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Supplemental Information

Viral Replication Complexes Are Targeted by LC3-Guided Interferon-Inducible GTPases

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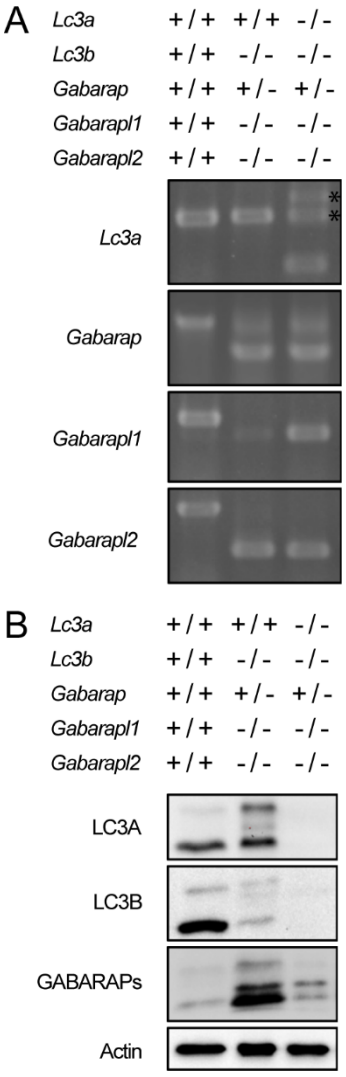

A
Lc3a + / + + / + - / -
Lc3b + / + - / - - / -
Gabarap + / + + / - + / -
Gabarapl1 + / + - / - - / -
Gabarapl2 + / + - / - - / -
Lc3a
Gabarap
Gabarapl1
Gabarapl2
B
Lc3a + / + + / + - / -
Lc3b + / + - / - - / -
Gabarap + / + + / - + / -
Gabarapl1 + / + - / - - / -
Gabarapl2 + / + - / - - / -
LC3A
LC3B
GABARAPs
Actin

Figure S1. LC3 Homologs Are Knocked-out, Related to Figure 1. (A and B) Genotyping PCR (A) and representative western blots (B) demonstrating the knocking-out of the indicated LC3 homologs in MEFs. * indicates PCR products from frameshift mutants with nonsense mutations (instead of deletion). N=3 replicates.

