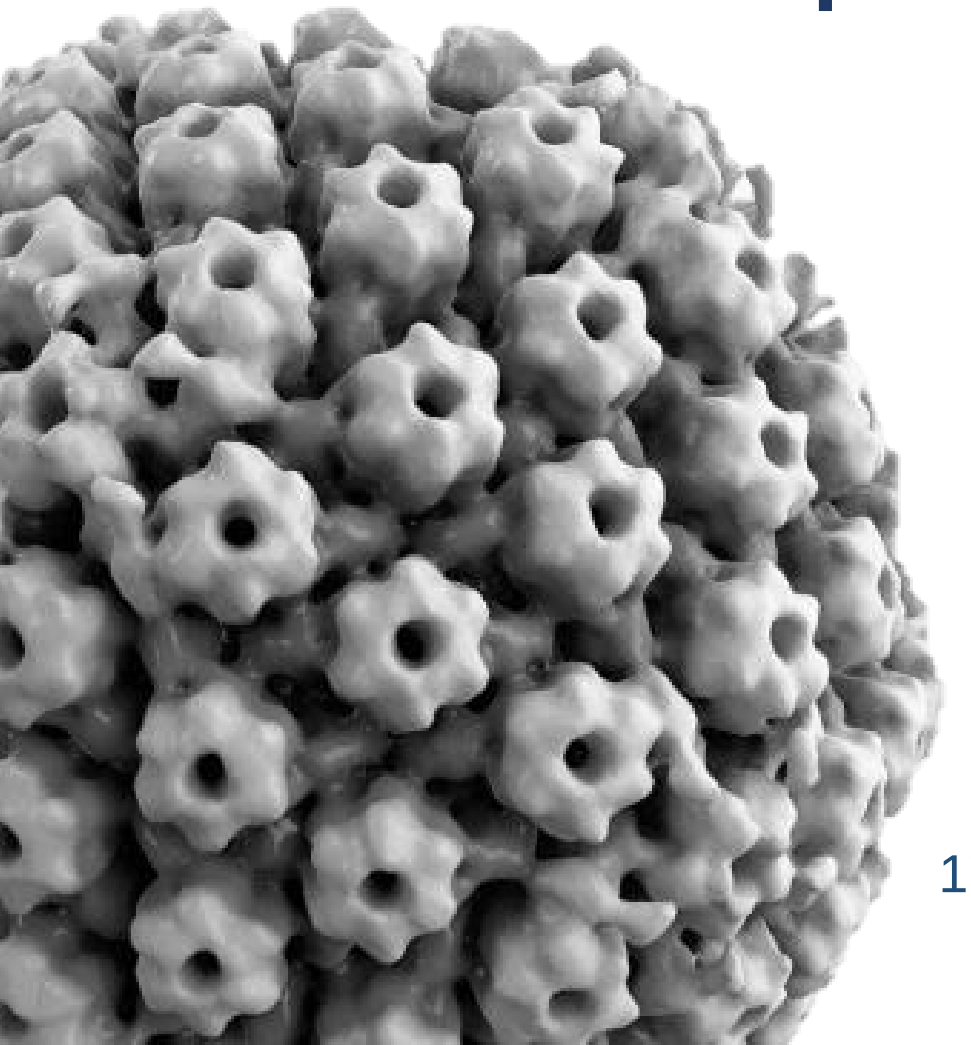
Searching for Substrates of the ICP0 Ubiquitin E3 Ligase of HSV-1



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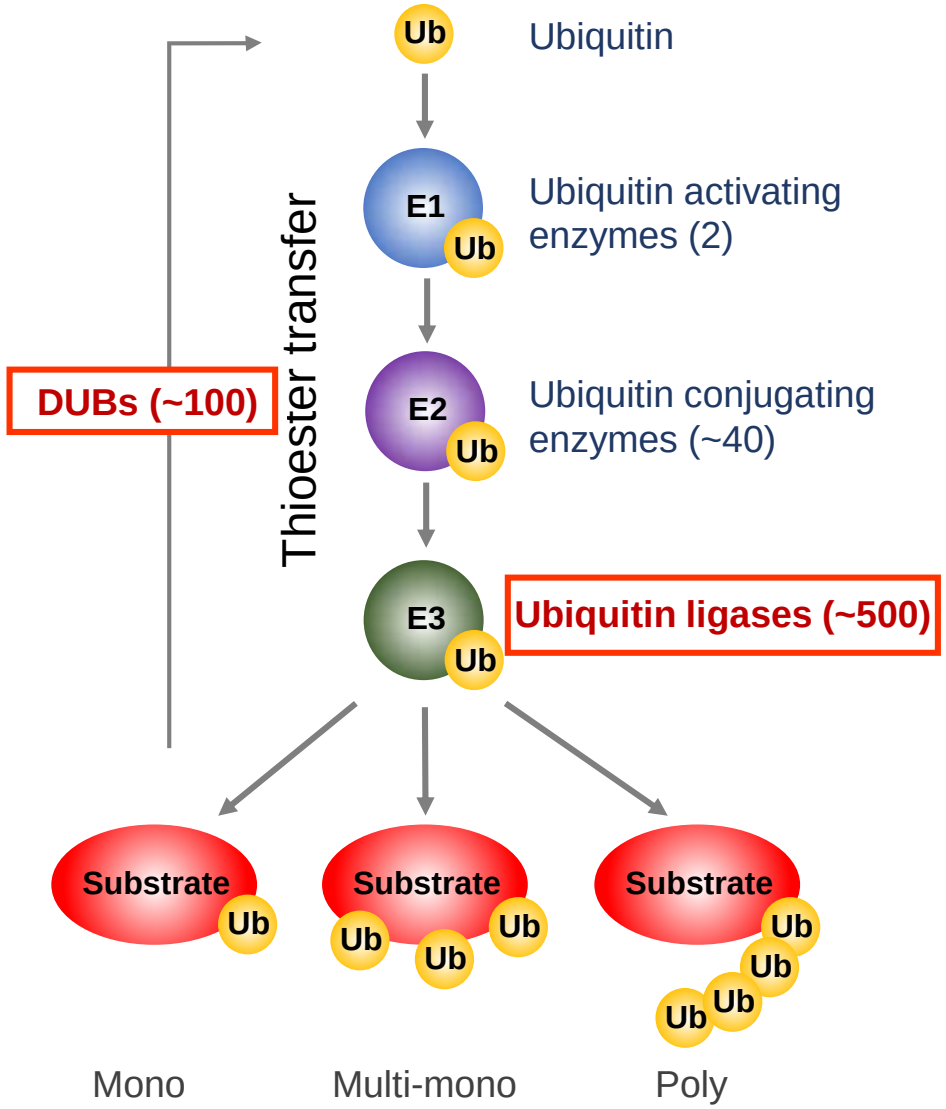
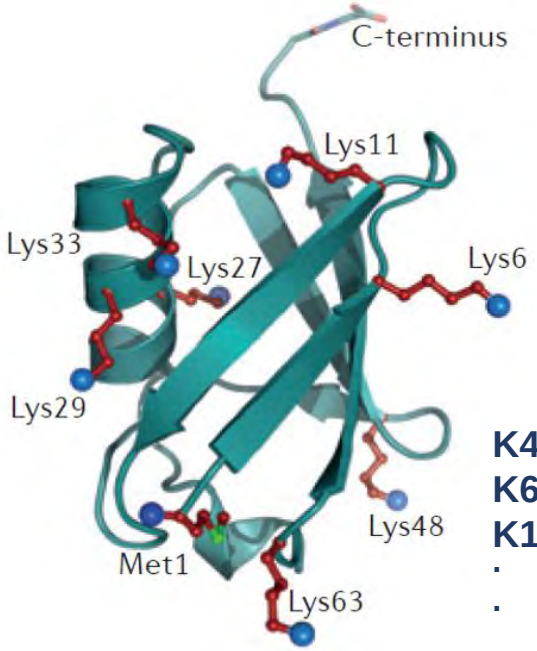
17th Annual Herpesvirus: Pathogenesis and Cancer Symposium

Ubiquitination





K48-linked: Proteasomal degradation
K63-linked: Cell signaling, DNA damage response
K11-linked: Cell cycle regulation
.
.

