Pathobiology of Hodgkin lymphoma

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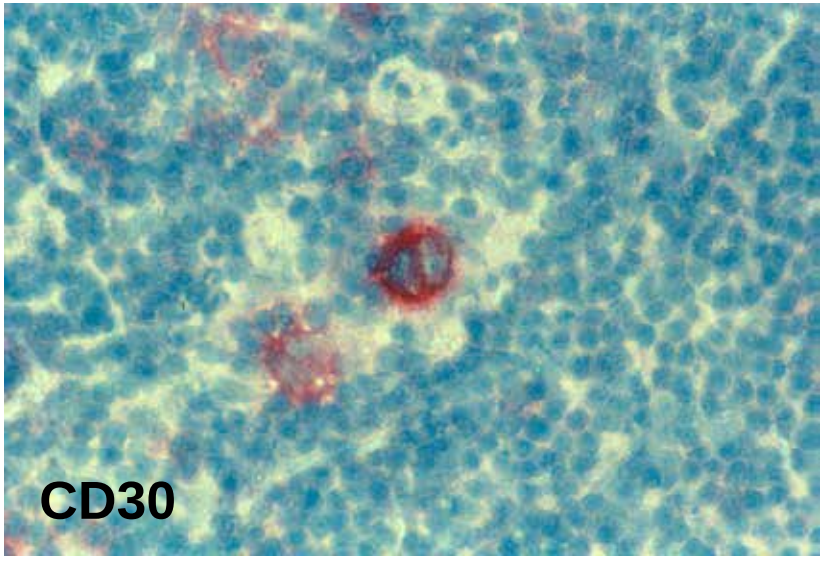
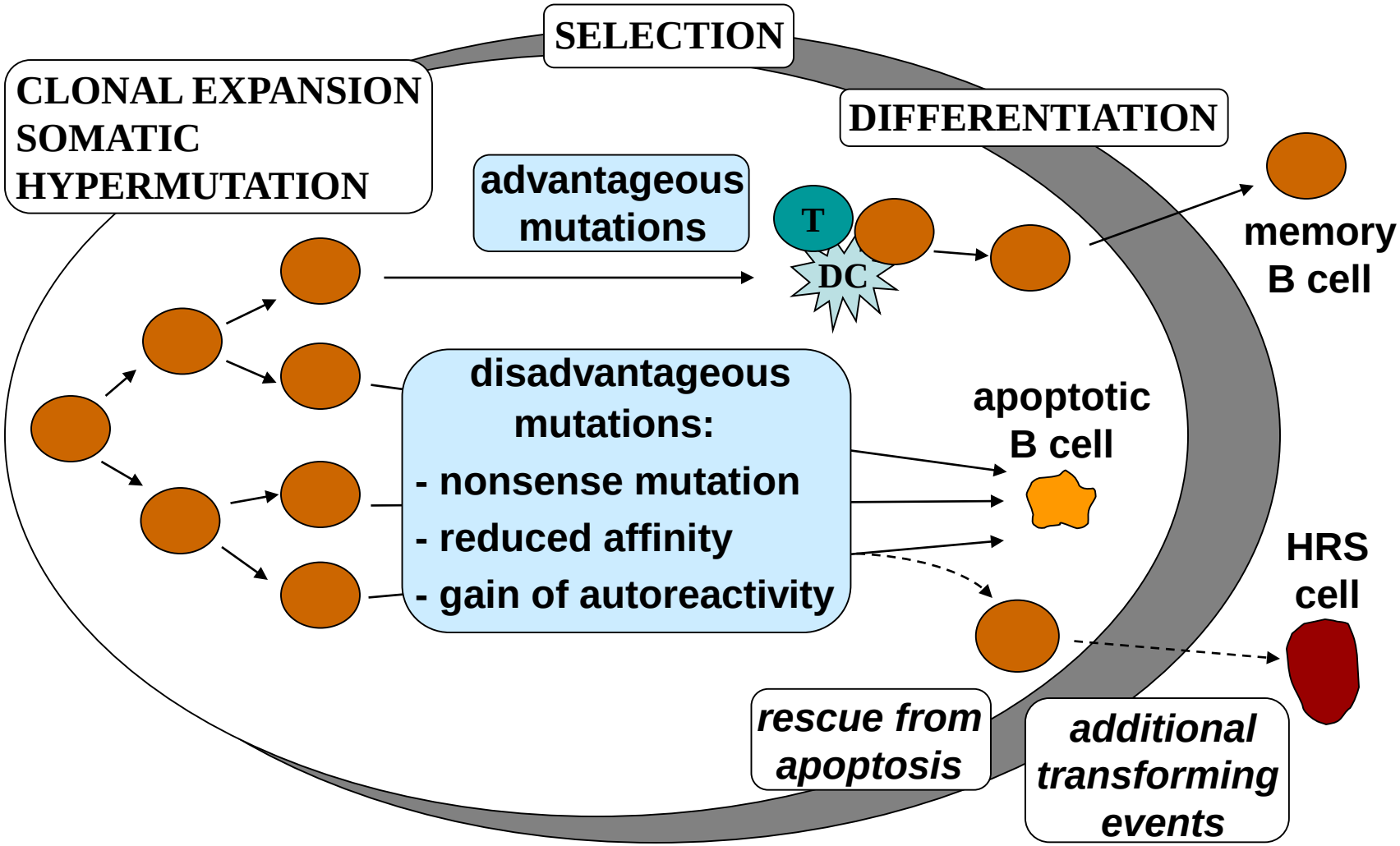
Hodgkin lymphoma

- One of the most frequent lymphomas in Western world
- Tumor cells are called Hodgkin and Reed/Sternberg (HRS) cells in classical HL
- Derivation of HRS cells from germinal center B cells
- HRS cells usually represent <1% of cells in tissue
- HRS cells always CD30-positive
- Activation of numerous signaling pathways



Scenario for HRS cell generation from “crippled“ GC B cells



Küppers & Rajewsky; Annu Rev Immunol. 1998

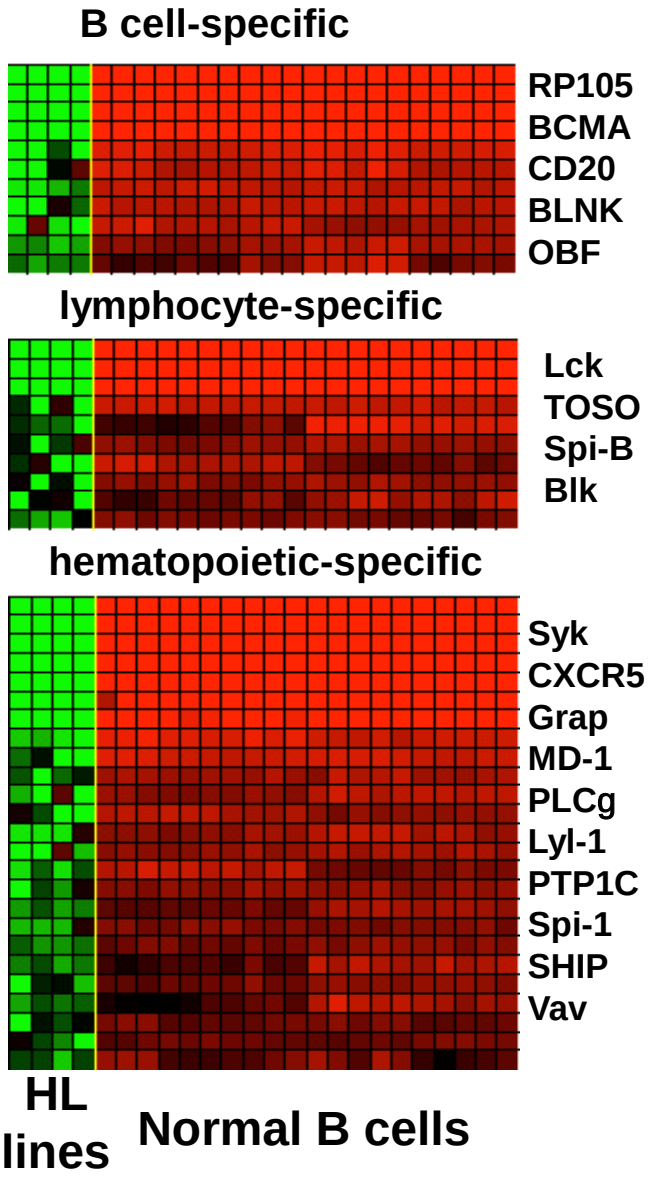
Topics

- **Lost B cell phenotype**
- **Genetic lesions**
- **Generation of multinuclear cells**
- **Normal CD30+ B cells and their relationship to HRS cells**
- **The role of BATF3 in Hodgkin lymphoma and ALCL**

The lost B cell identity of HRS cells of classical HL:
Deregulated transcription factor networks

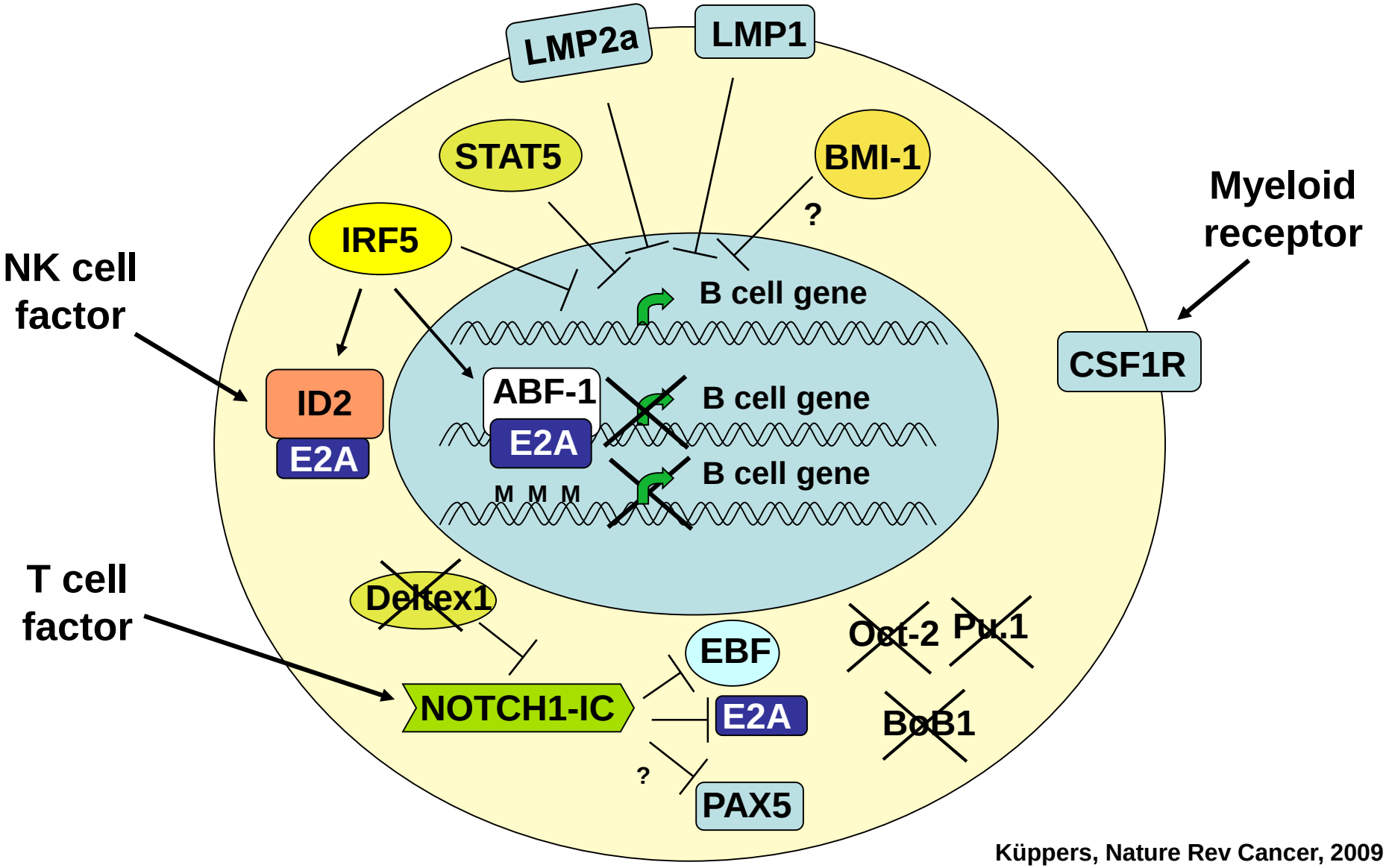
Lost B-cell identity of HRS cells

- Global gene expression analysis of HRS cell lines with microarrays
- Nearly complete loss of B cell specific gene expression program
- Retained expression of several B cell transcription factors (PAX5, E2A, EBF) and genes involved in interaction with CD4+ T cells
- Validated by immunohistochemistry and GEP of primary HRS cells



