

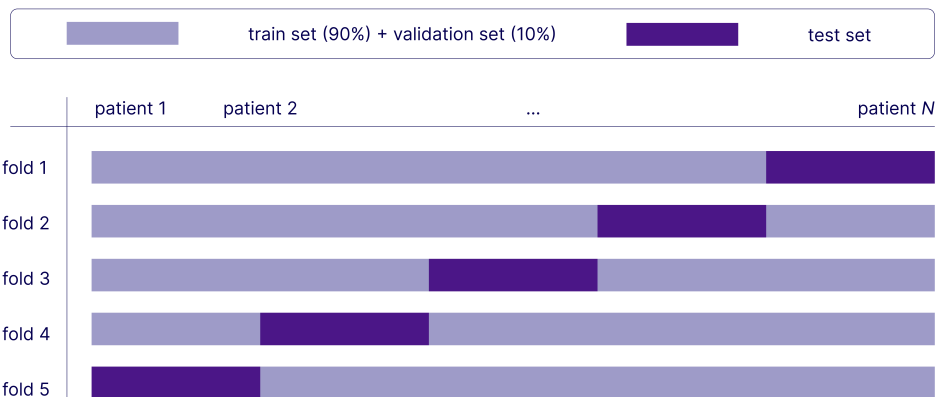
Supplementary tables and figures

Supplementary Table 1: Number of Whole Slide Images and unique number of patients used from each cancer type in TCGA. Source data are provided in the Source Data File.

| TCGA project | number WSIs | number patients |
|--------------|-------------|-----------------|
| TCGA-BRCA | 1,130 | 1,059 |
| TCGA-UCEC | 563 | 502 |
| TCGA-LUAD | 536 | 473 |
| TCGA-THCA | 517 | 504 |
| TCGA-KIRC | 514 | 508 |
| TCGA-LUSC | 510 | 476 |
| TCGA-SKCM | 474 | 432 |
| TCGA-HNSC | 464 | 442 |
| TCGA-COAD | 455 | 447 |
| TCGA-BLCA | 451 | 380 |
| TCGA-PRAD | 448 | 401 |
| TCGA-STAD | 411 | 386 |
| TCGA-LIHC | 373 | 359 |
| TCGA-KIRP | 299 | 275 |
| TCGA-GBM | 237 | 102 |
| TCGA-PAAD | 202 | 176 |
| total | 7,584 | 6,922 |

Supplementary Table 2: Number of Whole Slide Images and unique number of patients used from each cancer type in CPTAC. Source data are provided in the Source Data File.

| CPTAC project | number WSIs | number patients |
|---------------|-------------|-----------------|
| CPTAC-BRCA | 106 | 106 |
| CPTAC-CCRCC | 302 | 211 |
| CPTAC-COAD | 103 | 103 |
| CPTAC-GBM | 94 | 94 |
| CPTAC-LUAD | 222 | 222 |
| CPTAC-LSCC | 109 | 108 |
| CPTAC-PDA | 146 | 146 |
| total | 1,081 | 989 |



Supplementary Figure 1: Data splitting. First, five folds were made each of which consist of a ‘global’ train (80%) and test set (20%). The ‘global’ train set was further split into a train (90%) and validation (10%) set. In each fold i , validation set i was used to determine the optimal point to stop training model i , which was then evaluated on test set i . Afterwards, predictions on patients from test sets i ($i = 1..5$) were concatenated for performance evaluation.

Supplementary Table 3: Number of genes significantly well predicted in the TCGA test sets. Source data are provided in the Source Data File.

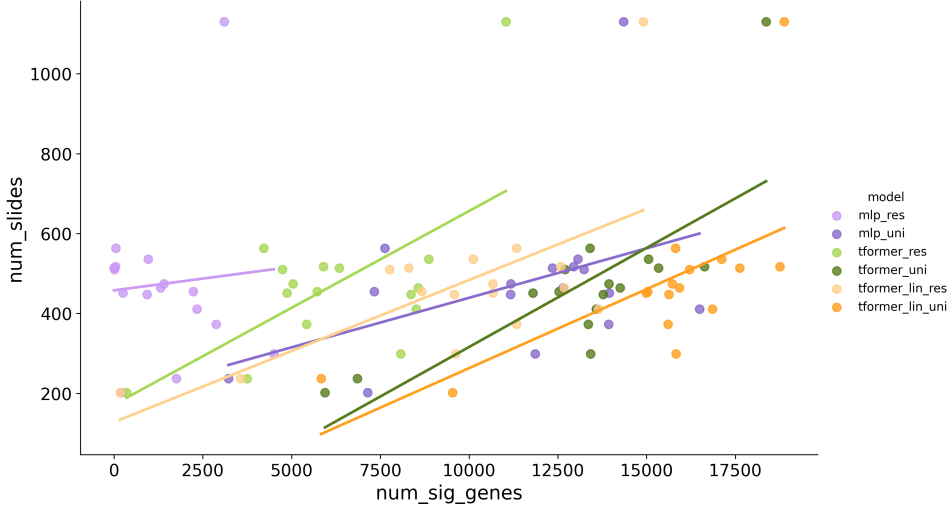
| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|---------|---------|---------|-------------|-------------|-----------------|-----------------|
| BRCA | 3,106 | 14,355 | 11,036 | 18,368 | 14,906 | 18,878 |
| THCA | 38 | 12,943 | 5,902 | 16,633 | 12,584 | 18,758 |
| KIRC | 0 | 12,344 | 6,342 | 15,337 | 8,300 | 17,623 |
| LUAD | 957 | 13,066 | 8,860 | 15,056 | 10,115 | 17,116 |
| STAD | 2,334 | 16,490 | 8,509 | 13,587 | 13,651 | 16,855 |
| LUSC | 11 | 13,238 | 4,740 | 12,706 | 7,769 | 16,210 |
| HNSC | 1,312 | 12,647 | 8,563 | 14,253 | 12,675 | 15,928 |
| KIRP | 4,500 | 11,861 | 8,071 | 13,424 | 9,620 | 15,828 |
| UCEC | 46 | 7,624 | 4,218 | 13,407 | 11,335 | 15,810 |
| SKCM | 1,403 | 11,179 | 5,041 | 13,937 | 10,670 | 15,728 |
| PRAD | 927 | 11,170 | 8,367 | 13,775 | 9,582 | 15,627 |
| LIHC | 2,874 | 13,927 | 5,418 | 13,355 | 11,334 | 15,604 |
| COAD | 2,231 | 7,331 | 5,723 | 12,530 | 8,668 | 15,028 |
| BLCA | 253 | 13,944 | 4,871 | 11,797 | 10,675 | 14,989 |
| PAAD | 219 | 7,144 | 355 | 5,942 | 170 | 9,535 |
| GBM | 1,750 | 3,223 | 3,749 | 6,854 | 3,559 | 5,831 |
| average | 1,372 | 11,405 | 6,235 | 13,185 | 9,725 | 15,334 |

Supplementary Table 4: Median correlation coefficient of top 1000 genes in TCGA test sets. (top 1000 genes defined independently within each model and cancer type). Source data are provided in the Source Data File.

| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|---------|---------|---------|-------------|-------------|-----------------|-----------------|
| THCA | 0.116 | 0.553 | 0.336 | 0.563 | 0.528 | 0.652 |
| LIHC | 0.227 | 0.506 | 0.283 | 0.480 | 0.418 | 0.560 |
| KIRP | 0.377 | 0.485 | 0.365 | 0.471 | 0.390 | 0.548 |
| KIRC | 0.080 | 0.418 | 0.225 | 0.462 | 0.332 | 0.537 |
| STAD | 0.265 | 0.500 | 0.346 | 0.405 | 0.407 | 0.536 |
| BRCA | 0.200 | 0.411 | 0.325 | 0.458 | 0.396 | 0.534 |
| PAAD | 0.149 | 0.494 | 0.168 | 0.371 | 0.140 | 0.523 |
| BLCA | 0.094 | 0.458 | 0.250 | 0.425 | 0.378 | 0.512 |
| PRAD | 0.126 | 0.464 | 0.321 | 0.417 | 0.345 | 0.503 |
| LUAD | 0.130 | 0.415 | 0.284 | 0.404 | 0.306 | 0.495 |
| SKCM | 0.166 | 0.420 | 0.225 | 0.449 | 0.366 | 0.483 |
| COAD | 0.215 | 0.328 | 0.263 | 0.385 | 0.345 | 0.480 |
| HNSC | 0.166 | 0.420 | 0.277 | 0.393 | 0.377 | 0.479 |
| LUSC | 0.094 | 0.360 | 0.197 | 0.337 | 0.266 | 0.443 |
| UCEC | 0.081 | 0.309 | 0.180 | 0.329 | 0.293 | 0.413 |
| GBM | 0.230 | 0.300 | 0.285 | 0.354 | 0.316 | 0.367 |
| average | 0.170 | 0.428 | 0.271 | 0.419 | 0.350 | 0.504 |

