

SUPPLEMENTARY TABLES & FIGURES

Symmans W.S., *et al.* Stage-related transcriptional changes in HR-positive/HER2-negative breast cancer.

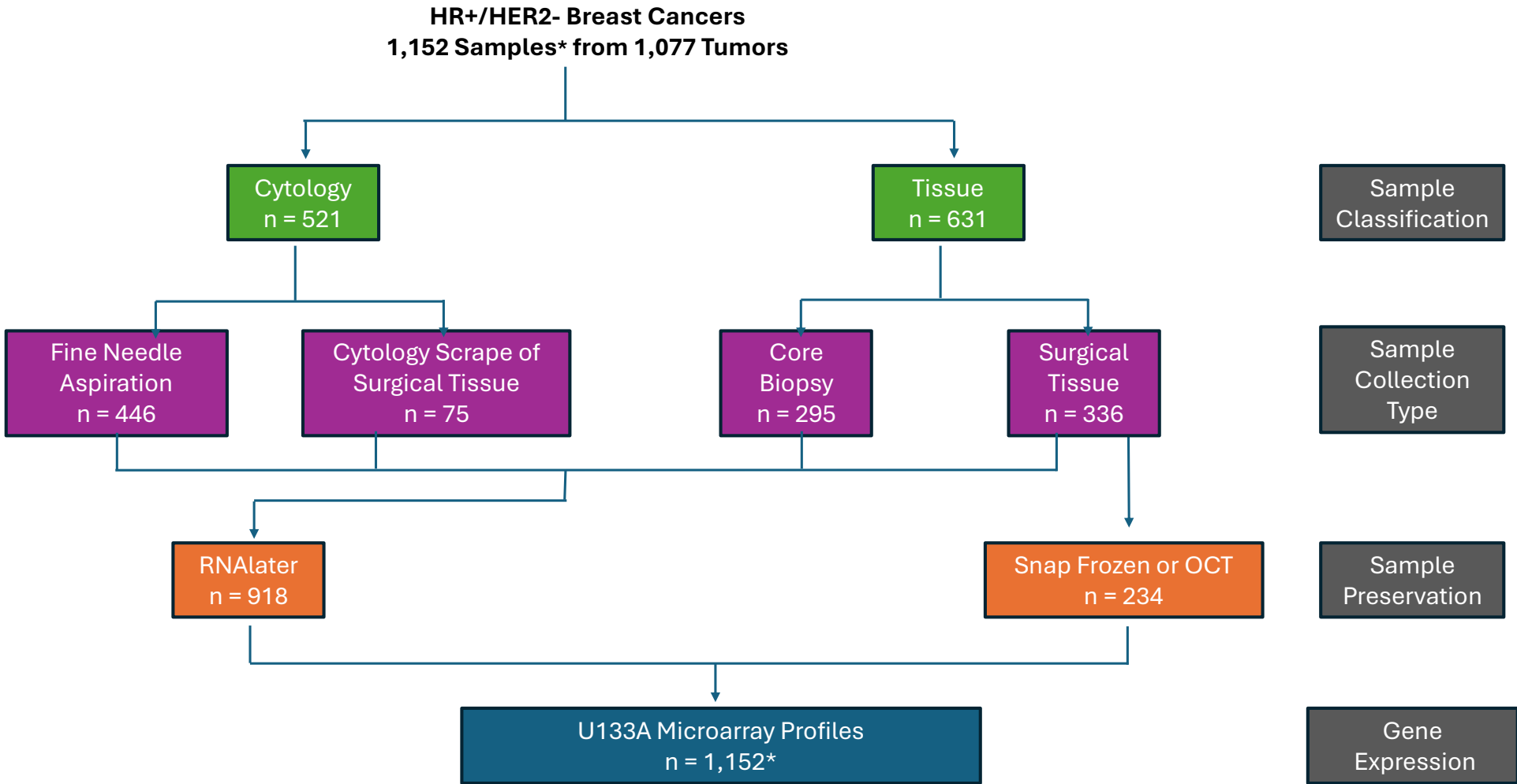
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Supplementary Figure S1. REMARK Diagram



Samples collected from 1,077 unique tumors. \*, 75 samples had matched pairs of cytology scrape and surgical tissue collected in RNAlater at time of surgery, per protocol, resulting in duplicate tumor profiling for comparison of gene expression concordance between sample collection type.

