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Supplemental Material to:

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MAPK15/ERK8 stimulates autophagy by interacting with LC3 and GABARAP proteins

Autophagy 2012; 8(12)
http://dx.doi.org/10.4161/auto.21857

www.landesbioscience.com/journals/autophagy/article/21857

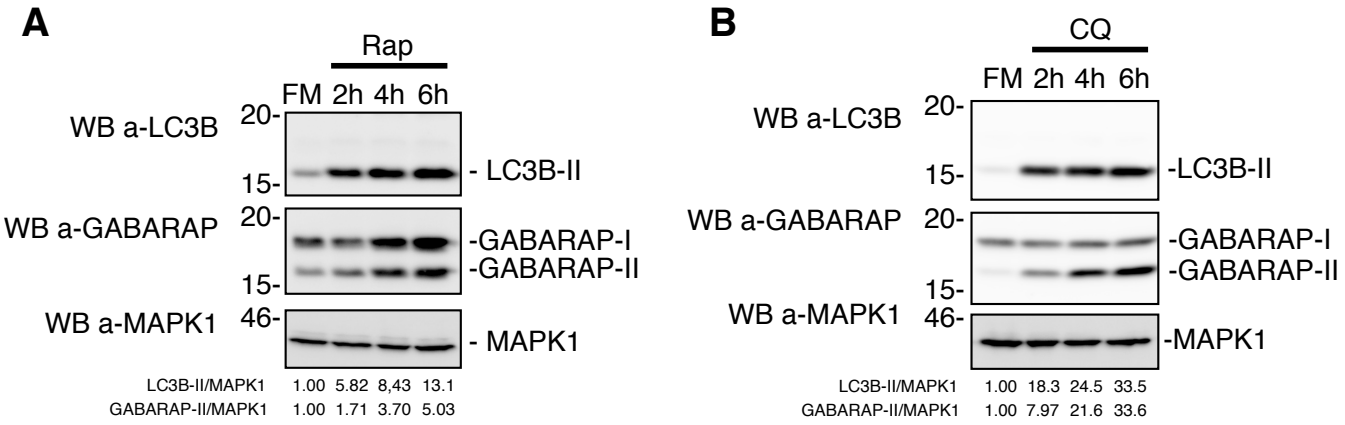

Fig S1. A, HeLa cells were treated with rapamycin (Rap, 200 nM) for the indicated periods. Lysates were analyzed by WB, with indicated antibodies. **B**, HeLa cells were treated with chloroquine (CQ, 10 μM) for the indicated periods, as indicated. Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools).

Fig. S1

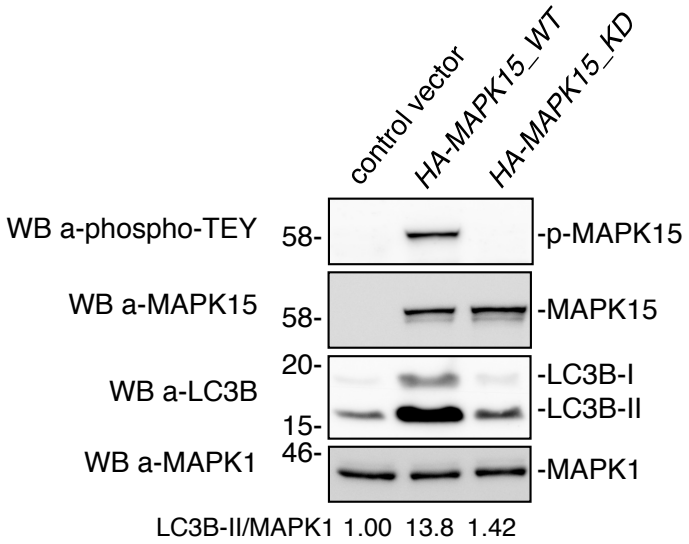

Fig S2. HeLa cells were transfected with control vector or *HA-MAPK15 wild type* (WT) or *HA-MAPK15 kinase dead* (KD). Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools).

