Expanded View Figures

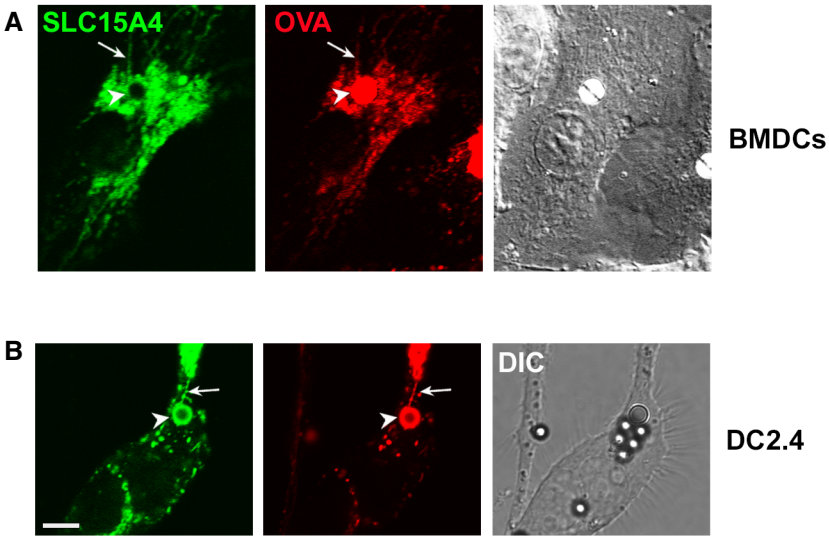

Figure EV1. SLC15A4 is recruited to phagosomes and phagosomal tubules and restrains autophagy induction in DCs.

A, B WT BMDCs (A) or DC line DC2.4 (B) expressing SLC15A4-GFP were pulsed with LPS/OVA-TxR beads and analyzed by live-cell imaging 2 h after the pulse. Representative images. DIC images show cell shape and outline. Arrowheads, phagosomes; arrows, phagosomal tubules. Scale bar, 6 μm.

C, D WT or SLC15A4^{feeble} BMDCs were infected with STm in the presence or absence of chloroquine (CHQ). Cell pellets collected at the indicated time points after STm infection were lysed, fractionated by SDS–PAGE, and immunoblotted for LC3 and actin. Representative immunoblots. (D) Quantification of band intensities for LC3-II normalized to LC3-I and actin from three independent experiments are shown as fold induction relative to time 0.

Data information: Data represent mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Two-tailed Student's t-test.
Source data are available online for this figure.

Figure EV2. SLC15A4 knock-down or *feeble* mutation does not significantly impair DC differentiation, maturation, LPS priming, or inflammasome stimulation with soluble ligands.

A–G WT BMDCs transduced with lentiviruses encoding non-target (ctrl), or any of three SLC15A4 shRNAs (A, B, D), were untreated (A, D) or treated with LPS for 3 h (B), or WT and SLC15A4^{feeble} BMDCs were untreated (E) or treated with LPS for 3 h (C) or 18 h (F), or intestinal DCs isolated from colon of WT or SLC15A4^{feeble} mice were untreated (G). (A) cDNA generated from isolated RNA was analyzed by RT–qPCR. Data from three independent experiments were normalized to the average of two housekeeping genes, and the ΔΔCt values were calculated and represented as mean ± SD fold change of mRNA in SLC15A4 shRNA-transduced cells relative to non-target ctrl-treated cells. (B) Cell supernatants collected 3 h after treatment were assayed for IL-6 by ELISA. Representative plot of three independent experiments. (C) Cell supernatants collected 6 h after poly(dA:dT)/LyoVec treatment or 1 h after Rod-Tox or LLO treatment were assayed for IL-1β by ELISA. Representative plot of three independent experiments. (D, E). Representative dot plots with the percentages of CD11b$^+$/CD11c$^+$ BMDCs indicated as markers of DC differentiation after 7 days. (F) Representative histograms with the percentages of CD40$^+$ or MHC-II$^+$ BMDCs as markers of DC maturation on day 7. Blue solid lines, untreated DCs; red solid lines, LPS-treated DCs; black solid lines, unstained controls. (G) Representative dot plots with the percentages of CD11c$^+$/CD103$^+$ DCs indicated as markers of intestinal DCs.

Data information: $^{**}P < 0.01$; $^{***}P < 0.001$. Two-tailed Student's *t*-test.
Source data are available online for this figure.

