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Viruses Across Life's Journey: Old, New, and Unforeseen

Inositol metabolism as a broad-spectrum antiviral target

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Outline

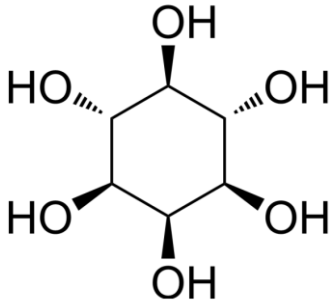
Introduction

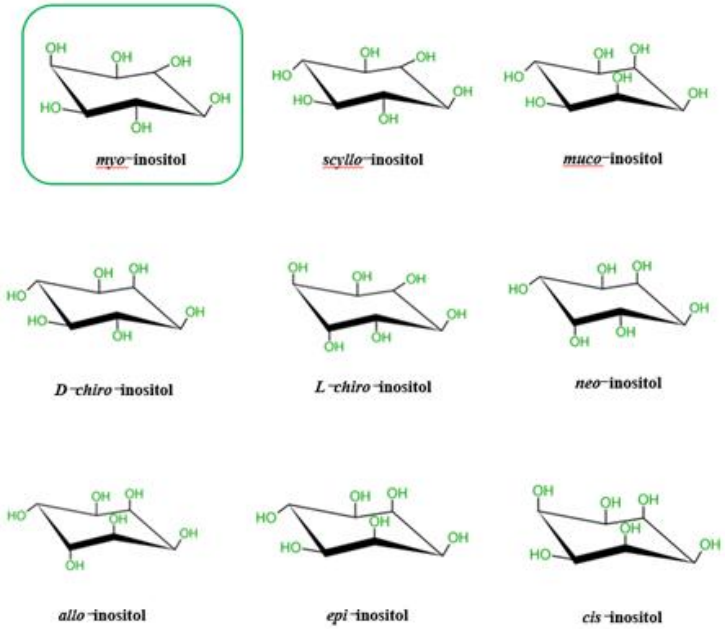
- Inositol and Phosphatidylinositol (PI)
- Phosphatidylinositol phosphates (PIPs)
- Phosphatidylinositol kinases (PIKs) and viral replication
- PIKs as an antiviral target

Inositol monophosphatase (IMPA) as a broad-spectrum antiviral target

- Identification of IMPA as a broad-spectrum antiviral target of ivermectin
- Future outlook

Inositol



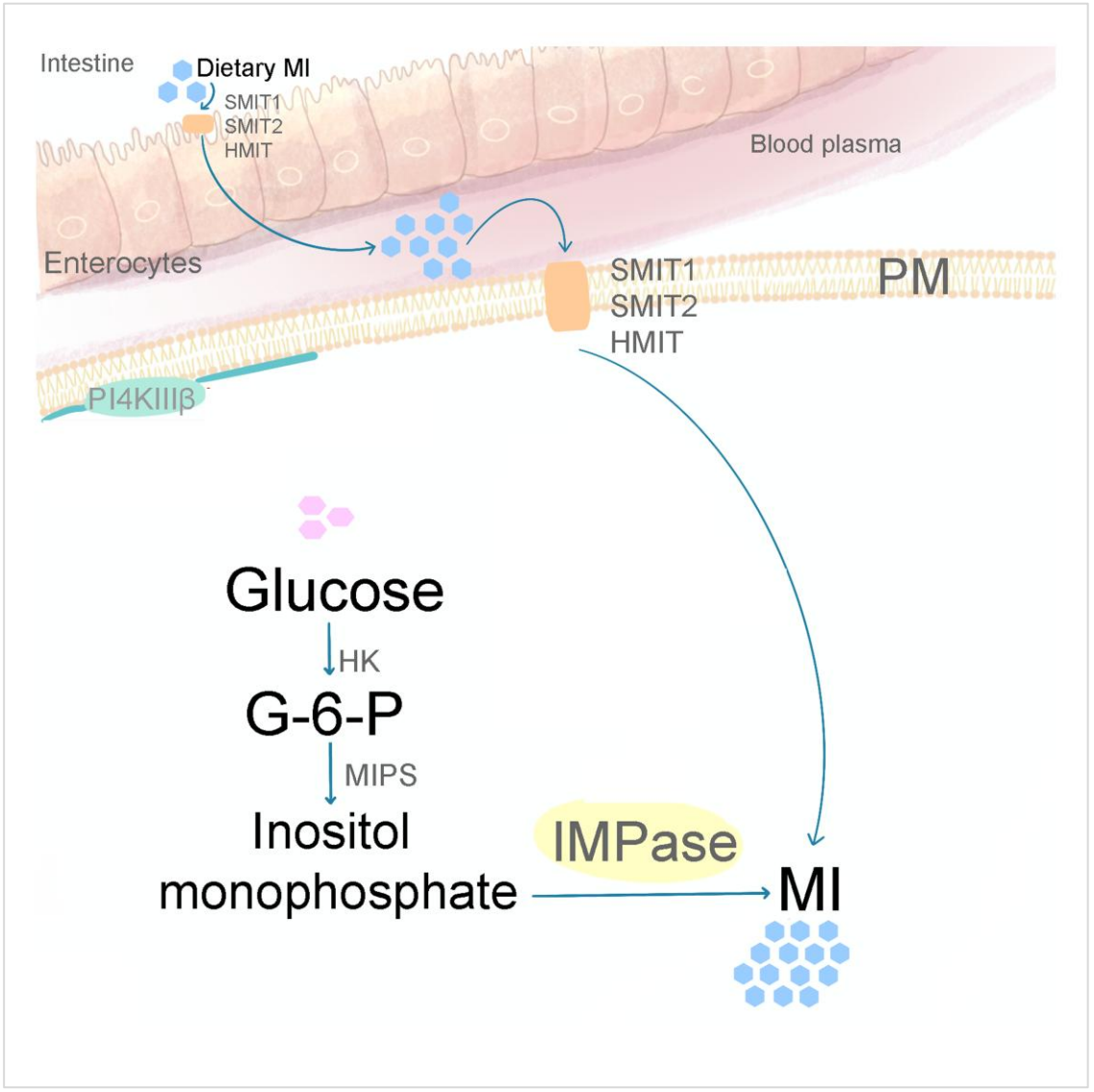


- A type of sugar, which composed of a six-carbon ring, and each carbon is hydroxylated.
- Nine stereoisomers of inositol (cis-, epi-, allo, myo-, neo-, scyllo-, L- chiro-, D- chiro-, and muco-inositol).
- Seven isomers are found in nature, except for epi- and allo-inositol, while **myo-inositol (MI)** is the most abundant form

Primary roles of inositol

- **Prokaryotes:** regulate physiological osmolarity and cellular pH
- **Eukaryotes:** osmoregulation to protect the cells from hyperosmolarity (mammalian brain and kidney cells)

Sources of myo-inositol (MI)



1) Acquired from food consumption (import via SMIT1, SMIT2, and HMIT transporters)
2) *De novo* synthesis from glucose
3) Catabolism of PI, PIPs, IP

Inositol can be generated *de novo* from glucose-6-phosphate (G6P) to inositol-3-phosphate [Ins(3)P] by myo-inositol 1-phosphate synthase (MIPS, *ISYNA1*).

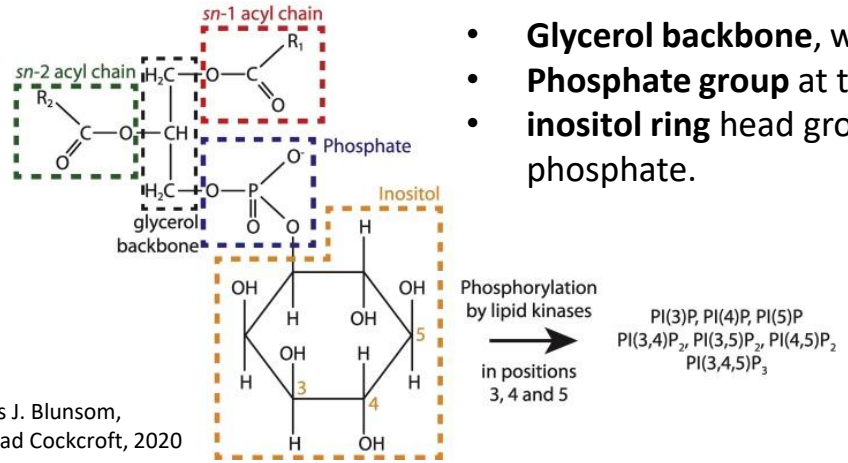
Then the phosphate moiety is removed **by inositol monophosphatase (IMPase**, *IMPA1*, *IMPA2*) into free myo-inositol

Myo-inositol-3-phosphate synthase (MIPS) and IMPase (IMPA), maintaining cellular free myo-inositol!

Inositol derivatives

- **Lipid-associated derivatives**:
  - Phosphatidylinositol (PI)
  - Phosphatidylinositol phosphates (PIPs) or phosphoinositides (PPIns)
- **Soluble derivatives:**
  - inositol phosphates (IP)
  - inositol pyrophosphates

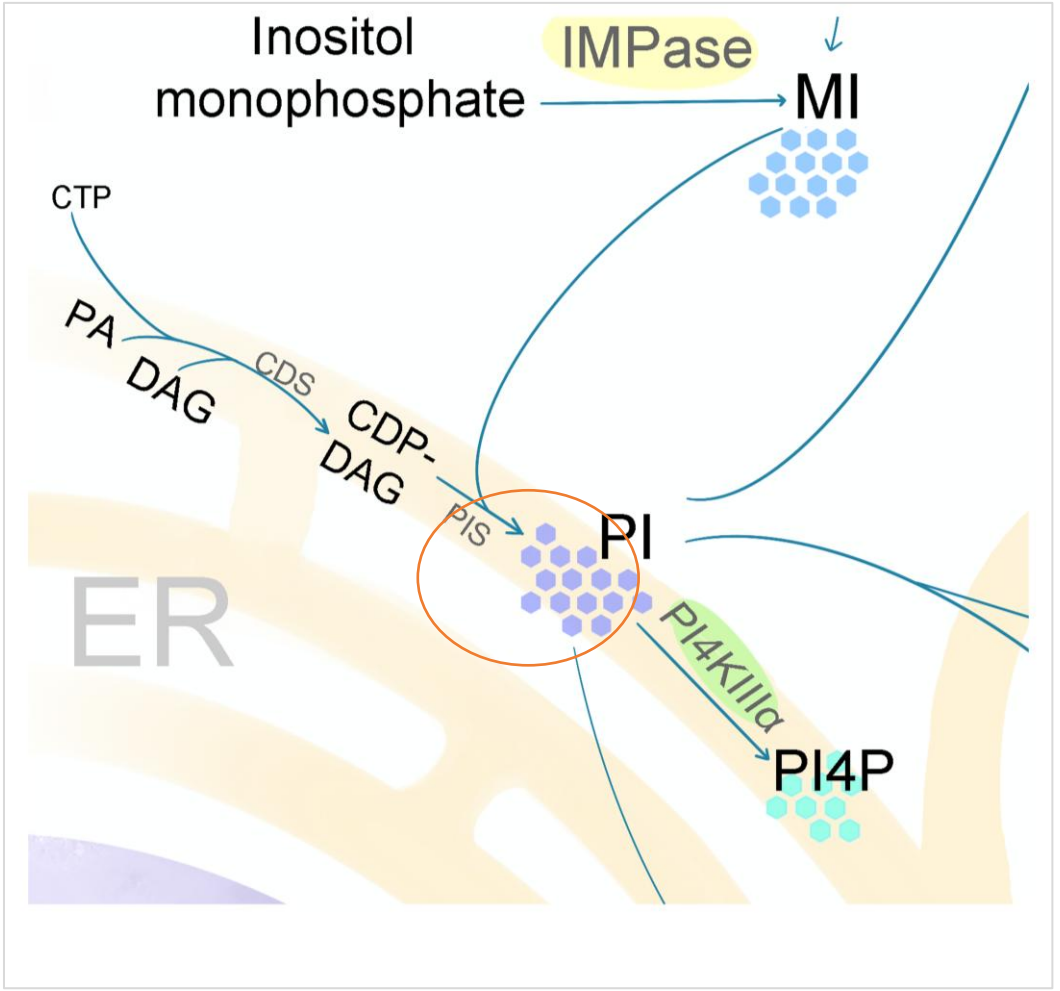
Phosphatidylinositol (PI)



Nicholas J. Blunsom, Shamshad Cockcroft, 2020

- **Glycerol backbone**, with two fatty acid chains
- **Phosphate group** at the third position
- **inositol ring** head group that is attached to the phosphate.

