Agilent gene expression results summary

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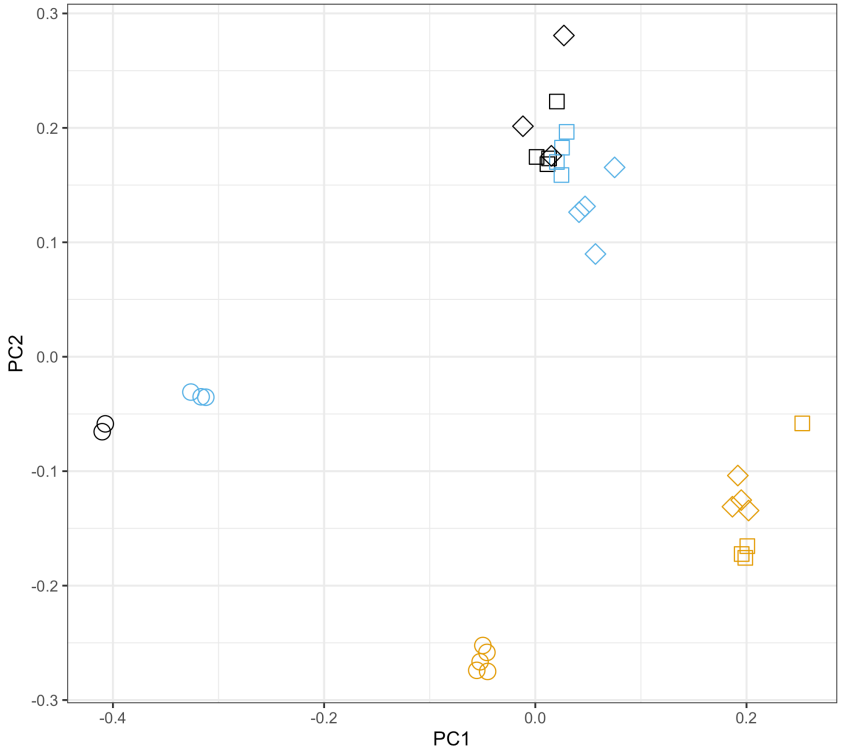
Preprocessing

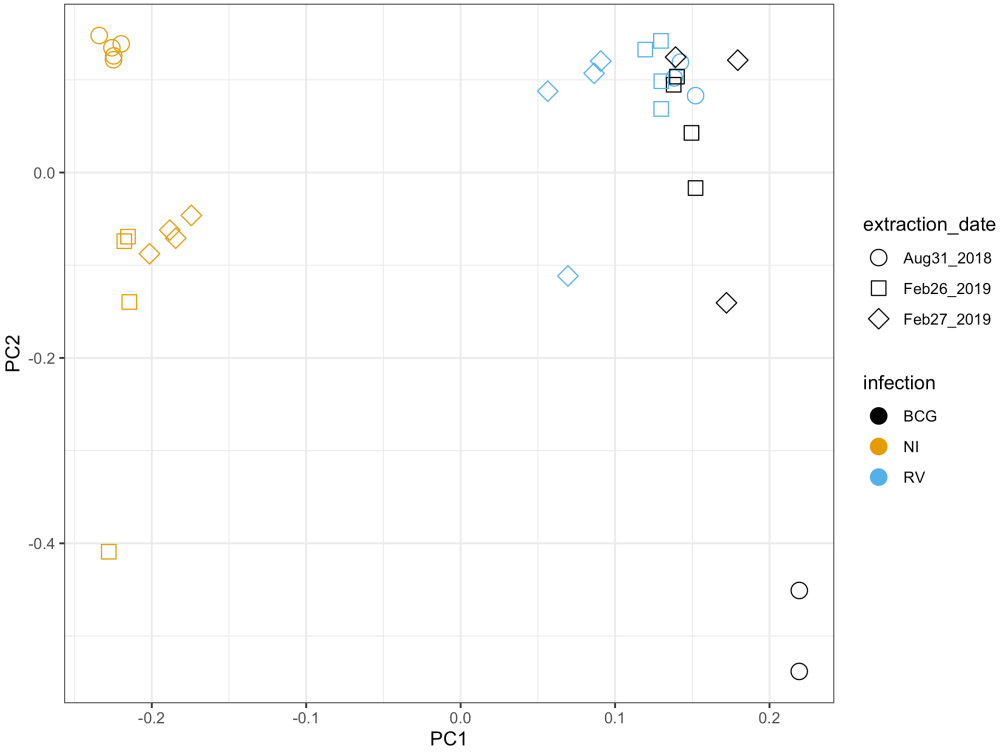
- The preprocessing method was kept the same as supplied by collaborators (background correction using normexp method and between array normalization by quantile normalization)
- QC using arrayQualityMetrics reports. Certain arrays flagged as potential outliers. All arrays were however kept for downstream analyses (except for the batch of 20 Sept which was requested for removal).
- Batch correction (using ComBat) was necessary to improve technical artifacts that corresponded with RNA extraction date
  - This had to be performed on astrocytes and neurons separately due to the study design (extraction dates don’t overlap between astrocytes and neurons, separate experiments)

