



RNA Isoforms and Their Relevance to MASLD

Paris MASH 2025

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VCU

GATEHOUSEBIO

WE ARE THE UNCOMMON.

Principal Question

Do changes in RNA isoform usage track MASLD progression and uncover targets, pathways, and biomarkers missed by analysis of canonical RNAs?

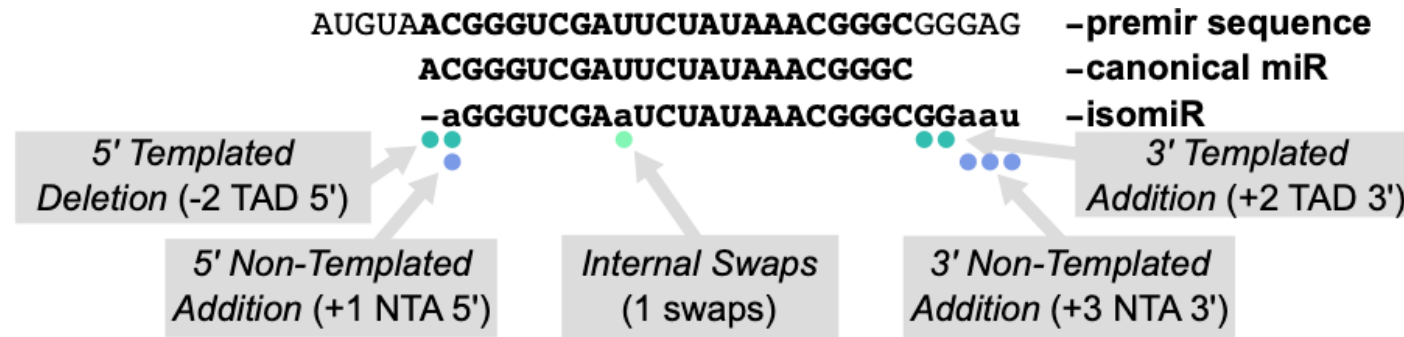
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What is a miRNA isoform (isomiR)?

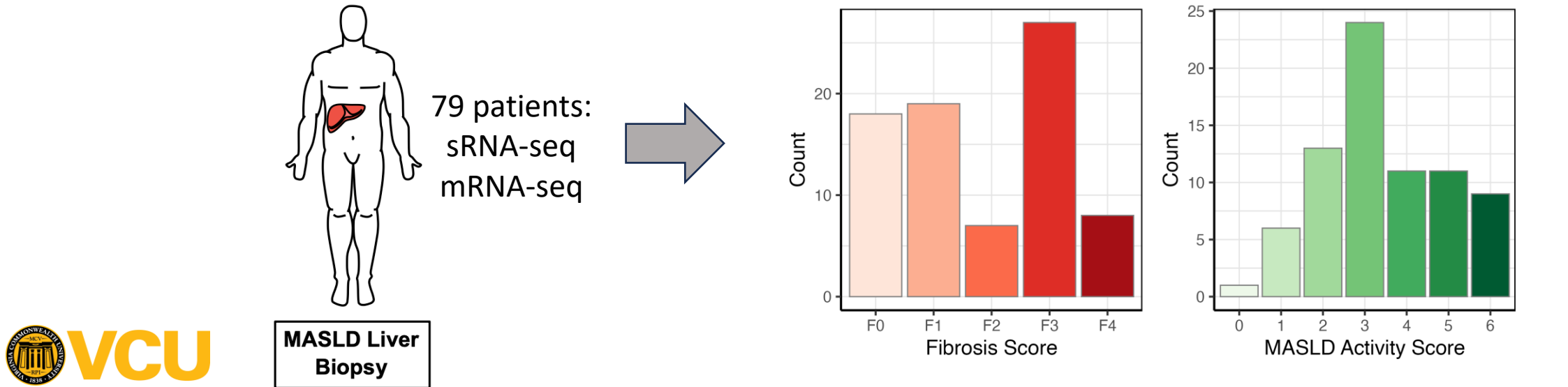
- Variations on canonical miRNA sequences that arise from variations in miRNA biogenesis. E.g., alternate cuts, RNA editing, SNPs, etc.
- They involve deletions, templated and non-templated additions, and internal swaps.



