# Supplementary Figures and Tables

## Radiosensitivity index emerges as a potential biomarker for combined radiotherapy and immunotherapy

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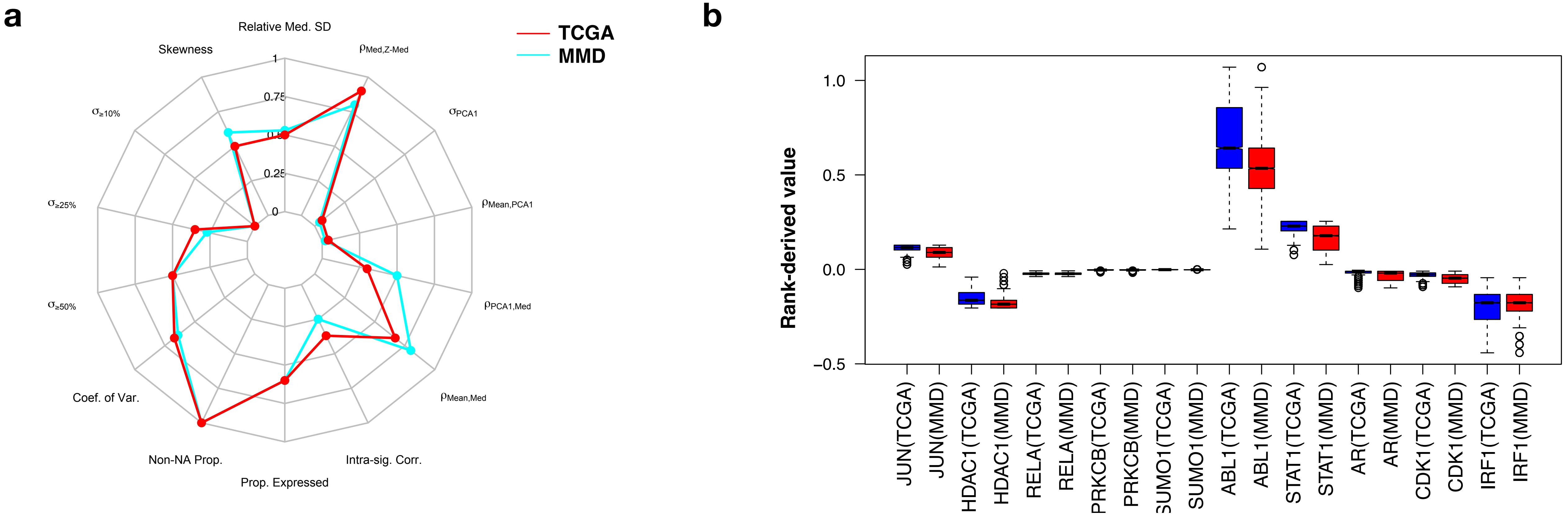
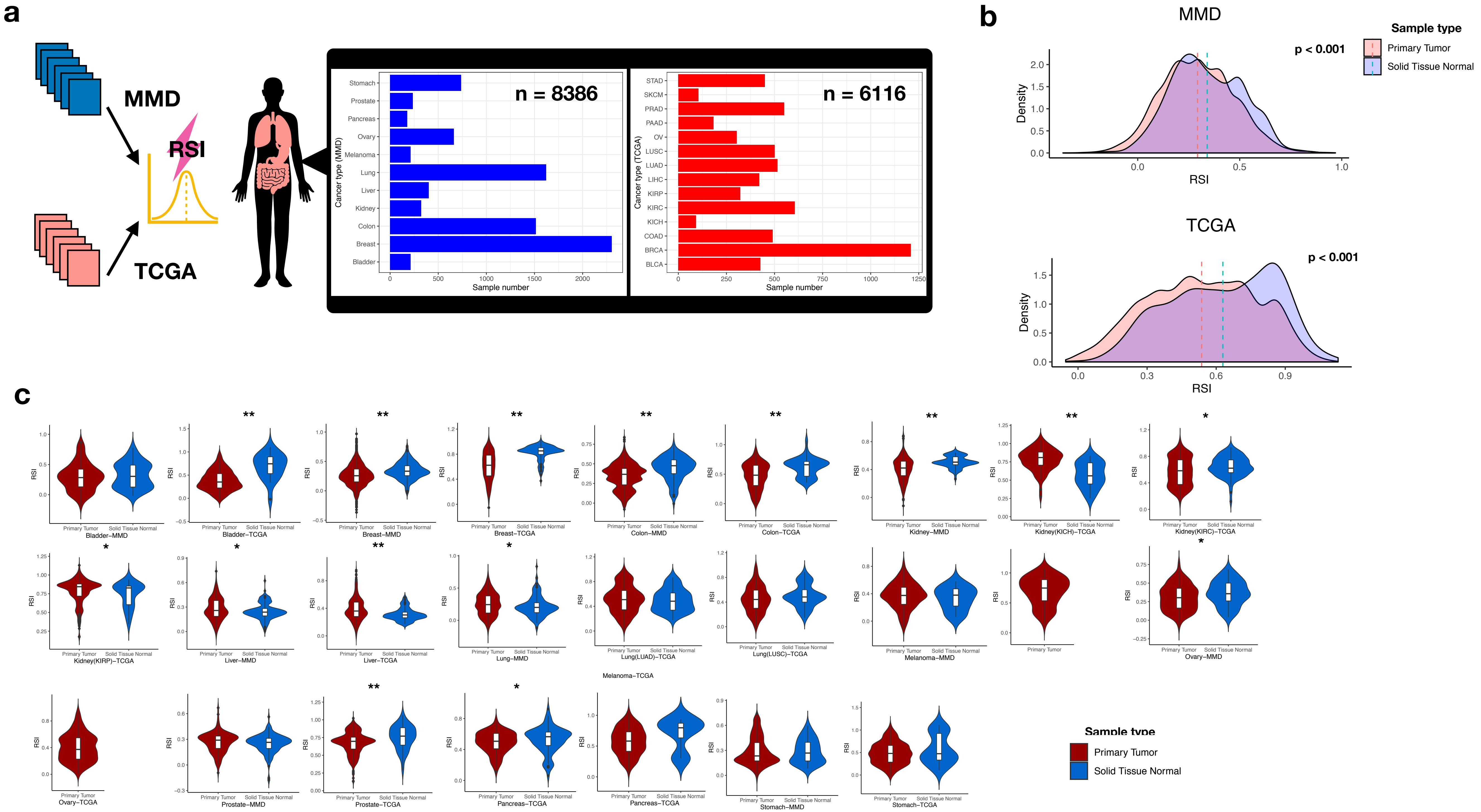
# Supplementary Figure 1



**Supplementary Figure 1: Signature quality control in TCGA and MMD.**

**a** Radar plot produced from sigQC. Each ray of the radar plot represents one of the summary metrics for RSI as a gene signature. **b** Distribution of each rank-transformed RSI genes in TCGA and MMD. The box hinges represent the 1st and 3rd quartiles, while the middle black horizontal lines indicate the median.

