“The Onassis Foundation
Science Lecture Series 2011 in Biology”

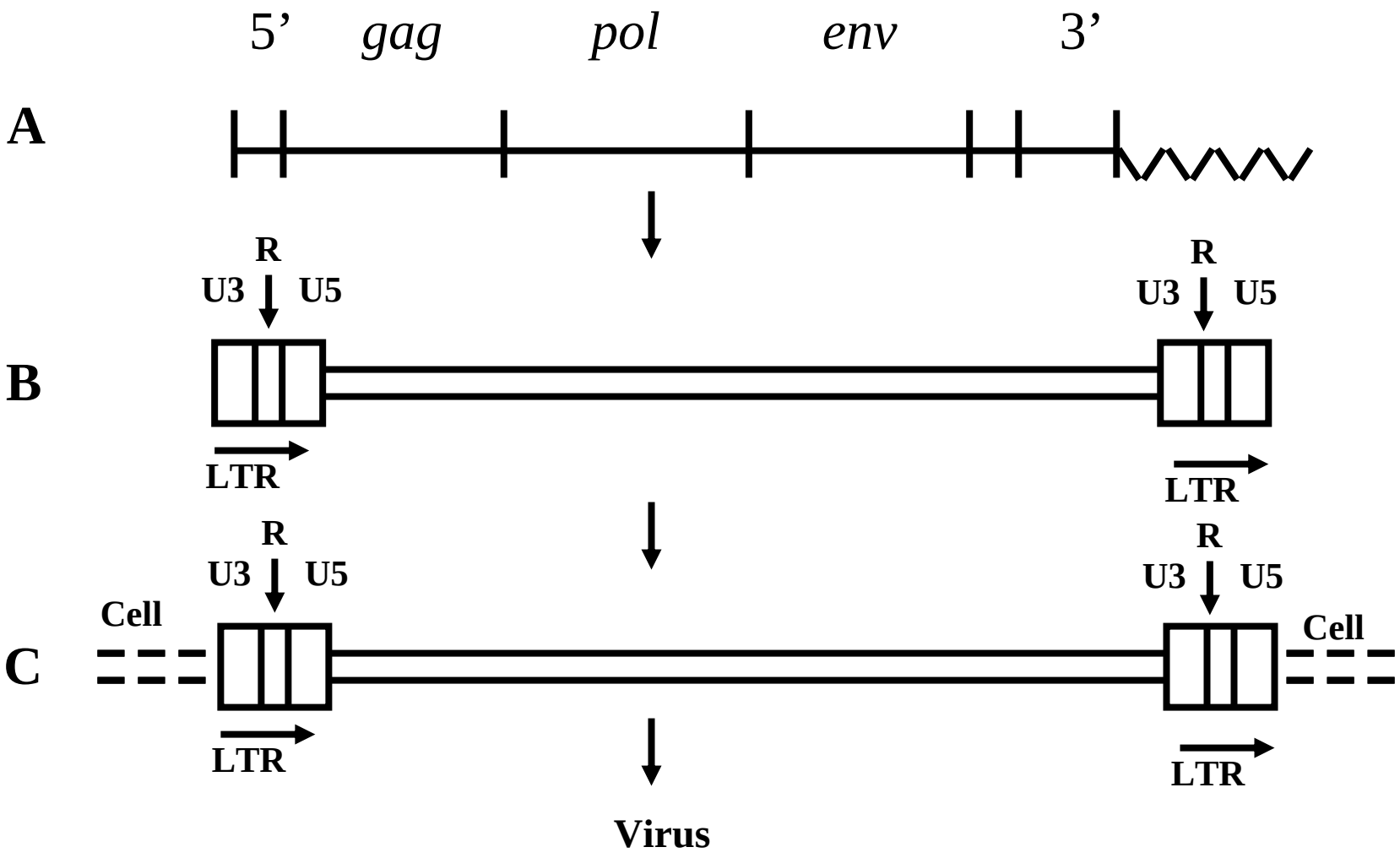
Basic and Applied Virology

July 14, 2011

Philip N. Tsichlis
Molecular Oncology Research Institute
Tufts Medical Center, Boston, MA

From viruses to cancer and from cancer back to viruses: Oncogenes activated by retroviruses give clues on the epigenetic regulation of DNA virus replication.

RETROVIRAL REPLICATION



Validation of the Insertional Mutagenesis Model of Oncogenesis

Cell, Vol. 23, 323–334, February 1981, Copyright © 1981 by MIT

Avian Leukosis Virus-Induced Tumors Have Common Proviral Integration Sites and Synthesize Discrete New RNAs: Oncogenesis by Promoter Insertion

Benjamin G. Neel and William S. Hayward
The Rockefeller University
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Harriet L. Robinson
The Worcester Foundation for Experimental Biology
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Joanna Fang and Susan M. Astrin
The Institute for Cancer Research
The Fox Chase Cancer Center
Philadelphia, Pennsylvania 19111

NATURE VOL. 302 31 MARCH 1983

A common region for proviral DNA integration in MoMuLV-induced rat thymic lymphomas

Philip N. Tsichlis, P. Gunter Strauss & Li Fu Hu

Laboratory of Tumor Virus Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205, USA

Cell, Vol. 31, 99–109, November 1982, Copyright © 1982 by MIT

Many Tumors Induced by the Mouse Mammary Tumor Virus Contain a Provirus Integrated in the Same Region of the Host Genome

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Amsterdam, The Netherlands

SnapShot: Histone-Modifying Enzymes

Cell

Tony Kouzarides
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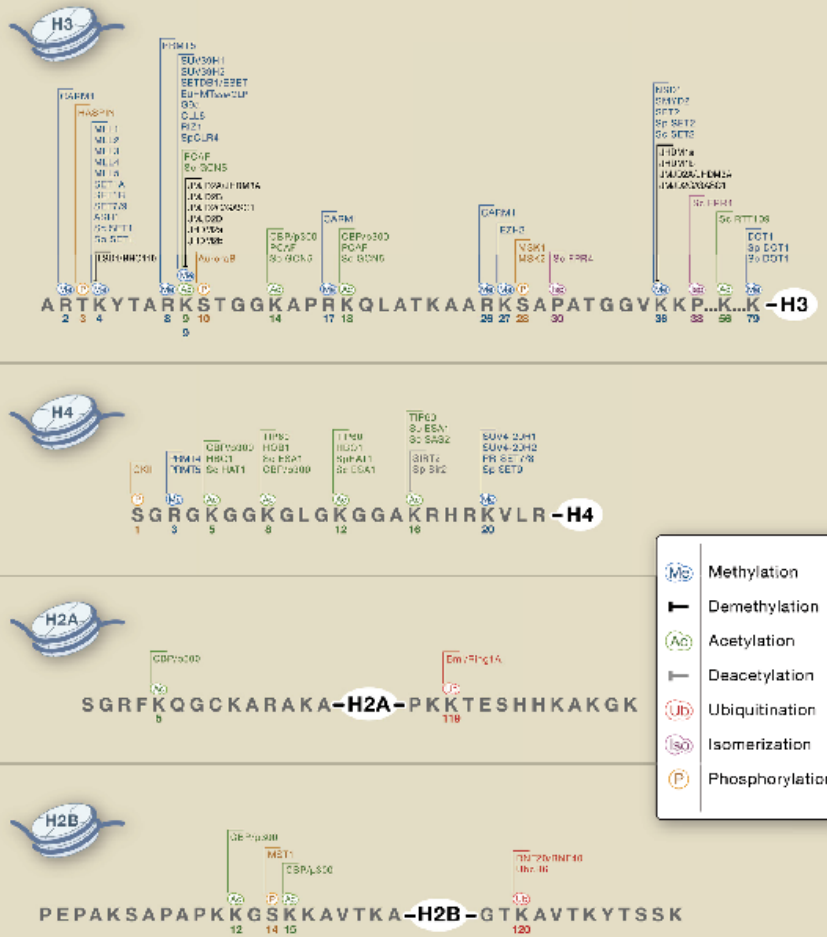
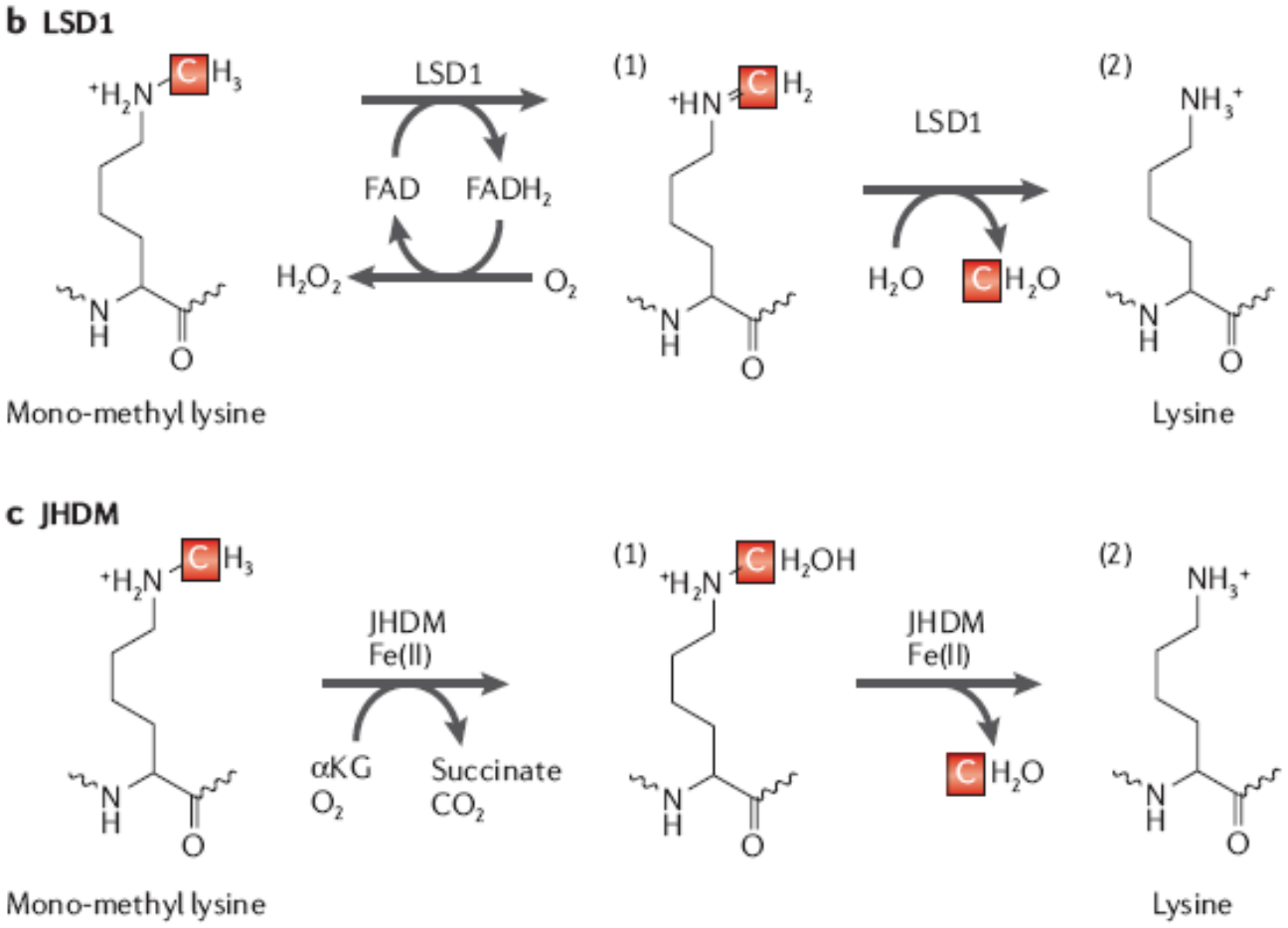


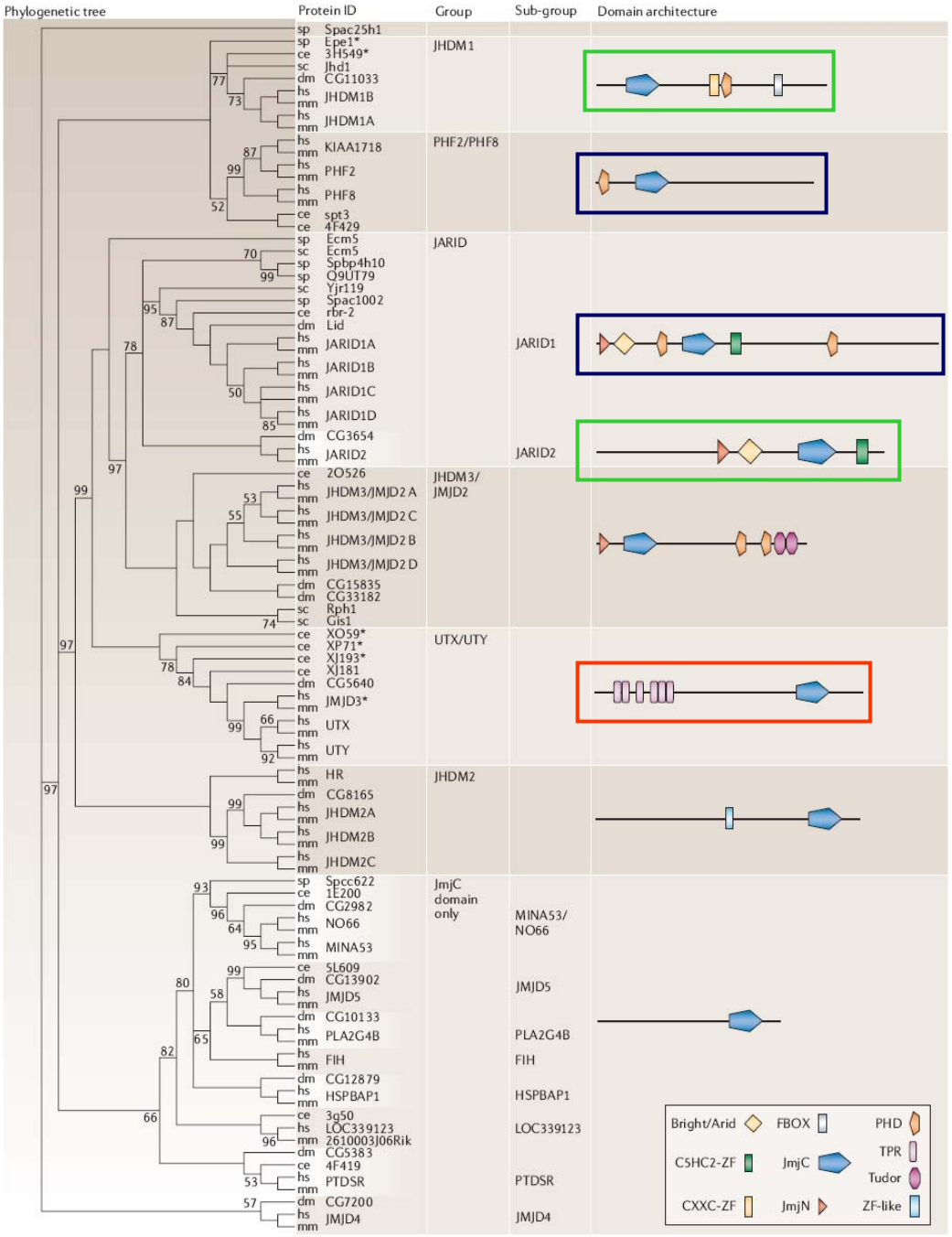
See online version for legend and references.

