Tumor abscopal responses induced by the TLR9 agonist, SD-101, are strongly potentiated by a HDAC class I inhibitor, Domatinostat

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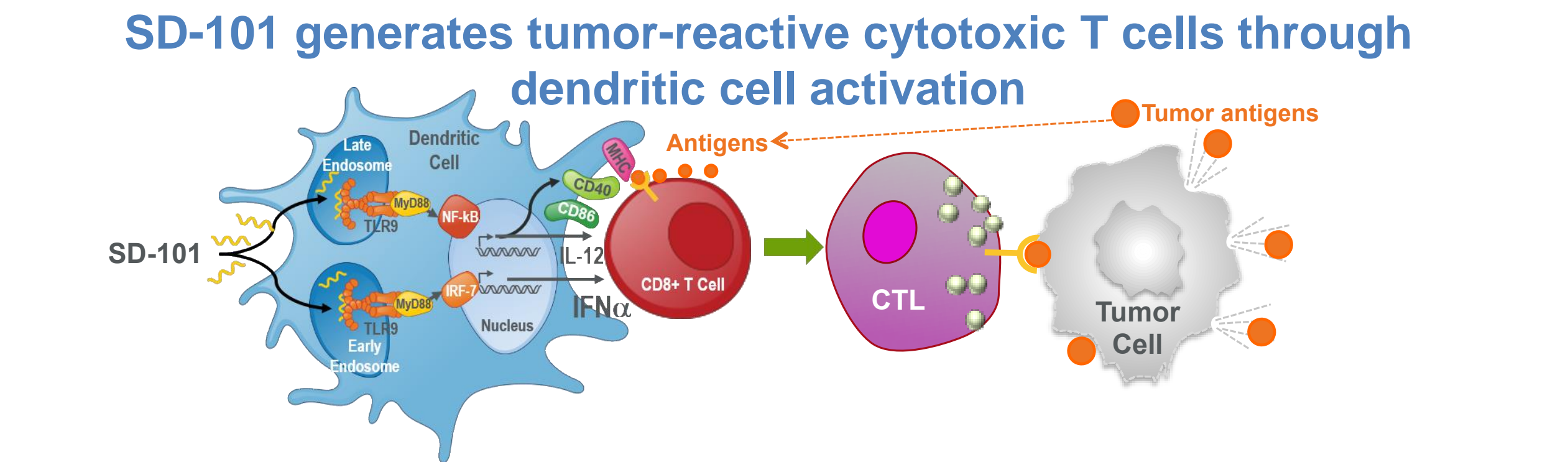
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Background

SD-101 generates tumor-reactive cytotoxic T cells through dendritic cell activation

Figure 1. SD-101 is a TLR9 agonist specifically developed for cancer based upon its ability to stimulate both IFN-α production and the maturation of plasmacytoid dendritic cells into antigen presenting cells. The activation of these two signaling pathways in DC produce the optimum conditions for the generation of high quality tumor antigen specific CD8+ T cells[1,2,3]. Importantly, in the tumor, IFNα induces NK activation, chemokine induction (CXCL9, CXCL10), M2 to M1 macrophage transition and rebalance of the CD4+ T cells: Th↑↑, Treg↓. SD-101 is currently under investigation in combination with Pembrolizumab in melanoma, advanced head and neck cancer, and in breast cancer in a neo-adjuvant setting.

[1]Wang S et al., Proc Natl Acad Sci USA 2016;113(46):E7240-E7249
[2]Gallotta M et al., Cancer Res 2018;78(17):4943-4956
[3]Sagiv-Barfi I et al., Sci Trans Med 2018; 10(426):aan4488
[4]Ribas A et al., Cancer Discov 2018; 8(10):1250
[5]Frank MJ et al., Cancer Discov 2018; 8(10):1258

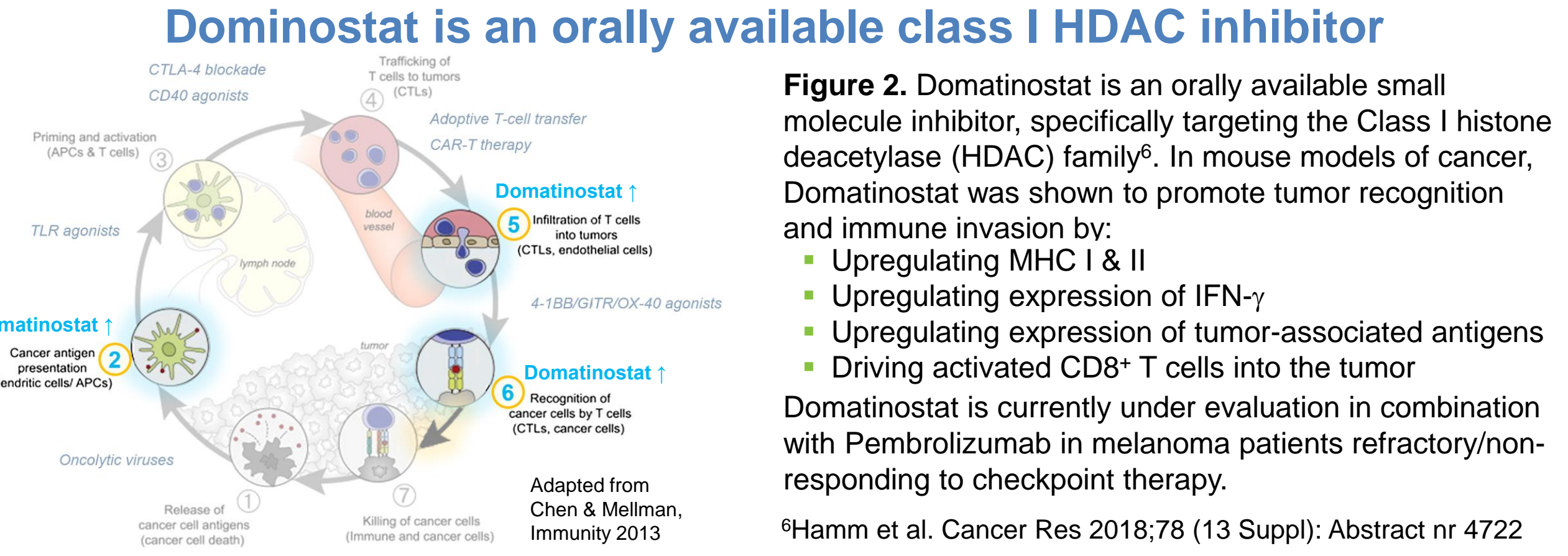### Dominostat is an orally available class I HDAC inhibitor

