BrdU RNA-seq

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Quality control - batch correction

Technical, non-biological variation due to RNA extraction or sorting introduces batch effects. Batch effects obscure biological variation. When RNA extraction or flow sorting batches are visualized on the PCA graph, a batch effect is clearly present (see Supplementary Figures). As a result of batch effect, the expression profiles of non-infected do not separate cleanly from infected animals (**Figure 1a**).

With batch correction non-infected and infected animals separate cleanly (**Figure 1b**). Subsequent analyses use batch-corrected reads.

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## Coefficients not estimable: animals_merged$infectedY batch21 batch23 batch24
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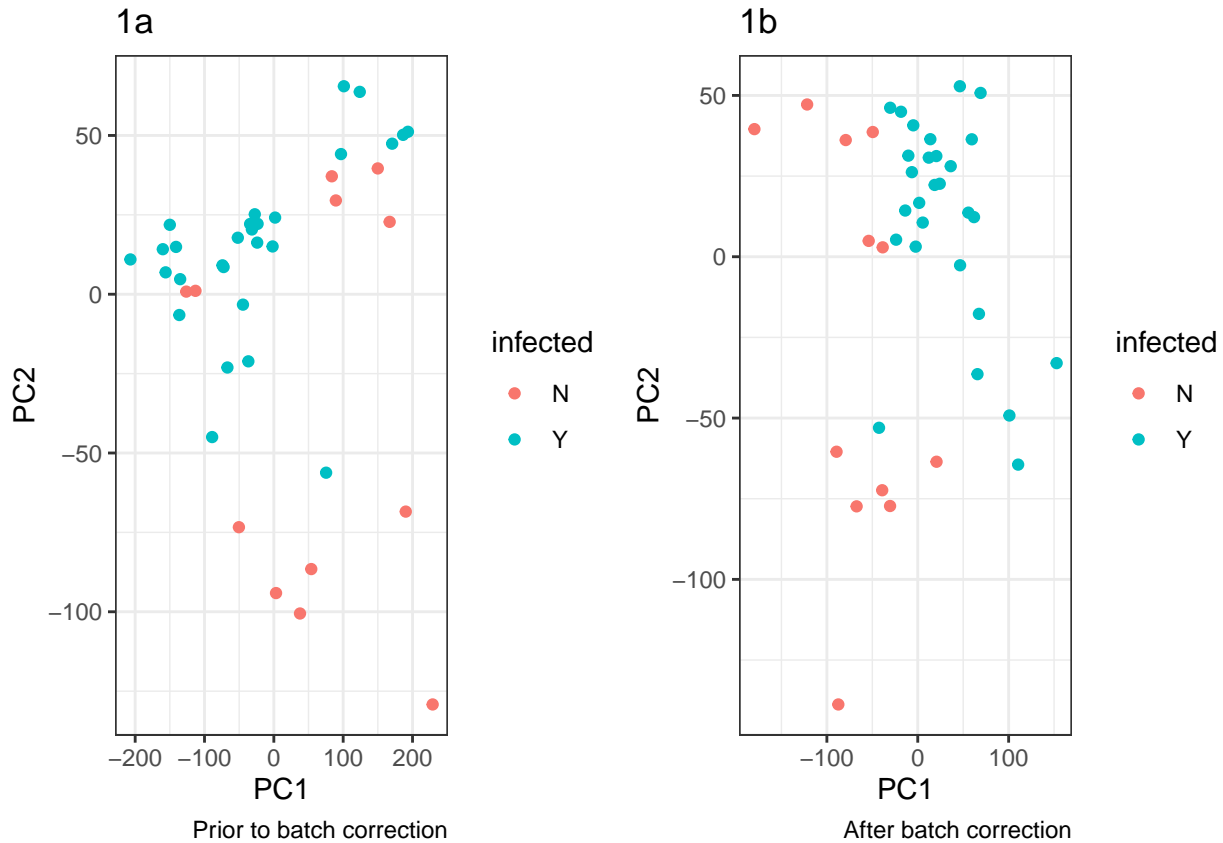

Figure 1: After batch correction, infected and non-infected animals cluster independently.