Supplementary Table 5: Normalized RMSE of top 1000 genes in TCGA test sets. (top 1000 genes defined independently within each model and cancer type). Source data are provided in the Source Data File.

| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|---------|---------|---------|-------------|-------------|-----------------|-----------------|
| COAD | 0.240 | 0.251 | 0.225 | 0.206 | 0.202 | 0.191 |
| UCEC | 0.234 | 0.180 | 0.211 | 0.181 | 0.190 | 0.166 |
| PAAD | 0.272 | 0.216 | 0.137 | 0.171 | 0.124 | 0.161 |
| KIRP | 0.242 | 0.234 | 0.175 | 0.167 | 0.162 | 0.153 |
| THCA | 0.309 | 0.268 | 0.223 | 0.181 | 0.182 | 0.146 |
| HNSC | 0.178 | 0.198 | 0.164 | 0.153 | 0.154 | 0.140 |
| LUSC | 0.224 | 0.197 | 0.160 | 0.168 | 0.159 | 0.139 |
| PRAD | 0.290 | 0.196 | 0.163 | 0.155 | 0.142 | 0.138 |
| KIRC | 0.189 | 0.205 | 0.148 | 0.159 | 0.148 | 0.138 |
| BLCA | 0.228 | 0.206 | 0.170 | 0.154 | 0.160 | 0.137 |
| LIHC | 0.220 | 0.202 | 0.164 | 0.138 | 0.156 | 0.123 |
| GBM | 0.221 | 0.232 | 0.098 | 0.115 | 0.093 | 0.122 |
| STAD | 0.180 | 0.198 | 0.149 | 0.142 | 0.142 | 0.119 |
| BRCA | 0.181 | 0.146 | 0.150 | 0.128 | 0.135 | 0.118 |
| SKCM | 0.131 | 0.169 | 0.136 | 0.113 | 0.135 | 0.112 |
| LUAD | 0.171 | 0.173 | 0.151 | 0.127 | 0.143 | 0.107 |
| average | 0.219 | 0.204 | 0.164 | 0.154 | 0.152 | 0.138 |



Supplementary Figure 2: Relation num_slides and num_sig_genes. Each dot represents for a particular model and in particular cancer type, how many WSIs were available and how many genes were well-predicted. The dots are colored according to the model type, and a regression line is plotted across dots within each model type. Source data are provided in the Source Data File.

Supplementary Table 6: Effect of downsampling training dataset size of TCGA-BRCA on performance metrics for SEQUOIA, with num_genes: number of significantly predicted genes, med_r and std_r: median and standard deviation of correlation coefficient of top 1000 genes, med_rmse and std_rmse: median and standard deviation on normalized RMSE of top 1000 genes. The percentage indicates the fraction of the slides that were retained. Source data are provided in the Source Data File.

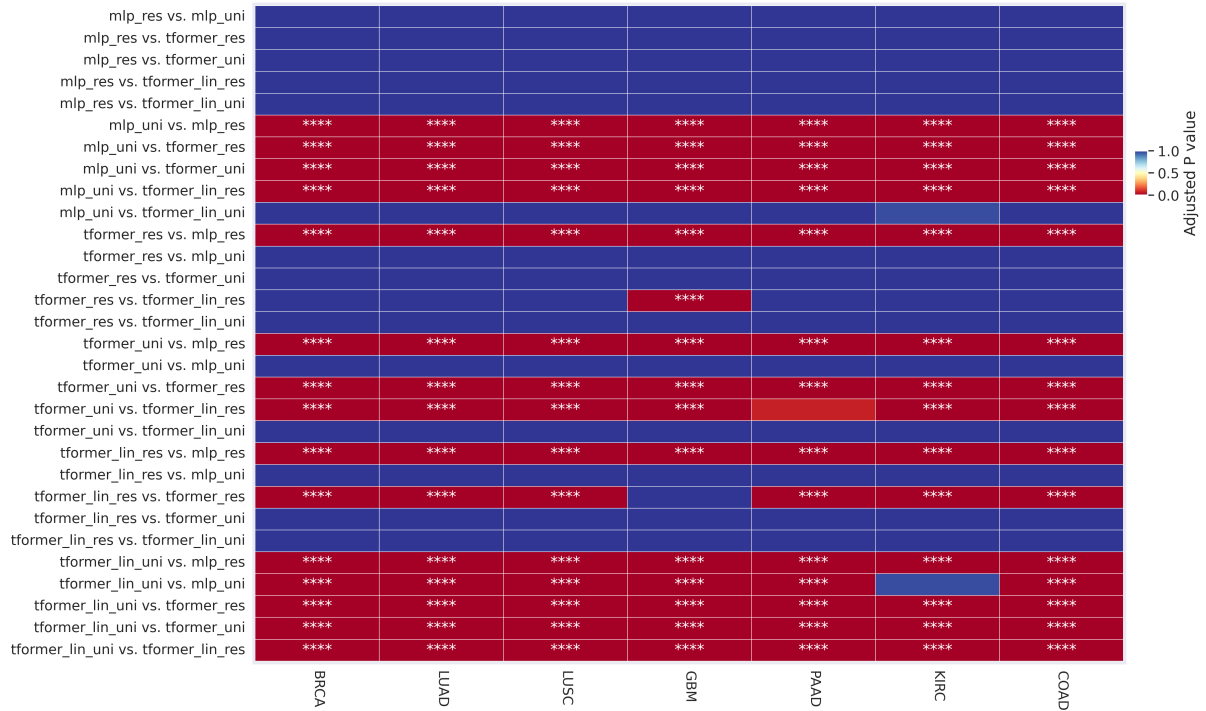
| | cancer | num_genes | med_r | std_r | med_rmse | std_rmse |
|---|----------|-----------|-------|-------|----------|----------|
| 0 | BRCA-40% | 15726 | 0.513 | 0.048 | 0.754 | 0.325 |
| 1 | BRCA-30% | 13684 | 0.509 | 0.054 | 0.776 | 0.345 |
| 2 | BRCA-20% | 9706 | 0.479 | 0.054 | 0.781 | 0.357 |

Supplementary Table 7: Median correlation coefficient of top 1000 genes in CPTAC. (top 1000 genes defined independently within each model and cancer type). Source data are provided in the Source Data File.

