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# Notch Signaling Pathway

## Important for Cancer & T Cell Research

### The Importance of Notch

The highly conserved Notch signaling pathway regulates many different cell fate decisions in both vertebrate and invertebrate species. It is important for pattern formation during development such as neurogenesis, angiogenesis or myogenesis and regulates T cell development and stem cell maintenance [1]. Notch signaling is also involved in cellular processes throughout adulthood [2]. Signaling via Notch occurs between receptors and its ligands, both at the surface of neighbouring cells (see Figure 1, Notch Receptors and Their Ligands). In mammals, expression of four Notch receptors (Notch1–4) and five canonical ligands [Delta-like ligand (DLL1, 3, 4) and Jagged (Jagged-1, -2) coordinate activation of this signaling pathway [3].

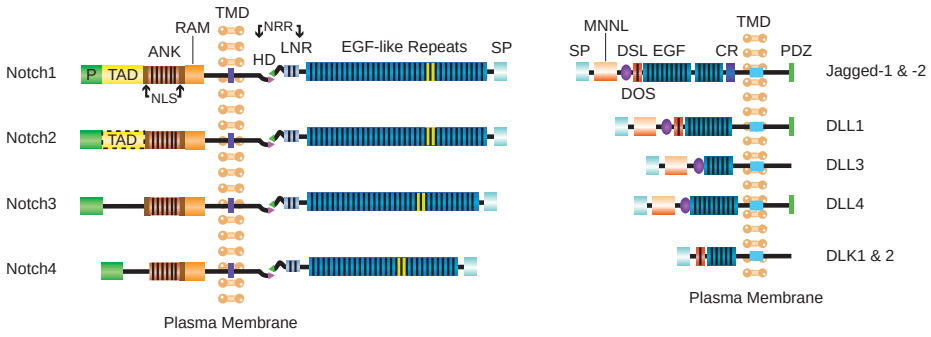



**FIGURE 1: Notch Receptors and their Ligands.**

Mammals possess four Notch receptors (Notch1–4) and five ligands including Jagged-1 and -2 and Delta-like (DLL) 1, 3 and 4. Additional noncanonical Notch ligands are DLK1, DLK2.

**ANK:** Ankyrin Repeats; **CR:** Cysteine-rich Domain: **DOS:** Delta and OSM-11-like Proteins Domain; **DSL:** Delta, Serrate and LAG-2 Domain; **EGF:** Epidermal Growth Factor-like Repeats; **HD:** Heterodimerization Domain; **LNR:** Cysteine-rich Lin12-Notch Repeats; **NRR:** Negative Regulatory Region; **MNNL:** Module at N-terminal Domain of Notch Ligands; **NLS:** Nuclear Localization Signal; **P:** PEST Domain; **PDZ:** PDZ Domain; **PM:** Plasma Membrane; **RAM:** RBPJ-associated Molecule; **SP:** Signal Peptide; **TAD:** Transactivation Domain; **TMD:** Transmembrane Domain

Adapted from: *The intracellular region of Notch ligands: does the tail make the difference?* A. Pintar, et al.; Biol. Direct **2,** 19 (2007), *The canonical Notch signaling pathway: unfolding the activation mechanism:* R. Kopan & M. X. Ilagan; Cell **137,** 216 (2009)

### CONTENTS

# Notch Receptors and Ligands Family

Mammalian Notch receptor homologs (Notch1 to 4) encode a Notch extracellular domain (NECD) that binds ligands, a transmembrane domain, and a Notch intracellular domain (NICD) that translocates to the nucleus to serve as a transcriptional cofactor. Mammalian NECDs consist of 29 to 36 EGF repeats followed by three Lin–Notch repeats (LNRs). EGF11 and 12 domains alone are sufficient for binding to Notch ligands (Jagged/DLL). All canonical Notch ligands are transmembrane proteins that share a largely similar structure, with an extracellular domain comprised primarily of multiple EGF repeats (6 for DLL3; 8 for DLL1 and DLL4; or 16 for Jagged-1 and Jagged-2), followed by "module at the N-terminus of Notch ligands" (MNNL) domain and by a "Delta/Serrate/Lag-2 (DSL) domain [1].

The non-canonical Notch ligands lack the DSL domain, among these are proteins delta homolog 1 and 2 (DLK1and DLK2) [4]. Some proteins including Contactin-3 and -6 and DNER have been postulated to act as Notch ligands, but confirmation of these observations are still needed [5].

