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Supplemental Material to:

Munir A. Al-Zeer, Hesham M. Al-Younes, Daniel Lauster, Mohammad Abu Lubad and Thomas F. Meyer

Autophagy restricts *Chlamydia trachomatis* growth in human macrophages via IFNG-inducible guanylate binding proteins

Autophagy 2012; 9(1)
http://dx.doi.org/10.4161/auto.22482

www.landesbioscience.com/journals/autophagy/article/22482

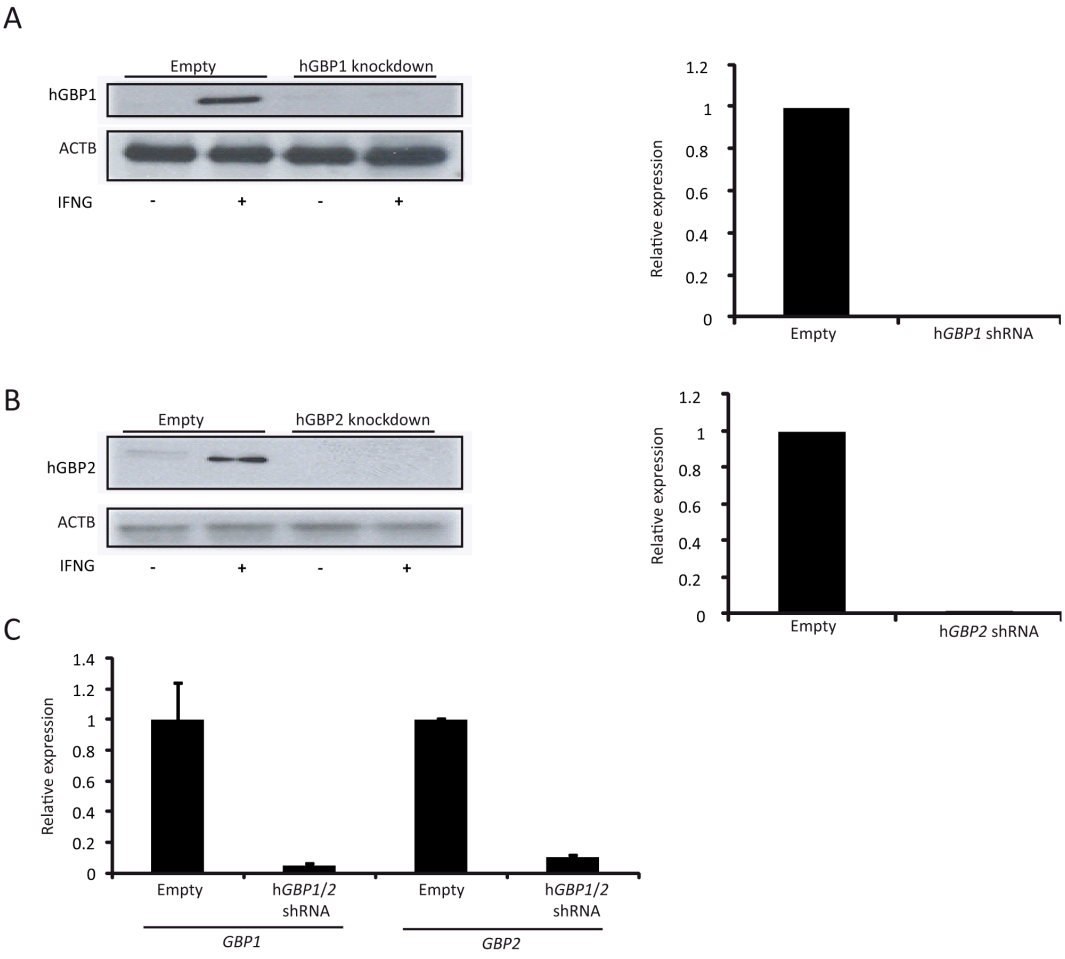

Figure S1

Knockdown validation of shRNA-h*GBP1* and h*GBP2* in THP1 cells using immunoblotting. Cells stably transfected with either h*GBP1* (A) or h*GBP2* (B) shRNA were then treated with 100 U IFNG for 24 h. Control cells were transfected with the empty vector. Anti-hGBP1 or hGBP2 immunoblots analyses of total lysates revealed that h*GBP1*- and h*GBP2*- shRNA efficiently reduced the amount of hGBP1 and hGBP2 protein in comparison to protein levels in cells transfected with the empty vector (A and B left panel) and mRNA as determined by qPCR (A and B right panels). (C) qPCR analysis revealed that h*GBP1/2* double knockdown efficiently reduced the amount of *GBP1* and *2* in comparison to cells transfected with the empty vector. Host ACTB was used as loading control.

