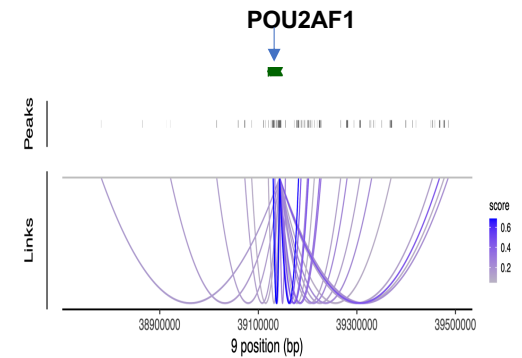


Extensive Identification of Expressed Regions and Regulatory Elements in Fetal, Adult, and Immune Cell Populations in the Pig

Christopher Tuggle
Iowa State University

FAANG Workshop 2023



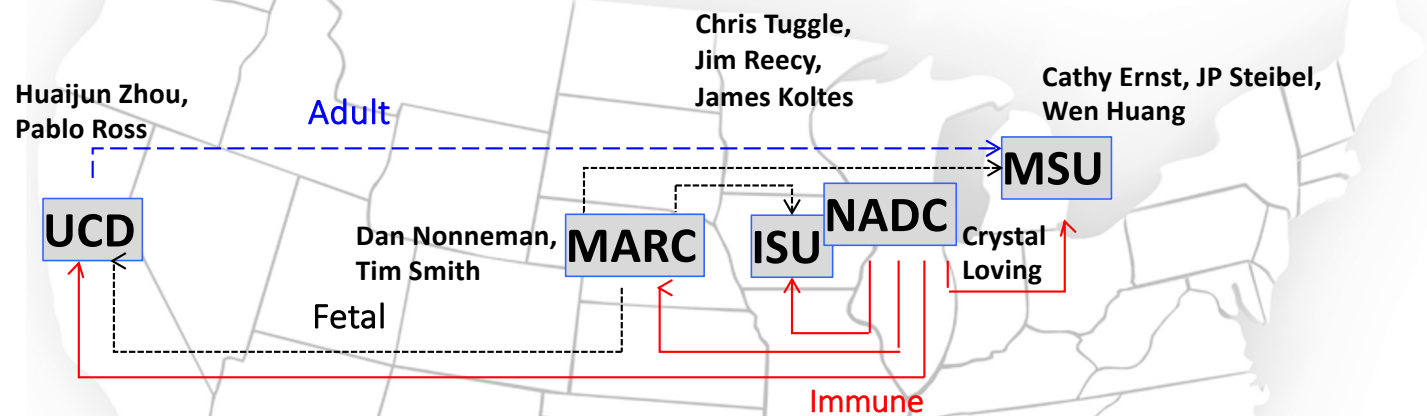
IOWA STATE UNIVERSITY



NIFA Project 2018-67015-2701

IOWA STATE UNIVERSITY
OF SCIENCE AND TECHNOLOGY

Pig FAANG: Community Sharing of Samples and Expertise



<u>Location</u>	<u>Tissue collection</u>	<u>Sample Analyses</u>
ISU	None	Histone CHIP, ATAC-Seq
MARC	Fetal*	RNAseq, IsoSeq
MSU	None#	DNA Methylation (WG-BS)
NADC	Immune	None
UCD	Adult#	Histone CHIP, ATAC-Seq, RAMPAGE

*Fetal Tissue already collected
 # Some adult tissues to be collected at MSU through 2016 AFRI project to UCD

Functional Annotation of the Porcine Genome

Overall goal: catalog functional elements in the porcine genome for >40 biological states, create Chromatin state map

Aim 1 Adult tissues

- extend FAANG pilot of 8 -> 25 tissues

Aim 2 Fetal tissues

- Identify and correlate allele-specific expression and AS chromatin modification
- Parent-of-origin effects (reciprocal crosses Meishan x WhiteCross)

Aim 3 Immune System

- Stimulated macrophages
- Single cell analysis of blood PMBC and immune tissues
- Epigenetics of circulating Blood Cells- flow-sorted into functional types

Herrera-Uribe PO0877

Yang PO0883

Corbett PE0876

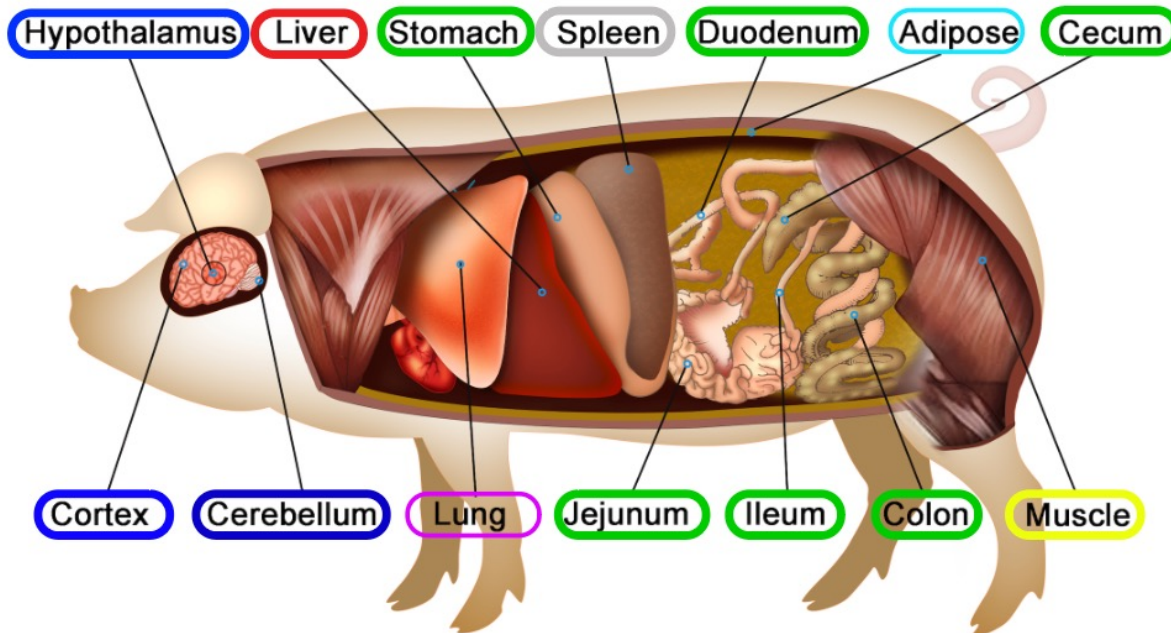
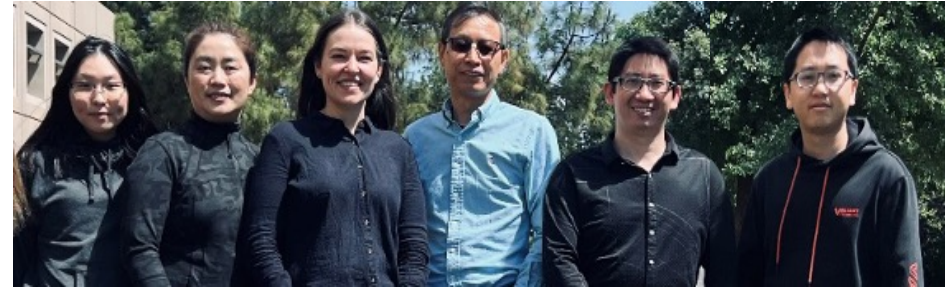
Aim 4 Data Integration

- Integrate all public and project data to develop a higher-order regulatory understanding of the porcine genome, including a predicted chromatin state map.

Aim 1: Adult tissues (UC-Davis)

- extend FAANG pilot of 8 -> 26 tissues

Liqi An, Ying WANG, Claire
PROUSE-WILKINS, Huaijun ZHOU,
Zhangyuan PAN, Dailu GUAN



Finished

Core tissues (8): cortex, cerebellum, hypothalamus, liver, lung, spleen, and adipose muscle (Kern, et. al., Nat Commun 2021).

Gut-associated tissues (6): stomach, duodenum, jejunum, ileum, cecum, colon (Pan Z. et al. Nat Commun 2021).