Histone demethylation
The demethylation reaction



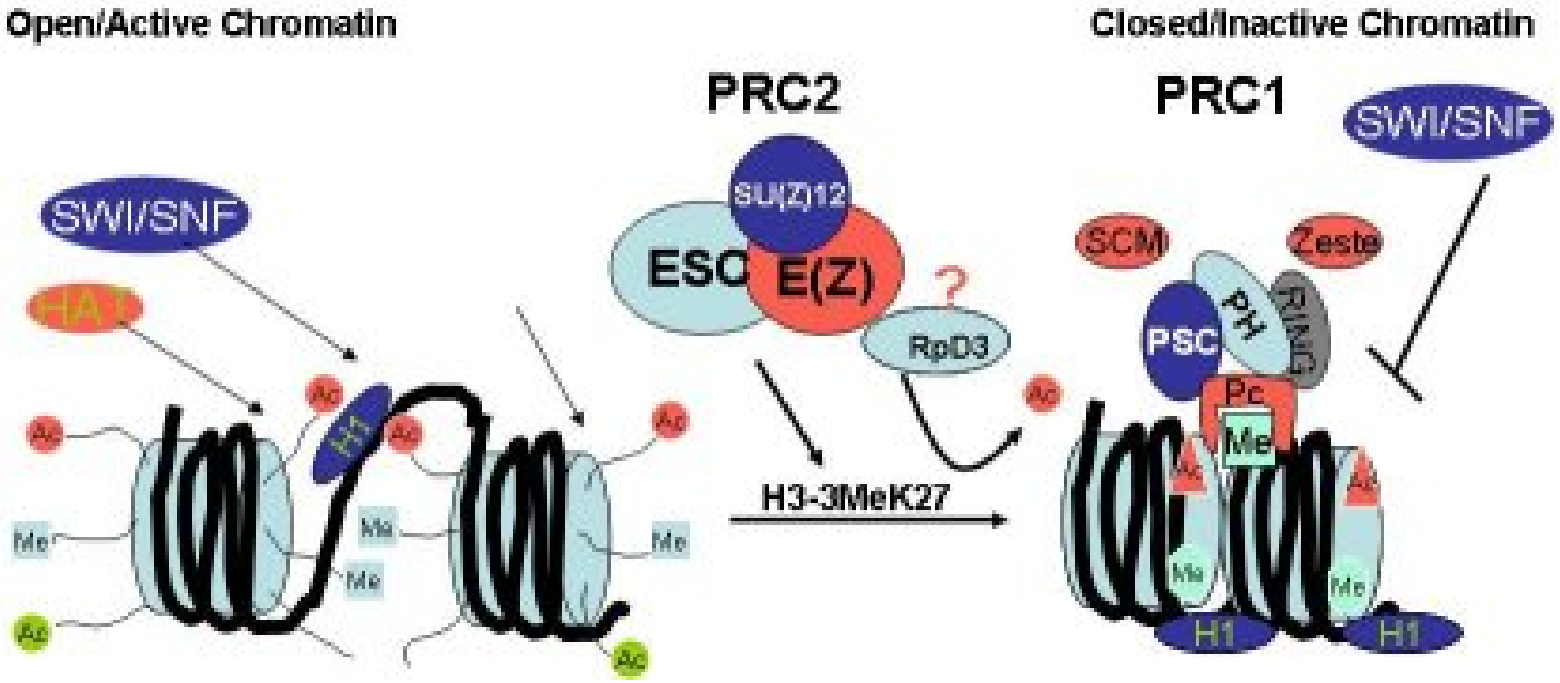
Nature Reviews Genetics 7: 715, 2006

Jumonji domain Family of histone demethylases

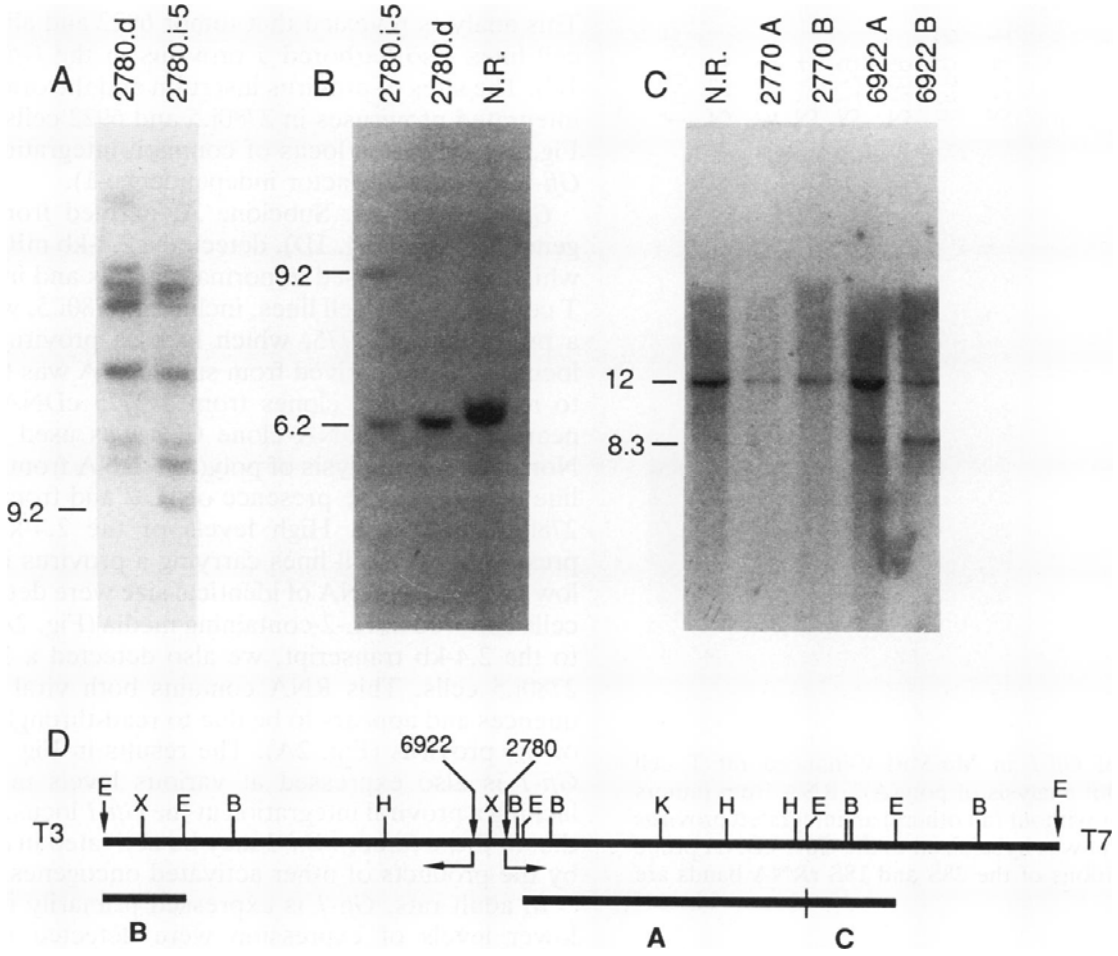


Nature Reviews Genetics 7: 715, 2006

PRC1 and PRC2



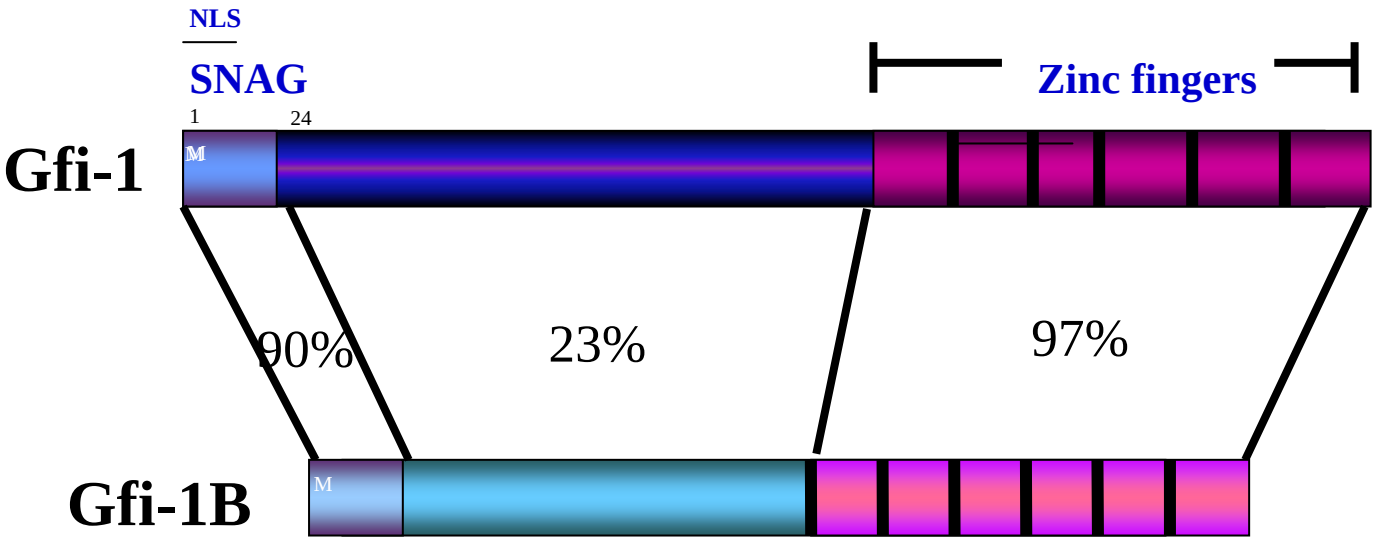
Cloning the Gfi1 locus

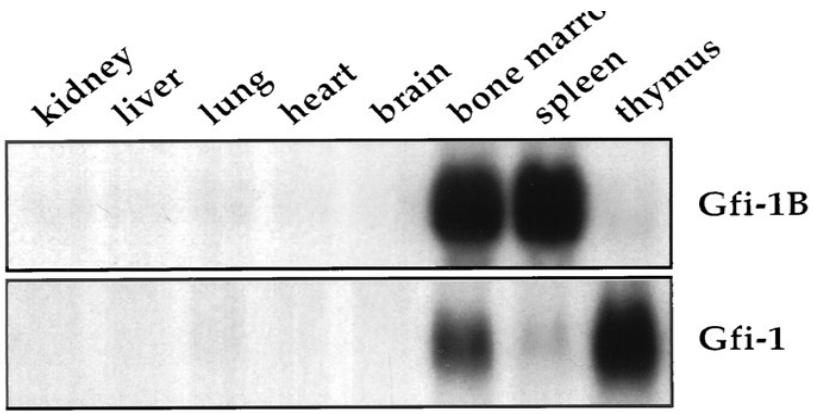


C. Blake Gilks

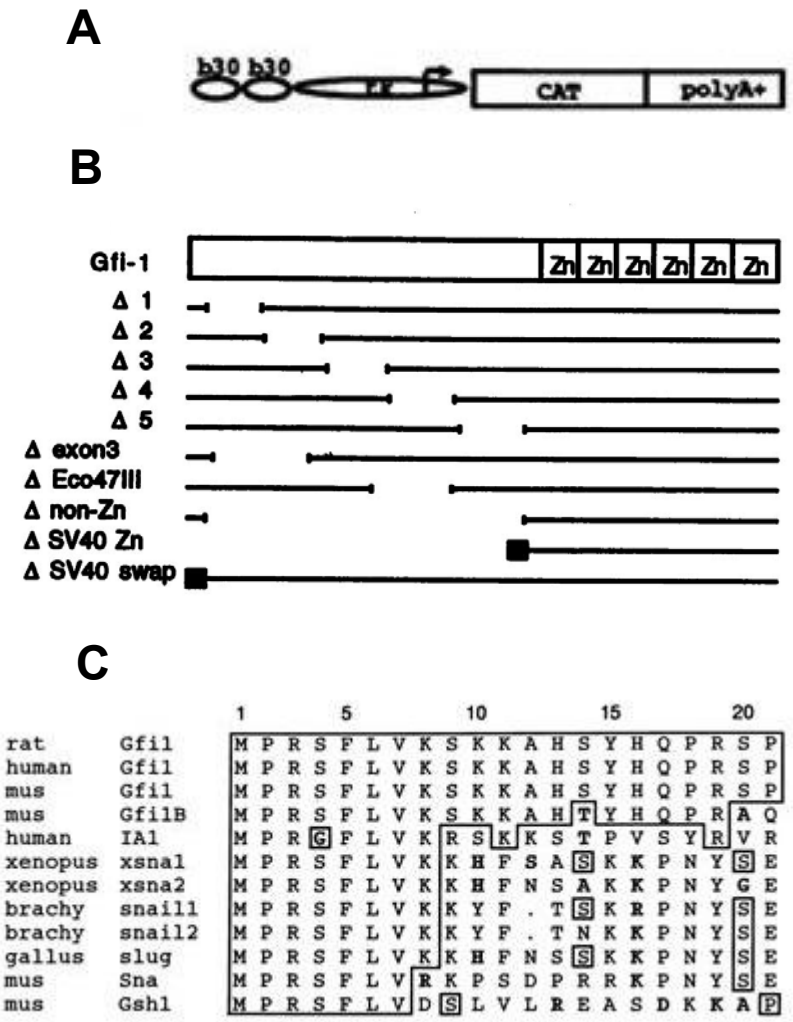
MCB 13:1759, 1993

Gfi1 family of transcriptional repressors
The SNAG domain



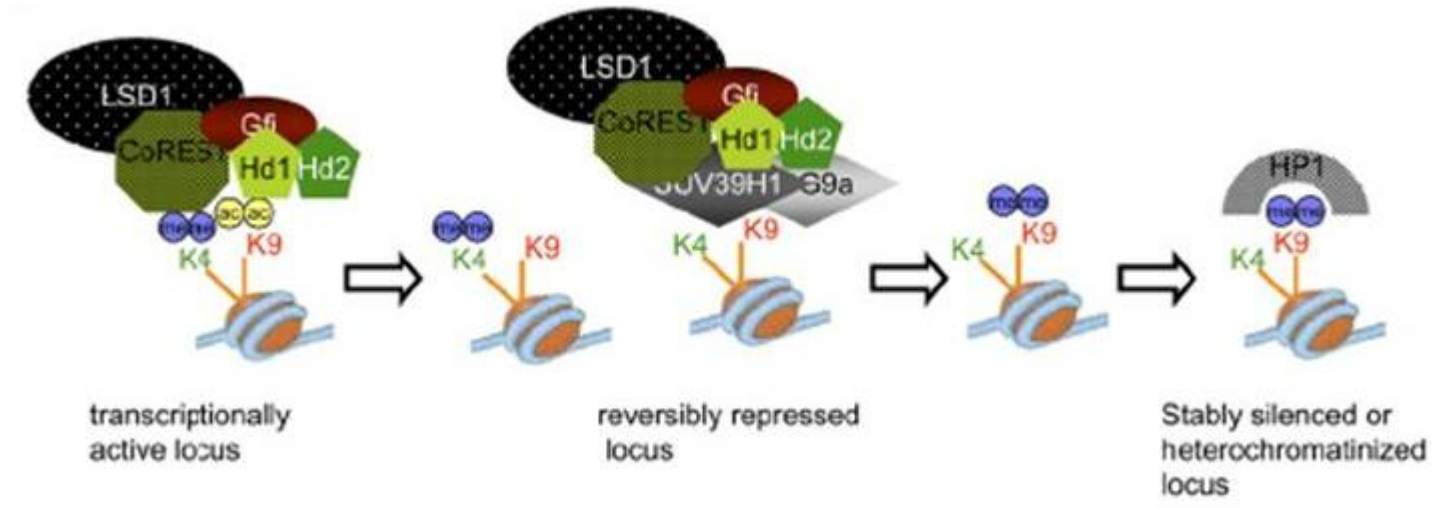


Gfi1 functions as a transcriptional repressor. Identification of the SNAG repressor domain