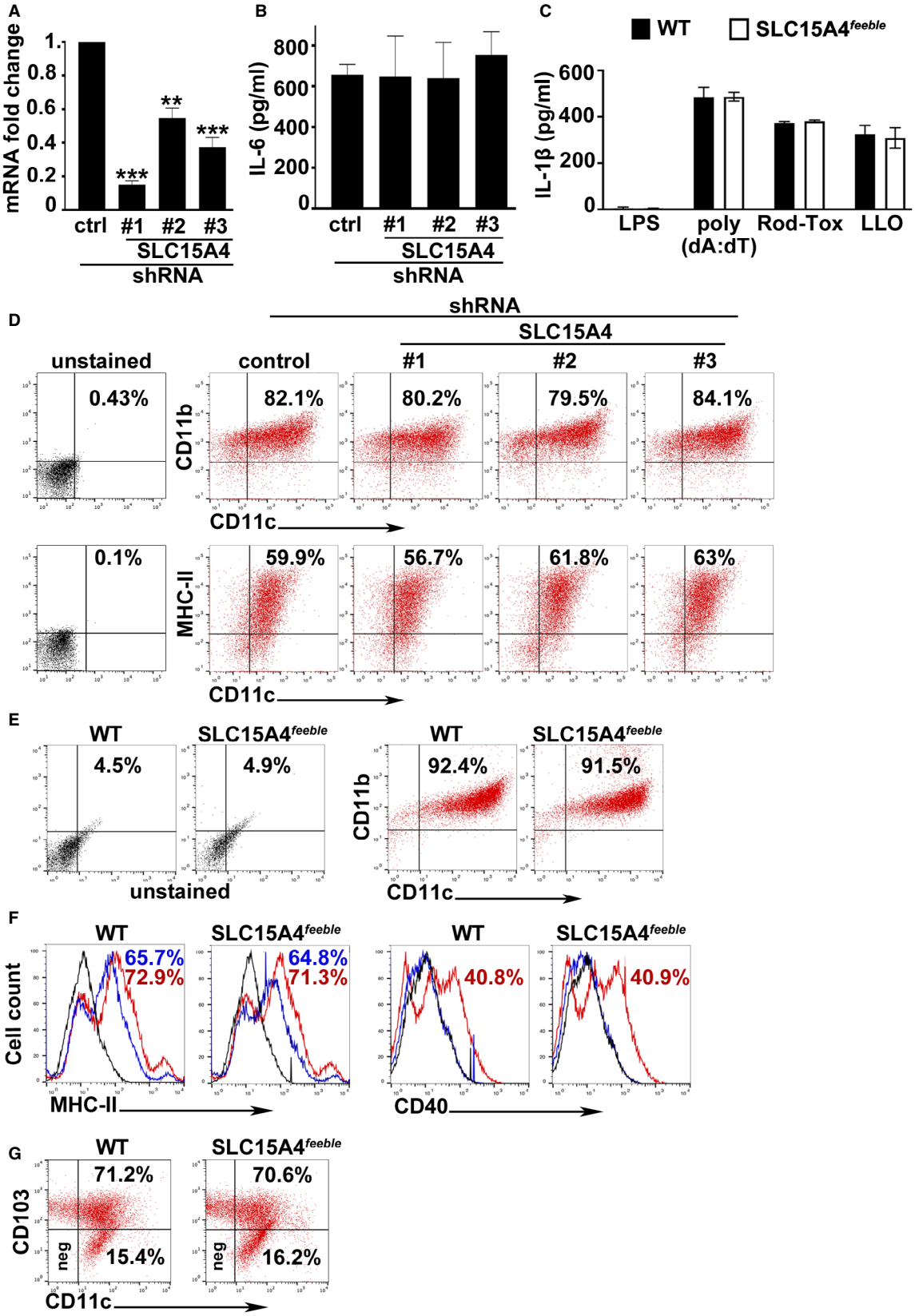
A
mRNA fold change
1.
0.8
0.6
0.4
0.2
0
**
***
***
ctrl #1 #2 #3
SLC15A4 shRNA
B
IL-6 (pg/ml)
800
600
400
200
0
ctrl #1 #2 #3
SLC15A4 shRNA
C
WT
SLC15A4feeble
IL-1β (pg/ml)
600
400
200
0
LPS
poly (dA:dT)
Rod-Tox
LLO
D
shRNA
SLC15A4
unstained
control
#1
#2
#3
0.43%
82.1%
80.2%
79.5%
84.1%
CD11b
CD11c
0.1%
59.9%
56.7%
61.8%
63%
MHC-II
CD11c
E
WT
SLC15A4feeble
4.5%
4.9%
unstained
92.4%
91.5%
CD11b
CD11c
F
WT
SLC15A4feeble
65.7%
72.9%
64.8%
71.3%
Cell count
MHC-II
40.8%
40.9%
CD40
G
WT
SLC15A4feeble
71.2%
70.6%
CD103
neg
15.4%
16.2%
CD11c

Figure EV2.