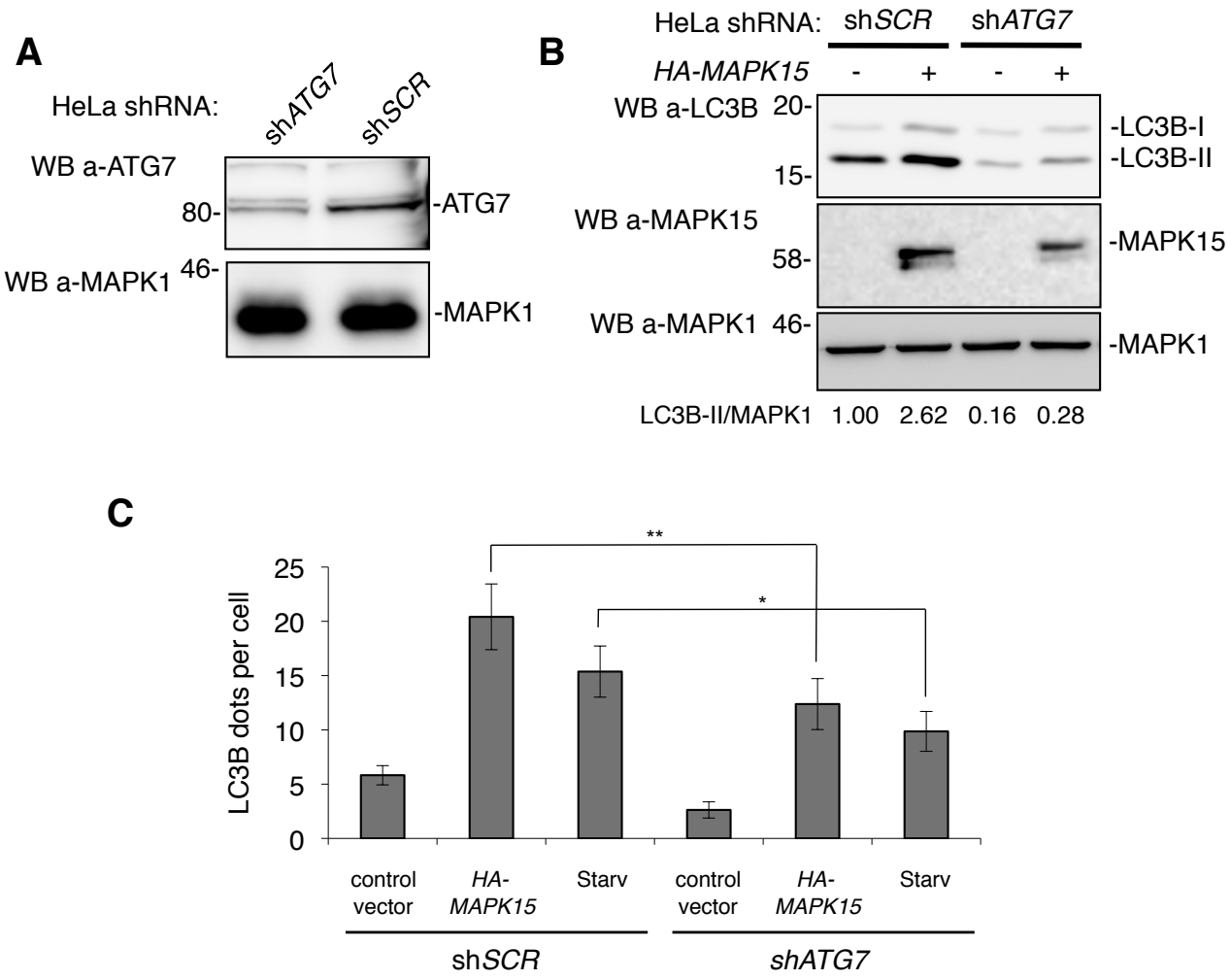

Fig S3. A, HeLa cells stably expressing sh*ATG7* and sh*SCR* were generated by lentiviral infection with pGIPZ_shRNA_*ATG7* and pGIPZ_shRNA_*SCR* and then selected with puromycin. Lysates were analyzed by WB, with indicated antibodies. **B**, sh*SCR* and sh*ATG7* HeLa cells were transfected with control vector or *HA-MAPK15*. Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools). **C,** sh*SCR* and sh*ATG7* HeLa cells were transfected with control vector or *HA-MAPK15*. Cells were fixed and then permeabilized with 100 µg/ml digitonin. Cells were stained with anti-LC3B (MBL) antibody and revealed with AlexaFluor488-conjugated antibody. The LC3B-positive dots per cell were quantified using Volocity software. Measures were obtained by analyzing at least 400 cells/sample from three different experiments (n=3). Measures were subjected to one-way ANOVA test. Asterisks were attributed for the following significance values: $p<0.05$ (*), $p<0.01$ (**).

Fig. S3

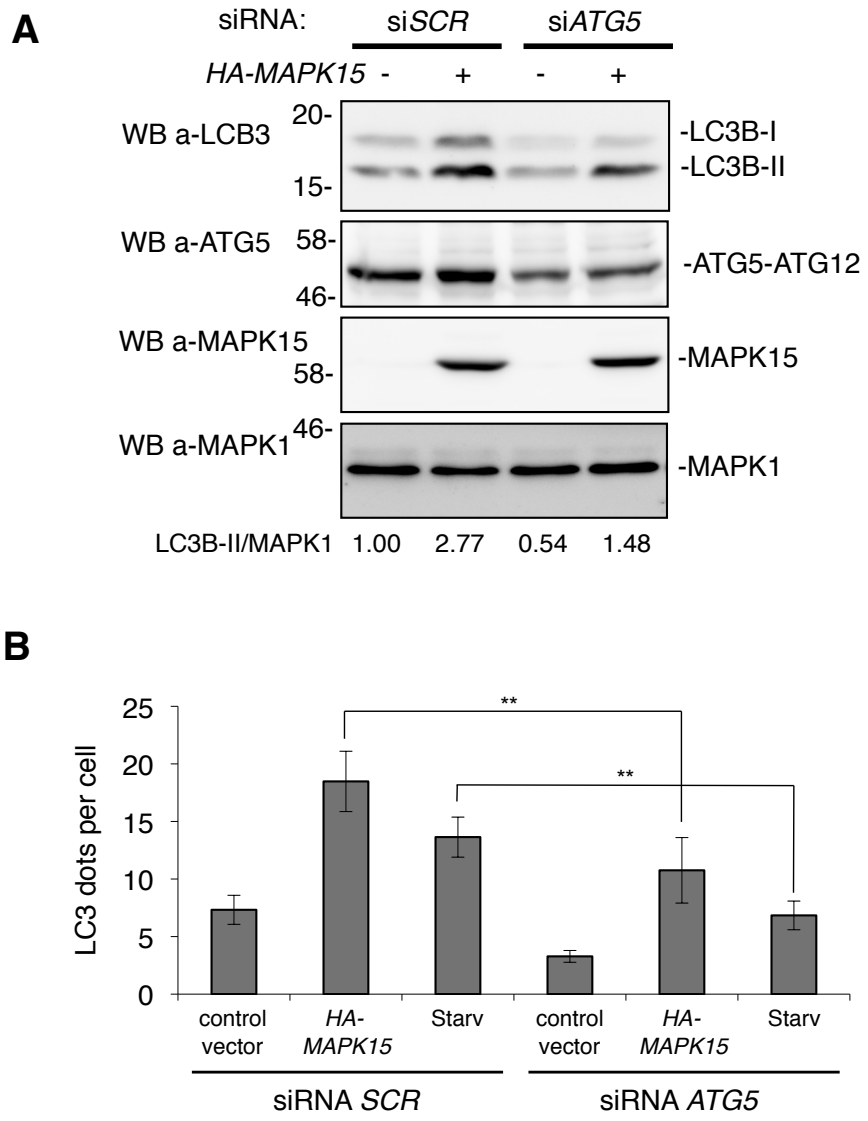

Fig S4. A, HeLa cells were transfected with non-silencing siRNA or with *ATG5*-specific siRNA. After 48 hours HeLa cells were transfected with control vector or *HA-MAPK15*. Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools). **B,** HeLa cells were transfected with non-silencing siRNA or with *ATG5*-specific siRNA. After 48 hours HeLa cells were transfected with control vector or *HA-MAPK15*. Cells were fixed and then permeabilized with 100 µg/ml digitonin. Cells were stained with anti-LC3B (MBL) antibody and revealed with AlexaFluor488-conjugated antibody. The LC3B-positive dots per cell were quantified using Volocity software. Measures were obtained by analyzing at least 400 cells/sample from three different experiments (n=3). Measures were subjected to one-way ANOVA test. Asterisks were attributed for the following significance value: $p<0.01$ (**).

