CHARITÉ CAMPUS BENJAMIN FRANKLIN

Analyse der Genexpression und Signalwege bei der akuten Campylobacter jejuni-Enteritis in der humanen Dickdarmmukosa

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1) RNA-Seq / Genexpression

2) Pathway & Regulator Anlayse (IPA)

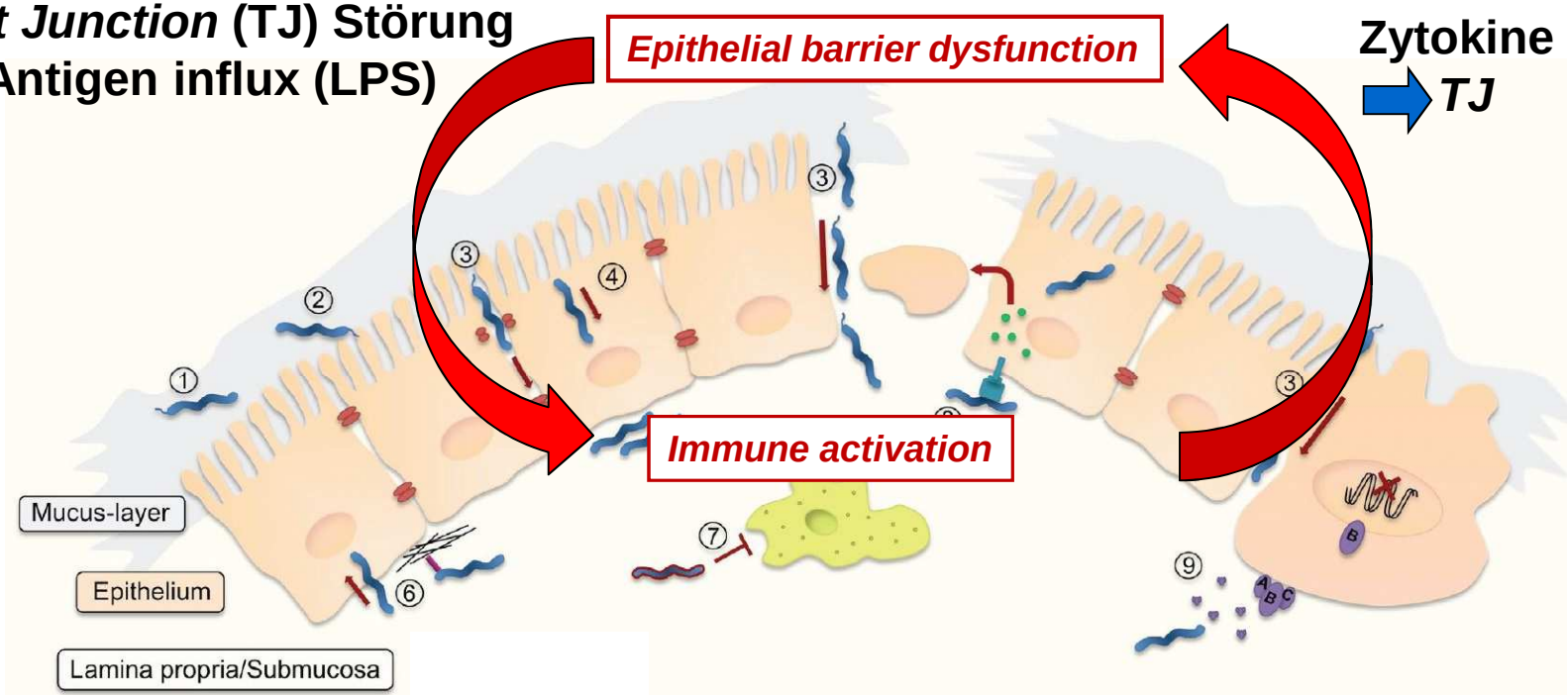
3) Pathway confirmation

Hintergrund

***Campylobacter jejuni* Infektion**

Leaky Gut

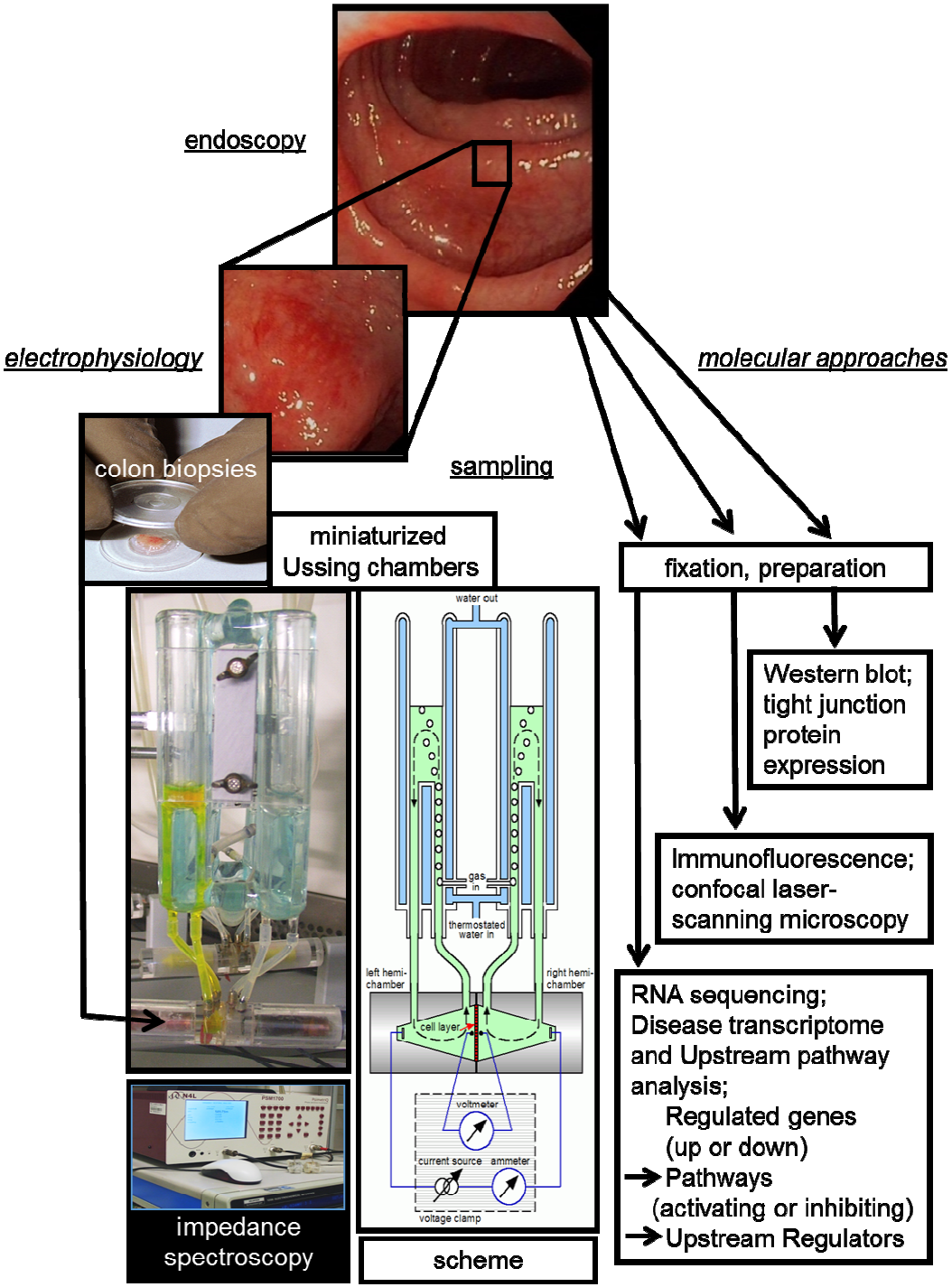
***Tight Junction* (TJ) Störung**
Antigen influx (LPS)



