FIGURE LEGENDS

Suppl. Fig. S1: The catalytic inactive mutants of CNFs do not induce STAT transcriptional activity and STAT3 phosphorylation at Tyr705.
Hek293 cells were transfected with pGRR5-Luc and pRL-TK followed by intoxication overnight with CNF1 or catalytically inactive CNF1-CS (light gray bars), CNFY or catalytically inactive CNFY-CS (dark gray bars), CNF3 or catalytically inactive CNF3-CS (black bars) 100 ng/ml each or left untreated (open bars). RLU values of firefly luciferase in the lysates were normalized to those of the internal control pRL-TK. Results are given as fold induction compared to the untreated control. Shown is one representative experiment out of at least 3, performed in triplicates ± SD (top).
Additionally, Tyr705 phosphorylation of STAT3 and total STAT3 was determined in cytosolic and nuclear fractions of the cells with specific antibodies by Western-blotting. Images are representatives of at least three independent experiments.

Suppl. Fig. S2: Shuffeled RhoA RNAi has no effect on RhoA content.
Hek293 cells were transfected with siRNA targeting RhoA (siRhoA) or the adequate control siRNA with two exchanged nucleotides (shuffeled RNAi, CoRhoA, CAGAU***A***CC***G***AUGUUAUACU) using the N-TER nanoparticle siRNA transfection system. After 24 h, cells were re-transfected with the respective siRNA and intoxicated with 100 ng/ml CNFY. 12 h after intoxication, cell lysates were prepared and immunoblotted with phosphospecific STAT3 antibody. Membranes were stripped and re-probed for total STAT3. Efficiency of RhoA knock-down was controled by immunoblotting using a RhoA antibody. Equal protein loading was verified by re-probing the membrane with GAPDH antibody. The image is representative for two independent experiments.

Suppl. Fig. S3: Rho kinase (ROCK) is involved in Rho-dependent STAT3 activation.
ROCK was inhibited with H1152. Luciferase assays were performed with lysates of Hek293 cells transfected with pRL-TK and pGRR5-Luc, left untreated or treated overnight with H1152 (100 nM) alone (open bars) or together with CNF1 (light gray bars), CNFY (dark gray bars) or CNF3 (black bars), respectively. RLU values of firefly luciferase were normalized to those of the internal control pRL-TK and the untreated control was set to one. Results were obtained from one representative experiment performed in triplicates ± SD.

Suppl. Fig. S4: Inhibition of Src and Src-like kinases with PP2 has no effect on RhoA-induced STAT3 phosphorylation.
Hek293 cells were transfected with pGRR5-Luc and pRL-TK followed by intoxication overnight with CNF1 (light gray bars), CNFY (dark gray bars), CNF3 (black bars) or left untreated (open bars). Additionally, cells were treated with PP2 (10 µM) as indicated. RLU values of firefly luciferase normalized to those of the internal control pRL-TK are indicated as fold induction compared to the untreated control. Shown is one representative experiment out of at least 3, performed in triplicates ± SD (top).
Additionally, Tyr705 phosphorylation of STAT3 and total STAT3 was determined in cytosolic and nuclear fractions of the cells. Images are representatives of at least three independent experiments (bottom).

Suppl. Fig. S5: CNF-induced AP1 activation is independent of protein secretion.
Secretion of proteins was blocked with Brefeldin A (BFA). Luciferase assays (top) were performed with cell lysates from Hek293 cells transfected with pRL-TK and pGRR5-Luc, then treated overnight with BFA (2.5 µM) alone (open bars) or together with CNF1 (light gray bars), CNFY (dark gray bars) or CNF3 (black bars), respectively. RLU values were

