

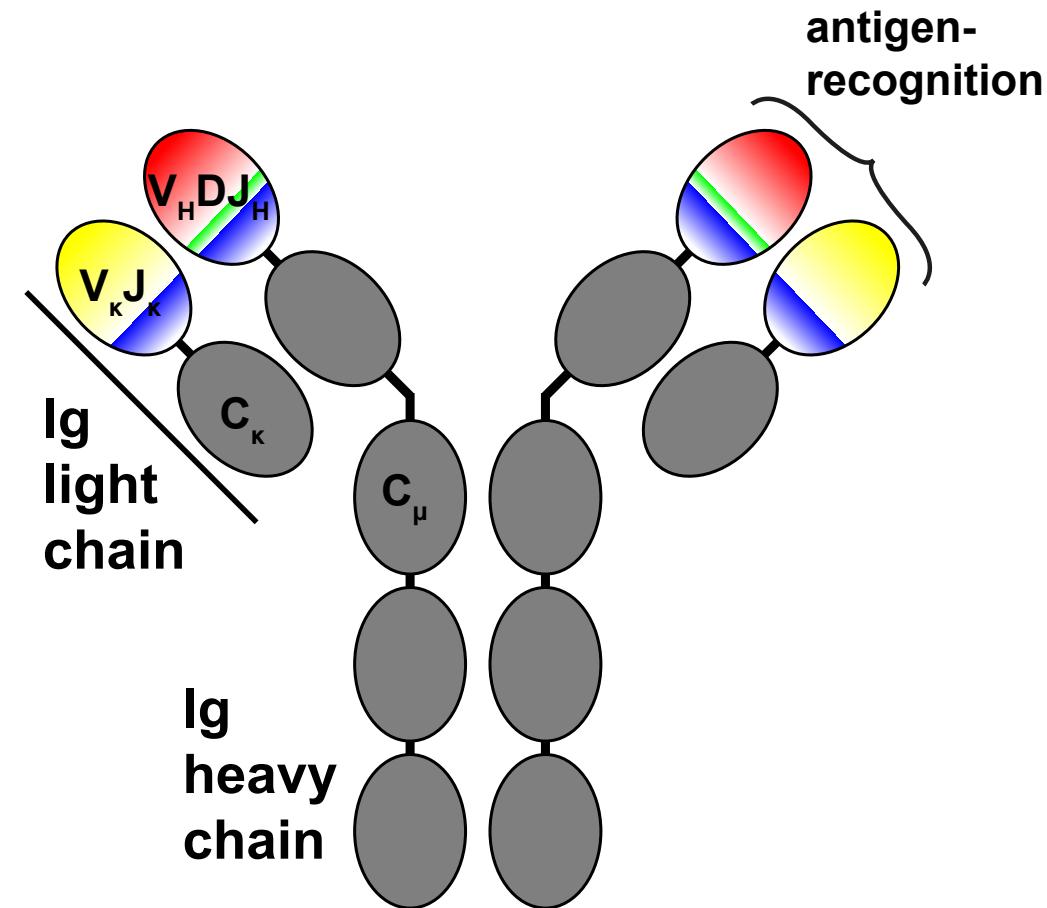
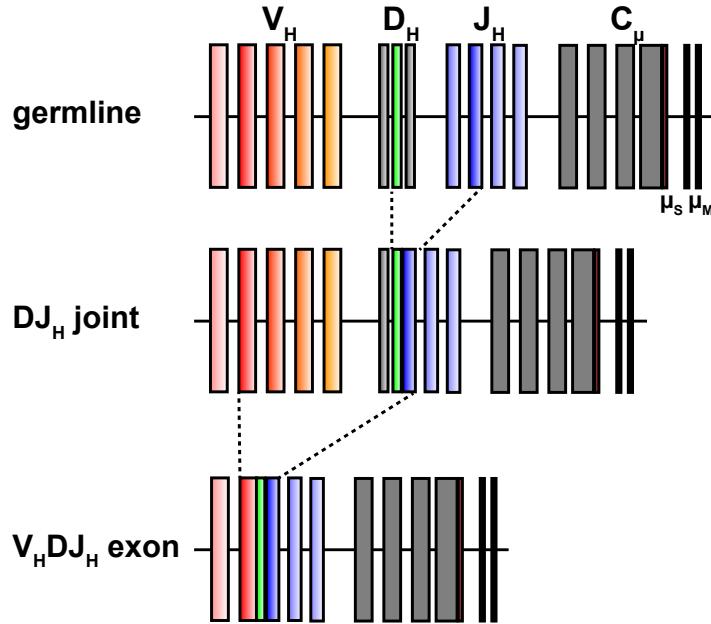
The role of Roquin proteins in B cell physiology and pathology

July 6th 2017

David Rieß

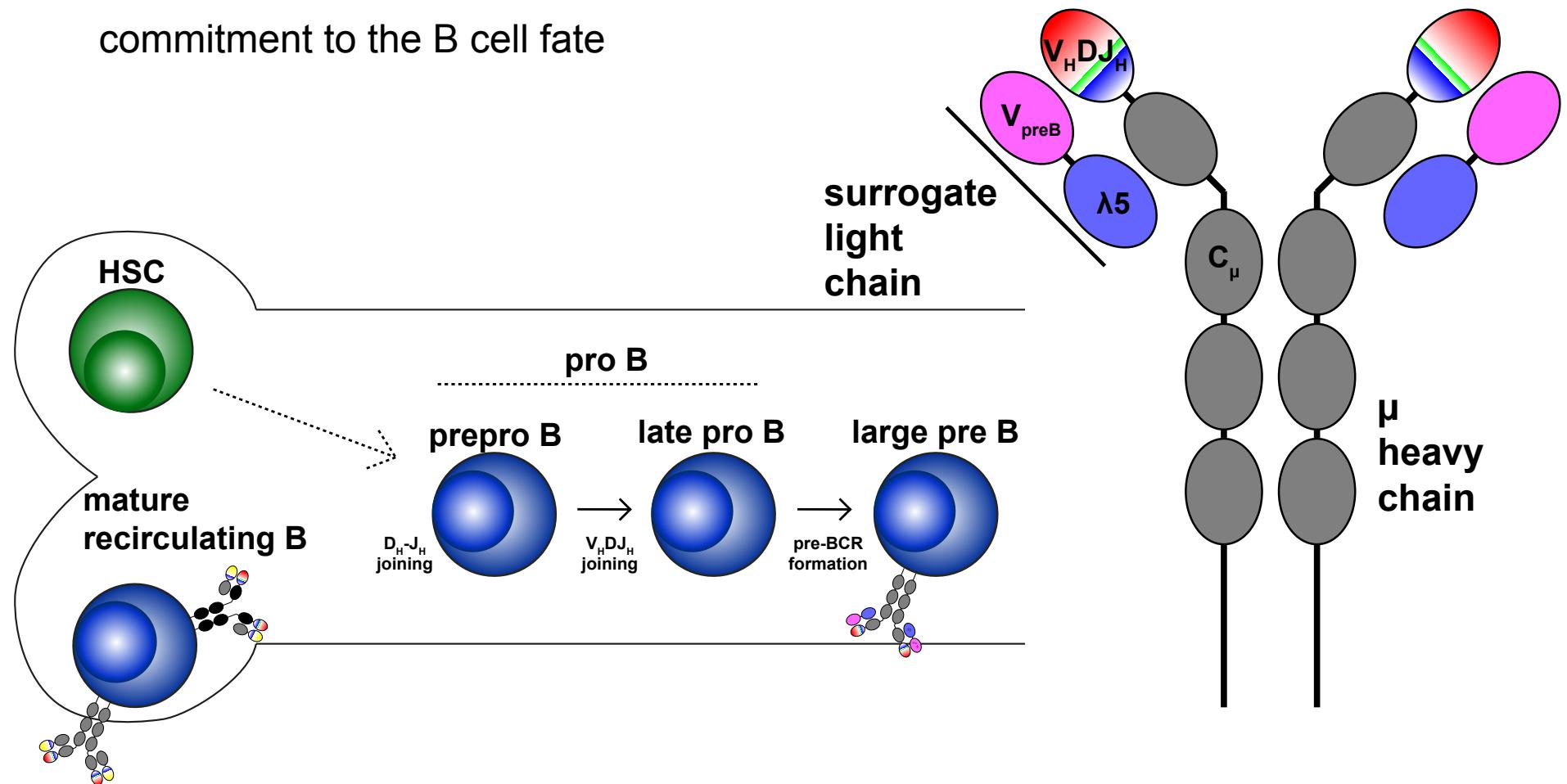
Antibody diversity relies on somatic rearrangements in developing B cells

Ig heavy chain (IgH)



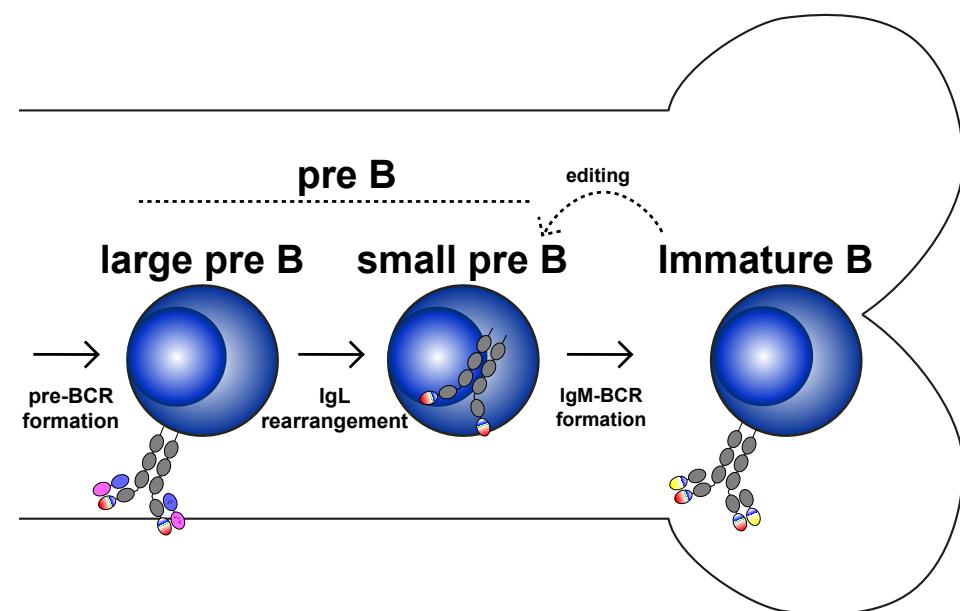
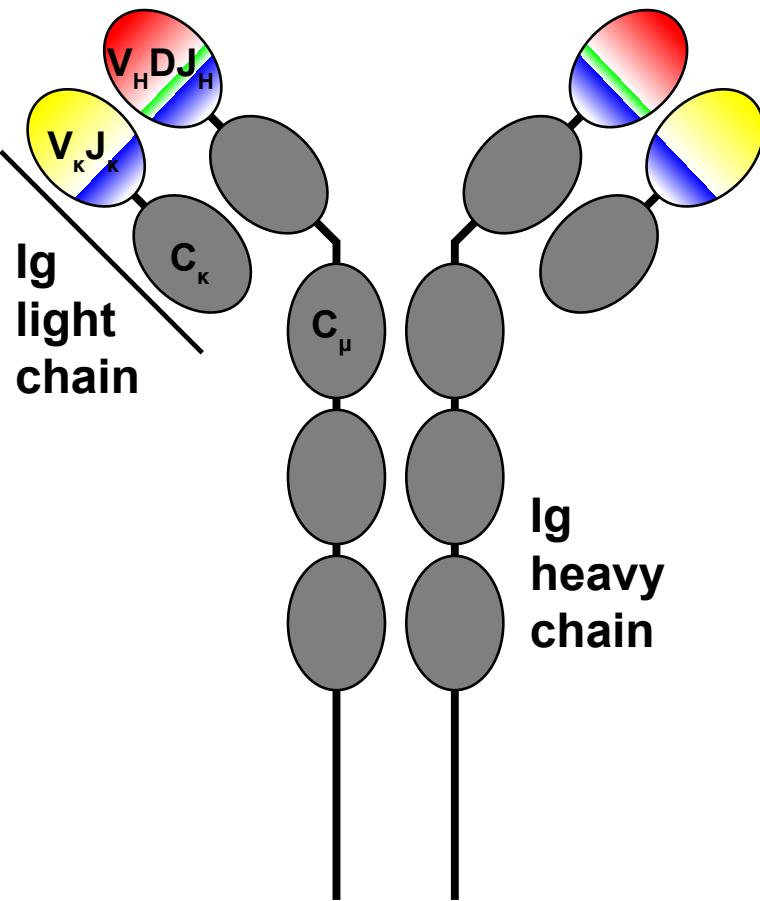
Control of IgH and IgL chain integrity is linked to B cell fates during their development

