Electronic Supplementary Material (ESI) for RSC Medicinal Chemistry.

Supplemental Figures for:

Deacylcortivazol-like pyrazole regioisomers reveal a more accommodating expanded binding pocket for the glucocorticoid receptor

Jessica A.O. Zimmerman[1,2], Mimi Fang[1], Bintou Doumbia[3], Alexis Neyman[3], Ji Hyeon Cha[3], Michael Thomas[3], Bonnie Hall[4], Meng Wu[1], Anne M. Wilson[3*], Miles A. Pufall[1*]

[1]Department of Biochemistry, Carver College of Medicine, University of Iowa, Iowa City, IA; [2]Stead Family Department of Pediatrics, Carver College of Medicine, University of Iowa, Iowa City, IA; [3]Department of Chemistry, Butler University, Indianapolis, IN; [4]Grand View University, Des Moines, IA.

Supplemental Table 1: Docking energy for each ligand into 3BQD

Ligand	ΔG of docking (kcal/mol)
Pred	-9.4
Dex	-10.1
DAC	-13.6
D1'A	-10.5
D1'H	-8.4
D1'P	-10.7
D1'PF	-9.6
D1'T	-8.3
D2'A	-11.7
D2'H	-11.1
D2'P	-12.4
D2'PF	-12.2

Compound	P value vs. dexamethasone
Prednisone	<0.0001
D2'P	<0.0001
D2'PF	<0.0001
D2'H	0.0009
D2'A	0.0212
D1'P	<0.0001
D1'PF	<0.0001
D1'T	0.9450
D1'A	0.9023
D1'H	N/A

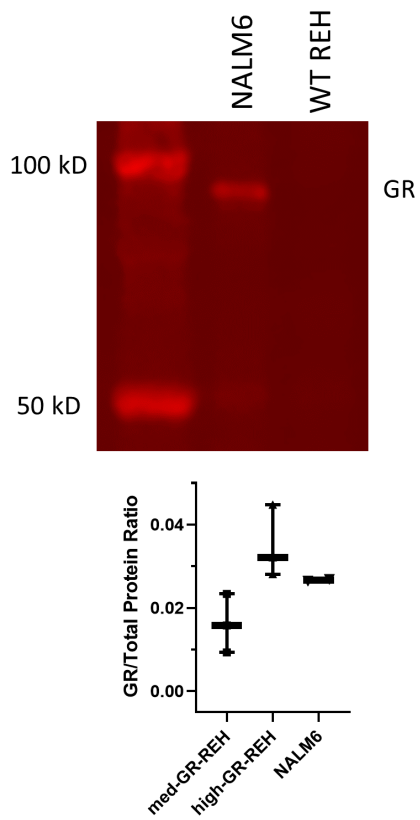
Supplemental Table 2. P values for EC50 of each DAC-like compound compared to EC50 of dexamethasone by one-way ANOVA (Brown-Forsythe and Welch) with Dunnett T3 multiple comparisons test. EC50 of D1'H cannot be determined.

Compound	P value vs. dexamethasone
Prednisone	0.9989
D2'P	>0.9999
D2'PF	0.9895
D2'H	0.9297
D2'A	<0.0001
D1'P	>0.9999
D1'PF	<0.0001
D1'T	0.4840
D1’H	0.8936
D1’A	N/A

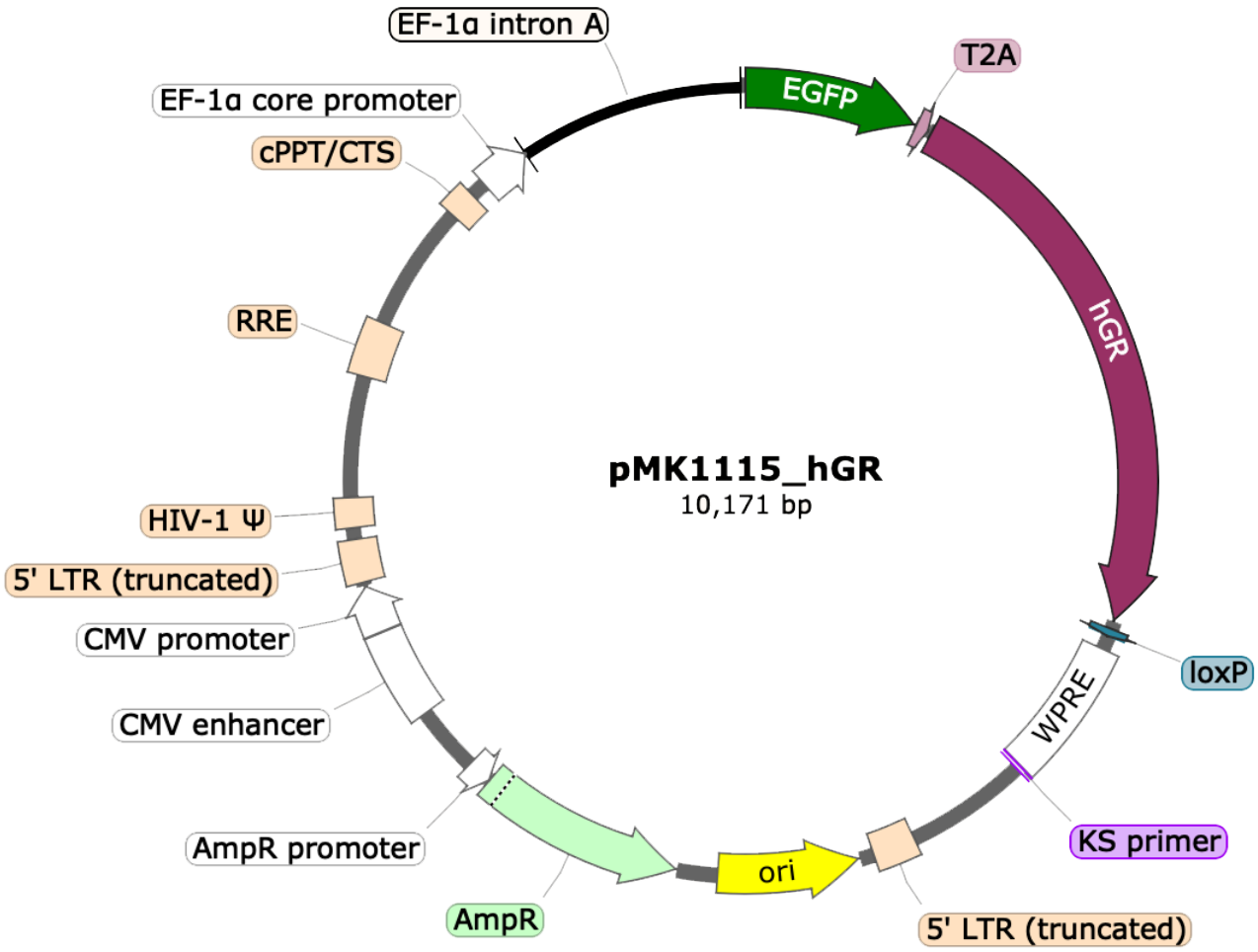
Supplemental Table 3. P values for $t_{1/2}$ of pred and each DAC-like compound compared to dexamethasone by one-way ANOVA (Brown-Forsythe and Welch) with Dunnett T3 multiple comparisons test. D1’A did not demonstrate significant translocation so is not included.

