VIRUSES SHAPE THE NUCLEAR ORGANIZATION FOR ONCOGENESIS

Yegor Vassetzky, CNRS UMR8126

UNIVERSITÉ PARIS SUD
FACULTÉ DE MÉDECINE
cnrs
GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS
Signalisation Noyaux Innovations en Cancérologie
UMR 8126

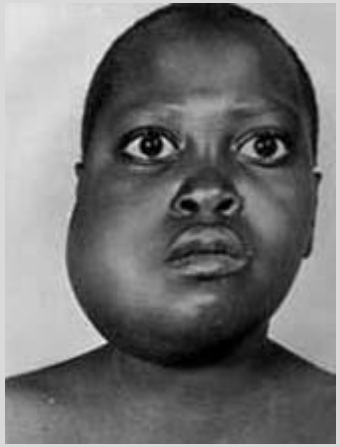
VIRAL THEORY OF CANCER: UPS AND DOWNS

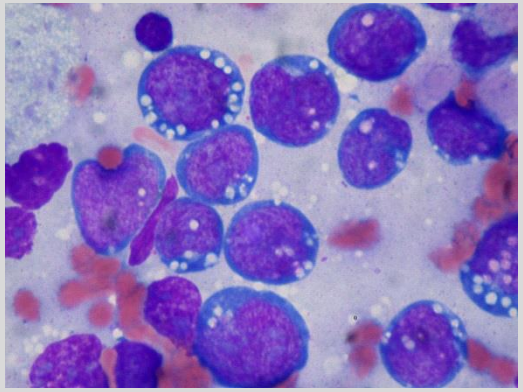
Peyton Rouss
1911: discovery of RSV

1966: Nobel Prize

Denis Burkitt
1957: discovery of Burkitt's lymphoma

Anthony Epstein **Yvonne Barr**
1964: discovery of EBV in Burkitt's lymphoma samples



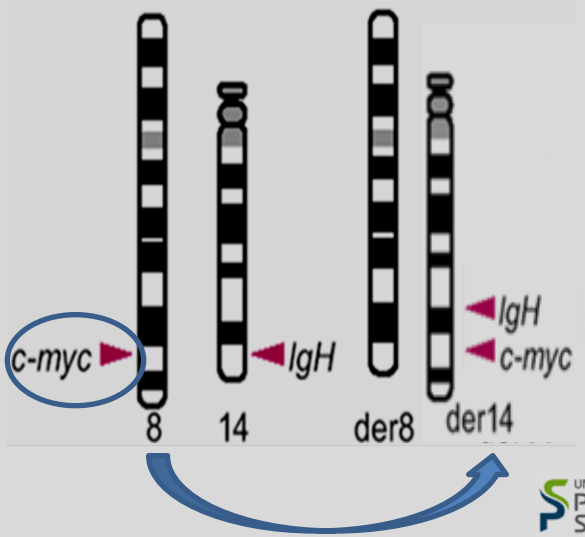


BURKITT LYMPHOMA

→A non-Hodgkin Lymphoma

→ Three forms:
- → An endemic form in Afrtica is 100% associated with EBV
- → A sporadic form in Europe and North America, rare and non-associated with EBV
- → **A form associated with HIV is frequent in Europe and the USA; it is found in up to 2% (!) of AIDS patients. 33% of deaths of Burkitt lymphoma are atributed to AIDS patients in the USA**

→In ~90% of the cases BL is linked to the t(8;14)(q24;q32) translocation of the *CMYC* gene locus next to the *IGH* gene locus leading to *CMYC* activation.



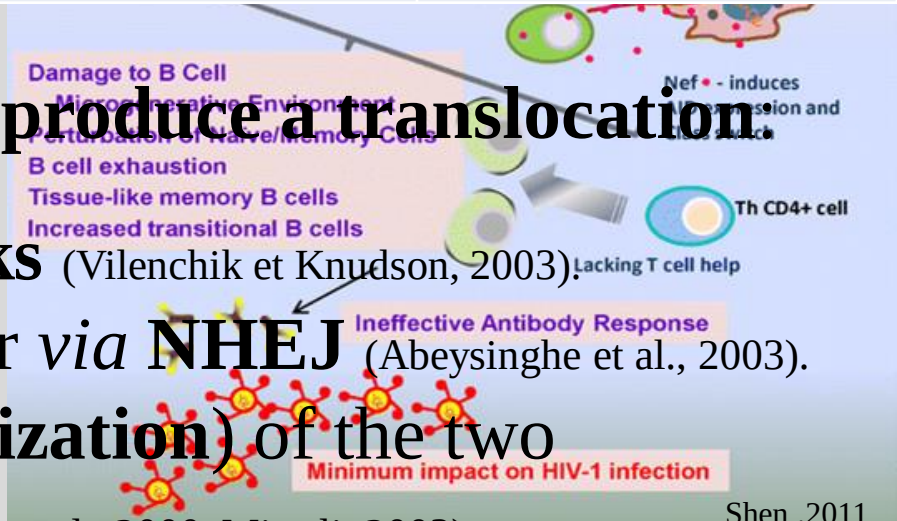
HIGH OCCURRENCE OF BURKITT'S LYMHOMA IN HIV-INFECTED PATIENTS: WHY?

Cancer	Frequency in the general poplation	Frequency in AIDS patients	Ratio
Burkitt's Lymhoma	**1:200 000**	**1: 4000**	**50**
Mantle Cell Lymphoma	**1:200 000**	**1:200 000**	**1**

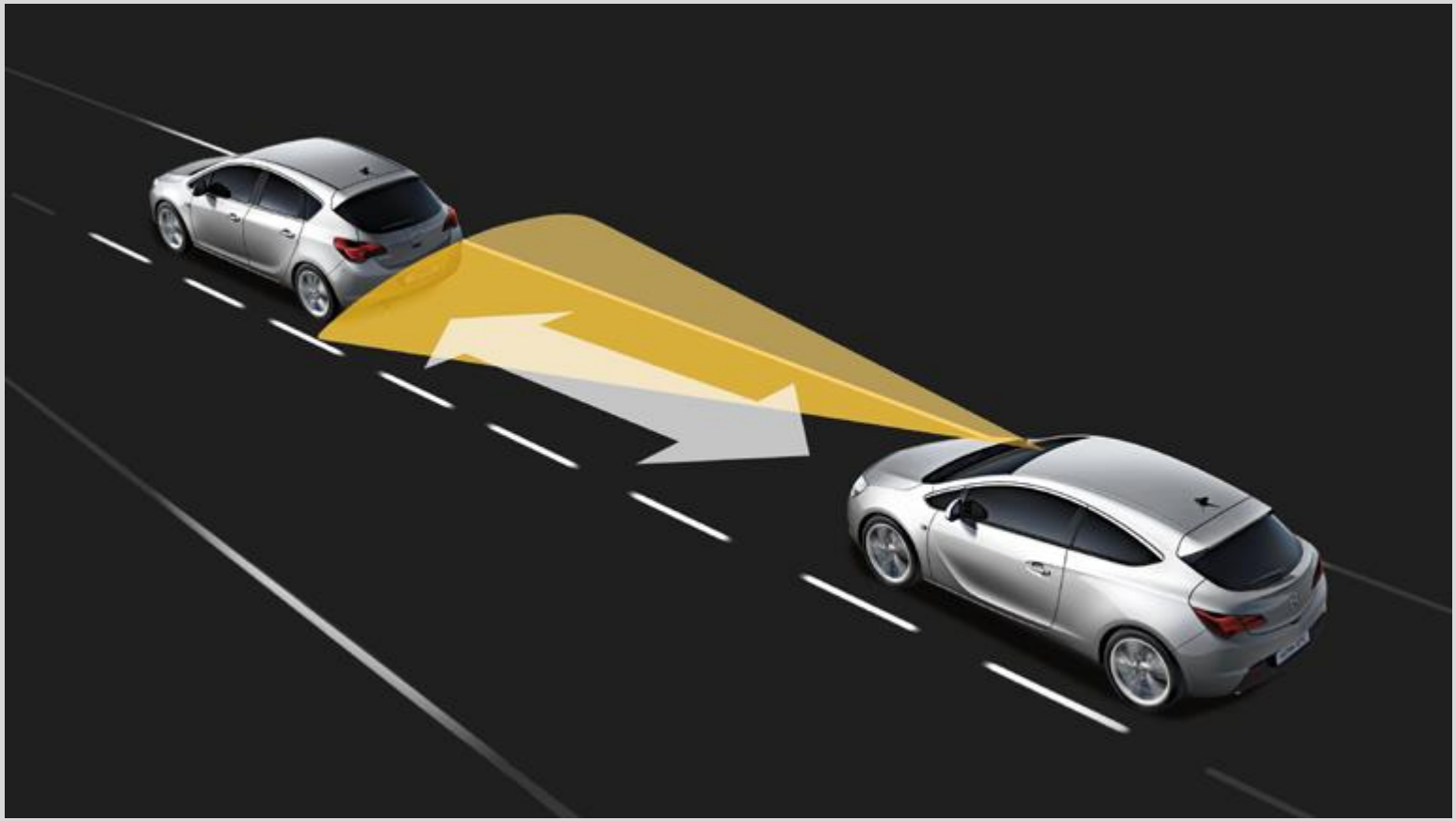
- ✓ gp-120 can interact with CD21 expressed on B cells *(Moir et al 2000)*
- ✓ HIV-1 causes B-cell hyperactivation *(Schnittan et al,1984)*
- ✓ Elevated class switch in B lymphocytes
- ✓ Induces B cell to proliferate (Nair MPN and al 1988)
- ✓ Causes B cell abnormal response
- ✓ The production of autoantibodies
- ✓ Aberrant B-cell surface markers: Change in B cell receptors

→Three events are necessary to produce a translocation.

- ✓ DNA **double strand breaks** (Vilenchik et Knudson, 2003).
- ✓ Double strand breaks repair *via* **NHEJ** (Abeysinghe et al., 2003).
- ✓ Spatial proximity (**colocalization**) of the two translocation partners. (Nikiforova et al., 2000, Misteli, 2003).



