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New frontiers in CLL Research
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ERIC
european research initiative on CLL

Microenvironmental interactions and signaling pathways in Chronic Lymphocytic Leukemia

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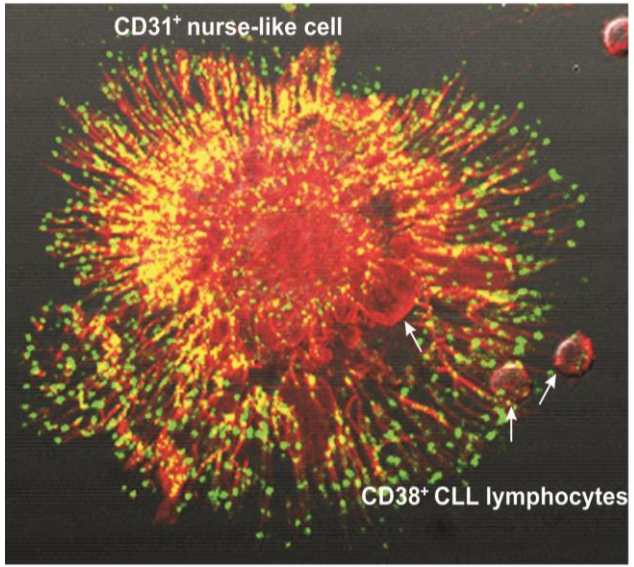
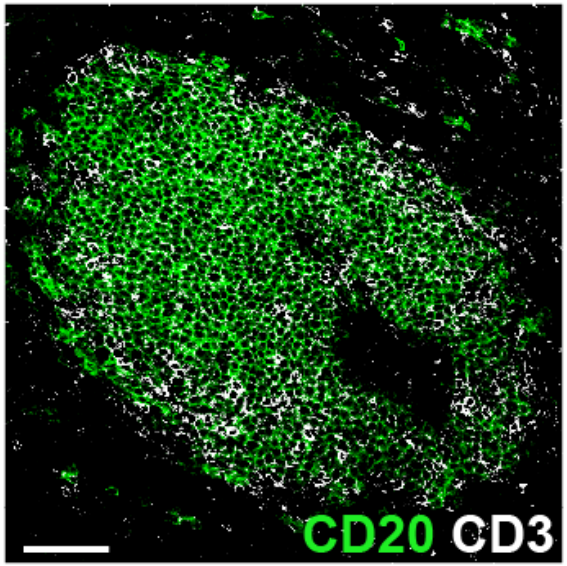
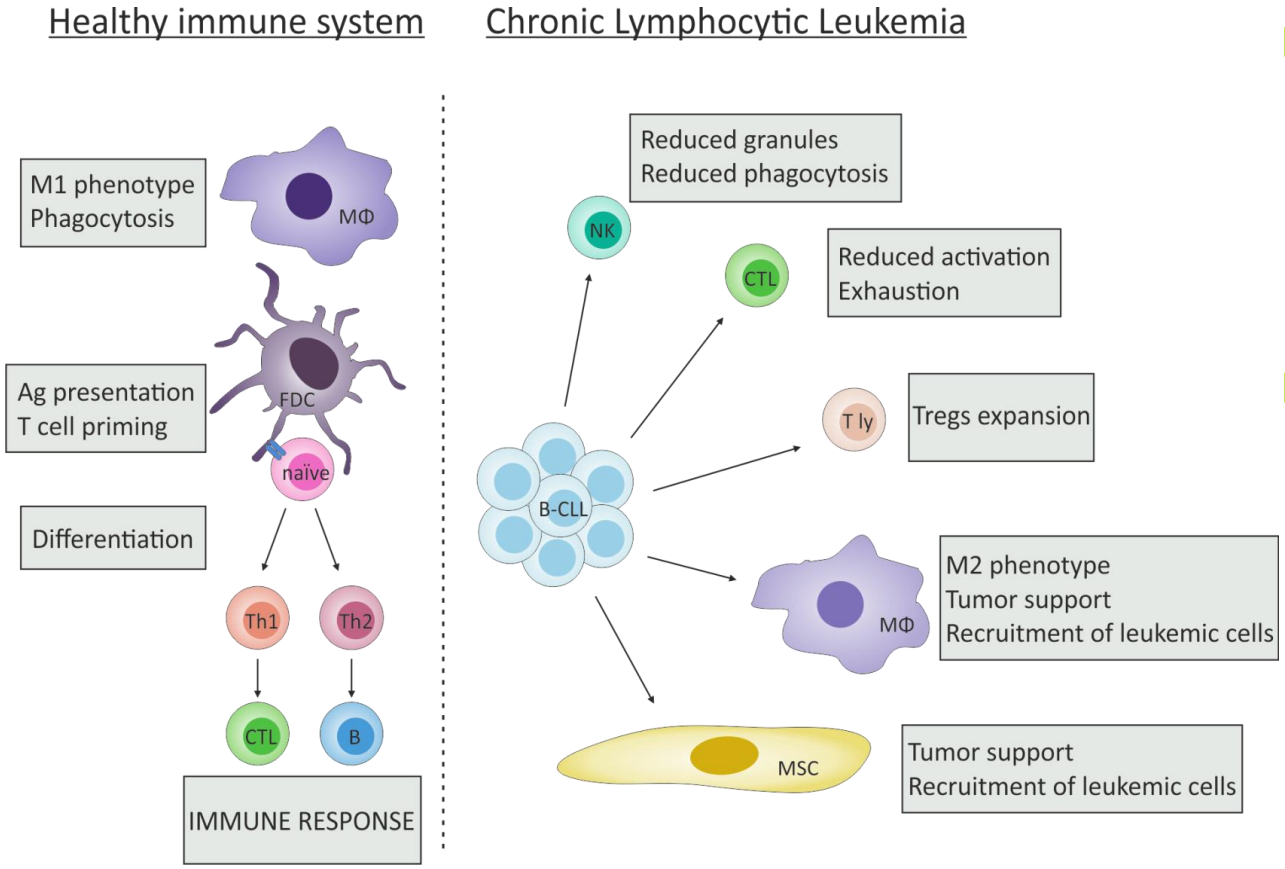
CLL cells need the microenvironment

- Purified CLL cells rapidly die *in vitro*
- Stromal cells, nurse-like cells and T cells prolong CLL survival *in vitro*
- CLL cell lines are difficult to stabilize
- Xenograft models require autologous T cells





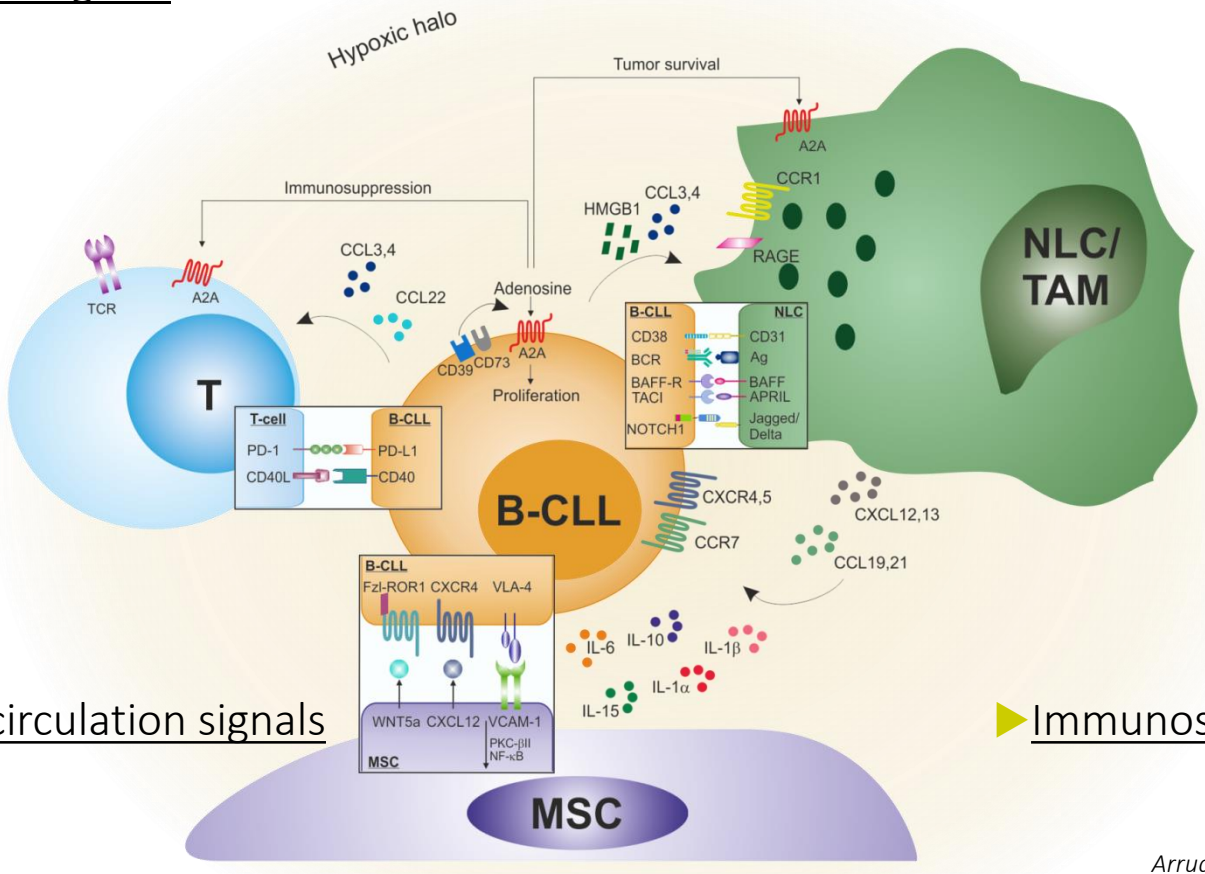
The microenvironment is changed by CLL cells



- Infections remain a major cause of morbidity and mortality in CLL patients
- Alterations of immune system in CLL are the result of infection-related mechanisms, chemotherapy and leukemia-driven re-shaping of cell functions occurring in the microenvironment

