Supplemental Materials

Molecular Biology of the Cell

Bhatnagar *et al*.

Supplementary Figures

Figure S1. Association of GFP-LC3, ubiquitin and p62 with ALIS: RAW GFP-LC3 cells were immunostained for p62 and ubiquitin to evaluate the localization of LC3, ubiquitin and p62 upon LPS stimulation at 8, 12 and 24 hours as compared to unstimulated cells. Scale bars, 10µm **(A)**. ALIS appeared to associate with phagosomes as detected in macrophage cells after stimulation with LPS (26h) **(B)**. Scale bars, 5µm. Occurrence of ALIS fusion is shown in the insets. Quantification of association of GFP-LC3 puncta smaller than 0.5µm clustering with STM upon 2hpi and 16hpi **(C)** Statistical significance was calculated on pooled data from experimental replicates using unpaired t-test. The experiments were performed in triplicates. A minimum 150 cells were counted for each repeat. (P) * < 0.05, (P) ** < 0.005, (P) *** <0.0005, (P) **** < 0.0001, ns = non-significant. Data are represented as mean ± SEM. Images from live cells are shown for indicated time points. LPS (48h) followed by infection with *M. smegmatis* mRFP for 48h **(D)**. Images from live cells are shown for indicated time points. Stabilization of GFP-LC3 puncta with bacteria **(Movie 3)**. Scale bars, 5µm. Line profile analysis of microtubule thickness from control cells and LPS (12h) treated cells **(E)**. Scale bars, 5µm.

Figure S2. Fold proliferation of *E. coli* K12 **(A)** and STM WT::LLO **(B)** in peritoneal macrophages (MOI = 10) (n=3, N=2 for K12 and n=3, N=3 for LLO). Statistical significance was calculated on experimental replicates using unpaired t-test. Quantification of mean intensity change of signal from STM-mCherry infected in RAW GFP-LC3 either unstimulated or pre-stimulated with LPS **(C)**. Statistical significance was calculated on pooled data from experimental replicates using one-way ANOVA with Sidak's multiple comparisons test upon comparing mean intensities per cell in p62-siRNA transfected cells vs non-target siRNA transfected cells. The experiments were performed in duplicates. A minimum 30 cells were counted for each repeat. (P) * < 0.05, (P) ** < 0.005, (P) *** <0.0005, (P) **** < 0.0001, ns = non-significant. Data are represented as mean ± SEM.

Figure S3. SIM images showing spatial localization, in unstimulated and LPS stimulated cells of p62 with GFP-LC3 **(A)**, and ubiquitin with GFP-LC3 **(B)**. RAW GFP-LC3 immunostained with anti-p62 and anti-Ub antibodies after 24 hours of LPS stimulation. ALIS

corresponding to different sizes with positive colocalizations of the three proteins is sequestered **(C)**. Scale bars, 2μm.

Figure S4. Graph to show changes in fluorescence intensity across sucrose density gradient fractions from supernatant **(A)** and pellet fractions **(B)**. Comparison between LPS treated lysate fraction and untreated fraction based on the cellular components **(C),** molecular functions **(D)**, and biological processes **(E).** Distribution of proteins from LPS treated fraction based on their cellular components **(F)** and molecular functions **(G)**.

Figure S5. Validation of association of AMPs identified from the mass spectrometry data with p62 in HeLa Kyoto cells: Cells transfected with AMPs and immunostained for the indicated tag of AMPs and endogenous p62. Nuclear staining was done using DAPI. For mCherry-Syk, cells were excited with 561 nm laser and emission spectra were collected ranging from wavelengths of 580-630 nm **(A-F)**. Scale bar, 5μm. LPS treated RAW GFP-LC3 cell lysates fractionated using a buffer containing 0.2, or 0.5, or 1 or 2% Triton X-100 and soluble and insoluble fractions were immunoblotted for IFITM2, IFITM3, Bst2, p62 and Tubulin **(G)**.

