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Phospho-Atg14 (Ser29) (D4B8M) Rabbit mAb

#92340



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Entrez-Gene ID #22863
UniProt ID #Q6ZNE5

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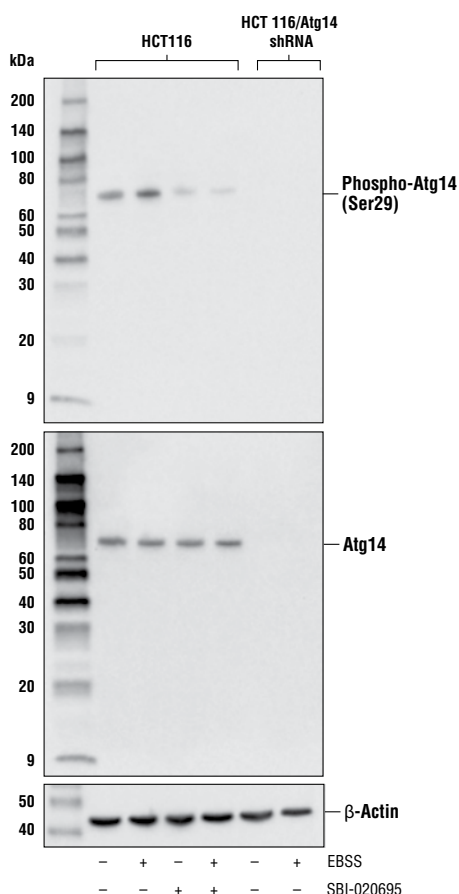
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Applications W, IF-IC Endogenous	Species Cross-Reactivity* H, M, R	Molecular Wt. 65 kDa	Isotype Rabbit IgG**
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Background: Autophagy is a catabolic process for the autophagosomal-lysosomal degradation of bulk cytoplasmic contents (1,2). Autophagy is generally activated by conditions of nutrient deprivation but is also associated with a number of physiological processes including development, differentiation, neurodegeneration, infection and cancer (3). The molecular machinery of autophagy was largely discovered in yeast and is directed by a number of autophagy-related (Atg) genes. These proteins are involved in the formation of autophagosomes, cytoplasmic vacuoles that are delivered to lysosomes for degradation. The class III type phosphoinositide 3-kinase (PI3K) Vps34 regulates vacuolar trafficking and autophagy (4,5). Multiple proteins associate with Vps34, including p105/Vsp15, Beclin-1, UVRAG, Atg14, and Rubicon, to determine Vps34 function (6-12). Atg14 and Rubicon were identified based on their ability to bind to Beclin-1 and participate in unique complexes with opposing functions (9-12). Rubicon, which localizes to the endosome and lysosome, inhibits Vps34 lipid kinase activity; knockdown of Rubicon enhances autophagy and endocytic trafficking (11,12). In contrast, Atg14 localizes to autophagosomes, isolation membranes and ER, and can enhance Vps34 activity. Knockdown of Atg14 inhibits starvation-induced autophagy (11,12). The serine/threonine kinase ULK1 phosphorylates Atg14 at Ser29 to promote autophagosome formation (13).

Specificity/Sensitivity: Phospho-Atg14 (Ser29) (D4B8M) Rabbit mAb recognizes endogenous levels of Atg14 protein only when phosphorylated at Ser29.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic phospho-peptide corresponding to residues surrounding Ser29 of human Atg14 protein.



Western blot analysis of extracts from HCT 116 and HCT 116/Atg14 shRNA knockout cells, untreated (-) or starved using Earle's Basic Salt Solution (EBSS, 2 hr; +) and the ULK1 inhibitor SBI-020695 #29089 (50 μM, 2 hr; +) as indicated, using Phospho-Atg14 (Ser29) (D4B8M) Rabbit mAb (upper), Atg14 (D1A1N) Rabbit mAb #96752 (middle), or β-Actin (D6A8) Rabbit mAb (lower). HCT 116/Atg14 shRNA knockout cells were kindly provided by Dr. Do-Hyung Kim, University of Minnesota, Minneapolis, MN.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

*Species cross-reactivity is determined by western blot.

