Supplementary Files

A high-throughput chemical screen identifies novel inhibitors and enhancers of anti-inflammatory functions of the glucocorticoid receptor

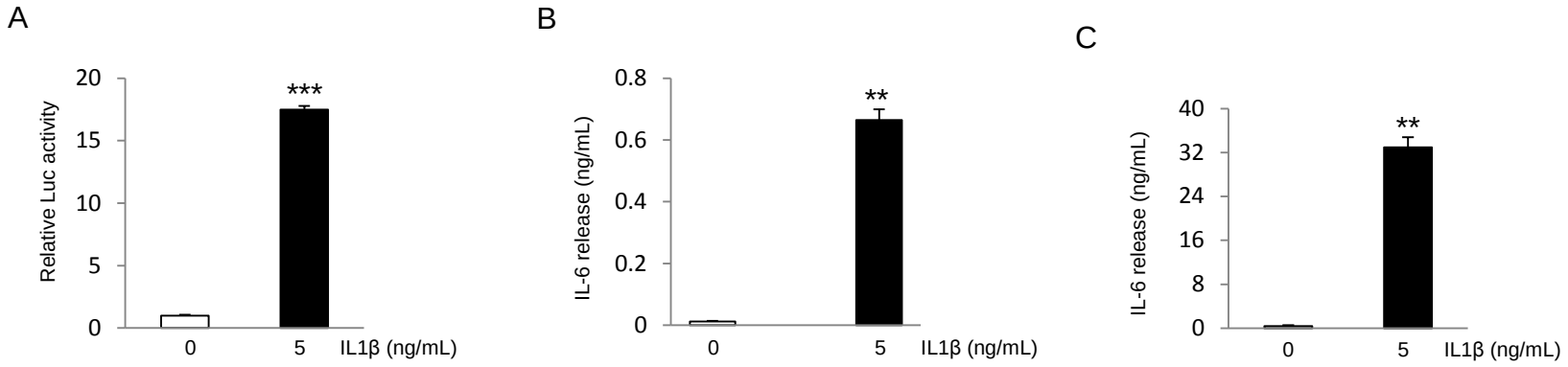
Xiaofeng Jiang[1], Amber Dahlin[2], Scott T. Weiss[2], Kelan Tantisira[2] and Quan Lu[1]*

Supplementary Table 1: Bioactivities and assay II/I ratios of GR inhibitors and enhancers.

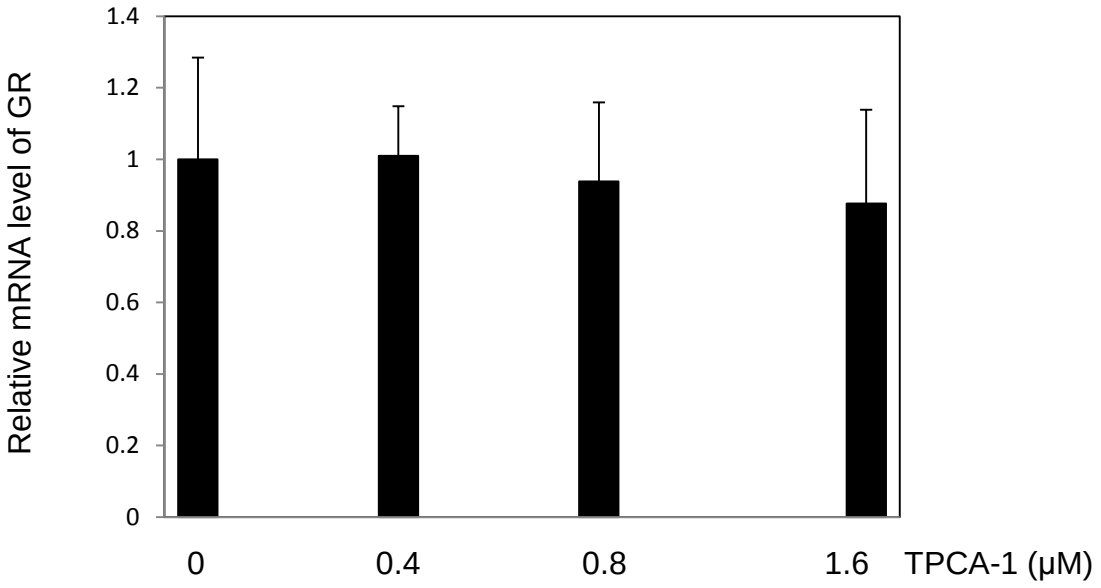
Compounds	Assay II/I ratio	Bioactivities
Alsterpaullone	0.70	GSK3β, Cdk1/cylin B inhibitor
RO0275062	0.85	GSK3β inhibitor
RO0317753	0.91	GSK3β inhibitor
Pyrromycin	0.77	DNA intercalation
Camptothecin	1.0	DNA topoisomerase I inhibitor
TPCA-1	0.28	IKK2 inhibitor
IKK2 inhibitor VI	0.30	IKK2 inhibitor

