Investigating gene function *in vivo*
- expectations and surprises -

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NIDDK, NIH

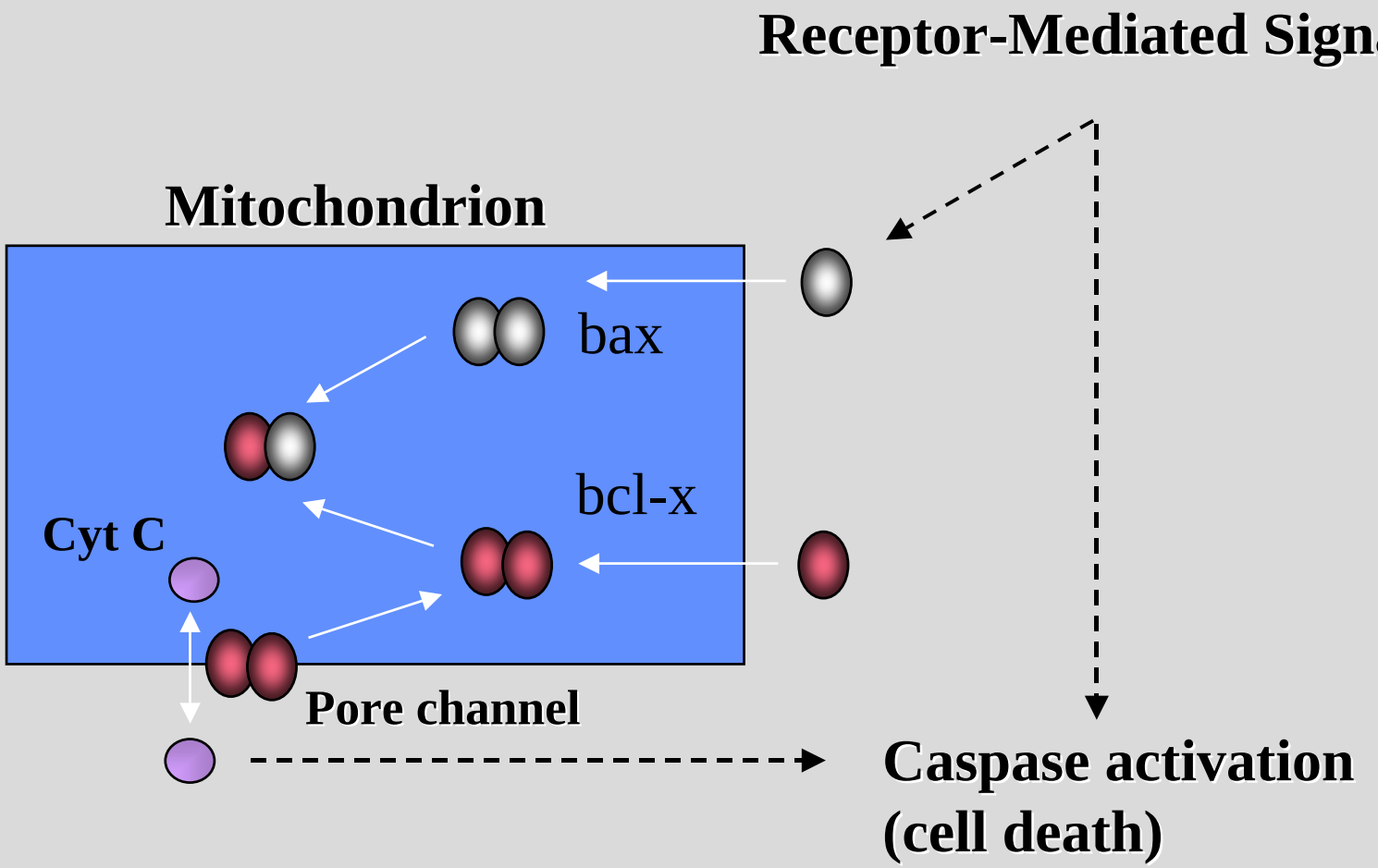
Bcl-2 Gene Family in Cell Survival and Death

Bcl-2: B-cell lymphoma breakpoint t(14;18)

15+ members in the family

Pro-apoptotic	**Bax**
Anti-apoptotic	**Bcl-x**

The Roles of Bcl-x and Bax in Cell Survival and Death



Bcl-x in Cell Survival and Development

Experimental strategies

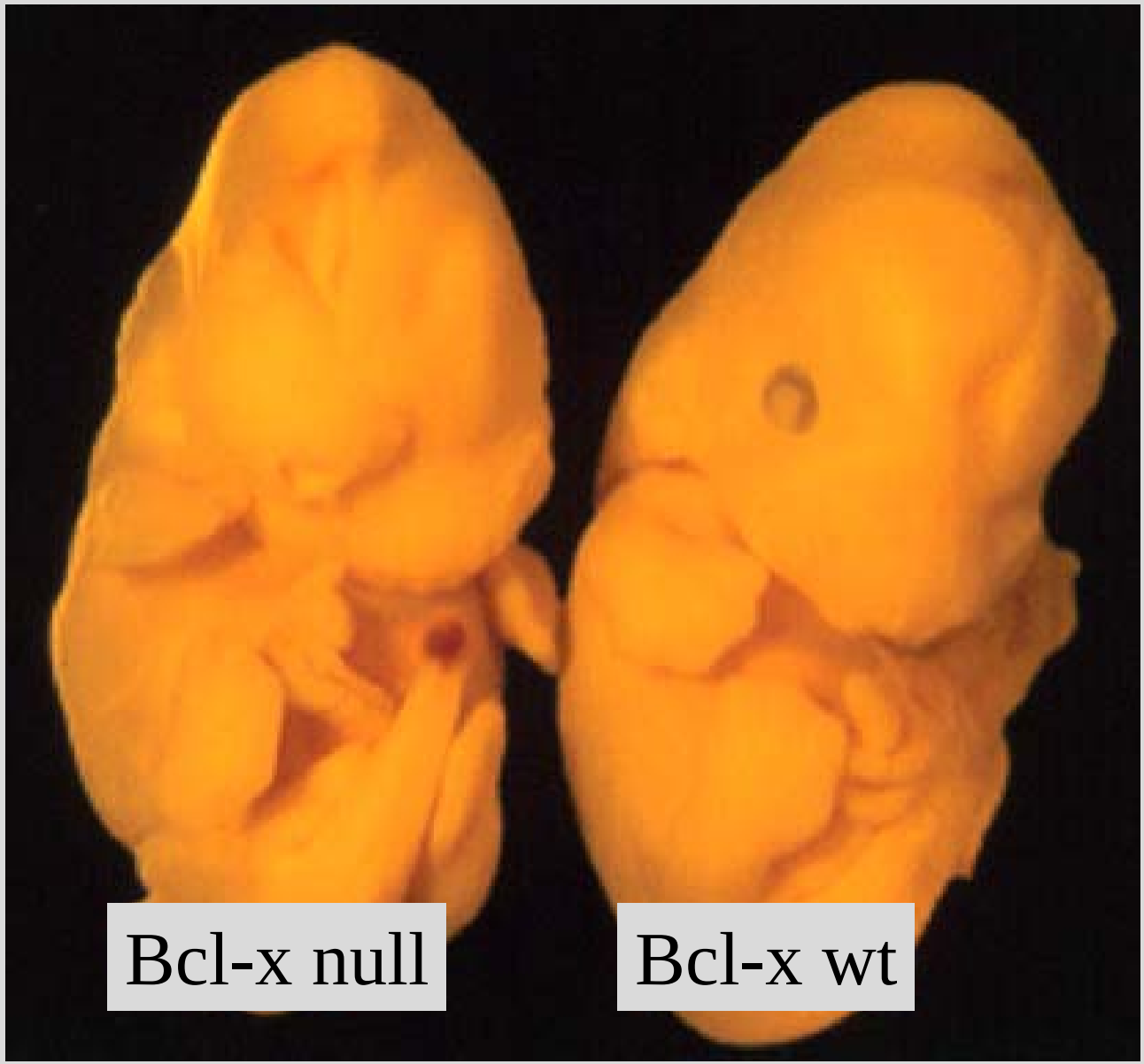
- Tissue culture cells
- Dominant gain-of-function
  - transgenic mice
- Loss-of-function (gene knock-out mice)
  - conventional
  - cell-specific

Publications on the Role of Bcl-x in Cell Survival During Normal Development and Cancer

Publications

- **tissue culture cells (kidney, prostate, mammary, etc) > 800**
- **transgenic mice 70**
- **gene knock-out mice 5**

