Dupont et al.

Supplementary methods

Quantitative real time RT-PCR

Total RNA was extracted from BMM and cDNA was synthetized using a Cells-to-Ct Kit (Applied Biosystems), according to the manufacturer's instructions. Real-time PCR was performed using SYBR Green Master Mix (Applied Biosystems), and products were detected on a Prism 5300 detection system (SDS, ABI/Perkin-Elmer). The relative extent of IL-1β expression was calculated using the 2eΔΔC(t) method. The results presented herein are from three independent experiments, each of which was performed in duplicate. Conditions for real time PCR were: initial denaturation for 10 min at 95°C, followed by 40 amplification cycles with 15s at 95°C and 1 min at 60°C. The sequences of the primers (IDT DNA Technologies, MA) were as follows: HPRT1 forward 5- GGA GCG GTA GCA CCT CCT -3; HPRT1 reverse 5- CTG GTT CAT CAT CGCTAATCA C -3; IL-1β reverse 5- TCT TCT TTG GGT ATT GCTTGG -3; IL-1β forward 5- TGT AAT GAA AGA CGG CAC ACC -3

Extracellular LDH release assay

Lactate dehydrogenase (LDH) release was measured using a cytotoxicity detection kit (Promega, Madison, WI). All LDH data are means ± standard error of the mean (s.e.m).

Supplementary discussion

Some of the nuclear staining with IL-1β in BMMs (e.g. in Suppl. Figs. S3 and S4) may be nonspecific, but we cannot rule out functional roles (a topic beyond the scope of our study). IL-1 family members such as IL-1α and IL-1β are known to be distributed between the cytoplasm and the nucleus, reflecting their dual functionality or to counter excessive inflammation (Luheshi et al, 2009a). Of further note is that IL-1β has been observed in the nucleus of microglia, a major macrophage cell type of the central nervous system (Luheshi et al, 2009b).

Supplementary references

Luheshi NM, Rothwell NJ, Brough D (2009a) Dual functionality of interleukin-1 family cytokines: implications for anti-interleukin-1 therapy. *Br J Pharmacol* **157:** 1318-1329

Luheshi NM, Rothwell NJ, Brough D (2009b) The dynamics and mechanisms of interleukin-1alpha and beta nuclear import. *Traffic* **10:** 16-25

Supplementary figures

Suppl. Fig. S1. A. $Atg5^{fl/fl}$ Cre^{-} and $Atg5^{fl/fl}$ Cre^{+} BMM were induced for autophagy by starvation in EBSS (Starv) or not (Full) for 1 h. Total cell extracts were analyzed by

immunobloting. **B.** LPS-pretreated BMM were stimulated with 20 μM nigericin (Nig) for 1 h with or without co-treatment with the mTor catalytic inhibitor pp242. IL-1β secretion was measured by ELISA. **C.** Bone marrow-derived macrophages **(**BMM), pretreated with 100 ng/ml LPS overnight to induce pro-IL-1β expression, were exposed to Alum (250 μg/ml) for 2 h with autophagic induction by starvation (Starv) in EBSS or were incubated in full medium (Full), and secreted IL-1β assayed. Data, means ± s.d. (n≥3); *, $p<0.05$. **D.** LPS-pretreated BMM were stimulated with 1 or 5 μM amyloid-β peptide fibrils (Aβ) for 2 h with or without autophagic induction by starvation and IL-1β secretion measured. **E.** BMM were transfected with scramble (Scr) control siRNA or siRNAs against ASC, NLRP3 or GRASP55. After 48 h following transfection, cells were treated overnight with LPS and IL-1β mRNA levels were quantified by real-time RT-PCR and internally normalized with respect to the *HPRT1* gene. Data, means ± s.d. (n=3). †, $p\geq0.05$. **F.** BMM were transfected with scrambled control siRNA (Scr) or siRNAs against ASC, NLRP3 or GRASP55. After 48 h following transfection, cells were treated overnight with LPS and pro-IL1β level assayed by immunoblots.

