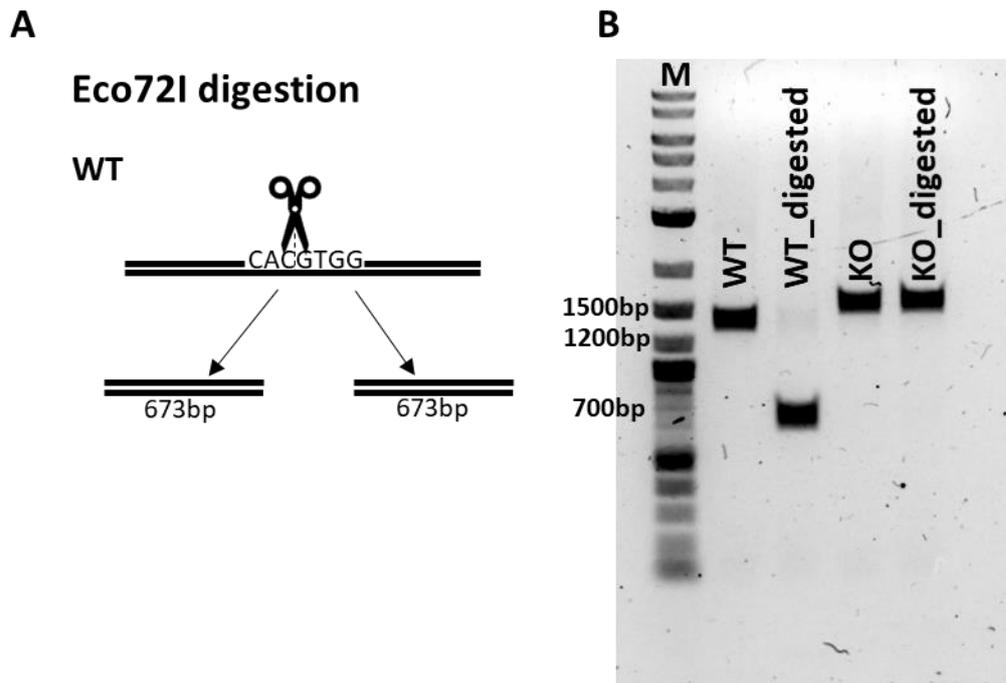


# Supplementray Material: Loss of the Tks4 scaffold protein induces epithelial-mesenchymal transition-like changes in human colon cancer cells



**Figure S1. Genotyping with Eco72I.** (A) Schematic representation of Eco72I digestion in wild-type alleles. Upon digestion PCR products with unmodified exon2 sequences are halved into two 673 basepair long fractions. (B) Comparison of WT and KO, undigested and digested sequences. Wild type sequences are effectively halved, while KO sequences remain intact. M= Quick-Load® Purple 1 kb Plus DNA Ladder (New England Biolabs).

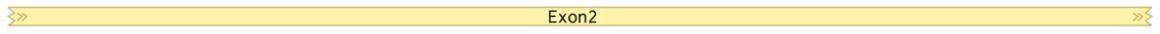
**A**

Allele1:

ATGCCGCCGCGCGCAGCATCGTGGAGGTGAAGGTGCTAGACGTGCAGAAGCGGGGTGCCCAACAAGCATTATGTCTACATCATCCGGTTCGCGCGAGCCCTGA  
M P P R R S I V E V K V L D V Q K R R V P N K H Y V Y I I R V A R A P D



TGCTCTTCGTCAGATCATCTGATCGACAAGACCGGCTTCCATCCGAGTACGTGCTCGCTCGATGCGATGTTTCGTTGGTGGTCAATGGCAGGTAGCCGGATC  
A L R P D H P D R Q D R L P S E Y V L A R C D V S L G G R M G R \*



