



Memorial Sloan Kettering  
Cancer Center

## **Notch signaling – from physiology to cancer biology**

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*Cancer Biology GSK course*  
*04/02/2026*

# Course outline

*April 2*

- Part I: Notch signaling – from physiology to cancer biology
- Part II: New insights into the role of Notch signaling in T and B cell immunity

*April 3*

- Part III: An introduction to early T cell development for cancer biologists
- Part IV: Paper discussion  
Herranz et al., *Nat Med* 2014  
A NOTCH1-driven MYC enhancer promotes T cell development, transformation and acute leukemia

# Learning objectives

- Understand the components of the Notch signaling pathway and the biochemical mechanisms of Notch activation
- Appreciate key physiological functions of Notch signaling in development and homeostasis
- Understand the role of Notch signaling in tumor biology, with a focus on both oncogenic and tumor suppressive functions

# Supporting information

Sprinzak and Blacklow. Biophysics of Notch Signaling. *Annu Rev Biophys*, 2021

Aster, Pear and Blacklow. The Varied Roles of Notch in Cancer. *Annu Rev Pathol Mech Dis*, 2017.

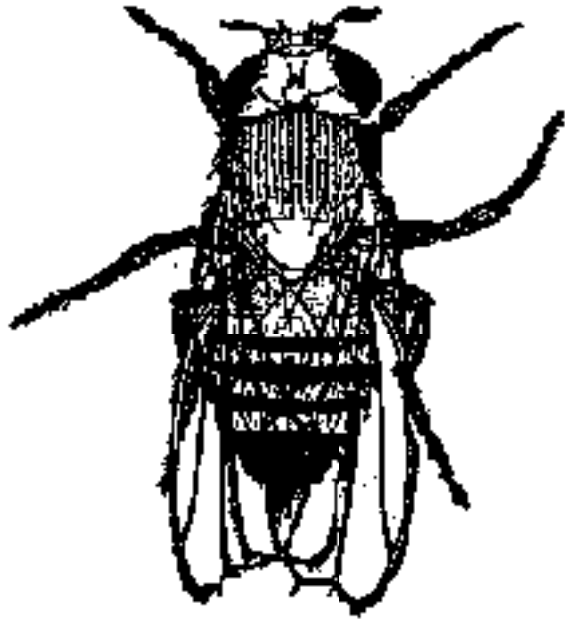
Ivan Maillard

RRL-417C

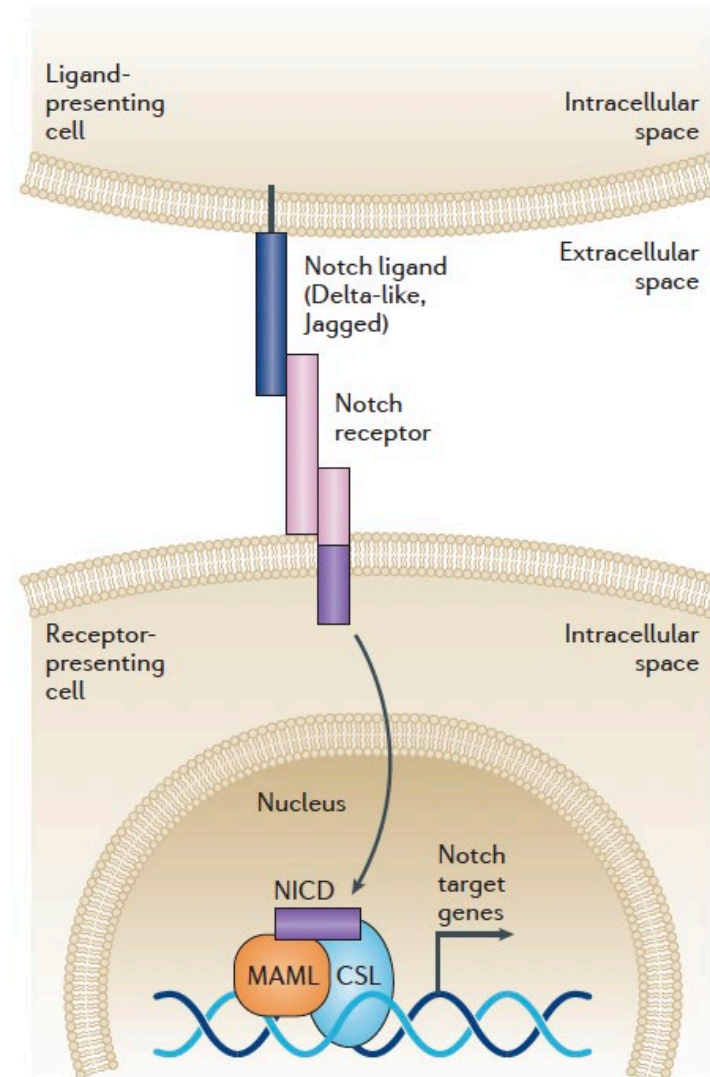
[maillai@mskcc.org](mailto:maillai@mskcc.org)

Please feel free to reach out!

# Notch signaling – a brief history



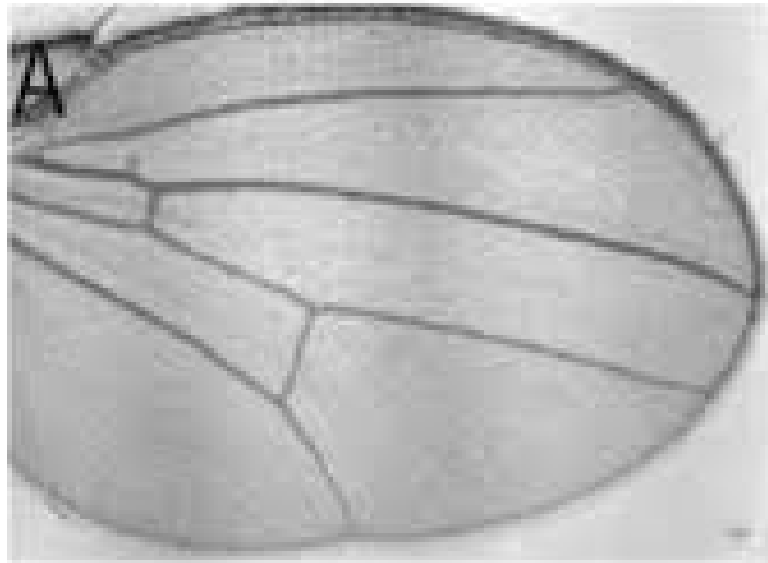
1917



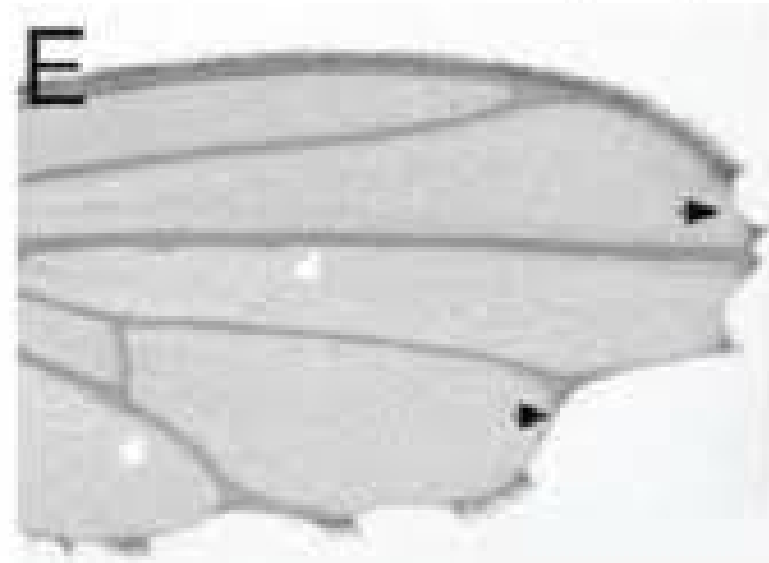
1983-present

# Notch loss-of-function mutations in *Drosophila*

Wild-type (normal)



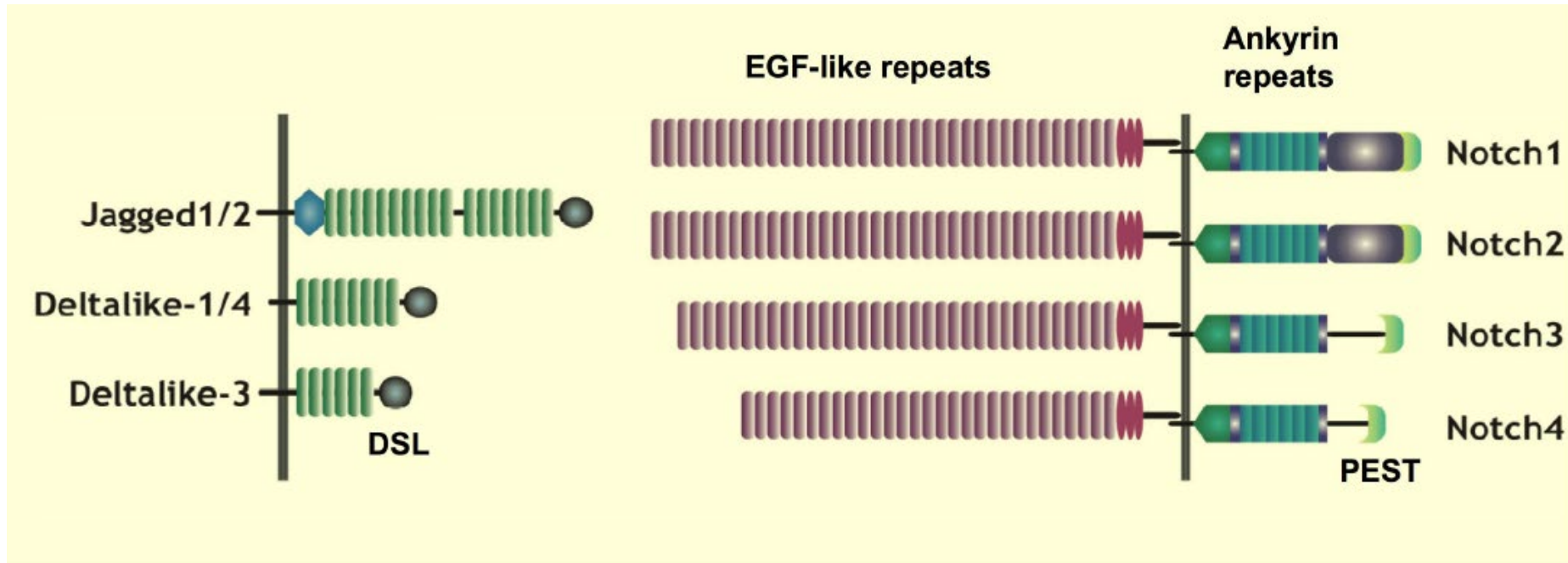
Notch mutant (+/-)



# Notch signaling over evolution

- Absent in yeast
- Emerging in primitive multicellular organisms, even ahead of body symmetry
- Highly conserved from primitive organisms to vertebrates
- Many important functions in human health and disease
- Multiple model organisms – *D. melanogaster*, *C. elegans*, *D. rerio*, etc.

