

Viruses and microRNAs

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Virally encoded pre-microRNAs

DNA Virus Families		Number of Known miRNAs
Alpha Herpesviruses	Herpes Simplex Virus Type 1	9
	Herpes Simplex Virus Type 2	9
Beta Herpesviruses	Human Cytomegalovirus	11
Gamma Herpesviruses	Kaposi's Sarcoma-Associated Herpesvirus	12
	Epstein-Barr Virus	25
	Rhesus Lymphocryptovirus	35
	Rhesus Rhadinovirus	15
Polyomaviruses	SV40, JC, BK etc	1
Adenoviruses	Human Adenovirus	2
Papillomaviruses	Human Papillomavirus	0
RNA Virus Families		
Retroviruses	Human Immunodeficiency Virus type 1	0
	Human T-Cell Leukemia Virus Type I	0
Flaviviruses	Hepatitis C Virus	0
	West Nile Virus	0

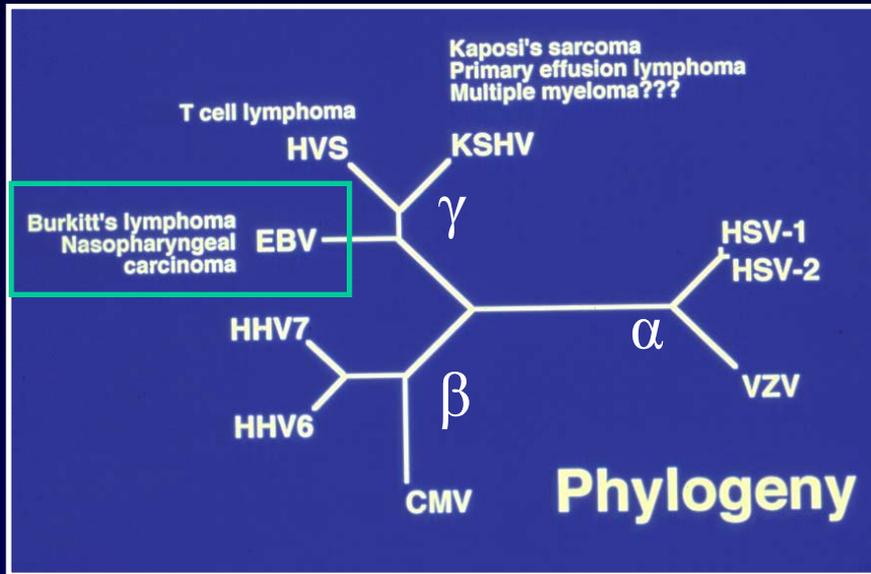
Viruses lacking known miRNAs

- The nuclear DNA viruses Hepatitis B Virus and Human Papilloma Virus and the cytoplasmic DNA virus Cowpox
- The nuclear RNA viruses influenza A virus, HIV-1 and HTLV-I.
- Several cytoplasmic RNA viruses including HCV, YFV, WNV, Dengue Virus, Polio Virus, Sendai Virus and Semliki Forest Virus.

EBV, microRNAs and Cancer

- **EBV is an important human transforming virus, with a clear etiological relationship to several forms of B-cell lymphoma as well as Nasopharyngeal Carcinoma (NPC).**
- **EBV can immortalize primary human B-cells in culture to give lymphoblastoid cell lines (LCLs).**
- **In infected LCLs, EBV expresses several viral miRNAs and EBV also strongly induces the expression of specific cellular miRNAs.**

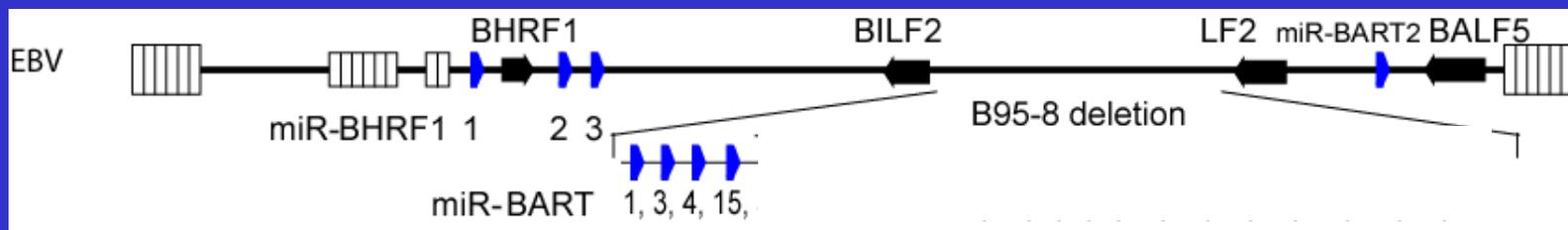
Primate Herpesviruses



>90% of adults are EBV+
infection is lifelong

1st human virus linked to cancer

1st virus shown to express miRNAs



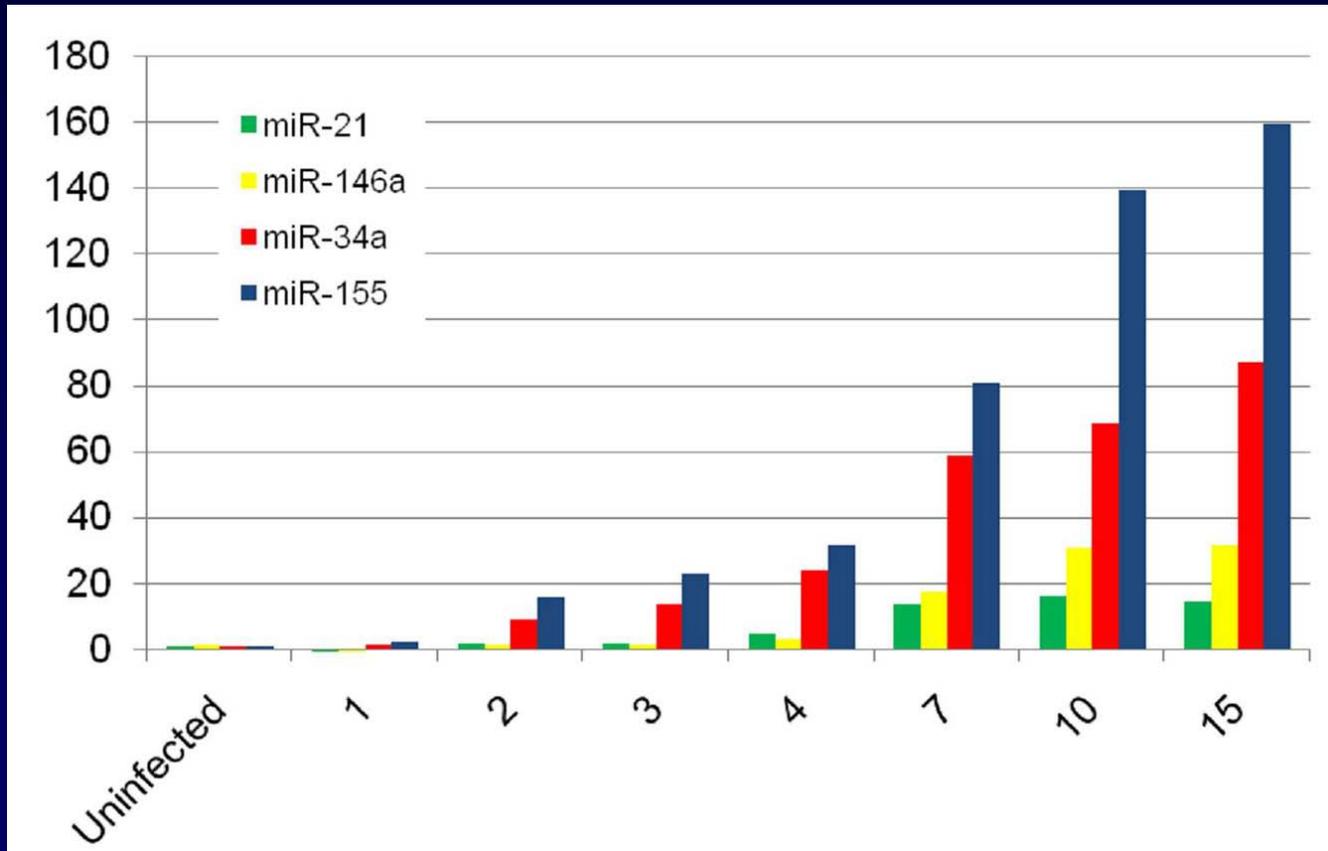
EBV Latency Programs

Type I	Type II	Type III
EBNA1	EBNA1	6 EBNAs
	LMP1	LMP1
	LMP2A/B	LMP2A/B
		<i>LCLs in vitro</i>
BARTs +	BARTs +++	BARTs +
		BHRF1s ++

EBV immortalizes B cells in vitro.

Lymphoblastoid cell lines (LCLs) are a model for EBV-induced lymphoproliferative diseases.

Induction of specific miRNAs after EBV infection of B cells



Why is miR-155 of potential importance?