Current processing (12 additional adult tissues)

Tissue	Animal	H3K4me3	H3K27me3	H3K27ac	H3K4me1	ATAC-seq	RNA-seq
Bone Marrow	P348	Completed	Completed	Completed	Completed	Completed	Completed
Bone Marrow	P350	Completed	Completed	Completed	Failed	Completed	Completed
Bladder	P348	Completed	Completed	Completed	Completed	Completed	Completed
Bladder	P350	Failed	Failed	Failed	Failed	Completed	Completed
Heart	P348	Completed	Completed	Completed	Completed	Completed	Completed
Heart	P350	Completed	Completed	Completed	Completed	Completed	Completed
Kidney	P348	Completed	Failed	Completed	Completed	Completed	Completed
Kidney	P350	Completed	Completed	Completed	Completed	Completed	Completed
Mammarygland	6796	Completed	Completed	Completed	Completed	Completed	Completed
Mammarygland	6797	Completed	Completed	Completed	Completed	Completed	Completed
Ovary	6796	Failed	Failed	Failed	Failed	Completed	Completed
Ovary	6797	Completed	Completed	Completed	Failed	Completed	Completed
Skin	P348	Completed	Completed	Completed	Completed	Completed	Completed
Skin	P350	Completed	Completed	Completed	Completed	Completed	Completed
Testis	6798	Completed	Completed	Completed	Completed	Completed	Completed
Testis	6800	Failed	Completed	Completed	Failed	Completed	Completed
Thyroid gland	P348	Completed	Failed	Failed	Failed	Completed	Completed
Thyroid gland	P350	Completed	Failed	Completed	Completed	Completed	Completed
Thymus	P348	Completed	Completed	Completed	Completed	Completed	Completed
Thymus	P350	Completed	Completed	Completed	Completed	Completed	Completed
Trachea	P348	Failed	Failed	Failed	Failed	Completed	Completed
Trachea	P350	Failed	Failed	Failed	Failed	Completed	Completed
Uterus	6796	Failed	Failed	Failed	Failed	Completed	Completed
Uterus	6797	Failed	Failed	Failed	Failed	Completed	Completed

Summary

Pig: P348, P350 and 6796, 6797 (for reproductive tissues)

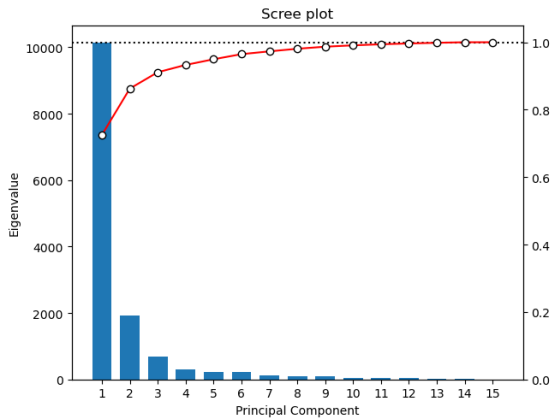
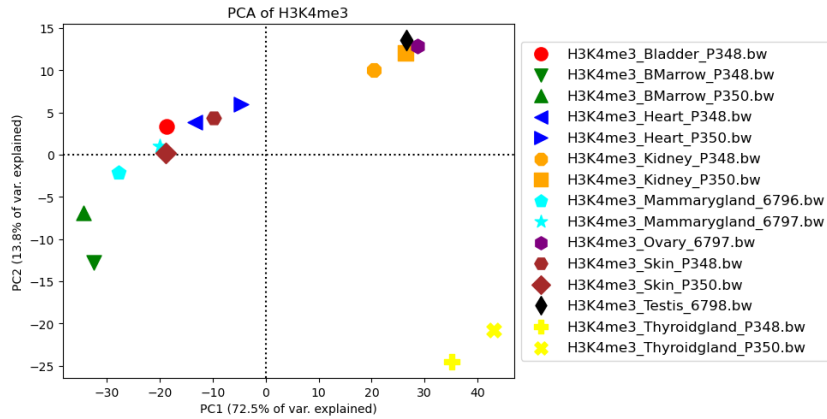
Tissue and assay: 12 tissues and 4 ChIP-seq assays

Library: 168 libraries (131 completed and 37 failed for 8 tissues)

Completed

Failed

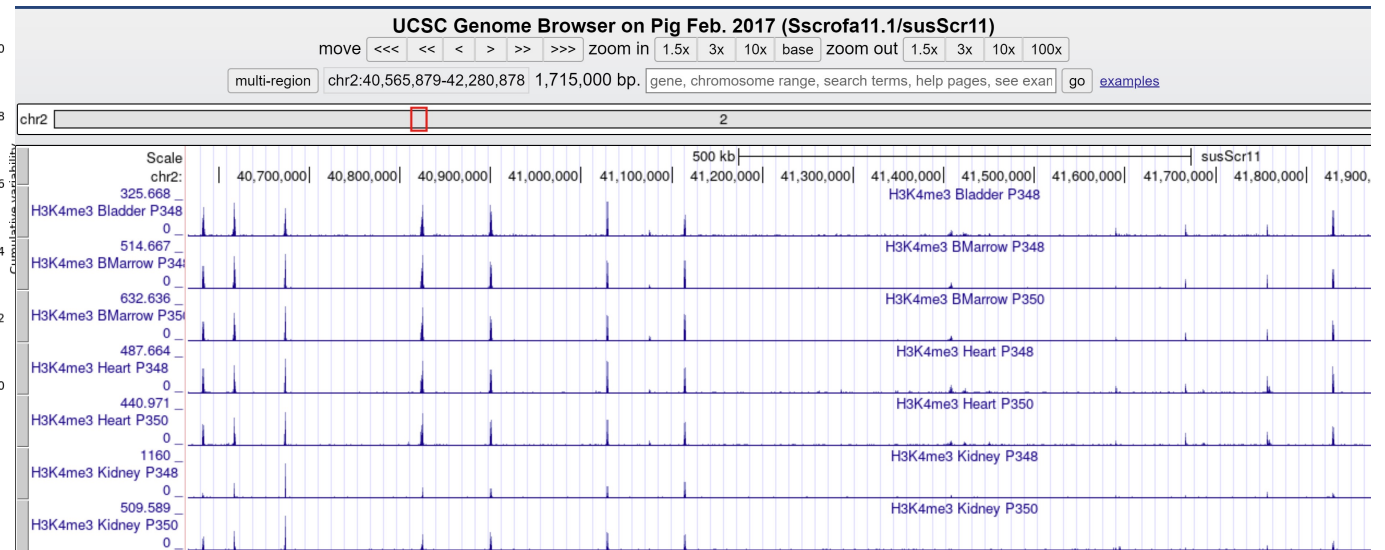
ChIP-seq assay quality controls (H3K4me3)



Future plans

- We will continuously work on the 37 failed libraries that covered 8 types of tissues and 4 pigs.
- They will be considered completed libraries when passing all QC parameters and getting the expected sequencing depth.
- All sequencing raw data will be submitted to ENA upon completion.

The peak enrichment on the genome browser

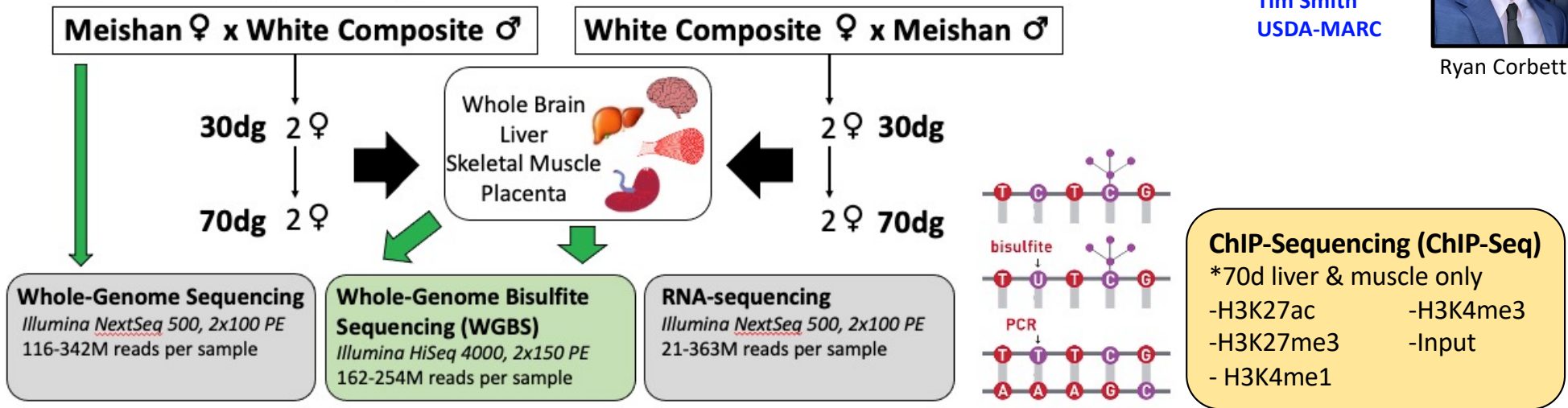


Aim 2: Characterization of allele-biased expression and epigenetic modifications in porcine fetal tissues