- Synthesized in ER
- Found in the cytoplasmic leaflet of the plasma membrane, membrane-bound organelles

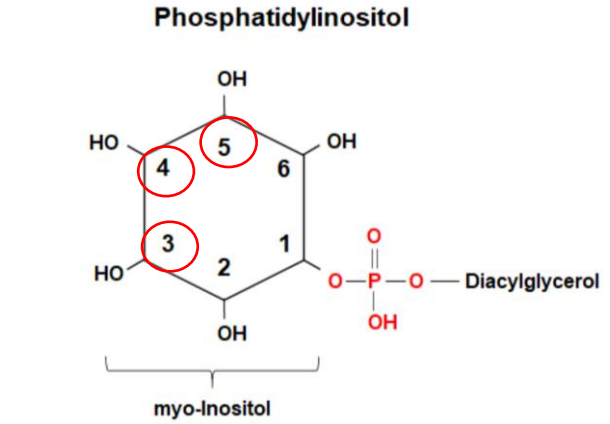


Phosphatidylinositol (PI)

A precursor of phosphatidylinositol phosphates (PIPs), which act as second messengers in multiple signaling pathways and are involved in diverse biological processes.

- Actin cytoskeletal organization
- Lipid metabolism and transport
- Coordinates the cellular response to nutrients and energy production via AMP-activated protein kinases (AMPK) and the mammalian target of rapamycin (mTOR)
- Immune cell functions
- Cellular stress response
- Apoptosis
- Secretory pathway

Phosphatidylinositol phosphates (PIPs)
or phosphoinositides (PPIns)



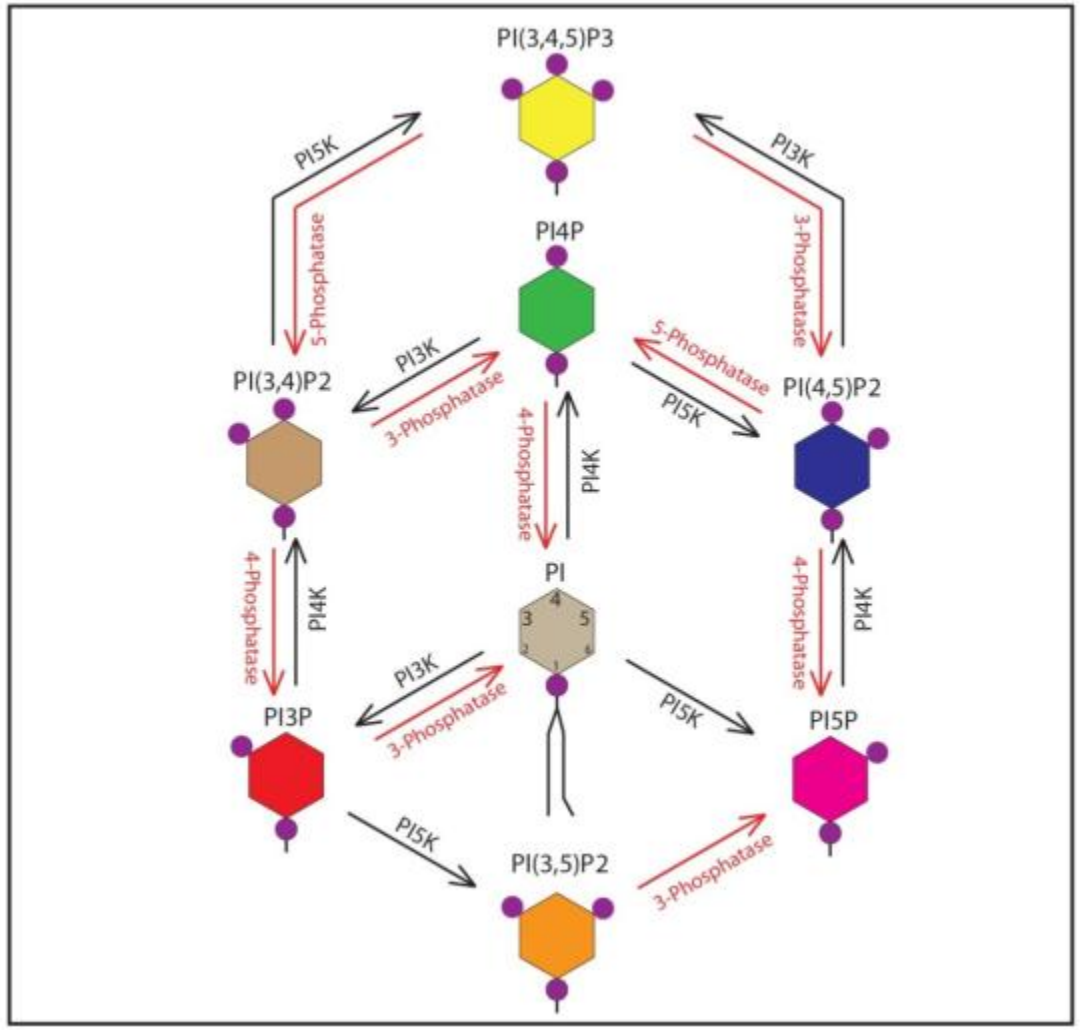
Maffucci T, Falasca M, 2020

PI with an inositol head group that can be phosphorylated at positions 3, 4, and 5.

Seven members of PIP

- Phosphatidylinositol 3-phosphate (PI3P)
- Phosphatidylinositol 4-phosphate (PI4P)
- Phosphatidylinositol 5-phosphate (PI5P)
- Phosphatidylinositol 3,4-bisphosphate [PI(3,4)P2]
- Phosphatidylinositol 3,5-bisphosphate [PI(3,5)P2]
- Phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2]
- Phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P3]

Biosynthesis and interconversion of PIPs



Bryan Bishé, et al, 2012

Regulated by

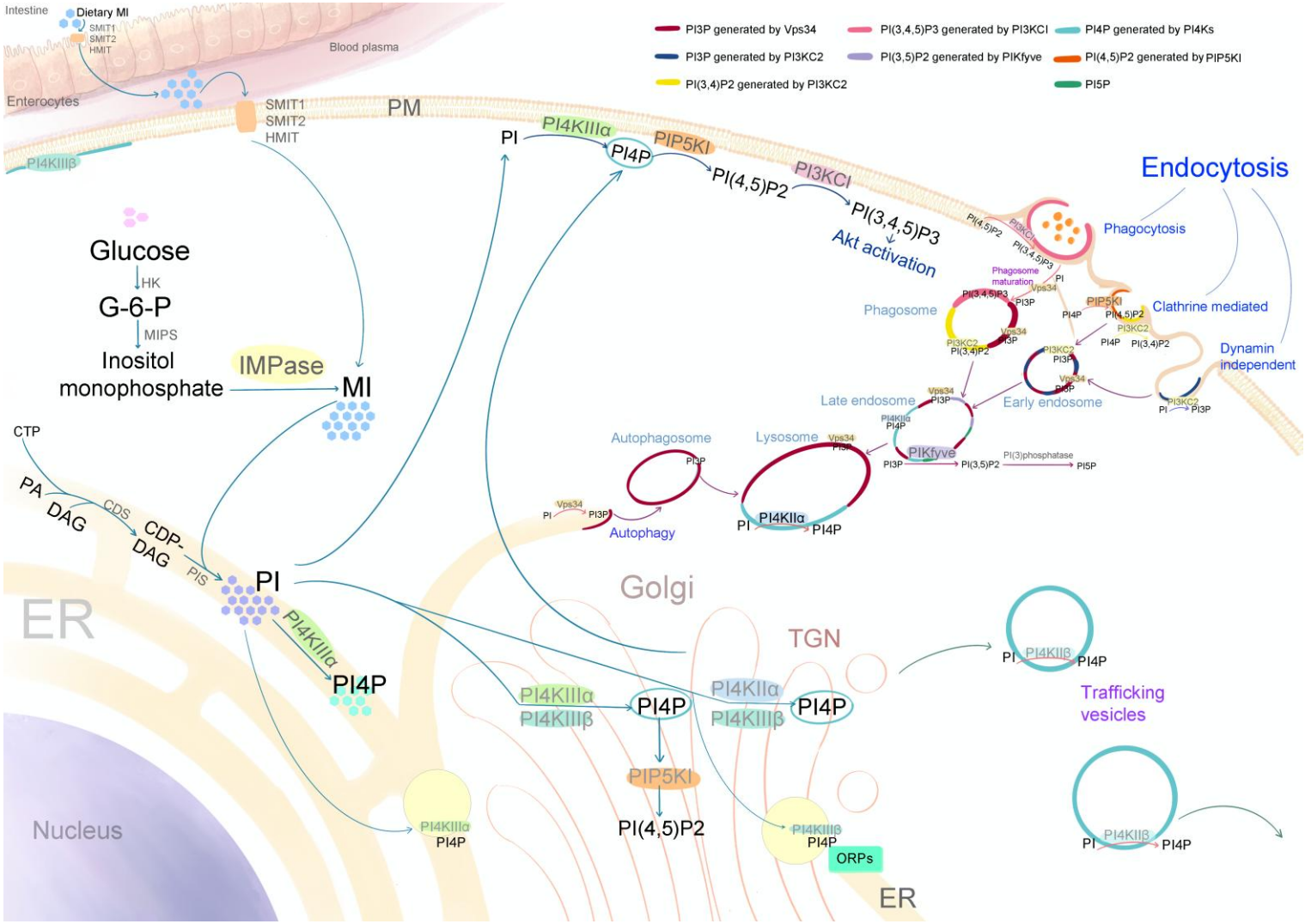
- **Phosphatidylinositol kinases (PIKs)**
- **Phosphatases**

PIKs

- **Phosphoinositide 3-kinases (PI3Ks)**
- **Phosphoinositide 4-kinases (PI4Ks)**
- **PIP4K/PIP5K family**

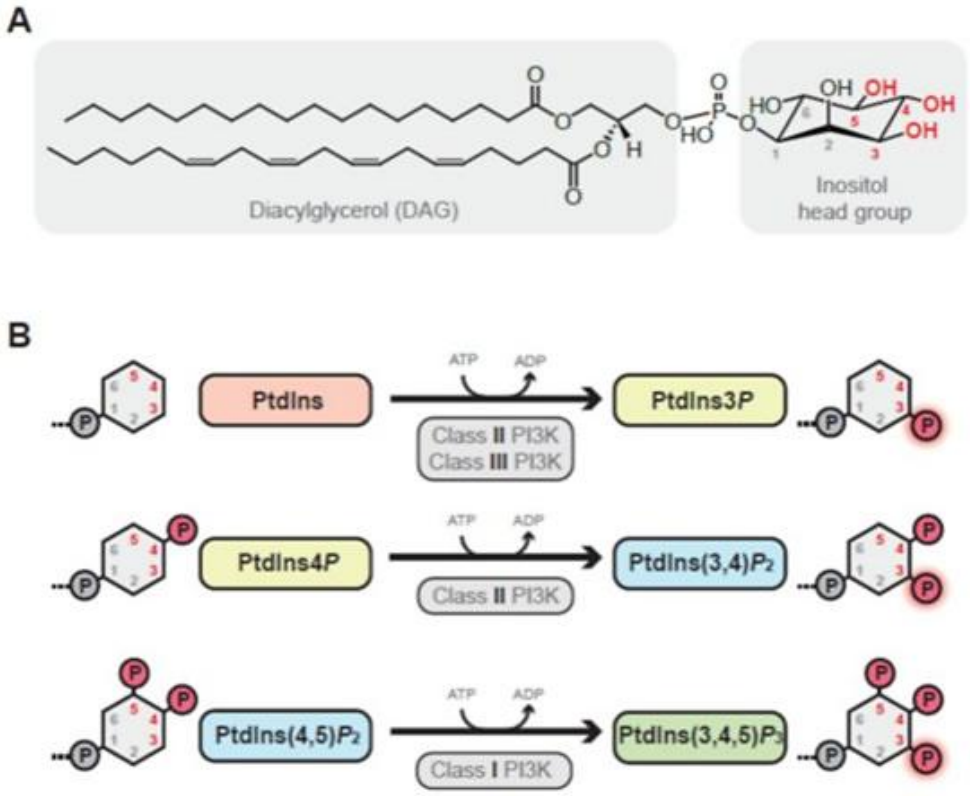
Distribution of PIKs and PIPs

- Phosphatidylinositol kinases (PIKs) and phosphatases differently distributed across various subcellular compartments.
- This distribution results in the different localization of PIPs and their roles in several metabolic pathways.



