

# Notch and mTOR Signaling Pathways Promote Human Gastric Cancer Cell Proliferation

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**Supplementary Table 1.** Patient information for human stomach tissue samples used in current study.

Patient	Age	Gender	Disease Pathology	Sample	Tissue Type	Tissue Use
1	42	Male	Moderately differentiated adenocarcinoma	H12	Gastric cancer	RNA, histology
				H13	Non-cancer	RNA, histology
2	68	Female	Adenocarcinoma, poorly cohesive type	H37	Non-cancer	Histology
				H38	Gastric cancer	Histology
3	54	Male	Invasive adenocarcinoma, tubular type	H39	Non-cancer	RNA, histology
				H40	Gastric cancer	RNA, histology
4	80+	Female	Invasive poorly differentiated adenocarcinoma	H41	Non-cancer	RNA, histology
				H42	Gastric cancer	RNA, histology
5	68	Male	Invasive moderately differentiated adenocarcinoma	H46	Non-cancer	RNA, histology, organoids
				H47	Gastric cancer	RNA, histology, organoids

**Supplementary Table 2.** List of antibodies used for immunostaining.

<b>Antibody</b>	<b>Host Species</b>	<b>Dilution</b>	<b>Source</b>
Cleaved Notch1 (NICD)	Rabbit polyclonal	1:50	Cell Signaling #4147S
HES1	Rabbit polyclonal	1:100	Abcam #ab108937
pS6	Rabbit monoclonal	1:300	Cell Signaling #2215S

**Supplementary Table 3.** List of antibodies used for western blot analysis.

<b>Antibody</b>	<b>Host Species</b>	<b>Dilution</b>	<b>Source</b>
pS6	Rabbit monoclonal	1:1000	Cell Signaling #2215S
S6	Mouse monoclonal	1:1000	Cell Signaling #2317S
pAKT	Rabbit polyclonal	1:1000	Cell Signaling #9271S
AKT	Mouse monoclonal	1:1000	Cell Signaling #2920S
P4EBP1	Rabbit monoclonal	1:1000	Cell Signaling #2855S
4EBP1	Rabbit monoclonal	1:1000	Cell Signaling #9644
Actin	Mouse	1:5000	Proteintech #60008-1

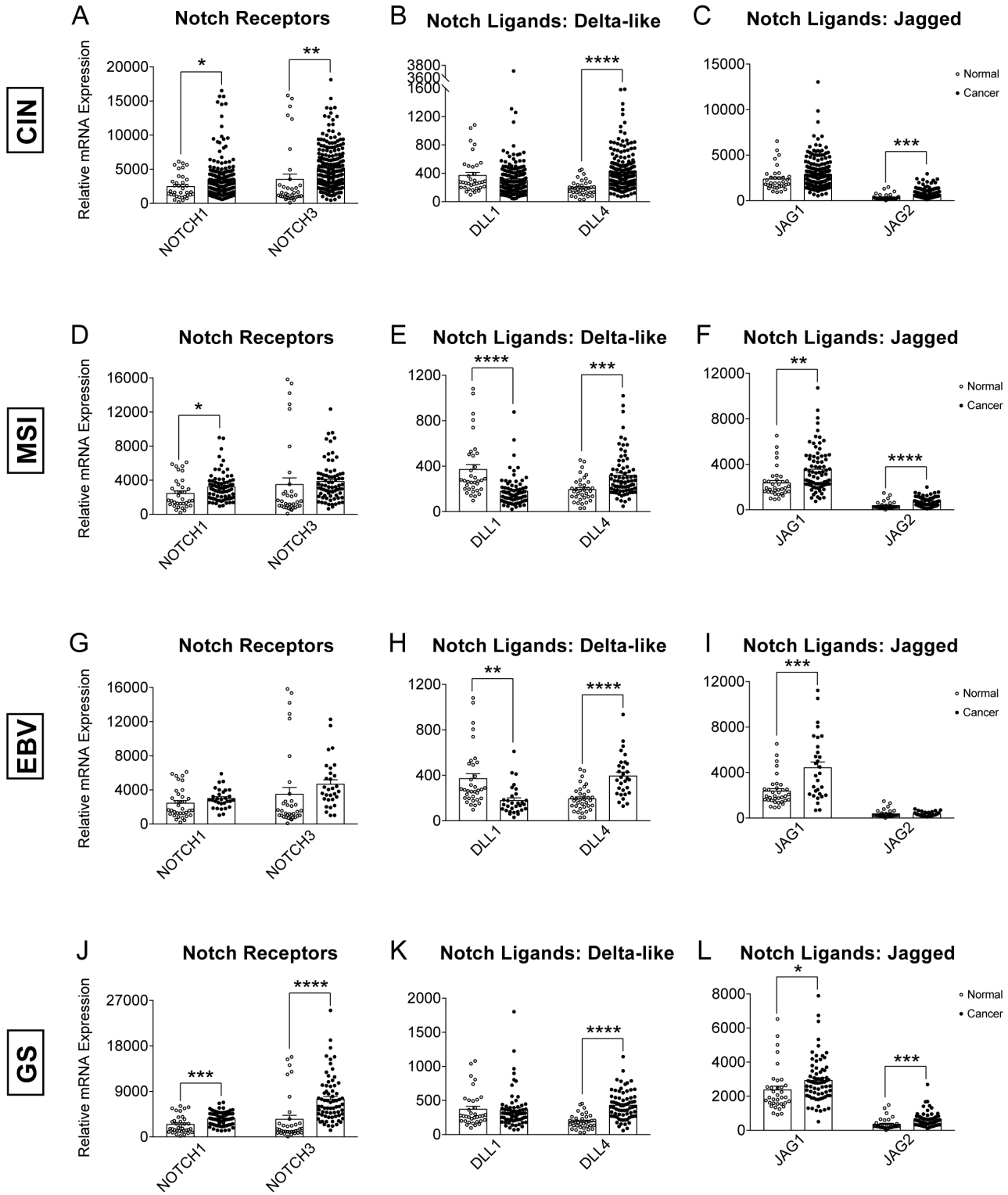
**Supplementary Table 4.** List of RT-qPCR primers.

