**Supplementary Tables**

**Supplementary Table 1:** Primer sequences used for qRT PCR analysis

GAPDH\_fw AAGGTCGGAGTCAACGGATTT

GAPDH\_rev ACCAGAGTTAAA AGCAGCCCTG

B2M\_fw TGCTGTCTCCATGTTTGATGTATCT

B2M\_rev TCTCTGCTCCCCACCTCTAAGT

GALNT5\_fw TTGGAACATACGACCCTGGC

GALNT5\_rev CACCACACATCCACACCTTG

HOXB7\_fw TTCCTTCAACATGCACTGCG

HOXB7\_rev TCAGTTCCTGAGCTTCGCAT

CPA4\_fw AGGACCTGCAGATTTACCACG

CPA\_rev GGGAGATCCACTCTCGGGAA

MUC13\_fw GCTGTAACCAGACTGCGGAT

MUC13\_rev TTGAGACTGGAAGCAACGCA

DKK1\_fw TGACAACTACCAGCCGTACC

DKK1\_rev CAGGCGAGACAGATTTGCAC

KRTAP2-3\_fw AGCTGATCCTCAAGCACGAA

KRTAP2-3\_rev GGGTGATGAGTCAGTGGGAC

KRTAP3-1\_fw CTGCTGCAAGCCTGATACCT

KRTAP3-1\_rev GGTTGATCCCACTCAGTCCG

Cyclin D1\_fw TGCCAACCTCCTCAACGAC

Cyclin D1\_rev TCTGTTCCTCGCAGACCT CC

CDKN1B\_fw TAATTGGGGCTCCGGCT AAC

CDKN1B\_rev GAAGAATCGTCGGTTGCAGGT

GATA6\_fw GAAGCGCGTGCCTTCATCA

GATA6\_rev CTGCGCCATAAGGTGGTAGT

HOXA9\_fw AAAAACAACCCAGCGAAGGC

HOXA9\_rev GGGTTATTGGGATCGATGGGG

HOXB7\_fw (human specific) TGGGAACTGAATCAGGAAACTCA

HOXB7\_rev (human specific) CCAAAATTTCTCCTTTCT CCCTCC

GALNT5\_fw (human specific) TCATCCAGAACTGGTGAATCACAT

GALNT5\_rev (human specific) TGGGTCACAGGCAGCTACT

**Supplementary Table 2;** Summary and comparison of clinico-pathological characteristics of the colorectal cancer patients in cohort 1 (n = 110) and cohort 2 (n = 182).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Cohort 1 (n=110) | Cohort 2 (n=182) | p-value |
|  |  | No (%) | No (%) |
| Age | median | 62 | 64 | P<0.05\* |
| minimum | 31 | 30 |
| maximum | 80 | 92 |
| Sex | female | 40 (36.4) | 83 (45.6) | P<0.05\*\* |
| male | 70 (63.4) | 99 (54.6) |
| Stage | stage II | 11 (10) | 71 (39) | P<0.05\*\* |
| stage III | 40 (36.4) | 51 (28) |
| stage IV | 59 (53.6) | 60 (31) |
|  |  | GX 4 (2.2) |
| Grading | G1 | 2 (1.8) | 45 (24.7) | P<0.05\*\* |
| G2 | 81 (73.6) | 98 (53.8) |
| G3+G4 | 27 (36.4) | 35 (19.2) |

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

**Supplementary table 3** Univariate analysis of clinico-pathological parameters for the prediction of 5-years overall survival in patients with colorectal cancer in cohort 1 (n=110) and cohort 2 (n=182).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameter | Univariate analysis | | | |
|  | **Cohort 1** | | **Cohort 2** | |
| **HR (95% CI)** | ***p*-value** | **HR (95% CI)** | ***p*-value** |
| Age at diagnosis (years; continuous) | 1.02 (0.99-1.04) | 0.230 | 1.001 (0.98-1.02) | 0.772 |
| Gender  Male  Female | 1 (reference)  0.88 (0.52-1.48) | 0.635 | 1 (reference)  0.69 (0.42-1.13) | 0.147 |
| Tumor grade  G1+G2  G3+G4 | 1 (reference)  2.58 (1.53-4.37) | <0.001 | 1 (reference)  1.46 (0.83-2.57) | 0.183 |
| Tumor Stage  I-III  IV | 1 (reference)  2.86 (1.67-4.9) | <0.001 | 1 (reference)  7.07 (4.23-11.8) | <0.001 |
| MiR-196b-5p  low  high | 1 (reference)  0.450 (0.268-0.758) | 0.003 | 1 (reference)  0.624 (0.38-0.98) | 0.048 |

