**Figure S1.** 4-12% SDS-PAGE gel showing T3SP preparations used to elicit antigen recall responses.

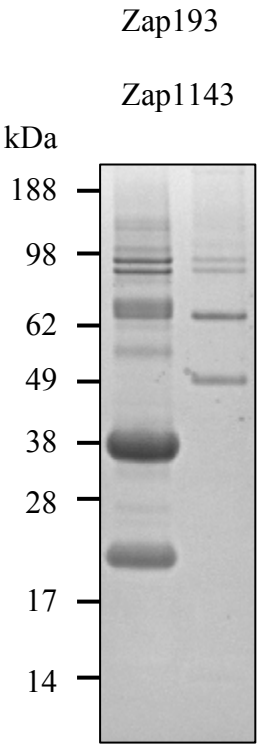
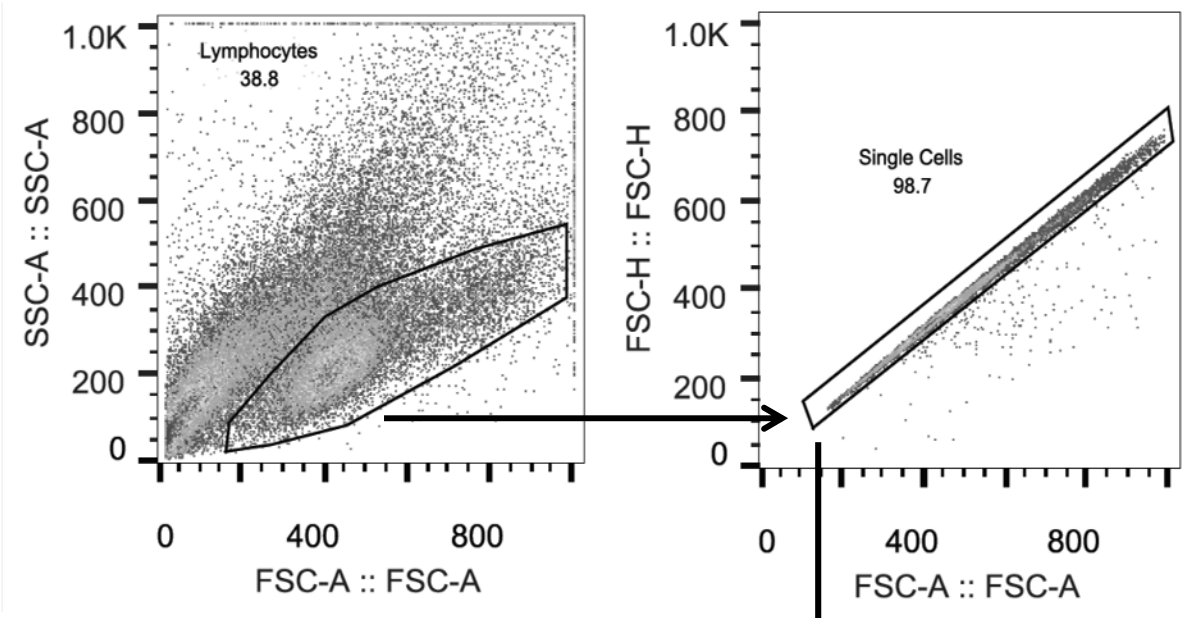
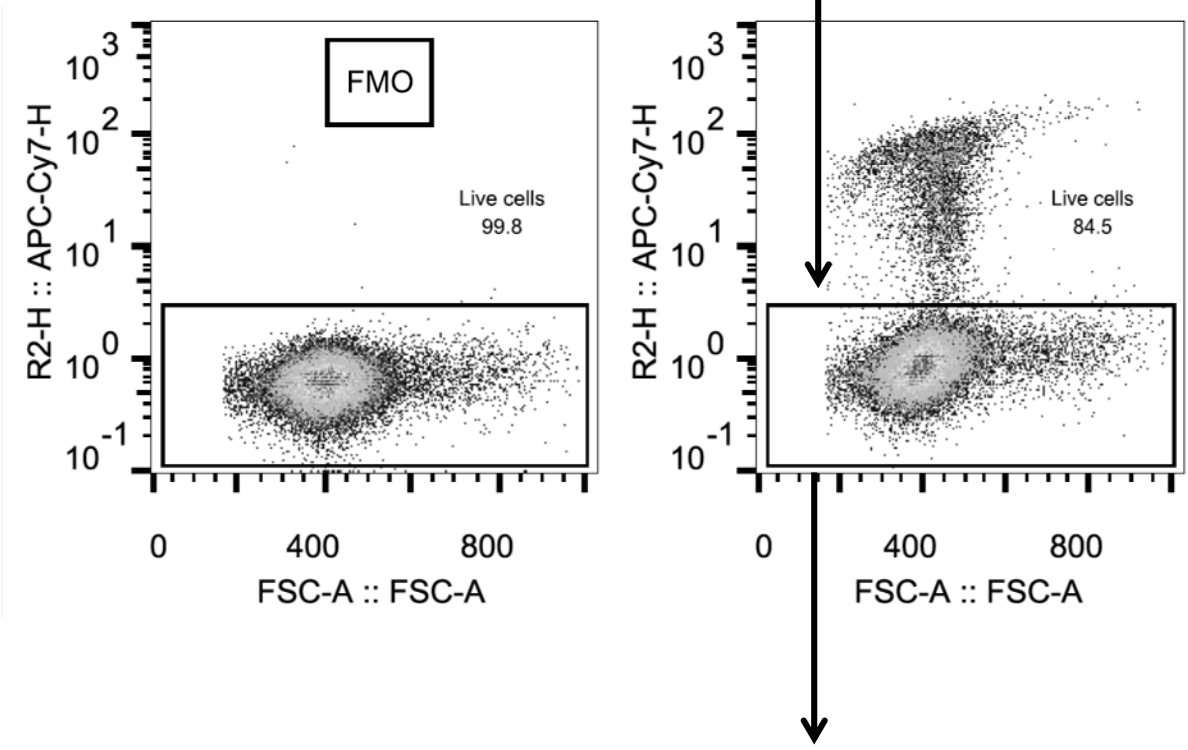