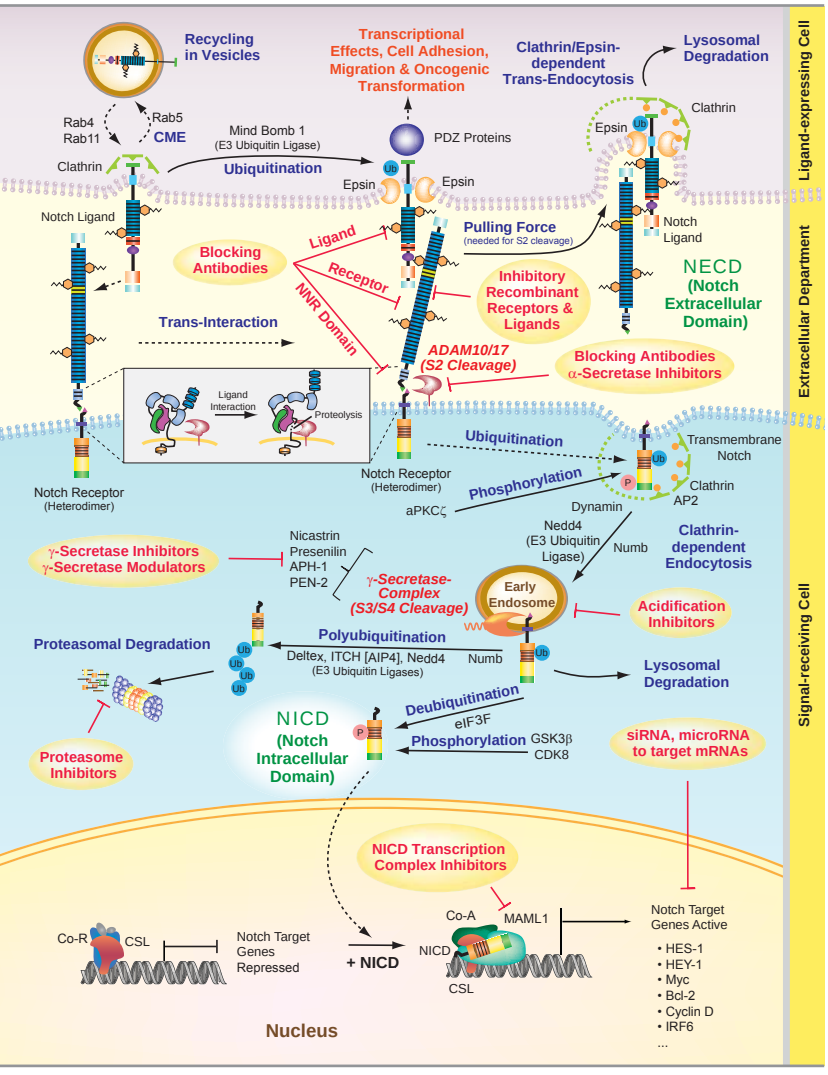



**FIGURE 2: Simplified Notch Signaling Pathway, including potential therapeutic target possibilities.**

**NUCLEUS: Co-A:** Co-Activator Proteins; **Co-R:** Co-Repressor Proteins; **CSL:** CBF1/Su(H)/LAG-1 Complex; **MAML1:** Mastermind-like 1. **SIGNALING: AP2:** Adaptor Protein 2; **E3 Ubiquitin Ligases:** Deltex, ITCH, Nedd4; **eIF3F:** Eukaryotic Translation Initiation Factor 3 Subunit F; **NRR:** Negative Regulatory Region

# Activation of Notch Signaling

The Notch receptors are synthesized as single precursor proteins that are cleaved during transport to the cell surface (at cleavage site S1, not shown in the Figure 2), where they are expressed as heterodimers. Notch signal transduction is initiated upon binding of a Notch receptor heterodimer to a ligand located on a neighbour cell (see Figure 2: Notch Signaling Pathway). Upon receptor-ligand binding, ubiquitination by RING E3 ligases (such as Mind bomb (Mib) or Neuralized), marks the ligands for Epsin-dependent endocytosis. This event generates a mechanical pulling force, which drives conformational changes of the Notch receptor and facilitates its sequential proteolytic cleavages [3]. The cleavage (at S2 site) which is triggered by ligand binding and mediated by a disintegrin and metalloproteinase (ADAM family, also called TACE, tumor necrosis factor-α-converting enzyme) family peptidase, releases the NECD, whereas the cleavage (at S3 /S4 sites) mediated by γ-secretase activity of a multiprotein complex (consisting of presenilin, nicastrin, APH1 and PEN2) releases the NICD. The Notch intracellular domain translocates to the nucleus where it binds with CSL/Rbpj (recombination signal binding protein for immunoglobulin κ j region) and recruits a transcriptional complex to activate the transcription of downstream targets, including Hairy/enhancer-of- split (Hes) and Hes-related with YRPW motif protein (Hey) family genes [6]. Activity of Notch receptors and ligands is profoundly affected by glycosylation of EGF repeats in the extracellular domain. O-fucosyltransferases, which add fucose to serine and threonine residues and O-glucosyltransferases, which add glucose to serine residues, followed by extension of the sugar by Fringe family GlcNAc-transferases are essential for modulating the binding avidity of ligand-receptor pairs. Other post-translational events, including mono- or polyubiquitination by specific E3 ubiquitin ligases and phosphorylation as well as endocytic trafficking, regulate the activities of both the Notch receptors and their ligands.

# Notch and Diseases

The Notch pathway plays an important role in many different processes in a wide range of tissues and deregulations in Notch signaling components have been associated with various human disorders such as cancer, immune disorders, developmental syndromes, stroke and cognitive symptoms. Other disorders affecting vertebral column such as scoliosis or the vasculature, hypertension and the developmental disorder Alagille syndrome are also caused by Notch defects [7].