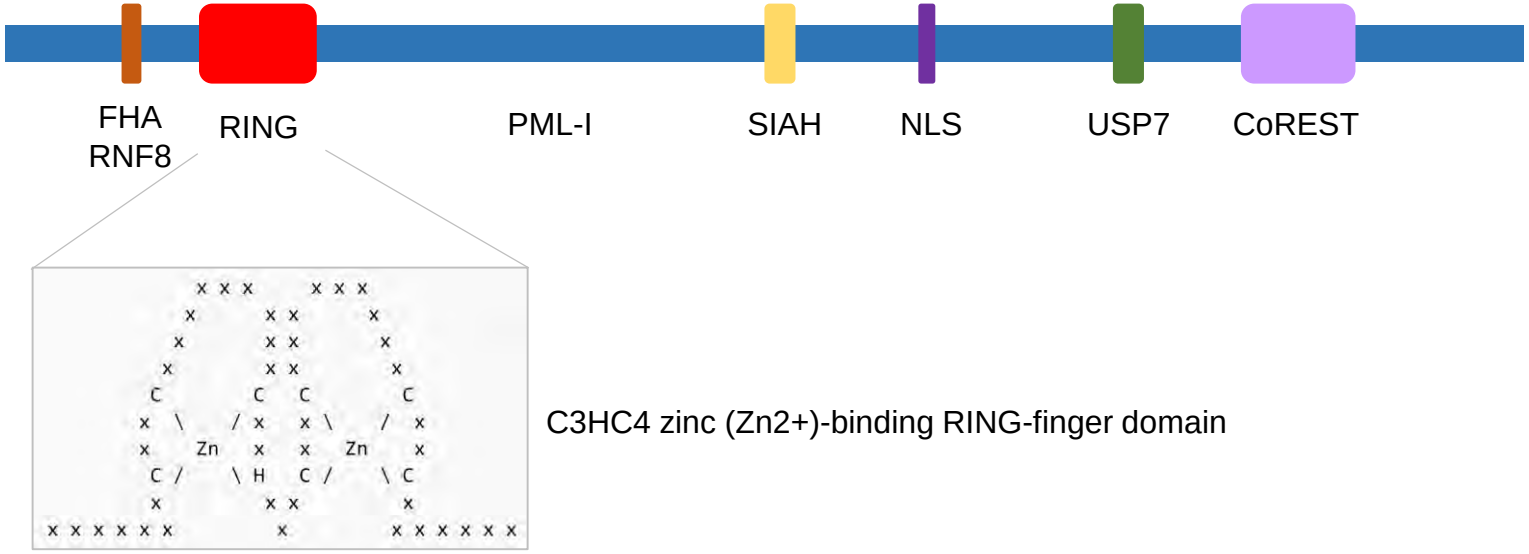
Many viruses encode E3s and DUBs
→ viral exploitation of the host ubiquitin system

HSV-1 proteins

- ICP0 - E3 ligase
- UL36 - DUB

ICP0 (Infected Cell Protein 0)

- An **immediate-early** protein of HSV-1
- A viral **transactivator**: a key regulator of the lytic phase and reactivation from latency
- An **ubiquitin E3 ligase** (RING finger type) for **proteasomal degradation**
- **Counteracts host intrinsic** and **innate immunity**
- ICP0 functions are dependent on its E3 ligase activity

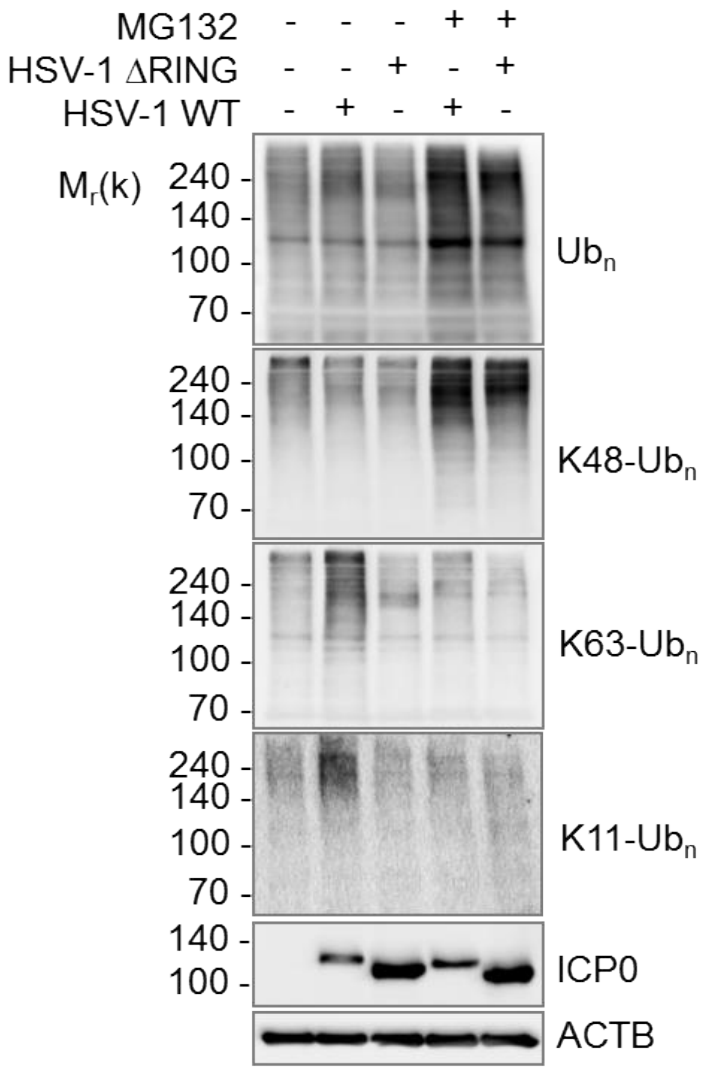


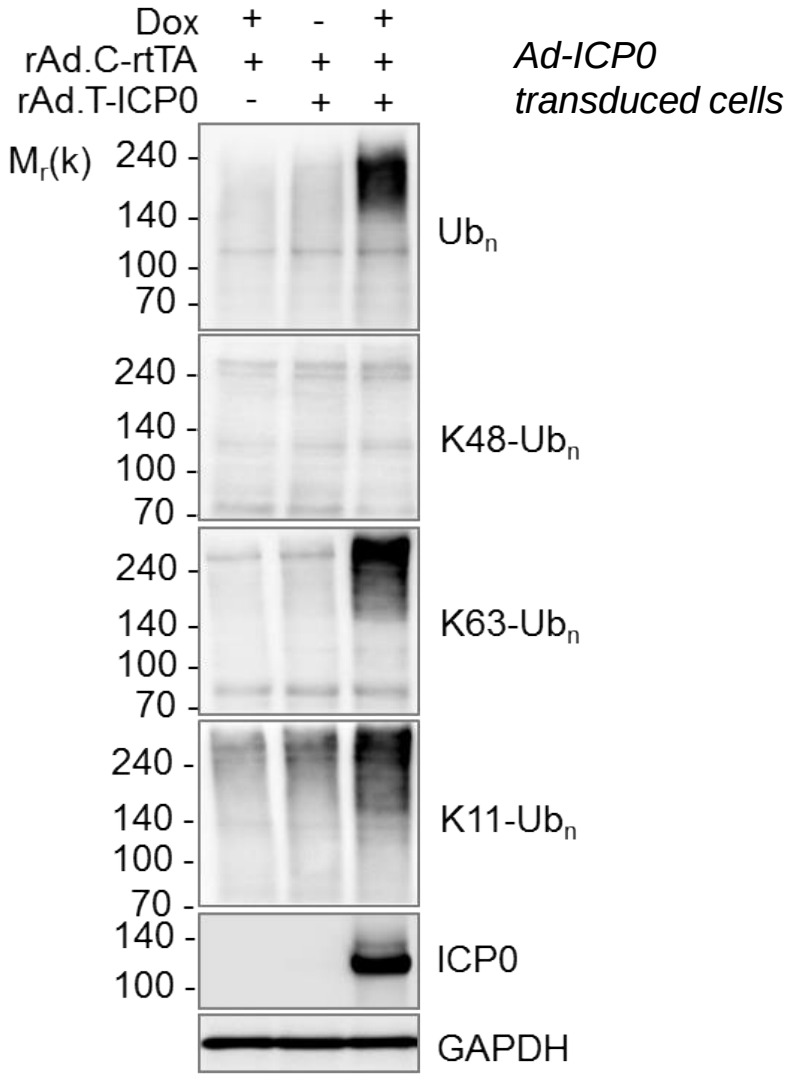
C3HC4 zinc (Zn2+)-binding RING-finger domain

