

EWS/FLI utilizes NKX2-2 to repress mesenchymal features of ewing sarcoma

Supplementary Material

Table S1: List of NKX2-2 Repressed and Activated Gene Targets. We found that NKX2-2 repressed 189 and activated 49 genes at fold change ≥ 1.5 ($|\log_2 T/C| \geq 0.585$) and maximum DESeq FDR ≥ 50 . “T/C” denotes the ratio of reads between “treatment” (NKX2-2 shRNA) and “control” (control shRNA). Genes in boldface are also EWS/FLI-regulated, i.e. genes that are in the Venn diagram intersections (Fig. 1c).

| <u>Repressed Genes</u> | | | |
|------------------------|---------------------|---------------|-----------------|
| Ensembl ID | Gene Symbol | Max DESeq FDR | $ \log_2(T/C) $ |
| ENSG00000175899 | A2M | 824.2042 | 2.5672 |
| ENSG00000171201 | SMR3B | 839.0181 | 2.4857 |
| ENSG00000130203 | APOE | 638.7277 | 2.2606 |
| ENSG00000109846 | CRYAB | 750.5163 | 2.0917 |
| ENSG00000113083 | LOX | 650.2720 | 2.0691 |
| ENSG00000120708 | TGFBI | 447.5006 | 1.8439 |
| ENSG00000182492 | BGN | 397.8915 | 1.7014 |
| ENSG00000168542 | COL3A1 | 388.0310 | 1.6943 |
| ENSG00000100292 | HMOX1 | 348.7940 | 1.6022 |
| ENSG00000140092 | FBLN5 | 287.2886 | 1.4978 |
| ENSG00000106333 | PCOLCE | 277.2993 | 1.4858 |
| ENSG00000164220 | F2RL2 | 224.5895 | 1.3074 |
| ENSG00000181104 | F2R | 197.7093 | 1.3003 |
| ENSG00000163017 | ACTG2 | 186.1635 | 1.2566 |
| ENSG00000224729 | RP13-530H6.2 | 198.2907 | 1.2327 |
| ENSG00000166426 | CRABP1 | 177.9007 | 1.2267 |
| ENSG00000152377 | SPOCK1 | 194.4080 | 1.2238 |
| ENSG00000050165 | DKK3 | 194.3323 | 1.2094 |
| ENSG00000162998 | FRZB | 177.4267 | 1.1972 |
| ENSG00000163661 | PTX3 | 161.1237 | 1.1891 |
| ENSG00000105270 | CLIP3 | 173.1340 | 1.1832 |
| ENSG00000122786 | CALD1 | 197.7093 | 1.1795 |
| ENSG00000174938 | SEZ6L2 | 177.9007 | 1.1714 |
| ENSG00000113721 | PDGFRB | 159.8752 | 1.1524 |
| ENSG00000138080 | EMILIN1 | 163.2299 | 1.1487 |

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|-----------------|----------------|----------|--------|
| ENSG00000255887 | HTR4 | 148.7123 | 1.1307 |
| ENSG00000132718 | SYT11 | 145.7101 | 1.1229 |
| ENSG00000140682 | TGFB1I1 | 178.7892 | 1.1204 |
| ENSG00000001036 | FUCA2 | 135.8940 | 1.1032 |
| ENSG00000163297 | ANTXR2 | 145.0749 | 1.0982 |
| ENSG00000137558 | PI15 | 135.0315 | 1.0911 |
| ENSG00000084636 | COL16A1 | 154.8618 | 1.0823 |
| ENSG00000170445 | HARS | 116.6256 | 1.0579 |
| ENSG00000140416 | TPM1 | 162.9830 | 1.0578 |
| ENSG00000159840 | ZYX | 146.2752 | 1.0574 |
| ENSG00000147100 | SLC16A2 | 129.2495 | 1.0493 |
| ENSG00000163359 | COL6A3 | 154.8618 | 1.0387 |
| ENSG00000185585 | OLFML2A | 139.5245 | 1.0355 |
| ENSG00000172020 | GAP43 | 138.2877 | 1.0349 |
| ENSG00000091129 | NRCAM | 131.3168 | 1.0223 |
| ENSG00000163346 | PBXIP1 | 118.8992 | 1.0092 |
| ENSG00000130176 | CNN1 | 157.1690 | 1.0060 |
| ENSG00000175868 | CALCB | 97.6286 | 1.0018 |
| ENSG00000007062 | PROM1 | 119.5708 | 0.9975 |
| ENSG00000143341 | HMCN1 | 107.9254 | 0.9945 |
| ENSG00000100095 | SEZ6L | 104.3363 | 0.9831 |
| ENSG00000172638 | EFEMP2 | 122.1316 | 0.9617 |
| ENSG00000113140 | SPARC | 113.8953 | 0.9529 |
| ENSG00000100968 | NFATC4 | 95.6476 | 0.9523 |
| ENSG00000148677 | ANKRD1 | 144.9075 | 0.9509 |
| ENSG00000174099 | MSRB3 | 119.5708 | 0.9492 |
| ENSG00000119630 | PGF | 112.9963 | 0.9484 |
| ENSG00000180573 | HIST1H2AC | 95.3523 | 0.9442 |
| ENSG00000142871 | CYR61 | 122.6468 | 0.9414 |
| ENSG00000141338 | ABCA8 | 102.8714 | 0.9372 |
| ENSG00000120896 | SORBS3 | 107.5048 | 0.9319 |
| ENSG00000117228 | GBP1 | 98.6774 | 0.9256 |
| ENSG00000115414 | FN1 | 93.2659 | 0.9066 |
| ENSG00000171195 | MUC7 | 108.4639 | 0.9031 |
| ENSG00000163395 | IGFN1 | 89.1615 | 0.8949 |
| ENSG00000174807 | CD248 | 97.7315 | 0.8923 |

