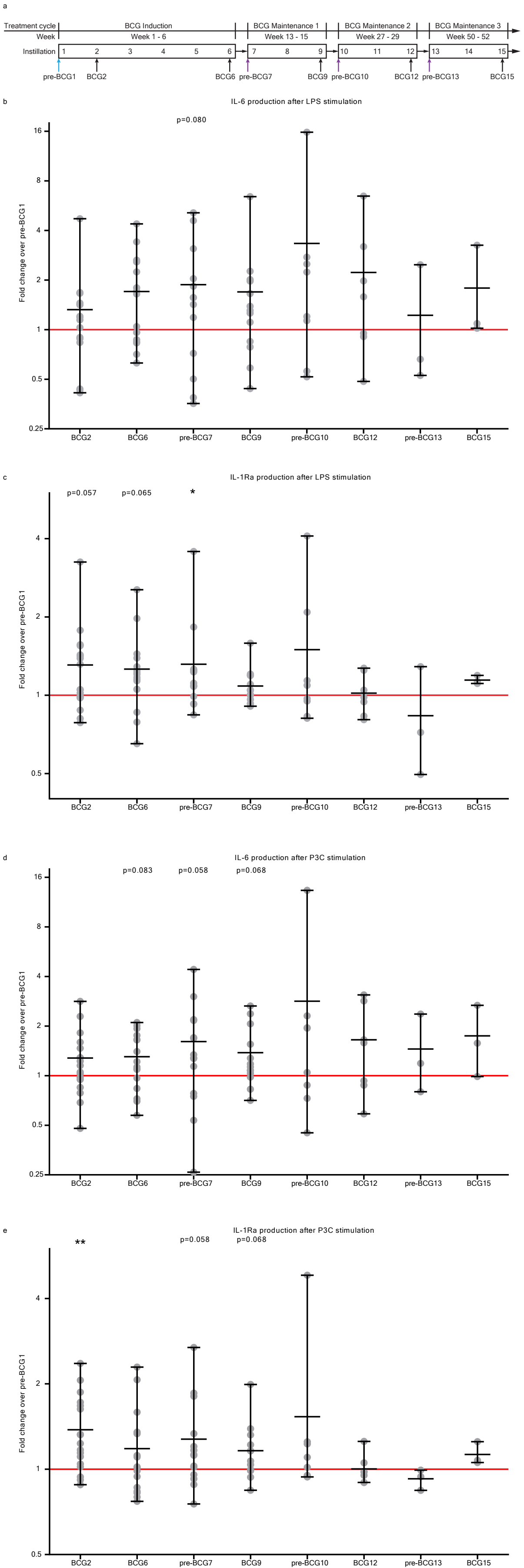


## Supplementary Figure 1



Supplementary Figure 1 – Production of IL-6 and IL-1Ra by PBMCs upon ex-vivo stimulation with heterologous stimuli after BCG instillations

**a:** Study schedule of the Tribute study. Blood was collected and PBMCs were isolated at three time points during the BCG induction cycle: pre-BCG1, BCG2 and BCG6; and two time points during each subsequent BCG maintenance cycle: pre-BCG7, BCG9, pre-BCG10, BCG12, and BCG15. Some patients did not receive BCG for weeks to months and thus represent the best 'innate immune memory' time points. Light blue arrow indicates pre-BCG1 time point which is used to calculate fold change in cytokine production. Purple arrows indicate important time points for TI, as patients did not receive BCG for weeks to months and thus represent the best 'innate immune memory' time points.

**b:** IL-6 production by PBMCs after 24 hour stimulation with LPS at 8 time points during BCG therapy compared to pre-BCG1.

**c:** IL-6 production by PBMCs after 24 hour stimulation with P3C at 8 time points during BCG therapy compared to pre-BCG1.

**d:** IL-1Ra production by PBMCs after 24 hour stimulation with LPS at 8 time points during BCG therapy compared to pre-BCG1.

**e:** IL-1Ra production by PBMCs after 24 hour stimulation with P3C at 8 time points during BCG therapy compared to pre-BCG1.

Individual patient fold change values are displayed as grey dots. Group values for each time point are displayed as median  $\pm$  range in fold change compared to pre-BCG1. Two tailed matched-pair Wilcoxon signed-rank test was used at  $p < 0.05$  and indicated as follows: \*  $p < 0.05$  and \*\*  $p < 0.01$ . \*\*\*  $p \leq 0.001$  \*\*\*\*  $p \leq 0.0001$ . Number of data points per time point for b: pre-BCG1: 17, BCG2: 17, BCG6: 16, pre-BCG7: 13, BCG9: 13, pre-BCG10: 8, BCG12: 7, pre-BCG13: 3, BCG15: 3. Number of data points per time point for c, d, e: pre-BCG1: 17, BCG2: 17, BCG6: 16, pre-BCG7: 14, BCG9: 13, pre-BCG10: 8, BCG12: 7, pre-BCG13: 3, BCG15: 3.