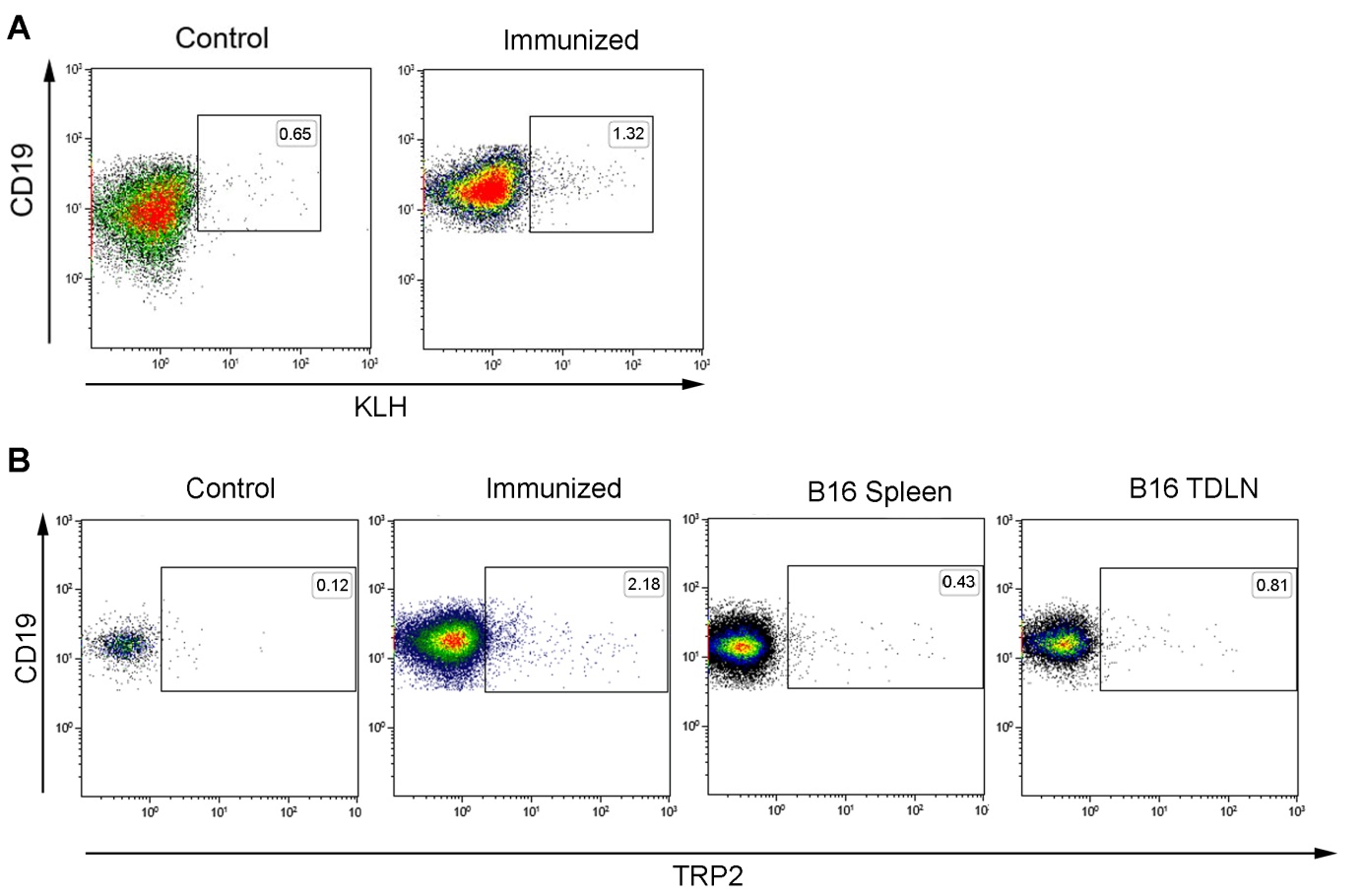
**Supplementary Information for Manuscript:**