# Mammalian Notch ligands and receptors



# Multiple functions of Notch in development and organ homeostasis

Heart and vessel formation

Angiogenesis

Gut epithelium differentiation

Skin differentiation

Pancreatic development

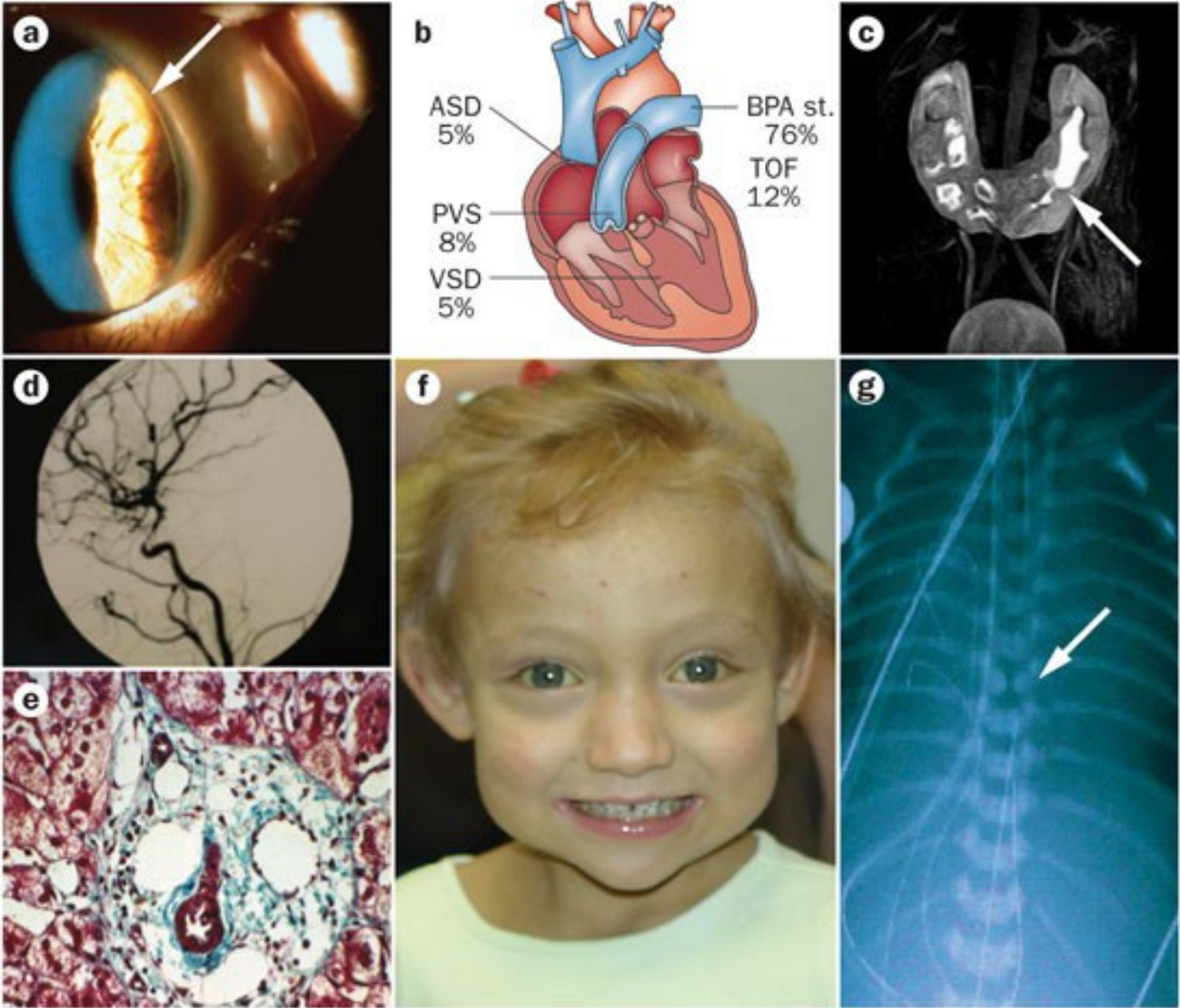
Biliary tract development

Hematopoietic and lymphoid development

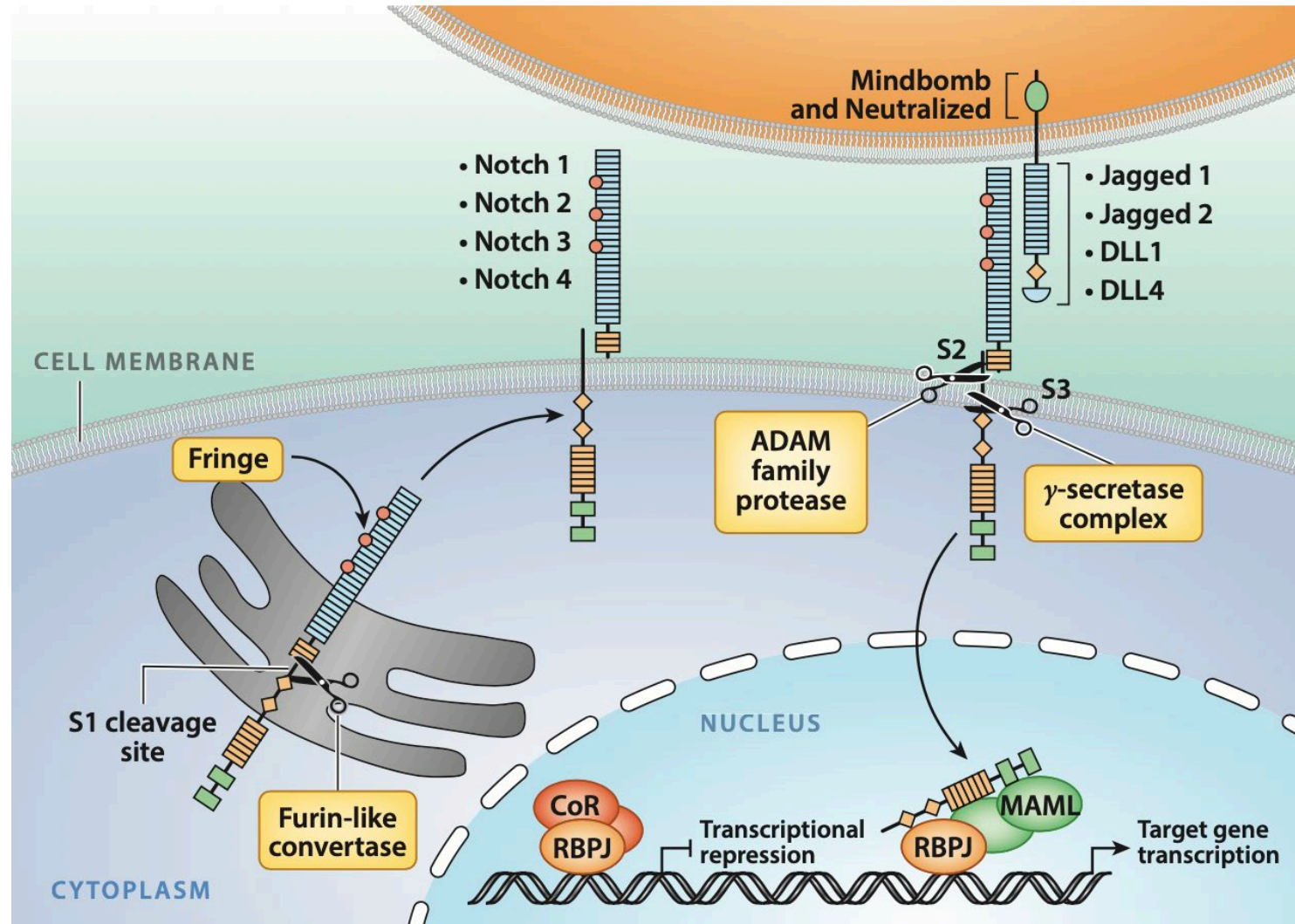
...

# Some examples of Notch-associated developmental phenotypes

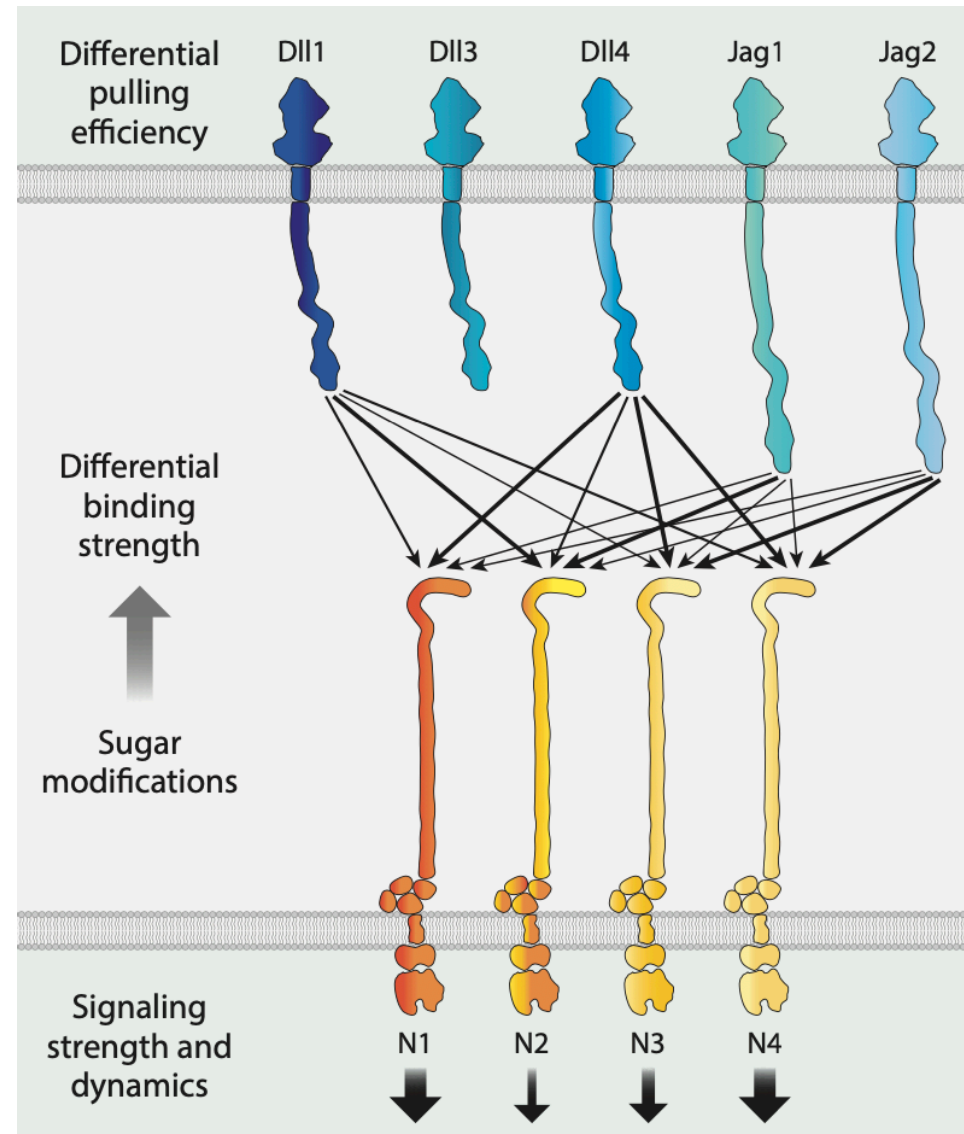
Alagille syndrome  
*JAG1* or *NOTCH2* mutations



