# Regulation of diversification and affinity maturation of antibodies


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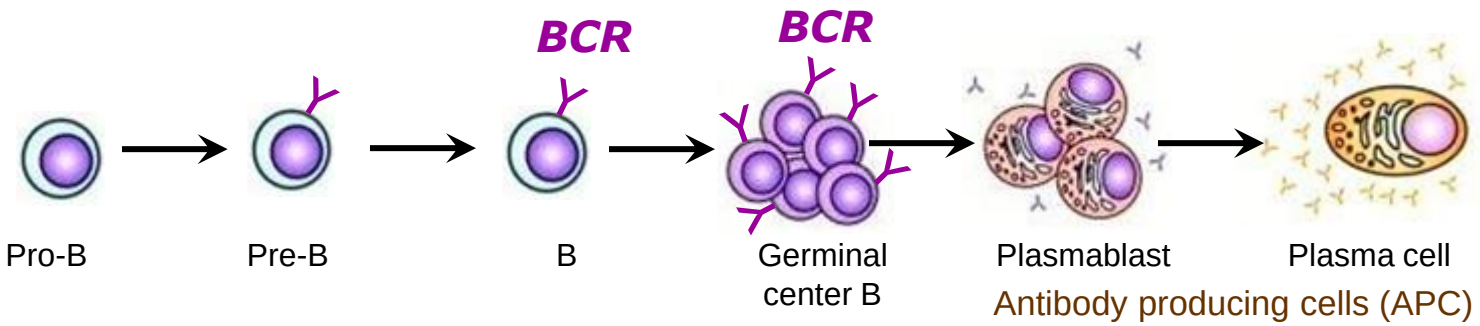



**Hypermutation & Class switching**

**Activation Induced cytidine Deaminase, AID**
(C ⇒ U, inducible)

# Control of the mutagen AID that changes C to U

- ~ 1 000 000 fold increased mutation rate in part of the **antibody genes** by the activation-induced cytidine deaminase, **AID**
- High specificity for **antibody genes**

# AID is of critical importance for the development of most B lymphocyte neoplasias

- ***Over-expression of AID in many B cell lymphomas***
- ***Translocations, which characterice most B cell tumors, are usually caused by errors in somatic hypermutation/class switching***
- ***Hypermutation by AID of many genes including oncogenes***

**Bcl6** ↑↑↑↑↑
**Pax5** ↑↑↑
**Myc** ↑↑↑
**Pim1** ↑↑↑
**...** ↑↑
**...** ↑

# Why is targeting of AID to antibody genes good but not perfect?

# Control of target specificity of AID

- **"The *trans*-acting factors mediating specific targeting of AID and thereby Somatic Hypermutation and Class Switch Recombination remain elusive**

- **The strongest link between gene specific transcription and specific targeting of AID is for E-boxes, the binding sites for many basic-helix-loop-helix proteins including E2A. However, no direct coupling between any transcription factor and the targeting of AID had been demonstrated, and how AID is recruited to its specific targets in antibody genes was still a big mystery in the field**

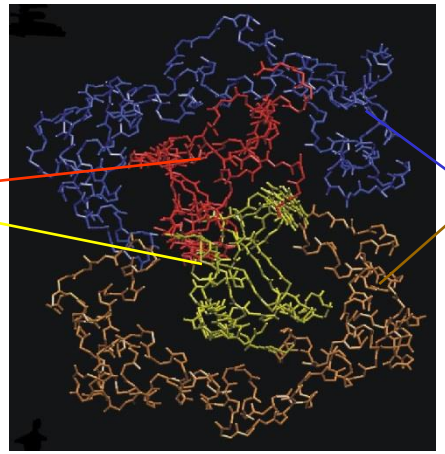
***Is E2A needed for the recruitment?***

**Tool:**

**Activation of the B cell receptor (BCR) inhibits DNA binding of E2A**

# Activation of the B cell receptor (BCR) inhibits DNA binding of the transcription factor E2A that is critical for B lymphocyte development

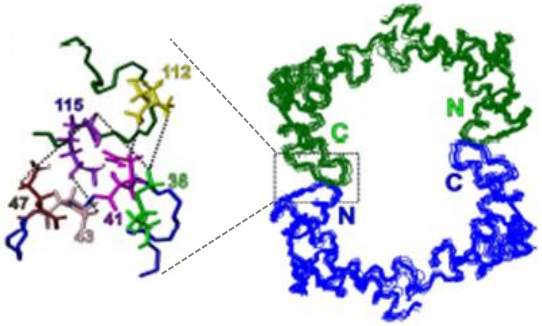
*DNA binding basic sequences of a bHLH protein such as E2A*

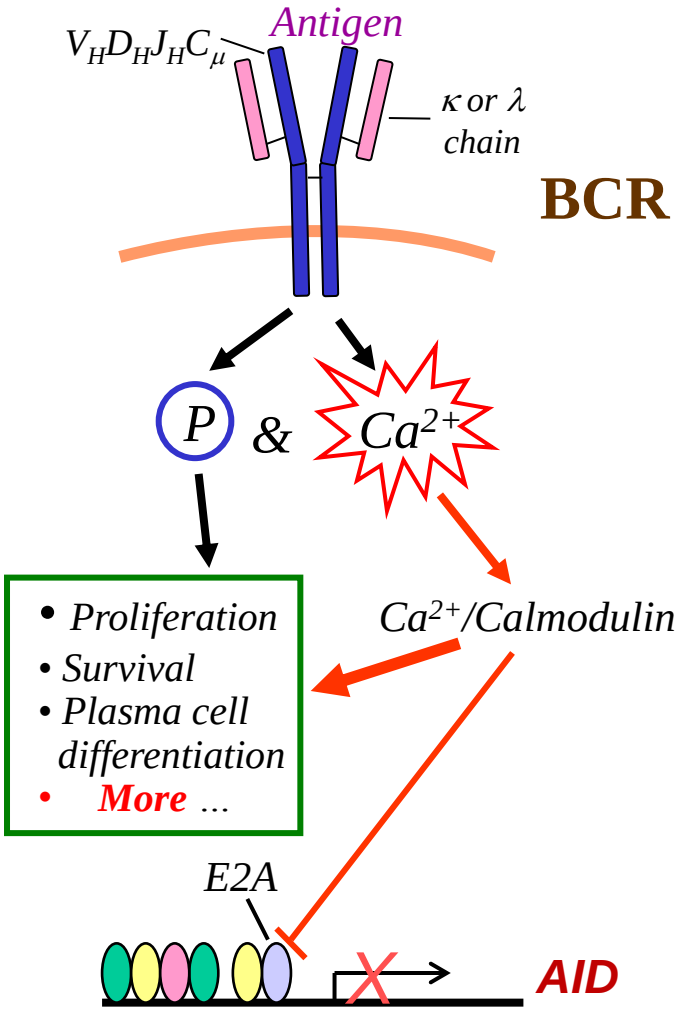


*$Ca^{2+}$/Calmodulin (CaM)*

*Two calmodulins form an open eight-shaped structure*

*The two basic DNA binding sequences are trapped inside*

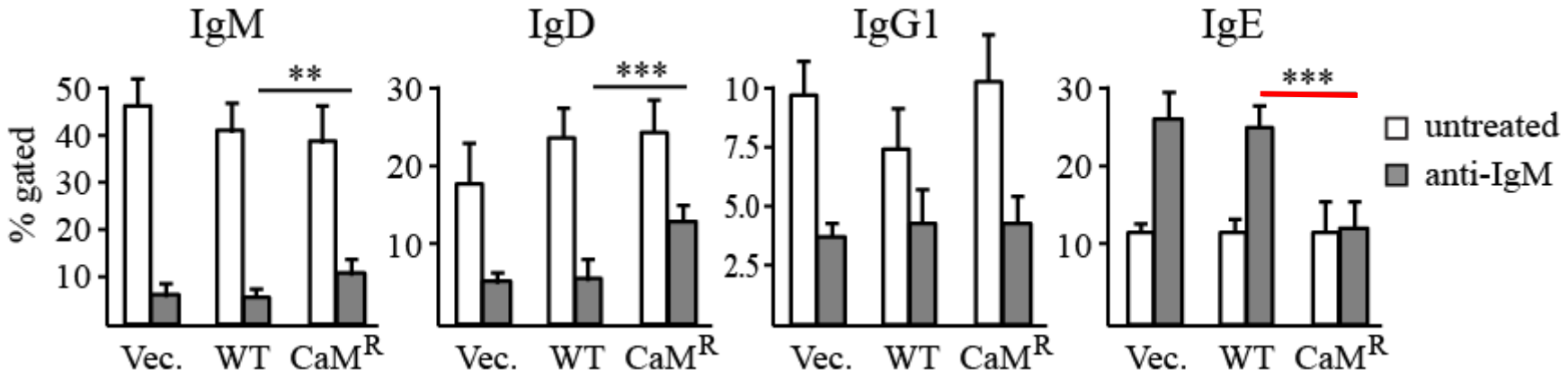


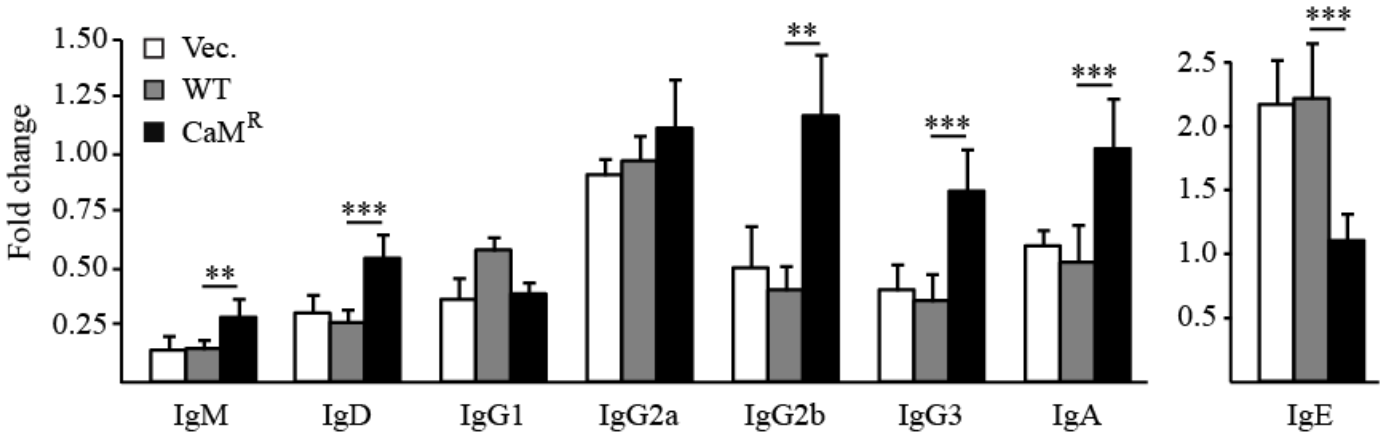

***AID expression is shut-off after BCR stimulation through CaM inhibition of E2A***

***Is targeting of class switch recombination to IgE by IL-4 and CD40 ligand plus BCR stimulation defect when E2A is mutated to calmodulin resistance?***

