SUPPLEMENTARY MATERIALS AND METHODS

Western blotting analysis. To study the effects of IL-1β, whole cell extracts were prepared using RIPA buffer (50 mM Tris pH 7.6, 150 mM NaCl, 1% Triton X100, 1mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS) containing protease inhibitor and phospho-Stop tablets (Roche, Indianapolis, IN). Protein was measured using a Bio-Rad DC method (Bio-Rad, CA). Equal amounts of protein (35 μg) were loaded and separated by SDS-polyacrylamide gel electrophoresis in 4-15% TGX gradient gel (Bio-Rad) and transferred onto PVDF (EMD Millipore). Blots were probed using rabbit mono- or polyclonal antibodies specific for MyD88, IRAK-4, pIRAK-4 (Thr 345/Ser346), IRAK-1, pTAK1 (Thr184/187), pTAK1 (Ser439), total TAK1, TRAF6, β-actin, and other signaling proteins. The protein bands were visualized by the Bio Rad XRS system or developed using films. Blots were stripped and re-probed with β-actin or other protein to ensure equal loading.

Western blots from rat joint homogenates were prepared using ankles from the rat AIA study as described earlier (12). Joint homogenates (30 μg) were used to analyze for the expression of pTAK1 Thr184/187, pTAK1 Ser439, TAK1, TRAF6, and β-actin. Densitometric analysis of the relative expression of each protein was done as described in our earlier studies (12, 13).

Immunoprecipitation Assay. RA-FLS were grown in 150 mm dish up to 80% confluence, then overnight starved with or without 20 μM EGCG, followed by IL-1β stimulation for 30 minutes. Cells were washed 2 times in ice cold 1X PBS, lysed in 500 μl of RIPA buffer as described earlier, utilized for immunoprecipitation assays. Clear lysate were subjected to protein estimation using Bio-Rad DC method. Equivalent protein (600 μg) from each sample was subjected for immunoprecipitation using mouse monoclonal antibody for FK-2 (Enzo Life Sciences), TRAF6 (Santa Cruz), K63-ubiquitin (Cell Signaling Technology), or K48-ubiquitin (Cell Signaling

Technology) depending on the experiment design. A similar amount of nonspecific IgG control antibody (Flag M2, Sigma) was used as isotype control. Antibody and whole cell extracts were incubated at 4°C on a rotor overnight, followed by the incubation with protein G Sepharose beads for 4 hours to capture the antibody and protein complex. Beads were then subjected to 3 washes with RIPA wash buffer followed by final wash with 1X PBS. The protein beads complex were eluted by boiling in 2X SDS sample buffer and then resolved on 4-15% Bio-Rad TGX gels. Western blot analysis was performed as described previously [12, 13]. For K63 IP assays, 120 µl bead volume equivalent of Dynabeads (Invitrogen) were subjected to crosslinking with 24 µl of K63 polyubiquitin antibody (Cell Signaling Technology) using BS3 cross linker (Thermo Scientific) as per manufacturer's instructions. Beads were equally divided in 4 samples for the IP assay. The protein complex was eluted from cross-linked beads using 2X sample buffer for 15 minutes at 70 °C.

Molecular Modeling Studies. *Ligand preparation:* EGCG ((2*R*,3*R*)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2*H*-1-benzopyran-3-yl 3,4,5-trihydroxybenzoate) was first optimized as a ligand by B3LYP/6-311++G** basis set using jaguar2014.3 then subjected to the ligand preparation in the LigPrep module of Schrodinger suit 2014.3.

Protein preparation: Since the crystal structure of IRAK1 is not known, we constructed a homologous 3D experimental structure model based on the crystal structure of IRAK4 (PDB ID: 2NRU), with loops further refined iteratively by using Modeller9.14. The sequence and domain information of IRAK1 (Homo sapiens) was obtained from UniProt (Accession No. P51617). The chosen model had less than 2.3% residues outside the allowed region of the Ramachandran plot and are away from the active site; hence, not contributing in the protein ligand interaction. The missing loop of 3D structures of TAK1 was first constructed. IRAK-1, TAK1, and TRAF6 were