**Using antigen-specific B cells to combine antibody and T cell based cancer immunotherapy**

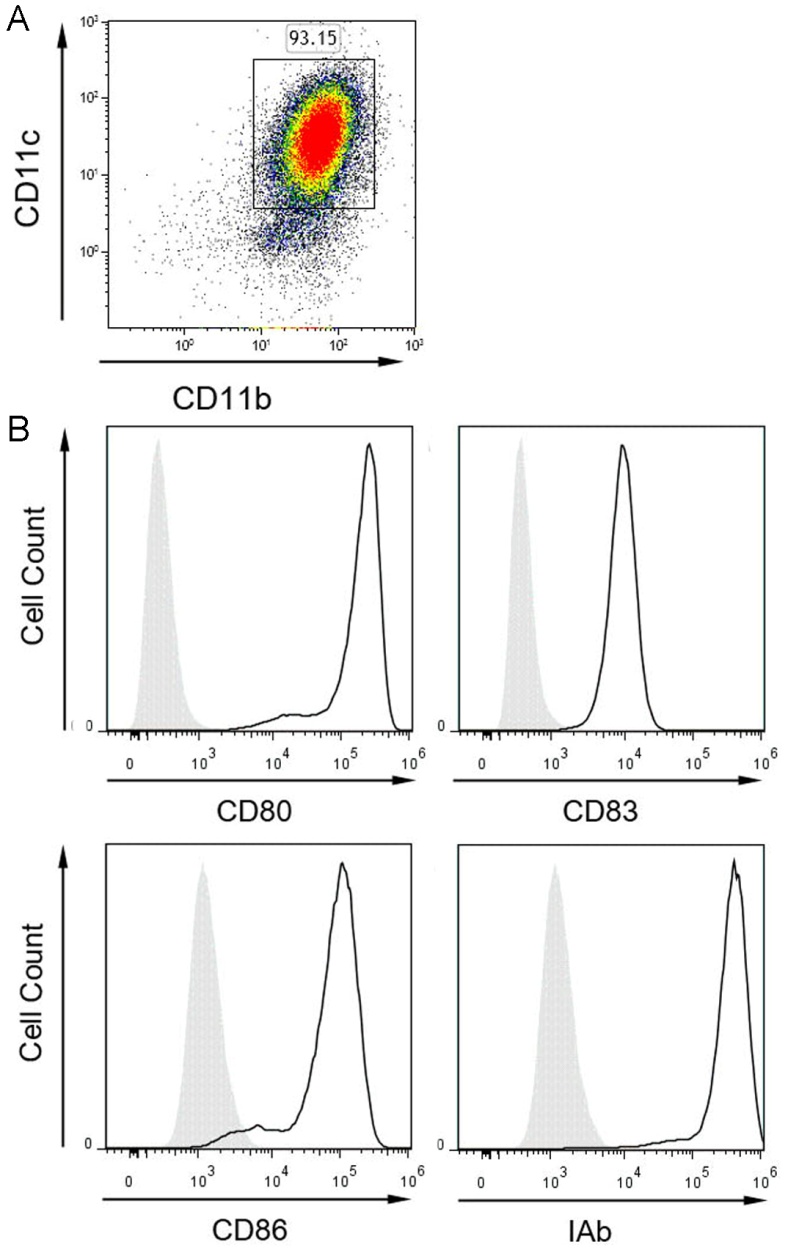
Kerstin Wennhold, Martin Thelen, Hans Anton Schlößer, Natalie Haustein, Sabrina Reuter, Maria Garcia-Marquez, Axel Lechner, Sebastian Kobold, Felicitas Rataj, Olaf Utermöhlen, Jan Becker, Geothy Chakupurakal, Sebastian Theurich, Michael Hallek, Hinrich Abken, Alexander Shimabukuro-Vornhagen, Michael von Bergwelt-Baildon

