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Rotavirus NSP4-mediated increase in intracellular calcium results in increased levels of the pro-inflammatory eicosanoid, prostaglandin E_2, during infection

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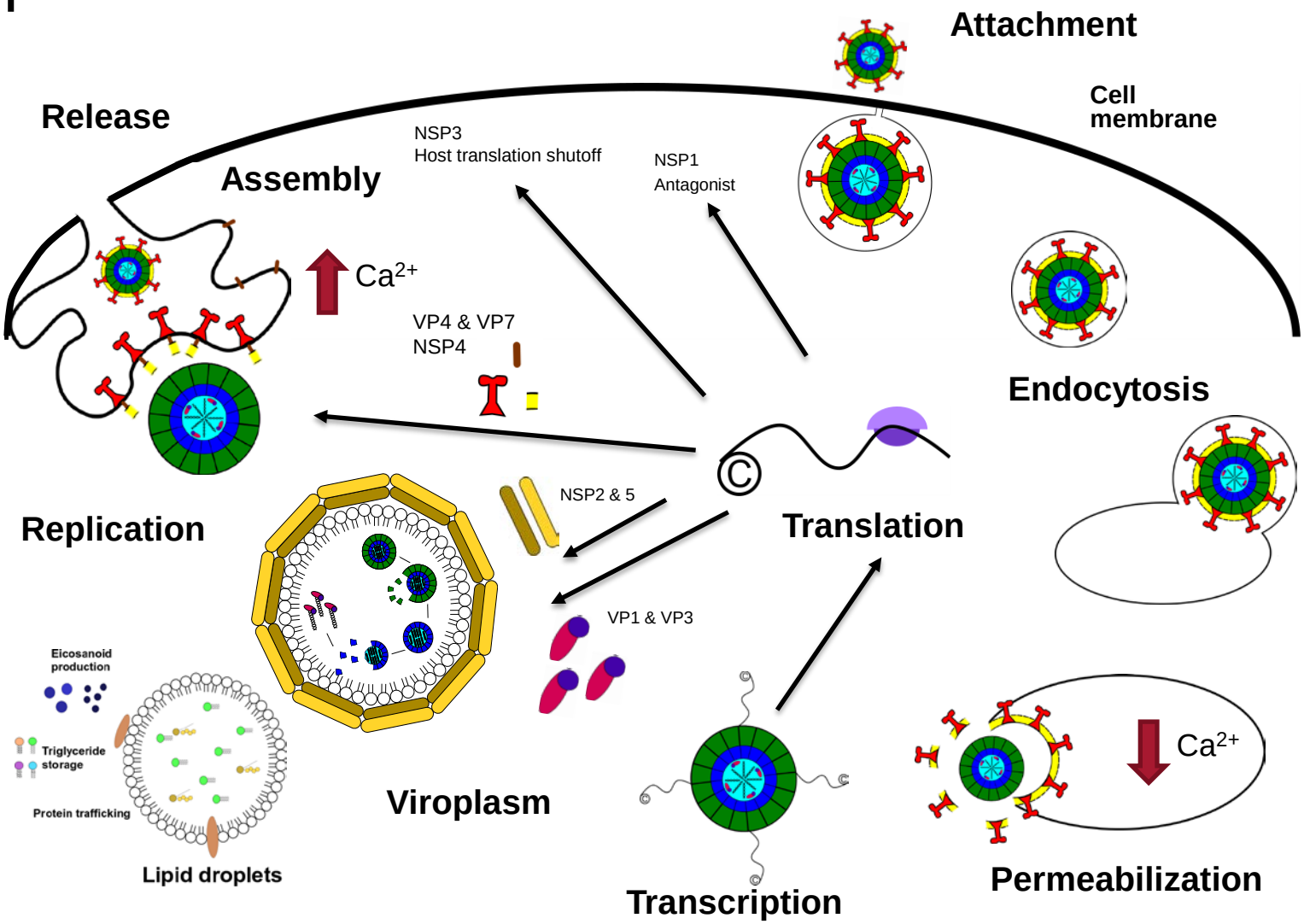
Replication: Overview



(Desselberger U. Virus Res. 2014;
Olzmann JA and Carvalho P. Nat Rev Mol Cell Biol. 2019)

Fatty acids are essential for rotavirus replication

- Supplementation of saturated fatty acids enhanced rotavirus infectivity in LLC-MK2 cells
- Compounds blocking or disrupting lipid droplets inhibited the formation of viroplasms and subsequently rotavirus replication
- The down regulation of fatty acid synthesis also inhibits rotavirus replication

(Superti F, *et al.* Comp Immunol Microbiol Infect Dis. 1995; Cheung W, *et al*. J Virol. 2010; Gaunt ER, *et al*. J Gen Virol. 2013)

