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Host long non-coding RNAs: key modulators in rotavirus infection dynamics

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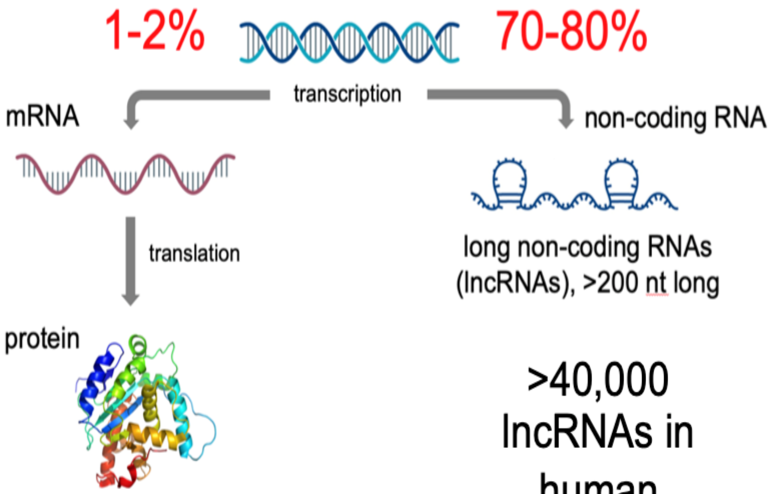
Long non coding RNA



1-2%

70-80%

transcription

mRNA

translation

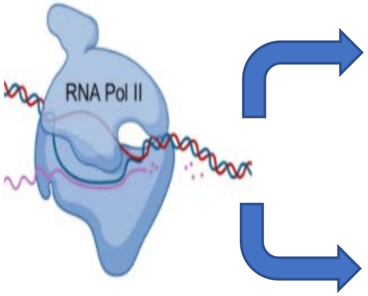
protein

non-coding RNA

long non-coding RNAs (lncRNAs), >200 nt long

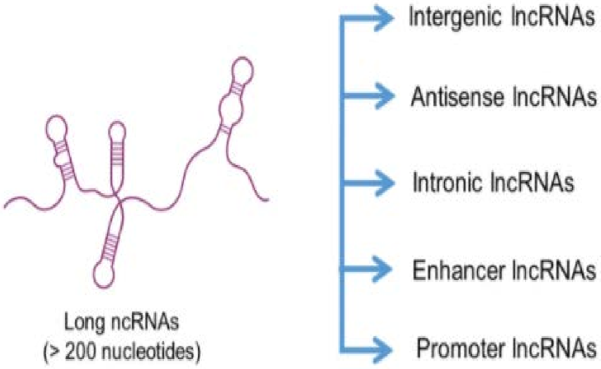
Dan Lim Lab at UCSF

>40,000 lncRNAs in human genome



capped at the 5′ end

polyadenylated at 3′end



Long ncRNAs (> 200 nucleotides)

Intergenic lncRNAs

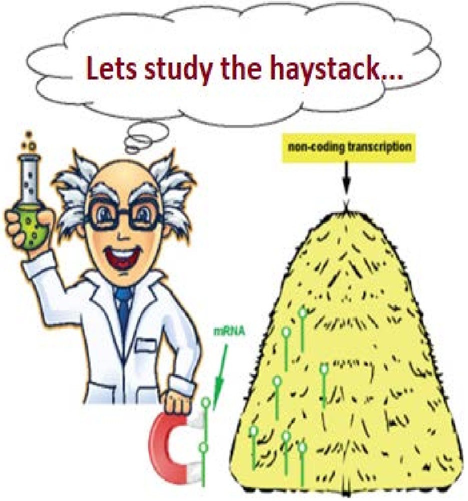
Antisense lncRNAs

Intronic lncRNAs

Enhancer lncRNAs

Promoter lncRNAs

Joshi et al.,2020, *Reprod Biol Endocrinol*



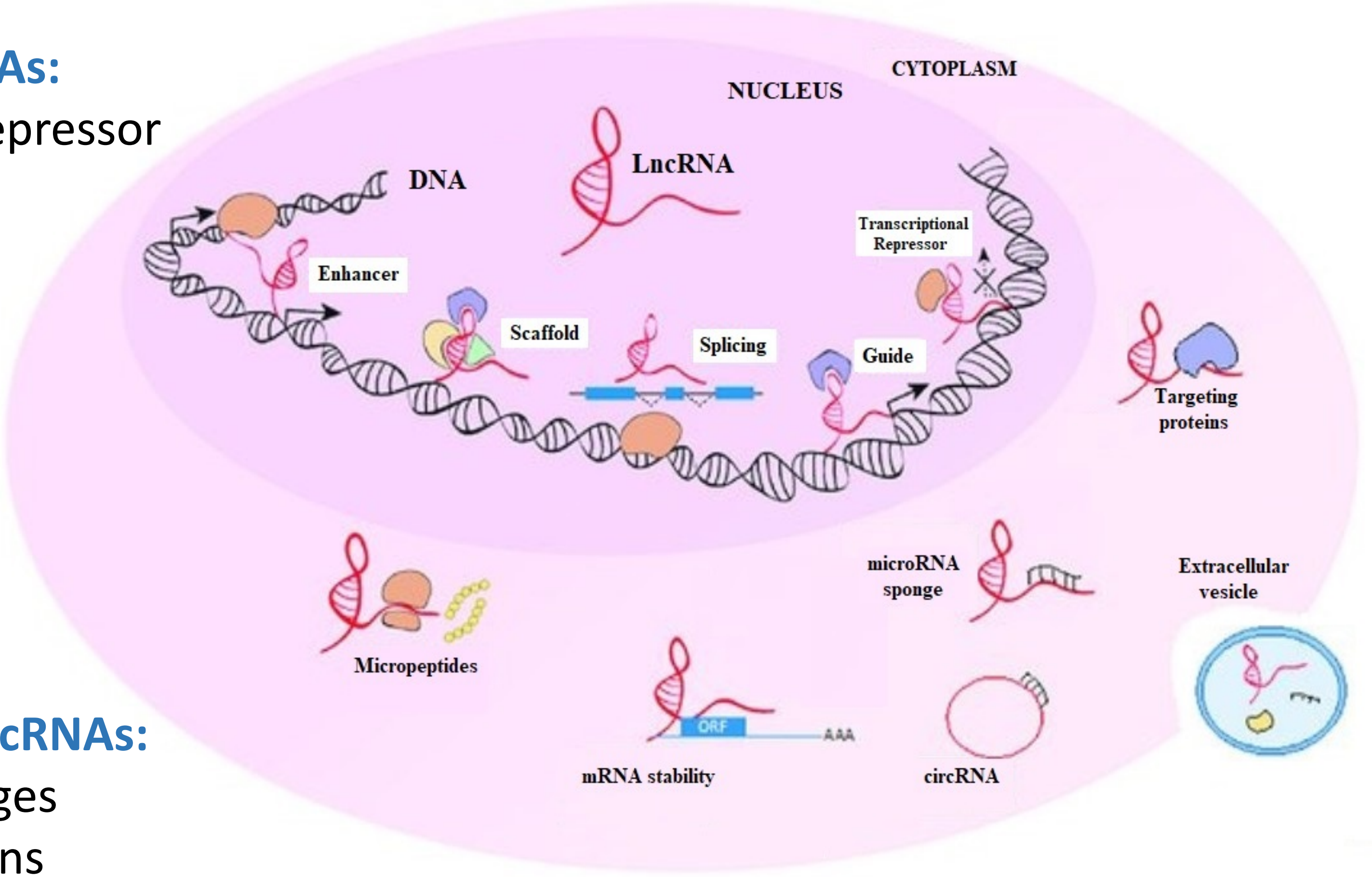
Sebastian's group: University of Copenhagen