**Supplementary figures**



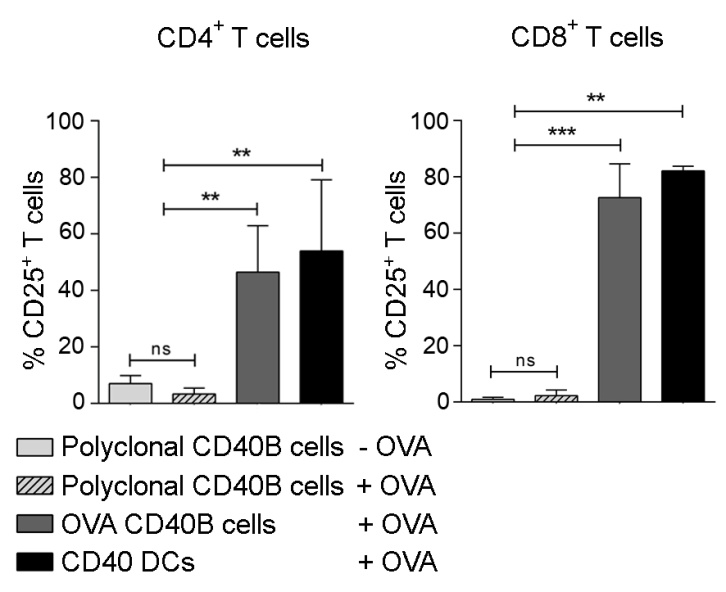
**Fig. S1. Flow cytometry staining of KLH and TRP2-specific B cells.**

(A) Mice were immunized with 10 µM KLH-protein in IFA twice in an interval of 7 days. 7 days after the last immunization, B cells were isolated from spleens and analyzed by flow cytometry for the presence of KLH-specific B cells. Representative plots of 5 independent experiments are shown. (B) Mice were either immunized with TRP2-Protein in IFA twice in an interval of 7 days (Immunized plot) or they received a single injection of B16.F1 melanoma cells s.c. (B16 spleen and B16 tumor draining lymph node (TDLN)). 7 days later B cells were isolated from spleens or TDLN and analyzed by flow cytometry for the presence of TRP2-specific B cells. Spleens from healthy untreated mice served as controls. Representative plots of 5 independent experiments are shown.



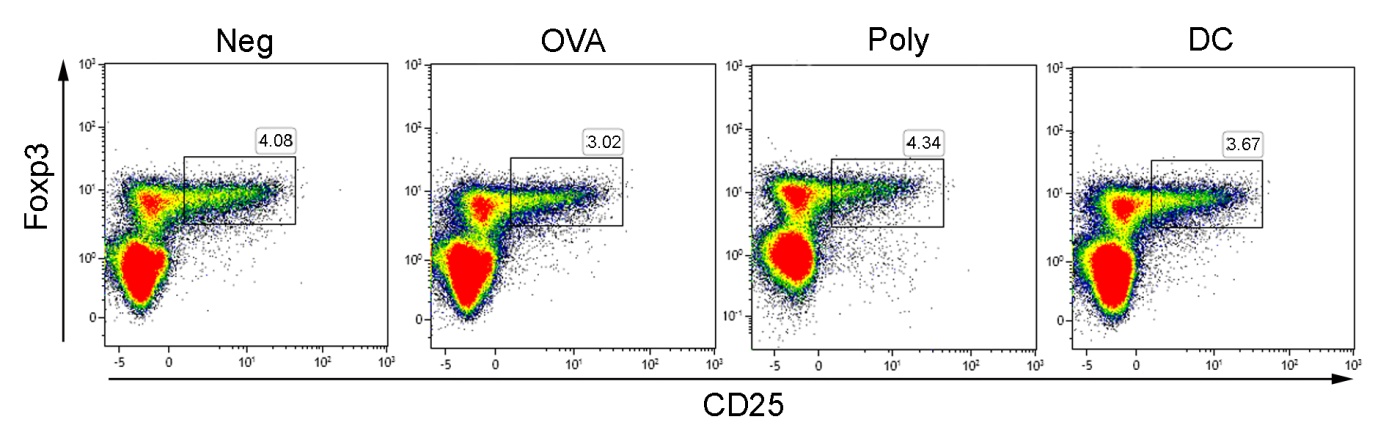
**Fig. S2. Quality control of CD40L-matured DCs.**

Representative flow cytometry analyses of CD40L-matured DCs out of at least five independent experiments are shown. Mature DCs were stained for (A) CD11b+CD11c+ cells and (B) among this population for the activation markers CD80, CD83, CD86 and IAb were indicated (black line) compared to an isotype control (filled line).

