Supplementary Materials

Antitumor Response and Immunomodulatory Effects of Sub-Microsecond Irreversible Electroporation and Its Combination with Calcium Electroporation

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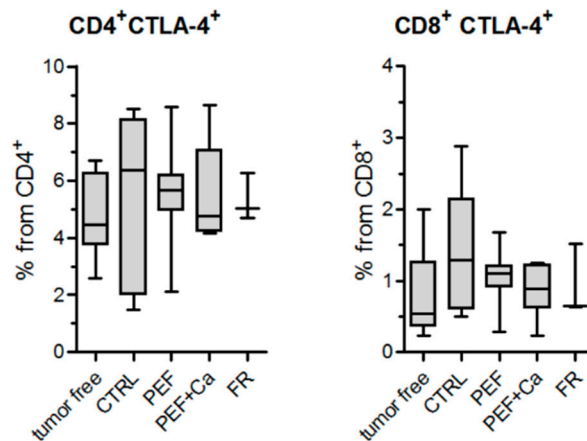

Figure S1. Flow cytometric analysis of CTLA expression on CD4 and CD8 T cells in spleen. Tumor free mice, CRTL (untreated and only CaCl2 treated mice), PEF – PEF2 treated mice, PEF+Ca- PEF2 and CaCl2 treated mice, FR (fully recovered). Gating strategy is presented in figure S3.
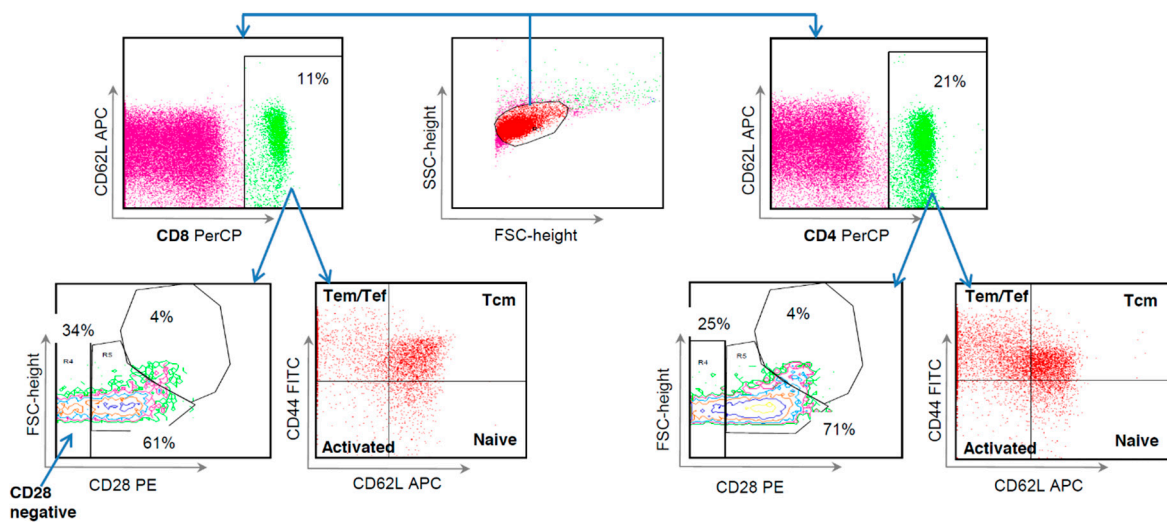