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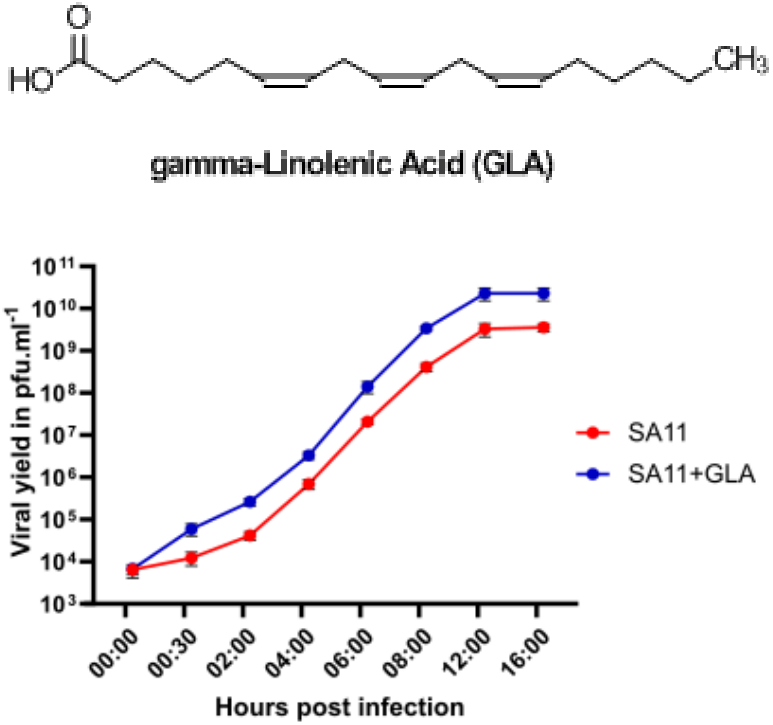
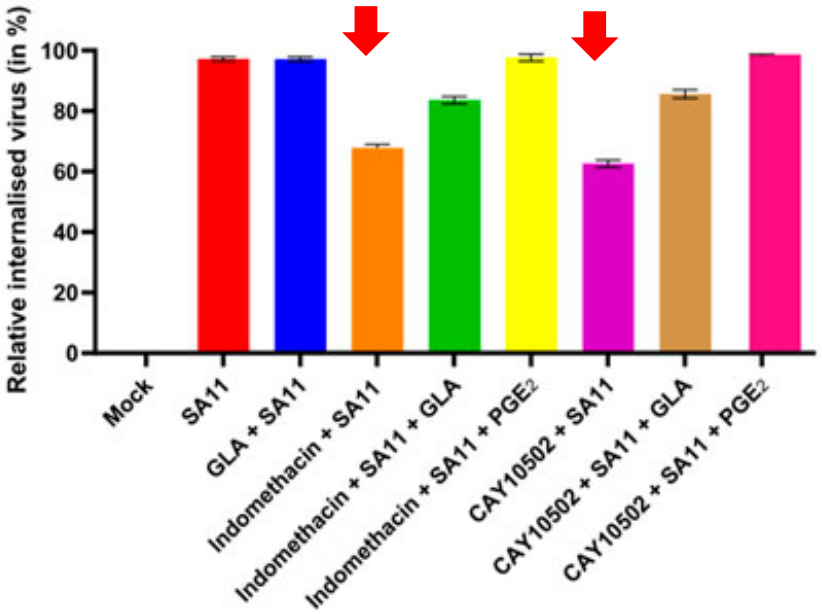
γ-Linolenic Acid (18:3) Enhances Viral Replication

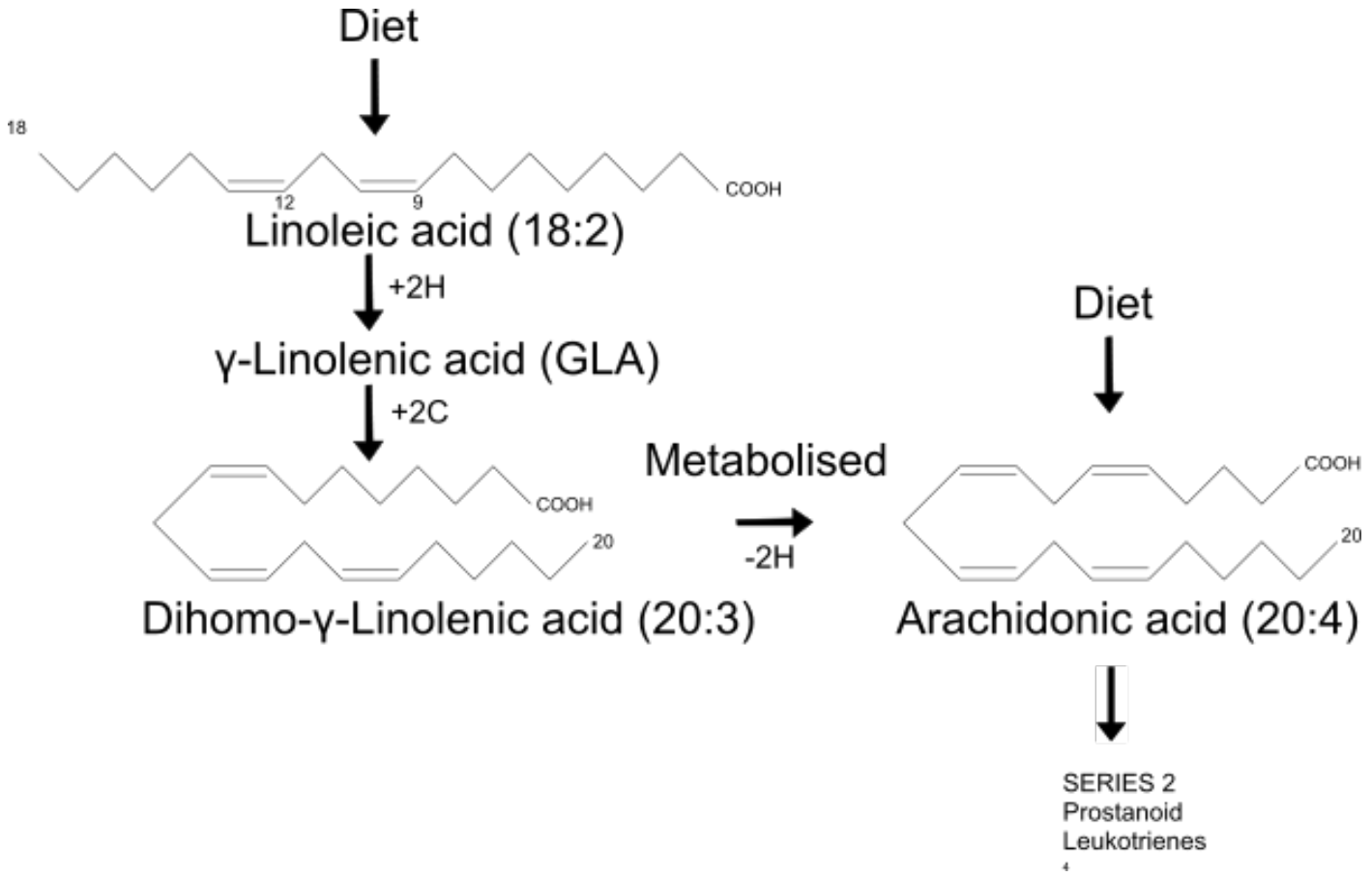




(Sander WJ, *et al*. Front Physiol. 2022)