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|-----------------|-----------------|----------|--------|
| ENSG00000026297 | RNASET2 | 84.3447 | 0.8848 |
| ENSG00000112769 | LAMA4 | 92.0180 | 0.8815 |
| ENSG00000213694 | S1PR3 | 93.4723 | 0.8723 |
| ENSG00000164488 | DACT2 | 91.3191 | 0.8670 |
| ENSG00000123384 | LRP1 | 94.8068 | 0.8653 |
| ENSG00000123933 | MXD4 | 92.1400 | 0.8647 |
| ENSG00000109576 | AADAT | 88.8271 | 0.8565 |
| ENSG00000232783 | AC073135.3 | 86.1990 | 0.8549 |
| ENSG00000132386 | SERPINF1 | 79.2795 | 0.8540 |
| ENSG00000103485 | QPRT | 80.5658 | 0.8507 |
| ENSG00000211445 | GPX3 | 95.0507 | 0.8500 |
| ENSG00000136235 | GPNMB | 125.6224 | 0.8428 |
| ENSG00000131724 | IL13RA1 | 92.2947 | 0.8420 |
| ENSG00000109323 | MANBA | 90.7411 | 0.8409 |
| ENSG00000115718 | PROC | 81.4366 | 0.8408 |
| ENSG00000168994 | C6orf145 | 80.7035 | 0.8352 |
| ENSG00000163485 | ADORA1 | 92.1477 | 0.8341 |
| ENSG00000164938 | TP53INP1 | 79.2954 | 0.8321 |
| ENSG00000171940 | ZNF217 | 74.9894 | 0.8251 |
| ENSG00000118733 | OLFM3 | 74.4940 | 0.8250 |
| ENSG00000126016 | AMOT | 79.1751 | 0.8215 |
| ENSG00000144730 | IL17RD | 90.7412 | 0.8207 |
| ENSG00000013016 | EHD3 | 90.2576 | 0.8147 |
| ENSG00000076706 | MCAM | 96.1121 | 0.8129 |
| ENSG00000180998 | GPR137C | 62.2068 | 0.8104 |
| ENSG00000133110 | POSTN | 93.8375 | 0.8089 |
| ENSG00000164692 | COL1A2 | 88.0884 | 0.8041 |
| ENSG00000169231 | THBS3 | 70.3029 | 0.8030 |
| ENSG00000168280 | KIF5C | 67.2596 | 0.8029 |
| ENSG00000155130 | MARCKS | 68.7284 | 0.8026 |
| ENSG00000162490 | C1orf187 | 88.9574 | 0.8025 |
| ENSG00000168487 | BMP1 | 78.8870 | 0.8007 |
| ENSG00000119522 | DENND1A | 69.3145 | 0.8002 |
| ENSG00000245532 | NEAT1 | 61.6564 | 0.7993 |
| ENSG00000168461 | RAB31 | 77.1856 | 0.7985 |
| ENSG00000176720 | BOK | 82.4016 | 0.7968 |

