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Supplemental Material to:

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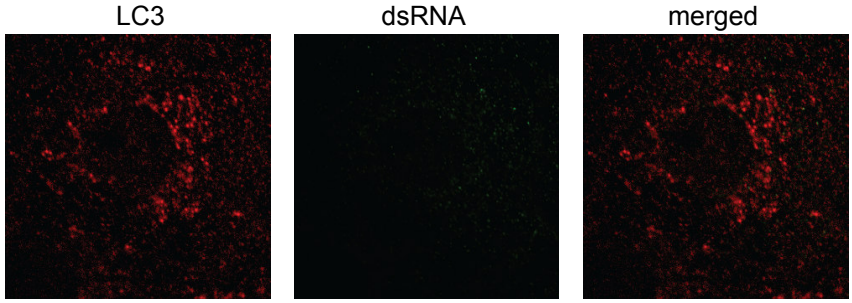
An autophagy-independent role for LC3 in equine arteritis virus replication

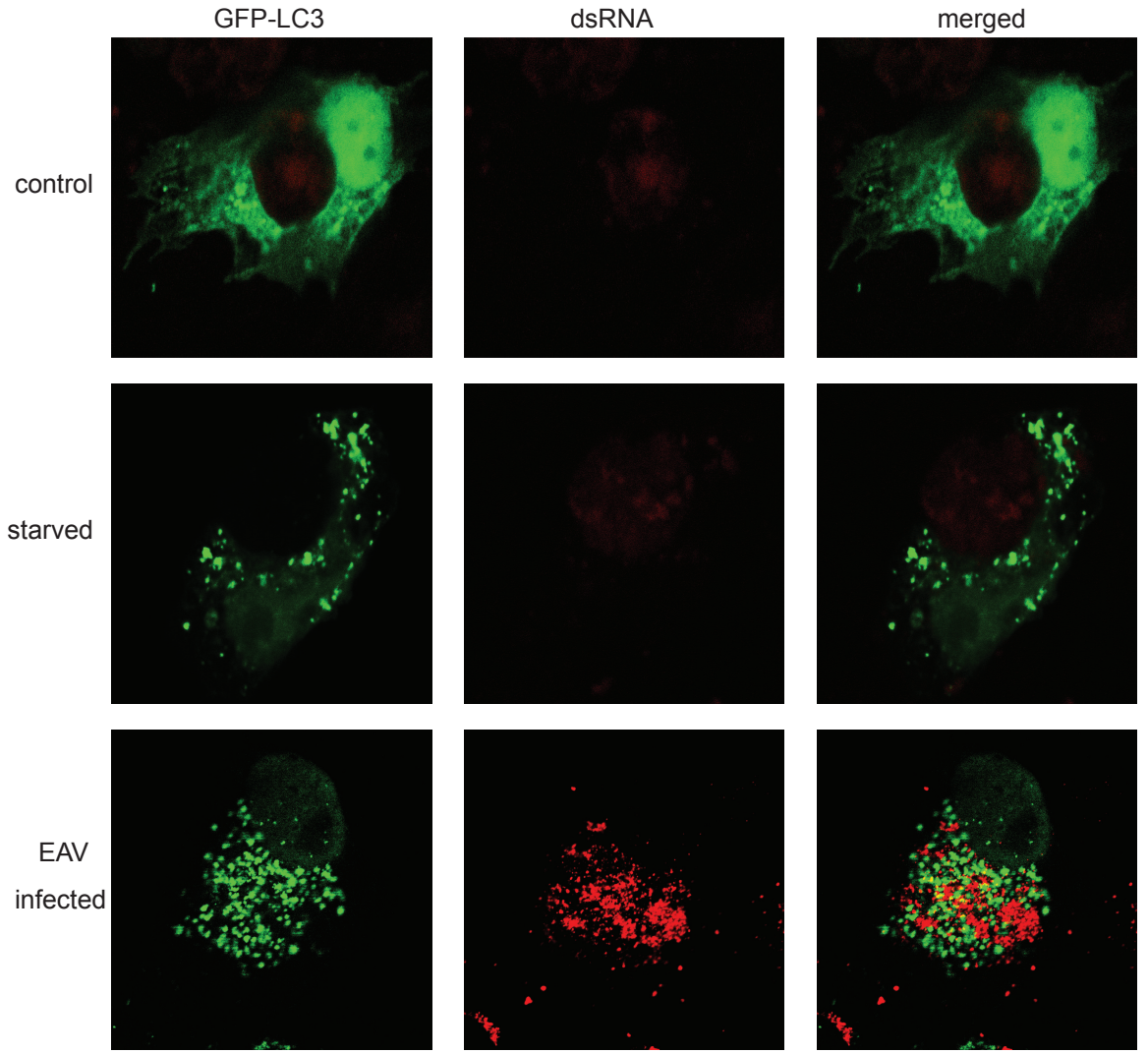
Autophagy 2012; 9(2)
http://dx.doi.org/10.4161/auto.22743

