Impact factor : 6.5
2023

AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Virology®

CELLULAR RESPONSE TO INFECTION

LncRNA446 Regulates Tight Junctions by Inhibiting the Ubiquitinated Degradation of Alix after Porcine Epidemic Diarrhea Virus Infection

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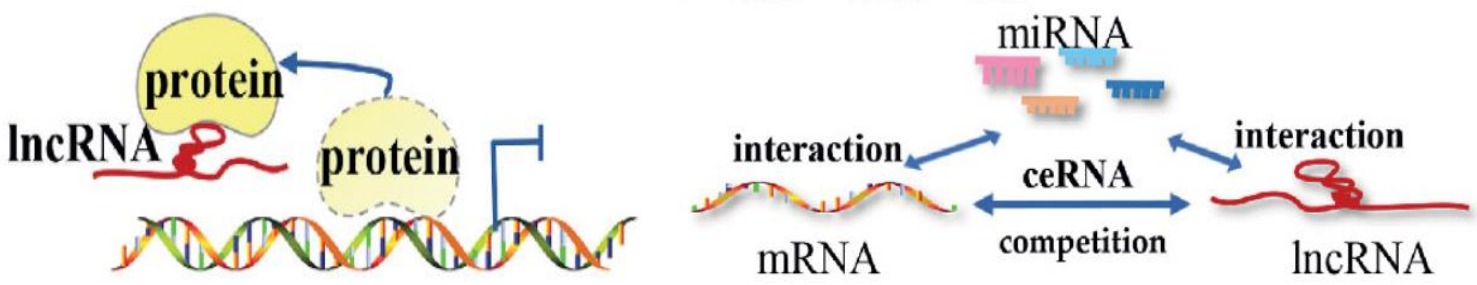
조건우
2023.08.02

Introduction

Long non-coding RNA

https://ksgd.org/webzine/2009/01.php



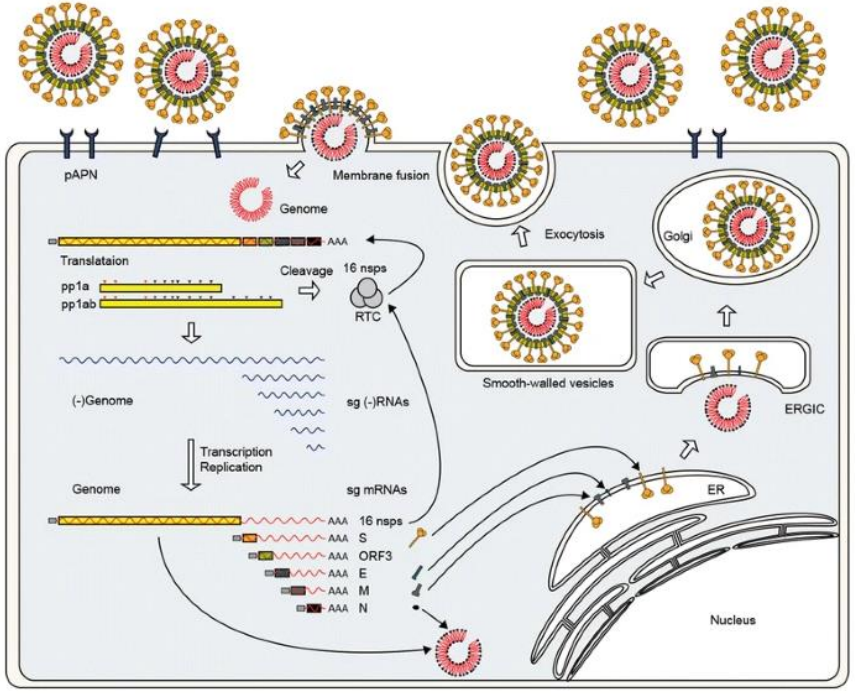
https://doi.org/10.3389/fonc.2020.598817

- LncRNA has more than 200nt
- Cell specific or condition specific expression
- Transcription regulation, proliferation, growth inhibition

Introduction

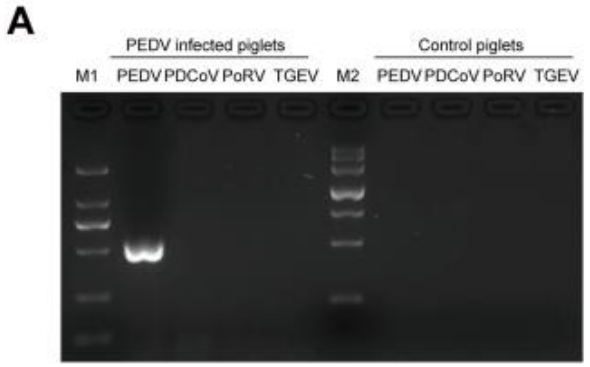
Porcine Epidemic Diarrhea virus

- enveloped, single-stranded, positive-sense RNA virus of family Coronaviridae, genus Alphacoronavirus
- Vommiting, decreased appetite, decreased expression of tight junction
- Receptor : pAPN, cell membrane cholesterol, sialic acid and occludin

