Building **OsteoCAR**, a Comprehensive Cross-Species Single-Cell Atlas of Osteosarcoma

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FACTOR 2025

THE OHIO STATE UNIVERSITY

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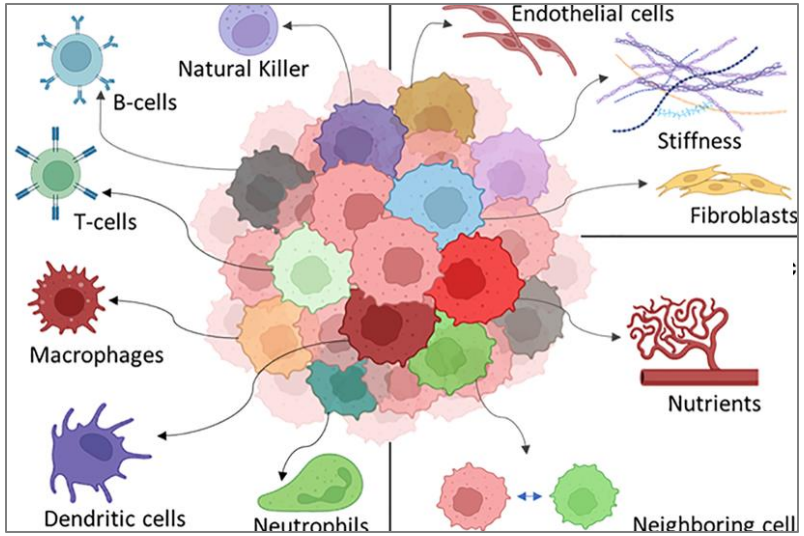
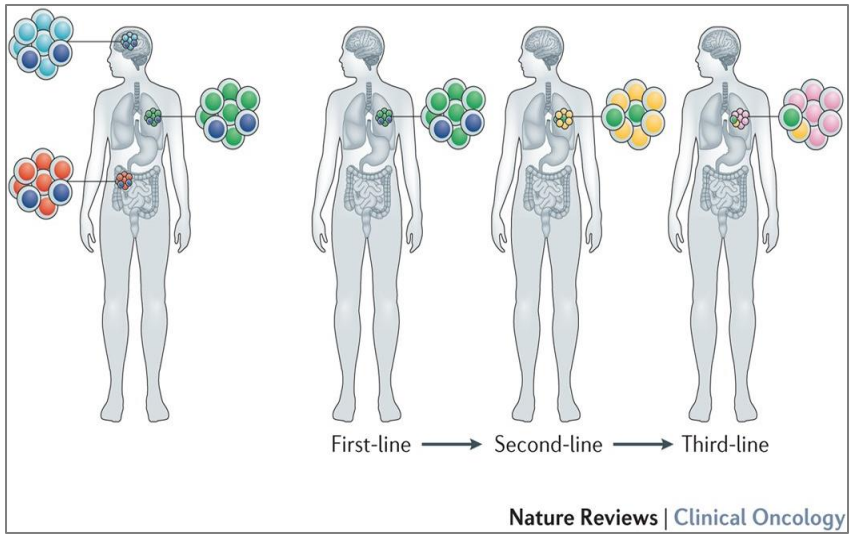
NATIONWIDE CHILDREN'S
When your child needs a hospital, everything matters.℠