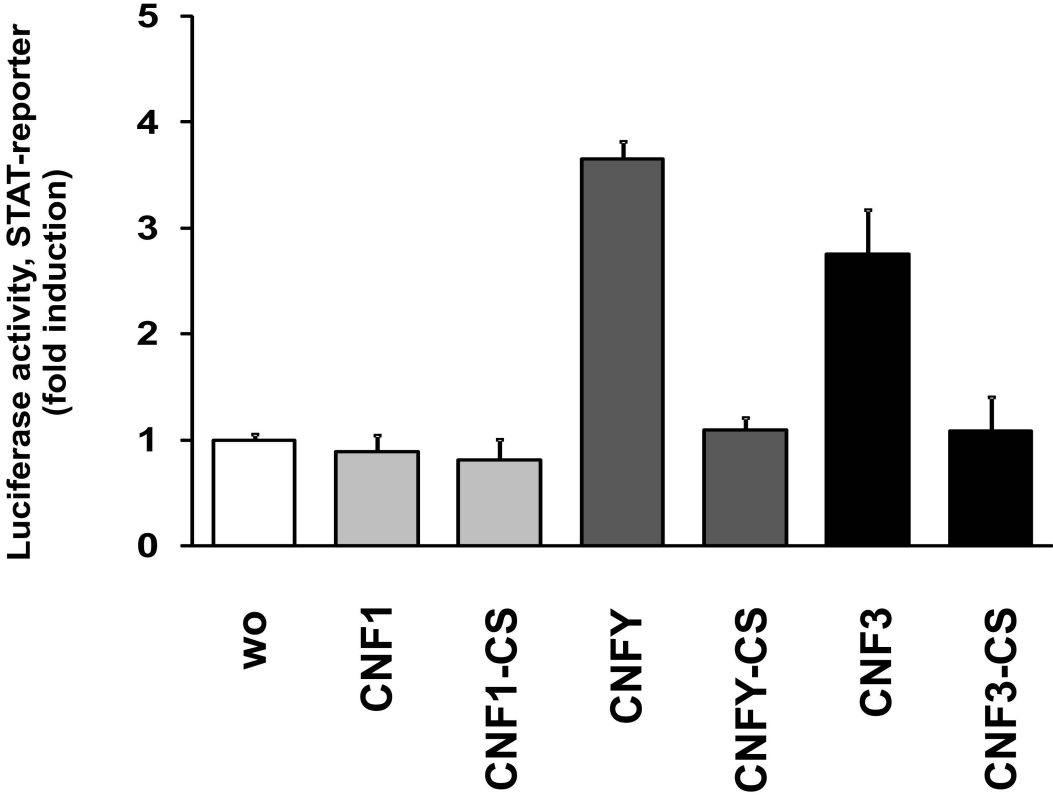
normalized to the internal control pRL-TK, signals of BFA-treated samples were calculated against the inhibitor control set to one. Results were obtained from one representative experiment performed in triplicates ± SD.

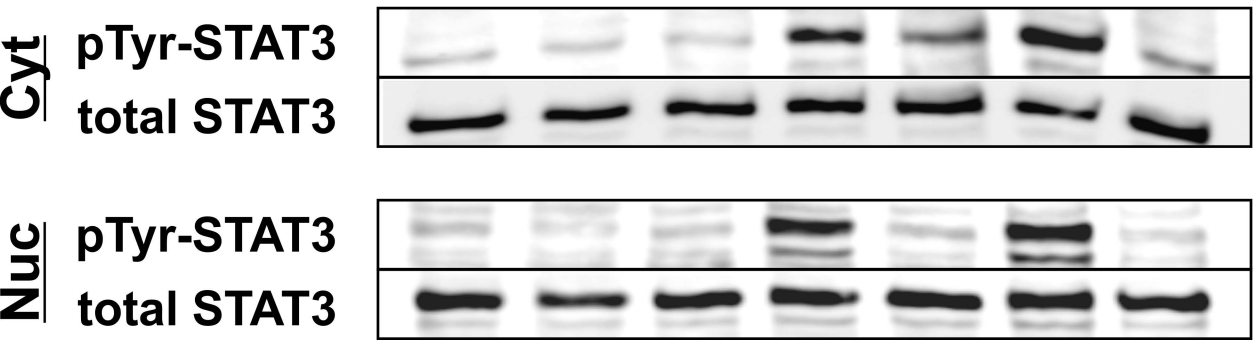
Suppl. Fig. S6: Constitutive active STAT3 stimulates STAT3-dependent, but not AP1-dependent transcription.
Hek293 cells were co-transfected with a vector expressing a dominant active form of STAT3 (c.a.) or empty control vector (mock) together with pGRR5-Luc or pAP1-Luc, as indicated. Luciferase activity in the cell lysates was measured and calculated with the mock-transfected control set to 1 (top). Additionally, expression of STAT3 was controled by Western-blotting with specific antibodies against STAT3 and GAPDH as loading control (bottom).