## Notch and Cancer

Components of the Notch signaling pathway are altered in diseases and cancers (T and B cell lymphoproliferative disorders, liver, breast, brain, bladder, lung and prostate). Notch can act either as an oncogene or tumor suppressor depending on the cellular context. Components of the Notch signaling are not frequently mutated in most tumor types, although mutations appear to accumulate during growth of tumors. However, there are exceptions with loss-of-function mutations in Notch receptors supporting their tumor-suppressive role in multiple malignancies, including bladder cancer and squamous cell carcinoma. Constitutive activation of the Notch receptors through gene rearrangements or gain-of-function mutations leads to Notch receptors' oncogenic function in T cell acute lymphoblastic leukemia, in chronic lymphocytic leukemia and in solid tumors such as lung adenocarcinoma. In breast and prostate cancer, Notch signaling frequently appears to be upregulated, and high levels of Jagged-1 expression correlate with poor prognosis of some tumors showing that the level of Notch signaling is critical in regulation of cell proliferation, survival or death.

Given that Notch signaling is dysregulated in different types of cancer, Notch inhibitors alone or in combination with chemotherapeutics are currently clinically evaluated and become an exciting new approach to fight cancer (see Figure 2).

## Notch and Regulation of Innate and Adaptive Immunity

Notch signaling plays an essential role during development and differentiation of hematopoietic cells [8]. During early stages of T cell development, Notch is required continuously in the thymus while in the bone marrow, it inhibits B cell development. Notch also plays essential roles later during lymphocyte development, in particular during T cell lineage commitment and maturation in the thymus and during marginal zone B (MZB) cell development in the spleen. Notch is also a key factor in dendritic cell (DC) homeostasis. Finally, Notch functions in the development of the newly described Innate Lymphoid Cells (ILCs) playing roles in innate immune responses to infectious microorganisms, in the generation of secondary lymphoid organs and in tissue remodeling after tissue injury or infection (see Figure 3).

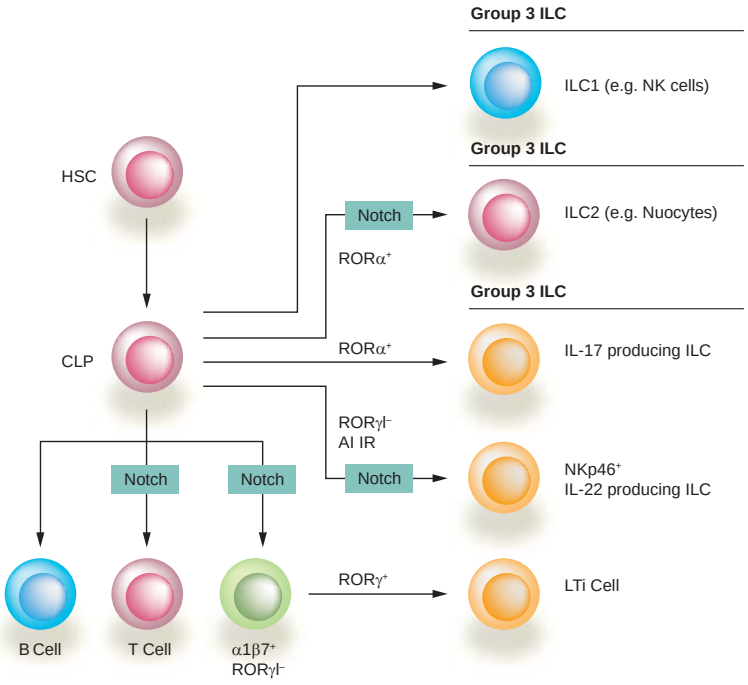


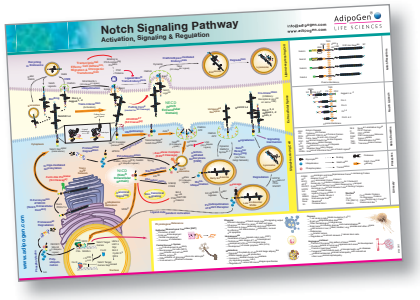

**FIGURE 3: The role of Notch Signaling in the development of innate lymphoid cells.**

Haematopoietic stem cell (HSC)-derived common lymphoid progenitors (CLPs) give rise to adaptive immune cells, such as T cells and B cells, as well as to innate lymphoid cells (ILCs). ILCs function in innate immune responses and are grouped into three major classes: group 1, group 2 and group 3. ILCs diverge in their requirement for Notch (as indicated). **AHR:** aryl hydrocarbon receptor; **IL:** interleukin; **LTi:** lymphoid tissue-inducer; **NK:** natural killer; **ROR:** retinoid-related orphan receptor.

