Also provided are antibodies that bind to Notch1 having a light chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 97. Further provided are antibodies that bind to Notch1 having a light chain variable region amino acid sequence as set forth in SEQ ID NO: 97.

Also provided are antibodies that bind to Notch1 having a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 111. Further provided are antibodies that bind to Notch1 having a heavy chain amino acid sequence as set forth in SEQ ID NO: 111.

Also provided are antibodies having a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 113. Further provided are antibodies having a light chain amino acid sequence as set forth in SEQ ID NO: 113.

In a further embodiment, the invention provides for antibodies that bind to Notch1, having a heavy chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 71; and a light chain variable amino acid sequence that is at least 90% identical to SEQ ID NO: 97. Further provided are antibodies that bind to Notch1 having a heavy chain variable region amino acid sequence as forth in SEQ ID NO: 71; and a light chain variable region amino acid sequence as set forth in SEQ ID NO: 97.

In a further embodiment, the invention provides for antibodies that bind to Notch1, having a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 111; and a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 113. Further provided are antibodies that bind to Notch1 having a heavy chain amino acid sequence as set forth in SEQ ID NO: 111, and a light chain amino acid sequence as set forth in SEQ ID NO: 113.

In a further embodiment, the invention provides for antibodies, that bind to human Notch1, wherein the antibodies bind an epitope having at least 8 amino acid residues selected from Asn 1461, Lys 1462, Val 1463, Cys 1464, Leu 1466, Leu 1580, Tyr 1621, Gly 1622, Met 1670, Asp 1671, Val 1672, Arg 1673, Leu 1707, Ala 1708, Leu 1710, Leu 1711, Leu 1712, Leu 1713, Leu 1716 and Leu 1718.

In another embodiment, the present invention provides for antibodies that bind to Notch1, having a heavy chain variable region having a CDR1 region, a CDR2 region, and a CDR3 region from the heavy chain variable region comprising SEQ ID NO: 115.

products were cloned into TOPO-Blunt cloning vector (Invitrogen) and sequenced by conventional methods.

Variable (V) region cDNAs from parental rat 438 and parental rat 351 (hereinafter "rat 438" and "rat 351", respectively) were subcloned into mammalian expression vectors wherein rat Variable Heavy chain (VH) were fused in frame with murine IgG1 (mIgG1), and rat Variable Light chain (VL) were fused with murine kappa. Similarly, for the generation of chimeric antibodies with rat V region and human IgG constant region, rat VH was fused in frame with human IgG1 (hIgG1), and VL with human kappa, respectively. Corresponding chimeric antibodies were generated from these constructs by transient transfection in COS cells and their binding and neutralizing activities were confirmed in assays.

Purified rat variable-mouse constant chimeric antibodies (hereinafter "rat 438-mIgG1" and "rat 351-mIG1") were further characterized in a series of *in vitro* and *in vivo* assays, including recombinant antigen and cell surface target binding, inhibition of Notch1 activity in the RGA and angiogenesis assays, and tumor growth inhibition in mouse models, as described in the Examples below. The lead antibodies, rat 438 and rat 351 were selected based on these studies. **Table 2** lists the amino acid and nucleic acid sequences of various regions of rat 438 and rat 351's variable regions and additional clones 90, 132, 132 (A12/G11) and 137.

Table 2: Rat variable region sequences

SEQ ID NO:		
13	438 Heavy Chain Variable Region amino acid sequence	AVQLVESGGGLVQPGRSLKLSCTASGFTFS SFAMAWVRQAPTKGLEWVASISYGGADTY YRDSVKGRFTISRDNAKSSLYLQMDSLRSE DTSTYYCAKDLPYYGYTPFVMDAWGQGTS VTVSS
14	438 Heavy Chain Variable Region nucleotide sequence	GCGGTACAGTTGGTGGAGTCTGGGGGAG GCTTAGTGCAGCCTGGAAGGTCCTTGAAA CTCTCCTGTACAGCCTCTGGATTCACTTT CAGTAGCTTTGCAATGGCCTGGGTCCGC CAGGCTCCAACGAAGGGGCTGGAGTGGG TCGCATCCATTAGTTATGGTGGTGCTGAC ACTTACTATCGAGACTCCGTGAAGGGCC GATTCACTATCTCCAGAGATAATGCAAAA AGCAGCCTATATTTGCAAATGGACAGTCT GAGGTCTGAGGACACGTCCACTTATTACT GTGCAAAAGACCTTCCATACTACGGATAT ACCCCCTTTGTTATGGATGCCTGGGGTCA GGGAACTTCAGTCACTGTCTCCTCA

back mutations in the human acceptor framework to rat 438 and rat 351 residues at the corresponding positions to optimize the activity and binding epitope.

Table 5: Back mutations in VH and VL of humanized 438 and humanized 351 variants.

438 VH Variant	1.0	1.1							
VH back mutation	none	R94K							
438 VL Variant	1.0	1.1	1.3	1.4	1.5	1.6	1.7	1.8	1.9
VL back mutation	none	M4L L46V Y49K	M4L	L46V	Y49K	M4L L46V	M4L Y49K	L46V Y49K	Q3M M4L L46V Y49K
351 VH Variant	1.0								
VH back mutation	none								
351 VL Variant	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	
VL back mutation	none	L46V Y49K	M4L	L46I	Y49K	M4L L46I	M4L Y49K	M4L L46V Y49K	

Example 3

Characterization of Anti-Notch1 Inhibitory Antibodies

A. Expression and Binding to Notch1

Relative expression yields of humanized 438 and humanized 351 variants were tested in a transient expression assay in COS cells. As shown in **Table 6,** a number of humanized 438 variants, including humanized 438 VH1.1/VL1.8, and a number of humanized 351 variants, including humanized 351 VH1.0/VL1.1, demonstrated significant yields of more than 30μg/ml.

Table 6. Relative expression yields in conditioned media of humanized 438 variants and humanized 351 variants in transient expression in COS cells.

438 Variant	VH1.0 /VL1.3	VH1.0/ VL1.4	VH1.0/ VL1.5	VH1.0/ VL1.6	VH1.0/ VL1.7	VH1.0/ VL1.8	VH1.0/ VL1.9	VH1.0/ VL1.10
438 Yield (μg/ml)	41.51	41.63	85.73	35.11	36.94	40.08	51.9	62.58

438 Variant	VH1.0/1.11	VH1.1/VL1.3	VH1.1/VL1.4	VH1.1/VL1.5	VH1.1/VL1.6	VH1.1/VL1.7	VH1.1/VL1.8	VH1.1/VL1.9
438 Yield (µg/ml)	45.86	23.2	49.47	65.93	47.72	31.95	66.65	14.12
438 Variant	VH1.1/VL1.10	VH1.1/VL1.11						
438 Yield (µg/ml)	36.7	37.54						
351 Variant	VH1.0/VL1.0	VH1.0/VL1.1	VH1.0/VL1.2	VH1.0/VL1.3	VH1.0/VL1.4	VH1.0/VL1.5	VH1.0/VL1.6	VH1.0/VL1.7
351 Yield (µg/ml)	33.5	26.66	24.52	25.1	35.20	29.25	28.93	33.54

Total expression levels of IgGs in conditioned media were measured by quantitative IgG ELISA, as described in Example 2. **Table 7** shows EC50 (nM) values calculated from cell surface Notch1 binding ELISAs for humanized 438 variants and rat 438, along with humanized 351 variants and rat 351.

The data demonstrates that multiple variants of humanized 438, including humanized 438 VH1.1/VL1.8, are similar to rat 438 in binding to full-length human Notch1 expressed on the cell surface of U-2 OS cells. Furthermore, **Table 7** shows that both humanized 438 VH1.1/VL1.8 and VH1.1/VL1.3 fully retained rat 438's cross-reactivity to mouse Notch1 expressed on the cell surface of U-2 OS cells.