Tumors Comprise Diverse Cells that Adapt and Function Cooperatively



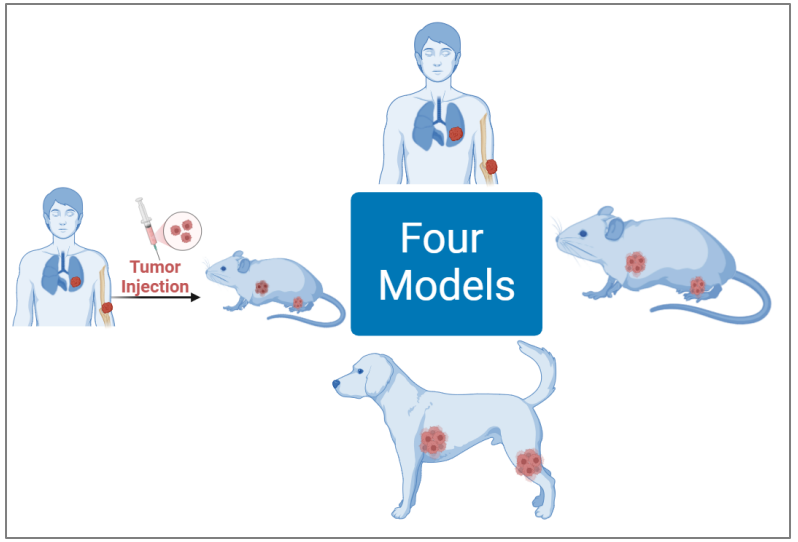
Intra-tumoral heterogeneity

DOI: 10.1038/nrclinonc.2017.166



Site-specific and Temporal heterogeneity

DOI:10.3389/fonc.2023.1164535



Inter-species heterogeneity

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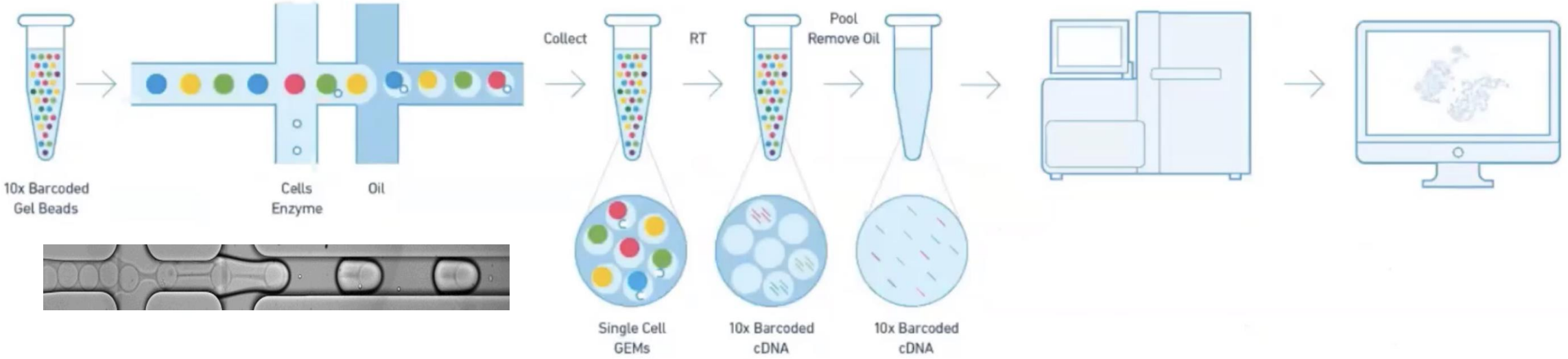
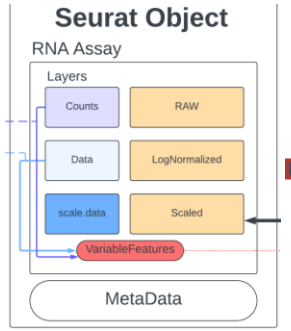
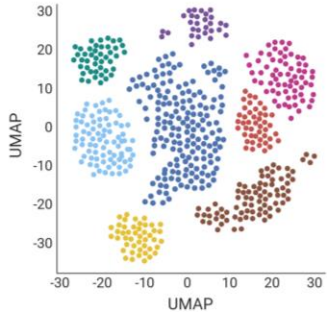
scRNAseq has Become a Powerful Tool for Studying Biology

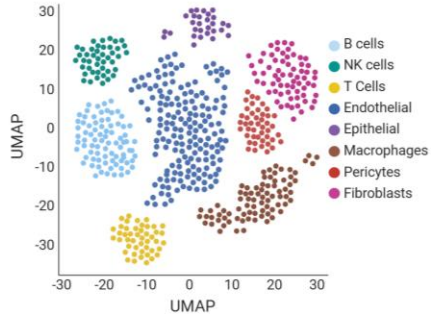


	Cell1	Cell2	...	CellN
Gene1	3	2	.	13
Gene2	2	3	.	1
Gene3	1	14	.	18
...	.	.	.	.
...	.	.	.	.
...	.	.	.	.
GeneM	25	0	.	0

Raw count matrix







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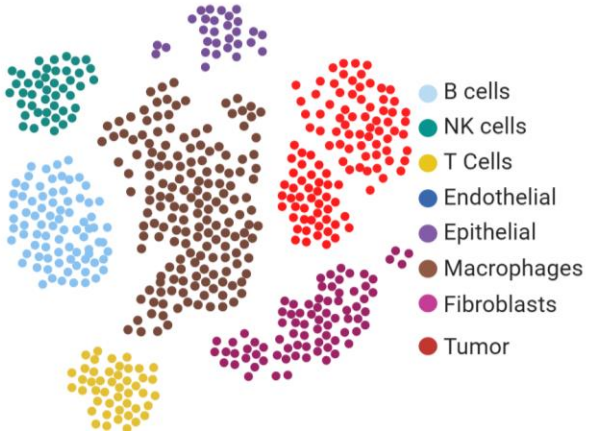
Aims and Significance



1. Build a tool to streamline data analysis

2. Facilitate collaboration

3. Allow exploration

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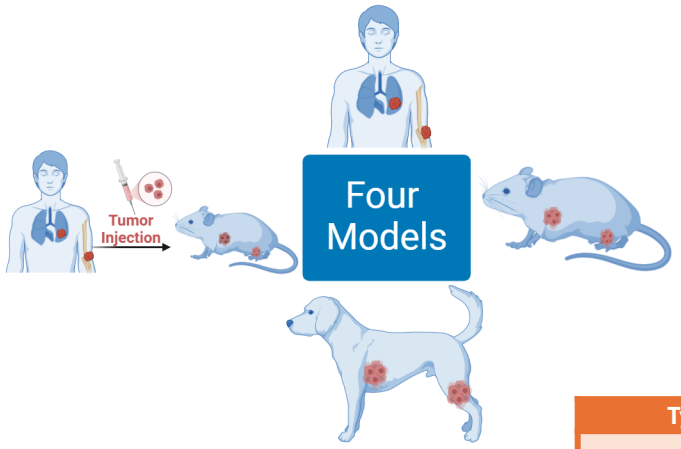
Assembling a Comprehensive Dataset for Atlas