Eicosanoid biosynthesis

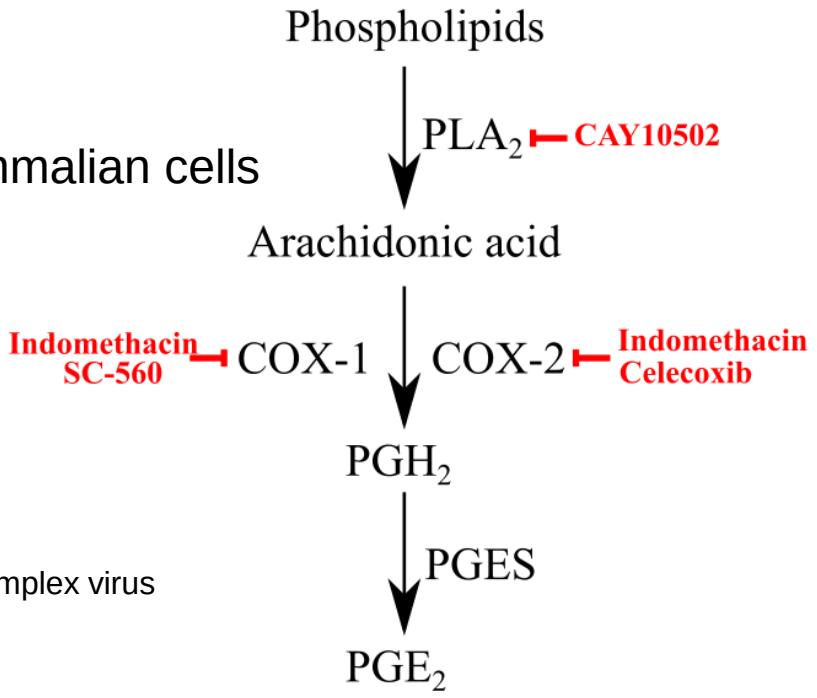
- signaling molecules derived from the oxidation of arachidonic acid



(Khanapure SP, *et al*. Curr Top Med Chem. 2007)

Prostaglandin E_2

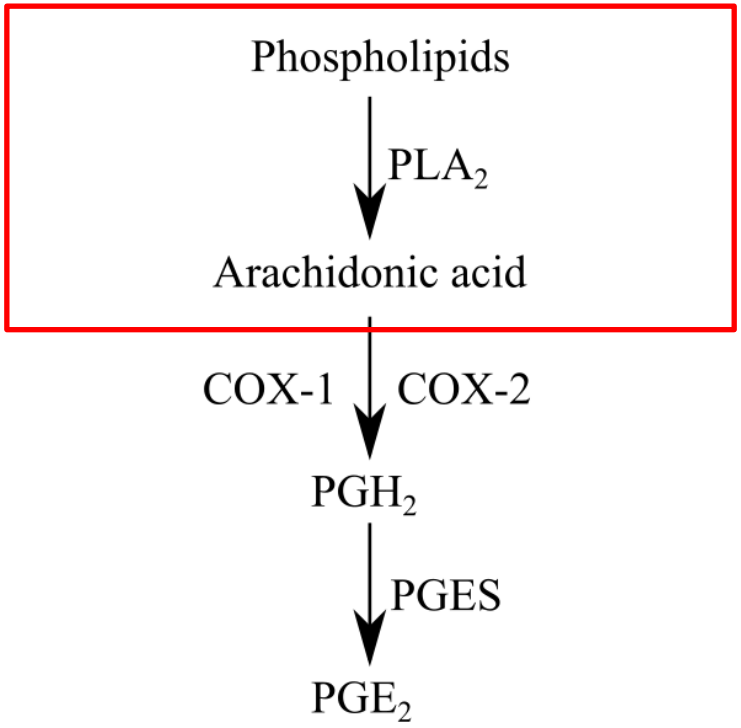
- Major eicosanoid produced by nearly all mammalian cells
- Physiological roles
  - Modulate inflammation and the immune response
  - Synthesized and stored within lipid droplets
- Modulates several viral infection including:
  - Human immunodeficiency virus, influenza virus and herpes simplex virus



(Park JY, *et al*. Clin Immunol. 2006; Sander WJ, *et al*. Front Physiol. 2017)

PGE_2 and Calcium

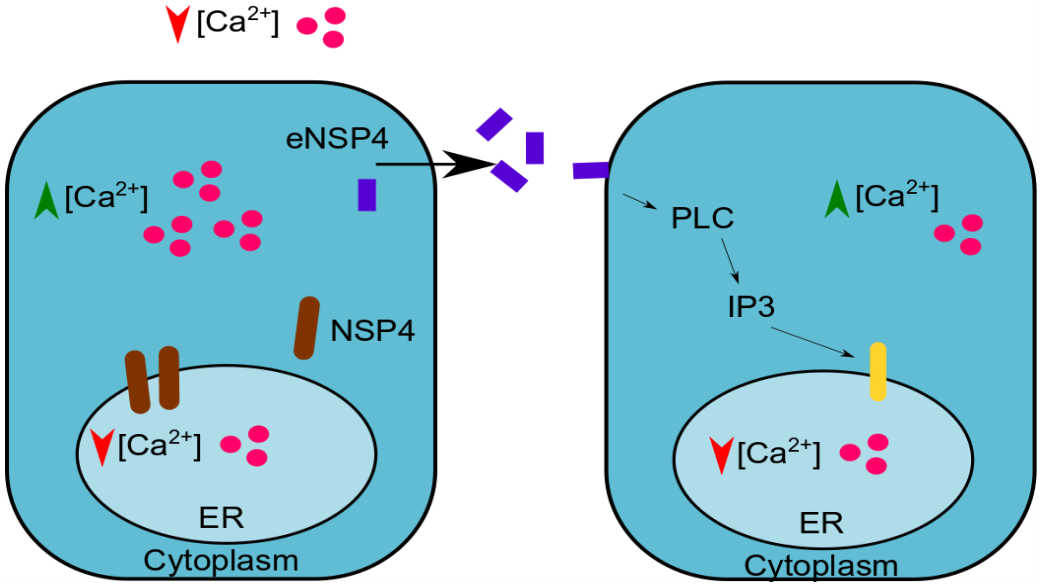
- PGE_2 biosynthesis is dependent on the activation of Phospholipase A_2
  - PLA_2 is activated in increased Ca^{2+} levels
- Elevated Ca^{2+} is crucial for rotavirus replication and morphogenesis



