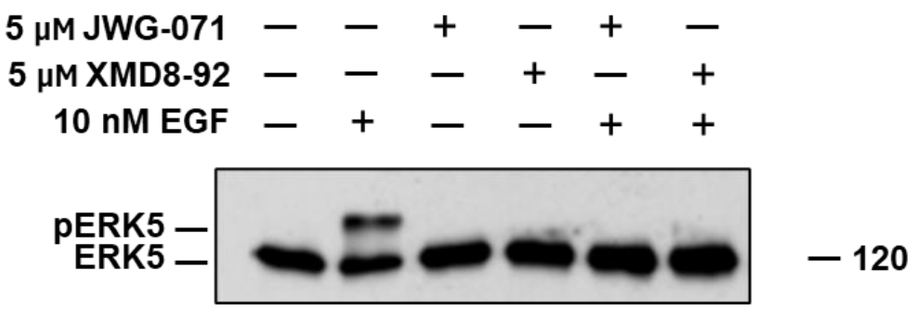
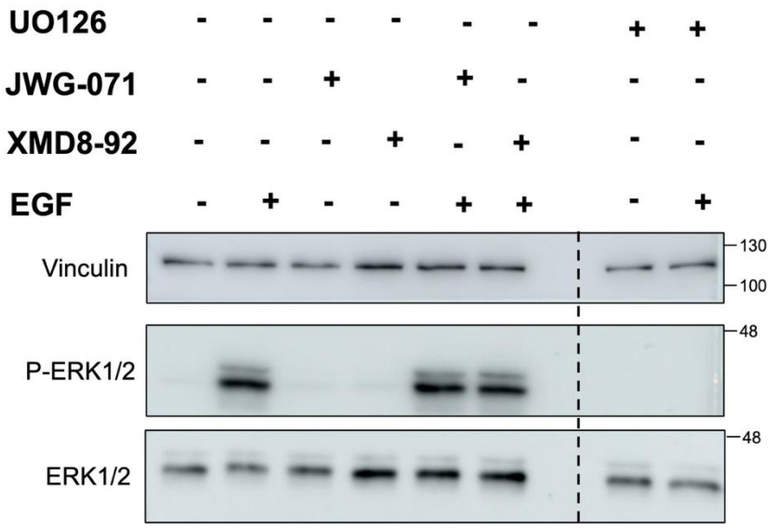
A