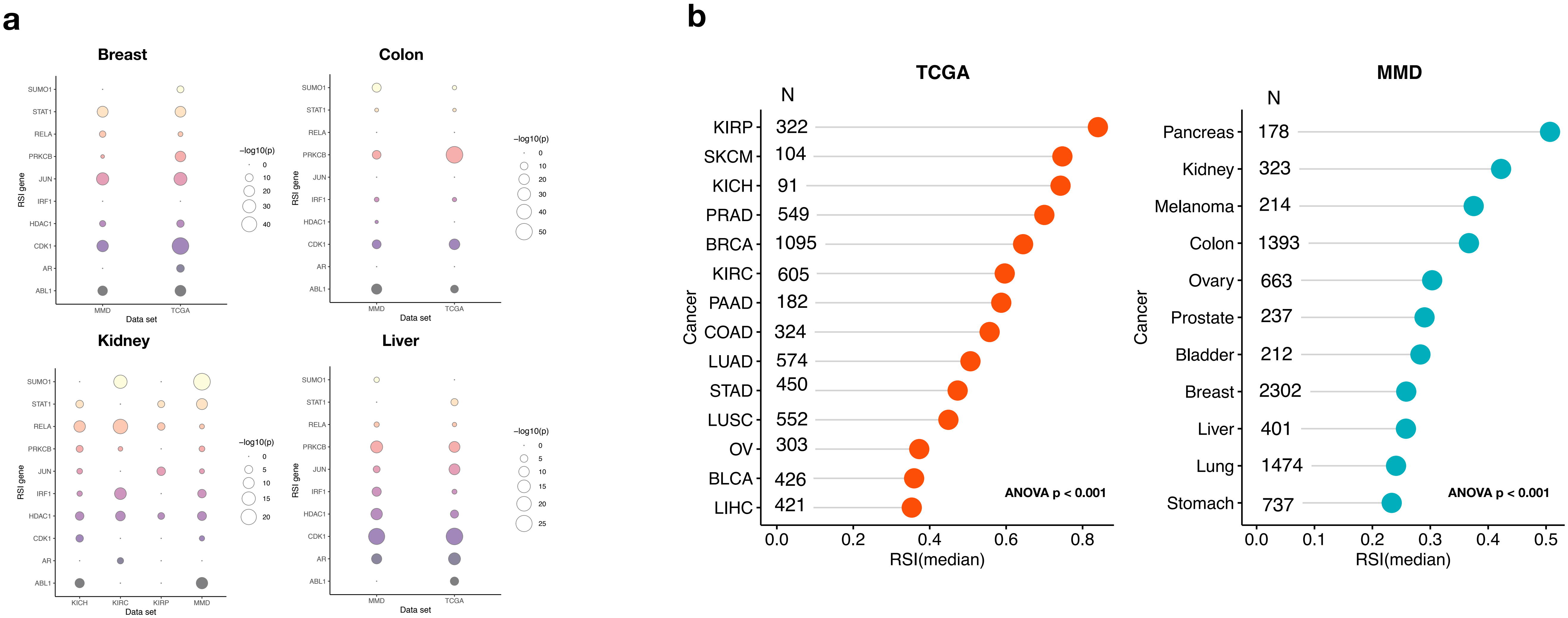
# Supplementary Figure 2



**Supplementary Figure 2: Variation of RSI in 11 major cancer types.**

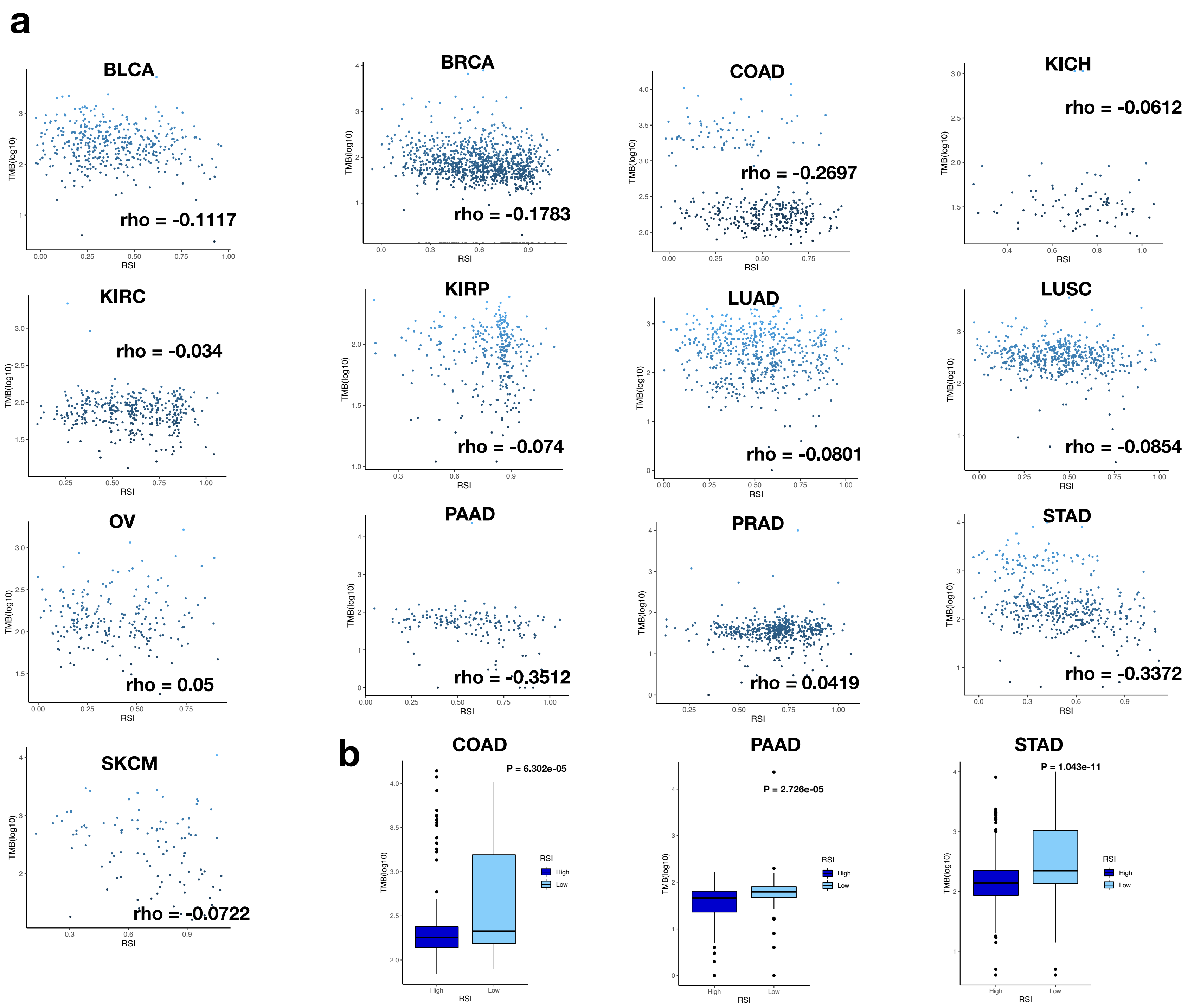
**a** Illustration of datasets used for characterization of RSI. **b** Density plot analyses depicting RSI distribution in tumour (MMD: n = 7286, TCGA: n = 5546 ) vs. normal tissues (MMD: n = 1100, TCGA: n = 570). Red and green horizontal dotted lines represent the mean RSI values in the primary tumour and normal tissues, respectively. **c** RSI in normal (solid normal tissue) vs. tumour (primary tumour) tissues across 11 cancer types. The black horizontal lines represent the median. Mann–Whitney U-test $***p < 0.001$, $**p < 0.01$, $*p < 0.05$. As melanoma cohort in TCGA does not contain normal tissues, only the tumour tissues are shown.

# Supplementary Figure 3



**Supplementary Figure 3: Differences of RSI between tumour and normal tissues and across cancer types. a** RSI gene differences between normal and tumour tissues. p value derived from the Mann–Whitney U-test is transformed to -log10(p). **b** Ranking of median RSI value across the 11 cancer types in TCGA (left panel) and MMD (right panel).