Compound	P value vs. dexamethasone
Prednisone	0.7872
D2'P	>0.9999
D2'PF	>0.9999
D2'H	0.9996
D2'A	0.1983
D1'P	>0.9999
D1'PF	<0.0001
D1'T	<0.0001
D1'H	<0.0001
D1'A	N/A

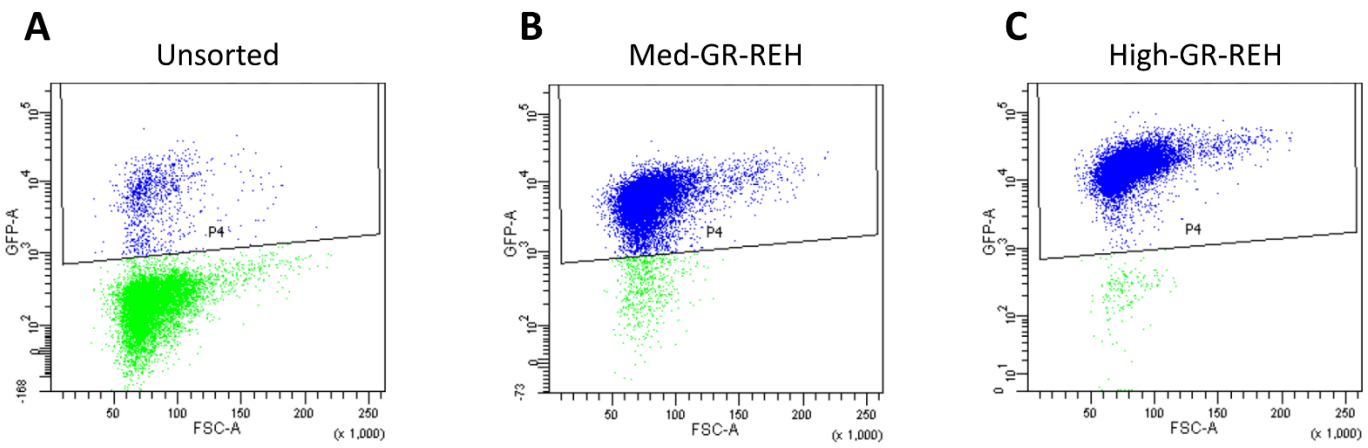
Supplemental Table 4. P values for effective K_D (K_{Deff}) of pred and each DAC-like compound compared to dexamethasone by one-way ANOVA (Brown-Forsythe and Welch) with Dunnett T3 multiple comparisons test. K_{Deff} of D1'A cannot be determined.