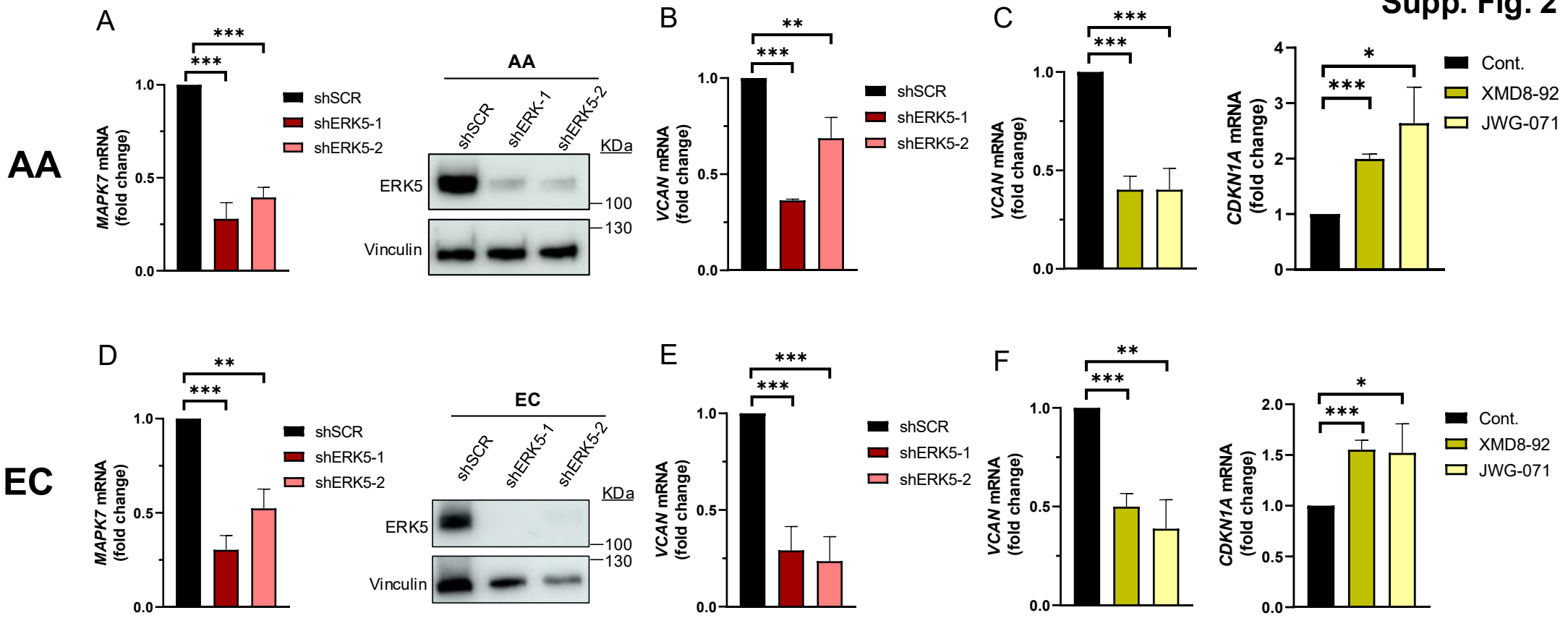



B



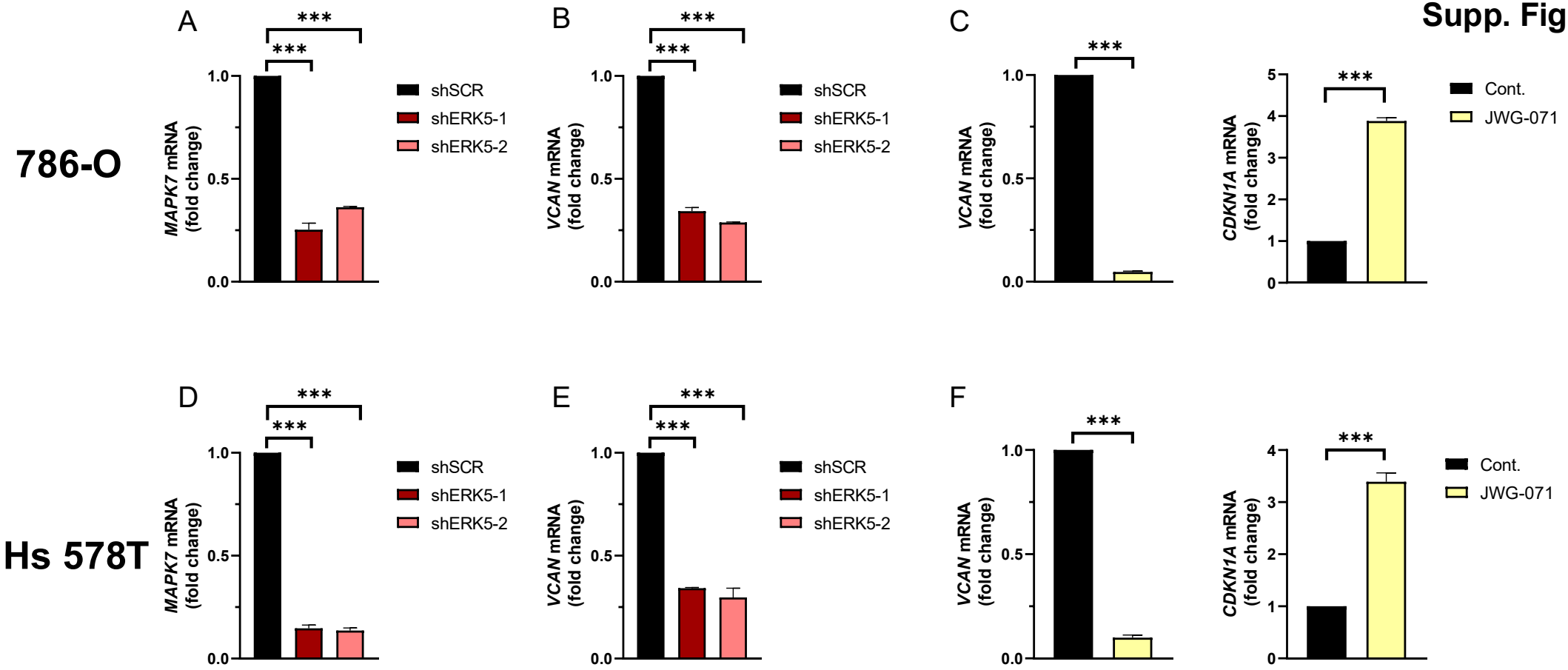

Supplementary Figure 1. Study of functionality and specificity of ERK5 inhibitors. A) SK-LMS-1 cells were starved overnight and then treated with either JWG-071 or XMD8-92 inhibitors both at 5 μM dose for 1 hour at 37ºC. Prior to lysis, cells were stimulated with 10 nM EGF for 15 minutes. Total ERK5 and pERK5 expression was determined by immunoprecipitating 1 mg of protein followed by Western Blotting with the anti-ERK5 antibody. B) SK-LMS-1 cells were starved overnight and then treated with either JWG-071, XMD8-92 or UO126 at 5 μM dose for 1 hour at 37ºC. Prior to lysis, cells were stimulated with 10 nM EGF for 15 minutes. Then 50μg of samples we blotted against indicated antibodies

**Supp. Fig. 2**

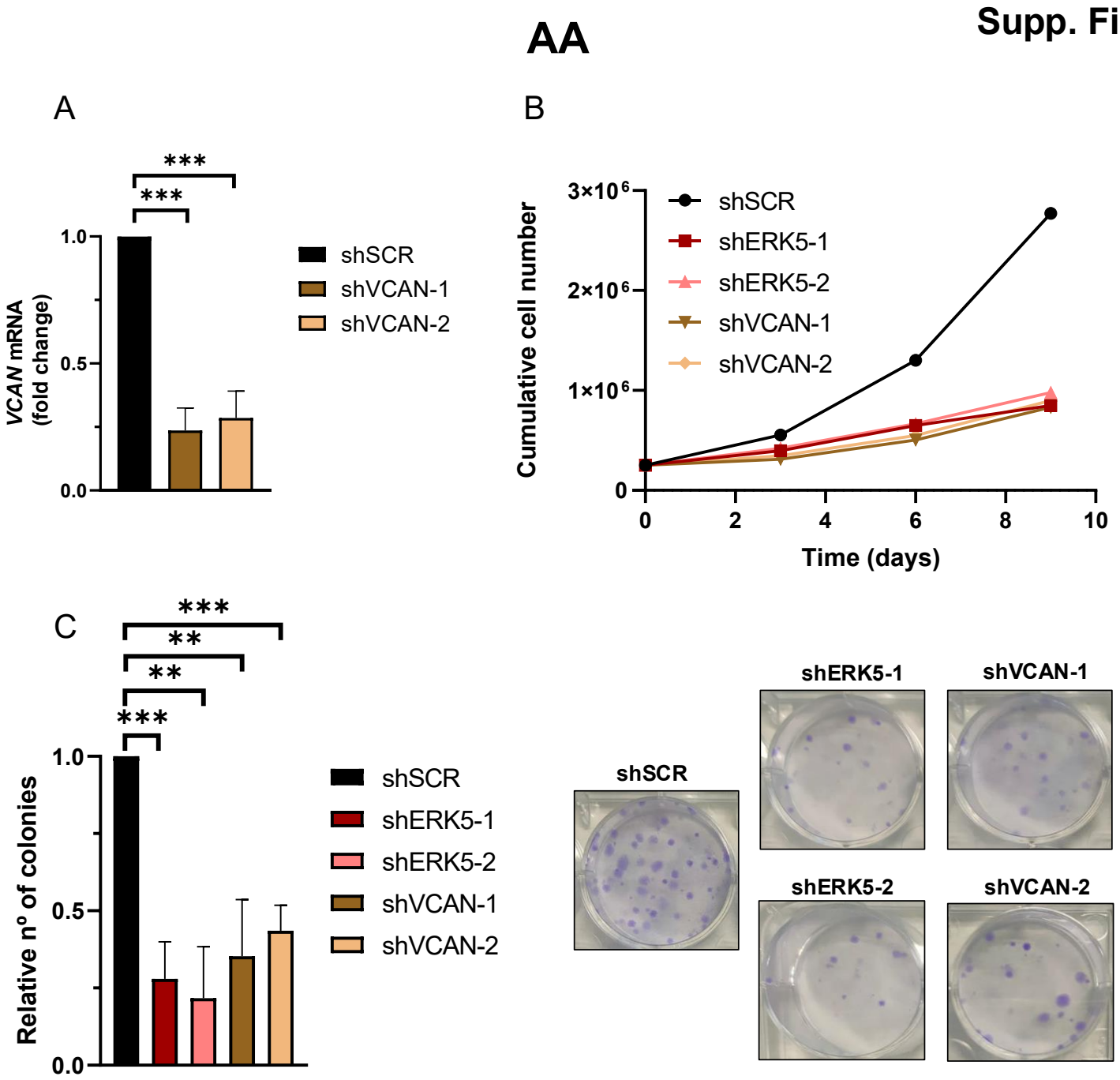



Supplementary Figure 2. ERK5 regulates VCAN mRNA levels in AA and EC cell lines. AA (A) and EC (D) cells were infected with lentiviruses carrying PLKO.1-shScramble vector (shSCR) or the human PLKO.1-shRNA ERK5 1/2 vectors (shERK5-1/2). MAPK7 mRNA levels were evaluated by RT-qPCR (left panel) and protein levels were evaluated by western blot (right panel). Vinculin was used as a loading control. VCAN mRNA levels analyzed by RT-qPCR in AA (B) and EC (E) cells infected with shSCR and shERK5-1/2 vectors. AA (C) and EC (F) cells were treated for 18 hours with the ERK5 chemical inhibitors XMD8-92 (5 μM) and JWG-071 (5 μM), and RT-qPCR was performed to analyze VCAN (left panels) and CDKN1A (right panels) mRNA levels. Graphics represent the mean +/- SD of 3 independent experiments from different pools of infection. The unpaired Student's t-test was used to assess statistical significance. **p<0.05; **p<0.01; ***p<0.001*.