AAGCGTATGCAGCCGCCATTGCATCAGCCATGATGGATACTTTCTCGGCAGGAGCAACGTGGTCCAGTGGCTCCACCGAGGCCATTTACCGCGCTACAGCAAGT



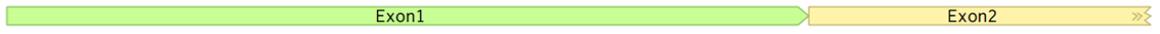
TTTTGACCTCCAG

Exon2 STOP(\*) codon distance from the final exon-junction complex: 1163bp

**B**

Allele2:

ATGCCGCCGCGCGCAGCATCGTGGAGGTGAAGGTGCTAGACGTGCAGAAGCGGGGTGCCCAACAAGCATTATGTCTACATCATCCGGTTCGCGCGAGCCCTCAAC  
M P P R R S I V E V K V L D V Q K R R V P N K H Y V Y I I R G P A A P P



GAGGCCATTTACCGCGCTACAGCAAGTTTTTGTGACCTCCAGATGCAGATGTTGGACAAATTTCCATGGAAGGAGGACAGAAGGACCCCAAGCAGCGGATCATCCC  
R P F T G A T A S F L T S R C R C W T N F P W K E D R R T P S S G S S



CTTTCTGCCAGTAAGATTCTCTTCCAGACGAAGCCACATCCGGGACGTGGCTGTCAAACGCCTGATACCAATTGATGAATACTGTAAG  
P F C Q V R F S S D E A T S G T W L S N A \*



STOP(\*) codon distance from the final exon-junction complex: 1059bp

**Figure S2. CRISPR/Cas9 modified alleles of SH3PXD2B gene pasted into the wild type open reading frame. (A) The modified second exon in Allele1 causes a premature STOP codon 1163 bases upstream from the final exon-junction complex. (B) The modified second exon in Allele2 causes a premature STOP codon 1059 bases upstream from the final exon-junction complex.**

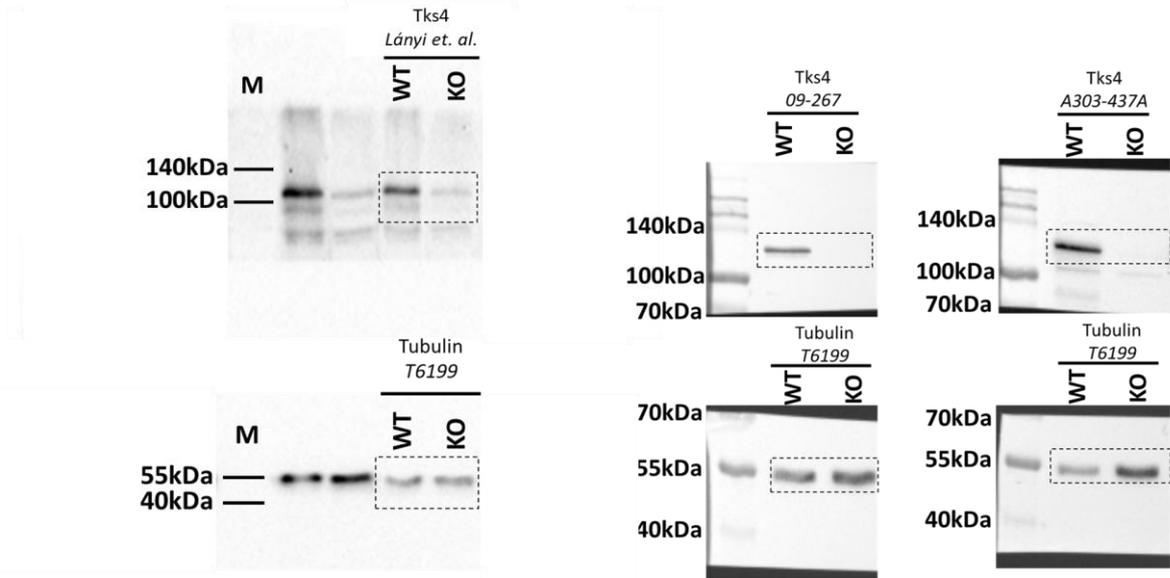


Figure S3. Original uncropped western blot pictures of membranes displayed in Figure 1

