Towards early cancer detection through EV analysis
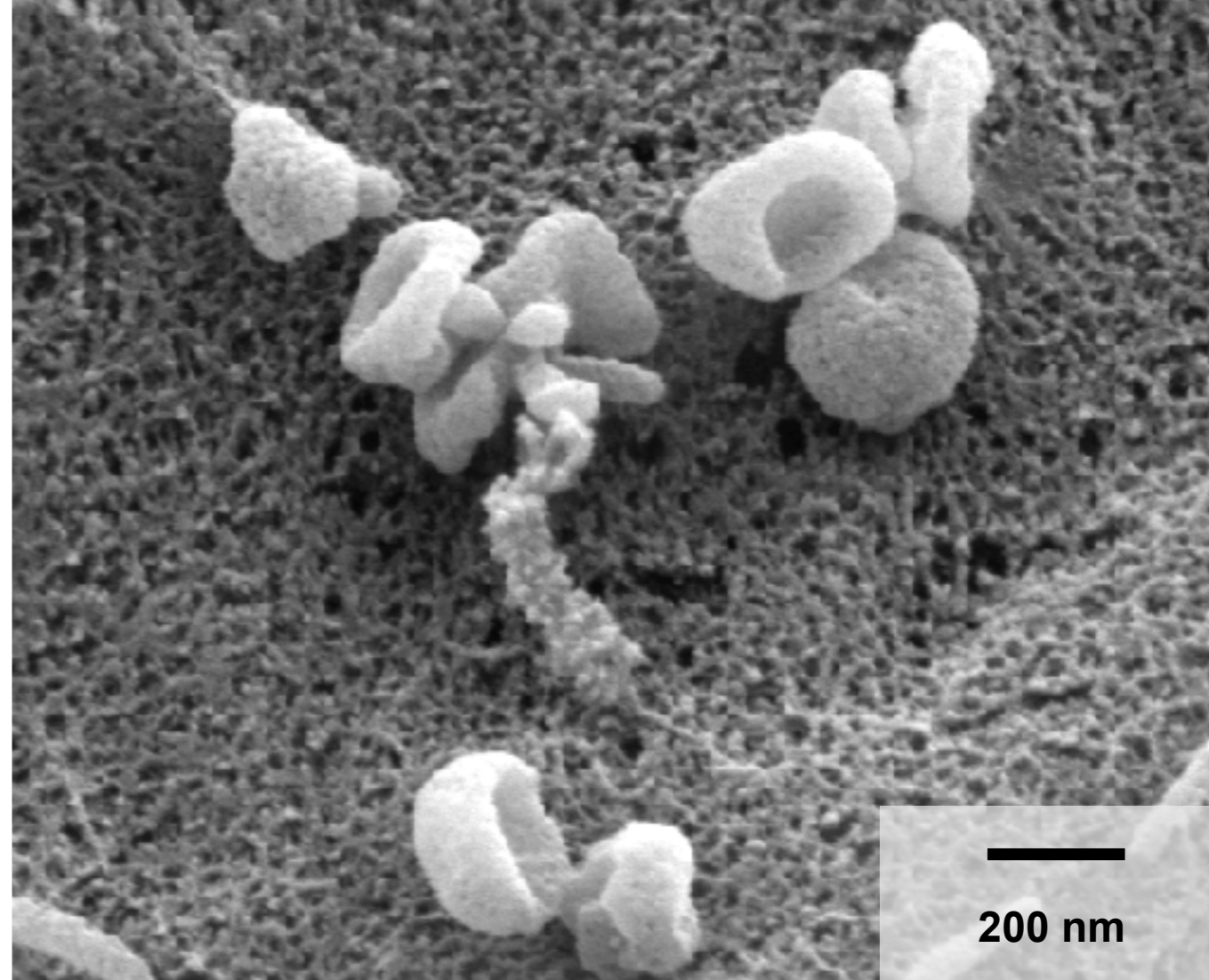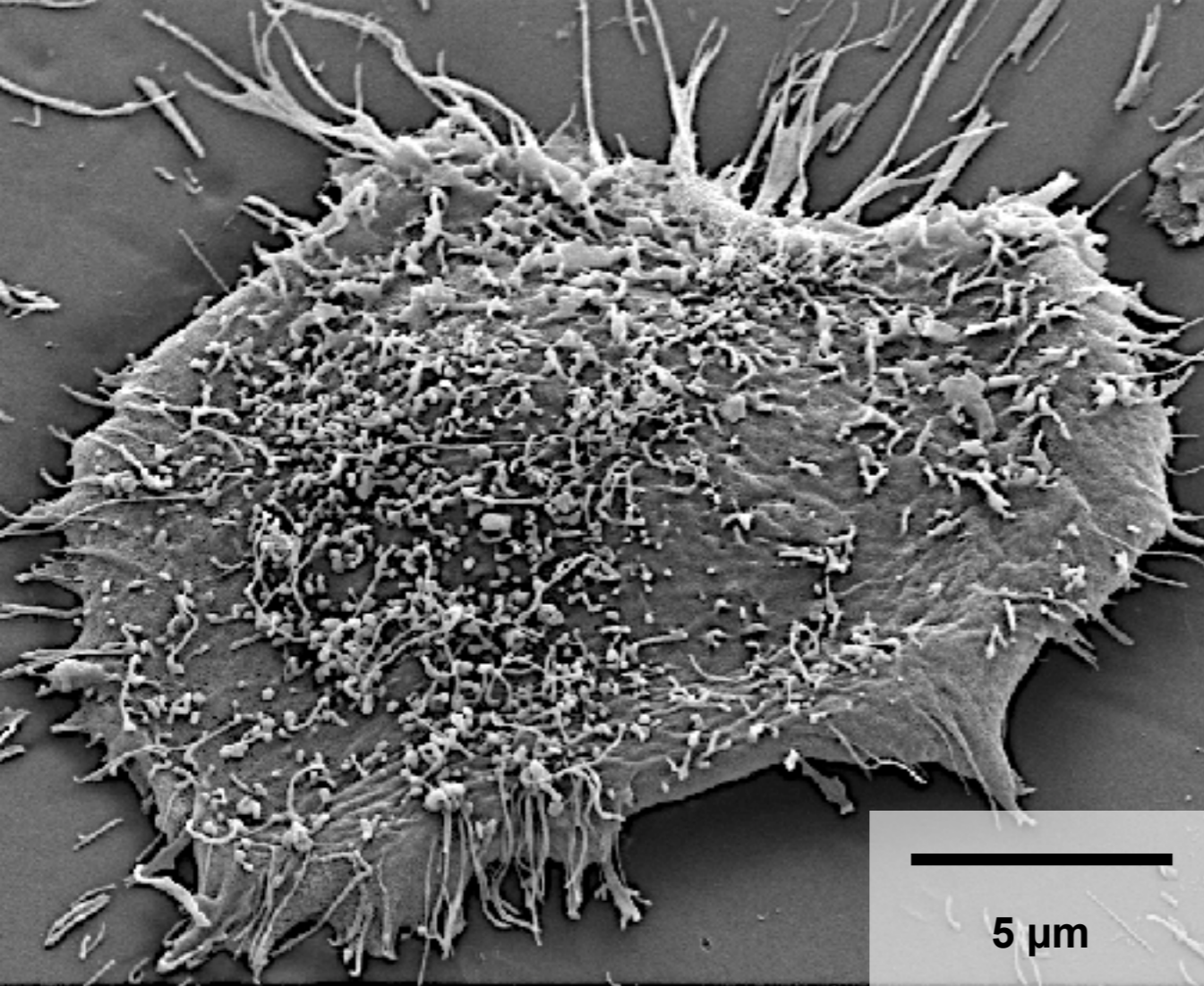
Ralph Weissleder