The CLL microenvironment: a crowded space

- BCR-mediated signals
- Accessory signals

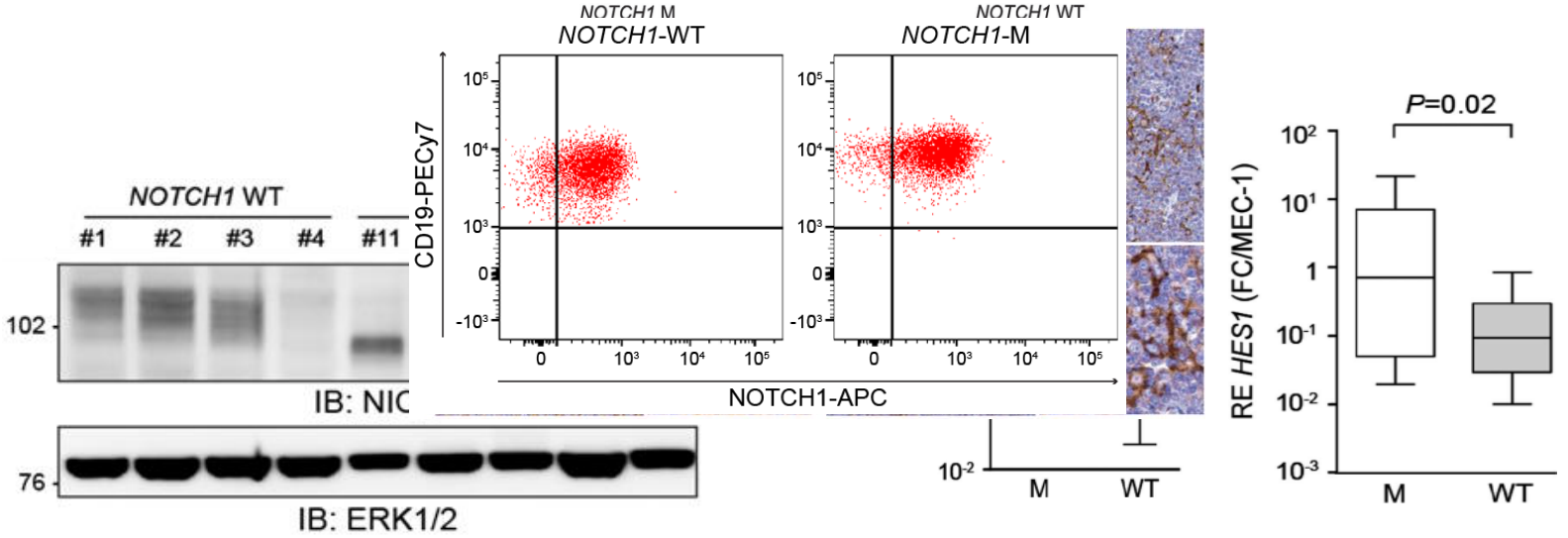


- Movement/recirculation signals
- Immunosuppressive signals

Arruga, F. Handbook Exp Pharm, 2017

Cherry picking NOTCH1

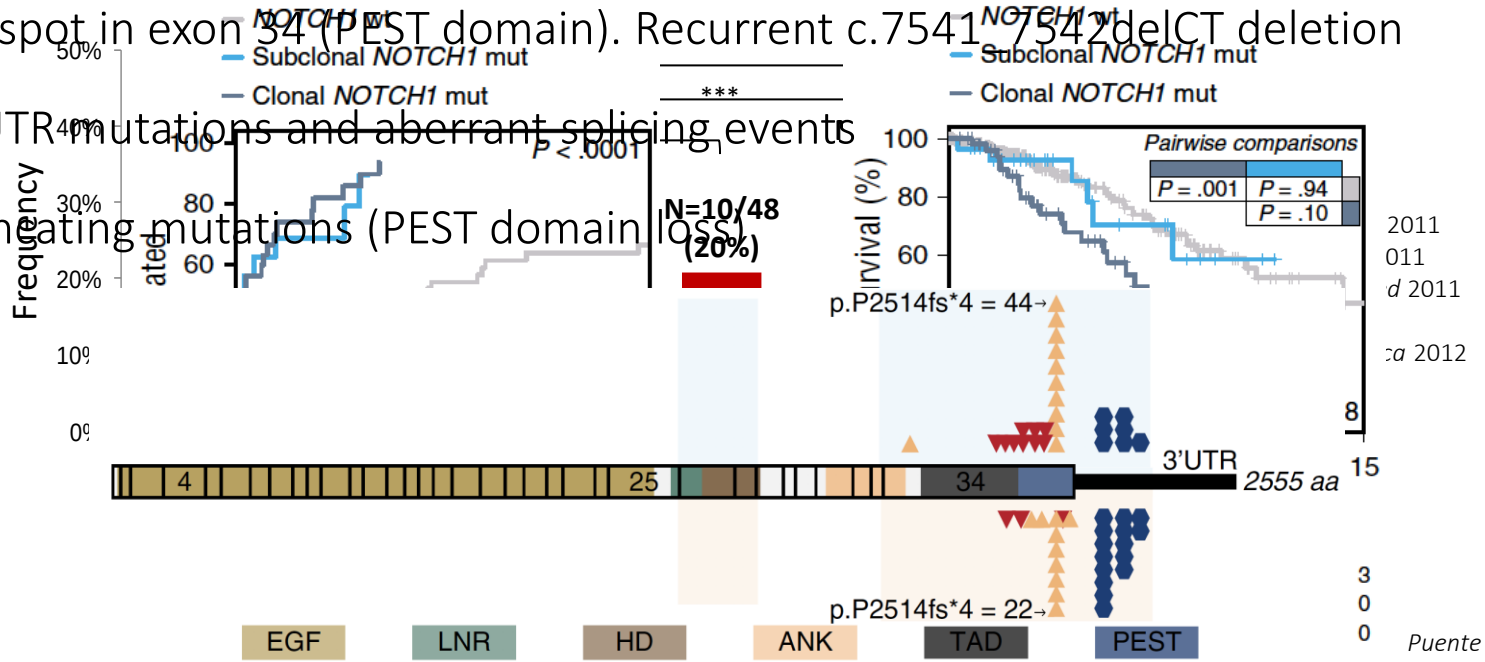
- All CLL cells express NOTCH1
- NOTCH1 ligands are abundantly present in the CLL niche
- There is evidence of NOTCH1 pathway constitutive activation



Arruga et al. Leukemia 2014

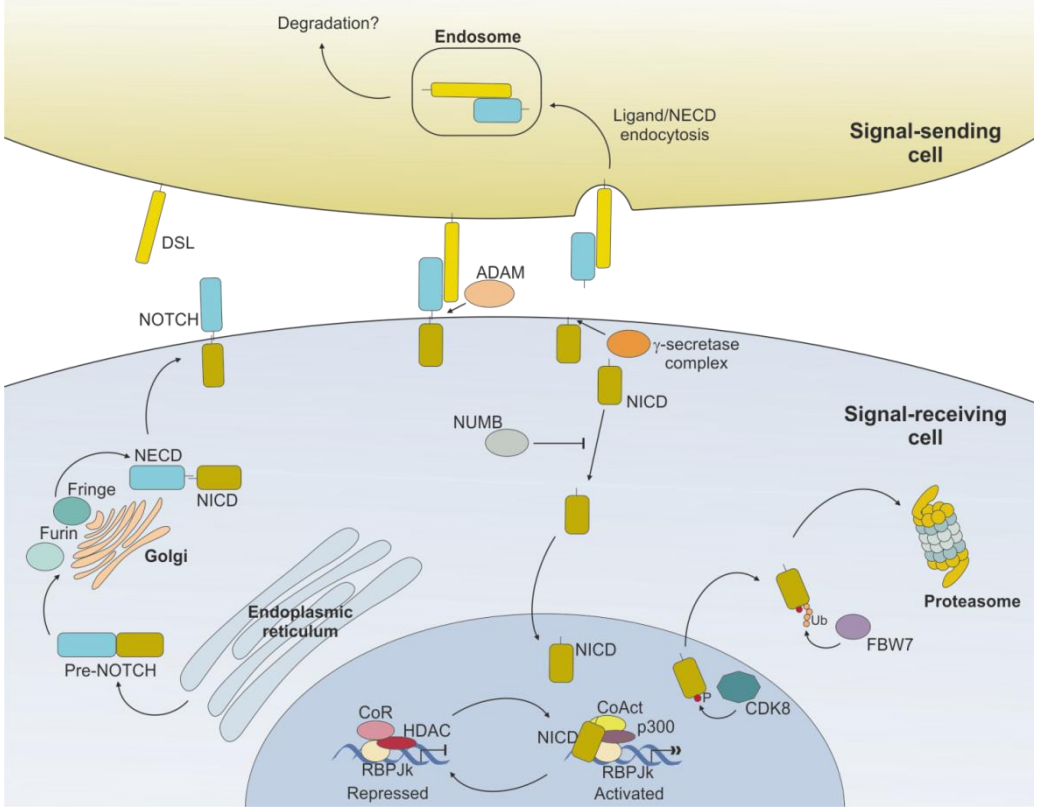
NOTCH1 is recurrently mutated in CLL patients

- Most frequent mutation in CLL at diagnosis
- Prevalence increases in chemorefractory/progressive CLL and is highest in Richter's
- Independent negative prognostic factor (OS, TTT, PFS)
- Hotspot in exon 34 (PEST domain). Recurrent c.7541_7542delCT deletion
- 3' UTR mutations and aberrant splicing events
- Truncating mutations (PEST domain loss)



Puente XS, et al. Nature 2015

The NOTCH1 signaling pathway



Arruga et al. submitted

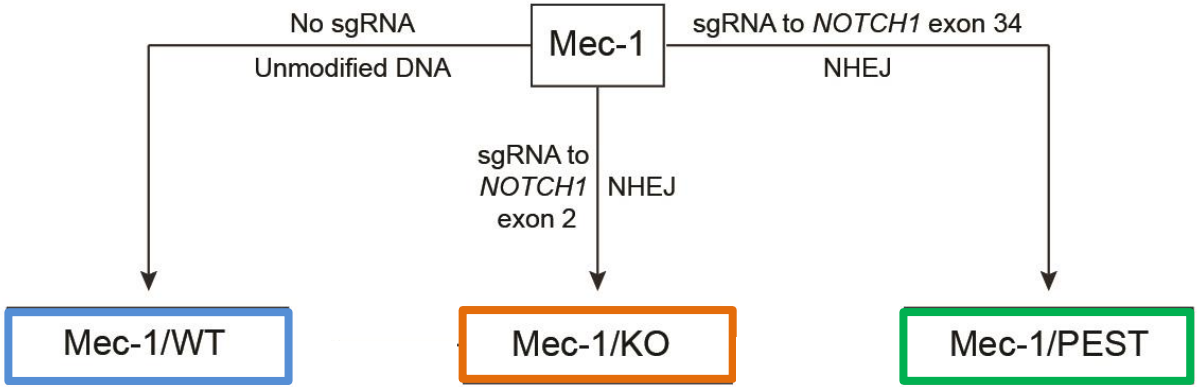
- Synthesis in the Endoplasmic Reticulum
- Transport to Golgi
  - glycosylation of serine and threonine residues (*Fringe*)
  - clevage of pre-NOTCH into the extracellular and intracellular domain (**S1 cleavage**)
- Heterodimeric receptor transported to plasma membrane
- Ligand binding leads to proteolytic cleavage
  - ADAM in the extracellular domain (**S2 cleavage**)
  - γ-secretase complex within the TMD (**S3 cleavage**)
- ICN (intracellular domain) translocates to the nucleus
  - interaction with the CSL transcription factor complex (CBF1 transcription factor)
  - recruitment of additional co-activators
- Phosphorylation of PEST domain of ICN by CDK8
  - ubiquitination and degradation via proteasome
  - switch off of NOTCH signalling

NOTCH1 signaling pathway controls cell fate by regulating cell **proliferation**, **survival**, and **differentiation**

