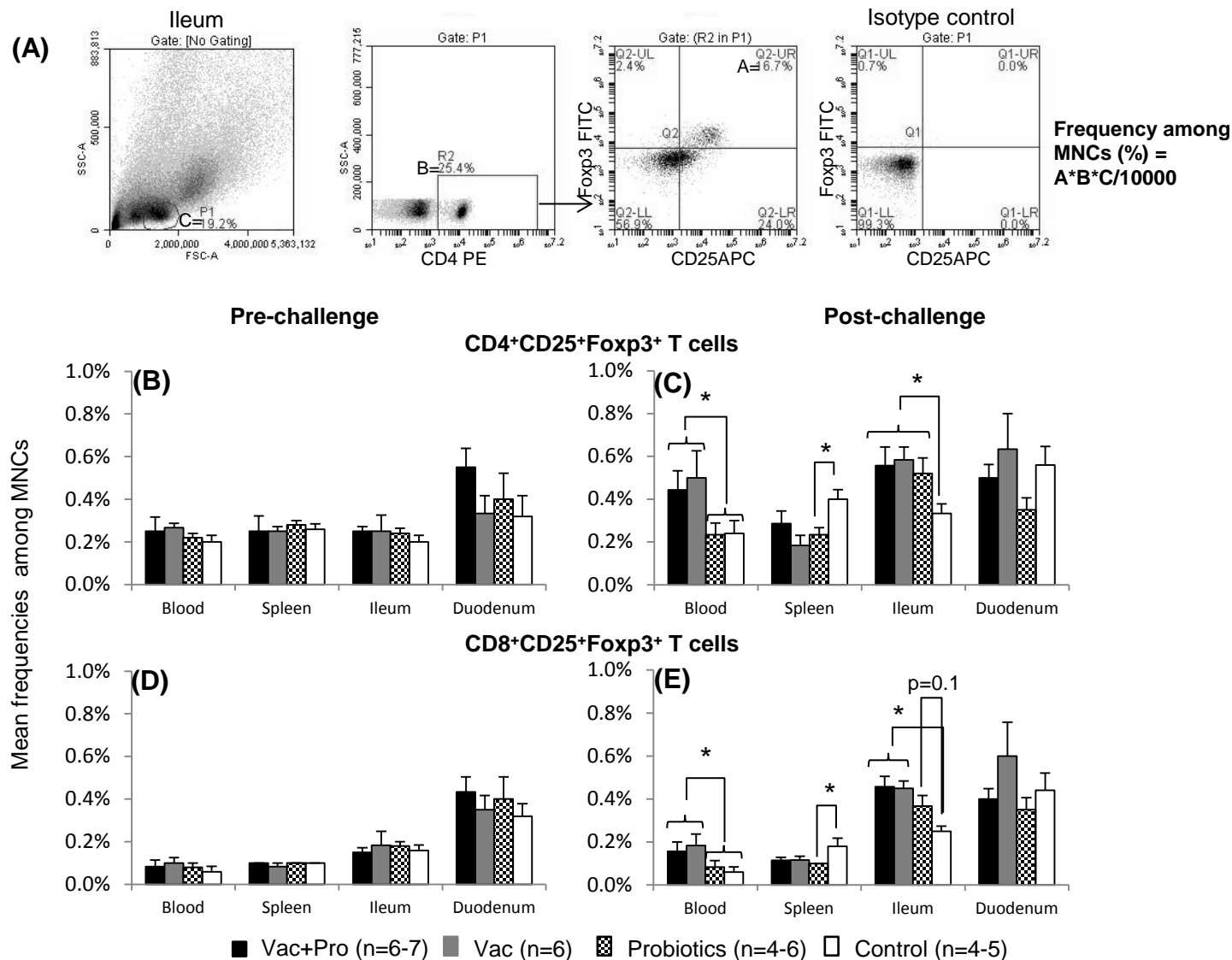
