Modulating T cell immunity in tumors by targeting tumor-associated antigens, PD-L1 and neoantigens using a versatile live attenuated oral *Salmonella* DNA vaccination platform

VAXIMM

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Background

VAXIMM's oral T-cell vaccine platform is based on the approved live attenuated *Salmonella* Typhi strain Ty21a vaccine, which has been administered in millions of individuals for prophylactic vaccination against typhoid fever. This strain has been thoroughly studied, and is safe and well tolerated. The bacteria are modified to deliver an eukaryotic expression plasmid which encodes the genetic information of a specific target antigen, via the oral route[1]. A proposed mechanism of action is described in **Figure 1**.
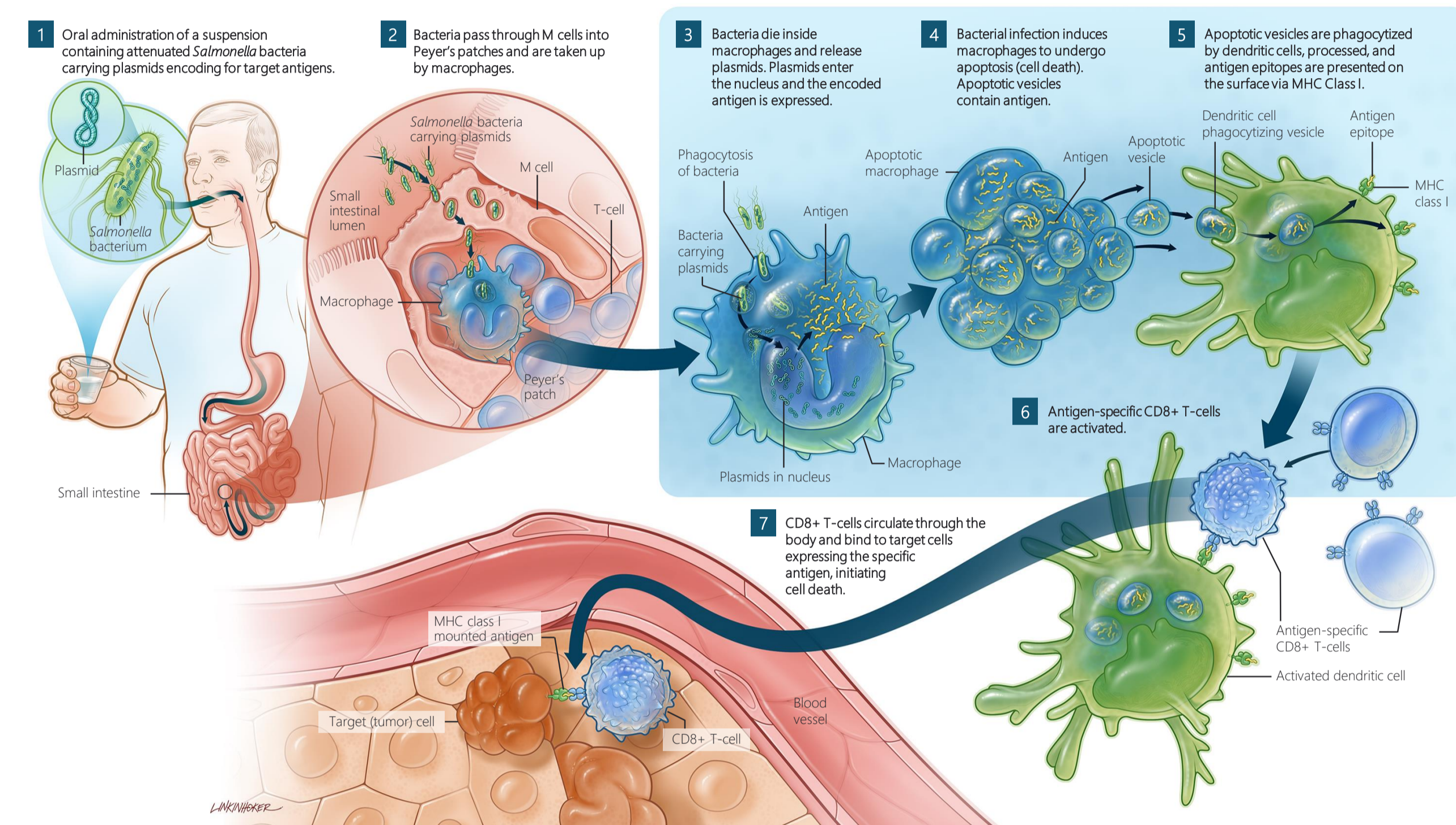
Figure 1. Intra-lymphatic delivery of *Salmonella* Typhi strain Ty21a T-cell vaccines via the oral route leading to target-specific T-cell activation.

VXM01 lead vaccine encodes vascular endothelium growth factor receptor 2 (VEGFR2) to evoke an immune response directed to the tumor vasculature. The murine analogue of VXM01 has shown consistent anti-angiogenic activity in different tumor models[2] and in several animal studies. VXM01 is currently in clinical development as a treatment for various solid cancers[3,4].

The current study summarizes the pre-clinical safety profile, the immunogenicity and the anti-cancer efficacy for the live attenuated *Salmonella* Typhimurium strain SL7207 based murine DNA vaccines **VXM04**, **VXM06**, **VXM10** and **VXMNEO** which encode Mesothelin, WT1, PD-L1 full-length or truncated proteins, and multi-epitope constructs respectively.

