



MHC-II expressing neutrophils circulate in blood and milk during mastitis and show high microbicidal activity

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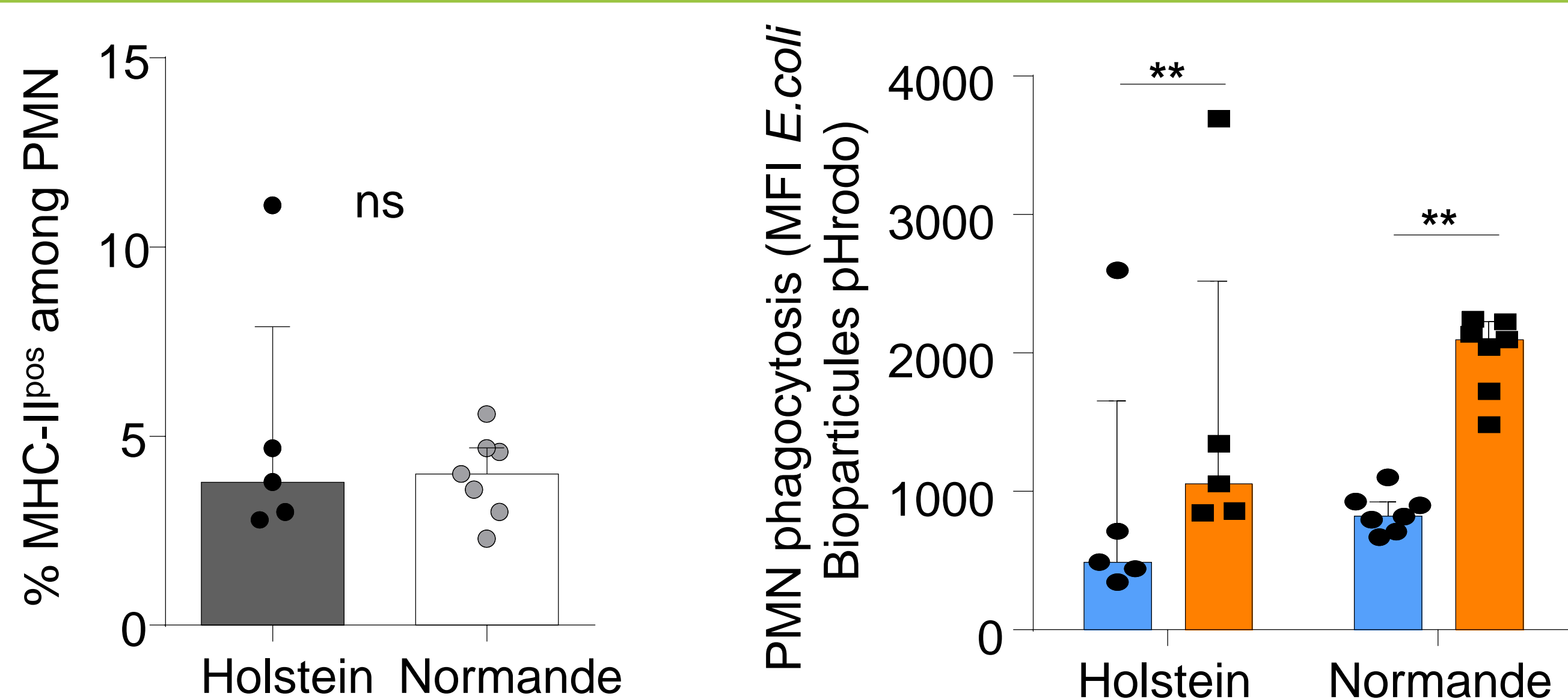
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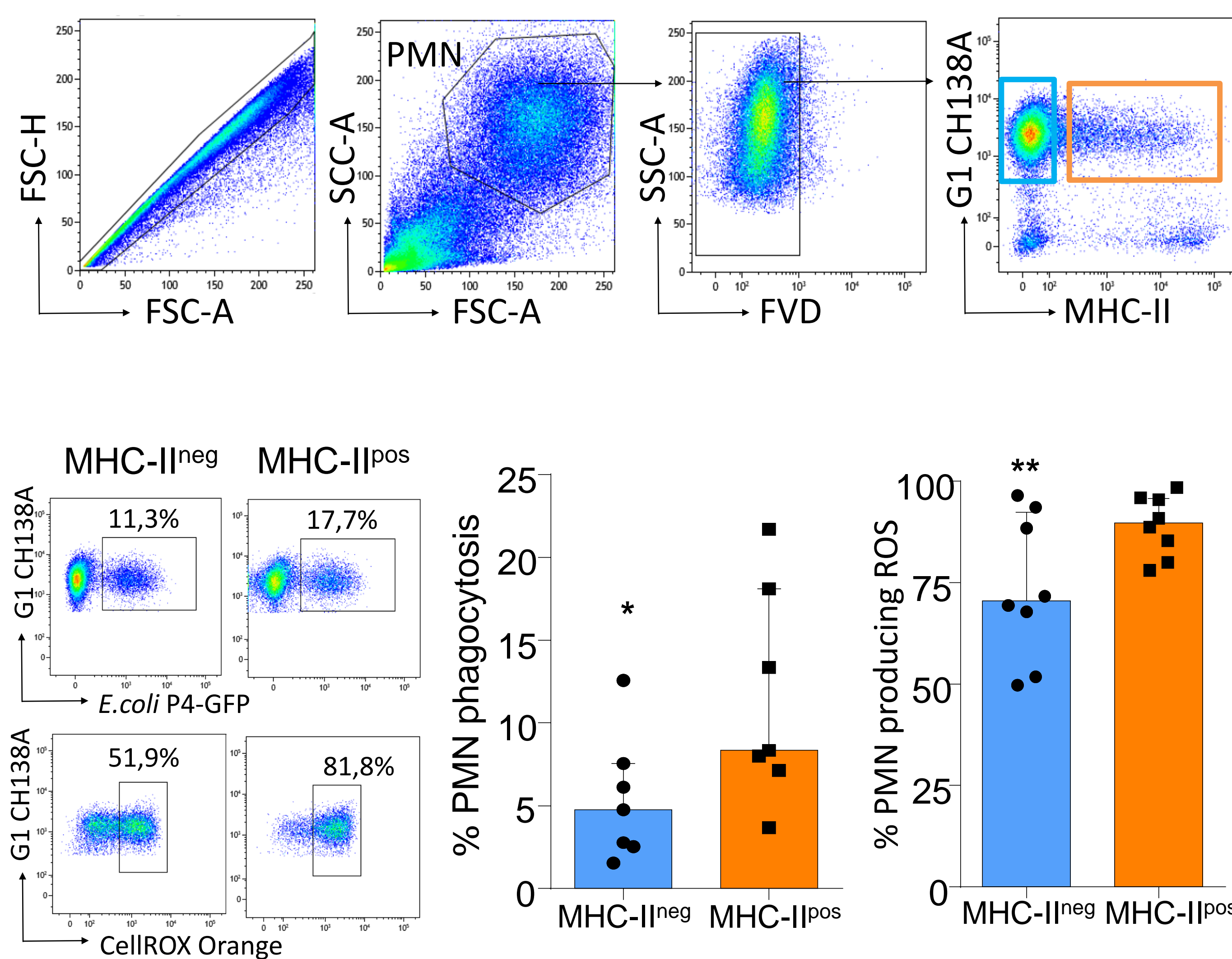
Bovine mastitis is mainly caused by **bacterial infection** and is responsible for important economic losses as well as alterations of the health and welfare of animals. The increase in the somatic cell count (SCC) in milk during mastitis is mainly due to the influx of **polymorphonuclear neutrophils** (PMN) which have a crucial role in the elimination of pathogens. For a long time, these first line defenders have been viewed as microbes' killers with limited role in the orchestration of the immune response. However, their role is more complex and we recently characterized an **MHC-II expressing neutrophil subset with regulatory capacities** in cattle. Here, we questioned the implication of different neutrophils subsets in the mammary gland immunity during clinical and subclinical mastitis.