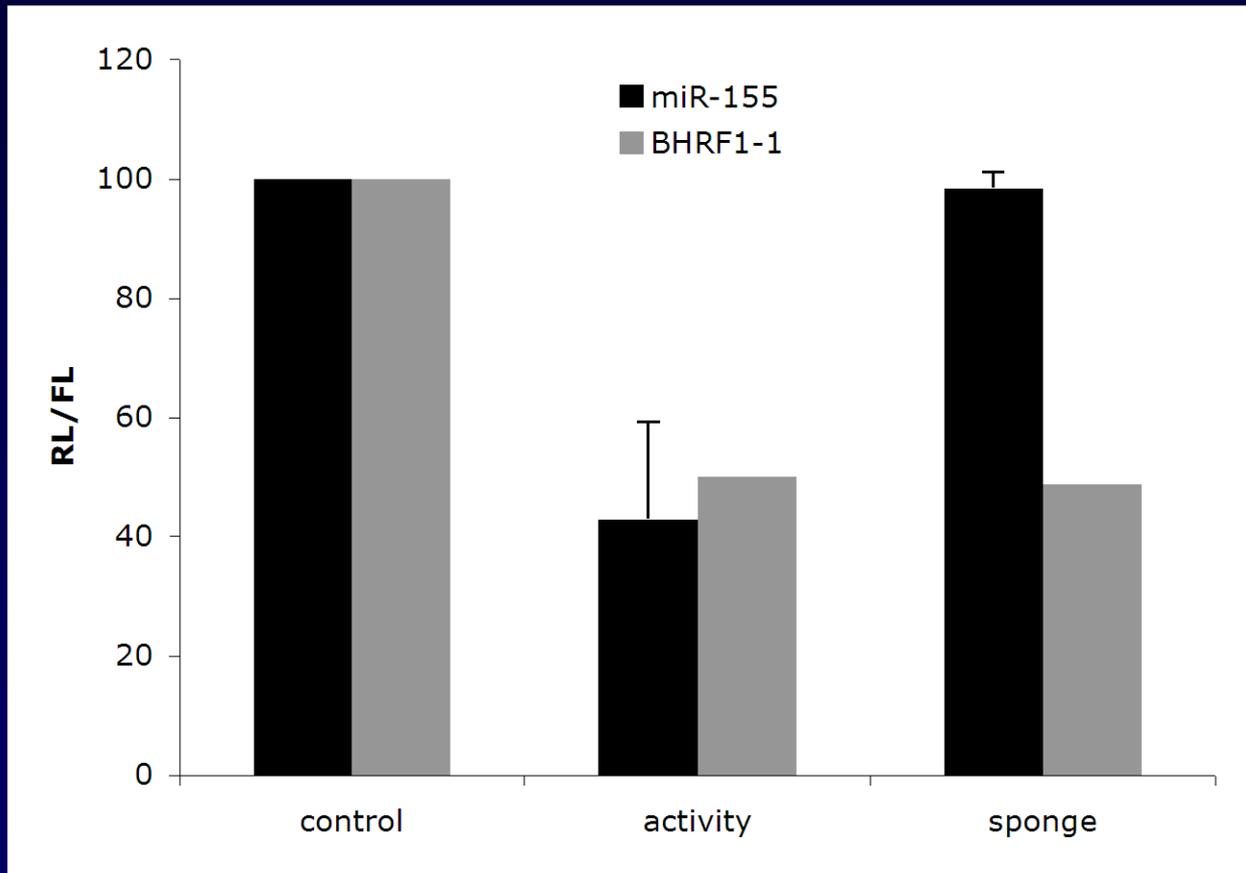
- miR-155 expression is induced in mature B and T cells by activation and miR-155(-/-) knock-out mice show impaired immune function.
- Both KSHV and the oncogenic avian herpesvirus MDV encode orthologs of cellular miR-155.
- miR-155 overexpression is etiologically linked to cancer:
 - 1) pri-miR-155 is a common integration site in ALV-induced B cell lymphomas
 - 2) pri-miR-155 transgenic mice develop B cell tumors
 - 3) miR-155 overexpression is observed in most lymphomas

KSHV encodes a miRNA, miR-K11, that functions as a homolog of cellular miR-155

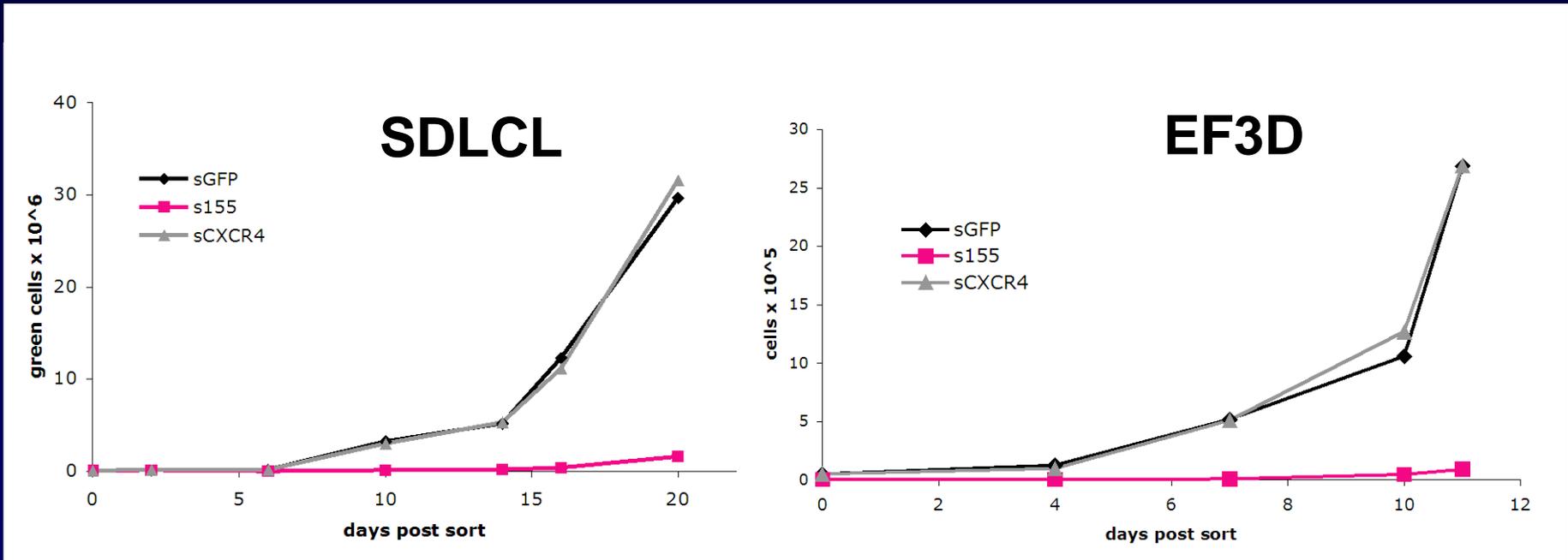
- KSHV miR-K11 and cellular miR-155 share an identical seed region, and miR-K11 and miR-155 target a similar set of mRNAs (Gottwein et al, 2007; Skalsky et al, 2007).