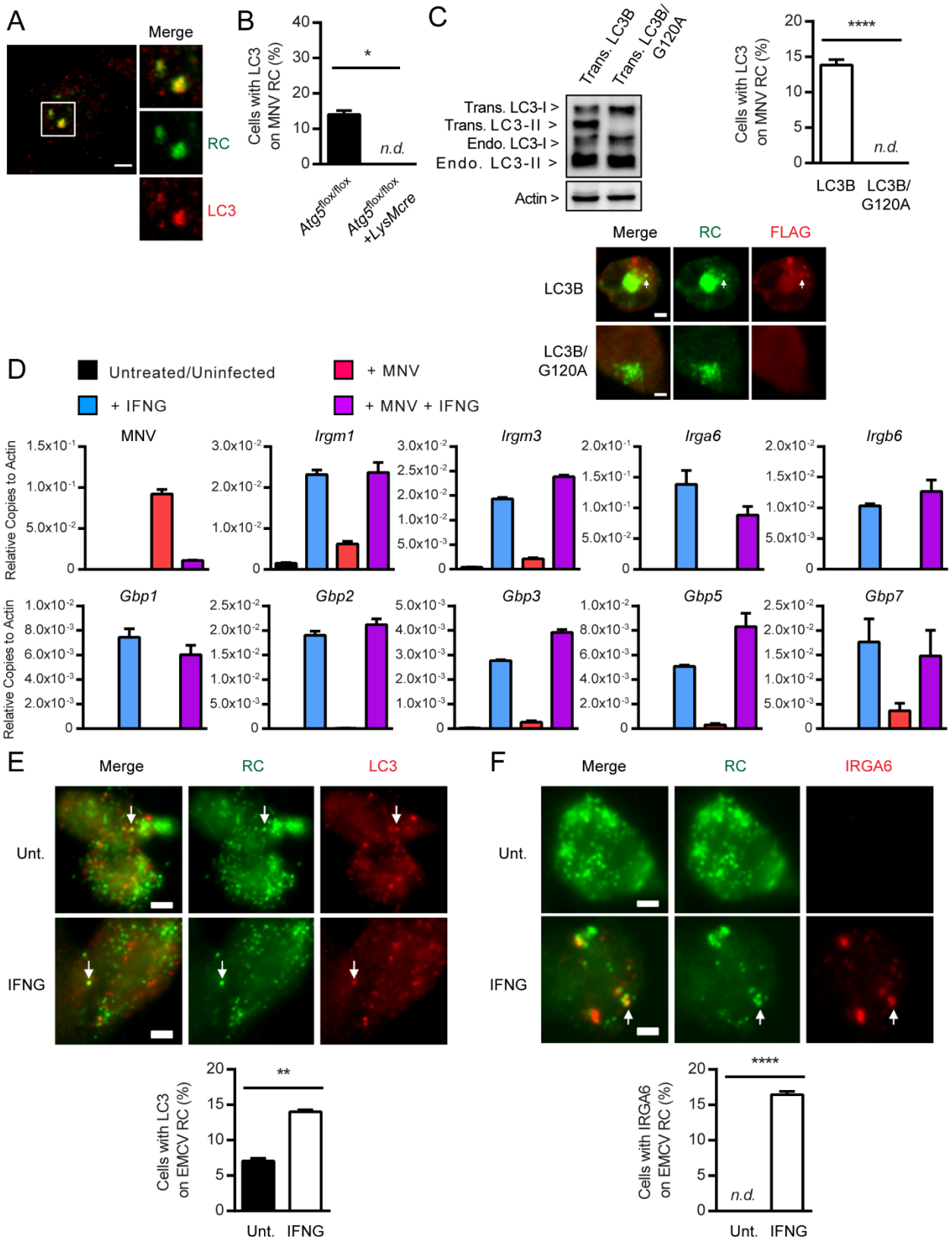
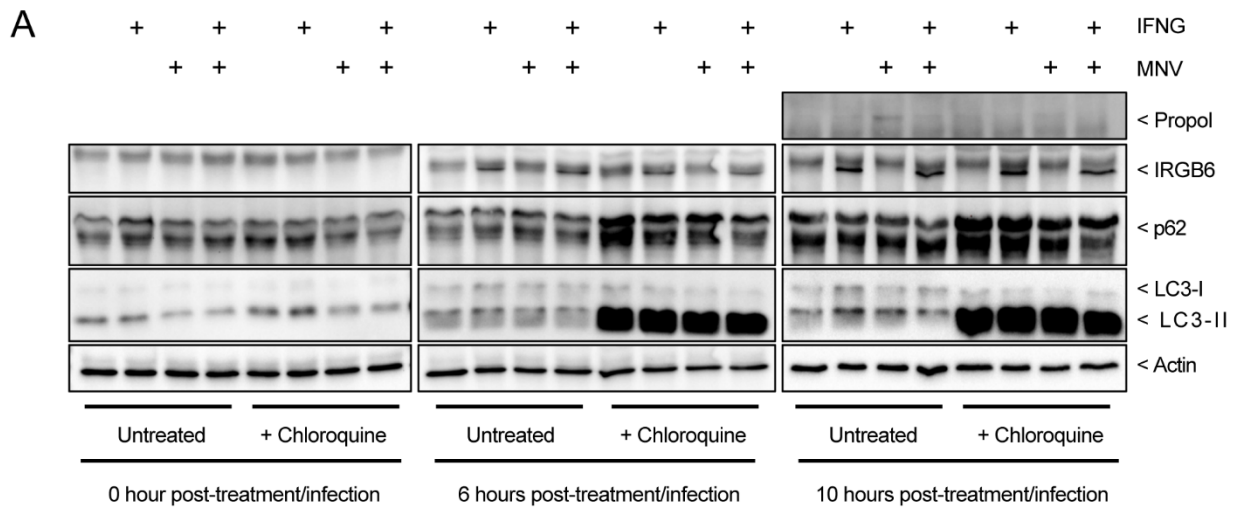
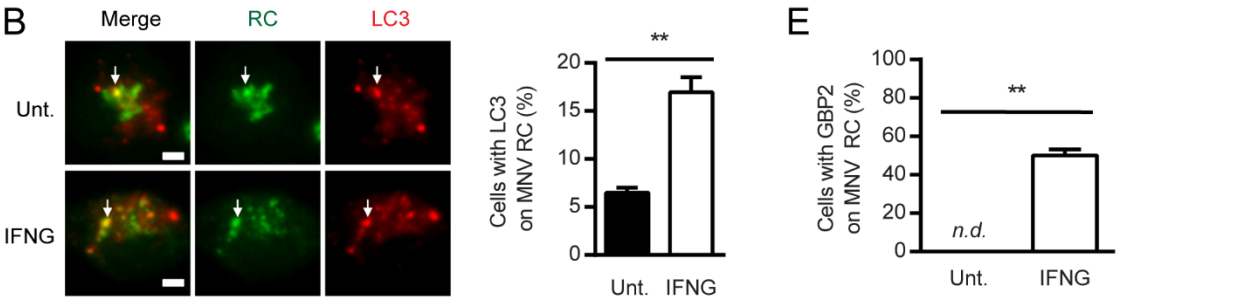

A
Merge
RC
LC3
B
Cells with LC3 on MNV RC (%)
*
n.d.
Atg5flox/flox
Atg5flox/flox +LysMcre
C
Trans. LC3B
Trans. LC3B/ G120A
Trans. LC3-I >
Trans. LC3-II >
Endo. LC3-I >
Endo. LC3-II >
Actin >
Cells with LC3 on MNV RC (%)
****
n.d.
LC3B
LC3B/ G120A
Merge
RC
FLAG
LC3B
LC3B/ G120A
D
Untreated/Uninfected
+ MNV
+ IFNG
+ MNV + IFNG
MNV
Irgm1
Irgm3
Irga6
Irgb6
Gbp1
Gbp2
Gbp3
Gbp5
Gbp7
Relative Copies to Actin
E
Merge
RC
LC3
Unt.
IFNG
Cells with LC3 on EMCV RC (%)
**
Unt.
IFNG
F
Merge
RC
IRGA6
Unt.
IFNG
Cells with IRGA6 on EMCV RC (%)
****
n.d.
Unt.
IFNG

