Genomic Characterization of Cancer-Adjacent Breast: Evidence of field effects and expression subtypes

Melissa Troester, PhD, MPH

What Predicts Breast Cancer Recurrence?

Veronesi et al. (2002) NEJM, 347(16): 1227.

- Recurrence rates are higher for breast conserving therapy.
- Local recurrence commonly occurs in the lumpectomy bed.
- Local recurrence rates are higher among basal-like breast cancers.

Field carcinogenic events

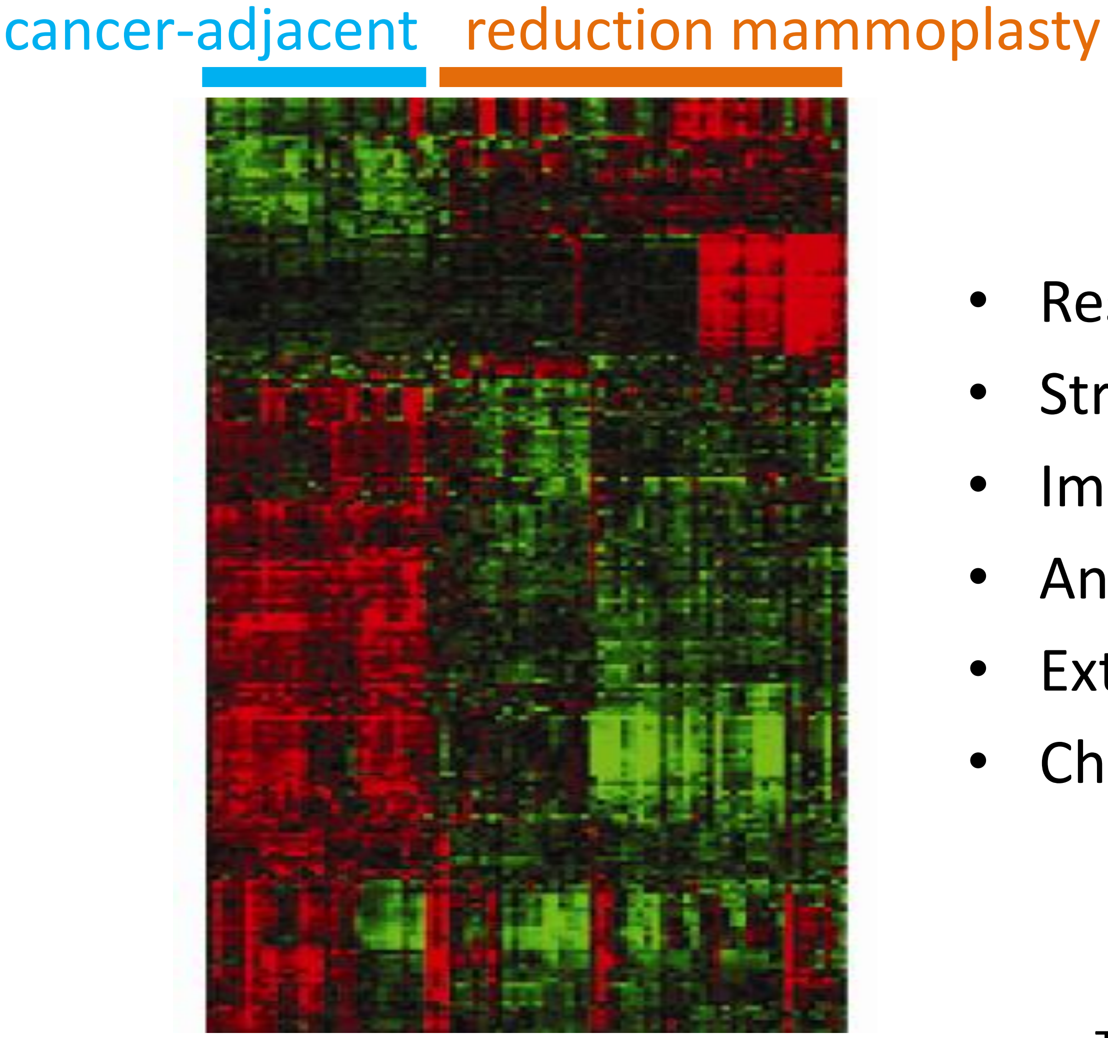
patch

field

cancer

- Slaughter *et al*. (1953) observed abnormal tissue surrounding oral squamous cell carcinoma
  - Field cancerization explains the development of multiple primaries and local recurrences.

How does cancer-adjacent tissue respond to tumor?

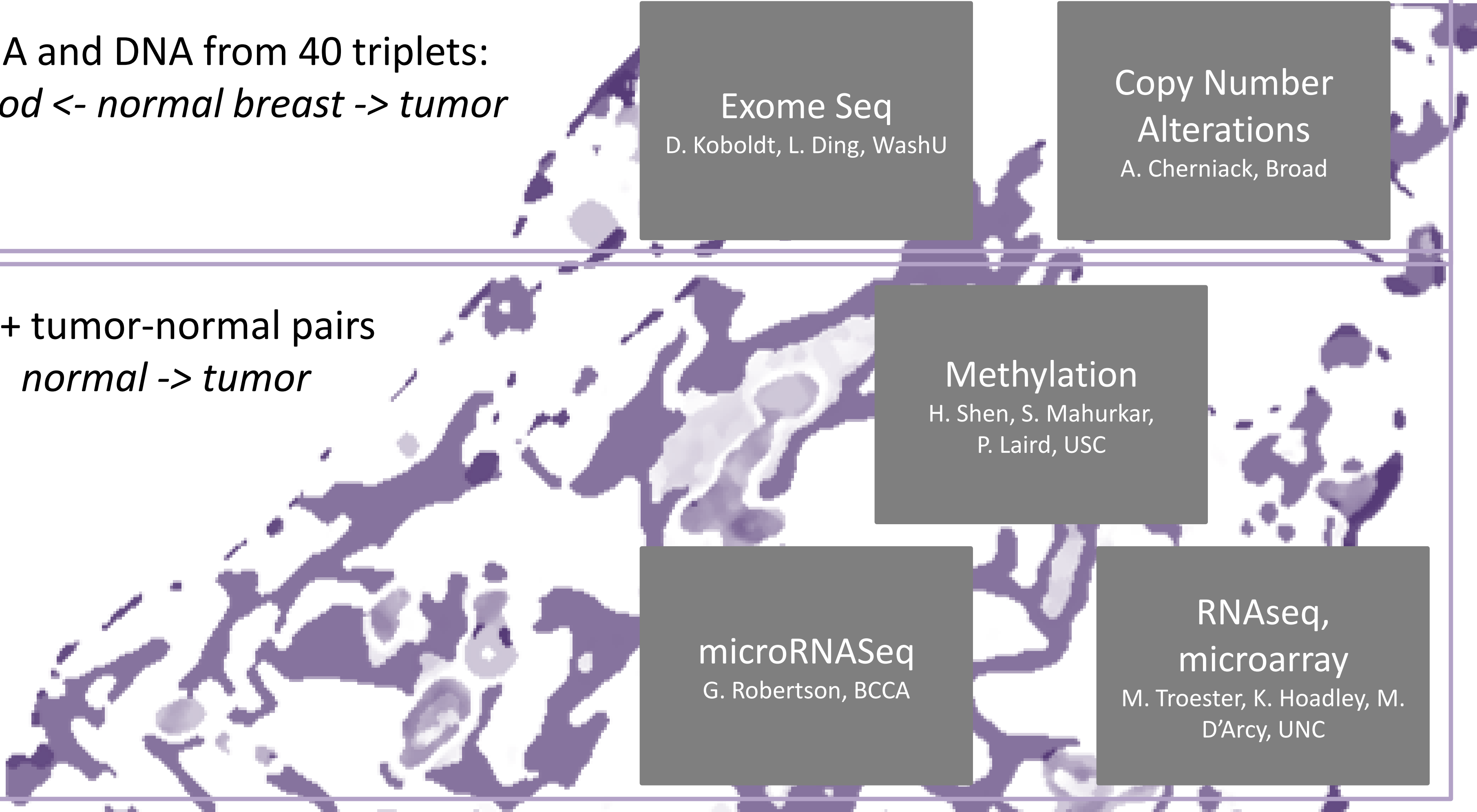


- Response to wounding
- Stress response
- Immune response
- Angiogenesis
- Extracellular matrix
- Chemotaxis