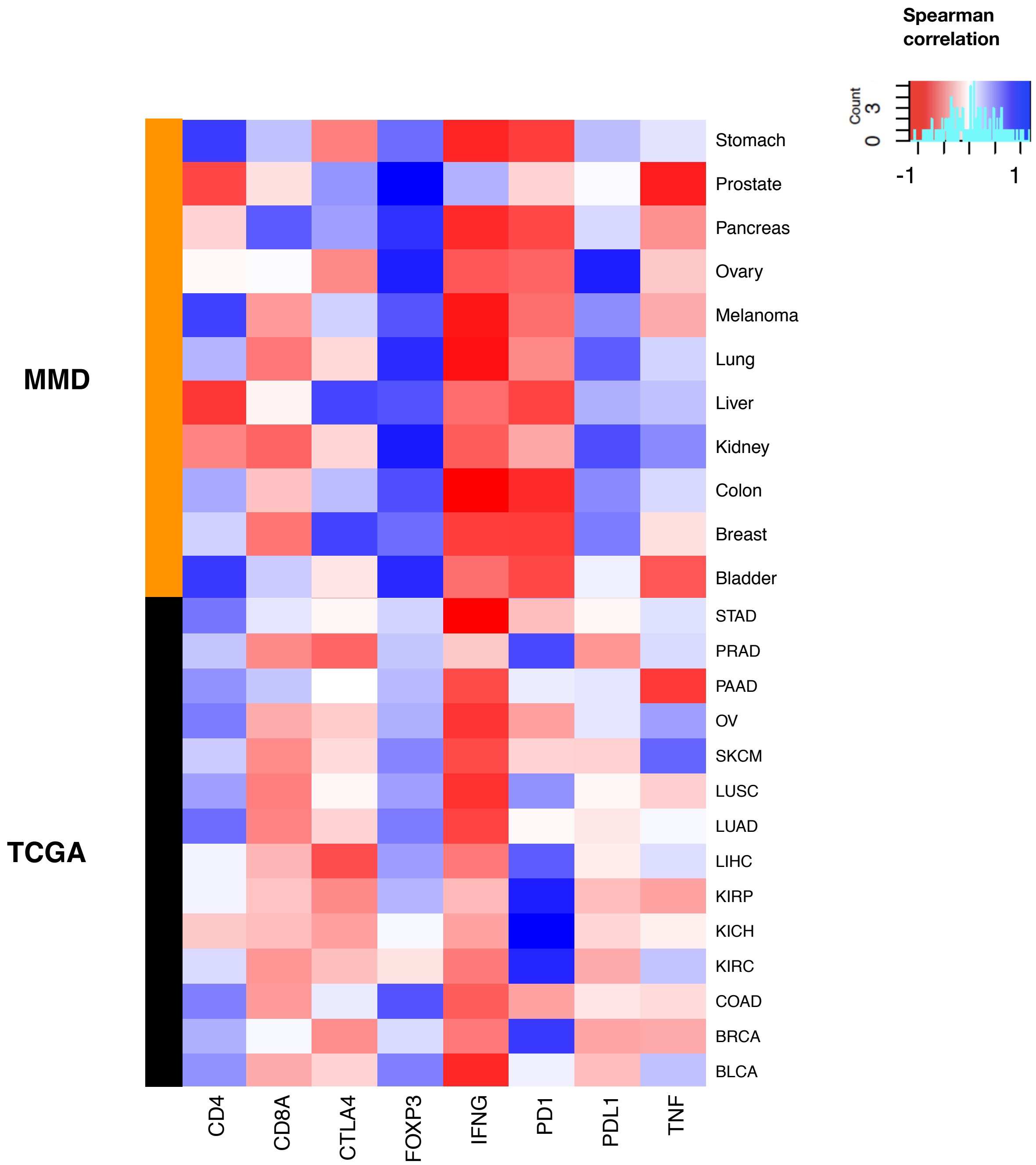
# Supplementary Figure 4



**Supplementary Figure 4: Correlation of RSI with TMB.**

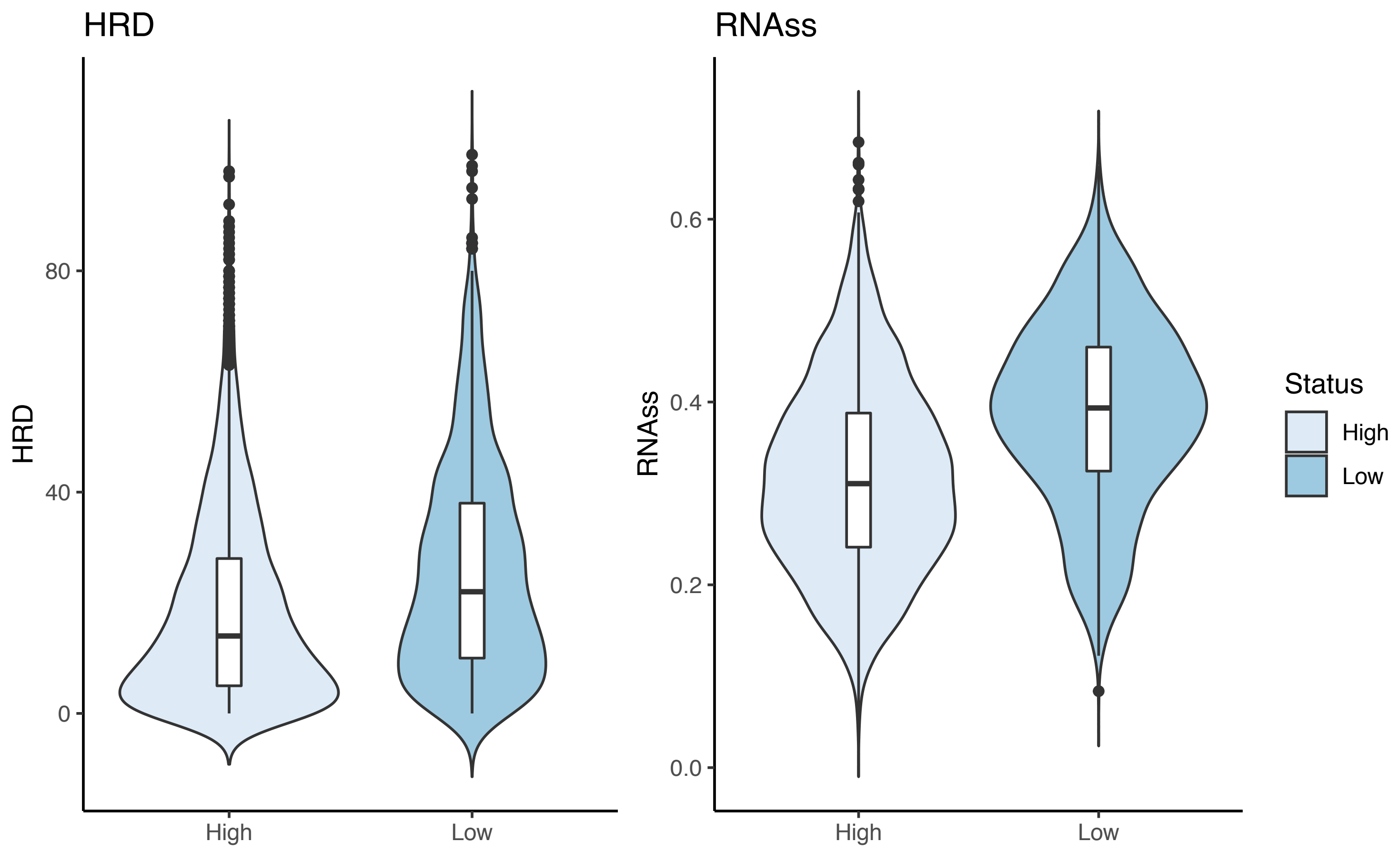
**a** Spearman's correlation between RSI and TMB in the TCGA. TMB was log10-transformed for plotting. **b** Distribution of log10(TMB) between RSI-High and RSI-Low tumours in COAD, PAAD and STAD.