CSB

MGH 1811

HARVARD MEDICAL SCHOOL

Outline

1. **Review EV analytical technologies**

2. PDAC/IPMN: from bulk to single EV analysis

3. Early stage BCA mRNA EV analysis

5 µm

200 nm

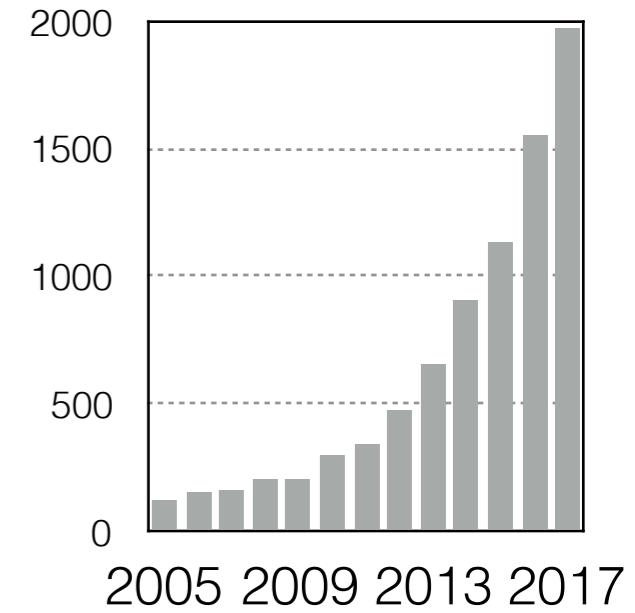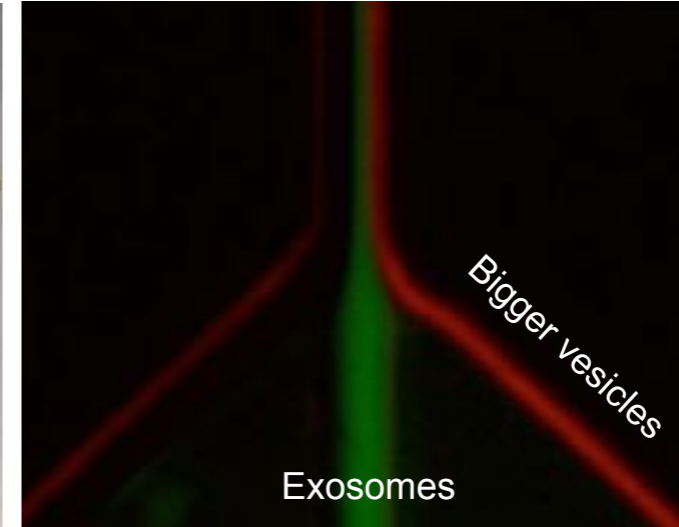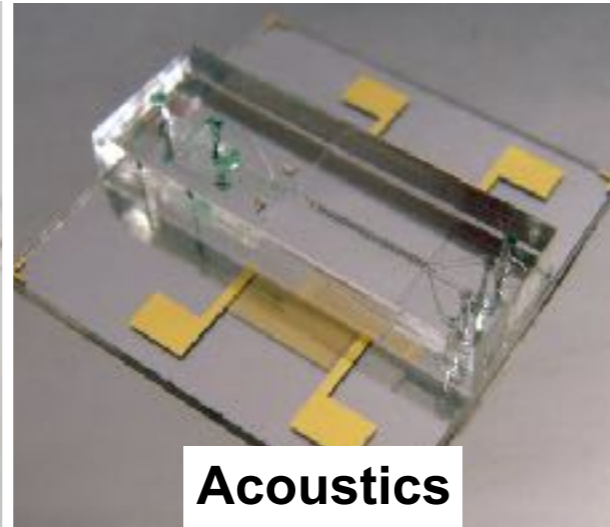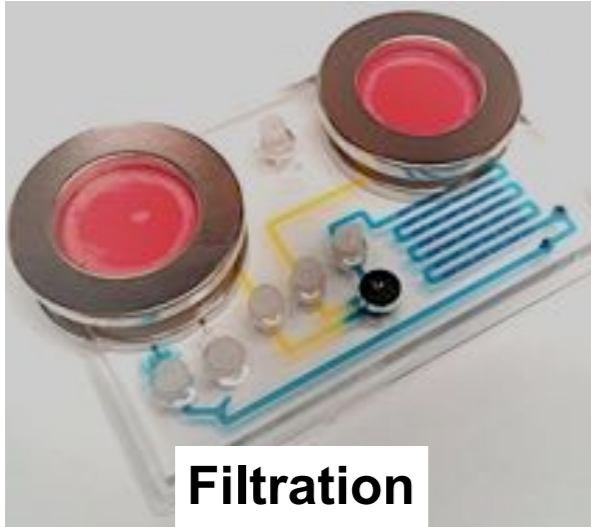## Characteristics
Abundant
Stable
Accessible
Protein, mRNA
Reflect cell of origin
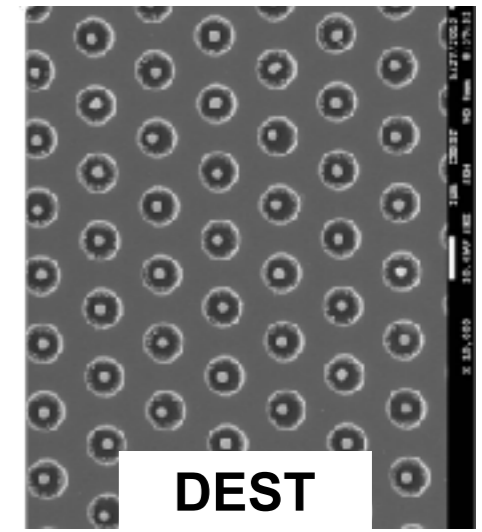Modulate cancer growth

EV challenges:
1. Which technology (speed/cost/accuracy/throughput) ?
2. Which EV (exo, MV, other) ?
3. How to separate TEV/HEV ?

