

# **microRNA-guided diagnostics for neuroendocrine tumors**

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**SEAMO Research and Innovation Showcase  
Isabel Bader Centre for the Performing Arts  
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# Neuroendocrine tumors

- Group of **histologically similar** neoplasms, likely arising from neuroendocrine cells
- Arise throughout the body, particularly in the **pancreas, gastrointestinal tract, lungs, and endocrine glands**, and also skin and other organs
- **Hard to diagnose, often metastatic** at presentation, and **hard to monitor**
- field desperately **needs tissue and circulating biomarkers** to guide clinical decision-making

# microRNAs

- are **small RNA** regulatory molecules
- are **abundantly** expressed
- exhibit **cell-type** and **disease-stage specificity**
- Are **stable** in **solid** (fresh and archived tissues and cell lines) and **liquid** (serum and plasma) clinical **samples**

# Proof-of-concept: GEP-NET study

- Obtained 81 tissue blocks from **27 pancreatic, 31 ileal, 15 appendiceal, and 8 rectal** NETs
- Isolated and quality controlled total RNA, generated microRNA profiles through **small RNA sequencing**
- Performed **data preprocessing**, removing three samples from further analysis
- Performed **high expression** analyses
- Generated a **microRNA-based classifier** for **classifying** and **(almost) grading** NETs

# Bar-coded small RNA library preparation with ext. standard

5 ng - 2 µg total RNA (no size fractionation necessary)

+ 5 fmol 22 nt RNA calibrator cocktail (or less)

5' p ————— OH 3' small RNAs

Rnl2(1-249)K227Q    5' App [blue box] [grey box] O-L 3' X    preadenylated 3' DNA adapters