The data further demonstrates that humanized 351 VH1.0/VL1.1 and VH1.1/VL1.4, are similar to rat 351 in binding to full-length human Notch1 expressed on the cell surface of U-2 OS cells. **Table 7** further shows that humanized 351 VH1.0/VL1.1 and VH1.1/VL1.4 fully retained rat 351's cross-reactivity to mouse Notch1 expressed on the cell surface of U-2 OS cells.

Table 7. EC50 (nM) values of cell surface Notch1 binding ELISAs for humanized 438 variants and rat 438, along with humanized 351 variants and rat 351.

EC50 (nM)								
Antibody	Rat 438	VH1.0/VL1.1	VH1.1/VL1.0	VH1.1/VL1.1	VH1.1/VL1.3	VH1.1/VL1.5	VH1.1/VL1.8	VH1.1/VL1.9
Human Notch1	0.2132	0.305	0.2956	0.1773	0.1516	0.2189	0.2025	0.1776
Mouse Notch1	0.1725	0.2287	0.1437	0.1291	0.09337	0.1457	0.1489	0.1374

EC50 (nM)			
Antibody	Rat 351	VH1.0/VL1.1	VH1.0/VL1.4
Human Notch1	0.10	0.15	0.10
Mouse Notch1	0.07	0.08	0.07

closest homologues of the Notch1 NRR region are Notch2 and Notch3 (~50% homology), and Notch4 is a more distant homologue (34% homology). Crossreactivity of anti-Notch1 antibodies to other members of the Notch family, especially Notch2, may lead to undesired effects in patients. Therefore, the potential crossreactivity of rat 438 and humanized 438, along with rat 351 and humanized 351 antibodies to other Notch family members were assessed.

Expression constructs encoding human Notch2 and Notch3 NRR regions, fused with human IgG1 Fc fragment were stably introduced into CHO-PACE cells. Conditioned media from these cells expressing NRR-Fc fusions were collected. Human Notch2 NRR-Fc and human and mouse Notch3 NRR-Fc were purified by protein A affinity followed by size exclusion chromatography (SEC). Purified preparations were dialysed into TBS with 1mM $CaCl_2$ and analyzed on analytical SEC to be >99% in purity.

As shown in **Table 10**, rat 438 lacked detectable binding to human Notch2 NRR-Fc fusion protein. Further shown in **Table 10**, humanized 438 variants lacked detectable binding to full-length human Notch3 expressed on U-2 OS cell surface, demonstrating that 438 did not cross-react with Notch3.

Table 10. Binding of rat-mIgG1 and humanized 438 variants to Notch2 NRR-Fc and Notch3 U-2 OS cells (N/B represents non-binding).

Binding to Notch2 NRR-Fc		**Binding to Notch3 U2-OS cells**	
Rat 438-mIgG1	N/B	Rat438-mIgG	N/B
Humanized 438 VH1.0/V1.1	N/B	A2	N/B
Humanized 438VH1.1/VL1.0	N/B		
Humanized 438VH1.1/VL1.3	N/B		
Humanized 438VH1.1/VL1.5	N/B		
Humanized 438VH1.1/VL1.8	N/B		
Humanized 438VHH1.1/VL1.9	N/B		
A2	N/B		

As shown in **Table 11**, humanized 351 VH1.0/VL1.1 lacked detectable binding to human Notch2 NRR-Fc fusion protein, and to both human and mouse Notch3 NRR-Fc fusion proteins. However, humanized 351 VH1.0/VL1.1 cross-reacted with human, mouse and cyno-Notch1 NRR.

The data shows that rat 438 and A2 bind overlapping but distinct surfaces within the Notch1 NRR. Both epitopes include the central HD domain. Rat 438 and A2 interact with LNR-A, however rat 438 interacts with a larger surface. Only rat 438 interacts with the S1 loop region and only A2 interacts with LNR-B. More specifically, the data shows that rat 438 binds to human Notch1 NRR residues Asn 1461, Lys 1462, Val 1463, Cys 1464, Leu 1466, Leu 1580, Tyr 1621, Gly 1622, Met 1670, Asp 1671, Val 1672, Arg 1673, Leu 1707, Ala 1708, Leu 1710, Leu 1711, Leu 1712, Leu 1713, Leu 1716 and Leu 1718.

The data also shows that rat 351 and A2 bind overlapping but distinct surfaces within the Notch1 NRR. In particular, only rat 351 interacts with the S1 loop region and only A2 interacts with LNR-B. Rat 351 and A2 both interact with LNR-A, however rat 351 interacts with a distinct subset of LNR-A amino acids. More specifically, the data shows that rat 351 binds to the human Notch1 NRR residues Asp 1458, Asn 1461, Val 1463, Cys 1464, Leu 1466, Leu 1580, Met 1581, Pro 1582, Tyr 1621, Gly 1622, Arg 1623, Asp 1671, Val 1672, Arg 1673, Gly 1674, Leu 1710, Gly 1711, Ser 1712, Leu 1713, Asn 1714, Ile 1715, Pro 1716, Lys 1718.

The x-ray crystal structure of rat 438 and rat 351 residues binding to Notch1 NRR were further analyzed using the program PISA. The data shows that rat 438 formed strong electrostatic interactions (salt bridges) with the residues Lys1718 and Arg1673. Notch1 NRR residues that formed hydrogen bonds with the rat 438 antibody were Asn1461, Asp1671, Arg1673, Leu1713, Lys1718, Cys1464, Ala1708, and Ser1712. The Notch1 NRR residues that contribute more than 40 Angstrom^2 of buried surface area upon formation of the complex with rat 438 antibody were Arg1673, Val1463, Lys1462, Gly1622, Asp1671 from the interaction with the heavy chain, and Leu1466, Lys1718, Gly1711, Cys1464, Pro1716, and Val1463 from the interaction with the light chain. Of the residues identified, rat 438 binds to human Notch1 NRR at least at residues Asn1461, Val1463, Lys1462, Asp1671, Arg1673, Leu1713, and Lys1718.

The data shows that rat 351 forms strong electrostatic interactions (salt bridges) with residues Asp1458 and Arg1673. Notch1 NRR residues that formed hydrogen bonds with the rat 351 antibody were Asp1458, Val1463, Cys1464, Ser1465, Tyr1621, Asp1671, Val1672, Arg1673, Gly1711, Ser1712, Leu1713, and Asn1714. The Notch1 NRR residues that contribute more than 40 Angstrom^2 of buried surface area upon formation of the complex with rat 351 were Val1463,

cells with a leucine to proline mutation at amino acid 1594 (L1594P), MOLT-4 cells with a leucine to proline mutation at amino acid 1601 (L1601P) and DND-41 cells with compound class 1 mutations of leucine to proline at amino acid position 1594 and aspartic acid to valine at amino acid position 1610 (L1594P/ D1610V). The CCRF-CEM cell line harbors a class 2 mutation and possesses a 12 amino acid insertion at position 1595. The Jurkat cell line harbors a class 3 JEM mutation that inserts 17 amino acids at position 1740.

Notch1 activation and Notch1^{ICD} release was examined by Western blot analysis of protein extracts generated from the T-ALL cell lines treated with increasing concentrations of rat 438-mIgG1, humanized 438 VH1.1/VH1.8, rat 351-mIgG1 or A2 antibodies, and control anti-E.tenella antibody. Released Notch1^{ICD} molecules initiating at a Valine residue are specifically detected with D3B8 antibody (anti-Notch1^{ICD}).