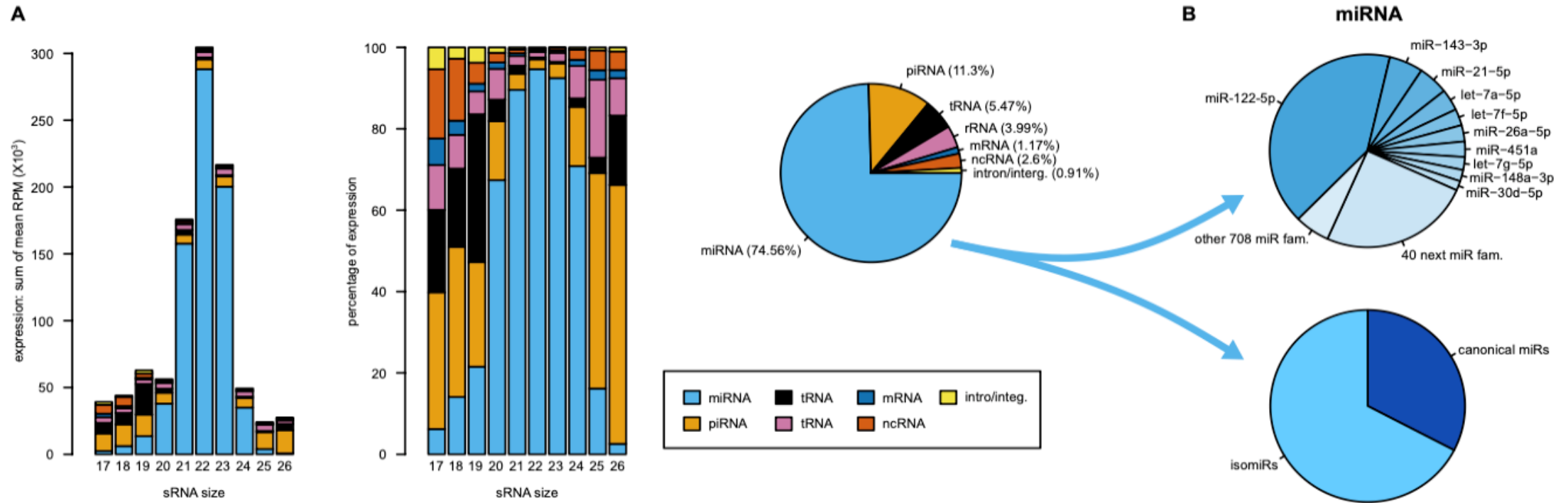
- Why they matter: Edits can change the seed, which can change the targetome. Variants can also affect miRNA stability & dynamics.

Why care about isomiRs in MASLD?

- miRNAs have been established as part of the homeostatic circuitry in the liver (e.g., miR-122), so changes to their regulation are a direct lever on disease biology.
- Measuring and manipulating isomiRs has potential implications for :
 - Therapeutic targeting
 - Biomarker candidates / biomarker refinement



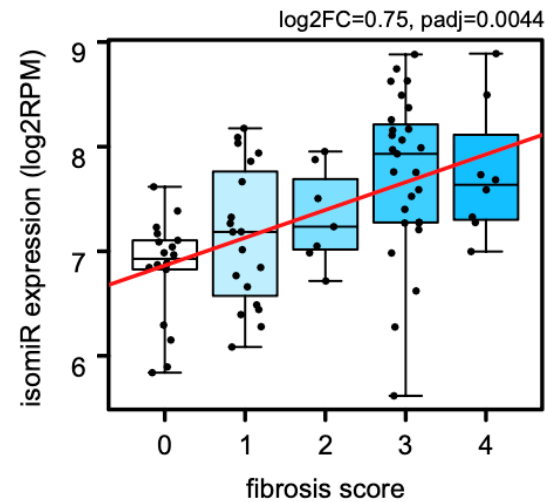
What the isomiR landscape looks like in liver



- Small RNAs (17-26 nts) are dominated by miRNAs
- A small number of miRNA families dominate the total expression of miRNAs (e.g., miR-122)
- Overall, isomiRs are more common than canonical miRNAs.
- 5' edits occur, but 3' edits are much more common

IsomiR-fibrosis association

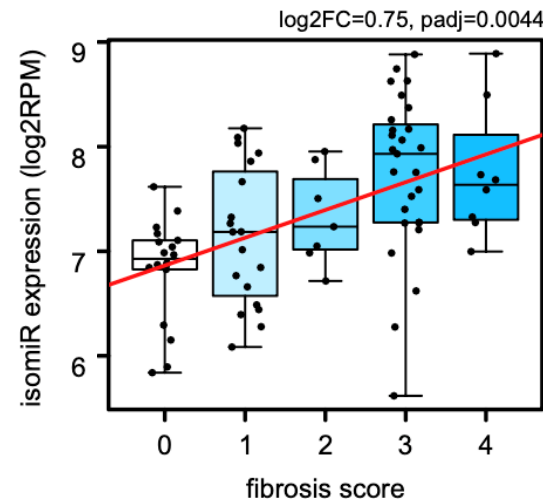
iso-miR-21-5p: aUAGCUUAUCAGACUGAUGUUGAC, 5' isoform seed