Figure EV3. SLC15A4 is not required for non-canonical inflammasome function in DCs or NLRC4 inflammasome activity in MΦs.

A–D WT or SLC15A4^{feeble} BMDCs were treated with LPS (100 ng/ml) overnight and infected with non-expressing flagellin STm [STm (−fla)] or STm lacking flagellin (ΔfliCΔfljB) (A–D) or flagellin-expressing STm (STm; control) (A) at MOI 5:1 for 4–24 h. A. Cells were pelleted and LDH release into the supernatant was measured in three independent experiments. Percent of cell death was normalized to release from uninfected cells by 1% Triton X-100 treatment (100% cell death). Representative experiment showing biological triplicates. (B, C) Cell pellets (B, C) or cell supernatants (C) collected at the indicated time points after STm infection were lysed, fractionated by SDS–PAGE, and immunoblotted for GSDMD, LC3, and actin (B), or pro-caspase 11, caspase 11, and actin (C). Representative immunoblots. (D) Quantification of band intensities for LC3-II normalized to LC3-I and actin from three independent experiments are shown as fold induction relative to time 0. Data represent mean ± SD.

E–G WT or SLC15A4^{feeble} BMMΦs were unstimulated or infected with flagellin-expressing STm for the indicated time points. (E) Cell supernatants collected after treatment were assayed for IL-1β by ELISA. Representative plot of three independent experiments. (F) Cell pellets collected at the indicated time points after STm infection were lysed, fractionated by SDS–PAGE, and immunoblotted for caspase-1, GSDMD, or LC-3. Representative immunoblots. (G) Quantification of band intensities for LC3-II normalized to LC3-I and actin from three independent experiments are shown as fold change relative to time 0.

Data information: Data represent mean ± SD.
Source data are available online for this figure.

