**SUPPLEMENTARY DATA**. *Stafford & McKenna, MiR-182 Is Upregulated in Prostate Cancer and Contributes to Tumor Progression by Targeting MITF*

**Table S1. Functional enrichment analysis of miR-182 related to TGF-β signaling**

Table shows the significant association of miR-182 target genes with Gene Set descriptions related to TGF-β signaling. Analysis performed using clusterProfiler in CancerMIRNome.

| Knowledge base | Gene Set ID | Description | Count / List Total | *p*-value | Adjusted *p*-value [1] | Gene Symbol |
|---|---|---|---|---|---|---|
| **TGF-β signaling** | | | | | | |
| GO-BP | GO:0030511 | Positive regulation of transforming growth factor beta receptor signaling pathway | 4/179 | $1.15 \times 10^{-4}$ | $6.64 \times 10^{-3}$ | EP300; SMAD4; THBS1; CITED2 |
| | GO:1903846 | Positive regulation of cellular response to transforming growth factor beta stimulus | 4/179 | $1.15 \times 10^{-4}$ | $6.64 \times 10^{-3}$ | EP300; SMAD4; THBS1; CITED2 |
| | GO:0071560 | Cellular response to transforming growth factor beta stimulus | 9/179 | $3.64 \times 10^{-4}$ | $1.02 \times 10^{-2}$ | EP300; CREB1; SMAD4; ACVR1B; THBS1; CITED2; NR3C1; ZFP36L1; LOX |
| | GO:0071559 | Response to transforming growth factor beta | 9/179 | $4.33 \times 10^{-4}$ | $1.17 \times 10^{-2}$ | EP300; CREB1; SMAD4; ACVR1B; THBS1; CITED2; NR3C1; ZFP36L1; LOX |
| | GO:0007179 | Transforming growth factor beta receptor signaling pathway | 7/179 | $1.91 \times 10^{-3}$ | $3.18 \times 10^{-2}$ | EP300; CREB1; SMAD4; ACVR1B; THBS1; CITED2; LOX |

GO-BP = Gene Ontology-Biological Process. [1] Adjusted *p*-value for multiple hypothesis correction used Benjamini and Hochberg procedure.