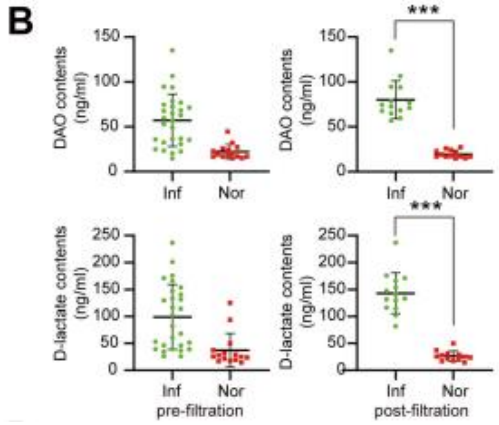


https://doi.org/10.1186/s12985-015-0421-2

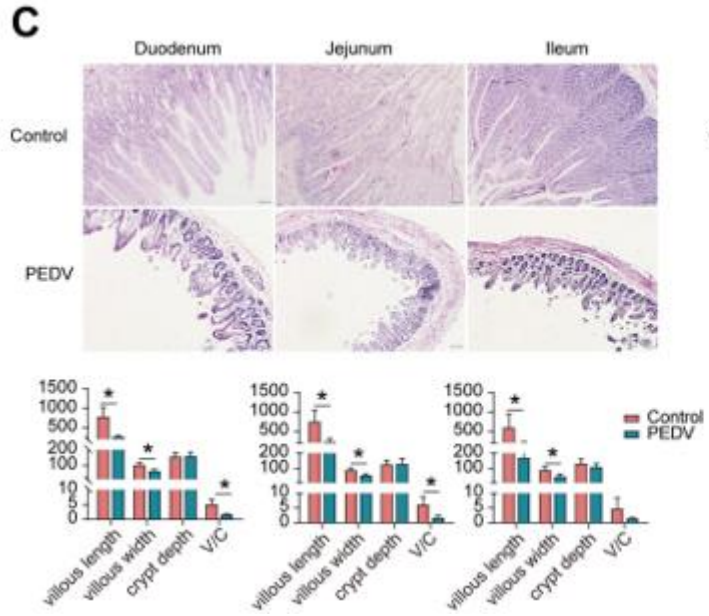
Figure 1. Screening and identification of extreme phenotypic individuals



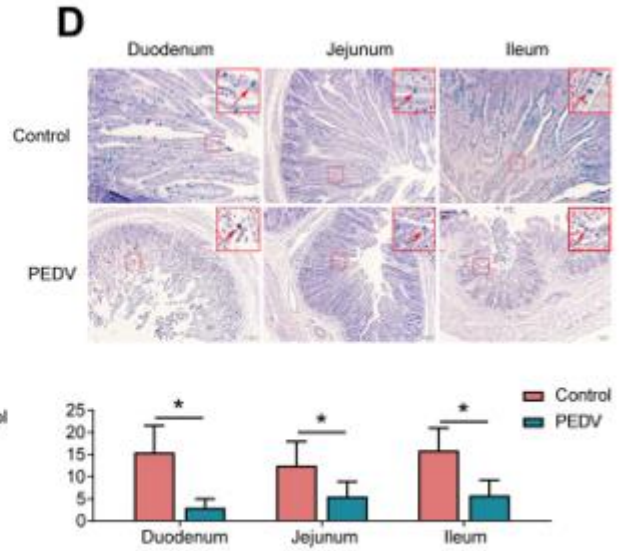
✓ Multiple coronavirus identification of piglet diarrhea



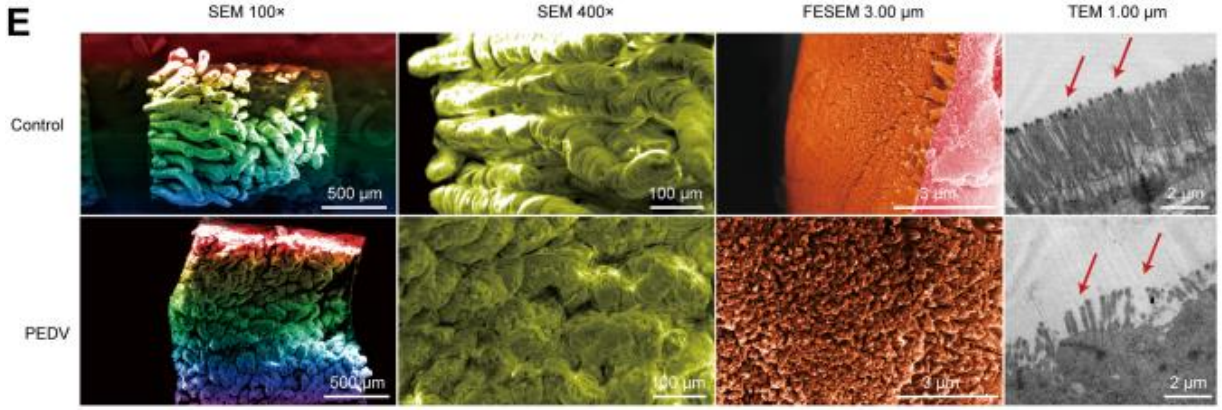
✓ Porcine serum D-lactate and DAO concentrations were measured by elisa