Suppl. Fig. S7: Induction of STAT3 phosphorylation by CCL1 is concentration dependent.
Hek293 cells were incubated with recombinant CCL1 (concentration as indicated) for 15 min and lysed. Tyr705 phosphorylation of STAT3 and total STAT3 was determined in the lysates of the cells by Western-blotting. Images are representatives of at least 2 independent experiments (bottom).

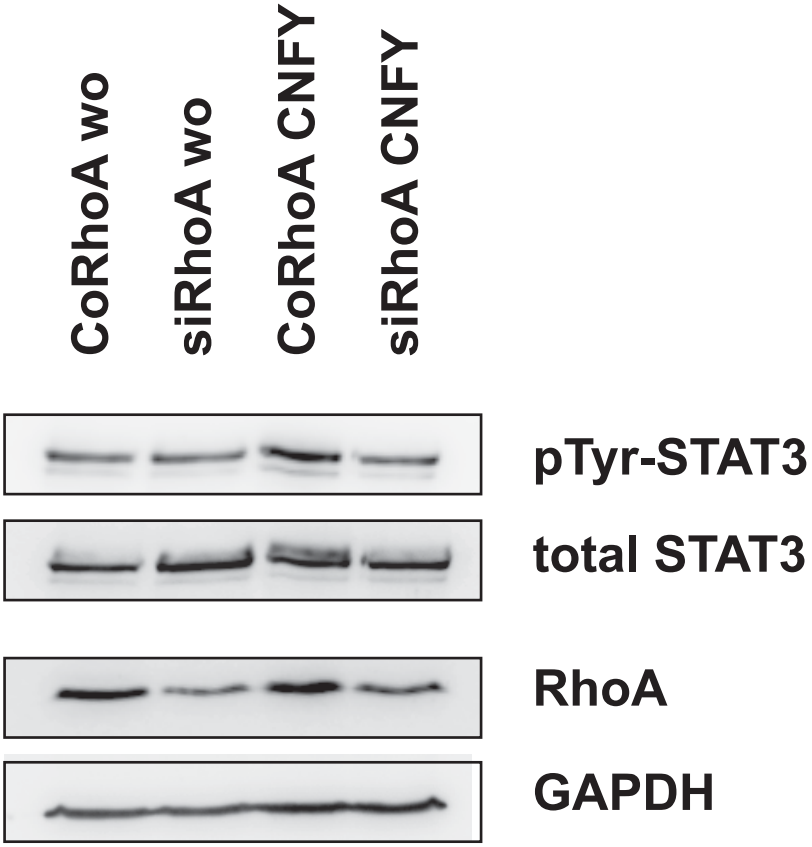
Suppl. T1: RhoA-dependent cytokine production and activity
Hek293 cells were incubated with CNFY (100 ng/ml) or left untreated. Up-regulation of cytokine genes was analysed by two different PCR arrays and indicated as fold induction (CNFY-treated versus untereated). Identified genes with more than 2 fold up-regulation were tested by QRT-PCR. Respective recombinant cytokines were analysed by their ability to induce STAT3 activation in the luciferase reporter assay and STAT3 phosphorylation by Western-blotting. Positive signals were indicated by (+), negative by (–) and (o) indicates not tested. All assays were repeated at least 2 times.

