

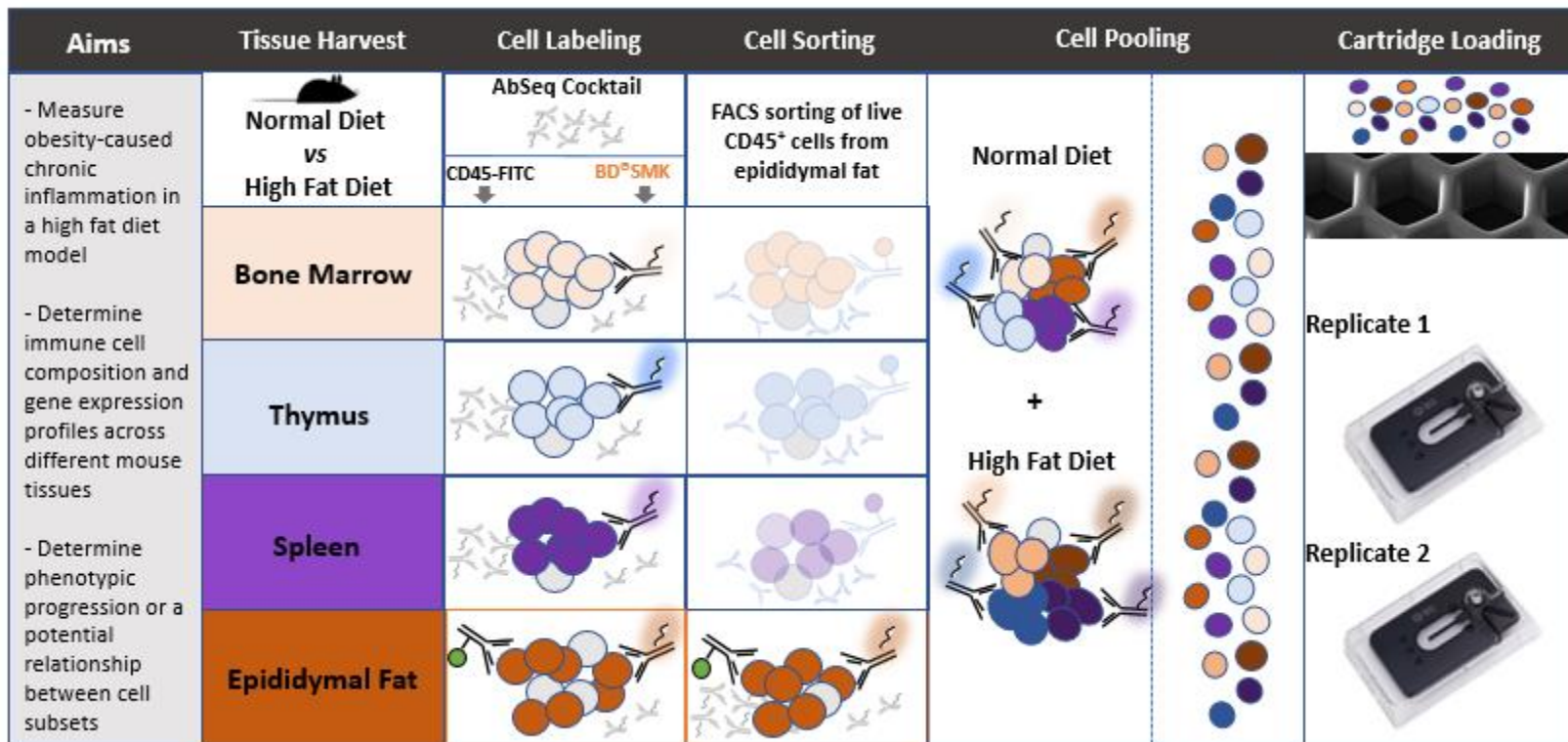


Single cell multiomics analysis of immune cells in obese mice

Background

- Diets high in fat can lead to obesity, which is a major risk factor for the development of various metabolic and inflammatory diseases.
- Here, we demonstrate the usage of the BD Rhapsody™ Single-Cell Analysis system along with BD® AbSeq assays, the BD Rhapsody™ Immune Response Panel and the BD® Mouse Immune Single-Cell Multiplexing Kit (SMK) to characterize obesity-caused chronic inflammation through analyses of immune-cell composition and gene expression profiles in high fat diet (HFD) mice.
- At six weeks of age, mice are placed on either a control diet (10% diet-induced obesity (DIO) or a high fat diet (60% DIO) for 17 weeks before the start of the experiment.

Experimental approach and workflow



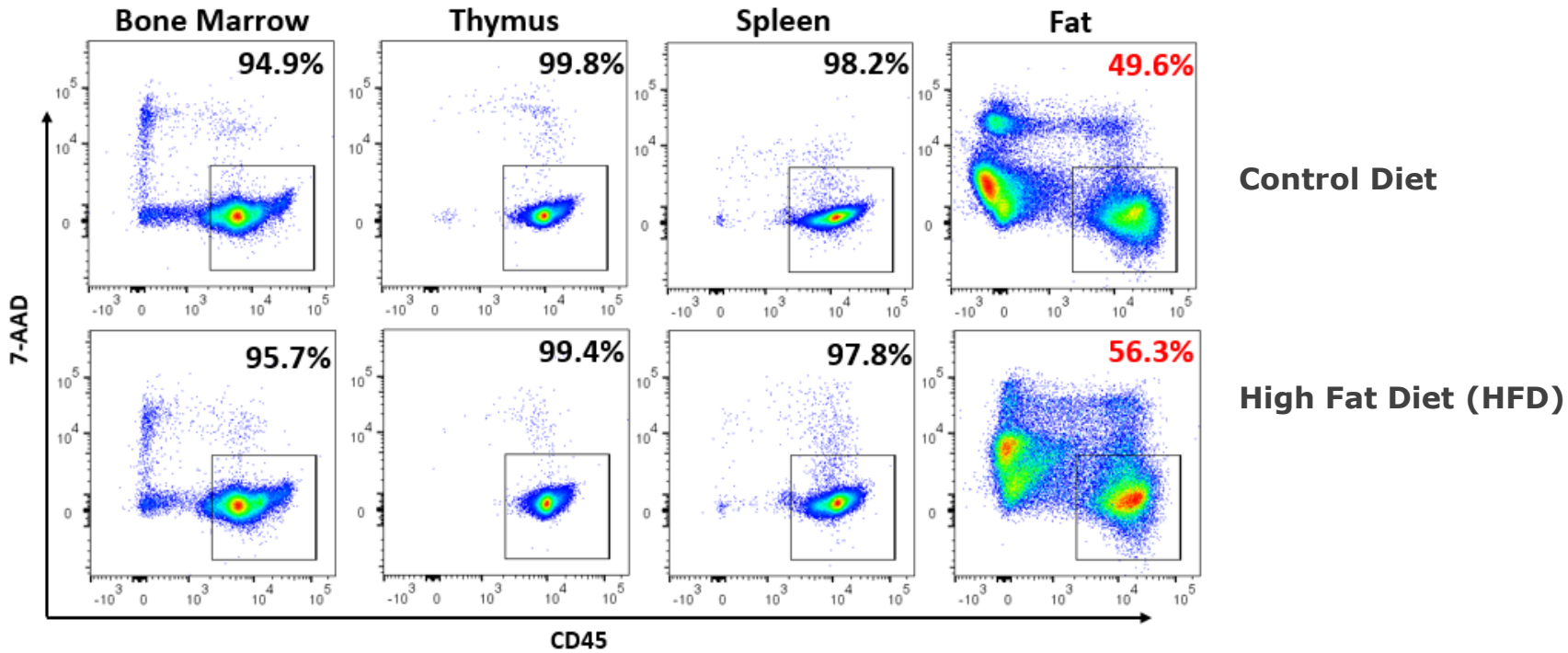
Panel design

30-plex BD® AbSeq Panel		
CD1d	CD25	CD184
CD4	CD44	CD197 (CCR7)
CD5	CD45R (B220)	CD223 (LAG-3)
CD8β	CD49a	CD274
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)	TCRβ

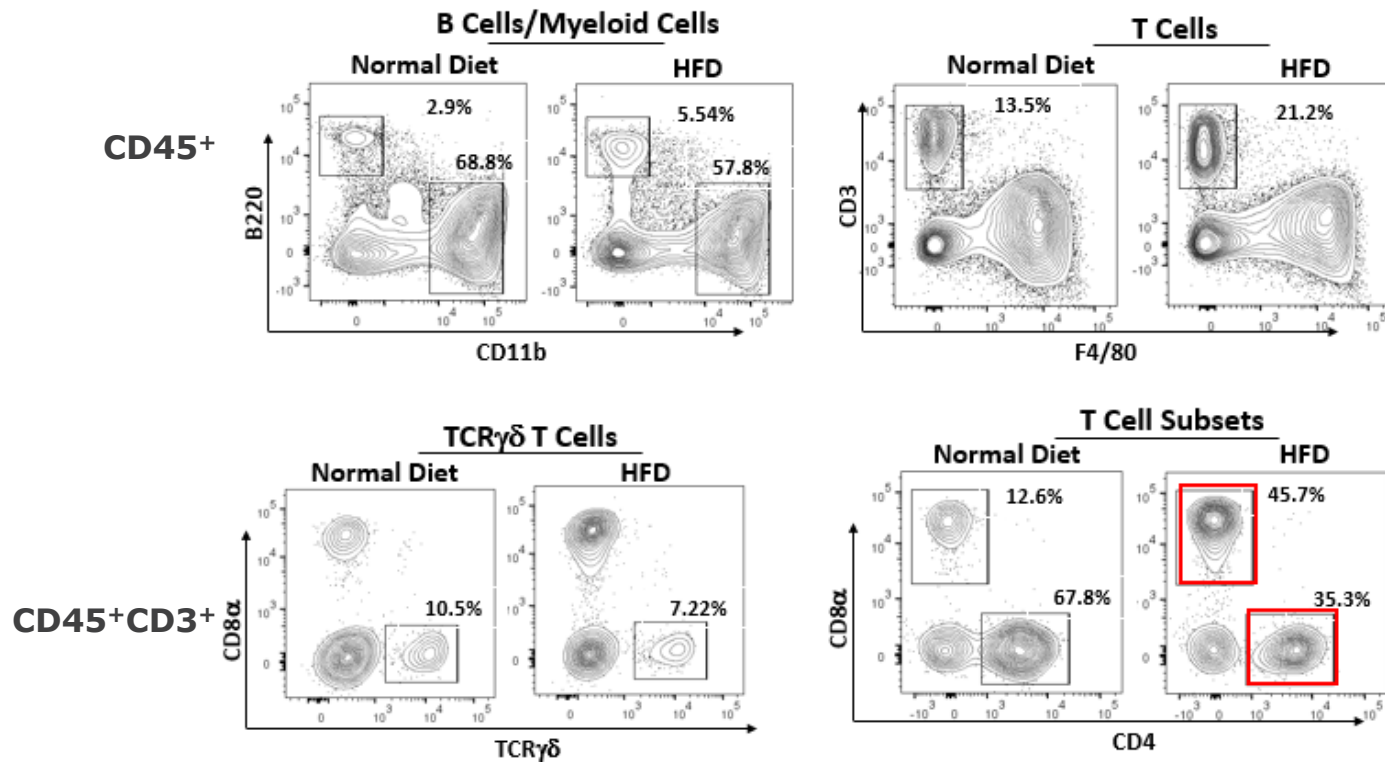
10-color Flow Cytometry Panel	
Marker	Fluorochrome
7-AAD	PerCP-Cy™5.5
B220	BUV737
CD3ε	APC
CD4	APC-H7
CD8α	BUV395
CD11b	APC-R700
CD45	FITC
CD69	BUV605
F4/80	PE
TCRγδ	BV480

- The BD AbSeq panel contains 30 proteins for cell lineage, differentiation, fate and function.
- A companion 10-color flow cytometry panel with selected, overlapping specificities was designed to assess flow cytometry and BD AbSeq concordance.
- The BD Rhapsody Immune Response Panel includes 400 genes involved in immune responses.

