Supporting Information

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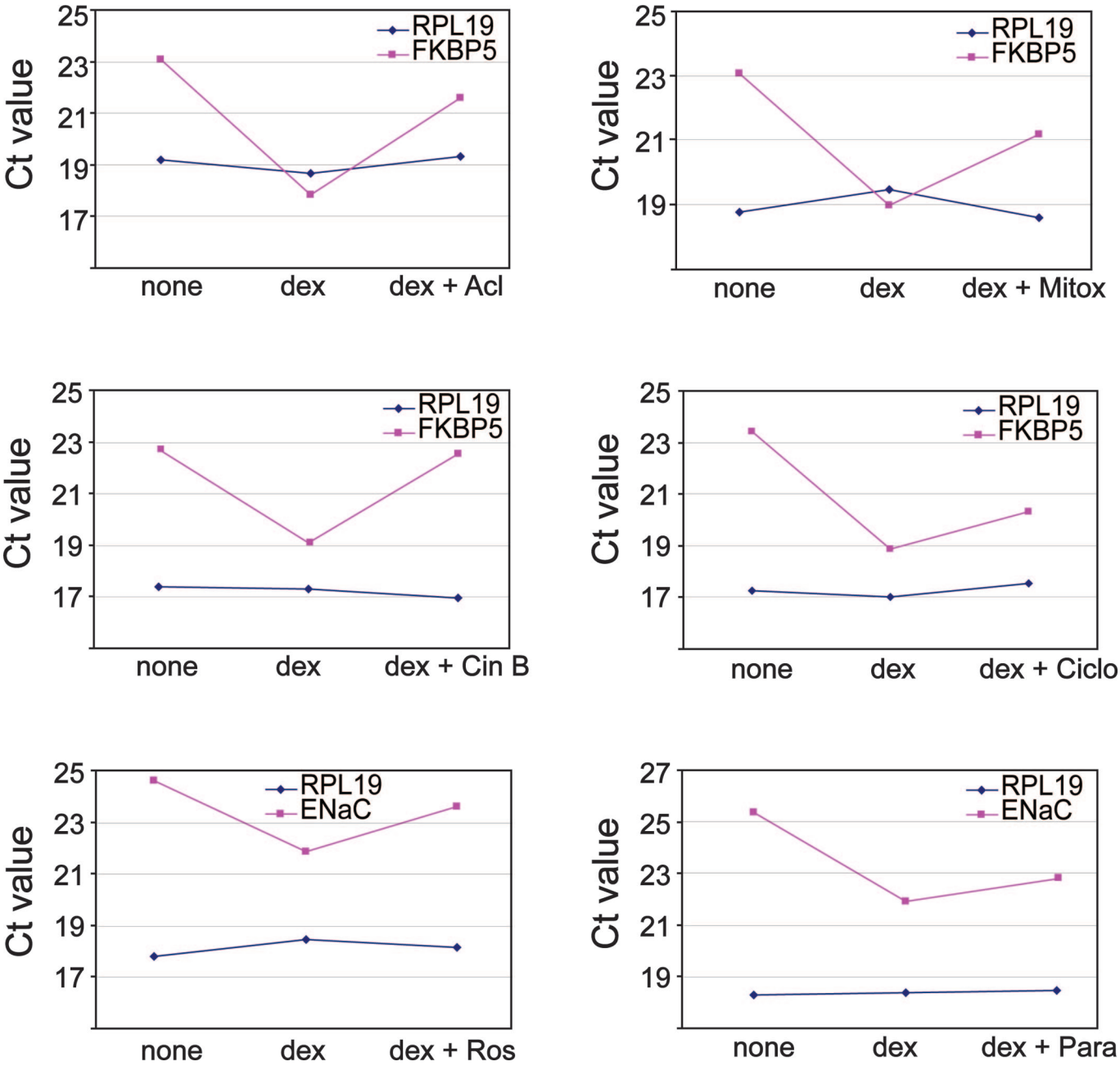

Fig. S1. Ct values for RPL19 and selected GR-regulated genes with various drug treatments. Cells were treated as indicated in the main text. Average Ct values obtained via qPCR for various treatments are shown. As the $\log_2$ of Ct values inversely correlate with message level, a decrease of 1 on the *y* axis represents a doubling in message levels. The control RPL19 Ct values were fairly constant with drug treatments, suggesting that the impact of the various drugs was not simply due to nonspecific effects on transcription. This is also reflected by the normalized data shown in Figs. 3, 4, and 5.