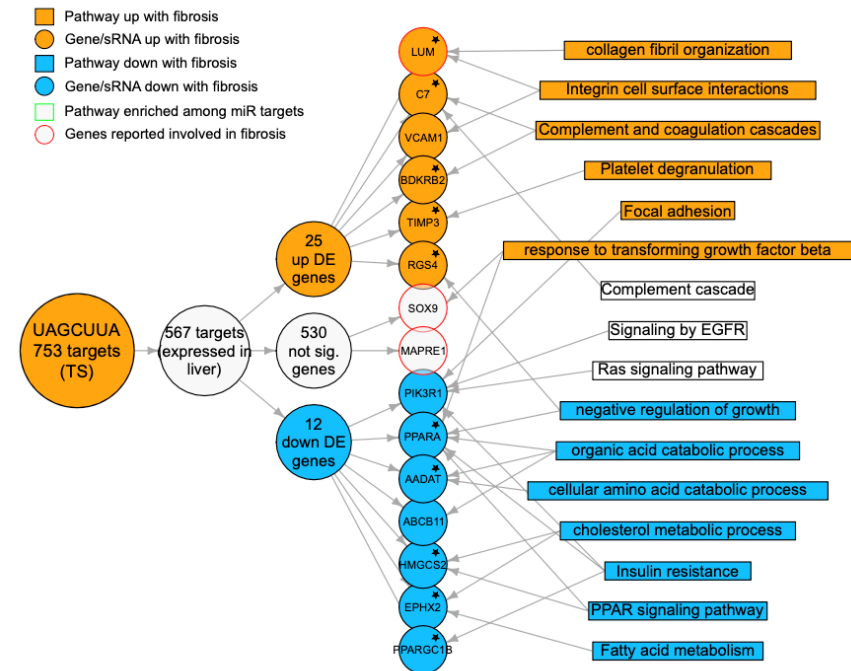
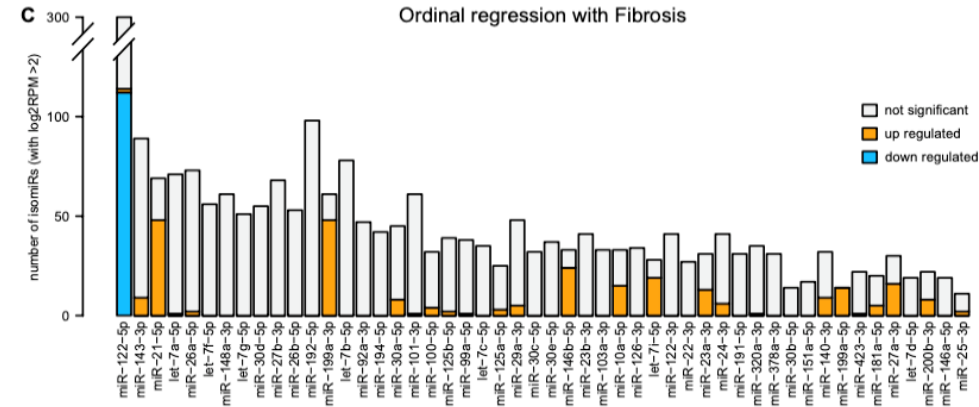
- Ordinal regression to identify isomiRs that are differentially regulated across the fibrosis spectrum (621 up, 141 down)

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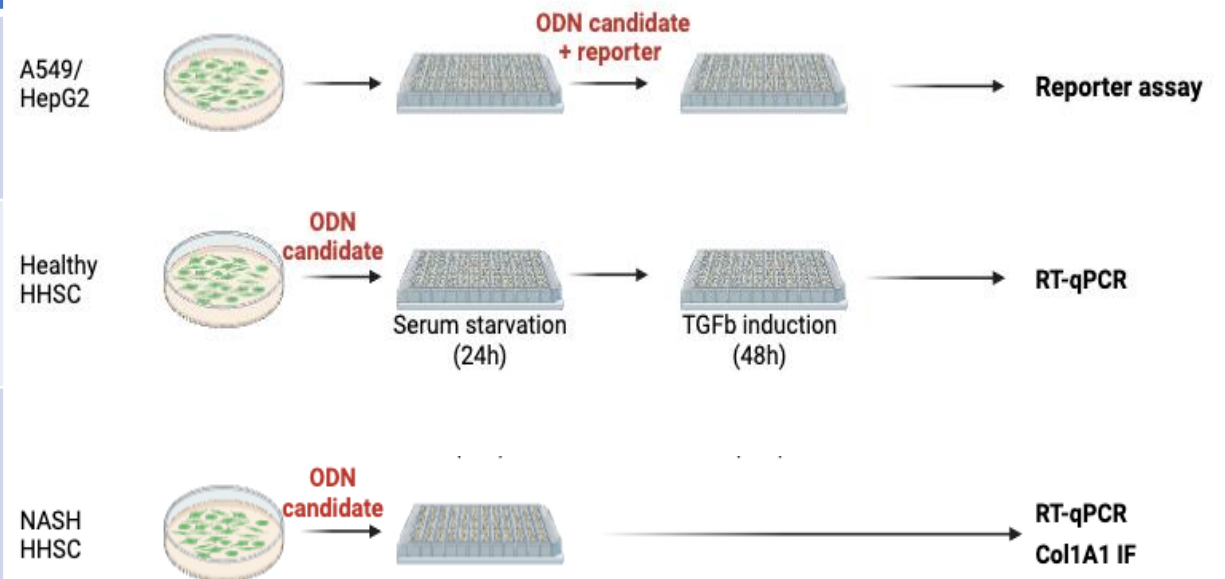


- Ordinal regression to identify isomiRs that are differentially regulated across the fibrosis spectrum (621 up, 141 down)
- Differentially expressed isomiRs tend to cluster within families.



