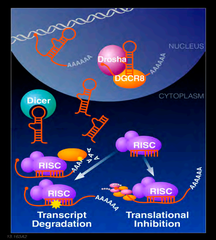
miR-155 Reinforces HIV Latency by Downregulating TRIM32

Debbie S. Ruelas[1,2], Jonathan K. Chan[1,2], Eugene Oh, Amy J. Heidersbach[1], Andrew M. Hebbeler[1,2], Leonard Chavez[1,2], Eric Verdin[1,2], Michael Rape[3], Warner C. Greene[1,2]

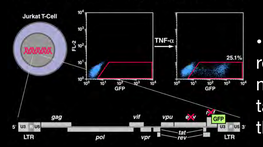
[1]University of California, San Francisco, [2]Gladstone Institute of Virology and Immunology, San Francisco, CA, [3]University of California, Berkeley

Introduction

- MicroRNAs are short RNAs of 19-25 nucleotides that control gene expression through translational repression or mRNA cleavage.
- J-Lat 5A8 cells are a cellular model of HIV latency that are capable of being induced by TCR stimulation.



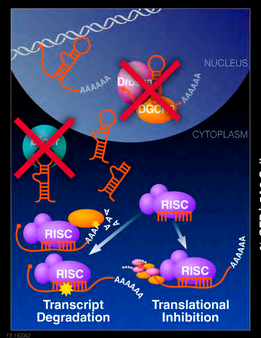
J-Lat Model of Post-integration HIV Latency

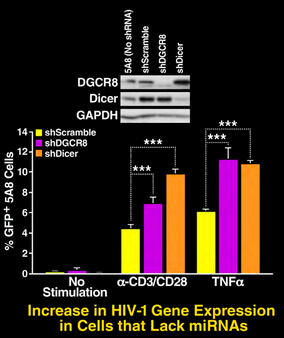


- Trim32 is an E3 ubiquitin ligase, responsible for attaching ubiquitin molecules to lysine residues of target proteins, in order to mark them for proteosomal degradation.

We are interested in how host miRNAs influence viral gene expression by targeting cellular host factors.

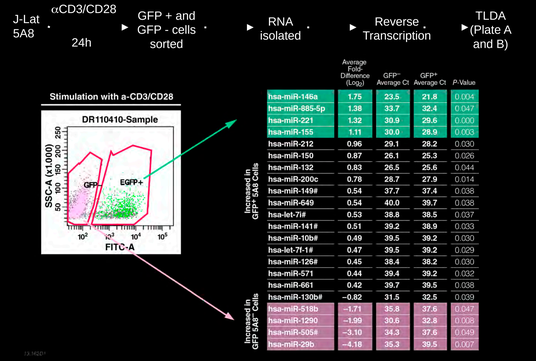
Knockdown of miRNA Machinery Results in Increased HIV-1 Reactivation





Increase in HIV-1 Gene Expression in Cells that Lack miRNAs

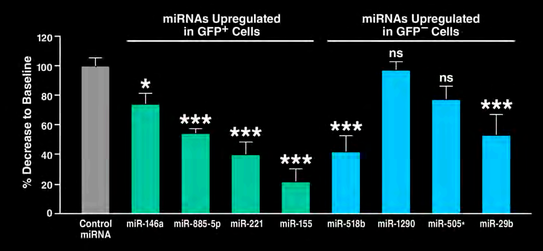
miR-155 is increased in GFP positive cells



	Average Fold-Difference (Log)	GFP⁻ Average Ct	GFP⁺ Average Ct	P-Value
hsa-miR-146a	1.75	23.5	21.8	0.004
hsa-miR-885-5p	1.38	33.7	32.4	0.047
hsa-miR-221	1.32	30.9	29.6	0.000
hsa-miR-155	1.11	30.0	28.9	0.003
hsa-miR-212	0.96	29.1	28.2	0.030
hsa-miR-150	0.87	26.1	25.3	0.026
hsa-miR-132	0.83	26.5	25.6	0.044
hsa-miR-200c	0.78	28.7	27.9	0.014
hsa-miR-149#	0.54	37.7	37.4	0.038
hsa-miR-649	0.54	40.0	39.7	0.038
hsa-let-7i#	0.53	38.8	38.5	0.037
hsa-miR-141#	0.51	39.2	38.9	0.033
hsa-miR-10b#	0.49	39.5	39.2	0.030
hsa-let-7f-1#	0.47	39.5	39.2	0.029
hsa-miR-126#	0.45	38.4	38.2	0.030
hsa-miR-571	0.44	39.4	39.2	0.032
hsa-miR-661	0.42	39.7	39.5	0.038
hsa-miR-130b#	−0.82	31.5	32.5	0.039
hsa-miR-619	−1.71	38.8	37.6	0.043
hsa-miR-1290	−1.99	30.6	32.8	0.006
hsa-miR-505#	−3.10	34.3	37.6	0.049
hsa-miR-29b	−4.16	35.3	39.5	0.007

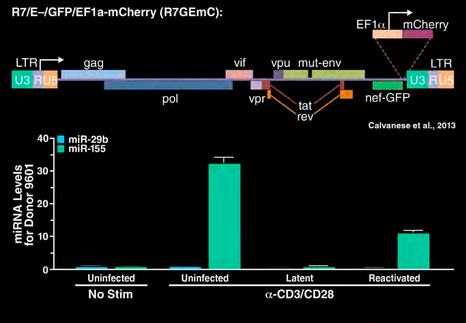
With Amy Heidersbach (Srivastava Lab)

