**Sex difference in innate inflammatory response and macrophage polarization in *Streptococcus agalactiae*-induced pneumonia and potential role of microRNA-223-3p**

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**Supplementary data**

**Figure S1.** Gating strategy followed in flow cytometry analysis of monocytes, macrophages, B cells, T cells and NK cells in the lungs of naive and GBS-infected mice.
Figure S2. Gating strategy followed in flow cytometry analysis of neutrophils and eosinophils in the lungs of naive and GBS-infected mice.

Figure S3. Sex difference in leukocyte cells in bronchoalveolar lavage (BAL) in naive and GBS-infected mice. C57BL/6 male (black) and female (white) mice were infected intranasally with ±10^8 CFUs of GBS. Age-matched naive male and female mice were used as controls (n=5 for each group). 24h pi, BALs were performed, and the recovered cells were fixed and stained with Diff-Quik staining kit. A minimum of 600 cells were counted by microscopy. Data are means ±SD. *p<0.05 (Mann-Whitney U test).
**Figure S4.** Gating strategy followed in flow cytometry analysis of CD80+CD206- macrophages (M1) and CD80-CD206+ macrophages (M2) in the lungs of naive and GBS-infected mice.

| Test 1 | Dilution factors | Mean CFUs count | number of GBS in 1 stock tube | Dilution factors | Mean CFUs count | number of GBS/mL |
|--------|------------------|-----------------|-------------------------------|------------------|-----------------|------------------|
|        | 6*10^6           | 145             | 8.17*10^8                     | 4*10^6           | 235             | 9.4*10^8         |
|        | 1*10^7           | 52              | 5.2*10^8                      | 4*10^7           | 24              | 9.6*10^8         |
| mean   |                  | **6.95*10^8**   |                               | mean             |                 | **9.5*10^8**     |

**Test 2**

| Dilution factors | Mean CFUs count | number of GBS/mL |
|------------------|-----------------|------------------|
| 2*10^7           | 75              | 1.5*10^9         |
| 2*10^8           | 8               | 1.6*10^9         |
| mean             |                 | **1.55*10^9**    |

**Table S1.** Representative data of CFUs (colony forming units) enumeration on Todd Hewitt Agar (THA) media plates with 15mg/mL nalidixic acid, after freezing and thawing and resuspension to the desired concentration.