*Adapted from: Regulation of innate and adaptive immunity by Notch: F. Radtke, et al.; Nat. Rev. Immunol. 13, 427 (2013)*


### REFERENCES

**[1]** Notch signaling at a glance: K. Hori, et al.; J. Cell Sci. **126,** 2135 (2016) • **[2]** Hematopoietic stem cells: to be or Notch to be: A. Bigas & L. Espinosa; Blood **119,** 3226 (2012) • **[3]** The Notch signalling system: recent insights into the complexity of a conserved pathway: K.G. Guruharsha, et al.; Nat. Rev. Genet. **9,** 654 (2012) • **[4]** Possible roles of DLK1 in the Notch pathway during development and disease: F.A. Falix, et al.; Biochim. Biophys. Acta **1822,** 988 (2012) • **[5]** Delta/Notch-Like EGF-Related Receptor (DNER) Is Not a Notch Ligand: M. Greene, et al.; PLoS One **11,** e0161157 (2016) • **[6]** Notch signalling in the nucleus: roles of Mastermind-like (MAML) transcriptional coactivators: M. Kitagawa; J. Biochem. **159,** 287 (2016) • **[7]** Therapeutic modulation of Notch signalling-are we there yet? E.R. Andersson & U. Lendahl; Nat. Rev. Drug Discov. **13,** 357 (2014) • **[8]** Regulation of innate and adaptive immunity by Notch: F. Radtke, et al.; Nat. Rev. Immunol. **13,** 427 (2013)


***Ask for our detailed Notch Signaling Wallchart or download it from www.adipogen.com***

# Notch Receptors Notch1 & Notch2

| ANTIBODIES | PID | SIZE | ISOTYPE | APPLICATION | SPECIES |
|---|---|---|---|---|---|
| **anti-Notch1 (mouse), mAb (22E5)** | AG-20B-0051 | 100 µg | Rat IgG2aκ | FACS | Ms |
| **anti-Notch1 (mouse), mAb (22E5) (Biotin)** | AG-20B-0051B | 100 µg | Rat IgG2aκ | FACS | Ms |
| **anti-Notch2, mAb (16F11)** | AG-20B-0052 | 100 µg | Rat IgG1κ | FACS | Ms |
| **anti-Notch2, mAb (16F11) (Biotin)** | AG-20B-0052B | 100 µg | Rat IgG1κ | FACS | Ms |

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|---|---|---|---|---|
| **Notch1 (mouse):Fc (human) (rec.)** | AG-40B-0109 | 50 µg \| 3 x 50 µg | CHO cells | <0.1EU/µg | Ms |
| **Notch2 (mouse):Fc (human) (rec.)** | AG-40B-0110 | 50 µg \| 3 x 50 µg | CHO cells | <0.01EU/µg | Ms |

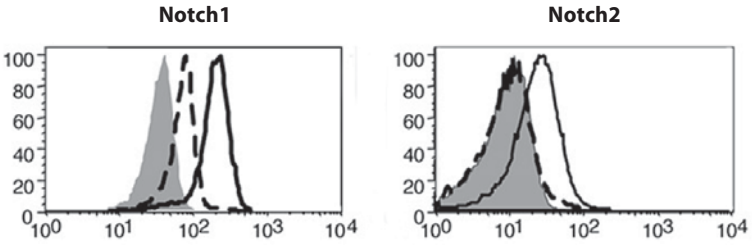



**FIGURE:** Detection of endogenous mouse Notch1 or Notch2 on resting and activated T cells with anti-Notch1 (mouse), mAb (22E5) (Prod. No. AG-20B-0051) and anti-Notch2, mAb (16F11) (Prod. No. AG-20B-0052), respectively.

**METHOD:** $CD4^+$ T cells from C57BL/6 mice were treated with anti-CD3 on plastic (solid line), IL-2 (dotted line) or medium alone as a negative control (shaded histogram) for 24h. The staining was revealed with a secondary anti-mouse IgG-PE (1/200) and then analyzed by flow cytometry.

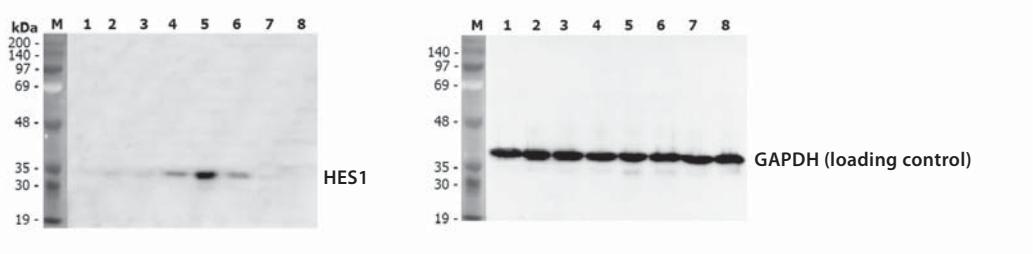
# Canonical Notch Ligands

## Notch Ligand DLL1 (Delta-like Protein 1)

BULK

| ANTIBODIES | PID | SIZE | ISOTYPE/SOURCE | APPLICATION | SPECIES |
|---|---|---|---|---|---|
| **anti-DLL1 (human), mAb (D1L165-6)** | AG-20A-0074 | 50 µg \| 100 µg | Mouse IgG1κ | ELISA, WB | Hu |
| **anti-DLL1 (mouse), mAb (D1L357-1-4)** | AG-20A-0085 | 50 µg \| 100 µg | Rat IgG2κ | ELISA, WB | Ms |
| **anti-DLL1 (mouse), mAb (30B11.1)** | AG-20B-0053 | 100 µg | Rat IgG2aκ | FACS, ICC | Ms |
| **anti-DLL1 (human), pAb** | AG-25A-0062 | 100 µg | Rabbit | ELISA, IHC, WB | Hu |
| **anti-DLL1 (human), pAb** | AG-25A-0079 | 100 µg | Rat | ELISA, WB | Hu |