* Assay II/I ratio for DMSO is 0.4

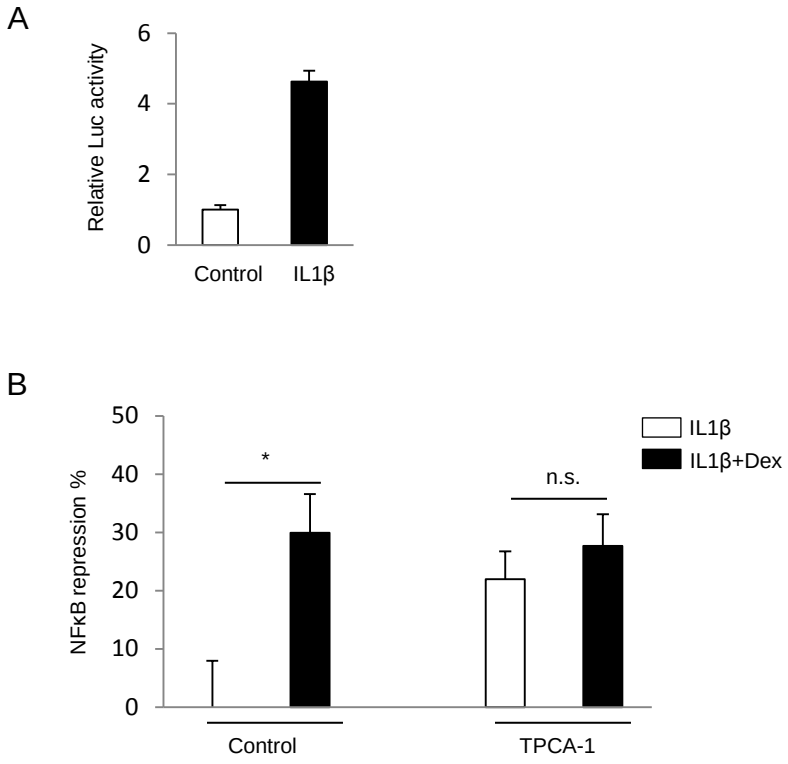
Supplementary Figure S1: Effects of IL1β on NFκB activation in A549 cells (A), on IL-6 release in A549 cells (B), and on IL-6 release in human airway smooth muscle cells (C).



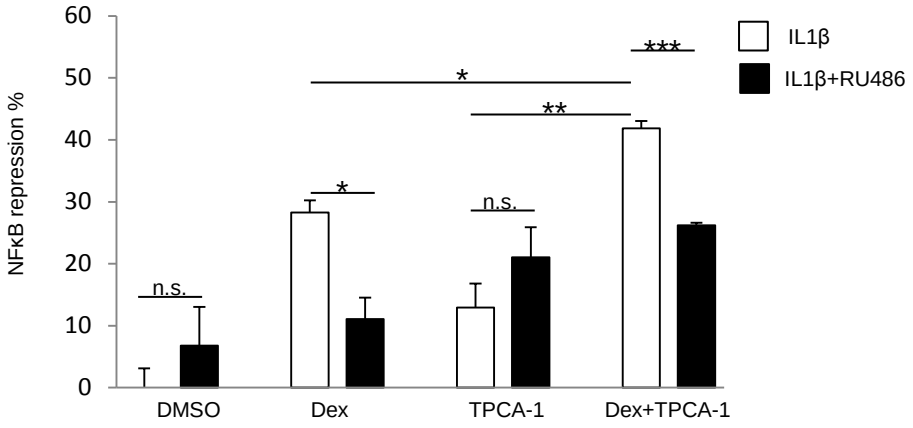
Supplementary Figure S2: Effects of TPCA-1 on GR mRNA expression. A549 cells were treated with different concentrations of TPCA-1 for 48 h, and qPCR was used to measure GR mRNA level.



