Supplementary information

Figure legends

Figure S1. Up-regulation of NLRP3, AIM2, and RIG-I inflammasome molecules in the NPC Affymetrix microarray database. The analysis of affymetrix microarray was according to Affymetrix HG U133 plus 2.0 set. Gene-specific probes were used in tumor and adjacent normal tissues.

Figure S2. Metacore network process analysis of iTRAQ candidates. The mitotic-process network shown by the Metacore software. The red spots indicate the candidates obtained from our iTRAQ analysis.

Figure S3. Knockdown of NLRP3, AIM2, RIG-I and EB1 in NPC TW02 cells. A) Cells were transfected with si-NLRP3 for 48 h, cell extracts were prepared, and RNA was collected for quantitative RT-PCR by specific primers. B, C and D) Cells were transfected with si-AIM2, si-RIG-I and si-EB1 for 48 h, and cell extracts were subjected to Western blot analysis using the indicated antibodies.

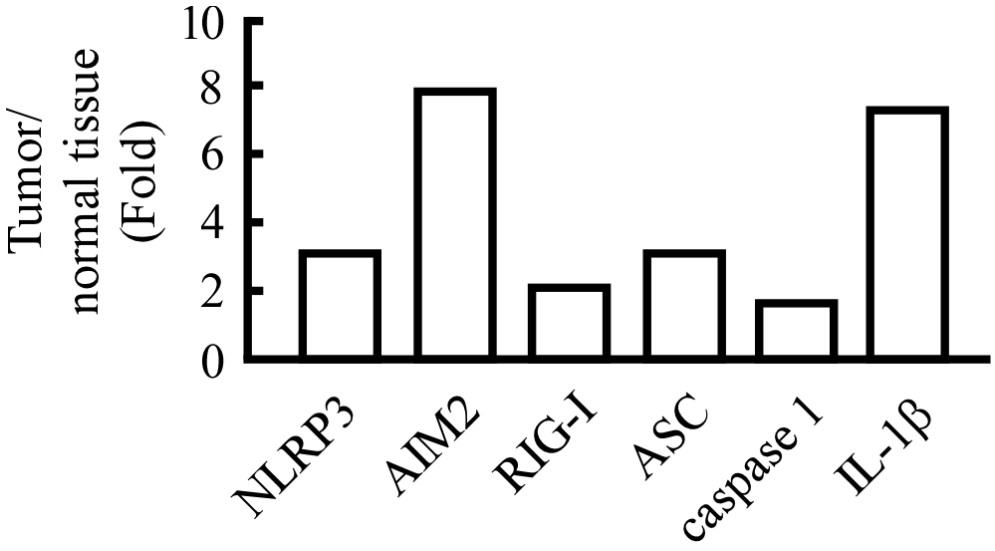
Figure S4. The association of EB1 with AIM2 inflammasome in different time points. NPC TW02 cells were transfected flag-AIM2 for 48h, then treated with poly (dA:dT). The cell extracts were collected at indicated time points and immunoprecipitated by anti-flang matrix. The indicated proteins were detected by specific antibodies.