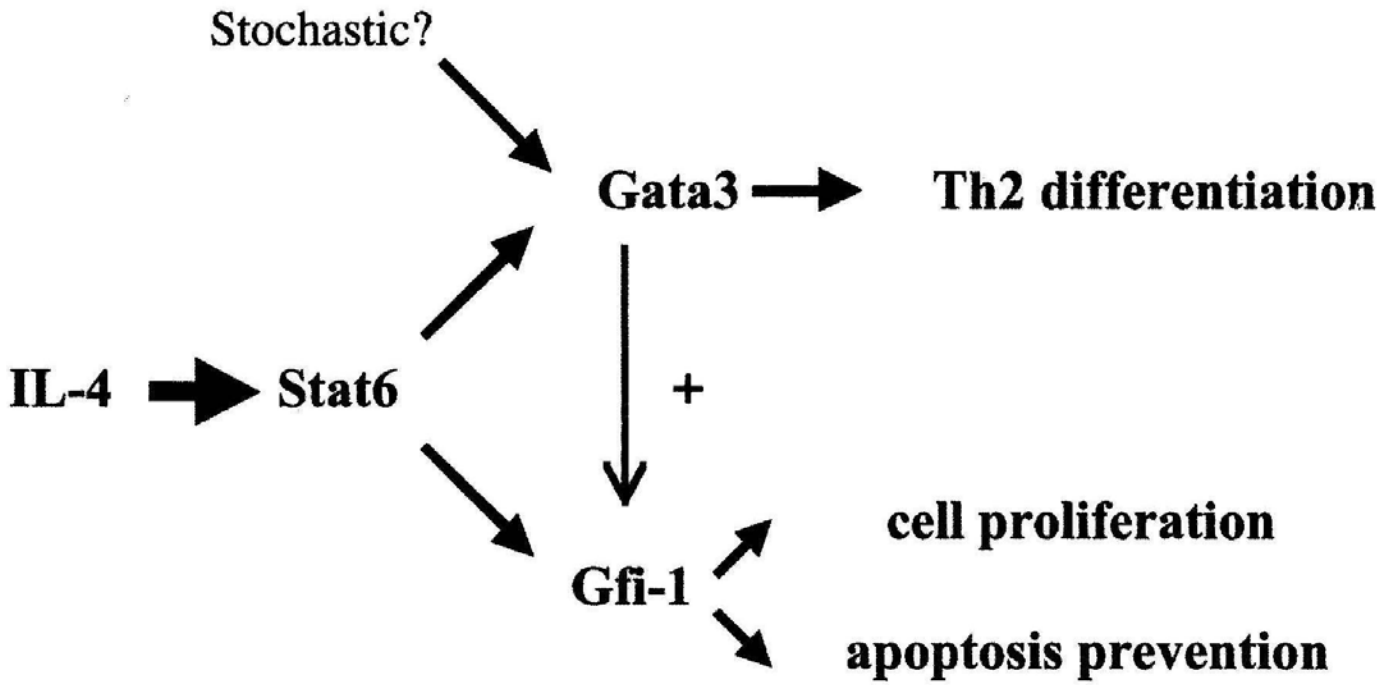
Grimes et al 16: 6263, 1996

Histone methylation status at Gfi1 target genes is regulated by LSD1



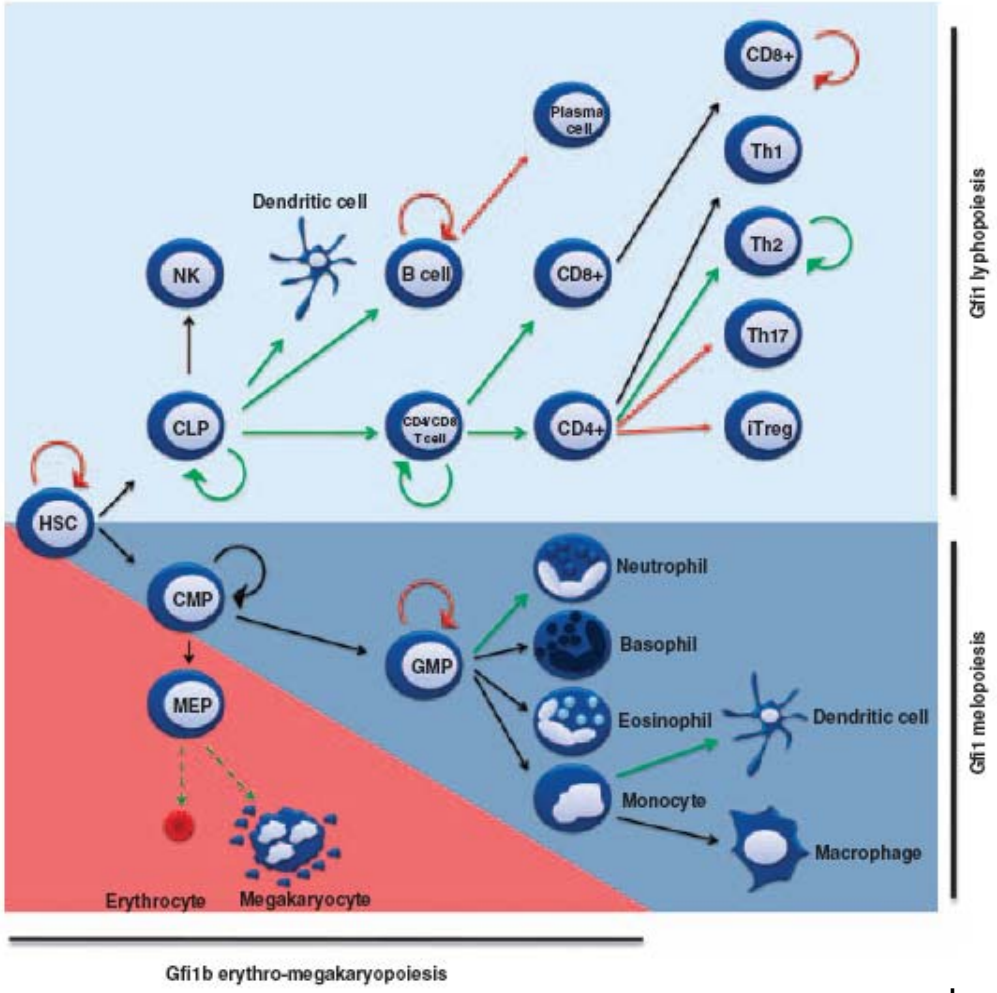
Mol Cell 27: 562, 2007

Gfi1 promotes the proliferation and survival of Th2 cells



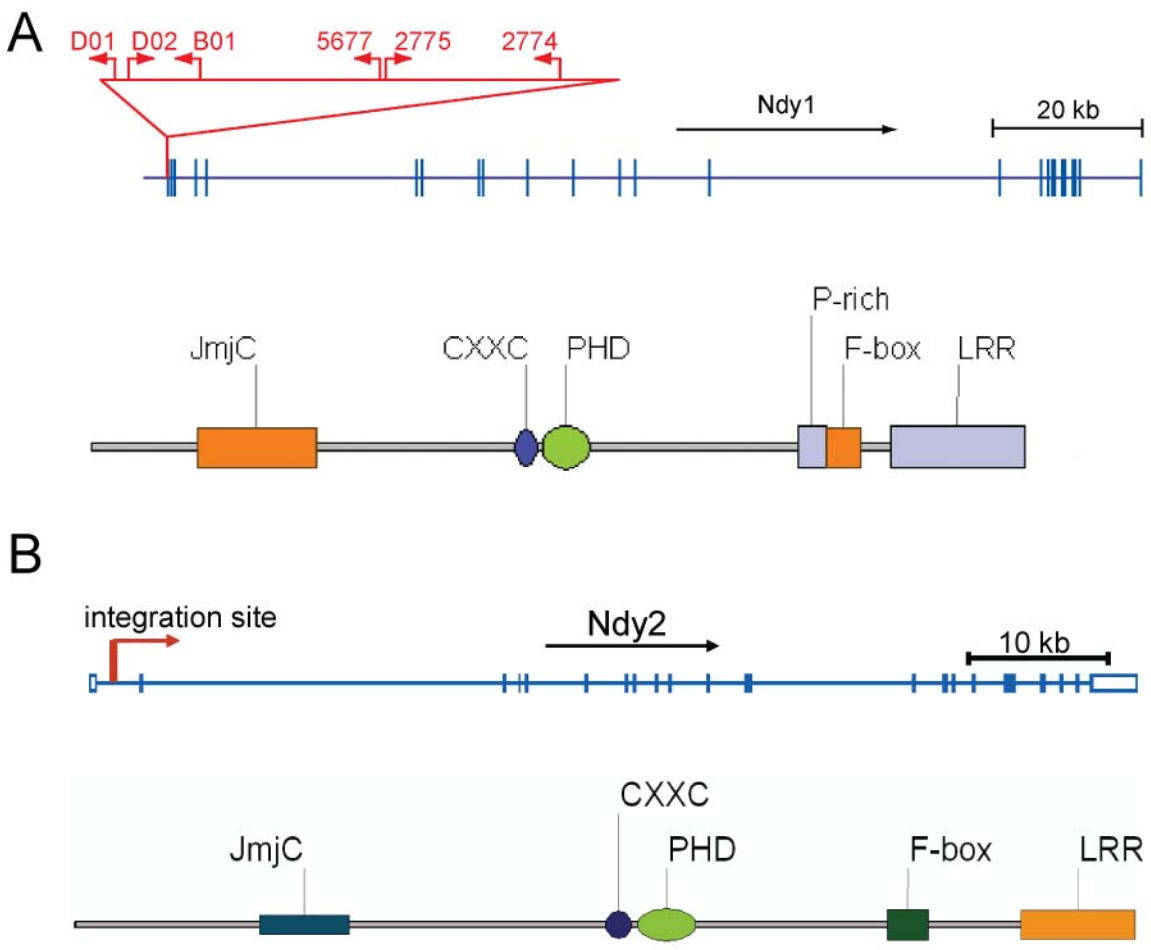
Immunity: 16: 733, 2002

Gfi1 and Gfi1b have broad roles in the regulation of hematopoiesis



Leukemia 24:1834, 2010

The JmjC domain histone demethylases Ndy1 and Ndy2 are activated by provirus integration in MoMuLV-induced rodent lymphomas

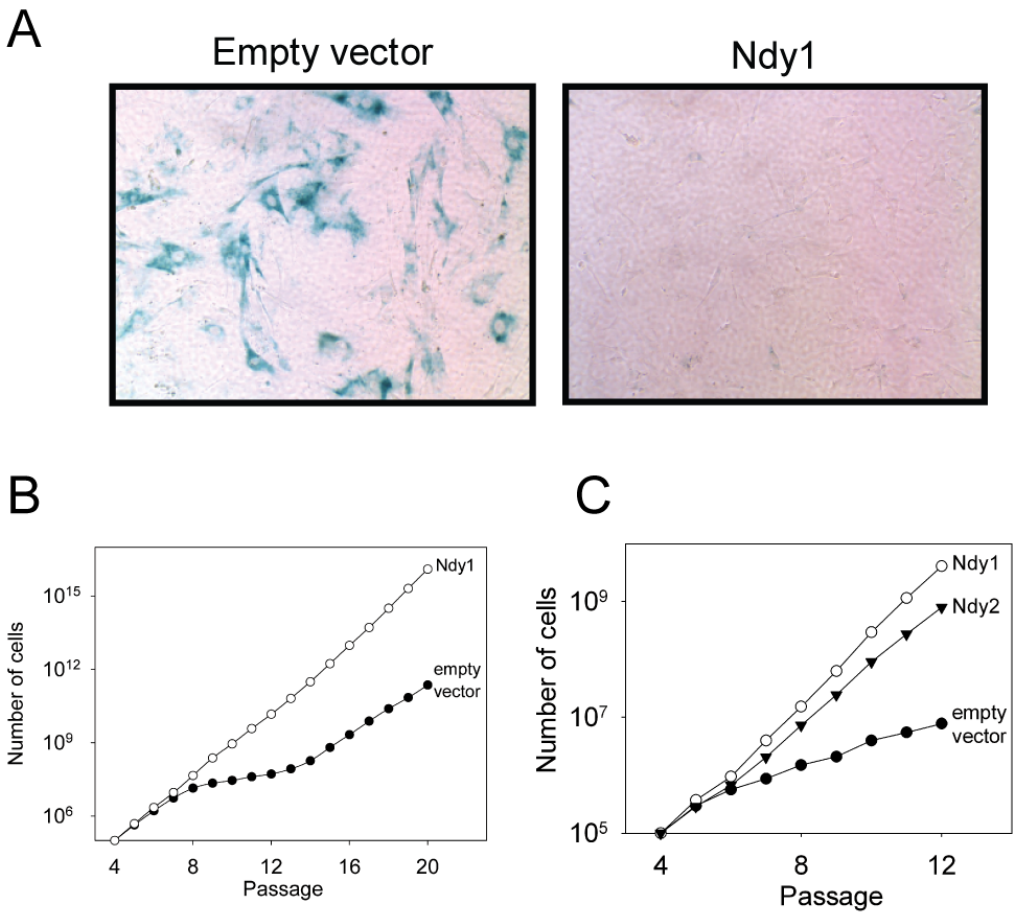


Susan E Bear

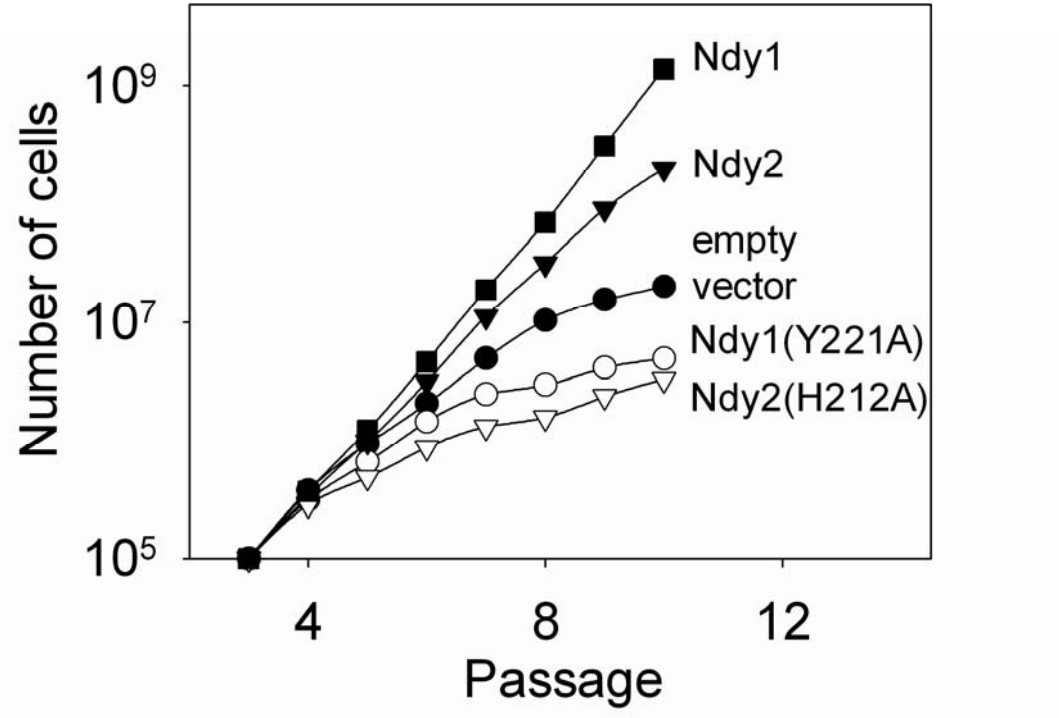
Ray Pfau

PNAS 105: 1907, 2008

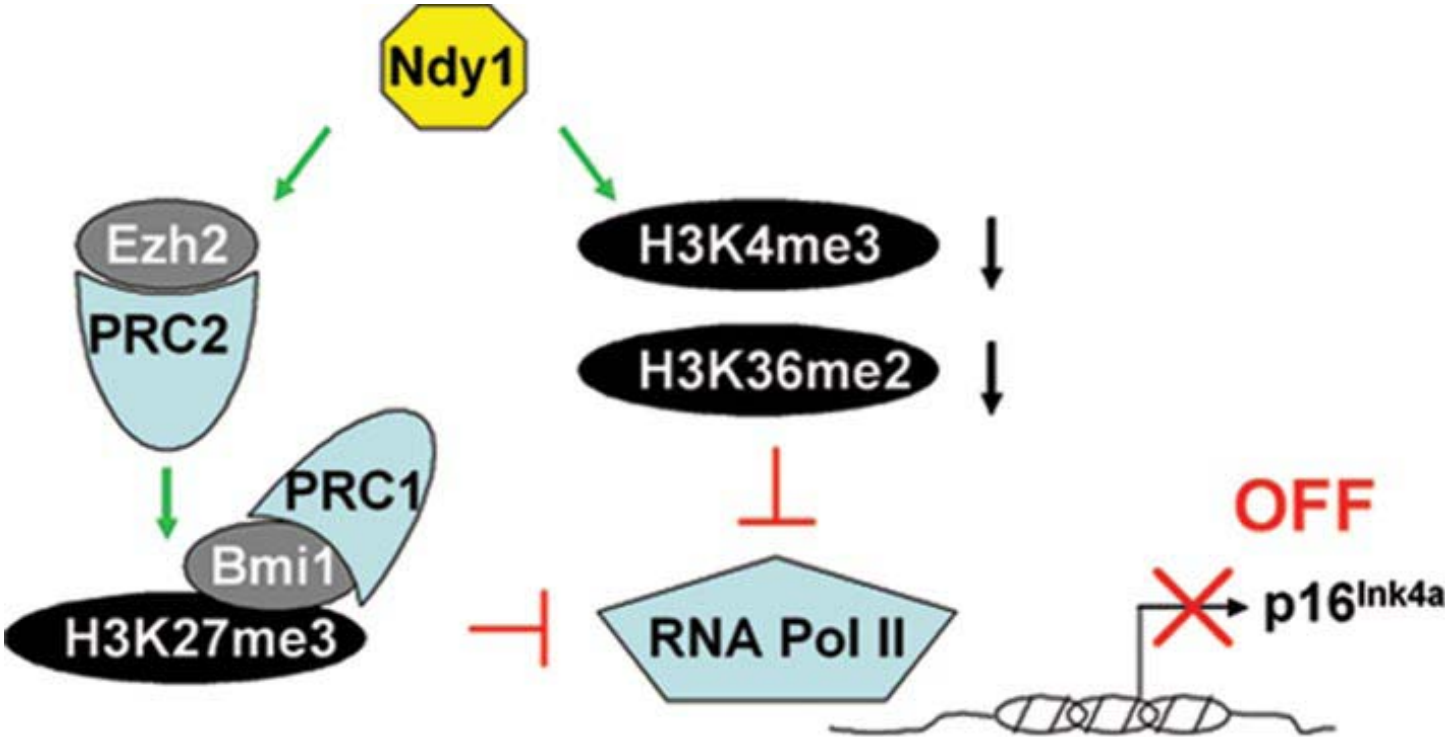
MEFs expressing exogenous Ndy1 or Ndy2 bypass replicative senescence



Ndy2-mediated MEF immortalization is also JmjC domain-dependent

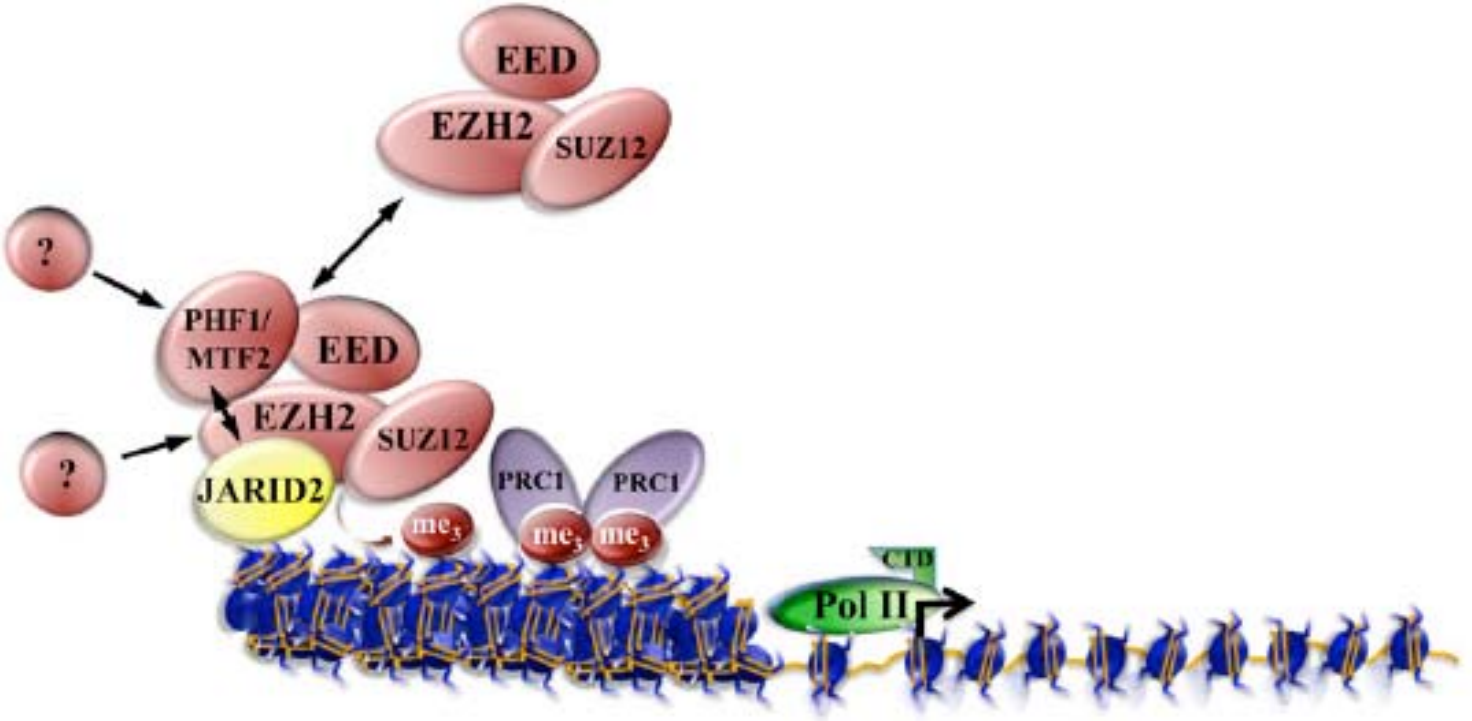


NDY1 represses p16^{INK4A} by coupling histone H3K36 me1 and H3K36me2 demethylation with histone H3K27 trimethylation and Histone H2A K119 ubiquitination



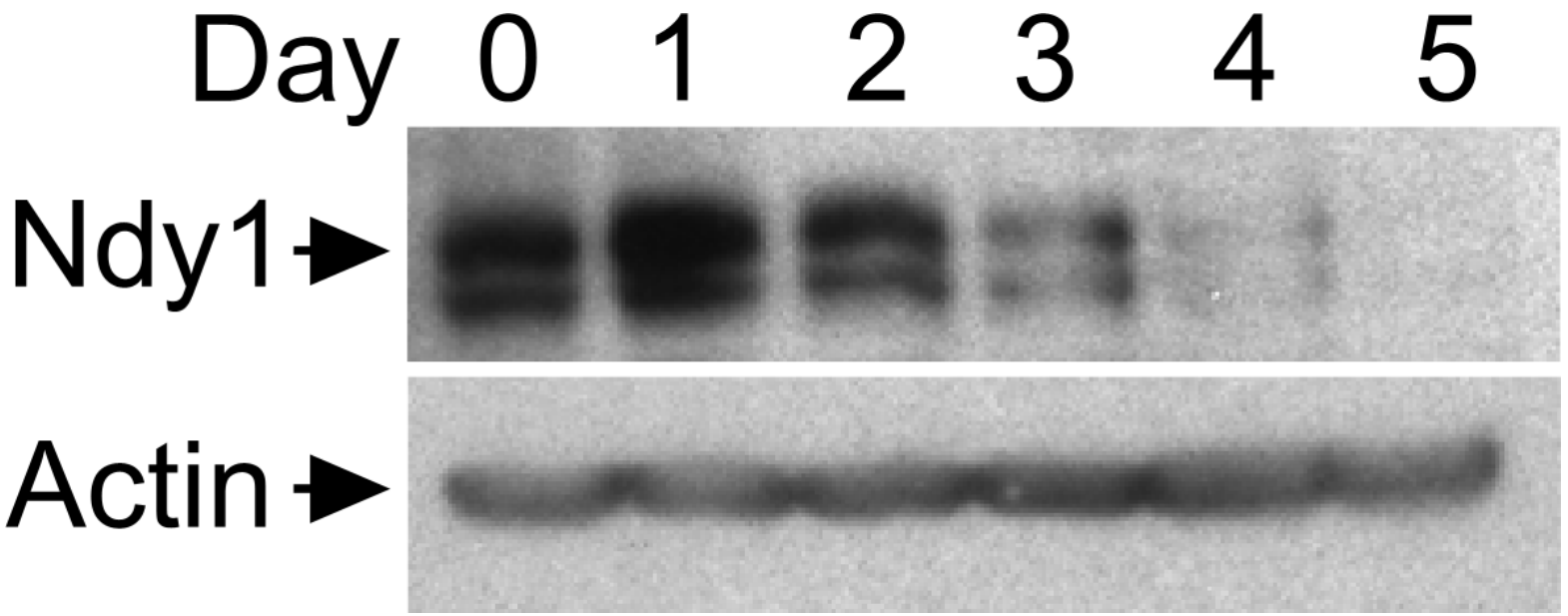
PNAS 106: 2641, 2009

JARID2 interacts with EZH2 and contributes to the PRC2-mediated transcriptional repression