Degradable targets of ICP0

- PML
- IFI16
- RNF8
- RNF168
- USP7
- IκBα
- E2FBP1
- CENPs
- SUMO
- TRIM27

ICP0 increases ubiquitin chains with multiple linkages





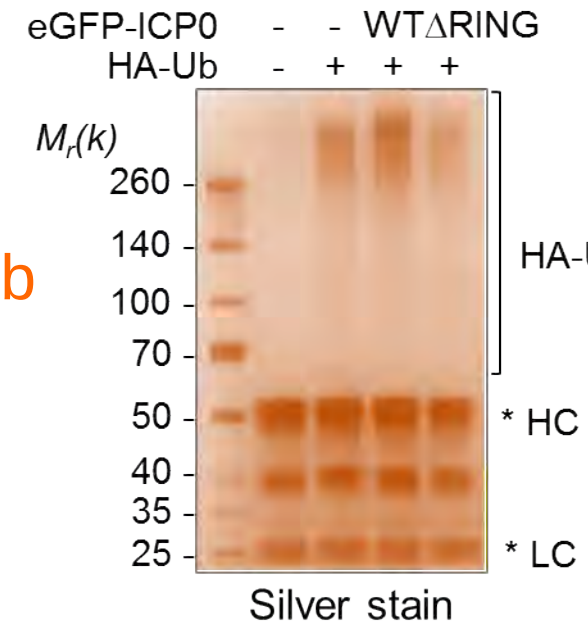
I. Searching for Non-Proteolytic Substrates of ICP0