Tools to study NOTCH1 signaling in CLL cells

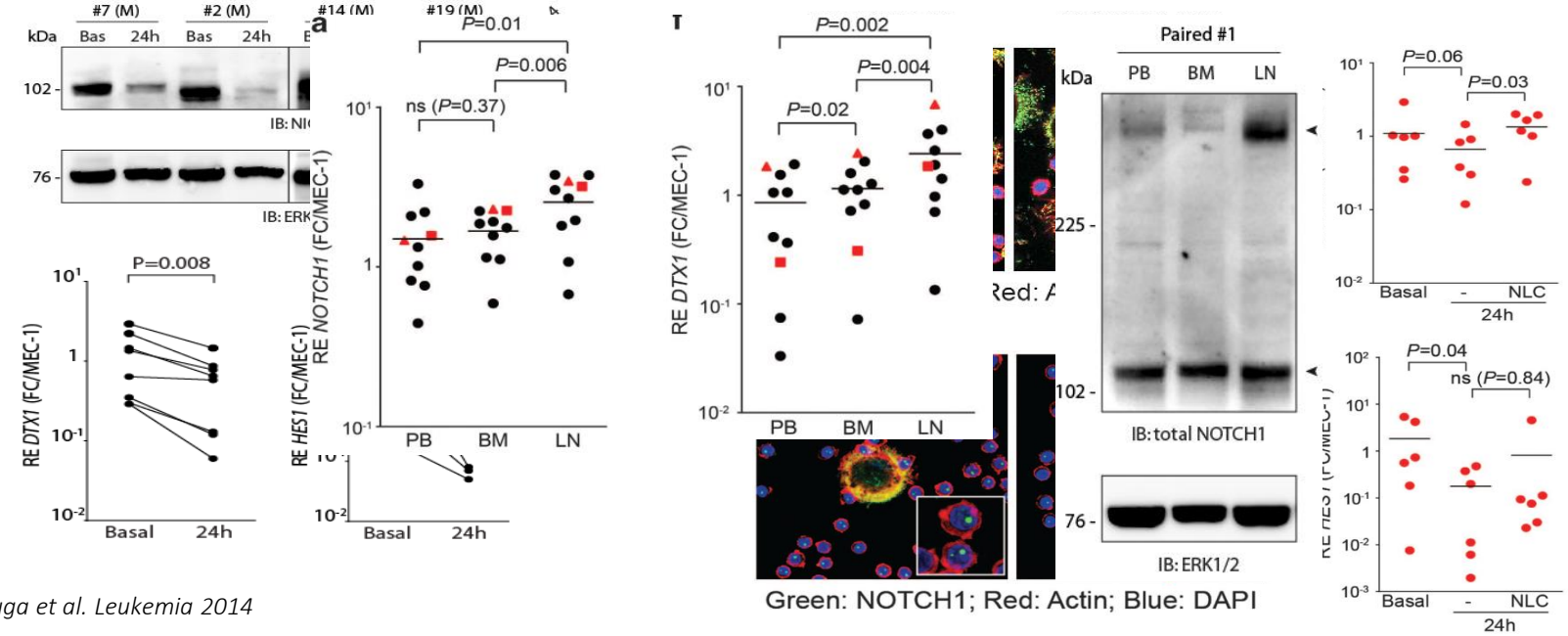
Primary cells

Cell lines



Signaling requires ligand-mediated triggering

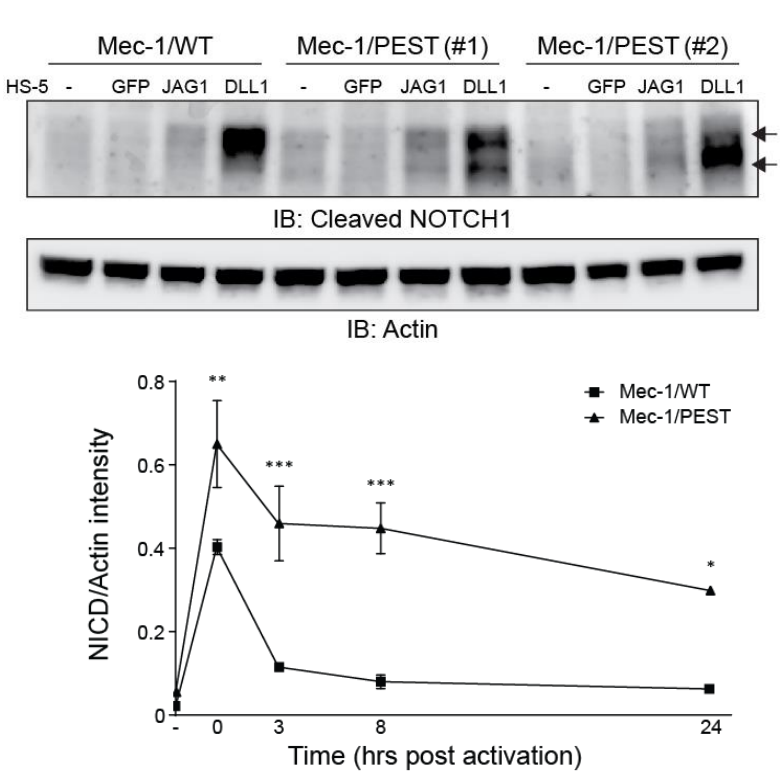
- In the absence of stimuli NOTCH1 activity is lost regardless of the mutational status
- Co-culture on ligand-expressing cells maintains NOTCH1 signaling activation
- *In vivo* NOTCH1 signaling is highest in the lymph nodes

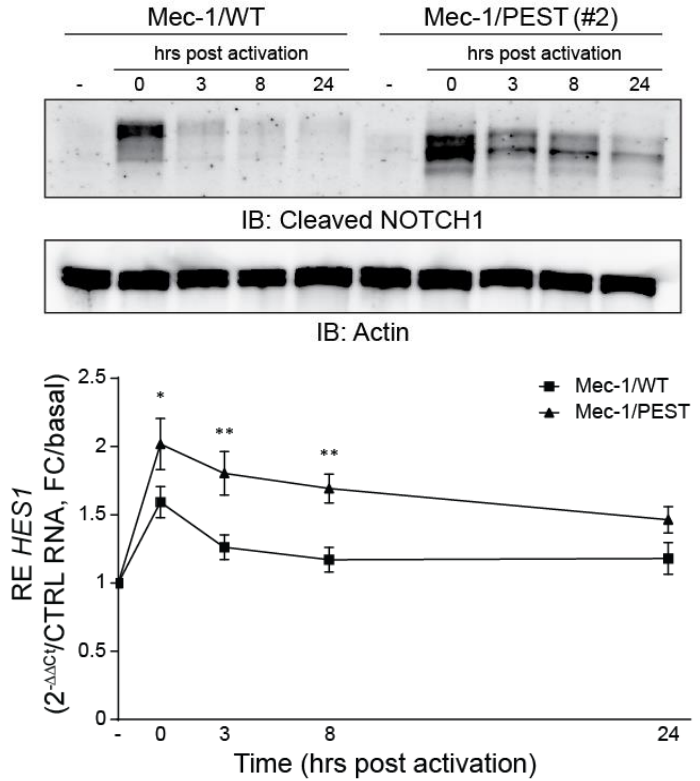


Arruga et al. Leukemia 2014

NOTCH1 mutations stabilize the NICD and prolong signaling

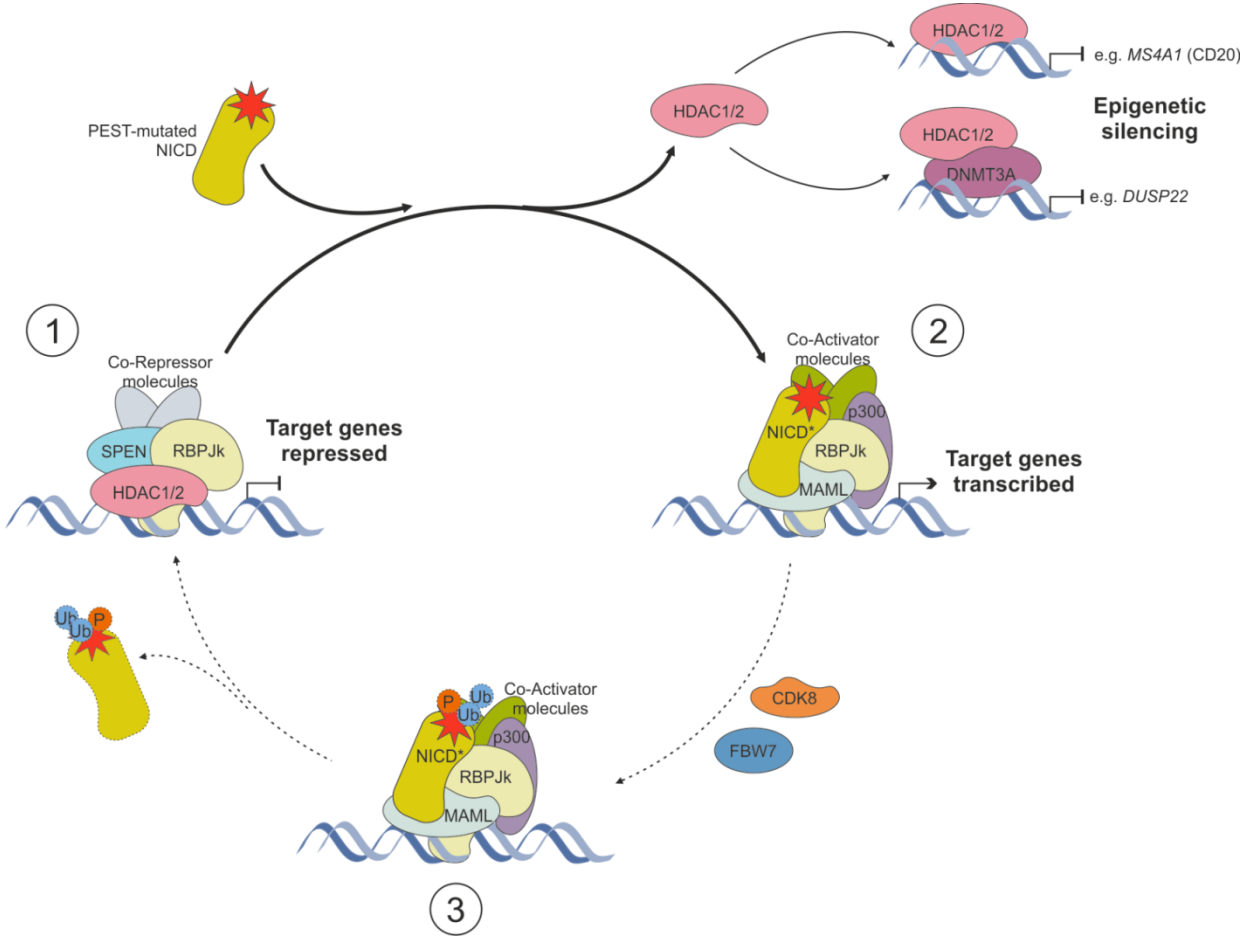
- PEST domain loss impairs NICD degradation upon ligand-induced cleavage





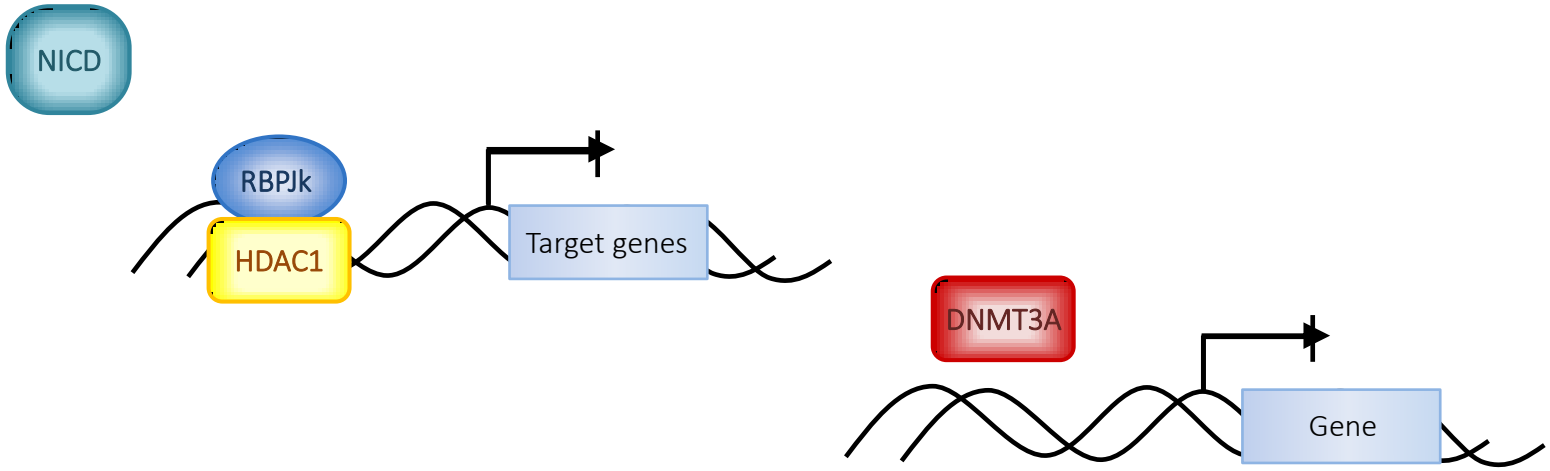
Arruga et al. Leukemia 2017

The mutant NICD alters complex nuclear balances



Arruga F et al, submitted

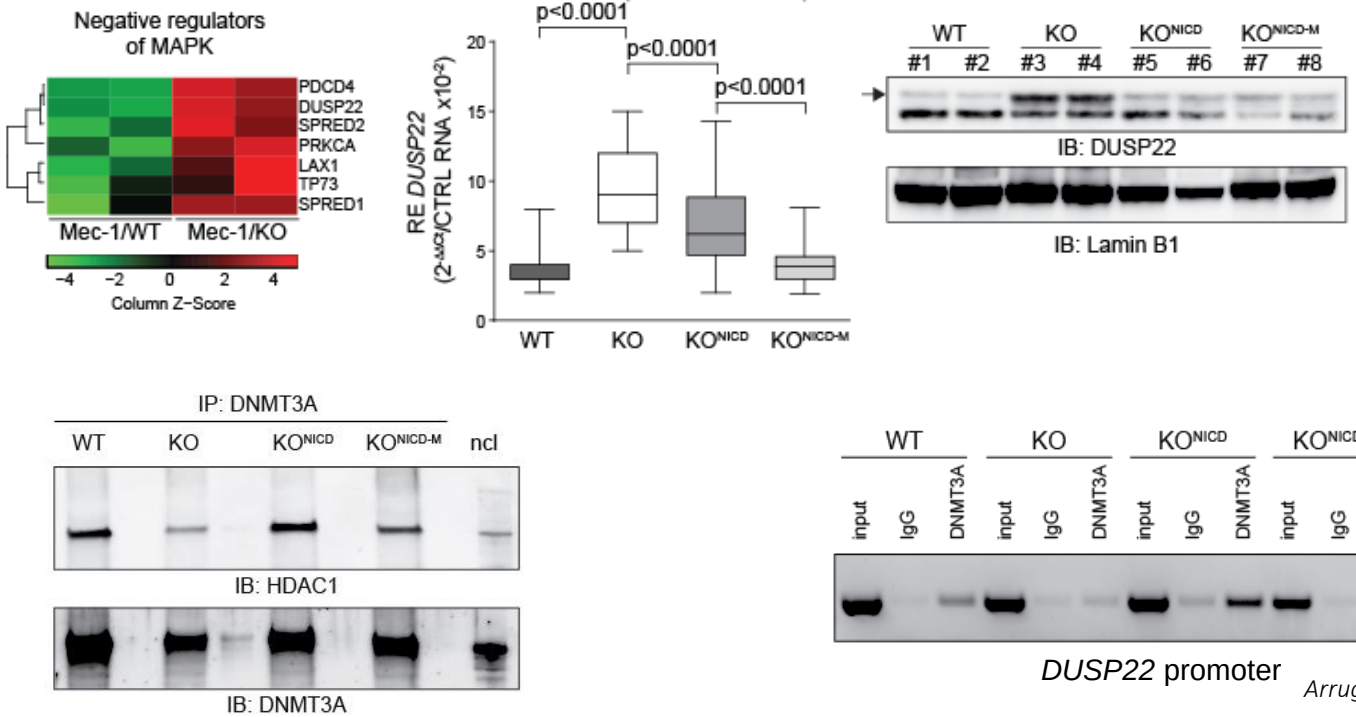
The mutated NICD alters complex nuclear balances



