Serving its own schemes: The manipulation of host cell signaling, stress responses and metabolism by HCMV

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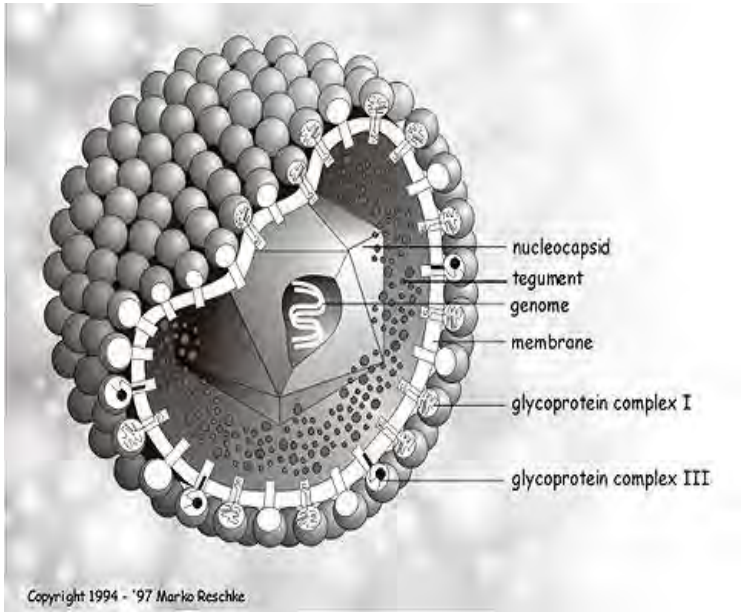
Penn Herpesvirus Pathogenesis and Cancer Symposium

Human Cytomegalovirus (HCMV)



- **The largest human herpesvirus.**
- **~230,000 bp linear ds DNA encoding at least 200 proteins which are temporally expresses: Immediate Early, Early and Late.**
- **Very long lytic cycle and can establish a latent/persistent state.**
- **Most common congenital viral infection in humans.**
- **Immunocompromised patients are at risk for developing HCMV disease.**
- **May be a subtle cofactor in many maladies, e.g. atherosclerosis and cancer.**
- **Many molecular and cell biological effects wrought by cancer are also wrought by HCMV infection.**

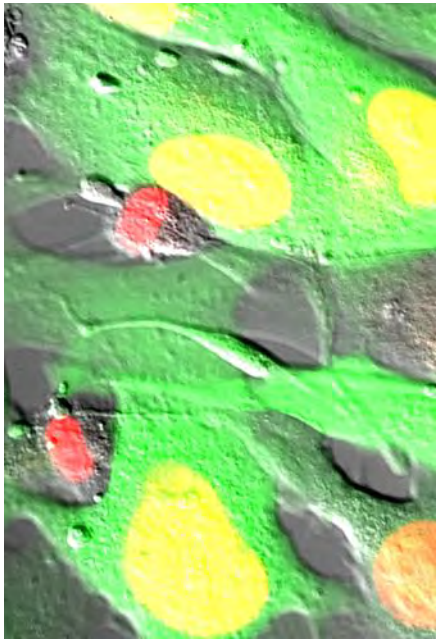
HCMV infection is stressful

During its slow lytic progression HCMV causes stress and induces many stress responses.

Thus it has evolved means to manipulate stress responses to its advantage in order to maintain host cells in a productive state that will support the infection.

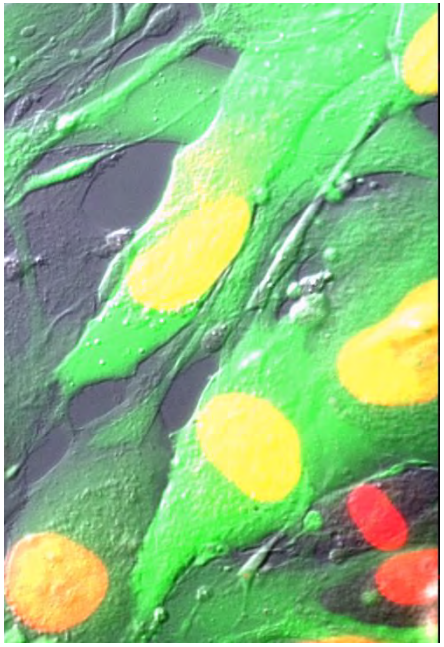
HCMV circumvents the effects of many stress responses

Normal



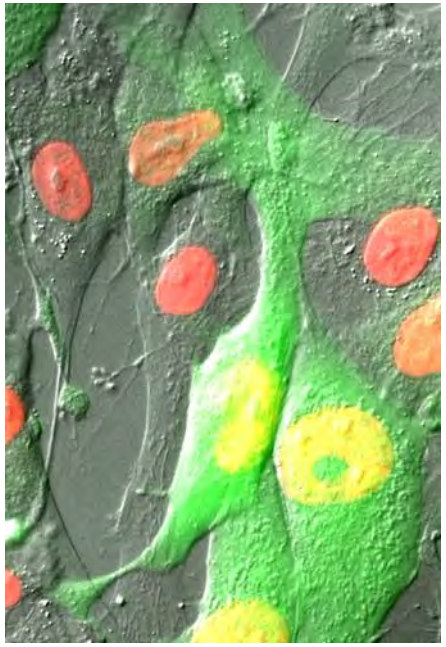
$+ O_2$ + Glucose

Hypoxia



$- O_2$ + Glucose

-Glucose



$+ O_2$ - Glucose

3-5 fold lowering of virus yield in hypoxia

Low yield of viruses but Infected cells remain viable

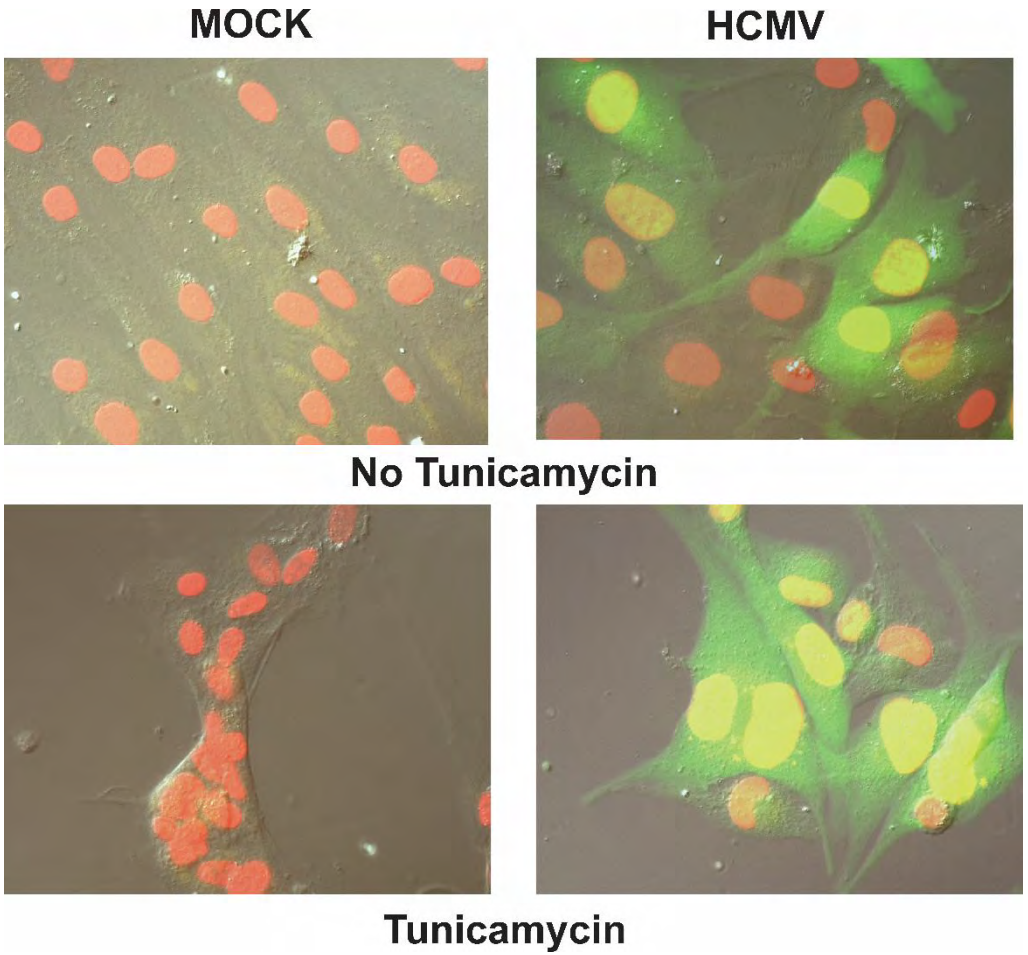
HCMV circumvents many stress responses

HCMV circumvents stress conditions that would normally inhibit replication, these include:

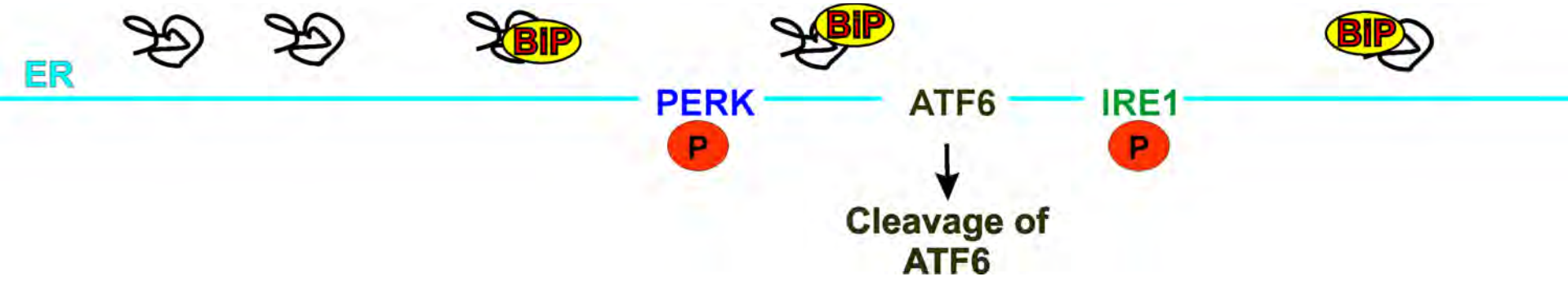
Amino acid deprivation
Nutrient deprivation
ATP depletion
ER stress
Hypoxia
ROS

HCMV circumvents the effects of many stress responses

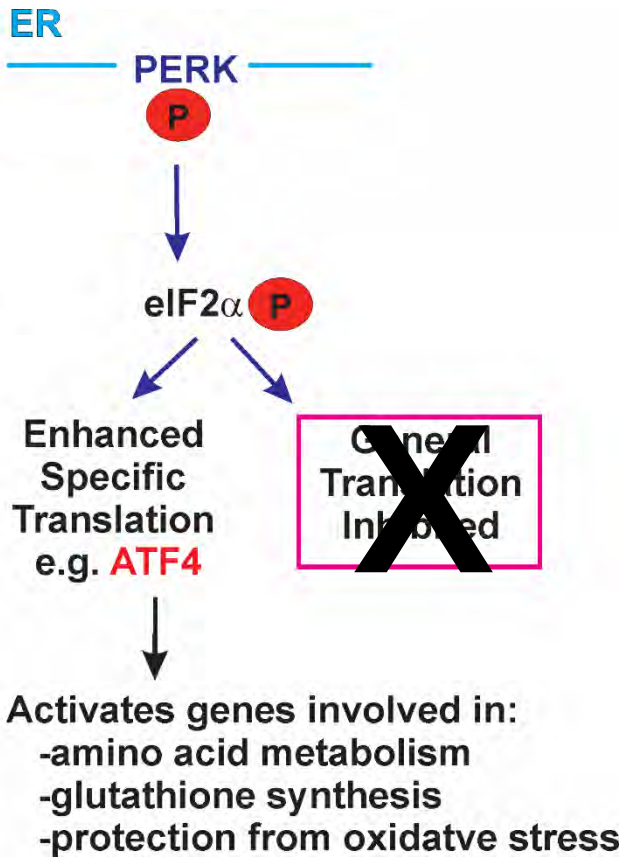
Unfolded Protein Response (UPR) / ER Stress

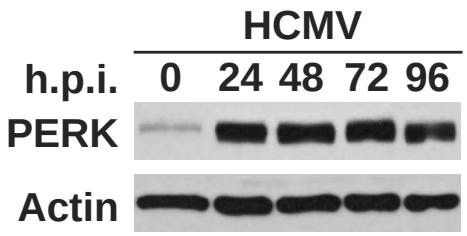


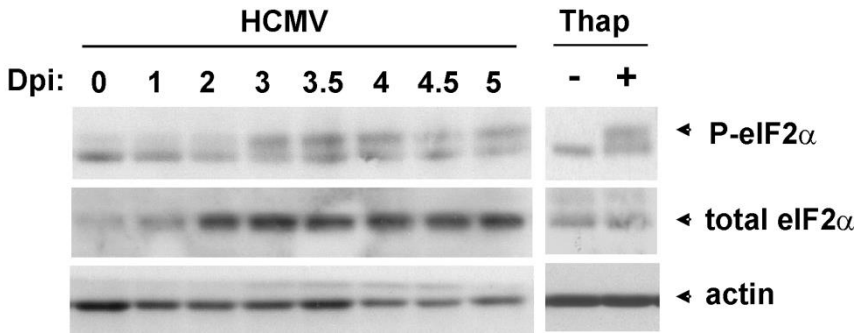
The UPR

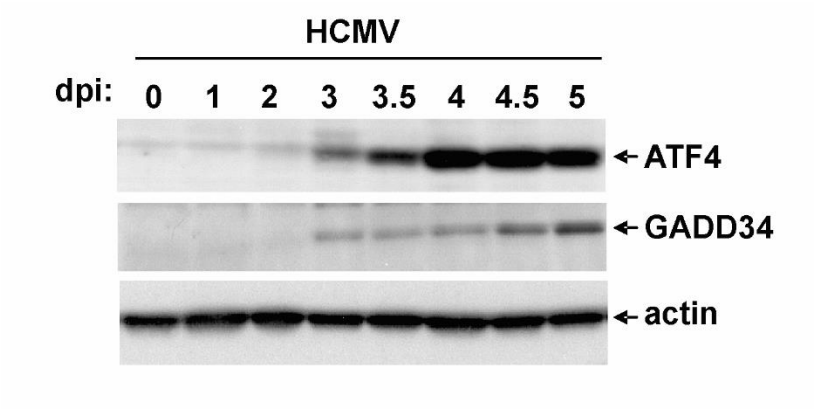


Functions of activated PERK

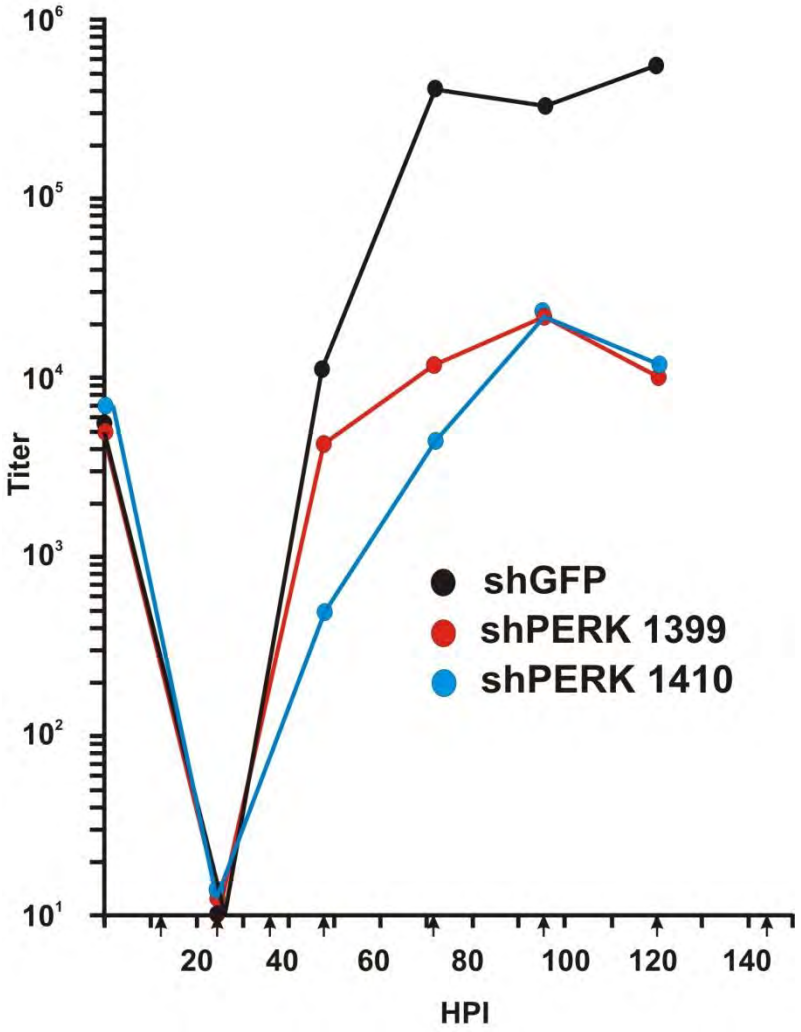








PERK is required for HCMV growth



PERK is a critical regulator in lipid metabolism and adipocyte differentiation

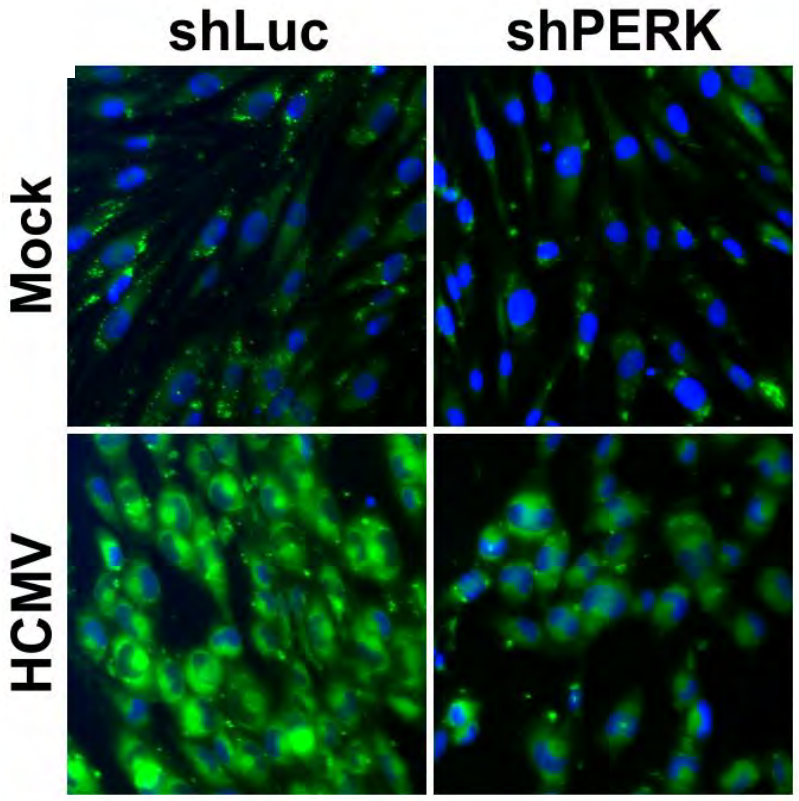
Diehl and colleagues (PNAS 105:16314) have shown:

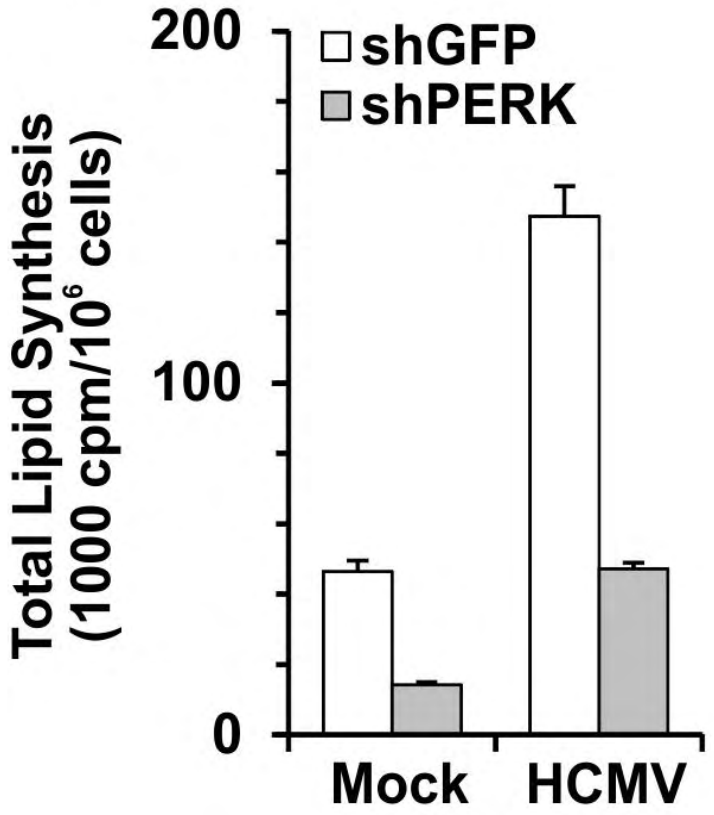
PERK regulates lipogenesis during mouse mammary gland development and adipocyte differentiation

PERK regulates SREBP1 activation. SREBP1 is a transcription factor which activates the promoters of genes encoding lypogenic enzymes.