Suppl. Fig. S2. A-B. Bone marrow-derived macrophages **(**BMM), pretreated with 100 ng/ml LPS overnight to induce pro-IL-1β expression, were exposed to nigericin (20 μM) for 20, 30 or 60 minutes with autophagic induction by starvation (Starv) in EBSS or were incubated in full medium (Full). IL-1β secretion (A) and LDH released (B) was measured by ELISA and by a cytotoxicity detection kit respectively. Data, means ± s.e.m. (n≥3); *, $p<0.05$. **C-D.** Bone marrow-derived macrophages **(**BMM), pretreated with 100 ng/ml LPS overnight to induce pro-IL-1β expression, were exposed to silica (250 μg/ml) for 20, 30 or 60 minutes with autophagic induction by starvation (Starv) in EBSS or were incubated in full medium (Full). IL-1β secretion (C) and LDH released (D) was measured by ELISA and by a cytotoxicity detection kit respectively. Data, means ± s.e.m. (n≥3); *, $p<0.05$; †, $p\geq0.05$. **E.** BMM from $Atg5^{fl/fl}$ Cre^{-} and $Atg5^{fl/fl}$ Cre^{+} mice were pretreated overnight with 100 ng/ml LPS, stimulated for 1 h with the inflammasome agonist nigericin (20 μM) in full medium (Full). Cell culture supernatants were assayed for murine IL-1β. Data, means ± s.d. (n≥3); *, $p<0.05$. **F***,* as in E, assayed for IL-18. Data, means ± s.d. (n≥3); *, $p<0.05$.

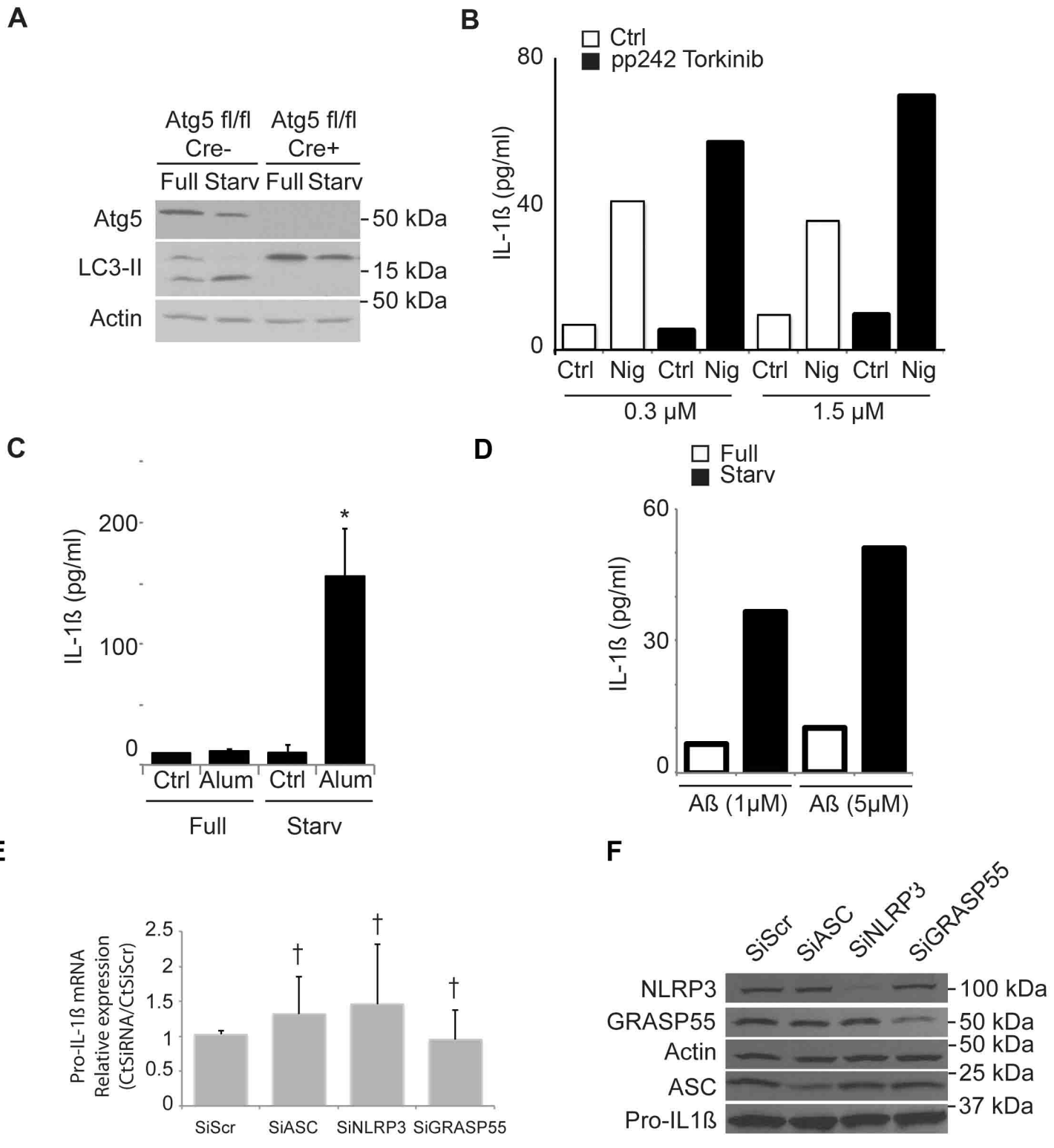
Suppl. Fig. S3. A. Colocalization analysis of IL-1β with the basal autophagic machinery factor LC3. Fluorescence: LC3 (green, Alexa 488); IL-1β (red, Alexa568). BMM from GFP-LC3 knock-in mice were pretreated with 100 ng/ml LPS overnight, stimulated for 30 min with the inflammasome agonist nigericin (20 μM) in full medium. BMM were prepared for immunofluorescence microscopy using fluorescently labeled antibodies against GFP and IL-1β. **B.** A line fluorescence tracing from images in A**. C.** Pearson's colocalization coefficients for IL-1β and LC3. Data, means ± s.e.m. (n≥3). **D.** LPS-pretreated BMM were starved (starv) for 1h with 20 μM nigericin and cathepsin B inhibitor CA-074 Me (10 μM) or with Bafilomycin A1 (Baf-100 nM). IL-1β mRNA levels were quantified by real-time RT-PCR, normalized with respect to the HPRT1 gene, and presented as fold increases. Data, means ± s.d. (n≥3); †, $p\geq0.05$.