- NICD displace HDACs from the complex repressing CSL (RBPJk)
- Free HDACs can bind to gene promoters and inhibit gene transcription (e.g. *MS4A1* encoding CD20, *Pozzo F., et al. Leukemia 2016*)
- HDACs can bind to and stabilize DNMTs promoting their activity (*Yang L., et al Nat Rev Can 2015*)

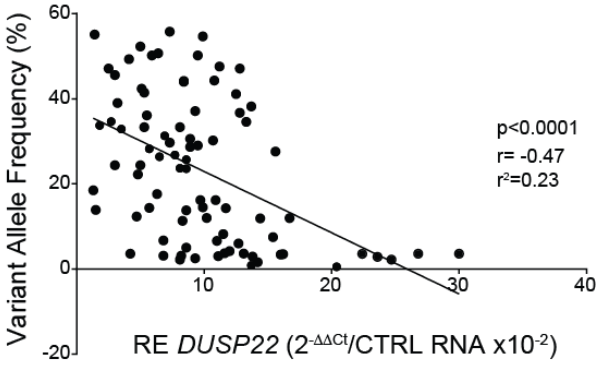
Functional interplay between NICD, RBPJ, HDACs and DNMT3A

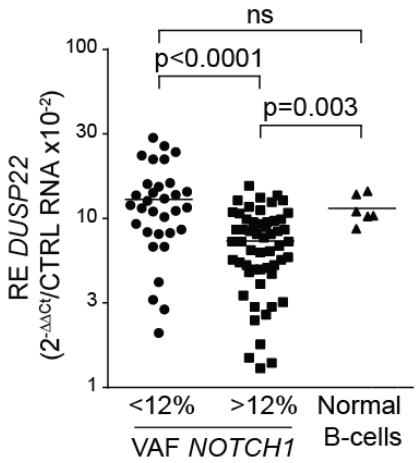
- NOTCH1-expressing cells show more DNMT3A on *DUSP22* promoter
- HDAC1 and DNMT3A interact in the nucleus in the presence of the NICD

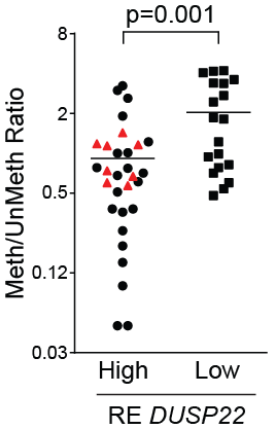


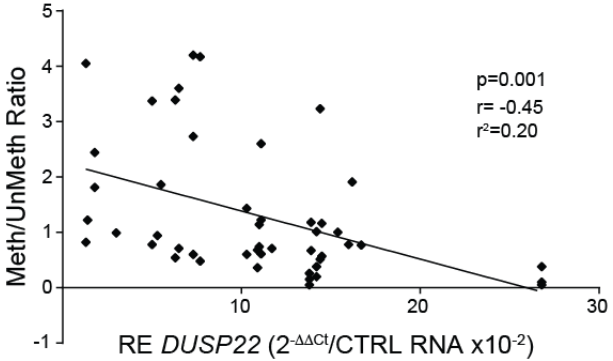
Arruga F et al, Leukemia 2017

DUSP22 expression in primary CLL cells is regulated through promoter methylation







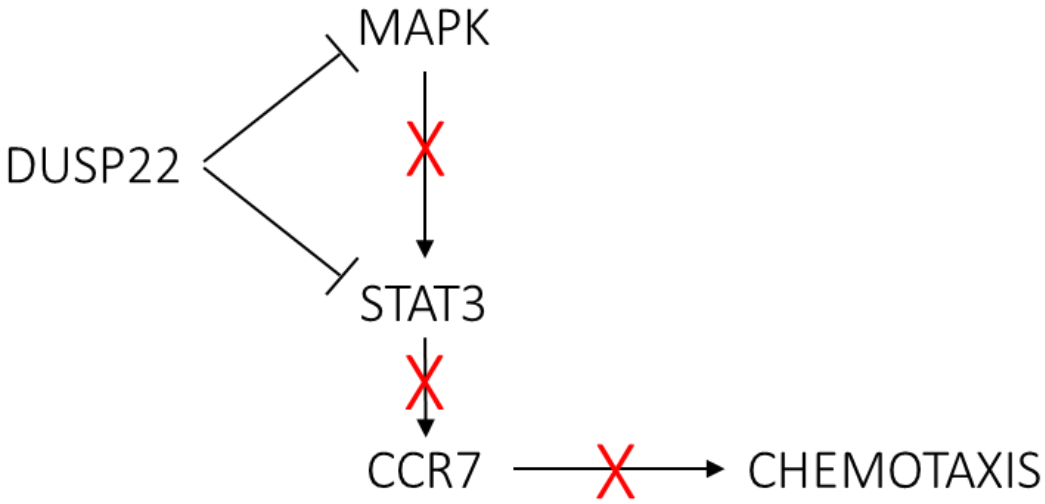


- *DUSP22* is variably expressed in CLL cells
- *DUSP22* expression is regulated through promoter methylation

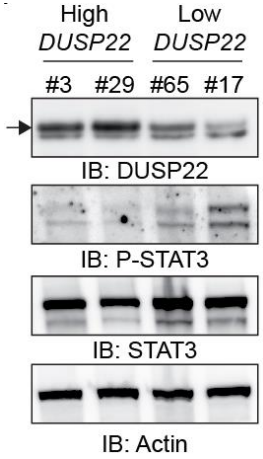
Arruga F et al, Leukemia 2017

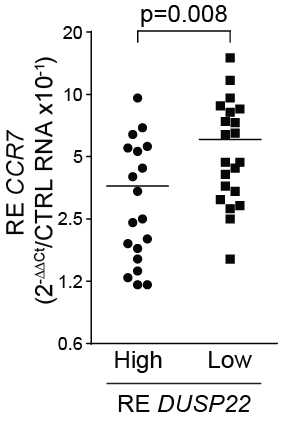
Chemotaxis may be regulated through DUSP22

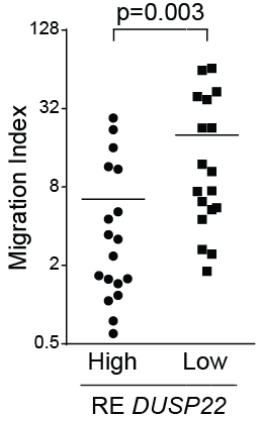
- DUSP22 is a *Du*al-*S*pecificity *P*hosphatase
- JNK, p38 and STAT3 are known DUSP22 targets
- DUSP22 is an oncosuppressor (Anaplastic Large Cell Lymphoma, colorectal cancer)



DUSP22 modulates a chemotactic circuit

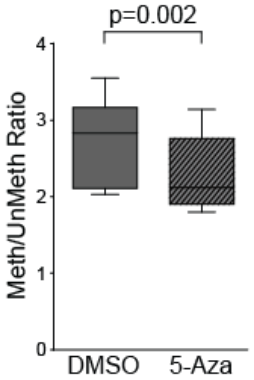


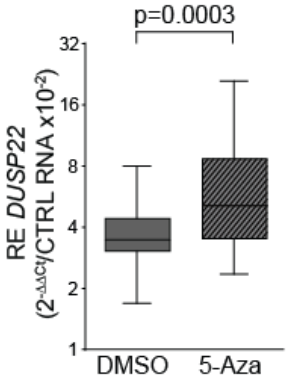


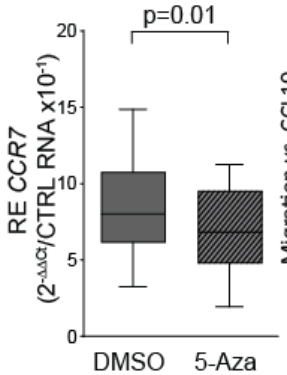


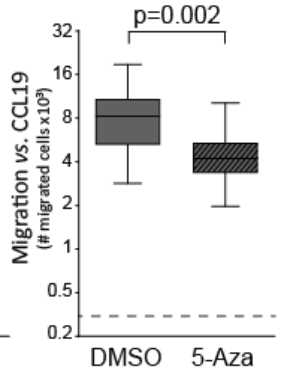
CLL cells with low DUSP22 expression show:

- Increased P-STAT3
- Increased CCR7 expression
- Increased chemotaxis towards CCL19





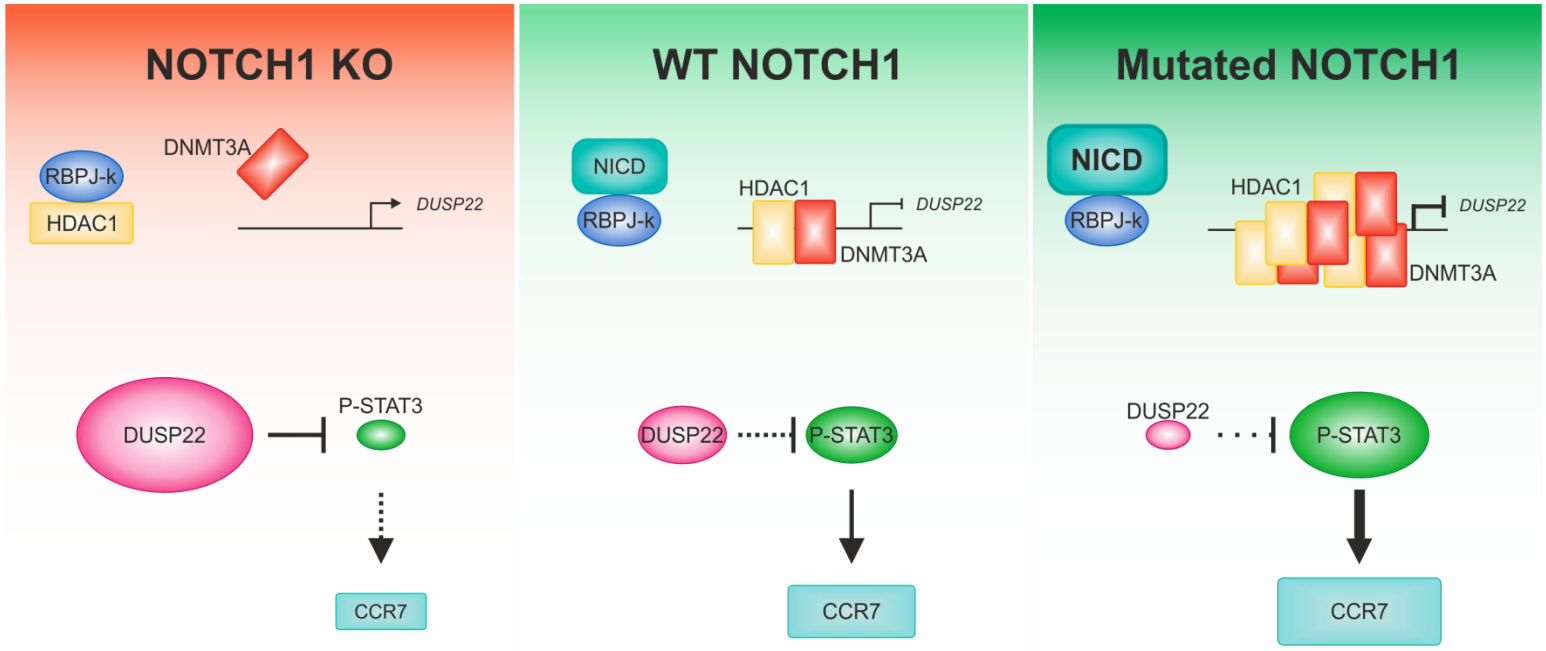




- Restoring DUSP22 expression decreases CLL migratory potential

Arruga F et al, Leukemia 2017

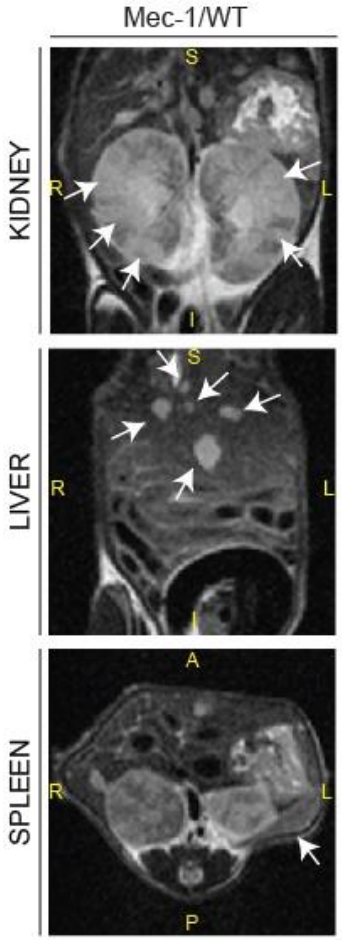
NOTCH1 affects migration through DUSP22-STAT3-CCR7 axis