- Previously, lncRNAs were considered as “dark matter” or “junk” in the genome.
- Now considered important physiological regulators of cell homeostasis, growth, and differentiation.

General molecular mechanism of lncRNAs

Nuclear lncRNAs:

- Enhancer/Repressor
- Splicing
- Scaffold
- Decoy
- Guide



Cytoplasmic lncRNAs:

- miRNA sponges
- Target proteins
- Promote or inhibit mRNA stability
- Secreted by extracellular vesicles for cell-cell communication

Significance of host lncRNAs in viral infection dynamics

- Viral infection leads to **large alterations in the host transcriptome** and stimulates an antiviral host immune response involving numerous host cellular components and signaling pathways.

- Recent data suggests that **host lncRNAs play crucial roles at the host pathogen interface** modulating viral infection.

- Reports suggests host lncRNAs being significantly modulated during infection with **SARS-CoV virus**(Peng et al.,2010), **HCV**(Zhang, 2016), **PEDV** (Chen et al.,2019), **ZIKV** (Hu et al.,2017), **IAV** (Wang & Cen, 2020) etc.

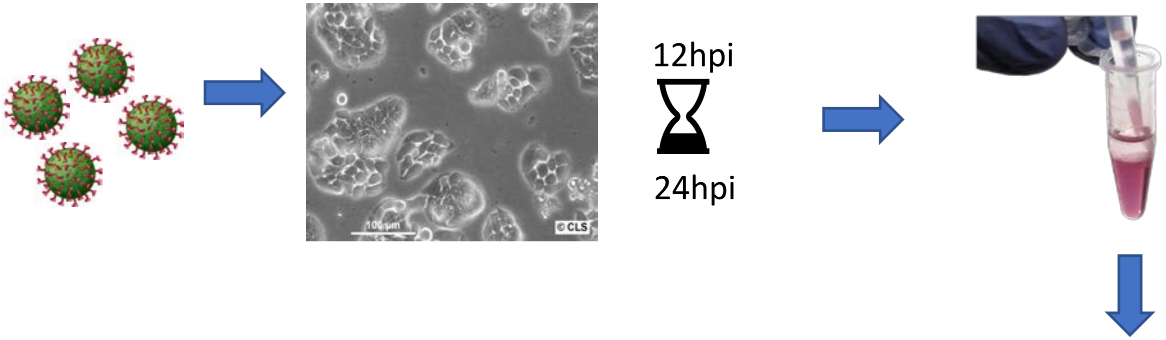
- **But no studies have previously been performed to examine lncRNA expression profiling during rotavirus infection.**

- LncRNAs are key modulators of transcriptional and post-transcriptional processes. A deeper understanding of their regulation in manipulating rotaviral infection will provide a better insight.

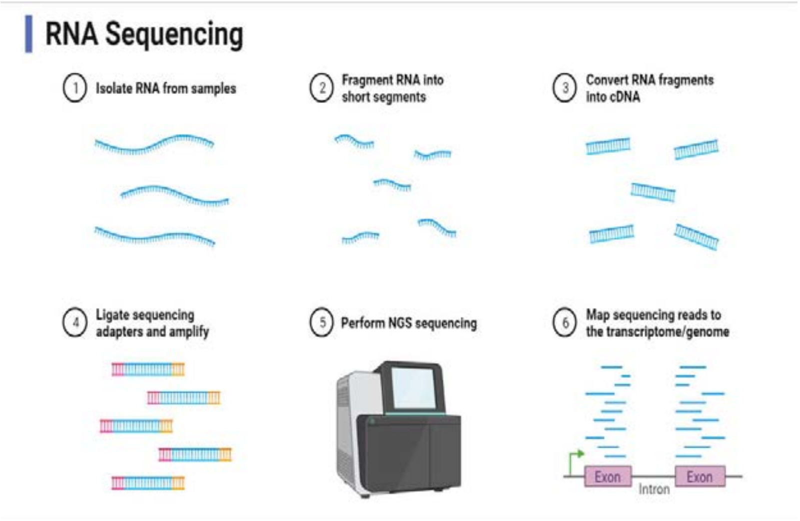
WORKPLAN AND METHODOLOGY

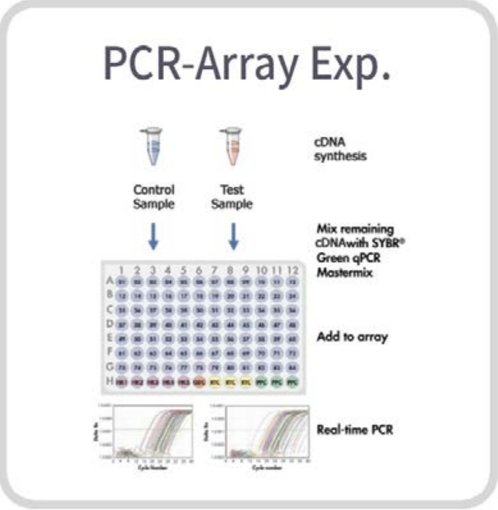
1. Expression profiling of lncRNAs in rotavirus infected cells:

- **HT-29 cells** were infected with the **RV-SA11** for two time points along with uninfected control.