Phosphatidylinositol kinases (PIKs)

Phosphatidylinositol 3-kinases (PI3Ks)



- Type I, II, and III with different structures and specific substrates
- Each type consists of several isoforms
- Generate PI3P, PI(3,4)P2, and PI(3,4,5)P3

PI3KC3 (Vsp34): formation of early endosomes and autophagosomes

PI3KC2: clathrin coated vesicle formation and maturations

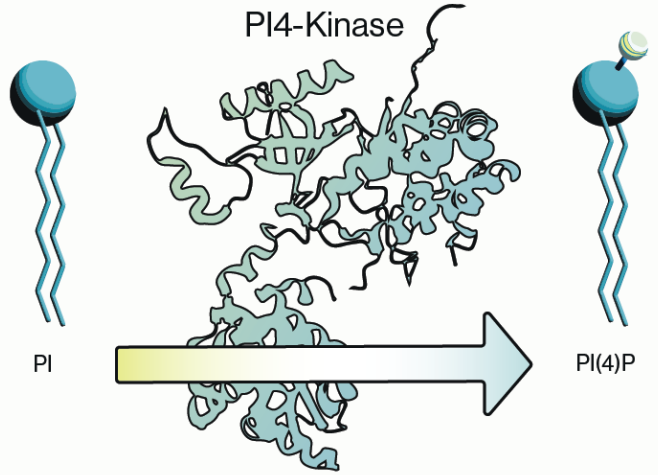
PI3KC1: Second messenger in PI3K/Akt/mTOR pathway

F. M. Gavgani, 2018

Phosphatidylinositol kinases (PIKs)

Phosphatidylinositol 4-kinases (PI4Ks)

Producing PI4P; precursor for other PIPs, lipid transport, membrane composition



2 Types

- **Type II** (**PI4KIIα**) (**PI4KIIβ**)
- **Type III** (**PI4KIIIα**) (**PI4KIIIβ**)

PI4KIIα (trans-Golgi network (TGN), late endosomes) function in cargo sorting during TGN to late endosome trafficking.

PI4KIIβ: (inactive in the cytosol) Upon palmitoylation, it associates with the membrane of the trafficking vesicles and become active.

PI4KIIIβ (Golgi, TGN, and Golgi derived vesicles)

- Both PI4KIIIβ and PI4Ks type II are mainly responsible for PI4P generation at the Golgi and TGN.
- This produced PI4P plays a key role in lipid transport

PI4KIIIα (ER, early cis-Golgi, and plasma membrane)

- Responsible for most of the PI4P generation at the plasma membrane.
- It can be recruited to the plasma membrane.
- Maintaining lipid composition and lipid transports.

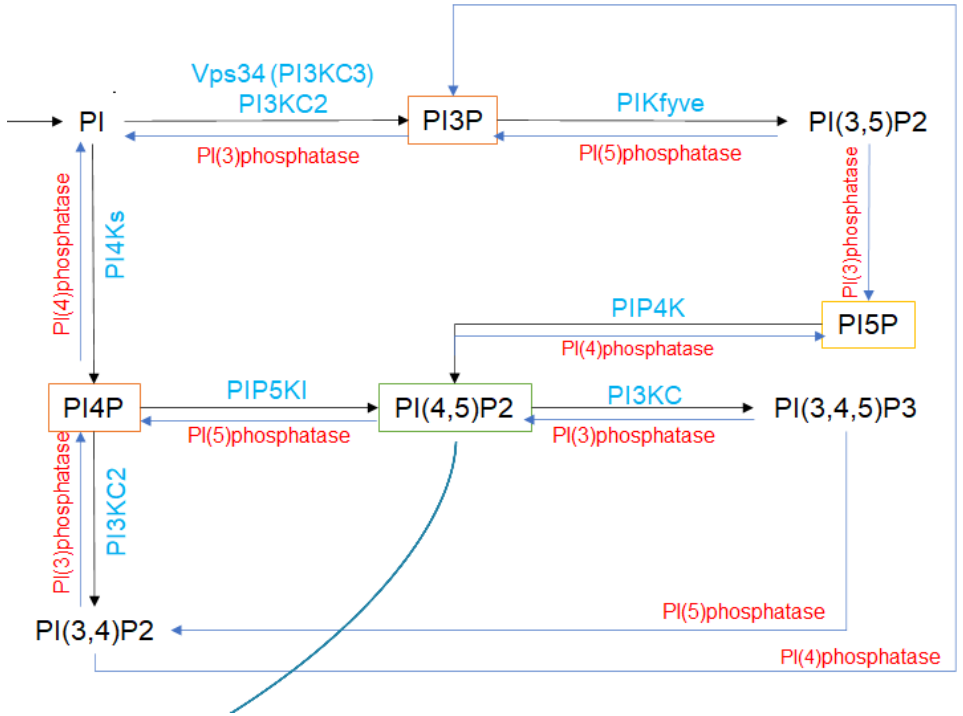
Phosphatidylinositol kinases (PIKs)

PIP4K/PIP5K family

- **Phosphatidylinositol-4-phosphate-5-kinase type I (PIP5KI)**
- **Phosphatidylinositol-5-phosphate 4-kinase type II (PIP4K)**
- **Phosphatidylinositol-3-phosphate 5- kinase type III (PIKfyve)**

Generation of **PI(4,5)P2.**

The most abundant PIPs and is mostly bi-phosphorylated PIP found at the plasma membrane.

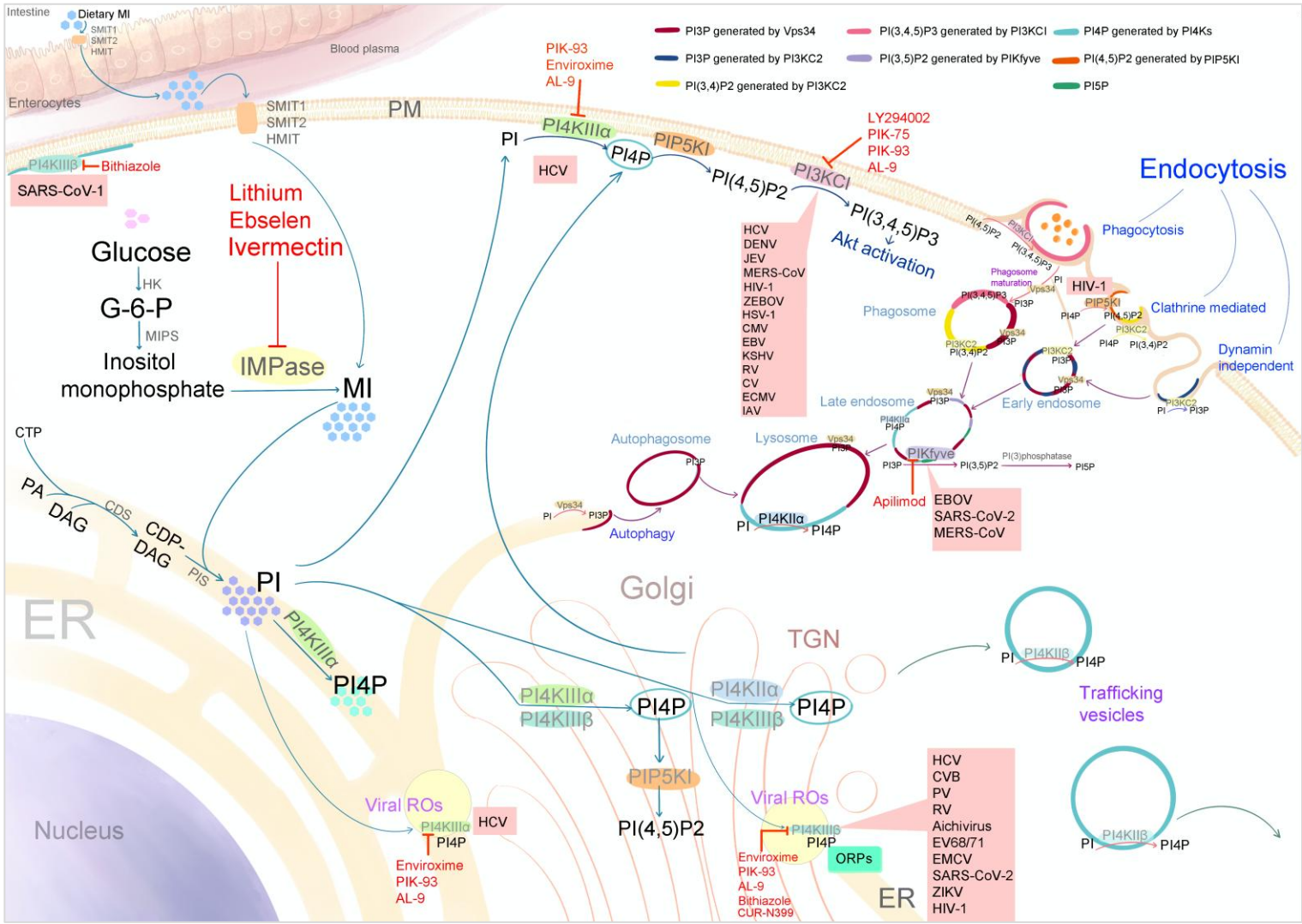


Generation of **PI(3,5)P2 and PI5P**

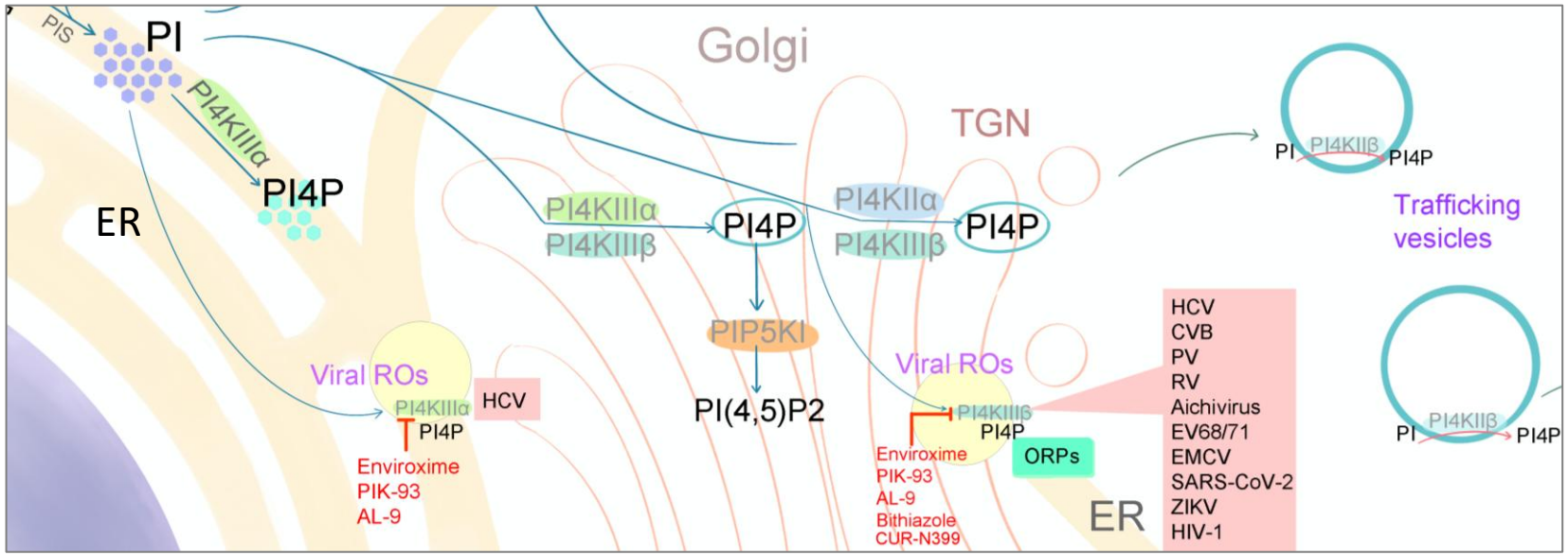
Maturation of endosomes from early endosomes to the TGN and lysosome transport

PIKs and viral replication

Viruses manipulate host cellular biological pathways involves **hijacking the function of PIKs.**



PI4Ks and virus replication



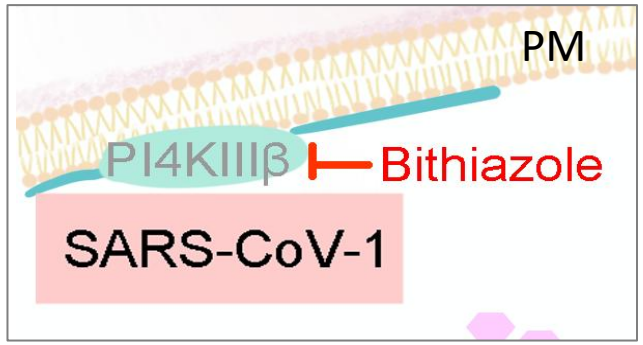
PI4KIIIα

- HCV recruits PI4KIIIα to the site of viral replication via interaction with NS5A, resulting in increased PI4P levels that facilitate the formation of viral replication organelles (ROs) known as the membranous web.