Figure S2. IFN-Inducible GTPases Are Targeted to Viral RCs via the LC3-Conjugation System, Related to Figure 2. (A and B) Immunofluorescence assay for the localization of LC3 with regard to the MNV RC in *Atg5*$^{flox/flox}$+*LysMcre* or control *Atg5*$^{flox/flox}$ BMDMs at 10 hour-post-infection (hpi) of MNV at the multiplicity of infection at 5 (MOI=5). Representative images in *Atg5*$^{flox/flox}$ BMDMs (A) and quantitation (B). Scale bar, 2 μm. Data as mean±SEM. N=3 replicates. n.d., not detected. Student's t-test. *, p<0.05. (C) Immunofluorescence assay for the localization of wild type (WT) LC3B or non-conjugatable mutant of LC3B (G120A) with regard to the MNV RC at 10 hpi of MNV at MOI=5 and simultaneous treatment of 100 U/ml IFNG in BV-2 cells transduced with lentiviruses expressing FLAG/HA-tagged WT LC3B and LC3B/G120A. A representative western blot of the transduced cells (top, left), representative images (bottom), and quantitation (top, right). White arrows indicate representative colocalization. Scale bar, 5 μm. Data as mean±SEM. N=3 replicates. n.d., not detected. Student's t-test. ****, p<0.0001. (D) Quantitative PCR analysis for the transcript levels of the indicated genes in WT BMDMs with or without 10 hrs infection of MNV at MOI=5 and/or 100 U/ml IFNG treatment. N=3 replicates. (E and F) Immunofluorescence assay for the localization of LC3 (E) and IRGA6 (F) with regard to the EMCV RC (via anti-dsRNA antibody) in WT BMDMs at 6 hpi of EMCV at MOI=500 with or without 8 hrs treatment of 100 U/ml IFNG. Representative images (top) and quantitation (bottom). White arrows indicate representative colocalization. Scale bars, 5 μm. Data as mean±SEM. N=3 replicates. n.d., not detected. Student's t-test. **, p<0.01; ****, p<0.0001. BMDMs for (A), (B), (D), (E), and (F) were from 2 mice for each genotype.


A
IFNG
MNV
< Propol
< IRGB6
< p62
< LC3-I
< LC3-II
< Actin
Untreated
+ Chloroquine
Untreated
+ Chloroquine
Untreated
+ Chloroquine
0 hour post-treatment/infection
6 hours post-treatment/infection
10 hours post-treatment/infection


B
Merge
RC
LC3
Unt.
IFNG
Cells with LC3 on MNV RC (%)
**
Unt.
IFNG
E
Cells with GBP2 on MNV RC (%)
**
n.d.
Unt.
IFNG