**Supplementary Table S1: All Other Signatures**

|                                  | Sample Matched Pairs |                |                   | Clinical Samples Cohort |                |                   |                   |                |                   |
|----------------------------------|----------------------|----------------|-------------------|-------------------------|----------------|-------------------|-------------------|----------------|-------------------|
|                                  | Cytology vs Tissue   |                |                   | Cytology vs Tissue      |                |                   | AJCC Stage Effect |                |                   |
| Signature                        | Estimate             | 95% CI         | p-value           | Estimate                | 95% CI         | p-value           | Estimate          | 95% CI         | p-value           |
| Endocrine Activity               |                      |                |                   |                         |                |                   |                   |                |                   |
| HALLMARK_ANDROGEN_RESPONSE       | -0.081               | -0.126, -0.035 | <b>0.001</b>      | -0.203                  | -0.232, -0.175 | <b>&lt; 0.001</b> | -0.007            | -0.018, 0.003  | 0.157             |
| HALLMARK_ESTROGEN_RESPONSE_EARLY | 0.011                | -0.054, 0.076  | 0.734             | -0.130                  | -0.160, -0.100 | <b>&lt; 0.001</b> | -0.036            | -0.046, -0.025 | <b>&lt; 0.001</b> |
| HALLMARK_ESTROGEN_RESPONSE_LATE  | 0.010                | -0.047, 0.066  | 0.733             | -0.108                  | -0.135, -0.080 | <b>&lt; 0.001</b> | -0.023            | -0.033, -0.013 | <b>&lt; 0.001</b> |
| Cell Signaling                   |                      |                |                   |                         |                |                   |                   |                |                   |
| BetaCatenin Signature            | -0.016               | -0.041, 0.009  | 0.196             | -0.036                  | -0.050, -0.023 | <b>&lt; 0.001</b> | -0.010            | -0.014, -0.005 | <b>&lt; 0.001</b> |
| HALLMARK_APICAL_JUNCTION         | -0.155               | -0.201, -0.109 | <b>&lt; 0.001</b> | -0.208                  | -0.227, -0.188 | <b>&lt; 0.001</b> | -0.003            | -0.010, 0.005  | 0.479             |
| HALLMARK_APICAL_SURFACE          | -0.172               | -0.228, -0.115 | <b>&lt; 0.001</b> | -0.093                  | -0.120, -0.066 | <b>&lt; 0.001</b> | -0.004            | -0.013, 0.006  | 0.455             |