(Gijón MA and Leslie CC. J Leukoc Biol. 1999; Ruiz MC, *et al*. Cell Calcium. 2000)

Calcium and NSP4

- Contains a viroporin domain that selectively conducts Ca^{2+}
- Two forms of NSP4
  - Expressed NSP4
    - Responsible for the release of intracellular calcium stores
  - Secreted NSP4 (eNSP4)
    - Induces release of intracellular calcium stores from neighbouring cells via PLC-IP3 signalling



(Berkova Z, *et* al. Cell Calcium. 2003; Didsbury A, *et al*. Virol J. 2011; Pham T, *et al*. Sci Rep. 2017)

PGE_2 and Rotavirus

- Increased PGE_2 found in stool of RV-infected children
  - Treatment with aspirin, decreased severity and duration of diarrhea
- Malnourished piglets have higher levels of PGE_2 during RV infection

- Co-localisation between viroplasms (NSP2 and NSP5), PGE_2
- Rotavirus increases PGE_2 in a time dependant-manner
- Inhibition of PGE_2 biosynthesis affects RV attachment and internalisation

frontiers in Physiology

ORIGINAL RESEARCH
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Rotavirus-Mediated Prostaglandin E_2 Production in MA104 Cells Promotes Virus Attachment and Internalisation, Resulting in an Increased Viral Load

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(Rossen JW, *et al*. J Virol. 2004; Yamashiro Y, *et al*. J Pediatr Gastroenterol Nutr. 1989; Zijlstra RT, *et al*. J Nutr. 1999; Sander WJ, *et al*. Front Physiol. 2022)

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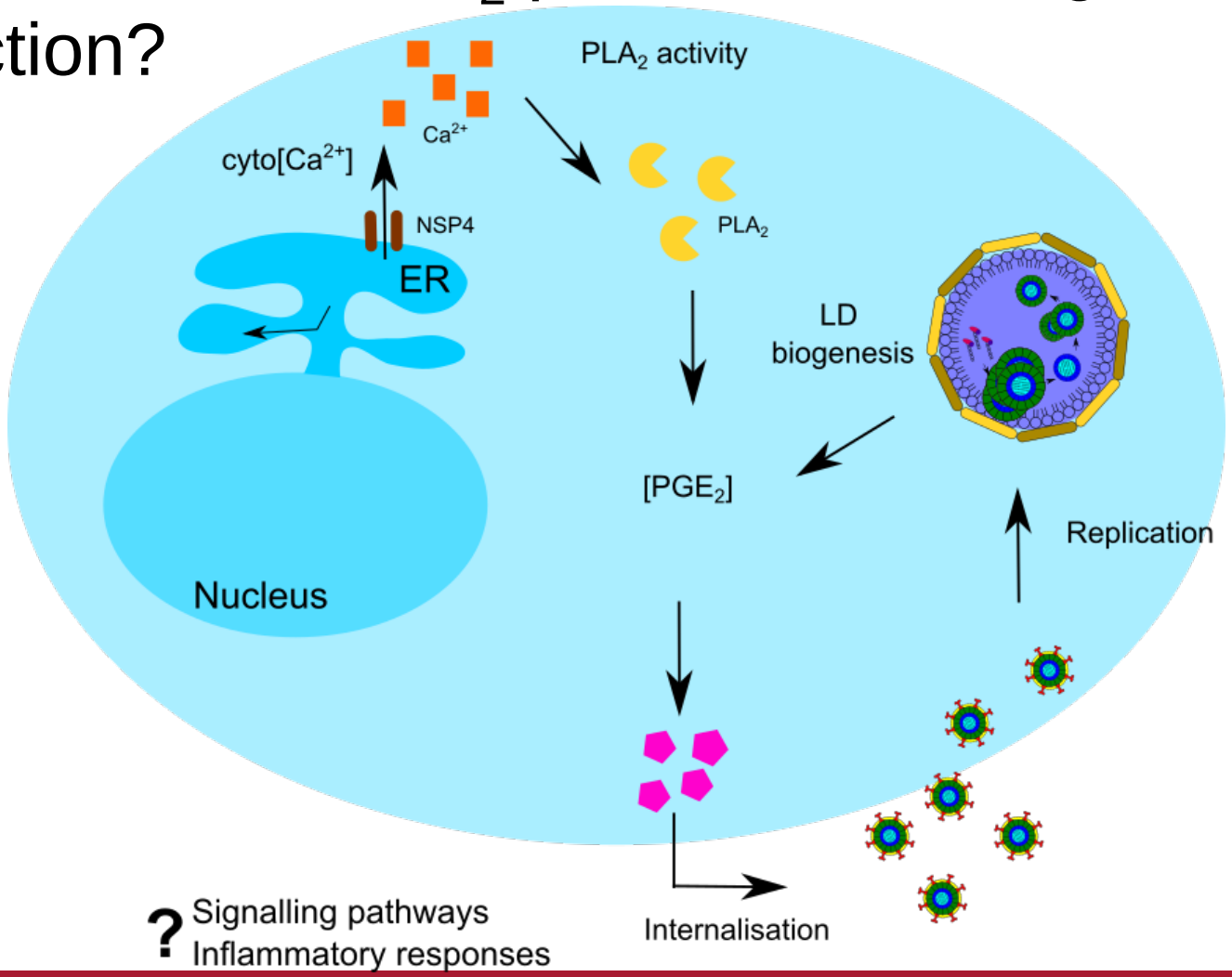
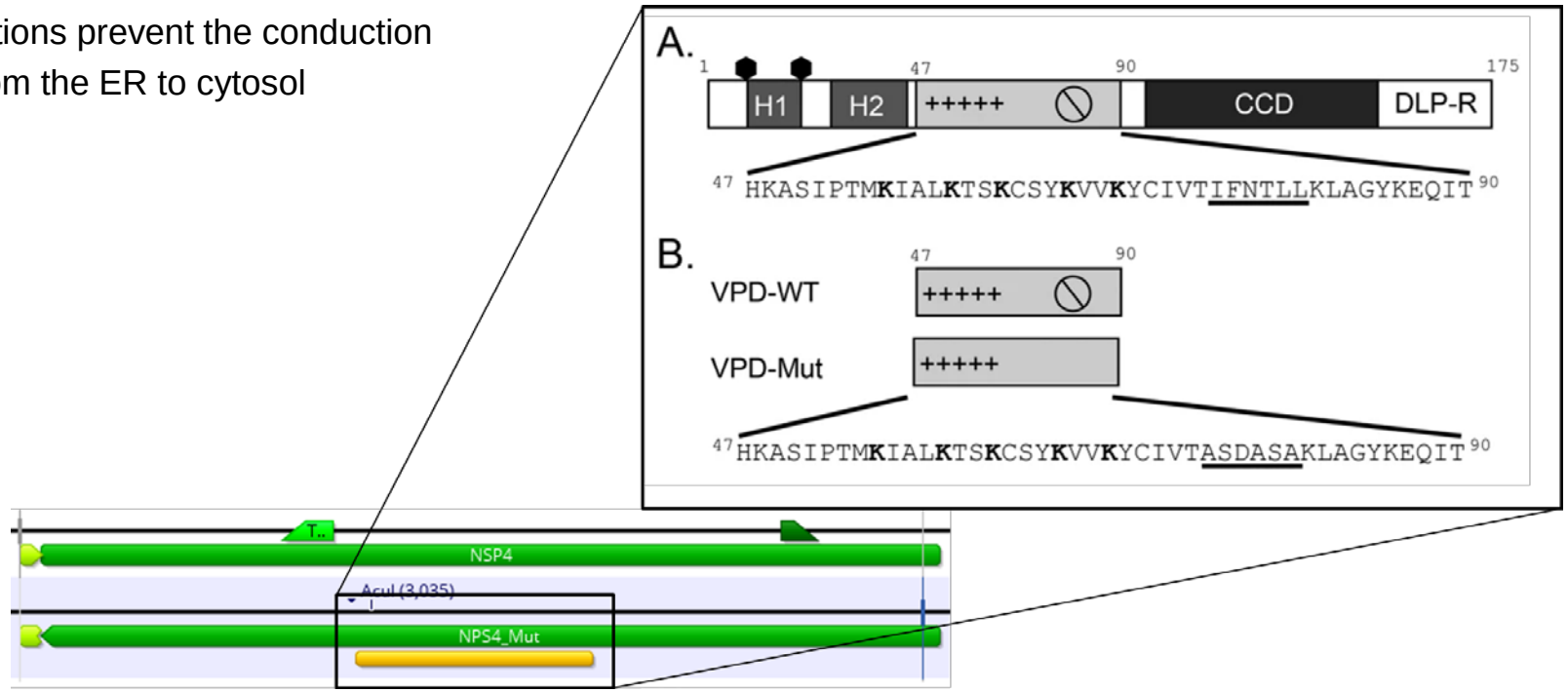
Does NSP4-induced changes in intracellular calcium effect PGE_2 production during rotavirus infection?