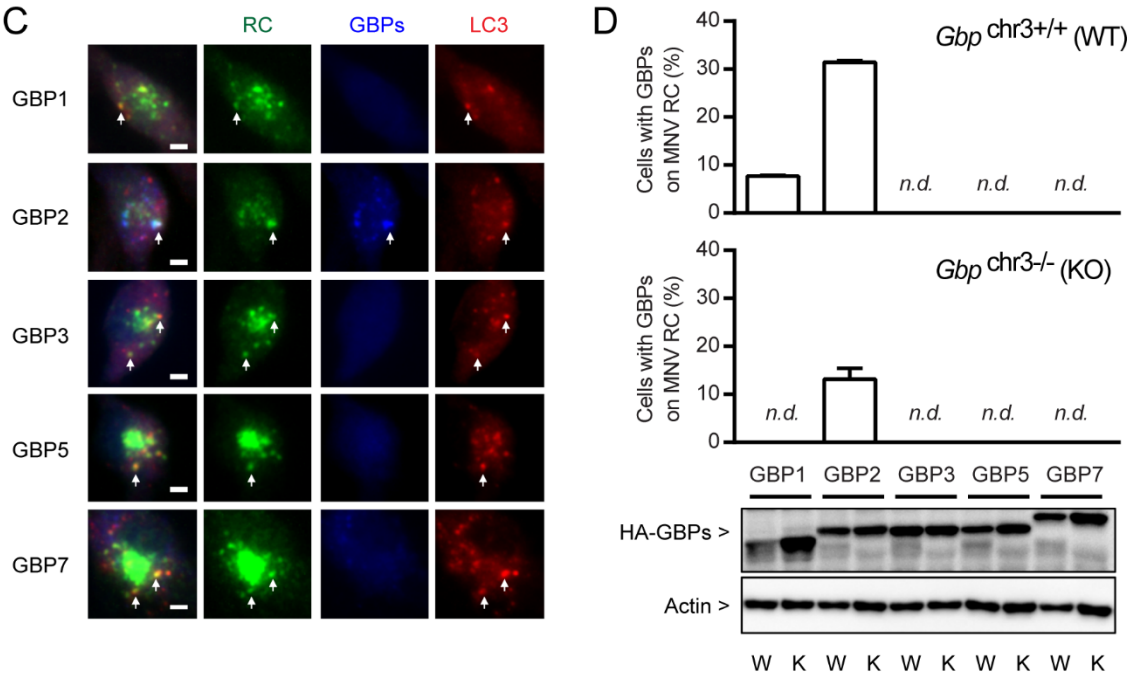

C
RC
GBPs
LC3
GBP1
GBP2
GBP3
GBP5
GBP7
D
Gbp chr3+/+ (WT)
Gbp chr3-/- (KO)
Cells with GBPs on MNV RC (%)
n.d.
GBP1
GBP2
GBP3
GBP5
GBP7
HA-GBPs >
Actin >
W K W K W K W K W K

Figure S3. IFN-Inducible GTPases Are Targeted to the MNV RC, Related to Figure 2. (A) A representative western blot of LC3 lipidation in WT BMDMs with or without 50 μM chloroquine treatment (to inhibit lysosomal degradation), upon infection of MNV at MOI=5 and/or treatment of 100 U/ml IFNG for 0, 6, 10 hours. Propol for MNV infection, IRGB6 for IFNG treatment, p62 for autophagy status, and Actin as loading control. N=3 replicates. (B) Immunofluorescence assay for the localization of LC3 with regard to the MNV RC in WT BMDMs at 10 hpi of MNV at MOI=5 with simultaneous treatment of none (Unt.) or 100 U/ml IFNG. Representative images (left) and quantitation (right). White arrows indicate representative colocalization. Scale bar, 5 μm. Data as mean±SEM. N=3 replicates. Student's t-test. **, $p<0.01$. (C and D) immunofluorescence analysis of GBPs with regard to the MNV RC in *Gbp*$^{chr3+/+}$ (W) and *Gbp*$^{chr3-/-}$ (K) BMDMs transduced with lentiviruses expressing the indicated GBPs at 10 hpi of MNV at MOI=5 and simultaneous treatment of 100 U/ml IFNG. Individual FLAG/HA-tagged GBP was detected by anti-FLAG (C) or anti-HA (D, bottom panel) antibodies. Representative images from the transduced *Gbp*$^{chr3-/-}$ BMDMs (C) and quantitation from both BMDMs (D). White arrows in (C) indicate representative colocalization. Scale bars, 5 μm. Data as mean±SEM. N=3 replicates. n.d., not detected. A representative western blot in D for the expression of individual GBPs. (E) Quantitation of immunofluorescence analysis of GBP2 localization to the MNV RC in WT BMDMs transduced with lentivirus expressing GBP2 at 10 hpi of MNV at MOI=5 with simultaneous treatment of none (Unt.) or 100 U/ml IFNG. Data as mean±SEM. N=3 replicates. n.d., not detected. Student's t-test. **, $p<0.01$. BMDMs were from 2 mice for each genotype.