- TF-orchestrated successive commitment to the B cell fate



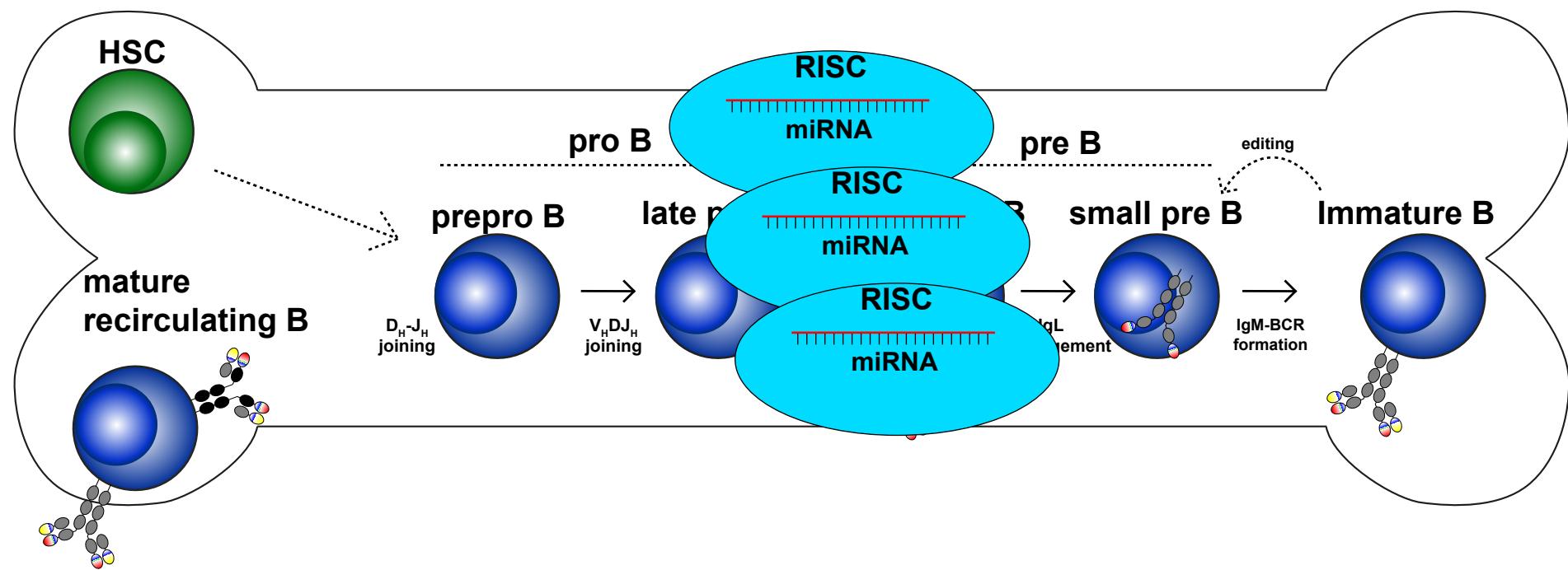
Control of IgH and IgL chain integrity is linked to B cell fates during their development

- TF-orchestrated successive commitment to the B cell fate



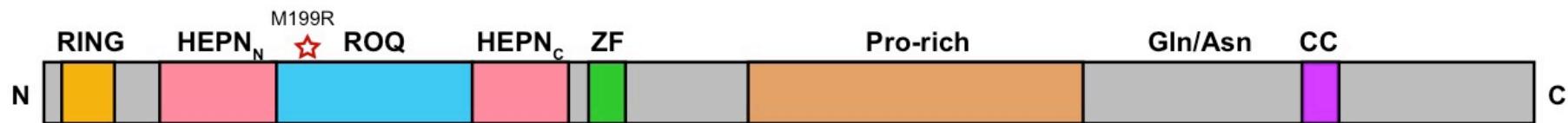
What is the role of specific RBPs in B cell development?

- TF-orchestrated successive commitment to the B cell fate
- Essential role of miRNAs