miR-155 can be effectively sponged in EBV infected B-cells

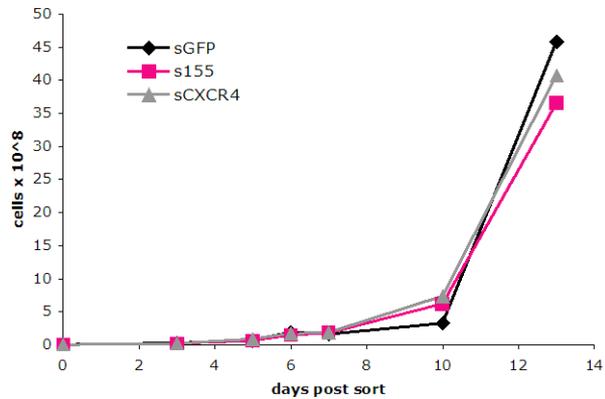


miR-155 is important for LCL growth

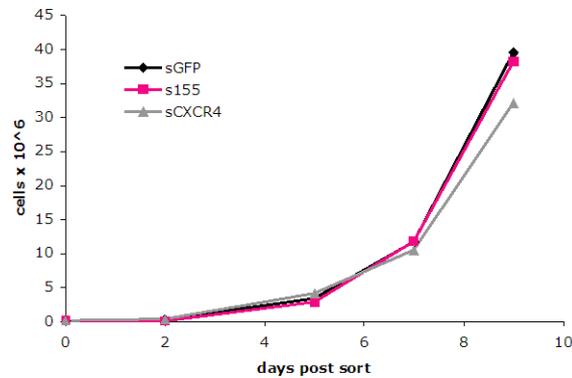


miR-155 is not important for growth of Burkitt's lymphoma cells

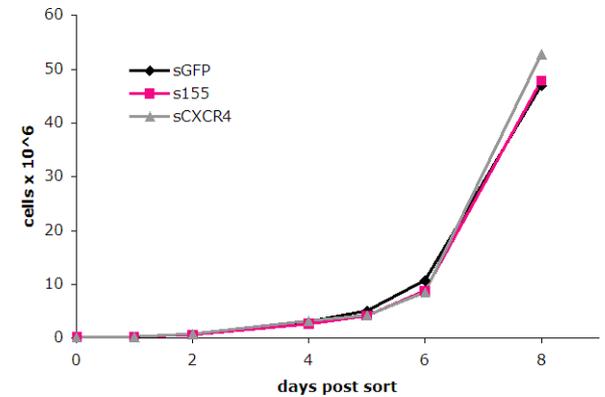
Namalwa



Raji

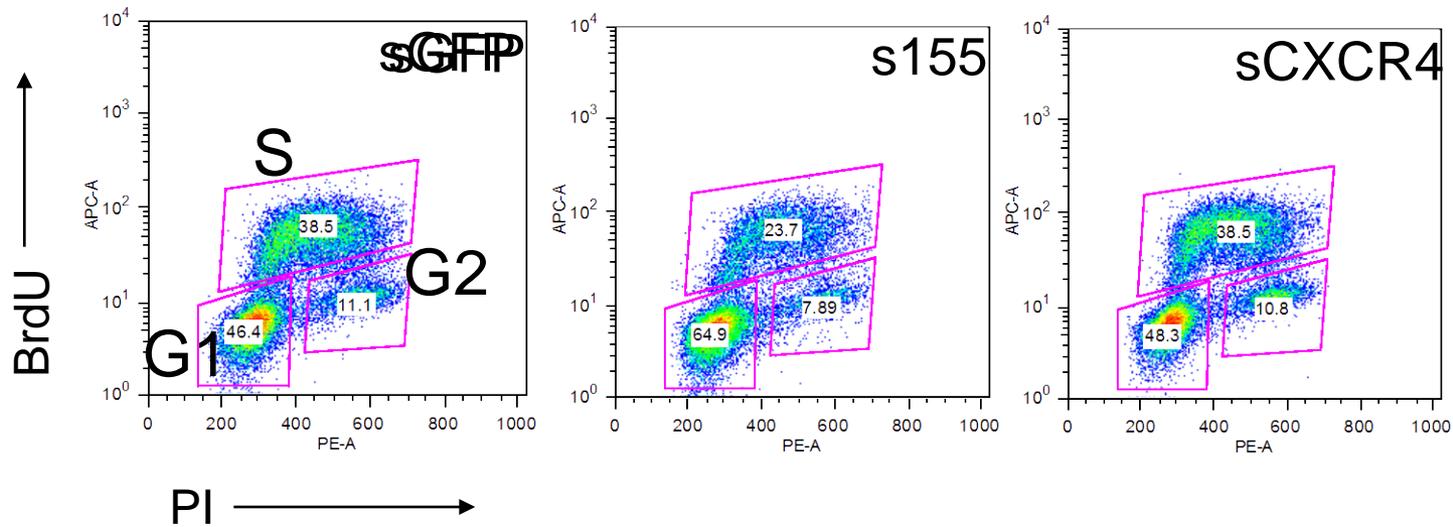


BJAB

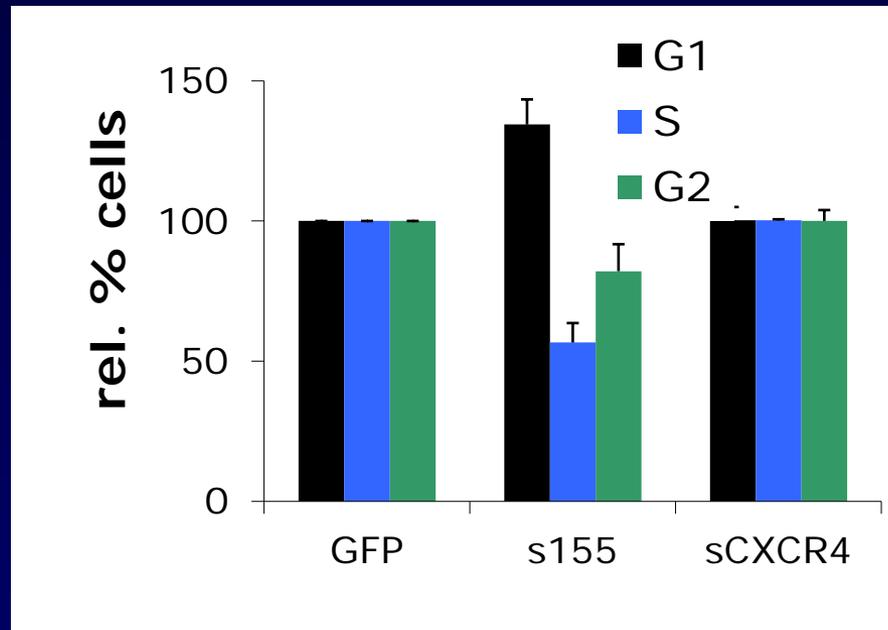


Cell cycle analysis of LCLs

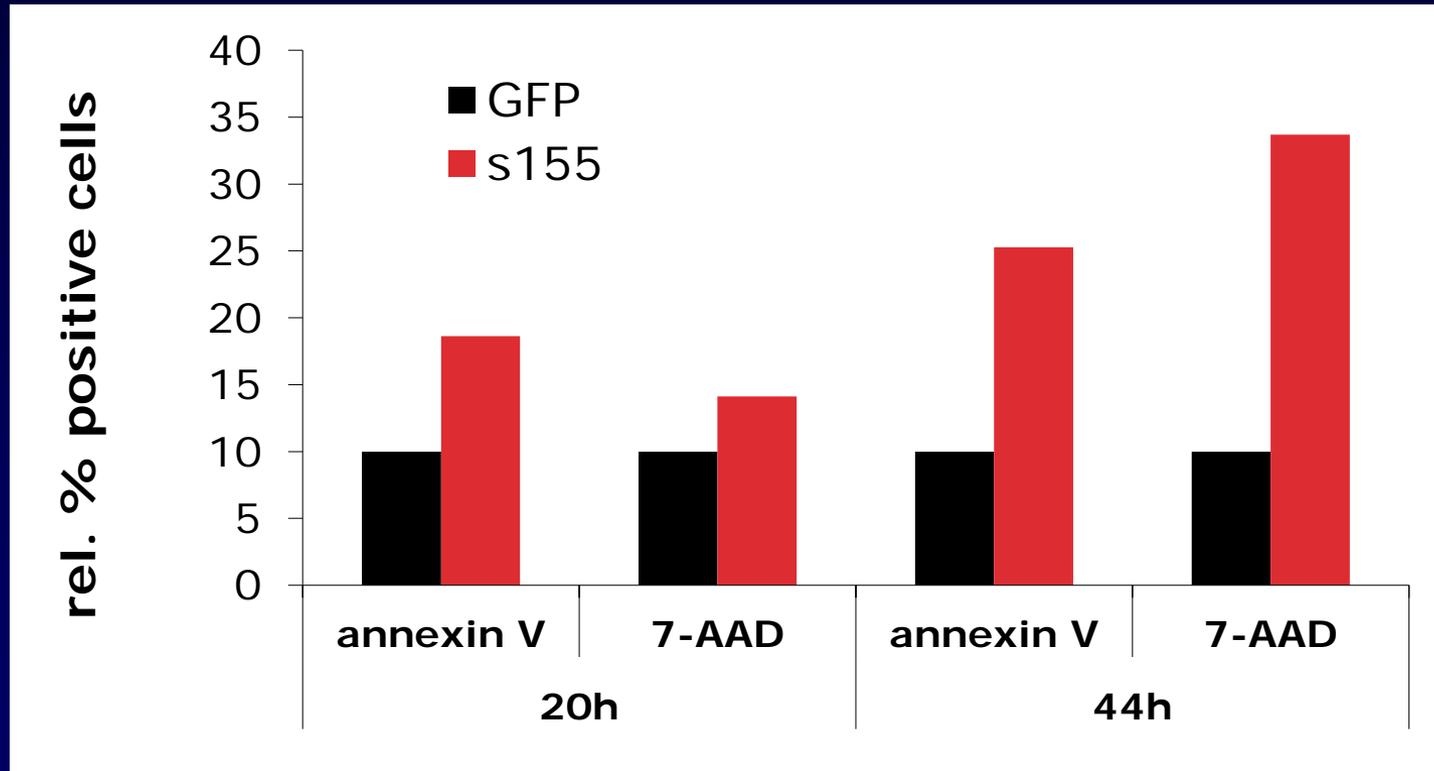
BrdU incorporation



LCLs lacking miR-155 function arrest in G1



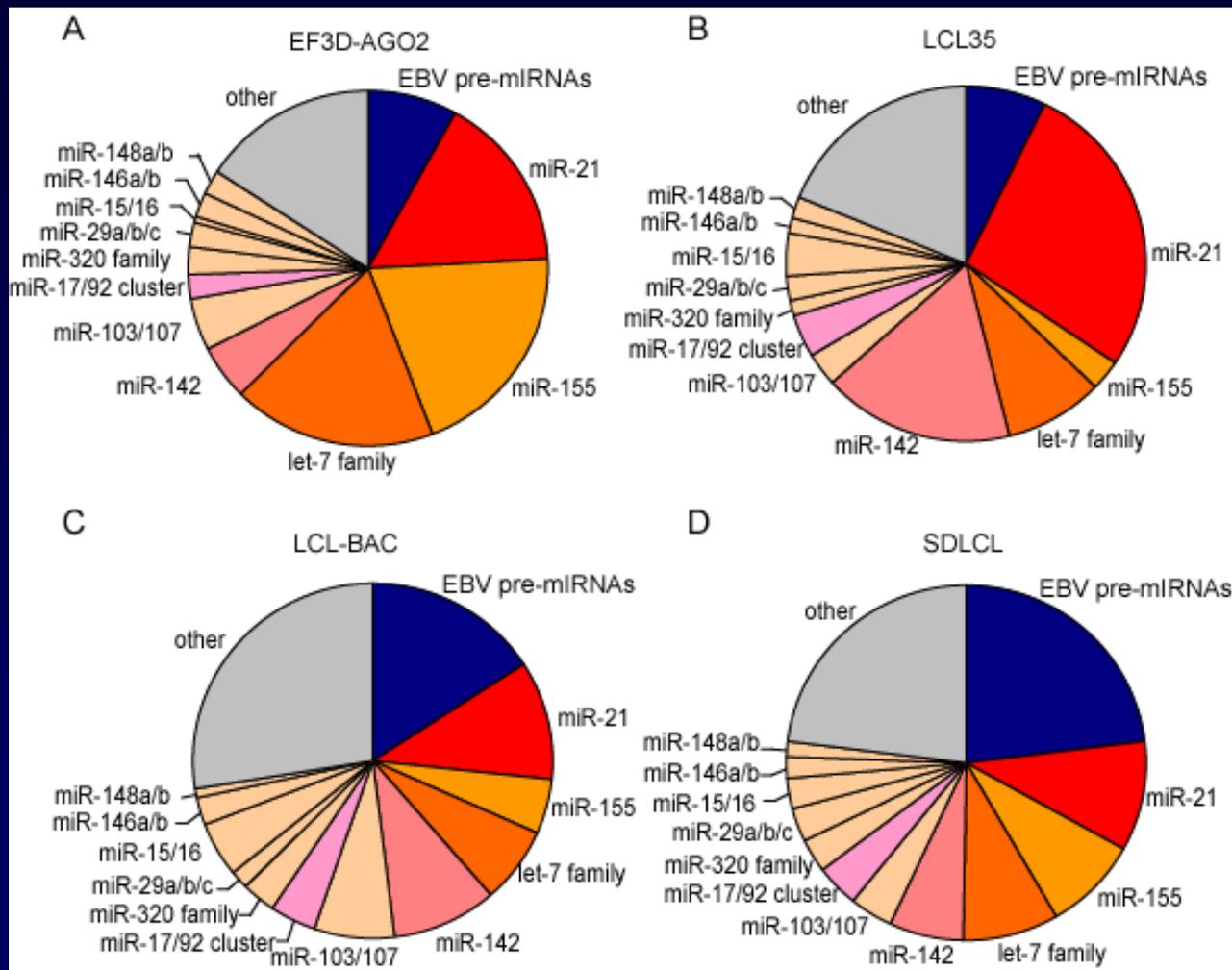