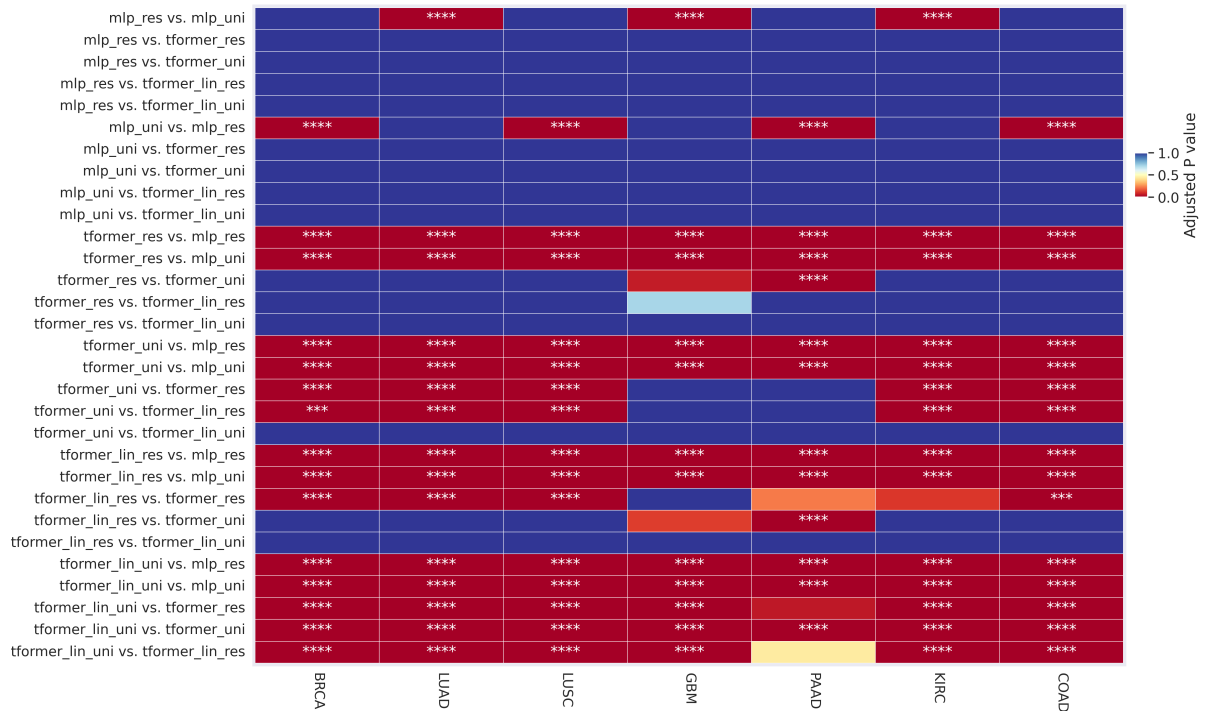
| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|--------------|---------|---------|-------------|-------------|-----------------|-----------------|
| BRCA | 0.466 | 0.596 | 0.544 | 0.582 | 0.562 | 0.636 |
| LUAD | 0.371 | 0.534 | 0.414 | 0.489 | 0.468 | 0.578 |
| LUSC (LSCC) | 0.235 | 0.509 | 0.316 | 0.490 | 0.343 | 0.543 |
| KIRC (CCRCC) | 0.233 | 0.528 | 0.337 | 0.471 | 0.370 | 0.526 |
| COAD | 0.262 | 0.426 | 0.272 | 0.379 | 0.326 | 0.499 |
| GBM | 0.210 | 0.403 | 0.254 | 0.294 | 0.237 | 0.422 |
| PAAD (PDA) | 0.194 | 0.249 | 0.203 | 0.221 | 0.221 | 0.321 |
| average | 0.282 | 0.464 | 0.334 | 0.418 | 0.361 | 0.504 |

Supplementary Table 8: Normalized RMSE of top 1000 genes in CPTAC. (top 1000 genes defined independently within each model and cancer type). Source data are provided in the Source Data File.

| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|--------------|---------|---------|-------------|-------------|-----------------|-----------------|
| PAAD (PDA) | 0.291 | 0.222 | 0.163 | 0.166 | 0.161 | 0.160 |
| LUAD | 0.190 | 0.215 | 0.178 | 0.167 | 0.171 | 0.150 |
| LUSC (LSCC) | 0.192 | 0.185 | 0.161 | 0.144 | 0.158 | 0.137 |
| KIRC (CCRCC) | 0.183 | 0.194 | 0.155 | 0.143 | 0.154 | 0.135 |
| BRCA | 0.179 | 0.163 | 0.147 | 0.138 | 0.141 | 0.131 |
| GBM | 0.215 | 0.242 | 0.124 | 0.126 | 0.125 | 0.116 |
| COAD | 0.152 | 0.147 | 0.132 | 0.122 | 0.130 | 0.114 |
| average | 0.200 | 0.195 | 0.151 | 0.144 | 0.149 | 0.135 |



Supplementary Figure 3: CPTAC evaluation (correlation coefficients). The table shows the P value calculated for each pairwise comparison of models using a one-sided Mann-Whitney U test for the hypothesis that values from model A are larger than values from model B. Legend: * for $p < 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$ and **** for $p \leq 0.0001$. Source data are provided in the Source Data File.



Supplementary Figure 4: CPTAC evaluation (normalized RMSE). The table shows the P value calculated for each pairwise comparison of models using a one-sided Mann-Whitney U test for the hypothesis that values from model A are smaller than values from model B. Legend: * for $p < 0.05$, ** for $p \leq 0.01$, *** for $p \leq 0.001$ and **** for $p \leq 0.0001$. Source data are provided in the Source Data File.

Supplementary Table 9: Number of genes that validate to both TCGA test set and CPTAC. Source data are provided in the Source Data File.

| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|---------|---------|---------|-------------|-------------|-----------------|-----------------|
| LUAD | 498 | 8,294 | 3,726 | 8,166 | 5,116 | 12,422 |
| KIRC | 0 | 6,899 | 2,532 | 8,698 | 3,937 | 10,477 |
| BRCA | 1,343 | 7,569 | 5,530 | 8,243 | 6,858 | 9,418 |
| LUSC | 0 | 6,128 | 780 | 5,914 | 2,029 | 8,610 |
| COAD | 140 | 1,944 | 242 | 2,798 | 1,159 | 5,784 |
| GBM | 9 | 1,039 | 94 | 495 | 90 | 1,816 |
| PAAD | 0 | 439 | 6 | 227 | 4 | 1,589 |
| average | 284 | 4,616 | 1,844 | 4,934 | 2,741 | 7,159 |

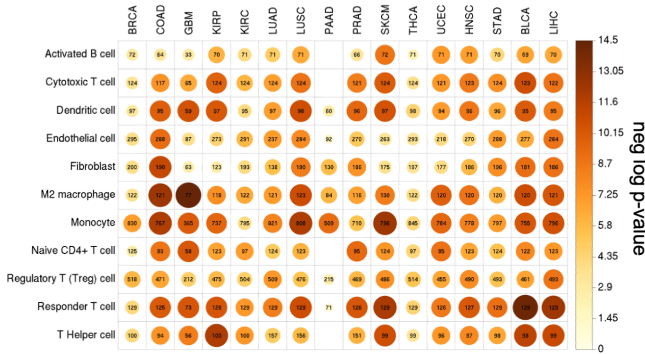
Supplementary Table 10: Median correlation coefficient of top 1000 genes for genes that validate to both TCGA test set and CPTAC (correlation coefficient from evaluation on CPTAC). Source data are provided in the Source Data File.

| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|---------|---------|---------|-------------|-------------|-----------------|-----------------|
| BRCA | 0.393 | 0.595 | 0.543 | 0.582 | 0.562 | 0.636 |
| LUAD | 0.333 | 0.531 | 0.406 | 0.488 | 0.463 | 0.578 |
| LUSC | 0.000 | 0.503 | 0.268 | 0.488 | 0.320 | 0.543 |
| KIRC | 0.000 | 0.525 | 0.308 | 0.470 | 0.356 | 0.525 |
| COAD | 0.245 | 0.391 | 0.260 | 0.365 | 0.298 | 0.498 |
| GBM | 0.245 | 0.333 | 0.254 | 0.271 | 0.258 | 0.391 |
| PAAD | 0.000 | 0.225 | 0.214 | 0.223 | 0.216 | 0.274 |
| average | 0.174 | 0.443 | 0.322 | 0.412 | 0.353 | 0.492 |

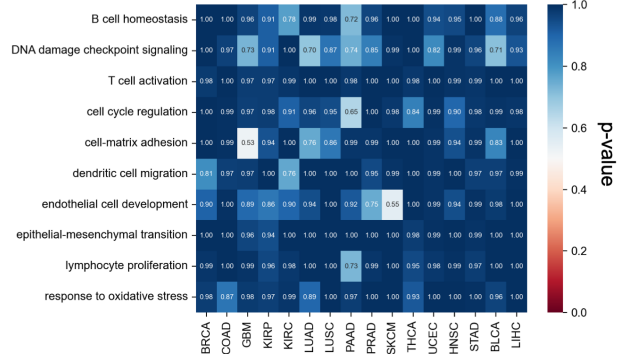
Supplementary Table 11: Normalized RMSE of top 1000 genes for genes that validate to both TCGA test set and CPTAC. (RMSE from evaluation on CPTAC). Source data are provided in the Source Data File.