Schwering et al., Blood, 2003; Tiacci et al., Blood 2012

Factors contributing to the downregulation of B cell genes in HRS cells



Küppers, Nature Rev Cancer, 2009
Kreher et al., PNAS, 2014

The lost B cell phenotype of HRS cells
- conclusions-

- **Multiple factors contributing to lost B cell program:**

  - downregulation of key B cell transcription factors

  - aberrant expression of master regulators of non-B cells

  - epigenetic silencing of B cell genes

- **Reexpression of B cell program toxic for HRS cells**

FOXO1: Xie, Blood 2012; PU.1: Yuki, Blood 2013; E2A: Guan, Oncotarget 2016

The lost B cell phenotype of HRS cells
- conclusions-

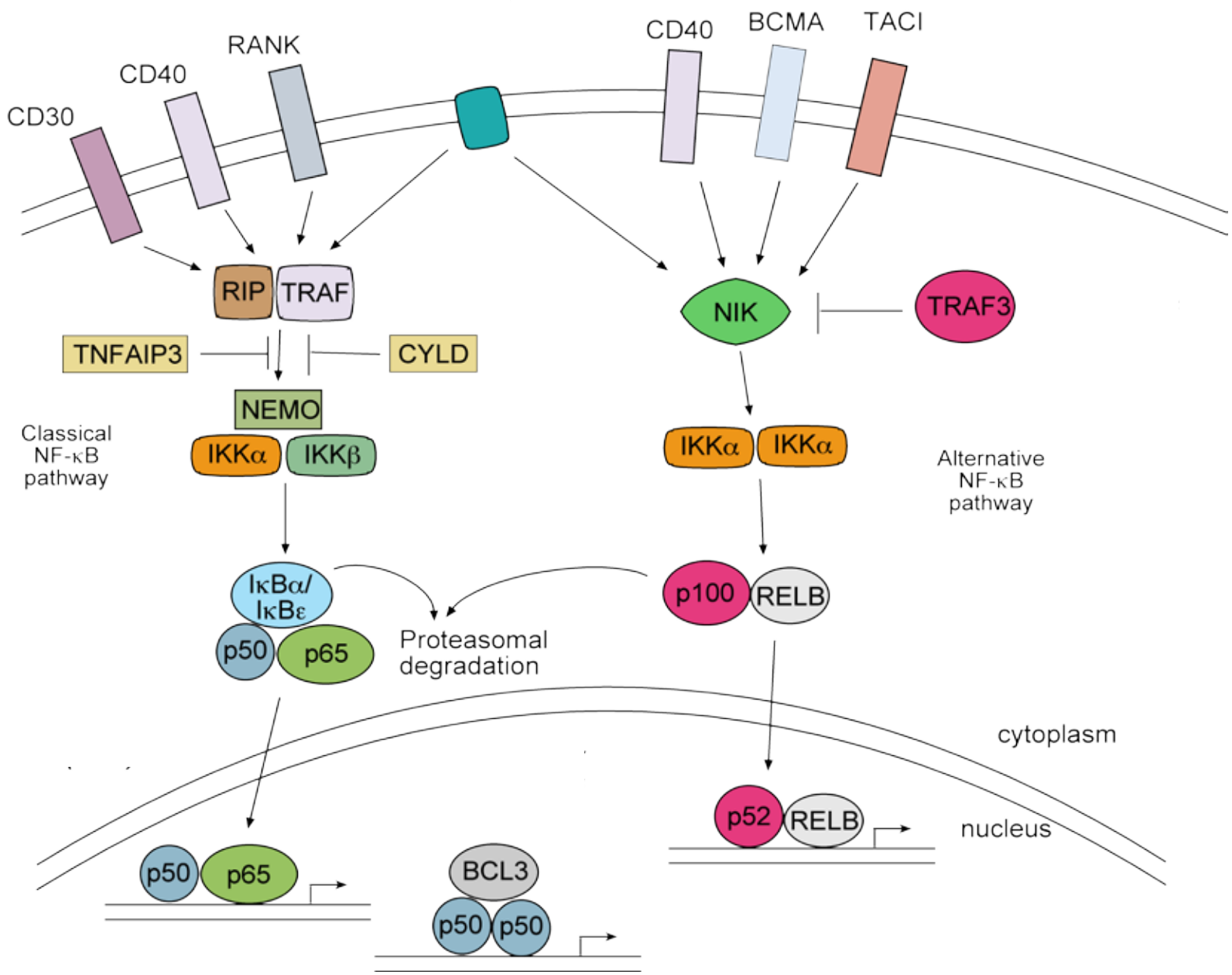
- **Multiple factors contributing to lost B cell program:**
  - **downregulation of key B cell transcription factors**
  - **aberrant expression of master regulators of non-B cells**
  - **epigenetic silencing of B cell genes**
- **Reexpression of B cell program toxic for HRS cells**

→ **Loss of B cell phenotype critical pathogenetic event**

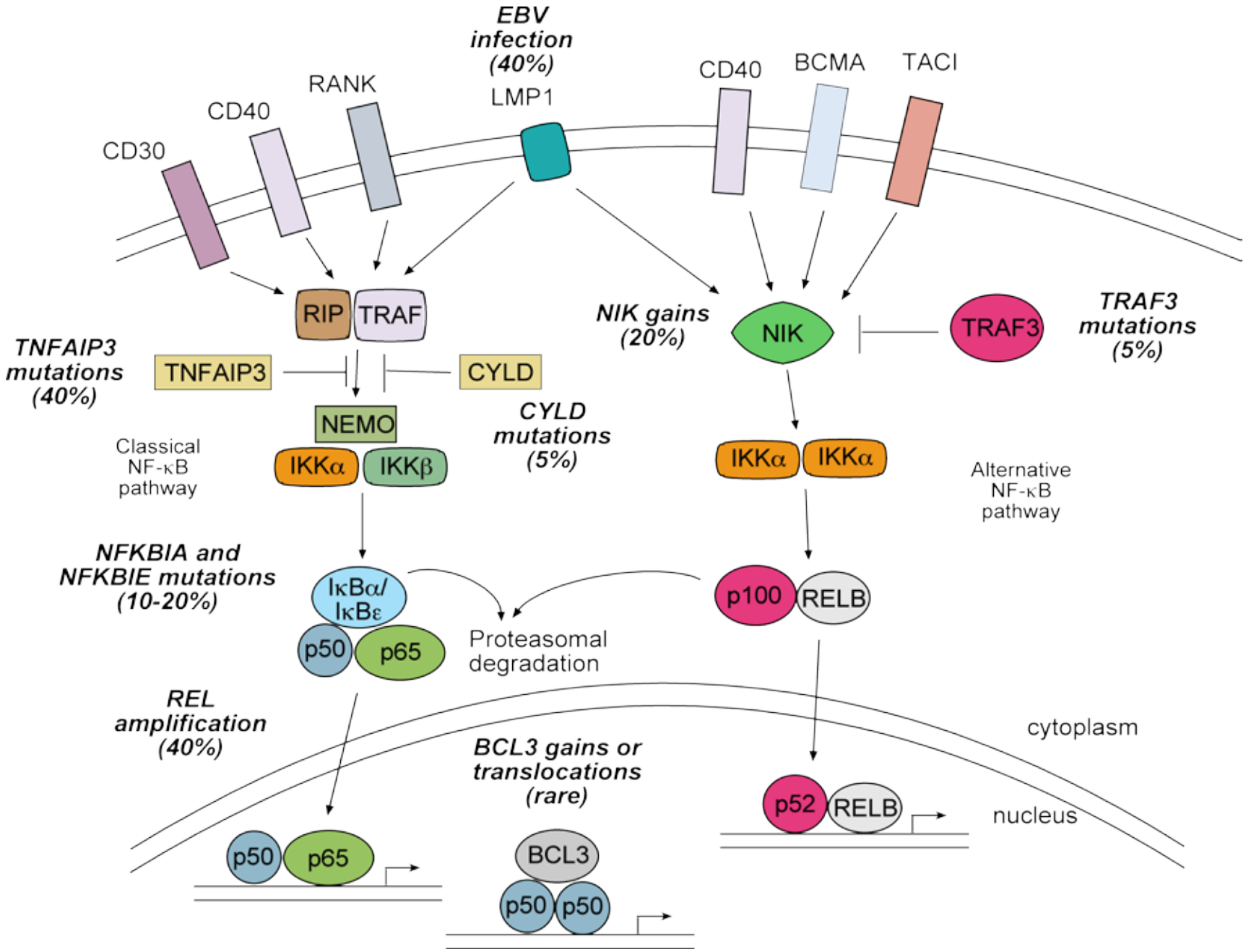
→ **Needed for HRS precursor cells to escape from apoptosis as crippled germinal center B cells?**

Genetic lesions in HRS cells

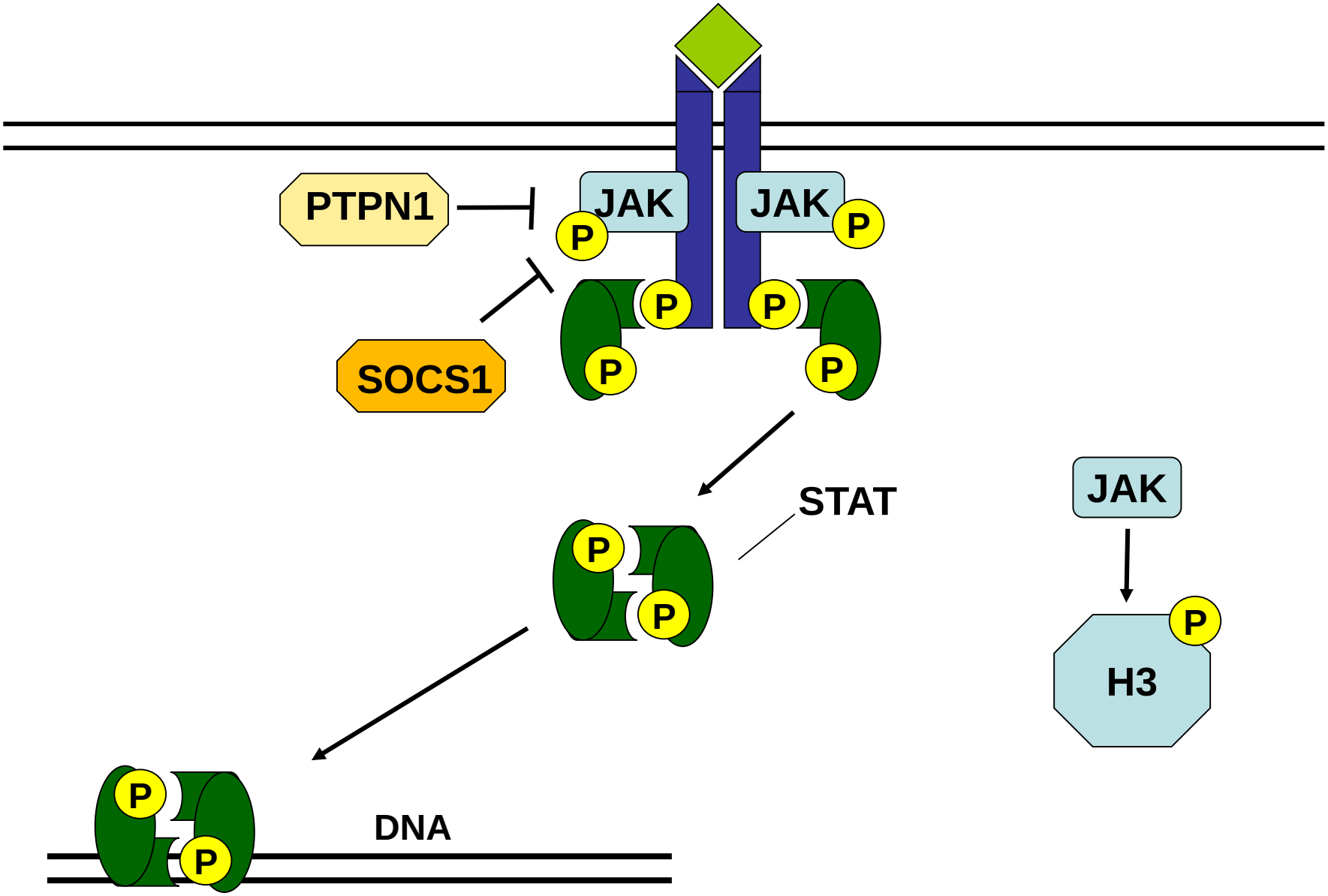
NF-kB pathway



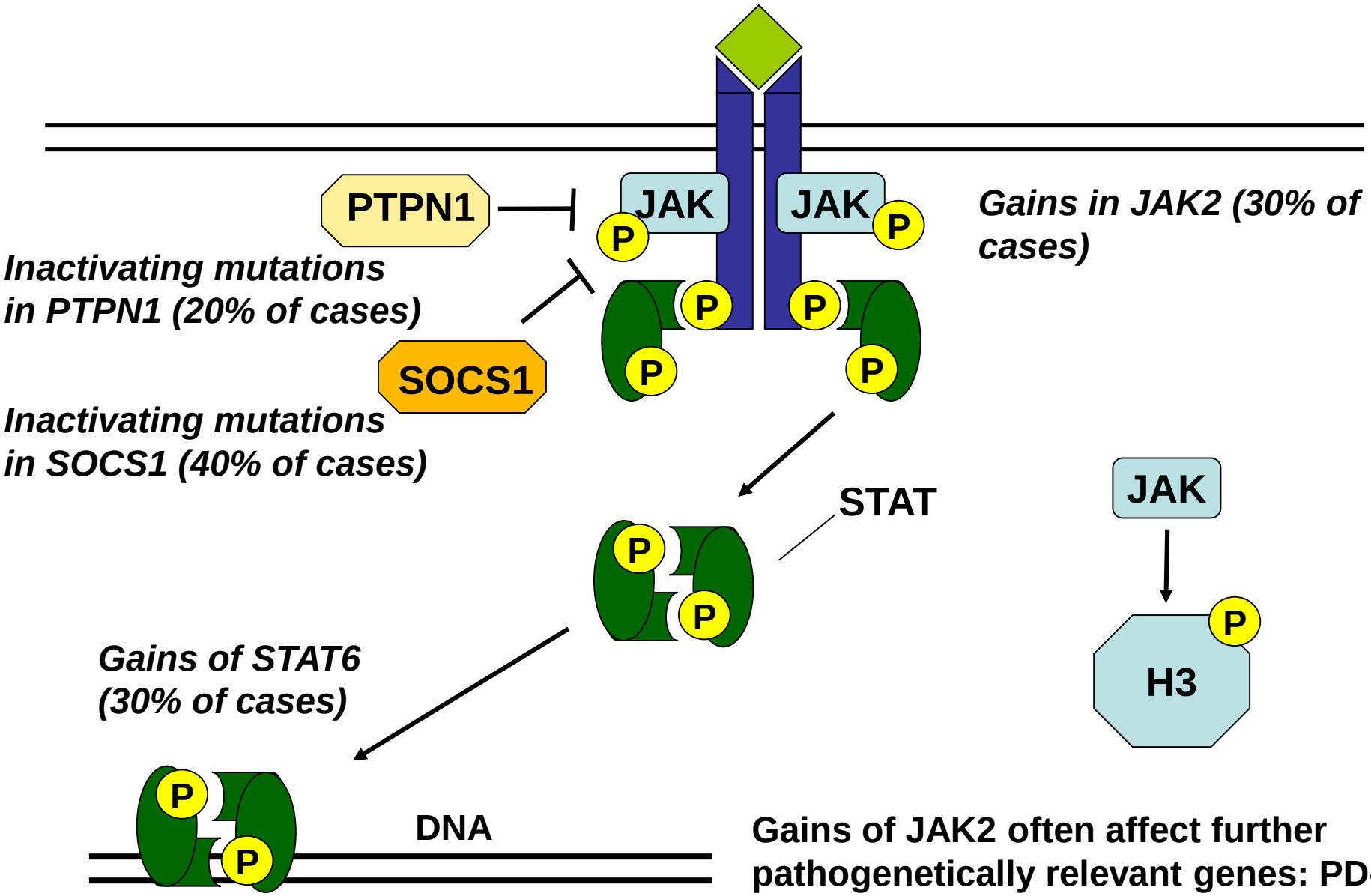
Multiple genetic lesions in the NF-kB pathway in HRS cells