Figure S1

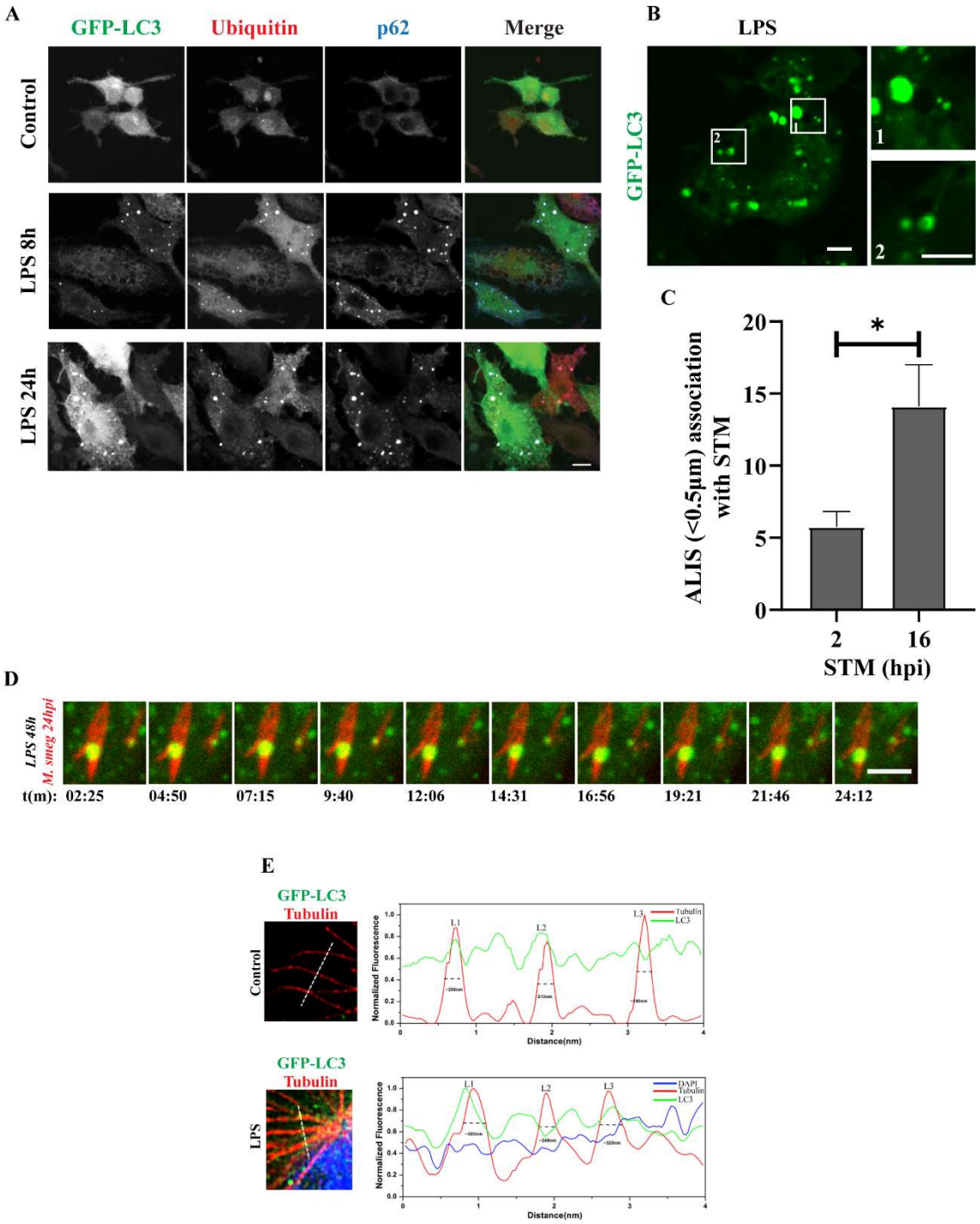

A
GFP-LC3
Ubiquitin
p62
Merge
Control
LPS 8h
LPS 24h
B
LPS
GFP-LC3
1
2
C
ALIS (<0.5μm) association with STM
20
15
10
5
0
*
2
16
STM (hpi)
D
LPS 48h
M. smeg 24hpi
t(m):
02:25
04:50
07:15
9:40
12:06
14:31
16:56
19:21
21:46
24:12
E
GFP-LC3
Tubulin
Control
LPS
L1
L2
L3
Tubulin
LC3
DAPI
Normalized Fluorescence
Distance(nm)