**Supp. Fig. 3**

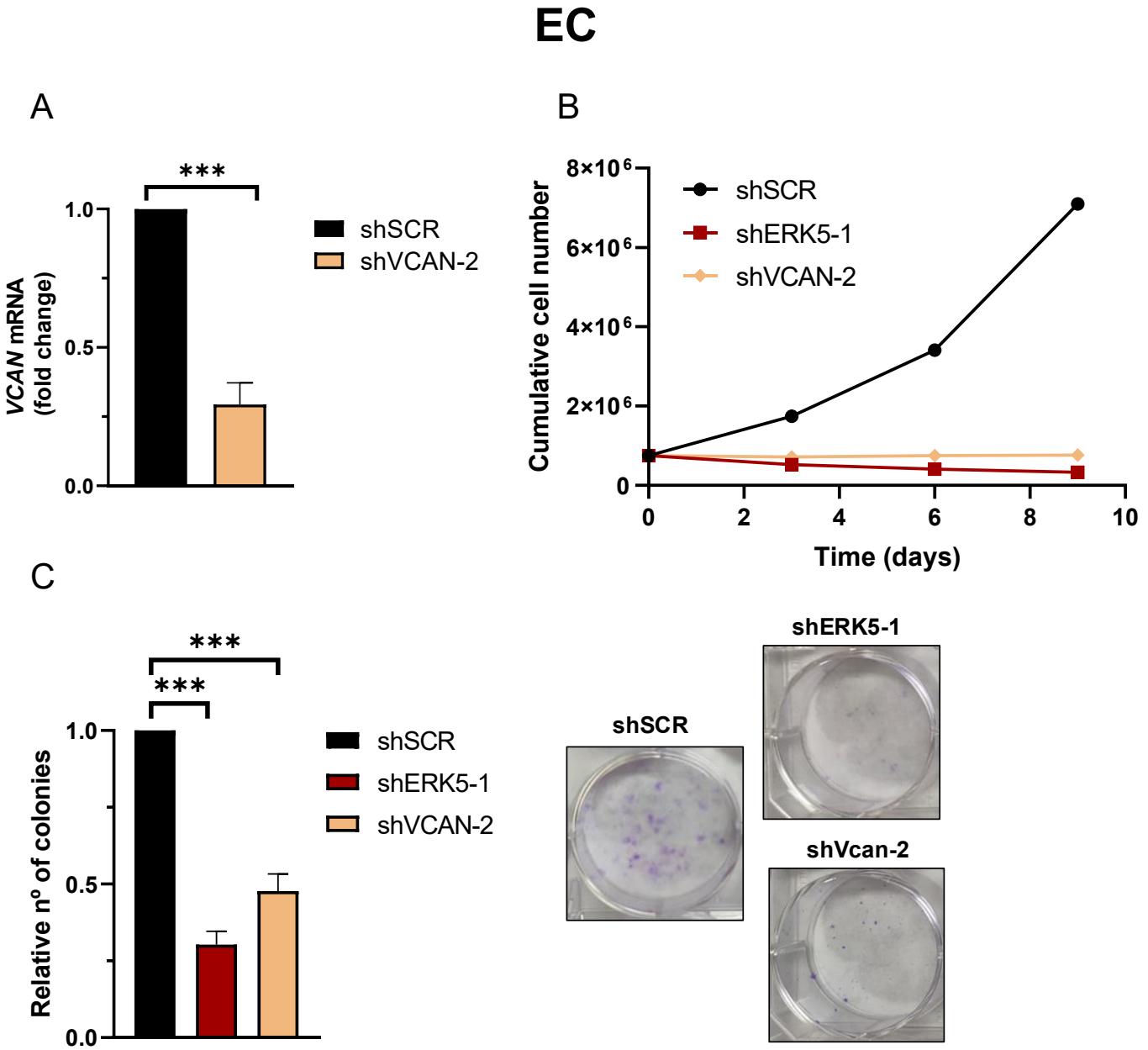



Supplementary Figure 3. ERK5 regulates VCAN mRNA levels in 786-O and Hs 578T cell lines. 786-O (A) and Hs 578T (D) cells were infected with lentiviruses carrying PLKO.1-shScramble vector (shSCR) or the human PLKO.1-shRNA ERK5 vector 1/2 (shERK5-1/2). MAPK7 mRNA levels were evaluated by RT-qPCR. VCAN mRNA levels analyzed by RT-qPCR in 786-O (B) and Hs 578T (E) cells infected with shSCR and shERK5-1/2. 786-O (C) and Hs 578T (F) cells were treated for 18 hours with the ERK5 chemical inhibitor JWG-071 (5 μM), and RT-qPCR was performed to analyze VCAN (left panels) and CDKN1A (right panels) mRNA levels. Graphics represent the mean +/- SD of 3 independent experiments from different pools of infections. The unpaired Student's t-test was used to assess statistical significance ****p<0.001*.