www.landesbioscience.com/journals/autophagy/article/22743



Monastyrska et al., Figure S1

A



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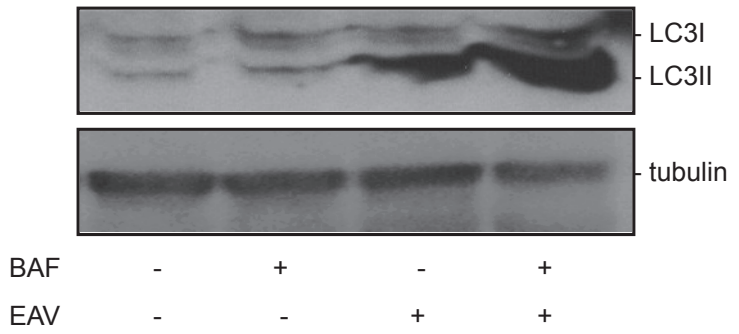
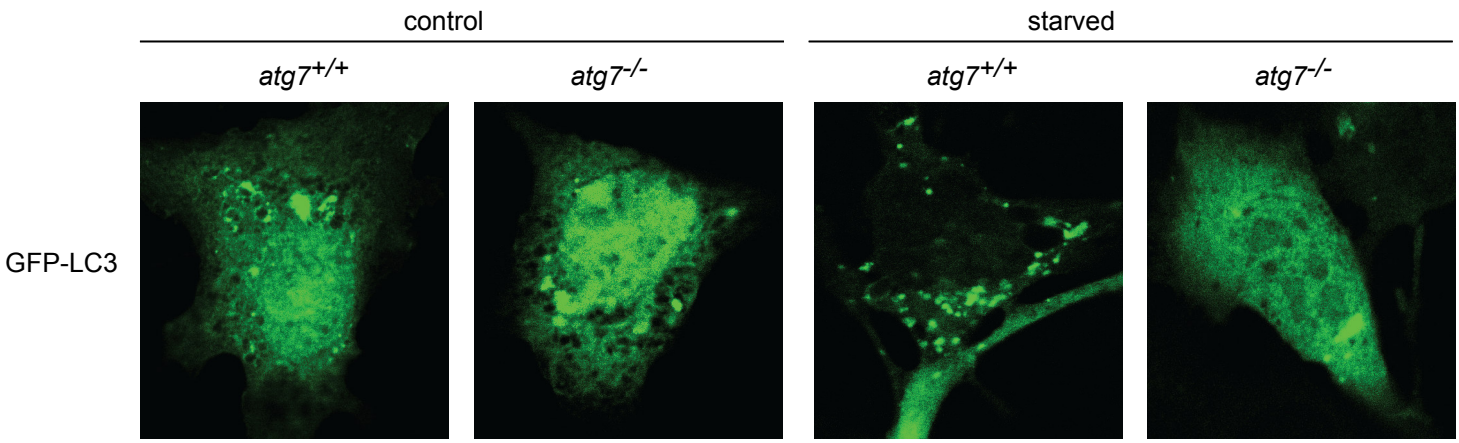
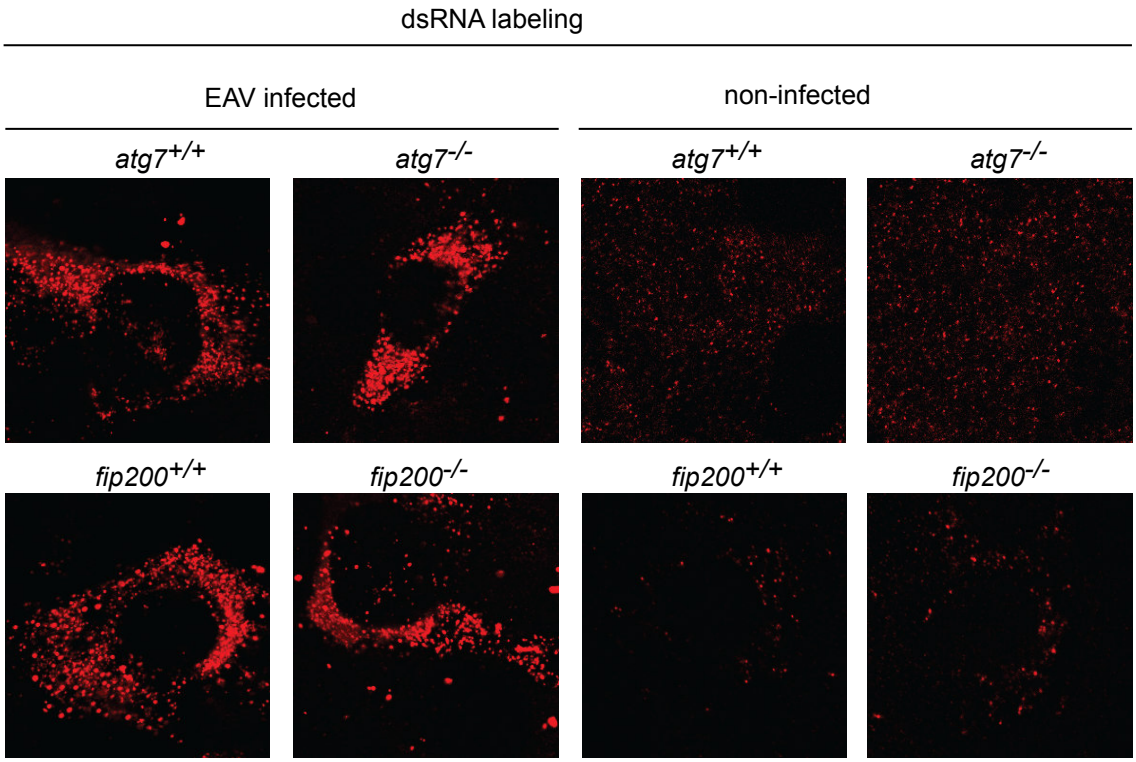