Suppl. Fig. S1

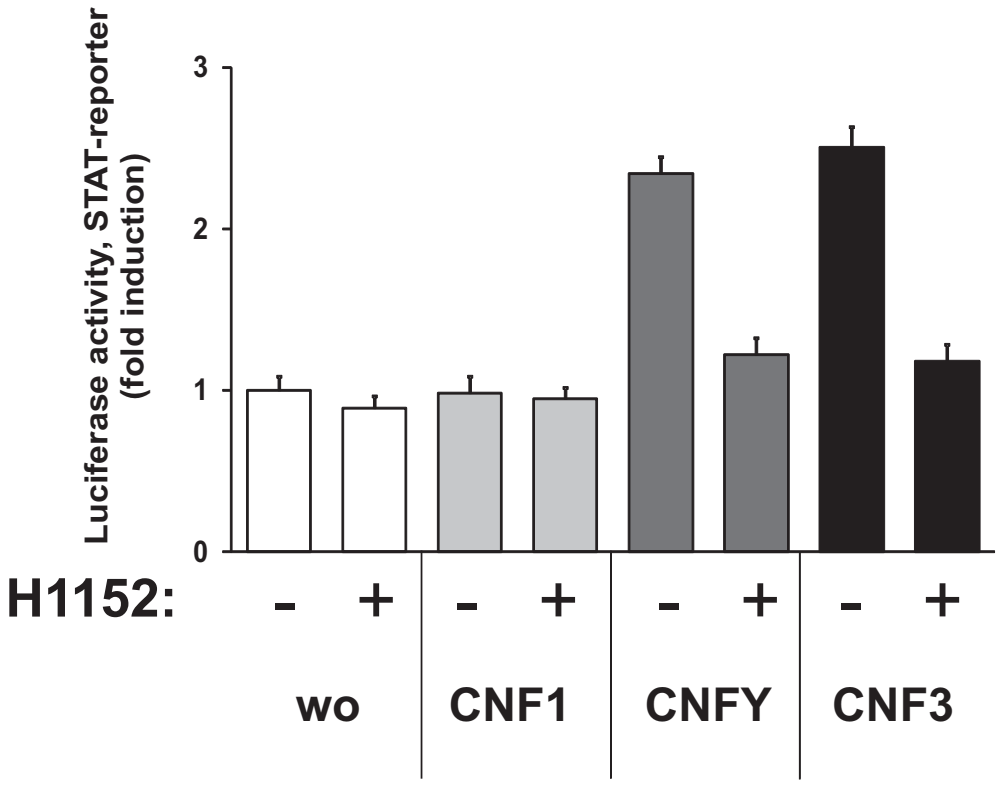

Luciferase activity, STAT-reporter (fold induction)
0
1
2
3
4
5
wo
CNF1
CNF1-CS
CNFY
CNFY-CS
CNF3
CNF3-CS