**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunofluorescence (IF-IC)	1:800
Fixative:	100% Methanol

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

Background References:

- (1) Reggiori, F. and Klionsky, D.J. (2002) *Eukaryot Cell* 1, 11-21.
- (2) Codogno, P. and Meijer, A.J. (2005) *Cell Death Differ* 12 Suppl 2, 1509-18.
- (3) Levine, B. and Yuan, J. (2005) *J Clin Invest* 115, 2679-88.
- (4) Corvera, S. (2001) *Traffic* 2, 859-66.
- (5) Yan, Y. and Backer, J.M. (2007) *Biochem Soc Trans* 35, 239-41.
- (6) Stack, J.H. et al. (1995) *J Cell Biol* 129, 321-34.
- (7) Zeng, X. et al. (2006) *J Cell Sci* 119, 259-70.
- (8) Liang, C. et al. (2006) *Nat Cell Biol* 8, 688-99.
- (9) Itakura, E. et al. (2008) *Mol Biol Cell* 19, 5360-72.
- (10) Sun, Q. et al. (2008) *Proc Natl Acad Sci U S A* 105, 19211-6.
- (11) Zhong, Y. et al. (2009) *Nat Cell Biol* 11, 468-76.
- (12) Matsunaga, K. et al. (2009) *Nat Cell Biol* 11, 385-96.
- (13) Park, J.M. et al. (2016) *Autophagy* 12, 547-64.

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IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.

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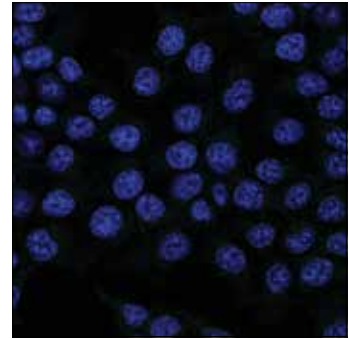
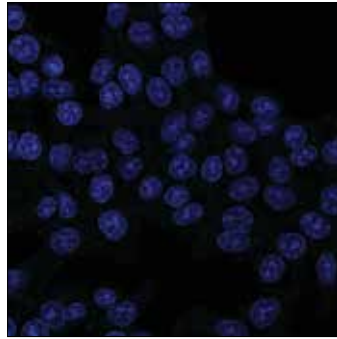
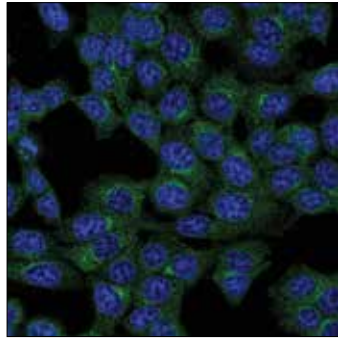
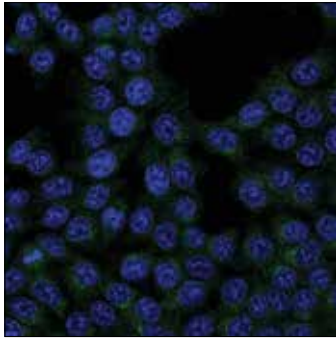
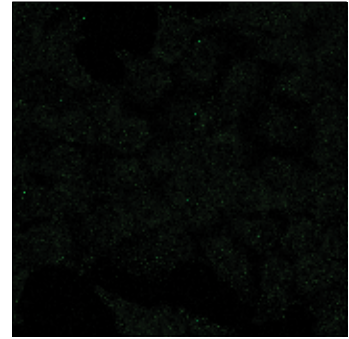
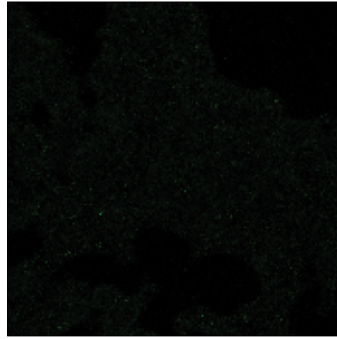
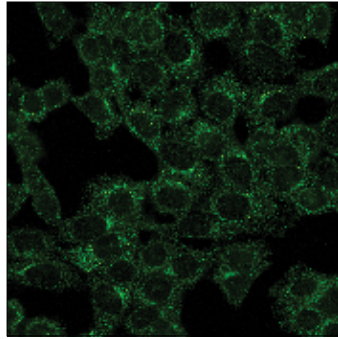
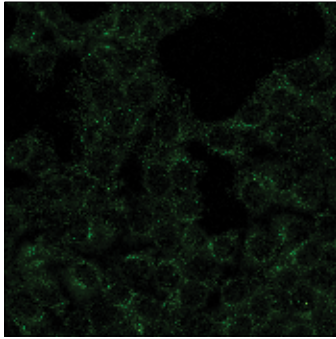
Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

