Viral FGARAT ORF75A promotes the specific infectivity of virus particles and gammaherpesvirus pathogenesis in mice

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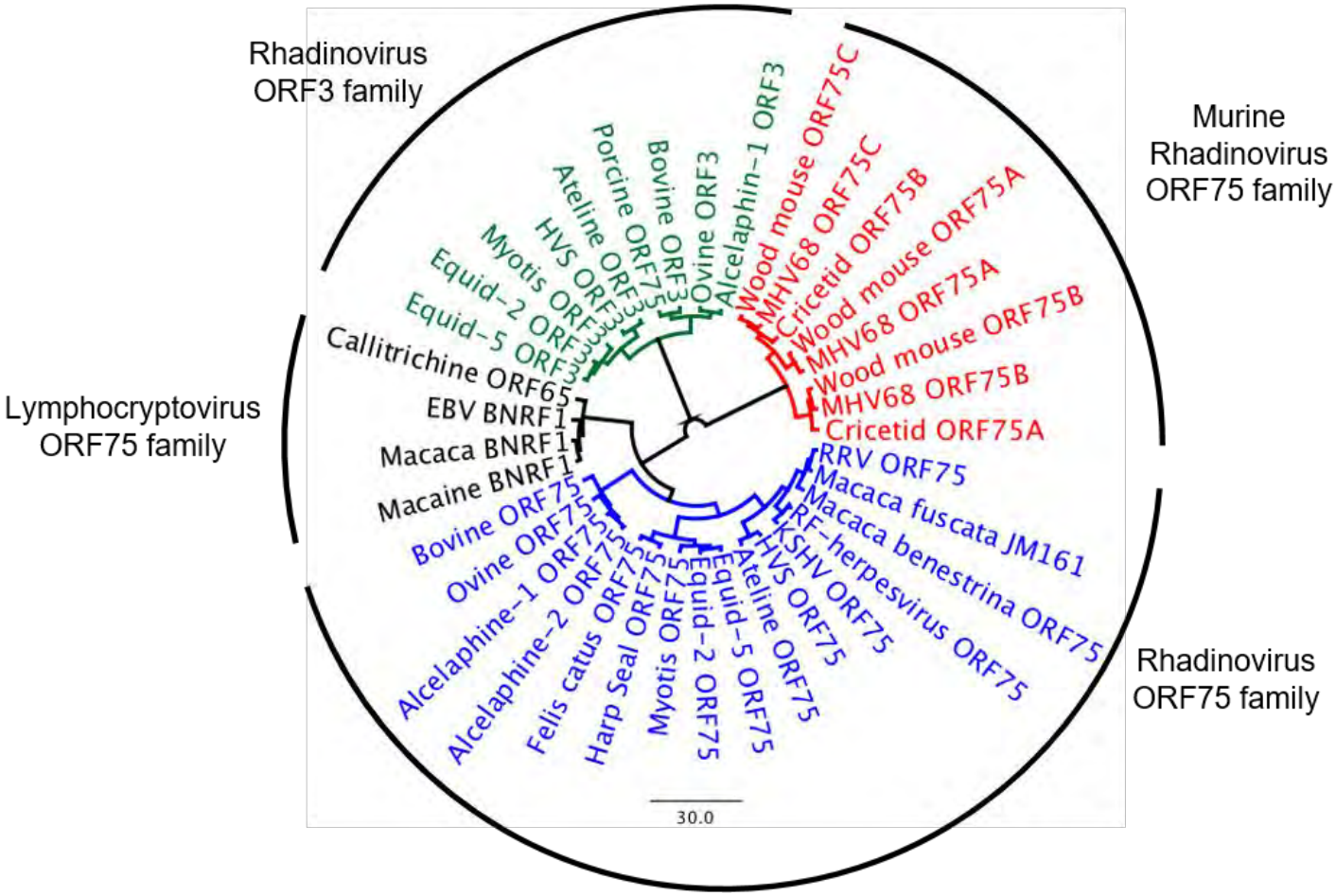
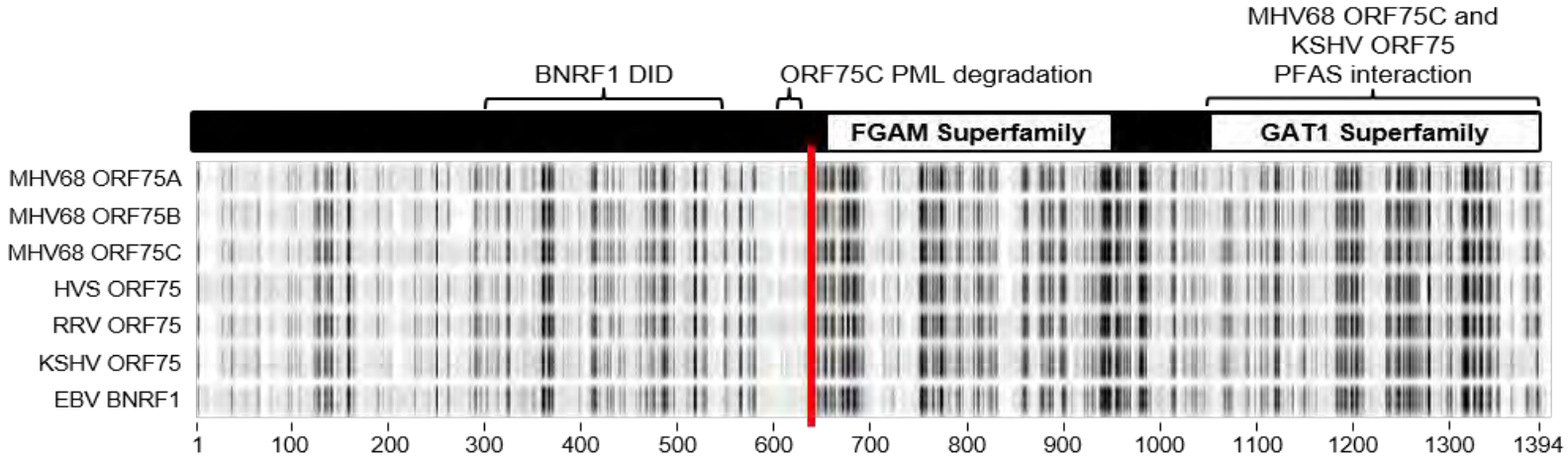
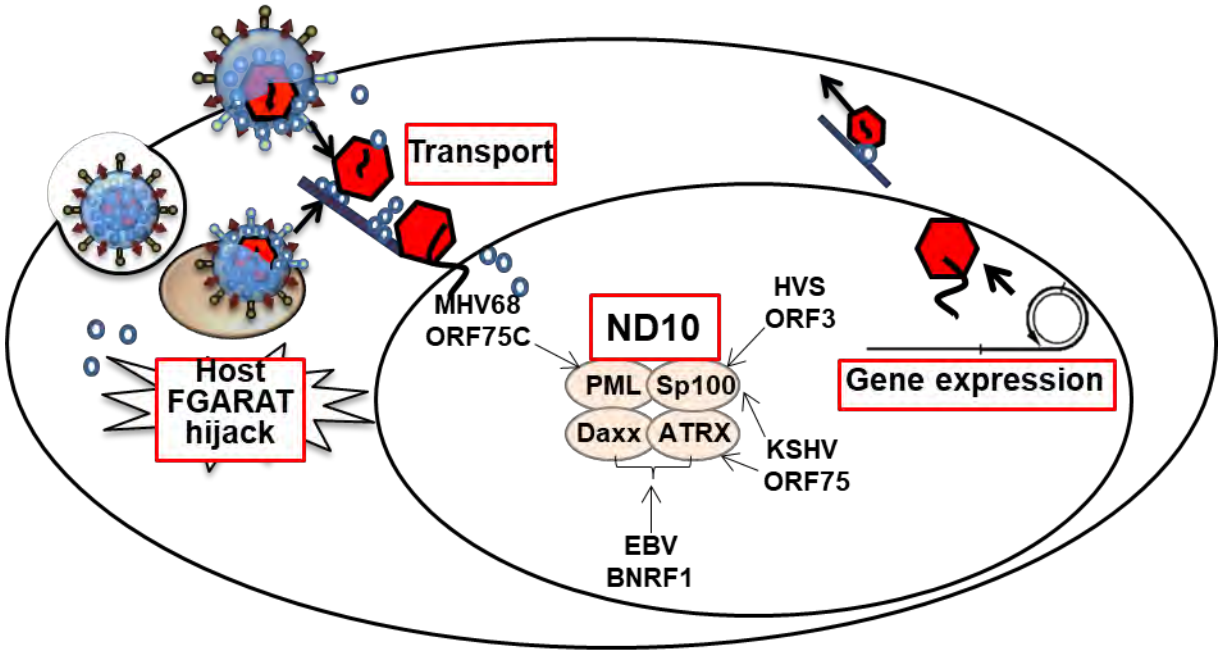
vFGARATs are a family of tegument proteins unique to gHV