Genetic lesions in the JAK-STAT pathway in HRS cells



Genetic lesions in the JAK-STAT pathway in HRS cells



Gains of JAK2 often affect further pathogenetically relevant genes: PD-1 ligands 1 and 2 and JMJD2C.

Further recently identified genetic lesions in HRS cells

- **Tanslocations affecting the MHC class II transactivator in ca. 15% of cHL** (Steidl et al., 2011)
- **Mutations in the MHC class I component b2 microglobulin in >50% of cHL** (Reichel et al., 2015)
- **Frequent mutations in CD58 gene in HL cell lines (3/7) and deletions in primary cHL cases (3/13)** (Schneider et al., 2015)

-> Immune evasion strategies of HRS cells?

Generation of bi- und multinuclear Reed-Sternberg cells from mononuclear Hodgkin cells

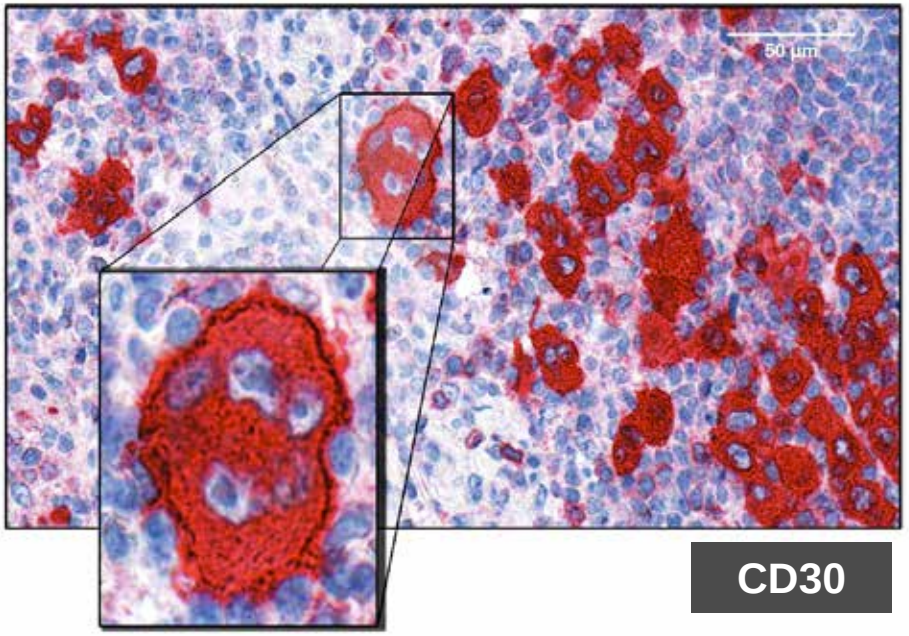
Relationship between Hodgkin and Reed-Sternberg cells

Key feature of classical HL:

Tumor cell population composed of mononuclear Hodgkin and bi- or multinucleated Reed-Sternberg cells

Mechanism?

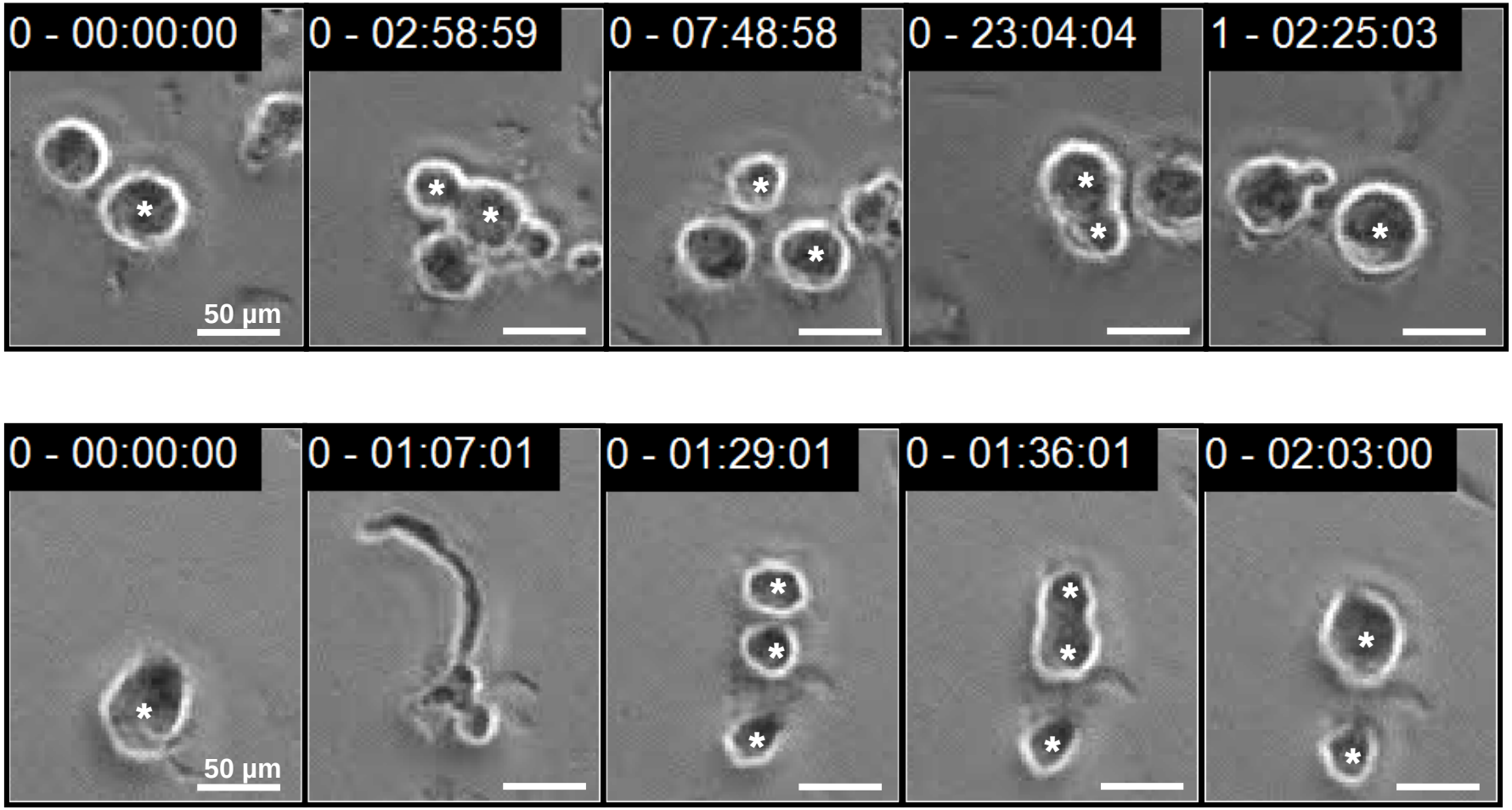
- **Fusion of two independent cells unlikely** **(Küppers et al., Blood 2000; Re et al., 2001)**
- **Mitosis without cell division (acytokinetic mitosis)?**
- **Other mechanism?**



Experiment:

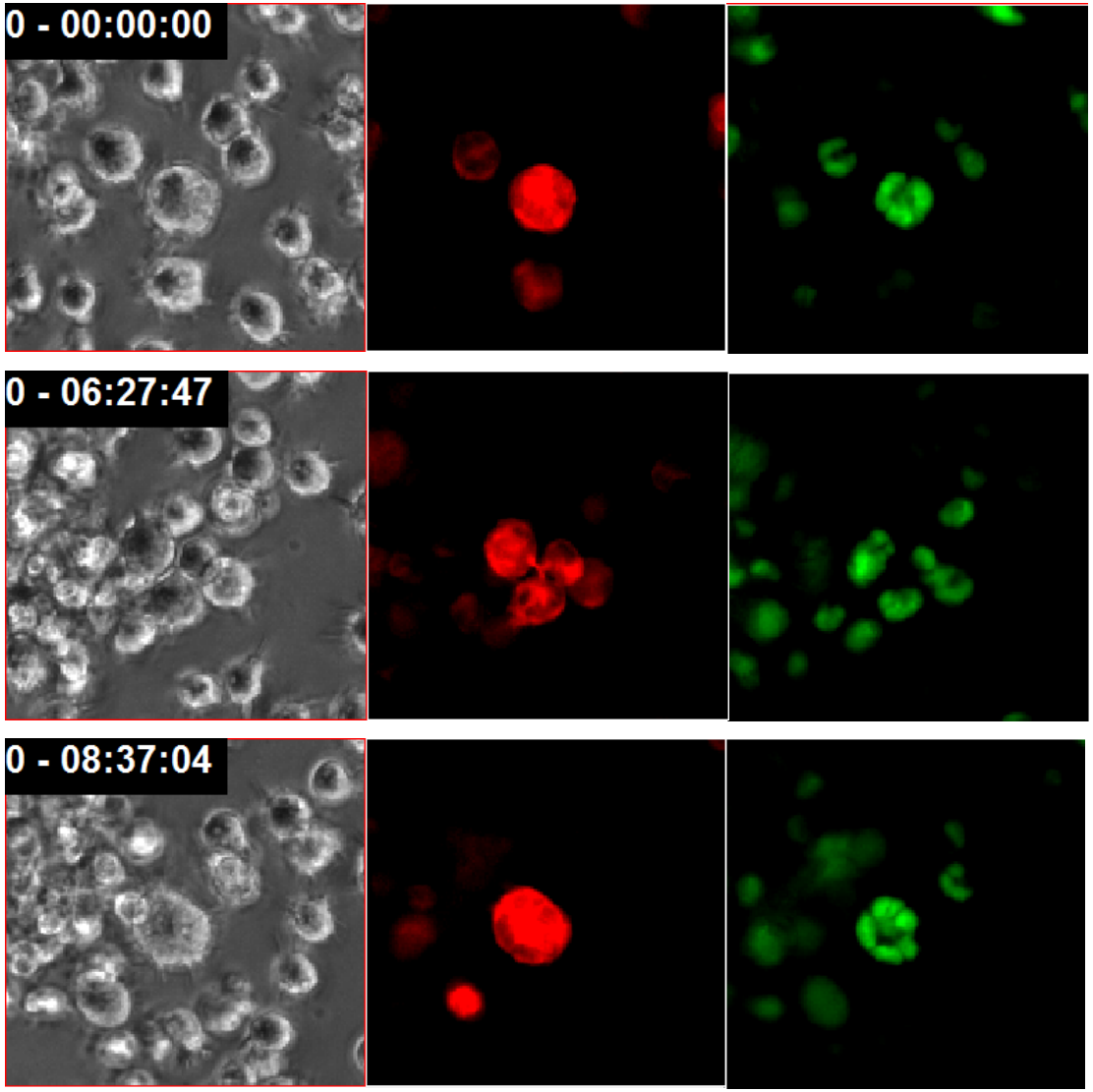
long-term time-lapse microscopy of HL cell lines

Time lapse microscopy of HL cell lines

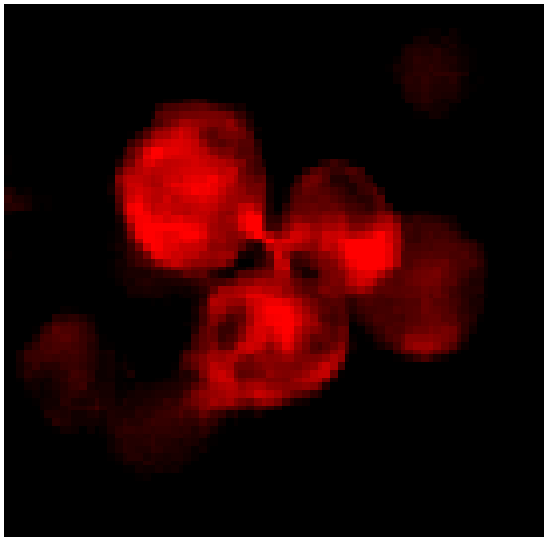


Rengstl, .., Rieger, Hansmann, PNAS, 2014

Complete or incomplete cytokinesis before refusion?