II. Searching for Proteolytic Substrates of ICP0

Identification of ICP0-mediated Ubiquitin targets

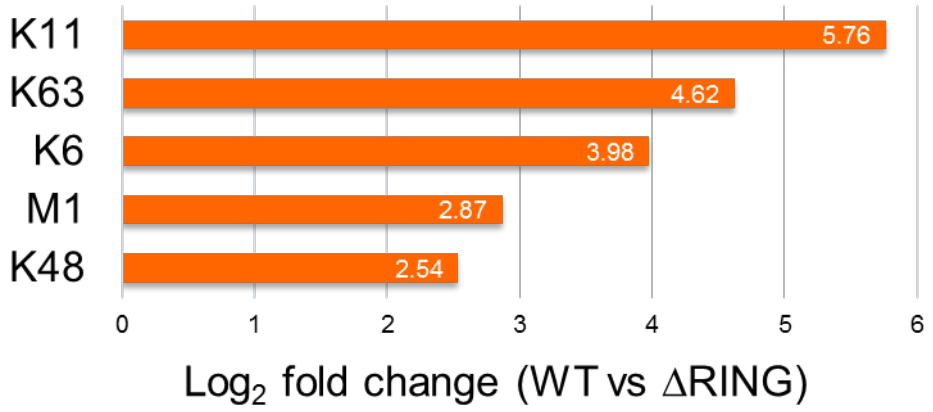
Cotransfection
GFP-ICP0 & HA-Ub

IP: HA-Ub

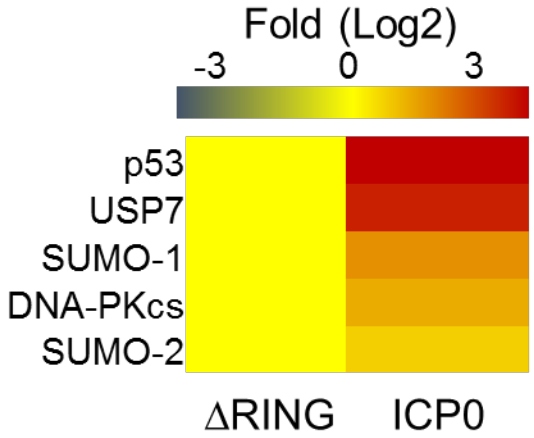


LC-MS/MS

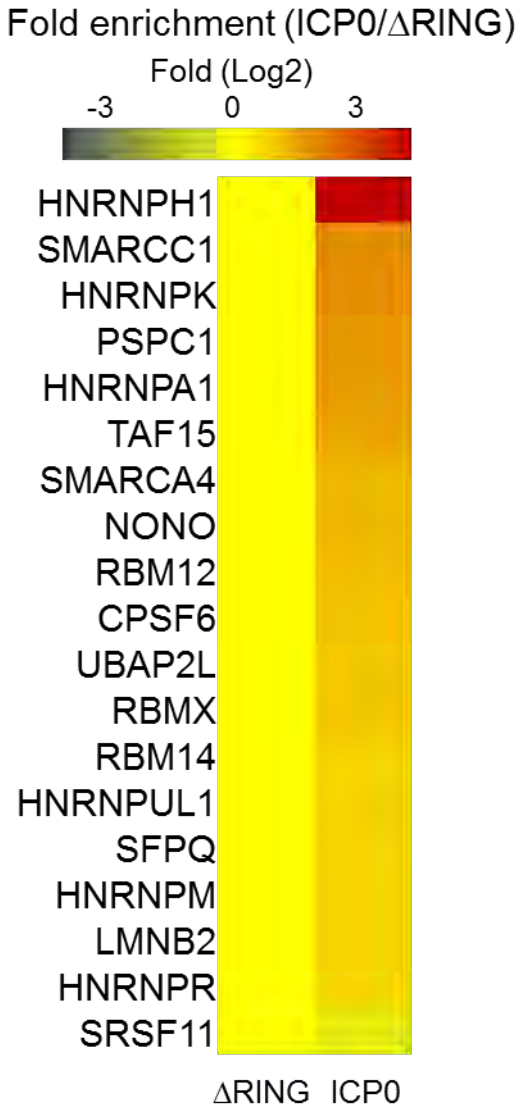
Ub linkages enriched by ICP0

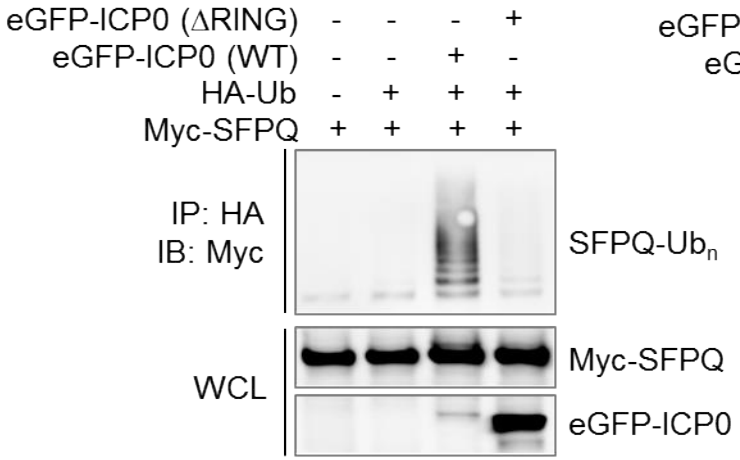


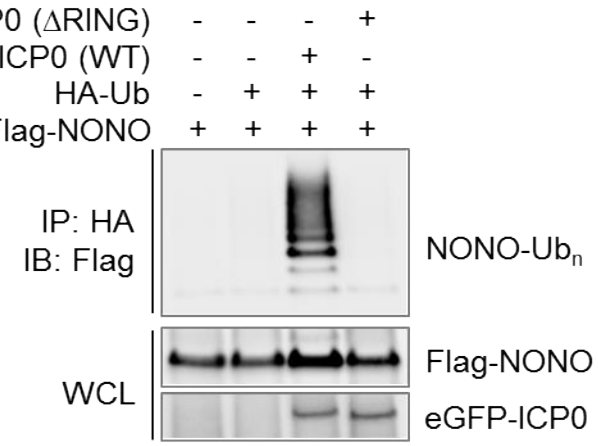
Previously known ICP0 substrates

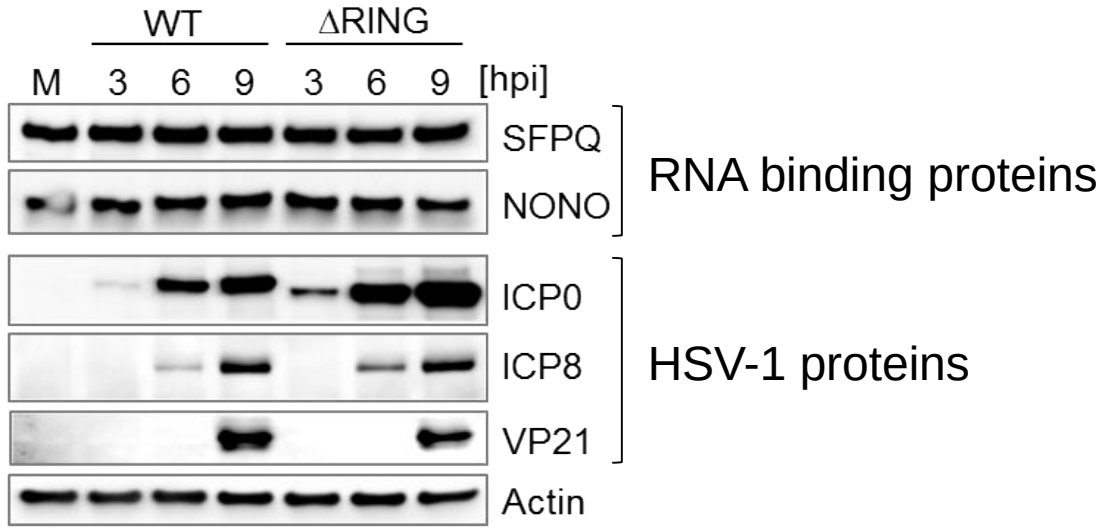


RNA binding proteins within the ICP0-mediated ubiquitome







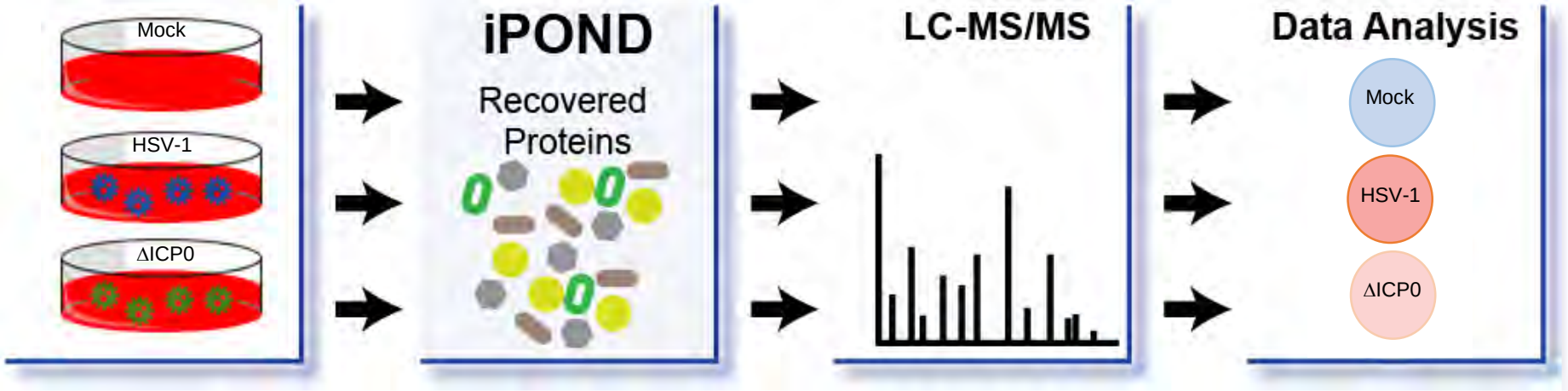


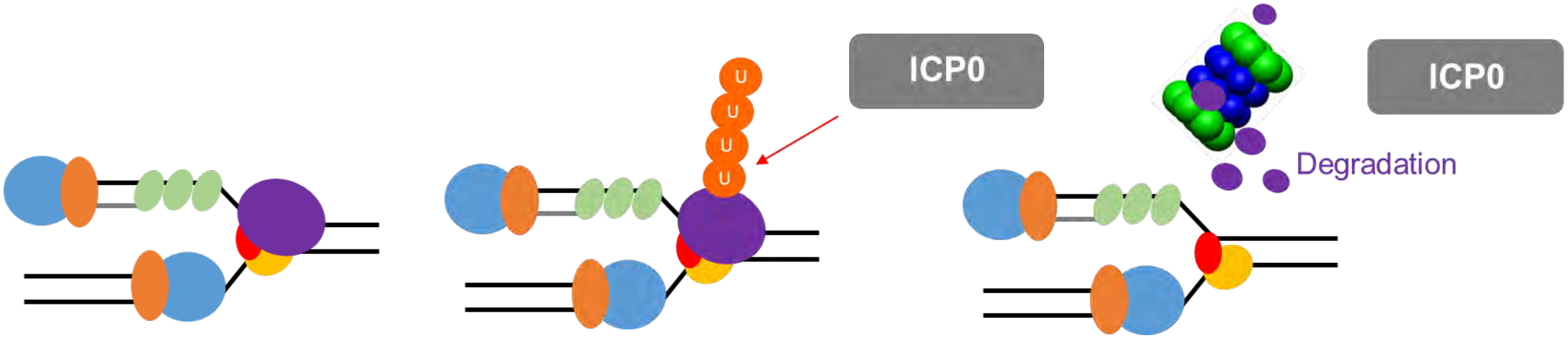
I. Searching for Non-Proteolytic Substrates of ICP0

II. Searching for Proteolytic Substrates of ICP0

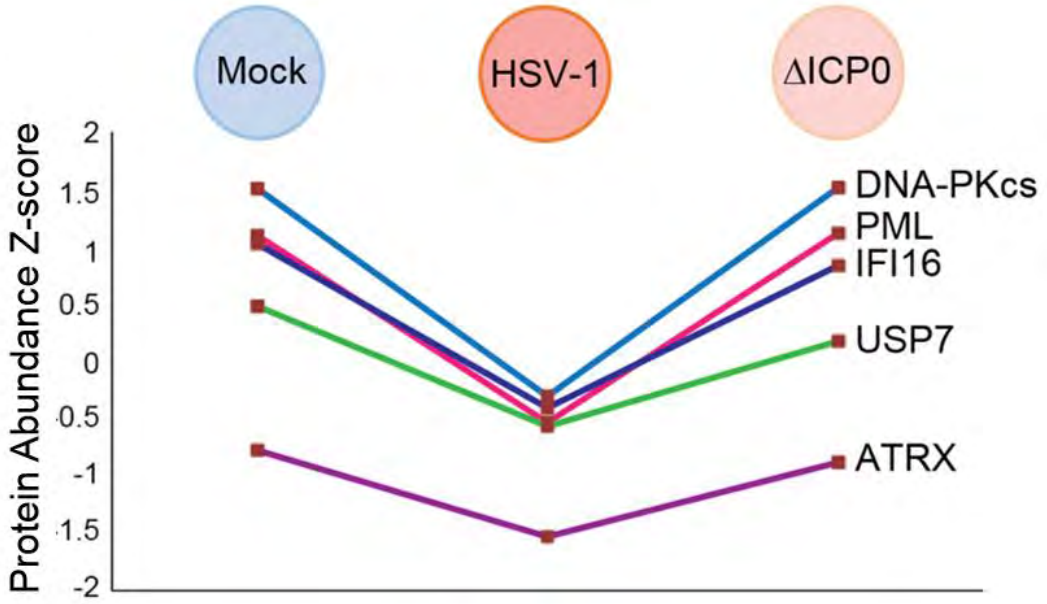
Identification of proteins associated with replicating viral DNA using iPOND (isolation of proteins on nascent DNA)