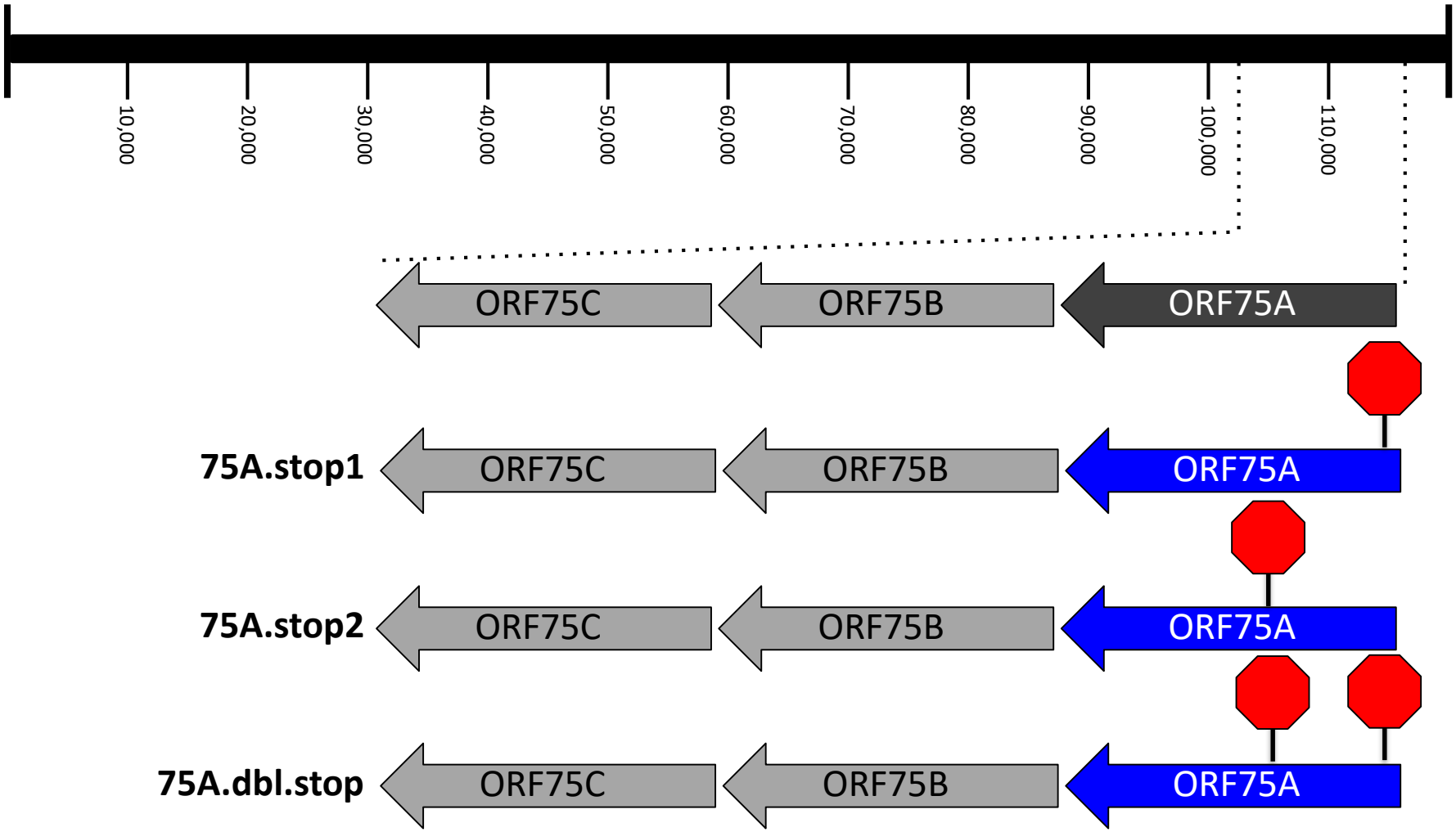
- Large 150 kDa tegument proteins <u>unique to gammaherpesviruses</u>
- Sequence homology to host FGARAT (formyl-glycineamide-phosphoribosyl-synthetase)
- Host FGARATs coordinate the 4th step in *de novo* purine synthesis
- vFGARATs do not retain catalytic FGARAT activity

