

Novel Metrics of HER2 Heterogeneity in HER2-Positive and HER2-Low Breast Cancer via High Dimensional Multiplexed Immunofluorescence Spatial Profiling



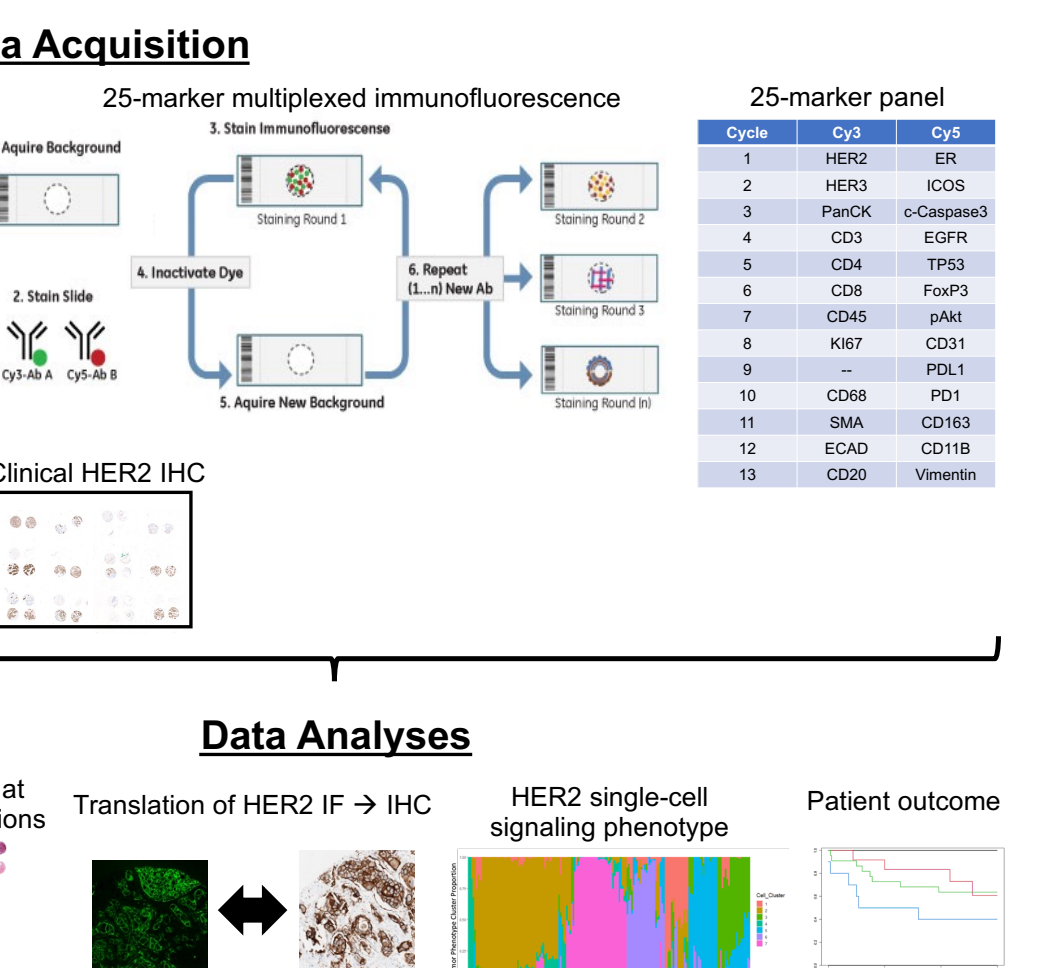
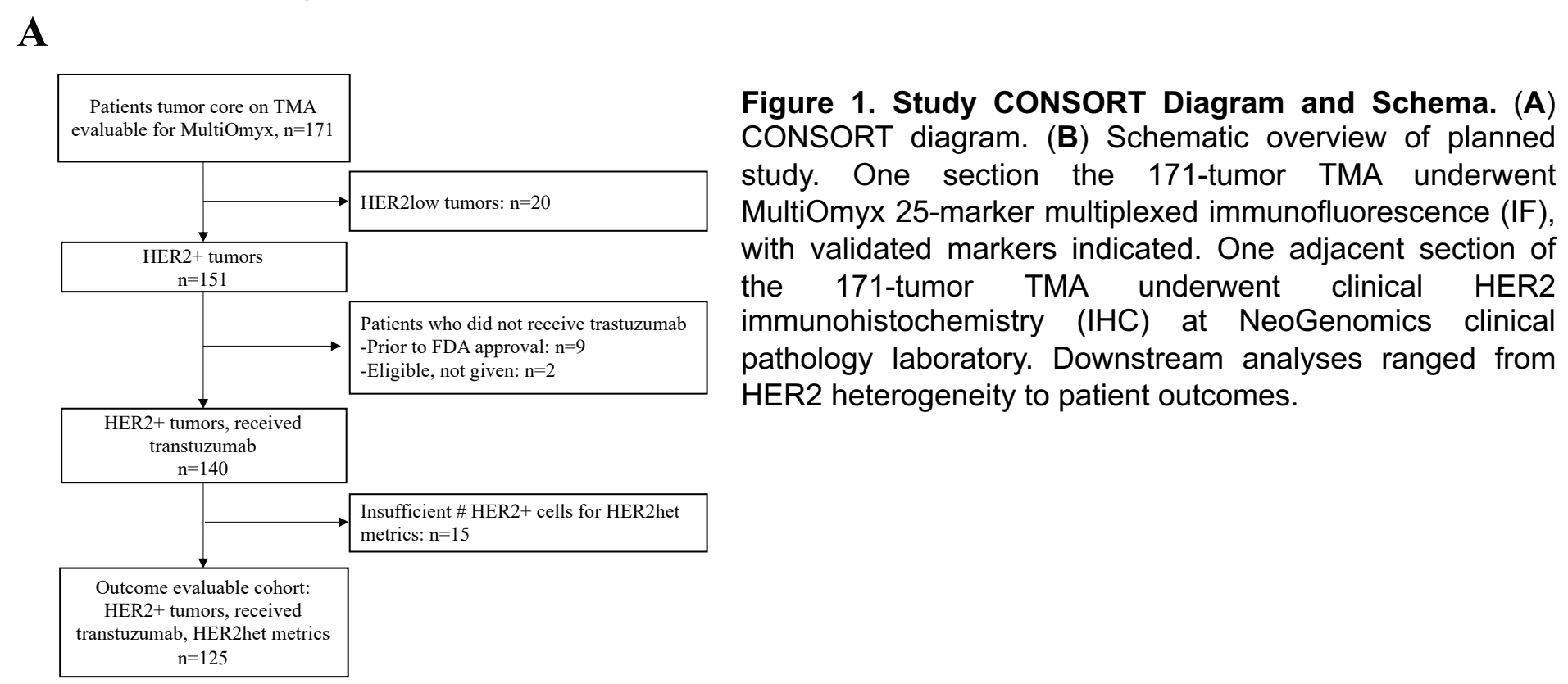
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Background/Approach