| | mlp_res | mlp_uni | tformer_res | tformer_uni | tformer_lin_res | tformer_lin_uni |
|---------|---------|---------|-------------|-------------|-----------------|-----------------|
| PAAD | 0.000 | 0.859 | 0.555 | 0.608 | 0.414 | 0.296 |
| GBM | 1.000 | 0.985 | 0.573 | 0.430 | 0.485 | 0.195 |
| LUAD | 0.395 | 0.236 | 0.206 | 0.175 | 0.191 | 0.152 |
| LUSC | 0.000 | 0.231 | 0.405 | 0.160 | 0.244 | 0.144 |
| KIRC | 0.000 | 0.224 | 0.214 | 0.152 | 0.190 | 0.139 |
| BRCA | 0.322 | 0.174 | 0.160 | 0.141 | 0.147 | 0.133 |
| COAD | 0.401 | 0.288 | 0.420 | 0.170 | 0.264 | 0.132 |
| average | 0.303 | 0.428 | 0.362 | 0.262 | 0.277 | 0.170 |

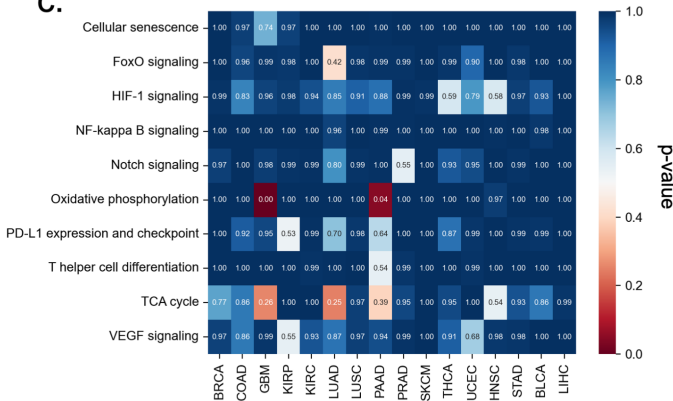
a. Cell-type signature



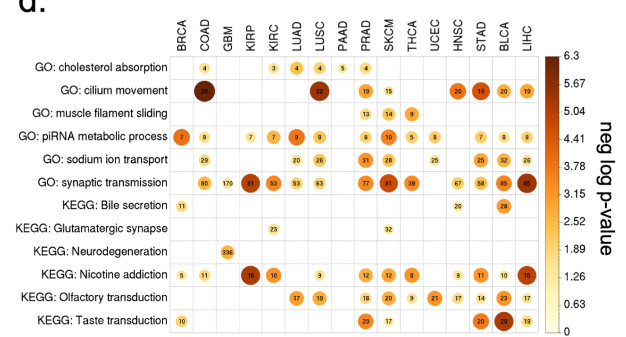
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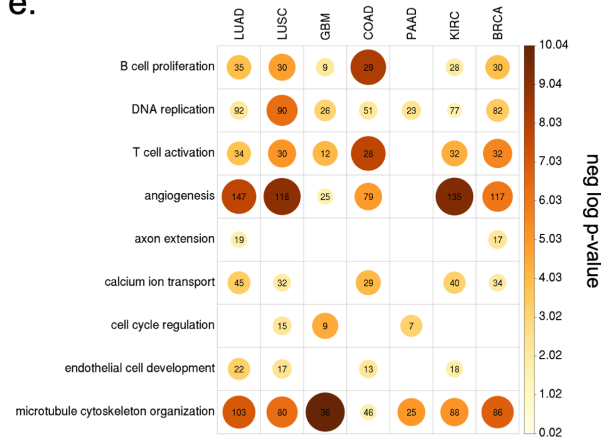
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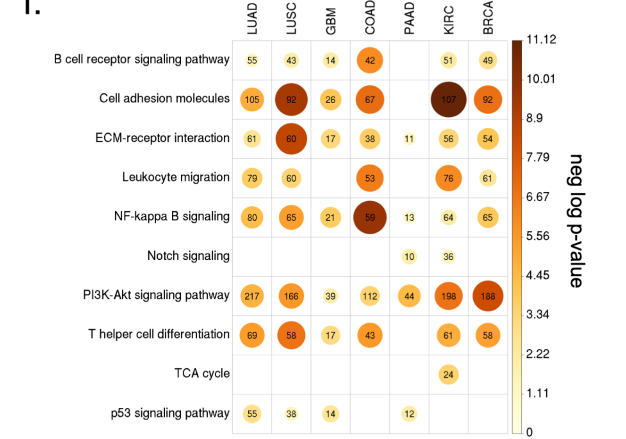
d.



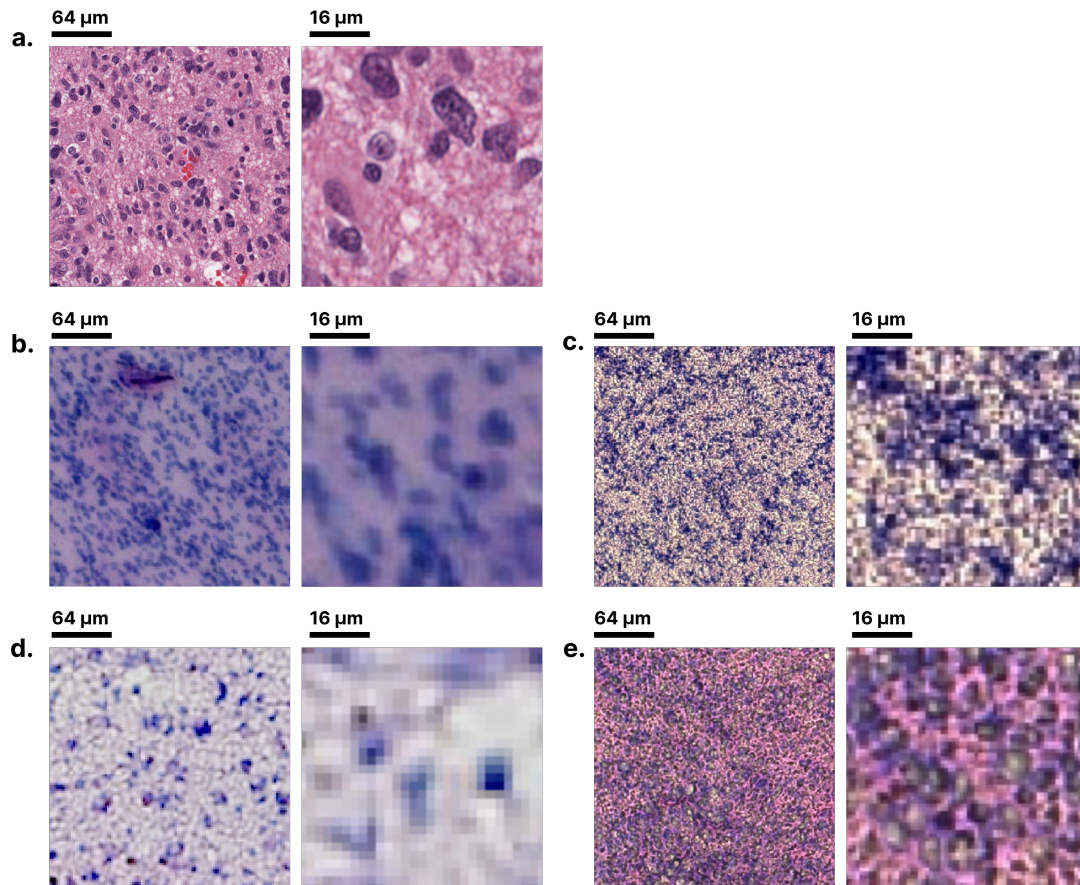
e.



f.