Collaboration:
Dan Nonneman,
Tim Smith
USDA-MARC



Ryan Corbett



Negative binomial regression model:

$$E = \mu + AG + PO + ID + \varepsilon$$

E = allele count

μ = population mean

AG = allelic genotype

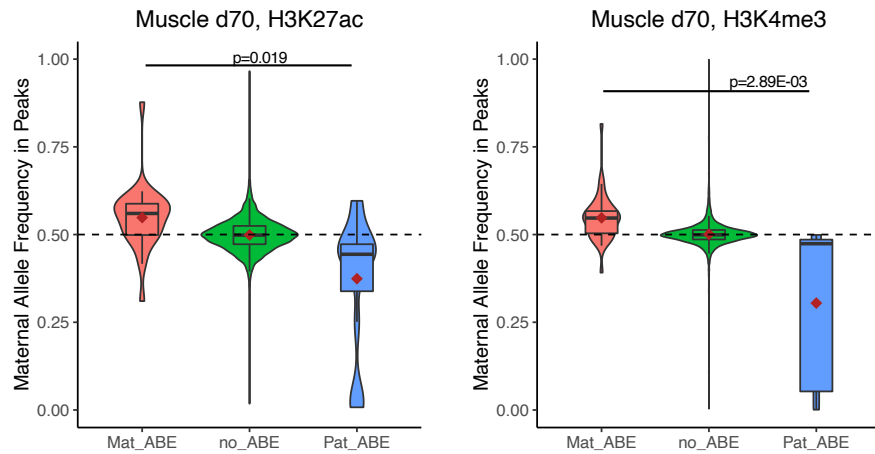
PO = parent of origin

ID = animal ID

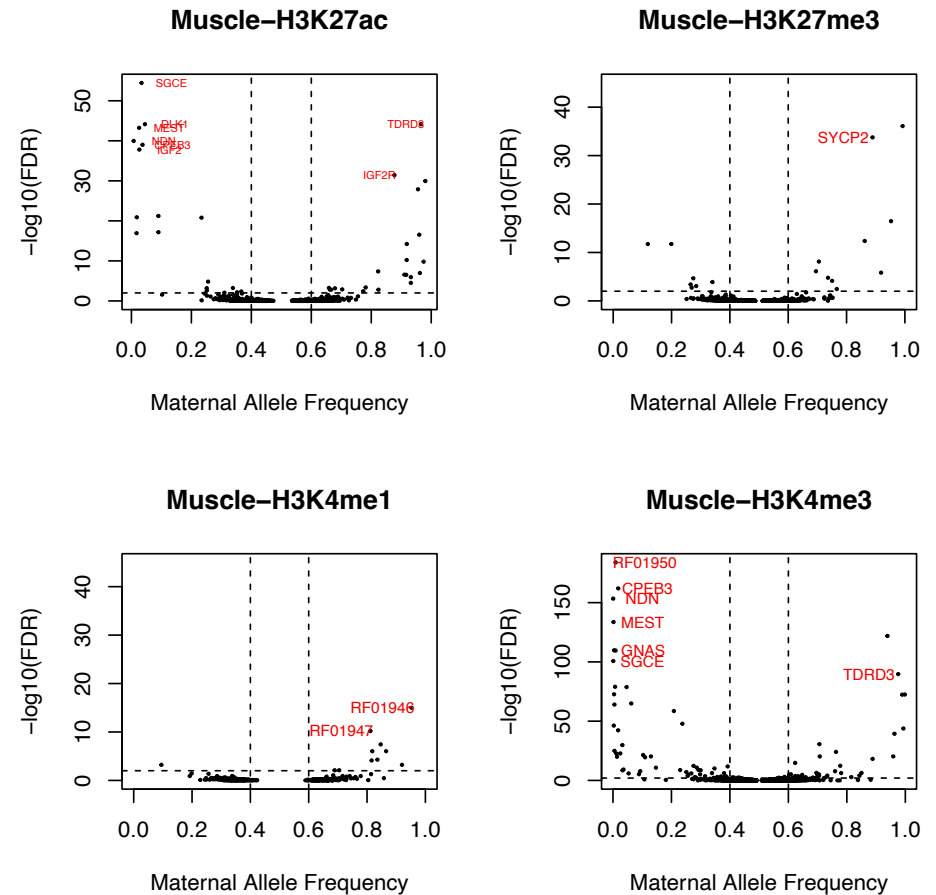
	WC ♀ X MS ♂		MS ♀ X WC ♂	
	WC ♀	MS ♂	MS ♀	WC ♂
AG	1	0	0	1
PO	0	1	0	1

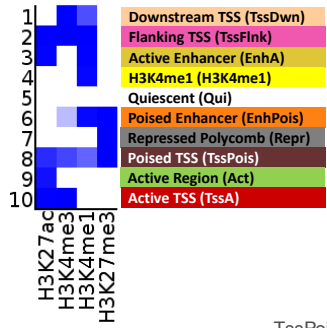
Allele biases in Chromatin Modifications: H3K27ac and H3K4me3 CHIP peak strength biases are prevalent in fetal liver and muscle and associated with ABE

- Parent-of-origin allele biased histone modifications (ABHM) are associated with validated imprinted genes
- Biases in H3K27ac, H3K4me3 peak allele counts are associated with genes exhibiting ABE



Frequency of maternal alleles in H3K27ac & H3K4me3 peaks overlapping promoters of genes exhibiting maternal, paternal, and no ABE in d70 muscle.

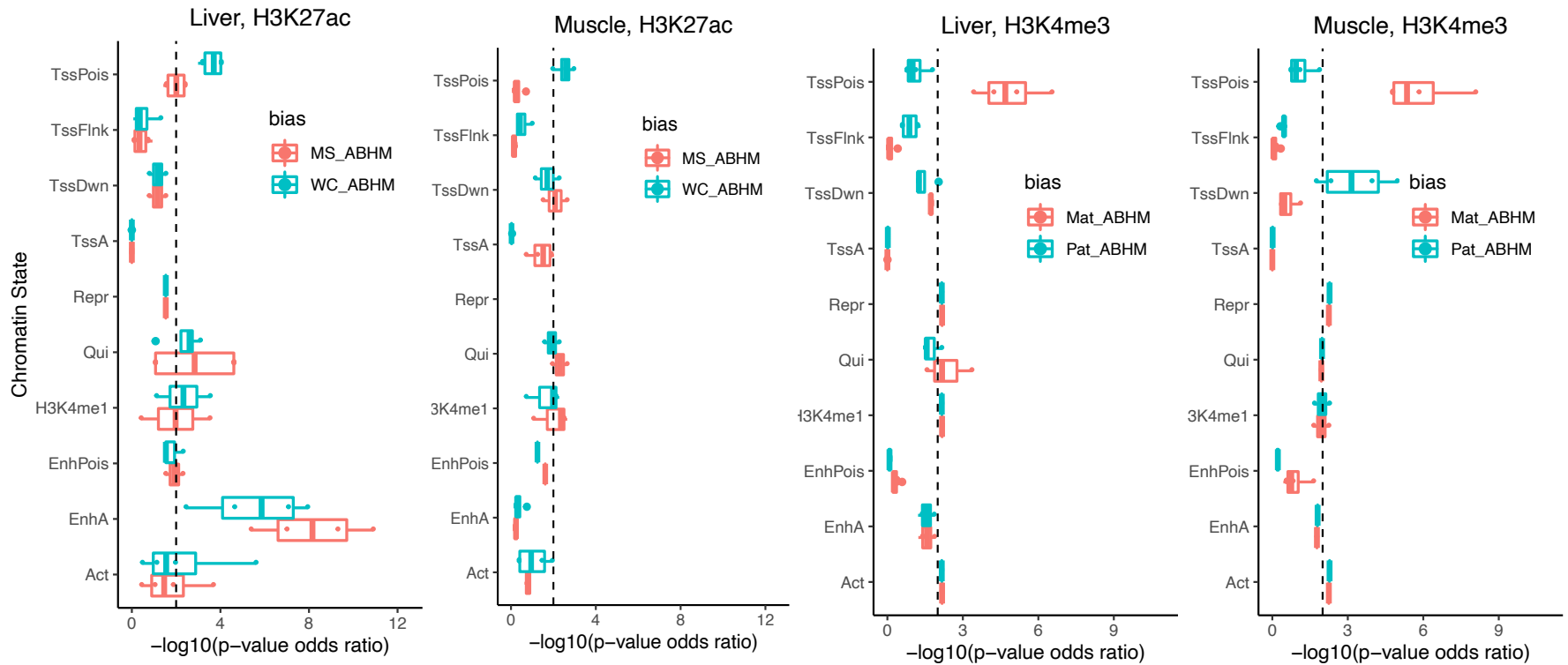




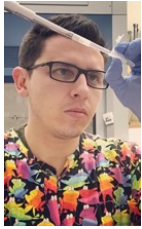
ABHM is enriched in enhancer and TSS chromatin states

- Breed H3K27ac ABHM is enriched in active enhancers

- Parent-of-origin H3K4ME3 ABHM is enriched in TSS regions



Aim 3: Integrative Profiling of Gene Expression and Chromatin Accessibility Elucidates Specific Transcriptional Networks in Porcine Neutrophils