✓ H&E staining and statistical analysis of small intestine



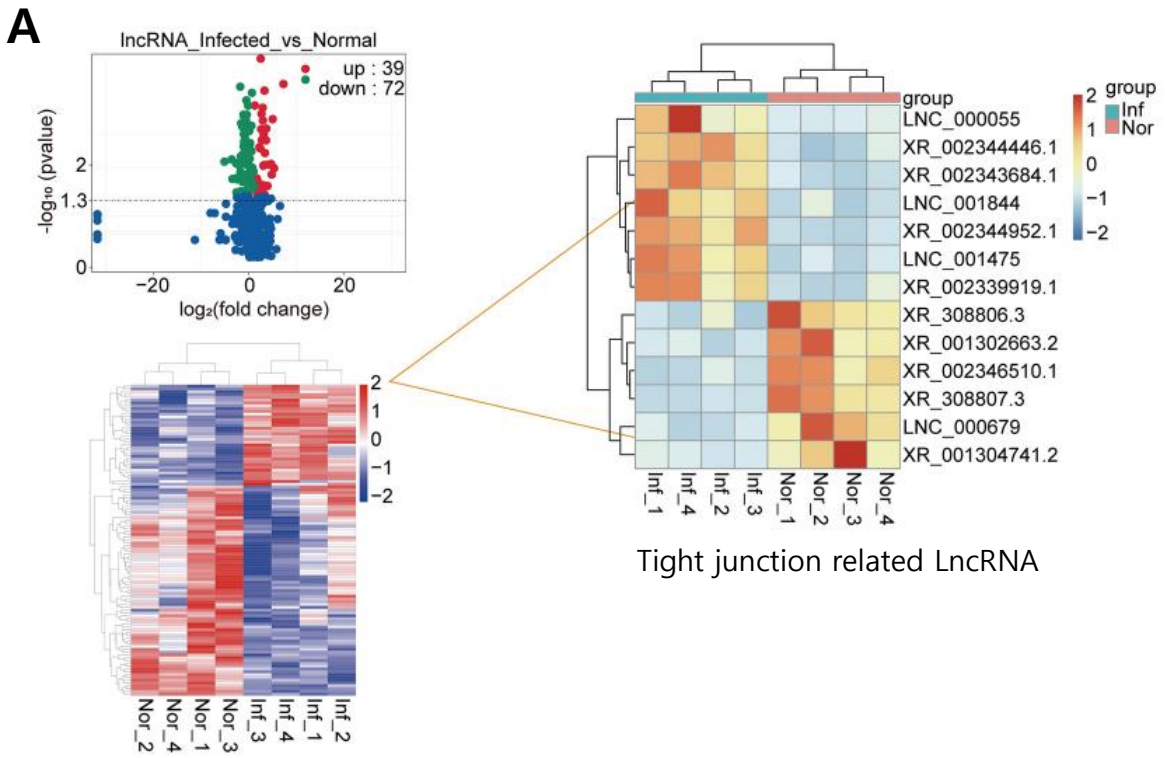
✓ AB-PAS staining and statistical analysis of small intestine