| HALLMARK_HEDGEHOG_SIGNALING      | -0.127               | -0.179, -0.075 | <b>&lt; 0.001</b> | -0.102                  | -0.127, -0.077 | <b>&lt; 0.001</b> | -0.011            | -0.020, -0.002 | 0.019             |
| HALLMARK_IL2_STAT5_SIGNALING     | -0.198               | -0.245, -0.151 | <b>&lt; 0.001</b> | -0.181                  | -0.203, -0.158 | <b>&lt; 0.001</b> | 0.011             | 0.003, 0.019   | <b>0.006</b>      |
| HALLMARK_IL6_JAK_STAT3_SIGNALING | -0.186               | -0.256, -0.116 | <b>&lt; 0.001</b> | -0.071                  | -0.102, -0.039 | <b>&lt; 0.001</b> | 0.012             | 0.001, 0.024   | 0.036             |
| HALLMARK_KRAS_SIGNALING_DN       | 0.053                | 0.018, 0.088   | <b>0.003</b>      | 0.174                   | 0.152, 0.197   | <b>&lt; 0.001</b> | 0.006             | -0.003, 0.014  | 0.172             |
| HALLMARK_KRAS_SIGNALING_UP       | -0.293               | -0.341, -0.244 | <b>&lt; 0.001</b> | -0.163                  | -0.187, -0.138 | <b>&lt; 0.001</b> | 0.005             | -0.003, 0.014  | 0.226             |
| HALLMARK_MTORC1_SIGNALING        | 0.054                | -0.025, 0.133  | 0.182             | -0.082                  | -0.121, -0.042 | <b>&lt; 0.001</b> | 0.021             | 0.007, 0.036   | <b>0.003</b>      |
| HALLMARK_NOTCH_SIGNALING         | -0.077               | -0.133, -0.020 | <b>0.008</b>      | -0.243                  | -0.270, -0.216 | <b>&lt; 0.001</b> | -0.008            | -0.018, 0.001  | 0.095             |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 0.014                | -0.033, 0.062  | 0.559             | -0.022                  | -0.044, -0.000 | 0.045             | 0.014             | 0.006, 0.022   | <b>0.001</b>      |
| HALLMARK_TGF_BETA_SIGNALING      | -0.186               | -0.250, -0.122 | <b>&lt; 0.001</b> | -0.335                  | -0.364, -0.307 | <b>&lt; 0.001</b> | -0.006            | -0.016, 0.004  | 0.267             |

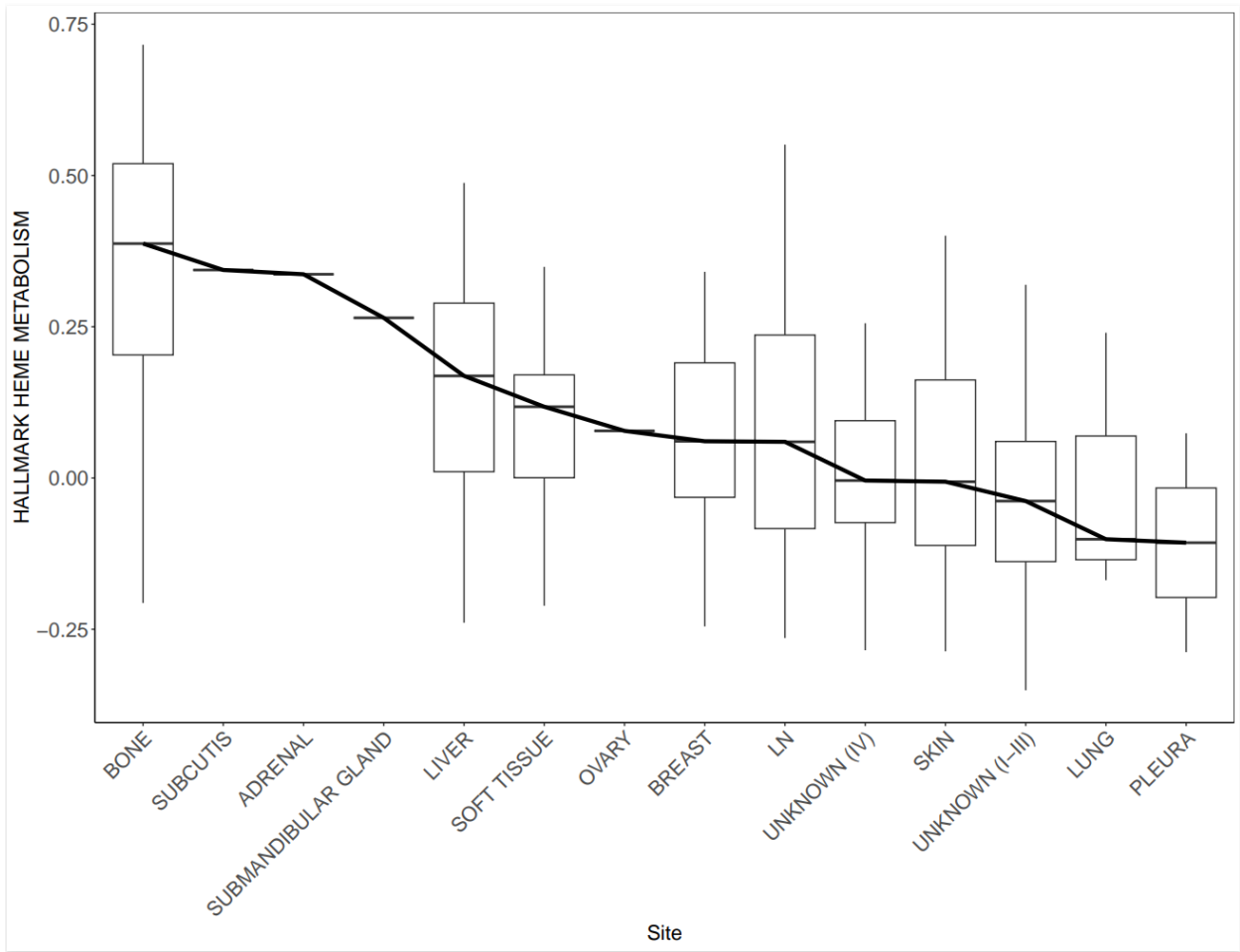
|                                         |        |                |         |        |                |         |        |                |         |
|-----------------------------------------|--------|----------------|---------|--------|----------------|---------|--------|----------------|---------|
| HALLMARK_TNFA_SIGNALING_VIA_NFKB        | -0.202 | -0.268, -0.135 | < 0.001 | -0.223 | -0.251, -0.195 | < 0.001 | -0.006 | -0.016, 0.004  | 0.241   |
| HALLMARK_WNT_BETA_CATENIN_SIGNALING     | -0.029 | -0.084, 0.026  | 0.298   | -0.089 | -0.112, -0.065 | < 0.001 | -0.002 | -0.010, 0.007  | 0.728   |
| PIK3cags                                | 0.006  | -0.049, 0.061  | 0.838   | -0.067 | -0.089, -0.046 | < 0.001 | -0.010 | -0.018, -0.003 | 0.009   |