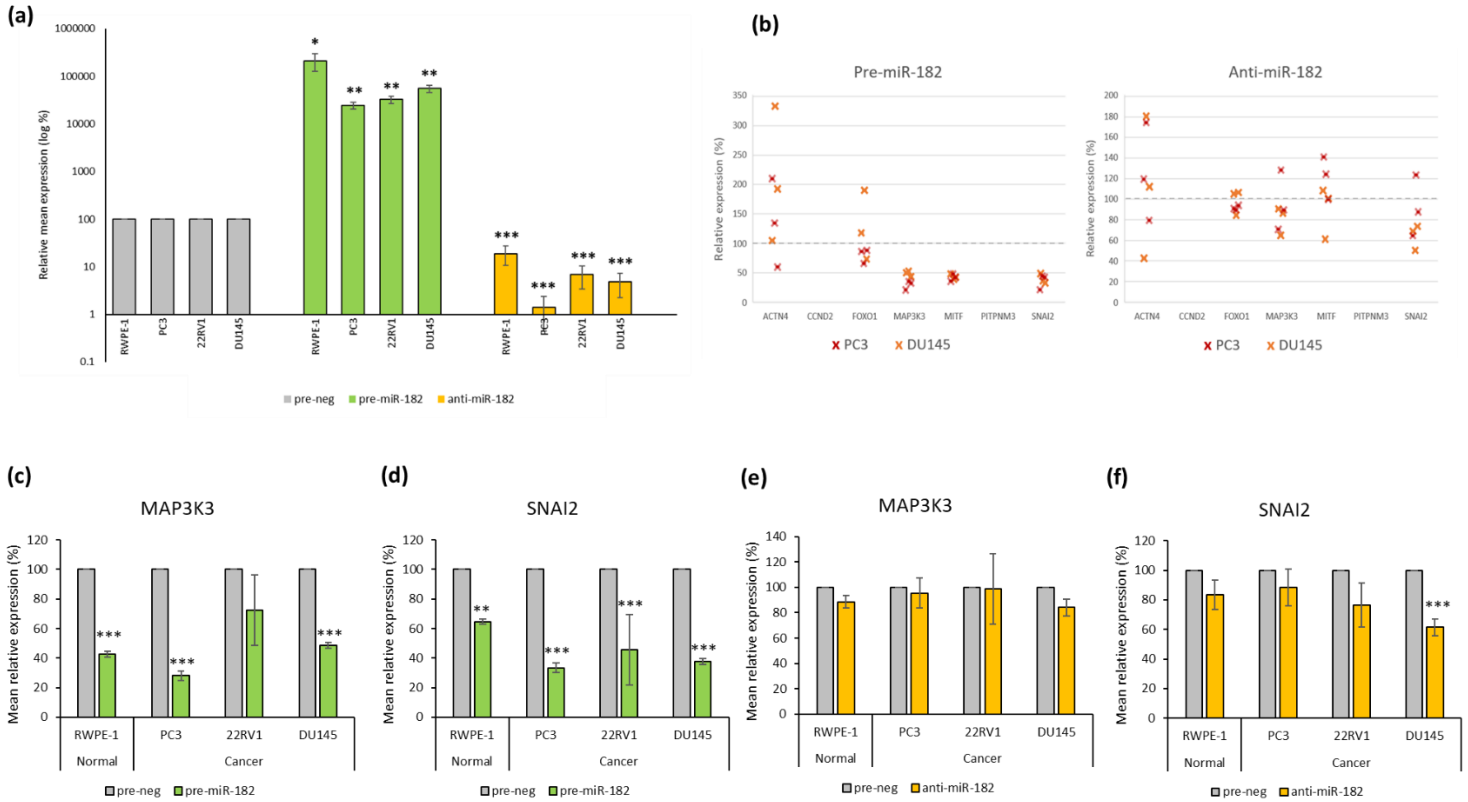


**Figure S1.** Effect of miR-182 expression on candidate targets. (a) RT-qPCR expression results of miR-182 48 hours post-transfection confirmed successful transfection of pre-miR-182 and anti-miR-182 in all cell-lines, where miR-182 was significantly upregulated (green) and downregulated (yellow) respectively, compared to control transfected cells ($n$ = 4, pre-neg: negative control, housekeeping: Snord48). (b) In vitro preliminary RT-qPCR results of the 7 candidate targets. In pre-miR-182 transfected PC3 and DU145 cells, MAP3K3, MITF and SNAI2 expressions were consistently decreased, while ACTN4 and FOXO1 showed a wide range of expressions. In anti-miR-182 transfected cells, results were less consistent. CCND2 and PITPNM3 were undetectable in both cell lines ($n$ = 3 per cell-line, each data point = one replicate vs pre-neg result normalised to 100%; housekeeping: ACTB). RT-qPCR shows over-expression of miR-182 causes significant down-regulation of (c) MAP3K3 and (d) SNAI2 in normal and cancerous prostate cell-lines ($n$ = 4; housekeeping: ACTB). However, RT-qPCR ($n$ = 4) shows inhibition of miR-182 does not cause up-regulation of (e) MAP3K3 and (f) SNAI2 in normal and cancerous prostate cell-lines ($n$ = 4; housekeeping: ACTB). For all bar graphs, p-values generated by paired $t$-test (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

