

# Assessing obesity-induced inflammation in mice through single cell multiomics analysis

Enabling simultaneous detection of protein and mRNA expression on a single cell level using the BD<sup>®</sup> AbSeq and BD Rhapsody<sup>™</sup> Mouse Immune Response Panel

## Features

- Interrogate changes in protein and mRNA expression simultaneously in single immune cells in a mouse receiving a high-fat diet (HFD)
- Investigate eight samples with one workflow using the BD<sup>™</sup> Mouse Immune Single-Cell Multiplexing Kit
- Determine HFD-induced differential gene expression in adipose tissue-resident immune cells

Obesity is a major risk factor for the development of various diseases such as type-2 diabetes, cardiovascular disease and cancer. The transition from a simple adiposity to a disease state correlates with an increase in local and systemic inflammation and is also marked by alterations in the number and function of immune cells. Macrophages are an important source of adipose tissue inflammation and several changes in T cell populations are also involved with the development of obesity in animal models. However, these changes have not been examined through an integrated analysis of mRNA and protein expression at a single cell level, hindering our understanding of the mechanism by which a HFD can induce inflammation and ultimately obesity-related disorders.



In this data sheet, we used a molecular cytometry approach to help better understand the perturbances caused by a HFD in the immune system. We analyzed 30 proteins and 399 targeted mRNAs in approximately 20,000 single cells derived from multiple tissues of mice that received different diets. Through analysis of cell differentiation and activation markers, we examined numerous cell compartments and observed distinct alterations associated with a HFD.

The HFD and control diet mice were obtained from Jackson Laboratories. Single cell suspensions were prepared from bone marrow, thymus, spleen and epididymal adipose tissue. The cells were co-stained with BD® AbSeq oligo-conjugated antibodies for protein detection and the BD™ Mouse Immune Single-Cell Multiplexing Kit (Sample Tag) for sample multiplexing. The reagents for the labeling of adipose tissue samples included BD Pharmingen™ FITC Rat Anti-Mouse CD45, allowing for the sorting of CD45<sup>+</sup> hematopoietic cells. All samples were pooled (2,500 cells from each tissue and mouse) before single cell capture on the BD Rhapsody™ Single-Cell Analysis System and Next Generation Sequencing (NGS) library was prepared using the BD Rhapsody™ Mouse Immune Response Panel.