Multivariate analysis (PCA): astrocytes (Sep20_2018 removed)

Normalized, no batch correction

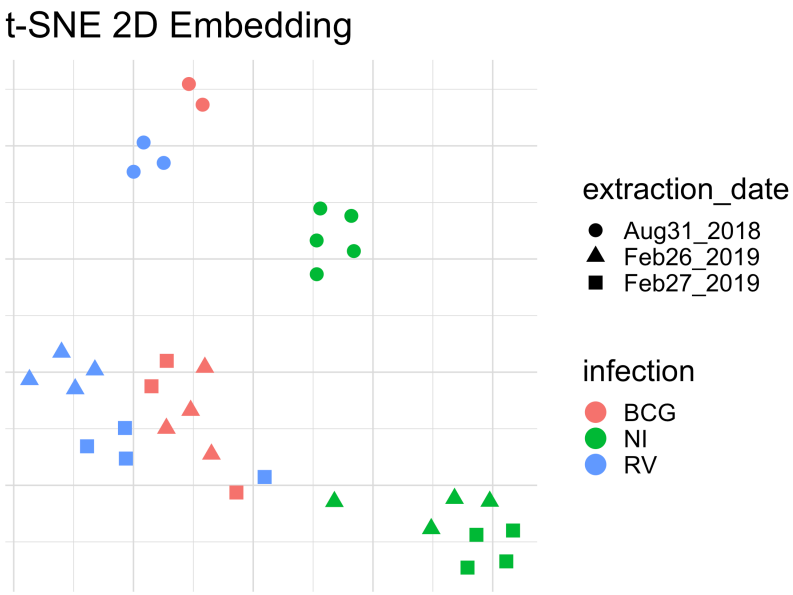


Normalized, with ComBat batch correction by extraction date

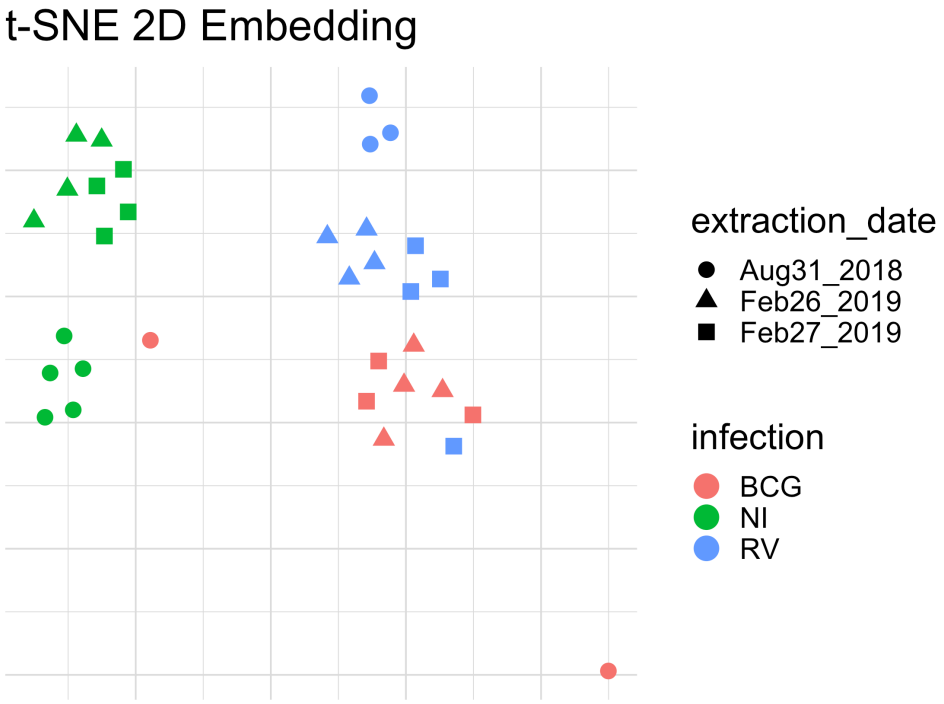


Multivariate analysis (tSNE): astrocytes (Sep20_2018 removed)