Fig. S4

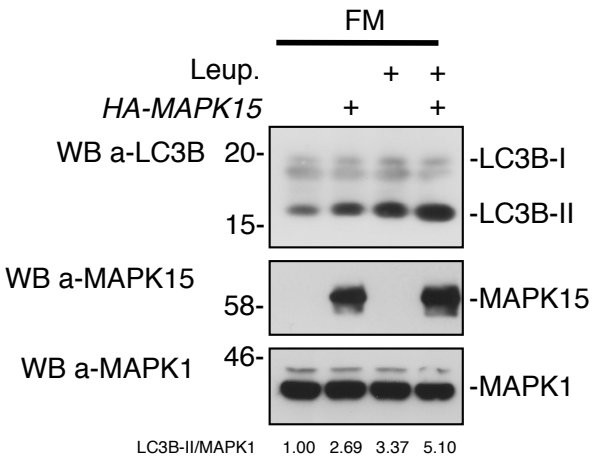

Fig. S5. HeLa cells were transfected with control vector or *HA-MAPK15*. One hour before harvesting, cells were treated with Leupeptin (Leup., 10 μg/ml), where indicated. Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools).

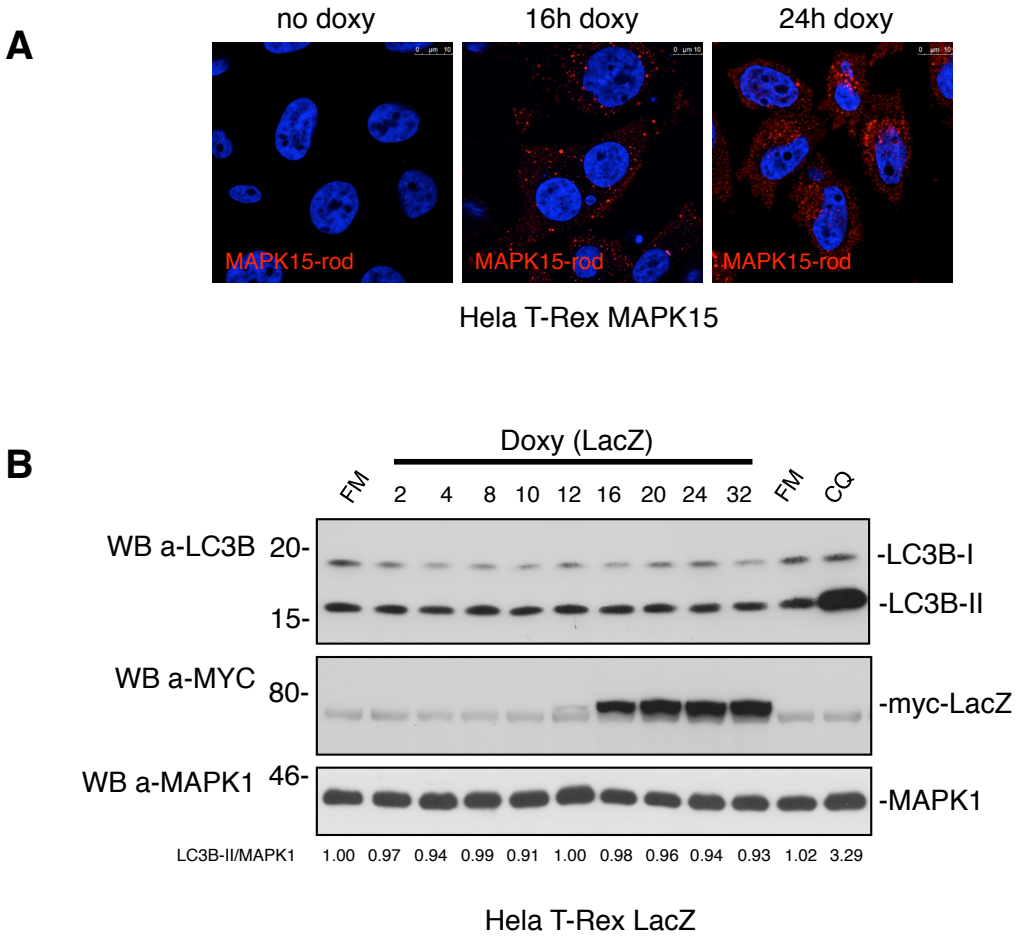

Fig. S6. A, HeLa T-Rex MAPK15 cells were treated with Doxy for 16 hrs or 24 hrs, where indicated. Cells were fixed and then permeabilized with 0.2% Triton X-100. Cells were stained with anti-MAPK15 antibody and revealed with AlexaFluor555-conjugated antibody. Nuclei were stained with DAPI. **B**, HeLa T-Rex LacZ cells were treated with Doxy for different periods. Two hours before harvesting, cells were treated with CQ, where indicated. Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools).