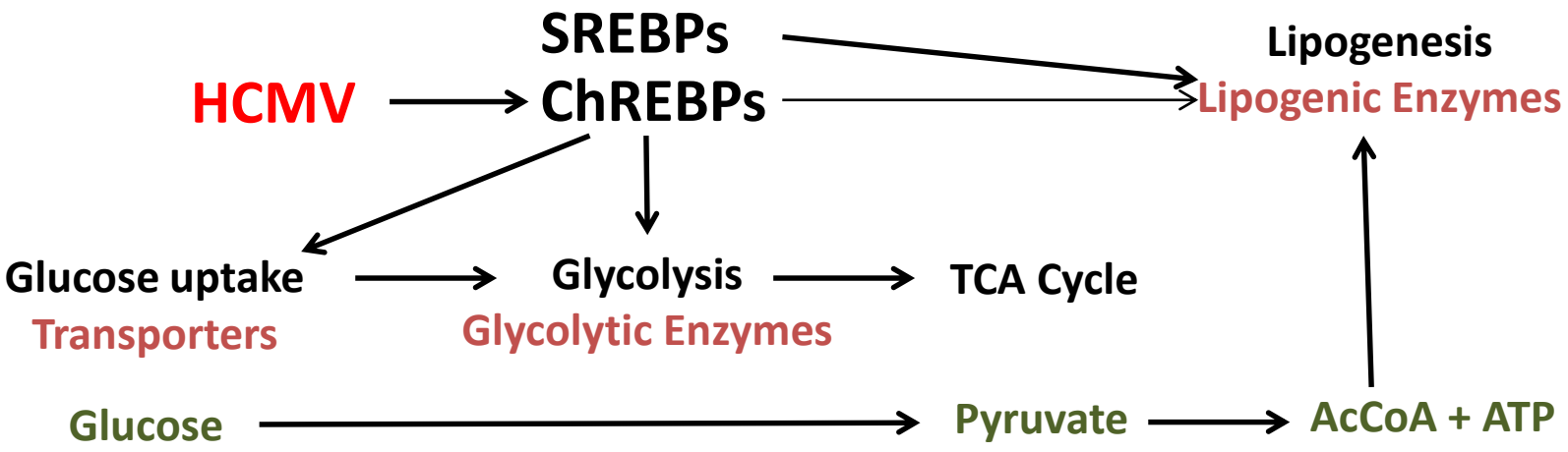
PERK mutation disrupts adipocyte differentiation.

Depletion of PERK inhibits lipogenesis in HCMV Infected cells

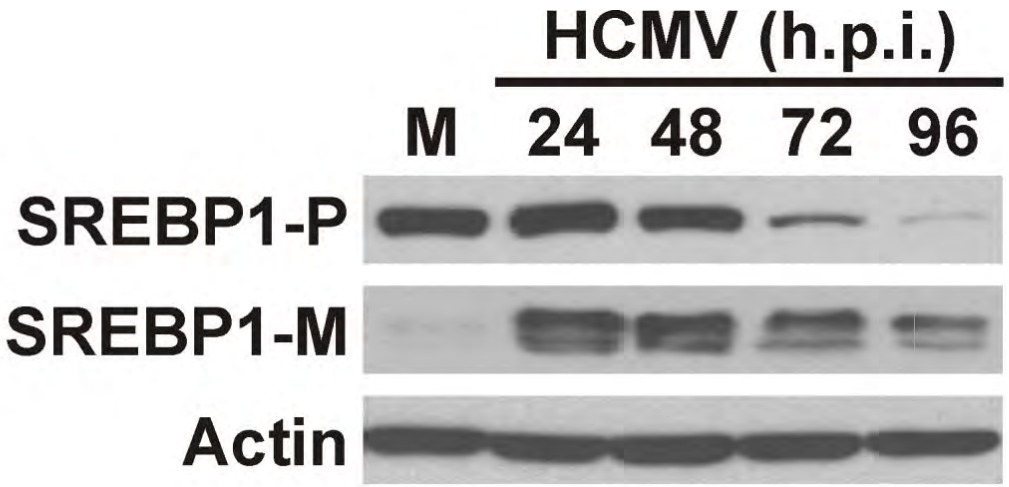




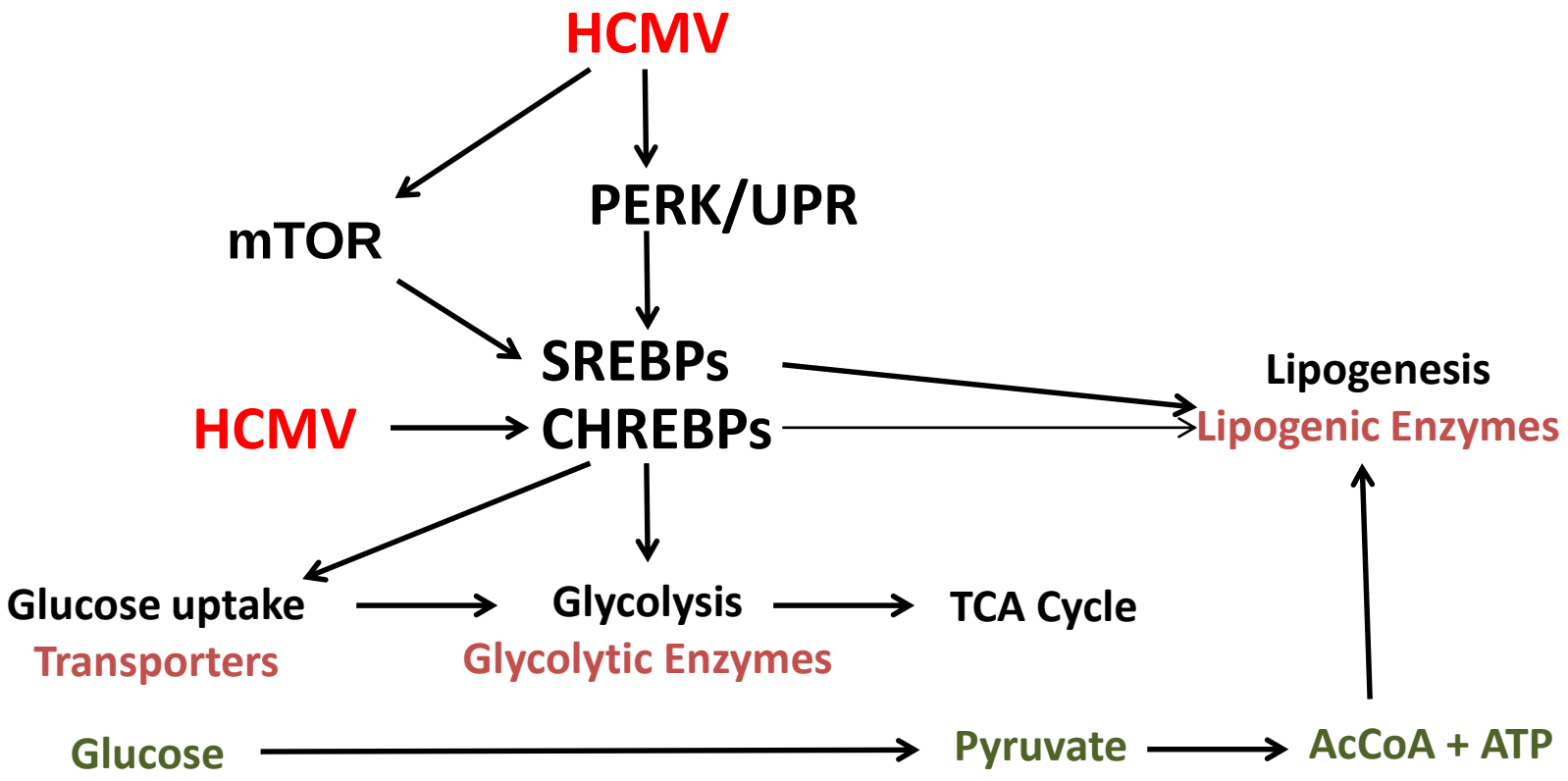
HCMV increases lipogenesis by activation of transcription factors



HCMV increases PERK to facilitate SREBP1 cleavage and activation



HCMV coordinates effects on mTOR, the UPR, SREBPs and CHREBPs to increase lipogenesis



Summary I

HCMV manipulated each arm of the UPR.

In each case HCMV inhibits aspects of the stress response that would be deleterious to infection while maintaining or activating aspects that benefit infection.

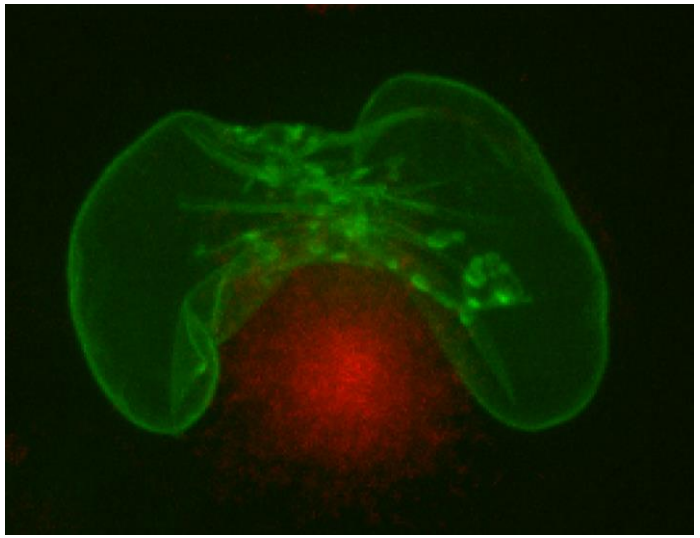
HCMV's effect on PERK is particularly important for the activation of lipogenesis which is critical for the success of the HCMV infection.

A Question

Why is so much lipogenesis needed in HCMV infected cells?

Increased lipogenesis is needed to supply the membranes required by infected cells

- infected cells and nuclei enlarge. $A=4\pi r^2$

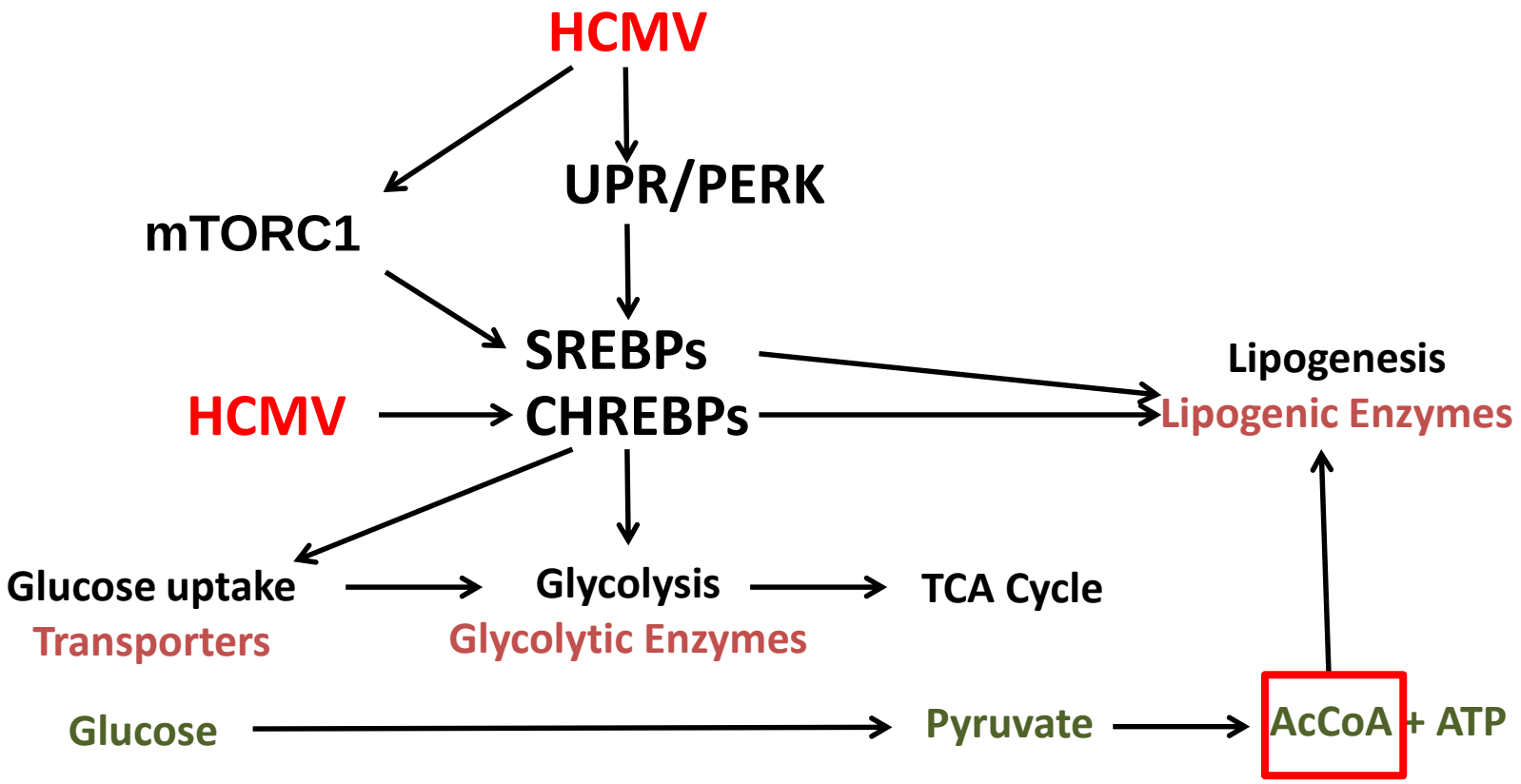


- the assembly compartment is made up of many small membranous vesicles derived from secretory organelles.

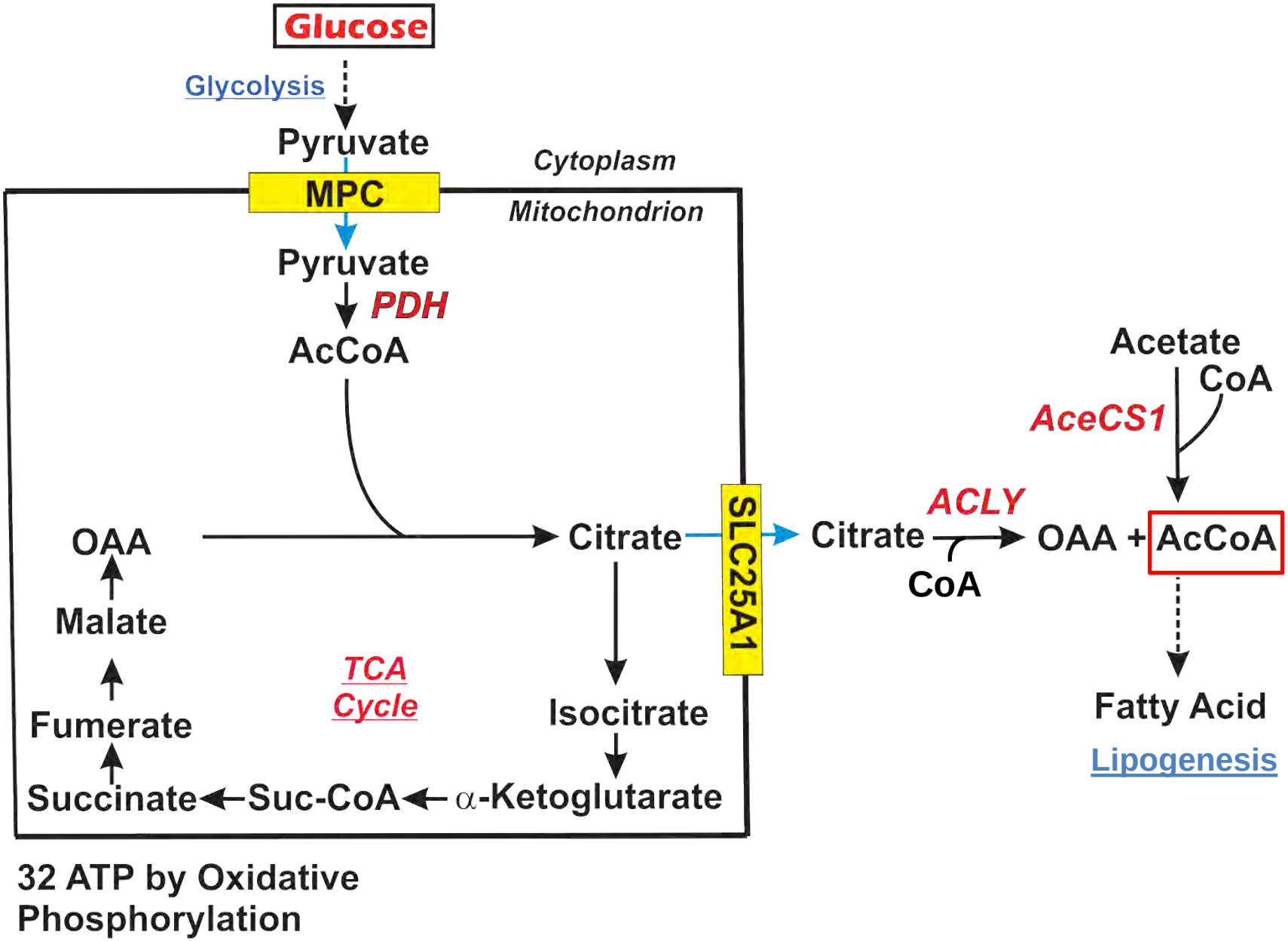
- virion envelopes.