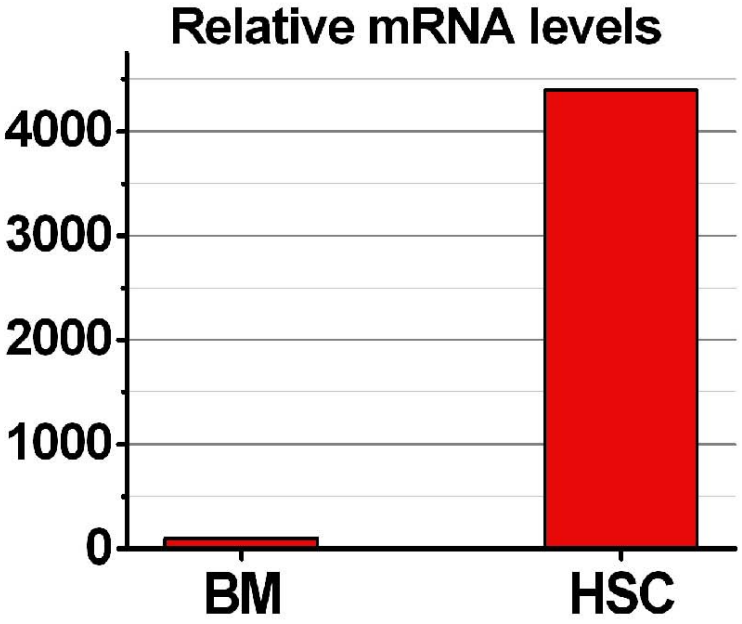


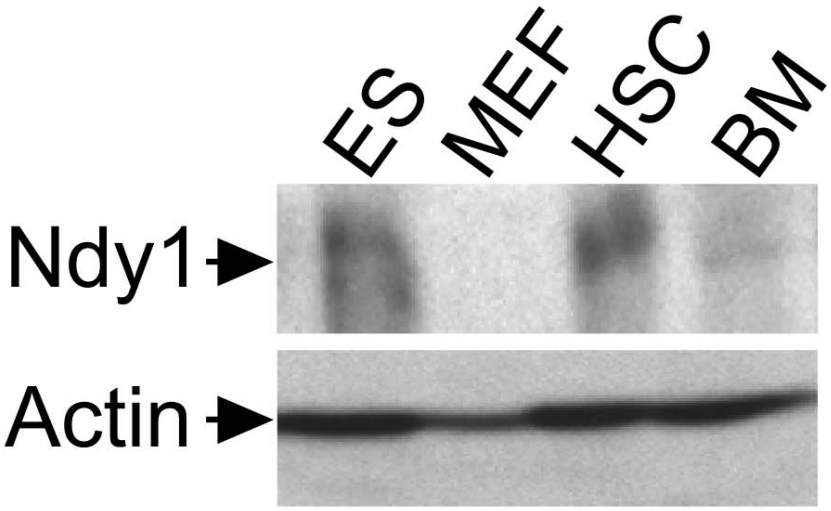
Genes and Development 24:857, 2010

Ndy1 is highly expressed in ES cells and its expression declines with differentiation

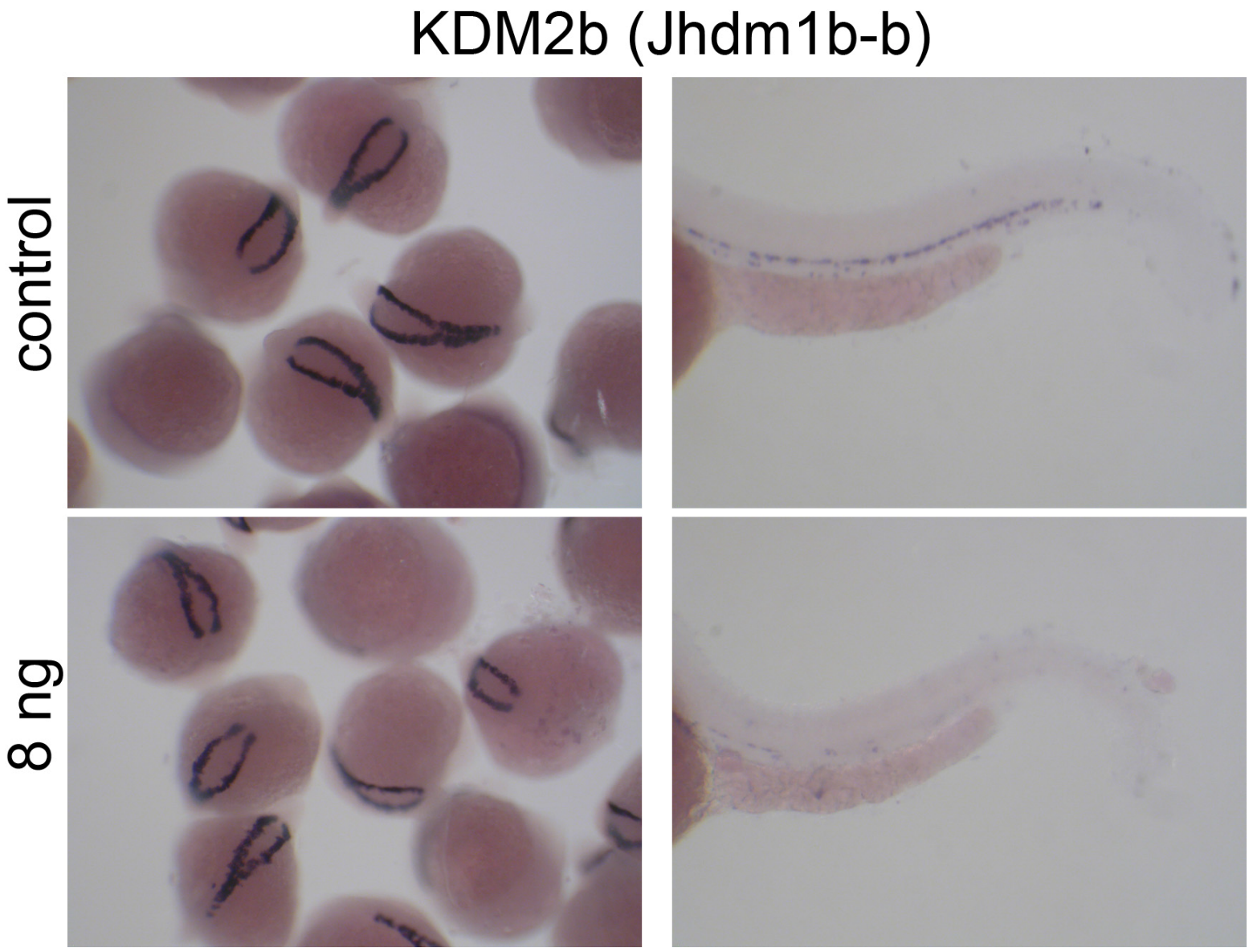


Ndy1 is highly expressed in c-Kit+/Sca1+ hematopoietic stem cells

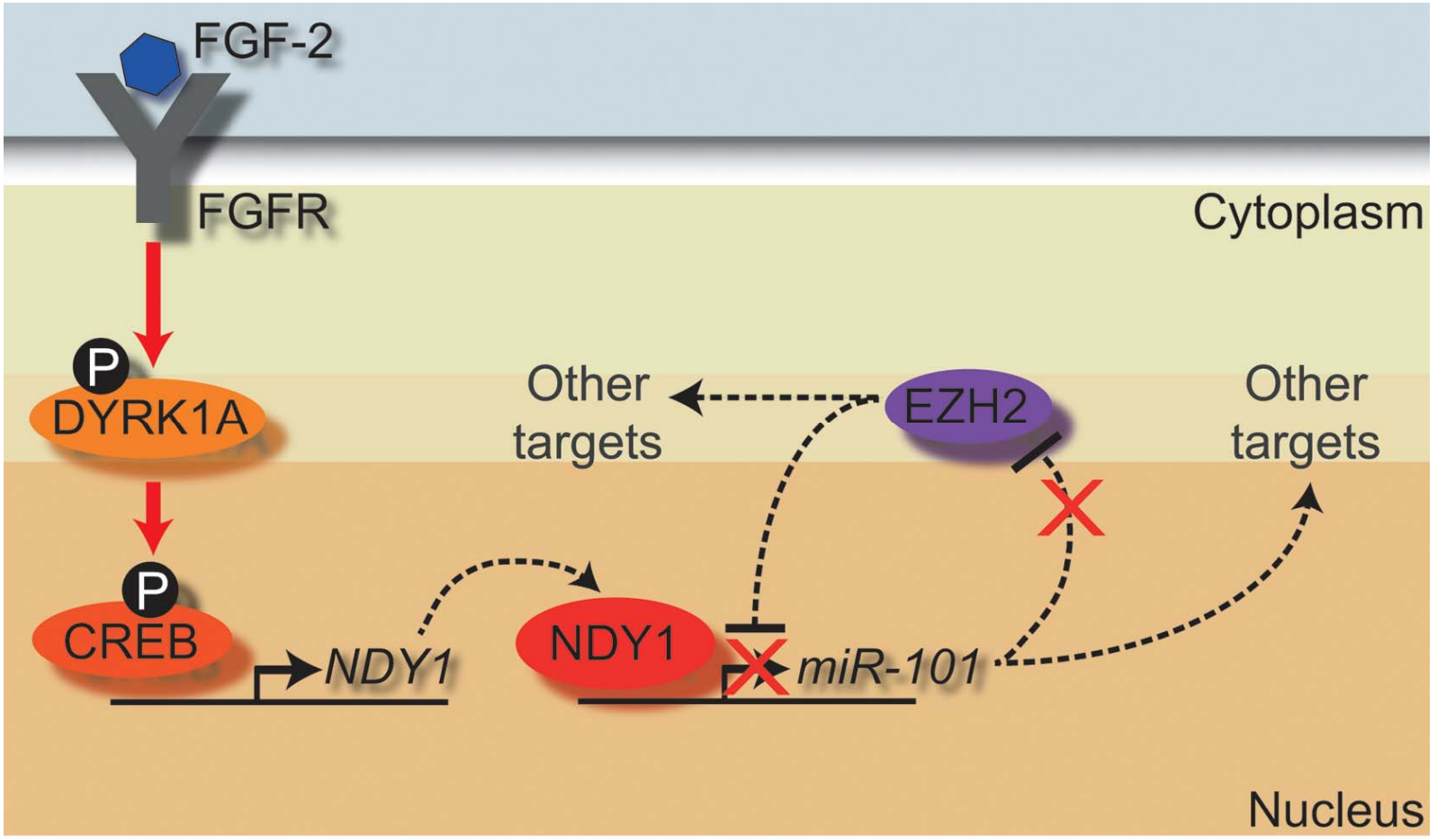




The knockdown of Ndy1/KDM2B gives rise to a hematopoietic stem cell and progenitor cell defect in zebra fish



The FGF2/EZH2-miR-101-EZH2 pathway. Mechanisms of NDY1-mediated transcriptional repression.



Filippos Kottakis et al. Mol Cell In press. July 22, 2011

HCMV

- HCMV is a double stranded DNA virus that belongs to the betaherpesvirus subfamily of herpesviruses