IsomiRs are functionally active and expand the potential therapeutic landscape

| Exp | Question | System | Endpoints |
|---------------------------------------|---|---|---|
| Measures baseline activity | <i>How does isomiR activity compare to canonical miRNAs?</i> | Dual-luciferase reporter with target site in A549 and HepG2 cells | Normalized luciferase activity as a proxy for miRNA / isomiR activity |
| Test the role of isomiRs in fibrosis | <i>Do isomiRs protect against TGFb-induced fibrosis?</i> | Healthy human stellate cells induced with TGFb | Fibrotic mRNA gene panel |
| Test therapeutic potential of isomiRs | <i>Can isomiRs inhibit Collagen and Fibrotic biomarkers in MASH patient stellate cells?</i> | Primary stellate cells from MASH patients | Fibrotic mRNA gene panel |



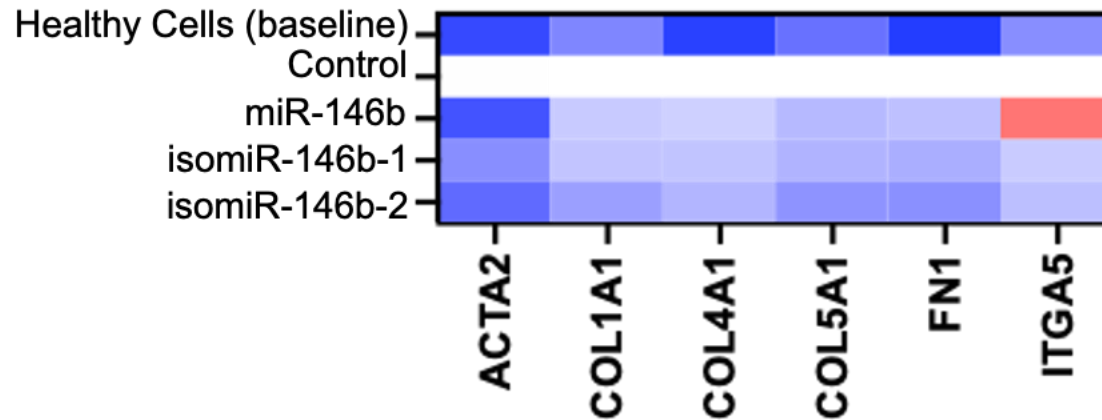
IsomiRs and canonical miRNAs from 6 families were selected based on their correlation to fibrotic scores in MASLD liver biopsies
 miR-164b (2 isomiRs); miR-21 (1 isomiR), miR-224 (3 isomiRs), miR-122 (2 isomiRs), miR-192 (1 isomiR), miR-200b (1 isomiR)

IsomiRs showed enhanced antifibrotic activity in MASLD stellate cells compared to their canonical miRNA counterpart

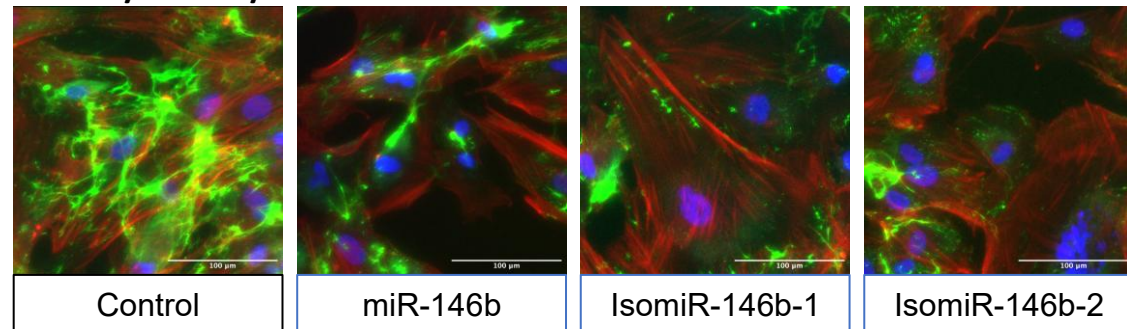
Primary, MASLD stellate cells were treated with oligos

After 48-hours cells were fixed and stained for Collagen (green), F-Actin (red), and Nuclei (DAPI, blue)

