Figure S1. Impact of chemotherapy on the activation of $CD4^+$ and $CD8^+$ T cells present in the tumor and spleen. (A and D) Representative dot plots showing the method for distinguishing effector and memory T cells in tumors (A) or spleens (D). Previous steps of gating strategy in flow cytometric analysis is presented in Fig. 3F (for tumors) and Fig. 4A (for spleens). The percentage of effector ($CD44^+CD62L^{neg}$) (B and E) and memory (CD44+CD62L+) (C and F) $CD4^+$ and $CD8^+$ T cells infiltrating into MC38 tumor tissue (B and C) or present in the spleen of MC38-tumor bearing mice. (E and F) In brief, mice with subcutaneously growing MC38 tumors received MTX or HES-MTX intravenously on the 14th day of experiment and three days later the tumor nodules and spleens were dissected for further analyses (scheme of treatment is presented in the Fig. 3A). Results are expressed as mean ± SD (5 mice per group were analyzed from one experiment). Splc ctrl, splenocytes isolated from spleen derived from healthy mice (i.e. without MC38-tumor). In all presented data, the differences between groups were calculated using the one-way ANOVA followed by Tukey's multiple comparison post-hoc test ($^*P<0.05$). HES, hydroxyethyl starch; MTX, methotrexate.

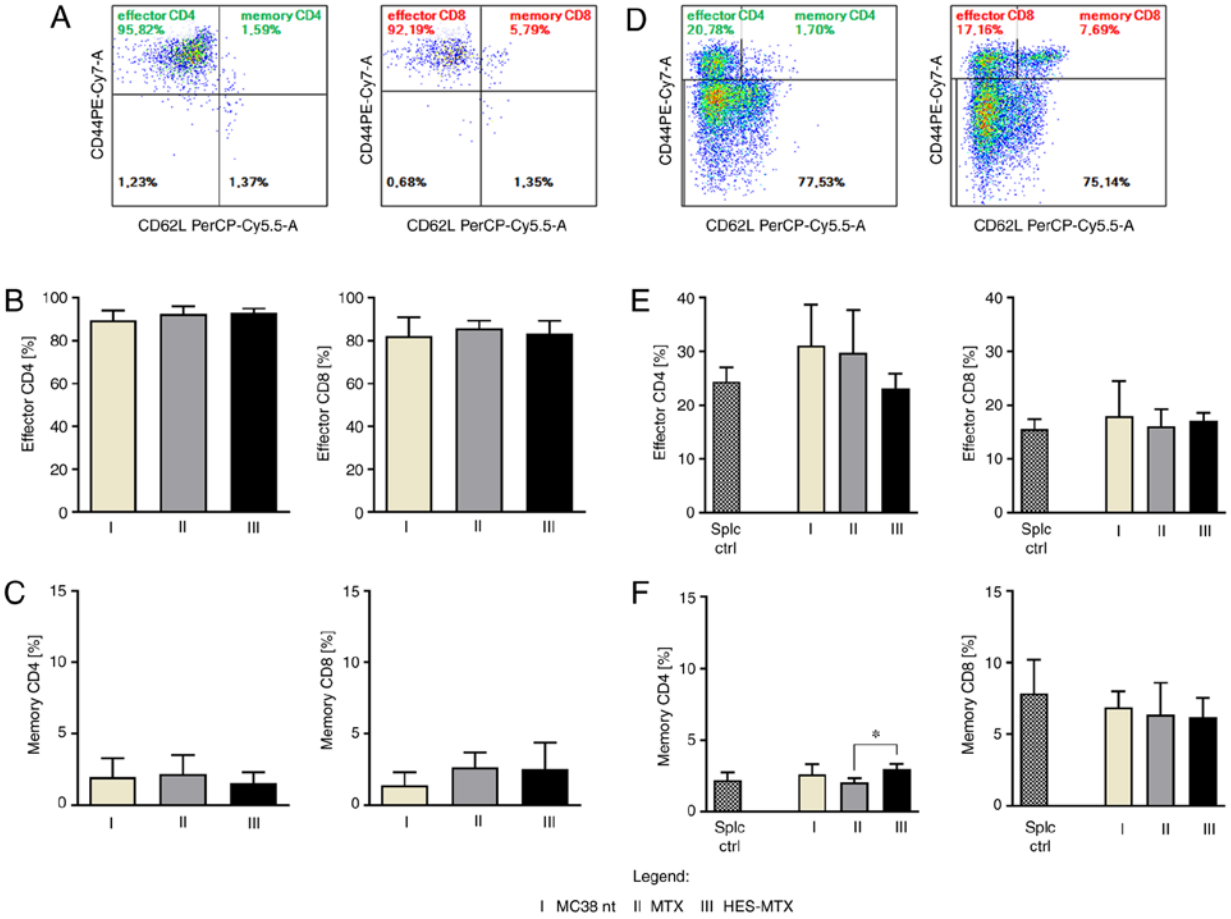

Figure S2. Effect of multiple injections of DC-based vaccines on tumor growth inhibition. (A) MC38 tumor growth inhibition (TGI) calculated on the 35th day of experiment in relation to the DC/TAg group. (B) Median tumor volume after chemoimmunotherapy on the 35th day of experiment. Differences between groups were calculated by two-way ANOVA followed by Bonferroni's multiple comparisons (not significant). HES, hydroxyethyl starch; MTX, methotrexate; DC, dendritic cell; TAg, tumor antigen.

A

Therapeutic groups	TGI [%]*
MTX+DC/TAg	−14
HES-MTX+DC/TAg	22

(*calculated for 35th day of exp)

B

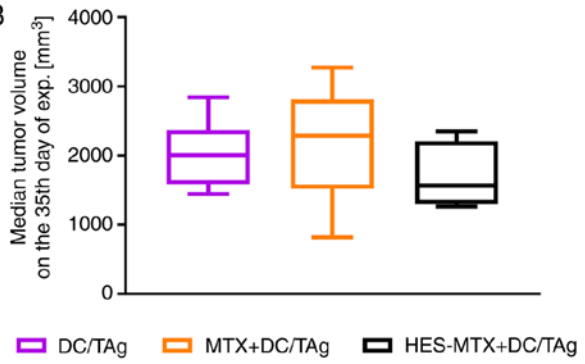

Figure S3. Schemes of multiparameter flow cytometric analyses showing the method for distinguishing myeloid and lymphoid cell subpopulation in tumors (A) and lymphoid cell subpopulation in spleens (B) dissected from MC38 tumor-bearing mice treated according to the scheme presented in Fig. 5A. HES, hydroxyethyl starch; MTX, methotrexate; DC, dendritic cell; TAg, tumor antigen.