# Supplementary Figure 5



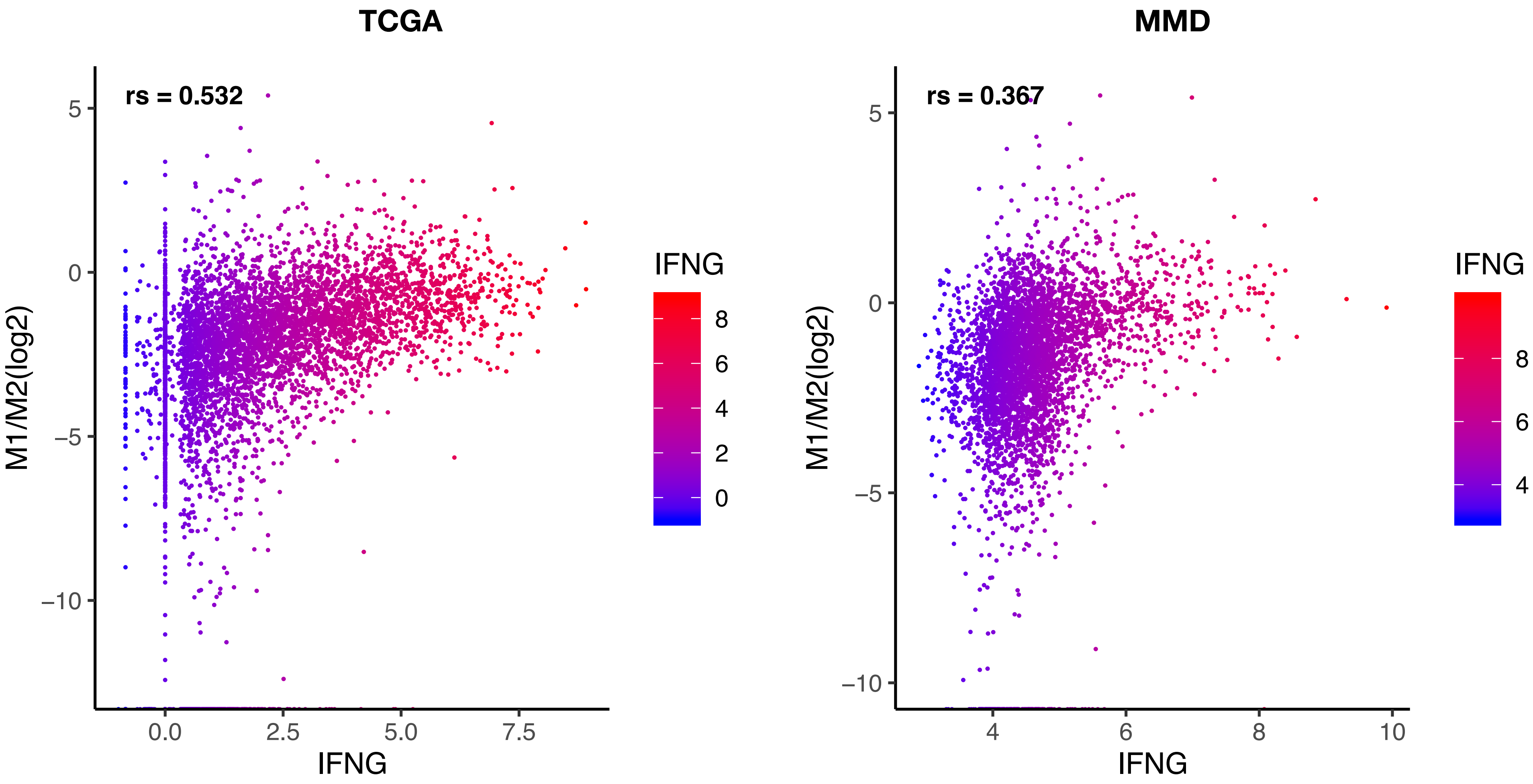
**Supplementary Figure 5: Heatmap depicting Spearman's correlation between RSI and expression of genes related to immunotherapy across 11 major cancer types in TCGA and MMD. Colour bar indicates Spearman's rs.**

# Supplementary Figure 6



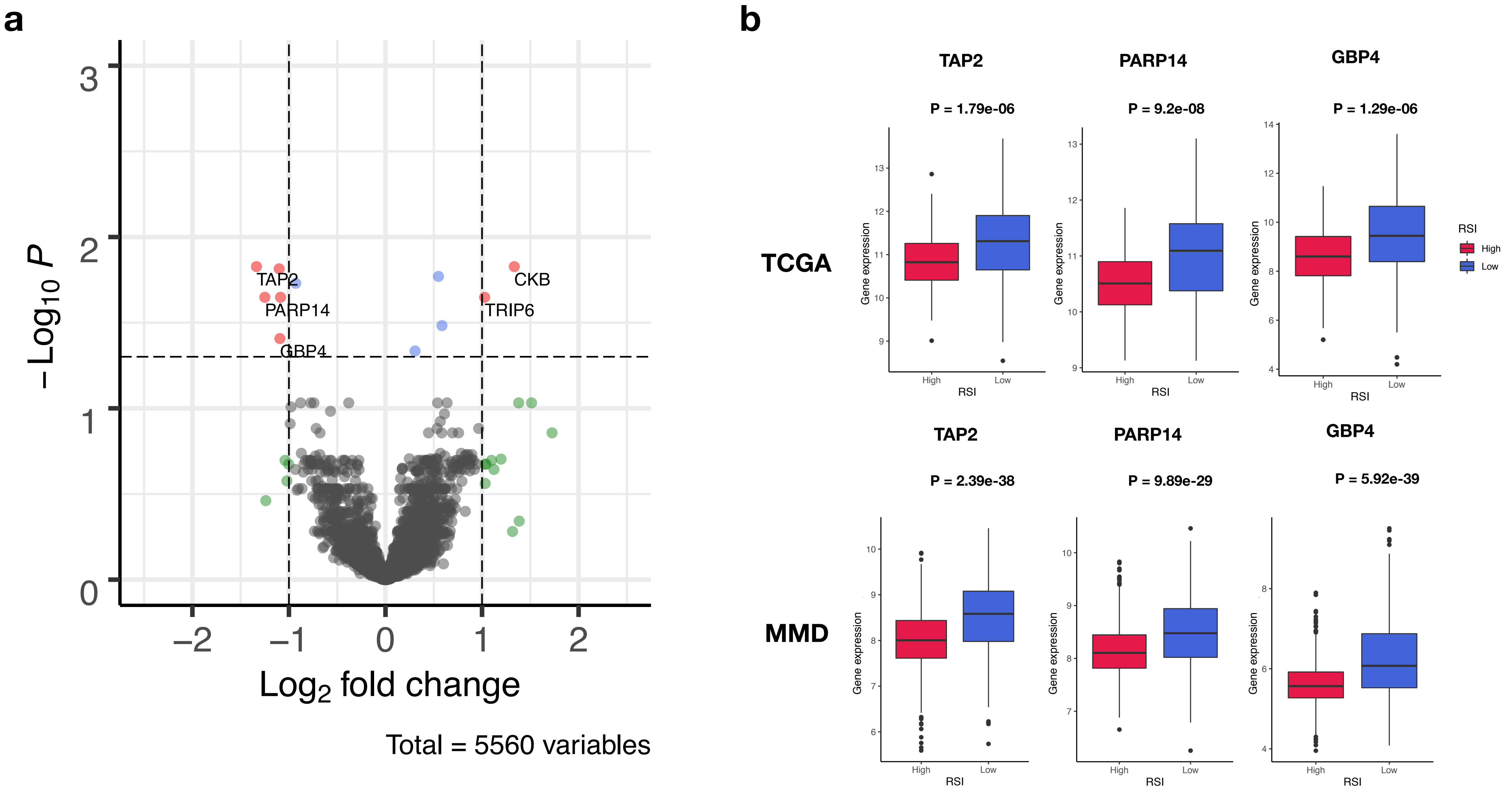
**Supplementary Figure 6: Violin plots showing distribution of HRD and RNAss scores between RSI-High and RSI-Low tumours in TCGA.** The black horizontal lines represent the median.