After 48-hours RNA was extracted and mRNA was analyzed by RT-qPCR



Col1A1/F-Actin/Nuclei



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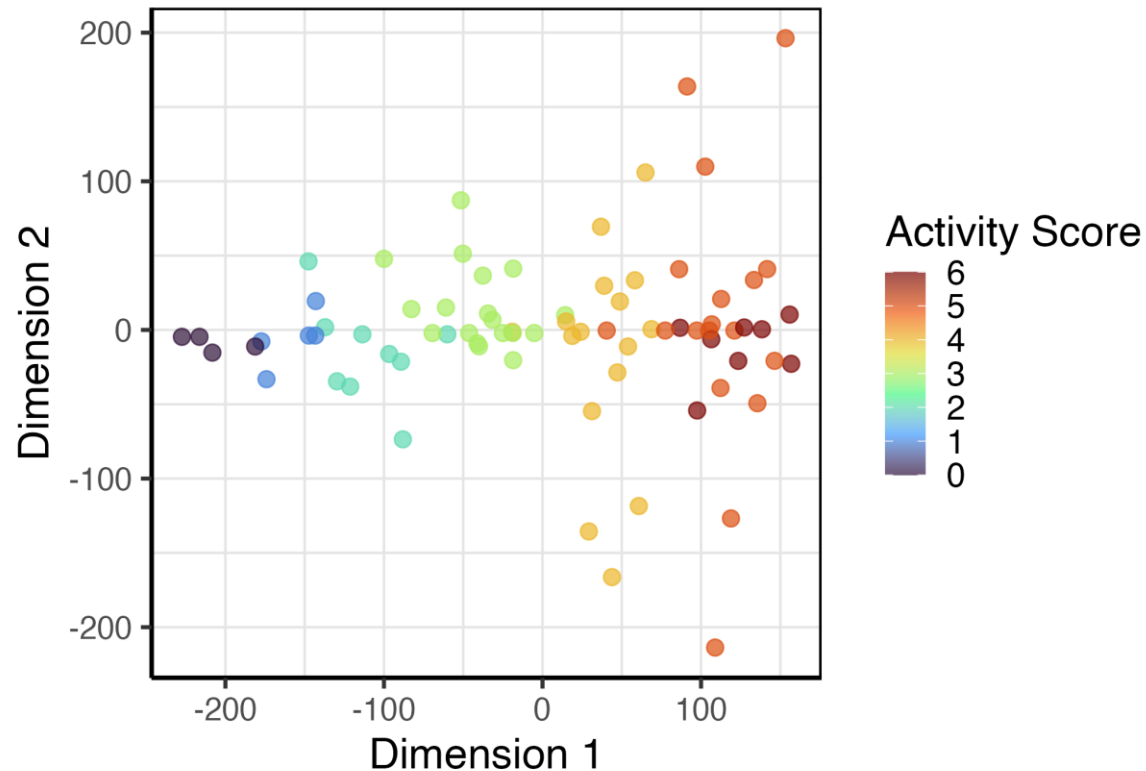
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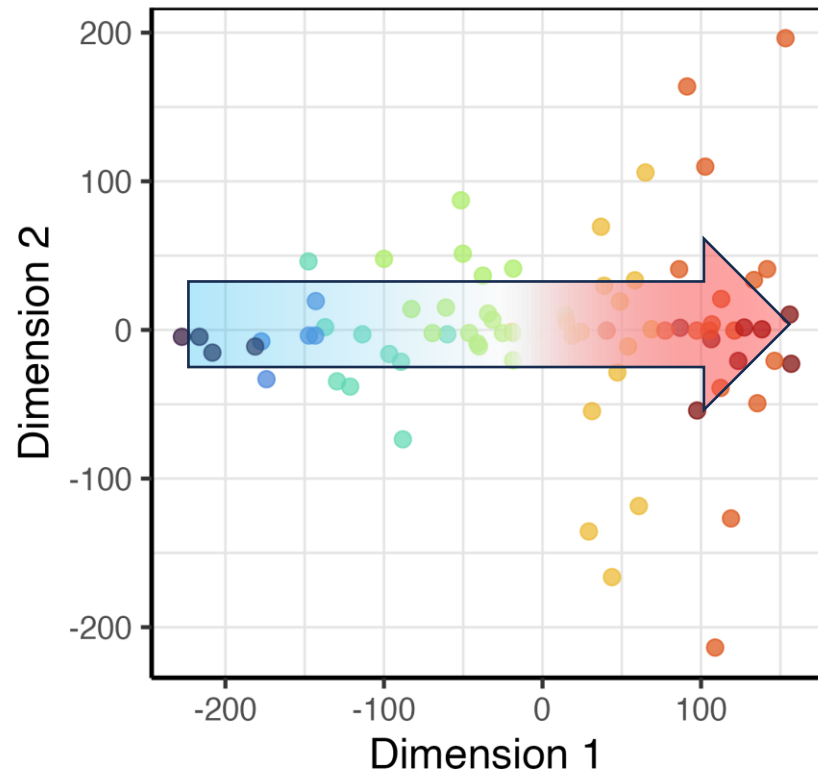
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Does the RNA isoform landscape encode trajectories of disease progression?

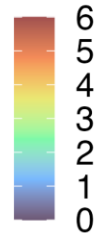


- Previous work has shown clear, reproducible, progressive changes in mRNA expression across the spectrum of disease severity (Hoang et al., 2019).