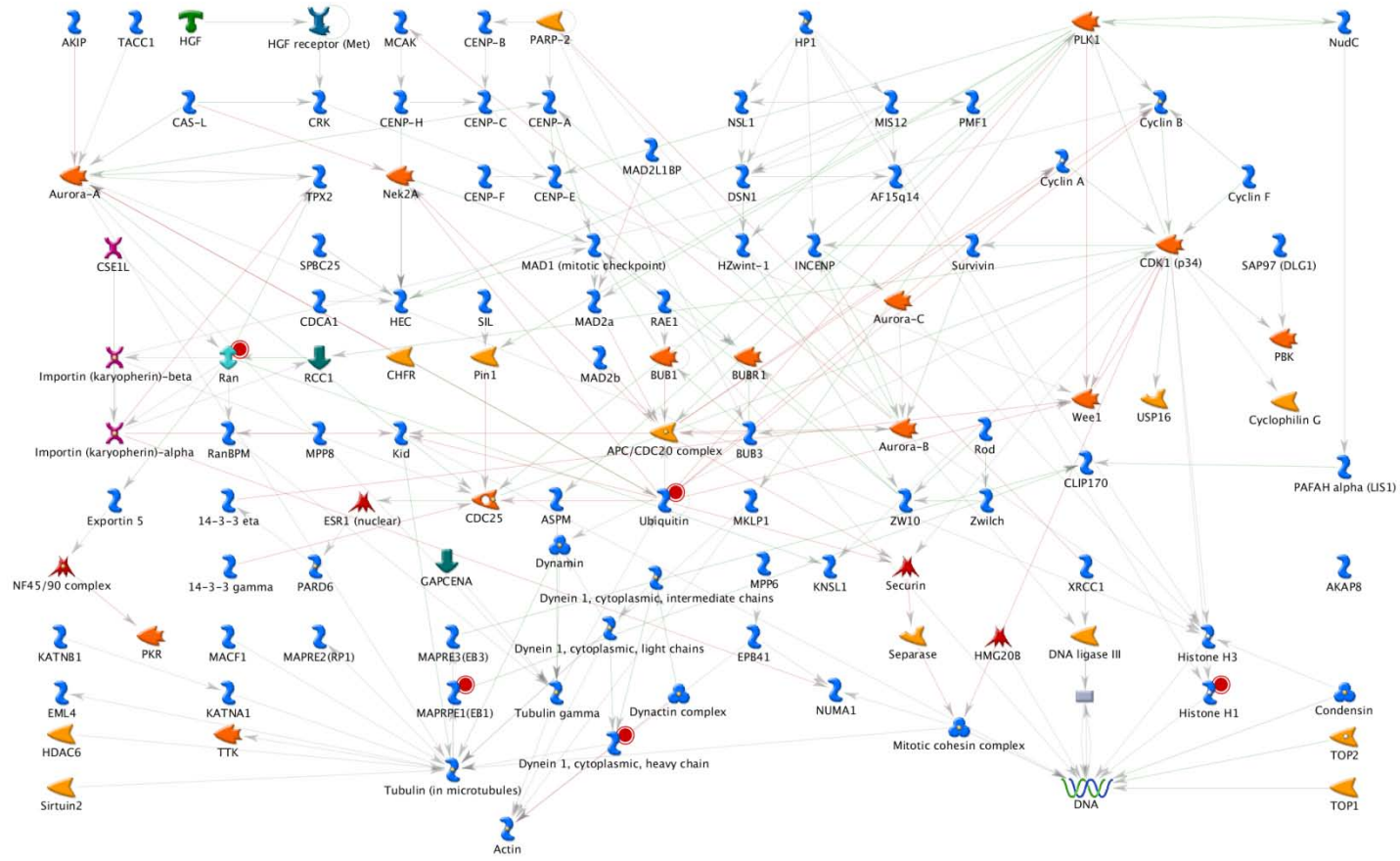
Figure S5. EB1 directly interacts with ASC through the CT domain. Purified GST, GST-EB1-WT, GST-EB1-CH and GST-EB1-CT fusion proteins were immobilized on glutathione agarose and separately incubated with purified His-ASC fusion proteins for 24 h. The bound proteins were washed and then analyzed by Western blotting with an anti-His antibody. The fold-change numbers were obtained from three independent experiments. The amounts of GST fusion proteins were assessed by Coomassie blue staining.

Figure S6. Knockdown of the small GTPase, RhoA, and inhibition of microtubule polymerization both decrease AIM2-mediated IL-1β secretion in

