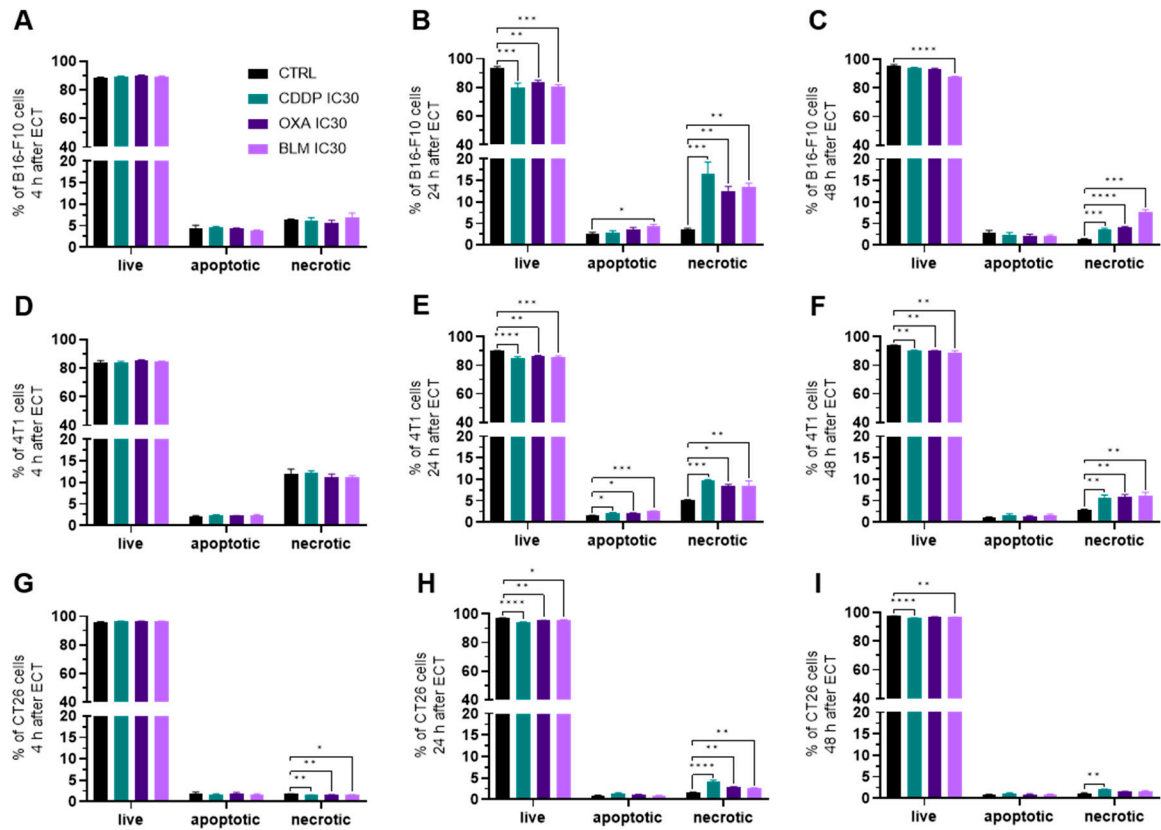


Figure S2: Cell viability at IC₃₀ concentrations. Cell viability in B16-F10 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. Cell viability in 4T1 cell line 4 h (D), 24 h (E) and 48 h (F) after ECT. Cell viability in CT26 cell line 4 h (G), 24 h (H) and 48 h (I) after ECT. The values are presented as the AM ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, n = 4.