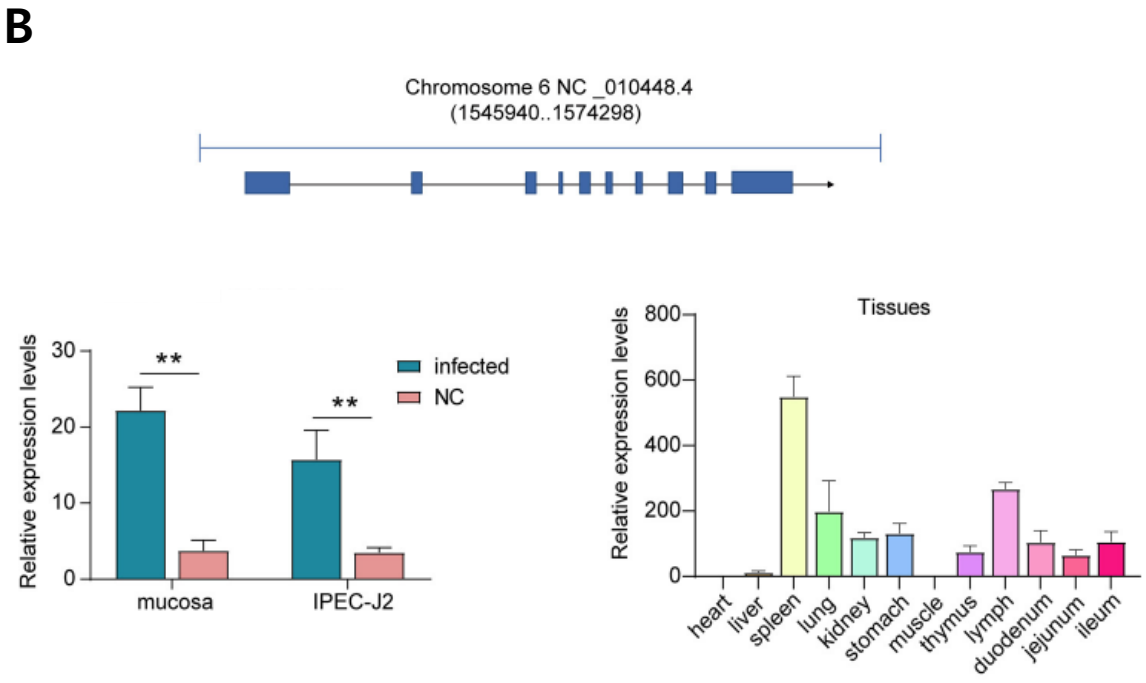
✓ SEM and TEM microscopy in jejunum

→Remove extreme individual

Figure 2. Identification of differentially expressed lncRNA



Tight junction related LncRNA



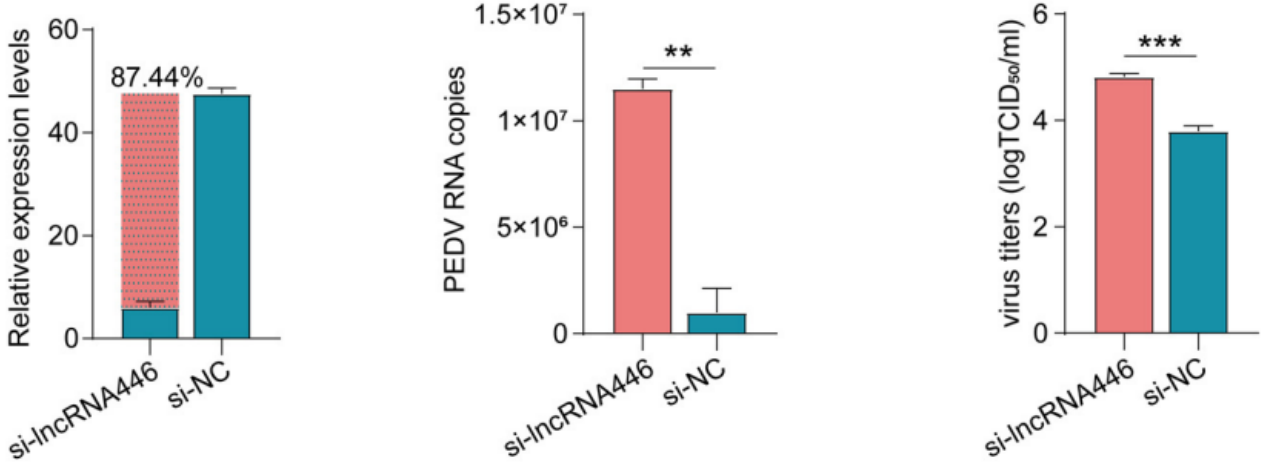
✓ Defferentiated expressed lncRNA between intact intestine of normal group and damaged intestine of pedv-infected group

✓ LncRNA446 expression level in tissue and cell

→LncRNA446 is significantly related to PEDV

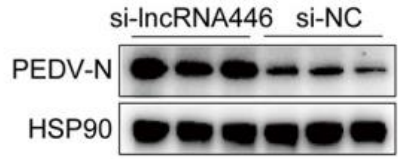
Figure 3. LncRNA 446 inhibits PEDV replication

A



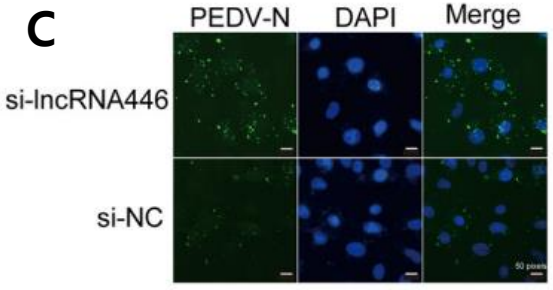
✓ When lncRNA 446 knockdowns, PEDV replication is promoted (IPEC-J2)

B



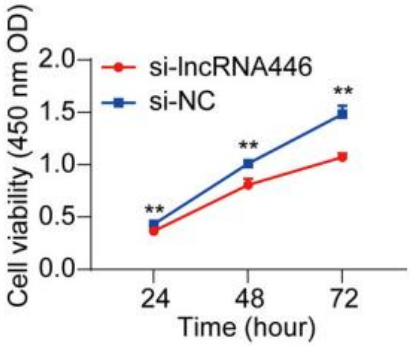
✓ LncRNA 446 inhibit PEDV replication (IPEC-J2 ,Western blot)

C



✓ LncRNA 446 inhibit PEDV replication (IPEC-J2 ,IFA)

D

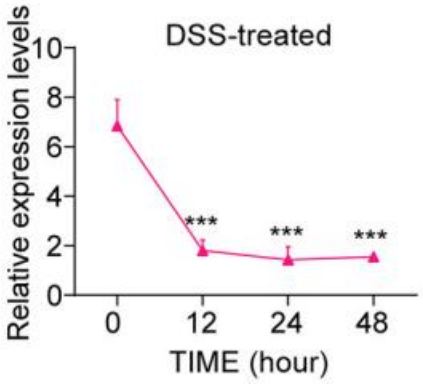


✓ LncRNA 446 promotes cell proliferation (IPEC-J2, CCK8 assay)

→LncRNA 446 knockdown promotes PEDV replication and inhibits the viability of IPEC-J2 cell

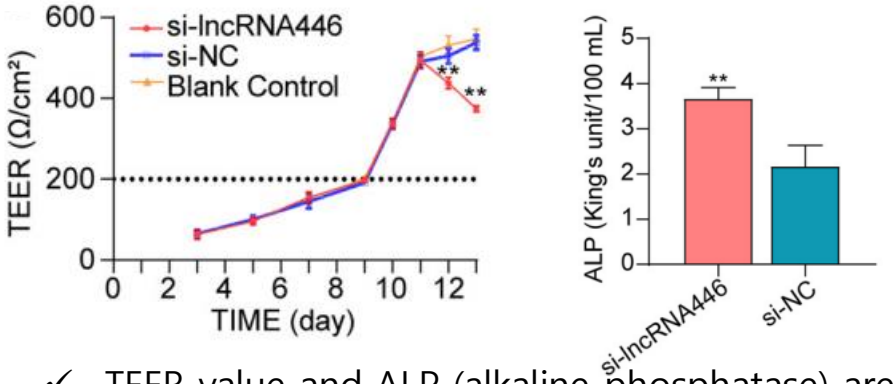
Figure 4. LncRNA446 maintains the structure of tight junctions