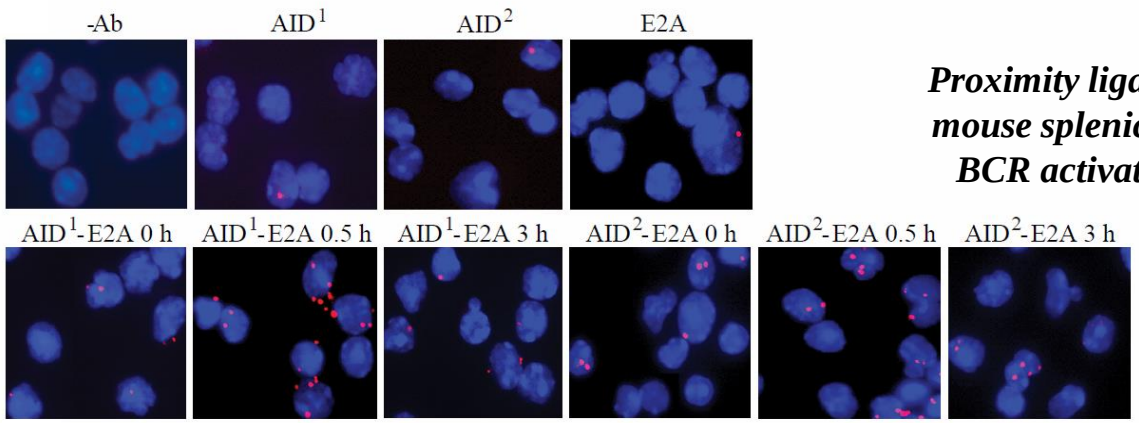
# *Defect calmodulin inhibition of E2A leads to reduced BCR-, IL4- plus CD40L-stimulated class-switch recombination to IgE*




# *... and instead in-appropriate CSR to other Ig classes*



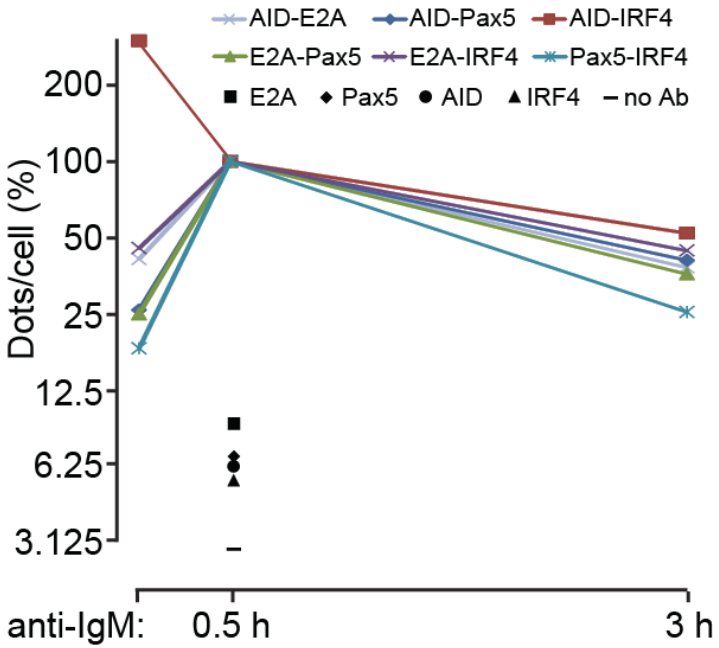
# *AID is in proximity with the transcription factors E2A, Pax5 and IRF4 in activated B cells*

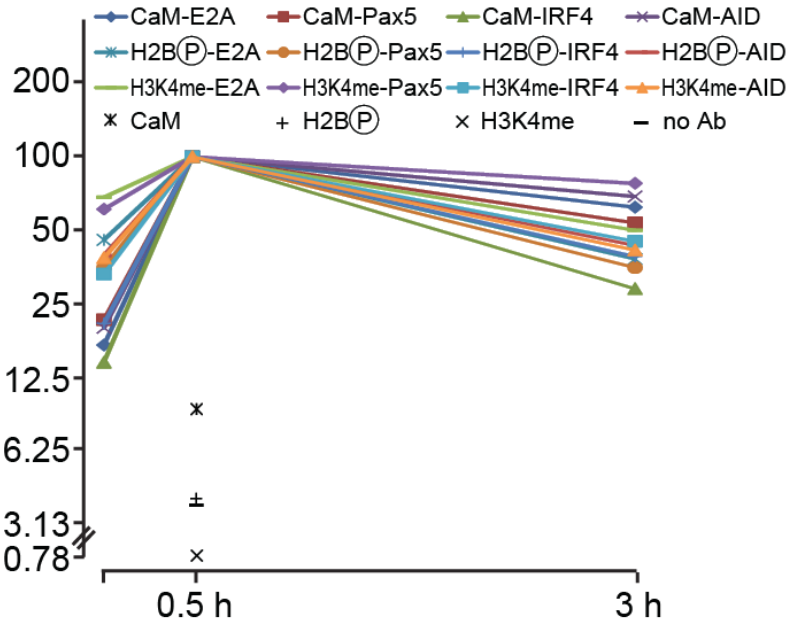



***Proximity ligation assay (PLA) of indicated proteins in mouse splenic B cells grown with CD40L and IL4 and BCR activated with anti-IgM for the indicated time***

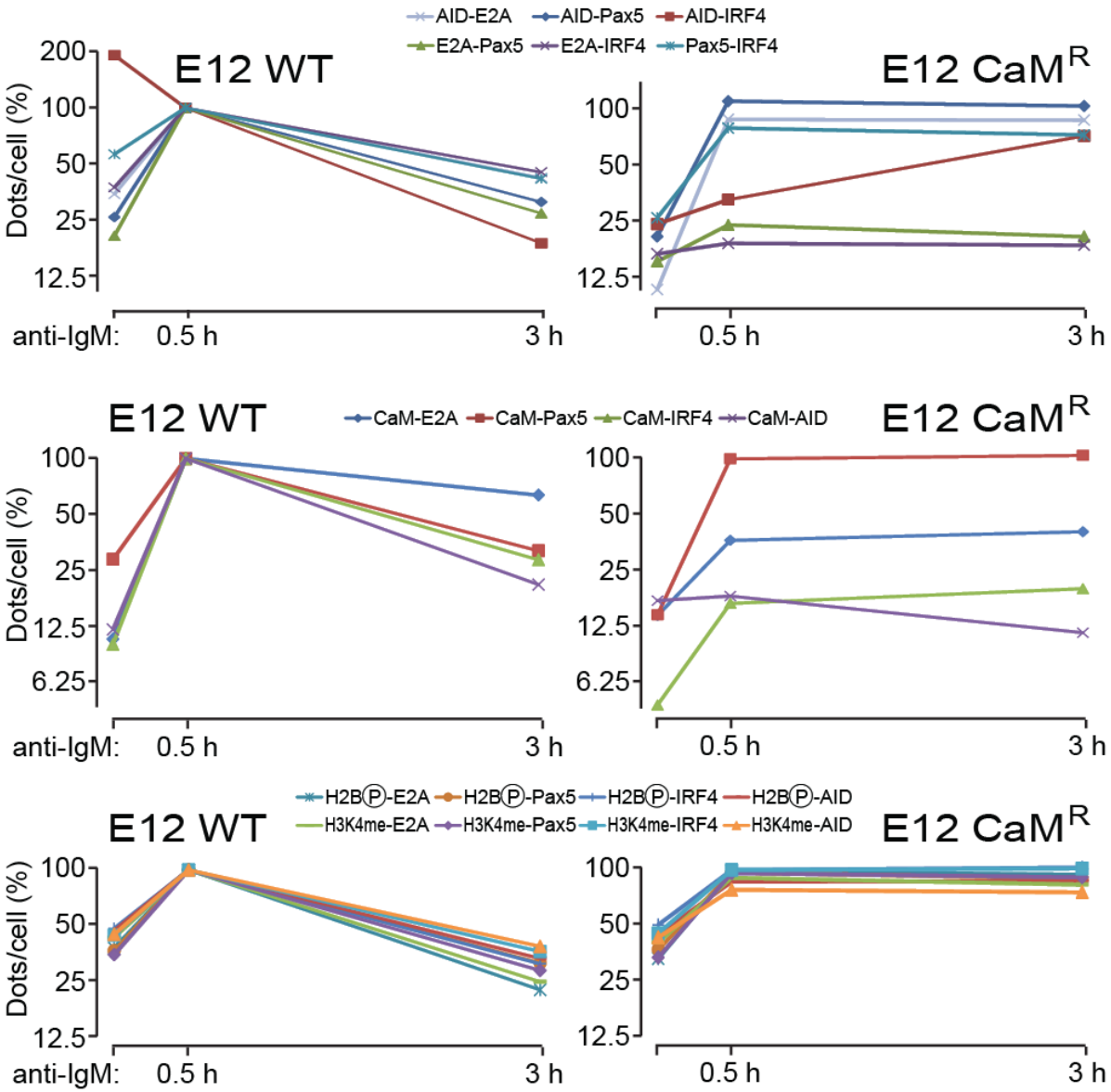
*... and with CSR/SH associated histone modifications*