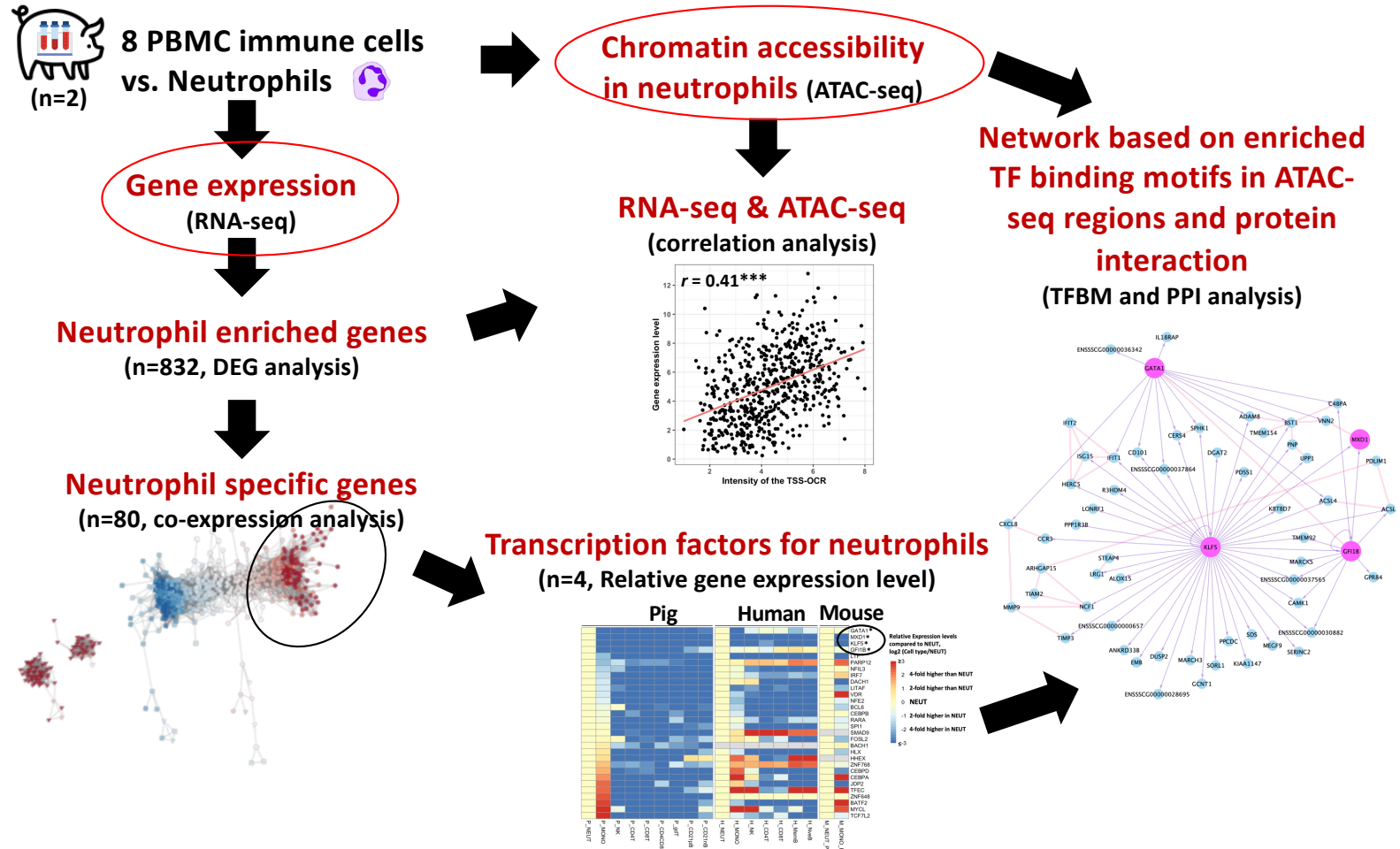


Juber Herrera
Uribe
C. Tuggle group



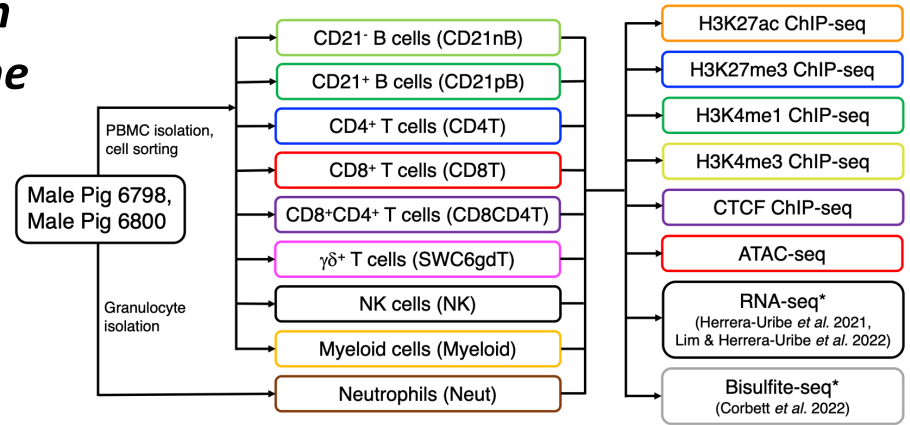
Kyu-sang Lim
J. Koltes group

Poster #PO0883
for details



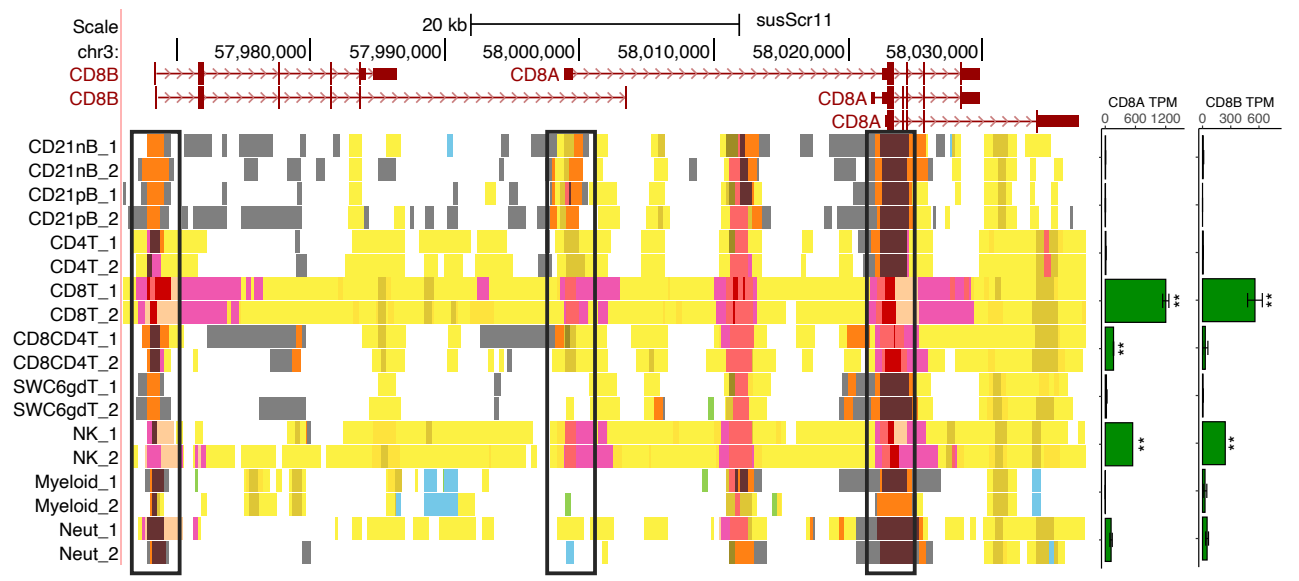
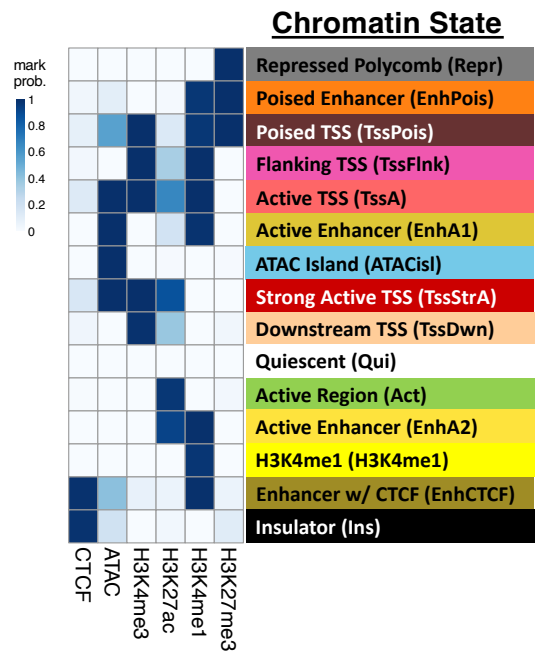
Aim 3: Functional Annotation of Circulating Porcine Immune Cell Populations