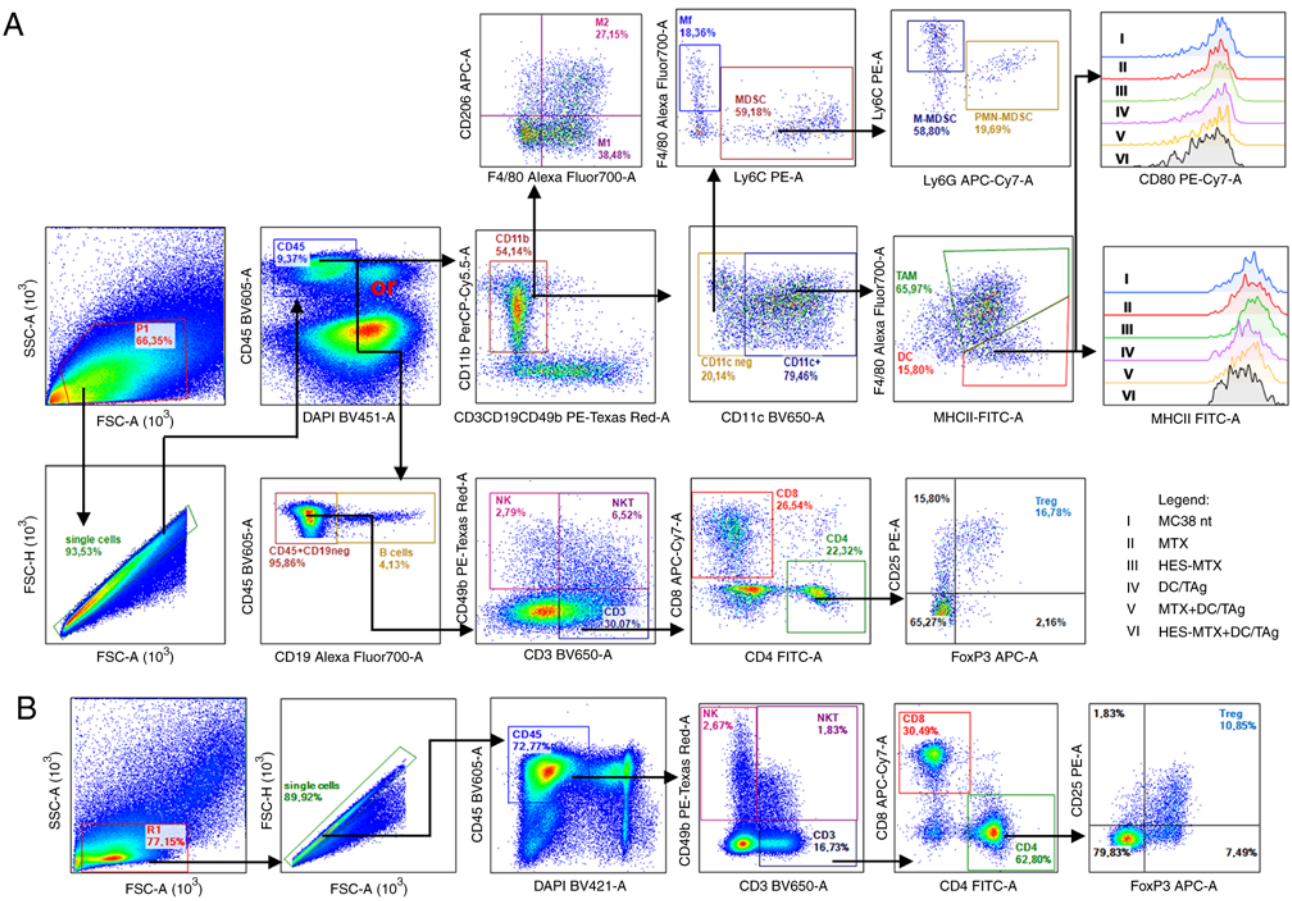

Figure S4. Impact of chemoimmunotherapy on the activation of $CD4^+$ and $CD8^+$ T cells present in the tumor and spleen. (A and D) Representative dot plots showing the method for distinguishing effector and memory T cells in tumors (A) or spleens. (D) Previous steps of gating strategy in flow cytometric analysis is presented in Fig. S3A (for tumors) and Fig. S3B (for spleens). The percentage of effector ($CD44^+CD62L^{neg}$) (B and E) and memory ($CD44^+CD62L^+$) (C and F) $CD4^+$ and $CD8^+$ T cells infiltrating into MC38 tumor tissue (B and C) or present in the spleen of MC38-tumor bearing mice. (E and F) In brief, mice with subcutaneously growing MC38 tumor received MTX or HES-MTX intravenously on the 14th day of the experiment and on the 17th, 24th and 31st day of the experiment tumor antigen-stimulated dendritic cell-based vaccines were applied peritumorally. After the end of the therapy, tumors and spleen were dissected for further analyses (scheme of treatment is presented in Fig. 5A). Results are expressed as mean ± SD (3-5 mice per group were analyzed from one experiment). Splc ctrl, splenocytes isolated from spleen derived from healthy mice (i.e. without MC38-tumor). In all presented data the differences between groups were calculated using the one-way ANOVA followed by Tukey's multiple comparison post-hoc test ($^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$). HES, hydroxyethyl starch; MTX, methotrexate; DC, dendritic cell; TAg, tumor antigen.