Komplikationen:
RDS CED RA

from: Sprenger, *et al. Eur. J. Microbiol. Immunol.* 2012

Biopsy sampling (colonoscopy)



***C. jejuni*-infected patients & controls**

day 4-7 p.i. (n=4-6)

Ziele

Klinische Studie – Campylobacter-Enteritis

- **Identifizierung von Genen & Regulatoren, die das Epithel und mukosale Immunzellen modulieren (direkt in der Kolonmukosa von *C.j.* Patienten)**
- **Bestätigung von *Campylobacter*-induzierten Veränderungen der Signalwege / differentielle Genexpression konsitent mit beschriebenen Signalwegen**
- **Upstream regulators, Regulator effects (Bioinformatik/Vorhersagen)**
- **Identifizierung von potentiellen therapeutischen Substanzen**

Methoden

RNA-Seq und Bioinformatik:

- **RNA Extraktion aus humanen Kolonbiopsie mit Trizol**
- **cDNA library Preparation und Sequenzierung (Illumina HiSeq2500)**
- **Read mapping gegen humane Genom GRCh37/hg19 mittels STAR**
- **Statistik mit R software.**
- **Upstream Regulators identifiziert mit Ingenuity Pathway Analysis (IPA) software (Qiagen Silicon Valley).**

INGENUITY PATHWAY ANALYSIS

RNA-Seq → **CPM (counts per million) der einzelnen Gentranskripten**

IPA Literatur-basierte Zell-Signal Netzwerk Platform

→ **Canonical pathways**

→ **Upstream regulators**

Ergebnisse

RNA-Seq: In der *C. jejuni*-infizierten humanen Kolonmukosa waren 2,988 Transkripte herunter-reguliert und 2,410 herauf-reguliert.

S100A8/9 (calprotectin)

CXCL (chemokines)

Mellits *et al.* 2009 *BMC Microbio.*

SCNN1 (ENaC subunit)