# Supplementary Figure 7



**Supplementary Figure 7: Scatter plots showing correlation between *IFNG* expression and M1/M2 (log2) in TCGA (upper panel) and MMD (lower panel).** M1: Macrophage M1; M2: macrophage M2.

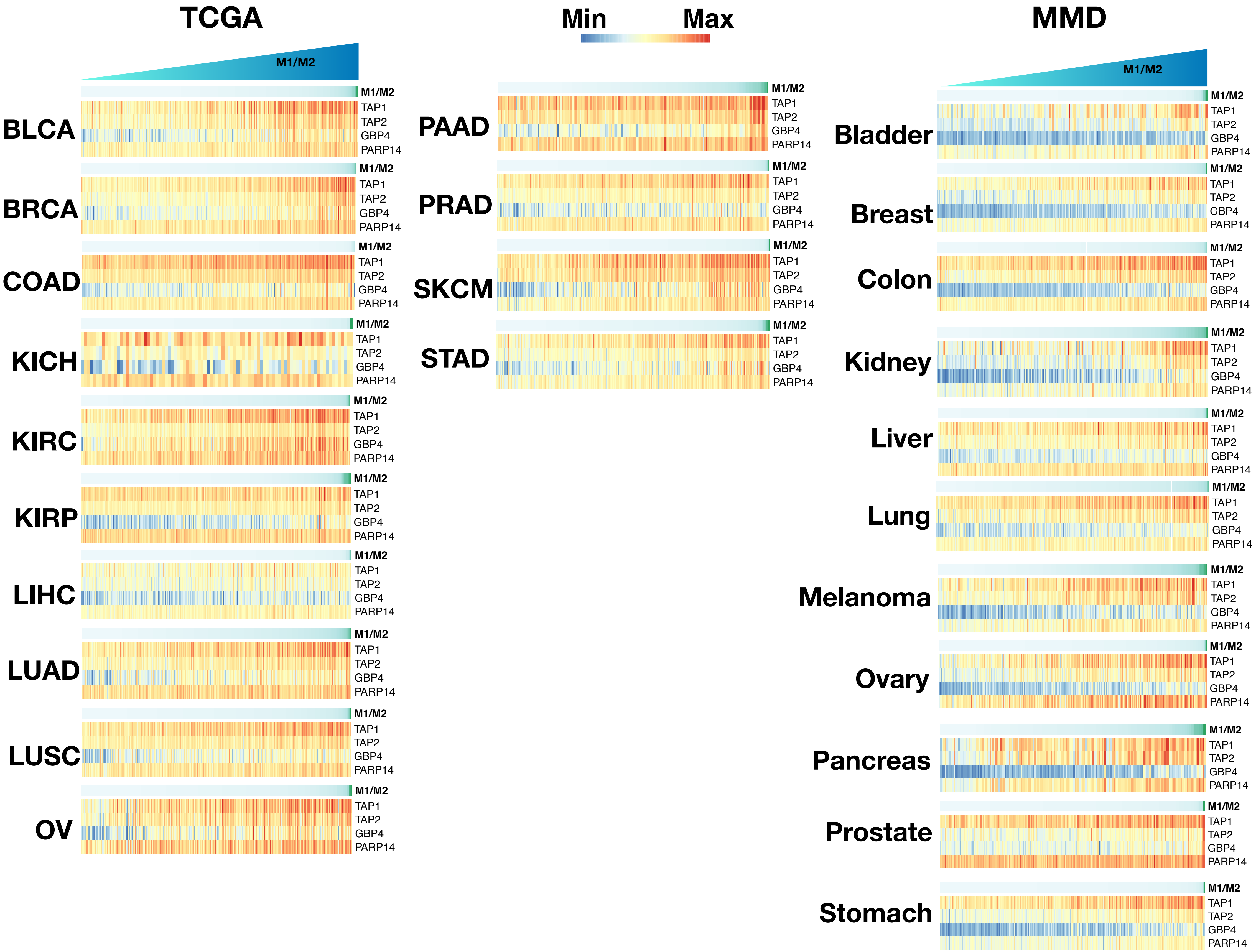
# Supplementary Figure 8



**Supplementary Figure 8: Proteomic analysis.**

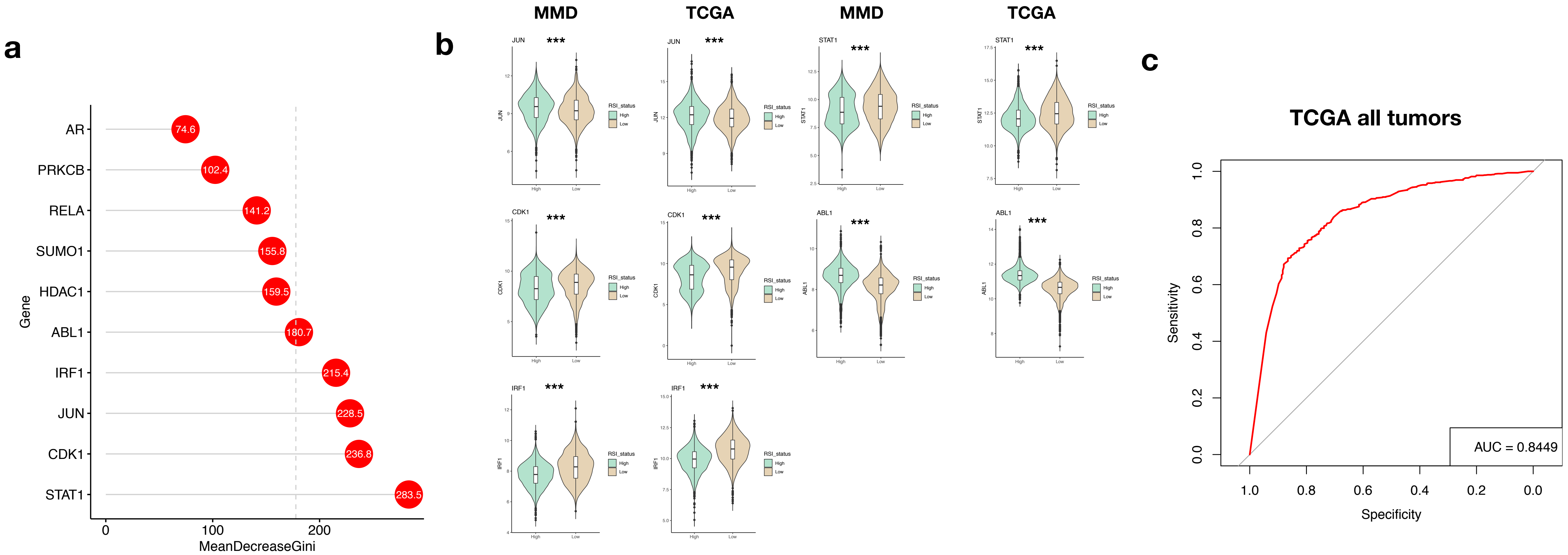
**a** Volcano plot illustrating differentially expressed proteins in the RSI-High and RSI-Low tumours. Red dots indicate proteins with FC > 1 or < -1 and adjusted *P* < 0.05. Blue dots represent proteins with -1 < FC < 1 and adjusted *P* < 0.05. Green dots represent proteins with FC > 1 or < -1 and adjusted *P* > 0.05. (B) Box plots showing expression of *TAP2*, *PARP14* and *GBP4* between RSI-High and RSI-Low tumours in TCGA (upper panel) and MMD (lower panel). The box hinges represent the 1st and 3rd quartiles, while the middle black horizontal lines indicate the median.