| SRC Signature                           | -0.021 | -0.040, -0.001 | 0.041   | -0.022 | -0.033, -0.012 | < 0.001 | -0.009 | -0.013, -0.005 | < 0.001 |
| STAT1 Signature                         | -0.160 | -0.265, -0.054 | 0.003   | 0.017  | -0.022, 0.057  | 0.391   | 0.016  | 0.001, 0.030   | 0.034   |
| <b>Proliferation and Grade</b>          |        |                |         |        |                |         |        |                |         |
| E2F3 Signature                          | 0.018  | 0.001, 0.035   | 0.043   | 0.011  | 0.003, 0.020   | 0.008   | -0.005 | -0.008, -0.002 | 0.001   |
| GENIUS                                  | 0.339  | 0.180, 0.497   | < 0.001 | 0.330  | 0.269, 0.391   | < 0.001 | 0.032  | 0.009, 0.054   | 0.006   |
| GGI                                     | 0.094  | 0.004, 0.184   | 0.042   | 0.132  | 0.097, 0.167   | < 0.001 | 0.033  | 0.020, 0.045   | < 0.001 |
| HALLMARK_E2F_TARGETS                    | 0.149  | 0.038, 0.260   | 0.009   | 0.017  | -0.029, 0.063  | 0.472   | 0.030  | 0.014, 0.047   | < 0.001 |
| HALLMARK_G2M_CHECKPOINT                 | 0.131  | 0.040, 0.222   | 0.005   | 0.001  | -0.038, 0.040  | 0.969   | 0.027  | 0.013, 0.041   | < 0.001 |
| HALLMARK_MYC_TARGETS_V1                 | 0.150  | 0.045, 0.254   | 0.005   | -0.099 | -0.150, -0.049 | < 0.001 | 0.021  | 0.002, 0.039   | 0.027   |
| HALLMARK_MYC_TARGETS_V2                 | 0.170  | 0.072, 0.267   | 0.001   | -0.026 | -0.071, 0.018  | 0.241   | 0.013  | -0.003, 0.029  | 0.116   |
| MAPK Signature                          | -0.019 | -0.032, -0.006 | 0.004   | 0.023  | 0.015, 0.031   | < 0.001 | -0.002 | -0.005, 0.001  | 0.251   |
| Ras Signature                           | -0.061 | -0.084, -0.037 | < 0.001 | -0.046 | -0.058, -0.034 | < 0.001 | -0.004 | -0.008, 0.000  | 0.071   |
| <b>Metabolism and Cellular Activity</b> |        |                |         |        |                |         |        |                |         |
| HALLMARK_ADIPOGENESIS                   | -0.066 | -0.118, -0.013 | 0.015   | -0.203 | -0.236, -0.170 | < 0.001 | -0.008 | -0.020, 0.004  | 0.167   |
| HALLMARK_BILE_ACID_METABOLISM           | -0.033 | -0.072, 0.006  | 0.095   | 0.021  | 0.002, 0.041   | 0.033   | -0.007 | -0.014, 0.000  | 0.063   |
| HALLMARK_CHOLESTEROL_HOMEOSTASIS        | 0.071  | 0.005, 0.136   | 0.034   | -0.092 | -0.128, -0.057 | < 0.001 | -0.000 | -0.013, 0.012  | 0.944   |
| HALLMARK_FATTY_ACID_METABOLISM          | 0.016  | -0.040, 0.073  | 0.562   | -0.095 | -0.126, -0.064 | < 0.001 | -0.005 | -0.016, 0.007  | 0.427   |
| HALLMARK_GLYCOLYSIS                     | -0.004 | -0.053, 0.044  | 0.861   | -0.117 | -0.143, -0.091 | < 0.001 | 0.002  | -0.008, 0.011  | 0.747   |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION      | 0.117  | 0.027, 0.207   | 0.011   | -0.138 | -0.188, -0.089 | < 0.001 | -0.001 | -0.019, 0.017  | 0.877   |