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	ensg	name	logFC	logCPM	PValue	Padj	[redacted]		readcount	[redacted]	readcount	[redacted]	readcount	readcount	readcount	readcount.
2	ENSG0000	REG1A	1,4E+08	5,81243	6,85E-41	4,16E-36	21563	10049	0	270	0	1508	0	0	0	0
3	ENSG0000	LCN2	5,44532	6,30563	9,36E-24	2,84E-19	17567	20551	51	978	135	3411	339	287	50	584
4	ENSG0000	DMBT1	5,21459	6,12326	1,02E-22	2,04E-18	9276	6961	166	2270	341	7138	80	151	89	295
5	ENSG0000	IGHG3	3,85399	4,24572	1,35E-22	2,04E-18	2106	2401	101	589	112	1704	224	117	17	513
6	ENSG0000	SAA1	8,53559	1,48482	8,43E-21	1,02E-16	591	141	0	15	0	528	0	3	0	3
7	ENSG0000	DUOXA2	5,48479	2,42574	1,62E-18	1,64E-14	1555	1147	8	32	3	199	16	16	6	38
8	ENSG0000	S100A9	6,25565	2,95323	7,80E-18	6,76E-14	549	316	8	564	4	386	17	20	5	26
9	ENSG0000	SAA2	1,4E+08	-0,6007	9,37E-18	7,11E-14	79	90	0	3	0	117	0	0	0	0
10	ENSG0000	CXCL9	7,0637	3,38306	6,88E-17	4,64E-13	3489	1815	27	2887	10	1134	98	18	3	141
11	ENSG0000	TNIP3	5,87102	0,67695	3,88E-16	2,35E-12	288	136	4	86	8	88	1	1	1	7
12	ENSG0000	REG1B	1,4E+08	3,18174	1,01E-15	5,57E-12	4954	142	0	4	0	158	0	0	0	0
13	ENSG0000	S100A8	5,86387	1,24812	4,22E-14	2,13E-10	174	109	1	183	5	187	7	3	3	21
14	ENSG0000	CHI3L1	7,22255	0,67487	5,99E-14	2,79E-10	422	78	1	97	2	13	1	2	0	4
15	ENSG0000	IDO1	4,36757	2,83923	1,25E-13	5,41E-10	790	408	35	323	17	680	102	24	3	113
16	ENSG0000	CXCL10	5,0834	1,81904	3,19E-13	1,29E-09	197	147	10	275	16	224	18	15	2	32
17	ENSG0000	PCK1	-3,74174	5,38128	4,36E-13	1,65E-09	385	322	3007	28	3746	229	1449	4723	328	9012
18	ENSG0000	FAM3B	6,71999	-0,50035	4,66E-13	1,66E-09	123	138	0	2	2	68	2	0	0	0
19	ENSG0000	PI3	4,0617	6,21898	7,23E-13	2,38E-09	19295	15485	284	339	871	4541	541	447	83	699
20	ENSG0000	GLRA2	-5,41569	0,43674	7,47E-13	2,38E-09	1	3	48	0	141	5	140	61	35	181
21	ENSG0000	SOCS3	3,05394	3,02342	3,20E-12	9,70E-09	880	1137	114	256	95	513	168	99	6	181
22	ENSG0000	SLC6A14	4,83171	1,60995	1,04E-11	3,00E-08	883	605	22	35	5	43	13	8	4	10
23	ENSG0000	CAPN13	-3,07449	3,78265	2,55E-11	7,04E-08	96	294	874	22	1050	80	475	1150	340	2102
24	ENSG0000	CXCL1	3,10233	2,38862	3,56E-11	9,39E-08	428	1066	26	137	23	354	68	107	26	132
25	ENSG0000	CXCL8	4,94041	0,33071	9,00E-11	2,27E-07	48	276	3	80	7	57	9	2	3	7
26	ENSG0000	CASP1	2,52343	4,17571	2,62E-10	6,35E-07	2423	2252	110	421	389	1113	337	242	57	1046
27	ENSG0000	CXCL11	4,41228	-0,75925	2,85E-10	6,66E-07	44	53	1	23	2	91	8	3	0	10
28	ENSG0000	DPP10-AS:	-4,46207	0,02161	7,34E-10	1,65E-06	2	9	67	2	74	0	42	105	23	137
29	ENSG0000	REG3A	1,4E+08	-1,30658	9,31E-10	2,02E-06	24	156	0	0	0	14	0	0	0	0
30	ENSG0000	FCN1	5,41612	0,91748	1,41E-09	2,95E-06	320	31	3	140	0	83	11	5	2	27
31	ENSG0000	SLC7A5	2,98697	1,47026	2,20E-09	4,46E-06	523	286	14	73	28	140	40	22	8	176
32	ENSG0000	FCGR3B	6,40631	-0,28231	6,50E-09	1,27E-05	58	28	4	102	0	40	0	1	1	6
33	ENSG0000	MST1L	-3,36067	2,72834	6,99E-09	1,32E-05	75	90	560	1	205	47	455	981	102	558
34	ENSG0000	LRRN2	-3,14152	3,27536	8,76E-09	1,57E-05	116	80	471	19	1297	68	321	401	100	2606
35	ENSG0000	IGKV2-29	7,8996	0,6759	8,78E-09	1,57E-05	164	3	1	191	0	19	2	0	0	7
36	ENSG0000	FCGR3A	5,73459	3,80769	1,07E-08	1,85E-05	767	220	7	1124	22	374	10	21	32	23
37	ENSG0000	TRIM40	2,63055	3,58341	1,22E-08	2,06E-05	1642	2155	105	270	282	450	168	102	75	276
38	ENSG0000	PADI2	-2,78895	6,20398	1,28E-08	2,10E-05	534	2121	4210	132	6476	491	2495	5483	1353	15501
39	ENSG0000	TRPM6	-3,16167	5,24486	1,76E-08	2,73E-05	406	516	3152	16	3468	416	1066	1953	669	8284
40	ENSG0000	SLC16A9	-4,22521	3,62844	1,80E-08	2,73E-05	67	114	357	0	1167	54	400	338	238	5412
41	ENSG0000	FPR1	6,0747	0,26133	1,78E-08	2,73E-05	87	25	0	113	0	109	2	4	4	4
42	ENSG0000	TREM1	6,57297	-2,18899	1,85E-08	2,74E-05	14	16	0	14	0	31	2	0	0	0
43	ENSG0000	RP3-407E4	-1,4E+08	-1,99389	2,32E-08	3,36E-05	0	0	5	0	26	0	24	12	2	37
44	ENSG0000	PLEK	3,32944	3,32457	3,24E-08	4,36E-05	1052	376	103	490	78	648	263	47	13	216
45	ENSG0000	CD38	3,28539	1,99439	3,16E-08	4,36E-05	534	231	27	176	27	255	84	14	6	216
46	ENSG0000	TLR8	4,72507	0,97567	3,20E-08	4,36E-05	136	35	9	190	7	83	21	4	4	25
47	ENSG0000	GPR84	5,75696	-2,13915	3,66E-08	4,83E-05	20	22	0	19	0	16	0	1	0	4
48	ENSG0000	HLA-DRB5	3,60187	4,80795	4,01E-08	5,17E-05	8332	524	343	826	141	736	233	122	67	728
49	ENSG0000	SCNN1B	-2,79782	3,38634	4,63E-08	5,85E-05	631	651	1259	63	4007	414	1873	2854	1419	4150
50	ENSG0000	DIO3	-4,71389	-0,34805	4,86E-08	6,01E-05	0	8	21	0	96	1	35	69	9	144