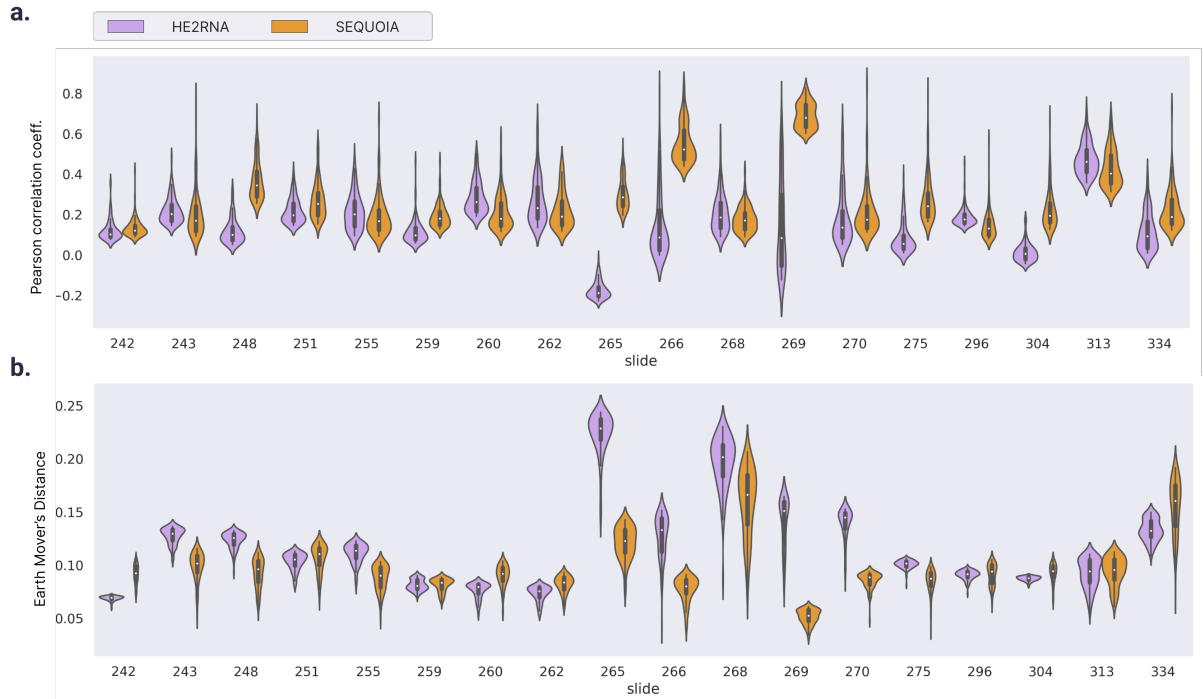
Supplementary Figure 5: Characterization of the well-predicted genes. a) Heatmap showing the significant P values for cell-type signatures enriched with the well-predicted genes. Color and size of the circles represent the negative log-transformed P values. Integers represent the absolute gene count in each category, and non-significant categories are left in blank. b-c) P values from the gene-set enrichment analysis of the inaccurately predicted genes using the gene sets from b) gene ontology and c) KEGG. d) Heatmap showing the P values for gene sets enriched with the inaccurately predicted genes across cancer types. Color and size of the circles represent the negative log-transformed P values. Integers represent the absolute gene count in each category, and non-significant categories are left in blank. e-f) Heatmaps showing the significant P values for pathways enriched with well-predicted genes in both the TCGA and CPTAC datasets using the e) gene ontology and f) KEGG pathways. Source data for all panels are provided in the Source Data File.



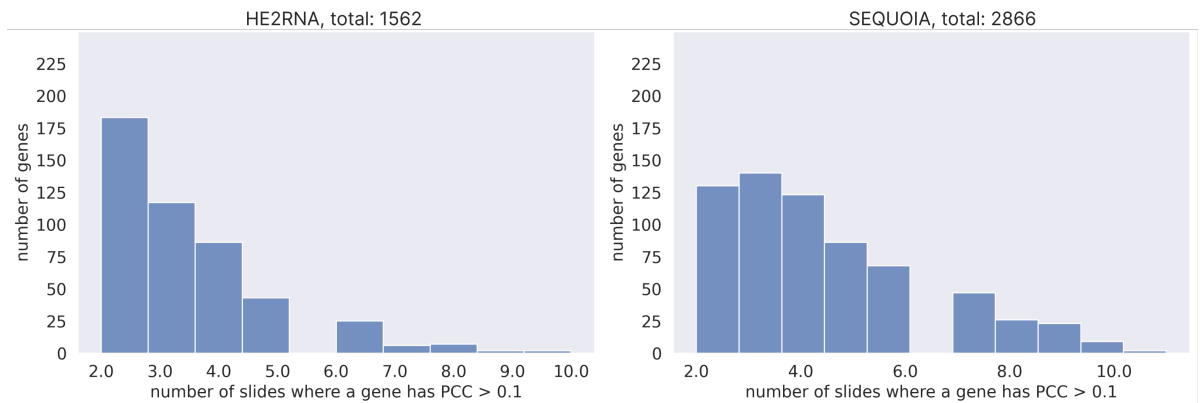
Supplementary Figure 6: Comparison of H&E quality of TCGA-GBM slides to slides from spatial GBM dataset. a) Example of quality of TCGA-GBM slide, with more zoomed-in version on the right. b-e) Four examples of quality from spatial GBM slides.

Supplementary Table 12: median EMD and Pearson correlation coefficient for top 100 genes for each model on the spatial GBM dataset. Source data are provided in the Source Data File.

| | emd_HE2RNA | emd_SEQUOIA | | corr_HE2RNA | corr_SEQUOIA |
|---------|------------|-------------|---------|-------------|--------------|
| 268 | 0.202 | 0.166 | 269 | 0.084 | 0.678 |
| 334 | 0.132 | 0.160 | 266 | 0.086 | 0.522 |
| 265 | 0.229 | 0.123 | 313 | 0.461 | 0.402 |
| 251 | 0.105 | 0.110 | 248 | 0.100 | 0.344 |
| 243 | 0.130 | 0.101 | 265 | -0.189 | 0.285 |
| 248 | 0.125 | 0.096 | 251 | 0.196 | 0.253 |
| 313 | 0.094 | 0.095 | 275 | 0.055 | 0.241 |
| 304 | 0.087 | 0.094 | 304 | 0.006 | 0.194 |
| 296 | 0.091 | 0.094 | 334 | 0.092 | 0.189 |
| 242 | 0.069 | 0.092 | 262 | 0.232 | 0.189 |
| 260 | 0.079 | 0.092 | 259 | 0.097 | 0.180 |
| 255 | 0.113 | 0.090 | 260 | 0.261 | 0.178 |
| 270 | 0.145 | 0.088 | 270 | 0.135 | 0.174 |
| 275 | 0.101 | 0.087 | 268 | 0.186 | 0.171 |
| 262 | 0.075 | 0.083 | 243 | 0.202 | 0.170 |
| 259 | 0.080 | 0.083 | 255 | 0.201 | 0.167 |
| 266 | 0.133 | 0.080 | 296 | 0.175 | 0.131 |
| 269 | 0.151 | 0.052 | 242 | 0.101 | 0.121 |
| average | 0.119 | 0.099 | average | 0.138 | 0.255 |