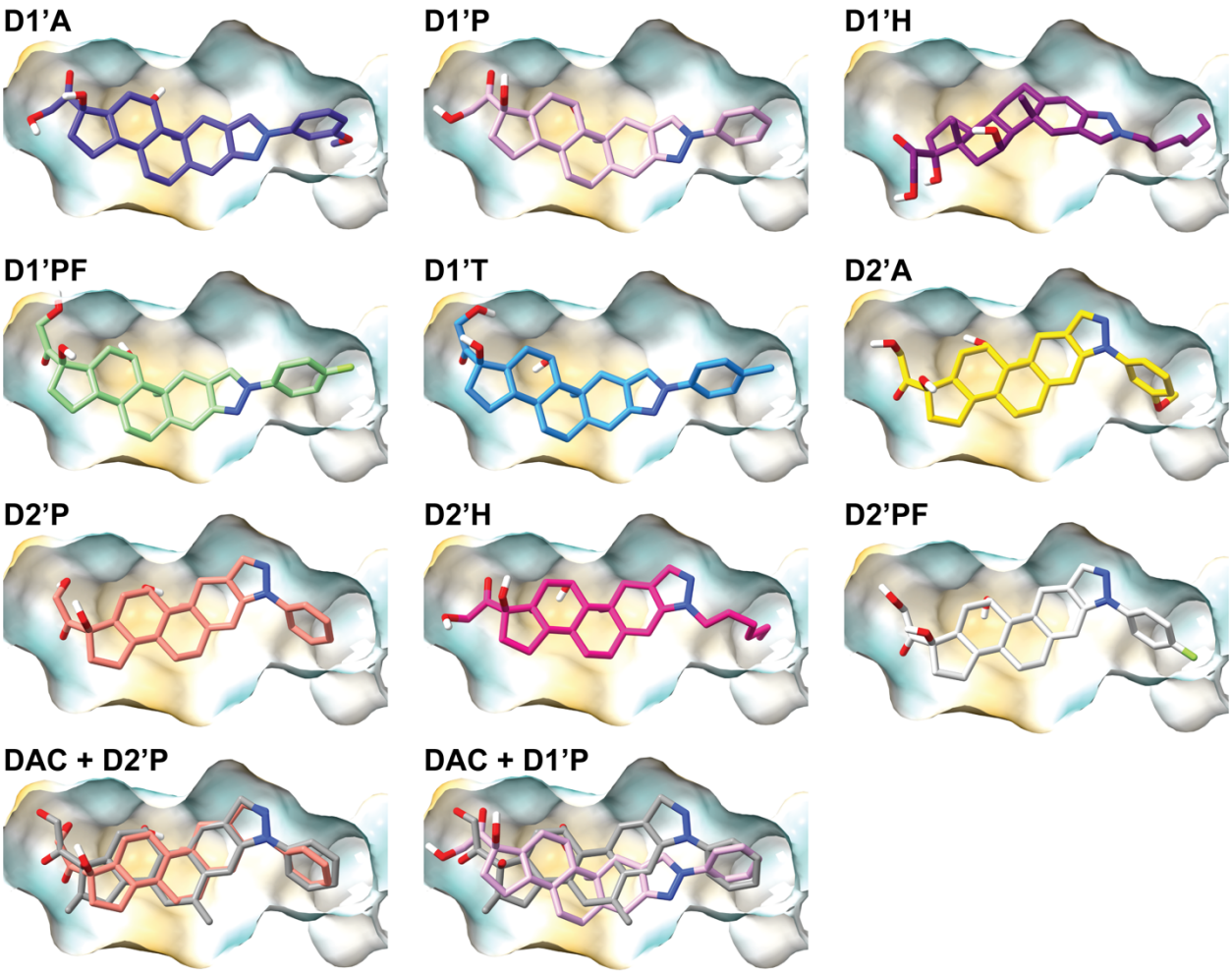
Supplemental Figure 1. REH cell do not express GR protein. (**A**) Western blot for GR in NALM6 cells and REH cells. **B**) Ratio of GR to total protein of infected REH cells compared to NALM6. High-GR-REH and NALM6 cells express a similar level of GR protein.



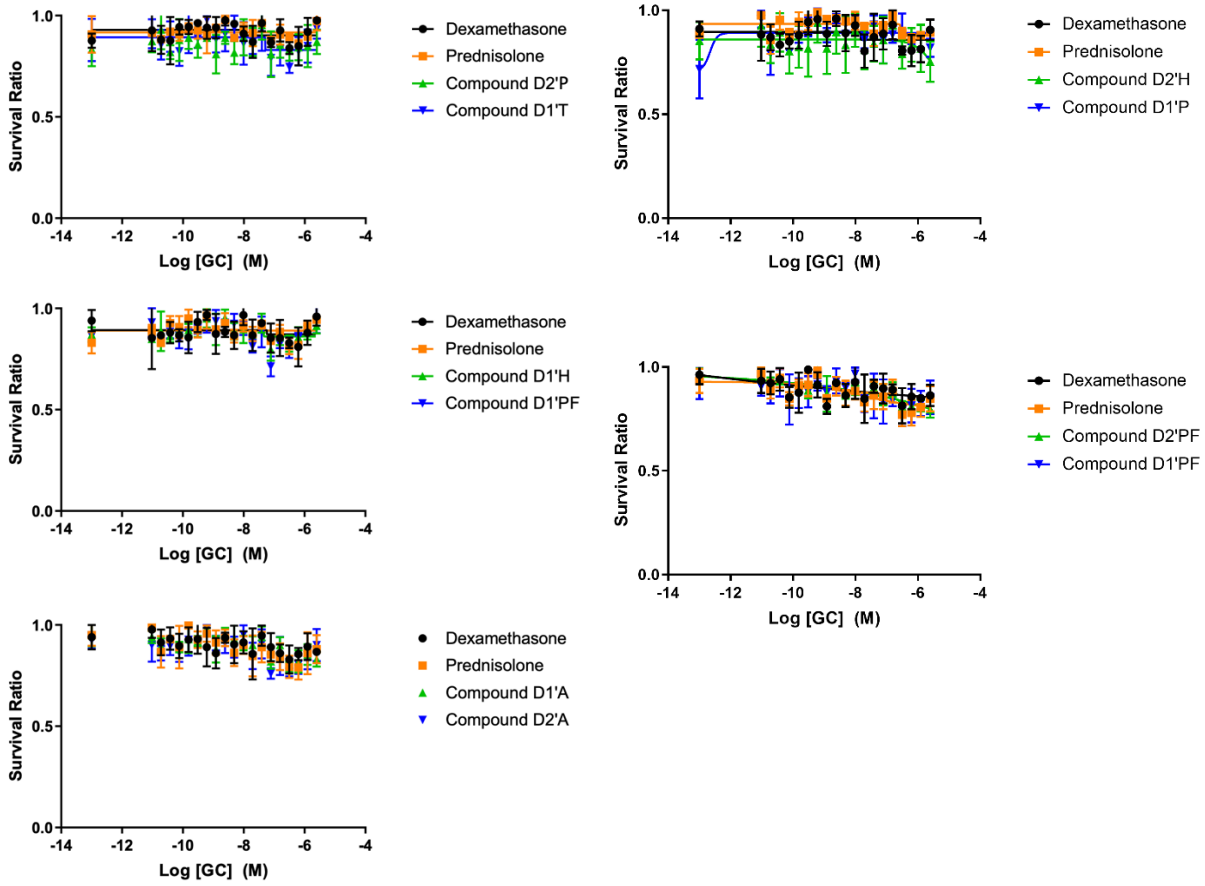
Supplemental Figure 2. Lentiviral vector pMK1115-GFP-GR. GFP and GR are transcribed from the same promoter, but translated as separate proteins because of the T2A site.