Pull-down of EdU-labeled viral DNA and protein complex

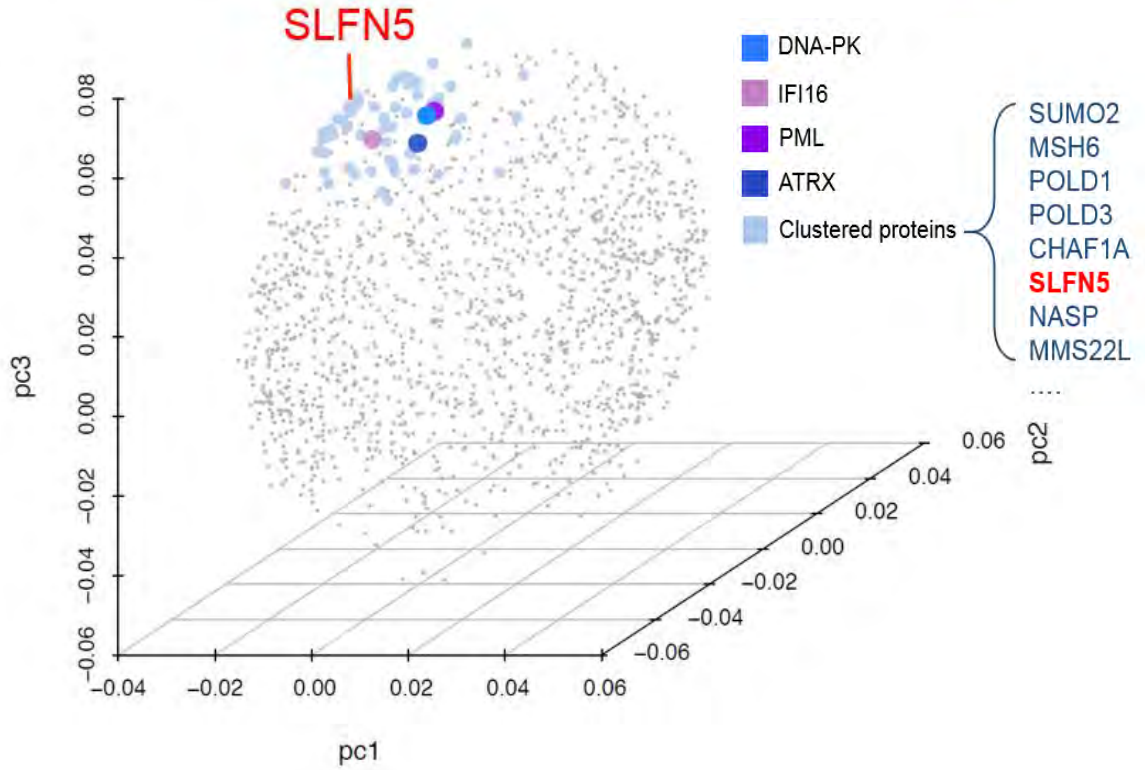




PCA clustering analysis of iPOND-proteome data uncovered SLFN5 as a novel degradation substrate for ICP0



Known ICP0 substrates are decreased on WT HSV genomes compared to HSV ΔICP0 mutant virus genomes

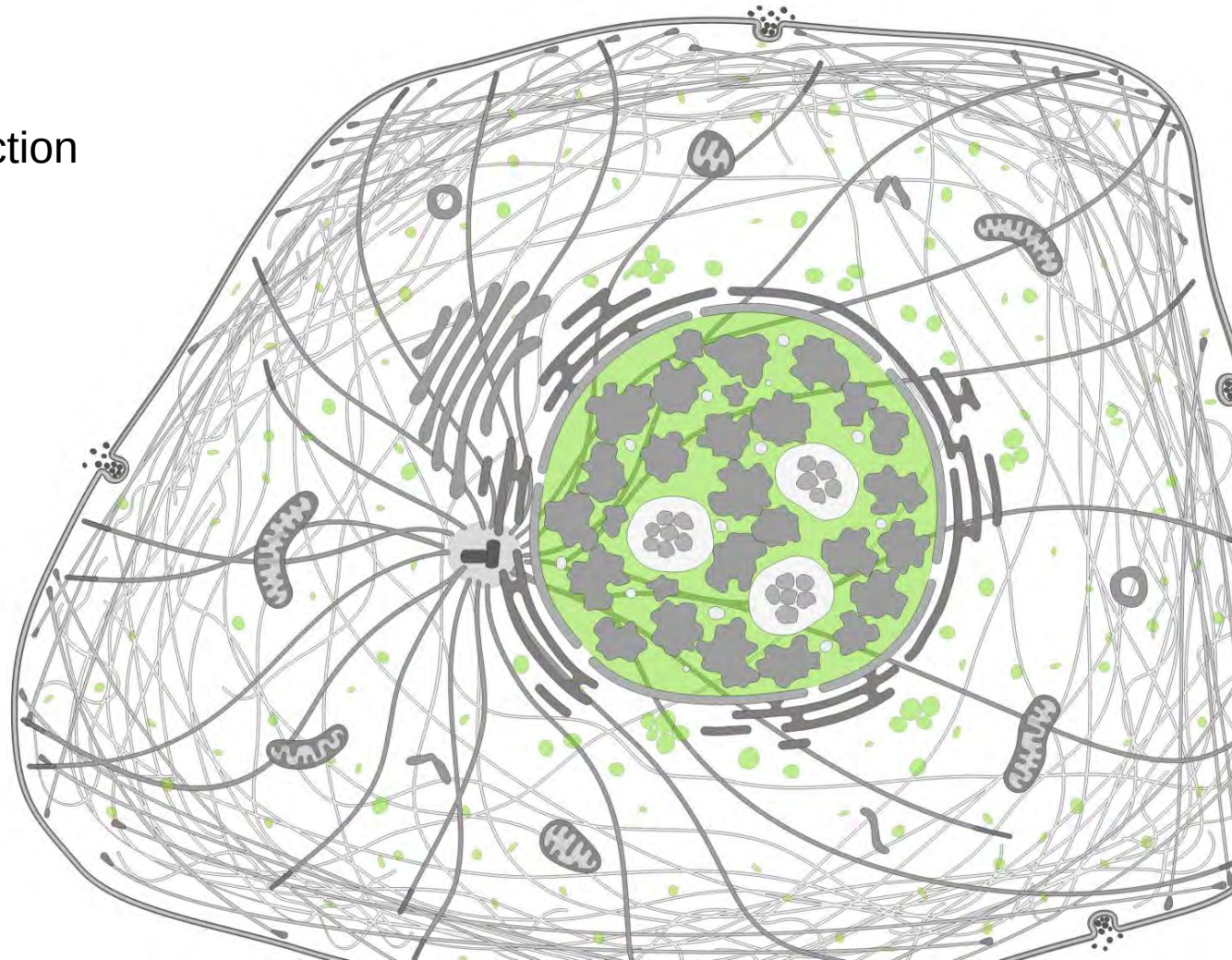


Clustering of proteins identified in iPOND proteome predicts additional ICP0 substrates

SLFN5 (Schlafen 5)

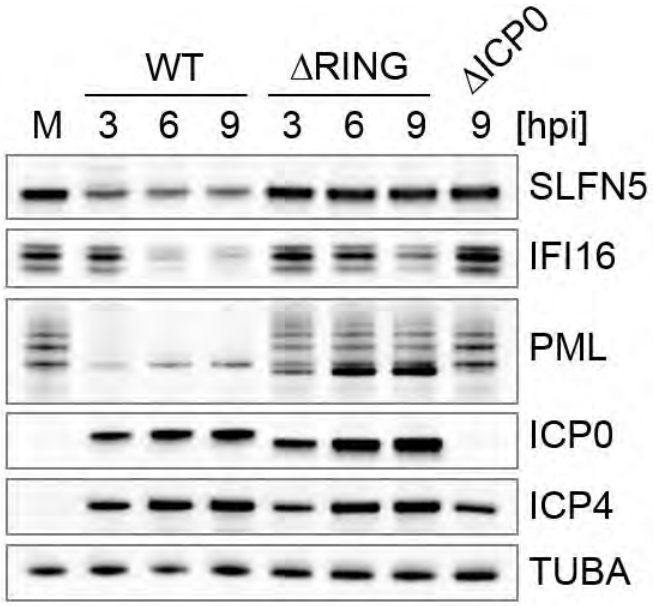
- May have roles in hematopoietic cell differentiation and in controlling motility and invasiveness of carcinoma cells
- Contains a divergent AAA domain that may function in GTP/ATP binding
- Predicted putative DNA/RNA helicase domain
- Member of a family of related SLFN proteins
- SLFN11 has anti-HIV activity
- SLFN5 has not been investigated during virus infection

SLFN5 detected in Nucleoplasm and Vesicles
(adapted from the human proteins ATLAS)