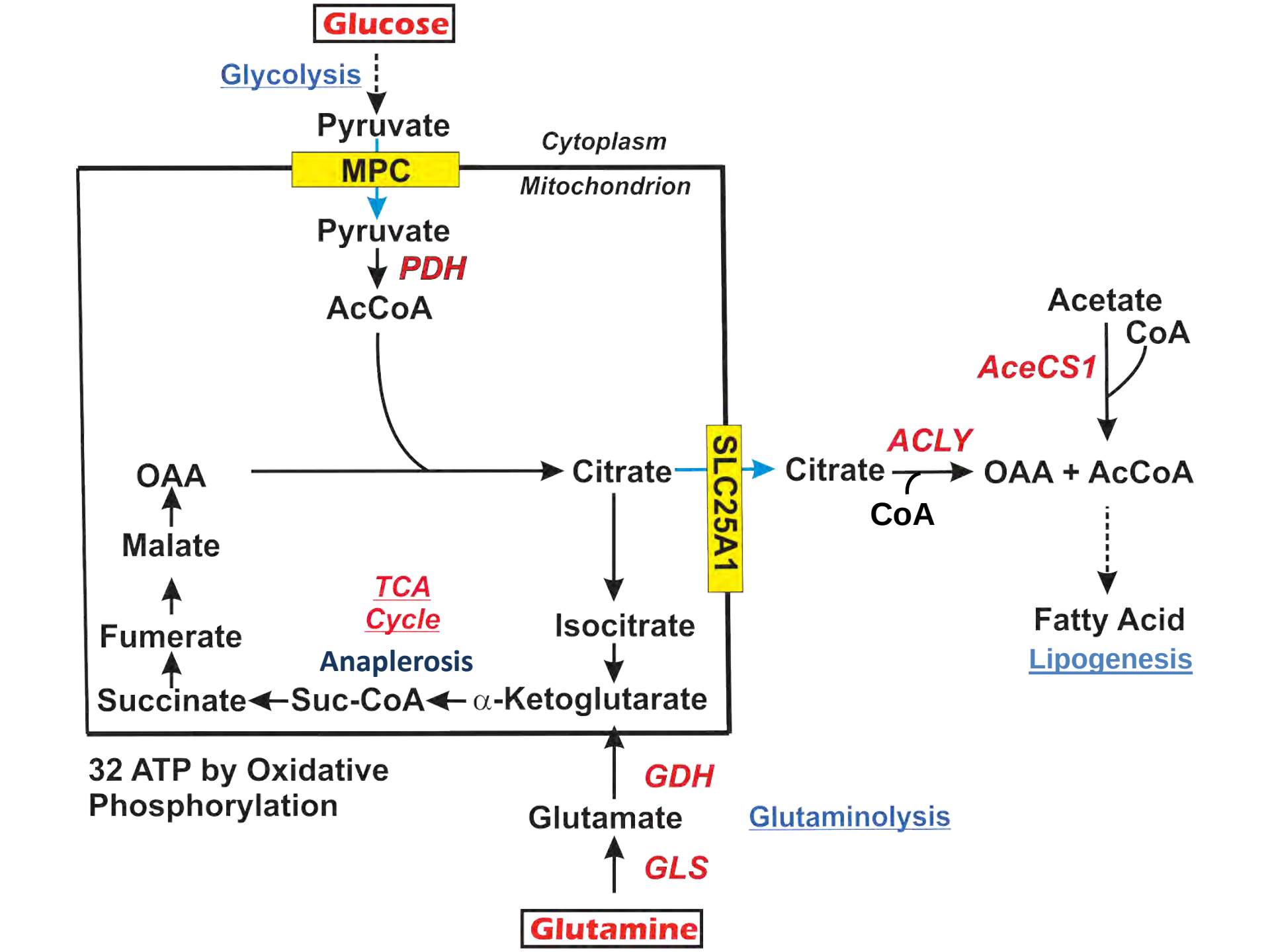
Without increased lipogenesis you get none of these.

HCMV coordinates effects on mTOR, the UPR, SREBPs and CHREBPs to increase lipogenesis



AcCoA levels are dramatically increased in HCMV-infected cells compared to actively growing cells.


Glucose
Glycolysis
Pyruvate
MPC
Cytoplasm
Mitochondrion
Pyruvate
PDH
AcCoA
OAA
Citrate
SLC25A1
Citrate
ACLY
CoA
OAA + AcCoA
Acetate
CoA
AceCS1
Malate
TCA
Cycle
Isocitrate
Fumerate
Fatty Acid
Lipogenesis
Succinate
Suc-CoA
α-Ketoglutarate
32 ATP by Oxidative
Phosphorylation


Glucose
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OAA + AcCoA
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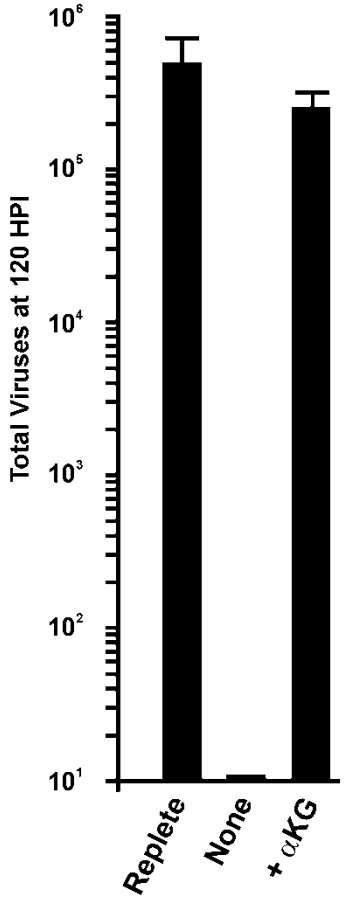
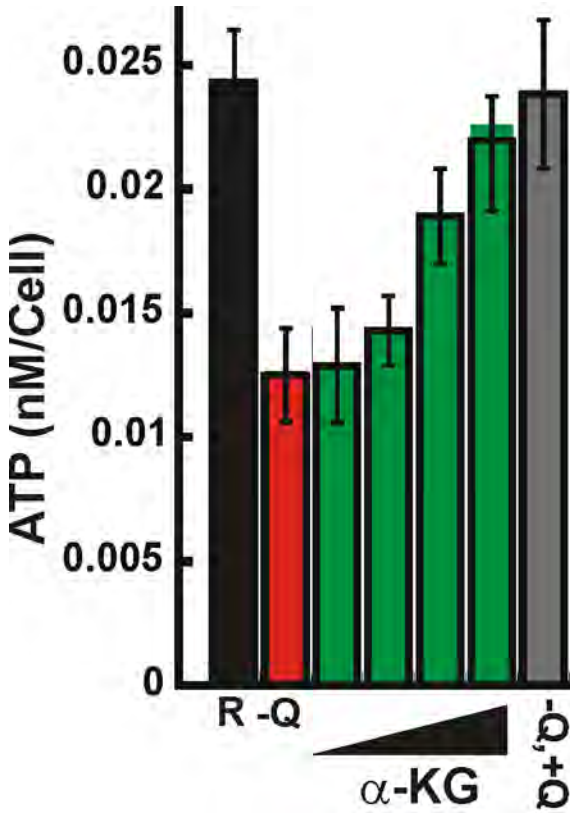
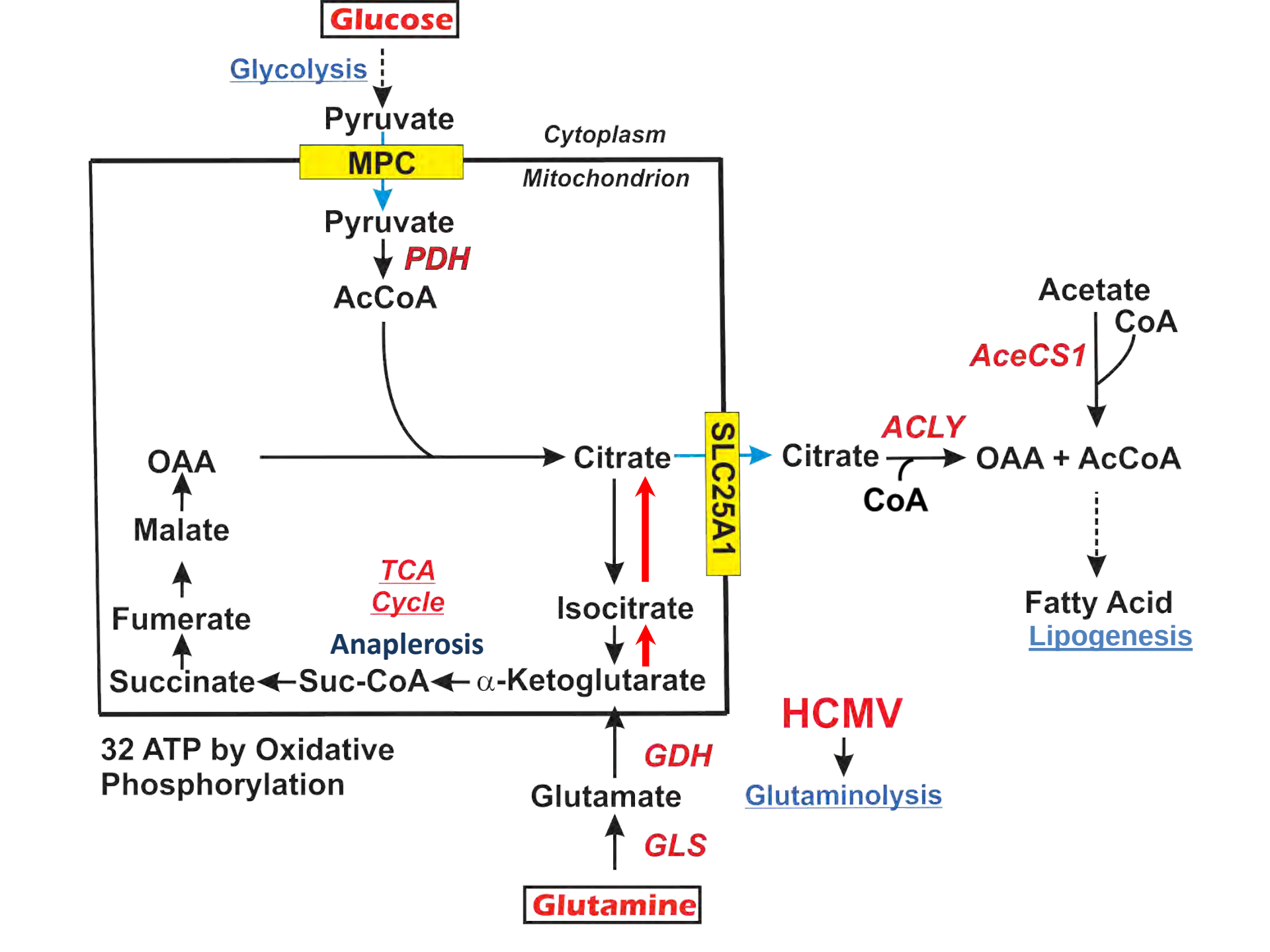
32 ATP by Oxidative
Phosphorylation
GDH
Glutamate
Glutaminolysis
GLS
Glutamine

Glutamine is necessary for HCMV infection and anaplerotically maintains the TCA cycle






Glucose
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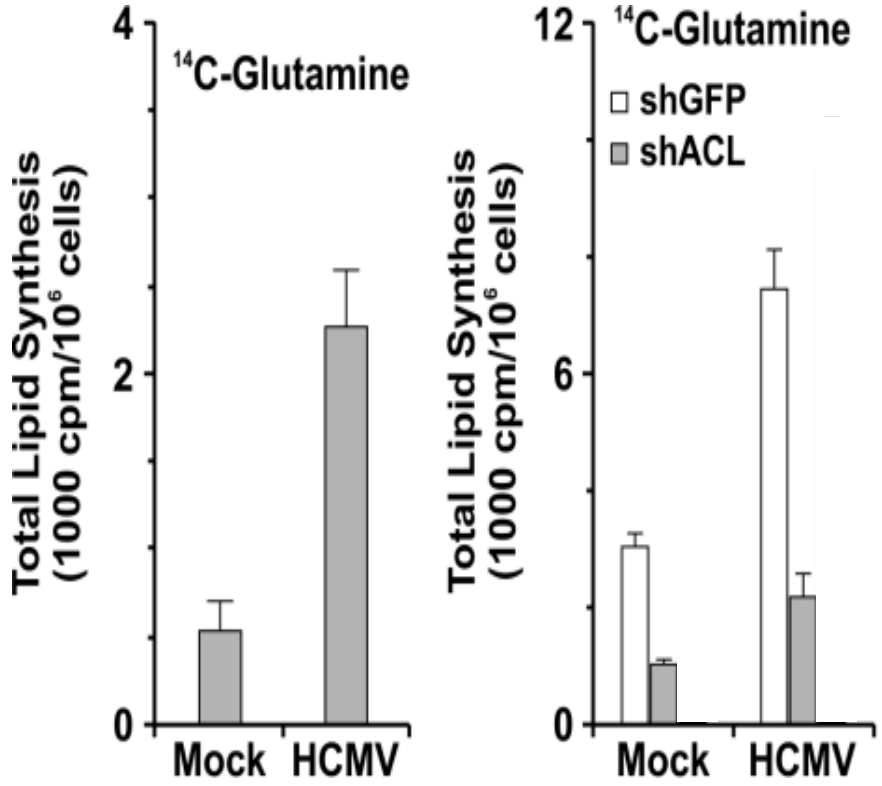
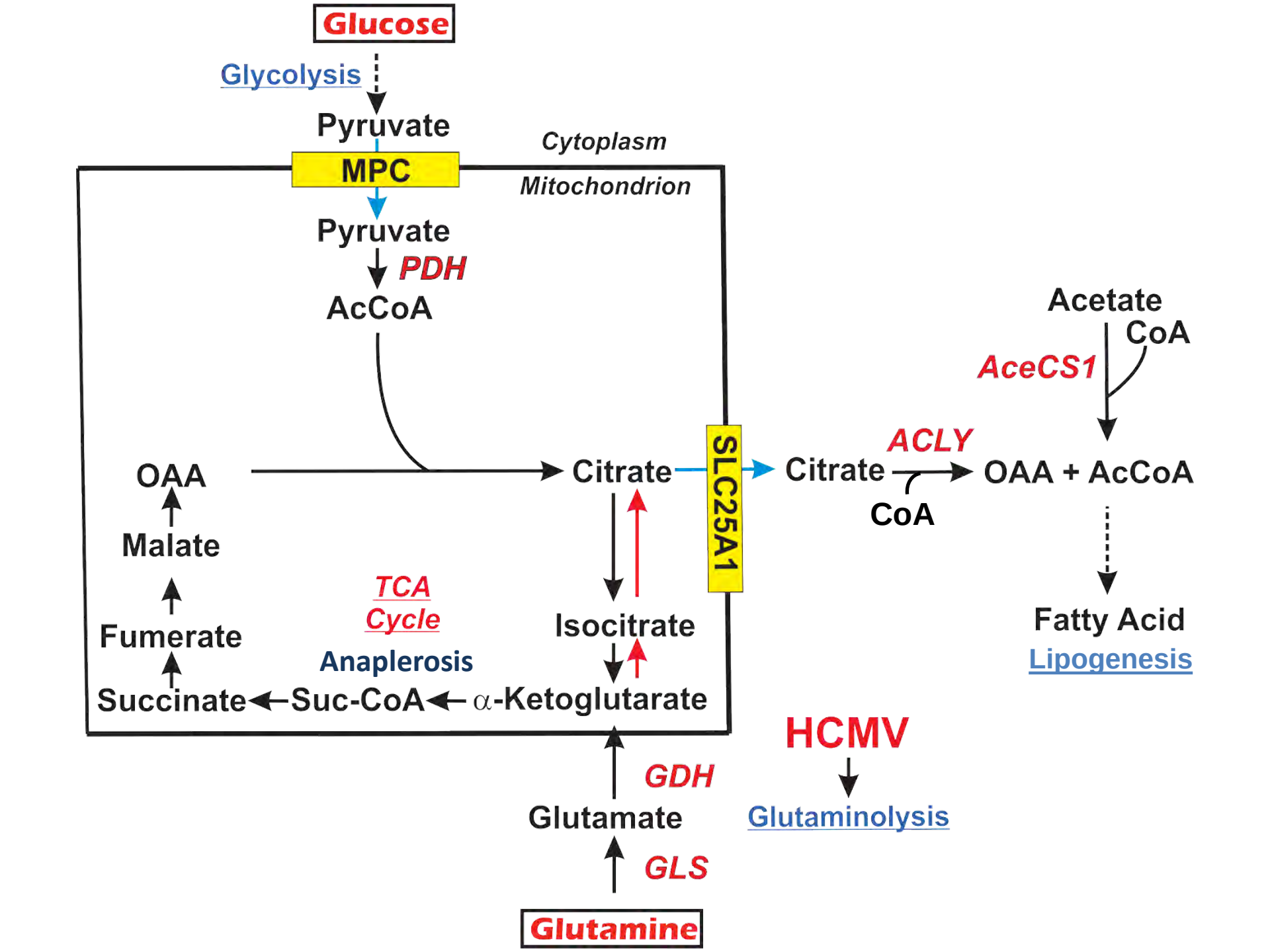
HCMV
32 ATP by Oxidative
Phosphorylation
GDH
Glutamate
Glutaminolysis
GLS
Glutamine