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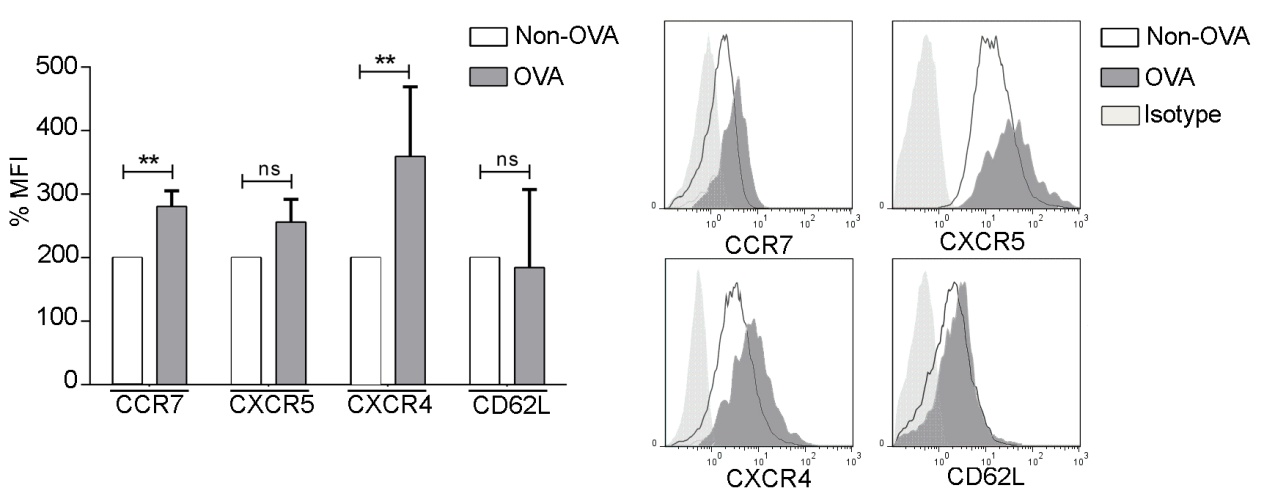
**Fig. S3.** **Antigen-specific CD40B cells activate specific T cells.**

In antigen-presentation assays, T cells from OT-II (CD4+ T cells) or OT-I (CD8+ T cells) mice were cocultured with different unpulsed or protein-pulsed APCs, i.e. polyclonal CD40B cells, OVA-CD40B cells and CD40L-matured DCs (CD40 DCs). The percentage of activated T cells was determined by expression of CD25. Significant differences calculated with one-way ANOVA are marked by an asterisk. ns = not significant, \*\* p ≤ 0.01, \*\*\* p ≤ 0.001.