Toxicology

The preclinical safety profile of VXM06 and the empty vector control was assessed in C57BL/6J mice after oral gavage administration of doses up to 10^8 colony-forming units (CFU) per occasion on days 1, 3, 5, 7, and once monthly thereafter during 13 weeks, followed by 6-week recovery, in a GLP-compliant toxicology study.
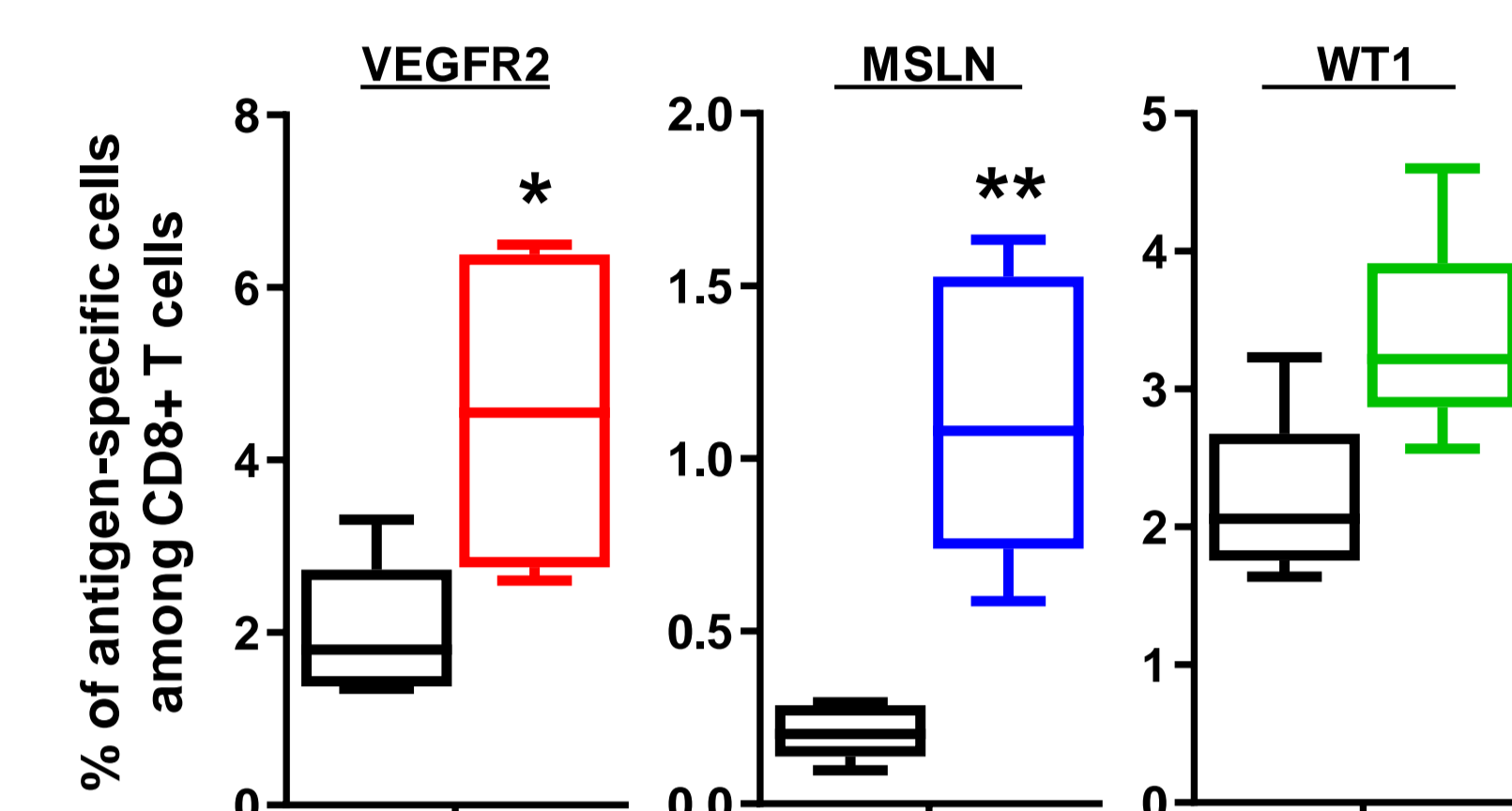
VXM06 was safe and well tolerated, there were no deaths related to empty vector or VXM06, and no clear treatment related clinical signs, nor bodyweight, food consumption, hematology, organ weight and macroscopic pathology findings. There was no evidence of proliferation of VXM06 in the feces or organs analyzed. Histopathology analyses revealed extra-intestinal manifestations restricted to the liver and the kidney in some animals (**Figure 2**).
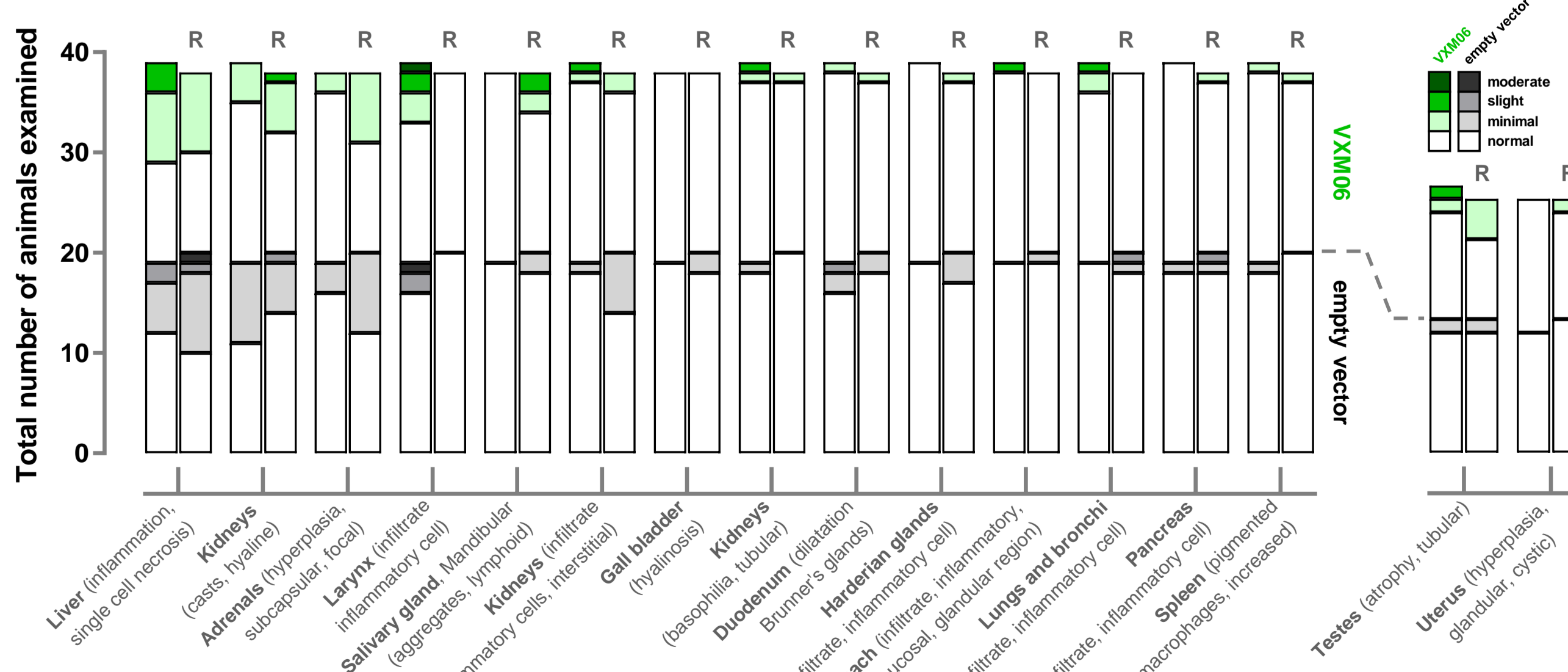
Figure 2. Histopathology findings observed in ≥2% of the animals examined, males and females collectively, after 13 weeks of treatment with empty vector (grey and black stacked bars) and VXM06 (green stacked bars) at 10^8 CFU per occasion (left bars) and after 6-week recovery (R; right bars).