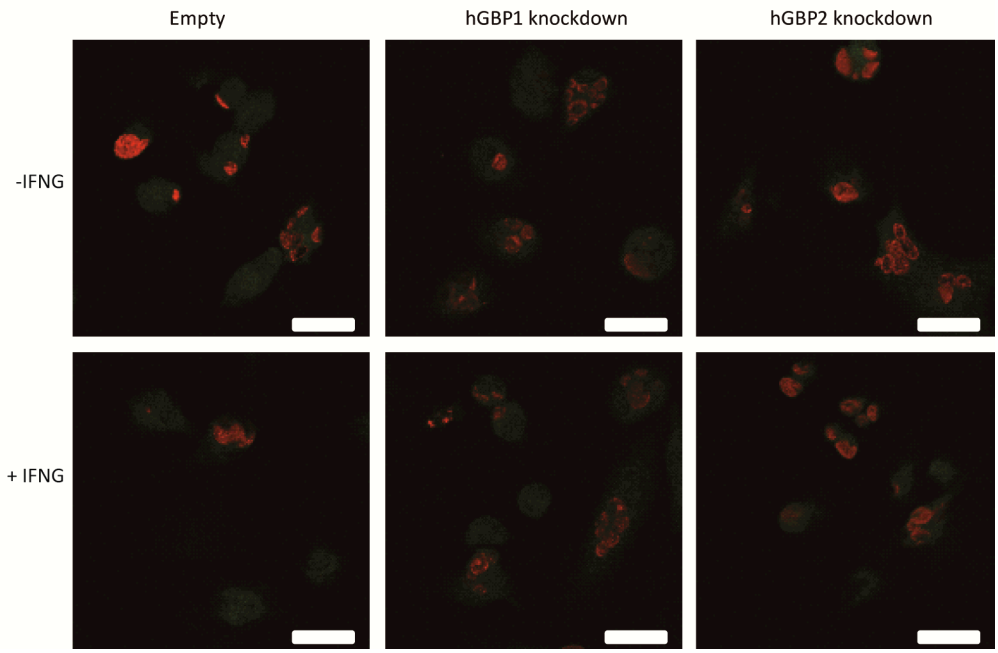

Figure S2

IFNG-induced inhibition of *C. trachomatis* growth was abrogated in cells lacking hGBP1 or hGBP2. Unstimulated and IFNG-stimulated host cells were infected for 48 h with *C. trachomatis* (MOI 5) in the presence or absence of IFNG. Immunofluorescence micrographs of cells infected with *C. trachomatis* (Red). IFNG stimulation did not suppress chlamydial growth in hGBP1 or hGBP2-deficient cells compared to control (Empty) - derived macrophages. Scale bars 30 µm.