Figure 2. Domatinostat is an orally available small molecule inhibitor, specifically targeting the Class I histone deacetylase (HDAC) family[6]. In mouse models of cancer, Domatinostat was shown to promote tumor recognition and immune invasion by:
- Upregulating MHC I & II
- Upregulating expression of IFN-γ
- Upregulating expression of tumor-associated antigens
- Driving activated CD8+ T cells into the tumor

Domatinostat is currently under evaluation in combination with Pembrolizumab in melanoma patients refractory/non-responding to checkpoint therapy.
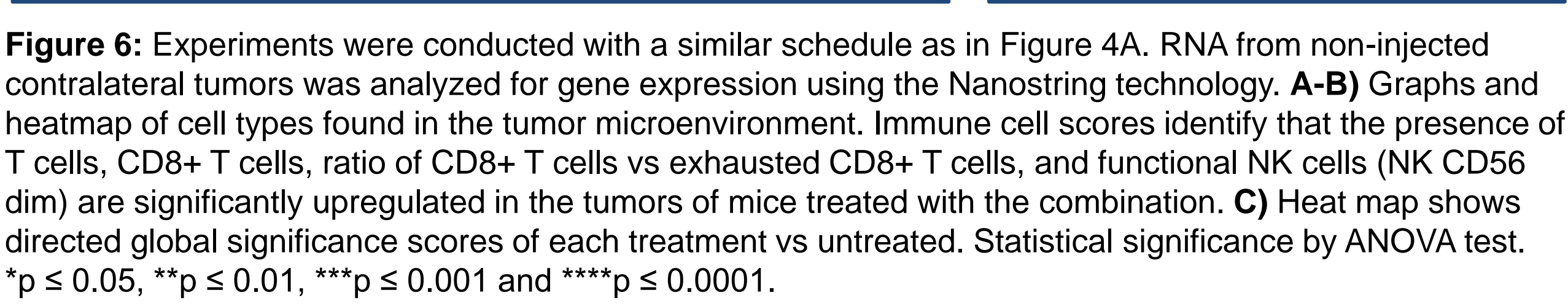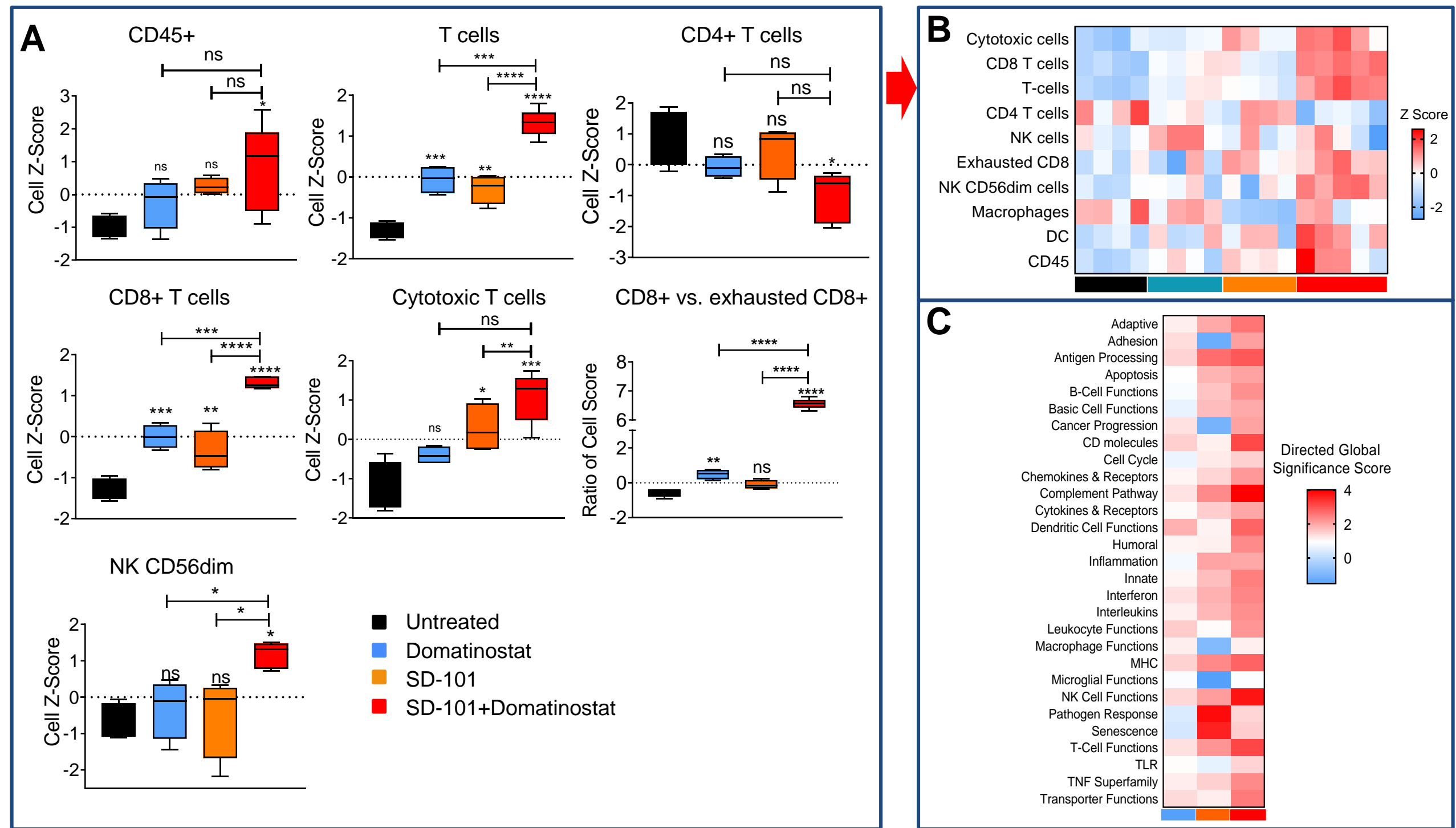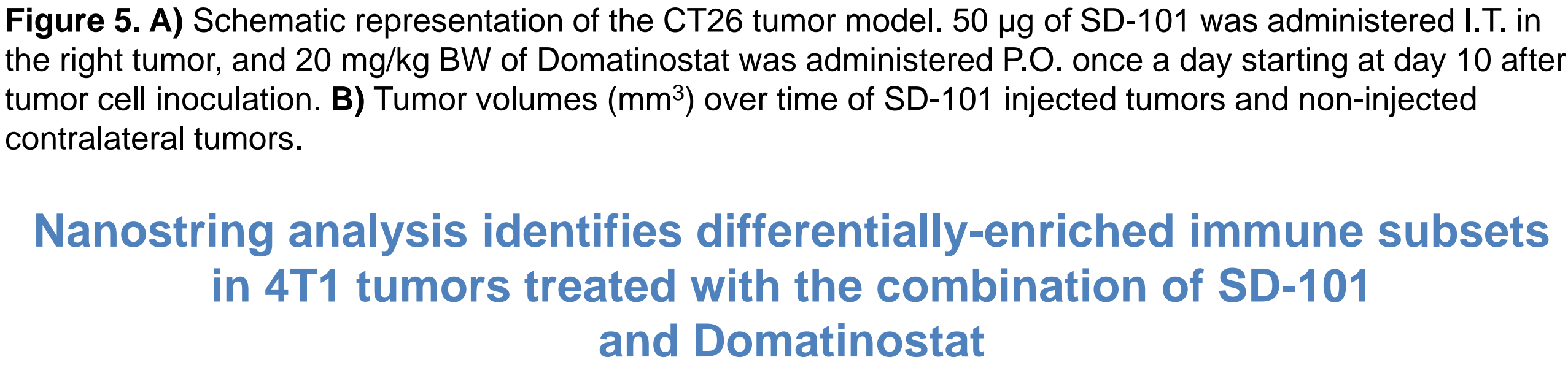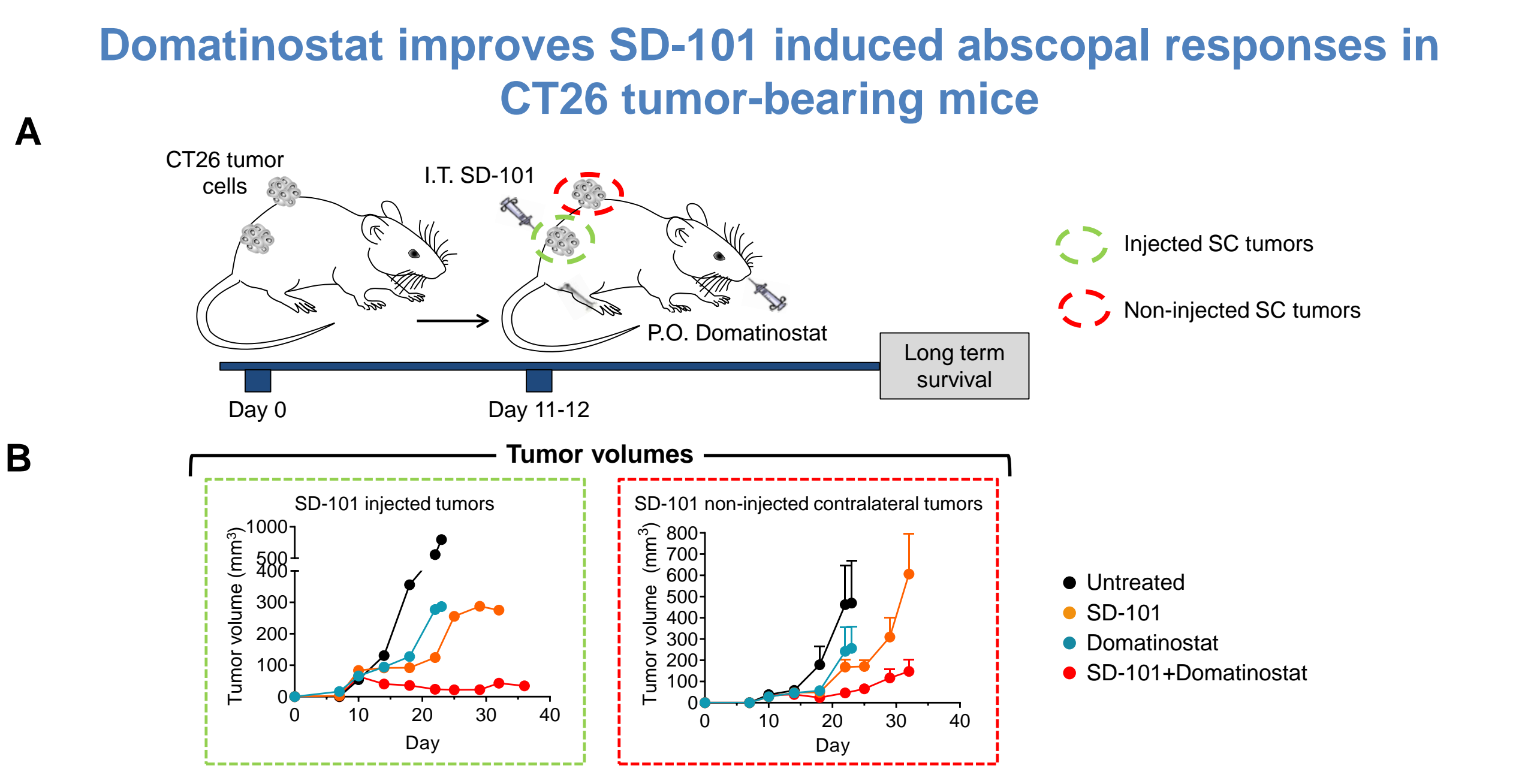
[6]Hamm et al. Cancer Res 2018;78 (13 Suppl): Abstract nr 4722