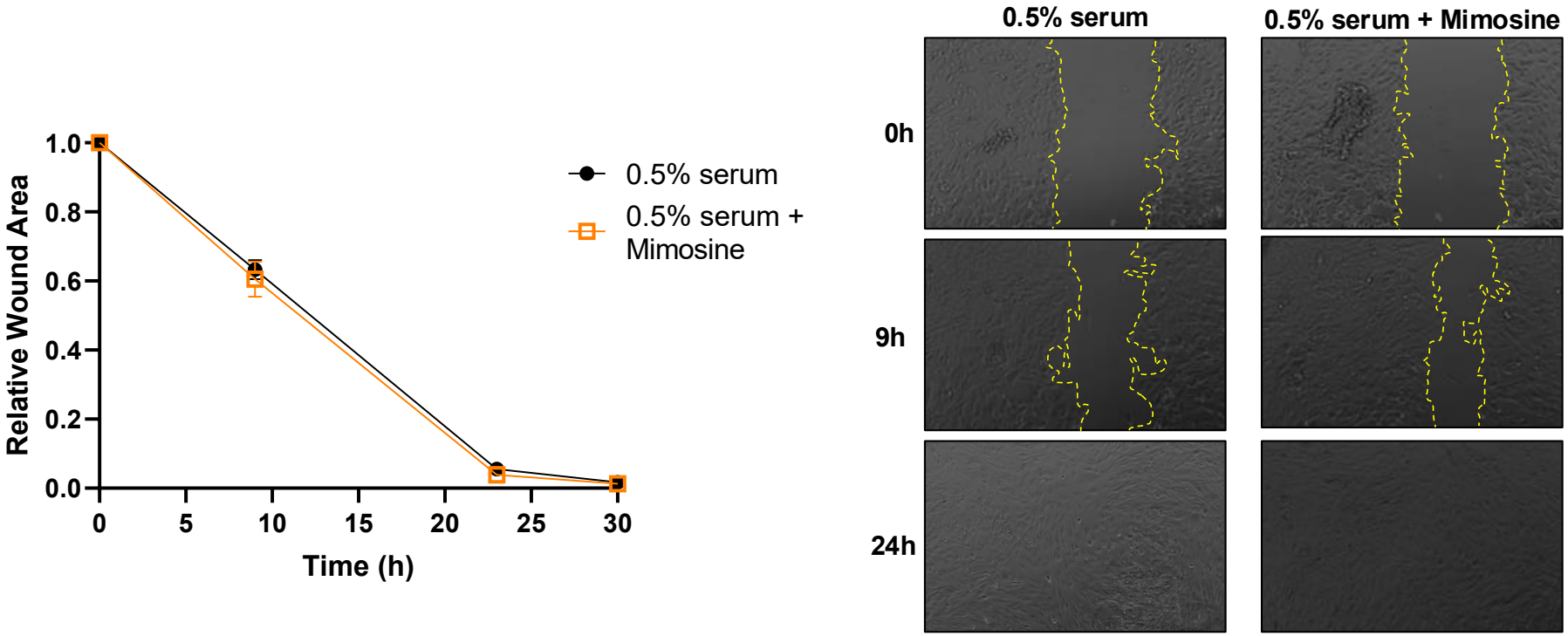

Supplementary Figure 4. ERK5 and VCAN contribute to the regulation of proliferation and colony formation capacity in AA cells. (A) AA cells were infected with lentiviruses carrying PLKO.1-shScramble vector (shSCR) or the human PLKO.1-shRNA VCAN 1/2 vectors (shVCAN-1/2). VCAN mRNA levels were evaluated by RT-qPCR. (B) For growth curves, 2.5 × 105 shSCR, shERK5-1/2 or shVCAN-1/2 AA cells were seeded in 100 mm plates. Every 3 days, cells were counted and replated in the same manner up to day 9. The graphic shows the cumulative cell number from a representative experiment out of 3 from different pools of infection with nearly identical results. (C) For evaluation of colony formation capacity, clonogenic assays were performed by seeding 400 cells/well in a 6_ well plate and they were revealed after 12-14 days. Relative number of colonies obtained in clonogenic assays of shSCR, shERK5-1/2 and shVCAN-1/2 AA cells (left panel). Representative images of clonogenic assays from the different cell lines (right panel). The graphic represents the mean +/- SD of 3 independent experiments from different pools of infections. The unpaired Student's t-test was used to assess statistical significance. **p<0.01; ***p<0.001*.

**Supp. Fig. 5**



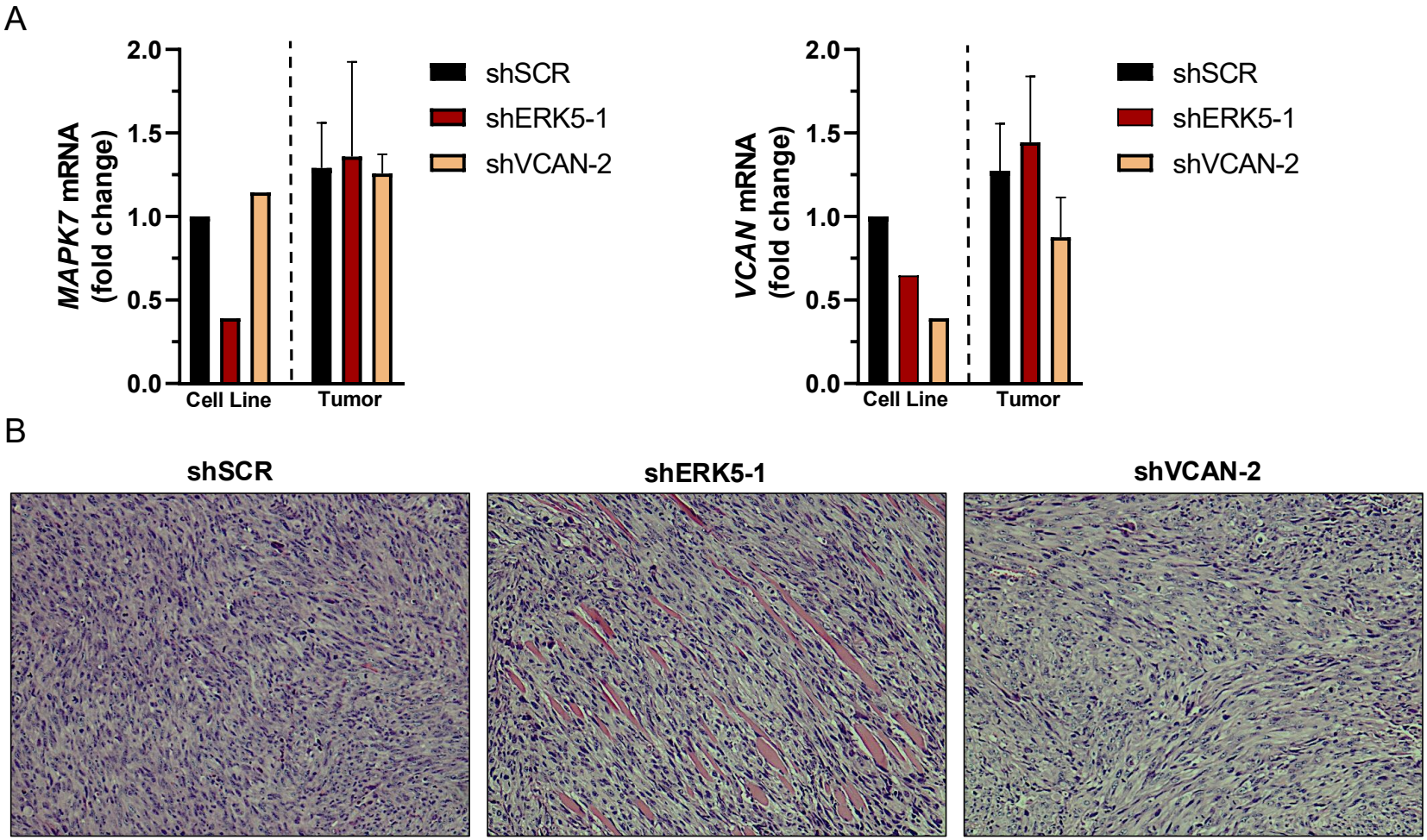

Supplementary Figure 5. ERK5 and VCAN contribute to the regulation of proliferation and colony formation capacity in EC cells. (A) EC cells were infected with lentiviruses carrying PLKO.1-shScramble (shSCR) or the human PLKO.1-shRNA VCAN-2 (shVCAN-2) vectors. VCAN mRNA levels were evaluated by RT-qPCR. (B) For growth curves, 7.5 × 105 shSCR, shERK5-1 or shVCAN-2 EC cells were seeded in 100 mm plates. Every 3 days, cells were counted and replated in the same manner up to day 9. Graphic shows the cumulative cell number from a representative experiment out of 3 from different pools of infection with nearly identical results. (C) For evaluation of colony formation capacity, clonogenic assays were performed by seeding 600 cells/well in a 6- well plate 6, and they were revealed after 12-14 days. Relative number of colonies obtained in clonogenic assays of shSCR, shERK5-1 and shVCAN-2 EC cells (left panel). Representative images of clonogenic assays from the three different cell lines (right panel). The graphic represents the mean +/- SD of 3 independent experiments from different pools of infections. The unpaired Student's t-test was used to assess statistical significance. ****p<0.001*.