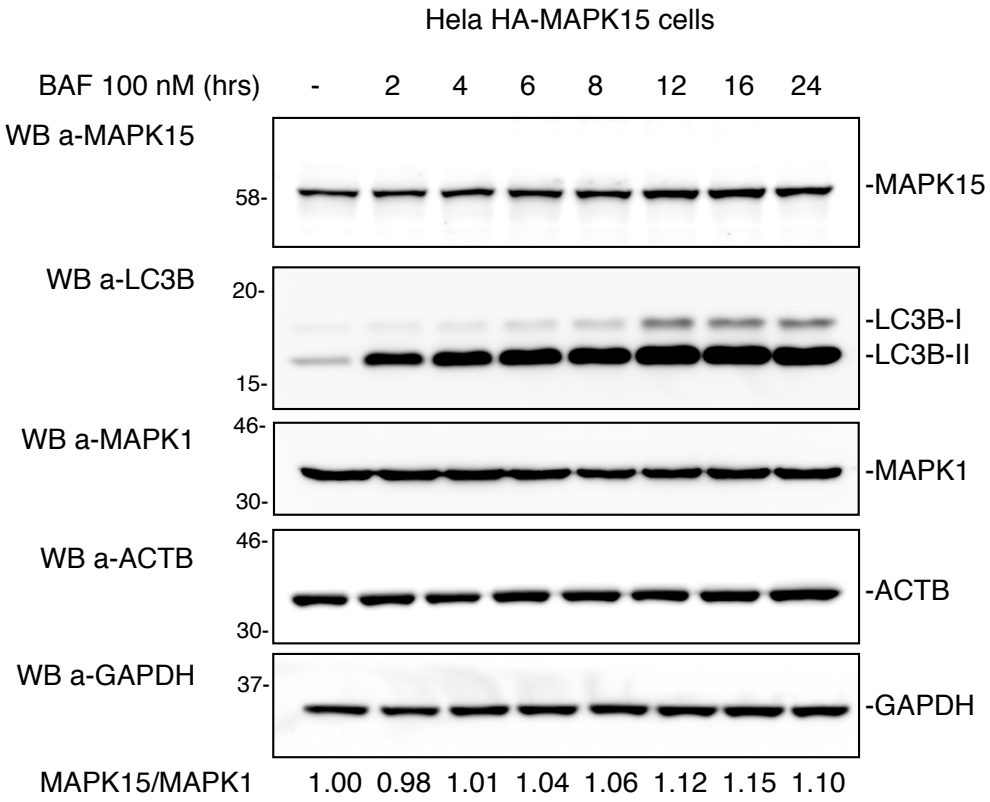

Fig. S7. Hela HA-MAPK15 cells were treated with Bafilomycin A_1 (BAF, 100 nM) for different periods. Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools).

Fig. S7

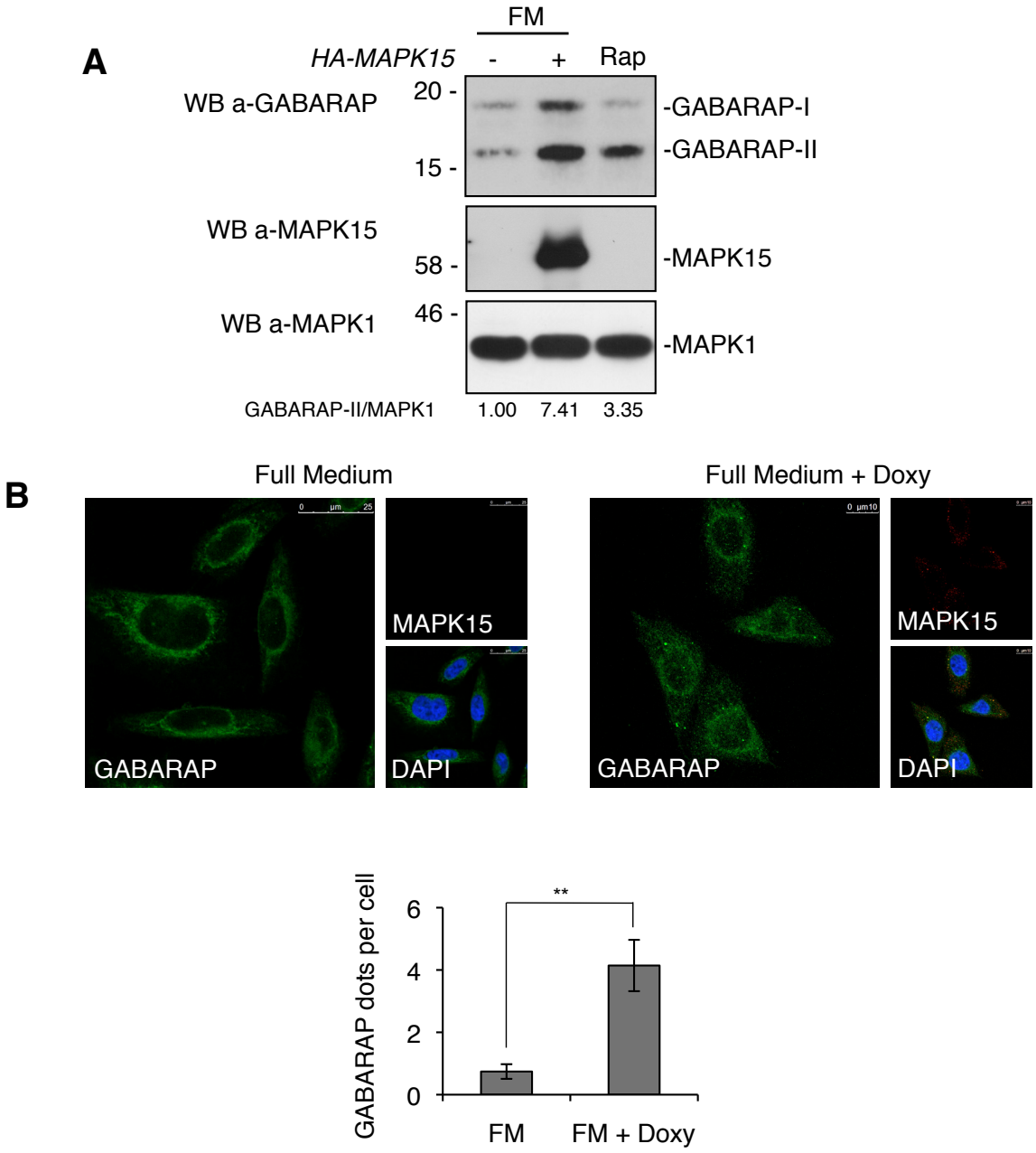

Fig. S8. A, HeLa cells were transfected with control vector or *HA-MAPK15*. Four hrs before harvesting, cells were treated with Rap, where indicated. Lysates were analyzed by WB with indicated antibodies. **B**, HeLa T-Rex MAPK15 cells were treated with Doxy for 16 hrs, where indicated. Cells were fixed and then permeabilized with 100 µg/ml digitonin. Cells were stained with anti-GABARAP and anti-MAPK15 antibodies and revealed with AlexaFluor488-conjugated and AlexaFluor555-conjugated secondary antibodies, respectively. Nuclei were stained with DAPI. The GABARAP-positive dots per cell were obtained by analyzing at least 400 cells/samples from three different experiments (n=3). Measures were subjected to one-way ANOVA test. Asterisks were attributed for the following significance values: $p<0.01$ (**).