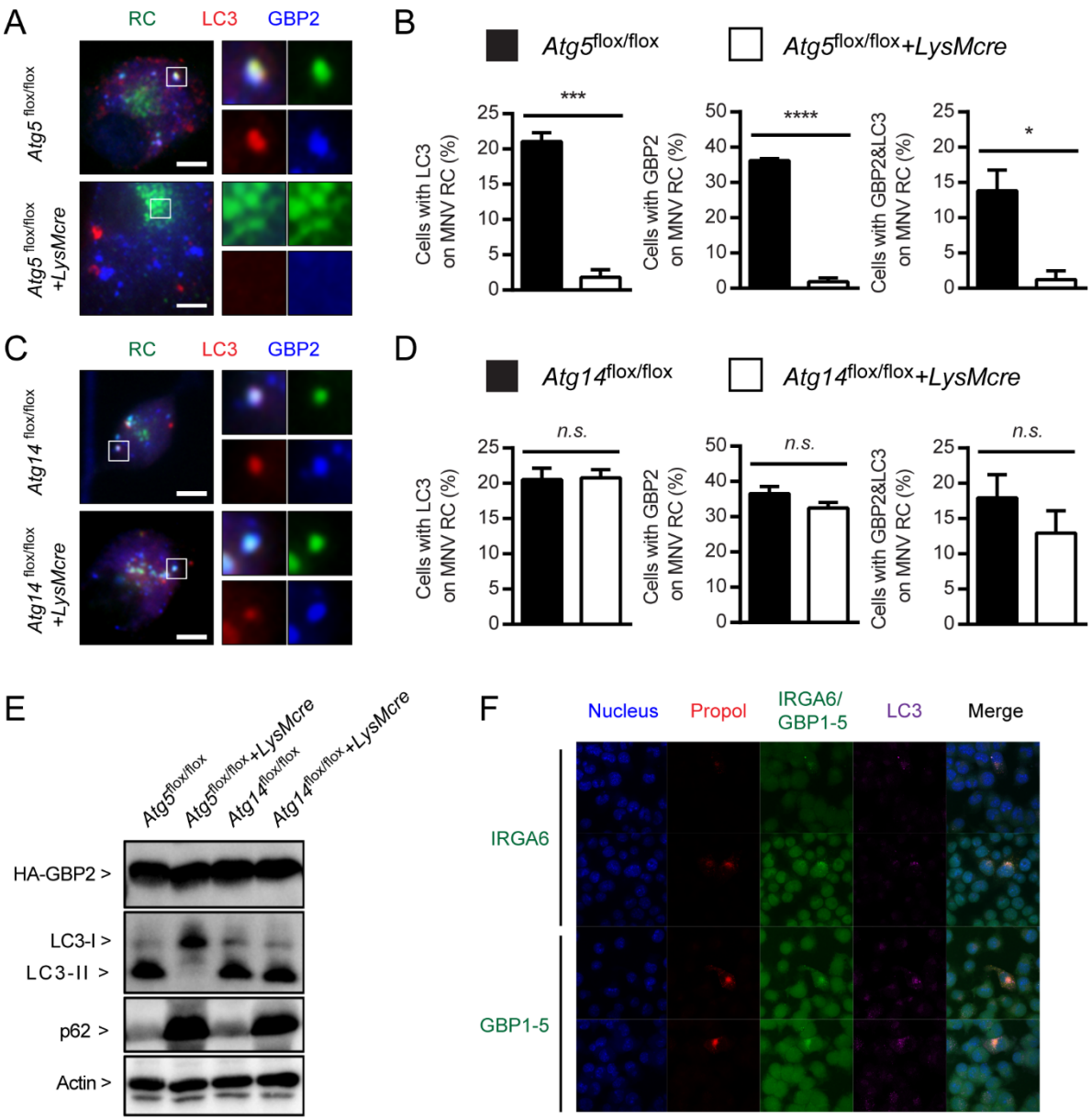

A
RC
LC3
GBP2
Atg5 flox/flox
Atg5 flox/flox +LysMcre
B
Atg5flox/flox
Atg5flox/flox+LysMcre
Cells with LC3 on MNV RC (%)
Cells with GBP2 on MNV RC (%)
Cells with GBP2&LC3 on MNV RC (%)
***
****
*
C
RC
LC3
GBP2
Atg14 flox/flox
Atg14 flox/flox +LysMcre
D
Atg14flox/flox
Atg14flox/flox+LysMcre
n.s.
n.s.
n.s.
E
Atg5flox/flox
Atg5flox/flox+LysMcre
Atg14flox/flox
Atg14flox/flox+LysMcre
HA-GBP2 >
LC3-I >
LC3-II >
p62 >
Actin >
F
Nucleus
Propol
IRGA6/ GBP1-5
LC3
Merge
IRGA6
GBP1-5

Figure S4. IFN-Inducible GTPases Are Targeted to the MNV RC via the LC3-Conjugation System, Related to Figures 2 and 3. (A and B) Immunofluorescence assay for the localization of LC3 and GBP2 with regard to the MNV RC in *Atg5*$^{flox/flox}$+*LysMcre* and *Atg5*$^{flox/flox}$ BMDMs transduced with lentiviruses expressing FLAG/HA-tagged GBP2 at 10 hpi of MNV at MOI=5 and simultaneous treatment of 100 U/ml IFNG. Representative images (A) and quantitation (B). Scale bars, 10 μm. Data as mean±SEM. N=3 replicates. Student's t-test. *, p<0.05; ***, p<0.001; ****, p<0.0001. (C and D) The same assays as described for (A) and (B) in *Atg14*$^{flox/flox}$+*LysMcre* and *Atg14*$^{flox/flox}$ BMDMs. n.s., not significant. N=3 replicates. (E) A representative western blot for the cells described in (A) and (C). N=3 replicates. (F) Representative immunofluorescence images for the localization of endogenous IRGA6 and GBP1-5 with regard to the MNV RC. BV-2 cells were infected with MNV at MOI=25 and simultaneously treated with 100 U/ml IFNG. At 10 hpi, cells were fixed and stained for MNV Propol (RC), LC3, and IRGA6 or GBP1-5. N=2 replicates. BMDMs for (A) to (E) were from 2 mice for each genotype.