Glutamine is used for lipogenesis in HCMV-infected cells

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HCMV
GDH
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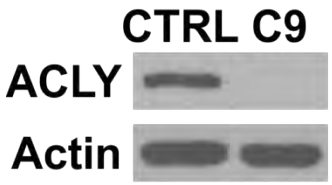
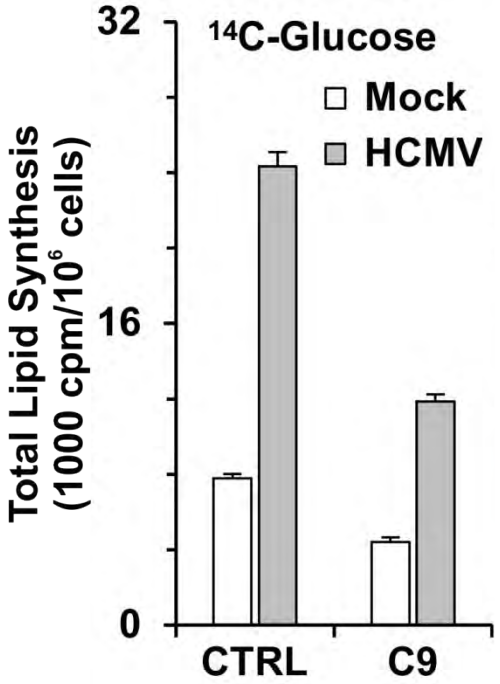
Glutamate
GLS
Glutamine

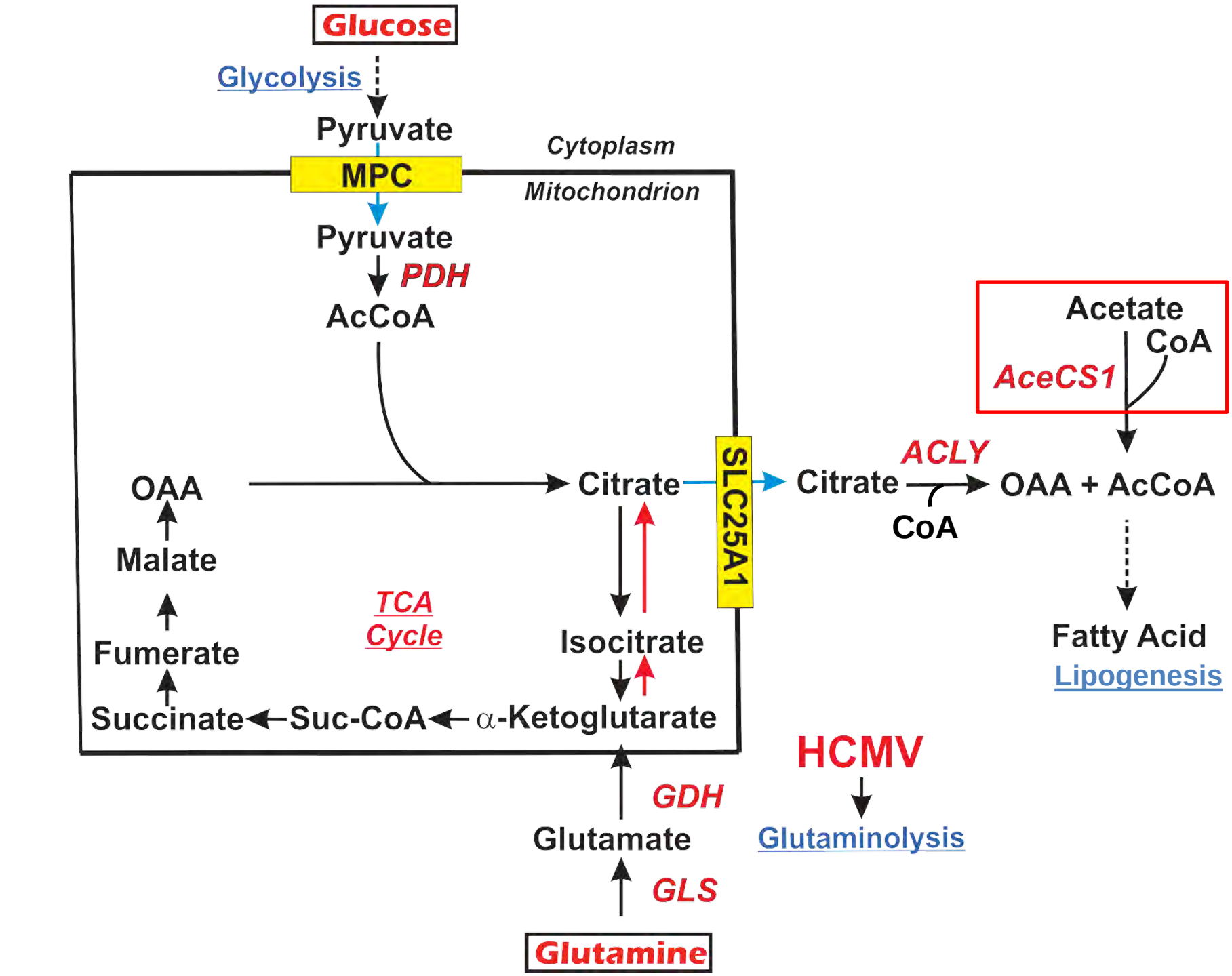
CRISPR/Cas9 knockout of ACL lowers glucose utilization for lipogenesis, but does not affect overall lipid levels or virus production





What is compensating?

How is the other 50% of glucose getting into lipids?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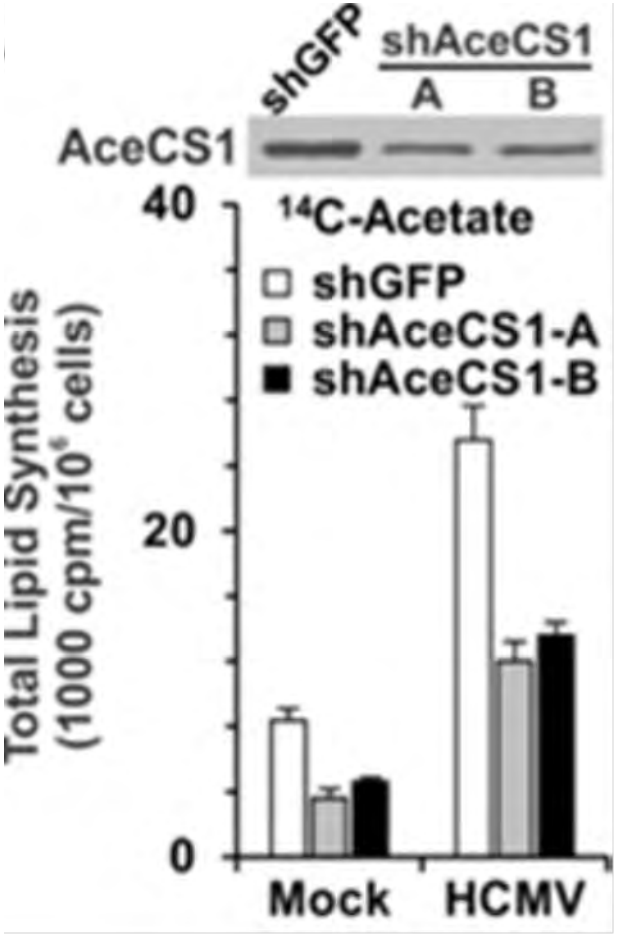
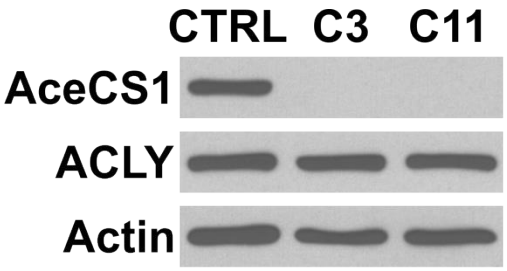
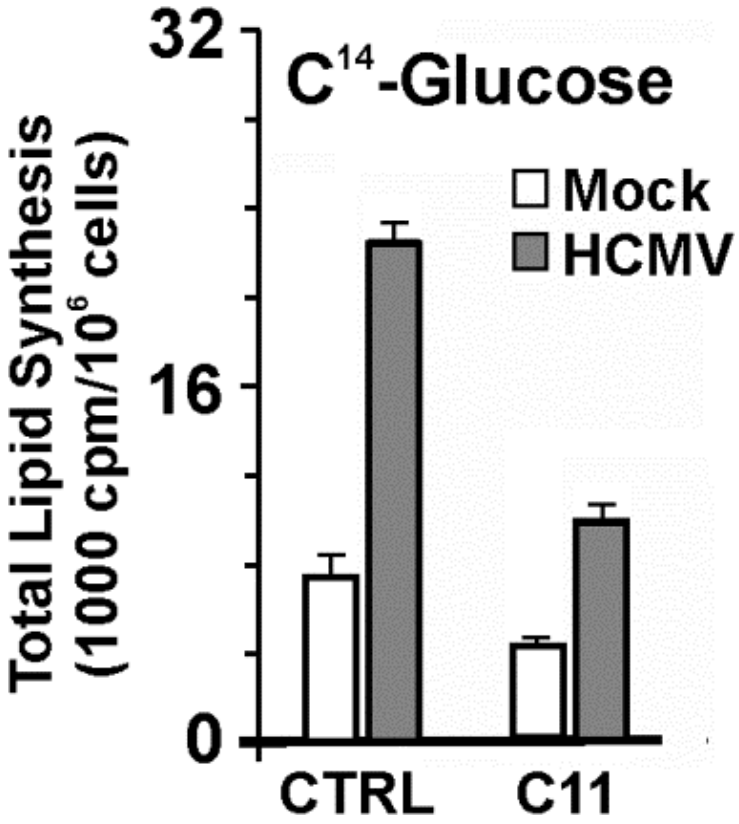
α-Ketoglutarate
HCMV
GDH
Glutaminolysis
Glutamate
GLS
Glutamine

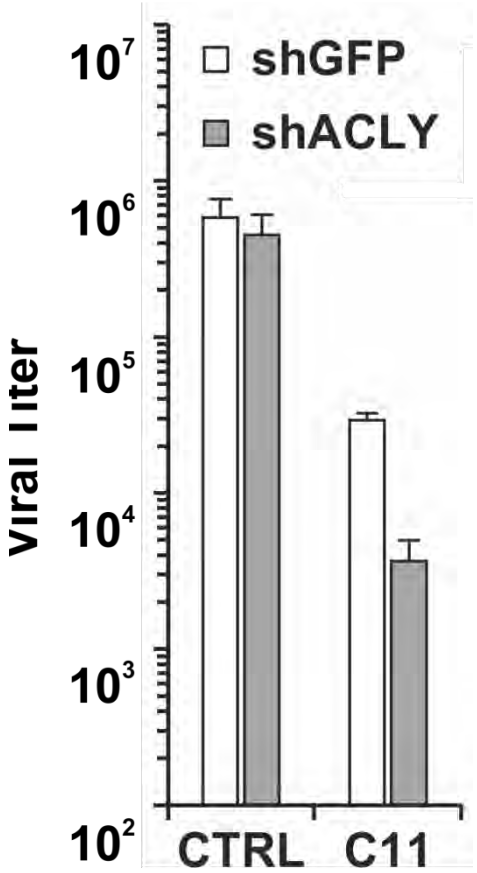
Increased use of acetate by Acetyl CoA synthetase in infected cells

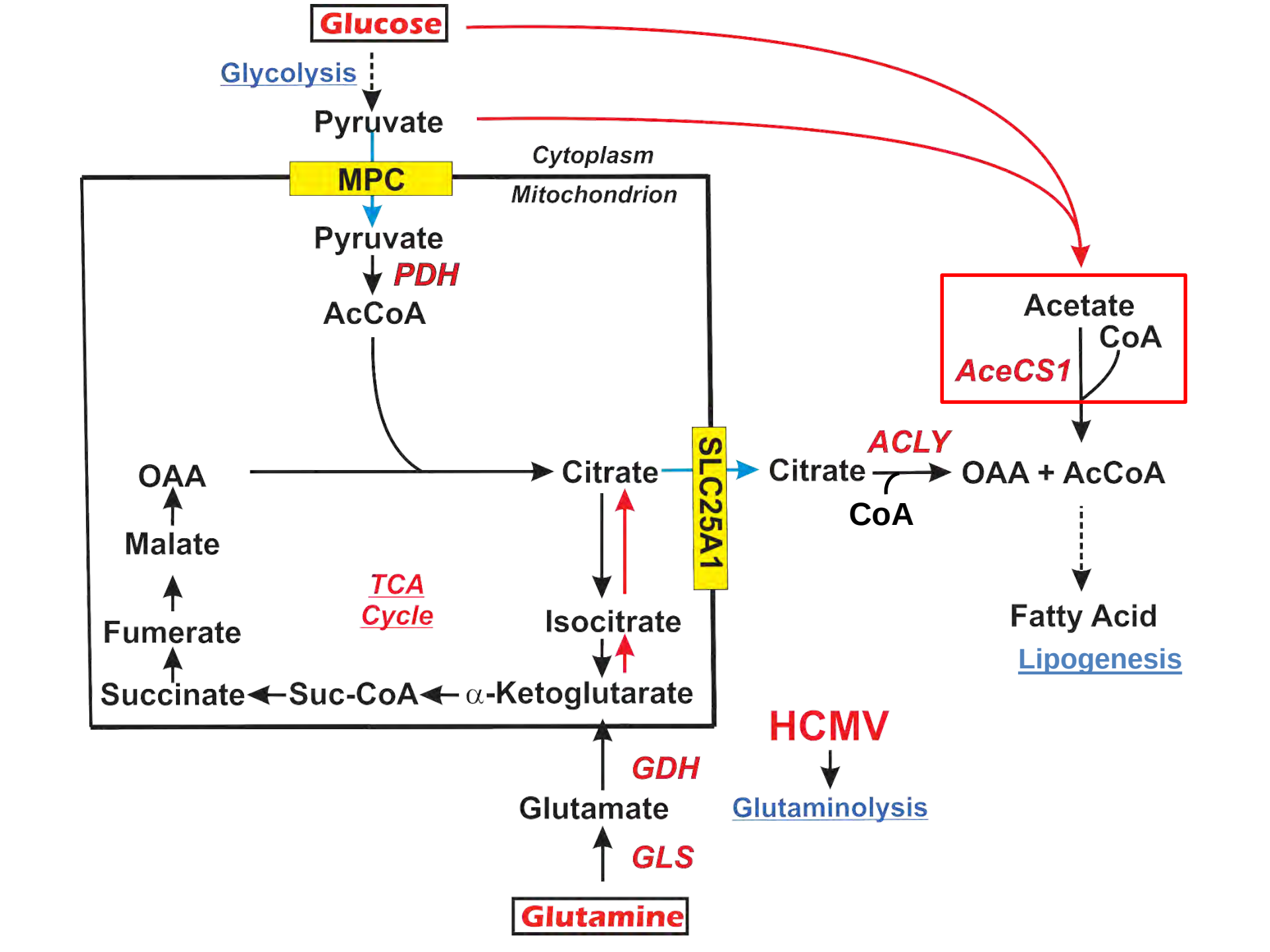


CRISPR/Cas9 knockout of AceCS1 lowers glucose utilization for lipogenesis and viral titer





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HCMV
GDH
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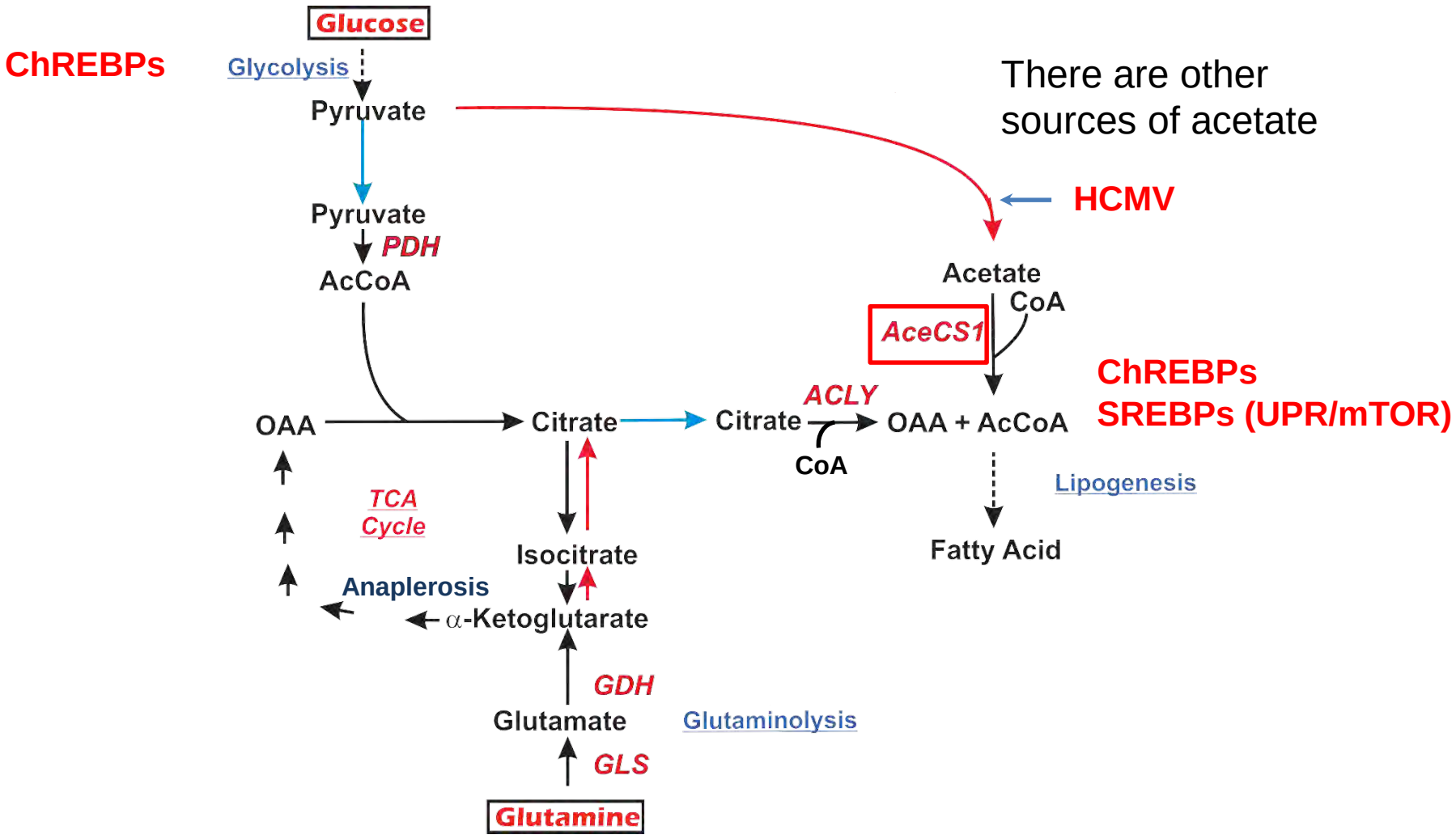
GLS
Glutamine

Glucose - derived pyruvate is a source of acetate

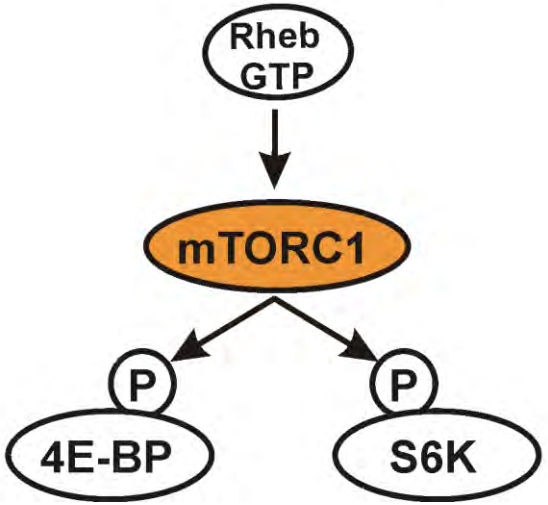
Vysochan, A., Sengupta, A., Weljie, A., Alwine, J.C. and Y. Yu. (2017). ACSS2-mediated Acetyl-CoA Synthesis from Acetate Is Necessary for Human Cytomegalovirus Infection. Proc. Natl. Acad. Sci. USA. 114: E1528-E1535.