time-lapse experiment with tubulin-RFP labeled HL cell lines



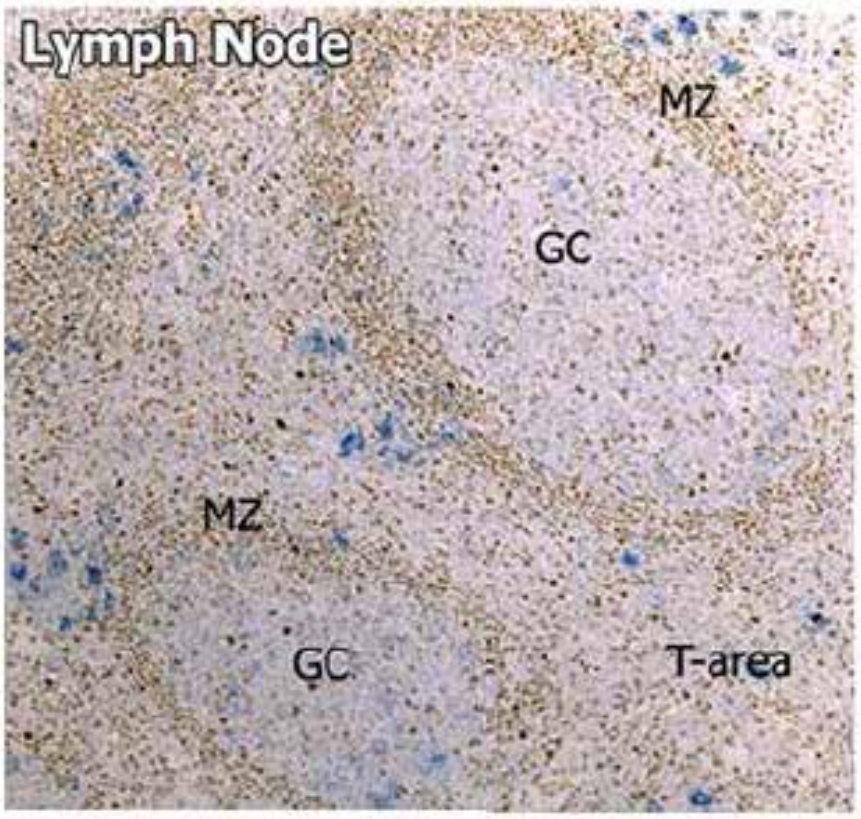
83% of refused cells with detectable persistent microtubule bonds

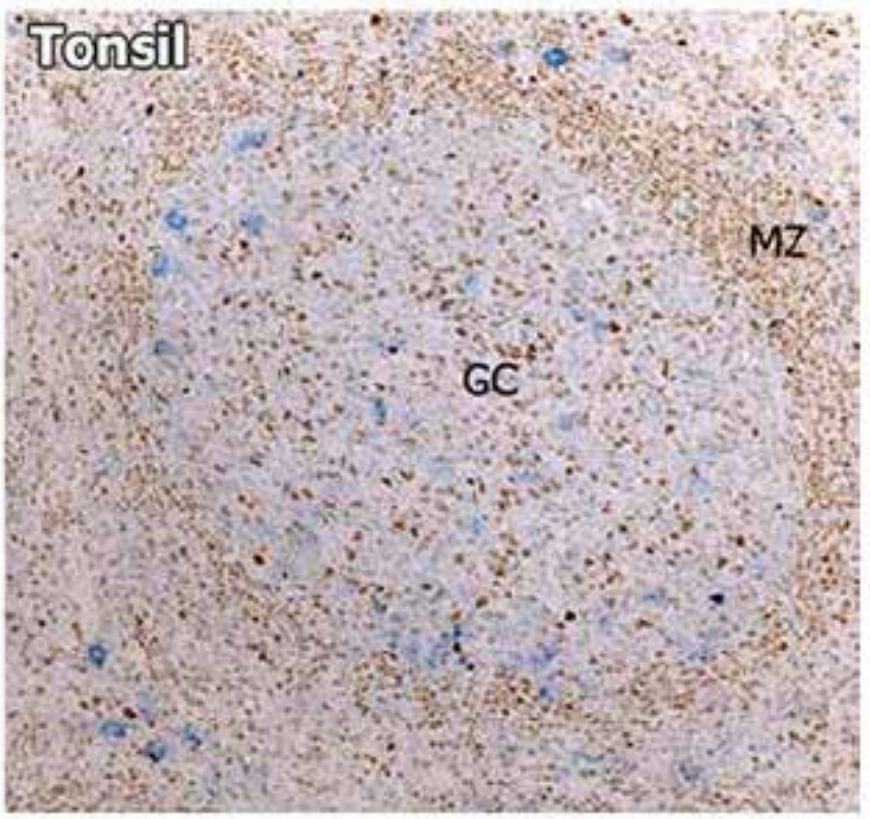
RS cell generation
- Summary and Conclusions -

- **Refusion as major route of RS cell generation from Hodgkin cells**
- **Refusion mostly if not always based on incomplete cytokinesis**
- **Mechanisms for failure to complete cytokinesis unknown**

Gene expression profiling analysis of CD30-positive B cells and their relationship to HRS cells of Hodgkin lymphoma

Extrafollicular and GC CD30-positive lymphocytes

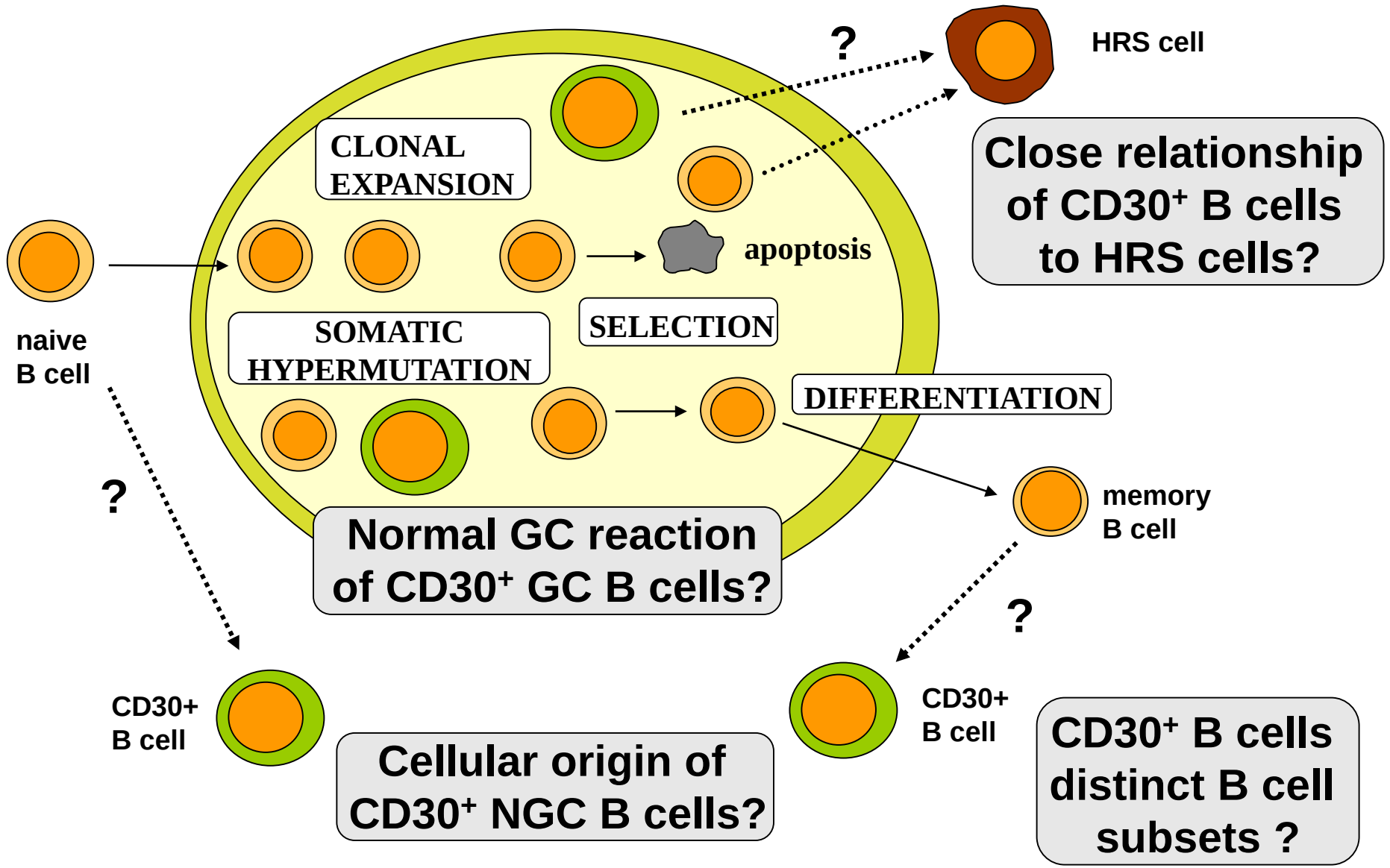




blue = CD30

J Mol Histol 2005;36:249

Human CD30⁺ B cells: open questions

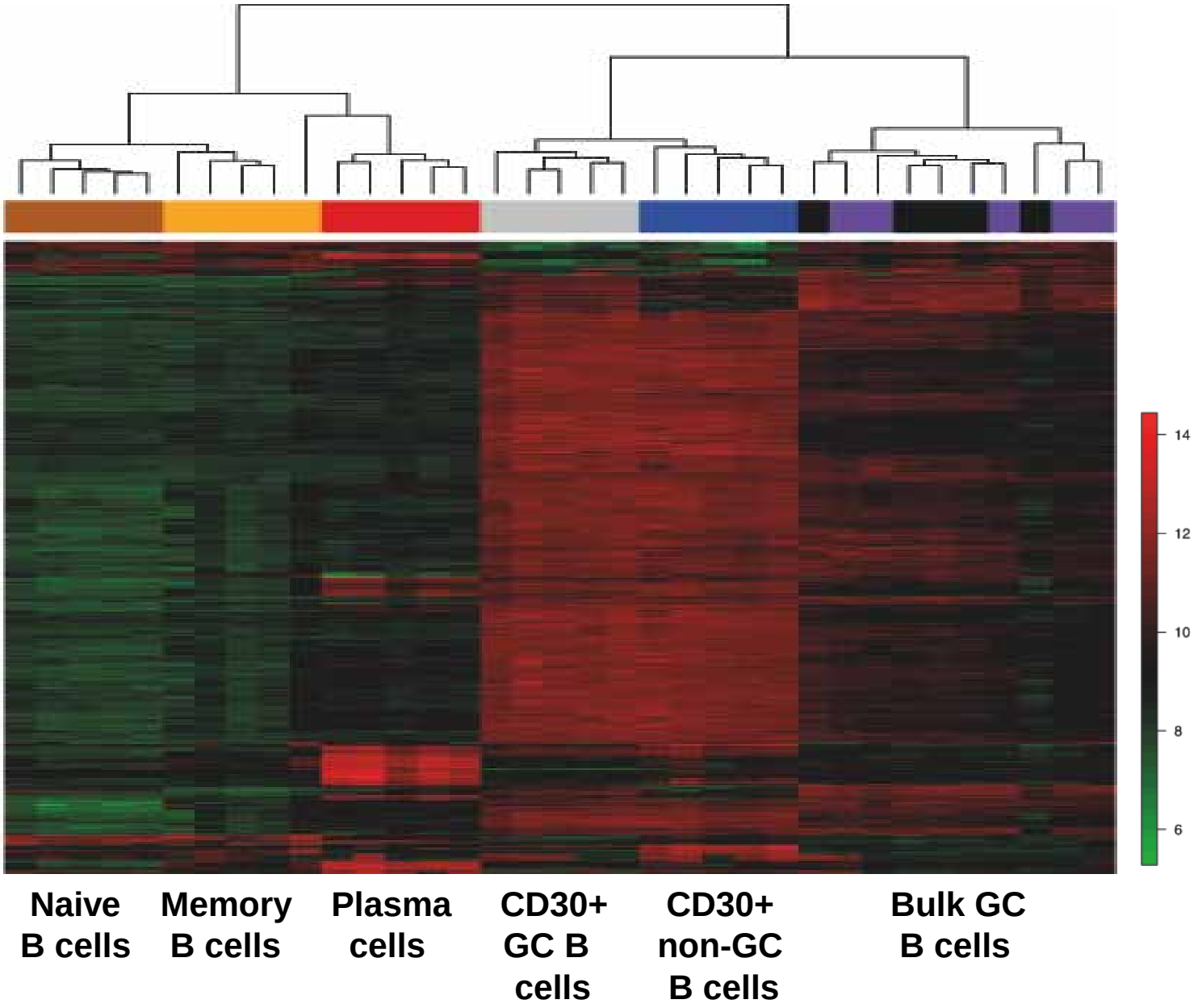


VH gene mutation analysis of CD30+ GC and non-GC B cells

Donor	CD30+ cells	Mutated sequences (%)	Aver. mutation frequency* (%)	R/S ratios FRs#
1	GC	30 / 30 (100)	5.9	1.4
2	GC	23 / 23 (100)	8.2	1.9
3	GC	28 / 28 (100)	4.6	2.3
4	GC	38 / 41 (93)	6.2	1.5
3	non-GC	16 / 20 (84)	3.9	1.6
4	non-GC	36 / 41 (88)	5.6	1.6
5	non-GC	22 / 26 (79)	8.6	1.4
6	non-GC	9 / 20 (42)	2.2	1.9