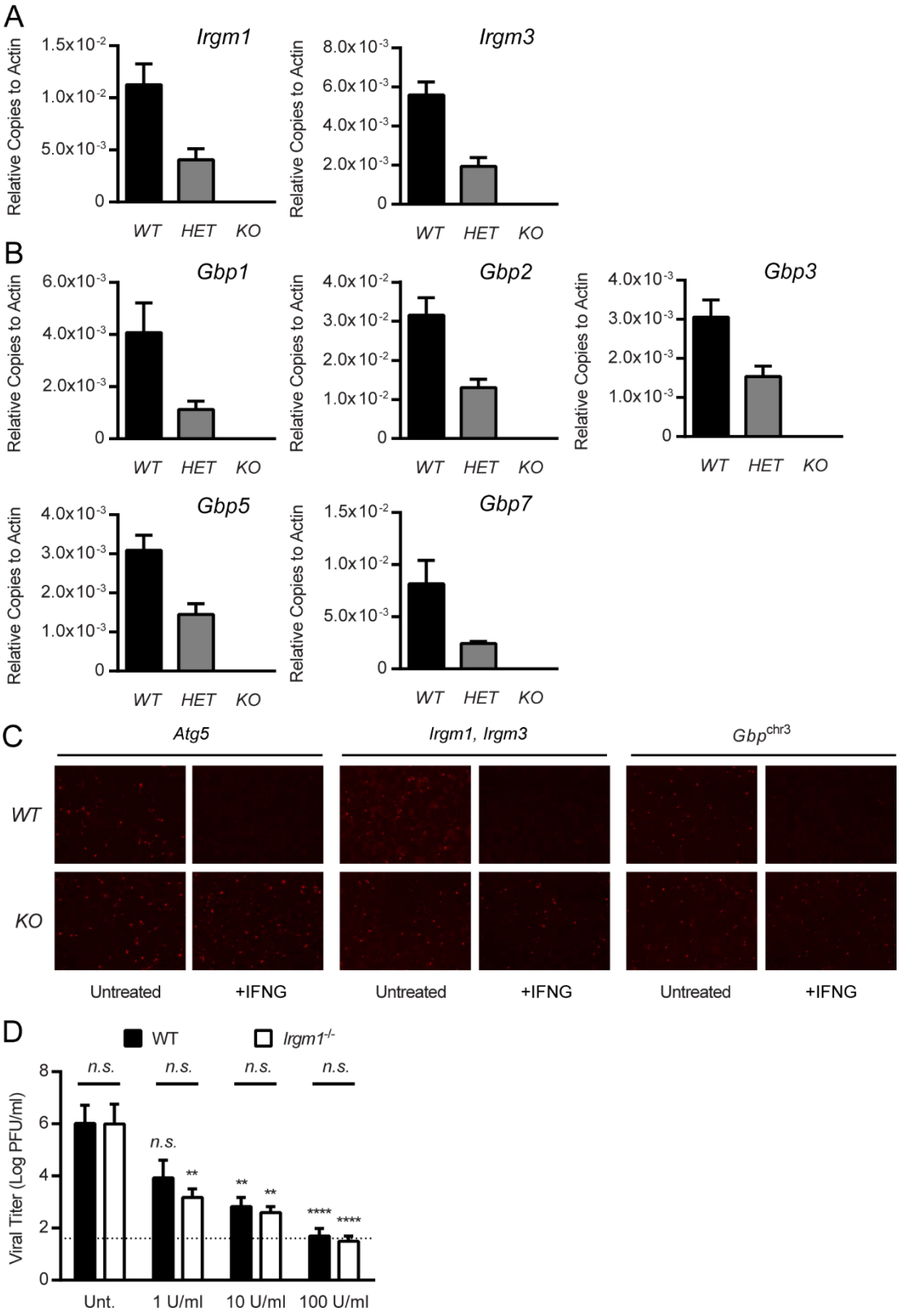

A
Irgm1
Irgm3
Relative Copies to Actin
WT HET KO
B
Gbp1
Gbp2
Gbp3
Gbp5
Gbp7
C
Atg5
Irgm1, Irgm3
Gbpchr3
WT
KO
Untreated
+IFNG
D
WT
Irgm1-/-
n.s.
**
****
Viral Titer (Log PFU/ml)
Unt.
1 U/ml
10 U/ml
100 U/ml

Figure S5. Analysis of IFN-inducible GTPase Defective cells, Related to Figure 4. (A) Quantitative PCR analysis for the transcript levels of *Irgm1* and *Irgm3* in BMDMs from *Irgm1*$^{-/-}$*Irgm3*$^{-/-}$ mice (KO), compared to wild type (WT) and heterozygote (HET) control mice at 12 hour-post-treatment (hpt) of 100 U/ml IFNG. N=3 replicates. (B) Quantitative PCR analysis for the transcript levels of *Gbp1, Gbp2, Gbp3, Gbp5, and Gbp7* in BMDMs from *Gbp*$^{chr3-/-}$ mice (KO) compared to wild type (WT) and heterozygote (HET) control mice at 12 hpt of 100 U/ml IFNG. N=3 replicates. (C) Representative images of MNV RCs in BMDMs from *Atg5*$^{flox/flox}$*+LysMcre*, *Irgm1*$^{-/-}$*Irgm3*$^{-/-}$, *GBP*$^{chr3-/-}$, and their respective littermate control mice. Cells were either untreated or treated with 100 U/ml IFNG for 12 hours before infection with MNV at MOI=5 and harvested and analyzed at 12 hpi with anti-Propol immunofluorescence. N=3 replicates. (D) Growth analysis of MNV in BMDMs from *Irgm1*$^{-/-}$ and littermate control mice. Cells were treated with none (Unt.) or indicated doses of IFNG for 12 hours and then infected with MNV at MOI=0.05. At 24 hpi, cells were harvested to titer infectious virus. Data as mean±SEM. N=3 replicates. One-way ANOVA with Tukey's multiple comparisons test; n.s., not significant; **, $p<0.01$; ****, $p<0.0001$. Dashed line indicates the limit of detection. BMDMs were from 2 mice of each genotype.