Troester et al. (2009) *Clin Cancer Res*

Double Normal Breast Committee

Chair: Melissa Troester, UNC



RNA and DNA from 40 triplets:
blood <- normal breast -> tumor

Exome Seq
D. Koboldt, L. Ding, WashU

Copy Number Alterations
A. Cherniack, Broad

40+ tumor-normal pairs
normal -> tumor

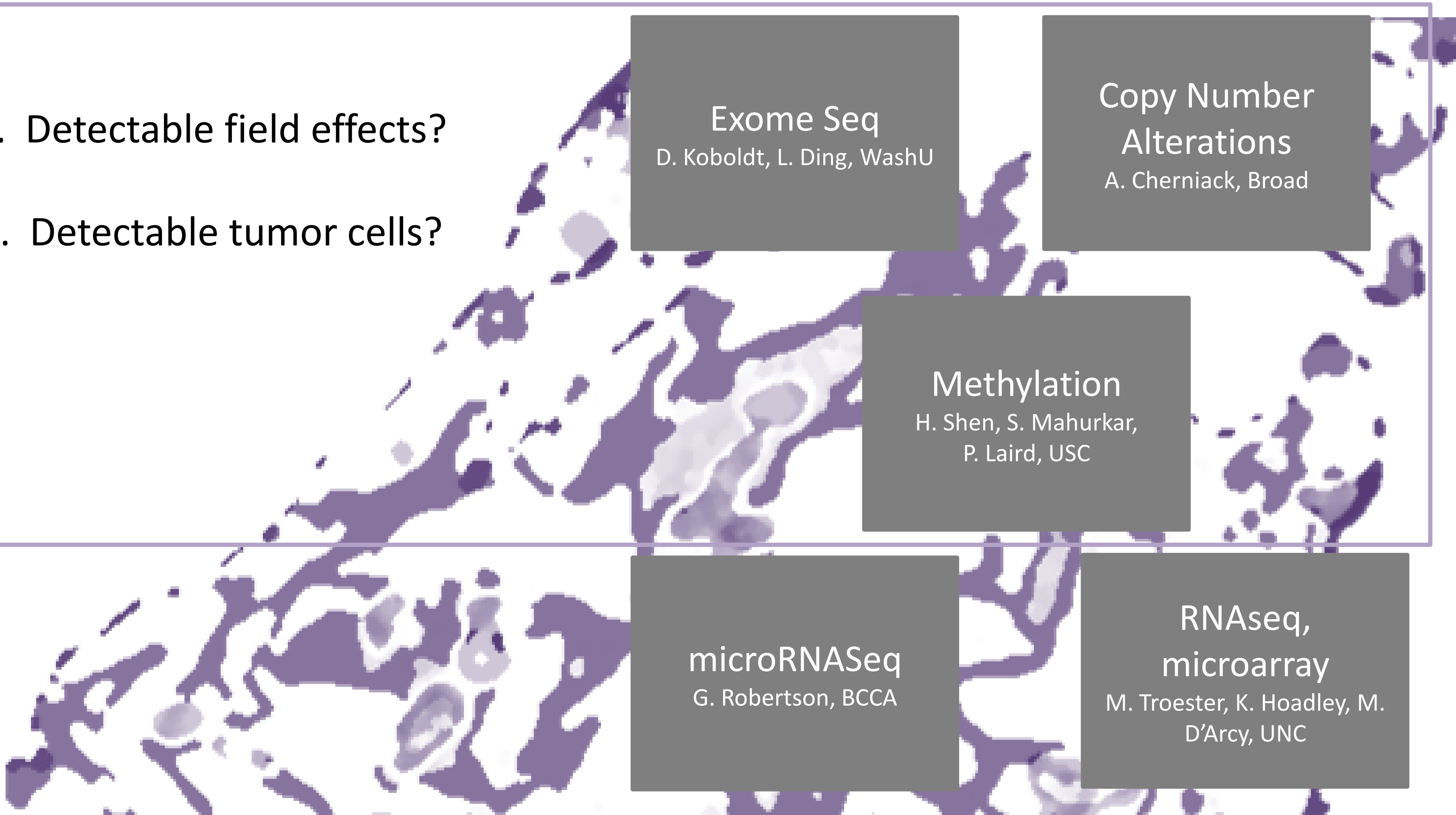
Methylation
H. Shen, S. Mahurkar, P. Laird, USC

microRNASeq
G. Robertson, BCCA

RNAseq, microarray
M. Troester, K. Hoadley, M. D’Arcy, UNC

Double Normal Breast Committee

Chair: Melissa Troester, UNC



Q1. Detectable field effects?

Q2. Detectable tumor cells?

Exome Seq
D. Koboldt, L. Ding, WashU

Copy Number Alterations
A. Cherniack, Broad

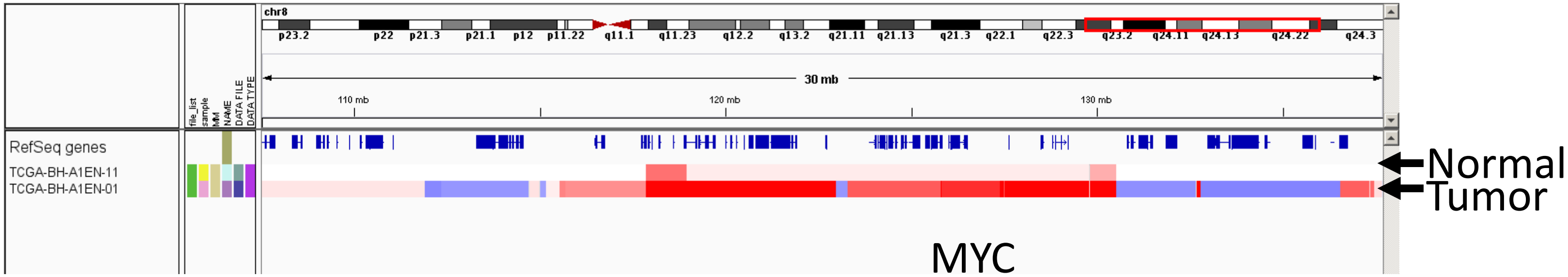
Methylation
H. Shen, S. Mahurkar, P. Laird, USC

microRNASeq
G. Robertson, BCCA

RNAseq, microarray
M. Troester, K. Hoadley, M. D'Arcy, UNC

Tumor-like copy number alterations

Chromosome 8

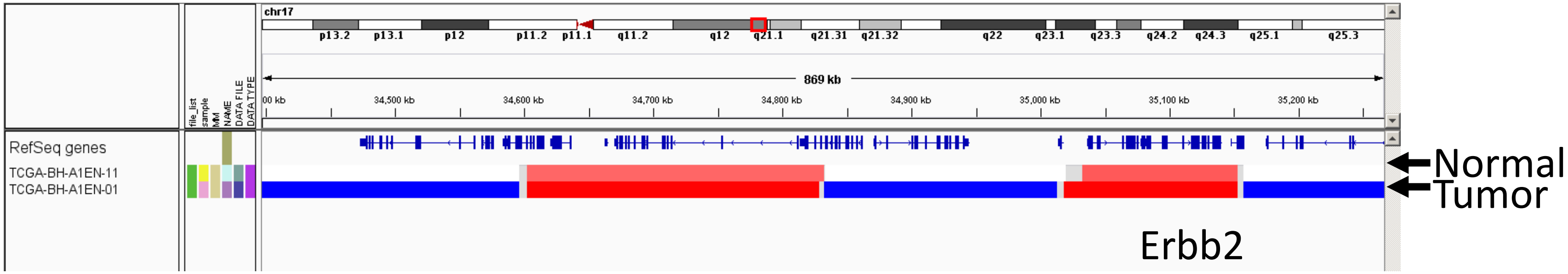


Normal

Tumor

MYC

Chromosome 17



Normal

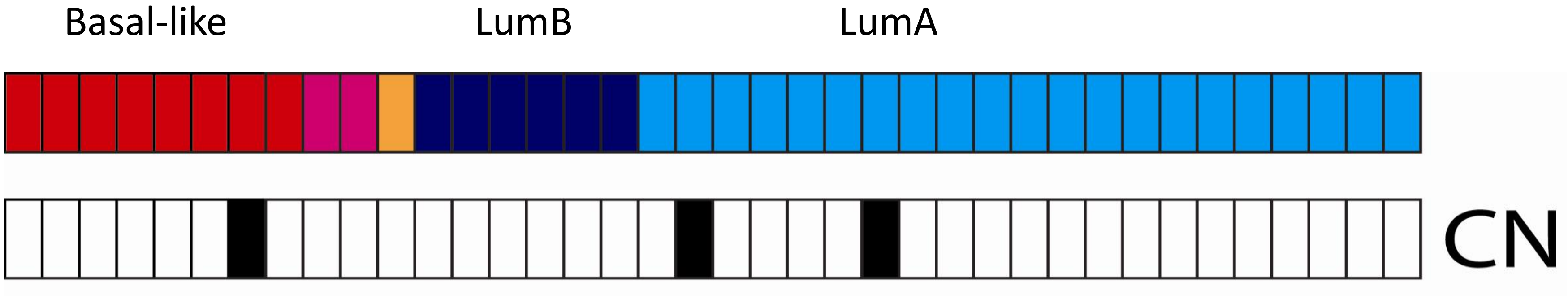
Tumor

Erbb2

Focal peak in chromosome 10 is also seen in normal.