Cyt
pTyr-STAT3
total STAT3
Nuc
pTyr-STAT3
total STAT3

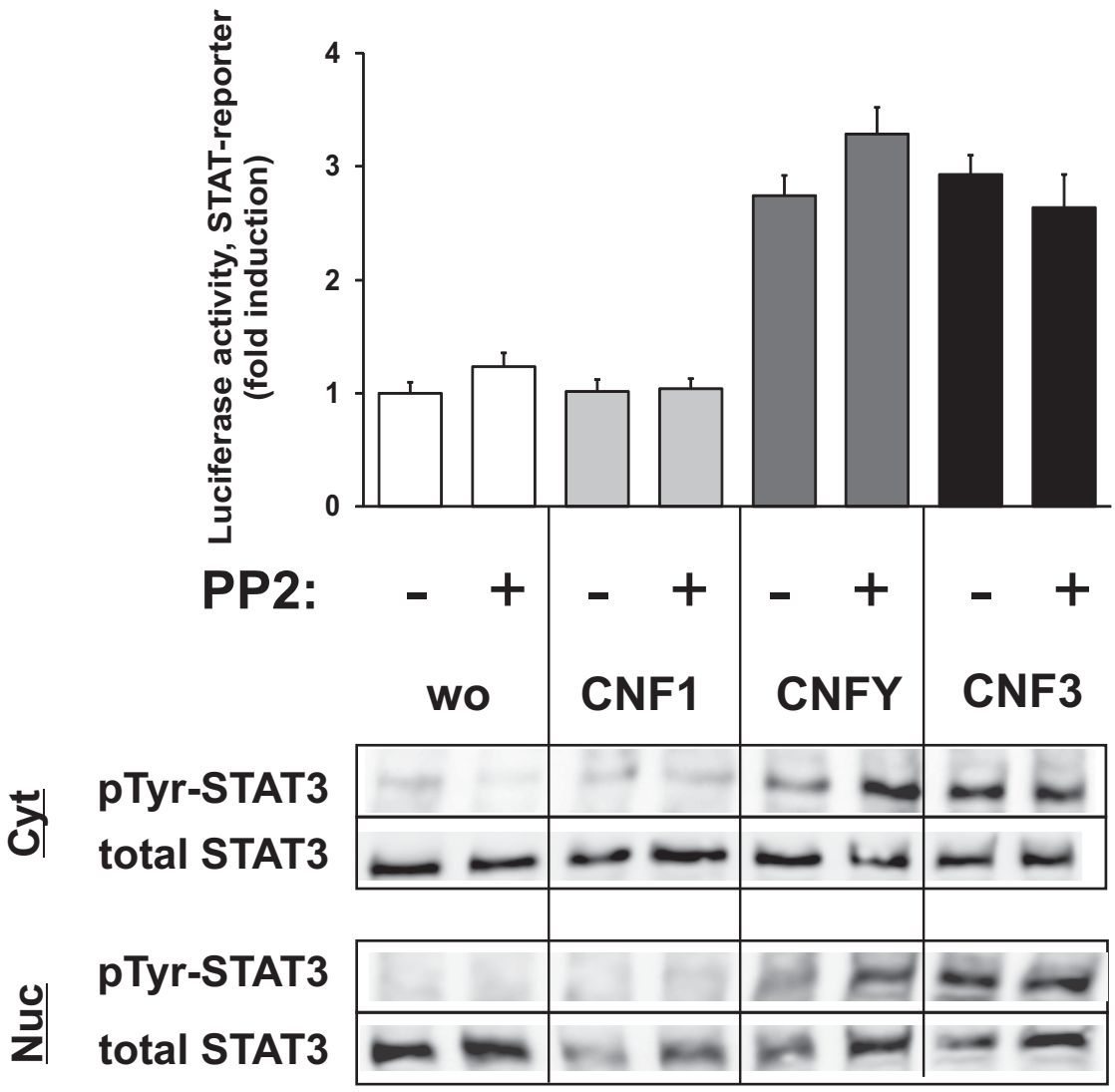
Suppl. Fig. S2


CoRhoA wo
siRhoA wo
CoRhoA CNFY
siRhoA CNFY
pTyr-STAT3
total STAT3