Campylobacter.differentialGeneE

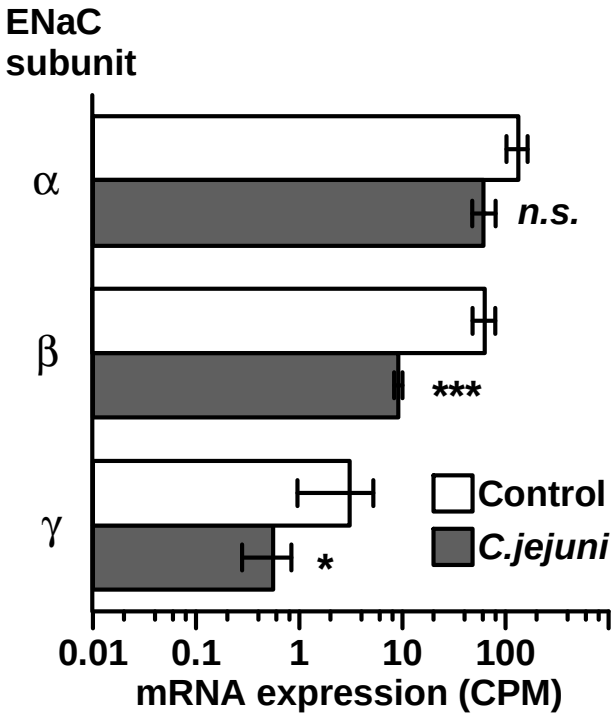
truncated

RNA sequencing data deposited in NCBI's Gene Expression Omnibus (GEO), Accession code: GSE88710

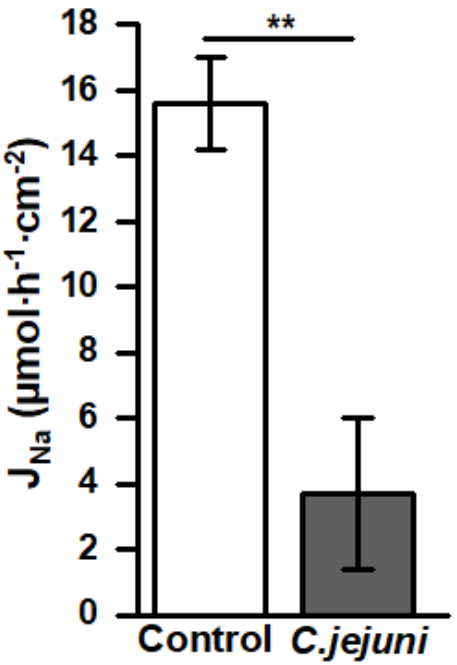
Epithelialer Natrium Kanal (ENaC)

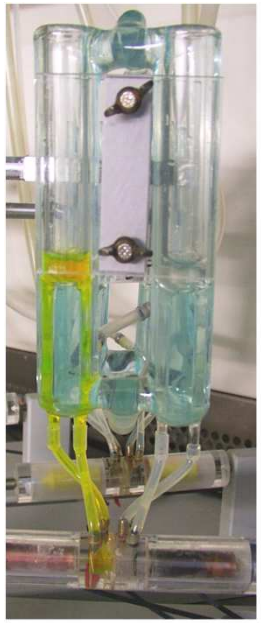
Kolon-Biopie aus *C. jejuni*-Patienten verglichen mit gesunden Kontrollen (n=4-6)

RNA-Seq Daten



ENaC Aktivität





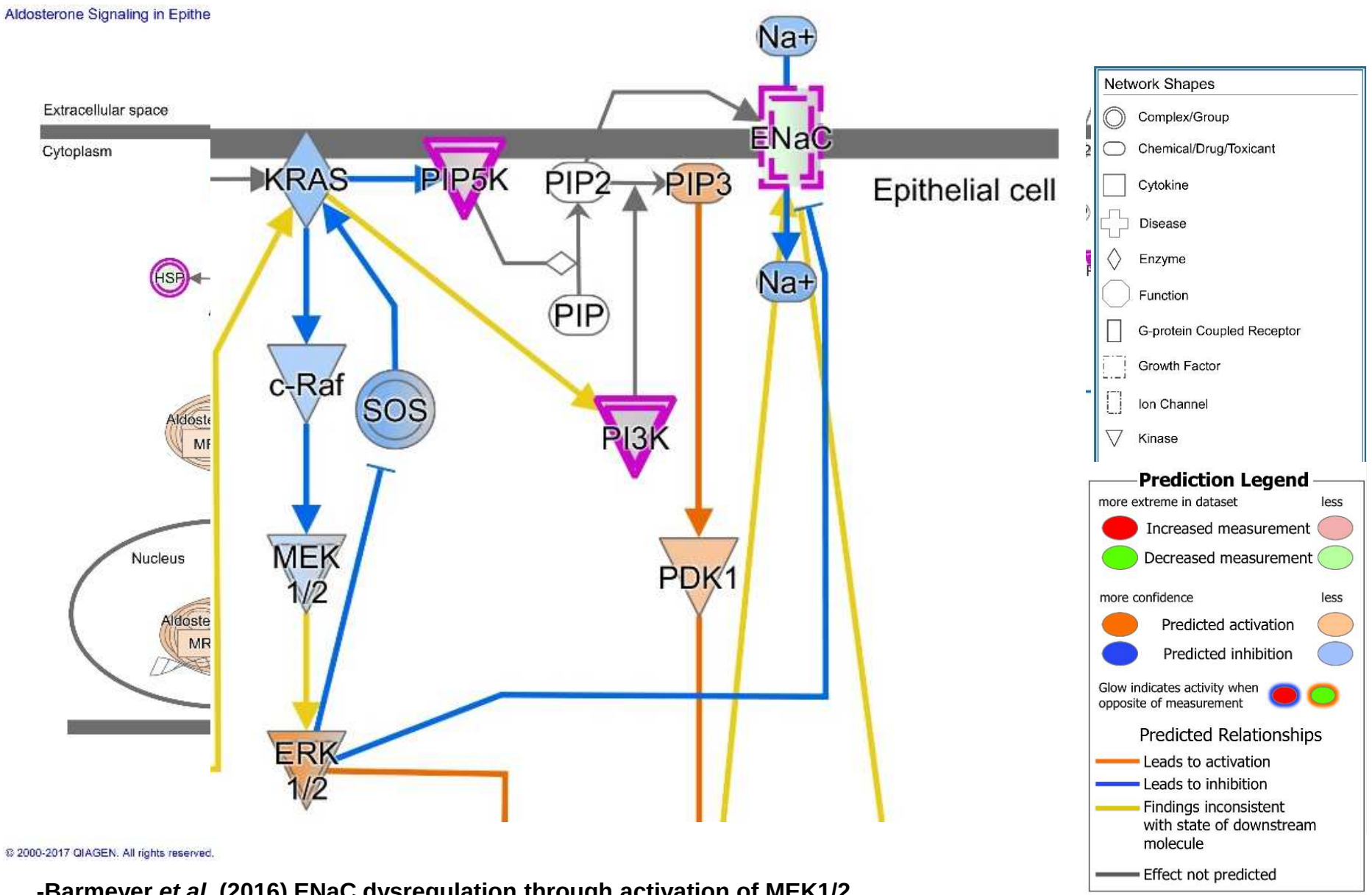
SCNN1 A / B / G
(ENaC subunits α / β / γ)