Figure S2

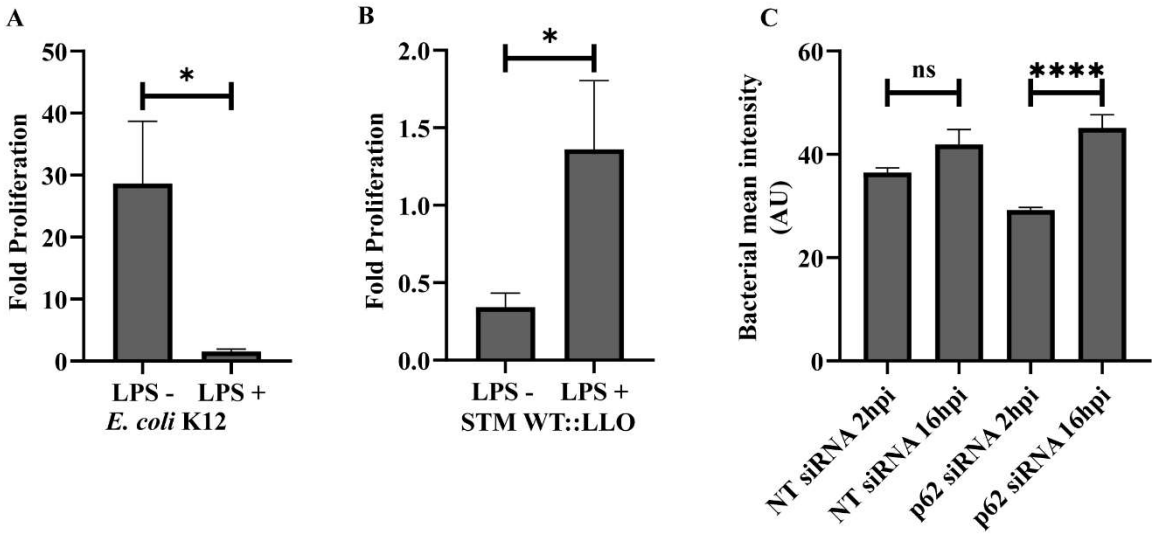

A
Fold Proliferation
50
40
30
20
10
0
*
LPS -
LPS +
E. coli K12
B
Fold Proliferation
2.0
1.5
1.0
0.5
0.0
*
LPS -
LPS +
STM WT::LLO
C
Bacterial mean intensity (AU)
60
40
20
0
ns
****
NT siRNA 2hpi
NT siRNA 16hpi
p62 siRNA 2hpi
p62 siRNA 16hpi