Figure 1

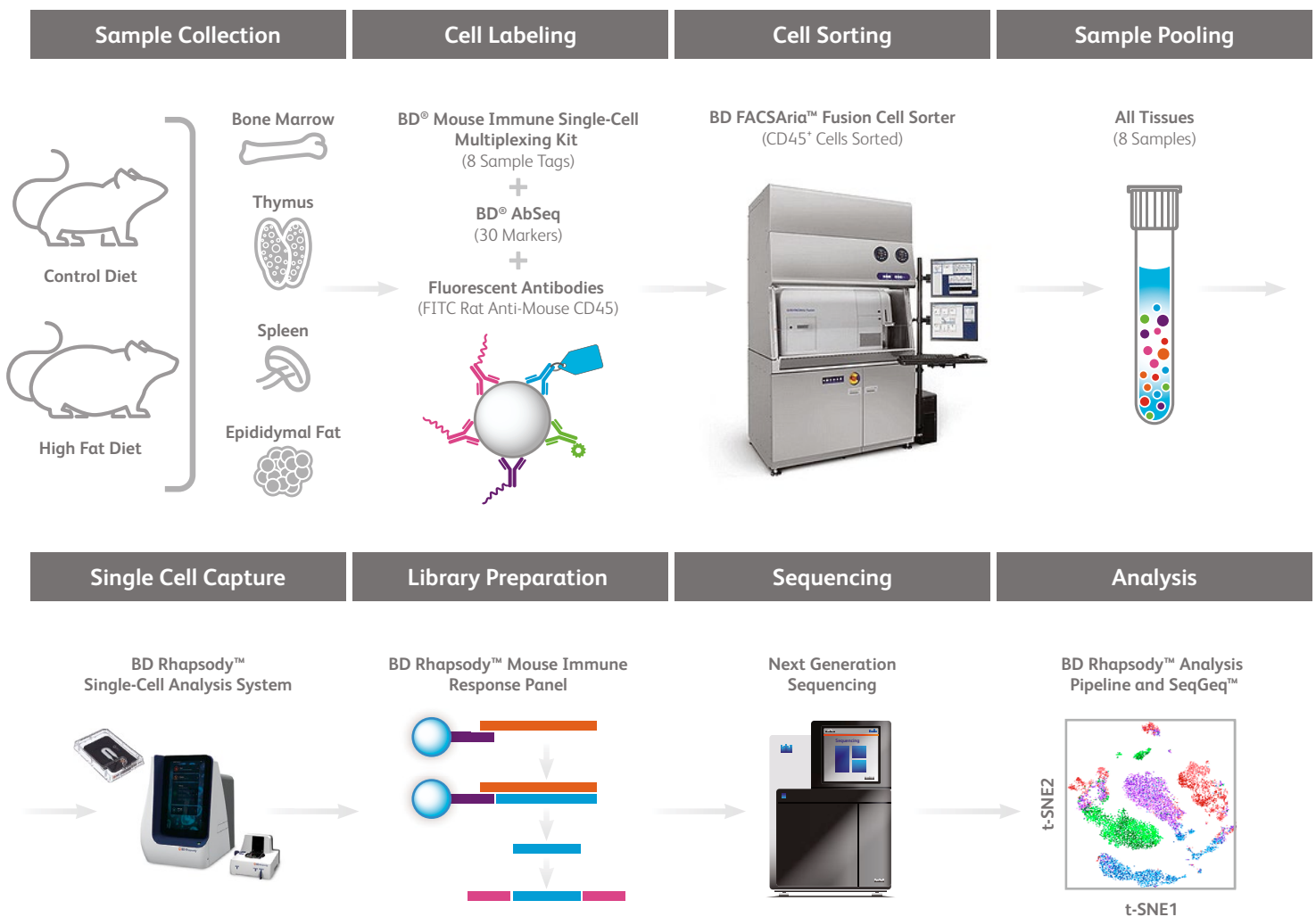


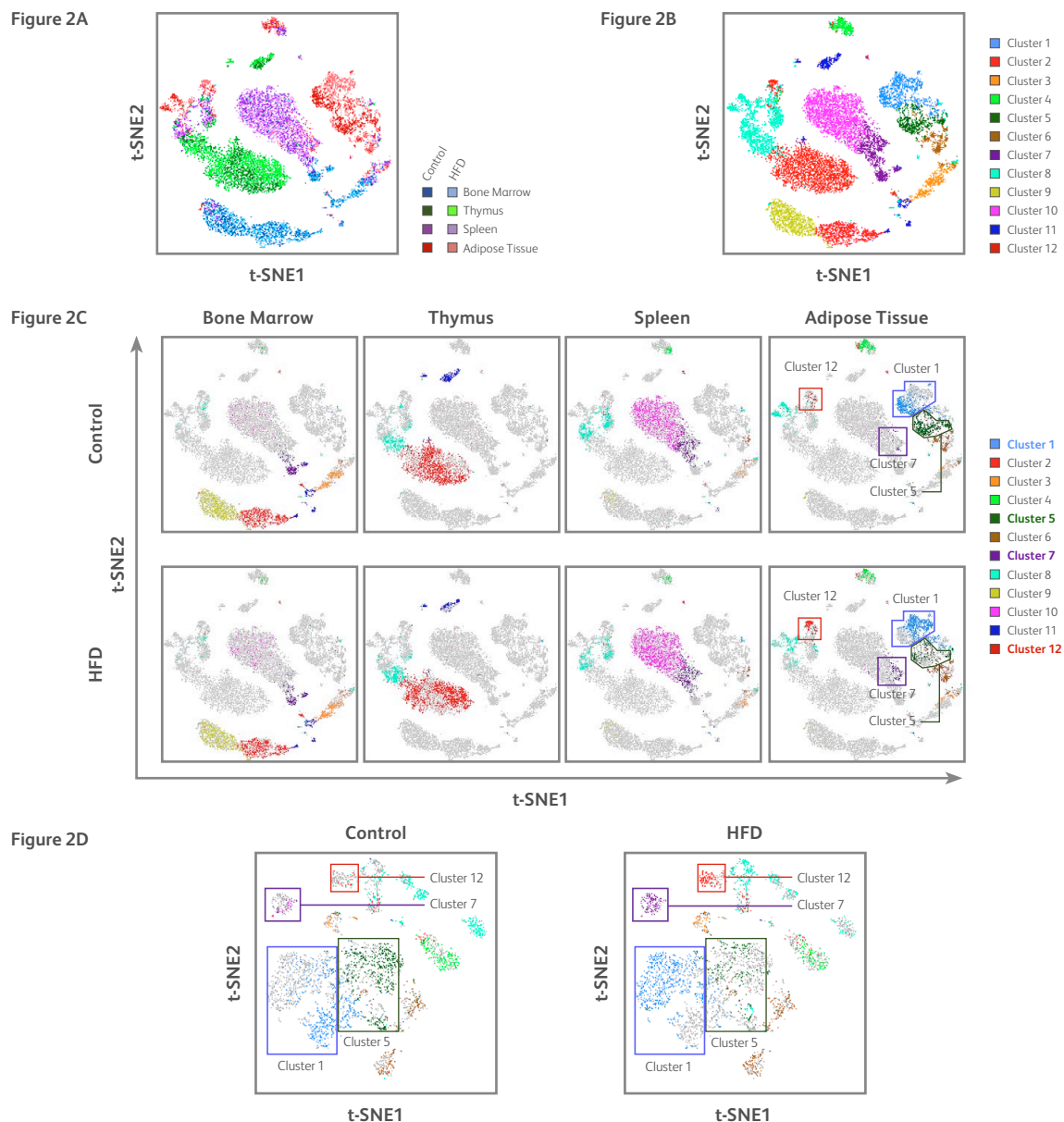
Figure 1. Experimental overview and the BD Rhapsody™ system workflow