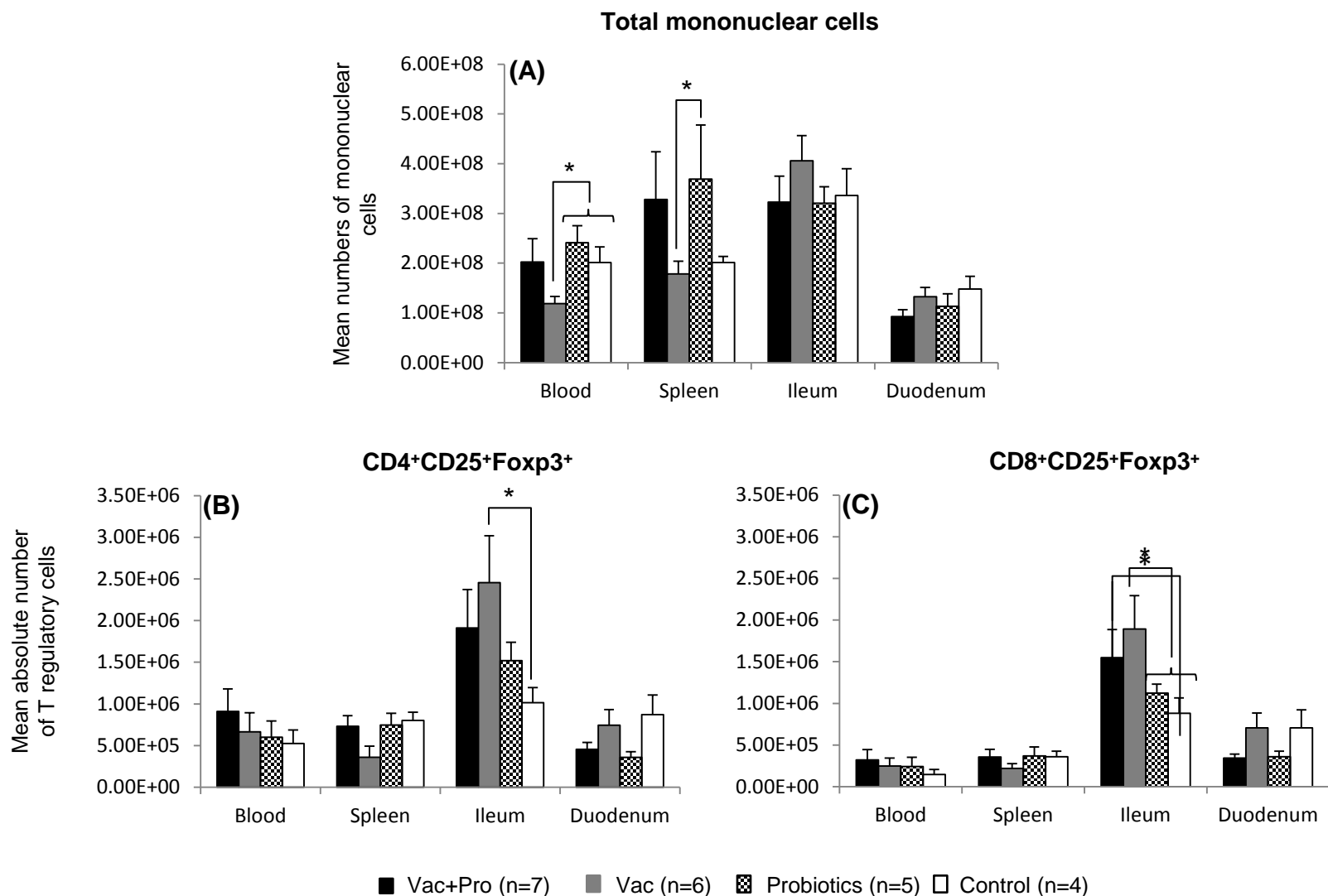