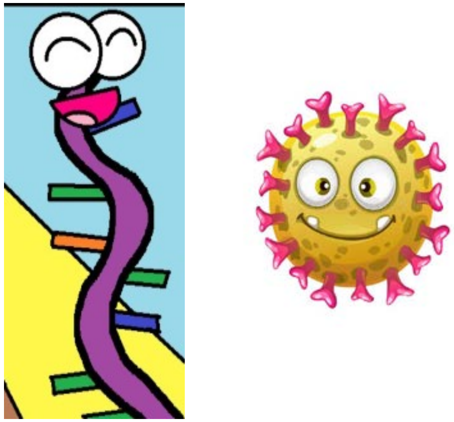
- After incubation total cell RNA was isolated and subjected to: **lncRNA PCR based array** and **RNA sequencing.**



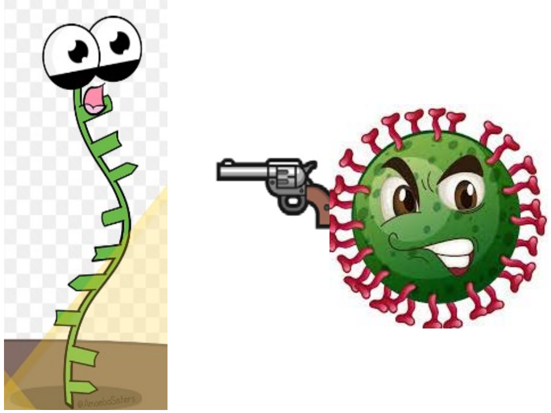


- Differentially regulated lncRNAs with fold change ≥5 in response to rotavirus infection were further validated and quantified by **qRT-PCR.**

2. Assessing the role of differentially expressed lncRNAs in the context of rotavirus infection biology:



Friend or Foe?



➢To confirm the role of dysregulated lncRNA:

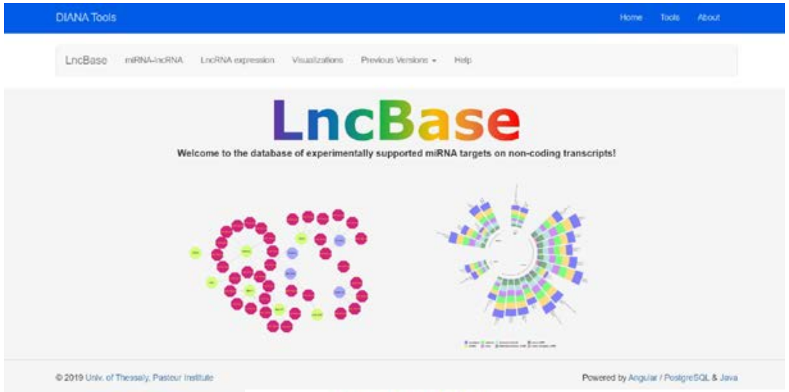
- knock-down of virus induced lncRNAs
- overexpression of virus repressed lncRNAs.

➢ To investigate the effects of these lncRNA knockdown/overexpression on viral infection and replication:

- **Western blot** to check **viral protein expression.**
- **qRT-PCR** to quantify **viral RNA synthesis.**
- Estimation of **infectious virus particle** by **plaque assay .**

3.Identification of <u>key cellular components </u>(DNA/mRNA/miRNA/proteins) targeted by differentially expressed lncRNAs and reveal their mechanisms of action in promoting or inhibiting rotavirus infection.

In-silico Identification of targets that associate with differentially expressed lncRNAs:



Welcome to the database of experimentally supported miRNA targets on non-coding transcripts!

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Powered by Angular / PostgreSQL & Java

Welcome to LncTarD 2.0

LncTarD is a comprehensive resource which aims to provide experimentally supported key lncRNA-target regulations, their influenced functions and lncRNA-mediated regulatory mechanisms in human diseases and several web-based tools based on single-cell RNA-seq and RNA-seq/microarray data. LncTarD can serve as a timely and valuable resource for understanding functions and molecular mechanisms of lncRNA deregulation in disease pathogenesis, which will help to identify novel and sensitive biomarkers and therapeutic targets in human diseases.

HOXA11-AS

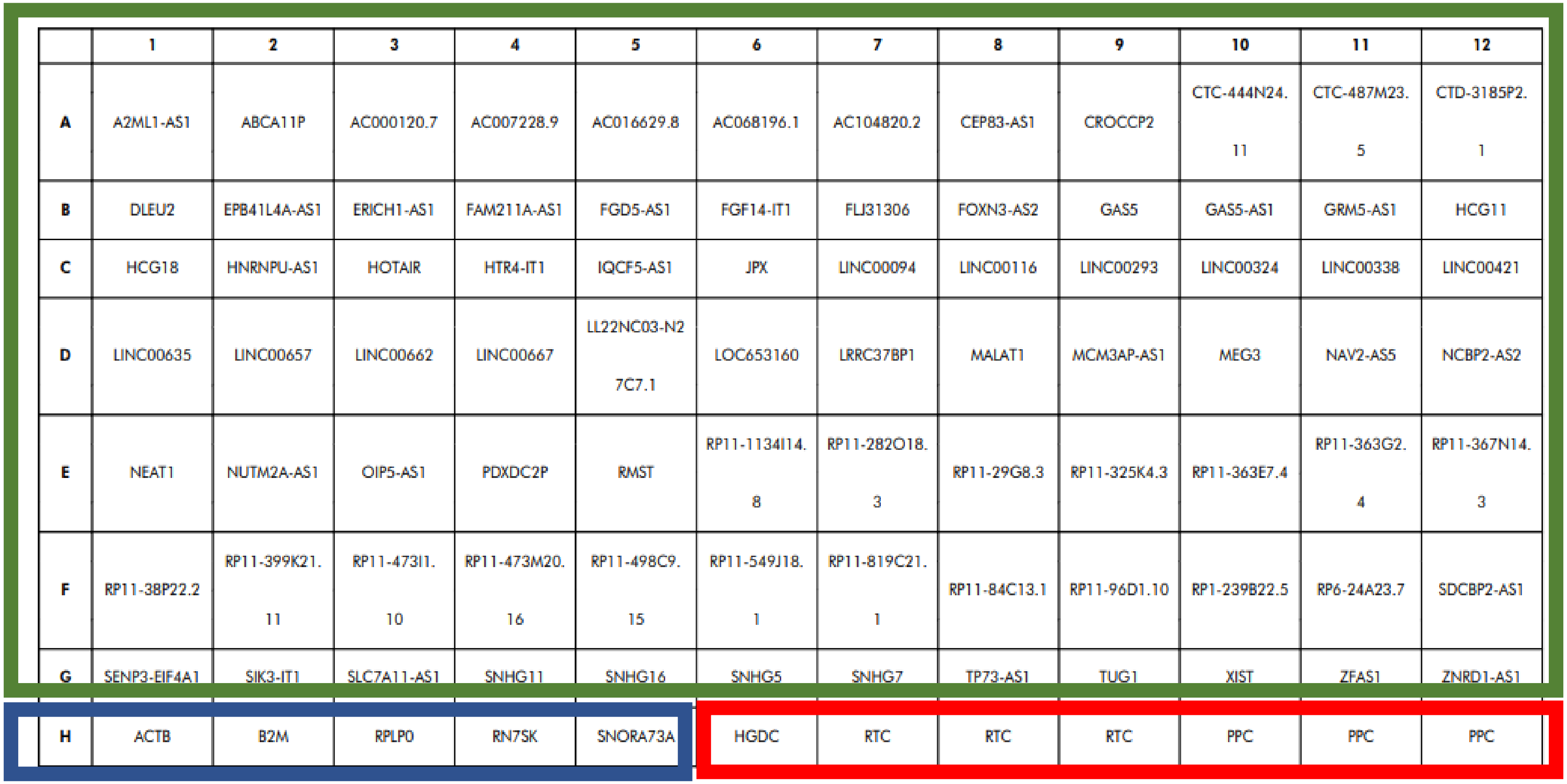
e.g. lncRNA: " HOXA11-AS " Disease: " Breast cancer " Function: " Angiogenesis "