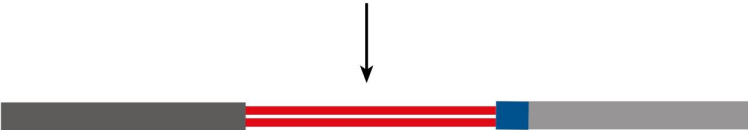
pool up to 20  
separate 3' ligations

5' p ————— [blue box] [grey box] O-L 3' X

5' RNA adapter

5' HO [grey box] OH 3'    Rnl1, ATP

5' HO [grey box] ————— [blue box] [grey box] O-L 3' X



Solexa sequencing,  
bioinformatic separation of libraries

## barcoded 3' adapter collection

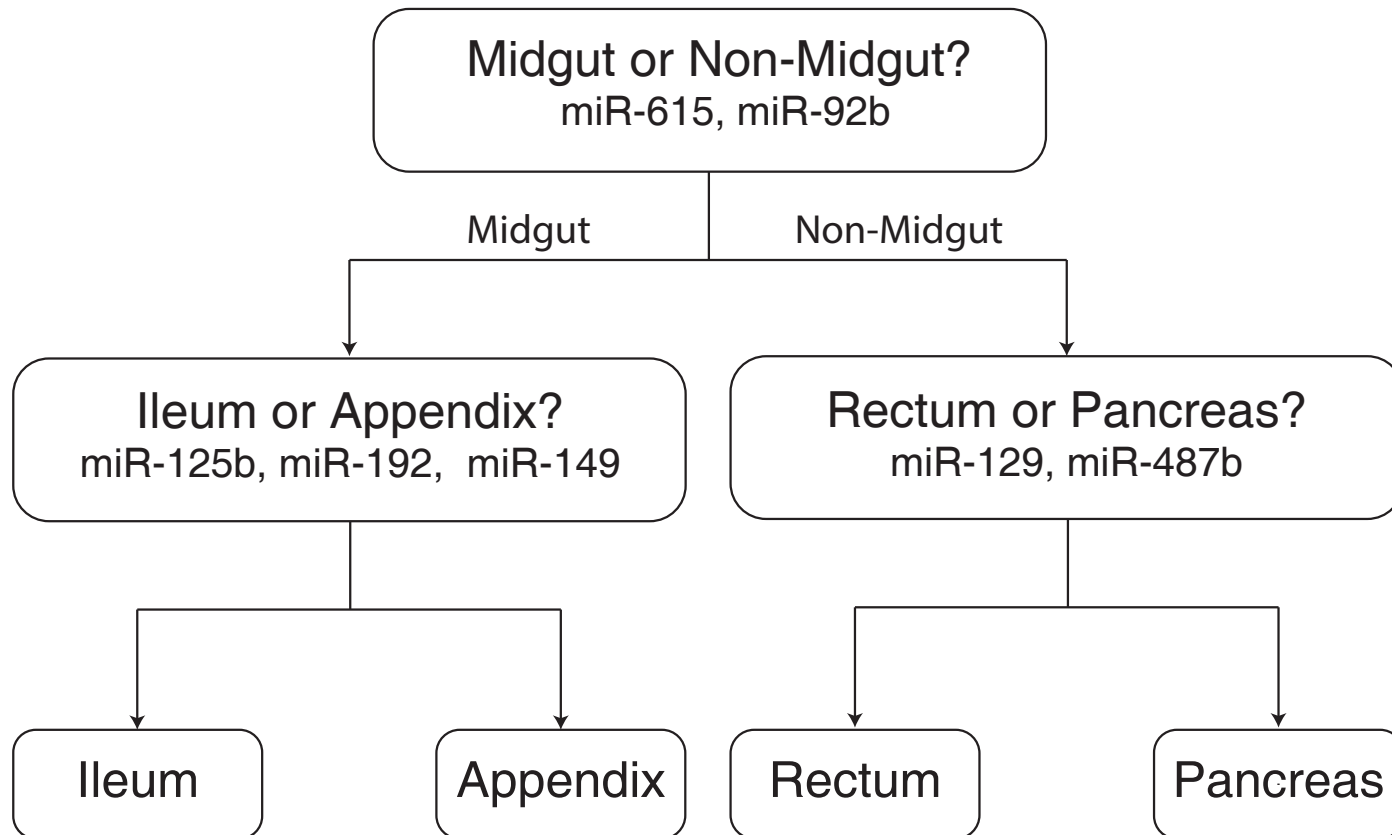
App	TCAC	TCG	TATG	CCGTCTTCTGCTTGT-L
App	TCATC			
App	TCCAC			
App	TCCGT			
App	TCCTA			
App	TCGAT			
App	TCGCG			
App	TCTAG			
App	TCTCC			
App	TCTGA			
App	TTAAG			
App	TAACG			
App	TAATA			
App	TAGAG			
App	TAGGA			
App	TATCA			
App	TGATG			
App	TGTGT			
App	TTACA			
App	TTCCG			
App	TTGGT			