Fig. S8

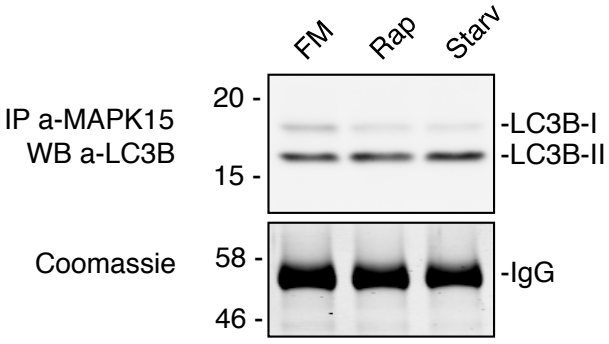

Fig. S9. Hela cells were treated with Rap for 4 hrs or starved for 1 hour. Cells were lysed in RIPA buffer and lysates (5 mg) were then immunoprecipitated with anti-MAPK15 antibody and analyzed by WB with anti-LC3B antibodies (Sigma Aldrich).

		Average	StDev
FM	coloc. Rate	27.0	3.2
FM	Pearson's coeff	0.2	0.0
Starv 1h	coloc. Rate	51.9	8.7
Starv 1h	Pearson's coeff	0.4	0.1
Rap 4h	coloc. Rate	36.4	6.2
Rap 4h	Pearson's coeff	0.3	0.1

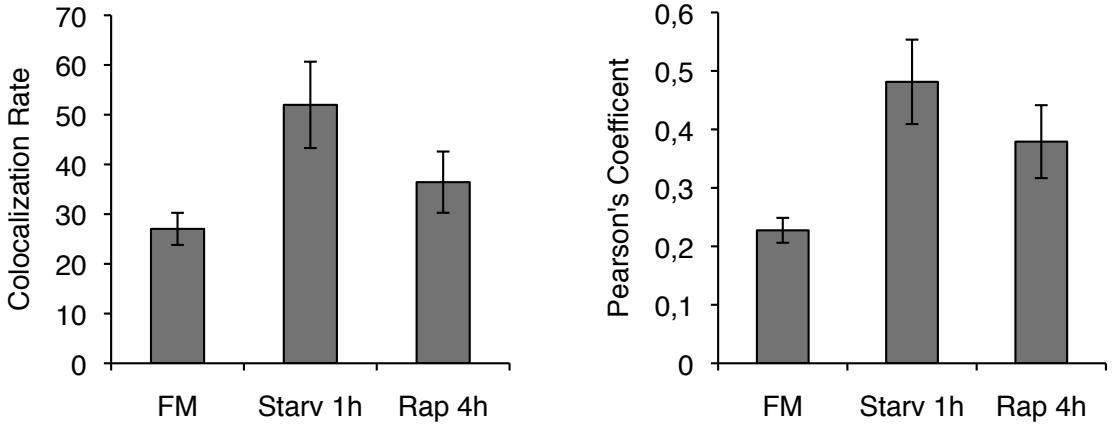

Fig. S10. HeLa cells stably expressing HA-MAPK15 were starved for 1 hour or treated with Rap for 4 hrs. Cells were permeabilized with 100 µg/ml digitonin and then stained with anti-LC3B (MBL) and anti-MAPK15 antibodies, revealed with AlexaFluor488- and AlexaFluor555-conjugated secondary antibodies. Nuclei were stained with DAPI. Colocalization rate and Pearson's coefficent of MAPK15 and LC3B, were respectively obtained by analyzing at least 400 cells/samples from three different experiments (n=3).

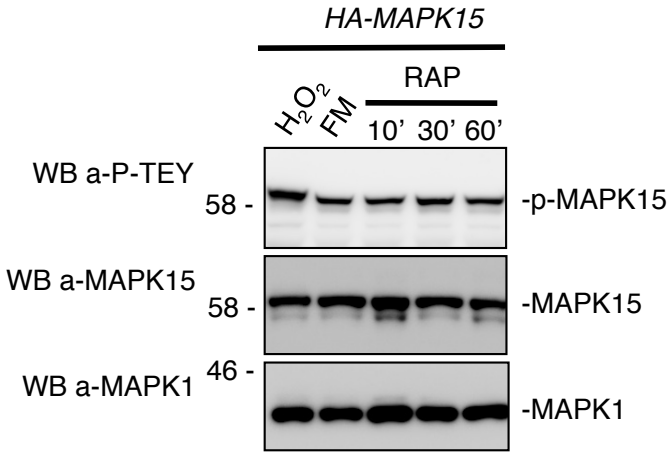

Fig. S11. HeLa cells were transfected with *HA-MAPK15*. Before harvesting, cells were treated with hydrogen peroxide (H_2O_2, 1 mM) for 10 minutes or with Rap for indicated periods. Lysates were analyzed by WB, with indicated antibodies.