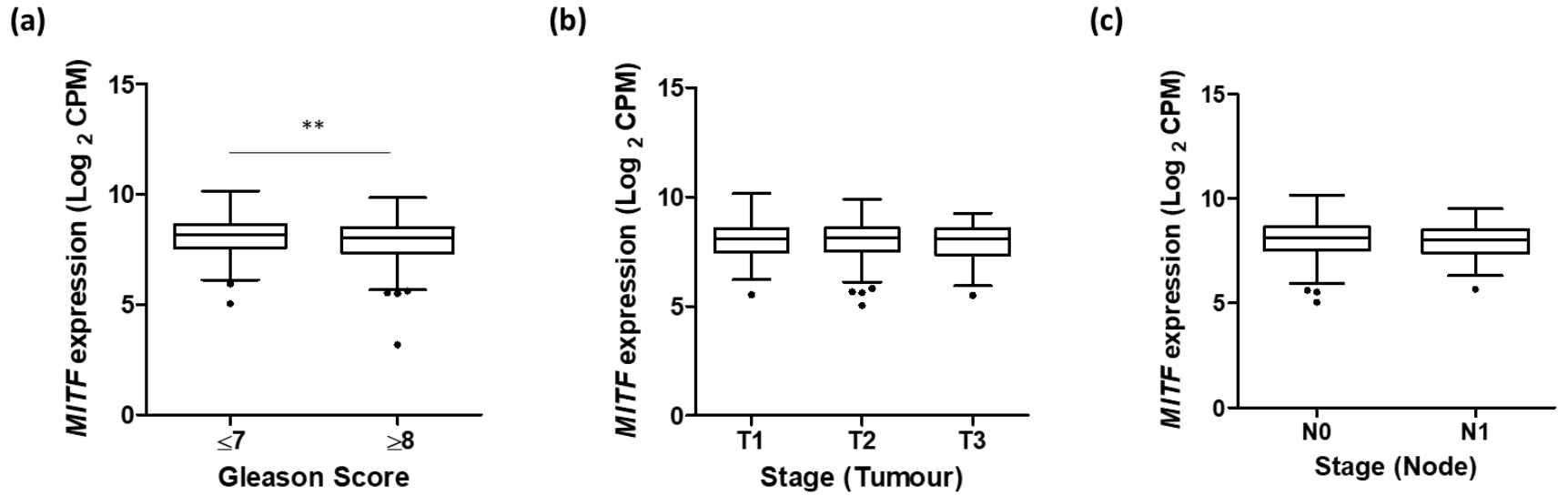


**Figure S2.** Correlation between MITF expression and clinicopathological markers of PCa progression

UCSC Xena analysis of TCGA PRAD samples shows expression of *MITF* is significantly lower in patients (a) with Gleason score ≥ 8 (*n* = 213) compared to those scored ≤ 7 (*n* = 337). There was no significant association between MITF expression and (b) pathological T-stage (*n*, T1 = 199, T2 = 190, T3 = 56)(One-way ANOVA with multiple comparison tests, *p* = ns) or (c) pathological N stage (*n*, N0 = 317, N1 = 66) (Welch's *t*-test, *p* = ns). All Boxplots show mean and Tukey whiskers. *n*, number; ns, non-significant.

**Table S2.** STRING functional enrichment analysis of MITF protein network using KEGG annotation. Table shows top 20 significantly enriched items ranked by Enrichment strength (ES)(Adjusted $p$-value < 0.05). Results are dominated by cancer-related annotations, including PCa (red), and EMT-related pathways (green) and function (purple).

| ID | Description | Count in network | ES | Adjusted p-value [1] | Protein labels |
|---|---|---|---|---|---|
| hsa05219 | Bladder cancer | 4 of 41 | 1.96 | $1.83 \times 10^{-6}$ | MAPK1, MDM2, MAP2K2, MAPK3 |
| hsa05211 | Renal cell carcinoma | 6 of 66 | 1.93 | $4.97 \times 10^{-9}$ | MAPK1, CREBBP, MAP2K2, MAPK3, EP300, TFE3 |
| hsa05216 | Thyroid cancer | 3 of 36 | 1.89 | $6.92 \times 10^{-5}$ | MAPK1, MAP2K2, MAPK3 |
| hsa04720 | Long-term potentiation | 5 of 64 | 1.86 | $2.19 \times 10^{-7}$ | MAPK1, CREBBP, MAP2K2, MAPK3, EP300 |
| hsa05221 | Acute myeloid leukaemia | 5 of 66 | 1.85 | $2.36 \times 10^{-7}$ | MAPK1, RPS6KB1, SPI1, MAP2K2, MAPK3 |
| hsa04916 | Melanogenesis | 7 of 95 | 1.84 | $1.55 \times 10^{-9}$ | MAPK1, CREBBP, MAP2K2, MAPK3, EP300, MITF, CREB1 |
| hsa05215 | **Prostate cancer** | 7 of 96 | 1.83 | $1.55 \times 10^{-9}$ | MAPK1, MDM2, CREBBP, MAP2K2, MAPK3, EP300, CREB1 |
| hsa05218 | Melanoma | 5 of 72 | 1.81 | $3.15 \times 10^{-7}$ | MAPK1, MDM2, MAP2K2, MAPK3, MITF |
| hsa04350 | **TGF-β signaling pathway** | 6 of 91 | 1.79 | $2.71 \times 10^{-8}$ | MAPK1, RPS6KB1, SMAD2, CREBBP, MAPK3, EP300 |
| hsa05210 | Colorectal cancer | 5 of 82 | 1.75 | $5.24 \times 10^{-7}$ | MAPK1, RPS6KB1, SMAD2, MAP2K2, MAPK3 |
| hsa04520 | **Adherens junction** | 4 of 67 | 1.75 | $8.95 \times 10^{-6}$ | MAPK1, CREBBP, MAPK3, EP300 |
| hsa04066 | **HIF-1 signaling pathway** | 6 of 106 | 1.72 | $5.19 \times 10^{-8}$ | MAPK1, RPS6KB1, CREBBP, MAP2K2, MAPK3, EP300 |
| hsa04068 | **FoxO signaling pathway** | 7 of 127 | 1.71 | $3.60 \times 10^{-9}$ | SIRT1, MAPK1, MDM2, CREBBP, MAP2K2, MAPK3, EP300 |
| hsa05214 | Glioma | 4 of 72 | 1.71 | $1.11 \times 10^{-5}$ | MAPK1, MDM2, MAP2K2, MAPK3 |
| hsa05212 | Pancreatic cancer | 4 of 73 | 1.71 | $1.14 \times 10^{-5}$ | MAPK1, RPS6KB1, SMAD2, MAPK3 |
| hsa05220 | Chronic myeloid leukaemia | 4 of 75 | 1.70 | $1.23 \times 10^{-5}$ | MAPK1, MDM2, MAP2K2, MAPK3 |
| hsa04960 | Aldosterone-regulated sodium reabsorption | 2 of 37 | 1.70 | 0.0028 | MAPK1, MAPK3 |
| hsa01522 | Endocrine resistance | 5 of 95 | 1.69 | $8.74 \times 10^{-7}$ | MAPK1, RPS6KB1, MDM2, MAP2K2, MAPK3 |
| hsa04370 | **VEGF signaling pathway** | 3 of 57 | 1.69 | 0.00021 | MAPK1, MAP2K2, MAPK3 |
| hsa05213 | Endometrial cancer | 3 of 57 | 1.69 | 0.00021 | MAPK1, MAP2K2, MAPK3 |

