Hunting for Hippo proteins

With highly specific ABfinity recombinant monoclonal antibodies.

The Hippo signaling pathway is an evolutionarily conserved pathway that has been shown to play a critical role in controlling organ size through the regulation of both cell proliferation and apoptosis. Dysregulation of the Hippo pathway results in aberrant cell growth and neoplasia. Given its involvement in these vital cell processes, it is not surprising that mutations in key Hippo pathway proteins are linked to a variety of cancers [1,2].

At the cellular level, the Hippo pathway integrates signals through several mechanisms, including G protein–coupled receptor (GPCR) signaling and the apicobasal polarity fundamental to epithelial cell function [3]. Kinase cascade and nuclear transcription modules form the backbone of the Hippo pathway. The kinase cascade includes serine/threonine kinases such as mammalian STE20–like protein kinases (MST1/2) and large tumor suppressors (LATS1/2), along with adaptor proteins including Salvador homolog 1 (SAV1), MOB kinase activator 1A (MOB1A), and MOB kinase activator 1B (MOB1B). The kinase cascade functions to restrict the activity of two transcriptional coactivators—Yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ)—which are further modified by several Hippo regulators.

Antibodies are essential to the study of pathway proteins. However, for many components of the Hippo pathway, specific antibodies are either unavailable or poorly characterized. To address this need, Thermo Fisher Scientific has made a concerted effort to develop highly specific, application-tested antibodies directed against key proteins in this pathway.

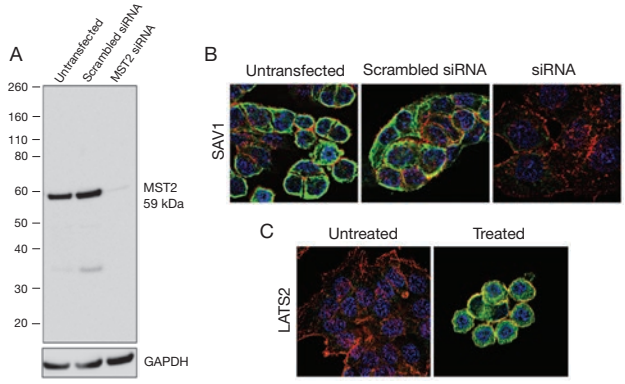

Figure 1. Antibodies directed against proteins in the Hippo pathway kinase cascade. (A) The specificity of Invitrogen™ MST2 Antibody (clone 19H19L39), ABfinity™ Rabbit Monoclonal (Cat. No. 703027) was determined by western blot analysis using siRNA-mediated MST2 knockdown in A549 cells. **(B)** The specificity of Invitrogen™ SAV1 Antibody (clone 6H5L16), ABfinity™ Rabbit Monoclonal (Cat. No. 703002) was demonstrated by immunofluorescence/immunocytochemistry (IF/ICC) using siRNA-mediated SAV1 knockdown in HCT116 cells. **(C)** The specificity of Invitrogen™ LATS2 Antibody (clone 17H14L2), ABfinity™ Rabbit Monoclonal (Cat. No. 703621) was demonstrated by IF/ICC; increased LATS2 expression was observed in U2OS cells treated with nocodazole. For IF/ICC, primary antibodies were detected with Invitrogen™ Goat Anti–Rabbit IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor™ 488 (green, Cat. No. A27034), nuclei were stained using Invitrogen™ ProLong™ Diamond Antifade Mountant with DAPI (blue, Cat. No. P36962), and cytoskeletal F-actin was labeled with Invitrogen™ Rhodamine Phalloidin (Cat. No. R415). Chemiluminescence detection was performed using Invitrogen™ Goat Anti–Rabbit IgG (H+L) Secondary Antibody, HRP (0.25 µg/mL, 1:4,000 dilution; Cat. No. A27036) and Invitrogen™ Novex™ ECL Chemiluminescent Substrate Reagent Kit (Cat. No. WP20005) on the Invitrogen™ iBright™ FL1000 Imaging System (Cat. No. A32752).