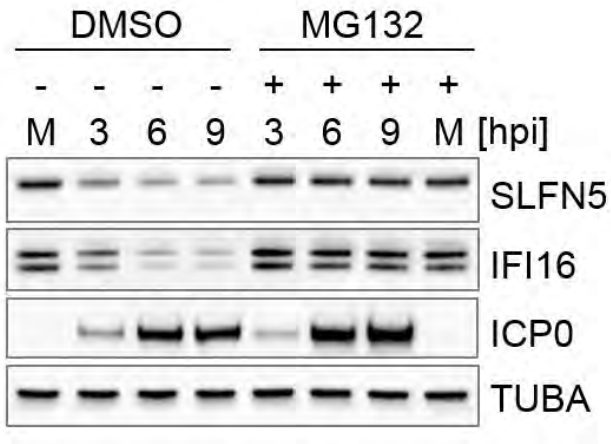


HSV-1 infection reduces SLFN5 through proteasomal degradation

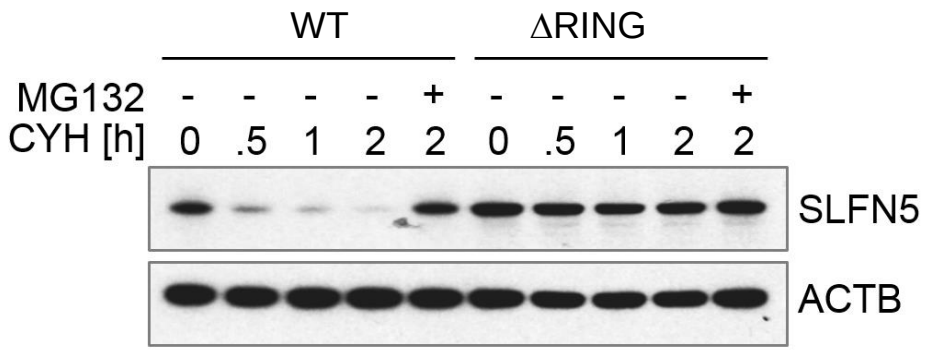
ICP0 RING domain dependent degradation

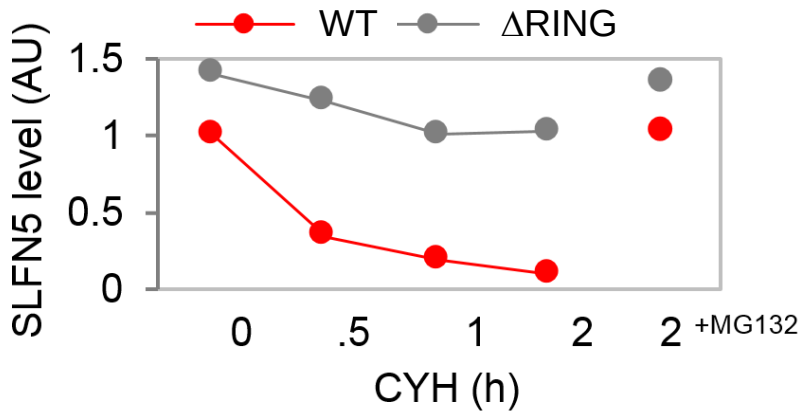


Proteasome dependent degradation



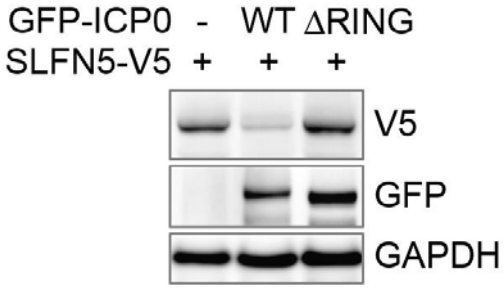
CYH chase assay



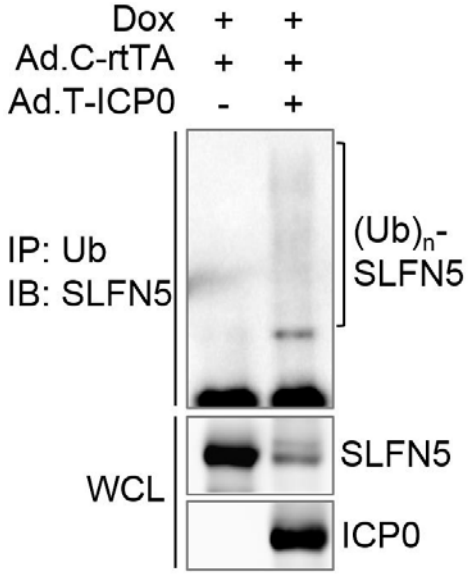


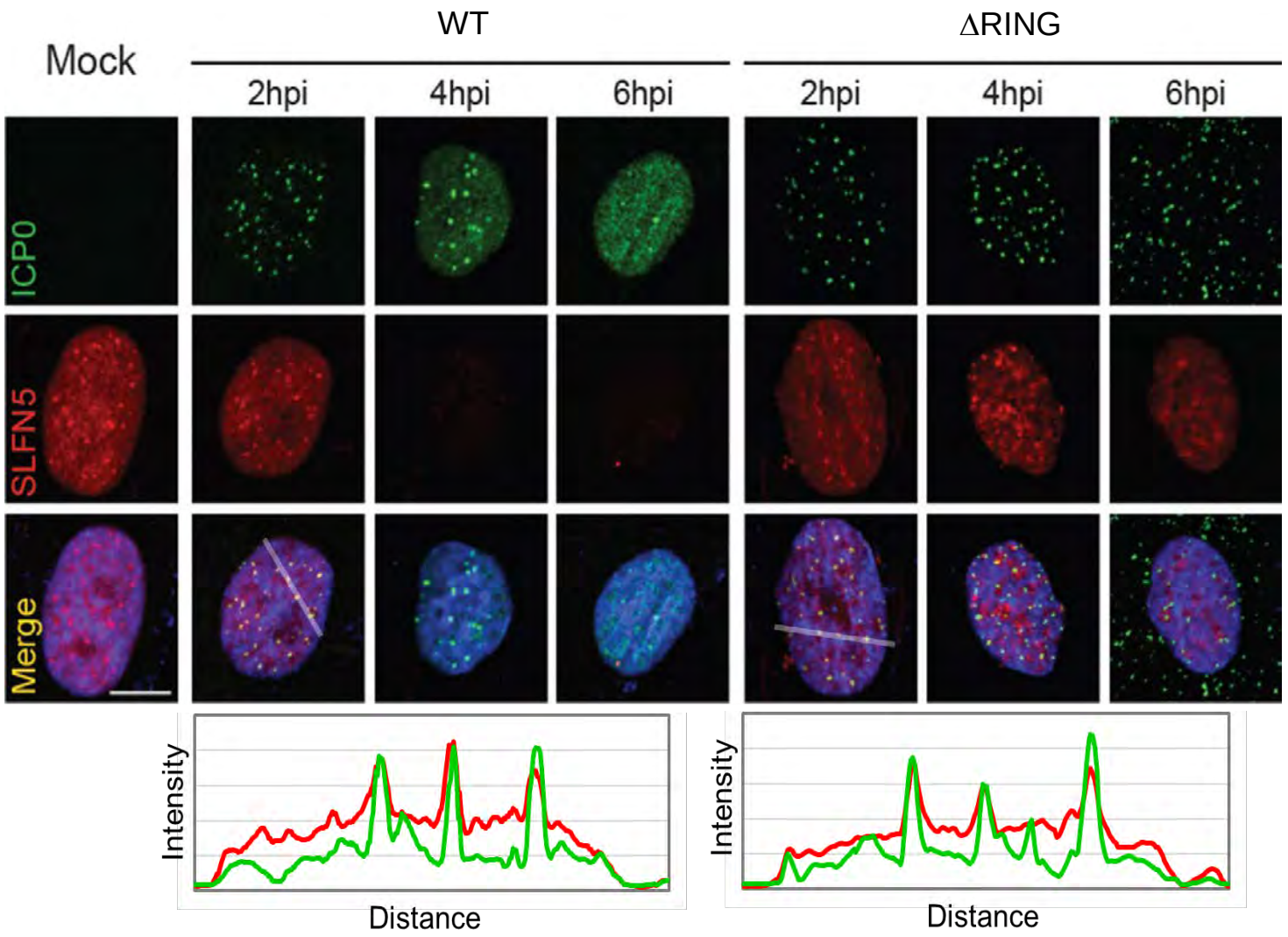
ICP0 ubiquitinates and degrades SLFN5

ICP0 cotransfection

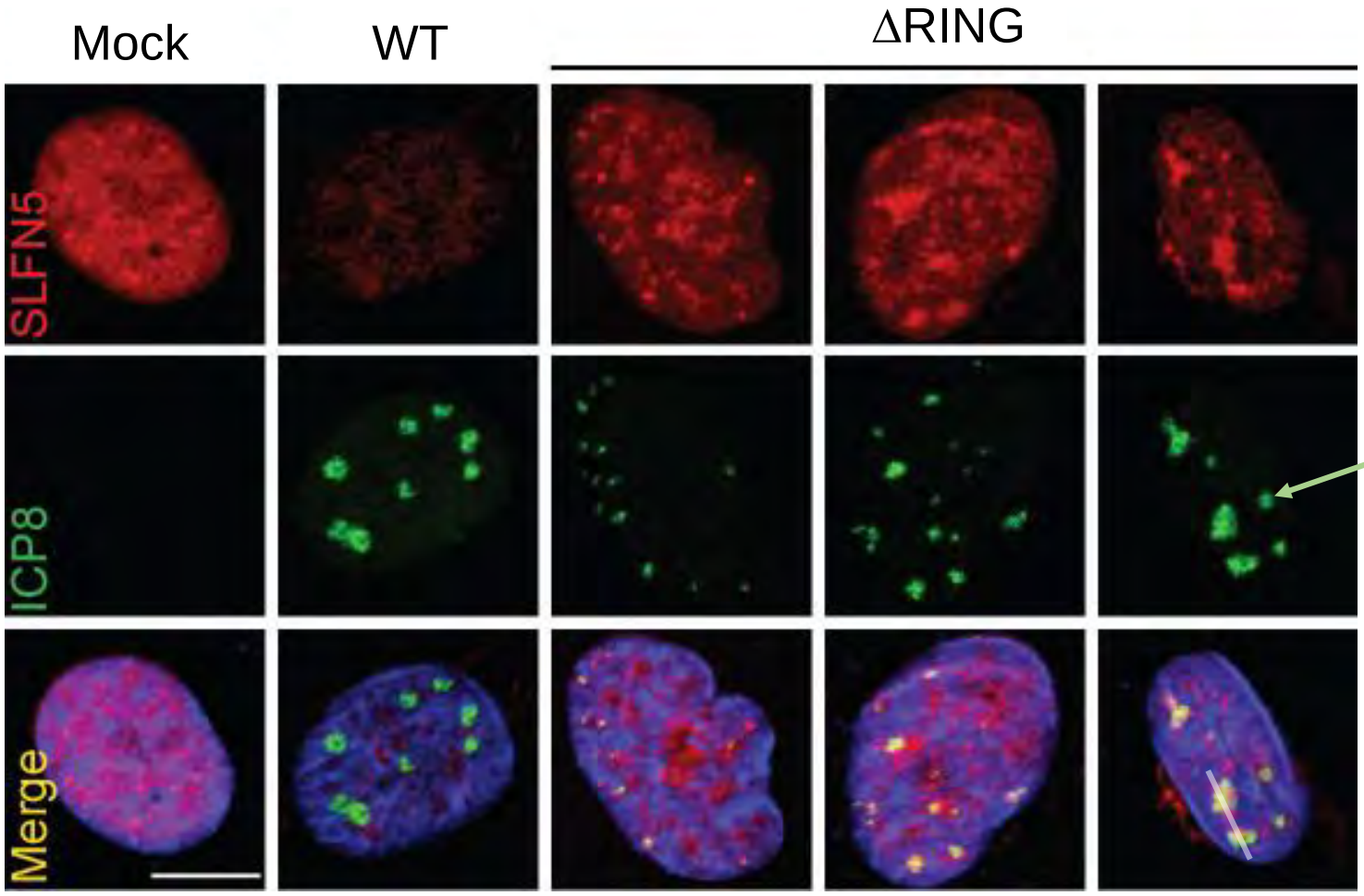


In vivo ubiquitination assay



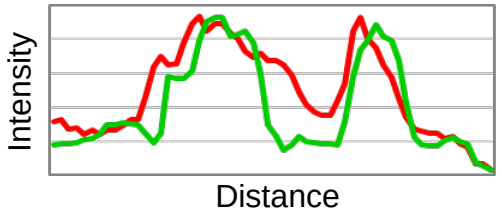