*... and after BCR stimulation also with calmodulin*



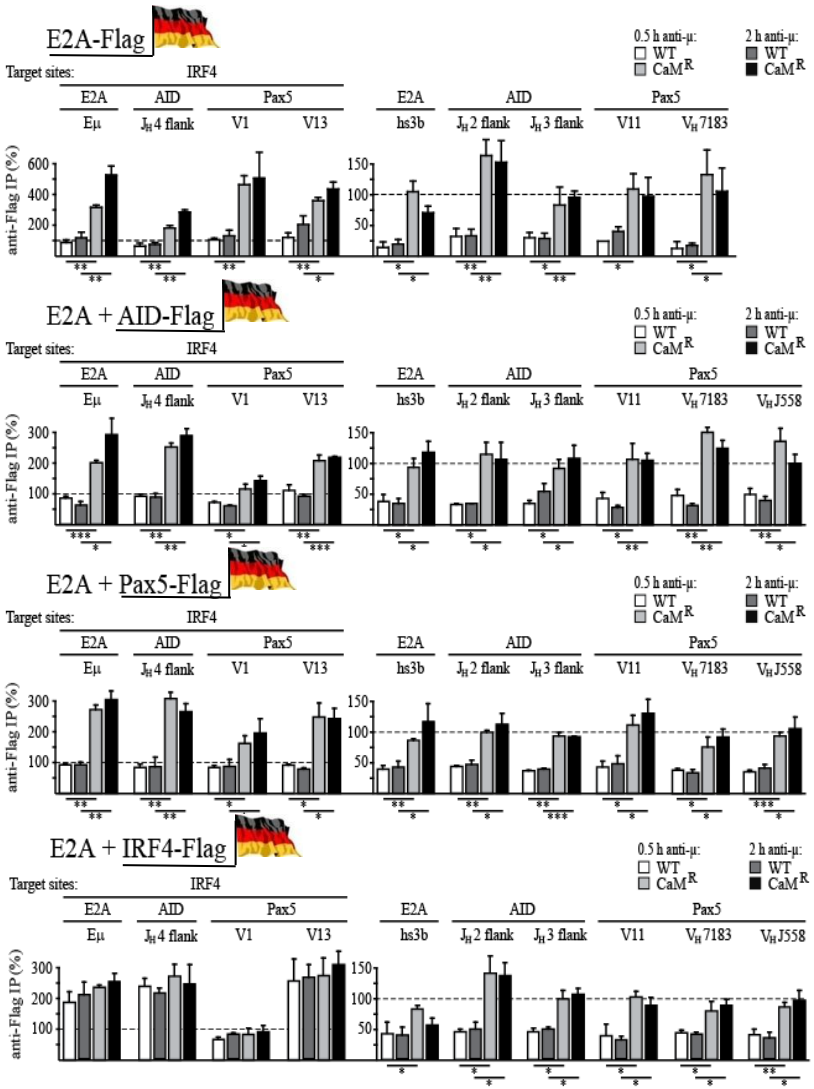



# *Calmodulin inhibition of E2A regulates the AID complex with E2A, Pax5 and IRF4*

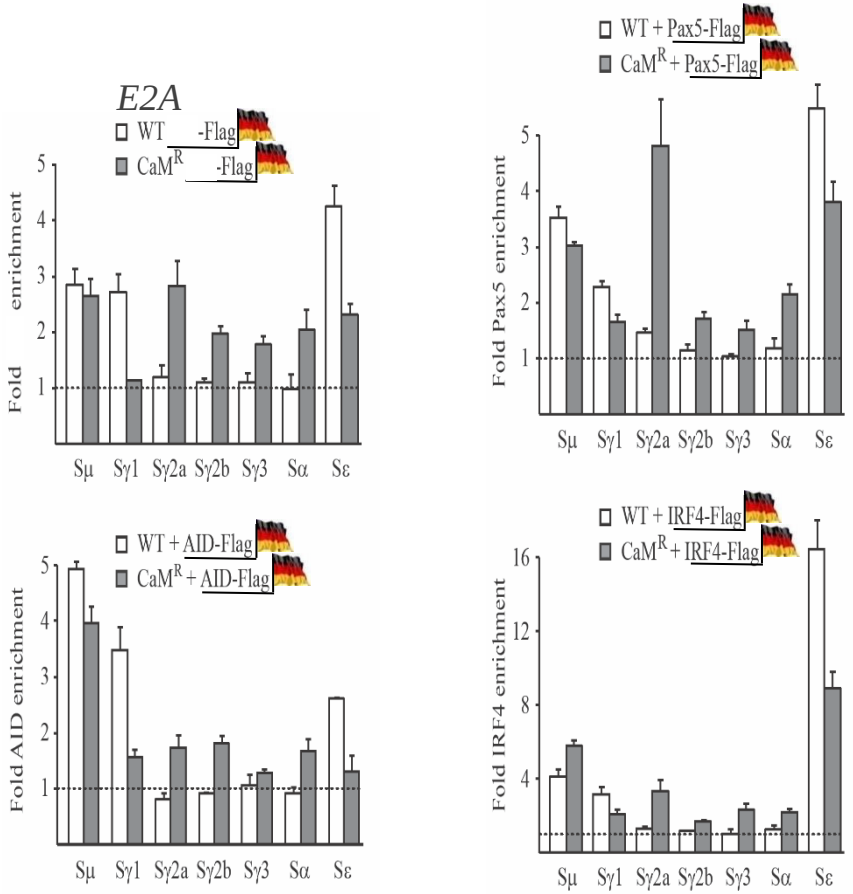



***PLA of indicated proteins in infected mouse splenic B cells grown with CD40L and IL4 and BCR activated with anti-IgM for the indicated times***

# *Regulated localisation of the AID complex with E2A, Pax5 and IRF4 at the Igh switch regions by calmodulin inhibition of E2A*

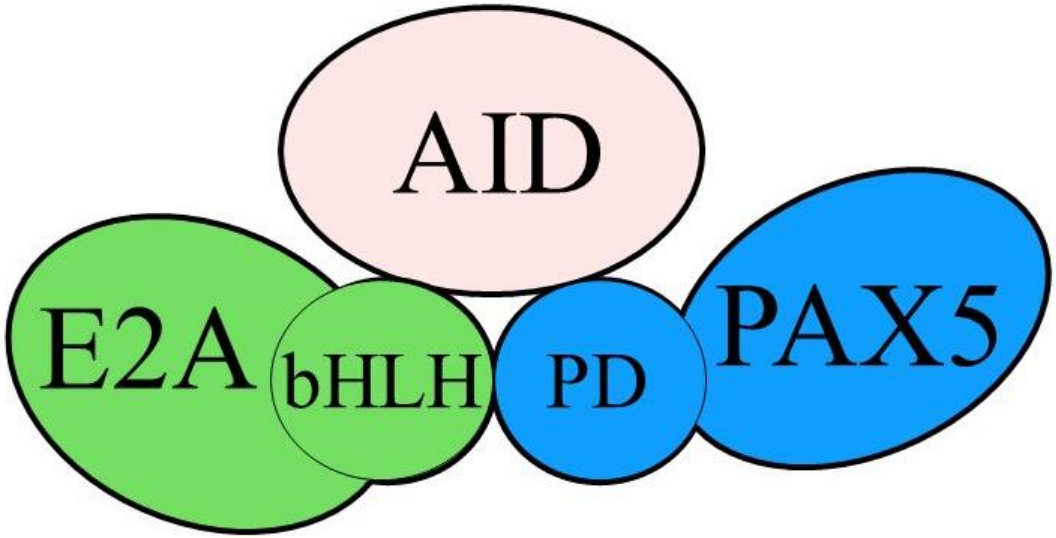


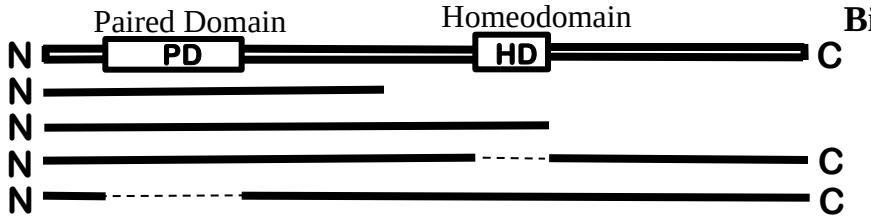

*Infection of splenic B cells with virus expressing Flagged proteins and anti-Flag ChIP of Pax5- and E2A-specific sites and AID target sites of the Igh locus*



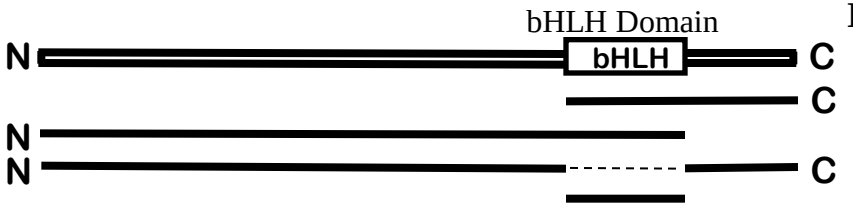

*Localization of the AID complex with E2A, Pax5 and IRF4 at the Igh locus is affected by calmodulin inhibition of E2A*