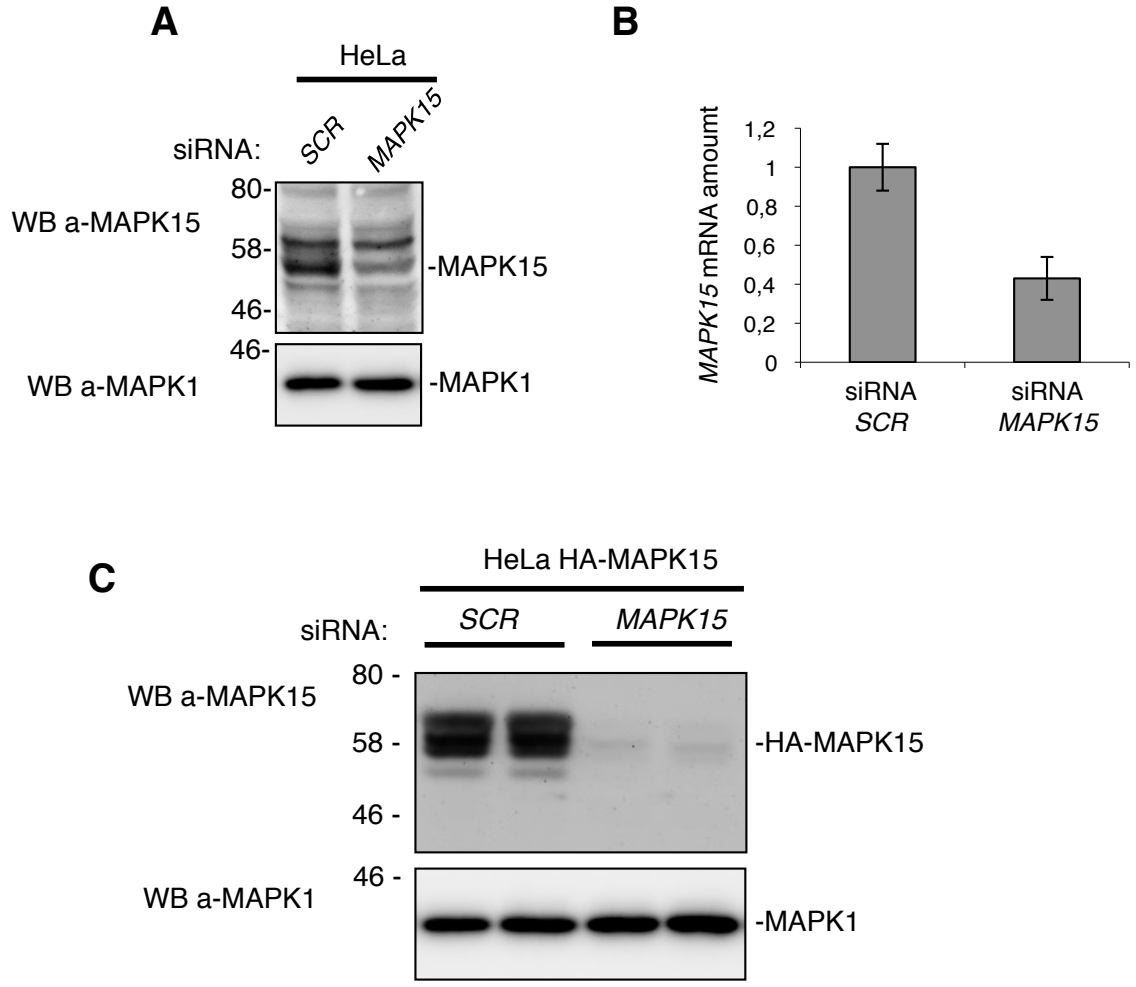

Fig. S12. A, HeLa cells were transfected with non-silencing siRNA or with *MAPK15*-specific siRNA. After 72 hours cells were harvested, washed and resuspended in RIPA buffer. Protein extracts (200 µg for each sample) were loaded on SDS-PAGE and analyzed by WB, with indicated antibodies. **B,** HeLa cells were transfected with non-silencing siRNA or with *MAPK15*-specific siRNA. After 48 hrs, total mRNA were collected and analyzed by RT-PCR. Relative *MAPK15* mRNA amount were determined using the following primers 5'-GGAGTTTGGGGACCATCC-3' and 5'-GCGTTCAGGTCAGTGTCC-3' and normalized for ACTB mRNA levels (5'-TGCGTGACATTAAGGAGAAG-3' and 5'-GCTCGTAGCTCTTCTCCA-3'). **C**, HeLa cells stably expressing HA-MAPK15 were transfected with non-silencing siRNA or with *MAPK15*-specific siRNA. After 72 hours cells were harvested, washed and resuspended in RIPA buffer. Protein extracts (30 µg for each sample) were loaded on SDS-PAGE and analyzed by WB, with indicated antibodies.

Fig. S12

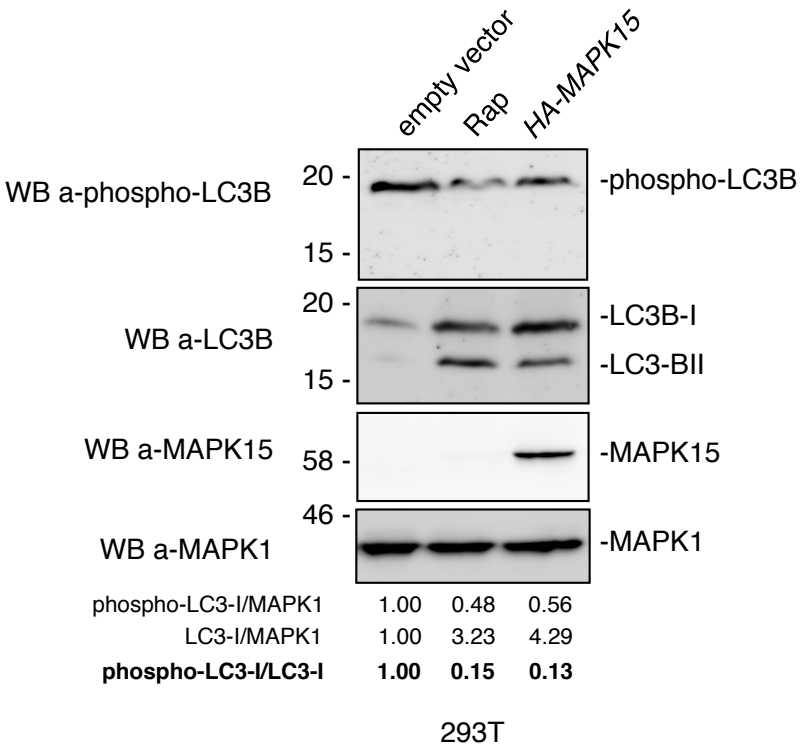

Fig S13. 293T cells were transfected with empty vector or *HA-MAPK15*. One hour before harvesting, cells were treated with Rap, where indicated. Lysates were analyzed by WB, with indicated antibodies. total LC3B was detected with anti-LC3B antibody (Sigma Aldrich).