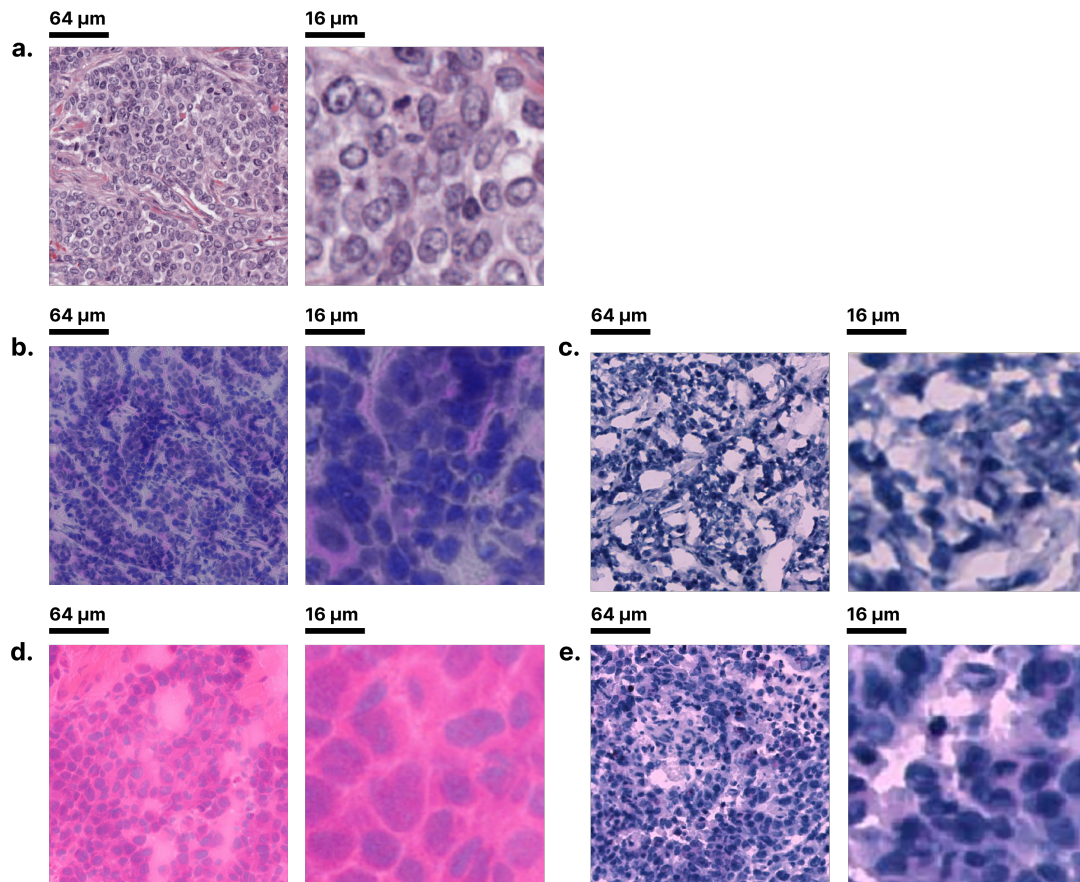
Supplementary Figure 7: Performance for gene expression prediction of the spatial GBM dataset. a) Distribution of Pearson correlation coefficients for the top 100 most accurately predicted genes with highest correlation coefficients obtained from each model. b) Distribution of EMD values for the top 100 most accurately predicted genes with lowest EMD values obtained from each model. Source data are provided in the Source Data File.



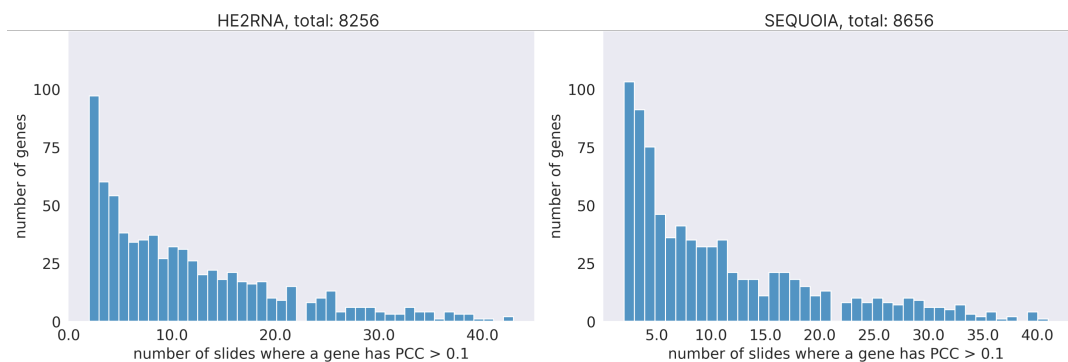
Supplementary Figure 8: Predictability of genes across > 2 slides in the spatial GBM cohort. Source data are provided in the Source Data File.

| gene | N | gene | N | gene | N | gene | N | gene | N |
|----------|----|----------|---|---------|---|------------|---|----------|---|
| PDIA4 | 11 | CSRP1 | 8 | HNMT | 7 | EPC1 | 6 | KIF2C | 5 |
| GALNT2 | 11 | INTS3 | 8 | TRIP6 | 7 | SOX4 | 6 | CADM2 | 5 |
| CTSH | 10 | BHLHE41 | 8 | CYBRD1 | 7 | PABPN1 | 6 | FZD7 | 5 |
| EXOSC10 | 10 | PLOD1 | 8 | IL13RA1 | 6 | MEX3A | 6 | PRR3 | 5 |
| S100A13 | 10 | CAVIN1 | 8 | FNDC3B | 6 | CTSB | 6 | SERPING1 | 5 |
| PLXDC2 | 10 | ACIN1 | 8 | ZNF711 | 6 | PHACTR3 | 6 | AMOTL2 | 5 |
| LMNA | 10 | HNRNPH1 | 8 | PCGF2 | 6 | EHD2 | 6 | EMP1 | 5 |
| PDLIM7 | 10 | STOM | 8 | LYN | 6 | LITAF | 6 | FOXK2 | 5 |
| PTBP1 | 10 | HLA-DRB1 | 7 | PHACTR4 | 6 | HES6 | 6 | NKIRAS2 | 5 |
| DVL2 | 10 | PTPRC | 7 | ATL1 | 6 | FAM102B | 6 | SMARCB1 | 5 |
| FCGBP | 10 | HLA-DPB1 | 7 | DKC1 | 6 | CASP7 | 6 | ELOVL2 | 5 |
| ALDH9A1 | 9 | MESD | 7 | TMEM248 | 6 | EZH2 | 6 | AIFM2 | 5 |
| COLGALT1 | 9 | FKBP10 | 7 | NBPF15 | 6 | IL4R | 6 | ATAD3B | 5 |
| LPCAT2 | 9 | HLA-DQB1 | 7 | GMPS | 6 | CYSTM1 | 6 | DHPS | 5 |
| ALDOC | 9 | ZNF274 | 7 | S100A16 | 6 | MYO1C | 6 | HEBP1 | 5 |
| TUBB | 9 | LAIR1 | 7 | BCL3 | 6 | TRIP10 | 6 | PIPOX | 5 |
| FAM3C | 9 | PIK3R1 | 7 | CHSY1 | 6 | DPYSL4 | 6 | RNF135 | 5 |
| TRIM24 | 9 | ARHGEF2 | 7 | NEIL2 | 6 | GALNT15 | 6 | RIN1 | 5 |
| TCF12 | 9 | PHF19 | 7 | RIN3 | 6 | TRO | 6 | ENPP4 | 5 |
| PLOD3 | 9 | MTA1 | 7 | PTGDS | 6 | SOX11 | 5 | KANK2 | 5 |
| FGFRL1 | 9 | RIN2 | 7 | B3GNT9 | 6 | RBM14 | 5 | SOX6 | 5 |
| COL6A1 | 9 | EFNB2 | 7 | IQGAP1 | 6 | TNR | 5 | FMNL1 | 5 |
| SERPINH1 | 9 | FAM20C | 7 | FGF1 | 6 | PDPN | 5 | PTK7 | 5 |
| TGFBI | 9 | MLLT11 | 7 | ADD3 | 6 | DPYD | 5 | ACSL3 | 5 |
| TENT5A | 9 | ACSL4 | 7 | ANXA1 | 6 | HNRNPD | 5 | HLA-DQA1 | 5 |
| TFDP2 | 9 | P4HB | 7 | CDCA5 | 6 | ARHGAP18 | 5 | PTPN18 | 5 |
| LAMC1 | 9 | ATL3 | 7 | SPOCD1 | 6 | SAT1 | 5 | STRIP1 | 5 |
| FXYD5 | 9 | CAPRIN2 | 7 | NCALD | 6 | F13A1 | 5 | GPR173 | 5 |
| C1R | 9 | BIN1 | 7 | DNAJC9 | 6 | CLU | 5 | FCHSD2 | 5 |
| SLC4A7 | 9 | VEZF1 | 7 | FUBP1 | 6 | SLC25A37 | 5 | LIG3 | 5 |
| RAB3GAP2 | 9 | P3H1 | 7 | PLCXD1 | 6 | ELAVL2 | 5 | TUT4 | 5 |
| VSIR | 9 | CPQ | 7 | FZD1 | 6 | RELB | 5 | XPO5 | 5 |
| CREBL2 | 9 | CAMK2D | 7 | AP3M1 | 6 | MFSD1 | 5 | POLD4 | 5 |
| GJA1 | 9 | ELK3 | 7 | SPRY2 | 6 | TNFAIP2 | 5 | RETREG1 | 5 |
| AKAP8 | 8 | LAMB2 | 7 | RPS6KA5 | 6 | MARCKSL1 | 5 | ATP6AP2 | 5 |
| CBX4 | 8 | THRA | 7 | LILRB4 | 6 | PTPN9 | 5 | RSBN1L | 5 |
| PCOLCE | 8 | TLR4 | 7 | RNF144A | 6 | TAGLN2 | 5 | NRP1 | 5 |
| FAM171B | 8 | CTSZ | 7 | SYTL2 | 6 | ACAP3 | 5 | SHROOM2 | 5 |
| PPP1R1B | 8 | PROSER1 | 7 | TRIM28 | 6 | CSNK2A1 | 5 | CYB5B | 5 |
| SMPD4 | 8 | SLC16A3 | 7 | RNPS1 | 6 | SNRPD3 | 5 | PPP1R3B | 5 |
| LOXL2 | 8 | PCGF3 | 7 | ANXA4 | 6 | GLIPR1 | 5 | CDC5L | 5 |
| DAZAP1 | 8 | NPEPL1 | 7 | INTS11 | 6 | PLEKHB1 | 5 | CEBPD | 5 |
| MMP14 | 8 | ATP6V1A | 7 | SPRED2 | 6 | PYGL | 5 | ANXA2 | 5 |
| ELAVL1 | 8 | OSBPL3 | 7 | MCM7 | 6 | TRADD | 5 | SIX5 | 5 |
| SFPQ | 8 | SLC12A7 | 7 | SRPK1 | 6 | SLC44A1 | 5 | ETV5 | 5 |
| PLS3 | 8 | ZYX | 7 | LRRC8D | 6 | AC018647.1 | 5 | CHURC1 | 5 |
| KPNA2 | 8 | RICTOR | 7 | SDC4 | 6 | SNX32 | 5 | CD163 | 5 |
| UGDH | 8 | RMND5A | 7 | SUCLG2 | 6 | ABHD15 | 5 | CHI3L1 | 5 |
| MYD88 | 8 | DPP10 | 7 | TJAP1 | 6 | SLA | 5 | BHLHE40 | 5 |
| TWSG1 | 8 | COL6A2 | 7 | ALCAM | 6 | KIF21B | 5 | C1RL | 5 |
| FURIN | 8 | B3GNT2 | 7 | SHOC2 | 6 | AQP1 | 5 | SETDB1 | 5 |
| NONO | 8 | TBXAS1 | 7 | MICALL2 | 6 | RAB40B | 5 | CHI3L2 | 5 |
| | | gene | N | | | | | | |
| | | S100A4 | 5 | | | | | | |