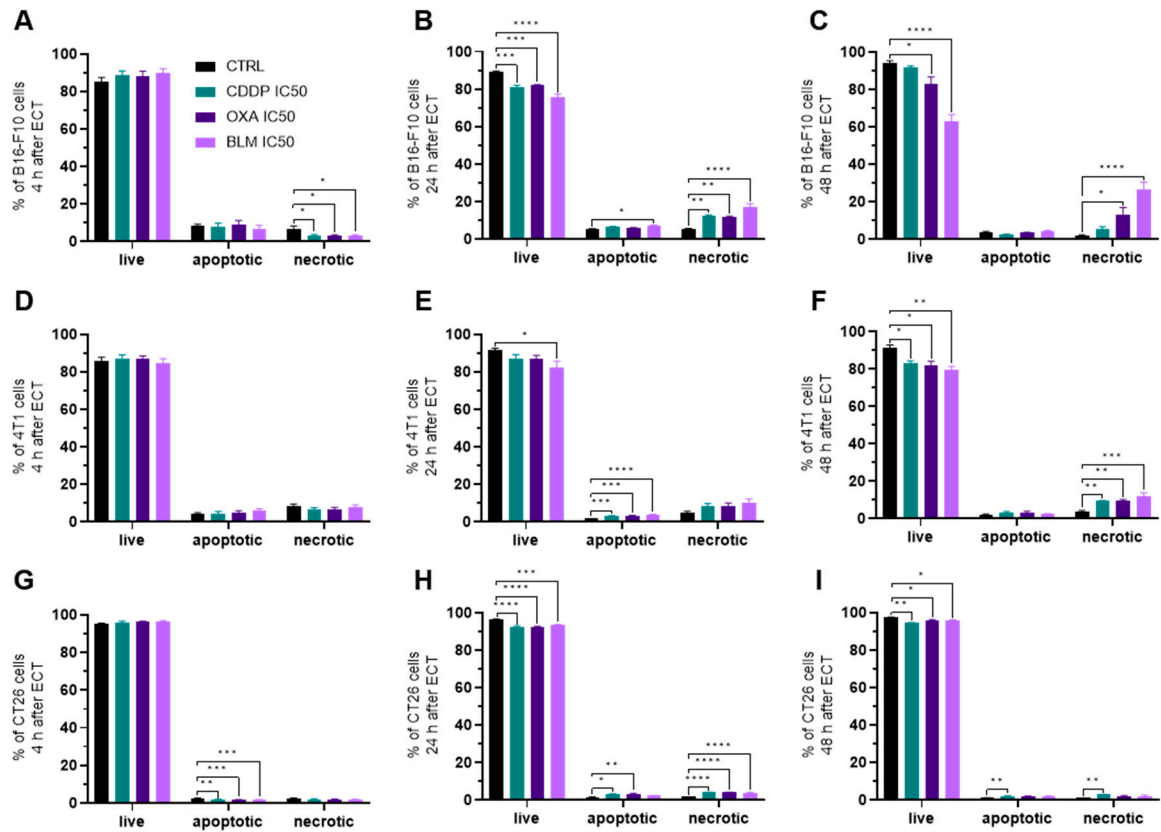


Figure S3: Cell viability at IC₅₀ concentrations. Cell viability in B16-F10 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. Cell viability in 4T1 cell line 4 h (D), 24 h (E) and 48 h (F) after ECT. Cell viability in CT26 cell line 4 h (G), 24 h (H) and 48 h (I) after ECT. The values are presented as the AM ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, n = 4.