**Supp. Fig. 6**



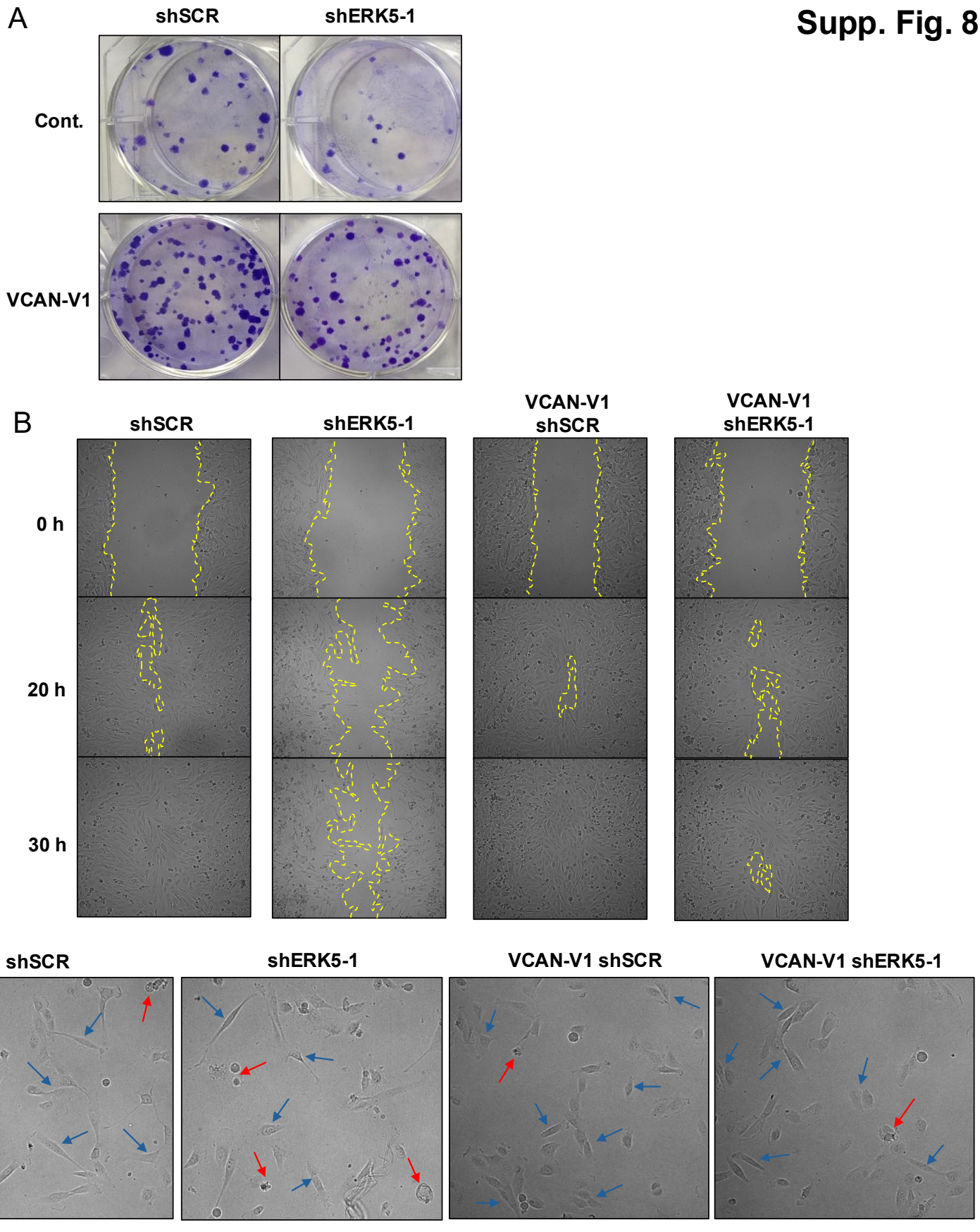

Supplementary Figure 6. Setting the conditions of wound healing assay in SK-LMS-1 cells. Relative wound area of SK-LMS-1 cells with 0,5% FBS concentration with/without mimosine treatment (400 μM) (left panel). Representative images from one out of 3 independent experiments (right panel).

**Supp. Fig. 7**



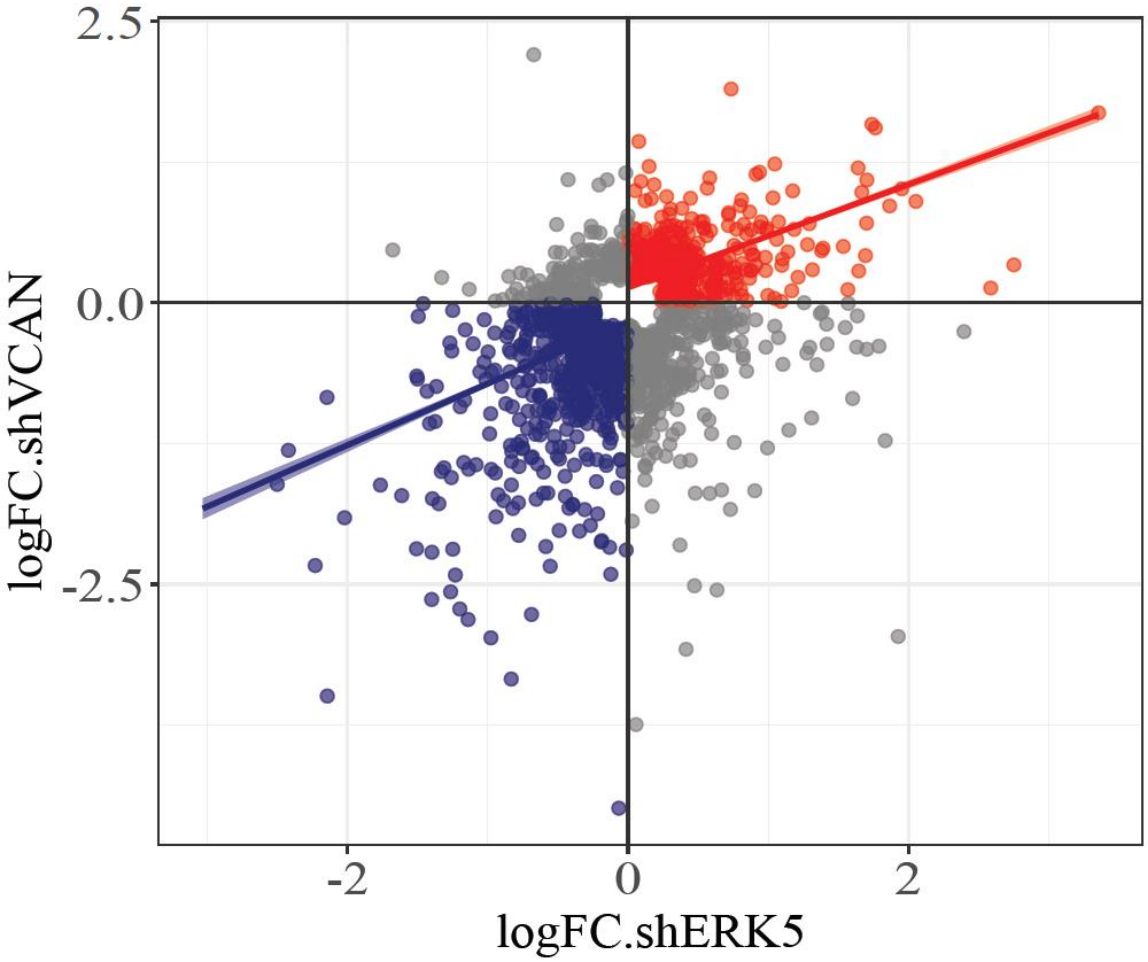

Supplementary Figure 7. Analysis of the tumors obtained in figure 4C. (A) MAPK7 (left panel) and VCAN (right panel) mRNA levels of SK-LMS-1 cells before injection (Cell Line) and from recovered tumors (Tumor), analyzed by RT-qPCR. The unpaired Student's t-test was used to assess statistical significance. (B) Representative images of hematoxylin and eosin histological study of tumors obtained from SK-LMS-1 derived cell lines, showing lesions with high cell density, spindle-shaped cells or occasionally with epithelioid appearance arranged in disorganized bundles. tumor cells also had vesicular nuclei with macronucleoli, extensive eosinophilic cytoplasm and atypical mitoses in all neoplasms. Pictures are shown at a 20X magnification.