- **HCMV-infected cells produce more glucose-derived pyruvate which can be converted to acetate through a heretofore uncharacterized non-enzymatic mechanism.**

Summary 2


ChREBPs
Glucose
Glycolysis
Pyruvate
Pyruvate
PDH
AcCoA
There are other sources of acetate
HCMV
Acetate
CoA
AceCS1
ChREBPs
SREBPs (UPR/mTOR)
ACLY
OAA
Citrate
Citrate
OAA + AcCoA
CoA
Lipogenesis
TCA Cycle
Isocitrate
Fatty Acid
Anaplerosis
α-Ketoglutarate
GDH
Glutamate
Glutaminolysis
GLS
Glutamine

mTOR kinase control



Translational Activation

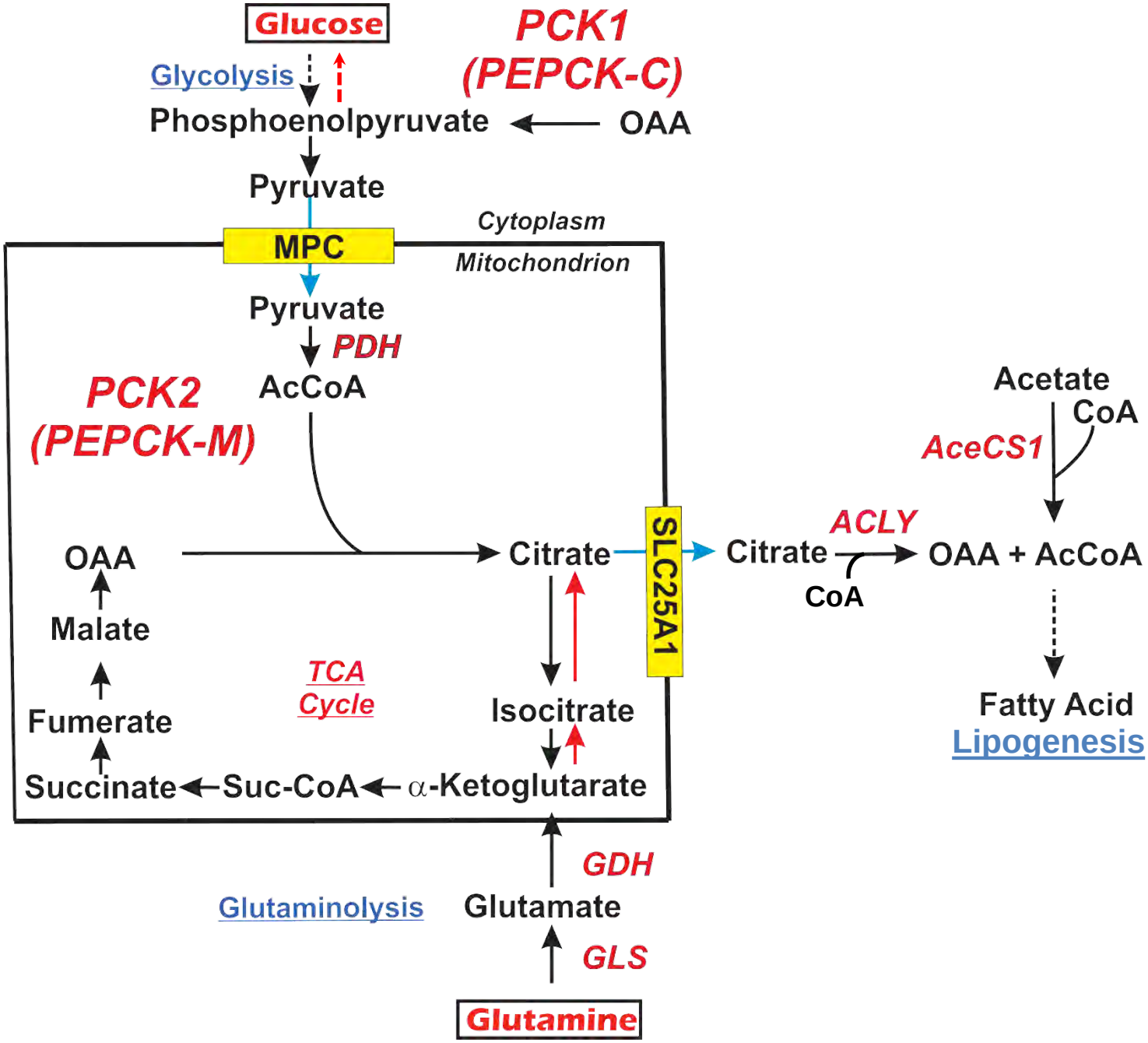
Activated Metabolism,
Fatty acid synthesis,
Translation

Inhibits Apoptosis
and Autophagy

Phosphoenolpyruvate Carboxykinase

Phosphoenolpyruvate Carboxykinase (PEPCK or PCK)

Catalyzes the first committed step in gluconeogenesis by decarboxylating and phosphorylating oxaloacetate (OAA) converting it to phosphoenolpyruvate (PEP), when GTP is present.


Glucose
PCK1
(PEPCK-C)
Glycolysis
Phosphoenolpyruvate
OAA
Pyruvate
Cytoplasm
MPC
Mitochondrion
Pyruvate
PDH
PCK2
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CoA
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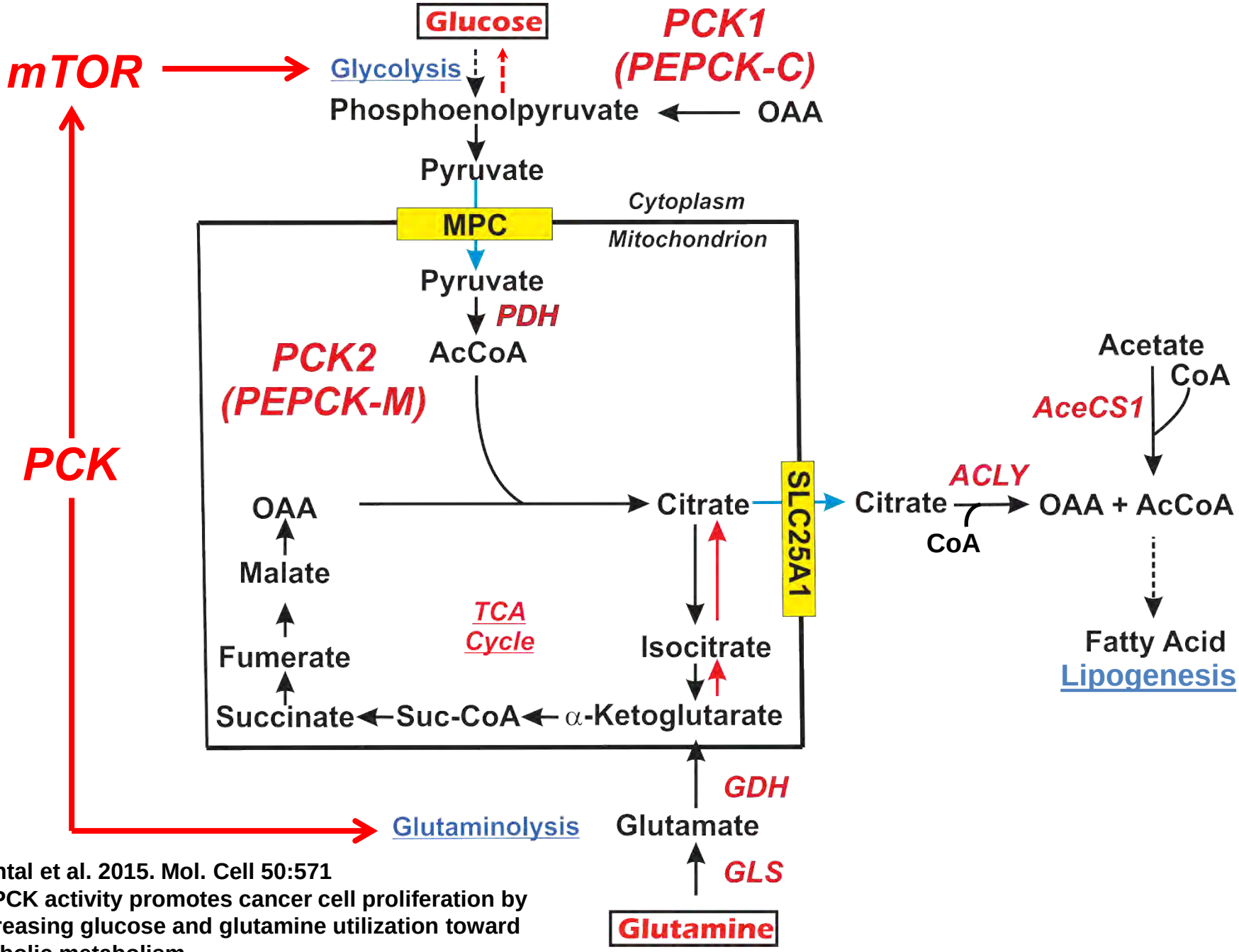
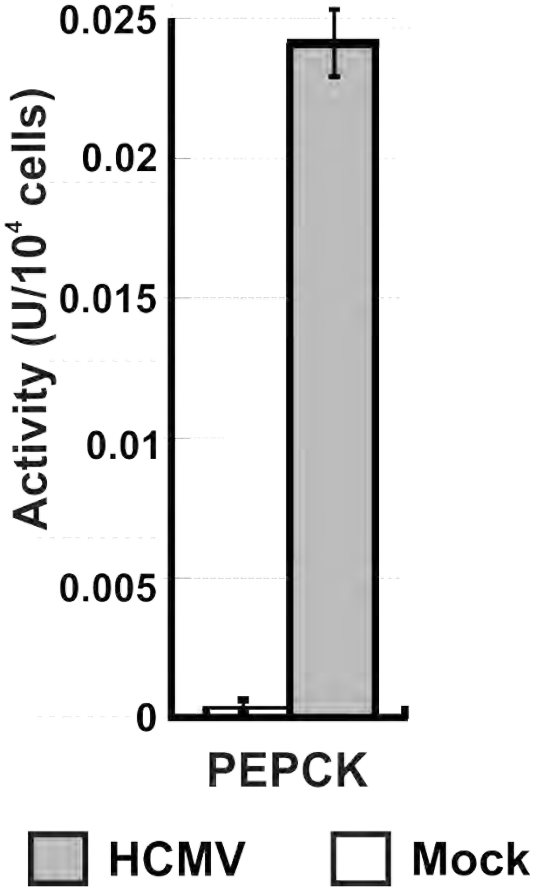
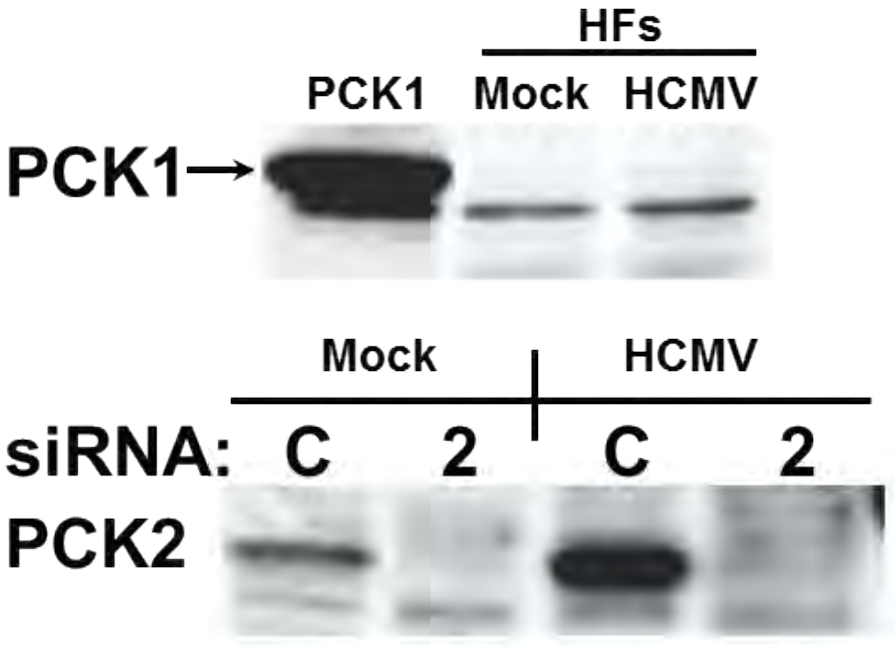
Glutamine



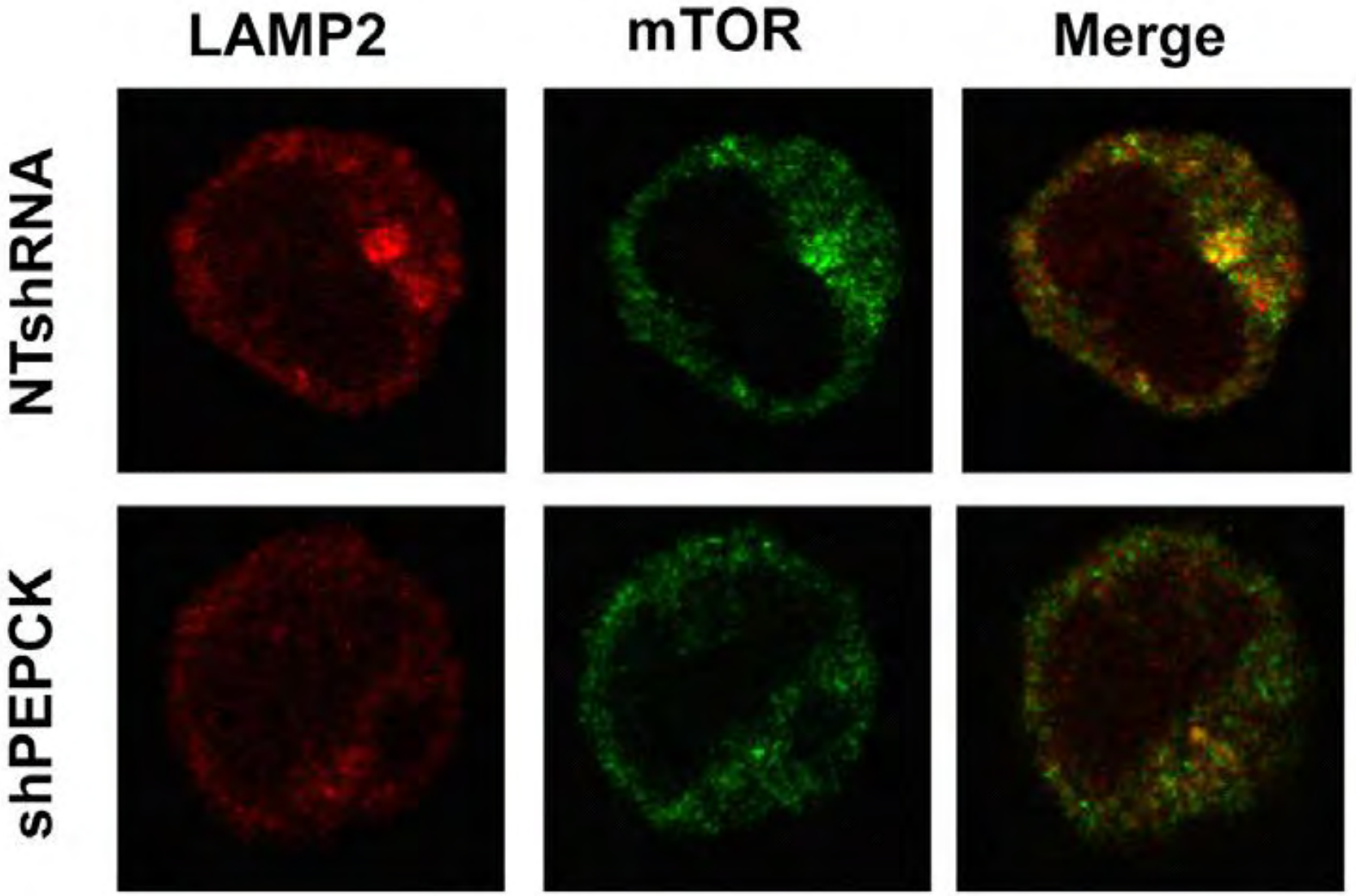
Montal et al. 2015. Mol. Cell 50:571
PEPCK activity promotes cancer cell proliferation by increasing glucose and glutamine utilization toward anabolic metabolism

Phosphoenolpyruvate Carboxykinase activity is increased in HCMV infected cells





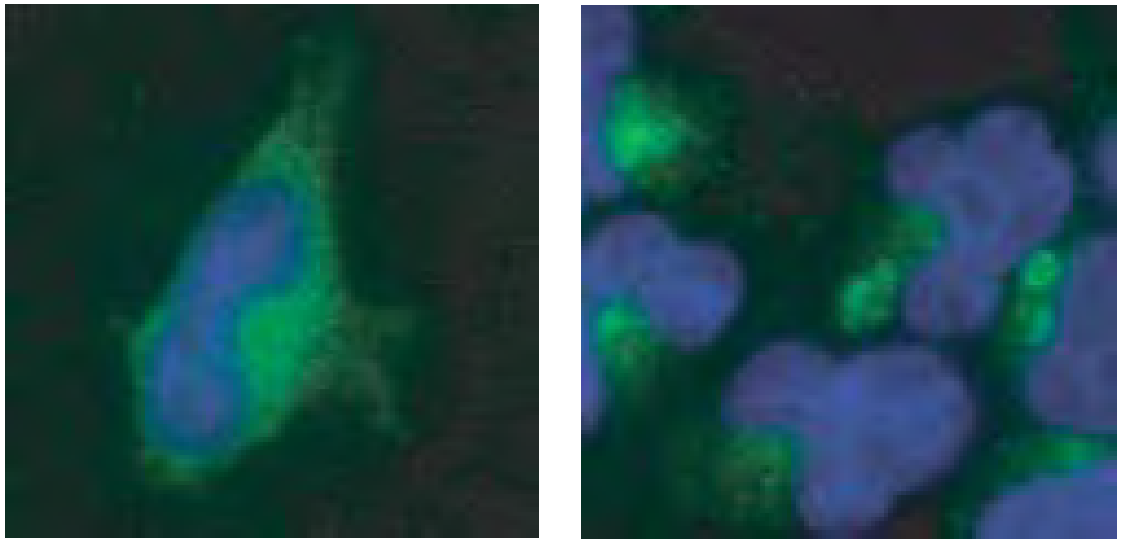
Does PCK facilitate mTOR perinuclear localization



