



ANTIBODIES, KITS, AND REAGENTS TO TARGET

# Developmental Signaling Pathways and the Link to Cancer



# Developmental Signaling Pathways and the Link to Cancer

Developmental signaling pathways, including Wnt, Hedgehog, Hippo, Notch, and BMP/TGF $\beta$ , play a fundamental role in the dynamic transformation of a single-celled zygote into a highly complex multicellular organism. These signaling pathways regulate the core cellular processes, including proliferation, differentiation, and migration, that collectively underlie organismal growth and development.<sup>1,2</sup> Importantly, the role of these signaling pathways is not restricted to early development. They also function to regulate cellular activity within adult stem cell niches, which serve to maintain the functional integrity of tissues and organs.<sup>3</sup> It is now widely recognized that aberrant regulation of these developmental signaling pathways can lead to disease states, including cancer.<sup>4,5</sup> The processes of tumorigenesis and metastasis are commonly viewed as uncontrolled manifestations of basic developmental processes, such as cell proliferation, epithelial-mesenchymal transition (EMT), and migration, which are regulated by core developmental signaling pathways.<sup>6</sup> Research studies that examine the fundamental mechanisms underlying normal development will therefore contribute to a greater understanding of mechanisms underlying the origin and progression of cancer. This will help lead to the discovery and development of novel strategies for therapeutic intervention.

## References:

1. Basson, M. A. (2012) *Cold Spring Harb Perspect Biol.* 4: a008151
2. Minseong, K. et al. (2014) *BMB Rep.* 47, 540–5
3. Sancho, R. et al. (2015) *Embo Rep.* 16, 571–81
4. Briscoe, J. (2013) *Nat Rev Mol Cell Biol.* 14, 416–29
5. Ring, A. et al (2014) *Stem Cell Rev.* 10: 512–25
6. Zavadil, J. et al. (2005) *Oncogene.* 24, 5764–74

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## Developmental Biology and Cancer

# CST has antibodies, kits, and reagents

for each stage of the  
experimental process.



### Primary Antibodies

Over 600 unconjugated and directly conjugated primary antibodies targeted against more than 300 proteins. The portfolio is continually expanding, so please check our website frequently for a complete, up-to-date product list.

### SimpleChIP® Chromatin IP Kits

These kits contain all the reagents needed to perform successful ChIP assays in cultured cells and tissue samples. ChIP buffer reagents for preparing, immunoprecipitating, and purifying DNA are also sold individually. PCR primers are available and can be used to amplify positive control sequences or used as a negative control to demonstrate antibody specificity.

### SignalSilence® siRNA

Rigorously validated siRNAs can be used to selectively silence a protein of interest.

### Experimental Controls

Cell extracts, proteins, blocking peptides, and isotype controls are available to help you verify antibody specificity.

### Companion Products

Secondary antibodies, growth factors and cytokines, loading controls, buffers, dyes, chemical modulators, detection reagents, protease inhibitors, proteases, and peptide standards are available to support your protocol.

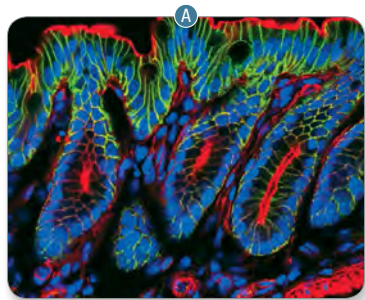
### Custom Products

Our custom department will work with you if you require a product in a specific size or formulation for your assay platform, or if you need a product validated using a specific measure or assay.

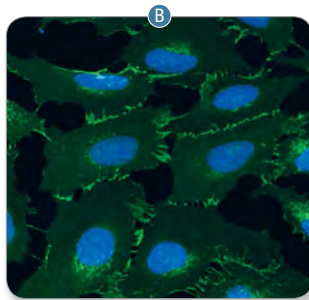
# Tools to Support Your Workflow

## PROTEIN EXPRESSION AND LOCALIZATION

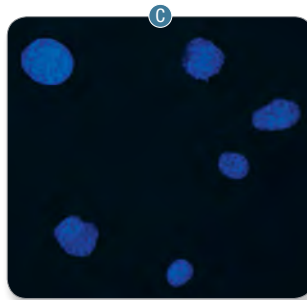
Antibodies to assess expression of key developmental biology targets



Mouse Colon



HeLa

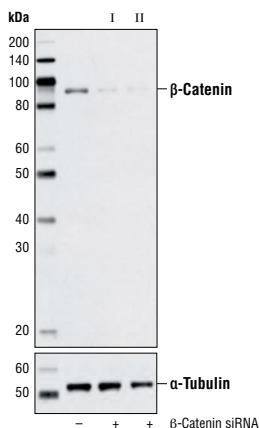


NCI-H28

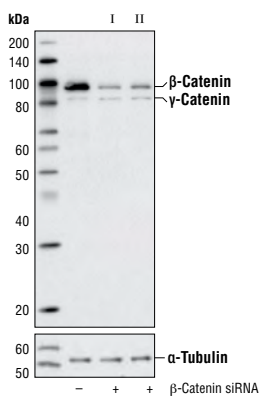
◀ **β-Catenin (D10A8) XP® Rabbit mAb #8480:** Confocal IF analysis of mouse colon (A), HeLa cells (β-Catenin-positive, B), and NCI-H28 cells (β-Catenin-negative, C) using #8480 (green). Actin filaments were labeled with DyLight™ 554 Phalloidin #13054 (red). Blue pseudocolor=DRAQ5® #4084 (fluorescent DNA dye).

## EXPERIMENTAL CONTROLS

SignalSilence® siRNAs to confirm target specificity



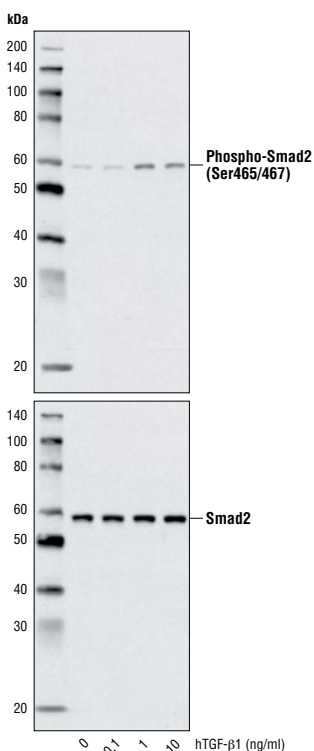
◀ **SignalSilence® β-Catenin siRNA I #6225:** WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), #6225 (+), or SignalSilence® β-Catenin siRNA II #6238 (+), using β-Catenin (6B3) Rabbit mAb #9582 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The β-Catenin antibody confirms silencing of β-Catenin expression, while the α-tubulin rabbit mAb is used as a loading control.



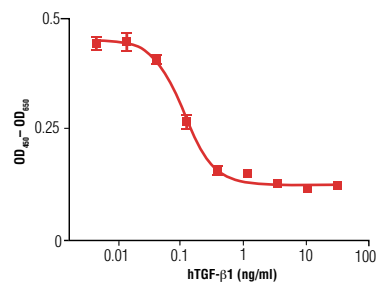
◀ **SignalSilence® β-Catenin siRNA I #6225:** WB analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), #6225 (+), or SignalSilence® β-Catenin siRNA II #6238 (+), using β-Catenin Antibody #9562 (upper) or α-Tubulin (11H10) Rabbit mAb #2125 (lower). The β-Catenin antibody recognizes both β-Catenin and γ-Catenin and demonstrates the specificity of β-Catenin expression silencing. The α-Tubulin rabbit mAb is used as a loading control.

## GROWTH FACTORS AND CYTOKINES

Treat cells with a chemical modulator to induce differentiation



◀ **Human Transforming Growth Factor β1 (hTGF-β1) #8915:** WB analysis of extracts from HeLa cells, untreated or treated with indicated concentration of #8915 for 25 min, using Phospho-Smad2 (Ser465/467) (138D4) Rabbit mAb #3108 (upper) and Smad2 (86F7) Rabbit mAb #3122 (lower).