# Mechanisms of Notch receptor processing and activation

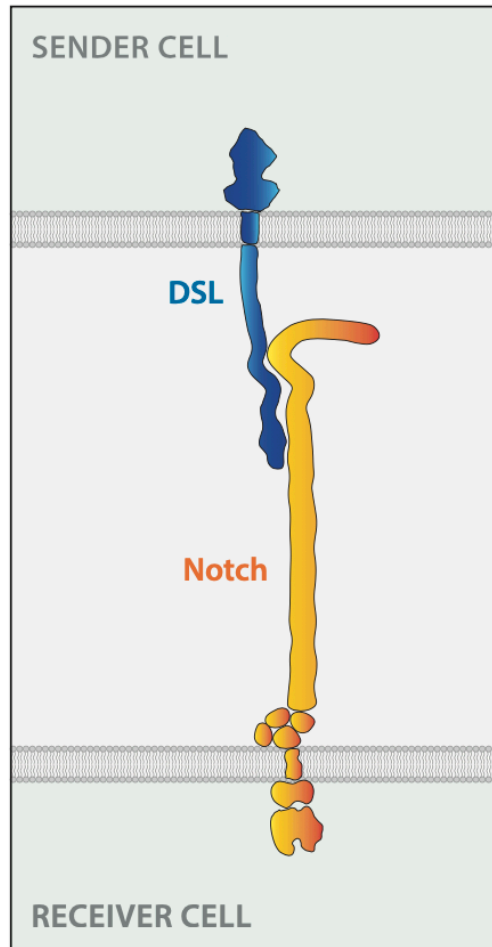


# Diversity of Notch ligand-receptor activation

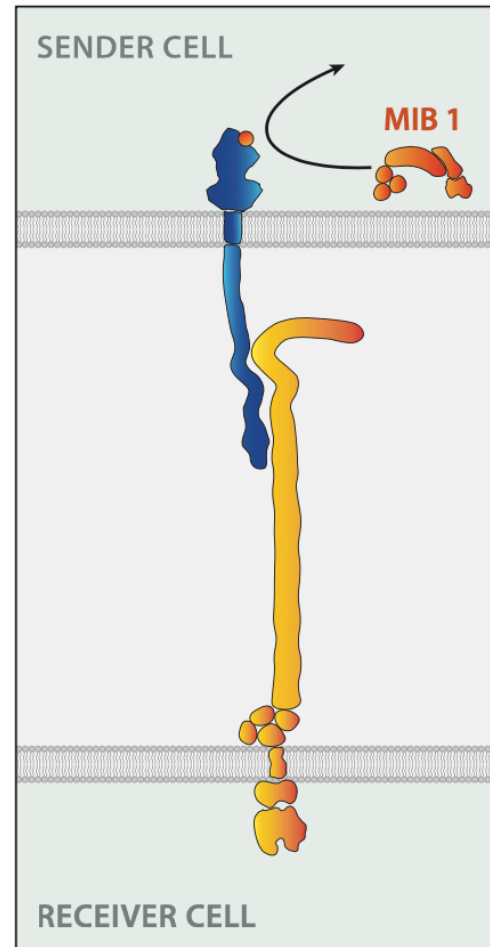


# Notch ligand-receptor binding generates a physical force and promotes receptor cleavage by ADAM10

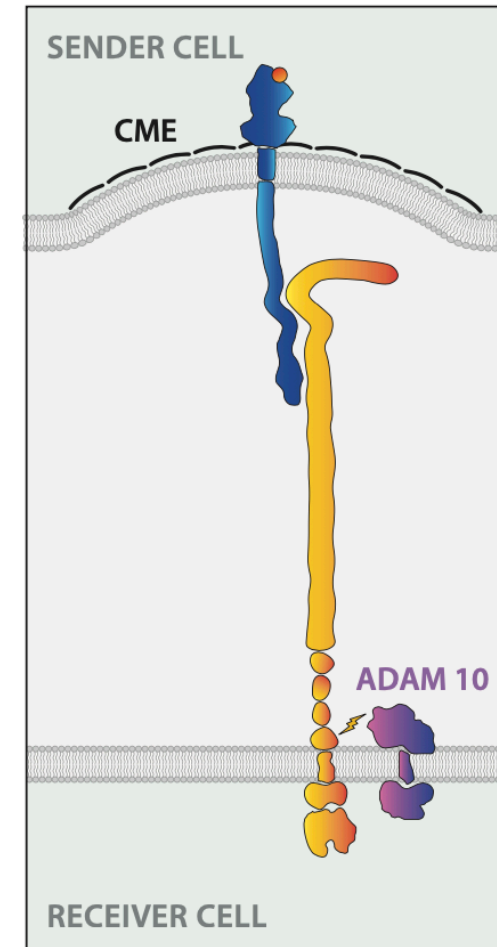
**a** Binding



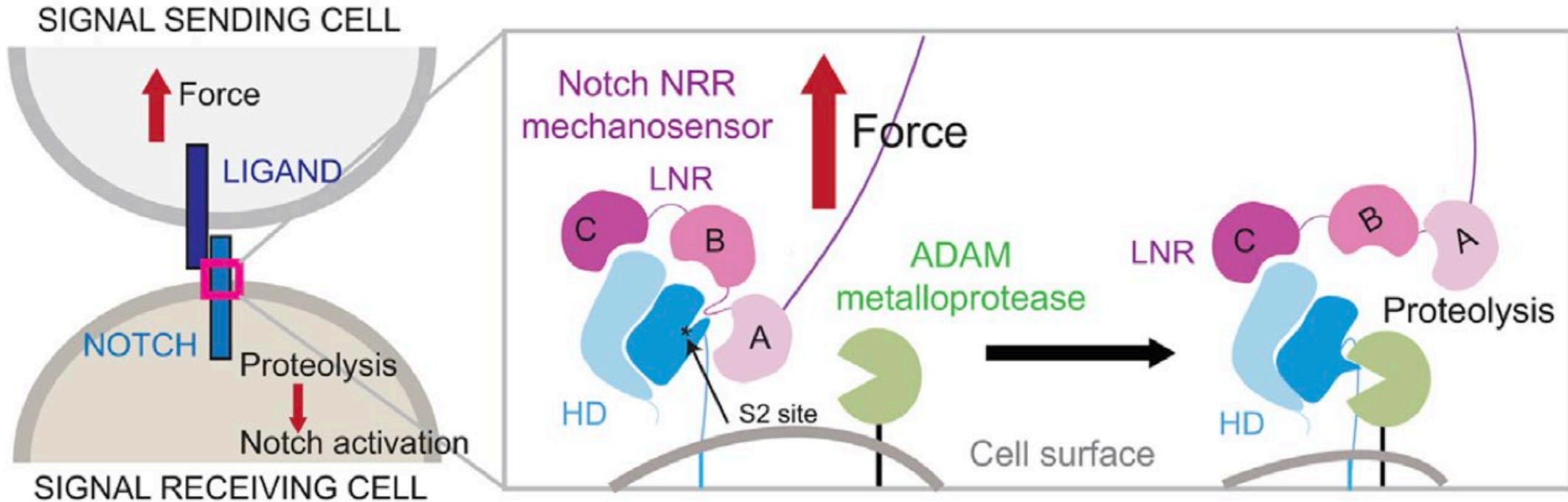
**b** Ubiquitylation



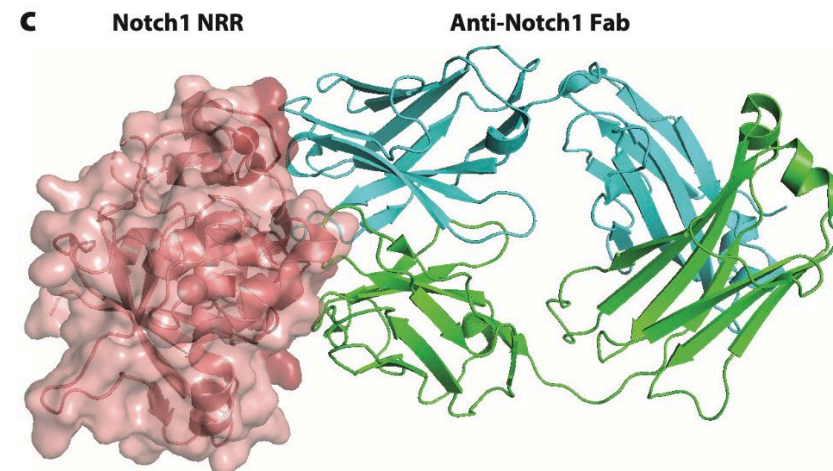
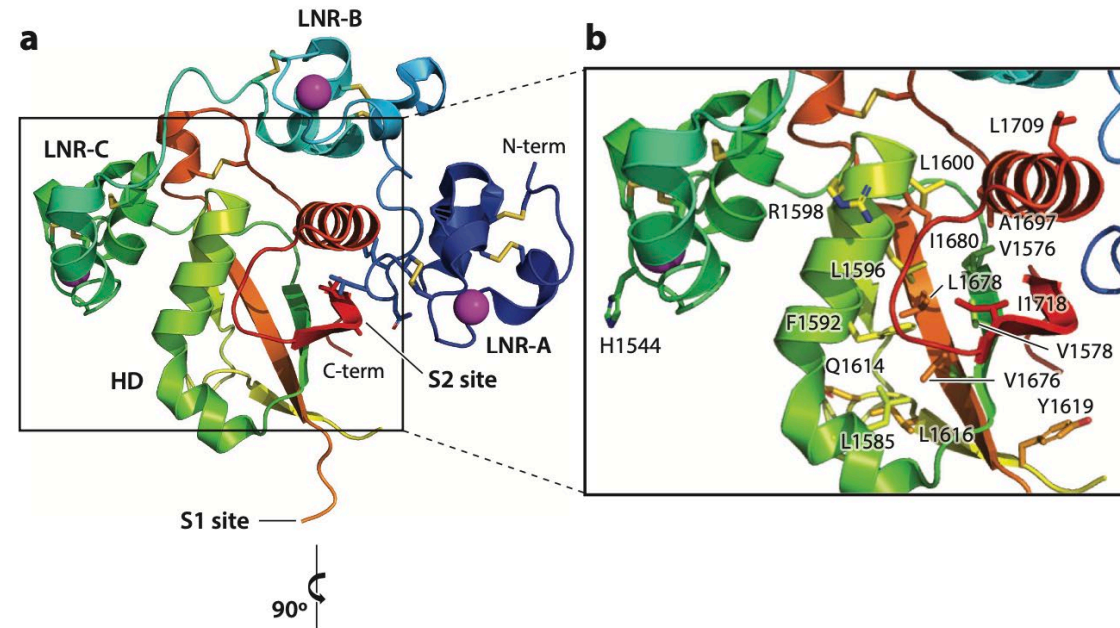
**c** Pulling



# Notch ligand-receptor binding generates a physical force and promotes receptor cleavage by ADAM10



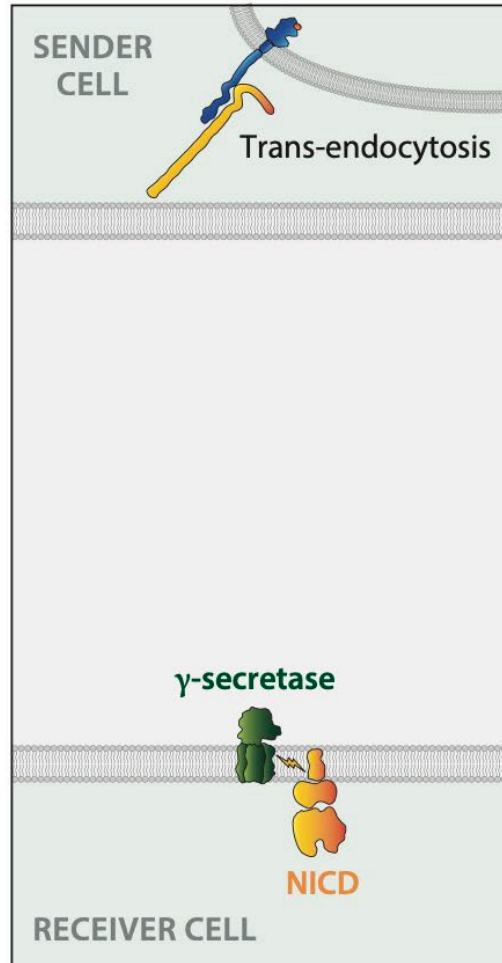
# Structure and function of the Notch negative regulatory region (NRR)



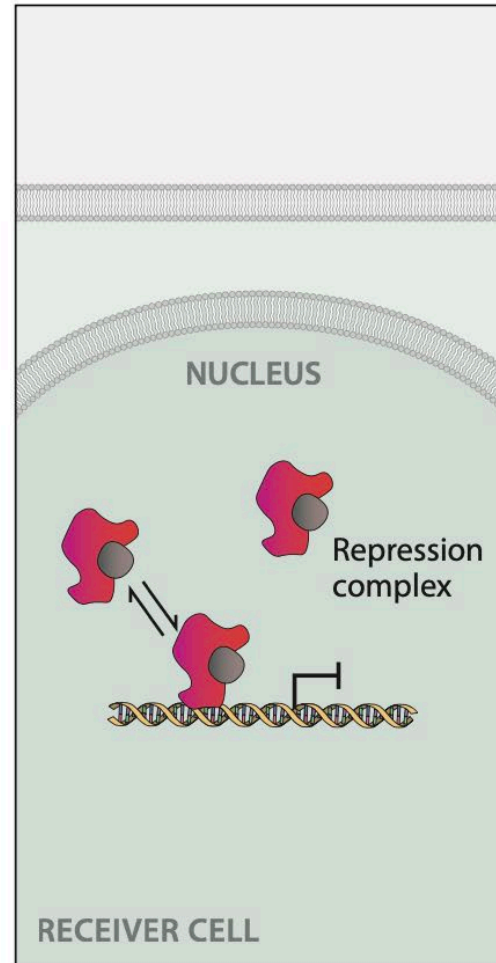
Sprinzak and Blacklow, 2017  
Structures from Blacklow and Siebel labs