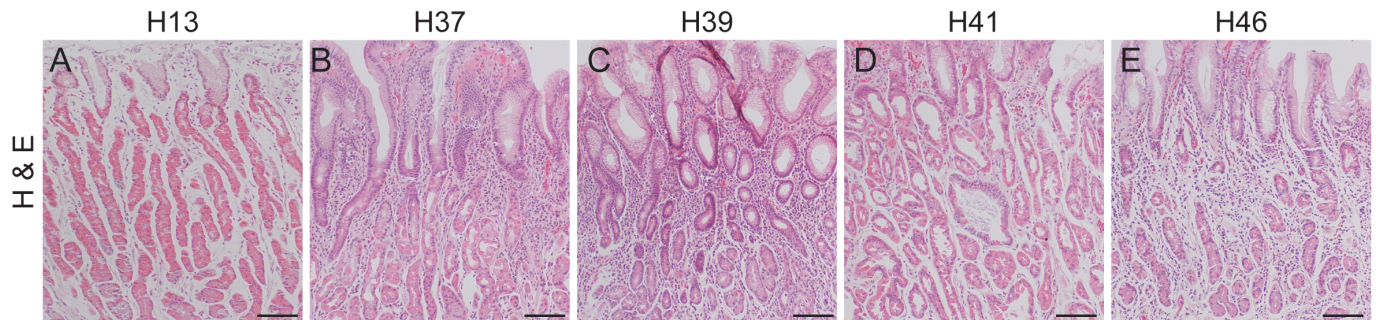
Gene	Forward (5'-3')	Reverse (5'-3')	Amplicon Size (bp)	Reference
<i>NOTCH1</i>	GACAGCCTCAACGGGTACAA	CACACGTAGCCACTGGTCAT	137	1
<i>NOTCH2</i>	CAACCGCCAGTGTGTTCAAG	GAGCCATGCTTACGCTTTTCG	240	1
<i>NOTCH3</i>	TCTTGCTGCTGGTCATTCTC	TGCCTCATCCTCTTCAGTTG	485	1
<i>NOTCH4</i>	TGAGGTGAATCCAGACAAC	ATACAGTCATCCAGGTTCTC	261	1
<i>DLL1</i>	GATTCTCCTGATGACCTCGCA	TCCGTAGTAGTGTTTCGTCACA	168	
<i>DLL3</i>	CACTCCCGGATGCACTCAAC	GATTCCAATCTACGGACGAGC	78	
<i>DLL4</i>	CTGCGAGAAGAAAGTGGACAGG	ACAGTCGCTGACGTGGAGTTCA	139	2
<i>JAG1</i>	GGGGCAACACCTTCAACCTC	CCAGGCGAAACTGAAAGGC	77	
<i>JAG2</i>	TGCAAAAACCTGATTGGCGG	CACACACTGGTACCCGTTCA	144	
<i>GAPDH</i>	GAGTCCACTGGCGTCTTCACC	GAGGCATTGCTGATGATCTTGAGG	164	1
<i>ACTB</i>	CATCGAGCACGGCATCGTCA	TAGCACAGCCTGGATAGCAAC	211	1

<sup>1</sup>Gifford GB, Demitrack ES, Keeley TM, et al. Notch1 and Notch2 receptors regulate mouse and human gastric antral epithelial cell homeostasis. *Gut* 2017;66:1001-1011.

