

Additional file 1

Growth behavior and mRNA expression profiling during growth of IPEC-J2 cells

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Text S1 Cell culture conditions and handling of IPEC-J2 cells

IPEC-J2 cells were incubated with 5% CO₂ and ~90% air humidity (Heracell VIOS160i, Thermo fisher scientific, Schwerte, Germany). Cells were cultured in cell culture dishes (diameter 35mm, 60mm, or 100mm, Greiner Bio-One, Kremsmünster, Germany) with Dulbecco's Modified Eagle Medium/F-12 Nutrient Mixture (Ham), [L] L-Glutamine (DMEM/F12, Gibco, Schwerte, Germany) including 5% fetal calf serum (FCS, Sigma, Hamburg, Germany) and 100 Units/ml Penicillin/Streptomycin (Sigma, Hamburg, Germany). Experiments were performed in 12-well cell culture dishes (Grainer, cellstar, Bio-one GmbH, Austria) and in 10E ECIS cell culture dishes (8W10E, ibidi, Gräfelfing, Germany). For any experiments and splitting, the cells were washed two times with pre-warmed Dulbecco's Phosphate Buffered Saline (DPBS, Sigma, Hamburg, Germany) and detached with pre-warmed trypsin (0.25% Trypsin/0.02% EDTA, Sigma, Hamburg, Germany). After detaching of the cells, trypsin activity was blocked with culturing medium, and cells were counted if necessary. Cells were pelleted (pelleting: centrifugation: 300 g RCF, 5 min), the medium was removed, and cells were diluted in the desired concentration and seeded. Cell counting was performed with a Neubauer-improved cell counting chamber. If a container or liquid came in contact with cells, the opening was conducted under a workflow bench, except for the NGS experiment, as RNA was extracted, respectively cells were counted but not further used.

Text S2 Electric cell-substrate sensing experiment (ECIS)

The timepoint of seeding of the cells was set to value zero hours, the baseline of all growth curves was shortened to 1-10 hours (depending on the measured baseline time) and the plateau phase was shortened to 70 hours after timepoint zero. Growth curves of all monitored wells were uploaded [1] and processed [2] in R, and the baseline was extended to -50 hours. Afterwards, the fitting of the curves was performed.

The experiment was repeated ten times on different days and with different suspensions of cells with two wells and two ECIS dishes for each repetition ($n = 10 \times 2 \times 2 = 400$) so that 400 growth curves were assessed in total. During each repetition, four wells were used as a negative control (only medium change, but no seeded cells) so that biological contaminations were excluded as far as possible.

Text S3 Next-generation sequencing experiment (NGS) and cell count

The QIAzol-treated cells were washed off and transferred to DNA LoBind tubes (Eppendorf, Hamburg, Germany). DNA LoBind tubes were vortexed roughly for 10 seconds and put directly on ice. Afterwards, they were stored immediately at -80°C until further processing. For all timepoints (TP1-TP3), one of the two QIAzol-treated wells was used for total RNA extraction with the Qiagen miRNeasy Mini Kit (Qiagen, miRNeasy Mini Kit, Hilden, Germany). Further, RNA quantity and quality were assessed with the Bioanalyzer (Bioanalyzer 2100, Agilent, USA, Agilent RNA-6000 Nano Kit, Frankfurt, Germany, Fig. S1). For further processing, the mRNA was enriched with a magnetic isolation module kit (NEBNext Poly (A) mRNA, 24 reactions, NewEngland BioLabs, Frankfurt, Germany). The library preparation was performed with the NEBNext Ultra II RNA Library Prep kit (with Sample Purification Beads, 24 reactions, NewEngland

BioLabs, Frankfurt, Germany) and the NEBNext Multiplex Oligos for Illumina kit (Dual Index Primers Set 1 NewEngland Biolabs, Frankfurt, Germany). NovaSeq 6000 S1 Reagent kit (100 cycles, 1x 100 bp, Illumina, New York, US) was performed prior to the NGS step.

