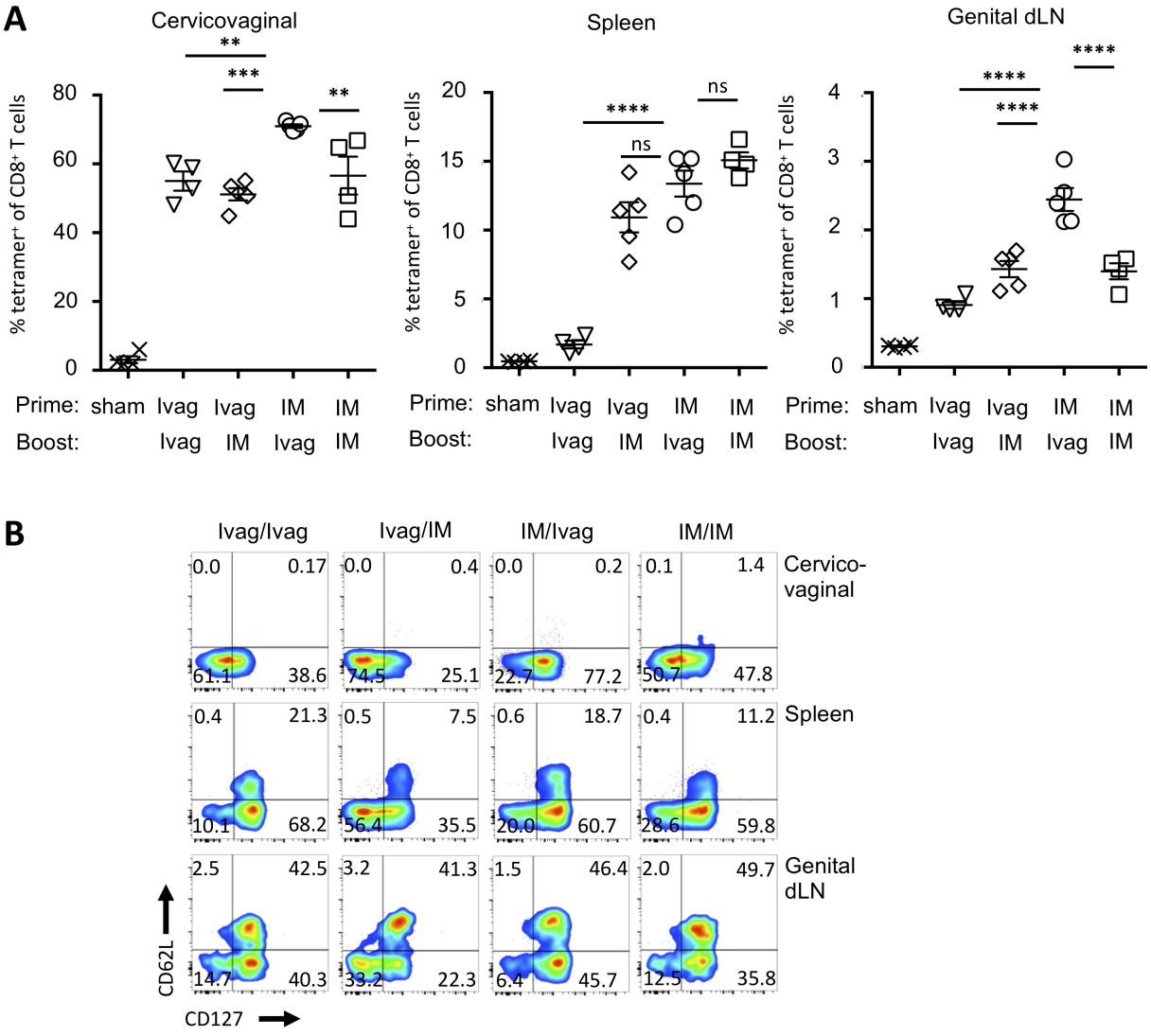