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|-----------------|---------------------|---------|--------|
| ENSG00000166750 | SLFN5 | 66.4069 | 0.7968 |
| ENSG00000170500 | LONRF2 | 62.5051 | 0.7967 |
| ENSG00000064042 | LIMCH1 | 65.5743 | 0.7945 |
| ENSG00000149212 | SESN3 | 68.1630 | 0.7911 |
| ENSG00000162493 | PDPN | 65.1762 | 0.7899 |
| ENSG00000173698 | GPR64 | 71.5143 | 0.7875 |
| ENSG00000162804 | SNED1 | 69.0734 | 0.7869 |
| ENSG00000075223 | SEMA3C | 67.8865 | 0.7862 |
| ENSG00000144455 | SUMF1 | 65.5743 | 0.7844 |
| ENSG00000169071 | ROR2 | 75.8909 | 0.7837 |
| ENSG00000256061 | DYX1C1-CCPG1 | 68.4338 | 0.7803 |
| ENSG00000134013 | LOXL2 | 80.7819 | 0.7742 |
| ENSG00000150593 | PDCD4 | 63.3693 | 0.7726 |
| ENSG00000166340 | TPP1 | 80.7120 | 0.7698 |
| ENSG00000007866 | TEAD3 | 67.3072 | 0.7641 |
| ENSG00000087303 | NID2 | 67.6544 | 0.7616 |
| ENSG00000166444 | ST5 | 63.5447 | 0.7592 |
| ENSG00000168016 | TRANK1 | 72.5045 | 0.7586 |
| ENSG00000090776 | EFNB1 | 63.4265 | 0.7585 |
| ENSG00000141756 | FKBP10 | 68.4422 | 0.7580 |
| ENSG00000138829 | FBN2 | 58.2532 | 0.7577 |
| ENSG00000168386 | FILIP1L | 69.6937 | 0.7573 |
| ENSG00000179820 | MYADM | 65.5680 | 0.7567 |
| ENSG00000135363 | LMO2 | 57.9719 | 0.7542 |
| ENSG00000137070 | IL11RA | 64.4975 | 0.7539 |
| ENSG00000119655 | NPC2 | 63.1146 | 0.7536 |
| ENSG00000088882 | CPXM1 | 66.3513 | 0.7523 |
| ENSG00000197444 | OGDHL | 69.8468 | 0.7505 |
| ENSG00000173548 | SNX33 | 61.2146 | 0.7388 |
| ENSG00000154262 | ABCA6 | 62.7327 | 0.7380 |
| ENSG00000107796 | ACTA2 | 69.8468 | 0.7357 |
| ENSG00000135406 | PRPH | 61.3089 | 0.7353 |
| ENSG00000102032 | RENBP | 61.7957 | 0.7351 |
| ENSG00000123200 | ZC3H13 | 59.6177 | 0.7334 |
| ENSG00000090238 | YPEL3 | 60.3276 | 0.7327 |
| ENSG00000154258 | ABCA9 | 61.8086 | 0.7305 |

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|-----------------|----------------------|---------|--------|
| ENSG00000019549 | SNAI2 | 72.7718 | 0.7304 |
| ENSG00000132563 | REEP2 | 59.5967 | 0.7296 |
| ENSG00000229036 | RP1-20N2.6 | 54.4579 | 0.7280 |
| ENSG00000187714 | SLC18A3 | 61.0677 | 0.7227 |
| ENSG00000185924 | RTN4RL1 | 57.4379 | 0.7204 |
| ENSG00000115363 | FAM176A | 64.7503 | 0.7196 |
| ENSG00000100196 | KDELR3 | 62.9979 | 0.7189 |
| ENSG00000129038 | LOXL1 | 72.0864 | 0.7175 |
| ENSG00000174233 | ADCY6 | 56.6107 | 0.7171 |
| ENSG00000173110 | HSPA6 | 70.8810 | 0.7158 |
| ENSG00000166147 | FBN1 | 70.4821 | 0.7138 |
| ENSG00000132205 | EMILIN2 | 66.4069 | 0.7129 |
| ENSG00000104325 | DECR1 | 51.8177 | 0.7125 |
| ENSG00000153208 | MERTK | 57.4382 | 0.7101 |
| ENSG00000180596 | HIST1H2BC | 51.0862 | 0.7096 |
| ENSG00000166033 | HTRA1 | 66.6696 | 0.7074 |
| ENSG00000073712 | FERMT2 | 63.8742 | 0.7049 |
| ENSG00000089169 | RPH3A | 58.0015 | 0.7037 |
| ENSG00000103710 | RASL12 | 52.0173 | 0.7024 |
| ENSG00000163430 | FSTL1 | 54.7960 | 0.6943 |
| ENSG00000184500 | PROS1 | 52.8782 | 0.6936 |
| ENSG00000254087 | LYN | 53.2529 | 0.6934 |
| ENSG00000180139 | RP11-399O19.5 | 59.4507 | 0.6920 |
| ENSG00000087245 | MMP2 | 74.3663 | 0.6888 |
| ENSG00000144668 | ITGA9 | 55.5170 | 0.6885 |
| ENSG00000137507 | LRRC32 | 64.4975 | 0.6875 |
| ENSG00000135424 | ITGA7 | 54.2254 | 0.6854 |
| ENSG00000035862 | TIMP2 | 59.4507 | 0.6841 |
| ENSG00000145703 | IQGAP2 | 55.9574 | 0.6839 |
| ENSG00000148926 | ADM | 54.9067 | 0.6836 |
| ENSG00000136153 | LMO7 | 69.2007 | 0.6835 |
| ENSG00000075213 | SEMA3A | 53.0944 | 0.6815 |
| ENSG00000024422 | EHD2 | 70.2261 | 0.6797 |
| ENSG00000146904 | EPHA1 | 52.8476 | 0.6784 |
| ENSG00000064666 | CNN2 | 51.0883 | 0.6764 |
| ENSG00000100097 | LGALS1 | 56.0601 | 0.6757 |