Normalized, no batch correction

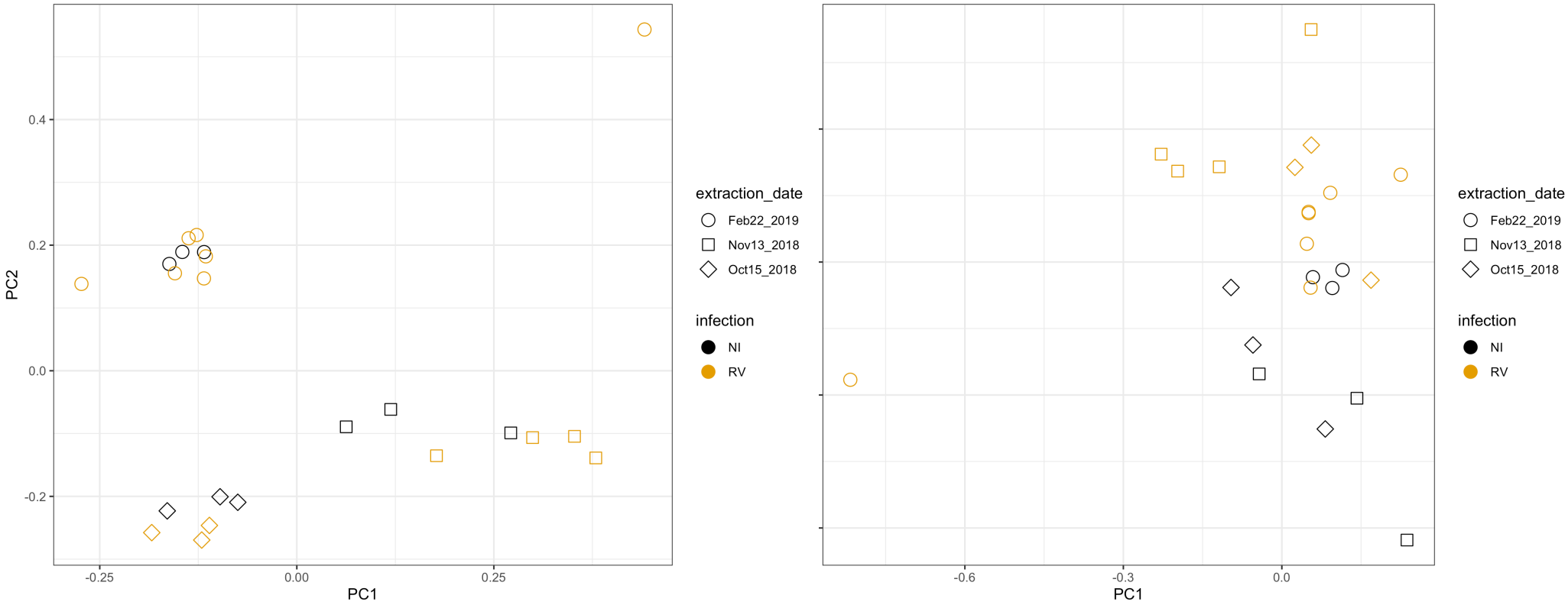


Normalized, with ComBat batch correction by extraction date



Multivariate analysis (PCA): neurons