*of mutated sequences; #of productive rearrangements

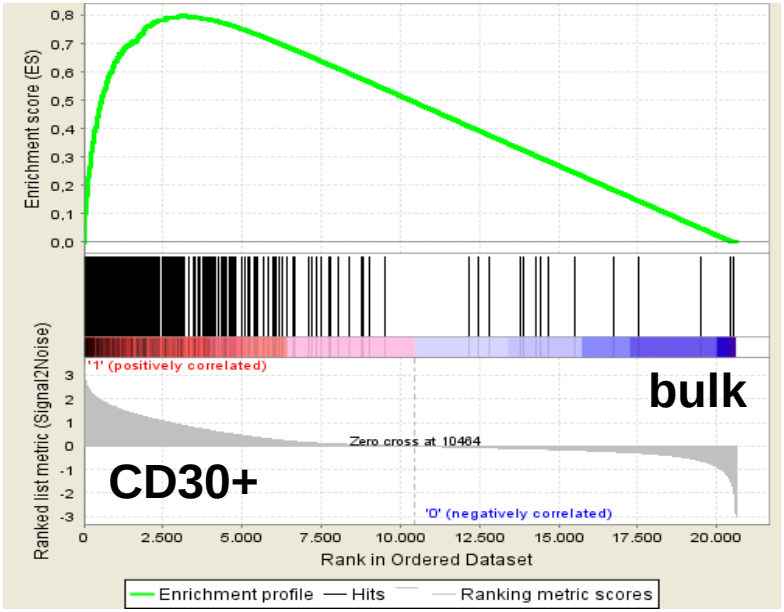
Unsupervised hierarchical clustering of normal B cell subsets



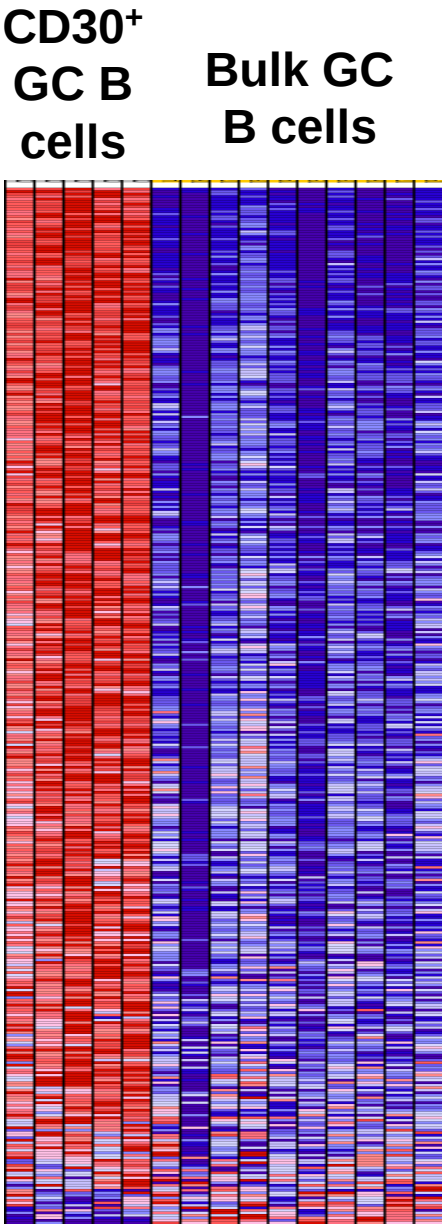
Manhatten distance
Average linkage
SD > 1
407 probesets

High MYC activity in CD30^{+} GC B cells

CD30+ GC B cells versus bulk GC B cells



Gene set	MYC targets Seitz; 2011 PLoS One
NES	2.105
Nominal p-value	0.0
FDR q-value	0.0



26 MYC gene sets among most significantly enriched gene sets in normal CD30^{+} vs. bulk GC B cells

Differential gene expression between CD30+ B cells and HRS cells

Higher expression in CD30+ B cells

- typical B cell genes
- signatures for strong proliferation (MYC, E2F)
- genes with roles in regulation of mitosis and DNA stability

Higher expression in HRS cells

- regulators of extracellular matrix
- many chemokines and cytokines
- non-B-cell genes (CD3D, granzyme B, ID2,..)
- several transcription factors: MAF, MAFB, STAT1, BATF3

⟶ Key features of HRS cells are disease-associated

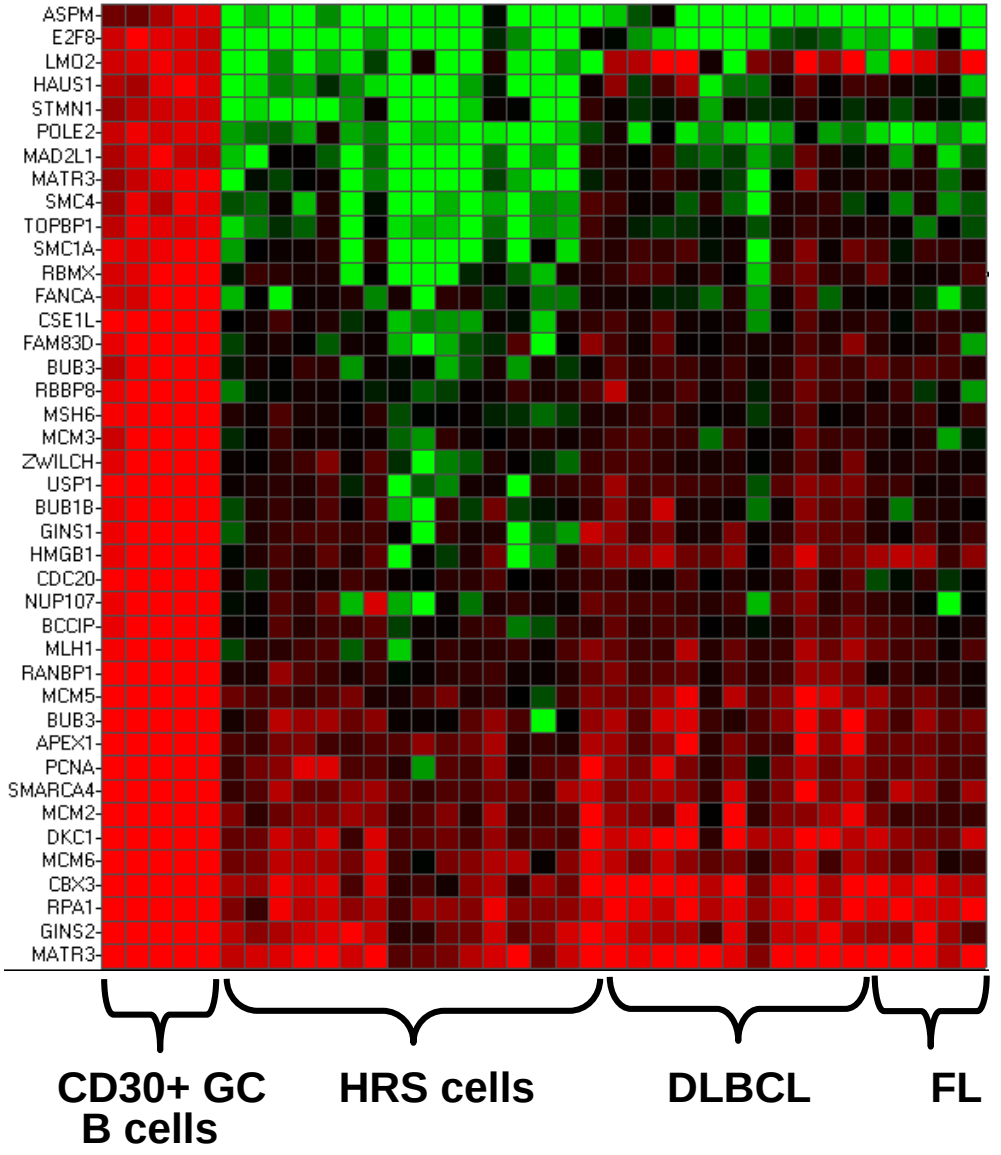
Potential mechanisms for genetic instability and disturbed cytokinesis in HRS cells

Supervised analysis of genes differentially expressed between HRS cells and CD30+ GC B cells

Among 207 genes downregulated at least 5-fold in HRS cells, there were 41 genes with functions in mitosis, cytokinesis, genomic stability, DNA repair

Such a downregulation is less pronounced in DLBCL and FL

Potential cause for the genomic instability of HRS cells and generation of multinucleated RS cells



Gene expression profiling and V gene mutation analysis of CD30+ B cells

- Identification of distinct CD30+ GC and non-GC B cell subsets with specific gene expression patterns.
- High MYC activity in CD30+ GC B cells -> GC B cells at transition from centrocytes back to centroblasts
- CD30+ non-GC B cells mostly post-GC B cells; highly activated and proliferating memory B cells
- HRS cells show significant similarities to normal CD30+ B cells (a “CD30 activation“ signature or indication for cell of origin?).
- Key features of HRS cells are disease-associated

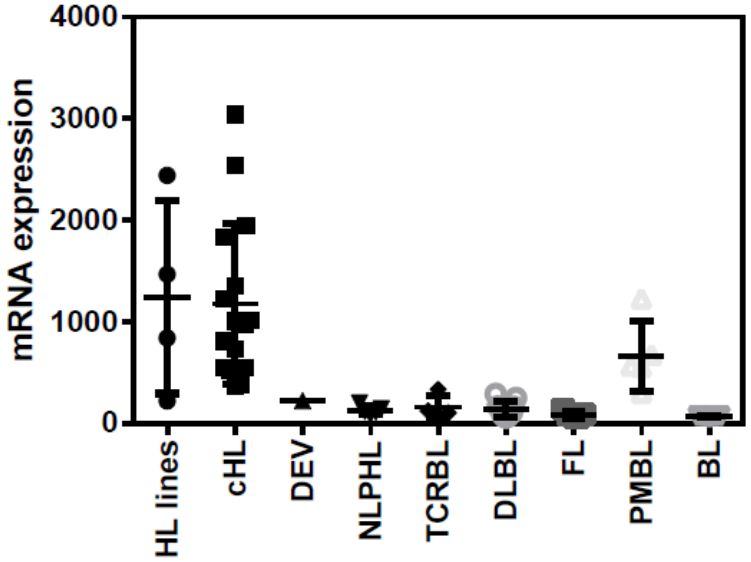
The AP-1 transcription factor BATF3 is constitutively expressed in classical Hodgkin lymphoma and contributes to tumor cell survival and proliferation

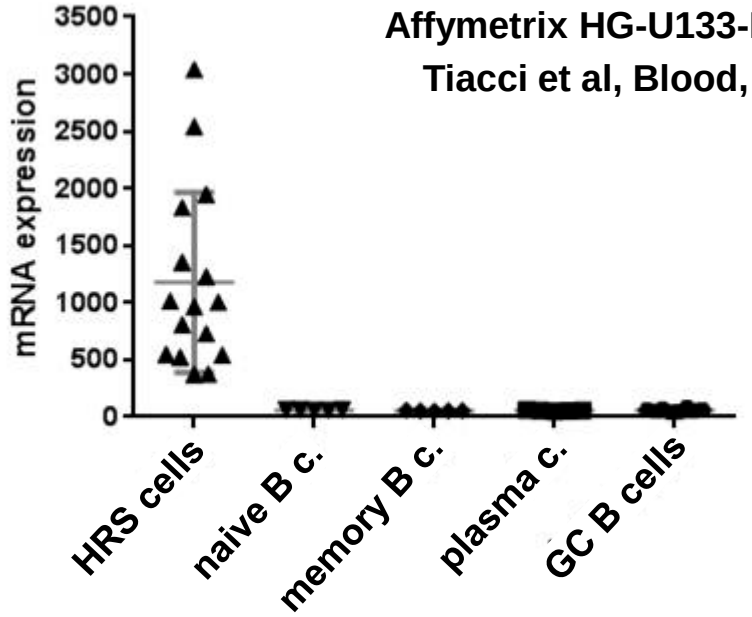
Gene expression profiling studies of HRS cells revealed high expression of BATF3 in primary HRS cells

RT-PCR of microdissected or sorted cells

Samples	BATF3 positive
HRS cells	
case 1	3/3
case 2	2/3
case 3	3/3
case 4	2/3
Non-HRS cells	**0/12**
GC B cells	**0/6**

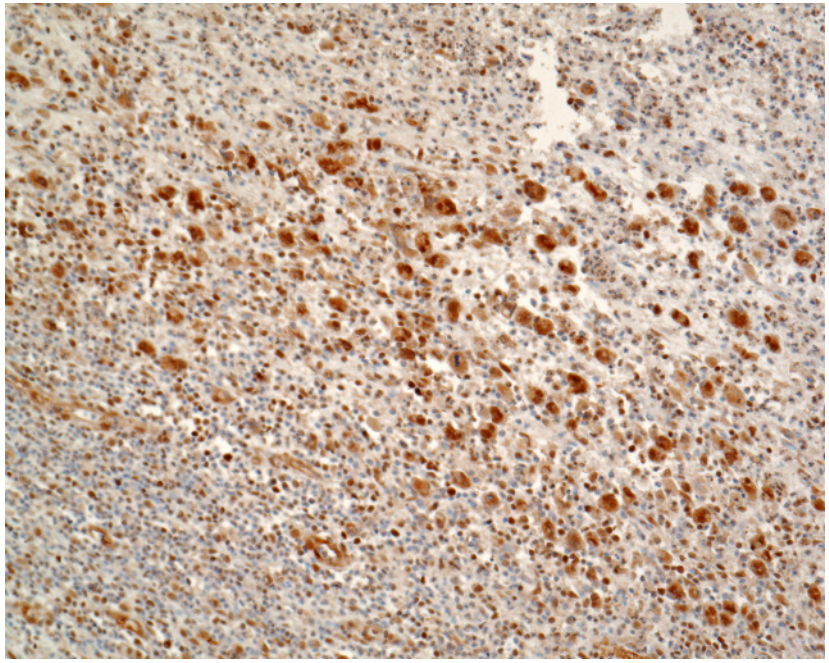
Schwering et al, Mol Med, 2003





Affymetrix HG-U133-Plus2.0
Tiacci et al, Blood, 2012

Frequent BATF3 protein expression in classical HL, PMBCL and ALCL



Lymphoma		BATF3 positive
classical Hodgkin lymphoma	EBV-	12 /20
	EBV+	1 / 10
PMBCL		8 / 9
ALCL*		40 / 40