The mRNA-binding proteins Roquin1 and 2

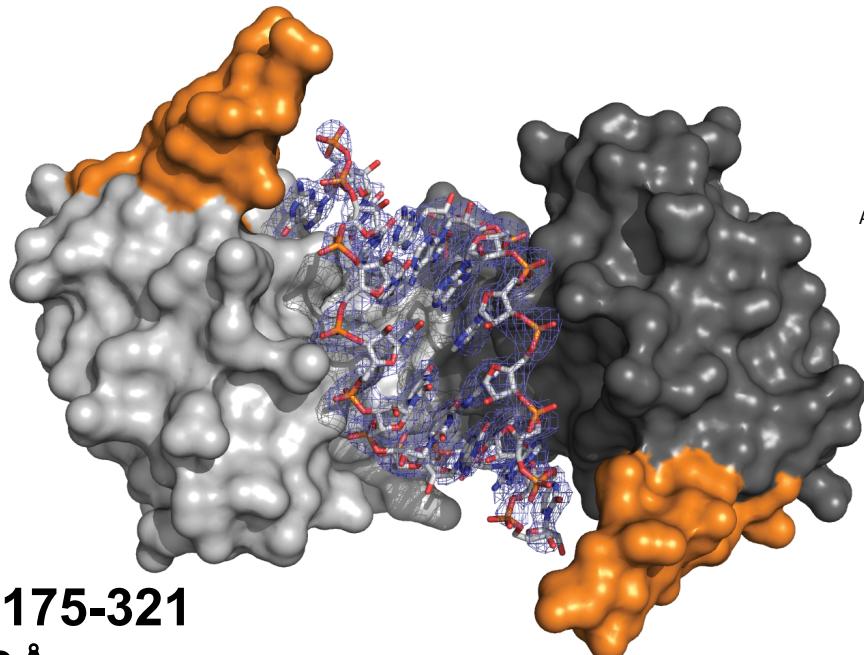
Roquin1



Roquin2

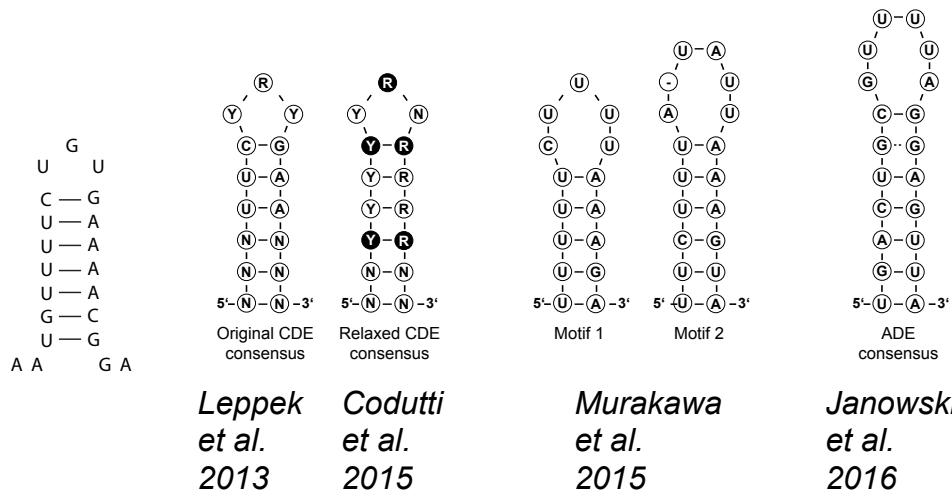


The ROQ domain recognizes stem-loop motifs



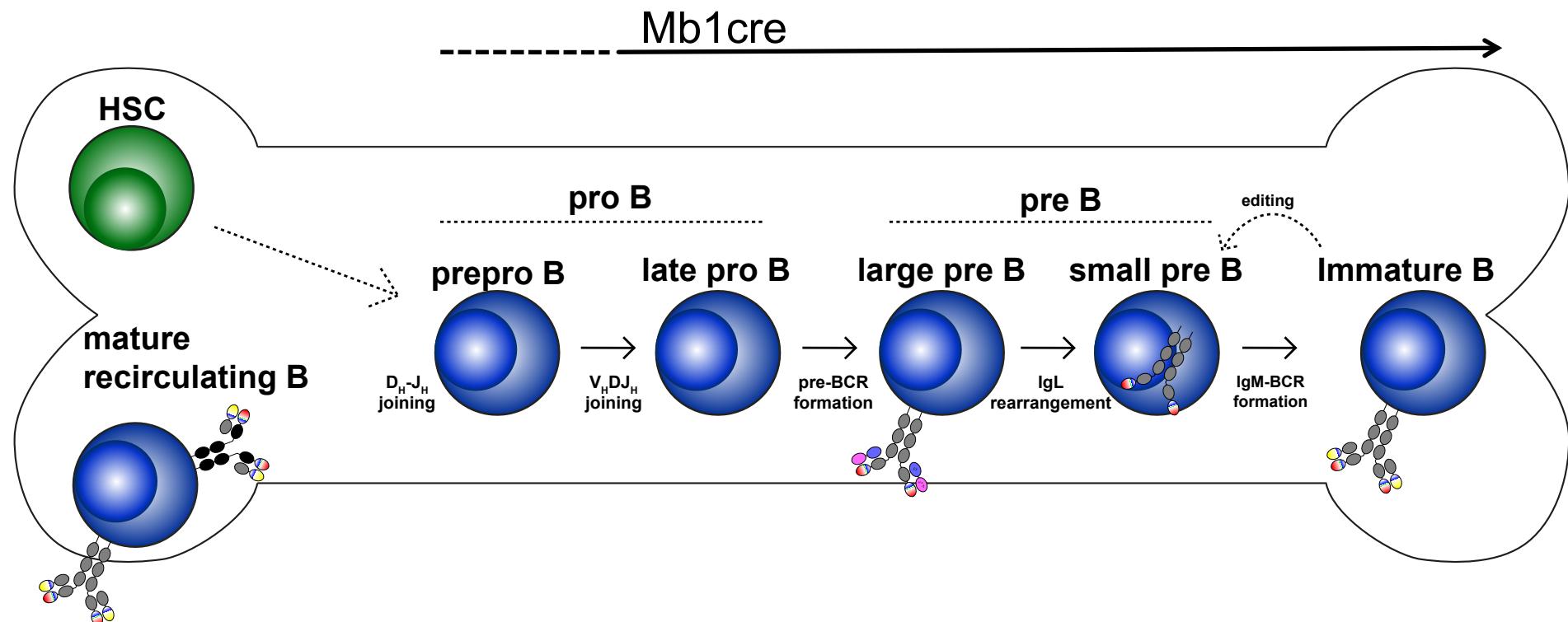
**aa175-321
3.3 Å**

In collaboration with Christian Benda

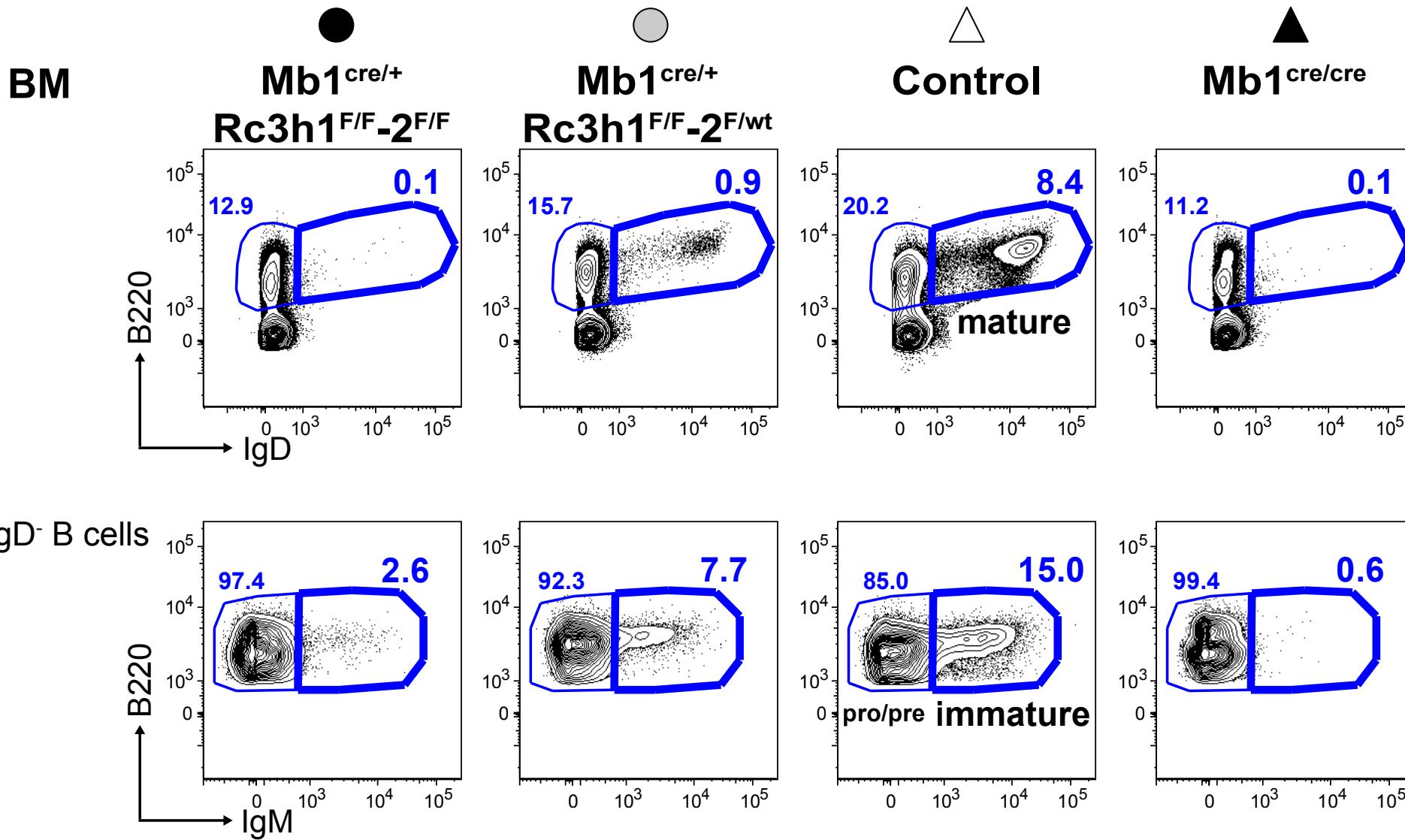


What is the role of the RBPs Roquin1 and 2 in B cell physiology?

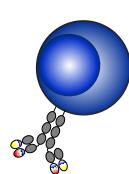
→ *in vivo* ablation of Roquin1 and 2 specifically in B cells



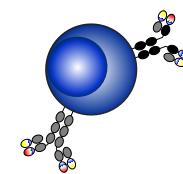
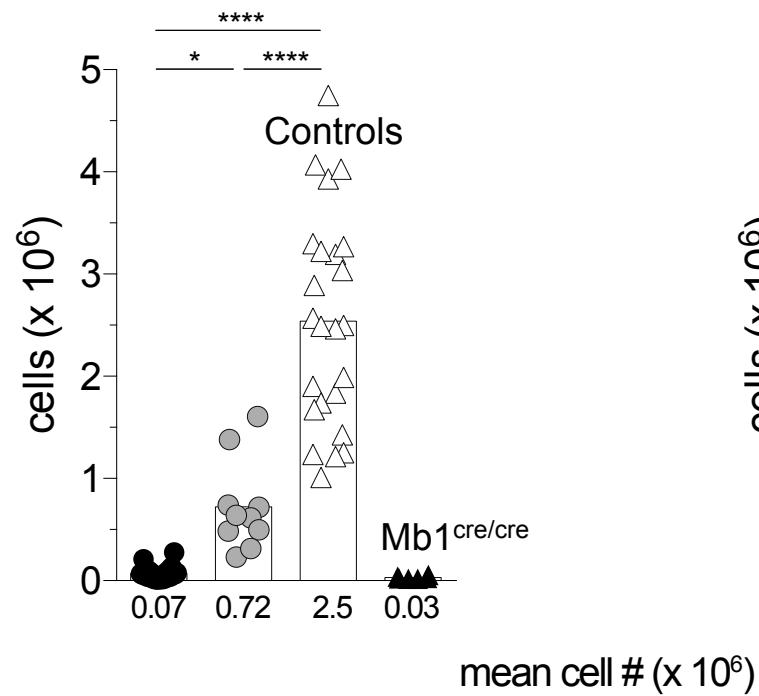
Absence of mature B cells in $\text{Mb1}^{\text{cre}/+}$ $\text{Rc3h1}^{\text{F/F}}\text{-}2^{\text{F/F}}$ mice



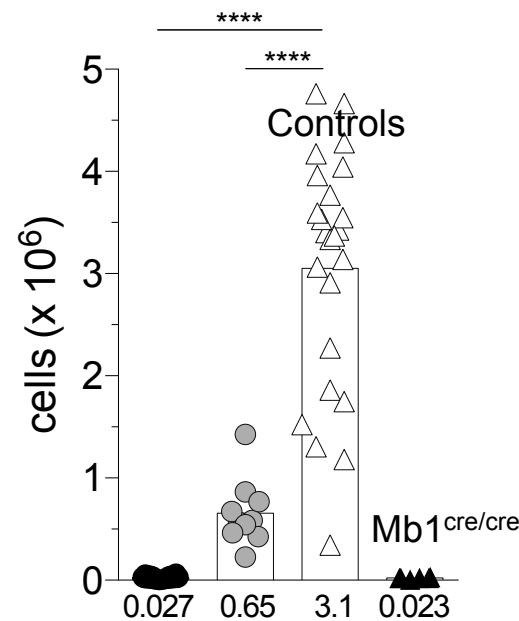
Loss of Roquin paralogs impedes B cell development



Immature B cells
 $B220^+$ IgD^- IgM^+



Mature B cells
 $B220^+$ IgD^+



● Mb1^{cre/+} Rc3h1^{F/F}-2^{F/F}

● Mb1^{cre/+} Rc3h1^{F/F}-2^{F/wt}

△ Controls

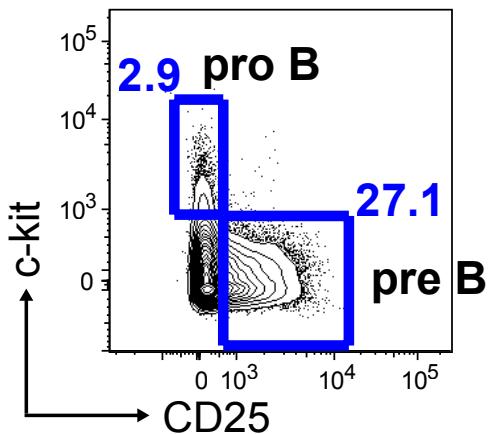
▲ Mb1^{cre/cre}

When is B cell development blocked?