A



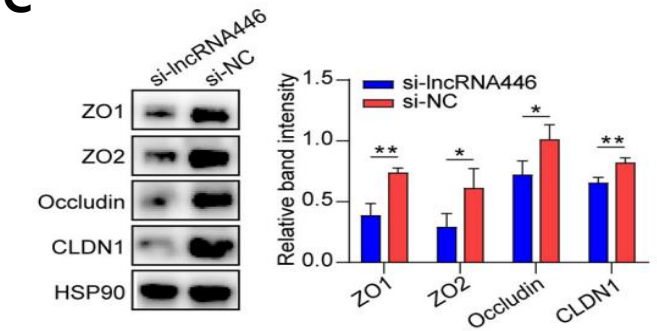
✓ LncRNA446 expression level (IPEC-J2)

B



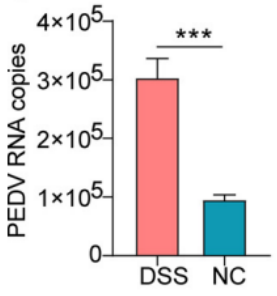
✓ TEER value and ALP (alkaline phosphatase) are measured using transwell (IPEC-J2)
day11 si-RNA infection

C



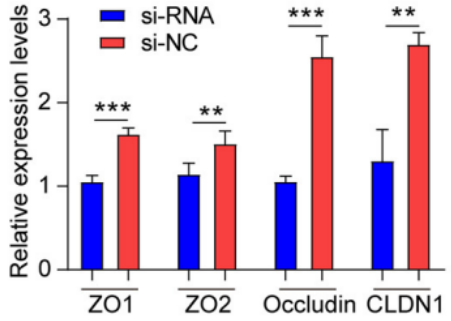
✓ Effect of knockdown lncRNA446 about tight junction (Western blot)

D



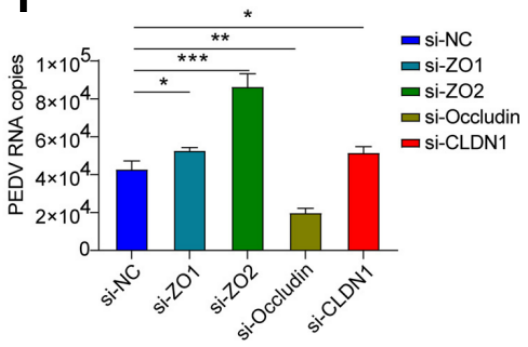
✓ PEDV gene expression with DSS treatment (IPEC-J2)

E



✓ Tight junction RNA level with si-lncRNA446b(RT-PCR)

F

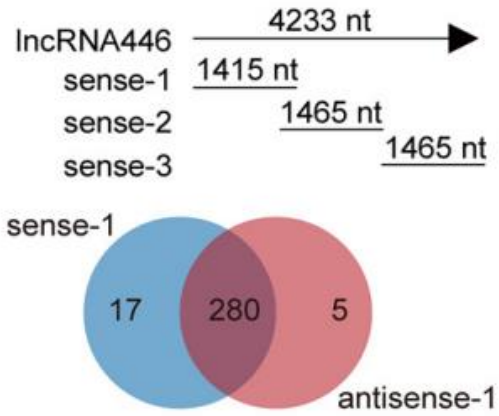


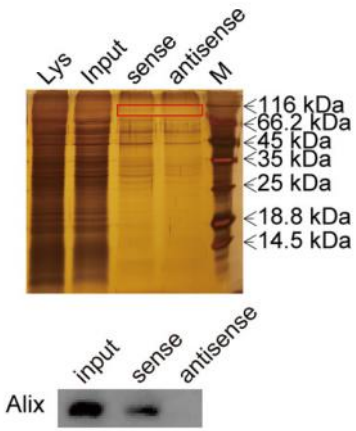
✓ Effect of tight junction related gene knockdown on PEDV

→LncRNA 446 knockdown disrupts tight junction structures

Figure 5. LncRNA 446 binds to Alix

A

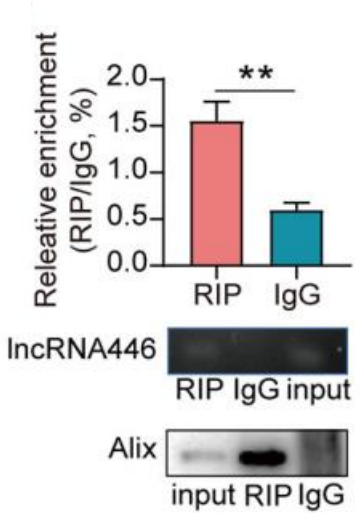




합성인지 sense1을 어떻게 만들었는데

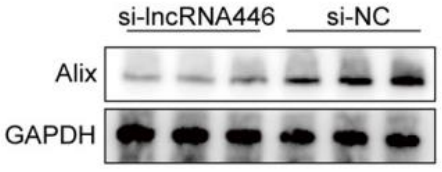
- ✓ RNA pulldown assay and analysis using MS and western blot
  Lys (cell lysate), M (protein marker), input (positive control), antisense (negative control)