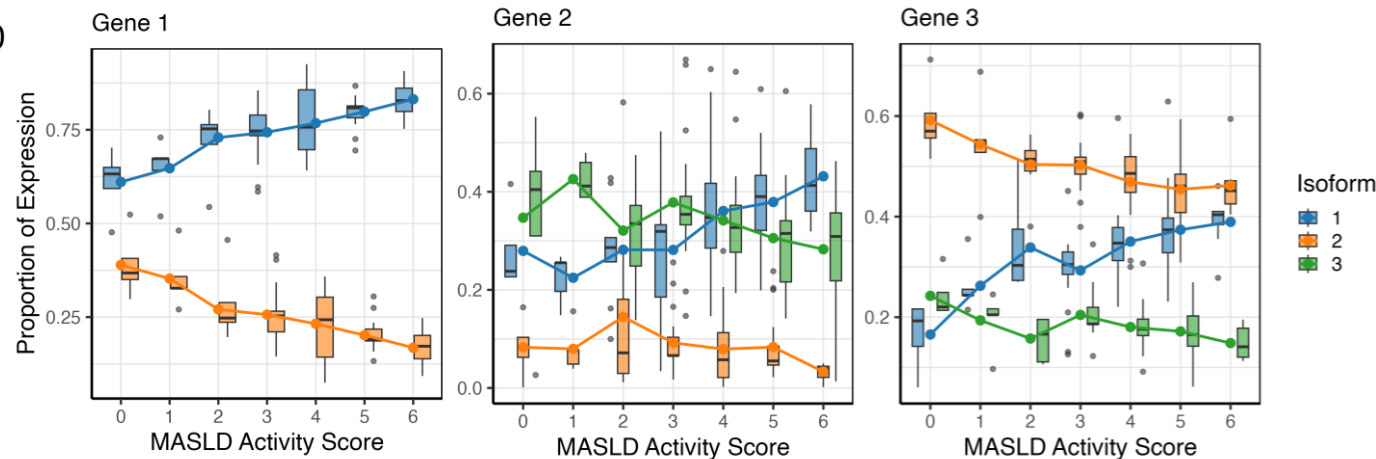
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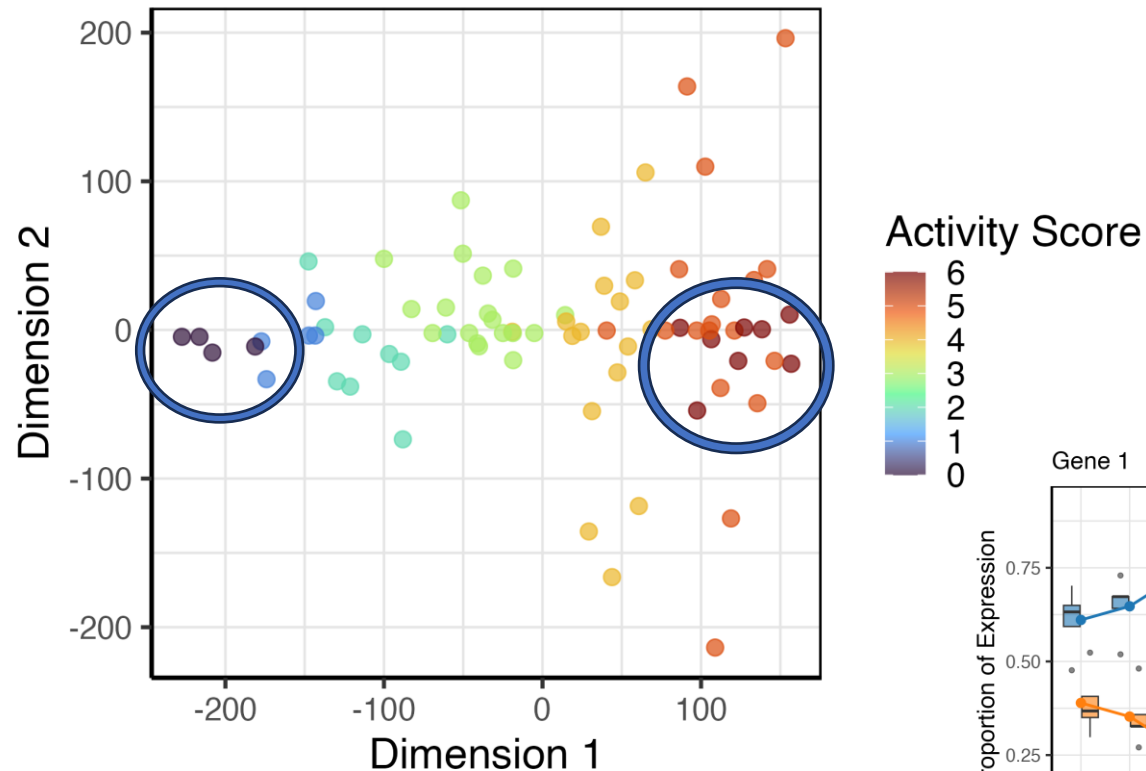
Activity Score



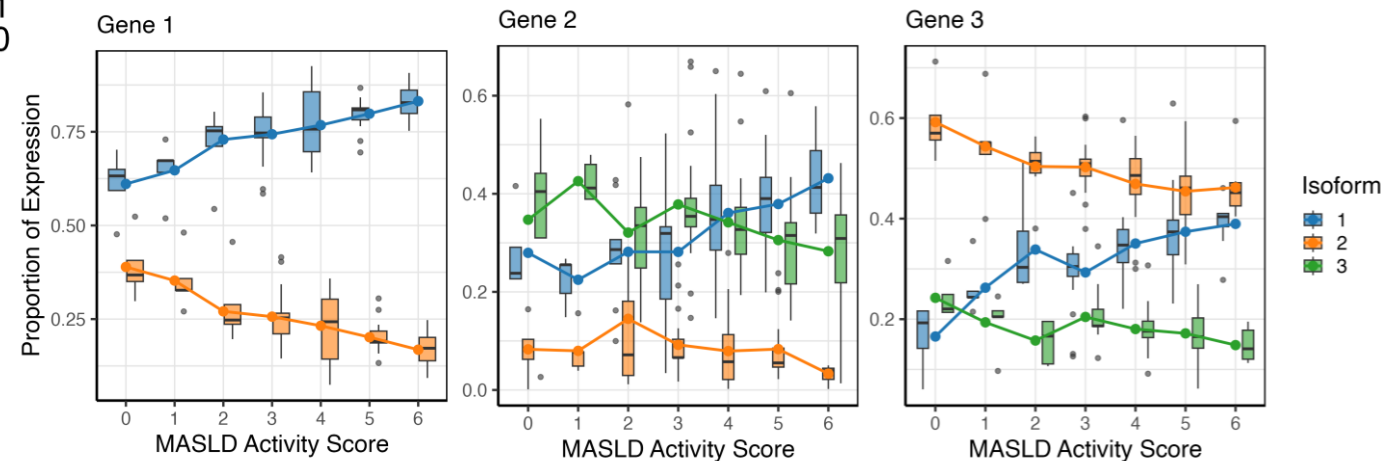
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- Hundreds of genes show compositional changes in isoform usage across the activity spectrum.