Shen ,2011



UNIVERSITÉ PARIS SUD
FACULTÉ DE MÉDECINE

cnrs

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Signalisation Noyaux Innovations en Cancérologie
UMR 8126

UNIVERSITÉ PARIS SUD
FACULTÉ DE MÉDECINE

cnrs

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Signalisation Noyaux Innovations en Cancérologie
UMR 8126

UNIVERSITÉ PARIS SUD
FACULTÉ DE MÉDECINE

cnrs

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Signalisation Noyaux Innovations en Cancérologie
UMR 8126

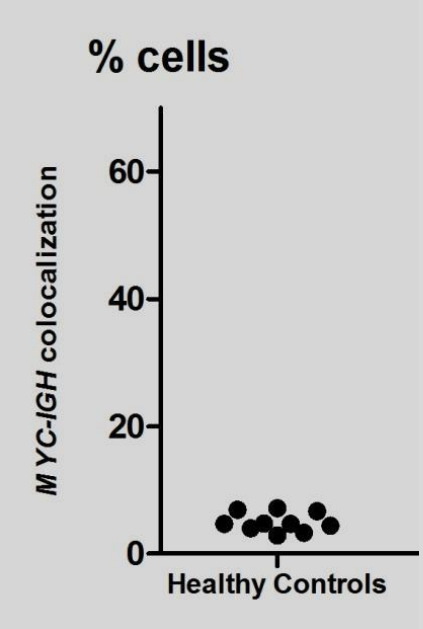
UNIVERSITÉ PARIS SUD
FACULTÉ DE MÉDECINE

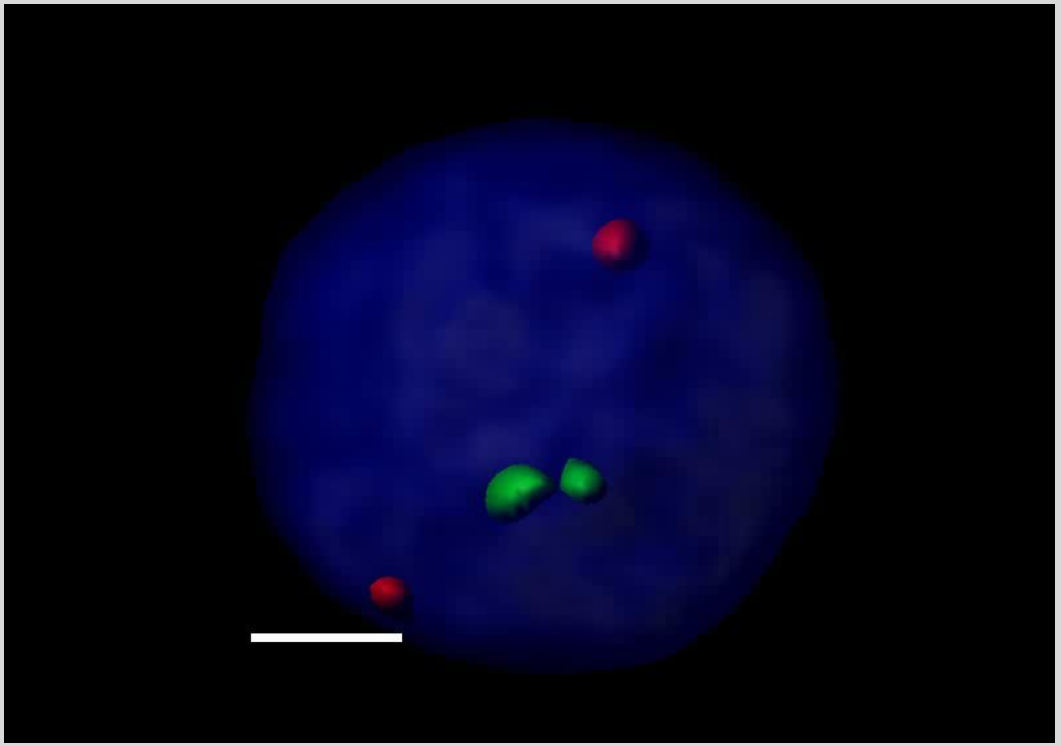
cnrs

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Signalisation Noyaux Innovations en Cancérologie
UMR 8126

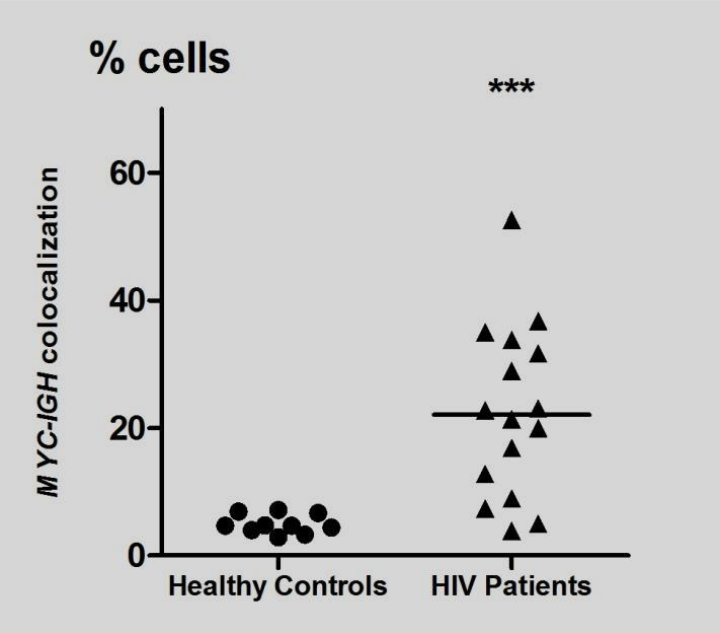
MYC AND *IGH* LOCI HAVE A DISTINCT LOCALIZATION IN B LYMPHOCYTES FROM HEALTHY DONORS

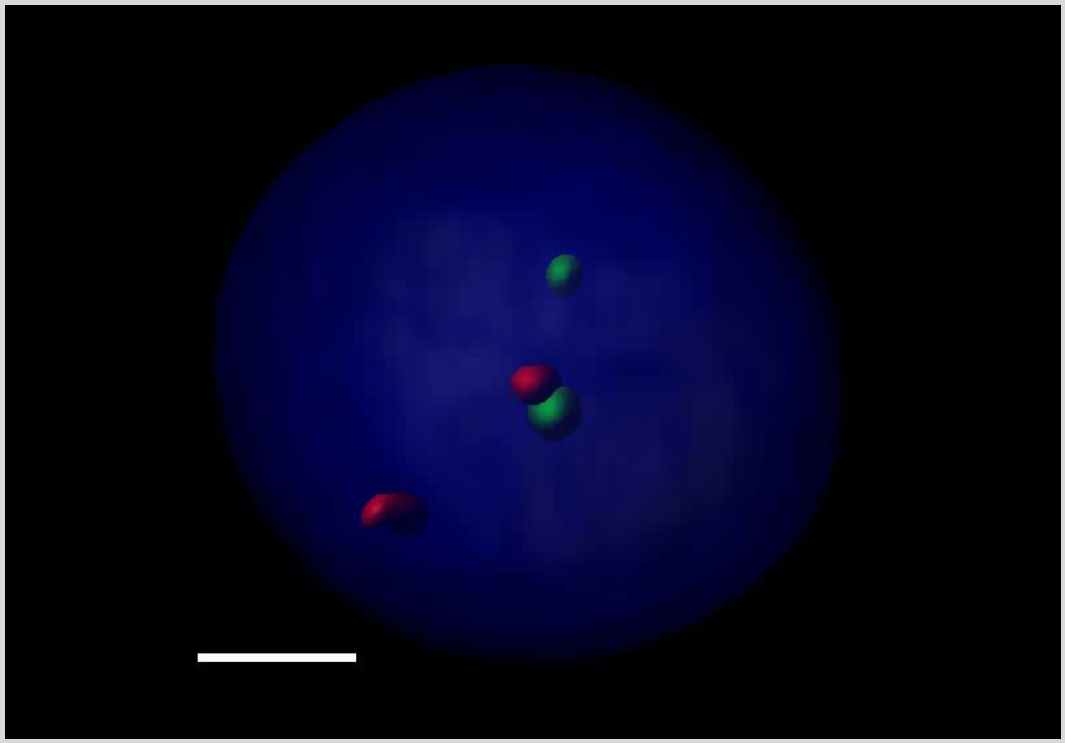




The *IGH* alleles are located more centrally while *MYC* alleles are close to the periphery in the B cell nuclei . The colocalizaton between the IGH and MYC loci is observed in 3-5% cells

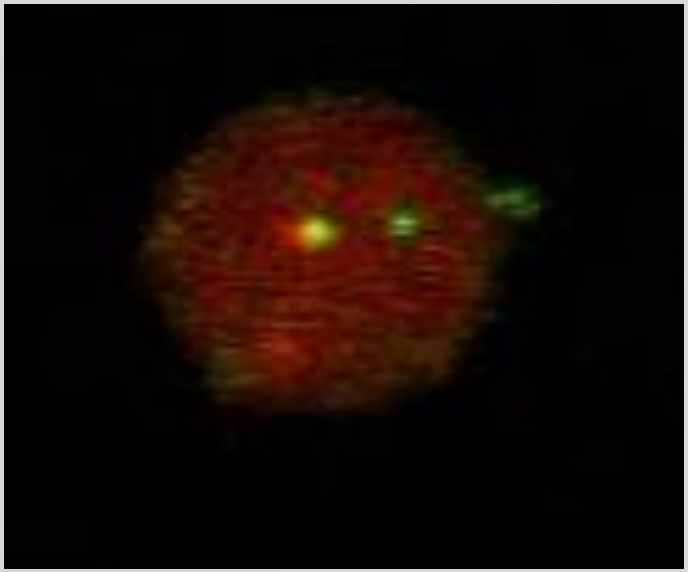
MYC AND *IGH* LOCI ARE FREQUENTLY COLOCALIZED IN CIRCULATING B CELLS FROM HIV-INFECTED PATIENTS