[1]Adjusted $p$-value for multiple hypothesis correction used Benjamini and Hochberg procedure.

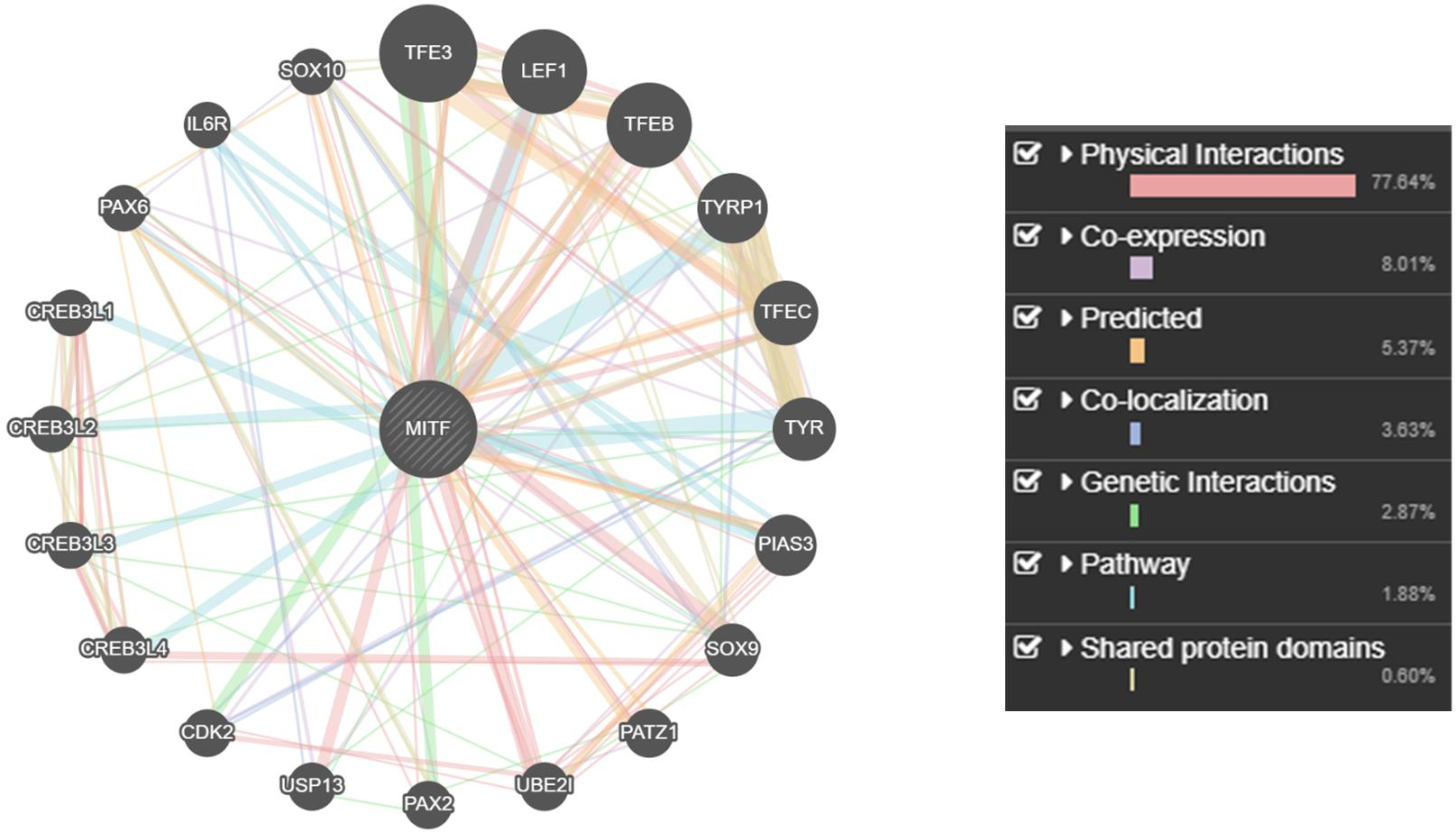


**Figure S3.** Network analysis of MITF interactions. Visualization by GeneMANIA.

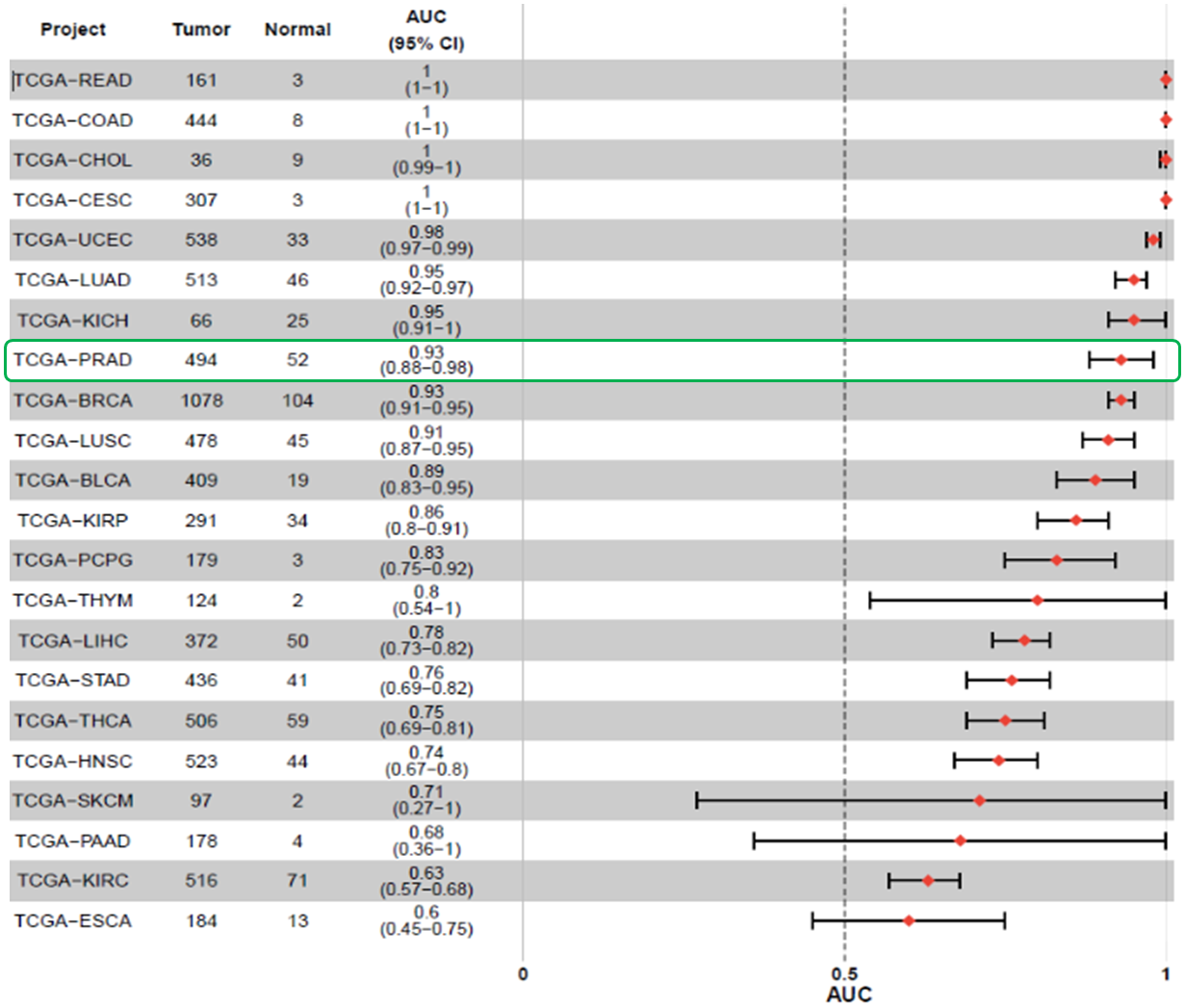


**Figure S4.** CancerMIRNome TCGA Pan-Cancer ranked forest plots of miR-182-5p ROC analysis revealed in general that miR-182-5p has significant diagnostic value in multiple cancer types (AUC = 0.6 - 1.0). Result for PCa is enclosed in green circle (TCGA-PRAD).