Categorizing breast cancer HER2/ERBB2 expression as “positive” or “negative” is no longer sufficient, with evidence that treatment response and outcomes are associated with HER2 “low” status and HER2 intratumoral heterogeneity. We **hypothesized** that interrogating HER2 heterogeneity (HER2het) across multiple spatial resolutions would more accurately capture HER2 diversity and be associated with clinical outcomes.



METHODS

- We interrogated tumor cell and microenvironmental features by profiling 1,113,204 single cells in tissue sections from 171 HER2+/HER2low cancers via custom 25-marker high dimensional multiplexed immunofluorescence (IF) using NeoGenomics MultiOmyx, with adjacent section HER2 immunohistochemistry (IHC).
- 1166 regions of interest were profiled from 208 unique tumors. Median follow-up from diagnosis was 143 months and 98.9% (n=183/185) received HER2-directed therapy in the (neo)adjuvant or metastatic setting.

Results

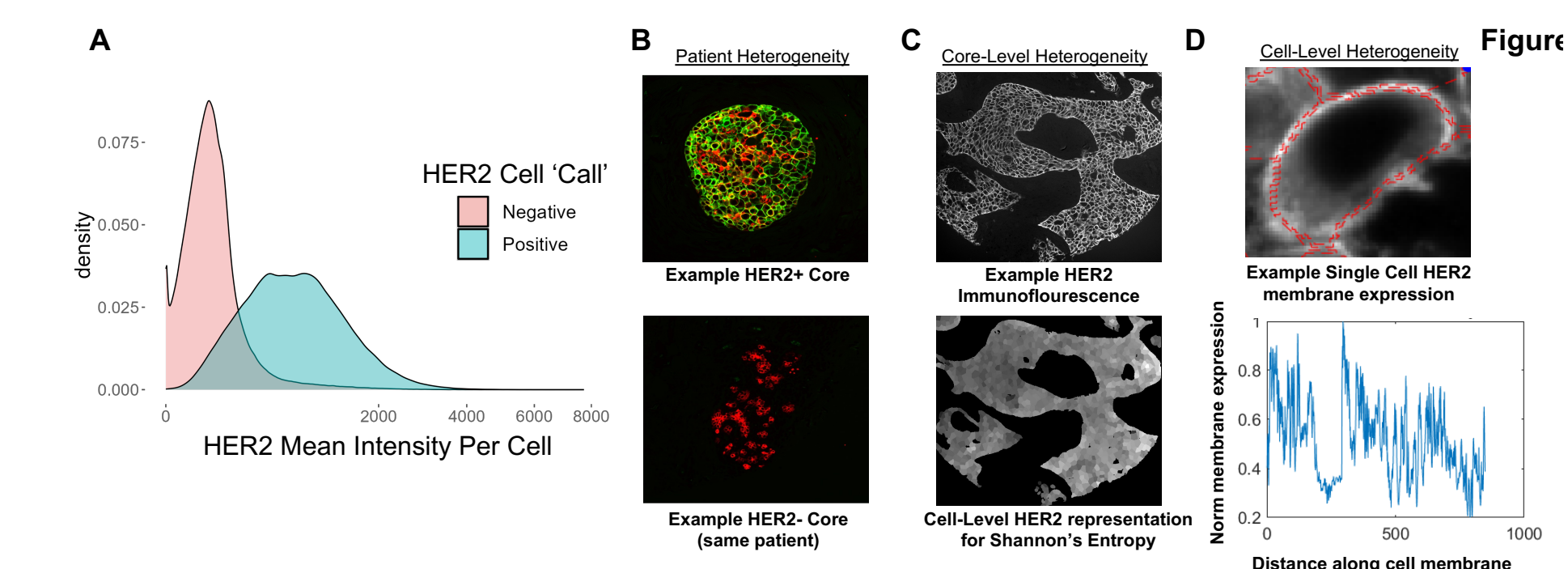


Figure 3. Application of Novel HER2 Heterogeneity Metrics. (A) HER2 mean membrane expression (y-axis) for paired tumor cores, with intraclass correlation coefficient (ICC) indicated. (B) Shannon's Entropy (y-axis) for paired tumor cores, with ICC indicated. (C) Correlation plot of each HER2het metric relative to each other metric with correlation from 1 (positive correlation; red) to -1 (negative correlation; blue); x indicates non-significant association (p>0.05). (D) Comparison of HER2het metrics and HER2 IHC receptor status, divided as 'negative' (HER2 IHC 1+/2+ and in-situ hybridization/ISH negative) versus positive (HER2 IHC 3+ and/or ISH positive). (E) Correlation plot of mean cell expression of 25 markers in multiplexed IF (x-axis) relative to mean membrane HER2 expression and HER2het metrics (y-axis) with correlation from 1 (positive correlation; red) to -1 (negative correlation; blue); x indicates non-significant association (p>0.05).

Results (cont)