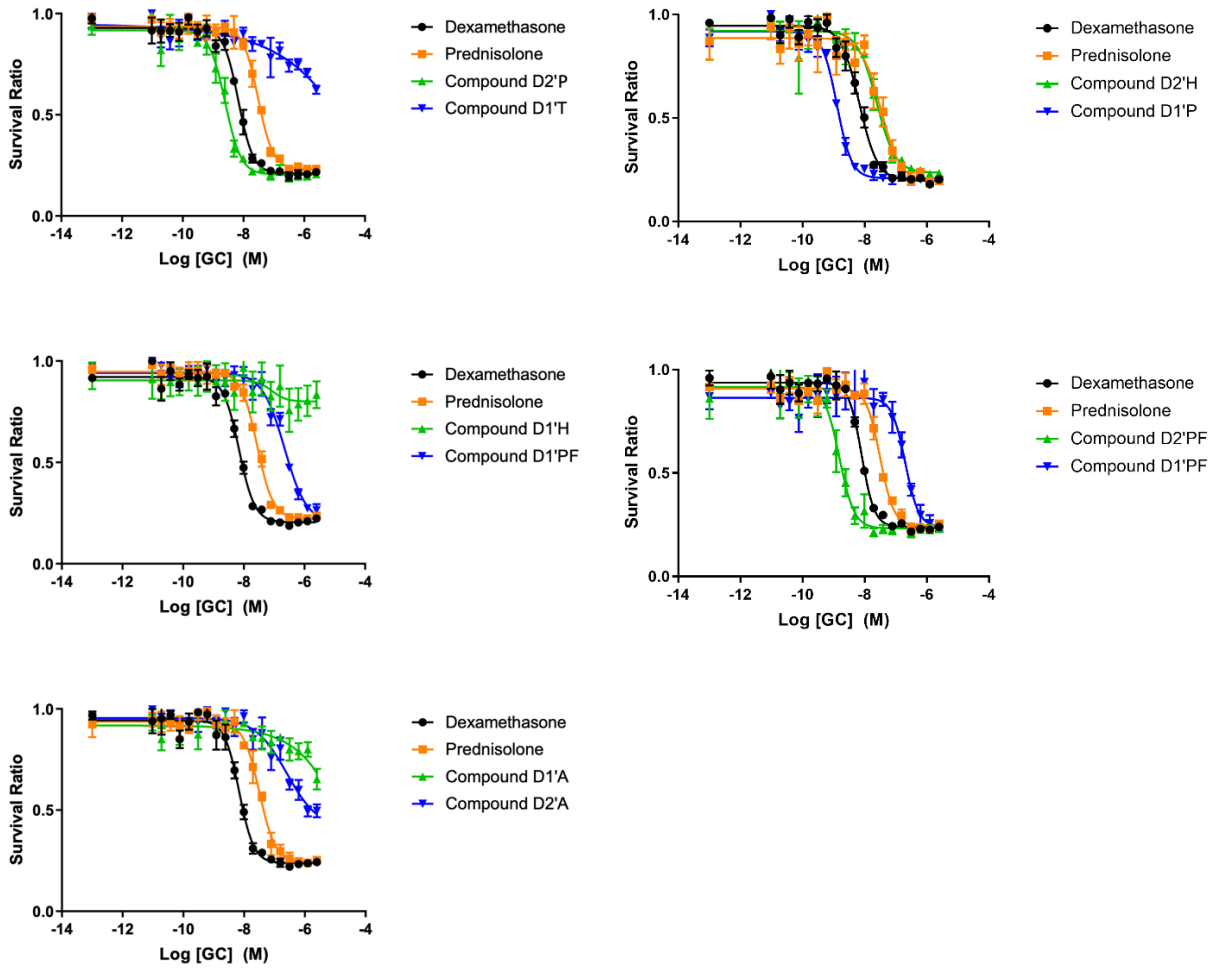
Supplemental Figure 3. GFP-GR-REH cells sorted into medium and high GR expression by GFP intensity. FACS sorting of REH cells infected with pMK1115-GR-GFP lentiviral vector either unsorted (**A**) or after sorting by medium (**B**) or high (**C**) GFP expression.