AUC: Area under the curve; OS: Overall survival; ROC: Receiver operating characteristic; BLCA: Bladder urothelial carcinoma; BRCA: Breast invasive carcinoma; CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL: Cholangiocarcinoma; COAD: Colon adenocarcinoma; ESCA: Esophageal carcinoma; HNSC: Head and neck squamous cell carcinoma; KICH: Kidney chromophobe; KIRC: Kidney renal clear cell carcinoma; KIRP: Kidney renal papillary cell carcinoma; LIHC: Liver hepatocellular carcinoma; LUAD: Lung adenocarcinoma; LUSC: Lung squamous cell carcinoma; PAAD: Pancreatic adenocarcinoma; PCPG: Pheochromocytoma and paraganglioma; PRAD: Prostate adenocarcinoma; READ: Rectal adenocarcinoma; SKCM: Skin cutaneous melanoma; STAD: Stomach adenocarcinoma; THCA: Thyroid carcinoma; THYM: Thymoma; UCEC: Uterine corpus endometrial carcinoma

**Table S3** KM plotter meta-analysis results of overall survival in various cancers comparing high/low expression of miR-182 and MITF. Table included results with log-rank $p < 0.05$. HR estimated for high compared to low expression (Cox proportional analysis, auto-selected cut-off).

| | *Cancer* | *n* | *HR** | *Log-rank p-value* |
|---|---|---|---|---|
| ***miR-182*** | Breast cancer | 1076 | 0.70 | 0.035 |
| | Head-neck squamous cell carcinoma | 522 | 0.70 | 0.013 |
| | Kidney renal clear cell carcinoma | 516 | 1.94 | $2.2 \times 10^{-5}$ |
| | Kidney renal papillary cell carcinoma | 290 | 0.52 | 0.037 |
| | Liver hepatocellular carcinoma | 371 | 1.56 | 0.012 |
| | Lung squamous cell carcinoma | 472 | 0.57 | 0.00016 |
| | Sarcoma | 259 | 1.78 | 0.0043 |
| | Stomach adenocarcinoma | 431 | 0.68 | 0.012 |
| | Uterine corpus endometrial carcinoma | 537 | 0.51 | 0.0021 |
| ***MITF*** | Cervical squamous cell carcinoma | 304 | 0.62 | 0.043 |
| | Esophageal adenocarcinoma | 80 | 0.37 | 0.0028 |
| | Kidney renal clear cell carcinoma | 530 | 0.55 | $8.3 \times 10^{-5}$ |
| | Kidney renal papillary cell carcinoma | 287 | 0.51 | 0.031 |
| | Liver hepatocellular carcinoma | 370 | 1.54 | 0.016 |
| | Lung adenocarcinoma | 504 | 0.66 | 0.019 |
| | Ovarian cancer | 373 | 1.47 | 0.0054 |
| | Pancreatic ductal adenocarcinoma | 177 | 2.09 | 0.0039 |
| | Rectum adenocarcinoma | 165 | 2.24 | 0.037 |
| | Sarcoma | 259 | 0.54 | 0.0019 |
| | Thymoma | 118 | 3.96 | 0.027 |

***** Red text indicates HR < 1; high expression of miR-182/MITF predicted favourable prognosis; blue text indicates HR > 1; high expression of miR-182/MITF predicted poor prognosis. *n* = number; HR = Hazard ratio; KM = Kaplan-Meier

