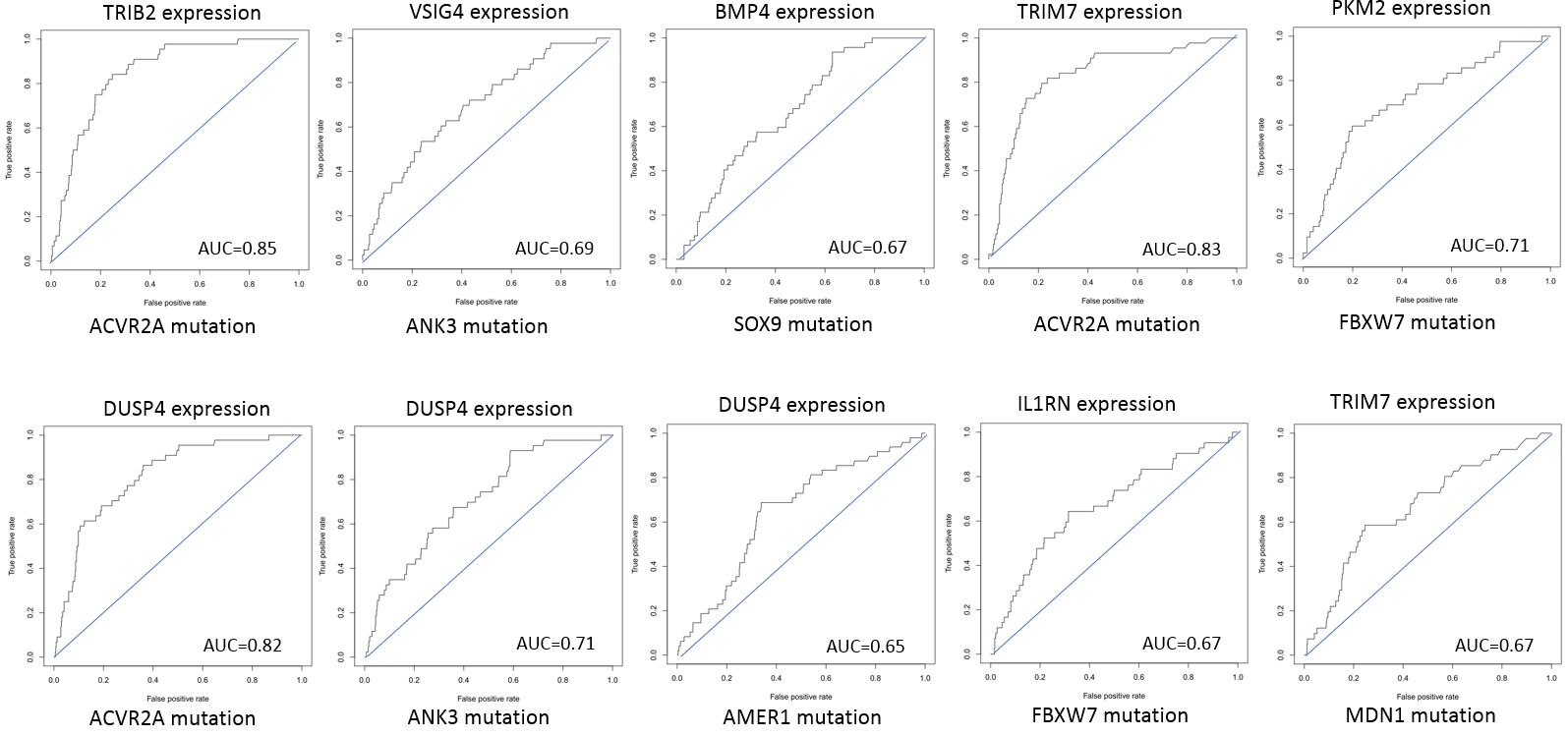
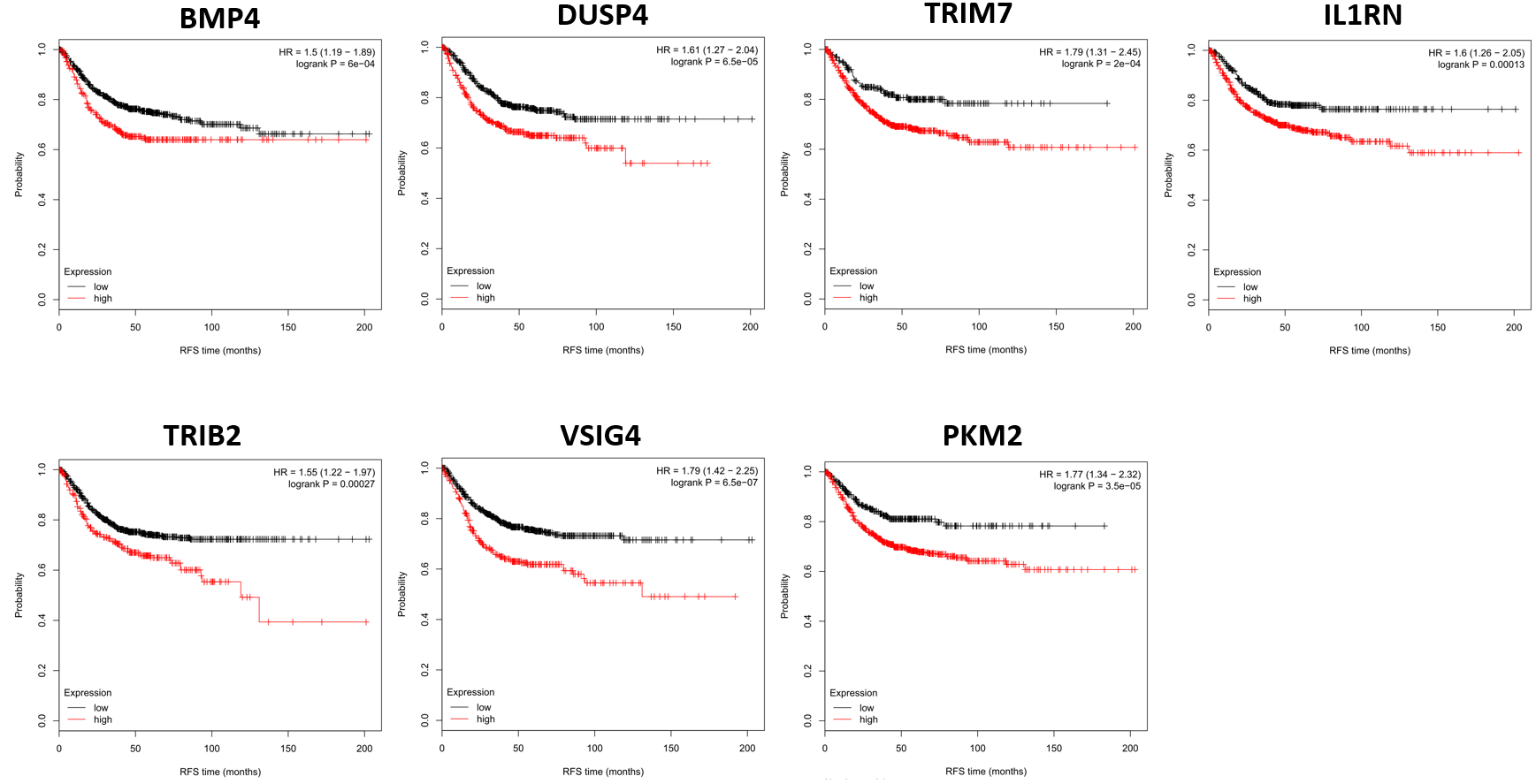
Supplementary Materials

Uncovering Potential Therapeutic Targets in Colorectal Cancer by Deciphering Mutational Status and Expression of Druggable Oncogenes

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**Figure S1.** Sensitivity and specificity of the seven selected potentially actionable genes associated with frequent disruptive mutations by ROC analysis.



**Figure S2.** High expression of the selected seven potentially actionable genes from the training dataset is linked to worse relapse-free survival based on Cox proportional hazard regressions at *p* < 0.05. The analysis was performed using the auto select best cutoff function, when all possible cutoff values between the upper and lower quartiles were computed and the best performing threshold was used as a cutoff.



**Figure S3.** Box plots indicating expression of the potentially actionable genes in the validation cohort. Two groups were stratified using the median value as a cutoff. Expression data was analyzed by Student’s *t*-test. *Y*-axis is defined as −ΔCT.