Results

Side by side comparison of multiple epigenetic modulators in combination with SD-101

Figure 3. Domatinostat strongly synergizes with SD-101 to control tumor growth and is superior compared to the other epigenetic drugs tested. **A)** Schematic representation of the 4T1 tumor model. 50µg of SD-101 was administered I.T. in the right tumor. Domatinostat (20 mg/kg BW), Entinostat (5 mg/kg BW) and Mocetinostat (50 mg/kg BW) were administered P.O. once a day except weekend. Azacitidine (0.8 mg/kg BW) and Vorinostat (50 mg/kg BW) were administered I.P. daily except weekend. **B)** Tumor volumes of contralateral non-injected tumors. **C)** Lung metastases were Log2 transformed to normalize the data and show cumulative data from four experiments. Mice with no lung metastases were reported as zeros. Data are cumulative of four experiments. Statistical significance by ANOVA test.
*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 and ****p ≤ 0.0001.

Domatinostat potentiates SD-101 induced abscopal response in 4T1 tumor-bearing mice

Figure 4. A) Schematic representation of the 4T1 tumor model. 50 μg of SD-101 was administered I.T. in the right tumor, and 20 mg/kg BW of Domatinostat was administered P.O. once a day starting at day 12 after tumor cell inoculation. **B)** Tumor volumes (mm³) of SD-101 injected tumors and contralateral (non-injected) tumors in the combination group demonstrate the induction of a stronger systemic immune response. **C)** Lung metastases were decreased in the combination treatment compared to either drug alone. **D and E)** CD8+ T cells are necessary for tumor growth and metastasis control in the combination treatment. Statistical significance by ANOVA test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 and ****p ≤ 0.0001.

Domatinostat improves SD-101 induced abscopal responses in CT26 tumor-bearing mice

Figure 5. A) Schematic representation of the CT26 tumor model. 50 µg of SD-101 was administered I.T. in the right tumor, and 20 mg/kg BW of Domatinostat was administered P.O. once a day starting at day 10 after tumor cell inoculation. **B)** Tumor volumes (mm³) over time of SD-101 injected tumors and non-injected contralateral tumors.

Nanostring analysis identifies differentially-enriched immune subsets in 4T1 tumors treated with the combination of SD-101 and Domatinostat

Figure 6: Experiments were conducted with a similar schedule as in Figure 4A. RNA from non-injected contralateral tumors was analyzed for gene expression using the Nanostring technology. **A-B)** Graphs and heatmap of cell types found in the tumor microenvironment. Immune cell scores identify that the presence of T cells, CD8+ T cells, ratio of CD8+ T cells vs exhausted CD8+ T cells, and functional NK cells (NK CD56 dim) are significantly upregulated in the tumors of mice treated with the combination. **C)** Heat map shows directed global significance scores of each treatment vs untreated. Statistical significance by ANOVA test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 and ****p ≤ 0.0001.

Domatinostat synergizes with SD-101 to increase the quality and quantity of the T cell response in abscopal sites

Figure 7: A) Mice bearing 4T1 tumors were treated as in Figure 4A and non-injected contralateral SC tumors and tumor bearing lungs were analyzed by flow cytometry. **B)** Phenotypic analysis of T cells from the various treatment groups is shown for the non-injected contralateral tumors (B) and for the tumor bearing lungs (C). Percentage CD8+ T cells (upper panel) and CD4+ T cells (upper panel) among CD45+ leukocytes. Dextramer specific for gp70 was used to identify a tumor associated antigen known to be expressed by 4T1 cells. Cytolytic activity (GranzB+ and CD107a+) is shown for PD-1+CD8+ T cells (B) In the tumor-bearing lungs PD-1 was used to identify antigen experienced CD8+ T cell subsets. **C)** Representative dot plots showing the expression of GranzB and CD107a . Data are representative of three experiments. Statistical significance by ANOVA test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 and ****p<0.0001.

Domatinostat in combination with SD-101 increases the generation of polyfunctional tumor antigen specific CD8+ T cells

Figure 8: A) Schematic representation of the EG7.OVA tumor model. SD-101 (50 ug) was administered I.T. in the right tumor, and Domatinostat was administered P.O. once a day 5 days a week. **B)** Tumor volumes (mm³) of SD-101 non-injected contralateral tumors. **C)** Survival represents mice that reject tumors in both flanks. **D-E)** Splenocytes were pulsed with 5nM OVA 257-264 peptide for 60 hours, and the phenotype of antigen specific OVA+ CD8+ T cells were analyzed by flow cytometry. Panel (D) demonstrates that OVA specific T cells from SD-101 or combination treated mice are equally capable to respond to peptide stimulation. (E) Shows that the percentage of OVA specific T cells producing cytokines is greatly increased following combination treatment. Statistical significance by Mantel-Cox test (C) and ANOVA test (D-E). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 and ****p < 0.0001. In (D), statistics placed on top of each bar compare each treatment with treatment in the 5nM concentration. No significance was observed between the different groups in the non-peptide pulsed condition. (E) Statistics on top of each bar compare each treatment with untreated.

The triple combination of SD-101, Domatinostat and anti-PD-1 strongly synergize to improve SD-101 abscopal response in a setting of high tumor burden

Figure 9: A) Schematic representation of the CT26 tumor model. **B)** Representative pictures of the lungs from each treatment. **C)** Mean number of lung metastasis is shown. In this model of heavy tumor burden, the triple combination exert superior antitumor activity on abscopal disease (lung tumors). Statistical significance by Chi Square T test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 and ****p<0.0001. Statistics on top of the histogram bar are calculated versus the untreated group.