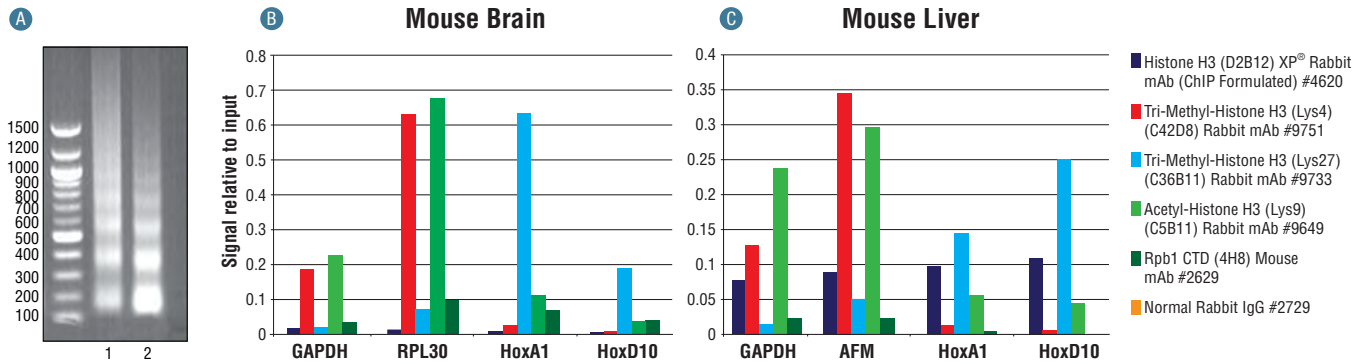


▶ **Human Transforming Growth Factor β1 (hTGF-β1) #8915:** The inhibition of IL-4 induced proliferation in HT-2 cells treated with increasing concentrations of #8915 was assessed. After 48 hour treatment with #8915, cells were incubated with a tetrazolium salt and the OD<sub>450</sub> - OD<sub>650</sub> was determined.



## QUANTITATIVE ASSAYS

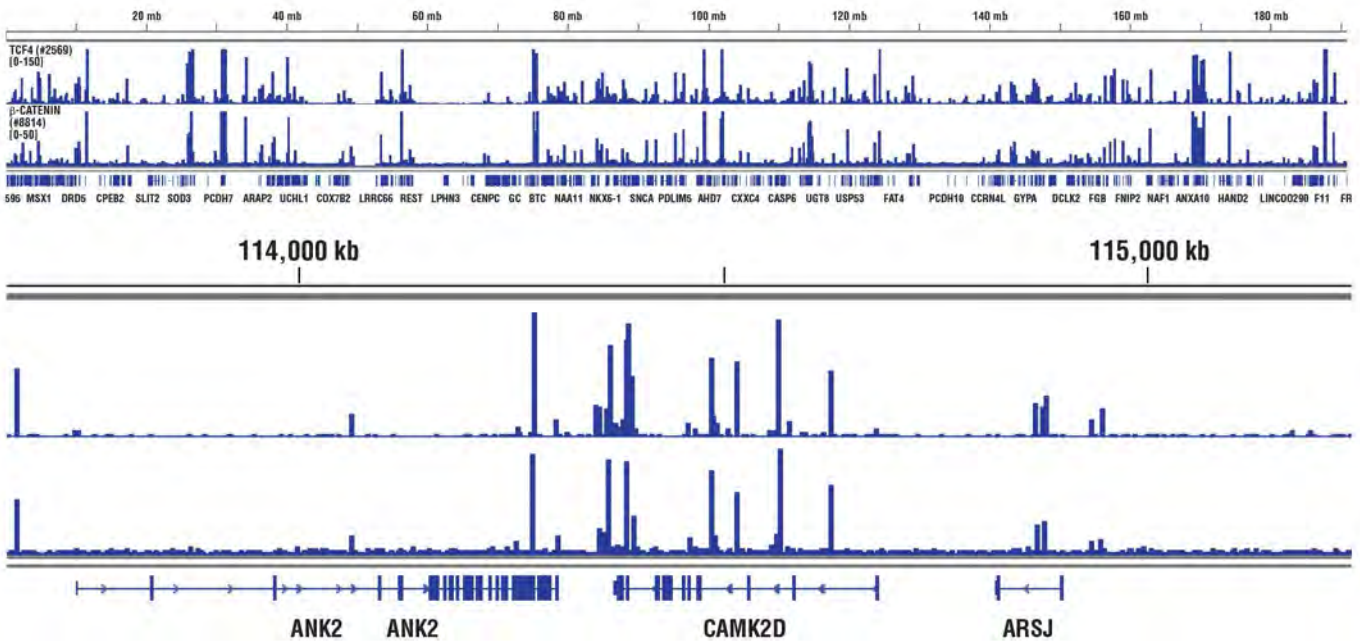
ChIP validated kits, primers, and antibodies to examine protein-DNA interactions



**SimpleChIP<sup>®</sup> Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005:** Mouse brain or mouse liver were cross-linked and disaggregated into a single-cell suspension using a Dounce homogenizer or tissue disaggregator, respectively. The chromatin was prepared and digested. DNA was purified, and 10  $\mu$ l was separated by electrophoresis on a 1% agarose gel and stained with ethidium bromide. The majority of chromatin from both brain (lane 1) and liver (lane 2) was digested to 1 to 5 nucleosomes in length (150 to 900 bp) (A).

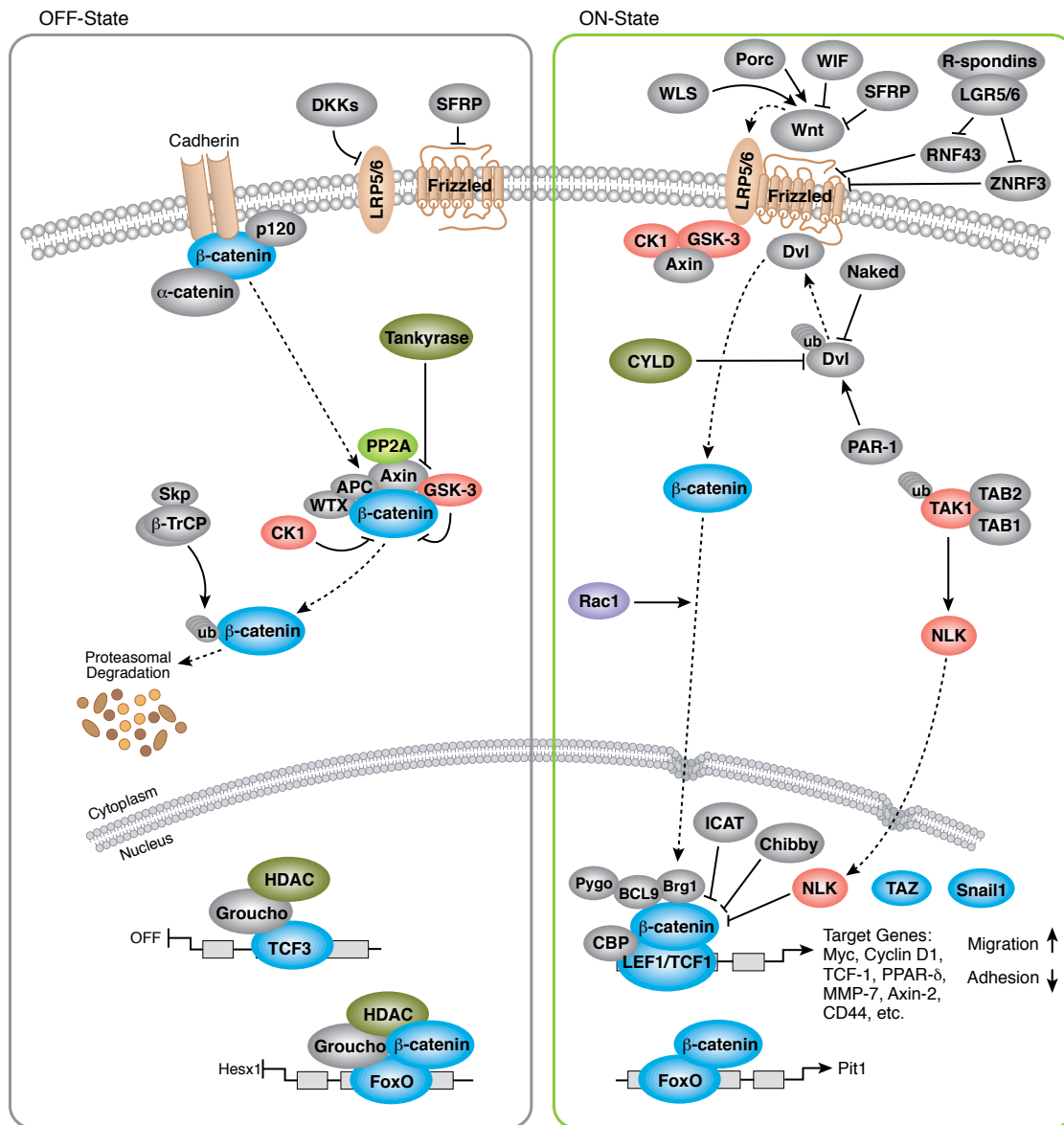
Chromatin immunoprecipitations were performed using the indicated ChIP-validated antibodies. Purified DNA was analyzed by quantitative real-time PCR using SimpleChIP<sup>®</sup> Mouse GAPDH Intron 2 Primers #8986, SimpleChIP<sup>®</sup> Mouse RPL30 Intron 2 Primers #7015, SimpleChIP<sup>®</sup> Mouse HoxA1 Promoter Primers #7341, and SimpleChIP<sup>®</sup> Mouse HoxD10 Exon 1 Primers #7429 in mouse brain (B) and using SimpleChIP<sup>®</sup> Mouse GAPDH Intron 2 Primers #8986, SimpleChIP<sup>®</sup> Mouse AFM Intron 2 Primers #7269, SimpleChIP<sup>®</sup> Mouse HoxA1 Promoter Primers #7341, and SimpleChIP<sup>®</sup> Mouse HoxD10 Exon 1 Primers #7429 in mouse liver (C). The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin (equivalent to 1).