**Supplementary Table 4** Multivariate analysis of clinico-pathological parameters for the prediction of 5-years overall survival in patients with colorectal cancer in cohort 1 (n=110) and cohort 2 (n=182).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Multivariate analysis** | | | |
|  | **Cohort 1** | | **Cohort 2** | |
| **HR (95% CI)** | ***p*-value** | **HR (95% CI)** | ***p*-value** |
| **Age at diagnosis** (years; continuous**)** | 1.01 (0.98-1.04) | 0.312 | 1.02 (0.99-1.05) | 0.115 |
| **Gender**  Male  Female | 1 (reference)  1.07 (0.62-1.84) | 0.793 | 1 (reference)  0.734 (0.44 – 1.21) | 0.229 |
| **Tumor grade**  G1+G2  G3+G4 | 1 (reference)  2.57 (1.50-4.38) | 0.001 | 1 (reference)  1.38 (0.77-2.46) | 0.274 |
| **Tumor Stage**  I-III  IV | 1 (reference)  2.69 (1.54-4.69) | <0.001 | 1 (reference)  8.92 (5.06-15.7) | <0.001 |
| **MiR-196b-5p**  low  high | 1 (reference)  0.50 (0.29-0.84) | 0.010 | 1 (reference)  1.06 (0.64-1.75) | 0.807 |

**Supplementary table 5:** Up- and downregulated transcripts obtained from a microarray whole transcriptome profiling analysis in three independent biological replicates comparing the HCT116 miR-196b-5p stably overexpressing cells against control cells