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| ENSG00000123989 | CHPF | 59.5704 | 0.6753 |
| ENSG00000109790 | KLHL5 | 50.8233 | 0.6749 |
| ENSG00000124749 | COL21A1 | 51.7971 | 0.6745 |
| ENSG00000168140 | VASN | 55.5914 | 0.6706 |
| ENSG00000134531 | EMP1 | 51.4953 | 0.6703 |
| ENSG00000197712 | FAM114A1 | 74.0854 | 0.6691 |
| ENSG00000170558 | CDH2 | 55.2606 | 0.6657 |
| ENSG00000136859 | ANGPTL2 | 64.4887 | 0.6649 |
| ENSG00000145248 | SLC10A4 | 54.7960 | 0.6649 |
| ENSG00000179954 | SSC5D | 55.6596 | 0.6588 |
| ENSG00000176046 | NUPR1 | 54.9144 | 0.6544 |
| ENSG00000064309 | CDON | 50.7290 | 0.6529 |
| ENSG00000106366 | SERPINE1 | 56.9858 | 0.6427 |
| ENSG00000131981 | LGALS3 | 52.5960 | 0.6402 |
| ENSG00000011028 | MRC2 | 56.4280 | 0.6401 |
| ENSG00000158089 | GALNT14 | 50.5565 | 0.6331 |
| ENSG00000118523 | CTGF | 52.6271 | 0.6294 |
| ENSG00000130635 | COL5A1 | 52.9122 | 0.6276 |
| ENSG00000163909 | HEYL | 51.0532 | 0.6096 |
| ENSG00000149591 | TAGLN | 50.0557 | 0.5875 |

Activated Genes

| Ensembl ID | Gene Symbol | Max DESeq FDR | Log₂(T/C) |
|-------------------|--------------------|----------------------|-------------------------------|
| ENSG00000128245 | YWHAH | 221.7330 | 1.4394 |
| ENSG00000158710 | TAGLN2 | 157.9439 | 1.2271 |
| ENSG00000064300 | NGFR | 146.8393 | 1.1719 |
| ENSG00000049540 | ELN | 127.8538 | 1.1311 |
| ENSG00000140497 | SCAMP2 | 117.3317 | 1.0755 |
| ENSG00000137309 | HMGA1 | 108.8962 | 1.0253 |
| ENSG00000166803 | KIAA0101 | 104.7058 | 0.9707 |
| ENSG00000009709 | PAX7 | 86.6807 | 0.9616 |
| ENSG00000101187 | SLCO4A1 | 89.0895 | 0.9250 |
| ENSG00000079215 | SLC1A3 | 86.1420 | 0.9049 |
| ENSG00000112759 | SLC29A1 | 81.4337 | 0.8922 |
| ENSG00000186867 | QRFPR | 87.2617 | 0.8907 |
| ENSG00000135447 | PPP1R1A | 81.5021 | 0.8899 |

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|-----------------|----------------------|---------|--------|
| ENSG00000158195 | WASF2 | 78.8870 | 0.8809 |
| ENSG00000169894 | MUC3A | 84.3078 | 0.8776 |
| ENSG00000124164 | VAPB | 77.2833 | 0.8664 |
| ENSG00000085552 | IGSF9 | 80.7035 | 0.8663 |
| ENSG00000138061 | CYP1B1 | 81.5021 | 0.8430 |
| ENSG00000159167 | STC1 | 73.5211 | 0.8410 |
| ENSG00000117289 | TXNIP | 67.0722 | 0.8307 |
| ENSG00000071655 | MBD3 | 66.4069 | 0.8216 |
| ENSG00000249906 | RP5-1029K10.2 | 70.9345 | 0.8201 |
| ENSG00000187867 | PALM3 | 68.1630 | 0.7994 |
| ENSG00000213390 | ARHGAP19 | 66.9043 | 0.7947 |
| ENSG00000081181 | ARG2 | 66.3513 | 0.7900 |
| ENSG00000166342 | NETO1 | 66.8842 | 0.7828 |
| ENSG00000173805 | HAP1 | 65.5680 | 0.7654 |
| ENSG00000198576 | ARC | 61.3460 | 0.7649 |
| ENSG00000166974 | MAPRE2 | 63.4265 | 0.7561 |
| ENSG00000080819 | CPOX | 58.0015 | 0.7519 |
| ENSG00000173890 | GPR160 | 53.8457 | 0.7515 |
| ENSG00000126785 | RHOJ | 54.9563 | 0.7467 |
| ENSG00000172757 | CFL1 | 53.0042 | 0.7441 |
| ENSG00000054611 | TBC1D22A | 50.5441 | 0.7417 |
| ENSG00000131238 | PPT1 | 53.0363 | 0.7396 |
| ENSG00000135446 | CDK4 | 55.7100 | 0.7380 |
| ENSG00000057294 | PKP2 | 55.8437 | 0.7289 |
| ENSG00000149269 | PAK1 | 55.1639 | 0.7271 |
| ENSG00000172236 | TPSAB1 | 54.3280 | 0.7254 |
| ENSG00000171766 | GATM | 54.9487 | 0.7203 |
| ENSG00000072736 | NFATC3 | 52.5705 | 0.7202 |
| ENSG00000128594 | LRRC4 | 52.9122 | 0.7202 |
| ENSG00000129484 | PARP2 | 53.2527 | 0.7194 |
| ENSG00000168765 | GSTM4 | 50.8257 | 0.7172 |
| ENSG00000181744 | C3orf58 | 54.6557 | 0.7170 |
| ENSG00000113368 | LMNB1 | 51.6650 | 0.7152 |
| ENSG00000165495 | PKNOX2 | 52.9258 | 0.7141 |
| ENSG00000164362 | TERT | 51.2488 | 0.7124 |
| ENSG00000165731 | RET | 52.0706 | 0.7109 |