Figure S2. Gating strategy of CD28-negative, and Tem/Tef and Tcm CD4 and CD8 T cells. Splenocytes wre stained with CD28-PE/ CD62L-APC/CD44-FITC in combination with CD4-PerCP or CD8-PerCP and analyzed by BD FACSCalibur and CellQuest software.
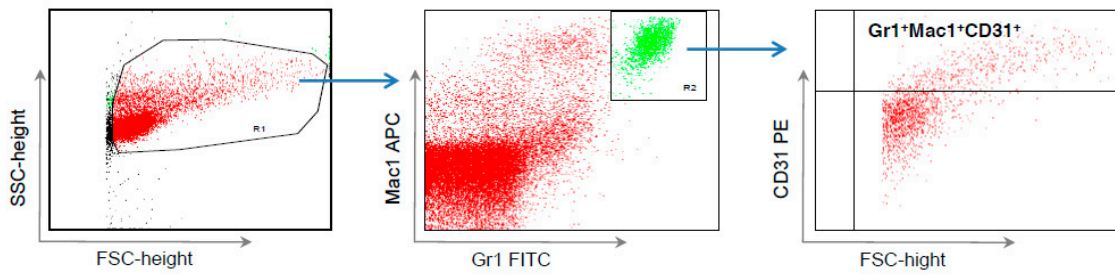
Figure S3. Gating strategy of myeloid suppressor Mac1+Gr1+CD31+ cells. Splenocytes were stained with Gr1-FITC/Mac1-APC/CD31-PE and analyzed by BD FACSCalibur and CellQuest software.
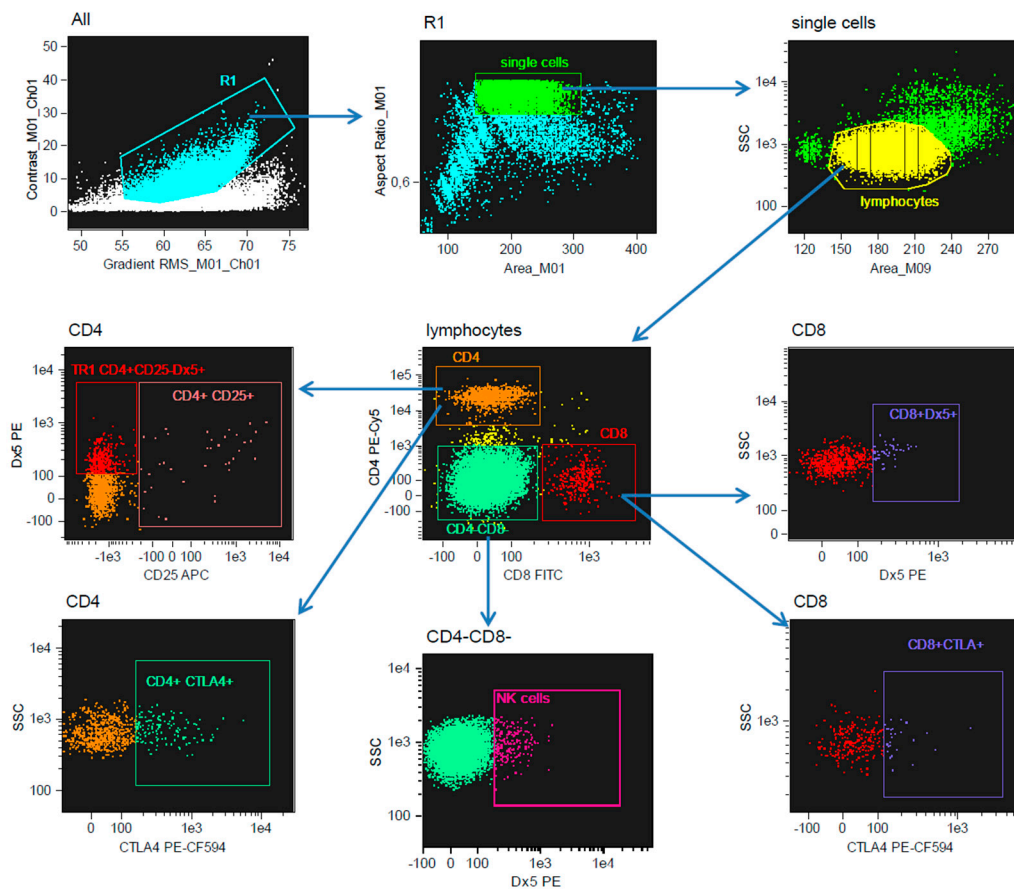

Figure S4. Gating strategy of CD4+CD25-Dx5+ (Tr1), CD4+CD25+, CD8+Dx5+, CD4+CTLA+, CD8+CTLA+ and NK cells. Splenocytes were stained with CD4-PE-Cy5/CD8-FITC/CD25-APC/Dx5-PE (for splenocytes) and CD4-PE-Cy5/CD8-FITC/CTLA4–PE-CF594 and analyzed by FlowSight (Amnis Millipore) cytometer and IDEAS software.

Table S1. Expression of CD28 on Tem/Tef and Tcm T cells.