# AID, PAX5 and E2A bind directly to each other



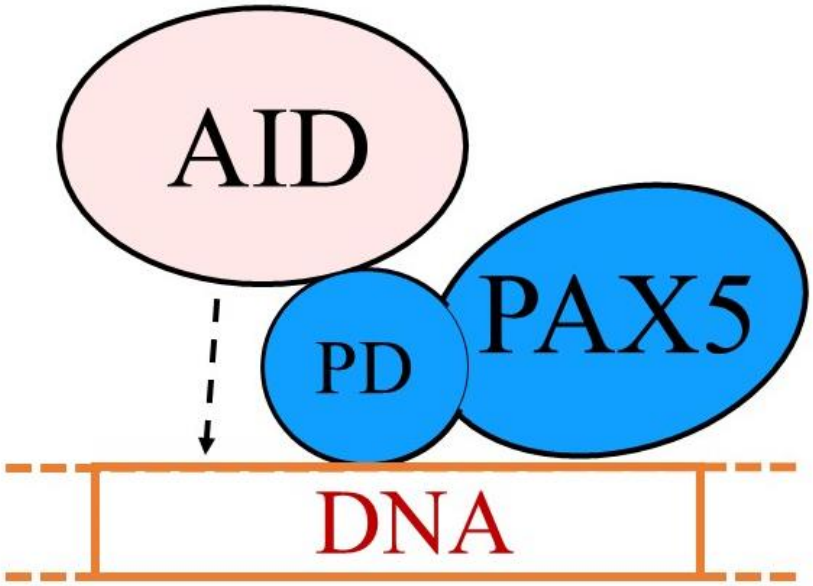



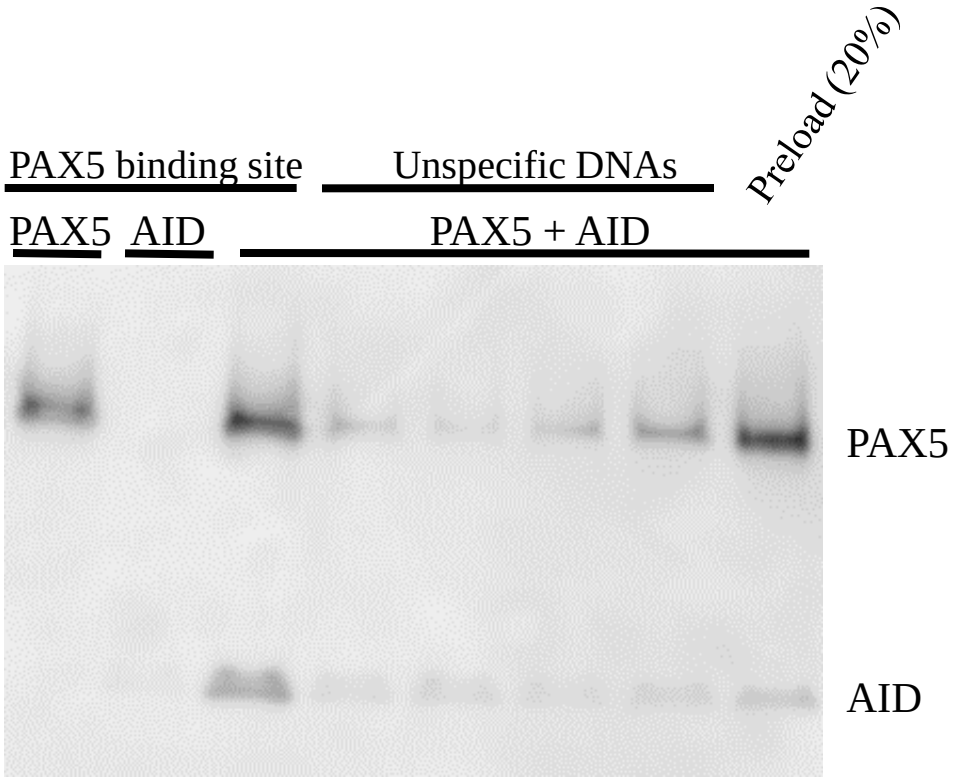

| Binding: | AID-Sepharose | E2A-Sepharose |
|---|---|---|
| Pax5 wt | ++ | ++ |
| Pax5 1-176 | ++ | ++ |
| Pax5 ΔC | ++ | ++ |
| Pax5 ΔHD | ++ | ++ |
| Pax5 ΔPD | - | - |




| Binding: | AID-Sepharose | PAX5-Sepharose |
|---|---|---|
| E12 wt | + | + |
| E12 ΔN | ++ | ++ |
| E12 ΔC | ++ | ++ |
| E12 ΔbHLH | - | - |
| E12 bHLH | ++ | ++ |

# PAX5 recruits AID to DNA with PAX5 binding sites



# *Summary and conclusions (part 1)*

## *Regulated localisation of an AID complex with E2A, Pax5 and IRF4 at the Igh locus*

- ***Defect calmodulin inhibition of E2A leads to reduced BCR-stimulated CSR to IgE***
- ***AID that initiates the CSR process is together with E2A, Pax5 and IRF4 in a complex on key sequences of the Igh gene in activated mouse splenic B cells***
- ***E2A, AID, Pax5 and IRF4 are components of a CSR and SH complex that is redistributed on the Igh gene by BCR signalling through calmodulin binding to E2A in the complex***
- ***AID, E2A and PAX5 bind directly to each other. The interactions are through the bHLH domain of E2A and the PH domain of PAX5***
- ***PAX5 recruits AID to PAX5 sites on DNA***

# Two hallmarks of cancer that enable acquisition of the other hallmarks of cancer:

- ***Tumour-promoting inflammation*:**

  *Chronic inflammations can aberrantly induce AID!*

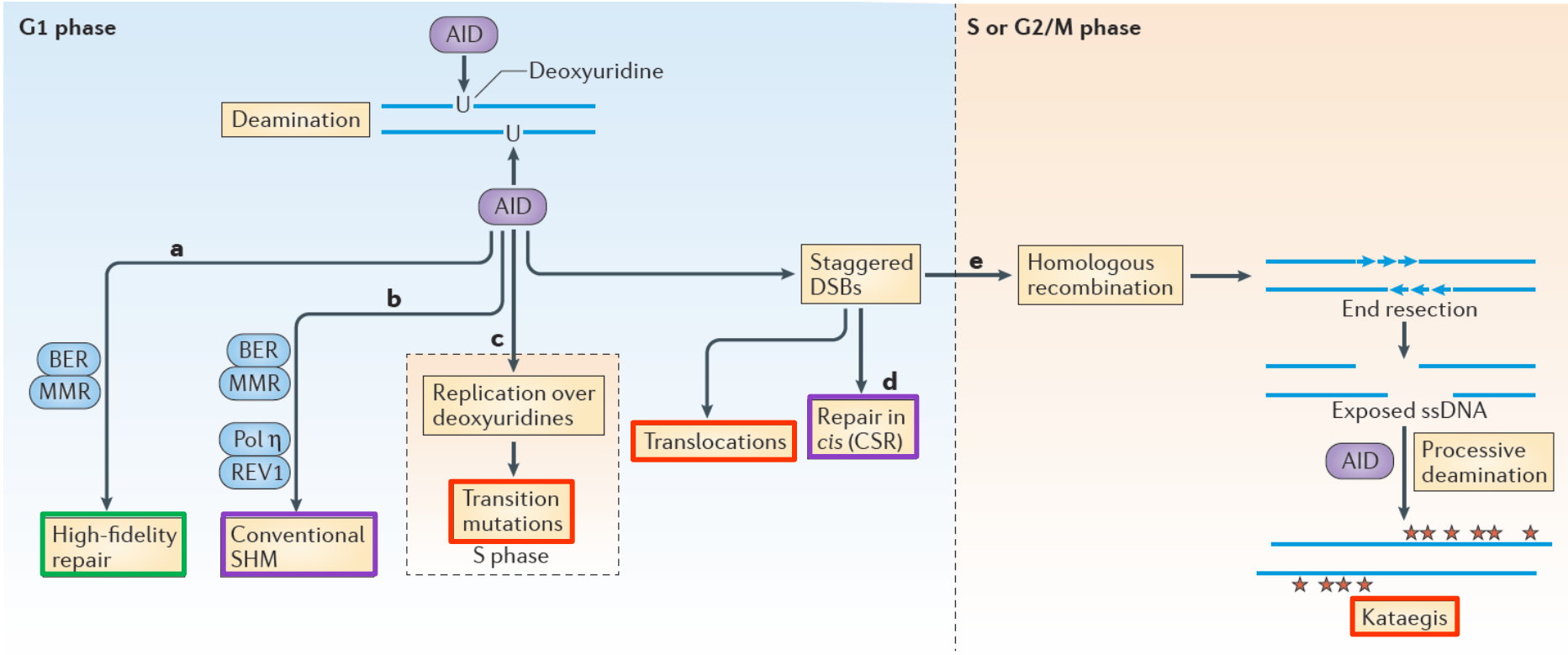
- ***Genome instability and mutation*:**

  *High rate of mutations that DNA repair doesn't take care of*

  Hundred or thousands and sometimes more than 100 000 mutations in cancers

  - Extensive exposure to mutagen
  - Defect in DNA repair enzyme
  - **Defect control of the DNA repair system ?**

# Differential processing of AID lesions




Casellas R. *et al*. Nature 216: 164-176

**Kataegis:** "**Mutation storms**". Clusters of mutations (mostly C to T) in the same DNA strand introduced in tumour genomes by cytidine deaminases: APOBEC enzymes in non-B cell tumours and AID in B cell lymphomas.