The T-ALL cell lines HPB-ALL, ALL-SIL, CCRF-CEM, MOLT-4, DND-41 and Jurkat cells were used instead of CCD1076SK fibroblasts as described above. Since T-ALL cells possess constitutively released Notch1^{ICD}, no exogenous DLL4 ligand was required. Suspension cultures of 2x10E6 T-ALL cells were mixed with 0, 0.001, 0.01, 0.1, 1 and 10 µg/ml of rat 438-mIgG1, humanized 438 VH1.1/VH1.8, rat 351-mIgG1 or A2 antibodies, and control anti-E.tenella antibody in RPMI 1640 medium containing 10% FBS, Pen/Strep/Glutamine. Cells were incubated with the antibodies for 24 hours at 37°C in 5% CO_2. Cells were harvested by centrifugation and medium was removed by aspiration. Cell pellets were lysed in 1% NP40, 0.5% sodium deoxycholate, 5 mM EDTA, 0.25 M NaCl, 0.025 M Tris-HCl, pH 7.5, containing Complete Mini Protease inhibitor cocktail (Roche). Extracts were resolved by SDS-PAGE on a 7.5% polyacrylamide gel and transferred to nitrocellulose paper using an iBlot Gel transfer system (Invitrogen). The released Notch1^{ICD} molecules initiating at a Valine residue are detected with anti-Notch1^{ICD} and, as a loading control, anti-beta-actin using standard western blot procedures. Densitometric analysis was performed on films that were scanned with a BioRad GS-800 Calibrated Densitometer and analyzed with Quantity One version 4.6.9 software (BioRad). Notch1^{ICD} levels were normalized to beta-actin in each sample and then compared to the untreated control.

As shown in **FIG. 19**, treatment of HBP-ALL cells with increasing concentrations of rat 438-mIgG1 and humanized 438 VH1.1/VL1.8 significantly

Table 28. Average mouse body weights (minus tumor weights) in 87393A1 NSCLC PDXs after treatment with rat 351-mIgG1.

	Average Mouse Body Weights in grams (minus tumor weights)				
Antibody	Day 0	Day 7	Day 14	Day 20	Day 26
Control Anti-E.tenella	23.9	23.1	23.4	23.3	22.9
Rat 351-mIgG1	23.4	23.0	23.1	23.3	23.5

C. *In vivo* growth inhibition studies for HPB-ALL Xenografts

Similar *in vivo* experiments were performed with the mutant Notch1 HPB-ALL cell line as performed with the 37622A1 NSCLC PDX and 87393A1 NSCLC PDX described above. To generate xenografts, nude female mice (Nu/Nu) were implanted subcutaneously with 8x10E6 HPB-ALL cells in 50% Matrigel (BD Biosciences). When the tumors reached a volume of 200 to 400 mm^3, the tumors were staged to ensure uniformity of the tumor mass among various treatment groups prior to the administration of anti-Notch1 and control D16A antibodies. The HPB-ALL model was dosed s.c. at 20 mg/kg of rat 438-mIgG1, 351-mIgG1, A2 or D16A antibody, 2 times a week for 2 weeks. Tumors were measured at least once a week and their volume was calculated with the formula: tumor volume (mm^3) = 0.5 x (tumor width^2)(tumor length). From 8-11 animals, mean tumor masses (± SEM) for each treatment group were calculated and compared to the control-treated.

Table 29 shows the efficacy of rat 438-mIgG1, rat 351-mIgG1 and A2 antibodies in HPB-ALL xenografts with L1575P mutation in Notch1 NRR. The data demonstrates that treatment with rat 438-mIgG1 and treatment rat 351-mIgG1 both inhibited *in vivo* growth of HPB-ALL cells compared to control D16A antibody, thus slowing tumor growth.

Table 29. Efficacy of rat 351-mIgG1, rat 438-mIgG1 and A2 antibodies in HPB-ALL xenografts with L1575P mutation in Notch1 NRR.

	Control D16A					
	Day 0	Day 1	Day 4	Day 8	Day 11	Day 14
Tumor Volume (mm^3)	175.3	201.9	261.2	522.0	735.9	1003.4
S.E.M.	6.4	4.4	11.4	44.1	82.3	162.2
	Rat 351-mIgG1					
	Day 0	Day 1	Day 4	Day 8	Day 11	Day 14
Tumor Volume (mm^3)	166.1	198.6	244.1	267.0	231.0	251.0

Table 30. Efficacy of rat 438-mIgG1 and A2 in Calu-6 lung cancer model.

	Day 0	Day 6	Day 9	Day 13	Day 16	Day 19
Anti-E.tenella (10 mg/kg)	258±17	491±34	740±64	1156±140	1260±171	1613±222
438 (3 mg/kg)	250±29	362±58	465±88	742±127	888±171	1279±219
438 (10 mg/kg)	263±26	383±40	448±51	566±68	667±87	904±129
438 (30 mg/kg)	242±27	343±58	445±82	566±106	601±120	720±152
A2 (3 mg/kg)	238±29	349±42	450±58	603±74	664±91	851±113
A2 (10 mg/kg)	250±34	297±35	348±47	444±64	480±70	603±101
A2 (30 mg/kg)	241±26	376±52	486±68	598±115	622±121	779±168

To determine if inhibition of Notch1 signaling alone effects body weight, mice were weighed during *in vivo* efficacy studies. **Table 31** shows that the average mouse body weights (minus tumor weights) of rat 438-mIgG1 and A2, and control anti-E.tenella treated groups over the course of the study, were not significantly different

Table 31. Average mouse body weights (minus tumor weights) of rat 438-mIgG1 and A2, and control anti-E.tenella treatments in Calu-6 model (n=10-11).

	Day 6	Day 9	Day 13	Day 16	Day 19
E.tenella (10 mg/kg)	24.6±1.56	25.2±1.42	24.3±1.64	24.7±1.74	24.0±1.77
Rat 438-mIgG1 (3 mg/kg)	24.5±2.18	25.8±1.74	25.8±1.67	26.4±1.60	25.7±1.66
Rat 438-mIgG1 (10 mg/kg)	25.1±2.06	25.1±2.21	24.6±1.88	25.1±1.71	24.5±1.54
Rat 438-mIgG1 (30 mg/kg)	24.7±2.21	25.7±2.10	25.2±2.48	25.8±2.18	25.3±2.23
A2 (3 mg/kg)	24.7±2.39	26.1±2.06	26.1±2.05	27.1±1.99	26.4±1.82
A2 (10 mg/kg)	24.5±1.63	25.2±1.74	25.1±1.86	25.3±1.79	24.7±1.69
A2 (30 mg/kg)	25.8±1.61	26.4±1.54	26.2±1.73	26.8±1.99	26.0±2.07

E. *In vivo* growth inhibition studies for breast cancer xenografts

The effects of humanized 438 VH1.1/VL1.8 was examined on the *in vivo* growth in triple negative breast cancer xenografts models, Sum149 and MDA-MB-231. Athymic female mice (Nu/Nu, 6-8 weeks) were obtained from Charles River Laboratories and housed in specific pathogen-free conditions, according to the guidelines of the Association for the Assessment and Accreditation for Laboratory Animal Care, International. Animals were provided sterile rodent chow and water ad libitum.

Cells for implantation into athymic mice were harvested and pelleted by centrifugation at 450Xg for 5-10 minutes. The cell pellets were washed once and re-suspended in sterile serum-free medium. Tumor cells were supplemented with 50%

crypts. Thus inhibition of Notch1 signaling alone increased goblet cell differentiation, however not to a level that significantly impacted body weight.

2. Image Analysis

Virtual images were opened in Image Pro-Plus Software (Media Cybernetics, Bethesda, MD). A manual outline Area of Interest (AOI) was created that included the intestinal tissue (crypt and villi), but excluded the smooth muscle, artifacts, folds, and debris. A threshold was created to identify Alcian Blue stain area and tissue area. The thresholds were applied to the AOIs and range statistics were exported to an Excel spreadsheet. In Excel, a mean Alcian Blue stain ratio was calculated for each animal from the two collected stain ratios to provide a single calculated Alcian Blue Stain Ratio per animal. A mean Alcian Blue Stain Ratio and standard deviation were created for each group. Statistics were performed using the JMP statistical software (JMP, Cary, NC).