**Figure S4.** Recurrence-free survival of the clinical validation cohort, stratifying the population into high and low risk using the Youden index as a cutoff.

**Table S1.** Primer sequences used in the qPCR validation.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene** | **Forward Primer** | **Tm** | **Reverse Primer** | **Tm** | **Length of Product** |
| *TRIB2* | GGACCTCAAGCTGCGGAAAT | 57.9 | GTAGGCGTCTTCCAGGCTTT | 57.3 | 79 |
| *DUSP4* | CCGACATCTGCCTGCTCAAA | 57.8 | GCCAGGGCCTTGGTTTTAGA | 57.8 | 82 |
| *PKM2* | TGAGATCCGAACTGGGCTCA | 58.1 | TTCTTCAGCTCCACCTCTGC | 57.0 | 57 |
| *VSIG4* | CAGCACAGCGACATTGTGAA | 56.1 | GGTGCCTCAGTCTTGGTCTT | 57.1 | 71 |
| *IL1RN* | GCTGGATACTTGCAAGGACCA | 57.3 | GCATGAGGCTCAATGGGTAC | 56.0 | 71 |
| *TRIM7* | AGCTCTTGGAGTCCAGGCT | 58.4 | CCTCACAGTCCTCCAGTTCC | 57.1 | 54 |
| *BMP4* | CAGATCCACAGCACTGGTCT | 56.8 | CTTCGTGGTGGAAGCTCCTC | 57.8 | 79 |

**Table S2.** Clinical characterization of colon and rectal cancer patient populations from the training datasets. N/A—data unavailable.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Characteristic** | | **Colon (%)** | **Rectal (%)** |  |
| **Sex** |  |  |  |  |
|  | Male | 52.9 | 54.1 |  |
|  | Female | 47.1 | 45.9 |  |
| **Age/years** | |  |  |  |
|  | <60 | 27.5 | 31.2 |  |
|  | ≥60 | 72.5 | 68.8 |  |
| **Race** |  |  |  |  |
|  | White/Caucasian | 46.6 | 48.2 |  |
|  | Asian | 2.4 | 0.6 |  |
|  | Black/African American | 12.9 | 3.5 |  |
|  | N/A | 38.1 | 47.6 |  |
| **Clinical stage** | |  |  |  |
|  | I | 16.6 | 19.4 |  |
|  | II | 38.8 | 30.0 |  |
|  | III | 28.1 | 30.6 |  |
|  | IV | 14.2 | 14.7 |  |
|  | N/A | 2.4 | 5.3 |  |
| **Histological type** | |  |  |  |
|  | adenocarcinoma | 85.4 | 88.8 |  |
|  | mucinous adenocarcinoma | 13.5 | 7.6 |  |
|  | N/A | 1.1 | 3.5 |  |
| **Residual tumor after surgery** | |  |  |  |
|  | R0 | 72.1 | 74.7 |  |
|  | R1 | 0.9 | 1.2 |  |
|  | R2 | 5.7 | 7.1 |  |
|  | RX | 5.4 | 2.9 |  |
|  | N/A | 15.9 | 14.1 |  |
| **Venous invasion** | |  |  |  |
|  | Present | 20.9 | 22.4 |  |
|  | Absent | 65.8 | 64.1 |  |
|  | N/A | 13.3 | 13.5 |  |
| **Lymphatic invasion** | |  |  |  |
|  | Present | 35.7 | 40.0 |  |
|  | Absent | 54.7 | 49.4 |  |
|  | N/A | 9.6 | 10.6 |  |
| **Microsatellite instability** | |  |  |  |
|  | Yes | 2.4 | 0.0 |  |
|  | No | 17.6 | 14.7 |  |
|  | N/A | 80.0 | 85.3 |  |
| **Follow-up** | |  |  |  |
|  | Median follow up (months) | 22.3 | 20.6 |  |
|  | Death event | 22.2% | 15.9% |  |

**Table S3.** Top 40 most frequently mutated genes (disruptive mutations exclusively) in the CRC patient population (*n* = 582).