Mutations introduced coding sequence of NSP4

- Lack of viroporin domain, decreases rotaviral yield and pathogenesis
  - Mutations prevent the conduction of Ca^{2+} from the ER to cytosol

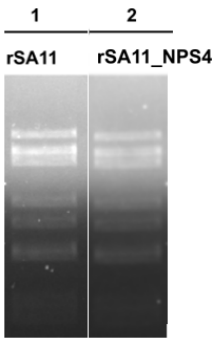


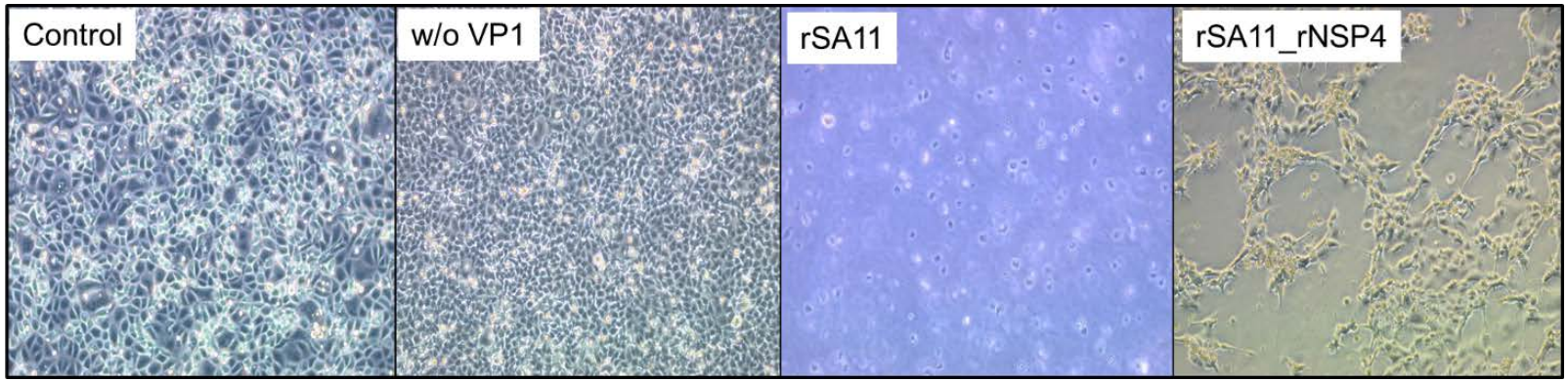
(Pham T, *et al*. Sci Rep. 2017 Mar)

Rescue of rSA11_rNSP4Mut

- Evaluation of cell death during viral passages

		Day 1	Day 2	Day 3
P1	MA104	-	-	-
	w/o pT7-VP1SA11	-	-	-
	rSA11	-	+	+++
P3	rSA11_NSP4	-	+/-	+