Immunogenicity

Immunokinetic studies were performed in C57BL/6 mice (n=5 per group) immunized 4 times every other day via the oral route with 10^{10} CFU of either VXM01, VXM04, VXM06 or the empty vector control. The frequency of antigen-specific T cells was measured at different time points in the spleen by flow cytometry using fluorescently labelled MHC class I/peptide pentamers.


Vaccination with VXM01, VXM04 and VXM06 induced a significant systemic antigen-specific CD8 T cell response, with a peak immune response detected 7 to 10 days after the final immunization, without in vitro antigenic stimulation (**Figure 3B**).

Figure 3. Frequency of antigen-specific after immunization with the empty vector (black box plots), VXM01 (red), VXM04 (blue) and VXM06 (green) measured at the peak immune response.

Antitumor efficacy

The anti-tumor efficacy of VXM01 and VXM04 was evaluated in a prophylactic setting in the Panc02 syngeneic model of pancreatic adenocarcinoma expressing MSLN[5]. Vaccination with VXM01 and VXM04 resulted in markedly reduced tumor growth (**Figure 4**). At the end of the study, the tumor growth inhibition relative to the control group reached 60.6% and 82.9% in the VXM01 and VXM04 vaccination groups respectively.
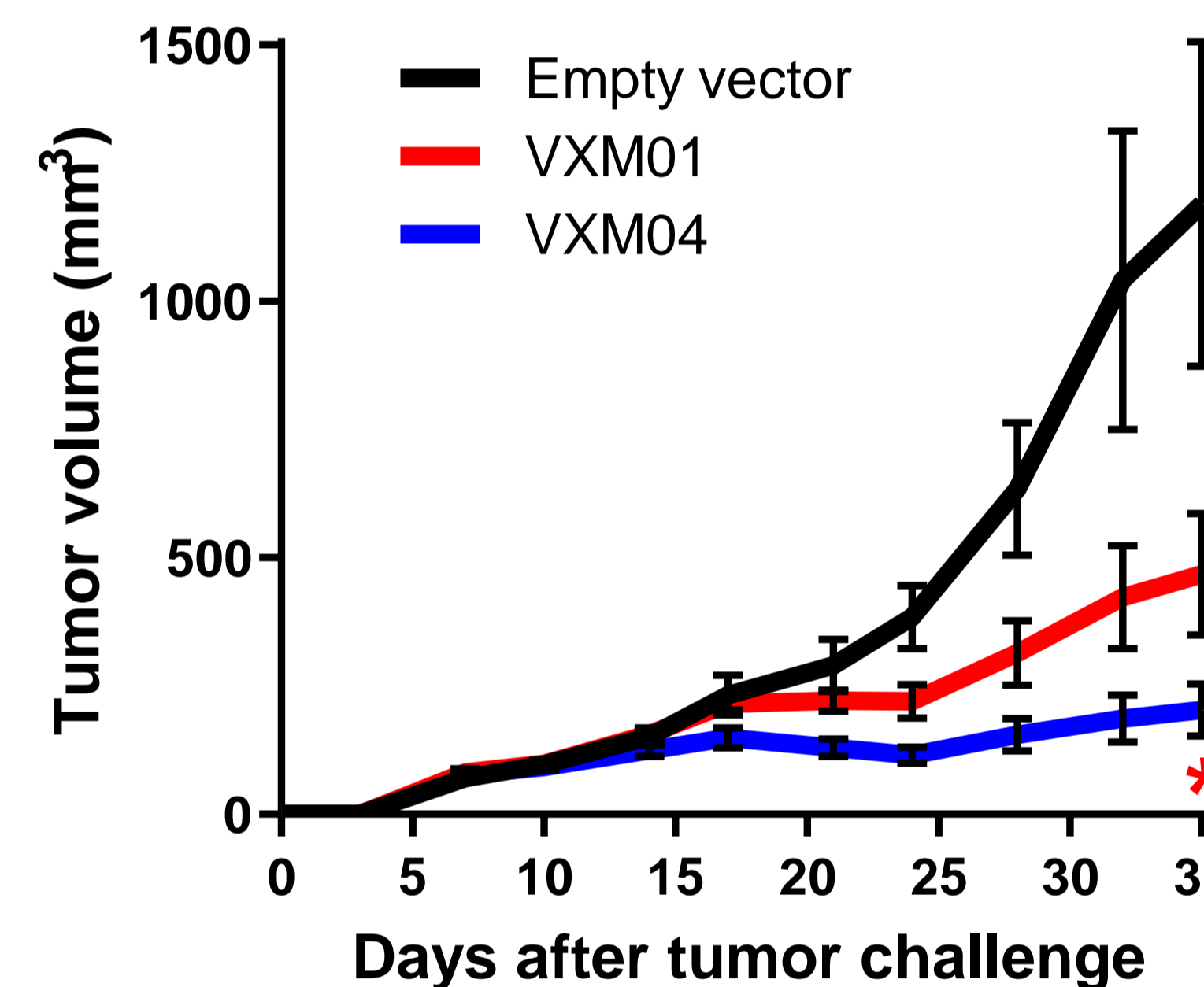
Figure 4. Tumor volumes (mean and SEM) measured after immunization with 10^8 CFU of VXM01 (red) or VXM04 (blue) on days 1, 3, 5 and 7 (prime), and on days 14, 28, 35, 42 and 49 (boosts), and subcutaneous challenge with 1×10^6 Panc02 cells on day 21.
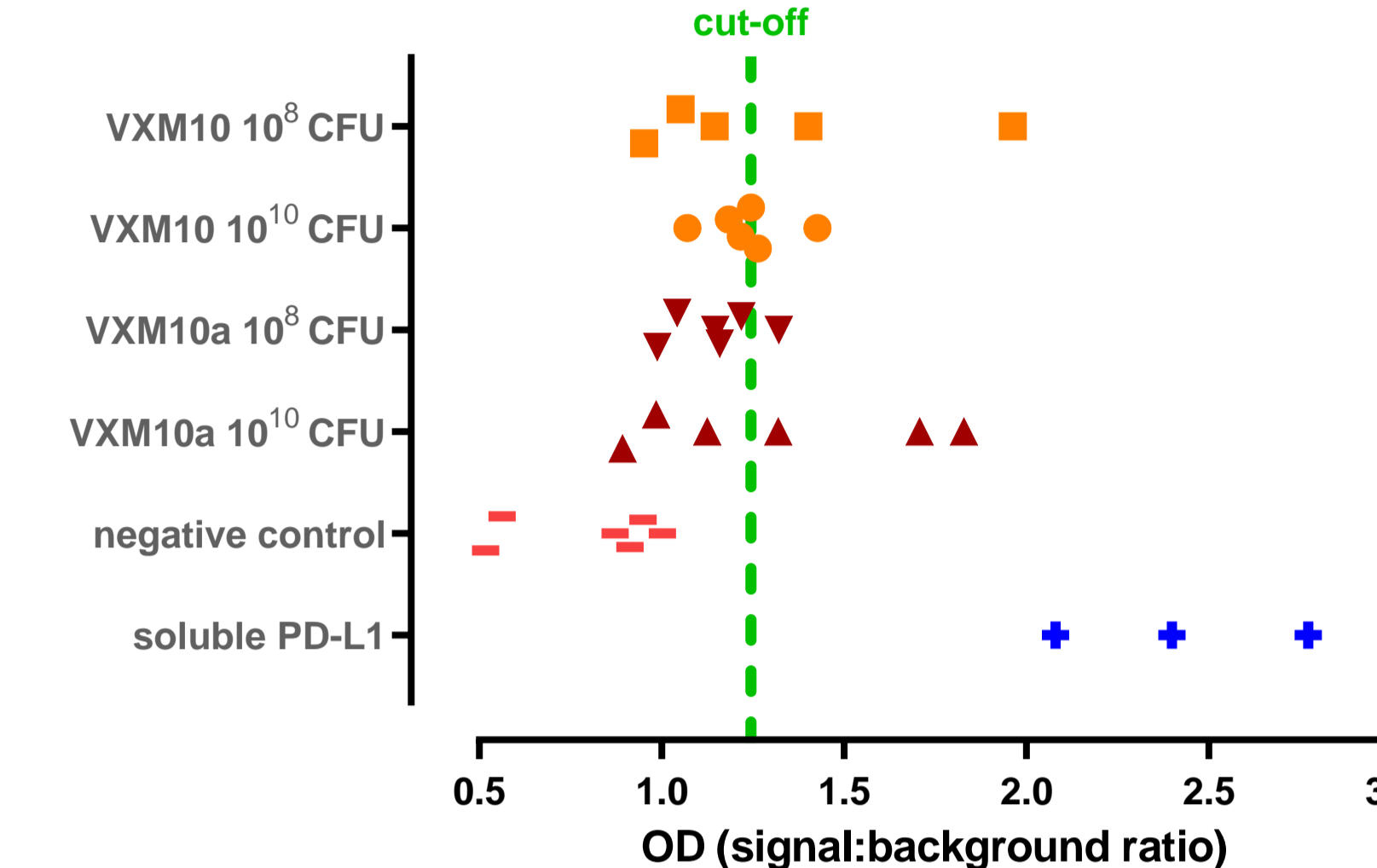
Anti-leukemia activity