### ChIP-seq analysis was performed using SimpleChIP<sup>®</sup> Enzymatic Chromatin IP Kit (Magnetic Beads) #9003



**Non-phospho (Active)  $\beta$ -Catenin (Ser33/37/Thr41) (D13A1) Rabbit mAb #8814:** ChIP was performed with cross-linked chromatin from  $4 \times 10^6$  HCT116 cells and either 10  $\mu$ l of TCF4 (C48H11) Rabbit mAb #2569 or 5  $\mu$ l of #8814, using SimpleChIP<sup>®</sup> Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared from 5ng enriched ChIP DNA using NEBNext<sup>®</sup> Ultra<sup>™</sup> II DNA Library Prep Kit for Illumina<sup>®</sup>, and sequenced on the Illumina NextSeq. TCF4 and  $\beta$ -Catenin are known to associate with each other on chromatin. The figure shows binding of both TCF4 and  $\beta$ -Catenin across chromosome 4 (upper), including CAMK2D (lower), a known target gene of both TCF4 and  $\beta$ -Catenin.

# Wnt/ $\beta$ -Catenin Signaling Pathway

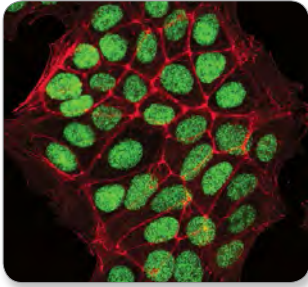


The Wnts comprise a family of secreted glycoproteins that bind to cell surface receptors and activate intracellular signaling pathways.<sup>1</sup> Wnt signaling is mediated through the canonical  $\beta$ -catenin pathway, the non-canonical planar cell polarity pathways, and the non-canonical Wnt/calcium pathway. Among these, the canonical Wnt/ $\beta$ -catenin signaling pathway is most frequently implicated in cancer.<sup>1,2</sup> Canonical Wnt signaling regulates myriad developmental and homeostatic functions, including cell proliferation, differentiation, and fate determination.<sup>1</sup> Wnt/ $\beta$ -catenin signaling also plays a vital role in promoting self-renewal and pluripotency of stem and progenitor cells during both embryonic development and adult tissue homeostasis.<sup>3</sup> In canonical Wnt signaling,  $\beta$ -catenin functions as a co-activator of the TCF/LEF family of transcription factors that regulate the expression of Wnt target genes. Mutations in  $\beta$ -catenin, or proteins that regulate  $\beta$ -catenin stability, lead to increased levels of nuclear  $\beta$ -catenin, and the activation of Wnt target genes, thereby predisposing cells to tumorigenesis.<sup>2</sup> Elevated Wnt signaling has been reported to enhance tumorigenesis by promoting cellular proliferation and EMT, thereby driving metastasis.<sup>4</sup> In addition, loss of function mutations or epigenetic silencing of genes repressing the Wnt/ $\beta$ -catenin signaling pathway have been implicated by research studies in the development of cancer, making this signaling pathway an attractive therapeutic target.<sup>5,6</sup>

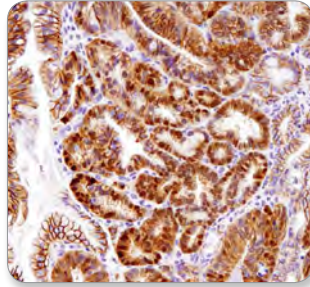
## References:

1. Angers, S. et al. (2009) *Nat Rev Mol Cell Biol.* 10, 468–77
2. MacDonald, B.T. et al. (2009) *Dev Cell.* 17, 9–26
3. Ring, A. et al (2014) *Stem Cell Rev.* 10: 512–25
4. Anastas J. N. (2013) *Nat Rev Can.* 13, 11–26
5. Zheng, K. et al. (2016) *Cancer Lett.* 376, 178–87
6. Polakis, P (2012) *Cold Spring Harb Perspect Biol.* 4, a008052

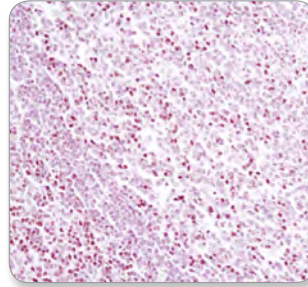
## PROTEIN EXPRESSION



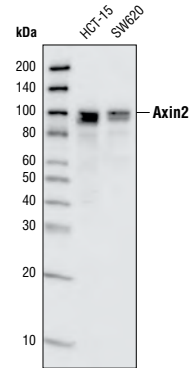
▲ **LEF1 (C12A5) Rabbit mAb #2230:** Confocal IF analysis of HCT-15 cells using #2230 (green). Actin filaments have been labeled with DyLight™ 554 Phalloidin #13054 (red).



▲ **Non-phospho (Active) beta-Catenin (Ser45) (D2U8Y) XP® Rabbit mAb #19807:** IHC analysis of paraffin-embedded Apc (Min/+) mouse intestinal adenoma using #19807.

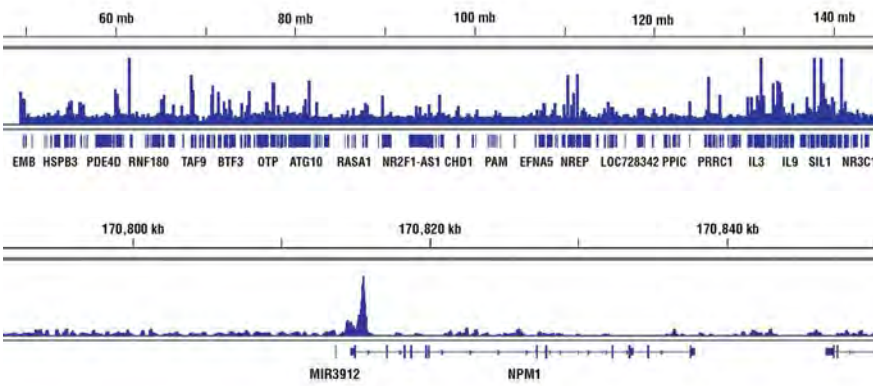


▲ **TCF1/TCF7 (C63D9) Rabbit mAb #2203:** IHC analysis of paraffin-embedded human tonsil using #2203.

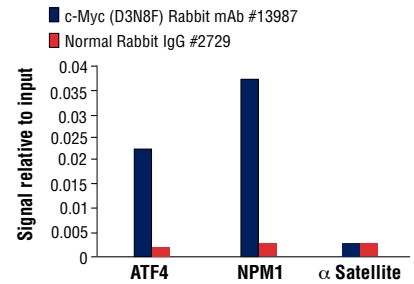


▲ **Axin2 (7666) Rabbit mAb #2151:** WB analysis of total cell lysates from HCT-15 and SW620 cells using #2151.