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|---|---|---|---|---|
| **DLL1 (human) (rec.)** | AG-40A-0073 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Hu |
| **DLL1 (human):Fc (human) (rec.)** | AG-40A-0116Y | 10 µg \| 50 µg | CHO cells | <0.01EU/µg | Hu |
| **DLL1 (mouse):Fc (human) (rec.)** | AG-40A-0148 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Ms |




Lanes:
M: Marker
1: hDLL1-Fc, 0 min
2: hDLL1-Fc, 10 min
3: hDLL1-Fc, 30 min
4: hDLL1-Fc, 1 h
5: hDLL1-Fc, 2 h
6: hDLL1-Fc, 4 h
7: hDLL1-Fc, 8 h
8: hDLL1-Fc, 24 h

**FIGURE:** DLL1 (human):Fc (human) (rec.) (AG-40A-0116Y) induces the Notch target gene HES1 when coated on a plate.

**METHOD:** A mouse preadipocyte cell line, 3T3L1, was stimulated with 1µg/ml of human DLL1:Fc as in indicated time points and each cell lysate was prepared and subjected to Western blot by using an anti-mouse HES1 or anti-mouse GAPDH specific antibody.



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**APPLICATIONS: FACS:** Flow Cytometry; **FUNC:** Functional Application; **ICC:** Immunocytochemistry; **IHC:** Immunohistochemistry **IP:** Immunoprecipitation; **WB:** Western blot
**SPECIES: Bv** = Bovine; **Dg** = Dog; **Dr** = Drosophila; **Hu** = Human; **Mk** = Monkey; **Ms** = Mouse; **Pg** = Pig; **Rt** = Rat; **Rb** = Rabbit; **Prm** = Primate

## Notch Ligands DLL3 & DLL4 (Delta-like Protein 3 & 4)

BULK

| ANTIBODIES | PID | SIZE | ISOTYPE | APPLICATION | SPECIES |
|---|---|---|---|---|---|
| **anti-DLL4 (human), mAb (DL86-3AG)** | AG-20A-0080 | 50 µg \| 100 µg | Mouse IgG1κ | ELISA, WB | Hu |
| **anti-DLL4 (mouse), mAb (9A1.5)** | AG-20B-0054 | 100 µg | Rat IgG1κ | FACS, ICC | Ms |

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|---|---|---|---|---|
| **DLL3 (human) (rec.)** | AG-40B-0151 | 10 µg \| 3 x 10 µg | HEK 293 cells | <0.02EU/µg | Hu |
| **DLL3 (extracellular domain) (mouse):Fc (human) (rec.)** | AG-40A-0178 | 10 µg | HEK 293 cells | <0.1EU/µg | Ms |
| **DLL4 (human):Fc (human) (rec.)** | AG-40A-0077Y | 10 µg \| 50 µg | HEK 293 cells | <0.01EU/µg | Hu |
| **DLL4 (mouse):Fc (human) (rec.)** | AG-40A-0145 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Ms |

Literature Citations in High Ranking Journals using AdipoGen's DLL4 (human):Fc (human) [PID# AG-40A-0077Y]:

1. Jagged2 acts as a Delta-like Notch ligand during early hematopoietic cell fate decisions: I. Van de Walle, et al.; Blood **117,** 4449 (2011)
2. Notch regulates BMP responsiveness and lateral branching in vessel networks via SMAD6: K.P. Mouillesseaux, et al.; Nat. Commun. **7,** 13247 (2016)

## Notch Ligands Jagged-1 & Jagged-2

BULK

| ANTIBODIES | PID | SIZE | ISOTYPE | APPLICATION | SPECIES |
|---|---|---|---|---|---|
| **anti-Jagged-1 (human), mAb (J1G53-3)** | AG-20A-0049 | 100 µg | Mouse IgG1κ | ELISA, FACS, IHC, WB | Hu |
| **anti-Jagged-1 (human), mAb (J1G53-3) (FITC)** | AG-20A-0049F | 50 µg | Mouse IgG1κ | FACS, WB | Hu |
| **anti-Jagged-1 (human), mAb (J1G74-7)** | AG-20A-0050 | 100 µg | Mouse IgG1κ | ELISA, FACS, WB | Hu |