# High expression analyses for GEP-NETs

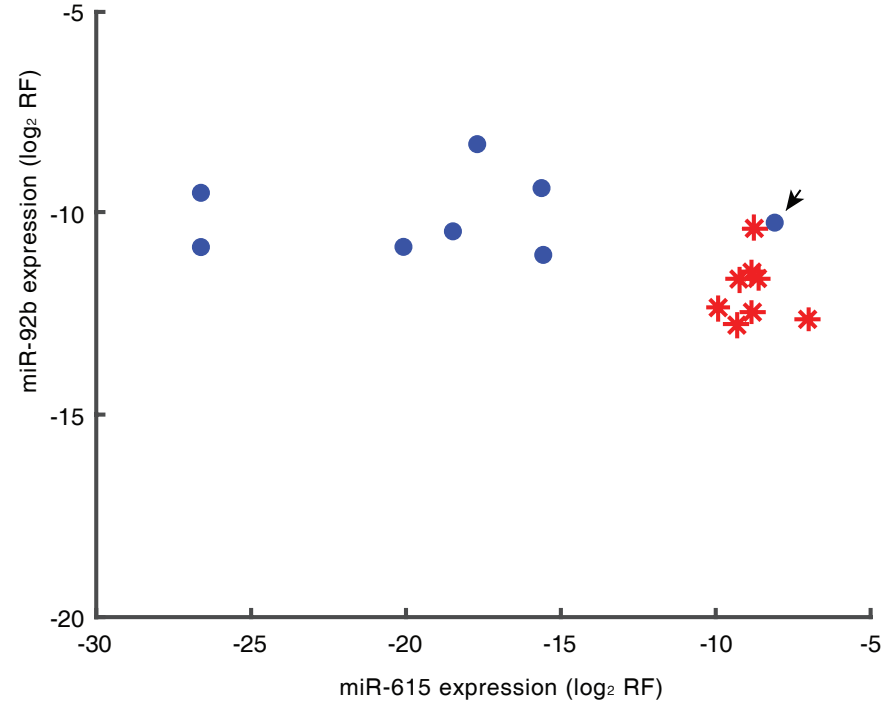
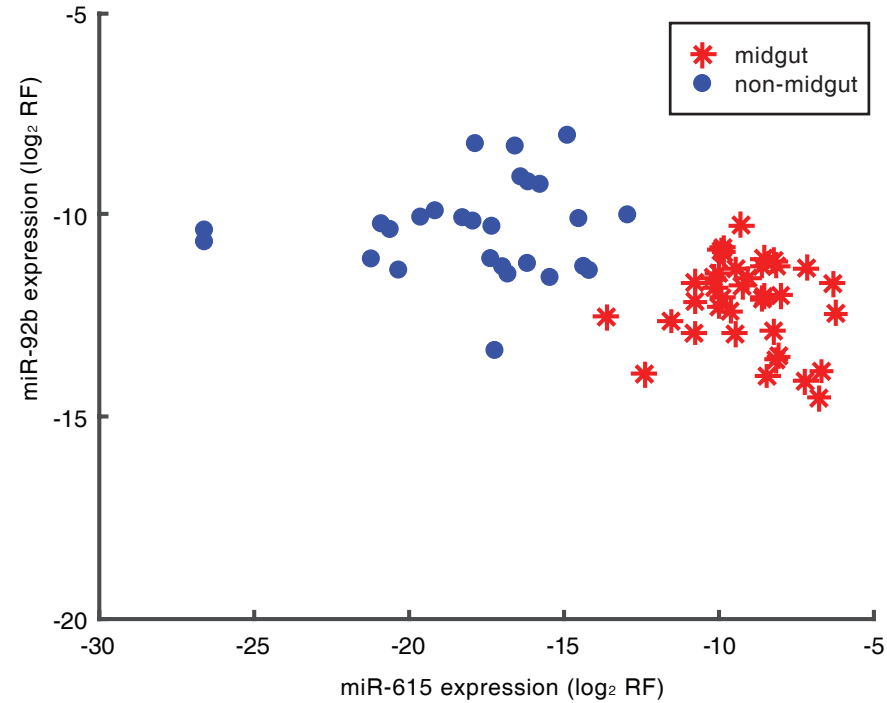
Discovery set		Validation set	
miRNA	median % of miRNA in all samples	miRNA	median % of miRNA in all samples
miR-375	16.6	miR-375	20.2
miR-143	4.9	miR-143	5.3
miR-7	3.9	miR-21	3.9
miR-21	3.3	miR-7	3.2
miR-26a	2.0	miR-192	1.9
miR-125b	1.4	miR-200a	1.4
miR-192	1.4	miR-141	1.4
let-7a	1.3	miR-26a	1.4
miR-29a	1.3	miR-125b	1.2
miR-101	1.2	miR-194	1.2
miR-125a	1.1	miR-27b	1.2
miRNA cistron	median % of miRNA cistron in all samples	miRNA cistron	median % of miRNA cistron in all samples
cluster-mir-375(1)	16.6	cluster-mir-375(1)	20.2
cluster-mir-98(13)	6.9	cluster-mir-143(2)	5.7
cluster-mir-143(2)	5.4	cluster-mir-98(13)	5.5
cluster-mir-7-1(3)	3.9	cluster-mir-21(1)	3.9