## QUANTITATIVE ASSAYS



▲ **c-Myc (D3N8F) Rabbit mAb #13987:** ChIP was performed with cross-linked chromatin from  $4 \times 10^6$  Daudi cells and 10  $\mu$ l of #13987 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared from 5 ng enriched ChIP DNA using NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®, and sequenced on the Illumina NextSeq. The figure shows binding across chromosome 5 (upper), including NPM1 (lower), a known target gene of c-Myc.



▲ **c-Myc (D3N8F) Rabbit mAb #13987:** ChIP was performed with cross-linked chromatin from  $4 \times 10^6$  Daudi cells and either 10  $\mu$ l of #13987 or 2  $\mu$ l of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human ATF4 promoter primers, SimpleChIP® Human NPM1 Intron 1 Primers #4779, and SimpleChIP® Human alpha Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

## WNT/ $\beta$ -CATENIN SIGNALING PATHWAY TARGETS

Axin: 1, 2  
 BCL9  
 Phospho-BCL9L (Ser915)  
 CACYBP  
 $\beta$ -Catenin  
 Acetyl- $\beta$ -Catenin (Lys49)  
 Non-phospho (Active)  $\beta$ -Catenin: (Ser33/Ser37/Thr41), (Thr41/Ser45)  
 Phospho- $\beta$ -Catenin: (Ser33/Ser37), (Ser33/Ser37/Thr41), (Ser37/Thr41/Ser45), (Ser552), (Ser675)

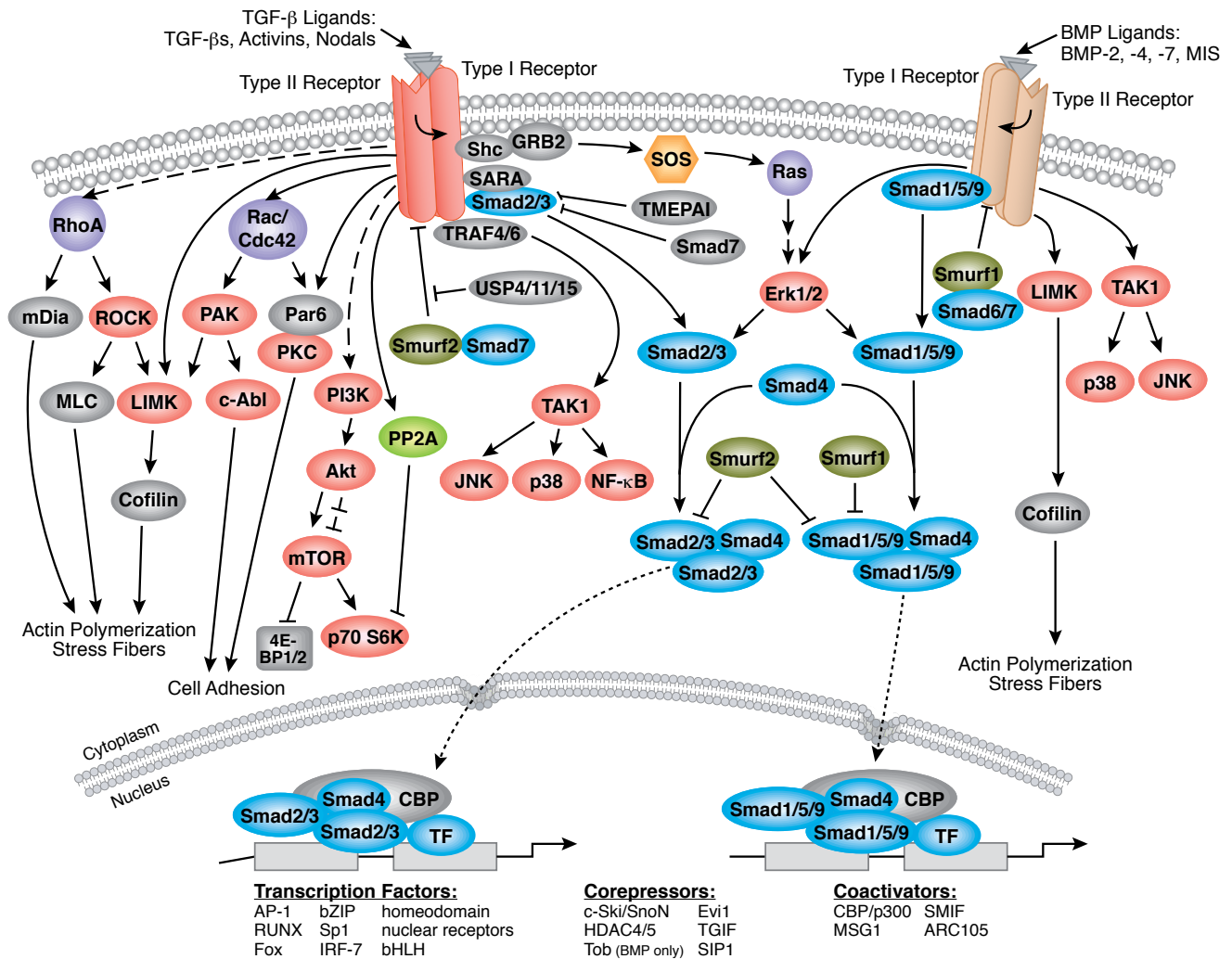
$\alpha$ -E-Catenin  
 Phospho- $\alpha$ -E-Catenin: (Ser652), (Ser655/Thr658)  
 $\alpha$ -N-Catenin  
 CDC73  
 CK1  
 DIXDC1  
 DKK: 1, 2  
 Dvl: 2, 3  
 Frizzled: 5, 6

LEF1  
 LRP: 5, 6  
 Phospho-LRP6 (Ser1490)  
 MITF  
 Naked: 1, 2  
 NDRG2  
 PTK7  
 Scribble  
 Phospho-Scribble (Ser1220)  
 SFRP1

Sox17  
 TCF: 3, 4, 7  
 TLE1/2/3/4  
 WIF1  
 Wnt: 3a, 5a, 5a/5b  
 WTX



# TGF- $\beta$ /Smad Signaling Pathway



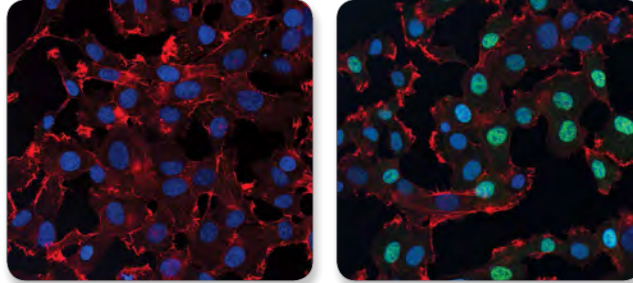
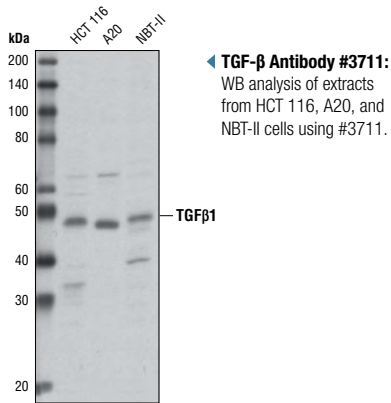
Transforming growth factor- $\beta$  (TGF $\beta$ ) superfamily signaling plays a critical role in a wide range of biological processes, including cell growth, differentiation, and development. In general, signaling is initiated by the binding of the ligand to the TGF $\beta$  receptor Type II and recruitment and activation of the TGF $\beta$  receptor Type I. This enables the recruitment and phosphorylation of the receptor-regulated SMADs (R-SMADs), which are distinct for each ligand/receptor class.<sup>1,2</sup> The activation of R-SMADs triggers them to form complexes with the co-SMAD, SMAD4, and subsequent translocation of the SMAD complex to the nucleus. Activated SMADs regulate diverse biological effects by partnering with transcription factors resulting in cell-state specific modulation of transcription.<sup>2,3</sup> The stability of TGF $\beta$  receptors and/or Smads is regulated by Smurf E3 ubiquitin ligases and USP4/11/15 deubiquitinases.<sup>4</sup> Disruption of the TGF $\beta$  pathway has critical roles in tumor initiation and development, induction of EMT, and metastasis. TGF $\beta$  signaling also plays an important role in maintaining immune homeostasis and modulating the tumor microenvironment.<sup>5,6</sup> Thus, the TGF $\beta$  signaling pathway is a promising therapeutic target for cancer therapy.