FIGURE S2

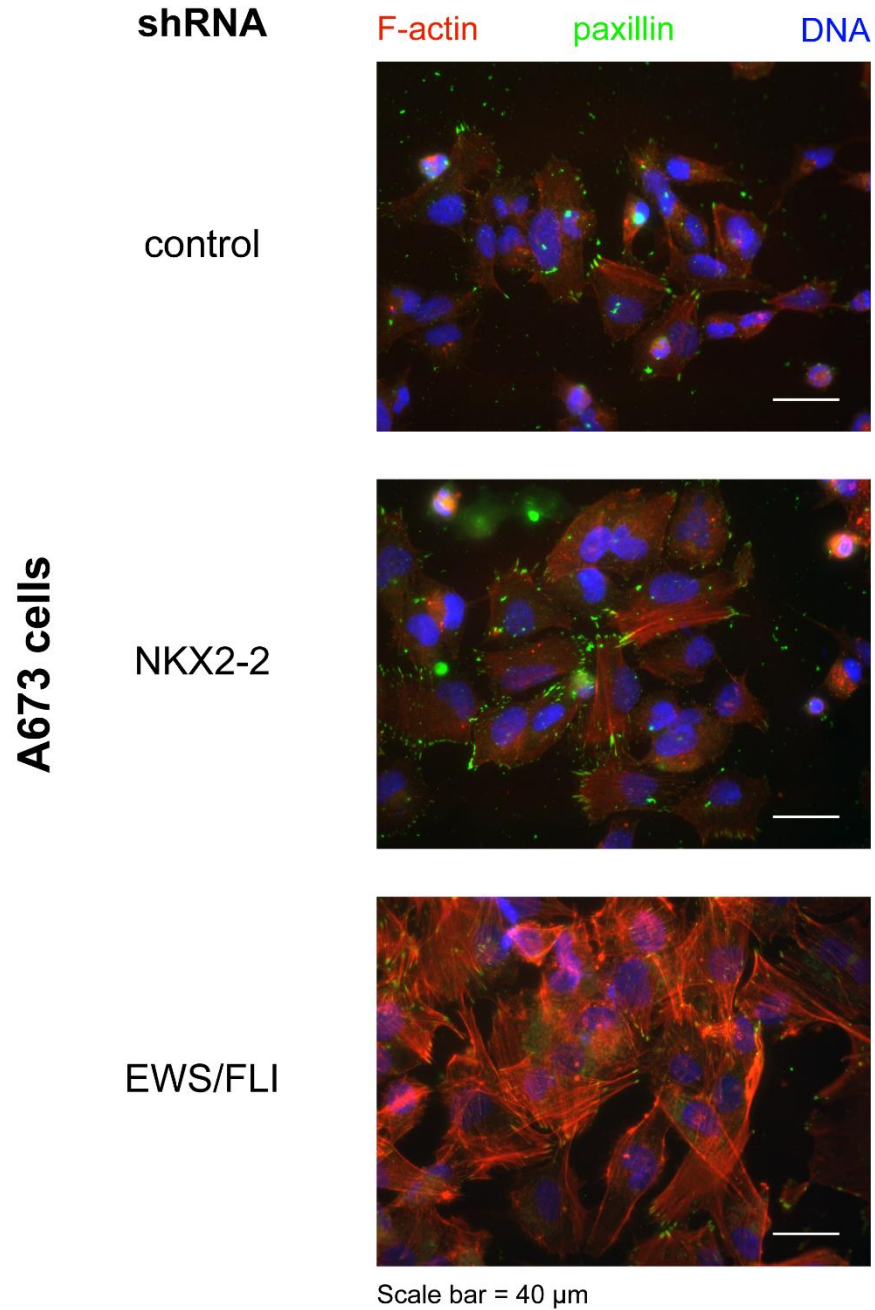


Figure S2: Immunofluorescence 40X fields of A673 cells harboring control, NKX2-2, or EWS/FLI shRNA. Merged images of IF staining for F-actin (red), paxillin (green), and DNA (blue) are shown.