EV Nano Analysis Platforms

Isolation
- Filtration
- Acoustics
- Bigger vesicles / Exosomes

Protein profiling
- µNMR
- nPLEX
- iMEX
- SEA
- DEST

RNA detection
- Magnetic beads — 500 nm
- ❶ Exosome enrichment
- ❷ RNA capture
- ❸ RNA reservoir
- ❹ qPCR chamber
- Inlet / Push valve
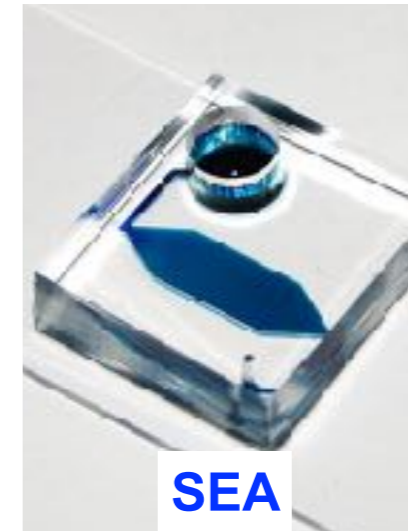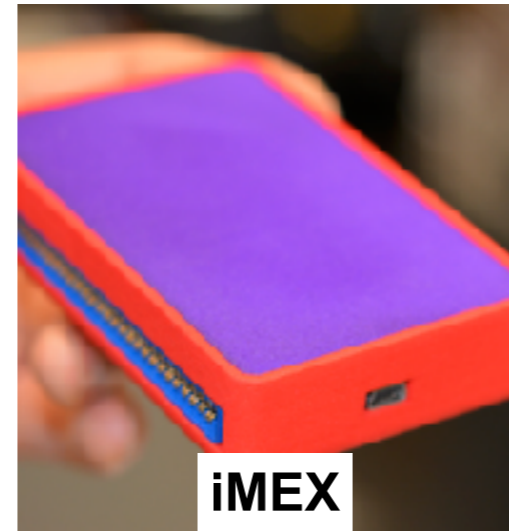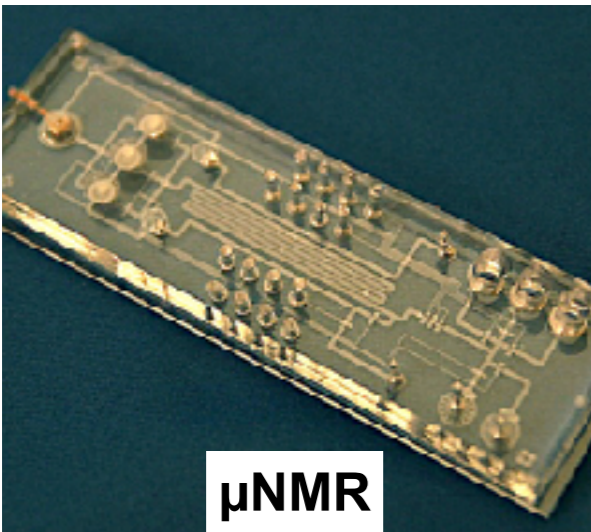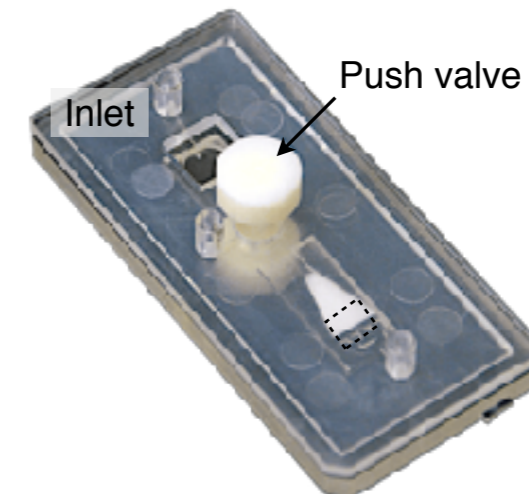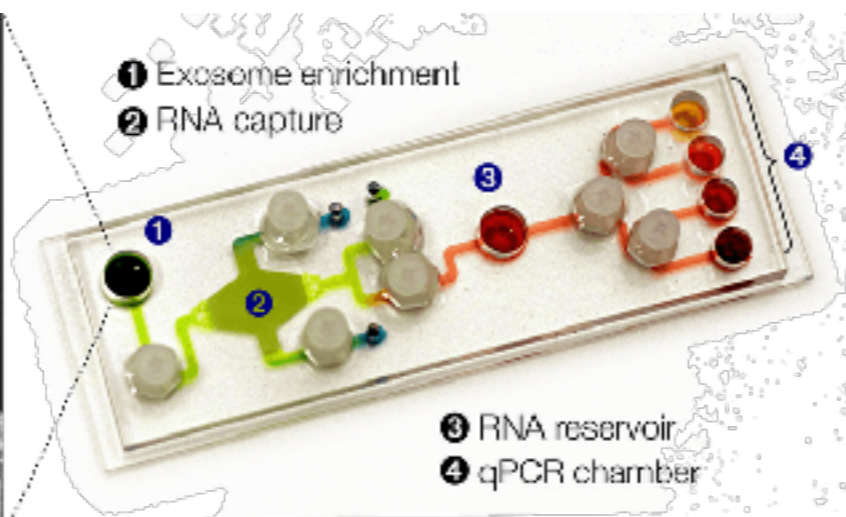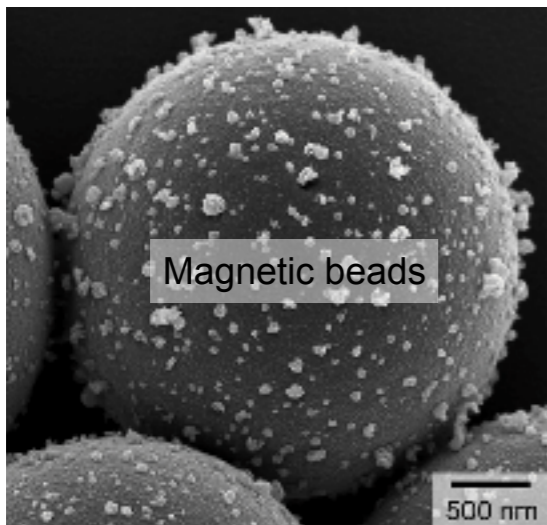
Many more + commercial tests

Types of secreted vesicles (EV)

Biogenesis

Secretory pathway

Lipid bilayer

Carrier vesicle

Secretory vesicle

Multivesicular bodies (MVB) from other organelles

Golgi

Nucleus

Endoplasmic reticulum

Early/sorting endosome

Receptors

Endocytosis

Fusion to create late endosomes

Multivesicular endosomes

Release

Lysosome

Degradation

Integrins

Microvesicles

CD40

Selectins (CD62)

Exosome-like vesicles

TNFRI

Exosomes

Tetraspanins (CD63/CD9)