# ADAM10 (S2) cleavage is followed by gamma-secretase-mediated release of the Notch intracellular domain (NICD)

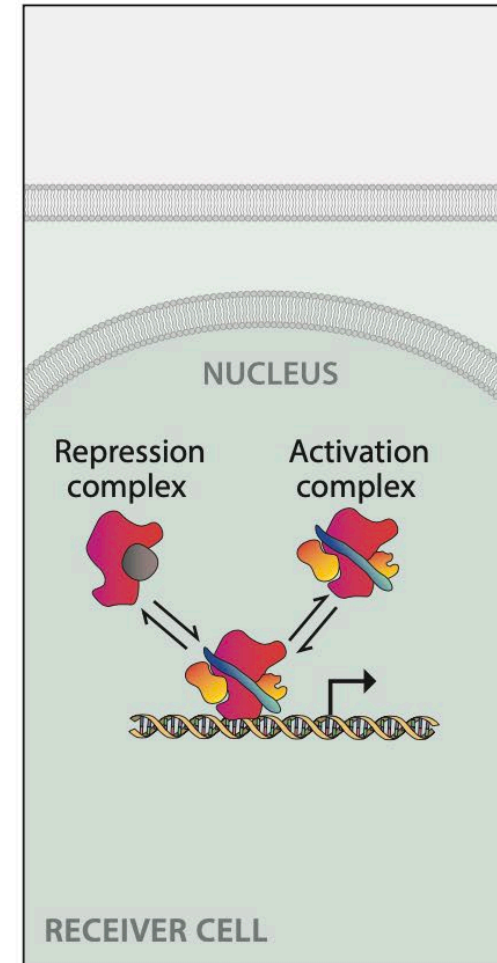
**d** NICD release



**e** Before activation



**f** After activation



# Structure of the Notch transcriptional activation complex

## Structural Basis for Cooperativity in Recruitment of MAML Coactivators to Notch Transcription Complexes

Yunsun Nam,<sup>1,4</sup> Piotr Sliz,<sup>2,4</sup> Luyan Song,<sup>3</sup> Jon C. Aster,<sup>3</sup> and Stephen C. Blacklow<sup>3,\*</sup>

<sup>1</sup> Biological and Biomedical Sciences Graduate Program in the Division of Medical Sciences, Harvard Medical School, Boston, MA 02115, USA

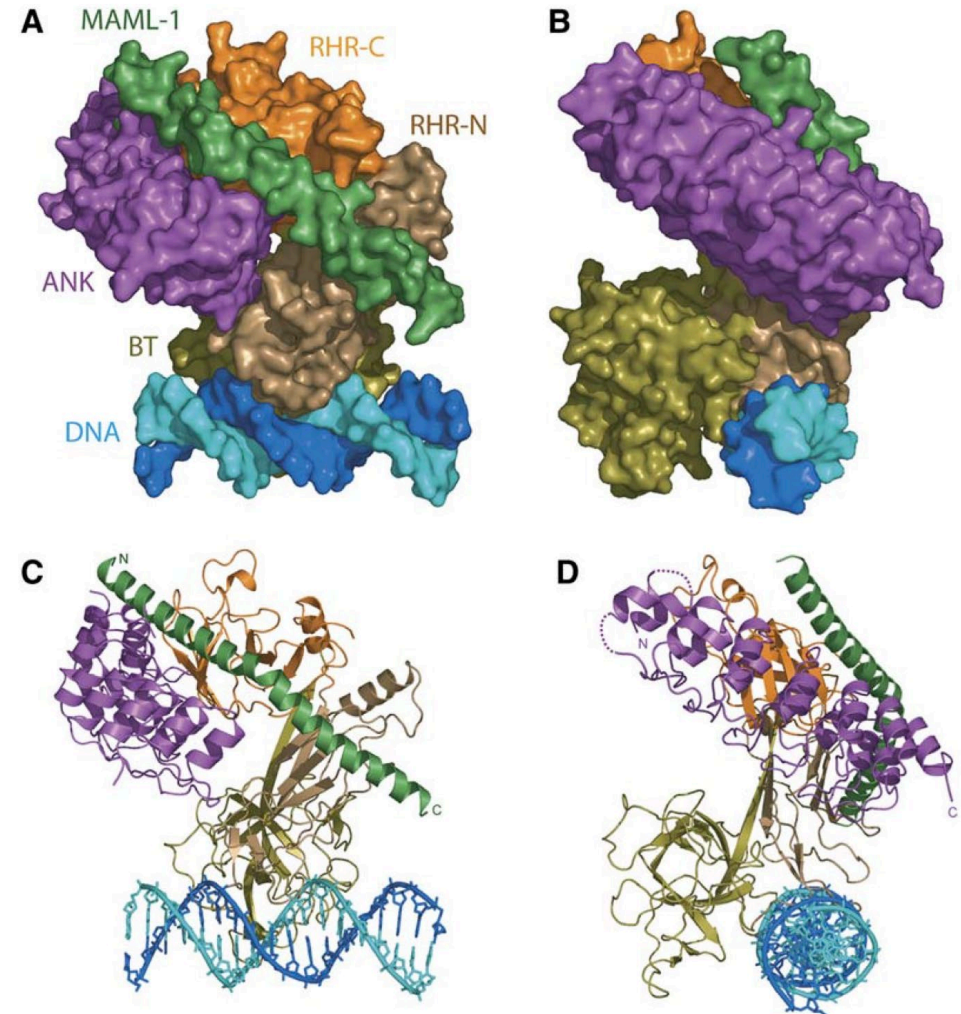
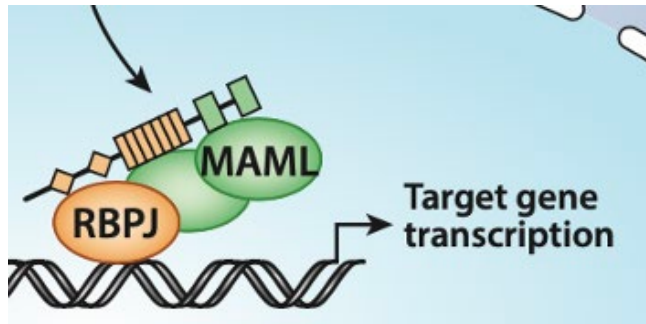
<sup>2</sup> Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School and Howard Hughes Medical Institute, 250 Longwood Ave., Boston, MA 02115, USA

<sup>3</sup> Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

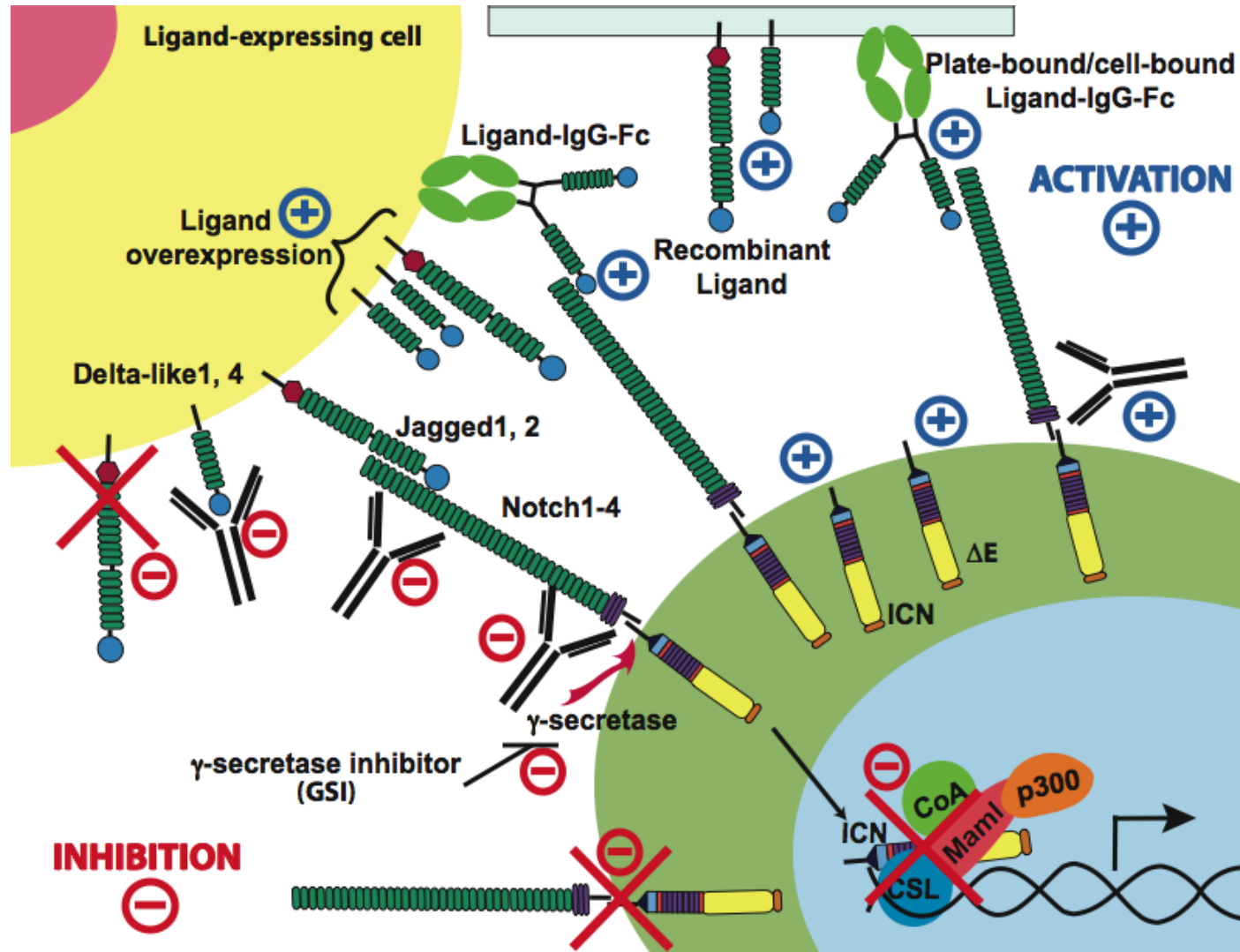
<sup>4</sup> These authors contributed equally to this work.