|  |  |
| --- | --- |
| Gene Symbol | Proportion of Patients |
| APC | 69.4% |
| TTN | 23.4% |
| TP53 | 11.9% |
| SYNE1 | 9.3% |
| AMER1 | 8.2% |
| SOX9 | 8.1% |
| ACVR2A | 7.7% |
| ANK3, ARID1A | 7.6% |
| FBXW7, MDN1 | 7.2% |
| SYNE2 | 6.9% |
| BMPR2, CCDC168, FAT4, LRP1B, RNF43 | 6.7% |
| CSMD3, DNAH11, KMT2C, ZFHX4 | 6.4% |
| DOCK3, MUC16 | 6.2% |
| ATM, RYR2 | 5.8% |
| PRKDC, SACS | 5.7% |
| ABCA13, ADGRV1, DNAH5 | 5.5% |
| SRCAP, USH2A | 5.3% |
| DNAH9 | 5.2% |
| MYCBP2 | 5.2% |
| COL6A3, FAT1, NBEA | 5.0% |
| BRCA2, DNAH7, DNAH8 | 4.8% |

**Table S6.** List of upregulated genes associated with worse clinical outcome (RFS, *p* < 0.05). Highlighted genes were validated in clinical specimens.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Mutation** | **Gene Symbol** | **Expression in Mutated** | **Expression in Wild Type** | **FC** | **MW *p*-Value** | **AUC** | **Sensitivity** | **Specificity** | **Potentially Actionable** | **Survival (RFS) *p*-Value** |
| ACVR2A | TRIB2 | 1272 | 440 | 2.89 | 1.35E-14 | 0.85 | 0.75 | 0.84 | yes | 0.00027 |
| ACVR2A | TRIM7 | 529.5 | 51 | 10.38 | 2.47E-13 | 0.83 | 0.76 | 0.82 | yes | 2.00E-04 |
| ACVR2A | DUSP4 | 1738.5 | 287.5 | 6.05 | 2.59E-12 | 0.82 | 0.64 | 0.86 | yes | 6.50E-05 |
| ACVR2A | FECH | 720.5 | 376.5 | 1.91 | 1.98E-14 | 0.85 | 0.82 | 0.75 | no | 0.0024 |
| ACVR2A | MBP | 1957 | 1144 | 1.71 | 6.14E-14 | 0.84 | 0.74 | 0.86 | no | 0.0088 |
| ACVR2A | GAS2L1 | 865.5 | 483 | 1.79 | 4.83E-13 | 0.83 | 0.72 | 0.84 | no | 0.036 |
| ACVR2A | FAM59A | 272.5 | 158 | 1.72 | 2.40E-12 | 0.82 | 0.74 | 0.86 | no | 0.017 |
| ACVR2A | GMEB1 | 322 | 220.5 | 1.46 | 3.22E-12 | 0.82 | 0.61 | 0.89 | no | 0.0053 |
| ACVR2A | TNNT1 | 116.5 | 10 | 11.65 | 1.84E-11 | 0.80 | 0.65 | 0.84 | no | 0.017 |
| ACVR2A | BLVRA | 790 | 337 | 2.34 | 2.35E-11 | 0.80 | 0.82 | 0.70 | no | 0.037 |
| ACVR2A | CIITA | 685 | 184.5 | 3.71 | 4.59E-11 | 0.80 | 0.73 | 0.77 | no | 0.00023 |
| AMER1 | DUSP4 | 707 | 298 | 2.37 | 7.00E-04 | 0.65 | 0.66 | 0.69 | yes | 6.50E-05 |
| AMER1 | F5 | 102 | 61 | 1.67 | 5.13E-03 | 0.62 | 0.42 | 0.81 | yes | 0.0057 |
| AMER1 | TCN1 | 171.5 | 62.5 | 2.74 | 5.76E-03 | 0.62 | 0.59 | 0.67 | yes | 0.039 |
| AMER1 | CARD6 | 263.5 | 178.5 | 1.48 | 7.20E-04 | 0.65 | 0.52 | 0.77 | no | 0.00016 |