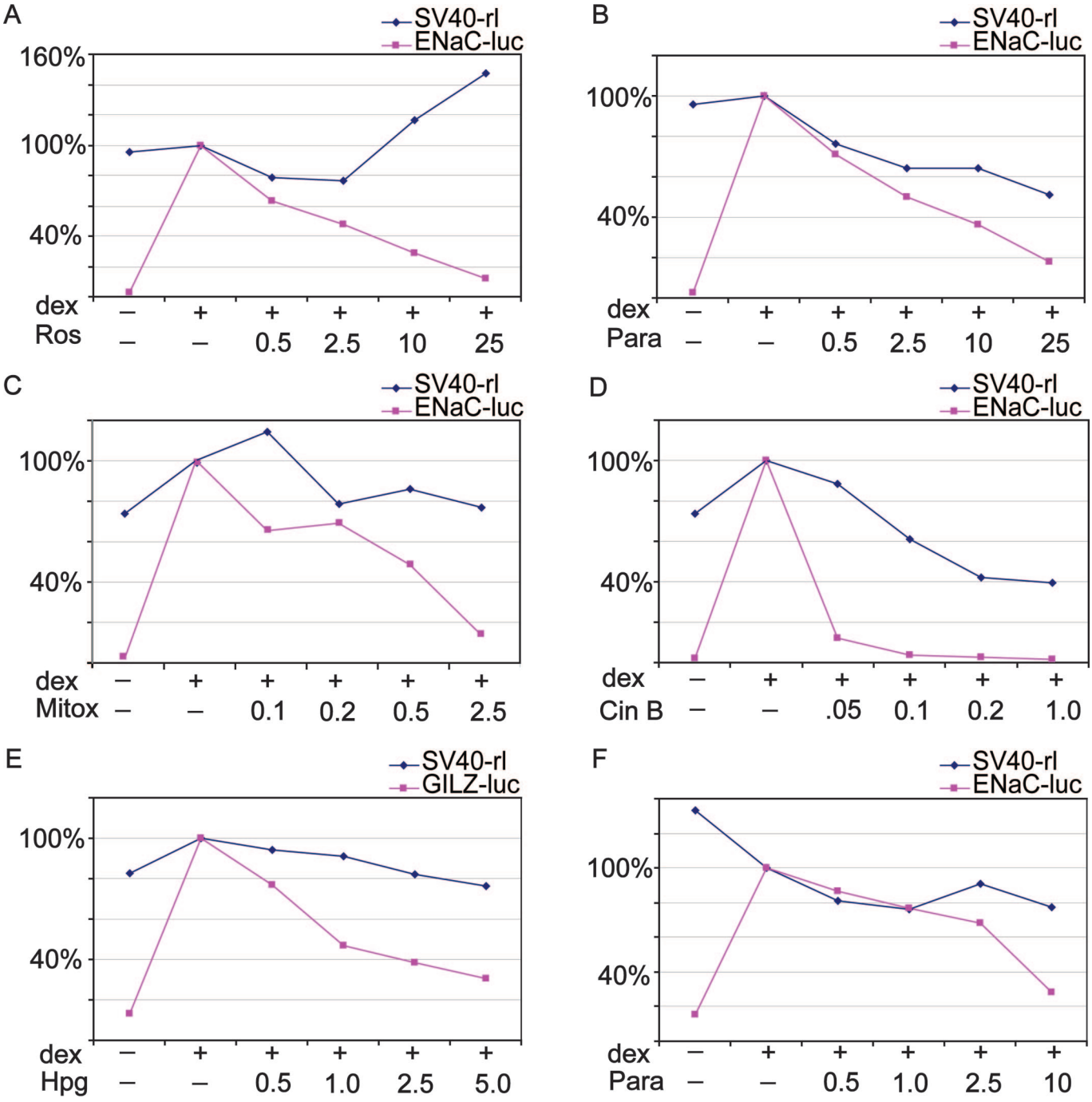

Fig. S2. GR-dependent firefly luciferase and GR-independent renilla-luciferase values with various drug treatments graphed individually. Luciferase assays were performed on A549 cells (*A–E*) and U2OS-GR cells (*F*) transfected with pENaC-luc and pSV40-rl (*A–D* and *F*) or pGILZ-luc and pSV40-rl (*E*). Before harvesting, cells were treated with dex and increasing concentrations of various drugs as indicated (doses in μM) for $\approx$24 h. Average absolute activities of the dex-responsive firefly luciferase reporters and the dex-unresponsive pSV40-rl reporter with dex treatment alone were independently normalized to 100%. These data further indicate that the various compounds impact GR-dependent reporters to a greater extent than the control SV40-renilla reporter.