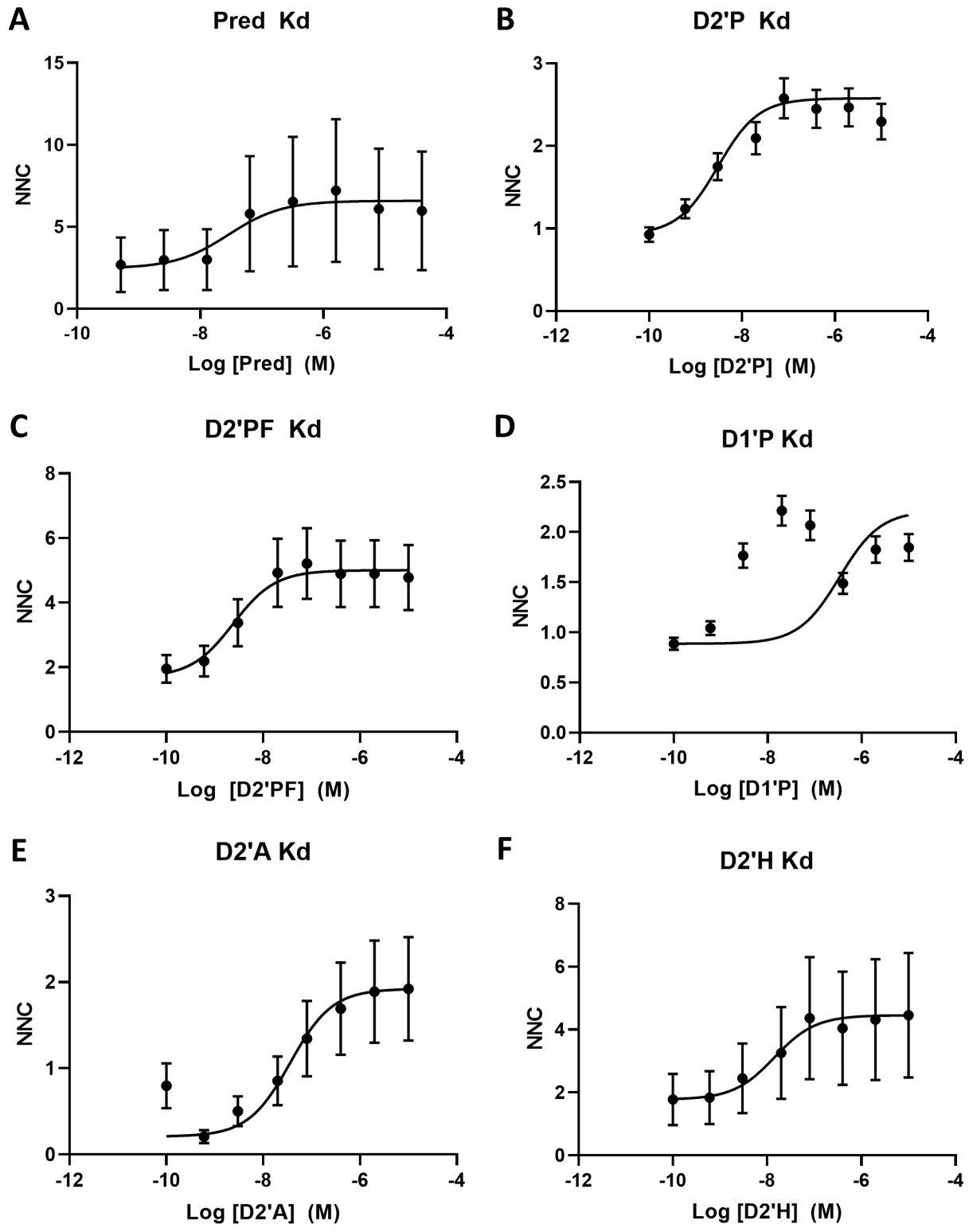
Supplemental Figure 4. Ligands docked into the binding pocket of the GR ligand binding domain. Each panel is a cutaway of the lowest energy docking position for each ligand synthesize. Docking was performed using AutoDock Vina (See Methods). Overlays of D2'P and DAC as well as D1'P and DAC are presented to highlight the overlap with the D2' compounds and the displacement of the D1' compounds in the pocket.