## References:

1. Massague, J. (2012) *Nat Rev Mol Cell Biol.* 13, 616–30
2. Mullen, A. C. et al. (2011) *Cell.* 147, 565–76
3. Trompouki, E. et al. (2011) *Cell.* 147, 577–89
4. Zhang, J. et al. (2014) *Protein Cell.* 5, 503–17
5. Zavadil, J. et al. (2005) *Oncogene.* 24, 5764–74
6. Moustakas, A. et al. (2014) *Biochim Biophys Acta.* 1840, 2621–34

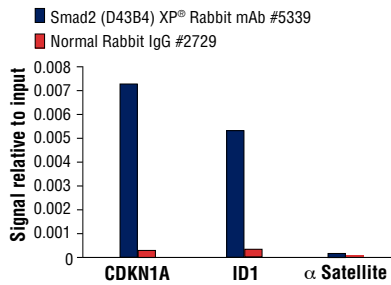


## PROTEIN EXPRESSION



**▲ Phospho-Smad1 (Ser463/465)/ Smad5 (Ser463/465)/ Smad9 (Ser465/467) (D5B10) Rabbit mAb #13820:** Confocal IF analysis of HT-1080 cells, serum-starved (overnight; left) or serum-starved and treated with Human BMP2 #4697 (50 ng/ml, 30 min; right), using #13820 (green) and  $\beta$ -Actin (13E5) Rabbit mAb (Alexa Fluor<sup>®</sup> 647 Conjugate) #8584 (red). Blue pseudo-color = Propidium iodide (PI)/RNase Staining Solution #4087.

## QUANTITATIVE ASSAYS



**◀ Smad2 (D43B4) XP<sup>®</sup> Rabbit mAb #5339:** ChIP was performed with cross-linked chromatin from  $4 \times 10^6$  HaCaT cells treated with Human TGF- $\beta$ 3 #8425 (7 ng/ml) for 1 h and either 10  $\mu$ l of #5339 or 2  $\mu$ l of Normal Rabbit IgG #2729 using SimpleChIP<sup>®</sup> Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP<sup>®</sup> Human CDKN1A Intron 1 Primers #4669, SimpleChIP<sup>®</sup> Human ID1 Promoter Primers #5139, and SimpleChIP<sup>®</sup> Human  $\alpha$  Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

## TGF- $\beta$ /SMAD SIGNALING PATHWAY TARGETS

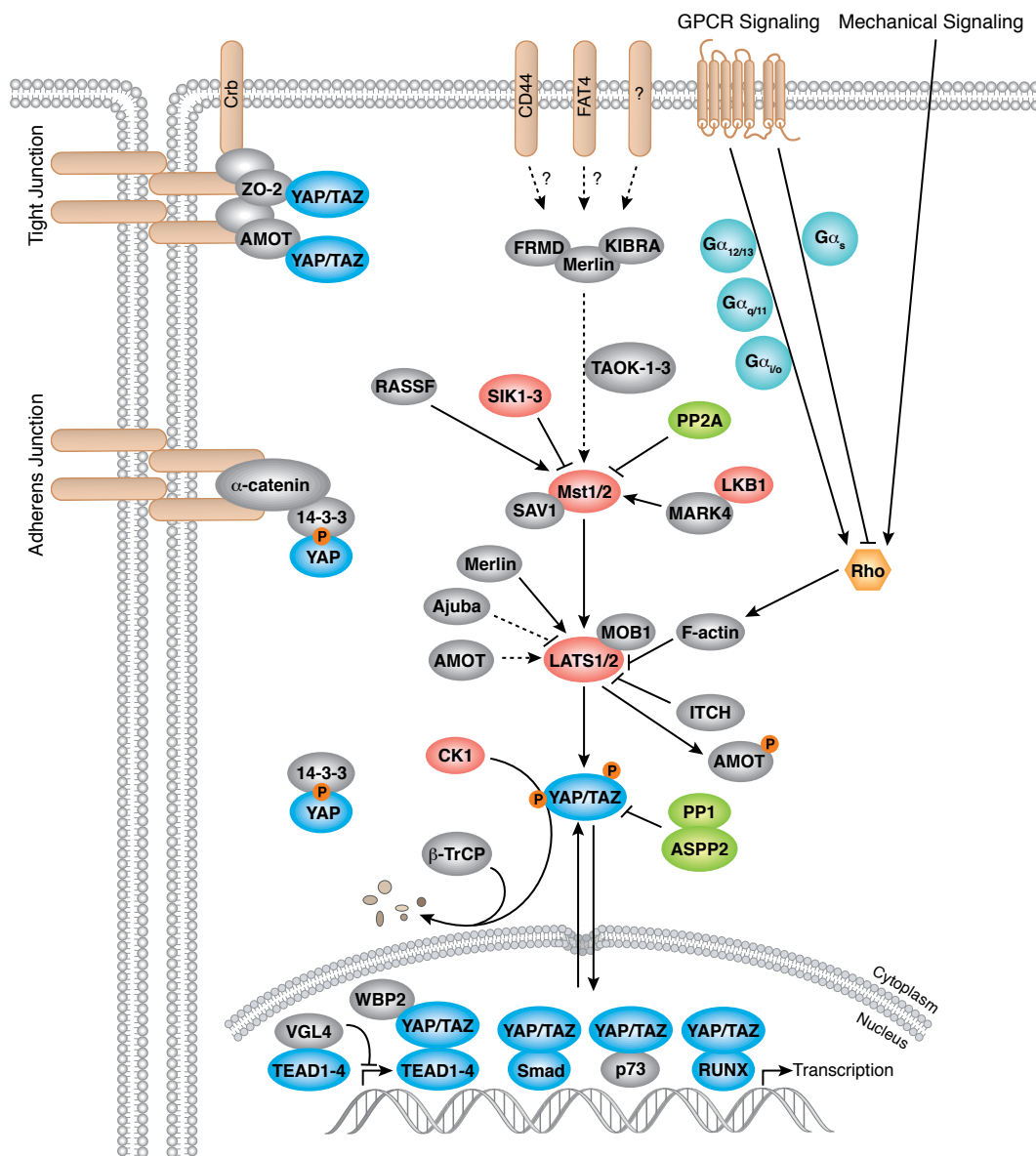
ACVR1  
BMP: 4, 6, 7  
BMPR2  
Brachyury  
Endoglin  
Endoglin (mouse specific)  
Gremlin  
Hic-5

$\beta$ IG-H3  
Mic1  
MISR2  
NFI-C  
PINCH  
Sara  
Smad: 1, 2, 3, 4, 5  
Smad 2/3

Phospho-Smad 1 (Ser206)  
Phospho-Smad 1/5 (Ser463/Ser465)  
Phospho-Smad 1 (Ser463/Ser465)/  
Smad5 (Ser463/Ser465)/Smad9  
(Ser426/Ser428)  
Phospho-Smad 2: (Ser245/Ser250/  
Ser255), (Ser465/Ser467)  
Phospho-Smad 2 (Ser465/Ser467)/  
Smad3 (Ser423/Ser425)

Phospho-Smad 3: (Ser422/Ser423/  
Ser425), (Ser423/Ser425)  
Smurf: 1, 2  
SnoN  
TGF- $\alpha$   
TGF- $\beta$  1/2/3  
TGF $\beta$  Receptor: I, II, III