Blood MHC-II^{pos} PMN display better phagocytosis activity as compared to MHC-II^{neg} PMN in Holstein and Normande cows



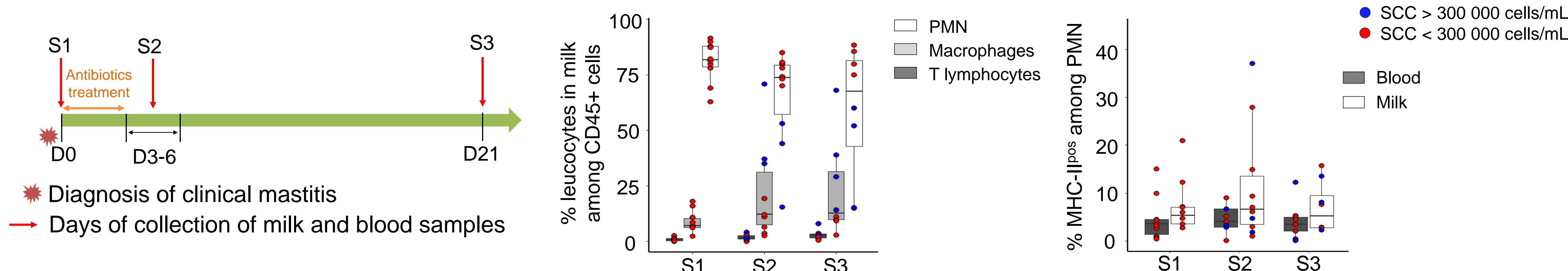
Percentage of MHC-II^{pos} PMN was determined by flow cytometry among live PMN and phagocytosis of pHrodo *E. coli* bioparticles by MHC-II^{pos} and MHC-II^{neg} PMNs was assessed by measuring fluorescence intensity directly correlated to ingested particles.