Ussing chamber (electrogenic Na^+ transport)

Na^+ Malabsorption

Bücker, *et al. Mucosal Immunology.* 2017

Zytokin-induzierte ENaC Dysregulation?

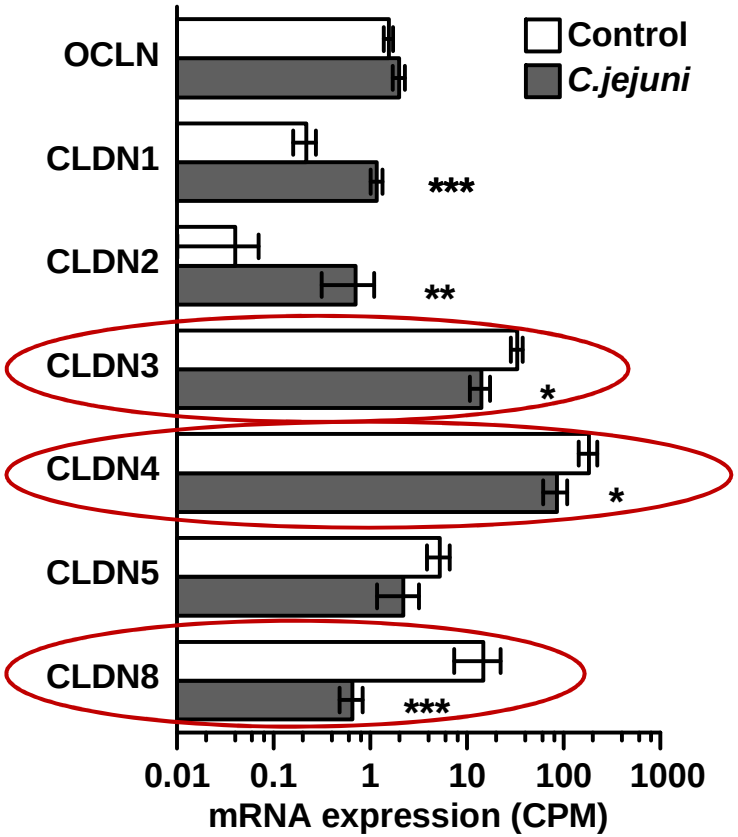


-Barmeyer *et al.* (2016) ENaC dysregulation through activation of MEK1/2 contributes to impaired Na+ absorption in lymphocytic colitis. *Inflamm. Bowel Dis.*

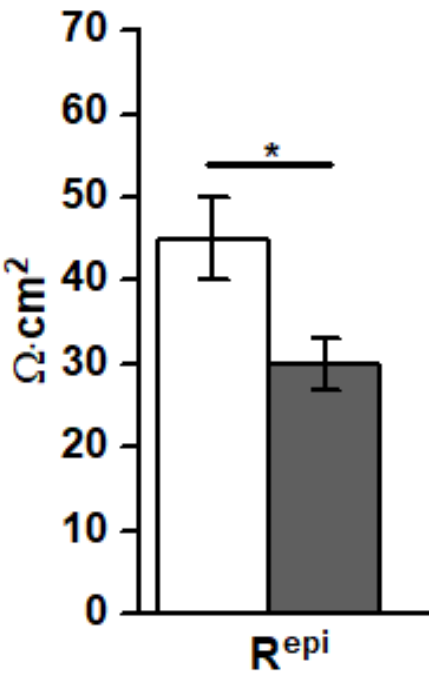
-Dames *et al.* (2015) Interleukin-13 affects the intestinal epithelial sodium channel (ENaC) by coordinated modulation of STAT6 and p38 MAPK activity. *J. Physiol. (Lond.)*

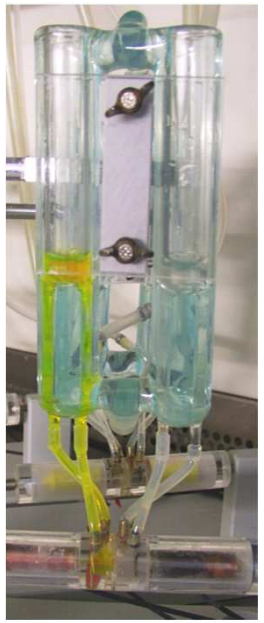
Tight Junction

RNA-Seq Daten



Epithelialer Widerstand





Ussing chamber (impedance spectroscopy)