	(a) No metastases ± SEM	(b) Mice with no metastases at sacrifice	(c) % mice that died of lung tumors before the end of the experiment
Untreated	77±29	0/15=0%	11/15=73%
SD-101	71±16	1/10=10%	2/10=20%
SD-101+Domatinostat	47±14	2/12=17%	2/12=17%
SD-101+Anti-PD-1	30±11	4/22=18%	8/22=36%
Domatinostat+Anti-PD-1	36±14	0/14=0%	7/14=50%
SD-101+Domatinostat+Anti-PD-1	4±2	14/22=63%	0/22=0%

Table 1: Triple combination of SD-101 + Domatinostat and anti-PD-1 significantly increase abscopal site disease control. Table 1 refers to experiments in Figure 9. **(a)** the number of metastases, **(b)** the % of mice that reached the end of the experiment with no visible lung metastases and **(c)** the % of mice that succumb of lung metastases before day 24.

The triple combination of SD-101, Domatinostat and anti-PD-1 synergize to improve SD-101 abscopal response in a spontaneous mammary carcinoma model

Figure 10: FVB/N-Tg(MMTV-PyVT) mammary carcinoma model carries the Polyoma Virus middle T antigen with the mouse mammary tumor virus (MMTV) promoter that drives mammary tissue-specific expression. This mouse transgenic strain is very aggressive, and palpable carcinoma is already present in the ten mammary glands at eight weeks of age. Because this breast cancer model develops carcinoma tumors in multiple mammary glands it is possible to inject one tumor with SD-101 and follow the tumor volume of the non-injected tumors to assess abscopal response. The data demonstrate that in this model of heavy tumor burden, the triple combination exerts superior antitumor activity on abscopal disease if compared to SD-101 given in combination with Domatinostat or in combination with anti-PD-1. **A)** Schematic representation of the model. At ten weeks of age mice were treated with SD-101 given I.T. at 50 μg per dose given biweekly. Only a single tumor was treated with I.T. SD-101. anti-PD-1 was administered by the I.P. route twice a week. Domatinostat was administered by the oral route once a day (excluding weekends). **B)** The mean of the cumulative tumor volume. **C)** Representative pictures of each treatment taken at week twelve. Statistical significance by two way ANOVA test. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 and ****p<0.0001.