**Figure S2.** Gating strategy used for analysis of Ki67 expression by *ex-vivo* re-stimulated rectal lymph node cells.

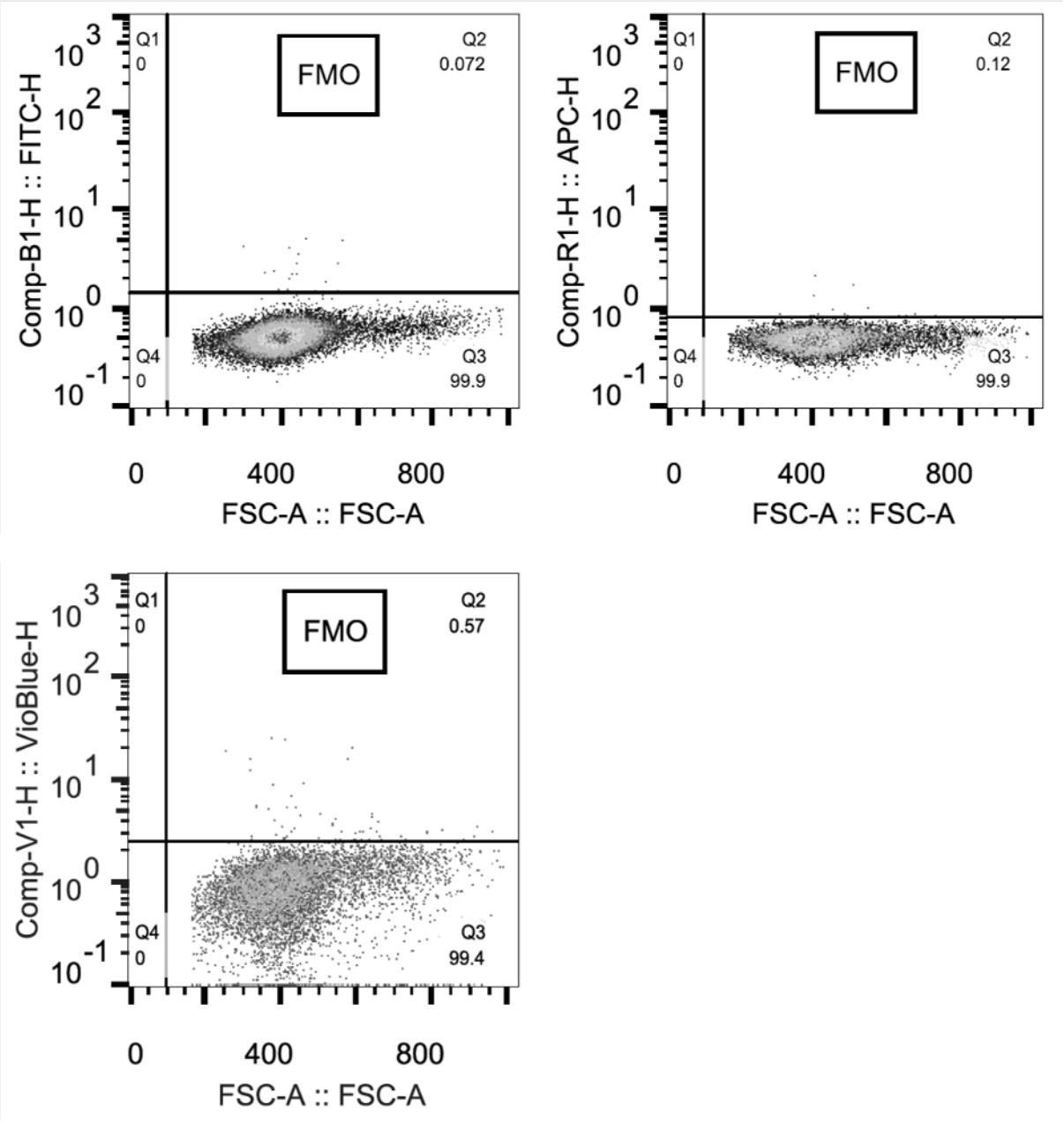
a. Morphology:



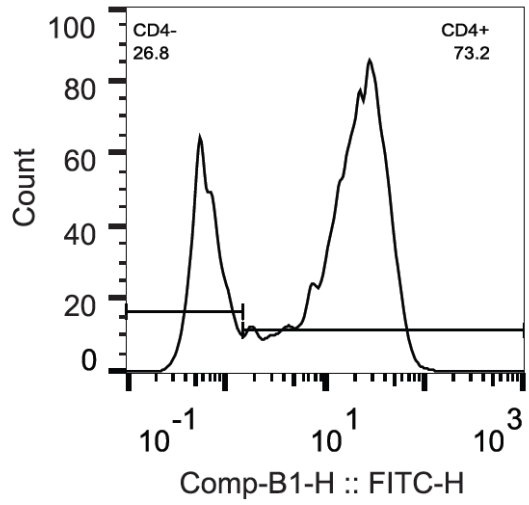

b. Vital stain:

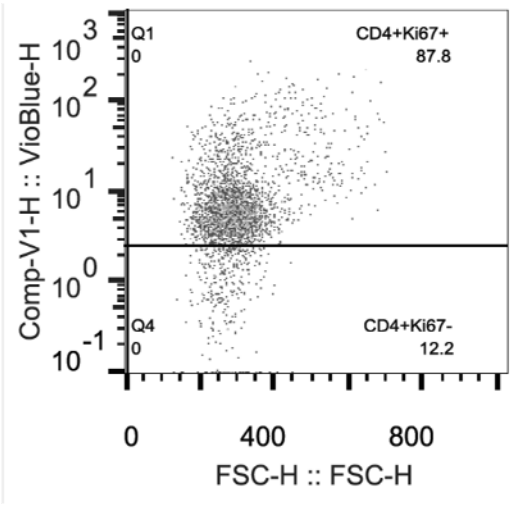


c. Fluorescence minus one (FMO) controls:

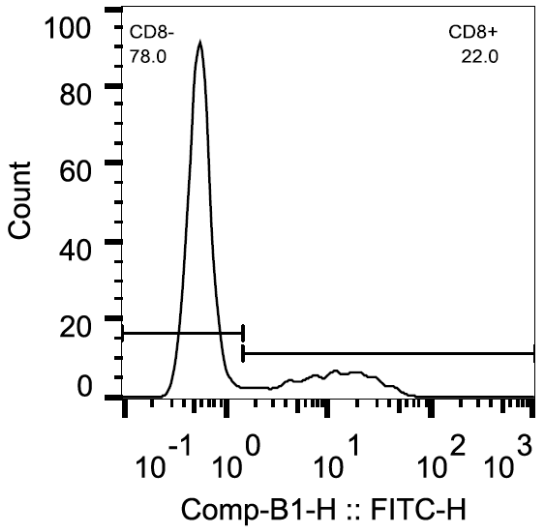


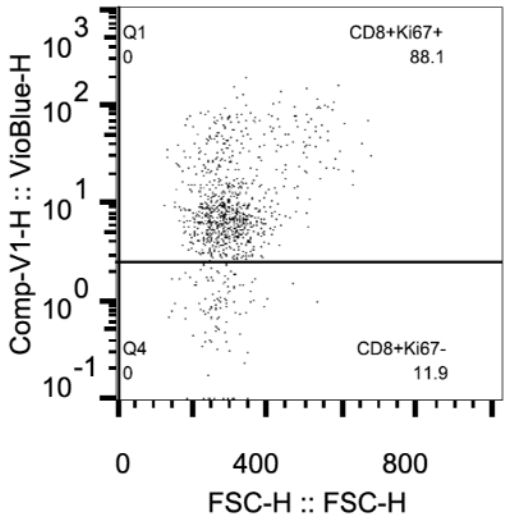
d. $CD4^+$ cell gating:



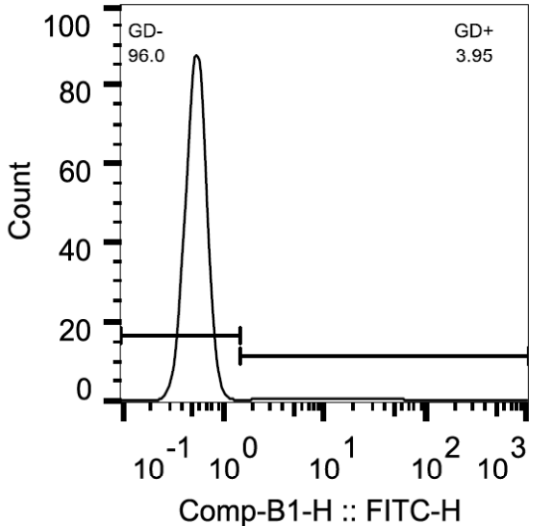



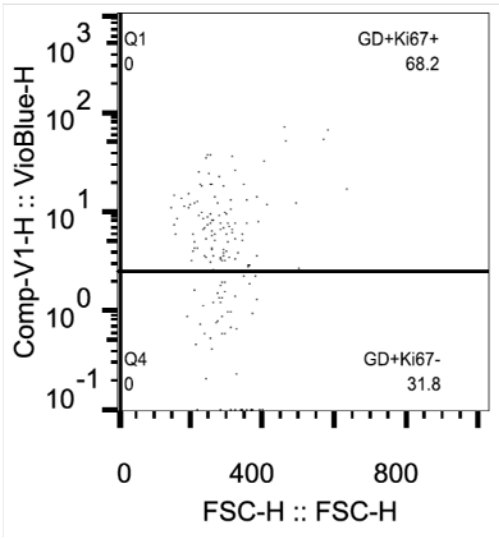

e. $CD8^+$ cell gating:



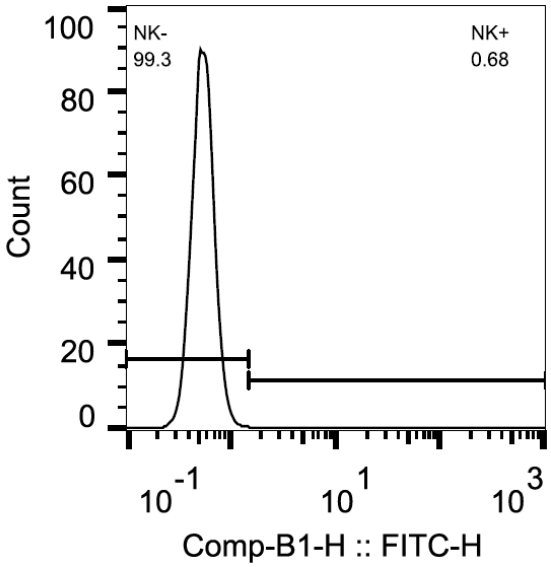




f. TcR1-N24(δ)$^+$ cell gating:



g. NKp46$^{+}$ cell gating:



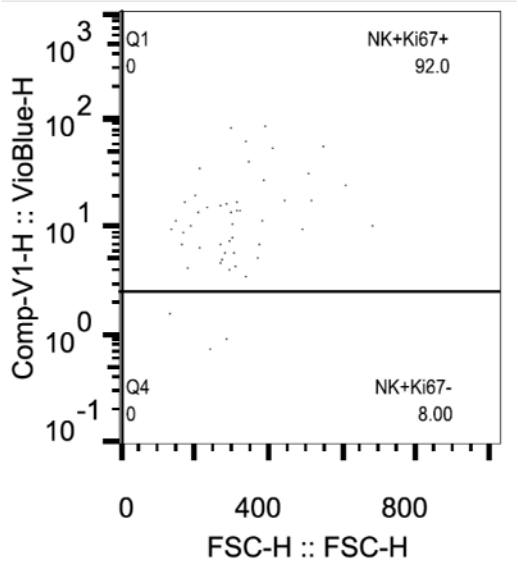



**Table S1.** External primers used for cloning of gene fragments for standard curve plasmid generation.

| Gene | Direction | Sequence |
|---|---|---|
| FoxP3 | F | GGAGTGGGTTTCCAGGGAGCCA |
| | R | TGCCTGGCAGTGCTTGAGGAA |
| T-bet | F | GGGCTTCCAACAATGCGACCCA |
| | R | AAGTTCTCCCGGAATCCTTTGGC |
| IL17 | F | CCGGAGCACAAACTCCAGAAGGC |
| | R | TGATGGTCCACCTTCCCTTCAGC |
| IL22 | F | CGAAGCGCTGCTACGTGCTGAA |
| | R | TCTGGATGTGCTGGTTGTCAGACTC |
| RORC | F | CCGGGCCTACAACGCTGACA |
| | R | AAAGGCCAGCTCCAGATTGCAC |
| GATA3 | F | ACCATACGTCCCCGAGTACAGC |
| | R | TACCTGCCCTTCTTGCTGCCGA |

**Table S2.** Internal primers used for RT-qPCR analysis of gene expression.

| Gene | Direction | Sequence | Reference |
|---|---|---|---|
| FoxP3 | F | GACAGCACCCTTTCGACTGT | |
| | R | CCACTTGCAGACGCCATTTG | |
| GATA3 | F | TGTGTGAACTGTGGGGCGACAT | |
| | R | TACACAGGTAATGCCCGGTTCCGT | |
| IFNγ | F | CTTGAATGGCAGCTCTGAGAAA | (1) |
| | R | TGCAGATCATCCACCGGAAT | |
| T-bet | F | GGGCTTCCAACAATGCGACCCA | |
| | R | AAGTTCTCCCGGAATCCTTTGGC | |
| IL17 | F | CCGGAGCACAAACTCCAGAAGGC | |
| | R | TGATGGTCCACCTTCCCTTCAGC | |
| RORC | F | GAGTGCCTTGCGCTTTTCA | (1) |
| | R | AAAGGCCAGCTCCAGATTGCAC | |
| TNFα | F | TCTACCAGGGAGGAGTCTTCCA | (1) |
| | R | TGCACCCTCACAGGGCGATGAT | |
| IL10 | F | GCGGCGCTGTCATCGCTTTC | |
| | R | GATGAAGATGTCAAACTCACTCATGG | (1) |
| IL4 | F | GCCACA CGTGCT TGAACAAA | (2) |
| | R | TGCTTGCCAAGC TGTTGAGA | |
| IL22 | F | GTGCTGTTCCCCCAATCTGA | |
| | R | AGCTTTTTGCTGAGCCTGGA | |
| IL13 | F | GGATGACACTGCAGTTGGAGA | |
| | R | TCTGCTACAGCCCTCAAGGA | |
| $TGF\beta_1$ | F | CGCTGCCCGAGGCCATACTG | |
| | R | GCTCAGGCTCCGTTTCGGCA | |

**Table S3.** qPCR standard curve dynamic range.

| Gene | Gene copies/well | | Regression $r^2$ range (all plates) |
|---|---|---|---|
| | **Top standard** | **Bottom standard** | |
| ATP5B | $10^6$ | $10^3$ | 0.990-0.995 |
| GAPDH | $10^6$ | $10^3$ | 0.998-0.999 |
| FoxP3 | $10^7$ | $10^2$ | 0.991-0.995 |
| GATA3 | $10^7$ | $10^3$ | 0.996-0.996 |
| IFNγ | $10^7$ | $10^2$ | 0.997-0.999 |
| T-bet | $10^7$ | $10^3$ | 0.995-0.998 |
| IL17 | $10^7$ | $10^4$ | 0.996-0.999 |
| RORC | $10^7$ | $10^2$ | 0.993-0.999 |
| TNFα | $10^7$ | $10^2$ | 0.994-0.999 |
| IL10 | $10^6$ | $10^2$ | 0.993-0.997 |
| IL4 | $10^7$ | $10^2$ | 0.994-0.998 |
| IL22 | $10^8$ | $10^3$ | 0.996-0.997 |
| IL13 | $10^7$ | $10^3$ | 0.983-0.993 |
| $TGF\beta_1$ | $10^7$ | $10^1$ | 0.994-0.996 |

**Table S4.** Test results for the fixed effects of the linear mixed models used to analyse changes in gene expression at the terminal rectum.