Table 35 shows an image quantitation of Alcian Blue stain ratio of rat 438-mIgG1 and A2, and control anti-E.tenella antibody treated mouse intestines in Calu-6 efficacy study. Compared to anti-E.tenella control at 10 mg/kg, there was a significant increase in the Alcian Blue stain ratio in rat 438-mIgG1 dosed at 10 mg/kg and 30 mg/kg. The quantitative image analysis of alcian blue stain confirms the increased differentiation of goblet cells over a larger region of the ileum in comparison to the region shown in **FIG. 27**.

Table 35 shows an image quantitation of Alcian Blue stain ratio of rat 438-mIgG1 and A2, and control anti-E.tenella antibody treated mouse intestines in Calu-6 efficacy study.

Treatment Group	Alcian Blue Stain Ratio	S.E.M.
ANTI-E.TENELLA, 10mg/kg	2.71	0.23
438, 3mg/kg	3.97	0.59
438, 10mg/kg	7.64	0.79
438, 30mg/kg	8.24	0.63
A2, 3mg/kg	7.38	1.44
A2, 10mg/kg	14.13	2.32
A2, 30mg/kg	11.63	1.07

Statistical analysis of pairwise comparisons between the average or mean Alcian Blue stain ratios from each treatment group are shown in **Table 36.** Ratios for

WHAT IS CLAIMED IS:

1. An antibody that binds to Notch1, comprising:
a heavy chain variable region having a CDR1 region, a CDR2 region, and a CDR3 region from the heavy chain variable region comprising SEQ ID NO: 71.

2. An antibody that binds to Notch1 comprising:
a light chain variable region having a CDR1 region, a CDR2 region, and a CDR3 region from the light chain variable region comprising SEQ ID NO: 97.

3. An antibody that binds to Notch1 comprising:
a heavy chain variable region having a CDR1 region, a CDR2 region, and a CDR3 region from the heavy chain variable region as shown in SEQ ID NO: 71, and
a light chain variable region having a CDR1 region, a CDR2 region, and a CDR3 region from the light chain variable region as shown in SEQ ID NO: 97.

4. The antibody according to any one of claims 1-3, comprising a heavy chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 71.

5. The antibody according to claim 4, comprising a heavy chain variable region amino acid sequence as set forth in SEQ ID NO: 71.

6. The antibody according to any one of claims 1-3, comprising a light chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 97.

7. The antibody according to claim 6, comprising a light chain variable region amino acid sequence as set forth in SEQ ID NO: 97.

8. The antibody according to any one of claims 1-3, comprising a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 111.

9. The antibody according to claim 8, comprising a heavy chain amino acid sequence as set forth in SEQ ID NO: 111.

10. The antibody according to any one of claims 1-3, comprising a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 113.

11. The antibody according to claim 10, comprising a light chain amino acid sequence as set forth in SEQ ID NO: 113.

12. An antibody that binds to Notch1, comprising:

a heavy chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 71; and

a light chain variable amino acid sequence that is at least 90% identical to SEQ ID NO: 97.

13. An antibody that binds to Notch1, comprising:

a heavy chain variable region amino acid sequence as set forth in SEQ ID NO: 71; and

a light chain variable amino acid sequence as set forth in SEQ ID NO: 97.

14. An antibody that binds to Notch1, comprising:

a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 111; and

a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 113.

15. An antibody that binds to Notch1, comprising:

a heavy chain amino acid sequence as set forth in SEQ ID NO: 111; and

a light chain amino acid sequence as set forth in SEQ ID NO: 113.

16. An antibody that binds to Notch1 and competes for binding to Notch1 with the antibody of claims 1, 2 or 3.

17. An antibody or antigen binding portion thereof, that binds to Notch1, wherein the antibody binds an epitope comprising at least 7 amino acid residues selected from Asn 1461, Lys 1462, Val 1463, Cys 1464, Leu 1466, Leu 1580, Tyr 1621, Gly 1622, Met 1670, Asp 1671, Val 1672, Arg 1673, Leu 1707, Ala 1708, Leu 1710, Leu 1711, Leu 1712, Leu 1713, Leu 1716 and Leu 1718.

18. The antibody according to any one of claims 1-17, wherein said antibody is an IgA, IgD, IgE, IgG, or IgM molecule, or is derived therefrom.

19. The antibody according to claim 18, wherein the antibody is an IgG, having a subclass selected from the group consisting of IgG1, IgG2, IgG3 and IgG4, or is derived therefrom.

20. The antibody according to claim 19, wherein the subclass is derived from IgG1.

21. A nucleic acid that encodes the antibody according to any one of claims 1-20.

22. A nucleic acid comprising the sequence as set forth in SEQ ID NO: 112.

23. A nucleic acid comprising the sequence as set forth in SEQ ID NO: 114.

24. A vector comprising the nucleic acid according to any one of claims 21-23.

25. A host cell comprising the vector of claim 24.

26. A process for producing an antibody, comprising cultivating the host cell of claim 25 and recovering the antibody from the culture media.

27. A host cell that recombinantly produces the antibody according to any one of claims 1-20.

28. A pharmaceutical composition comprising the antibody according to any one of claims 1-20 and a pharmaceutically acceptable carrier.

29. A method of treating a disorder in a subject in need thereof, comprising administering to said subject the antibody according to any one of claims 1-20, or the pharmaceutical composition of claim 28.

30. The method of claim 29, wherein said disorder is associated with abnormal activation of Notch1.

31. The method of claim 29 or 30, wherein said disorder is selected from the group consisting of T-cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC) and breast cancer in a subject in need thereof.

32. The antibody of any one of claims 1-20 for use in therapy.

33. The use of an antibody of any one of claims 1-20 for the manufacture of a medicament.

34. The use according to claim 32 or 33, wherein said use is for the treatment of T-cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC) and breast cancer in a subject in need thereof.

35. An antibody that binds to Notch1, comprising:

a heavy chain variable region amino acid sequence as set forth in SEQ ID NO: 115; and

a light chain variable amino acid sequence as set forth in SEQ ID NO: 129.

36. An antibody that binds to Notch1, comprising:

a heavy chain amino acid sequence as set forth in SEQ ID NO: 149; and

a light chain amino acid sequence as set forth in SEQ ID NO: 151.

37. An antibody that binds to human Notch1, wherein the antibody binds an epitope comprising at least 8 amino acid residues selected from Asp 1458, Asn 1461, Val 1463, Cys 1464, Leu 1466, Leu 1580, Met 1581, Pro 1582, Tyr 1621, Gly 1622,

Arg 1623, Asp 1671, Val 1672, Arg 1673, Gly 1674, Leu 1710, Gly 1711, Ser 1712, Leu 1713, Asn 1714, Ile 1715, Pro 1716 and Lys 1718.

38. An antibody that demonstrates higher inhibition of Notch1 activation of a mutant Notch1 receptor compared to inhibition of Notch1 activation of a native Notch1 receptor.

39. A pharmaceutical composition comprising the antibody according to any one of claims 35-38 and a pharmaceutically acceptable carrier.

40. A method of treating a disorder in a subject in need thereof, comprising administering to said subject the antibody according to any one of claims 35-38, or the pharmaceutical composition of claim 39.

41. The method of claim 40, wherein said disorder is associated with abnormal activation of Notch1.

42. The method of claim 40 or 41, wherein said disorder is selected from the group consisting of T-cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC) and breast cancer in a subject in need thereof.

43. The antibody of any one of claims 35-38 for use in therapy.

44. The use of an antibody of any one of claims 35-38 for the manufacture of a medicament.

45. The use according to claim 43 or 44, wherein said use is for the treatment of T-cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC) and breast cancer in a subject in need thereof.

Also provided are antibodies that bind to Notch1 having a light chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 97. Further provided are antibodies that bind to Notch1 having a light chain variable region amino acid sequence as set forth in SEQ ID NO: 97.

Also provided are antibodies that bind to Notch1 having a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 111. Further provided are antibodies that bind to Notch1 having a heavy chain amino acid sequence as set forth in SEQ ID NO: 111.

Also provided are antibodies having a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 113. Further provided are antibodies having a light chain amino acid sequence as set forth in SEQ ID NO: 113.