\*Contact: sblacklow@rics.bwh.harvard.edu

DOI 10.1016/j.cell.2005.12.037



# The Notch signaling toolkit



# Emerging Synthetic Notch Agonists

nature chemical biology



Article

<https://doi.org/10.1038/s41589-025-02030-y>

## Engineering synthetic agonists for targeted activation of Notch signaling

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Published online: 24 September 2025

David H. Perez<sup>Ⓞ</sup>, Daniel Antfolk<sup>Ⓞ</sup>, Shiun Chang, Xiomar E. Bustos<sup>Ⓞ</sup>,  
Elliot Medina<sup>Ⓞ</sup>, Ahmed A. Ramadan, David Gonzalez-Perez<sup>Ⓞ</sup>,  
Daniel Abate-Daga, Paulo C. Rodriguez<sup>Ⓞ</sup> & Vincent C. Luca<sup>Ⓞ</sup> ✉

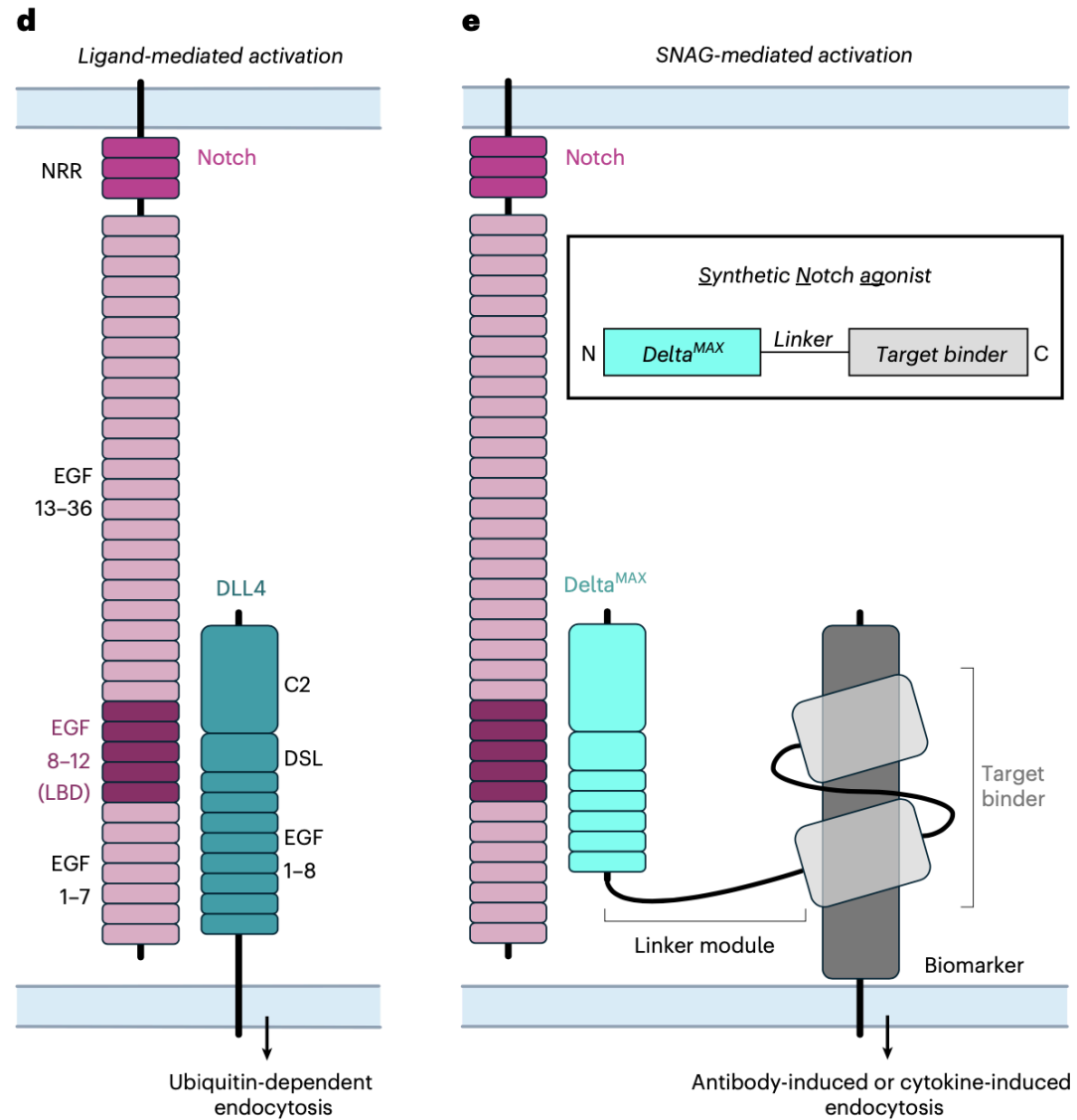


Article

## Design of soluble Notch agonists that drive T cell development and boost immunity

Rubul Mout,<sup>1,2,3</sup> Ran Jing,<sup>1,2,3,16</sup> Mayuri Tanaka-Yano,<sup>1,16</sup> Emily D. Egan,<sup>2</sup> Helen Eisenach,<sup>5,6</sup> Martin A. Kononov,<sup>1</sup> Roland Windisch,<sup>1,2,3</sup> Mohamad Ali Toufic Najja,<sup>1,2,7,8</sup> Allison Tompkins,<sup>1</sup> Luca Hensch,<sup>1</sup> Trevor Bingham,<sup>1</sup> Rajesh Gunage,<sup>1,4</sup> Yunliang Zhao,<sup>1</sup> Natasha I. Edman,<sup>5,6,9</sup> Christopher Li,<sup>1,2</sup> Dahai Wang,<sup>1</sup> Thorsten M. Schlaeger,<sup>1</sup> Leonard I. Zon,<sup>1,3,4,10</sup> Trista E. North,<sup>1</sup> Urban Lendahl,<sup>11</sup> R. Grant Rowe,<sup>1,3,12,13</sup> David Baker,<sup>5,6,14</sup> Stephen C. Blacklow,<sup>2,15</sup> and George Q. Daley<sup>1,2,3,17,\*</sup>

# Example of a Synthetic Notch Agonist (“SNAG”)



# Synthetic Notch receptors (“Syn-Notch”)

## Engineering T Cells with Customized Therapeutic Response Programs Using Synthetic Notch Receptors

Kole T. Roybal,<sup>1,2,3</sup> Jasper Z. Williams,<sup>1,2,3</sup> Leonardo Morsut,<sup>1,2,3</sup> Levi J. Rupp,<sup>1,2,3</sup> Isabel Kolinko,<sup>1,2,3</sup> Joseph H. Choe,<sup>1,2,3</sup> Whitney J. Walker,<sup>1,2,3</sup> Krista A. McNally,<sup>1,2,3</sup> and Wendell A. Lim<sup>1,2,3,4,\*</sup>

<sup>1</sup>Department of Cellular & Molecular Pharmacology, University of California San Francisco, San Francisco, CA 94158, USA

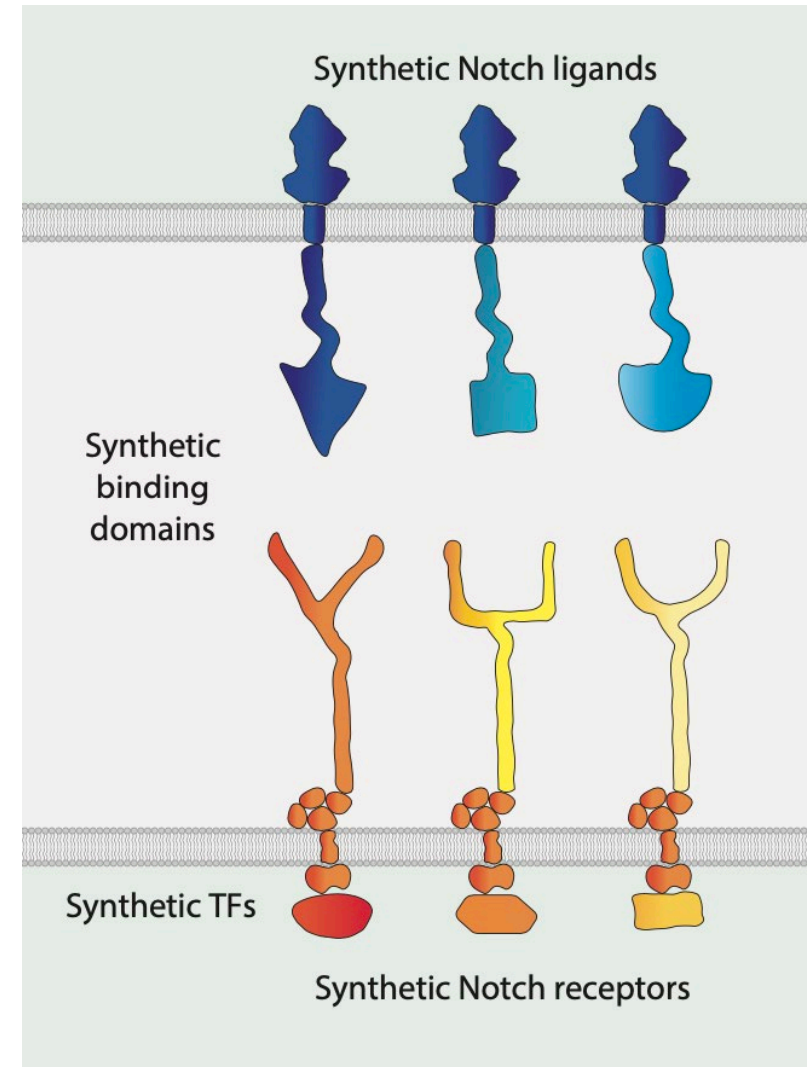
<sup>2</sup>UCSF Center for Systems and Synthetic Biology, San Francisco, CA 94158, USA

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<sup>4</sup>Lead Contact

\*Correspondence: [wendell.lim@ucsf.edu](mailto:wendell.lim@ucsf.edu)

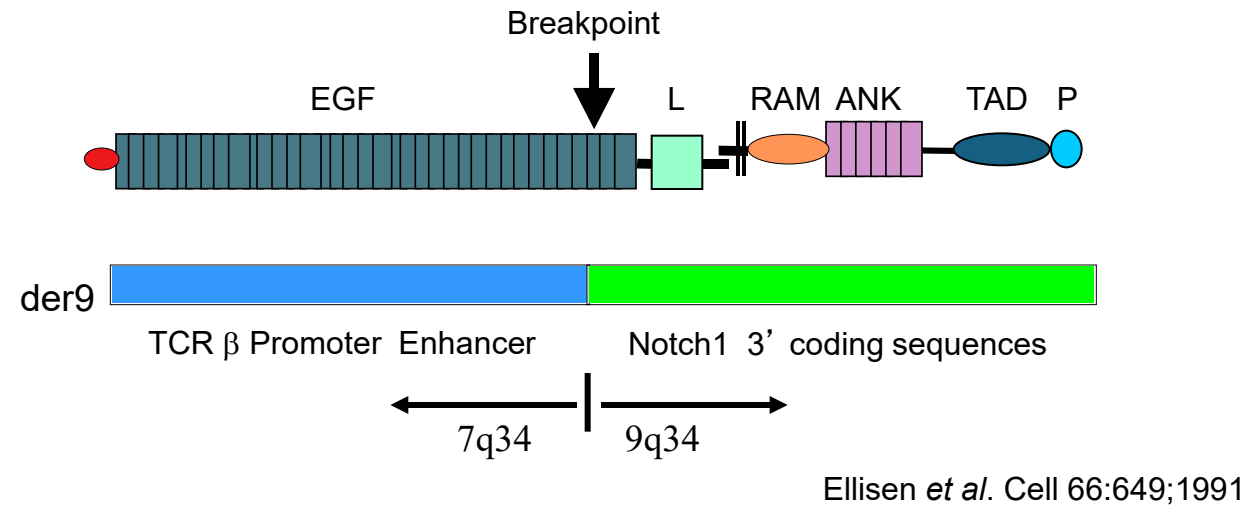
<http://dx.doi.org/10.1016/j.cell.2016.09.011>



Sprinzak and Blacklow, 2017

**What about the role of Notch signaling  
in tumor biology?**

# Identification of *NOTCH1* as an oncogene in T cell leukemia



- *NOTCH1* placed under the transcriptional control of *TCRB* regulatory sequences
- Creates a dominant gain-of-function allele
- Very rare

# Insertional mutagenesis disrupts *NOTCH4* in mouse breast cancer

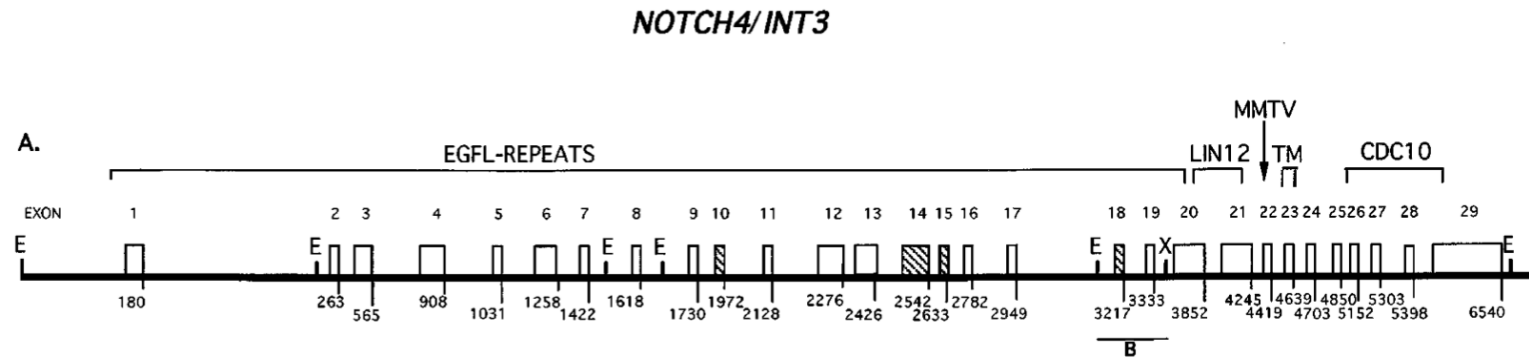
*Oncogene* (1997) 14, 1883–1890  
© 1997 Stockton Press All rights reserved 0950–9232/97 \$12.00