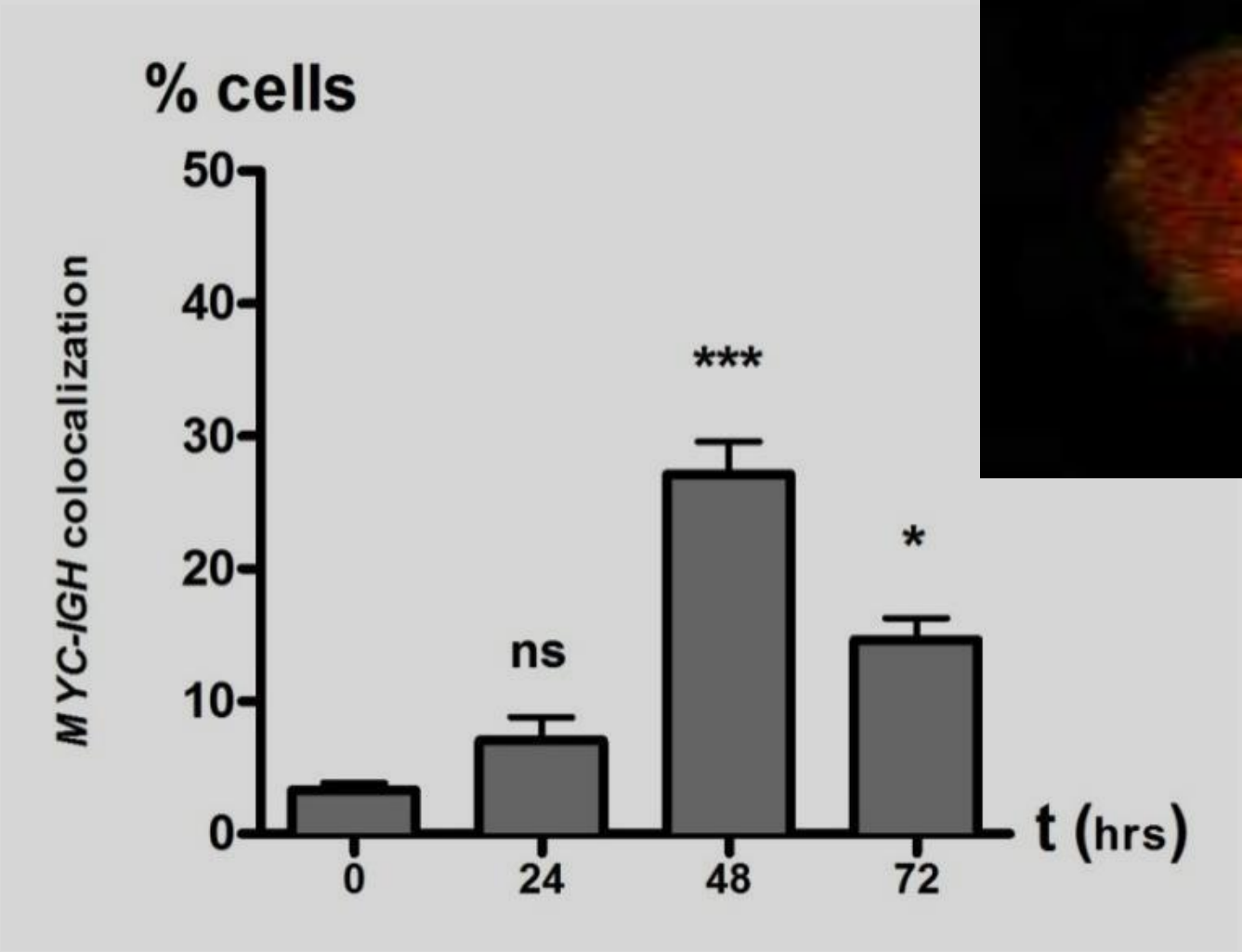




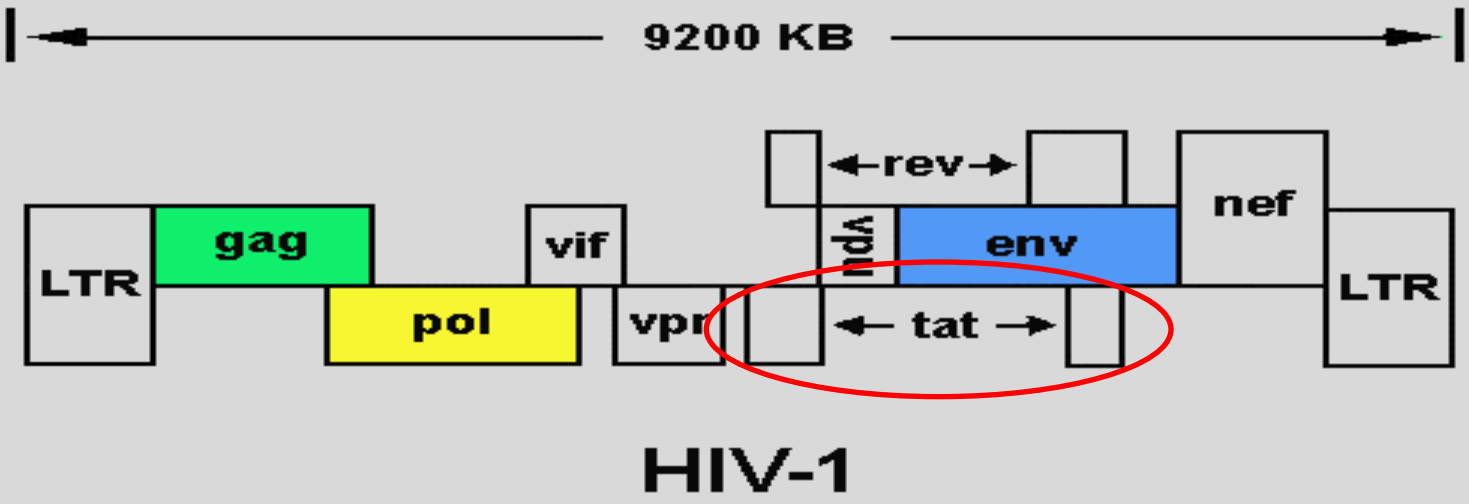
- In some HIV-1-infected patients, *MYC* and *IGH* loci are colocalized in a half of the circulating B cells

HIV-1 INDUCES *MYC/IGH* COLOCALIZATION IN B CELLS FROM HEALTHY DONORS *EX VIVO*





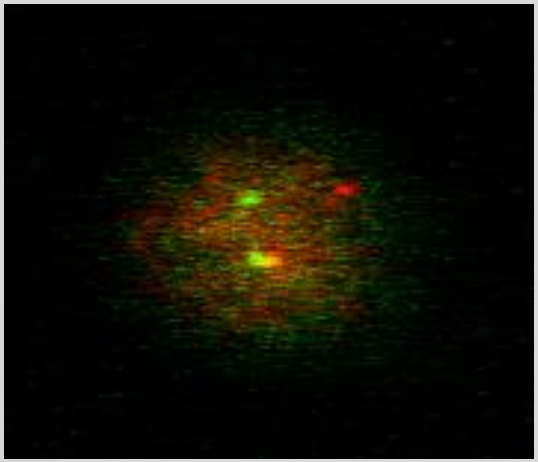
HIV GENOME

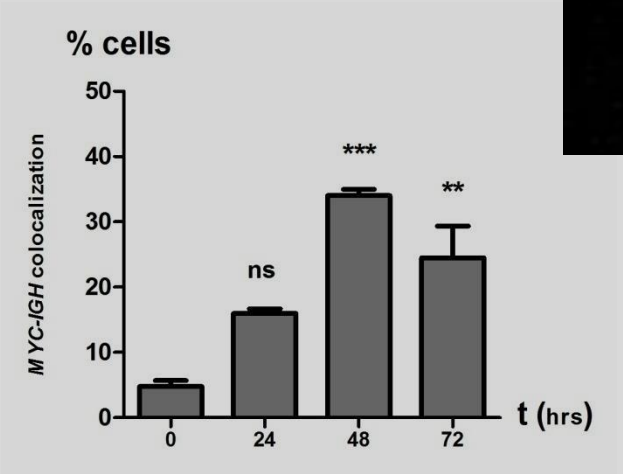


→ 9 genes encoding 3 structural, 2 envelope, and 6 regulatory proteins in addition to 3 enzymes

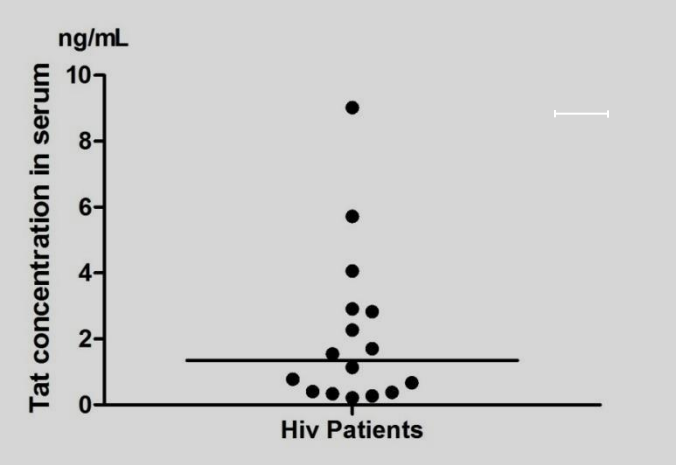
→ **TAT** is the transactivator of transcription encoded by 2 different exons. The 102 aa Tat is responsible for activation of viral Tat is secreted into the circulation and is capable to penetrate into cells. Produced in excess in infected cells. Tat is present in blood of AIDS patients.

MYC/IGH COLOCALIZATION IS INDUCED BY HIV-1 AND ITS PROTEIN TAT



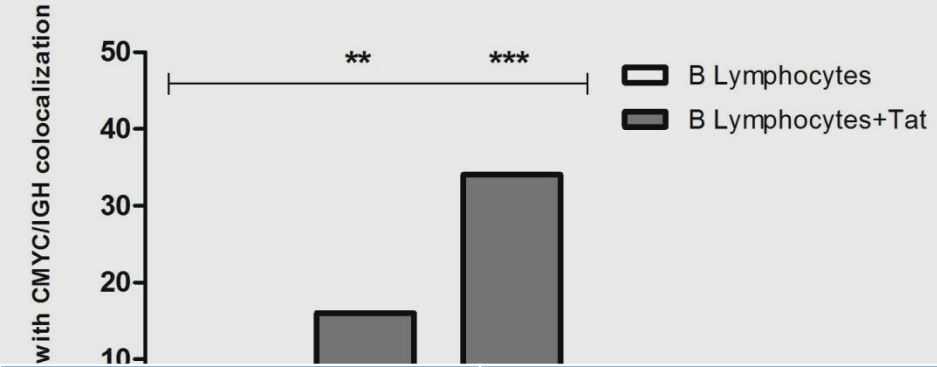


HIV-1 protein Tat induces *MYC/IGH* colocalization in normal B-cells *ex vivo*

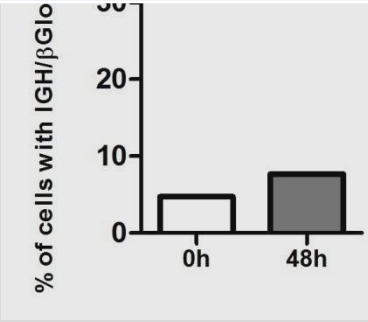


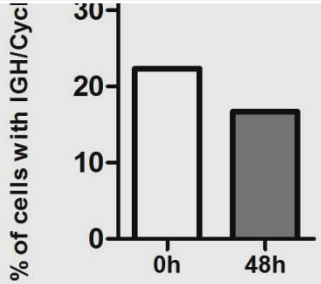
Tat is present in AIDS patients' sera

HIV Tat PROVOKES A SPECIFIC COLOCALIZATION OF THE *IGH* AND *CMYC* LOCI IN THE NUCLEAR SPACE OF B CELLS



Cancer	Frequency in the general poplation	Frequency in AIDS patients	Ratio
Burkitt's Lymhoma	1:200 000	1: 4000	50
Mantle Cell Lymphoma	1:200 000	1:200 000	1





Tat does not induce *IGH/β-globin* or *IGH/CCND1* colocalization in B lymphocytes

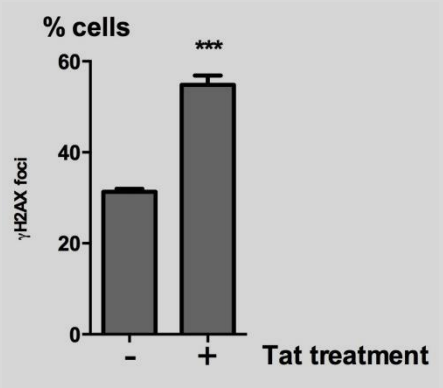
UNIVERSITÉ PARIS SUD FACULTÉ DE MÉDECINE

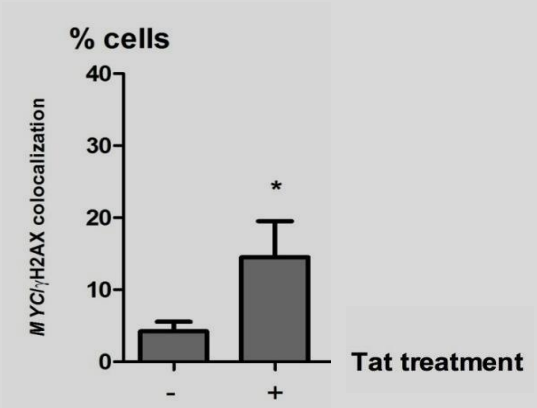
cnrs

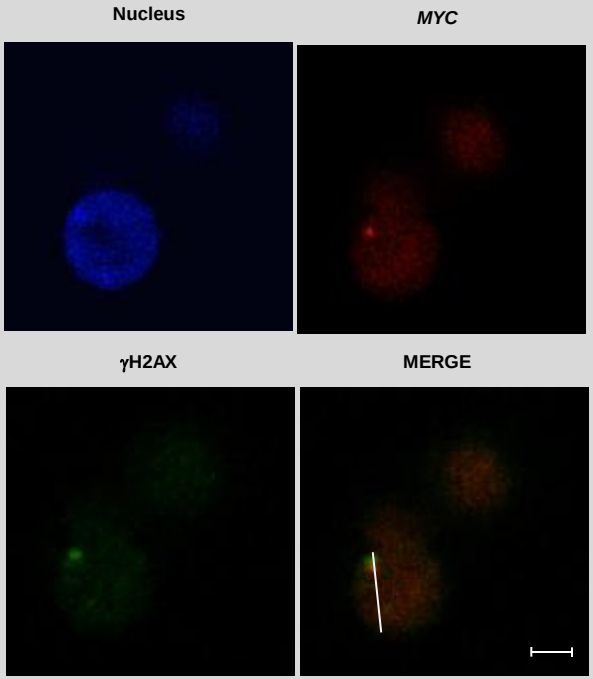
GUSTAVE ROUSSY CANCER CAMPUS GRAND PARIS