Analysis tools in LncTarD 2.0

Target Validation:

- **RNA-Immunoprecipitation** was done to study the **target protein interaction** with lncRNA.
- **Quantitative real-time PCR (qRT-PCR)** was performed to check the expression of lncRNA-regulated **mRNA or miRNA** in virus infected cells.

Differential expression of cellular lncRNAs in response to RV infection are screened and identified by a qPCR based microarray:

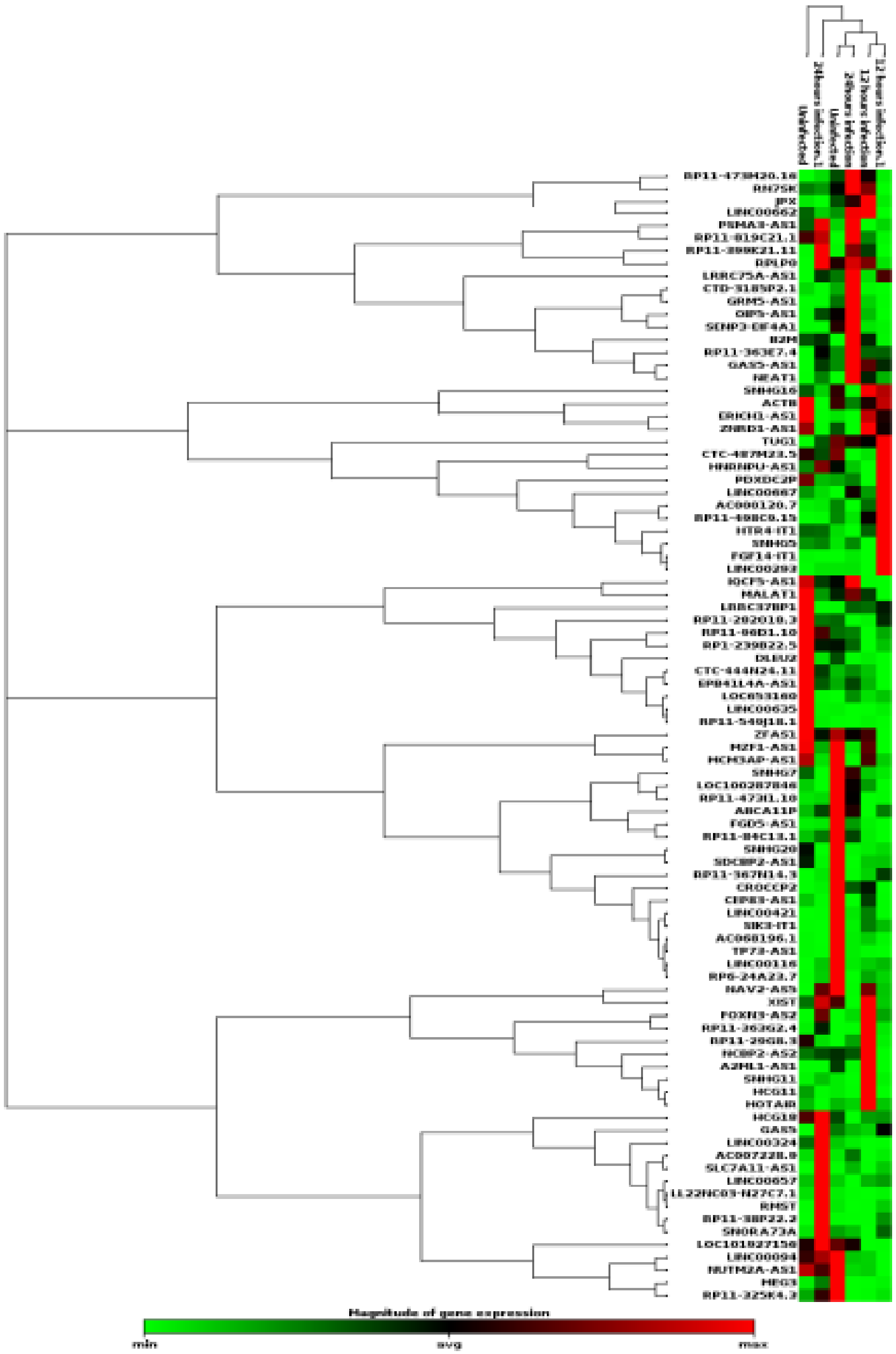


	1	2	3	4	5	6	7	8	9	10	11	12
A	A2ML1-AS1	ABCA11P	AC000120.7	AC007228.9	AC016629.8	AC068196.1	AC104820.2	CEP83-AS1	CROCCP2	CTC-444N24.11	CTC-487M23.5	CTD-3185P2.1
B	DLEU2	EPB41L4A-AS1	ERICH1-AS1	FAM211A-AS1	FGD5-AS1	FGF14-IT1	FLJ31306	FOXN3-AS2	GAS5	GAS5-AS1	GRM5-AS1	HCG11
C	HCG18	HNRNPU-AS1	HOTAIR	HTR4-IT1	IQCF5-AS1	JPX	LINC00094	LINC00116	LINC00293	LINC00324	LINC00338	LINC00421
D	LINC00635	LINC00657	LINC00662	LINC00667	LL22NC03-N27C7.1	LOC653160	LRRC37BP1	MALAT1	MCM3AP-AS1	MEG3	NAV2-AS5	NCBP2-AS2
E	NEAT1	NUTM2A-AS1	OIP5-AS1	PDXDC2P	RMST	RP11-1134I14.8	RP11-282O18.3	RP11-29G8.3	RP11-325K4.3	RP11-363E7.4	RP11-363G2.4	RP11-367N14.3
F	RP11-38P22.2	RP11-399K21.11	RP11-473I1.10	RP11-473M20.16	RP11-498C9.15	RP11-549J18.1	RP11-819C21.1	RP11-84C13.1	RP11-96D1.10	RP1-239B22.5	RP6-24A23.7	SDCBP2-AS1
G	SENP3-EIF4A1	SIK3-IT1	SLC7A11-AS1	SNHG11	SNHG16	SNHG5	SNHG7	TP73-AS1	TUG1	XIST	ZFAS1	ZNRD1-AS1
H	ACTB	B2M	RPLP0	RN7SK	SNORA73A	HGDC	RTC	RTC	RTC	PPC	PPC	PPC