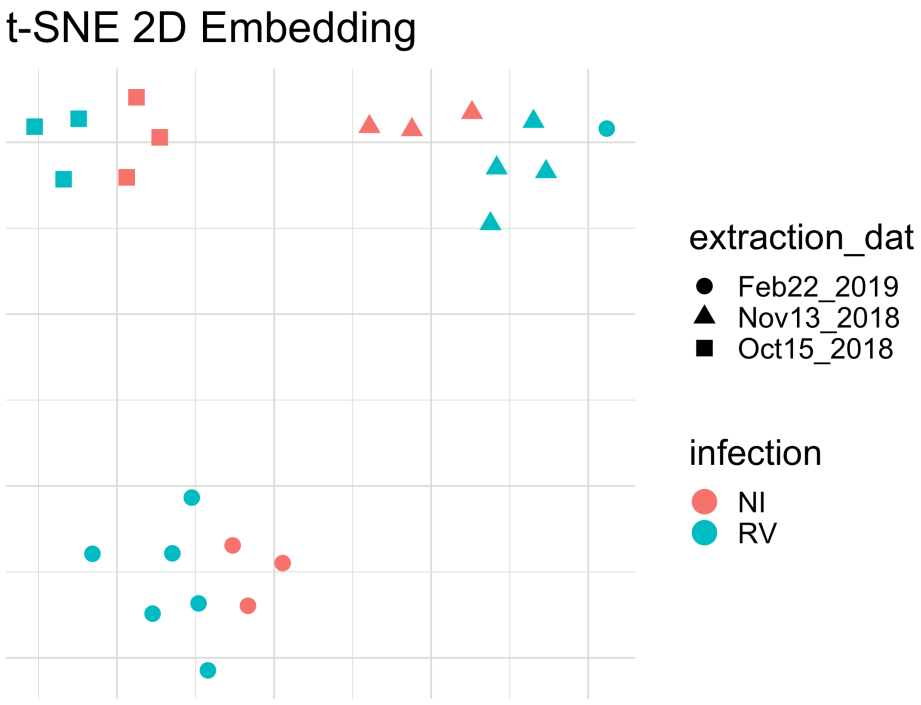
Normalized, no batch correction



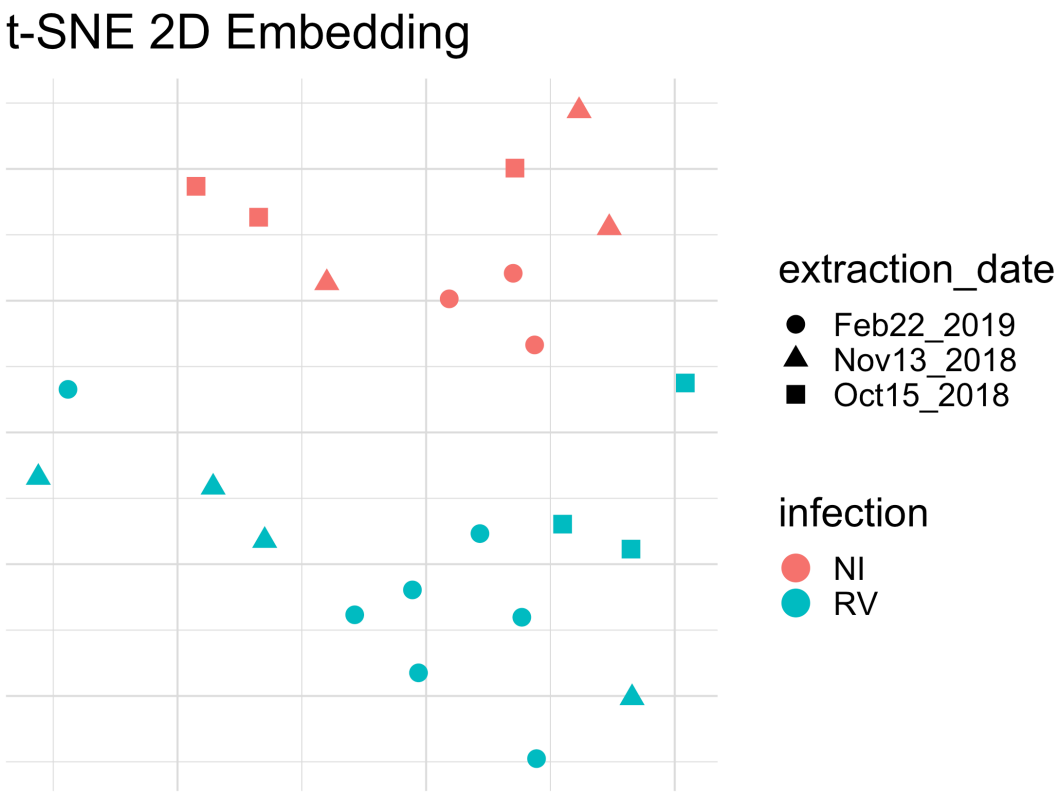
Normalized, with ComBat batch correction