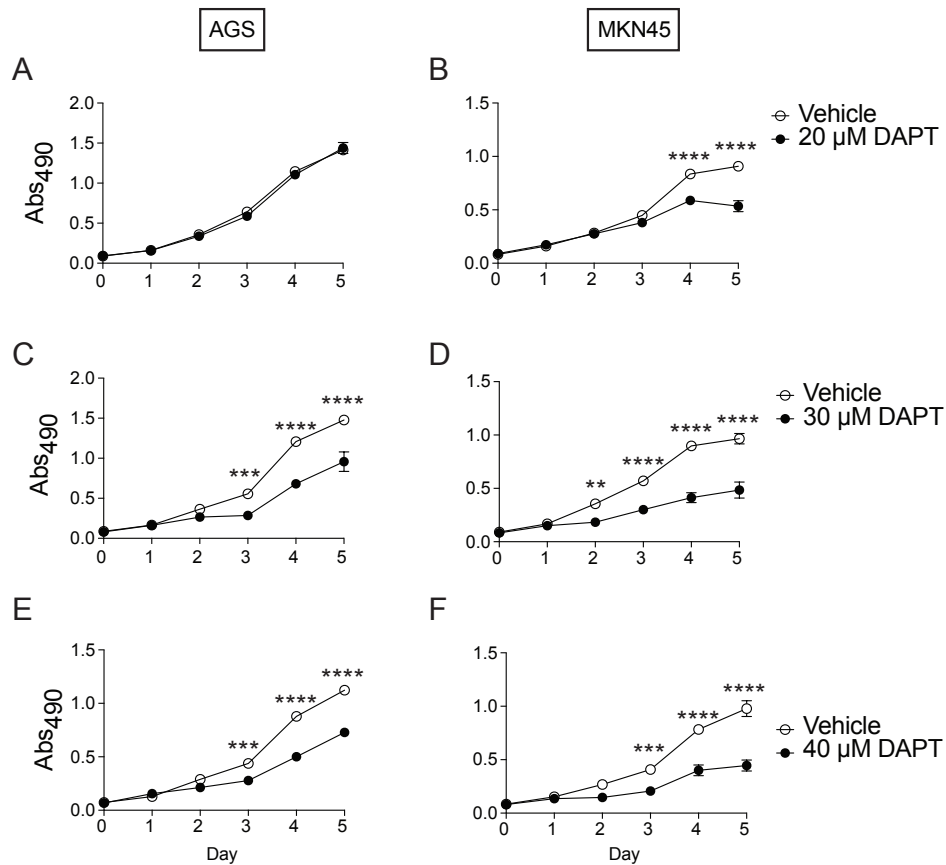
<sup>2</sup>Weidenbusch M, Rodler S, Song S et al. Gene expression profiling of the Notch-AhR-IL22 axis at homeostasis and in response to tissue injury. *Biosci Rep* 2017;37:1-14.

**Supplementary Figure 1.** Association of Notch pathway activation with molecular subtypes of human gastric cancer. Normalized RNAseq data from the 4 subtypes of gastric adenocarcinoma in The Cancer Genome Atlas (TCGA) database were examined for upregulated expression of Notch receptors (NOTCH1 and NOTCH3) and ligands (DLL1, DLL4, JAG1 and JAG2). This analysis included 35 normal stomach (white circles) compared to gastric adenocarcinomas (black circles), including: (A-C) 210 chromosomal instability (CIN), (D-F) 76 microsatellite instability (MSI), (G-I) 30 Epstein-Barr virus positive (EBV) and (J-L) 70 genomically stable (GS) subtypes. Data are presented as mean  $\pm$  SEM. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001 vs. normal.





**Supplementary Figure 2.** Histology of non-cancer control tissues. Histological analysis of patient-matched non-cancer tissues to the 5 primary gastric adenocarcinoma samples used in the study assessed by H&E staining. Scale bars: 100  $\mu$ m.



**Supplementary Figure 3.** Gastric cancer growth response to Notch inhibition. Gastric cancer cell lines AGS (A,C,E) and MKN45 (B,D,F) were treated with 20, 30 or 40  $\mu$ M of the Notch inhibitor DAPT or vehicle (Veh) daily, as marked. Cell growth was measured using a colorimetric assay kit. Veh, open circles; DAPT, filled circles. N-4 technical replicates for each sample. Data are presented as mean  $\pm$  SEM. \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001 vs. vehicle by 2-way ANOVA.