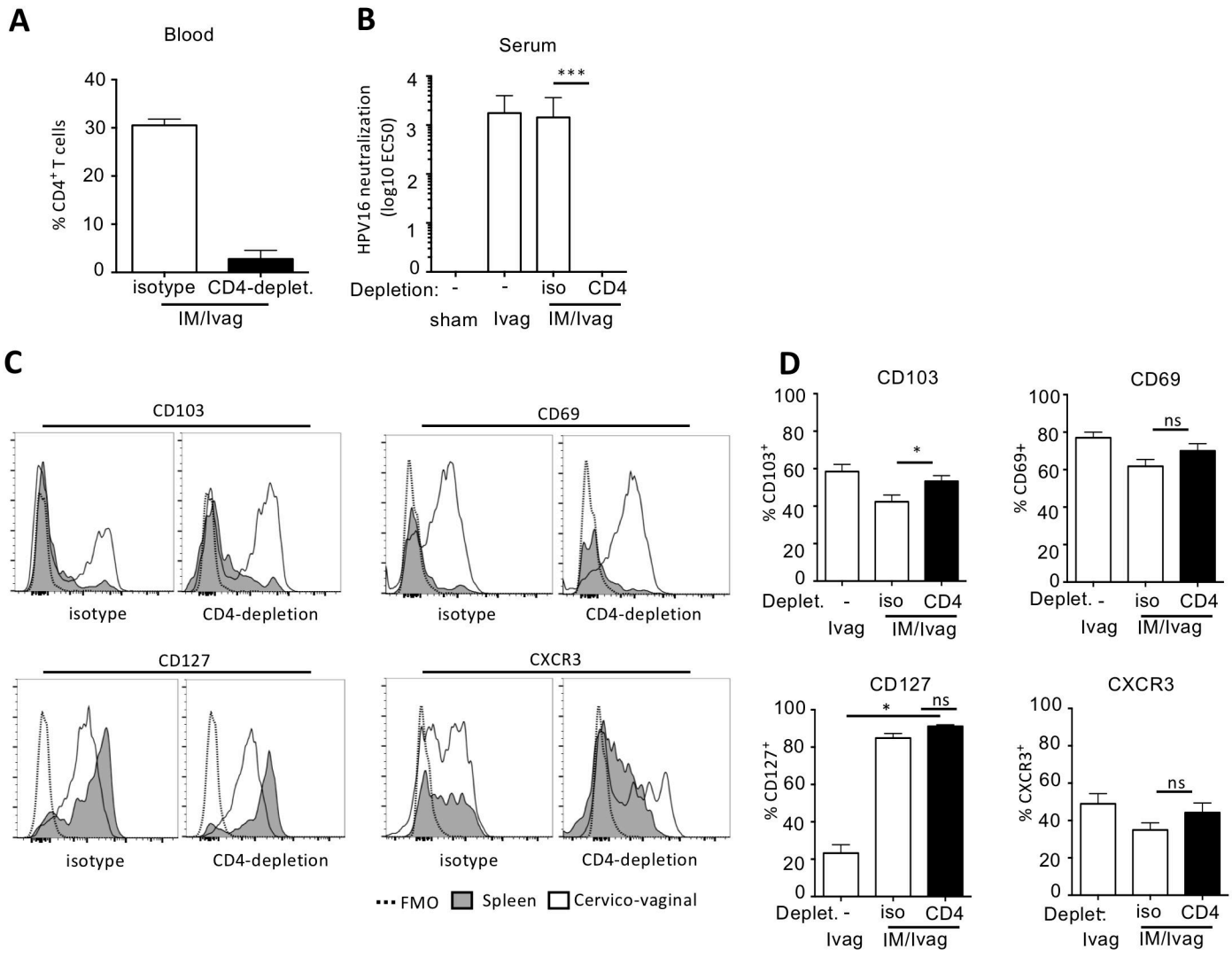
Supplemental Figure 1



