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## Neutrophils encompass a regulatory subset suppressing T cells at steady state in cattle

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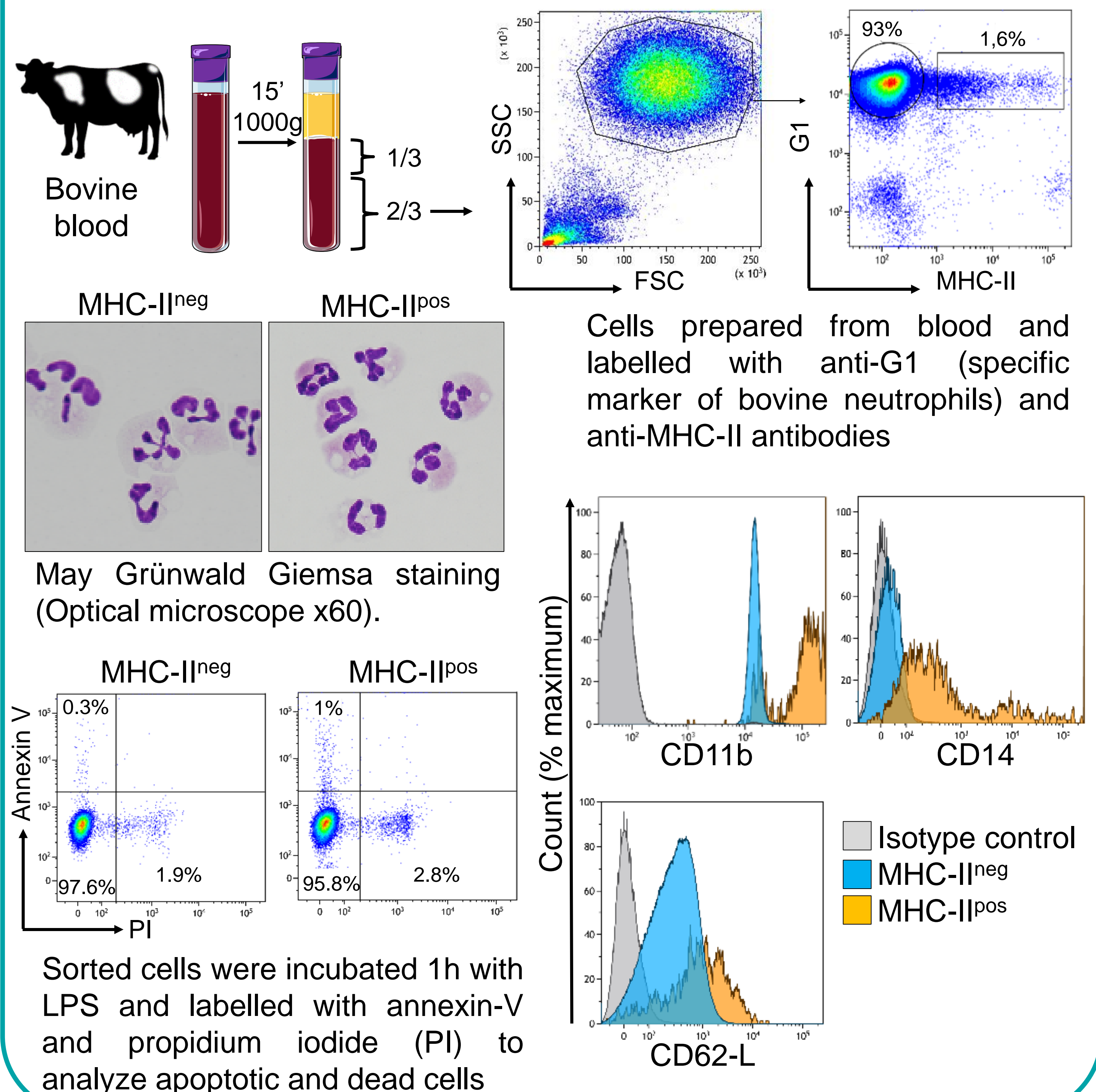
## Neutrophils in cattle

Neutrophils are major partners of the innate immune system and are considered the first line of defense against microbes. These phagocytes recognize invading microbes and produce large amounts of reactive oxygen species into phagocytic vacuoles to kill microbes. New roles for neutrophils bridging innate and adaptive immunity have also emerged. Neutrophils play an essential role in the control of infectious diseases in cattle, especially mastitis. As a biomarker, they are also the major indicator of inflammation during subclinical mastitis. Neutrophils are the main cell type responsible for the rise of milk somatic cell count during mammary infection.