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|---|---|---|---|---|
| **Jagged-1 (human):Fc (human) (rec.)** | AG-40A-0081 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Hu |
| **Jagged-1 (mouse):Fc (human) (rec.)** | AG-40A-0157T | 10 µg \| 50 µg | HEK 293 cells | <0.01EU/µg | Ms |
| **Jagged-2 (human):Fc (human) (rec.)** | AG-40A-0155Y | 10 µg | HEK 293 cells | <0.1EU/µg | Hu |
| **Jagged-2 (mouse):Fc (human) (rec.)** | AG-40A-0183 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Ms |

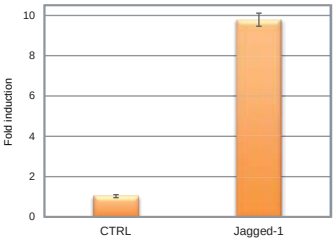


**FIGURE:** Induction of IL-6 expression in human dermal fibroblasts by Jagged-1 (human):Fc (human) (rec.) (Prod. No. AG-40A-0081).

**METHOD:** Jagged-1 (human):Fc was coated on a 12-well plate at 1µg/ml overnight at 4°C. Human dermal fibroblasts were cultured in the presence or absence of Jagged-1 (human):Fc for 72 hours. Real time quantitative PCR was used to quantify the expression of IL-6.

*Picture courtesy of the lab of Prof. Gian-Paolo Dotto, Department of Biochemistry, University of Lausanne*

## LATEST INSIGHT

### New role of Jagged-1 & OX40L in the selective induction of Treg proliferation

P. Kumar, et al. reported that the Notch ligand Jagged-1 and the TNF superfamily ligand OX40L induce selective proliferation of functional regulatory T cells (Tregs) independent of canonical TCR signaling (in the absence of anti-CD3/CD28 activation) when used together as soluble recombinant ligands. This activation of Tregs by Jagged/OX40L works in an IL-2 dependent way without activating effector T cells. This novel "TCR-independent" strategy using Jagged-1, OX40L and IL-2 for the selective expansion of functional Tregs could have therapeutic implications in various autoimmune diseases including T1D.

**LIT:** Soluble OX40L and JAG1 Induce Selective Proliferation of Functional Regulatory T-Cells Independent of canonical TCR signaling: P. Kumar, et al.; Sci. Rep. **7,** 39751 (2017)

# Non-Classical Notch Ligands

## DLK1 & DLK2 (Protein Delta Homolog 1)

| ANTIBODIES | PID | SIZE | ISOTYPE/SOURCE | APPLICATION | SPECIES |
|---|---|---|---|---|---|
| **anti-DLK1 (human), mAb (PF13-3)** | AG-20A-0069 | 50 µg \| 100 µg | Mouse IgG1κ | ELISA, FACS, IHC, WB | Hu |
| **anti-DLK1 (human), mAb (PF299-1)** | AG-20A-0070 | 50 µg \| 100 µg | Mouse IgG1κ | ELISA, FACS, IHC, WB | Hu |
| **anti-DLK1 (mouse), mAb (PF105B)** | AG-20A-0057 | 50 µg \| 100 µg | Rat IgG2aκ | ELISA, WB | Ms |
| **anti-DLK1 (mouse), mAb (PF183E)** | AG-20A-0058Y | 50 µg \| 100 µg | Rat IgG2aκ | ELISA, WB | Ms |
| **anti-DLK1 (human), pAb** | AG-25A-0091 | 100 µg | Rat | ELISA, FACS, WB | Hu |
| **anti-DLK1 (human), pAb** | AG-25A-0092 | 100 µg | Rabbit | ELISA, IHC, WB | Hu |

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|---|---|---|---|---|
| **DLK1 (human) (rec.)** | AG-40A-0133 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Hu |
| **DLK1 (human):Fc (human) (rec.)** | AG-40B-0152 | 10 µg \| 3 x 10 µg | HEK 293 cells | <0.01EU/µg | Hu |
| **DLK1 (mouse):Fc (human) (rec.)** | AG-40A-0107Y | 10 µg \| 3 x 10 µg | HEK 293 cells | <0.1EU/µg | Ms |
| **DLK2 (human):Fc (human) (rec.)** | AG-40A-0158 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Hu |

### DLK1, Soluble (human) ELISA Kit

AG-45A-0032Y 96 wells

**Species reactivity:** Human
**Sensitivity:** 336 pg/ml
**Range:** 0.47 to 30 ng/ml
**Assay type:** Sandwich
**Sample type:** Serum, Cell Culture Supernatant


# Non-Confirmed Notch Ligand

## DNER (Delta and Notch-like Epidermal Growth Factor-related Receptor)

| ANTIBODIES | PID | SIZE | ISOTYPE/SOURCE | APPLICATION | SPECIES |
|---|---|---|---|---|---|
| **anti-DNER (human), mAb (DR324-4)** | AG-20A-0078 | 50 µg \| 100 µg | Mouse IgG2aκ | ELISA, WB | Hu |
| **anti-DNER (human), pAb** | AG-25A-0102 | 100 µg | Rabbit | ELISA, WB | Hu |

| PROTEINS | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|---|---|---|---|---|
| **DNER (extracellular domain) (human) (rec.)** | AG-40A-0137Y | 10 µg \| 3 x 10 µg | HEK 293 cells | <0.1EU/µg | Hu |
| **DNER (extracellular domain) (human):Fc (human) (rec.)** | AG-40A-0119 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Hu |
| **DNER (extracellular domain) (mouse):Fc (human) (rec.)** | AG-40A-0177 | 10 µg \| 50 µg | HEK 293 cells | <0.1EU/µg | Ms |