|                                           |        |                |                   |        |                |                   |        |                |                   |
|-------------------------------------------|--------|----------------|-------------------|--------|----------------|-------------------|--------|----------------|-------------------|
| HALLMARK_PANCREAS_BETA_CELLS              | -0.058 | -0.109, -0.007 | 0.026             | 0.078  | 0.053, 0.102   | <b>&lt; 0.001</b> | 0.010  | 0.001, 0.019   | 0.034             |
| HALLMARK_PEROXISOME                       | 0.059  | 0.012, 0.106   | 0.014             | -0.030 | -0.055, -0.004 | 0.021             | -0.003 | -0.012, 0.006  | 0.526             |
| HALLMARK_PROTEIN_SECRETION                | -0.034 | -0.102, 0.034  | 0.324             | -0.156 | -0.192, -0.119 | <b>&lt; 0.001</b> | 0.003  | -0.010, 0.017  | 0.615             |
| <b>Genome Instability and Mutagenesis</b> |        |                |                   |        |                |                   |        |                |                   |
| CIN70 Signature                           | 0.126  | 0.036, 0.216   | <b>0.006</b>      | 0.111  | 0.075, 0.148   | <b>&lt; 0.001</b> | 0.025  | 0.012, 0.038   | <b>&lt; 0.001</b> |
| HALLMARK_DNA_REPAIR                       | 0.115  | 0.048, 0.183   | <b>0.001</b>      | -0.068 | -0.102, -0.033 | <b>&lt; 0.001</b> | 0.008  | -0.004, 0.021  | 0.200             |
| HALLMARK_MITOTIC_SPINDLE                  | 0.006  | -0.045, 0.058  | 0.814             | -0.122 | -0.147, -0.098 | <b>&lt; 0.001</b> | 0.008  | -0.000, 0.017  | 0.063             |
| HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY  | -0.012 | -0.083, 0.060  | 0.748             | -0.138 | -0.174, -0.102 | <b>&lt; 0.001</b> | 0.008  | -0.005, 0.021  | 0.246             |
| HALLMARK_SPERMATOGENESIS                  | 0.063  | 0.021, 0.105   | <b>0.003</b>      | 0.182  | 0.162, 0.202   | <b>&lt; 0.001</b> | 0.014  | 0.007, 0.021   | <b>&lt; 0.001</b> |
| HALLMARK_UV_RESPONSE_DN                   | -0.248 | -0.303, -0.192 | <b>&lt; 0.001</b> | -0.351 | -0.374, -0.329 | <b>&lt; 0.001</b> | -0.019 | -0.028, -0.011 | <b>&lt; 0.001</b> |
| HALLMARK_UV_RESPONSE_UP                   | 0.008  | -0.041, 0.058  | 0.741             | -0.092 | -0.117, -0.067 | <b>&lt; 0.001</b> | 0.010  | 0.001, 0.019   | 0.035             |
| <b>Resisting Cell Death</b>               |        |                |                   |        |                |                   |        |                |                   |