Figure S3

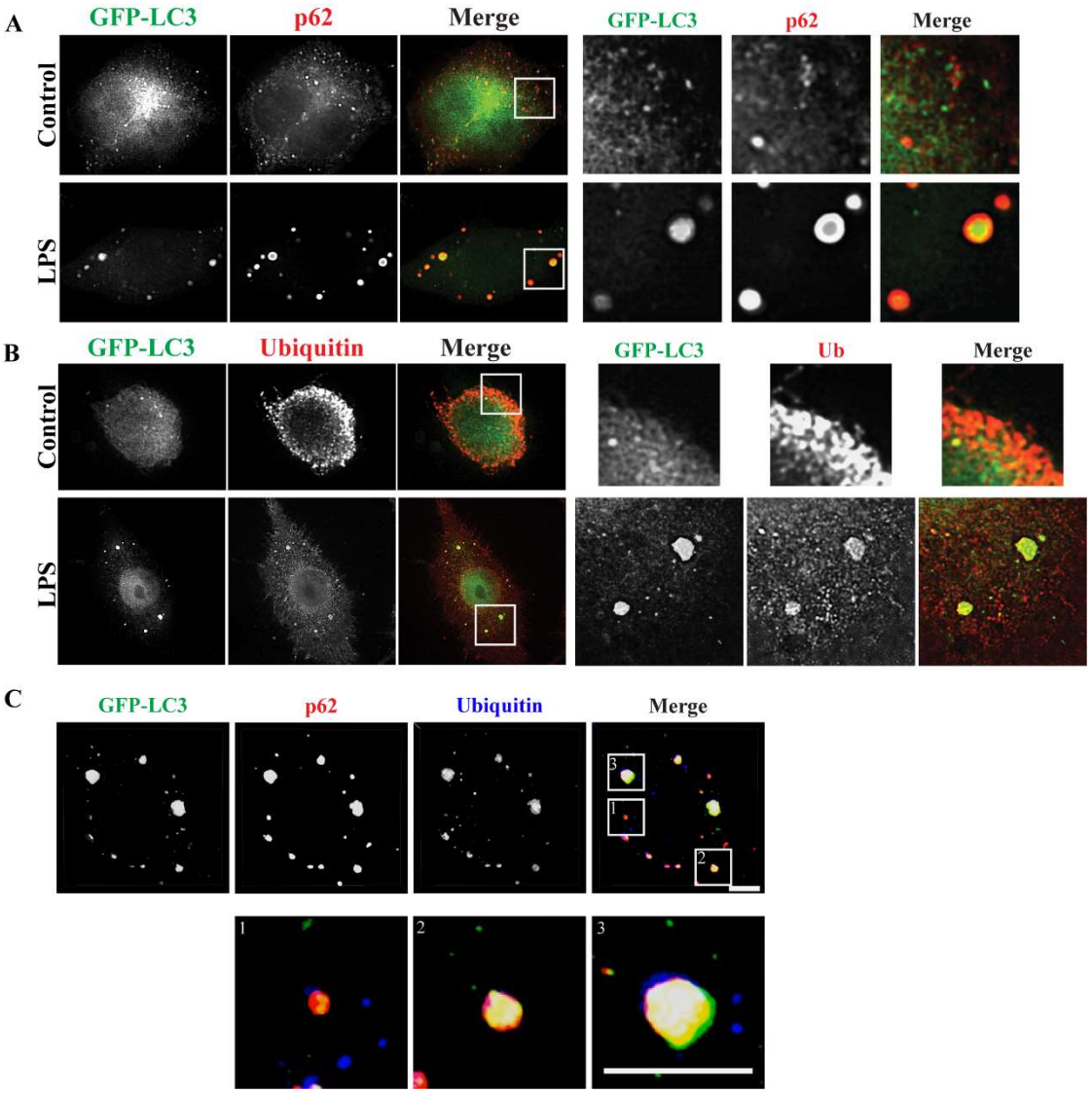

A
GFP-LC3
p62
Merge
GFP-LC3
p62
Merge
Control
LPS
B
GFP-LC3
Ubiquitin
Merge
GFP-LC3
Ub
Merge
Control
LPS
C
GFP-LC3
p62
Ubiquitin
Merge
3
1
2
1
2
3