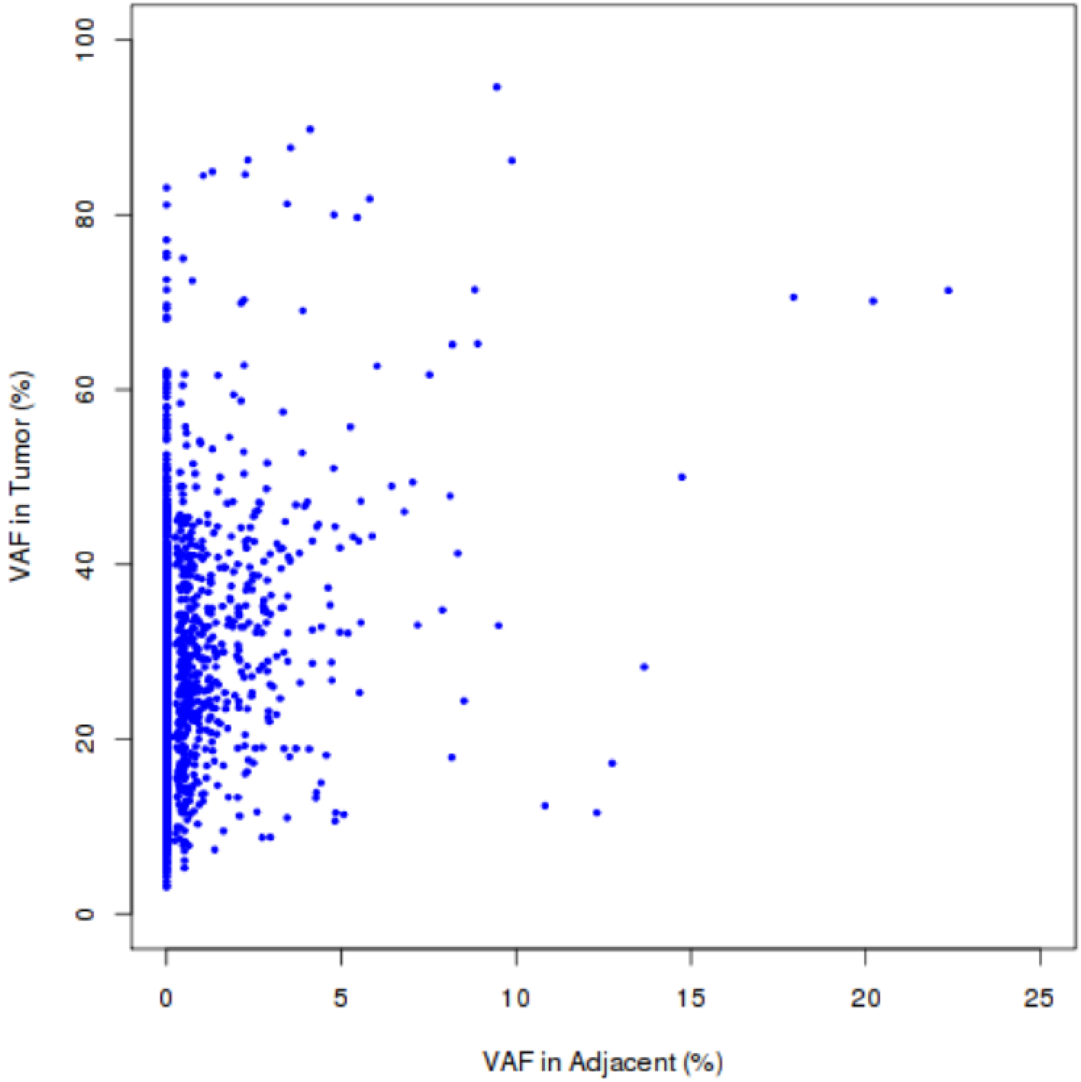
Courtesy of Andy Cherniack

Tumor-like copy number alterations



7% with ‘field effect’ OR tumor contamination

Does VAF in Adjacent Correlate with VAF in Tumor?

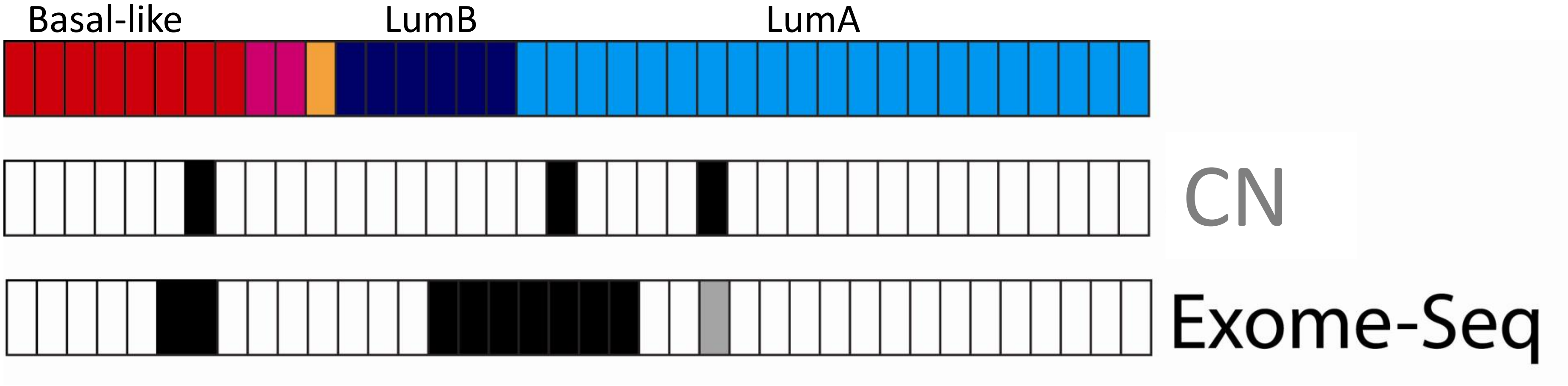


- 1,760 mutations from 41 cases
- R^2=0.288

10 cases (25%) had strong evidence of field effect (many mutations with VAF >=2% in the adjacent normal).