vFGARATs are a family of tegument proteins unique to gHV

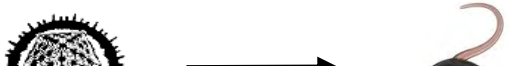




Recombinant ORF75A viruses used in this study

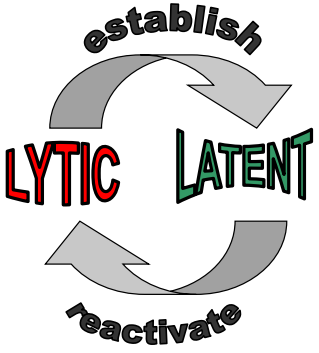


Murine gammaherpesvirus 68 pathogenesis in mice



Fibroblasts

Fibroblasts



Macrophage

B lymphocyte

memory
B lymphocyte

ıocyte

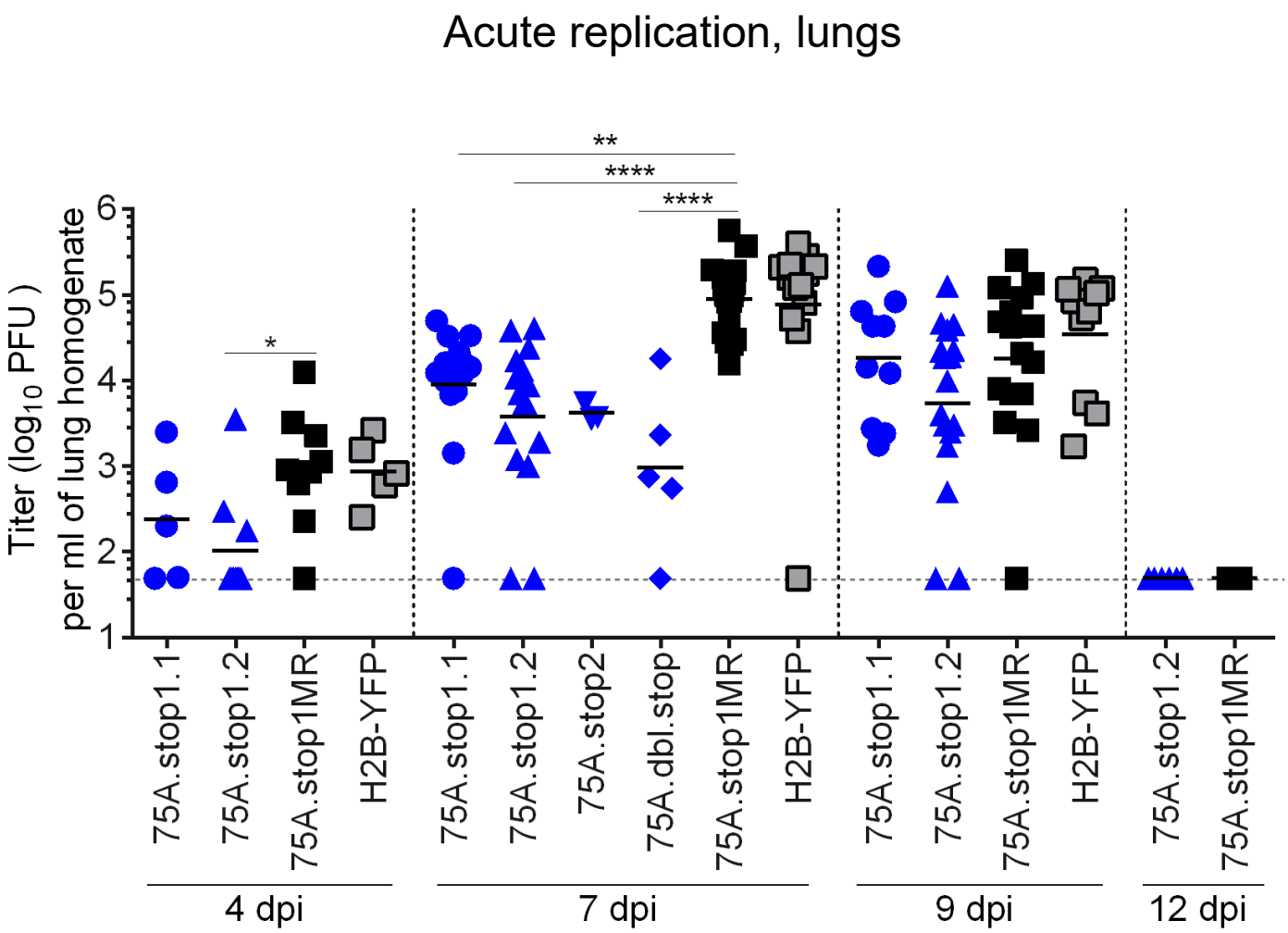
Dendritic cell

Epithelial and endothelial cells

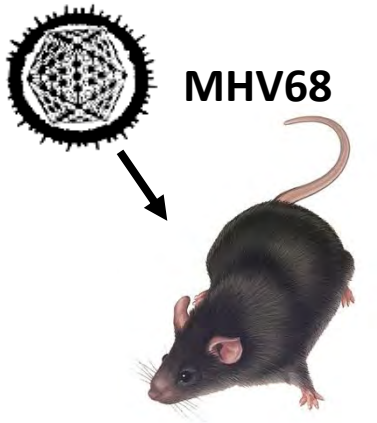
Epithelial

Lymphomas

ORF75A promotes replication in culture and in the respiratory tract

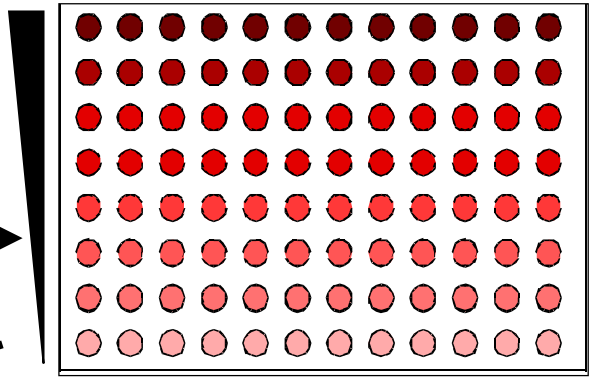


Experimental Approaches



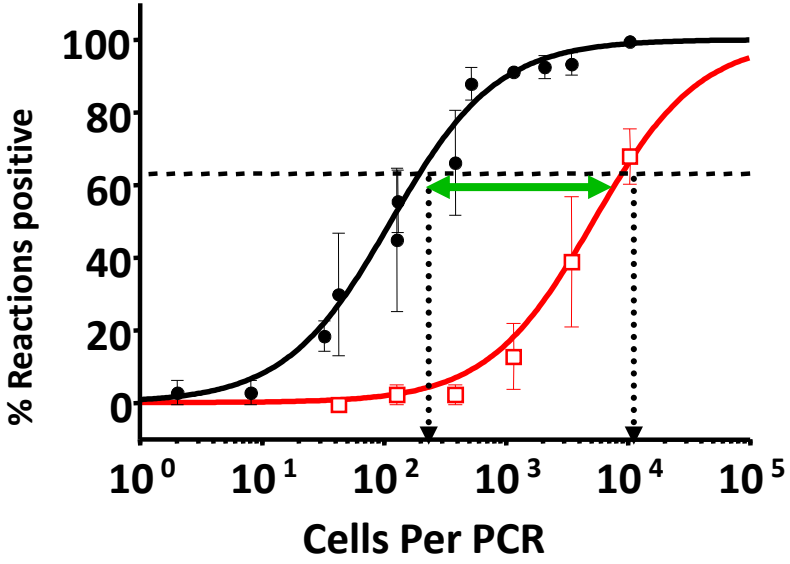
16 dpi

Splenocytes



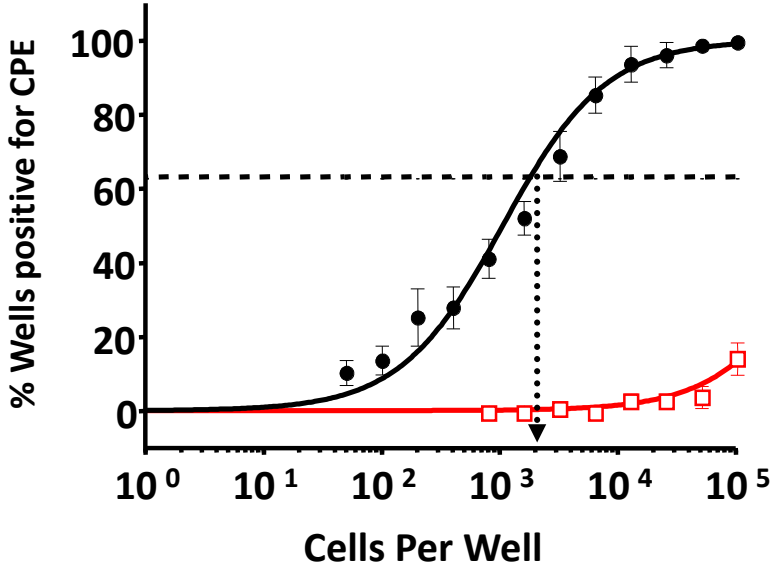
Frequency of Latency:

Limiting Dilution PCR

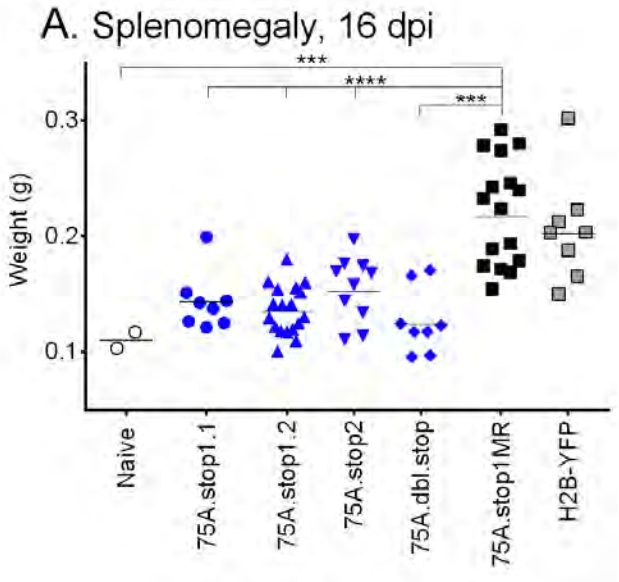


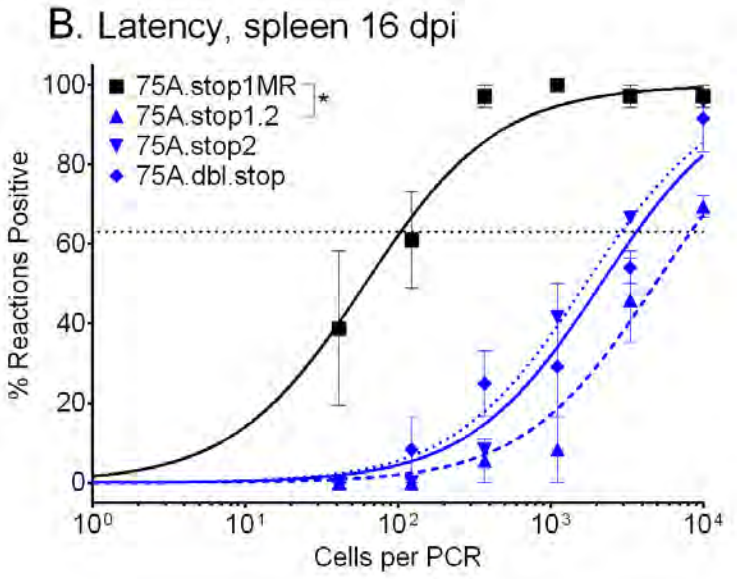
Frequency of Reactivation:

Limiting Dilution on Indicator MEFs

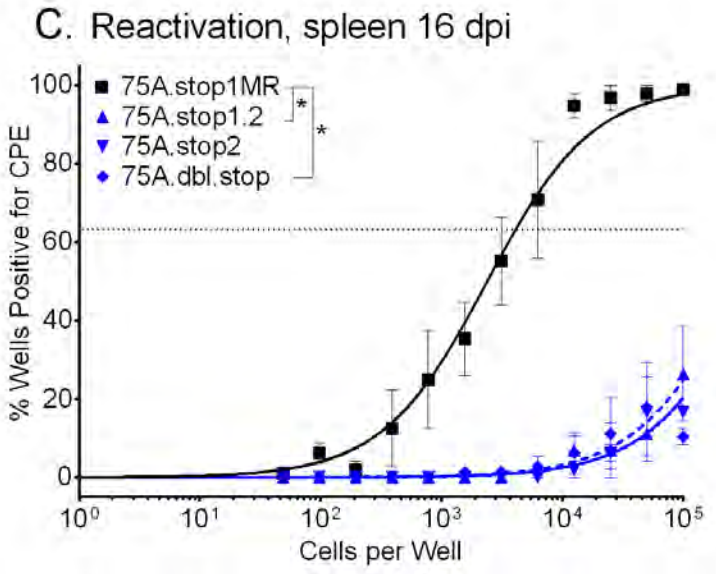


ORF75A promotes latency in spleens of infected mice

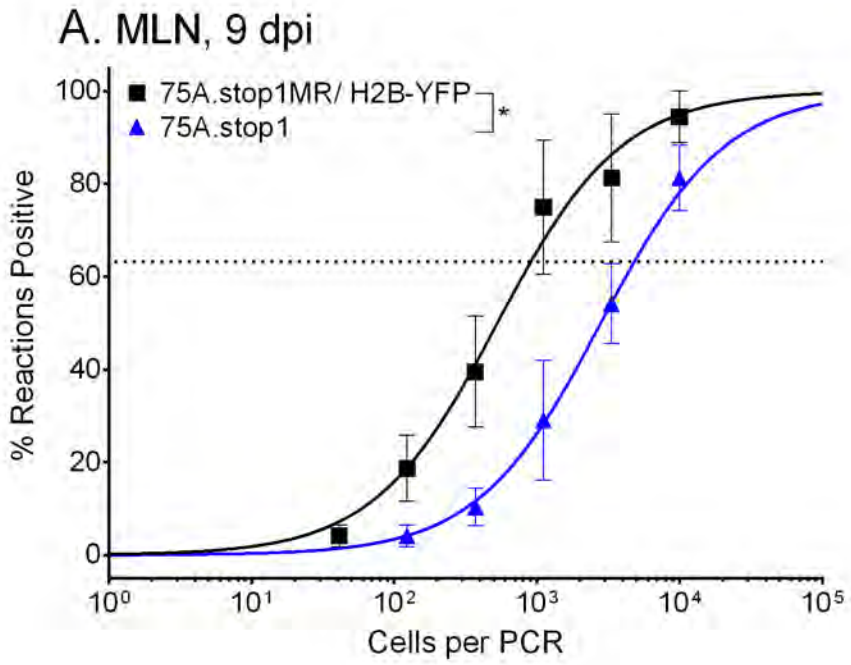


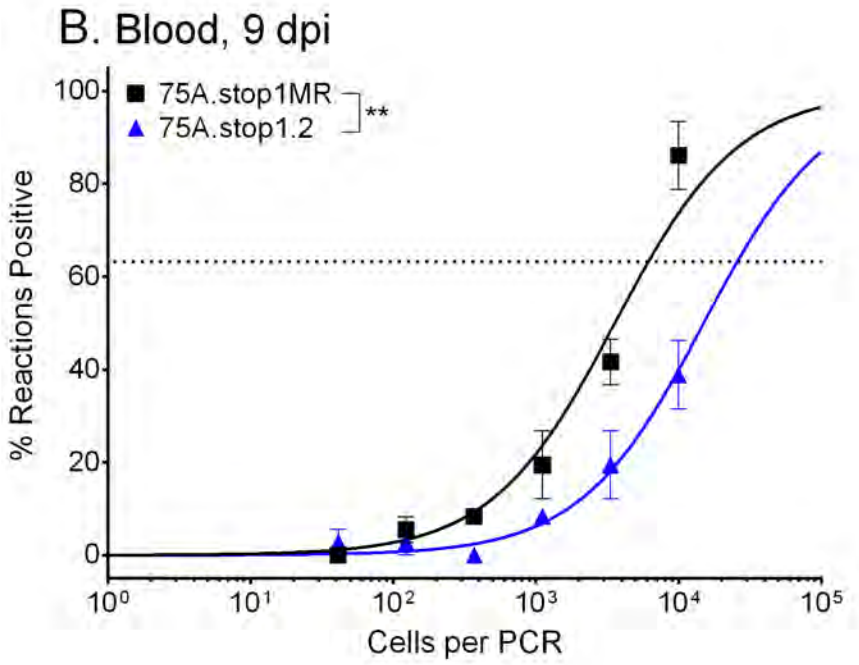


Intranasal inoculation



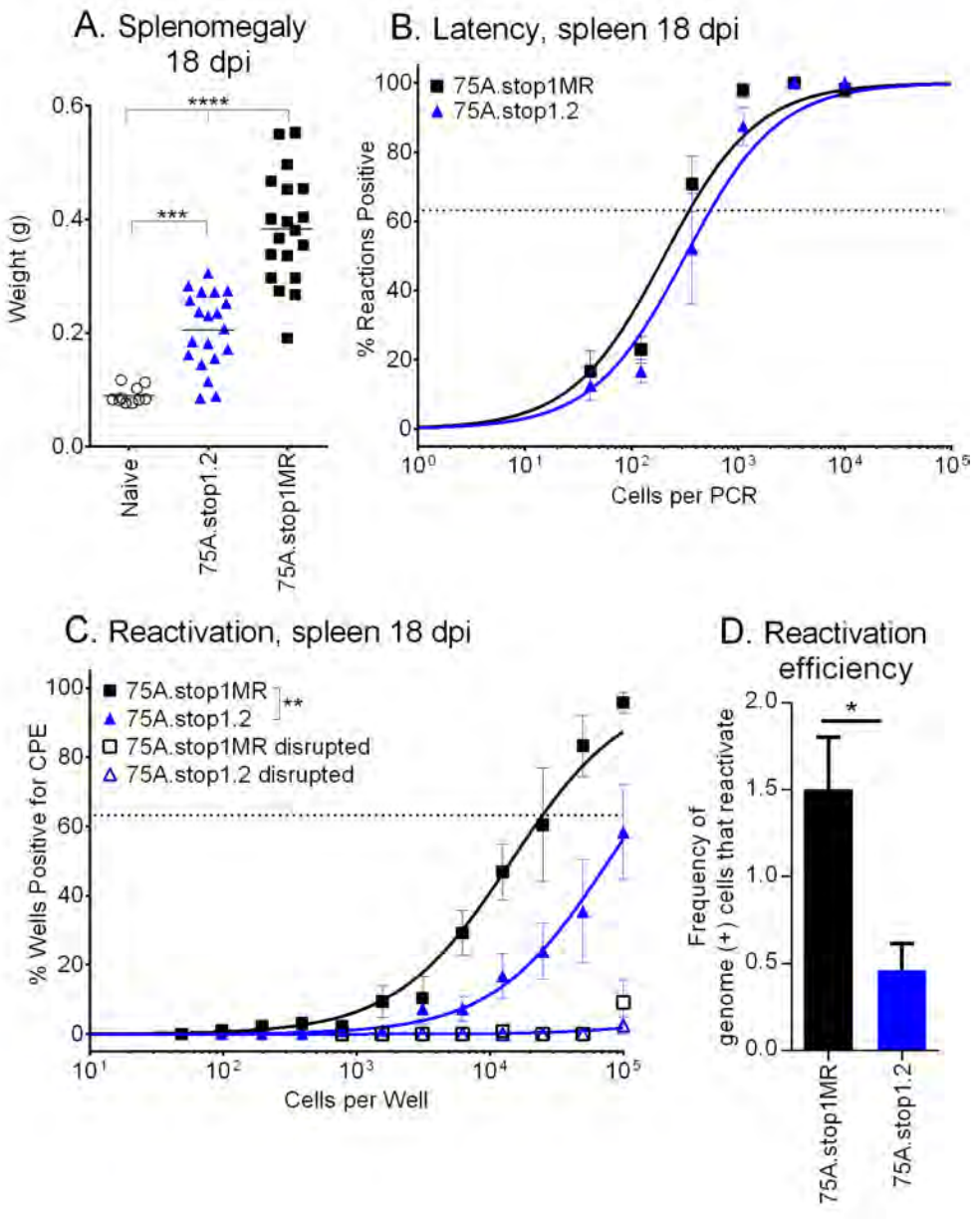
Tissue-specific roles for ORF75A in the establishment of latency





