Retinoid X Receptor α Attenuates Host Antiviral Response by Suppressing Type I Interferon

Feng Ma[1,7], Su-Yang Liu[1,7], Bahram Razani[1], Neda Arora[1], Bing Li[2], Hiroyuki Kagechika[3], Peter Tontonoz[4,5], Vanessa Núñez[6], Mercedes Ricote[6], & Genhong Cheng[1,*]

Supplementary figures

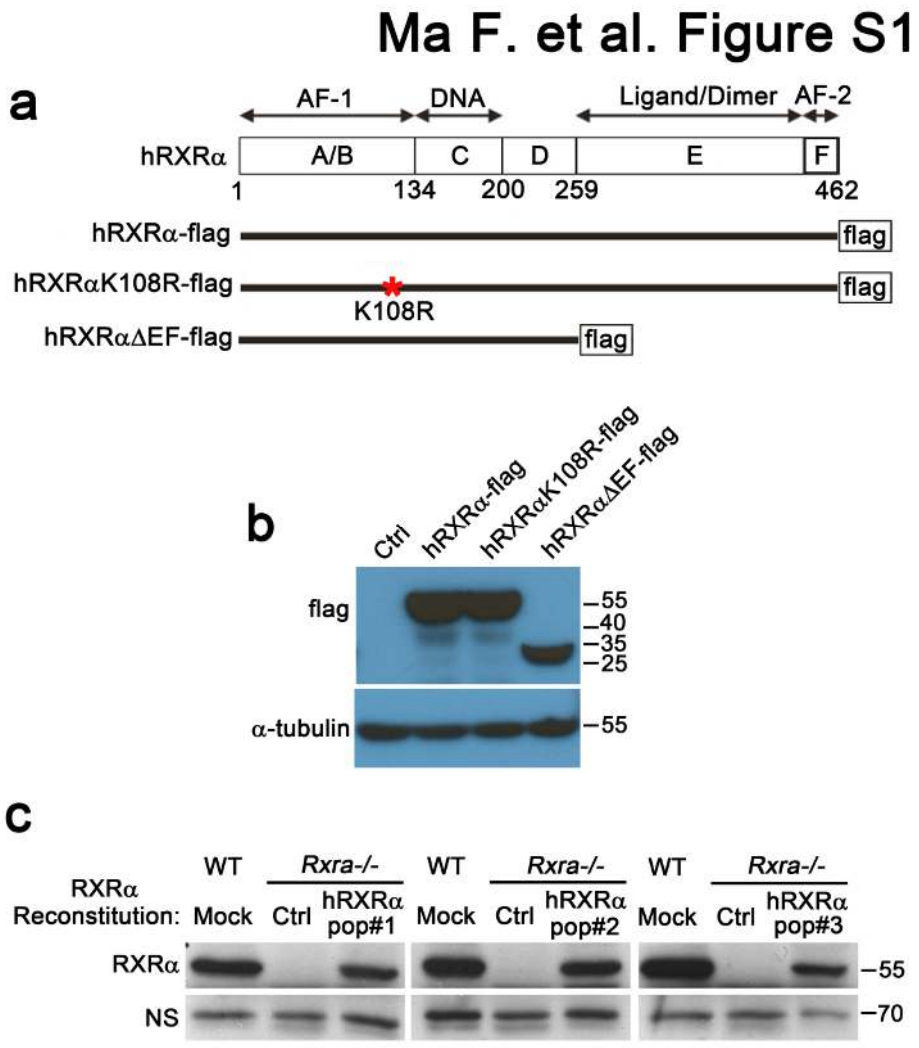

Figure S1 RXRα mutant constructs and RXRα reconstitution in F9 *Rxra-/-* cells. (**a**) Schematic map for RXRα mutants. hRXRα-flag, full length wild type human RXRα construct with flag tag; hRXRαK108R-flag, point mutation of the construct hRXRα-flag, the amino acid in position 108 of human RXRα, lysine, was mutated to arginine; hRXRαΔEF-flag, the ligand binging/dimeriaztion domain (E) and AF2 domain (F) was deleted from the construct hRXRα-flag. The backbone of the above three construct was pBABE vector. (**b**) Immunoblot of flag tag expression in RAW264.7 cells stably transfected with pBABE-empty vector (Ctrl), hRXRα-flag, hRXRαK108R-flag, and hRXRαΔEF-flag. (**c**) *Rxra*-/- F9 cells were reconstituted with pBABE-hRXRα (hRXRα) or pBABE-empty vector (Ctrl) by retroviral infection. Three independent hRXRα-overexpressing populations (pop#1~3) were made, RXRα expression in these cells and in WT cells was confirmed by immunoblot, non-specific band (NS) was taken as a loading control. Data of (**b**) and (**c**) are representative of three independent experiments.