PI4KIIIβ

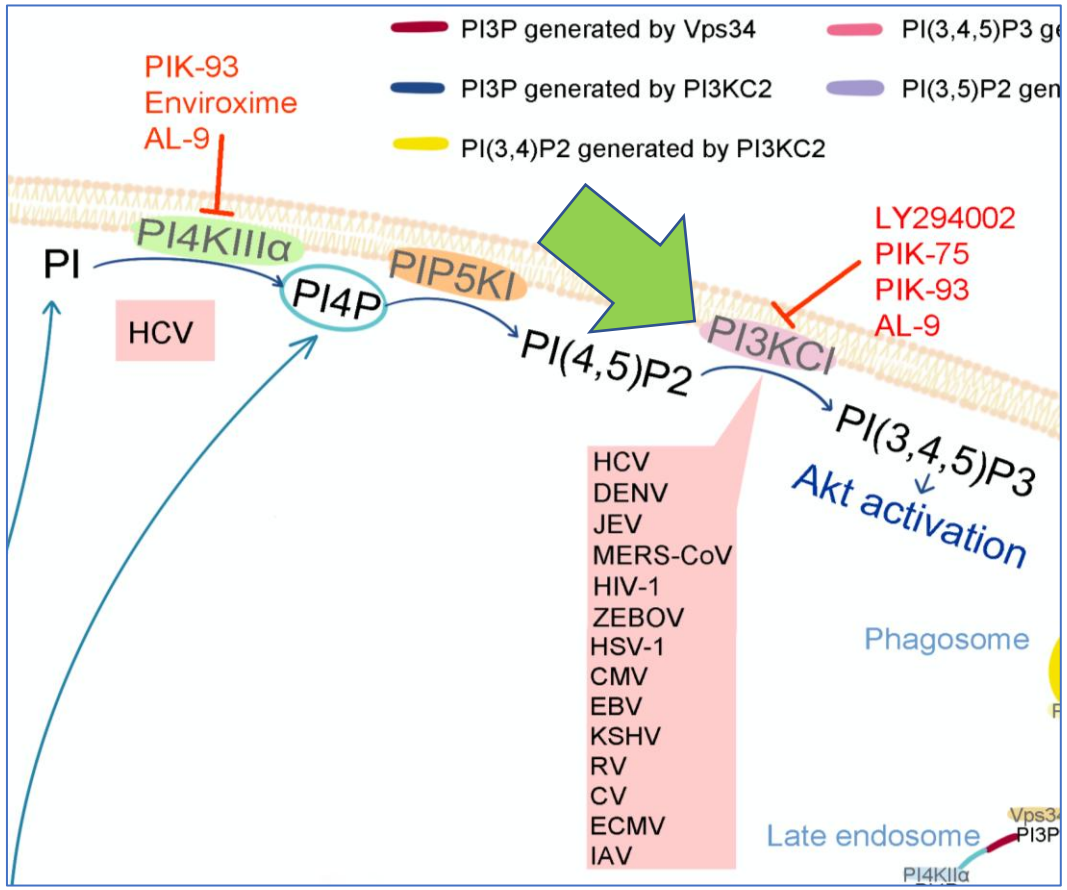
- **HCV:** essential for the replication of some HCV genotypes.
- **Picornaviruses:** The viral 3A protein associates with PI4KIIIβ, increasing PI4P levels at the ROs to facilitate lipid transport.
- **ZIKV:** PI4P is enriched at ROs, facilitating ROs formation.
- **SARS-CoV-2:** PI4KIIIβ regulating lipid membrane composition; an increase in PI4P levels in the organelle membrane is favorable for viral replication.

PI4Ks and virus replication



SARS-CoV-1:
PI4KIIIβ was identified as being involved in the entry of SARS-CoV-1, mediated by angiotensin I-converting enzyme 2 (ACE2) receptors

PI3Ks and virus replication



PI3K type I (PI3KCI): viruses activate the PI3K/Akt signaling pathway for entry, regulate cell survival, or gene expression

Flaviviruses:

- HCV: drive cell survival and suppress apoptosis.
- DENV-2, JEV: suppresses apoptosis at an early stage of infection.

Coronaviruses:

- MERS-CoV: regulating cell proliferation and apoptosis.

Retroviruses:

- HIV-1: requires for viral entry and fusion.

Ebolaviruses:

- ZEBOV: requires for viral entry

Herpesviruses:

- HSV-1: persistent PI3K activation to maintain latent stage
- CMV: essential for early gene expression and genome replication.
- EBV, KSHV: PI3K/Akt activation has been observed in malignancies associated with these viruses.

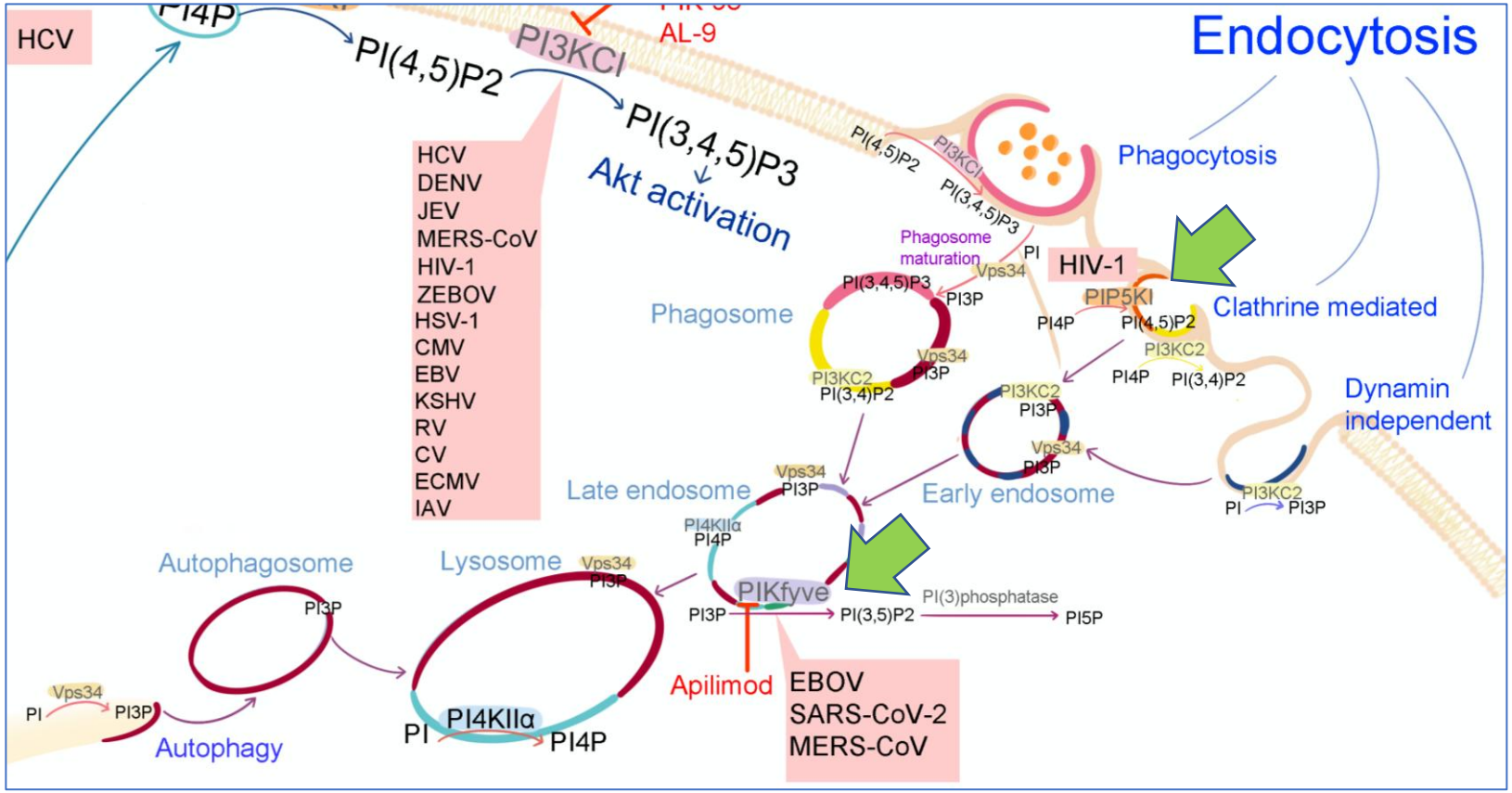
Picornaviruses:

- RV: attachment and entry
- CV, ECMV: inhibiting apoptosis and promoting viral replication

Orthomyxoviruses:

- IAV: requires for facilitate viral internalization

PIP5K, PIKfyve and virus replication



PIP5KI:

- Responsible for PI(4,5)P2 production
- Involved in HIV-1 entry and assembly

PIKfyve:

- Responsible for PI(3,5)P2 production
- Regulates early endosome to late endosome maturation
- Crucial for SARS-CoV-2, MERS-CoV, murine hepatitis virus (MHV), EBOV entry.

Phosphatidylinositol kinases as an antiviral target

PIKs is crucial for viral entry, fusion, genome replication, translation, assembly, and release across multiple viral families, highlighting PIKs as promising antiviral targets.

Inhibitors of phosphatidylinositol kinases with board-spectrum antiviral activity

Inhibitors	Targeted PIKs	Virus group and species or strain	Findings
LY294002	PI3Kα, PI3Kδ, PI3Kβ	**HAdV 2**	Inhibit viral entry, conc. 100 μM (SW480 cells)
		HAdV-19	Inhibit Akt activation, conc. 20 μM (HCF cells)
		HSV-1	Inhibits viral entry, conc. 0.05-0.5 mM (RPE, HeLa and CF cells)
		HCMV	Inhibits virus replication, conc. 10 μM (HFFF2 cells)
			conc. 1-20 μM (HEL fibroblasts)
		CyHV-2	Inhibits virus replication and protein expression (GiCF cells)
		RVA-SA11	Inhibits virus replication, conc. 1, 5 μM (Caco2 cells)
		CVB	Promotes CVB3-induced CPE and apoptosis
		IAV A/WSN/33 (H1N1)	Reduces virus replication, attenuates lung injury in mice
		HIV-1	Inhibits viral entry, conc. 3-10 μM (TZM-bl cells)

Unfavorable pharmacological properties
- limited solubility
- short half-life
- off-target activities

Inhibitors of phosphatidylinositol kinases with board-spectrum antiviral activity

Inhibitors	Targeted PIKs	Virus group and species or strain	Findings
PIK-75	PI3Kα	**IAV**	Inhibits virus replication in A549 cells
		A/Anhui/1/2013 (H7N9)	IC_{50}: 0.04 μM
		A/California/04/09 (pdmH1N1)	IC_{50}: 0.32 μM
		A/Philippines/2/82-X79 (H3N2)	IC_{50}: 0.40 μM
		HIV-1	Inhibits viral entry, conc. 3-30 nM (TZM-bl cells)

- Targets other kinases, including DNA-dependent protein kinase (DNA-PK), raising concerns about off-target effects.
- Poor solubility, which hinder the achievement of therapeutic concentrations.
- The development of a PIK-75 nanosuspension improved solubility and enhanced activity in both in vitro assay and mouse models.

Inhibitors of phosphatidylinositol kinases with board-spectrum antiviral activity

PIK-93	PI4KIIIβ, PI3Kγ, PI3Kα	**AiV**	inhibits virus replication, EC_{50}: 0.60 μM (HeLa cells)
		CVB3	Inhibits virus replication, conc. 1 μM (BGM kidney cells)
		EV71	Inhibits virus replication, conc. 0.25 μM (RD cells)
		HRV	Inhibits virus replication(Cells) mean EC_{50}±SD
		HRVc15	(HeLa) 285 ± 258 nM, (HAE) 225 ± 103 nM
		HRVc11	(HeLa cells) 90 ± 10 nM, (HAE) 342 ± 81 nM
		HRVc25	(HeLa) 57 ± 33 nM
		HRVc24	(HeLa) 75 ± 9 nM
		HRVA16	(HeLa) 574 ± 115 nM, (HAE) 127 ± 50 nM
		PV	Inhibits virus replication, Mean EC_{50}±SD: 0.14±0.0086 μM (RD cells)
		HCV	Inhibits virus replication, (Huh-7.5) mean IC_{50}±SD, (Huh-7) mean EC_{50}±SD
		Genotype 1a	(Huh-7.5) 0.098±0.05 μM, (Huh-7) 0.47 ± 0.1 μM
		Genotype 1b	(Huh-7.5) 0.05±0.01 μM
		Genotype 1b (Huh 5-2)	(Huh-7) 0.28 ± 0.07 μM
		Genotype 1b (Huh 9-13)	(Huh-7) 0.17 ± 0.1 μM
		Genotype 2a	(Huh-7.5) 0.39±0.04 μM, (Huh-7) 5.8 ± 0.5 μM
		Genotype 4a	(Huh-7) 0.72 ± 0.01 μM
		SARS-CoV-2	Inhibits viral entry, conc. 0.1-10 μmol/L (293T-ACE2 stable cell lines)

- The IC50 or EC50 below 1 μM
- Data on its Cmax are still lacking.

