**Table S1.** Summary and comparison of clinico-pathological characteristics of the colorectal cancer patients in the screening (n = 228) and validation cohort (n = 332).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Screening cohort | Validation cohort | p-value |
|  |  | No (%) | No (%) |
| Age | median | 70 | 64 | p=4.7e-08\* |
| minimum | 34 | 18 |
| maximum | 90 | 92 |
| Sex | female | 112 (49) | 138 (42) | p=0.0928\*\* |
| male | 116 (51) | 194 (58) |
| Stage | stage I | 51 (22) | 39 (12) | p=1.6e-06\*\* |
| stage II | 80 (35) | 83 (25) |
| stage III | 56 (25) | 91 (27) |
| stage IV | 41 (18) | 119 (36) |
| Location | colon | 186 (82) | 296 (89) | p=0.0155\*\* |
| rectum | 42 (18) | 36 (11) |

\*Mann-Whitney-Wilcoxon test
\*\*chi-square test

**Table S2** List of all microRNAs that are significantly (p<0.05) associated with survival in the colorectal cancer screening cohort (cohort 1; n = 228) calculated by univariate Cox proportional model. Yellow marked are the ones that were further explored in the validation cohort.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRNA | HR | lower95%CI | upper95%CI | p value |
| hsa.mir.425\_MIMAT0001343 | 2.364164 | 1.345653129 | 4.153573985 | 0.002767 |
| hsa.mir.195\_MIMAT0004615 | 1.945962 | 1.07978293 | 3.506973078 | 0.026733 |
| hsa.mir.3610\_MIMAT0017987 | 1.914306 | 1.052975757 | 3.480199608 | 0.033236 |
| hsa.mir.188\_MIMAT0004613 | 1.874051 | 1.21968087 | 2.879497837 | 0.004155 |
| hsa.mir.221\_MIMAT0004568 | 1.782102 | 1.050126835 | 3.0242905 | 0.032256 |
| hsa.mir.129.1\_MIMAT0000242 | 1.712541 | 1.073807128 | 2.731214614 | 0.023885 |
| hsa.mir.331\_MIMAT0000760 | 1.695194 | 1.036829648 | 2.771604116 | 0.035365 |
| hsa.mir.195\_MIMAT0000461 | 1.66737 | 1.017733301 | 2.731681947 | 0.042382 |
| hsa.mir.497\_MIMAT0002820 | 1.646315 | 1.001009748 | 2.707619103 | 0.049537 |
| hsa.mir.147b\_MIMAT0004928 | 1.573449 | 1.016532898 | 2.435476519 | 0.041999 |
| hsa.let.7e\_MIMAT0004485 | 1.551875 | 1.016760374 | 2.368616937 | 0.041649 |
| hsa.mir.92b\_MIMAT0003218 | 0.496884 | 0.284619065 | 0.86745369 | 0.013889 |
| hsa.mir.1976\_MIMAT0009451 | 0.528338 | 0.292163225 | 0.955428652 | 0.034789 |