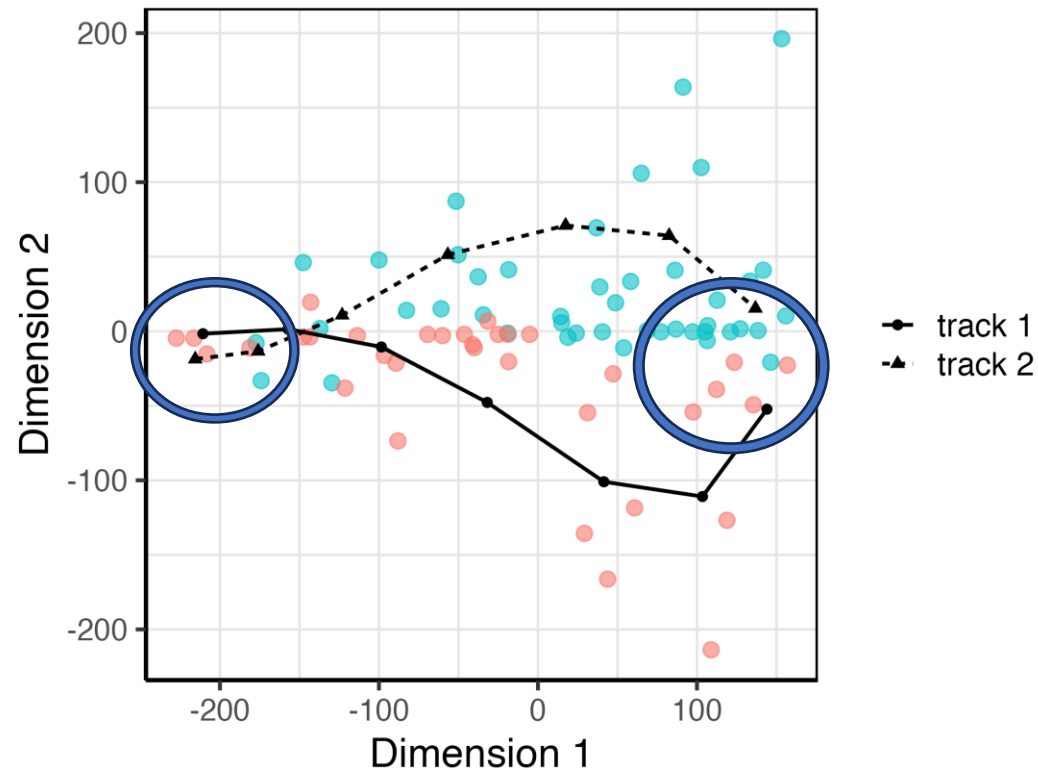
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- A low-dimensional projection of the mRNA isoform landscape hints at attractor states.

