

Loss of LSR affects epithelial barrier integrity and tumor xenograft growth of CaCo-2 cells

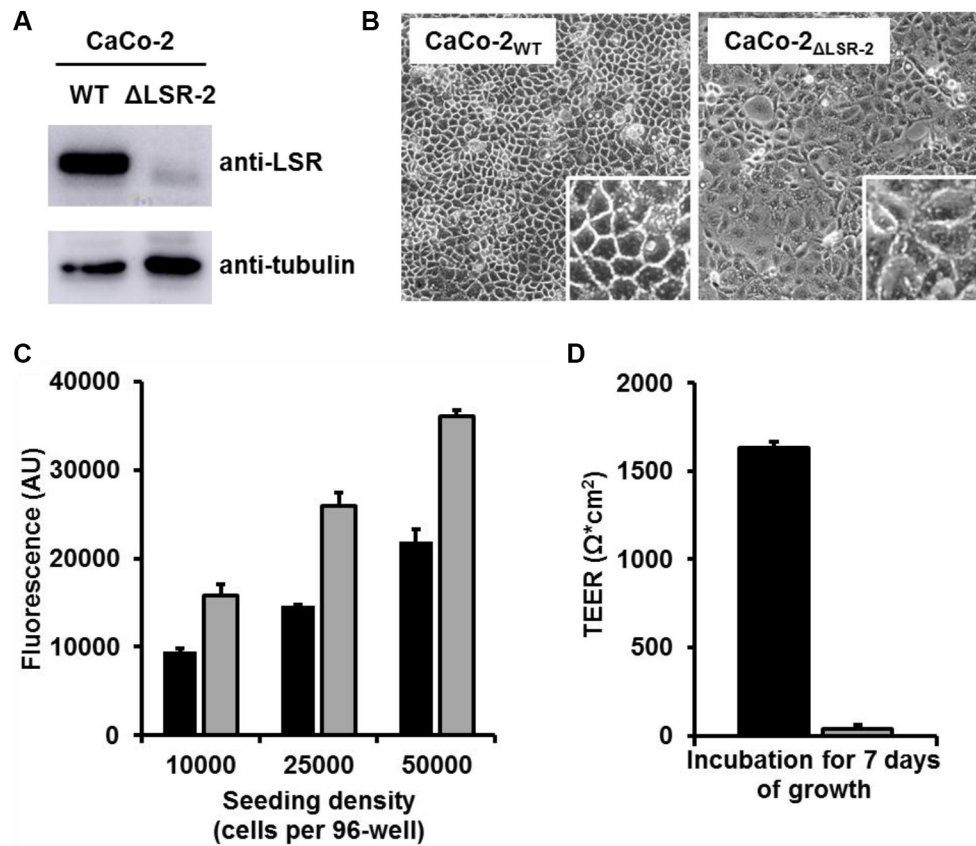
Supplementary Materials



LSR-exon 2

CTCCTGCCAGGGCCATCCAGGTGACCGTGTCCAACCCCTACCACGTGGTGATCCTCTTCCAGCCTGTGACCCCT
 GCCCTGTACCTACCAGATGACCTCGACCCCCACGCAACCCATCGTCATCTGGAAGTACAAGTCTTTCTGCCGG
 GACCGCATCGCCGATGCCTTCTCCCCGGCCAGCGTCGACAACCAGCTCAATGCCAGCTGGCAGCCGGGAACC
 CAGGCTACAACCCCTACGTTGAGTGCCA**GGACAGCGTGC****GCACCGTCAGGG**TCGTGGCCACCAAGCAGGGCAA
 CGCTGTGACCCCTGGGAGATTACTACCAG**GGCCGGAGGATTACCATCACCGGAA**

Supplementary Figure S1: Targeting sequences of guide RNAs for CRISPR/Cas9-mediated DNA cleavage. Gene architecture of human LSR gene (exons 1 to 10) is shown (upper panel) and DNA sequence of LSR exon 2 with highlighted sequences indicating targets of the guide RNAs for Cas9 (lower panel). Red, Cas9 targeting sequence for generating CaCo-2_{ΔLSR}. Green, Cas9 targeting sequence for generating CaCo-2_{ΔLSR-2}.



Supplementary Figure S2: Evaluation of CaCo-2 _{Δ LSR-2} cells. (A) LSR immunoblot with whole-cell lysates from CaCo-2_{WT} (WT) and CaCo-2 _{Δ LSR-2} (Δ LSR-2) cells (top panel). Equal loading of samples was verified by detecting tubulin with a specific antibody (bottom panel). (B) Transmitted light microscopy images of confluent cell monolayers from CaCo-2_{WT} and CaCo-2 _{Δ LSR-2} cells. (C) Cell viability assay. Absolute resorufin fluorescence values are presented in arbitrary units (AU). Black bars represent fluorescence values obtained with CaCo-2_{WT} and grey bars with CaCo-2 _{Δ LSR-2} cells, respectively. Bar graphs represent quantitative analysis of triplicates. The values represent mean \pm SEM. (D) CaCo-2_{WT} and CaCo-2 _{Δ LSR-2} cells were seeded into transwells and incubated for growth. Transepithelial electrical resistance (TEER) was measured after day 7 with a Volt-Ohm meter. TEER results are expressed as absolute TEER values (Ω^*cm^2) with black bars representing CaCo-2_{WT} and grey bars representing CaCo-2 _{Δ LSR-2} cells. Bar graphs represent quantitative analysis of triplicates. The values represent mean \pm SEM.