Courtesy of Dan Koboldt/Li Ding

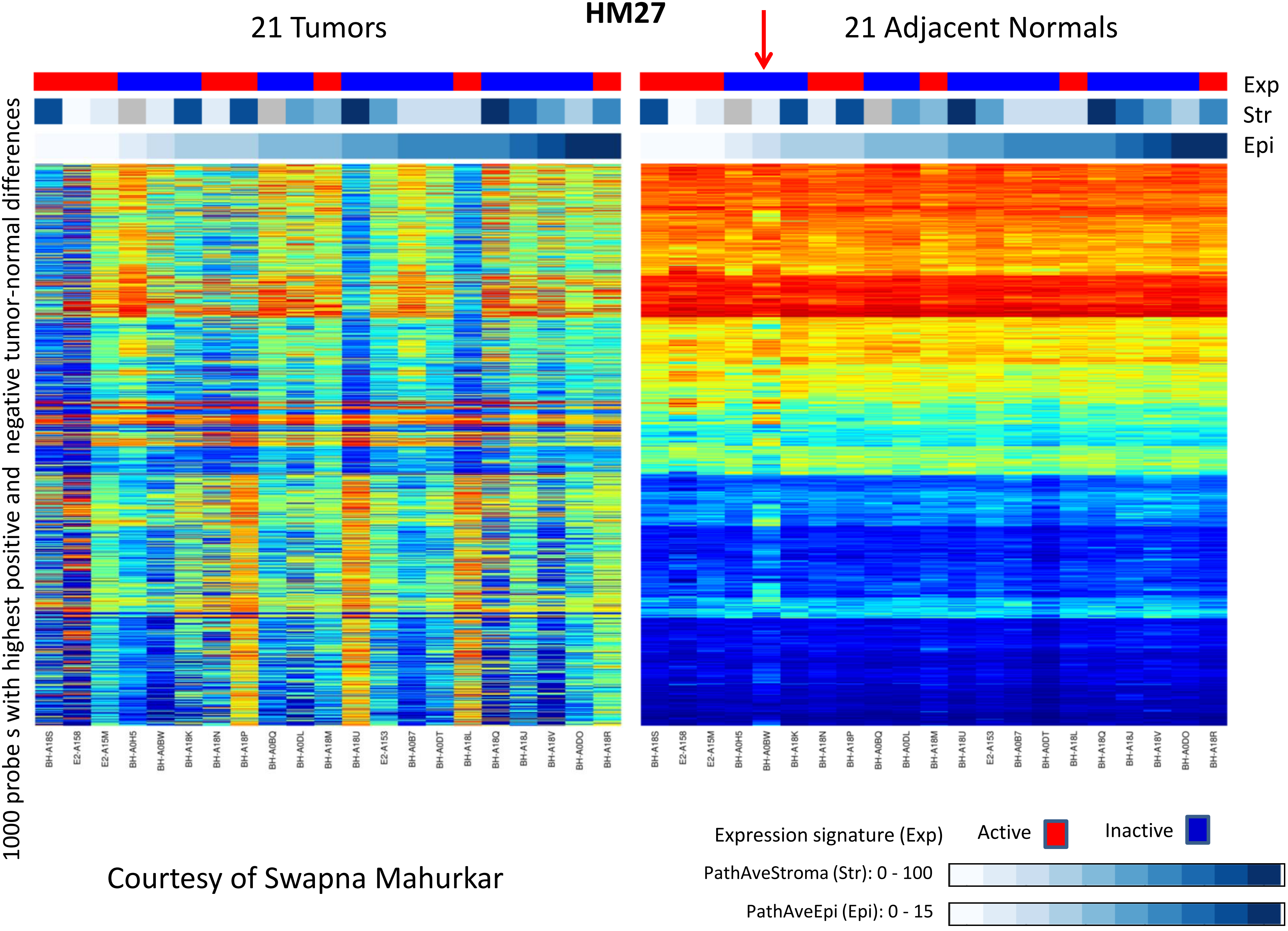
Tumor-like mutations



7% with ‘field effect’ OR tumor contamination

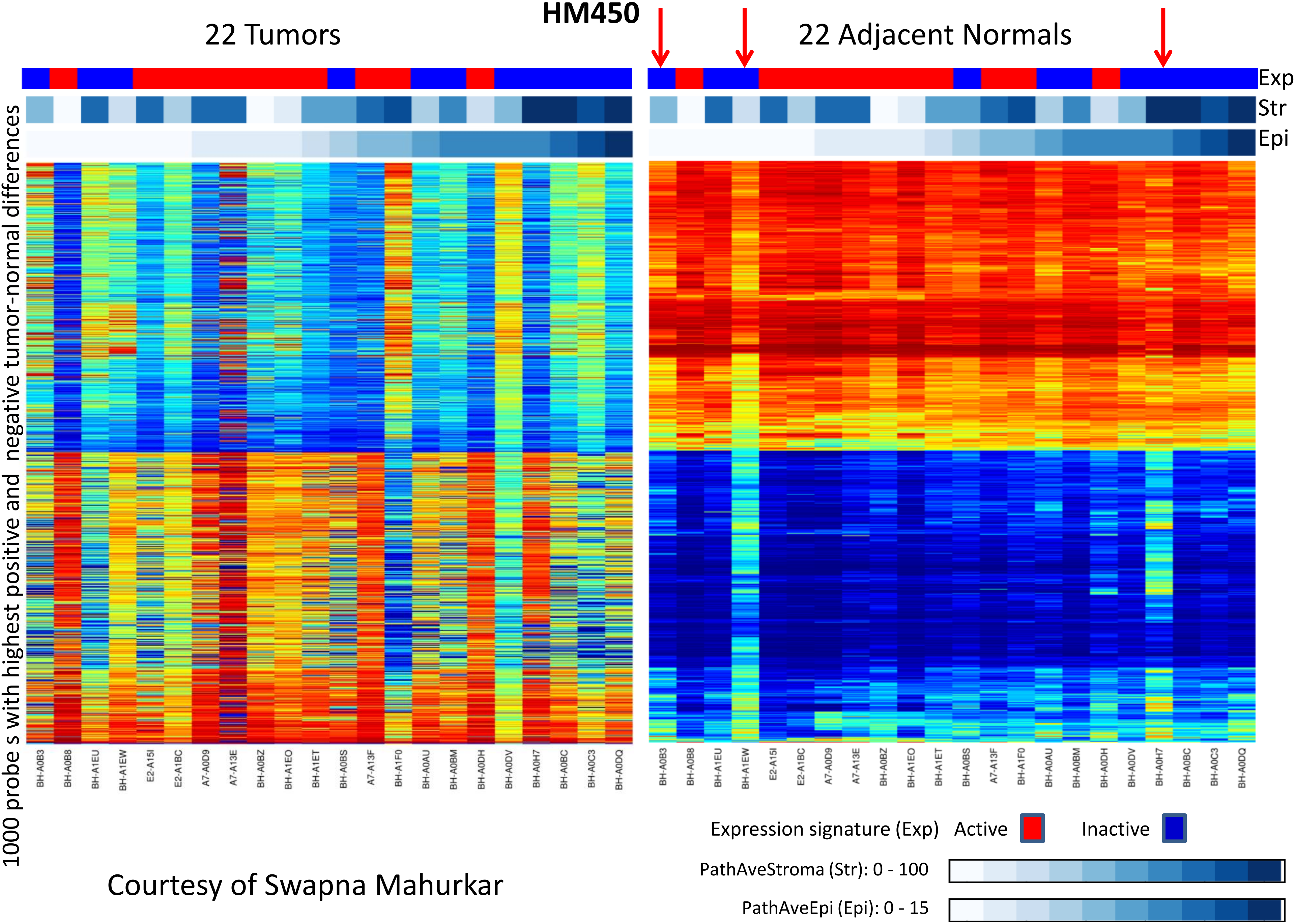
25% with ‘field effect’ OR tumor contamination

HM27



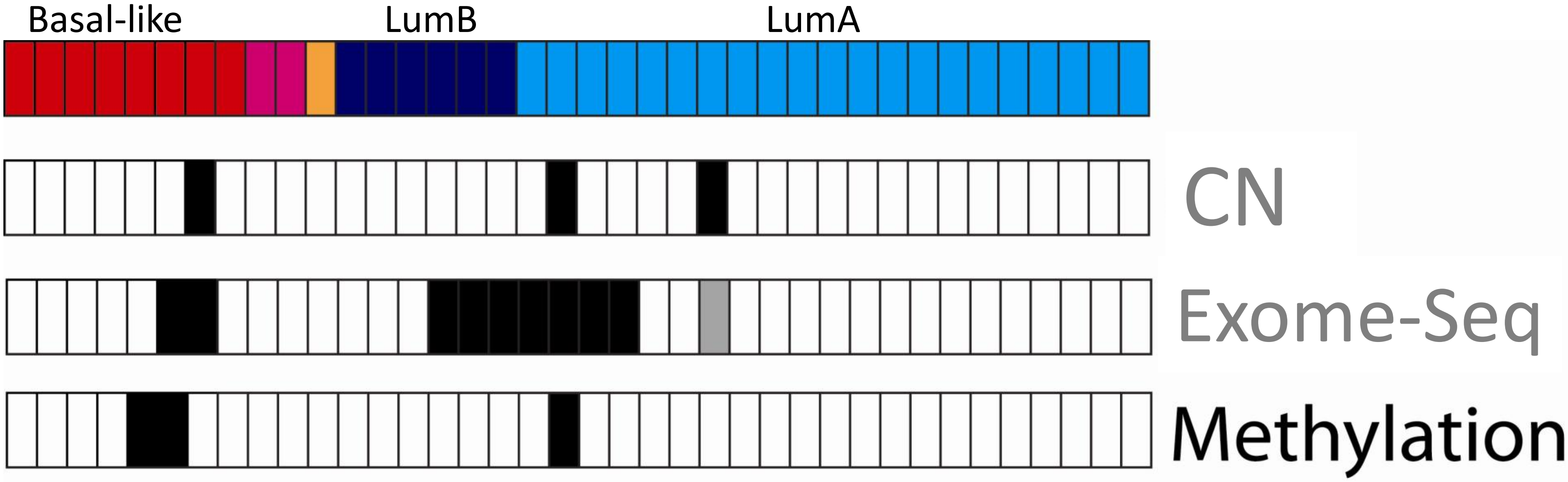
Courtesy of Swapna Mahurkar

HM450



Courtesy of Swapna Mahurkar

Tumor-like methylation patterns



7% with ‘field effect’ OR tumor contamination

25% with ‘field effect’ OR tumor contamination

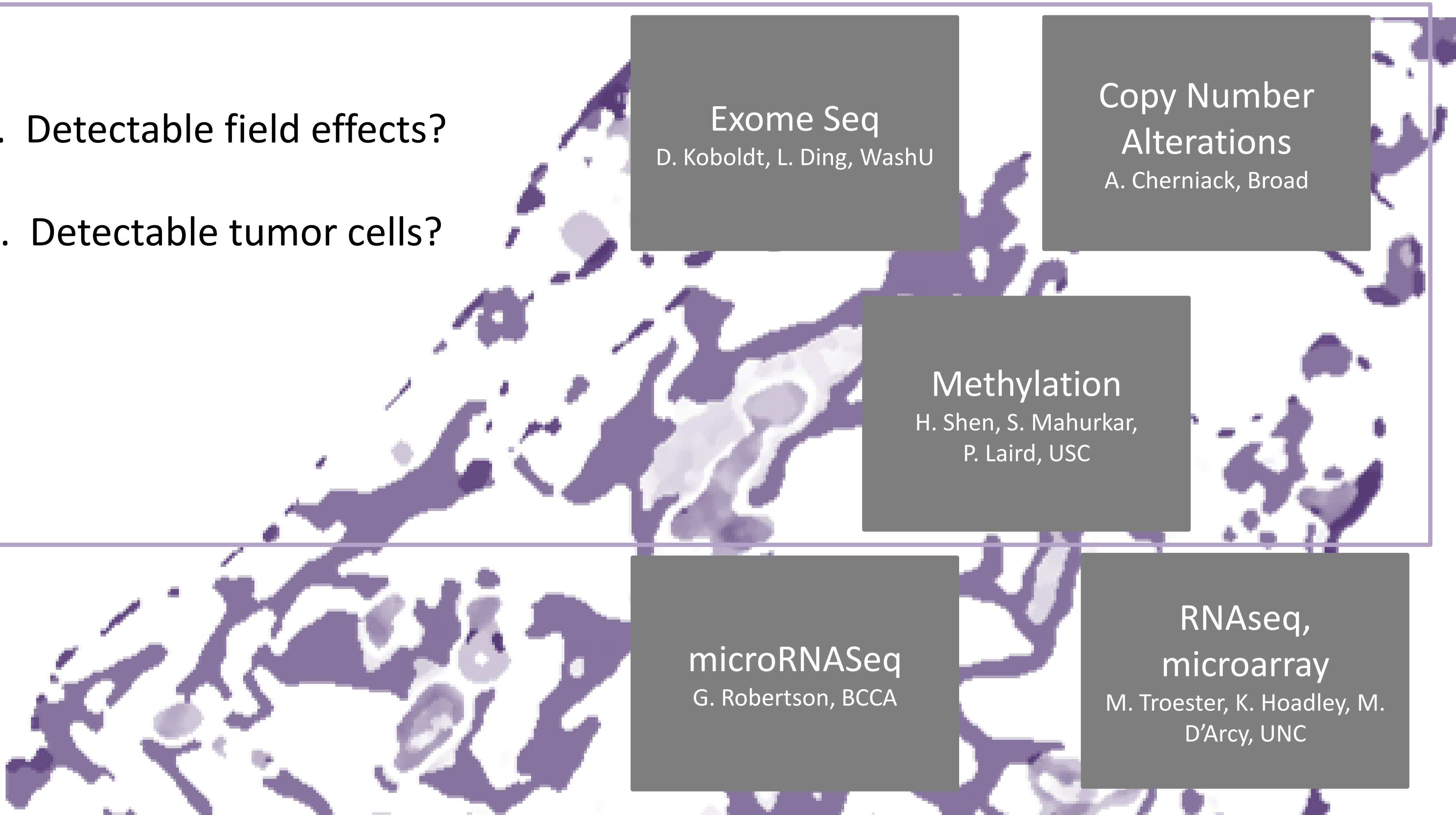
7-10% with ‘field effect’ OR tumor contamination

Double Normal Breast Committee

Chair: Melissa Troester, UNC

Q1. Detectable field effects?