Alix
TSG101
ESCRT
mitochondrial DNA
mRNA
miRNA

CD133

Membrane particles

Types/Characteristics

Microvesicles
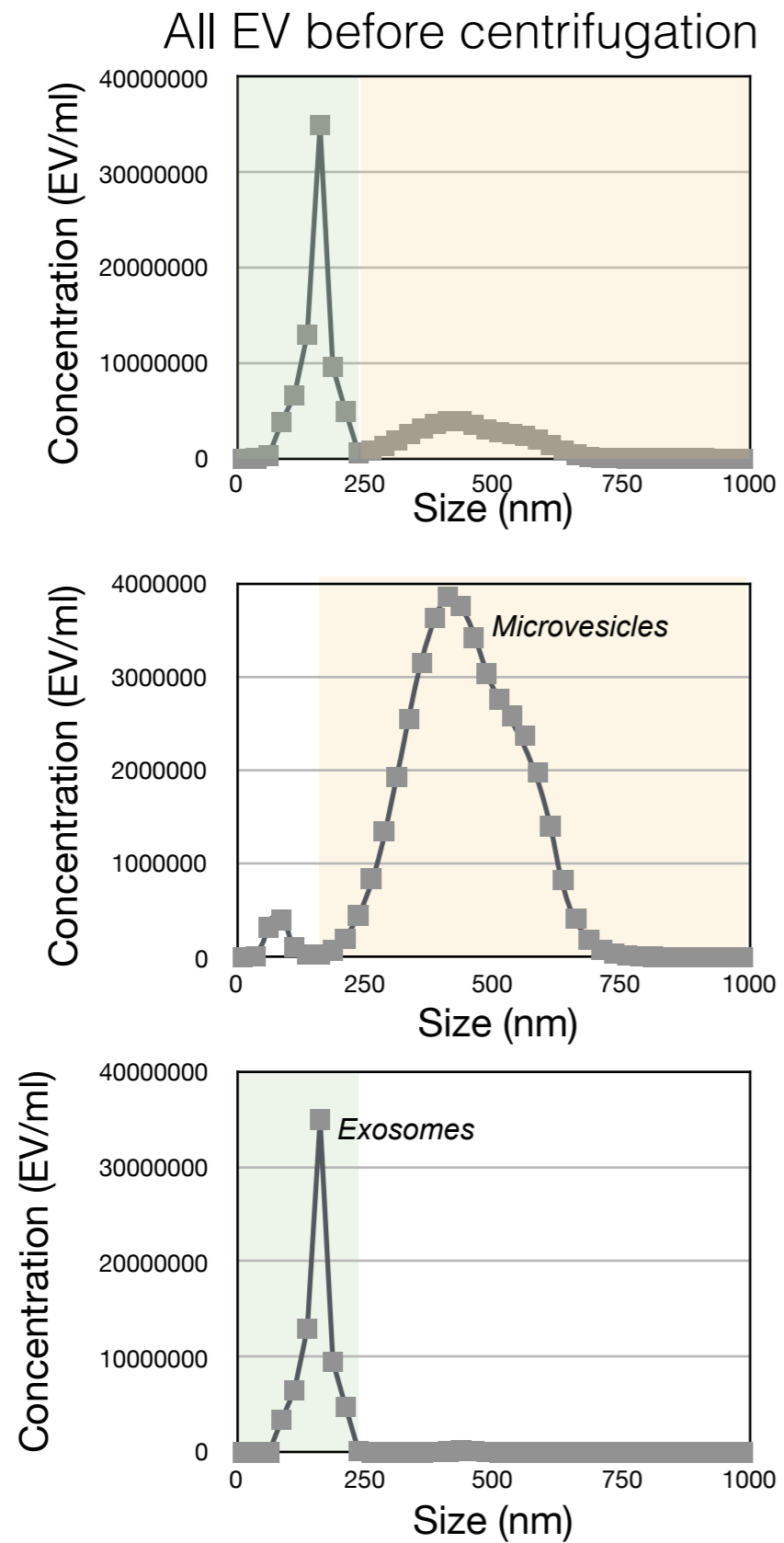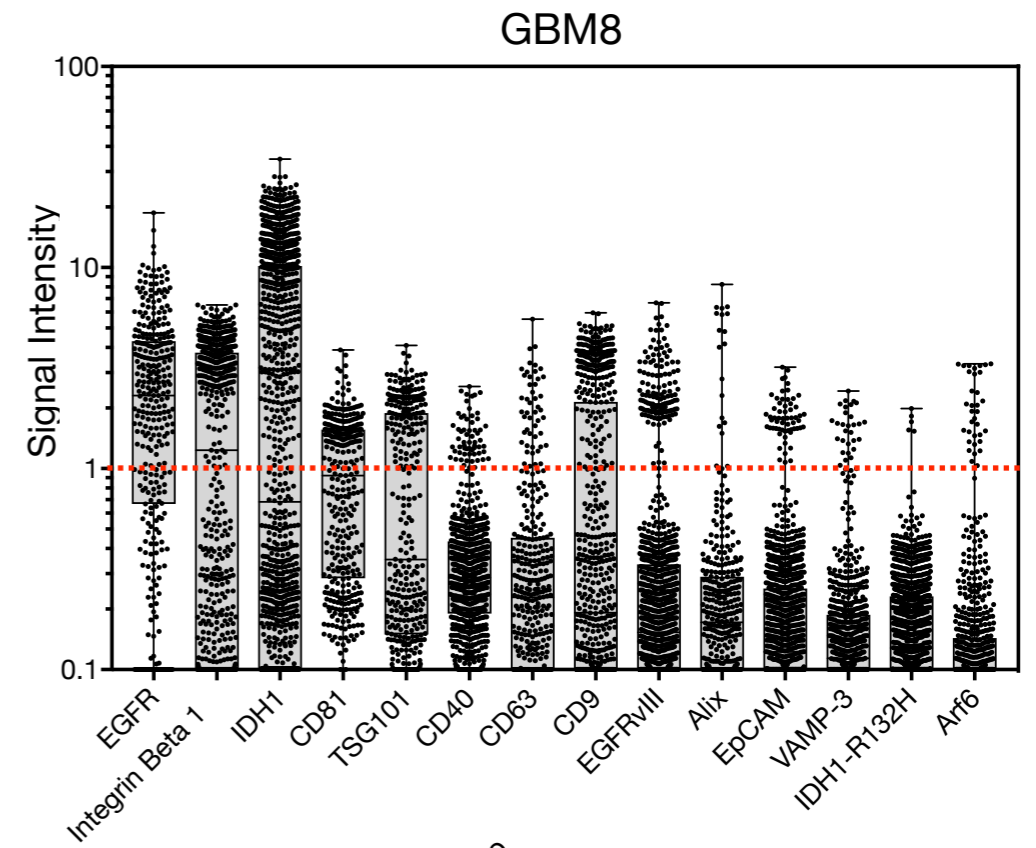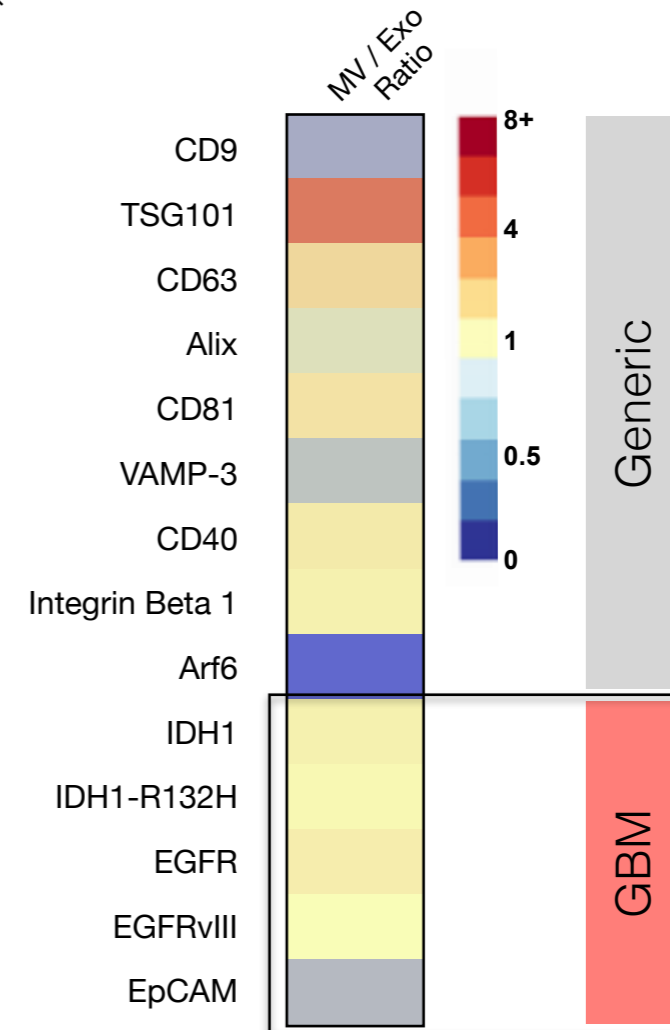|	
Size:	100-1000nm
Shape:	Irregular
Markers:	Intergins, selectins, CD40 ligand
Lipids:	Phosphatidylserine
Origin:	Plasma membrane

Exosome-like vesicles
Size:	20-50nm
Shape:	Irregular
Markers:	TNFRI
Lipids:	No lipid rafts
Origin:	MVB from other organelles?

Exosomes
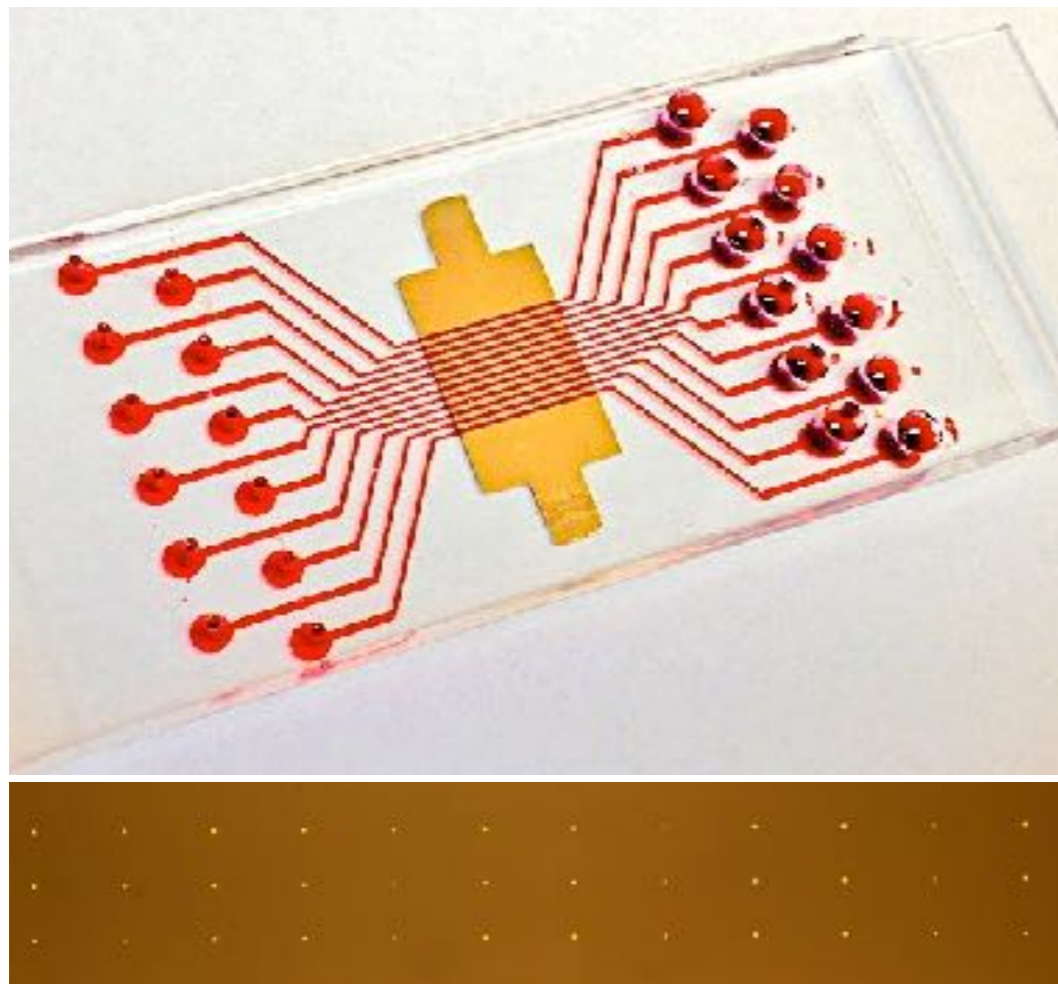|	
Size:	50-100nm
Shape:	Cup shaped
Markers:	Tetraspanins (CD63/CD9), Alix, TSG101, ESCRT
Lipids:	Cholesterol, sphingomyelin, ceramide, lipid rafts, phosphatidylserine
Origin:	Multivesicular endosomes