A

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17   RQLGQGAYGIVWKA 31
21   QGAYGIVWKAVDRR 34
51   KTDAQRTFREITLL 64
111  DVHVRSIFYQLLRA 124
112  VHVRSIFYQLLRAT 125
333  PEYRSRVYQMILEC 346
518  LGGYSQAYGTVCHS 532
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B

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MAPK15   331  --SVPEYRSRVYQMILECGGSS 350
NBR1     723  SQSSASSED--YIIILPECFDT 742
SQSTM1   327  SDNCSGGDD-DWTHLSSKEVDP 348
CBL      791  SDISNASSSFGWLSLDGDPTTN 812
BNIP3L   27   -PPPAGLNS-SWVELPMNSSNG 48
OPTN     169  -NSSGSSED-SFVEIRMAEGEA 188
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Fig. S14. A, Regions of MAPK15 protein sequence containing putative LIR motifs. Residue important for LIR motif are highlighted by colors: cyan for the conserved residues of W/Y/F-X-X-L/I/V motif, green for serine and purple for acidic. **B,** Alignment of the most conserved LIR contained in MAPK15 and in already characterized human proteins. Residue important for LIR motif are highlighted by colors: cyan for the conserved residues of W/Y/F-X-X-L/I/V motif, green for serine and purple for acidic.

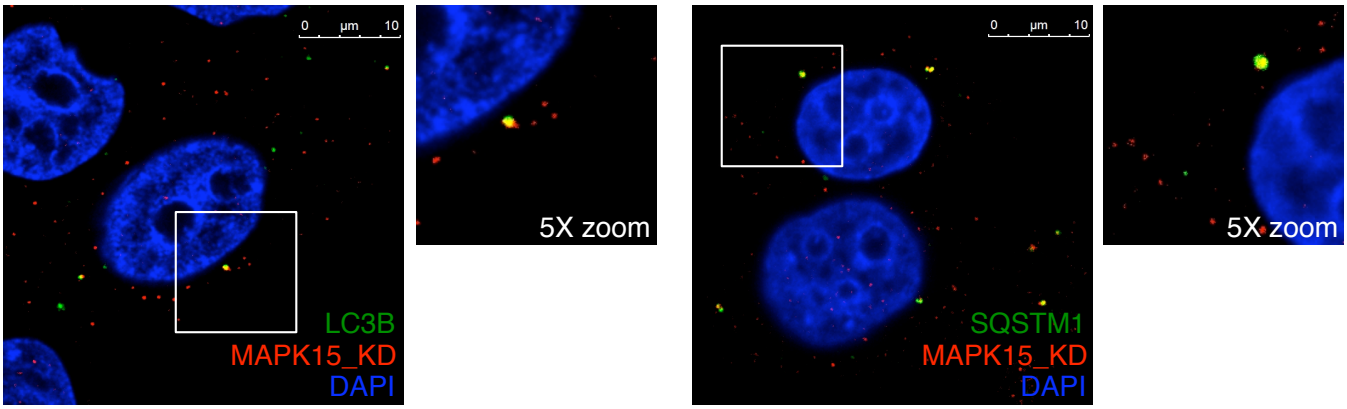

Fig. S15. Clones of HeLa cells stably expressing HA-MAPK15_KD were permeabilized with 100 µg/ml digitonin. Cells were stained with appropriate antibodies (anti-MAPK15, anti-LC3B MBL, and anti-SQSTM1) and revealed with AlexaFluor488- and AlexaFluor555-conjugated secondary antibodies. Nuclei were stained with DAPI. The region enclosed in the white square has been enlarged in the smaller panels for better appreciation of the colocalizations. Similar results were obtained in at least 3 independent clones.

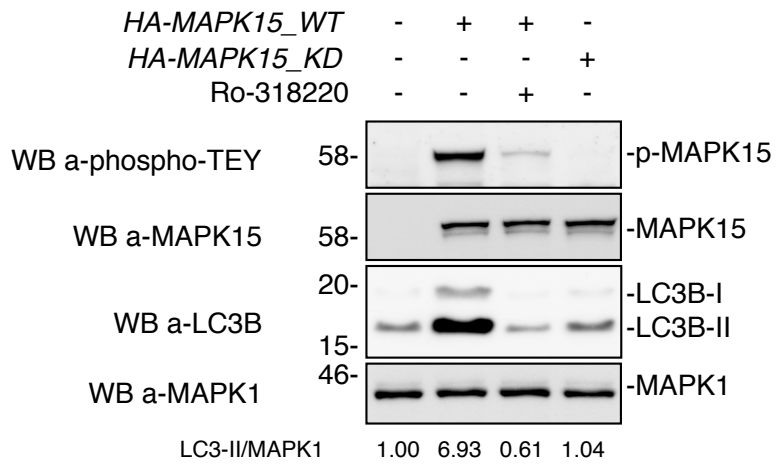

Fig. S16. HeLa cells were transfected with control vector or *HA-MAPK15_WT* or *HA-MAPK15_KD*. Cells were treated with MAPK15 inhibitor, Ro-318220 (1 μM) for 1 hour, where indicated. Lysates were analyzed by WB, with indicated antibodies. LC3B was detected with anti-LC3B antibody (Nanotools).