Frequency of hematopoietic cells in mouse lymphoid and epididymal adipose tissues

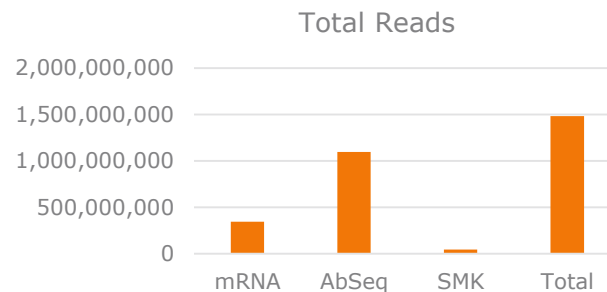
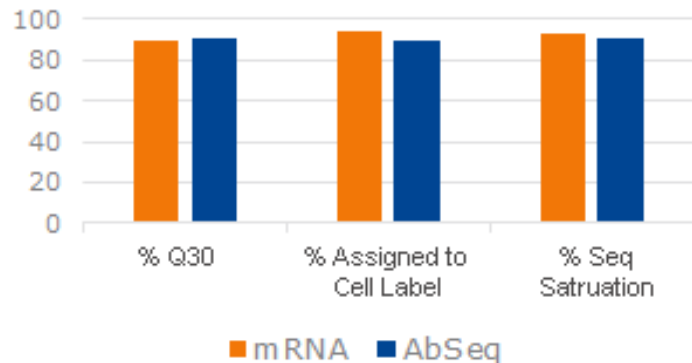


Characterization of immune cell populations in adipose tissue



A HFD causes a decrease in the ratio of CD4/CD8 in adipose tissue.

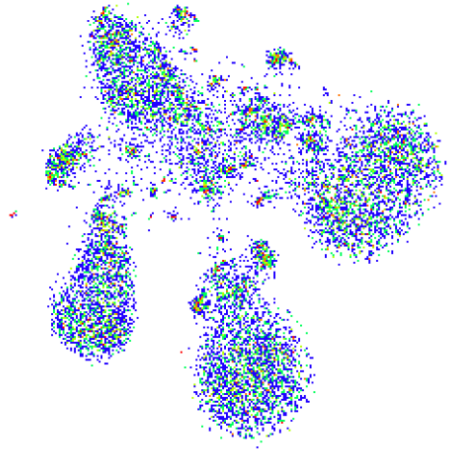
Sequencing metrics



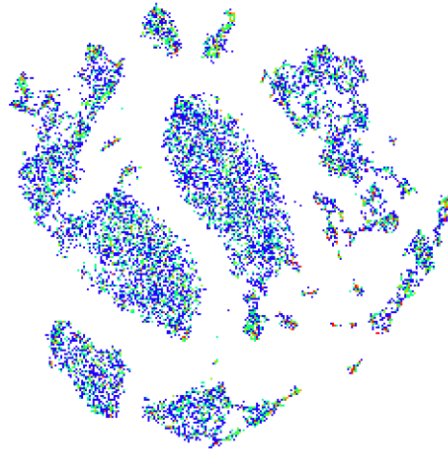
Sample Tag Metrics

Sample Name	ST Cell #	Rep1	Rep2	Total
Control Adipose Tissue	ST 1	972	928	1900
Control Bone Marrow	ST 2	1501	1527	3028
Control Spleen	ST3	1566	1337	2903
Control Thymus	ST4	1074	1006	2080
HFD Adipose Tissue	ST5	1196	1029	2225
HFD Bone Marrow	ST6	1471	1308	2779
HFD Spleen	ST7	1389	1229	2618
HFD Thymus	ST8	1129	1039	2168
	Multiplet	1048	818	1866
	Undetermined	72	42	114
	Total	11418	10263	21681

The combination of BD AbSeq assays and the BD Rhapsody Immune Response Panel improves cell clustering



t-SNE: Ab Alone



t-SNE: mRNA Alone



t-SNE: Abs + mRNA

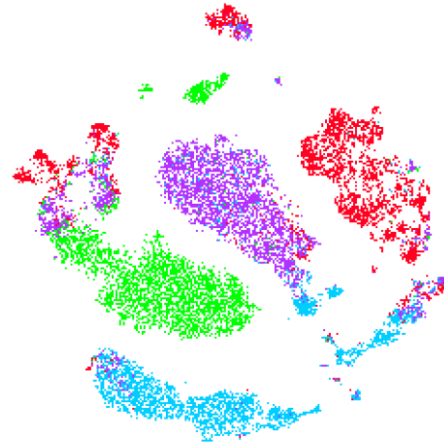
Dimensionality reduction using t-SNE plots

All Tissues (HFD and Control Diet)



Key:

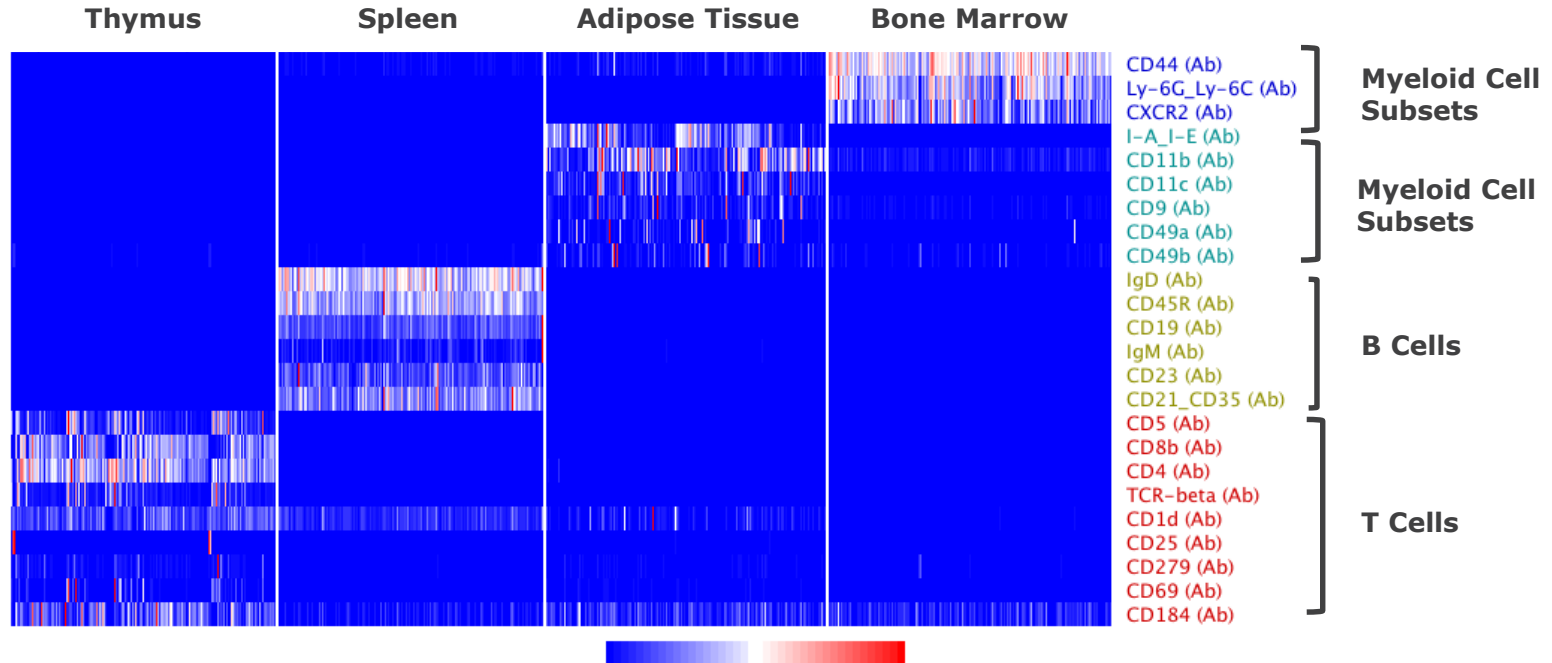
- Thymus
- Spleen
- Adipose Tissue
- Bone Marrow



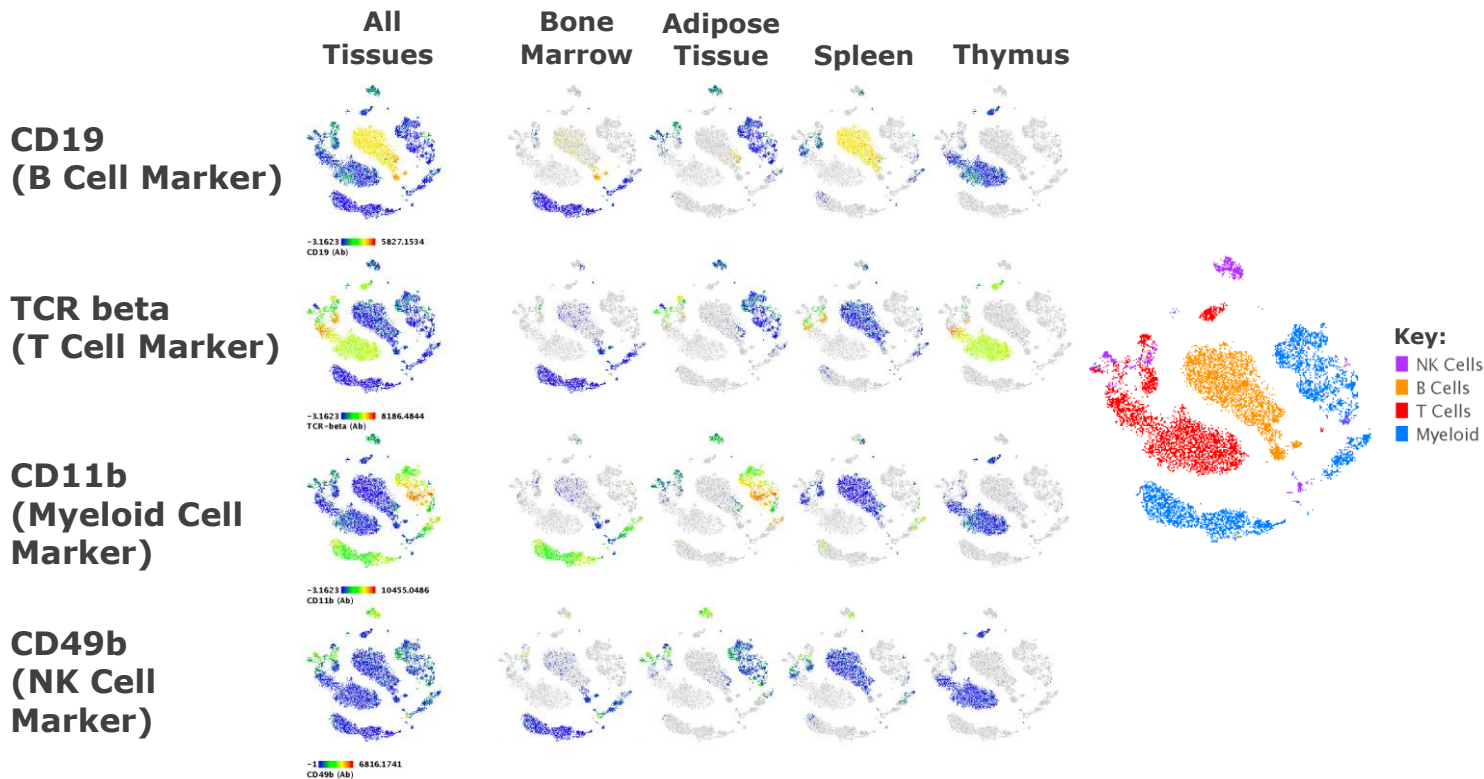
Cell Islands Created From
Concatenation of All Tissues