Membrane particles
Size:	50-80mn
Shape:	Round
Markers:	CD133, no CD63
Lipids:	Unknown
Origin:	Plasma membrane

Adapted from www.abcam.com

A All EV before centrifugation

Microvesicles

Exosomes

B GBM8

C MV / Exo Ratio

Generic

GBM

Remember
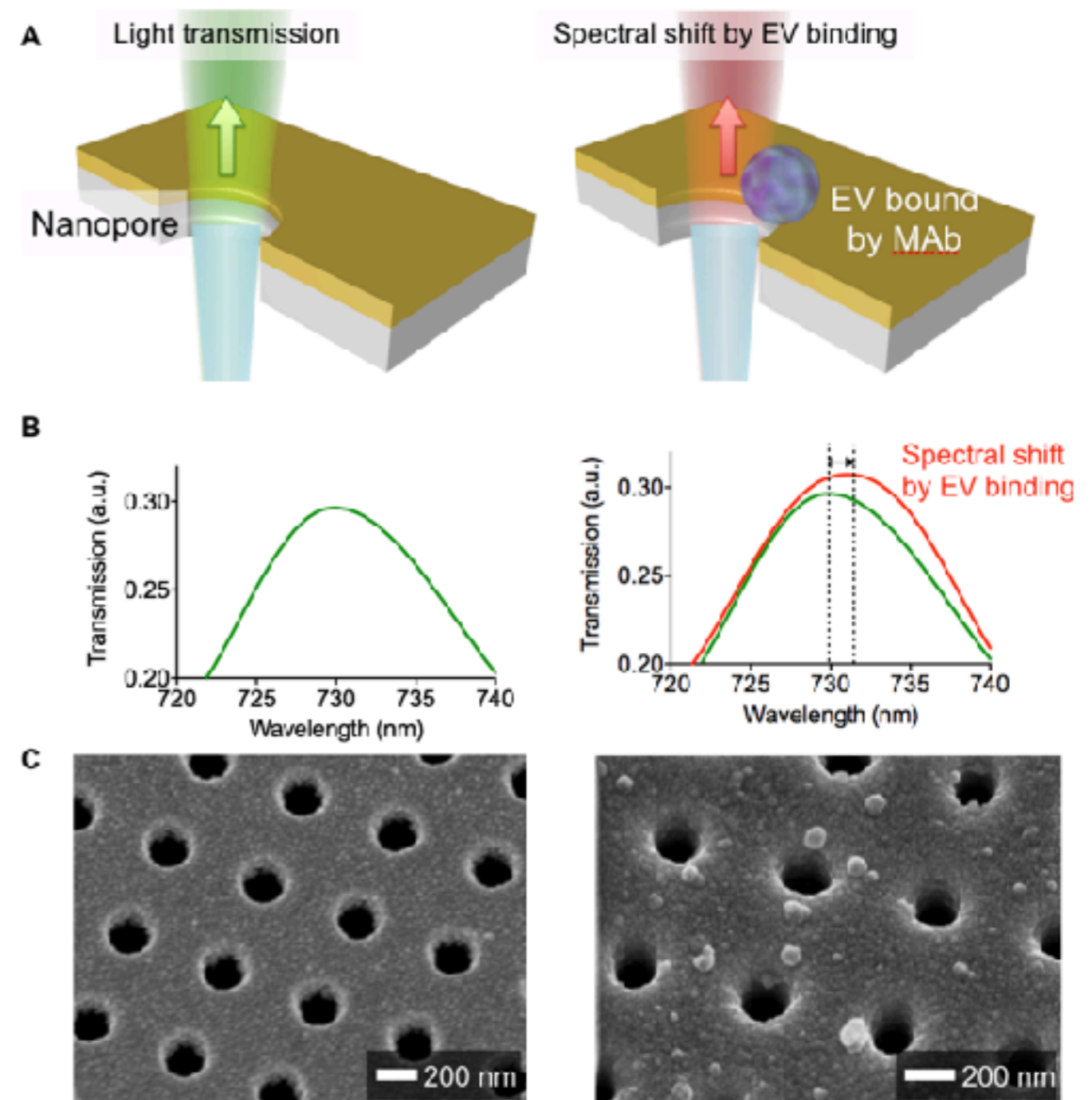Analyze all "EV" (< 800 nm) since they all have dx information

Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor

Hyungsoon Im[1,3], Huilin Shao[1,3], Yong Il Park[1], Vanessa M Peterson[1], Cesar M Castro[1], Ralph Weissleder[1,2] & Hakho Lee[1]
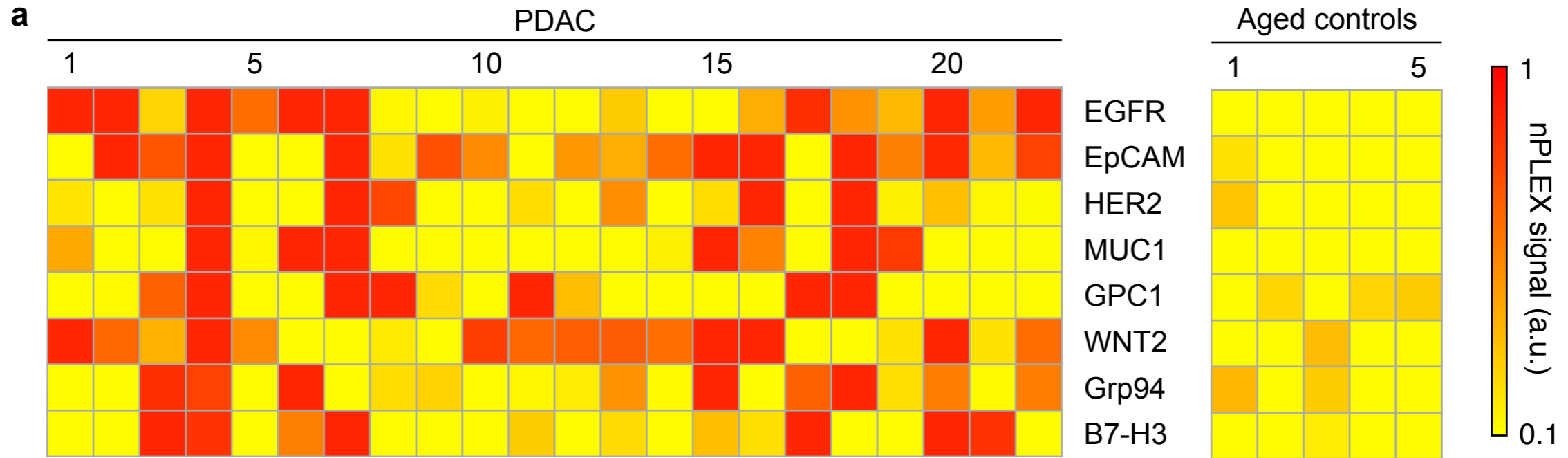
Pancreatic cancer patients

a

PDAC

Aged controls

EGFR
EpCAM
HER2
MUC1
GPC1
WNT2
Grp94
B7-H3

nPLEX signal (a.u.)

PDAC^{EV} signature: EGFR + EpCAM + MUC1 + GPC1 + WNT2

b

PDAC^{EV} signature (a.u.)

****

PDAC   Healthy controls

c

PDAC^{EV} signature (a.u.)