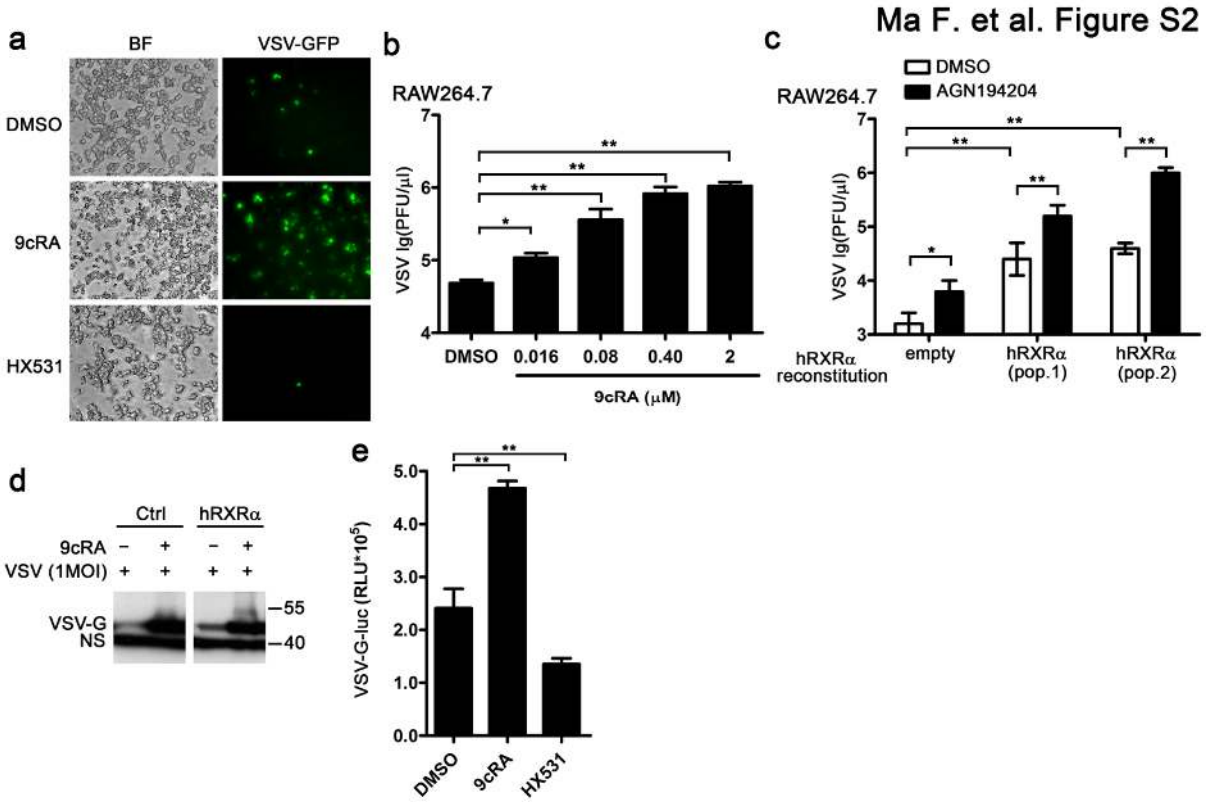

Figure S2 Host susceptibility VSV infection after pretreating with RXR agonist or antagonist. (**a**) RAW264.7 cells were pretreated with DMSO, 9cRA (100 nM), and HX531 (1 μM) for 16 h and infected by VSV-GFP (MOI = 0.01). The representative images of three independent experiments for the VSV-GFP-infected cells (9 h.p.i) were shown (BF, bright field). (**b**) RAW264.7 cells were pretreated with DMSO or indicated concentration of 9cRA for 16 h and subsequently infected by VSV (MOI = 0.01). The supernatants were collected at 14 h.p.i and viral titer was detected by plaque assay. (**c**) Two independent populations of RAW264.7 cells were reconstituted with pBABE-hRXRα (pop.1~2) or pBABE-empty vector (empty). The cells were pretreated with DMSO or AGN194204 (100 nM) for 16 h and then infected by VSV (MOI = 0.01). The supernatants were collected at 14 h.p.i and viral titer

was detected by plaque assay. (**d**) RAW264.7 cells expressing pBABE control vector (Ctrl) or hRXRα were pretreated DMSO or 9cRA (100 nM) for 16 h and infected by VSV, cell lysates were collected at 8 h.p.i and VSV-G protein level was detected by immunoblot, α-tubulin was shown as a loading control. Data are representative of three independent