BD AbSeq assays identify major cell populations in each tissue

The heat map shows the most highly expressed proteins in each tissue.



Robust identification of target cell populations

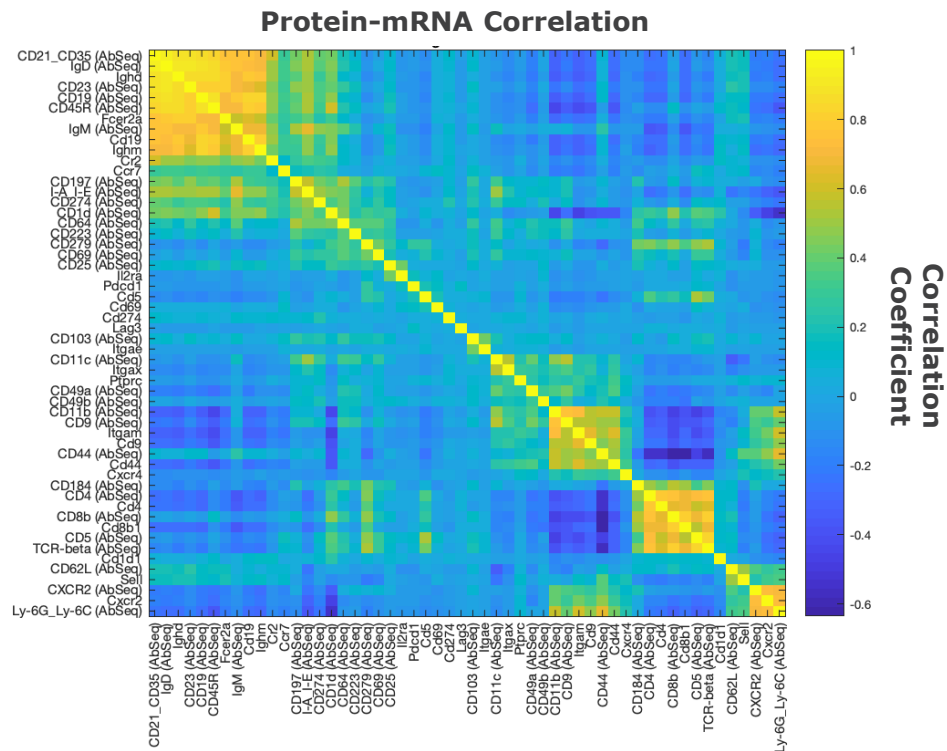


BD, the BD Logo and Rhapsody are trademarks of Becton, Dickinson and Company or its affiliates. All other trademarks are the property of their respective owners. © 2019 BD. All rights reserved.



Single cell protein and mRNA correlation

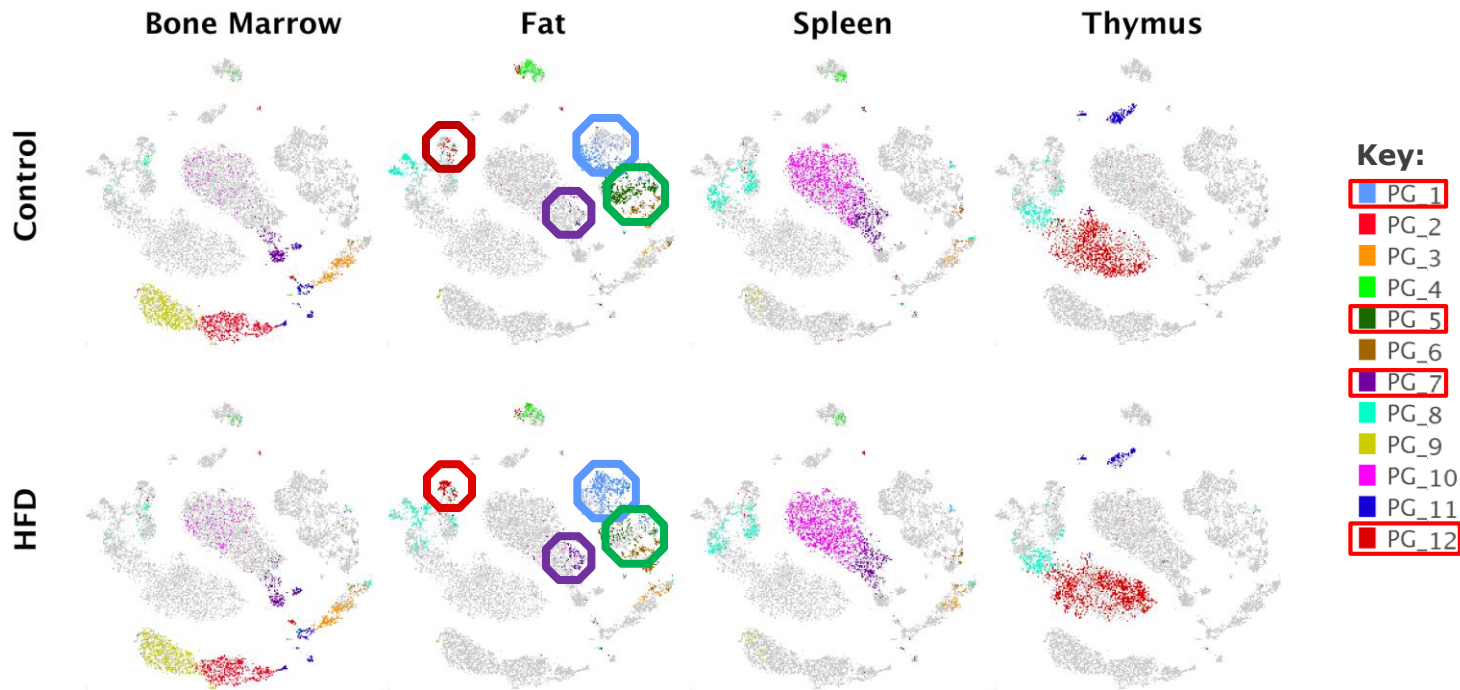
AbSeq (Protein)	mRNA
CD1d	Cd1d1
CD4 (AbSeq)	Cd4
CD5 (AbSeq)	Cd5
CD8b (AbSeq)	Cd8b1
CD9 (AbSeq)	Cd9
CD11b	Itgam
CD11c	Itgax
CD19	Cd19
CD21_CD35	Cr2
CD23	Fcer2a
CD25	Il2ra
CD44	Cd44
CD45R (AbSeq)	Ptprc
CD49a (AbSeq)	Itga1
CD49b (AbSeq)	Itga2
CD62L (AbSeq)	Sell
CD69 (AbSeq)	Cd69
CD103	Itgae
CD182 (CXCR2) (AbSeq)	Cxcr2
CD184	Cxcr4
CD197	Ccr7
CD223	Lag3
CD274	Cd274
CD279	Pdcd1
I-A_I-E (AbSeq)	H2-Ab
IgD (AbSeq)	Ighd
IgM (AbSeq)	Ighm
Ly-6G_Ly-6C (AbSeq)	Ly6g_Ly6c
TCR-beta (AbSeq)	Tcr



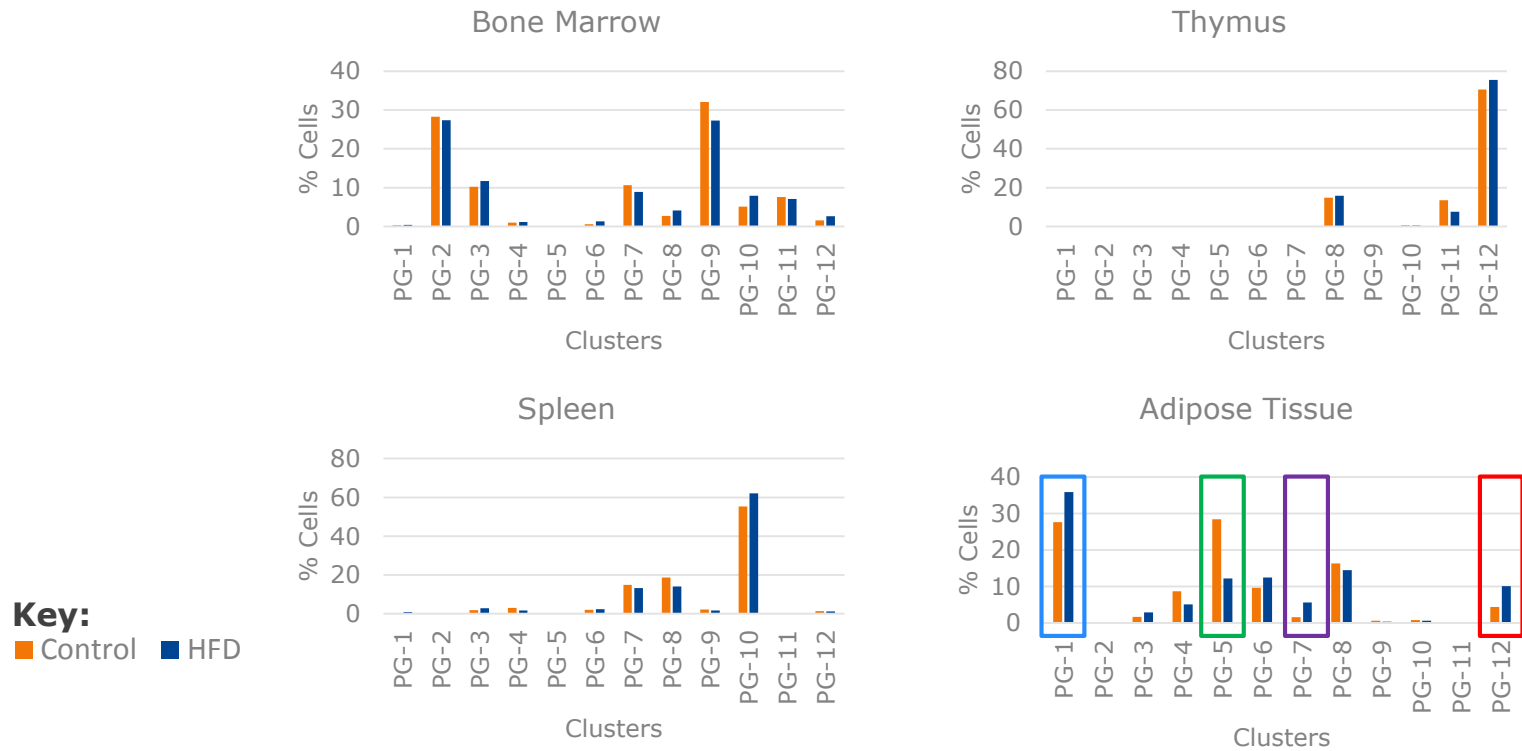
Unbiased cell clustering identifies multiple cell phenotypes across different samples