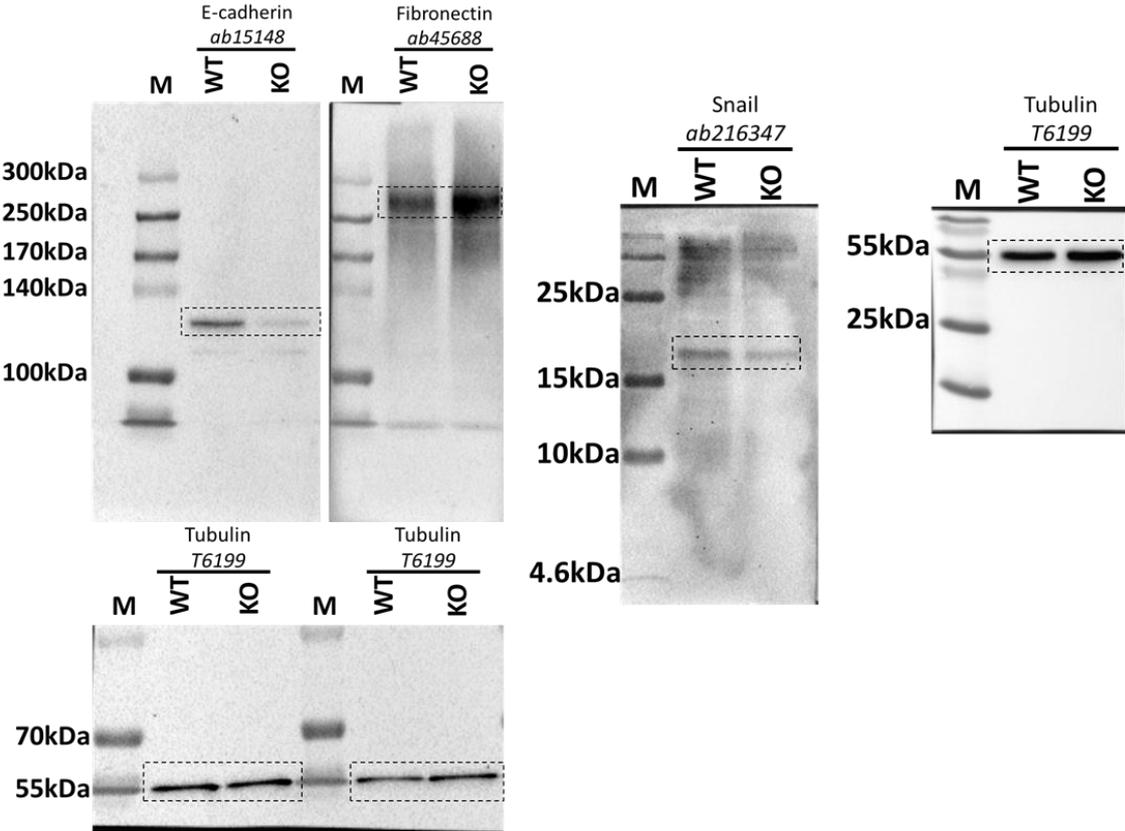
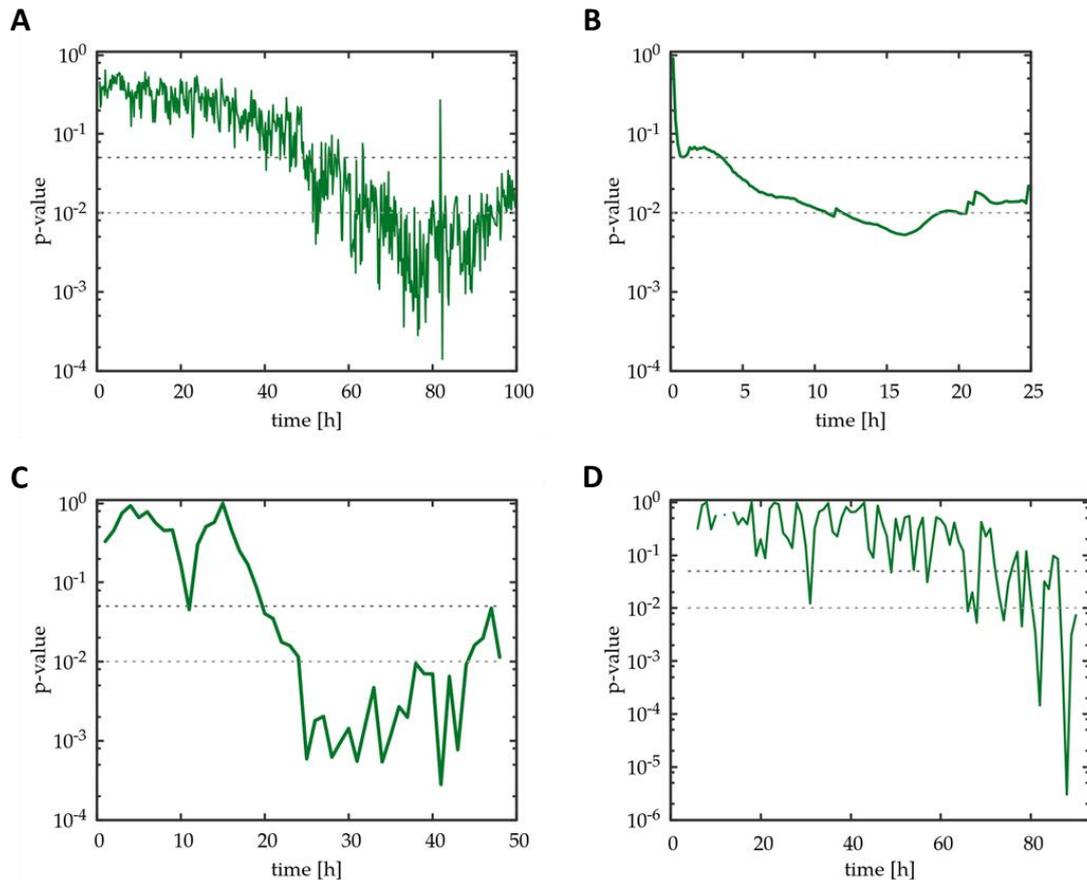