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**APPLICATIONS: FACS:** Flow Cytometry; **FUNC:** Functional Application; **ICC:** Immunocytochemistry; **IHC:** Immunohistochemistry **IP:** Immunoprecipitation; **WB:** Western blot
**SPECIES: Bv** = Bovine; **Dg** = Dog; **Dr** = Drosophila; **Hu** = Human; **Mk** = Monkey; **Ms** = Mouse; **Pg** = Pig; **Rt** = Rat; **Rb** = Rabbit; **Prm** = Primate

# Notch Target HES1 (Hairy and Enhancer of Split 1)

Hes genes, encoding basic helix–loop–helix (HLH) transcriptional repressors, are seven members in human, expressed in many tissues and playing various roles mainly in development. Hes1, Hes5, and Hes7 are downstream effectors of canonical Notch signaling. Hes1 plays a crucial role in the control and regulation of cell cycle, proliferation, cell differentiation, survival and apoptosis in neuronal, endocrine and T-lymphocyte progenitors as well as various cancers and is a key target gene of the Notch signaling pathway.

| ANTIBODY | PID | SIZE | ISOTYPE | APPLICATION | SPECIES |
|---|---|---|---|---|---|
| **anti-HES1, mAb (7H11)** | AG-20T-0400 | 100 µg | Mouse IgG2bκ | ELISA, FACS, IP, WB | Hu, Ms, Rt |

| PROTEIN | PID | SIZE | SOURCE | ENDOTOXIN | SPECIES |
|---|---|---|---|---|---|
| **HES1 (human) (rec.) (His)** | AG-40A-0180 | 10 µg \| 50 µg | E. coli | n.a. | Hu |

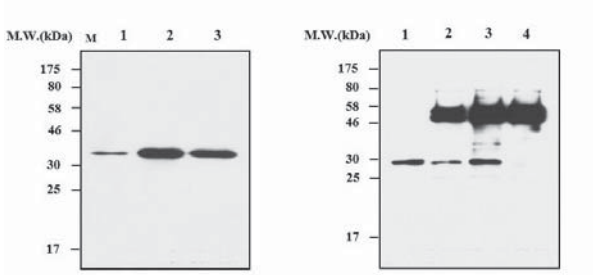



**FIGURE (LEFT):** Western blot analysis on cell lysates HeLA cells (lane1), HT-29 cell (lane2) and BeWo cell (lane 3) using anti-HES1, mAb (7H11) at 1µg/ml.

**FIGURE (RIGHT):** Immunoprecipitation analysis on BeWo cell lysate using anti-HES1, mAb (7H11).

Lane 1: BeWo cell lysate
Lane 2: Precipitation from BeWo cell lysate (400µg) at 2µg
Lane 3: Precipitation from BeWo cell lysate (400µg) at 5µg
Lane 4: Precipitation from PBS at 5µg

# E3 Ubiquitin Ligases (E3UBLs) & Deubiquitinating Enzymes (DUBs)

In contrast to other signaling pathways, where a cascade of second messengers is at stake, the activated Notch receptor is itself transformed into a transcriptional activator, NICD. As a consequence, affecting NICD production and quantity directly affects Notch-dependent response. Therefore, regulating this pathway means controlling spatiotemporal production and maintenance of active receptors and ligands at the cell surface, efficiency of signal transduction and stability of NICD. These key steps all involve ubiquitination events. Dysregulations of these events might be involved in various pathological processes in which the Notch signaling is disrupted. Recently, USP12 has been shown to be important for Notch degradation. The deubiquitinating complex USP12/UAF1 is recruited by Itch to non-activated Notch and regulates Notch trafficking toward lysosomal degradation.

**LIT:** Ubiquitinations in the Notch signaling pathway: J. Moretti & C. Brou; Int. J. Mol. Sci. **14,** 6359 (2013)

| KIT | PID | SIZE | SOURCE | SPECIES | TYPE |
|---|---|---|---|---|---|
| **ITCH (human) Ubiquitination Kit** | AG-44T-0117 | Kit | | Hu | E3UBLs |

