

Gene	Primers	Annealing temperature (°C)	Cycle number	Amplicon size (bp)
Notch1	Forward: AGGACCTCATCAACTCACACGC Reverse: TCTTTGTTAGCCCCGTTCTTCAG	66	30	130*
Notch2	Forward: GCTGCAAGTATGCATCAGTGC Reverse: GATTTGAGCATCACAGCCAATT	60	27	952
Notch3	Forward: CTTGCAGCGTGACCGAGA Reverse: CAGGCAGCTCCTCTTCTTGTAGTC	64	30	171*
Notch4	Forward: CATGGTTGGGATGTCCTGA Reverse: CGAGCCTGCAGACAAGCT	60 +5%DMSO	35	858
Nodal	Forward: CTTCTCCTTCCTGAGCCAACAAGAGG Reverse: GGTGACCTGGGACAAAGTGACAGTG	64	30	202
GAPDH	Forward: TGAAGGTCGGAGTCAACGGATTTGGT Reverse: CATGTGGGCCATGAGGTCCACCAC	68	25	983

*Watanabe *et al*, J Cell Biol. 2009

Supplemental Table 1: Primer pairs and conditions utilized in semi-quantitative PCR.

Primary Antibody	Dilution (Application)	Company
Rabbit anti-Notch1	1:1000 (WB)	Abcam, Cambridge, MA
Rabbit anti-Notch2	1:1000 (WB)	Abcam
Rabbit anti-Notch3	1:1000 (WB)	Abcam
Goat anti-Notch4 (C-19)	1:1000 (WB) 1:100 (IF)	Santa Cruz Biotechnology, Santa Cruz, CA
Goat anti-Notch4 (N-17)	1:60 (B/N-VM/CL) 1:40 (B/N-OA)	Santa Cruz Biotechnology
Rabbit anti-Notch4 (H-225)	1:150 (IHC)	Santa Cruz Biotechnology
Rabbit anti-Nodal (H-110)	1:2000 (WB) 1:100 (IF)	Santa Cruz Biotechnology
Mouse anti-Nodal	1:250 (IHC)	Abcam
Mouse anti-Actin	1:10,000 (WB)	Chemicon/Millipore, Billerica, MA
Rabbit anti-HistoneH3	1:1000 (WB)	Cell Signaling Technology, Danvers, MA
Rabbit anti-phospho-HistoneH3 (Ser10)	1:1000 (WB)	Cell Signaling Technology
Mouse anti-PCNA (PC10)	1:5000 (WB)	Santa Cruz Biotechnology
Rabbit anti-PARP	1:1000 (WB)	Cell Signaling Technology
Rabbit anti-cleaved PARP (Asp214)	1:1000 (WB)	Cell Signaling Technology
Mouse anti-cadherin5 (VE-cadherin)	1:1000 (WB)	BD Biosciences, San Jose, CA

Key: WB, western blotting; IF, immunofluorescence; B/N-VM/CL, blocking/neutralizing (vasulogenic mimicry/clonogenic assays); B/N-OA, blocking/neutralizing (other assays); IHC, immunohistochemistry

Supplemental Table 2: Primary antibodies utilized in this study.

STAGE	PROTEIN	EXPRESSION			
		NONE	WEAK	MODERATE	STRONG
I-II (EARLY)	NODAL	0/36	5/36	18/36	13/36
	NOTCH4	4/36	8/36	18/36	6/36
III-IV (ADVANCED)	NODAL	0/25	2/25	7/25	16/25
	NOTCH4	1/25	6/25	8/25	10/25

Supplemental Table 3: Summary of Nodal and Notch4 immunohistochemistry analyses in melanoma tissue samples. Protein expression is designated as none, weak (<25%), moderate (25-50%), or strong (>50%). Staging is as indicated on the tissue array data sheet.

		<i>Gene expression compared to control (1.0)</i>			
<i>Cell line</i>	<i>siRNA</i>	Notch1	Notch2	Notch3	Notch4
C8161	none	1.0	1.0	NE	1.0
C8161	Negative control	1.07	1.01	NE	0.74
C8161	Notch1	0.48	0.93	NE	0.88
C8161	Notch2	1.44	0.32	NE	0.89
C8161	Notch3	1.04	1.18	NE	0.81
C8161	Notch4	1.29	1.12	NE	0.26

		<i>Gene expression compared to control (1.0)</i>			
<i>Cell line</i>	<i>siRNA</i>	Notch1	Notch2	Notch3	Notch4
MV3	none	1.0	1.0	1.0	1.0
MV3	Negative control	1.13	1.04	0.95	0.93
MV3	Notch1	0.45	1.05	0.90	0.93
MV3	Notch2	1.05	0.24	0.72	1.01
MV3	Notch3	1.06	1.00	0.52	1.10
MV3	Notch4	1.05	1.03	1.20	0.35

Key: NE, not expressed

Supplemental Table 4: Notch siRNA specificity for respective target genes. RNA was isolated from cells transfected with siRNA to each Notch receptor, and then analyzed for expression of all four Notch receptors compared with untreated control cells. Expression of each targeted receptor is shown in bold.