FIGURE S3

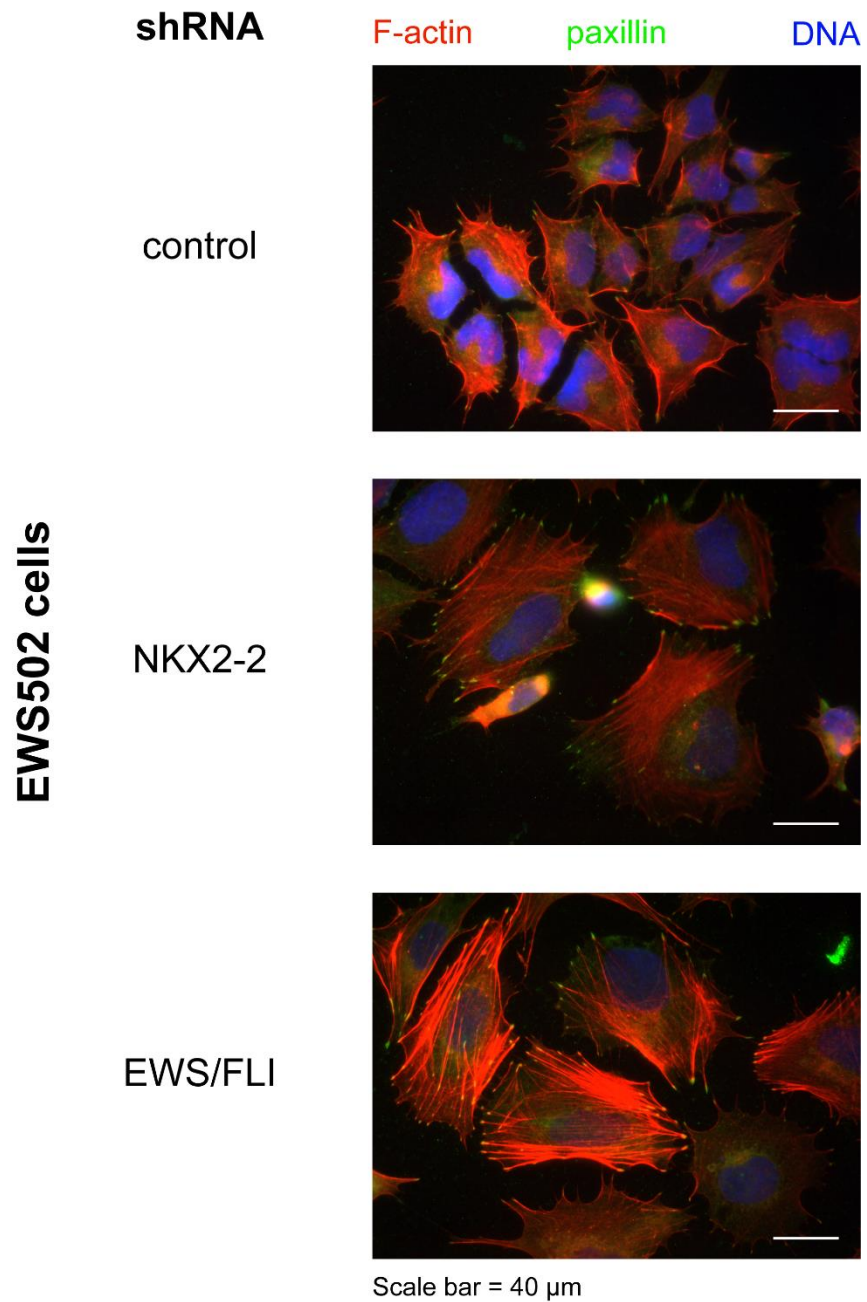


Figure S3: Immunofluorescence 40X fields of EWS502 cells harboring control, NKX2-2, or EWS/FLI shRNA. Merged images of IF staining for F-actin (red), paxillin (green), and DNA (blue) are shown.