# Two hallmarks of cancer that enable acquisition of the other hallmarks of cancer:

- ***Tumour-promoting inflammation*:**

  *Chronic inflammations can aberrantly induce AID!*

- ***Genome instability and mutation*:**

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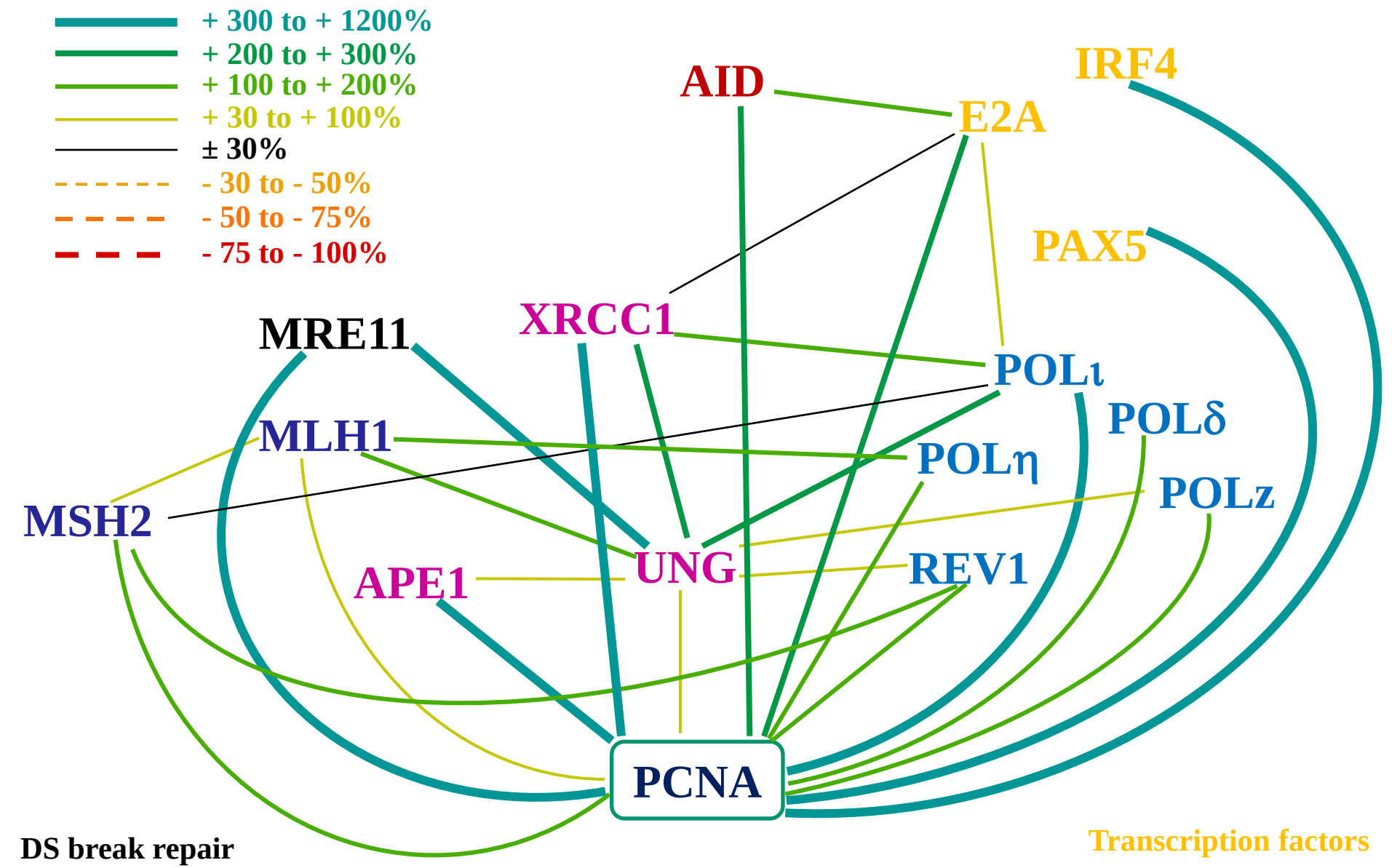
  Hundred or thousands and sometimes more than 100 000 mutations in cancers

**A mutasome?**

# Activation of mutagenesis and CSR increases the interactions




**DS break repair**

**Mismatch repair**

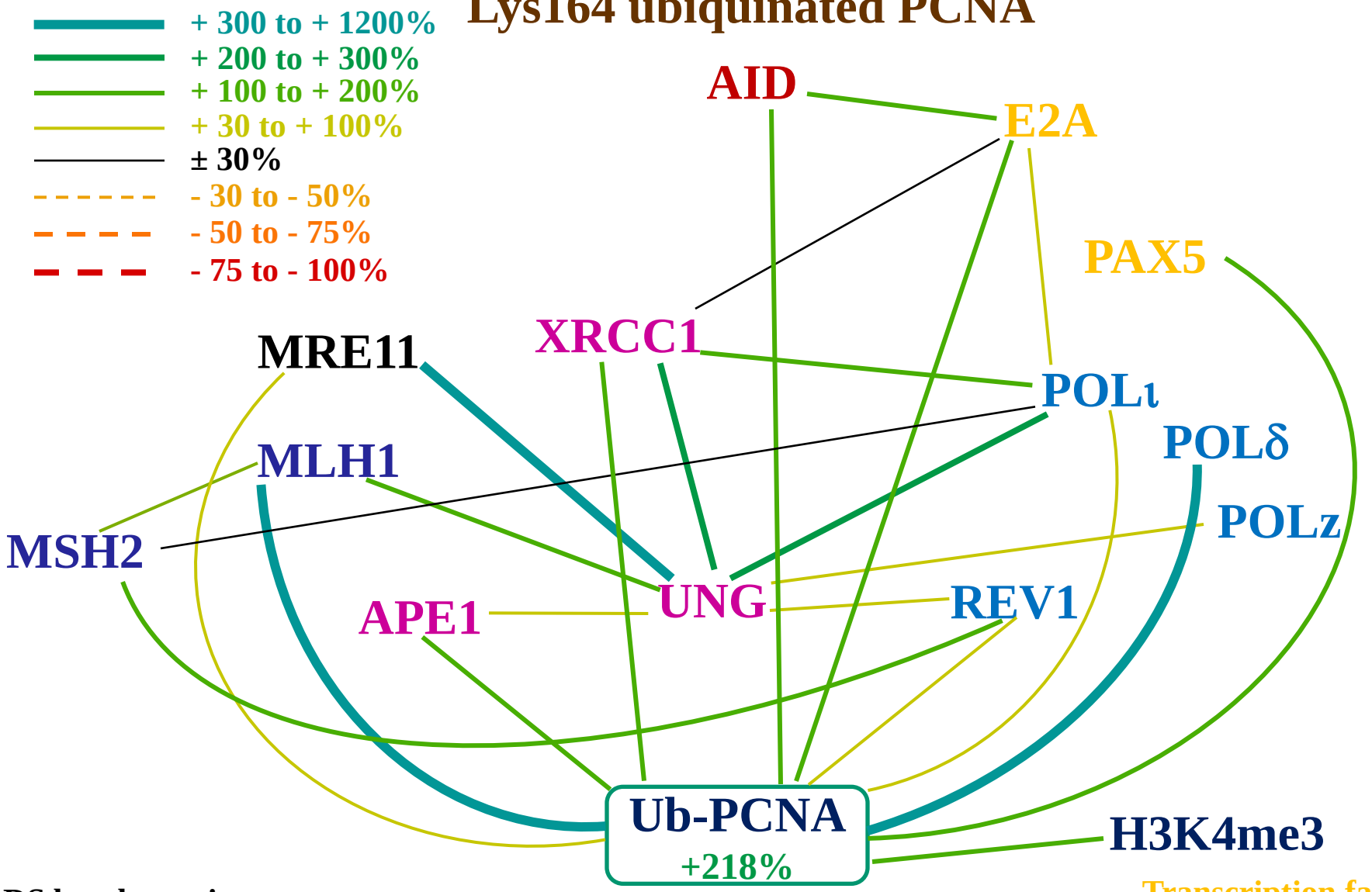
**Base excision repair** **DNA Polymerases**