- PDAC (n = 22)
- Healthy controls (n = 5)

Serum samples (n = 27)

d

Sensitivity (%)

1-Specificity (%)

	AUC
Signature	1
EGFR	0.90
EpCAM	0.84
WNT2	0.78
GPC1	0.54
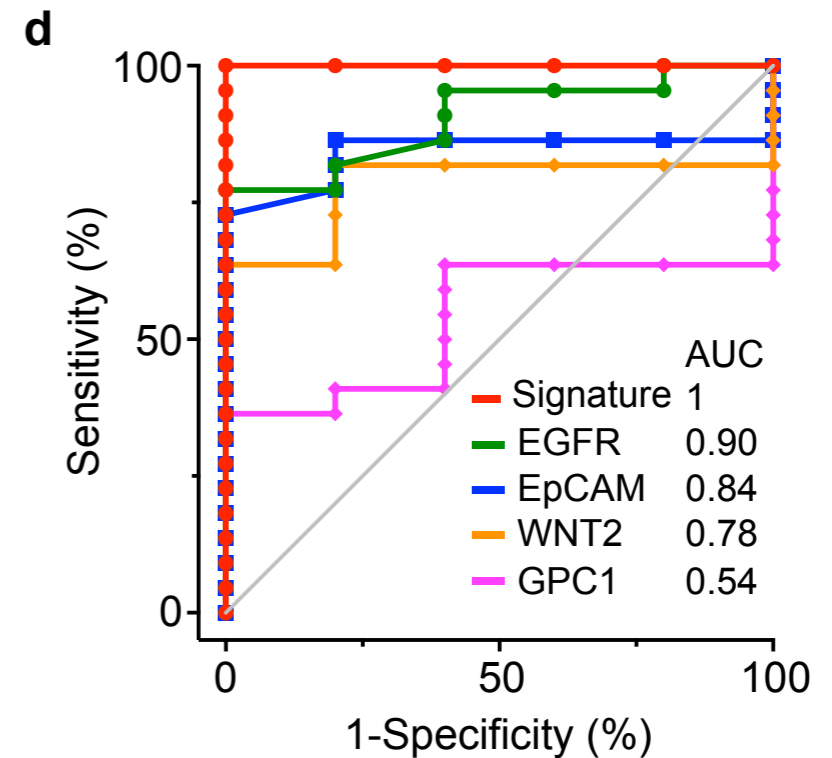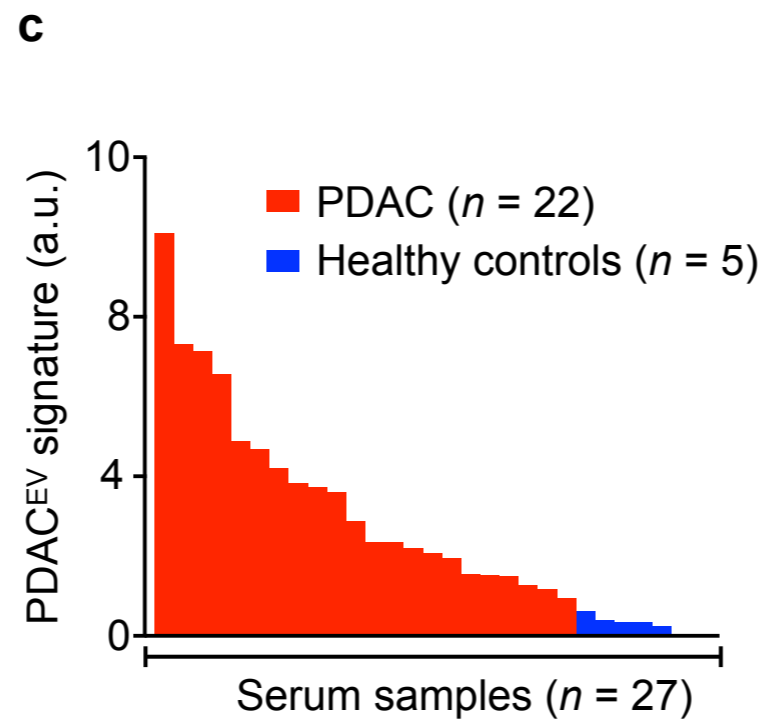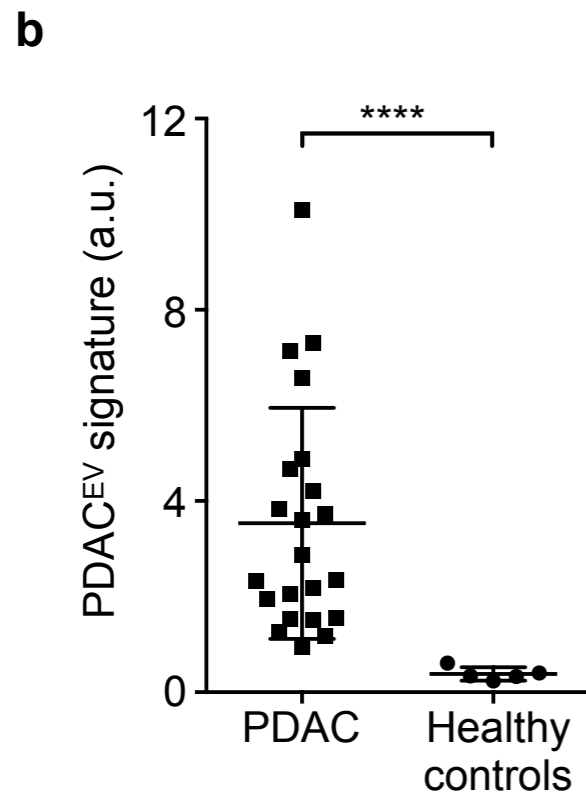
Pancreatic cancer detection
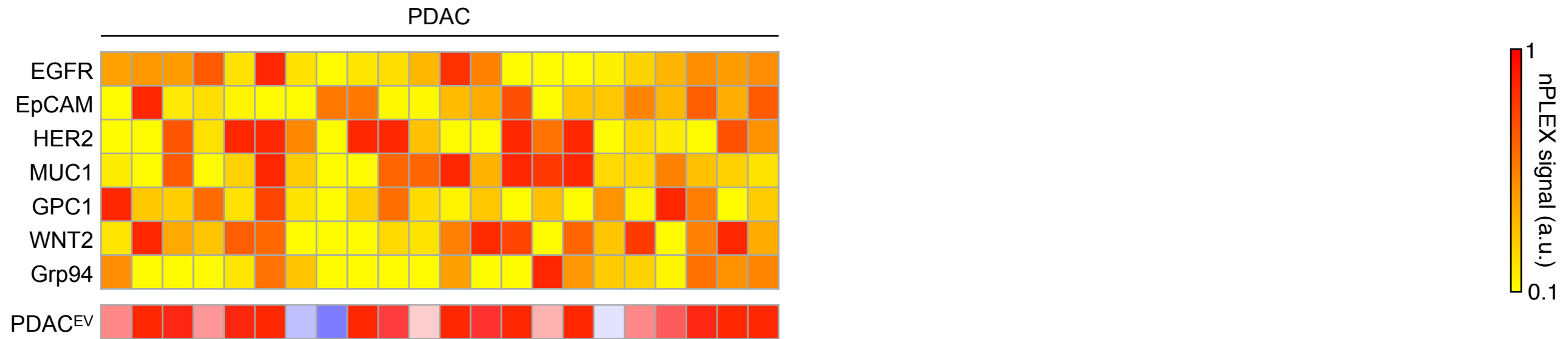
n = 146 patients; PDAC^{EV} signature Sens: 91%; Spec: 85%