Seven weeks old mice were placed on either a control diet or HFD for 17 weeks. Following tissue collection and dissociation, the 8 samples indicated in the figure were stained with one of the 12 unique DNA-barcoded antibodies from the BD™ Mouse Immune Single-Cell Multiplexing Kit and 30 BD® AbSeq mouse antibodies. BD Pharmingen™ FITC Rat Anti-Mouse CD45 antibody was included in the staining of adipose tissue cells and CD45<sup>+</sup> cells were sorted on the BD FACSAria™ Fusion cell sorter. The 8 samples were pooled and loaded onto the BD Rhapsody™ Single-Cell Analysis System. After sample retrieval, BD® AbSeq, Sample Tag and mRNA (BD Rhapsody™ Mouse Immune Response Panel) libraries were prepared for sequencing. The sequencing results were analyzed with the BD Rhapsody™ Analysis Pipeline and SeqGeq™.

**Table 1. List of BD<sup>®</sup> AbSeq antibodies used**

30-plex BD <sup>®</sup> AbSeq Panel		
CD1d	CD25	CD184 (CXCR4)
CD4	CD44	CD197 (CCR7)
CD5	CD45R (B220)	CD223 (LAG-3)
CD8b	CD49a	CD274 (PD-L1)
CD9	CD49b	CD279 (PD-1)
CD11b	CD62L	I-A/I-E
CD11c	CD64	IgD
CD19	CD69	IgM
CD21/CD35	CD103	Ly-6C/Ly-6G
CD23	CD182 (CXCR2)	TCR $\beta$

We used t-Distributed Stochastic Neighbor Embedding (t-SNE) for data visualization and an unbiased clustering algorithm PhenoGraph, SeqGeq™ plug-in, for detection of cell populations. PhenoGraph identified 12 cell clusters across the four different tissue types (Figures 2A and 2B). Of the 12 clusters, 4 showed differences in cell density and distribution in the adipose tissue of HFD mouse compared to control, clusters 1, 5, 7 and 12 (Figures 2C and 2D).



**Figure 2. Unsupervised cell clustering analysis identifies 12 distinct clusters across different tissues from control and HFD mice**

**A.** t-SNE map colored according to tissue types. **B.** Unsupervised clustering of all single cells in the tissues using PhenoGraph identified 12 cell clusters projected on the t-SNE map. **C.** Cluster analyses of the individual tissues displaying the major differences between control and HFD mice. **D.** t-SNE visualization of PhenoGraph clusters in adipose tissue only, showing a detailed view of clusters 1, 5, 7 and 12 and revealing the differences in cell density and distribution between control and HFD mice.