The expression profile of BrdU+ monocytes change substantially with infection.

Two graphs are presented. On the left, the PCA graph has been colored by day post infection; 0dpi corresponds to non-infected animals (**Figure 2a**). On the right, the PCA graph has been colored by BrdU+ vs. BrdU- monocytes (**Figure 2b**. The expression profile of BrdU+ monocytes change substantially with infection, while BrdU-negative monocytes are more similar between non-infected and infected animals.
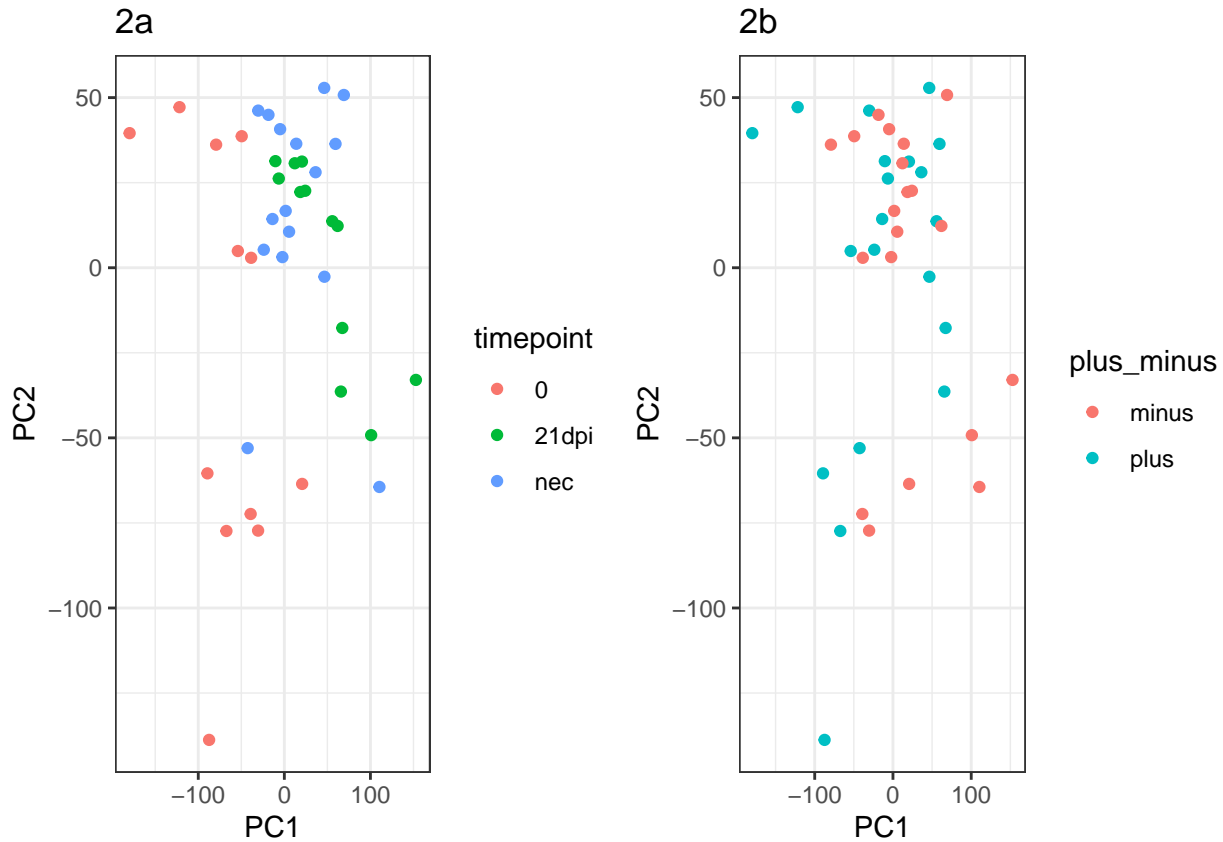

Figure 2: Changes in BrdU+ and BrdU- expression profiles with infection

BrdU+ expression profiles at 21 days post-infection predict SIVE development

SIV-infected animals with increased %BrdU+ monocytes at days 21 post infection rapidly progress to AIDS and develop severe encephalitis. The identity of BrdU+ monocytes is undefined. In this section, PCA graphs of BrdU+ monocytes from SIV-infected animals are presented.