LCLs lacking miR-155 function undergo apoptosis



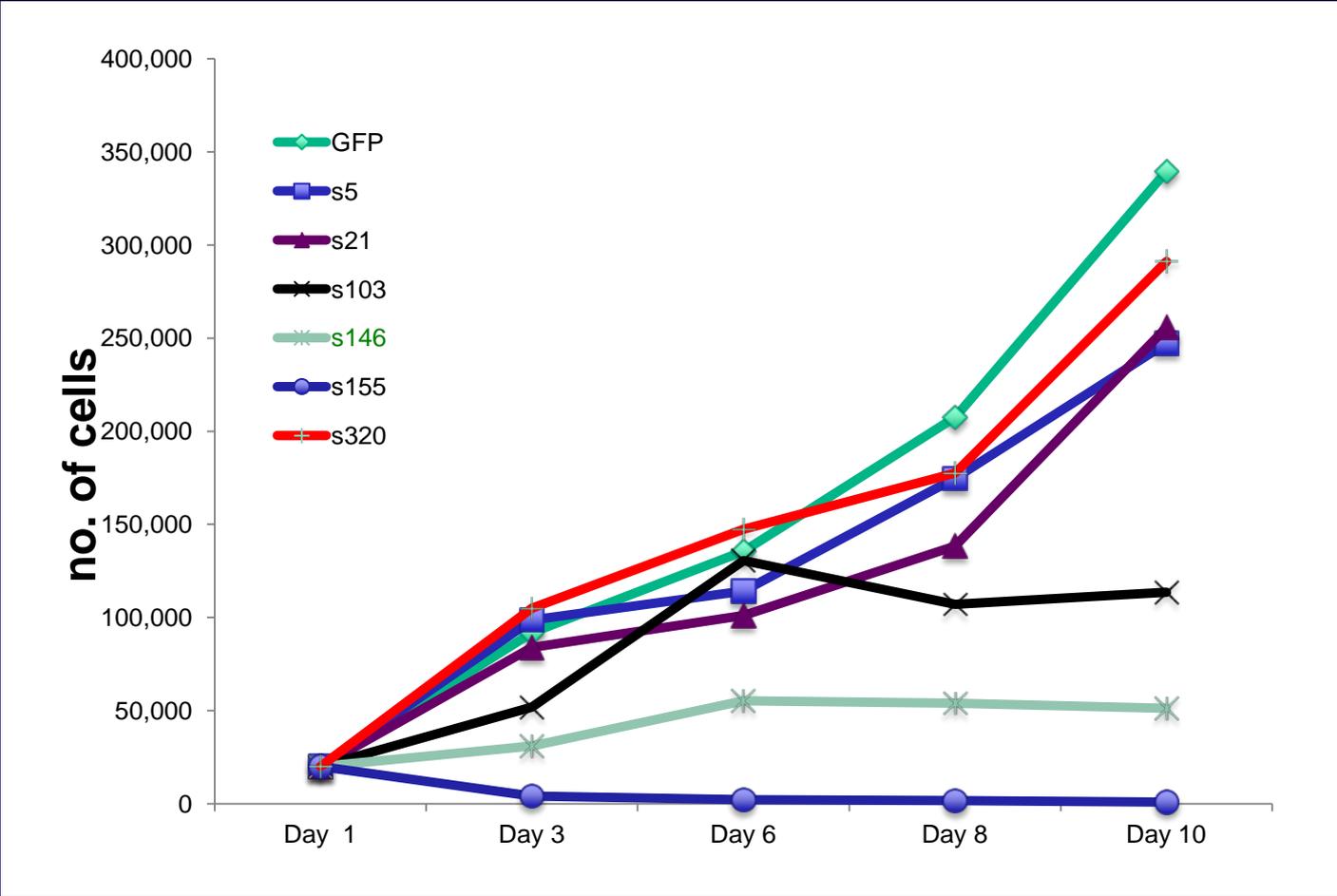
Cellular miR-155 plays a key role in B-cell transformation by EBV

- **High level expression of cellular miR-155 is critical for the growth and survival of EBV-infected LCLs.**
- **LCLs lacking miRNA-155 show enhanced levels of apoptosis and tend to arrest in G1.**
- **Are other miRNAs expressed in LCLs, of either cellular or viral origin, also important for cell growth and survival?**

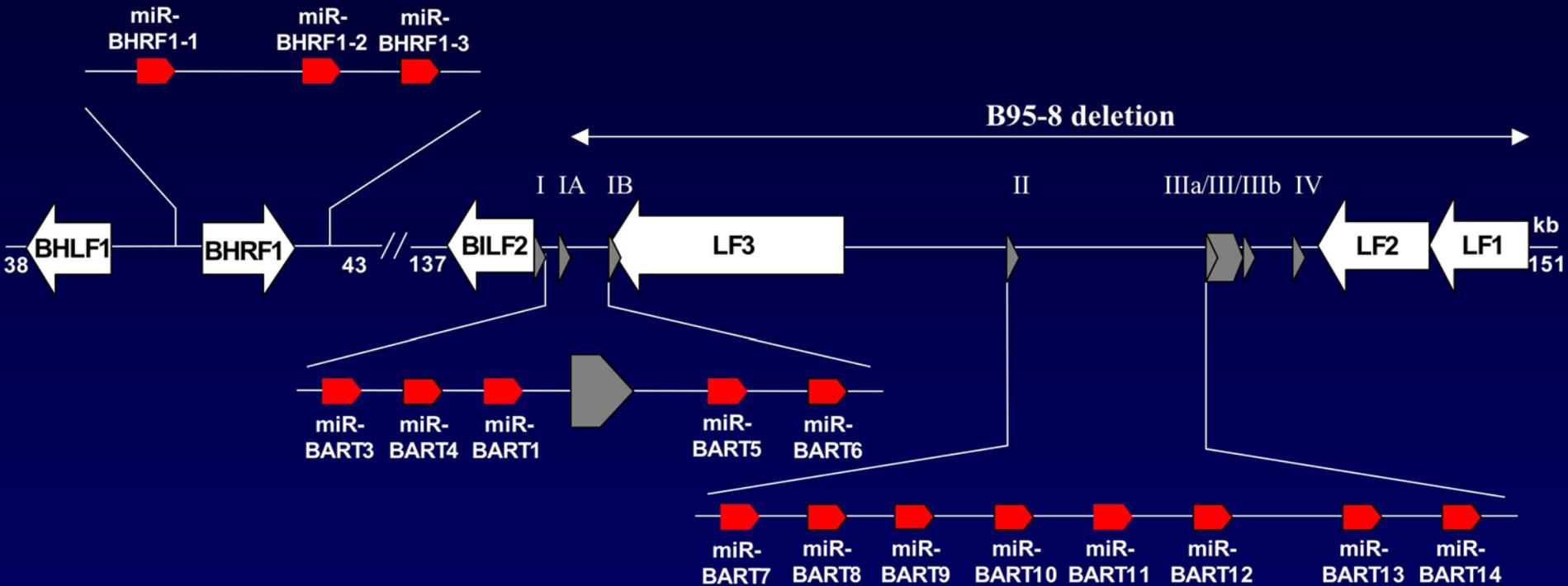
EBV miRNAs represent ~7-22% of all miRNAs detected in EBV-B95-8 LCLs