Suppl. Fig. S4. A. Colocalization analysis of Rab8a with the basal autophagic machinery factor LC3 and IL-1β. Fluorescence; LC3 (green, Alexa488), IL-1β (red,

Alexa568), Rab8a (blue, Alexa633). BMM from GFP-LC3 knocking mice were pre-treated with LPS and stimulated for 30 min with 20 μM nigericin in full medium. BMM were prepared for immunofluorescence microscopy. White arrows, triple colocalization. **B.** A line fluorescence tracing from images in A. **C.** Pearson's colocalization coefficients for IL-1β and Rab8a. **D.** BMM were transfected twice with scramble (Scr) control siRNA or siRNAs against Rab8a. After 48 h following transfection, cells were treated overnight with LPS and IL-1β mRNA levels were quantified by real-time RT-PCR, normalized with respect to the HPRT1 gene, and presented as fold increases. Data are means +/- SDV. **E.** Colocalization of Sec6 with LC3 and IL-1β. BMM from GFP-LC3 knock-in mice were pre-treated with LPS and analyzed by immunofluorescence microscopy. White arrows, triple colocalization (Sec6-IL-1β-LC3; turquoise arrows, LC3-Sec6 colocalization. **F.** Line tracing, fluorescence signal coincidence analysis. **G.** Pearson's colocalization coefficients for IL-1β and sec6. **H.** Colocalization analysis of Sec6 with LC3 and IL-1β (as in A). White arrows, triple colocalization. **I.** A line fluorescence tracing from images in H. **J**. Pearson's colocalization coefficients for IL-1β and sec6. Pearson's coefficients bars and error bars, means ± s.d. (n=3).

Suppl. Fig. S5. A,B. BMM cells were transfected with siRNAs or scrambled (Scr) control siRNA. After 48 h of transfection, cells were treated with LPS and the day after subjected to 20 μM nigericin in full medium, and secreted IL-1β (A) and IL-18 (B) were measured. Data, means ± s.d. (n≥3); *, $p<0.05$. The number of cells in the experiment in panel A ($2x10^5$ cells) was 2.8 times higher than in Fig. 4A ($0.7x10^5$ cells). **C**. Effect of GRASP65 on autophagy induction by measuring LC3-II. BMM cells were transfected with GRASP65 or with GRASP65 and GRASP55 siRNAs or scramble (Scr) control. At 72 h post-transfection, cells were induced for autophagy, treated or not with Bafilomycin A1 (Baf) to inhibit autophagic degradation. All lanes are from the same gel (one intervening lane was removed to avoid redundancy).