A more comprehensive investigation of clusters 1 and 5 based on the combined analysis of specific mRNAs and proteins identified two distinct changes in myeloid cell populations in the adipose tissue from the HFD mouse: an accumulation of *Adgre1*-expressing cells that resemble adipose tissue macrophages (ATM) and a loss of CD11c<sup>+</sup> cells (Figure 3A). Differential gene and protein expression analyses revealed an upregulation of molecules involved in inflammation (*Cd9*, *Cxcl1*, and *Il1b*) in ATM from the HFD mouse whereas an anti-inflammatory gene (*Cd163*) was down-regulated (Figure 3B). In CD11c<sup>+</sup> cells, genes involved in adipogenesis (*Lgals1*) as well as dendritic cell activation and regulation (*Clec10a*, *Clec4e*) were up-regulated (Figure 3C).

Figure 3A

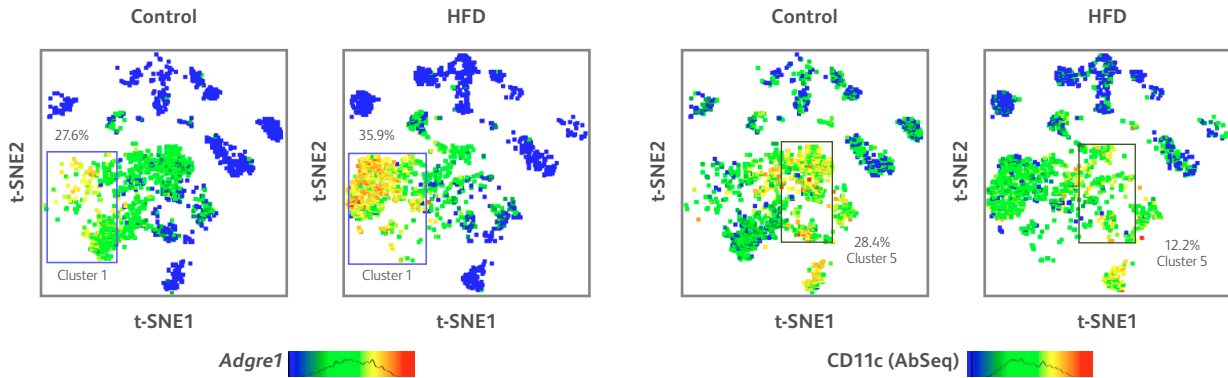


Figure 3B

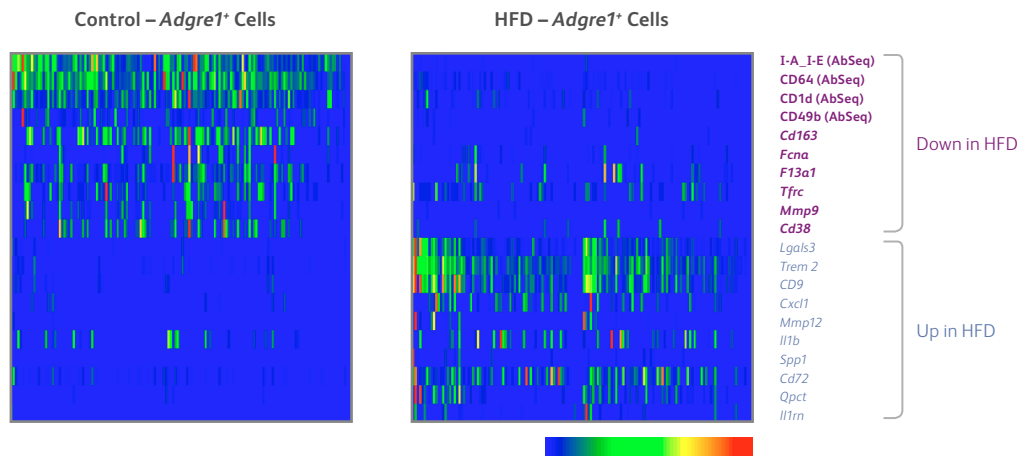


Figure 3C

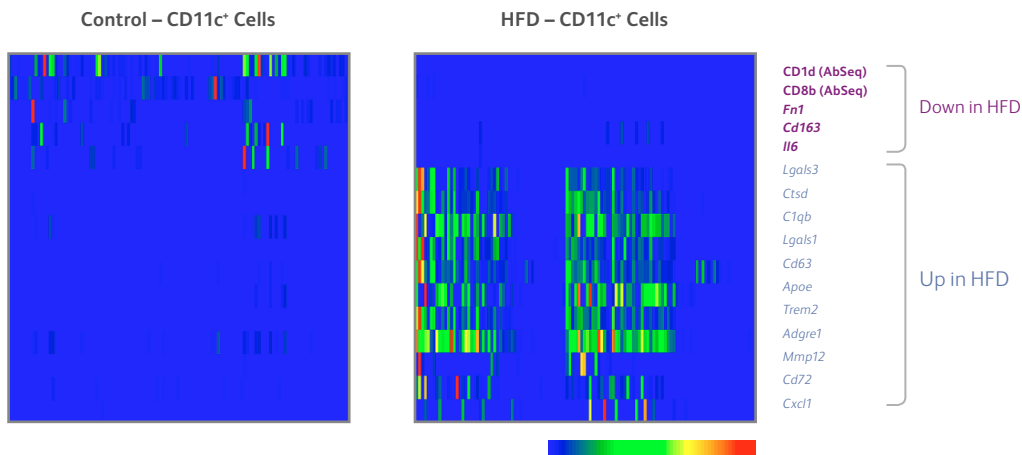


Figure 3. Single cell AbSeq and targeted mRNA-Seq analyses of myeloid cell populations localized in adipose tissue from control and HFD mice