Montal et al (2015) Mol. Cell **50**:571

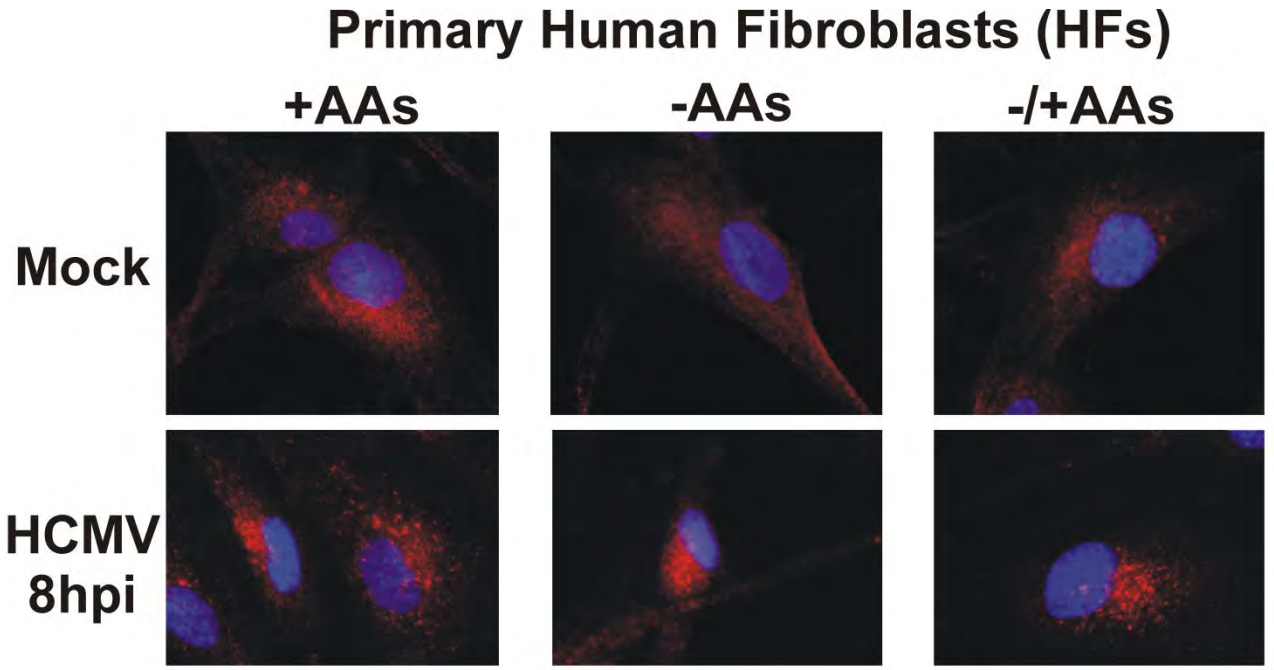
Perinuclear localization of mTOR

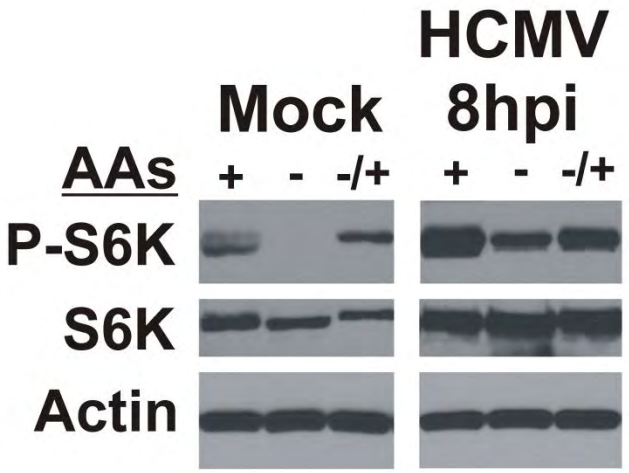
-AAs **+AAs**



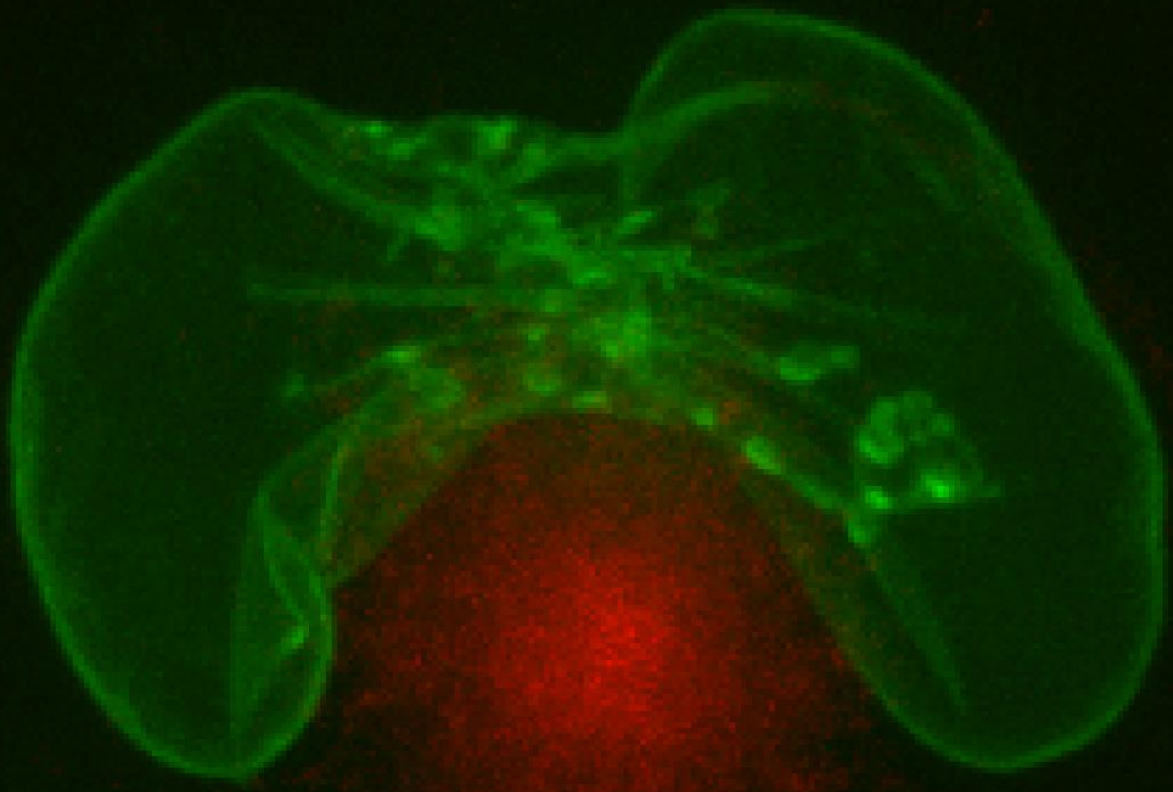
Sancak *et al. Science* (2008)

mTOR perinuclear localization and resistance to AA depletion in HCMV infected cells

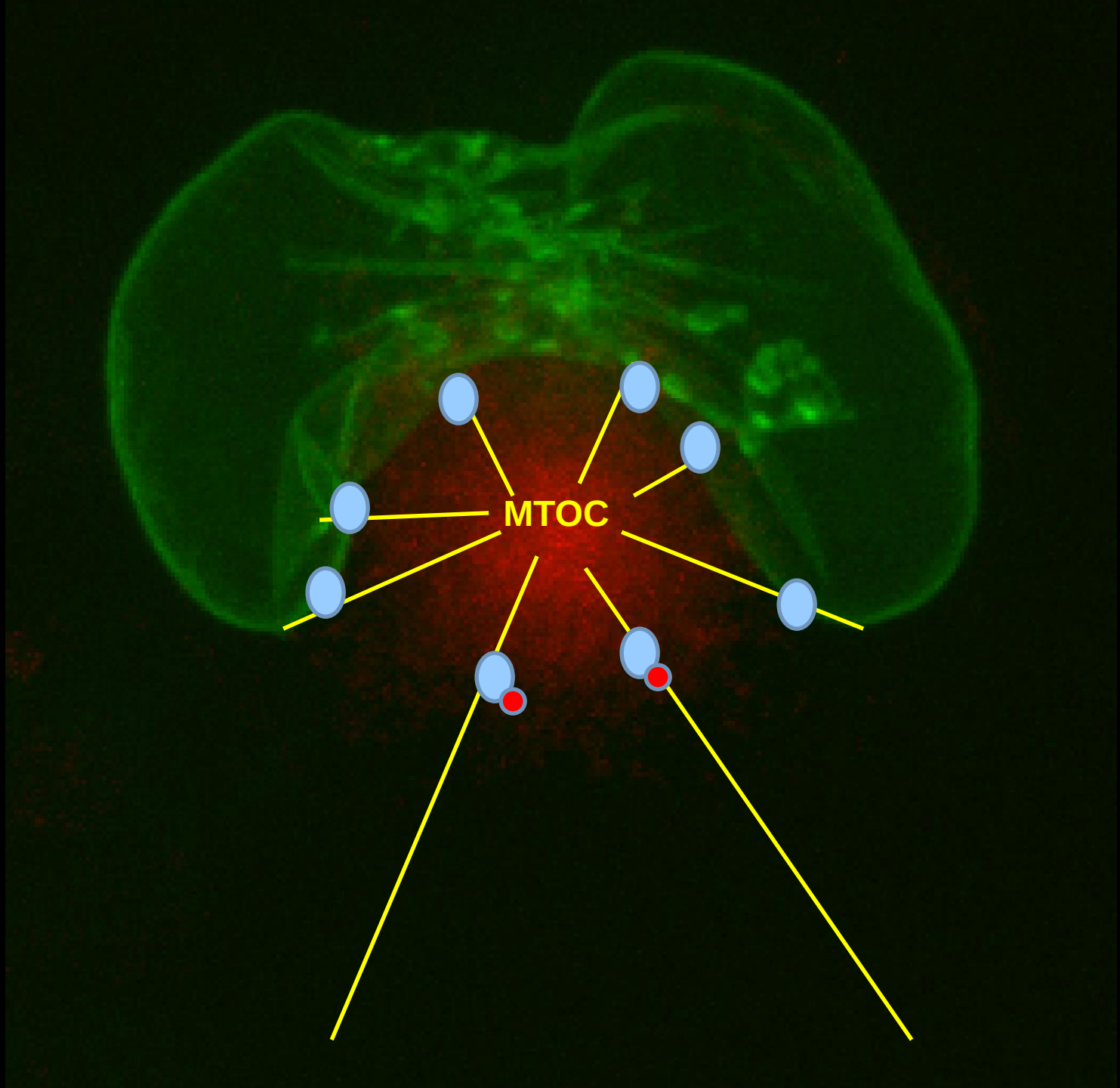




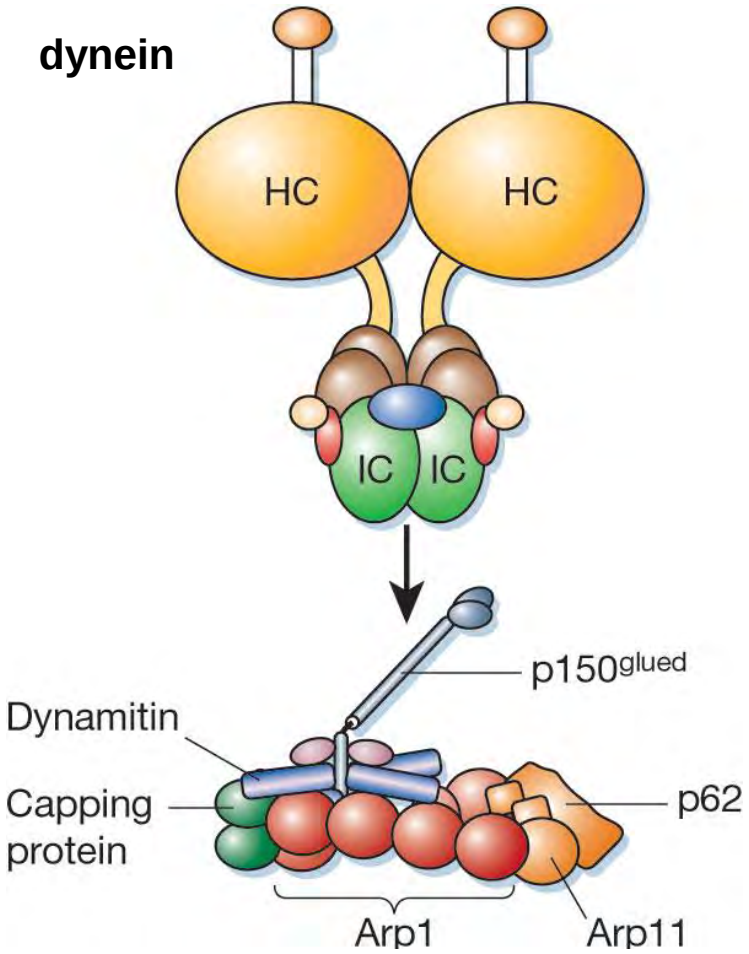
Enlarged kidney-shaped nucleus



Cytoplasmic Assembly Compartment


MTOC

Dynein and Nuclear Envelope Breakdown

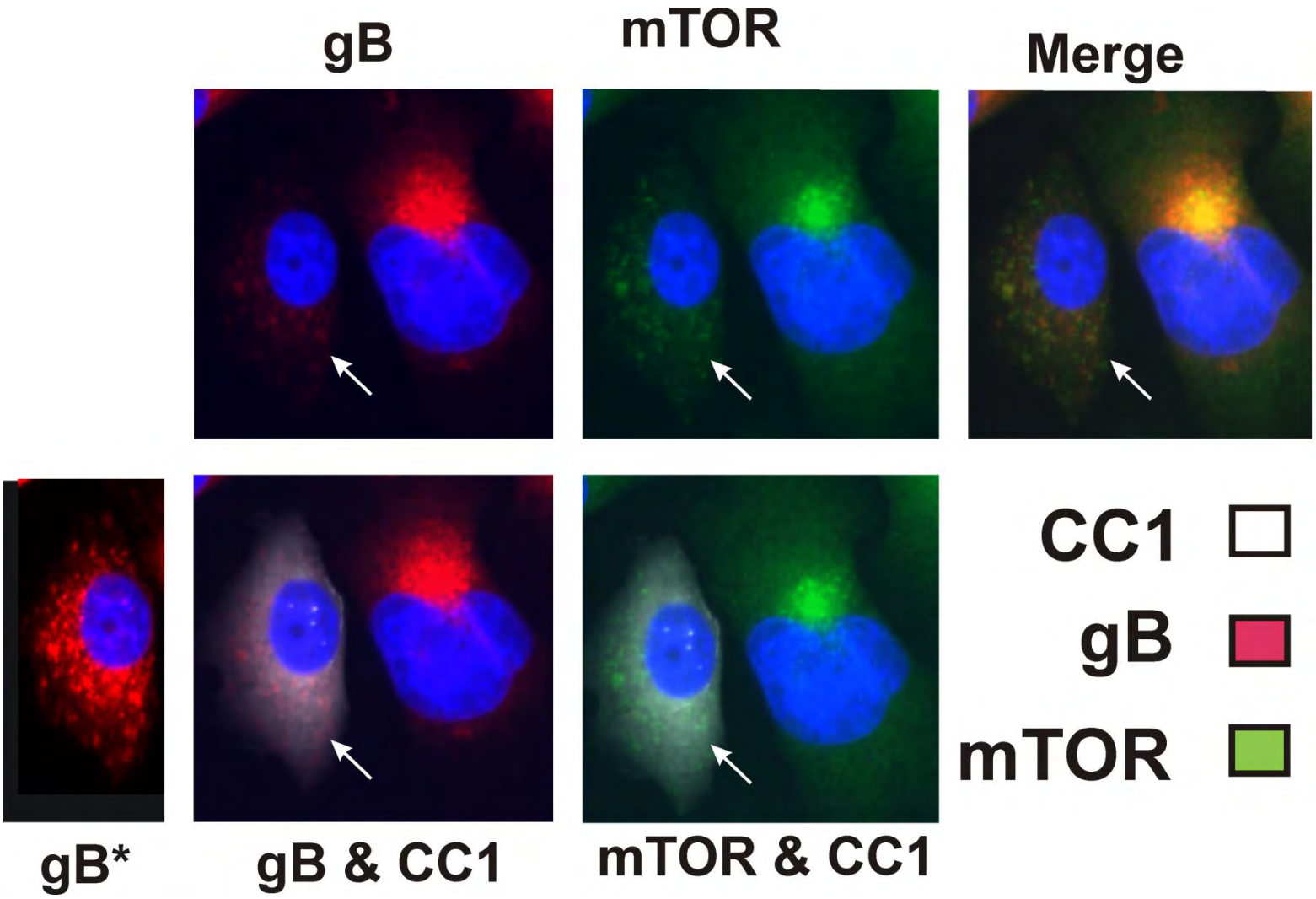


Dynein is the minus-end directed motor and has been shown to be necessary for the formation of the AC.

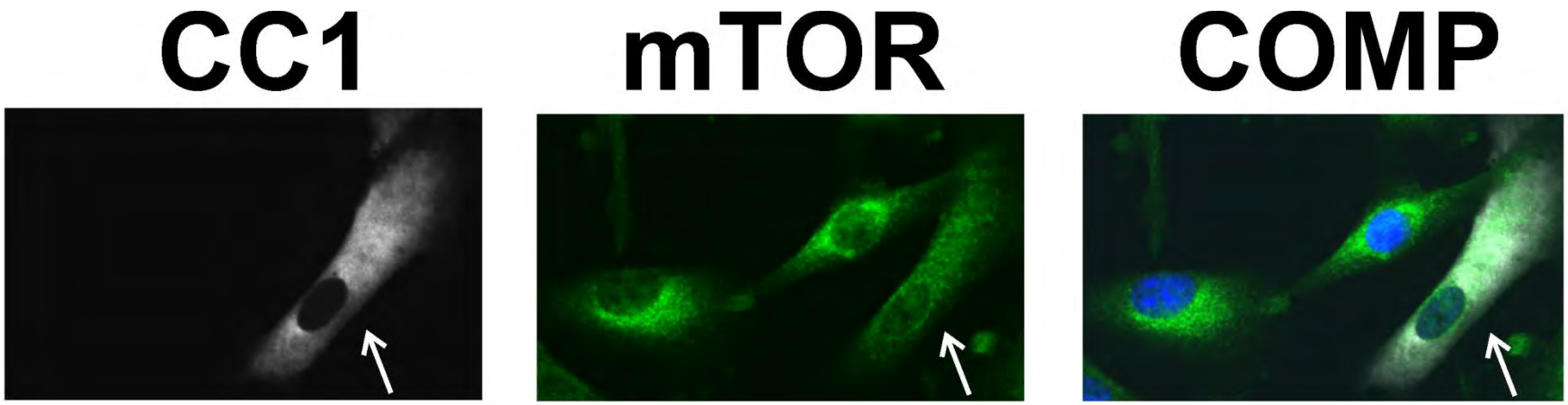
Dynein interacts with cargo via dynactin.