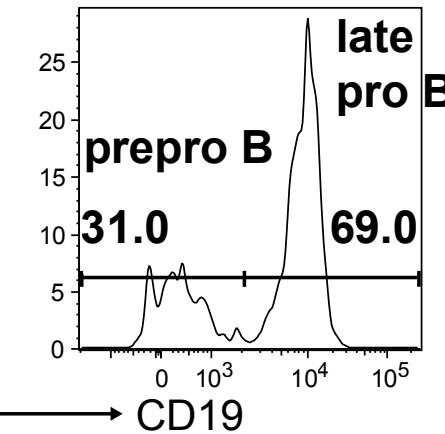
BM

IgD⁻
IgM⁻
B220^{int}
Pro/pre
B cells

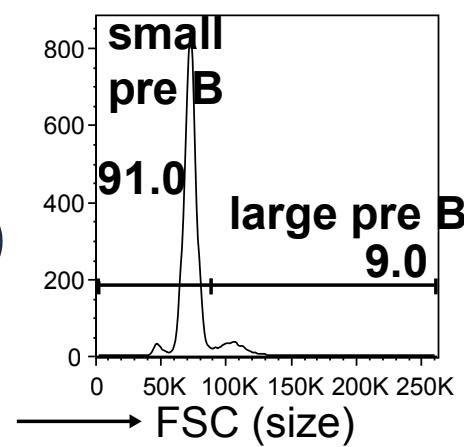
Control



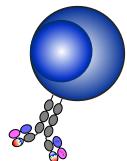
Pro B cells



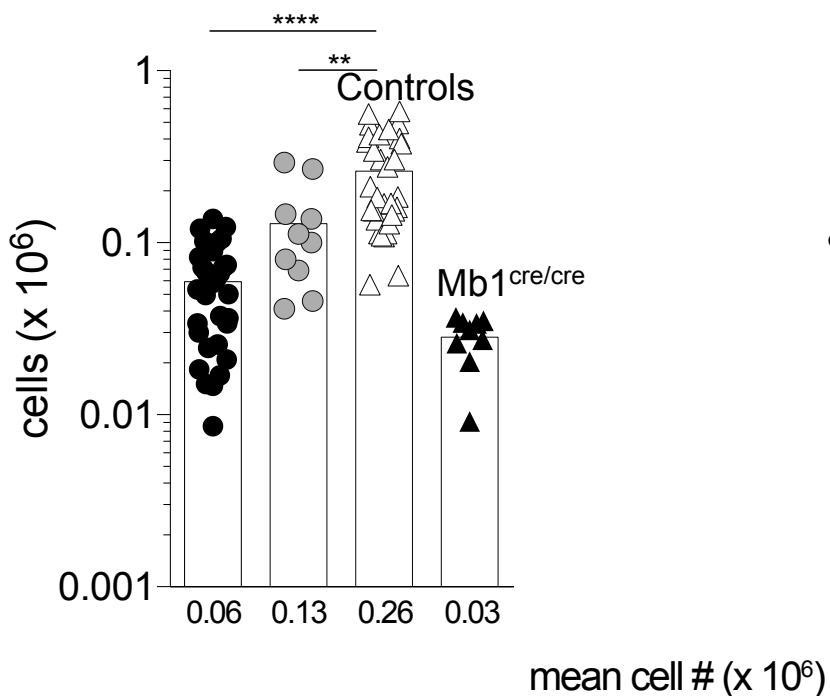
Pre B cells



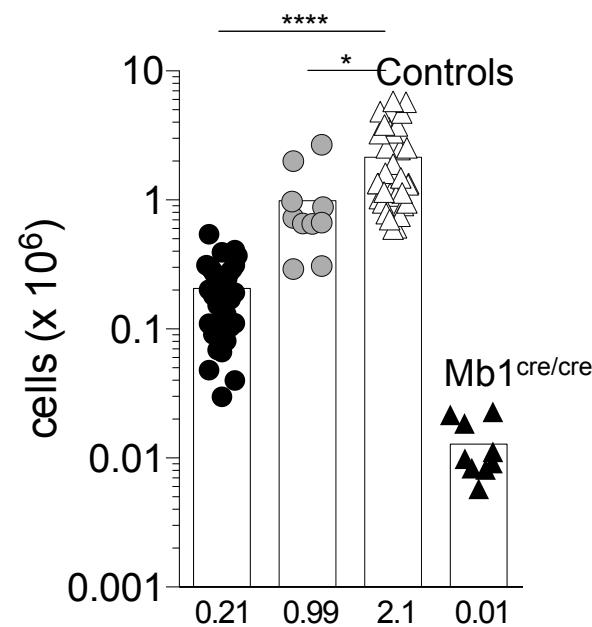
Block of B cell development at the pre B cell stage in $Mb1^{cre/+}$ $Rc3h1^{F/F}$ - $2^{F/F}$ mice



Large pre B cells
 IgD⁻ IgM⁻ B220^{lo} CD25⁺ FSC^{hi}



Small pre B cells
 IgD⁻ IgM⁻ B220^{lo} CD25⁺ FSC^{lo}



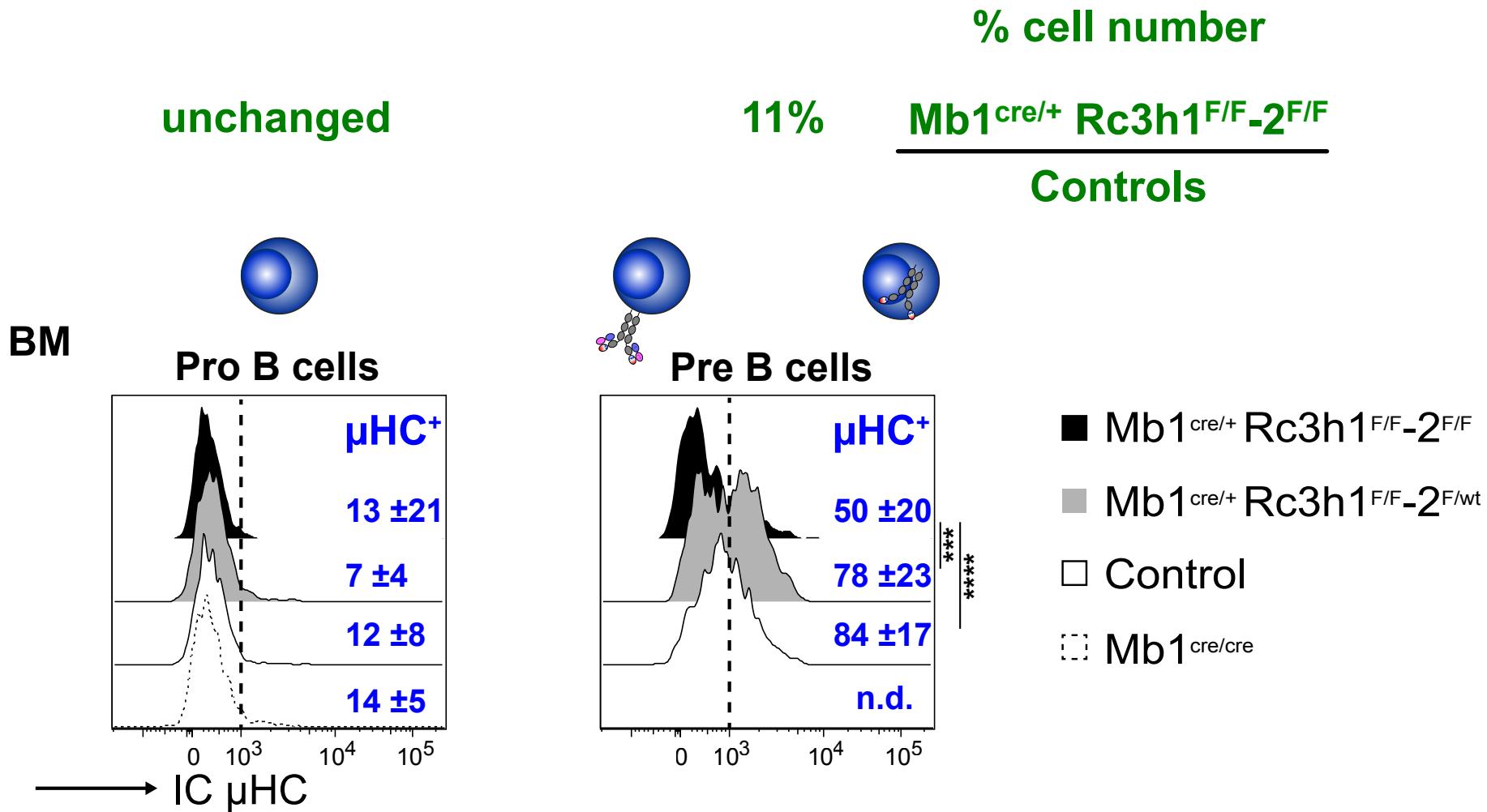
- $Mb1^{cre/+}$ $Rc3h1^{F/F}$ - $2^{F/F}$