Knockdown of cellular miRNAs

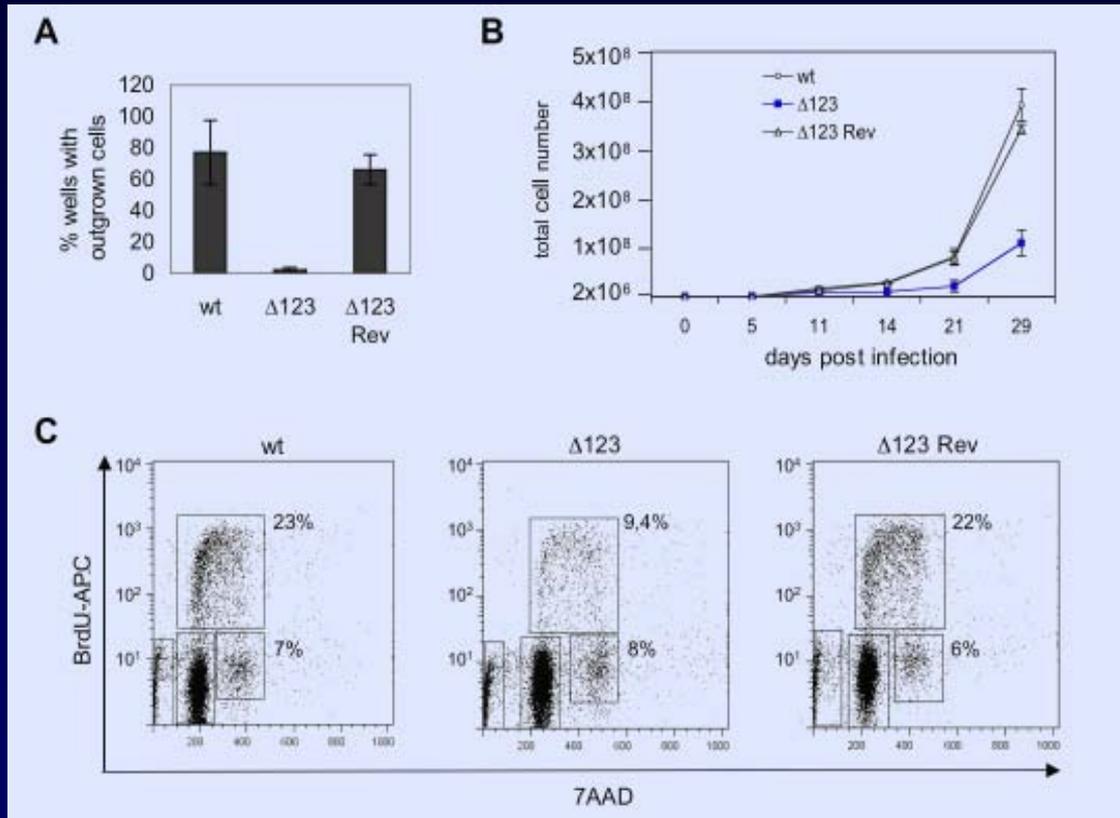


Localization of miRNA precursors within the EBV Genome

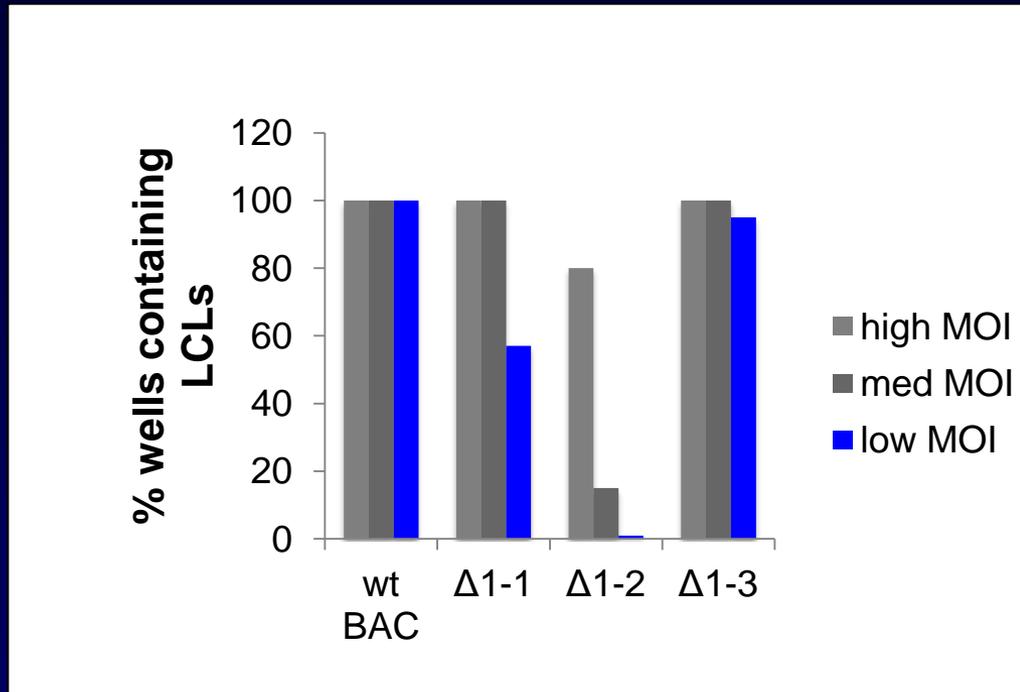


 other EBV genes
  BART Exons
  miRNA precursor

Viral miRNAs play a key role in B cell transformation by EBV



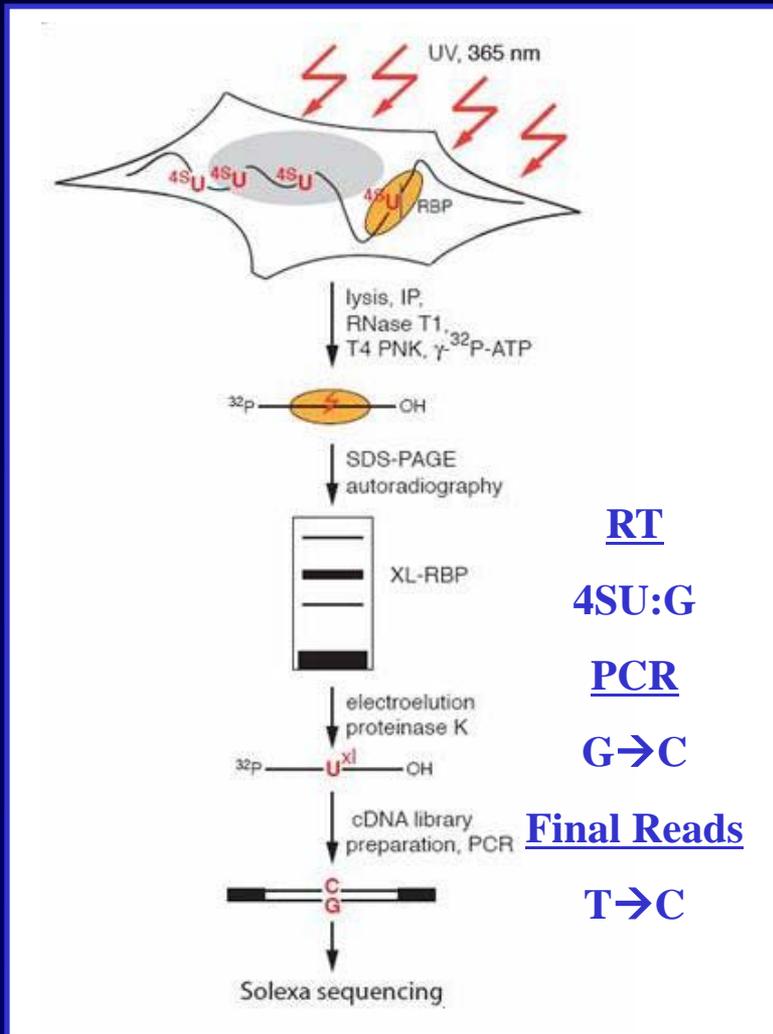
The EBV miR-BHRF1 miRNAs promote LCL outgrowth