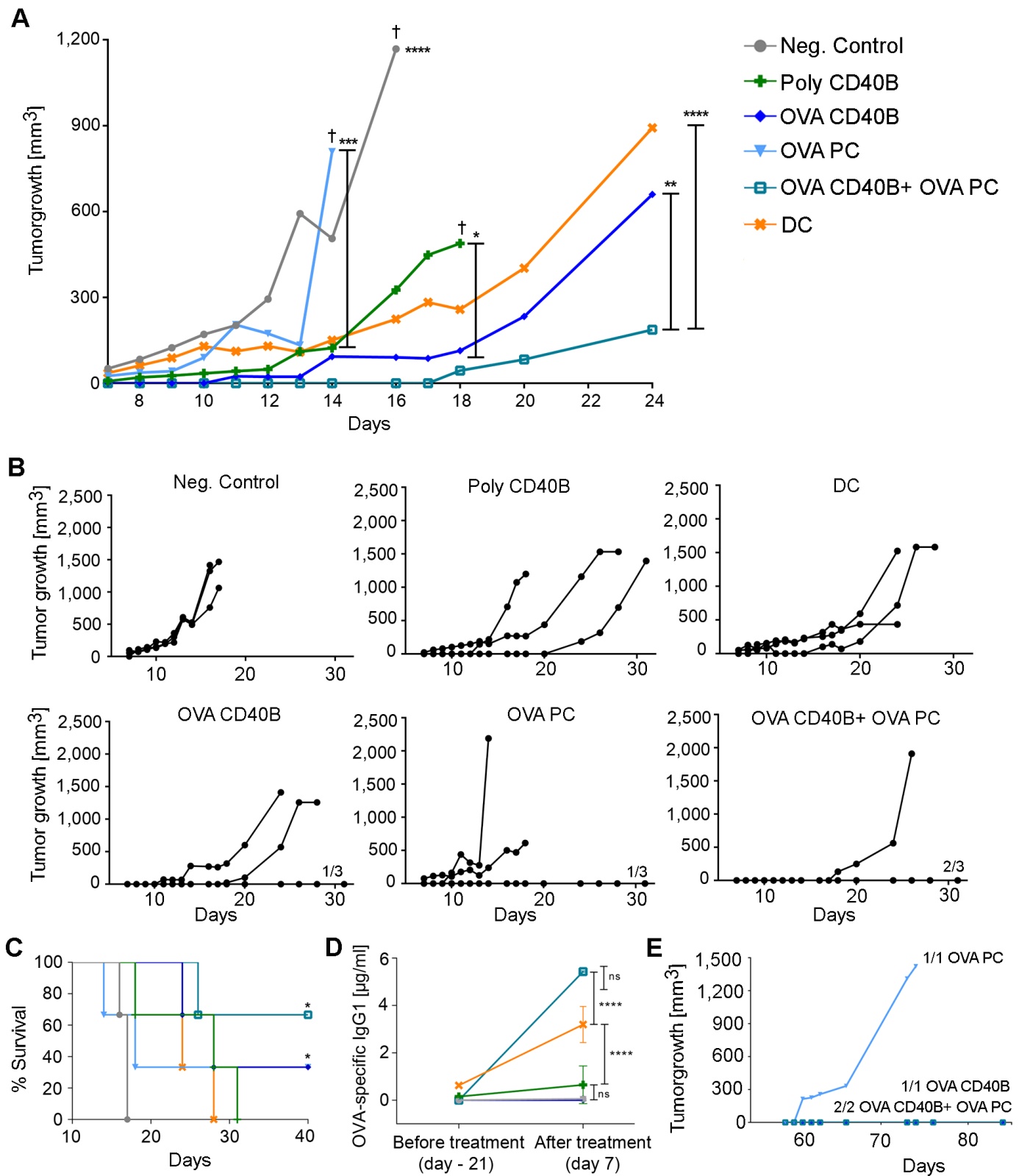
**Fig. S4. T cells of immunized mice show normal percentages of Tregs.**

B6 mice were immunized with protein-pulsed APCs, i.e. OVA-CD40B cells (OVA), polyclonal CD40B cells (Poly), CD40-matured DCs. PBS served as negative control (Neg). CD3+ CD4+ T cells from vaccinated mice were stained for the presence of CD25+ Foxp3+ Tregs. Density plots show representative analyses out of three independent experiments.