Figure S4

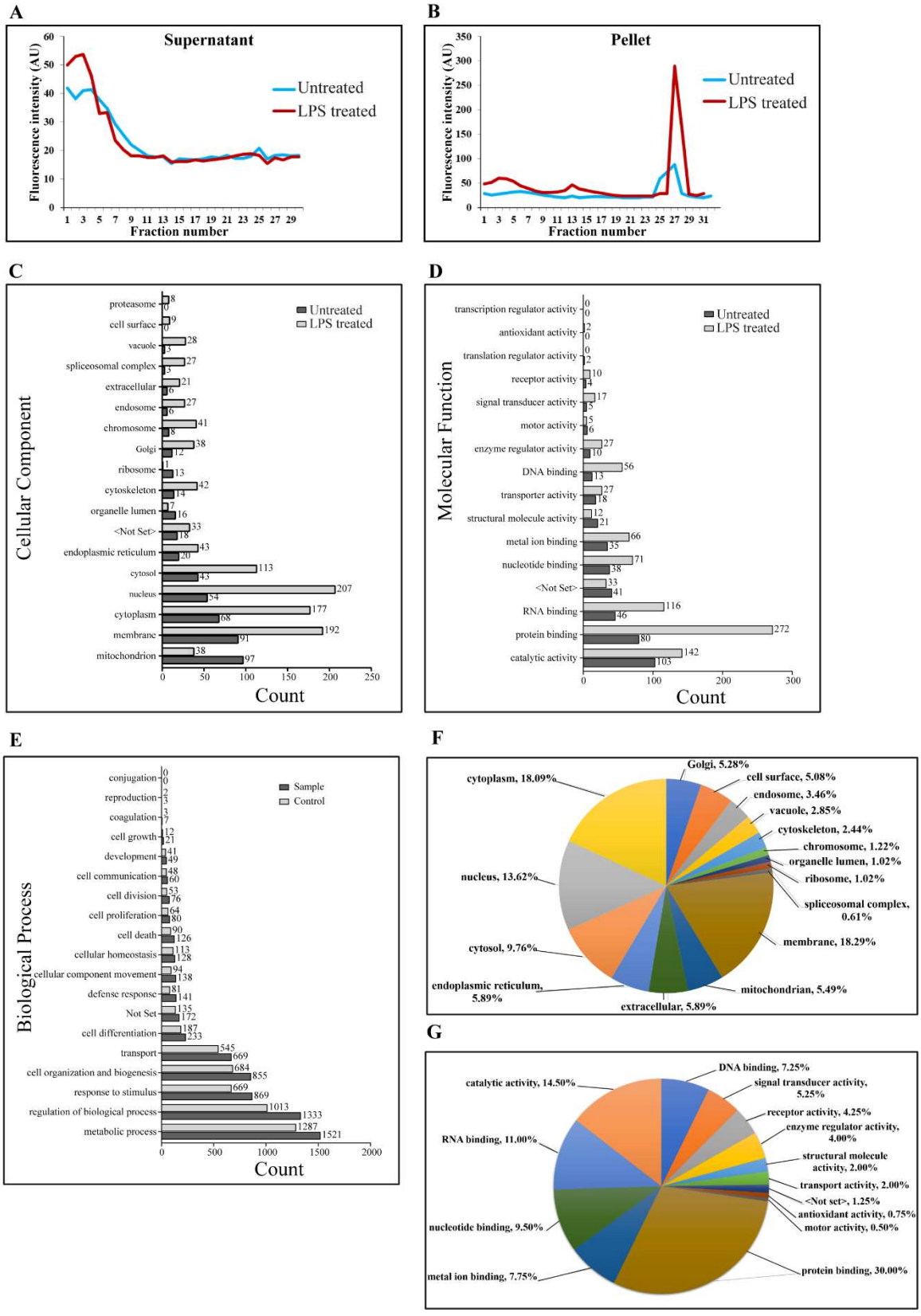
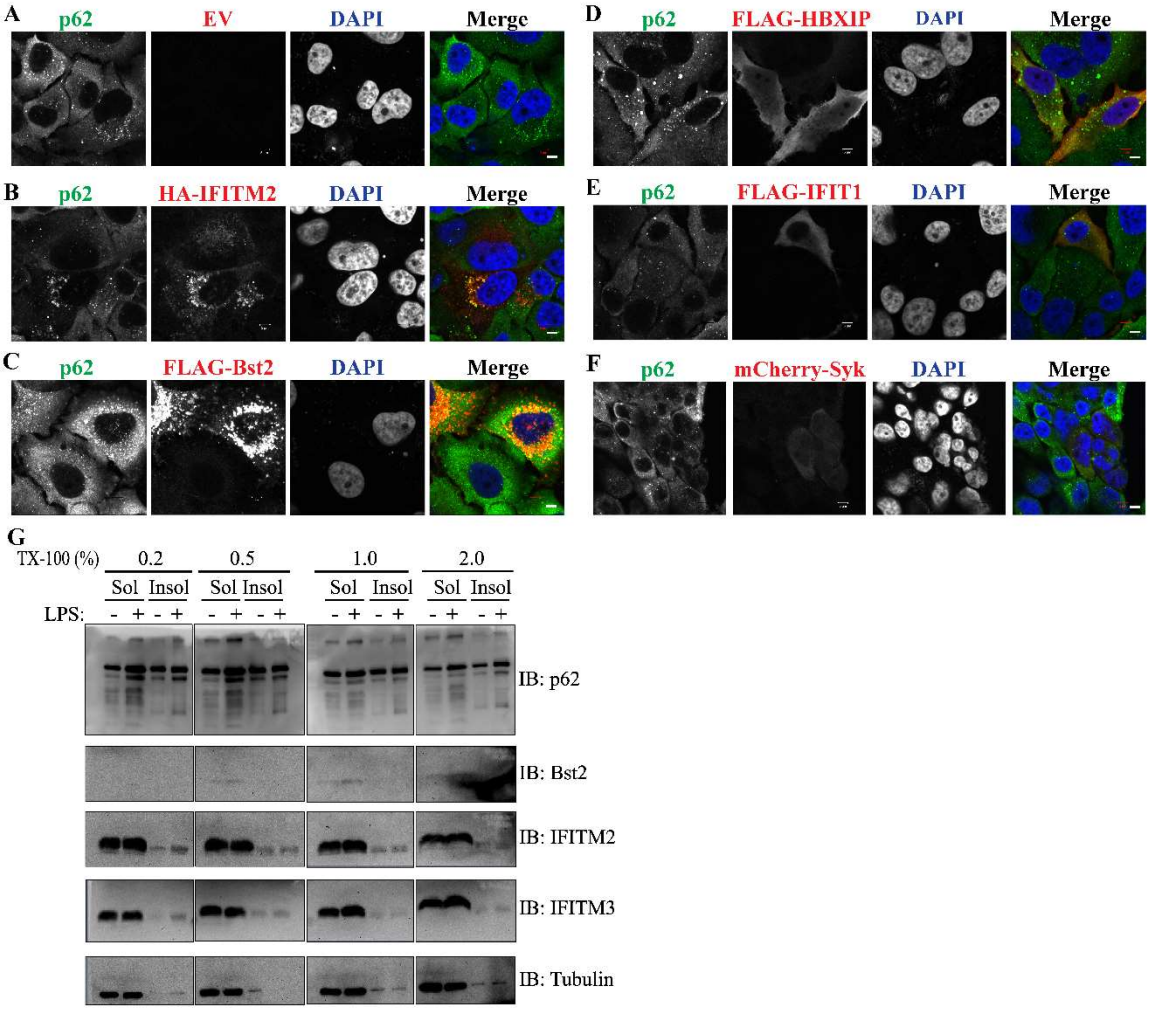

