

## **Supplementary information**

### **Combination of STING and TLR 7/8 agonists as vaccine adjuvants for cancer immunotherapy**

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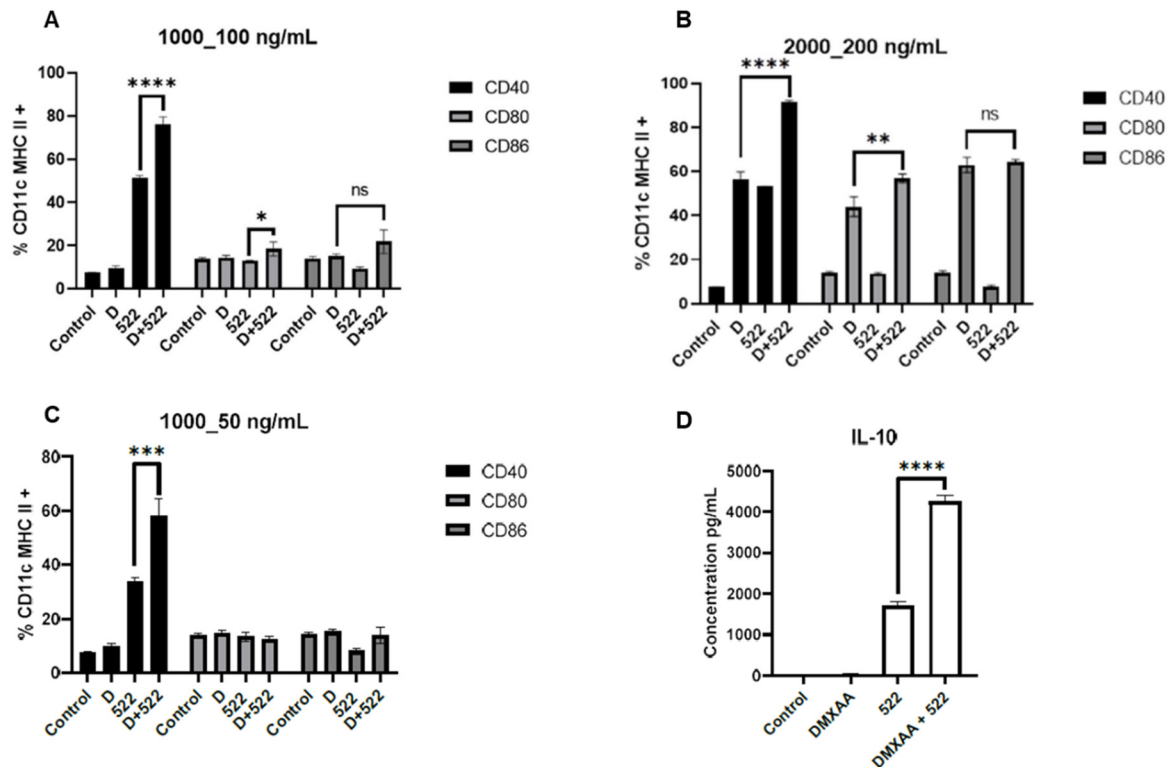
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Marker	Immune Cell Subsets	Function
CD45	All immune cells	Leukocyte marker
CD3	T cells	Lineage Marker
CD49	NK cells	Lineage marker
CD4	CD4 T cells	Lineage marker
CD8	CD8 T cells	Lineage Marker
PD-1	T cells	Checkpoint molecule
CD69	T, NK	Activation marker
CD44	T cells	Memory & Activation
Tetramer	CD8 T cells	OVA-specific CTLs
NKG2D	NK cells	Activation marker
CD11c	DCs – high, Mφ, B cells, Monocytes – low	Lineage marker
F4/80	Mφ	Lineage Marker
CD19	B cells	Lineage marker
H-2K <sup>b</sup> SIINFEKL	DCs, Mφ, B cells	Binds to OVA on MHC I expressed by APCs
PD-L1	APCs and tumor cells	Checkpoint molecule
CD40	DCs, Mφ, B cells	Activation marker
CD80	DCs, Mφ, B cells	Activation, M1 marker (F4/80+ CD206- CD80+)
CD86	DCs, Mφ, B cells	Activation marker
CD206	Immunosuppressive Mφ	M2 marker

Cell Subset	Markers
DCs	CD45+ F4/80- CD11c+
B cells	CD45+ F4/80 – CD19+
F4/80	CD45+ F4/80+ CD11c-
M2 macrophages	CD45+ F4/80+ CD80- CD206+
CD4 T cells	CD45+ CD3+ CD4+ CD8-
CD8 T cells	CD45+ CD3+ CD4- CD8+
NK Cells	CD45+ CD3- CD49b+ NKG2D+
Tumor cells	CD45-