Hodgkin lymphoma, BATF3 staining

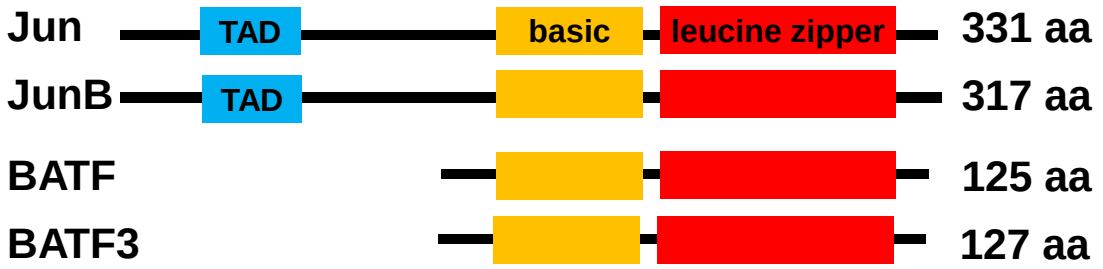
*Eckerle et al., Leukemia, 2009

- Frequent BATF3 protein expression in all three types of lymphomas
- EBV+ HRS cells rarely positive
- Lymphomas share CD30 positivity of tumor cells & JAK/STAT activity

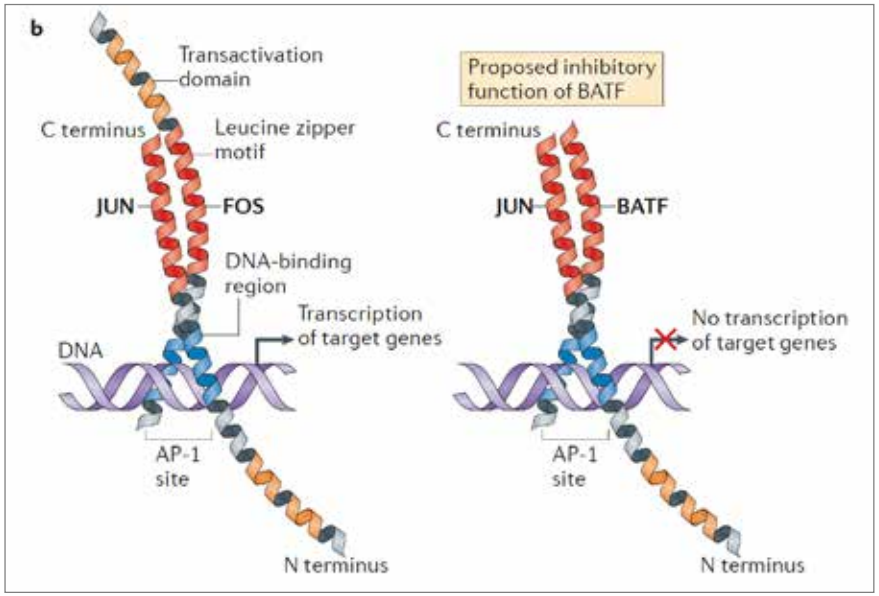
Basic facts about BATFs

- Members of the AP-1 family
- form heterodimers with other AP-1 family members
- form composite elements with IRFs
- Can have inhibitory and activating functions
- BATF3 mainly expressed in TH1 cells and subsets of dendritic cells

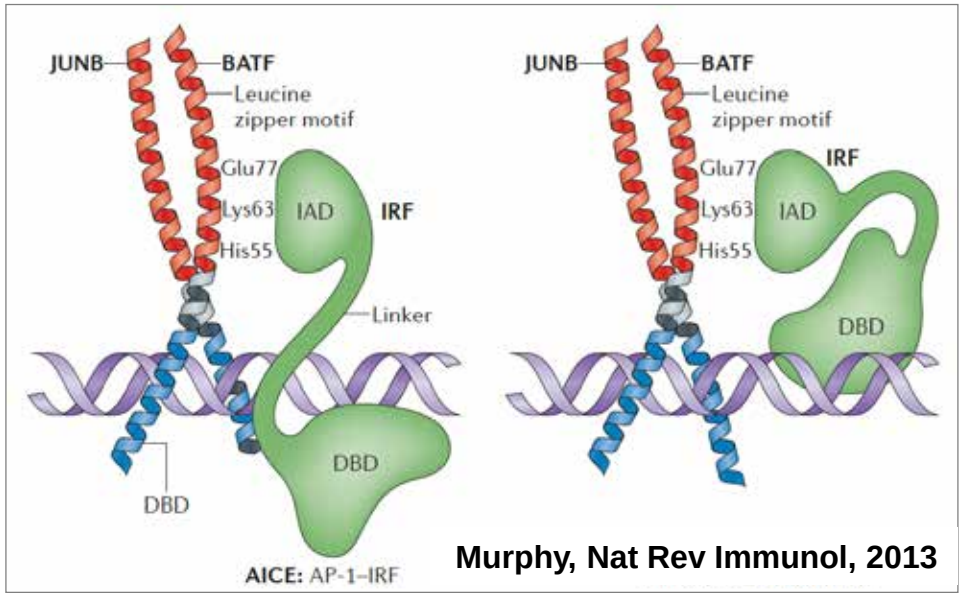
Transactivation, DNA binding and dimerization domains



Proposed inhibitory function of BATF

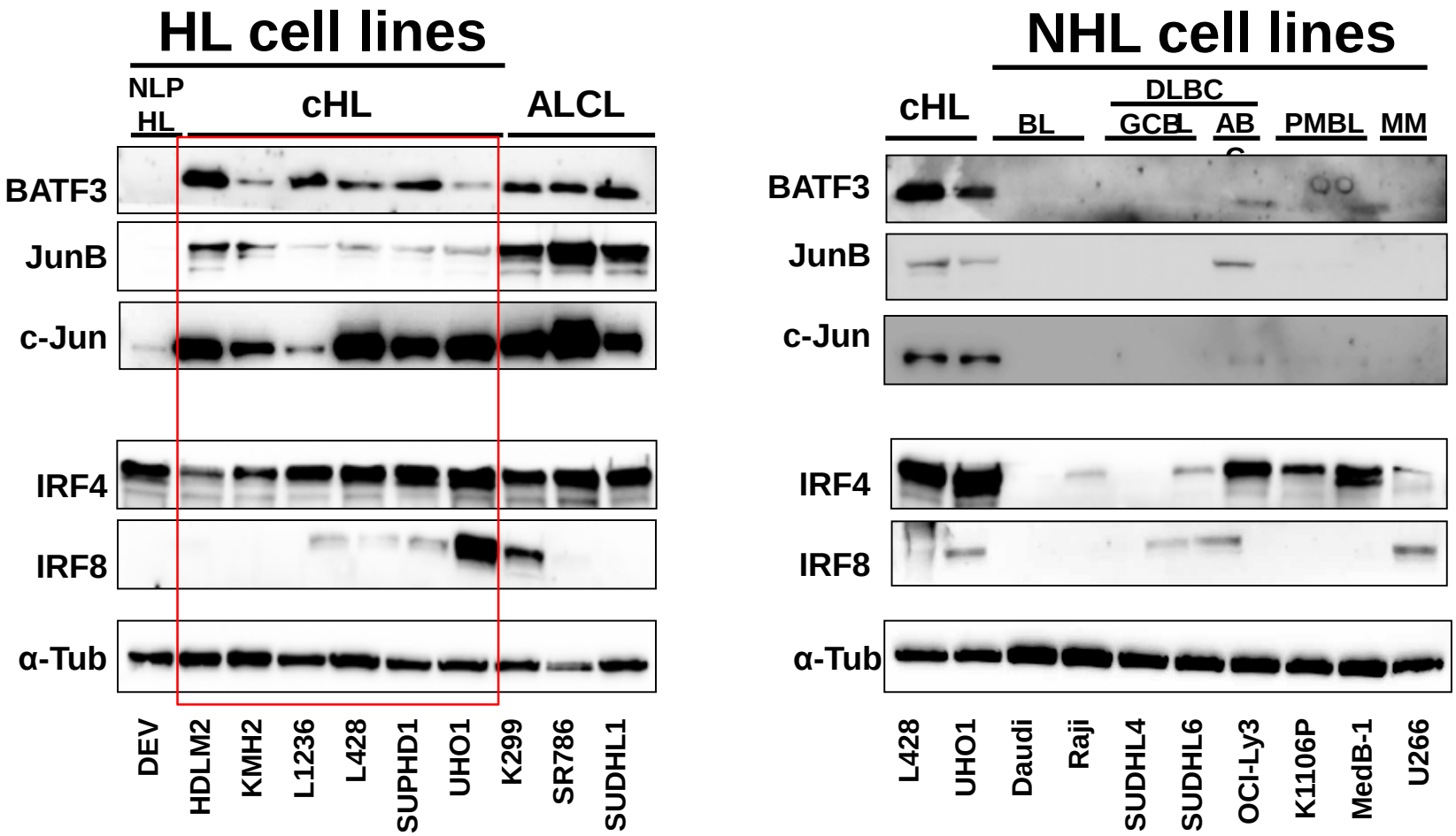


Interaction at AP-1-IRF composite elements



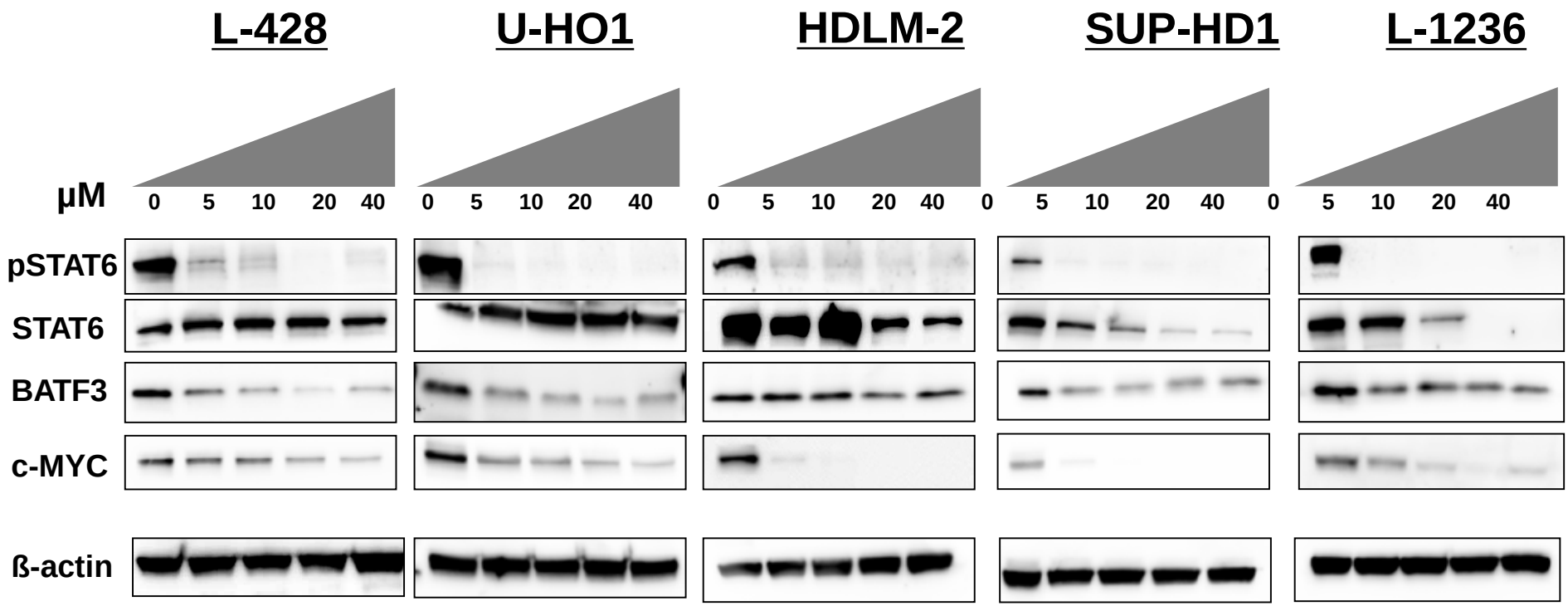
Murphy, Nat Rev Immunol, 2013

High expression of AP-1 and IRF factors in classical HL and ALCL cell lines



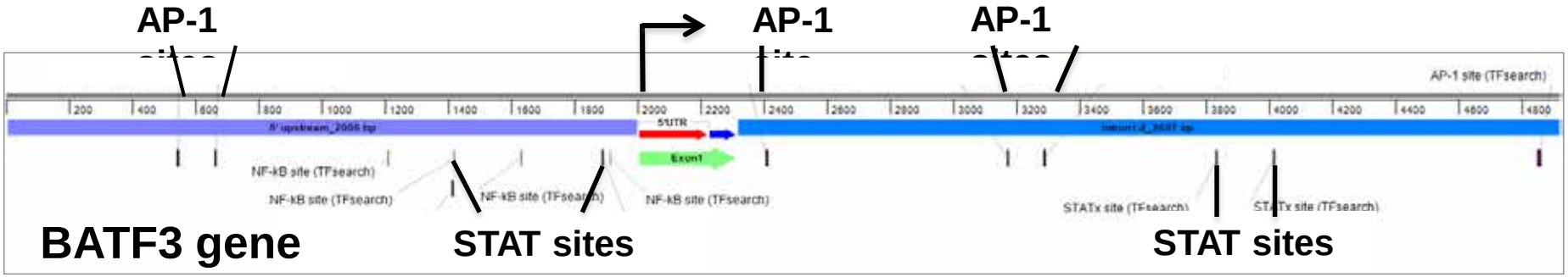
Inhibition of JAK2 leads to downregulation of BATF3 in 4/5 cHL cell lines