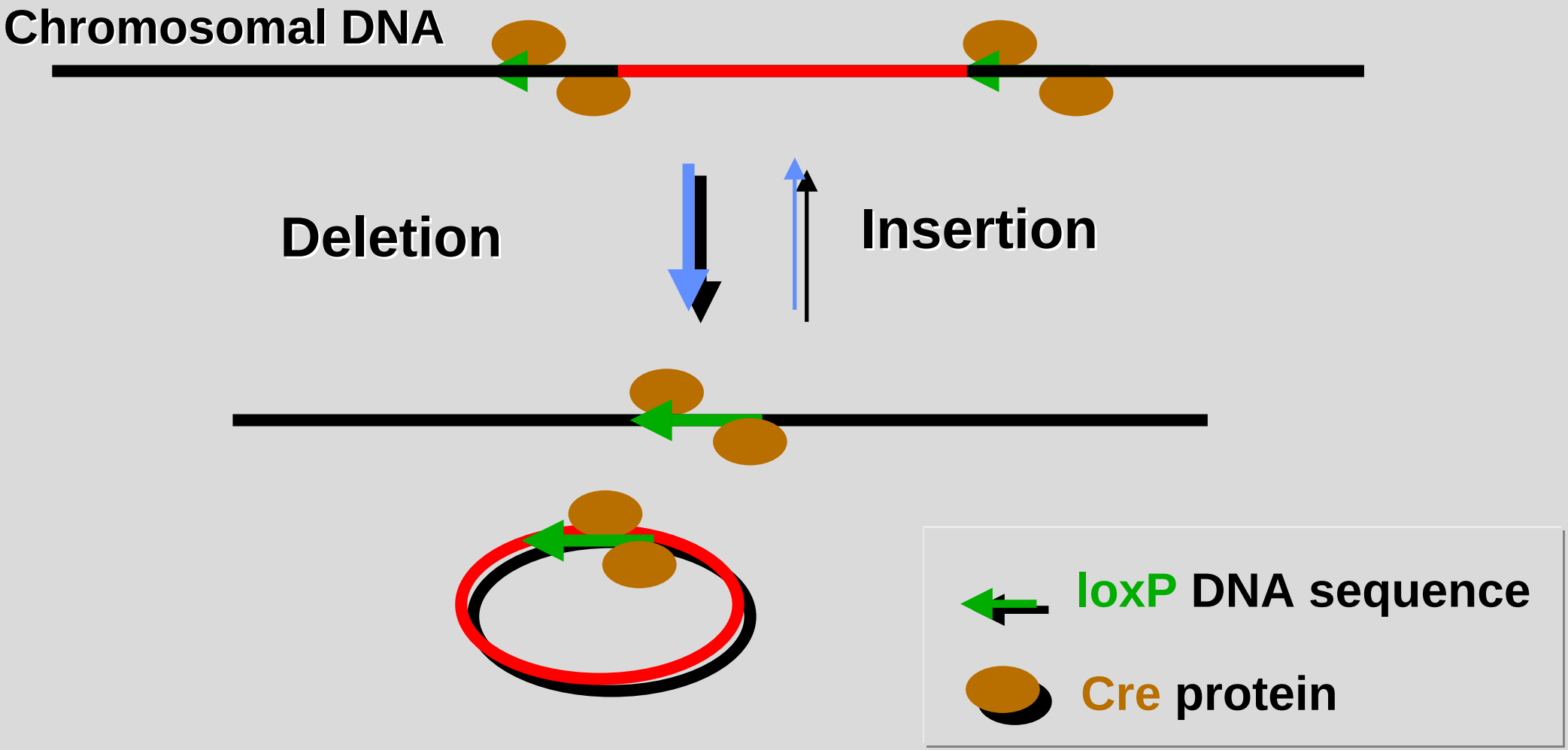
Bcl-x is Required for Fetal Development



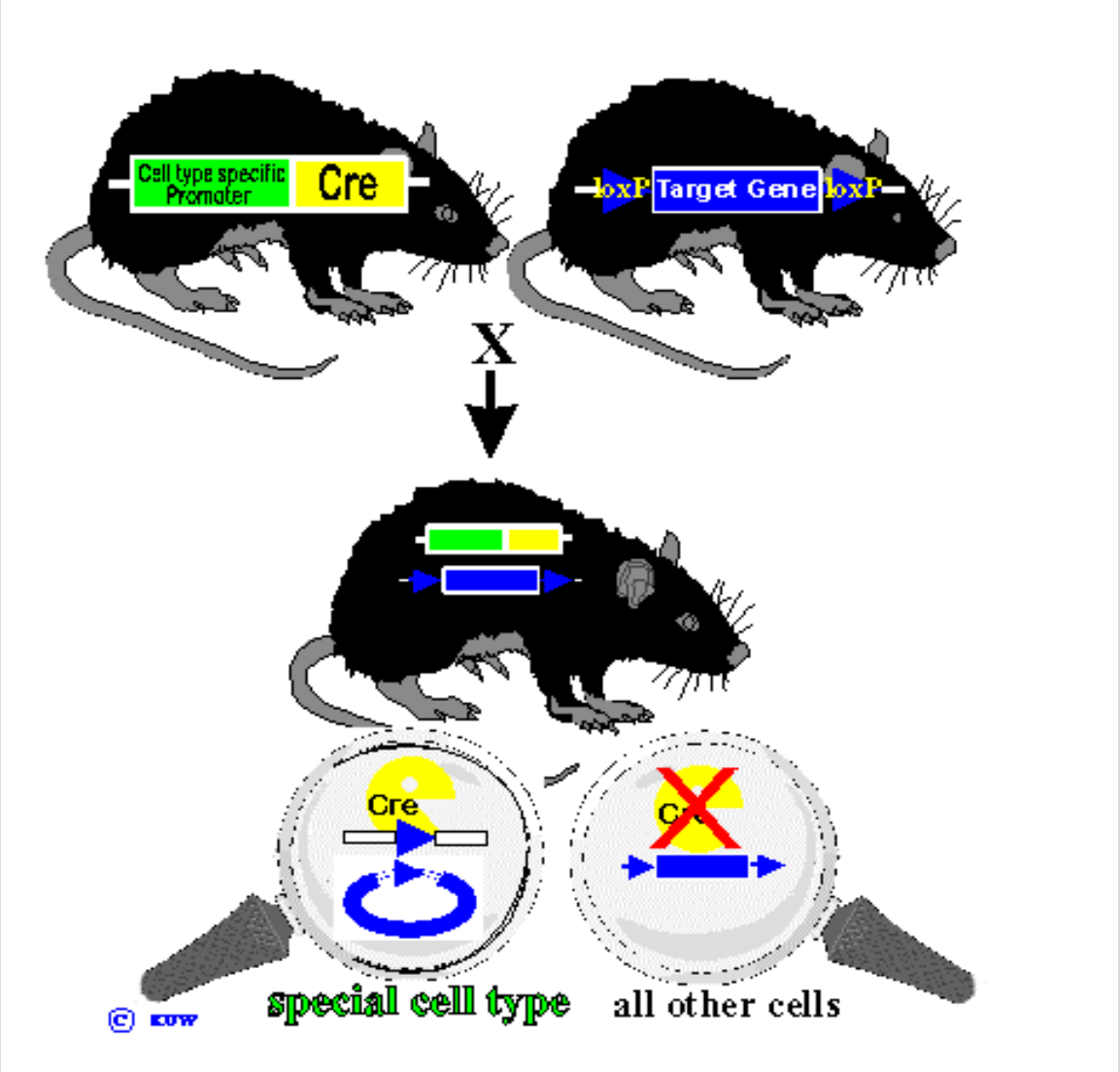
E 12.5 days

Cre - lox Recombination

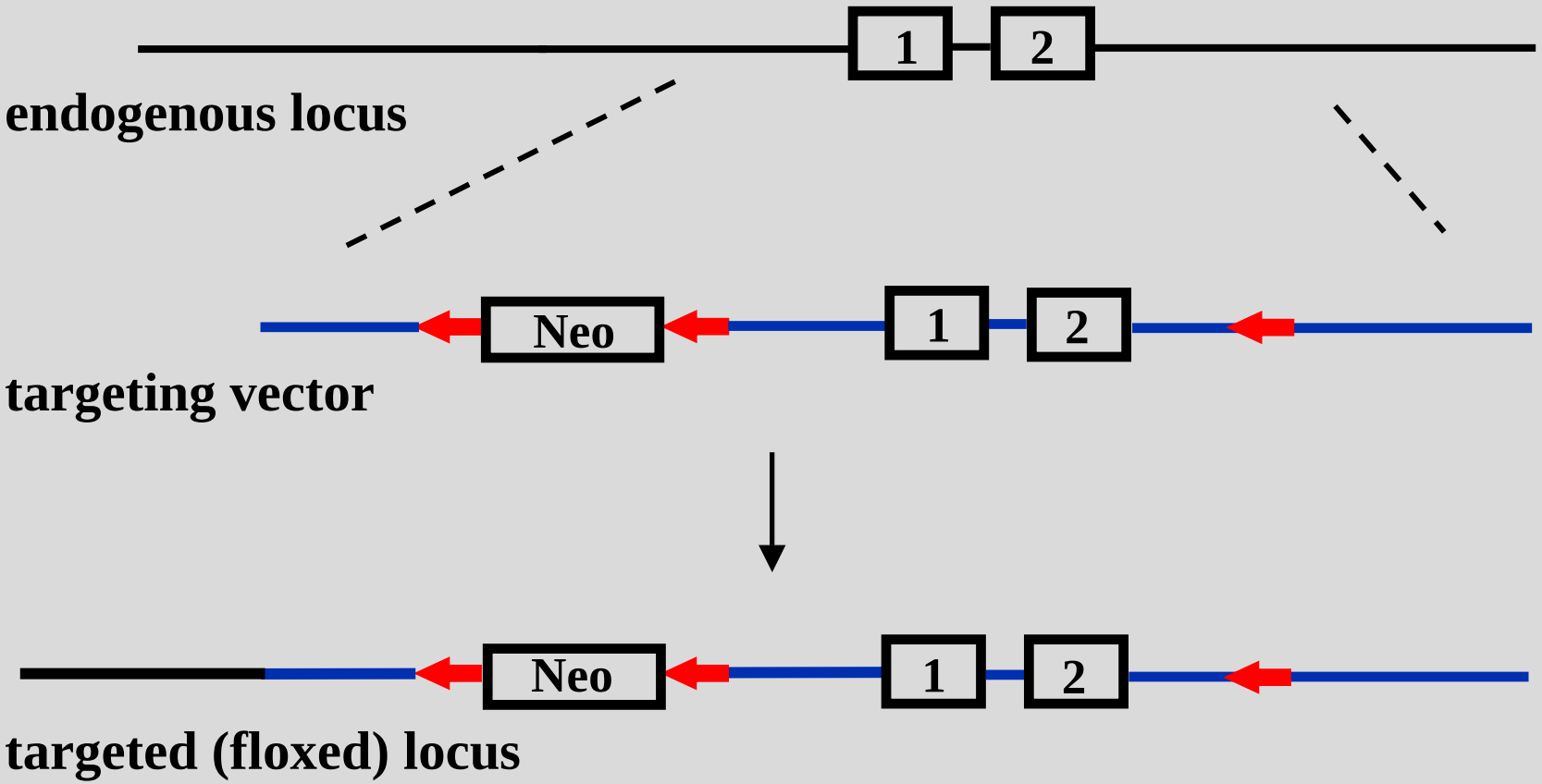
- the biochemistry -



Cre - lox Recombination