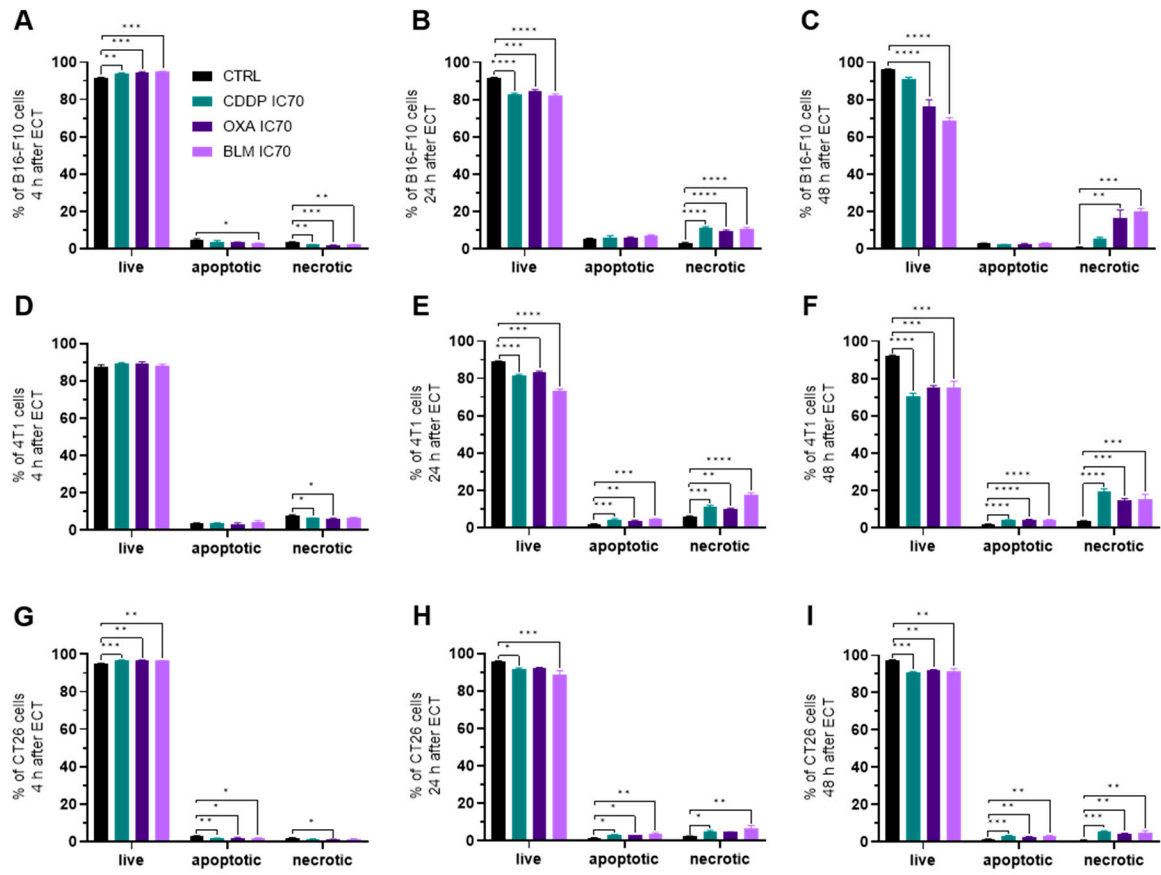


Figure S4: Cell viability at IC₇₀ concentrations. Cell viability in B16-F10 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. Cell viability in 4T1 cell line 4 h (D), 24 h (E) and 48 h (F) after ECT. Cell viability in CT26 cell line 4 h (G), 24 h (H) and 48 h (I) after ECT. The values are presented as the AM ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, n = 4.

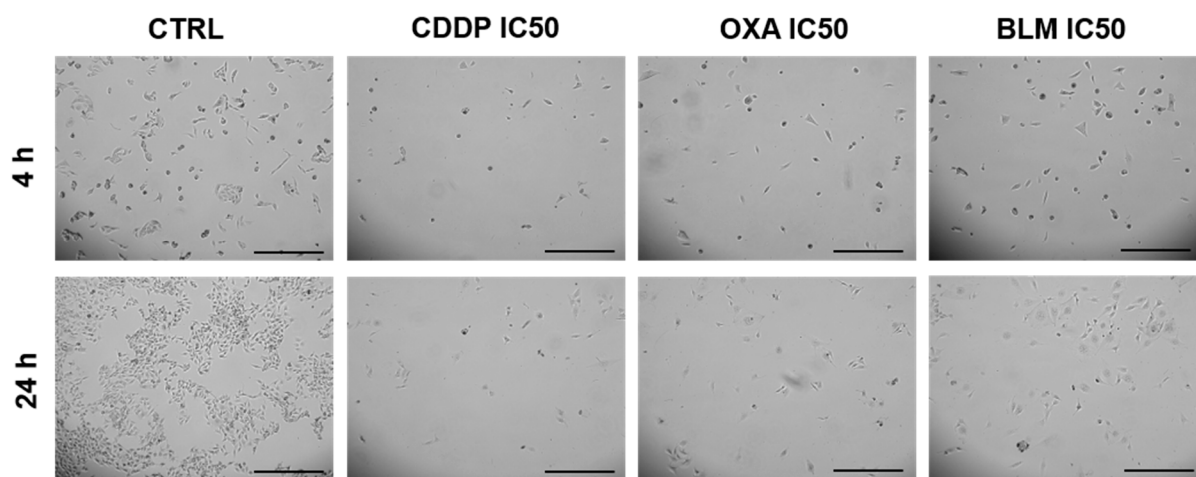


Figure S5: Cell number changes after ECT in the B16-F10 cell line. Cells 4 or 24 h after ECT with IC₅₀ concentrations of CDDP, OXA or BLM in comparison with untreated control cells. Scale bar: 0.5 mm.