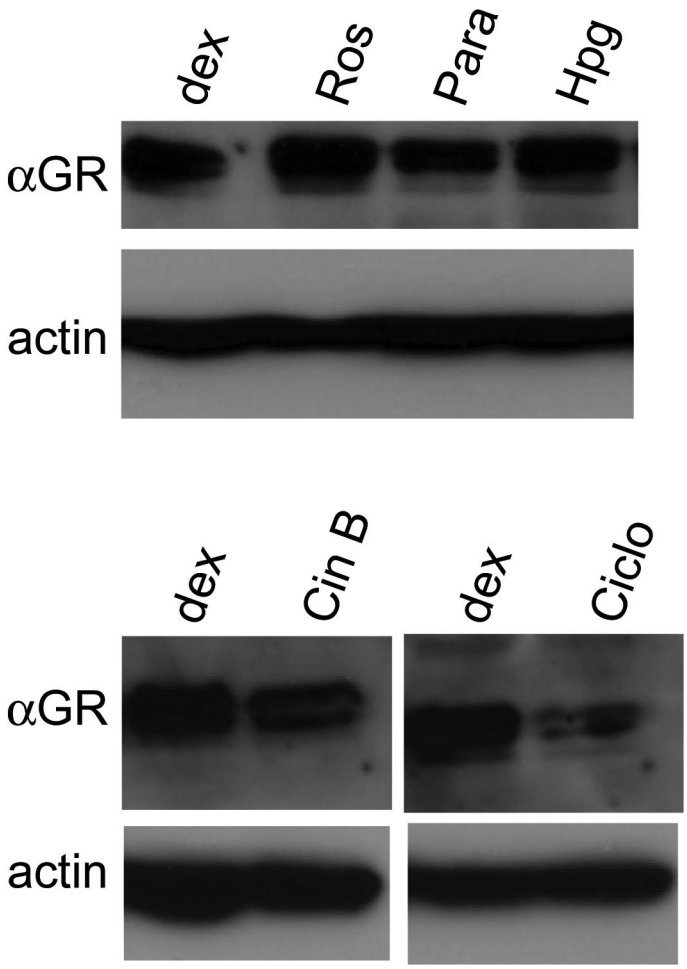

Fig. S3. Effects of selected compounds on GR protein levels in A549 cells. Cell lysates were prepared from A549 cells that were treated with dex alone (100 nm) or dex and Ros (10 μM), Para (10 μM), Hpg (2.5 μM), Cin B (50 nM), or Ciclo (25 μM) as indicated for 24 h. Western blot analysis was performed by using standard techniques; GR antibody (Abcam) was used at a 1/1,000 dilution; actin (Sigma) was used at 1/5,000.

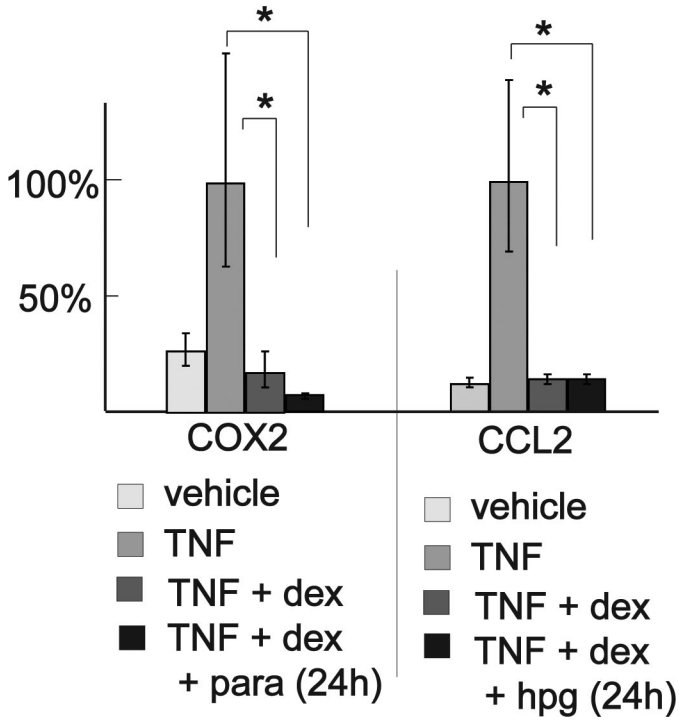

Fig. S4. Hpg and Para do not prevent repression of additional TNF-α-regulated cytokines. Relative mRNA levels of the indicated target genes were determined by using qPCR. Cells were treated for 24 h. Doses used were TNF-α (2.5 ng/ml); Para (10 μM) and Hpg (2.5 μM). *, $P \leq 0.05$.