Q2. Detectable tumor cells?



Exome Seq
D. Koboldt, L. Ding, WashU

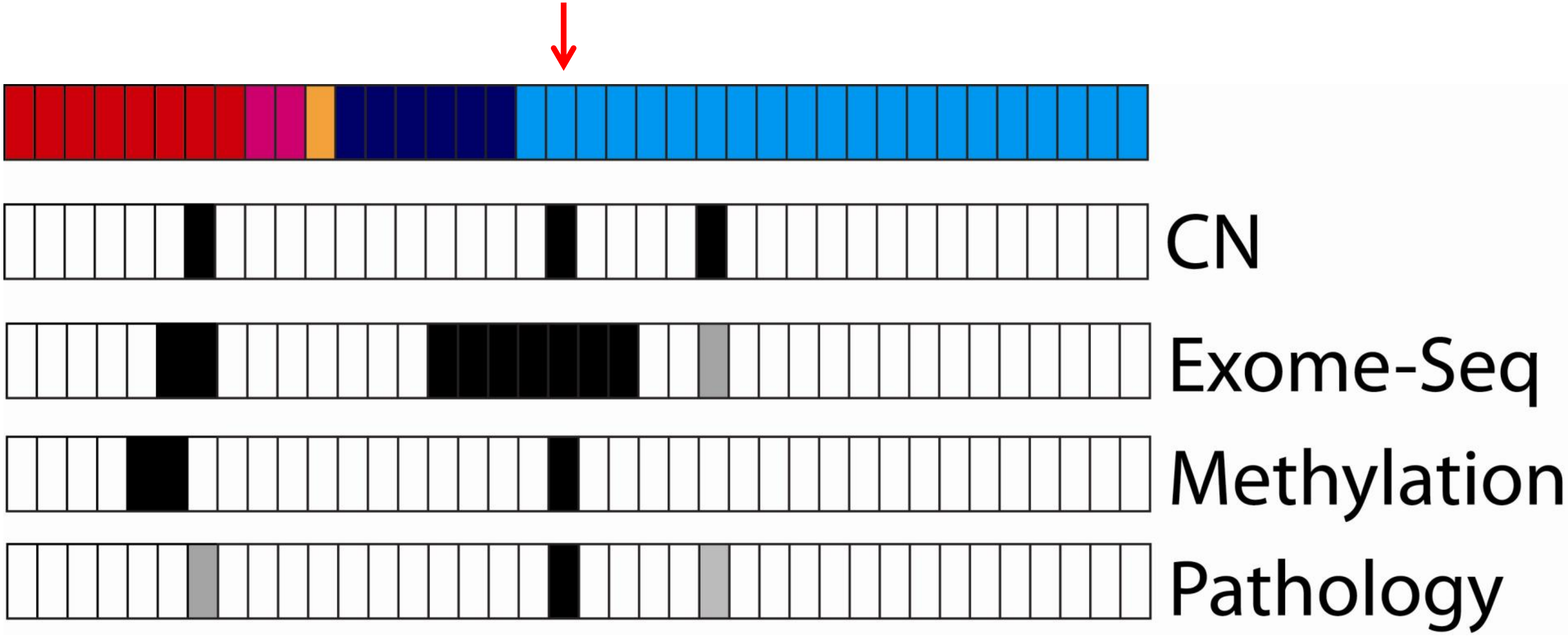
Copy Number Alterations
A. Cherniack, Broad

Methylation
H. Shen, S. Mahurkar, P. Laird, USC

microRNASeq
G. Robertson, BCCA

RNAseq, microarray
M. Troester, K. Hoadley, M. D’Arcy, UNC

DNA data types: Comparison & Validation



A ‘positive control’ – all three DNA platforms detected the sample with tumor contamination

Histopathologic Assessment

Melissa Troester, UNC
Rupninder Sandhu, UNC
Andy Beck, Harvard
Nicole Johnson, Harvard
Kim Allison, U of Wash

<u>SCORING:</u>
Pathology (tumor, benign)
Immune infiltrations
Percent Composition:
e.g. 30% Stroma
63% Adipose
7% Epithelium

Methylation Reflecting Composition

- Epithelial Content on HM450 platform (qvalue<0.05).
  - 13000 probes were positively correlated
  - 12500 probes were negatively correlated

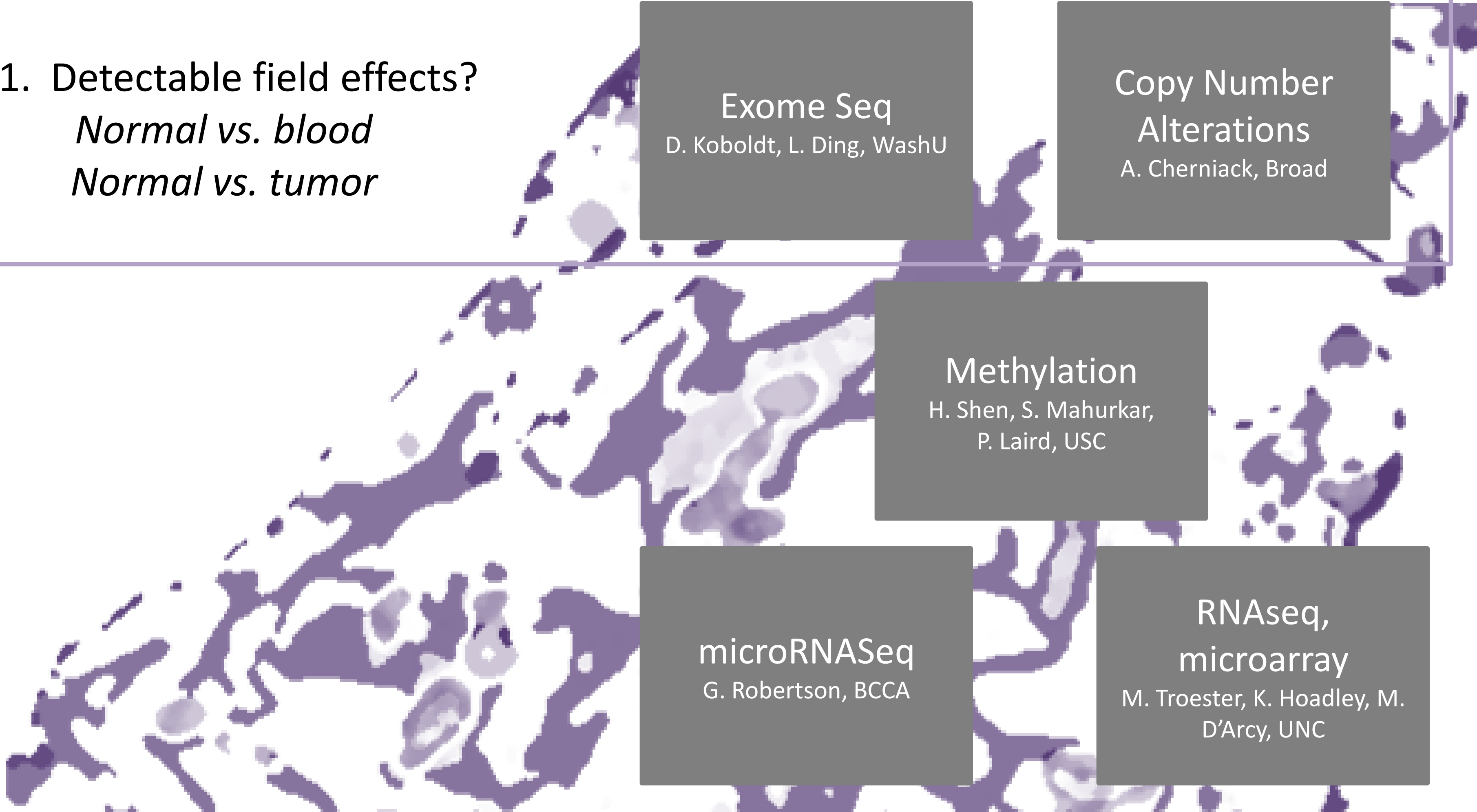
- Stromal Content on HM450 platform (qvalue<0.05):
  - 5700 probes were positively correlated
  - 2300 probes were negatively correlated

- Correlation composition and DNA methylation on 27k was weak. This needs further investigation.