In a further embodiment, the invention provides for antibodies that bind to Notch1, having a heavy chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 71; and a light chain variable amino acid sequence that is at least 90% identical to SEQ ID NO: 97. Further provided are antibodies that bind to Notch1 having a heavy chain variable region amino acid sequence as forth in SEQ ID NO: 71; and a light chain variable region amino acid sequence as set forth in SEQ ID NO: 97.

In a further embodiment, the invention provides for antibodies that bind to Notch1, having a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 111; and a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 113. Further provided are antibodies that bind to Notch1 having a heavy chain amino acid sequence as set forth in SEQ ID NO: 111, and a light chain amino acid sequence as set forth in SEQ ID NO: 113.

In a further embodiment, the invention provides for antibodies, that bind to human Notch1, wherein the antibodies bind an epitope having at least 8 amino acid residues selected from Asn 1461, Lys 1462, Val 1463, Cys 1464, Leu 1466, Leu 1580, Tyr 1621, Gly 1622, Met 1670, Asp 1671, Val 1672, Arg 1673, Leu 1707, Ala 1708, Leu 1710, Gly 1711, Ser 1712, Leu 1713, Pro 1716 and Lys 1718.

In another embodiment, the present invention provides for antibodies that bind to Notch1, having a heavy chain variable region having a CDR1 region, a CDR2 region, and a CDR3 region from the heavy chain variable region comprising SEQ ID NO: 115.

products were cloned into TOPO-Blunt cloning vector (Invitrogen) and sequenced by conventional methods.

Variable (V) region cDNAs from parental rat 438 and parental rat 351 (hereinafter "rat 438" and "rat 351", respectively) were subcloned into mammalian expression vectors wherein rat Variable Heavy chain (VH) were fused in frame with murine IgG1 (mIgG1), and rat Variable Light chain (VL) were fused with murine kappa. Similarly, for the generation of chimeric antibodies with rat V region and human IgG constant region, rat VH was fused in frame with human IgG1 (hIgG1), and VL with human kappa, respectively. Corresponding chimeric antibodies were generated from these constructs by transient transfection in COS cells and their binding and neutralizing activities were confirmed in assays.

Purified rat variable-mouse constant chimeric antibodies (hereinafter "rat 438-mIgG1" and "rat 351-mIgG1") were further characterized in a series of *in vitro* and *in vivo* assays, including recombinant antigen and cell surface target binding, inhibition of Notch1 activity in the RGA and angiogenesis assays, and tumor growth inhibition in mouse models, as described in the Examples below. The lead antibodies, rat 438 and rat 351 were selected based on these studies. **Table 2** lists the amino acid and nucleic acid sequences of various regions of rat 438 and rat 351's variable regions and additional clones 90, 132, 132 (A12/G11) and 137.

Table 2: Rat variable region sequences

SEQ ID NO:		
13	438 Heavy Chain Variable Region amino acid sequence	AVQLVESGGGLVQPGRSLKLSCTASGFTFS SFAMAWVRQAPTKGLEWVASISYGGADTY YRDSVKGRFTISRDNAKSSLYLQMDSLRSE DTSTYYCAKDLPYYGYTPFVMDAWGQGTS VTVSS
14	438 Heavy Chain Variable Region nucleotide sequence	GCGGTACAGTTGGTGGAGTCTGGGGGAG GCTTAGTGCAGCCTGGAAGGTCCTTGAAA CTCTCCTGTACAGCCTCTGGATTCACTTT CAGTAGCTTTGCAATGGCCTGGGTCCGC CAGGCTCCAACGAAGGGGCTGGAGTGGG TCGCATCCATTAGTTATGGTGGTGCTGAC ACTTACTATCGAGACTCCGTGAAGGGCC GATTCACTATCTCCAGAGATAATGCAAAA AGCAGCCTATATTTGCAAATGGACAGTCT GAGGTCTGAGGACACGTCCACTTATTACT GTGCAAAAGACCTTCCATACTACGGATAT ACCCCCTTTGTTATGGATGCCTGGGGTCA GGGAACTTCAGTCACTGTCTCCTCA

back mutations in the human acceptor framework to rat 438 and rat 351 residues at the corresponding positions to optimize the activity and binding epitope.

Table 5: Back mutations in VH and VL of humanized 438 and humanized 351 variants.

438 VH Variant	1.0	1.1							
VH back mutation	none	R94K							
438 VL Variant	1.0	1.1	1.3	1.4	1.5	1.6	1.7	1.8	1.9
VL back mutation	none	M4L L46V Y49K	M4L	L46V	Y49K	M4L L46V	M4L Y49K	L46V Y49K	Q3M M4L L46V Y49K
351 VH Variant	1.0								
VH back mutation	none								
351 VL Variant	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	
VL back mutation	none	L46V Y49K	M4L	L46I	Y49K	M4L L46I	M4L Y49K	M4L L46V Y49K	

Example 3

Characterization of Anti-Notch1 Inhibitory Antibodies

A. Expression and Binding to Notch1

Relative expression yields of humanized 438 and humanized 351 variants were tested in a transient expression assay in COS cells. As shown in **Table 6,** a number of humanized 438 variants, including humanized 438 VH1.1/VL1.8, and a number of humanized 351 variants, including humanized 351 VH1.0/VL1.1, demonstrated significant yields.

Table 6. Relative expression yields in conditioned media of humanized 438 variants and humanized 351 variants in transient expression in COS cells.

438 Variant	VH1.0 /VL1.3	VH1.0/ VL1.4	VH1.0/ VL1.5	VH1.0/ VL1.6	VH1.0/ VL1.7	VH1.0/ VL1.8	VH1.0/ VL1.9	VH1.0/ VL1.10
438 Yield (µg/ml)	41.51	41.63	85.73	35.11	36.94	40.08	51.9	62.58

438 Variant	VH1.0/ VL1.11	VH1.1/ VL1.3	VH1.1/ VL1.4	VH1.1/ VL1.5	VH1.1/ VL1.6	VH1.1/ VL1.7	VH1.1 /VL1.8	VH1.1/ VL1.9
438 Yield (µg/ml)	45.86	23.2	49.47	65.93	47.72	31.95	66.65	14.12
438 Variant	VH1.1/ VL1.10	VH1.1/ VL1.11						
438 Yield (µg/ml)	36.7	37.54						
351 Variant	VH1.0/ VL1.0	VH1.0/ VL1.1	VH1.0/ VL1.2	VH1.0/ VL1.3	VH1.0/ VL1.4	VH1.0/ VL1.5	VH1.0/ VL1.6	VH1.0/ VL1.7
351 Yield (µg/ml)	33.5	26.66	24.52	25.1	35.20	29.25	28.93	33.54

Total expression levels of IgGs in conditioned media were measured by quantitative IgG ELISA, as described in Example 2. **Table 7** shows EC50 (nM) values calculated from cell surface Notch1 binding ELISAs for humanized 438 variants and rat 438, along with humanized 351 variants and rat 351.

The data demonstrates that multiple variants of humanized 438, including humanized 438 VH1.1/VL1.8, are similar to rat 438 in binding to full-length human Notch1 expressed on the cell surface of U-2 OS cells. Furthermore, **Table 7** shows that both humanized 438 VH1.1/VL1.8 and VH1.1/VL1.3 fully retained rat 438's cross-reactivity to mouse Notch1 expressed on the cell surface of U-2 OS cells.

The data further demonstrates that humanized 351 VH1.0/VL1.1 and VH1.1/VL1.4, are similar to rat 351 in binding to full-length human Notch1 expressed on the cell surface of U-2 OS cells. **Table 7** further shows that humanized 351 VH1.0/VL1.1 and VH1.1/VL1.4 fully retained rat 351's cross-reactivity to mouse Notch1 expressed on the cell surface of U-2 OS cells.

Table 7. EC50 (nM) values of cell surface Notch1 binding ELISAs for humanized 438 variants and rat 438, along with humanized 351 variants and rat 351.