| PROTEINS | PID | SIZE | SOURCE | SPECIES | TYPE |
|---|---|---|---|---|---|
| **ITCH Isoform 2 (human) (rec.)** | AG-40T-0284 | 100 µg | E. coli | Hu | E3UBLs |
| **MDM2 (human) (rec.)** | AG-40T-0289 | 50 µg | E. coli | Hu | E3UBLs |
| **MDM2 (human) (rec.) (GST)** | AG-40T-0290 | 50 µg | E. coli | Hu | E3UBLs |
| **UAF1 (human) (rec.) (His)** | AG-40T-0406 | 50 µg | Sf21 cells | Hu | DUBs |
| **USP1/UAF1 Complex (human) (rec.) (His)** | AG-40T-0536 | 50 µg | Sf21 cells | Hu | DUBs |
| **USP9x Isoform 2 (human) (rec.) (His)** | AG-40T-0545 | 25 µg | Sf21 cells | Hu | DUBs |
| **USP12 (human) (rec.) (His)** | AG-40T-0570 | 50 µg | Sf21 cells | Hu | DUBs |
| **USP12/UAF1 Complex (human) (rec.) (His)** | AG-40T-0571 | 1 vial | Sf21 cells | Hu | DUBs |
| **USP28 (human) (rec.) (His)** | AG-40T-0541 | 100 µg | Sf21 cells | Hu | DUBs |

# ADAM17 – Important Sheddase in the Notch Pathway

ADAM17 (Disintegrin and metalloproteinase domain-containing protein 17), also called TACE (Tumor Necrosis Factor-α-Converting Enzyme) is the prototype of the ADAM family of ectodomain shedding proteases (sheddase). ADAM17 is responsible for the processing of a diverse variety of membrane-anchored cytokines, cell adhesion molecules, receptors, ligands and enzymes, including processing of tumor necrosis factor α at the surface of the cell and extracellular Notch Receptor 1. As the proteolytic cleavage is an indispensable activation event for many of these substrates, ADAM17 has emerged as an attractive therapeutic target for the treatment of inflammatory diseases (e.g. rheumatoid arthritis) or inflammation associated cancer.

NEW

### anti-ADAM17 (human), mAb (rec.) (blocking) (D1(A12)) (preservative-free)

AG-27B-6000PF 100 µg

### anti-ADAM17 (human), mAb (rec.) (blocking) (D1(A12)) (Fab Fragment) (His) (preservative-free)

AG-27B-6003PF 100 µg

Recognizes the catalytic and non-catalytic domain of human ADAM17 (TACE) through its variable light (VL) domain and variable heavy (VH) domain, respectively. Does not bind recombinant mouse ADAM17 ectodomain.

**Functional Application (Blocking):** Inhibits ADAM17 activity at 15µg/ml (200nM).

**LIT:** Cross-domain inhibition of TACE ectodomain: C.J. Tape, et al.; PNAS **108,** 5578 (2011)

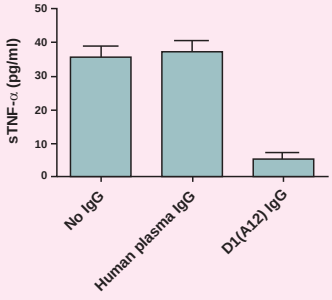



**FIGURE:** D1(A12) IgG inhibits constitutive shedding of TNF-α from IGROV1 (human ovarian cancer cell line) into culture medium. Medium was collected after 48 hours of incubation with or without IgGs at 200nM.

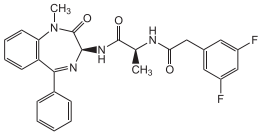
# Notch Processing / γ-Secretase Inhibitors

### Compound E

AG-CR1-0081 250 µg | 1 mg | 5 mg

**Formula:** $C_{27}H_{24}F_2N_4O_3$
**MW:** 490.5
**CAS:** 209986-17-4

Non-competitive γ-secretase inhibitor. Notch processing inhibitor.

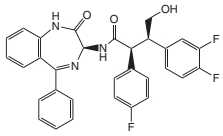


### Compound 34

AG-CR1-0007 200 µg | 1 mg

**Formula:** $C_{31}H_{24}F_3N_3O_3$
**MW:** 543.5
**CAS:** 564462-36-8

Cell permeable, highly potent inhibitor of γ-secretase ($IC_{50}$= 0.06nM).

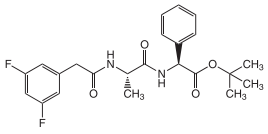


### DAPT

AG-CR1-0016 5 mg | 25 mg

**Formula:** $C_{23}H_{26}F_2N_2O_4$
**MW:** 432.5
**CAS:** 208255-80-5

Cell permeable γ-secretase inhibitor. Notch processing inhibitor.



NEW

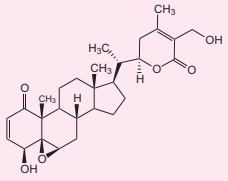
### Withaferin A

AG-CN2-0490 1 mg | 5 mg | 10 mg

**Formula:** $C_{28}H_{38}O_6$
**MW:** 470.6
**CAS:** 5119-48-2

Notch receptors modulator.

**LIT:** J. Lee, et al.; Breast Cancer Res. Treat. **136,** 45 (2012)



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**EUROPE/REST OF WORLD**
**AdipoGen Life Sciences**
TEL +41-61-926-60-40
FAX +41-61-926-60-49
info@adipogen.com

**NORTH & SOUTH AMERICA**
**Adipogen Corp.**
TEL +1-858-457-8383
FAX +1-858-457-8484
info-us@adipogen.com

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