Figure 1

# miRNA-based classification of GI-NETs

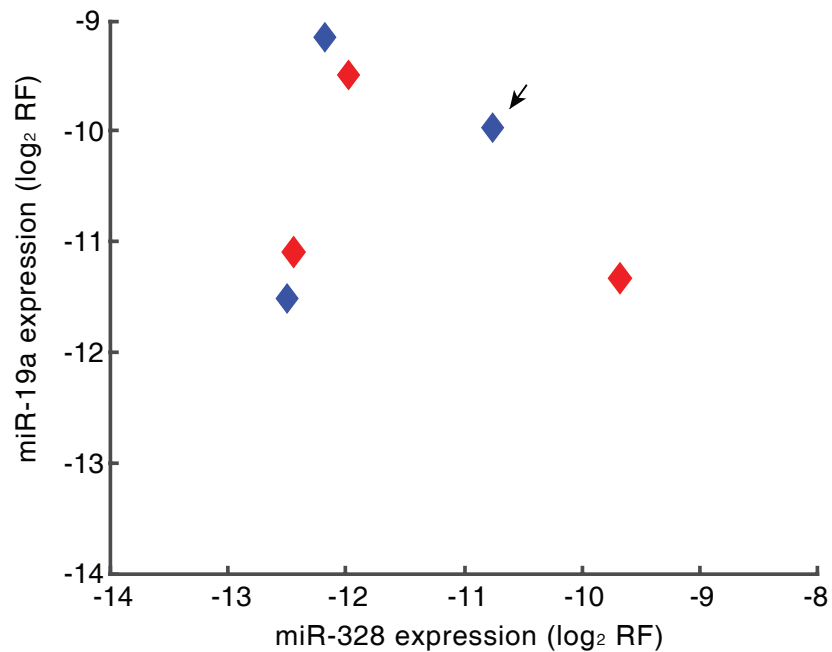
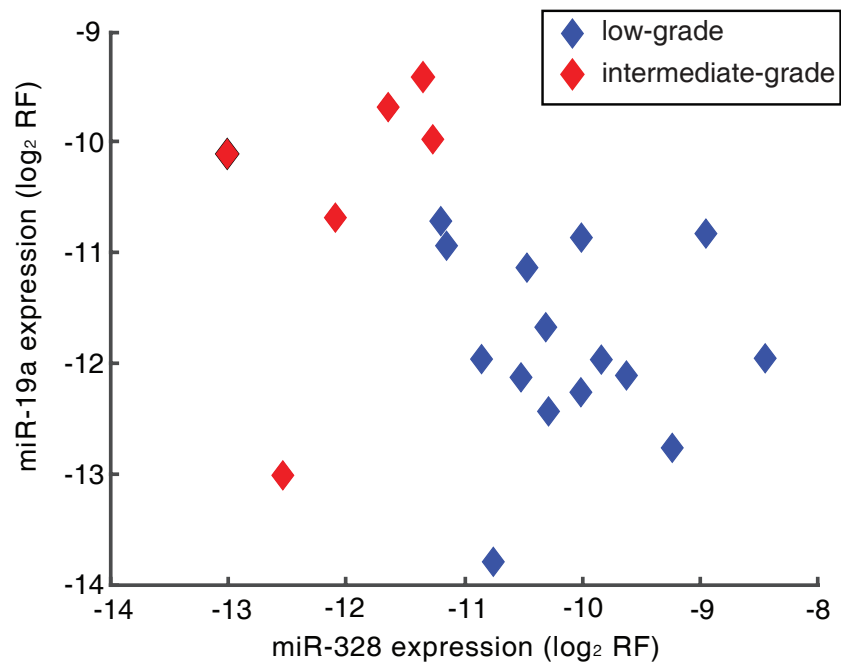