CCL19-DRIVEN CHEMOTAXIS

Arruga et al. Leukemia 2017

NOTCH1 mutated cells show different spreading in xenografts



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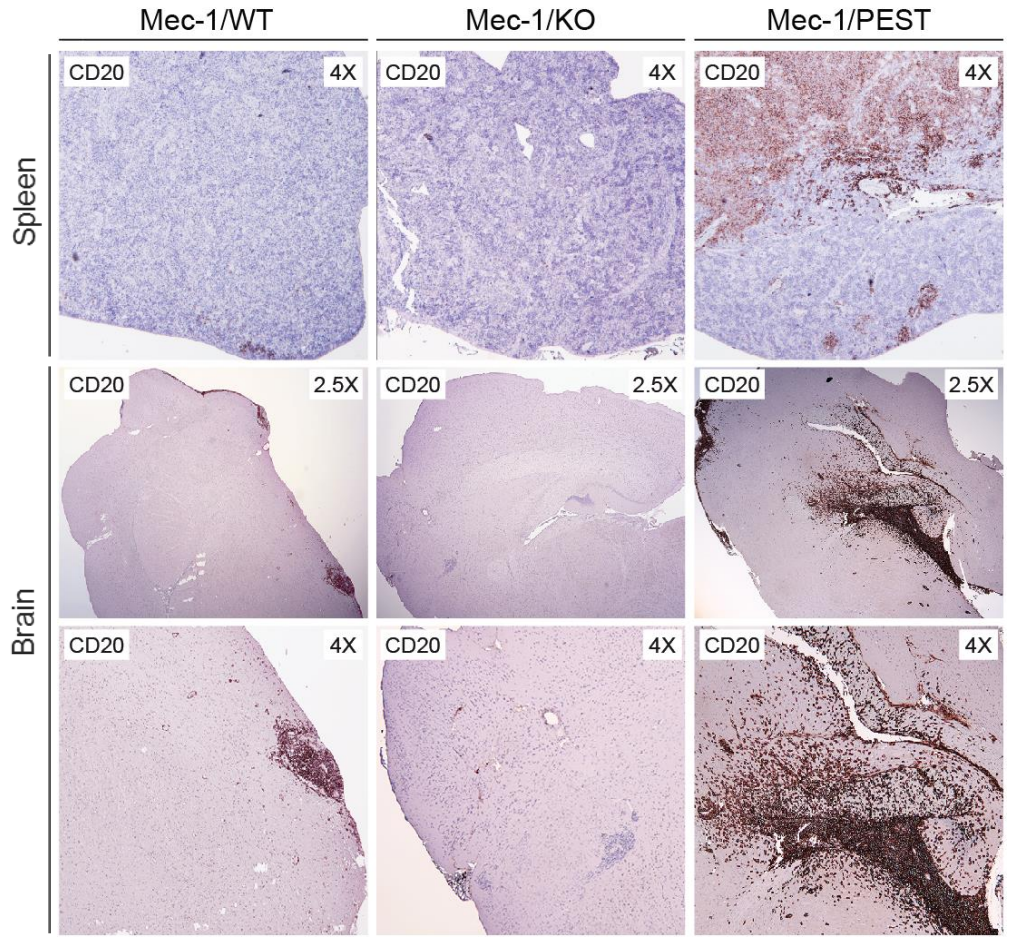
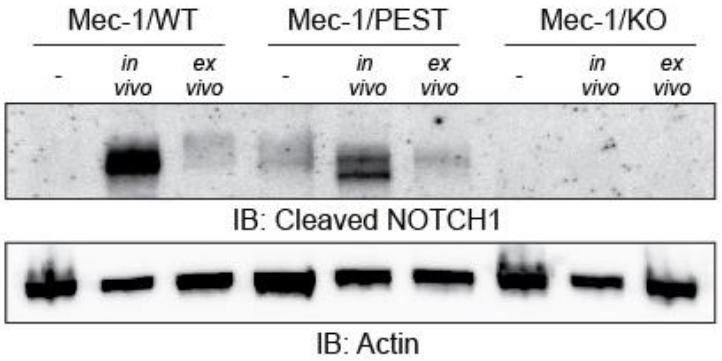
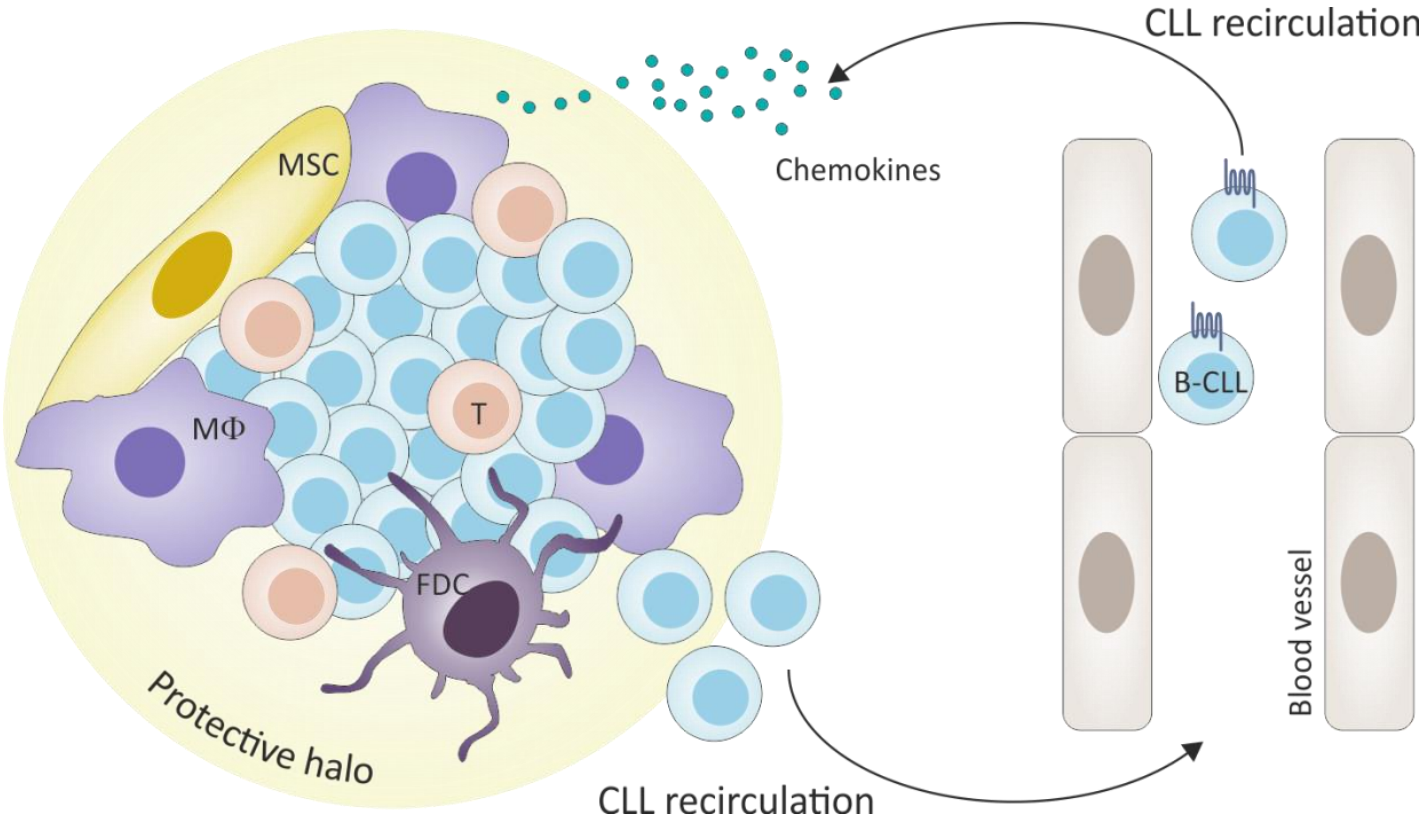




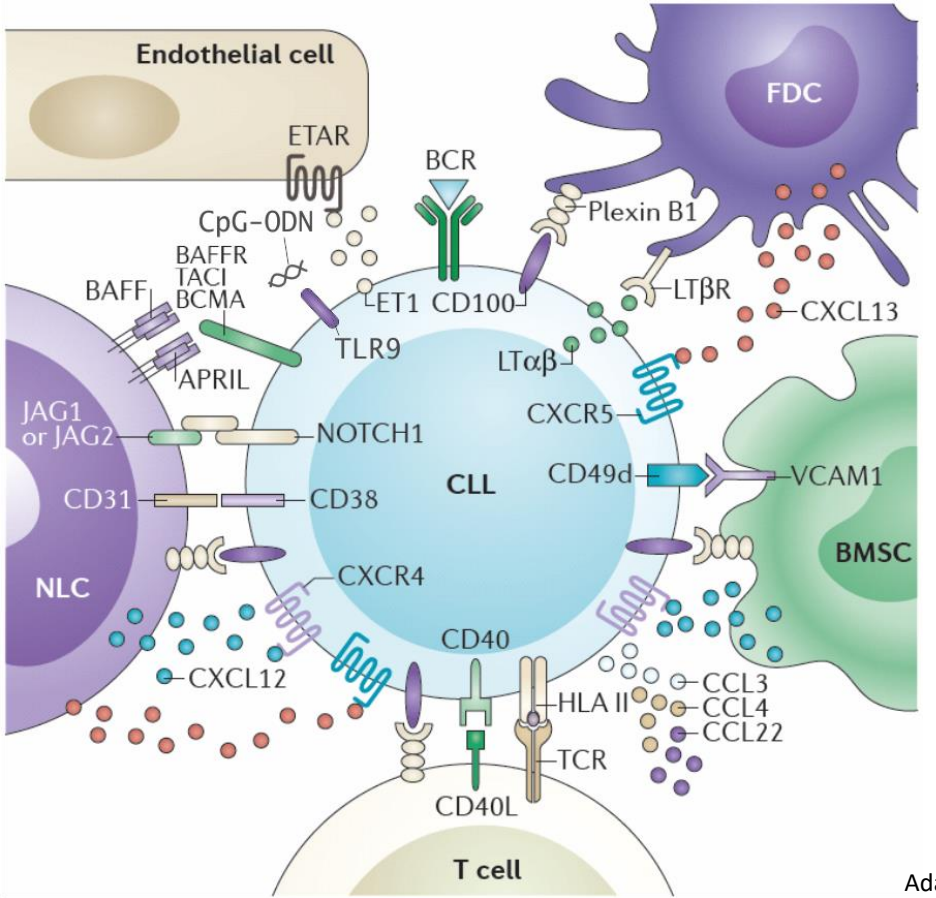
- Massive spleen and brain infiltration by *NOTCH1*-M cells
- Evidence of *in vivo* NOTCH activation

NOTCH1-M cells may be more prone to reach privileged niches



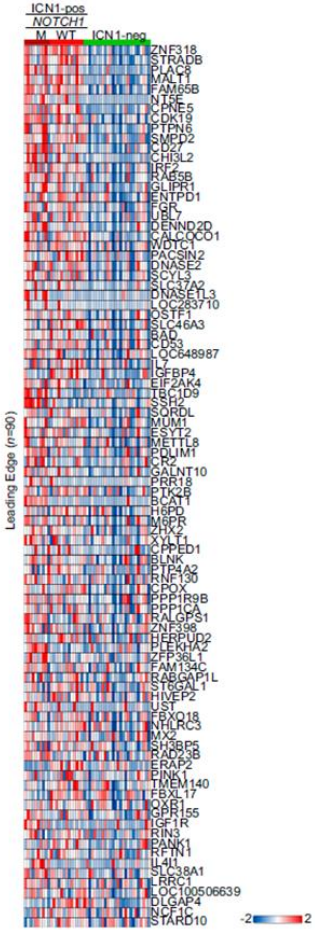
Adapted from *Vaisitti T et al, IJMS 2018*

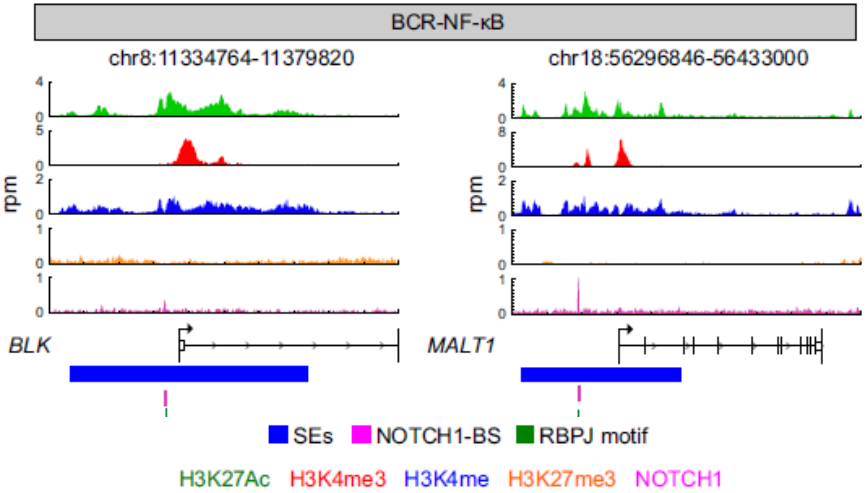
Is there an interplay among microenvironment-driven pathways?