HCT 116/Atg14 WT

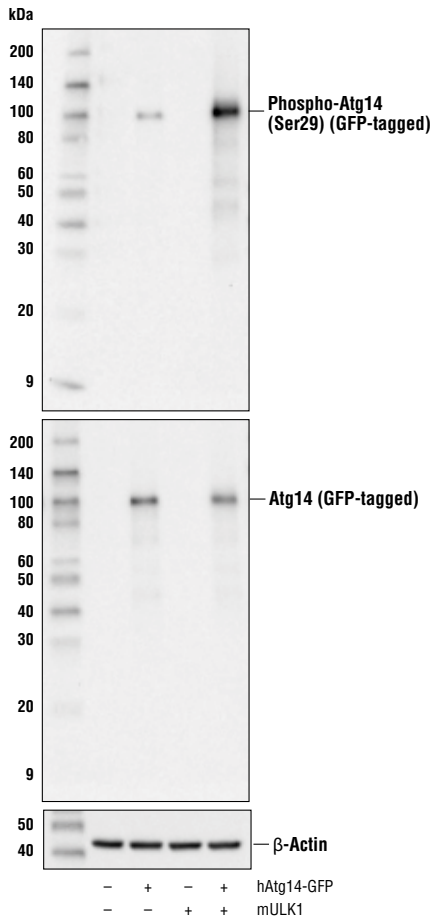
HCT 116/Atg14 WT + Torin-1

HCT116/Atg14 KO + Torin-1

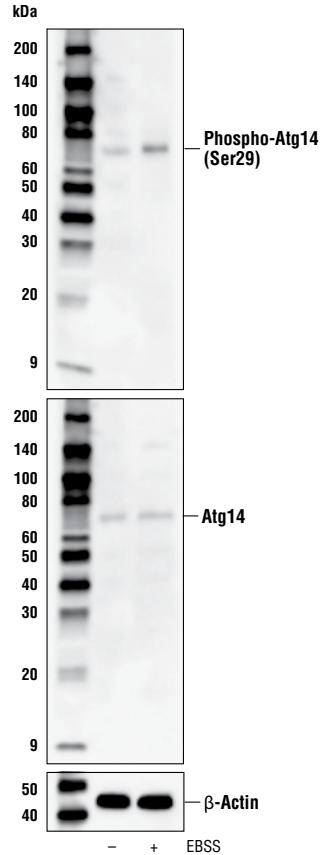
HCT 116/Atg14 WT + λ -phosphatase



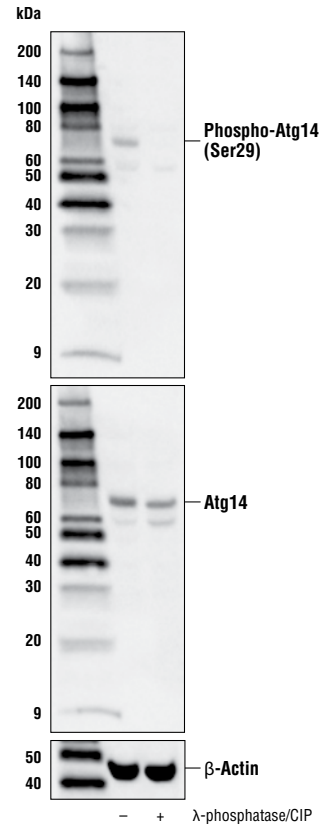
Confocal immunofluorescent analysis of HCT 116 Atg14 wild-type cells, untreated (left, low-expressing) or treated with Torin 1 #14379 (250 nM, 2 hr, middle-left, high-expressing), HCT 116/Atg14 shRNA knock-out cells treated with Torin 1 (middle-right, negative), or HCT 116 Atg14 wild-type cells post-processed with λ -phosphatase (2 hr; right, negative), using Phospho-Atg14 (Ser29) (D4B8M) Rabbit mAb (green). Samples were mounted in ProLong[®] Gold Antifade Reagent with DAPI #8961 (blue). HCT 116/Atg14 shRNA knockout cells were kindly provided by Dr. Do-Hyung Kim, University of Minnesota, Minneapolis, MN.



Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with constructs expressing GFP-tagged human Atg14 protein (hAtg14-GFP; +) and/or mouse ULK1 protein (mULK1; +), using Phospho-Atg14 (Ser29) (D4B8M) Rabbit mAb (upper), Atg14 (D1A1N) Rabbit mAb (middle), or β -Actin (D6A8) Rabbit mAb #8457 (lower).



Western blot analysis of extracts from Saos-2 cells, untreated (-) or starved using Earles Basic Salt Solution (EBSS, 2 hr), using Phospho-Atg14 (Ser29) (D4B8M) Rabbit mAb (upper), Atg14 (D1A1N) Rabbit mAb #96752 (middle), and β -Actin (D6A8) Rabbit mAb #8457 (lower).



Western blot analysis of extracts from HCT 116 cells, untreated (-) or treated with lambda-phosphatase and calf intestinal phosphatase (λ -phosphatase/CIP; +), using Phospho-Atg14 (Ser29) (D4B8M) Rabbit mAb (upper), Atg14 (D1A1N) Rabbit mAb #96752 (middle), and β -Actin (D6A8) Rabbit mAb #8457 (lower).

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