Multivariate analysis (tSNE): neurons

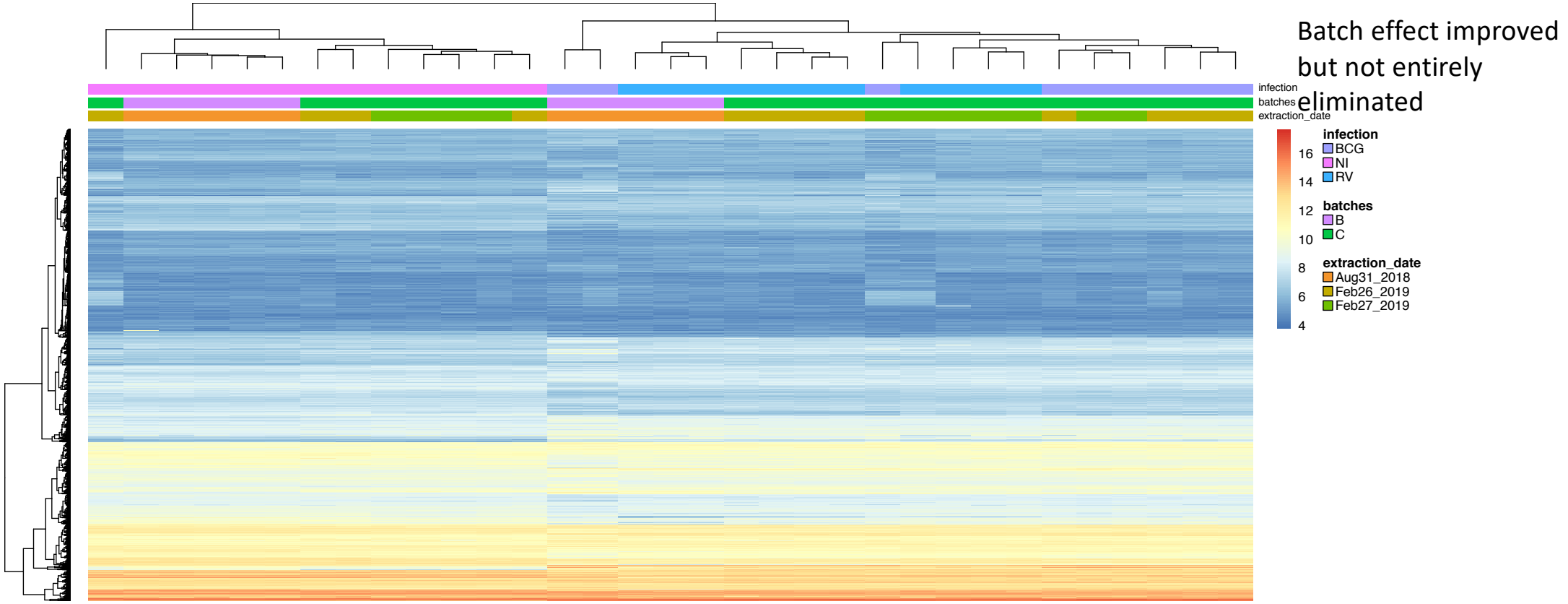
Normalized, no batch correction



Normalized, with ComBat batch correction

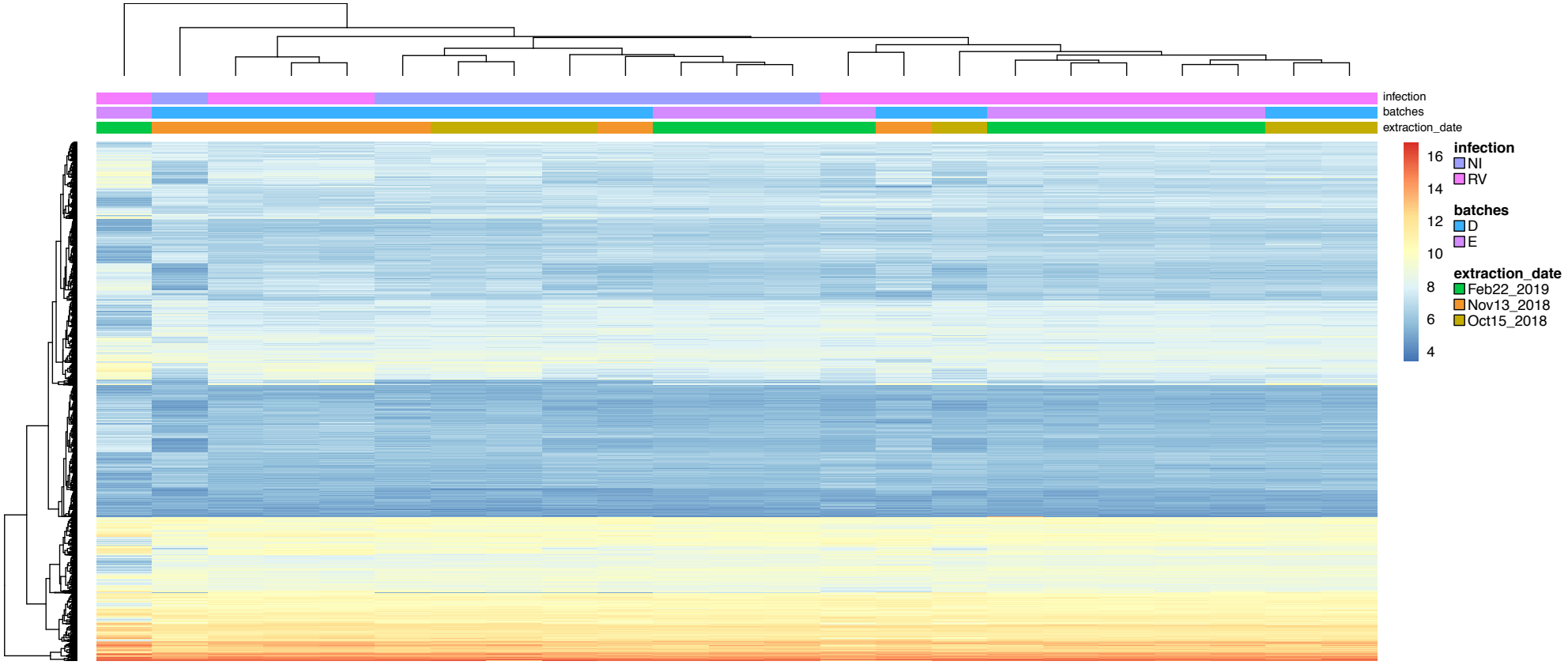