Double Normal Breast Committee

Chair: Melissa Troester, UNC

Q1. Detectable field effects?
Normal vs. blood
Normal vs. tumor



Exome Seq
D. Koboldt, L. Ding, WashU

Copy Number Alterations
A. Cherniack, Broad

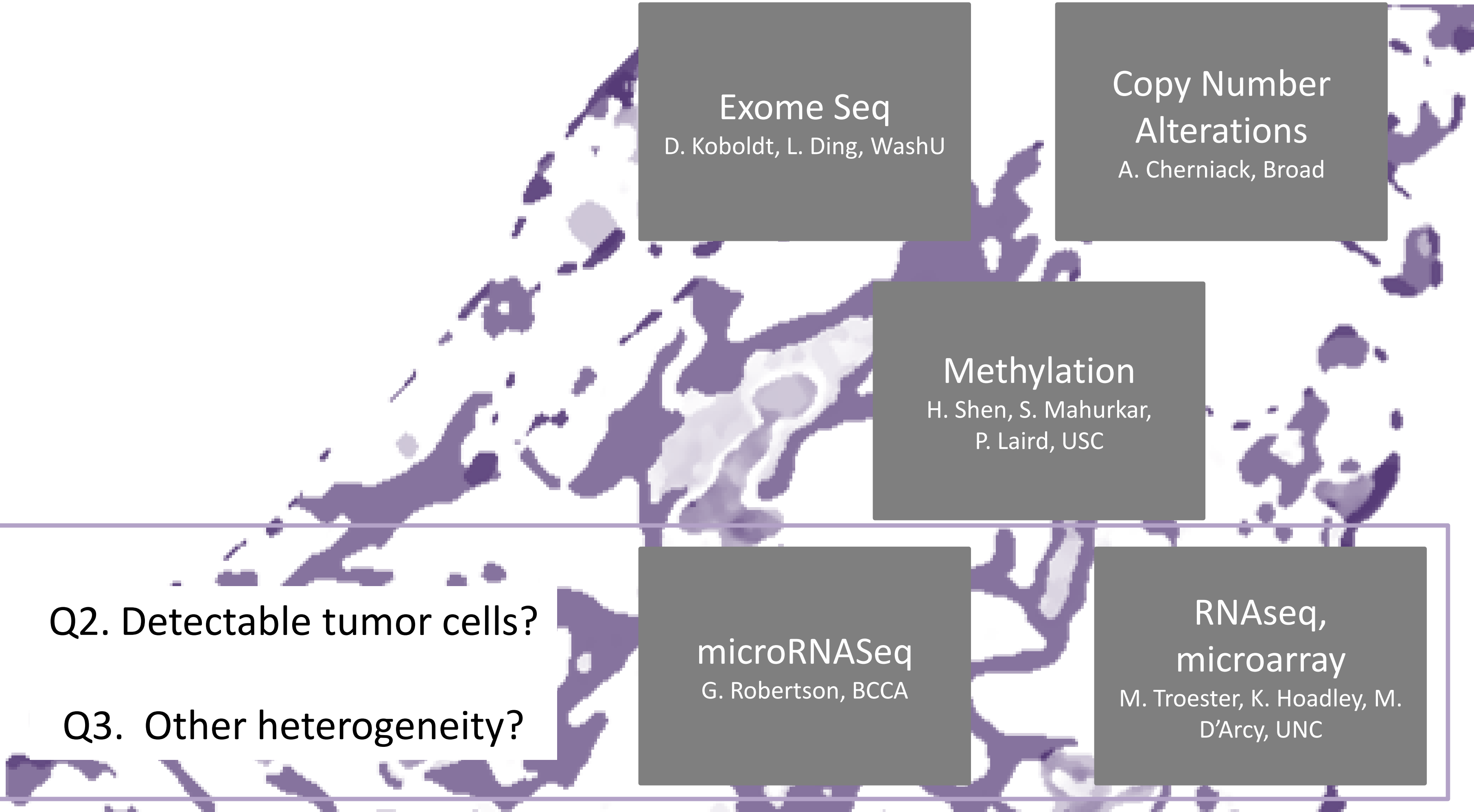
Methylation
H. Shen, S. Mahurkar, P. Laird, USC

microRNASeq
G. Robertson, BCCA

RNAseq, microarray
M. Troester, K. Hoadley, M. D’Arcy, UNC

Double Normal Breast Committee

Chair: Melissa Troester, UNC



Exome Seq
D. Koboldt, L. Ding, WashU

Copy Number Alterations
A. Cherniack, Broad

Methylation
H. Shen, S. Mahurkar, P. Laird, USC

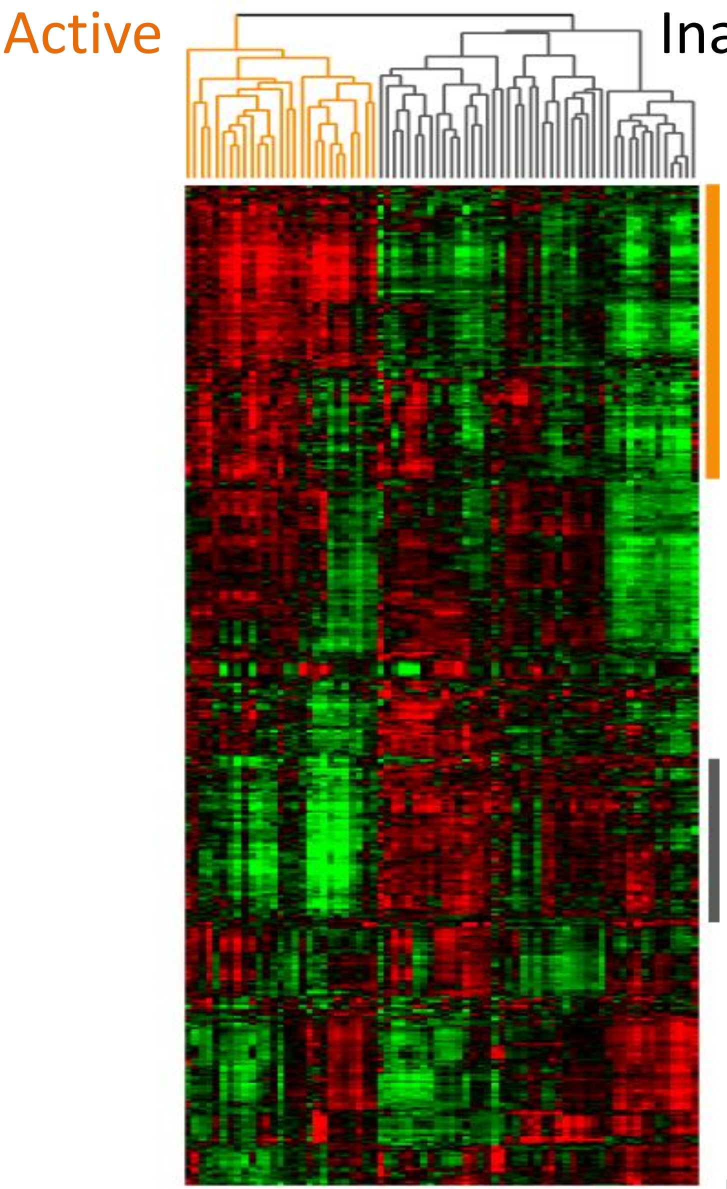
Q2. Detectable tumor cells?

Q3. Other heterogeneity?