then submitted to the protein preparation wizard of Schrodinger suite 2014.3. The non-polar hydrogens were merged and OPLS2005 force field was applied. The suitable binding site cavity for the ligand (EGCG) was chosen after analyzing the cavities present in the proteins using Computed Atlas of Surface Topography of Proteins (CASTp) server on the basis of area and volume. After finding the probable binding site of the ligand the 20Å size grid was generated from the binding site center of all three proteins, followed by ligand subjected to dock into it using GLIDE module. The docking was performed first using the default setting of standard precision (SP), followed by the conformations obtained from SP were used as input for extra precision (XP). Hydrophobic and hydrophilic maps were generated in order to visualize solvent accessible regions to analyze the chemical nature of binding cavity. The 10 best conformations of the ligand were generated. The selection of the best pose was presented on the basis of energy, cluster RMSD, and interaction with the respective protein.

Human Phospho-kinase antibody array: RA-FLS were grown in 100 mm dishes until >85% confluent. Cells were starved overnight with or without 20 μM EGCG, followed by IL-1β (10 ng/ml) stimulation for 30 minutes. Treated RA-FLS were lysed using 200 μl lysis buffer provided with the proteome profiler kit (Human Phospho-kinase Antibody Array Kit; Cat #ARY003B; R&D Systems, MN). Samples from 3 donors were pooled and 500 μg of whole cell extract for each treatment was incubated with each set of membrane (set A and B) overnight in the cold room. The method of detection and streptavidin HRP incubation were done as per the manufacturer's instructions. The final images were developed on GelDoc (XRS System, Bio-Rad).

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1: Repeated EGCG administration at nanomolar concentrations inhibits IL-1β-induced IL-6 and IL-8 production in RA-FLS. RA-FLS were treated daily with EGCG (1-1000 nM) for 7 days prior to IL-1β (10 ng/ml) stimulation for 8 and 24 hours. **(A and C)** IL-6 and **(B and D)** IL-8 production was determined in the conditioned media using commercially available ELISA kits. The values are represented as mean ±SEM of n=4 experiments using different donors. **p<0.01 for IL-1β vs IL-1β+EGCG (nM).

Fig. S2A & S2B: Effect of EGCG on phosphorylation of kinases in RA-FLS. RA-FLS were pretreated with EGCG, followed by IL-1β (10 ng/ml) stimulation for 30 minutes. Cell lysates were pooled from 3 different RA-FLS donors and utilized as described in the 'Materials and Methods' section. S2A showed a set A of kinase panel probed with lysates from NS, IL-1β, and EGCG + IL-1β treatments. A reference panel for each of the array is also provided.

Fig. S3: Densitometric analysis of the proteins modulated by EGCG treatment in rat AIA. Statistical analysis was performed for the expression profile of (*A*) K48-linked ubiquitination, (*B*) K63-linked ubiquitination, and (*C*) pTAK1 (Thr184/187), (*D*) pTAK1 (Ser439), (*E*) TAK1, (*F*) TRAF6, and (*G*) Deubiquitinase expression in the joint homogenates from naïve, AIA, and AIA+EGCG groups. Values in the graphs represent mean ±SEM of six ankles from each group. $^{\#\#}$p<0.01, $^{\#}$p<0.05 for Naïve *vs* AIA group; *p<0.05, **p<0.01 for AIA *vs* AIA+EGCG group.