84 LncRNAs , 5 House-keeping genes and 7 other control genes for Human genomic DNA contamination, Reverse transcription control, positive PCR control primer pairs were coated inside the 96 well-qPCR array plate.

Clustergram of differential expression of lncRNAs in mock infected and rotavirus infected cells at 12 hpi and 24 hpi. **The red colour represents upregulation whereas the green represents downregulation of lncRNAs during rotavirus infection.**

Ct values were normalized against housekeeping genes (ACTB).



Rotavirus infection triggered **downregulation of 19 lncRNAs** and **upregulation 15 lncRNAs** among 84 lncRNAs at both 12 hpi and 24 hpi.

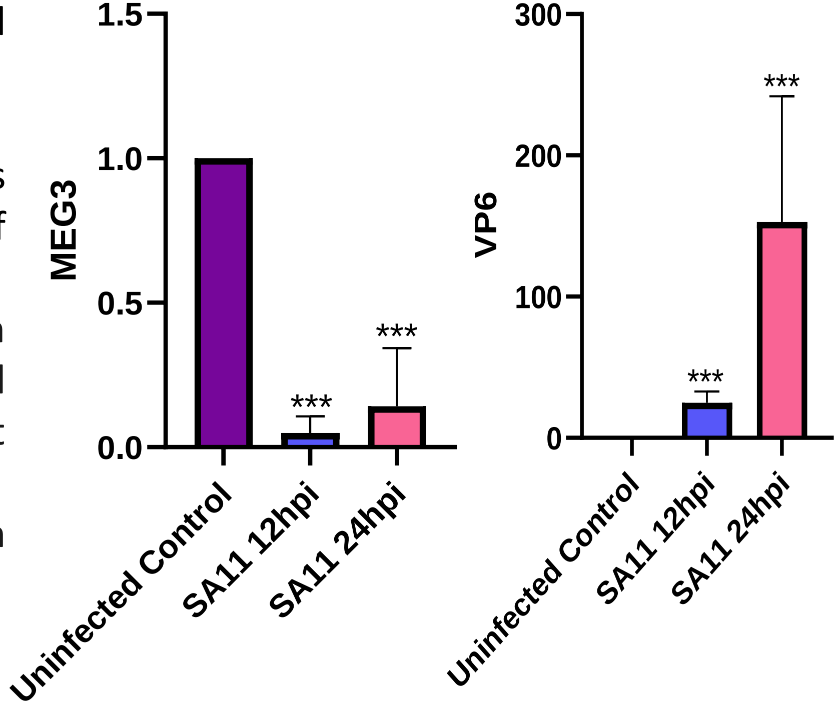
	Position	Gene Symbol	Fold regulation(comparing to control group)	
Downregulated genes			12 hpi	24hpi
1	A01	A2ML1-AS1	-6.36	-3.29
2	A05	MZF1-AS1	-11.27	-11.74
3	A06	AC068196.1	-3.26	-15.74
4	A08	CEP83-AS1	-20.4	-158.97
5	B01	DLEU2	-67.49	-31.8
6	B12	HCG11	-3.17	-26.78
7	C07	LINC00094	-11.64	-2.33
8	C11	SNHG20	-11.58	-49.11
9	D01	LINC00635	-58.3	-806.94
10	D10	MEG3	-50.36	-36.04
11	E02	NUTM2A-AS1	-10.12	3.26
12	E06	LOC100287846	-10.68	-8.13
13	E07	RP11-282O18.3	-5.71	-20.45
14	E09	RP11-325K4.3	-15.77	-7.03
15	F06	RP11-549J18.1	-3.5	-11.76
16	F12	SDCBP2-AS1	-4.66	-14.05
17	G07	SNHG7	-5.92	-3.8
18	G08	TP73-AS1	-5359.62	-1398.37
19	D08	MALAT1	-5.59	-1.67

	Position	Gene Symbol	Fold regulation(comparing to control group)	
Upregulated genes			12hpi	24hpi
1	B06	FGF14-IT1	3.77	2.24
2	B08	FOXN3-AS2	1597.76	820.31
3	B10	GAS5-AS1	3.46	3.96
4	B11	GRM5-AS1	3.69	56.26
5	C09	LINC00293	2.53	5.14
6	E05	RMST	2.27	4.62
7	E10	RP11-363E7.4	4.15	9.19
8	E11	RP11-363G2.4	137.89	4.41
9	F01	RP11-38P22.2	7.09	13.06
10	F02	RP11-399K21.11	3.41	75.87
11	F04	RP11-473M20.16	5.6	11.95
12	G03	SLC7A11-AS1	466.5	10217.95
13	G09	TUG1	3.5	2.28
14	D04	LINC00667	3.61	2
15	E01	NEAT1	1.75	2.51

These results suggest rotavirus infection dysregulate the expression of lncRNAs which may have important role in rotavirus replication and pathogenesis.