Supplementary Figure S3: *A*: Effect of IL-1β (5 ng/mL) treatment on NF-κB activation. *B*: Effect of TPCA-1 (0.8 μM) on NF-κB repression by Dex (0.5 nM) in A549/NF-κB-luc cells. All treatments were done for 4 h. Luciferase assays were performed after 4 h treatment.

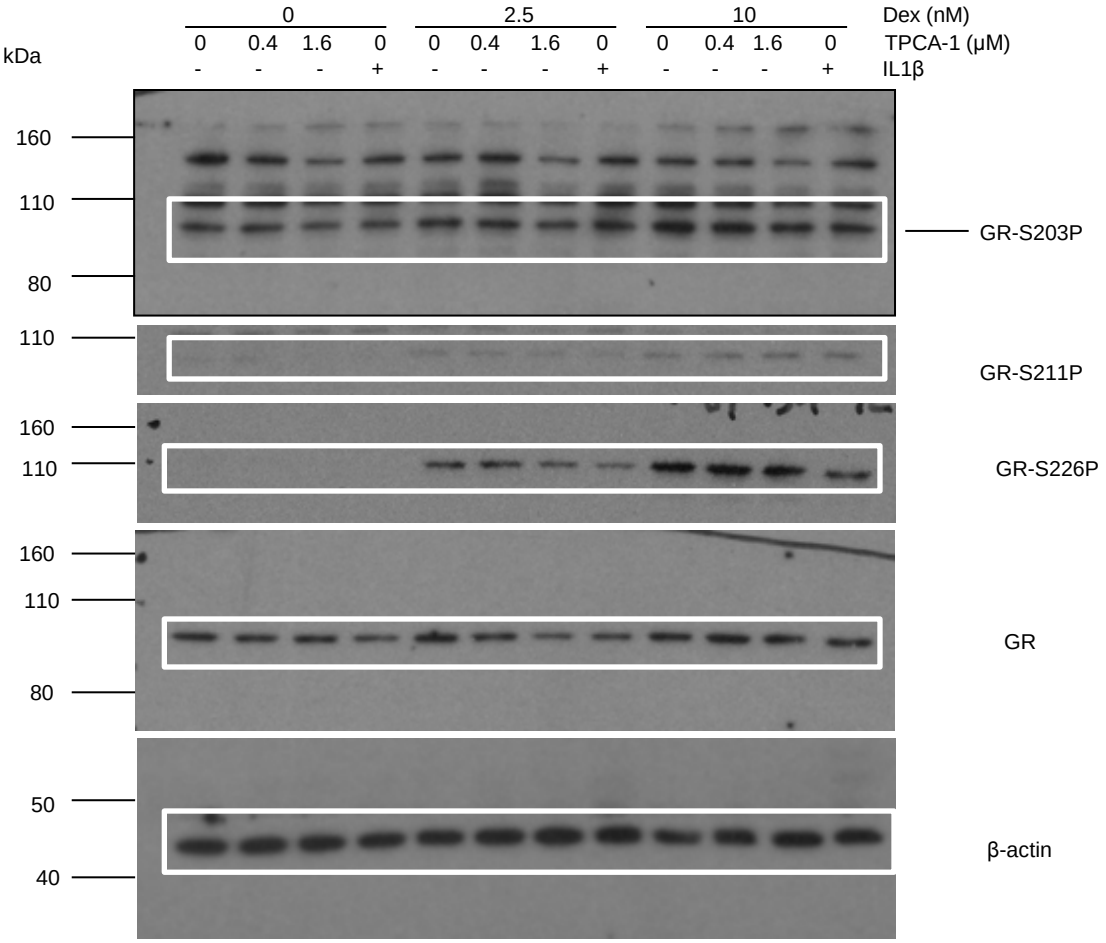


Supplementary Figure S4: GR antagonist RU486 attenuates the effect of Dex and TPCA-1 on NFκB repression. The reporter cells were treated with 5ng/mL IL-1β ± 0.5 nM Dex, 0.4 μM TPCA-1 or 5 nM RU486 as indicated for 18 h. Luciferase assays were performed, and NF-κB repression was calculated relative to the cells treated with IL1β. n.s., no significance. *, P <0.05. **, P < 0.01, ***P<0.005.