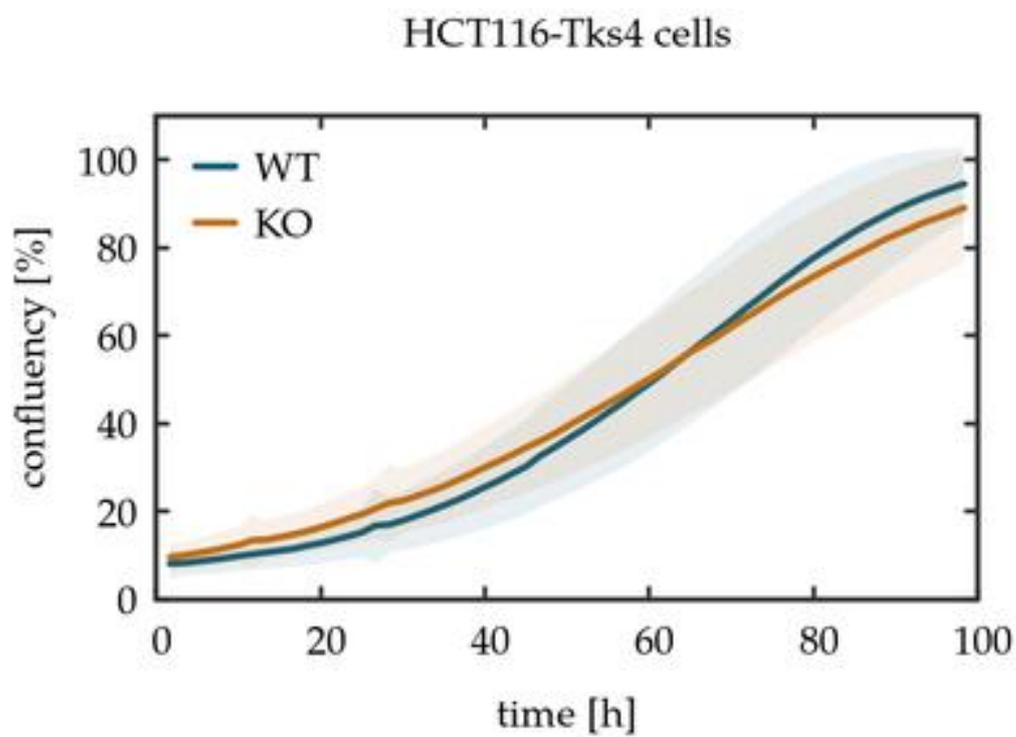