Type	Sub-Type	No. of Samples	Data Sources	No. of Cells	Methods
Human Patient	Primary	25	GEO, SJ	152,770	SC, SN
	Metastatic	36	GEO, NCH, NCI, SJ	221,271	SC, SN
Dog Patient	Primary	17	CSU, TU, UoM	110,827	SC
	Metastatic	16	CSU, TU	78,323	SC, SN
Human Xenograft	Primary	12	NCH, SJ	72,265	SC, SN
	Metastatic	12	NCH, SJ	59,207	SC, SN
Syngeneic Mouse	Primary	3	NCH, SJ	16,579	SC
	Metastatic	8	NCH	64,199	SC, SN
TOTAL	**ALL**	**129**	**ALL**	**775,441**	**ALL**

Data Sources: Gene Expression Omnibus (GEO), St. Jude Children's Research Hospital (SJ), Nationwide Children's Hospital (NCH), National Cancer Institute (NCI), Colorado State University (CSU), Tufts University (TU), University of Minnesota (UoM).

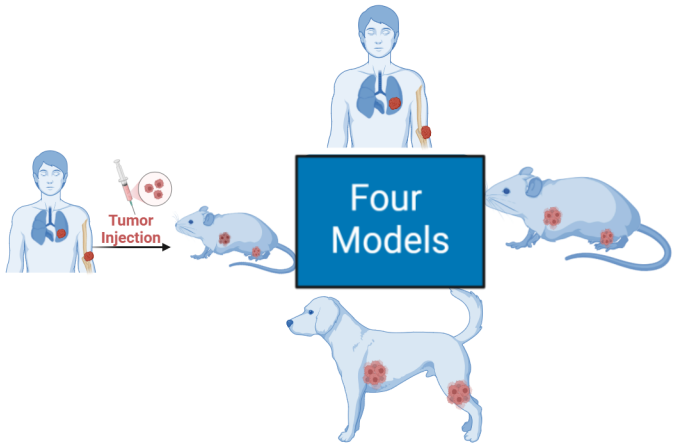
Methods: Single Cell (SC), Single Nucleus (SN)

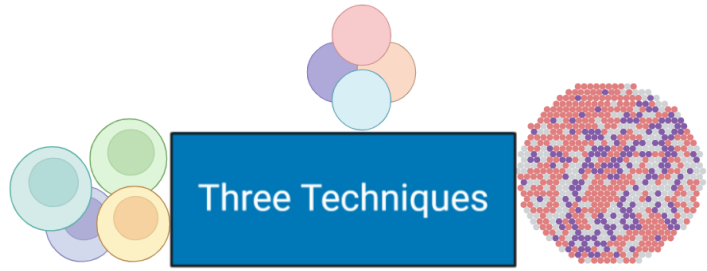
*Note: Over **1,000,000** cells, including the samples used for tumor cell identification and reference validation.*

About 100 Samples

About 1,000,000 Cells

Presenting Osteo-CAR
(Osteosarcoma Cell-type Annotation Reference)





About 100 Samples

About 1,000,000 Cells

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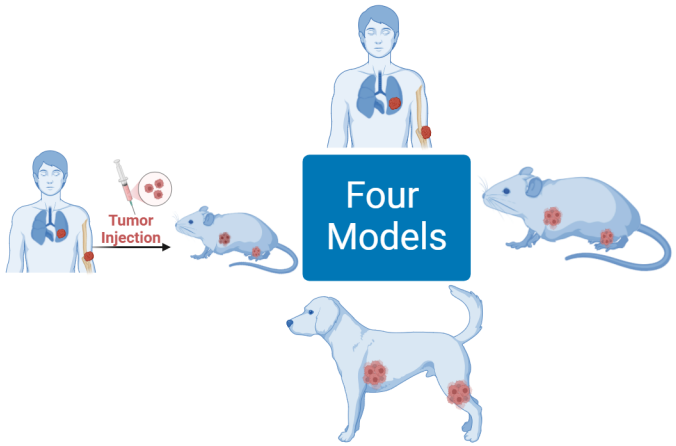
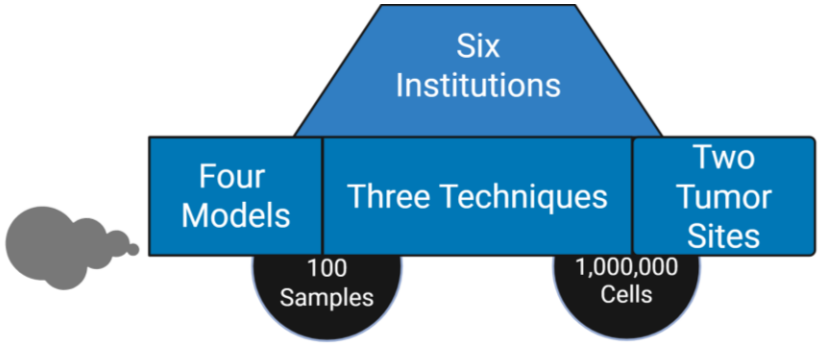
Presenting Osteo-CAR