**Supp. Fig. 8**



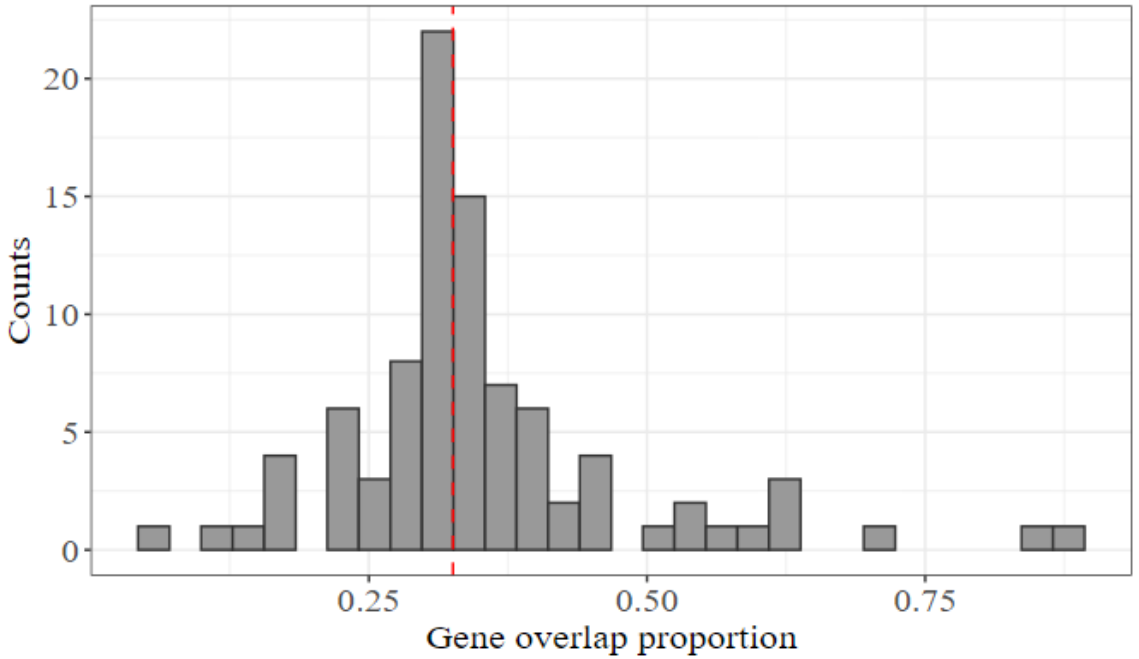

Supplementary Figure 8. Images of results presented in Figure 5. (A) Representative images of clonogenic assays from SK-LMS-1 derived cells. (B) Representative images from wound healing assays in SK-LMS-1 derived cells. (C) Representative images of SK-LMS-1 derived cells taken 300 minutes after seeding on a collagen-pretreated surface; blue arrows mark fully adherent and expanded cells; red arrows mark not attached cells

**Supp. Fig. 9**



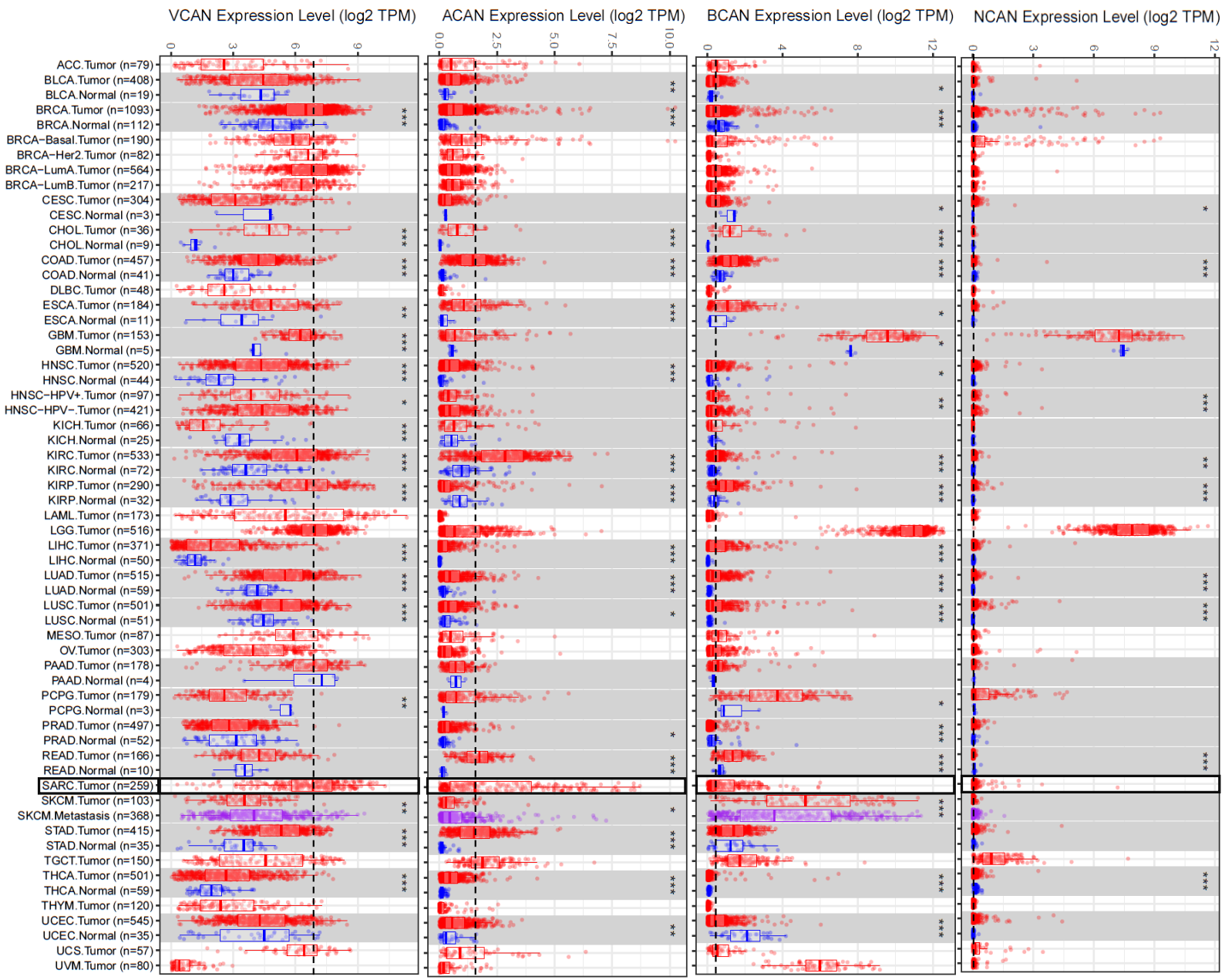

Supplementary Figure 9. Scatter plot of logFC in gene expression following VCAN versus ERK5 suppression. Each point represents a gene that was differentially expressed in at least one of the two conditions. Genes upregulated in both conditions are shown in red, those downregulated in both are shown in blue. The Pearson correlation coefficients for commonly upregulated and downregulated genes are 0.53 and 0.42, respectively ($p < 0.001$ for both).