The leftmost graph shows all BrdU+ monocytes from SIV-infected animals, annotated by day post infection (**Figure 3a**). The middle graph shows only 21dpi BrdU+ monocytes and is annotated by SIVE status(**Figure 3b**). The rightmost graph displays only necropsy timepoint BrdU+ monocytes and is annotated by SIVE status (**Figure 3c**).

BrdU+ monocytes at 21dpi and necropsy cluster separately (**Figure 3a**). At 21dpi, Animals that develop SIVE vs. SIVnoE cluster separately on the basis of BrdU+ monocyte expression profiles (**Figure 3b**). At necropsy, BrdU+ expression profiles are similar between SIVE and SIVnoE animals (**Figure 3c**). This suggests early monocyte dysregulation plays a key role in SIVE. This notion is consistent with Burdo (2011). The similarity of monocyte expression profiles at necropsy is consistent with Nowlin (2018).
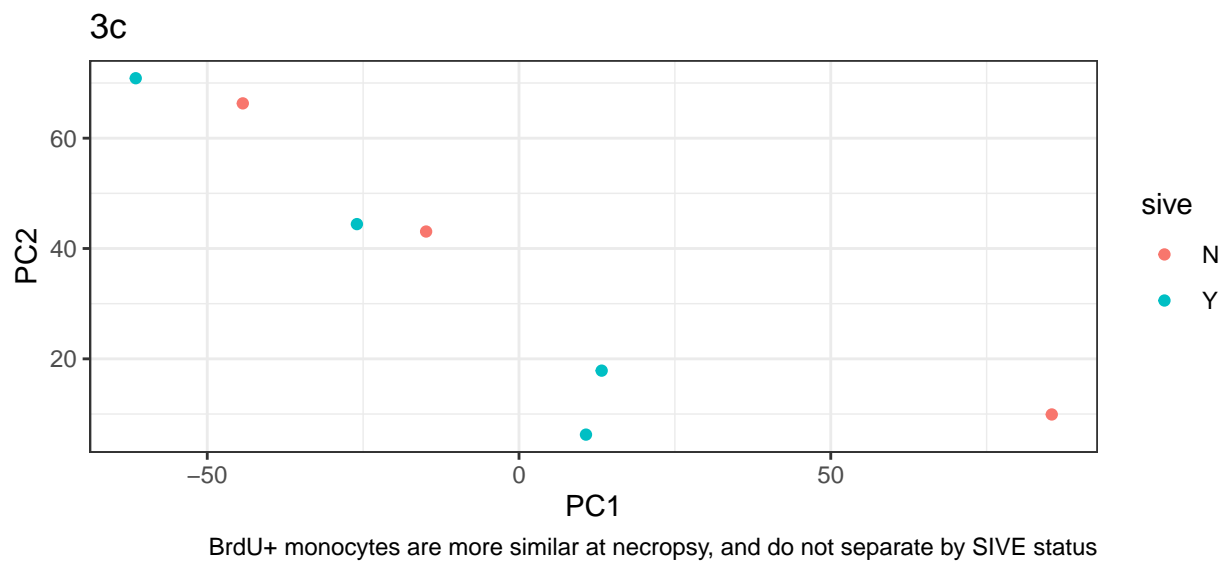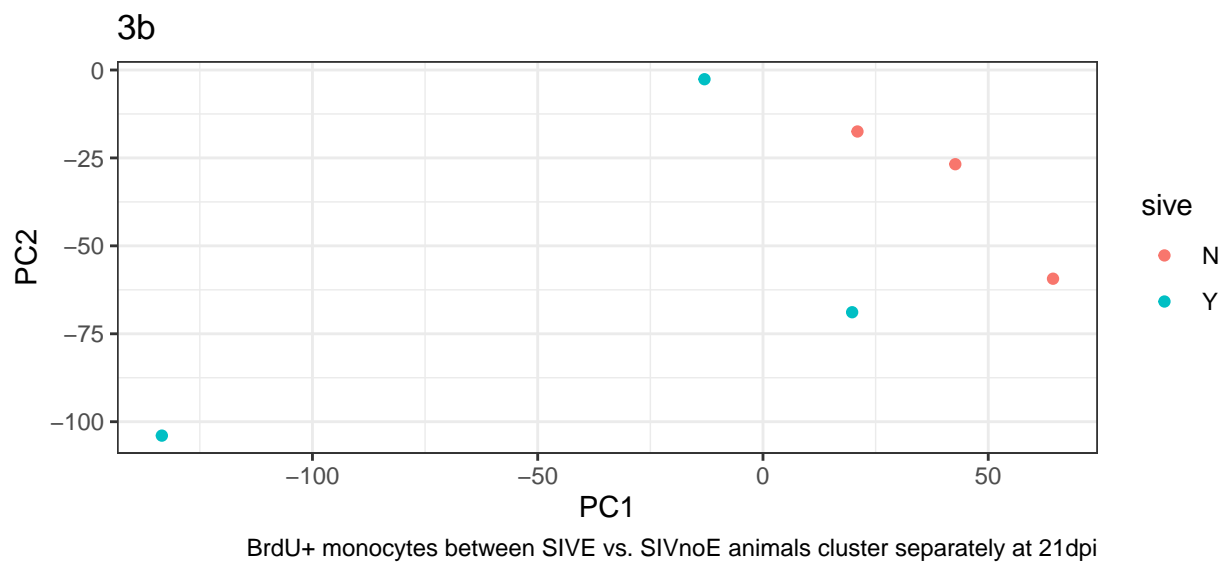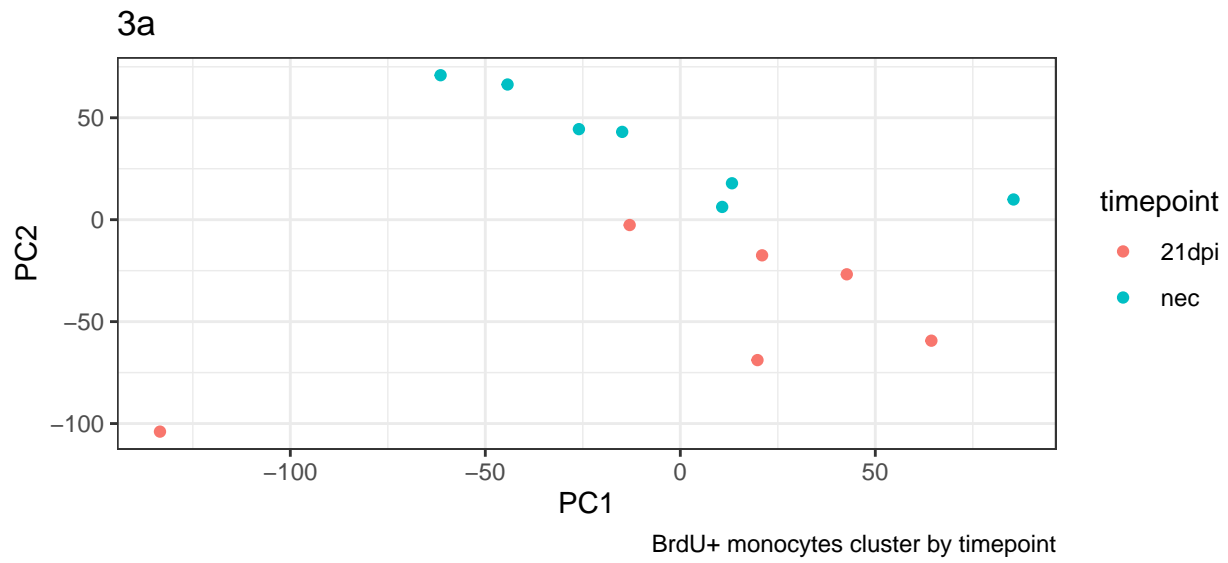
3a