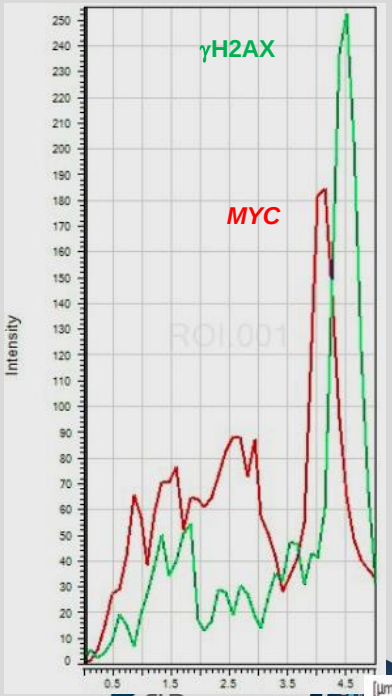
Signalisation Noyaux Innovations en Cancérologie UMR 8126

HIV TAT INDUCES SPECIFIC DNA DAMAGE IN THE *MYC* LOCUS *VIA* ABERRANT ACTIVATION OF RAG1









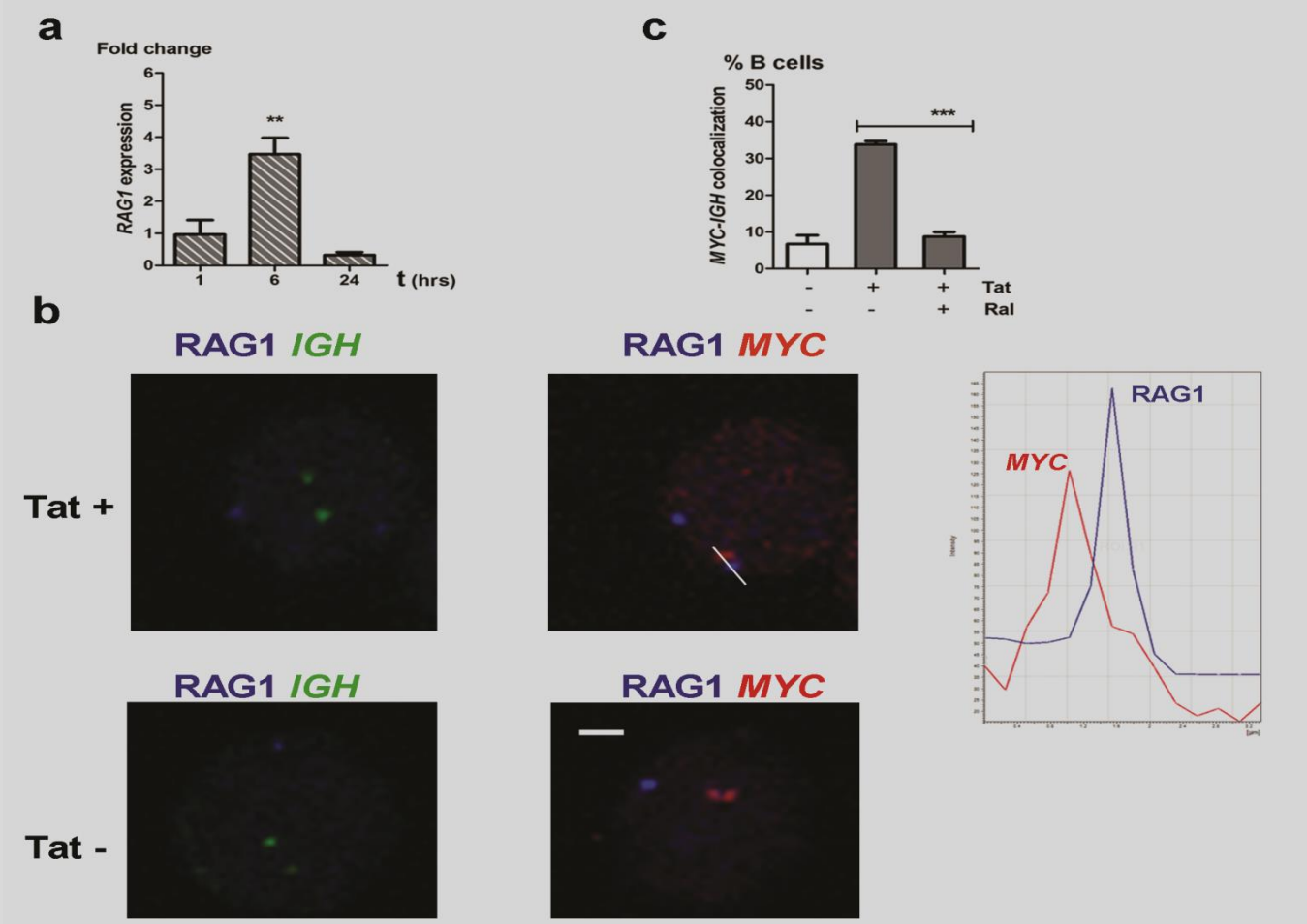
FACULTÉ DE MÉDECINE

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Signalisation Noyaux Innovations en Cancérologie
UMR 8126

HIV TAT INDUCES SPECIFIC DNA DAMAGE IN THE *MYC* LOCUS *VIA* ABERRANT ACTIVATION OF RAG1

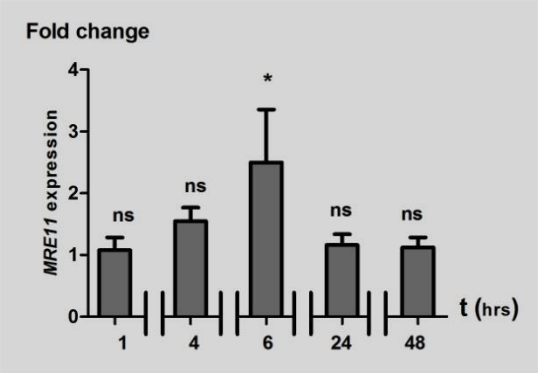
→ When **overexpressed, RAG1** may **provoke DNA double strand breaks** at a variety of genomic locations, including **MYC genes** (Bernard et al., 1988)

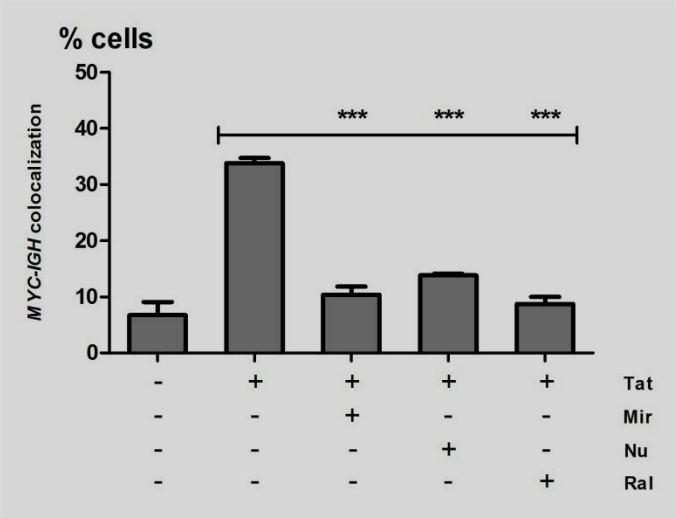


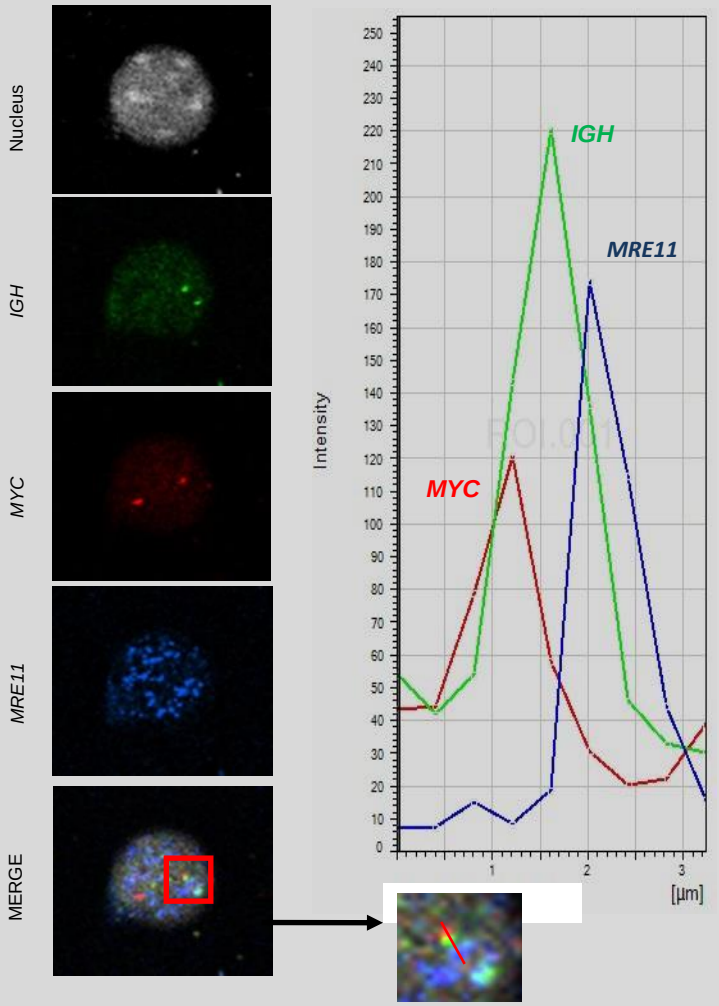
→RAG1 is overexpressed after Tat treatment

→High level of damage induced by Tat in the *MYC* locus is due to RAG1 activation

TAT-INDUCED DNA DAMAGE ACTIVATES DNA REPAIR AND RELOCALIZATION OF *MYC* TOWARDS THE *IGH* LOCUS



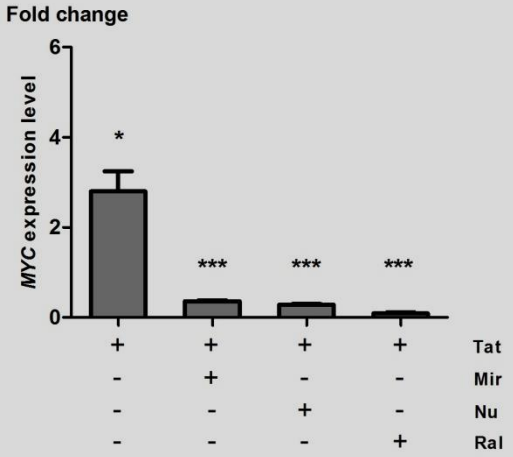


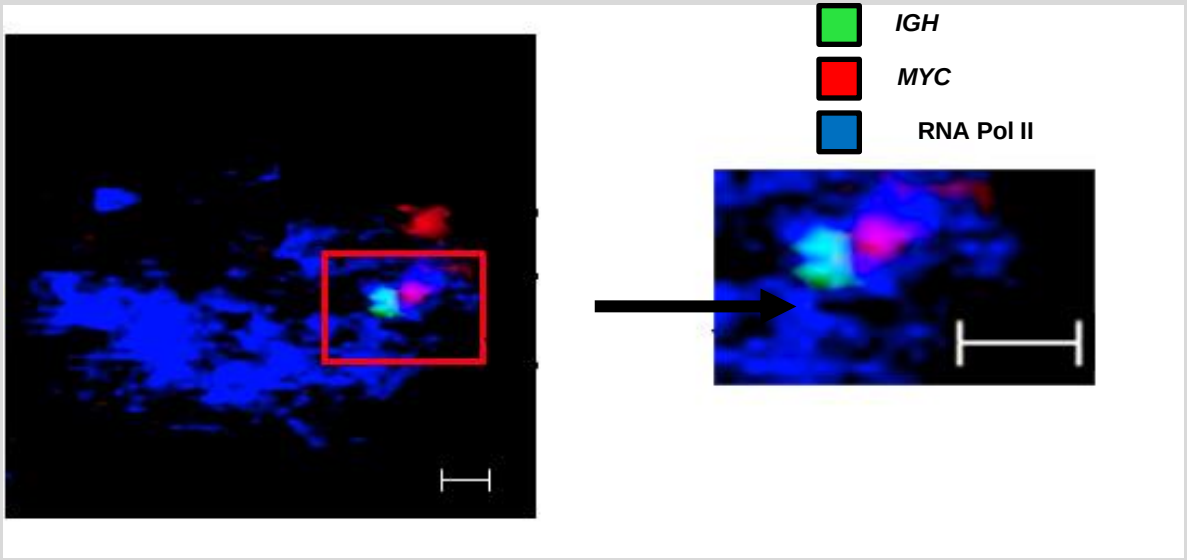


→ **Tat and DNA damage stimulate the expression of MRE11**, a protein involved in non-homologous end joining