A. t-SNE visualization of *Adgre1* (encodes F4/80) and CD11c expression revealing distinct populations (clusters 1 and 5) of myeloid cells in the adipose tissue of HFD mouse. B. Heatmap of differentially expressed mRNAs and proteins in *Adgre1*<sup>+</sup> cells (ATM) from control and HFD mice. C. Heatmap of differentially expressed mRNAs and proteins in CD11c<sup>+</sup> myeloid cells from control and HFD mice.

Characterization of cluster 12 revealed a high expression level of CD8b on the surface of these cells, suggesting this cluster was primarily comprised of CD8<sup>+</sup> T cells (Figure 4A). Notably, cluster 12 of HFD mouse presented a higher concentration of CD8<sup>+</sup> T cells than the control mouse. Differential mRNA and protein expression analyses of this cluster showed an elevated expression of *Pdcd1* (CD279, PD-1 mRNA), *Lag3* and *Tigit*, which are putative markers of cells undergoing exhaustion (Figure 4B).

Figure 4A

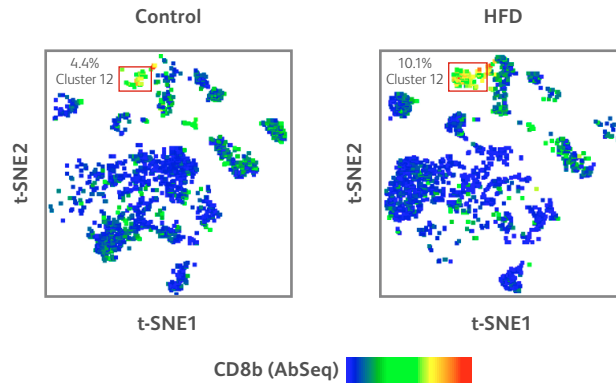


Figure 4B

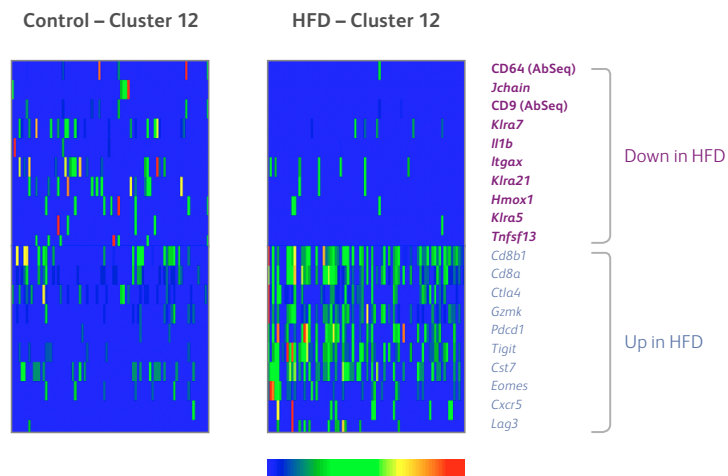


Figure 4. Single cell protein and targeted mRNA-Seq analyses of CD8<sup>+</sup> T cells in adipose tissue from control and HFD mice

A. t-SNE visualization of CD8b expression showing an increase in CD8<sup>+</sup> T cells in the adipose tissue of HFD mouse. B. Heatmap of top differentially expressed mRNAs and proteins in cluster 12 from control and HFD mice.