EC50 (nM)								
Antibody	Rat 438	VH1.0/ VL1.1	VH1.1/ VL1.0	VH1.1/ VL1.1	VH1.1/ VL1.3	VH1.1/ VL1.5	VH1.1/ VL1.8	VH1.1/ VL1.9
Human Notch1	0.2132	0.305	0.2956	0.1773	0.1516	0.2189	0.2025	0.1776
Mouse Notch1	0.1725	0.2287	0.1437	0.1291	0.09337	0.1457	0.1489	0.1374

EC50 (nM)			
Antibody	Rat 351	VH1.0/ VL1.1	VH1.0/ VL1.4
Human Notch1	0.10	0.15	0.10
Mouse Notch1	0.07	0.08	0.07

closest homologues of the Notch1 NRR region are Notch2 and Notch3 (~50% homology), and Notch4 is a more distant homologue (34% homology). Crossreactivity of anti-Notch1 antibodies to other members of the Notch family, especially Notch2, may lead to undesired effects in patients. Therefore, the potential crossreactivity of rat 438 and humanized 438, along with rat 351 and humanized 351 antibodies to other Notch family members were assessed.

Expression constructs encoding human Notch2 and Notch3 NRR regions, fused with human IgG1 Fc fragment were stably introduced into CHO-PACE cells. Conditioned media from these cells expressing NRR-Fc fusions were collected. Human Notch2 NRR-Fc and human and mouse Notch3 NRR-Fc were purified by protein A affinity followed by size exclusion chromatography (SEC). Purified preparations were dialysed into TBS with 1mM $CaCl_2$ and analyzed on analytical SEC to be >99% in purity.

As shown in **Table 10**, rat 438 lacked detectable binding to human Notch2 NRR-Fc fusion protein. Further shown in **Table 10**, humanized 438 variants lacked detectable binding to full-length human Notch3 expressed on U-2 OS cell surface, demonstrating that 438 did not cross-react with Notch3.

Table 10. Binding of rat-mIgG1 and humanized 438 variants to Notch2 NRR-Fc and Notch3 U-2 OS cells (N/B represents non-binding).

Binding to Notch2 NRR-Fc		**Binding to Notch3 U2-OS cells**	
Rat 438-mIgG1	N/B	Rat438-mIgG	N/B
Humanized 438 VH1.0/VL1.1	N/B	A2	N/B
Humanized 438VH1.1/VL1.0	N/B		
Humanized 438VH1.1/VL1.3	N/B		
Humanized 438VH1.1/VL1.5	N/B		
Humanized 438VH1.1/VL1.8	N/B		
Humanized 438VHH1.1/VL1.9	N/B		
A2	N/B		

As shown in **Table 11**, humanized 351 VH1.0/VL1.1 lacked detectable binding to human Notch2 NRR-Fc fusion protein, and to both human and mouse Notch3 NRR-Fc fusion proteins. However, humanized 351 VH1.0/VL1.1 cross-reacted with human, mouse and cyno-Notch1 NRR.

The data shows that rat 438 and A2 bind overlapping but distinct surfaces within the Notch1 NRR. Both epitopes include the central HD domain. Rat 438 and A2 interact with LNR-A, however rat 438 interacts with a larger surface. Only rat 438 interacts with the S1 loop region and only A2 interacts with LNR-B. More specifically, the data shows that rat 438 binds to human Notch1 NRR residues Asn 1461, Lys 1462, Val 1463, Cys 1464, Leu 1466, Leu 1580, Tyr 1621, Gly 1622, Met 1670, Asp 1671, Val 1672, Arg 1673, Leu 1707, Ala 1708, Leu 1710, Gly 1711, Ser 1712, Leu 1713, Pro 1716 and Lys 1718.

The data also shows that rat 351 and A2 bind overlapping but distinct surfaces within the Notch1 NRR. In particular, only rat 351 interacts with the S1 loop region and only A2 interacts with LNR-B. Rat 351 and A2 both interact with LNR-A, however rat 351 interacts with a distinct subset of LNR-A amino acids. More specifically, the data shows that rat 351 binds to the human Notch1 NRR residues Asp 1458, Asn 1461, Val 1463, Cys 1464, Leu 1466, Leu 1580, Met 1581, Pro 1582, Tyr 1621, Gly 1622, Arg 1623, Asp 1671, Val 1672, Arg 1673, Gly 1674, Leu 1710, Gly 1711, Ser 1712, Leu 1713, Asn 1714, Ile 1715, Pro 1716, Lys 1718.

The x-ray crystal structure of rat 438 and rat 351 residues binding to Notch1 NRR were further analyzed using the program PISA. The data shows that rat 438 formed strong electrostatic interactions (salt bridges) with the residues Lys1718 and Arg1673. Notch1 NRR residues that formed hydrogen bonds with the rat 438 antibody were Asn1461, Asp1671, Arg1673, Leu1713, Lys1718, Cys1464, Ala1708, and Ser1712. The Notch1 NRR residues that contribute more than 40 Angstrom^2 of buried surface area upon formation of the complex with rat 438 antibody were Arg1673, Val1463, Lys1462, Gly1622, Asp1671 from the interaction with the heavy chain, and Leu1466, Lys1718, Gly1711, Cys1464, Pro1716, and Val1463 from the interaction with the light chain. Of the residues identified, rat 438 binds to human Notch1 NRR at least at residues Asn1461, Val1463, Lys1462, Asp1671, Arg1673, Leu1713, and Lys1718.

The data shows that rat 351 forms strong electrostatic interactions (salt bridges) with residues Asp1458 and Arg1673. Notch1 NRR residues that formed hydrogen bonds with the rat 351 antibody were Asp1458, Val1463, Cys1464, Ser1465, Tyr1621, Asp1671, Val1672, Arg1673, Gly1711, Ser1712, Leu1713, and Asn1714. The Notch1 NRR residues that contribute more than 40 Angstrom^2 of buried surface area upon formation of the complex with rat 351 were Val1463,

cells with a leucine to proline mutation at amino acid 1594 (L1594P), MOLT-4 cells with a leucine to proline mutation at amino acid 1601 (L1601P) and DND-41 cells with compound class 1 mutations of leucine to proline at amino acid position 1594 and aspartic acid to valine at amino acid position 1610 (L1594P/ D1610V). The CCRF-CEM cell line harbors a class 2 mutation and possesses a 12 amino acid insertion at position 1595. The Jurkat cell line harbors a class 3 JEM mutation that inserts 17 amino acids at position 1740.

Notch1 activation and Notch1^{ICD} release was examined by Western blot analysis of protein extracts generated from the T-ALL cell lines treated with increasing concentrations of rat 438-mIgG1, humanized 438 VH1.1/VL1.8, rat 351-mIgG1 or A2 antibodies, and control anti-E.tenella antibody. Released Notch1^{ICD} molecules initiating at a Valine residue are specifically detected with D3B8 antibody (anti-Notch1^{ICD}).

The T-ALL cell lines HPB-ALL, ALL-SIL, CCRF-CEM, MOLT-4, DND-41 and Jurkat cells were used instead of CCD1076SK fibroblasts as described above. Since T-ALL cells possess constitutively released Notch1^{ICD}, no exogenous DLL4 ligand was required. Suspension cultures of 2x10E6 T-ALL cells were mixed with 0, 0.001, 0.01, 0.1, 1 and 10 µg/ml of rat 438-mIgG1, humanized 438 VH1.1/VL1.8, rat 351-mIgG1 or A2 antibodies, and control anti-E.tenella antibody in RPMI 1640 medium containing 10% FBS, Pen/Strep/Glutamine. Cells were incubated with the antibodies for 24 hours at 37°C in 5% CO_2. Cells were harvested by centrifugation and medium was removed by aspiration. Cell pellets were lysed in 1% NP40, 0.5% sodium deoxycholate, 5 mM EDTA, 0.25 M NaCl, 0.025 M Tris-HCl, pH 7.5, containing Complete Mini Protease inhibitor cocktail (Roche). Extracts were resolved by SDS-PAGE on a 7.5% polyacrylamide gel and transferred to nitrocellulose paper using an iBlot Gel transfer system (Invitrogen). The released Notch1^{ICD} molecules initiating at a Valine residue are detected with anti-Notch1^{ICD} and, as a loading control, anti-beta-actin using standard western blot procedures. Densitometric analysis was performed on films that were scanned with a BioRad GS-800 Calibrated Densitometer and analyzed with Quantity One version 4.6.9 software (BioRad). Notch1^{ICD} levels were normalized to beta-actin in each sample and then compared to the untreated control.