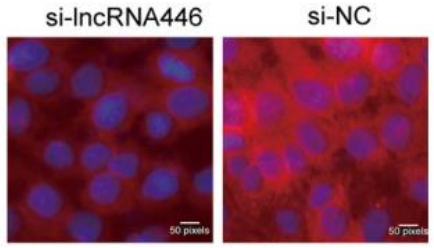
B



- ✓ LncRNA 446 directly interact with Alix (RNA immunoprecipitation, rtPCR, Western blot)

C



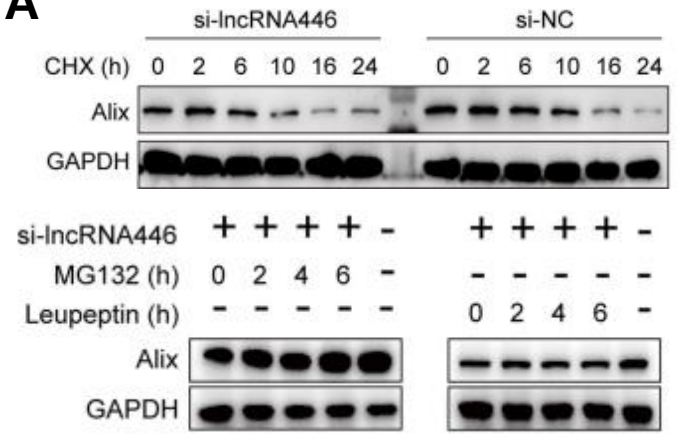


- ✓ LncRNA446 improves Alix

→LncRNA446 binds to Alix and prevent disappearance

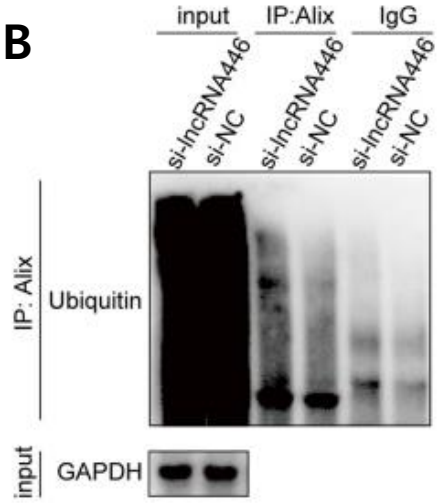
Figure 6. LncRNA 446 regulate tight junctions by binding to Alix

A



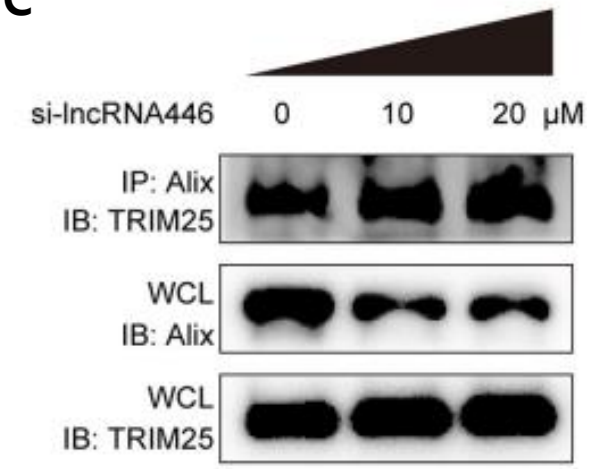
- ✓ LncRNA 446 affects degradation of Alix
  MG132 (proteasome inhibitor), Leupepitn (lysosome inhibitor)

B



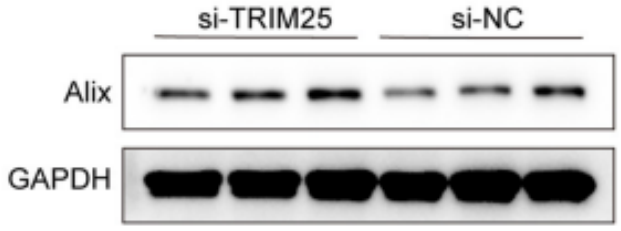
- ✓ Ubiquitin binds to Alix
  Input (positive control), IP (immunoprecipitation)

C



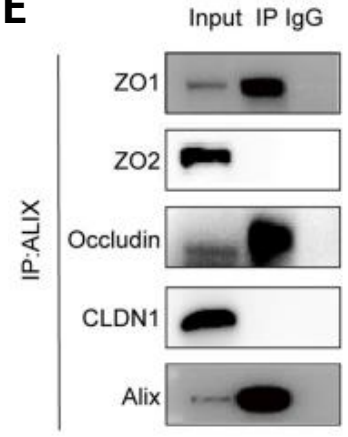
- ✓ Trim25 is potential helper of ubiquitination and binds to Alix
  WCL (whole cell lysate)