**Supp. Fig. 10**

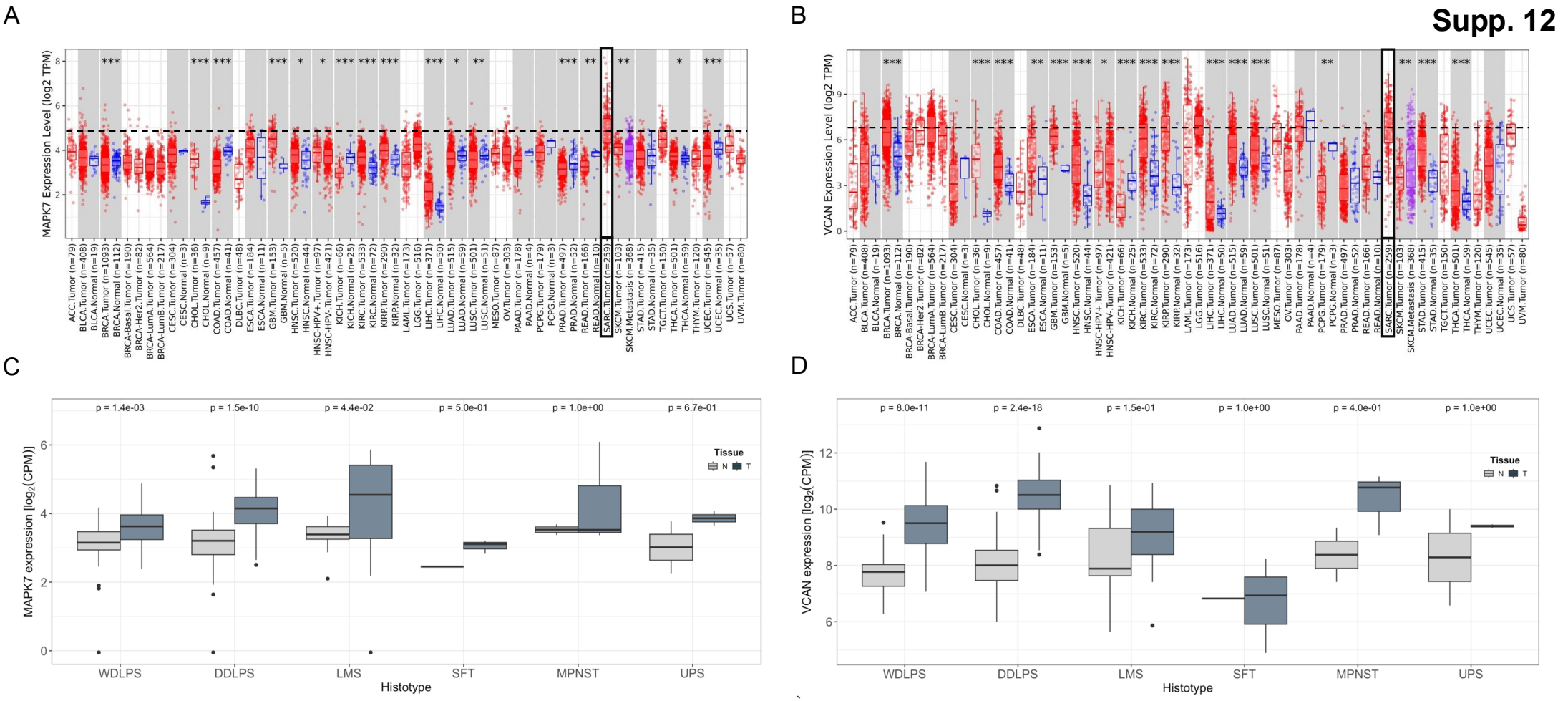



Supplementary Figure 10. Overlap of enriched biological processes between MAPK7 and VCAN interferences. Histogram shows the proportion of overlapping genes in the enriched biological processes shared between MAPK7 and VCAN interferences. For each category, the proportion is calculated by dividing the number of genes identified in the MAPK7 and VCAN deletion enrichments by the total number of genes enriched after MAPK7 deletion, with MAPK7 deletion set as the reference: P=G_(MAPK7∩VCAN)/G_MAPK7, where G_(MAPK7∩VCAN) is the number of overlapping genes between MAPK7 and VCAN deletion enrichments and G_MAPK7 is the total number of genes enriched after MAPK7 deletion. The dashed red line indicates the median overlap ratio across all categories.

# Supp. Fig. 11

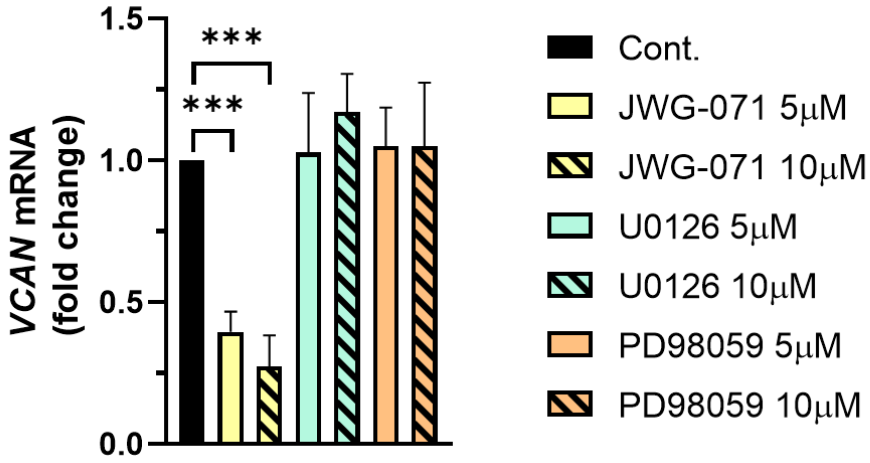



Supplementary Figure 11. mRNA expression (log2 TPM) analysis of VCAN, ACAN, BCAN and NCAN expression in samples from TCGA series using TIMER 2.0 platform. Sarcoma series (SARC Tumor) is marked with a black rectangle and the average mRNA level of each gene in sarcoma is marked with a discontinuous black line. Tumor samples depicted in red and healthy samples depicted in blue. Statistical significance of differential expression evaluated using the Wilcoxon test.




Supplementary Figure 12. mRNA expression (log2 TPM) analysis of MAPK7 (A) and VCAN (B) in the TCGA series using TIMER 2.0 platform. Soft Tissue Sarcoma series (SARC Tumor) is marked with a black rectangle and the average MAPK7 and VCAN mRNA levels in sarcoma are marked with discontinuous black lines. Comparison of MAPK7 (C) and VCAN (D) mRNA expression levels (log2 CPM) in retroperitoneal soft-tissue sarcomas (blue boxes) and paired normal tissue (grey boxes) from the SARCOMICS study by RNA-Seq. Wilcoxon test was used to address statistical significance.

WDLPS: Well-differentiated liposarcoma; DDLPS: dedifferentiated liposarcoma; LMS: leiomyosarcoma; MPNST: malignant peripheral nerve sheath tumor; UPS: undifferentiated pleomorphic sarcoma; SFT: solitary fibrous tumor.




Supplementary figure 13. Effect of ERK1/2 inhibitors on VCAN expression. Subconfluent cultures of SK-LM-S1 cells were incubated with 5 and 10 μM of ERK1/2 inhibitors (U0126 and PD98059), or with the ERK5 inhibitor JWG-071 (5 and 10 μM) for 24 hours, as described in Figure 2. Samples were collected and processed for qRT-PCR to evaluate VCAN mRNA levels under the different conditions, using GAPDH as the housekeeping gene. Figure represents average of 2 independent experiments . Bars means S.D. The unpaired Student's t-test was used to assess statistical significance. **p<0.05; **p<0.01; ***p<0.001*.