Heatmap astrocytes (filtered by median absolute deviation N= 13921/55681 retained)



Batch effect improved but not entirely eliminated

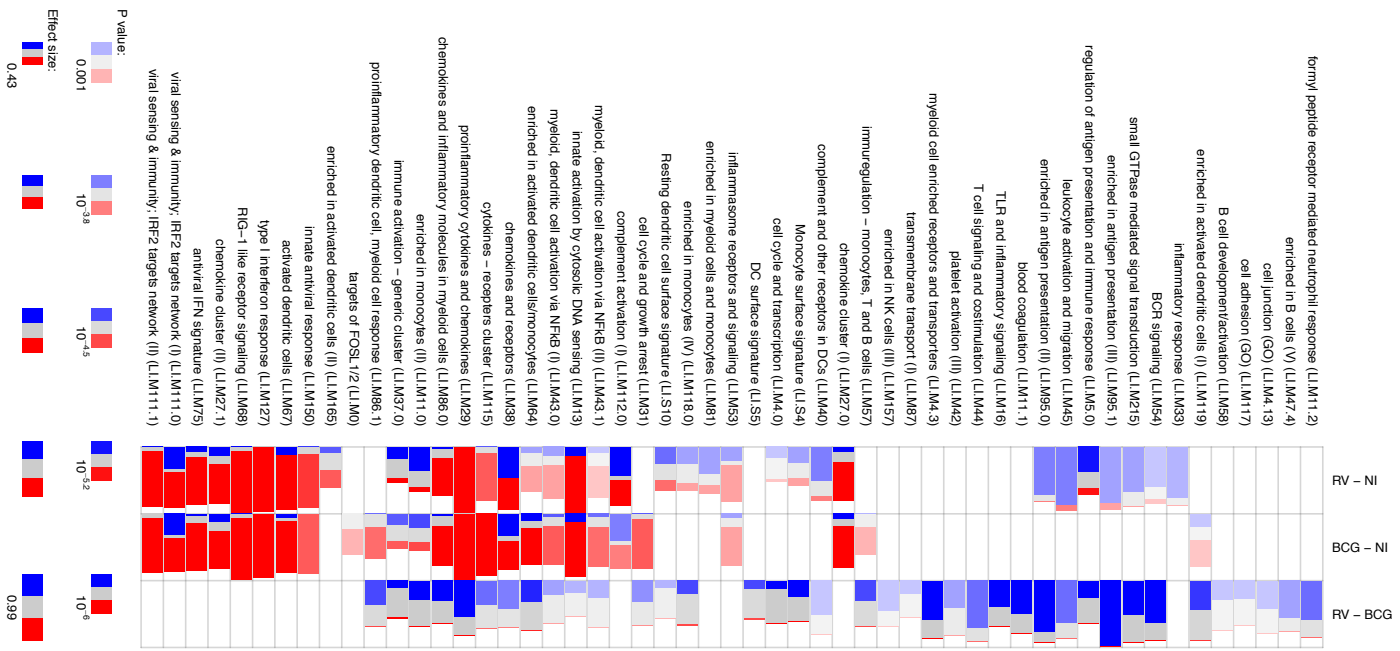
Heatmap neurons (filtered by median absolute deviation N= 13921/55681 retained)