microRNASeq
G. Robertson, BCCA

RNAseq, microarray
M. Troester, K. Hoadley, M. D’Arcy, UNC

Two Subtypes of Cancer-Adjacent Tissue



increased

cell movement

inflammation

fibrosis

chemotaxis

decreased

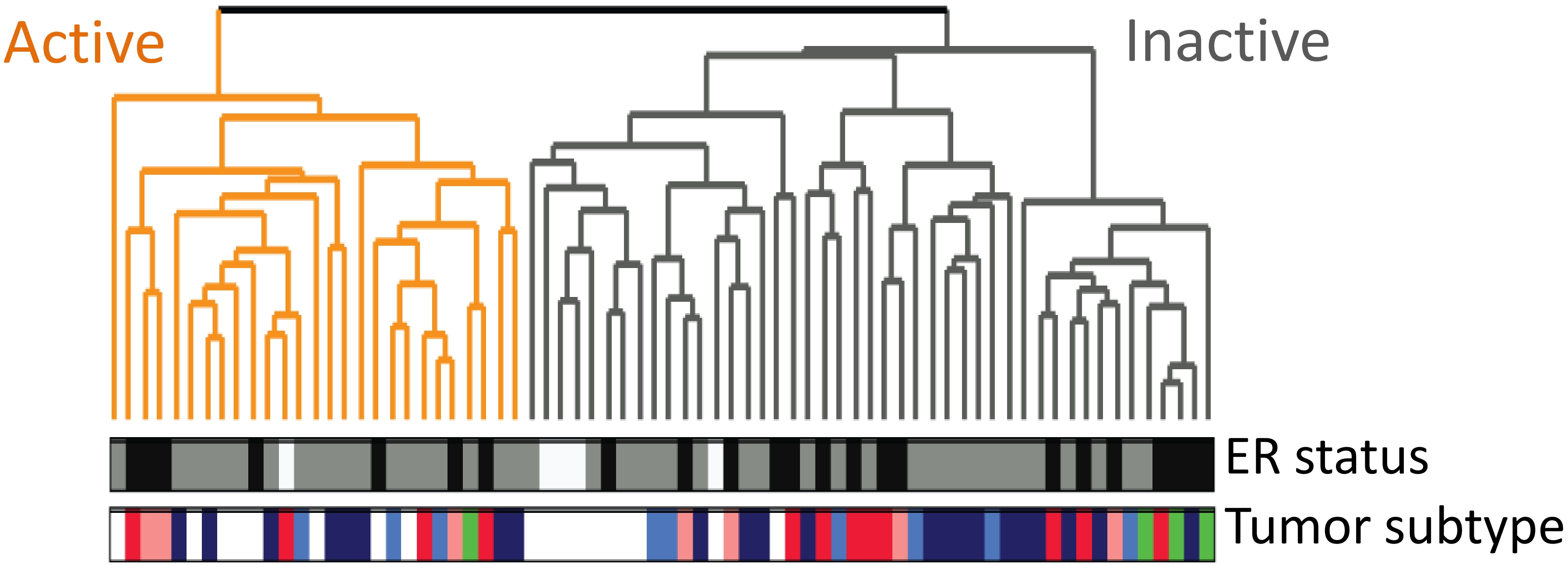
cell adhesion

differentiation

cell-cell contact

Roman-Perez et al. (2012) *Breast Cancer Res*

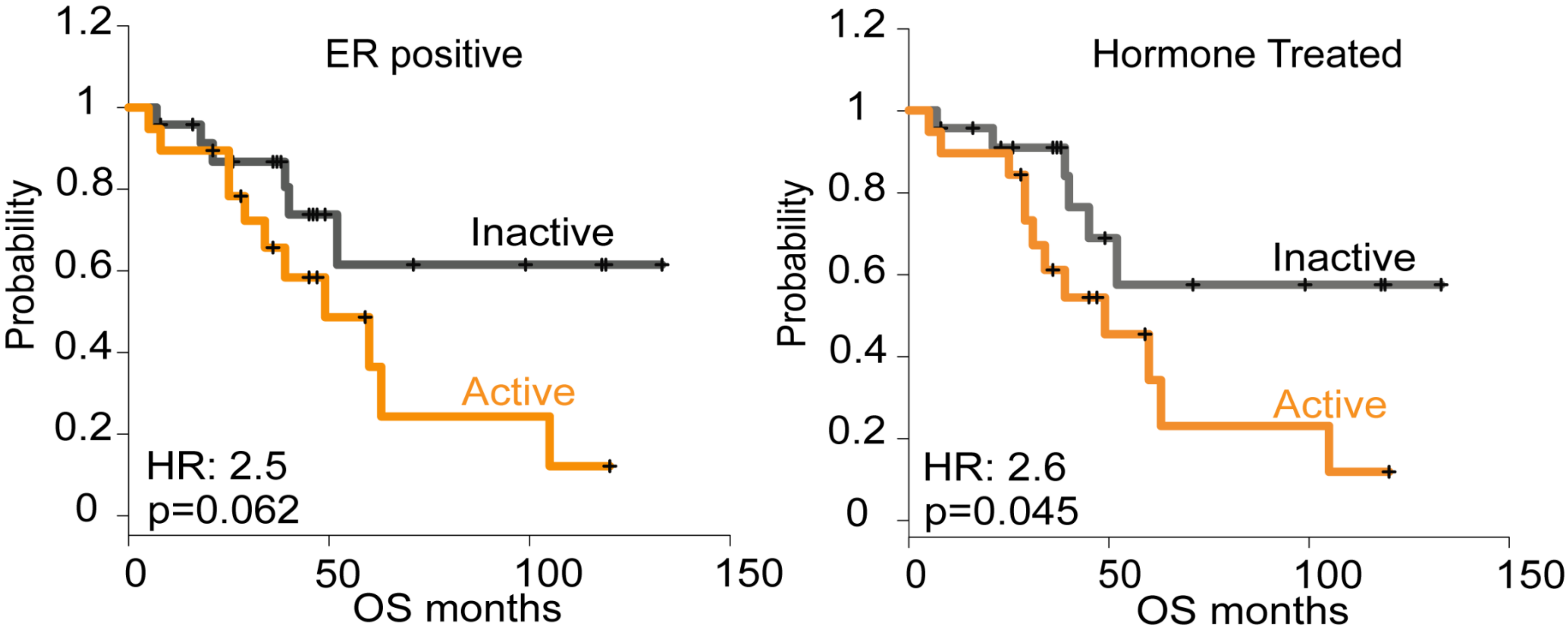
Cancer-Adjacent Subtype vs. Tumor Subtype



Active microenvironment occurs in all tumor subtypes

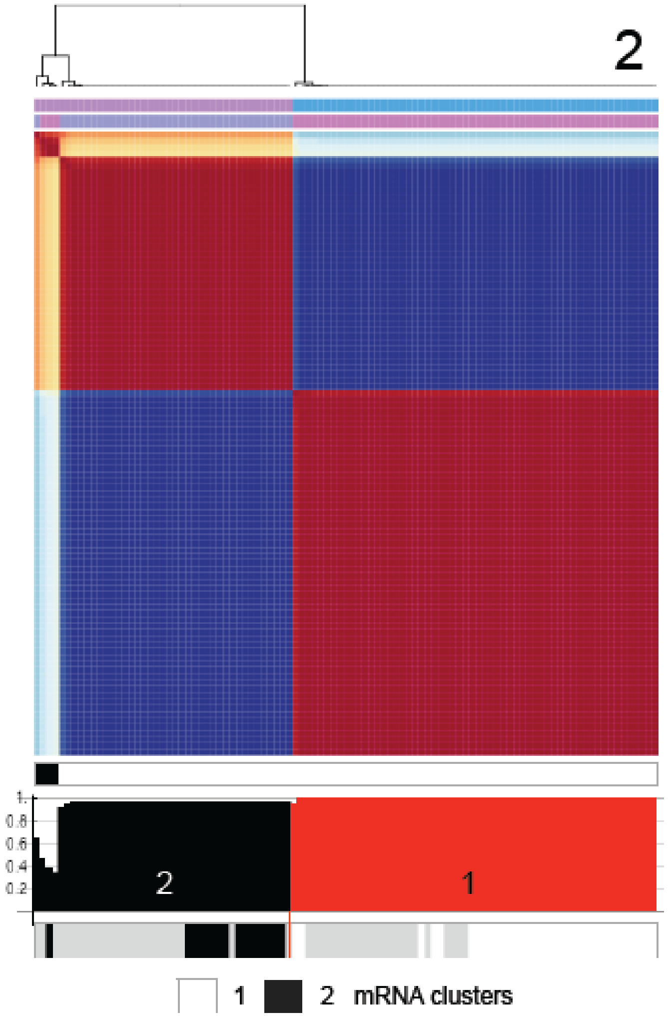
Roman-Perez et al. (2012) *Breast Cancer Res*

Active Microenvironment Predicts Survival



Roman-Perez et al. (2012) Breast Cancer Res

mRNA and microRNA subtypes



- RNA expression clusters
  - Two main clusters by microRNA-seq
  - Two main clusters by RNA-seq

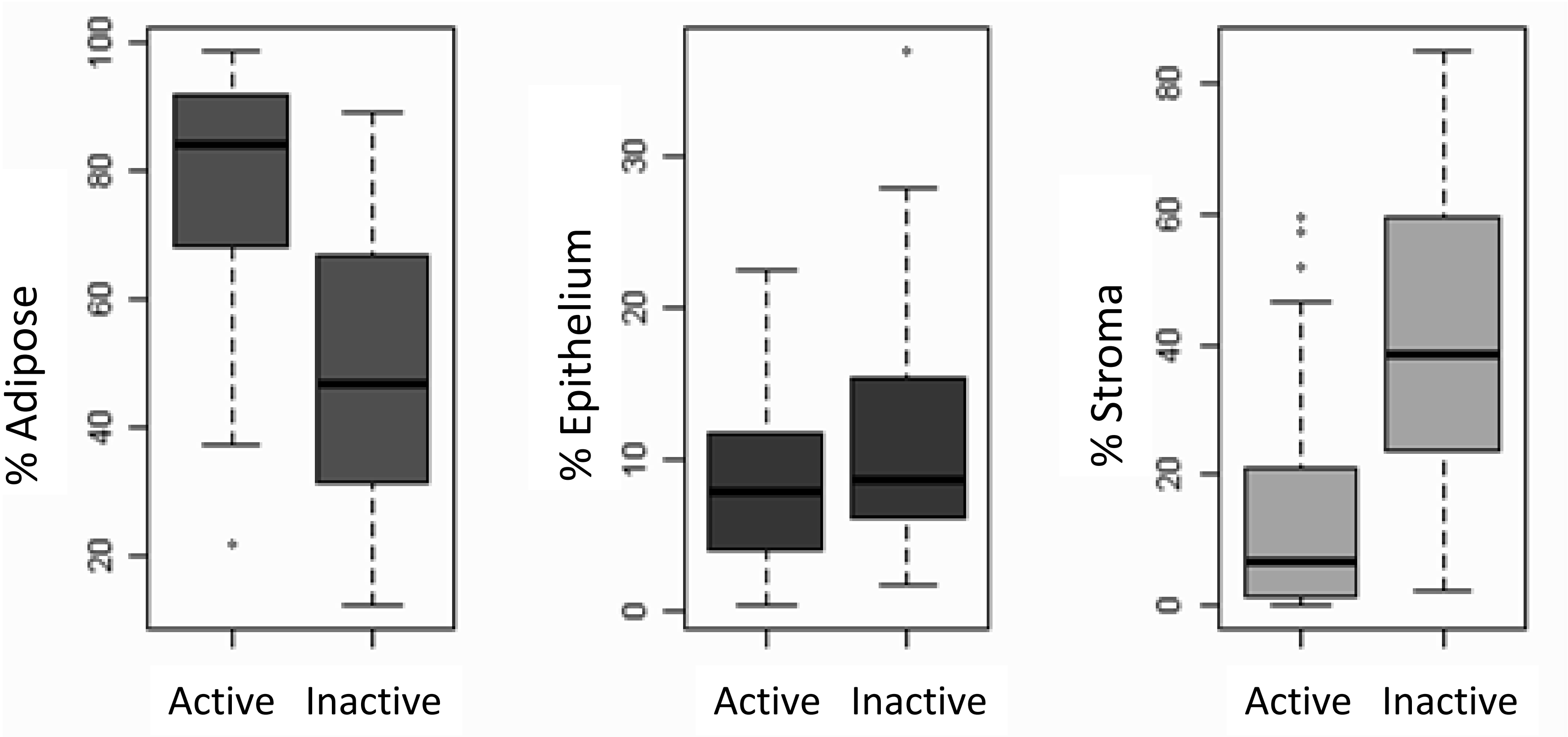
- RNA and miRNA concordance

- Tumor characteristics (ER status, intrinsic subtype, etc.) not strongly associated with main clusters

- 'Probable contamination' samples not readily detected.