Supplementary Table 13: Genes that have $PCC > 0.1$ in N slides in the spatial GBM cohort. Only shown for $N \geq 5$. Source data are provided in the Source Data File.



Supplementary Figure 9: Comparison H&E quality of TCGA-BRCA slides to slides from spatial BRCA dataset. a) Example of quality of TCGA-BRCA slide, with more zoomed-in version on the right. b-e) Four examples of quality from spatial BRCA slides.



Supplementary Figure 10: Predictability of genes across > 2 slides in the spatial BRCA cohort. Source data are provided in the Source Data File.

Supplementary Table 14: median PCC for top 100 genes for HE2RNA ('HE2') versus SEQUOIA ('SEQ') on the spatial BRCA dataset. Source data are provided in the Source Data File.

| study | corr_HE2 | corr_SEQ | | corr_HE2 | corr_SEQ | | corr_HE2 | corr_SEQ |
|--------|----------|----------|--------|----------|----------|---------|----------|----------|
| SPA145 | 0.507 | 0.512 | SPA114 | 0.146 | 0.156 | | | |
| SPA143 | 0.507 | 0.496 | SPA75 | 0.268 | 0.150 | | | |
| SPA146 | 0.517 | 0.485 | SPA95 | 0.100 | 0.149 | SPA54 | 0.124 | 0.114 |
| SPA148 | 0.508 | 0.456 | SPA76 | 0.189 | 0.148 | SPA88 | 0.047 | 0.112 |
| SPA134 | 0.368 | 0.373 | SPA92 | 0.148 | 0.147 | SPA106 | 0.101 | 0.112 |
| TENX53 | 0.301 | 0.362 | SPA58 | 0.048 | 0.146 | SPA85 | 0.150 | 0.110 |
| SPA142 | 0.399 | 0.346 | SPA70 | 0.110 | 0.141 | SPA65 | 0.108 | 0.109 |
| SPA140 | 0.440 | 0.341 | SPA94 | 0.114 | 0.140 | SPA109 | 0.040 | 0.109 |
| TENX13 | 0.182 | 0.317 | SPA67 | 0.156 | 0.139 | SPA113 | 0.152 | 0.108 |
| TENX39 | 0.254 | 0.301 | SPA62 | 0.063 | 0.136 | SPA105 | 0.063 | 0.105 |
| TENX14 | 0.273 | 0.289 | SPA68 | 0.135 | 0.135 | SPA84 | 0.069 | 0.102 |
| SPA138 | 0.403 | 0.286 | SPA100 | 0.109 | 0.134 | SPA72 | 0.081 | 0.099 |
| TENX68 | 0.313 | 0.285 | SPA121 | 0.088 | 0.134 | SPA108 | 0.112 | 0.099 |
| SPA117 | -0.041 | 0.281 | SPA91 | 0.114 | 0.133 | SPA89 | 0.095 | 0.099 |
| SPA133 | 0.318 | 0.280 | SPA55 | 0.109 | 0.131 | SPA118 | 0.137 | 0.098 |
| SPA135 | 0.191 | 0.272 | SPA56 | 0.097 | 0.131 | SPA86 | 0.054 | 0.095 |
| SPA60 | 0.292 | 0.270 | SPA126 | 0.107 | 0.131 | SPA64 | 0.058 | 0.093 |
| SPA139 | 0.406 | 0.257 | SPA90 | 0.118 | 0.129 | SPA96 | 0.139 | 0.089 |
| SPA149 | -0.056 | 0.248 | SPA127 | 0.111 | 0.128 | SPA57 | 0.072 | 0.086 |
| SPA141 | 0.389 | 0.221 | SPA101 | 0.113 | 0.127 | SPA122 | 0.130 | 0.085 |
| SPA111 | 0.032 | 0.206 | SPA87 | 0.058 | 0.127 | SPA119 | 0.133 | 0.084 |
| SPA115 | 0.091 | 0.198 | SPA69 | 0.087 | 0.124 | SPA130 | 0.075 | 0.082 |
| SPA61 | 0.180 | 0.187 | SPA73 | 0.108 | 0.124 | SPA93 | 0.144 | 0.081 |
| SPA116 | 0.021 | 0.187 | SPA98 | 0.168 | 0.122 | SPA110 | 0.119 | 0.075 |
| SPA102 | 0.198 | 0.183 | SPA79 | 0.066 | 0.121 | SPA63 | 0.086 | 0.074 |
| SPA53 | 0.121 | 0.182 | SPA51 | 0.069 | 0.120 | SPA104 | 0.063 | 0.071 |
| SPA128 | 0.160 | 0.181 | SPA129 | 0.118 | 0.119 | SPA59 | 0.075 | 0.052 |
| SPA131 | 0.137 | 0.176 | SPA77 | 0.174 | 0.117 | SPA83 | -0.003 | 0.046 |
| SPA151 | 0.221 | 0.167 | SPA123 | 0.046 | 0.116 | SPA82 | -0.008 | 0.045 |
| SPA107 | 0.074 | 0.162 | SPA154 | 0.103 | 0.116 | SPA81 | -0.043 | 0.027 |
| SPA152 | 0.138 | 0.161 | SPA124 | 0.137 | 0.116 | average | 0.154 | 0.168 |
| SPA153 | 0.248 | 0.160 | SPA125 | 0.158 | 0.116 | | | |

Supplementary Table 15: median EMD for top 100 genes for HE2RNA ('HE2') versus SEQUOIA ('SEQ') on the spatial BRCA dataset. Source data are provided in the Source Data File.