## The mouse mammary tumor associated gene *INT3* is a unique member of the *NOTCH* gene family (*NOTCH4*)

Daniel Gallahan<sup>1</sup> and Robert Callahan

*Laboratory of Tumor Immunology and Biology, National Cancer Institute, NIH, Bethesda, Maryland 20892, USA*

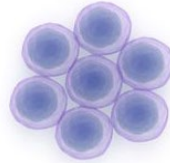


# NOTCH1 as an oncogene: lessons from mouse models



Transduction

HSCs



Transplantation

- 100% of animals moribund with T-ALL by 8-12 weeks
- Tumor development is thymus-independent
- No tumors in genetic backgrounds that do not support T cell development

Pear *et al.*, J Exp Med 1996, PMID: 8642337



Take home messages:

1. Among bone marrow progenitors, Notch is only transforming within cells adopting T cell fate
2. Cell autonomous Notch signaling drives cells to T cell fate, expanding the pool of cells that are at risk for Notch-induced T-ALL

Notch GOF Mutation

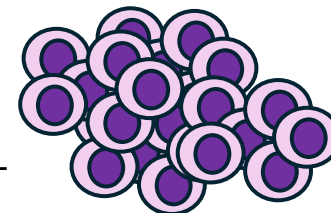


Hematopoietic Progenitor



Additional studies: GEMM T-ALL models not driven by Notch virtually always acquire spontaneous GOF mutations in murine Notch1, thus, there is selection for Notch GOF in T-ALL regardless of ordering of events

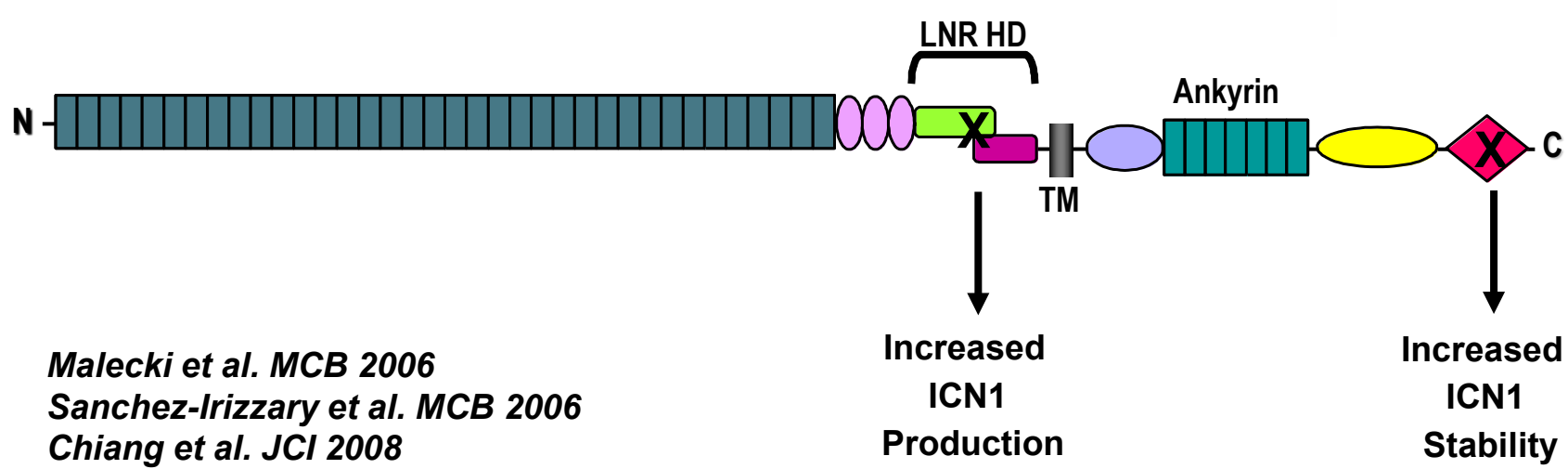
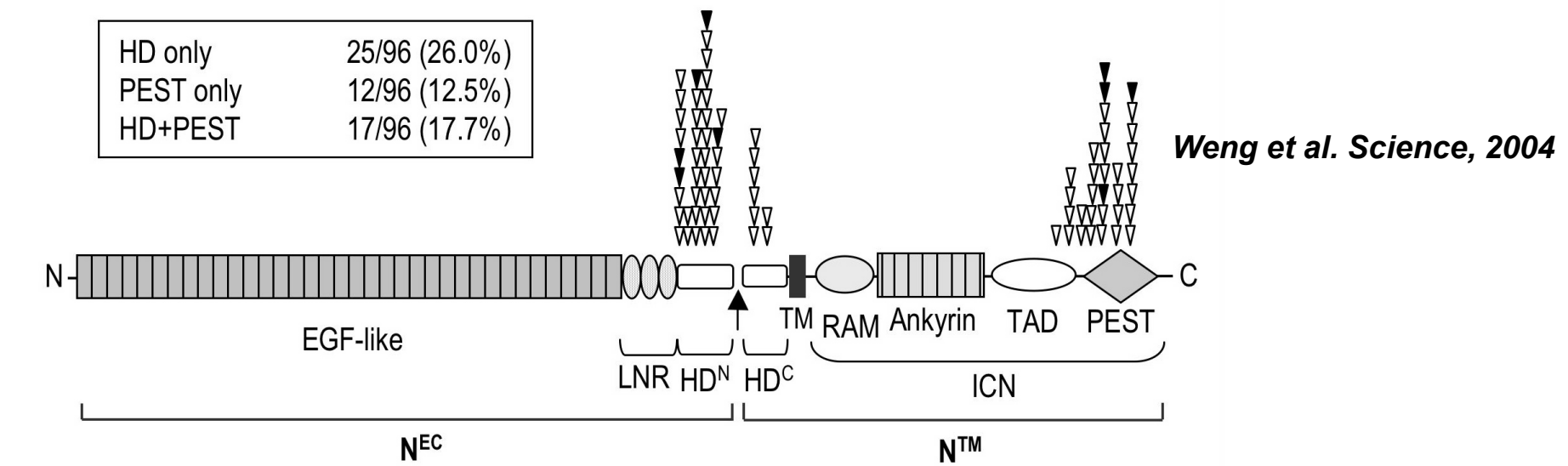
Secondary Events