- $Mb1^{cre/+}$ $Rc3h1^{F/F}$ - $2^{F/wt}$

- △ Controls

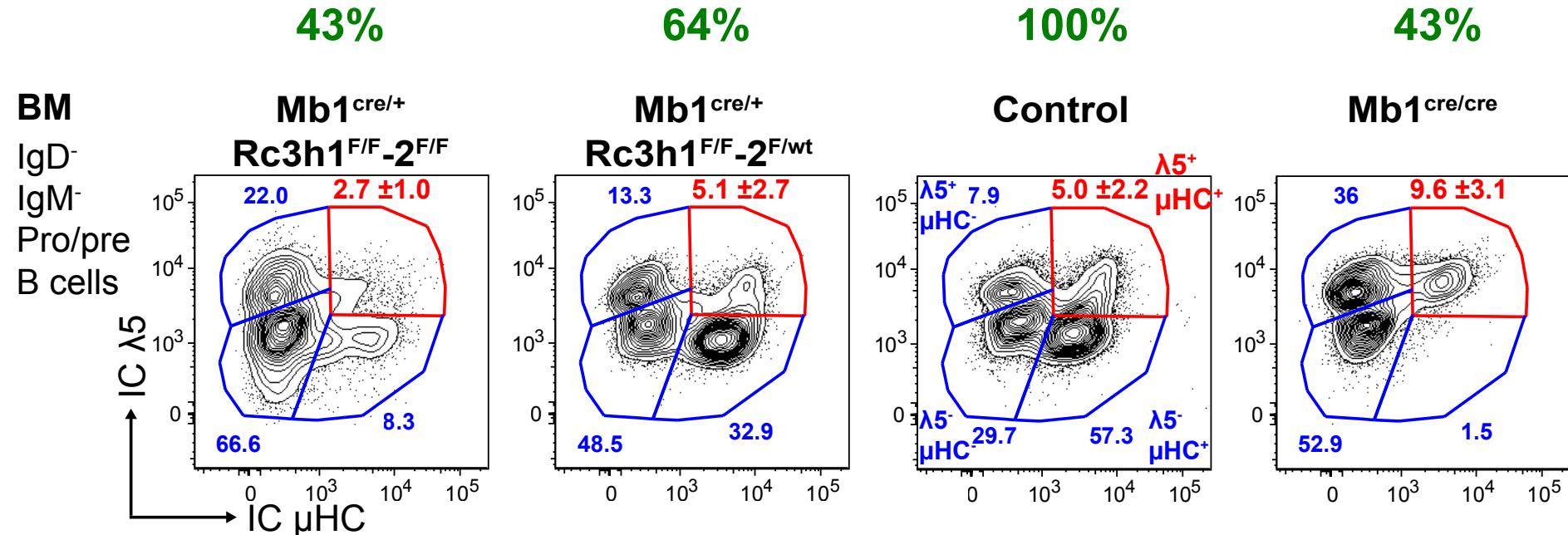
- ▲ $Mb1^{cre/cre}$

Defective μ HC expression in Roquin1/2-deficient pre B cells

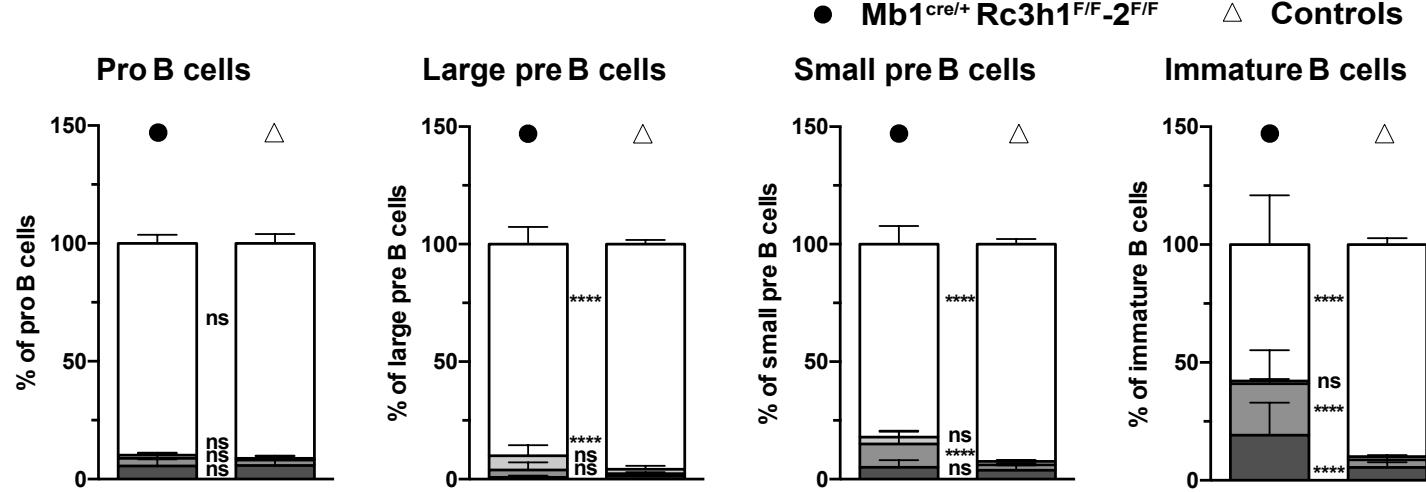
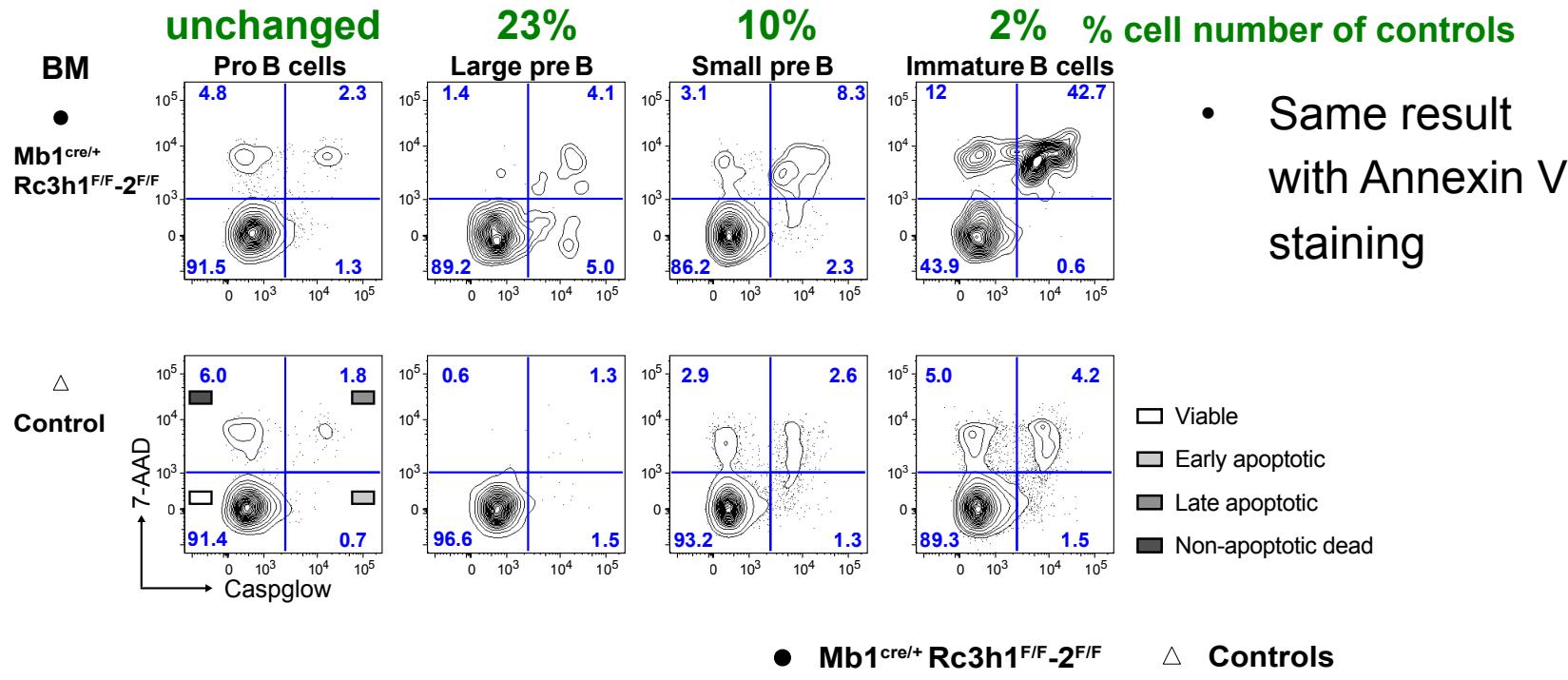


Intact pre-BCR expression in Roquin1/2-deficient pre B cells

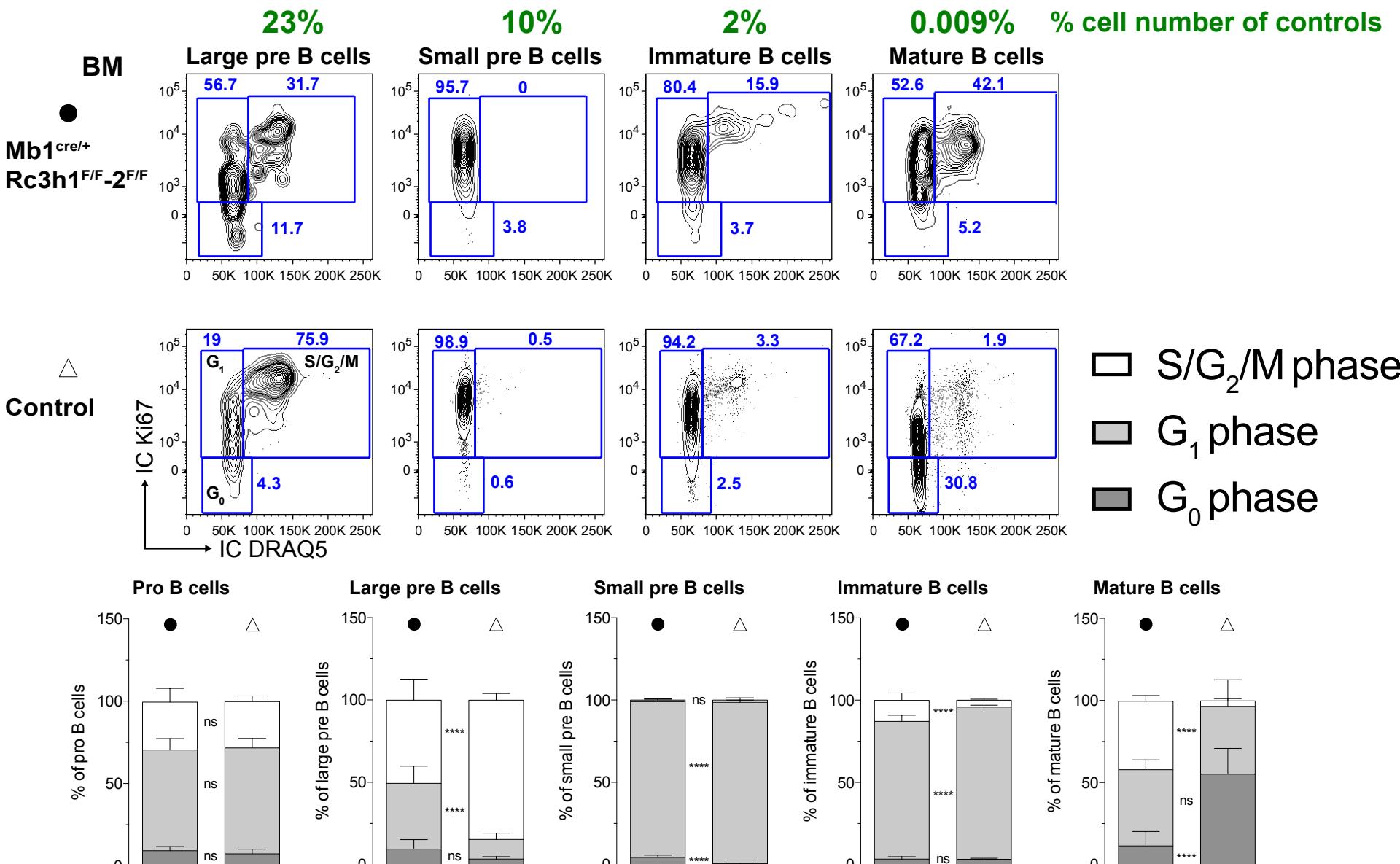
% cell number of controls



Apoptosis is largely unaltered in ex vivo pro & pre B cells of $\text{Mb1}^{\text{cre}/+}$ $\text{Rc3h1}^{\text{F/F}}$ - $\text{2}^{\text{F/F}}$ mice