MEG3: Maternally expressed gene 3 transcript variant 1

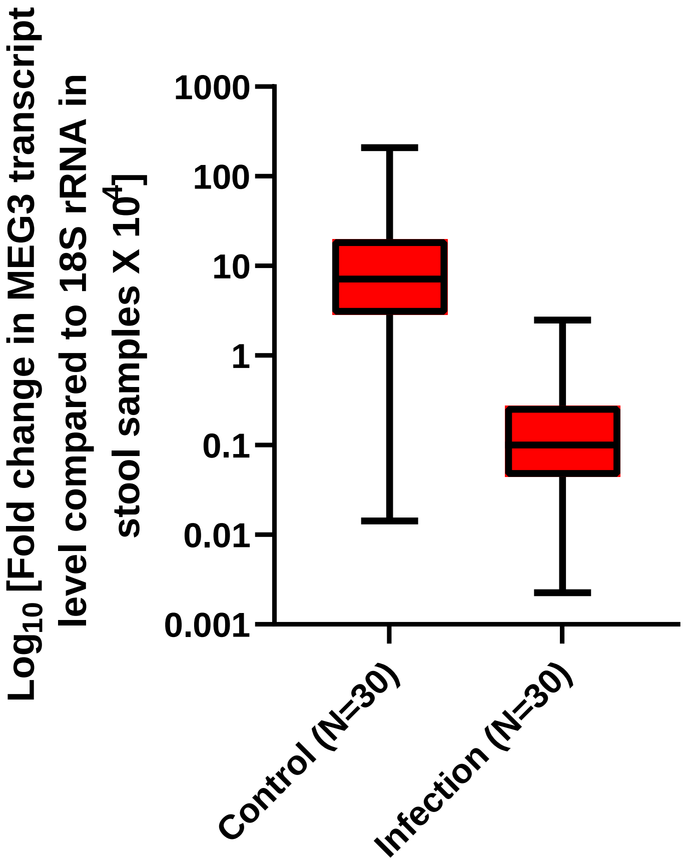
- This gene is a maternally expressed imprinted gene.
- 1,595 bp linear ncRNA
- Multiple alternatively spliced transcript variants have been transcribed from this gene and all of them are lncRNAs.
- Several functional studies in cancer have shown that this lncRNA controls expression of several tumor suppressor genes and oncogenes (Xu et al.,2022)
- MEG3 was reported to play antiviral function in RSV infection(Tao et al.,2018)



Transcript expression detected by qRT-PCR

Gene symbol	Fold regulation in RV compared to uninfected control	
	SA11 12hpi	**SA11 24hpi**
MEG3	**-50.36**	**-36.04**

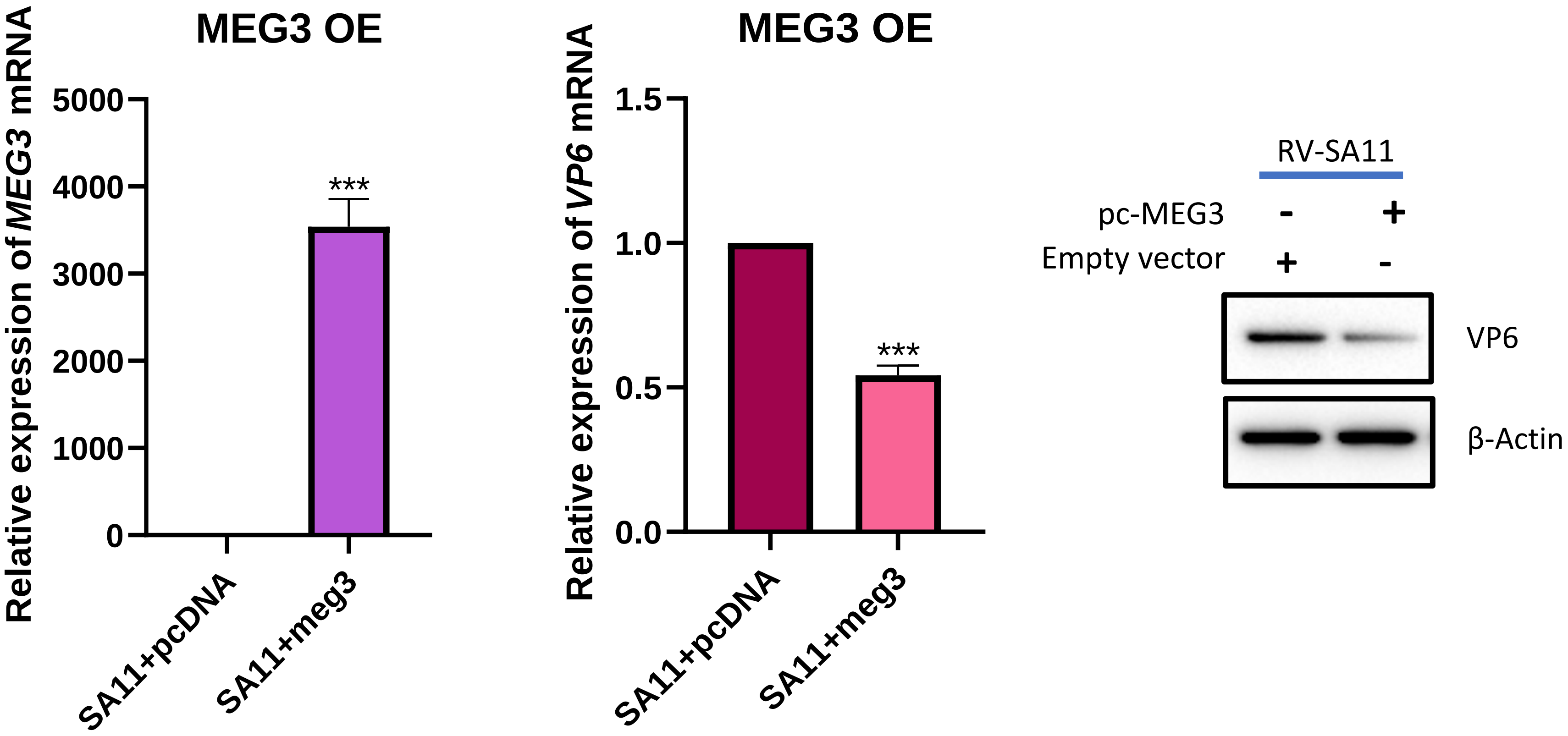
RNA from patient stool samples were tested for MEG3 expression :