Courtesy of Gordon Robertson

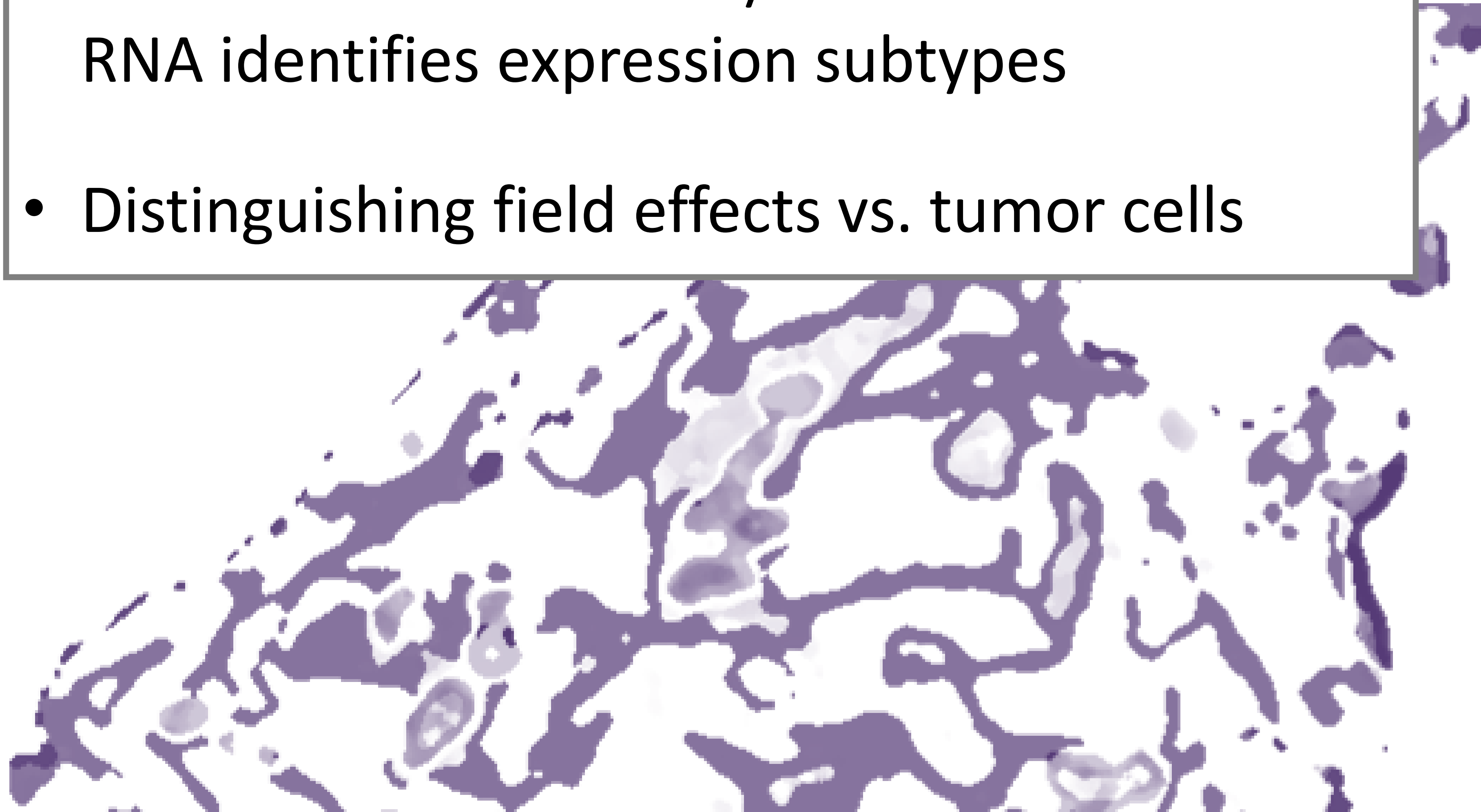
RNA Expression Subtype vs. Composition



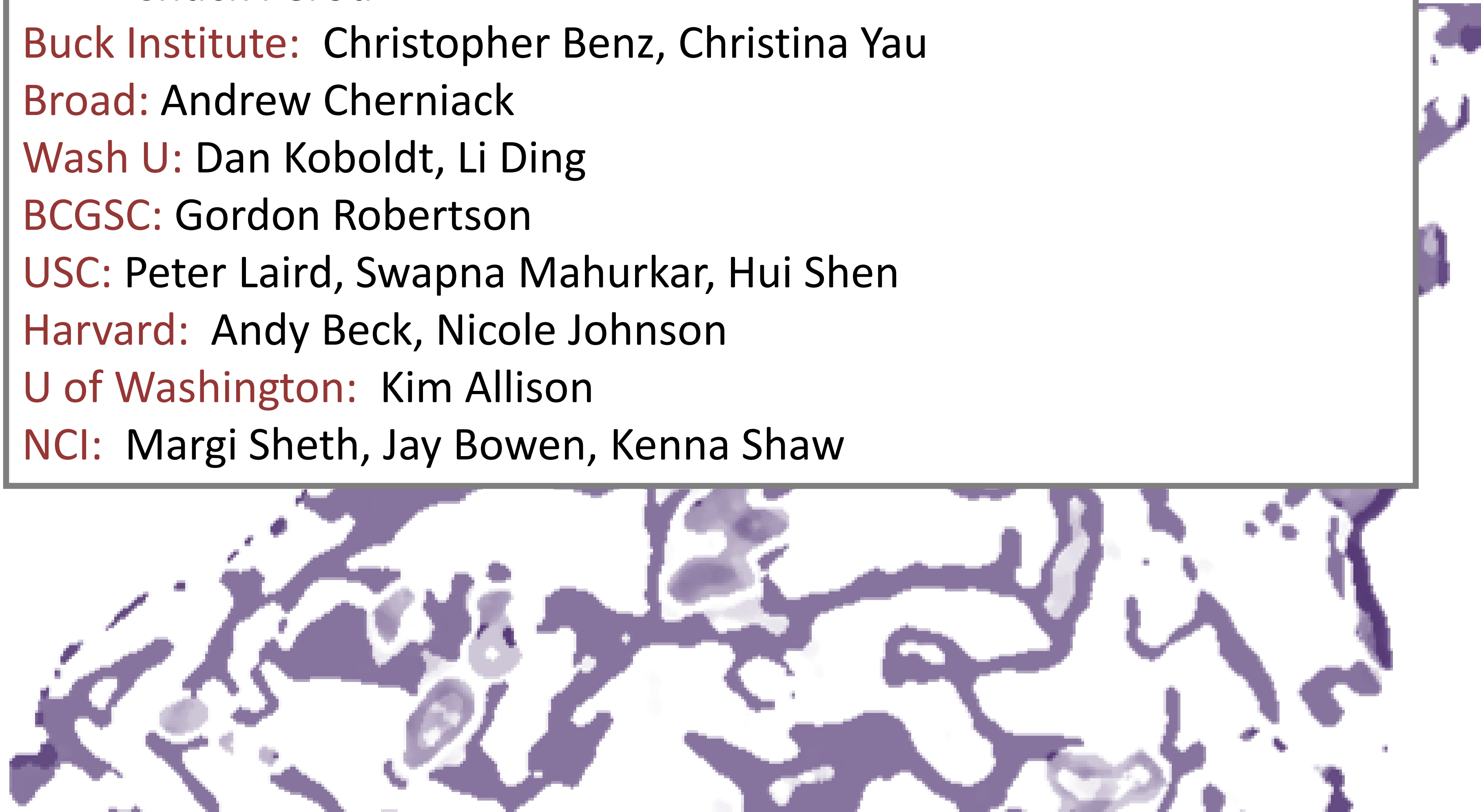
Conclusions & Future Directions

- DNA shows field effects/tumor contamination
  RNA identifies expression subtypes

- Distinguishing field effects vs. tumor cells



Acknowledgments



UNC: Melissa Troester, Katie Hoadley, Monica D’Arcy, Rupan Sandhu, Chuck Perou

Buck Institute: Christopher Benz, Christina Yau

Broad: Andrew Cherniack

Wash U: Dan Koboldt, Li Ding

BCGSC: Gordon Robertson

USC: Peter Laird, Swapna Mahurkar, Hui Shen

Harvard: Andy Beck, Nicole Johnson

U of Washington: Kim Allison

NCI: Margi Sheth, Jay Bowen, Kenna Shaw