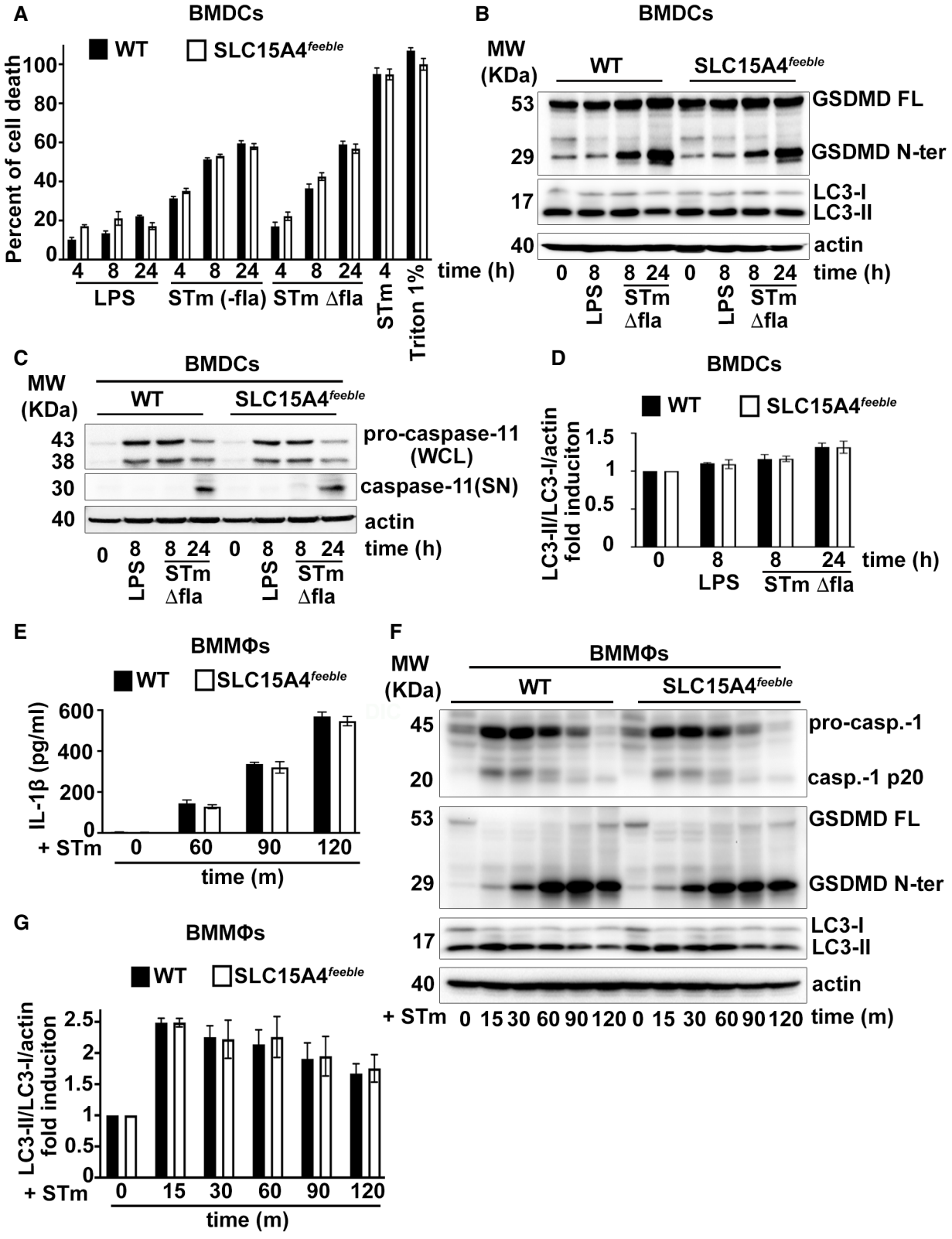
A
BMDCs
WT
SLC15A4feeble
Percent of cell death
100
80
60
40
20
0
4 8 24 4 8 24 4 8 24 4 8 24
LPS
STm (-fla)
STm Δfla
STm
Triton 1%
time (h)
B
BMDCs
MW (KDa)
WT
SLC15A4feeble
53
29
17
40
GSDMD FL
GSDMD N-ter
LC3-I
LC3-II
actin
0 8 8 24 0 8 8 24 time (h)
LPS
STm Δfla
LPS
STm Δfla
C
BMDCs
MW (KDa)
WT
SLC15A4feeble
43
38
30
40
pro-caspase-11 (WCL)
caspase-11(SN)
actin
0 8 8 24 0 8 8 24 time (h)
LPS
STm Δfla
LPS
STm Δfla
D
BMDCs
WT
SLC15A4feeble
LC3-II/LC3-I/actin fold induciton
1.5
1
0.5
0
0 8 8 24 time (h)
LPS
STm Δfla
E
BMMΦs
WT
SLC15A4feeble
IL-1β (pg/ml)
600
400
200
0
+ STm 0 60 90 120
time (m)
F
BMMΦs
MW (KDa)
WT
SLC15A4feeble
45
20
53
29
17
40
pro-casp.-1
casp.-1 p20
GSDMD FL
GSDMD N-ter
LC3-I
LC3-II
actin
+ STm 0 15 30 60 90 120 0 15 30 60 90 120 time (m)
G
BMMΦs
WT
SLC15A4feeble
LC3-II/LC3-I/actin fold induciton
2.5
2
1.5
1
0.5
0
+ STm 0 15 30 60 90 120
time (m)

Figure EV3.