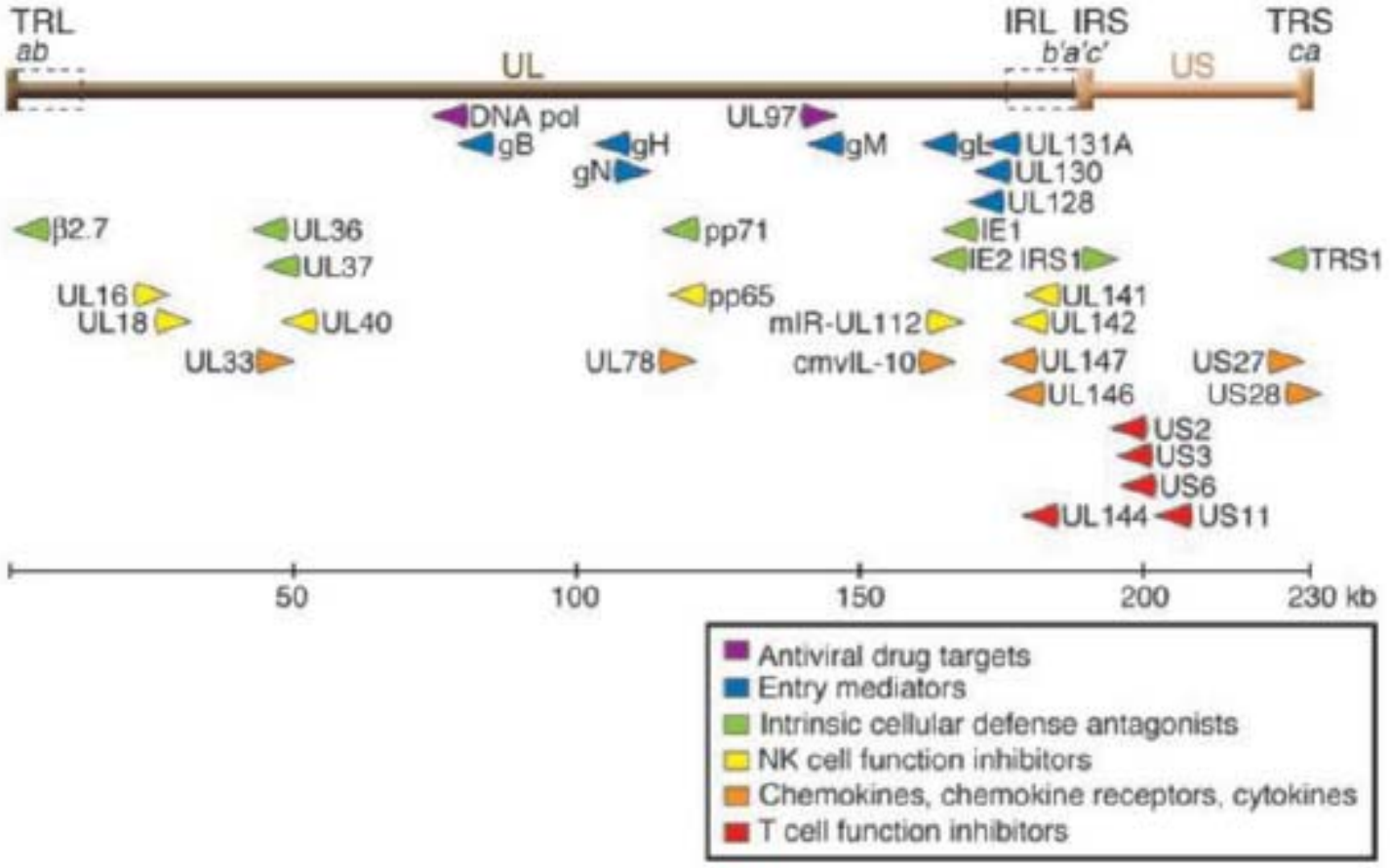
Classes of herpesviruses

Alphaherpesviruses - HSV-1, HSV-2, VZV

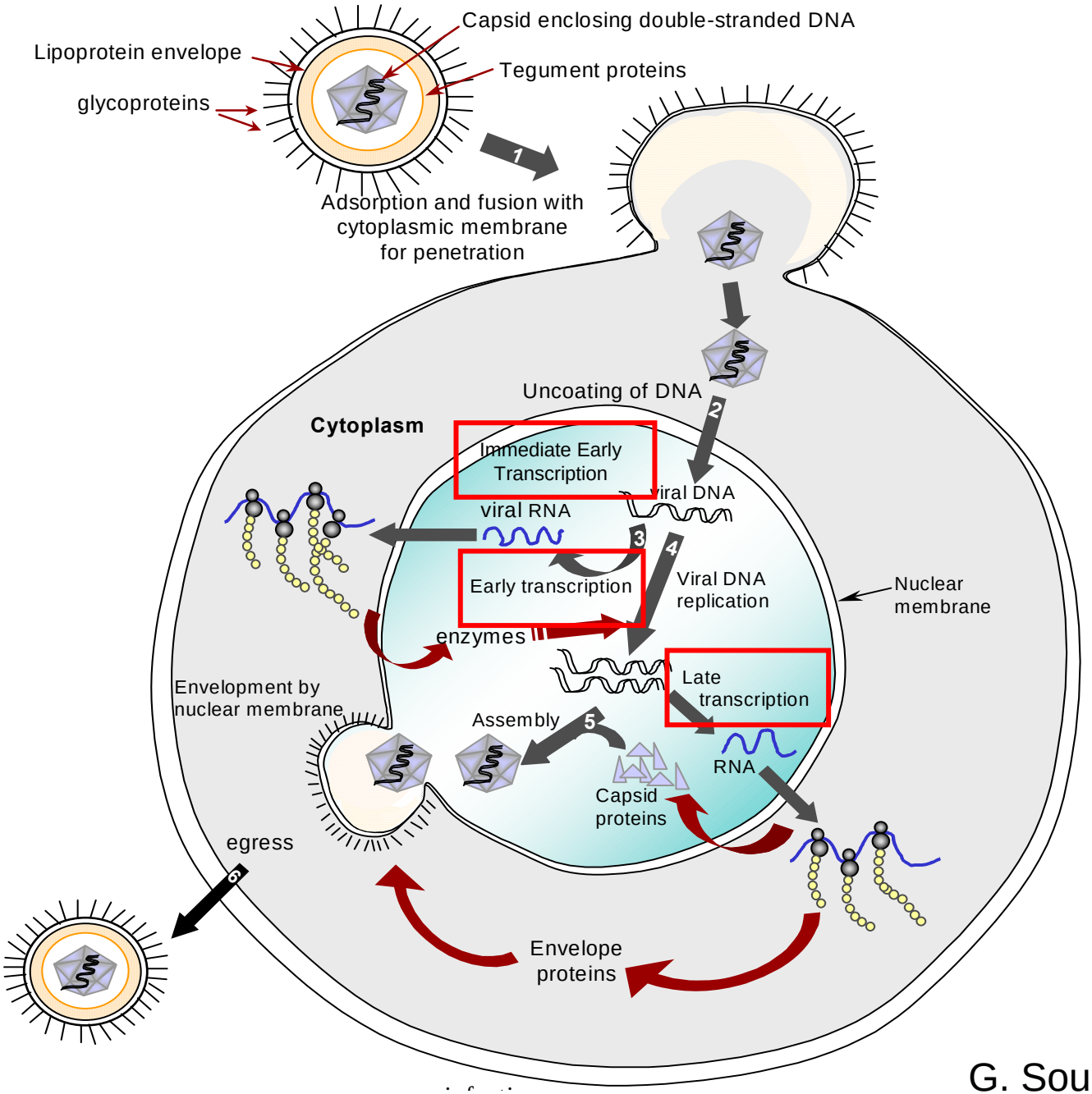
Betaherpesviruses - CMV, HHV-6, HHV-7

Gammaherpesviruses - EBV, HHV-8

The HCMV genome

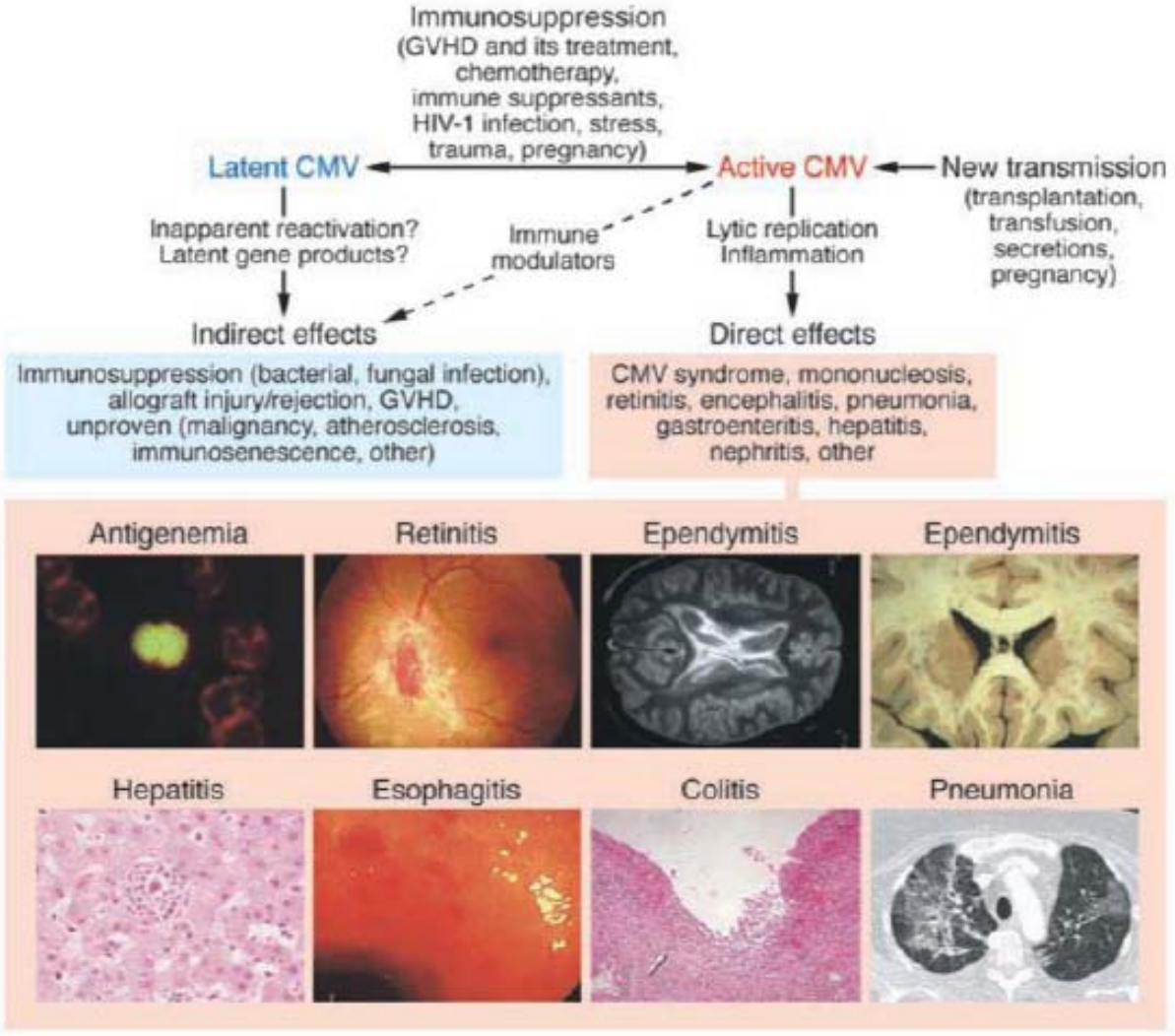


JCI 121: 1673, 2011


Capsid enclosing double-stranded DNA
Lipoprotein envelope
Tegument proteins
glycoproteins
1
Adsorption and fusion with
cytoplasmic membrane
for penetration
Uncoating of DNA
2
Cytoplasm
Immediate Early
Transcription
viral DNA
viral RNA
3
4
Early transcription
Viral DNA
replication
Nuclear
membrane
enzymes
Late
transcription
Envelopment by
nuclear membrane
Assembly
5
RNA
Capsid
proteins
egress
6
Envelope
proteins

G. Sourvinos

HCMV disease mechanisms



JCI 121: 1673, 2011

Productive infection and reactivation from latency can be affected by the differentiation status of the cells

NDY1/KDM2B And EZH2 are selectively required for the infectionof human foreskin fibroblasts with HCMV

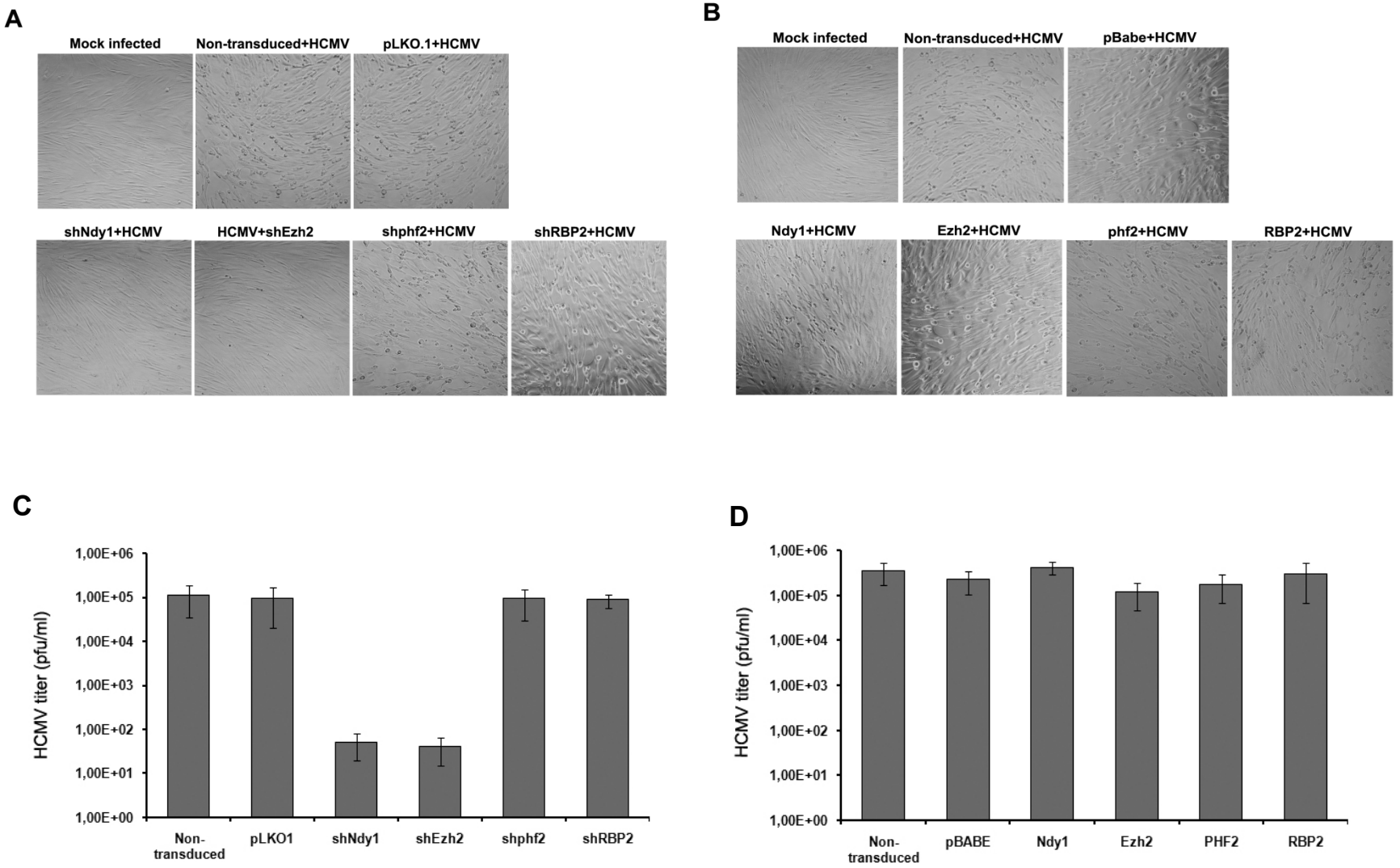


Figure 1

Infection of human foreskin fibroblasts with HCMV depends on H3K27 trimetylation

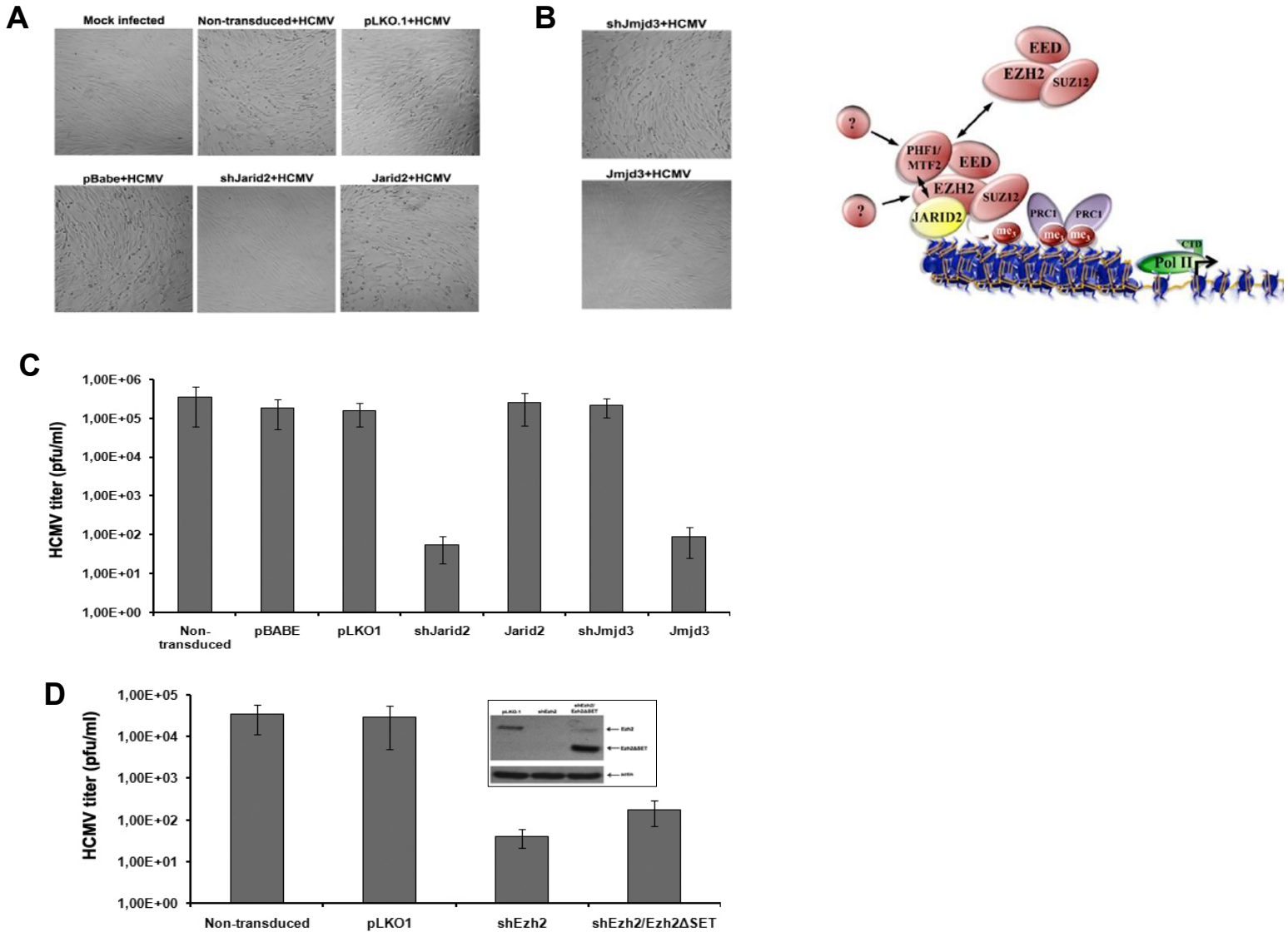


Figure 2

The knockdown of NDY1, EZH2 and JARID2 does not interfere with viral entry

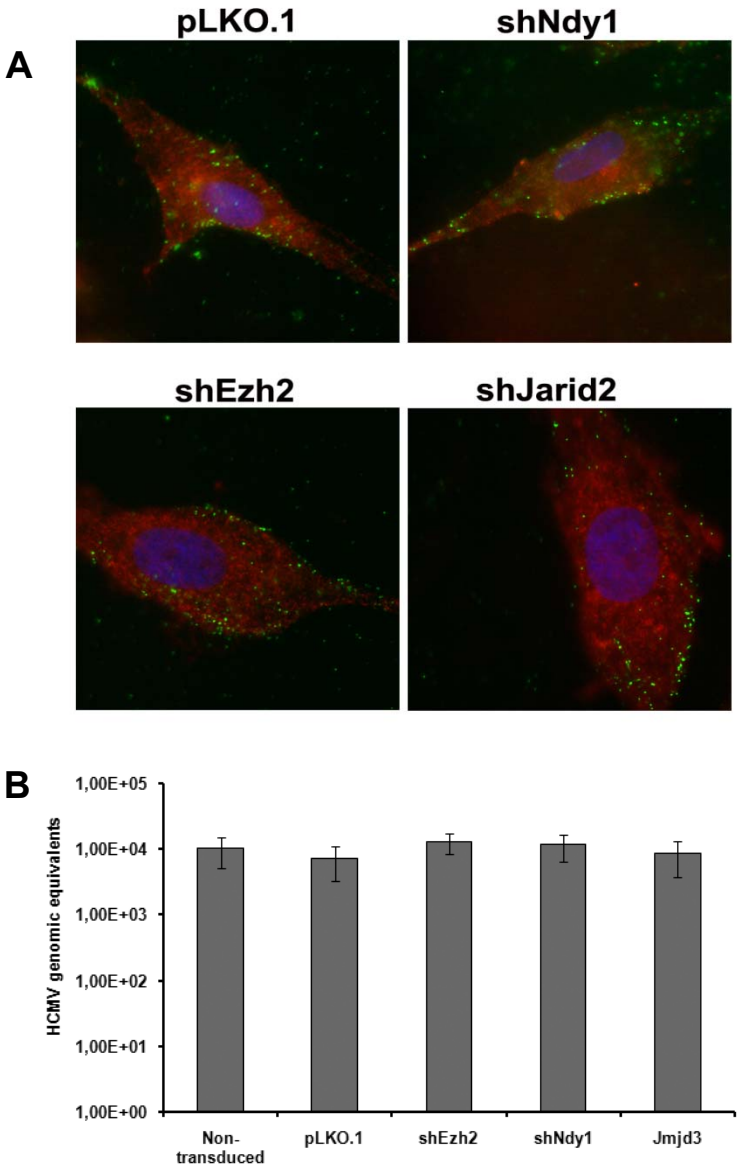
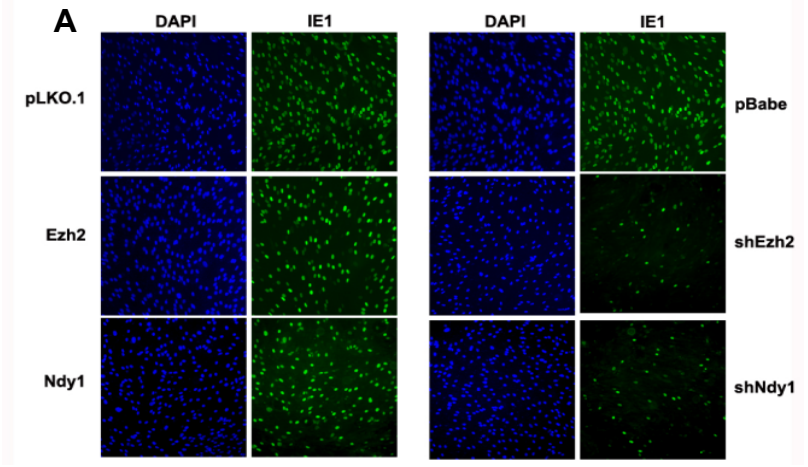
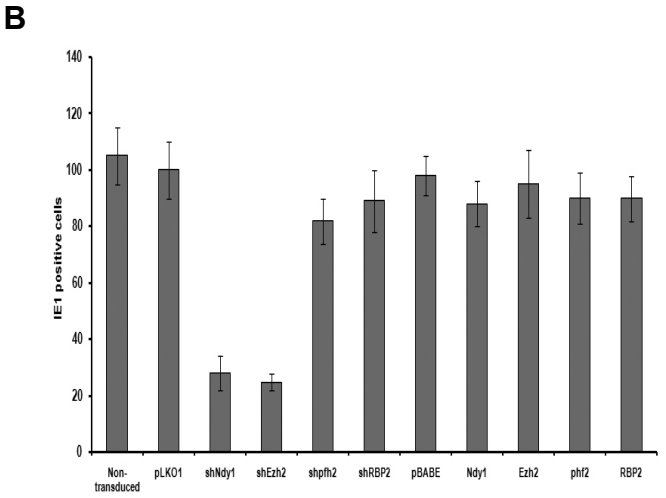
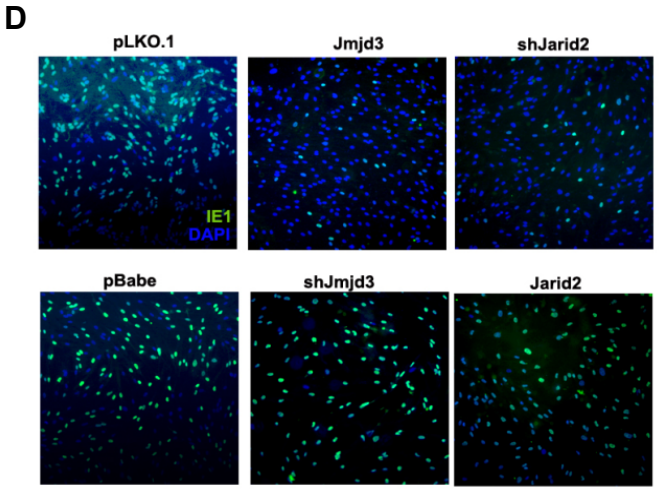