BATF3 downregulation upon JAK2 inhibition in a PMBCL line (Rui et al., Cancer Cell 2010)

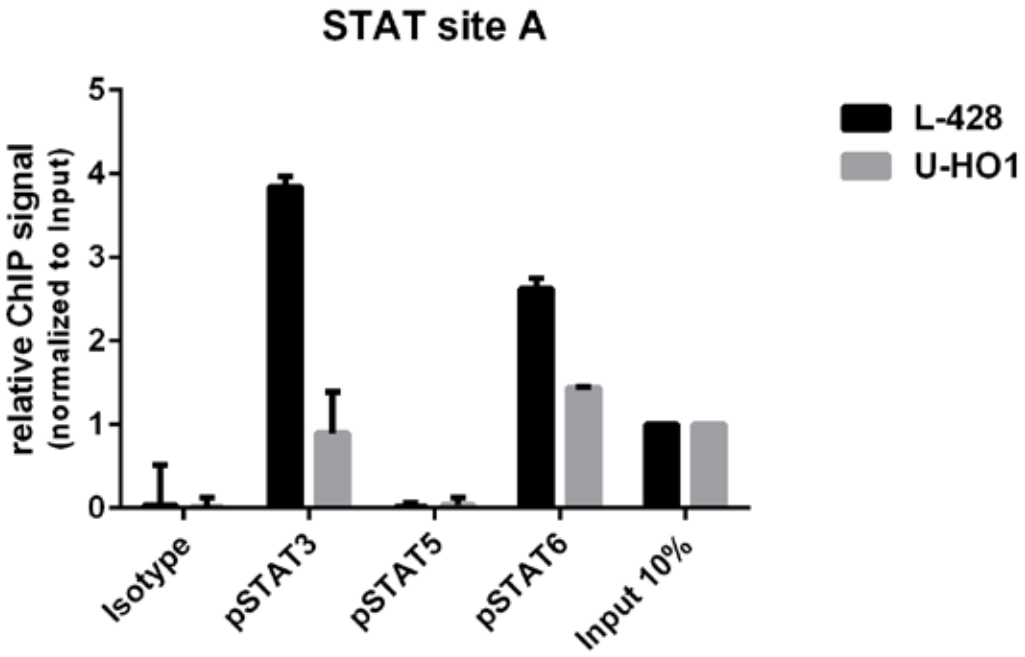


TG101348/ Fedratinib (Jak2 inhibitor)

STAT factors bind to the BATF3 promoter

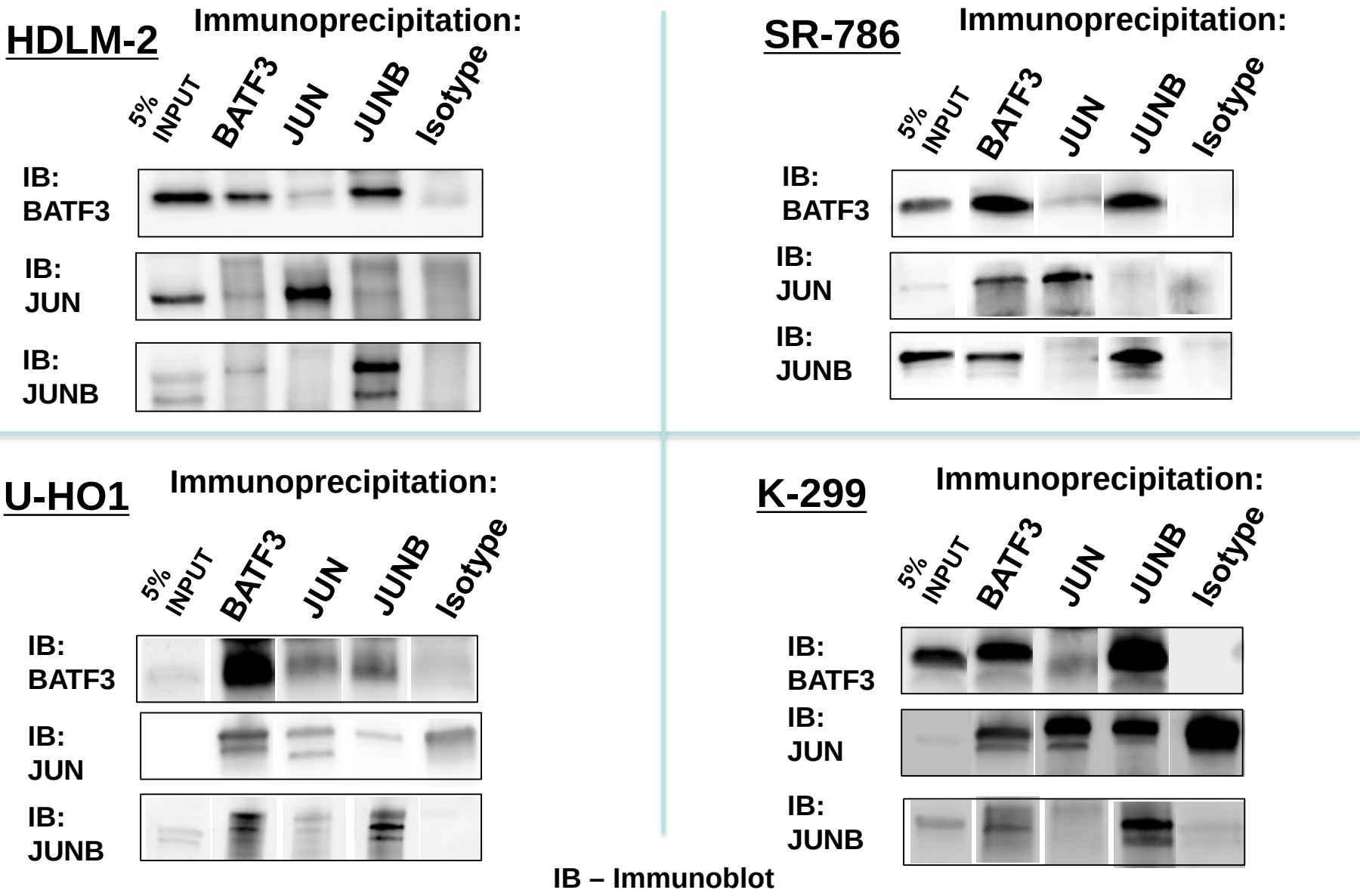


Chromatin immunoprecipitation and quantitative PCR to study binding of pSTAT3, pSTAT5 and pSTAT6 to a putative STAT binding site in the BATF3 promoter in the cHL cell lines L-428 and U-HO1.



→ **Binding of pSTAT3 and pSTAT6 to the BATF3 promoter in cHL cell lines**

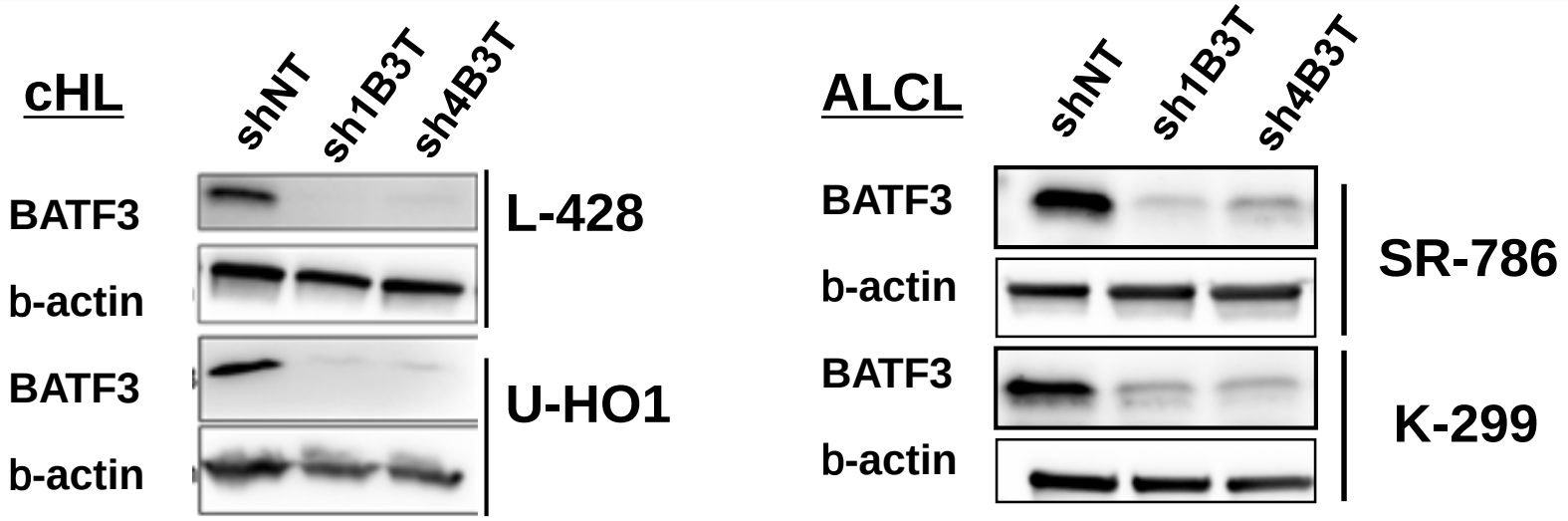
The AP-1 family members JUN and JUNB form heterodimers with BATF3 (co-immunoprecipitation studies)



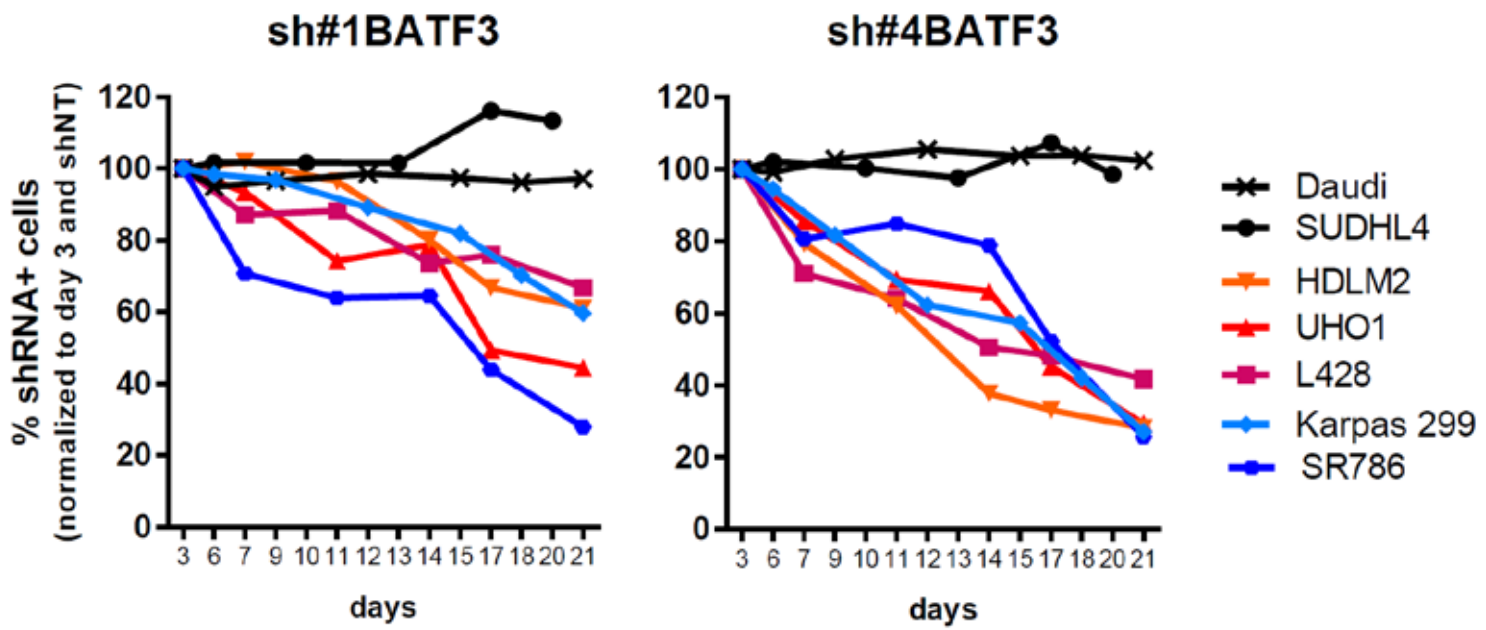
IB – Immunoblot

shRNA-mediated down-regulation of BATF3 leads to growth disadvantages of HRS and ALCL cells