experiments. (**e**) hRXRα-overexpressing RAW264.7 cells were pretreated with DMSO, 9cRA (100 nM) and HX531(1 μM) for 16 h and infected by replication-incompetent VSV pseudovirus encoding VSV-G luciferase reporter (VSV-G-luc). The lysates were collected at 24 h.p.i and the luciferase activity was quantified. RLU is represented as mean ± SD in biological quadruplicates. **, $p < 0.01$(Student's *t*-test), data are representative of three independent experiments. Data of (**b**) and (**c**) are shown as mean ± SD (n = 3) in one representative experiment. Similar results were obtained in three independent experiments. *, $p < 0.05$ and **, $p < 0.01$ (Student's *t*-test).

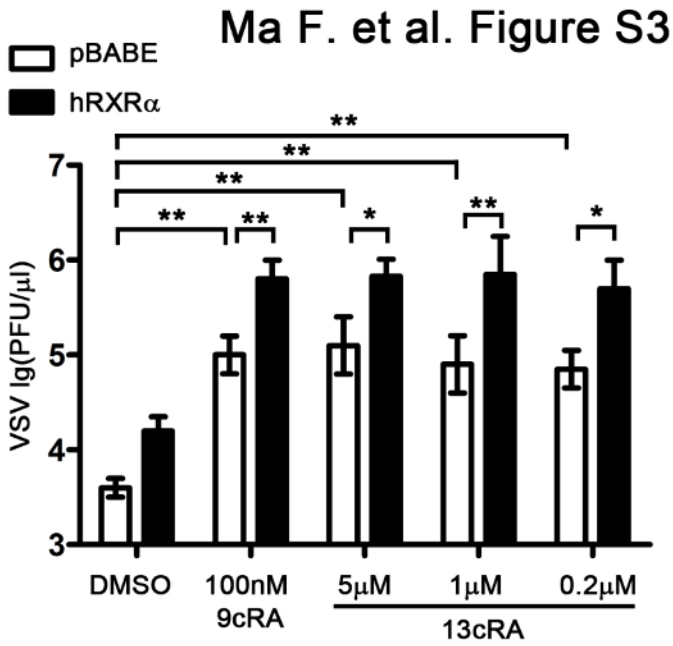

Figure S3 13cRA treatment increases the host susceptibility to VSV infection. RAW264.7 cells were reconstituted with pBABE-hRXRα or pBABE-empty vector (pBABE), the cells were treated with DMSO, or indicated concentration of 9cRA or 13cRA for 16 h and subsequently infected with VSV (MOI = 0.01). The supernatants were collected at 14 h.p.i and viral titer was detected by plaque assay. Data are shown as mean ± SD (n = 3) in one representative experiment. Similar results were obtained in three independent experiments. *, $p < 0.05$ and **, $p < 0.01$ (Student's *t*-test).