# Midgut vs non-midgut NETs





# Low- vs intermediate-grade pancreatic NETs



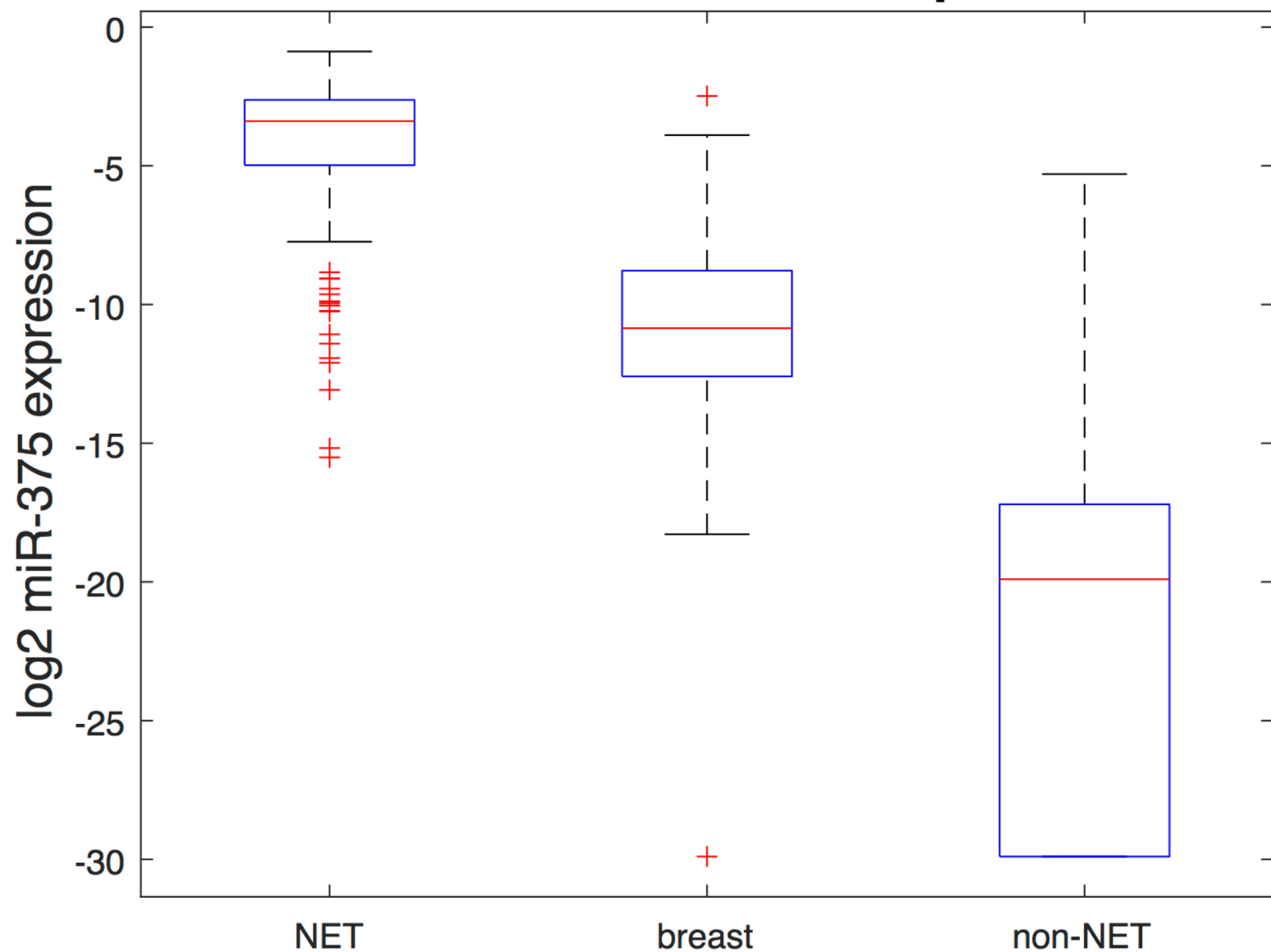
# Summary

- **microRNAs** can be used to **classify** GEP-NETs by anatomic site-of-origin
- microRNAs can likely be used to **grade** pancreatic NETs but further testing is required
- miR-375 is abundantly expressed in all GEP-NETs and **may be valuable tissue and circulatory biomarkers** for this disease