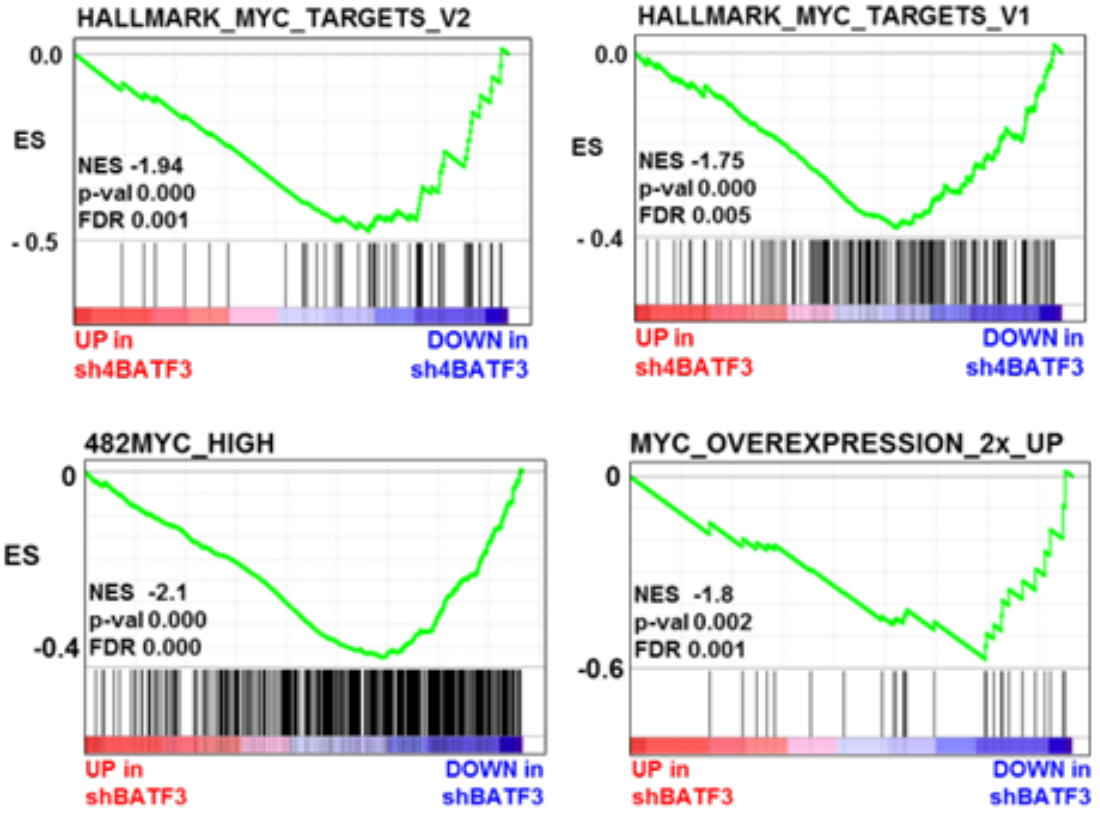
A)



B)



Downregulation of positive MYC target genes in L428 HRS cells upon downregulation of BATF3

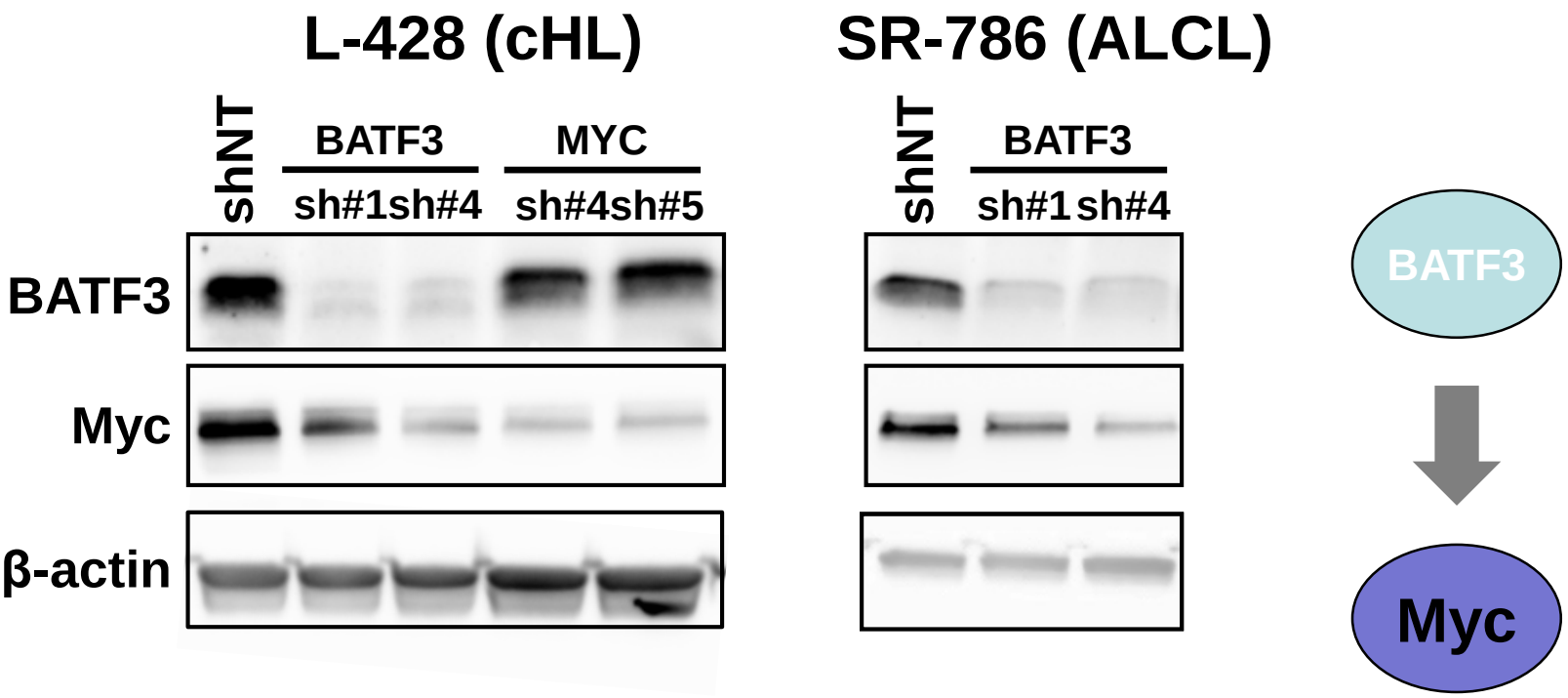


Gene sets	NES	p-val	FDR
gene sets enriched in sh4BATF3			
HALLMARK_INTERFERON_GAMMA_RESPONSE	2,17	0	0
HALLMARK_INTERFERON_ALPHA_RESPONSE	2,12	0	0
HALLMARK_IL6_JAK_STAT3_SIGNALING	1,97	0	0
HALLMARK_TNFA_SIGNALING_VIA_NFKB	1,94	0	0,001

Gene sets	NES	p-val	FDR
gene sets enriched in shNT			
HALLMARK_MYC_TARGETS_V2	-1,94	0	0,001
HALLMARK_MYC_TARGETS_V1	-1,75	0	0,005

p<0.01, FDR<0.05

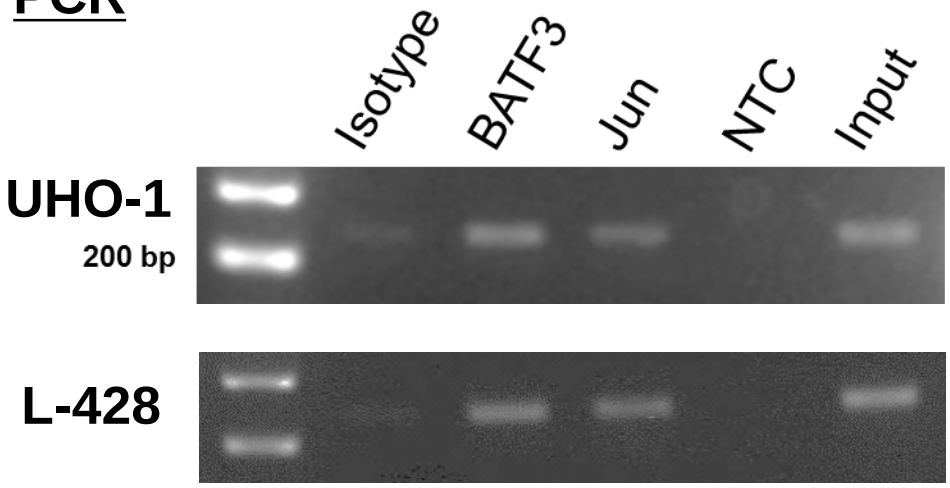
Downregulation of BATF3 causes downregulation of MYC



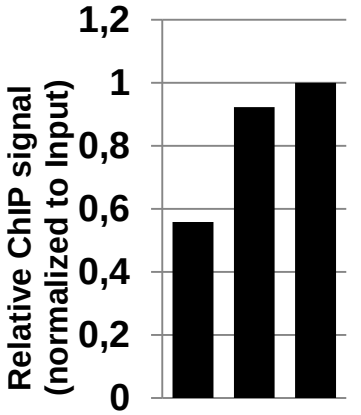
BATF3 and JUN bind to the MYC promoter, indicating direct regulation of MYC expression

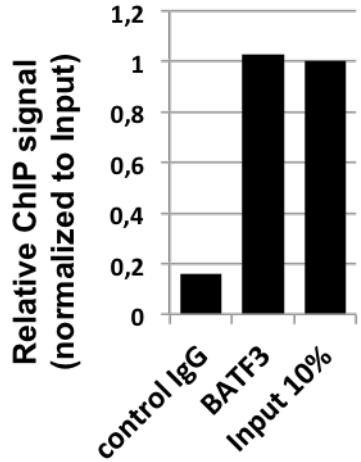
Chromatin immunoprecipitation for BATF3 and JUN

PCR



qPCR

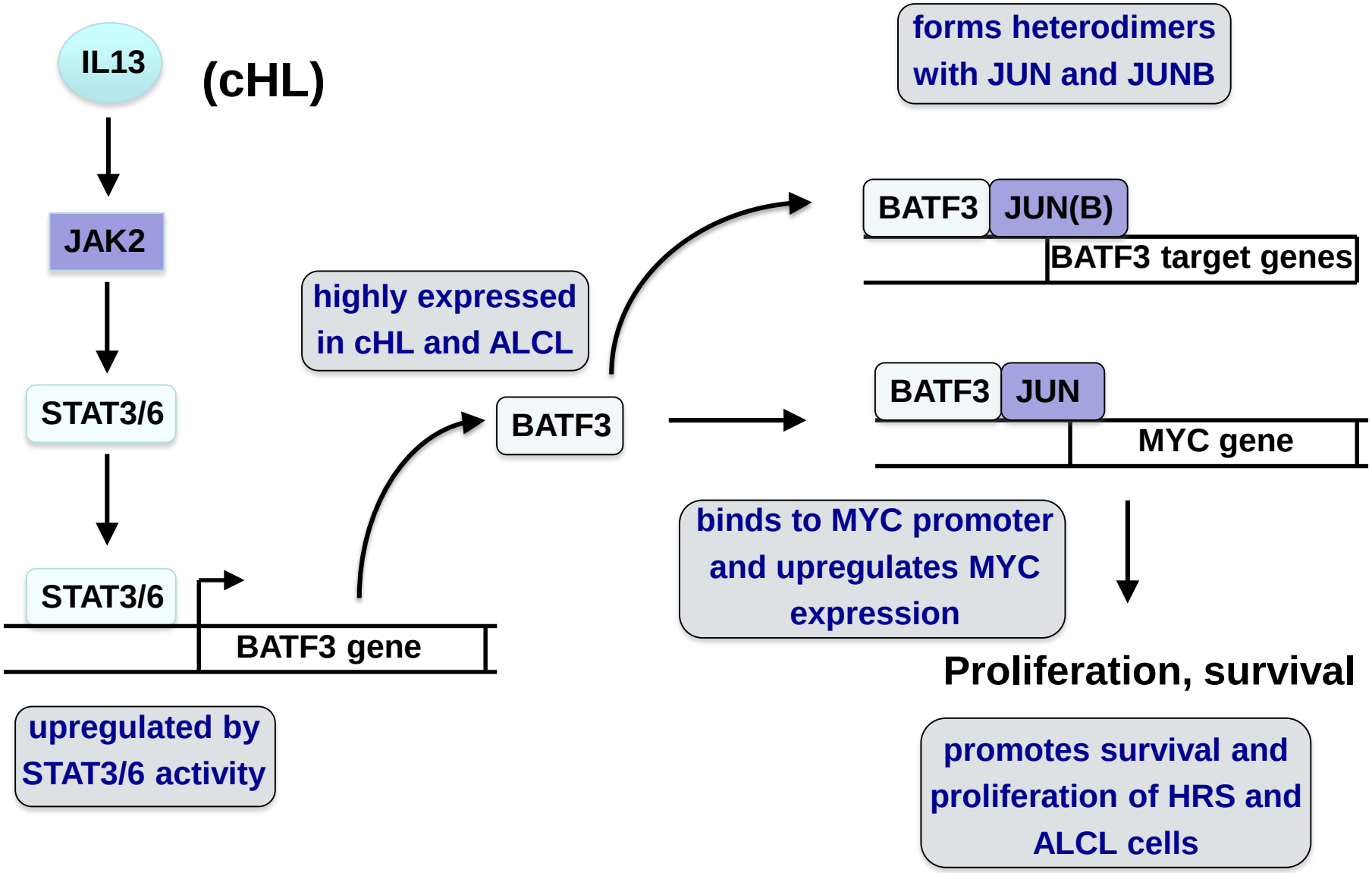




JUN and JUND binding known (Iavarone et al., J Biol Chem 2003)

BATF3 in Hodgkin lymphoma and ALCL

- Summary and Conclusions -



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