Where: HR hazard ratio and CI confidence interval

**Table S3** List of all microRNAs that are significantly (p<0.05) associated with survival in the colon cancer only screening cohort (cohort 1; n = 186) calculated by univariate Cox proportional model. Yellow marked are the ones that are further validated in the validation cohort.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRNA | HR | lower.95%CI | upper.95%CI | p value |
| hsa-mir-331\_MIMAT0000760 | 2.297344 | 1.271467516 | 4.150945004 | 0.005857 |
| hsa-mir-425\_MIMAT0001343 | 2.164009 | 1.242505403 | 3.768944613 | 0.006392 |
| hsa-mir-188\_MIMAT0004613 | 2.007447 | 1.266909656 | 3.180844187 | 0.003004 |
| hsa-mir-3607\_MIMAT0017985 | 2.004293 | 1.040685249 | 3.860138956 | 0.037597 |
| hsa-mir-221\_MIMAT0004568 | 1.980054 | 1.151587704 | 3.404527778 | 0.013497 |
| hsa-mir-497\_MIMAT0002820 | 1.895216 | 1.13666149 | 3.159994029 | 0.014244 |
| hsa-mir-195\_MIMAT0000461 | 1.845756 | 1.07835644 | 3.15926722 | 0.025413 |
| hsa-mir-455\_MIMAT0003150 | 1.779172 | 1.091786031 | 2.899333923 | 0.020755 |
| hsa-mir-455\_MIMAT0004784 | 1.778764 | 1.076350881 | 2.93956392 | 0.024638 |
| hsa-mir-326\_MIMAT0000756 | 1.665797 | 1.084566556 | 2.558514766 | 0.019767 |
| hsa-mir-129-1\_MIMAT0000242 | 1.662969 | 1.027604971 | 2.691175687 | 0.038374 |
| hsa-mir-147b\_MIMAT0004928 | 1.64919 | 1.028162273 | 2.645329416 | 0.037971 |
| hsa-mir-29c\_MIMAT0000681 | 1.62336 | 1.029026488 | 2.560961225 | 0.037253 |
| hsa-mir-374a\_MIMAT0004688 | 0.484745 | 0.235754184 | 0.99670507 | 0.04896 |
| hsa-mir-320a\_MIMAT0000510 | 0.593477 | 0.373943358 | 0.941895002 | 0.026831 |

Where: HR hazard ratio and CI confidence interval

**Table S4** Univariate analysis of clinico-pathological parameters for the prediction of overall survival in patients with colorectal cancer in the screening set (n=228) and validation set (n=332).

|  |  |
| --- | --- |
| Parameter | Univariate analysis |
|  | **Screening set** | **Validation set** |
| **HR (95% CI)** | ***p*-value** | **HR (95% CI)** | ***p*-value** |
| Age at diagnosis (years; continuous) | 1.043 (1.004-1.083) | 0.029 | 1.002 (0.987-1.016) | 0.825 |
| GenderMaleFemale | 1 (reference)0.873 (0.385-1.980)  | 0.746 | 1 (reference)0.788 (0.570-1.09) | 0.151 |
| Tumor LocationColonRectum | 1 (reference)0.441 (0.130-1.497) | 0.189 | 1 (reference)1.297 (0.868-1.938) | 0.204 |
| Tumor StageI-IIIIV | 1 (reference)3.635 (1.589-8.317) | 0.002 | 1 (reference)4.93 (3.56-6.832) | <0.001 |
| MiR-188-3p (median)lowhigh | 1 (reference)2.717 (1.135-6.507) | 0.025 | 1 (reference)1.768 (1.283-2.436) | <0.001 |

