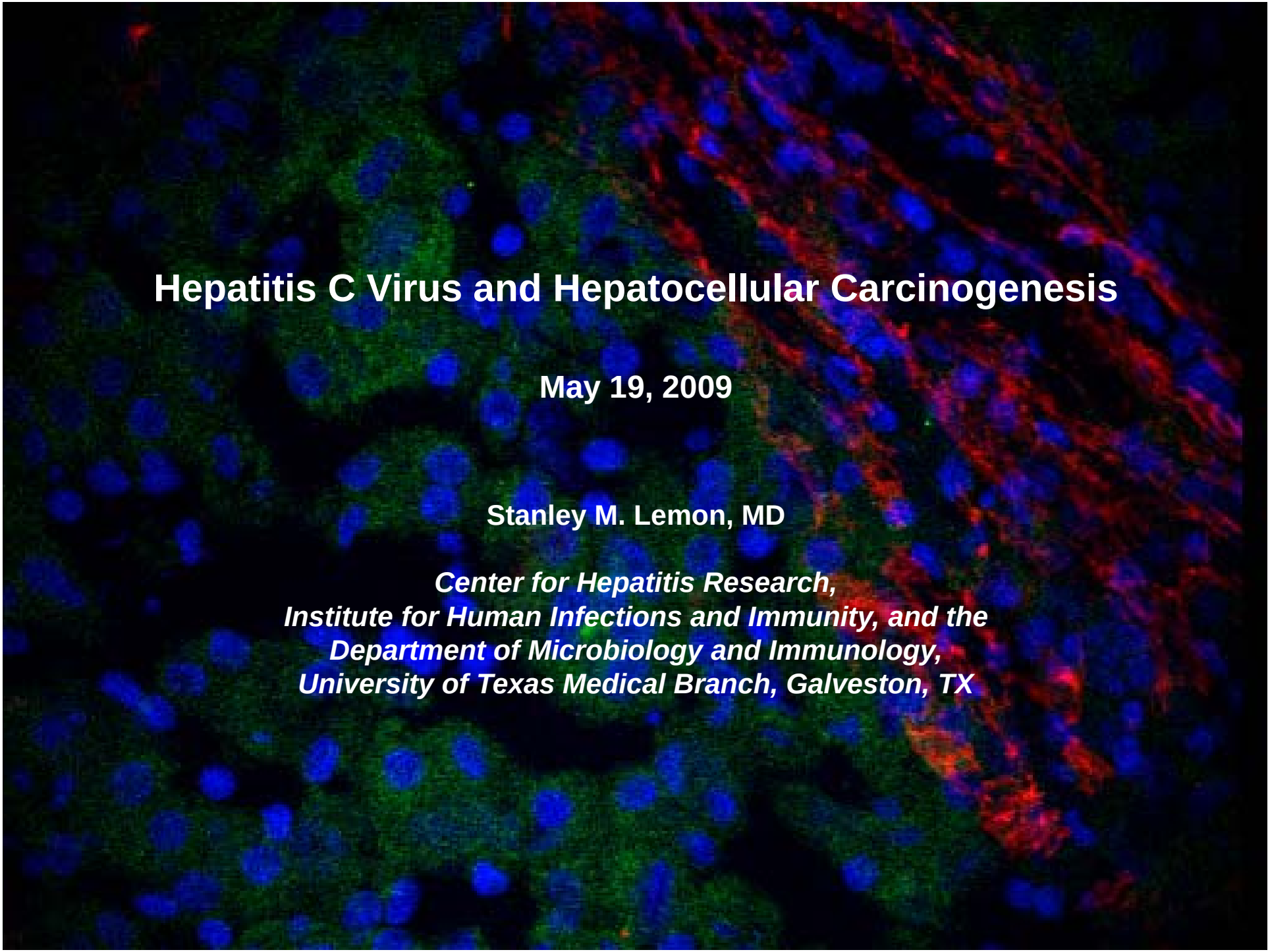


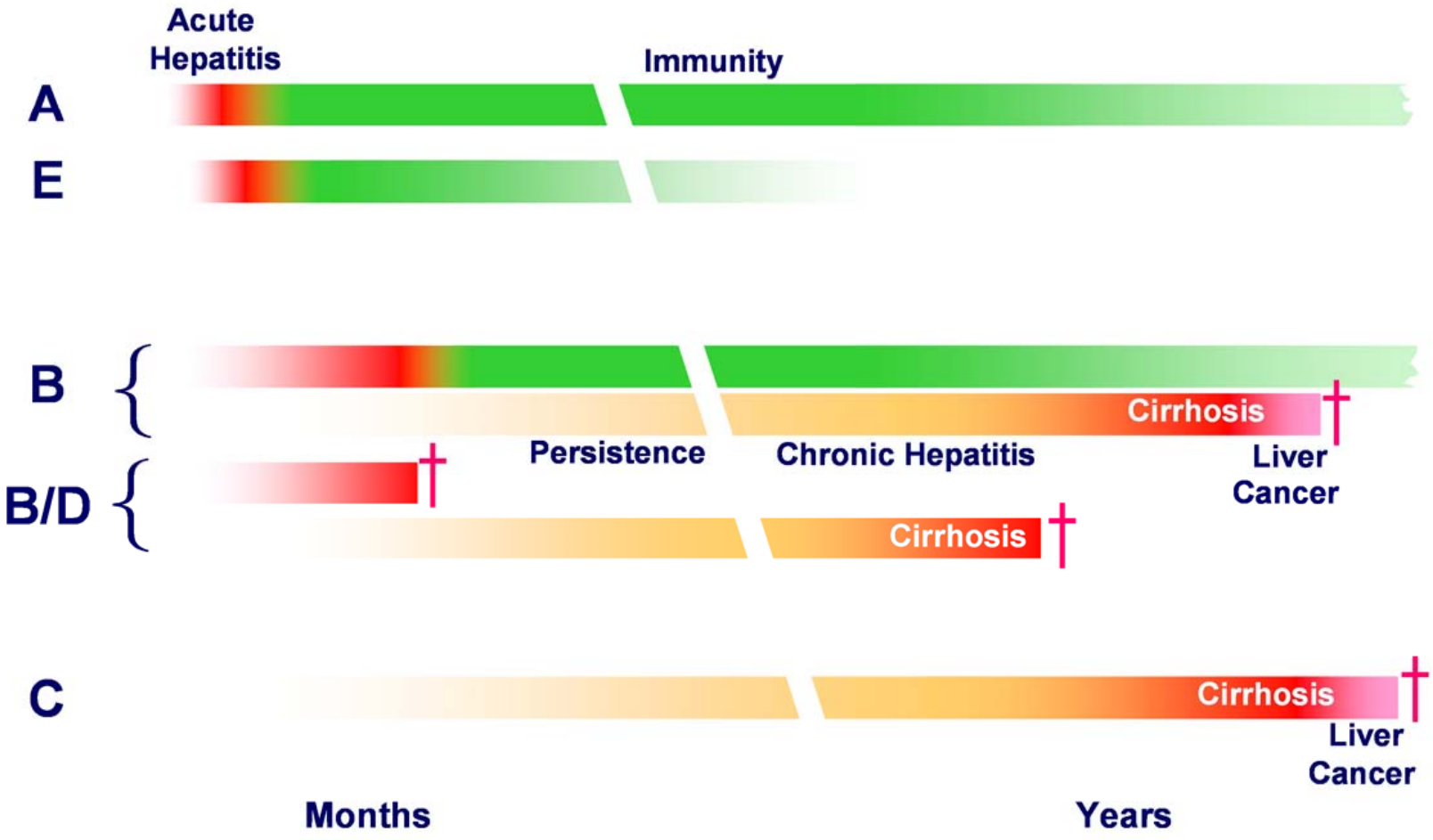
# Hepatitis C Virus and Hepatocellular Carcinogenesis

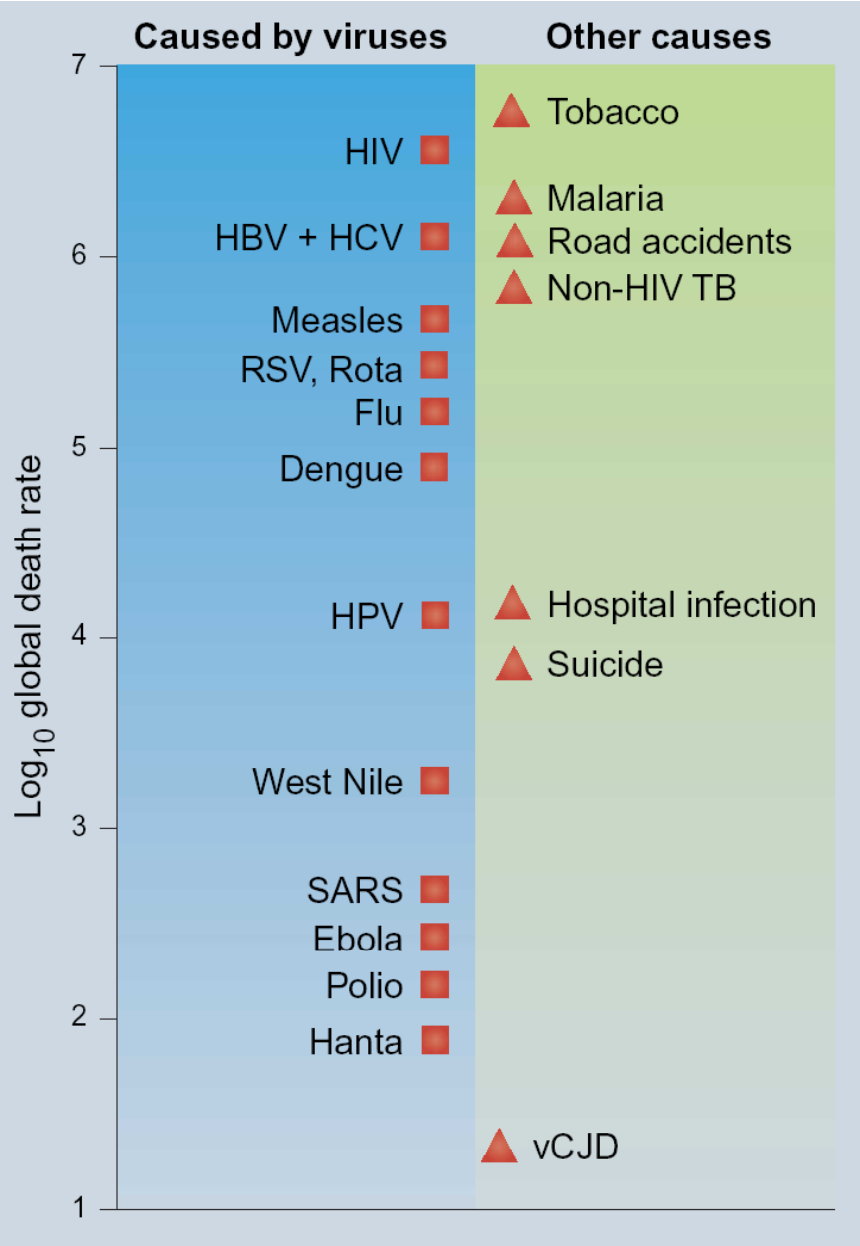
**May 19, 2009**

**Stanley M. Lemon, MD**

***Center for Hepatitis Research,***
***Institute for Human Infections and Immunity, and the***
***Department of Microbiology and Immunology,***
***University of Texas Medical Branch, Galveston, TX***

# The Spectrum of Hepatitis Viruses



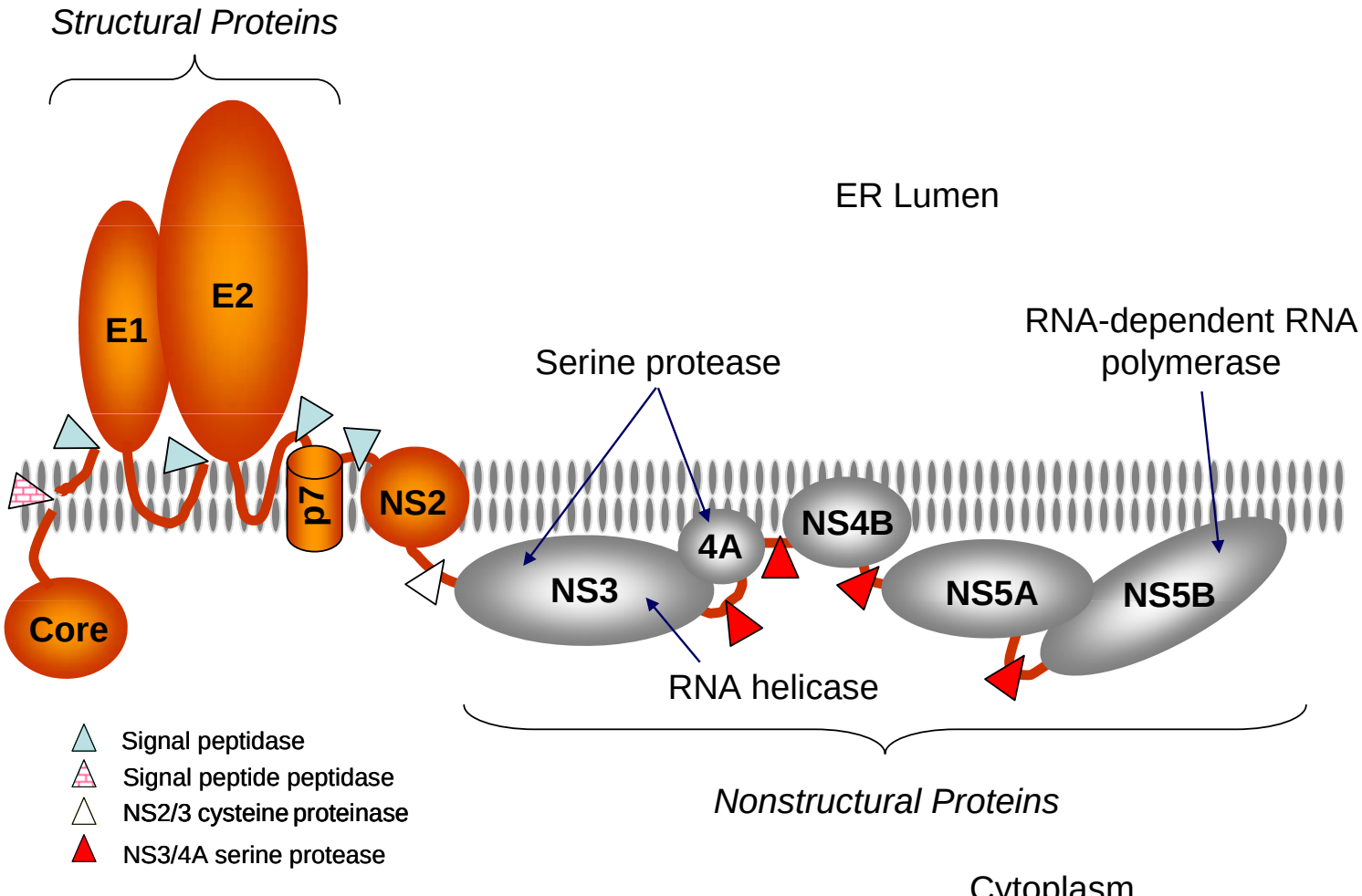



# A "Richter Scale" of Global Mortality Rates

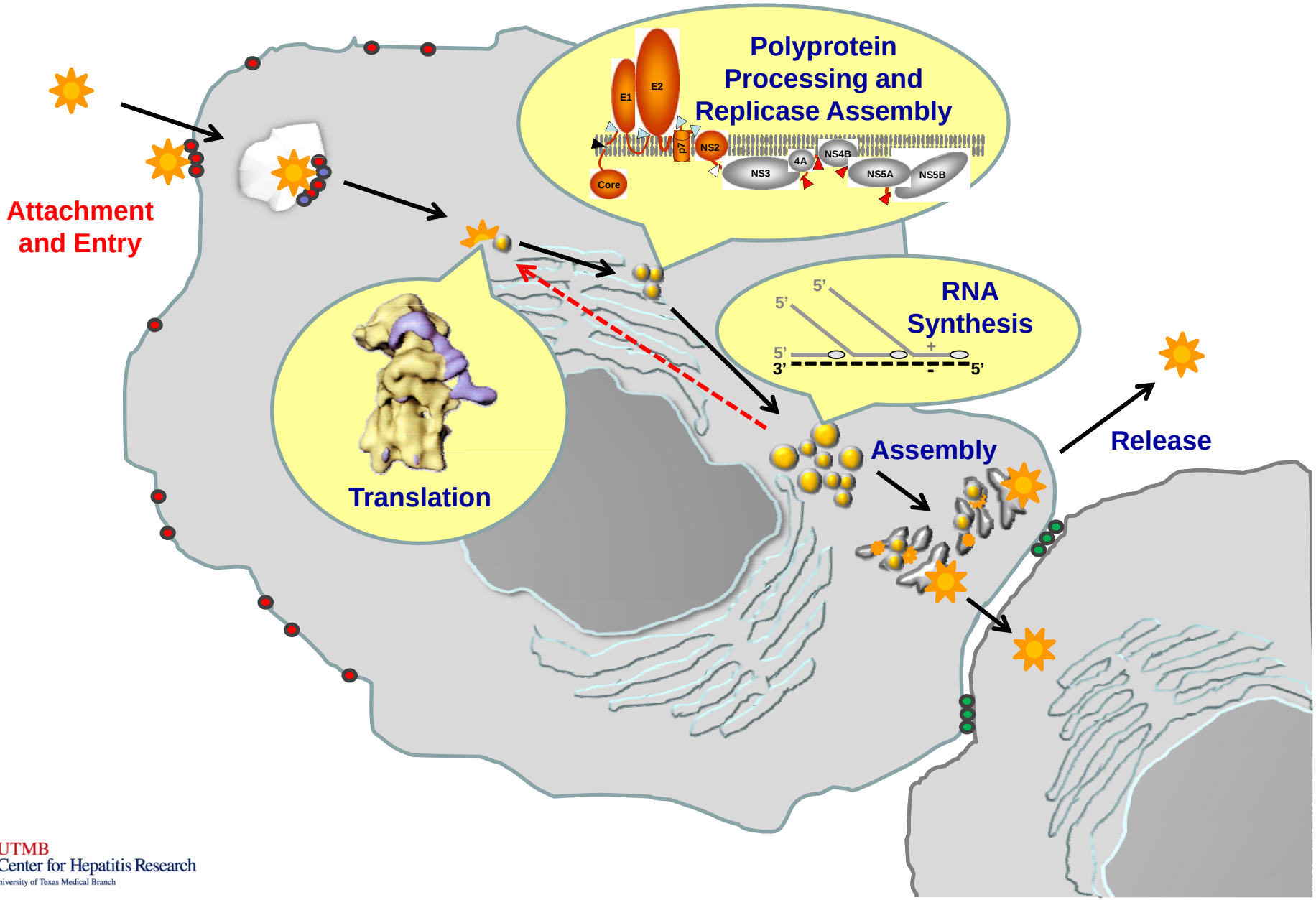


Weiss and McMichael, Nat Medicine *10:S70-76, 2004*

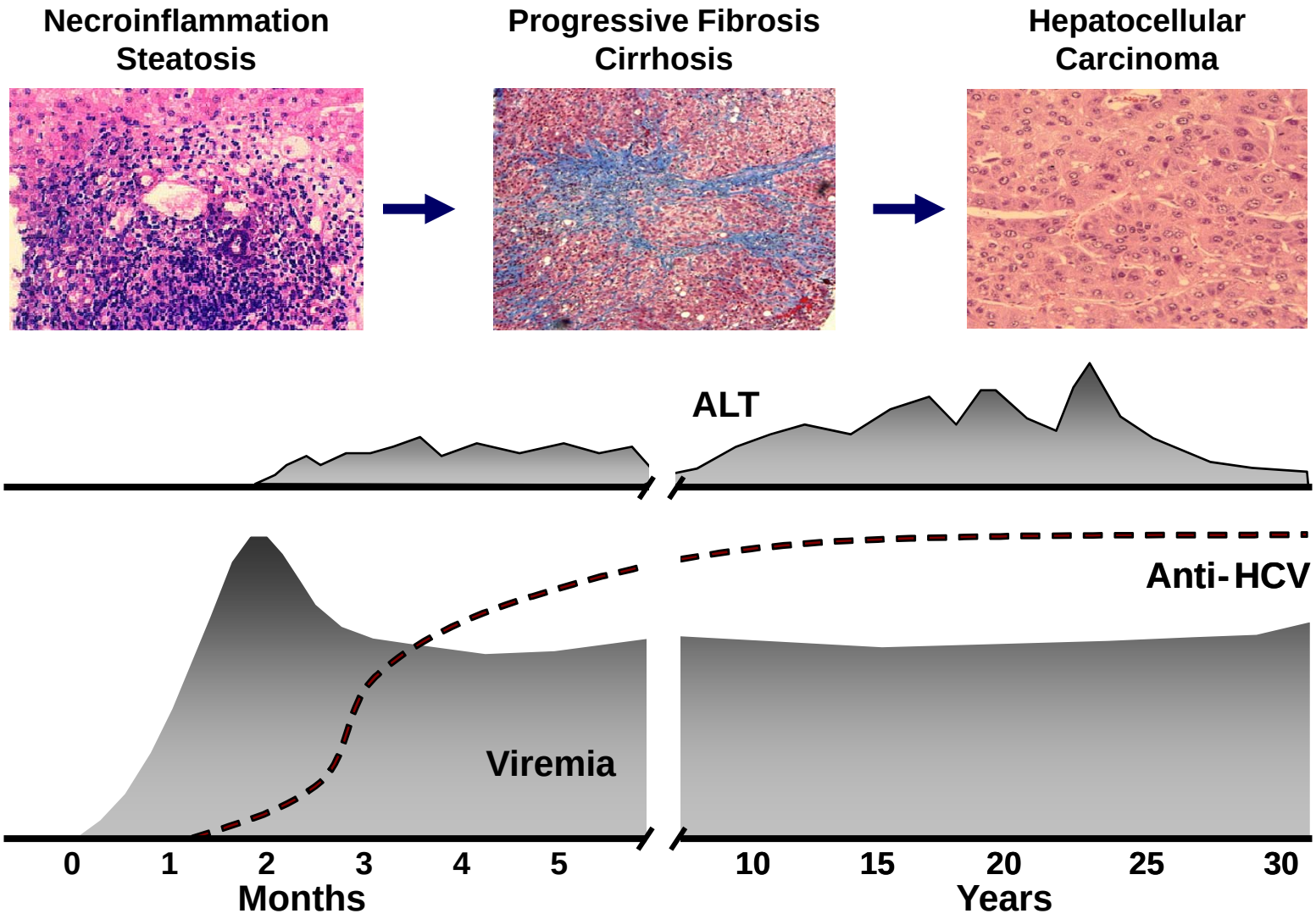
# Hepatitis C Virus

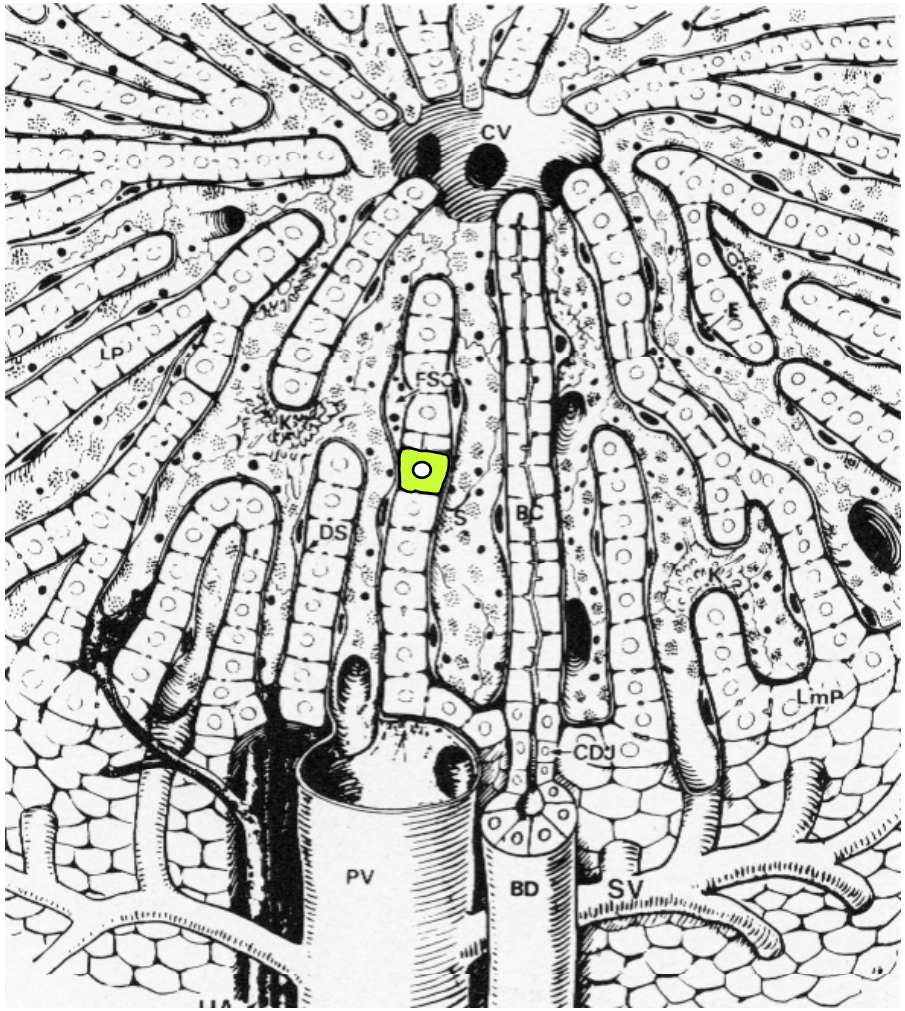
# The Hepatitis C Virus Life Cycle



# Natural History of Hepatitis C



# Hepatitis C Pathogenesis

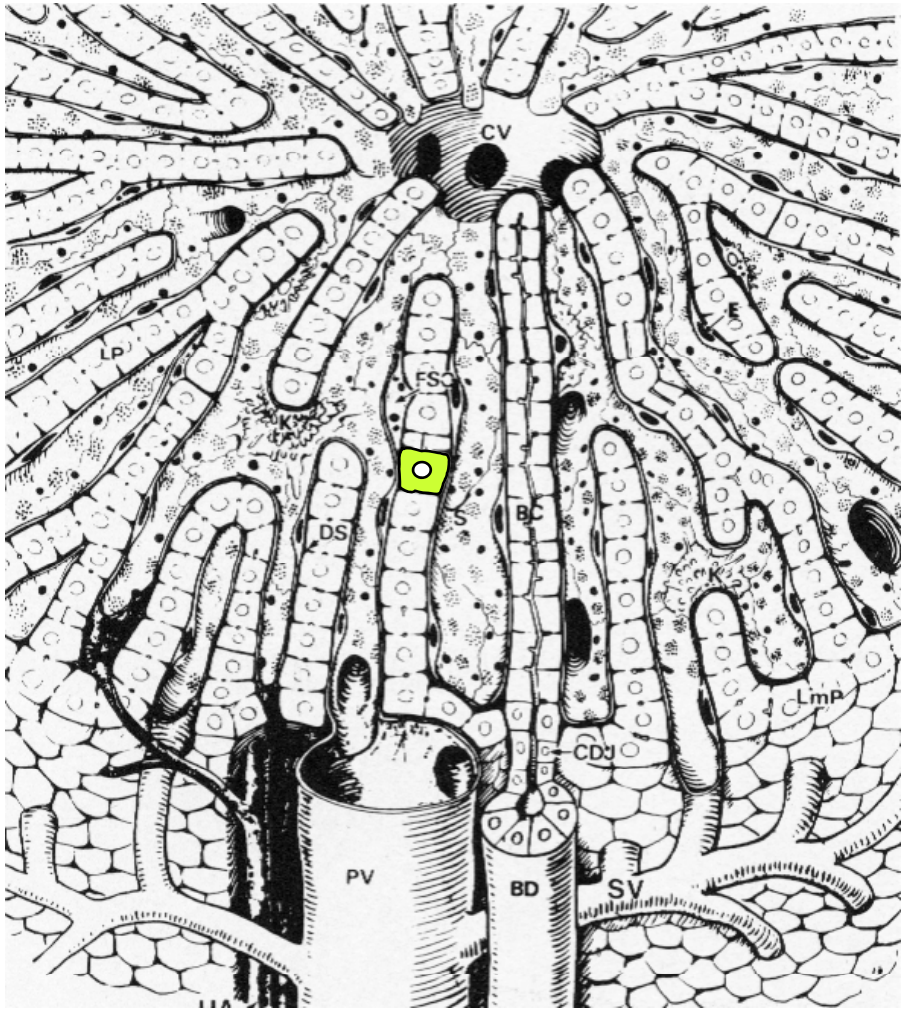



**Virus-Cell Interactions**
- Perturbed intracellular milieu
- Innate antiviral defenses

**System Level Interactions**
- Innate immune responses
  - NK, NKT cells, cyto/chemokines
- Adaptive immune responses
- Inflammation
- Steatosis
- HSC activation, fibrosis (ROS)
- Carcinogenesis (DNA damage)

**Host Consequences**
- Hepatic failure
- Cryoglobulinemia

# Hepatitis C Pathogenesis




**Virus-Cell Interactions**
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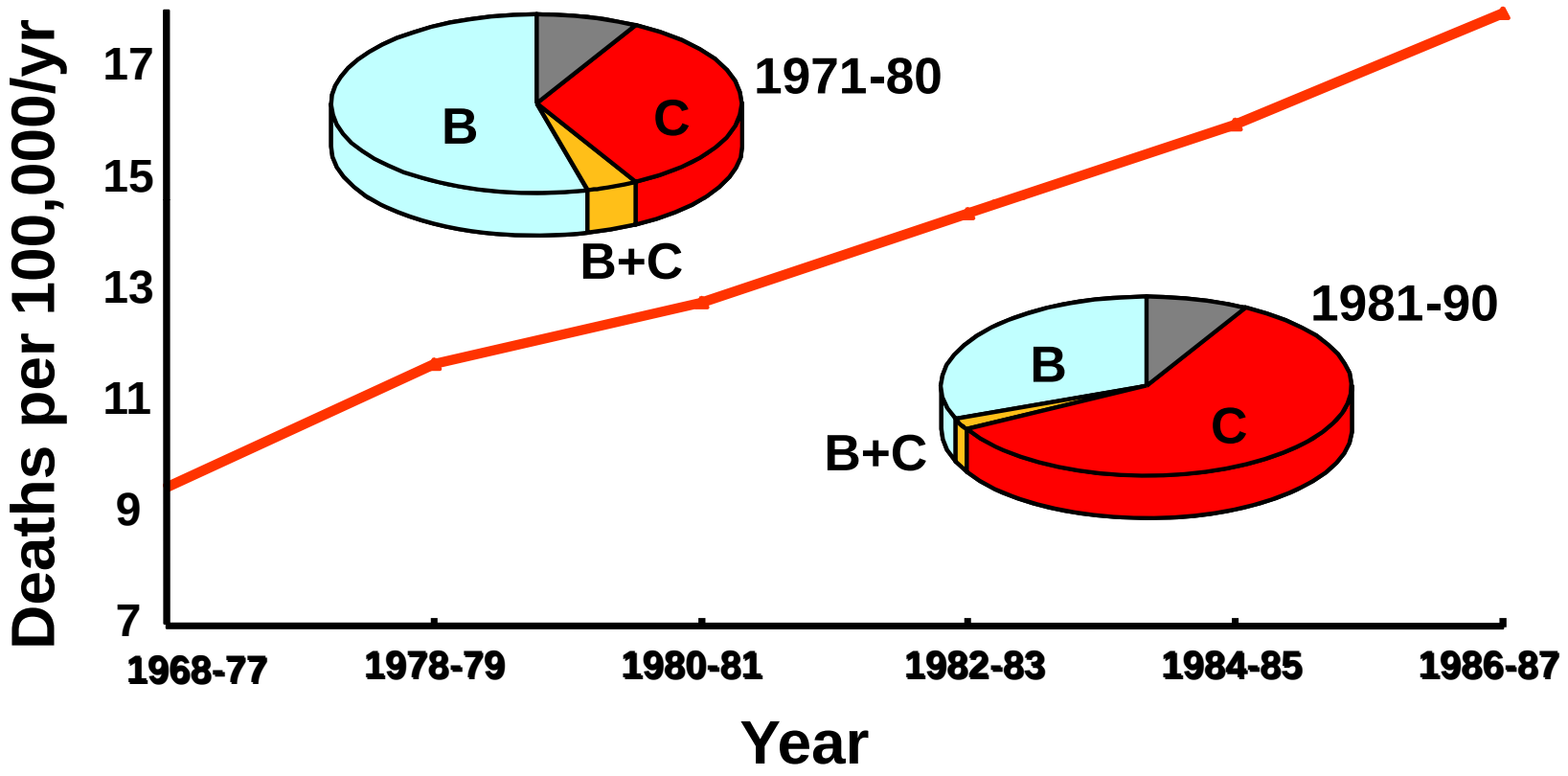
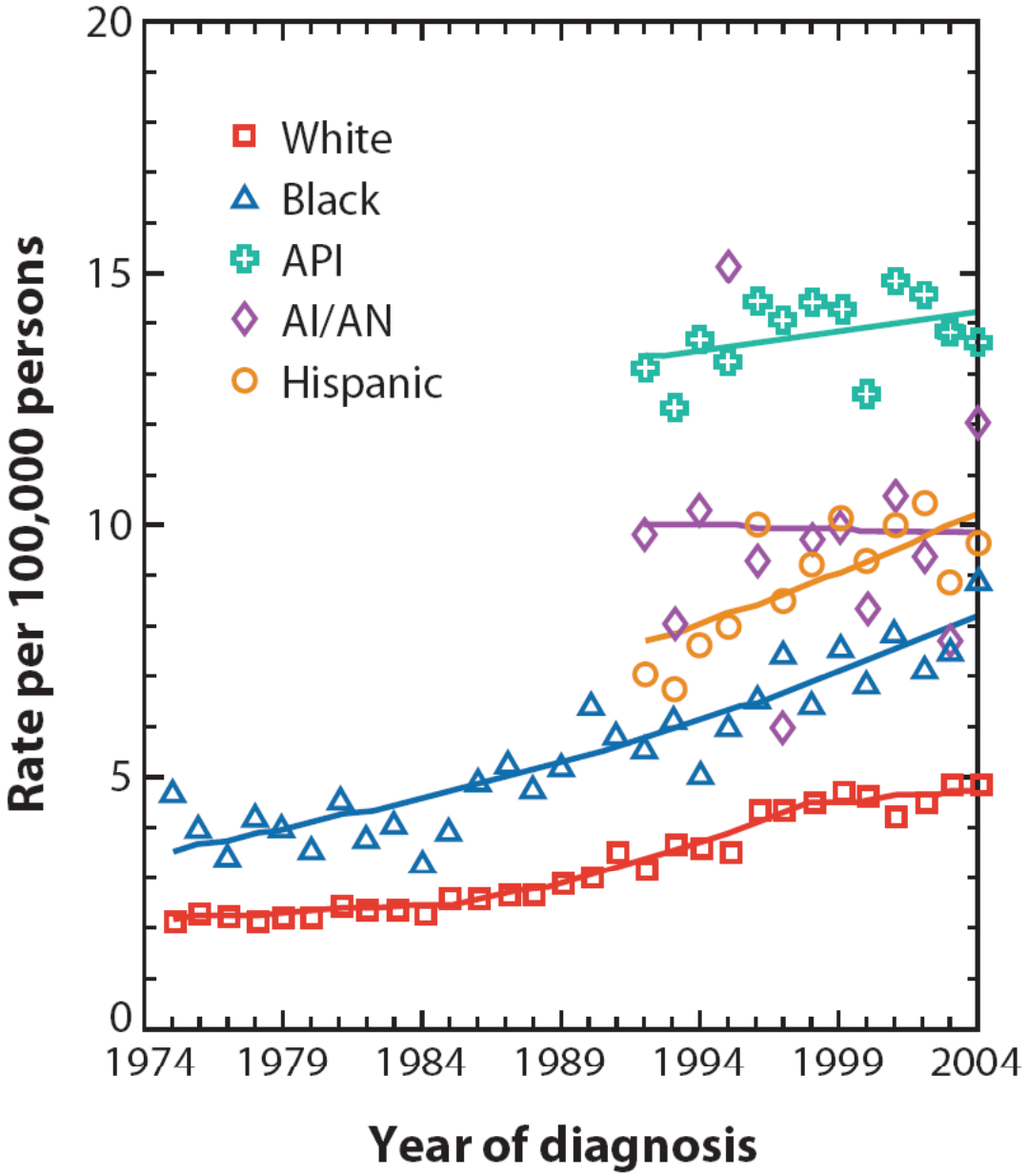
- Carcinogenesis (DNA damage)

**Host Consequences**
- Hepatic failure
- Liver cancer
- Cryoglobulinemia

# Emergence of HCV-Related Liver Cancer
## Japan 1971-1990




20
15
10
5
0
Rate per 100,000 persons
White
Black
API
AI/AN
Hispanic
1974
1979
1984
1989
1994
1999
2004
Year of diagnosis

# How does HCV cause liver cancer?

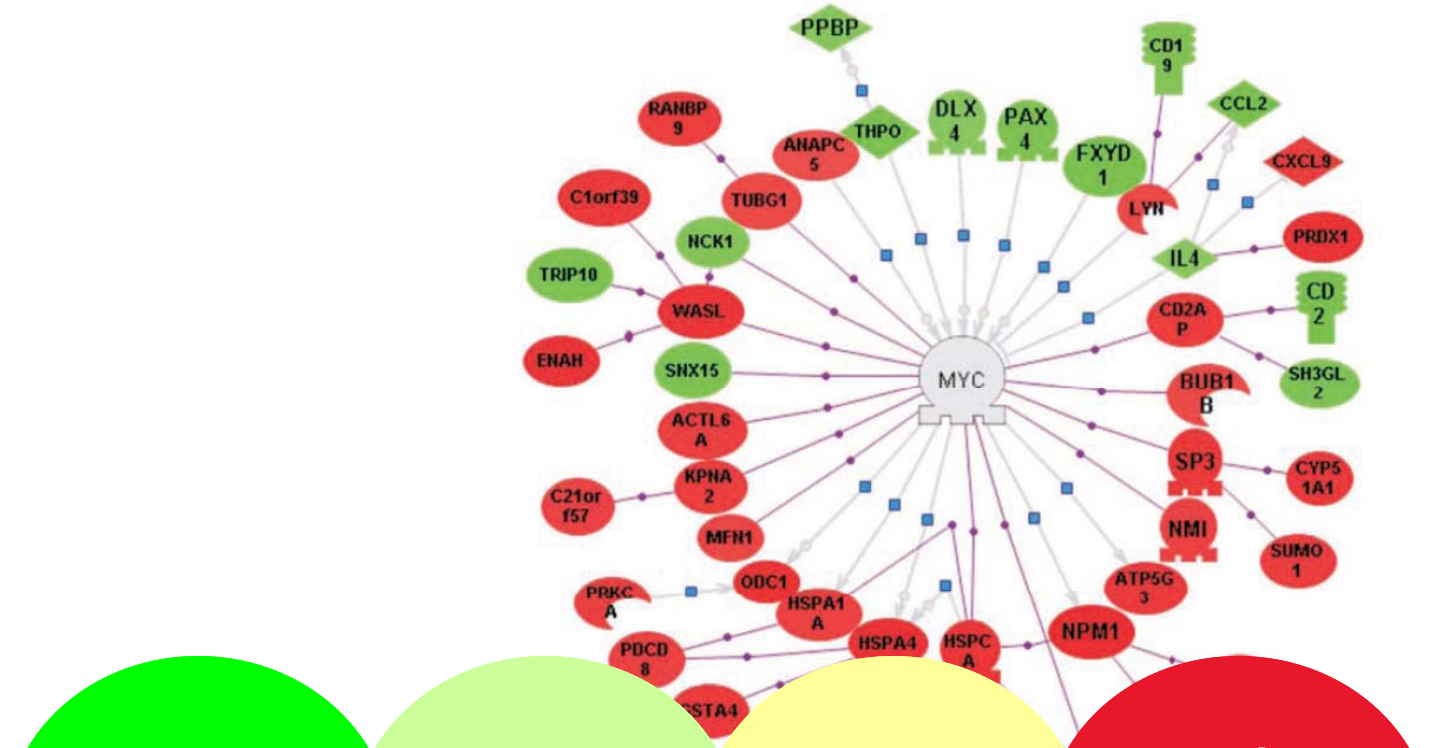
- Any hypothesis must consider:
  - Contribution of chronic inflammation
  - Increased risk with EtOH consumption
  - HCV transgenic mice develop HCC
  - Association with cirrhosis
  - Genetically heterogeneous nature of HCC

# How does HCV cause liver cancer?

- Any hypothesis must consider:
  - Contribution of chronic inflammation
  - Increased risk with EtOH consumption
  - HCV transgenic mice develop HCC
  - Association with cirrhosis
  - Genetically heterogeneous nature of HCC

- Other potential causes of HCC:
  - Aflatoxin
  - Hepatitis B virus
  - Helicobacter sp.
  - Hereditary hemachromatosis (30%)
  - $\alpha$1-antitrypsin deficiency

# Central Role of c-Myc Responsive Genes in Malignant Conversion of Dysplastic Nodules to Early HCC



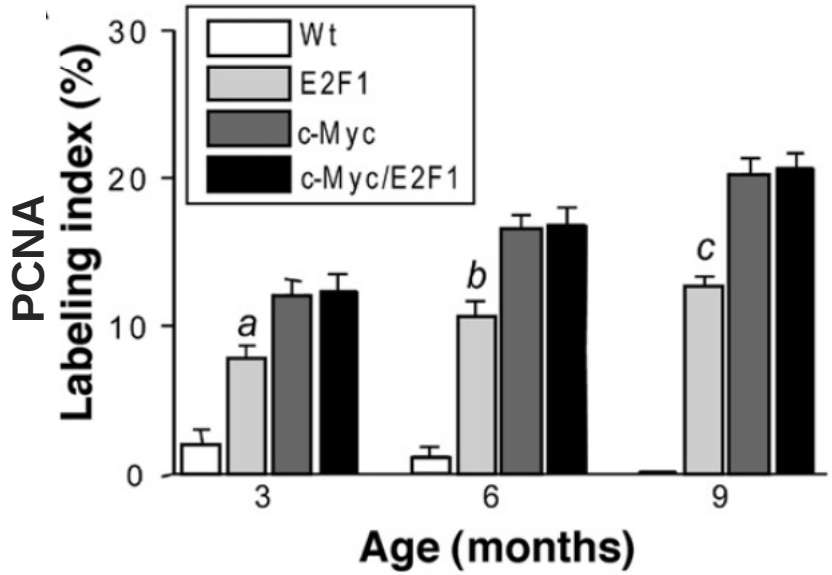

- Accumulating genetic and epigenetic modifications
- Increasing c-Myc copy number with 8q duplications



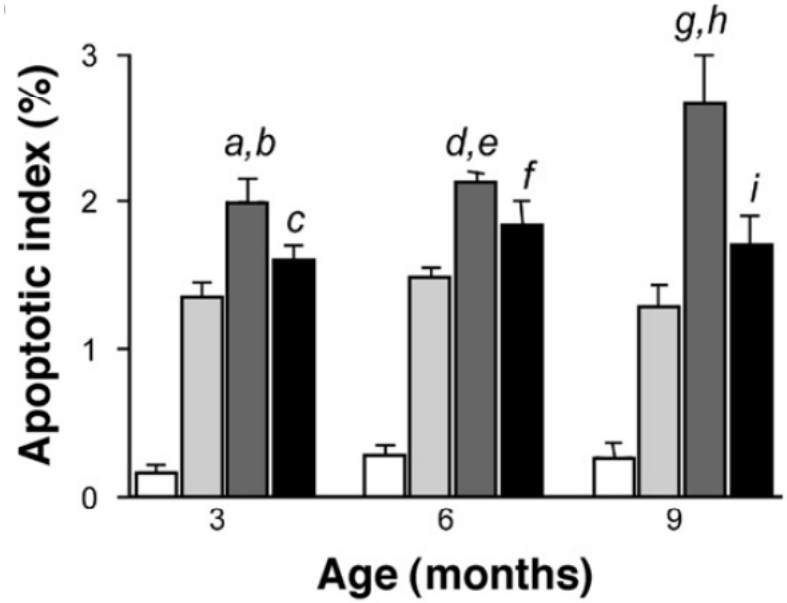
Kaposi-Novak et al. *Cancer Res*, 69:2775-2782, 2009

# c-Myc and E2F1 Cooperatively Promote Hepatocellular Carcinogenesis in Double Transgenic Mice

## Proliferation



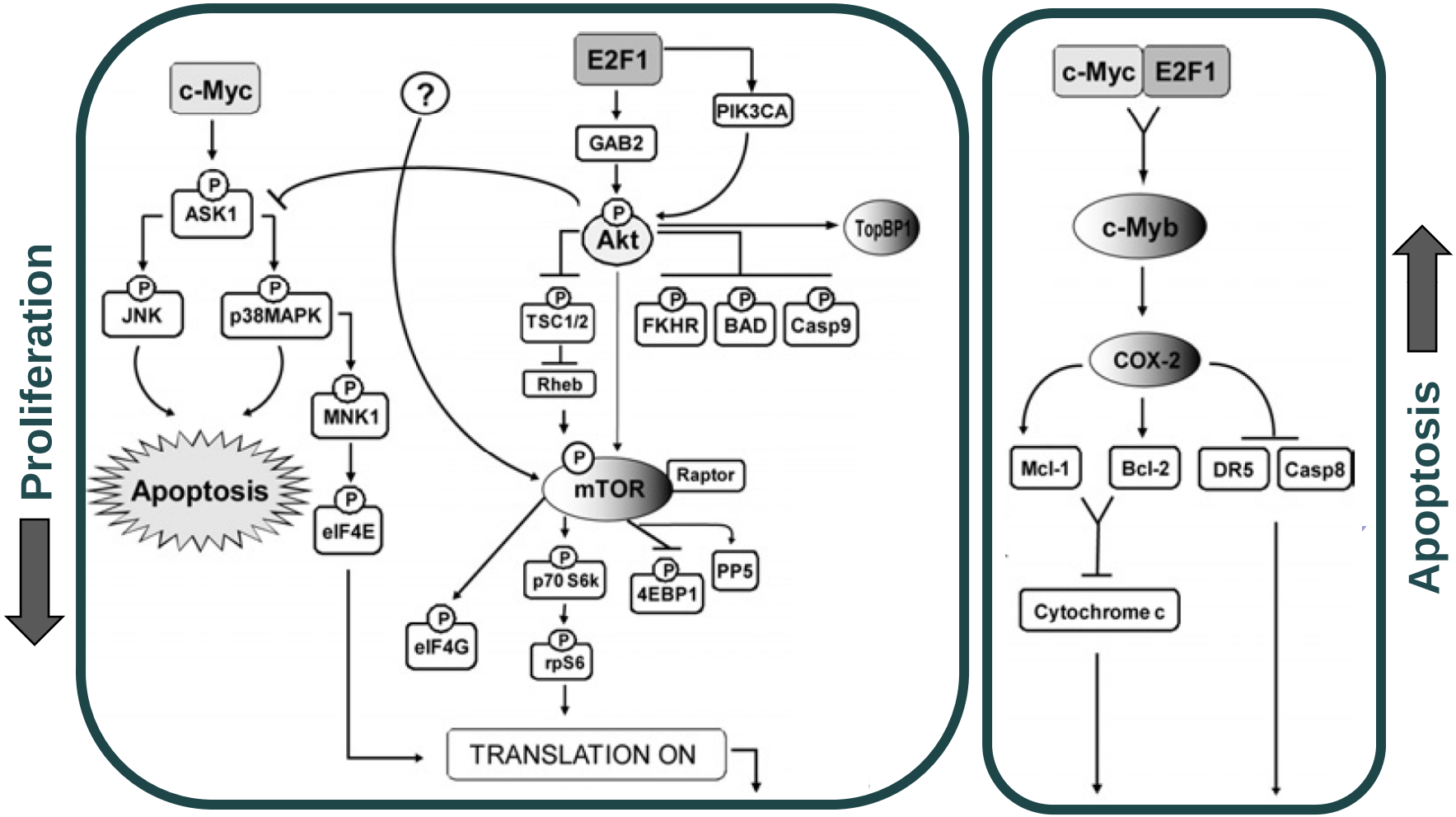

## Apoptosis






S. Ladu et al. *Gastroenterology* 135:1322-1332, 2008

# Cooperative Actions of c-Myc and E2F1

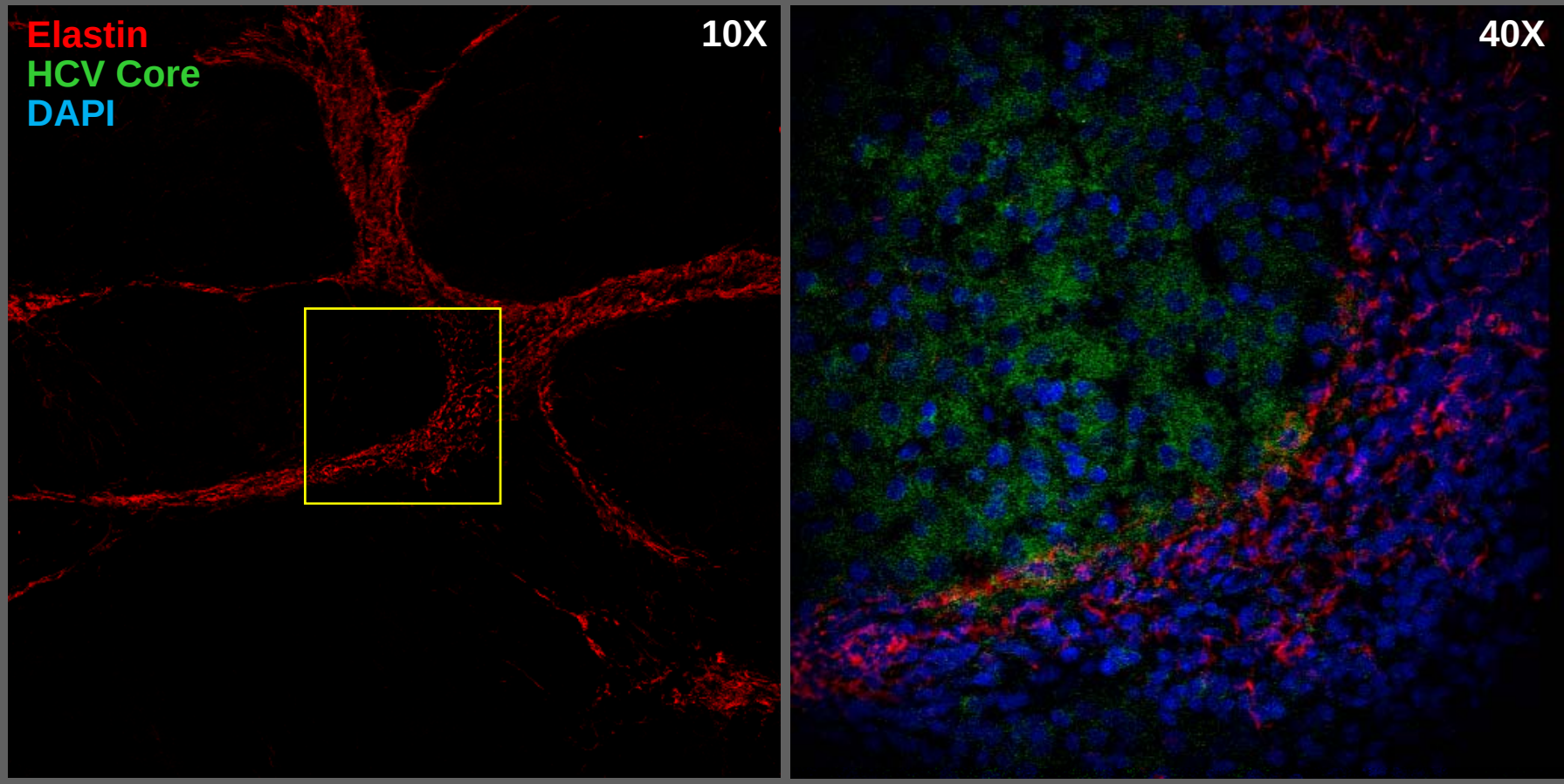



**Hepatocellular Proliferation and Survival**



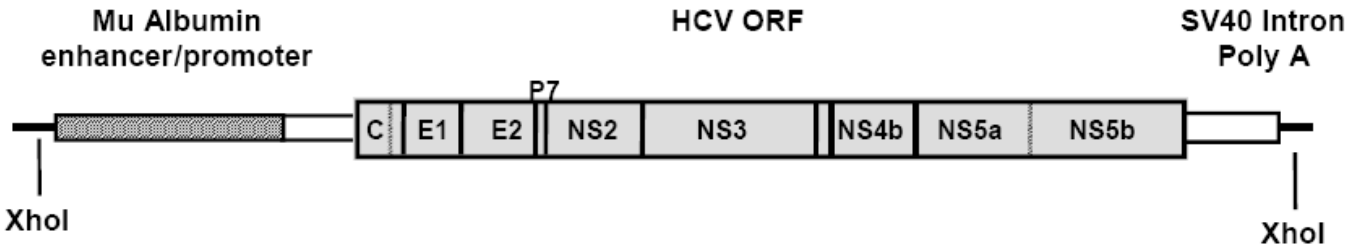
S. Ladu et al. *Gastroenterology* 135:1322-1332, 2008

# HCV-infected Cells within a Regenerative Nodule Imaged by 2PE Microscopy



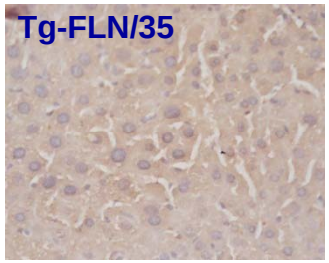

Y. Liang et al., Submitted, 2009

# Transgenic FL-N/35 Mice

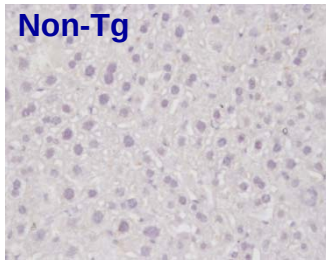



**NS3**

**Tg-FLN/35**



**Non-Tg**

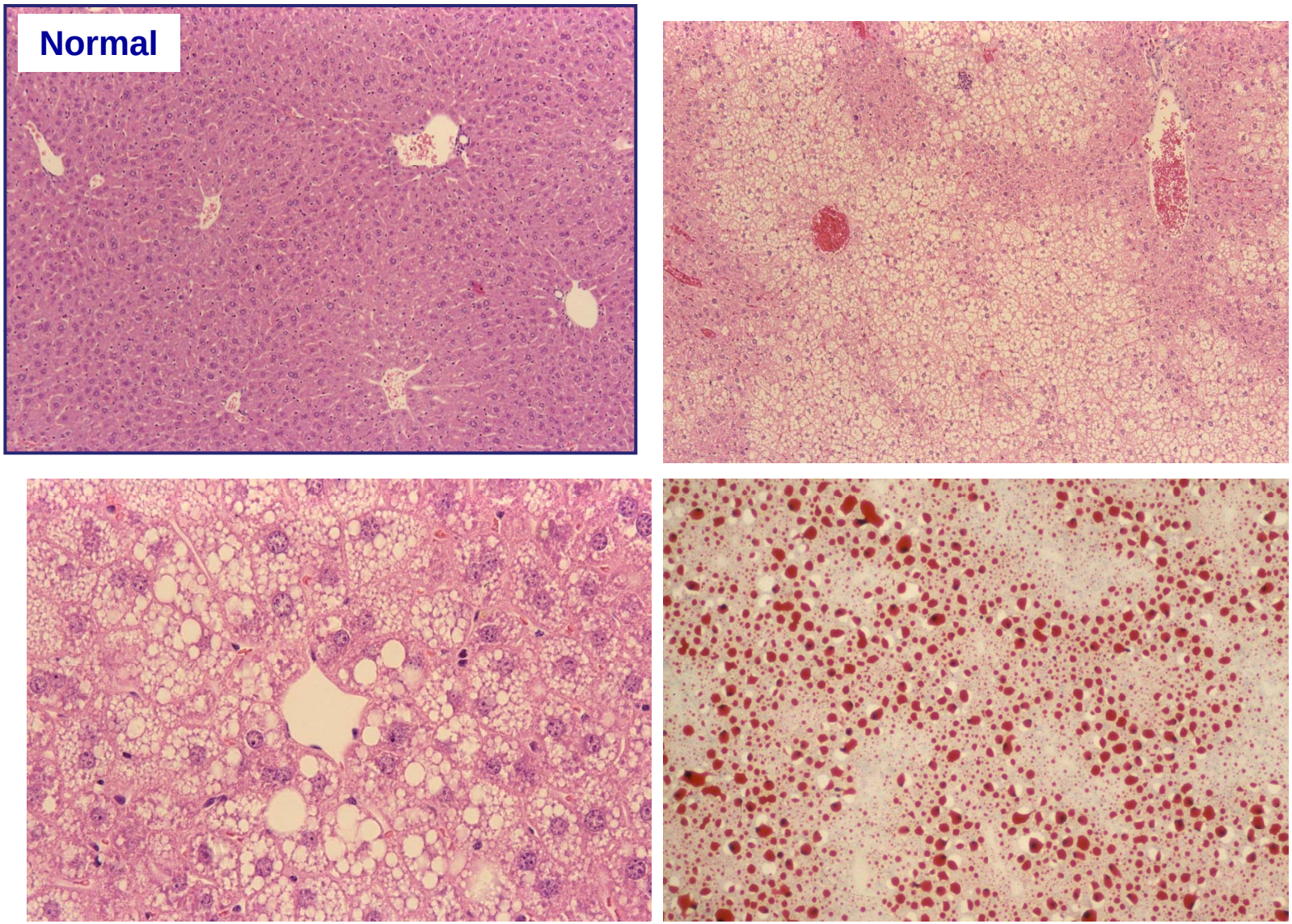




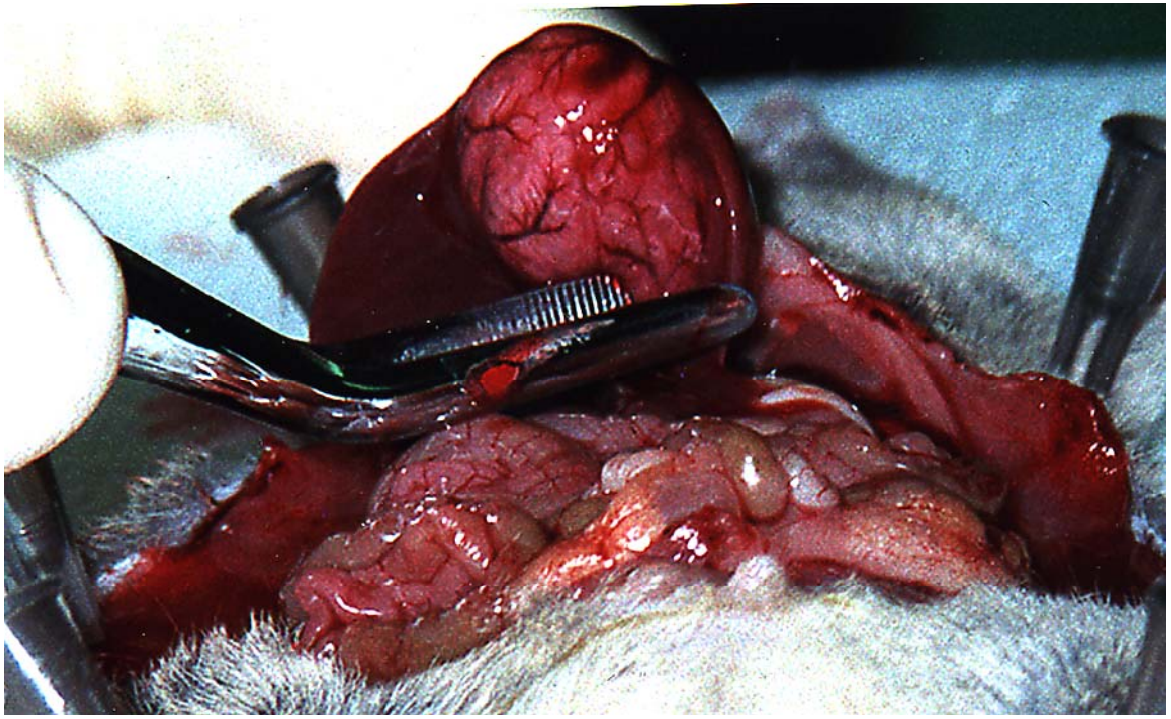
Lerat et al., *Gastroenterology* 122: 352-365, 2002

# Centrilobular Hepatic Steatosis

## FL-N/35 Lineage, 11-12 months old



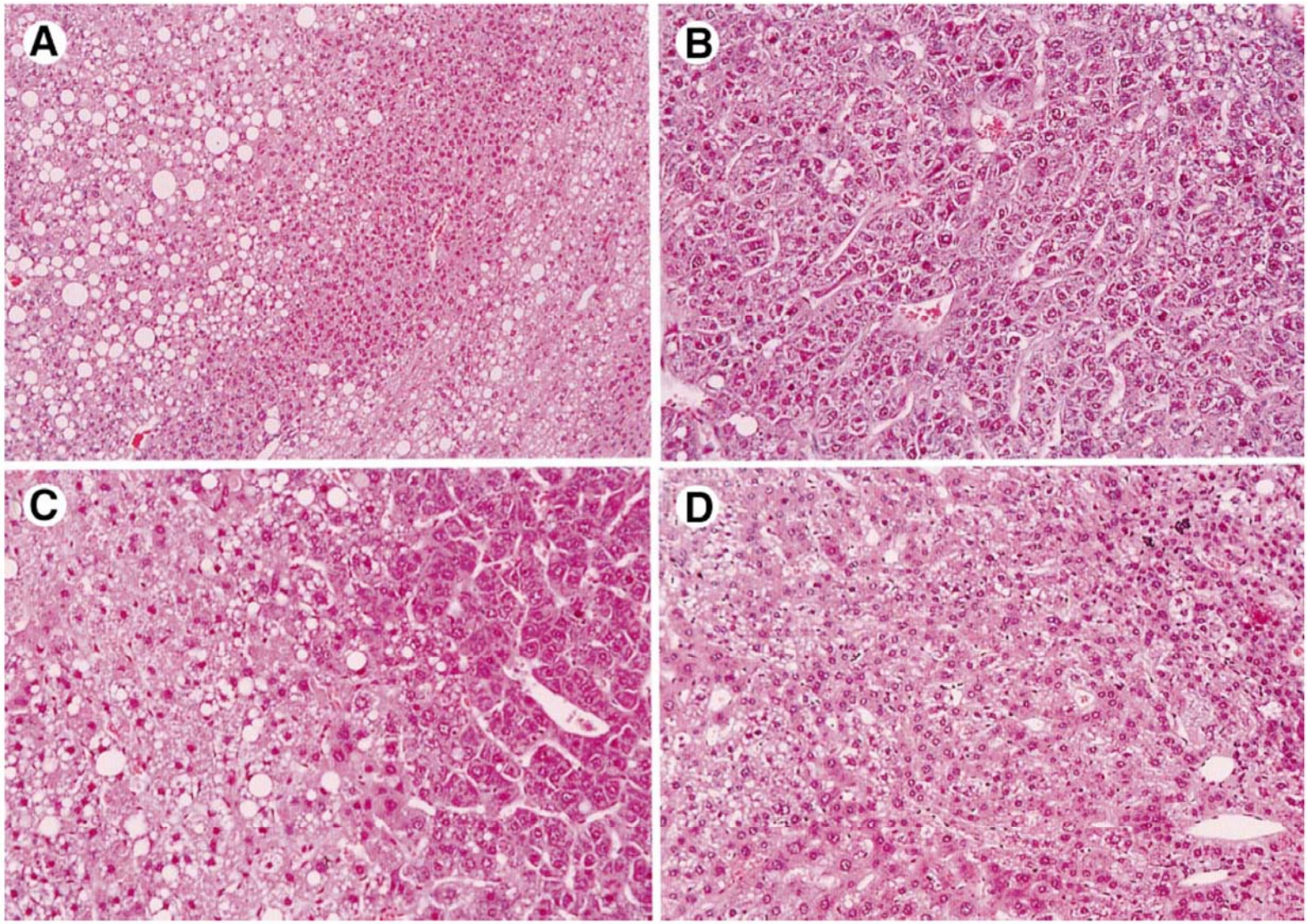
# Hepatic Tumor in an FL-N/35 HCV Transgenic Mouse
## (FL-N/35-171, 13 mo Male)





Lerat et al., *Gastroenterology* 122: 352-365, 2002

# Hepatic Adenoma and Hepatocellular Carcinomas in HCV Transgenic FL-N/35 Mice

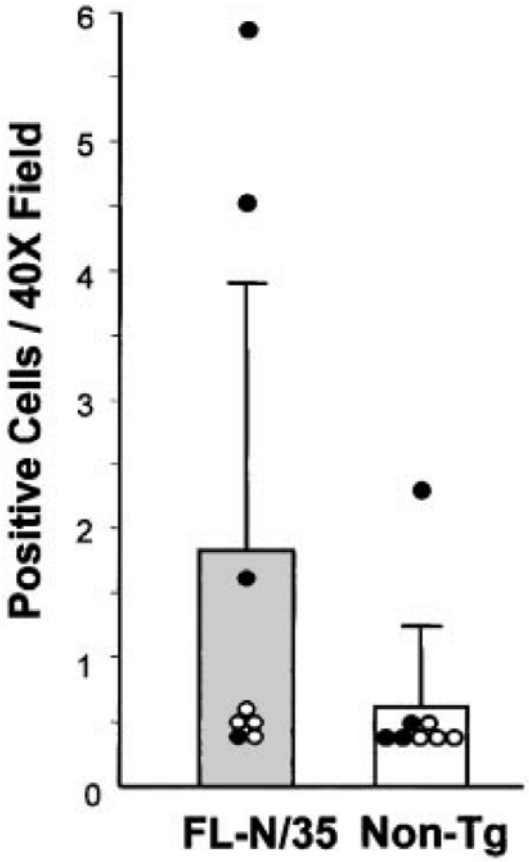


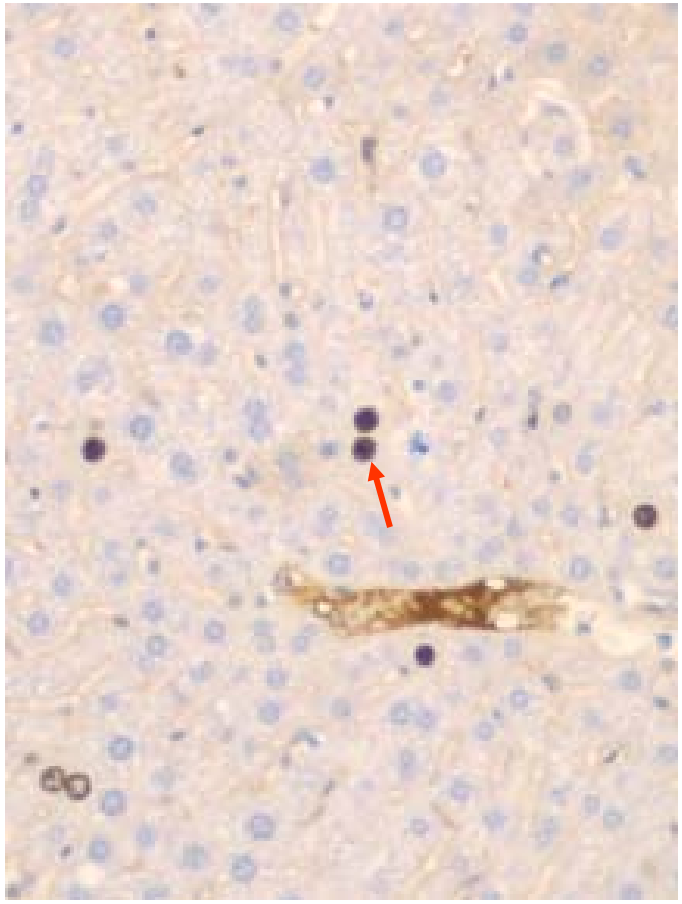



Lerat et al., *Gastroenterology* 122: 352-365, 2002

# BrDU Staining for DNA Synthesis

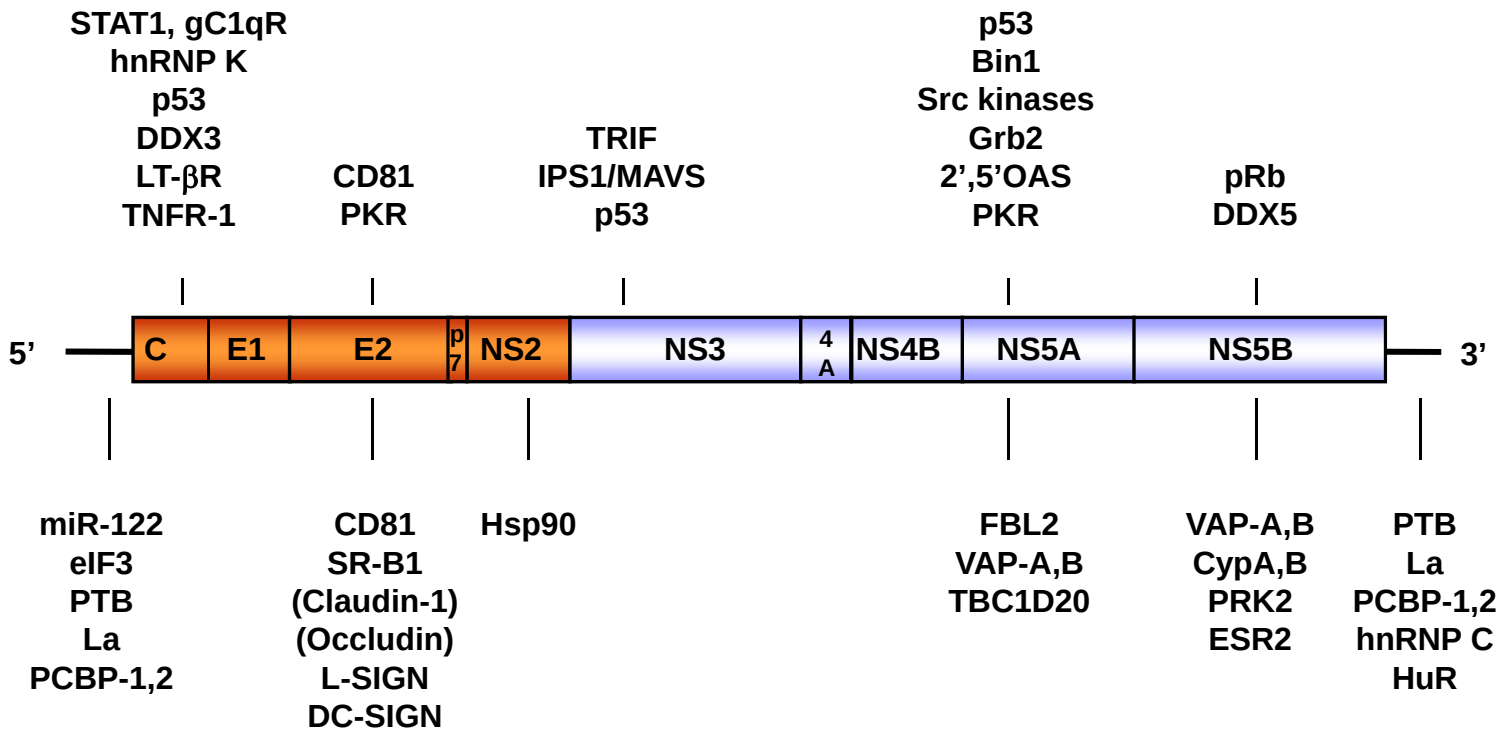
## FL-N/35 Lineage



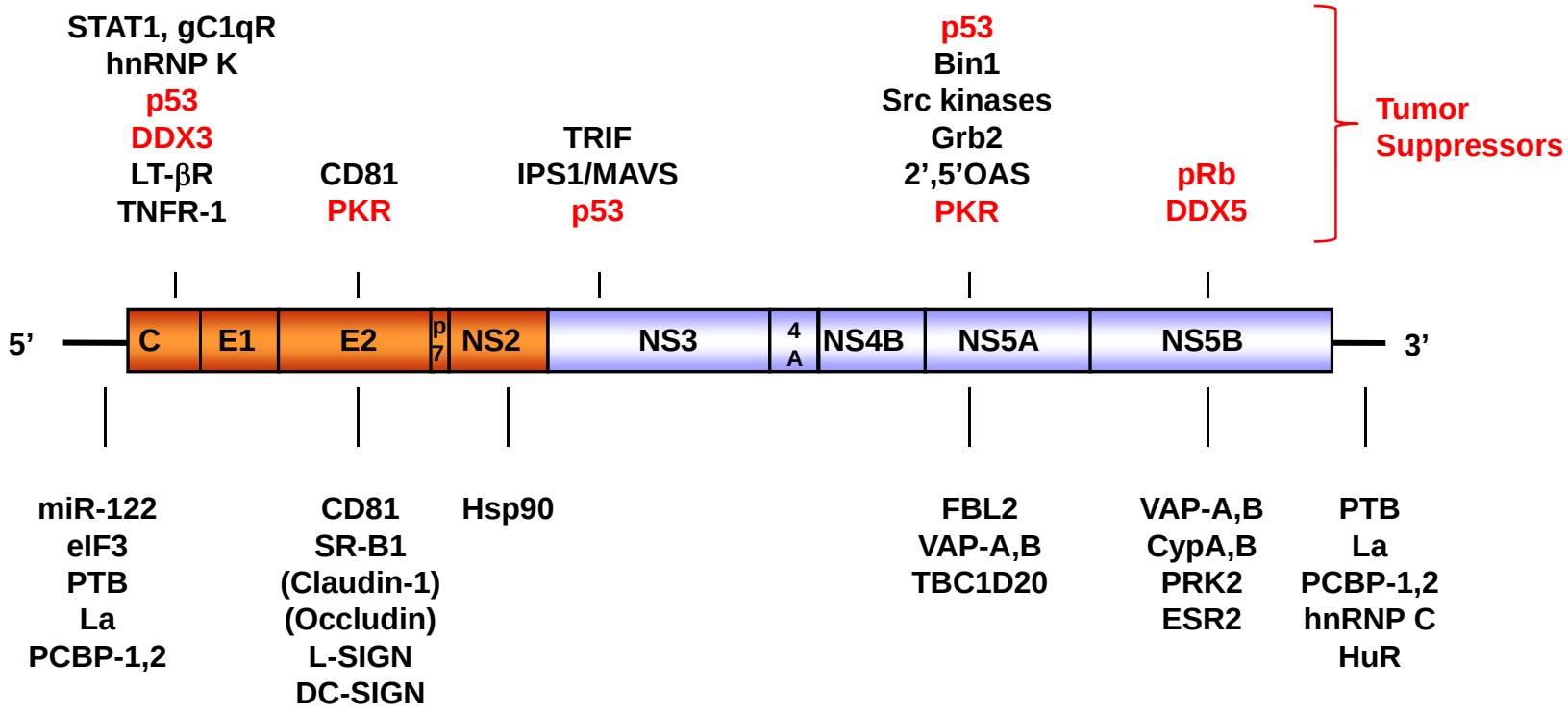



Lerat et al., *Gastroenterology* 122: 352-365, 2002

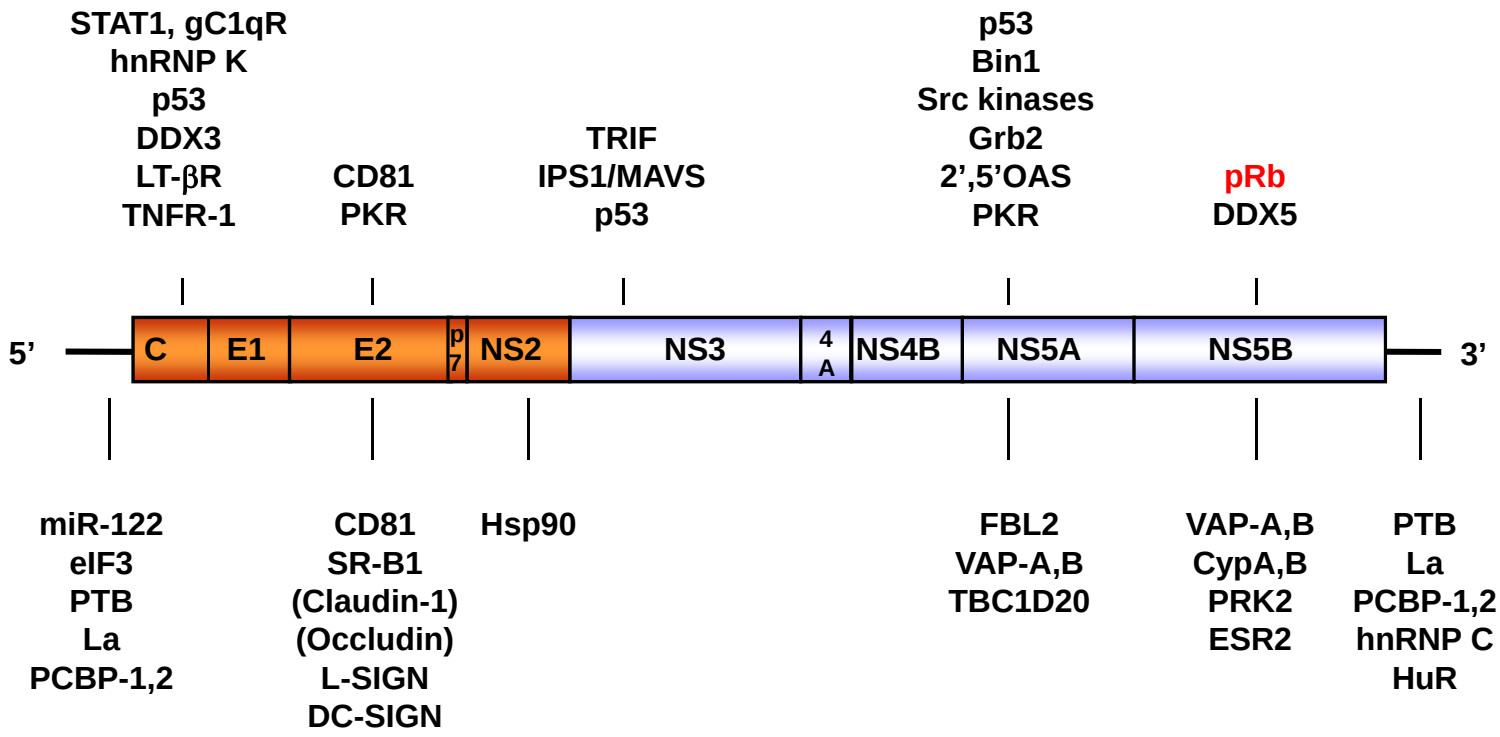
# Host Factors in HCV Replication and Pathogenesis



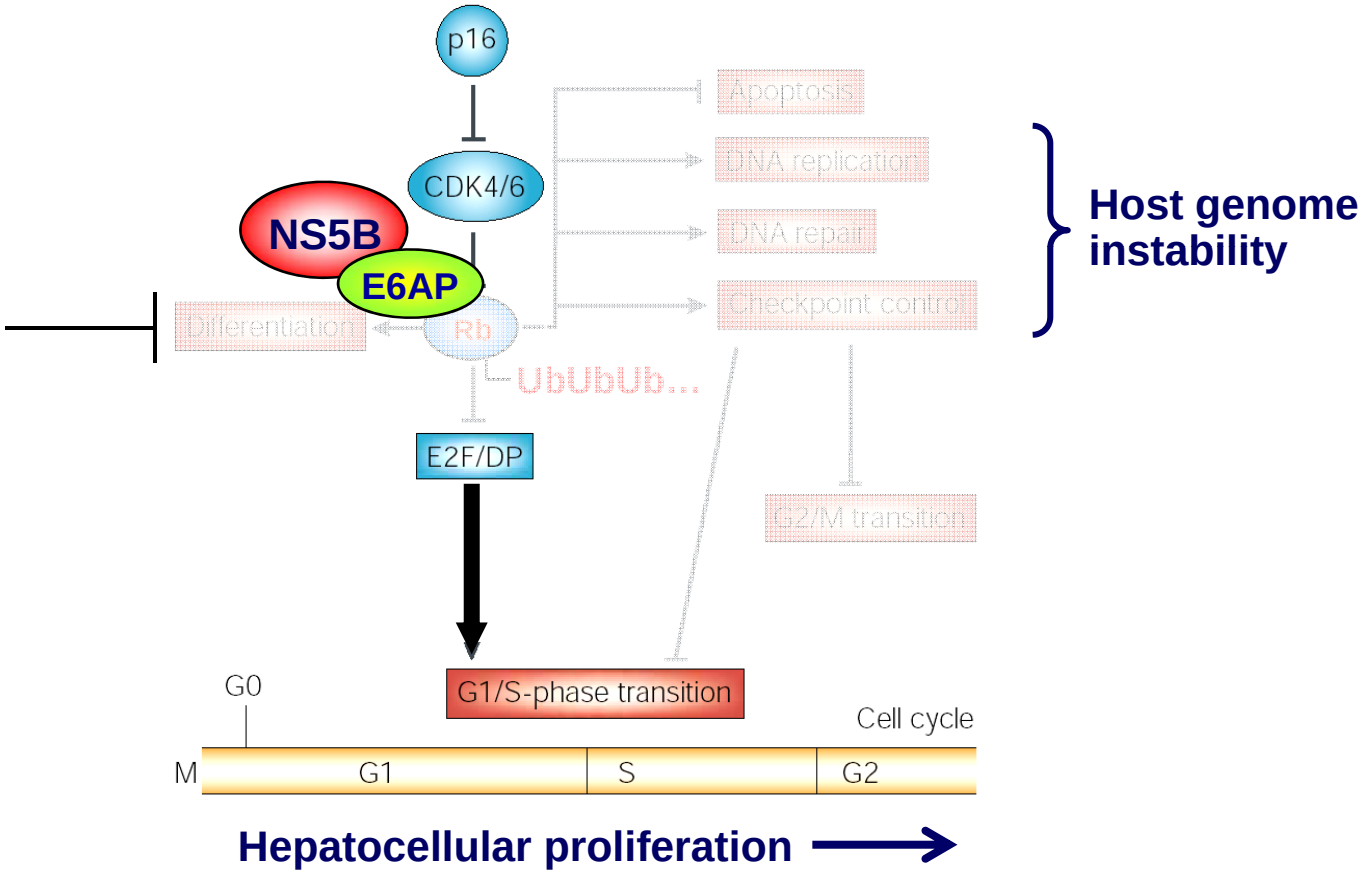
# Host Factors in HCV Replication and Pathogenesis



# Host Factors in HCV Replication and Pathogenesis



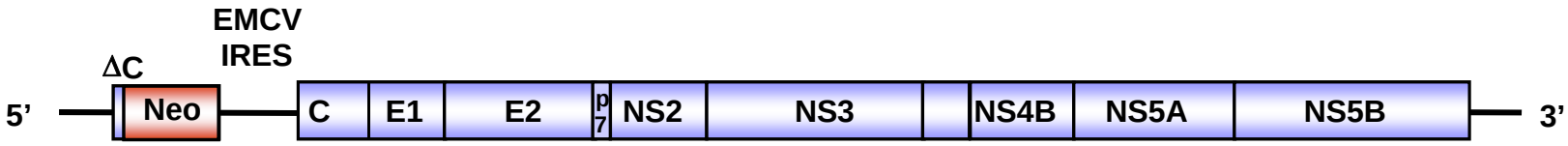
# NS5B Regulation of pRb



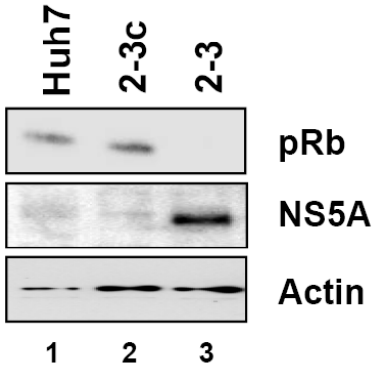



Modifed from Classon and Harlow, *Nature Rev Cancer* 2: 910, 2002

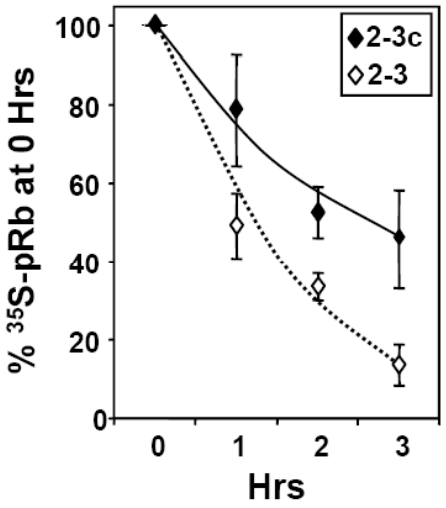
# pRb Abundance is Post-Transcriptionally Down-Regulated in HCV RNA Replicon Cells



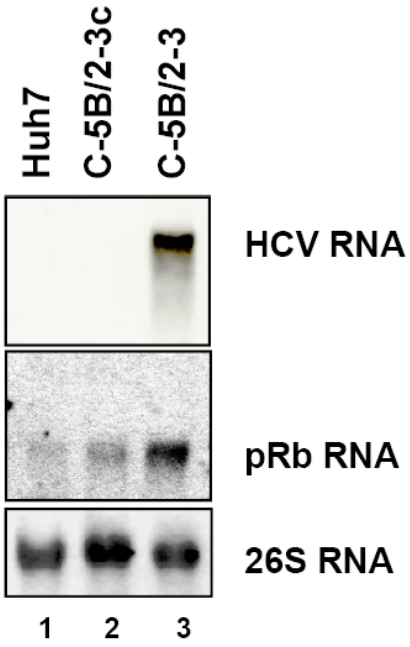

**Immunoblot**




**Pulse-chase**



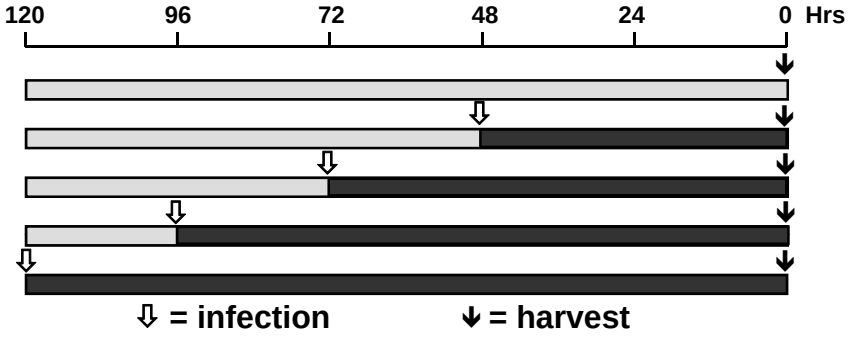

**Northern**

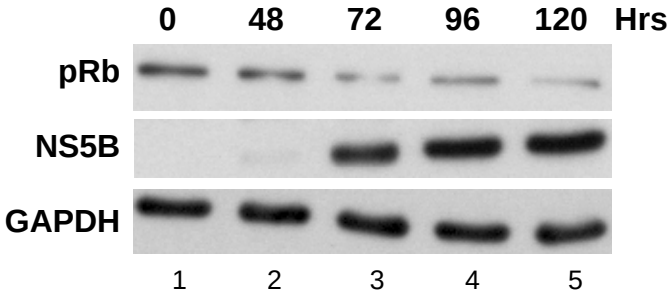


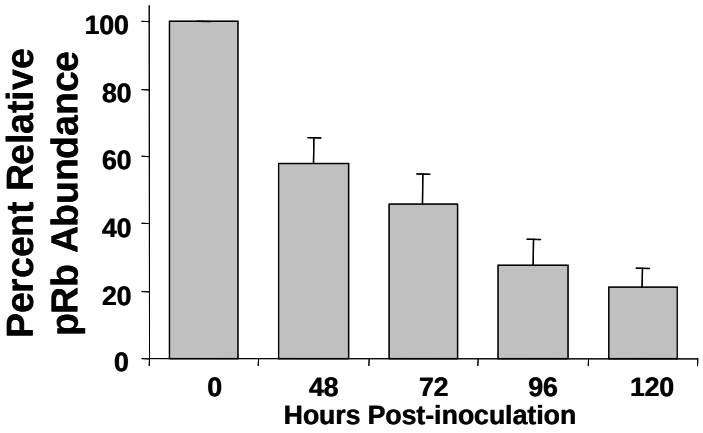



Munakata et al., PNAS 102:18159-64, 2005

# pRb Abundance is Reduced by Genotype 2a JFH1 Virus Infection of Huh-7.5 Cells



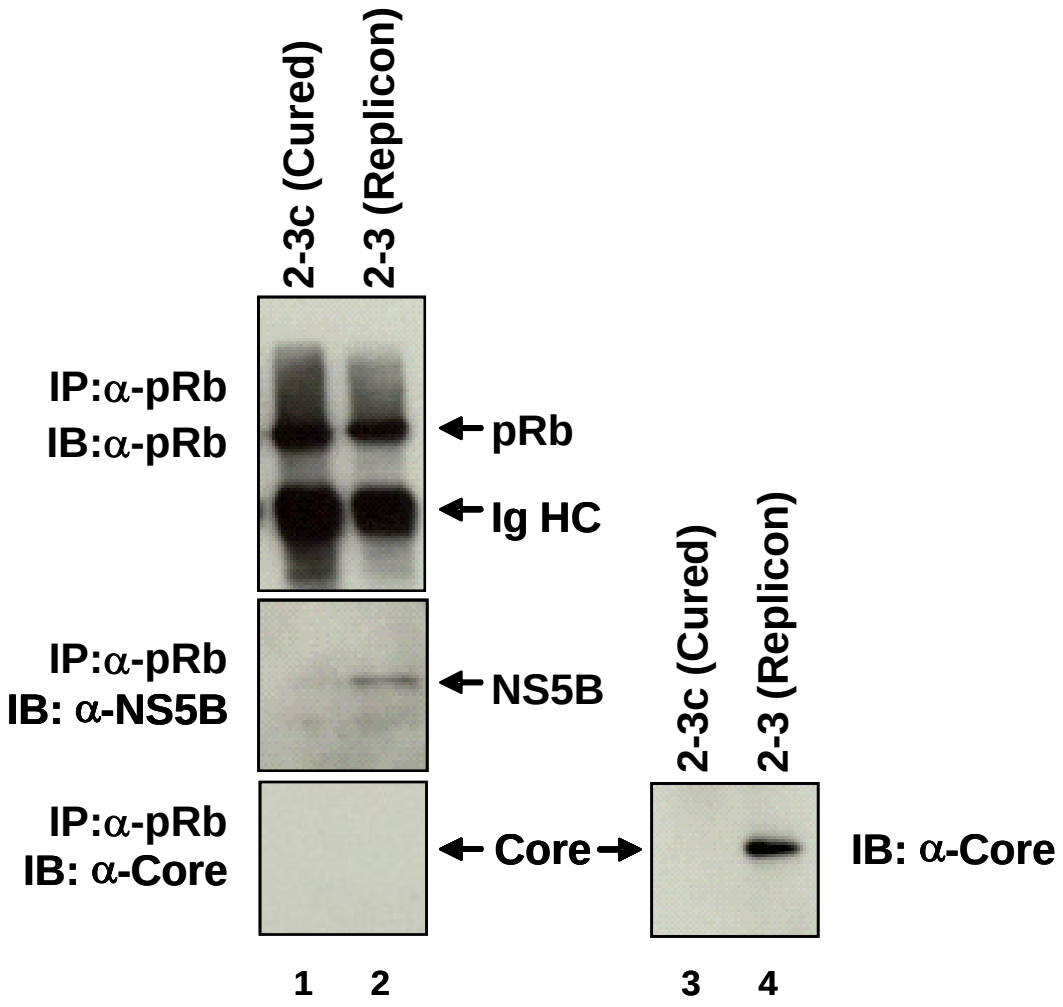






Munakata et al., PLoS Pathogens, 3(9): e139, 2007

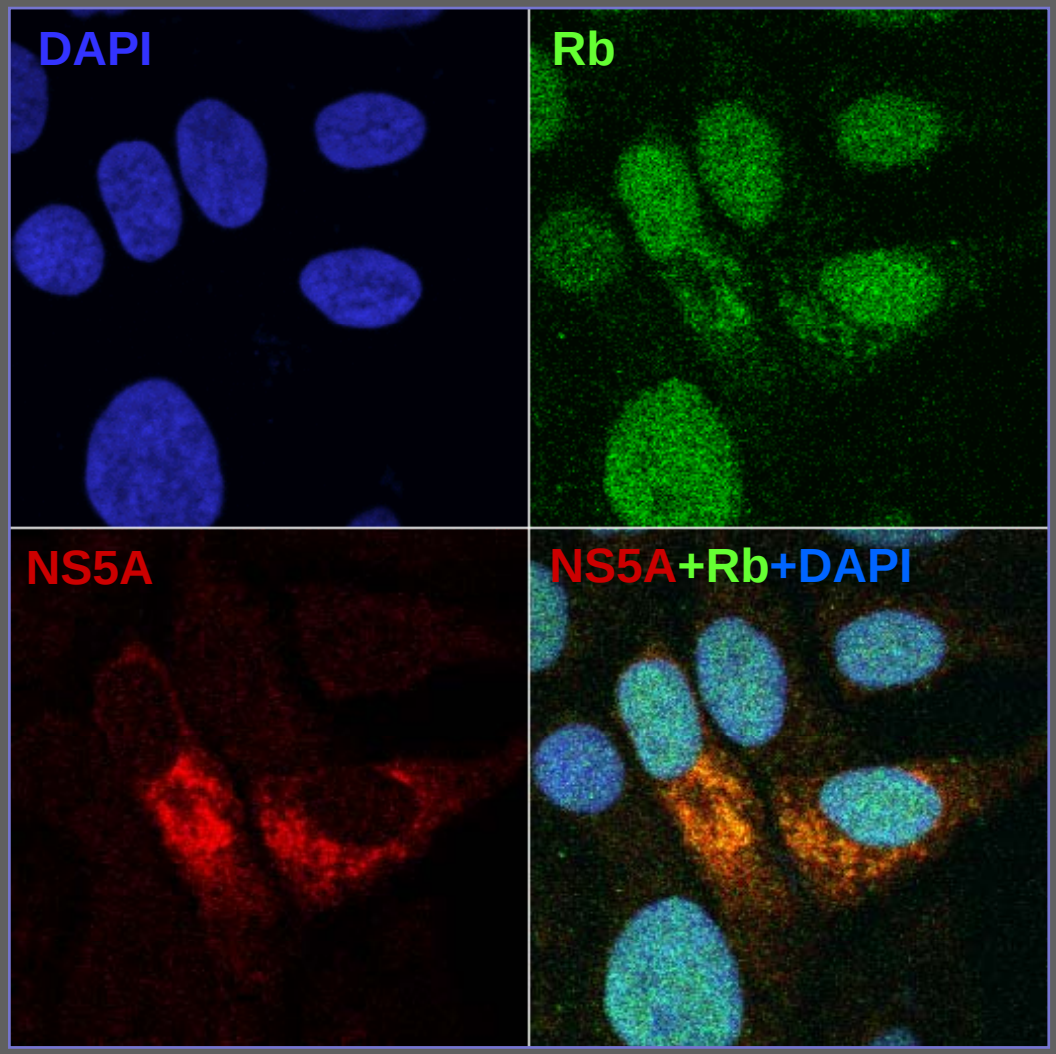
# NS5B Forms a Complex With pRb





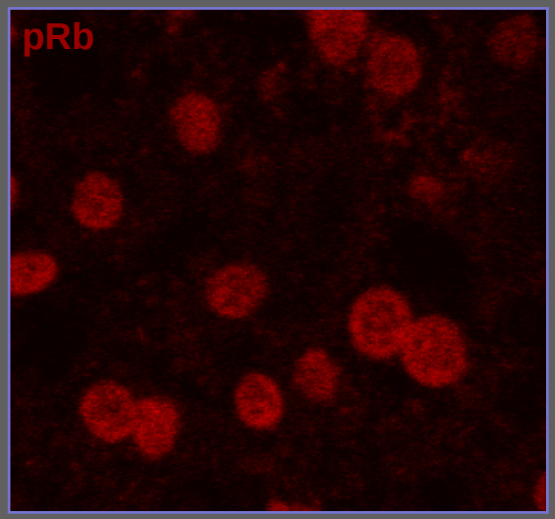

Munakata et al., PNAS 102:18159-64, 2005

# Cytoplasmic Colocalization of pRb and NS5A (replicase) in Huh-7.5 cells 48 hrs Post-infection with JFH1 Virus



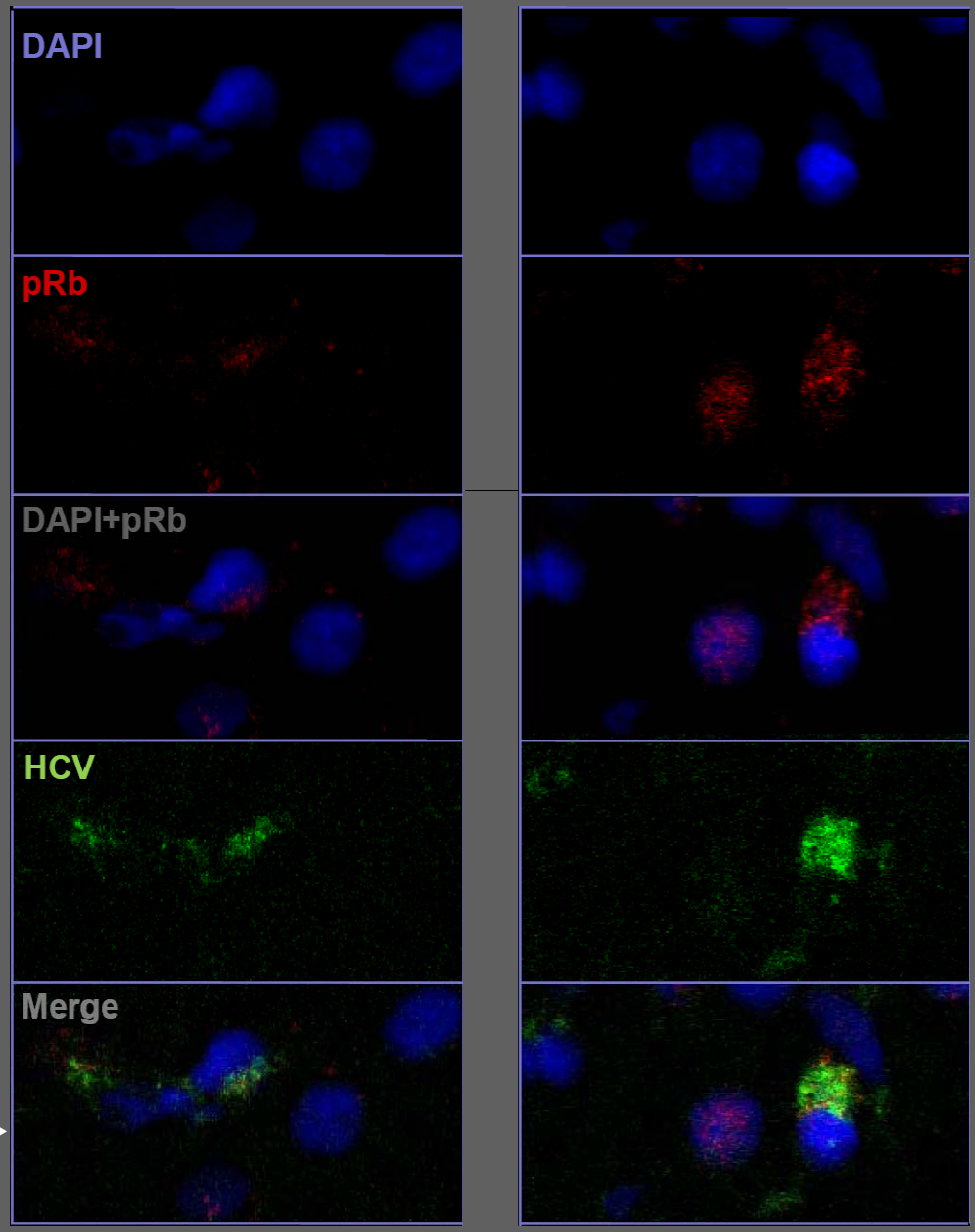

Munakata et al., PLoS Pathogens, 3(9): e139, 2007

# HCV Regulation of pRb in Human Liver



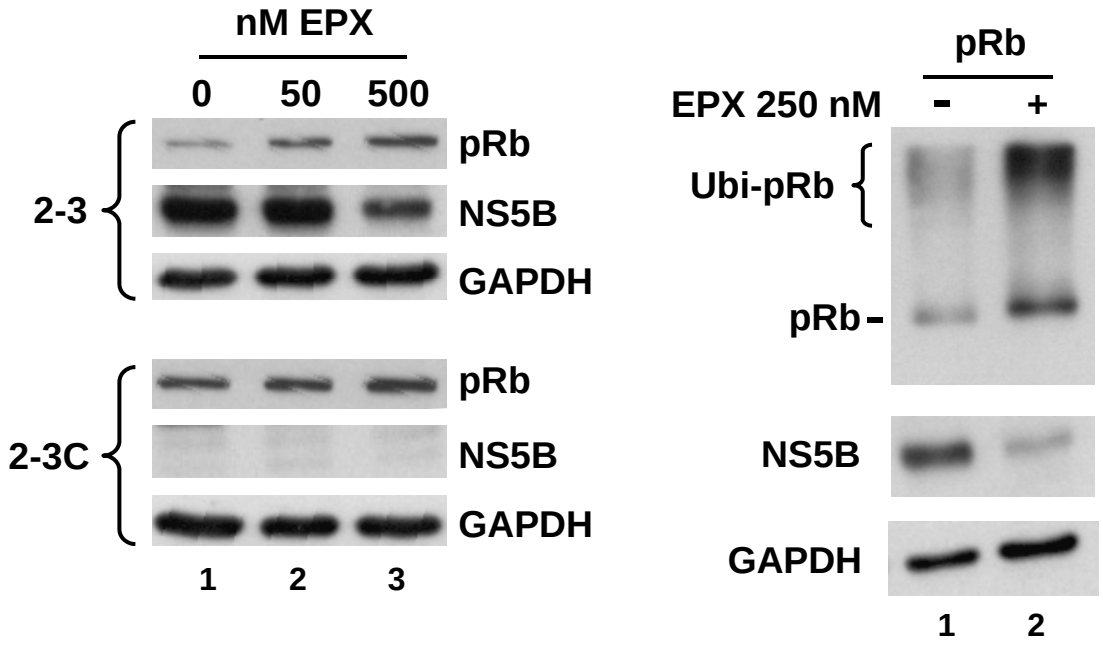

Normal liver

Chronic Hepatitis C




Y. Liang

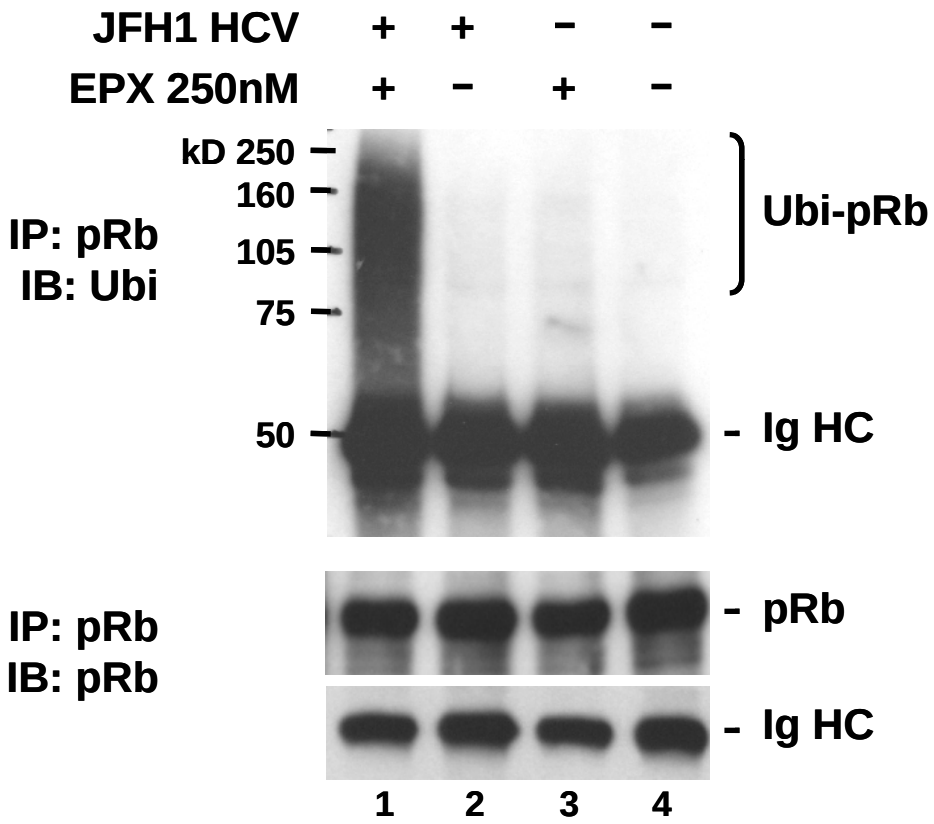
# Epoxomicin, a Potent Inhibitor of the Proteasome, Restores pRb and $PO_4$-pRb Abundance






Munakata et al., PLoS Pathogens, 3(9): e139, 2007

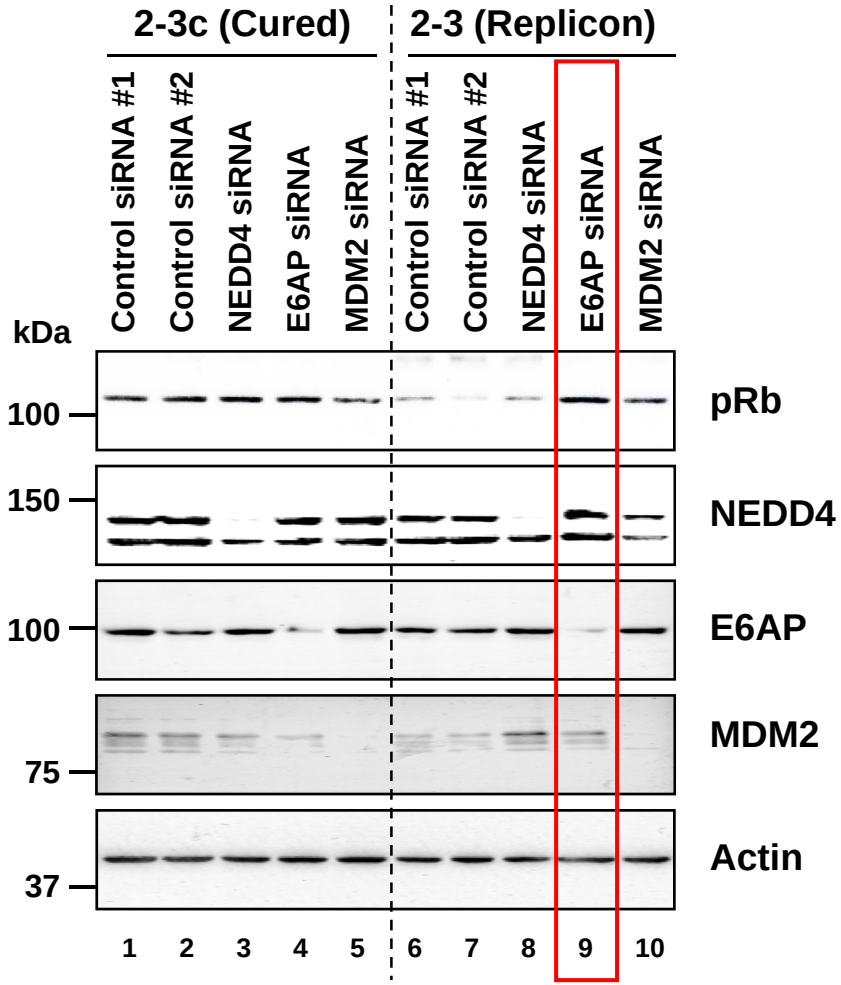
# HCV Infection Induces Ubiquitination of pRb in Cultured Hepatoma Cells






Munakata et al., PLoS Pathogens, 3(9): e139, 2007

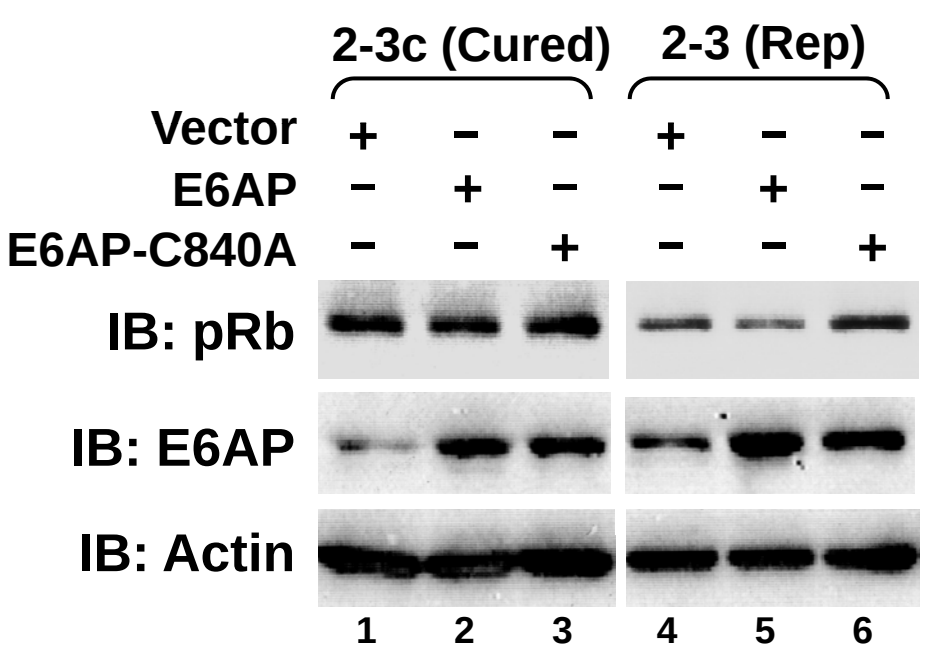
# siRNA Knock-down of E6AP Restores pRb Abundance in Replicon Cells

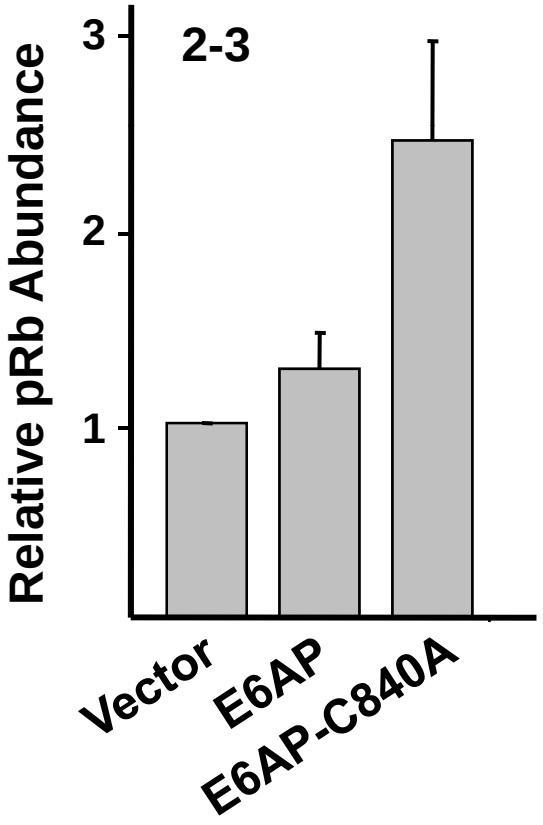





Munakata et al., PLoS Pathogens, 3(9): e139, 2007

# Over-expression of Dominant-negative E6AP-C840A Partially Restores pRb Abundance in 2-3 Cells

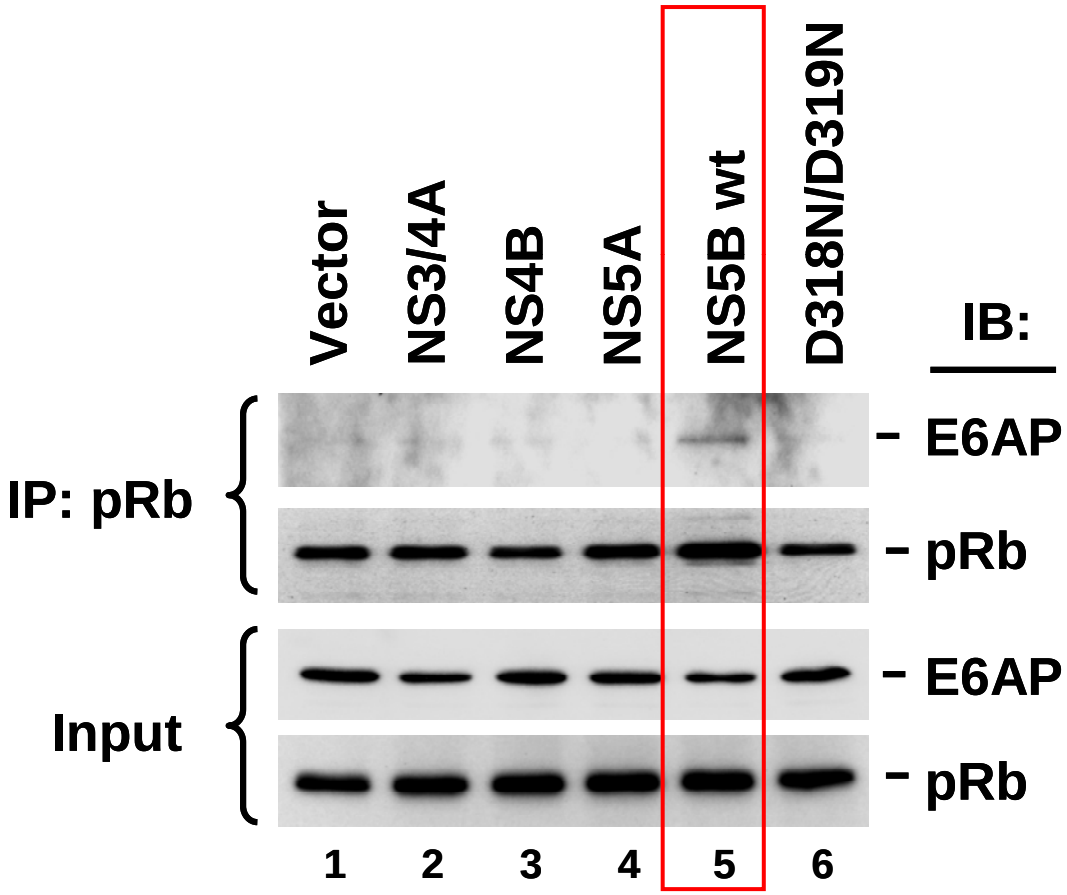





Munakata et al., PLoS Pathogens, 3(9): e139, 2007

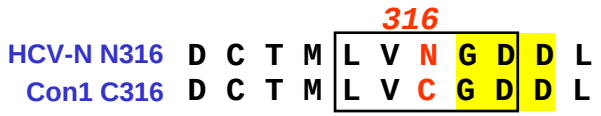
# E6AP-pRb Complex in Cells Ectopically Expressing wt NS5B

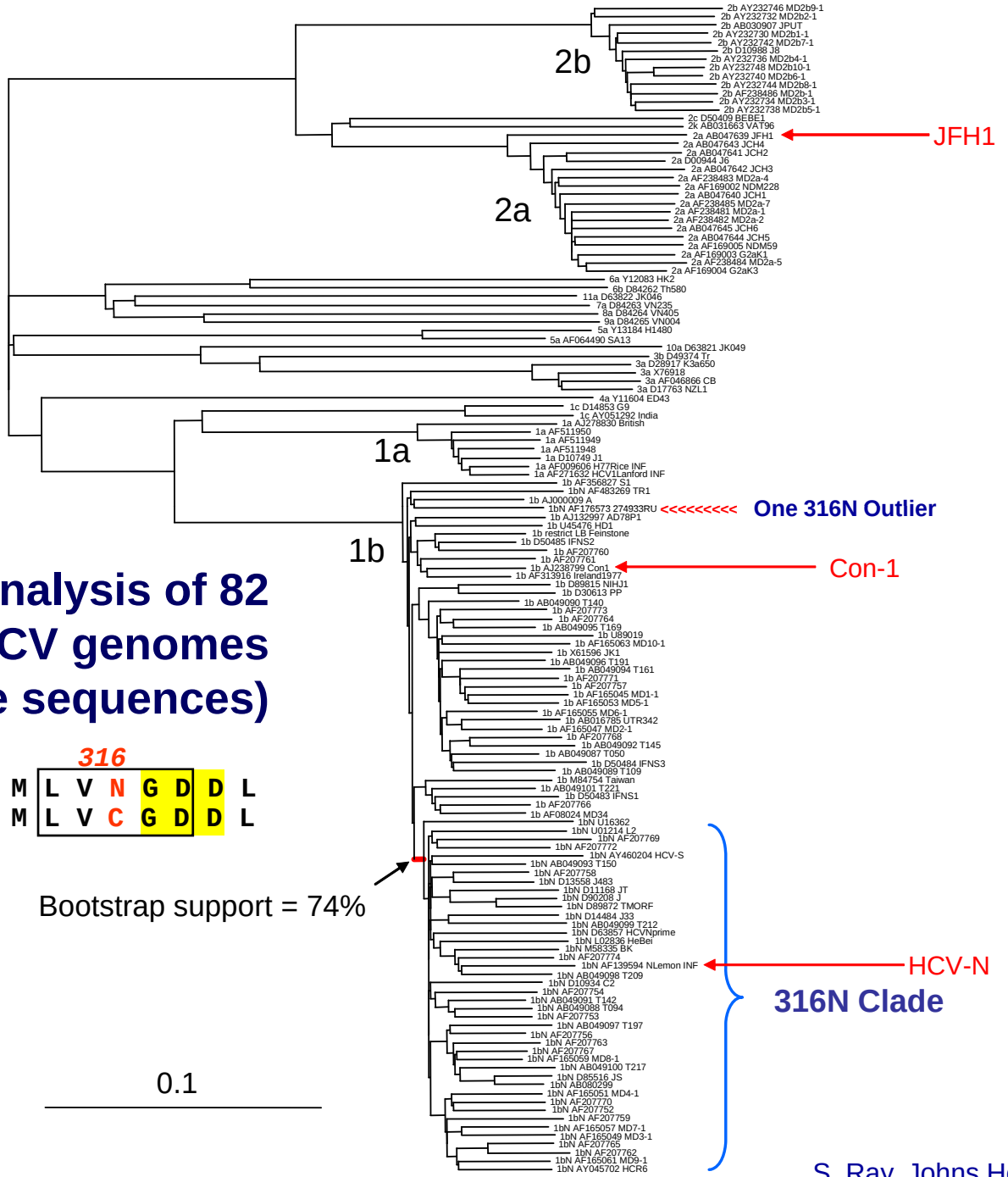





Munakata et al., PLoS Pathogens, 3(9): e139, 2007

# Phylogenetic analysis of 82 full-length HCV genomes (nucleotide sequences)



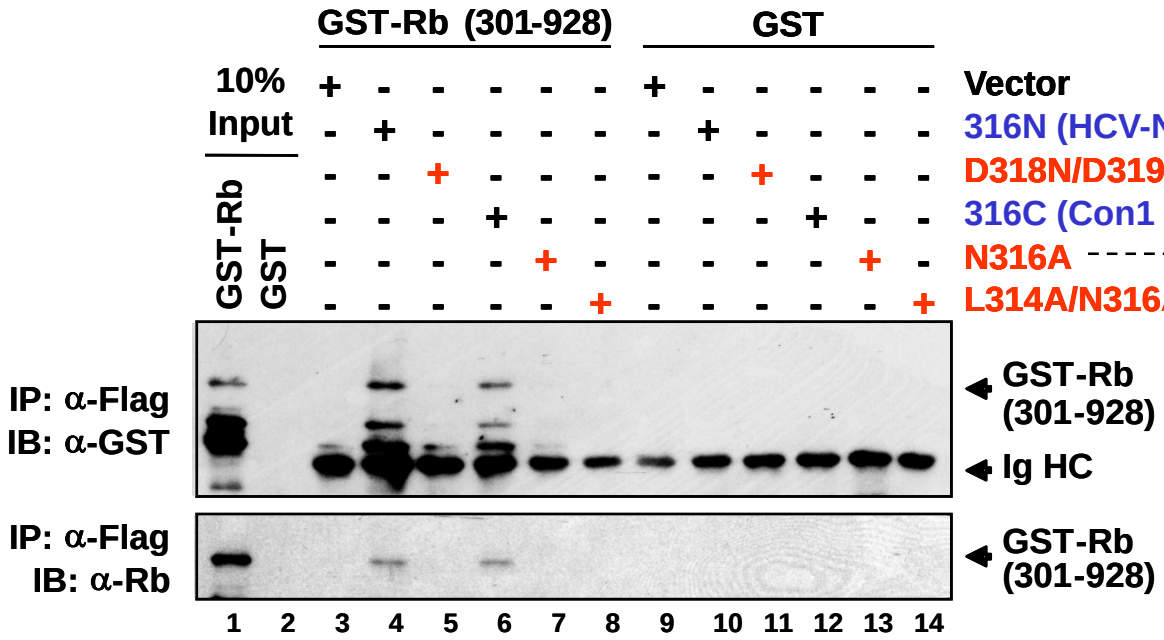




UTMB Center for Hepatitis Research
The University of Texas Medical Branch

S. Ray, Johns Hopkins Univ.

# The NS5B LxCxE-homology Domain is Required for NS5B Binding to pRb

## NS5B-Flag Pull-Down of GST-pRb(301-928)




## Flag-NS5B Mutants

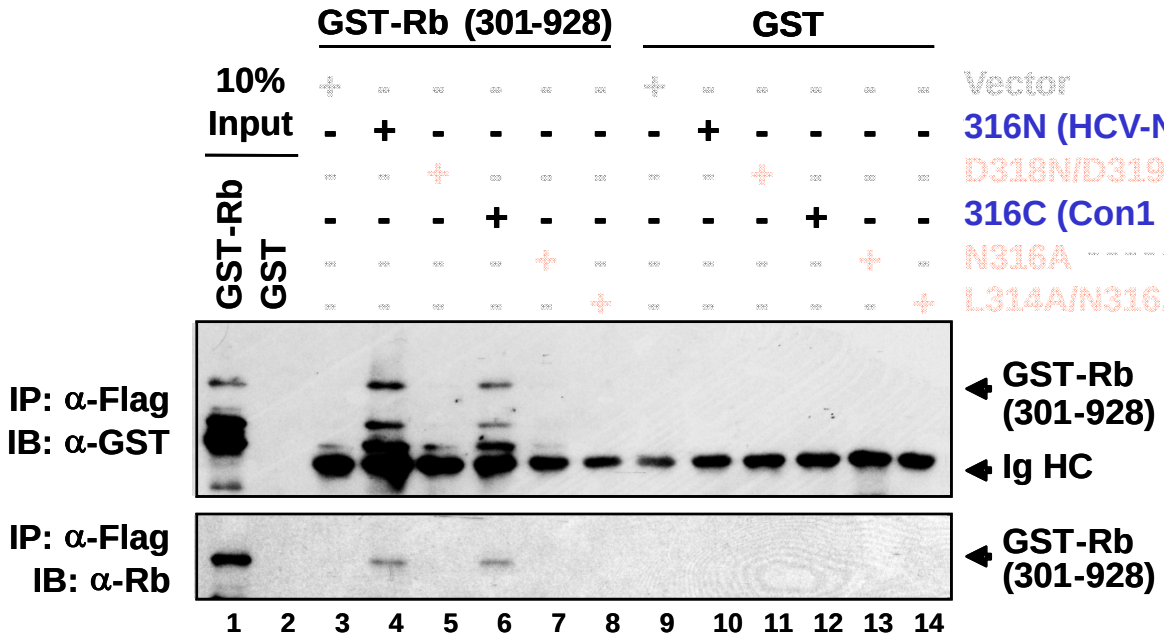
| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | LxCxE | | | | | | | | |
| 316N (HCV-N wt) | D | C | T | M | L | V | N | G | D | D | L |
| D318N/D319N | D | C | T | M | L | V | N | G | N | N | L |
| 316C (Con1 wt) | D | C | T | M | L | V | C | G | D | D | L |
| N316A | D | C | T | M | L | V | A | G | D | D | L |
| L314A/N316A | D | C | T | M | A | V | A | G | D | D | L |



Munakata et al., PNAS 102:18159-64, 2005

# The NS5B LxCxE-homology Domain is Required for NS5B Binding to pRb

## NS5B-Flag Pull-Down of GST-pRb(301-928)




### Flag-NS5B Mutants

| | LxCxE | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Vector | | | | | | | | | | | |
| 316N (HCV-N wt) | D | C | T | M | L | V | N | G | D | D | L |
| D318N/D319N | D | C | T | M | L | V | N | G | N | N | L |
| 316C (Con1 wt) | D | C | T | M | L | V | C | G | D | D | L |
| N316A | D | C | T | M | L | V | A | G | D | D | L |
| L314A/N316A | D | C | T | M | A | V | A | G | D | D | L |



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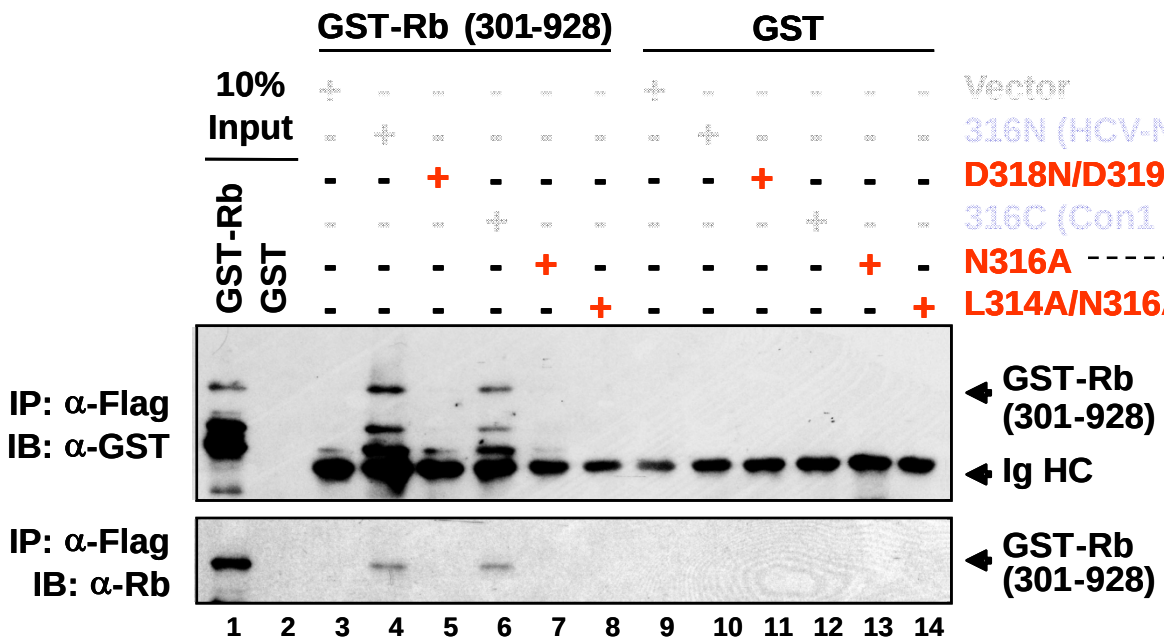


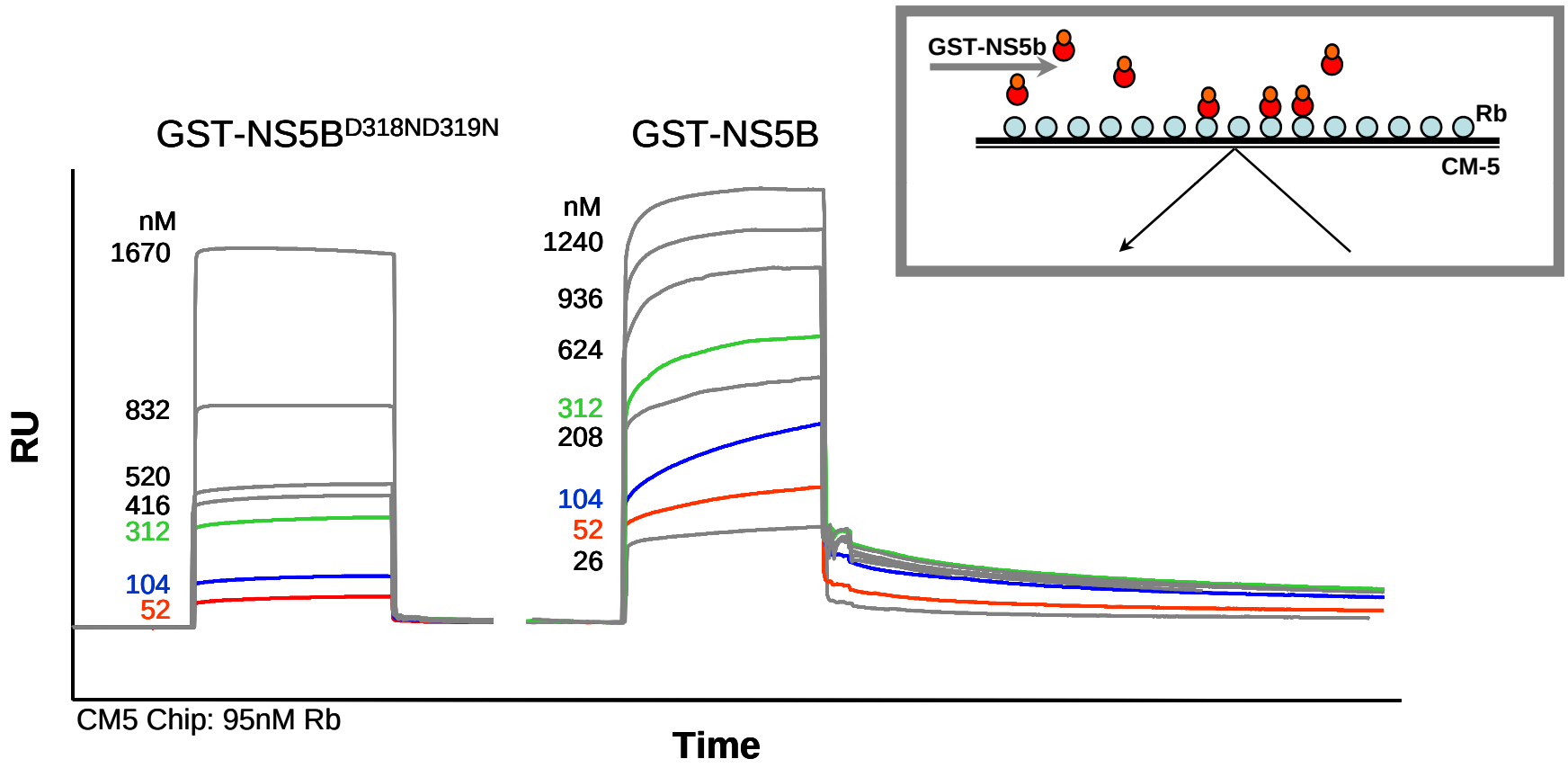

## Flag-NS5B Mutants

| | LxCxE | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Vector | | | | | | | | | | | | |
| 316N (HCV-N wt) | D | C | T | M | L | V | N | G | D | D | L | |
| D318N/D319N | D | C | T | M | L | V | N | G | N | N | L | |
| 316C (Con1 wt) | D | C | T | M | L | V | C | G | D | D | L | |
| N316A | D | C | T | M | L | V | A | G | D | D | L | |
| L314A/N316A | D | C | T | M | A | V | A | G | D | D | L | |

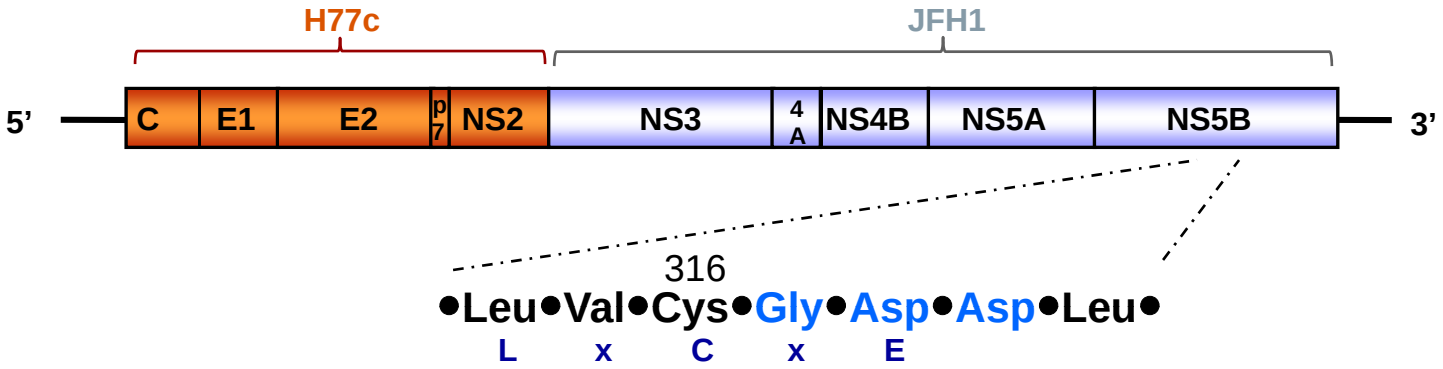


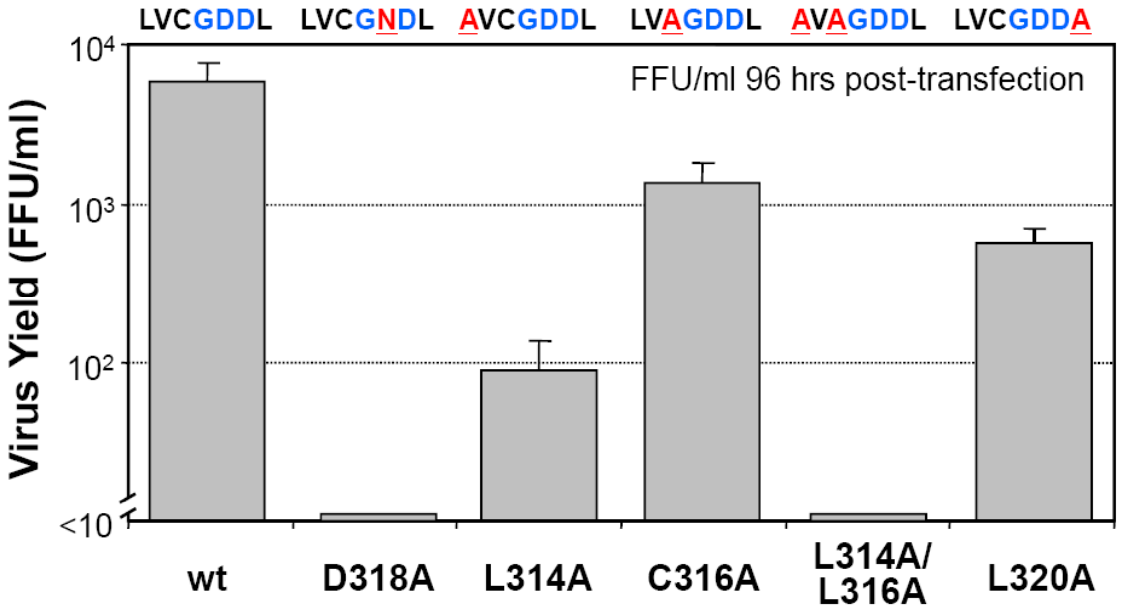
Munakata et al., PNAS 102:18159-64, 2005

# Surface Plasmon Resonance Measurements Demonstrate NS5B Interacts Physically with pRb in an LxCxE-motif Dependent Manner



# HJ3/5 Chimeric HCVs With NS5B LxCxD Domain Mutations Are Viable But Handicapped

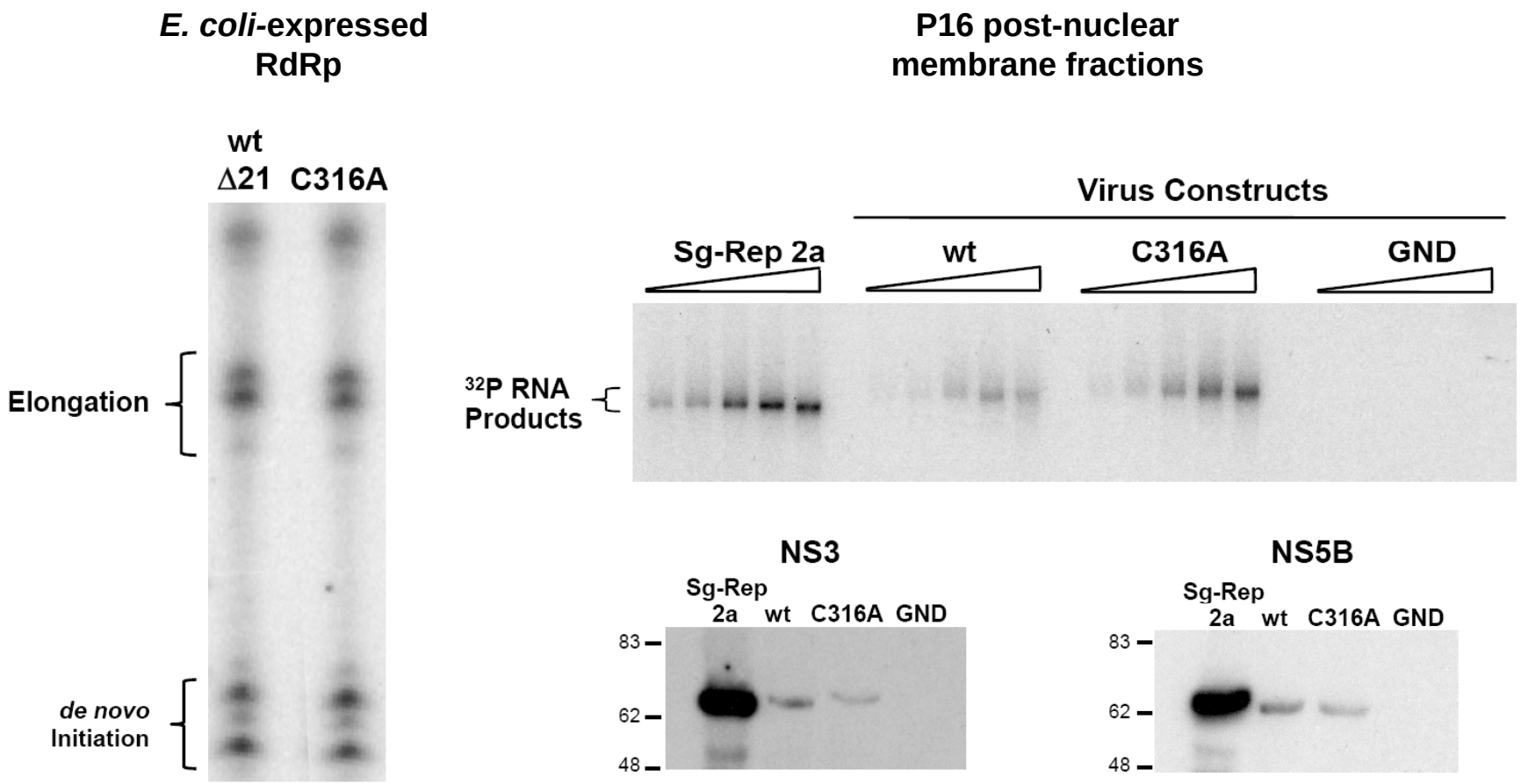





D.R. McGivern et al., J. Virol. In press, 2009

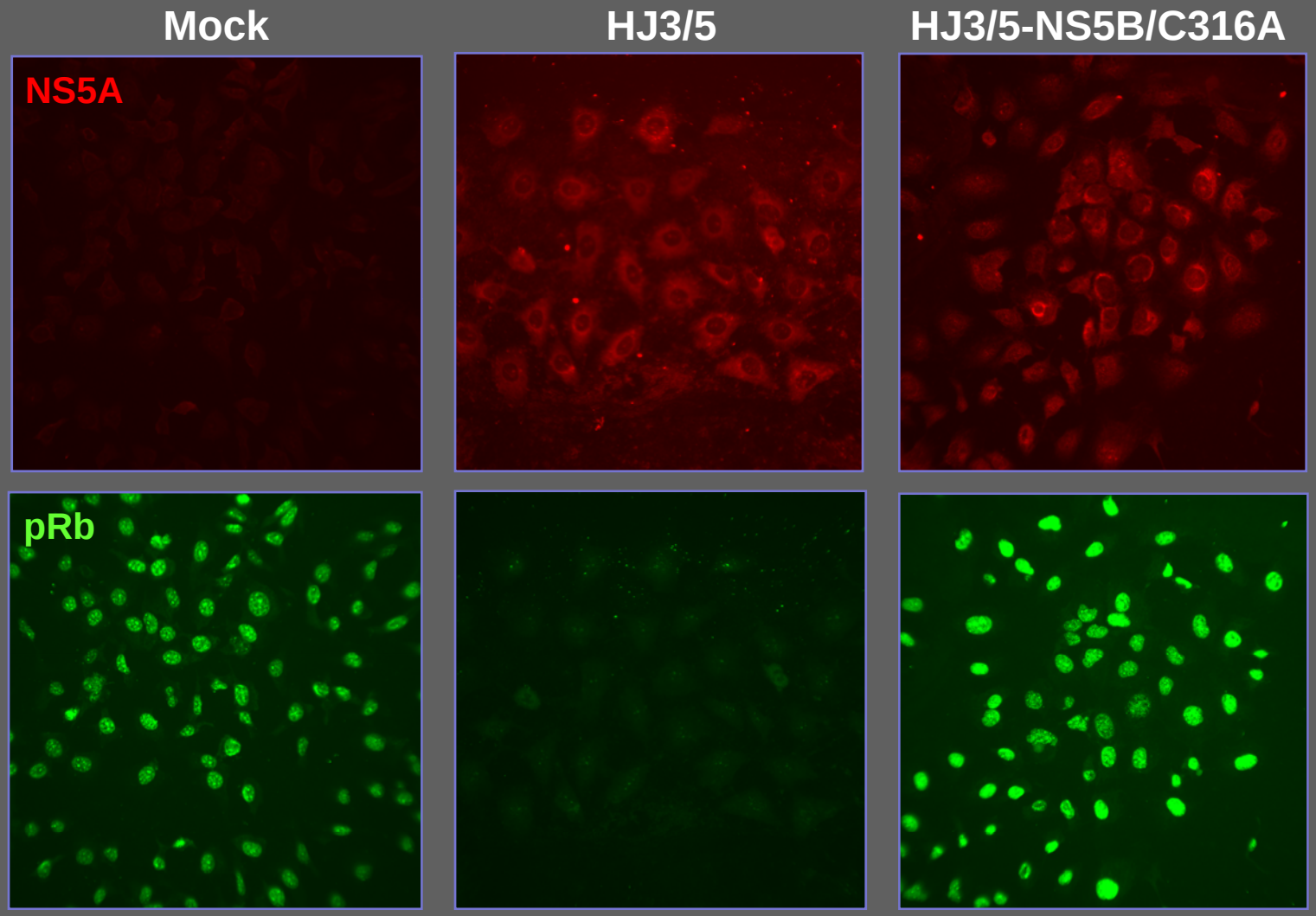
# Polymerase Activities of wt vs. C316A NS5B





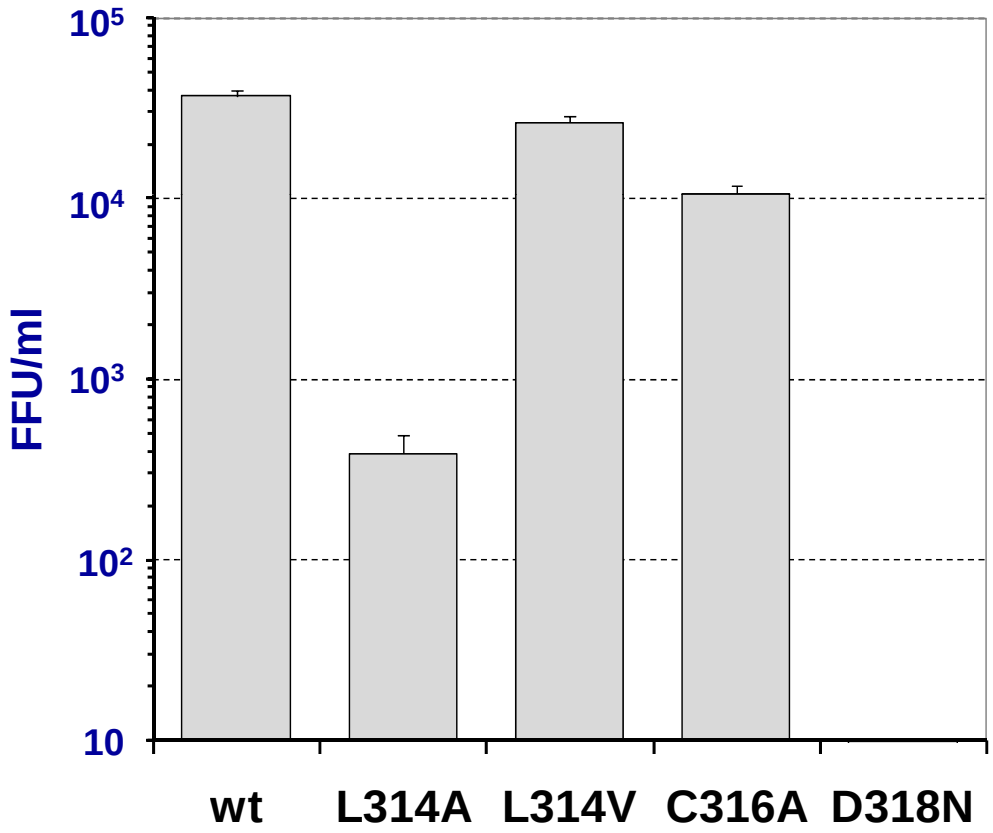

D.R. McGivern et al., J. Virol. In press, 2009

# pRb Expression in Cells Infected with HJ3/5 vs. HJ3/5-C316A Viruses




D.R. McGivern et al., J. Virol. In press, 2009

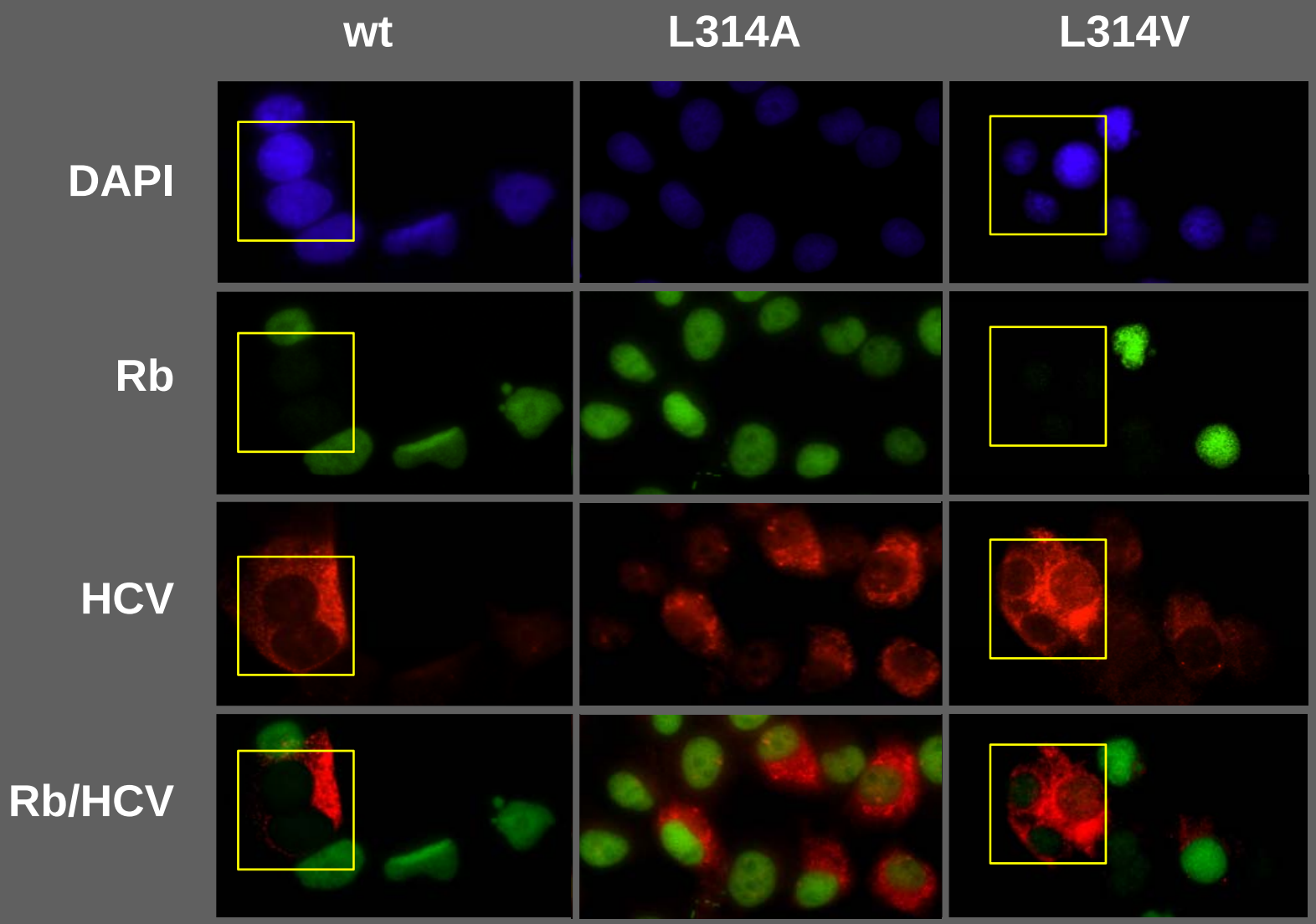
# Passage of L314A led to Emergence of L314V which Restored Replication Capacity





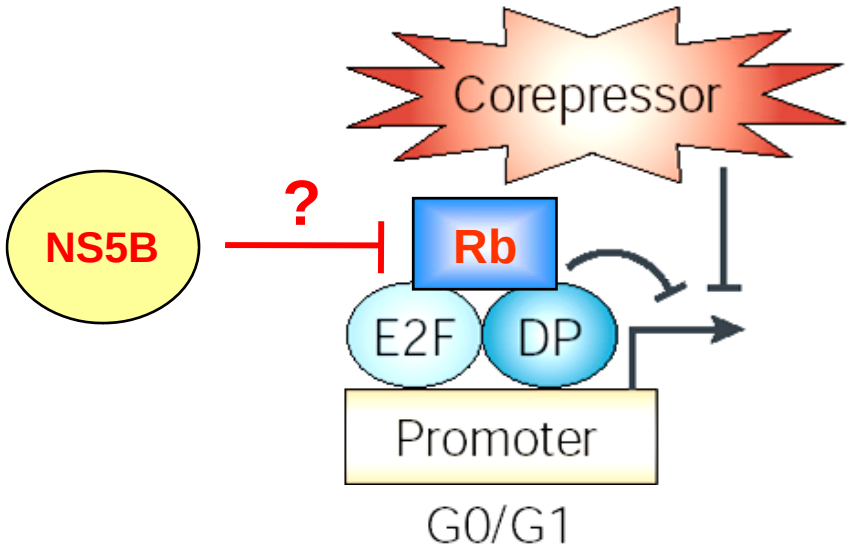

D.R. McGivern et al., J. Virol. In press, 2009

# The L314V Revertant Regulates Rb




D.R. McGivern et al., J. Virol. In press, 2009

# Does NS5B Regulation of pRb Attenuate pRb Repression of E2F Transcription Factors?

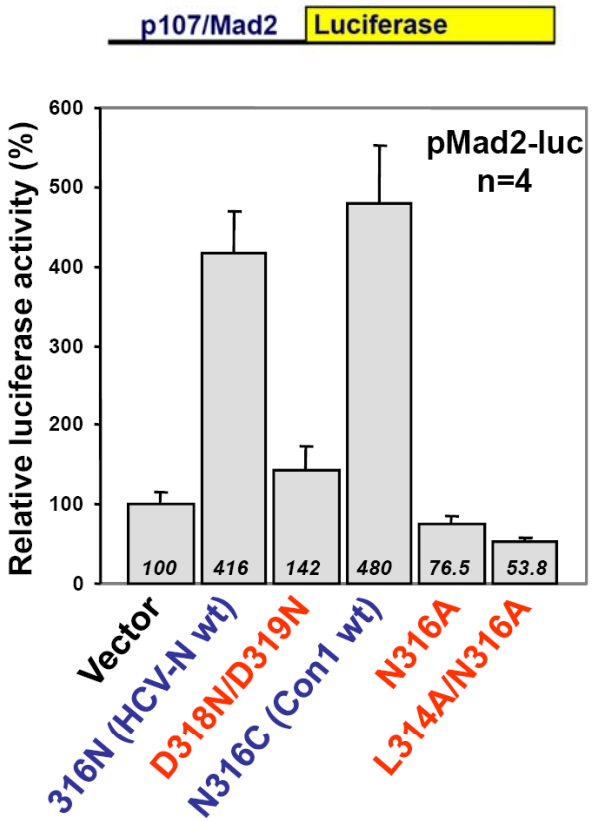




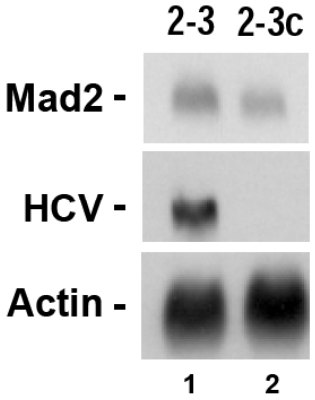

Modified from Classon and Harlow, Nature Rev Cancer 2: 910-917, 2002

# NS5B Activates E2F-Responsive Promoters in an LxCxE Domain-Dependent Fashion

**Ectopic NS5B Expression in Huh7 Cells**



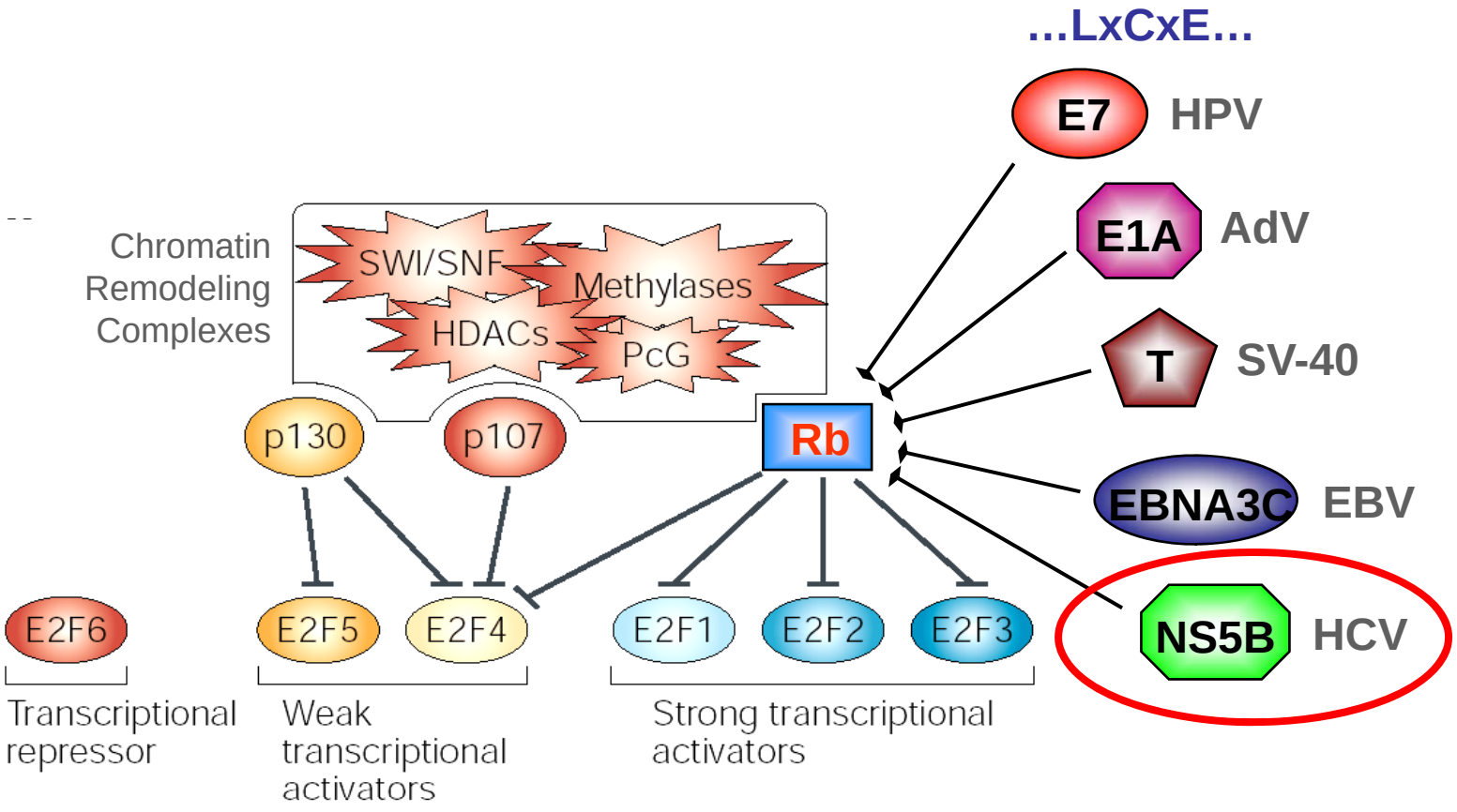

**Mad2 Transcripts in HCV Replicon Cells**






Munakata et al., PNAS 102:18159-64, 2005
Lauren Evers, David McGivern

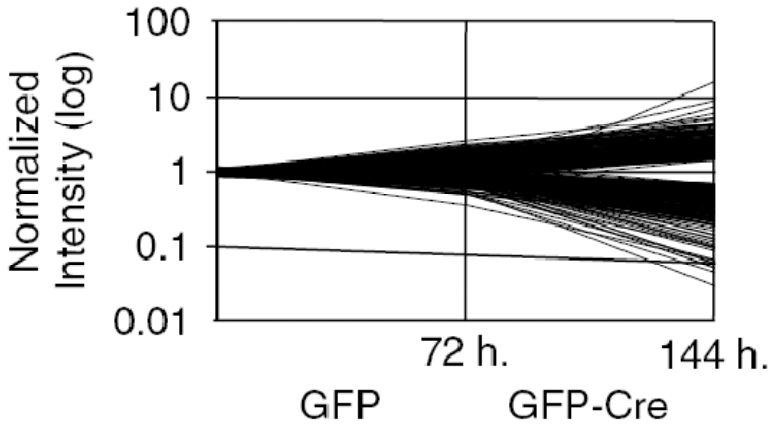
# Viral Proteins That Bind pRb and Suppress pRb Function

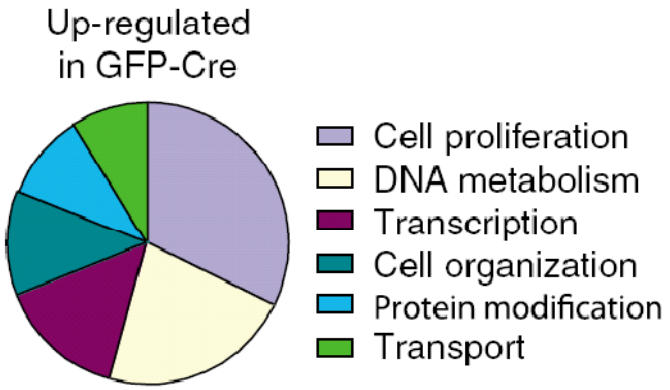


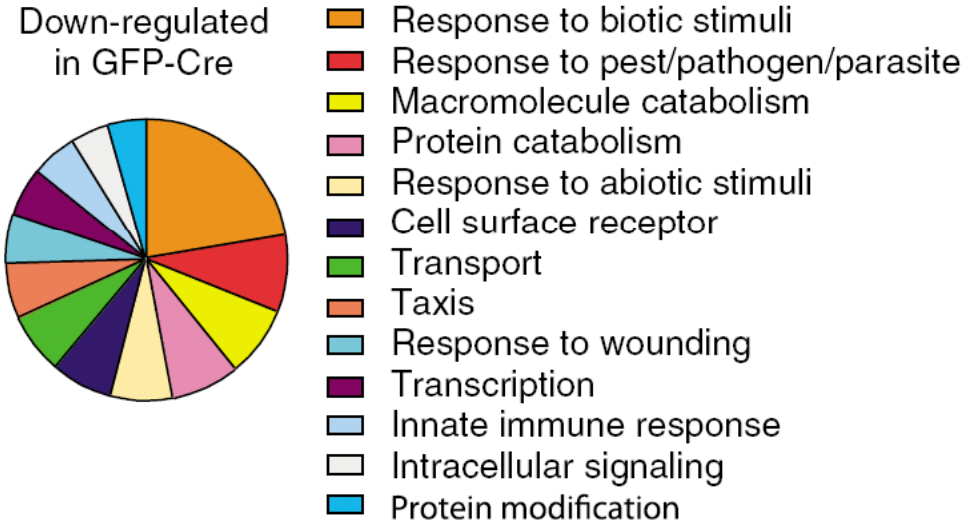



Modified from Classon and Harlow, Nature Rev Cancer 2: 910-917, 2002

# Acute pRb Loss De-Regulates Many Genes



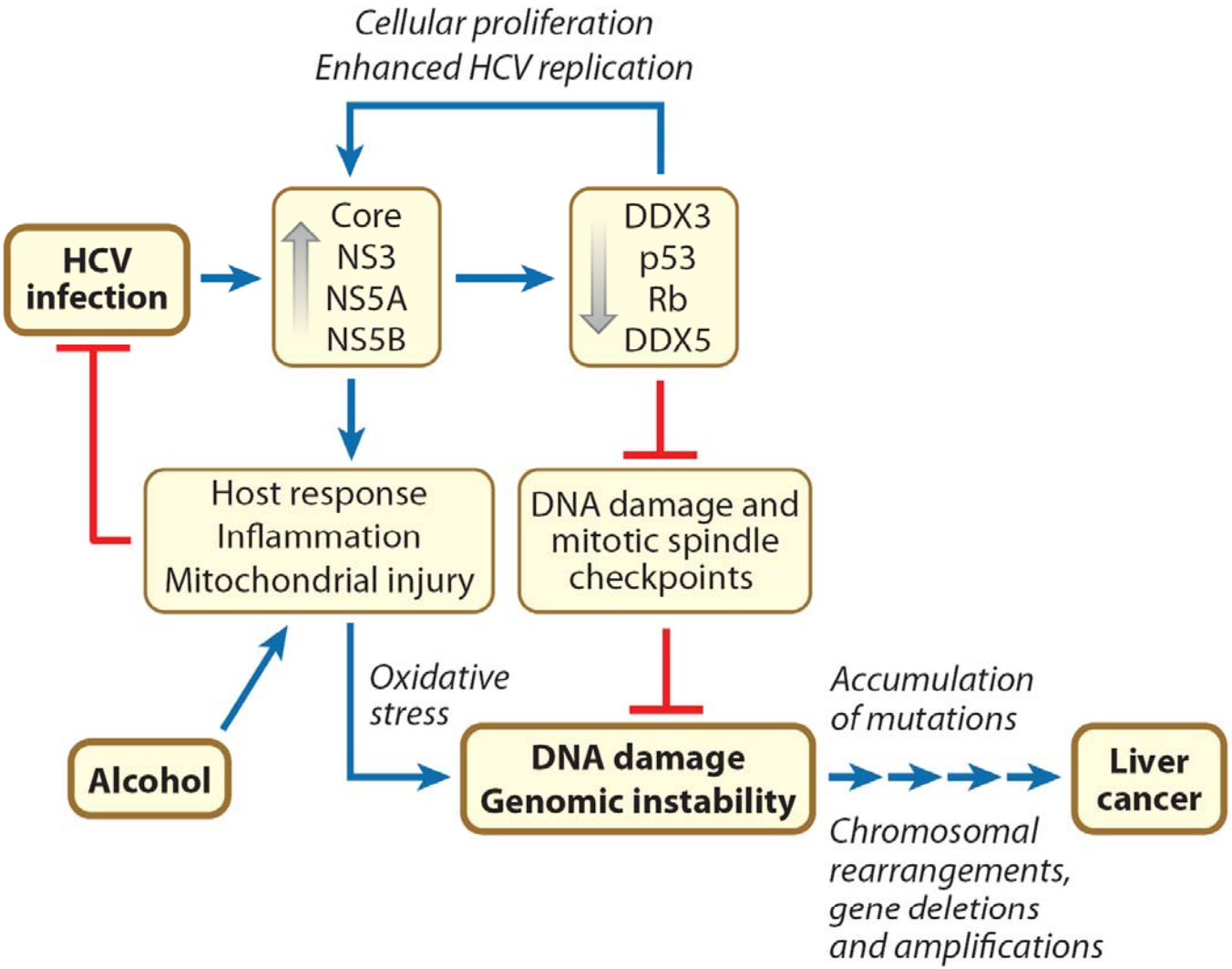






MP Markey et al., *Oncogene* 10.1038/sj.onc.1210450

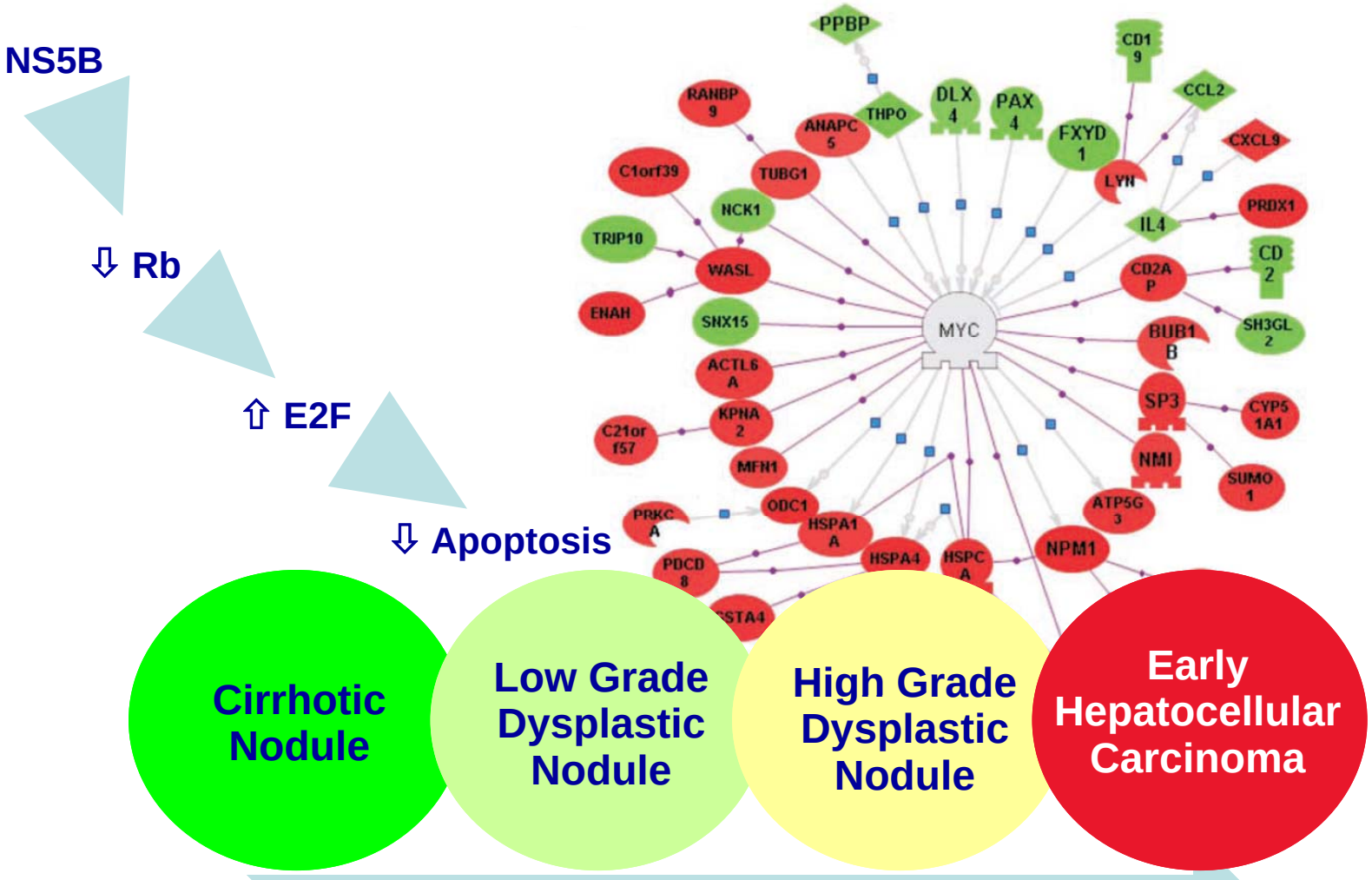
# HCV Interactions withTumor Suppressors and HCC






D.R. McGivern & S,M. Lemon, *Annu Rev Pathol Mech Dis* 4:399-415, 2008

# Potential Protective Role of NS5B in c-Myc Malignant Conversion of Dysplastic Nodules to Early HCC






Kaposi-Novak et al. *Cancer Res*, 69:2775-2782, 2009

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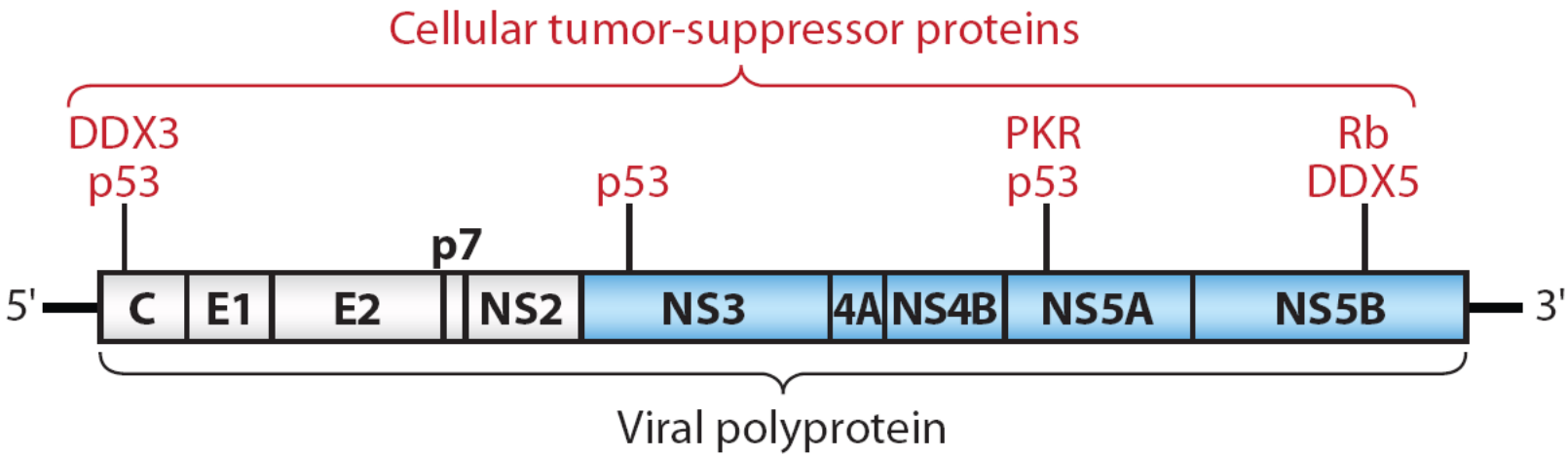
UTMB Center for Hepatitis Research
The University of Texas Medical Branch

# Putative HCV Interactions with p53

| HCV protein interacting with p53 | Experimental system | Cell type | Observations |
|---|---|---|---|
| Core | Proteins expressed in vitro | Not applicable | Interaction detected by GST pull-down using in vitro translated proteins or by "far-western" blot using proteins expressed in bacteria |
| Core | Transient overexpression of core with GST-p53 fusions | COS-7 | Interaction detected by GST pull-down |
| Core | Proteins expressed in vitro | Not applicable | Interaction detected by GST pull-down using in vitro translated proteins |
| Core | Transient overexpression with wt p53 | HepG2, HeLa | Colocalization of core and p53 detected by immunofluorescence analysis |
| NS3 | Transient overexpression with wt p53 | HeLa, BHK-21 | Interaction detected by coimmunoprecipitation |
| NS5A | Proteins expressed in vitro | Not applicable | Interaction detected by GST pull-down using in vitro translated NS5A and either p53 expressed from bacteria or HepG2 nuclear extracts |
| NS5A | Transient overexpression | HepG2 | Interaction detected by coimmunoprecipitation or mammalian two-hybrid; colocalization of NS5A and p53 detected by immunofluorescence |






D.R. McGivern & S,M. Lemon, *Annu Rev Pathol Mech Dis* 4:399-415, 2008