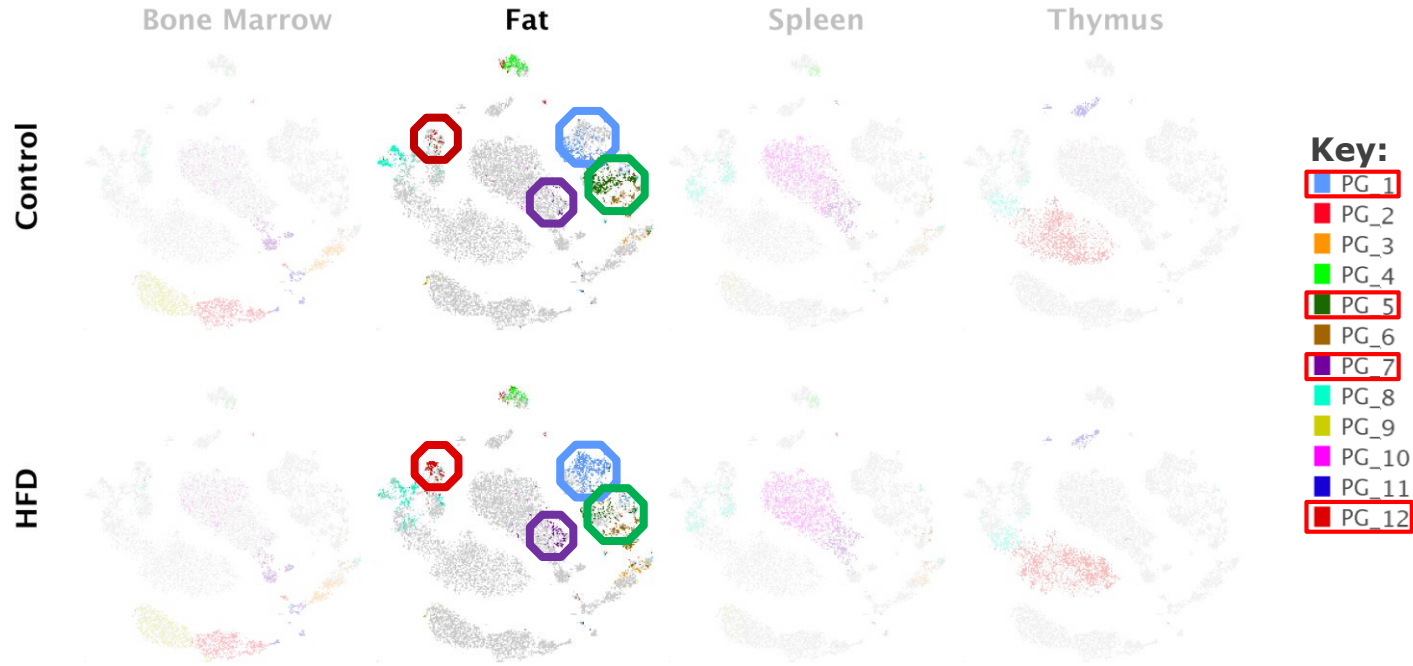
Unbiased clustering reveals disease-associated cell phenotypes



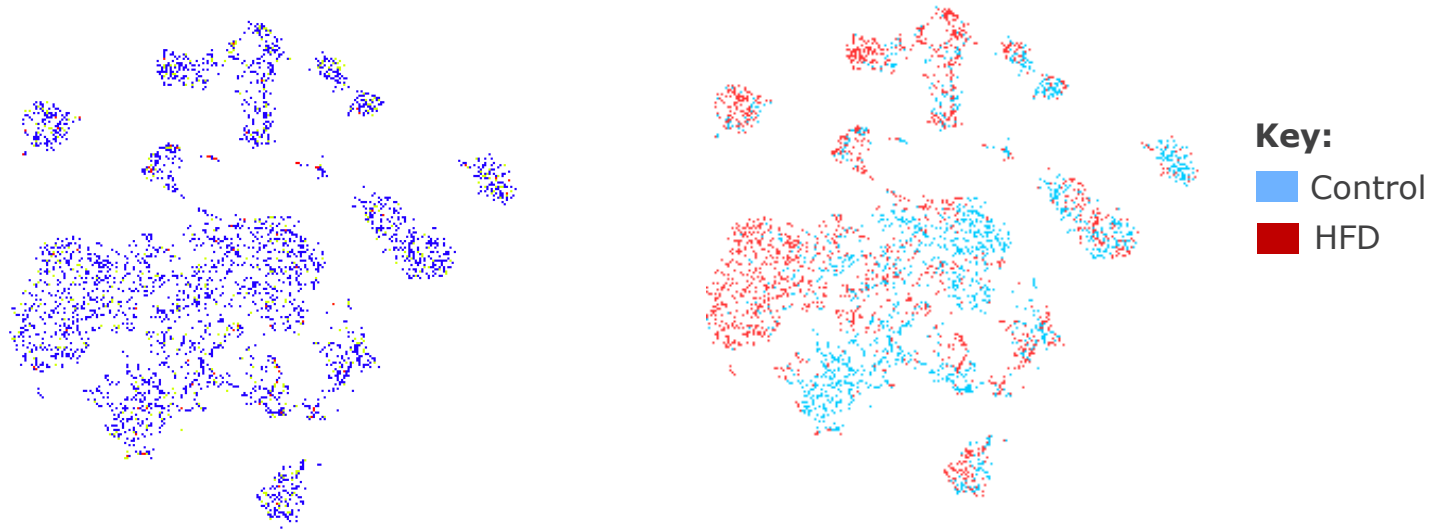
Subsets of clusters show differences in cell number for adipose tissues of HFD mice