**Table S5:** Integrated function and pathway analysis using DAVID bioinformatics resources shows a number of potential miR-188-3p interactors. We focused on genes of three colorectal cancer related pathways (underlined bold letters) and based on literature search for negative regulators of migration, we selected NLK, CTNNA2 and MLLT4 for further validation.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| data base | pathway | Count | P-value | Genes |
| KEGG | Aldosterone-regulated sodium reabsorption | 13 | 0.000638 | PRKCA, IRS2, PIK3CD, ATP1B4, NR3C2, IGF2, ATP1A2, IRS1, PRKCB, KRAS, NEDD4L, PIK3R3, INSR |
| KEGG | Phosphatidylinositol signaling system | 18 | 0.001166 | PRKCA, PIK3CD, DGKI, CDS1, PRKCB, CDS2, CALML3, DGKD, CALM3, IPPK, PIP4K2A, PIK3R3, ITPK1, INPP5B, PLCB2, PIP4K2C, INPP5A, PIP4K2B |
| KEGG | Axon guidance | 26 | 0.00136 | PLXNC1, PLXNA1, PLXNA2, ABLIM3, EFNA3, PPP3R1, CXCL12, SEMA5A, EPHB6, KRAS, UNC5B, SEMA3G, UNC5D, NGEF, ROCK1, LIMK2, LIMK1, EFNB1, MET, CHP2, NTN1, SLIT2, SLIT3, SEMA4F, SRGAP3, EFNA5 |
| REACTOME | Signalling by NGF | 29 | 0.001549 | ADCY1, ADCY2, APH1A, ADCYAP1R1, ADCY6, MAPKAPK3, CLTC, SORCS3, PRKAR2B, PRKAR2A, KRAS, RALB, RALA, PRKACB, ARHGDIA, AKT2, IRS2, MAP2K1, PRKCI, MAPK11, IRS1, RPS6KA3, AP2A2, HDAC2, MAPK13, MAPK14, CALM3, NGFR, DNAL4 |
| KEGG | SNARE interactions in vesicular transport | 11 | 0.004245 | SNAP29, STX6, STX3, STX18, VAMP7, BET1L, VAMP4, VAMP1, YKT6, STX1B, VTI1A |
| KEGG | **Wnt signaling pathway** | 27 | 0.006047 | WNT5A, PRKCA, PPARD, WNT10B, APC2, VANGL1, ROCK1, **NLK**, VANGL2, CHP2, PPP3R1, CXXC4, FZD5, DAAM2, PRKCB, FZD6, SENP2, CSNK2A1, CCND2, JUN, PRICKLE2, FRAT1, FRAT2, PRKACB, WNT6, TBL1X, PLCB2 |
| KEGG | Neurotrophin signaling pathway | 23 | 0.007811 | IRS2, MAP2K1, PIK3CD, MAPK11, IRS1, PTPN11, RPS6KA3, YWHAG, CRKL, KRAS, CALML3, MAPK13, MAPK14, JUN, BCL2, CALM3, SH2B3, SORT1, RAP1B, NGFR, PIK3R3, ARHGDIA, AKT2 |
| KEGG | Long-term potentiation | 15 | 0.008717 | PRKCA, RPS6KA3, ADCY1, KRAS, MAP2K1, GRIA2, CALML3, PPP1R1A, CHP2, PPP3R1, CALM3, RAP1B, PRKACB, PLCB2, PRKCB |
| KEGG | Leukocyte transendothelial migration | 22 | 0.008916 | PRKCA, ITGAL, ROCK1, PIK3CD, ACTN1, MAPK11, CLDN10, VAV2, CXCL12, VASP, PXN, PTPN11, CTNNA2, PRKCB, RASSF5, MAPK13, MAPK14, CLDN2, ESAM, RAP1B, PIK3R3, MLLT4 |
| KEGG | Insulin signaling pathway | 24 | 0.010794 | IRS2, MAP2K1, PRKAB2, CBL, PIK3CD, HK2, PRKCI, IGF2, ACACB, IRS1, PRKAR2B, PPP1R3D, PRKAR2A, CRKL, KRAS, PPP1R3B, SLC2A4, CALML3, CALM3, PRKACB, PTPN1, PIK3R3, INSR, AKT2 |
| REACTOME | Signal attenuation | 5 | 0.012486 | DOK1, IRS2, IGF2, INSR, IRS1 |