Suppl. Fig. S6. Fluorescent confocal microscopy images: GM130 (green, Alexa488), GRASP55 (red, Alexa568). **A.** Control, resting BMM (). **B.** Nigericin, BMM treated with 20 μM nigericin.


A
Atg5 fl/fl Cre-
Atg5 fl/fl Cre+
Full Starv Full Starv
Atg5
LC3-II
Actin
50 kDa
15 kDa
50 kDa
B
Ctrl
pp242 Torkinib
IL-1ß (pg/ml)
80
40
0
Ctrl Nig Ctrl Nig Ctrl Nig Ctrl Nig
0.3 µM
1.5 µM
C
IL-1ß (pg/ml)
200
100
0
*
Ctrl Alum Ctrl Alum
Full
Starv
D
Full
Starv
IL-1ß (pg/ml)
60
30
0
Aß (1µM)
Aß (5µM)
E
Pro-IL-1ß mRNA
Relative expression
(CtSiRNA/CtSiScr)
2.5
2
1.5
1
0.5
0
†
†
†
SiScr
SiASC
SiNLRP3
SiGRASP55
F
SiScr
SiASC
SiNLRP3
SiGRASP55
NLRP3
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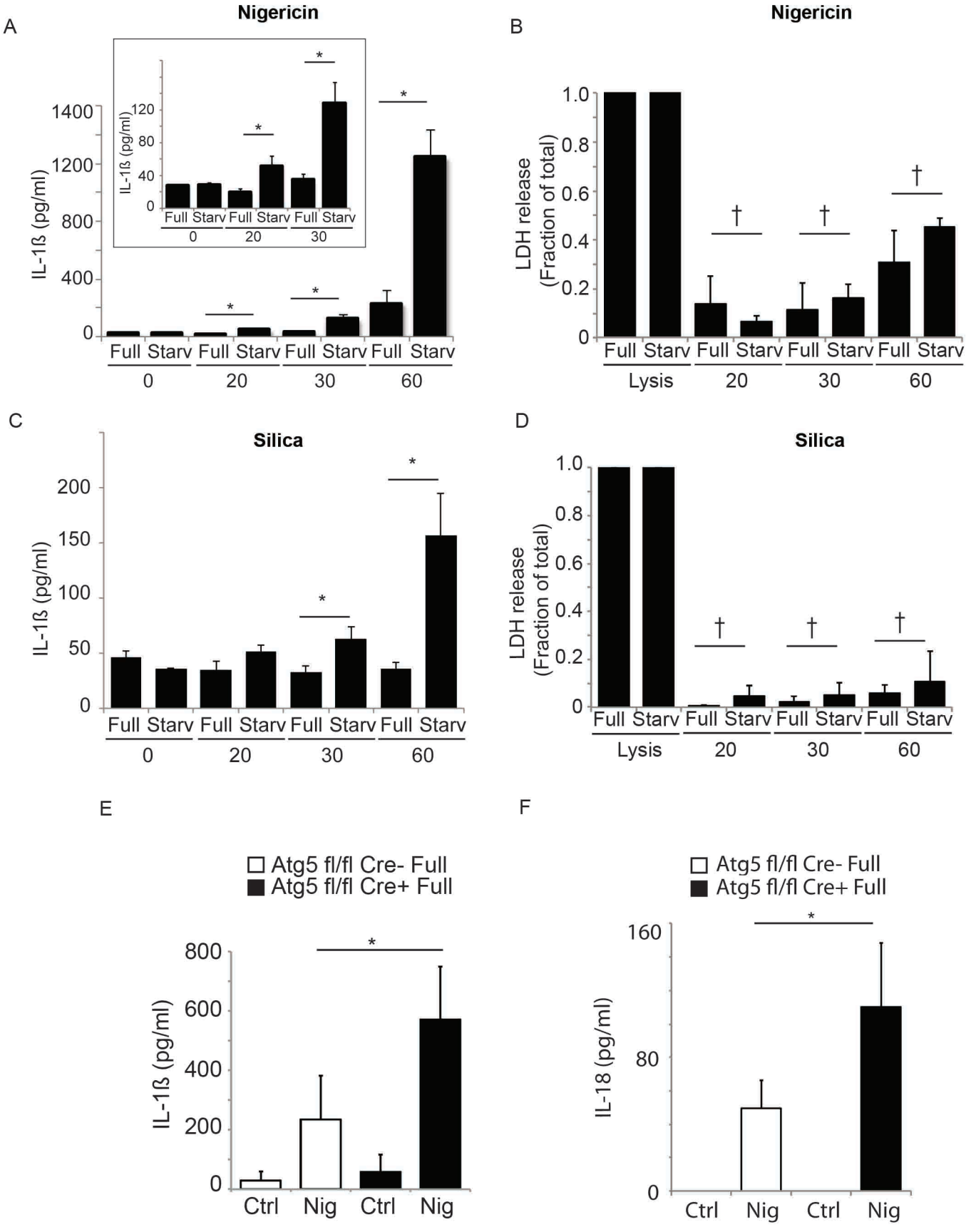
ASC
Pro-IL1ß
100 kDa
50 kDa
50 kDa
25 kDa
37 kDa