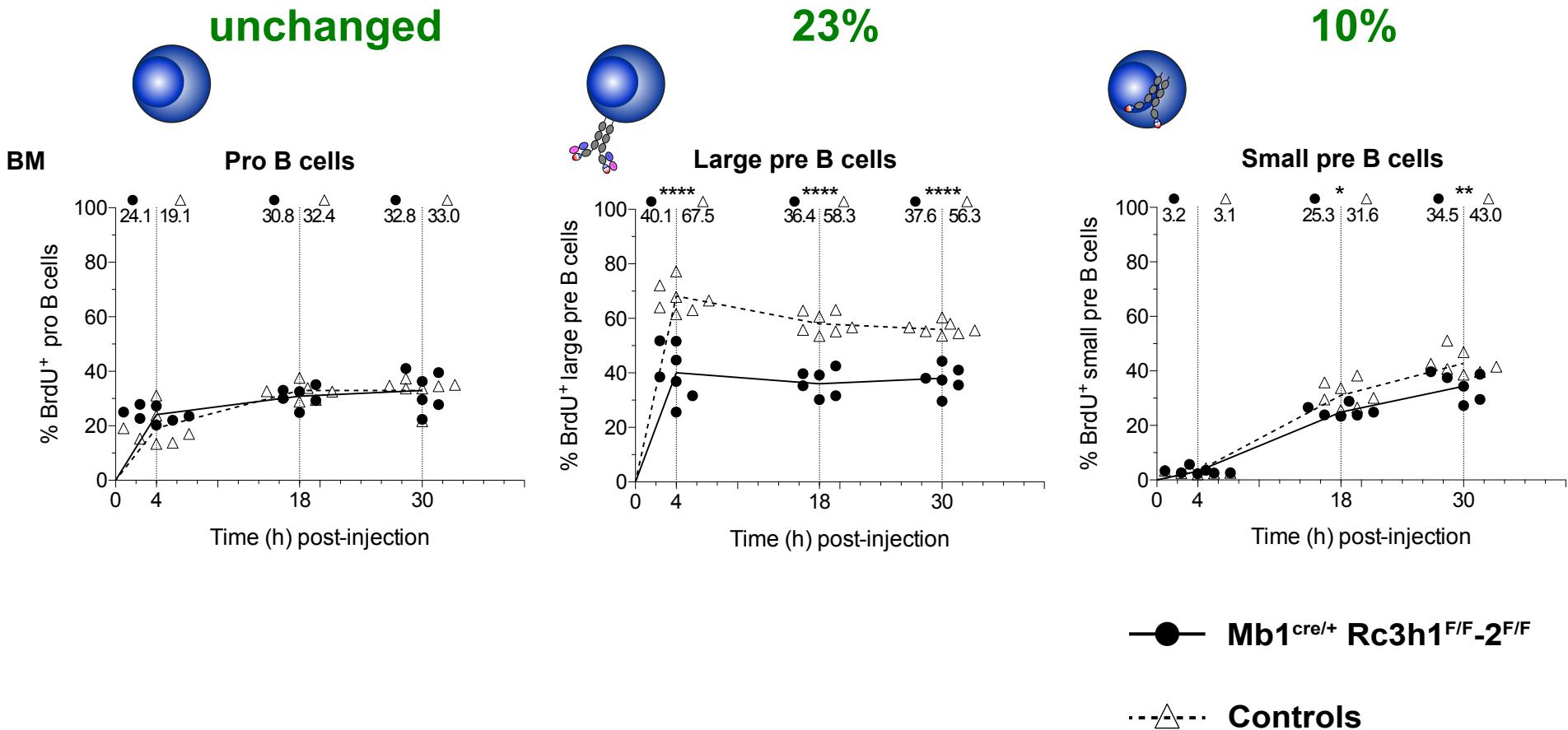


Impaired proliferation of Roquin1/2-deficient large pre B cells

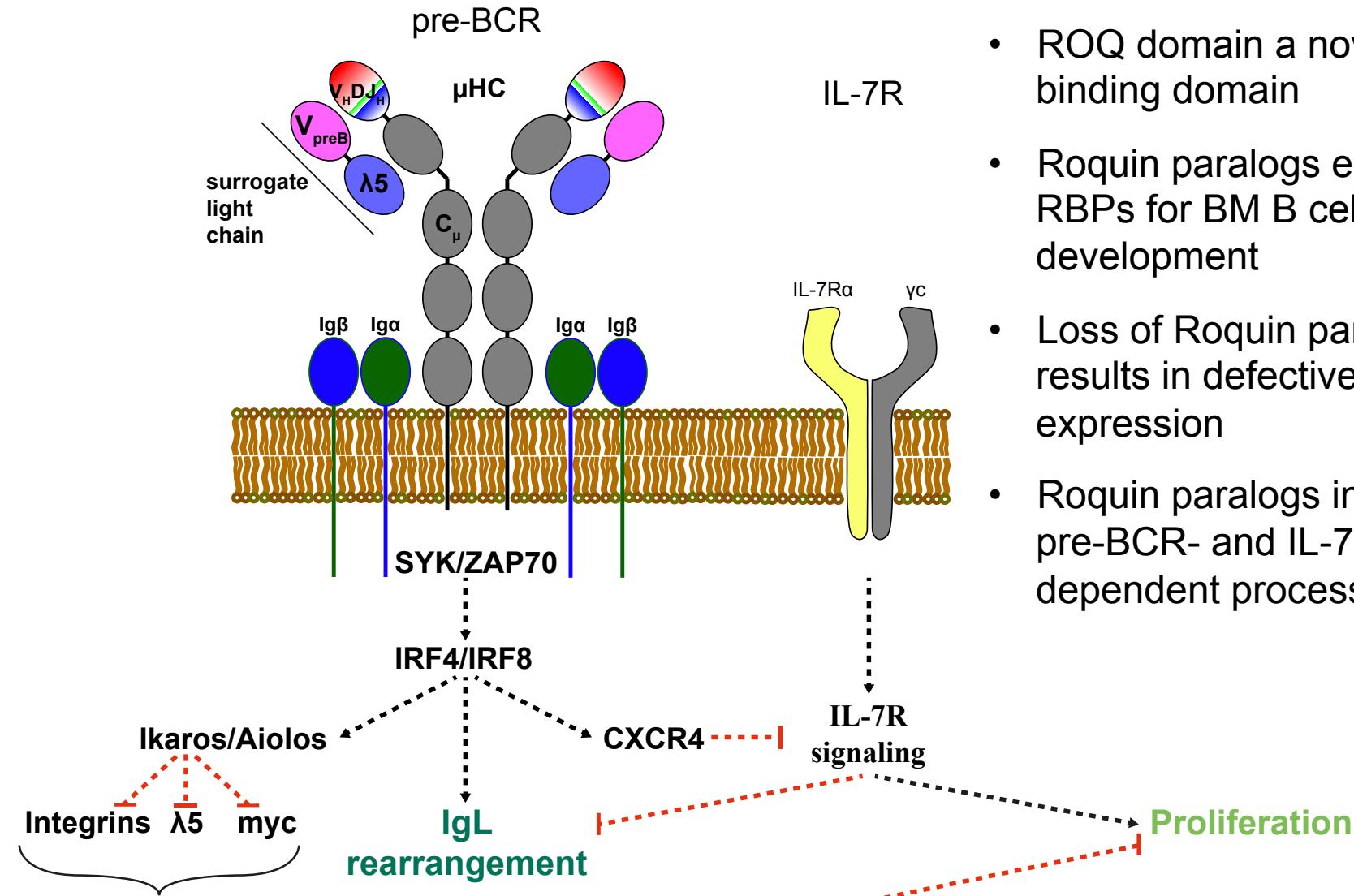


Impaired proliferation of Roquin1/2-deficient large pre B cells

% cell number of controls



Summary



Data Mb1^{cre/+} BM B cells

Efficient and stringent deletion of *Rc3h1* and *Rc3h2*

unchanged

11%

2%

% cell number of controls

BM

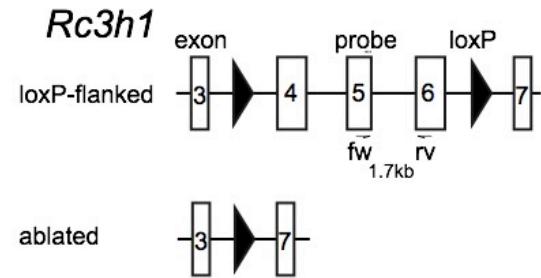
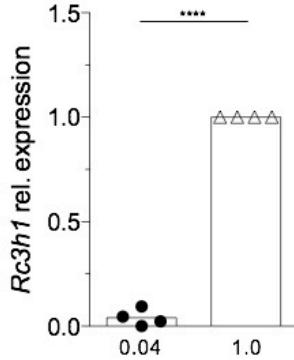
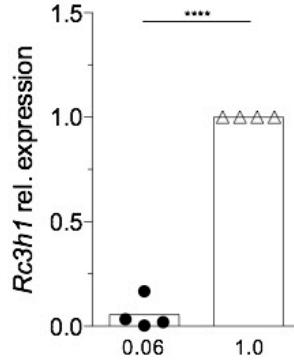
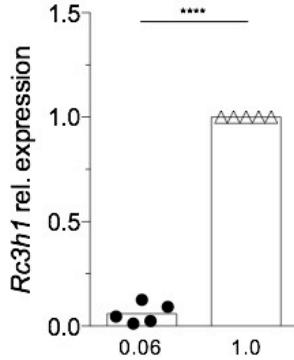
Pro B cells
IgD⁻ IgM⁻ B220^{lo} c-kit⁺

Pre B cells
IgD⁻ IgM⁻ B220^{lo} CD25⁺

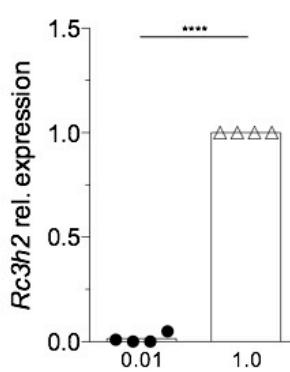
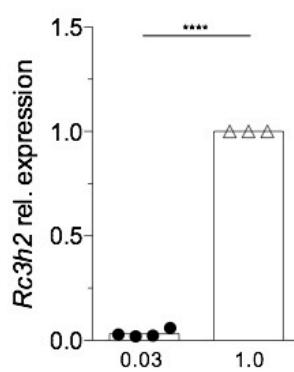
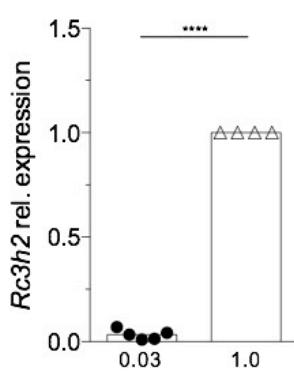
Immature B cells
B220⁺ IgD⁻ IgM⁺

- Mb1^{cre/+} *Rc3h1*^{F/F-2F/F} ($n=4-5$)
- △ Mb1^{cre/+} controls ($n=3-5$)