Figure 3

NDY1, EZH2 and H3K27 trimethylation are required for Immediate early gene transcription







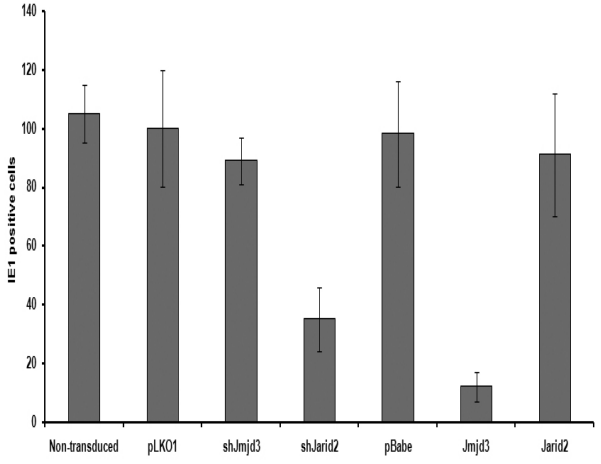


Figure 4

Knockdown of NDY1 or EZH2 inhibits the transcriptional activity of the HCMV Immediate early promoter

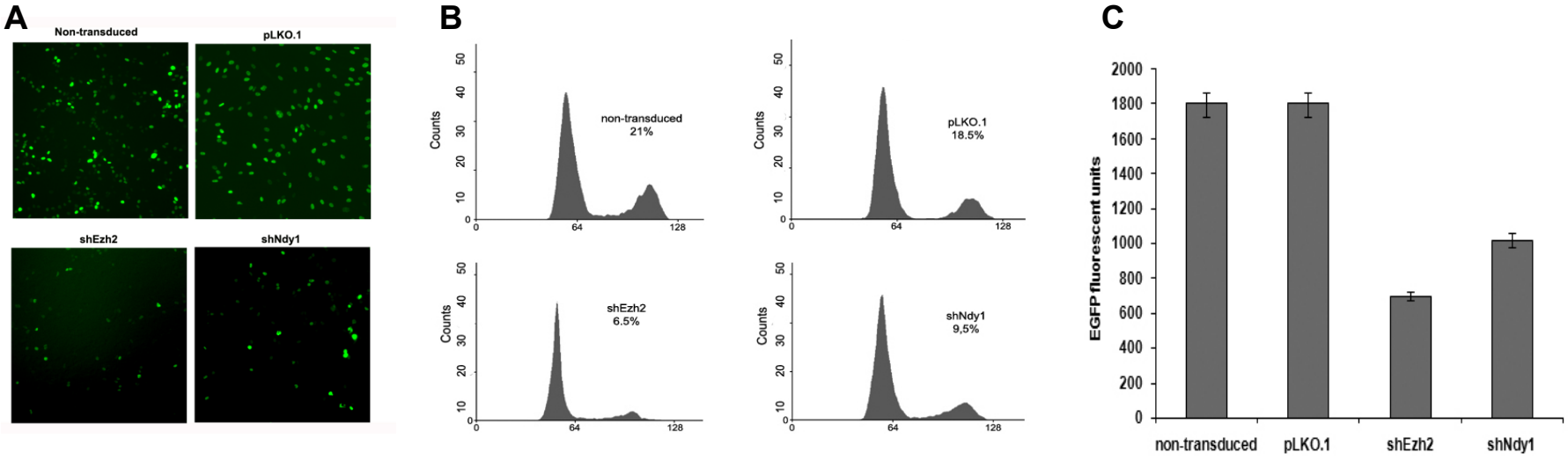
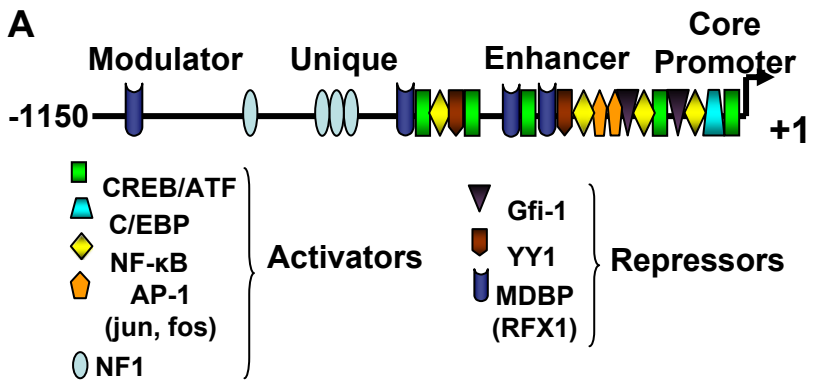


Figure 5

The knockdown of NDY1, EZH2 and JARID2 and the overexpression of JMJD3 selectively upregulate the expression of Gfi1



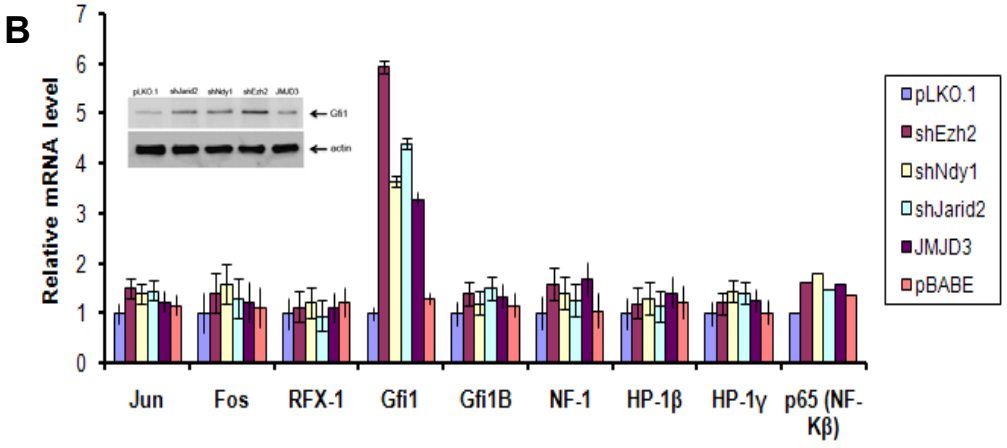


Figure 6

Immediate early upregulation of Ezh2 and Jarid2 and downregulation of Jmjd3 in the course of HCMV infection

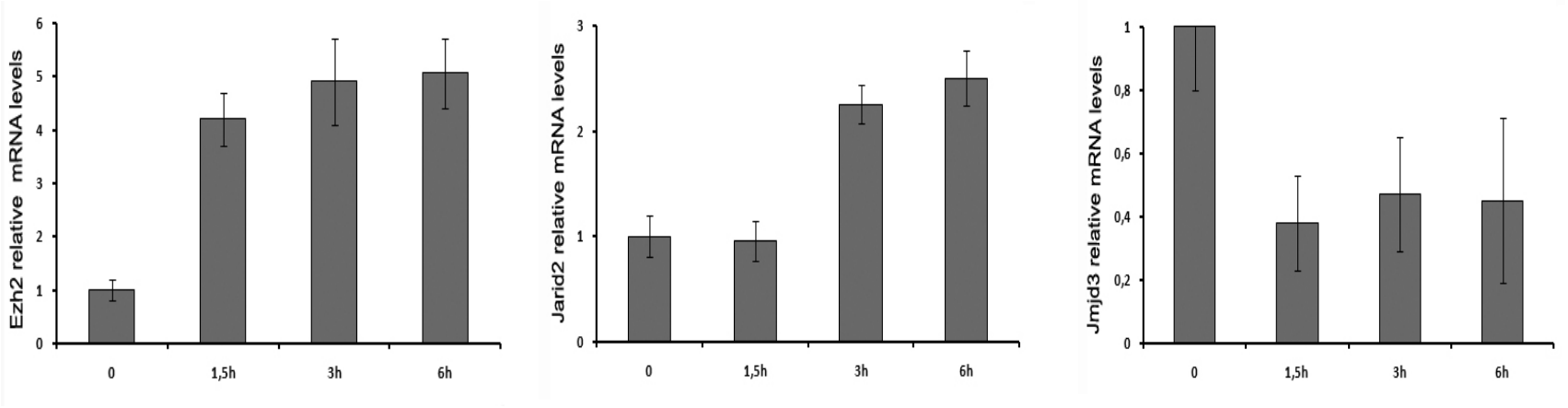
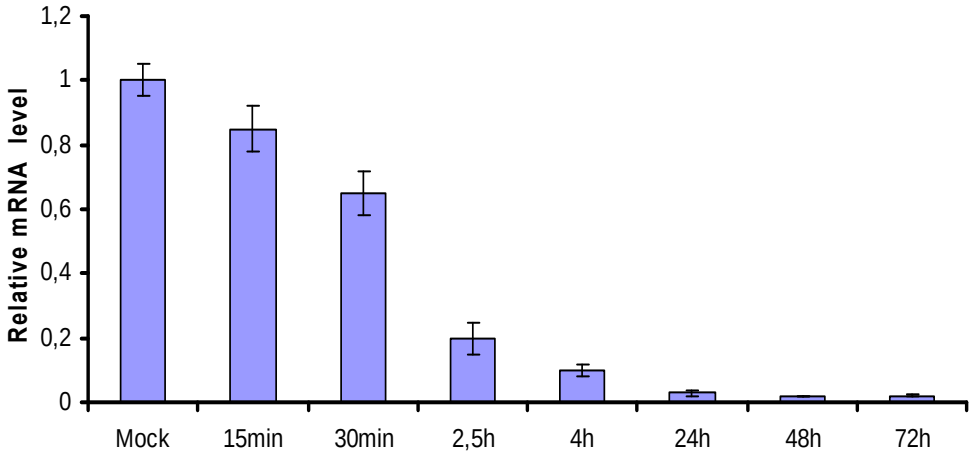
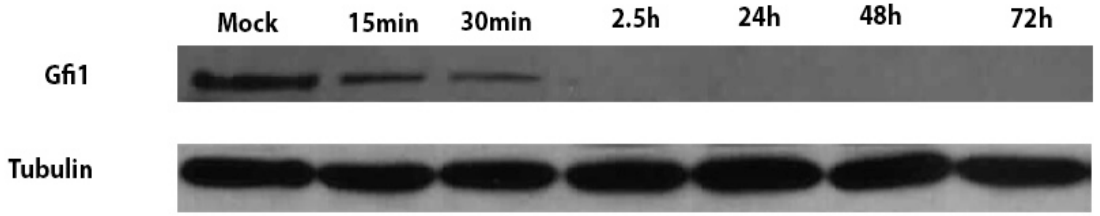


Figure 7

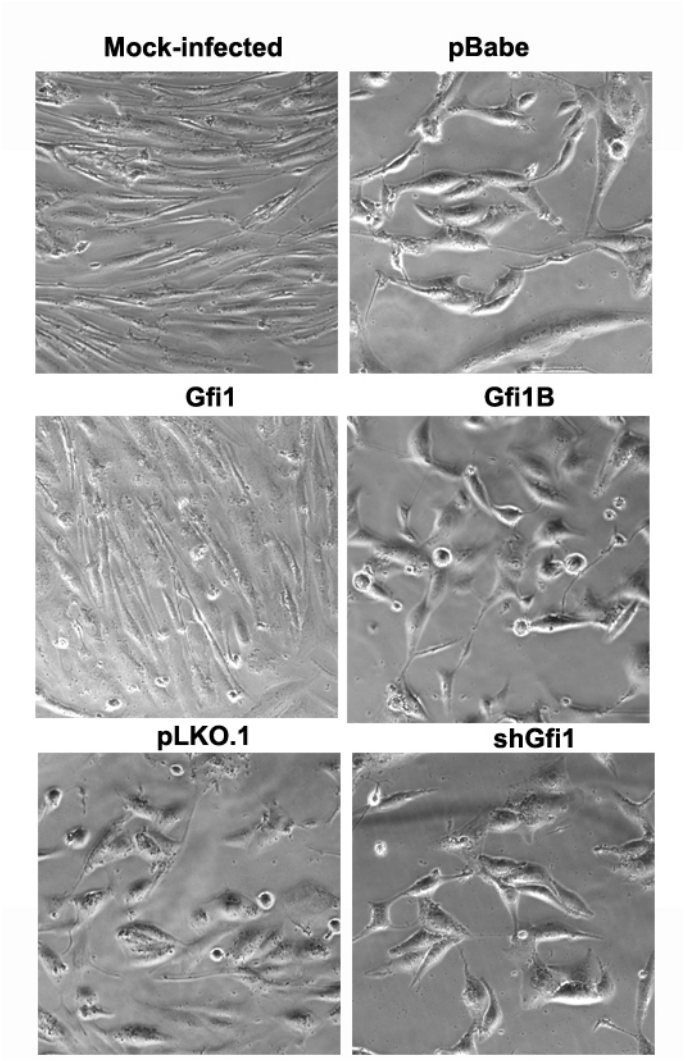
Immediate early downregulation of Gfi1 in the course of HCMV infection



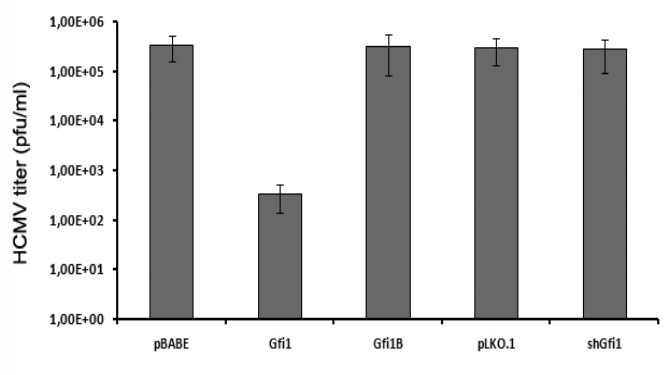


The initiation of HCMV infection depends on Gfi1 but not on Gfi1B

A



B



C

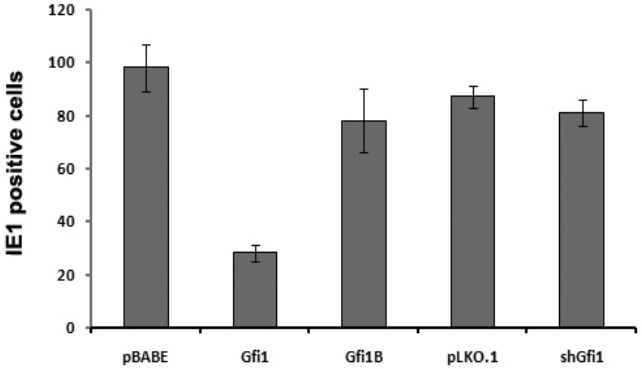
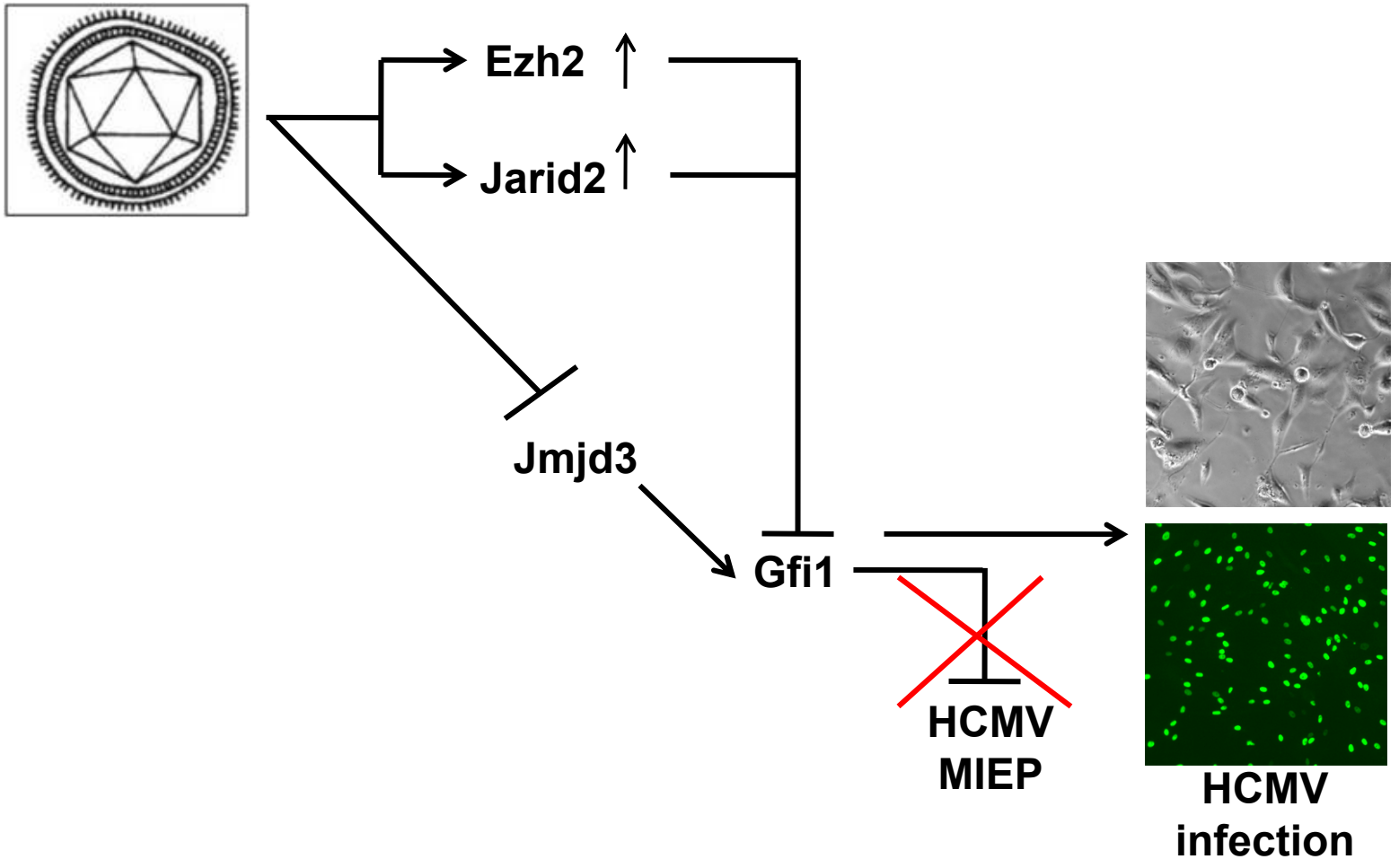
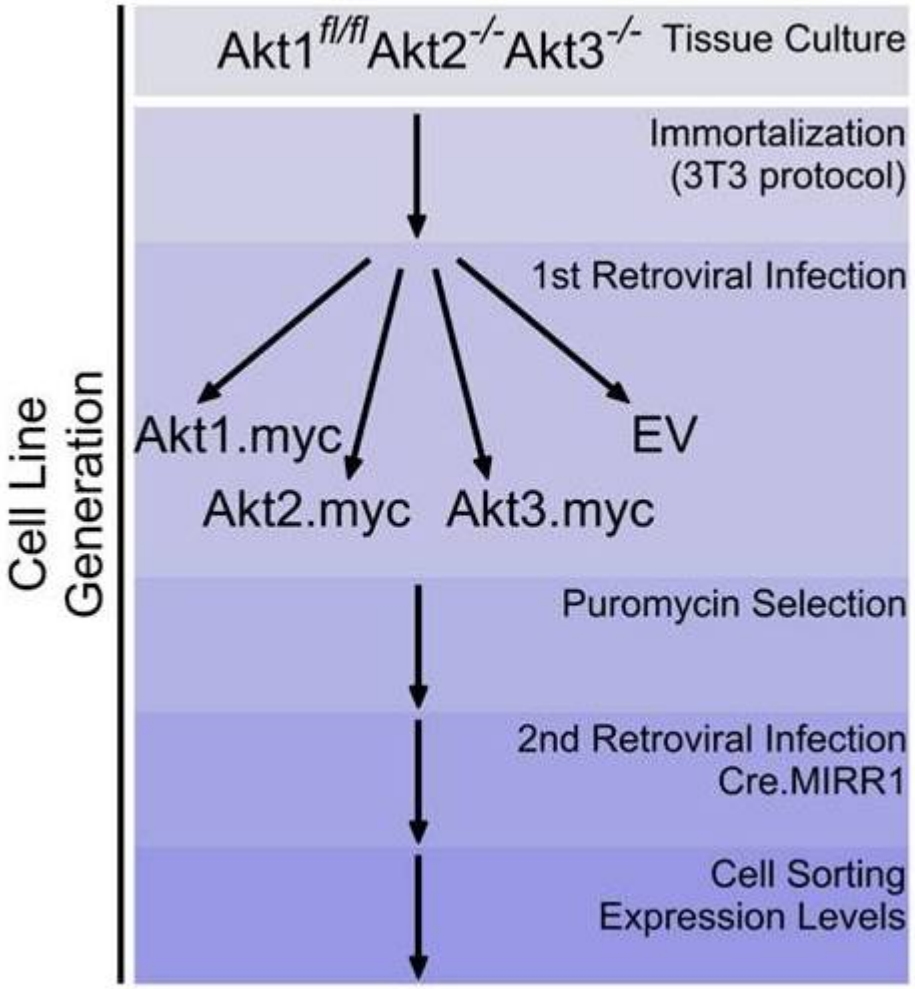


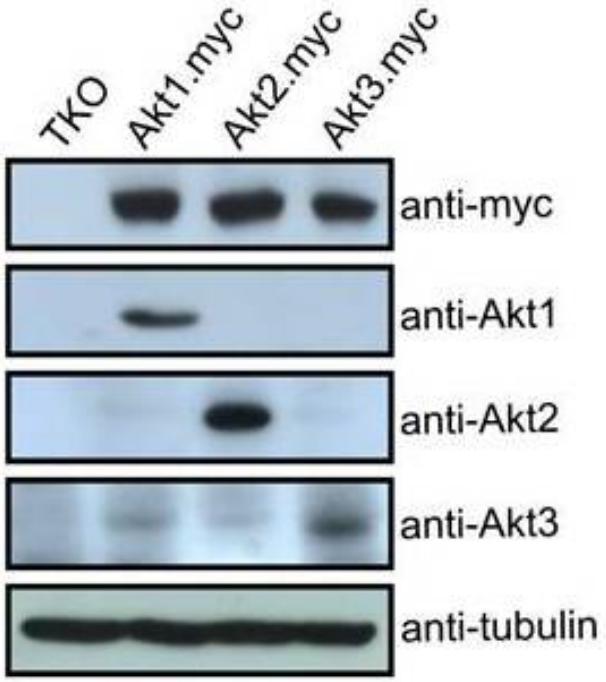
Figure 7

Infection by HCMV depends on NDY1/KDM2B, EZH2 and JARID2, which epigenetically regulate GFI1, a repressor of immediate early gene transcription



Development of a new platform for the study of Akt isoform-specific properties

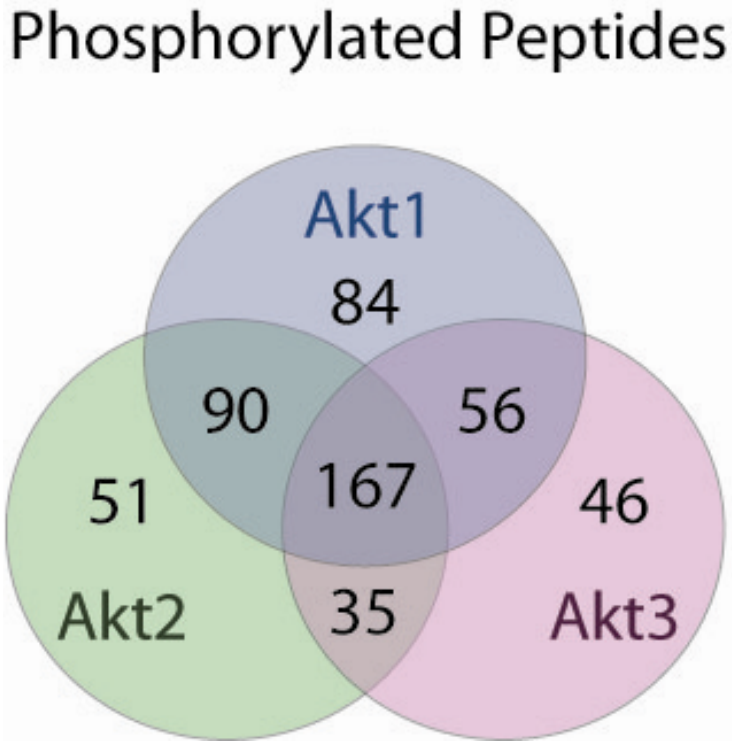


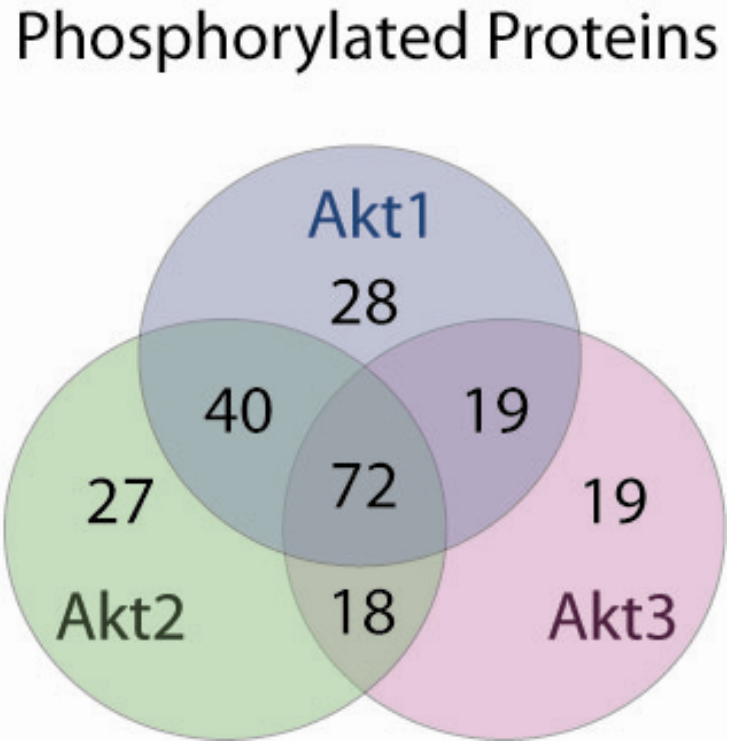


Iliopoulos et al Science Signaling: 2: ra62, 2009

Akt1 is not Akt2
Akt1 is not Akt3
Akt2 is not Akt3

Common-Individual Targets

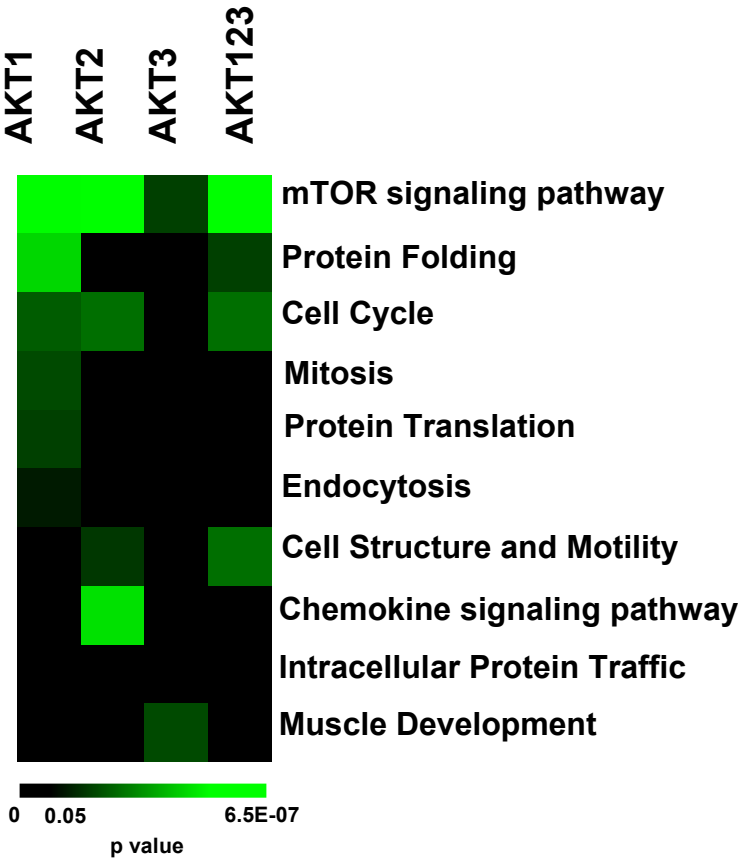




Polytarchou et al Unpublished

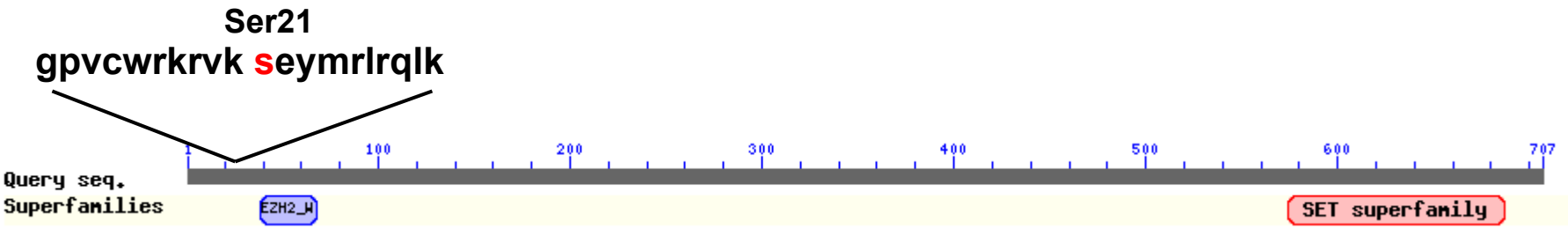
Akt1 is not Akt2
Akt1 is not Akt3
Akt2 is not Akt3

Gene Ontology HeatMap of AKT Phosphoproteomics



Polytarchou et al Unpublished

EZH2 is phosphorylated preferentially by Akt1 and Akt2 at Ser21



Phosphorylation at Ser21 inhibits the EZH2 methyltransferase activity

Figure3

EZH2 is required and constitutively active Akt1 and Akt2 inhibit the initiation of HCMV infection

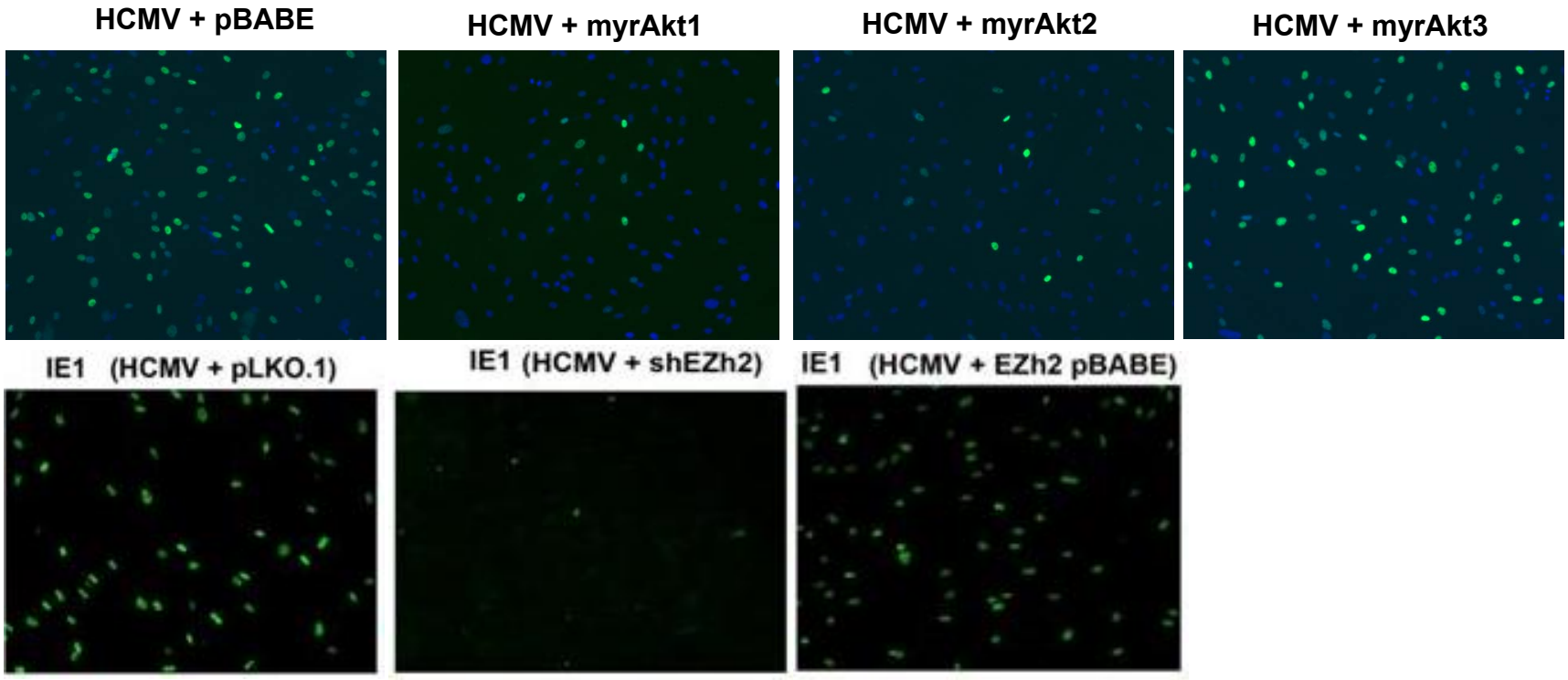


Figure 2A

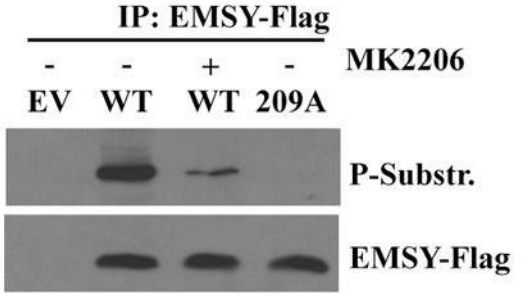
EMSY is preferentially phosphorylated by Akt1 at Ser209

Scott Ezell

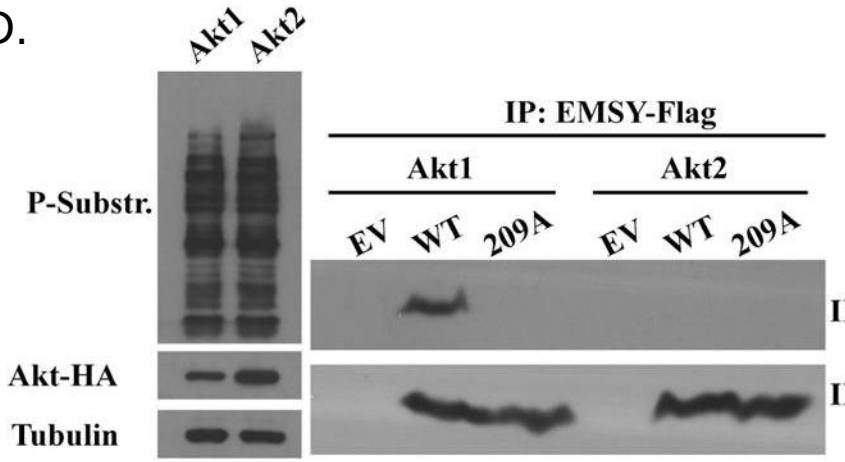
B.

H. sapiens:	EKPRKRRTNSSSSSPVVLKE
M. musculus:	EKPRKRRTNSSSSSPVVLKE
R. norvegicus:	EKPRKRRTNSSSSSPVVLKE
D. rerio:	EKPRKRRTSSSSSSPVLLKE
X. tropicalis:	EKPRKRRTNSNSSSPVVLKE

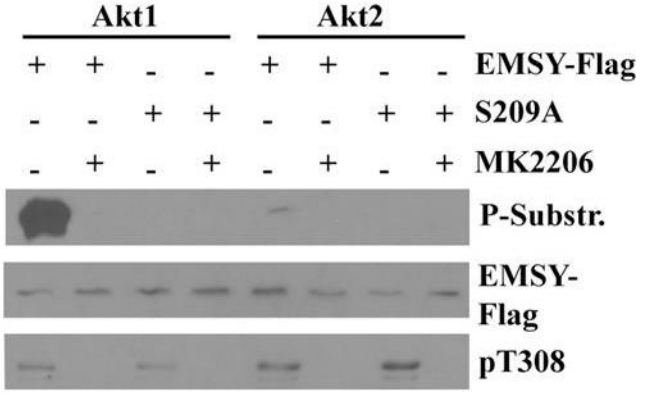
C.



D.

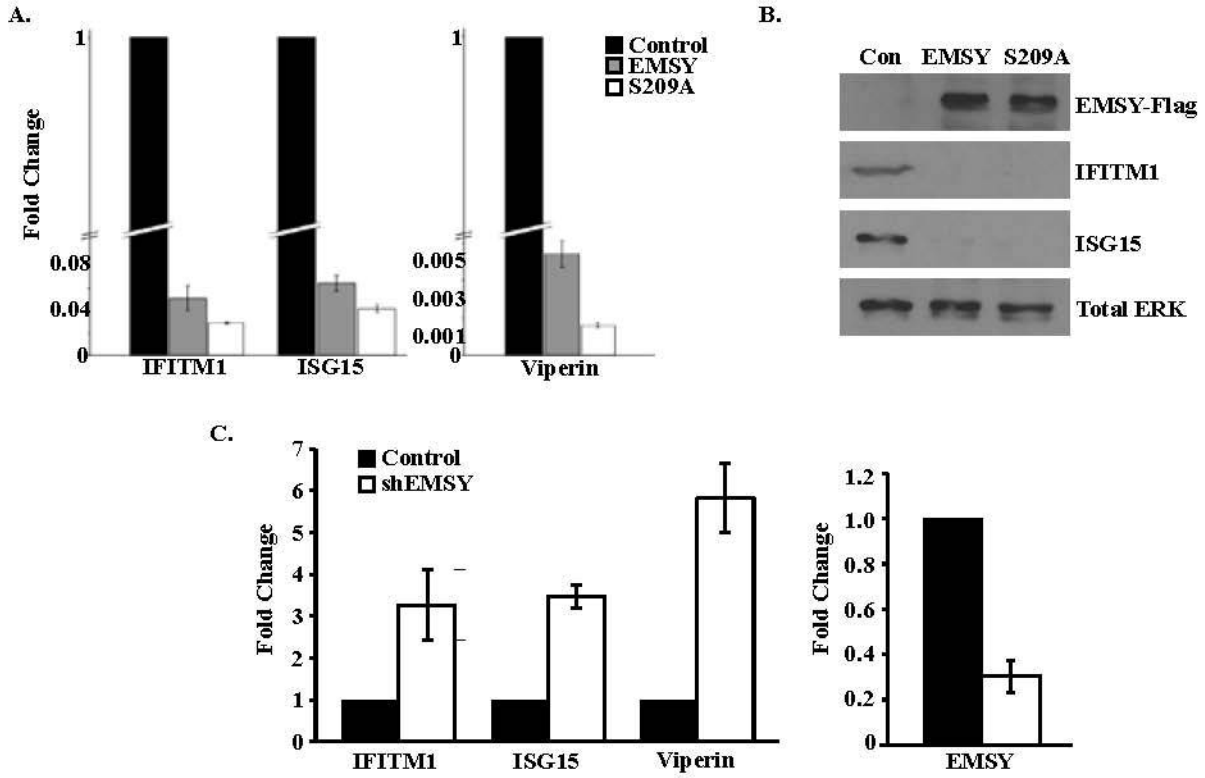


E.



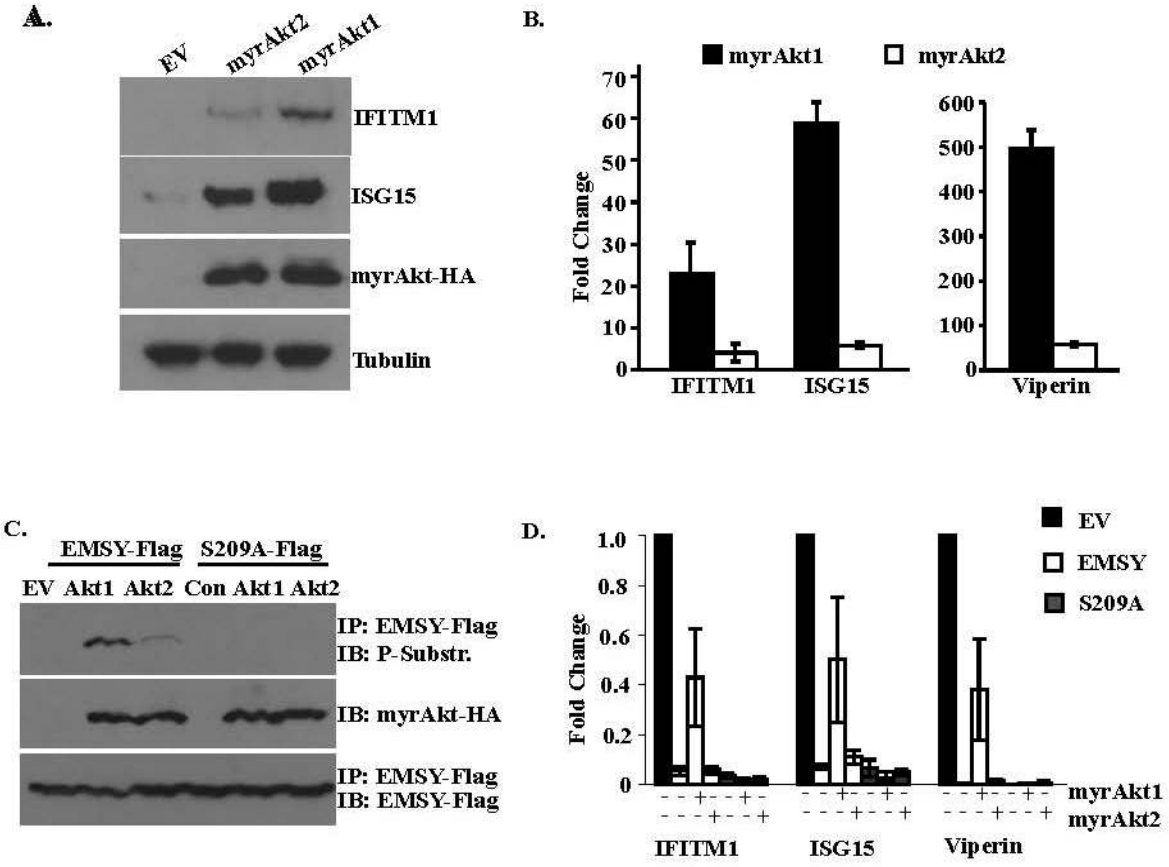
Ezell, et al. Figure 1

EMSY functions as a repressor of IFN-stimulated genes



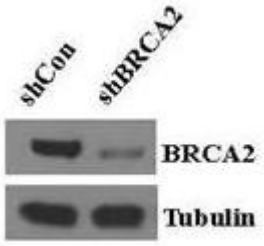
Ezell, et al. Figure 2

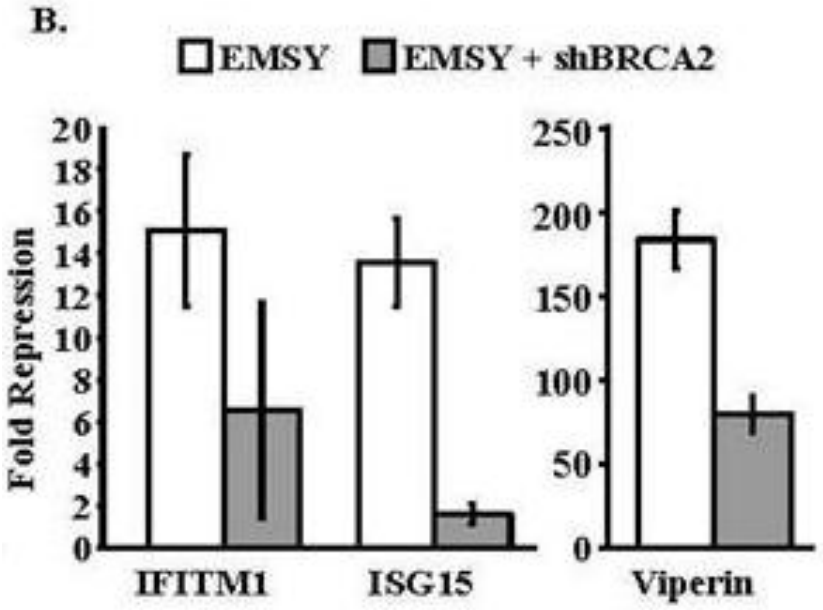
Akt1 selectively relieves the repression of ISGs via EMSY phosphorylation at Ser209



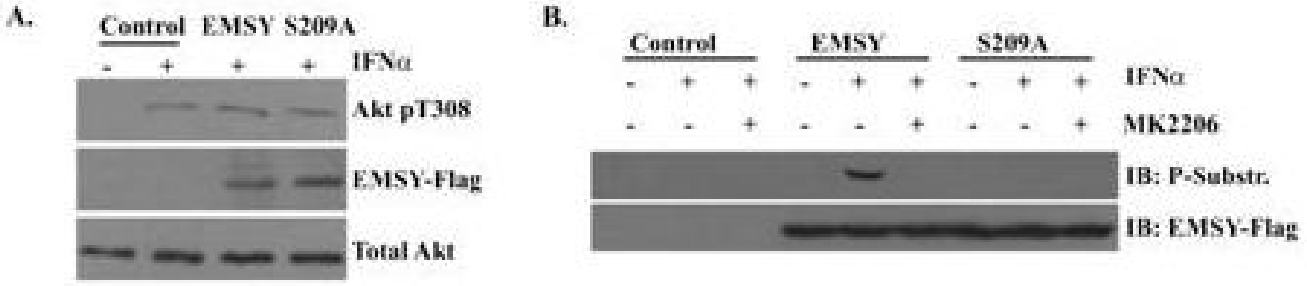
Ezell, et al. Figure 3

The repression of ISG expression by EMSY is BRCA2-dependent

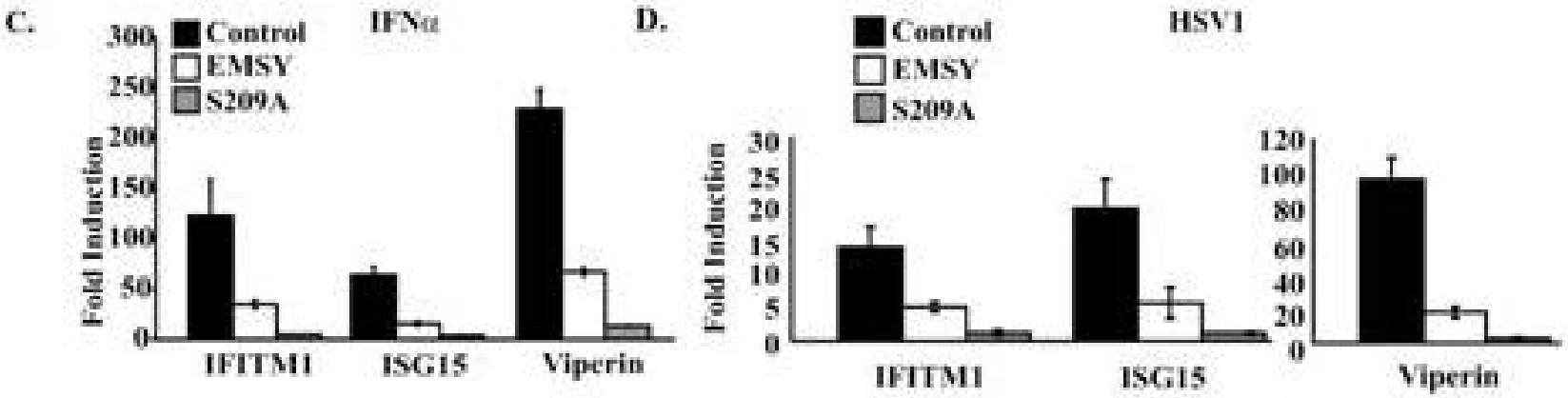




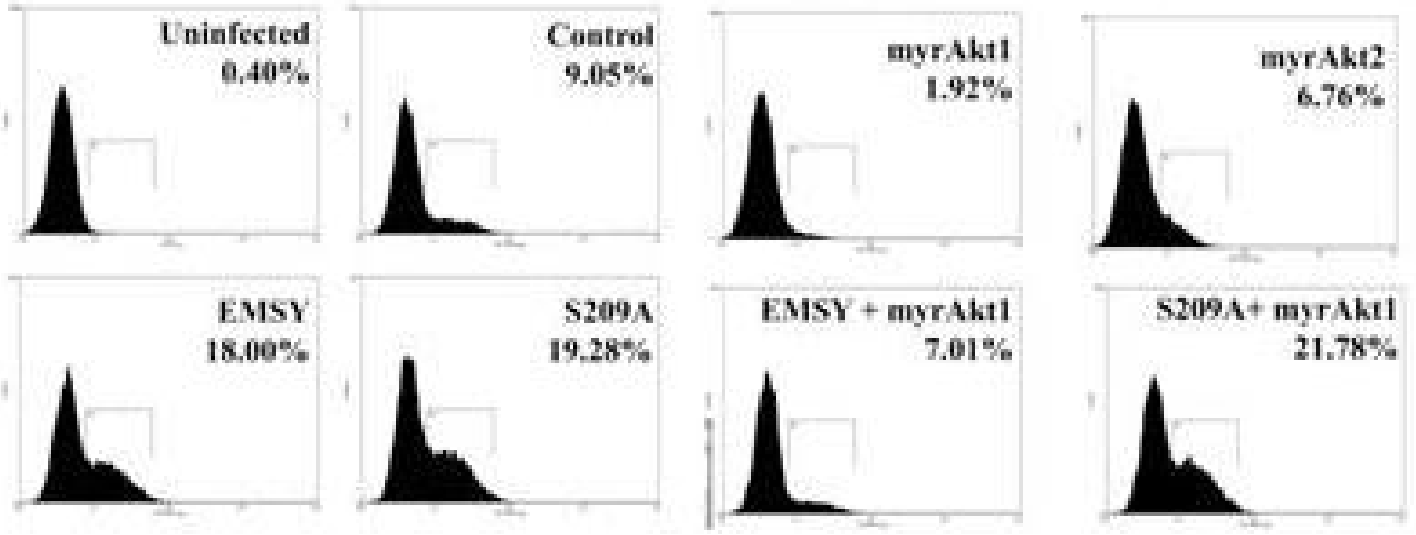
IFN-α promotes Akt activation and EMSY phosphorylation at Ser209



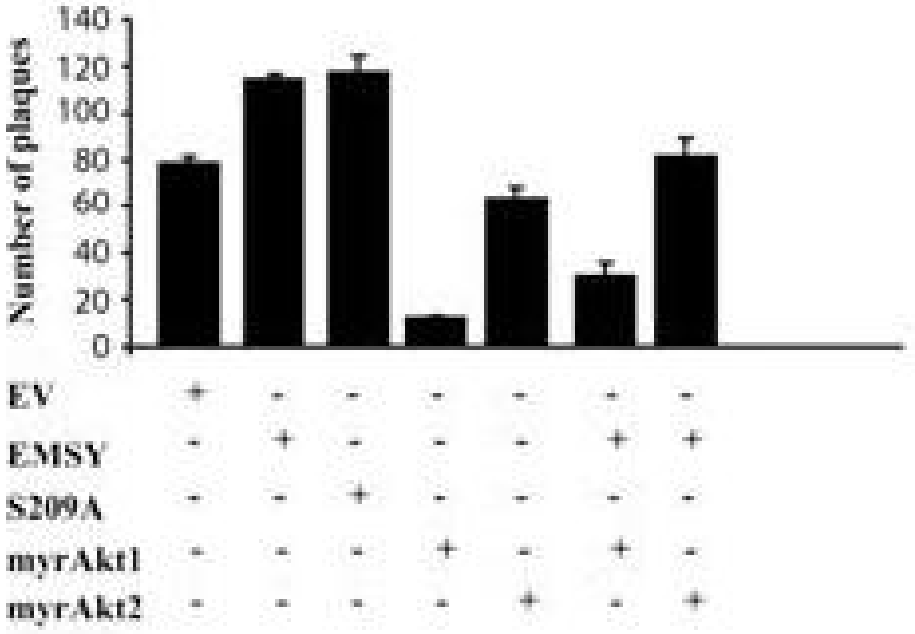
IFN-α and HSV1 induce robust expression of ISGs in control and wild type EMSY-transduced cells but not in cells transduced with EMSYS209A



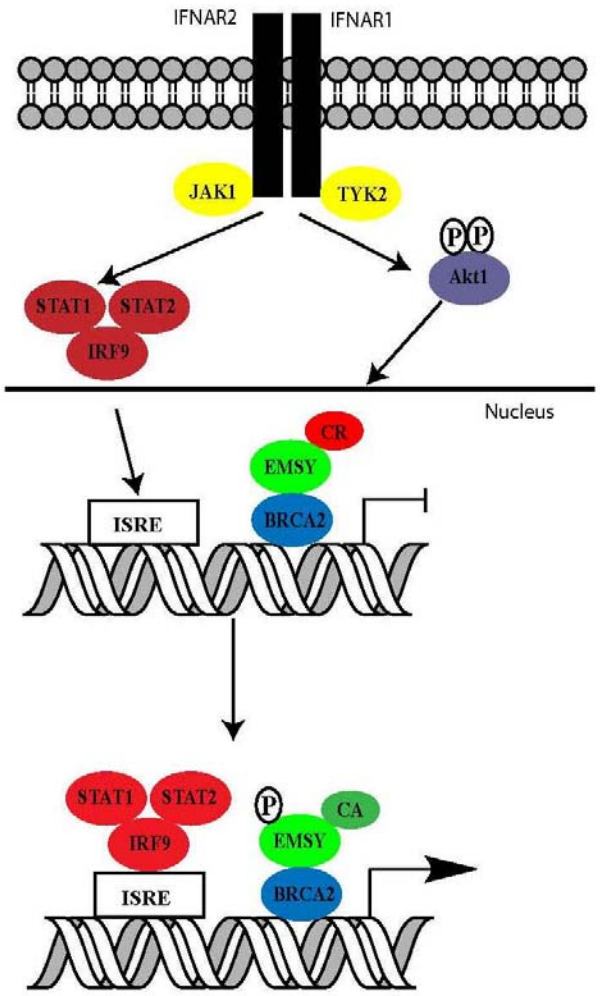
Akt1 selectively inhibits HSV1 infection via EMSY phosphorylation at Ser209



Akt1 selectively inhibits VSV infection via EMSY phosphorylation at Ser209



A model for the functional role of EMSY, BRCA2 and Akt1 in the regulation of IFN-stimulated genes



SUMMARY

- HCMV immediate early gene expression during infection requires the repression of Gfi1.
- Gfi1 repression depends on histone H3K27 trimethylation, which is due to the concerted action of NDY1, EZH2 and JARID2
- Akt1 inhibits HCMV infection through phosphorylation of EZH2 at Ser21, which inhibits the enzymatic activity of the protein

- EMSY, a BRCA2 interacting protein, is a repressor of Interferon stimulated genes (ISGs) and its repression function is inhibited by phsphorylation at Ser209 by Akt1 and Akt3.
- By regulating the induction of ISGs in the course of viral infection, EMSY promotes and Akt1 inhibits infection by HSV1 and VSV

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- Patrick Zweidler-McKay
- Betty Tong

- Susan Bear
- Ray Pfau
- Christos Polytarchou
- Alexandros Tzatsos
- Sotiris Kampranis
- Filippos Kottakis

- George Sourvinos
- Scott Ezell