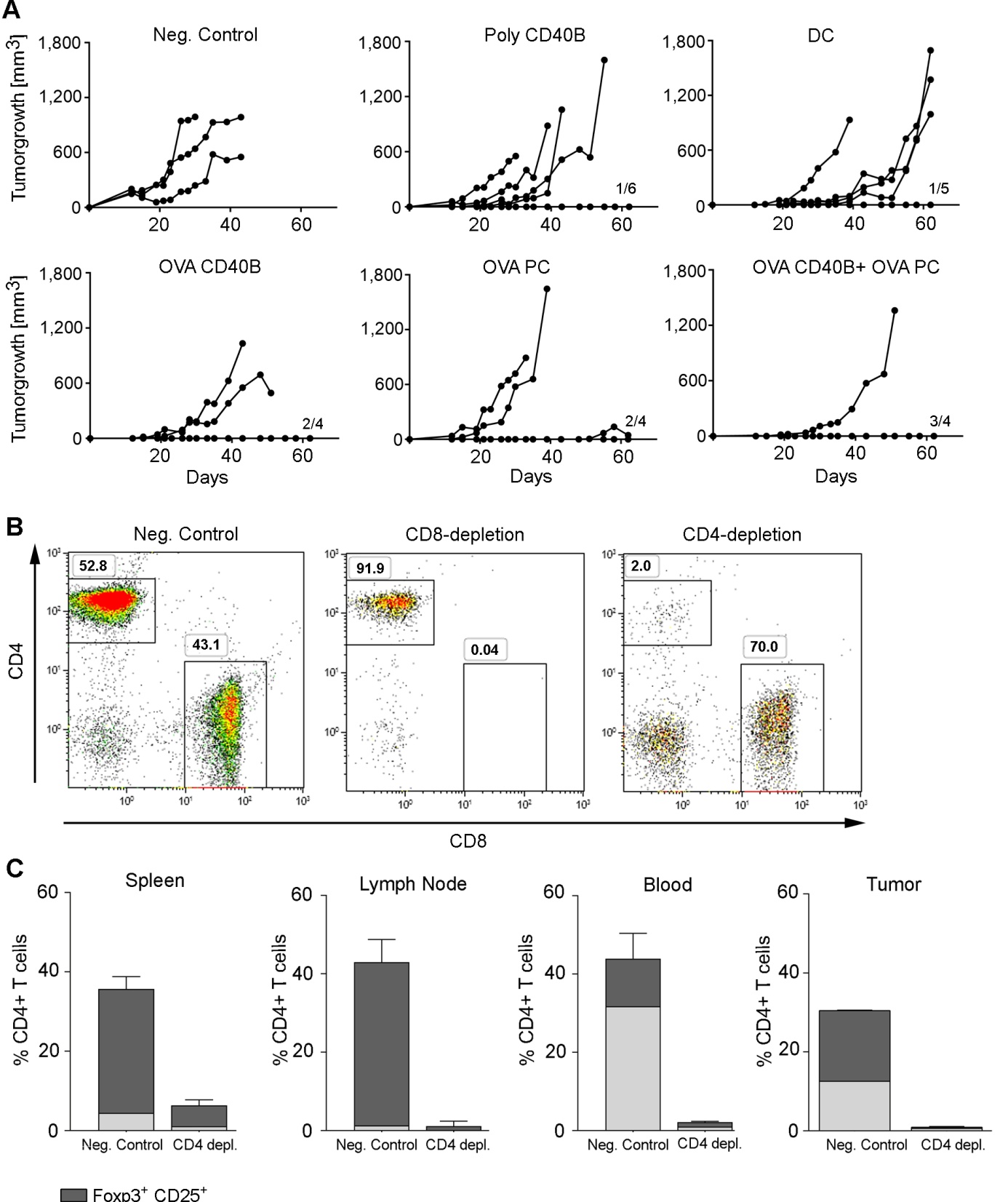
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**Fig. S5.** **Unstimulated OVA-B cells express homing molecules.**

OVA-B cells from Luc+ mice were analyzed by flow cytometry for the expression of the migratory markers CCR7, CXCR5, CXCR4 and CD62L. The MFI was normalized to values of non-specific B cells of the same mice. Significant differences calculated with a two-tailed t-test are marked by an asterisk. ns = not significant, \*\* p ≤ 0.01. Histograms show representative analyses compared to an isotype control.