Tissue-specific roles for ORF75A in the establishment of latency

Intraperitoneal inoculation



Summary of 75A.stop virus *in vivo*

Intranasal

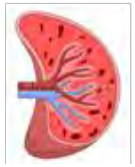
Lungs

Replication ↓

MLN

Blood

Spleen



Seeding of multiple reservoirs ↓

Intraperitoneal

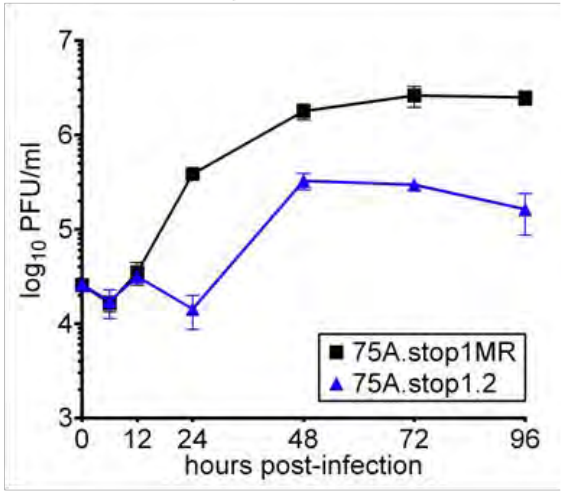
Spleen

Latency ✓

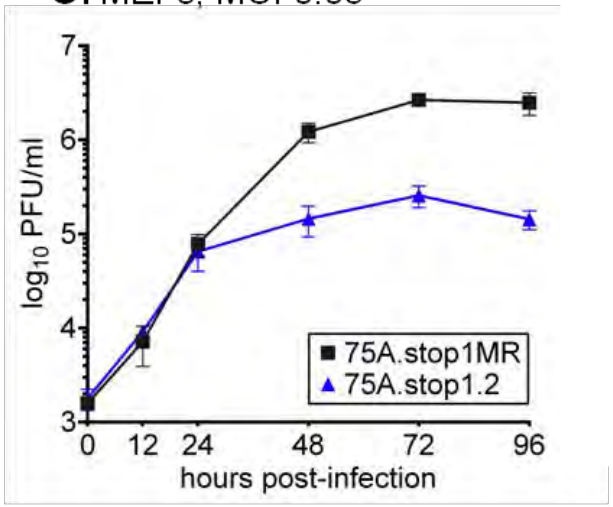
Reactivation ↓

Loss of ORF75A impairs replication and enhances protein expression

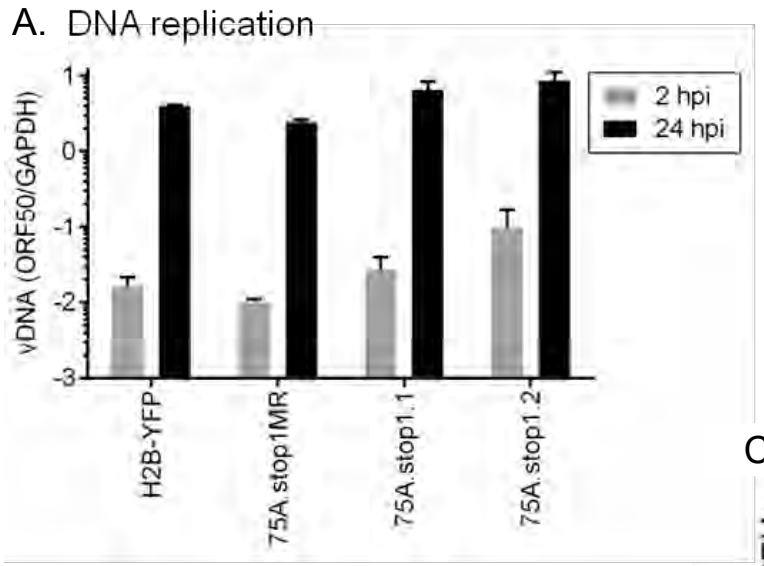
A. BMDMs, MOI 5

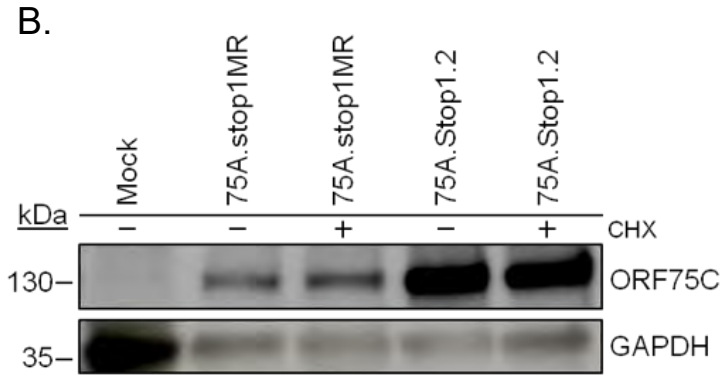


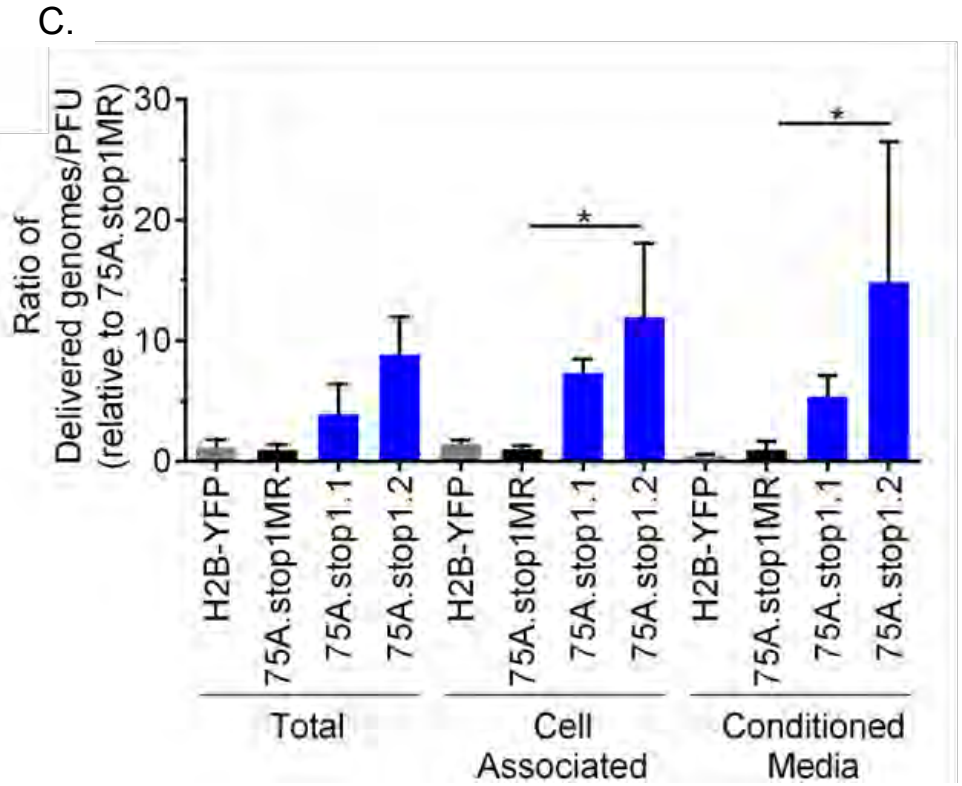
C. MEFs, MOI 0.05



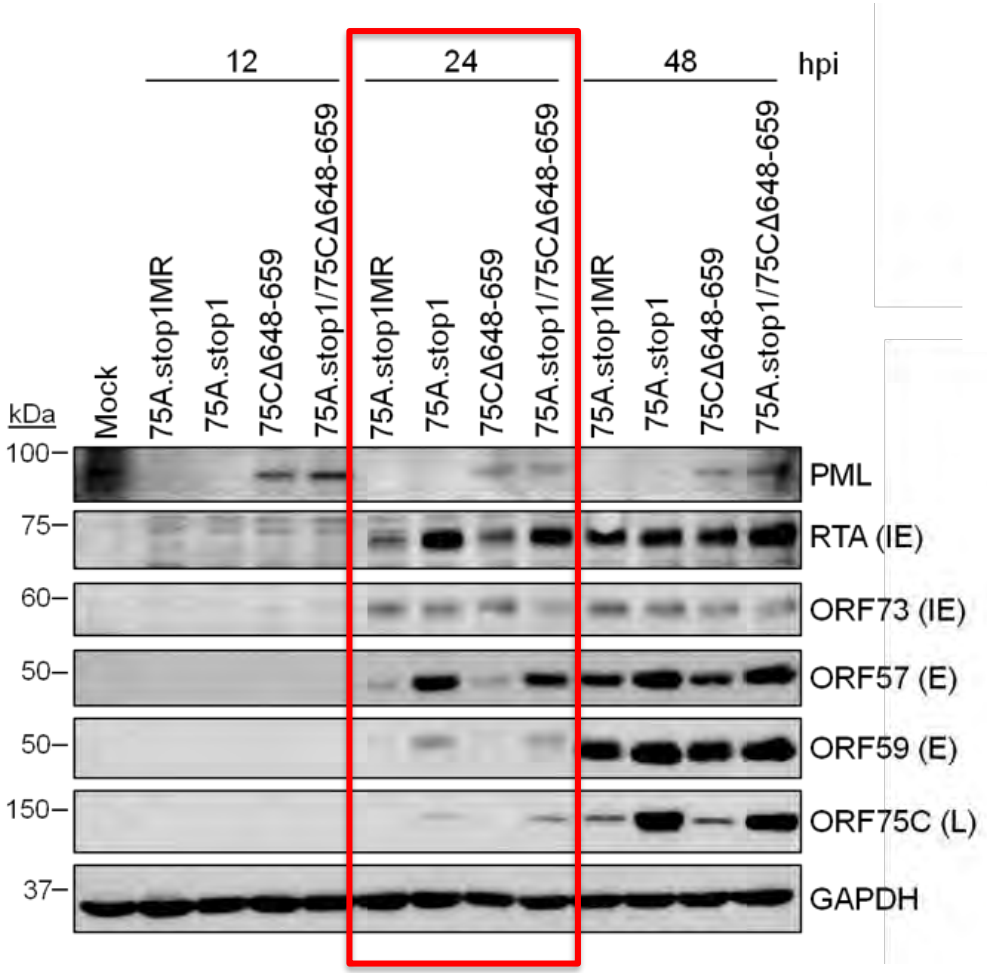
Loss of ORF75A decreases the specific infectivity of MHV68