| Gene | Fixed Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|---|
| FoxP3 | Log10(Expression pre-challenge) | 1 | 6 | 5.31 | 0.0607 |
| FoxP3 | Experimental group | 2 | 6 | 1.63 | 0.2729 |
| FoxP3 | Days post challenge | 3 | 21 | 1.92 | 0.1575 |
| FoxP3 | Days*group interaction | 6 | 21 | 2.35 | 0.0679 |
| GATA3 | Log10(Expression pre-challenge) | 1 | 6 | 29.71 | 0.0016 |
| GATA3 | Experimental group | 2 | 6 | 0.75 | 0.5105 |
| GATA3 | Days post challenge | 3 | 21 | 4.64 | 0.0122 |
| GATA3 | Days*group interaction | 6 | 21 | 1.09 | 0.402 |
| IFNγ | Log10(Expression pre-challenge) | 1 | 6 | 9.87 | 0.02 |
| IFNγ | Experimental group | 2 | 6 | 3.25 | 0.1107 |
| IFNγ | Days post challenge | 3 | 21 | 1.49 | 0.245 |
| IFNγ | Days*group interaction | 6 | 21 | 4.11 | 0.007 |
| IL10 | Log10(Expression pre-challenge) | 1 | 6 | 15.96 | 0.0072 |
| IL10 | Experimental group | 2 | 6 | 3.76 | 0.0875 |
| IL10 | Days post challenge | 3 | 21 | 3.02 | 0.0528 |
| IL10 | Days*group interaction | 6 | 21 | 2.98 | 0.0288 |
| IL17 | Log10(Expression pre-challenge) | 1 | 6 | 31.23 | 0.0014 |
| IL17 | Experimental group | 2 | 6 | 3.53 | 0.097 |
| IL17 | Days post challenge | 3 | 21 | 2.45 | 0.0917 |
| IL17 | Days*group interaction | 6 | 21 | 0.26 | 0.9506 |
| IL22 | Log10(Expression pre-challenge) | 1 | 6 | 21.39 | 0.0036 |
| IL22 | Experimental group | 2 | 6 | 4.81 | 0.0566 |
| IL22 | Days post challenge | 3 | 21 | 2.97 | 0.0551 |
| IL22 | Days*group interaction | 6 | 21 | 0.69 | 0.6567 |
| RORC | Log10(Expression pre-challenge) | 1 | 6 | 101.22 | <.0001 |
| RORC | Experimental group | 2 | 6 | 0.56 | 0.5959 |
| RORC | Days post challenge | 3 | 21 | 2.27 | 0.1105 |
| RORC | Days*group interaction | 6 | 21 | 0.29 | 0.9354 |
| Tbet | Log10(Expression pre-challenge) | 1 | 6 | 36.15 | 0.001 |
| Tbet | Experimental group | 2 | 6 | 10.66 | 0.0106 |
| Tbet | Days post challenge | 3 | 21 | 8.46 | 0.0007 |
| Tbet | Days*group interaction | 6 | 21 | 11.77 | <.0001 |
| TGFβ1 | Log10(Expression pre-challenge) | 1 | 6 | 5.54 | 0.0567 |
| TGFβ1 | Experimental group | 2 | 6 | 25.44 | 0.0012 |
| TGFβ1 | Days post challenge | 3 | 21 | 5.36 | 0.0067 |
| TGFβ1 | Days*group interaction | 6 | 21 | 15.27 | <.0001 |
| TNFα | Log10(Expression pre-challenge) | 1 | 6 | 13.9 | 0.0098 |
| TNFα | Experimental group | 2 | 6 | 4.34 | 0.0683 |
| TNFα | Days post challenge | 3 | 21 | 1.48 | 0.2496 |

| | | | | | |
|---|---|---|---|---|---|
| TNFα | Days*group interaction | 6 | 21 | 0.32 | 0.9188 |

**Table S5.** Predicted means and 95% confidence intervals from the linear mixed models used to analyse changes in gene expression at the terminal rectum.