About 100 Samples

About 1,000,000 Cells

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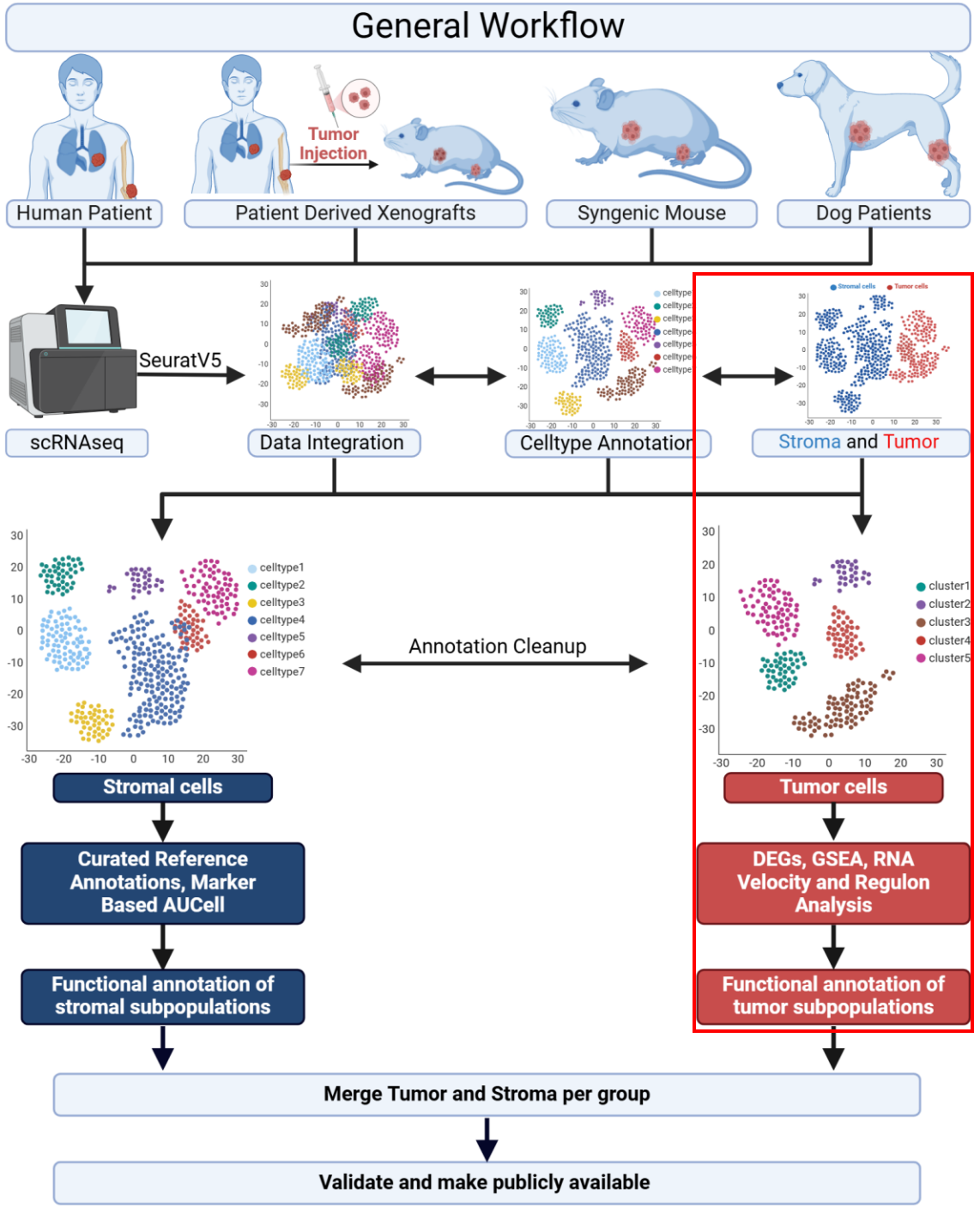
Data Analysis and Cell Type Annotation Pipeline

For simplification, this talk will focus on human patient data



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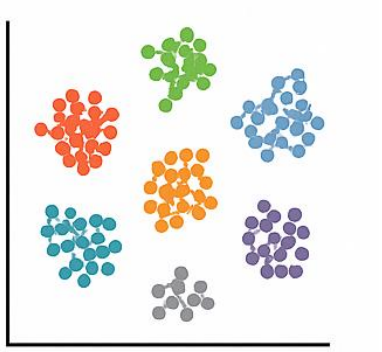
Inconsistent Results from Conventional Tumor Cell Identification Methods Led to the Development of Custom Method(s)

Conventional methods

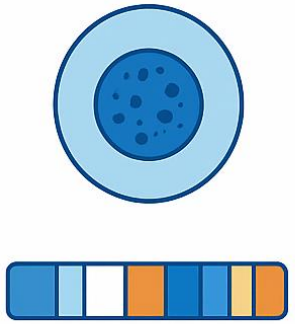
Novel method

scATOMIC



single **c**ell **A**nnotation of **T**um**O**ur **M**icro-environments **I**n pan-**C**ancer settings