Supplementary Fig 1: Probiotic colonization did not affect activated CD8 T cells pre-challenge and CD4 T cells post-challenge in vaccinated animals.

Mean frequencies of CD8⁺CD25⁺Foxp3⁻ (pre-challenge, A) and CD4⁺CD25⁺Foxp3⁻ (post-challenge, B) T cells among CD4⁺ and CD8⁺ T cells subsets (\pm SEM) respectively in blood, spleen, ileum and duodenum. Vac+Pro = 3X AttHRV vaccinated + probiotic colonized (LGG+Bb12), Vac = 3X AttHRV vaccinated only, Probiotics = probiotic colonized only, Control = non-vaccinated and non-colonized control.



Supplementary Fig 2: Probiotic colonization and/or vaccination increased CD4 and CD8 Treg in blood and ileum post-VirHRV challenge. Representative dot plot of frequencies of ileal CD4⁺CD25⁺Fopx3⁺ T cells from Vac+Pro group (A) and formula for determining frequencies of these cells among total MNCs. Mean frequencies (n=4-7/group pre and post-challenge) of CD4⁺CD25⁺Fopx3⁺ (B = pre-challenge, C = post-challenge), CD8⁺CD25⁺Fopx3⁺ (D = pre-challenge, E = post-challenge) and CD4⁺CD25⁻Fopx3⁺ T cells (F = pre-and post-challenge) ± SEM in blood, spleen, ileum and duodenum. Significant differences between groups are indicated with * (p<0.05), as determined by non-parametric Kruskal-wallis rank sum test. Vac+Pro = 3X AttHRV vaccinated + probiotic colonized (LGG+Bb12), Vac = 3X AttHRV vaccinated only, Probiotics = probiotic colonized only, Control = non-vaccinated and non-colonized control.



Supplementary Fig. 3: Vaccination increased absolute numbers of intestinal natural CD4 and CD8 T regulatory cells post-challenge.

Mean absolute numbers of mononuclear cells (A), CD4+CD25+Foxp3+ (B) and CD8+CD25+Foxp3+ (C) cells \pm SEM post-challenge in blood, spleen, ileum and duodenum in different groups. Significant differences between groups are indicated with * ($p < 0.05$), as determined by non-parametric Kruskal-wallis rank sum test. Vac+Pro = 3X AttHRV vaccinated + probiotic colonized (LGG+Bb12), Vac = 3X AttHRV vaccinated only, Probiotics = probiotic colonized only, Control = non-vaccinated and non-colonized control

1 **Supplementary Table I:** Mean probiotic (LGG+Bb12) counts (\pm standard deviation) in small and large intestines of probiotic
 2 colonized groups.

| Intestinal sections | Pre-challenge (CFU/g)* | | Post-challenge (CFU/g)* | |
|------------------------|---|---|---|---|
| | PID27/PCD0** | | PID34/PCD7** | |
| | Vac+Pro (n=5) | Pro (n=5) | Vac+Pro [†] (n=3) | Pro (n=6) |
| Duodenum | $5.5 \times 10^4 (\pm 4.2 \times 10^4)$ | $7.8 \times 10^5 (\pm 1.2 \times 10^6)$ | $7.9 \times 10^4 (\pm 6.9 \times 10^4)$ | $1.7 \times 10^7 (\pm 2.0 \times 10^7)$ |
| Jejunum | $1.5 \times 10^7 (\pm 2.2 \times 10^7)$ | $9.6 \times 10^5 (\pm 1.0 \times 10^6)$ | $1.1 \times 10^4 (\pm 1.3 \times 10^4)$ | $1.4 \times 10^7 (\pm 1.7 \times 10^6)$ |
| Ileum | $7.2 \times 10^6 (\pm 7.5 \times 10^6)$ | $3.8 \times 10^6 (\pm 2.9 \times 10^6)$ | $1.4 \times 10^5 (\pm 3.7 \times 10^4)$ | $1.5 \times 10^8 (\pm 6.1 \times 10^7)$ |
| cecum | $1.7 \times 10^{10} (\pm 1.6 \times 10^{10})^A$ | $5.2 \times 10^{10} (\pm 5.9 \times 10^{10})^A$ | $2.4 \times 10^7 (\pm 1.2 \times 10^7)^B$ | $1.0 \times 10^7 (\pm 5.5 \times 10^7)^B$ |
| colon | $1.3 \times 10^{10} (\pm 1.4 \times 10^{10})^A$ | $1.7 \times 10^{10} (\pm 2.0 \times 10^{10})^A$ | $6.6 \times 10^7 (\pm 8.8 \times 10^7)^B$ | $8.3 \times 10^5 (\pm 2.6 \times 10^8)^B$ |

3 * CFU/g = Colony forming units/gram

4 ** Probiotic counts for different tissues were compared within the group (Vac+Pro or Pro) at pre- (PID27/PCD0) and post-challenge
 5 (PID34/PCD7) time-points. Different superscript upper-case letters differ significantly (determined by non-parametric Kruskal-wallis
 6 rank sum test)

7 [†] In Vac+Pro group, intestinal tissues were collected from only 3 out of 7 pigs-