RhoA
GAPDH

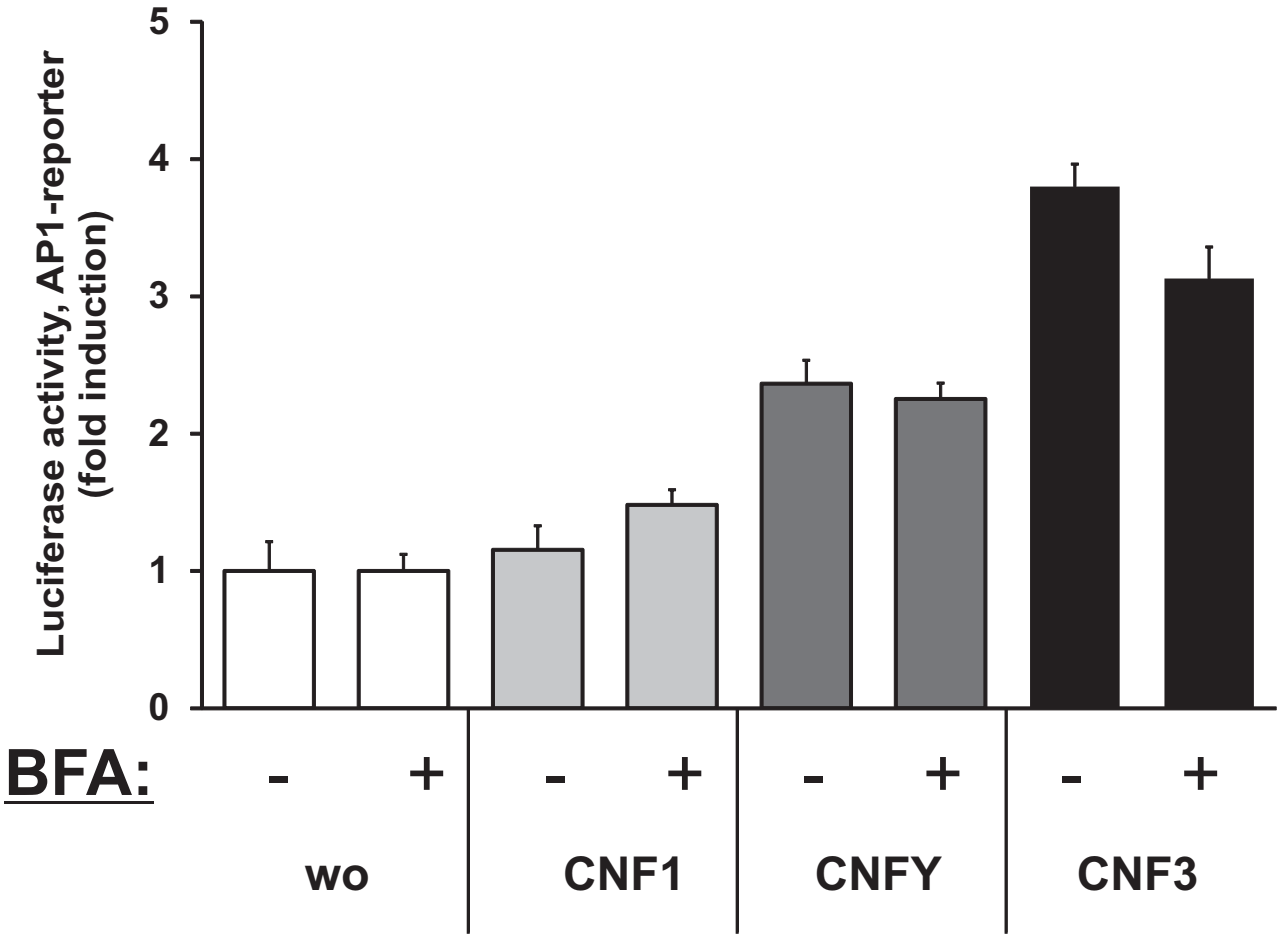
Suppl. Fig. S3


Luciferase activity, STAT-reporter
(fold induction)
3
2
1
0
H1152:
-
+
-
+
-
+
-
+
wo
CNF1
CNFY
CNF3