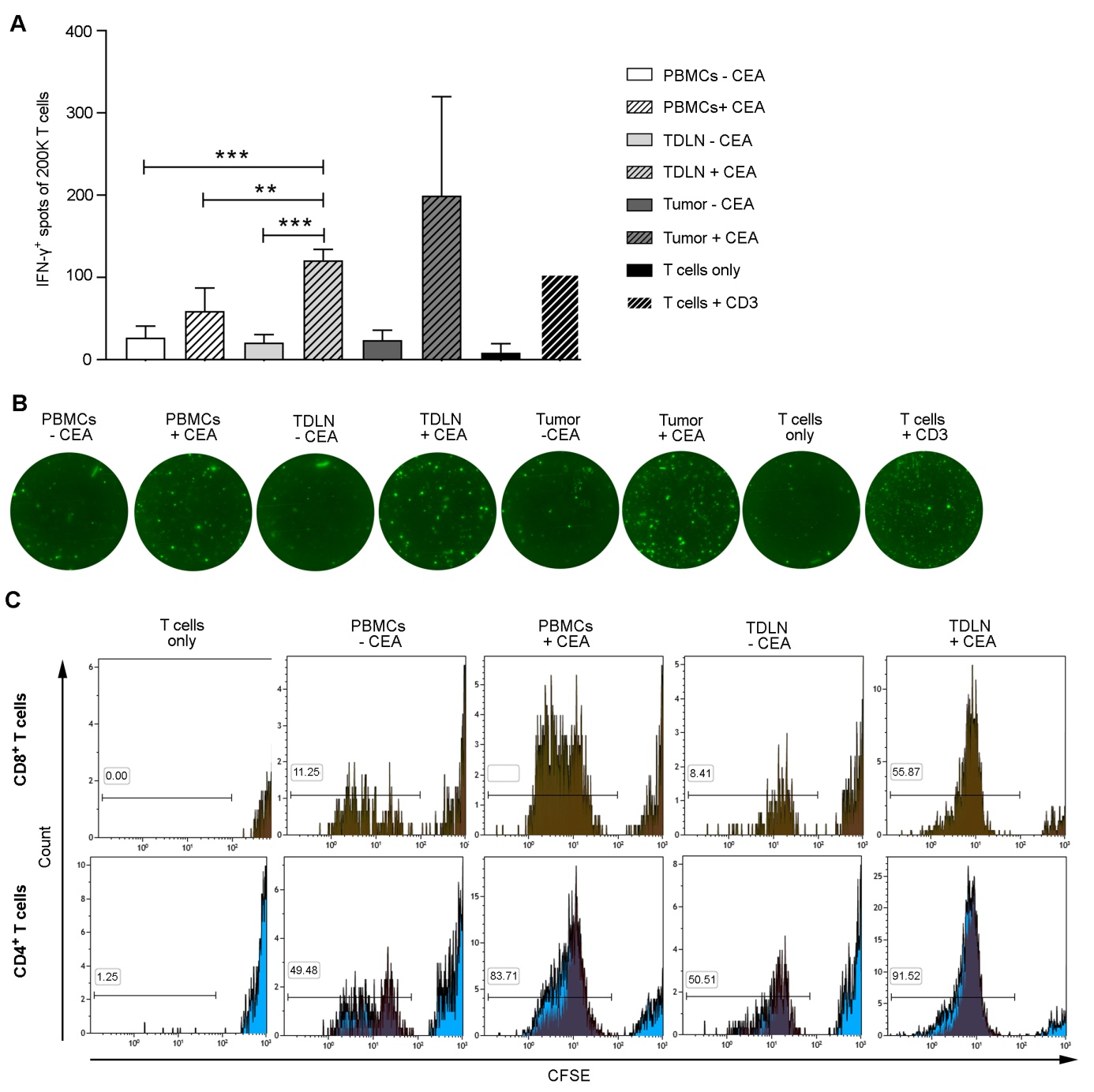


**Fig. S6. Preventive combinational immune intervention protects from tumor growth.** Mice were treated with 0.1-2 x 106 APCs, i.e. polyclonal CD40B cells (Poly CD40B), OVA-CD40B cells (OVA CD40B), DCs; PBS+IFA as negative controls (Neg. Control); OVA-specific PCs alone (OVA PC) or in combination with OVA-CD40B cells (OVA CD40B+ OVA PCs), thrice i.v. every seven days. On day 28, mice were challenged with 0.4 x 106 E.G7 cells s.c. Growth curves show (A) mean increase or (B) single increase of tumor volume in mm3 of one representative experiment out of three with three mice per group.. Significant differences calculated with a two-way ANOVA are marked by an asterisk. \* p ≤ 0.05, \*\* p ≤ 0.007, \*\*\* p ≤ 0.0005, \*\*\*\* p ≤ 0.0001. (C) Survival curves show means of one representative experiment out of three with three mice per group. Significant differences were calculated with the log-rank test: \* p ≤ 0.02. (D) Serum samples of mice were taken before treatment and on the day of tumor challenge. ELISA for OVA-specific IgG1 antibodies was performed. Significant differences calculated with two-way ANOVA are marked by an asterisk. ns = not significant, \*\*\*\* p ≤ 0.0001. (E) On day 50 after the first tumor cell injection, tumor-free mice were rechallenged with 0.4 x 106 E.G7 lymphoma cells sc. Tumor growth was measured until day 84.

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**Fig. S7.** **Combinational immunotherapy induces T cell-dependent anti-tumor immunity**

(A)On day 7 and 15, after Panc02OVA tumors became palpable, mice were treated i.v. with 0.1-2 x 106 APCs, i.e. polyclonal CD40B cells (Poly CD40B), OVA-CD40B cells (OVA CD40B), DCs, OVA-specific PCs alone (OVA PC) or in combination with OVA-CD40B cells (OVA CD40B+ OVA PCs), PBS as negative controls (Neg. Control). Growth curves show increase of tumor volume in mm3 in single mice of one representative experiment out of three experiments with 3-6 mice per group. Tumor growth is shown as 0.5 times the product of (length diameter x width diameter2). (B) On day 5 and 25 after Panc02Ova challenge, mice were treated with 500 µg of anti-CD4 (α-CD4) or