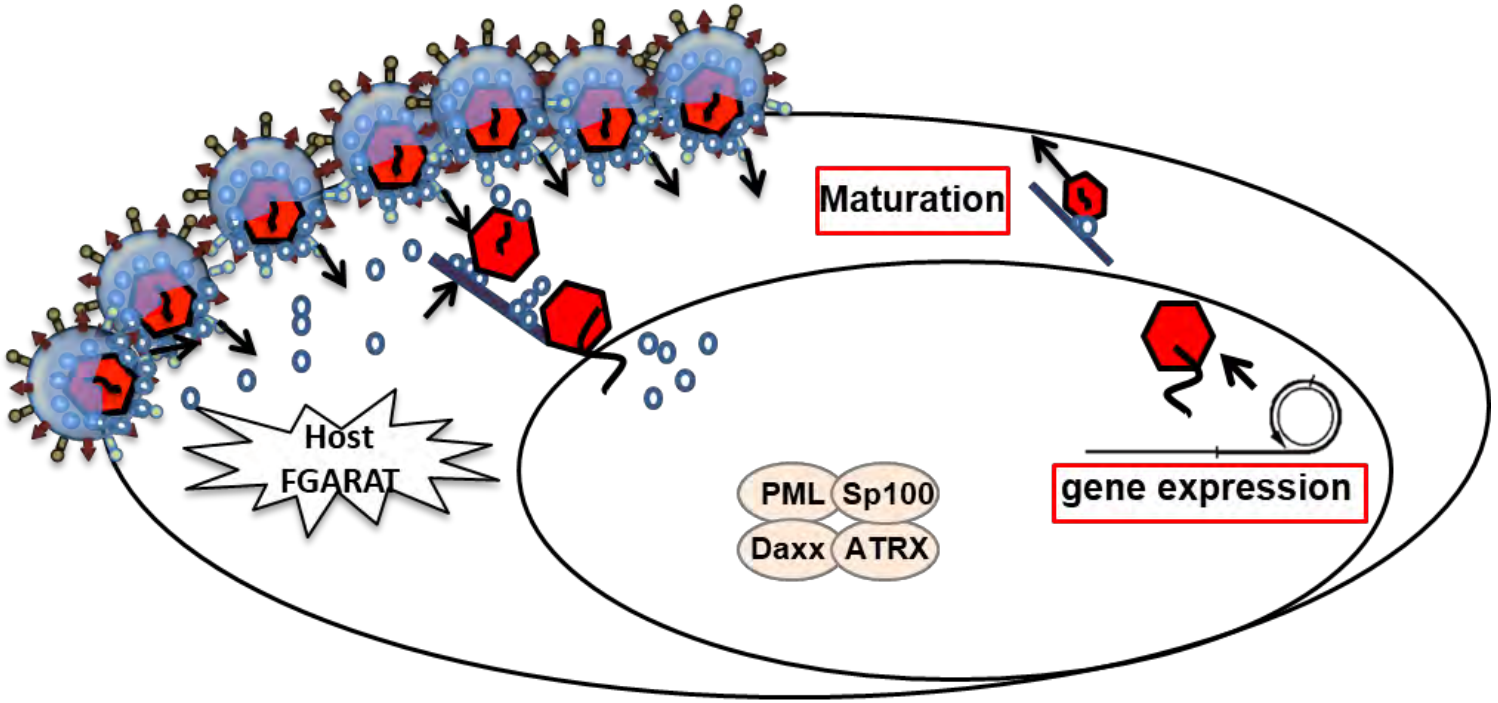


Enhanced viral protein expression and replication are independent from the PML degradation function of ORF75C



Model for ORF75A function in cell culture

- ORF75A does not primarily localize to the nucleus during infection.
- The loss of ORF75A is characterized by a decrease in viral replication, but an increase in viral protein expression.
- Loss of ORF75A leads to increased deposition of ORF75C at early time points
- Loss of ORF75A leads to a ten-fold decrease in the specific infectivity of ORF75A.stop viruses.
- The defects in viral replication and protein expression are independent from the PML degradation function of ORF75C.