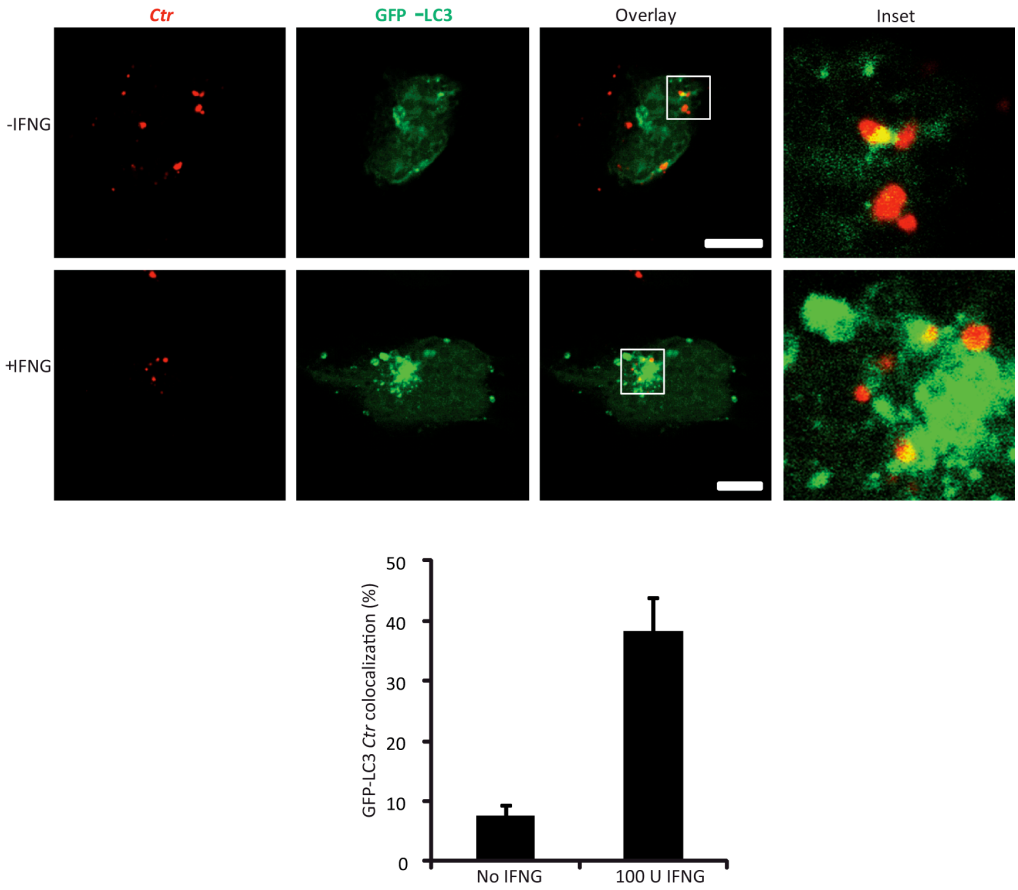

Figure S3

IFNG induced colocalization of autophagosomes with inclusions. Macrophages were transfected for 24 h with the autophagosome membrane marker GFP-LC3 and then exposed to 100 U/ml IFNG for an additional 24 h. Cells were then infected with *C. trachomatis* (MOI 20) for 3 h. IFNG-untreated control cells were similarly infected. LC3 (green) localized to bacterial inclusions (red) in response to IFNG stimulation (A). (B) Quantification of GFP-LC3-positive *C. trachomatis* inclusions in the presence or absence of IFNG. Approximately 300 bacterial inclusions were examined for LC3 sequestration per sample. Quantification results shown as mean percentage normalized to control from two independent experiments. Error bars ± SD.

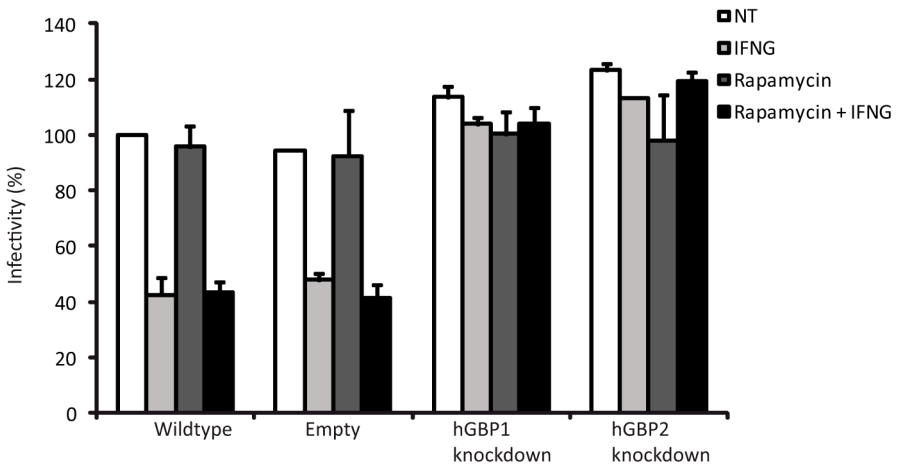

Figure S4

Rapamycin treatment did not affect chlamydial growth in human macrophages. Wild-type, empty, h*GBP1* or h*GBP2* shRNAs human macrophages were infected with Chlamydia (MOI 5) for 2 h and then treated with rapamycin (5 nm) alone or rapamycin and 100 U IFNG or left untreated as control. The yield of infectious progeny (48 h p.i.) did not change upon rapamycin stimulation in the presence or absence of IFNG. Infectivity expressed as a percentage of control cells ± standard deviation (SD) from three independent experiments (n=3).