Milk MHC-II^{pos} PMN have a higher bactericidal capacities compare to MHC-II^{neg} PMN.



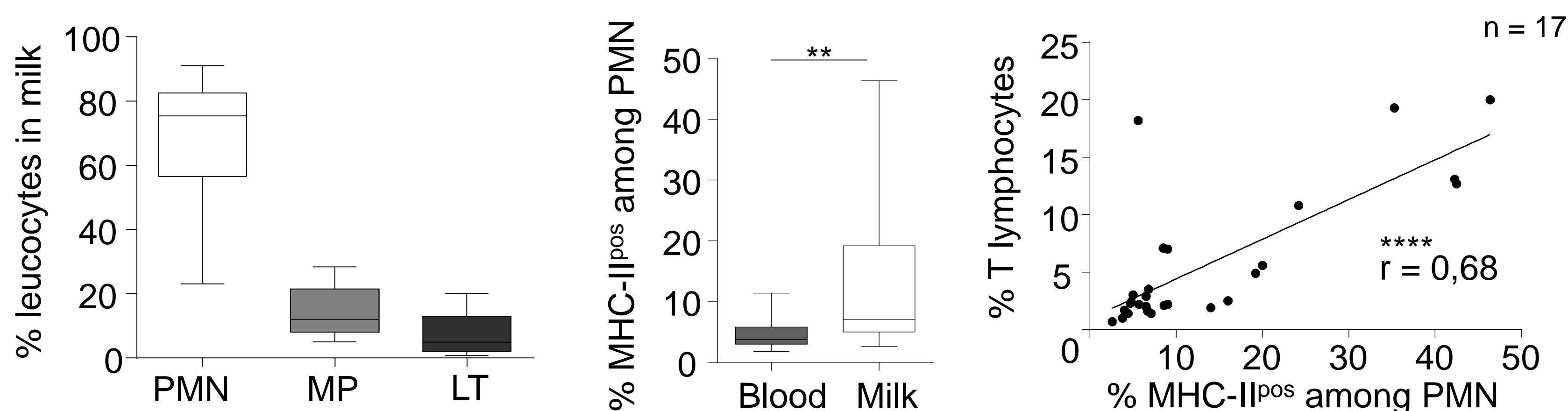
After milk cells labelling, **phagocytosis** by MHC-II^{pos} or MHC-II^{neg} PMN was assessed using *E. coli* P4-GFP strain at 37°C. PMNs phagocytosis were normalized on PMNs stimulated with *E.coli* P4-GFP at 4°C. **Oxidative stress** was measured in MHC-II^{pos} and MHC-II^{neg} neutrophils using the **CellROX Orange** probe that reacts with all ROS species. Cells were activated with TBHP and levels of ROS were measured by flow cytometry.

MHC-II^{pos} PMN are present in blood and milk during clinical mastitis and their proportion does not vary over time



S1 = first blood and milk sample from the infected quarter immediately collected after the diagnosis of the clinical mastitis S2 = second blood and milk samples collected between day 3 and day 6 (the day was choose to avoid week-ends). S3 = third blood and milk samples collected at day 21. Different cells populations in blood and in milk were identify by flow cytometry.

MHC-II^{pos} PMN accumulate in milk during subclinical mastitis and correlate with T cells.



Percentage of PMN, macrophages (MP) and T lymphocytes (LT) among CD45+ cells in milk, MHC-II^{pos} PMN among PMN in blood and in milk, during subclinical mastitis.

Conclusion : MHC-II^{pos} and MHC-II^{neg} PMN subsets were present in milk from healthy cows or during mastitis, and we observed higher bactericidal capacities of milk MHC-II^{pos} PMNs as compared to their classical counterparts. Interestingly, we observed a positive correlation between counts of MHC-II^{pos} PMNs and T cells in milk during subclinical mastitis. This could also open the way to the discovery of new biomarkers of mastitis.

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