**Transcription factors**

**PCNA:** The clamp around the DNA

**AID:** Activation-induced cytidine deaminase (the mutagen)

# Activation of mutagenesis and CSR increases interactions with Lys164 ubiquinated PCNA

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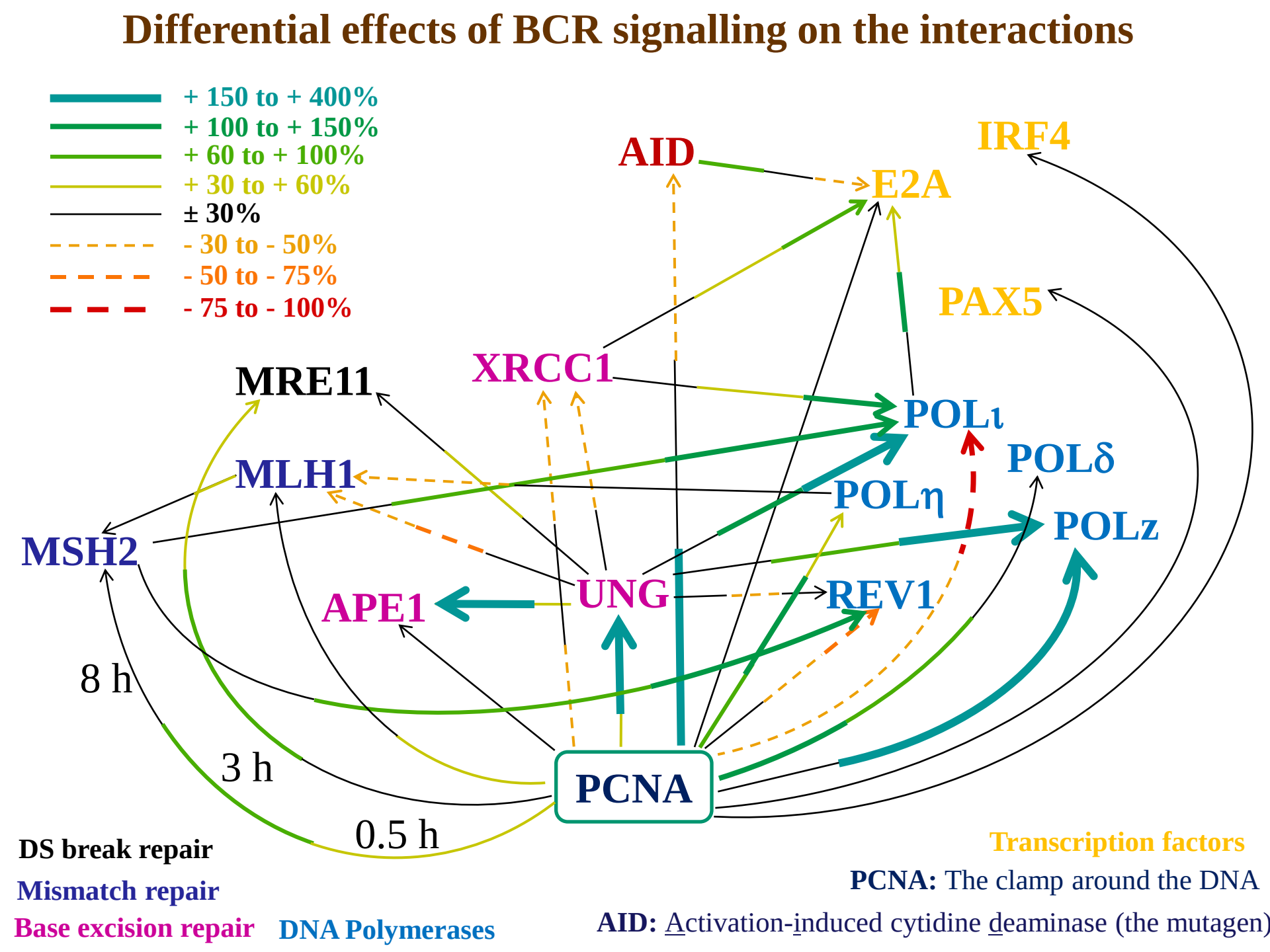
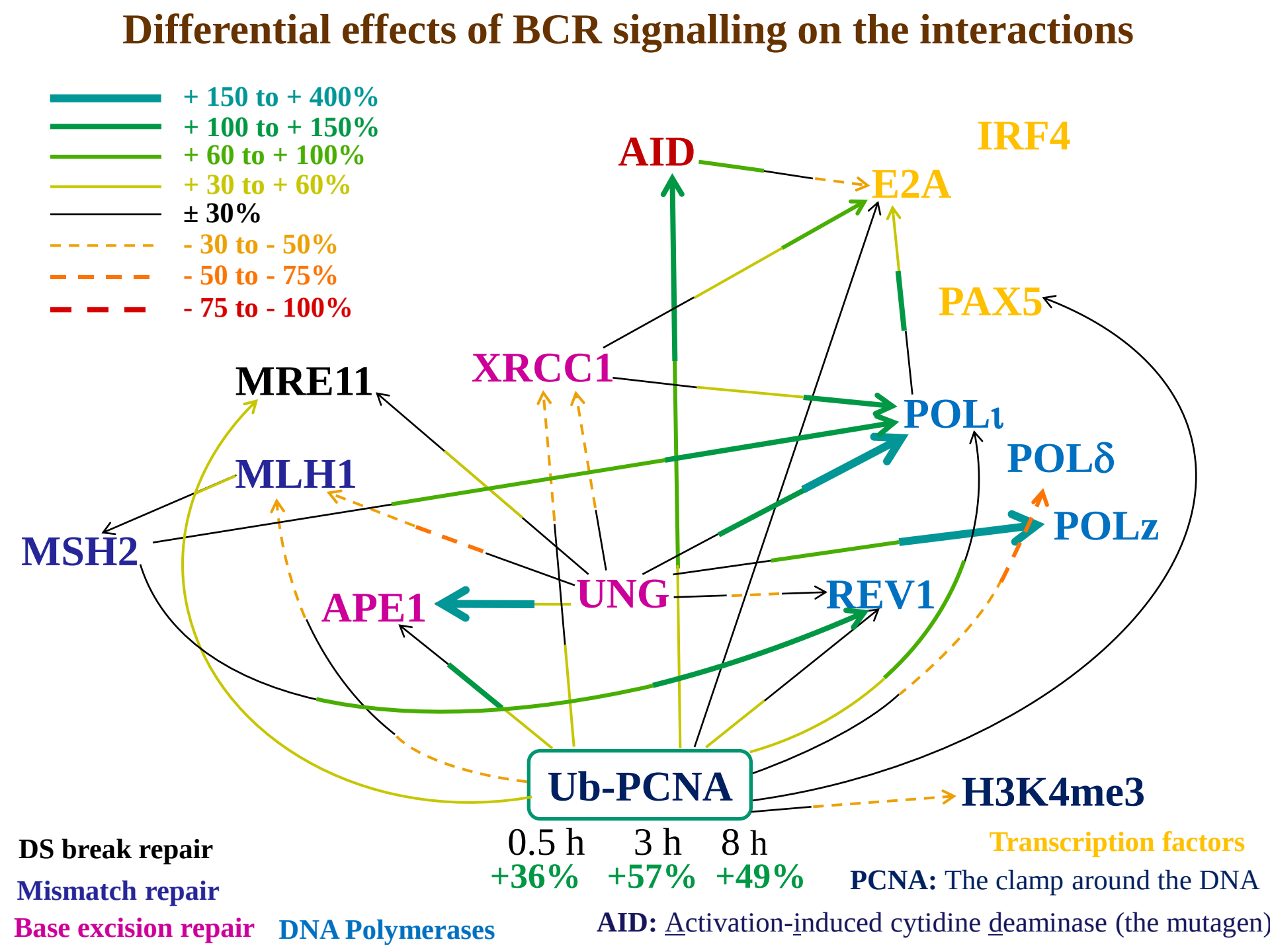
# Co-Immunoprecipitation analyses of interactions

## Corresponding increases

# Differential effects of BCR signalling on the interactions

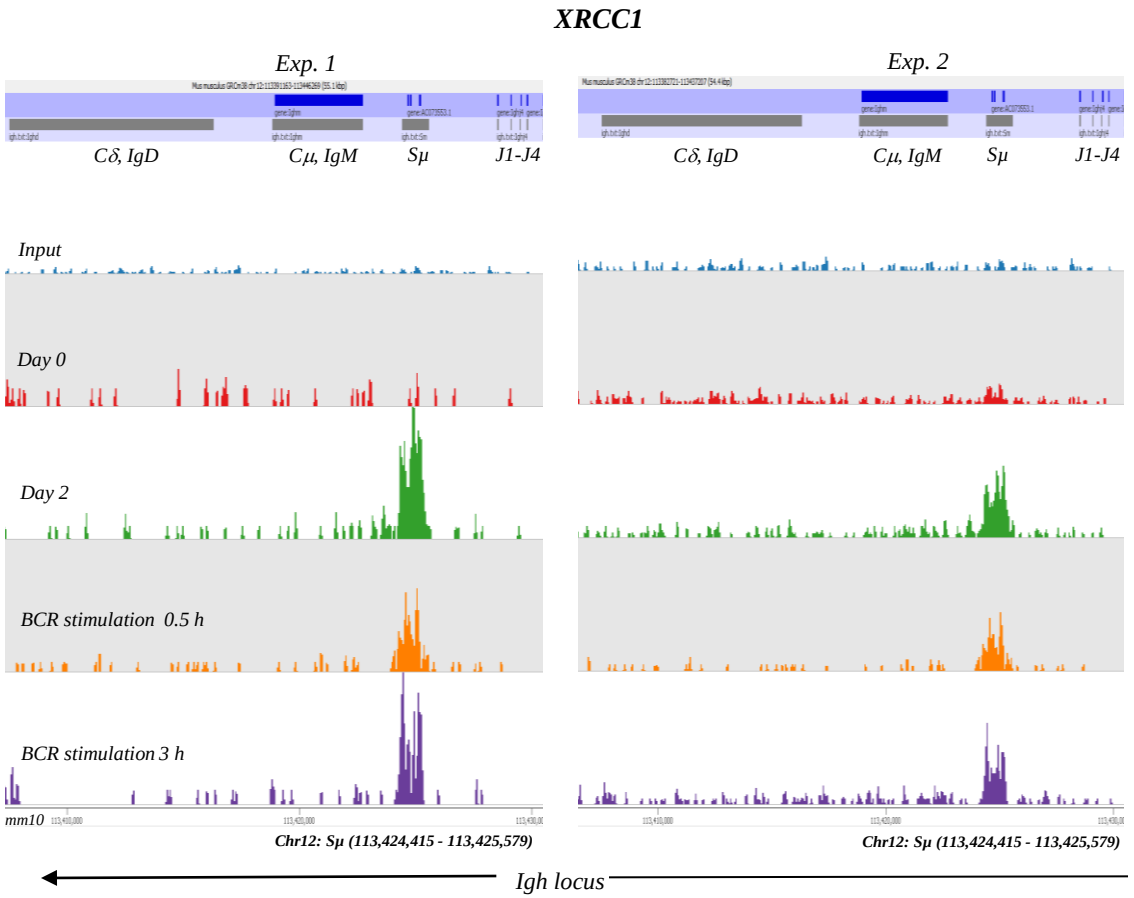


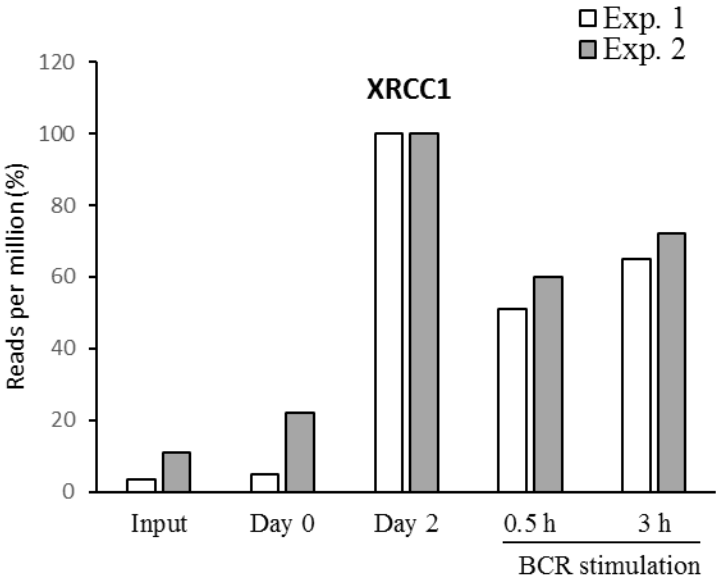
# Differential effects of BCR signalling on the interactions



