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Supplementary Materials for

Lymphangiogenesis-inducing vaccines elicit potent and long-lasting T cell immunity against melanomas

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Figs. S1 to S5

Supplementary Materials

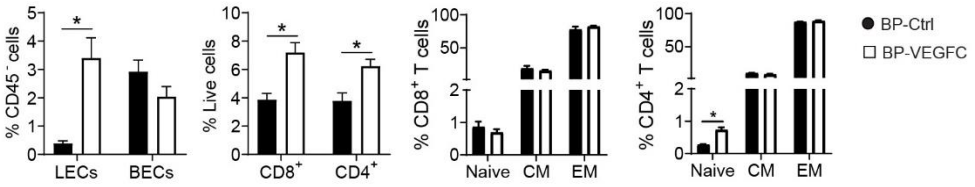

Fig. S1. Irradiated VEGF-C-overexpressing BP cells induce local lymphangiogenesis and T cell infiltration. Frequency of lymphatic endothelial cells (LECs), blood endothelial cells (BECs) and T cells in the injection site of irr BP-Ctrl or BP-VEGFC cells at day 8 post i.d. injection, as assessed by flow cytometry. Naïve=$CD62L^+$ $CD44^-$, CM (central memory)= $CD62L^+$ $CD44^+$, EM (effector and effector memory)= $CD62L^-$ $CD44^+$. Values are reported as mean ± SEM, n=4. Statistical analyses were performed with two-tailed Student's t test.

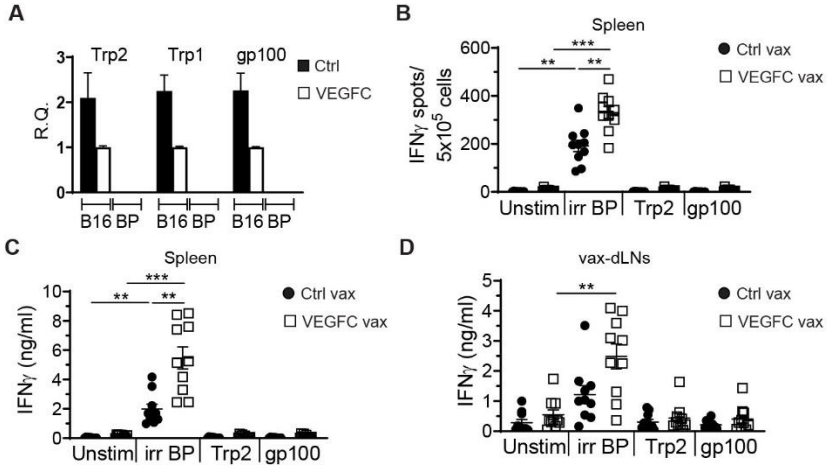

Fig. S2. BP cell lines express very low to undetectable levels of melanoma tissue-associated antigens. (A) Expression of melanoma tissue-associated antigens in B16 and BP cell lines in vitro, measured by RT-qPCR (mean ± SEM, n=2-4)**.** R.Q.=relative quantification, normalized to expression levels in B16-VEGFC. (**B-D)** Mice were vaccinated with Ctrl vax or VEGFC vax, composed of irradiated BP-Ctrl or B16-VEGFC cells , respectively, plus IMQ and αCD40 antibody, as described in Fig 3G. At day 17 from vaccination, splenocytes and LN cells were stimulated ex vivo with irradiated parental BP cell, Trp2 or gp100 peptides, or left unstimulated. **(B)** Frequency of IFNγ-producing splenocytes determined by ELISPOT. **(C)** ELISA quantification of IFNγ production in the spleen. **(D)** ELISA quantification of IFNγ production in the vaccine-draining LNs. Pooled data from 2 independent experiment. Individual values and mean ± SE (n=10). **P <0.01, ***P < 0.001., one-way Anova with Welch's correction for unequal SD and Dunnett's T3 multiple comparisons test.

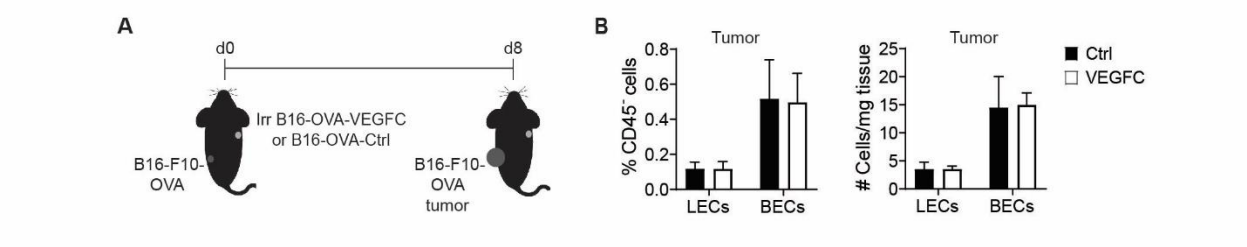

Fig. S3. Irradiated VEGF-C-overexpressing cells do not induce lymphangiogeneesis in distant tumors. Mice were injected i.d. with irradiated B16-OVA-Ctr or B16-OVA-VEGFC on the right side of the back, close to the shoulder area, and with non-irradiated B16-F10-OVA on the left side at the middle of the back. Presence of VEGF-C induced lymphangiogenesis and angiogenesis in B16-F10-OVA tumors was assessd at day 8 post injection. **(A)** treatment schematic. (**B)** Quantification of lymphatic endothelial cells (LECs) and blood endothelial cells (BECs) in the tumor by flow cytometry. mean ± SEM, n=3.