Supplemental Figure 1. The order of route of immunization determines the tissue localization of CD8$^+$ T cell responses. BALB/c mice were prime-boost immunized one month apart via Ivag or IM routes with HPV ($5x10^7$ IU) and Ad5 ($5x10^7$ PFU) vectors expressing the RSV fusion protein MM2, respectively. (**A, B**) Two weeks after the final immunization, cell suspensions from cervicovaginal tissues, spleen and genital draining lymph nodes were analyzed by FACS for the presence of H2-K^d/$M2_{82-90}$ tetramer$^+$ CD8$^+$ T cells (**A**) and for the expression of CD62L and CD127 (**B**). Representative FACS plot (**B**) of expression of CD62L and CD127 by M2-specific CD8$^+$ T cells in the indicated tissues. Histogram bars represent percentage +/- SEM of M2-specific CD8$^+$ T cells. P values (**P≤0.01, ***P≤0.001, ****P≤0.0001) were determined by one-way ANOVA with post-hoc Tukey analysis.

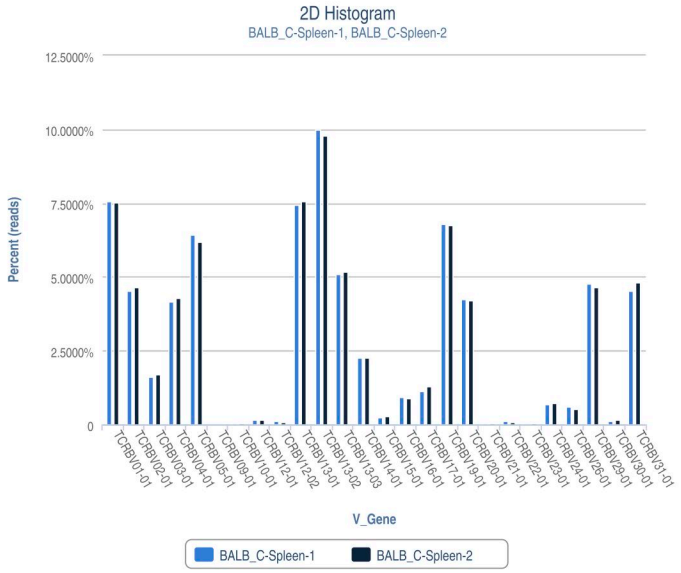
Supplemental Figure 2



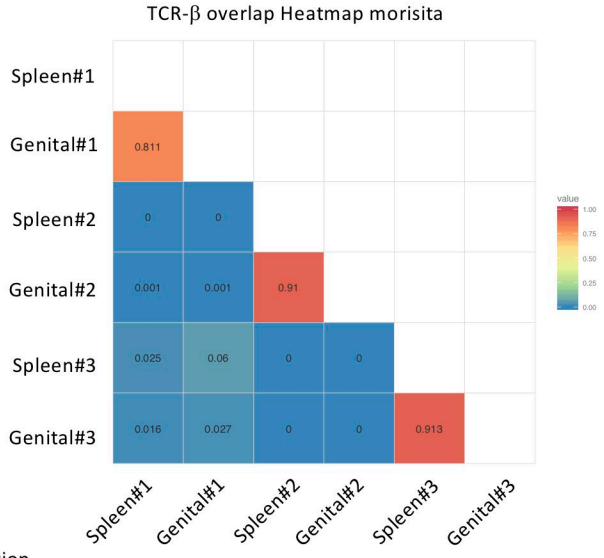
Supplementary Figure 2. Induction of cervicovaginal resident memory CD8⁺ T cells does not require CD4⁺ T cell help. BALB/c mice were primed IM with Ad5-MM2 vector and one month later received a booster Ivag with HP16-MM2 in CD4⁺ T cells depleted (GK1.5-treated) and isotype control-treated mice. (**A-C**) One month after the final immunization, cervicovaginal and spleen cell suspensions were analyzed by FACS for the presence of H2-K^d/$M2_{82-90}$ tetramer⁺CD8⁺ T lymphocytes. (**A**) CD4 depletion in blood samples. (**B**) Neutralization titers against HPV PsV capsid. (**C**) FACS histograms plots represent mean percentage of expression CD103, CD69, CD127 and CXCR3 expression by spleen (shaded area) and cervicovaginal (open area) M2-specific CD8⁺ T cells. (**D**) Histogram bars +/- SEM represents the mean percent of expression of CD103, CD69, CD127 and CXCR3 expression by and cervicovaginal M2-specific CD8⁺ T cells. *P* values (*$P \leq 0.05$, **$P \leq 0.01$) were determined by one-way ANOVA with post-hoc Tukey analysis.