→ **The inhibition of DNA repair and RAG1** significantly diminish the level of Tat-induced MYC/IGH colocalization

TAT-INDUCED DNA DAMAGE AVTIVATES DNA REPAIR AND RELOCALIZATION OF *MYC* TOWARDS THE *IGH* LOCUS

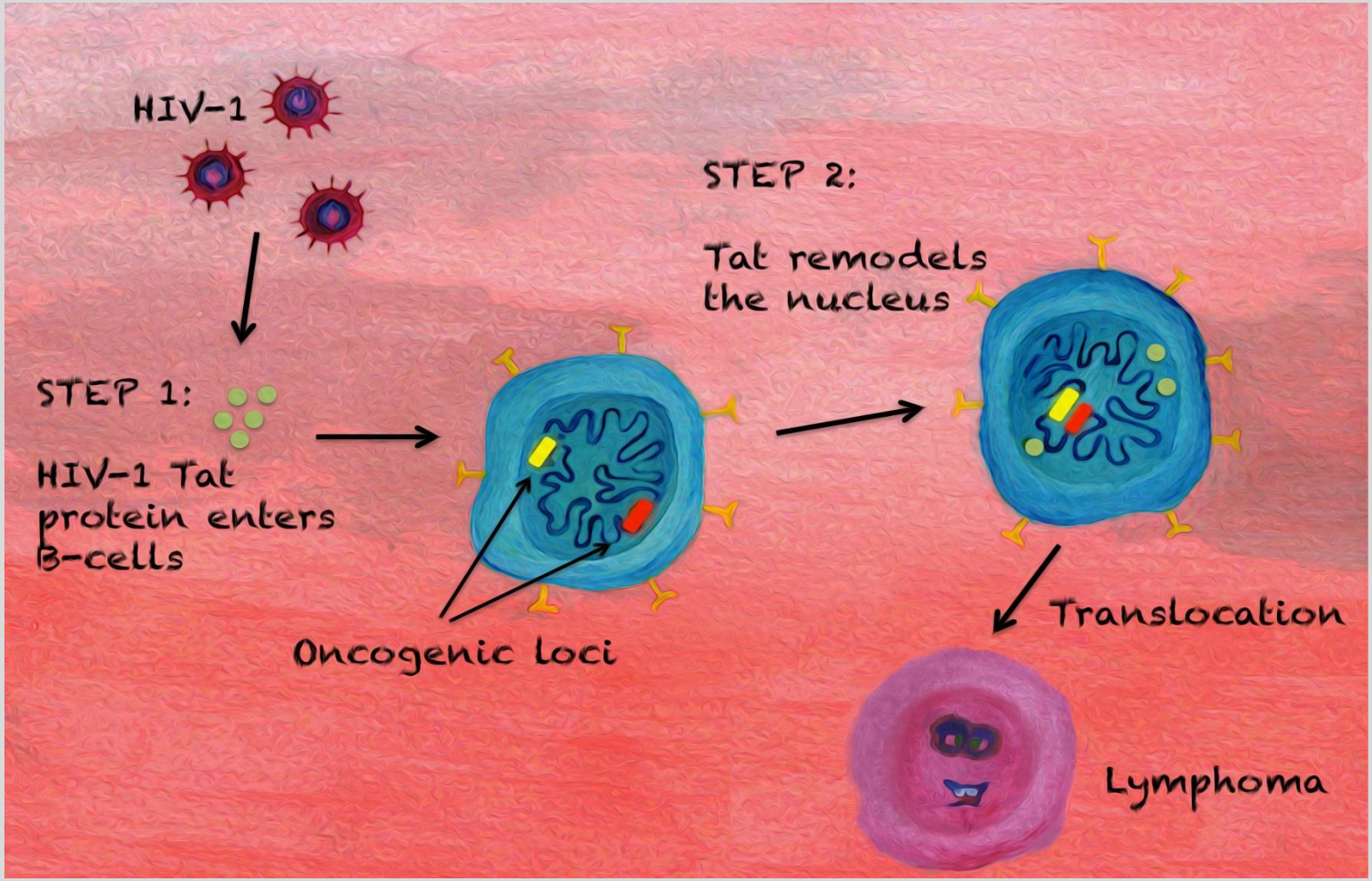




→ **Tat, DNA damage and repair stimulate *MYC* expression**

→ **Activated *MYC* moves into the transcription factory occupied by *IGH***

HIV TAT: A ROLE IN INTRANUCLEAR REORGANIZATION AND IN GENERATION OF SPECIFIC TRANSLOCATIONS



HIV Tat → NFkB → RAG → DSB → NHEJ → CMYC relocalization

Tat C22 Transription | **RAGi** | **Mirin NU7026**

Germini et al., ***Leukemia,*** 2017

EBV AND BURKITT'S LYMPHOMA

→ EBV is 100% associated with the endemic form in Afrtica

→Malaria and the use of latex-producing plants are additional risc factors in Africa

→EBV is an innocent passenger in tumour cells?

→EBV plays a role in initial transformation?

→ EBNA1 Stabilizes B-lymphocytes

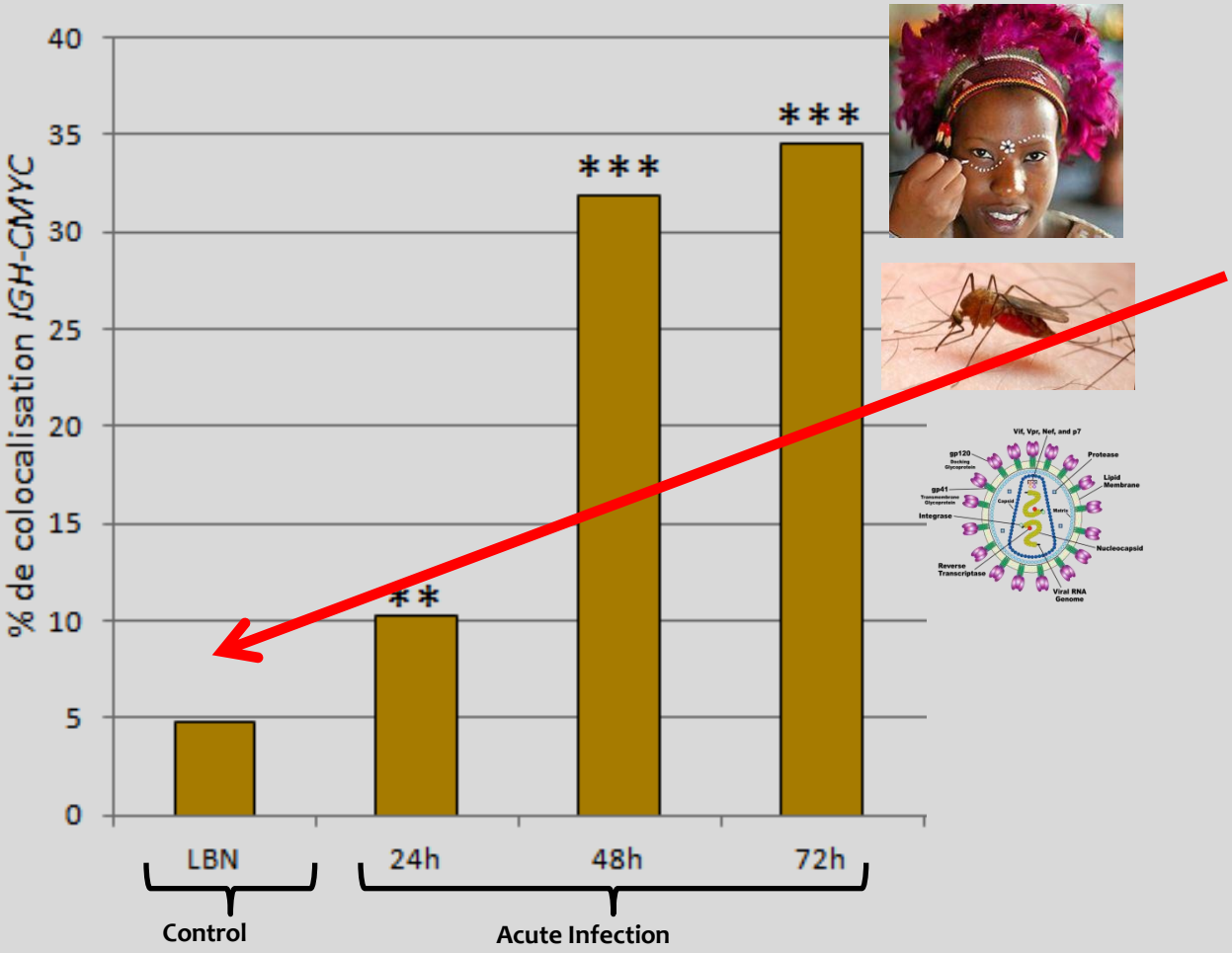
→A role or EBV in sustenance of the tumour?

→A role of non-coding RNAs (EBER)?