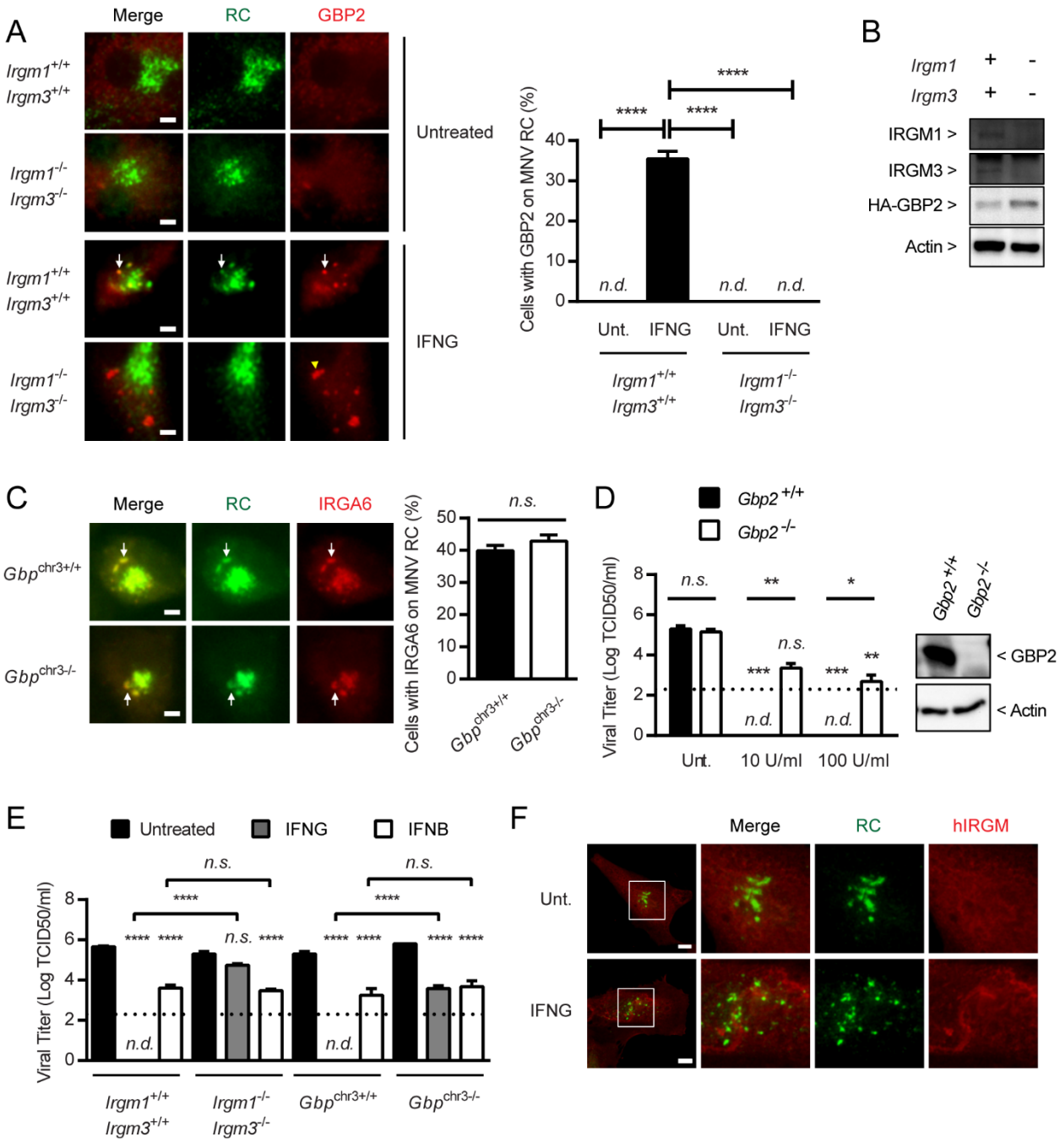

A
Merge
RC
GBP2
Irgm1+/+ Irgm3+/+
Irgm1-/- Irgm3-/-
Untreated
IFNG
Cells with GBP2 on MNV RC (%)
****
****
****
n.d.
n.d.
n.d.
Unt.
IFNG
Unt.
IFNG
Irgm1+/+ Irgm3+/+
Irgm1-/- Irgm3-/-
B
Irgm1
Irgm3
IRGM1 >
IRGM3 >
HA-GBP2 >
Actin >
C
Merge
RC
IRGA6
Gbpchr3+/+
Gbpchr3-/-
Cells with IRGA6 on MNV RC (%)
n.s.
D
Gbp2 +/+
Gbp2 -/-
Viral Titer (Log TCID50/ml)
n.s.
**
*
n.s.
***
***
**
n.d.
n.d.
Unt.
10 U/ml
100 U/ml
< GBP2
< Actin
E
Untreated
IFNG
IFNB
Viral Titer (Log TCID50/ml)
n.s.
n.s.
****
****
n.d.
n.d.
Irgm1+/+ Irgm3+/+
Irgm1-/- Irgm3-/-
Gbpchr3+/+
Gbpchr3-/-
F
Merge
RC
hIRGM
Unt.
IFNG