Single EV analysis in early PDAC

Breast cancer (mRNA EV)
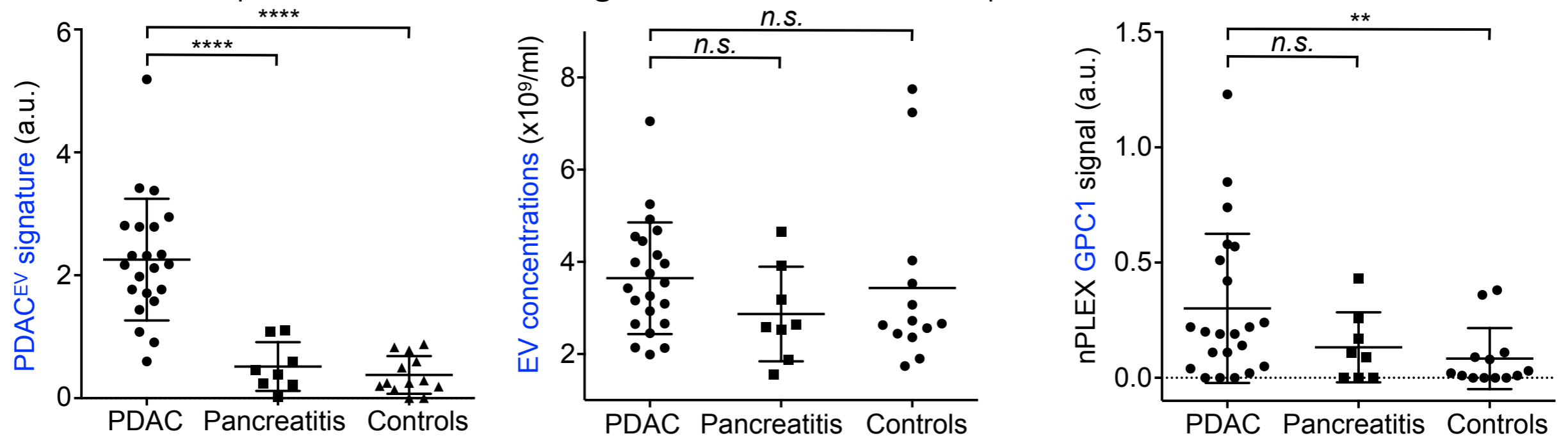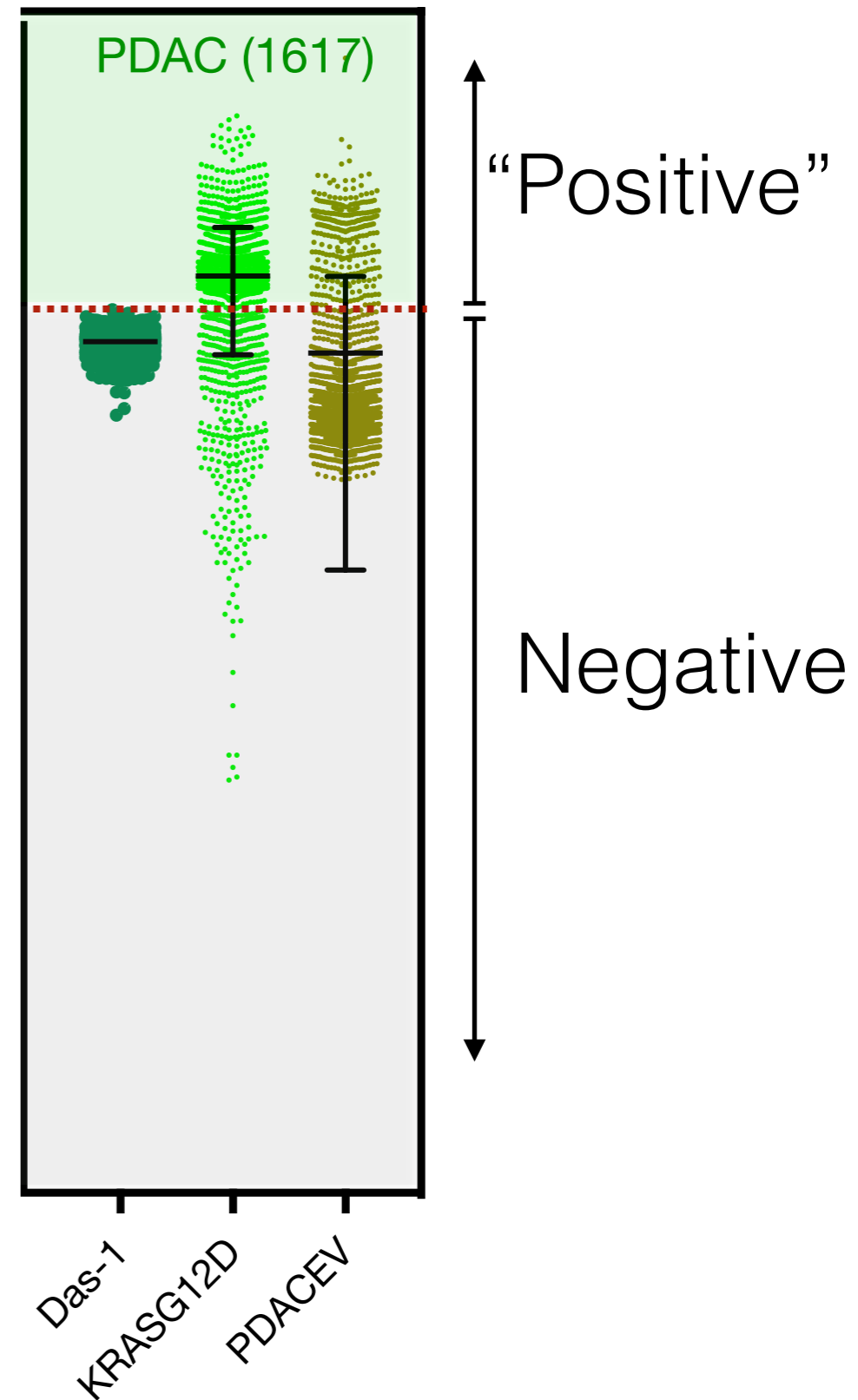

17,000 of 19,950 mRNA detected (85%)