- tissue-specific gene deletion -

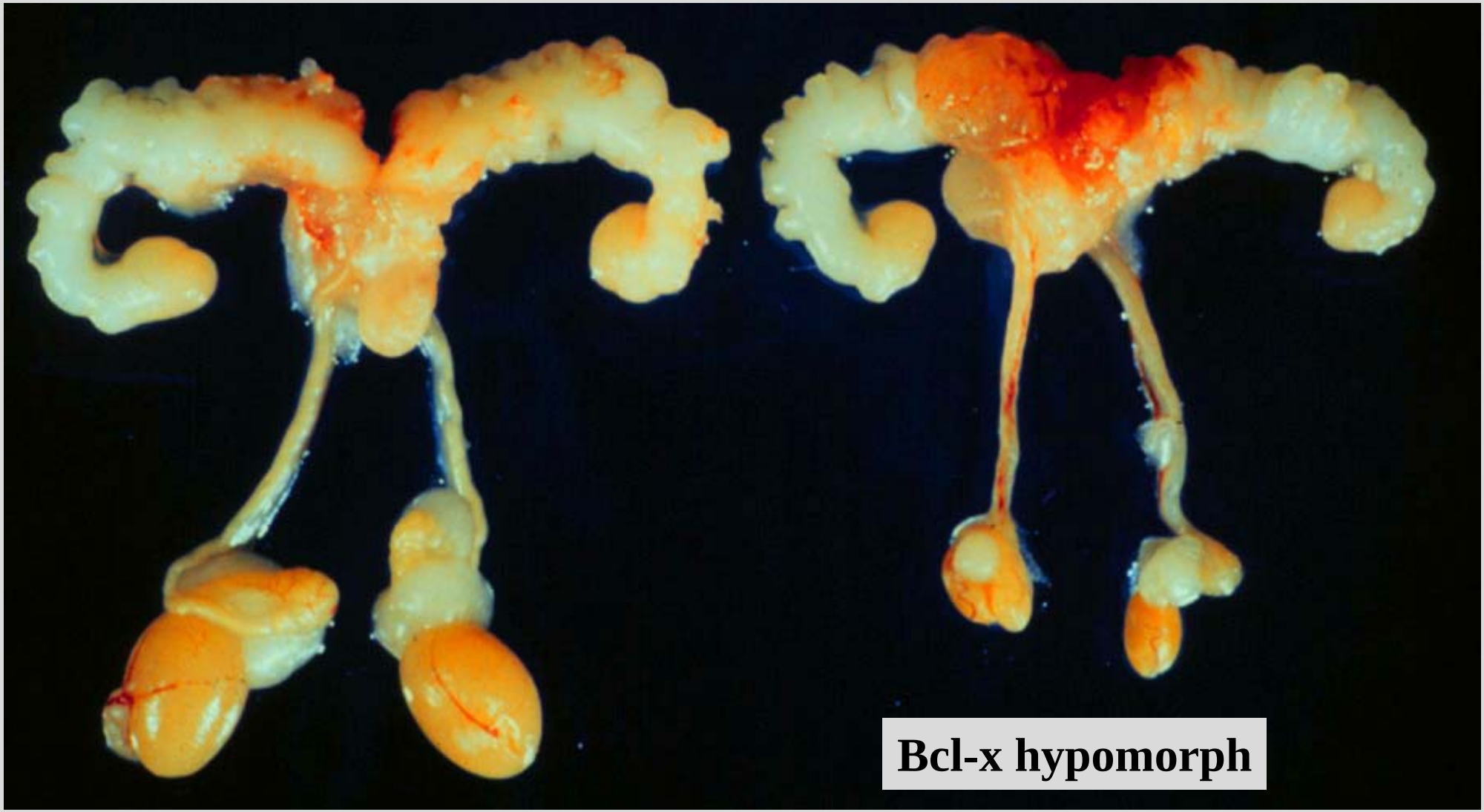


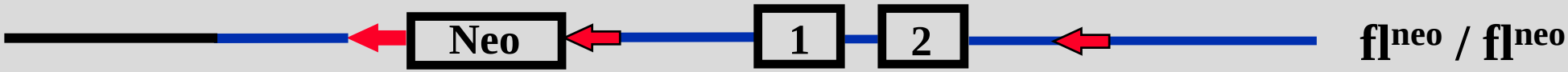
Targeting the *bcl-x* Locus



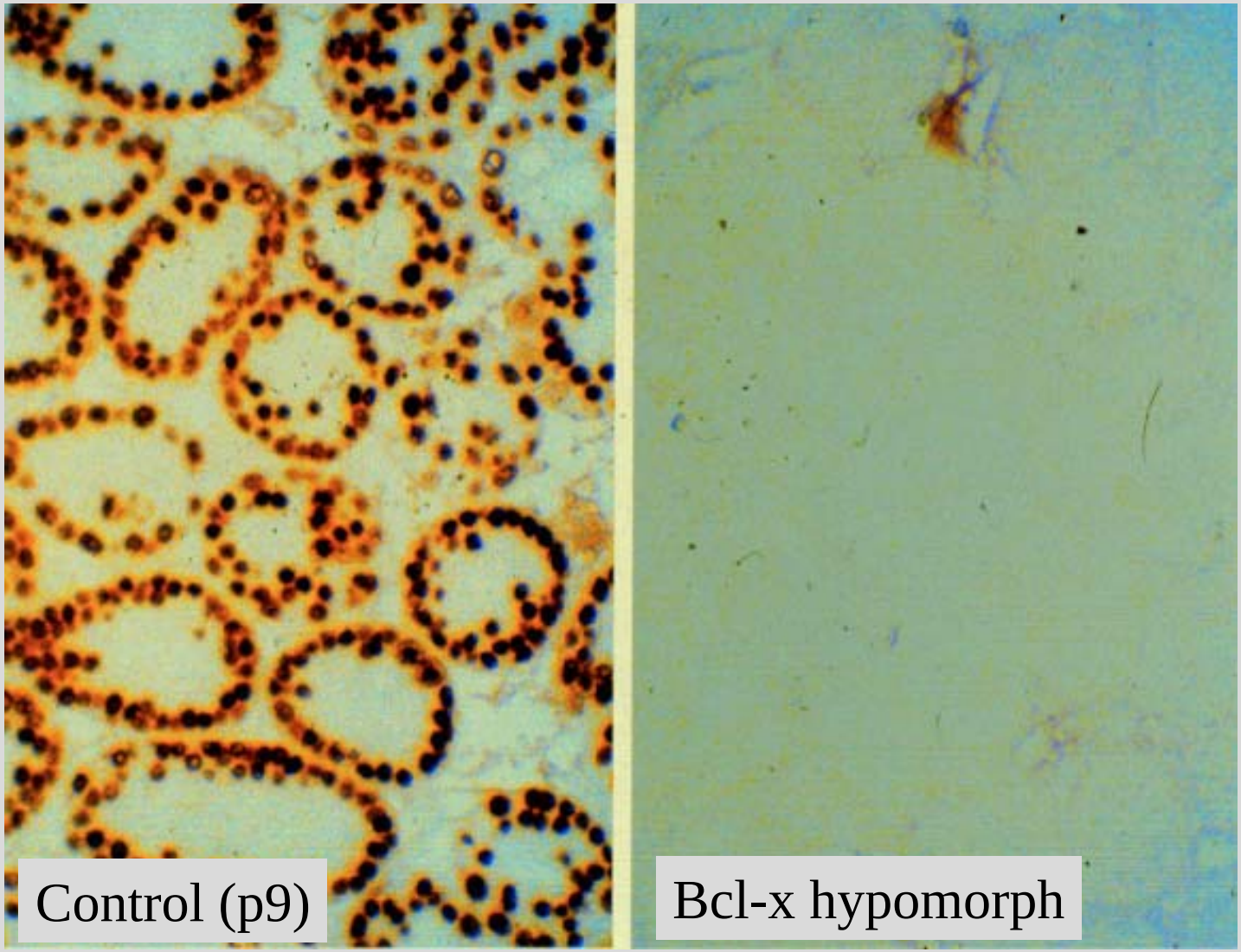
loxP site

The First Surprise: a *bcl-x* Hypomorph