	CD28 -/low	CD28 medium	CD28 high	CD28 -/low	CD28 medium	CD28 high	CD28 -/low	CD28 medium	CD28 high	CD28 -/low	CD28 medium	CD28 high
	% from CD4+ CD44+CD62L- (CD4 Tem/Tef)			% from CD4+ CD44+CD62L+ (CD4 Tcm)			% from CD8+ CD44+CD62L- (CD8 Tem/Tef)			% from CD8+ CD44+CD62L+ (CD8 Tcm)		
Spleen												
CTRL wt	25,5 ±2,8	64,7 ±4,7	7,6 ±1,9	20,9 ±4,2	53,3 ±7,1	23,4 ±5,7	15,9 ±5,4	56,9 ±6,2	24,6 ±9,9	31,1 ±5,3	56,7 ±3,9	12,4 ±3,5
CTRL	24,9 ±3,0	68,5 ±5,0	6,1 ±1,9	17,1 ±5,9	52,5 ±3,2	27,9 ±6,2	14,2 ±2,2	58,9 ±5,3	23,2 ±2,9	30,2 ±11,9	51,8 ±3,3	17,0 ±12,1
PEF2	22,8 ±3,1	**60,2 ±5,2**	**10,9 ±3,8**	19,4 ±3,4	49,8 ±2,9	27,2 ±3,5	14,8 ±5,4	52,6 ±7,6	29,3 ±11,0	32,8 ±3,6	56,2 ±4,6	10,9 ±2,8
PEF2+Ca	22,7 ±4,6	60,1 ±4,5	**12,0 ±6,4**	18,0 ±3,0	40,7 ±15,8	30,6 ±6,0	16,4 ±5,9	55,0 ±6,9	26,0 ±10,6	35,6 ±6,9	51,3 ±5,9	12,8 ±4,3
Lymph nodes												
CTRLwt	37,1 ±6,3	57,8 ±3,6	4,4 ±2,3	43,2 ±9,9	51,7 ±7,2	5,0 ±3,4	34,1 ±5,9	61,0 ±4,6	4,7 ±2,2	42,8 ±6,1	56,9 ±5,5	1,2 ±0,7
CTRL	35,1 ±8,5	62,4 ±7,4	2,6 ±1,6	39,6 ±14,8	57,7 ±14,4	3,4 ±1,7	39,7 ±14,5	57,2 ±11,6	3,3 ±2,3	45,8 ±15,3	54,3 ±14,0	1,2 ±1,4
PEF2	31,1 ±9,5	58,0 ±7,8	9,3 ±11,1	**27,5 ±11,9**	55,0 ±15,3	15,7 ±21,9	36,1 ±4,7	59,2 ±4,1	4,5 ±4,2	39,0 ±16,7	50,6 ±13,3	8,7 ±20,8
PEF2+Ca	32,1 ±3,7	60,3 ±3,1	6,5 ±5,6	32,3 ±6,2	58,9 ±3,9	8,1 ±5,3	36,8 ±5,6	58,1 ±5,2	4,9 ±2,4	41,6 ±3,7	57,7 ±2,3	1,6 ±1,4
Tumor infiltrated lymphocytes (TIL)												
CTRL	24,8 ±2,9	71,3 ±3,0	4,2 ±3,2	28,4 ±14,6	59,1 ±15,6	11,5 ±8,0	32,6 ±4,6	66,4 ±5,0	1,9 ±1,5	31,3 ±1,4	53,1 ±12,0	15,6 ±11,4
PEF2	17,5 ±8,0	64,2 ±9,2	5,1 ±2,9	14,9 ±12,2	60,3 ±9,6	18,0 ±8,4	**13,9 ±13,4**	41,5 ±33,0	**39,4 ±42,7**	**12,6 ±12,7**	61,5 ±12,5	24,1 ±18,5
PEF2+Ca	21,6 ±6,1	**60,6 ±6,2**	7,2 ±5,8	**13,9 ±7,5**	56,0 ±13,1	20,0 ±11,1	16,6 ±15,6	45,9 ±21,8	**37,2 ±37,3**	**12,9 ±17,0**	49,3 ±10,8	35,6 ±22,4

Tef/Tem -T effector memory cells, Tcm - T central memory cells. Significant differences (p<0.05) between the mice groups according to Mann Whitney test are in bold, where red - significant versus CTRL wt; blue - significant versus CTRL. CTRL wt – untreated mice without tumors; CTRL – tumor bearing control mice without treatment; CTRL+Ca – tumor bearing mice treated with $CaCl_2$; PEF1 and PEF2 – tumor bearing mice treated with PEF2 protocol –12 kV/cm × 500 ns × 500. PEF2+Ca – tumor-bearing mice treated with PEF2 and $CaCl_2$.