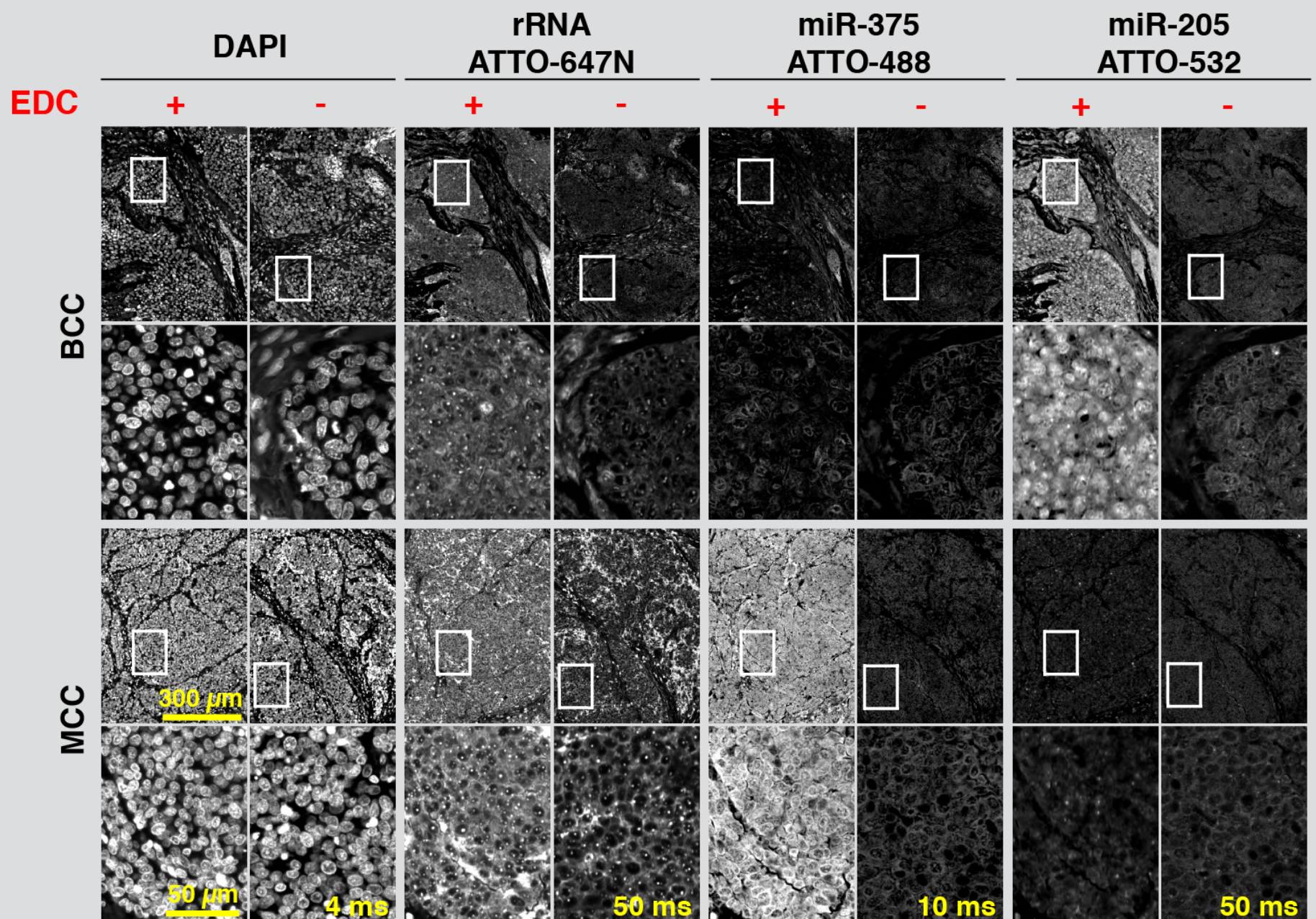
# Showcase

- small RNA profiling of all NETs
- microRNA fluorescence *in situ* hybridization
- plasma microRNA profiling
- microRNA sequence reannotation
- functional characterization of relevant microRNAs

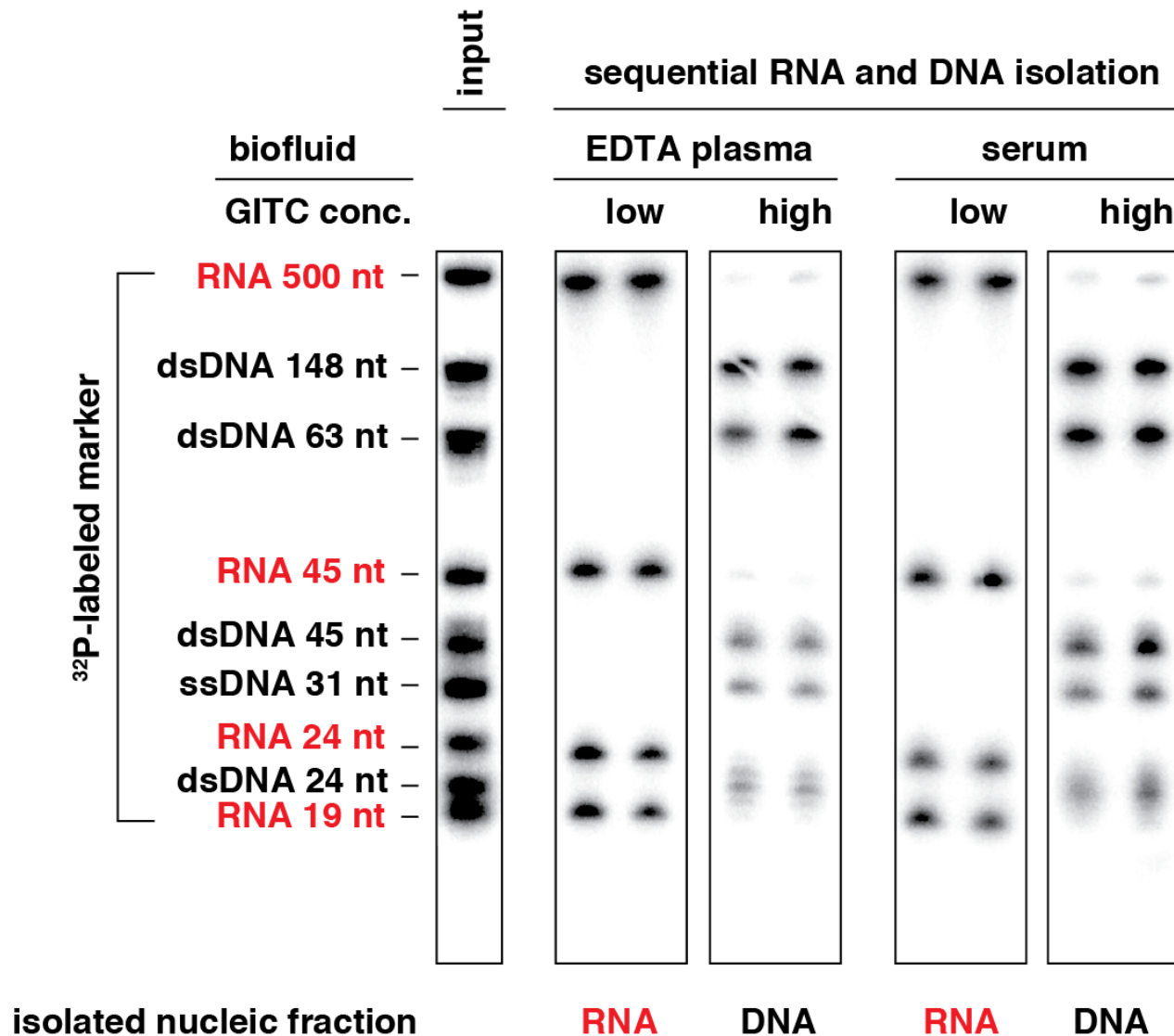
# NET vs non-NET, Kruskal Wallis p= 1.0215e-202