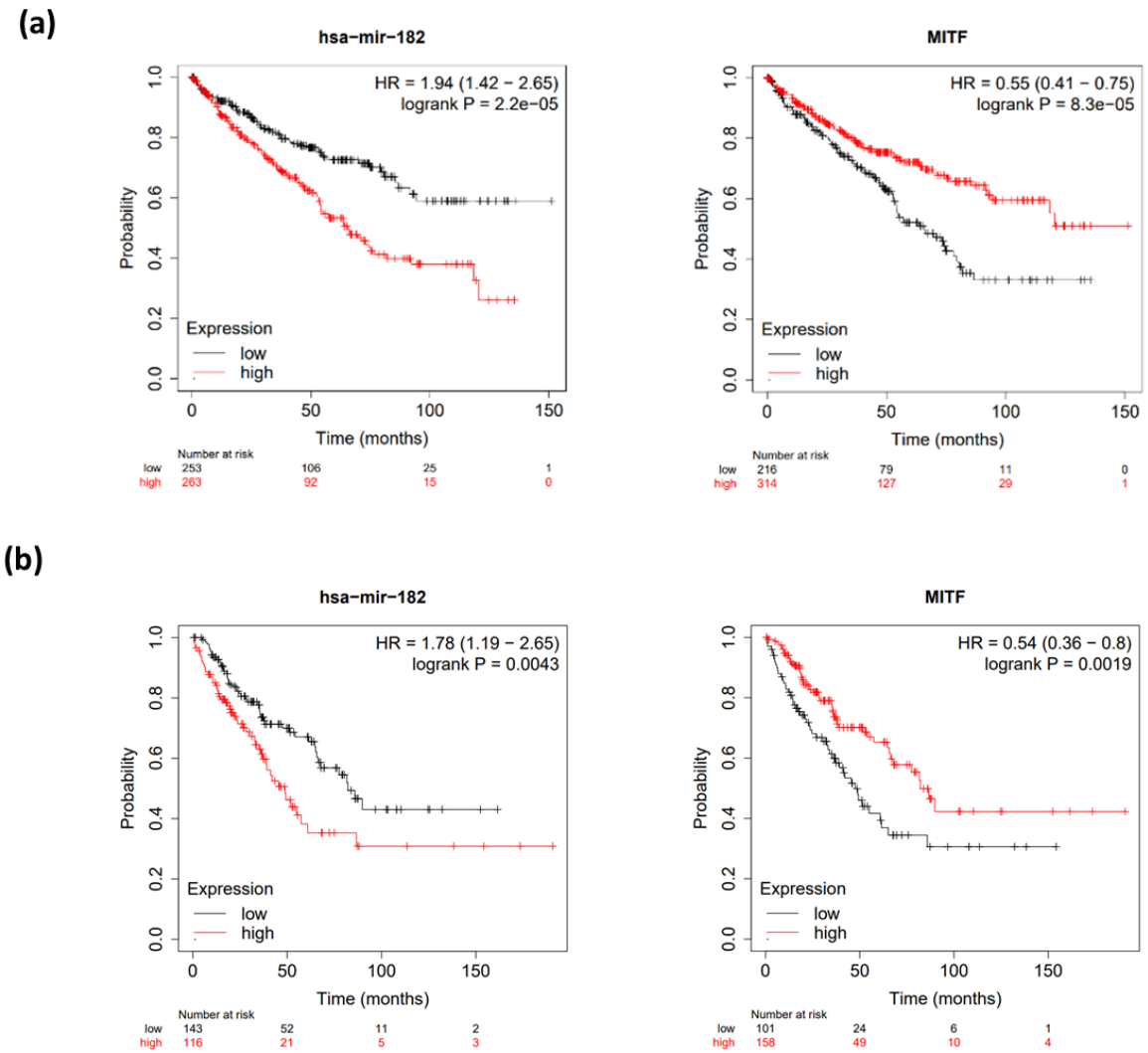


**Figure S5.** KM survival plots showing significant inverse associations between miR-182 and MITF expressions in **(a)** Kidney renal clear cell carcinoma (*n*: miR-182 = 516, MITF = 530), and **(b)** Sarcoma (*n*: miR-182 = 259, MITF = 259). In both cases, high miR-182 expression is significantly associated with poor prognosis, while high MITF is significantly associated with favourable prognosis. Visualization by KM Plotter. *n* = number; KM = Kaplan-Meier