| AMER1 | WDR72 | 549.5 | 245.5 | 2.24 | 2.62E-03 | 0.63 | 0.63 | 0.63 | no | 0.046 |
| ANK3 | DUSP4 | 854 | 301 | 2.84 | 4.65E-06 | 0.71 | 0.41 | 0.93 | yes | 6.50E-05 |
| ANK3 | TRIM7 | 217 | 53 | 4.09 | 5.96E-06 | 0.71 | 0.58 | 0.79 | yes | 2.00E-04 |
| ANK3 | MAPK11 | 153 | 89 | 1.72 | 8.83E-06 | 0.70 | 0.68 | 0.70 | yes | 0.02 |
| ANK3 | MAPK12 | 195 | 107 | 1.82 | 1.51E-05 | 0.70 | 0.70 | 0.60 | yes | 0.04 |
| ANK3 | PLA2G4A | 488 | 112 | 4.36 | 1.88E-05 | 0.70 | 0.69 | 0.67 | yes | 0.044 |
| ANK3 | VSIG4 | 430 | 184 | 2.34 | 2.26E-05 | 0.69 | 0.76 | 0.53 | yes | 6.50E-07 |
| ANK3 | FECH | 619 | 382 | 1.62 | 1.78E-07 | 0.74 | 0.78 | 0.60 | no | 0.0024 |
| ANK3 | C12ORF57 | 2357 | 1415 | 1.67 | 2.47E-06 | 0.72 | 0.69 | 0.67 | no | 0.0026 |
| ANK3 | KRT7 | 243 | 101 | 2.41 | 5.28E-06 | 0.71 | 0.73 | 0.60 | no | 4.50E-05 |
| ANK3 | GAS2L1 | 722 | 490 | 1.47 | 2.59E-05 | 0.69 | 0.63 | 0.72 | no | 0.036 |
| APC | C13ORF23 | 3725 | 2575 | 1.45 | 7.67E-12 | 0.68 | 0.54 | 0.77 | no | 0.007 |
| APC | SYT7 | 2288 | 1420 | 1.61 | 3.28E-11 | 0.67 | 0.59 | 0.67 | no | 0.0081 |
| APC | MEX3A | 508 | 272 | 1.87 | 1.21E-10 | 0.67 | 0.54 | 0.75 | no | 4.80E-03 |
| APC | EPB41L1 | 2881 | 1966 | 1.47 | 2.70E-08 | 0.64 | 0.55 | 0.70 | no | 0.012 |
| APC | ASCL2 | 3459 | 2008 | 1.72 | 2.90E-08 | 0.64 | 0.40 | 0.90 | no | 3.90E-06 |
| ARID1A | CHST6 | 111 | 46 | 2.41 | 5.67E-08 | 0.75 | 0.60 | 0.84 | yes | 0.0015 |
| ARID1A | SPNS2 | 3704 | 2170 | 1.71 | 3.22E-07 | 0.73 | 0.63 | 0.81 | yes | 0.018 |
| ARID1A | CFD | 783 | 307 | 2.55 | 1.83E-05 | 0.70 | 0.77 | 0.56 | yes | 0.013 |
| ARID1A | MAPK12 | 237 | 106 | 2.24 | 2.89E-05 | 0.69 | 0.80 | 0.53 | yes | 0.04 |
| ARID1A | ITGB4 | 17840 | 11977 | 1.49 | 4.33E-05 | 0.69 | 0.88 | 0.44 | yes | 0.014 |
| ARID1A | SEMA4B | 4887 | 3007 | 1.63 | 5.02E-05 | 0.69 | 0.68 | 0.70 | yes | 0.0098 |
| ARID1A | CDC42EP1 | 6413 | 3655 | 1.75 | 1.07E-08 | 0.76 | 0.57 | 0.84 | no | 0.0028 |
| ARID1A | GAS2L1 | 789 | 489 | 1.61 | 1.69E-08 | 0.76 | 0.73 | 0.70 | no | 0.036 |
| ARID1A | FLNB | 17616 | 12092 | 1.46 | 2.60E-07 | 0.74 | 0.70 | 0.72 | no | 0.024 |
| ARID1A | ZMYND15 | 112 | 73 | 1.53 | 1.04E-05 | 0.70 | 0.71 | 0.60 | no | 0.00026 |
| ARID1A | CTXN1 | 115 | 52 | 2.21 | 3.26E-05 | 0.69 | 0.71 | 0.63 | no | 0.016 |