Fig. S1

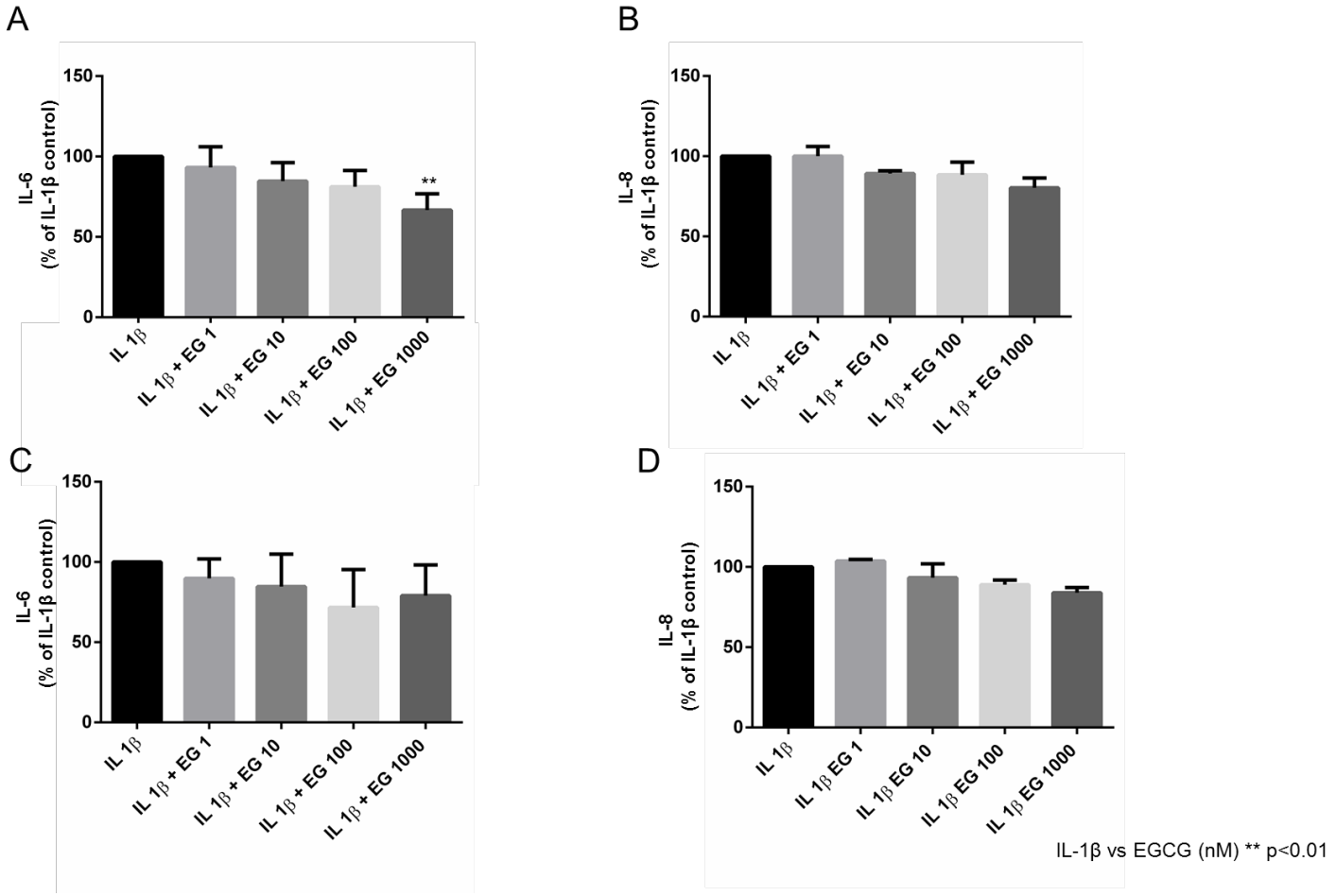
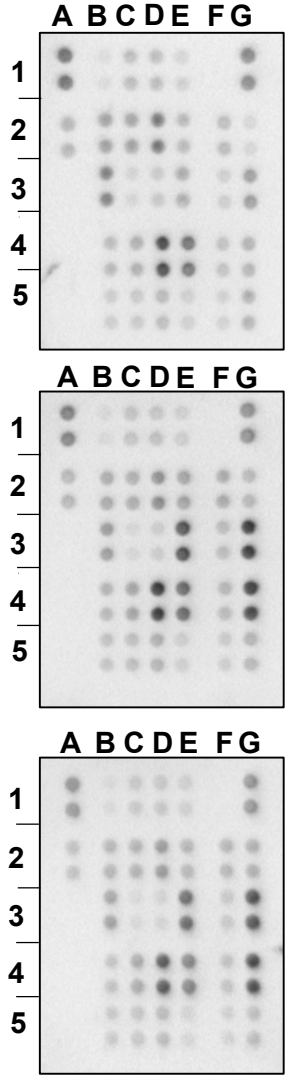