Conclusions

- SD-101 in combination with Domatinostat strongly synergize to control tumor growth in syngeneic mouse models.
- Domatinostat works on SD-101 generated tumor specific CD8+ T cells to increase their number and their functionality. This results in an enlarged pool of CTL accumulating and rejecting tumors distant from the SD-101 injected site.
- The addition of PD-1 blockade to SD-101 plus Domatinostat drugs regimen further increases tumor control in abscopal sites in models with high metastases.
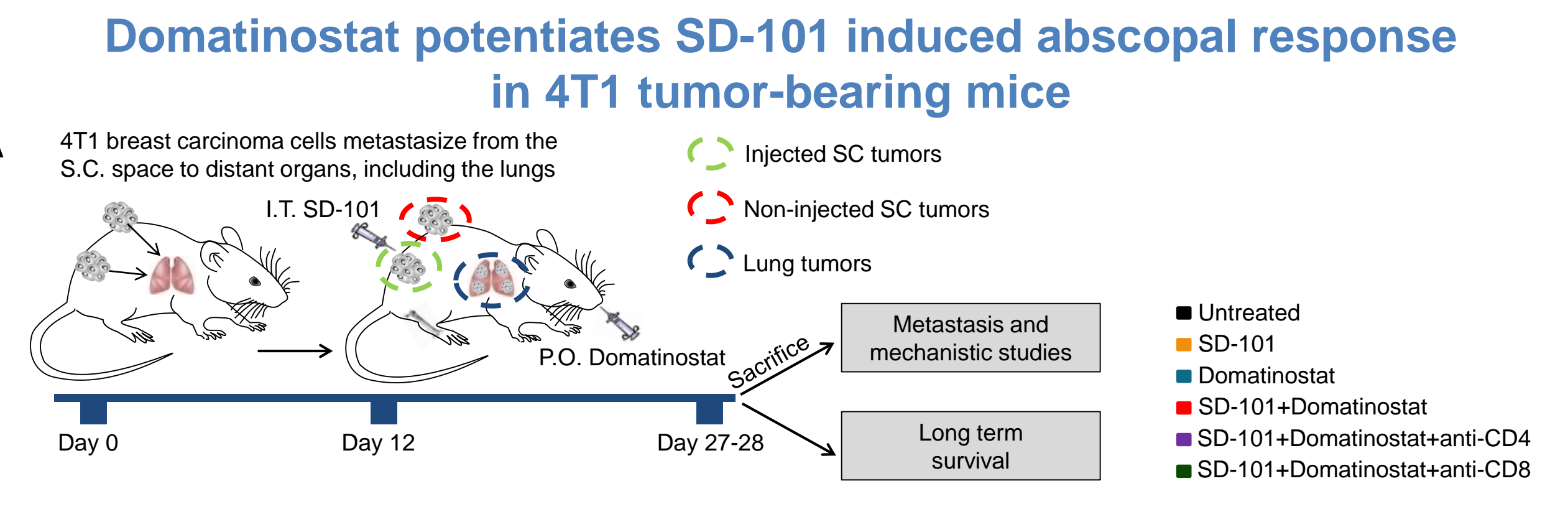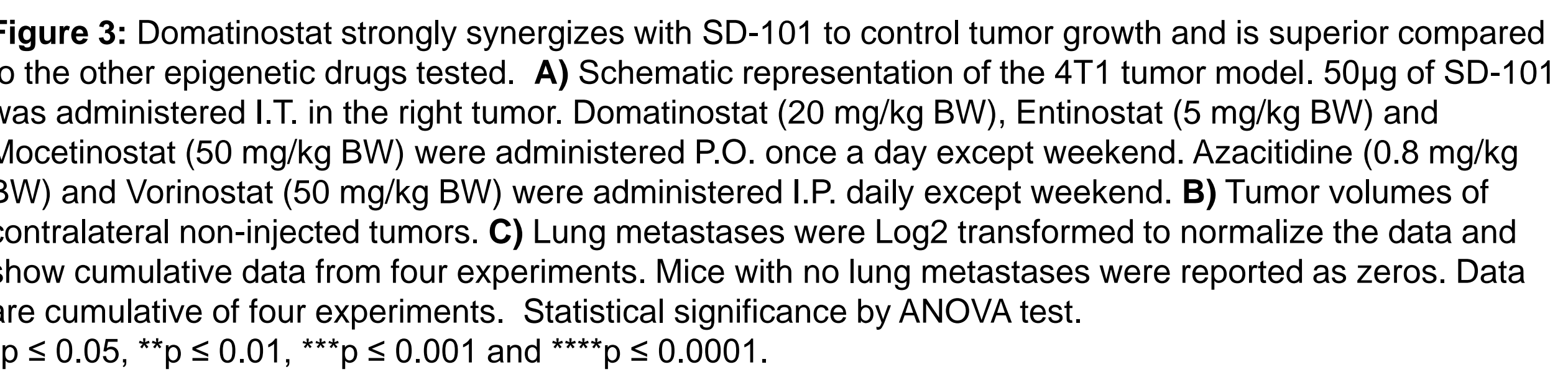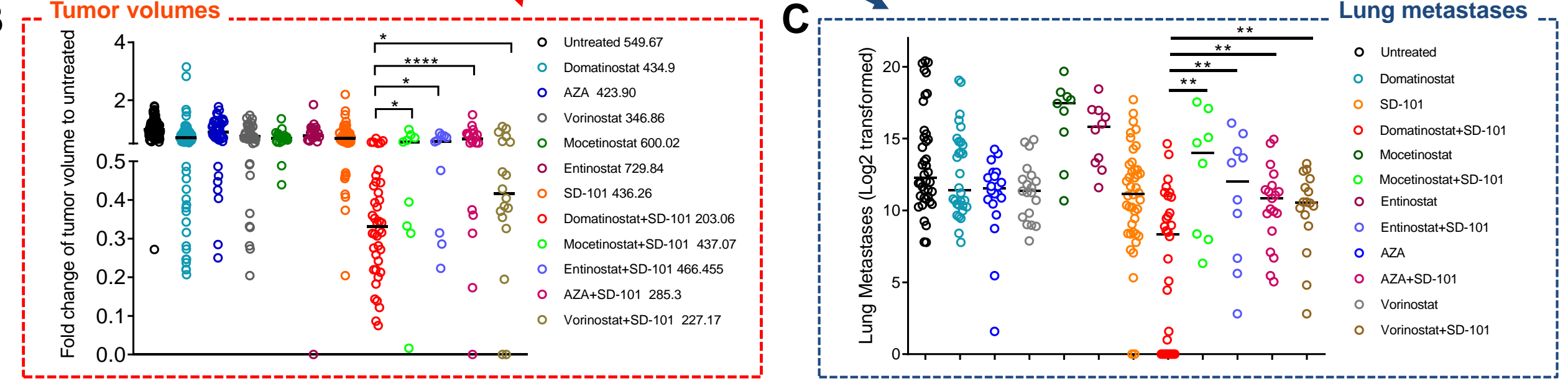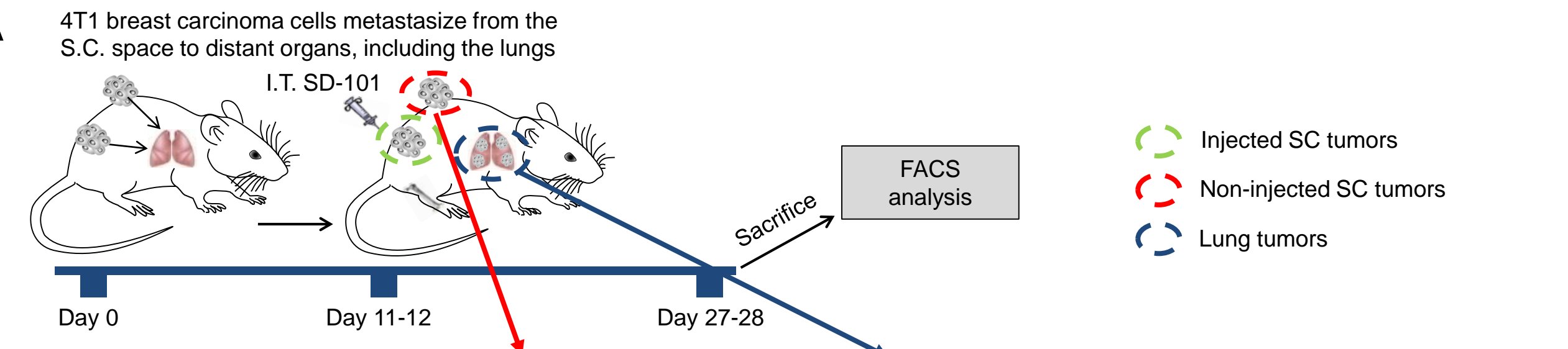