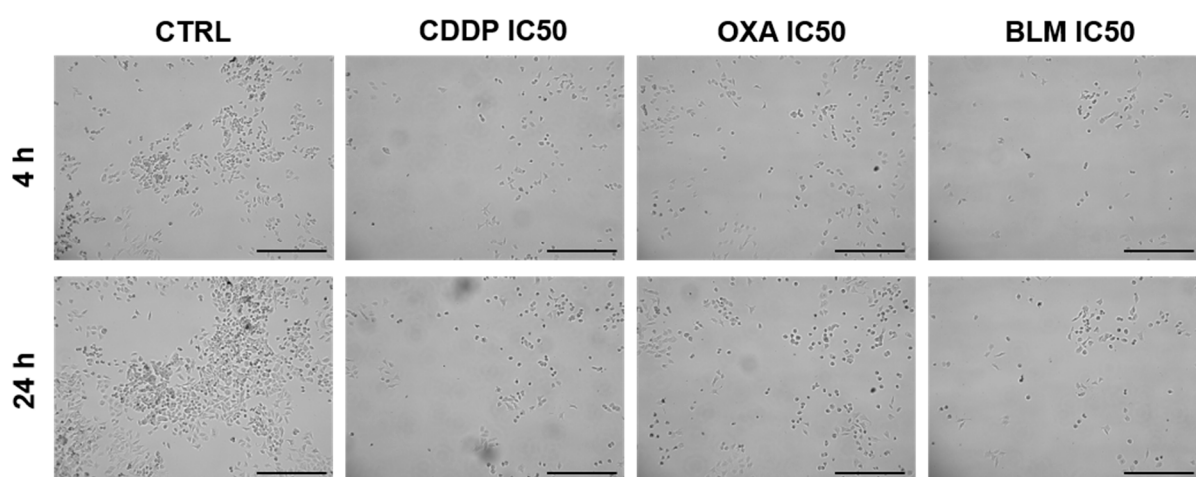


Figure S6: Cell number changes after ECT in the 4T1 cell line. Cells 4 or 24 h after ECT with IC₅₀ concentrations of CDDP, OXA or BLM in comparison with untreated control cells. Scale bar: 0.5 mm.

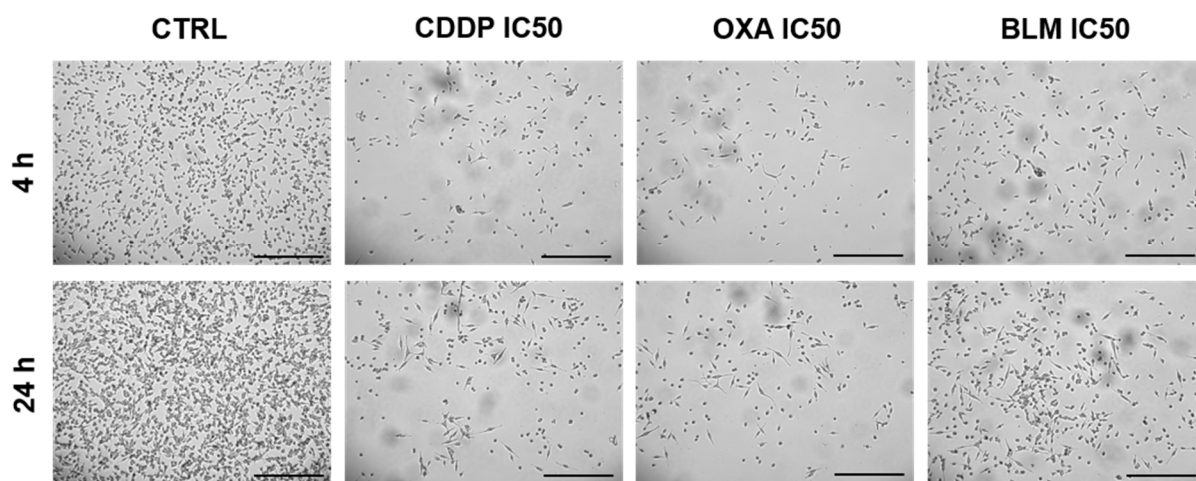


Figure S7: Cell number changes after ECT in the CT26 cell line. Cells 4 or 24 h after ECT with IC₅₀ concentrations of CDDP, OXA or BLM in comparison with untreated control cells. Scale bar: 0.5 mm.