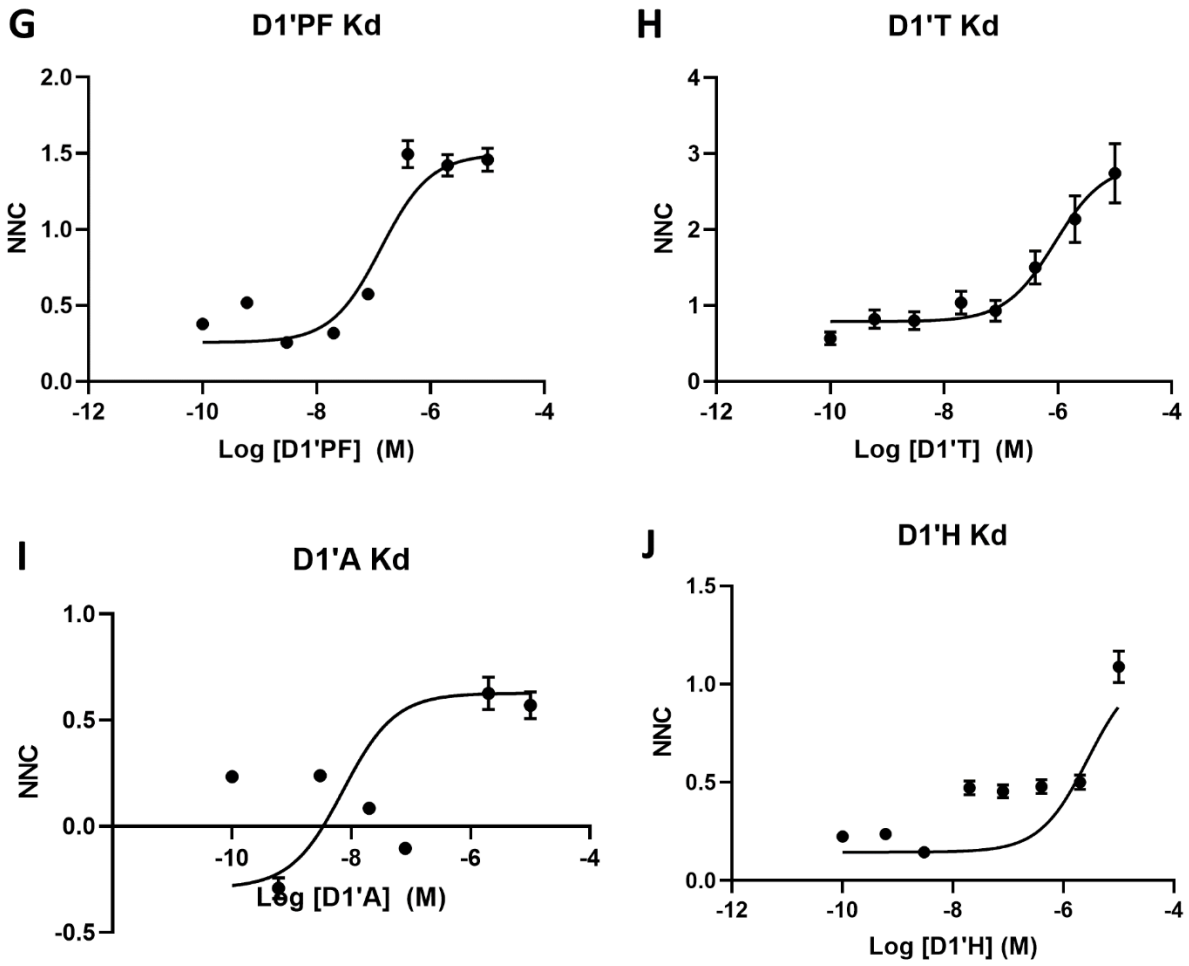


Supplemental Figure 5. GCs do not induce cell death in REH cells in the absence of GR. Death curves of REH cells infected with pMK1115 empty vector and treated with DAC derivatives. In all panels, dexamethasone treatment is shown in black and prednisone treatment is shown in orange.

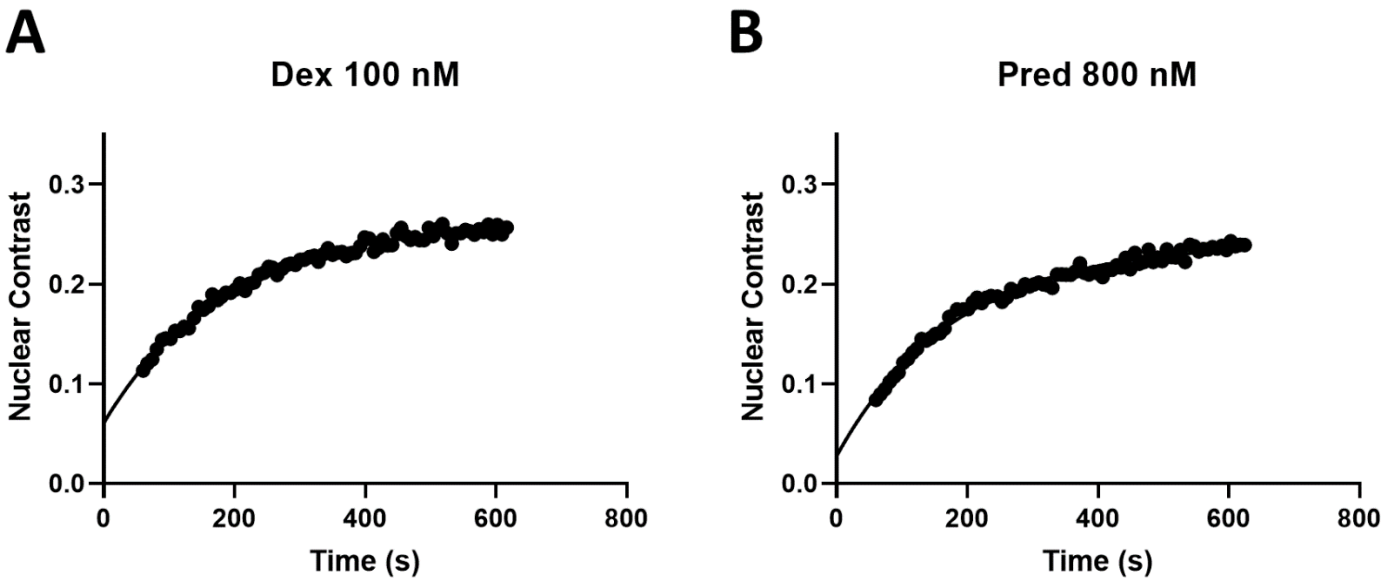


Supplemental Figure 6. 1'- and 2'- pyrazole substituted DAC-like compounds induce cell death in REH cells expressing GR. Death curves of REH cells infected with pMK1115-GR-GFP non-fusion and treated with DAC derivatives. In all panels, dexamethasone treatment is shown in black and prednisone treatment is shown in orange.