Unbiased clustering reveals disease-associated cell phenotypes

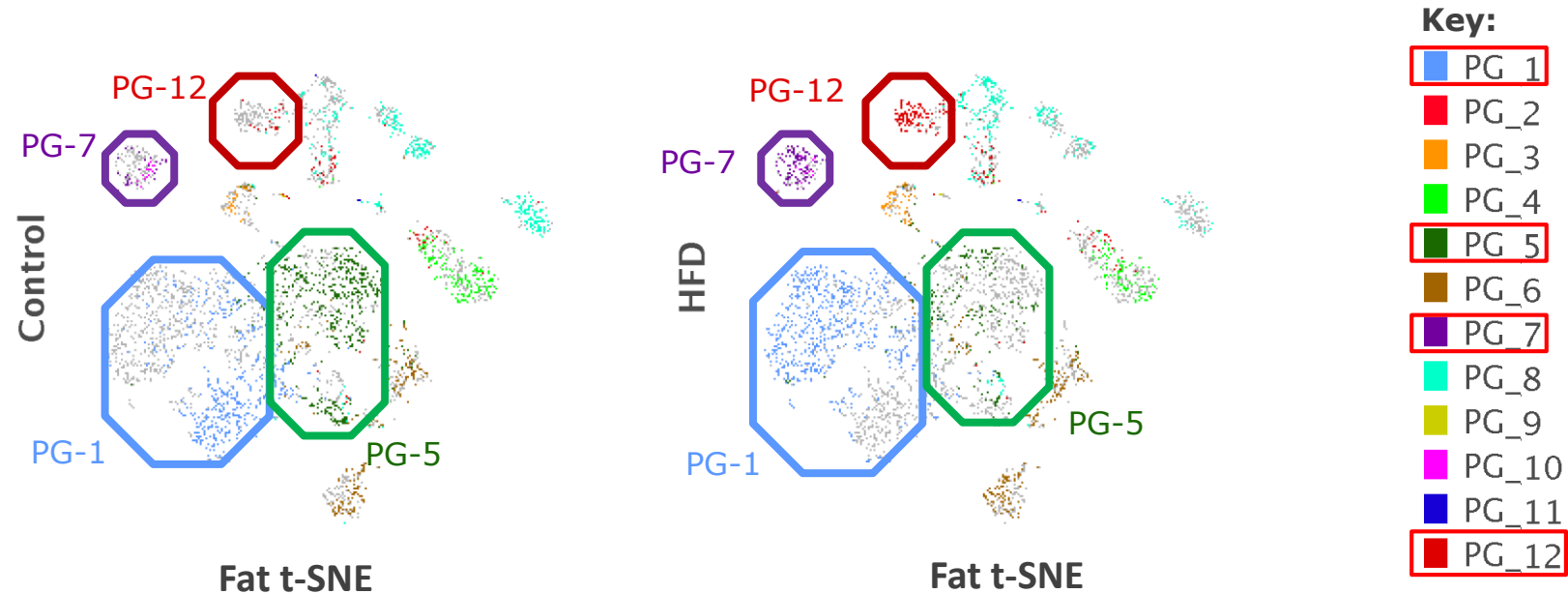


t-SNE plots of adipose tissue of control and HFD mice

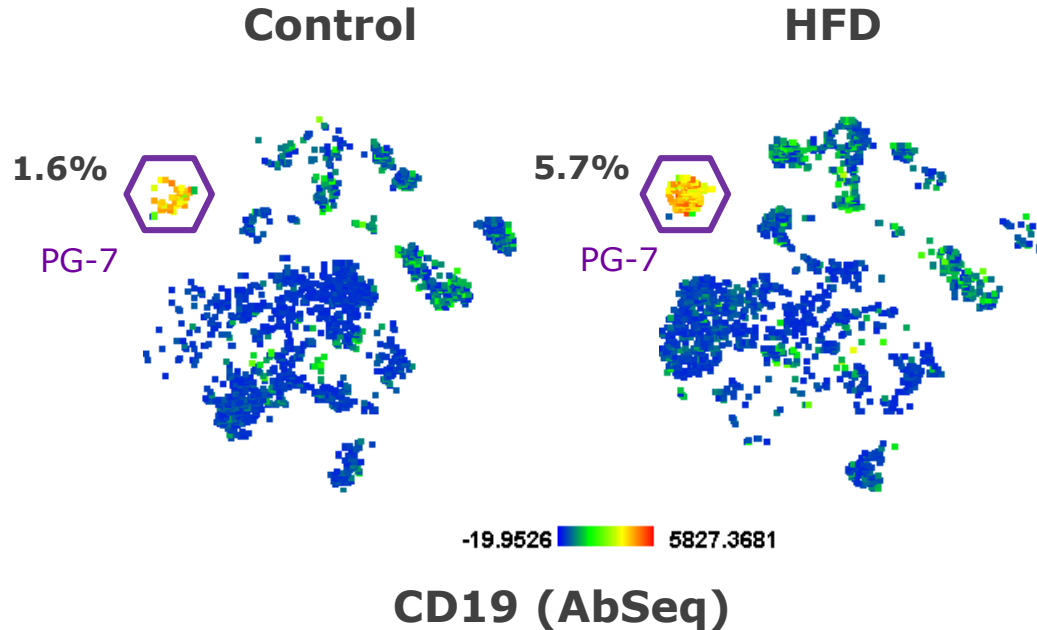


Adipose Tissue t-SNE Plots

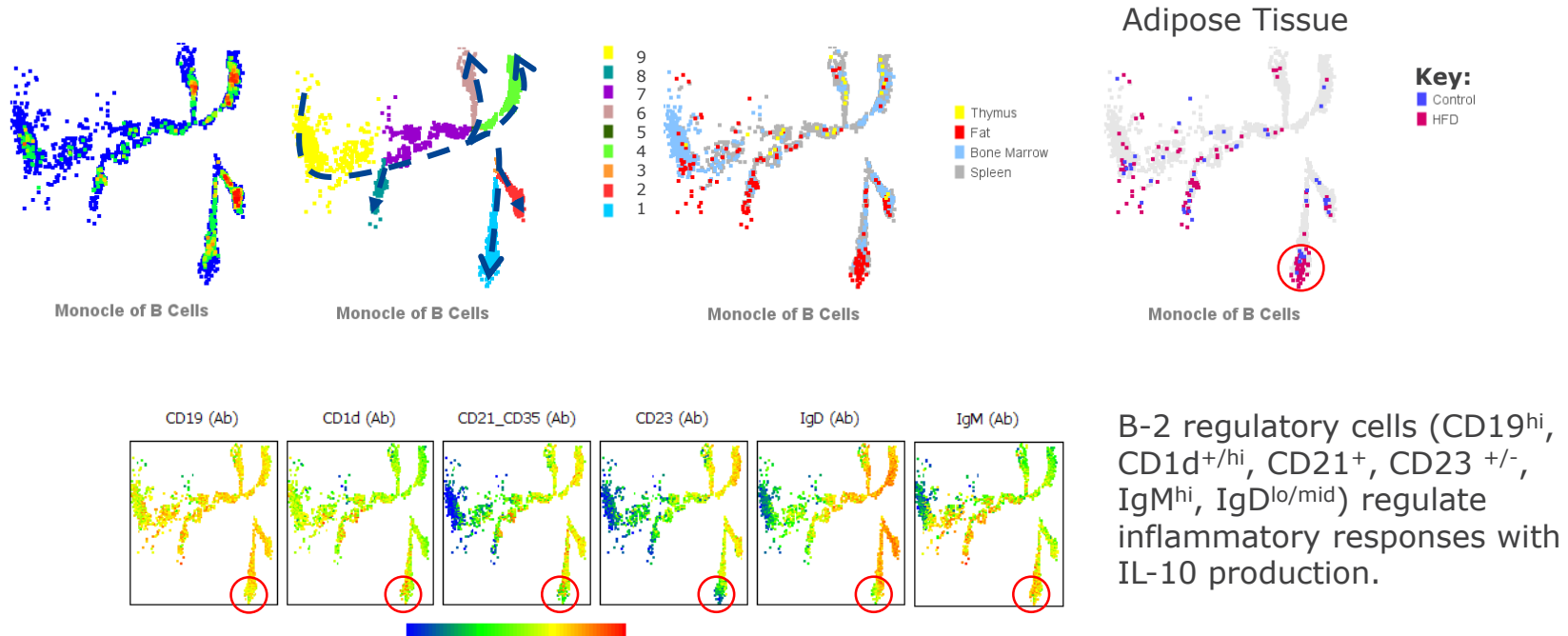
HFD causes drastic changes to immune cell composition in adipose tissue



HFD increases the frequency of B cells in adipose tissue

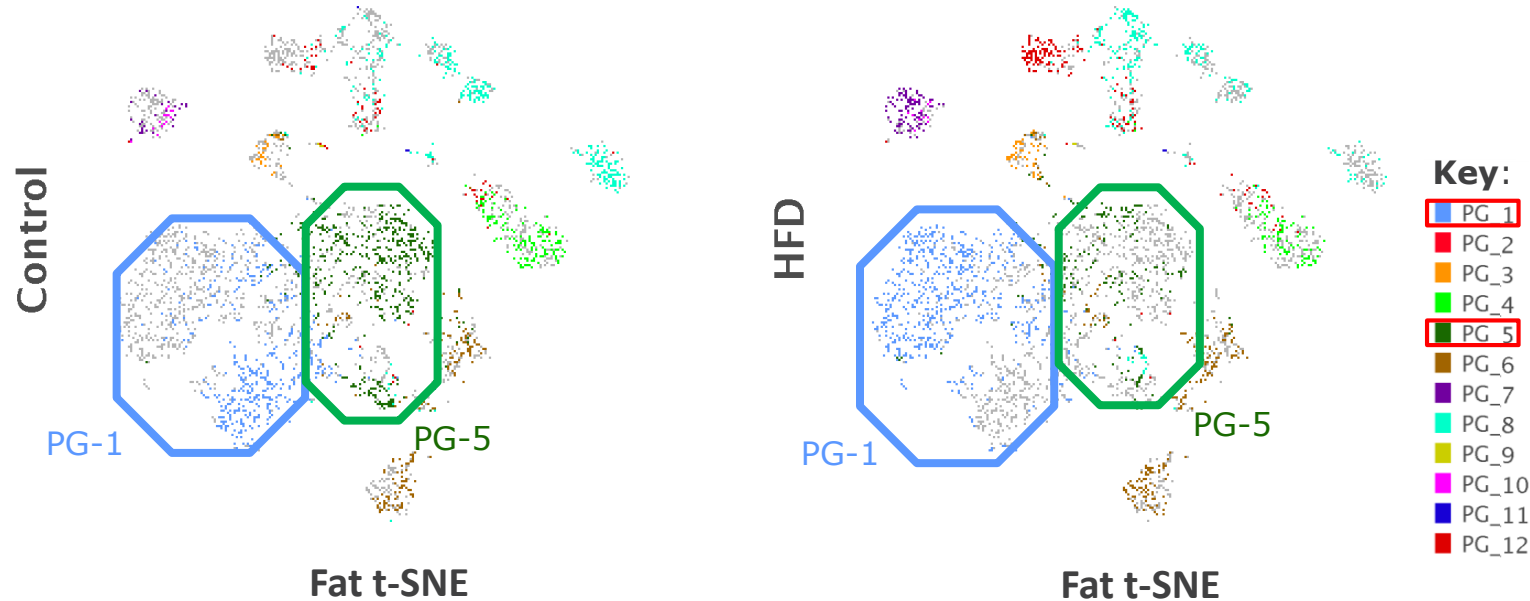


HFD induces the B cells similar to B-2 regulatory cells



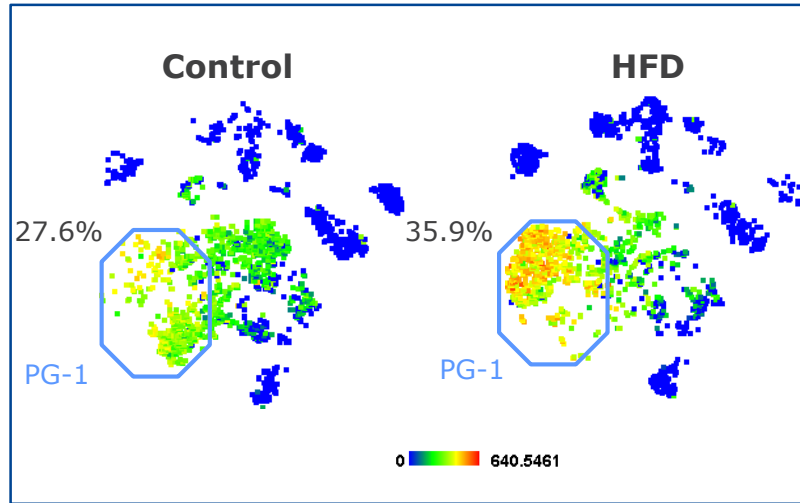
Shaikh *et al.*, 2014

HFD causes drastic changes in immune cell composition in adipose tissue



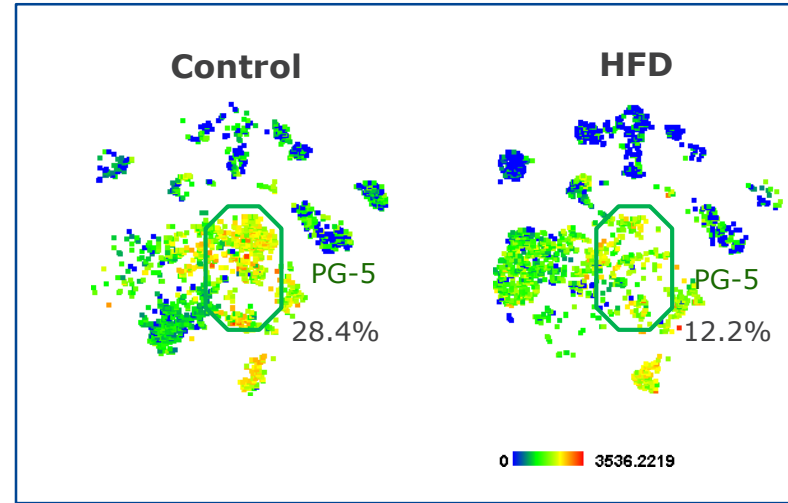
Distinct populations of myeloid cells in the adipose tissue of obese mouse