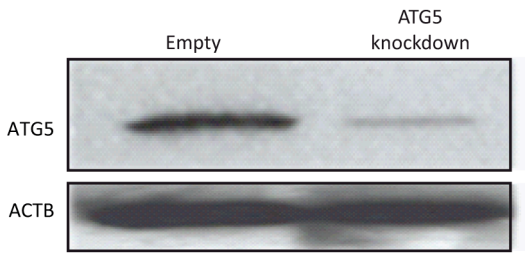

Figure S5

Knockdown validation of shRNA-*ATG5* in THP1 cells using immunoblotting. Cells were stably transfected with *ATG5* shRNA while control cells were transfected with the empty vector. Anti-ATG5 immunoblot analysis of total cell lysates revealed that shRNA efficiently reduced level of ATG5 protein in comparison to cells transfected with the empty vector. Host ACTB is used as loading control.

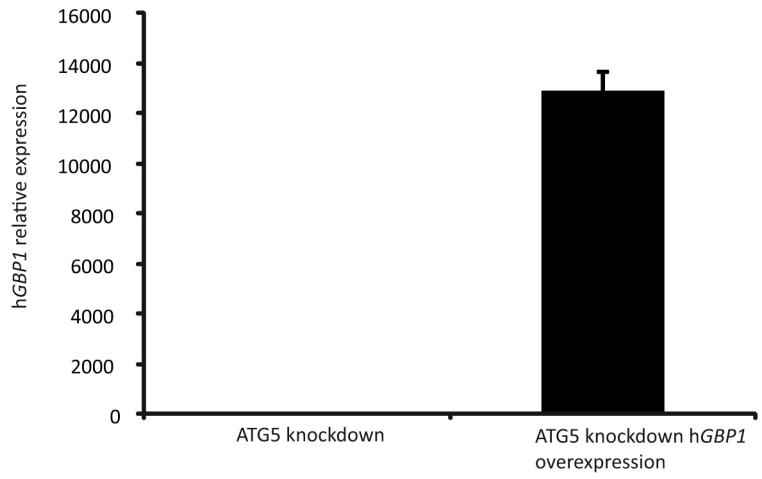

Figure S6

qPCR analysis revealed that h*GBP1* efficiently overexpressed in ATG5 knockdownTHP1 stably overexpressing h*GBP1* in comparison to control cells. Data are displayed as mean values ± SD of three replicates.