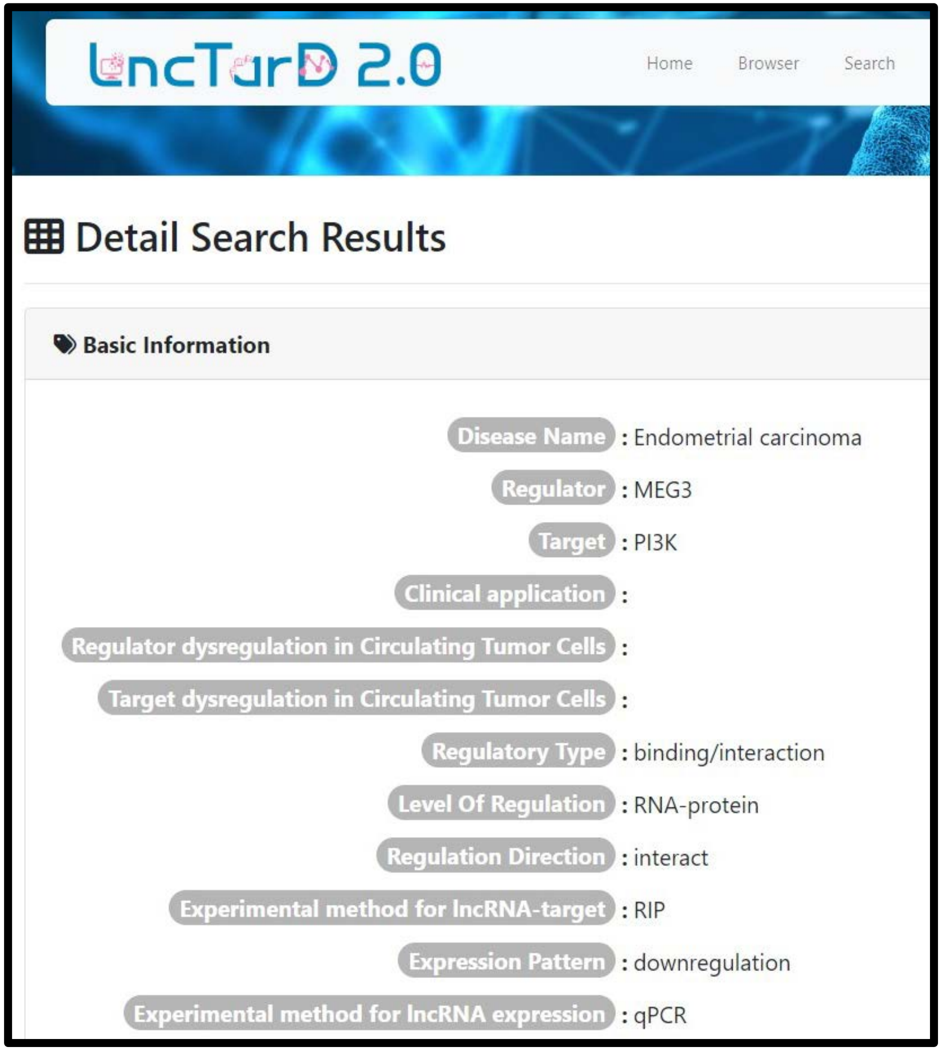
RNA was isolated from 30 Rota positive and 30 Rota negative diarrheal stool samples and real-time RT-PCR was done for MEG3.

MEG3 transcripts were **low in expression in rota-positive** samples than in rota-negative samples.

MEG3 overexpression reduced rotavirus infection :



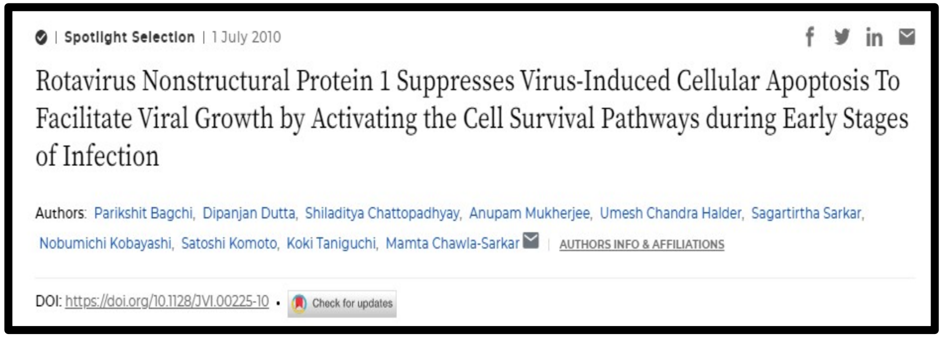
In silico study revealed association of MEG3 with PI3K



Detail Search Results

Basic Information

Disease Name : Endometrial carcinoma
Regulator : MEG3
Target : PI3K
Clinical application :
Regulator dysregulation in Circulating Tumor Cells :
Target dysregulation in Circulating Tumor Cells :
Regulatory Type : binding/interaction
Level Of Regulation : RNA-protein
Regulation Direction : interact
Experimental method for lncRNA-target : RIP
Expression Pattern : downregulation
Experimental method for lncRNA expression : qPCR



Spotlight Selection | 1 July 2010

Rotavirus Nonstructural Protein 1 Suppresses Virus-Induced Cellular Apoptosis To Facilitate Viral Growth by Activating the Cell Survival Pathways during Early Stages of Infection

Authors: Parikshit Bagchi, Dipanjan Dutta, Shiladitya Chattopadhyay, Anupam Mukherjee, Umesh Chandra Halder, Sagartirtha Sarkar, Nobumichi Kobayashi, Satoshi Komoto, Koki Taniguchi, Mamta Chawla-Sarkar ✉ | AUTHORS INFO & AFFILIATIONS

DOI: https://doi.org/10.1128/JVI.00225-10 • Check for updates

PLOS PATHOGENS

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RESEARCH ARTICLE

Activation of PI3K, Akt, and ERK during early rotavirus infection leads to V-ATPase-dependent endosomal acidification required for uncoating

Mahmoud Soliman, Ja-Young Seo, Deok-Song Kim, Ji-Yun Kim, Jun-Gyu Park, Mia Madel Alfajaro, Yeong-Bin Baek, Eun-Hyo Cho, Joseph Kwon, Jong-Soon Choi, Mun-Il Kang, Sang-Ik Park, Kyoung-Oh Cho

Version 2 Published: January 19, 2018 • https://doi.org/10.1371/journal.ppat.1006820

Research Paper

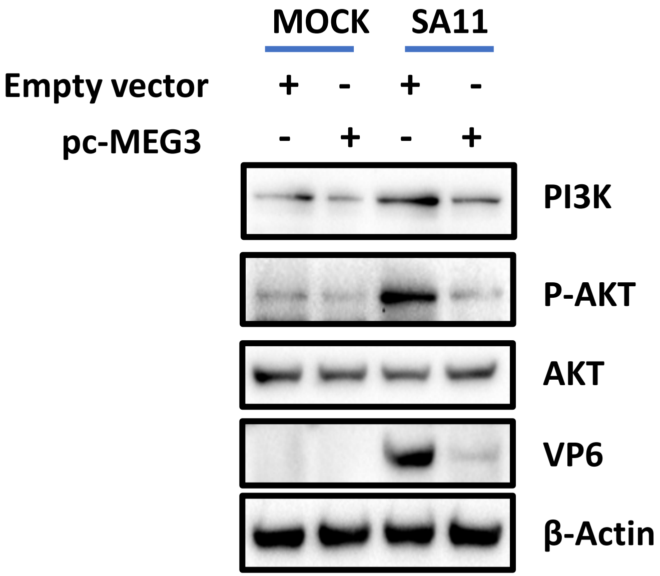
PI3K-Akt-mTOR axis sustains rotavirus infection via the 4E-BP1 mediated autophagy pathway and represents an antiviral target