# Hippo Signaling Pathway

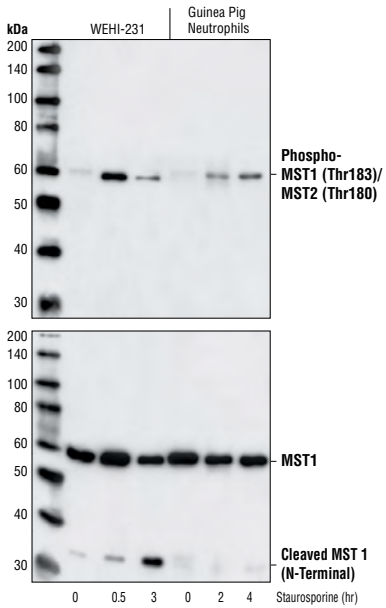


Hippo signaling is an evolutionarily conserved pathway that controls organ size by regulating cell proliferation and cell death<sup>1</sup> in response to multiple factors, including cell density, mechanical sensation, and G-protein-coupled receptor (GPCR) signaling.<sup>2</sup> Core to the Hippo pathway is a kinase cascade, wherein MST1/2 (ortholog of *Drosophila* Hippo) kinases and SAV1 form a complex to phosphorylate and activate LATS1/2 kinases. LATS1/2 in turn phosphorylates the transcriptional coactivators YAP and TAZ, leading to their functional inhibition through multiple mechanisms, including proteosomal degradation. These two major downstream effectors of the Hippo pathway are widely activated in human malignancies<sup>3</sup> YAP and TAZ interact with TEAD transcription factors to coactivate expression of target genes that promote cell proliferation.<sup>4,5</sup> Activation of YAP/TAZ induces cancer stem cell attributes and has been shown to be essential in metastasis.<sup>6</sup> The Hippo pathway is only one of the tumor suppressor pathways regulating YAP/TAZ in normal tissues. Others, including Wnt, Notch, and TGF $\beta$ , may also converge with YAP/TAZ on multiple levels.<sup>5,7</sup> Dysregulation of the Hippo pathway has been shown in research studies to contribute to cancer development, thereby making components of the Hippo pathway promising therapeutic targets.<sup>7,8</sup>

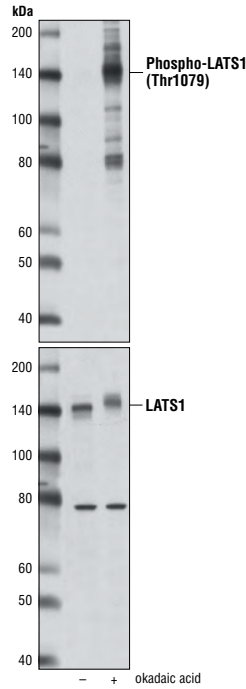
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2. Mo, J. S. et al. (2014) *Embo Rep.* 15, 642–56
3. Yu, F. et al. (2012) *Cell.* 150, 780–91
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5. Zhang, K. et al. (2015) *Biochem.* 54, 6555–66
6. Zanconato, F. et al. (2016) *Cancer Cell.* 29, 783–803
7. Yu, F. X. et al. (2015) *Cell.* 163, 811–28
8. Santucci, M. et al. (2015) *J Med Chem.* 58, 4857–73

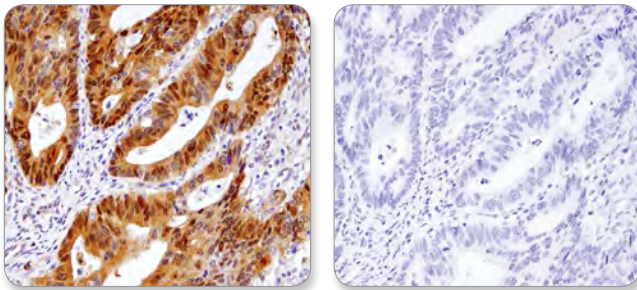
## PROTEIN EXPRESSION



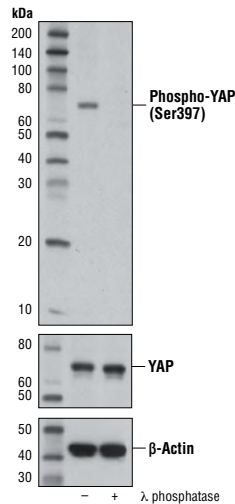
◀ **Phospho-MST1 (Thr183)/MST2 (Thr180) Antibody #3681:** WB analysis of extracts from WEHI-231 cells, and guinea pig neutrophils treated with Staurosporine #9953 for the indicated times, using #3681 (upper) and MST1 Antibody #3682 (lower).



◀ **Phospho-LATS1 (Thr1079) (D57D3) Rabbit mAb #8654:** WB analysis of extracts from HeLa cells, untreated (-) or okadaic acid-treated (+), using #8654 (upper) or LATS1 (C66B5) Rabbit mAb #3477 (lower).

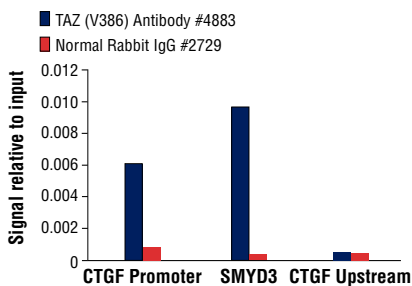


▲ **Phospho-YAP (Ser127) (D9W21) Rabbit mAb #13008:** IHC analysis of paraffin-embedded human colon adenocarcinoma, control (left) or λ-phosphatase treated (right), using #13008.



◀ **Phospho-YAP (Ser397) (D1E7Y) Rabbit mAb #13619:** WB analysis of extracts from Hep G2 cells untreated (-), or λ-phosphatase-treated (+), using #13619 (upper), YAP Antibody #4912 (middle), and β-Actin (D6A8) Rabbit mAb #8457 (lower).

## QUANTITATIVE ASSAYS



◀ **TAZ (V386) Antibody #4883:** ChIP was performed with cross-linked chromatin from  $4 \times 10^6$  NCI-2052 cells and either 10  $\mu$ l of #4883 or 2  $\mu$ l of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human CTGF Promoter Primers #14927, human SMYD3 intron 2 primers, and SimpleChIP® Human CTGF Upstream Primers #14928. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

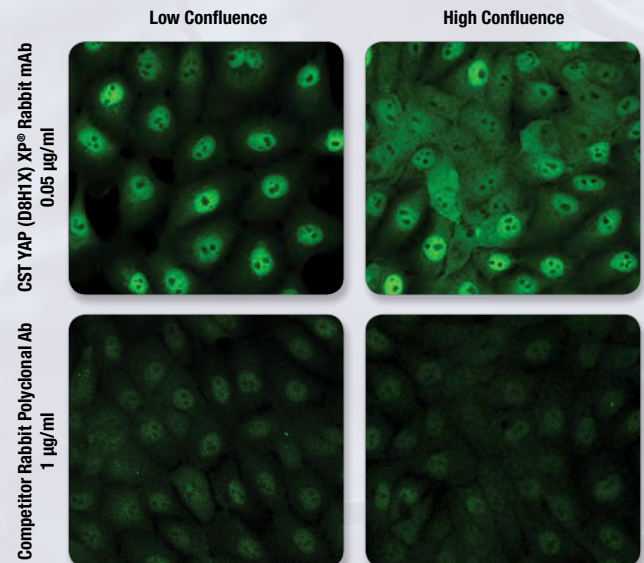


# Hippo Signaling Pathway

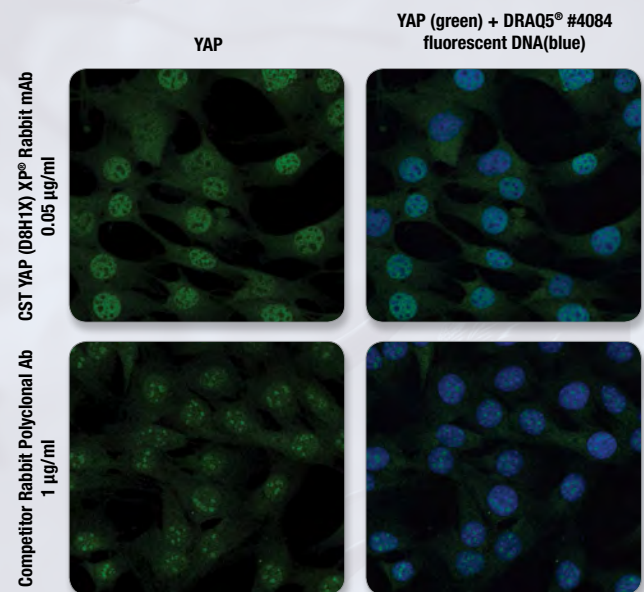
Don't let your antibody be a variable in your experiment. Use an antibody that works.