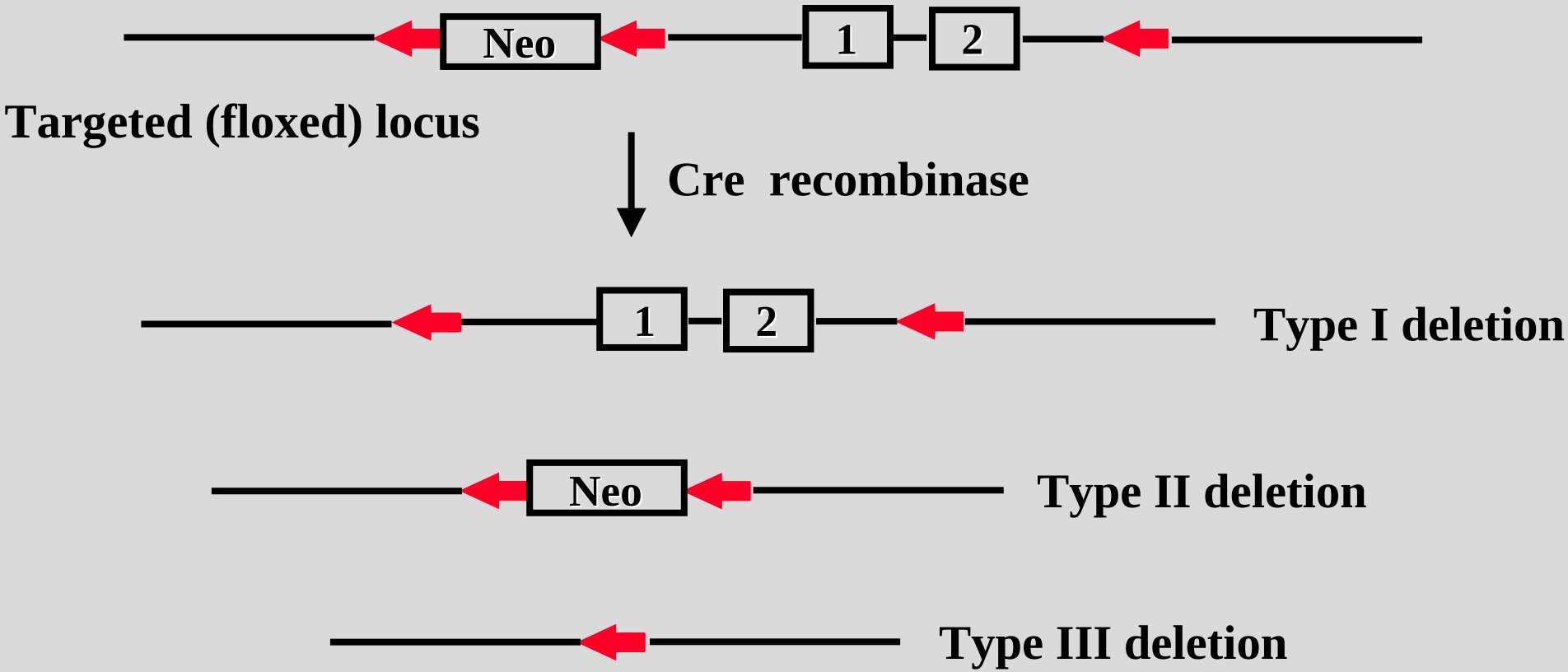


Normal Expression of Bcl-x is Required for Germ Cell Survival

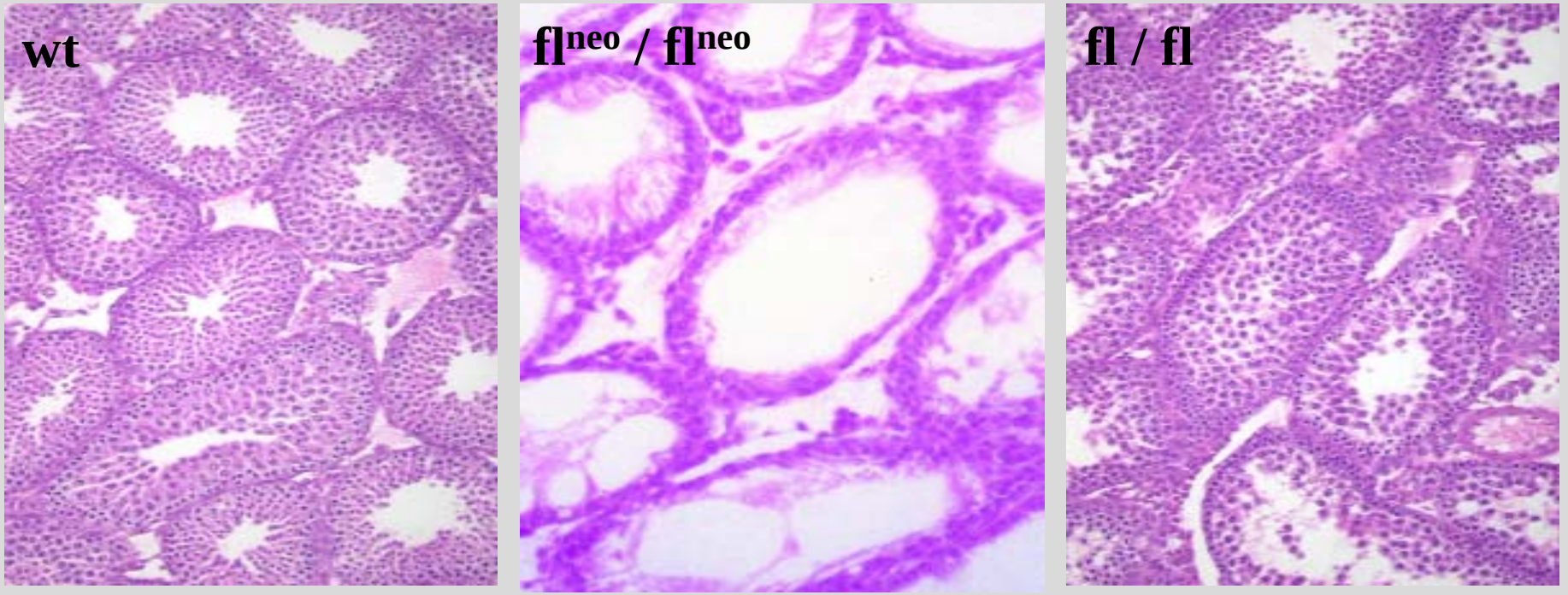


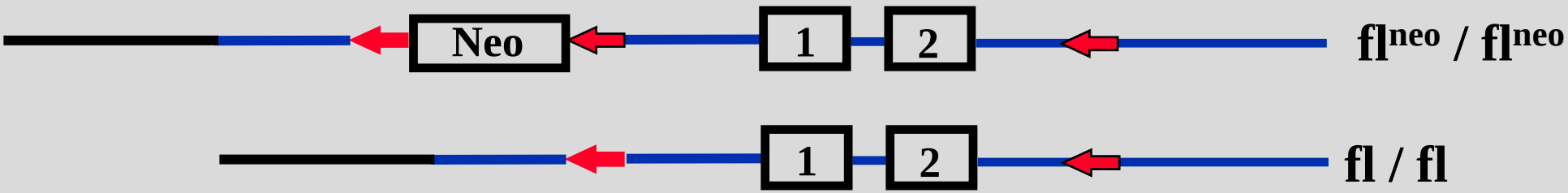
Germ Cell Nuclear Antigen (GCNA) staining