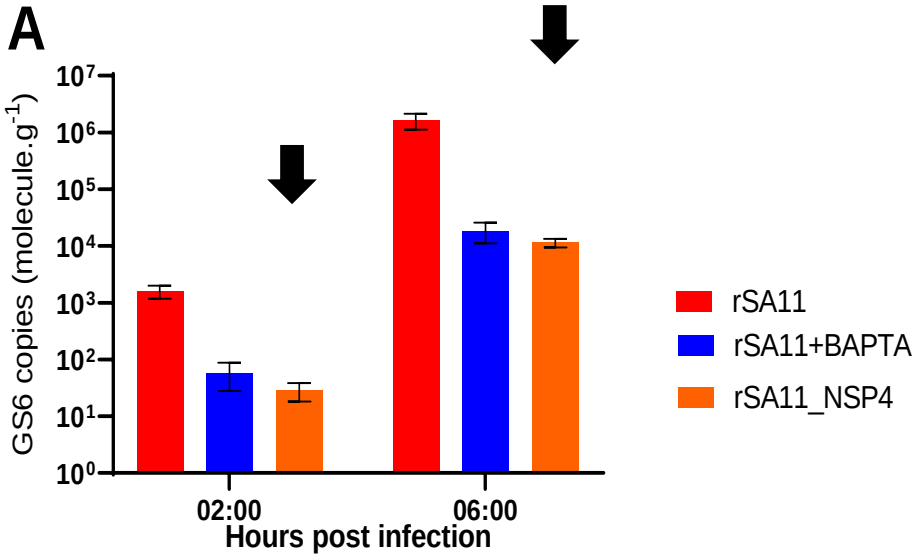


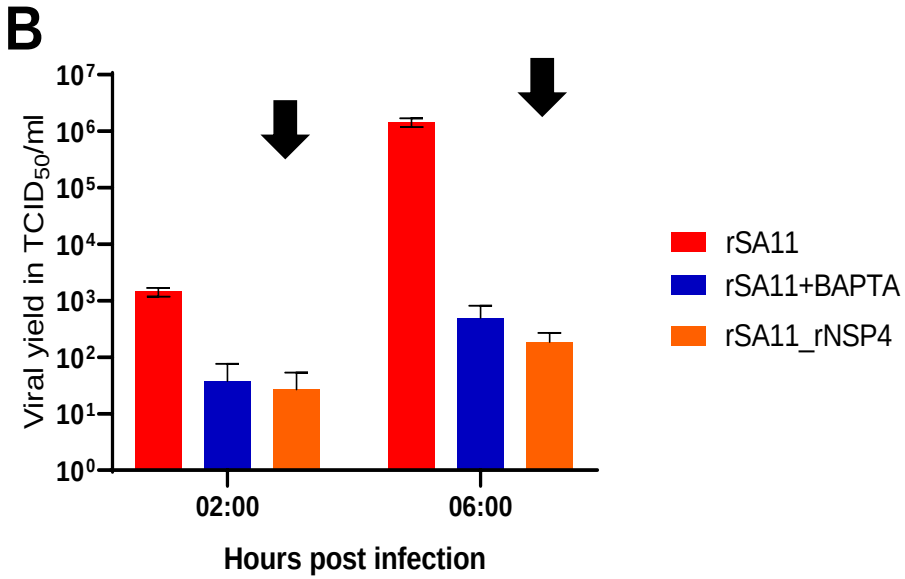
- Confirmed using NGS analysis

1. Mutations in the viroporin domain decreases copy number of GS6 and viral progeny

Infect for 6 hours → Harvest supernatant → $TCID_{50}$ / RT-qPCR

BAPTA





2. Mutations in the viroporin domain modifies intracellular calcium concentration during infection

Load cells with 10µM Fura-dextran

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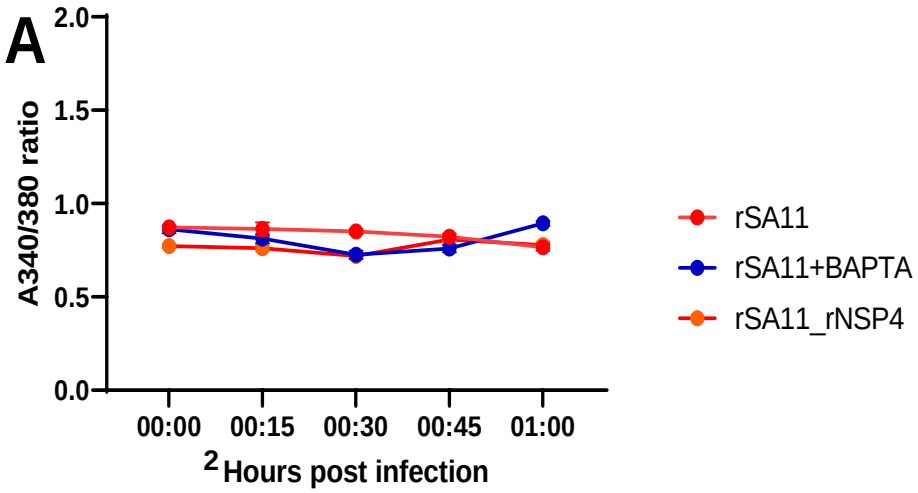
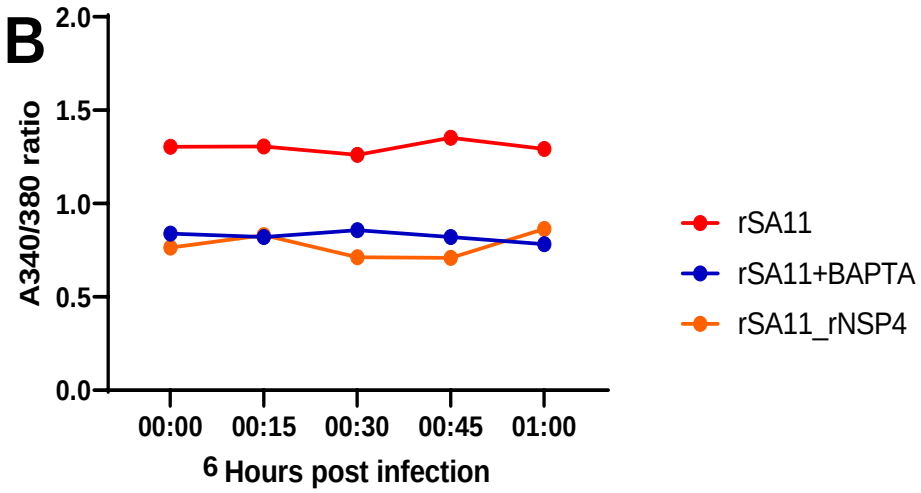
Infect with MOI of 5