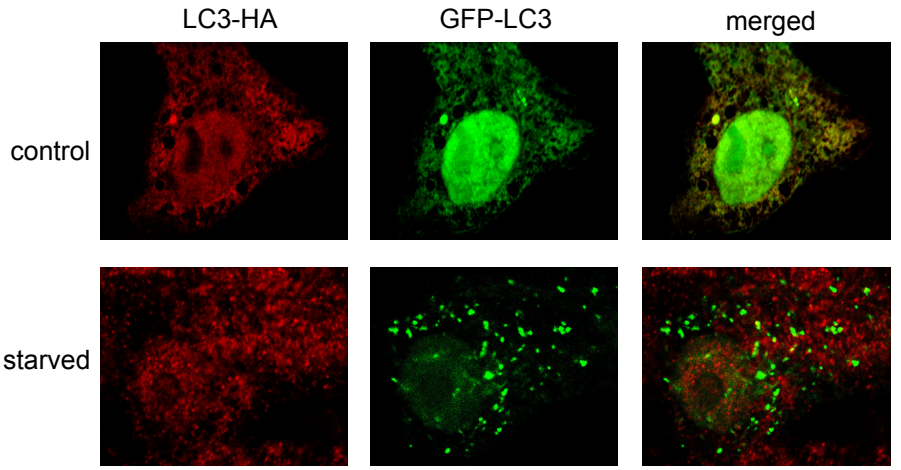
Monastyrska et al., Figure S2



Monastyrska et al., Figure S3



Monastyrska et al., Figure S4



Monastyrska et al., Figure S5

Figure S1. Control labeling of non-infected cells with antibodies against LC3 and dsRNA. Vero E6 cells were fixed and processed for immunofluorescence analysis using LC3- and dsRNA-specific antibodies.

Figure S2. EAV infection induces autophagy. Vero E6 cells were transfected with a plasmid expressing N-terminally GFP-tagged LC3 (GFP-LC3). A. The next day, cells were cultured for 2 h in media with (control) or without (starved) FCS or infected with EAV for 16 h. Cells were subsequently fixed and processed for immunofluorescence analysis using the anti-dsRNA antibody. B. Cells were (mock)-infected with EAV in the absence or presence of bafilomycin A (BAF). After 16 h of infection cells were lysed and processed for Western blot analysis using antibodies against LC3 and tubulin (loading control) .

Figure S3. Autophagosomes are not formed in Atg7-deficient cells. *atg7*$^{+/+}$ and *atg7*$^{-/-}$ MEFs were transfected with a plasmid expressing N-terminally GFP-tagged LC3 (GFP-LC3). The next day, cells were cultured for 2 h in media with (control) or without (starved) FCS. Cells were subsequently fixed and processed for immunofluorescence analysis.

Figure S4. EAV replication does not require the Atg5/Atg7-independent autophagy. *atg7*$^{+/+}$, *atg7*$^{-/-}$, *fip200*$^{+/+}$ and *fip200*$^{-/-}$ MEFs were (mock-) infected with EAV for 16 h and processed for immunofluorescence analysis using antibodies against dsRNA.

Figure S5. Non-lipidated LC3-I does not associate with autophagosomes. Vero E6 cells were transfected with the plasmids expressing either C-terminally HA-tagged non-lipidated LC3-I (LC3-HA) or N-terminally GFP-tagged LC3 (GFP-LC3). The next day, cells were cultured for 2 h in media with (control) or without (starved) FCS and subsequently processed for immunofluorescence analysis.