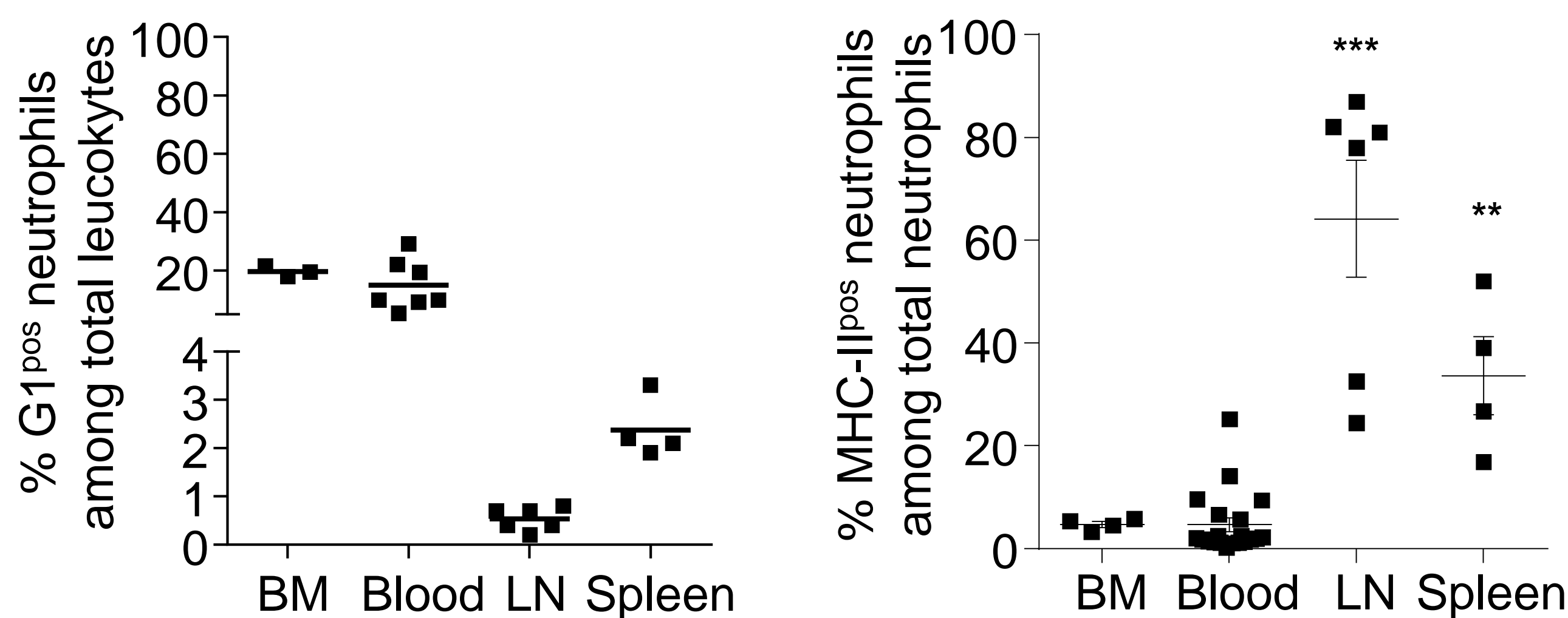
## Plasticity of neutrophils

Despite the major importance of neutrophils in bovine infectious diseases, neutrophils are also a double-edged sword. If their recruitment is not controlled, they can cause tissue damage. New populations of neutrophils have been recently described with role in regulation of inflammation and adaptative immune response. Although neutrophils diversity and plasticity are now well documented for mouse and human neutrophils, they remain unknown for domestic species including cattle. Here, we discovered and characterized a new population of regulatory neutrophils that displayed suppressive activity on T-cells at steady state in cattle.