Read at 2 and 6 hours post infection every 15 min @ 340 and 380 nm

Calculate 340/380 ratio





3. Lower levels of intracellular calcium decreases the activity of $cPLA_2$

Infect for 6 hours → Lyse cells with hypotonic non-detergent buffer → Incubate with:
- Bromoenol lactone
- Thioetheramide-PC

→ Assay

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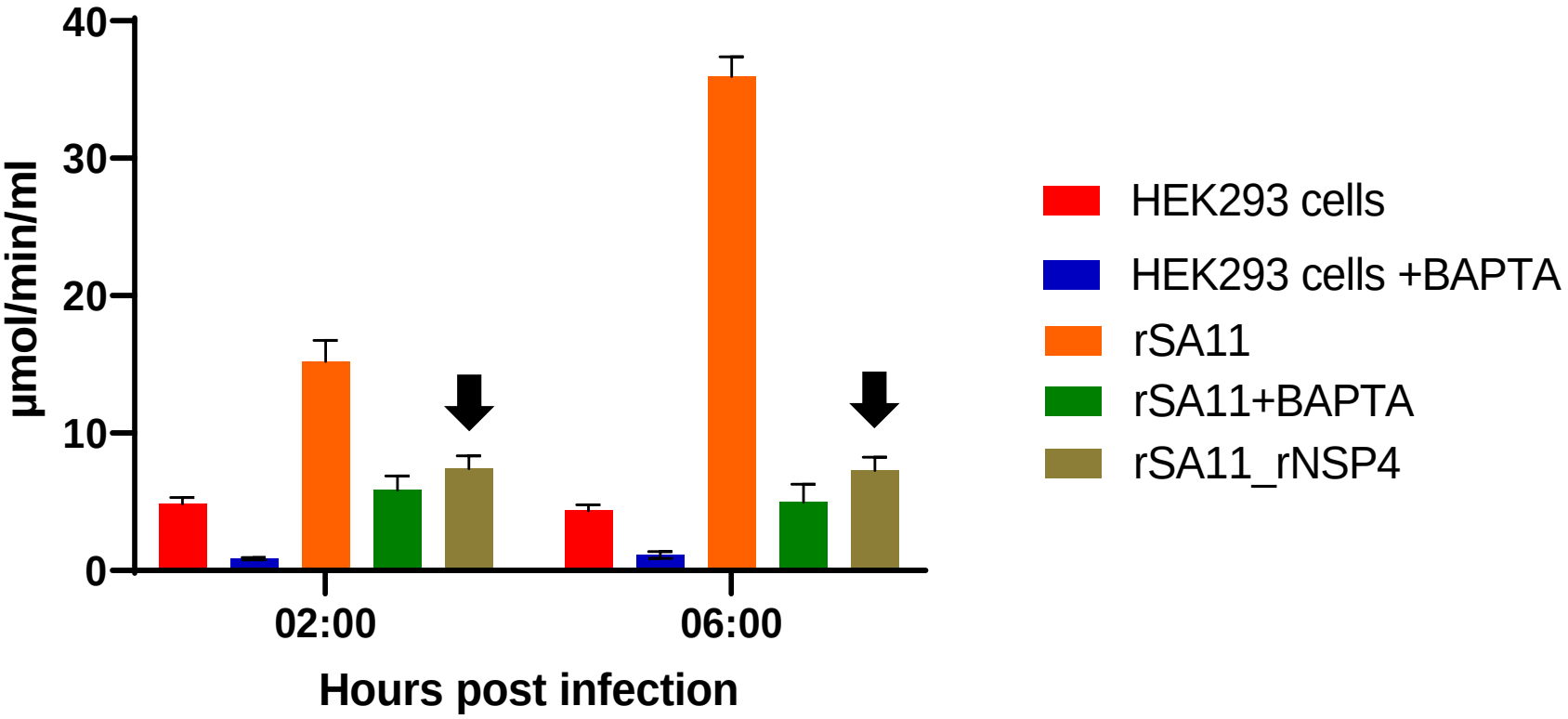
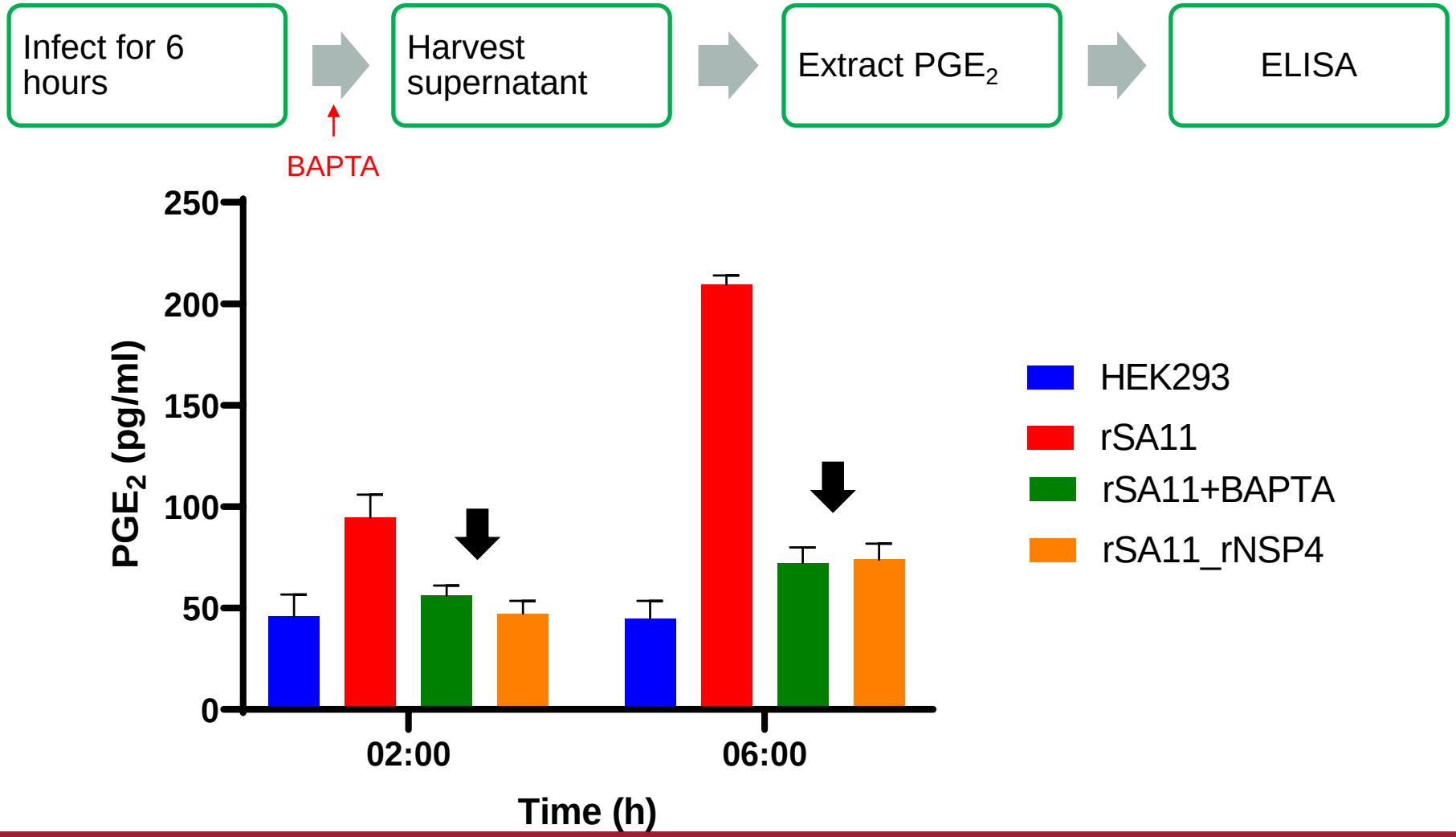