Cre-Mediated Deletion of the Neomycin Gene

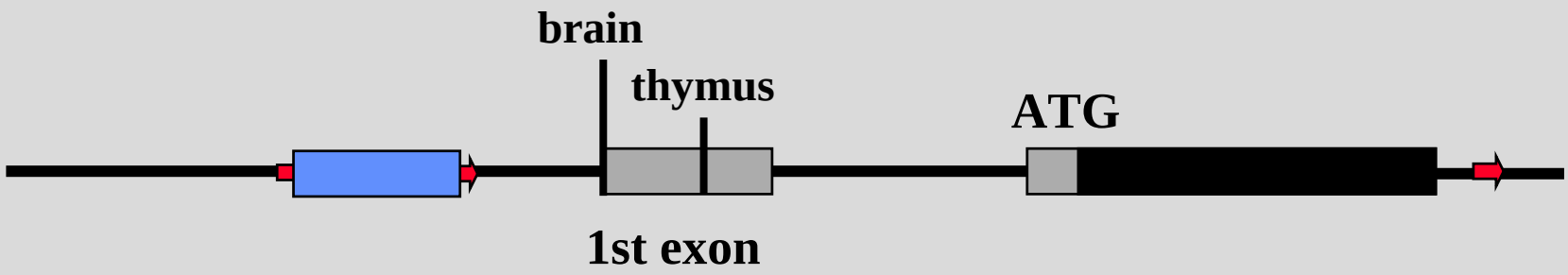


Deletion of the neomycin gene rescues the Bcl-x hypomorph

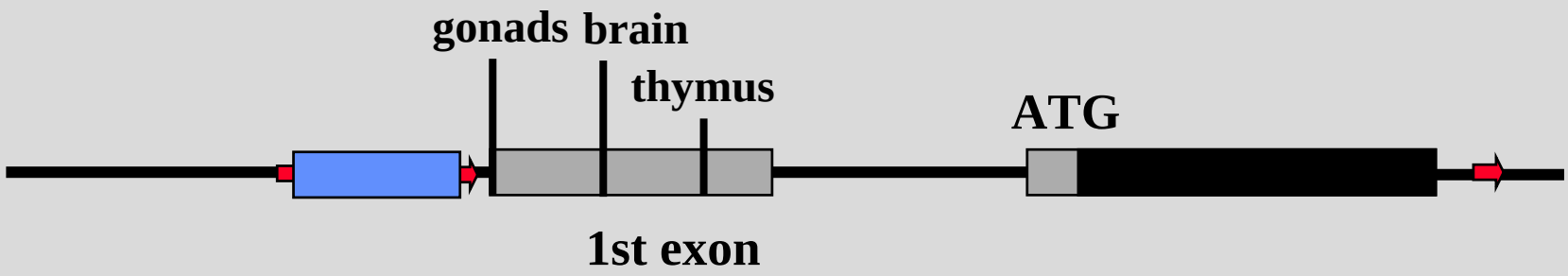




The *bcl-x* Gene Promoter Contains Tissue-specific Start Sites

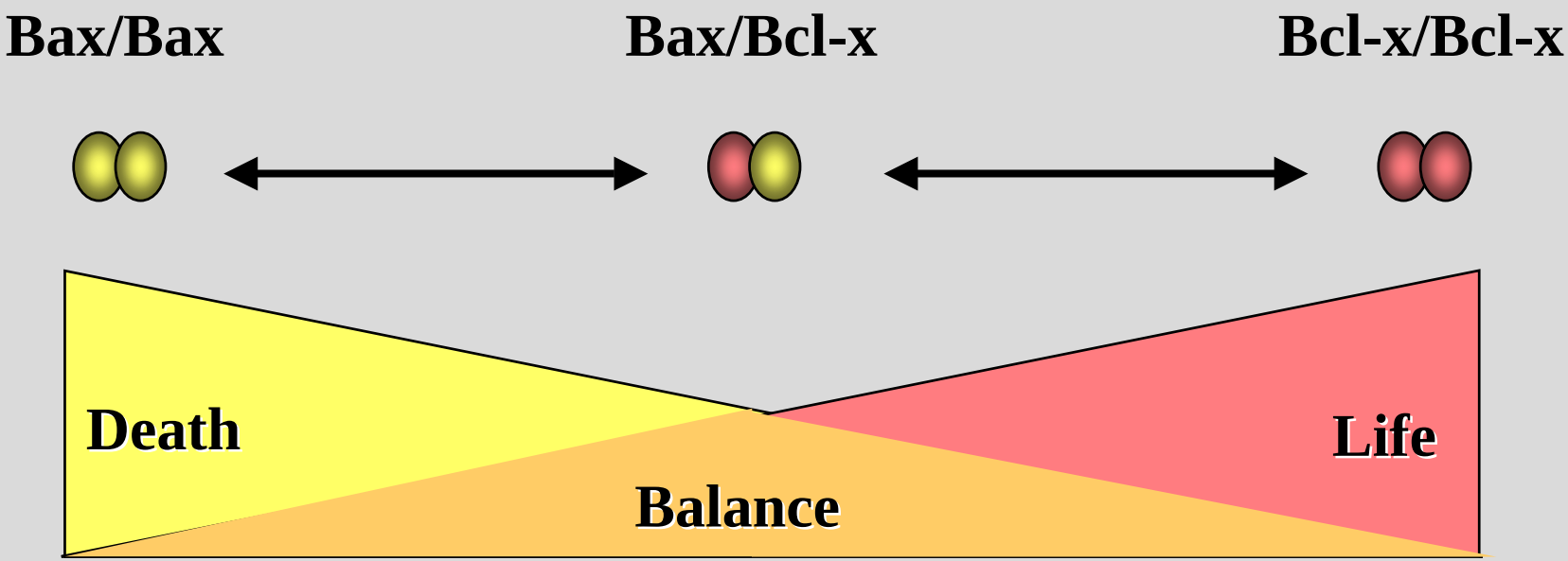


The *bcl-x* Gene Promoter has a Gonad-specific Start Site

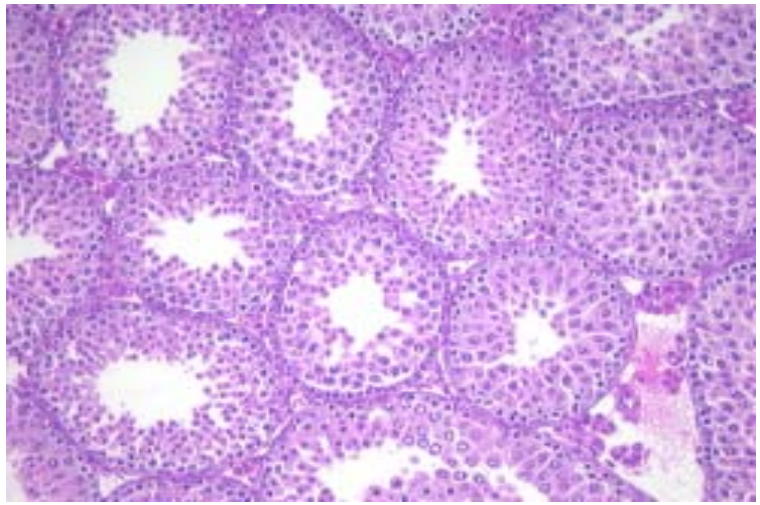


Cell Specificity of Bcl-x / Bax in Apoptosis

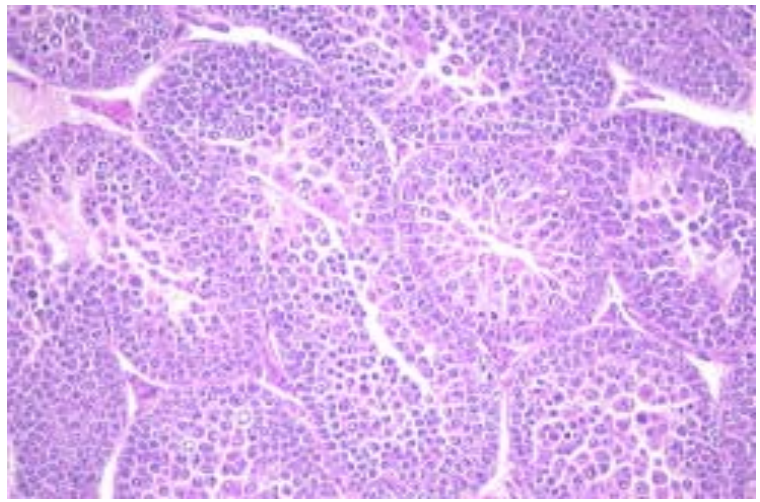
- germ cells versus erythroid and mammary cells -



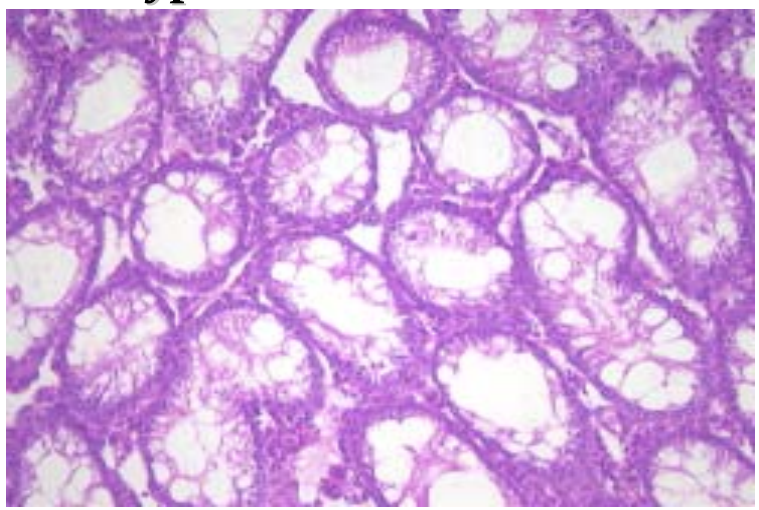
Deletion of BAX reverses Germ Cell Loss In the Bcl-x Hypomorph



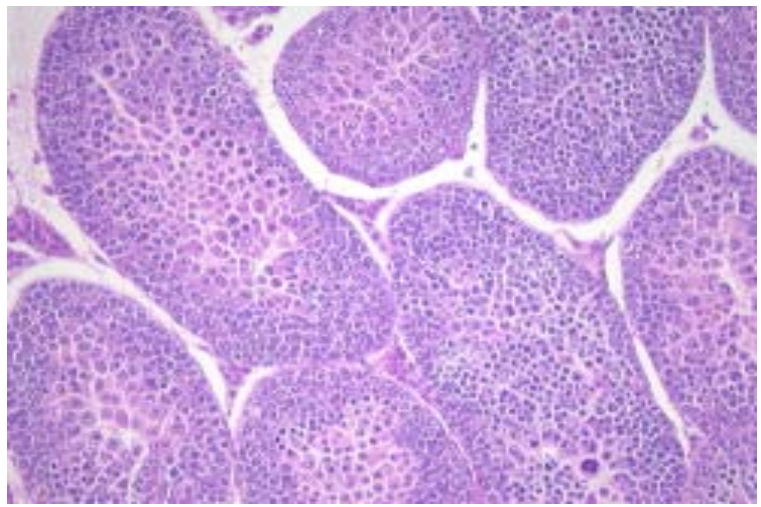
wild-type



bax KO



bcl-x mutant



bax KO; bcl-x mutant

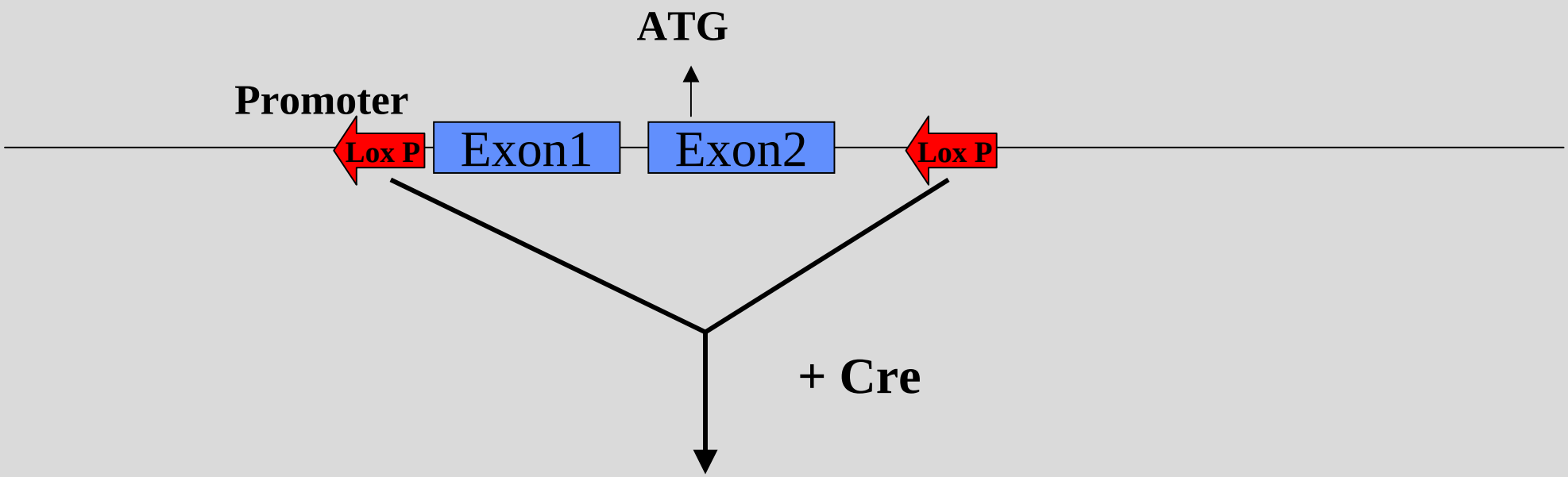
Cell Specificity of Bcl-x and Bax Function