## Neutrophils represent discrete populations in cattle blood



## MHC-II<sup>pos</sup> neutrophils are enriched in lymphoid organs

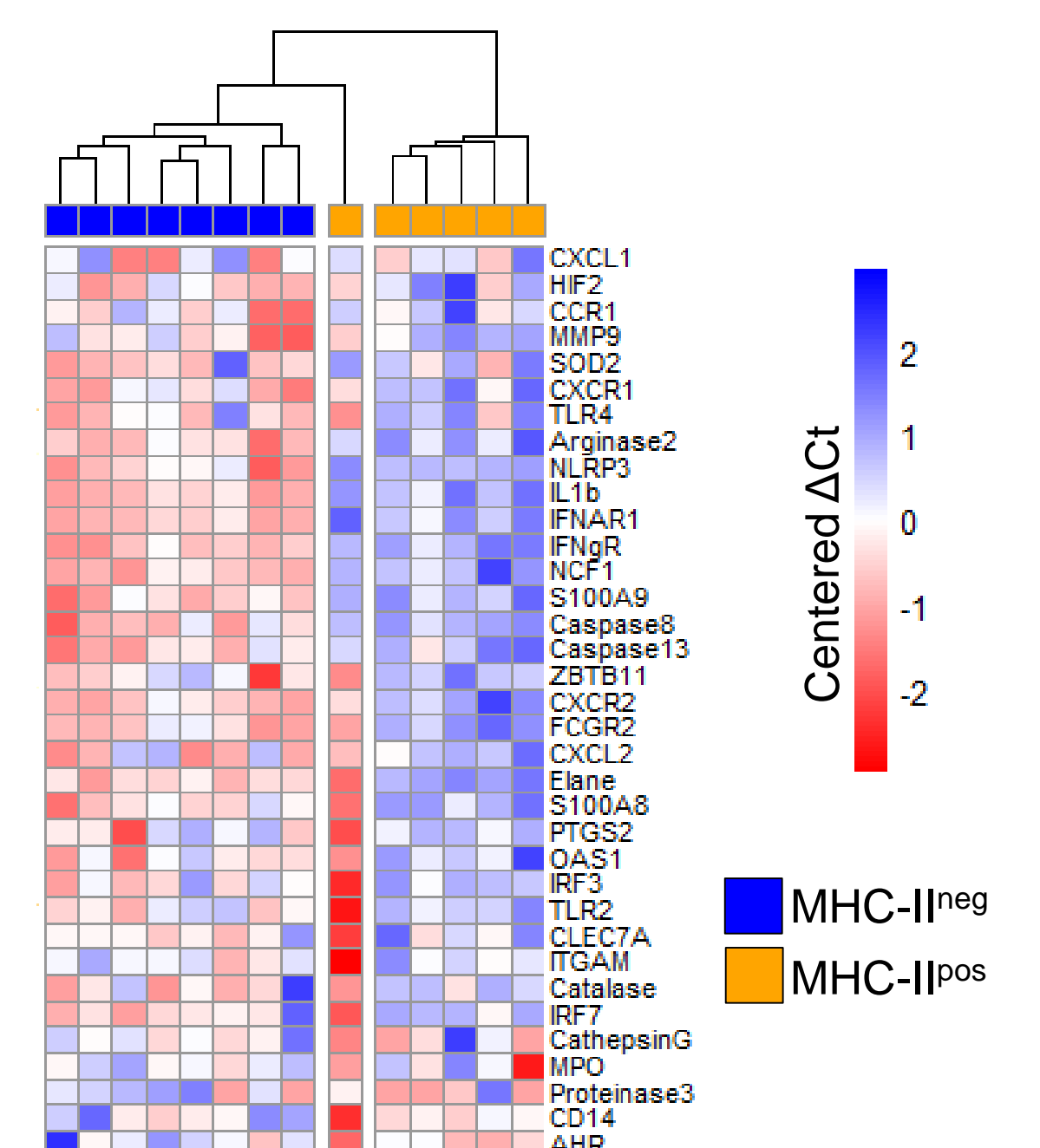


Cells collected and prepared from blood, bone marrow (BM), tracheobronchial lymph nodes (LN) and spleen, then labelled with anti-G1 and anti-MHC-II antibodies