# Supplementary Figure 9



**Supplementary Figure 9: Heatmaps showing gene expression level of *TAP1*, *TAP2*, *PARP14*, and *GBP4*.** Columns have been ordered based on increasing M1/M2 ratio. M1: Macrophage M1; M2: macrophage M2.

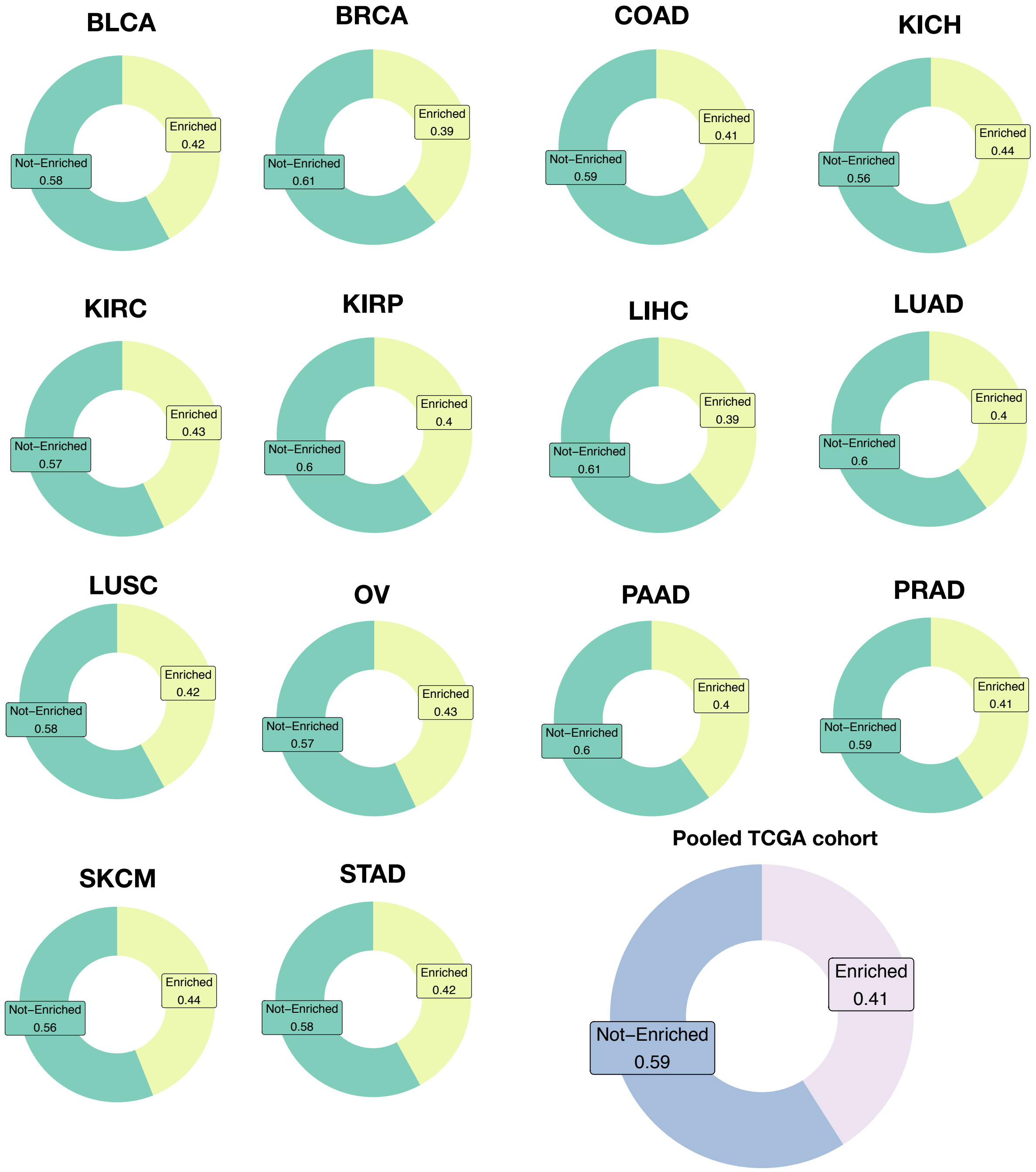
# Supplementary Figure 10



**Supplementary Figure 10: Classifier of C2 immune subtype.**

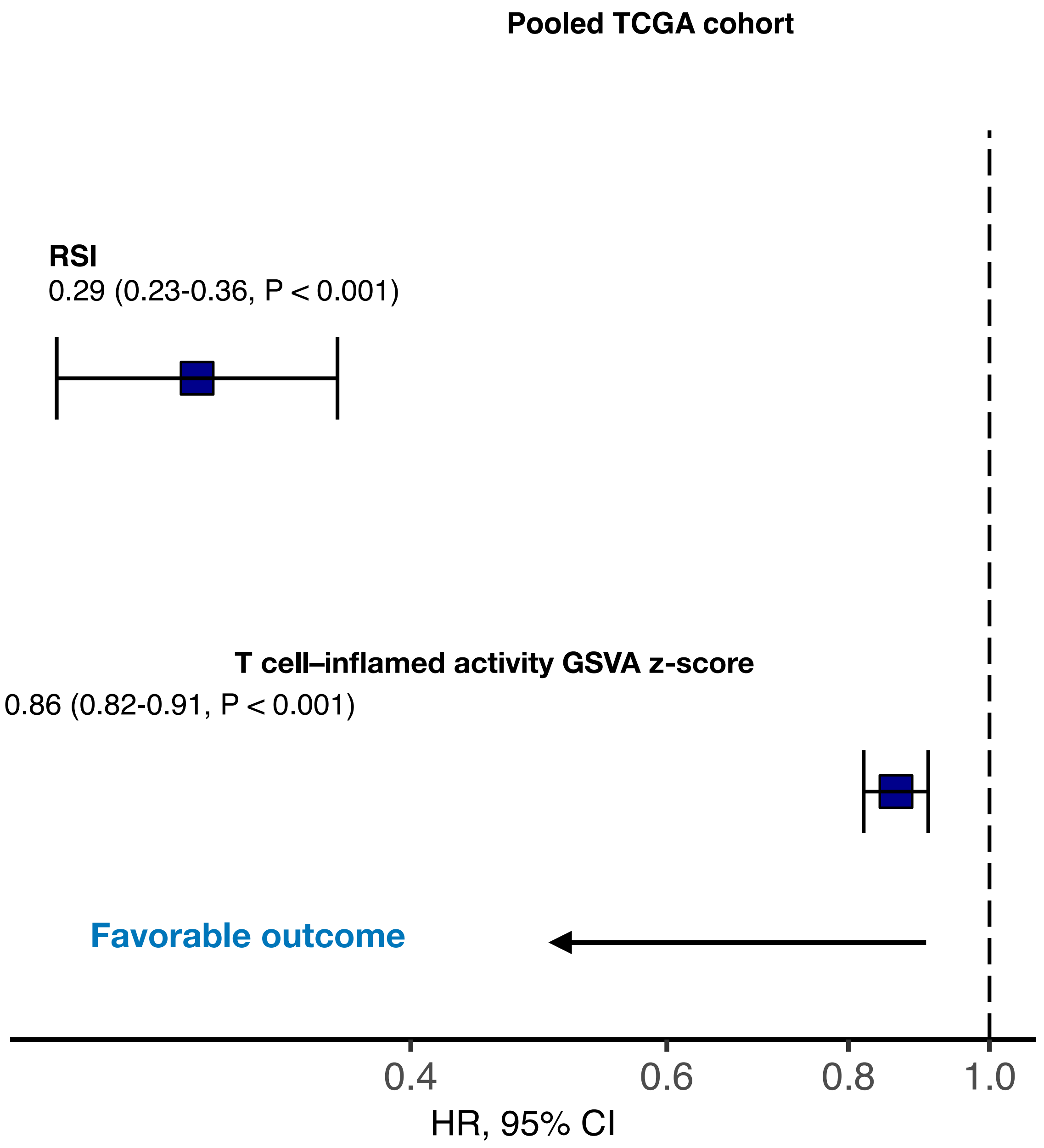
**a** Mean decrease in Gini for the 10 RSI genes in classification of C2 subtype. Gray horizontal dotted line indicates a MDG of 177.8. **b** Expression of *JUN*, *CDK1*, *IRF1*, *STAT1* and *ABL1* between RSI-High and RSI-Low tumours in the TCGA (left panel) and MMD (right