In order to further investigate the CD8<sup>+</sup> T cells infiltrates in the adipose tissue, we initially gated on TCRβ<sup>+</sup> cells and then CD8b<sup>+</sup> cells from all 4 tissues using conventional manual gating (Figure 5A). The analysis of the correlated expression of CD279 (PD-1) versus CD103 in the gated CD8<sup>+</sup> T cells confirmed that the adipose tissue of HFD mouse had higher numbers of CD8<sup>+</sup>PD-1<sup>+</sup> T cells than the control mouse. Conversely, these cells did not express CD103 (Figure 5A). Next, we determined the developmental trajectories of all CD8<sup>+</sup> T cells in the tissues using the algorithm Monocle (another SeqGeq plug-in). This analysis revealed a trajectory that goes from position 1 to position 3, in which a majority of CD8<sup>+</sup> T cells from bone marrow, thymus and spleen localized closer to position 1 (Figure 5B, left). The CD8<sup>+</sup> T cells in the adipose tissue reached positions 2 and 3, however, the furthest developmental state, position 3, contained mostly cells from the HFD mouse (Figure 5B, right).

Specific analysis of CD8<sup>+</sup> T cells in the adipose tissue using Monocle discriminated five developmental states. The CD8<sup>+</sup> T cells from the control mouse belonged to states 1 and 2 whereas cells from the HFD further followed a trajectory towards states 3, 4, and 5. States 2, 3, 4 and 5 from HFD mouse presented an elevated expression of exhaustion markers such as CD279 (PD-1) protein and mRNA (*Pdcd1*) as well *Tigit* (Figure 6).