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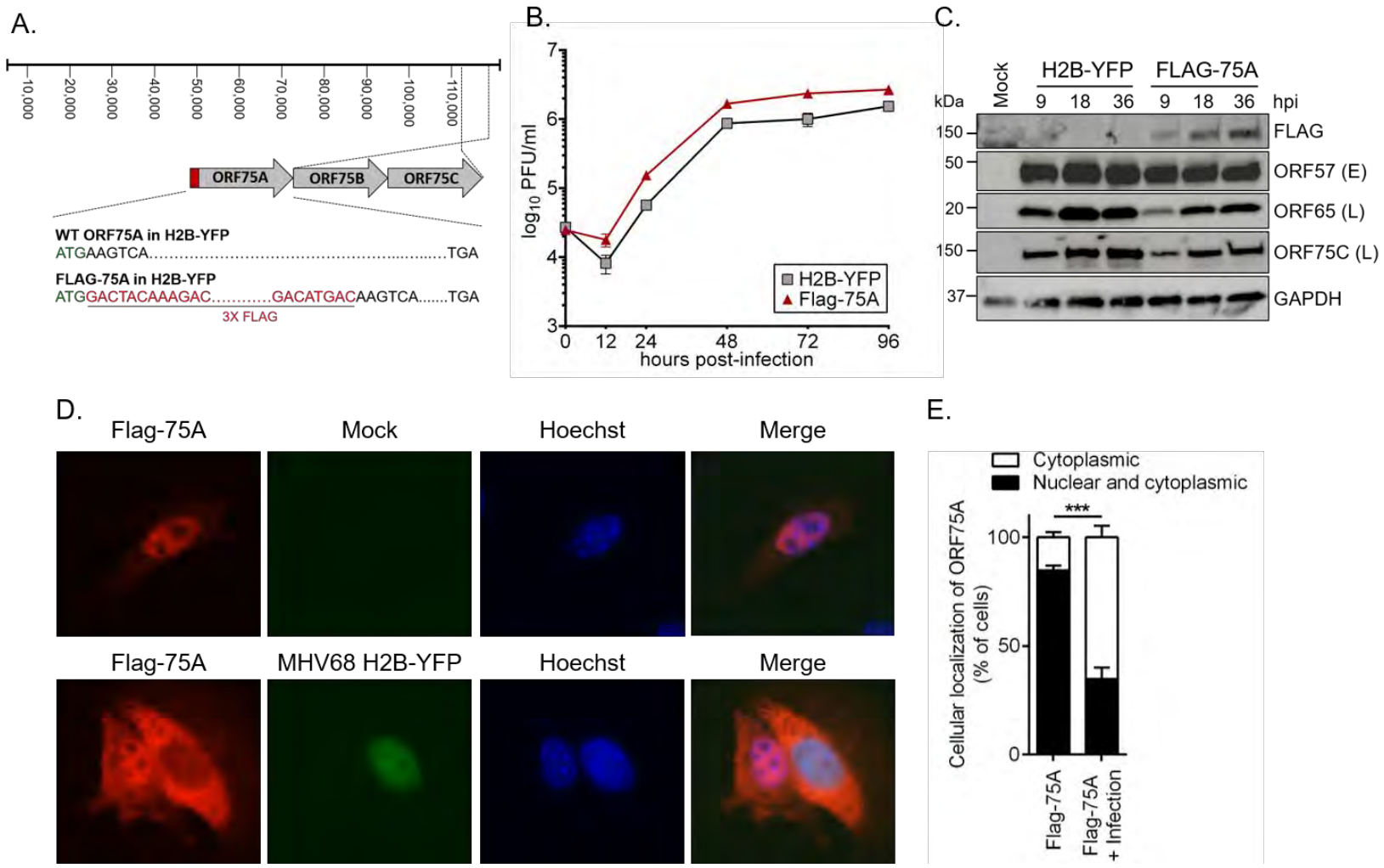
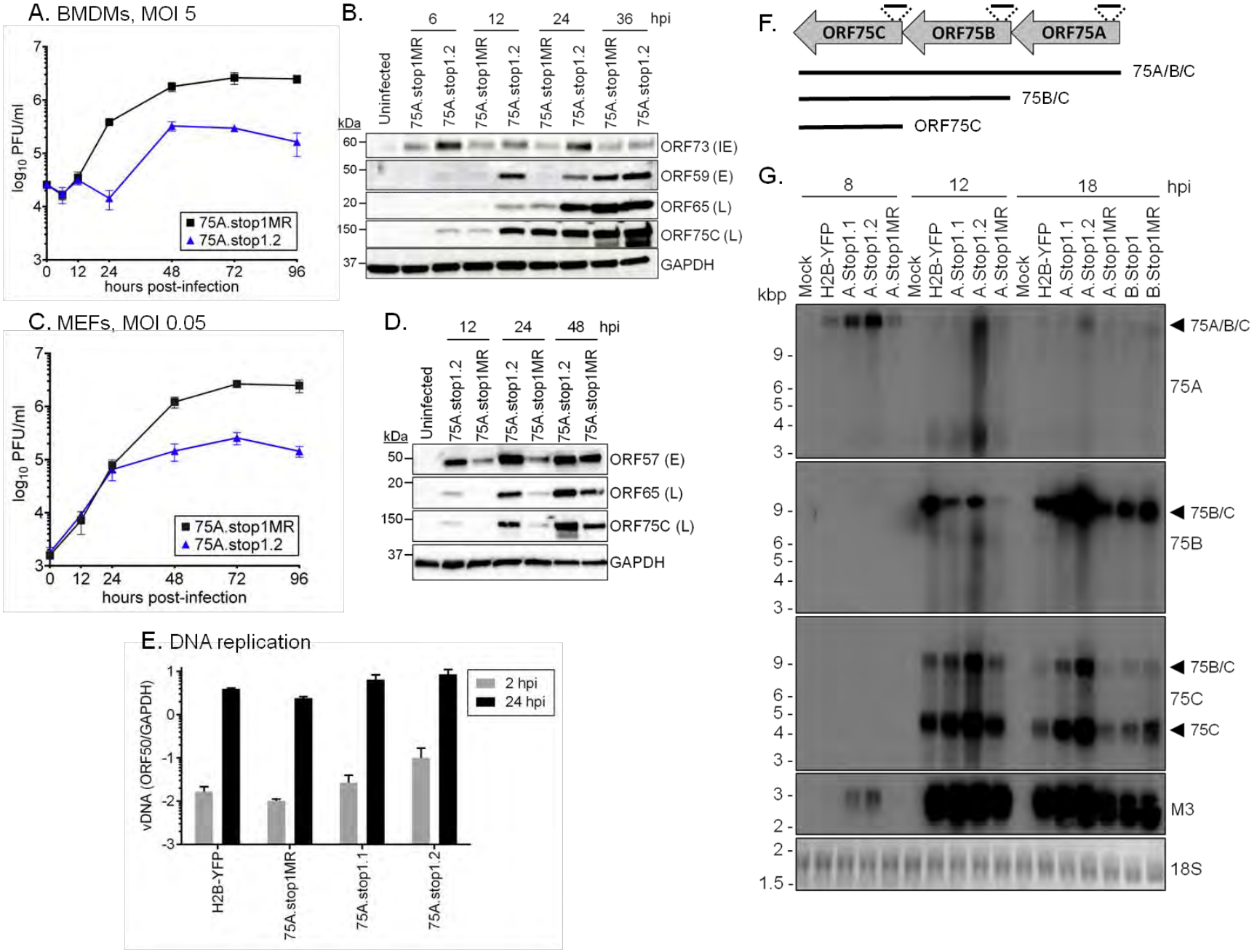
ORF75A is expressed during lytic infection




A. BMDMs, MOI 5
log10 PFU/ml
hours post-infection
75A.stop1MR
75A.stop1.2
B.
6
12
24
36
hpi
Uninfected
75A.stop1MR
75A.stop1.2
kDa
ORF73 (IE)
ORF59 (E)
ORF65 (L)
ORF75C (L)
GAPDH
C. MEFs, MOI 0.05
D.
12
24
48
hpi
ORF57 (E)
E. DNA replication
vDNA (ORF50/GAPDH)
2 hpi
24 hpi
H2B-YFP
75A.stop1MR
75A.stop1.1
75A.stop1.2
F.
ORF75C
ORF75B
ORF75A
75A/B/C
75B/C
ORF75C
G.
8
12
18
hpi
kbp
Mock
H2B-YFP
A.Stop1.1
A.Stop1.2
A.Stop1MR
B.Stop1
B.Stop1MR
75A/B/C
75A
75B/C
75B
75C
M3
18S