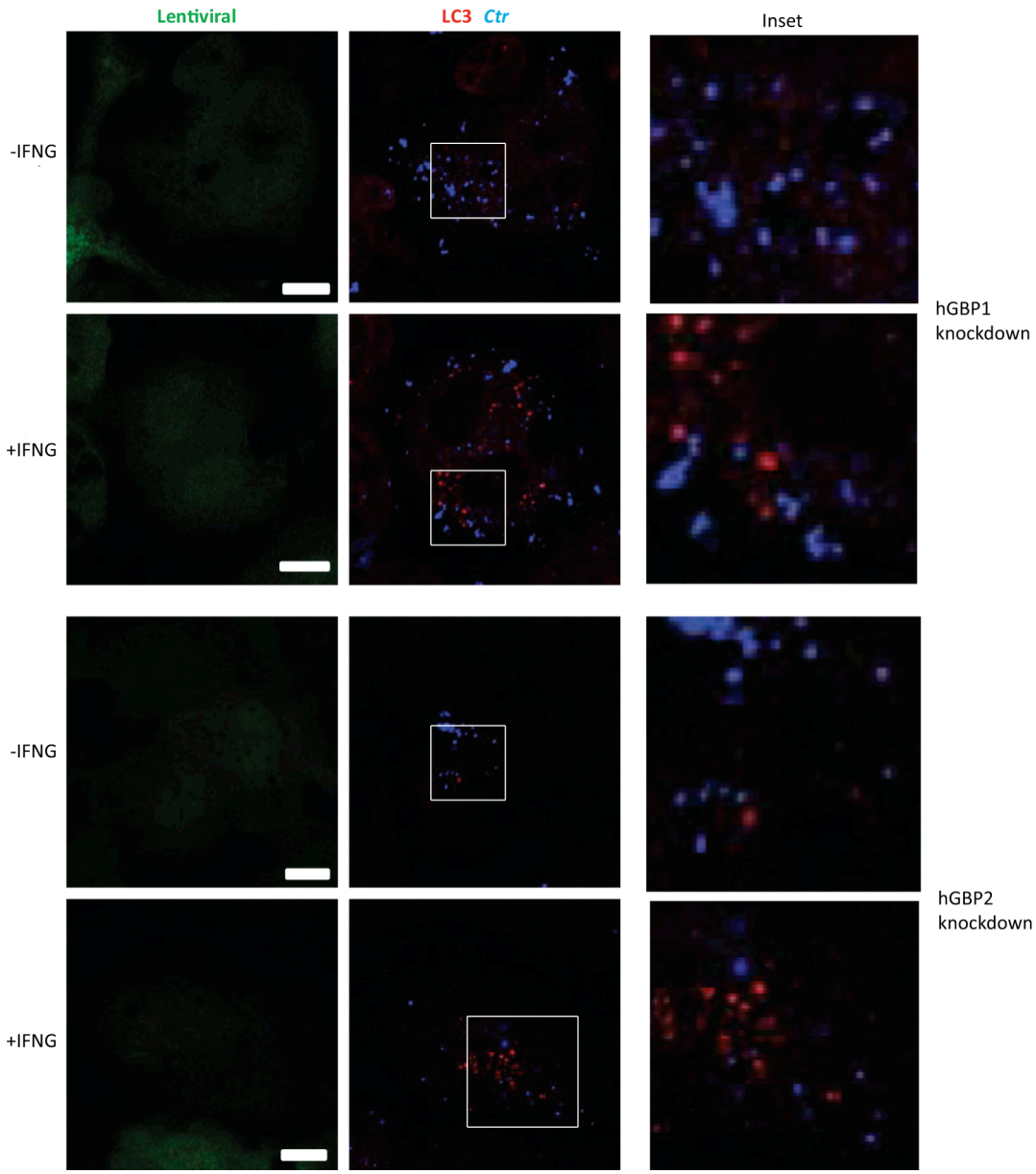

Figure S7

hGBP1 and hGBP2-stable knockdown THP1-derived macrophage monolayers were prestimulated for 24 h with 100 U/ml IFNG and then infected with *C. trachomatis* as described in Figure 2. IFNG untreated control cells were similarly infected. Double immunofluorescence labelling 3 h p.i. demonstrated no recruitment of LC3 (red) to inclusions (blue) in cells deficient of either hGBP1 or hGBP2. Images are representative of two independent experiments. Higher magnification insets are shown on the right. Scale bars 10 µm.

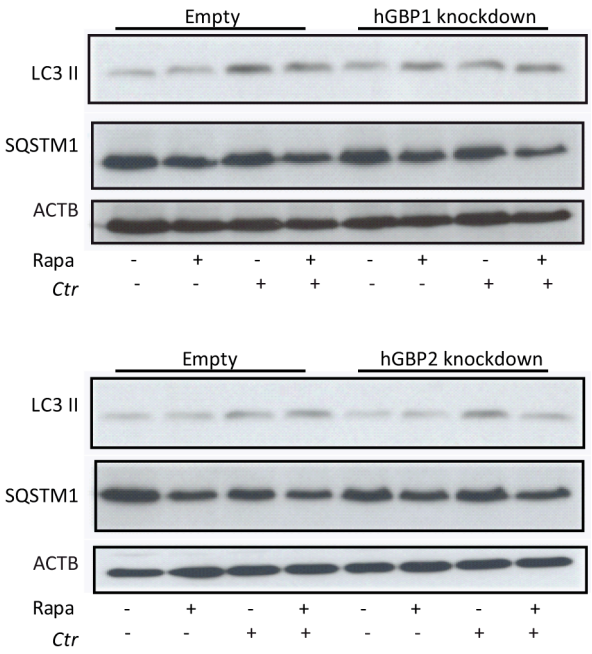

Figure S8

Anti-LC3 and SQSTM1 immunoblots analyses of total lysates from uninfected WT, hGBP1 or -2 KD THP1 cells from cultures infected for 6 h. Some uninfected cell cultures were exposed to 5 nM rapamycin (Rapa) for 3 h. Other monolayers were pretreated with 5 nM rapamycin prior to infection and then infected in the presence of chemicals. Autophagy activation is reflected by the increased cellular level of LC3-II and the decreased levels of SQSTM1. ACTB was used to control equal loading of proteins. Rapamycin treatment induced autophagy activation in WT and hGBP1 or 2 knockdown cells. SQSTM1 levels decreased in rapamycin treated h*GBP1* shRNAs (A) and h*GBP2* shRNAs cells (B) similar to control cells.