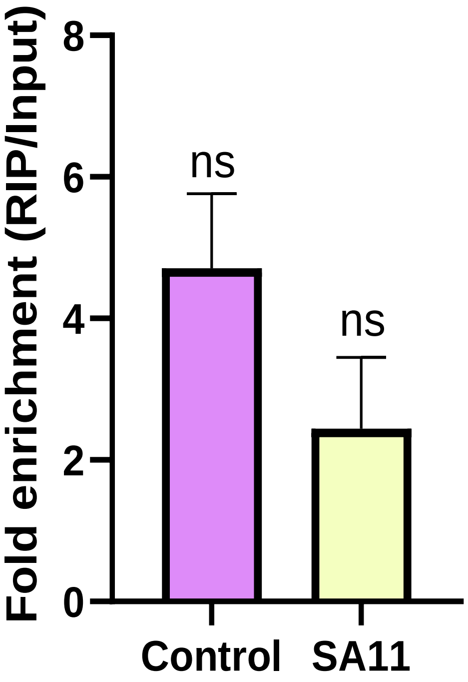
Yuebang Yin, Wen Dang, Xinying Zhou, Lei Xu, Wenshi Wang, Wanlu Cao, ...show all

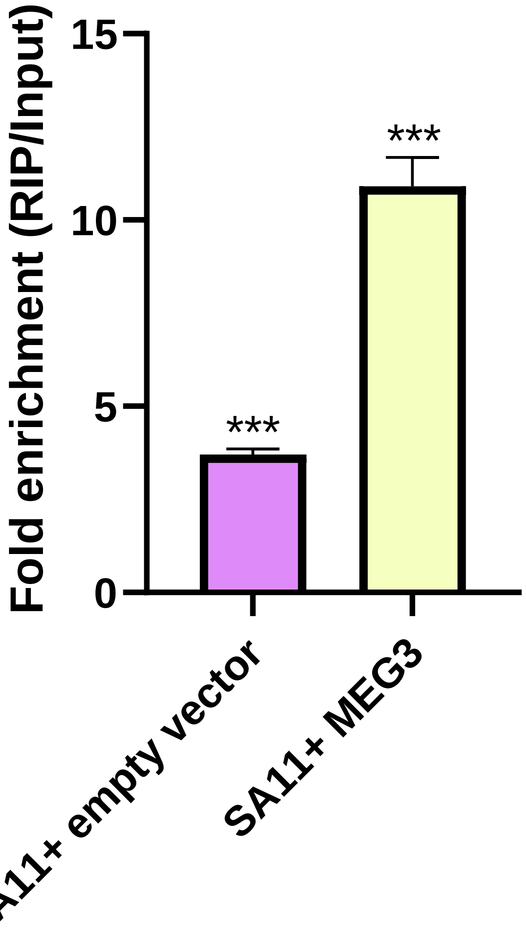
Pages 83-98 | Received 17 Jan 2017, Accepted 29 Apr 2017, Accepted author version posted online: 05 May 2017, Published online: 01 Jun 2017

Download citation https://doi.org/10.1080/21505594.2017.1326443 Check for updates

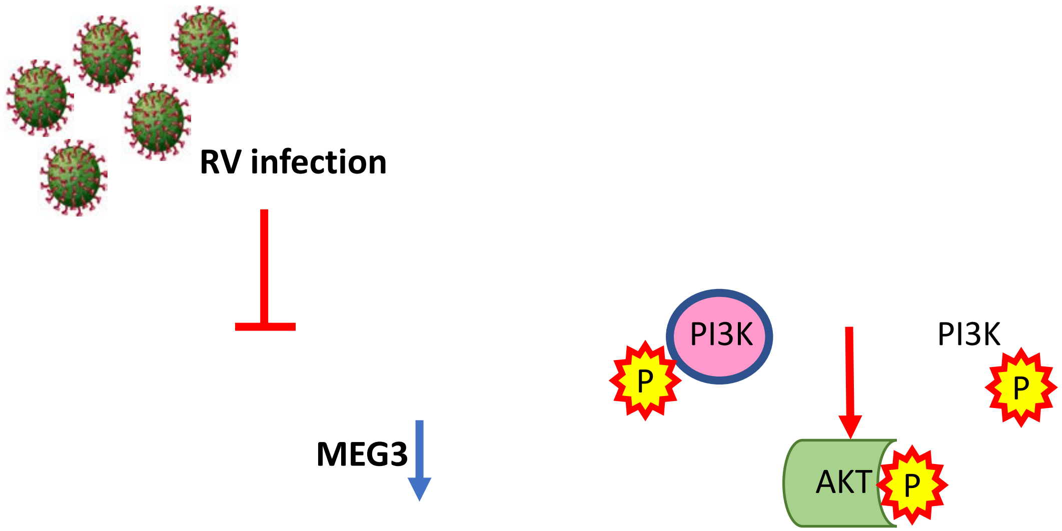
MEG3 overexpression resulted in downregulation of rotavirus induced PI3K-AKT pathway



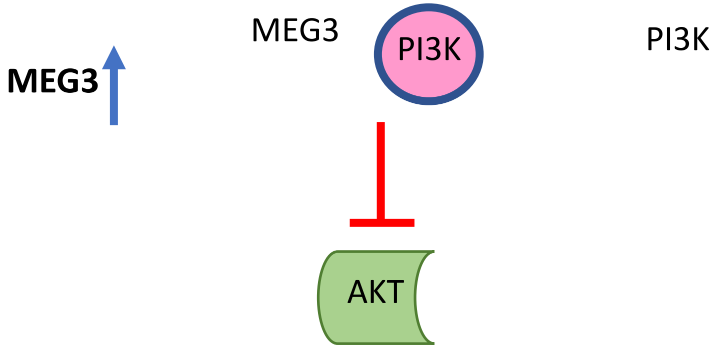




MEG3 interacts with PI3K directly and downregulate its activation



RV infected condition:
MEG3 expression is reduced and
PI3K-AKT signalling is
activated by RV.



MEG3 Overexpression:
MEG3 binds to PI3K.
PI3K-AKT signalling is
downregulated

Acknowledgement

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INDIAN COUNCIL OF MEDICAL RESEARCH
NATIONAL INSTITUTE OF CHOLERA AND ENTERIC DISEASES

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Thank you