The anticancer activity of VXM06 was evaluated in the FBL-3 disseminated model of leukemia expressing WT1[6]. Prophylactic vaccination with VXM06 generated a rapid and sustained anti-leukemia activity with 100% of surviving animals 80 days after leukemia challenge (**Figure 5A**). Importantly, 100% of surviving mice resisted re-challenge with FBL-3 cells at least 100 days after leukemia re-challenge, demonstrating that vaccination with VXM06 generated a potent memory T cell response against the leukemia (**Figure 5A**). Therapeutic vaccination with VXM06 induced the full leukemia control, with 100% of surviving animals 94 days after leukemia challenge (**Figure 5B**).
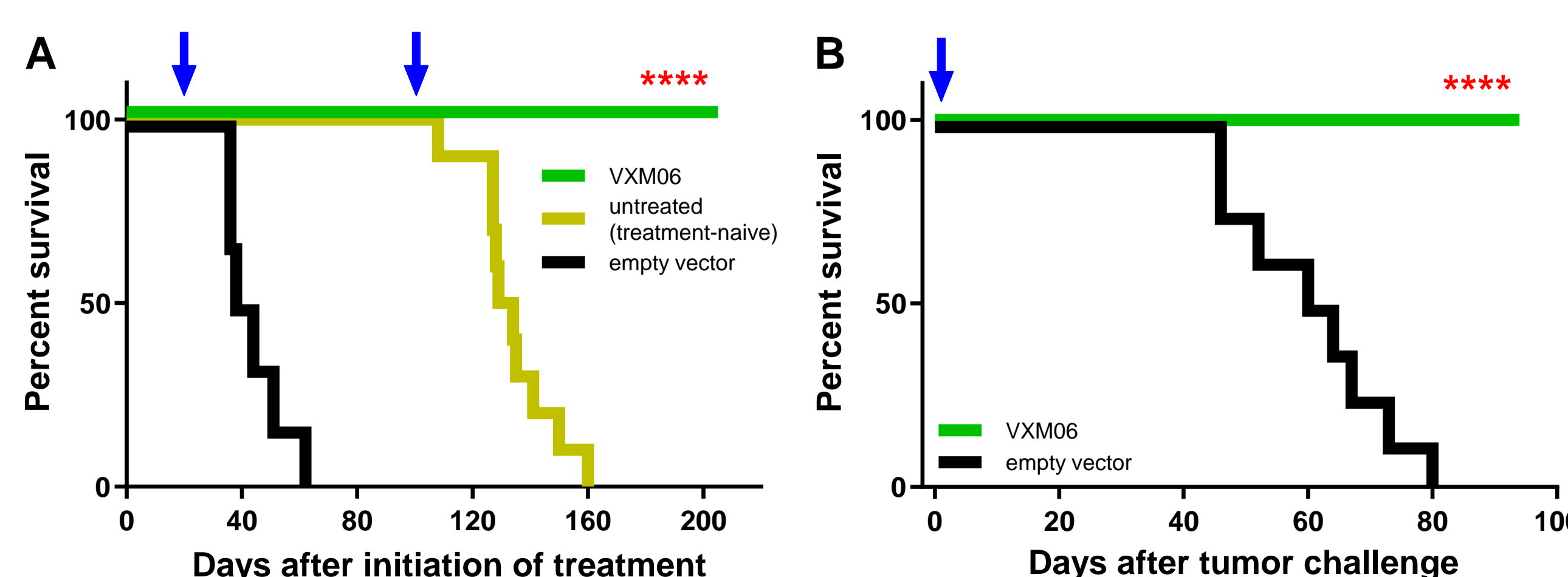
Figure 5. Overall survival in the prophylactic (A) and therapeutic (B) settings, after immunization with 10^8 (A) or 10^9 CFU (B) of VXM06 via the oral route on days 1, 3, 5 and 7 (prime), and on days 14 and 21 (boosts), and challenge with 5×10^6 of FBL-3 cells (blue arrows) on day 20 and 100 (A) or on day 0 (B).