panel). **c** Receiver operating characteristic curve for the established model in predicting C2 subtype.

# Supplementary Figure 11



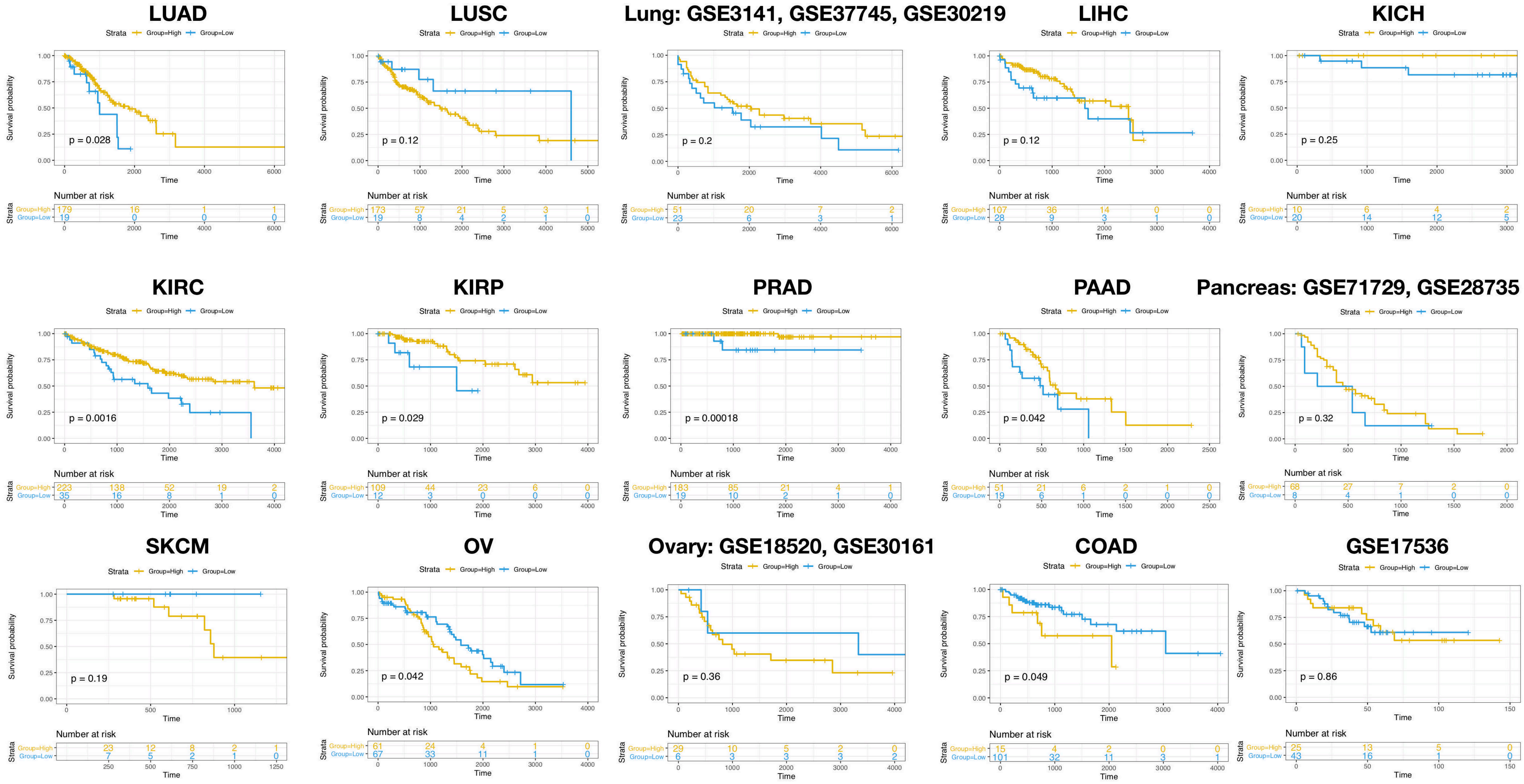
**Supplementary Figure 11: Donut plots depicting percentages of TCIA-enriched and not-enriched tumour samples in the 11 TCGA cancer cohorts.**

# Supplementary Figure 12



**Supplementary Figure 12: Hazard ratio plot indicating favourable overall survival with high RSI and TCIA in the pooled TCGA cohort with the 11 cancer types.**

# Supplementary Figure 13



**Supplementary Figure 13: KM plots for lung, liver, kidney, prostate, pancreas and melanoma in TCGA and several independent GSE datasets**