gene ko	mammary	germ cells	erythroid cells	T- and B-cells
bcl-x		• loss of germ cells		
bax		excess germ cells		
bcl-x & bax		excess germ cells		

balance

Whose Survival Depends on Bcl-x ?

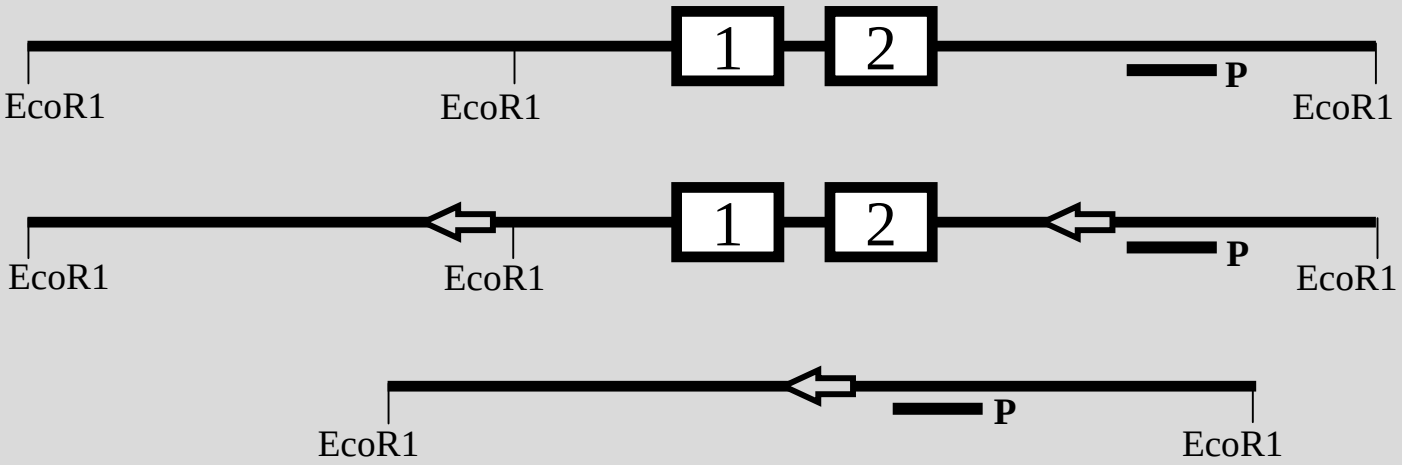
- Targeting the *bcl-x* Locus -

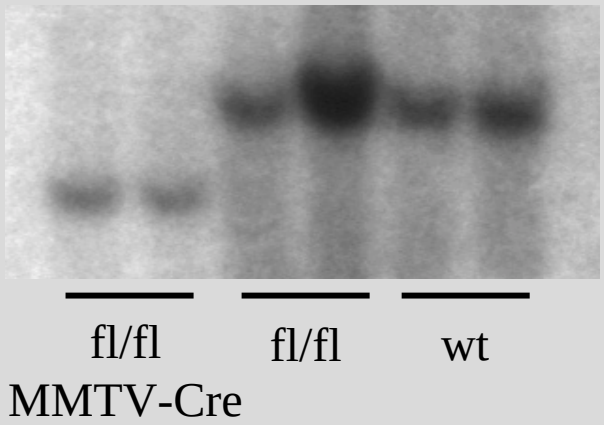


germ cells **hematopoietic cells** **mammary cells**

Deletion of the *bcl-x* Gene in Erythroid Cells

Deletion of the *bcl-x* Gene in the Erythroid Lineage

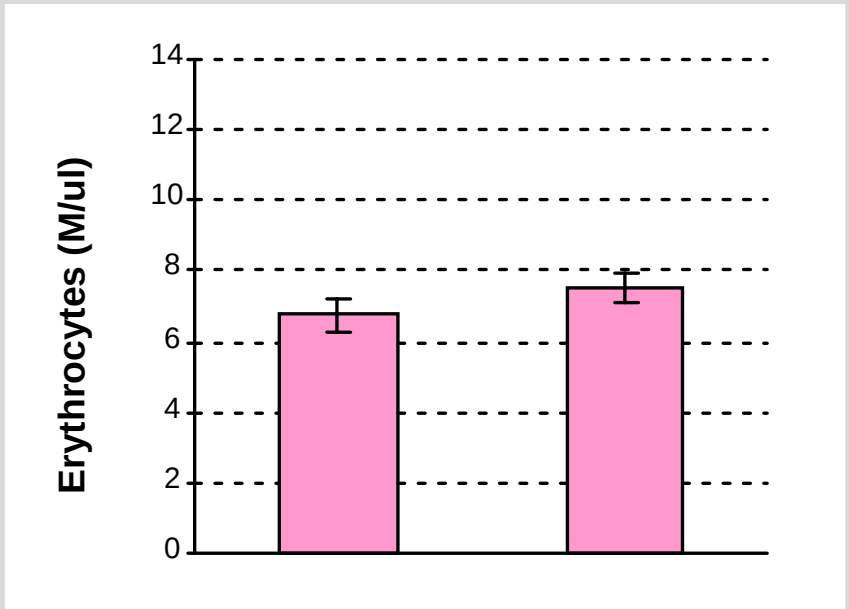




Southern blot

Loss of Circulating Erythrocytes in Bcl-x Mutants

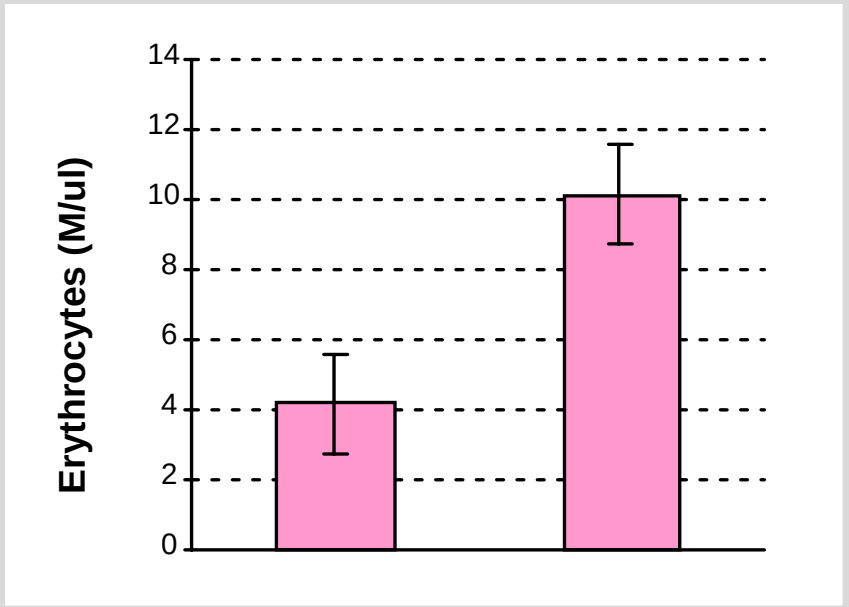
5 weeks



Bcl-x $^{fl/fl}$ MMTV-Cre **n=3**

Bcl-x $^{fl/fl}$ **n=3**

3 months

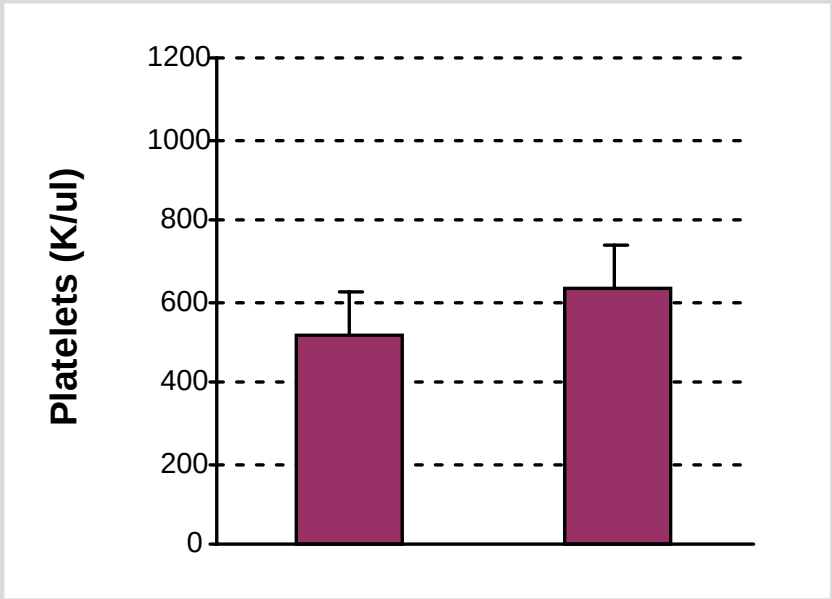