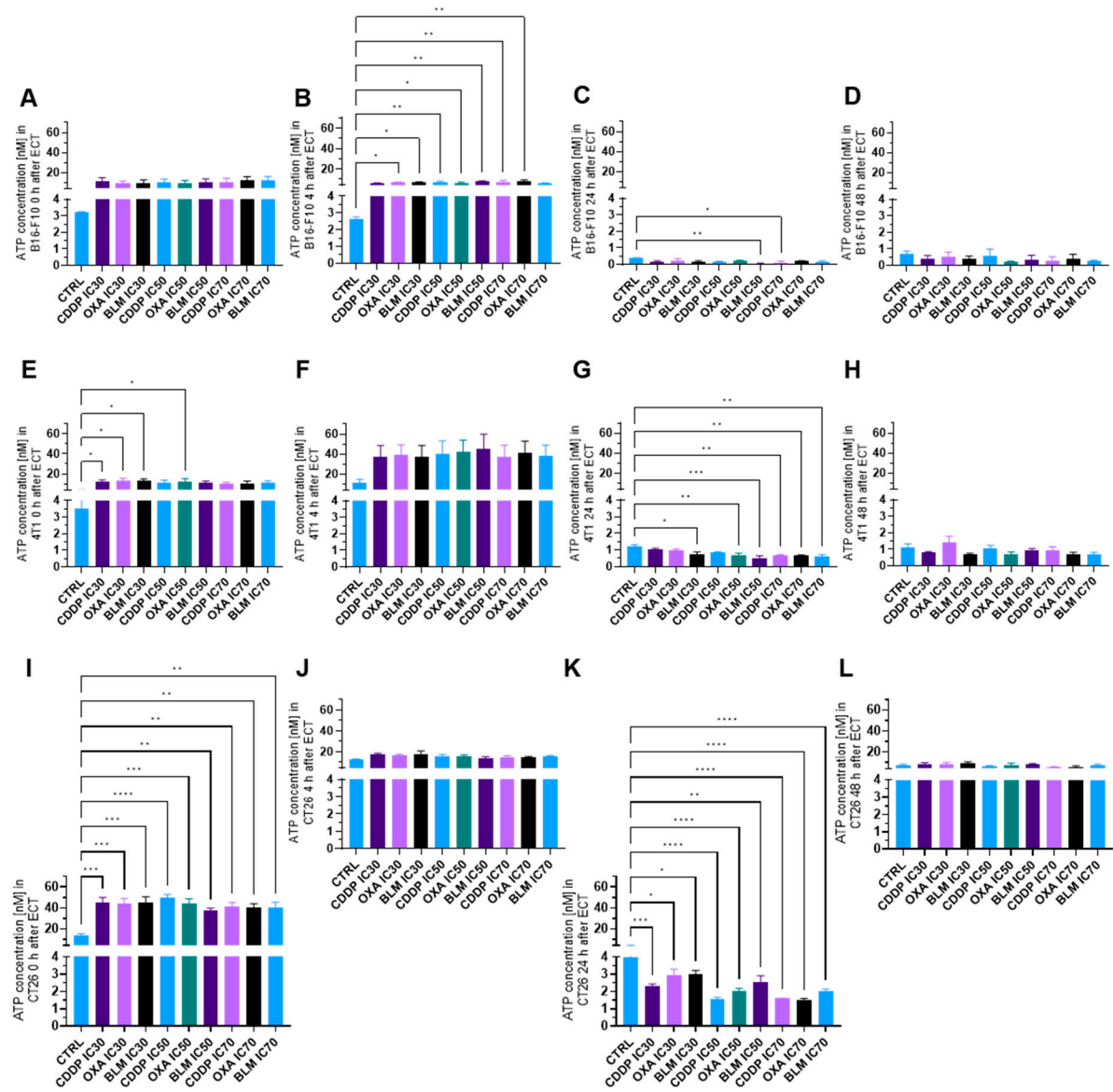


Figure S8: Released ATP concentration in B16-F10, 4T1 and CT26 cell lines. Released ATP concentration in B16-F10 cell line 0 h (A), 4 h (B), 24 h (C) and 48 h (D) after ECT. Released ATP concentration in 4T1 cell line 0 h (E), 4 h (F), 24 h (G) and 48 h (H) after ECT. Released ATP concentration in CT26 cell line 0 h (I), 4 h (J), 24 h (K) and 48 h (L) after ECT. The values are presented as the AM \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, $n = 3$.