## MHC-II<sup>neg</sup> and MHC-II<sup>pos</sup> neutrophils display distinct gene expression profiles

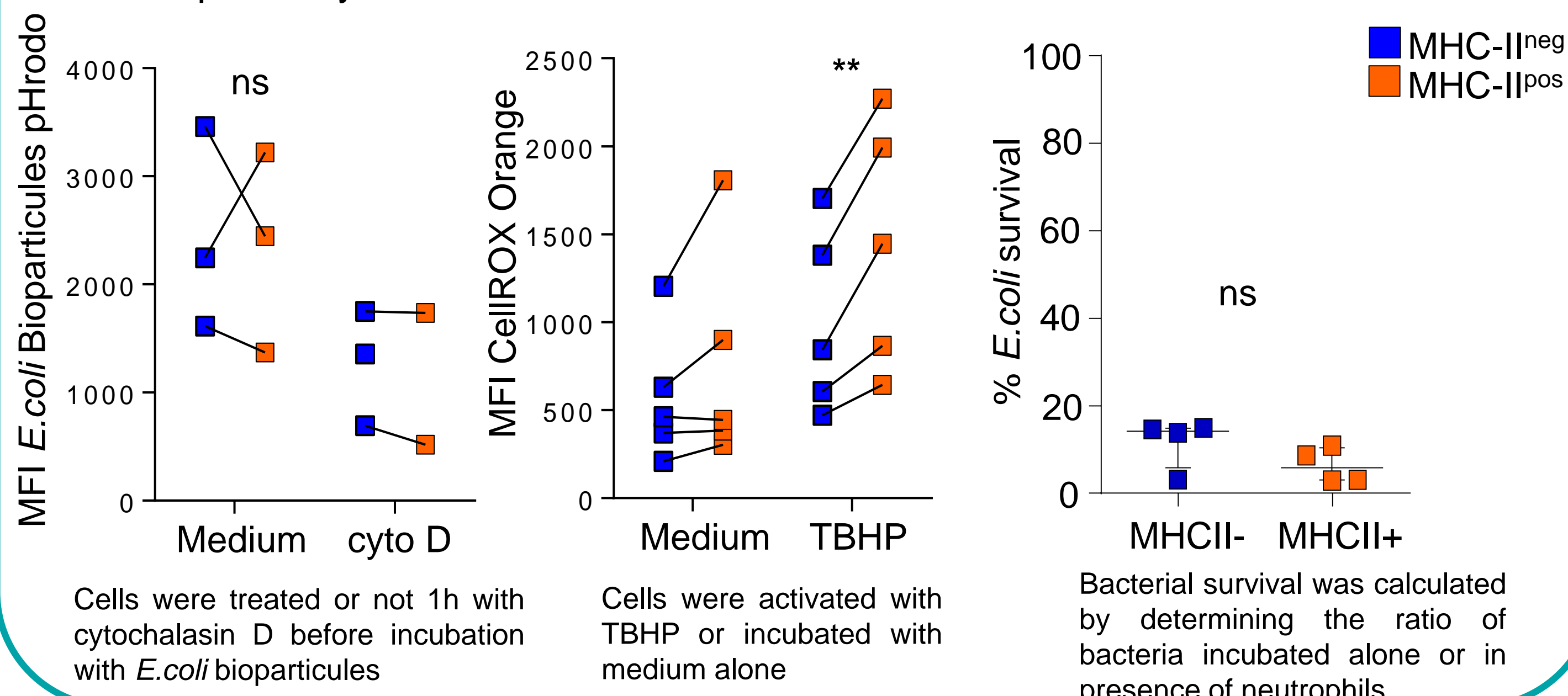
- ✓ Real Time PCR (Fluidigm biomark)
- ✓ mRNA expression normalized to the expression of three housekeeping genes to calculate the  $\Delta C_t$  values.
- ✓ Hierarchical clustering of gene expression performed on median centered  $\Delta C_t$  values, using the ward.D2 method. For each gene,  $\Delta C_t$  values were centered to the median  $\Delta C_t$  value.

n=8 for MHC-II<sup>neg</sup> ; n=6 for MHC-II<sup>pos</sup>



## MHC-II<sup>pos</sup> neutrophils produce higher levels of ROS than MHC-II<sup>neg</sup> but similarly phagocytose bioparticles and kill bacteria

Capacities of **phagocytosis**, **production of ROS** and **killing** of MHC-II<sup>neg</sup> and MHC-II<sup>pos</sup> neutrophils was measured after purification by cells sorting and using *E.coli* bioparticules pHrodo, CellROX detection kit (flow cytometry) and *E.coli* P4 strain respectively.