Suppl. Fig. S1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Nigericin
IL-1ß (pg/ml)
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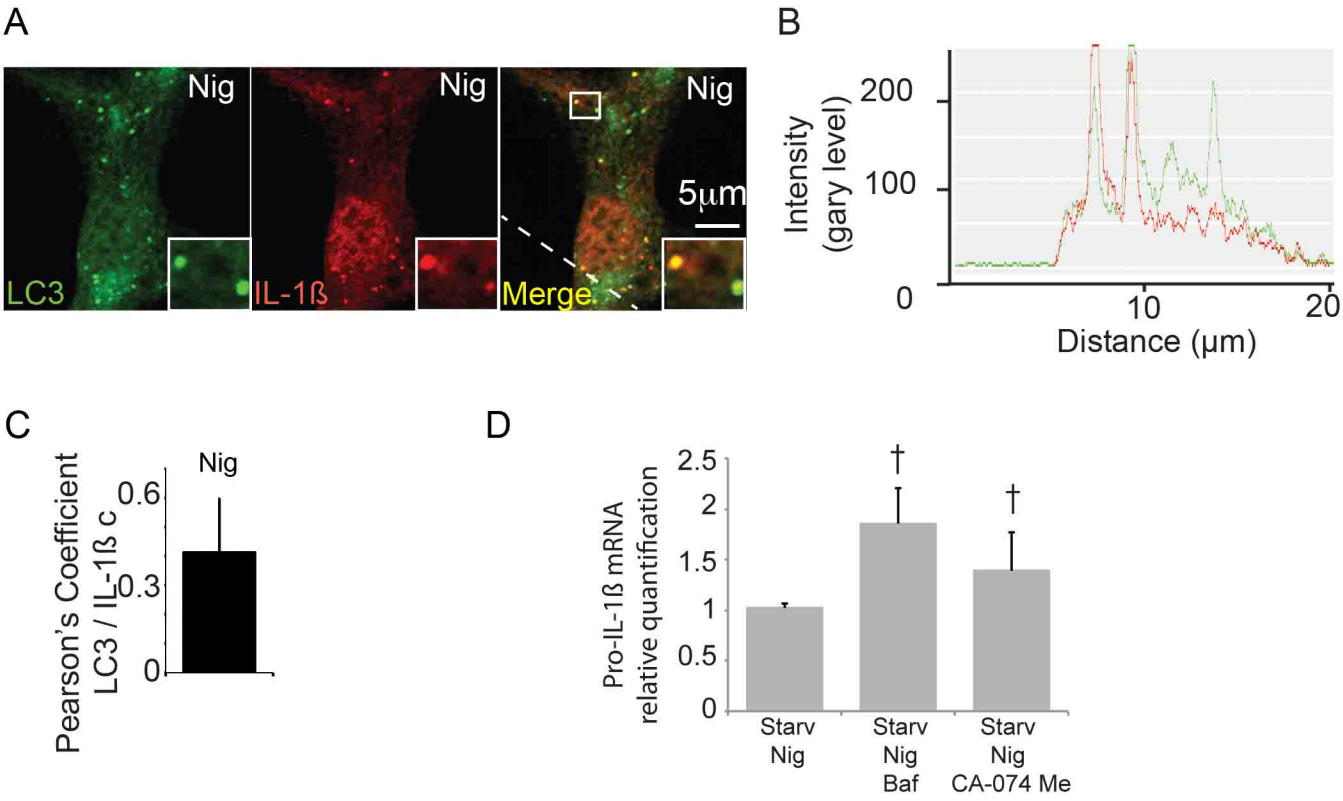
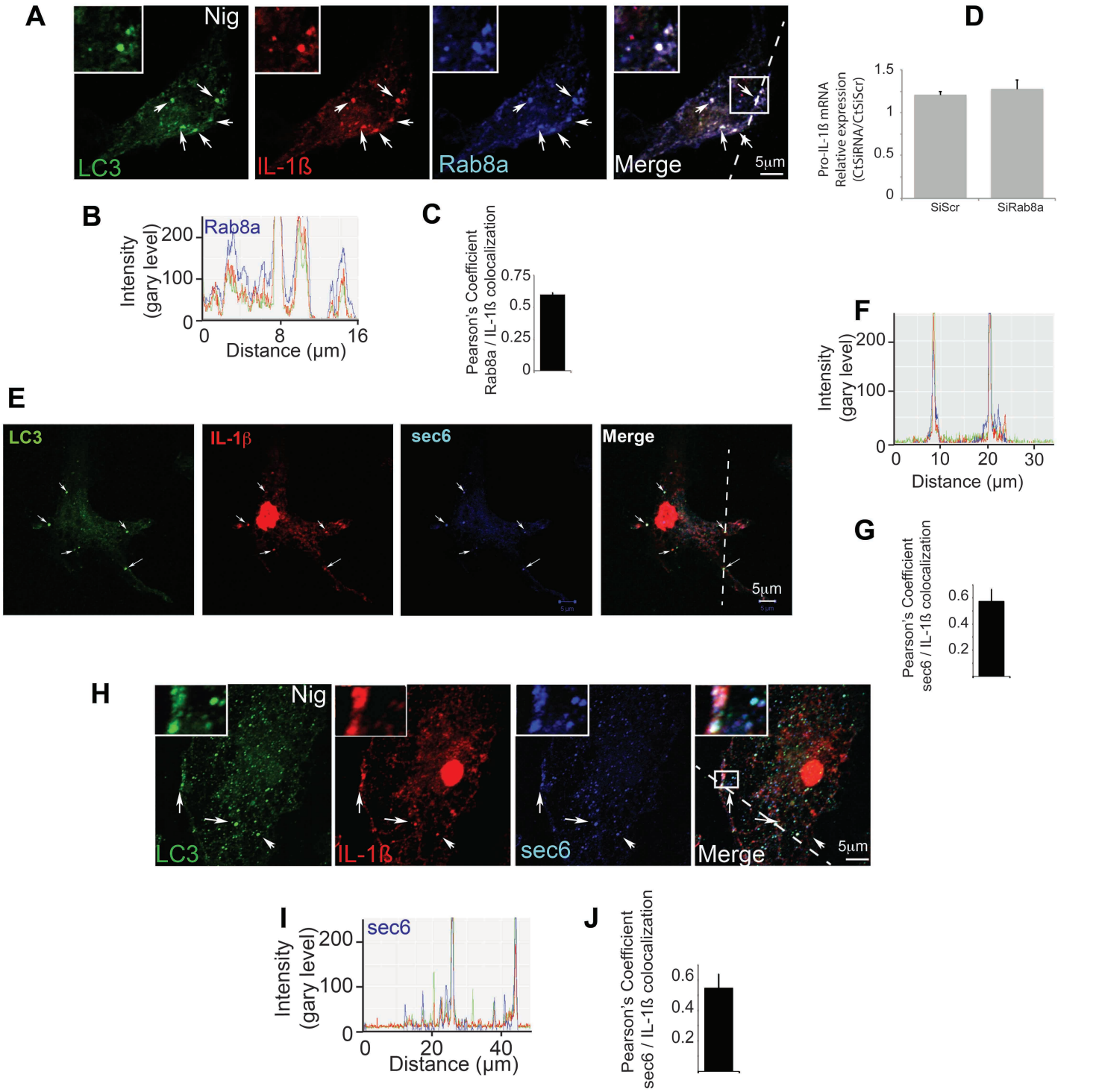
1200
800
400
0
Full Starv Full Starv Full Starv Full Starv
0 20 30 60
*
IL-1ß (pg/ml)
120
80
40
0
Full Starv Full Starv Full Starv
0 20 30
B
Nigericin
LDH release (Fraction of total)
1.0
0.8
0.6
0.4
0.2
0
†
Full Starv Full Starv Full Starv Full Starv
Lysis 20 30 60
C
Silica
IL-1ß (pg/ml)
200
150
100
50
0
*
Full Starv Full Starv Full Starv Full Starv
0 20 30 60
D
Silica
LDH release (Fraction of total)
1.0
0.8
0.6
0.4
0.2
0
†
Full Starv Full Starv Full Starv Full Starv
Lysis 20 30 60
E
Atg5 fl/fl Cre- Full
Atg5 fl/fl Cre+ Full
IL-1ß (pg/ml)
800
600
400
200
0
*
Ctrl Nig Ctrl Nig
F
Atg5 fl/fl Cre- Full
Atg5 fl/fl Cre+ Full
IL-18 (pg/ml)
160
80
0
*
Ctrl Nig Ctrl Nig