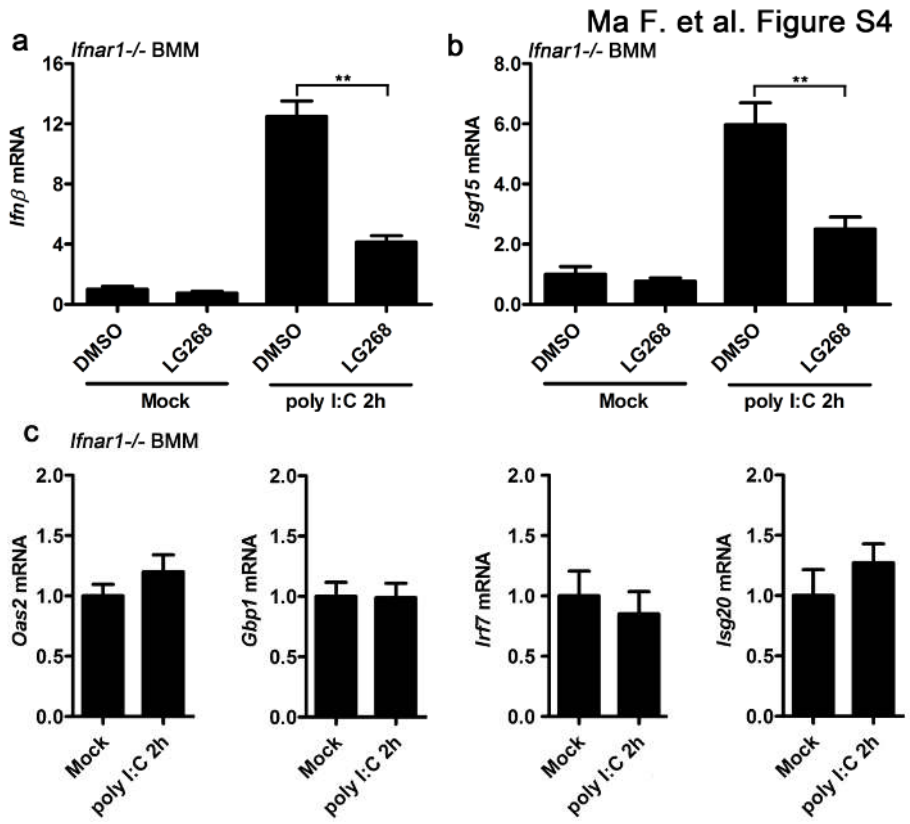

Figure S4 Activation of RXR by LG268 inhibits the induction of *Ifnβ* and *Isg15*. *Ifnar-/-* BMMs were pretreated with DMSO or LG268 (100 nM) for 24 h and transfected with 1 μg/ml polyI:C for 2 h. *Ifnβ* (**a**) and *Isg15* (**b**) mRNA level were quantified by qPCR. (**c**) *Ifnar-/-* BMMs were transfected with 1 μg/ml polyI:C for 2 h, *Oas2*, *Gbp1*, *Irf7*, and *Isg20* mRNA levels were quantified by qPCR. Data are shown as mean ± SD (n = 3) of one representative experiment, similar results were obtained in three independent experiments, **, $p < 0.01$ (Student's *t*-test).