15%

85%
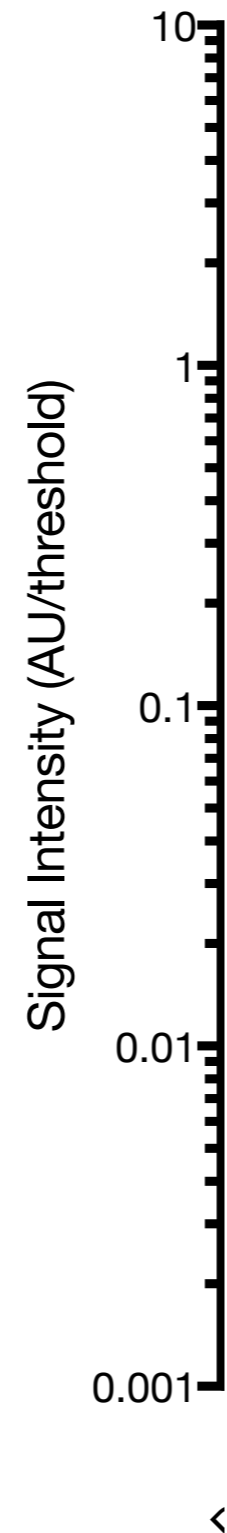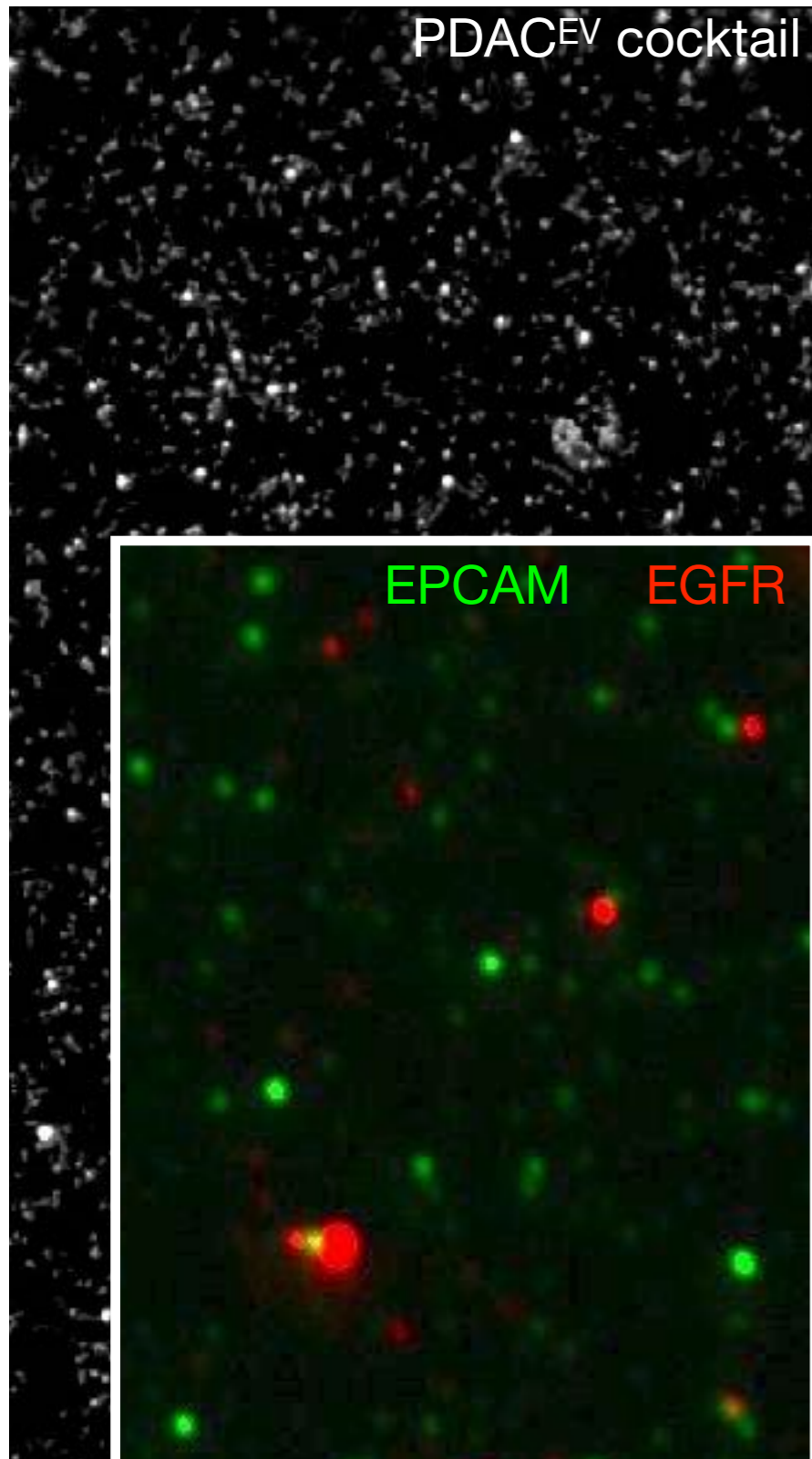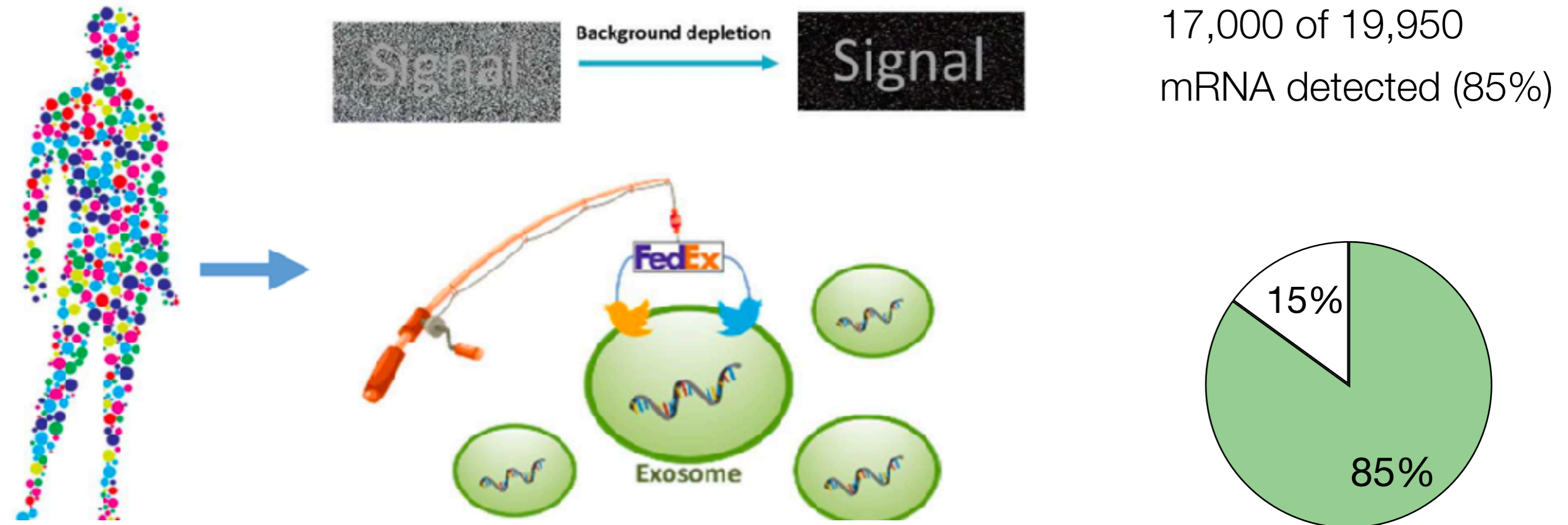
- **EDDE platform**: positive and negative selections
- 1 mL of plasma is used for EV mRNA analysis
- ~300-400 $ in sequencing costs
- Tested in early stage breast cancer patients
- Detects ~85% of mRNA in EV (c/w tumor tissue)

exosome**dx**

Breast cancer (stage1)

mRNA genes up-regulated in plasma EV of stage 1 BCA patients

BrCa
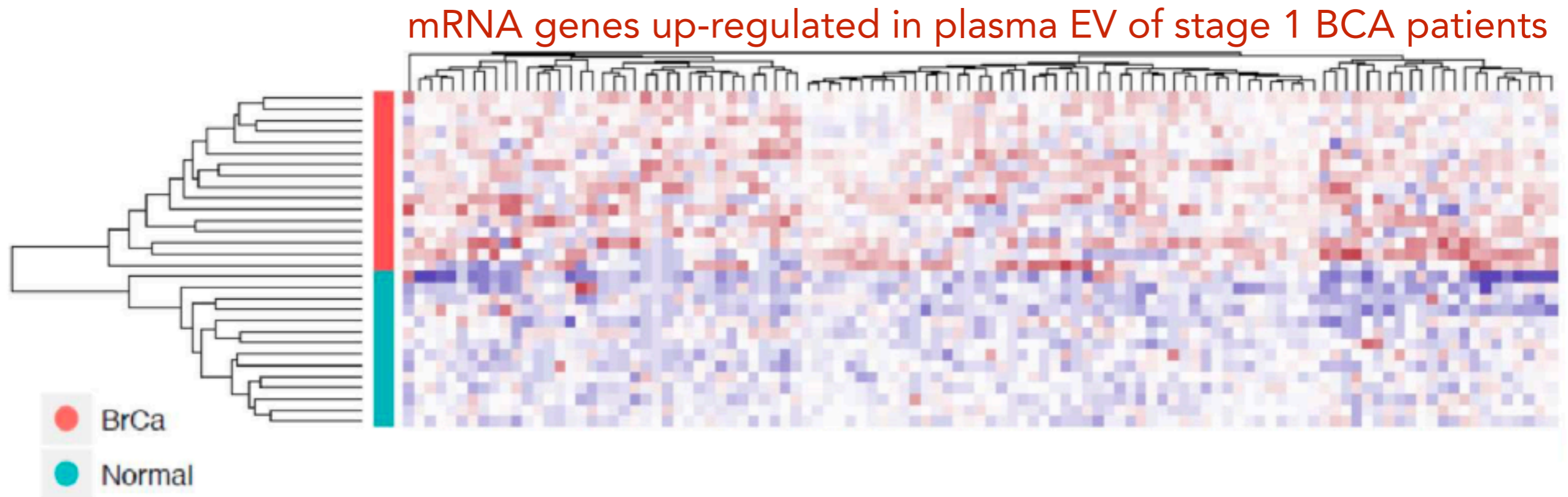
Normal

- 1 mL of plasma was used for EV mRNA analysis (positive and negative selection)
- Early stage BCA is distinct from normal plasma EV profile
- Small test trial of ~30 patients:

  Sensitivity 88%, Specificity 100%

  PPV 100%, NPV 88%

exosomedx

Summary: overarching themes

- EV analysis is diagnostically promising: *abundance, stability*

- *Challenges*: TEV/HEV differentiation; heterogeneity of individual vesicles; biomarker validation; protein vs. mRNA analysis

- Clinical priorities: need for validated, easy-to-use systems with multiplexing capabilities (commercial systems); *high-sensitivity systems* will require more research (miniaturization, nanotechnology)

- *Single EV studies* in primary patient samples are needed

- *Evidence for utility in early cancer: well controlled prospective studies needed; more biomarker research needed*

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