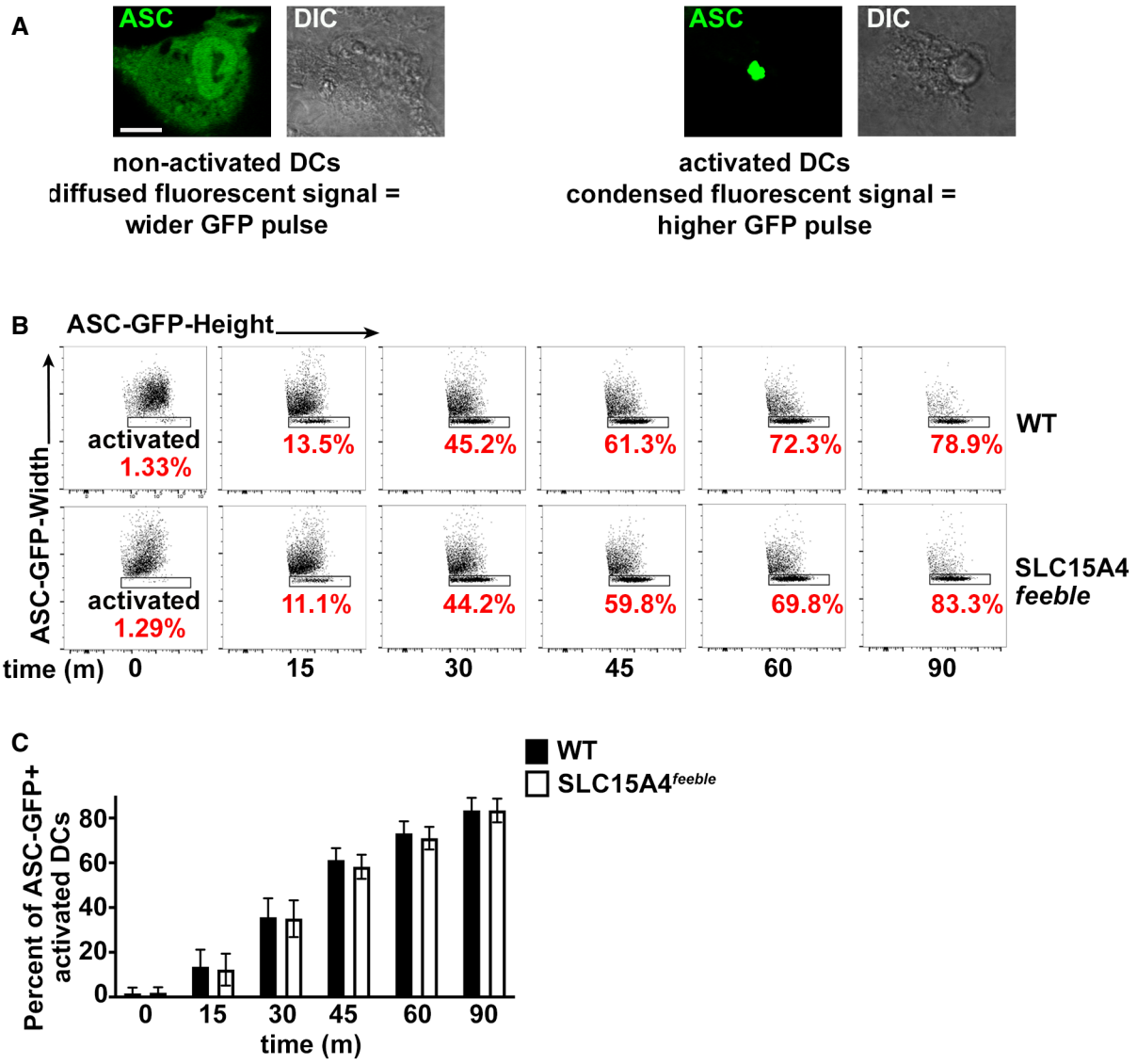

Figure EV4. SLC15A4 does not affect the kinetics of ASC speck formation.

WT or SLC15A4^{feeble} BMDCs expressing ASC-GFP were infected with flagellin-expressing STm. Cells were fixed at the indicated time points after infection and analyzed by flow cytometry measuring GFP-width and GFP-height pulses.

A Cytosolic-diffused ASC-GFP in non-activated cells correlates to a wider GFP signal compared to condensed ASC-GFP in activated cells. Representative images. DIC images show cell shape and outline. Scale bar, 6 μm.

B Representative dot plots, after gating on GFP$^+$ cells. Note that the height of the GFP pulse is more prominent (*rectangular shape*) relative to the width of the GFP signal as the number of cells bearing ASC specks (activated DCs) increases over time. Percent of GFP$^+$ activated DCs are indicated in red.

C Plot represents three independent experiments.

Data information: Data represent mean ± SD.
Source data are available online for this figure.