Supplemental Figure 3

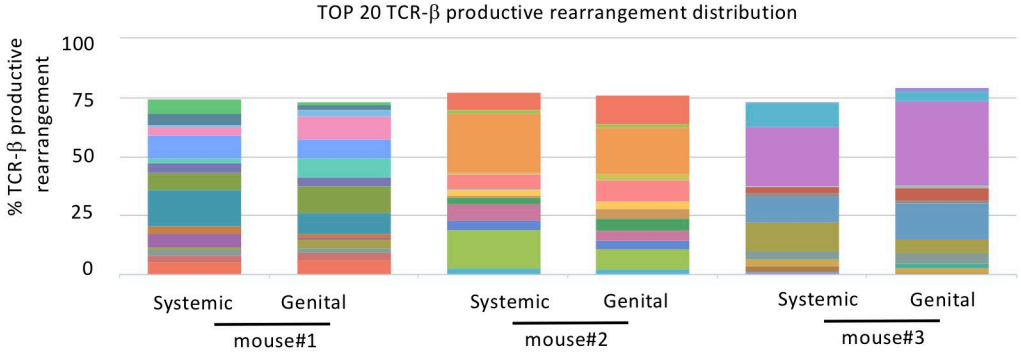
A



B



C

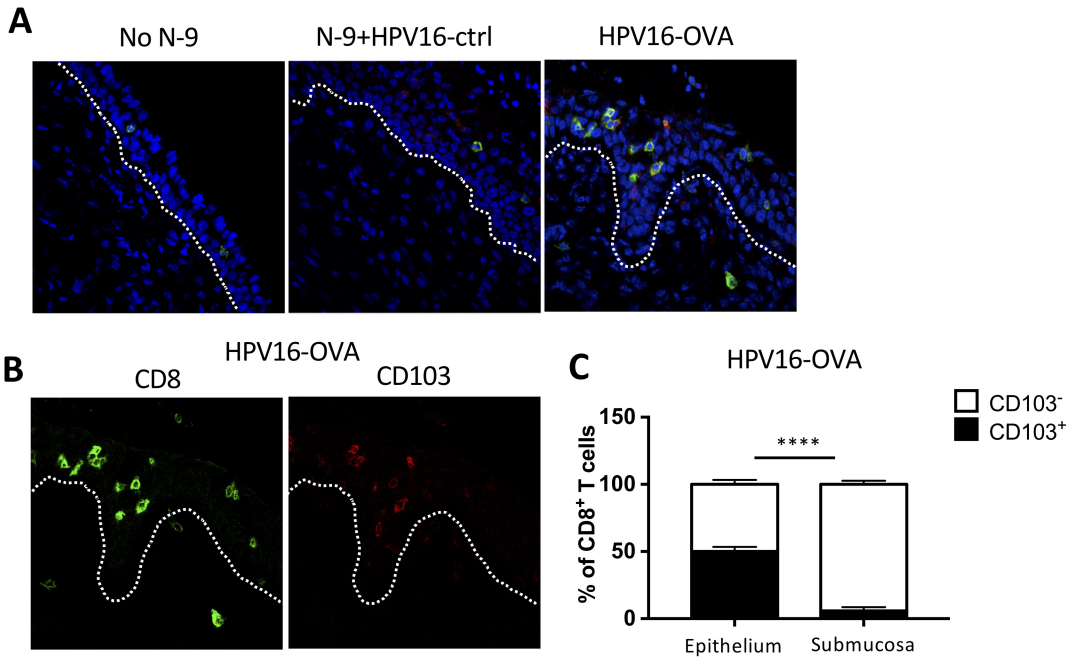


D

Tissue	CDR3 (amino acid)	Frequency	Rank	CDR3 (amino acid)	Frequency	Rank	CDR3 (amino acid)	Frequency	Rank
Spleen	CASGGTGGGNQAPLF	15.5	1	CASGPGTGFAEQFF	25.1	1	CASGVTGGGNQAPLF	25.4	1
	CASGGGGAGYAEQFF	9.5	2	CASGARDWGGSYEQYF	16.4	2	**CASGGGTGYAEQFF**	15.6	2
	CASRDRGEQYF	7.2	3	CASSAGNSGNTLYF	7.3	3	CASGAGTGYAEQFF	12.4	3
	CASGGGTGYAEQFF	6.2	4	CASRTTSQNTLYF	6.7	4	CASGTGENSPLYF	10.1	4
	CASGAGTGFAEQFF	6.0	5	CASSVAGANTGQLYF	6.2	5	CASGPGGTEVFF	4.0	5
	CASGPGTGYAEQFF	5.5	6	CASRGQYEQYF	4.0	6	CASGGGTGGFAEQFF	2.7	6
	CASGGGTGGARAEQFF	5.2	7	CASGATGYYAEQFF	2.9	7	*CASGTGWDTEVFF*	2.5	7
	CASGPVTGQLYF	4.8	8	*CASRDTYEQYF*	2.6	8	*CASGGTGGGYEQYF*	1.7	8
	CASGDGWGITLYF	3.9	9	CASGDGKGEQYF	2.5	9	*CASGTGGVSYEQYF*	1.3	9
	CASSPTGDAEQFF	3.8	10	**CASGPGTGYAEQFF**	2.1	10	*LCQQQGASGNTLYF*	1.1	10
Genital	CASRDRGEQYF	11.3	3	CASGPGTGFAEQFF	19.4	1	CASGVTGGGNQAPLF	35.5	1
	CASSPTGDAEQFF	9.7	10	CASSAGNSGNTLYF	12.5	3	CASGAGTGYAEQFF	16.1	3
	CASGGTGGGNQAPLF	9.0	1	CASSVAGANTGQLYF	8.9	5	**CASGGGTGYAEQFF**	10.2	2
	CASGGGGAGYAEQFF	8.3	2	CASGARDWGGSYEQYF	8.2	2	CASGPGGTEVFF	5.9	5
	CASGAAEQFF	8.1	-[d]	CASGATGYYAEQFF	5.3	7	CASGTGENSPLYF	4.1	4
	CASGGGTGYAEQFF	7.4	4	**CASGPGTGYAEQFF**	4.5	10	CASGGGTGGFAEQFF	2.5	6
	CASGGGTGGARAEQFF	7.2	7	CASRTTSQNTLYF	4.1	4	*CASGAGEVFF*	2.2	-
	CASGDGWGITLYF	3.5	9	CASRGQYEQYF	3.8	6	*CASGASGTEVFF*	1.5	-
	CASSDGGNEQYF	3.4	-	CASGDGKGEQYF	3.1	9	*CASGSYEQYF*	1.4	-
	CASGPLGGEQYF	2.9	-	*CASGSPNSDYTF*	2.9	-	*CASGDAWGVAEQFF*	1.4	-