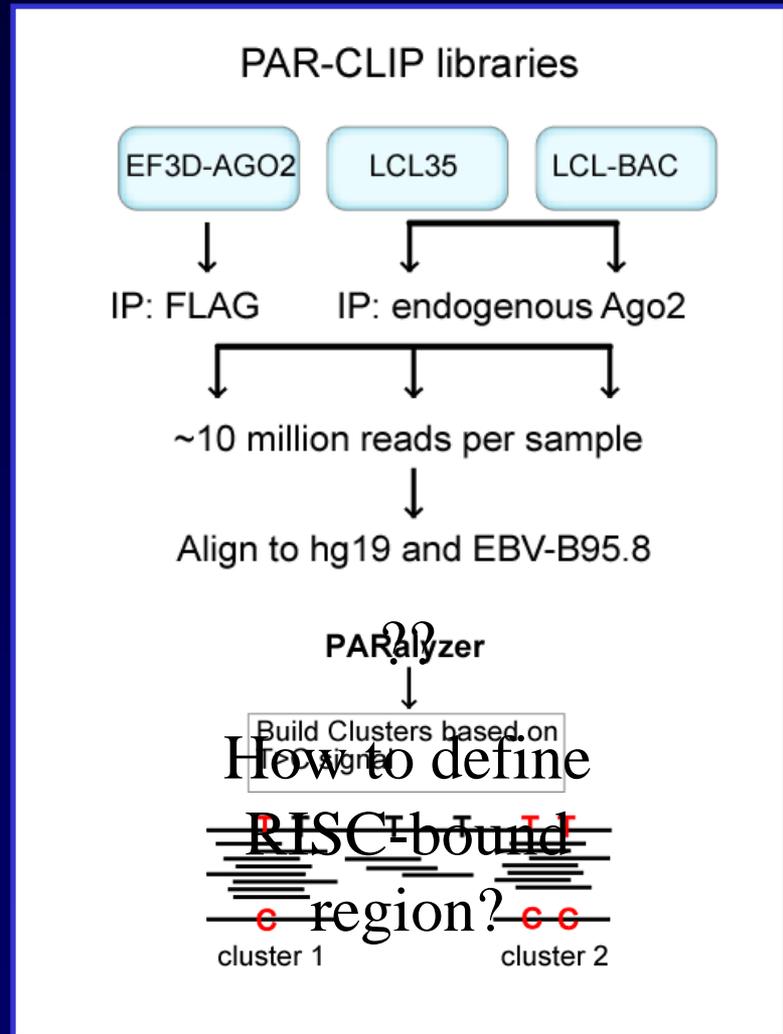
What are the functions of EBV miRNAs?

Objective: Comprehensively identify the mRNA targets of EBV miRNAs and EBV-induced cellular miRNAs to elucidate their roles in viral replication and pathogenesis

Photoactivatable-ribonucleoside enhanced cross-linking and immunoprecipitation (PAR-CLIP)



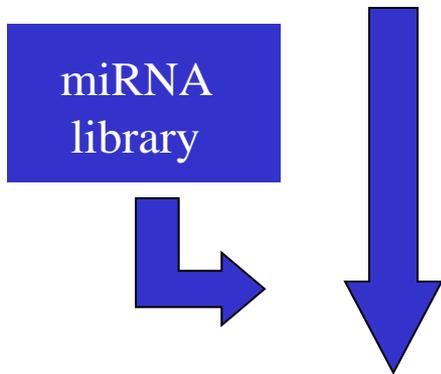
Hafner et al., Cell, 2010



Corcoran et al., Genome Biology, 2011

494 3'UTRs are putative EBV miRNA targets

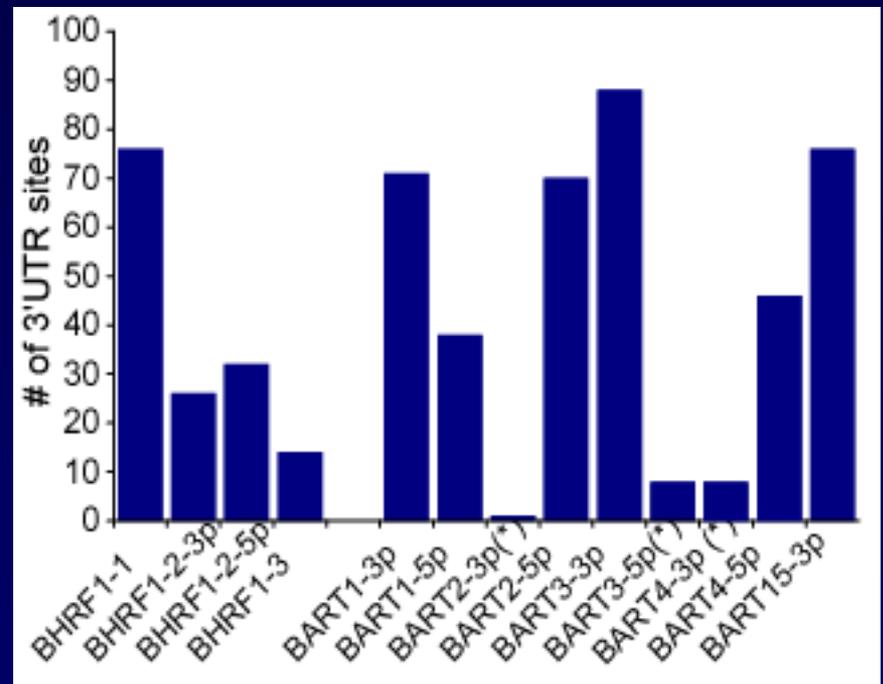
30,432 clusters in 3'UTRs
Assign to a miRNA?



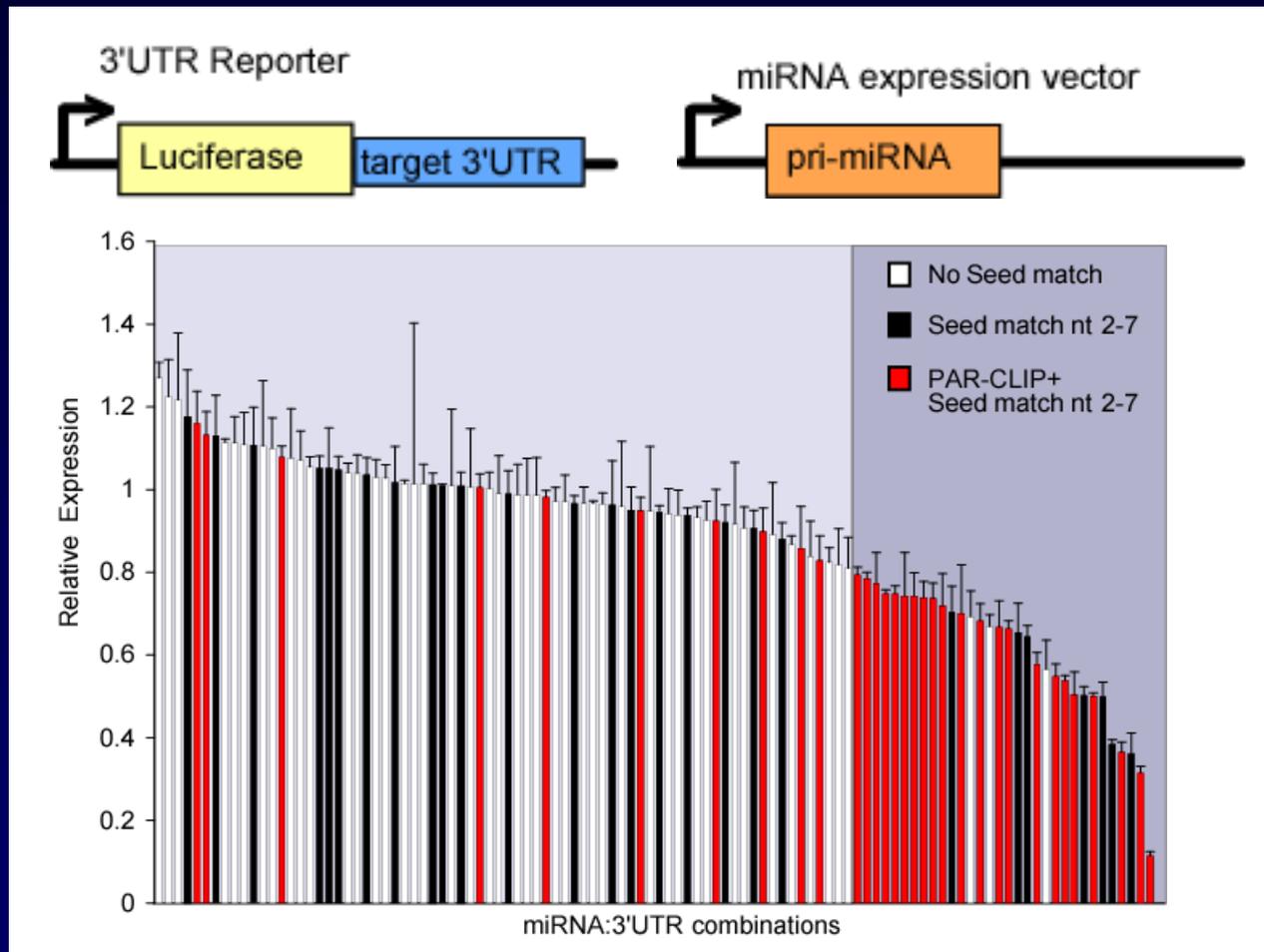
23,240 clusters (76%) have a seed match to a miRNA