Inhibitors of phosphatidylinositol kinases with board-spectrum antiviral activity

Enviroxime (LY122772)	PI4KIIIβ, PI4KIIIα	**MPXV**	(VeroE6) EC_{50}: 4.75 μM
		CVB1	Combination of 50 mg/kg enviroxime and 3.125-6.25 mg/kg disoxaril synergistically inhibits virus replication in mice
		CVB3	Inhibits virus replication, EC_{50}: 0.7 μM
		EV68	(HeLa) EC_{50}: 0.154 μM
		EV71	EC_{50}: 0.0303 μM
		HRV14	EC_{50}: 0.11 μM
		HRV16	(HeLa) EC_{50}: 0.042 μM
		HRV54	EC_{50}: 0.120 μM
		PV1	EC_{50}: 0.19 μM
		PV	(L2OB and RD) MIC of 0.06 μg/ml
		Rubella virus	(HeLa and WISH) MIC of 0.125 μg/ml
		HCV	Inhibits virus replication (Huh-7) mean EC_{50}±SD or only EC_{50} indicated
		Genotype 1a	0.49 ± 0.07 μM
		Genotype 1b	0.22 μM
		Genotype 1b (Huh 5-2)	0.33 ± 0.1 μM
		Genotype 1b (Huh 9-13)	0.22 ± 0.06 μM
		Genotype 2a	2.3 ± 0.8 μM
		Genotype 4a	0.20 ± 0.1 μM
		HCoV-229E	(Huh7) EC_{50}: 4.75 μM
		SARS-CoV-2	(VeroE6) EC_{50}: 0.57 μM

- Targets the viral 3A protein of rhinoviruses and enteroviruses.
- Unfavorable pharmacokinetics, undesirable side effects, and limited efficacy.
- Plasma levels of enviroxime were notably low, with concentrations around 4 ng/ml (approximately 0.01 μM)

Inhibitors of phosphatidylinositol kinases with board-spectrum antiviral activity

Bithiazole derivatives • IC50 values in the low micromolar range	PI4KIIIβ	Different substituents on bithiazole derivatives showed varying antiviral activity across various viruses and cell types. (Cells) Ranges of EC_{50} or IC_{50} values	
		MPXV	(VeroE6) EC_{50}: 3-11 µM
		EV68	(HeLa) EC_{50}: 0.41-3.22 µM
		EV71	(VeroE6) EC_{50}: 0.05-0.03 µM
		HRV2	(HeLa) IC_{50}: 0.39-9.70 µM
		HRV14	(HeLa) IC_{50}: 0.48-15.30 µM
		HRV16	(HeLa) EC_{50}: 0.145-1.6 µM
		YFV	(VeroE6) EC_{50}: 1.05-1.52 µM
		ZIKV	(VeroE6) EC_{50}: 1.88-4.59 µM (Huh-7) EC_{50}: 1.64-6.51 µM (Huh-7) IC_{50}: 0.51-13.79 µM
		HCoV-229E	(Huh-7) EC_{50}: 0.55-0.94 µM
		SARS-CoV-2	(VeroE6) EC_{50}: 0.57-9.67 µM (Calu3) EC_{50}: 2.71-11.2 µM (Calu3) IC_{50}: 1.57-7.45 µM
CUR-N399 • IC50 in low nanomolar • mild toxicity • a phase I clinical trial (NCT05016687).	PI4KIIIβ	**Enterovirus A, B, C, D**	EC_{50}: 2.5 – 53 nM
		Human rhinovirus A, B	EC_{50}: 2.8-53 nM

Inhibitors of phosphatidylinositol kinases with board-spectrum antiviral activity

Apilimod Poor pharmacokinetics • low plasma concentration • poor bioavailability	PIKfyve	**HRV14**	Inhibits virus replication, (HeLa) IC_{50}: 12.3 µM
		HRV1B	(HeLa) IC_{50}: 0.52 µM
		HCoV-229E	IC_{50}: 0.04 µM
		HCoV-OC43	IC_{50}: 0.007 µM
		MERS-CoV	Reduce viral entry, conc. 10-1000 nM, (HeLa/hDPP4 cells)
		SARS-CoV-2	(VeroE6) EC_{50} < 6.9 nM
			(VeroE6) IC_{50} ~10 nM
			Reduces viral entry, conc. 10-1000 nM, (293/hACE2 cells)
		MHV	Reduce viral entry, conc. 10-1000 nM (HeLa/mCEACAM cells)
		ZEBOV	Reduces viral entry, IC_{50} ~50 nM ,(MA104 cells)
		Various IAV strains	(MDCK) IC_{50}: 3.8-24.6 µM
		IAV **PR8** A/Puerto Rico/8/1934(H1N1)	Inhibits body weight loss in BALB/c mice, Dose: 2 mg/mL daily
		IBV Florida/4/2006	(MDCK) IC_{50}: 16.4 µM
		RSV A2	Inhibits body weight loss in BALB/c mice, Dose: 2 mg/mL daily (HEp-2) IC_{50}: 19.6 µM
		PIV3 C243 strain	(LLC-MK2 7.1) IC_{50}: 31.1 µM

Phosphatidylinositol kinases as an antiviral target

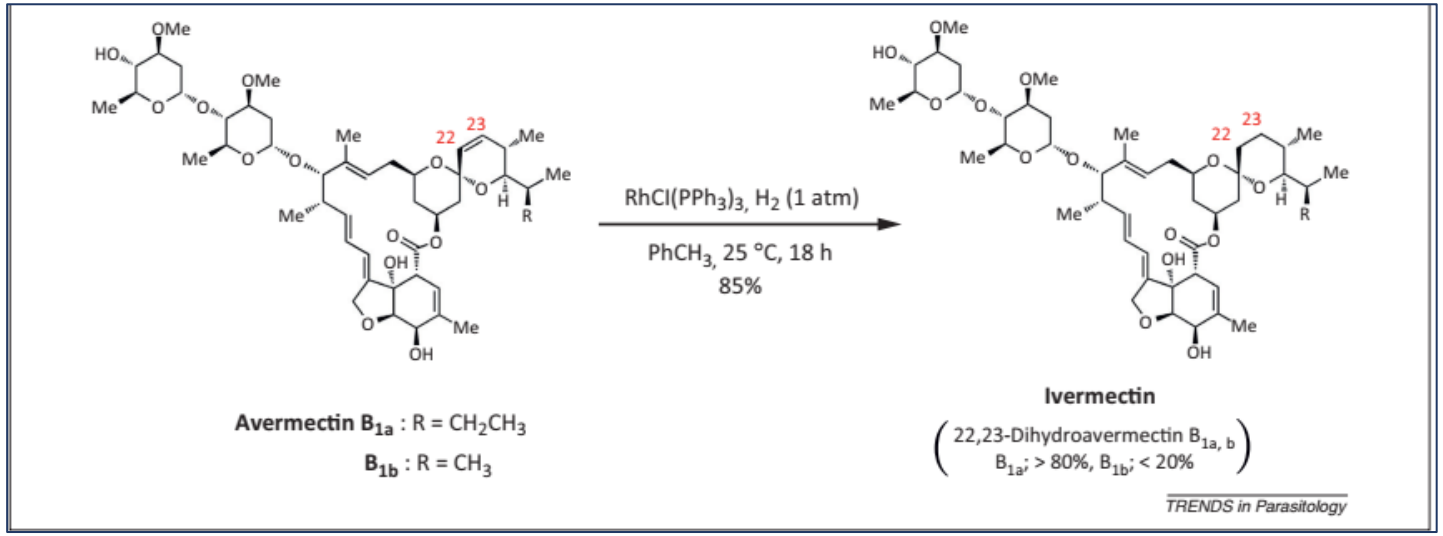
PIK inhibitors: **PIK-75, PIK-93, bithiazole derivatives, and CUR-N399**

- Antiviral activity against various viruses
- Favorable pharmacological properties.
- Most reported: picornaviruses; certain flaviviruses; coronaviruses; influenza viruses; other RNA viruses, such as ZEBOV, rubella virus, human parainfluenza virus, and respiratory syncytial virus (RSV); retroviruses such as HIV-1; and DNA viruses including HSV, HCMV, and MPXV.

Limitations of PIKs as a Broad-Spectrum Antiviral Target

- Viruses within the same genus may share similarities, their replication depends on distinct PIK subtypes.
    - DENV and WNV, both flaviviruses, do not require PI4K activity, unlike HCV.
    - PI3K inhibition has been shown to enhance WNV production by suppressing PI3K signaling, which in turn impairs the IFN-I response.
    - PI3K inhibitor LY294002 increases HBV replication
    - Viral genotype influences PIK dependency: different HCV genotypes exhibit differing reliance on PI4KIIIα or PI4KIIIβ isoforms, resulting in variable sensitivity to their corresponding PI4KIII inhibitors.

Identification of IMPA as a broad-spectrum antiviral target of ivermectin



Ivermectin (IVM)

Anti-parasite medication

- a derivative of avermectin (Macrocyclic lactones from *Streptomyces avermectinius*)
- MW: 875.1 g/mol
- Chemical Formula: $C_{48}H_{74}O_{14}$
- high lipid solubility

- Also possess **broad-spectrum antiviral activity**

Identification of IMPA as a broad-spectrum antiviral target of ivermectin

IVM: Broad spectrum antiviral activity

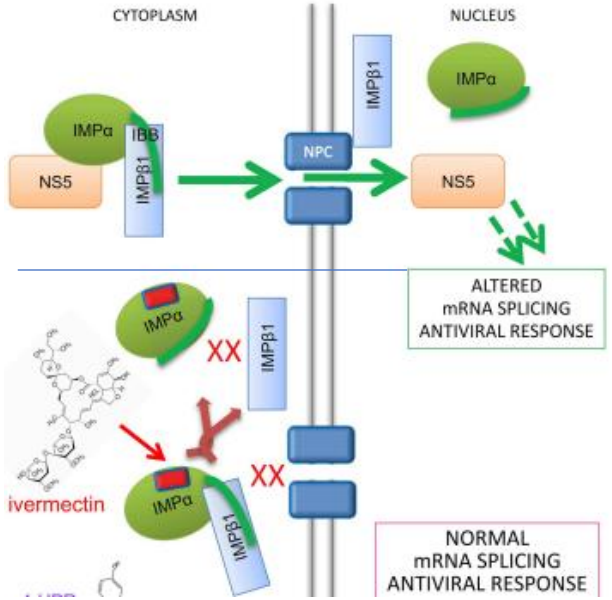
Proposed mechanism >> targets the host nuclear transport **importin α/β1** heterodimer, therefore inhibit nuclear import of various viral proteins.

DENV, WNV, ZIKV > inhibit NS5 nuclear import
(Yang, et al. 2020, Tay MY, et al. 2013, Lopez-Denman AJ, et al. 2018)

HIV-1 > inhibit integrase nuclear import
(Wagstaff et al., 2012)

Influenza > inhibit nuclear import of vRNPs
(Götz V et al, 2016)

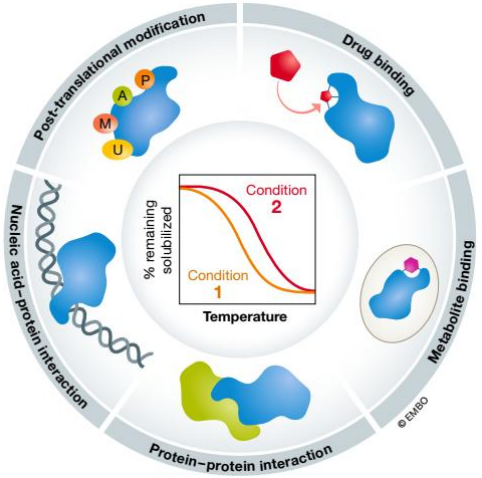
SARS-CoV-2 > the non-structural protein 9 (Nsp9) exhibited the strongest affinity to IVM
(Faizul Azam et al, 2022, *in-silico* analysis)



These mechanisms are applicable only for viruses that require nuclear entry or have specific structures targetable by the drug.

Identification of IMPA as a broad-spectrum antiviral target of ivermectin

- Its main broad-spectrum antiviral mechanism had not been identified.
- We reasoned that a broad-spectrum antiviral activity against various unrelated viruses is likely to target a host mechanism that is commonly used by many viruses, and therefore performed a search for cellular target of IVM



Thermal proteome profiling (TPP)

The thermal shift assay + Mass spectrometry

The thermal shift assay is based on the principle that proteins denature and become insoluble when subjected to heat.

Proteins can change their thermal stability upon interactions with small molecules (such as drugs or metabolites), nucleic acids or other proteins.

Binding of a drug to a protein leads to a thermal stabilization of the protein.

The remaining soluble protein fraction is determined by MS to identified drug targeted proteins.