|  |  |  |
| --- | --- | --- |
| Gene Symbol | Gene Assignment | Fold-Change(miR-196b-5p OE vs. OE Control) |
| PRF1 | NM\_001083116 | 2,24563 |
| MBNL3 | NM\_001170704 | 1,9677 |
| RNU7-55P | ENST00000459426 | 1,95474 |
| HIF1A-AS2 | ENST00000554254 | 1,86226 |
| SAT1 | NM\_002970 | 1,79687 |
| METTL7A | NM\_014033 | 1,70887 |
| CALCRL | ENST00000409998 | 1,68066 |
| LEMD1 | NM\_001001552 | 1,67825 |
| LOC151760 | XR\_171398 | 1,66902 |
| PELI2 | NM\_021255 | 1,66612 |
| NR2F1 | NM\_005654 | 1,66005 |
| B3GALT1 | NM\_020981 | 1,64909 |
| GRIN2B | NM\_000834 | 1,64811 |
| MAPK8IP1 | NM\_005456 | 1,64206 |
| ZNF221 | NM\_013359 | 1,62754 |
| IL23A | ENST00000390418 | 1,6275 |
| KLRK1 | ENST00000396451 | 1,62234 |
| TM4SF18 | NM\_001184723 | 1,61283 |
| FAT4 | NM\_001291285 | 1,60888 |
| OR2A42 | NM\_001001802 | 1,607 |
| NFIB | NM\_001190737 | 1,59117 |
| TANK | NM\_001199135 | 1,59064 |
| ACTR3C | ENST00000478393 | 1,58428 |
| CNTNAP3B | OTTHUMT00000129769 | 1,58131 |
| SESN3 | NM\_001271594 | 1,58118 |
| RNA5SP221 | ENST00000411271 | 1,57367 |
| CLCNKB | NM\_001165945 | 1,56909 |
| LOC100506127 | ENST00000530460 | 1,5645 |
| TNFSF18 | NM\_005092 | 1,56157 |
| SESTD1 | NM\_178123 | 1,56049 |
| MIR548I2 | NR\_031688 | 1,55382 |
| OR2A1 | NM\_001005287 | 1,54072 |
| GRAMD1C | NM\_001172105 | 1,53522 |
| PBX1 | NM\_001204961 | 1,51931 |
| RNU7-57P | ENST00000459540 | 1,51705 |
| TNRC6C-AS1 | NR\_040071 | 1,51501 |
| MIR548I2 | NR\_031688 | 1,50798 |
| TAS2R10 | ENST00000240619 | 1,50222 |
| MIR548L | NR\_031630 | -1,50385 |
| MIR4486 | NR\_039706 | -1,50391 |
| CGN | AF263462 | -1,50964 |
| GSTTP1 | NR\_003081 | -1,51934 |
| ZBED2 | ENST00000317012 | -1,52435 |
| RNA5SP361 | ENST00000362686 | -1,53585 |
| RNA5SP409 | ENST00000516815 | -1,53643 |
| RNU4-63P | ENST00000411313 | -1,5407 |
| ZNF804A | NM\_194250 | -1,54416 |
| LOC101060542 | NR\_110764 | -1,54904 |
| HOXB7 | NM\_004502 | -1,55316 |
| IPW | ENST00000547292 | -1,55897 |
| RNA5SP207 | ENST00000390874 | -1,56357 |
| TRGV5 | ENST00000390344 | -1,57482 |
| MIR130B | NR\_029845 | -1,58401 |
| CCDC106 | NM\_013301 | -1,5897 |
| TXLNGY | ENST00000445715 | -1,59438 |
| MIR544B | NR\_036088 | -1,6022 |
| TXLNGY | NR\_045128 | -1,60561 |
| LOC101927171 | XR\_242040 | -1,61848 |
| LOC100130172 | ENST00000500267 | -1,64255 |
| GAS6-AS2 | NR\_044993 | -1,66338 |
| MIR149 | NR\_029702 | -1,68 |
| RNU6ATAC18P | ENST00000408413 | -1,70476 |
| ARL14EPL | NM\_001195581 | -1,71038 |
| DKK1 | NM\_012242 | -1,77465 |
| MUC13 | NM\_033049 | -1,80149 |
| KIR2DS1 | NM\_014512 | -1,82954 |
| RNU6-736P | ENST00000516436 | -1,83458 |
| TTTY15 | NR\_001545 | -1,89931 |
| ANXA10 | NM\_007193 | -1,90447 |
| LOC101928687 | NR\_120641 | -2,05617 |
| LOC101928102 | XR\_248922 | -2,09693 |
| RNA5SP295 | ENST00000362655 | -2,11431 |
| GALNT5 | NM\_014568 | -2,13099 |
| RNU6-893P | ENST00000458841 | -2,30349 |
| KRTAP2-3 | NM\_001165252 | -2,4029 |
| KRTAP3-1 | NM\_031958 | -2,44953 |
| CPA4 | NM\_001163446 | -2,60247 |
| DDX3Y | NM\_004660 | -2,66755 |
| HIST1H2BB | NM\_021062 | -3,05063 |

**Supplementary Table 6:** Down-regulated genes identified in the microarray analysis underwent in silico target prediction analysis according to the details described in the methods section. The table shows only downregulated genes with potentially miR-196b-5p interaction sites.

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene name** | **log2 FoldChange** | **Number of prediction tools that identified a putative target** | **Seed match count in 3'UTR (based on ENSEMBL database)** |
| DDX3Y | -2.67 | **2** | 0 |
| CPA4 | -2.6 | **4** | 2 |
| KRTAP3-1 | -2.45 | **2** | 0 |
| KRTAP2-3 | -2.4 | **1** | 0 |
| GALNT5 | -2.13 | **NA** | 2 |
| LOC101928102 | -2.1 | **1** | 0 |
| ANXA10 | -1.9 | **3** | 0 |
| MUC13 | -1.8 | **2** | 0 |
| DKK1 | -1.77 | **3** | 0 |
| ARL14EPL | -1.71 | **1** | 0 |
| HOXB7 | -1.55 | **9** | 2 |
| ZNF804A | -1.54 | **1** | 0 |
| HDGFRP3 | -1.52 | **2** | 0 |
| ZBED2 | -1.52 | **2** | 0 |
| CGN | -1.51 | **4** | 1 |