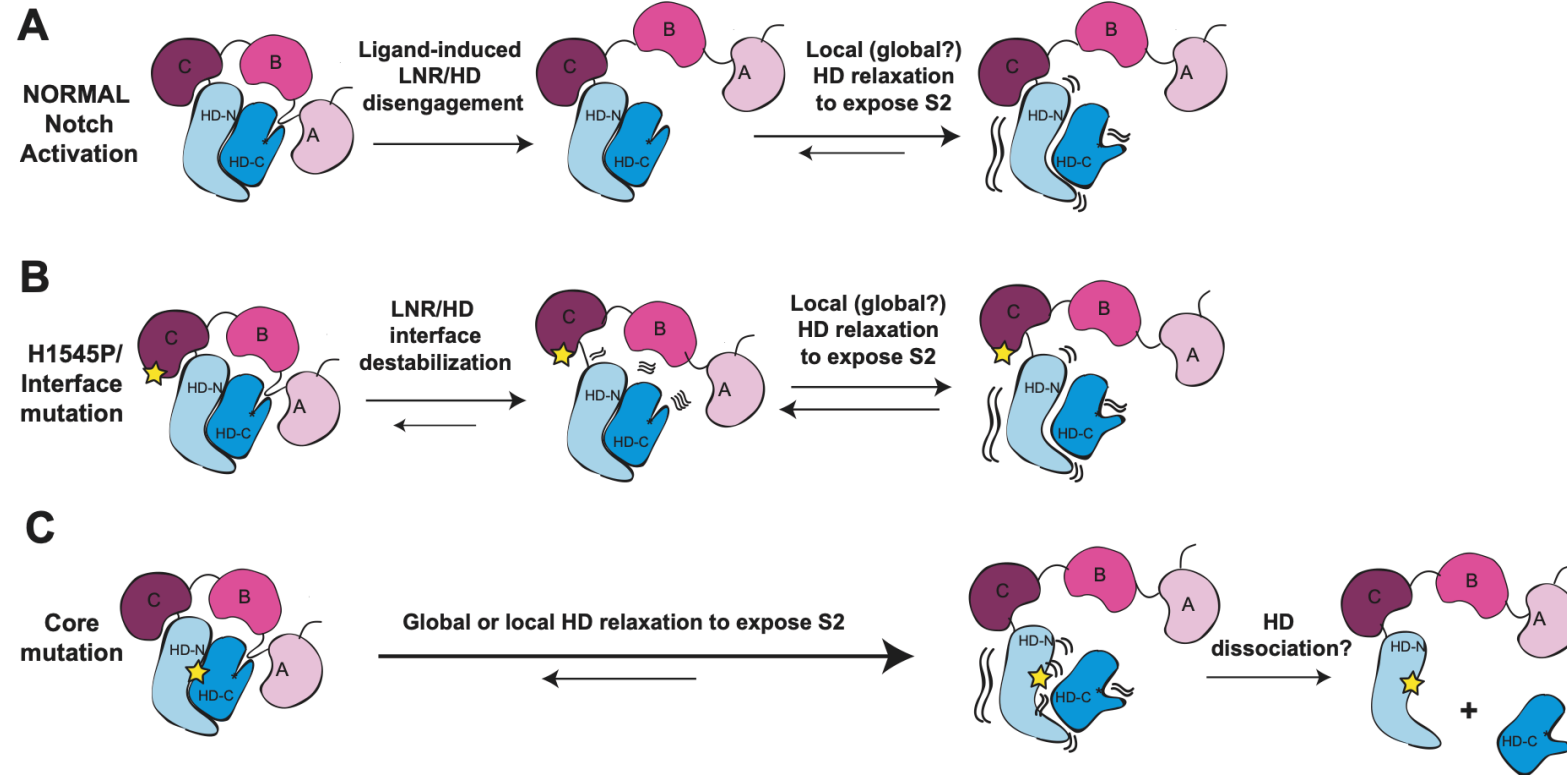
T-ALL

Slide courtesy Dr. Jon Aster

# Frequent gain-of-function *NOTCH1* Mutations in Human T-ALL



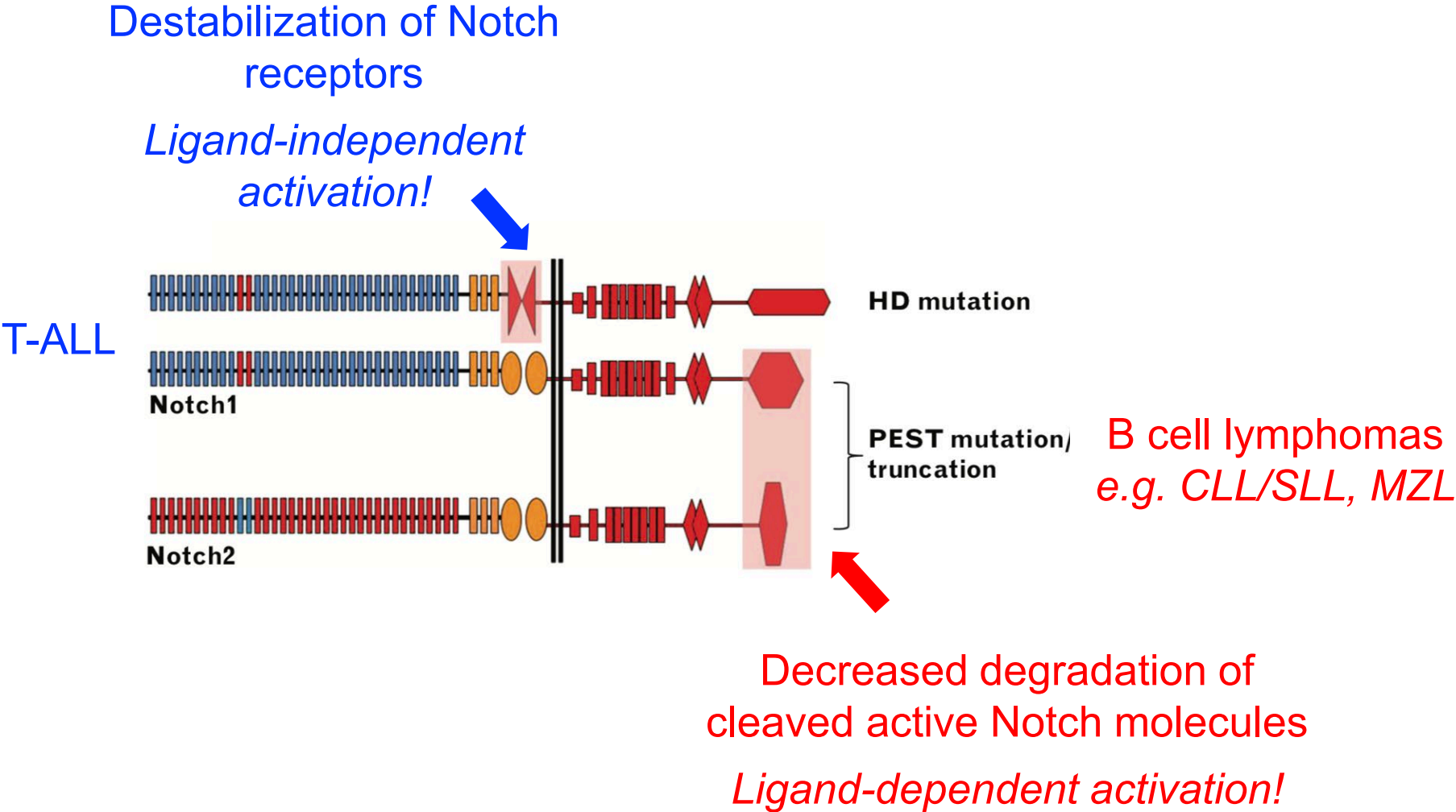
# Mechanisms of oncogenic Notch activation – HD mutations



# Examples of oncogenic Notch mutations in human cancer

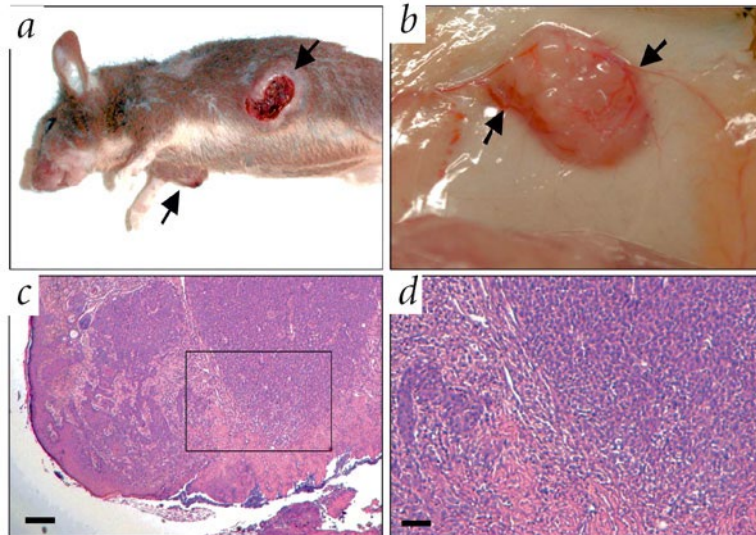
- T cell acute lymphoblastic leukemia and T cell lymphoblastic lymphoma (T-ALL/T-LBL)
  - *NOTCH1* mutations, *FBW7* mutations >60%
  - HD domain, PEST domain or both
  - Other less frequent mechanisms of *NOTCH* activation
- Chronic lymphocytic leukemia and mantle cell lymphoma
  - *NOTCH1* PEST mutations 5-20%
  - Other less frequent mechanisms of *NOTCH* activation
  - Non-mutational Notch activation
- Marginal zone lymphoma
  - *NOTCH2* PEST mutations 25% of splenic marginal zone lymphoma
  - Non-mutational Notch activation
- Breast cancer
  - Activating gene fusions, <5%

# Patterns of oncogenic *NOTCH1/2* mutations in human leukemias and lymphomas



# Notch signaling as a tumor suppressor

- Conditional floxed *Notch1* mice crossed with a keratin-specific Cre promoter to target skin
- Block in terminal differentiation
- Development of skin cancers

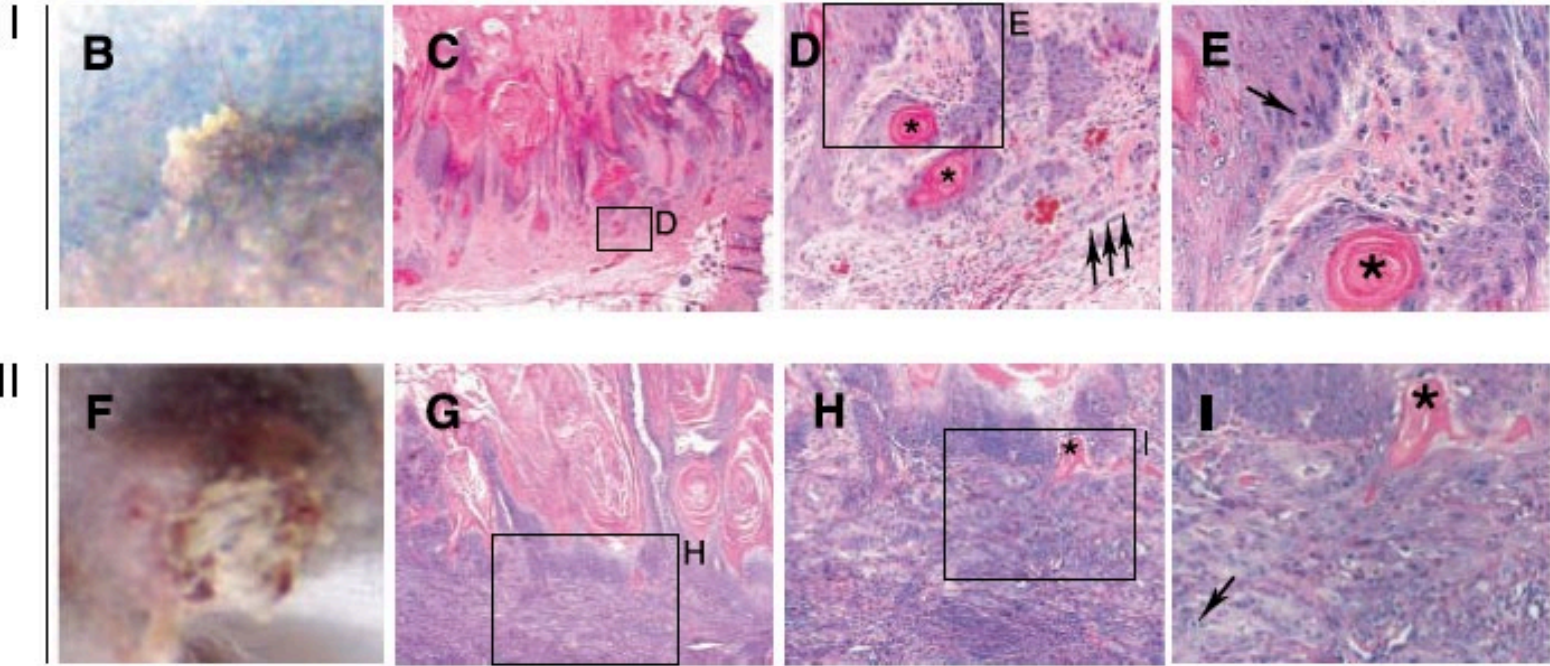
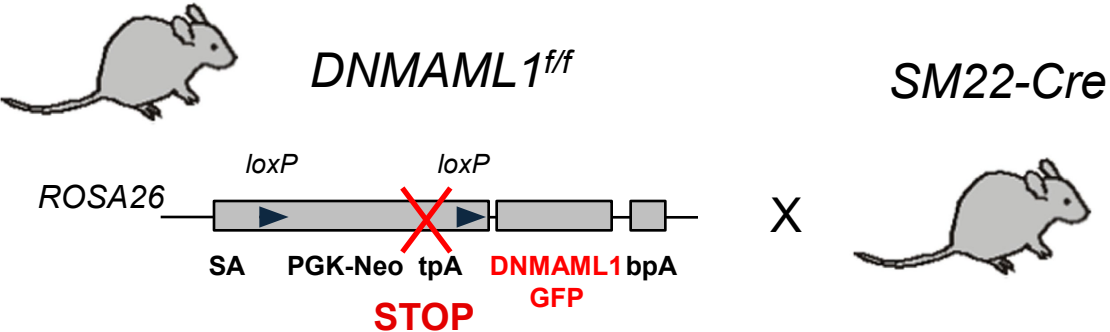
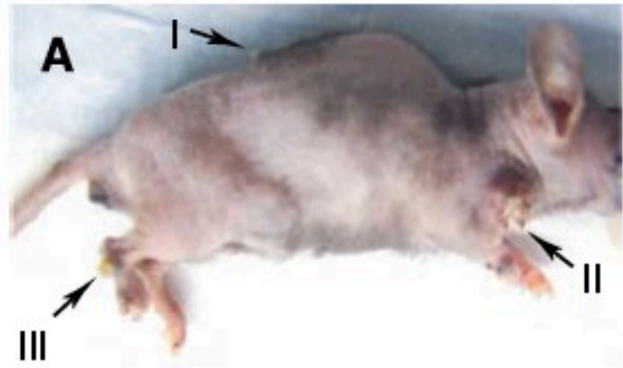


**Table 1 • Histological diagnosis of wild-type and *Notch1*<sup>-/-</sup> mice**

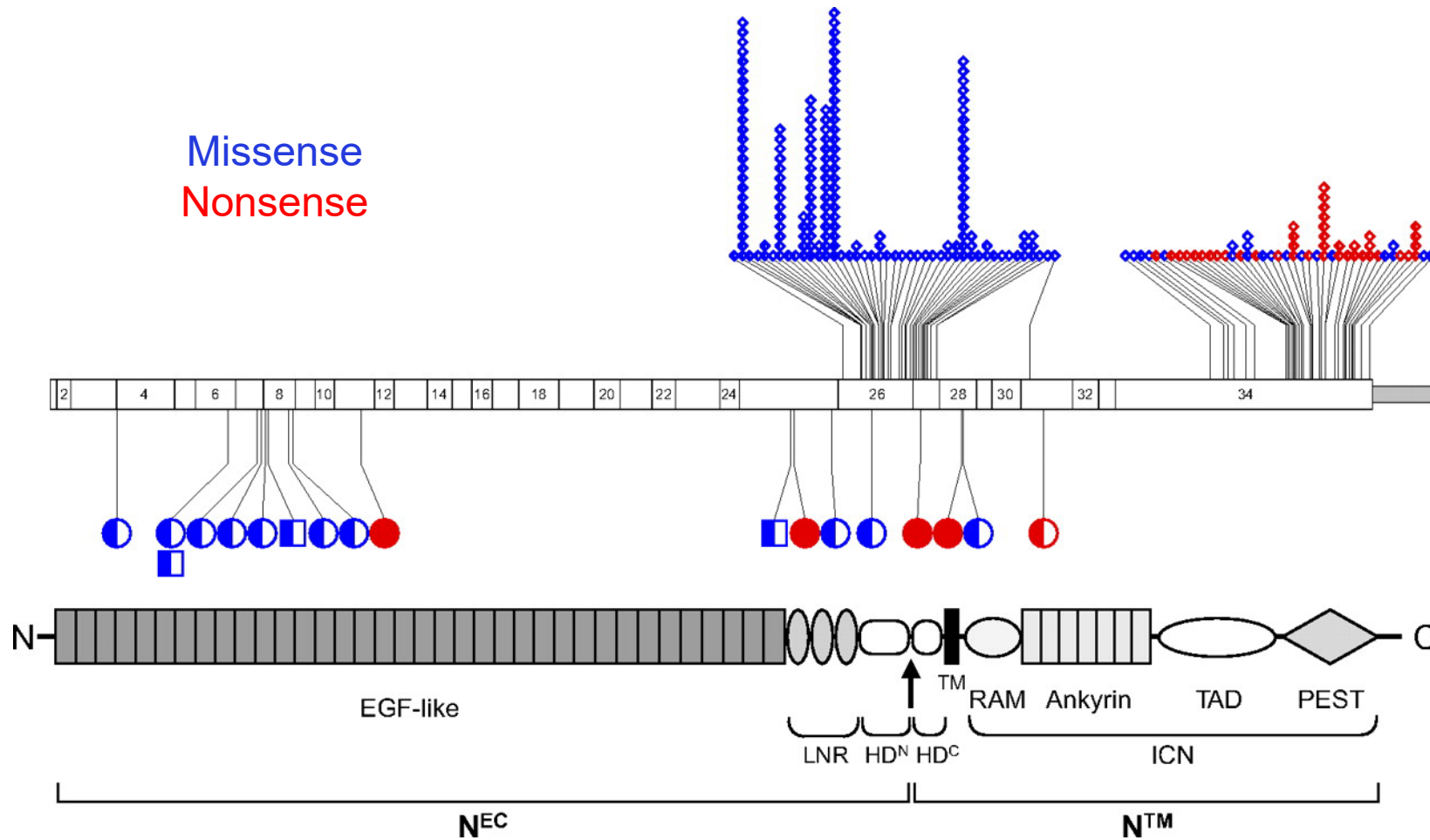
Diagnosis	Wild-type	<i>Notch1</i> <sup>-/-</sup>
Papilloma	1	105
Dysplastic lesion	0	29
BCC	0	7
SCC, grade I-III	0	6
Total	1	147