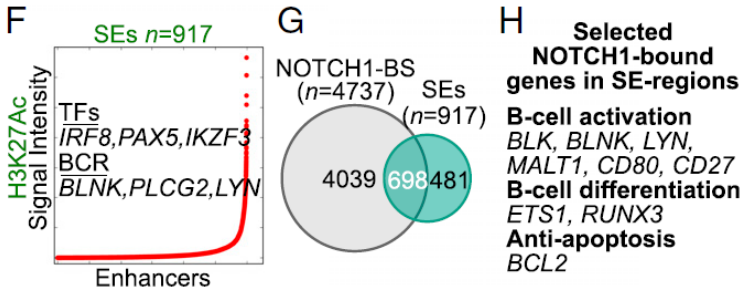


Adapted from *Fabbri, G. Nat Rev Cancer 2016*

NOTCH1 regulates genes belonging to the BCR pathway





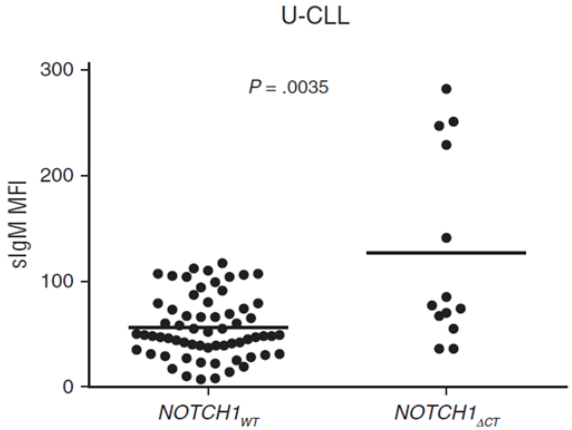


- NOTCH1 directly regulates genes belonging to:

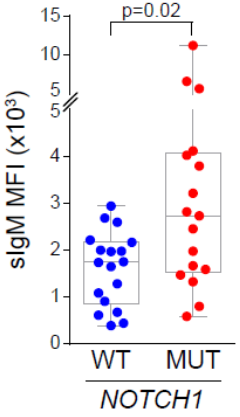
- **BCR signaling pathway**
- MAPK effectors
- NFkB cascade
- Chemotaxis

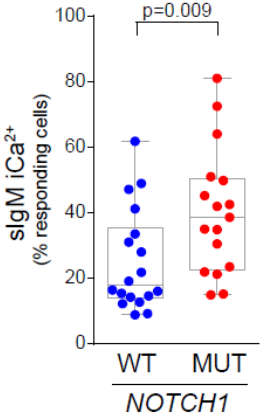
Fabbri G, et al. PNAS 2017

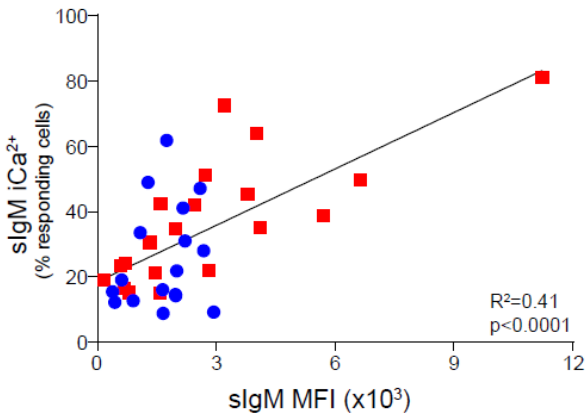
NOTCH1-M samples show increased BCR signaling capacity



D'Avola et al., Blood 2016

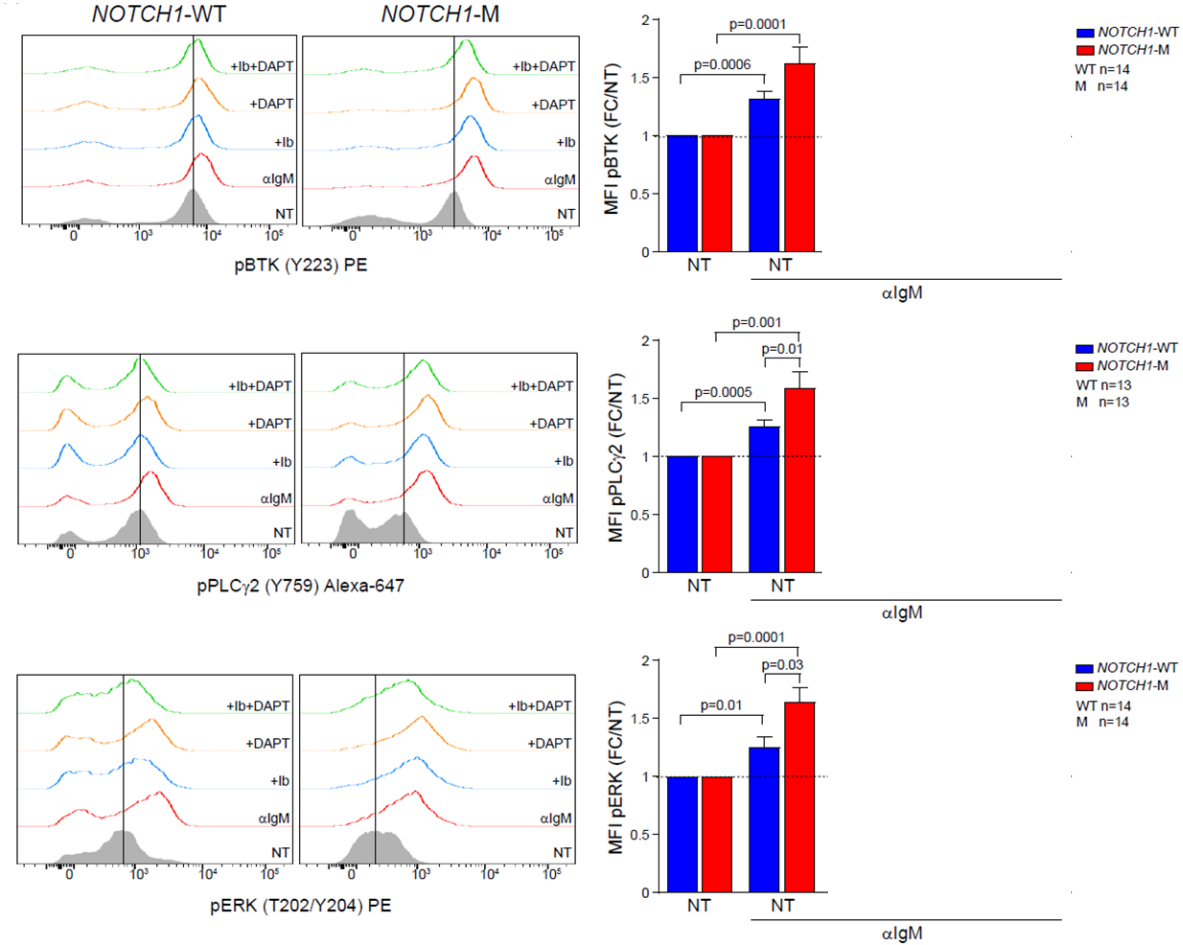






- Direct correlation between clonality of *NOTCH1* mutations and sIgM expression and signaling capacity

NOTCH1-M samples show increased BCR signaling capacity



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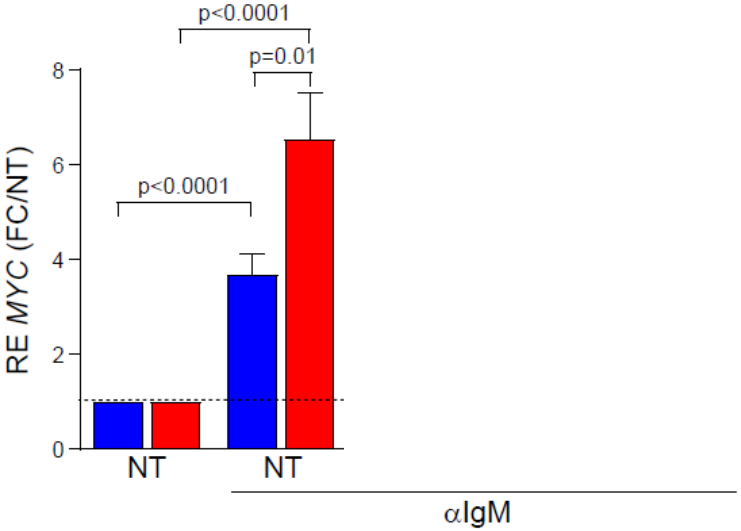
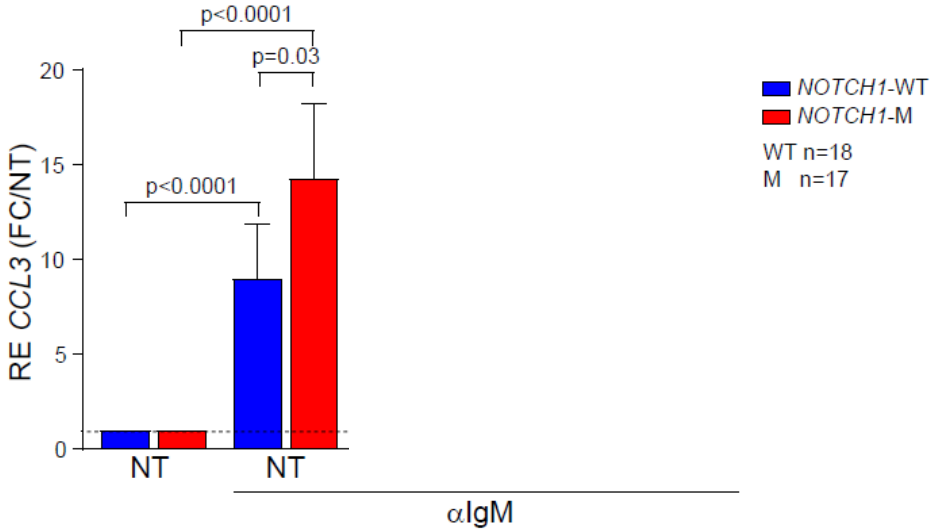
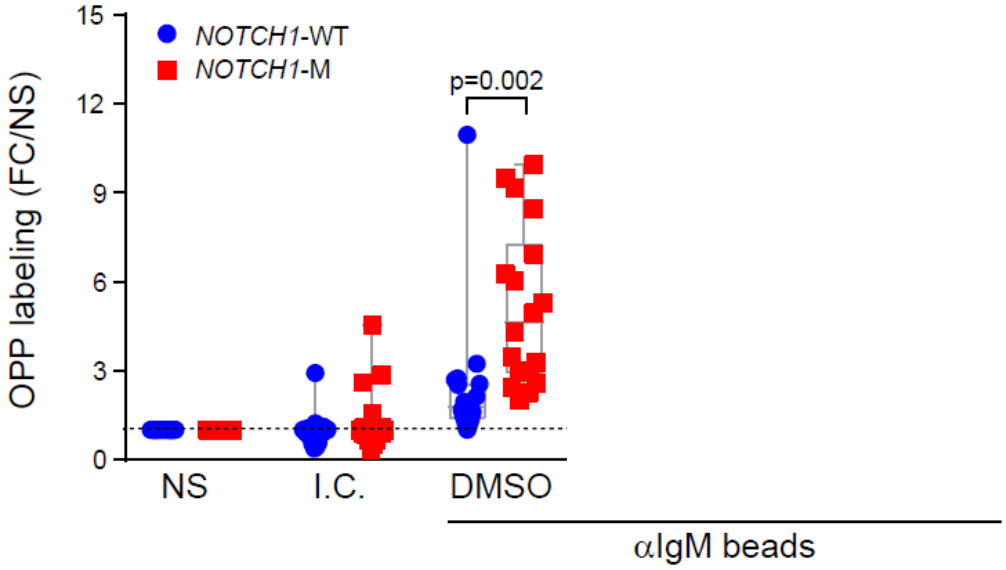
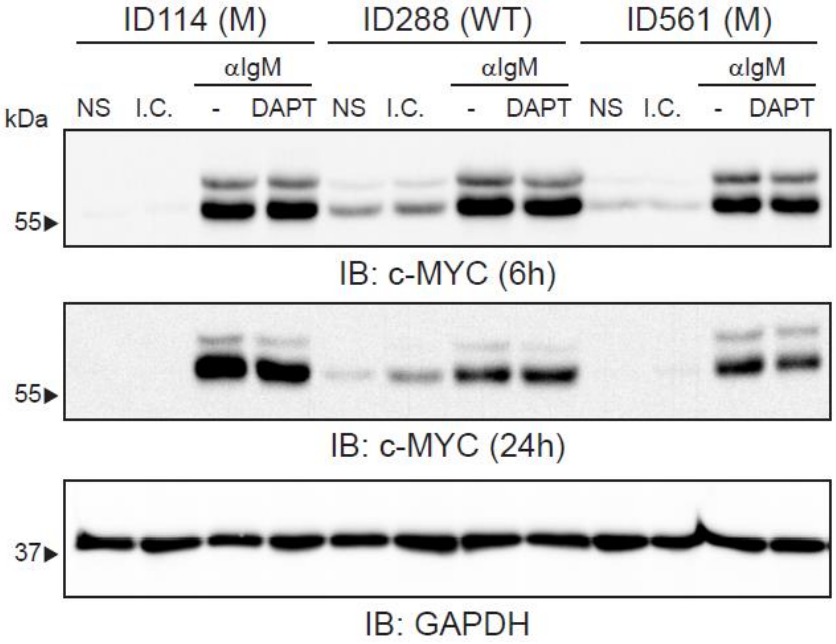