Figure 5A

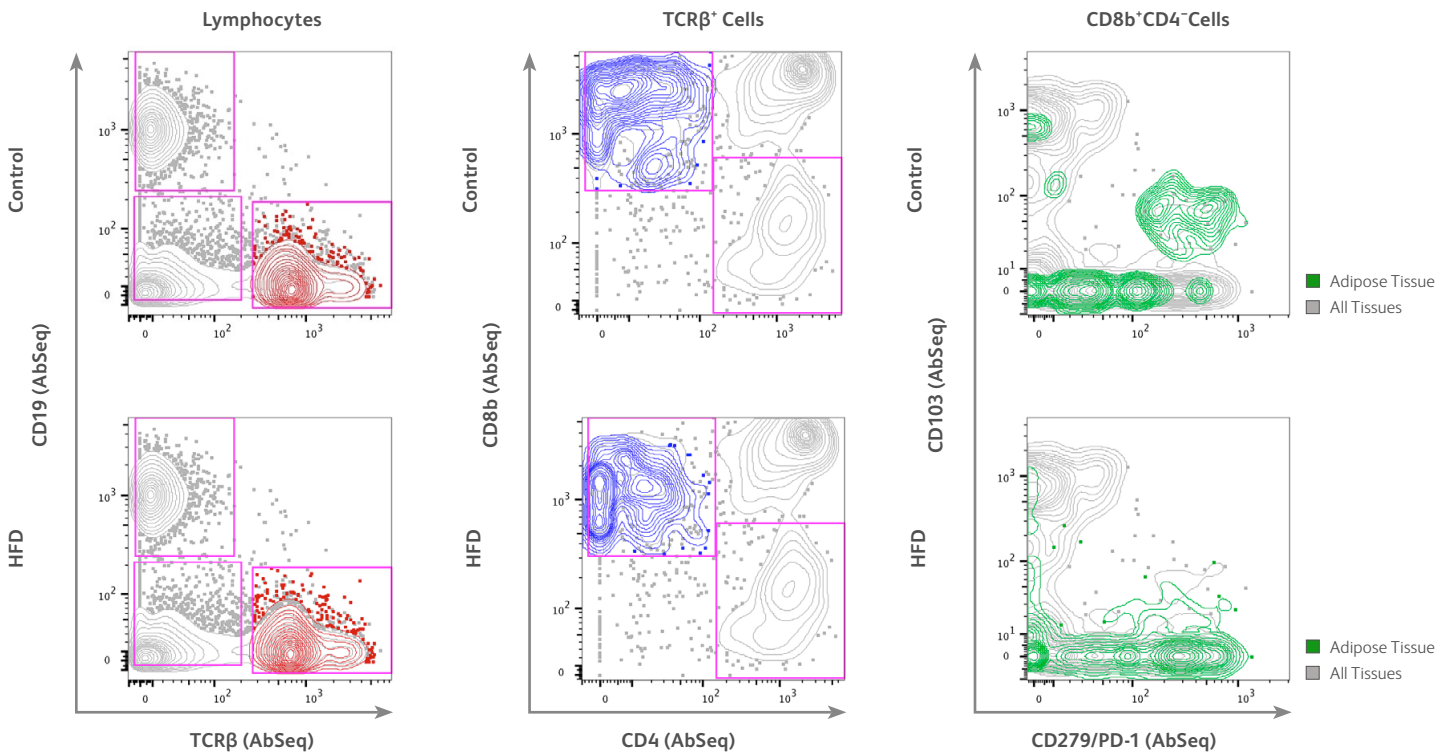


Figure 5B

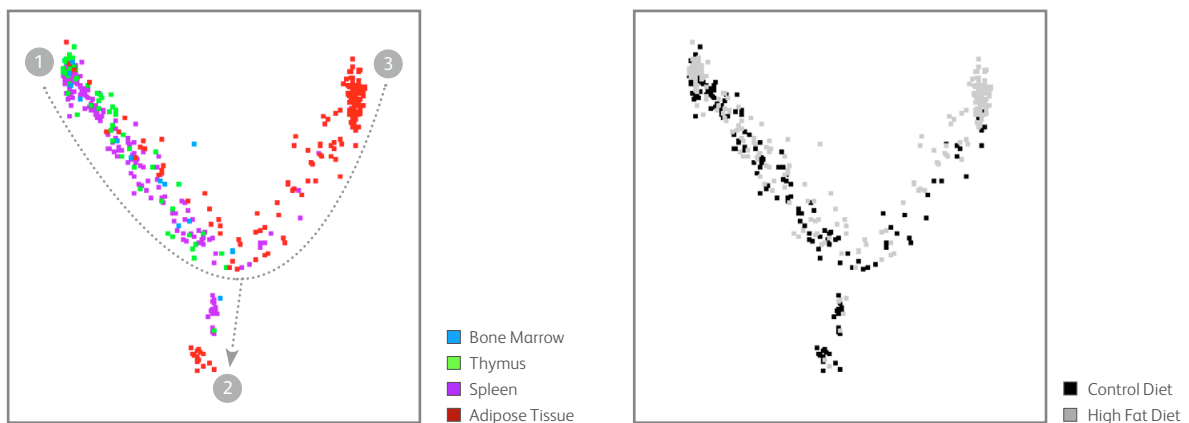


Figure 5. Developmental trajectories of CD8<sup>+</sup> T cells across tissues from control and HFD mice

A. Cells from all tissues were first gated based on TCRβ expression and then CD8b expression. The correlated expression of CD279 (PD-1) and CD103 on gated CD8<sup>+</sup> T cells is shown in green (adipose tissue) and grey (all tissues). B. Monocle trajectory for CD8<sup>+</sup> T cells from all tissues from least (upper left corner, position 1) to most differentiated (upper right corner, position 3) states. The most differentiated CD8<sup>+</sup> T cells located at the end of the trajectory (position 3) corresponds to cells in the adipose tissue.