A
Supernatant
Fluorescence intensity (AU)
Fraction number
Untreated
LPS treated
B
Pellet
Fluorescence intensity (AU)
Fraction number
Untreated
LPS treated
C
Cellular Component
Count
Untreated
LPS treated
proteasome 8 0
cell surface 9 0
vacuole 3 28
spliceosomal complex 3 27
extracellular 6 21
endosome 6 27
chromosome 8 41
Golgi 12 38
ribosome 1 13
cytoskeleton 14 42
organelle lumen 7 16
<Not Set> 18 33
endoplasmic reticulum 20 43
cytosol 43 113
nucleus 54 207
cytoplasm 68 177
membrane 91 192
mitochondrion 38 97
D
Molecular Function
Count
Untreated
LPS treated
transcription regulator activity 0 0
antioxidant activity 12 0
translation regulator activity 0 2
receptor activity 14 10
signal transducer activity 5 17
motor activity 6 5
enzyme regulator activity 10 27
DNA binding 13 56
transporter activity 18 27
structural molecule activity 21 12
metal ion binding 35 66
nucleotide binding 38 71
<Not Set> 41 33
RNA binding 46 116
protein binding 80 272
catalytic activity 103 142
E
Biological Process
Count
Sample
Control
conjugation 0 0
reproduction 3 2
coagulation 7 3
cell growth 21 12
development 49 41
cell communication 60 48
cell division 76 53
cell proliferation 80 64
cell death 126 90
cellular homeostasis 128 113
cellular component movement 138 94
defense response 141 81
Not Set 172 135
cell differentiation 233 187
transport 669 545
cell organization and biogenesis 684 855
response to stimulus 869 669
regulation of biological process 1333 1013
metabolic process 1521 1287
F
Golgi, 5.28%
cell surface, 5.08%
endosome, 3.46%
vacuole, 2.85%
cytoskeleton, 2.44%
chromosome, 1.22%
organelle lumen, 1.02%
ribosome, 1.02%
spliceosomal complex, 0.61%
membrane, 18.29%
mitochondrian, 5.49%
extracellular, 5.89%
endoplasmic reticulum, 5.89%
cytosol, 9.76%
nucleus, 13.62%
cytoplasm, 18.09%
G
DNA binding, 7.25%
signal transducer activity, 5.25%
receptor activity, 4.25%
enzyme regulator activity, 4.00%
structural molecule activity, 2.00%
transport activity, 2.00%
<Not set>, 1.25%
antioxidant activity, 0.75%
motor activity, 0.50%
protein binding, 30.00%
metal ion binding, 7.75%
nucleotide binding, 9.50%
RNA binding, 11.00%
catalytic activity, 14.50%

Figure S5


A
p62
EV
DAPI
Merge
B
p62
HA-IFITM2
DAPI
Merge
C
p62
FLAG-Bst2
DAPI
Merge
D
p62
FLAG-HBXIP
DAPI
Merge
E
p62
FLAG-IFIT1
DAPI
Merge
F
p62
mCherry-Syk
DAPI
Merge
G
TX-100 (%)
0.2
0.5
1.0
2.0
Sol
Insol
LPS:
IB: p62
IB: Bst2
IB: IFITM2
IB: IFITM3
IB: Tubulin