→Tumour formation due to other cellular changes

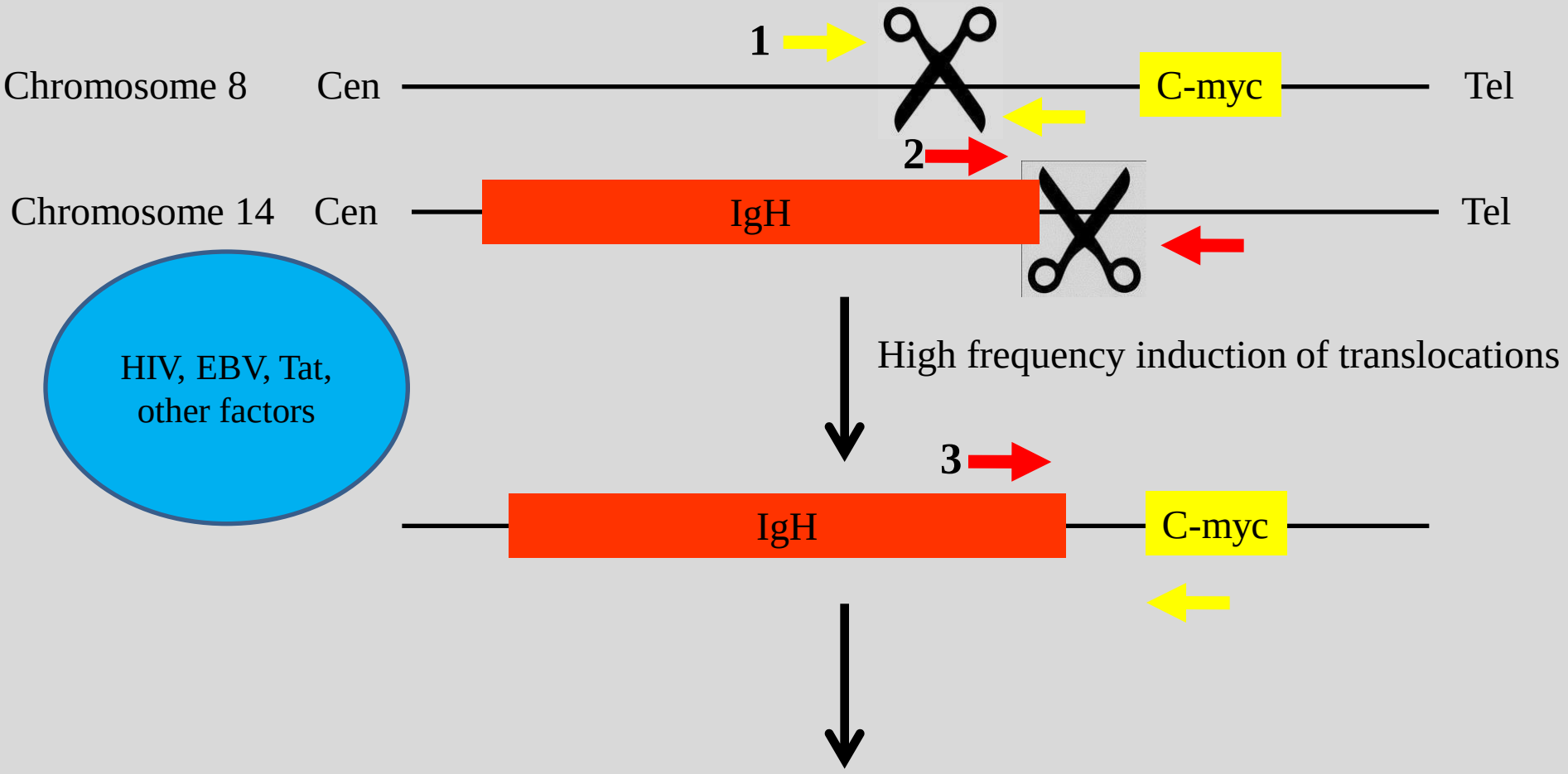
→ **Does EBV infection affect the nuclear architecture?**

EBV INFECTION INDUCES SPATIAL PROXIMITY OF THE *CMYC* AND *IGH* LOCI

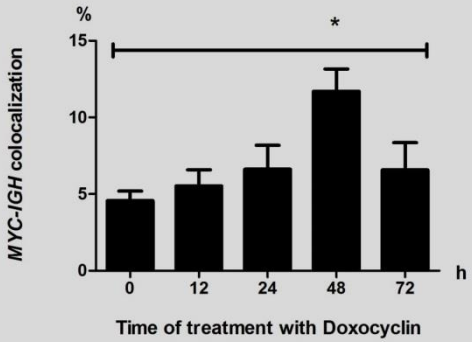


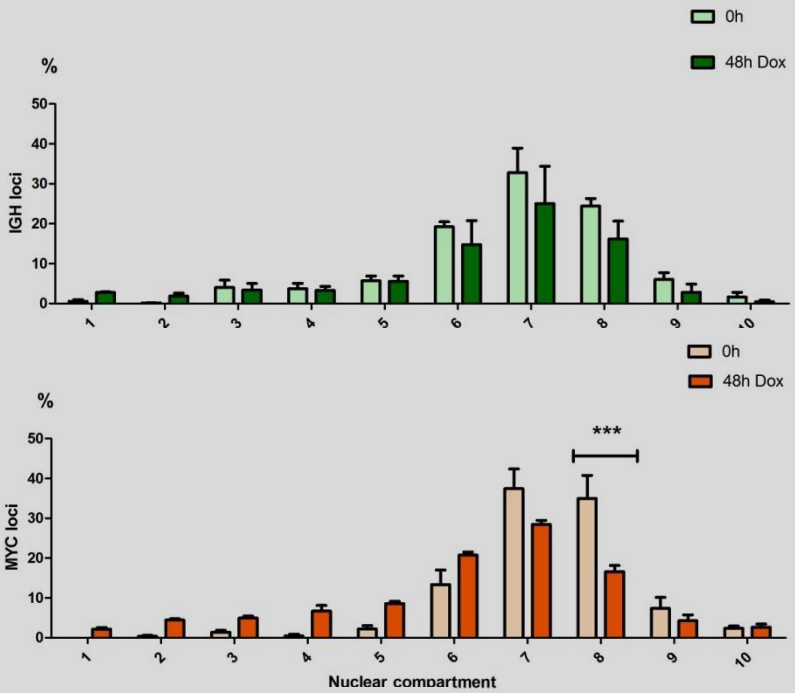
X7

AN EXPERIMENTAL SYSTEM FOR INDUCTION OF TRANSLOCATIONS IN LCL USING CRISPR/Cas9



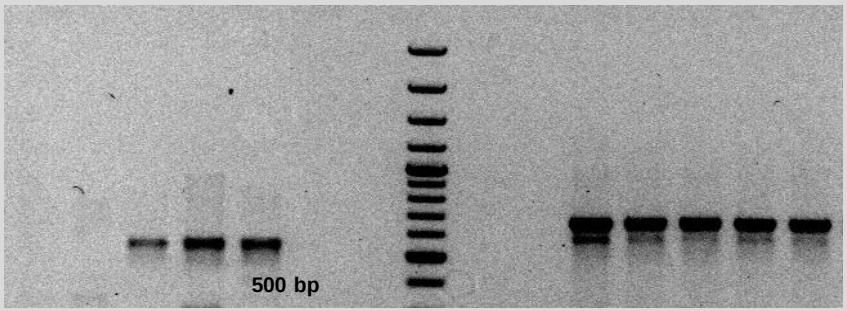
AN EXPERIMENTAL SYSTEM FOR INDUCTION OF TRANSLOCATIONS IN LCL USING CRISPR/Cas9

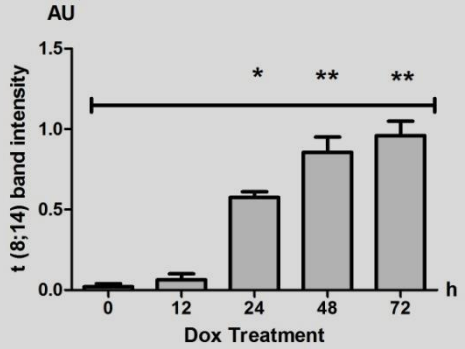




TRANSLOCATON
0 12 24 48 72

MYC
0 12 24 48 72h





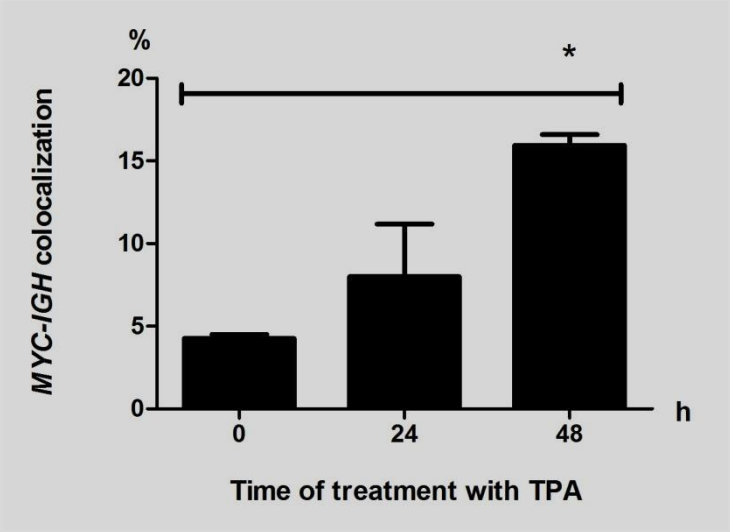
UNIVERSITÉ PARIS SUD
FACULTÉ DE MÉDECINE