| KEGG | Ubiquitin mediated proteolysis | 24 | 0.012817 | UBE2A, UBE2Z, XIAP, UBE2G1, CBL, PML, SAE1, UBE2I, NHLRC1, UBE3C, UBE2H, UBE2L3, UBE2R2, UBE2N, CUL3, FANCL, CUL2, UBE2O, UBE2D3, MGRN1, DDB2, DET1, UBE2W, NEDD4L |
| REACTOME | Signaling by BMP | 7 | 0.013304 | ACVR2A, ACVR2B, UBE2D3, SMAD9, CHRDL1, BMPR2, GREM2 |
| KEGG | Endocytosis | 30 | 0.013352 | LDLR, CHMP4B, STAM2, VPS37B, ASAP1, CLTC, CHMP2B, ACVR1B, DAB2, SMAP2, RAB11A, STAM, NEDD4L, EHD1, TGFBR1, MET, TGFBR2, CBL, PRKCI, PSD2, RAB11FIP4, AP2A2, RAB11FIP2, RAB11FIP3, PSD, RAB22A, GRK6, RAB11FIP1, PIP4K2B, SH3GL1 |
| PANTHER | Synaptic\_vesicle\_trafficking | 11 | 0.013469 | SYP, SNAP29, RAB3B, RAB3D, SYT11, SYT2, SYT7, VAMP1, STX1B, RIMS4, RIMS3 |
| KEGG | Pancreatic cancer | 15 | 0.014377 | ACVR1B, E2F3, KRAS, MAP2K1, RALBP1, TGFBR1, TGFBR2, PIK3CD, RALB, RALA, CDK6, RB1, STAT1, PIK3R3, AKT2 |
| KEGG | MAPK signaling pathway | 40 | 0.015704 | FGF19, TRAF2, FGF5, ZAK, PDGFB, PPP3R1, MAPKAPK3, PPM1A, SRF, ACVR1B, KRAS, MAP3K2, RASGRP1, MAPT, PRKACB, FGF1, AKT2, PTPN7, PRKCA, MAP2K1, CACNG8, NLK, TGFBR1, PTPN5, TGFBR2, CHP2, MAPK11, CACNG2, TAB1, STK4, PRKCB, CACNA2D4, DUSP5, RPS6KA3, CRKL, RASGRF2, MAPK13, MAPK14, JUN, RAP1B |
| KEGG | Chemokine signaling pathway | 30 | 0.016483 | ADCY1, CCL3, ADCY2, CXCL5, ADCY6, CX3CL1, CXCL12, PXN, CCL7, DOCK2, KRAS, CCL3L1, PRKACB, PIK3R3, PLCB2, GNG7, AKT2, MAP2K1, ROCK1, PIK3CD, STAT1, VAV2, PRKCB, CCL11, CCL13, CRKL, CCR6, GNB1, GRK6, RAP1B |
| KEGG | Chronic myeloid leukemia | 15 | 0.020221 | E2F3, MAP2K1, TGFBR1, CBL, PIK3CD, TGFBR2, CDK6, RB1, PTPN11, ACVR1B, CRKL, HDAC2, KRAS, PIK3R3, AKT2 |
| KEGG | Non-small cell lung cancer | 12 | 0.0205 | PRKCA, E2F3, RASSF5, KRAS, MAP2K1, PIK3CD, CDK6, RB1, PIK3R3, STK4, PRKCB, AKT2 |
| PANTHER | Metabotropic glutamate receptor group III pathway | 16 | 0.020757 | SNAP29, GRIK3, GRIN3A, GRIA4, PRKG1, STX1B, VTI1A, PRKAR2B, PRKAR2A, SLC1A3, GRIA2, GNB1, GRM6, PRKACB, VAMP1, GNG7 |
| REACTOME | Signaling by EGFR | 10 | 0.021144 | AP2A2, KRAS, MAP2K1, ARHGEF7, STAM2, CBL, STAM, CLTC, PXN, PTPN11 |
| KEGG | **Colorectal cancer** | 16 | 0.02418 | APC2, MAP2K1, TGFBR1, CYCS, PIK3CD, MET, TGFBR2, MLH1, FZD5, FZD6, ACVR1B, KRAS, JUN, BCL2, PIK3R3, AKT2 |
| KEGG | **Adherens junction** | 15 | 0.02501 | WASF3, TGFBR1, **NLK**, MET, TGFBR2, LMO7, ACTN1, **CTNNA2**, ACVR1B, CSNK2A1, PVRL1, PVRL2, PTPN1, INSR, **MLLT4** |
| PANTHER | Beta1 adrenergic receptor signaling pathway | 11 | 0.025081 | SNAP29, PRKAR2B, PRKAR2A, ADCY2, STX3, GNB1, RYR3, GNAS, PRKACB, VAMP1, GNG7 |
| KEGG | Renal cell carcinoma | 14 | 0.025706 | PDGFB, MAP2K1, MET, PIK3CD, PTPN11, CUL2, CRKL, KRAS, ETS1, JUN, SLC2A1, RAP1B, PIK3R3, AKT2 |
| KEGG | Glioma | 13 | 0.026203 | PRKCA, E2F3, KRAS, MAP2K1, PDGFB, CALML3, PIK3CD, CALM3, CDK6, RB1, PIK3R3, PRKCB, AKT2 |