As shown in **FIG. 19**, treatment of HBP-ALL cells with increasing concentrations of rat 438-mIgG1 and humanized 438 VH1.1/VL1.8 significantly

Table 28. Average mouse body weights (minus tumor weights) in 87393A1 NSCLC PDXs after treatment with rat 351-mIgG1.

	Average Mouse Body Weights in grams (minus tumor weights)				
Antibody	Day 0	Day 7	Day 14	Day 20	Day 26
Control Anti-E.tenella	23.9	23.1	23.4	23.3	22.9
Rat 351-mIgG1	23.4	23.0	23.1	23.3	23.5

C. *In vivo* growth inhibition studies for HPB-ALL Xenografts

Similar *in vivo* experiments were performed with the mutant Notch1 HPB-ALL cell line as performed with the 37622A1 NSCLC PDX and 87393A1 NSCLC PDX described above. To generate xenografts, nude female mice (Nu/Nu) were implanted subcutaneously with 8x10E6 HPB-ALL cells in 50% Matrigel (BD Biosciences). When the tumors reached a volume of 200 to 400 mm^3, the tumors were staged to ensure uniformity of the tumor mass among various treatment groups prior to the administration of anti-Notch1 and control D16A antibodies. The HPB-ALL model was dosed s.c. at 20 mg/kg of rat 438-mIgG1, rat 351-mIgG1, A2 or D16A antibody, 2 times a week for 2 weeks. Tumors were measured at least once a week and their volume was calculated with the formula: tumor volume (mm^3) = 0.5 x (tumor $width^2$)(tumor length). From 8-11 animals, mean tumor masses (± SEM) for each treatment group were calculated and compared to the control-treated.

Table 29 shows the efficacy of rat 438-mIgG1, rat 351-mIgG1 and A2 antibodies in HPB-ALL xenografts with L1575P mutation in Notch1 NRR. The data demonstrates that treatment with rat 438-mIgG1 and treatment rat 351-mIgG1 both inhibited *in vivo* growth of HPB-ALL cells compared to control D16A antibody, thus slowing tumor growth.

Table 29. Efficacy of rat 351-mIgG1, rat 438-mIgG1 and A2 antibodies in HPB-ALL xenografts with L1575P mutation in Notch1 NRR.

	Control D16A					
	Day 0	Day 1	Day 4	Day 8	Day 11	Day 14
Tumor Volume (mm^3)	175.3	201.9	261.2	522.0	735.9	1003.4
S.E.M.	6.4	4.4	11.4	44.1	82.3	162.2
	Rat 351-mIgG1					
	Day 0	Day 1	Day 4	Day 8	Day 11	Day 14
Tumor Volume (mm^3)	166.1	198.6	244.1	267.0	231.0	251.0

Table 30. Efficacy of rat 438-mIgG1 and A2 in Calu-6 lung cancer model.

	Day 0	Day 6	Day 9	Day 13	Day 16	Day 19
Anti-E.tenella (10 mg/kg)	258±17	491±34	740±64	1156±140	1260±171	1613±222
Rat 438-mIgG1 (3 mg/kg)	250±29	362±58	465±88	742±127	888±171	1279±219
Rat 438-mIgG1 (10 mg/kg)	263±26	383±40	448±51	566±68	667±87	904±129
Rat 438-mIgG1 (30 mg/kg)	242±27	343±58	445±82	566±106	601±120	720±152
A2 (3 mg/kg)	238±29	349±42	450±58	603±74	664±91	851±113
A2 (10 mg/kg)	250±34	297±35	348±47	444±64	480±70	603±101
A2 (30 mg/kg)	241±26	376±52	486±68	598±115	622±121	779±168

To determine if inhibition of Notch1 signaling alone effects body weight, mice were weighed during *in vivo* efficacy studies. **Table 31** shows that the average mouse body weights (minus tumor weights) of rat 438-mIgG1 and A2, and control anti-E.tenella treated groups over the course of the study, were not significantly different

Table 31. Average mouse body weights (minus tumor weights) of rat 438-mIgG1 and A2, and control anti-E.tenella treatments in Calu-6 model (n=10-11).

	Day 6	Day 9	Day 13	Day 16	Day 19
E.tenella (10 mg/kg)	24.6±1.56	25.2±1.42	24.3±1.64	24.7±1.74	24.0±1.77
Rat 438-mIgG1 (3 mg/kg)	24.5±2.18	25.8±1.74	25.8±1.67	26.4±1.60	25.7±1.66
Rat 438-mIgG1 (10 mg/kg)	25.1±2.06	25.1±2.21	24.6±1.88	25.1±1.71	24.5±1.54
Rat 438-mIgG1 (30 mg/kg)	24.7±2.21	25.7±2.10	25.2±2.48	25.8±2.18	25.3±2.23
A2 (3 mg/kg)	24.7±2.39	26.1±2.06	26.1±2.05	27.1±1.99	26.4±1.82
A2 (10 mg/kg)	24.5±1.63	25.2±1.74	25.1±1.86	25.3±1.79	24.7±1.69
A2 (30 mg/kg)	25.8±1.61	26.4±1.54	26.2±1.73	26.8±1.99	26.0±2.07

E. *In vivo* growth inhibition studies for breast cancer xenografts

The effects of humanized 438 VH1.1/VL1.8 was examined on the *in vivo* growth in triple negative breast cancer xenografts models, Sum149 and MDA-MB-231. Athymic female mice (Nu/Nu, 6-8 weeks) were obtained from Charles River Laboratories and housed in specific pathogen-free conditions, according to the guidelines of the Association for the Assessment and Accreditation for Laboratory Animal Care, International. Animals were provided sterile rodent chow and water ad libitum.

Cells for implantation into athymic mice were harvested and pelleted by centrifugation at 450Xg for 5-10 minutes. The cell pellets were washed once and re-suspended in sterile serum-free medium. Tumor cells were supplemented with 50%

crypts. Thus inhibition of Notch1 signaling alone increased goblet cell differentiation, however not to a level that significantly impacted body weight.

2. Image Analysis

Virtual images were opened in Image Pro-Plus Software (Media Cybernetics, Bethesda, MD). A manual outline Area of Interest (AOI) was created that included the intestinal tissue (crypt and villi), but excluded the smooth muscle, artifacts, folds, and debris. A threshold was created to identify Alcian Blue stain area and tissue area. The thresholds were applied to the AOIs and range statistics were exported to an Excel spreadsheet. In Excel, a mean Alcian Blue stain ratio was calculated for each animal from the two collected stain ratios to provide a single calculated Alcian Blue Stain Ratio per animal. A mean Alcian Blue Stain Ratio and standard deviation were created for each group. Statistics were performed using the JMP statistical software (JMP, Cary, NC).

Table 35 shows an image quantitation of Alcian Blue stain ratio of rat 438-mIgG1 and A2, and control anti-E.tenella antibody treated mouse intestines in Calu-6 efficacy study. Compared to anti-E.tenella control at 10 mg/kg, there was a significant increase in the Alcian Blue stain ratio in rat 438-mIgG1 dosed at 10 mg/kg and 30 mg/kg. The quantitative image analysis of alcian blue stain confirms the increased differentiation of goblet cells over a larger region of the ileum in comparison to the region shown in **FIG. 27**.