Suppl. Fig. S2


A
Nig
Nig
Nig
5µm
LC3
IL-1ß
Merge
B
Intensity (gary level)
200
100
0
10
20
Distance (µm)
C
Nig
Pearson's Coefficient LC3 / IL-1ß c
0.6
0.3
0
D
Pro-IL-1ß mRNA relative quantification
2.5
2
1.5
1
0.5
0
†
†
Starv Nig
Starv Nig Baf
Starv Nig CA-074 Me

Suppl. Fig. S3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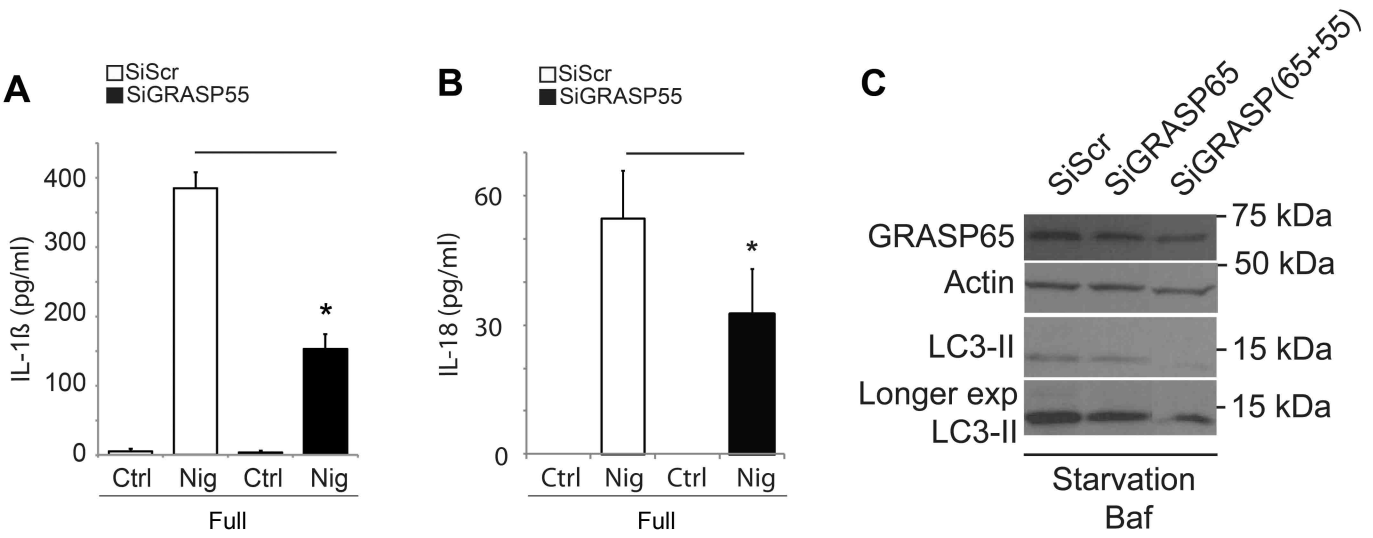
Nig
LC3
IL-1ß
Rab8a
Merge
5µm
B
Rab8a
Intensity (gary level)
200
100
0
0
8
16
Distance (µm)
C
Pearson's Coefficient Rab8a / IL-1ß colocalization
0.75
0.5
0.25
0
D
Pro-IL-1ß mRNA Relative expression (CtSiRNA/CtSiScr)
1.5
1
0.5
0
SiScr
SiRab8a
E
LC3
IL-1β
sec6
Merge
5µm
F
Intensity (gary level)
200
100
0
0
10
20
30
Distance (µm)
G
Pearson's Coefficient sec6 / IL-1ß colocalization
0.6
0.4
0.2
H
Nig
LC3
IL-1ß
sec6
Merge
5µm
I
sec6
Intensity (gary level)
200
100
0
0
20
40
Distance (µm)
J
Pearson's Coefficient sec6 / IL-1ß colocalization
0.6
0.4
0.2