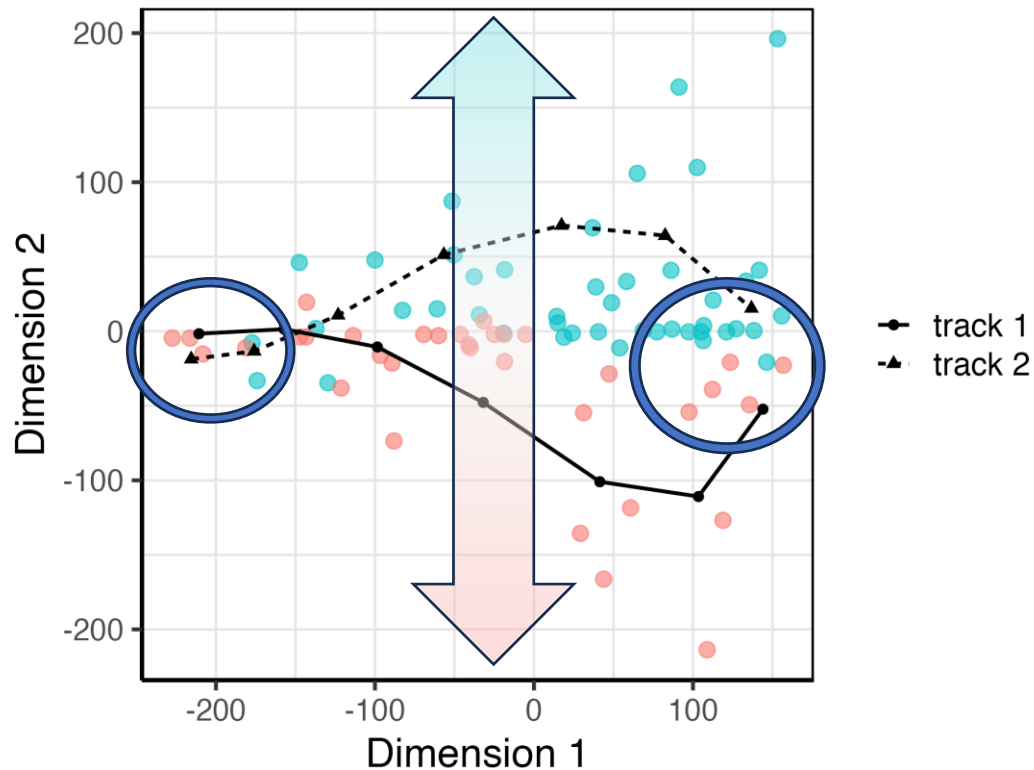


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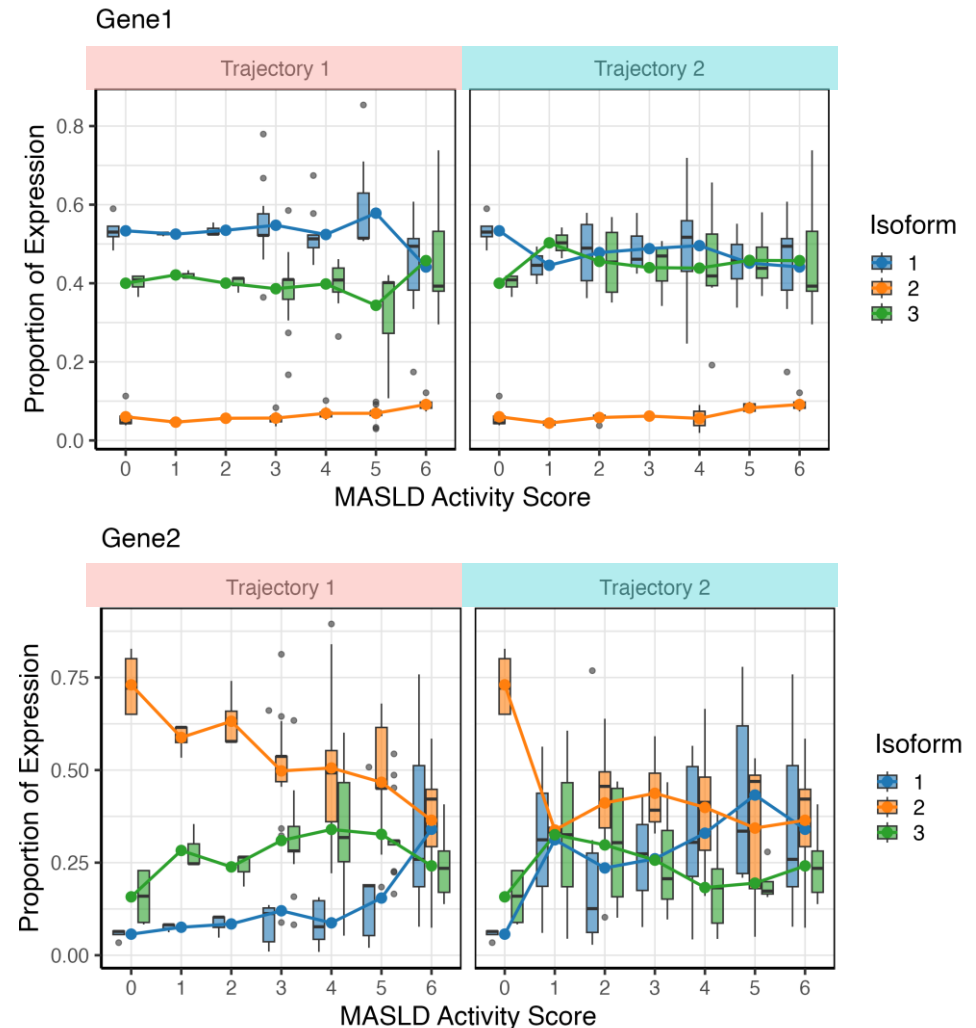


- Samples divided into two clusters corresponding to distinct trajectories through isoform expression space

Does the RNA isoform landscape encode trajectories of disease progression?



T1 vs T2 is strongly enriched for growth, lipid metabolism, and inflammatory pathways



Conclusions

1. The RNA isoform landscape shows promise as a “metric space” for identifying different disease trajectories and patient subtypes.
2. Changes in the isomiR landscape correlate with MASLD severity; individual isomiRs functionally intersect with disease biology.
3. The set of RNA isoforms extends the molecular landscape under consideration for biomarkers and therapeutic target discovery.

Next Steps

1. Replicate in independent cohorts with larger sample sizes. Link trajectory clusters to clinical endpoints and longitudinal data.
2. Further validation of additional isomiRs that have potential therapeutic effects.
3. Use of single-cell technologies to address the inherent limitations imposed by bulk averaging.

Acknowledgements



STRAVITZ-SANYAL INSTITUTE
FOR LIVER DISEASE AND METABOLIC HEALTH

- Arun Sanyal
- Steve Hoang
- Ronnie Li
- Farid Mirshahi
- Mohammad Siddiqui
- Amon Asgharpour

GATEHOUSE BIO

- David Salzman
- Neal Foster
- Christian Brion
- Matthew Long
- Guangliang Wang
- Jessie Ang
- Zheng Zhu
- Bhanu Sakhamuri
- Molly Srouer

Preprint on
isomiR work:

