# Roles and regulation of microRNAs in viral infections

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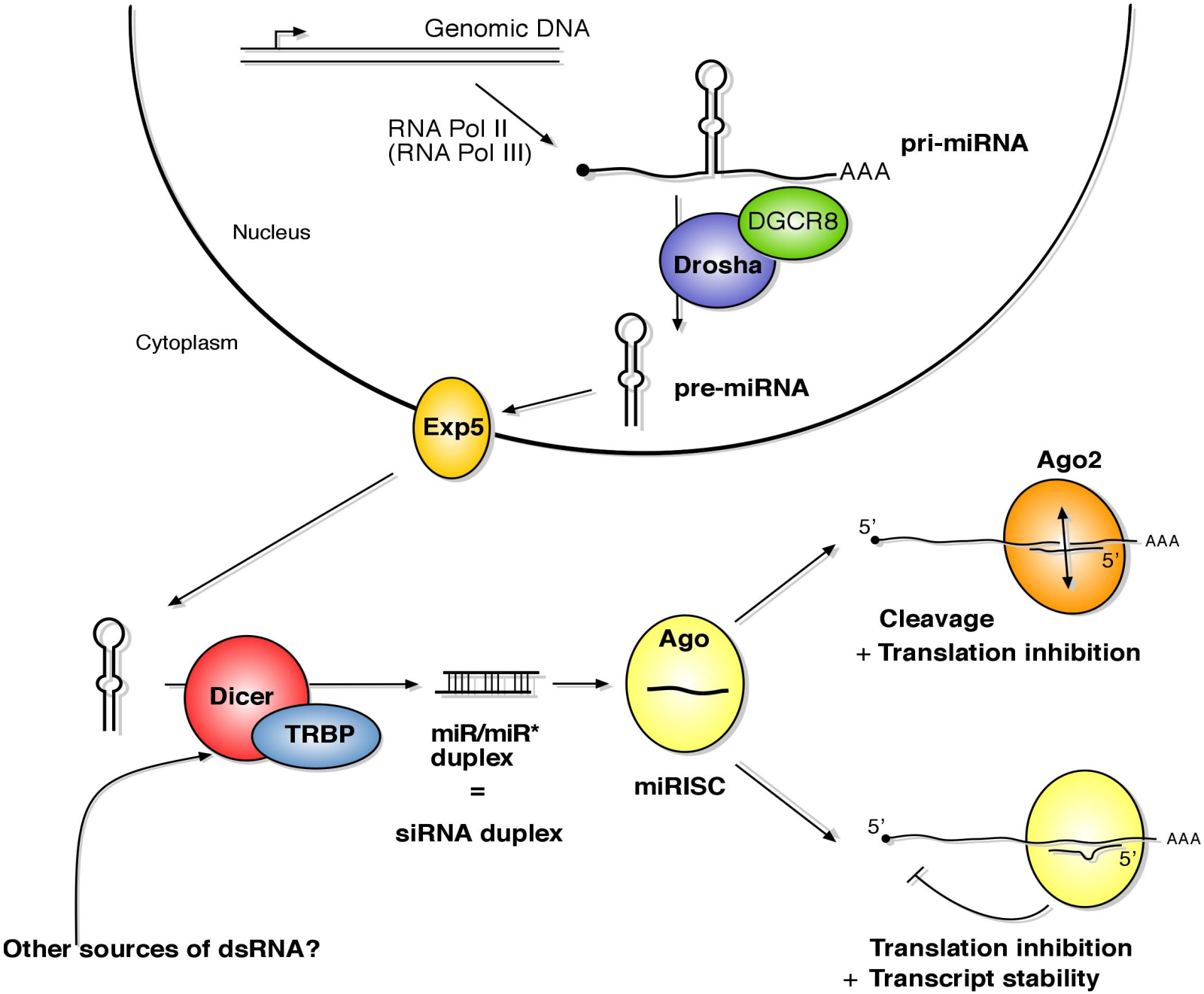
# miRNA biogenesis



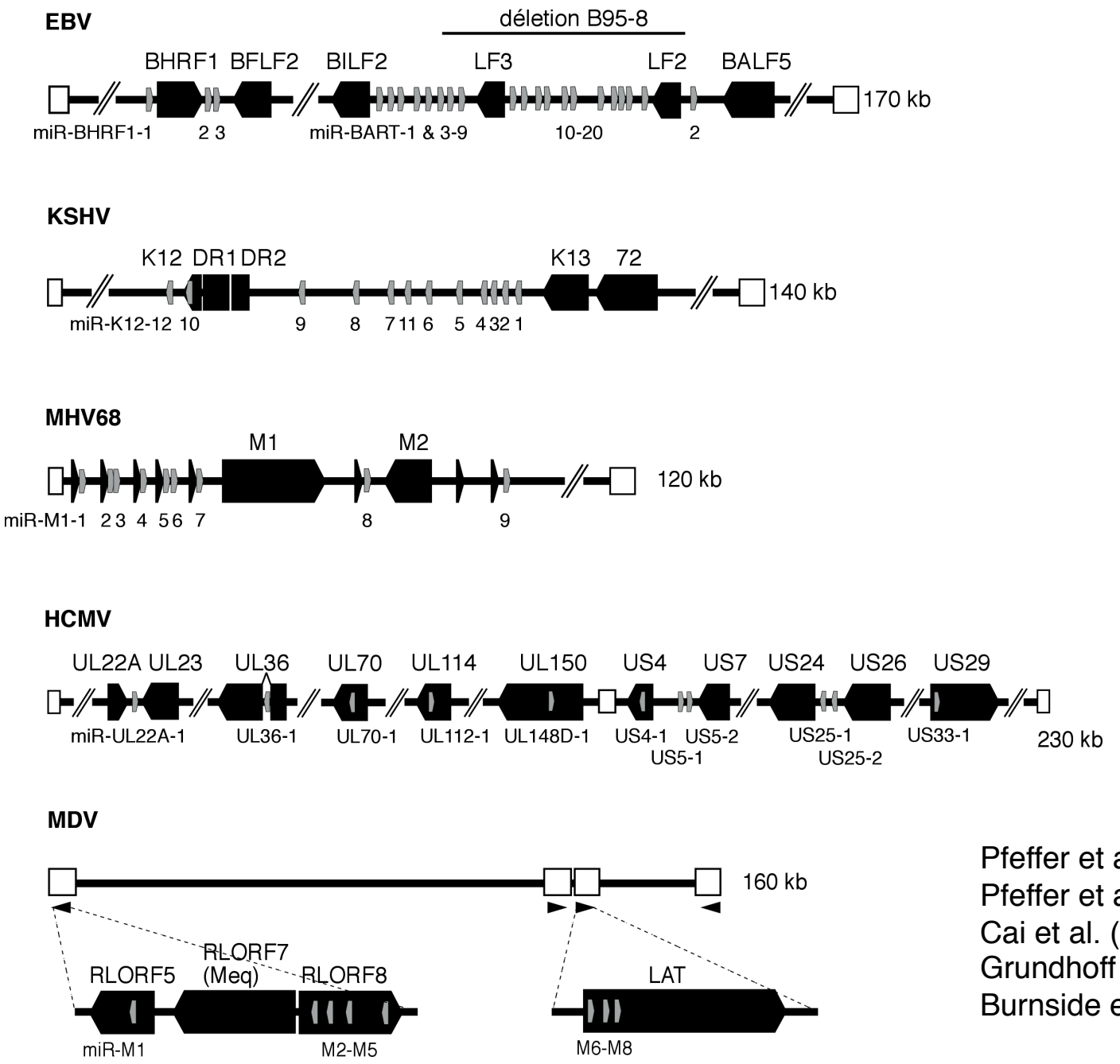
# Number of miRNAs identified to date

April 2011 release of miRbase

| Specie | miRNAs number |
|---|---|
| *D. melanogaster* | 238 |
| *C. elegans* | 207 |
| *G. gallus* | 499 |
| *H. sapiens* | 1424 |
| *M. musculus* | 720 |
| *R. norvegicus* | 408 |
| *A. thaliana* | 232 |

http://www.mirbase.org/

# Mammalian DNA viruses encode miRNAs




Pfeffer et al. (2004)
Pfeffer et al. (2005)
Cai et al. (2006)
Grundhoff et al. (2006)
Burnside et al. (2006)

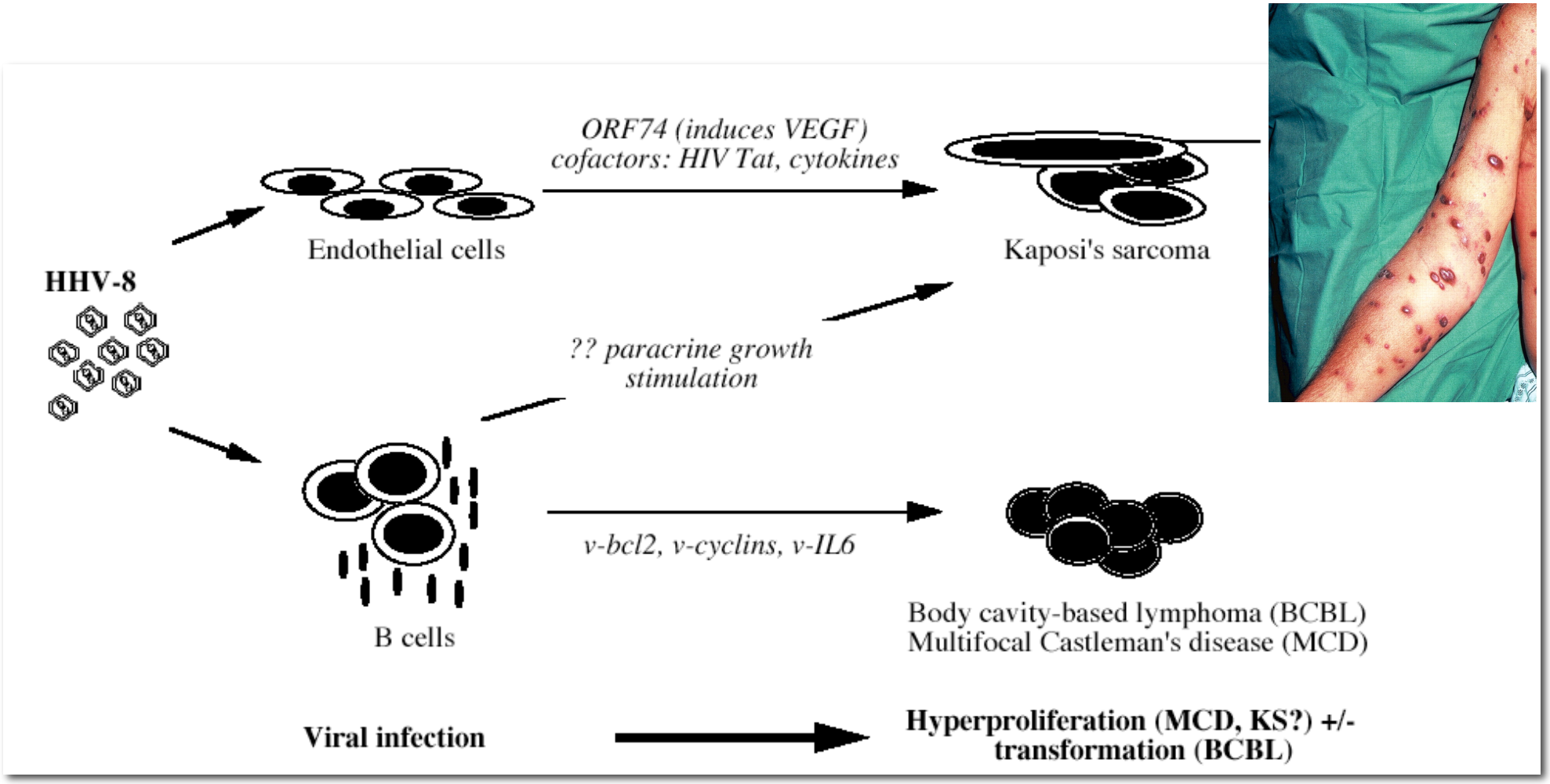
# Viral miRNAs target viral genes…

| Virus | miRNA | Target | Function |
|---|---|---|---|
| **SV40** | miR-S1 | T antigen | Early protein |
| **BKV** | miR-B1 | T antigen | Early protein |
| **JCV** | miR-J1 | T antigen | Early protein |
| **MCV** | miR-M1 | T antigen | Early protein |
| **EBV** | miR-BART2 | BALF5 | DNA polymerase |
| | miR-BART1-5p, -16, 17 | LMP1 | Signaling molecule, TNF receptor mimic |
| **HCMV** | miR-UL112-1 | UL112/113, UL120/121<br>UL123 (IE72, IE1)<br><br>UL114<br>UL117 | IE protein<br>IE protein, viral transactivator<br>Uracil DNA glycosylase<br>Maturation of replication compartments |
| **HSV-1** | miR-H2-3p<br>miR-H6 | ICP0<br>ICP4 | IE protein<br>IE protein |
| **HSV-2** | miR-I<br>miR-II, miR-III | ICP34.5<br>ICP34.5, ICP0 | Pathogenicity factor |
| **HvAv** | miR-1 | ORF1 | DNA polymerase |
| **KSHV** | miR-K12-9* | ORF50/Rta | Viral transactivator |
| **ILTV** | miR-I5 | ICP4 | IE viral transactivator |

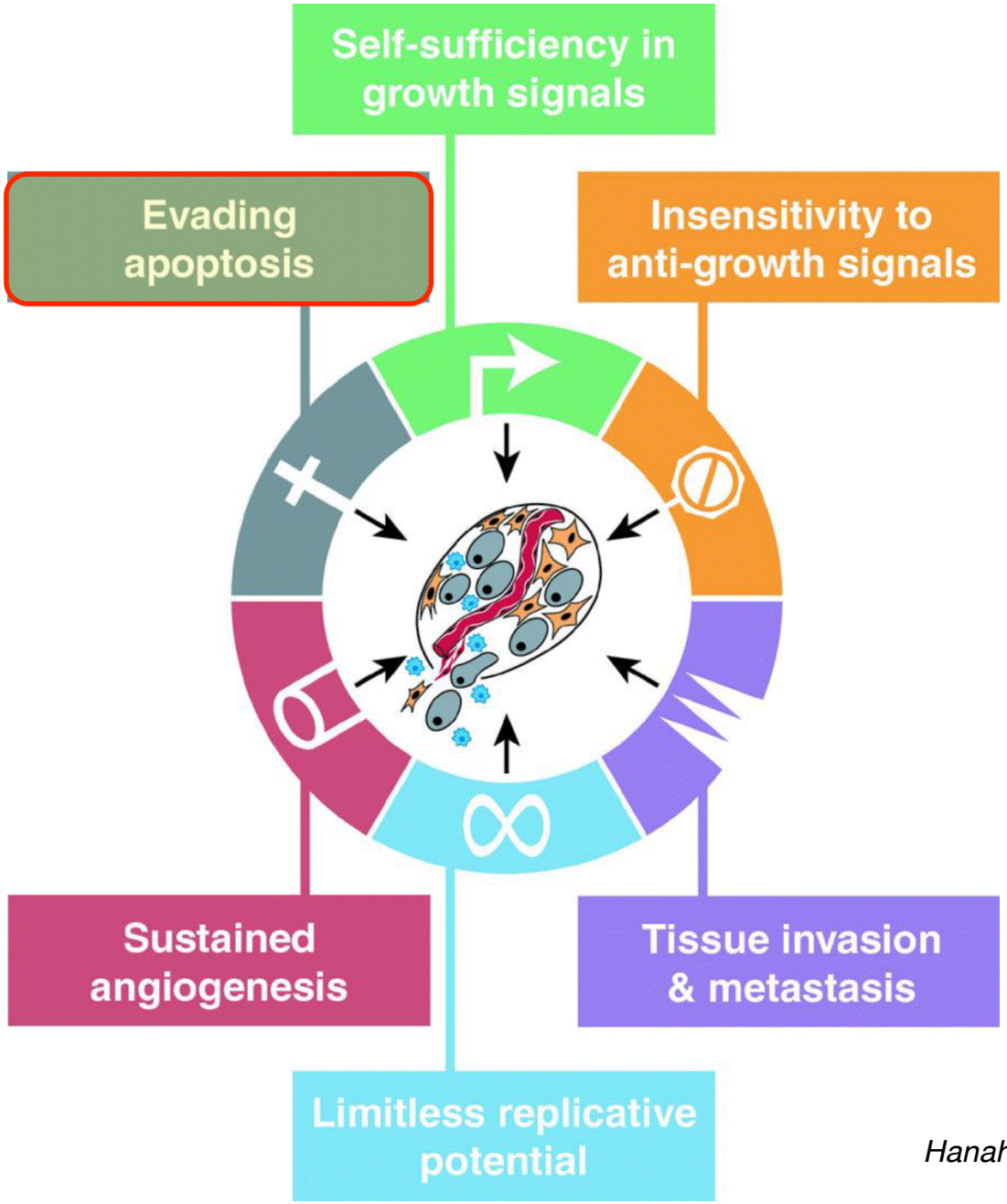
# … and cellular genes

| Virus | miRNA | Target | Function |
|---|---|---|---|
| **KSHV** | miR-K12-6-3p and others | THBS1 | Angiogenesis regulator |
| | miR-K12-11 | BACH1, Fos | Transcription factors |
| | miR-K12-5 | BCLAF1 | Pro-apoptotic protein |
| | miR-K12-11, -6 | MAF | Transcription factor |
| | miR-K12-7 | MICB | NK cell ligand |
| | miR-K12-10a | TWEAKR | Pro-apoptotic protein |
| **EBV** | miR-BHRF1-3 | CXCL-11 | Chemokine |
| | miR-BART5 | PUMA | Pro-apoptotic protein |
| | miR-BART2 | MICB | NK cell ligand |
| **HCMV** | miR-UL112-1 | MICB | NK cell ligand |
| | miR-US25-1 | CCNE2 | G1/S cyclin E2 |
| | | H3F3B | H3 histone family 3B |
| | | TRIM28 | Transcriptionnal corepressor |
| **MDV** | miR-M4 | PU.1 | Transcription factor |

# Kaposi's sarcoma herpesvirus (KSHV or HHV8)

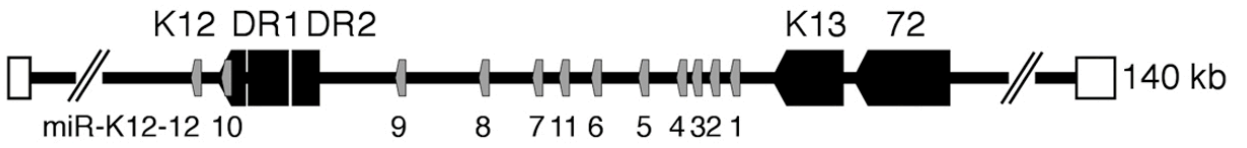


# Hallmarks of cancer




*Hanahan & Weinberg, Cell 2000*

# Generation of KSHV miRNAs expressing lines

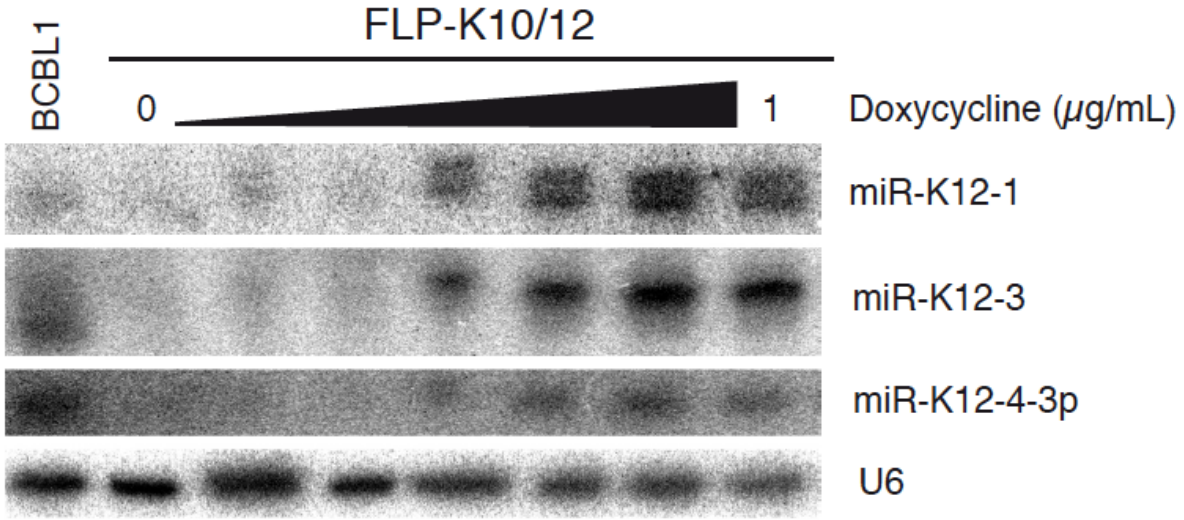



K10/12 (intronic miRNAs)

✓ Dox Inducible FLP HEK293 cells

✓ Stable DG75 cells

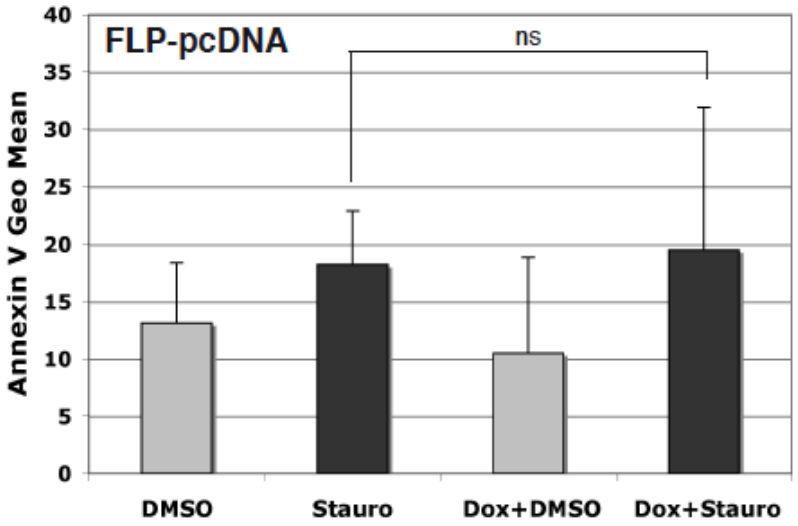
✓ Stable EaHy926 cells

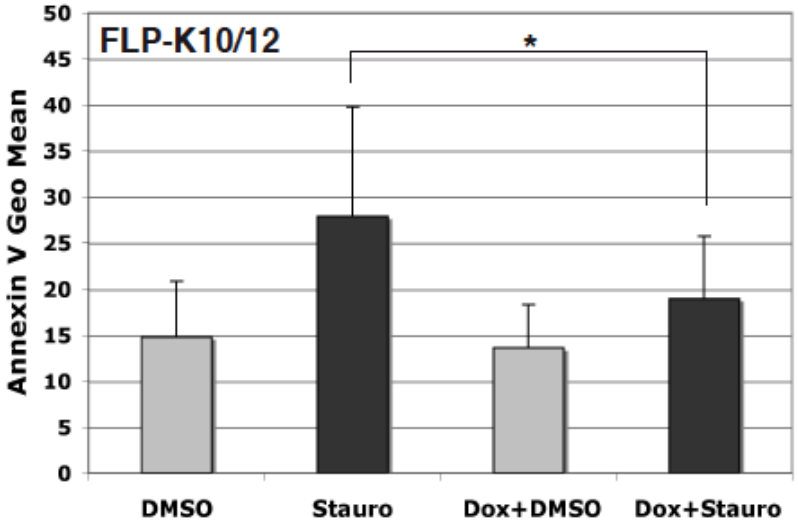


# Effect of KSHV miRNAs on apoptosis

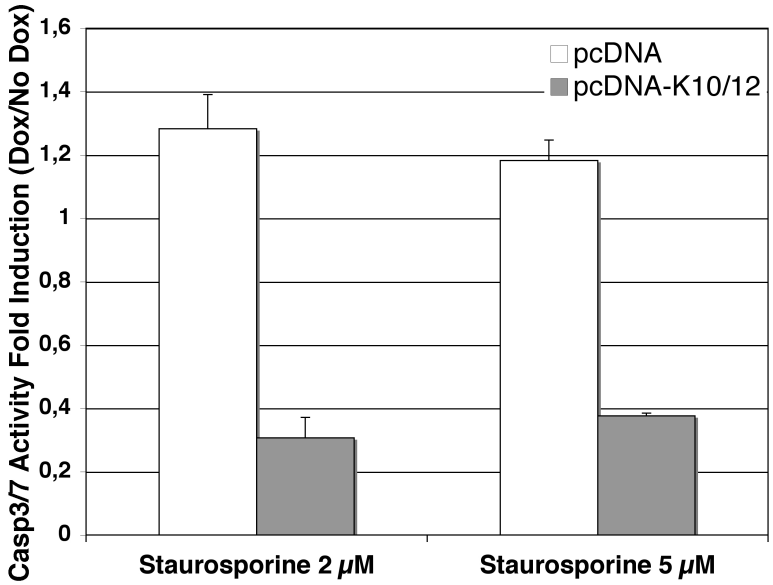
In HEK293 FLP K10/12 cells

Annexin V measurement after Staurosporine treatment

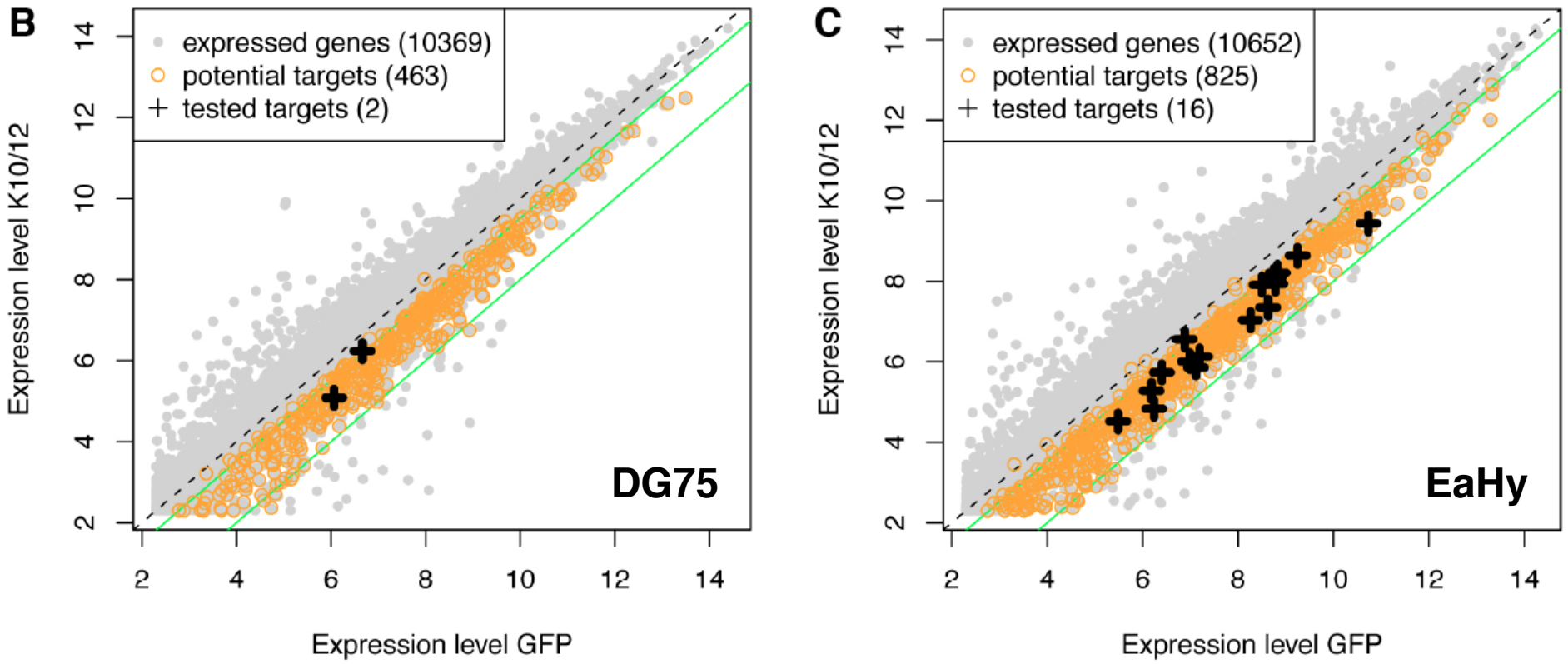



Casp3/7 activity after Staurosporine treatment



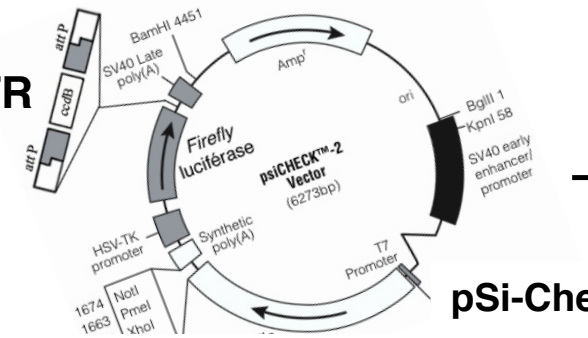
# Affymetrix analysis of DG75 and EaHy K10/12 cells




**Search for KSHV miRNAs binding sites in regulated transcripts**

# Validation of KSHV miRNAs putative targets

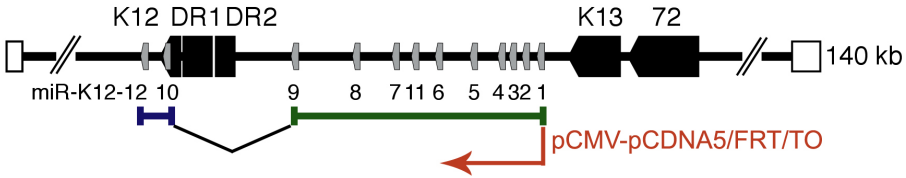
3' UTR

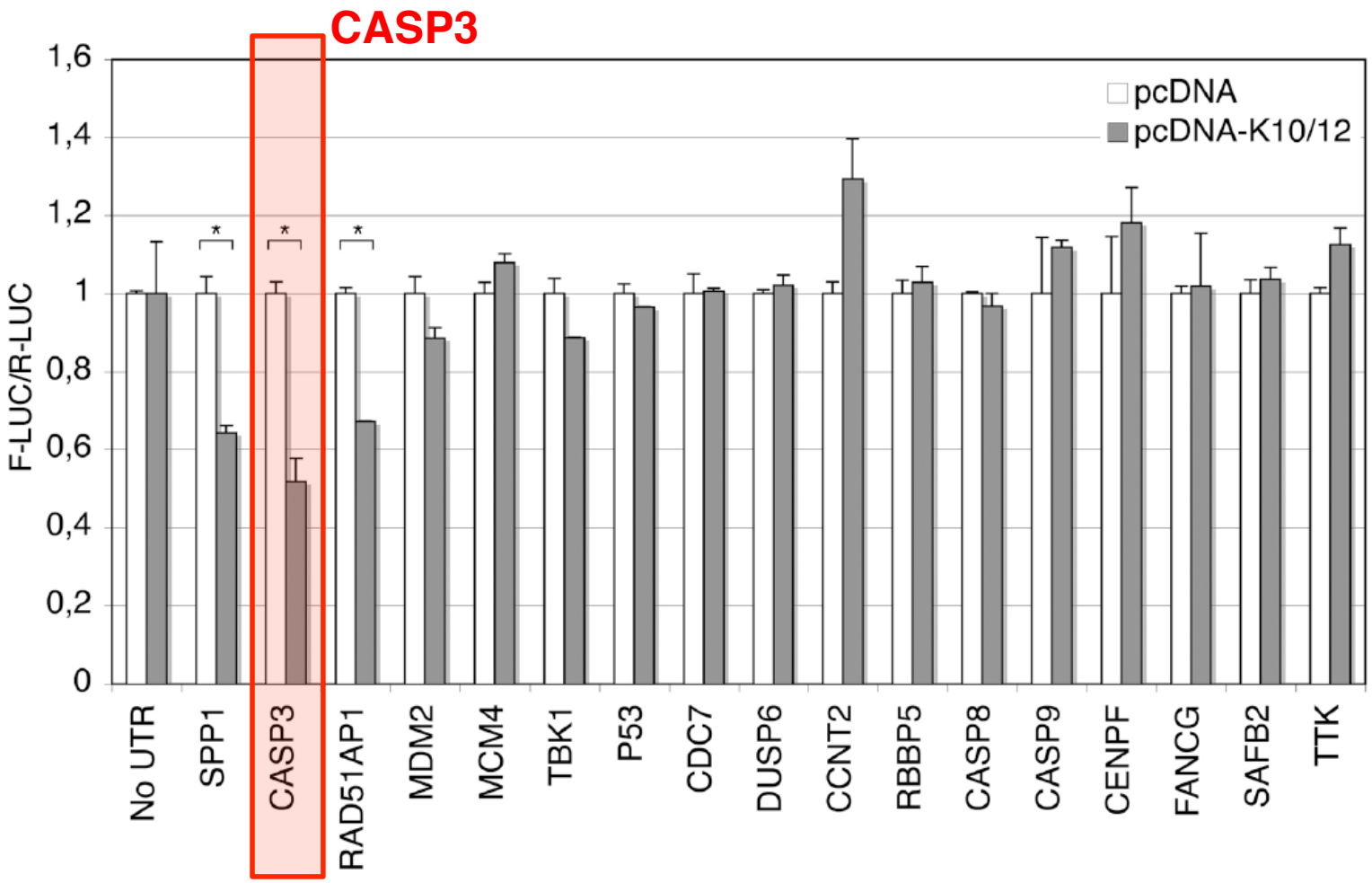


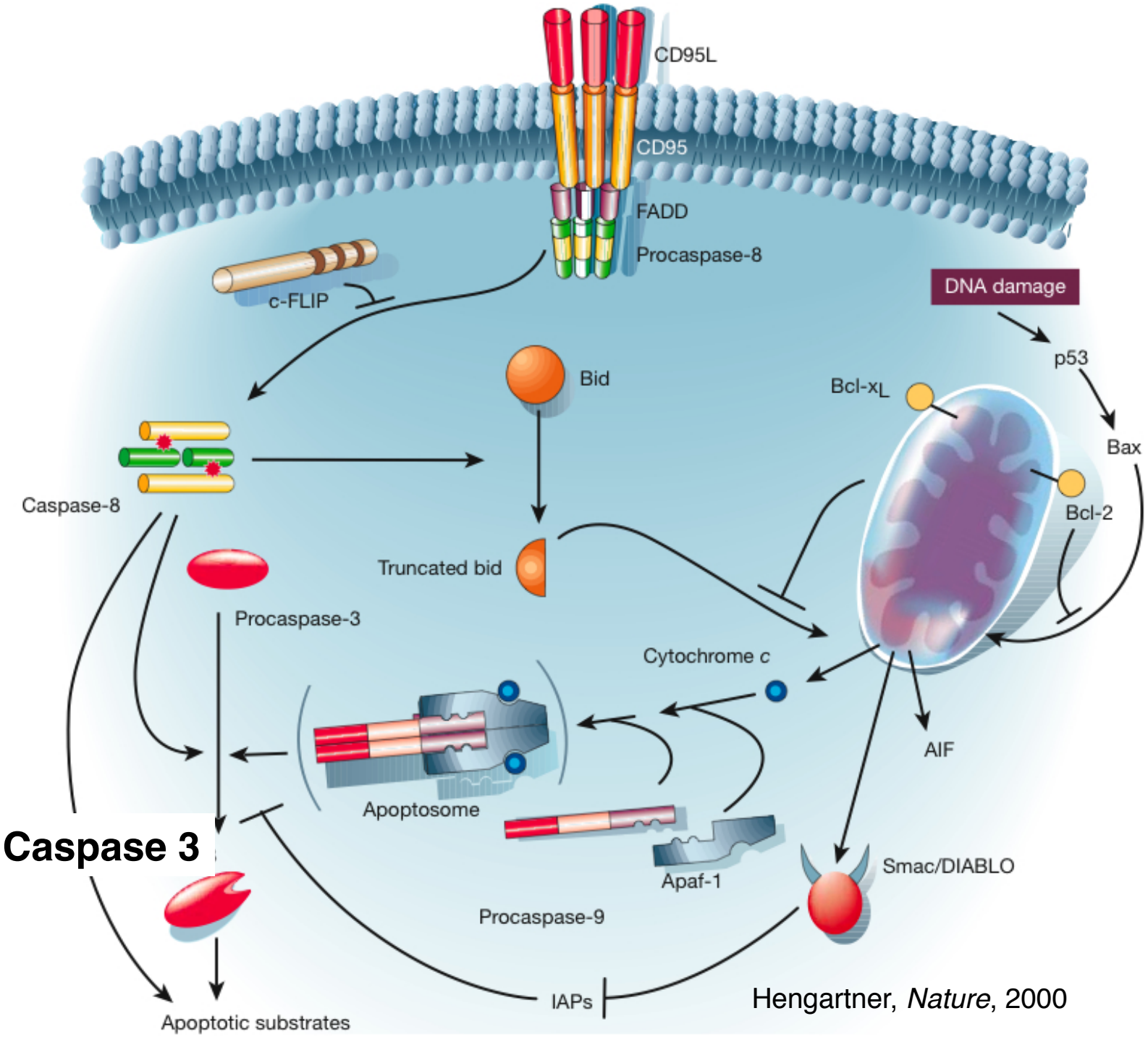

pSi-Check2

+

pcDNA-K10/12



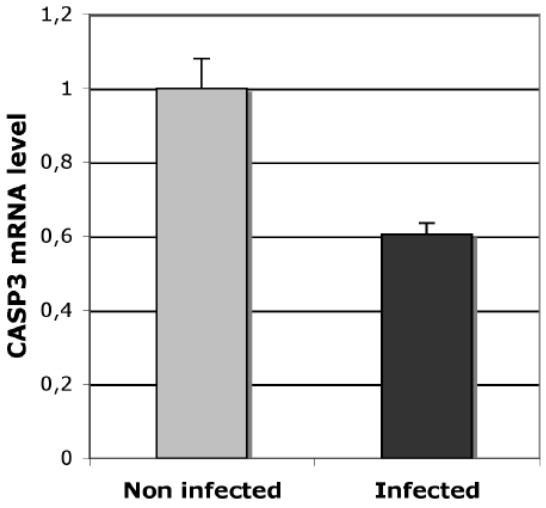



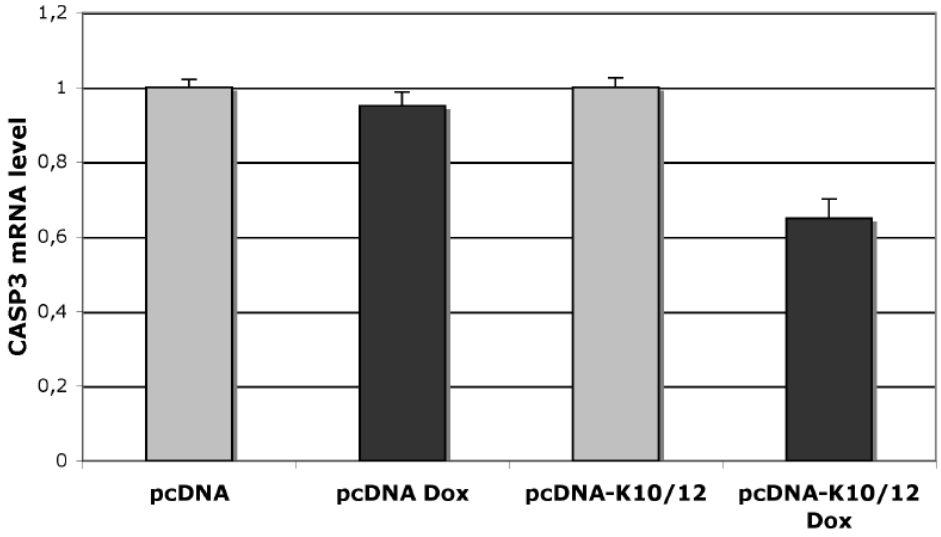



Hengartner, *Nature*, 2000

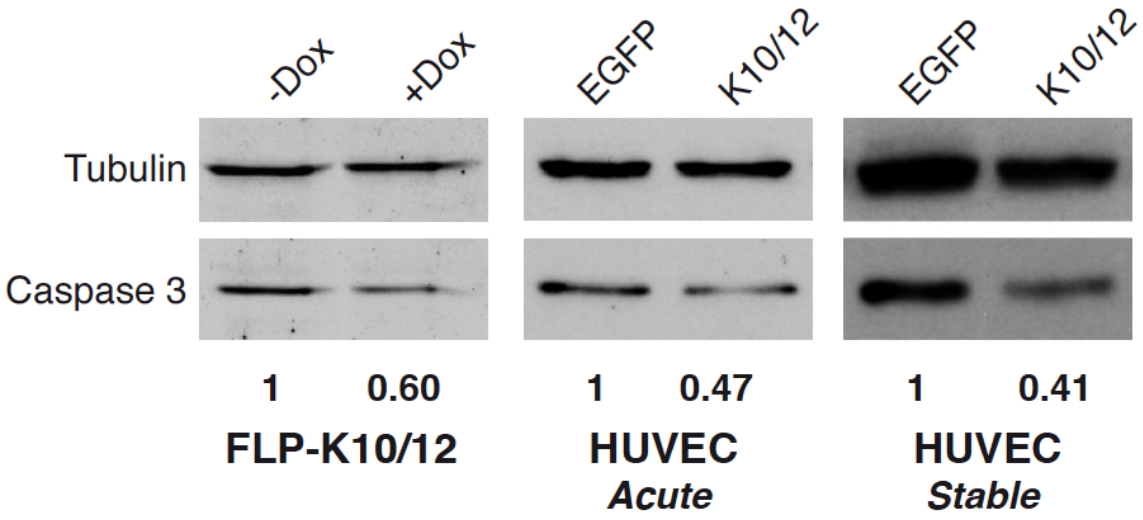
# KSHV miRNAs target endogenous Caspase 3

Casp3 mRNA

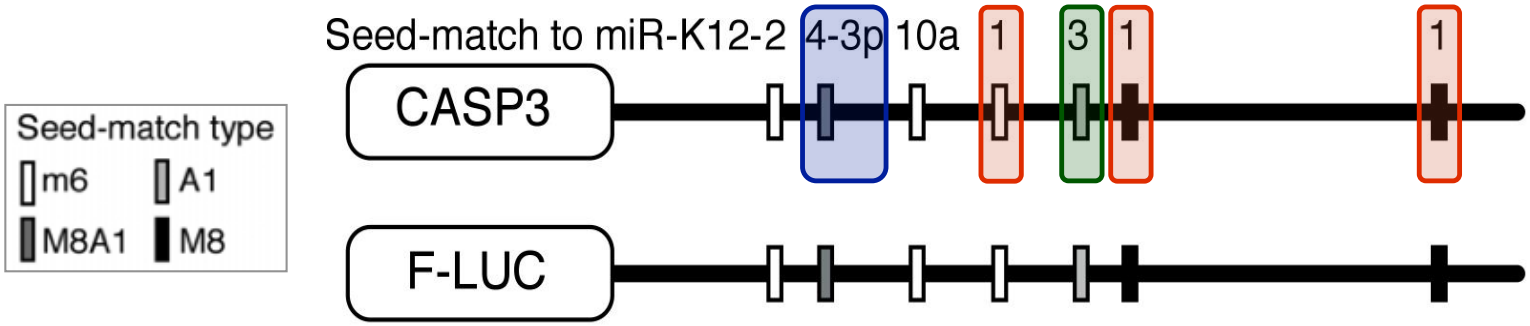


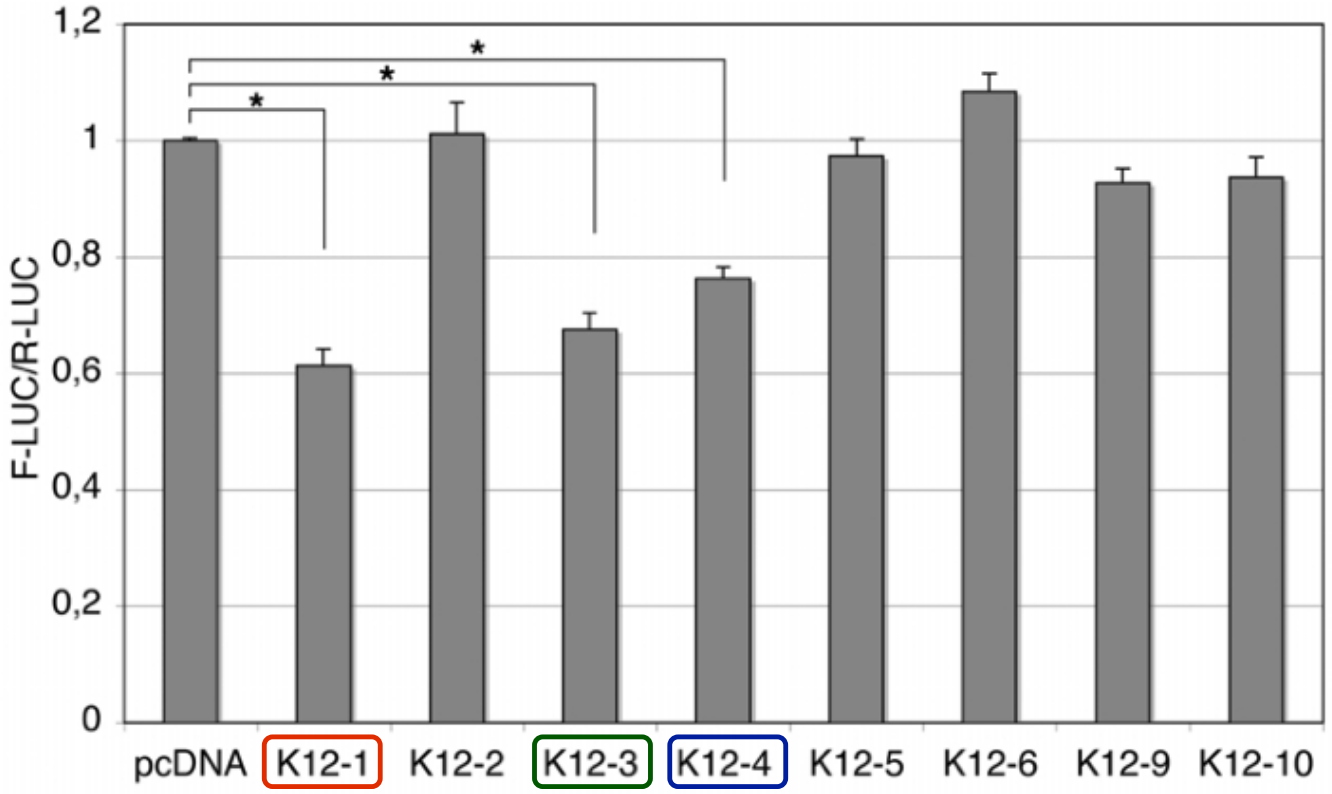




Casp3 protein

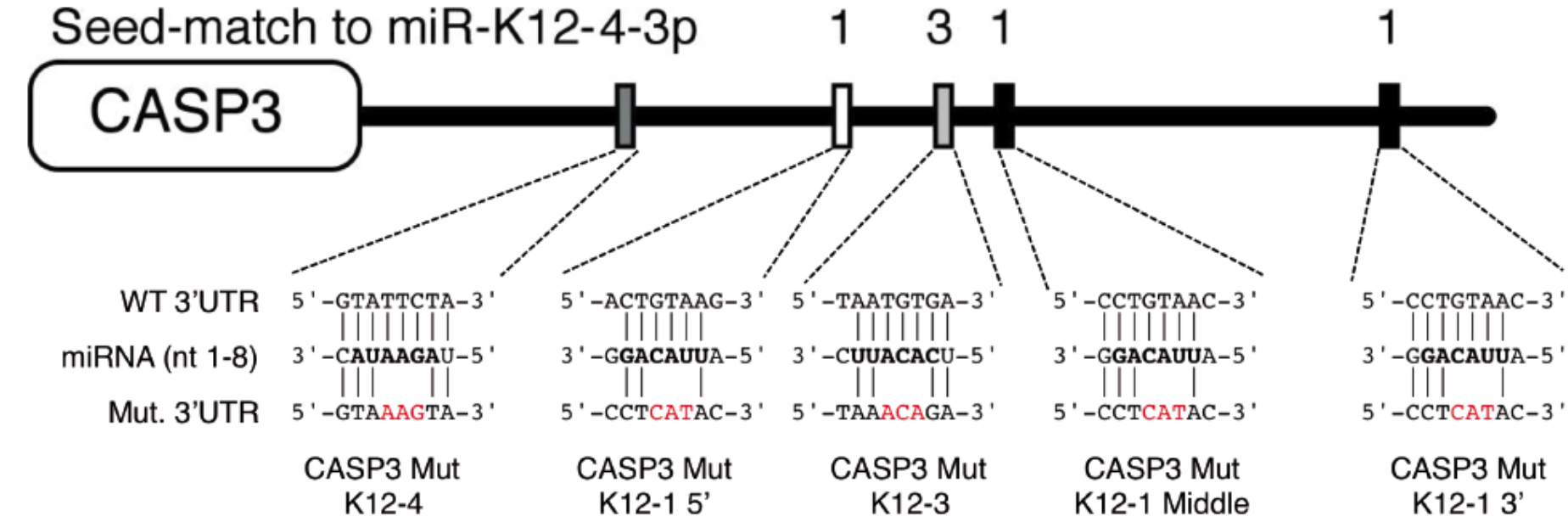


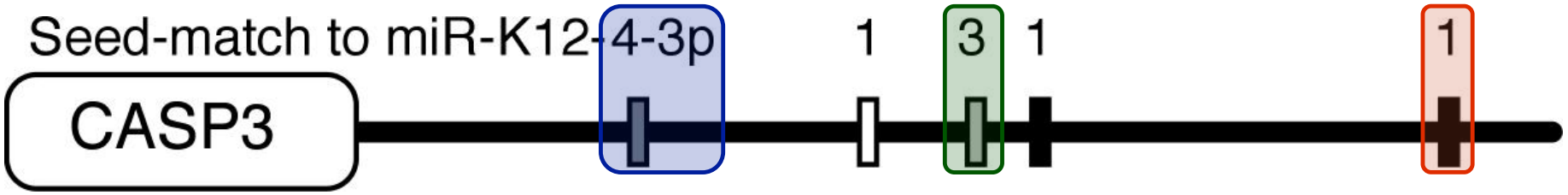
# KSHV miRNAs K12-1, 3 & 4 target CASP3



# Mutagenesis of miRNAs binding sites

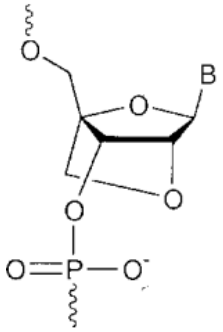


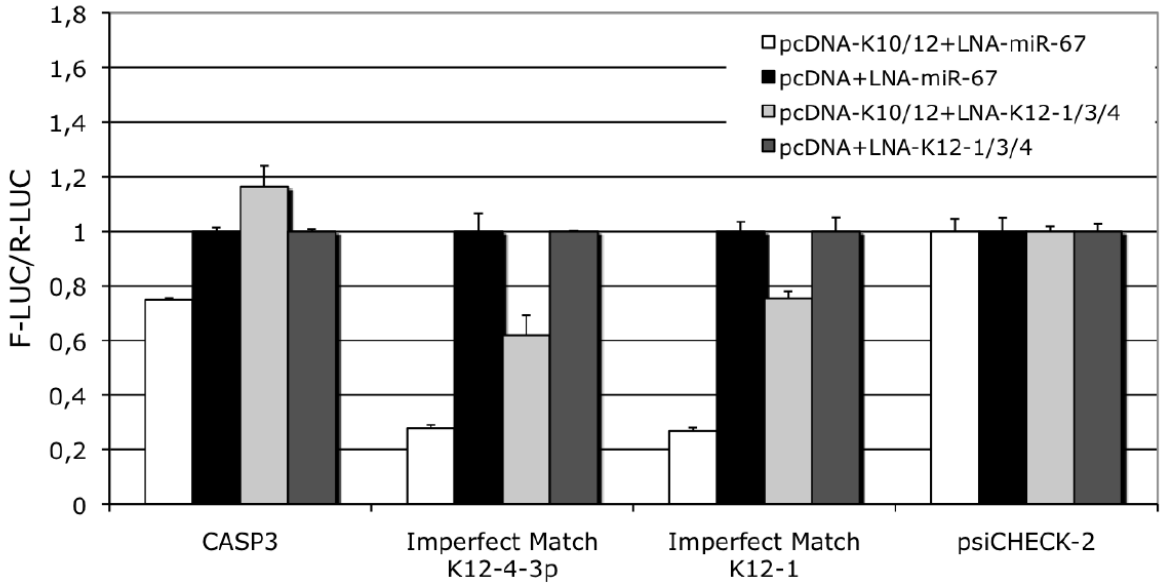



# Blocking KSHV miRNAs in KSHV infected BC3 cells

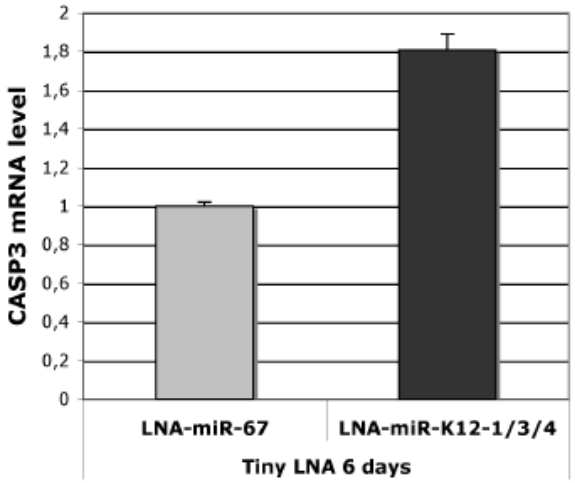
**Locked Nucleic Acid antisense oligo (tiny LNA)**

AATGTCCT
||||||||
5′ AUUACAGGAAACUGGGGUGUAAGC 3′

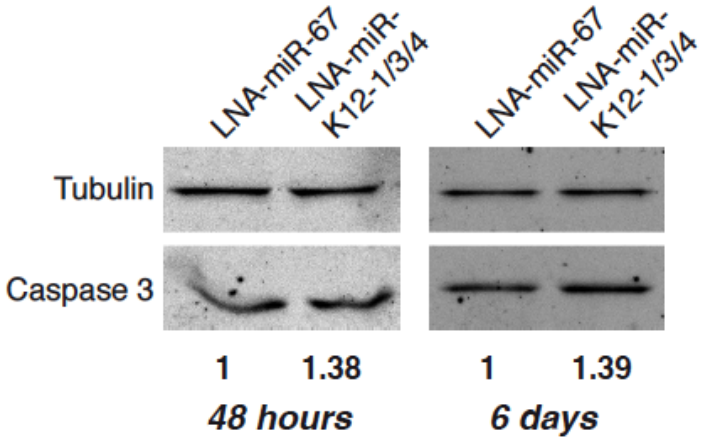



Casp3 mRNA

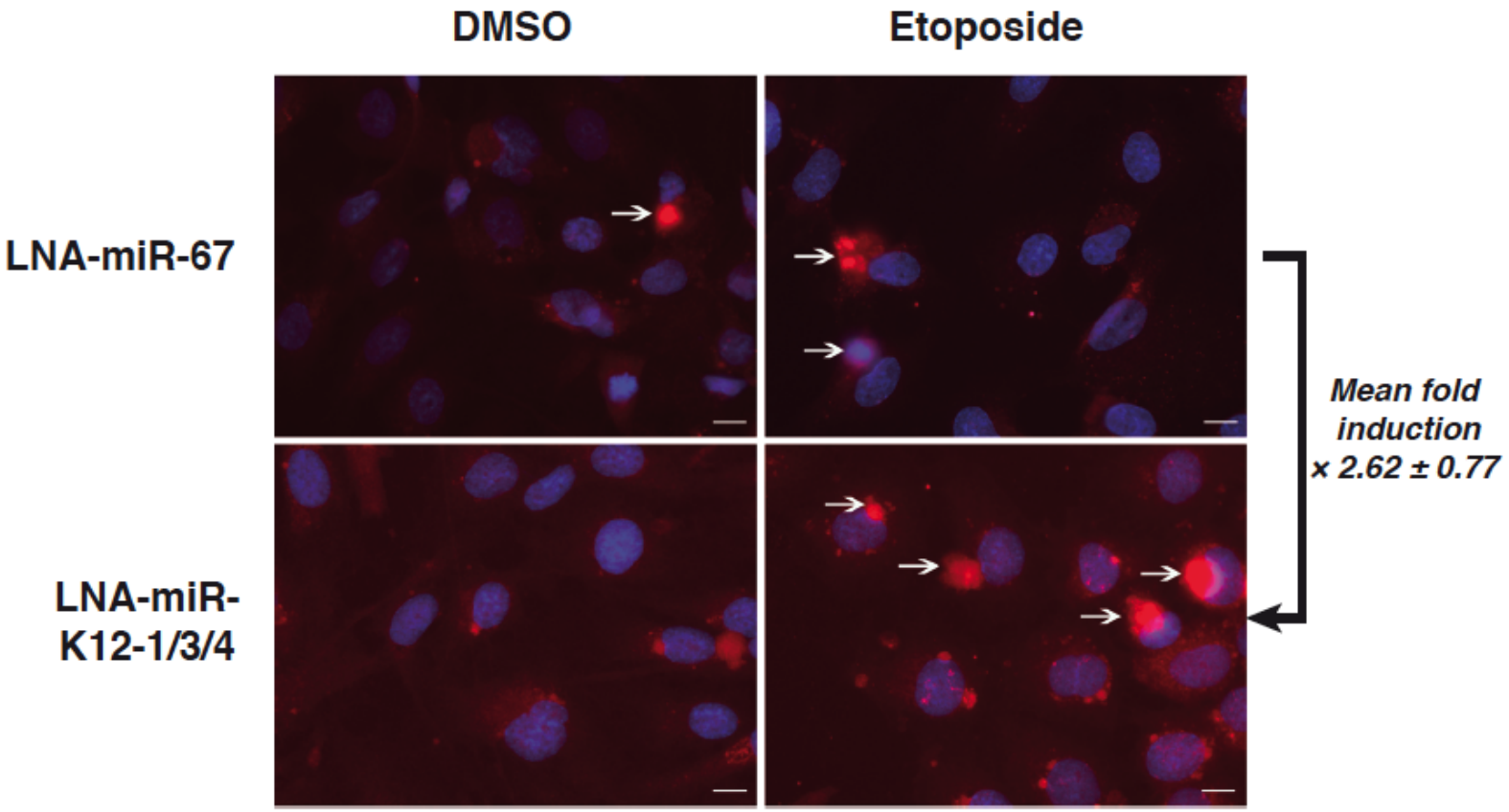



Casp3 protein



# Blocking KSHV miRNAs in KSHV+ iLECs cells

TUNEL measurement after Etoposide treatment



# Summary

- Viruses interact extensively with the miRNA machinery in mammals
- They modify the expression profile of cellular miRNAs
- DNA viruses usurp the host machinery by expressing their own miRNAs
- Apoptosis is a target of viral miRNAs during KSHV infection
- Casp3 validated as a KSHV miRNA target by luciferase assay
- Site-directed mutagenesis identifies 3 binding sites for miR-K12-1, 3 and 4-3p
- Endogenous Casp3 mRNA and protein are regulated by KSHV miRNAs
- Blocking miR-K12-1, 3 and 4 in KSHV-infected cells results in increase in Casp3 levels and in apoptosis

# Acknowledgements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