# EDC fixation improves retention of long and short RNAs



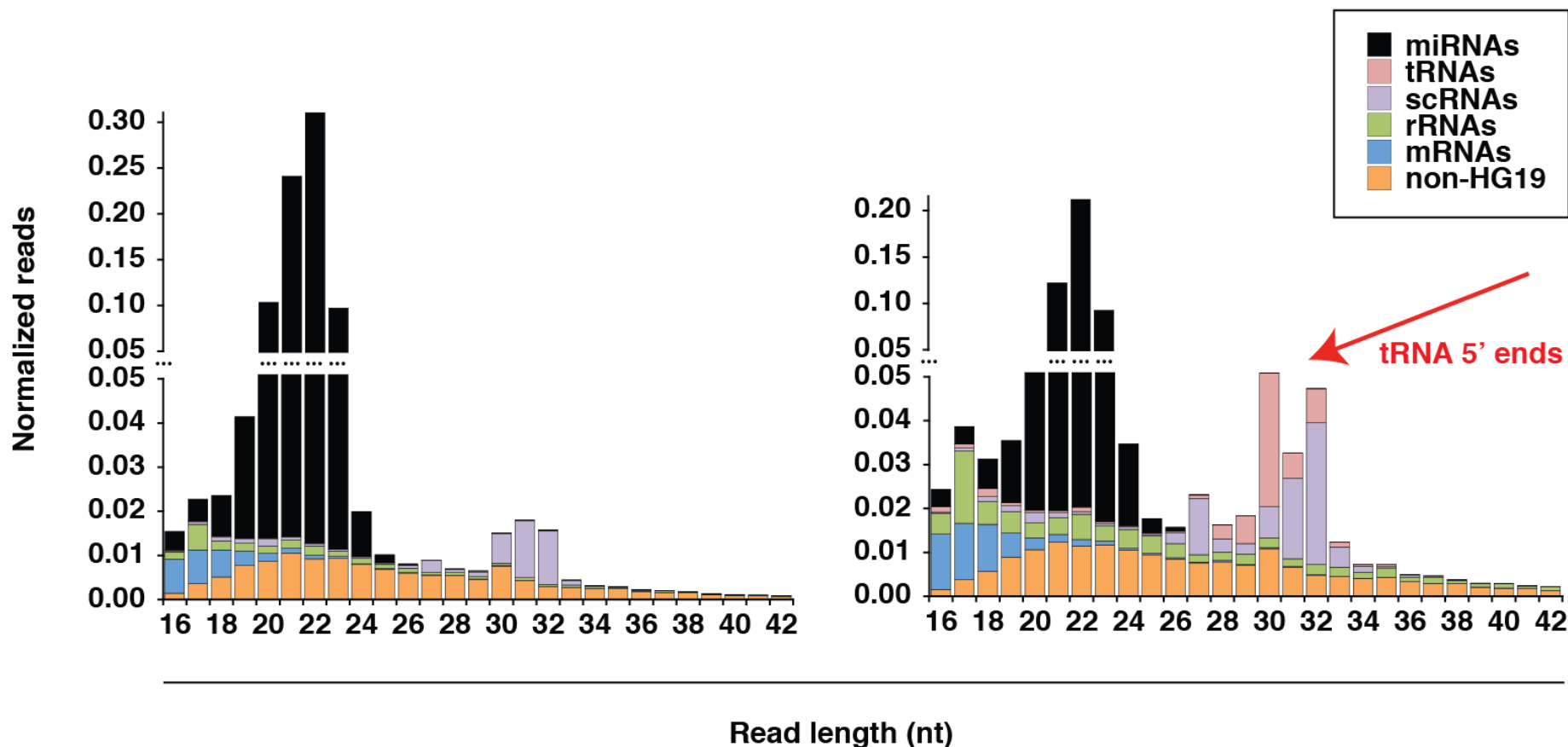
# Quality control of RNA and DNA recovery and separation