Poster PE0876



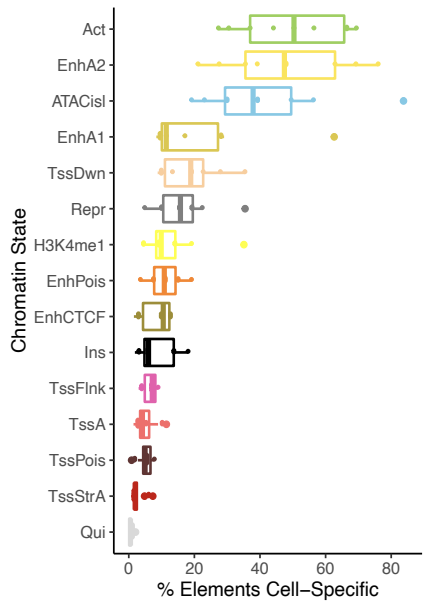
Ryan Corbett
Juber Herrera Uribe

Collaboration:
Lingzhao Fang, et al. Aarhus U
Crystal Loving, USDA-NADC
Cathy Ernst, Michigan State

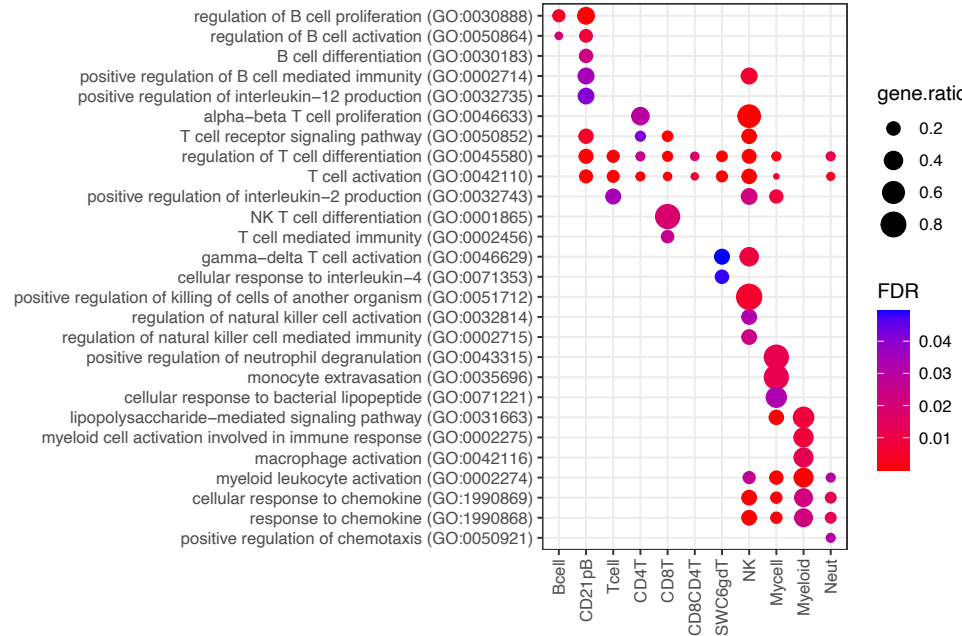


Common and cell-specific enhancer <-> target prediction in porcine immune cells

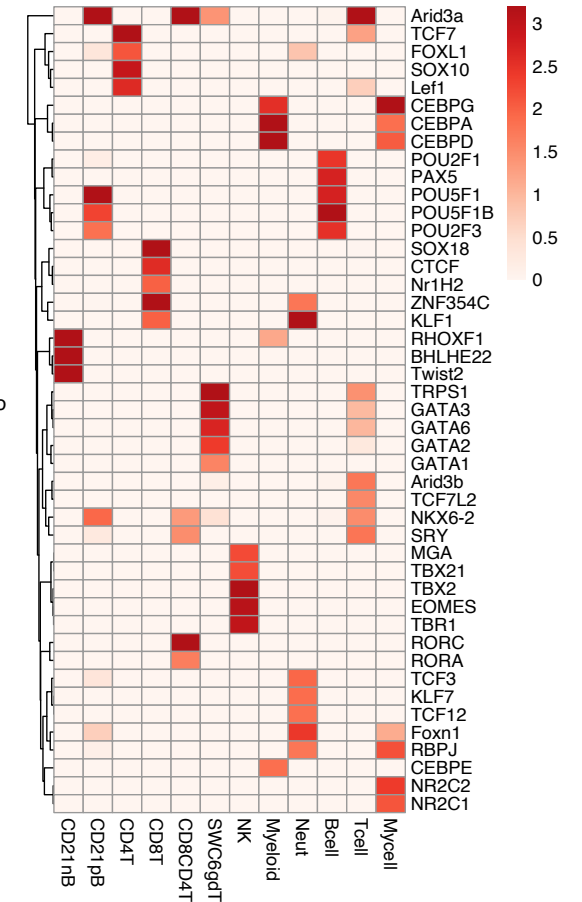
Sig. Enhancer-target Pairs*	No. Enhancers	No. Target Genes
47,674	28,142	9,845



GO enrichment for genes with cell-specific active enhancers (EnhA1) in each cell population

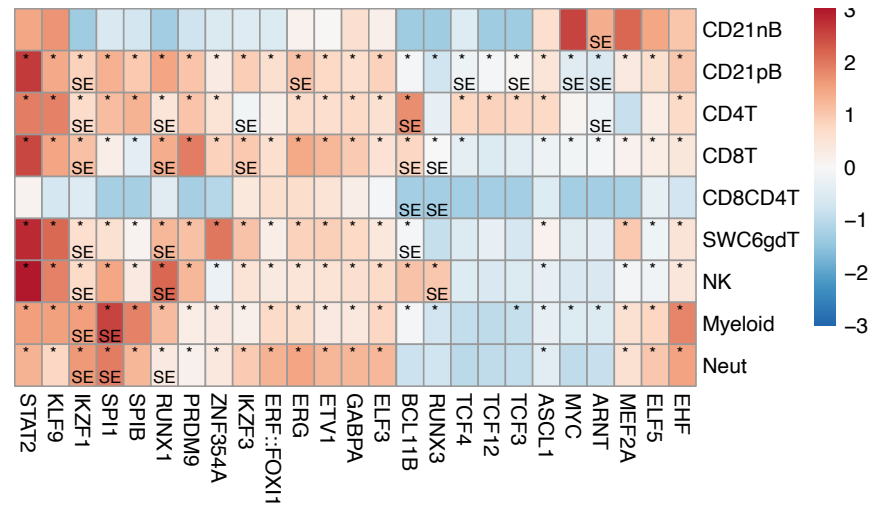
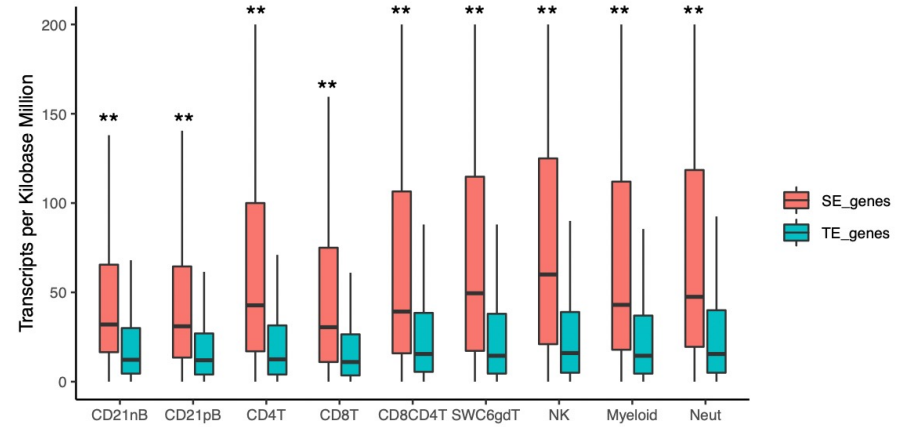
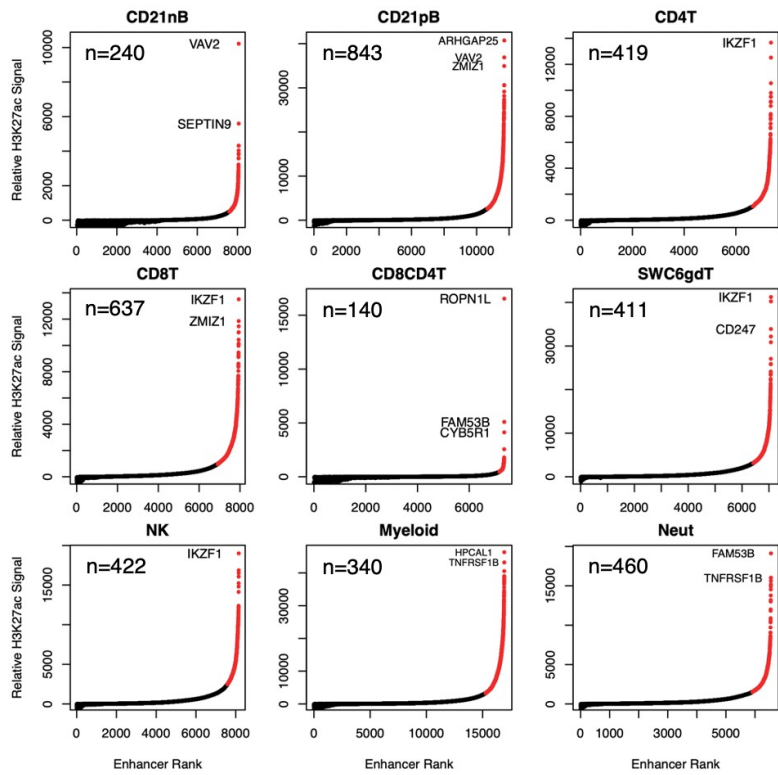


Enriched TF binding motifs among cell- and lineage-specific EnhA1 states.