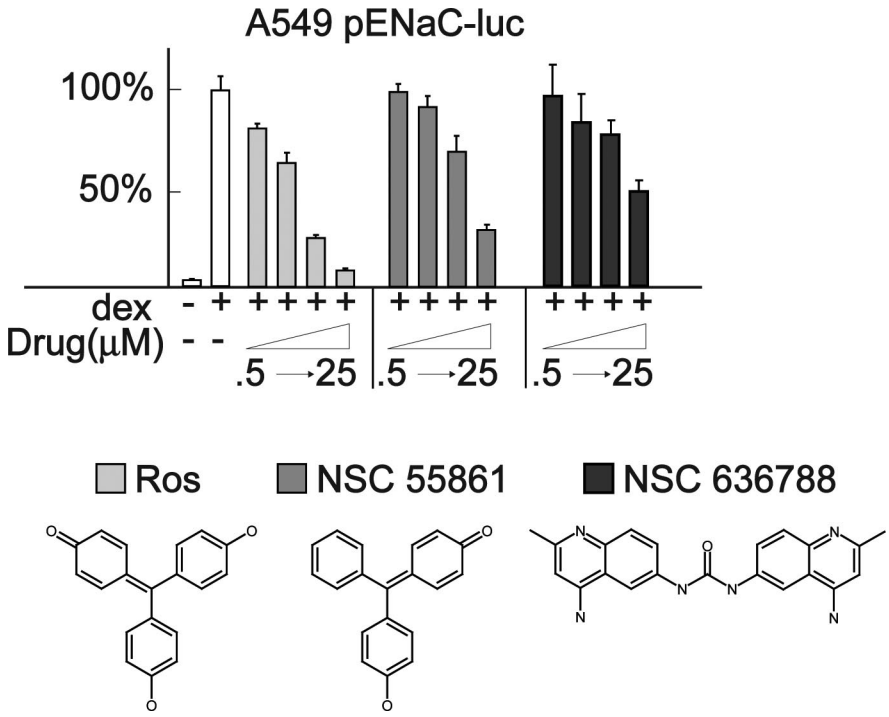

Fig. S5. Additional rosolic acid-like compounds repress GR activity. Luciferase assays were performed by using A549 cells transfected with pENaC-luc and pSV40-rl after treatment with increasing concentrations of drugs (0.5, 2.5, 10, and 25 μM) for 18 h. The structure of each drug is shown. Ros is rosolic acid.

Table S1. Tabulated results of primary screen

Hit type	Total	No. of reporters scored as hits		
		1 reporter	2 reporters	3 reporters
Primary hits	66	20	34	12
Known NR ligands	14	6	5	3
Other	52	14	29	9

Table S2. Effects of known glucocorticoids that scored as hits in primary screen

Glucocorticoid	ENaC-CFP	GILZ-YFP	FKBP5-OFP
Prednisolone acetate	No effect	↓ ↓ *	No effect
Trimacinolone acetonide	No effect	↑ ↑ †	No effect
Trimacinolone diacetate	No effect	No effect	↓ ↓
Fluradrenolide	No effect	↑ ↑	No effect
Flunisolide	↑ ‡	↑ ↑	↑
Amcinonide	No effect	↑ ↑	↑
Clobetasol propionate	No effect	↑	↑ ↑ ↑ §

These 7 known glucocorticoids scored positive in the primary screen. As with all of the library compounds, they were tested at a concentration of 2.5 μM in the presence of 100 nm of dex. In these conditions, they modulated the activity of the various reporters as indicated. Although further analysis was not pursued, these results suggest that many already developed GR ligands cause differential activation of different promoters.

*More than 1.5 SD less than the mean.

†More than 1.5 SD greater than the mean.

‡Indicates 0.8–1.5 SD greater than the mean.

§More than 3.0 SD greater than the mean.