Antibodies for kinase cascade proteins

In the kinase arm of the Hippo pathway, MST1/2-mediated phosphorylation of SAV1 and MOB1A/B leads to the recruitment, phosphorylation, and subsequent activation of LATS1/2. In conjunction with MOB1, the activated LATS1/2 kinases phosphorylate YAP/TAZ. These phosphorylation events result in the cytoplasmic sequestration and degradation of YAP/TAZ, mediated by 14-3-3 proteins. Figures 1A and 1B demonstrate the specificity of Invitrogen™ anti-MST1/2 and anti-SAV1 antibodies in western blot and immunofluorescence/immunocytochemistry (IF/ICC) applications using siRNA-mediated knockdown. In response to nocodazole treatment, LATS2 translocates from the cytoplasm into the nucleus, then binds to and activates p53, inducing LATS2 expression [4]. Figure 1C shows the specificity of the Invitrogen™ anti-LATS2 antibody in IF/ICC using this nocodazole-induced upregulation of LATS2 expression.

Antibodies for transcription factors and Hippo regulators

In the nuclear transcription module of the Hippo pathway, several DNA-binding proteins regulate the transcription of genes encoding Hippo pathway proteins and also undergo modification themselves. When not phosphorylated, YAP and TAZ translocate to the nucleus and serve as transcriptional coactivators for Hippo pathway genes. Their most significant interacting partners are the TEA-domain family member transcription factors (TEAD1–4) [5]. Figure 2A shows the specificity of the Invitrogen™ anti-TEAD4 antibody in western blot analysis using siRNA-mediated knockdown. Another transcription regulator, VGLL4, selectively binds to TEAD and interferes with the YAP–TEAD interaction. The specificity of the Invitrogen™ anti-VGLL4 antibody has been demonstrated with IF/ICC using siRNA-mediated knockdown (Figure 2B).

Angiomotin proteins (AMOT), which play a central role in tight junction maintenance, are also important negative regulators of YAP/TAZ,