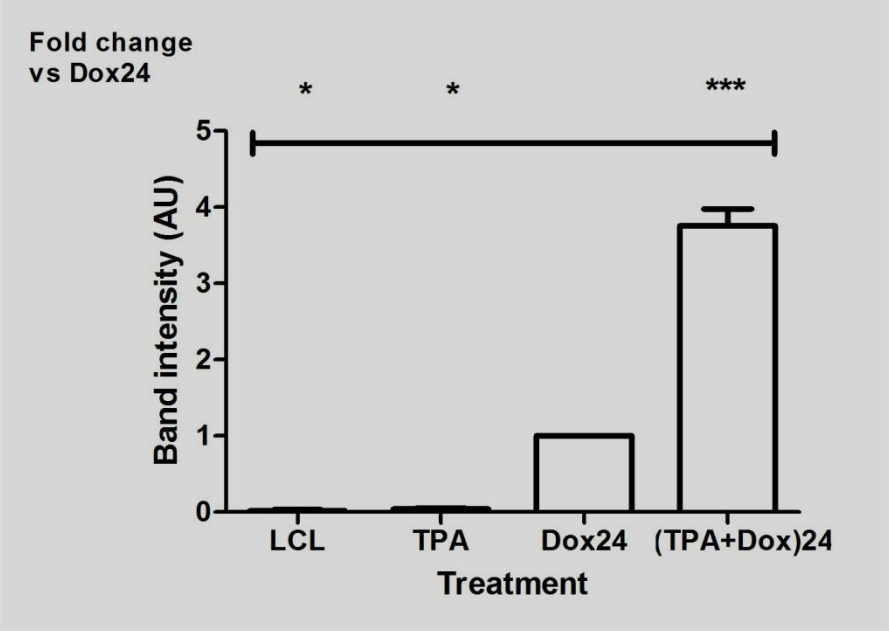
cnrs

GUSTAVE ROUSSY
CANCER CAMPUS GRAND PARIS

Signalisation Noyaux Innovations en Cancérologie
UMR 8126

INDUCTION OF THE EBV LYTIC CYCLE INCREASES THE RATE OF t(8;14) in LYMPHOBLASTOID CELLS





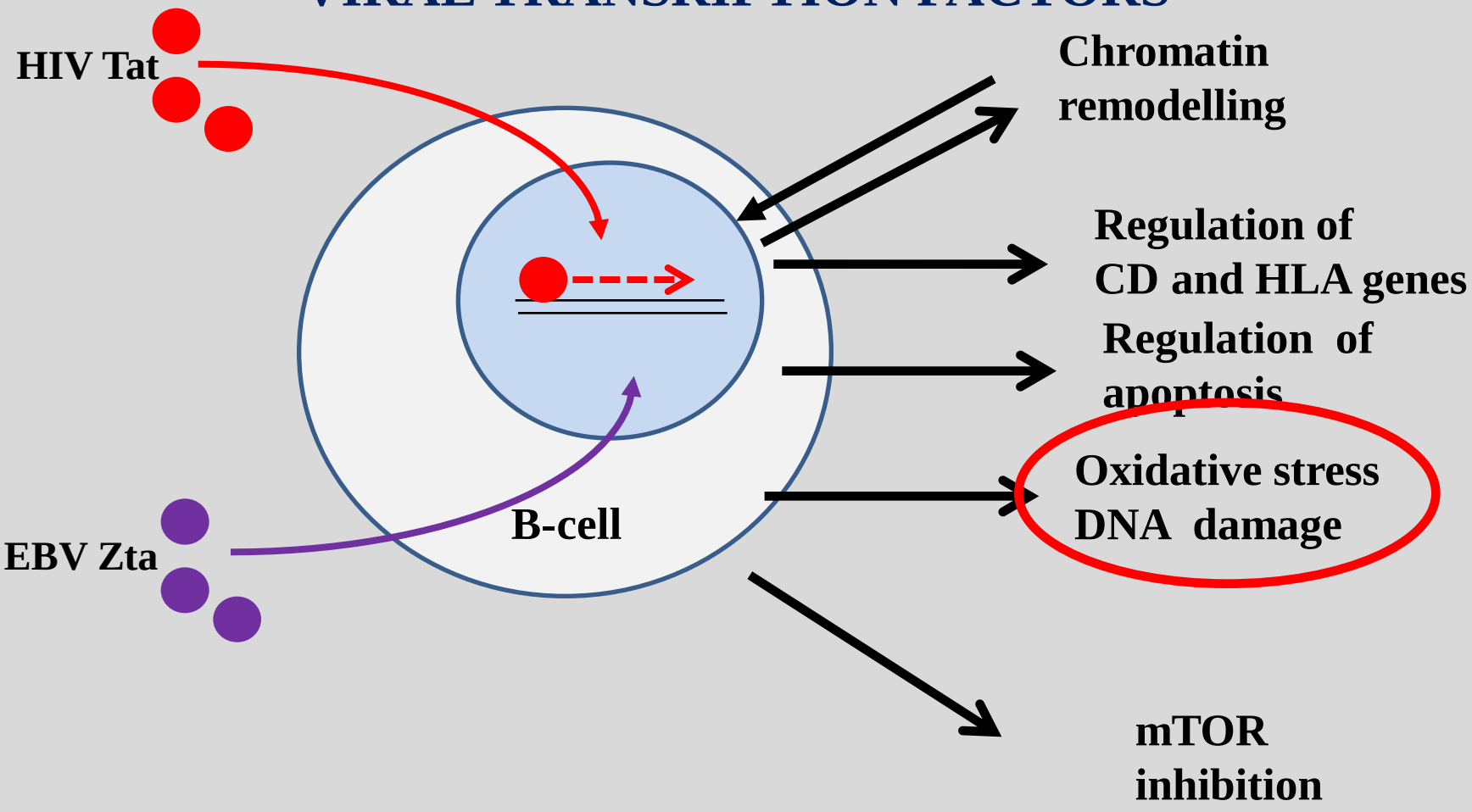
→**Induction of lytic cycle by TPA leads to an increased *IGH/MYC* colocalization and t(8;14) rate in RPMI8866 cells**

→ ***IGH/MYC* colocalization correlates with the increase in the induced t(8;14) rate**

HIGH OCCURRENCE B-CELL LYMPHOMAS IN HIV-INFECTED AND EBV-INFECTED PATIENTS

HIV	EBV
Burkitt Lymphoma t (8:14) in the majority of cases	**Burkitt Lymphoma** t (8:14) in the majority of cases
Diffuse Large B-cell Lymphoma No specific translocations	**Diffuse Large B-cell Lymphoma** No specific translocations
Hodgkin Lymphoma No specific translocations	**Hodgkin Lymphoma** mixed-cellularity (MCHL), lymphocyte-depleted (LDHL)

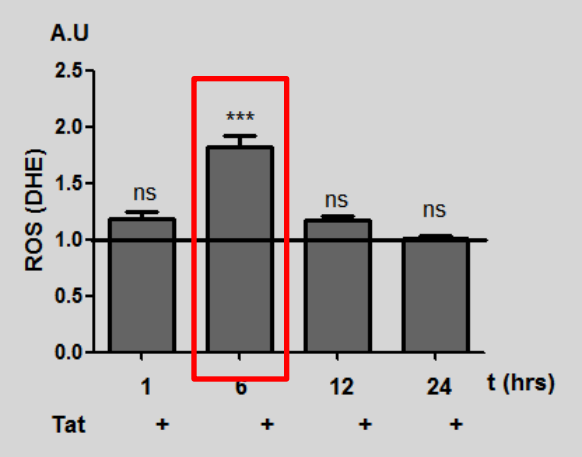
REGULATION OF CELLULAR GENES BY CIRCULATING VIRAL TRANSRIPTION FACTORS

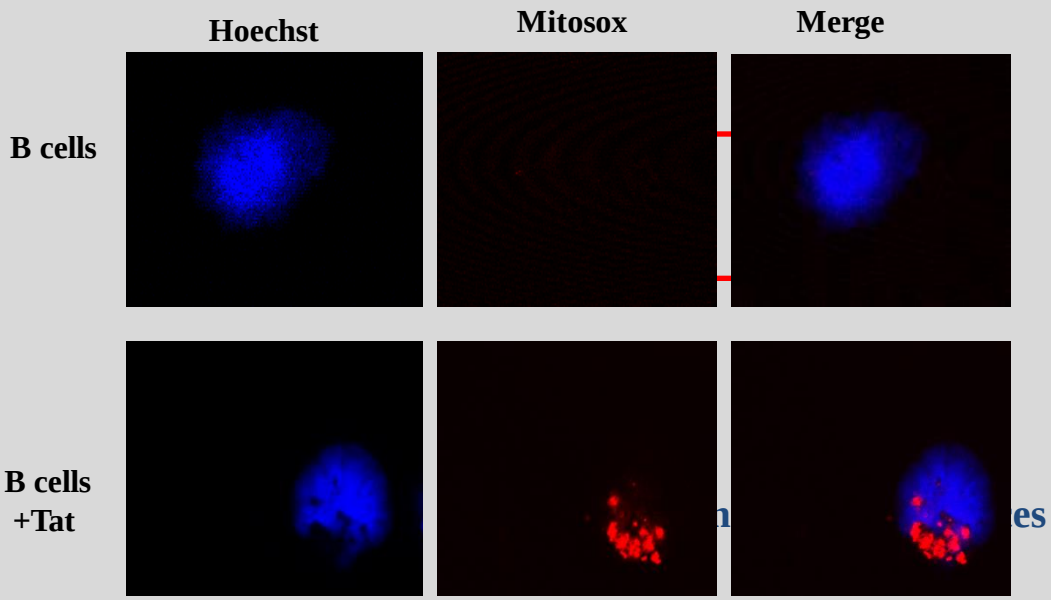


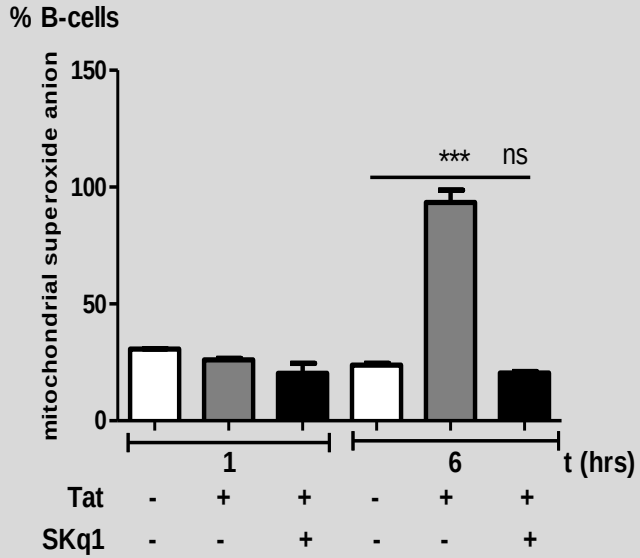
→ HIV Tat is present in HIV-infected patients' serum

→ Zta (ZEBRA) protein shares a significant homology to HIV Tat and is present in serum