| CASP3 Signature                           | -0.013 | -0.048, 0.023  | 0.478             | 0.071  | 0.051, 0.091   | <b>&lt; 0.001</b> | 0.004  | -0.003, 0.011  | 0.274             |
| HALLMARK_APOPTOSIS                        | -0.191 | -0.234, -0.149 | <b>&lt; 0.001</b> | -0.238 | -0.261, -0.216 | <b>&lt; 0.001</b> | -0.003 | -0.011, 0.006  | 0.525             |
| HALLMARK_P53_PATHWAY                      | -0.088 | -0.128, -0.048 | <b>&lt; 0.001</b> | -0.189 | -0.210, -0.168 | <b>&lt; 0.001</b> | -0.008 | -0.015, -0.000 | 0.049             |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE        | 0.099  | 0.032, 0.166   | <b>0.004</b>      | -0.157 | -0.194, -0.120 | <b>&lt; 0.001</b> | 0.003  | -0.011, 0.016  | 0.691             |
| HALLMARK_XENOBIOTIC_METABOLISM            | -0.071 | -0.110, -0.033 | <b>&lt; 0.001</b> | -0.091 | -0.112, -0.071 | <b>&lt; 0.001</b> | -0.002 | -0.009, 0.006  | 0.648             |
| TAMR13                                    | 2.248  | 1.626, 2.871   | <b>&lt; 0.001</b> | 2.180  | 1.919, 2.440   | <b>&lt; 0.001</b> | 0.223  | 0.128, 0.318   | <b>&lt; 0.001</b> |
| <b>Immune Activity</b>                    |        |                |                   |        |                |                   |        |                |                   |
| HALLMARK_ALLOGRAFT_REJECTION              | -0.218 | -0.301, -0.134 | <b>&lt; 0.001</b> | -0.058 | -0.093, -0.022 | <b>0.001</b>      | 0.016  | 0.003, 0.029   | 0.014             |
| HALLMARK_COMPLEMENT                       | -0.224 | -0.286, -0.163 | <b>&lt; 0.001</b> | -0.159 | -0.187, -0.131 | <b>&lt; 0.001</b> | 0.015  | 0.004, 0.025   | <b>0.005</b>      |
| HALLMARK_INTERFERON_ALPHA_RESPONSE        | -0.134 | -0.254, -0.014 | 0.028             | -0.163 | -0.216, -0.110 | <b>&lt; 0.001</b> | 0.027  | 0.008, 0.047   | <b>0.005</b>      |

|                                            |        |                |                   |        |                |                   |        |                |              |
|--------------------------------------------|--------|----------------|-------------------|--------|----------------|-------------------|--------|----------------|--------------|
| HALLMARK_INTERFERON_GAMMA_RESPONSE         | -0.201 | -0.300, -0.102 | <b>&lt; 0.001</b> | -0.183 | -0.225, -0.140 | <b>&lt; 0.001</b> | 0.028  | 0.012, 0.043   | <b>0.001</b> |
| IMMUNE Signature                           | -1459  | -1946, -971    | <b>&lt; 0.001</b> | 93     | -150, 336      | 0.452             | 146    | 56, 234        | <b>0.001</b> |
| <b>Invasion and Microenvironment</b>       |        |                |                   |        |                |                   |        |                |              |