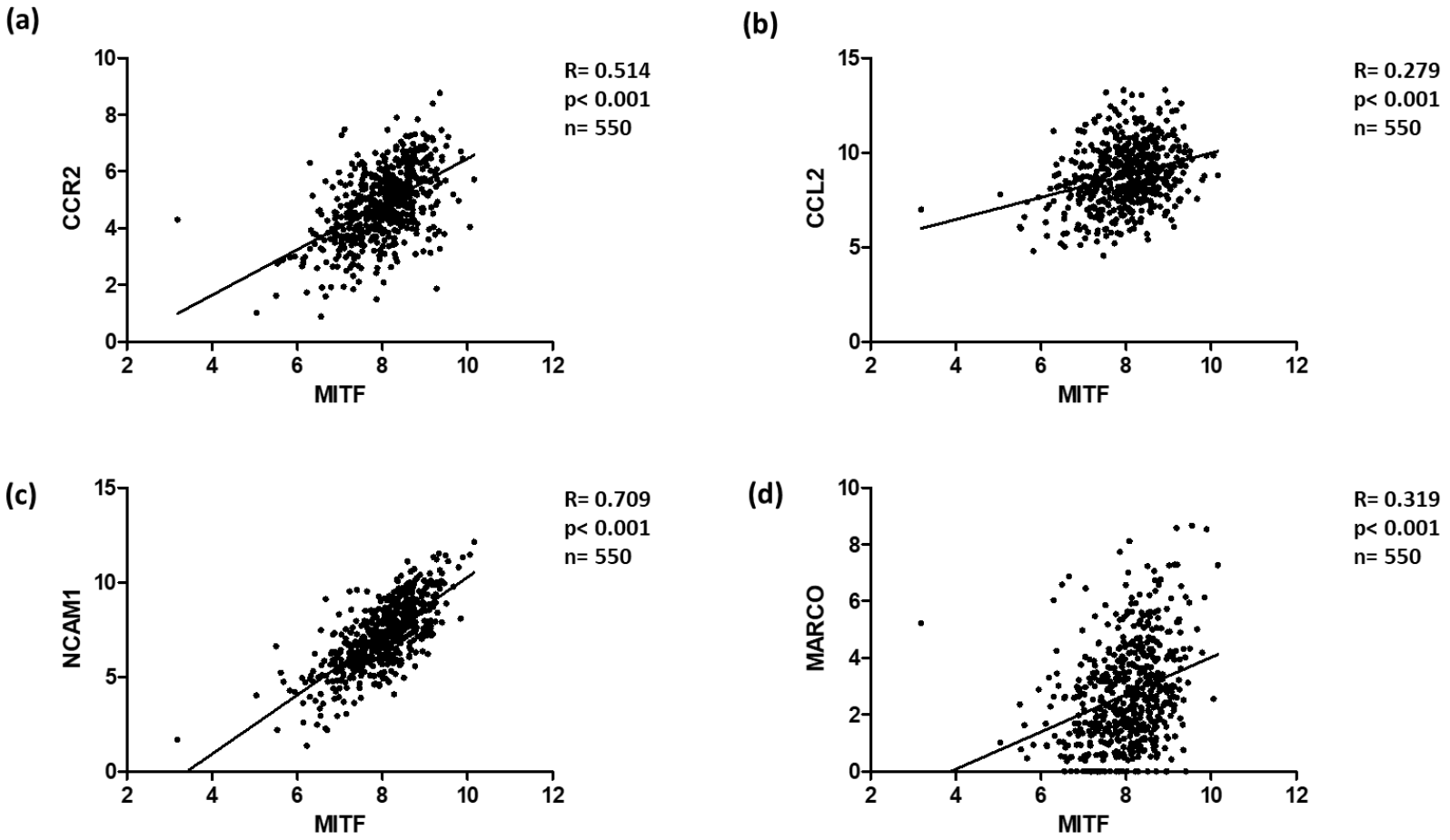


**Figure S6. Correlation of MITF with gene markers of tumor-associated macrophages.** UCSC Xena analysis of TCGA prostate tissue samples ($n$ = 550) , shows the expression of MITF is significantly positively correlated with (a) CCR2 (b) CCL2 (c) NCAM1 (d) MARCO (Pearson correlation, $p < 0.001$). $n$ = number

**Table S4.** Diagnostic and prognostic studies of miR-182-5p in prostate cancer.

| miR-182-5p | Source | Diagnostic | Prognostic | | Reference |
|---|---|---|---|---|---|
| Up | Plasma | 65 PCa vs 58 BPH<br>AUC = 0.61<br>($p$ = 0.0365) | (NA) | | *[59] Abramovic et al., 2021* |
| Up | Tissue | 82 PCa vs 24 BPH<br>AUC = 0.95<br>($p$ < 0.001) | **BCR-free survival**<br>34 AA PCa:<br>HR = 2.93<br>($p$ = 0.041) | 22 EA PCa:<br>HR = 0.83<br>($p$ = 0.86) | *[60] Shiina et al., 2021* |
| Up | Tissue | 98 PCa vs 15 Normal<br>AUC=0.81<br>($p$ = 0.0001) | (NA) | | *[61] Bidarra et al., 2019* |
| | Plasma | 252 PCa vs 52 Normal<br>AUC = 0.64<br>($p$ = 0.0021) | **MFS** (252 PCa)<br>Univariate:<br>Log-rank $p$ = 0.0206 | Multivariate:<br>(Not significant) | |
| Up | Urine<br>Tissue | (Urine)<br>47 PCa vs 45 Normal<br>OR = 0.976<br>($p$ = 0.717) | (Tissue, 272 PCa)<br>**Biochemical PFS**<br>Log-rank $p$ = 0.026<br>**Clinical PFS**<br>Log-rank $p$ = 0.043 | | *[62] Casanova-Salas et al., 2014* |
| Up | Tissue | (NA) | **BCR-free survival** (100 PCa)<br>Univariate OR = 1.91 ($p$ = 0.11)<br>**MFS** (74 PCa)<br>Univariate OR = 1.07 ($p$ = 0.93) | | *[63] Wallis et al., 2015* |

AA = African Americans; AUC = Area under the curve; BCR = Biochemical recurrence; BPH = Benign prostatic hyperplasia; EA = European Americans; MFS = Metastatic-free survival; NA = Not applicable; OR = Odds ratio; PFS = Progression-free survival