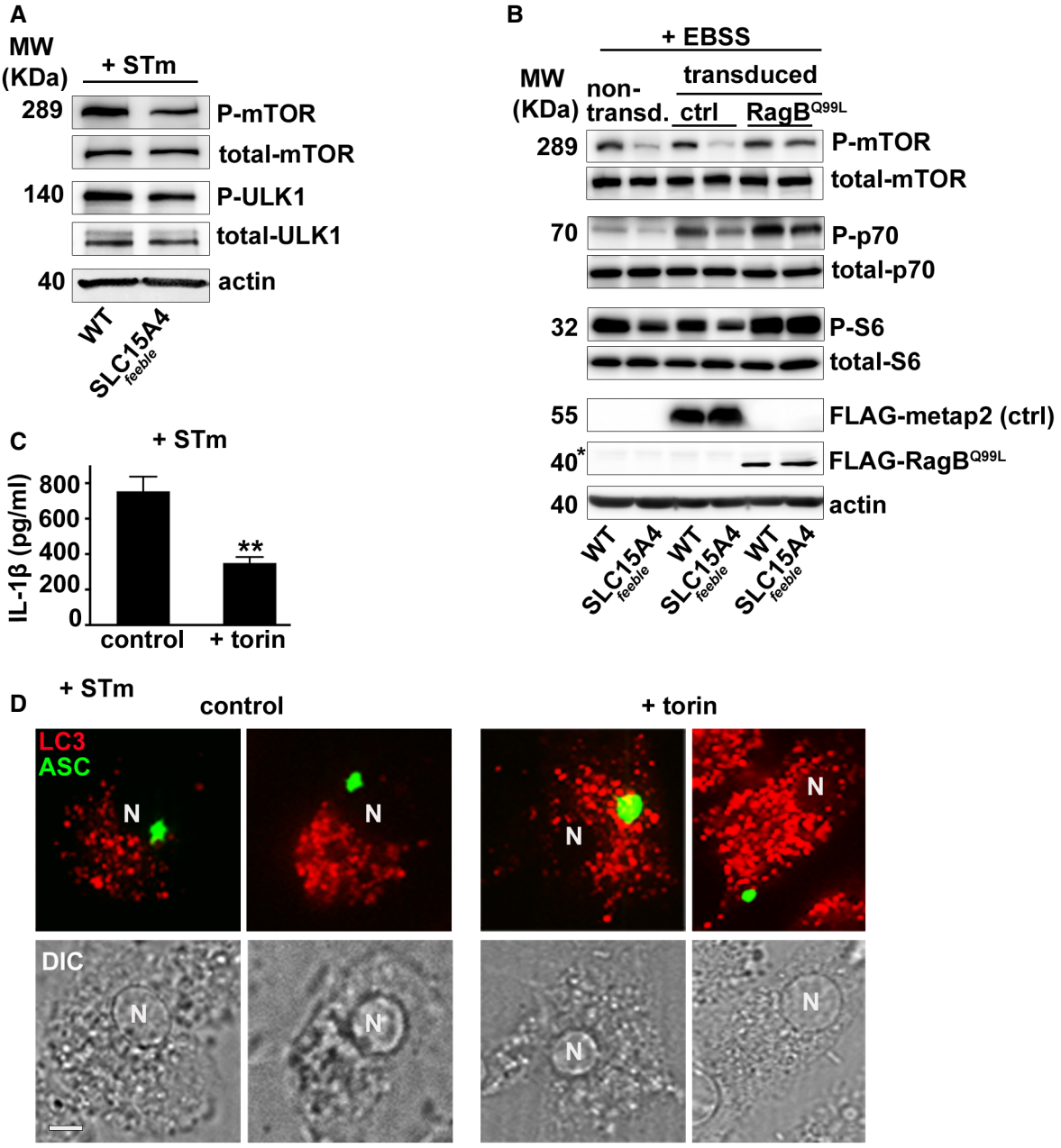

Figure EV5. Constitutively active mTOR rescues defective mTOR signaling, and mTOR inhibition in WT DCs phenocopies SLC15A4^{feeble} defects in inflammasome activity and positioning.

A–D WT or SLC15A4^{feeble} BMDCs were untreated (A–D) or pre-treated with torin (C, D), non-transduced (A), or transduced with FLAG-metap2 (ctrl) or FLAG-RagB^{Q99L} (B) or ASC-GFP and mcherry-LC3 (D) and infected with STm (A, C, D) or starved in EBSS for 4 h (B). (A, B). Cell pellets were lysed, fractionated by SDS–PAGE, and immunoblotted for phospho (P) and total mTOR, ULK1, p70 kinase or S6, FLAG, or actin. Representative immunoblots of three independent experiments. (B) Non-specific band right above FLAG-RagB^{Q99L} is indicated with an asterisk. (C) Cell supernatants collected 1 h after infection were assayed for IL-1β by ELISA. Representative plot of three independent experiments. (D) Cells were fixed and analyzed by fluorescence microscopy 1 h after infection. Representative images showing ASC speck (green) relative to LC3 (red). Note peripheral ASC speck positioning in torin-treated DCs. Corresponding DIC images show nuclear position. N, nucleus. Scale bar, 6 μm.

Data information: Data represent mean ± SD. $^{**}P < 0.01$. Two-tailed Student's *t*-test.
Source data are available online for this figure.