# ChIP-sequencing

## XRCC1

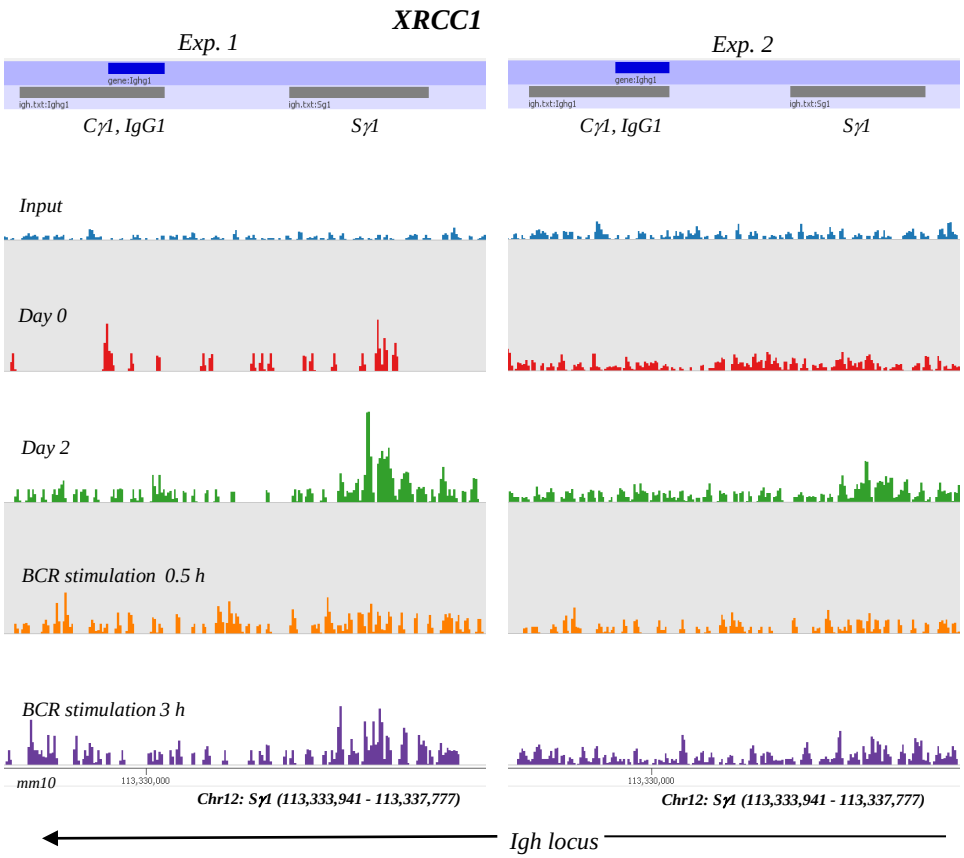


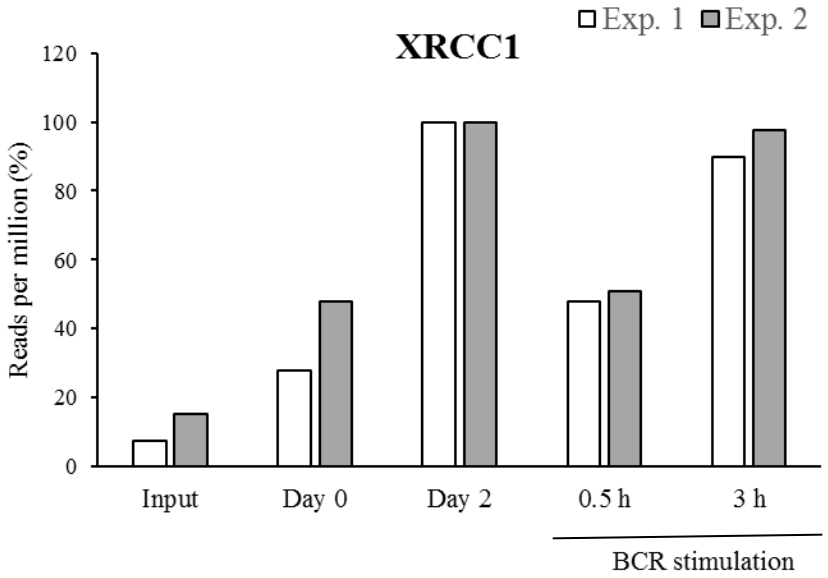




*XRCC1 is recruited to the CSR site Sμ*

# *ChIP-sequencing*

## *XRCC1*

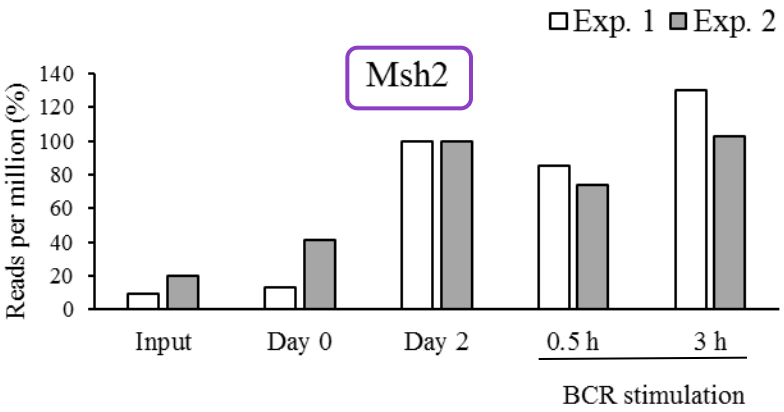


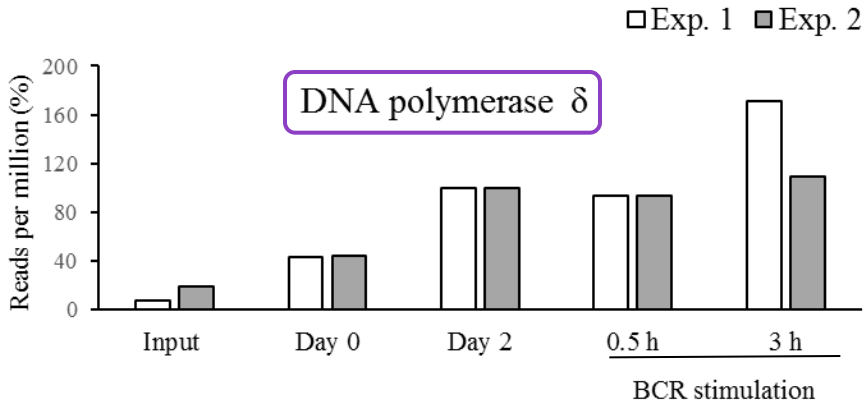




*XRCC1 is also recruited to the CSR site Sγ1*

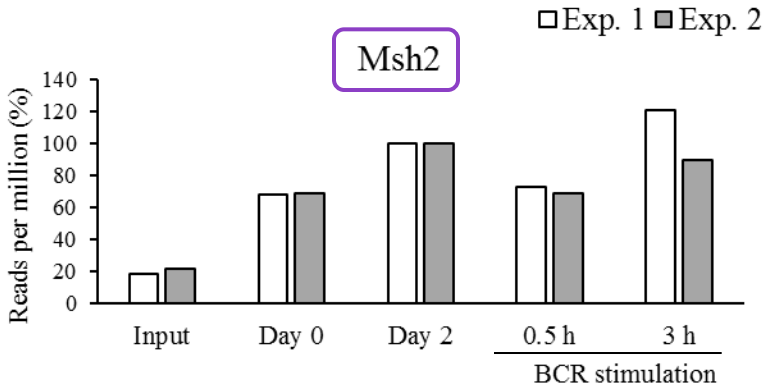
# *ChIP-sequencing*

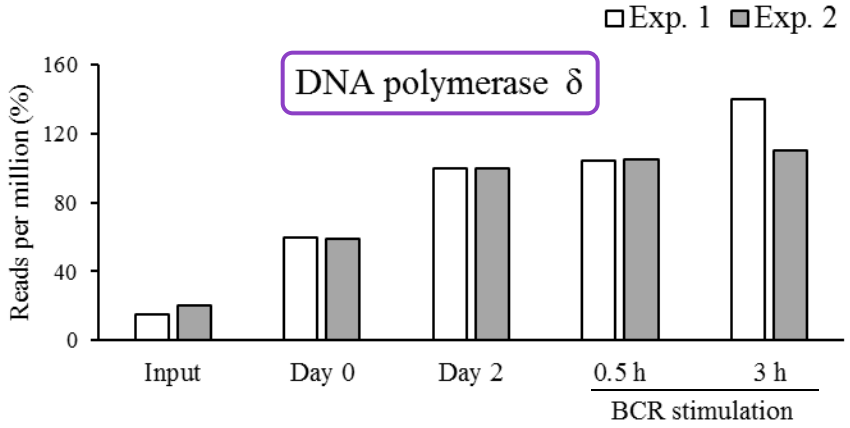
*sμ*



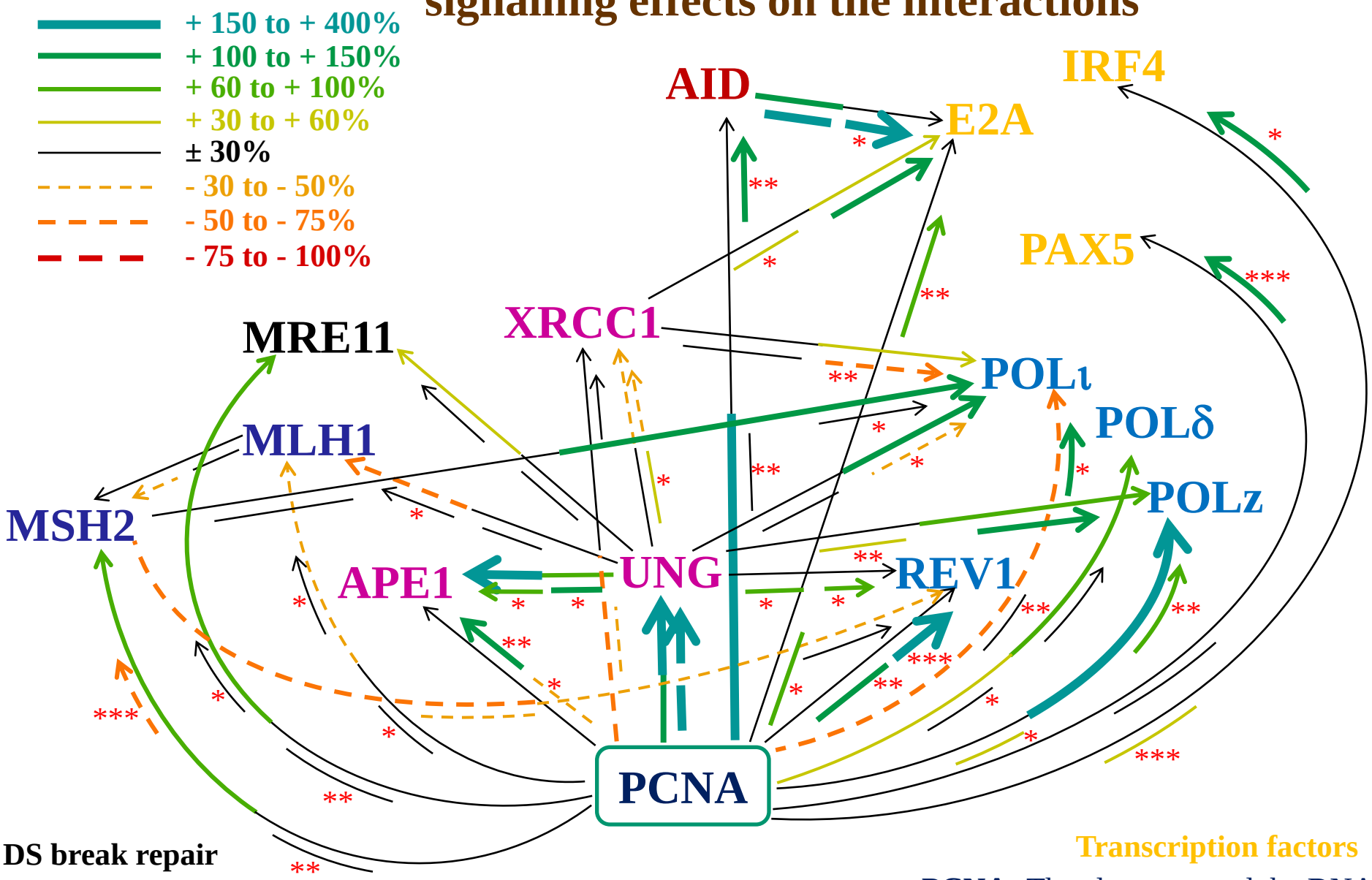

*sγ1*




*Msh2, DNA polymerase δ and others are also recruited to both the Sμ and Sγ1 CSR sites*