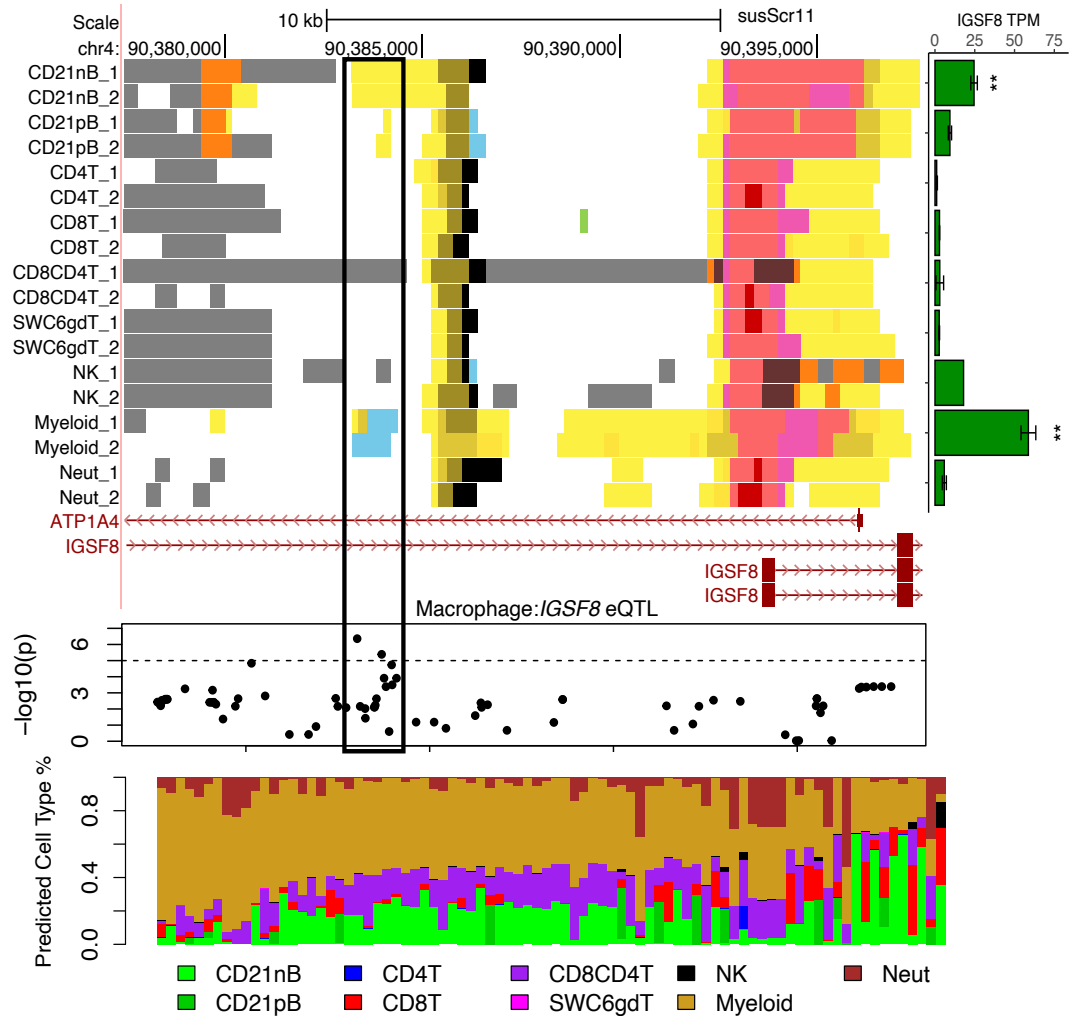
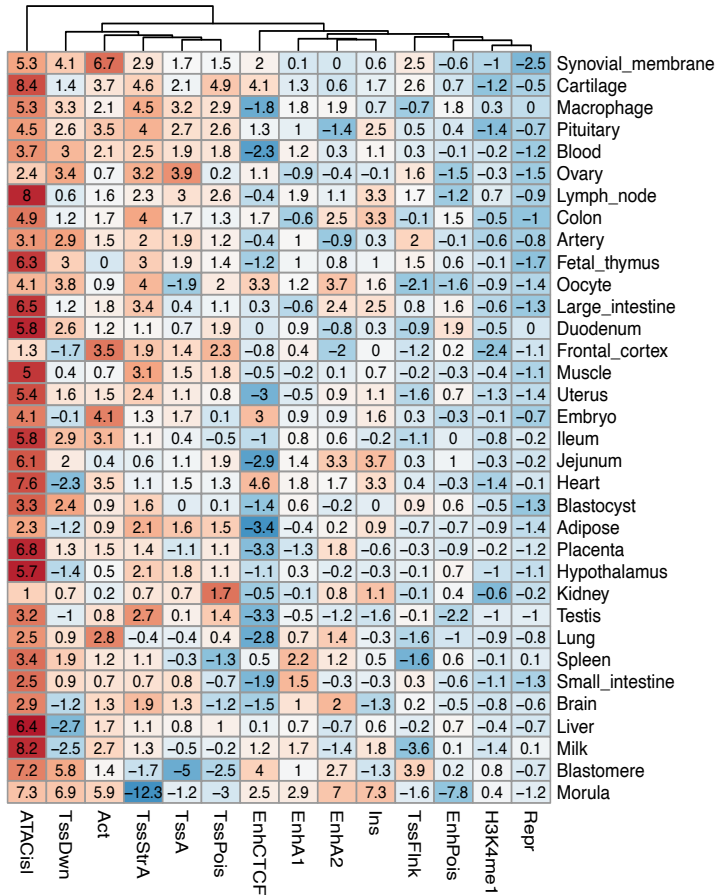
*based on correlations between enhancer ATAC signal and gene TPM within TAD boundaries.

Super-enhancer prediction in sorted porcine immune cells

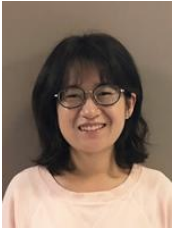


Poster PE0876, 'Characterization and Applications of Advanced Regulatory Element Annotation in Circulating Porcine Immune Cells'

Active chromatin states are enriched for Pig GTEx eQTL in immune tissues

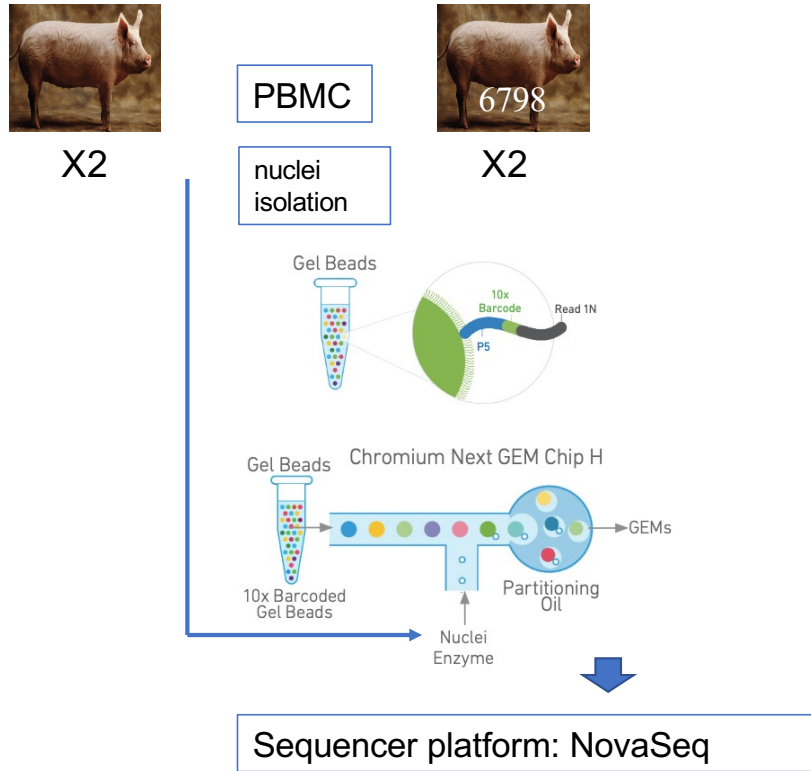


Poster PE0876, 'Characterization and Applications of Advanced Regulatory Element Annotation in Circulating Porcine Immune Cells'