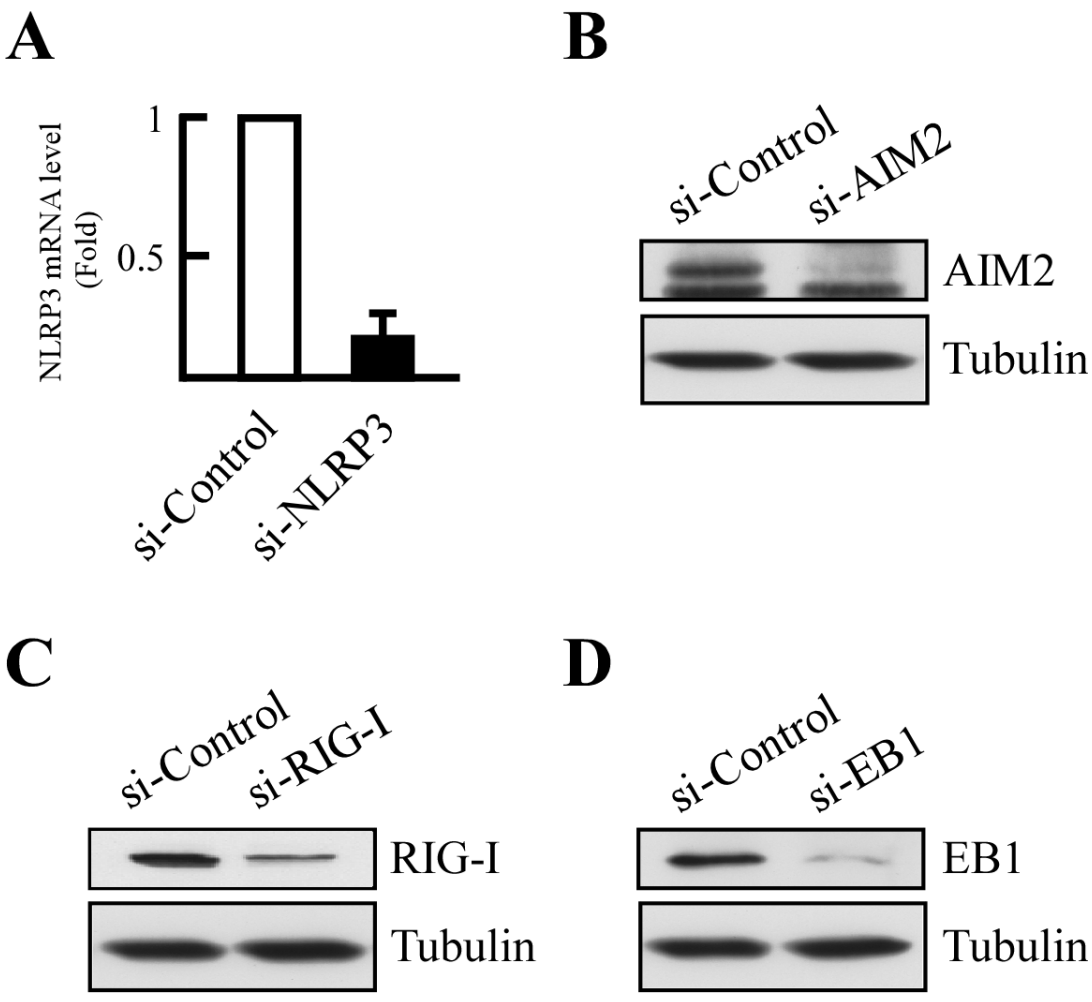
HK1 cells. A) Cells were transfected with si-control, si-Rac1, si-RhoA or si-cdc42 for 48 h, treated with poly (dA:dT) for 2 h, and further incubated for 10 h. Media and cell extracts were then collected for ELISA of IL-1β and western blot. B) Cells were pre-treated with nocodazole (2 μg/μl) or paclitaxel (5 μM) for 2 h, treated with poly (dA:dT) for 2 h, and further incubated for 10 h. Media were collected for ELISA against IL-1β. Symbols: *, $P < 0.05$ and **, $P < 0.01$. The results are presented as the means ± SD of three experiments.