Accumulation of Adipose Tissue Macrophages in HFD



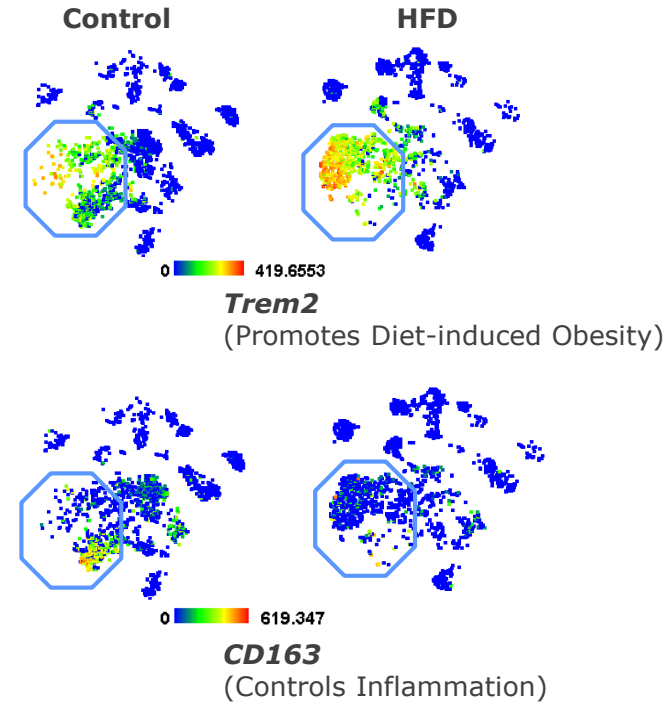
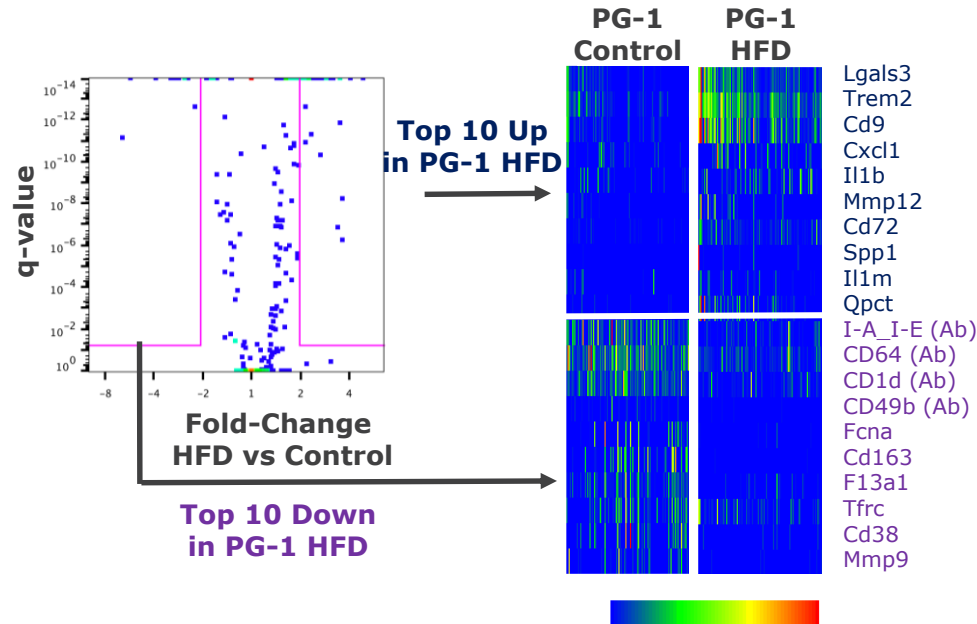
Adgre1

Loss of CD11c⁺ Cell Subsets in HFD



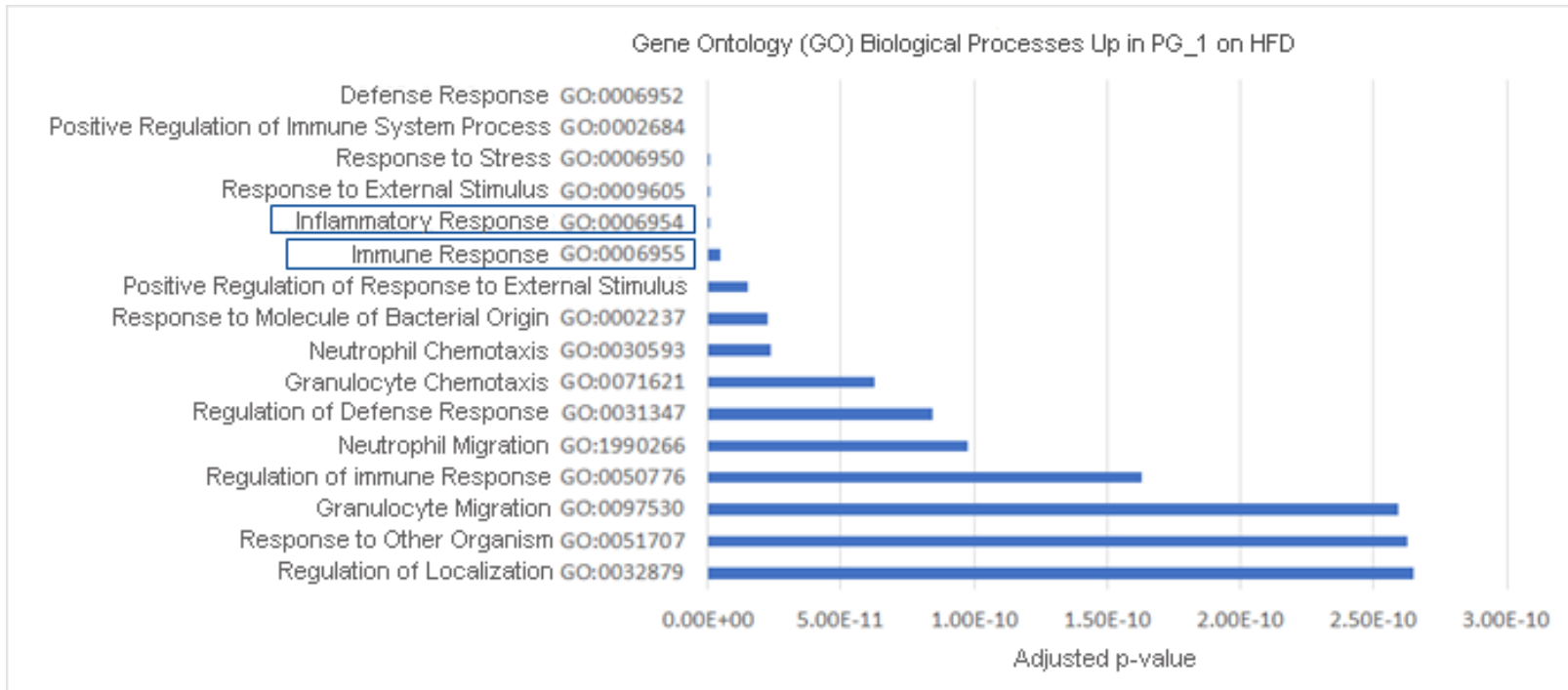
CD11c (AbSeq)

Adipose tissue macrophages in obese mouse exhibit an inflammatory gene signature

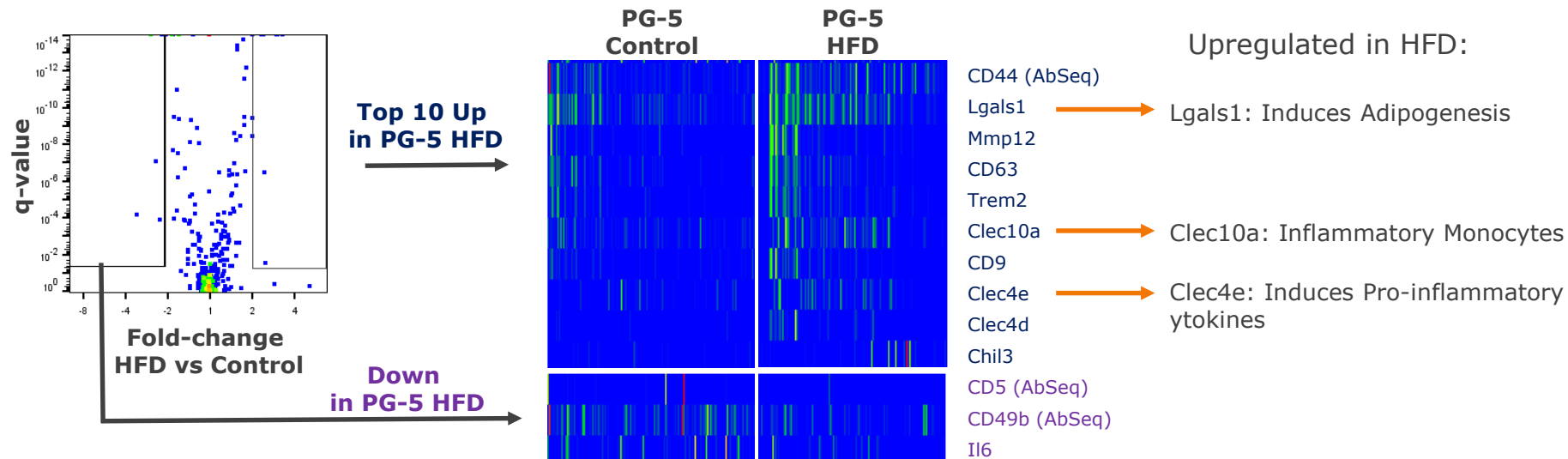


Park M. et al, *Diabetes*, 2015
 Junghwan Baek, *Diabetes*, 2018
 David A. et al, *PNAS*, 2018

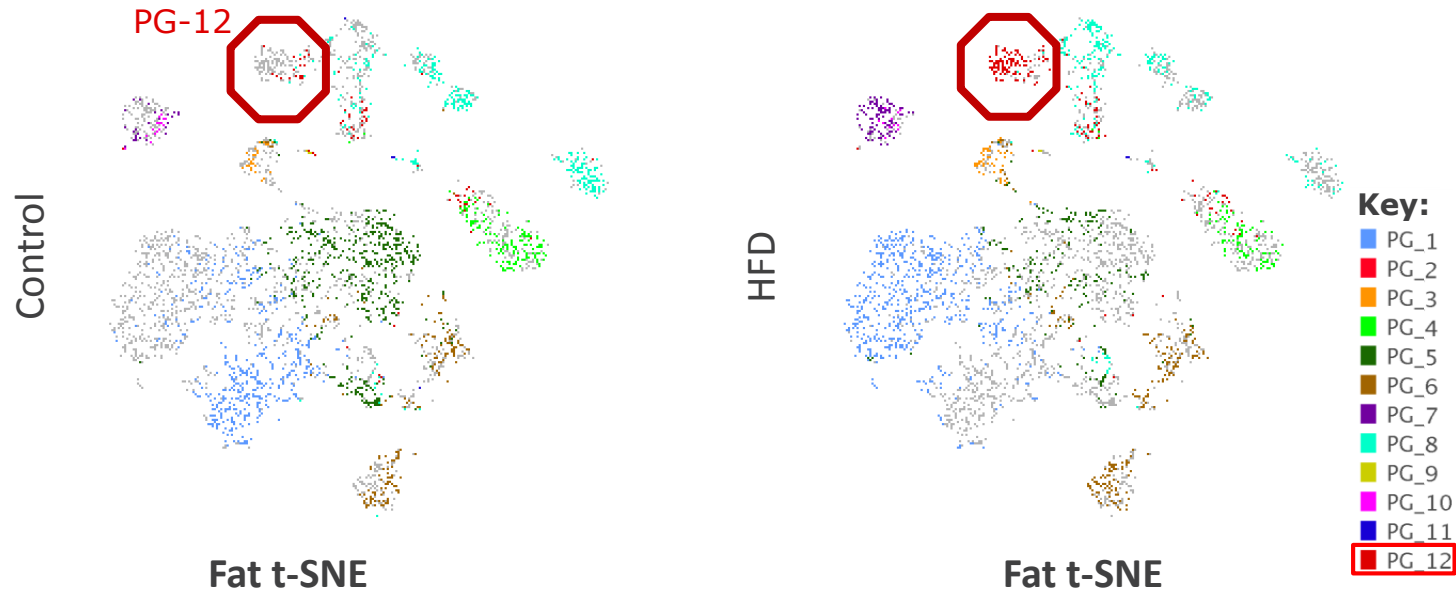
HFD causes upregulation of genes associated with inflammation in adipose tissue macrophages