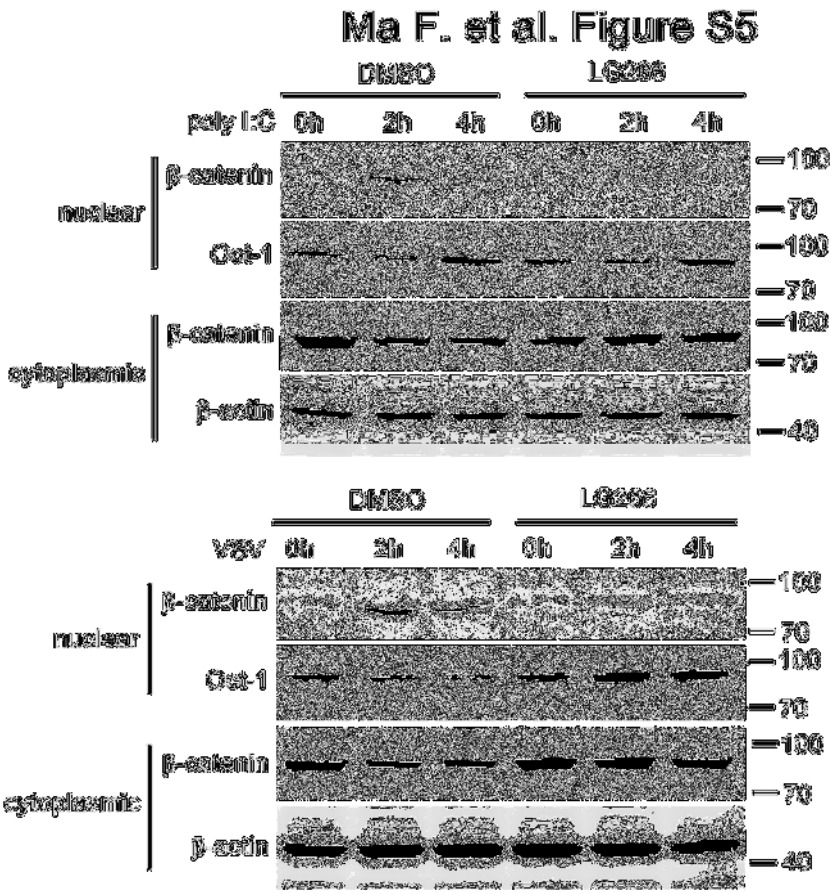

Figure S5 Activation of RXR by LG268 inhibits β-Catenin nuclear translocation. J2 BMMs were pretreated with DMSO or LG268 (100 nM) for 16 h, and then the cells were transfected with 3 μg/ml polyI:C or infected by 1 MOI VSV for indicated time point. Nuclear and cytoplasmic β-Catenin were detected by immunoblot, Oct1 and β-actin were shown as loading controls. Data are representative of three independent experiments.

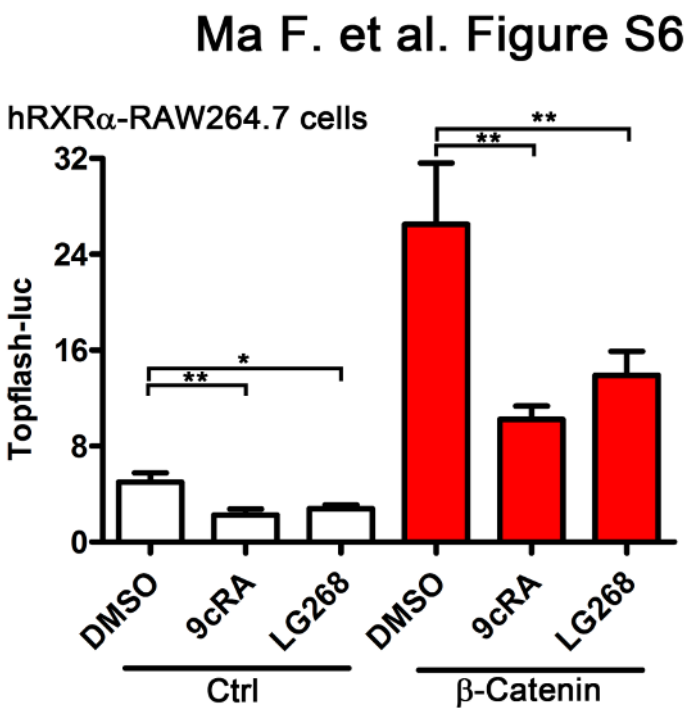

Figure S6 Ligand activation of RXR inhibits β-Catenin transcription activity. hRXRα-stably transfected RAW264.7 cells were cotransfected with β-Catenin luciferase reporter construct (Topflash-luc) and pcDNA 3.1control vector, or β-Catenin overexpressing vector for 12 h, and then these cells were treated with DMSO, 9cRA (100 nM), or LG268 (100 nM) for another 12 h. The lysates were collected and luciferase reporter activity was measured by luminescence assay. RLU is represented as mean ± SD (n = 6) and is representative of three experiments. *, $p < 0.05$ and **, $p < 0.01$ (Student's *t*-test).