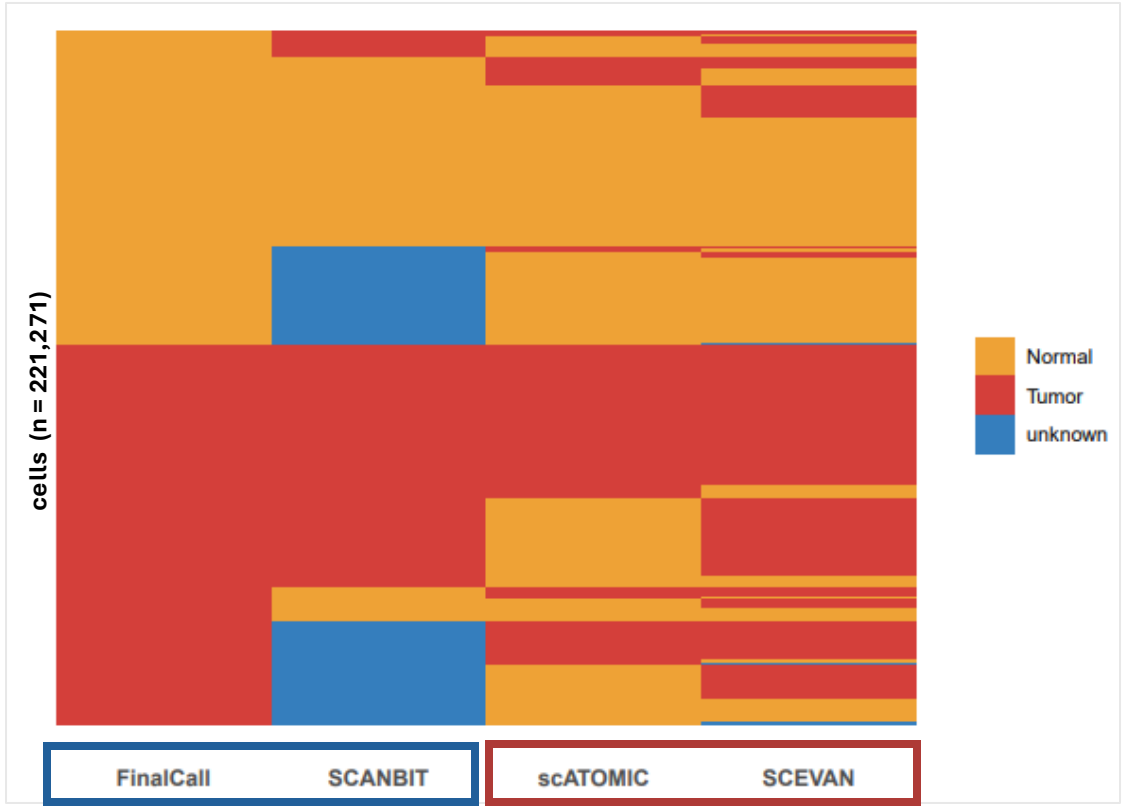
SCEVAN



Single **CE**ll **V**ariational **AN**euploidy analysis

SCAN-BIT

Single **C**ell **A**ltered **N**ucleotide-**B**ased **I**nference of **T**umor



Novel methods being developed in the lab

Conventional methods

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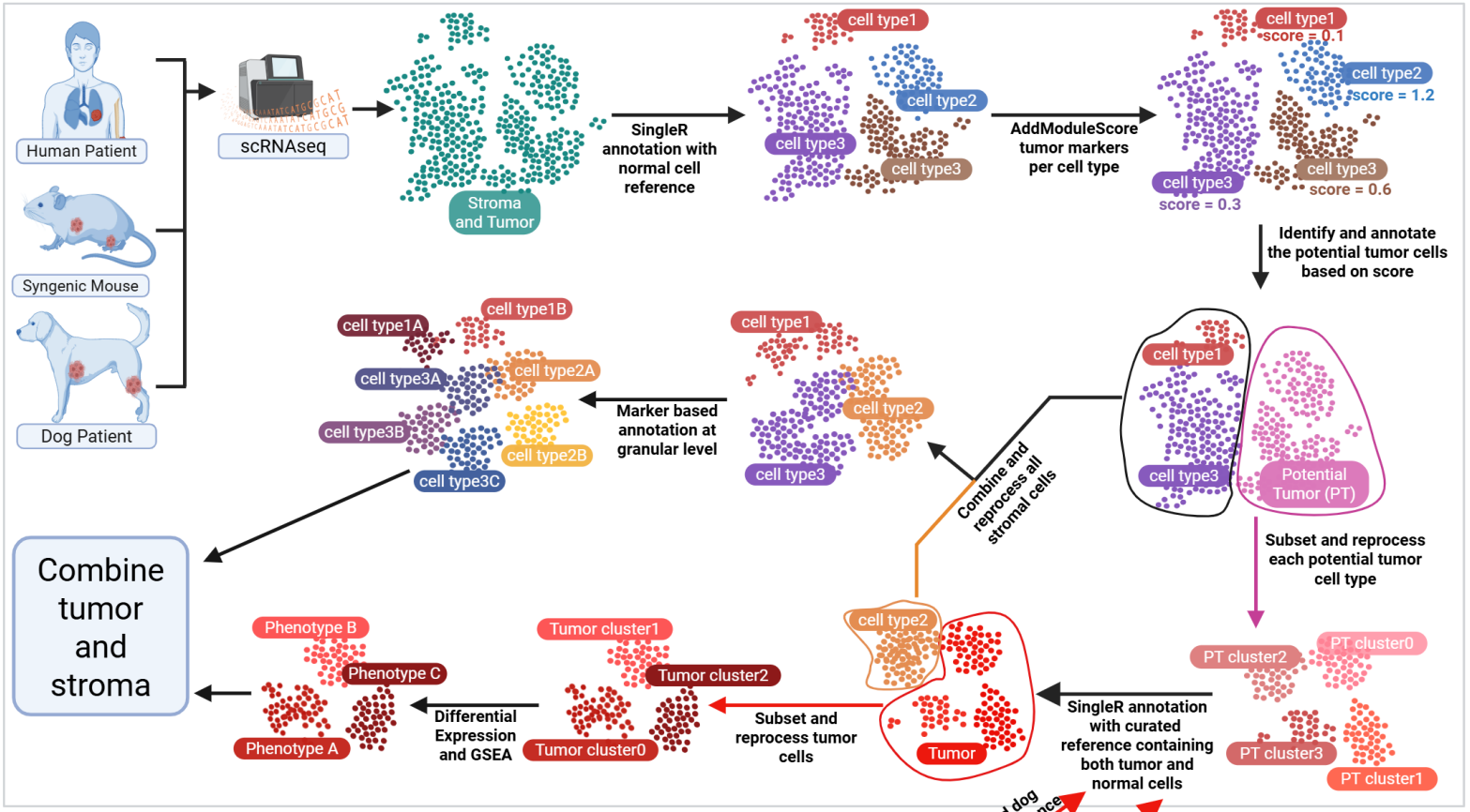