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RESEARCH ARTICLE

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Identification of inositol monophosphatase as a broad-spectrum antiviral target of ivermectin

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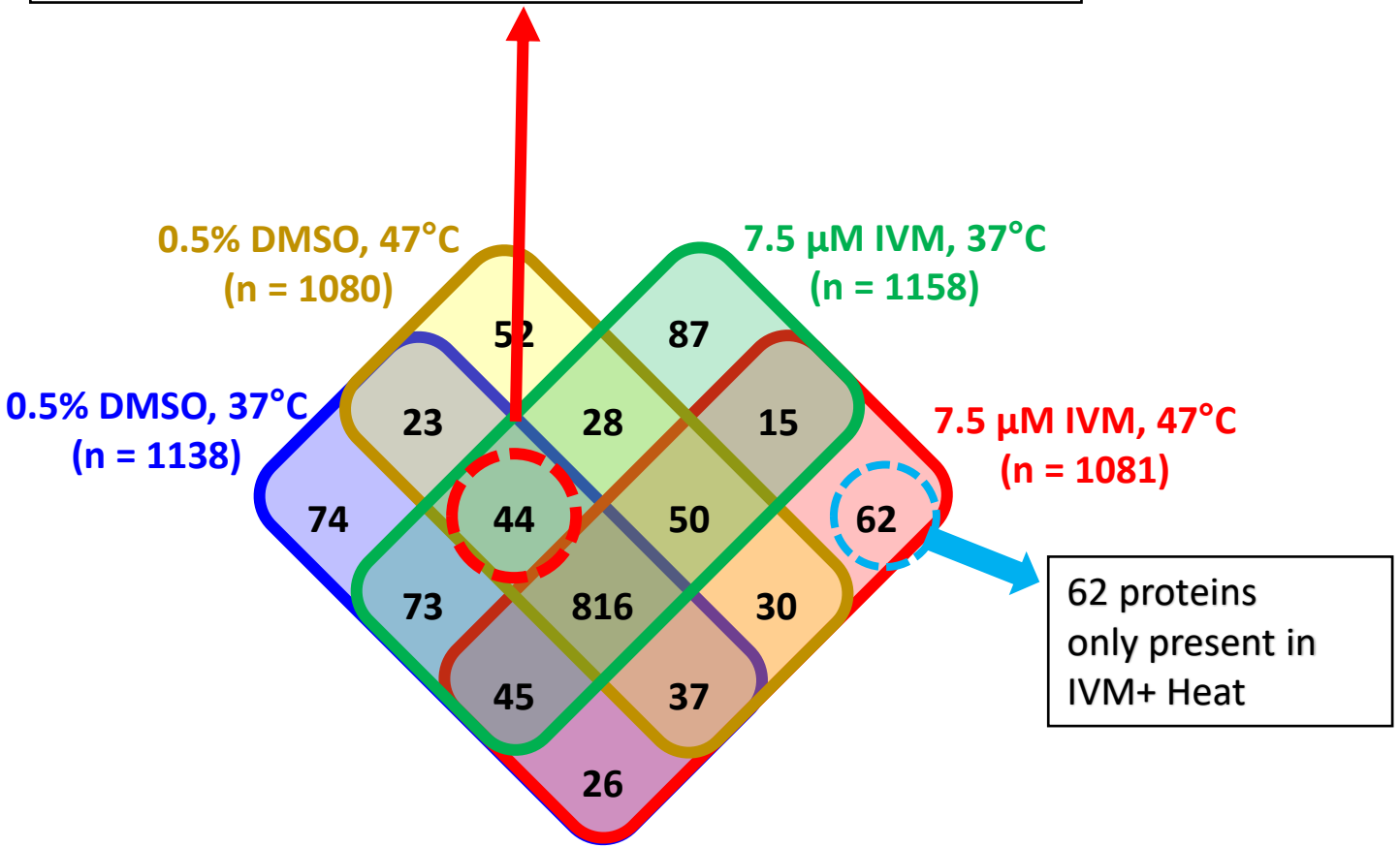
Identification of IMPA as a broad-spectrum antiviral target of ivermectin

44 proteins are:

- Present in
  - Control cells
  - IVM alone
  - Heat alone
- Absent (tentatively precipitated) in IVM + Heat



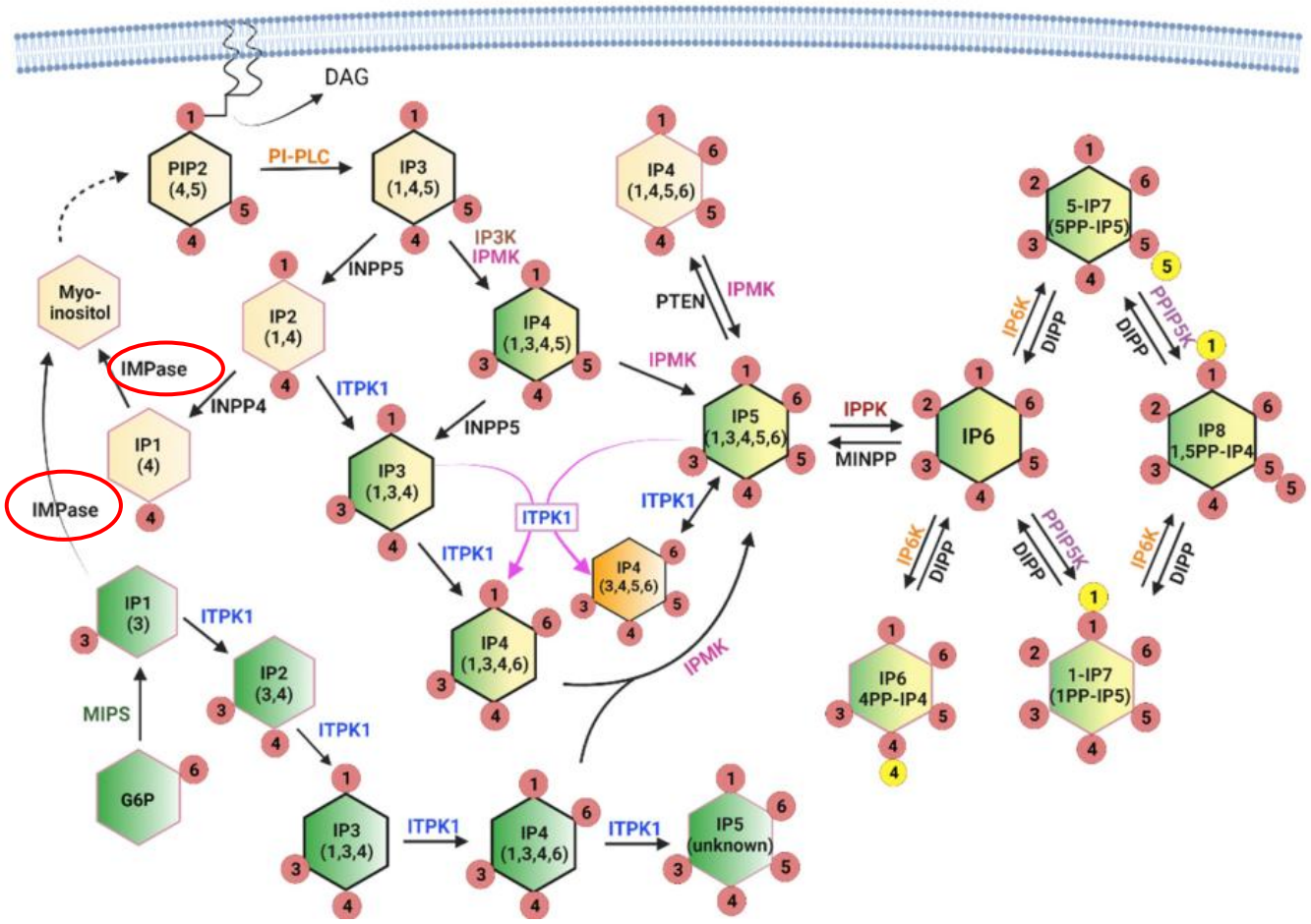
62 proteins
only present in
IVM+ Heat

Identification of IMPA as a broad-spectrum antiviral target of ivermectin

IVM interacting candidates

- **Inositol monophosphatase (IMPA, IMPase)** dephosphorylates myo-inositol monophosphate to generate free myo-inositol, a precursor of phosphatidylinositol synthesis.

- *de novo* inositol biosynthesis
- catabolism of PIPs, IP, and inositol pyrophosphates



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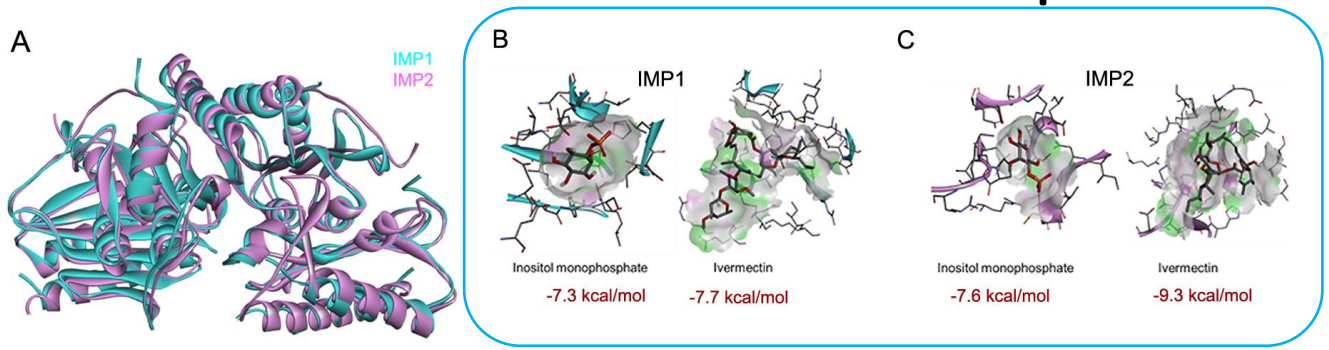
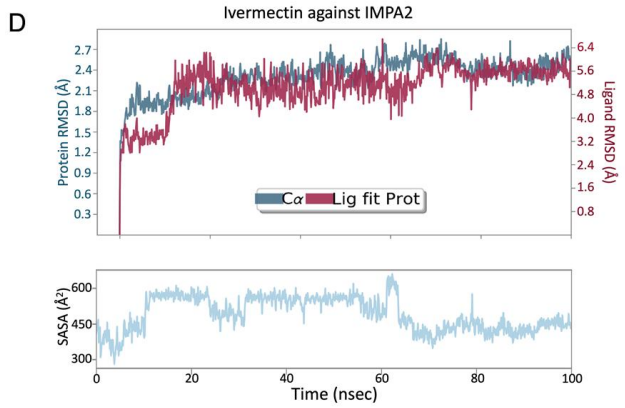
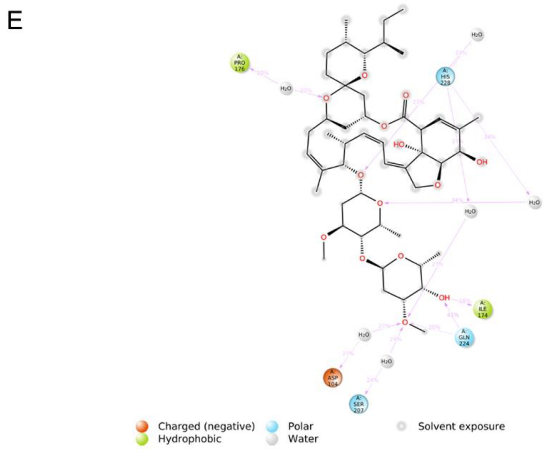
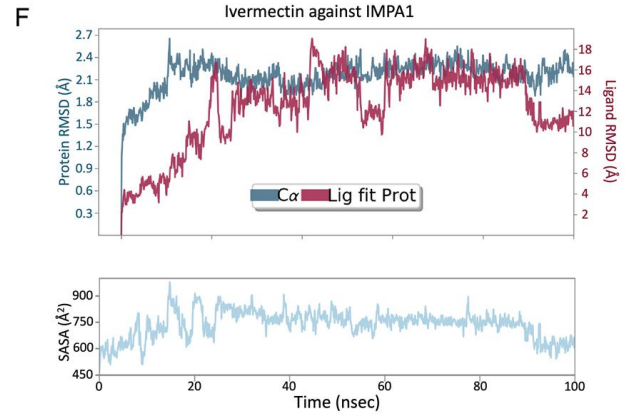


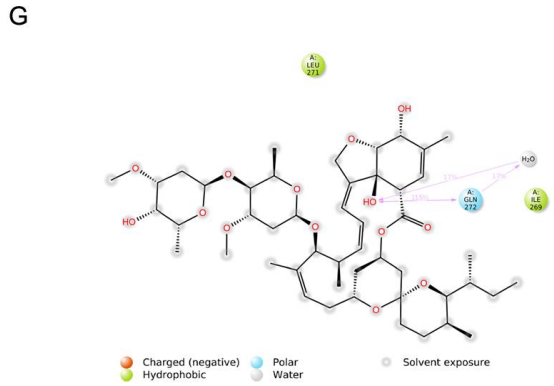
IMPA isoforms: IMPA1 & IMPA2
53.65% sequence identity





Molecular docking of IMPs and their substrates.
(A) Superimposed crystal structures of IMP1 (PDB ID: 4AS4) and IMP2 (PDB ID:2DDK). The structures of IMP1 and IMP2 are shown in cyan and pink, respectively.
(B) Docking of inositol monophosphate and IVM to IMP1.
(C) Docking inositol monophosphate and IVM to IMP2.