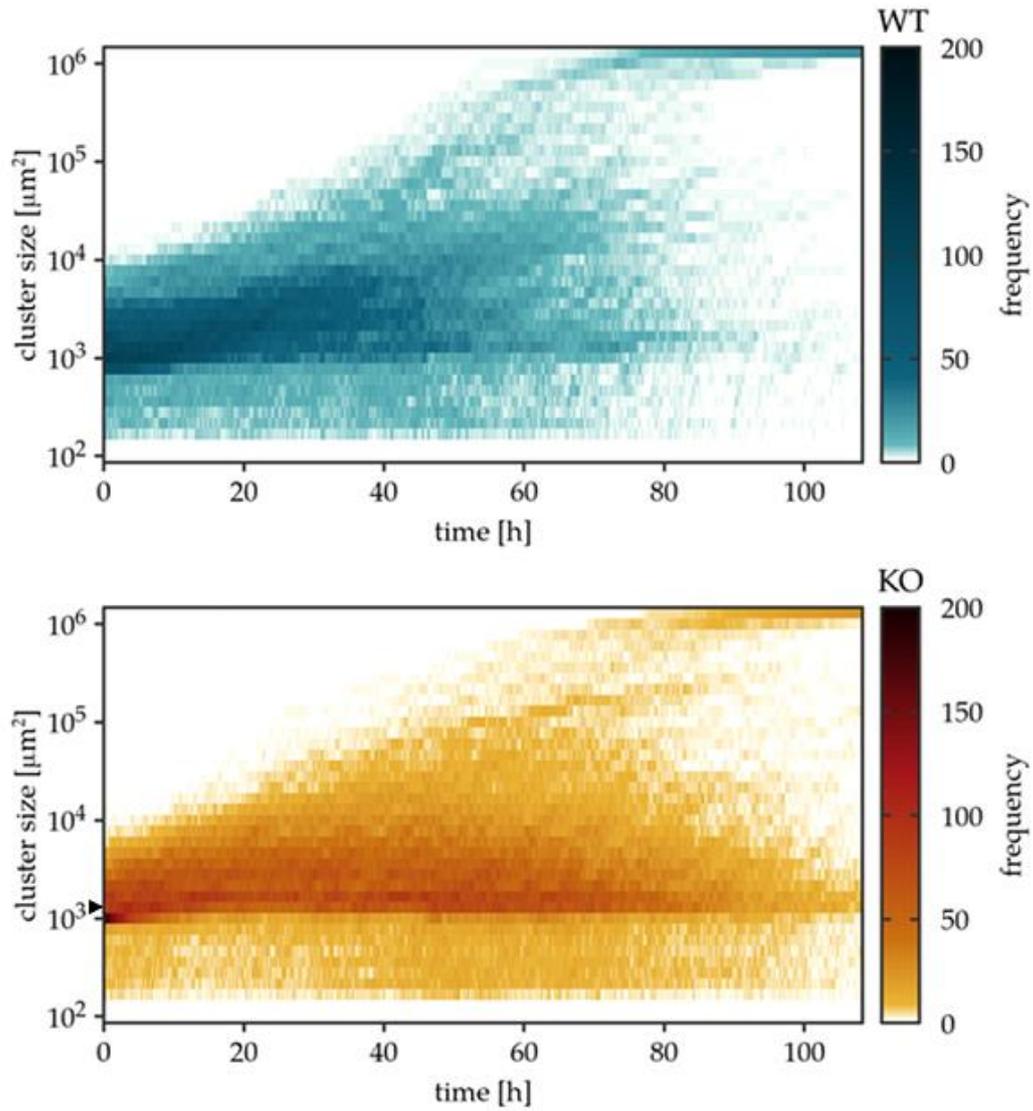
Figure S4. Original uncropped western blot pictures of membranes displayed in Figure 2.