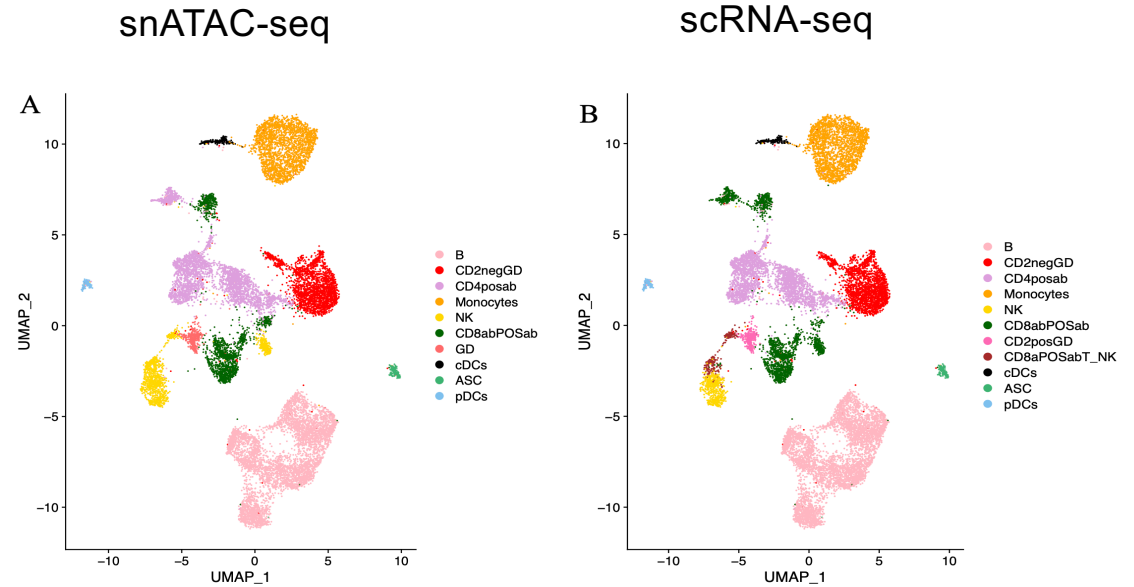


Pengxin Yang

snATAC-seq porcine PBMC chromatin accessibility is powerful for identifying regulatory elements



snATAC-seq and scRNA-seq have similar power to annotate cell types



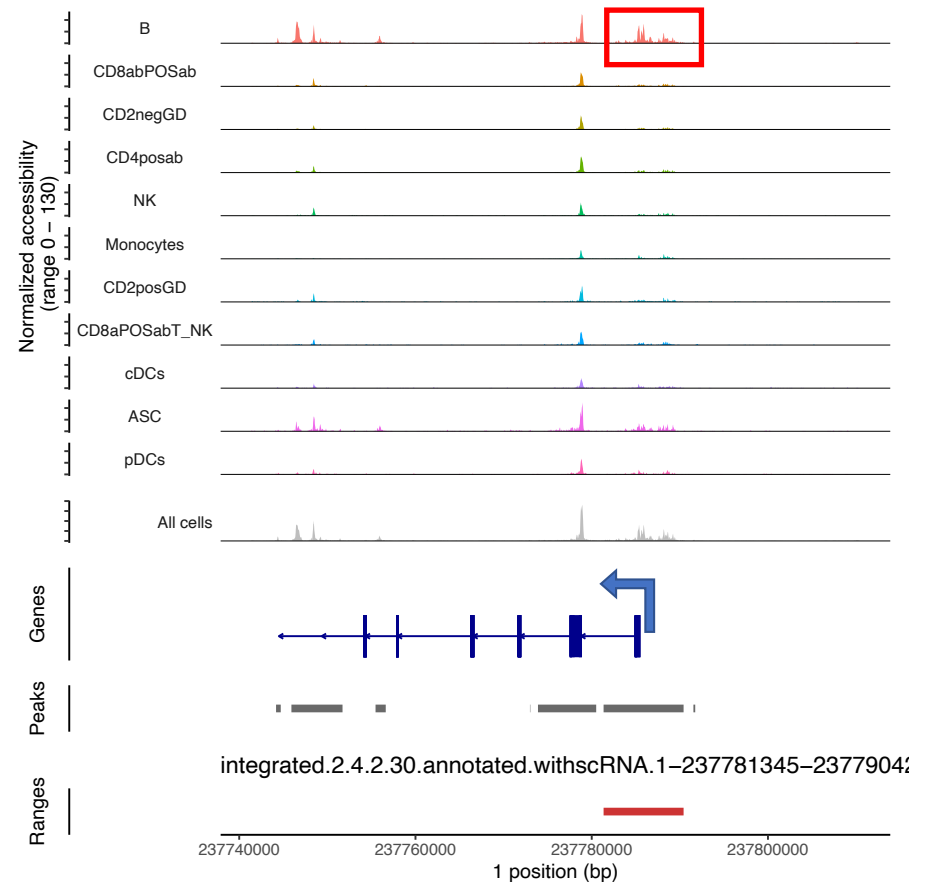
36 clusters were identified for 17230 nuclei

Cell type specific differentially accessible peaks (DAPs): clues to immune regulatory control

14,092 unique cell type-specific DAPs

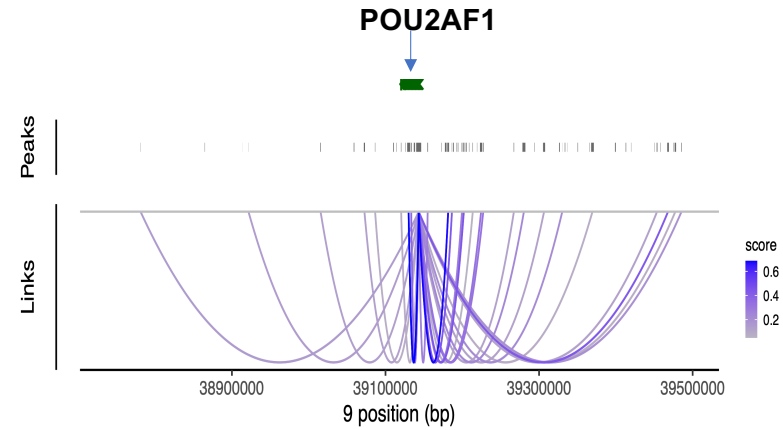
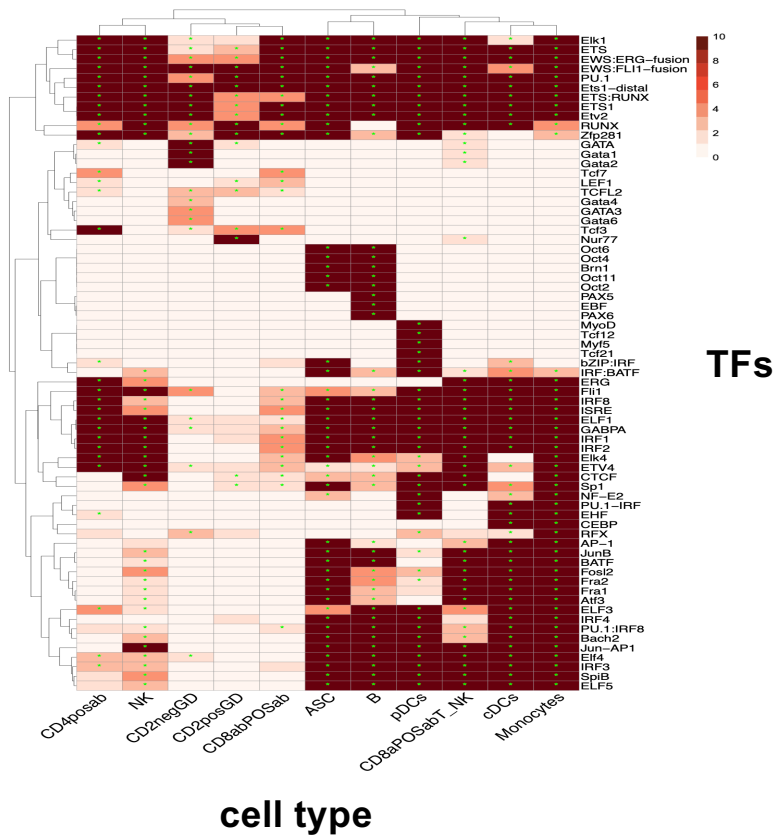
Cell type	# DAPs	# closest genes
cDCs	1481	1178
Monocytes	2524	1943
CD4posab	557	429
CD2negGD	572	435
B	1114	855
ASC	1455	1109
CD2posGD	602	456
CD8abPOSab	342	256
NK	1157	909
pDCs	2402	1890
CD8aPOSabT_NK	1220	968

DAP analysis identifies a cis-element within PAX5 for B cells, which specifically express PAX5