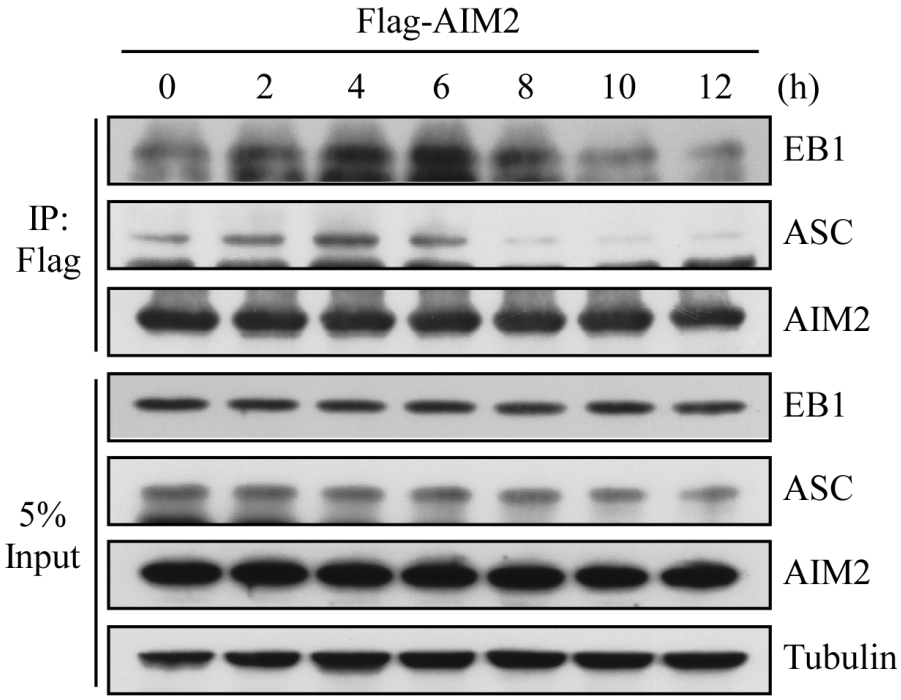
Figure S7. The effect of knockdown EB1 on AIM2 inflammsome activation in THP-1 cells. Cells were treated with PMA (2 μM) for 6h then treated with LPS (2 μg) for 24h. Cells transfected with si-control, si-AIM2 or si-EB1 for 24 h, then treated with poly (dA:dT) for 4 h. Media and cell extracts were collected for IL-1β ELISA and western blot. Symbols: *, $P < 0.05$ and **, $P < 0.01$. The results are presented as the means ± SD of three experiments.