Figure 11: The actions of SD-101 are focused on the injected tumor and tumor-draining lymph nodes. Both innate and adaptive immune responses are increased by intratumoral injection of SD-101. SD-101 induces high levels of Type I IFN secretion from plasmacytoid dendritic cells (PDC) which is a potent immunomodulatory cytokine that boosts NK cytotoxic activity and induces recruitment of T cells and differentiation of CTLs. In addition, SD-101 promotes DC maturation of dendritic cells and their ability to cross-present tumor associated antigens, thereby promoting a high quality T cells response. An important feature of the intended mechanism is the generation of systemic anti-tumor immunity, mediated by T cells that migrate and attack non-injected tumor sites. The combination of SD-101 and systemic Domatinostat boosts the quality and quantity of SD-101 induced CTL response, deepening SD-101 abscopal responses. These responses can be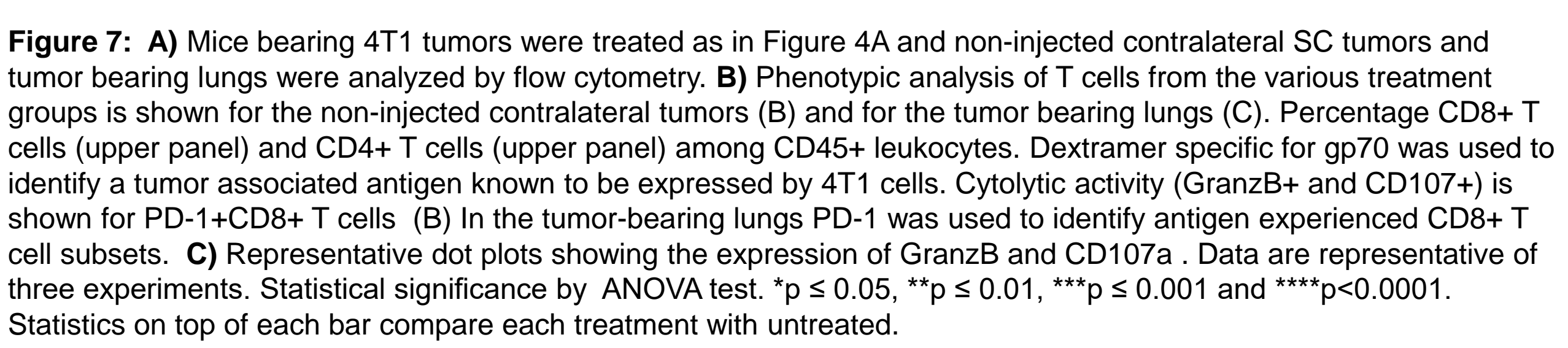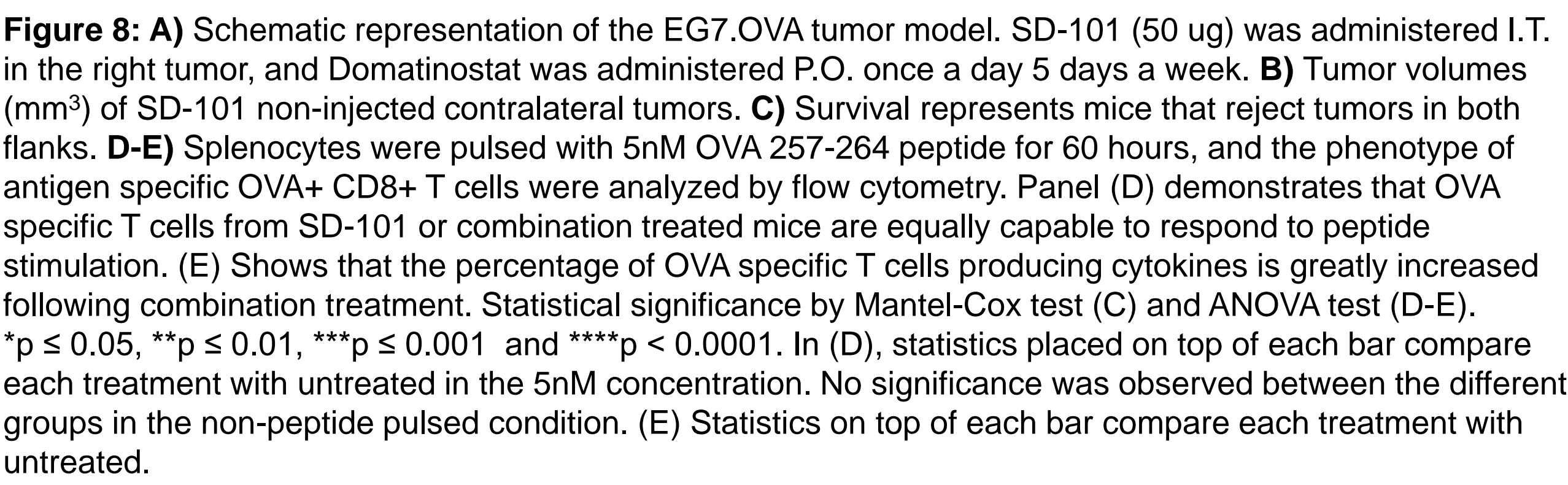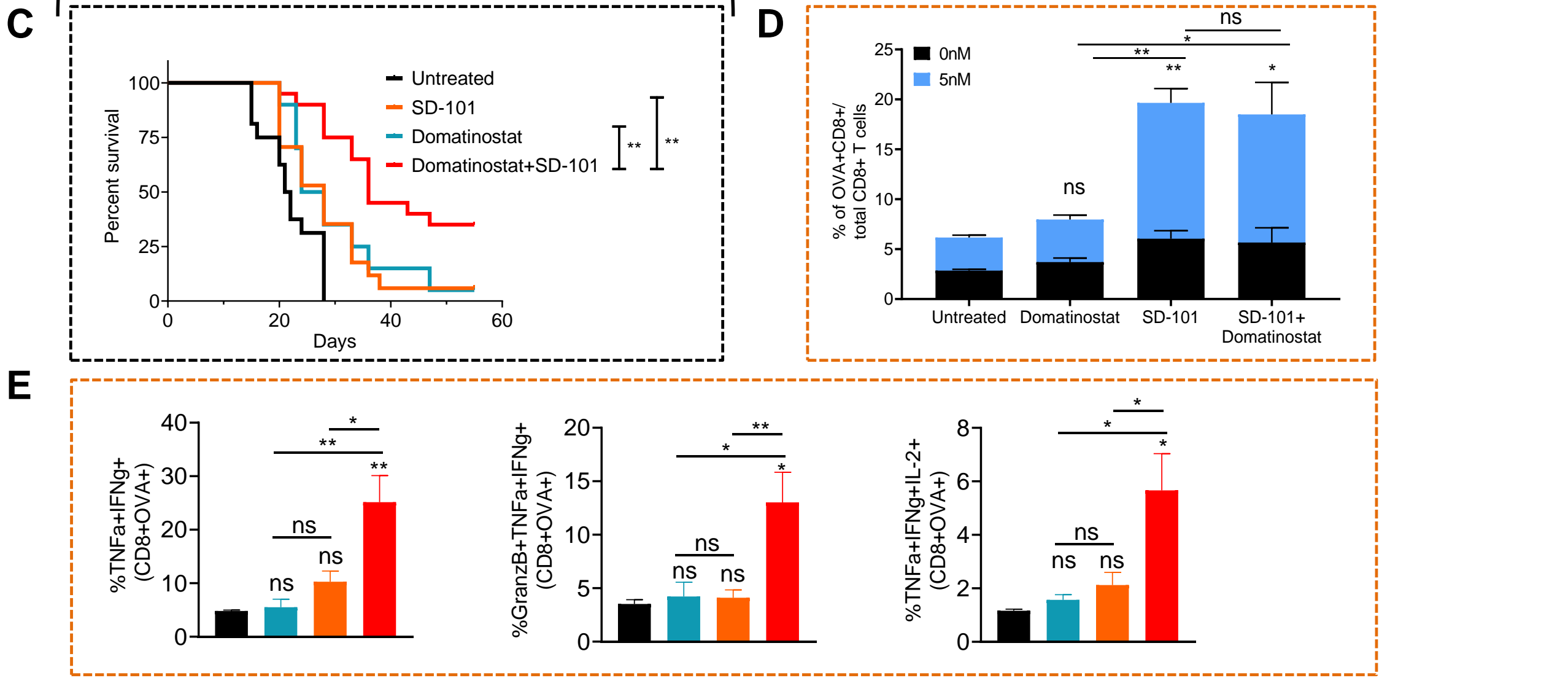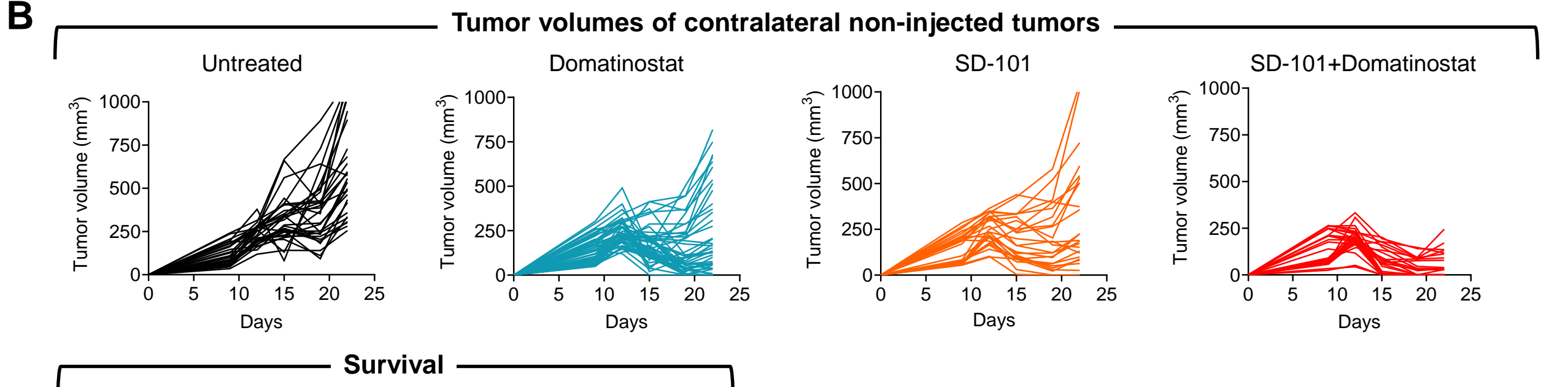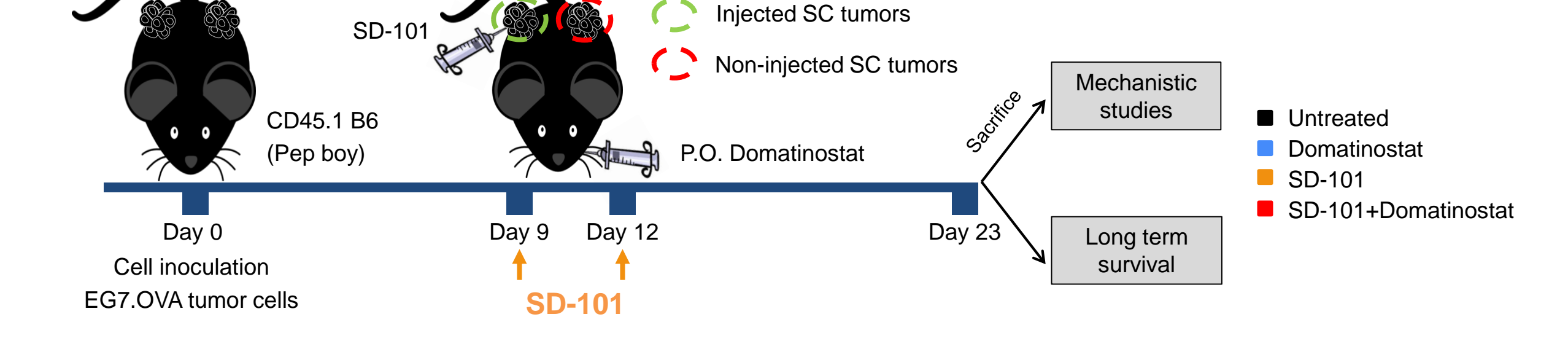 further increased by the addition of PD-1 blockade.