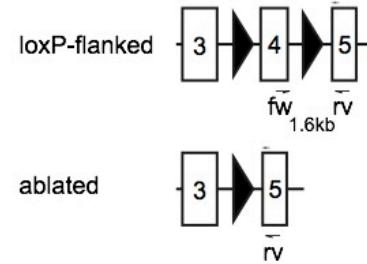
Rc3h1



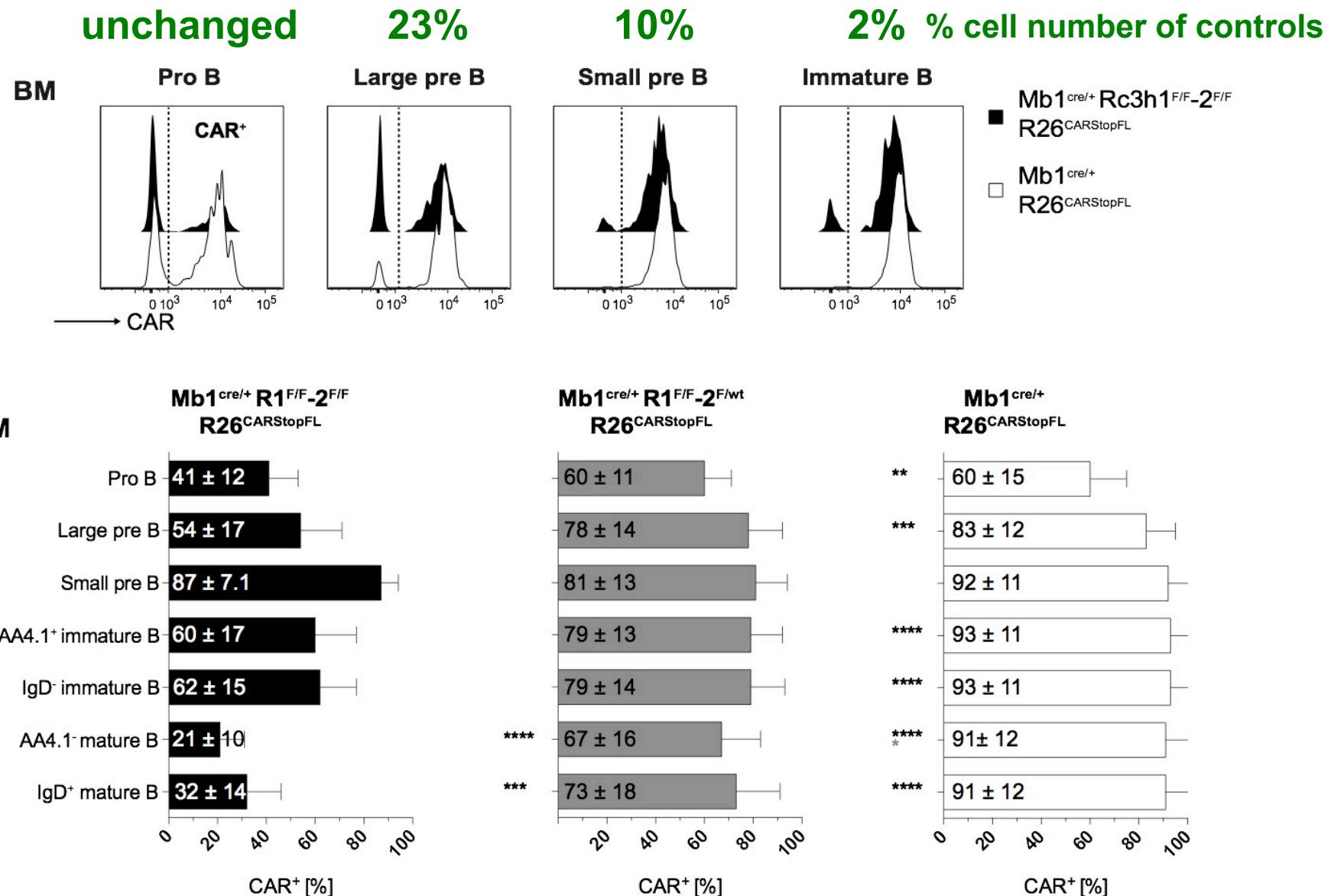
Rc3h2



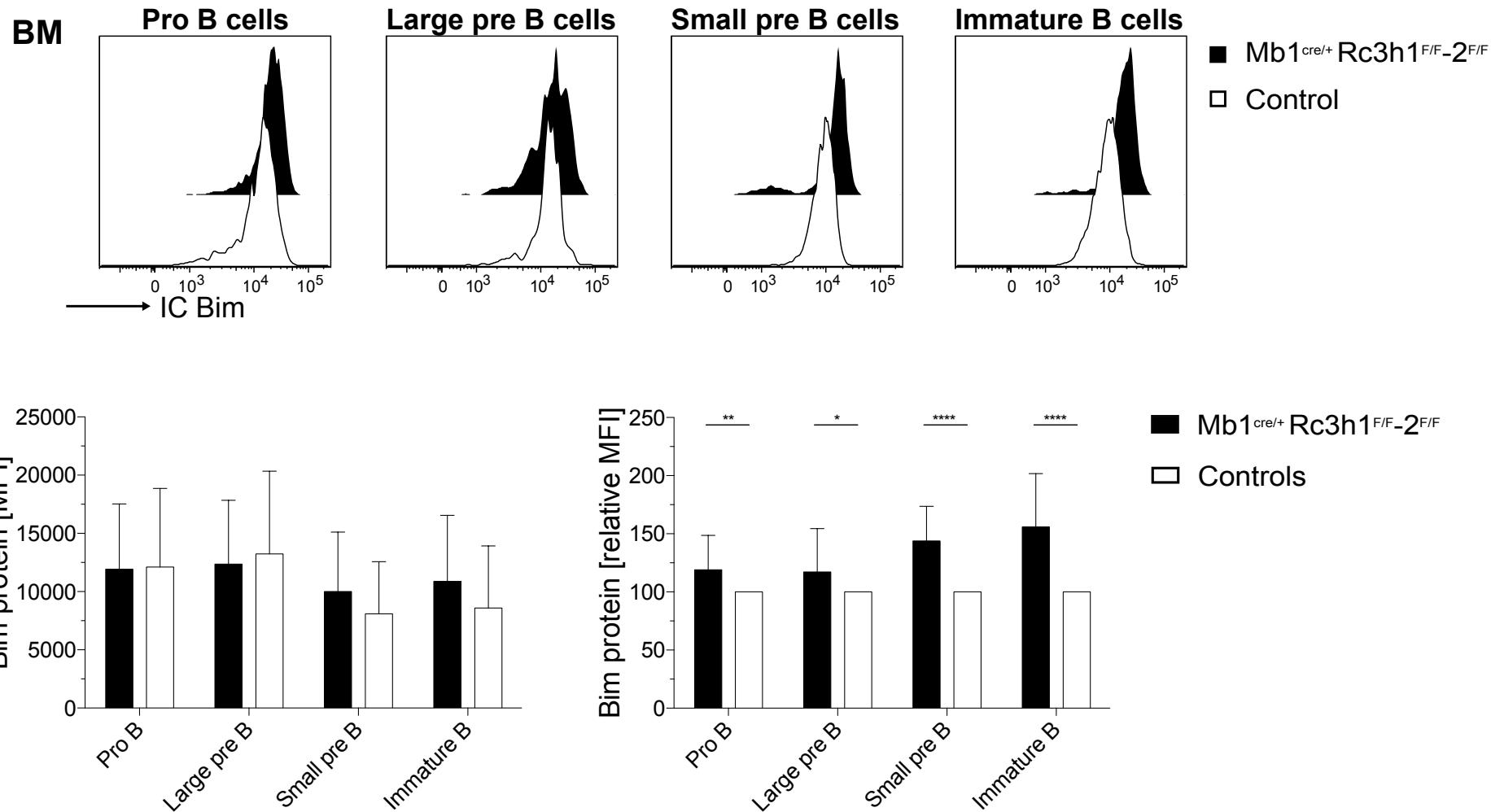
Rc3h2



Efficient and stringent deletion of Rc3h1 and Rc3h2



Apoptosis is largely unaltered in *ex vivo* pro & pre B cells of $\text{Mb1}^{\text{cre/+}} \text{Rc3h1}^{\text{F/F}}\text{-}2^{\text{F/F}}$ mice



Data Mb1^{cre/+} R1-2^{F/F} IgH^{MOG}

BM in $\text{Mb1}^{\text{cre/+}}$ R1-2^{F/F} IgH^{MOG} mice

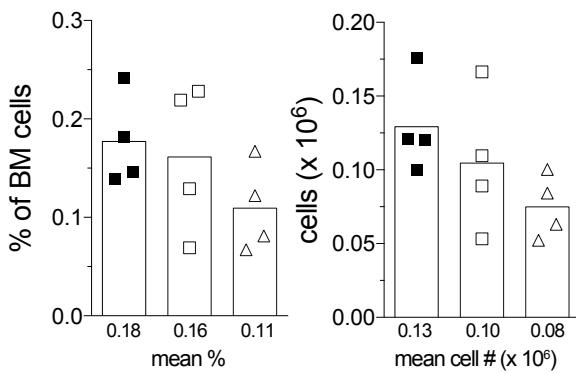
$\text{Mb1}^{\text{cre/+}}$ R1-2^{F/F} IgH^{MOG}