**YAP (D8H1X) XP® Rabbit mAb #14074** does not stain the nucleolus of murine cells like the competitor antibody, demonstrating superior specificity.

## MCF10A Human Mammary Epithelial Cells



## C2C12 Mouse Myoblast Cells



▲ **YAP (D8H1X) XP® Rabbit mAb #14074:** IF-IC analysis of MCF10A (upper) and C2C12 (lower) cells using #14074 and an antibody from another company.

## HIPPO SIGNALING PATHWAY TARGETS

Ajuba  
FRMD6  
ITCH  
KIBRA  
LATS1  
Phospho-LATS1: (Ser909), (Thr1079)

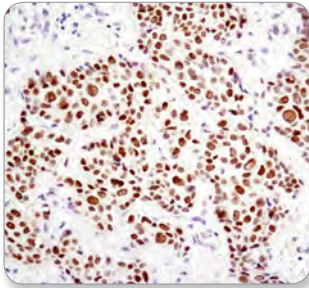
LATS2  
LIMD1  
MOB1  
Phospho-MOB1: (Thr12), (Thr35)  
MST: 1, 2

Phospho-MST1 (Thr183)/MST2 (Thr180)  
PTPN14  
SAV1  
Scribble  
TAZ

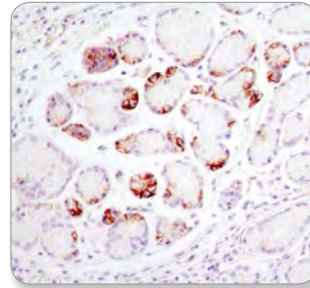
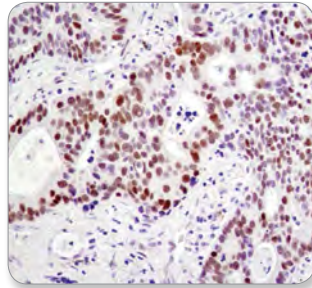
TEAD3  
Pan-TEAD  
YAP  
Phospho-YAP: (Ser127), (Ser397)  
YAP/TAZ  
WBP2

# Notch Signaling Pathway

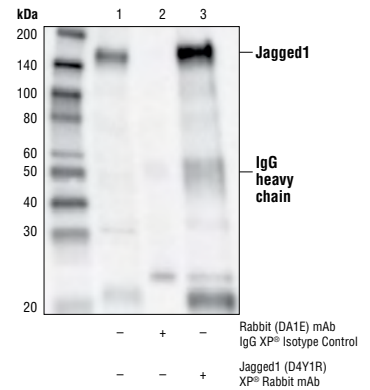
## PROTEIN EXPRESSION



**HES1 (D6P2U) Rabbit mAb #11988:** IHC analysis of paraffin-embedded human breast carcinoma (left) and paraffin-embedded human lung carcinoma (right) using #11988.

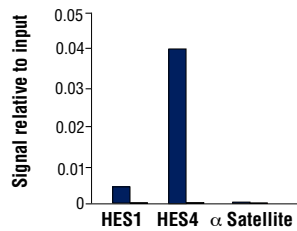
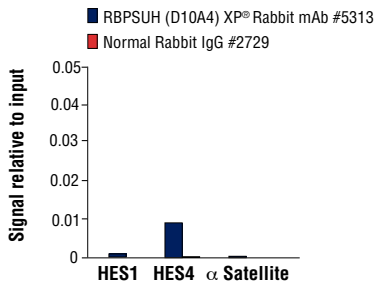


**Notch1 (D1E11) XP® Rabbit mAb #3608:** IHC analysis of paraffin-embedded human stomach adjacent to MALT (mucosa-associated lymphoid tissue) lymphoma using #3608.

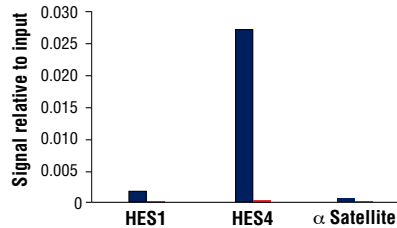
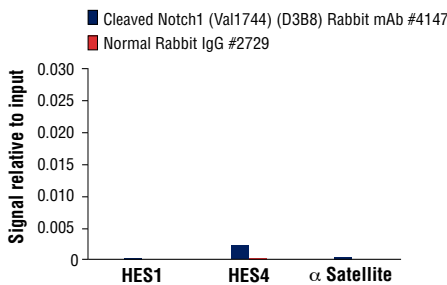


**Jagged1 (D4Y1R) XP® Rabbit mAb #70109:** IP of Jagged1 protein from HT-29 cell extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) XP® IgG Isotype Control #3900, and lane 3 is #70109. WB analysis was performed using #70109.

## QUANTITATIVE ASSAYS



◀ **RBPSUH (D10A4) XP® Rabbit mAb #5313:** CUTLL1 cells were cultured in media with  $\gamma$ -secretase inhibitor (1  $\mu$ M, 3 d) and then either harvested immediately (left panel) or washed and cultured in fresh media for 3 hr (right panel). ChIP was performed with cross-linked chromatin from  $4 \times 10^6$  cells and 10  $\mu$ l of #5313 or 2  $\mu$ l of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human HES1 promoter primers, SimpleChIP® Human HES4 Promoter Primers #7273, and SimpleChIP® Human  $\alpha$  Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.



◀ **Cleaved Notch1 (Val1744) (D3B8) Rabbit mAb #4147:** CUTLL1 cells were cultured in media with  $\gamma$ -secretase inhibitor (1  $\mu$ M) for 3 days and then either harvested immediately (left panel) or washed and cultured in fresh media for 3hr (right panel). ChIP was performed with cross-linked chromatin from  $4 \times 10^6$  cells and 2.5  $\mu$ l of #4147 or 2  $\mu$ l of Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using human HES1 promoter primers, SimpleChIP® Human HES4 Promoter Primers #7273, and SimpleChIP® Human  $\alpha$  Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

## NOTCH SIGNALING PATHWAY TARGETS

Deltex-2

DKL1

DLL: 1, 3, 4

HES1

Jagged: 1, 2

Lunatic Fringe

MAML: 1, 2

Notch: 1, 2, 3, 4

Cleaved Notch1 (Val1744)

Numb

Phospho-Numb (Ser276)

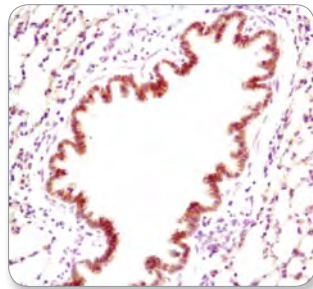
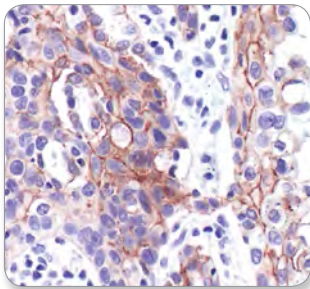
RBPSUH

TRIB2

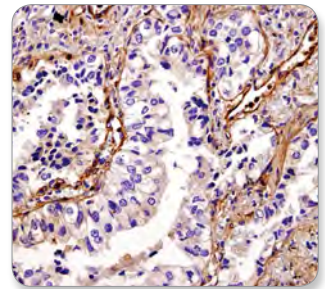
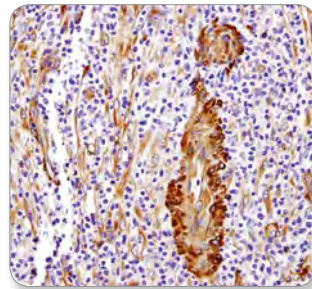


# Epithelial-Mesenchymal Transition (EMT) Signaling

## PROTEIN EXPRESSION

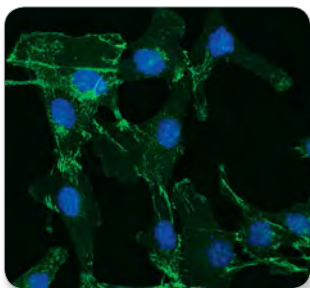


**E-Cadherin (24E10) Rabbit mAb #3195:** IHC analysis of paraffin-embedded human lung carcinoma (left) and paraffin-embedded mouse lung (right) using #3195.