References

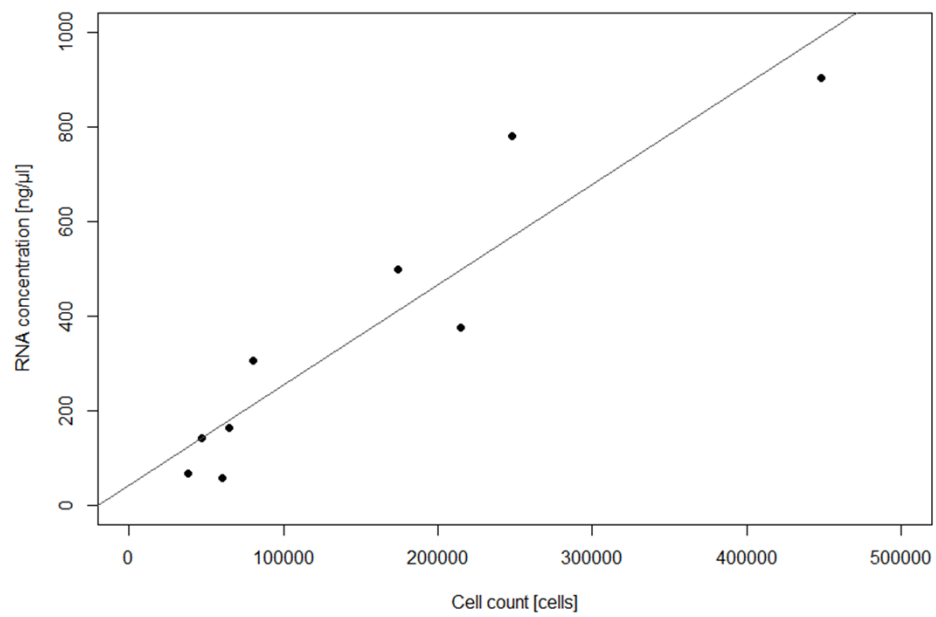
1. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. <https://cran.r-project.org/>.
2. Binder ARD, Spiess A-N, Pfaffl MW. Modelling and Differential Quantification of Electric Cell-Substrate Impedance Sensing Growth Curves. *Sensors*. 2021;21:5286.

Table S1: The 10 most relevant pathways detected by Reactome for comparison of time-point 6h vs. 36h (TP1/TP2), respectively 36h vs. 60h (TP2/TP3)

Compared time-point	Pathway name	Entities (found)	Reactions (found)
TP1/TP2	Cell cycle	292/734	433/451
TP1/TP2	Cell cycle, mitotic	244/596	338/352
TP1/TP2	Cell cycle, checkpoints	118/279	54/56
TP1/TP2	TP53 Regulates Transcription of Genes Involved in G1 Cell Cycle Arrest	17/20	17/17
TP1/TP2	Mitotic Spindle Checkpoint	55/111	7/7
TP1/TP2	DNA strand elongation	25/38	14/15
TP1/TP2	Diseases of mitotic cell cycle	25/38	4/5
TP1/TP2	Aberrant regulation of mitotic cell cycle due to RB1 defects	24/36	2/3
TP1/TP2	rRNA modification in the nucleus and cytosol	39/72	7/8
TP1/TP2	Activation of ATR in response to replication stress	25/39	9/9
TP2/TP3	rRNA modification in the nucleus and cytosol	32/72	5/8
TP2/TP3	rRNA processing in the nucleus and cytosol	51/208	12/15
TP2/TP3	Major pathway of rRNA processing in the nucleolus and cytosol	47/189	7/7
TP2/TP3	rRNA processing	53/246	14/21
TP2/TP3	GRB7 events in ERBB2 signaling	5/6	1/1
TP2/TP3	FOXO-mediated transcription of cell cycle genes	10/27	18/22
TP2/TP3	Metabolism of RNA	116/829	106/189
TP2/TP3	RNA Polymerase III Transcription Termination	8/23	2/2
TP2/TP3	FOXO-mediated transcription of cell death genes	8/23	14/15
TP2/TP3	ISG15 antiviral mechanism	18/83	6/16

P-value for all pathways (found entities) was <0.01

Fig. S1 RNA concentration [ng/μl] in relation to the counted cells



R^2 : 0.87, adjusted R^2 : 0.85.