IgH^{MOG} controls

Controls

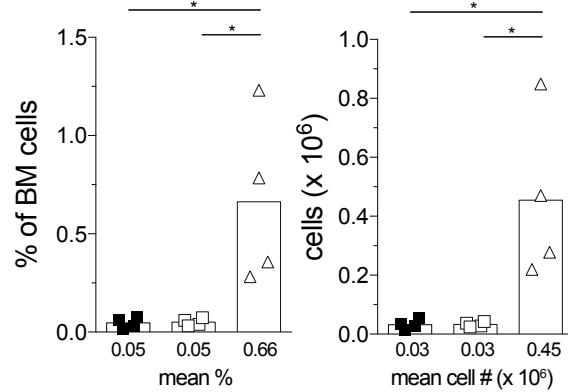
Prepro B cells

IgD⁻ IgM⁻ B220^{lo} c-kit⁺ CD19⁻



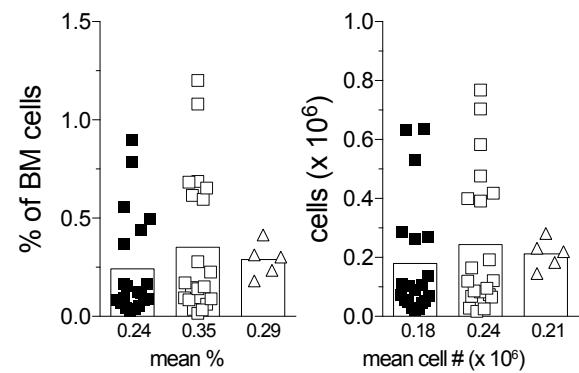
Late pro B cells

IgD⁻ IgM⁻ B220^{lo} c-kit⁺ CD19⁺



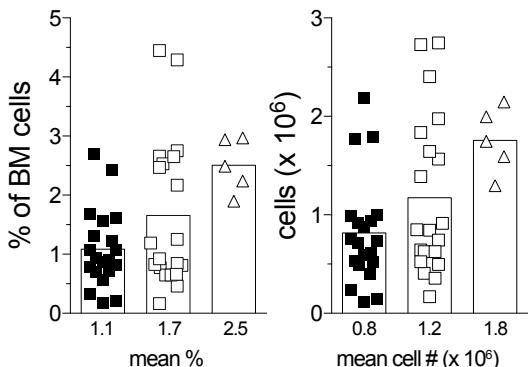
Large pre B cells

B220⁺ IgM⁻ CD25⁺ FSC^{hi}



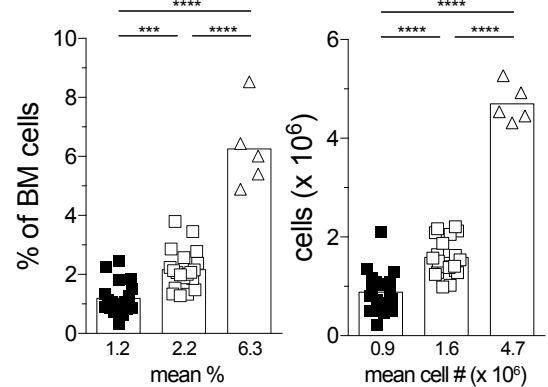
Small pre B cells

B220⁺ IgM⁻ CD25⁺ FSC^{lo}



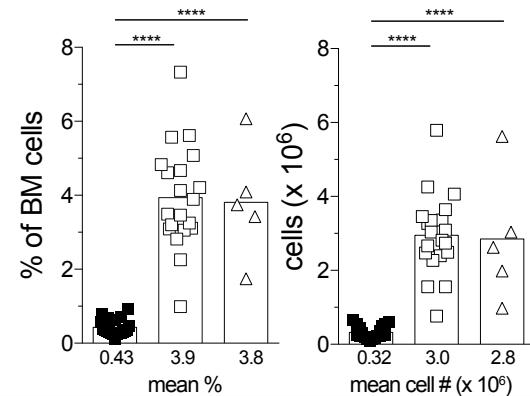
Immature B cells

B220⁺ IgM⁺ AA4.1⁺



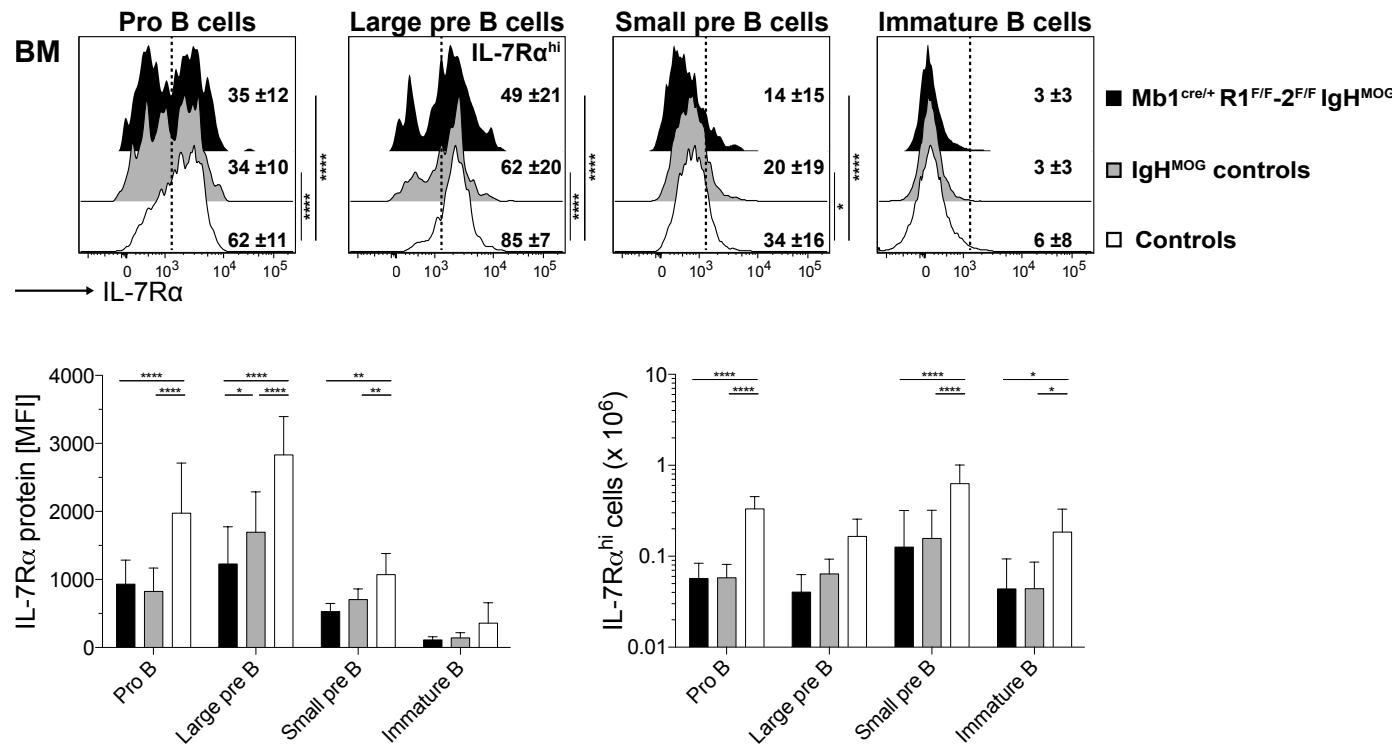
Mature B cells

B220⁺ IgM⁺ AA4.1⁻

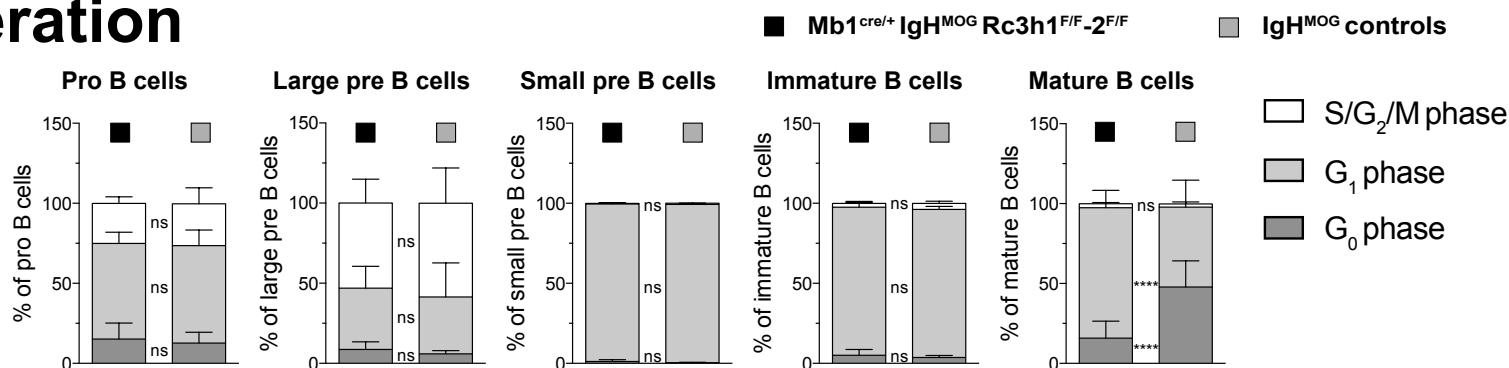


IL-7R in $\text{Mb1}^{\text{cre/+}}$ R1-2^{F/F} IgH^{MOG} mice

IL-7R

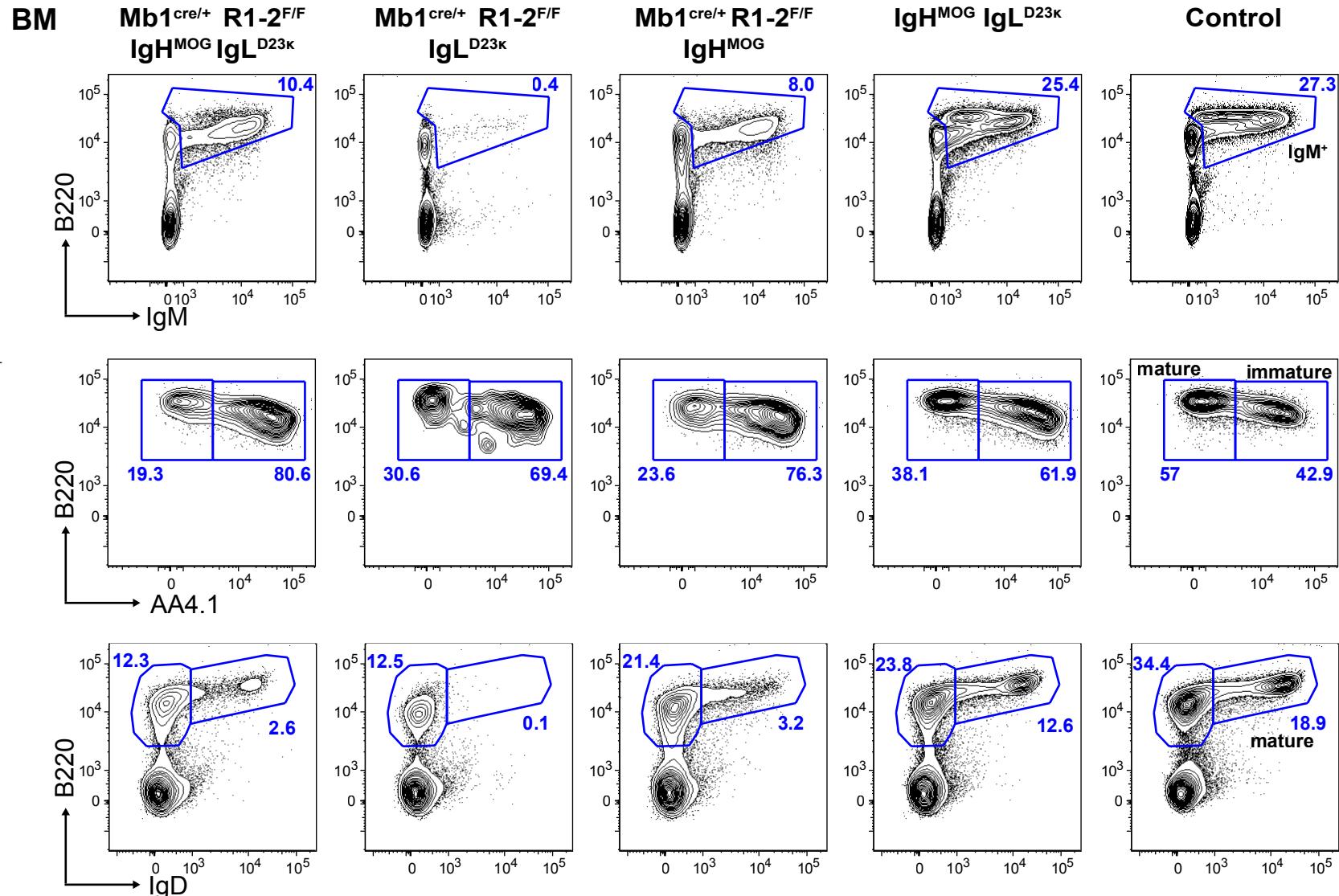


Proliferation



Data Mb1^{cre/+} R1-2^{F/F} IgL^{D23κ} IgH^{MOG}

BM B cells in $\text{Mb1}^{\text{cre/+}}$ R1-2^{F/F} IgL^{D23k} IgH^{MOG} mice



BM B cells in Mb1^{cre/+} R1-2^{F/F} IgL^{D23k} IgH^{MOG} mice