SASA plot over the simulation time for ivermectin complexes of (D) IMPA2 and (F) IMPA1. The interaction maps detail contact frequency and contact interaction types between ivermectin and the residues of (E) IMPA2 and (G) IMPA1.

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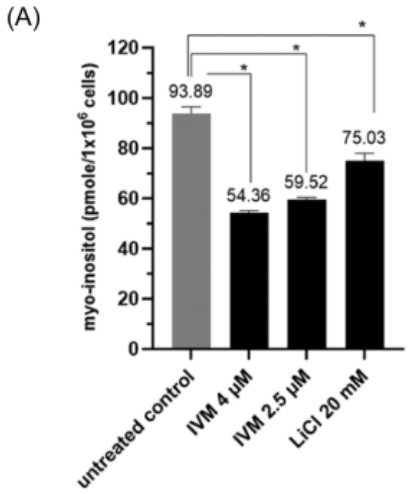
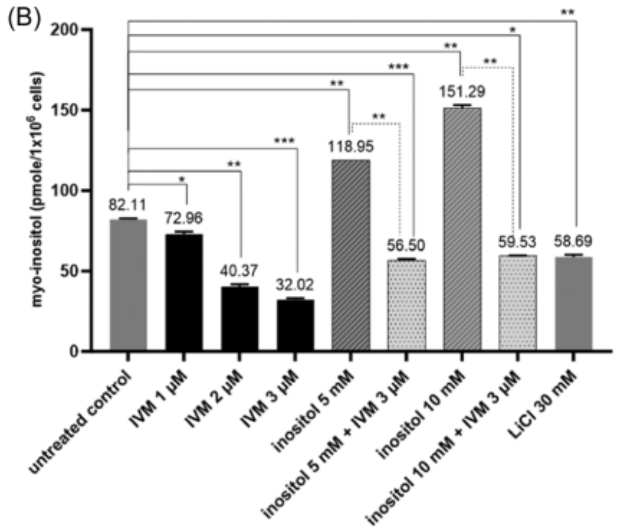
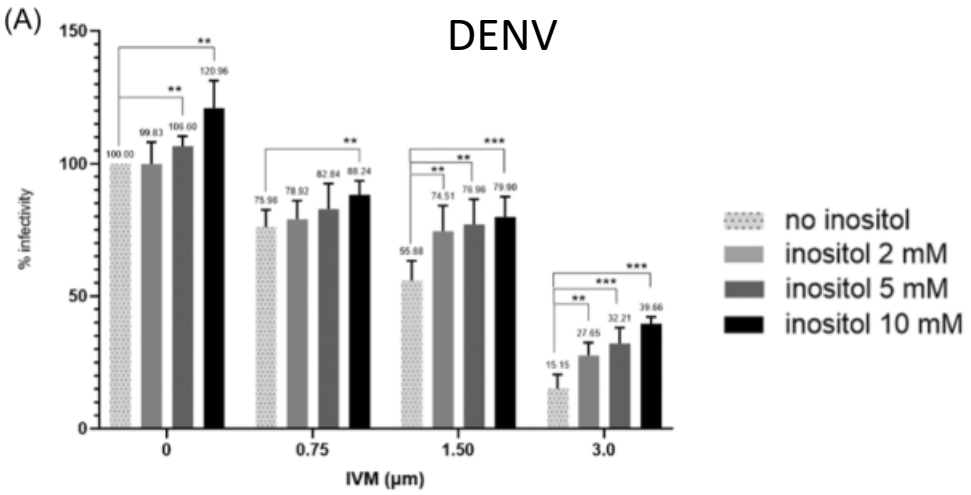
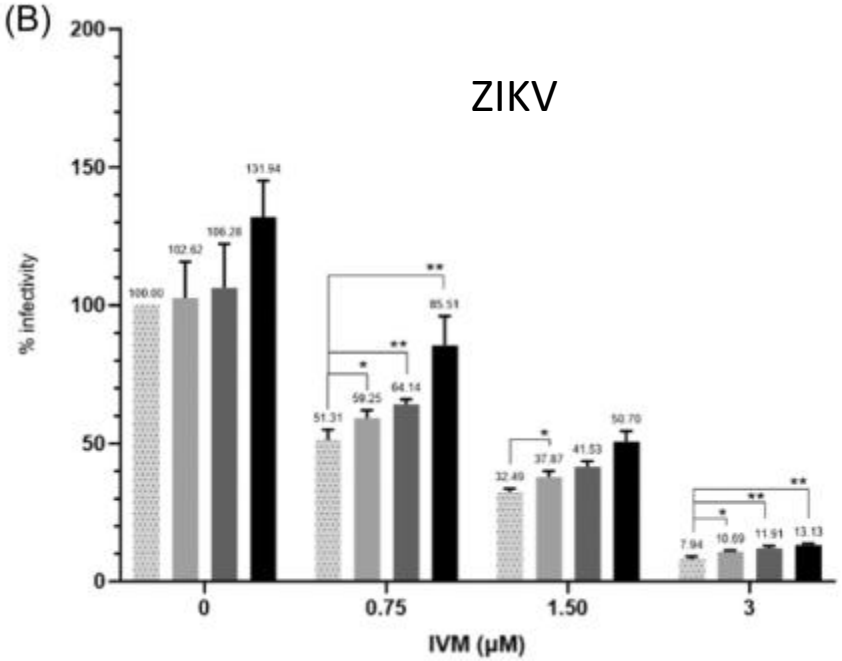




IVM reduced cellular myo-inositol level

Reversion of IVM antiviral activity by inositol





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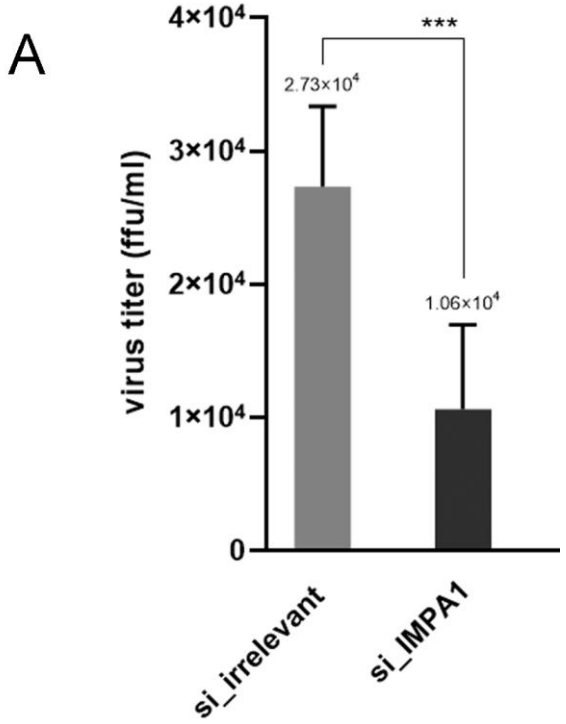
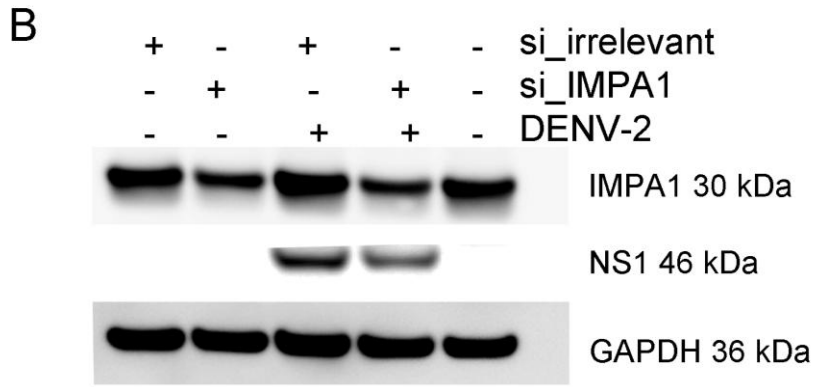




Silencing IMPA1 expression reduces virus production.

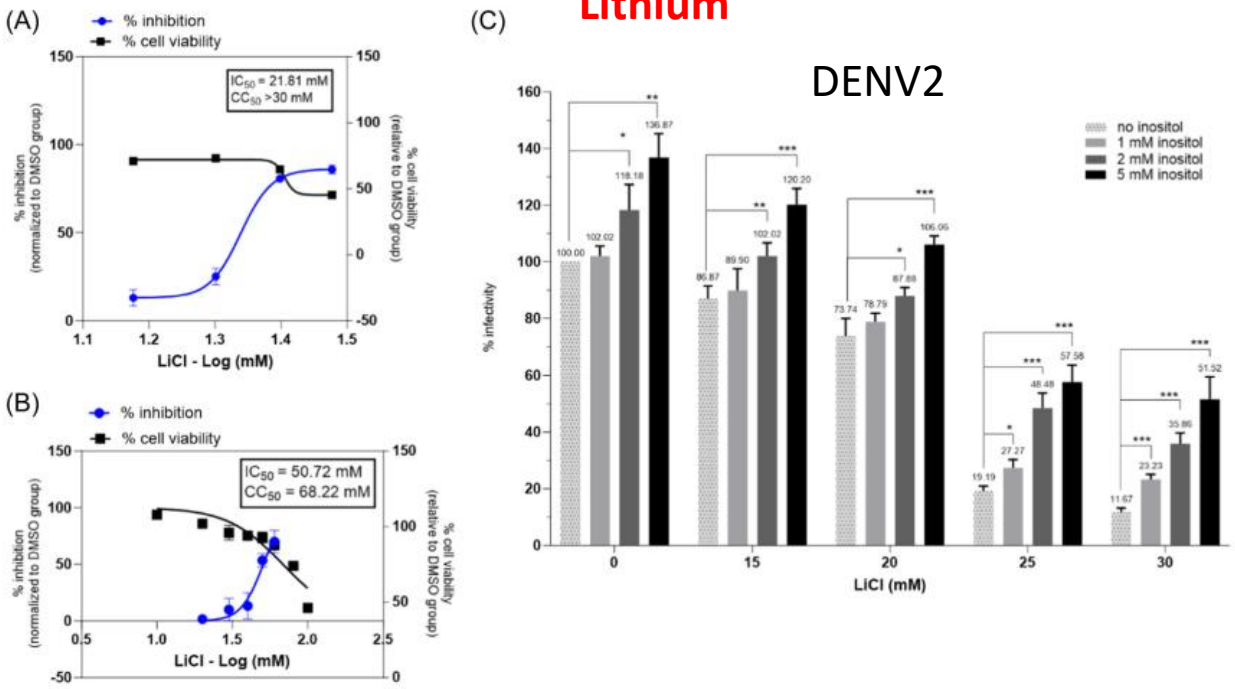
Inositol monophosphatase (IMPA) can be a broad-spectrum antiviral target