SLFN5 localizes to viral replication centers when not degraded during HSV-1 infection

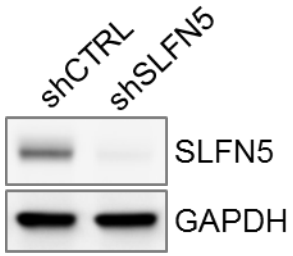


MOI 3 at 6 hpi

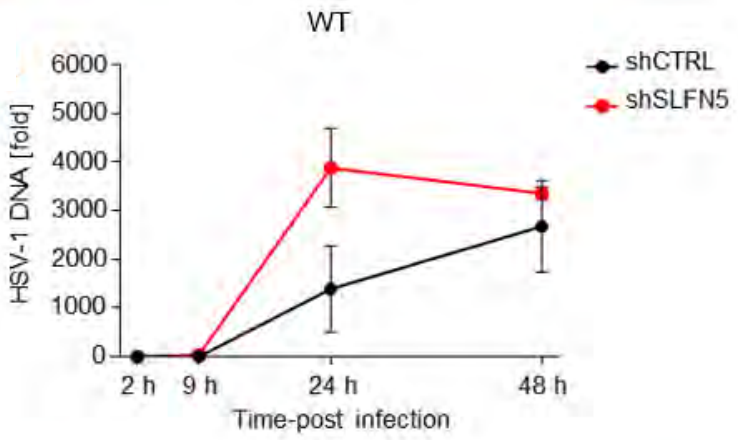
Virus Replication Center (VRC)



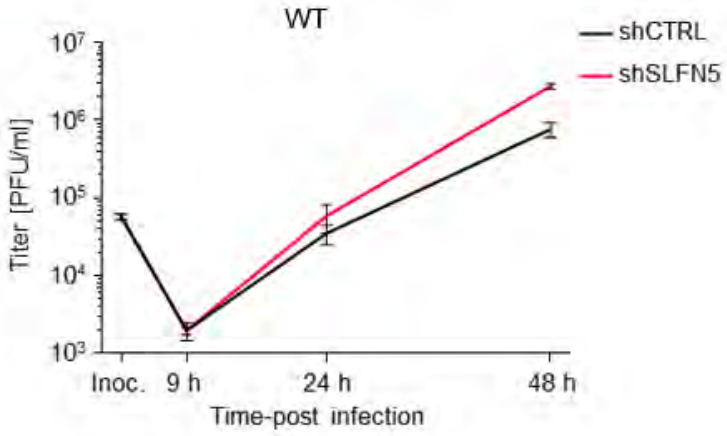
Depletion of SLFN5 increases HSV-1 DNA replication and virus progeny production

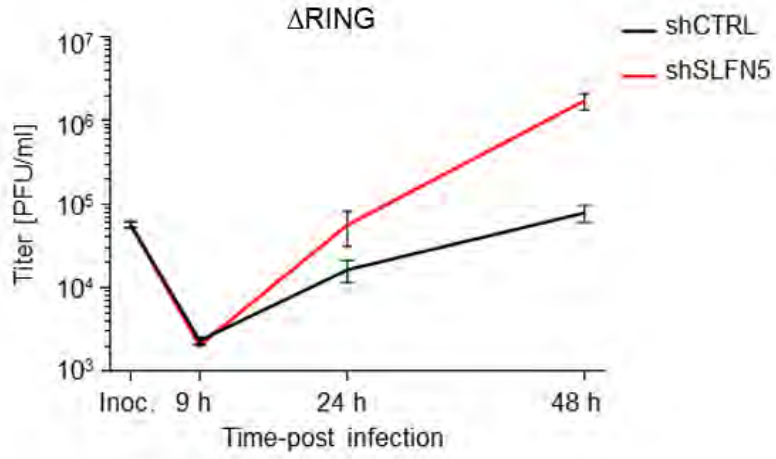


vDNA qPCR



Viral titer





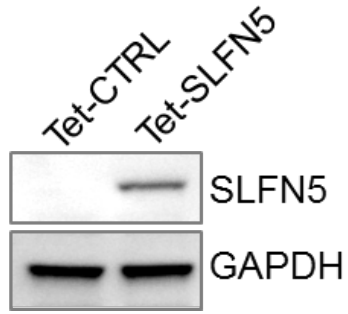
- SLFN5 is inhibitory to HSV-1 replication
- Overcome by the degradation of SLFN5 during WT virus infection