| | emd_HE2 | emd_SEQ | | emd_HE2 | emd_SEQ | | emd_HE2 | emd_SEQ |
|--------|---------|---------|--------|---------|---------|---------|---------|---------|
| SPA129 | 0.098 | 0.140 | SPA119 | 0.161 | 0.111 | | | |
| SPA117 | 0.224 | 0.139 | SPA84 | 0.133 | 0.110 | | | |
| SPA61 | 0.169 | 0.135 | SPA64 | 0.104 | 0.110 | SPA135 | 0.105 | 0.091 |
| SPA128 | 0.111 | 0.133 | SPA93 | 0.159 | 0.110 | SPA51 | 0.085 | 0.090 |
| SPA89 | 0.097 | 0.132 | SPA75 | 0.096 | 0.109 | TENX68 | 0.125 | 0.089 |
| SPA102 | 0.111 | 0.132 | SPA151 | 0.122 | 0.109 | SPA55 | 0.102 | 0.089 |
| SPA109 | 0.119 | 0.129 | SPA108 | 0.087 | 0.109 | SPA138 | 0.107 | 0.089 |
| SPA131 | 0.105 | 0.128 | SPA60 | 0.158 | 0.108 | SPA133 | 0.088 | 0.089 |
| SPA154 | 0.162 | 0.127 | SPA121 | 0.180 | 0.107 | SPA122 | 0.125 | 0.089 |
| SPA118 | 0.168 | 0.126 | SPA110 | 0.094 | 0.105 | SPA145 | 0.107 | 0.088 |
| SPA116 | 0.203 | 0.123 | SPA95 | 0.180 | 0.105 | SPA96 | 0.124 | 0.088 |
| SPA87 | 0.100 | 0.123 | SPA105 | 0.153 | 0.105 | SPA57 | 0.106 | 0.087 |
| SPA113 | 0.110 | 0.123 | SPA91 | 0.140 | 0.105 | SPA54 | 0.095 | 0.087 |
| SPA86 | 0.128 | 0.122 | SPA142 | 0.129 | 0.103 | SPA53 | 0.115 | 0.086 |
| SPA130 | 0.105 | 0.122 | SPA81 | 0.141 | 0.102 | SPA56 | 0.085 | 0.085 |
| SPA94 | 0.170 | 0.122 | SPA139 | 0.092 | 0.100 | SPA127 | 0.117 | 0.084 |
| SPA111 | 0.105 | 0.120 | SPA148 | 0.113 | 0.100 | SPA68 | 0.109 | 0.084 |
| SPA88 | 0.115 | 0.119 | SPA79 | 0.148 | 0.099 | TENX53 | 0.100 | 0.084 |
| SPA104 | 0.106 | 0.119 | SPA146 | 0.100 | 0.098 | SPA69 | 0.075 | 0.084 |
| SPA62 | 0.150 | 0.118 | SPA76 | 0.114 | 0.097 | SPA124 | 0.129 | 0.083 |
| SPA72 | 0.131 | 0.117 | SPA63 | 0.093 | 0.097 | SPA70 | 0.083 | 0.083 |
| SPA114 | 0.112 | 0.117 | SPA58 | 0.092 | 0.097 | SPA125 | 0.085 | 0.083 |
| SPA92 | 0.125 | 0.116 | SPA90 | 0.146 | 0.097 | SPA101 | 0.118 | 0.083 |
| SPA141 | 0.124 | 0.115 | SPA77 | 0.116 | 0.096 | SPA98 | 0.127 | 0.082 |
| SPA152 | 0.147 | 0.115 | SPA153 | 0.136 | 0.095 | SPA123 | 0.137 | 0.079 |
| SPA65 | 0.089 | 0.114 | SPA149 | 0.198 | 0.095 | SPA126 | 0.088 | 0.077 |
| SPA107 | 0.139 | 0.113 | SPA67 | 0.118 | 0.094 | TENX13 | 0.107 | 0.077 |
| SPA106 | 0.153 | 0.113 | SPA73 | 0.129 | 0.093 | TENX39 | 0.085 | 0.077 |
| SPA115 | 0.108 | 0.112 | SPA85 | 0.129 | 0.093 | SPA100 | 0.087 | 0.077 |
| SPA83 | 0.124 | 0.112 | SPA143 | 0.106 | 0.092 | TENX14 | 0.100 | 0.075 |
| SPA82 | 0.138 | 0.112 | SPA134 | 0.102 | 0.092 | average | 0.121 | 0.103 |
| SPA59 | 0.119 | 0.111 | SPA140 | 0.104 | 0.091 | | | |

| gene | N | gene | N | gene | N | gene | N | gene | N |
|----------|----|---------|----|---------|----|----------|----|---------|----|
| YWHAZ | 41 | COL1A2 | 32 | CD6 | 28 | ZNF703 | 25 | TSPAN13 | 22 |
| DCN | 40 | ILF2 | 32 | CD79A | 28 | GATA3 | 25 | ENO1 | 22 |
| HLA-DPB1 | 40 | ERBB3 | 31 | CST3 | 28 | CSTB | 25 | YBX1 | 22 |
| CYB5R1 | 40 | TUBB | 31 | LTB | 28 | RRM2 | 25 | TIMP2 | 21 |
| TMSB10 | 40 | LIMD2 | 31 | SLC44A4 | 28 | SNRPG | 24 | CD7 | 21 |
| AEBP1 | 38 | SDC1 | 31 | SF3B3 | 28 | ZG16B | 24 | RBM17 | 21 |
| NASP | 38 | STMN1 | 31 | IL32 | 27 | AGR2 | 24 | MSN | 21 |
| HDGF | 37 | HTRA1 | 31 | HLA-DMB | 27 | AR | 24 | TAP2 | 21 |
| PCNA | 36 | IGFBP4 | 30 | GAPDH | 27 | HMGB2 | 24 | TUBA1C | 21 |
| LRP1 | 36 | SOD2 | 30 | CD37 | 27 | C1S | 24 | MCM3 | 21 |
| TPI1 | 36 | LAMB2 | 30 | SERBP1 | 27 | IVD | 24 | SFT2D2 | 21 |
| COL1A1 | 36 | CCL5 | 30 | TLE3 | 27 | REEP5 | 24 | LRRC32 | 21 |
| HMGA1 | 35 | HSPD1 | 30 | COTL1 | 27 | MMP2 | 23 | RPS7 | 21 |
| LUM | 35 | C1R | 30 | TMPO | 26 | TOP2A | 23 | TMEM119 | 21 |
| SF3B6 | 34 | JAK3 | 29 | TSC22D3 | 26 | ITGB5 | 23 | CXXC5 | 21 |
| MCM7 | 34 | EVL | 29 | RHOB | 26 | LDHB | 23 | PIM2 | 21 |
| CCT5 | 34 | CORO1A | 29 | POSTN | 26 | HLA-DQA1 | 23 | CDT1 | 20 |
| UQCRH | 33 | MRPS5 | 29 | ANTXR1 | 26 | CDC123 | 23 | RANBP1 | 20 |
| COL3A1 | 33 | KDM4B | 29 | TFF3 | 26 | RNF166 | 23 | PFKP | 20 |
| MAGED2 | 33 | CEBPB | 29 | HID1 | 26 | ARSG | 23 | PSMB2 | 20 |
| FAM174B | 33 | GSTP1 | 29 | DEK | 26 | CD27 | 23 | PAICS | 20 |
| ALG3 | 33 | PLOD1 | 29 | LOXL1 | 25 | SLC39A6 | 23 | BTG2 | 20 |
| XBP1 | 33 | EMILIN1 | 29 | SNRPD1 | 25 | KLF2 | 22 | TPX2 | 20 |
| CIRBP | 33 | WFS1 | 28 | MCM6 | 25 | TMEM123 | 22 | CDC25B | 20 |
| SPARC | 32 | EHD2 | 28 | CTSC | 25 | DCAF13 | 22 | RRP1 | 20 |
| SERPINF1 | 32 | COL5A1 | 28 | BIRC5 | 25 | MRPL37 | 22 | FOXM1 | 20 |
| MXRA8 | 32 | COL6A3 | 28 | KPNA2 | 25 | MLPH | 22 | MYBL2 | 20 |

Supplementary Table 16: Genes that have $PCC > 0.1$ in N slides in the spatial BRCA cohort. Only shown for $N \geq 20$. Source data are provided in the Source Data File.