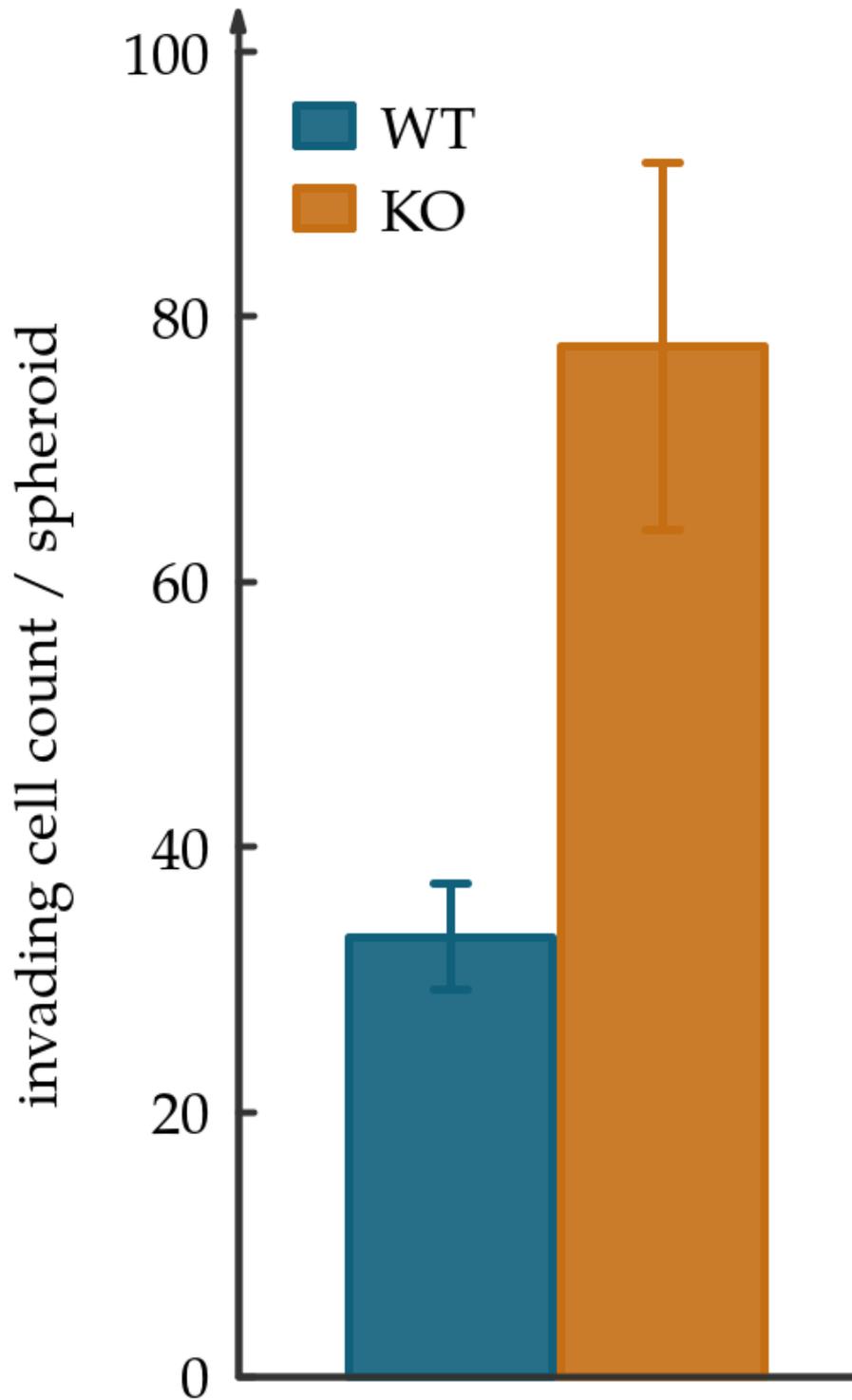
**Figure S5. P-values of cell motility measurements and spheroid analysis experiments.** (A) P-values of experimental data displayed in Figure 3A. Significance (p-value) of difference between the average speeds of wild type and Tks4-KO cells, calculated for each time point of two dimensional random motility assays. P-values were obtained using unpaired Student's t-tests, calculated for a pool of  $n=27$  microscopic fields. (B) P-values of experimental data displayed in Figure 3B. Significance (p-value) of difference between the average net cell displacements of wild type and Tks4-KO cells, calculated for each time point of two dimensional random motility assays. P-values were obtained using unpaired Student's t-tests, calculated for a pool of  $n=20$  manually followed cells. (C) P-values of experimental data displayed in Figure 4B. Significance (p-value) of difference between the normalized perimeter of wild type and Tks4-KO cells, calculated for each time point of spheroid formation assays. P-values were obtained using unpaired Student's t-tests, calculated for a pool of  $n=9$  microscopic fields. (D) P-values of experimental data displayed in Figure 4D Significance (p-value) of difference between the average speeds of wild type and Tks4-KO cells, calculated for each time point of three dimensional collagen invasion assays. P-values were obtained using unpaired Student's t-tests, calculated for a pool of  $n=3$  wild type and  $n=6$  Tks4-KO microscopic fields.