| ARID1A | POLR2A | 9019 | 5694 | 1.58 | 4.42E-05 | 0.69 | 0.79 | 0.58 | no | 0.0015 |
| ARID1A | FECH | 593 | 386 | 1.54 | 4.86E-05 | 0.69 | 0.65 | 0.72 | no | 0.0024 |
| FBXW7 | MAP3K6 | 807.5 | 504.5 | 1.6 | 3.63E-07 | 0.74 | 0.52 | 0.88 | yes | 0.033 |
| FBXW7 | PKM2 | 53636 | 34701 | 1.55 | 3.82E-06 | 0.71 | 0.80 | 0.60 | yes | 3.50E-05 |
| FBXW7 | TRIB2 | 822.5 | 463 | 1.78 | 1.20E-04 | 0.68 | 0.86 | 0.45 | yes | 0.00027 |
| FBXW7 | IL1RN | 742 | 327 | 2.27 | 2.57E-04 | 0.67 | 0.69 | 0.64 | yes | 0.00013 |
| FBXW7 | CDC42EP1 | 5535 | 3667 | 1.51 | 6.55E-07 | 0.73 | 0.54 | 0.86 | no | 0.0028 |
| FBXW7 | GJB5 | 151 | 16.5 | 9.15 | 1.01E-06 | 0.73 | 0.75 | 0.62 | no | 0.02 |
| FBXW7 | GAS2L1 | 776.5 | 491 | 1.58 | 1.42E-05 | 0.70 | 0.79 | 0.57 | no | 0.036 |
| FBXW7 | KRT6A | 100 | 25 | 4 | 2.79E-05 | 0.69 | 0.69 | 0.67 | no | 4.30E-09 |
| FBXW7 | PYGL | 1650 | 828 | 1.99 | 2.88E-05 | 0.69 | 0.69 | 0.62 | no | 0.016 |
| FBXW7 | ENO2 | 1027 | 597 | 1.72 | 4.50E-05 | 0.69 | 0.74 | 0.62 | no | 0.0028 |
| FBXW7 | CDR2L | 799.5 | 464.5 | 1.72 | 5.11E-05 | 0.69 | 0.71 | 0.67 | no | 1.40E-06 |
| FBXW7 | TRIM29 | 4084.5 | 1967.5 | 2.08 | 5.15E-05 | 0.69 | 0.53 | 0.79 | no | 0.046 |
| FBXW7 | RHOF | 1338.5 | 776.5 | 1.72 | 5.23E-05 | 0.69 | 0.76 | 0.57 | no | 0.00039 |
| FBXW7 | BAG2 | 544.5 | 237 | 2.3 | 1.38E-04 | 0.68 | 0.59 | 0.74 | no | 0.022 |
| FBXW7 | ACSL1 | 1774.5 | 1198 | 1.48 | 1.46E-04 | 0.68 | 0.67 | 0.64 | no | 0.023 |
| FBXW7 | PIK3AP1 | 1030.5 | 613 | 1.68 | 2.14E-04 | 0.67 | 0.57 | 0.79 | no | 0.032 |
| FBXW7 | GJB3 | 1360.5 | 863 | 1.58 | 2.17E-04 | 0.67 | 0.71 | 0.62 | no | 7.90E-06 |
| FBXW7 | FECH | 577.5 | 389 | 1.48 | 2.60E-04 | 0.67 | 0.88 | 0.43 | no | 0.0024 |
| FBXW7 | BLVRA | 598.5 | 346.5 | 1.73 | 2.71E-04 | 0.67 | 0.66 | 0.69 | no | 0.037 |
| FBXW7 | ZNF385A | 513.5 | 291 | 1.76 | 2.90E-04 | 0.67 | 0.84 | 0.48 | no | 2.50E-05 |
| MDN1 | FCGR2C | 149 | 54 | 2.76 | 1.45E-05 | 0.70 | 0.61 | 0.76 | yes | 0.0016 |
| MDN1 | TRIM16L | 411 | 180 | 2.28 | 1.17E-04 | 0.68 | 0.50 | 0.83 | yes | 0.047 |
| MDN1 | FCGR2B | 188 | 88 | 2.14 | 1.37E-04 | 0.68 | 0.62 | 0.76 | yes | 3.60E-06 |
| MDN1 | TUBB2A | 2099 | 1147 | 1.83 | 2.50E-04 | 0.67 | 0.51 | 0.78 | yes | 0.0017 |
| MDN1 | TRIM7 | 235 | 54 | 4.35 | 3.30E-04 | 0.67 | 0.75 | 0.59 | yes | 2.00E-04 |