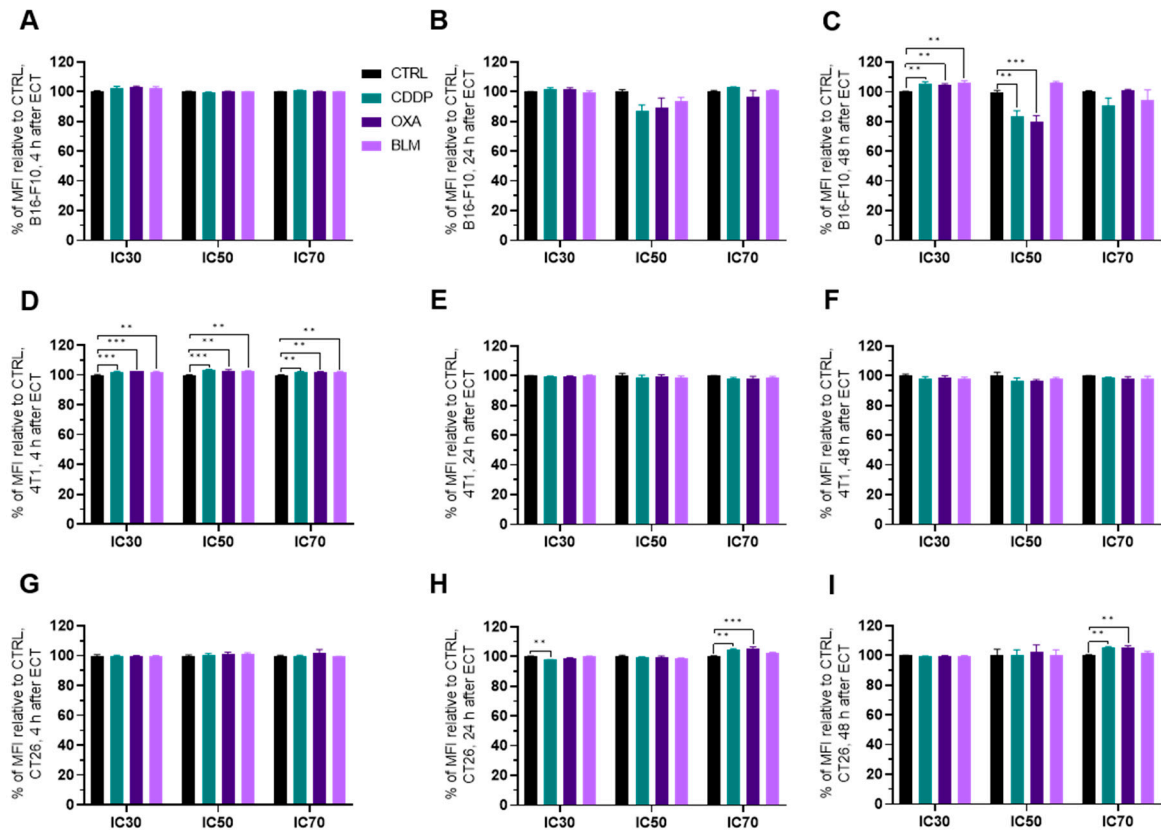


Figure S9: CRT expression after ECT. Calreticulin expression in B16-F10 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. Calreticulin expression in 4T1 cell line 4 h (D), 24 h (E) and 48 h (F) after ECT. Calreticulin expression in CT26 cell line 4 h (G), 24 h (H) and 48 h (I) after ECT. The values are presented as the AM ± SEM. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001, n = 4.