OCLN = occludin
CLDN = claudin

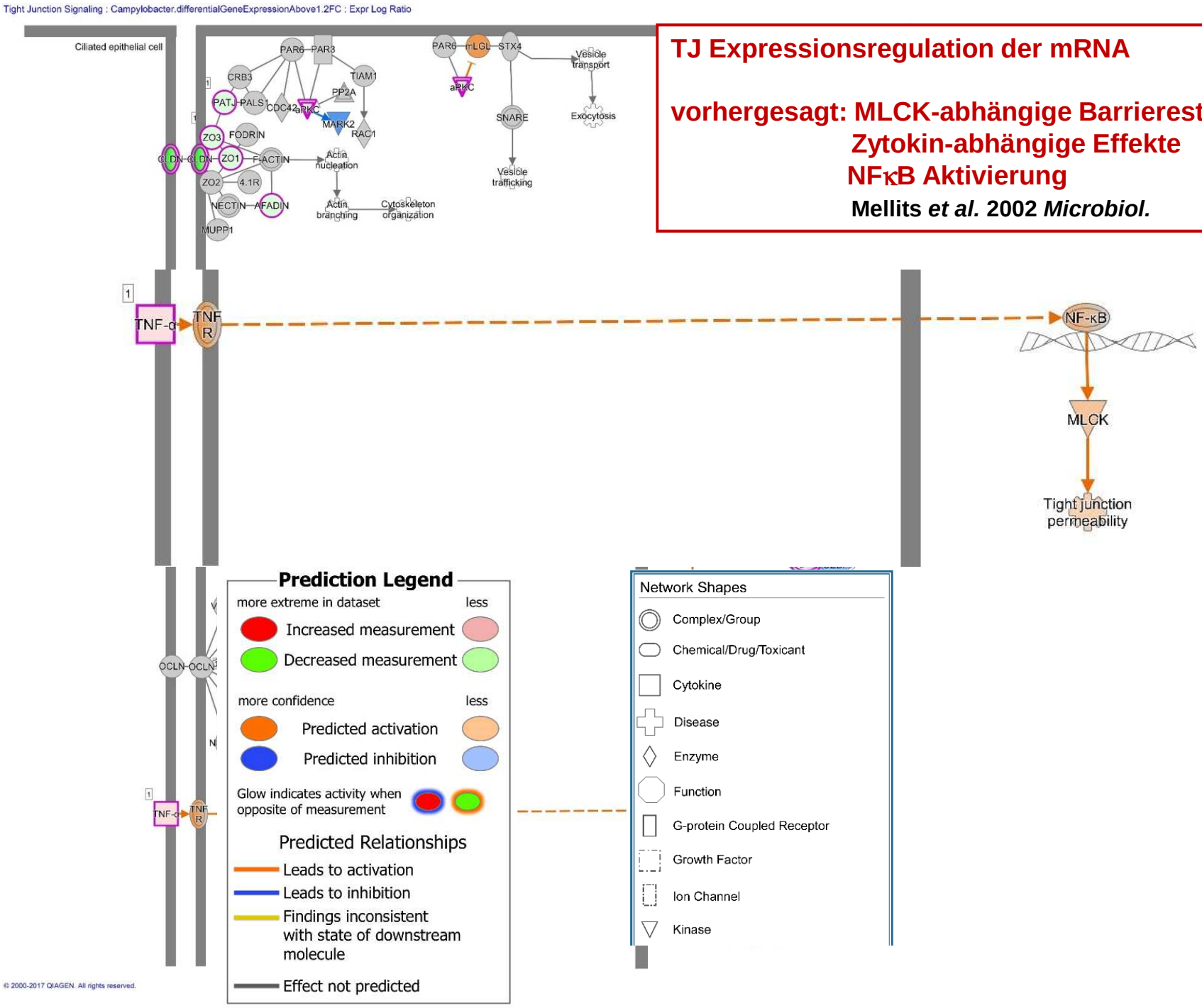
Epitheliale Barriere-Dysfunktion

Bücker, *et al. Mucosal Immunology.* 2017

TJ Expressionsregulation der mRNA

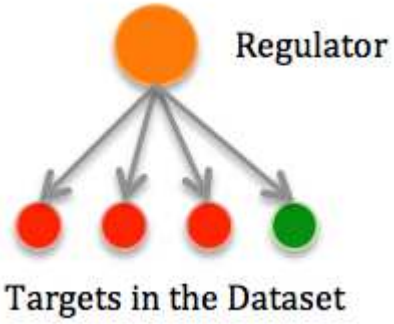
vorhergesagt: MLCK-abhängige Barrierestörung
Zytokin-abhängige Effekte
NFκB Aktivierung