Bcl-x $^{fl/fl}$ MMTV-Cre **n=8**

Bcl-x $^{fl/fl}$ **n=6**

Loss of Platelets in Bcl-x Mutants

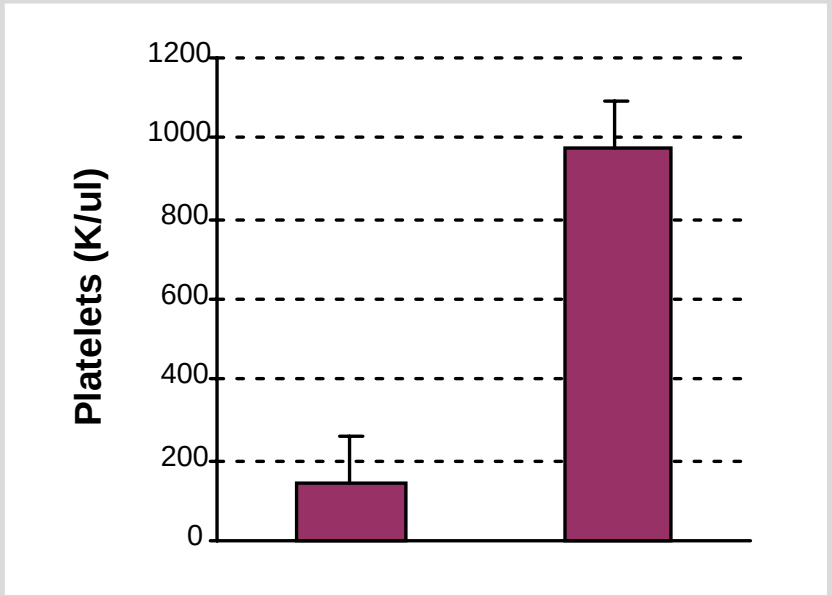
5 weeks



Bcl-x $^{fl/fl}$ MMTV-Cre n=3

Bcl-x $^{fl/fl}$ n=3

3 months



Bcl-x $^{fl/fl}$ MMTV-Cre n=8

Bcl-x $^{fl/fl}$ n=6

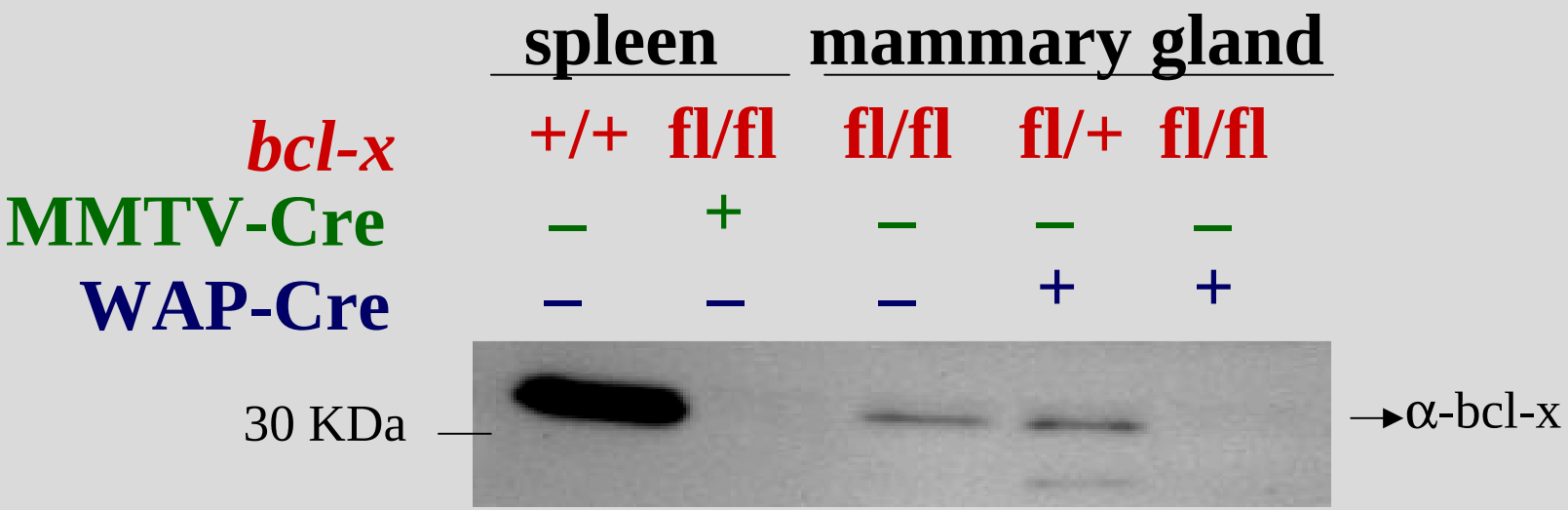
Cell Specificity of Bcl-x and Bax Function

gene ko	mammary	germ cells	erythroid cells	T- and B-cells
bcl-x		• loss of germ cells	• reduced red cells • excess immature erythrocytes	
bax		excess germ cells	normal	
bcl-x & bax		excess germ cells	• reduced red cells • excess immature	

balance NO balance

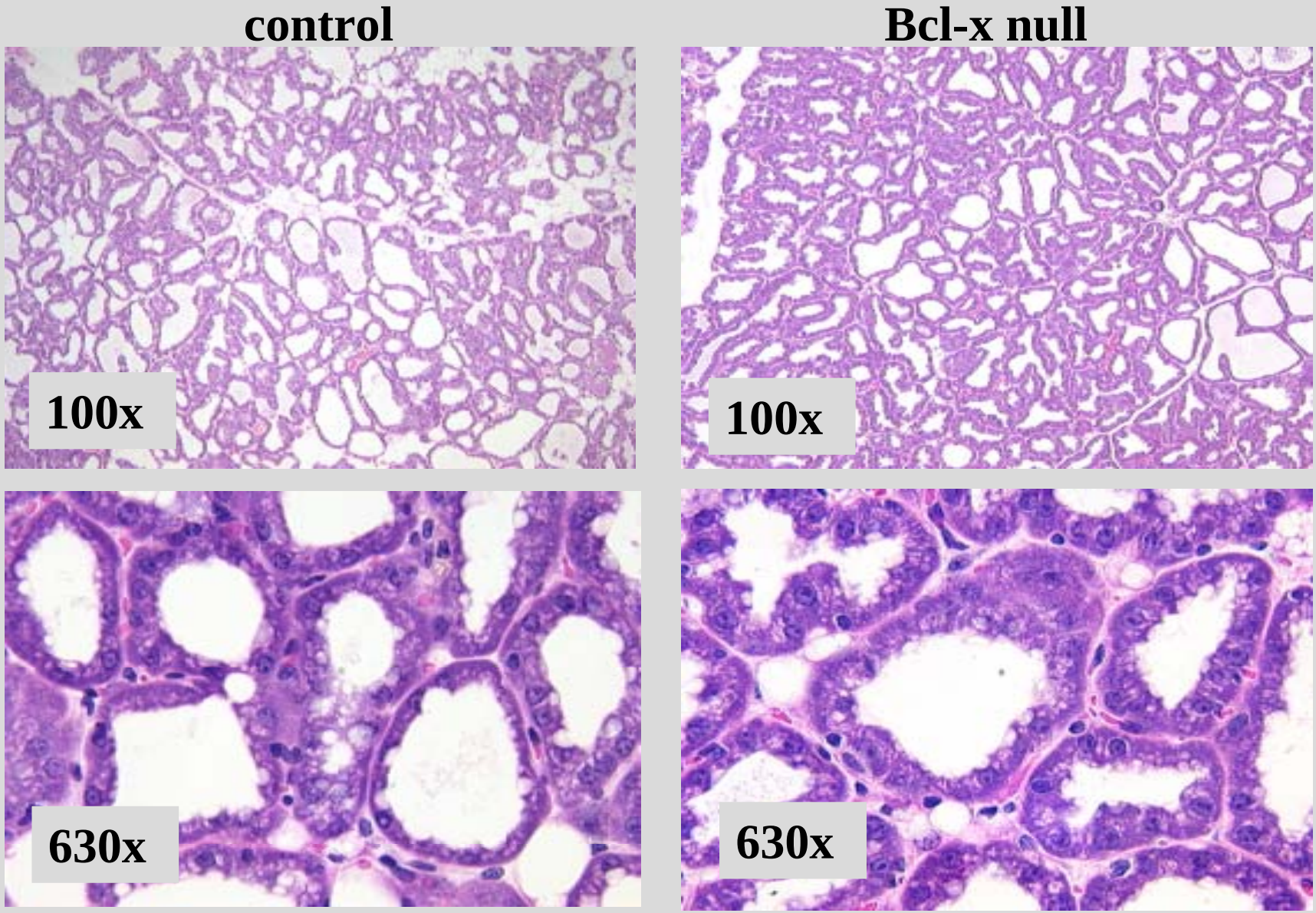
Deletion of the *bcl-x* Gene in Mammary Epithelial Cells