Differential gene expression (limma) analysis summary

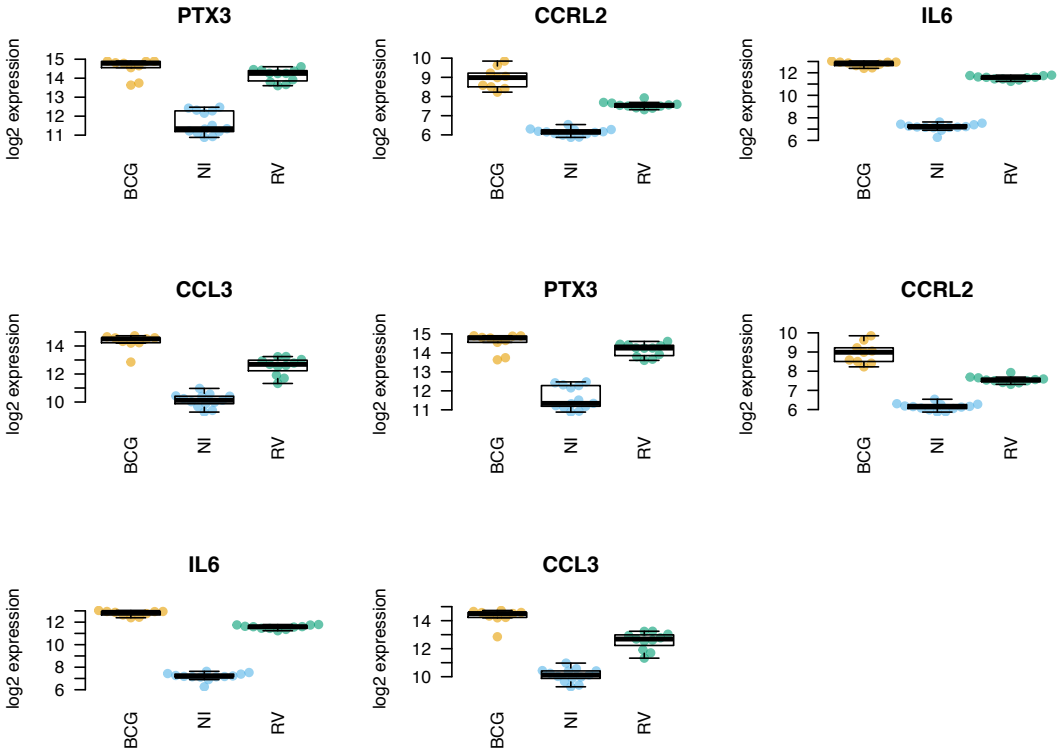
- Astrocytes:
  - RV vs. NI 1368 significant at FC ≥ 2 and adj. p-value ≤ 0.05
  - BCG vs. NI 1794 significant at FC ≥ 2 and adj. p-value ≤ 0.05
  - RV vs BCG 209 significant at FC ≥ 2 and adj. p-value ≤ 0.05
- Neurons:
  - RV vs. NI 340 significant at FC ≥ 2 and adj. p-value ≤ 0.05

Transcription module testing: astrocytes (stringent filter to limit sig. modules displayed)



Example gene-wise breakdown of significant transcription module: astrocytes

‘proinflammatory cytokines and chemokines’ module



Transcription module testing: neurons (stringent filter to limit sig. modules displayed)

Example gene-wise breakdown of significant transcription module: neurons

‘RIG−1 like receptor signaling’ module