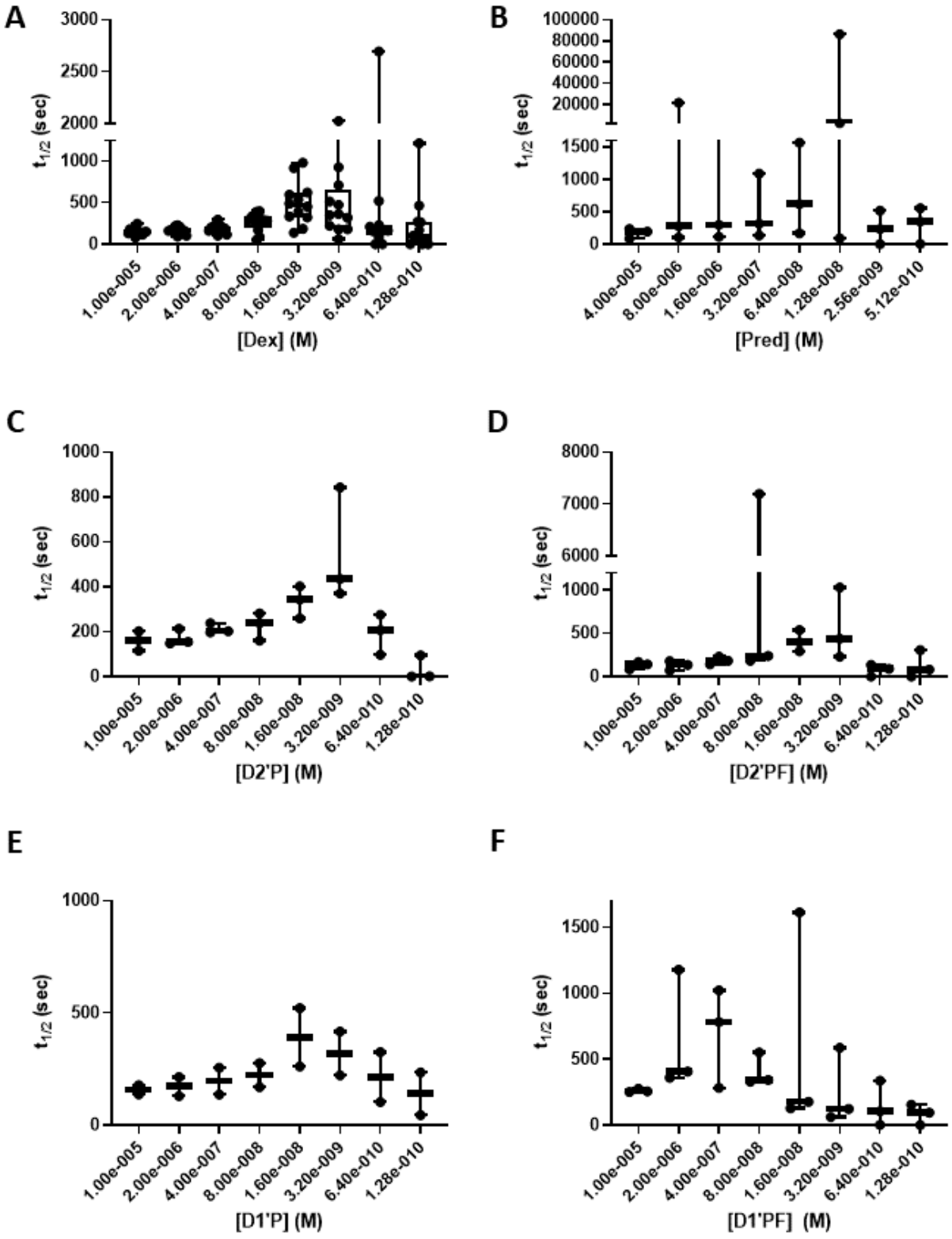

A
Pred Kd
NNC
Log [Pred] (M)
B
D2'P Kd
NNC
Log [D2'P] (M)
C
D2'PF Kd
NNC
Log [D2'PF] (M)
D
D1'P Kd
NNC
Log [D1'P] (M)
E
D2'A Kd
NNC
Log [D2'A] (M)
F
D2'H Kd
NNC
Log [D2'H] (M)