| Gene | Group | Days post challenge | Estimated Fold Change | 95% CIs | | t Value | p |
|---|---|---|---|---|---|---|---|
| | | | | Lower | Upper | | |
| IFNg | Control | 7 | 0.73 | 0.36 | 1.51 | -0.89 | 0.3837 |
| IFNg | PT2128 | 7 | 0.96 | 0.59 | 1.58 | -0.15 | 0.8815 |
| IFNg | PT32 | 7 | 2.79 | 1.72 | 4.52 | 4.41 | 0.0002 |
| IFNg | Control | 14 | 1.14 | 0.55 | 2.34 | 0.37 | 0.7146 |
| IFNg | PT2128 | 14 | 0.81 | 0.49 | 1.32 | -0.90 | 0.3802 |
| IFNg | PT32 | 14 | 2.35 | 1.45 | 3.81 | 3.67 | 0.0014 |
| IFNg | Control | 21 | 1.08 | 0.53 | 2.23 | 0.23 | 0.8185 |
| IFNg | PT2128 | 21 | 2.47 | 1.51 | 4.04 | 3.83 | 0.001 |
| IFNg | PT32 | 21 | 1.62 | 1.00 | 2.63 | 2.09 | 0.0492 |
| IFNg | Control | 31 | 1.15 | 0.56 | 2.36 | 0.40 | 0.6906 |
| IFNg | PT2128 | 31 | 1.16 | 0.71 | 1.90 | 0.63 | 0.5357 |
| IFNg | PT32 | 31 | 0.86 | 0.53 | 1.40 | -0.63 | 0.5328 |
| Tbet | Control | 7 | 0.38 | 0.21 | 0.69 | -3.42 | 0.0026 |
| Tbet | PT2128 | 7 | 1.69 | 1.11 | 2.59 | 2.60 | 0.0169 |
| Tbet | PT32 | 7 | 2.93 | 1.93 | 4.44 | 5.37 | <.0001 |
| Tbet | Control | 14 | 1.11 | 0.62 | 2.00 | 0.38 | 0.7079 |
| Tbet | PT2128 | 14 | 0.50 | 0.33 | 0.76 | -3.40 | 0.0027 |
| Tbet | PT32 | 14 | 0.61 | 0.40 | 0.92 | -2.49 | 0.0214 |
| Tbet | Control | 21 | 0.22 | 0.12 | 0.39 | -5.39 | <.0001 |
| Tbet | PT2128 | 21 | 2.09 | 1.37 | 3.19 | 3.63 | 0.0016 |
| Tbet | PT32 | 21 | 0.55 | 0.36 | 0.83 | -3.03 | 0.0064 |
| Tbet | Control | 31 | 0.53 | 0.29 | 0.95 | -2.26 | 0.0346 |
| Tbet | PT2128 | 31 | 0.34 | 0.23 | 0.53 | -5.25 | <.0001 |
| Tbet | PT32 | 31 | 0.58 | 0.38 | 0.88 | -2.70 | 0.0133 |
| TNFa | Control | 7 | 0.97 | 0.58 | 1.64 | -0.11 | 0.9146 |
| TNFa | PT2128 | 7 | 0.99 | 0.68 | 1.43 | -0.07 | 0.9442 |
| TNFa | PT32 | 7 | 1.29 | 0.89 | 1.87 | 1.42 | 0.1706 |
| TNFa | Control | 14 | 1.28 | 0.76 | 2.16 | 0.99 | 0.3324 |
| TNFa | PT2128 | 14 | 1.05 | 0.73 | 1.53 | 0.30 | 0.7696 |
| TNFa | PT32 | 14 | 1.26 | 0.86 | 1.82 | 1.27 | 0.2182 |
| TNFa | Control | 21 | 0.75 | 0.44 | 1.26 | -1.16 | 0.2577 |
| TNFa | PT2128 | 21 | 0.97 | 0.67 | 1.41 | -0.16 | 0.8772 |
| TNFa | PT32 | 21 | 1.38 | 0.95 | 2.00 | 1.79 | 0.0887 |
| TNFa | Control | 31 | 0.74 | 0.44 | 1.24 | -1.22 | 0.2342 |
| TNFa | PT2128 | 31 | 0.81 | 0.55 | 1.17 | -1.20 | 0.2419 |
| TNFa | PT32 | 31 | 0.94 | 0.65 | 1.37 | -0.33 | 0.7434 |
| GATA3 | Control | 7 | 1.33 | 0.57 | 3.13 | 0.70 | 0.4897 |
| GATA3 | PT2128 | 7 | 1.11 | 0.60 | 2.06 | 0.35 | 0.7277 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| GATA3 | PT32 | 7 | 0.76 | 0.45 | 1.29 | -1.07 | 0.2989 |
| GATA3 | Control | 14 | 0.94 | 0.40 | 2.19 | -0.16 | 0.872 |
| GATA3 | PT2128 | 14 | 1.96 | 1.06 | 3.64 | 2.27 | 0.0337 |
| GATA3 | PT32 | 14 | 1.25 | 0.74 | 2.12 | 0.88 | 0.3894 |
| GATA3 | Control | 21 | 1.34 | 0.57 | 3.13 | 0.70 | 0.4886 |
| GATA3 | PT2128 | 21 | 2.23 | 1.20 | 4.14 | 2.70 | 0.0134 |
| GATA3 | PT32 | 21 | 2.65 | 1.56 | 4.50 | 3.83 | 0.001 |
| GATA3 | Control | 31 | 0.51 | 0.22 | 1.19 | -1.66 | 0.1109 |
| GATA3 | PT2128 | 31 | 1.34 | 0.72 | 2.48 | 0.97 | 0.3414 |
| GATA3 | PT32 | 31 | 0.85 | 0.50 | 1.44 | -0.66 | 0.5168 |
| IL17 | Control | 7 | 0.73 | 0.42 | 1.28 | -1.17 | 0.2558 |
| IL17 | PT2128 | 7 | 1.08 | 0.73 | 1.60 | 0.39 | 0.699 |
| IL17 | PT32 | 7 | 1.32 | 0.89 | 1.97 | 1.46 | 0.1596 |
| IL17 | Control | 14 | 0.81 | 0.46 | 1.41 | -0.80 | 0.4314 |
| IL17 | PT2128 | 14 | 0.80 | 0.54 | 1.18 | -1.19 | 0.2466 |
| IL17 | PT32 | 14 | 0.88 | 0.59 | 1.31 | -0.66 | 0.5146 |
| IL17 | Control | 21 | 0.63 | 0.36 | 1.10 | -1.72 | 0.0992 |
| IL17 | PT2128 | 21 | 0.85 | 0.57 | 1.26 | -0.88 | 0.3862 |
| IL17 | PT32 | 21 | 0.87 | 0.59 | 1.30 | -0.72 | 0.4823 |
| IL17 | Control | 31 | 0.57 | 0.32 | 0.99 | -2.11 | 0.0467 |
| IL17 | PT2128 | 31 | 0.55 | 0.37 | 0.81 | -3.16 | 0.0047 |
| IL17 | PT32 | 31 | 0.70 | 0.47 | 1.04 | -1.90 | 0.0713 |
| IL22 | Control | 7 | 1.22 | 0.42 | 3.54 | 0.38 | 0.706 |
| IL22 | PT2128 | 7 | 0.43 | 0.20 | 0.89 | -2.40 | 0.0257 |
| IL22 | PT32 | 7 | 0.65 | 0.31 | 1.34 | -1.24 | 0.2271 |
| IL22 | Control | 14 | 0.73 | 0.25 | 2.11 | -0.62 | 0.541 |
| IL22 | PT2128 | 14 | 0.78 | 0.37 | 1.63 | -0.71 | 0.4876 |
| IL22 | PT32 | 14 | 1.95 | 0.94 | 4.04 | 1.89 | 0.0722 |
| IL22 | Control | 21 | 1.52 | 0.52 | 4.42 | 0.82 | 0.4238 |
| IL22 | PT2128 | 21 | 0.64 | 0.31 | 1.34 | -1.25 | 0.2252 |
| IL22 | PT32 | 21 | 1.55 | 0.75 | 3.23 | 1.25 | 0.2245 |
| IL22 | Control | 31 | 0.55 | 0.19 | 1.61 | -1.15 | 0.2633 |
| IL22 | PT2128 | 31 | 0.36 | 0.17 | 0.76 | -2.86 | 0.0094 |
| IL22 | PT32 | 31 | 0.44 | 0.21 | 0.92 | -2.31 | 0.0314 |
| FoxP3 | Control | 7 | 4.67 | 1.50 | 14.54 | 2.82 | 0.0102 |
| FoxP3 | PT2128 | 7 | 0.35 | 0.13 | 0.96 | -2.16 | 0.0423 |
| FoxP3 | PT32 | 7 | 1.62 | 0.67 | 3.89 | 1.14 | 0.2675 |
| FoxP3 | Control | 14 | 1.89 | 0.61 | 5.89 | 1.17 | 0.2564 |
| FoxP3 | PT2128 | 14 | 1.48 | 0.54 | 4.06 | 0.80 | 0.4307 |
| FoxP3 | PT32 | 14 | 2.14 | 0.89 | 5.16 | 1.81 | 0.0851 |
| FoxP3 | Control | 21 | 0.98 | 0.32 | 3.06 | -0.03 | 0.9736 |
| FoxP3 | PT2128 | 21 | 0.44 | 0.16 | 1.22 | -1.67 | 0.1091 |
| FoxP3 | PT32 | 21 | 2.77 | 1.15 | 6.67 | 2.42 | 0.0249 |
| FoxP3 | Control | 31 | 1.11 | 0.36 | 3.45 | 0.19 | 0.8511 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| FoxP3 | PT2128 | 31 | 0.56 | 0.20 | 1.54 | -1.20 | 0.2441 |
| FoxP3 | PT32 | 31 | 1.08 | 0.45 | 2.61 | 0.19 | 0.8519 |
| IL10 | Control | 7 | 0.67 | 0.38 | 1.18 | -1.48 | 0.1528 |
| IL10 | PT2128 | 7 | 0.58 | 0.40 | 0.84 | -3.04 | 0.0062 |
| IL10 | PT32 | 7 | 1.08 | 0.73 | 1.60 | 0.40 | 0.693 |
| IL10 | Control | 14 | 0.65 | 0.37 | 1.14 | -1.60 | 0.1252 |
| IL10 | PT2128 | 14 | 0.81 | 0.56 | 1.16 | -1.22 | 0.2352 |
| IL10 | PT32 | 14 | 0.99 | 0.67 | 1.47 | -0.04 | 0.9695 |
| IL10 | Control | 21 | 1.59 | 0.90 | 2.81 | 1.69 | 0.1051 |
| IL10 | PT2128 | 21 | 0.66 | 0.46 | 0.96 | -2.34 | 0.0295 |
| IL10 | PT32 | 21 | 1.46 | 0.98 | 2.17 | 1.99 | 0.0593 |
| IL10 | Control | 31 | 0.42 | 0.24 | 0.74 | -3.17 | 0.0047 |
| IL10 | PT2128 | 31 | 1.02 | 0.71 | 1.48 | 0.12 | 0.9045 |
| IL10 | PT32 | 31 | 0.92 | 0.62 | 1.36 | -0.45 | 0.6563 |
| TGFb | Control | 7 | 12.64 | 4.89 | 32.70 | 5.55 | <.0001 |
| TGFb | PT2128 | 7 | 0.05 | 0.02 | 0.11 | -8.14 | <.0001 |
| TGFb | PT32 | 7 | 1.27 | 0.81 | 1.98 | 1.12 | 0.2759 |
| TGFb | Control | 14 | 5.46 | 2.11 | 14.13 | 3.72 | 0.0013 |
| TGFb | PT2128 | 14 | 0.30 | 0.14 | 0.63 | -3.32 | 0.0032 |
| TGFb | PT32 | 14 | 2.38 | 1.53 | 3.71 | 4.07 | 0.0005 |
| TGFb | Control | 21 | 7.87 | 3.04 | 20.35 | 4.52 | 0.0002 |
| TGFb | PT2128 | 21 | 0.09 | 0.04 | 0.20 | -6.45 | <.0001 |
| TGFb | PT32 | 21 | 5.49 | 3.53 | 8.56 | 7.99 | <.0001 |
| TGFb | Control | 31 | 2.70 | 1.04 | 6.97 | 2.17 | 0.0416 |
| TGFb | PT2128 | 31 | 0.21 | 0.10 | 0.46 | -4.23 | 0.0004 |
| TGFb | PT32 | 31 | 2.48 | 1.59 | 3.86 | 4.25 | 0.0004 |
| RORC | Control | 7 | 1.03 | 0.57 | 1.85 | 0.10 | 0.9193 |
| RORC | PT2128 | 7 | 0.85 | 0.56 | 1.30 | -0.79 | 0.4387 |
| RORC | PT32 | 7 | 0.79 | 0.52 | 1.20 | -1.17 | 0.2541 |
| RORC | Control | 14 | 0.50 | 0.28 | 0.89 | -2.48 | 0.0218 |
| RORC | PT2128 | 14 | 0.56 | 0.37 | 0.85 | -2.88 | 0.0089 |
| RORC | PT32 | 14 | 0.57 | 0.38 | 0.87 | -2.79 | 0.0111 |
| RORC | Control | 21 | 0.61 | 0.34 | 1.10 | -1.74 | 0.0957 |
| RORC | PT2128 | 21 | 0.57 | 0.38 | 0.87 | -2.78 | 0.0112 |
| RORC | PT32 | 21 | 0.77 | 0.51 | 1.17 | -1.30 | 0.2076 |
| RORC | Control | 31 | 0.48 | 0.27 | 0.86 | -2.61 | 0.0164 |
| RORC | PT2128 | 31 | 0.61 | 0.40 | 0.92 | -2.50 | 0.0206 |
| RORC | PT32 | 31 | 0.60 | 0.39 | 0.91 | -2.56 | 0.0183 |