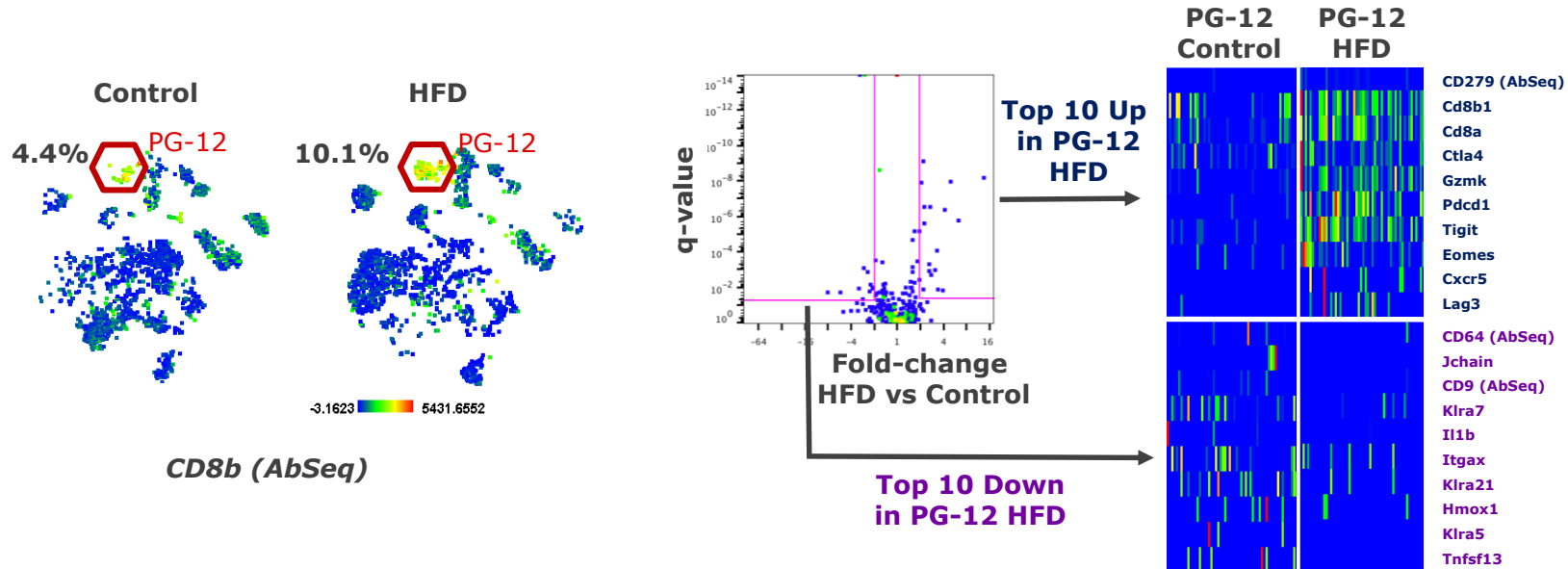
CD11c⁺ cells express genes related to immunoregulation, cell migration and adipogenesis



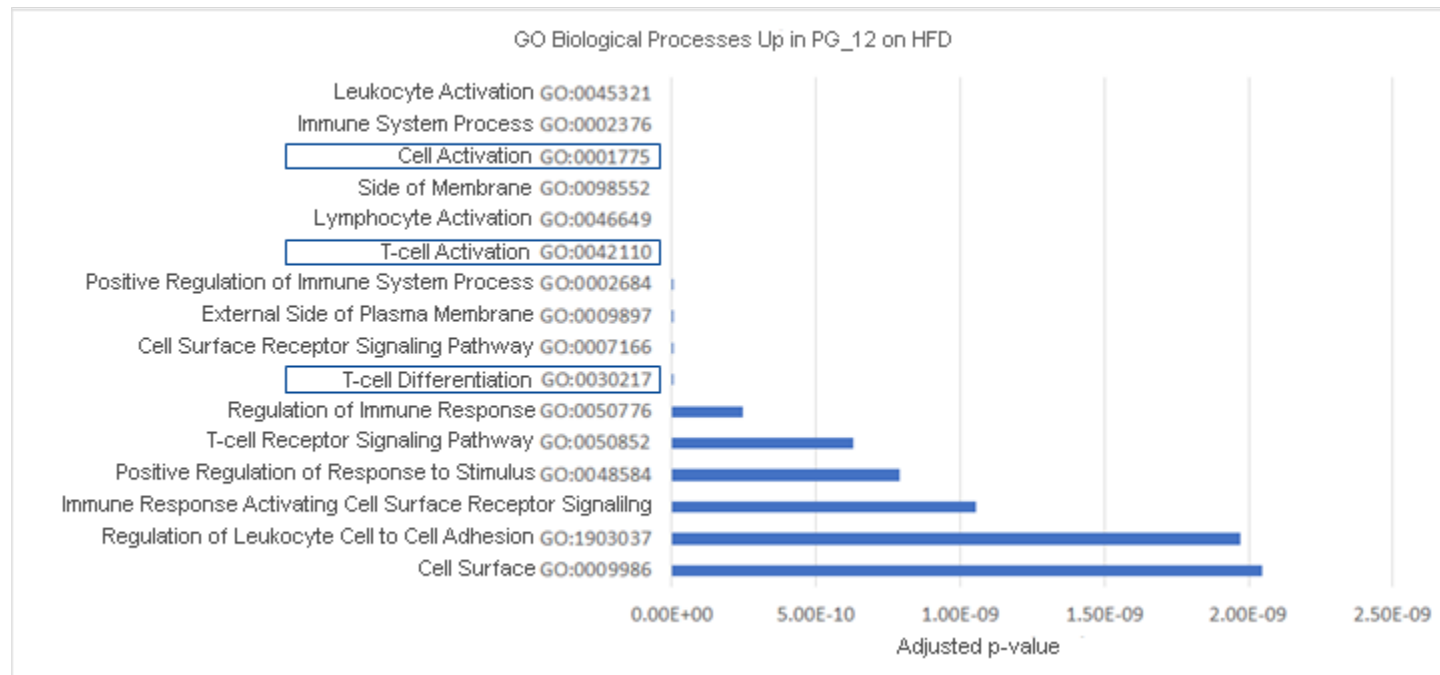
HFD causes drastic changes in immune cell composition in the epididymal adipose tissue



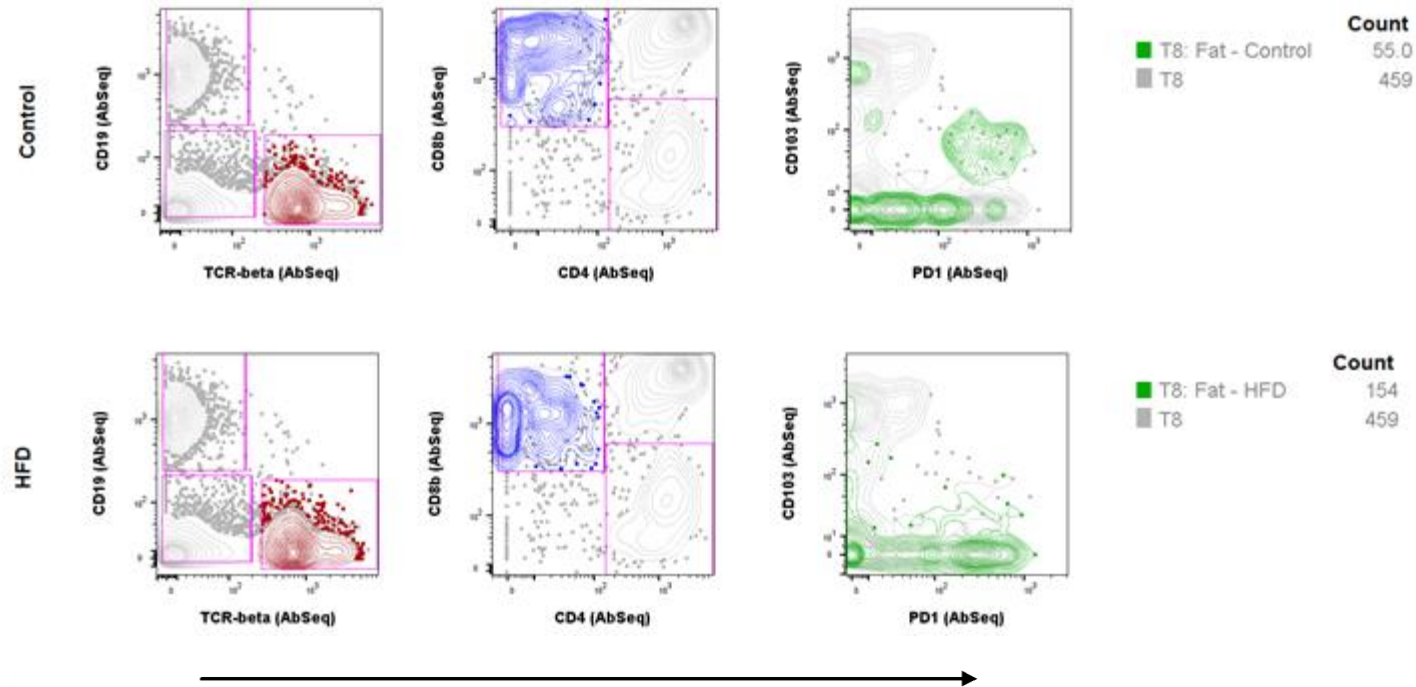
Unique population of cytotoxic cells in the adipose tissue of an obese mouse exhibits signs of exhaustion



A HFD causes upregulation of genes associated with T-cell activation in cytotoxic cells

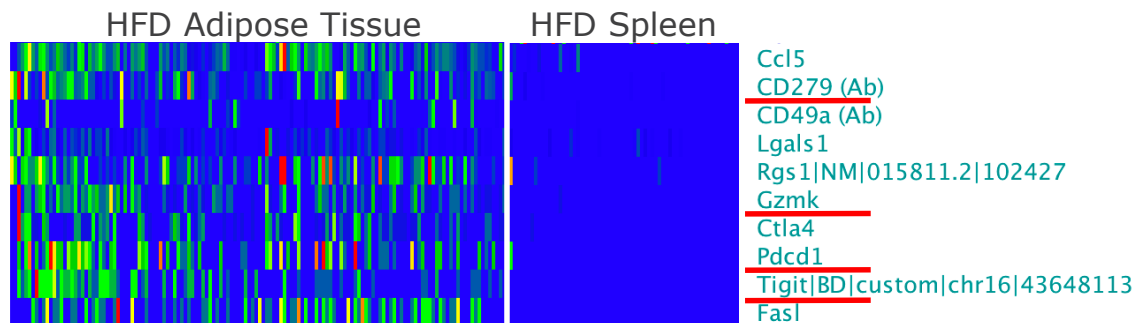


Cytotoxic cells infiltrate the adipose tissue of obese mice and acquire a profile of exhausted cells