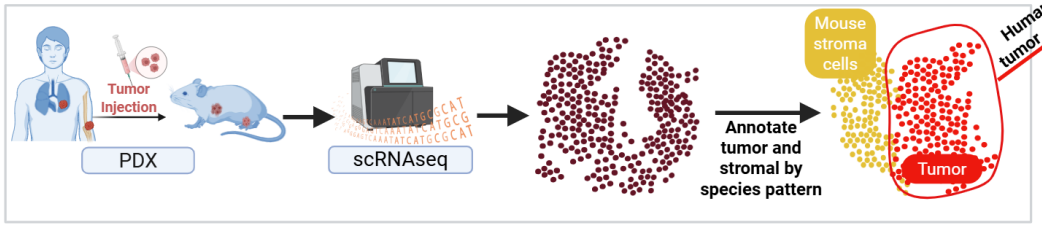
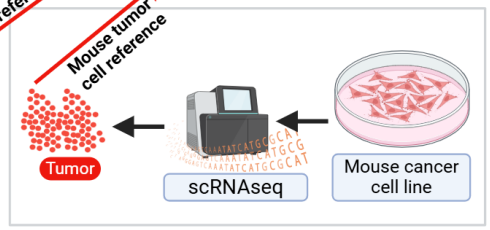
Our Custom Method of Identifying Tumor Cells Leverages the Cross-Species Data

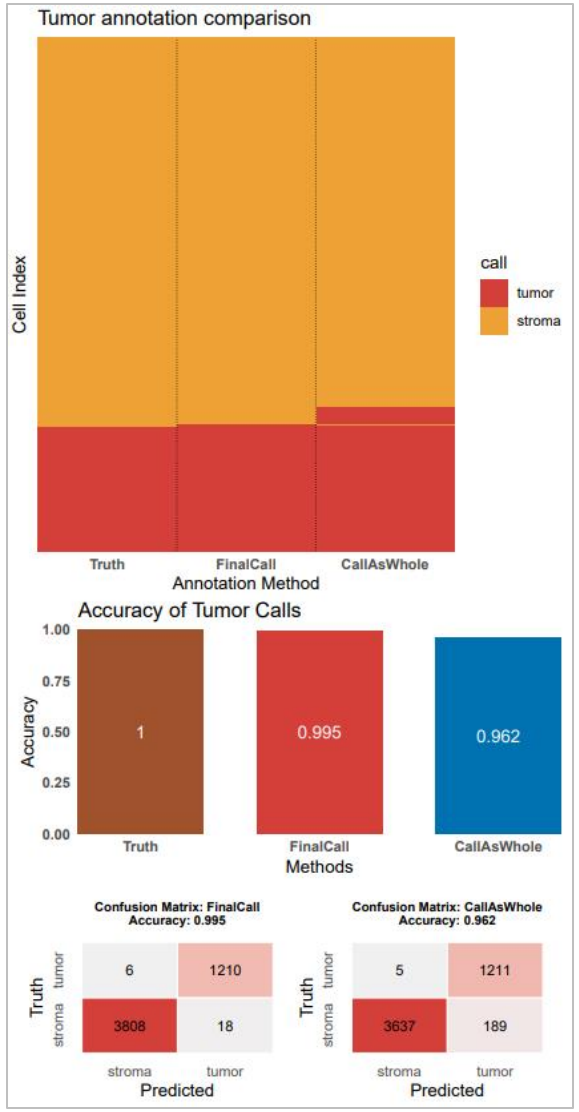




Ground truth tumor reference for human and dog (human-dog gene ortholog conversion)