Immunity to PD-L1

Live attenuated *Salmonella* Typhimurium DNA vaccines VXM10 and VXM10a are transformed with eukaryotic expression plasmids encoding the full-murine programmed death-ligand 1 (PD-L1) protein and a truncated form of PD-L1 respectively. The deletion of the signal peptide (SP) in VXM10a prevents the proper localization of the native PD-L1 protein to the cell membrane.


The antibody response was evaluated by ELISA in the serum of animals 79 days after the final immunization. Anti-PD-L1 antibodies were detected in animals vaccinated with VXM10 and VXM10a, and the response was more pronounced in the highest dose vaccination groups, with 50% of the animals showing signal-to-background ratio above the cut-off value (**Figure 6**).

Figure 6. Anti-PD-L1 antibody response in the sera of FBL3-bearing animals, collected 79 days after the final immunization. The green dashed line represents the cut-off value. In the positive control group mice were immunized with soluble recombinant murine PD-L1 and CFA/IFA (blue).

T-cell response to PD-L1 epitopes was measured in C57BL/6 mice immunized 4 times every other day via the oral route with 10^{10} CFU of either VXM10, VXM10a or the empty vector control. The level of TNFα, and to a lesser extent IFNγ, was significantly increased in the supernatant of splenocytes derived from animals vaccinated with VXM10a, and stimulated with PD-L1 peptides (**Figure 7**).
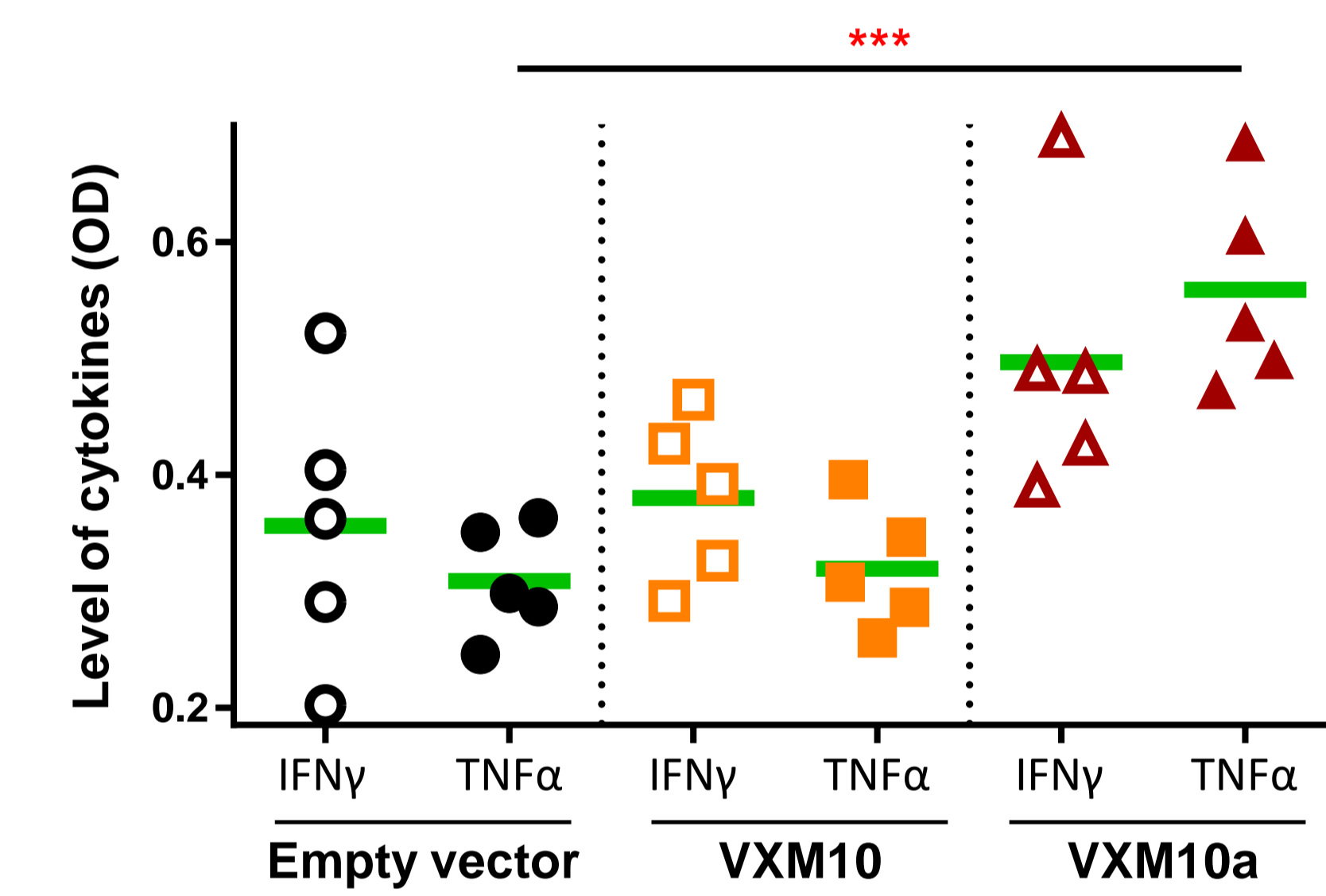
Figure 7. Mean level of IFNγ (open symbols) and TNFα (closed) secreted by splenocytes 10 days after the last immunization, and stimulated over 6 days with a pool of 5 peptides derived from PD-L1, as measured in the culture supernatant by ELISA.