| HALLMARK_ANGIOGENESIS                      | -0.395 | -0.460, -0.330 | <b>&lt; 0.001</b> | -0.433 | -0.462, -0.404 | <b>&lt; 0.001</b> | -0.003 | -0.013, 0.008  | 0.614        |
| HALLMARK_COAGULATION                       | -0.275 | -0.322, -0.228 | <b>&lt; 0.001</b> | -0.256 | -0.279, -0.233 | <b>&lt; 0.001</b> | -0.006 | -0.014, 0.002  | 0.166        |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | -0.473 | -0.550, -0.396 | <b>&lt; 0.001</b> | -0.569 | -0.602, -0.536 | <b>&lt; 0.001</b> | -0.016 | -0.028, -0.004 | 0.011        |
| HALLMARK_HYPOXIA                           | -0.184 | -0.231, -0.137 | <b>&lt; 0.001</b> | -0.241 | -0.263, -0.218 | <b>&lt; 0.001</b> | -0.001 | -0.009, 0.007  | 0.770        |
| HALLMARK_INFLAMMATORY_RESPONSE             | -0.210 | -0.275, -0.144 | <b>&lt; 0.001</b> | -0.056 | -0.085, -0.027 | <b>&lt; 0.001</b> | 0.011  | 0.001, 0.022   | 0.036        |
| HALLMARK_MYOGENESIS                        | -0.169 | -0.218, -0.119 | <b>&lt; 0.001</b> | -0.159 | -0.181, -0.137 | <b>&lt; 0.001</b> | -0.000 | -0.008, 0.008  | 0.931        |
| PLAU Signature                             | -0.311 | -0.373, -0.250 | <b>&lt; 0.001</b> | -0.343 | -0.371, -0.315 | <b>&lt; 0.001</b> | -0.009 | -0.019, 0.001  | 0.090        |
| STROMA Signature                           | -2486  | -2819, -2152   | <b>&lt; 0.001</b> | -2221  | -2391, -2052   | <b>&lt; 0.001</b> | -60    | -120, 4        | 0.066        |
| VEGF Signature                             | 0.126  | 0.086, 0.167   | <b>&lt; 0.001</b> | -0.010 | -0.031, 0.011  | 0.338             | -0.006 | -0.014, 0.002  | 0.119        |

These cancer biology signatures from the 50 listed in the Broad Institute Gene Set Enrichment Analysis were included in this Supplementary Table S1 if they: 1) had stage effect with significance level  $p \geq 0.01$ , 2) were redundant with a different biological signature that was already presented in Table 1 of the manuscript, or 3) demonstrated substantial difference in measurements from matched pairs of tissue and cytology samples. For the clinical samples cohort, this meant that the gradient of the regression line across staging was not significantly up or down. The “estimate” column quantifies the difference in means between cytology and tissue for matched pair samples, with a positive value indicating that the cytology was higher than tissue. “Estimate” in the clinical cohort is the slope of the regression line with increasing stage category as an integer.

Supplementary Figure S2. Heme Metabolism Expression by Site of Metastasis



Box plots showing mean expression values and interquartile range of heme metabolism in different tissues. The black line connects the mean values from all samples.