The coil-coiled domain of the dynactin subunit p150^{Glued} (termed CC1) inhibits dynactin-dynein binding, thus inhibiting cargo loading.

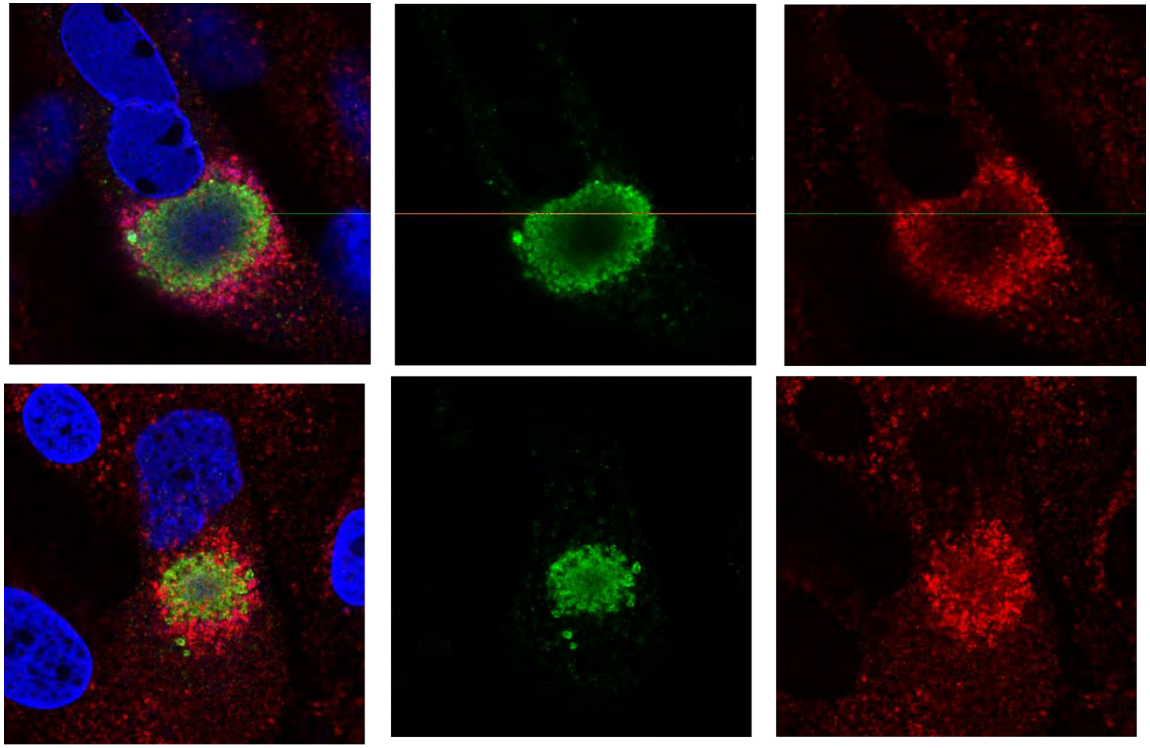
Dynein maintains mTOR in the AC



Dynein inhibition disrupts mTOR localization in normal HFs



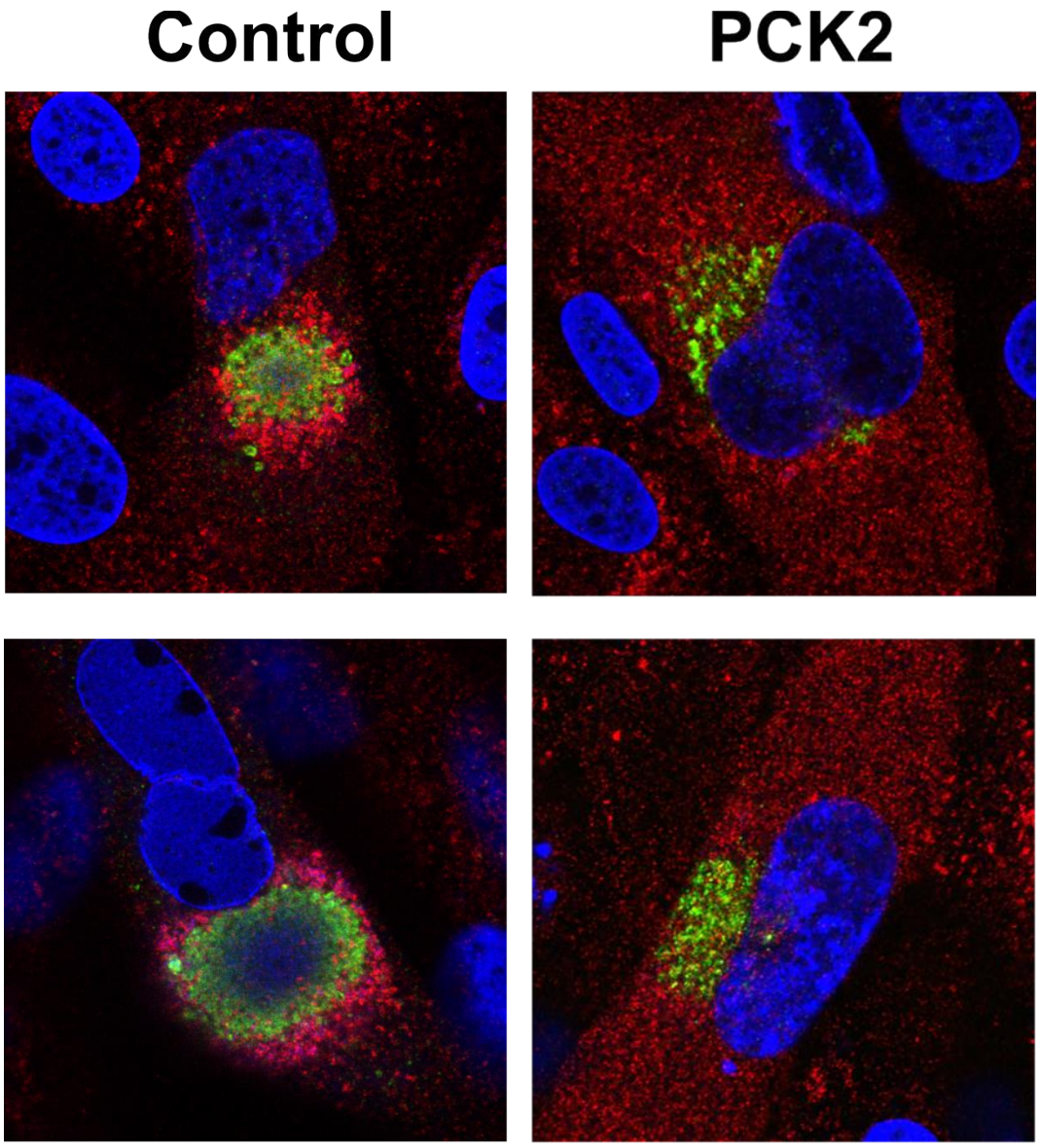
mTOR localizes to the AC



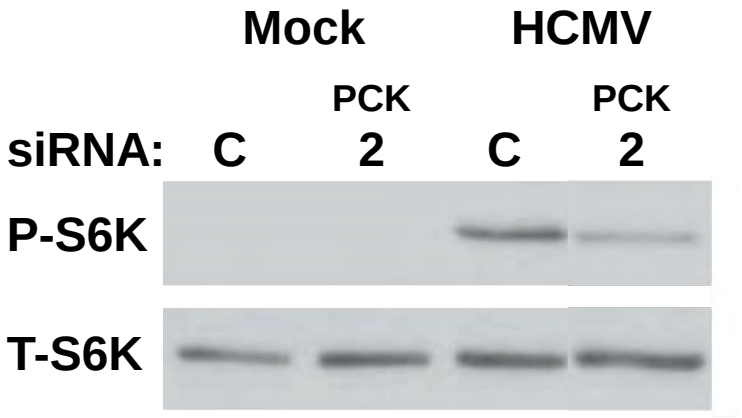
Merge + Nucs **pp28** **mTOR**

In the AC, mTOR is active and resistant to inhibition by many stress responses.

Depletion of PCK2 affects AC formation

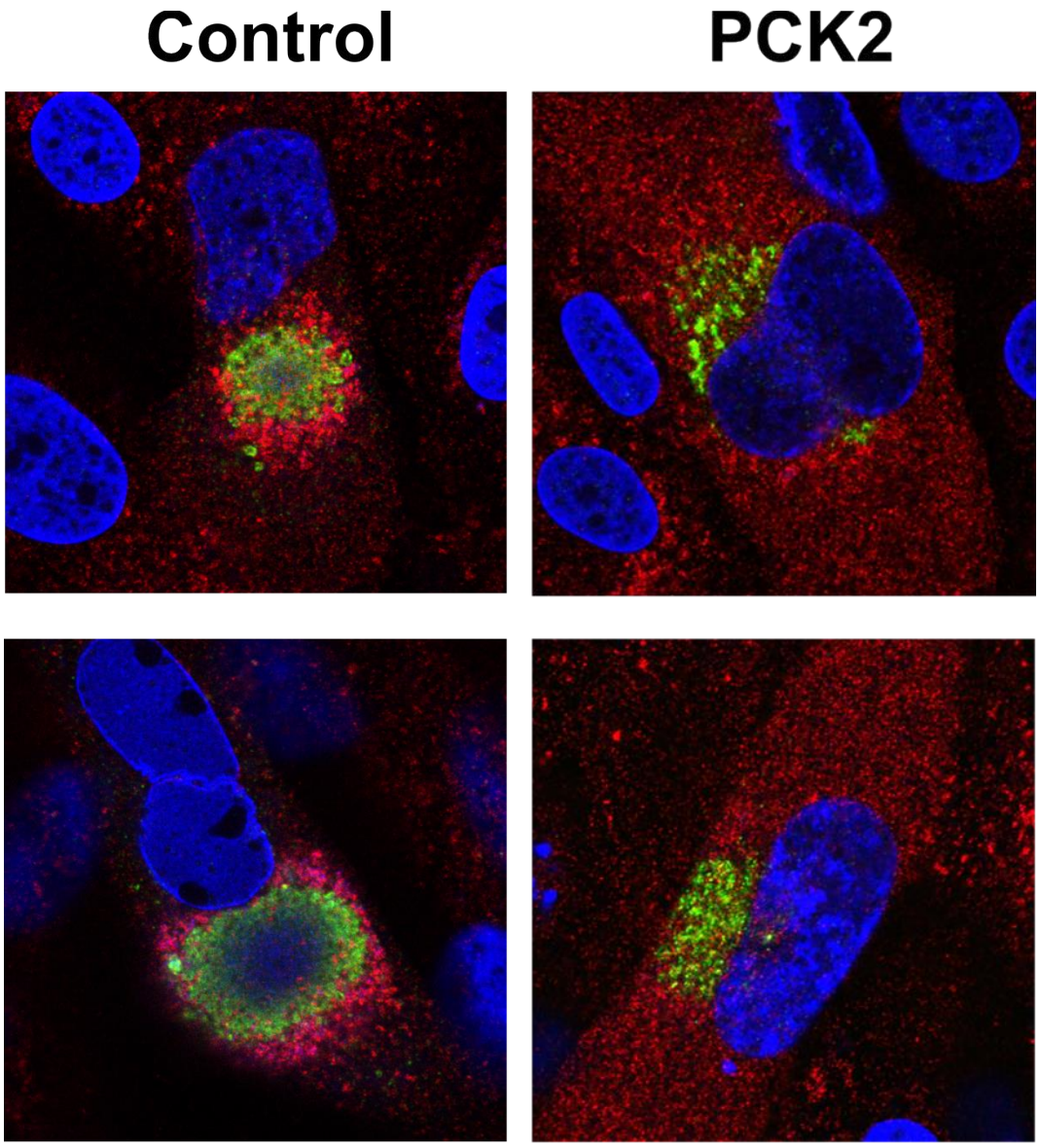


PCK2 depletion reduces mTOR activity and slows viral growth



PCK2 depletion slows viral growth and reduces final titer.

Depletion of PCK2 affects AC formation



Summary III

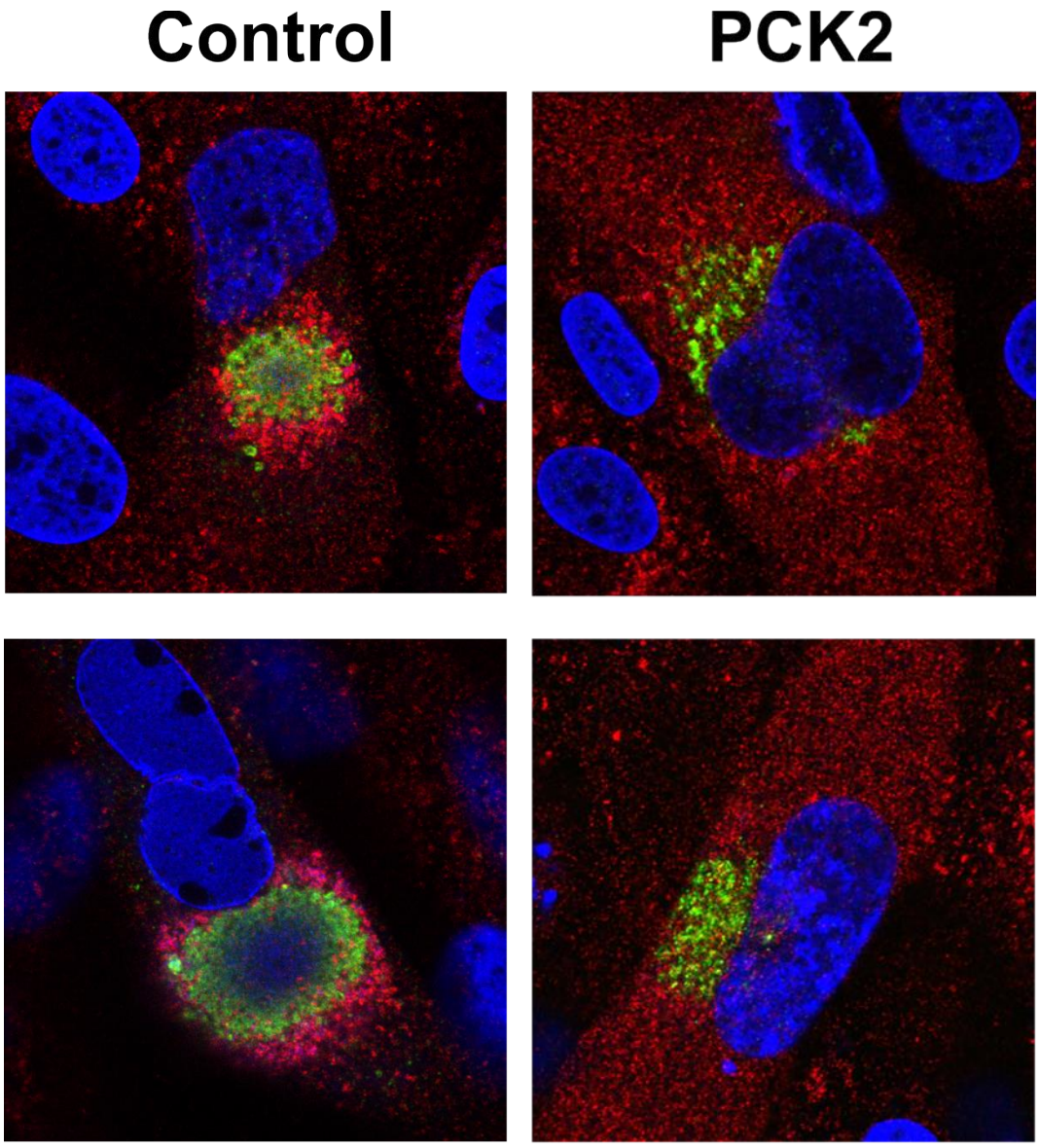
PCK2, a mitochondrial metabolic enzyme, is needed by HCMV for effective AC formation.

PCK2 levels and activity are increased during infection.

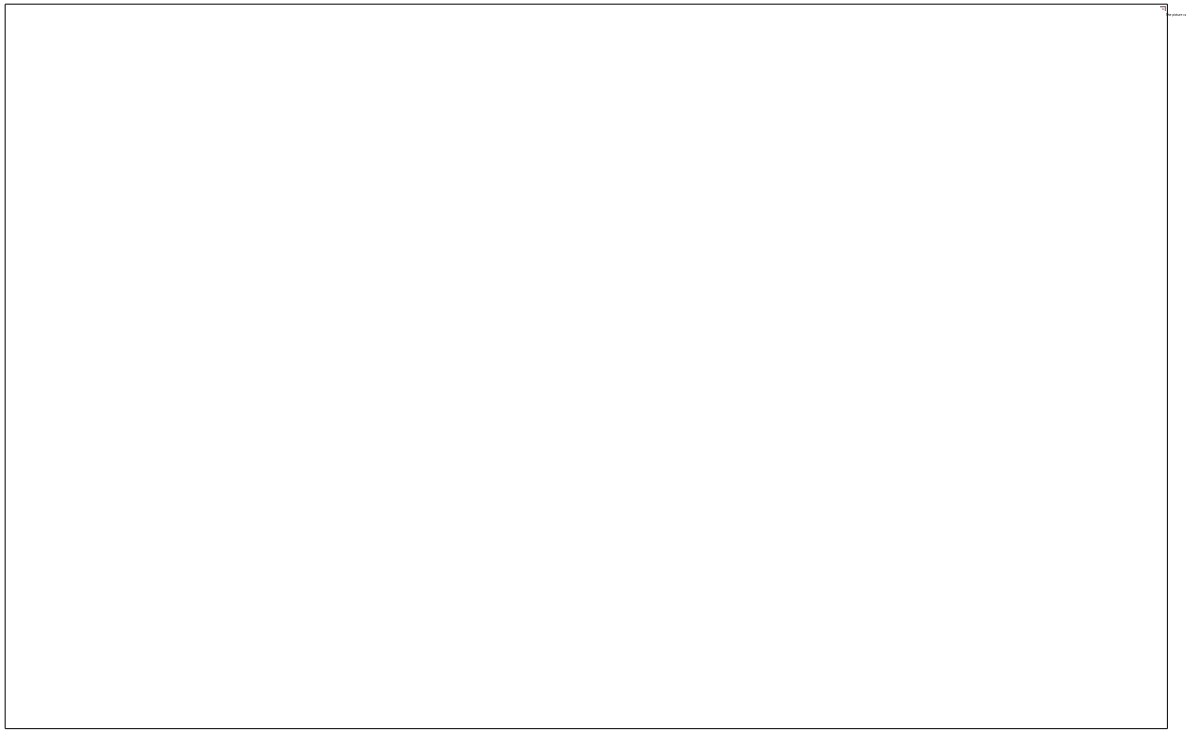
Depletion of PCK2 results in lowered mTOR activity and aberrant ACs that function inefficiently.