Finally, the anticancer activity of VXM10 and VXM10a was evaluated in the FBL-3 disseminated model of leukemia, which also expresses a high level of PD-L1[7]. Prophylactic vaccination with VXM10 and VXM10a generated a rapid and sustained anti-leukemia effect with 100% of surviving animals 80 days after leukemia challenge in the highest dose groups (**Figure 8A**). Importantly, 100% of surviving mice resisted re-challenge with FBL-3 cells in the high dose groups (**Figure 8A**), demonstrating that vaccination with VXM10 and VXM10a generated a potent memory T-cell response against the leukemia. Therapeutic vaccination with VXM10 and VXM10a induced full leukemia control, with 100% of surviving animals 94 days after leukemia challenge (**Figure 8B**).
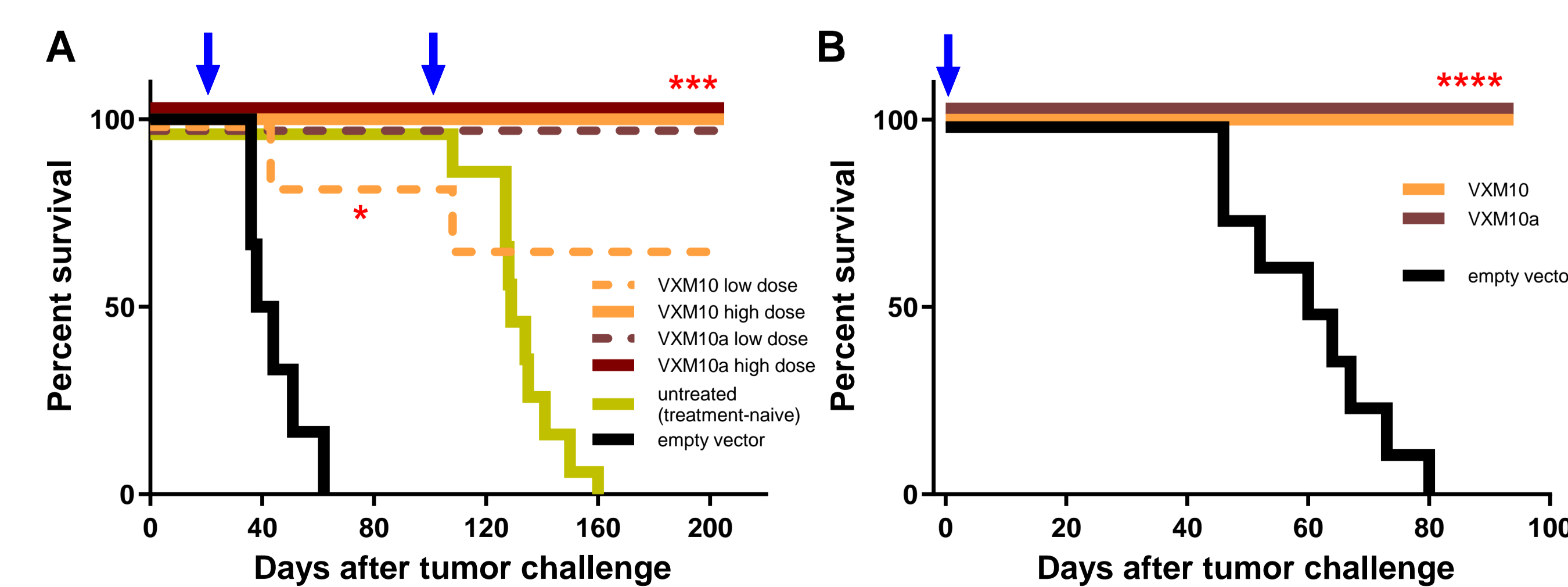

Figure 8. Overall survival in the prophylactic setting (A) after immunization with 10^8 (low dose) or 10^{10} CFU (high dose) of the indicated vaccine via the oral route on days 1, 3, 5 and 7 (prime) and on days 14 and 21 (boosts), and challenge with 5×10^6 of FBL-3 cells (blue arrows) on day 20 and 100. In the therapeutic setting (B), mice were challenged on day 0 with 5×10^6 of FBL-3 cells, and vaccinated with 10^9 CFU of the indicated vaccine via the oral route on days 1, 3, 5 and 7 (prime) and on days 14 and 21 (boosts).