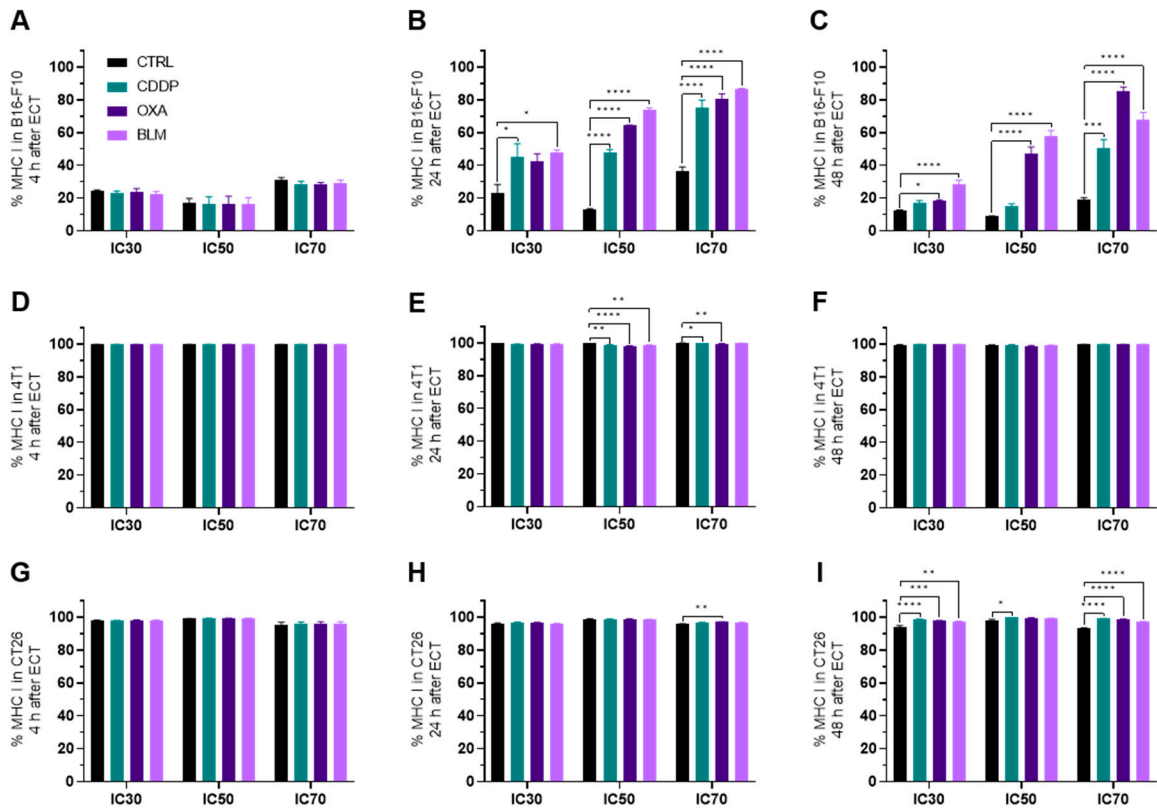


Figure S10: MHC I expression after ECT. Percent of MHC I positive cells is shown. MHC I expression in B16-F10 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. MHC I expression in 4T1 cell line 4 h (D), 24 h (E) and 48 h (F) after ECT. MHC I expression in CT26 cell line 4 h (G), 24 h (H) and 48 h (I) after ECT. The values are presented as the AM \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, $n = 4$.

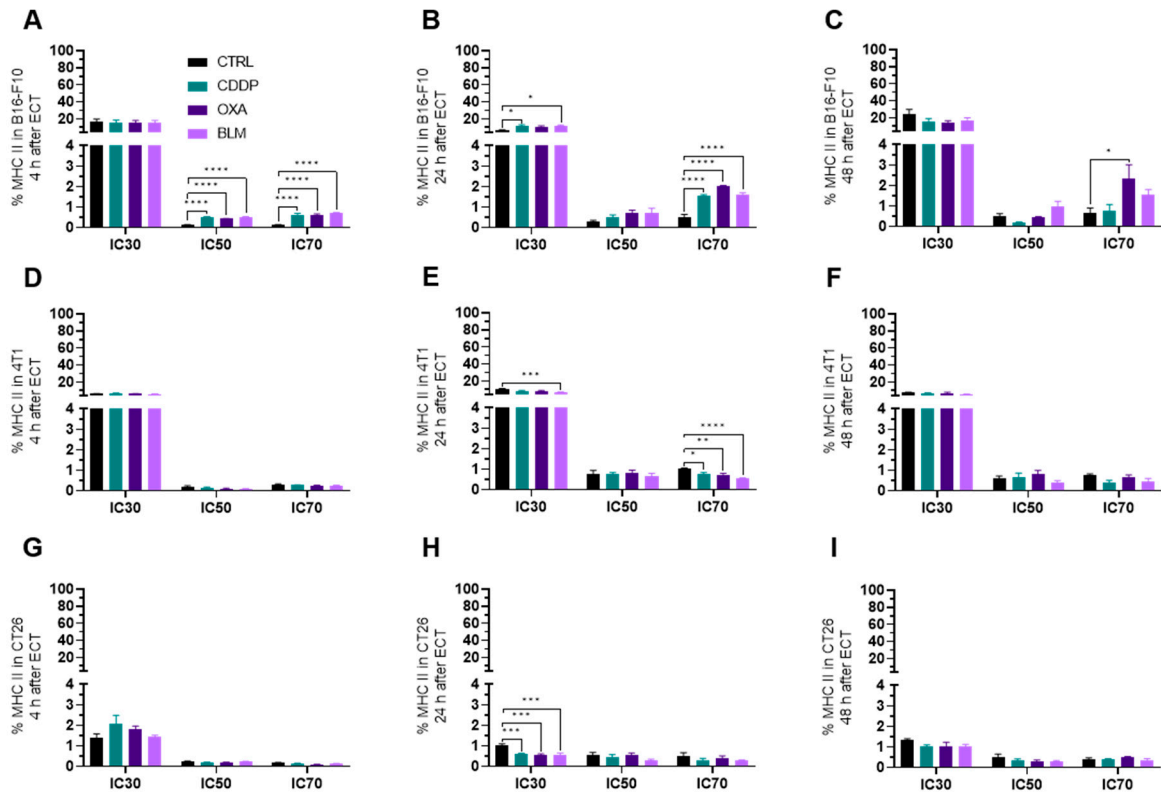


Figure S11: MHC II expression after ECT. Percent of MHC II positive cells is shown. MHC II expression in B16-F10 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. MHC II expression in 4T1 cell line 4 h (D), 24 h (E) and 48 h (F) after ECT. MHC II expression in CT26 cell line 4 h (G), 24 h (H) and 48 h (I) after ECT. The values are presented as the AM \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, $n = 4$.