| MDN1 | HLA-DQB2 | 185 | 71 | 2.61 | 2.75E-05 | 0.70 | 0.56 | 0.78 | no | 0.011 |
| MDN1 | EMR2 | 462 | 300 | 1.54 | 4.02E-05 | 0.69 | 0.65 | 0.68 | no | 0.028 |
| MDN1 | FECH | 573 | 384 | 1.49 | 4.31E-05 | 0.69 | 0.64 | 0.73 | no | 2.40E-03 |
| MDN1 | MORF4 | 108 | 6 | 18 | 2.82E-04 | 0.67 | 0.62 | 0.71 | no | 0.00072 |
| MDN1 | TNFSF12-TNFSF13 | 419 | 125 | 3.35 | 2.93E-04 | 0.67 | 0.65 | 0.63 | no | 0.0011 |
| MDN1 | LILRB3 | 117 | 60 | 1.95 | 3.14E-04 | 0.67 | 0.70 | 0.61 | no | 0.00022 |
| SOX9 | BMP4 | 3036 | 1769 | 1.72 | 1.59E-04 | 0.67 | 0.37 | 0.94 | yes | 6.00E-04 |
| SOX9 | SEMA4B | 4392 | 3004 | 1.46 | 6.36E-04 | 0.65 | 0.68 | 0.62 | yes | 0.0098 |
| SOX9 | PADI2 | 1834 | 1040 | 1.76 | 4.76E-04 | 0.65 | 0.37 | 0.89 | no | 0.022 |
| SOX9 | GPT | 278 | 144 | 1.93 | 5.72E-04 | 0.65 | 0.58 | 0.72 | no | 0.022 |
| SOX9 | RASGRF2 | 154 | 97 | 1.59 | 7.17E-04 | 0.65 | 0.67 | 0.64 | no | 1.80E-06 |
| SYNE1 | TRIM16L | 438.5 | 173.5 | 2.53 | 2.41E-07 | 0.71 | 0.51 | 0.91 | yes | 0.047 |
| SYNE1 | TRIM7 | 229.5 | 53 | 4.33 | 2.72E-06 | 0.69 | 0.76 | 0.61 | yes | 2.00E-04 |
| SYNE1 | MAPK12 | 229.5 | 106 | 2.17 | 4.97E-06 | 0.69 | 0.79 | 0.52 | yes | 0.04 |
| SYNE1 | RELT | 485 | 323.5 | 1.5 | 5.04E-06 | 0.69 | 0.81 | 0.59 | yes | 0.024 |
| SYNE1 | FCGR2C | 153.5 | 55 | 2.79 | 9.87E-06 | 0.68 | 0.67 | 0.65 | yes | 0.0016 |
| SYNE1 | SLC4A11 | 285.5 | 135 | 2.11 | 1.39E-05 | 0.68 | 0.58 | 0.78 | yes | 0.00016 |
| SYNE1 | IL4I1 | 375.5 | 202 | 1.86 | 1.48E-05 | 0.68 | 0.79 | 0.54 | yes | 0.013 |
| SYNE1 | FECH | 599 | 378.5 | 1.58 | 2.33E-07 | 0.71 | 0.61 | 0.74 | no | 0.0024 |
| SYNE1 | GAS2L1 | 746.5 | 489 | 1.53 | 5.55E-07 | 0.71 | 0.68 | 0.70 | no | 0.036 |
| SYNE1 | C12ORF57 | 2216.5 | 1405.5 | 1.58 | 4.11E-06 | 0.69 | 0.57 | 0.74 | no | 0.0026 |
| SYNE1 | LOC154761 | 111.5 | 33 | 3.38 | 2.13E-05 | 0.68 | 0.52 | 0.85 | no | 0.0039 |
| SYNE1 | AGPAT4 | 267 | 143.5 | 1.86 | 2.32E-05 | 0.67 | 0.77 | 0.54 | no | 0.018 |
| TP53 | CSF2RA | 140 | 96.5 | 1.45 | 8.99E-03 | 0.60 | 0.61 | 0.60 | yes | 0.00049 |
| TP53 | SDS | 223 | 133 | 1.68 | 1.25E-03 | 0.62 | 0.65 | 0.65 | no | 9.10E-05 |
| TP53 | ZNF304 | 106 | 61 | 1.74 | 1.91E-03 | 0.62 | 0.74 | 0.49 | no | 2.50E-05 |

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