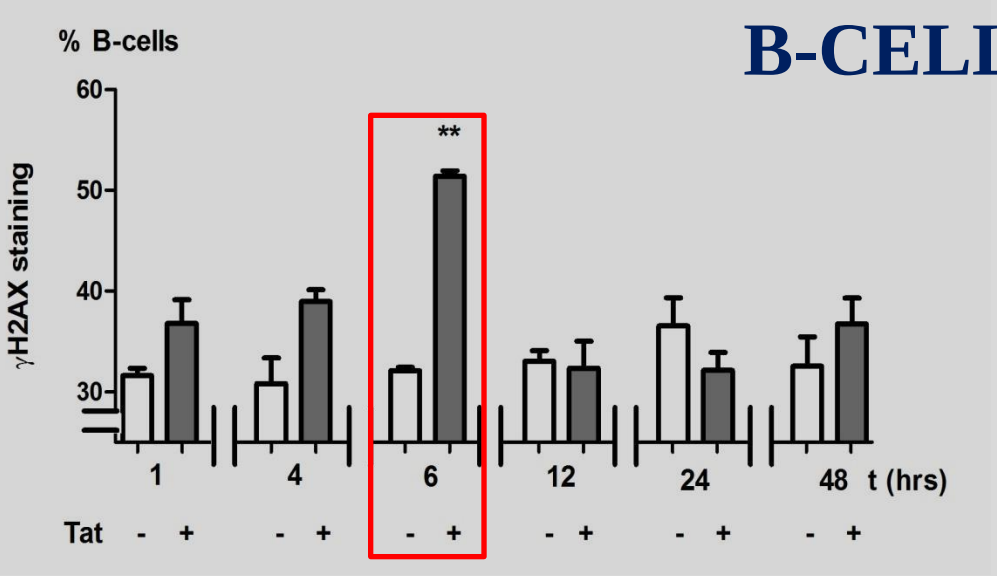
TAT INDUCES MITOCHONDRIAL OXIDATIVE STRESS IN B-CELLS

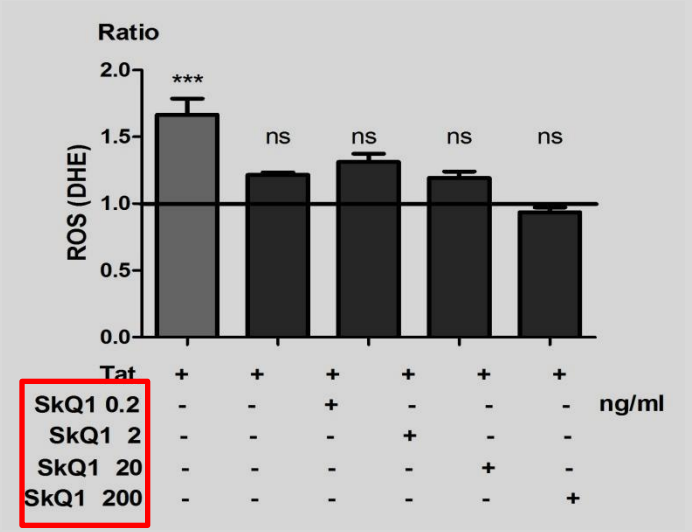


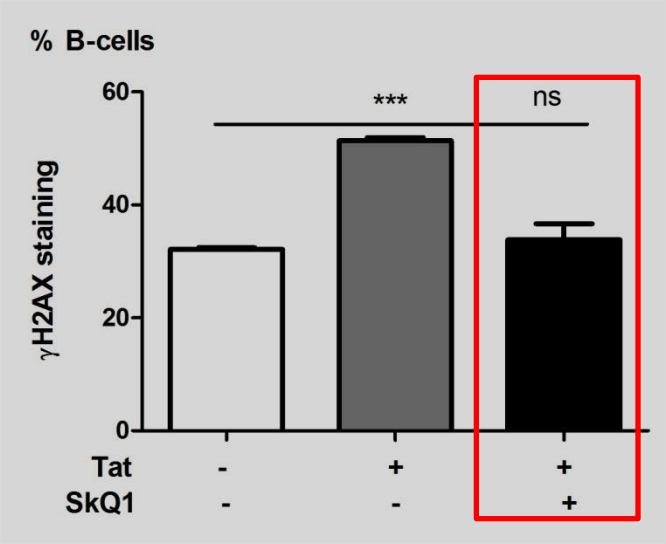


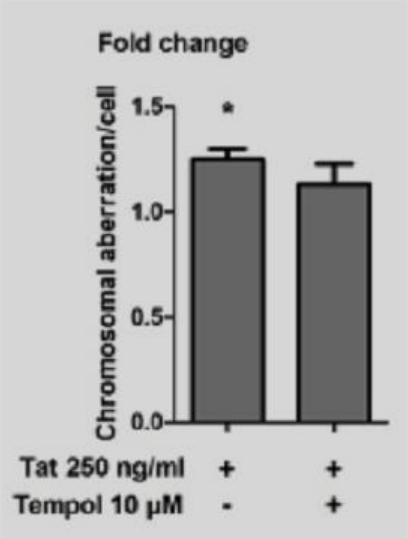


OXIDATIVE DNA DAMAGE IN TAT-TREATED B-CELLS



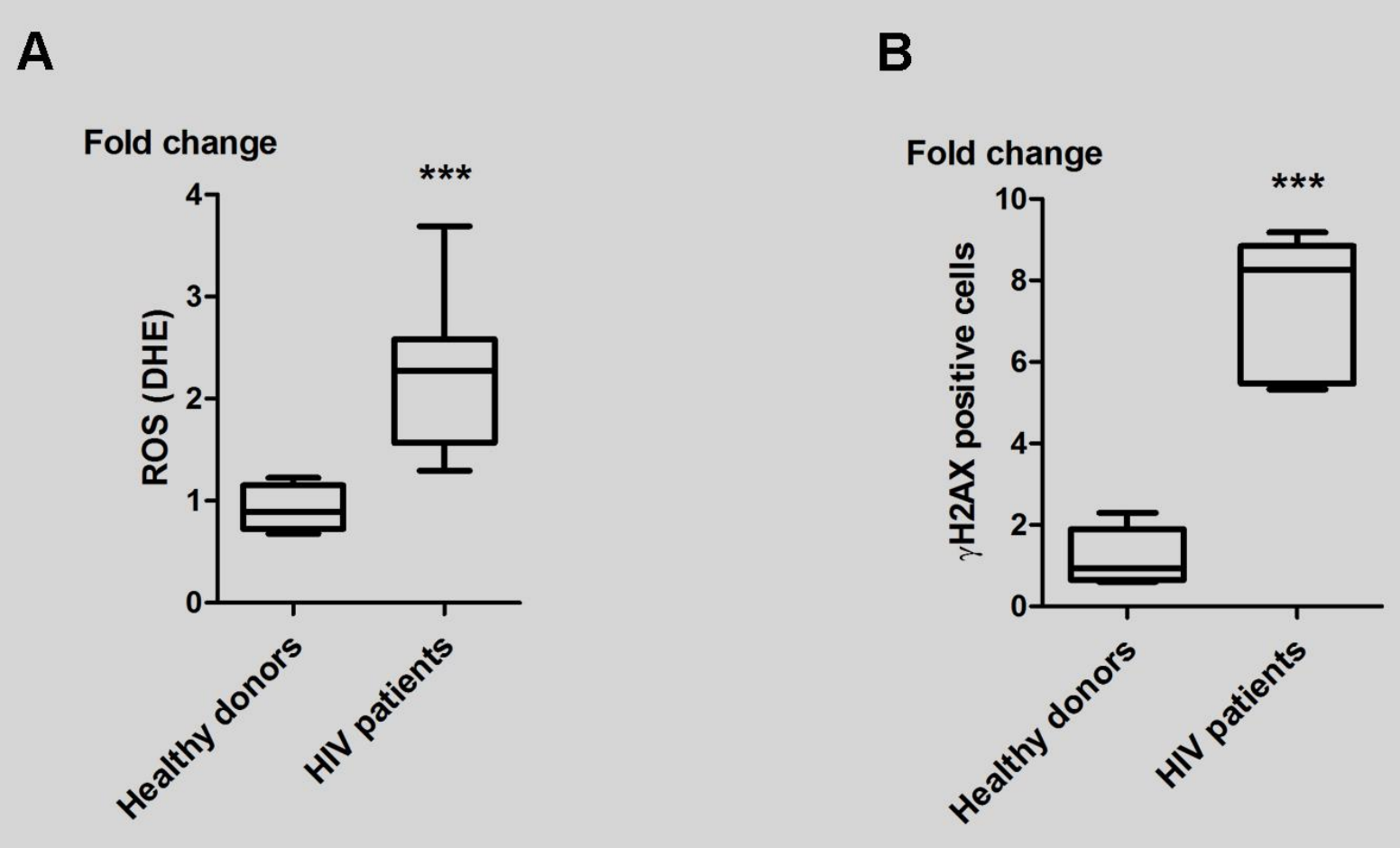


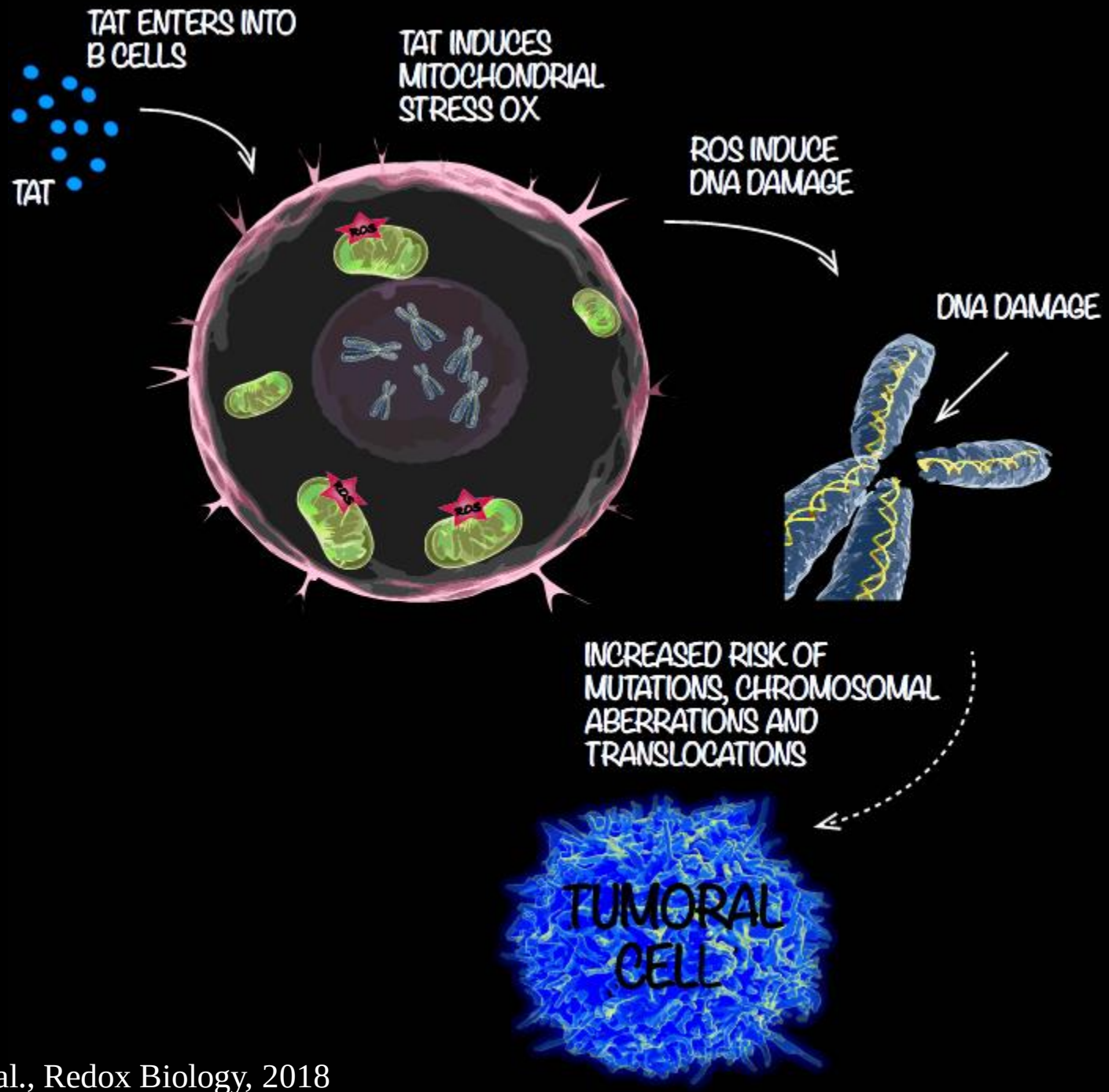




→**Tat induces the DNA damage and chromosomal aberrations in B cells *via* mitochondrial ROS production**

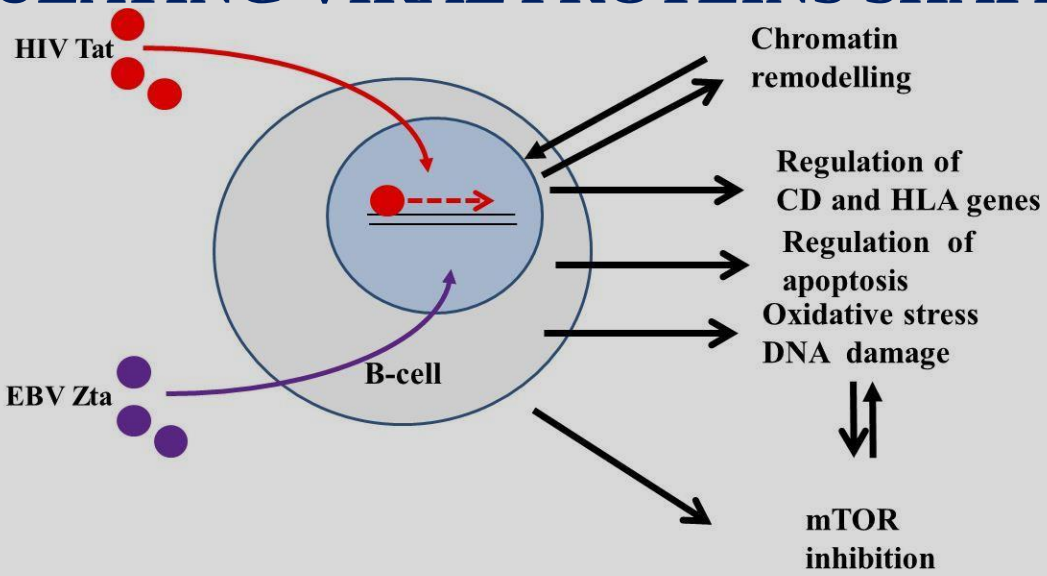
B -CELLS FROM HIV-INFECTED PATIENTS HAVE ELEVATED LEVELS OF OXIDATIVE STRESS AND DNA DAMAGE





El Amine et al., Redox Biology, 2018

HOW CIRCULATING VIRAL PROTEINS SHAPE B-CELLS?



→ Changes in the nuclear architecture (HiC, ChiP-Seq)

→ Induction of oxidative stress, DNA damage and chromosomal aberrations

→ Contribution to immune evasion

→ Modulation of mTOR and metabolic pathways

→ Synergistic effect of Tat and Zta?

Do viruses need to infect B-cells to induce lymphomagenesis???

Nuclear Organization and Pathologies (UMR-8126, IGR, Villejuif, France):

- **Marc Lipinski,** DREM CNRS
- **Diego Germini**, postdoctorant
- **Yinxing Ma**, postdoctorante
- **Fabi Sall**, doctorante
- **Carla Dib**, doctorante
- **Reynand Canoy**, doctorant
- **Burkitkan Akbay**, doctorant

Past lab members:

- **Tatana Tsfasman**
- **Vlada Zakharova**
- **Rawan El Amine**

Collaborators:

- **Eric Oksenhendler**, HSL, Paris
- **Vincent Ribrag,** IGR, Villejuif
- **Sergey Razin,** IBG, Moscow