Identification of IMPA as a broad-spectrum antiviral target of ivermectin

IMPA inhibitors also inhibit various viruses

- **Lithium**
- **L-690330**

Lithium



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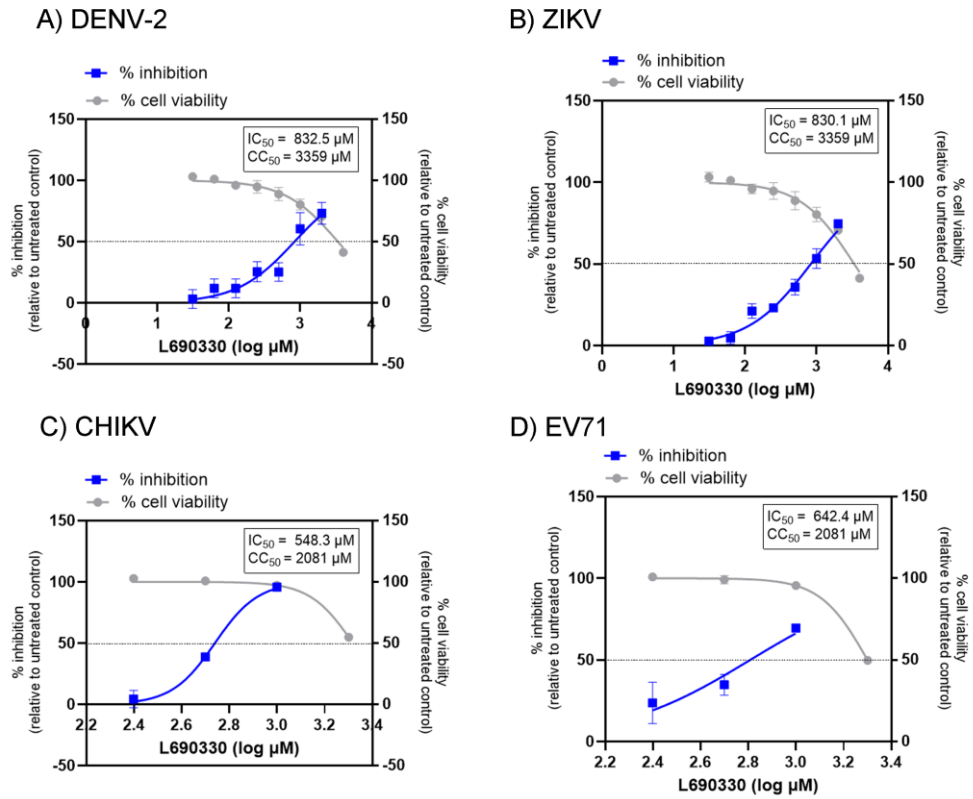
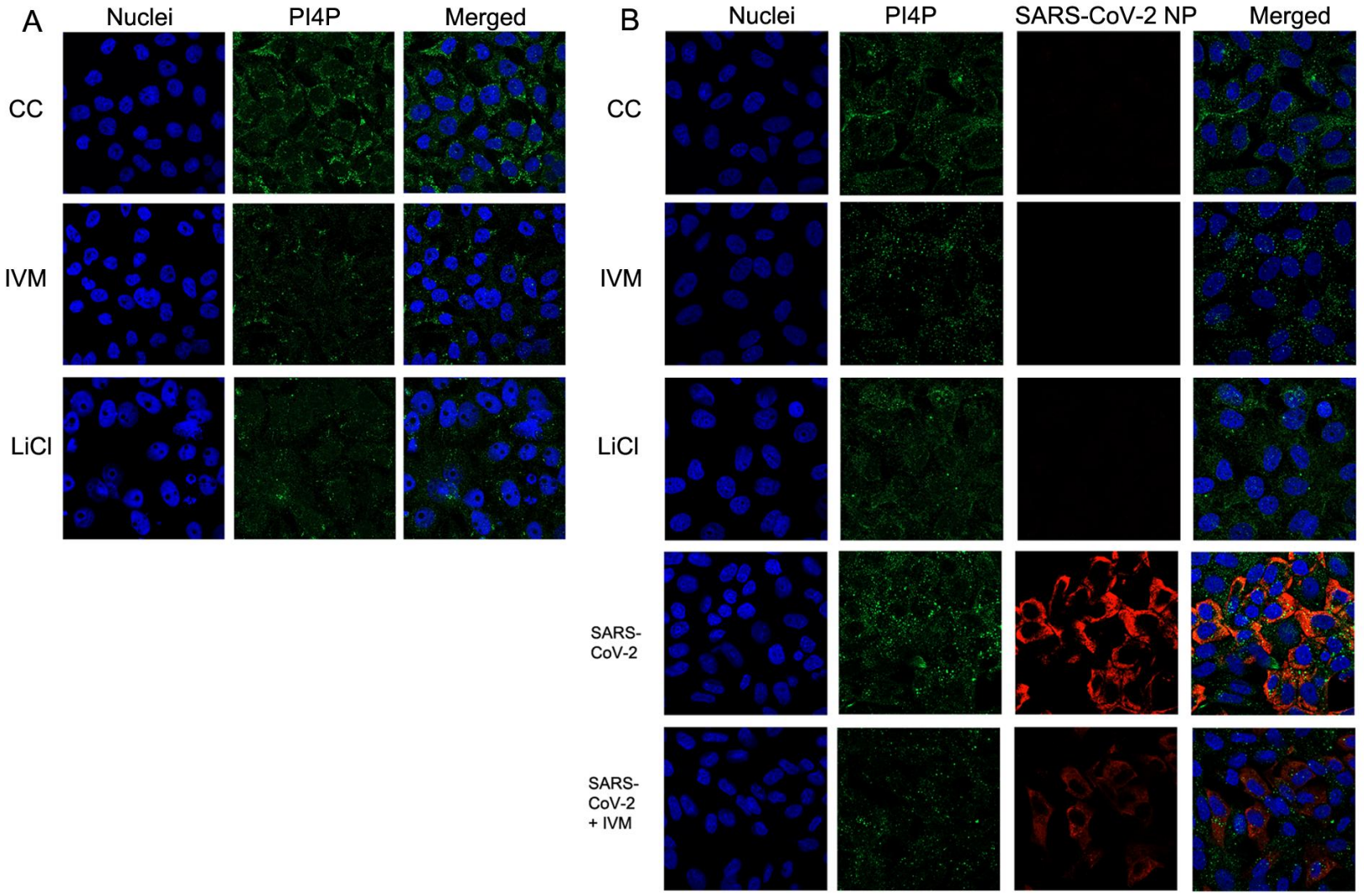


Identification of IMPA as a broad-spectrum antiviral target of ivermectin

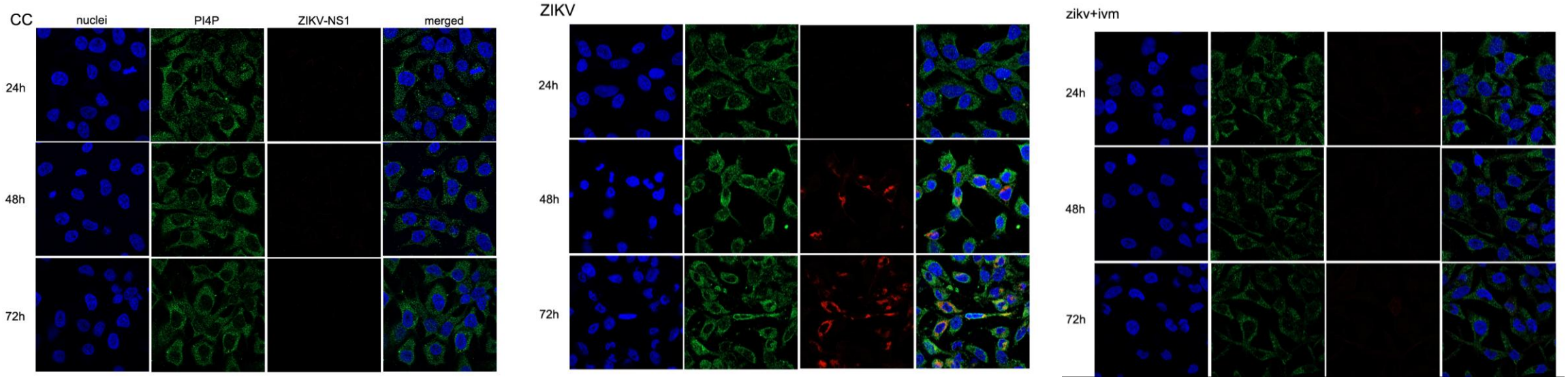
The reduction of cellular myo-inositol levels impacts the subsequent biosynthesis of PIPs

SARS-CoV-2



Identification of IMPA as a broad-spectrum antiviral target of ivermectin

ZIKV



- IVM binds to IMPA and inhibits its activity, resulting in the overall reduction of cellular myo-inositol levels, and inhibits virus replication of DENV-2, ZIKV, and SARS-CoV-2, which modulate PIKs differently.

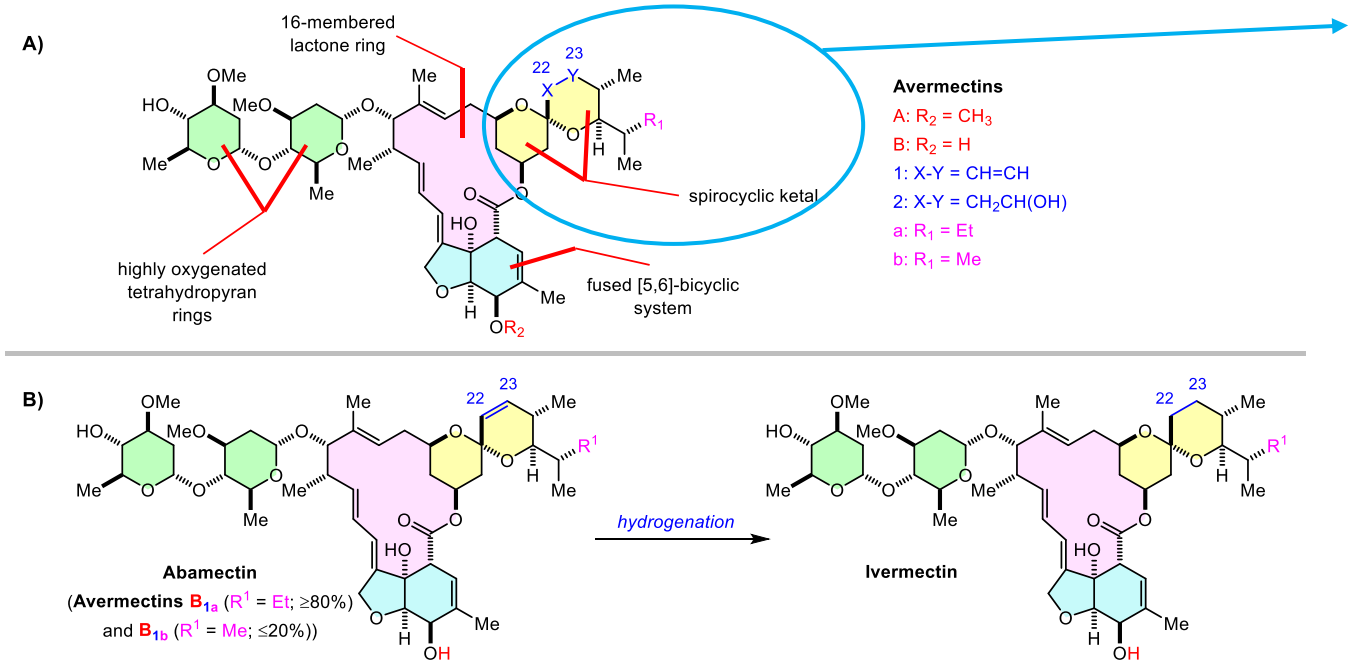
- **The inhibition of IMPA activity might provide a broader antiviral approach.**
- **IMPA can be a broad-spectrum antiviral target.**

Future outlook

Inositol monophosphatase (IMPA) as a broad-spectrum antiviral target

- Compare to the use of PIK inhibitors, which may face an issue with the complexity of PIK subtypes, targeting IMPA may provide a benefit of broader coverage, as it affects all types of PIPs.

Structure-based drug design target IMPA



Spirocyclic compounds

Antiviral activity

- *In vitro* assays
- Animals models

Anti-DENV-2 activity of spirocyclic compounds

Compounds	IC50 (µg/ml)	CC50 (µg/ml)	Selectivity index (CC50/IC50)
Compound 1	0.09	1.57	18.24
Compound 2	0.10	1.99	20.24
Compound 3	0.10	2.10	20.04
Compound 4	0.11	1.55	14.38
Compound 5	0.11	1.88	17.29
Compound 6	0.19	2.36	12.09
Compound 7	0.22	1.95	8.78
Compound 8	0.23	1.29	5.62
Compound 9	0.37	1.71	4.61
Compound 10	0.44	1.26	2.84
Compound 11	0.49	3.04	6.21
Compound 12	0.63	14.35	22.78
ivermectin	0.86	3.24	4.15
Compound 13	1.15	1.86	1.62
Compound 14	1.64	>2.26	>1.37
Compound 15	1.65	2.25	1.36
Compound 16	>0.65	2.65	<4.07
Compound 17	>1.62	1.62	<0.99
Compound 18	>1.76	1.76	<1.00

Antiviral activity of Spirocyclic compounds against various viruses

Compounds	Viruses	Cells	Antiviral activity		
			(IC_{50}, µg/ml)	(CC_{50}, µg/ml)	Selectivity index (SI)
Ivermectin	ZIKV	imHC	0.71	3.14	4.42
	CHIKV	Vero	1.12	5.91	5.28
	IAV (H1N1 2009)		1.77	3.13	1.77
	RSV	HEp-2	0.86	3.21	3.73
Compound 2	ZIKV	imHC	0.40	7.68	19.20
	CHIKV	Vero	0.79	> 9.63	> 12.19
	IAV (H1N1 2009)		1.67	> 9.63	> 5.77
	SARS2		< 0.73	> 23.40	> 32.05
Compound 4	ZIKV	imHC	0.20	6.82	34.10
	CHIKV	Vero	0.49	> 10.26	> 20.94
	IAV (H1N1 2009)		2.76	> 10.26	> 3.72
	SARS2		< 0.82	> 26.16	> 32.63
	RSV	HEp-2	1.19	13.95	11.72

Spirocyclic compounds: lower IC50 (increase inhibitory effects), lower cytotoxicity, compared to IVM

Next: Animal study (pharmacokinetics)

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Thank you for attention

Q&A