Addition of Candidate miRNA Mimics into 5A8shDicer Cells Decreases the Level of Reactivation



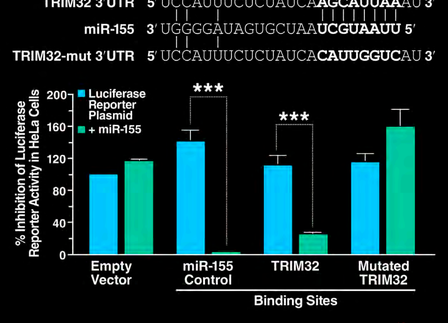
Most candidate miRNAs reinforce HIV-1 latency.

miR-155 Expression Is Increased in Reactivated Primary Cells



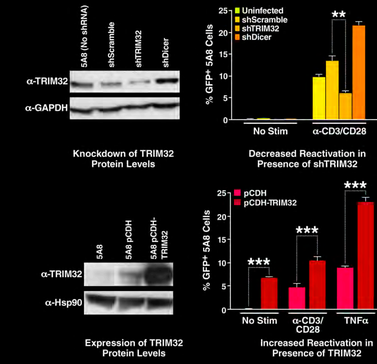
With Leonard Chavez (Verdin Lab)

miR-155 directly targets TRIM32

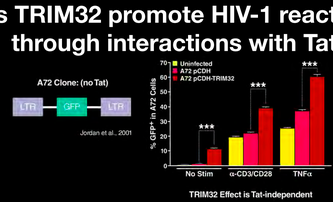


With Amy Heidersbach (Srivastava Lab)

TRIM32 Regulates HIV-1 Reactivation

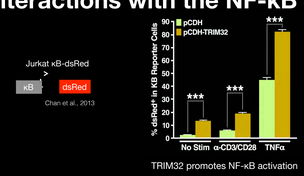


Does TRIM32 promote HIV-1 reactivation through interactions with Tat?



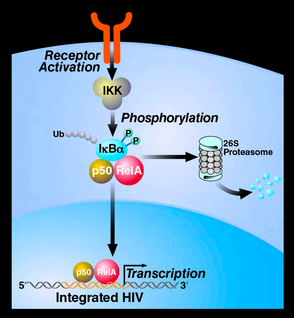
TRIM32 Effect is Tat-independent

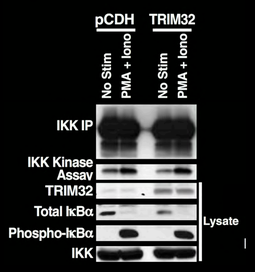
Does TRIM32 promote HIV-1 reactivation through interactions with the NF-κB pathway?



TRIM32 promotes NF-κB activation

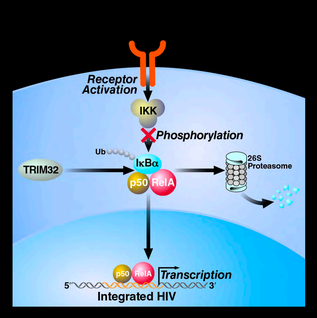
IKK Kinase Assay Shows No Increase in IKK Activity

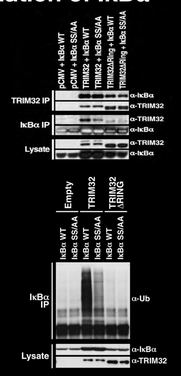




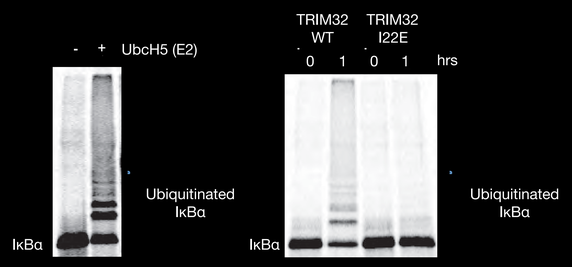
With Jonathan Chan (Greene Lab)

TRIM32 Activates the NF-κB Path via Binding and Ubiquitination of IκBα





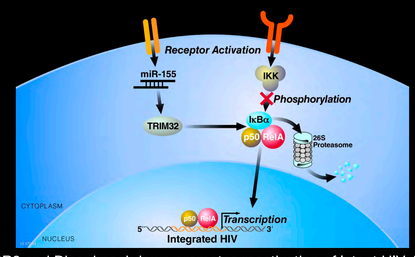
In vitro Ubiquitination Assay Shows that TRIM32 Directly Ubiquitinates IκBα



Wild type TRIM32, but not TRIM32 I22E, directly ubiquitinates IκBα.

With Eugene Oh (Rape Lab)

HIV-1 Latency Regulation by miR-155 and TRIM32



- DGCR8 and Dicer knockdown promotes reactivation of latent HIV-1
- miR-155 promotes reestablishment of HIV latency in activated T cells
- miR-155 suppresses expression of TRIM32, an HIV activator
- TRIM32 ubiquitinates IκBα promoting IKK-independent activation of NF-κB