**Table S6.** Stimulation Index Tukey comparisons for data in Figure 4A. Pairwise comparisons were made between the Δ*sepL* and WT T3SP preparations at each lymph node (RLN/MLN/PsLN) and the same T3SP preparation between each lymph node.

| **Contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
|---|---|---|---|---|
| SepL,RLN-WT,RLN | 1.773215 | 1.162678 | 3.8 | 0.0058 |
| SepL,MLN-WT,MLN | 2.829324 | 1.162678 | 6.9 | <.0001 |
| SepL,PsLN-WT,PsLN | 1.463793 | 1.162678 | 2.528 | 0.1391 |
| | | | | |
| SepL,RLN-SepL,MLN | 1.762583 | 1.284968 | 2.26 | 0.2342 |
| SepL,RLN-SepL,PsLN | 15.71546 | 1.284968 | 10.986 | <.0001 |
| SepL,MLN-SepL,PsLN | 8.91615 | 1.284968 | 8.726 | <.0001 |
| | | | | |
| WT,RLN-WT,MLN | 2.81236 | 1.284968 | 4.124 | 0.0024 |
| WT,RLN-WT,PsLN | 12.97315 | 1.284968 | 10.222 | <.0001 |
| WT,MLN-WT,PsLN | 4.612903 | 1.284968 | 6.098 | <.0001 |

**Table S7.** IFNγ Release Index Tukey comparisons for data in Figure 4B. Pairwise comparisons were made between the Δ*sepL* and WT T3SP preparations across all lymph nodes combined and for both T3SP preparations combined between each lymph node (RLN/MLN/PsLN).

| **Contrast** | **Estimate (fold change)** | **SE** | **z** | **p.value** |
|---|---|---|---|---|
| sepL-WT | 2.43204 | 1.2198 | 4.473 | <0.0001 |
| RLN-MLN | 1.68822 | 1.58056 | 1.144 | 0.61659 |
| RLN-PsLN | 7.22104 | 1.58056 | 4.319 | <0.0001 |
| MLN-PsLN | 4.2773 | 1.58056 | 3.175 | 0.00578 |

**Table S8.** Thymidine incorporation Tukey comparisons for data in Figure 4C. Pairwise comparisons were made between each experimental group (Control/PT2128/PT32) for both T3SP preparations combined within each lymph node (RLN/MLN/PsLN).

| **Node = RLN:** | | | | |
|---|---|---|---|---|
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.751470835 | 1.56838435 | -0.635 | 0.8024 |
| Control-PT32 | 1.985953658 | 1.56838435 | 1.525 | 0.2964 |
| PT2128-PT32 | 2.642755407 | 1.44406353 | 2.645 | 0.0355 |
| | | | | |
| **Node = MLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 1.38363787 | 1.56838435 | 0.722 | 0.7531 |
| Control-PT32 | 1.252827276 | 1.56838435 | 0.501 | 0.8715 |
| PT2128-PT32 | 0.905458938 | 1.44406353 | -0.27 | 0.9606 |
| | | | | |
| **Node = PsLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.491327429 | 1.56838435 | -1.579 | 0.2725 |
| Control-PT32 | 1.673746493 | 1.56838435 | 1.144 | 0.4962 |
| PT2128-PT32 | 3.406580609 | 1.44406353 | 3.336 | 0.0071 |

**Table S9.** IFNγ release Tukey comparisons for data in Figure 4D. Pairwise comparisons were made between each experimental group (Control/PT2128/PT32) within each combination of lymph node (RLN/MLN/PsLN) and *ex vivo* stimulant (ConA/Media/WT/WT LPS/sepL/sepL LPS).