anti-CD8 (α-CD8) antibody. Efficient T-cell depletion was determined by staining blood samples taken 5 days after first and second T-cell depletion for the presence of CD3+CD4+ or CD3+ CD8+ T cells. (C) On day 14 after Panc02OVA challenge, mice were treated with 500 µg of anti-CD4 (α-CD4). Five days later, tumors, spleens, TDLN and blood samples were removed and stained for the presence of CD4+ T cells and Foxp3+CD25+ subpopulations. Shown are means + SD in negative controls, which received PBS (Neg. Control) and CD4-depleted mice, each with 4 mice per group.



**Fig. S8** **Human antigen-specific CD40B cells induce specific T-cell responses.** (A+B) CD19+ purified B cells from PBMCs or single cell suspensions from TDLN or tumors of patients with colorectal cancer were cultivated for 14 days in the presence of the CD40L. Afterwards, 0.2 x 106 negatively isolated T cells from PBMCs were cocultured together with 0.1 x 106 protein-pulsed (+CEA) or unpulsed (-CEA) CD40B cells. Unstimulated T cells served as negative controls (T cells only); T cells + anti-CD3 served as positive controls. Fluorospot analysis was performed 20 hours later. (A) Means of IFN-γ+ spots per 0.2 x 106 (200K) T cells + SD are shown. PBMCs and TDLN: *n* = 2; and Tumor *n* = 1. Significant differences calculated with a one-way ANOVA are marked by an asterisk. \*\* p ≤ 0.008, \*\*\* p ≤ 0.0006. Tumor samples were excluded from statistical analysis. (B) Representative fluorospot pictures of IFN-γ signal in FITC are shown. Fluorospots with tumor-infiltrating B cells were performed with one high responding donor. (C) CD19+ purified B cells from PBMCs or single cell suspensions from TDLN or tumors of patients with colorectal cancer were cultivated for 14 days in the presence of the CD40L. Afterwards, 0.2 x 106 negatively isolated CFSE+ T cells from PBMCs were cocultured together with 0.1 x 106 protein-pulsed (+CEA) or unpulsed (-CEA) CD40B cells. Unstimulated T cells served as negative controls (T cells only). After 7 days of culture, proliferation of T cells was analyzed by a decrease in CFSE labeling by flow cytometry. Shown are representative CFSE plots gated on CD4+ or CD8+ T cells.