Mellits *et al.* 2002 *Microbiol.*

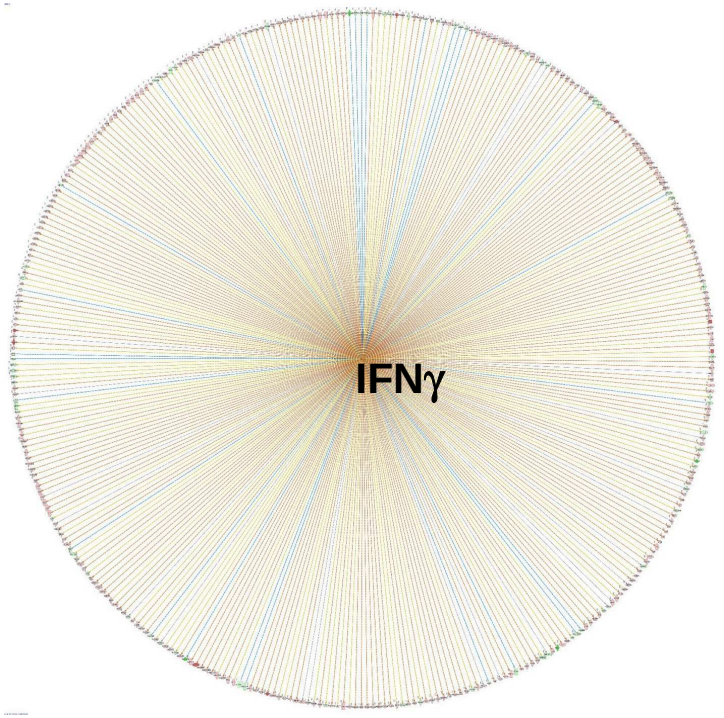


Upstream Regulators

INGENUITY PATHWAY ANALYSIS

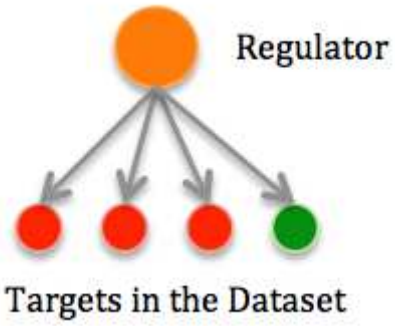


- **Über 1,000 identifizierte *Upstream Regulatoren* mit signifikant veränderten *Downstream Targets* in der *C. jejuni*-infizierten humanen Mukosa**
- **Top *Upstream Regulators* mit am stärksten aktivierten Signalwegen sind proinflammatorische Zytokine TNFα, IFNγ, IL13, IL6, IL1β, GM-CSF (Granulocyte Macrophage-Colony Stimulating Factor)**
- **Interaktion zwischen Zellen**
- **Chemische Regulatoren (Pharmaka)**



Upstream Regulators

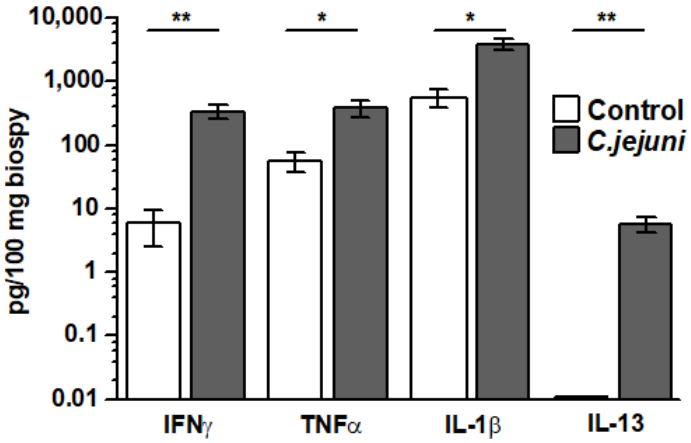
INGENUITY PATHWAY ANALYSIS



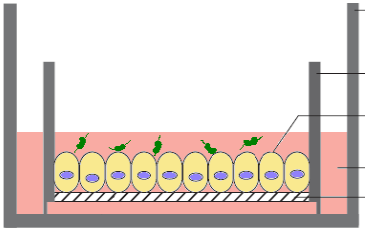
Most significant activating regulators → barrier dysfunction and immune activation

Upstream regulator	Overlap *P*-value	Activation z-score	Number of genes that have expression direction consistent with activation of the regulator
LPS	$3.22E^{-66}$	11.94	343 (of 501 affected downstream targets)
IFNγ	$3.95E^{-44}$	9.62	253 (of 370 affected downstream targets)
CSF2	$2.58E^{-42}$	10.15	144 (of 182 affected downstream targets)
TNFα	$3.23E^{-41}$	9.00	279 (of 462 affected downstream targets)
IL6	$1.18E^{-38}$	7.25	134 (of 236 affected downstream targets)
IL13	$1.39E^{-30}$	2.12	87 (of 145 affected downstream targets)
IL1β	$1.54E^{-24}$	8.57	165 (of 236 affected downstream targets)

The activation z-score determines that an upstream transcription regulator has significantly more "activated" predictions (z>0) than "inhibited" predictions (z<0).



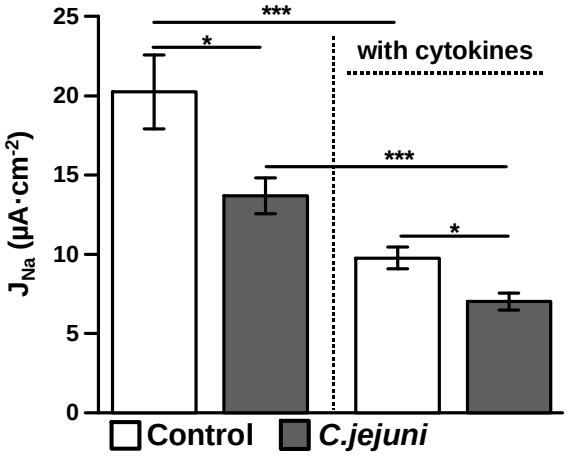
Cytometric bead array (CBA)
Released cytokines in human mucosal supernatant



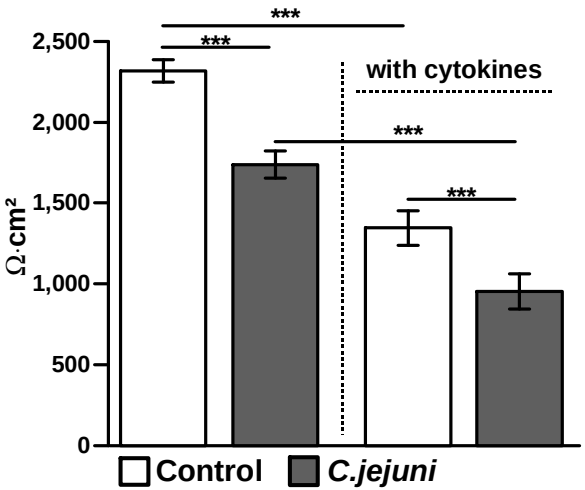
Elektrophysiologische Analysen
humaner intestinaler HT-29/B6 Zell Monolayer