- Expression levels of *MYC* and *CCL3* are markedly upregulated upon triggering of BCR
- *NOTCH1*-M samples show significantly stronger induction of BCR target genes expression
- Pretreatment with DAPT limits upregulation of *MYC* and *CCL3* in the *NOTCH1*-M subset
- The addition of DAPT to ibrutinib further inhibits BCR target genes expression

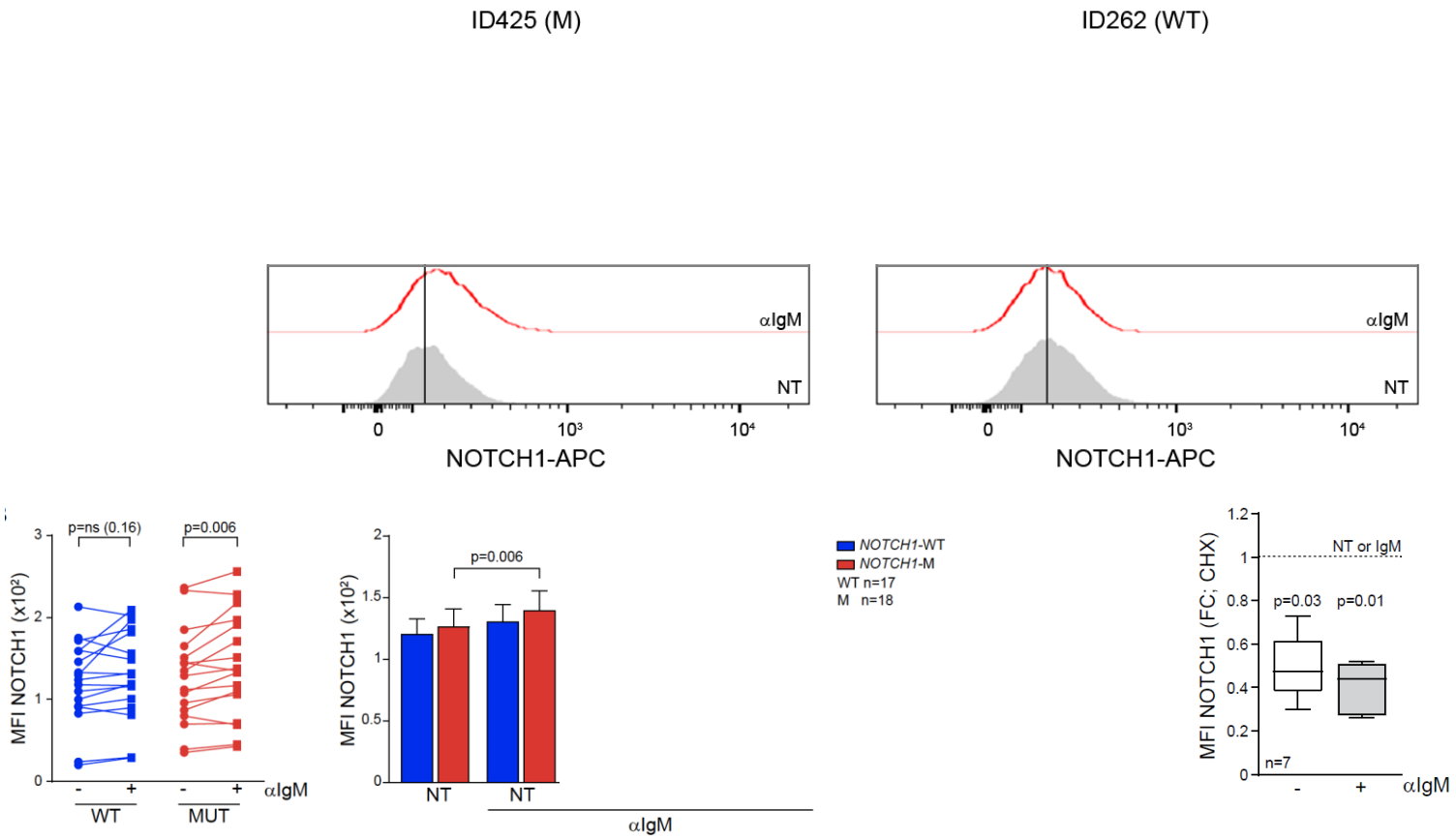
NOTCH1-M samples show enhanced global mRNA translation in response to BCR triggering



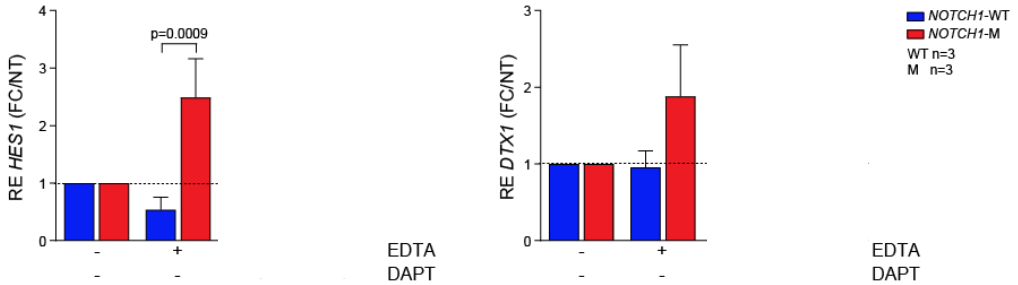


- BCR signaling activation stimulates mRNA translation in CLL cells and to a greater extent in *NOTCH1*-M samples, possibly due to a stronger MYC induction

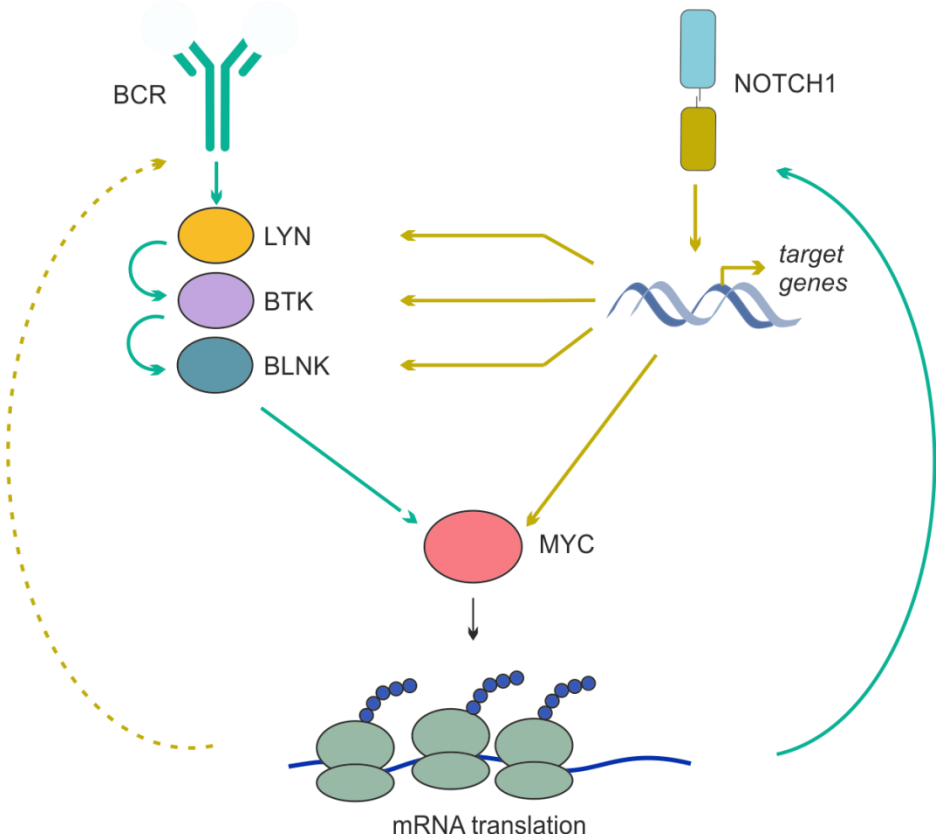
BCR signaling increases NOTCH1 expression



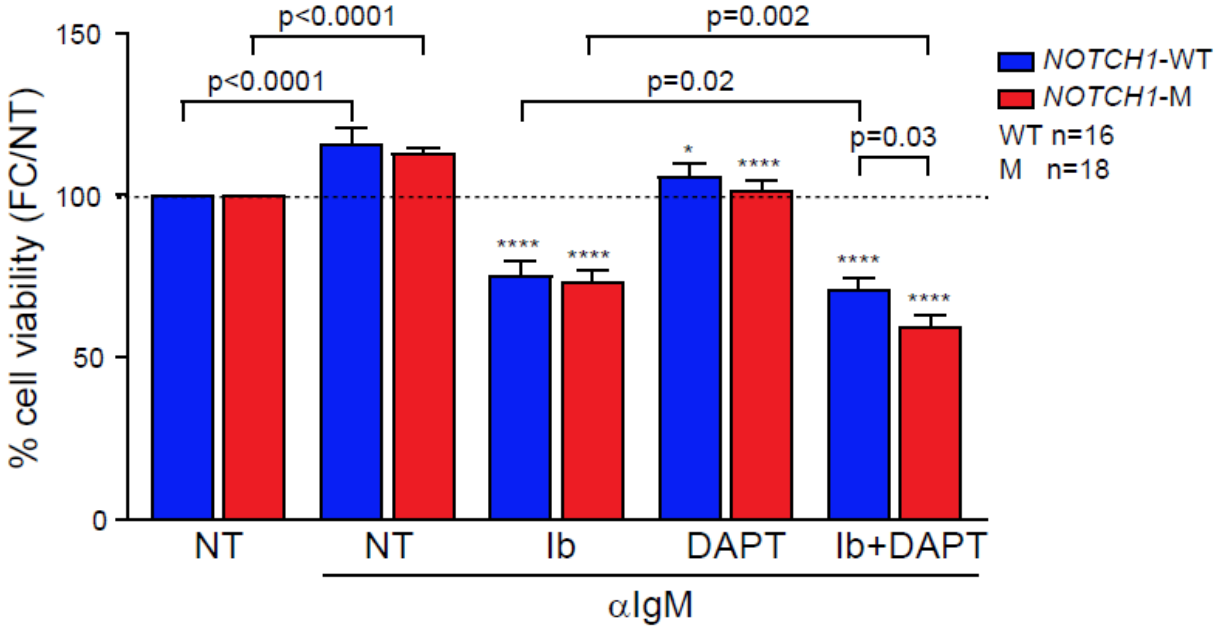
NOTCH1 regulates the expression of BCR signaling members