BrdU+ monocytes cluster by timepoint

3b

BrdU+ monocytes between SIVE vs. SIVnoE animals cluster separately at 21dpi

3c

BrdU+ monocytes are more similar at necropsy, and do not separate by SIVE status

Figure 3: BrdU+ monocyte expression profile at 21dpi predicts SIVE

Supplementary data

Batch correction

Samples extracted 2024-06-28 (cyan) and 2024-05-28 (red) cluster very far apart. These batches are balanced with respect to infection and therefore should cluster together. A similar effect is present for flow sorting batches.
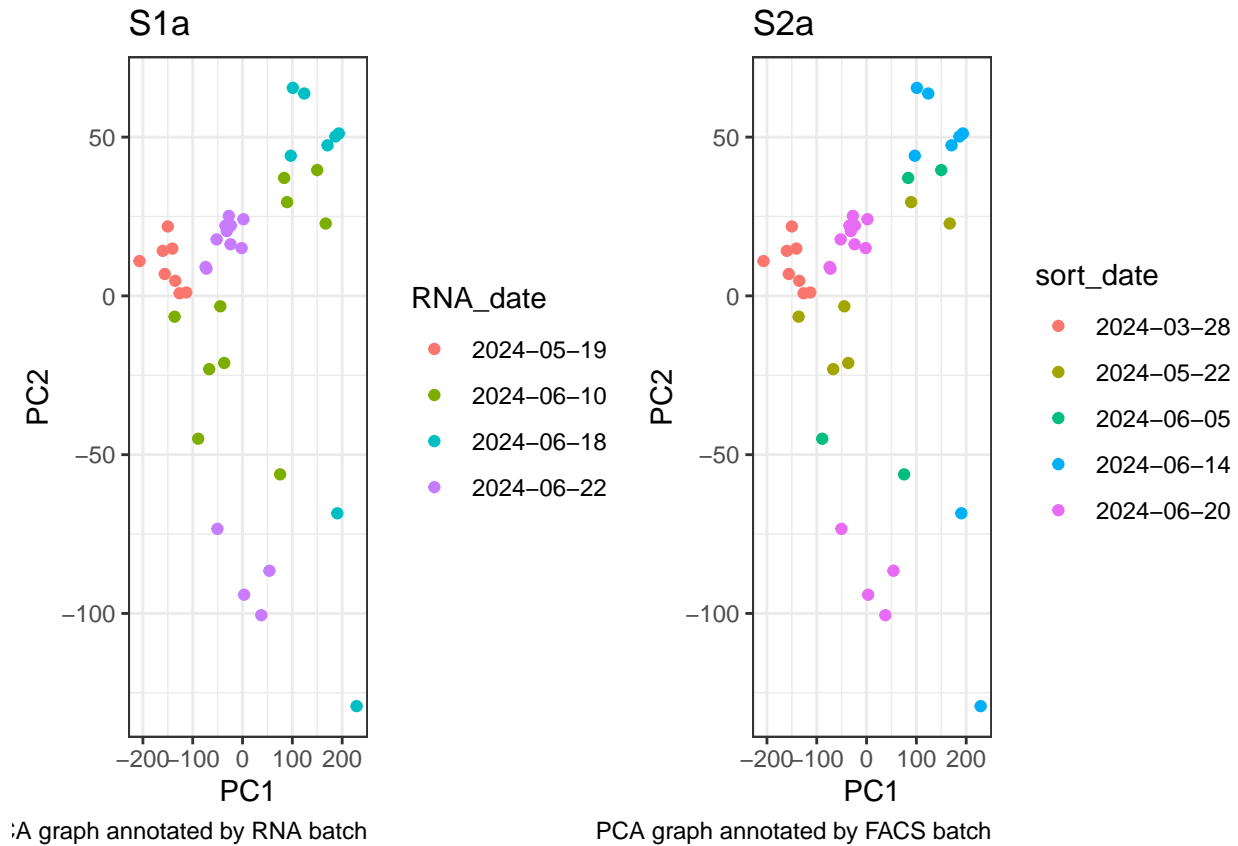

Figure 4: batch effect from RNA extraction