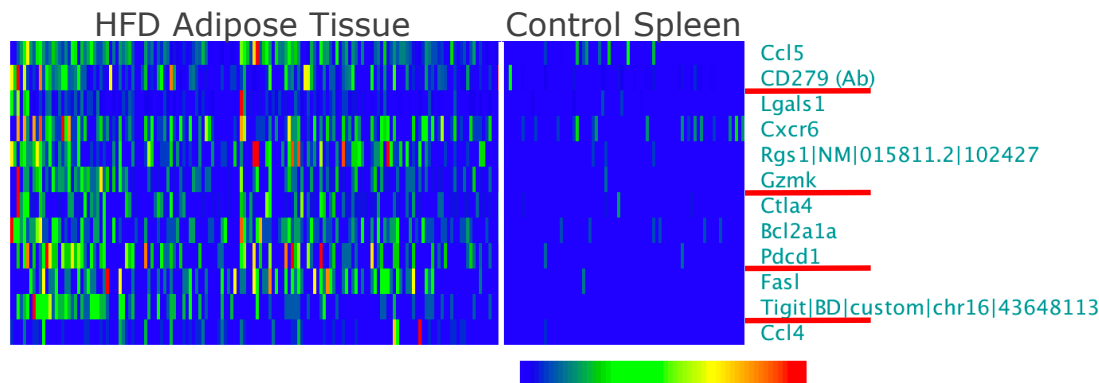


Cytotoxic cells from epididymal adipose tissue, but not spleen tissue, exhibit signs of exhaustion in obese mice

Up-regulated in HFD adipose tissue compared to HFD spleen

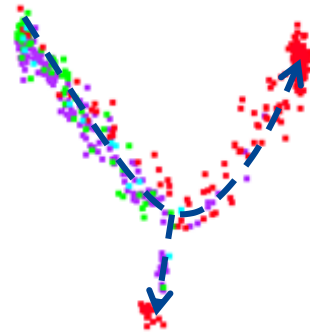


Up-regulated in HFD adipose tissue compared to control spleen



Analysis of cytotoxic cell states across different tissues

A high fat diet induces the transition to an exhausted cell phenotype.



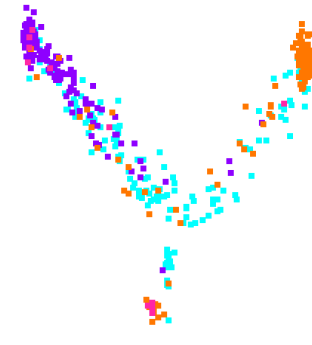
Tissue

	Count
Bone Marrow	13.0
Fat	181
Thymus	66.0
Spleen	199



Diet

	Count
HFD T8 Cells	254
Control T8 Cells	205

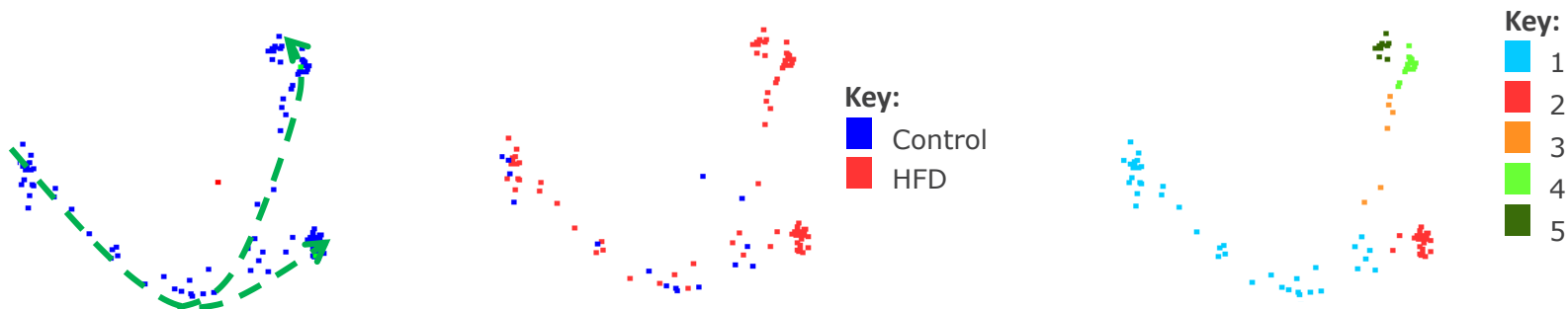


Exhausted/Activated

	Count
PD-1+, ITGAE+	12.0
PD-1+, ITGAE-	108
PD-1-, ITGAE+	161
PD-1-, ITGAE-	172

Adipose tissue-infiltrating cytotoxic cells from HFD mouse co-express PD-1 and *Tigit*

Monocle of Adipose Tissue CD8⁺ Cells



CD103 (AbSeq)

PD-1 (AbSeq)

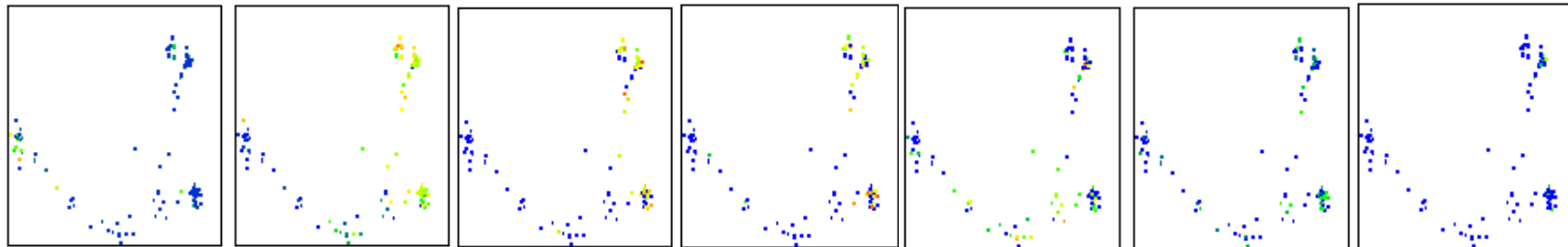
Pdcd1

Tigit

CD49a (AbSeq)

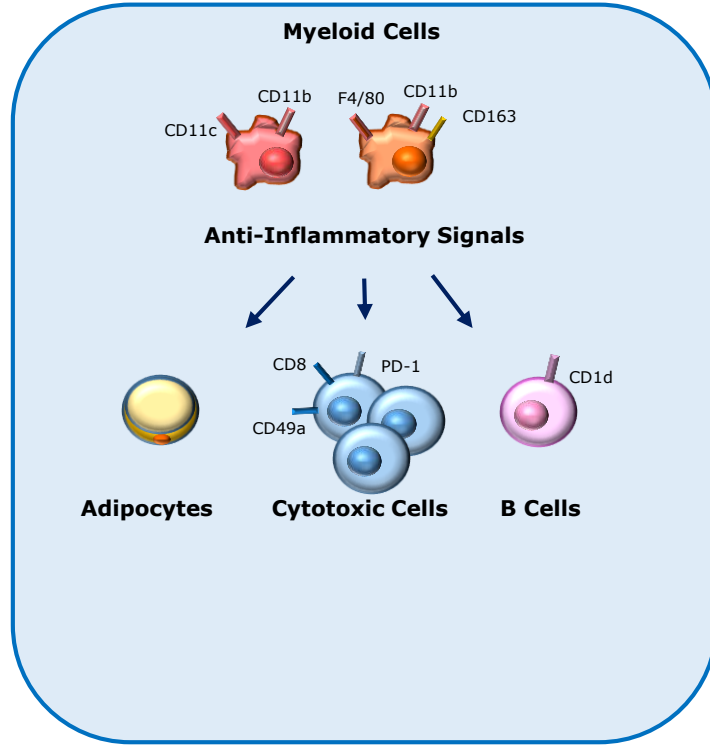
CD69 (AbSeq)

Lag3

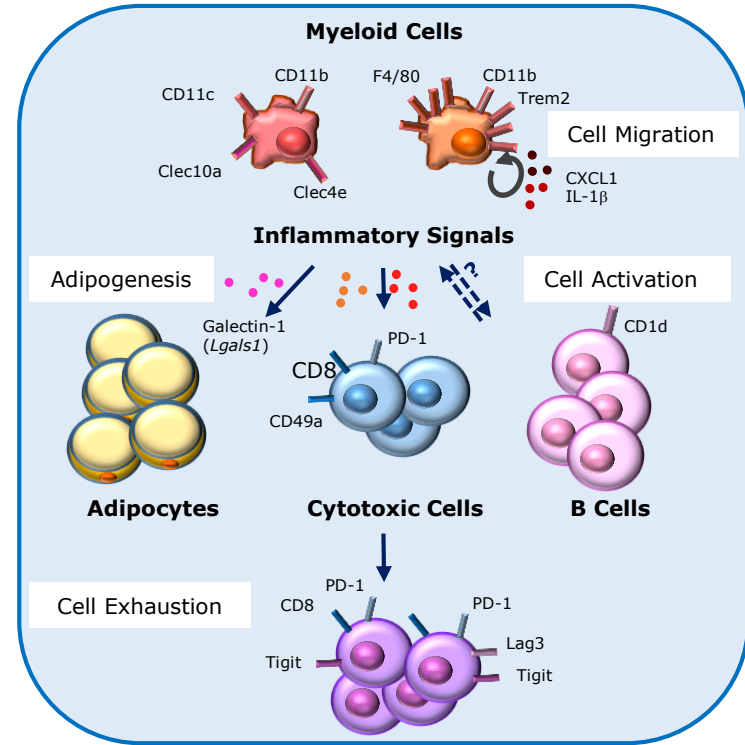


Working model

Healthy Adipose Tissue



Low-Grade Chronic Inflammation in Adipose Tissue



Summary

- The BD Single-Cell Multiplexing Kit allows assessment of immune cells from bone marrow, spleen, thymus and epididymal adipose tissue from control and high fat diet mice simultaneously.
- The BD Single-Cell Multiplexing Kit provides high-confidence information on relationships between samples without batch effects.
- BD AbSeq assays allow effective cell clustering, cell type identification and obesity related phenotyping of immune cells.
- B cells similar to B-2 regulatory cells in adipose tissue increased in mice given the high fat diet.
- Inflammatory and adipogenic genes were increased in adipose tissue myeloid cells in mice given the high fat diet.
- Cytotoxic cells were activated/exhausted in adipose tissue of mice given the high fat diet.

Thank you!



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

BD, the BD Logo and Rhapsody are trademarks of Becton, Dickinson and Company or its affiliates. All other trademarks are the property of their respective owners. © 2019 BD. All rights reserved.