A
IL-6
(% of IL-1β control)
150
100
50
0
**
IL 1β
IL 1β + EG 1
IL 1β + EG 10
IL 1β + EG 100
IL 1β + EG 1000
B
IL-8
(% of IL-1β control)
150
100
50
0
IL 1β
IL 1β + EG 1
IL 1β + EG 10
IL 1β + EG 100
IL 1β + EG 1000
C
IL-6
(% of IL-1β control)
150
100
50
0
IL 1β
IL 1β + EG 1
IL 1β + EG 10
IL 1β + EG 100
IL 1β + EG 1000
D
IL-8
(% of IL-1β control)
150
100
50
0
IL 1β
IL 1β EG 1
IL 1β EG 10
IL 1β EG 100
IL 1β EG 1000
IL-1β vs EGCG (nM) ** p<0.01

Fig. S2A

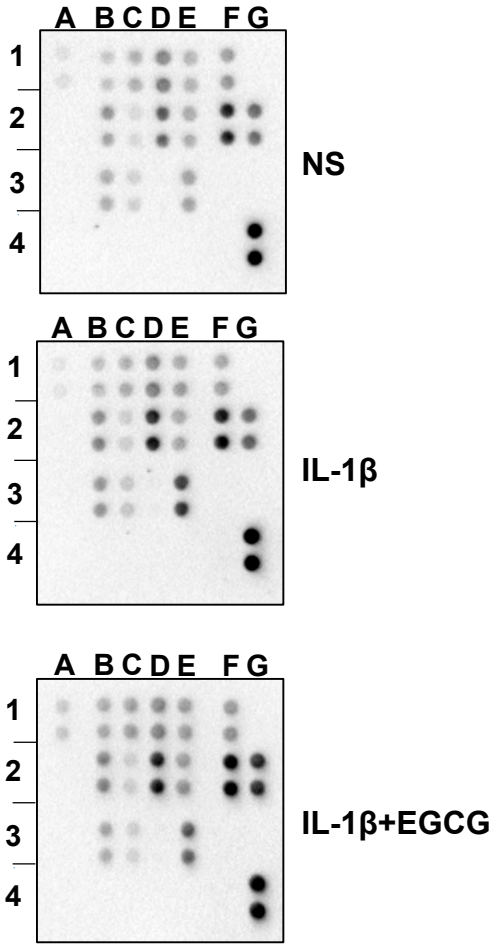
Set A : Human Phospho-Kinase assay



A1	Ref Spot
A2	PRAS40 (T246)
B1	Hck (Y411)
B2	Chk-2 (T68)
B3	FAK (Y397)
B4	PDGF R beta (Y751)
B5	STAT5a/b (Y694/Y699)
C1	Fyn (Y420)
C2	Yes (Y426)
C3	Fgr (Y412)
C4	STAT6 (Y641)
C5	STAT5b (Y699)
D1	Src (Y419)
D2	Lyn (Y397)
D3	Lck (Y394)
D4	STAT2 (Y689)
D5	STAT5a (Y694)
E1	TOR (S2448)
E2	CREB (S133)
E3	HSP27 (S78/S82)
E4	AMPK alpha2 (T172)
E5	beta-Catenin
F2	EGF R (Y1086)
F3	MSK1/2 (S376/S360)
F4	AMPK alpha1 (T183)
F5	Akt (S473)
G1	Ref Spot
G2	p38 alpha (T180/Y182)
G3	ERK1/2 (T202/Y204, T185/Y187)
G4	JNK pan (T183/Y185, T221/Y223)
G5	GSK-3 alpha/beta (S21/S9)

