

Supplemental Tables

Table S1: Analysis of Notch gene rearrangements in triple negative breast cancer subtypes

<i>NOTCH1</i> or <i>NOTCH2</i>	Basal	Immune	Luminal AR	Mesenchymal Stem Like
WT	27 (87%)	24 (100%)	4 (66%)	5 (100%)
Rearranged	4 (13%)	0	2 (33%)	0

Table S2: Genes whose expression correlates with Notch gene-rearrangement status in cancer cell lines

Gene	Notch wild type (N=603)*	Notch-gene-rearranged (N=6)	STD_WT	STD_GR	p value
<i>HES4</i>	1.78	2.28	0.16	0.07	5.53E-14
<i>HES5</i>	1.42	1.87	0.16	0.54	3.15E-10
<i>PTCRA</i>	1.41	1.86	0.17	0.54	6.46E-10
<i>NOTCH1</i>	2.65	3.18	0.27	0.35	3.12E-06
<i>DTX1</i>	1.45	2.41	0.53	0.58	1.22E-05
<i>NOTCH3</i>	1.62	2.53	0.40	0.29	4.17E-05
<i>PSEN2</i>	2.26	1.74	0.34	0.84	0.00022

*Average log₁₀ expression; STD_WT and STD_GR, standard deviations of wild type and Notch gene-rearranged cell lines, respectively.

Table S3: Genes whose expression correlates with Notch gene-rearrangement in triple negative breast cancer

Gene	Notch wild type (N=207)*	Notch-gene-rearranged (N=13)*	STD_WT	STD_GR	p value
'HES4'	1.81	2.17	0.14	0.2	3.89E-15
'HES5'	1.31	1.72	0.2	0.58	3.99E-09
'NRARP'	2.56	3.07	0.32	0.39	1.33E-07
'NOTCH1'	2.83	3.16	0.24	0.34	3.05E-06
'HEY2'	2.29	2.89	0.47	0.58	1.76E-05
'MYC'	2.71	3.11	0.34	0.3	4.67E-05
'HEY2'	2.37	2.82	0.46	0.5	0.00072
'JAG1'	1.16	1.42	0.25	0.35	0.00076
'HES1'	2.72	2.9	0.19	0.19	0.00094

*Average log10 expression; STD_WT and STD_GR, standard deviations of wild type and Notch gene-rearranged cell lines, respectively.

Table S4. Novel mutations identified in the NRR and PEST domains of NOTCH1 in solid tumors (N=4007).

Receptor	Mutation	Sequence	Location	Tumor Site	Tumor Type
Notch1	I1680S	LE <u>S</u> DN	NRR	BREAST	Breast,TN
Notch1	I1680N	LE <u>N</u> DN	NRR	H&N	Adenoid Cystic
Notch1	E1567K	VP <u>K</u> RL	NRR	Breast	Breast, Luminal B
Notch1	A1552G	NSA <u>E</u> C	NRR	BREAST	Breast,TN
Notch1	A1552V	NS <u>V</u> EC	NRR	ENDOMETRIUM	Adenocarcinoma
Notch1	A1570G	RL <u>G</u> AG	NRR	KIDNEY	RCC, Clear Cell Type
Notch1	R1627L	EL <u>L</u> KH	NRR	Stomach	Carcinoma, Neuroendocrine
Notch1	V1575A	TL <u>A</u> VV	NRR	Skin	Melanoma
Notch1	V1599M	SR <u>M</u> LH	NRR	BRAIN	Glioblastoma
Notch1	A1707T	AL <u>T</u> SL	NRR	CERVIX	Adenocarcinoma
Notch1	R1683W	DN <u>W</u> QC	NRR	COLON	Adenocarcinoma
Notch1	V1676I	SI <u>I</u> YL	NRR	PANCREAS	Carcinoma, Anaplastic
Notch1	2426_2431del	del(SGHLGR)	PEST	BREAST	Breast,TN
Notch1	S2228Y	SP <u>S</u> VP	PEST	BREAST	Breast,Luminal B

NRR, Negative Regulatory Region; PEST, PEST degron domain. Mutations in Breast and adenoid cystic carcinoma are highlighted in bold. H&N, head and neck; RCC, renal cell carcinoma.