# Average sample composition in normal study subjects

Plasma

Serum





# microRNA reannotation

>hsa-mir-205

TCCTTCATTCCACCGAGTCTGT	53756	1
TCCTTCATTCCACCGAGTCTG	49335	1
TCCTTCATTCCACCGAGTCT	16835	1
CTTCATTCCACCGAGTCTGT	1658	1
CTTCATTCCACCGAGTCTG	1585	1
TCCTTCATTCCACCGAGTC	879	1
TCCTTCATTCCACCGAGT	656	1
CTTCATTCCACCGAGTCT	487	1
	441	1
	182	1
	83	1
TCCTTCATTCCACCGAGTCTGTC	67	1
TCCTTCATTCCACCGAG	47	1
TTCCACCGAGTCTGT	36	1
CCCTTCATTCCACCGAGTCTGT	34	1
ATTCCACCGAGTCTGT	29	1
CTTCATTCCACCGAGTC	25	1
CCCTTCATTCCACCGAGTCTG	24	1
TCCTTCATTCCACCGAGTCTGTCT	23	1
ATTCCACCGAGTCTG	13	1
	13	1
TCCTTCATTCCACCGGA	11	1
CCCTTCATTCCACCGAGTCT	10	1
CTTCATTCCACCGAGT	9	1
TTTCATTCCACCGAGTCTG	8	1
CATTCCACCGAGTCTG	5	1
	5	1
TCATTCCACCGAGTCTG	5	1
TTTCATTCCACCGAGTCTGT	5	1
	4	1
TCATTCCACCGAGTCT	4	1
CATTCCACCGAGTCTGT	4	1
CATTCCACCGAGTCT	3	1
TTTCATTCCACCGAGTCT	3	1
	3	1
CTTCATTCCACCGAGTCTGTCT	2	1
	2	1
CTTCATTCCACCGAG	1	1
	1	1
	1	1
	1	1
TGTCCTTCATTCCACCGAGTCT	1	1

GATTTCAGTGGAGTGAAGTTCA
GATTTCAGTGGAGTGAAGTTTC
ATTTTCAGTGGAGTGAAGTTCA
AGTGGAGTGAAGTTCA
TTTCAGTGGAGTGAAGTTCA
ATTTTCAGTGGAGTGAAGTTTC
GATTTCAGTGGAGTGAAGTT
CCAGATTTTCAGTGGAGTGAAGTT
AGATTTTCAGTGGAGTGAAGTTTC
TCAGTGGAGTGAAGTTTC

GCTTCTCTTGCTTCATTCCACCGAGTCTGTCTCATACCAACCAGATTTTCAGTGGAGTGAAGTTTCAGGAGGCATG

sequence evidence criteria supporting microRNA existence include:

non-mapping to other non-coding RNAs  
sufficient read mapping  
bimodal read distribution  
supportive RNA folding  
5' end uracil  
5' end processing  
evolutionary conservation

5' UCCUUC AUUCCACCGGAGUCUGU  
CCUUGAAGUGAGGUGACUUUAG 5'



# Acknowledgments

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