Figure 6A

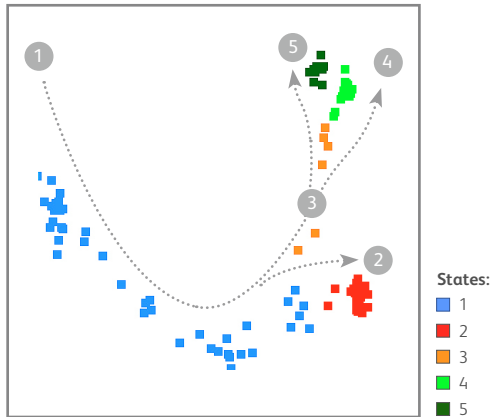


Figure 6B

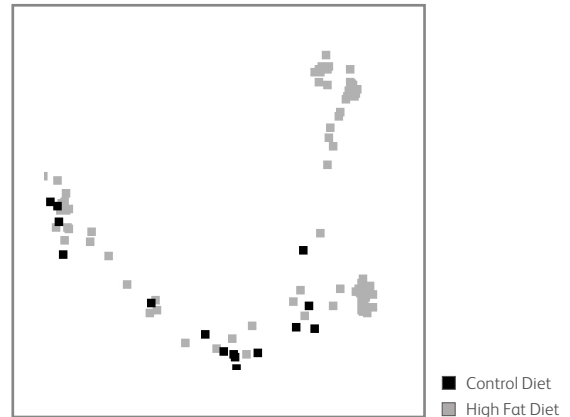
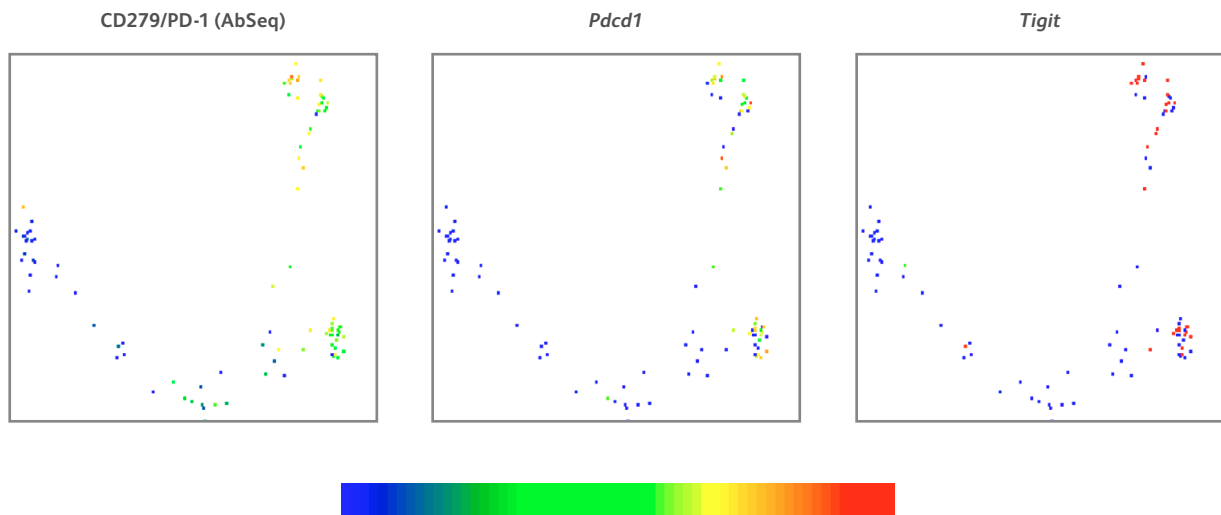


Figure 6C



**Figure 6. Monocle analysis of CD8<sup>+</sup> T cells from HFD adipose tissue suggests that these cells underwent cell exhaustion**

**A.** Monocle trajectory of adipose tissue CD8<sup>+</sup> T cells showing five differentiation states. The most differentiated cells are located at position 5. **B.** CD8<sup>+</sup> T cells trajectory based on diet shows that states 2-5 are detected almost exclusively in HFD mouse. **C.** The expression of exhaustion markers including PD-1 protein (CD279) and mRNA (*Pdcd1*) are detected in the latest differentiation states of CD8<sup>+</sup> T cells.

In conclusion, these results demonstrate that single cell analysis performed on the BD Rhapsody™ Single-Cell Analysis System using BD® AbSeq and BD Rhapsody™ Mouse Immune Response Panel can provide previously unseen immune cell characterization than with mRNA expression or protein analysis alone. This comprehensive single cell analysis platform in combination with powerful bioinformatic tools revealed unique disease state immune cell profiles including signs of CD8<sup>+</sup> T cells exhaustion as well as new insights for HFD-induced inflammation and obesity in mice.

## Ordering information

### Systems and software

#### Description

BD Rhapsody™ Single-Cell Analysis System

BD FACSAria™ Fusion Cell Sorter

BD Rhapsody™ Analysis Pipeline

SeqGeq™

### Reagents

#### Description

BD® AbSeq

BD® Mouse Immune Single-Cell Multiplexing Kit

BD Rhapsody™ Mouse Immune Response Panel

BD Rhapsody™ Targeted mRNA and AbSeq Reagent Kit

BD Pharmingen™ FITC Rat Anti-Mouse CD45

Class 1 Laser Product.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

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BD Life Sciences, San Jose, CA, 95131, USA

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