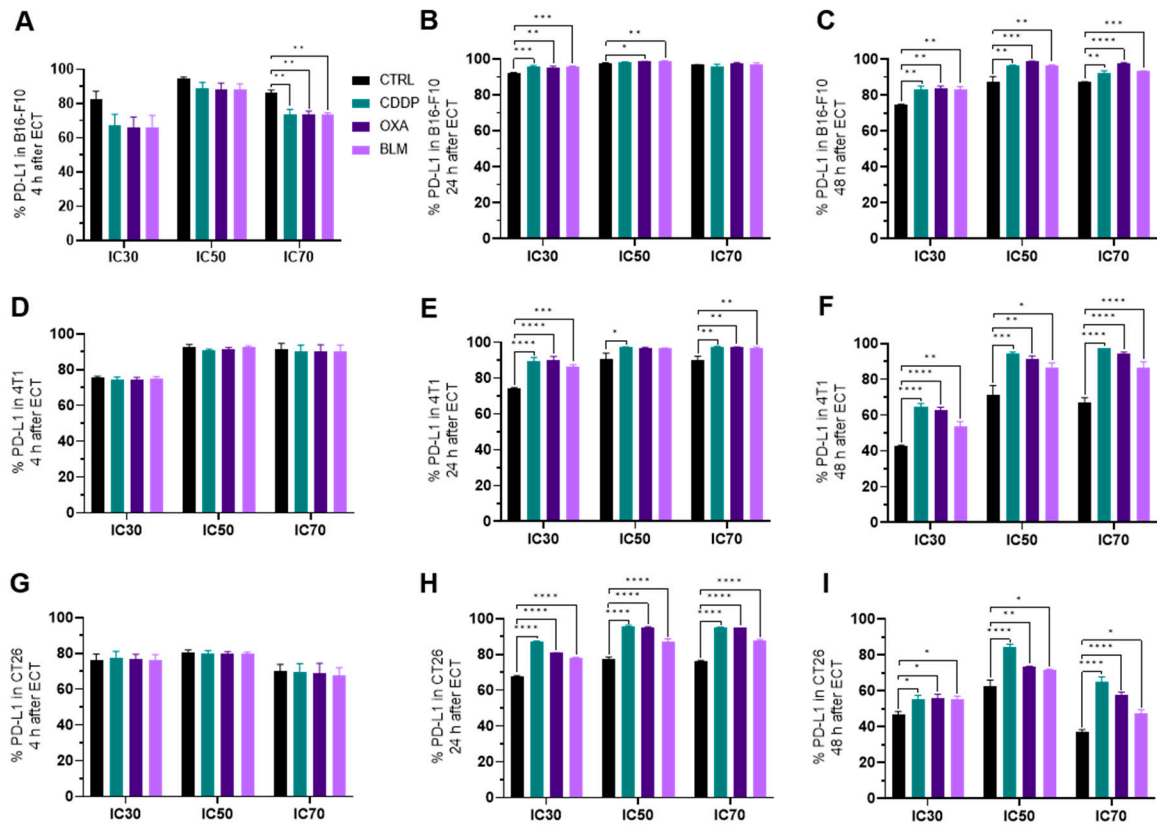


Figure S12: PD-L1 expression after ECT. PD-L1 expression in B16-F10 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. PD-L1 expression in 4T1 cell line 4 h (D), 24 h (E) and 48 h (F) after ECT. PD-L1 expression in CT26 cell line 4 h (G), 24 h (H) and 48 h (I) after ECT. The values are presented as the AM \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, $n = 4$.

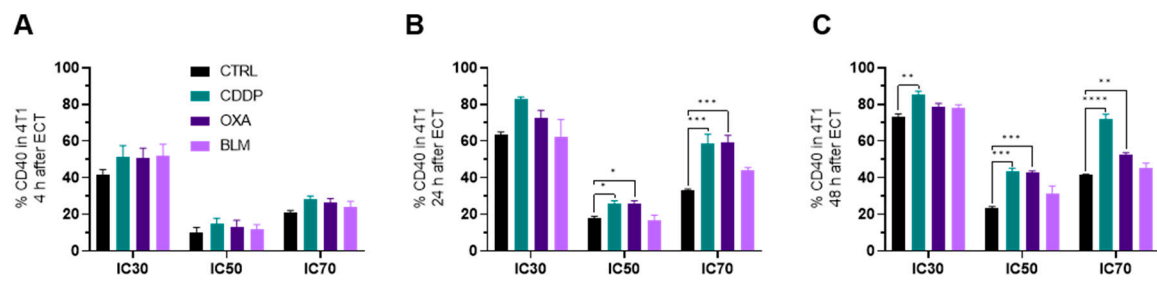


Figure S13: CD40 expression in the 4T1 cell line 4 h (A), 24 h (B), 48 h (C) after ECT. The values are presented as the AM \pm SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, $n = 4$.