BCR and NOTCH1 signaling are joined in a loop that is further enhanced in the presence of stabilizing NOTCH1 mutations

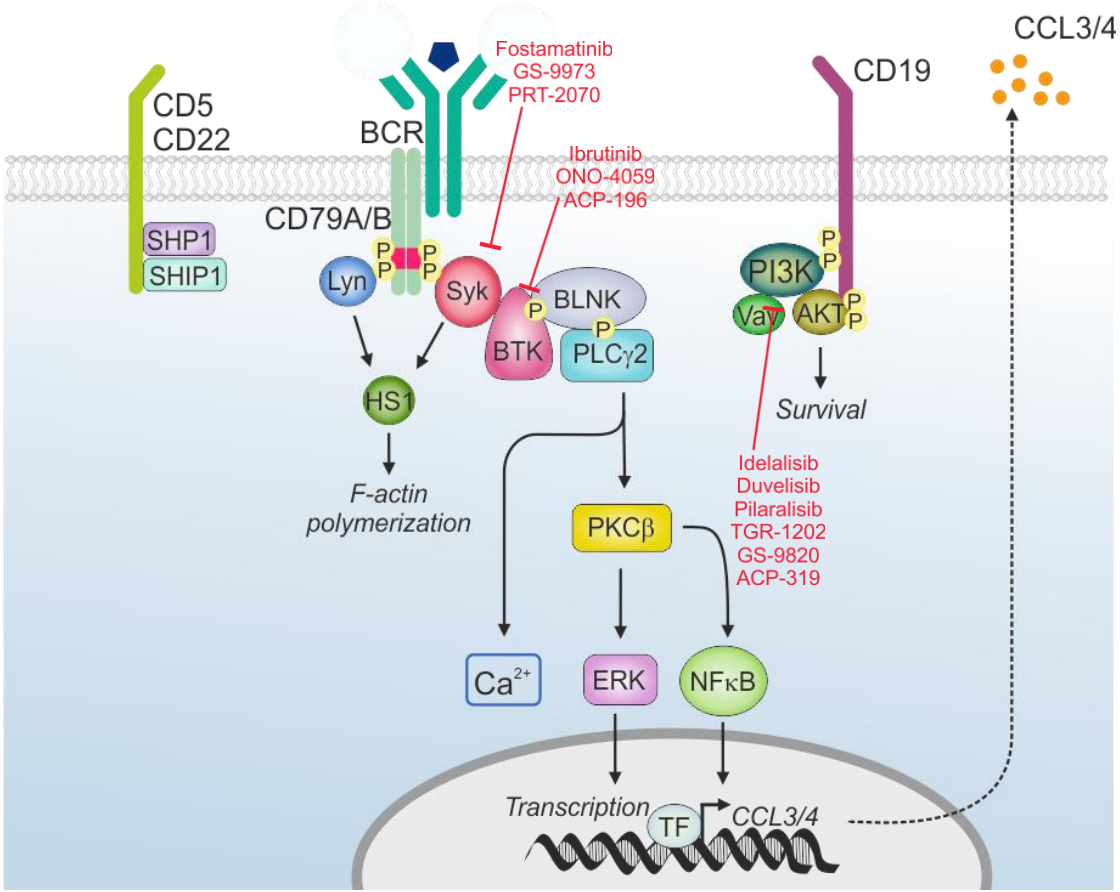


Inhibiting NOTCH1 enhances ibrutinib-mediated apoptosis



- Concomitant inhibition of BCR and NOTCH1 signaling results in enhanced apoptotic response that is even more effective in the *NOTCH1*-M subset

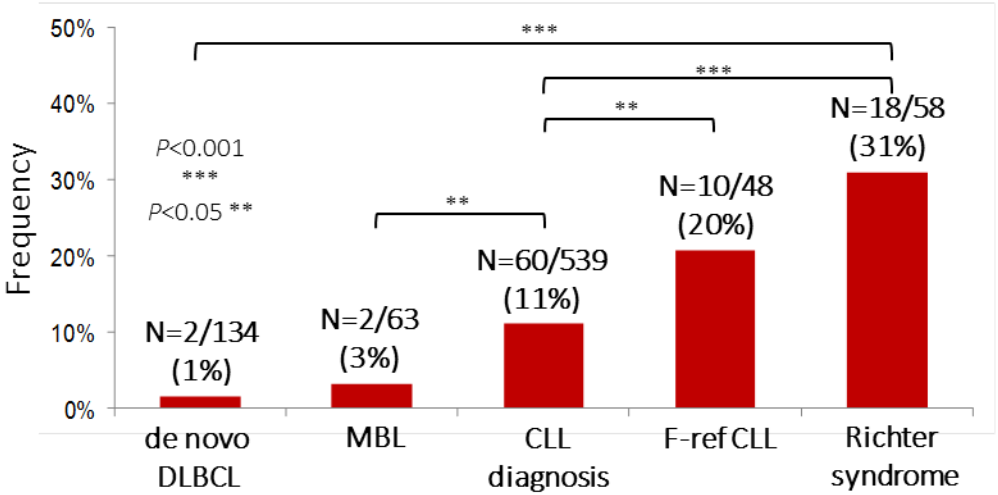
Target therapy is becoming standard of care for CLL patients

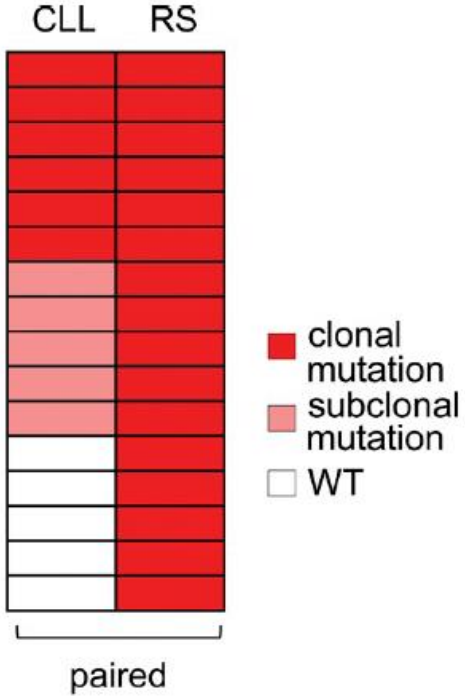


Modified from *Arruga, F. Handbook Exp Pharm, 2017*

What is the role of NOTCH1 in RS?

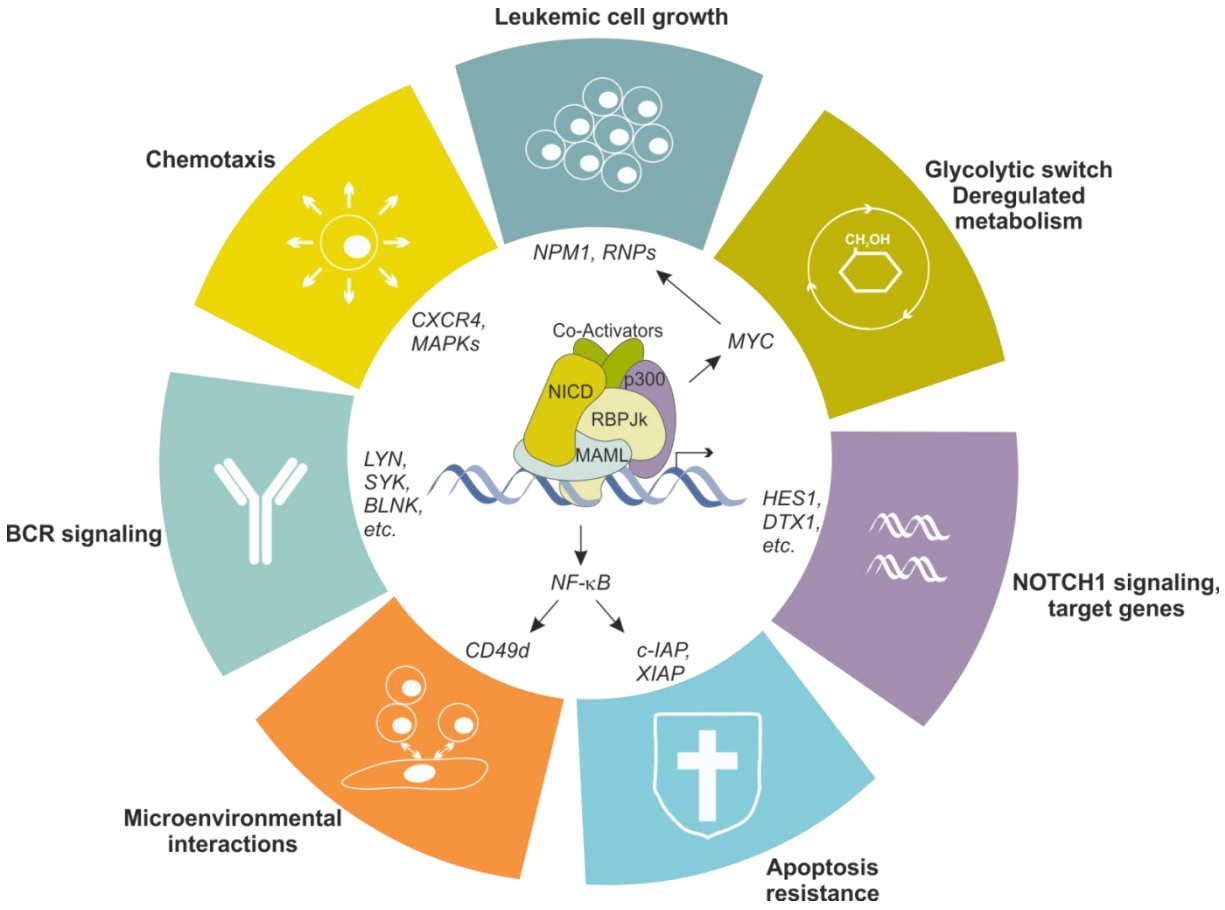
- Maybe not for CLL...but for Richter?





Courtesy of Dr. D. Rossi
Fabbri G, et al. JEM 2011

What does NOTCH1 do in the CLL microenvironment?



Arruga F et al, submitted

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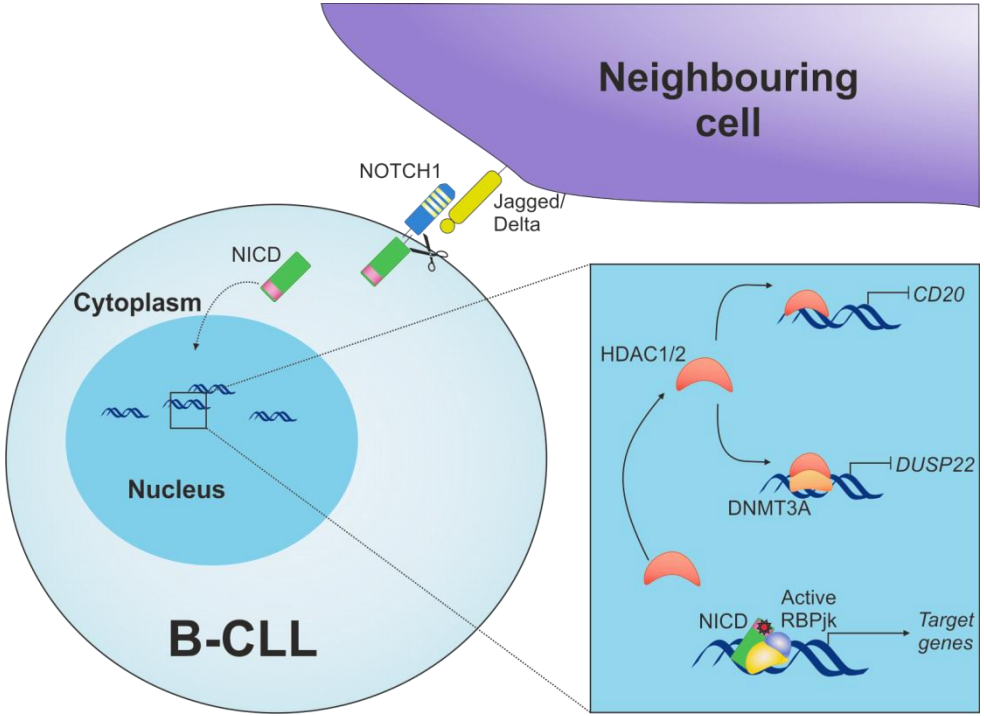
What does a mutant NICD do?

- The prolonged activation of the NICD results in modified composition of nuclear protein complexes, involving HDACs and DNMT3A, ultimately modifying gene transcription



Activation of NOTCH1 is associated with increased sIgM