How does mitochondrial PCK2 do this?

Depletion of PCK2 affects AC formation



Depletion of Lis1 affects AC formation

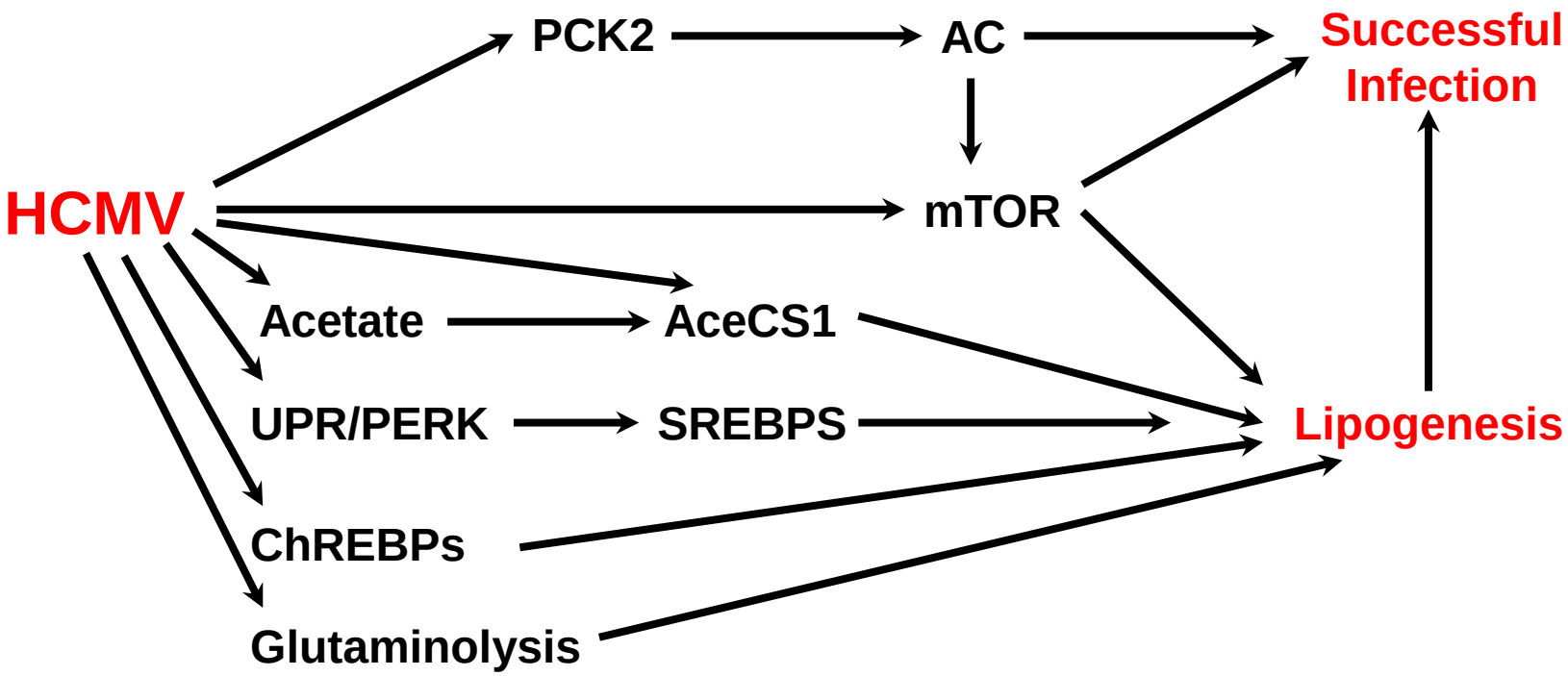


HCMV siLIS-1

Do metabolic conditions mediated by PCK2 communicate with motor proteins to control intracellular transport as needed by the cells metabolic status?

Does HCMV manipulate PCK2 to promote motor functions that will facilitate AC formation.

Serving its own Schemes



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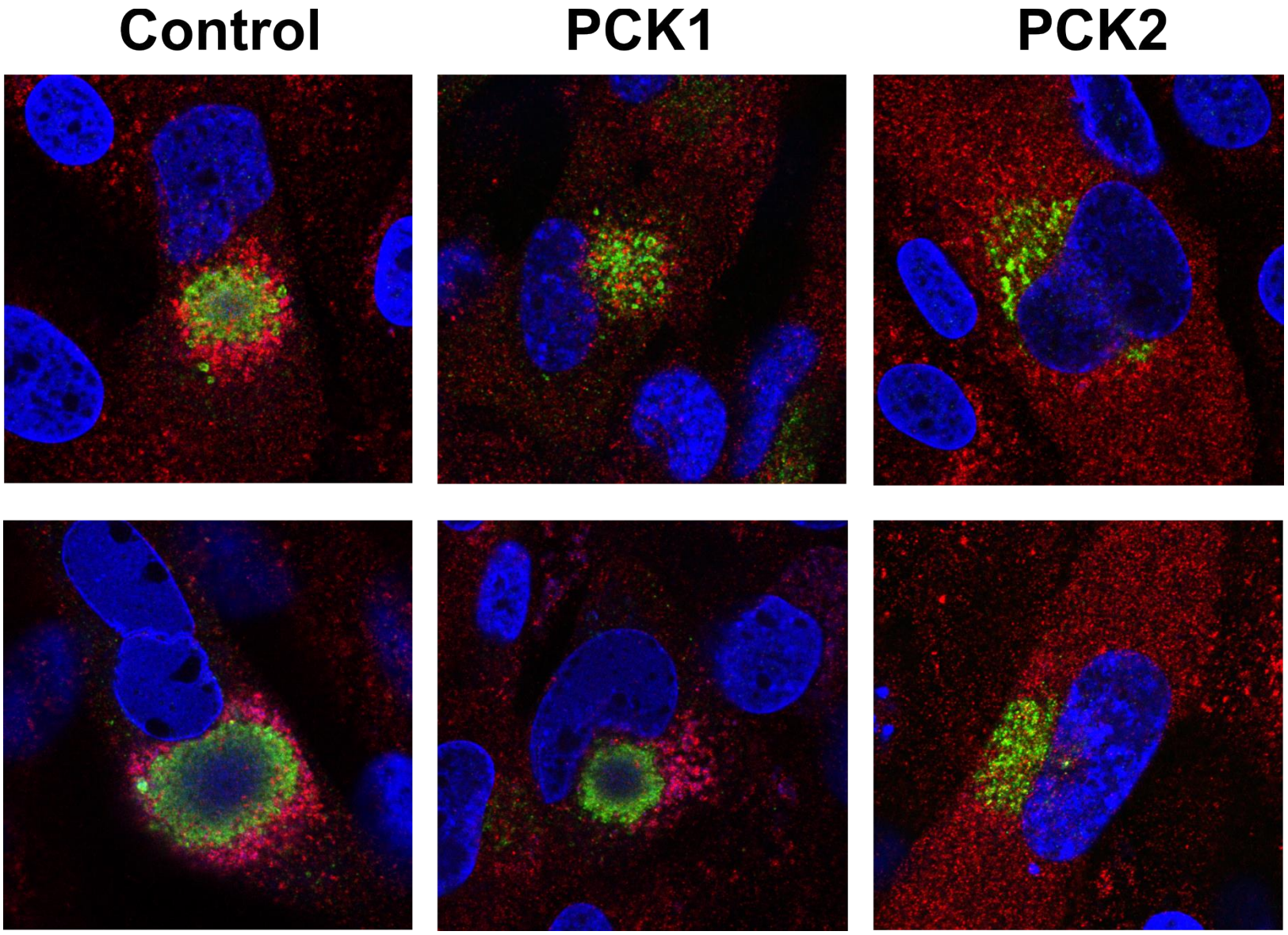
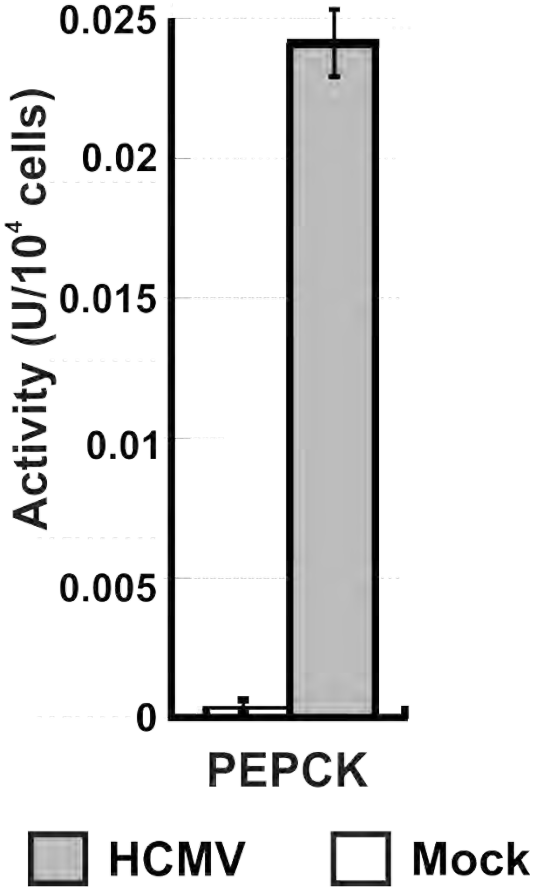
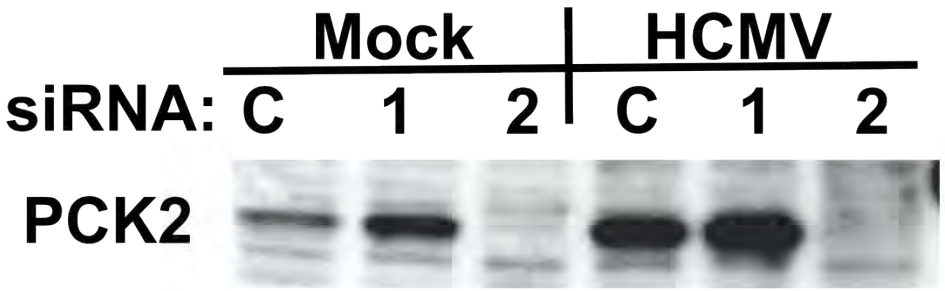
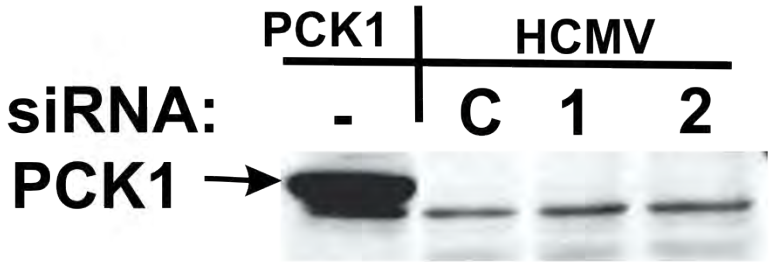
Depletion of PCK2 affects AC formation



Phosphoenolpyruvate Carboxykinase activity is increased in HCMV infected cells







Allosteric Regulation

Allosteric regulation is the regulation of an enzyme by binding an effector molecule at a site, an allosteric site, which is not the enzyme's active site.

Effector binding results in a conformational change which can enhance or decrease the enzyme's activity.

HCMV must maintain mechanistic target of rapamycin (mTOR) kinase activity

mTOR kinase promotes growth by activating:

-Translation

-Lipogenesis

-Glycolysis

and inhibiting

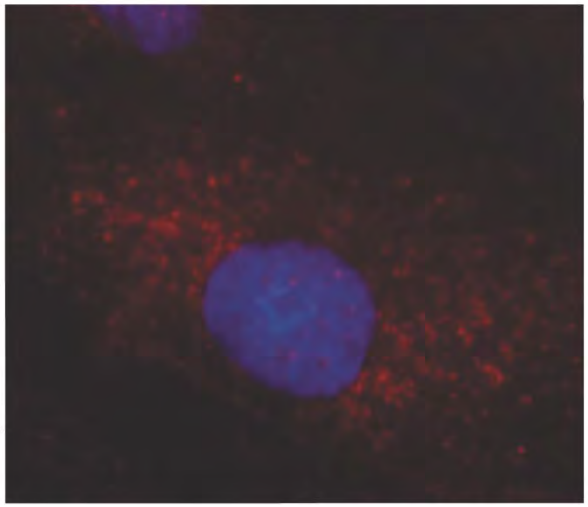
-Autophagy

-Apoptosis

Many cellular stress responses that signal growth inhibition will target mTOR kinase for inactivation. HCMV needs to counteract this.

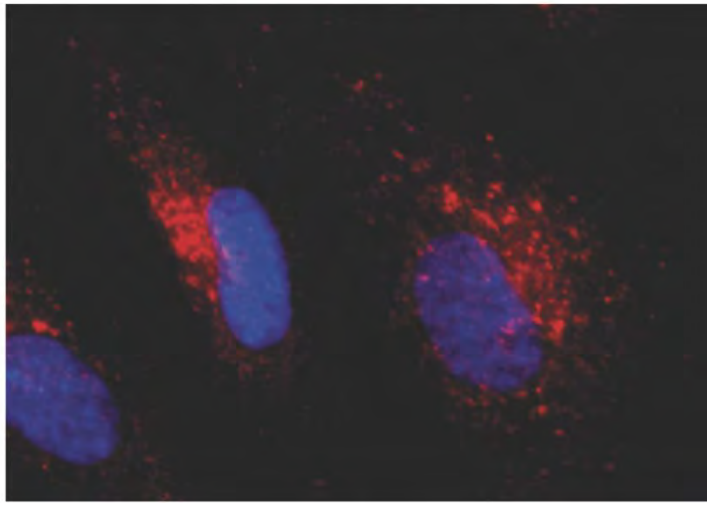
mTOR localizes to a perinuclear position early in infection

Quiescent HFs



mTOR inactive

HFs HCMV 8 HPI

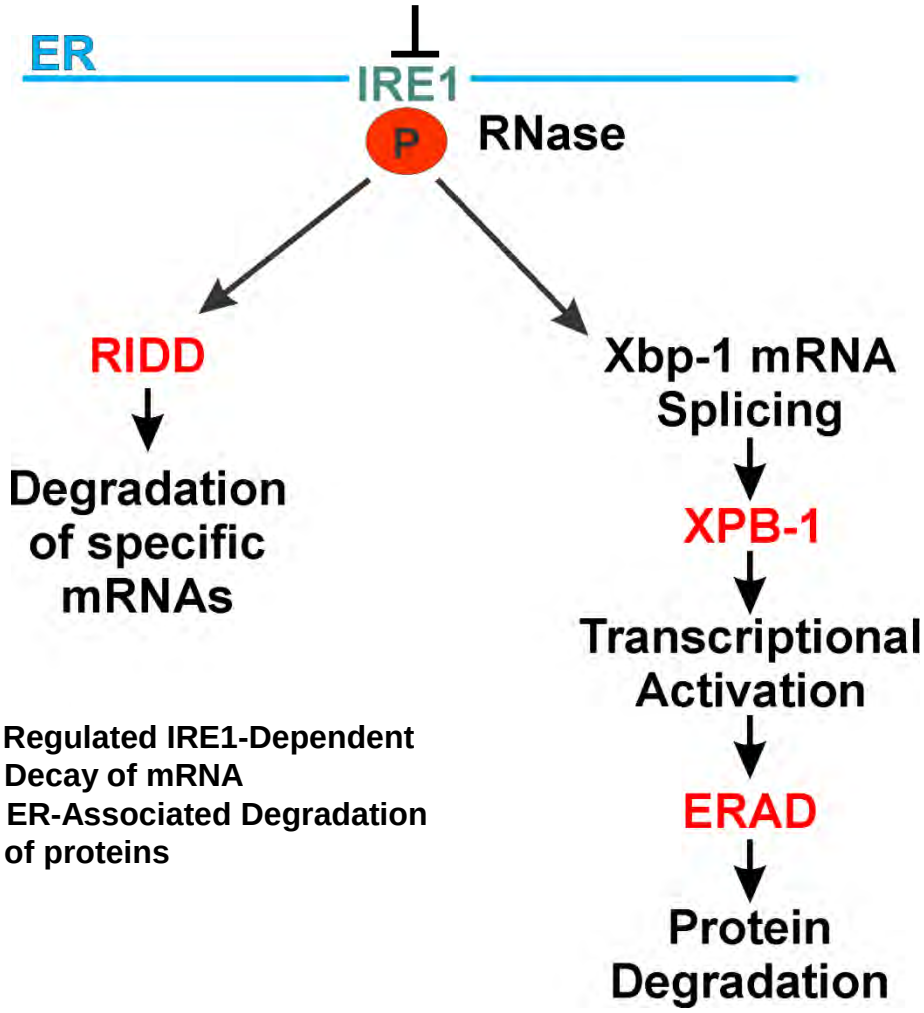


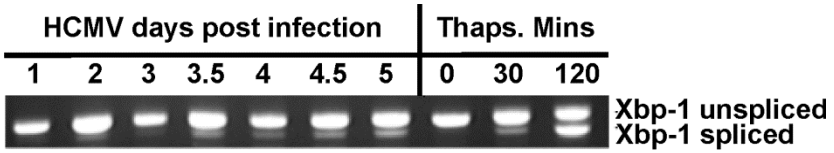
mTOR active

The molecular motor dynein brings mTOR to the perinuclear position

IRE1 activation is highly regulated

UL50 is associated with loss of IRE1





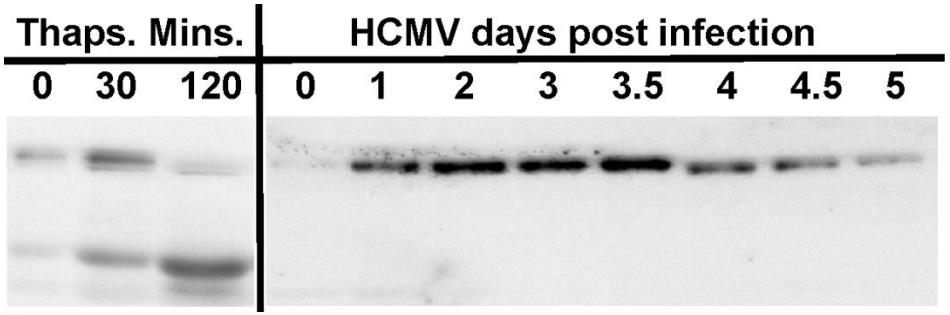
RIDD: Regulated IRE1-Dependent Decay of mRNA
ERAD: ER-Associated Degradation of proteins

No transcriptional activation of ERAD associated genes

ATF6 is not activated

ER

ATF6



Actives genes encoding:
Chaperones (including BiP) ←
Folding enzymes (e.g. PDI) ←
ERAD Components
Xbp-1

HCMV activates