**Figure S6.** Average confluency of Tks4-WT and Tks4-KO HCT116 cultures, as a function of time. Solid lines represents an average of  $n=3$  independent measurements, each containing 27 microscopic fields. shaded area represents SEM.



**Figure S7. Time development of cluster size distribution in Tks4-WT and Tks4-KO monolayer cultures.** The shade of color indicates cluster counts according to the calibration palette. Single cells and small cell clusters are prevalent in Tks4-KO cultures, as reflected by the persistent peak of the distribution at  $1000 \mu\text{m}^2$  (arrowhead).



**Figure S8.** Number of invading cells from the central aggregate at 3.5 days. Error bars represent SEM, sample size is  $n=18$  and  $n=14$ , for wild type and Tks4-KO spheroids respectively. Statistical analysis yielded  $p<0.0036$ , by using unpaired Student's t-test.

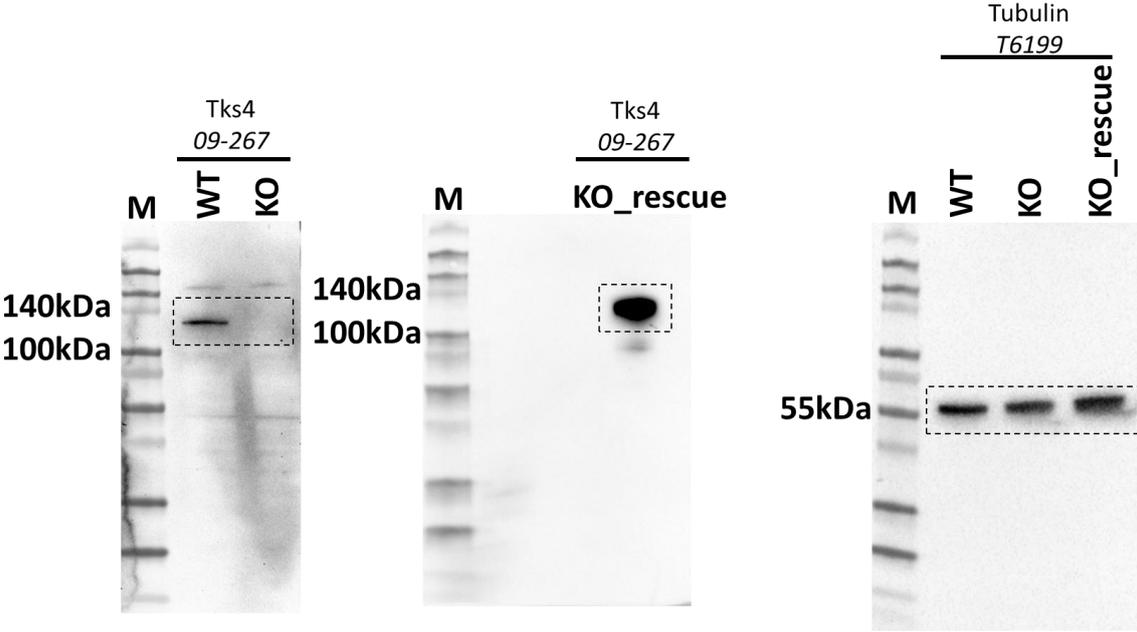


Figure S9. Original uncropped western blot pictures of membranes displayed in Figure 5C.

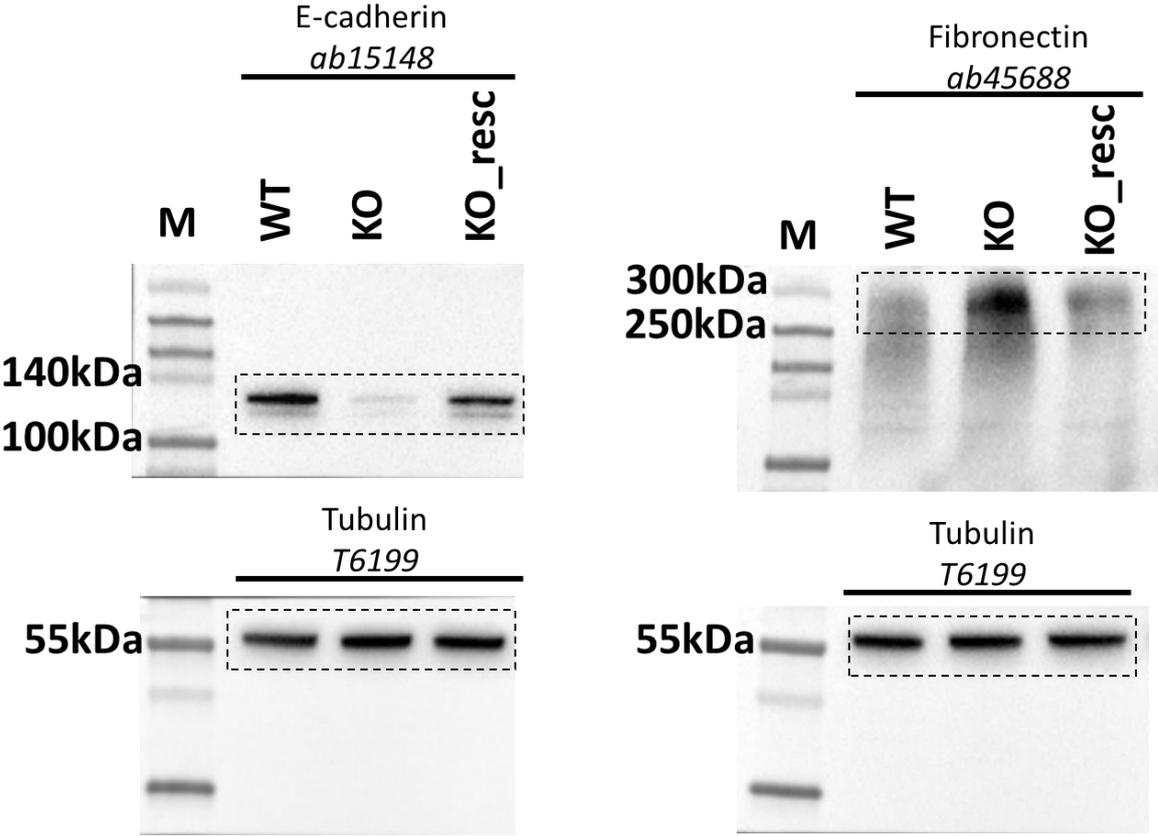


Figure S10. Original uncropped western blot pictures of membranes displayed in Figure 5D.