Ectopic expression of SLFN5 inhibits HSV-1 replication

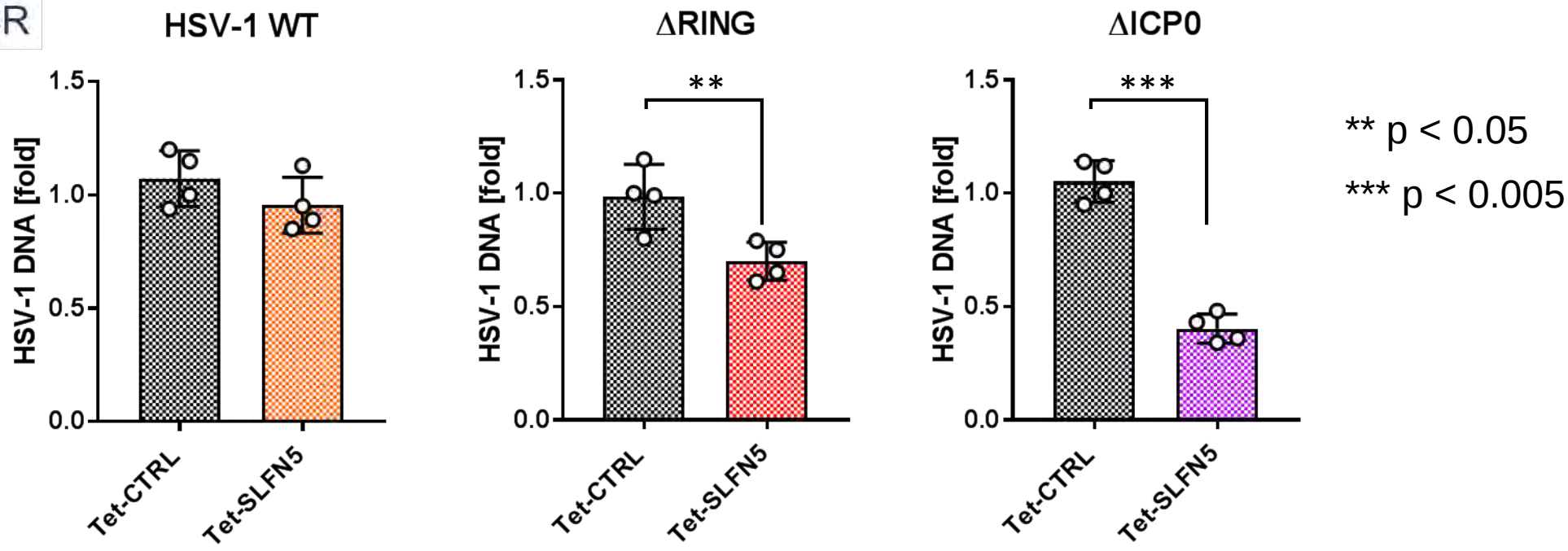
SLFN5 depletion
by CRISPR/Cas9

➡

Reconstitued with Tet-SLFN5-HA
by lentiviral vector transduction



vDNA qPCR



** p < 0.05

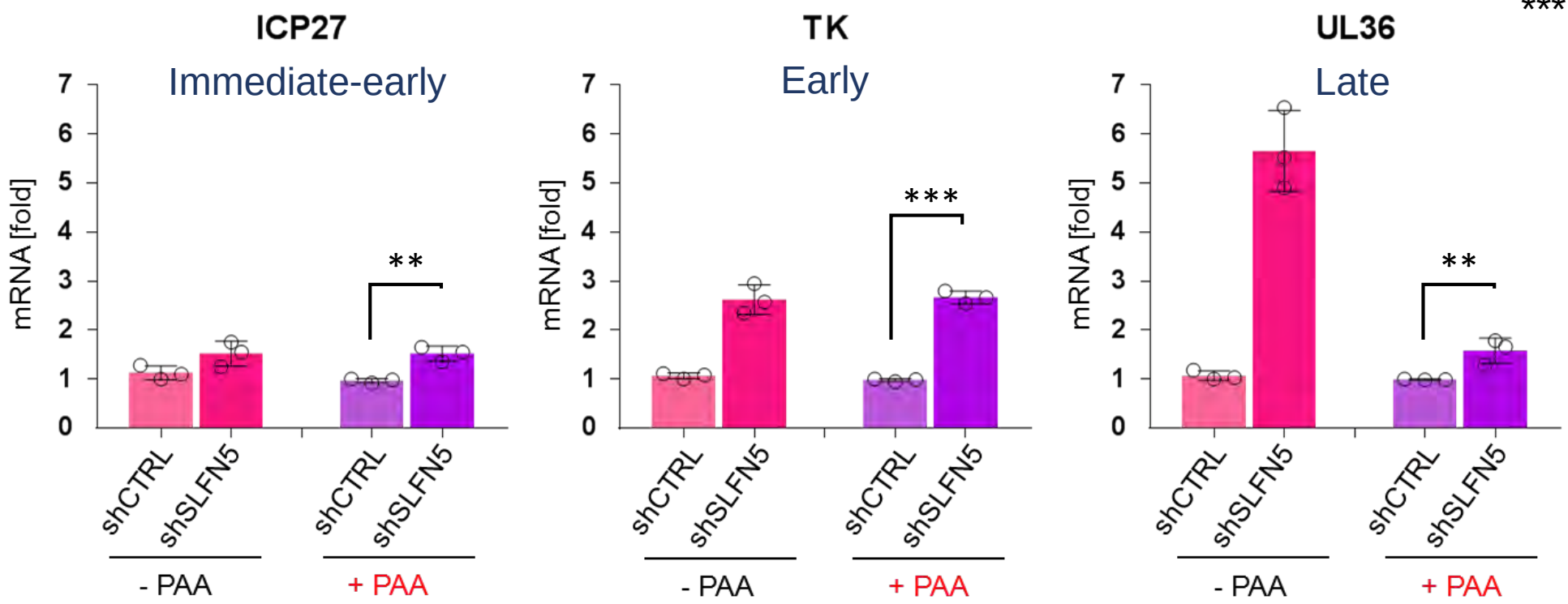
*** p < 0.005

SLFN5 suppresses transcription of viral genes

HSV-1 ΔRING
MOI of 0.5 for 8 h

** p < 0.05

*** p < 0.005



** PAA (Phosphonoacetic acid): a viral DNA polymerase inhibitor*

FINDINGS

I. HSV-1 E3 ligase ICP0 induces various non-proteolytic ubiquitination as well as proteolytic ubiquitination

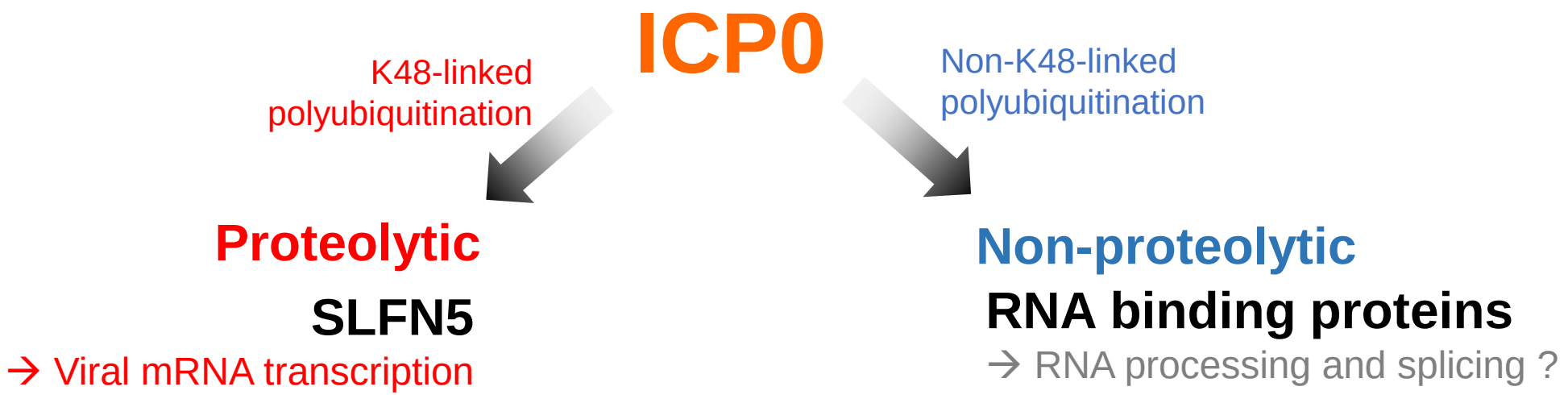
II. ICP0 modifies RNA binding proteins with non-proteolytic ubiquitination

III. Using PCA-based clustering of iPOND data, we identified SLFN5 as a potential ICP0 target

IV. ICP0 ubiquitinates and degrades SLFN5 via the proteasome

V. SLFN5 represses HSV-1 replication

VI. SLFN5 down-regulates viral genes transcription during HSV-1 infection



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