Table 35 shows an image quantitation of Alcian Blue stain ratio of rat 438-mIgG1 and A2, and control anti-E.tenella antibody treated mouse intestines in Calu-6 efficacy study.

Treatment Group	Alcian Blue Stain Ratio	S.E.M.
ANTI-E.TENELLA, 10mg/kg	2.71	0.23
Rat 438-mIgG1, 3mg/kg	3.97	0.59
Rat 438-mIgG1, 10mg/kg	7.64	0.79
Rat 438-mIgG1, 30mg/kg	8.24	0.63
A2, 3mg/kg	7.38	1.44
A2, 10mg/kg	14.13	2.32
A2, 30mg/kg	11.63	1.07

Statistical analysis of pairwise comparisons between the average or mean Alcian Blue stain ratios from each treatment group are shown in **Table 36.** Ratios for

WHAT IS CLAIMED IS:

1. An antibody that binds to Notch1, comprising:

a heavy chain variable region comprising a CDR1, a CDR2, and a CDR3, wherein the heavy chain variable region is selected from the group consisting of SEQ ID NOS: 69 and 71, and

a light chain variable region comprising a CDR1, a CDR2, and a CDR3, wherein the light chain variable region is selected from the group consisting of SEQ ID NOS: 83, 85, 87, 89, 91, 93, 95, 97, 105, 107 and 109.

2. The antibody according to claim 1, comprising:

(a) a heavy chain variable region CDR1 as set forth in SEQ ID NO: 73,

(b) a heavy chain variable region CDR2 as set forth in SEQ ID NO: 77,

(c) a heavy chain variable region CDR3 as set forth in SEQ ID NO: 81,

(d) a light chain variable region CDR1 as set forth in SEQ ID NO: 99,

(e) a light chain variable region CDR2 as set forth in SEQ ID NO: 101, and

(f) a light chain variable region CDR3 as set forth in SEQ ID NO: 103.

3. An antibody that binds to Notch1, comprising:

a heavy chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 71; and

a light chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 97.

4. The antibody according to claim 3, comprising:

a heavy chain variable region amino acid sequence as set forth in SEQ ID NO: 71; and

a light chain variable region amino acid sequence as set forth in SEQ ID NO: 97.

5. An antibody that binds to Notch1, comprising:

a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 111; and

a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 113.

6. The antibody according to claim 5, comprising:

a heavy chain amino acid sequence as set forth in SEQ ID NO: 111; and

a light chain amino acid sequence as set forth in SEQ ID NO: 113.

7. An antibody that binds to Notch1, wherein the antibody binds an epitope comprising at least 7 amino acid residues selected from Asn 1461, Lys 1462, Val 1463, Cys 1464, Leu 1466, Leu 1580, Tyr 1621, Gly 1622, Met 1670, Asp 1671, Val 1672, Arg 1673, Leu 1707, Ala 1708, Leu 1710, Gly 1711, Ser 1712, Leu 1713, Pro 1716 and Lys 1718.

8. An antibody that binds to Notch1, comprising:

a heavy chain variable region comprising a CDR1, a CDR2, and a CDR3, wherein the heavy chain variable region is set forth in SEQ ID NO: 115, and

a light chain variable region comprising a CDR1, a CDR2, and a CDR3, wherein the light chain variable region is selected from the group consisting of SEQ ID NOS: 127, 129, 137, 139, 141, 143, 145 and 147.

9. The antibody according to claim 8, comprising:

(a) a heavy chain variable region CDR1 as set forth in SEQ ID NO: 117,
(b) a heavy chain variable region CDR2 as set forth in SEQ ID NO: 121,
(c) a heavy chain variable region CDR3 as set forth in SEQ ID NO: 125,
(d) a light chain variable region CDR1 as set forth in SEQ ID NO: 131,
(e) a light chain variable region CDR2 as set forth in SEQ ID NO: 133, and
(f) a light chain variable region CDR3 as set forth in SEQ ID NO: 135.

10. An antibody that binds to Notch1, comprising:

a heavy chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 115; and

a light chain variable region amino acid sequence that is at least 90% identical to SEQ ID NO: 129.

11. The antibody according to claim 10, comprising:

a heavy chain variable region amino acid sequence as set forth in SEQ ID NO: 115; and

a light chain variable region amino acid sequence as set forth in SEQ ID NO: 129.

12. An antibody that binds to Notch1, comprising:

a heavy chain amino acid sequence that is at least 90% identical to SEQ ID NO: 149; and

a light chain amino acid sequence that is at least 90% identical to SEQ ID NO: 151.

13. The antibody according to claim 12, comprising:
a heavy chain amino acid sequence as set forth in SEQ ID NO: 149; and
a light chain amino acid sequence as set forth in SEQ ID NO: 151.

14. An antibody that binds to Notch1, wherein the antibody binds an epitope comprising at least 8 amino acid residues selected from Asp 1458, Asn 1461, Val 1463, Cys 1464, Leu 1466, Leu 1580, Met 1581, Pro 1582, Tyr 1621, Gly 1622, Arg 1623, Asp 1671, Val 1672, Arg 1673, Gly 1674, Leu 1710, Gly 1711, Ser 1712, Leu 1713, Asn 1714, Ile 1715, Pro 1716 and Lys 1718.

15. The antibody according to any one of claims 1-14, wherein the antibody is an IgA, IgD, IgE, IgG, or IgM molecule, or is derived therefrom.

16. The antibody according to claim 15, wherein the antibody is an IgG, having a subclass selected from the group consisting of IgG1, IgG2, IgG3 and IgG4, or is derived therefrom.

17. The antibody according to claim 16, wherein the subclass is IgG1.

18. The antibody according to any one of claims 8-17, wherein the antibody demonstrates higher inhibition of Notch1 activation of a mutant Notch1 receptor compared to inhibition of Notch1 activation of a native Notch1 receptor.

19. An antibody that binds to Notch1 and competes for binding to Notch1 with the antibody of any one of claims 1-18.

20. A nucleic acid that encodes the antibody according to any one of claims 1-19.

21. A nucleic acid comprising a sequence that is selected from the group consisting of SEQ ID NOS: 72, 98, 112, and 114.

22. A nucleic acid comprising a sequence that is selected from the group consisting of SEQ ID NOS: 116, 130, 150 and 152.

23. A vector comprising the nucleic acid of any of any one of claims 20-22.

24. A host cell comprising the vector of claim 23.

25. A process for producing an antibody, comprising cultivating the host cell of claim 24 and recovering the antibody from the culture media.

26. A host cell that recombinantly produces the antibody according to any one of claims 1-19.

27. A pharmaceutical composition comprising the antibody according to any one of claims 1-19 and a pharmaceutically acceptable carrier.

28. A pharmaceutical composition for treating a disorder associated with abnormal activation of Notch1 in a subject, comprising the antibody according to any one of claims 1-19 and a pharmaceutically acceptable carrier.

29. A pharmaceutical composition for treating a disorder selected from the group consisting of T-cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC), breast cancer, ovarian cancer, and colon cancer in a subject, comprising the antibody according to any one of claims 1-19 and a pharmaceutically acceptable carrier.

30. The antibody of any one of claims 1-19 for use in therapy.

31. The use of an antibody of any one of claims 1-19 for the manufacture of a medicament.

32. The use according to claim 30, wherein the use is for the treatment of cancer.

33. The use according to claim 32, wherein the cancer is selected from the group consisting of T-cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC), breast cancer, ovarian cancer, and colon cancer.

34. Use of an antibody according to any one of claims 1-19 for the manufacture of a medicament for the treatment of a disorder.

35. Use of a pharmaceutical composition according to any one of claims 27-29, for the manufacture of a medicament for the treatment of a disorder.

36. The use of claim 34 or 35, wherein said disorder is associated with abnormal activation of Notch1.

37. The use of claim 35, wherein said disorder is cancer.

38. The use of claim 37, wherein the cancer is selected from the group consisting of T-cell acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer (NSCLC), breast cancer, ovarian cancer, and colon cancer.