Figure S6. IFNG Requires the IFN-Inducible GTPases to Inhibit MNV Replication *in vitro* and *in vivo,* Related to Figures 4, 5, and 6. (A) Immunofluorescence analysis of GBP2 in *Irgm1*$^{+/+}$*Irgm3*$^{+/+}$ and *Irgm1*$^{-/-}$*Irgm3*$^{-/-}$ BMDMs transduced with lentiviruses expressing FLAG/HA-tagged GBP2 at 10 hpi of MNV at MOI=5 with simultaneous treatment of none (Untreated) or 100 U/ml IFNG. Representative images (left) and quantitation (right). White arrows indicate representative colocalization and yellow arrowhead indicates a representative aggregate of GBP2. Scale bars, 5 μm. Data as mean±SEM. N=3 replicates. Student's t-test.; ****, p<0.0001. n.d., not detected. (B) A representative western blot of cells described in (A). N=3 replicates. (C) The same analysis described in (A) for endogenous IRGA6 in *Gbp*$^{chr3+/+}$ and *Gbp*$^{chr3-/-}$ BMDMs. Data as mean±SEM. N=3 replicates. Student's t-test. n.s., not significant. (D) Growth analysis of MNV in WT and *Gbp2*$^{-/-}$ BMDMs. Cells were treated with none (Unt.), 10 U/ml or 100 U/ml IFNG for 24 hours and then infected with MNV at MOI=0.05. At 24 hpi, cells were harvested to titer infectious virus. Data as mean±SEM. N=4 replicates. n.d., not detected. One-way ANOVA with Tukey's multiple comparisons test; n.s., not significant; *, p<0.05; **, p<0.01; ***, p<0.001. Dashed line indicates the limit of detection. A representative western blot shown (right). (E) Growth analysis of MNV in BMDMs from *Irgm1*$^{-/-}$*Irgm3*$^{-/-}$, *GBP*$^{chr3-/-}$, and their respective littermate control mice. Cells were treated with none (Untreated), 100 U/ml of IFNG, or 100 U/ml of IFN-beta (IFNB) for 24 hours and then infected with MNV at MOI=0.05. At 24 hpi, cells were harvested to titer infectious virus. Data as mean±SEM. N=3 replicates. n.d., not detected. One-way ANOVA with Tukey's multiple comparisons test; n.s., not significant; ****, p<0.0001. Dashed line indicates the limit of detection. (F) Immunofluorescence assay for the localization of endogenous human IRGM (Red) with regard to the MNV RC (Green) in HeLa cells transfected with a plasmid expressing MNV ORF1 for 6 hours followed by treatment with 100 U/ml IFNG for an additional 18 hours. Representative images shown here. Scale bars, 20 μm. N=3 replicates. BMDMs for (A) to (E) were from 2 mice for each genotype.

Table S1. Quantitative PCR primers used to analyze the transcription of genes, Related to Figures 1, S2 and S5.

Genes	Forward Primer	Reverse Primer
MNV	agctcaggatggtctcggat	tcaagagcaaggtcgaaggg
Irgm1	tgctccactactccccaacat	gctcctactgacctcaggtaac
Irgm3	ctcatcagcccgtggtctaaa	caccgccttaccaatatcttcaa
Irga6	caggacatccgccttaactgt	aggaagtaagtacccattagcca
Irgb6	aggcatgtaagcacctccac	ggacagagaggcaggttcac
Gbp1	acctggagacttcactggct	tttattcagctggtcctcctgtatcc
Gbp2	acctggaacattccctgacc	acagctcctcctcccgcagag
Gbp3	ccagaaaaccaactggaacggaa	tctccagacaaggcacagtc
Gbp5	cactcagcaacgaggagctgaact	tgttctctatggaaggcagagcc
Gbp7	ttgaggaaatgccagaggaccagt	gtctccactattgatagcatccacg
Ulk1	tgcccttgatgagatgttcc	agtctcctctcaatgcacagc
Ulk2	tcgtttatcgctaccacaagg	gcctgctactcacacagttgc
Actin	gccttccttcttgggtatgg	gcactgtgttggcatagagg

Table S2. Sequence of guide RNAs to delete DNA and primers to screen the deletion, Related to Figure 1 and Figure S1.

Gene	Guide RNA	Primers for Screening		Expected Size of Product from WT Genome
Lc3a	gtgatcatcgagcgctacaa	Forward	gaccgctgtaaggaggtgc	678
	tgagttgcaggcggcgcctg	Reverse	tcagaagccgaaggtttcttg	
Gabarap	cggggccggtccgatggtcg	Forward	gtctgcctttccccaacgta	854
	agcgtcagcgacgctagcct	Reverse	gatcctagaacggcgcatca	
Gabarapl1	tagtgcggcgactggcgcgt	Forward	ccagctcttgggaaaagcca	696
	tcgaaagcgcagcgtgcgcg	Reverse	atgggcacaaggctcatctc	
Gabarapl2	gcgacgactactccacaggc	Forward	acagcgcttggaacaggtta	867
	cggacccgtgtcgccctacg	Reverse	gaactcggtcggggtacttc	