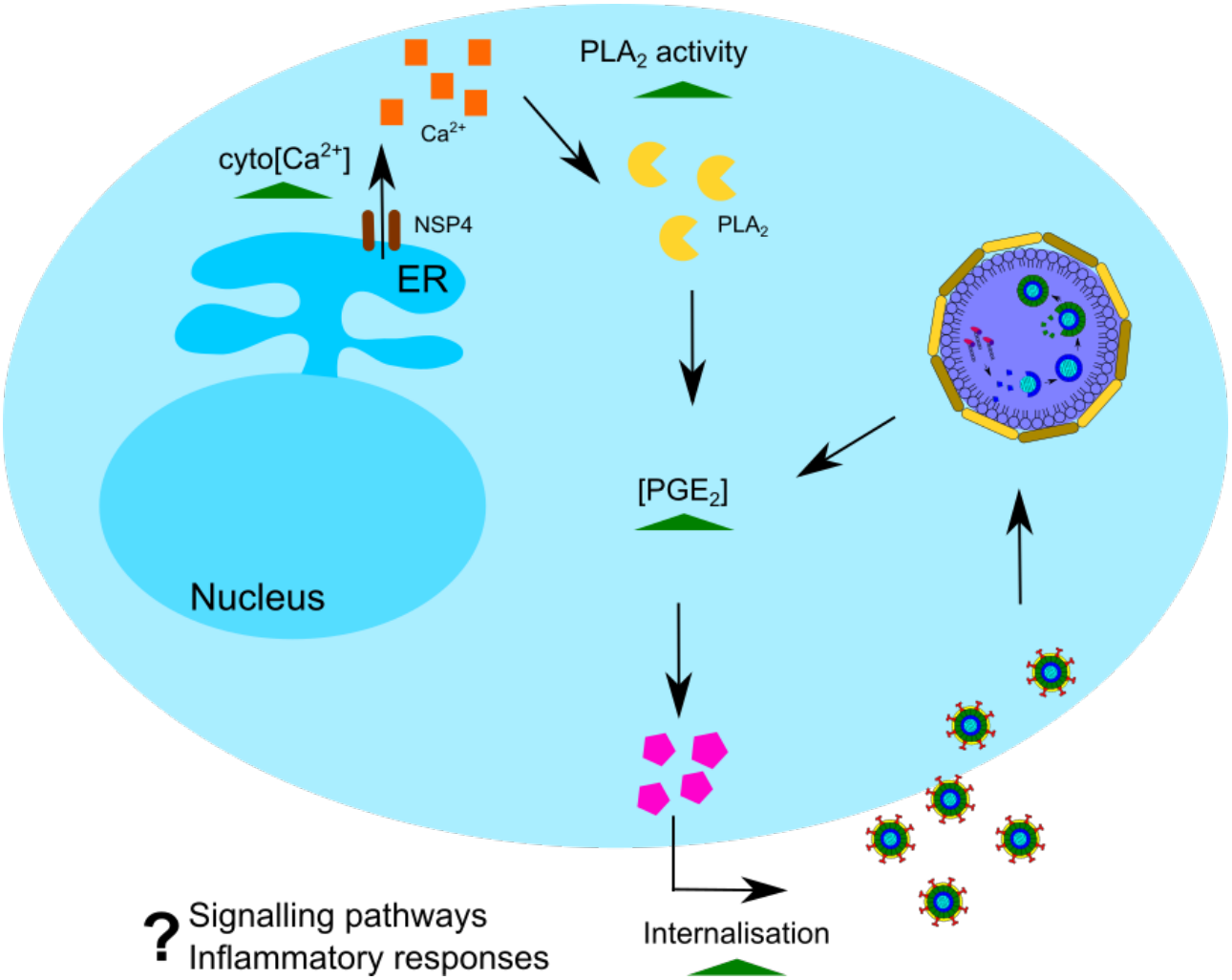


4. Decreases in the activity of $cPLA_2$ lowers the amount of PGE_2 produced during infection



Conclusions

- Increased intracellular calcium levels during RV infection increases the activity of PLA_2 subsequently increasing PGE_2 production
- The PGE_2 biosynthetic pathway is therefore a promising target for anti-viral treatment



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