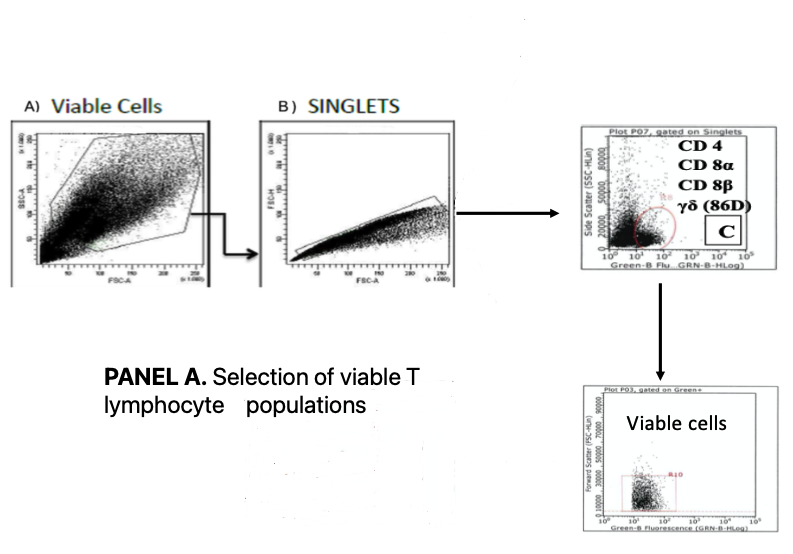
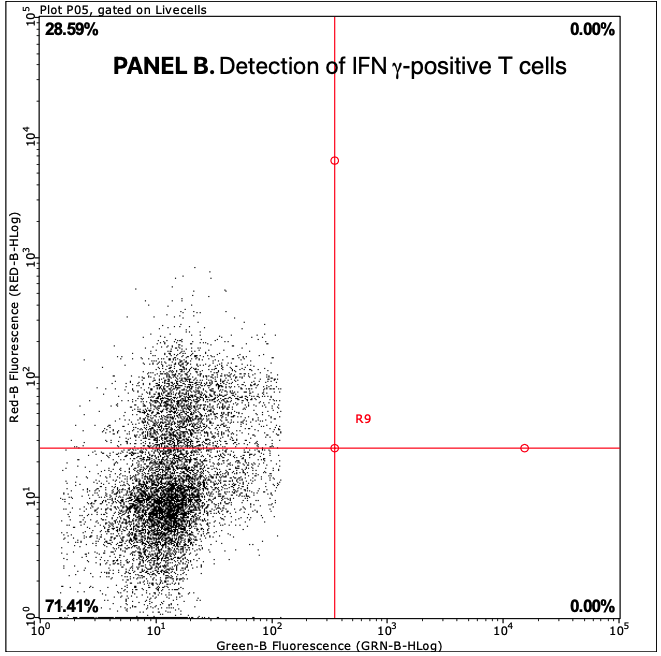
**Suppl. File 2**

**Detection of IFN g-positive T cell populations: protocol and gating strategy**

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**PCV2-specific, IFN-γ-positive T lymphocytes.**  PBMC of PCV2-vaccinated and/or infected pigs were grown over 24 hours with and without purified, Baculovirus-expressed PCV2 capsid protein (2 mg/mL). Different aliquots of PCV2-stimulated and control cells were surface-stained with monoclonal antibodies to porcine CD4, CD8a, CD8b and T cell Receptor (TcR) g/d, respectively, followed by Alexa Fluor® 488 F(ab')2 fragment of goat, anti-mouse IgG, IgM (H+L). Next, cells were fixed, permeabilized and stained with mAb CC302 to porcine IFN-g (BIO-RAD, code MCA 1783), conjugated with PE-Cy5.5 (Lightning-Link PE-Cy5.5 Antibody Labeling Kit, code 761-0010, Novus Biologicals Europe, Abingdon, UK). Cells were gated (Panel A) by a combination of forward and side scatter (A). Next, doublet and multiplet cells were discriminated from single cells in a FSC height / FSC area cytogram (B). After selecting T lymphocyte populations in a SSC x green fluorescence cytogram (C), viable cells were further selected in a FSC x green fluorescence cytogram as previously described (Walravens et al., 2002). Finally, such viable cells were checked for expression of IFN-γ in a green x red fluorescence cytogram (Panel B). The prevalence of IFN g-positive in ORF2 antigen/stimulated and control cultures was investigated by Fisher’s exact test. To define a positive response, the adopted threshold amounted to a 0.8% difference in prevalence between ORF2 antigen-stimulated and control T cells, which corresponds to P< 0.05 (significance threshold) for 5,000 cells examined on average.

Walravens K, Wellemans V, Weynants V, Boelaert F, deBergeyck V, Letesson JJ, et al. Analysis of the antigen-specific IFN-gamma producing T-cell subsets in cattle experimentally infected with Mycobacterium bovis. Vet Immunol Immunopathol 2002 Jan 1;84(1-2):29-41.