**Supplementary Table 1. List of antibodies used.**

| Antibody | Dilution | Manufacture | Reference | Use |
|---|---|---|---|---|
| ERK5 | 1:1000 | Cell Signaling Technology | 3372S | Western blot |
| VCAN | 1:5000 | Abcam | Ab19345 | Western blot |
| MEK5 | 1:1000 | Enzo Life Science | ADI-KAP-MA003-E | Western blot |
| HA | 1:1000 | Sigma-Aldrich | H9658 | Western blot |
| Vinculin | 1:3000 | Sigma-Aldrich | V9264 | Western blot |
| Nidogen 1 | 1:3000 | R&D Systems | AF2570 | Western blot |
| Calnexin | 1:1000 | Stressgen Bioreagents | SPC-127 | Western blot |
| ERK1/2 | 1:2000 | Cell Signaling Technology | 4696T | Western blot |
| Phospho-ERK1/2 | 1:2000 | Cell Signaling Technology | 4370S | Western blot |
| ERK5 | 1μg | In house | PMID: 17317102 | IP |
| Anti-mouse | 1:3000 | Cell Signaling Technology | 7076S | Western blot |
| Anti-rabbit | 1:3000 | Cell Signaling Technology | 7074S | Western blot |
| ERK5 | 1:500 | Abcam | ab196609 | Immunohistochemistry |
| VCAN | 1:2000 | Abcam | ab270445 | Immunohistochemistry |

**Supplementary Table 2. List of primers used.**

| Primer | Forward | Reverse |
|---|---|---|
| human *MAPK7* | AGCACTTTAAACACGACAAC | TAGACAGATTTGAATTCGCC |
| human *VCAN* | AGGTGGTCTACTTGGGGTGA | TGGTTGTAGCCTCTTTAGGTTT |
| human *CDKN1A* | ACTCTCAGGGTCGAAAACGG | CTTCCTGTGGGCGGATTAGG |
| human *GAPDH* | TCGTGGAAGGACTCATGACCA | CAGTCTTCTGGGTGGCAGTGA |
| mouse *Mapk7* | AGATCTGTCTATGTGGTACTG | CTGGTACAGGAAGTATCTCAC |
| mouse *Vcan* | AGGCGTCTACCGATGTGATG | CAGCGGCAAAGTTCAGAGTG |
| mouse *B2m* | GGTCTTTCTGGTGCTTGTCTCA | GTTCGGCTTCCCATTCTCC |

**Supplementary Table 3. Clinical and pathological characteristics of 222 patients with either primary retroperitoneal sarcoma (RPS) or primary extremity soft tissue sarcomas (ESTS) who were included in the retrospective cohort of the SARCOMCS clinical trial and were profiled with RNA-sequencing**. WDLPS: Well-differentiated liposarcoma; DDLPS: dedifferentiated liposarcoma; PLPS: pleomorphic liposarcoma; MLPS: myxoid liposarcoma; LMS: leiomyosarcoma; MPNST: malignant peripheral nerve sheath tumor; UPS: undifferentiated pleomorphic sarcoma; SS: synovial sarcoma; AS: angiosarcoma; SFT: solitary fibrous tumor; MFS: myxofibrosarcoma.

| **Site** | RPS | ESTS | All |
|---|---|---|---|
| **Sex** | | | |
| Female | 46 (34%) | 38 (44%) | 84 (38%) |
| Male | 89 (66%) | 49 (56%) | 138 (62%) |
| **Age (median, IQR)** | 63 (54.5-72) | 59 (46-71.5) | 62 (50-72) |
| **Histology** | | | |
| WDLPS | 40 (30%) | 0 | 40 (18%) |
| DDLPS | 69 (51%) | 4 (5%) | 73 (33%) |
| PLPS | 0 | 5 (6%) | 5 (2%) |
| MLPS | 0 | 14 (16%) | 14 (6%) |
| LMS | 17 (13%) | 7 (8%) | 24 (11%) |
| MPNST | 3 (2%) | 2 (2%) | 5 (2%) |
| UPS | 2 (1%) | 23 (26%) | 25 (11%) |
| SS | 0 | 3 (3%) | 3 (2%) |
| AS | 0 | 1 (2%) | 1 (1%) |
| SFT | 4 (3%) | 7 (8%) | 11 (5%) |
| MFS | 0 | 21 (24%) | 21 (9%) |
| **Grading** | | | |
| I | 44 (33%) | 21 (24%) | 65 (29%) |
| II | 57 (42%) | 20 (23%) | 77 (35%) |
| III | 34 (25%) | 46 (53%) | 80 (36%) |
| **Neoadjuvant treatment** | | | |
| Chemotherapy | 7 (5%) | 15 (17%) | 22 (9%) |
| Radiotherapy | 15 (11%) | 9 (10%) | 24 (11%) |

**Supplementary Table 4. Clinicopathological data from Complejo Hospitalario Universitario de Albacete sarcoma patients cohort.**

| ID | Age | Sex | Diagnosis | Location | ERK5 Intensity | VCAN Intensity |
|---|---|---|---|---|---|---|
| **Patient 01** | 77 | M | Leiomyosarcoma | Soft parts of the lower extremity | +++ | +++ |
| **Patient 02** | 68 | F | Leiomyosarcoma | Uterus | +++ | ++ |
| **Patient 03** | 64 | F | Leiomyosarcoma | Mediastinum | 0 | 0 |
| **Patient 04** | 44 | M | Leiomyosarcoma | Rectum | + | 0 |
| **Patient 05** | 58 | M | Leiomyosarcoma | Axilla | 0 | 0 |
| **Patient 06** | 48 | F | Leiomyosarcoma | Uterus | 0 | 0 |
| **Patient 07** | 49 | F | Leiomyosarcoma | Lung | + | + |
| **Patient 08** | 35 | F | Leiomyosarcoma | Uterus | + | +++ |
| **Patient 09** | 58 | F | Leiomyosarcoma | Uterus | + | 0 |
| **Patient 10** | 56 | F | Leiomyosarcoma | Uterus | + | 0 |
| **Patient 11** | 55 | M | Undifferentiated pleomorphic sarcom | Soft parts of the chest wall | ++ | ++ |
| **Patient 12** | 79 | F | Undifferentiated pleomorphic sarcom | Soft parts of the lower extremity | ++ | ++ |
| **Patient 13** | 72 | F | Undifferentiated pleomorphic sarcom | Meninges | 0 | 0 |
| **Patient 14** | 71 | M | Undifferentiated pleomorphic sarcom | Pelvis | + | + |
| **Patient 15** | 70 | M | Undifferentiated pleomorphic sarcom | Soft parts of the upper extremity | ++ | 0 |
| **Patient 16** | 70 | M | Undifferentiated pleomorphic sarcom | Rectum | ++ | ++ |
| **Patient 17** | 48 | F | Undifferentiated pleomorphic sarcom | Soft parts of the lower extremity | +++ | +++ |
| **Patient 18** | 76 | M | Undifferentiated pleomorphic sarcom | Soft parts of the lower extremity | +++ | ++ |
| **Patient 19** | 78 | M | Undifferentiated pleomorphic sarcom | Soft parts of the neck region | ++ | + |