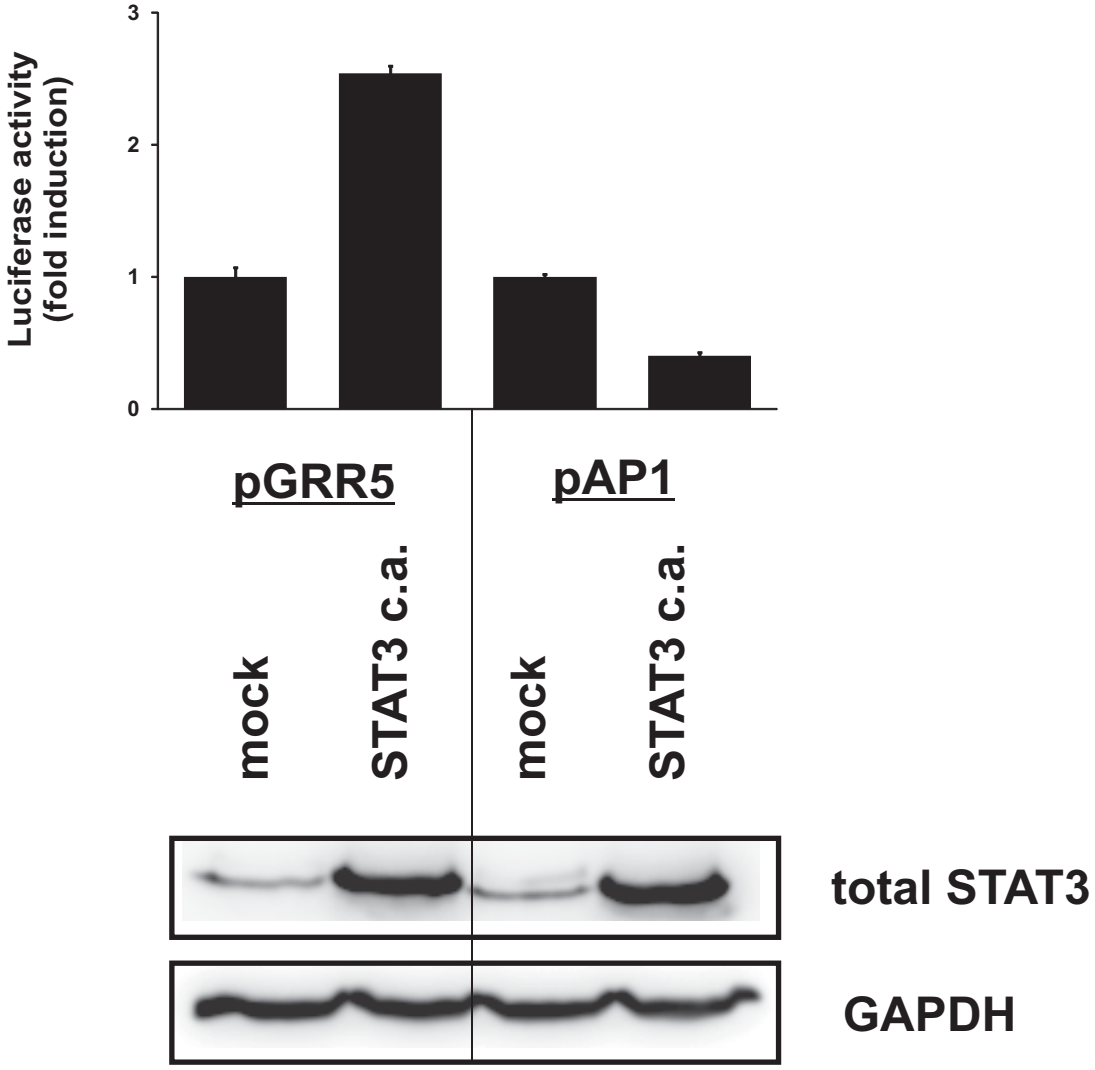
Suppl. Fig. S4


Luciferase activity, STAT-reporter (fold induction)
4
3
2
1
0
PP2: - + - + - + - +
wo
CNF1
CNFY
CNF3
Cyt
pTyr-STAT3
total STAT3
Nuc
pTyr-STAT3
total STAT3