Zytokin-Cocktail: IFNγ, TNFα, IL1β, IL13

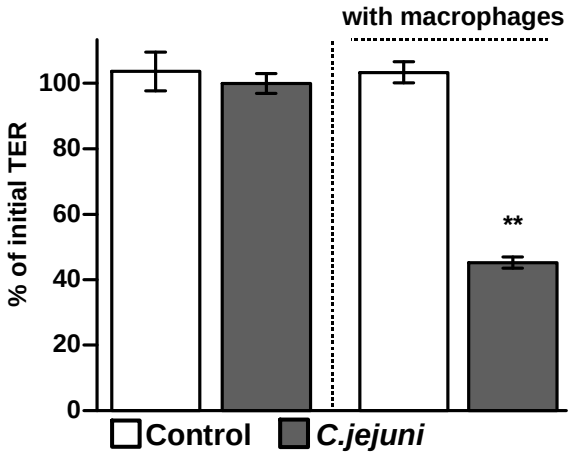
ENaC Aktivität



Epithelialer Widerstand



Co-culture model 24 h p.i.
CSF2 (GM-CSF) -> M1 macrophages



Predicted Drugs

Rapamycin (mTOR)
Sun *et al.* 2012
Gastroenterology

PI3K-Inhibitor
Hu *et al.* 2006
Microb. Pathogen.

Active vitamin D

Upstream Regulator	Predicted Activation State	p-value of overlap	Target molecules in dataset
LPS	Activated	3.22E-66	ABCB1,ABCC1,ABCC3,ABCC5,ABCC6,ABCG1,ACP5,ACVR2A,ADA,ADGRE1,...
IFNγ	Activated	3.95E-44	ABCA6,ABCB1,ABLIM3,ADIPOQ,AIF1,AIF1L,AIM2,ALOX5AP,APOBEC3G,...
CSF2	Activated	2.58E-42	ABCG1,ACP5,ADA,ADRBK2,ALOX5AP,ANLN,ANXA1,AOC1,AURKA,BCL2A1,...
TNFα	Activated	3.23E-41	ABCC1,ABCC3,ABCG2,ABR,ABTB2,ACADS,ACADVL,ACKR2,ACP5,ADAMTS4,..
IL6	Activated	1.18E-38	ABCC1,ABCC3,ABCC5,ABCG2,ACKR2,ACVR2A,ADAMTS4,ADGRE1,AHNAK,...
IL13	Activated	1.39E-30	ACADVL,ADA,ALDH1A2,ALOX5AP,ARNTL2,BATF,BCL2A1,BID,BLVRA,C3AR1,..
calcitriol	Inhibited	8.97E-25	ABCB1,ABCC3,ACKR4,ADAM19,AGR2,ALDH1A2,ALPI,ANLN,APOA1,BIRC5,...
IL1β	Activated	1.54E-24	ABCC3,ABCG2,ACHE,ACKR2,ACPP,ADAMTS4,AIF1,AKR1B1,ANXA1,APOE,...
sirolimus	Inhibited	3.03E-17	ABHD11,ACADVL,ADA,ANXA5,AQP3,ARHGDIB,ASS1,BCL2A1,BID,BIRC5,...
peptidoglycan	Activated	1.70E-14	BCL2A1,CCL18,CCL3,CCL3L3,CCL4,CD274,CD40,CD80,CD86,CORO1A,...
E. coli LPS	Activated	2.82E-13	ATG7,CCL3,CCL4,CCR7,CD40,CD80,CD83,CD86,CXCL1,CXCL10,CXCL2,...
LY294002	Inhibited	3.43E-11	ABCB1,ABCC1,ACSS2,ADH1C,APOE,AQP3,AURKA,AXIN1,BHLHE40,BIRC3,...
glucocorticoid	Inhibited	1.49E-09	ABCC8,ACHE,ANXA1,CCL3,CD163,CD2,CD69,CDK4,CLU,CSF1R,CSF3,...
vitamin D	Inhibited	5.46E-05	BIRC5,CCL3L3,CDK1,CYP24A1,CYR61,DEFB4A/DEFB4B,IFNG,IGFBP1,IL1B,...
quercetin	Inhibited	1.95E-05	ACPP,BIRC5,CXCL10,CXCL8,CYP2E1,EDN1,EGFR,FOS,HIF1A,HMGCS2,...
zinc	Affected	1.83E-04	ASPG,CCNB1,CD86,CDK4,CETP,ENO1,GUCA2B,IL17A,IL1B,IL6,KPNA2,LIPA,...

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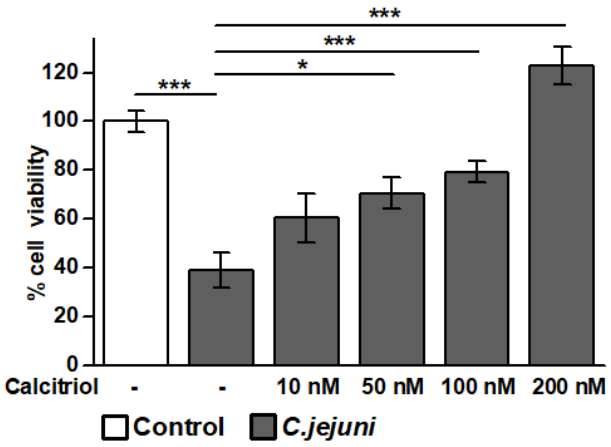
HT-29/B6 Zellen



Zusammenfassung – Take home messages

Klinische Studie – Campylobacter-Enteritis

- **Diarrhö der *C. jejuni* Infektion im Kolon basiert auf Na^+ Malabsorption (ENaC) und epitheliale Barrierestörung (*TJs*)**
- **Epithel-beeinflussende Zytokine (IFNγ, TNFα, IL13, IL1β) sind die Hauptregulatoren der pathophysiologischen Konsequenzen**
- **Signalwege von Immunzell - Epithelzell -Interaktionen sind potentielle Ziele für therapeutische Substanzen (Vit. D, …)**
- **Verminderung des *Leaky Gut* ➡ Reduktion von Komplikationen**

DFG Deutsche Forschungsgemeinschaft

Federal Ministry of Education and Research

ONE HEALTH APPROACH CAMPYLOBACTER Preventing and Combating Infections

PAC-Campy - Consortium

Universität Köln
Cologne Center for Genomics

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