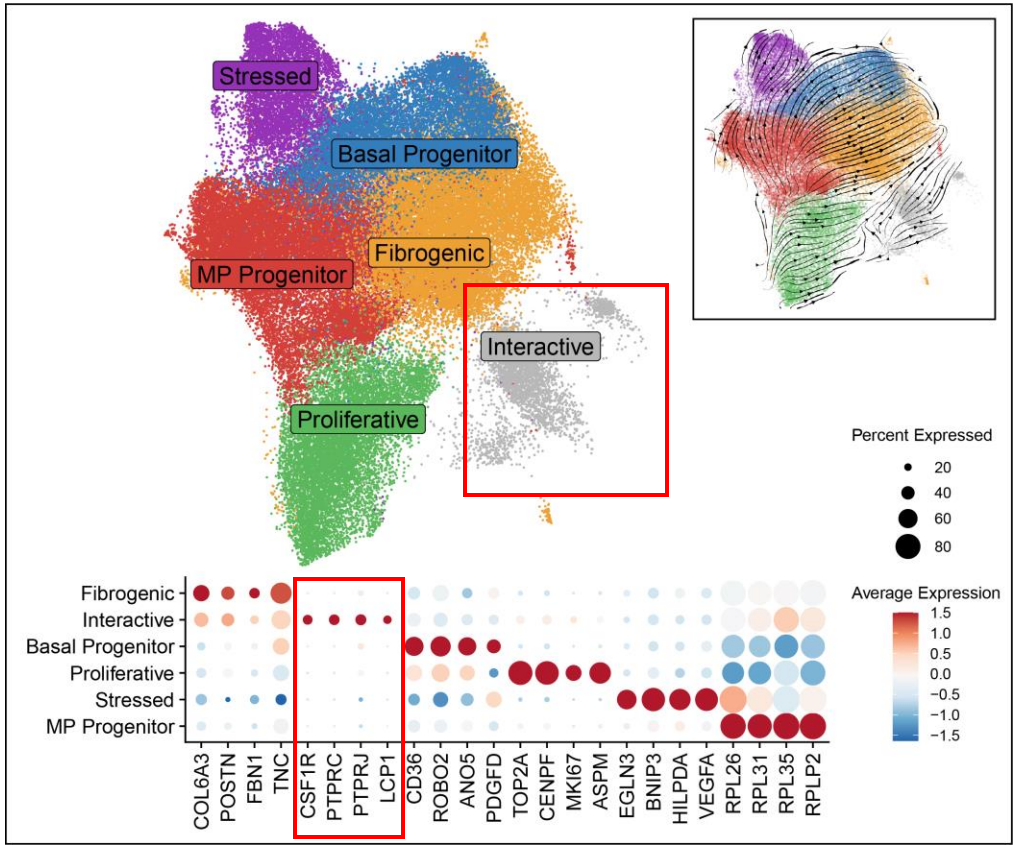


Ground truth tumor reference for mouse

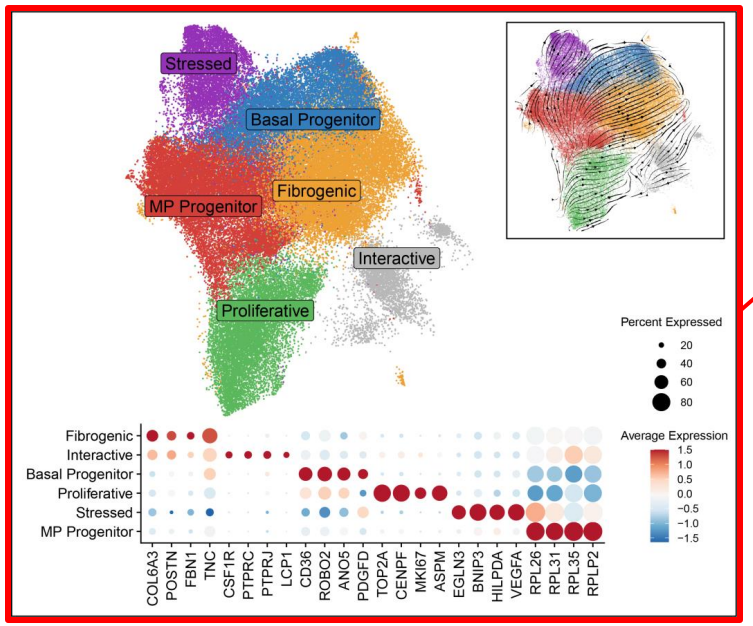


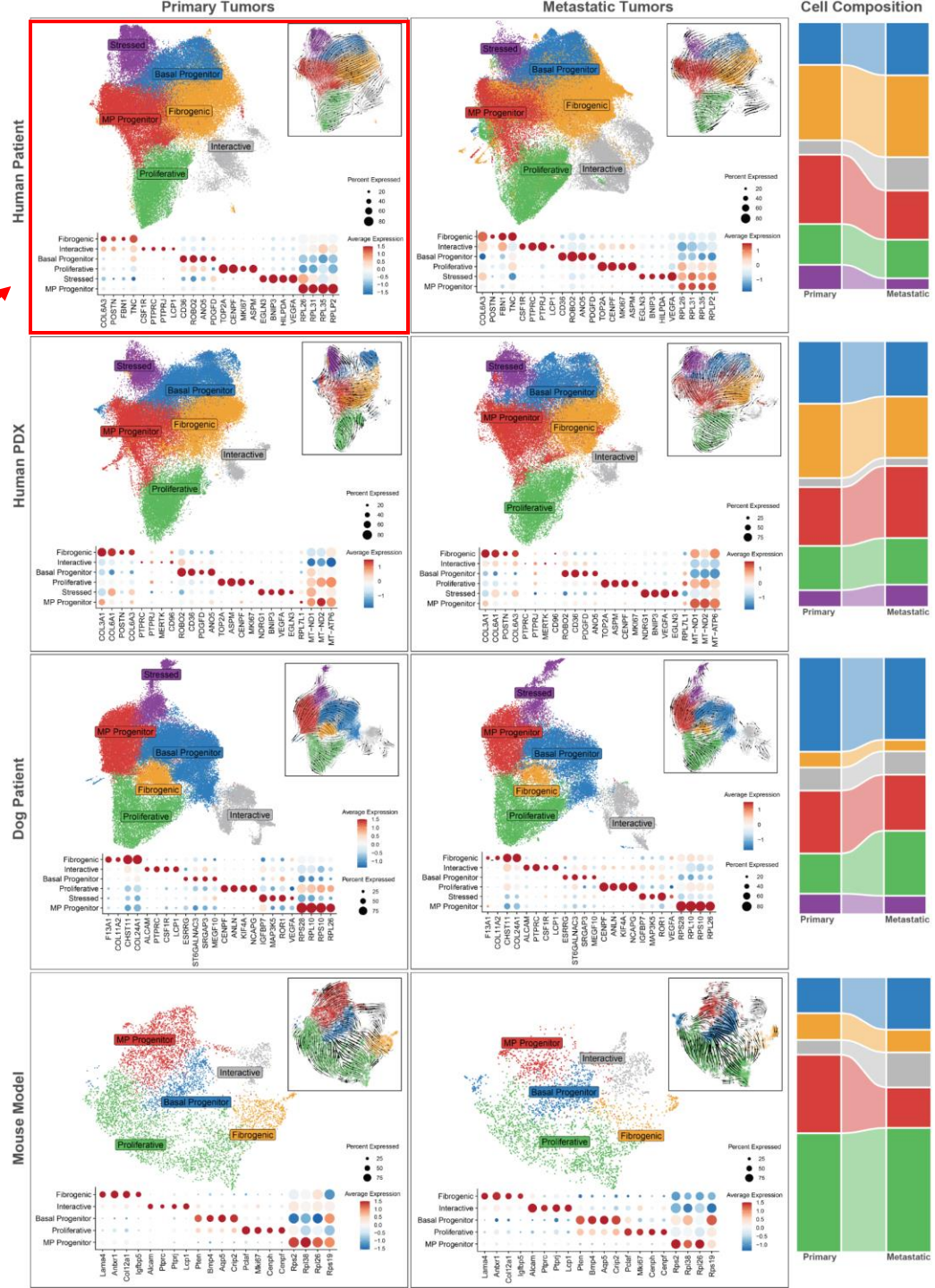
Tumor Subpopulations are Described by Specific Pathways

- **Proliferative**
  - Cell cycle pathways
- **Stressed**
  - Hypoxia and angiogenic pathways
- **Basal Progenitor**
  - Fewer pathways
- **Metabolically Primed (MP) Progenitor**
  - OxPhos and Metabolic pathways
- **Fibrogenic**
  - ECM and fibrotic pathways
- **Interactive**
  - Inflammatory and Immune-related pathways



Osteosarcoma Tumors Exhibit Conserved Cellular Heterogeneity Across Sites and Species





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Acknowledgments

Thank you for listening!

- Roberts Lab
  - **Ryan D. Roberts, MD, PhD**
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  - Daniel Regan, DVM, PhD (CSU)
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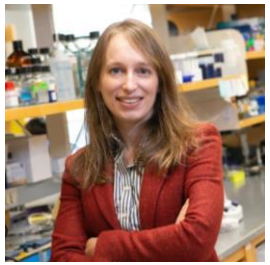
Dr. Anand Patel

Dr. Troy McEachron

Dr. Daniel Regan

Dr. Jaime Modiano



Dr. Heather Gardner

Alex's Lemonade Stand Foundation for Childhood Cancer™

National Cancer Institute

Hyundai Hope On Wheels® Helping Kids Fight Cancer

osteosarcoma INSTITUTE concentrating on the cure

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Animal Cancer Foundation Uniting Pet & Human Research for a Cure™