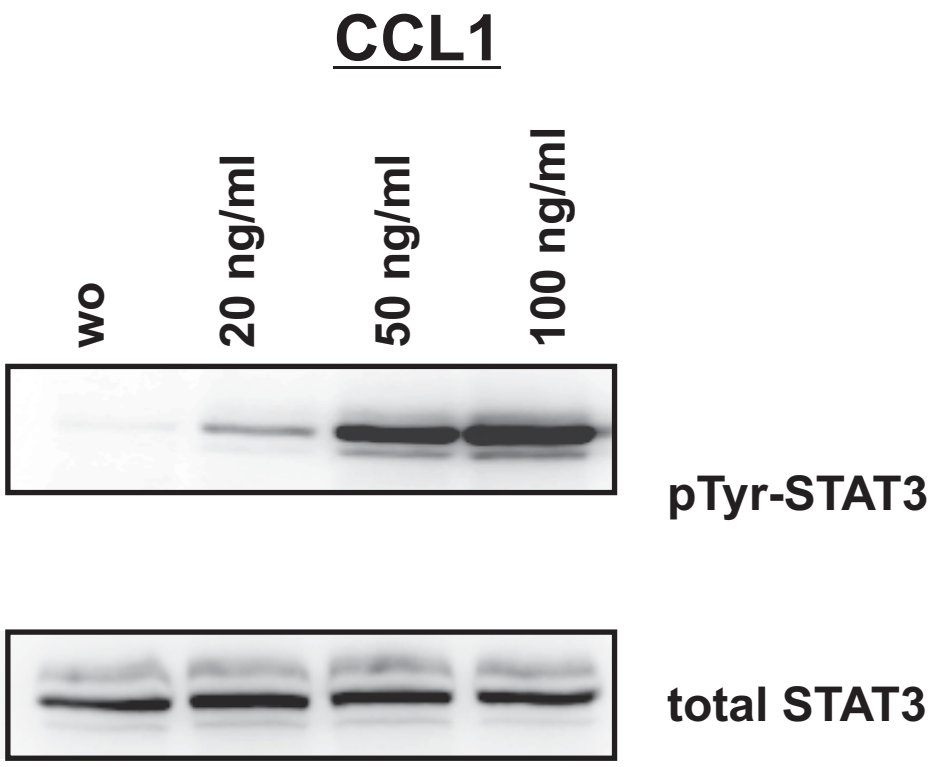
Suppl. Fig. S5


Luciferase activity, AP1-reporter
(fold induction)
5
4
3
2
1
0
BFA:
-
+
-
+
-
+
-
+
wo
CNF1
CNFY
CNF3

Suppl. Fig. S6


Luciferase activity
(fold induction)
3
2
1
0
pGRR5
pAP1
mock
STAT3 c.a.
mock
STAT3 c.a.
total STAT3
GAPDH

Suppl. Fig. S7



Suppl. T1

PCR Array “Common Cytokines”				
	PCR Array	**Verification by QRT-PCR**	**Induction of STAT transcriptional activity**	**Detection of phosphorylated STAT3**
BMP2	13.1	-	-	-
IL8	6.3	+	-	-
TNFalpha	4.1	o	-	o
IL24	3.8	+	-	-
TGFB2	3.6	+	-	-
PCR Array ”Chemokines and Receptors”				
	PCR Array	**Verification by QRT-PCR**	**Induction of STAT transcriptional activity**	**Detection of phosphorylated STAT3**
CCL7/MCP-3	4.9	o	-	-
CCL8/MCP-2	4.3	+	-	-
CCL3/MIP-1a	3.2	o	-	-
CCL1/I-309	2.1	+	+	+