FIGURE S4

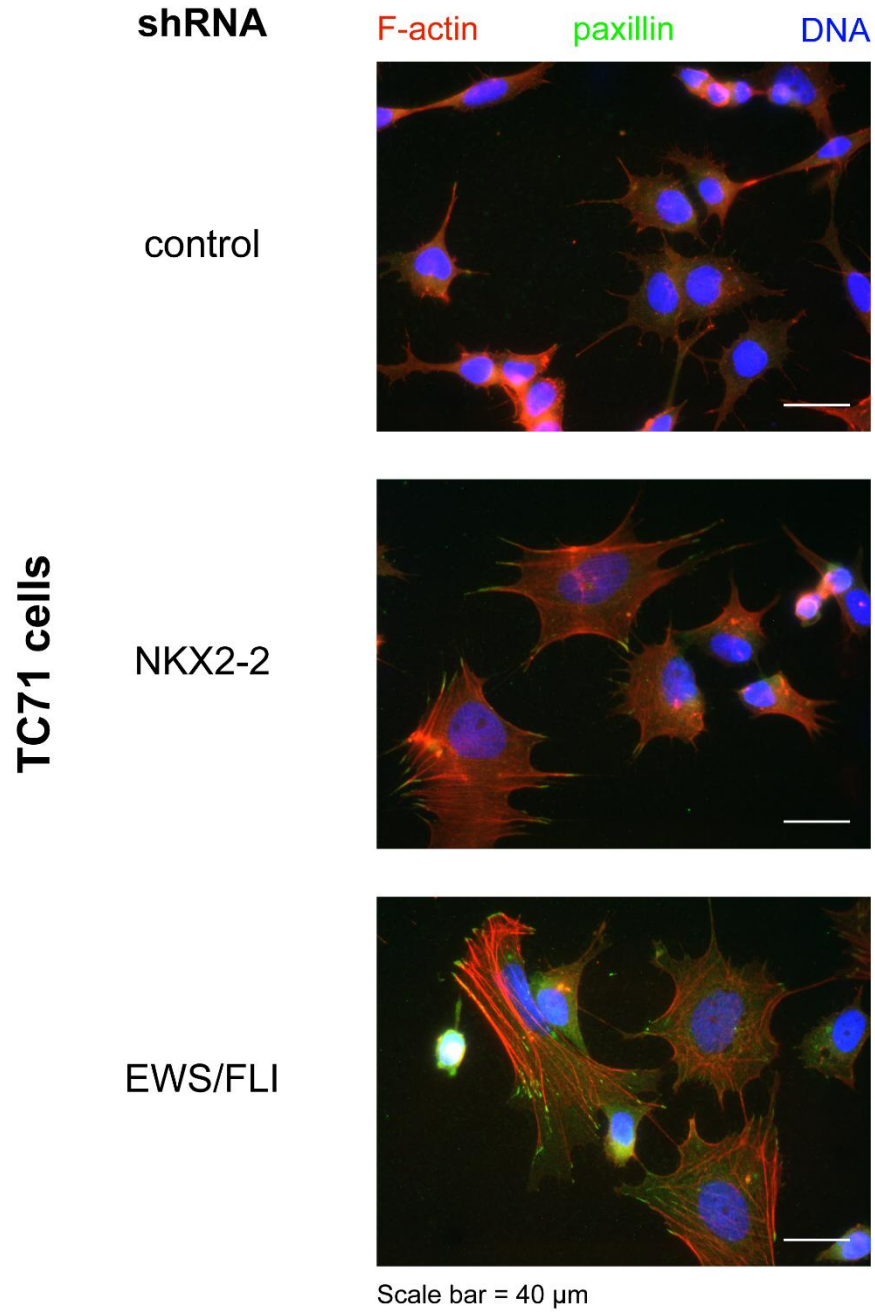


Figure S4: Immunofluorescence 40X fields of TC71 cells harboring control, NKX2-2, or EWS/FLI shRNA. Merged images of IF staining for F-actin (red), paxillin (green), and DNA (blue) are shown.

FIGURE S5

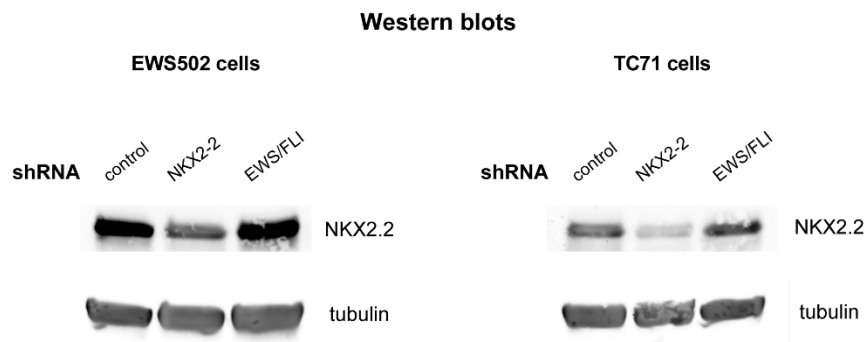
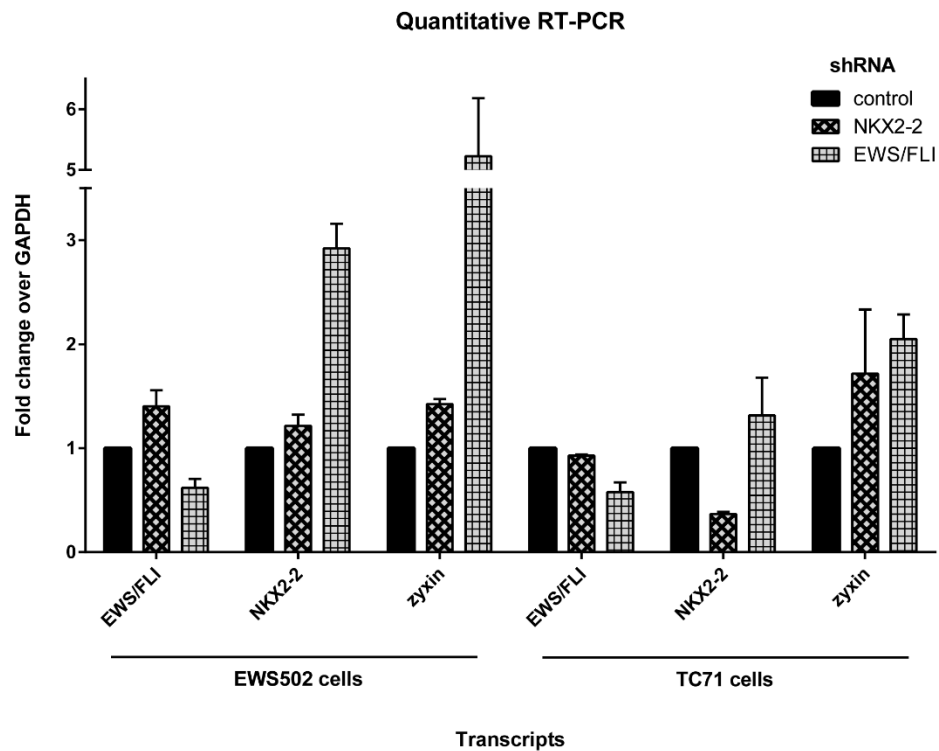