**Figure S1.** List of markers used for flow cytometry studies and gating strategies for different cell populations.



**Figure S2.** BMDC costimulatory molecule expression and cytokine secretion. BMDCs were incubated with DMXAA (1000 ng/ml, 2000 ng/ml), 522 (50 ng/ml, 100 ng/ml, 200 ng/ml) and DMXAA + 522 (1000\_100, 1000\_50, 2000\_200) for 24 h and costimulatory molecules CD40, CD80 and CD86 were measured by flow cytometry (A-C). CD11c and MHCII double positive cells identified as BMDCs in the population and were gated further for analysis. A population was selected and gated on the MHCII vs CD11c axis. Double positive events were analyzed for CD86, CD80 and CD40 expression. BMDCs were incubated with DMXAA (2000 ng/ml), 522 (50 ng/ml) and DMXAA + 522 (2000\_50 ng/ml) for 24 h and IL-10 were measured by ELISA (D). Results are reported as mean  $\pm$  SD,  $n=3$ , \*\*\*\* $p<0.0001$ , \*\*\* $p<0.001$ , One-way ANOVA.

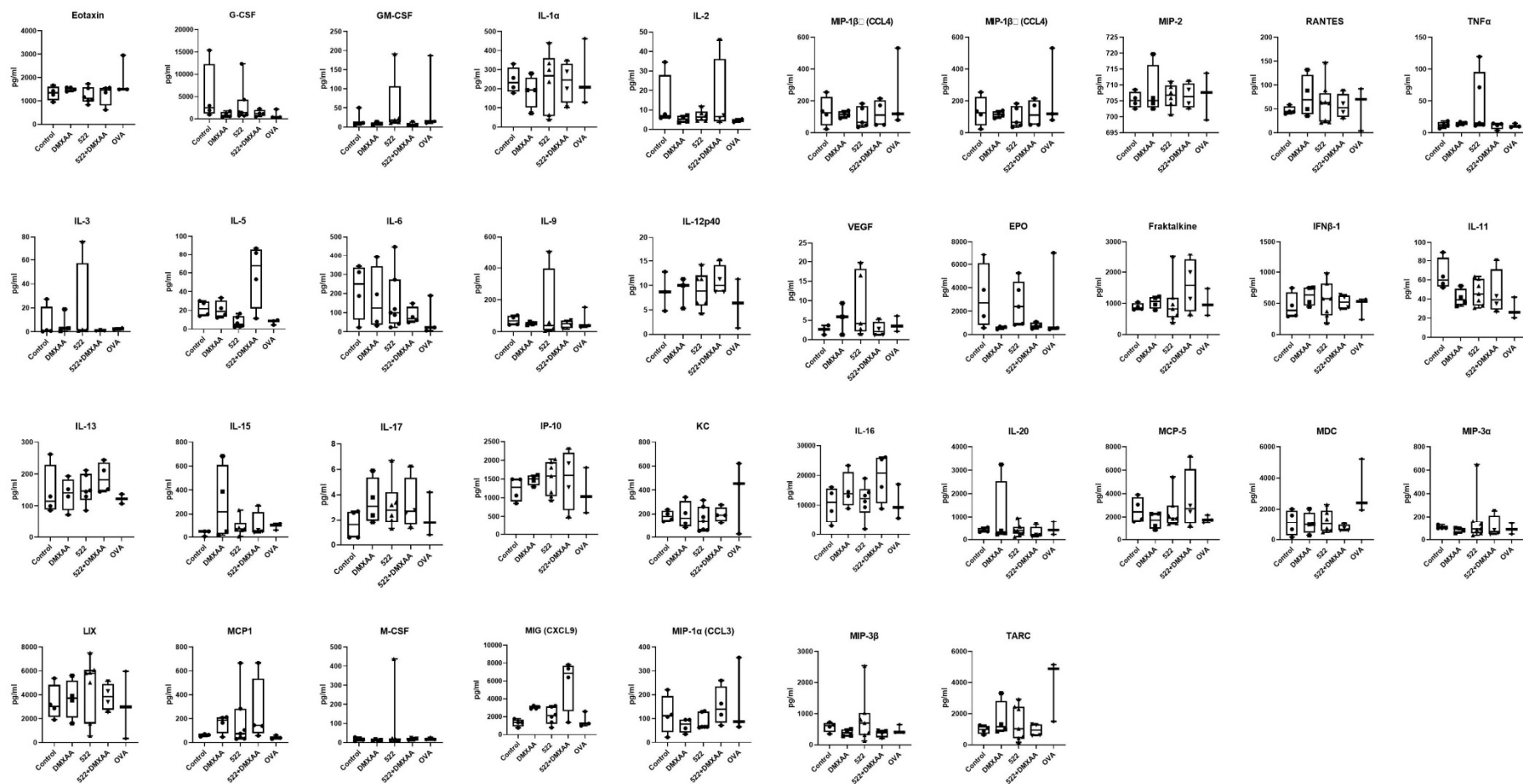


Figure S3. Different cytokines measured in the blood serum on day 7 after five consecutive doses day 1 through day 5.