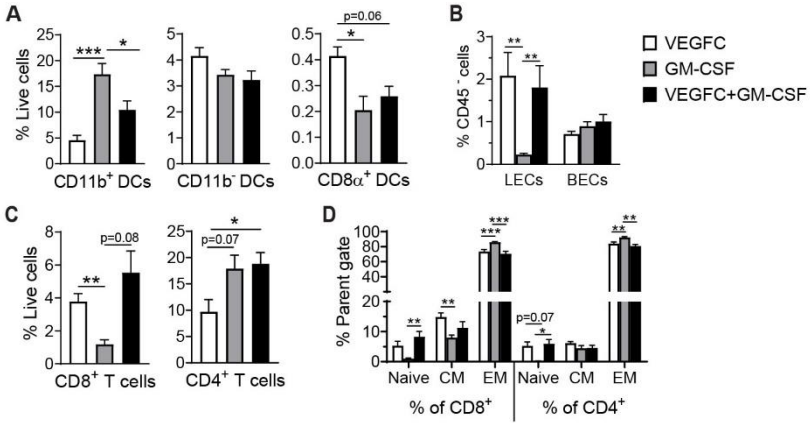

Fig. S4. Irradiated GM-CSF and VEGF-C-overexpressing cells drive distinct patterns of myeloid cell and T cell infiltration in the skin injection site. Naïve mice were injected i.d. in the back with either irradiated B16-VEGFC cells, irradiated B16-GM-CSF cells or a 1:1 mixture of both cell lines. Presence of lymphangiogenesis and immune cell infiltration in the cell injection site in the skin was characterized at day 8 post injection by flow cytometry. **(A)** Dendritic cell subsets infiltrating the irradiated cell injection site. **(B)** Lymphatic endothelial cells (LECs) and blood endothelial cells (BECs) in the skin, in the site of irradiated cell injection. **(C)** total $CD8^+$ and $CD4^+$ T cells infiltrating the skin injection site. **(D)** Frequency of different T cell subsets among the $CD8^+$ and CD4+ T cell compartments. Naïve=$CD62L^+$ $CD44^-$, CM (central memory)= $CD62L^+$ $CD44^+$, EM (effector and effector memory)= $CD62L^-$ $CD44^+$. Legend in B applies to all the figure. Values are reported as mean ± SEM, n=5. Statistical analyses were performed with one-way Anova with Tukey's multiple comparisons test. *P < 0.05, **P <0.01, ***P < 0.001.

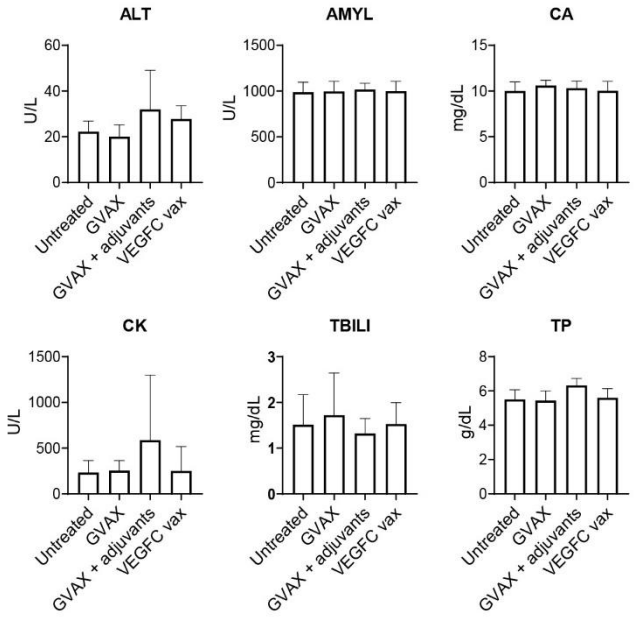

Fig. S5. VEGFC vax does not induce systemic toxicity. Mice were treated with either VEGFC vax, GVAX or GVAX further adjuvanted by addition of IMQ and MB-αCD40, administered topically as for VEGFC vax (GVAX+ adjuvants), or left untreated. The concentration of markers of hepatic, pancreatic, kidney and cardiac toxicity was measured in the serum after 4 days from the completion of the vaccination protocol. ALT= Alanine Transaminase, AMYL= amylase, CA= calcium, CK= creatinine kinase, TBILI= total billirubin, TP= total protein concentration. Values are reported as mean ± SD, n=8-11 mice per group.