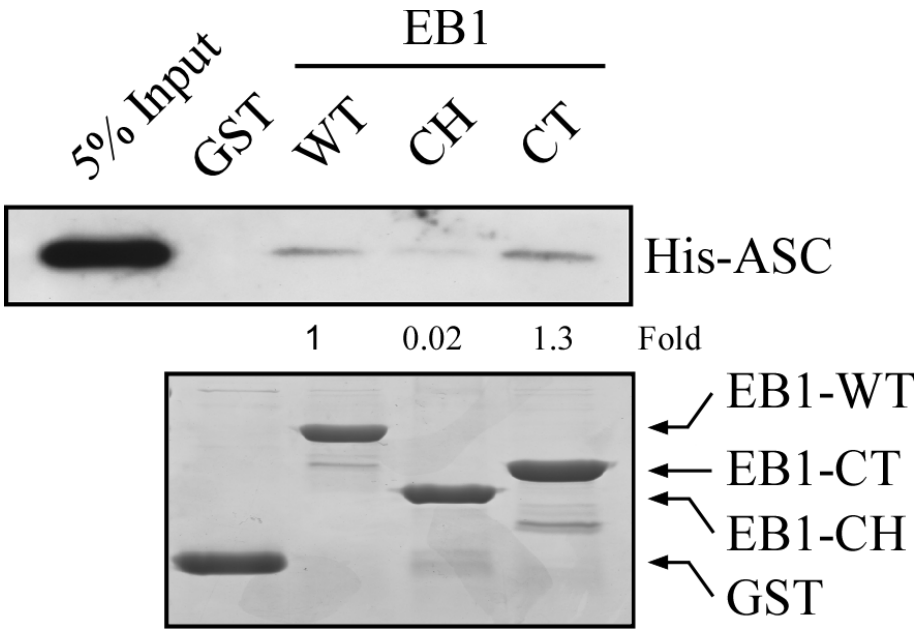

Tumor/
normal tissue
(Fold)
10
8
6
4
2
0
NLRP3
AIM2
RIG-I
ASC
caspase 1
IL-1β

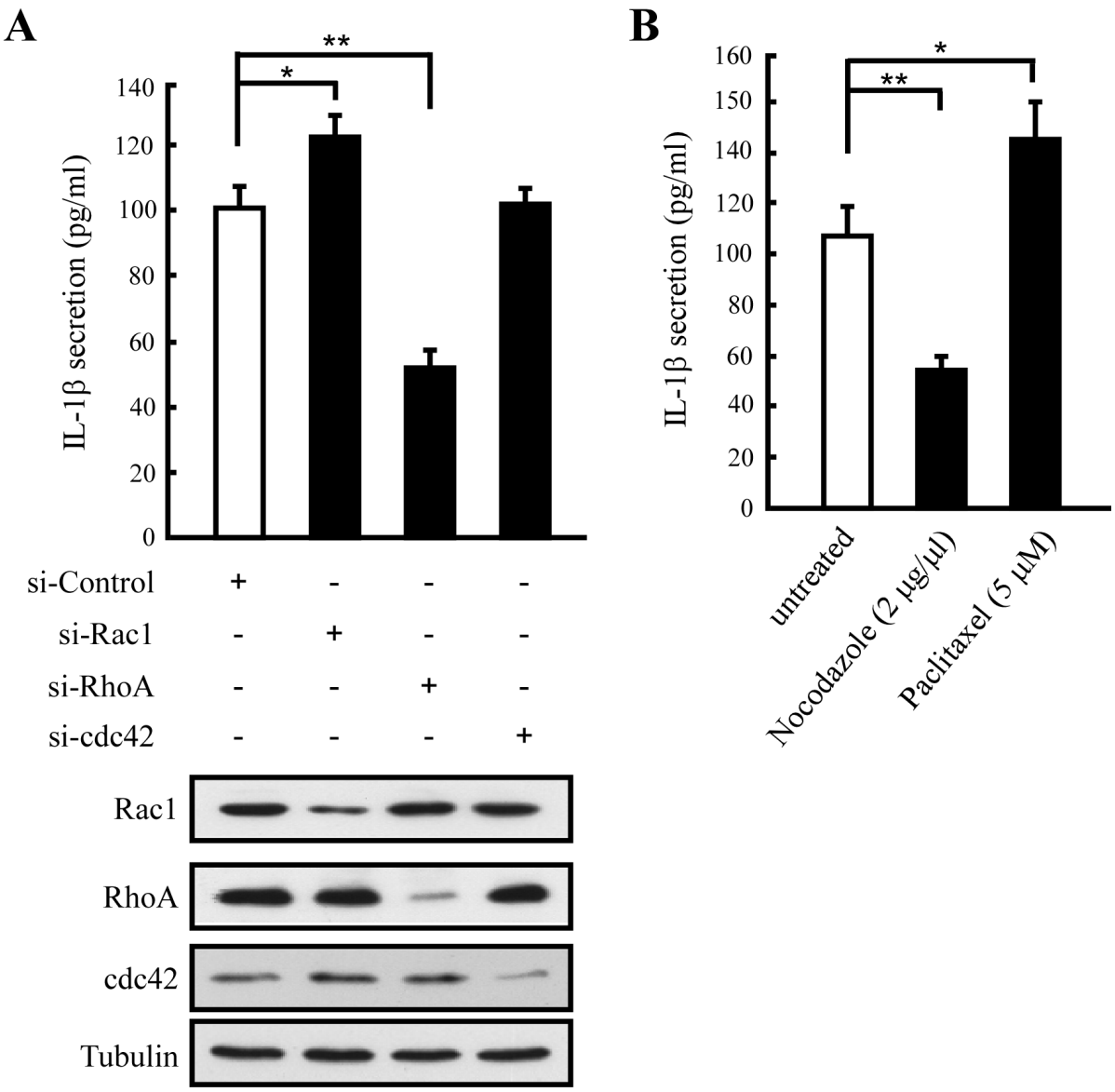


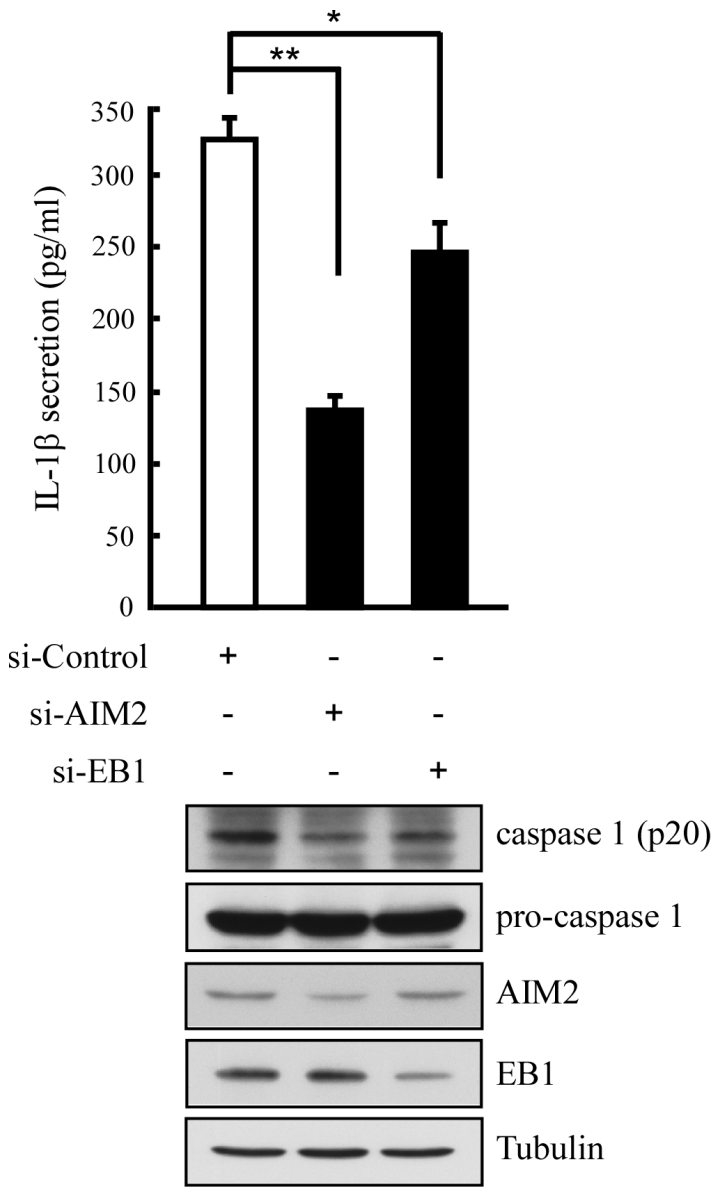
- G protein regulator
- Transcription factor
- Receptor ligand
- Receptor
- Channel
- Enzyme
- Kinase
- Phosphatase
- Protease
- Metalloprotease
- Binding protein
- Transporter
- RAS GTPase


A
NLRP3 mRNA level (Fold)
1
0.5
si-Control
si-NLRP3
B
si-Control
si-AIM2
AIM2
Tubulin
C
si-Control
si-RIG-I
RIG-I
Tubulin
D
si-Control
si-EB1
EB1
Tubulin


Flag-AIM2
0
2
4
6
8
10
12
(h)
IP:
Flag
EB1
ASC
AIM2
5%
Input
EB1
ASC
AIM2
Tubulin


EB1
5% Input
GST
WT
CH
CT
His-ASC
1
0.02
1.3
Fold
EB1-WT
EB1-CT
EB1-CH
GST


A
IL-1β secretion (pg/ml)
0
20
40
60
80
100
120
140
*
**
si-Control + - - -
si-Rac1 - + - -
si-RhoA - - + -
si-cdc42 - - - +
Rac1
RhoA
cdc42
Tubulin
B
IL-1β secretion (pg/ml)
0
20
40
60
80
100
120
140
150
160
*
**
untreated
Nocodazole (2 μg/μl)
Paclitaxel (5 μM)


*
**
350
300
250
200
150
100
50
0
IL-1β secretion (pg/ml)
si-Control + - -
si-AIM2 - + -
si-EB1 - - +
caspase 1 (p20)
pro-caspase 1
AIM2
EB1
Tubulin