Supplementary Figure 3. TCR-Vβ repertoire of CD8⁺ T cells from naïve BALB/c mice and M2-specific CD8+ T cells after IM/Ivag immunization. (**A**) Analysis of TCR-Vβ repertoire was performed using the ImmunoseQ software and a shared data set for BALB/c naïve CD8⁺ T cells. (**B**) Heat map plot of $K^d/M2_{82\text{-}90}$ tetramer⁺CD8⁺ T cells TCR-β rearrangement usage at the nucleotide levels between samples. (**C**) Distribution of the 20 most represented TCR-β rearrangement of M2-specific CD8⁺ T cells in each sample. (**D**) Ten most represented TCR-β CDR3 sequences in spleen samples. Color coding according to rank CDR3 abundance within spleen. CDR3 absent in spleen (white); CDR3 present in two mice or more (bold); CDr3 present in one sample (italicized).

Supplemental Figure 4



Supplementary Figure 4. Preferential expression of CD103 by intraepithelial OT-I $CD8^+$ T cells after Ivag booster immunization. In vitro activated $CD45.1^+$OT-I $CD8^+$ T cells were transferred into naïve C57BL/6 mice ($5x10^6$/mouse) one day prior to ivag immunization with HPV PsV expressing OVA or a control plasmid or no treatment (n=3) mice per group. On day 4 after immunization, (**A-B**) cervicovaginal tissue were analyzed by confocal microscopy to assess CD103 expression by intraepithelial and submucosal infiltrating $CD8^+$ T cells. (**C**) Percentage of CD103 expressing cells by intraepithelial and submucosal $CD8^+$ T cells in HPV16-OVA immunized mice. Analysis of CD103 expression by intraepithelial and submucosal cervicovaginal $CD8^+$ T cells was performed on 300 $CD8^+$ counted cells per tissue. *P* values (****P≤0.0001) were determined by Mann-Whitney U.