**Current miRNA targetome in LCLs:
3,492 genes**

Clusters with seed matches to EBV miRNAs



Validation of PAR-CLIP-identified miRNA interaction sites in target 3'UTRs

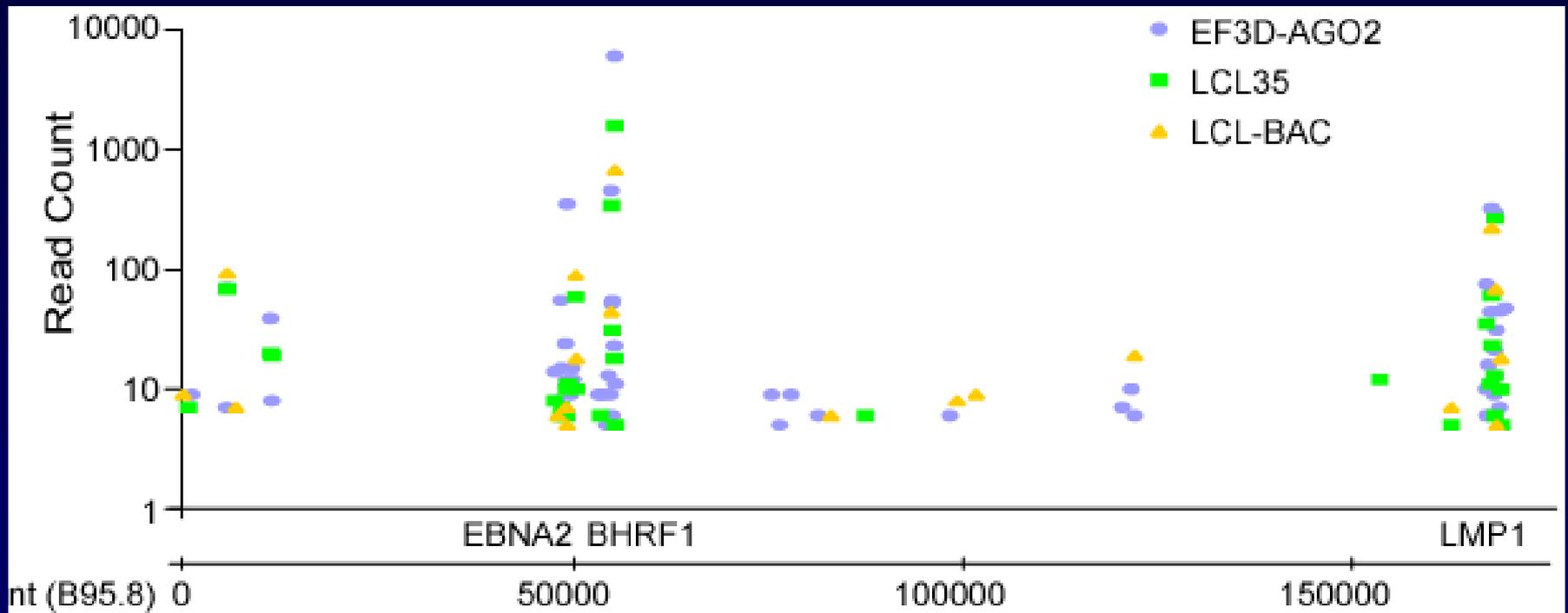


~70% of sites
can be
confirmed

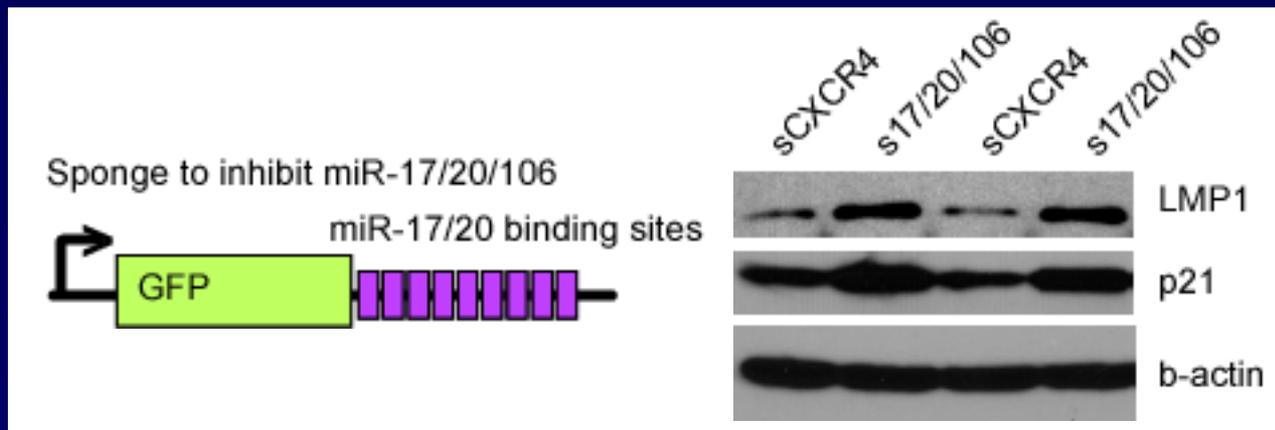
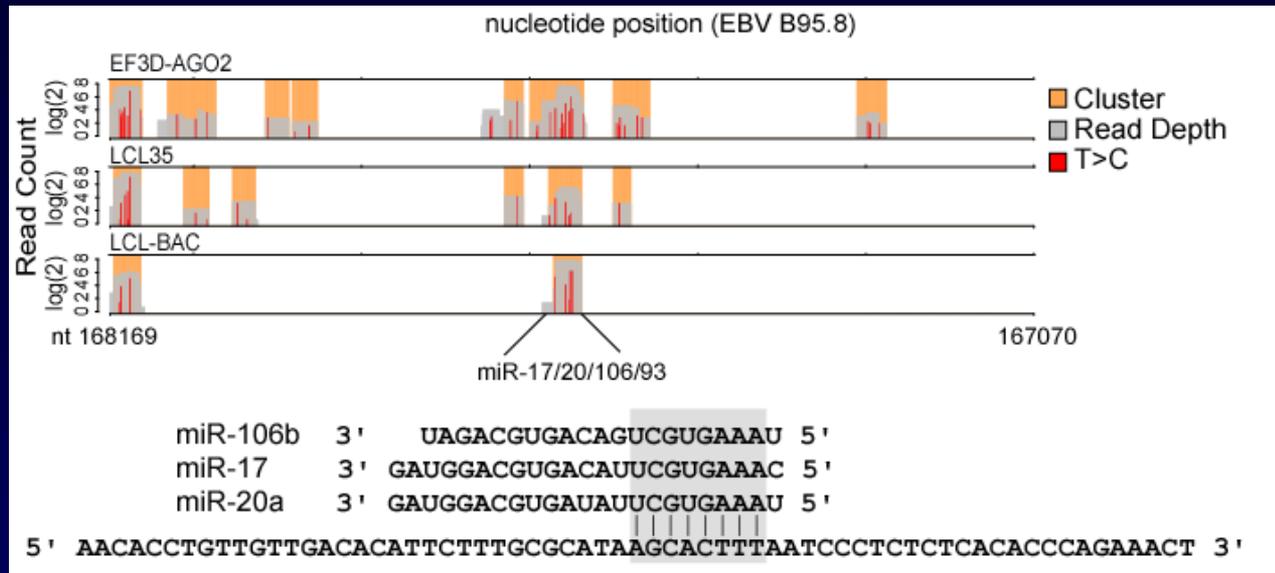
>100 miRNA:3'UTR combinations
(13 3'UTRs, 11 miRNAs)

Are viral transcripts targets of miRNAs?