Results shown are from 148 lesions from mice treated topically with a single initiation dose of 25  $\mu$ g DMBA followed by 4  $\mu$ g TPA twice a week for 15 weeks.

# Notch and Tumor Suppression in squamous cell carcinoma



# Patterns of NOTCH1 mutations in leukemias vs. squamous cell carcinomas



Leukemias  
-activating mutations!

Squamous cell  
Carcinomas  
-inactivating mutations!

# Examples of Notch signaling as a tumor suppressor in human cancer

- Non-melanoma skin cancer
  - *NOTCH1* 60-70%, *NOTCH2/3* less frequently
  - Truncating/inactivating mutations
- Head and neck squamous cell carcinoma
  - Inactivating *NOTCH1* mutations 15-20%
- Tumors with neuroendocrine differentiation (e.g. lung, prostate, neuroblastoma)
  - DLL3 upregulation (natural Notch inhibitor)
  - DLL3 now a promising therapeutic target for immunotherapy
- ? Myeloid leukemias

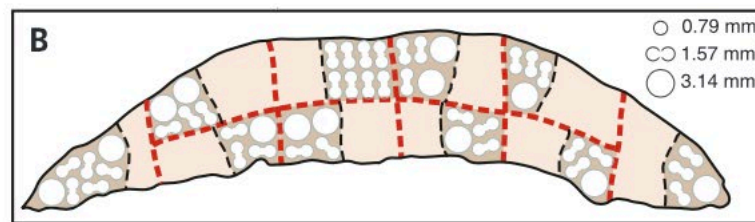
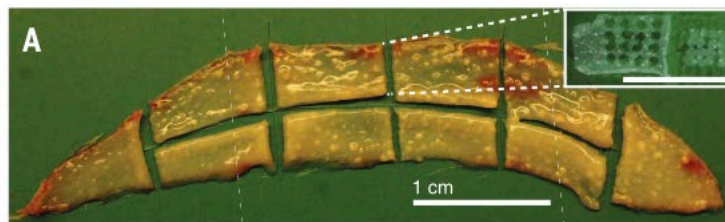
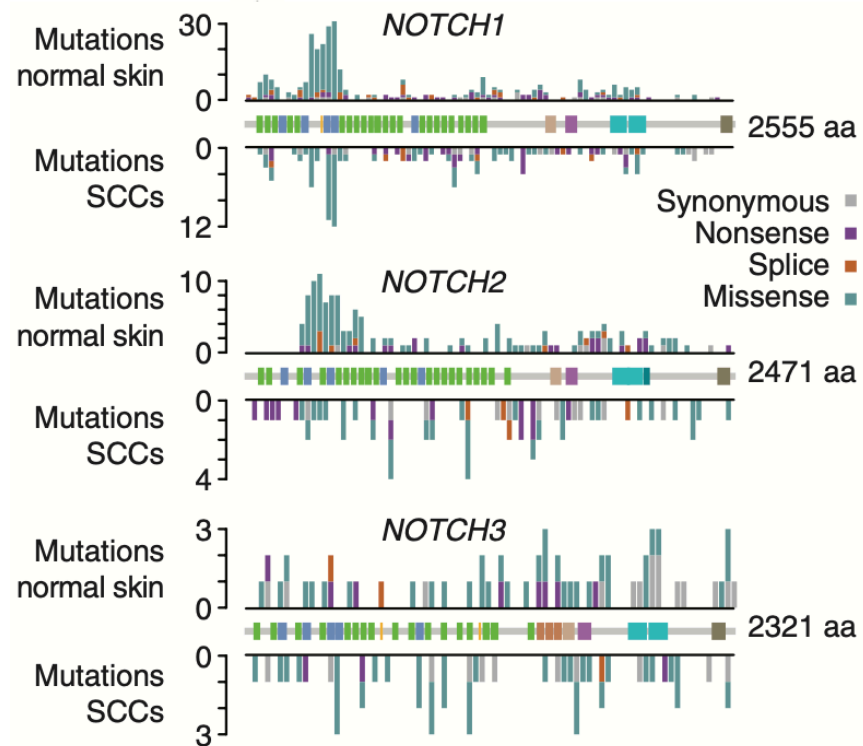
# Frequent *NOTCH1* mutations in sun-exposed normal human skin

## TUMOR EVOLUTION

## High burden and pervasive positive selection of somatic mutations in normal human skin

Iñigo Martincorena,<sup>1</sup> Amit Roshan,<sup>2</sup> Moritz Gerstung,<sup>1</sup> Peter Ellis,<sup>1</sup> Peter Van Loo,<sup>1,3,4</sup> Stuart McLaren,<sup>1</sup> David C. Wedge,<sup>1</sup> Anthony Fullam,<sup>1</sup> Ludmil B. Alexandrov,<sup>1</sup> Jose M. Tubio,<sup>1</sup> Lucy Stebbings,<sup>1</sup> Andrew Menzies,<sup>1</sup> Sara Widaa,<sup>1</sup> Michael R. Stratton,<sup>1</sup> Philip H. Jones,<sup>2\*</sup> Peter J. Campbell<sup>1,5\*</sup>

Science 2015



# Notch as an oncogene and a tumor suppressor in the same tumors?

## Intratumoural heterogeneity generated by Notch signalling promotes small-cell lung cancer

Jing Shan Lim<sup>1,2</sup>, Alvaro Ibaseta<sup>1,2</sup>, Marcus M. Fischer<sup>3</sup>, Belinda Cancilla<sup>3</sup>, Gilbert O'Young<sup>3</sup>, Sandra Cristea<sup>1,2</sup>, Vincent C. Luca<sup>4,5,6</sup>, Dian Yang<sup>1,2</sup>, Nadine S. Jahchan<sup>1,2</sup>, Cécile Hamard<sup>7,8</sup>, Martine Antoine<sup>7,8</sup>, Marie Wislez<sup>7,8</sup>, Christina Kong<sup>9</sup>, Jennifer Cain<sup>3</sup>, Yu-Wang Liu<sup>3</sup>, Ann M. Kapoun<sup>3</sup>, K. Christopher Garcia<sup>4,5,6</sup>, Timothy Hoey<sup>3</sup>, Christopher L. Murriel<sup>3</sup> & Julien Sage<sup>1,2</sup>

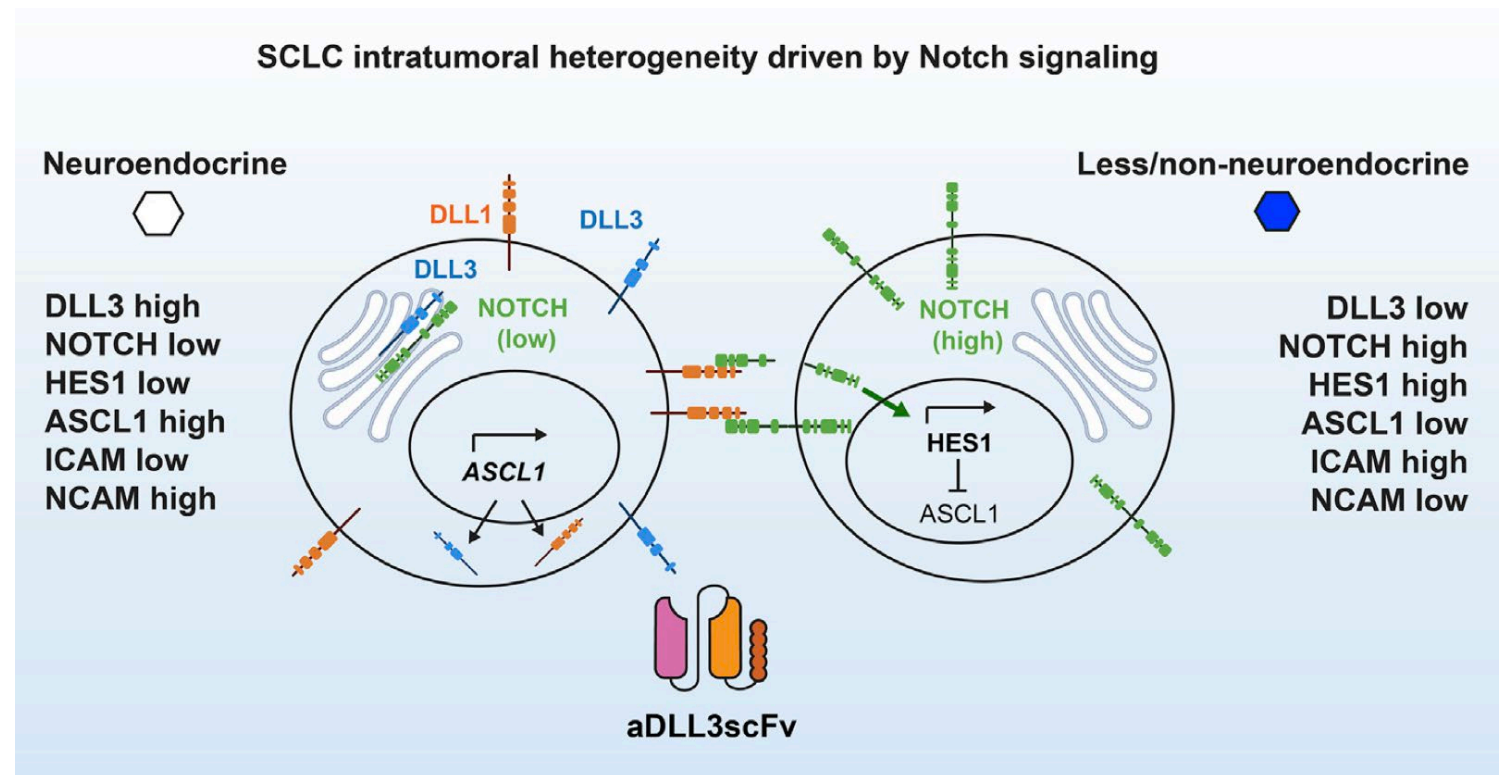
*Nature* 2017

Article

DLL3 regulates Notch signaling in small cell lung cancer

Jun W. Kim,<sup>1,2,3</sup> Julie H. Ko,<sup>1,2,3</sup> and Julien Sage<sup>1,2,4,\*</sup>

*iScience* 2022



# Learning objectives

- Understand the components of the Notch signaling pathway and the biochemical mechanisms of Notch activation
- Appreciate key physiological functions of Notch signaling in development and homeostasis
- Understand the role of Notch signaling in tumor biology, with a focus on both oncogenic and tumor suppressive functions