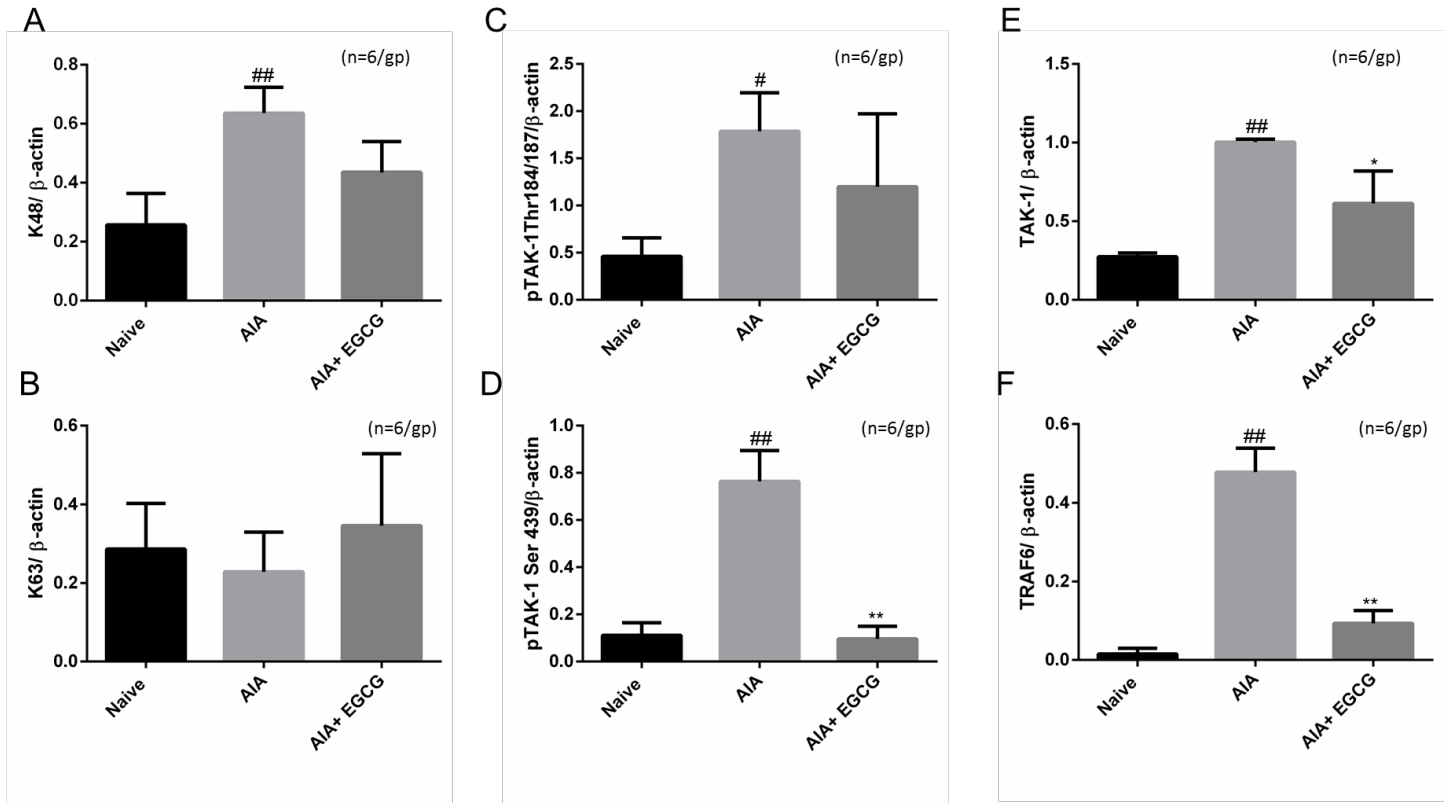
Fig. S2B

Set B : Human Phospho-Kinase assay



A1	HSP60
B1	STAT3 (S727)
B2	WNK-1 (T60)
B3	Pyk2 (Y402)
C1	STAT3 (Y705)
C2	p27 (T198)
C3	PLC gamma-1 (Y783)
D1	p70 S6 Kinase (T421/S424)
D2	RSK1/2/3 (S380/S386/S377)
D3	eNOS (S1177)
E1	p70 S6 Kinase(T389)
E2	p53 (S15)
E3	c-Jun (S63)
F1	Akt (T308)
F2	p53 (S46)
G2	p53 (S392)
G4	Ref Spot

Fig. S3


A
K48/ β-actin
(n=6/gp)
##
Naive
AIA
AIA+ EGCG
B
K63/ β-actin
(n=6/gp)
Naive
AIA
AIA+ EGCG
C
pTAK-1Thr184/187/β-actin
(n=6/gp)
#
Naive
AIA
AIA+ EGCG
D
pTAK-1 Ser 439/β-actin
(n=6/gp)
##
**
Naive
AIA
AIA+ EGCG
E
TAK-1/ β-actin
(n=6/gp)
##
*
Naive
AIA
AIA+ EGCG
F
TRAF6/ β-actin
(n=6/gp)
##
**
Naive
AIA
AIA+ EGCG