Table S5: Notch NRR and PEST domain mutation frequency

Cancer types	Notch1	Notch2	Notch3	Tumors sequenced	Mutation frequency
Cutaneous SCC	1	2	1	7	57%
Mantle cell lymphoma	1	0	0	2	50%
Adenoid cystic carcinoma	1	0	0	3	33%
Cervical carcinoma	5	2	1	49	16%
Gastric carcinoma	3	1	0	30	13%
Head-Neck SCC	2	2	2	65	9%
Pancreatic carcinoma	3	4	0	94	7%
Chronic lymphocytic leukemia	8	1	0	126	7%
Glioblastoma, Astrocytoma	1	1	1	36	8%
Endometrial carcinoma	14	7	4	344	7%
Melanoma	8	5	0	222	6%
Prostatic carcinoma	1	2	0	53	6%
Ovarian serous carcinoma	5	3	0	147	5%
Colonic carcinoma	16	9	4	504	6%
Ovarian mucinous carcinoma	1	1	0	38	5%
Triple negative breast carcinoma	4	1	0	105	5%

SCC, squamous cell carcinoma

Table S6: *NOTCH1* mutational status, N1-ICD levels, MYC expression in adenoid cystic carcinoma models

ACC model	<i>NOTCH1</i> mutation*	Exon	Amino acid change	Mutated region	N1-ICD levels (% positive cells)	MYC IHC (% positive cells)
ACCX14	WT	-	-	-	5-10%	50%
ACCX16	WT	-	-	-	30%	30-40%
ACCX5M1	C3011T	Exon 19	S1004L	EGF domain	30%	60-70%
ACCX6	WT	-	-	-	30%	80-90%
ACCX9	T5039A	Exon 27	I1680N	NRR region	100%	90%
POS-0912	G1094A	Exon 6	R365H	EGF domain	5%	20-30%

*Numbers correspond to codons in the *NOTCH1* coding sequence; WT, wild type.

Table S7: Primary antibodies and Catalog Numbers

Antibody	Cell Signaling Technologies	Santa Cruz	Abcam
MYC	9402		
p-Rb	9307		
N1-ICD	4147		
p21	2947		
p-ERK	4370		
CLEAVED PARP	5625		
Notch 1		sc-6014	
GAPDH	5174		
p-PRAS40	2997		
AKT	9272		
β -actin			ab8226

Table S8: RT-PCR Primers

Gene	Cat #
<i>CD24</i>	Hs02379687_s1
<i>CD44</i>	Hs01075861_m1
E-Cadherin	Hs01023894_m1
N-Cadherin	Hs00362037_m1
P-Cadherin	Hs00999918_m1
<i>DTX1</i>	Hs00269995_m1
<i>EGFR</i>	Hs01076078_m1
<i>EPCAM</i>	Hs00901885_m1
<i>FN1</i>	Hs00365052_m1
<i>FOXC1</i>	Hs01071515_s1
<i>GAPDH</i>	4352934-1101034
<i>GSC</i>	Hs00906630_g1
<i>HES1</i>	Hs00172878_m1
<i>HES4</i>	Hs00368353_g1

<i>HES5</i>	Hs01387464_g1
<i>HEY2</i>	Hs00232622_m1
<i>HEYL</i>	Hs00232718_m1
<i>IL6</i>	Hs00985639_m1
<i>KRT14</i>	Hs00265033_m1
<i>KRT19</i>	Hs00761767_s1
<i>KRT8</i>	Hs01595539_g1
<i>MME</i>	Hs00153510_m1
<i>MYC</i>	Hs99999003_m1
<i>NOTCH1</i>	Hs01062011_m1
<i>NRARP</i>	Hs01104102_s1
<i>PTCRA</i>	Hs00300125_m1
<i>SHQ1</i>	Hs00250772_m1
<i>SMA4</i>	Hs02341953_m1
<i>SNAI1</i>	Hs00195591_m1
<i>SNAI2</i>	Hs00950344_m1
<i>SNAI3</i>	Hs01018996_m1
<i>SOX10</i>	Hs00366918_m1
<i>SPARC</i>	Hs00234160_m1
<i>TGFB1</i>	Hs00998133_m1
<i>TP63</i>	Hs00978343_m1
<i>TWIST1</i>	Hs01675818_s1
<i>VIM</i>	Hs00185584_m1
<i>ZEB1</i>	Hs00232783_m1