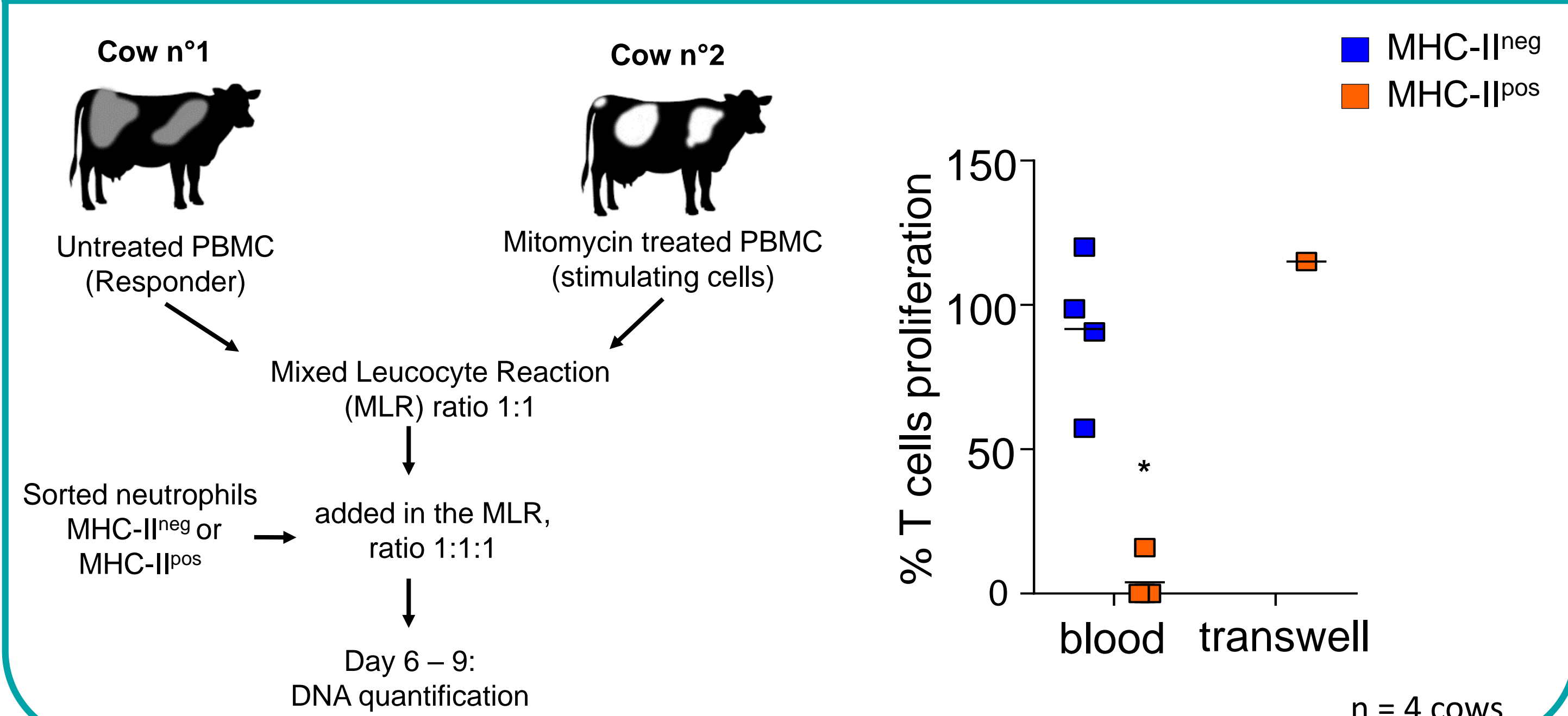


Cells were treated or not 1h with cytochalasin D before incubation with *E.coli* bioparticules

Cells were activated with TBHP or incubated with medium alone

Bacterial survival was calculated by determining the ratio of bacteria incubated alone or in presence of neutrophils

## MHC-II<sup>pos</sup> but not MHC-II<sup>neg</sup> neutrophils exert suppression on T cells at steady state



n = 4 cows

**Conclusion:** we characterized a new subset of regulatory neutrophils, able to suppress T cells. Next, we will investigate how they behave during clinical conditions such as mastitis, which remains one of the most important issue in dairy farming.