| ***Ex vivo* stimulant = ConA, Node = RLN:** | | | | |
|---|---|---|---|---|
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 1.155679661 | 2.377298 | 0.167 | 0.9847 |
| Control-PT32 | 2.3756318 | 2.377298 | 0.999 | 0.5811 |
| PT2128-PT32 | 2.055614441 | 2.056001 | 1 | 0.5814 |
| | | | | |
| ***Ex vivo* stimulant = Media, Node = RLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.243083723 | 2.377298 | -1.633 | 0.2421 |
| Control-PT32 | 0.739760128 | 2.377298 | -0.348 | 0.9355 |
| PT2128-PT32 | 3.043231916 | 2.056001 | 1.544 | 0.2818 |
| | | | | |
| ***Ex vivo* stimulant = WT, Node = RLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.262015926 | 2.377298 | -1.547 | 0.279 |
| Control-PT32 | 3.204437312 | 2.377298 | 1.345 | 0.3781 |
| PT2128-PT32 | 12.22993362 | 2.056001 | 3.474 | 0.0035 |
| | | | | |
| ***Ex vivo* stimulant = WT LPS, Node = RLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.47105741 | 2.377298 | -0.869 | 0.6622 |
| Control-PT32 | 2.132288281 | 2.377298 | 0.874 | 0.659 |
| PT2128-PT32 | 4.526599593 | 2.056001 | 2.095 | 0.1039 |
| | | | | |
| ***Ex vivo* stimulant = sepL, Node = RLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.53463088 | 2.377298 | -0.723 | 0.7511 |
| Control-PT32 | 3.044201951 | 2.377298 | 1.286 | 0.4104 |
| PT2128-PT32 | 5.694025661 | 2.056001 | 2.413 | 0.0525 |
| | | | | |
| ***Ex vivo* stimulant = sepL LPS, Node = RLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.361006457 | 2.377298 | -1.177 | 0.473 |
| Control-PT32 | 1.360236424 | 2.377298 | 0.355 | 0.9329 |
| PT2128-PT32 | 3.767900537 | 2.056001 | 1.84 | 0.1698 |
| | | | | |

| ***Ex vivo* stimulant = ConA, Node = MLN:** | | | | |
|---|---|---|---|---|
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 2.889143678 | 2.377298 | 1.225 | 0.4446 |
| Control-PT32 | 0.939340094 | 2.377298 | -0.072 | 0.9971 |
| PT2128-PT32 | 0.325127512 | 2.056001 | -1.559 | 0.2753 |
| | | | | |
| ***Ex vivo* stimulant = Media, Node = MLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.60769764 | 2.377298 | -0.575 | 0.834 |
| Control-PT32 | 0.29250592 | 2.377298 | -1.42 | 0.3394 |
| PT2128-PT32 | 0.481334621 | 2.056001 | -1.014 | 0.5722 |
| | | | | |
| ***Ex vivo* stimulant = WT, Node = MLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.655027239 | 2.377298 | -0.489 | 0.8771 |
| Control-PT32 | 1.267055125 | 2.377298 | 0.273 | 0.9597 |
| PT2128-PT32 | 1.934354865 | 2.056001 | 0.915 | 0.6339 |
| | | | | |
| ***Ex vivo* stimulant = WT LPS, Node = MLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 1.177620914 | 2.377298 | 0.189 | 0.9805 |
| Control-PT32 | 0.843120502 | 2.377298 | -0.197 | 0.9788 |
| PT2128-PT32 | 0.715952385 | 2.056001 | -0.464 | 0.8886 |
| | | | | |
| ***Ex vivo* stimulant = sepL, Node = MLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 1.336551569 | 2.377298 | 0.335 | 0.9401 |
| Control-PT32 | 1.203697033 | 2.377298 | 0.214 | 0.9751 |
| PT2128-PT32 | 0.90059902 | 2.056001 | -0.145 | 0.9884 |
| | | | | |
| ***Ex vivo* stimulant = sepL LPS, Node = MLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.902498835 | 2.377298 | -0.118 | 0.9923 |
| Control-PT32 | 0.537846244 | 2.377298 | -0.716 | 0.7552 |
| PT2128-PT32 | 0.595952272 | 2.056001 | -0.718 | 0.7543 |
| | | | | |
| ***Ex vivo* stimulant = ConA, Node = PSLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.907359102 | 2.377298 | -0.112 | 0.9931 |
| Control-PT32 | 4.37622608 | 2.377298 | 1.705 | 0.2143 |
| PT2128-PT32 | 4.823036515 | 2.056001 | 2.183 | 0.0866 |
| | | | | |

| ***Ex vivo* stimulant = Media, Node = PSLN:** | | | | |
|---|---|---|---|---|
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.190852393 | 2.377298 | -1.913 | 0.1466 |
| Control-PT32 | 1.362735438 | 2.377298 | 0.357 | 0.9321 |
| PT2128-PT32 | 7.140258419 | 2.056001 | 2.727 | 0.0251 |
| | | | | |
| ***Ex vivo* stimulant = WT, Node = PSLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.205716639 | 2.377298 | -1.826 | 0.1725 |
| Control-PT32 | 5.902994791 | 2.377298 | 2.05 | 0.1117 |
| PT2128-PT32 | 28.69478533 | 2.056001 | 4.657 | 0.0001 |
| | | | | |
| ***Ex vivo* stimulant = WT LPS, Node = PSLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.369841447 | 2.377298 | -1.149 | 0.4896 |
| Control-PT32 | 3.927955328 | 2.377298 | 1.58 | 0.2645 |
| PT2128-PT32 | 10.62064689 | 2.056001 | 3.278 | 0.0061 |
| | | | | |
| ***Ex vivo* stimulant = sepL, Node = PSLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.419754905 | 2.377298 | -1.002 | 0.5791 |
| Control-PT32 | 5.607820191 | 2.377298 | 1.991 | 0.1258 |
| PT2128-PT32 | 13.35974904 | 2.056001 | 3.597 | 0.0025 |
| | | | | |
| ***Ex vivo* stimulant = sepL LPS, Node = PSLN:** | | | | |
| **contrast** | **Estimate (fold change)** | **SE** | **t.ratio** | **p.value** |
| Control-PT2128 | 0.283437109 | 2.377298 | -1.456 | 0.3214 |
| Control-PT32 | 2.50573437 | 2.377298 | 1.061 | 0.543 |
| PT2128-PT32 | 8.840530361 | 2.056001 | 3.024 | 0.0119 |

**Table S10.** Ki67$^+$ versus Ki67$^-$ rectal lymph node cell generalised linear model analysis of deviance tables.

| **CD4$^+$** | Df | Deviance | Residual Df | Residual Deviance | F | Pr(>F) |
|---|---|---|---|---|---|---|
| NULL | | | 59 | 49000 | | |
| Group | 2 | 1728 | 57 | 47272 | 13.5664 | 2.14E-05 |
| Stimulant | 3 | 44127 | 54 | 3145 | 230.9522 | 2.20E-16 |
| Group:Stimulant | 6 | 215 | 48 | 2930 | 0.5614 | 0.7587 |
| | | | | | | |
| **CD8$^+$** | Df | Deviance | Residual Df | Residual Deviance | F | Pr(>F) |
| NULL | 59 | 22757.1 | | | | |
| Group | 2 | 828.2 | 57 | 21928.9 | 7.5486 | 0.001411 |
| Stimulant | 3 | 19247.2 | 54 | 2681.7 | 116.9524 | 2.20E-16 |
| Group:Stimulant | 6 | 64.1 | 48 | 2617.5 | 0.1948 | 0.976762 |
| | | | | | | |
| **γδTCR$^+$** | Df | Deviance | Residual Df | Residual Deviance | F | Pr(>F) |
| NULL | 58 | 2197.29 | | | | |
| Group | 2 | 172.15 | 56 | 2025.14 | 6.3101 | 0.003737 |
| Stimulant | 3 | 1315.93 | 53 | 709.21 | 32.1573 | 1.88E-11 |
| Group:Stimulant | 6 | 64.99 | 47 | 644.22 | 0.7941 | 0.57926 |
| | | | | | | |
| **NKp46$^+$** | Df | Deviance | Residual Df | Residual Deviance | F | Pr(>F) |
| NULL | 59 | 468.48 | | | | |
| Group | 2 | 59.699 | 57 | 408.78 | 7.3698 | 0.001617 |
| Stimulant | 3 | 188.32 | 54 | 220.46 | 15.4986 | 3.50E-07 |
| Group:Stimulant | 6 | 51.205 | 48 | 169.26 | 2.1071 | 0.069641 |