Suppl. Fig. S4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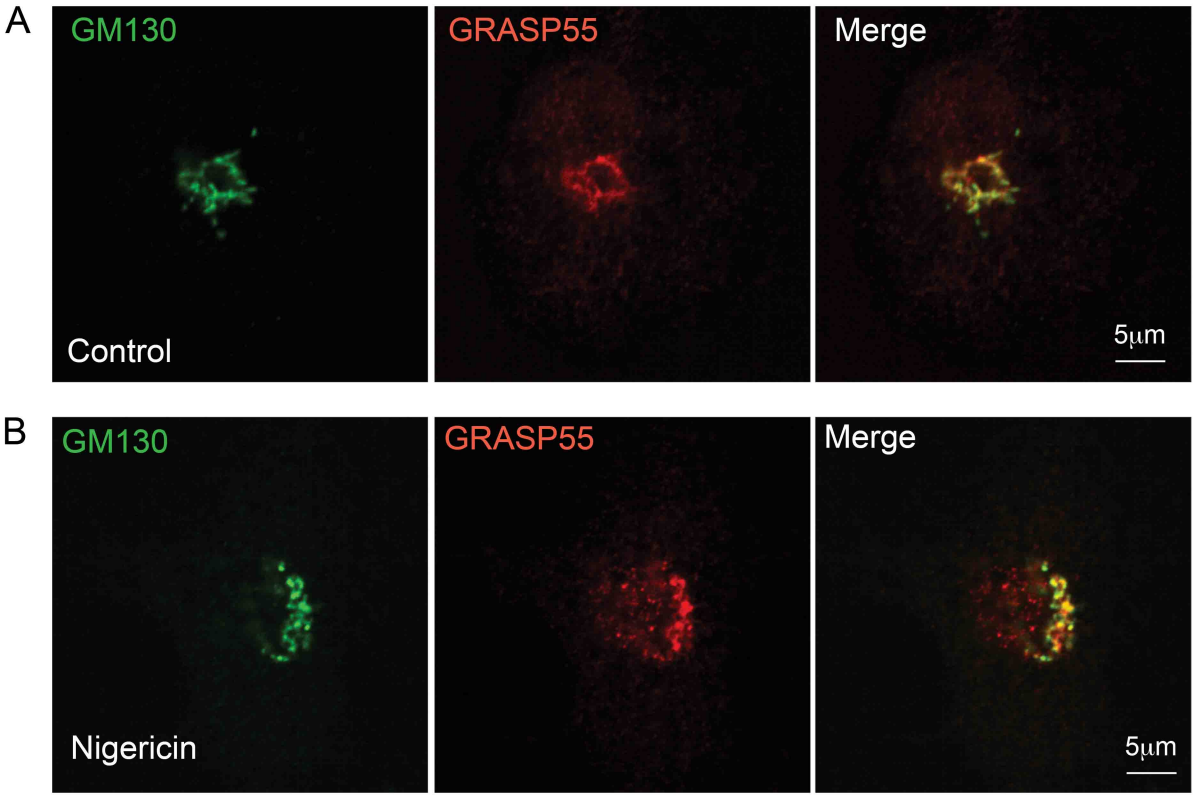
SiScr
SiGRASP55
IL-1ß (pg/ml)
400
300
200
100
0
*
Ctrl
Nig
Ctrl
Nig
Full
B
SiScr
SiGRASP55
IL-18 (pg/ml)
60
30
0
*
Ctrl
Nig
Ctrl
Nig
Full
C
SiScr
SiGRASP65
SiGRASP(65+55)
GRASP65
Actin
LC3-II
Longer exp
LC3-II
75 kDa
50 kDa
15 kDa
15 kDa
Starvation
Baf

Suppl. Fig. S5


A
GM130
GRASP55
Merge
Control
5μm
B
GM130
GRASP55
Merge
Nigericin
5μm

Suppl. Fig. S6