Extended Data

Extended Data Figure 1. Schematic overview of whole blood samples used for transcriptomics analysis. Each green and purple box represents a whole blood sample for RNA-sequencing, with each participant contributing 10 unique samples to the model.

Extended Data Figure 2. Genes significantly differentially expressed for the *BCG: Influenza* interaction and their associations with anti-mycobacterial immune responses. For each gene, the log2 fold change (LFC) and Benjamini-Hochberg (BH) adjusted p value (adj. p) are listed for the variables: *BCG:Influenza* interaction, *BCG*, *Influenza* and *BCG+ BCG:Influenza*. *BCG+ BCG:Influenza* describes the effect of BCG when influenza is also present. Where adj. p≤0.05, the box is shaded blue.

Extended Data Figure 3. Nine gene clusters identified by maSigPro. In total, 2,140 genes were identified as significantly differentially expressed (SDE) over time and between pre- *versus* post-influenza (adj. $p \leq 0.05$, $R^2>0.6$)). The coefficients obtained were used to group together SDE genes into clusters with similar temporal expression patterns. Summary plots of gene expression over time for the BCG-infected pre-influenza (dark blue), BCG-infected post-influenza (pink), BCG-uninfected pre-influenza (light blue) and BCG-uninfected post-influenza (red) groups are shown. Solid lines connect the median expression to show the trends for each group, and the dashed lines show the regression curves fitted to the data.

Extended Data Figure 4. **Cytokine responses to BCG *lux* infection, pre- and post-influenza.** Concentrations of IL-10, TNF-α, IL-1β, IL 17-A/F, IL-22 and IL-23 were measured in the supernatants from BCG lux-uninfected blood at baseline (0 h incubation) and BCG *lux*-infected blood at 6, 24 and 72 h incubation. Plots showing baseline and maximal concentrations. Box and whiskers plots are shown for 22 influenza PCR+ and 6 PCR- participants, with the horizontal lines showing the medians. Paired comparisons of medians were made using two-tailed Wilcoxon rank sum between the pre- versus post-influenza samples, and baseline versus maximal concentrations, in the PCR+ and PCR- groups. In all plots, false discovery rate (FDR)-corrected p values are shown: ns p >0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

Extended Data Figure 5. **Changing frequencies of cell subsets over time.** Plots showing the frequency of (A) IFN-α+ intermediate monocytes, (B) IFN-α+ non-classical monocytes, (C) IFN-α+ NK cells, (D) TNF-α+ classical monocytes, (E) IL-10+ classical monocytes over time. For each cellular subtype, box and whiskers plots of frequency in BCG *lux*-uninfected blood at baseline (0h) and BCG *lux*- uninfected and infected blood at 6 h (C, D) and 24 h (A, B, E) are shown for 16 influenza PCR+ participants, before and after influenza infection, with the horizontal lines showing the medians. Paired comparisons of medians were made using two-tailed Wilcoxon rank sum between pre- *versus* post-influenza samples, and BCG *lux*-infected *versus* -uninfected samples. In all plots, false discovery rate (FDR)-corrected p values are shown: ns p >0.05, *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

Extended Data References

Supplementary Tables

Supplementary Table 1. Demographics of influenza challenge patients testing PCR-positive (+) and PCR-negative (-) for Influenza A (H3N2) virus.

Supplementary Table 2. Comparisons of baseline (pre-influenza) BCG lux $GR_{72\,h}$ and $\Delta GR_{72\,h}$ for demographics, viral load and symptom scores.

Supplementary Table 3. Significantly differentially expressed genes identified in DESeq2 for the variable *BCG*.

Supplementary Table 4. Significantly differentially expressed genes identified in DESeq2 for the variable *Influenza*.

Supplementary Table 5. Significant pathways identified using IPA for *BCG: Influenza*.

Supplementary Table 6. Significant pathways identified using IPA for *BCG*.

Supplementary Table 7. Significant pathways identified using IPA for *Influenza*.

Supplementary Table 8. Significantly differentially expressed genes identified in DESeq2 with the cell-adjusted model for the variable *BCG: Influenza*.

Supplementary Table 9. Significantly differentially expressed genes identified in DESeq2 with the cell-adjusted model for the variable *BCG*.

Supplementary Table 10. Significantly differentially expressed genes identified in DESeq2 with the cell-adjusted model for the variable *Influenza*.

Supplementary Table 11. Significant pathways identified using IPA for *BCG: Influenza* (cell-adjusted model).

Supplementary Table 12. Significant pathways identified using IPA for *BCG* (cell-adjusted model).

Supplementary Table 13. Significant pathways identified using IPA for *Influenza* (cell-adjusted model).

Supplementary Table 14. Significantly differential expressed genes identified by maSigPro.

Supplementary Tables 15-21: Significant pathways identified using IPA for the nine gene clusters