Loss of Bcl-x in Mammary Tissue



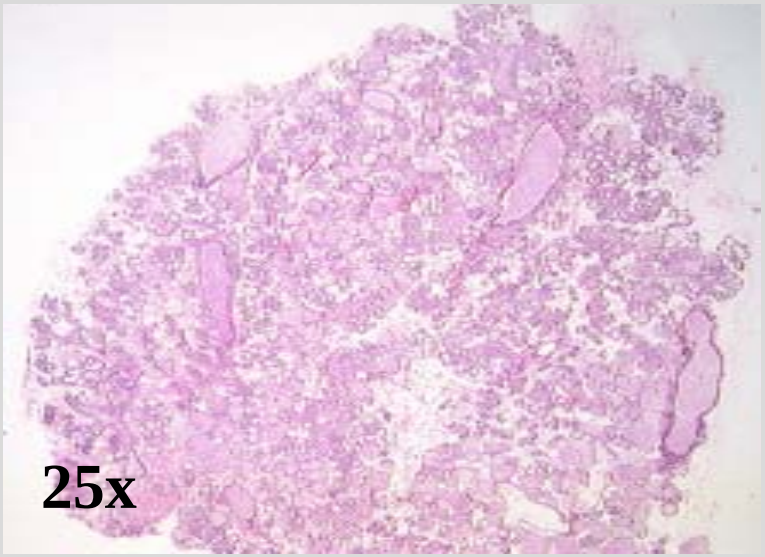
western blot

Normal Mammary Gland Development in the Absence of Bcl-x

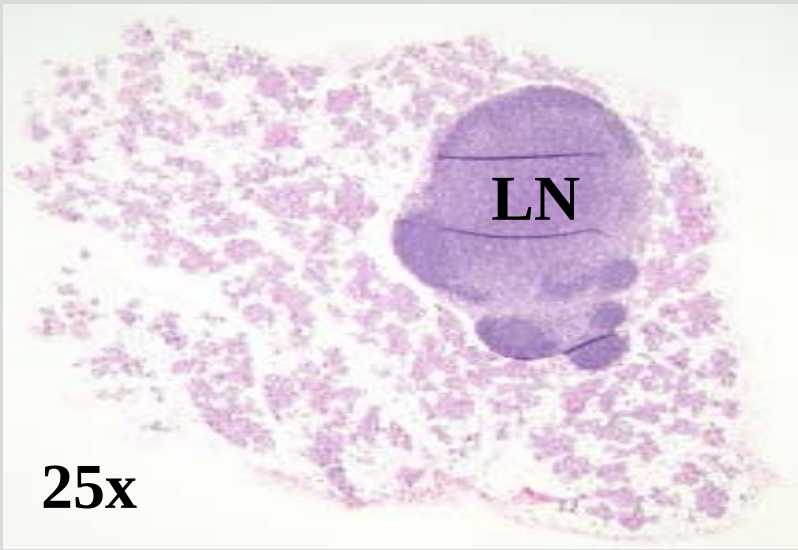


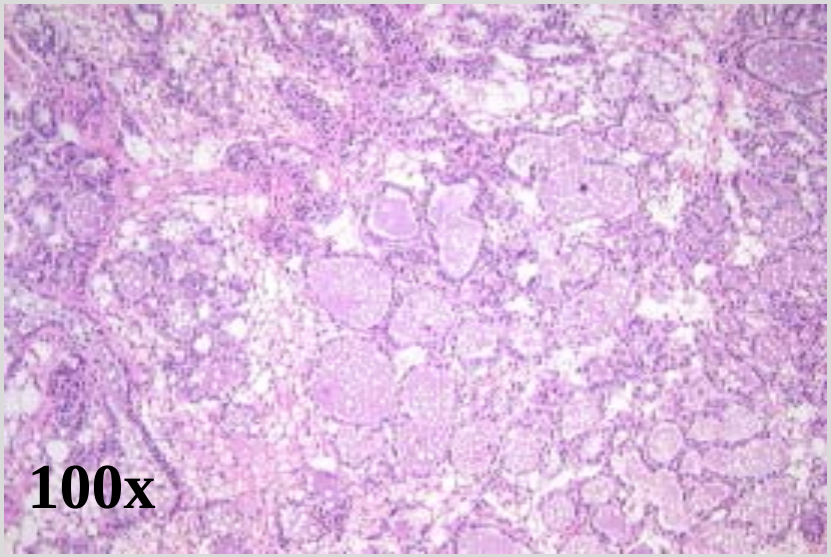
Involution is Accelerated in the Absence of Bcl-x

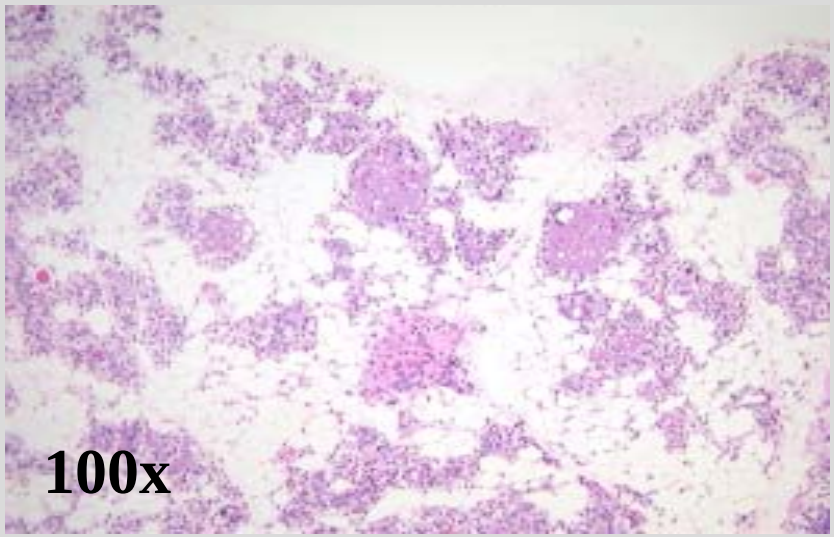
control



Bcl-x null

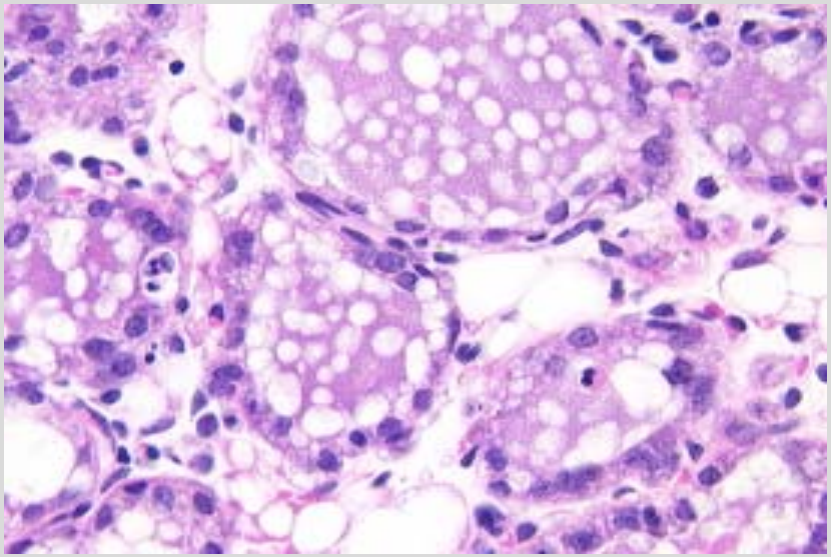






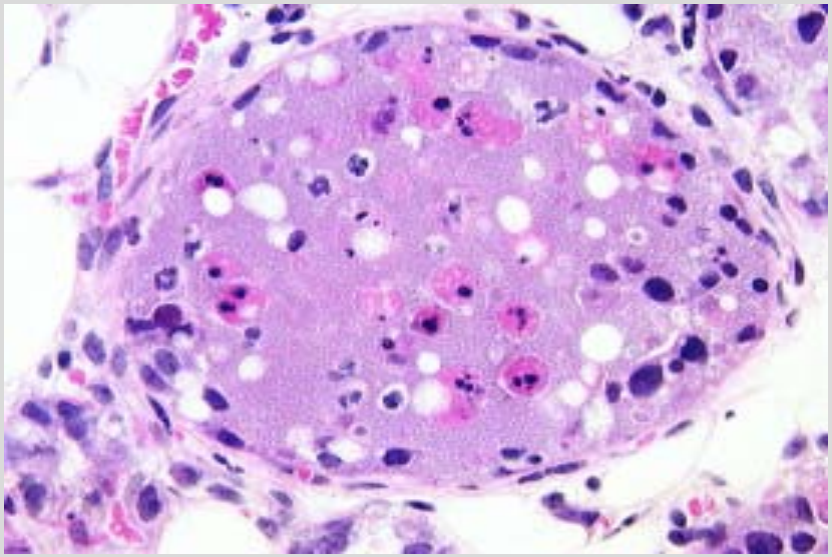
Increased Apoptosis in the Absence of Bcl-x

control



630x

Bcl-x bull



630x

Cell Specificity of Bcl-x and Bax Function

gene ko	mammary	germ cells	hematopoiesis	T- and B-cells
bcl-x	• functional development • accelerated apoptosis (remodeling)	• loss of germ cells	• reduced red cells • excess immature erythrocytes	reduced life span
bax	• functional development	excess germ cells	normal	normal
bcl-x & bax	•functional development • accelerated apoptosis (remodeling)	excess germ cells	• reduced red cells • excess immature	reduced life span
	NO balance	balance	NO balance	NO balance

Conditional Inactivation of the *bcl-x* Gene

Greg Riedlinger	**oocytes and granulosa cells**
Ed Rucker	**primordial germ cells**
Patricia Dierisseau	**primordial germ cells**
Kay-Uwe Wagner	**erythroid cells**
Kate Walton	**mammary epithelium**

Jodi Flaws

Lisa Garrett

Estefania Vazquez

Ulrich Siebenlist

http://mammary.nih.gov

Patricia Dierisseau

Ed Rucker

Kay-Uwe Wagner

Greg Riedlinger