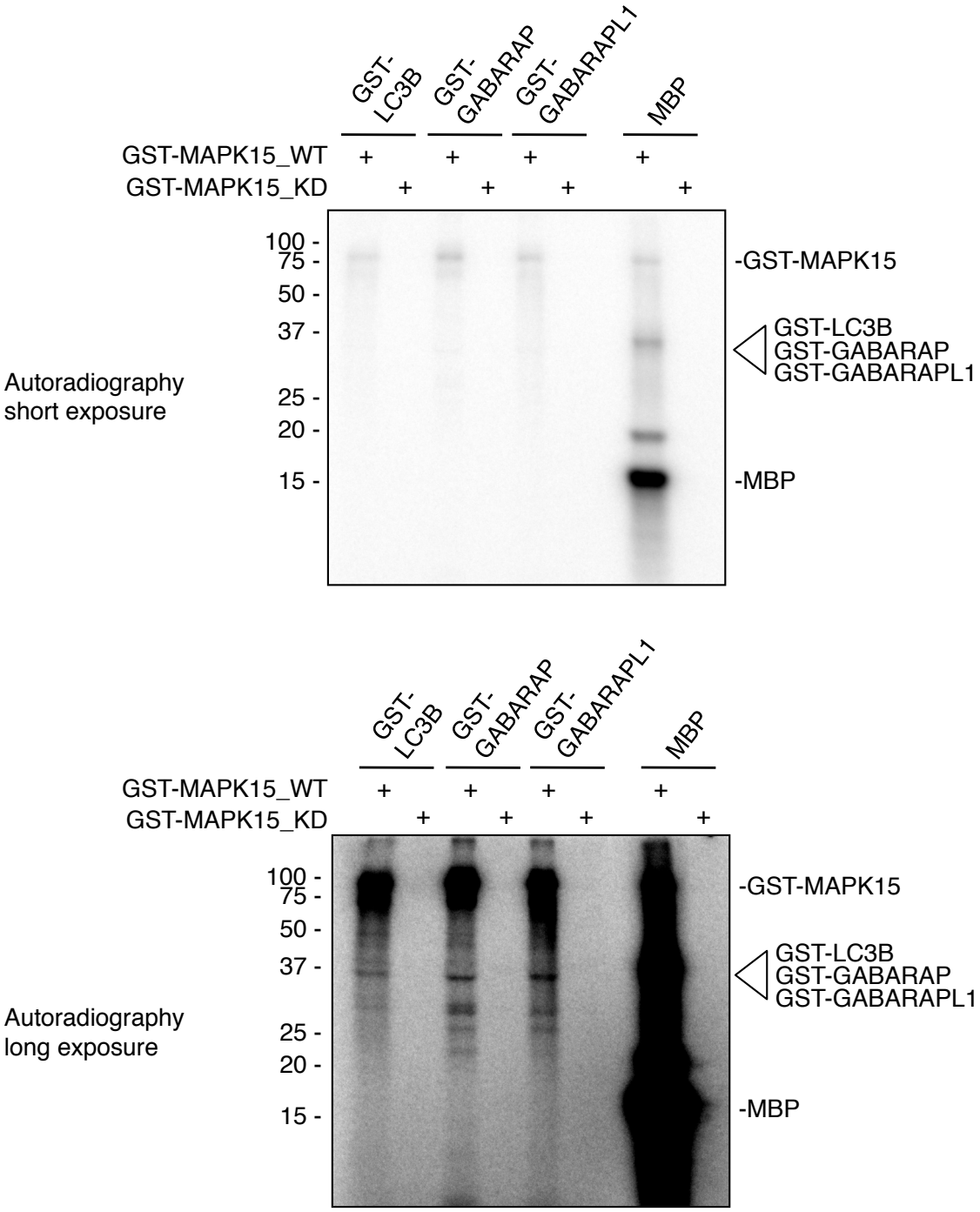

Fig. S17. Purified GST-fusion protein (50 ng/sample) was incubated 30 min at 30°C in kinase buffer [25 mM HEPES (pH 7.6), 0.1 mM Na_3VO_4, 20 mM β-glycerophosphate, 2mM DTT, 20 mM $MgCl_2$] with 2.5 µCi [γ-^{32}P]ATP (Perkin Elmer, NEG002A500UC) and unlabeled ATP (final concentration 5 µM) and with protein substrates: 5 µg/sample of Myelin Basic Protein (MBP) (Sigma, M1891) or with 5 µg/sample of GST-LC3B, GST-GABARAP or GST-GABARAPL1. Reaction was stopped adding 5X Laemmli buffer and resolved by SDS-PAGE. Dried gels were then exposed to Phosphorimager (Typhoon 8600 Molecular Dynamics) for autoradiography and ^{32}P incorporation on substrates was estimated by densitometry (ImageQuant TL Software, GE Healthcare).

Fig. S17

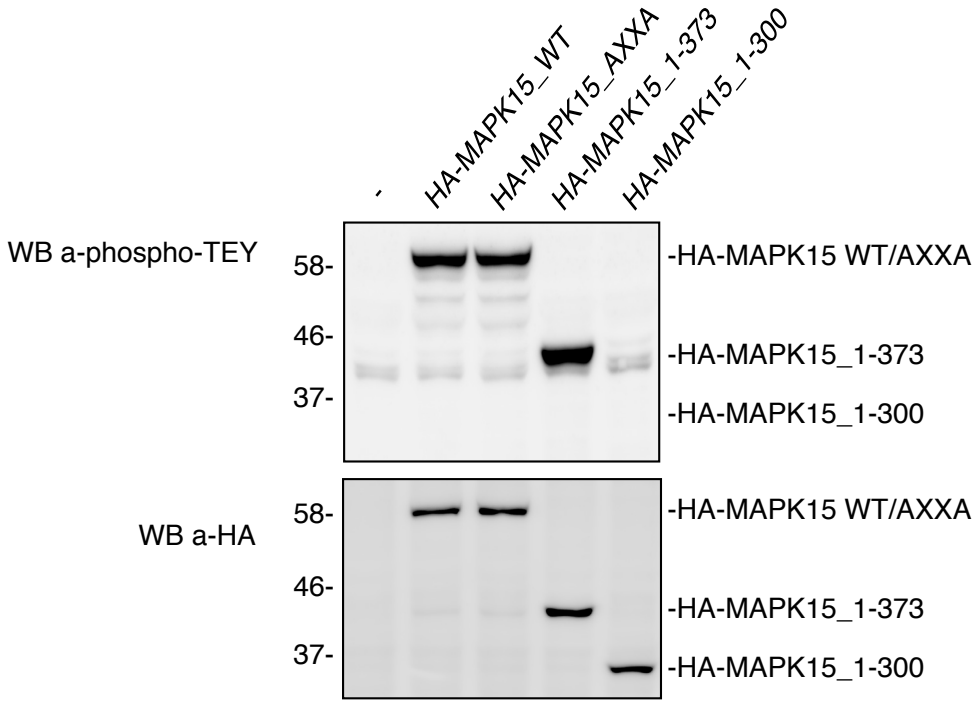

Fig. S18. HeLa cells were transfected with control vector or *HA-MAPK15_WT* or HA-tagged *MAPK15* mutants (*AXXA, 1-373, 1-300*). Lysates were analyzed by WB, with indicated antibodies.