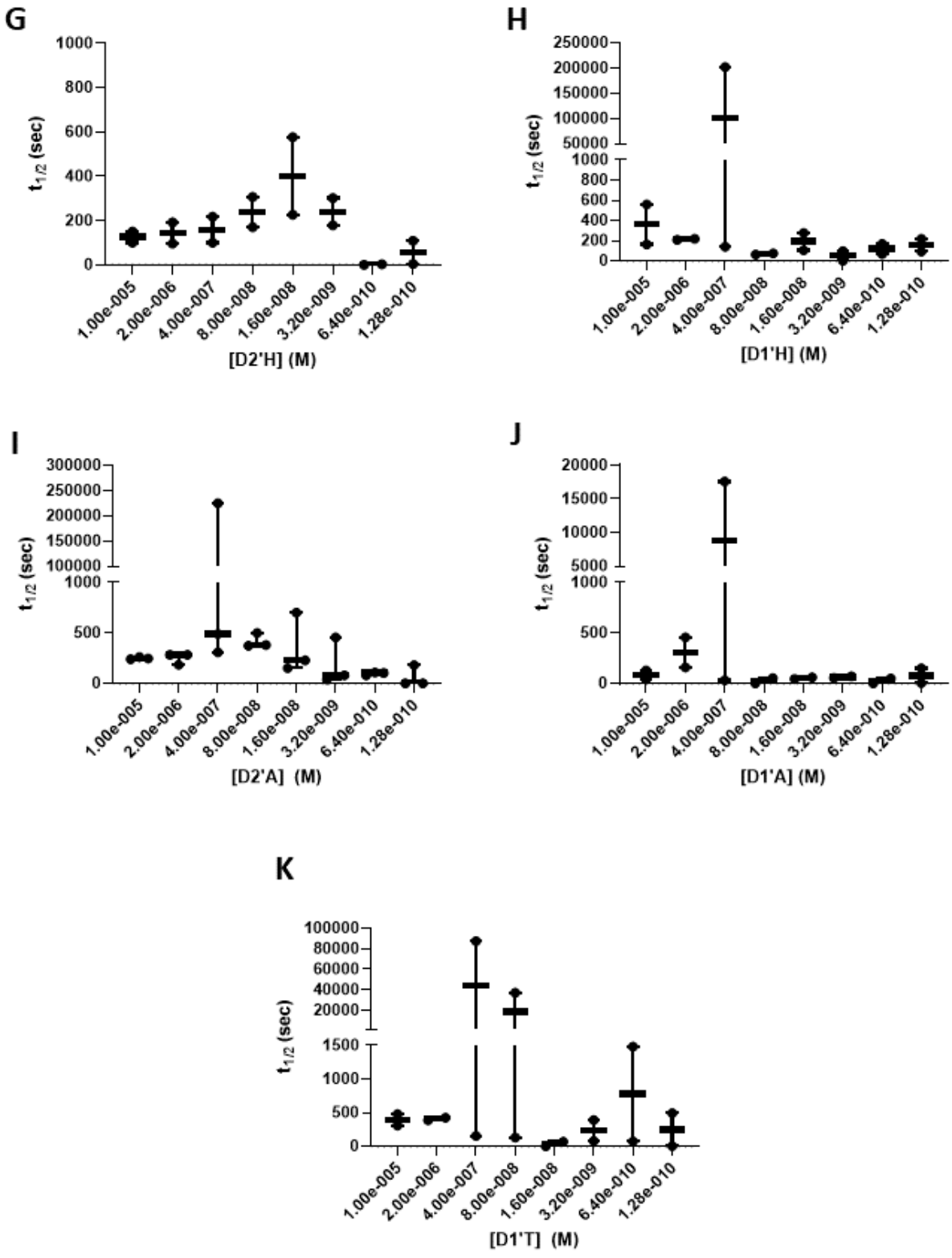


Supplemental Figure 7. The effective K_D of DAC-like ligands calculated by concentration at half translocation of GFP-GR. Representative curves to determine K_{Deff} of deacylcortivazol-like ligands, as shown with dexamethasone in **Figure 4C**. (**A**) prednisone, (**B-D**) ligands determined to be more potent than dexamethasone by EC50s in REH cells, (**E-H**) ligands less potent than dexamethasone by EC50s in REH cells, (**I**) D1'A, in which K_{Deff} could not be determined, and (**J**) D1'H, which was unable to determine EC50 in REH cells. NNC, normalized nuclear contrast.



Supplemental Figure 8. Dexamethasone and prednisolone induce the same rate of GR translocation. Nuclear accumulation of GFP-GR measured over time in the presence of dexamethasone (**A**) and prednisolone (**B**) measured by nuclear contrast. Measurements were taken every 7-8 seconds for ~10 minutes. $t_{1/2}$ was similar for both dexamethasone and prednisolone at these concentrations (dex = 146 +/- 22 seconds; pred = 142 +/- 12 seconds).


A
3000
2500
2000
1000
500
0
$t_{1/2}$ (sec)
1.00e-005
2.00e-006
4.00e-007
8.00e-008
1.60e-008
3.20e-009
6.40e-010
1.28e-010
[Dex] (M)
B
100000
80000
60000
40000
20000
1500
1000
500
0
$t_{1/2}$ (sec)
4.00e-005
8.00e-006
1.60e-006
3.20e-007
6.40e-008
1.28e-008
2.56e-009
5.12e-010
[Pred] (M)
C
1000
800
600
400
200
0
$t_{1/2}$ (sec)
1.00e-005
2.00e-006
4.00e-007
8.00e-008
1.60e-008
3.20e-009
6.40e-010
1.28e-010
[D2'P] (M)
D
8000
7000
6000
1000
500
0
$t_{1/2}$ (sec)
1.00e-005
2.00e-006
4.00e-007
8.00e-008
1.60e-008
3.20e-009
6.40e-010
1.28e-010
[D2'PF] (M)
E
1000
500
0
$t_{1/2}$ (sec)
1.00e-005
2.00e-006
4.00e-007
8.00e-008
1.60e-008
3.20e-009
6.40e-010
1.28e-010
[D1'P] (M)
F
1500
1000
500
0
$t_{1/2}$ (sec)
1.00e-005
2.00e-006
4.00e-007
8.00e-008
1.60e-008
3.20e-009
6.40e-010
1.28e-010
[D1'PF] (M)



Supplemental Figure 9. Half-times of dexamethasone, prednisone, and all deacylcortivazol-like ligands. Half-times ($t_{1/2}$) for dexamethasone (**A**), prednisone (**B**), D2'P

(**C**), D2'PF (**D**), D1'P (**E**), D1'PF (**F**), D2'H (**G**), D1'H (**H**), D2'A (**I**), D1'A (**J**), and D1'T (**K**) at all concentrations tested, demonstrating consistent half-times at the highest concentrations of each ligand regardless of potency. Half-times of dexamethasone at 2 μM and 400 nM were not significantly different from 10 μM dex by one-way ANOVA.