# Differential effects of CaM-resistance of E2A on the BCR signalling effects on the interactions




**DS break repair**

**Mismatch repair**

**Base excision repair** **DNA Polymerases**

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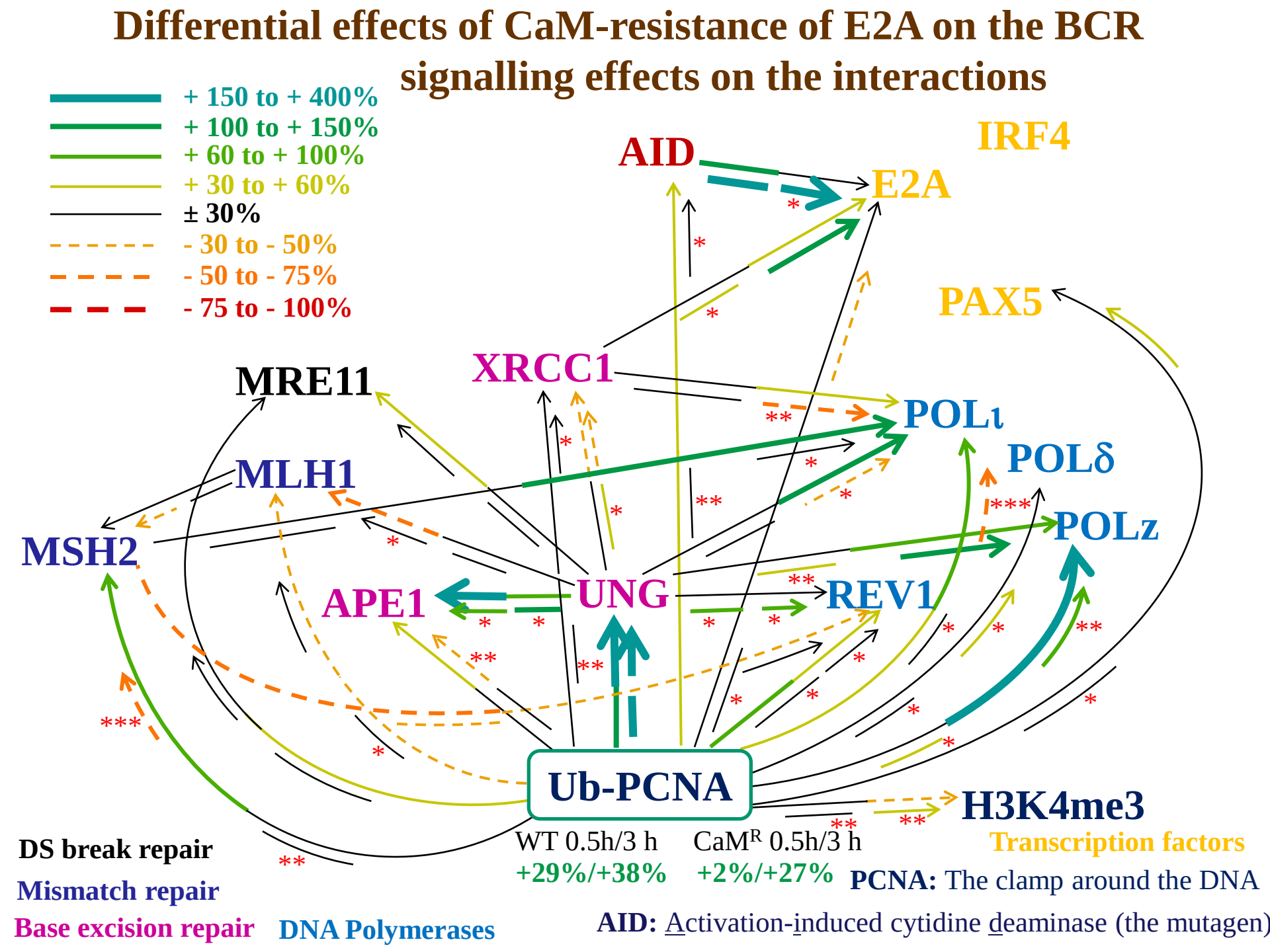

# Conclusions *(part 2)*

- **Activation of mutagenesis and CSR increases the interactions, including with Lys164 ubiquinated PCNA**
- **Differential effects of BCR signalling on the interactions: fast and slow increases and fast and slow reductions**
- **Some effects of BCR signalling on interactions are the same for ubiquinated PCNA as for total bulk PCNA but others are not**
- **The DNA repair proteins, replication proteins and transcription factors showed inducible localisation at the Sμ and Sγ1 switch regions when CSR and SHM is induced**
- **BCR stimulation that differentially affect CSR and SHM differentially affects the localisations**
- **CaM-resistance of E2A increases the BCR signalling effects on some interactions whereas others are reduced by CaM-resistance of E2A**
- **Inhibition of E2A by CaM is only one of several signalling pathways that regulate the mutasome**
- **Since several signalling pathways need to regulate correctly for good repair, it is not strange that mutation rates are high in cancers where many pathways are not regulated correctly**

## *Questions:*

- **Can increased mutation rate in cancer be reversed?**
  - **Which pathway(s) does then have to be corrected?**
    - **What protein(s) should be targeted?**

# Acknowledgements

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***Anshu Priya***

***Ramesh Kumar***

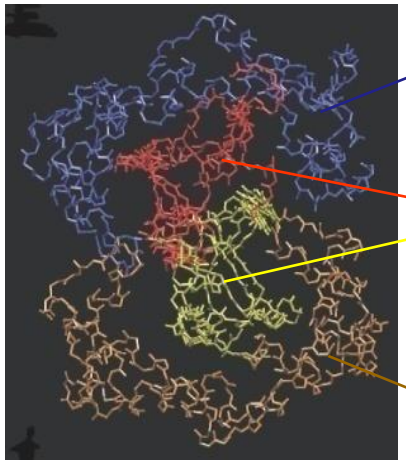
***Tanzeel Ahmed***

*Department of Molecular Biology*
*Umeå University*

# Other studies

- **Feedback inhibition** of antigen receptors and their signalosomes through CaM inibition of E2A enables selection of the good antibodies during the mutagenesis
- E2A represses expression and localisation at the *Igh* locus of **Uracil-DNA glycosylase (UNG),** the enzyme that takes away the U that AID makes
- CaM inhibition of E2A is essential for rapid down-regulation of immediate early genes after BCR stimulation in initiation of **plasma cell differentiation**

**Most effects of antigen receptor stimulation on gene expression are dependent on CaM inhibition of E2A**

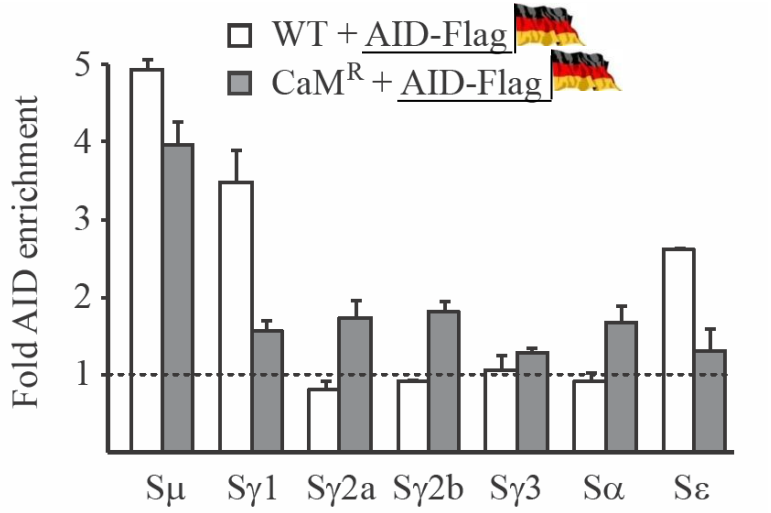


# *Regulated localisation of an AID complex with E2A, Pax5 and IRF4 at the Igh locus*

- ***Defect calmodulin inhibition of E2A leads to reduced BCR-, IL4- plus CD40L-stimulated correct CSR and instead aberrant CSR to other Ig classes***
- ***AID that initiates the CSR process is together with E2A, Pax5 and IRF4 in a complex on key sequences of the Igh gene in activated mouse splenic B cells***
- ***Calmodulin is in proximity with each of AID, E2A, Pax5 and IRF4 after BCR stimulation***
- ***BCR signalling reduces binding of the proteins to some of the target sites on the Igh locus, and calmodulin resistance of E2A blocks reduction of binding to these target sites and increases binding to other target sites***
- ***E2A, AID, Pax5 and IRF4 are components of a CSR and SH complex that is redistributed on the Igh gene by BCR signalling through calmodulin binding to E2A***
- ***AID, E2A and PAX5 bind directly to each other***
- ***The interactions are throught the bHLH domain of E2A and the PH domain of PAX5***

# *Defect calmodulin inhibition of E2A leads to relocalisation of AID to inappropriate switch regions*

***Anti-FLAG ChIP of infected mouse splenic B cells grown with CD40L and IL4 and BCR activated with anti-IgM for 2 h***




# *... and over-expression of AID leads to inappropriate CSR*