**Table S11.** Ki67$^{+}$ versus Ki67$^{-}$ rectal lymph node cell Tukey comparison tables (logit scale) for data in Figure 5. Pairwise comparisons were made between each *ex vivo* stimulant (ConA/Media/WT/LPS) within each experimental group.

| **CD4** | | | | |
|---|---|---|---|---|
| Group = Control | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 4.078103 | 0.659439 | 6.184198 | <.0001 |
| ConA-WT | 2.409936 | 0.455698 | 5.288448 | <.0001 |
| ConA-LPS | 3.539619 | 0.711232 | 4.976746 | <.0001 |
| Media-WT | -1.66817 | 0.758423 | -2.19952 | 0.1234 |
| Media-LPS | -0.53848 | 0.934556 | -0.57619 | 0.9392 |
| WT-LPS | 1.129683 | 0.803863 | 1.405318 | 0.496 |
| | | | | |
| Group = PT2128 | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 3.791895 | 0.344895 | 10.99435 | <.0001 |
| ConA-WT | 1.901821 | 0.189423 | 10.04009 | <.0001 |
| ConA-LPS | 3.528389 | 0.362439 | 9.735134 | <.0001 |
| Media-WT | -1.89007 | 0.361278 | -5.23163 | <.0001 |
| Media-LPS | -0.26351 | 0.475397 | -0.55429 | 0.9454 |
| WT-LPS | 1.626568 | 0.378062 | 4.30238 | 0.0001 |
| | | | | |
| Group = PT32 | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 3.567561 | 0.455581 | 7.830791 | <.0001 |
| ConA-WT | 2.406768 | 0.292653 | 8.223953 | <.0001 |
| ConA-LPS | 3.432117 | 0.460661 | 7.450416 | <.0001 |
| Media-WT | -1.16079 | 0.509831 | -2.27682 | 0.1035 |
| Media-LPS | -0.13544 | 0.621683 | -0.21787 | 0.9964 |
| WT-LPS | 1.025349 | 0.514375 | 1.993387 | 0.1905 |
| | | | | |
| **CD8** | | | | |
| Group = Control | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 3.540999 | 0.826799 | 4.28278 | 0.0001 |
| ConA-WT | 2.698219 | 0.547318 | 4.929893 | <.0001 |
| ConA-LPS | 3.483781 | 0.775991 | 4.489461 | <.0001 |
| Media-WT | -0.84278 | 0.836516 | -1.00749 | 0.745 |
| Media-LPS | -0.05722 | 1.001181 | -0.05715 | 0.9999 |
| WT-LPS | 0.785562 | 0.786336 | 0.999016 | 0.75 |
| | | | | |

| Group = PT2128 | | | | |
|---|---|---|---|---|
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 3.239418 | 0.332213 | 9.751028 | <.0001 |
| ConA-WT | 2.198237 | 0.260266 | 8.446124 | <.0001 |
| ConA-LPS | 3.396709 | 0.348758 | 9.73945 | <.0001 |
| Media-WT | -1.04118 | 0.290367 | -3.58575 | 0.0019 |
| Media-LPS | 0.157291 | 0.371761 | 0.423097 | 0.9745 |
| WT-LPS | 1.198472 | 0.309159 | 3.876555 | 0.0006 |
| | | | | |
| Group = PT32 | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 3.010154 | 0.520678 | 5.781225 | <.0001 |
| ConA-WT | 2.095138 | 0.317463 | 6.599625 | <.0001 |
| ConA-LPS | 3.441522 | 0.51134 | 6.730398 | <.0001 |
| Media-WT | -0.91502 | 0.518703 | -1.76405 | 0.2909 |
| Media-LPS | 0.431368 | 0.655544 | 0.65803 | 0.9127 |
| WT-LPS | 1.346383 | 0.509329 | 2.643446 | 0.041 |
| | | | | |
| **γδTCR$^+$** | | | | |
| Group = Control | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 0.050874 | 0.654942 | 0.077677 | 0.9998 |
| ConA-WT | -0.91047 | 0.550979 | -1.65245 | 0.3493 |
| ConA-LPS | -0.52537 | 0.645408 | -0.81401 | 0.8479 |
| Media-WT | -0.96134 | 0.524664 | -1.8323 | 0.2581 |
| Media-LPS | -0.57624 | 0.623094 | -0.92481 | 0.7916 |
| WT-LPS | 0.385097 | 0.512713 | 0.751096 | 0.8762 |
| | | | | |
| Group = PT2128 | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | -0.40109 | 0.218584 | -1.83496 | 0.2569 |
| ConA-WT | -1.10958 | 0.184644 | -6.00928 | <.0001 |
| ConA-LPS | -0.16425 | 0.238394 | -0.68898 | 0.9013 |
| Media-WT | -0.70848 | 0.205295 | -3.45105 | 0.0031 |
| Media-LPS | 0.236844 | 0.254725 | 0.929805 | 0.7889 |
| WT-LPS | 0.945328 | 0.226272 | 4.177834 | 0.0002 |

| | | | | |
|---|---|---|---|---|
| Group = PT32 | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 0.08338 | 0.304656 | 0.273686 | 0.9928 |
| ConA-WT | -1.24554 | 0.204482 | -6.09117 | <.0001 |
| ConA-LPS | -0.08588 | 0.294945 | -0.29116 | 0.9914 |
| Media-WT | -1.32892 | 0.291707 | -4.55566 | <.0001 |
| Media-LPS | -0.16926 | 0.360933 | -0.46894 | 0.9659 |
| WT-LPS | 1.159659 | 0.281549 | 4.118856 | 0.0002 |
| | | | | |
| **NKp46$^{+}$** | | | | |
| Group = Control | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 0.663033 | 0.45719 | 1.450237 | 0.468 |
| ConA-WT | -0.78495 | 0.541547 | -1.44947 | 0.4684 |
| ConA-LPS | 0.271985 | 0.496263 | 0.548066 | 0.9471 |
| Media-WT | -1.44799 | 0.48703 | -2.9731 | 0.0156 |
| Media-LPS | -0.39105 | 0.436121 | -0.89665 | 0.8066 |
| WT-LPS | 1.05694 | 0.523883 | 2.017513 | 0.1815 |
| | | | | |
| Group = PT2128 | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | -0.06269 | 0.355501 | -0.17636 | 0.9981 |
| ConA-WT | -0.80107 | 0.354101 | -2.26226 | 0.107 |
| ConA-LPS | -0.4181 | 0.393787 | -1.06175 | 0.7129 |
| Media-WT | -0.73837 | 0.341278 | -2.16355 | 0.1336 |
| Media-LPS | -0.35541 | 0.382298 | -0.92967 | 0.7889 |
| WT-LPS | 0.382964 | 0.380997 | 1.005164 | 0.7464 |
| | | | | |
| Group = PT32 | | | | |
| contrast | estimate | SE | z.ratio | p.value |
| ConA-Media | 0.835225 | 0.384027 | 2.174914 | 0.1303 |
| ConA-WT | -1.2282 | 0.442151 | -2.77778 | 0.0281 |
| ConA-LPS | 0.785173 | 0.379409 | 2.069464 | 0.1632 |
| Media-WT | -2.06342 | 0.401976 | -5.1332 | <.0001 |
| Media-LPS | -0.05005 | 0.33172 | -0.15089 | 0.9988 |
| WT-LPS | 2.013372 | 0.397567 | 5.064234 | <.0001 |

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