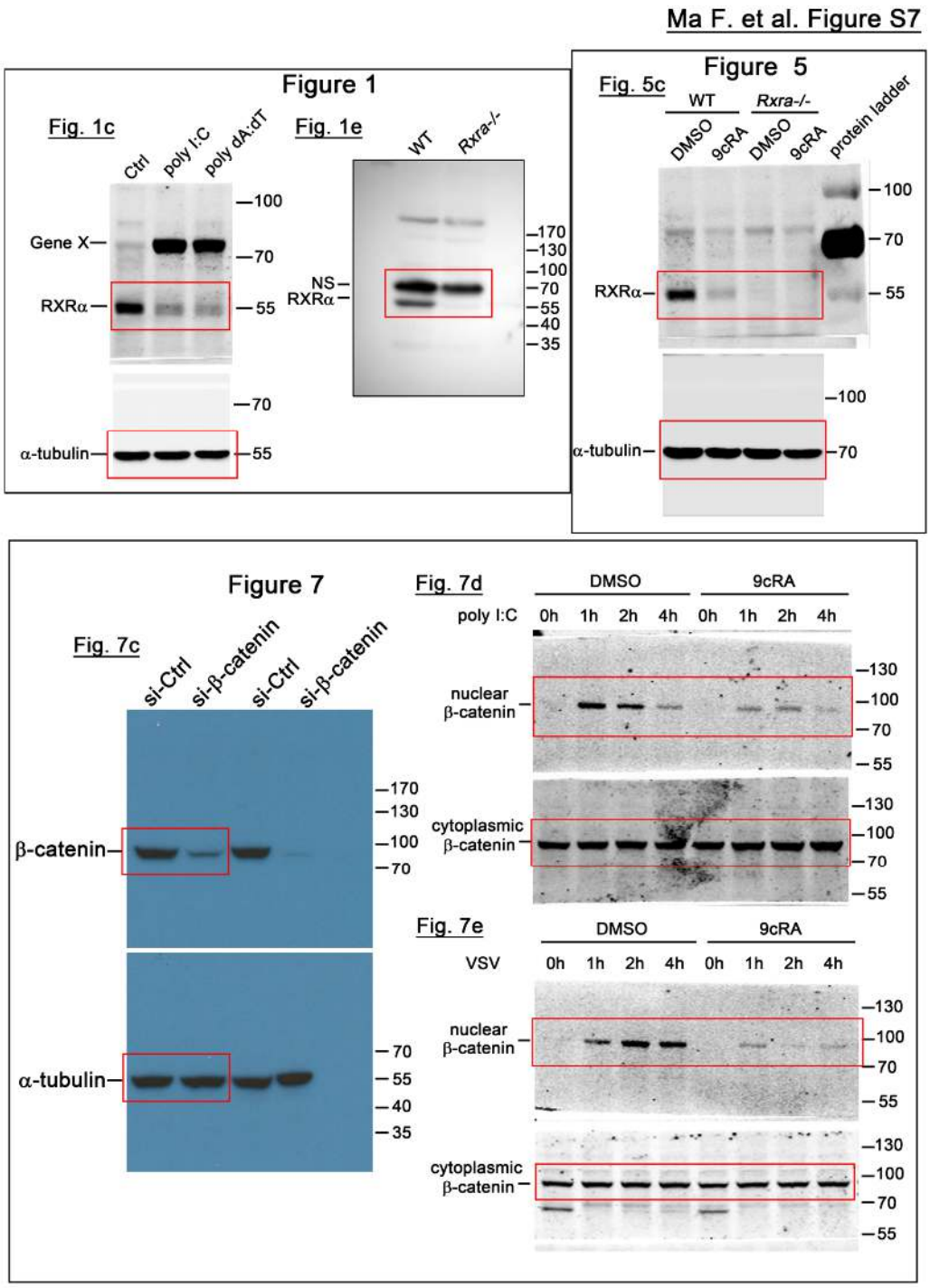

Figure S7 Uncropped gels and western blots. The red boxes indicate the cropped regions.