Figure S5: qRT-PCR and western blots of EWS502 and TC71 cells. *Top*, qRT-PCR results using EWS/FLI-, NKX2-2-, and zyxin-specific primer sets. Shown are fold-change values, normalized against GAPDH levels, for cells harboring control, NKX2-2 or EWS/FLI shRNA for either cell line. *Bottom*, NKX2.2 and tubulin protein levels for each knockdown condition in either cell line.

FIGURE S6

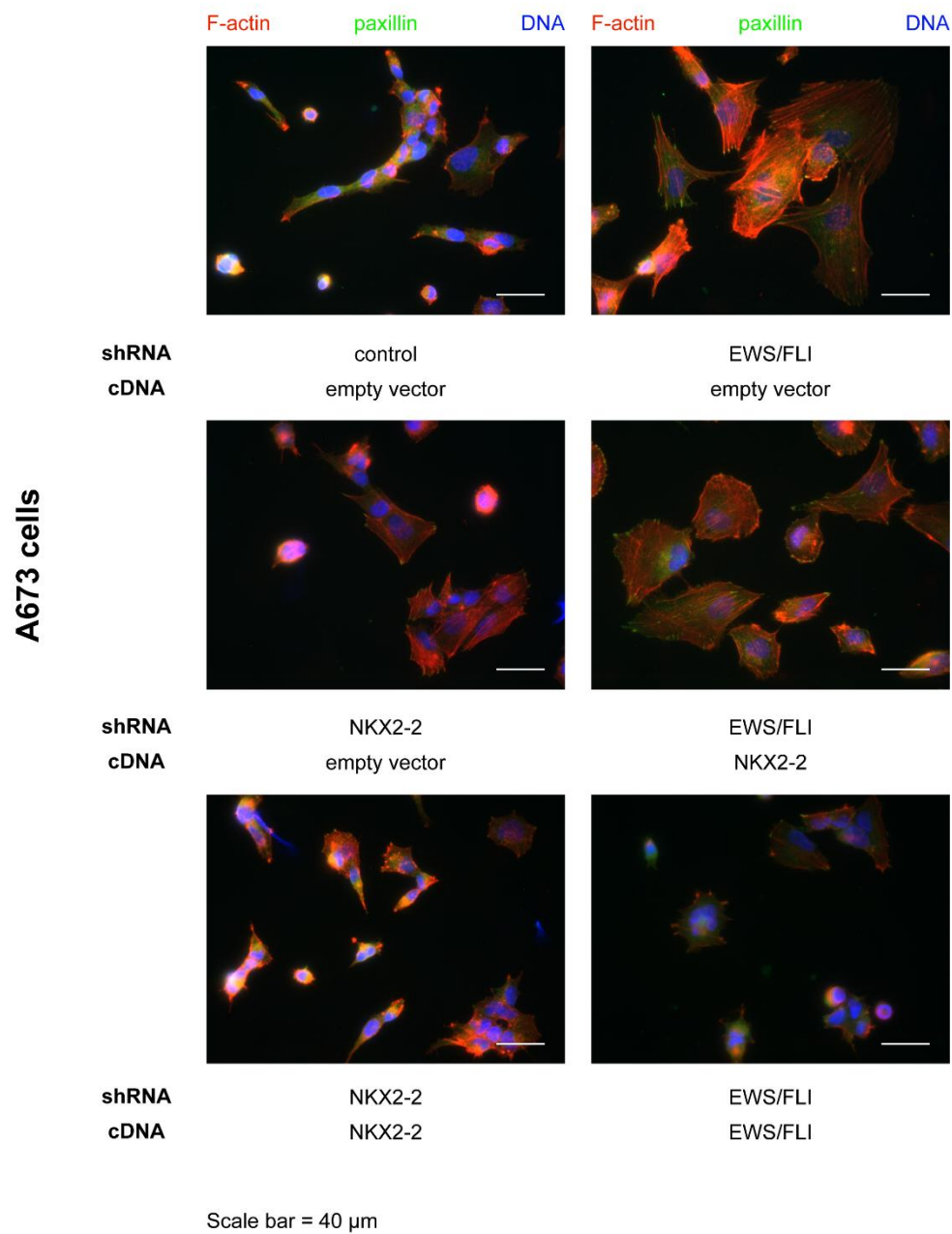


Figure S6: Immunofluorescence 40X fields of A673 knockdown-rescue cells. Cells harboring control, NKX2-2, or EWS/FLI shRNA, and empty vector (pMSCV-hygro) or cDNA for 3XFLAG::NKX2-2 or 3XFLAG::EWS/FLI, as indicated. Merged images of IF staining for F-actin (red), paxillin (green), and DNA (blue) are shown.