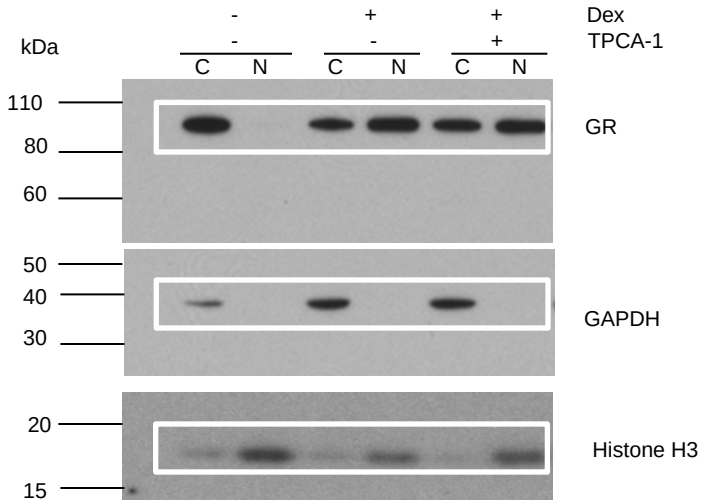


Supplementary Figure S5: Full-length blots for Fig.4

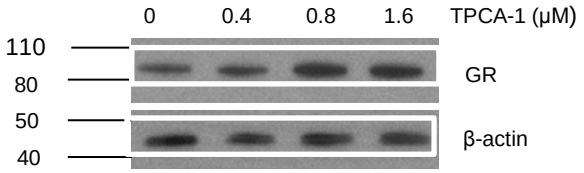
A



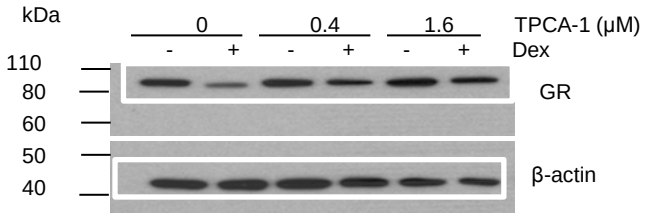
B


kDa
-
-
+
-
+
+
Dex
TPCA-1
C
N
C
N
C
N
110
80
60
50
40
30
20
15
GR
GAPDH
Histone H3

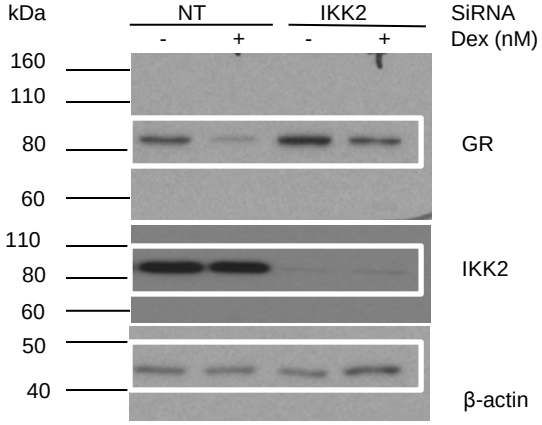
C


0
0.4
0.8
1.6
TPCA-1 (μM)
110
80
50
40
GR
β-actin

D


kDa
0
0.4
1.6
TPCA-1 (μM)
-
+
-
+
-
+
Dex
110
80
60
50
40
GR
β-actin

E


kDa
NT
IKK2
SiRNA
-
+
-
+
Dex (nM)
160
110
80
60
110
80
60
50
40
GR
IKK2
β-actin