# Supplementary Table 1

| Gene | logFC | AveExpr | t | P.Value | adj.P.Val | B |
|---|---|---|---|---|---|---|
| **TAP2** | -1.33635858039912 | 7.10180369178457 | -5.11273123854195 | 2.89055437531139E-06 | 0.0148879408907804 | 4.1468920630629 |
| **PARP14** | -1.24968104709633 | 7.47887540925286 | -4.47535117580896 | 3.05853208850455E-05 | 0.0224818108451525 | 2.21254110804622 |
| **MUC5B** | -1.23948925911655 | 7.88186030972953 | -2.38345382119777 | 0.0200122762472588 | 0.345763214669471 | -3.37630331791356 |
| **GBP1** | -1.10098891036227 | 8.110312201045 | -4.83292146198769 | 8.27350604401098E-06 | 0.0153335645349003 | 3.46223052006926 |
| **GBP4** | -1.09360011426999 | 5.5409463892827 | -4.24043684911711 | 7.04094578702984E-05 | 0.0391476585758859 | 1.31701594031414 |
| **RNF213** | -1.08685217483235 | 9.58144205106456 | -4.45974864744149 | 3.23479292736007E-05 | 0.0224818108451525 | 2.11348967238193 |
| **PARP9** | -1.04034227475808 | 6.61737476219293 | -3.23671570492533 | 0.00188608139222394 | 0.201202774300534 | -1.35269161283532 |
| **OAS2** | -1.0214865963295 | 5.99474331809927 | -2.69841907169059 | 0.00882110189611531 | 0.265250375564611 | -2.58502642187831 |
| **DDX60** | -1.00284567795184 | 6.59803792963941 | -2.99419713225748 | 0.00386044765501153 | 0.211937027554233 | -1.9301218249963 |
| **HMGCS2** | 1.72493369206382 | 8.13507237087143 | 3.5036321728 | 0.000825699589811233 | 0.139117870283347 | -0.635191248904549 |
| **DPEP1** | 1.51267783629714 | 6.62112429112653 | 3.82506076617208 | 0.00029091648498259 | 0.0929517040292831 | 0.135648011947727 |
| **FCGBP** | 1.38495196886674 | 9.96849827015208 | 2.09622043278546 | 0.039868513722781 | 0.455407305085665 | -4.36422096278626 |
| **AMACR** | 1.37938425012813 | 6.68174738248085 | 3.8438754480137 | 0.000273262116239093 | 0.0929517040292831 | 0.179160483378131 |
| **CKB** | 1.3336970142633 | 8.35457843800319 | 4.94940567399612 | 5.35537442114403E-06 | 0.0148879408907804 | 3.82443185620885 |
| **CLCA1** | 1.31572439269828 | 8.31327930865293 | 1.86973594807095 | 0.0659205369499829 | 0.523651212473593 | -4.32987512594169 |
| **CES1** | 1.19643096492599 | 7.9362310078049 | 3.26258325465329 | 0.00174392060847022 | 0.197881603736621 | -1.27630600890553 |
| **FBN2** | 1.12336671810833 | 6.85852022804601 | 2.8553219694421 | 0.00572841257413255 | 0.227434234390211 | -2.24598057823757 |
| **EPB41L1** | 1.09824612068415 | 6.43169502363832 | 3.22313827935965 | 0.00196498660760428 | 0.201202774300534 | -1.41203185340778 |
| **PTK7** | 1.04292763813684 | 7.00302726599441 | 3.03885384417029 | 0.00339206335657135 | 0.211937027554233 | -1.82660192599739 |
| **ANPEP** | 1.03264175954616 | 6.74636232542563 | 2.66910230053988 | 0.00954580772926303 | 0.274653401837071 | -2.64615703549093 |
| **LTBP2** | 1.03036057310203 | 7.12019301455174 | 2.98140415713139 | 0.00400530792343813 | 0.211937027554233 | -1.95774735288577 |
| **TRIP6** | 1.02714214311488 | 5.64410483473386 | 4.48091858415269 | 2.99792515338385E-05 | 0.0224818108451525 | 2.06292403610948 |

**Supplementary Table 1: Differential expression analysis for proteomics in colorectal cohort in TCGA. Genes with absolute logFC > 1 are shown.**

# Supplementary Table 2

| Cancer_type | STAT1 | PRKCB | IRF1 |
|---|---|---|---|
| **BLCA (TCGA)** | 0.78 | 0.56 | 0.8 |
| **Bladder (MMD)** | 0.49 | 0.56 | 0.58 |
| **BRCA (TCGA)** | 0.68 | 0.82 | 0.75 |
| **Breast (MMD)** | 0.71 | 0.81 | 0.76 |
| **COAD (TCGA)** | 0.71 | 0.7 | 0.78 |
| **Colon (MMD)** | 0.53 | 0.68 | 0.65 |
| **KICH (TCGA)** | 0.15 | 0.44 | 0.5 |
| **KIRC (TCGA)** | 0.67 | 0.7 | 0.8 |
| **KIRP (TCGA)** | 0.36 | 0.64 | 0.64 |
| **Kidney (MMD)** | 0.7 | 0.79 | 0.8 |
| **LIHC (TCGA)** | 0.57 | 0.79 | 0.62 |
| **Liver (MMD)** | 0.6 | 0.74 | 0.56 |
| **LUAD (TCGA)** | 0.7 | 0.66 | 0.77 |
| **LUSC (TCGA)** | 0.71 | 0.72 | 0.83 |
| **Lung (MMD)** | 0.65 | 0.69 | 0.8 |
| **SKCM (TCGA)** | 0.79 | 0.71 | 0.93 |
| **Melanoma** | 0.79 | 0.59 | 0.85 |
| **OV (TCGA)** | 0.54 | 0.49 | 0.56 |
| **Ovary (MMD)** | 0.63 | 0.52 | 0.64 |
| **PAAD (TCGA)** | 0.56 | 0.77 | 0.56 |
| **Pancreas (MMD)** | 0.66 | 0.84 | 0.74 |
| **PRAD (TCGA)** | 0.61 | 0.5 | 0.68 |
| **Prostate (MMD)** | 0.34 | 0.36 | 0.41 |
| **STAD (TCGA)** | 0.61 | 0.54 | 0.74 |
| **Stomach (MMD)** | 0.64 | 0.67 | 0.66 |
| **Overall (TCGA)** | 0.6 | 0.64 | 0.71 |
| **Overall (MMD)** | 0.6 | 0.67 | 0.66 |

**Supplementary Table 2: Correlation of TCIA and three RSI genes across tumours.**

# Supplementary Table 3

| Cancer types | RSI |
|---|---|
| BLCA (TCGA) | 0.29 |
| Bladder (GSE31684) | 0.13 |
| BRCA (TCGA) | 0.56 |
| Breast (GSE20711, GSE42568) | 0.22 |
| LUAD (TCGA) | 0.15 |
| LUSC (TCGA) | 0.1 |
| Lung (GSE3141, GSE37745, GSE30219) | 0.25 |
| LIHC (TCGA) | 0.2 |
| OV (TCGA) | 0.28 |
| Ovary (GSE18520, GSE30161) | 0.1 |
| COAD (TCGA) | 0.67 |
| Colorectum (GSE17536) | 0.54 |
| KICH (TCGA) | 0.81 |
| KIRC (TCGA) | 0.26 |
| KIRP (TCGA) | 0.42 |
| PAAD (TCGA) | 0.26 |
| Pancreas (GSE71729, GSE28735) | 0.58 |
| PRAD (TCGA) | 0.44 |
| STAD (TCGA) | 0.43 |
| Stomach (GSE15459, GSE34942) | 0.14 |
| SKCM (TCGA) | 0.31 |

**Supplementary Table 3: Optimal cut-off RSI values for OS.**

# Supplementary Table 4

| Cancer types | N | Median RSI |
|---|---|---|
| **Bladder** | 79 | 0.27 |
| **Breast** | 155 | 0.35 |
| **Lung** | 42 | 0.41 |
| **Liver** | 10 | 0.33 |
| **Ovary** | 24 | 0.35 |
| **Colon** | 20 | 0.32 |
| **Kidney** | 12 | 0.22 |
| **Pancreas** | 25 | 0.36 |
| **Prostate** | 155 | 0.28 |
| **Stomach** | 15 | 0.49 |
| **Melanoma** | 44 | 0.38 |

**Supplementary Table 4: Median RSI value for metastatic tissues.**