TFBM analysis on the cell type specific open chromatin region identifies relevant TF for cell type networks

Linking regulatory regions with target genes: cis co-accessibility network (CCAN) at a DEG



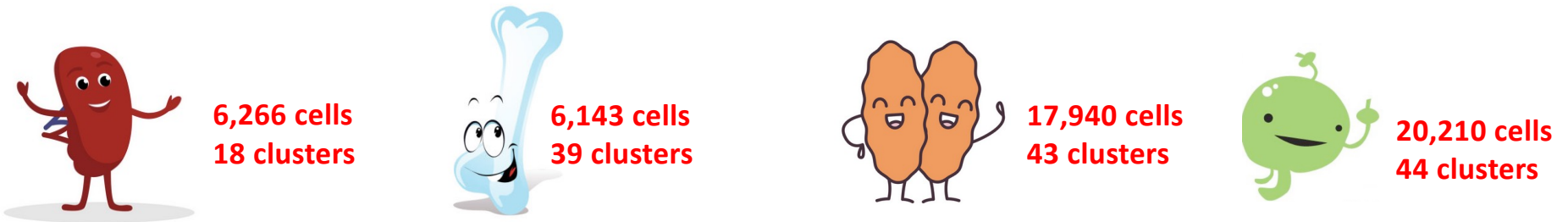
Pengxin Yang
 Poster #PO0883
Definition of regulatory elements and transcription factors controlling immune cell gene expression at single cell resolution using single nucleus ATAC-seq

Aim3: Summary of single cell Immune Tissue Atlas



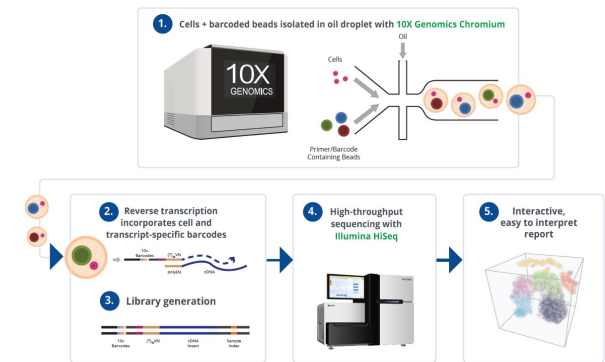
Lance Daharsh
C. Tuggle group

Kristen Byrne
C. Loving group



Global summary:

Used clustering analysis, canonical markers, porcine PBMC data, and human tissue specific data to annotate a porcine immune cell atlas





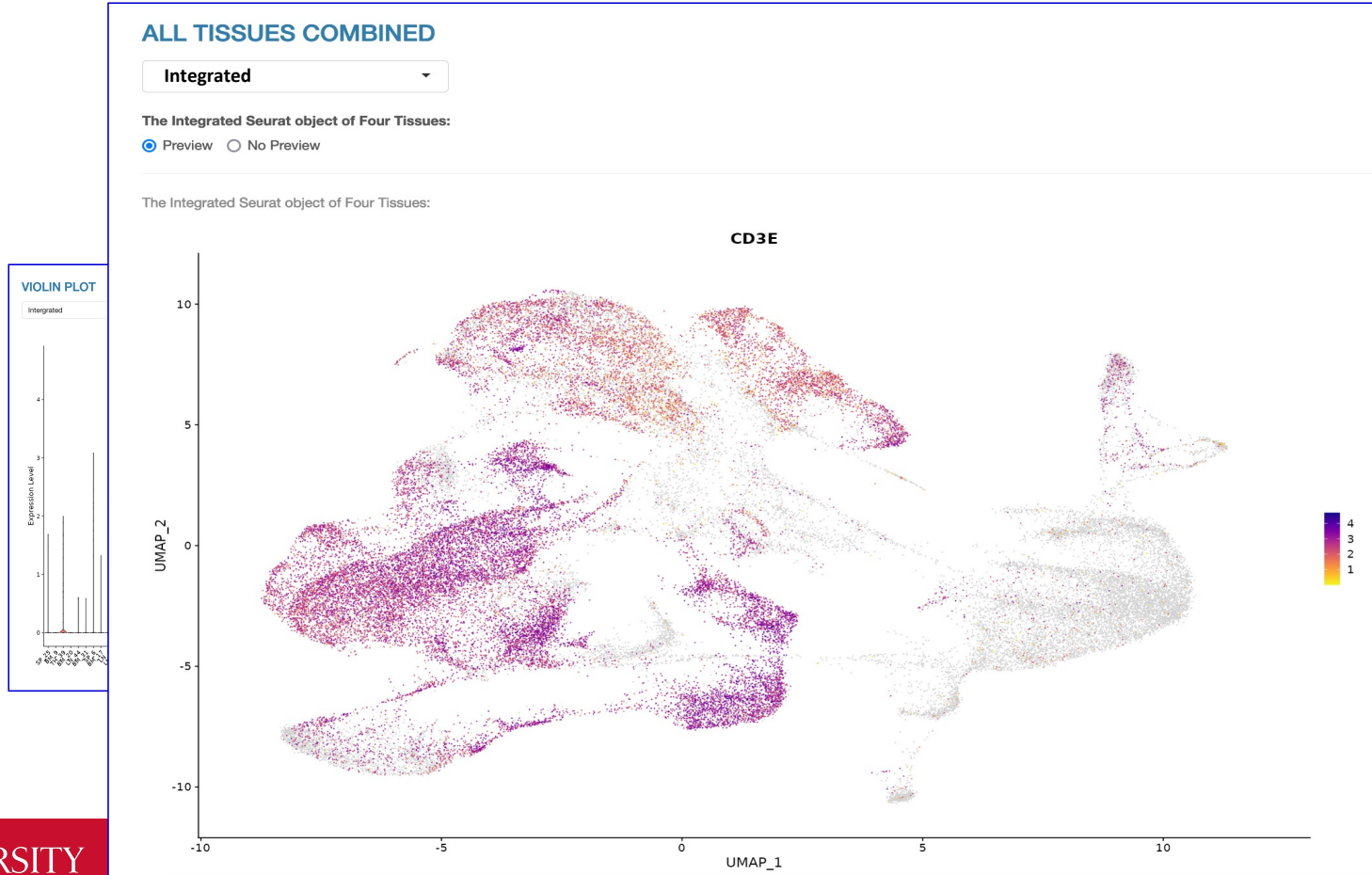
Muskan Kapoor

A Shiny-PIGGI tool for Exploration of these data

We are developing a simple public data exploration tool

<https://shinypiggi.ansci.iastate.edu/>

- Example: CD3E
- T cell gene marker
- Next:
- Cluster annotation tool



Aim 3 data status summary

Alveolar Mac +/- stim → published

Sorted cells:

RNA seq → published
Methyl-seq → published
Histone/CTCF → data complete + submitted, data analysis started
ATAC-seq → data complete + submitted, data analysis started

Neutrophils: → data complete+ submitted, manuscript submitted

Single cells:

PBMC: scRNAseq → published
 scATACseq → data complete, [in submission process](#), data analysis complete
Tissues: scRNAseq → data complete, [in submission process](#), initial analysis complete

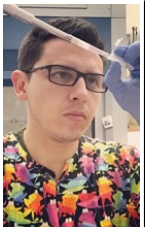
Next steps

- Complete Aim 1 and 3
 - Complete analyses on existing data
 - Contribute to pig TissueAtlas
 - Annotate initial Pig scImmuneAtlas
- Complete Aim 2 (Fetal) → R.Corbett + further work
- Initiate Aim 4 (Integration) across groups and samples
 - Bruna Petry (James Koltes group): within USDA project
 - Compare adult/fetal/bulk/single cell expression patterns → annotation
 - Compare regulatory relationships between sc and sorted cells in blood, tissues
 - With EU Project (Gene-SWitCH)- **complementary** tissues and stages
 - With FARM-GTEx- linking epigenetics to genetic variation
 - With AG2PI effort?

Acknowledgments- PIGGI@iastate.edu group (that's Pig ImmunoGenomics and Genetics in Iowa!)



Christopher Tuggle



Juber Herrera Uribe



Ryan Corbett



Lance Daharsh



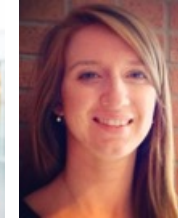
Pengxin Yang



Crystal Loving



Jayne Wiarda



Kristen Byrne



Sathesh Sivasankaran



Zahra Olson



James Koltes



Bruna Petry



Kyu-sang Lim

IOWA STATE UNIVERSITY



Houcheng Li
Lingzhao Fang
Aarhus University

We thank all pig FAANG collaborators!

Huaijun Zhou, et al.

Cathy Ernst, et al.

Tim Smith, Dan Nonneman
MARC

Joan Lunney
BARC

Jinyan Teng
Zhe Zhang
So China Ag University



United States Department of Agriculture

National Institute of Food and Agriculture

NIFA Project 2018-67015-2701