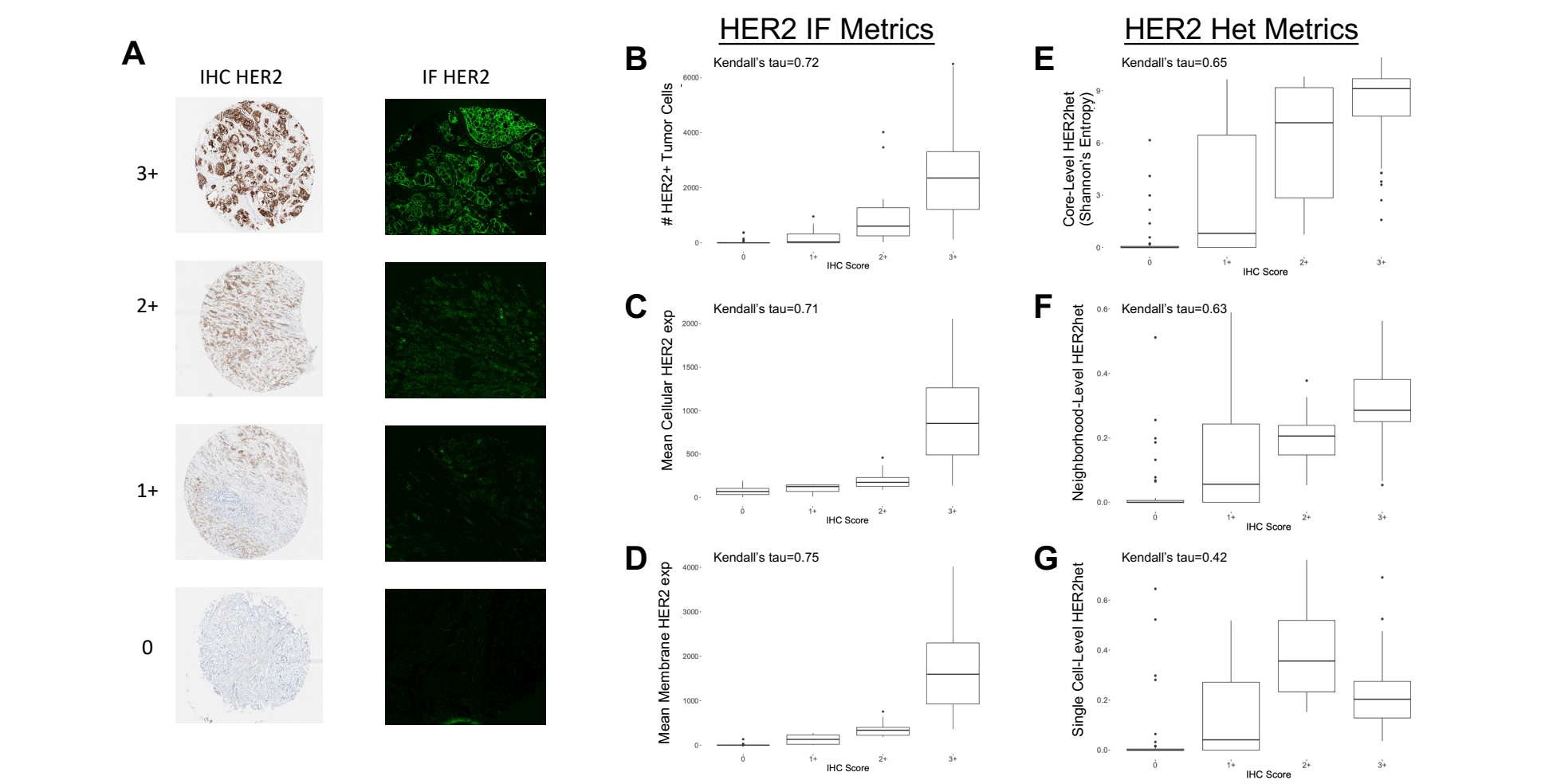


Figure 4. Translating HER2 Immunofluorescence to HER2 Immunohistochemistry. (A) Representative images HER2 IHC (left images) and adjacent section HER2 IF (right). (B-D) HER2 IHC 0/1+/2+/3+ relative to number of HER2+ tumor cells (B), mean total cellular HER2 expression (exp; C), mean membrane HER2 expression (D); ordinal correlation by Kendall's tau presented. (E-G) HER2 IHC 0/1+/2+/3+ relative to HER2 heterogeneity (het) metrics: core-level HER2het as Shannon's Entropy (E), neighborhood-level HER2het (F), single cell-level HER2het (G); ordinal correlation by Kendall's tau presented.

Results (cont)