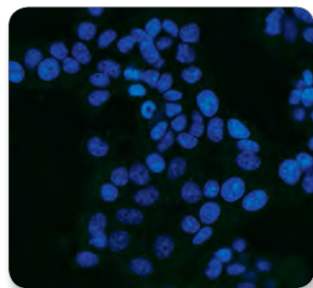


**Vinculin (E1E9V) XP® Rabbit mAb #13901:** IHC analysis of paraffin-embedded human breast ductal carcinoma *in situ* (left) and paraffin-embedded human non-small cell lung carcinoma (right) using #13901.

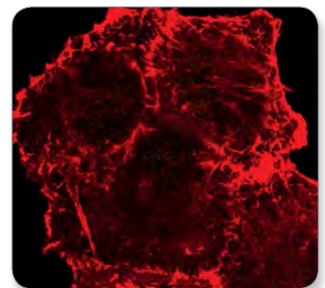
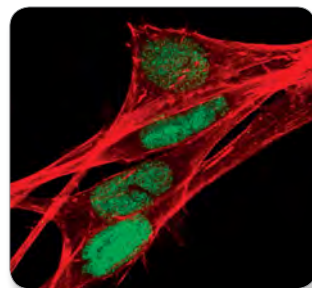
A172



MCF7



**N-Cadherin (D4R1H) XP® Rabbit mAb #13116:** Confocal IF analysis of A172 (left, positive) and MCF7 (right, negative) cells using #13116 (green). Blue pseudocolor= DRAQ5® #4084 (fluorescent DNA dye).



**Slug (C19G7) Rabbit mAb #9585:** Confocal IF analysis of A-204 cells (left, positive) and PANC-1 cells (right, negative) using #9585 (green). Actin filaments have been labeled with DyLight™ 554 Phalloidin #13054 (red).

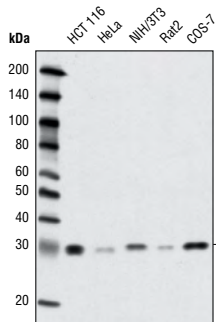
## EMT SIGNALING IN NORMAL DEVELOPMENT AND CANCER

Epithelial-mesenchymal transition (EMT) is central to both developmental and pathological processes. It is an important phenomenon in normal cell differentiation during early development, but can become pathological with the reactivation of developmental programs in adults.<sup>1</sup> During EMT, differentiated epithelial cells acquire mesenchymal fibroblast-like properties and display reduced intercellular adhesion and increased motility. During normal development, these cellular changes are fundamental for enabling tissue morphogenesis and are mediated by select transcription factors, including Snail, Slug, TCF8/ZEB1, and Twist.<sup>2</sup> Each of these factors is regulated at the transcriptional, translational, and post-translational levels.<sup>2</sup> Due to the acquisition of migratory, invasive, and stem cell properties, the mesenchymal state allows cells to migrate, thereby contributing to tissue morphogenesis. However, under pathological conditions, EMT-like changes can contribute to initiation of metastasis. EMT is thus utilized by malignant epithelial tumors to spread beyond their origin and can be driven by TGF $\beta$  and other pathways.<sup>2,3,4</sup> Reduced expression of cell adhesion molecules is a key feature of cells undergoing EMT. For example, E-cadherin is a critical component of adherens junctions and can actively suppress invasion and growth of epithelial cancers.<sup>5</sup> Research studies have shown that cancer cells typically downregulate expression of E-cadherin and upregulate expression of N-cadherin.<sup>4,5</sup> This is referred to as the cadherin switch and is one of the hallmarks of EMT.<sup>6</sup> Understanding the molecular events that promote EMT is important in designing therapies against human cancer.

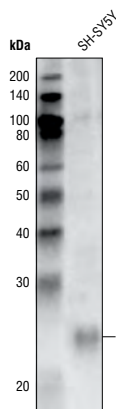
## EMT SIGNALING PATHWAY TARGETS

E-Cadherin	CYR61	Integrin $\alpha$ 5	MMP: 2, 3, 7, 9, 14	RBPSUH	Smad: 1, 2, 3, 4, 5, 8/9	SPARC
N-Cadherin	Desmoplakin	Jagged1	Moesin	Sara		TAZ
Caldesmon-1	FoxC2	Keratin 19	MTI-MMP	PAI-1	Smurf: 1, 2	TCF4
$\beta$ -Catenin	GLI: 1, 2	LEF1	Notch1	Shh	Snai1	TCF8/ZEB1
Caveolin-2	GSK-3 $\beta$	MITF	PTCH: 1, 2	Slug	Sox: 9, 10	TEAD: 1, 3





◀ **Snail (C15D3) Rabbit mAb #3879:** WB analysis of extracts from various cell lines using #3879.



◀ **TWIST1 Antibody #46702:** WB analysis of extract from SH-SY5Y cells using #46702.

#### References:

1. Kalluri, R. et al. (2009) *J Clin Invest.* 119, 1420–28
2. Lamouille, S. et al. (2014) *Nat Rev Mol Cell Biol.* 15, 178–96
3. Tsai, J. H. et al. (2013) *Genes and Development.* 27, 2192–206
4. Zavadil, J. et al. (2005) *Oncogene.* 24, 5764–74
5. Rodriguez, F. J. et al. (2012) *Biochem Biophys Acta.* 1826, 23–31
6. Lehenbre, F. et al. (2008) *EMBO J.* 27, 2603–15

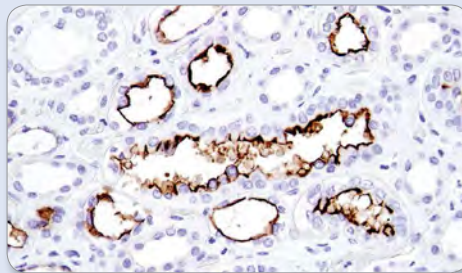
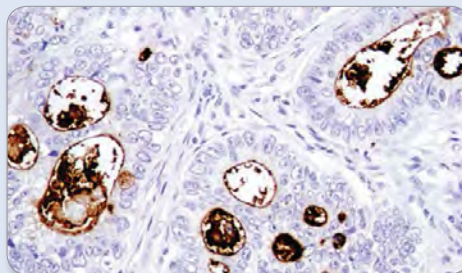
Pan-TEAD	Wnt5: a, b
TGF- $\beta$ 1/2/3	YAP
TIMP1	Zyxin
Twist1	
Vimentin	

## Cancer Stem Cells

The growth and maintenance of some tumor types is hypothesized to be driven by a self-renewing population of tumor-initiating cells, termed cancer stem cells (CSCs).<sup>1</sup> While the precise origins of CSCs have been a source of considerable debate, research studies increasingly support a role for CSCs in tumor progression, including the development of tumor heterogeneity, which represents a significant therapeutic obstacle in oncology.<sup>1,2</sup> Moreover, many putative CSCs exhibit resistance to traditional therapies, thereby promoting tumor recurrence following cessation of treatment.<sup>2,3</sup> Studies focused on identifying and characterizing the origin, behavior, and regulation of CSCs could therefore contribute to the development of novel anti-tumor therapies that deliver sustained positive outcomes.

#### References:

1. Clevers, H. (2011) *Nat Med.* 17, 313–19
2. Plaks, V. et al. (2015) *Cell Stem Cell.* 16, 225–38
3. Dawood, S. et al. (2014) *Oncology* 12, 1101–7



▲ **CD133 (D4W4N) XP® Rabbit mAb #86781:** IHC analysis of paraffin-embedded human colon carcinoma (upper) and paraffin-embedded human kidney (lower) using #86781.

## Antibodies for the Study of Cancer Stem Cells

#86781	CD133 (D4W4N) XP® Rabbit mAb
#45208	TIM-3 (D5D5R™) XP® Rabbit mAb
#3570	CD44 (156-3C11) Mouse mAb
#42078	ABCG2 (D5V2K) XP® Rabbit mAb
#39141	Olfm4 (D6Y5A) XP® Rabbit mAb (Mouse Specific)
#36746	EpCAM (D4K8R) XP® Rabbit mAb
#36671	ALDH1A1 (D9J7R) XP® Rabbit mAb
#4744	SSEA1 (MC480) Mouse mAb



Troy, Product Scientist, has been with CST since 2010.

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COVER IMAGE: Three-dimensional cancer cell rendering. Jovan Vitanovski/Shutterstock

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