D



- ✓ Alix level improves when TRIM25 knockdowns

E

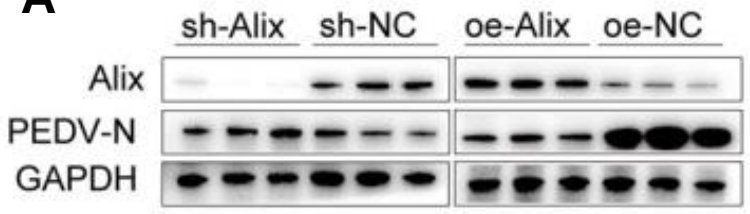


- ✓ Alix binds to ZO1 and Occludin

→Alix is degraded by ubiquitination and lncRNA 446 inhibit Alix degradation

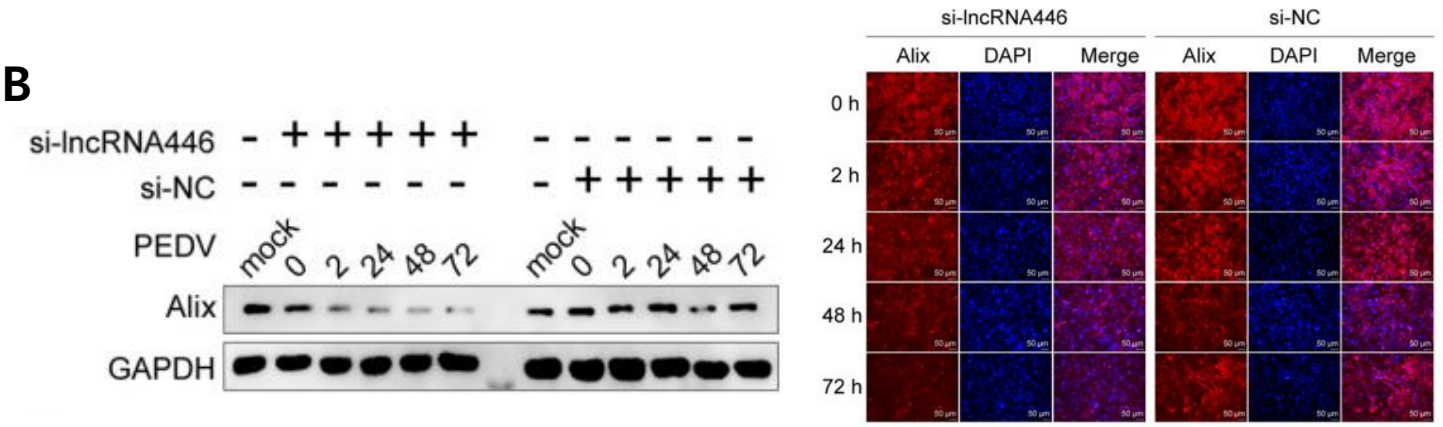
Figure 7. Alix inhibit PEDV replication

A



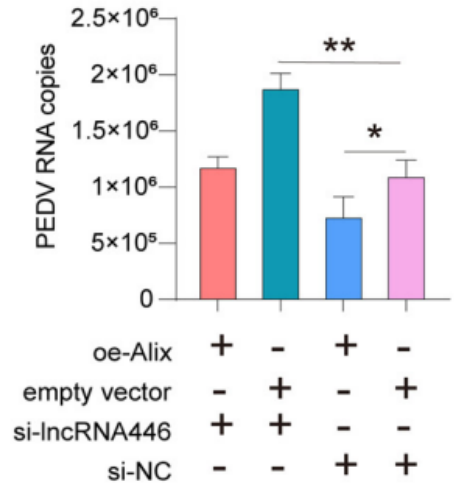
✓ Alix prevents PEDV replication
sh-Alix (shorhairpin-Alix)
oe-Alix (overexpressed-Alix)

B



✓ LncRNA446 prevents degradation of Alix when infected by PEDV

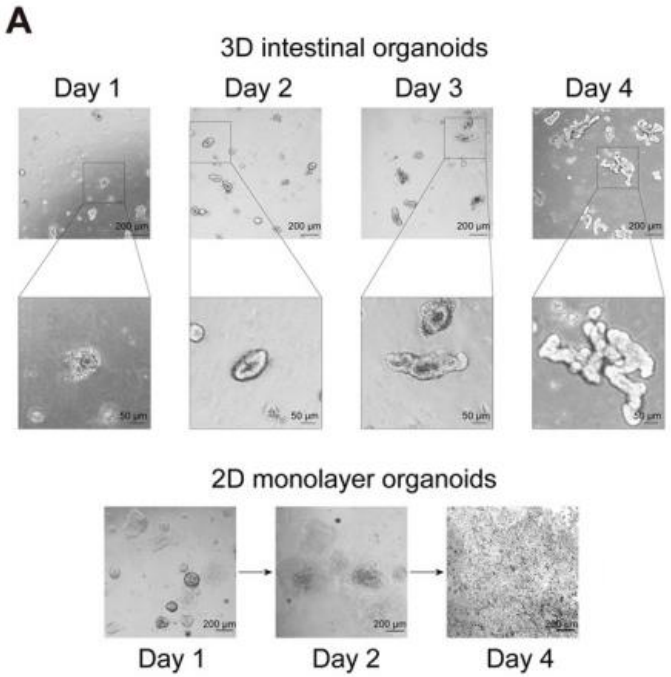
C



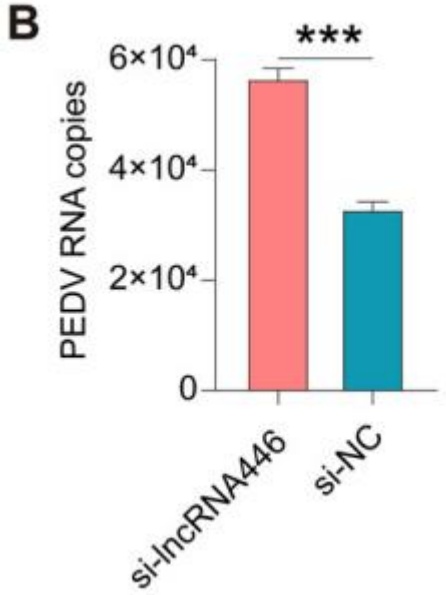
✓ LncRNA 446 and Alix inhibit PEDV replication