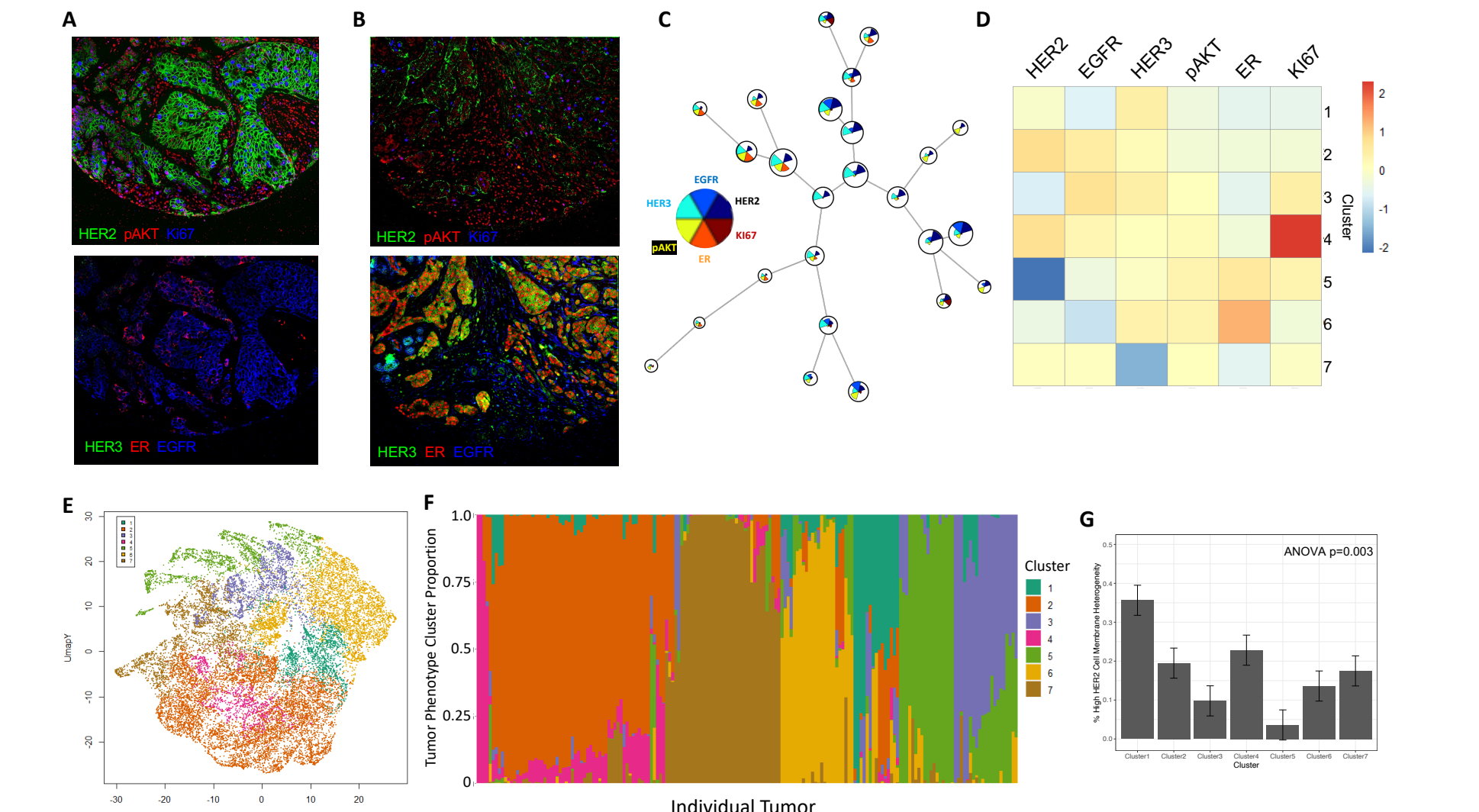


Figure 5. HAIQu: Translating HER2 Immunofluorescence to HER2 Immunohistochemistry. (A) HAIQu schematic overview of classification of IF images at single cell level, based on cell mask, HER2 membrane expression (central images), then binned based on intensity and completeness of membrane intensity (right), with percent of tumor and bin intensity integrated to correspond to IHC 0/1+/2+/3+ (bottom right). (B) F1 score of HAIQu by TMA slide. (C) Performance of HAIQu (x-axis) relative to clinical IHC (y-axis).

Conclusions

- We present novel metrics of HER2 heterogeneity via HDmIF, which offer detailed characterization of the diversity of HER2exp in a large, clinically-annotated cohort with long-term follow-up.
- Single-cell phenotypic analyses of 392,984 HER2+/PanCK+ tumor cells' concurrent expression of six HER2-positive breast cancer related proteins (HER2, HER3, EGFR, pAKT, ER, Ki67) resulted in 7 HER2 signaling cell phenotypes.
- Our HAIQu scoring system effectively translated adjacent section HER2 IF to IHC with K-folds cross validation of 11 TMA blocks demonstrating good performance of HER2 mask model (train F1 score 0.74; test F1 0.76).

Support