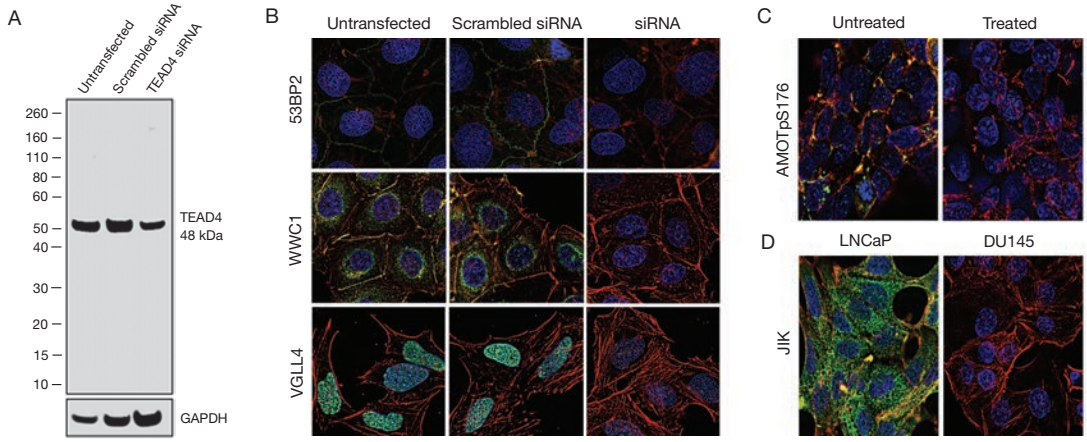

Figure 2. Antibodies that recognize Hippo pathway regulators. (A) The specificity of Invitrogen™ TEAD4 Polyclonal Antibody (Cat. No. 720430) was determined by western blot analysis using siRNA-mediated TEAD4 knockdown in HeLa cells. **(B)** The specificity of Invitrogen™ 53BP2 Antibody (clone 8H3L19), ABfinity™ Rabbit Monoclonal (Cat. No. 703010) and Invitrogen™ WWC1 Antibody (clone 1H4L22), ABfinity™ Rabbit Monoclonal (Cat. No. 703009) was demonstrated by immunofluorescence/immunocytochemistry (IF/ICC) using siRNA-mediated knockdown in Caco-2 cells. The specificity of Invitrogen™ VGLL4 Antibody (clone 16H12L24), ABfinity™ Rabbit Monoclonal (Cat. No. 703012) was demonstrated by IF/ICC using siRNA-mediated VGLL4 knockdown in HCT116 cells. **(C)** The specificity of Invitrogen™ Phospho-AMOT (Ser176) Antibody (clone 18H4L17), ABfinity™ Rabbit Monoclonal (Cat. No. 702980) was demonstrated by IF/ICC using untreated and serum-starved HEK293 cells, which exhibit reduced AMOTpS176 expression. **(D)** The specificity of Invitrogen™ JIK Antibody (clone 1HCLC), ABfinity™ Rabbit Oligoclonal (Cat. No. 712043) was demonstrated by IF/ICC to observe the differential basal expression of JIK in androgen-dependent (LNCaP) and -independent (DU145) cells. Fluorescent and chemiluminescent labeling reagents are detailed in Figure 1.

either by binding YAP/TAZ or by promoting their inhibitory phosphorylation. Phosphorylated AMOT (ser176) is downregulated during serum starvation, a characteristic that was used to show the specificity of the Invitrogen™ anti-AMOTpS176 antibody in IF/ICC (Figure 2C). In contrast to AMOT, 53BP2 (ASPP2) is a positive regulator of YAP, facilitating the dephosphorylation of YAP/TAZ. Specificity of the Invitrogen™ anti-53BP2 antibody has been demonstrated in IF/ICC using siRNA-mediated knockdown (Figure 2B).

WWC1 (KIBRA), a mechanical regulator that associates with tight junctions and other cell polarity complexes, can induce the phosphorylation of LATS1/2 [6]. The specificity of the Invitrogen™ anti-WWC1 antibody is shown in IF/ICC using siRNA-mediated knockdown (Figure 2B). The Hippo kinase cascade can also be initiated by TAO kinases, which phosphorylate MST1/2 [7]. TAO kinases, including TAO3 and JIK, are differentially expressed in androgen-dependent and -independent cell lines, a feature that was used to show the specificity of the Invitrogen™ anti-JIK antibody (Figure 2D).

Invitrogen antibodies for Hippo pathway proteins	Tested application*	Quantity	Cat. No.
Antibodies for Hippo pathway kinases			
SAV1 Antibody (clone 6H5L16), ABfinity™ Rabbit Monoclonal	IF, ICC	100 µg	703002
MST2 Antibody (clone 19H19L39), ABfinity™ Rabbit Monoclonal	WB	100 µg	703027
LATS2 Antibody (clone 17H14L2), ABfinity™ Rabbit Monoclonal	IF, ICC	100 µg	703621
Antibodies for Hippo pathway transcription regulators			
TEAD4 Polyclonal Antibody	WB	100 µg	720430
VGLL4 Antibody (clone 16H12L24), ABfinity™ Rabbit Monoclonal	IF, ICC	100 µg	703012
Phospho-AMOT (Ser176) Antibody (clone 18H4L17), ABfinity™ Rabbit Monoclonal	IF, ICC	100 µg	702980
53BP2 Antibody (clone 8H3L19), ABfinity™ Rabbit Monoclonal	IF, ICC	100 µg	703010
JIK Antibody (clone 1HCLC), ABfinity™ Rabbit Oligoclonal	IF, ICC, WB	100 µg	712043
WWC1 Antibody (clone 1H4L22), ABfinity™ Rabbit Monoclonal	IF, ICC	100 µg	703009

*IF = immunofluorescence, ICC = immunocytochemistry, WB = western blot. The use or any variation of the word "validation" refers only to research use antibodies that were subject to functional testing to confirm that the antibody can be used with the research techniques indicated. The product(s) was not validated for clinical or diagnostic use.

Find Hippo pathway antibodies

Visit **thermofisher.com/antibodies** to find specific, application-tested antibodies—including Invitrogen™ ABfinity™ recombinant antibodies—that recognize proteins in the Hippo signaling pathway. ■

References

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