Multi-epitope vaccines

The immunogenicity of different polyepitope vaccines, based on the VXMNEO platform, was assessed in healthy C57BL/6 mice. The frequency of specific T cells for each individual epitope encoded was measured 10 days after the final vaccination in the spleen by flow cytometry using fluorescently labelled MHC class I/peptide pentamers. The different constructs encode multiple CD8 and CD4 epitopes from VEGFR2 (KDR2 and KDR3), Mesothelin (MSLN), WT1, CEA, and Ovalbumin (OVA), linked via different spacers, e.g. "string-of-beads", and in different orders.

VXMNEO vaccines induced a substantial systemic T-cell response for up to 6 out of 9 CD8 epitopes. Importantly the dose, treatment schedule, ordering and linkage strategy greatly influence the immunogenicity of the encoded polyepitope (**Figure 9**).
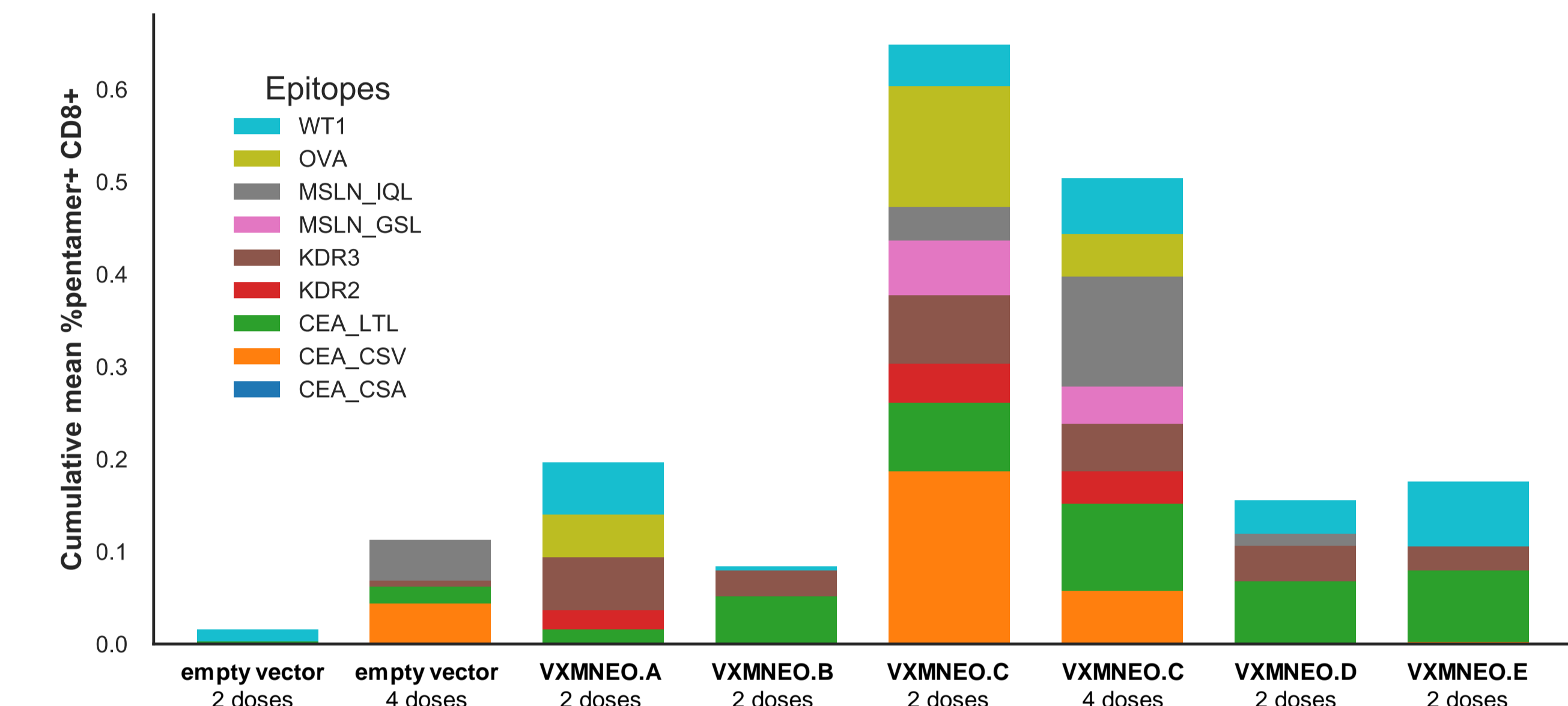
Figure 9. Cumulative mean frequency of the indicated epitope-specific CD8+ T cell population in the splenocytes of C57BL/6 mice immunized via the oral route with doses up to 10^{10} CFU of different VXMNEO constructs.

Conclusions

- VMX06 was well tolerated at the effective doses.
- VXM01, VXM04 and VXM06 induced significant systemic antigen-specific T cell responses in animals, and demonstrated consistent anti-cancer activities in different tumor models.
- VXM10 vaccines stimulate both humoral and cellular immunities against antigens of the checkpoint regulatory protein PD-L1.
- VXM06 and VXM10 vaccines induced a rapid and sustained anti-leukemia activity in both the prophylactic and therapeutic settings, and a potent memory T-cell response.
- VXMNEO platform can be employed to stimulate T cell responses against multiple antigens encoded by polyepitope constructs, and potentially neoantigens.
- This studies pave the way for advancing the development of tumor-associated antigen vaccines VXM04 and VXM06, PD-L1 antigen vaccine VXM10 and neoantigens using the VMXNEO platform, and combination thereof, into clinical development.

References

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