→PEDV is inhibited by lncRNA 446 and Alix

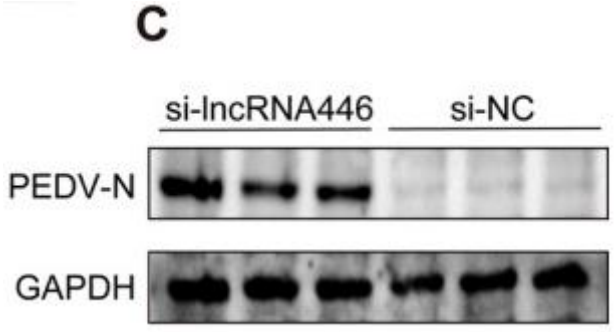
Figure 8. PEDV replication in 2D monolayer organoids



- ✓ Porcine 3D intestinal organoid culture and 2D monolayer organoid culture



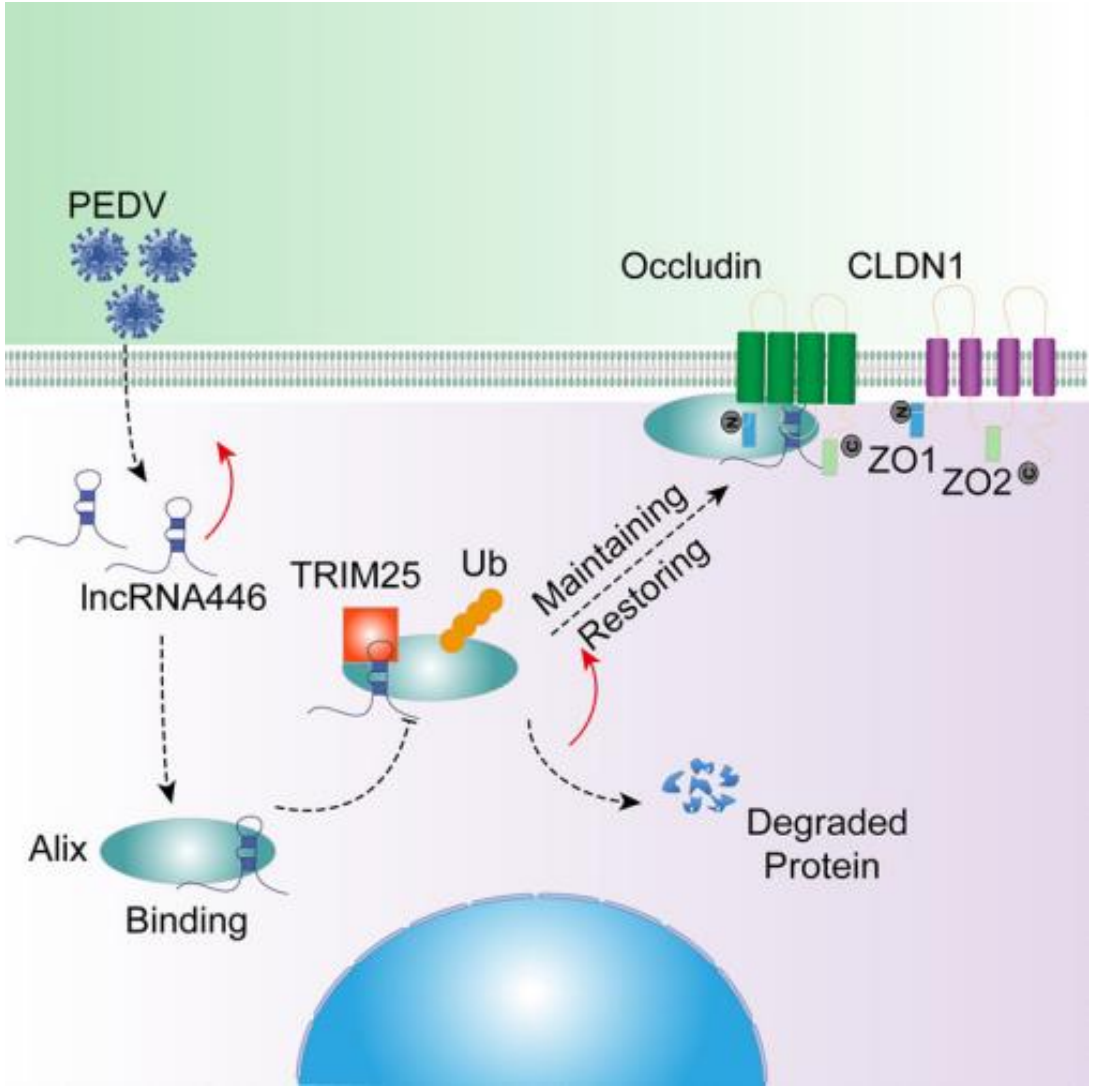
- ✓ RT-PCR using 2D monolayer organoid culture



- ✓ Western blot using 2D monolayer organoid culture

→LncRNA 446 inhibits PEDV replication using organoid

Conclusion



Summary

PEDV infection induces lncRNA446

→ LncRNA446 binds to Alix and inhibit degradation of Alix

→ Alix restore tight junctions

→ PEDV replication is inhibited