Clusters aligning to EBV-B95.8

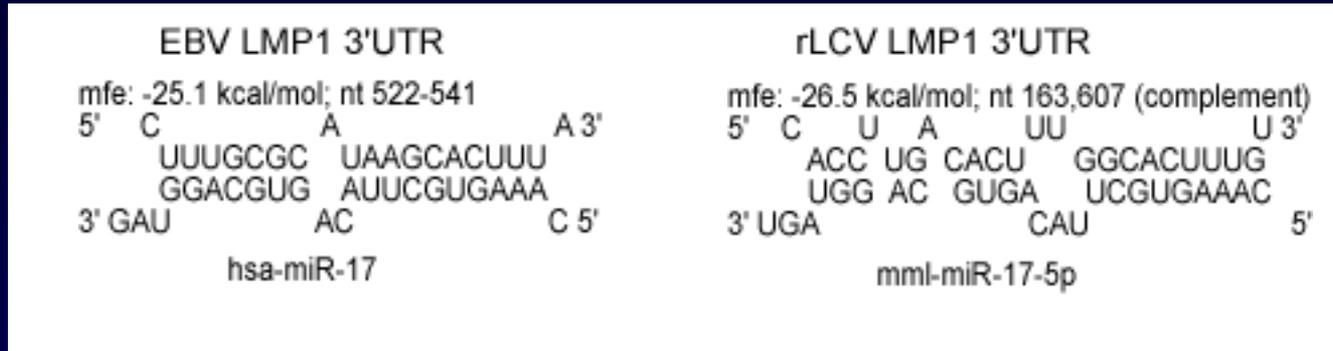


LMP1 is targeted by myc-regulated miR-17/20/106/93

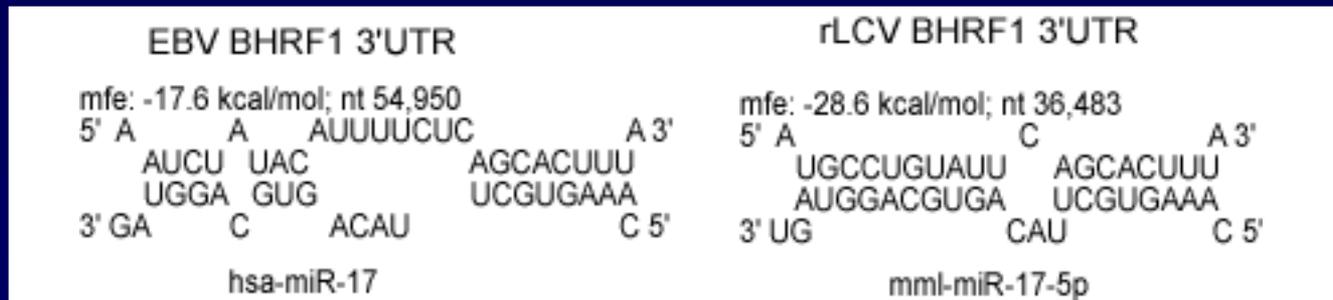


miR-17/20/106 binding sites are conserved in rLCV

LMP1



BHRF1

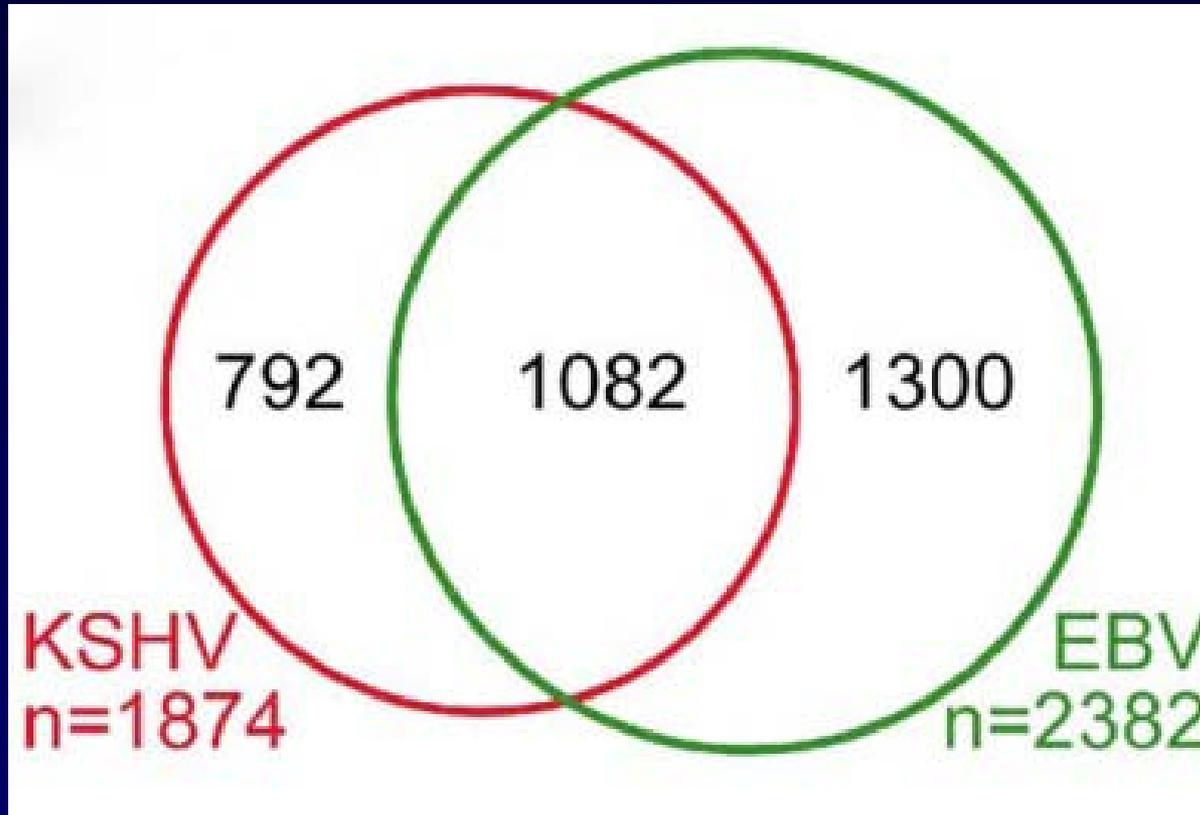


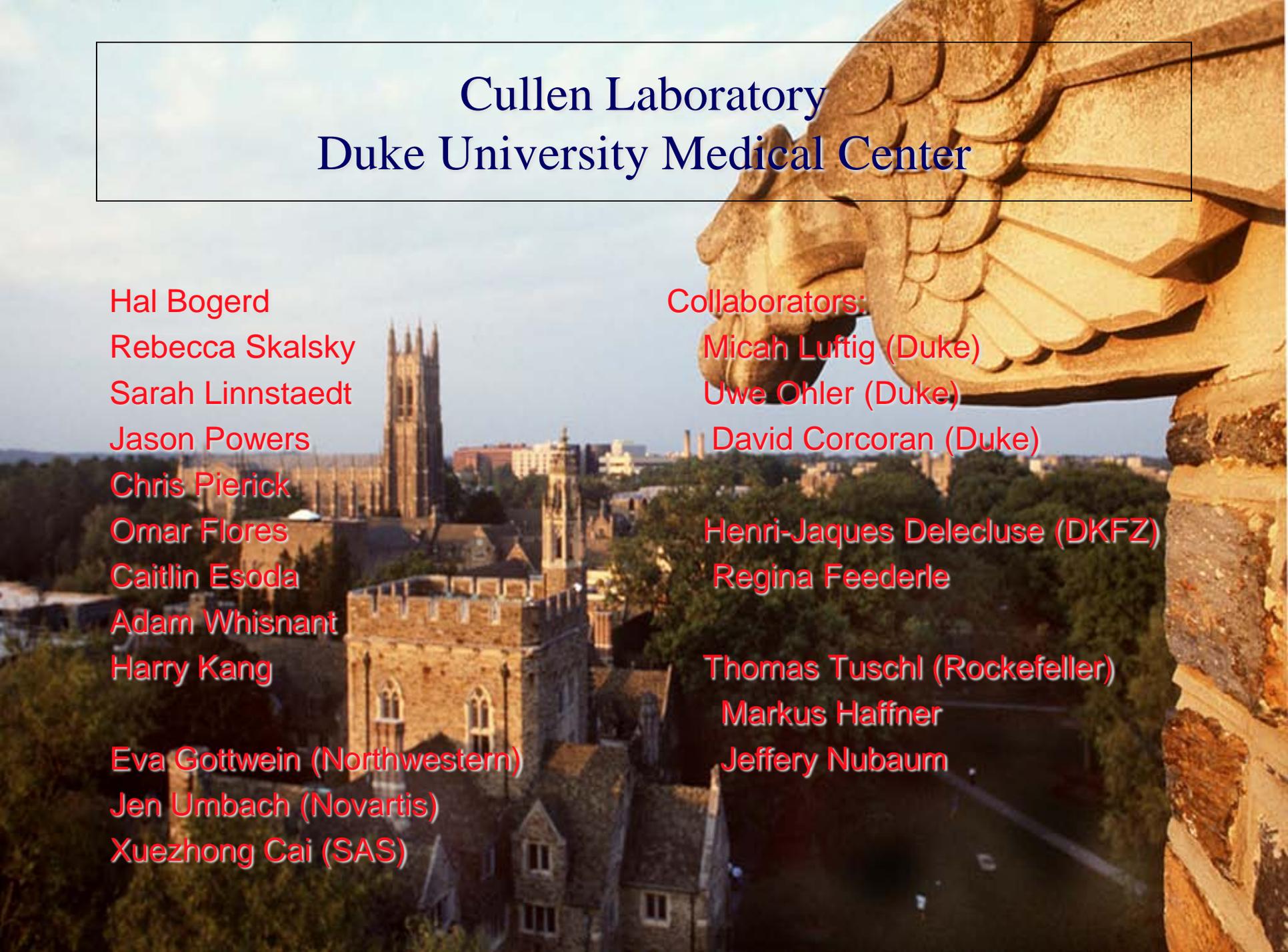
Ongoing: Exploring the interactions between myc-regulated miR-17/20/106 and EBV transcripts

Conclusions

- **miRNA targetome in EBV+ LCLs: 5,711 genes**
- **25% (1,406 3'UTRs) are putative EBV miRNA targets**
- **PANTHER analysis: Targets of EBV miRNAs function in multiple cellular processes that are directly relevant to EBV infection (cell survival and proliferation, immune evasion)**
- **So far confirmed 15 mRNAs targets of EBV miRNAs**
 - immunomodulatory roles (PML bodies, C-type lectin receptors, NK ligands) and many are also targets for KSHV miRNAs.
- **Next steps:**
 - More indicator assays and Westerns
 - Phenotypic assays

PAR-CLIP analysis of EBV and KSHV miRNA targets in co-infected PELs





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