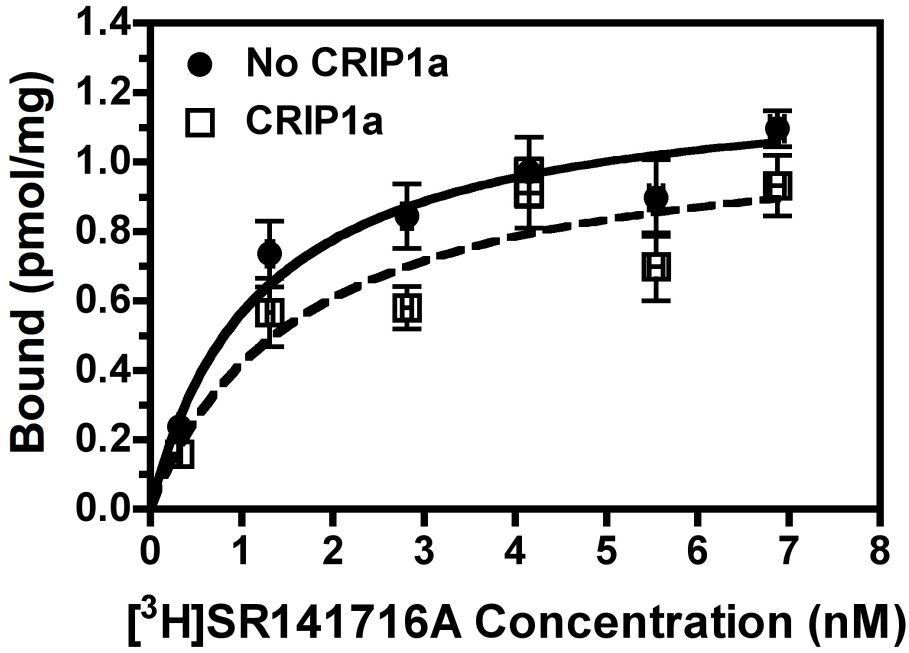
Smith, T.H., Blume, L.C., Straiker, A. David, B.G., McVoy, J.S., Cox, J.O., Sayers, K.W., Poklis, J.L., Abdullah, R.A., Egertová, M., Chen, C.K., Mackie, K. Elphick, M.R., Howlett, A.C. and Selley, D.E. Cannabinoid receptor interacting protein 1a ($CRIP_{1a}$) modulates CB_1 receptor signaling and regulation. *Molecular Pharmacology*.



Supplemental Figure 1. Effect of $CRIP_{1a}$ over-expression on [^{3}H]SR141716A saturation binding in CB_1-HEK cell membranes. Membranes from CB_1-HEK or CB_1-HEK-$CRIP_{1a}$ cells with were incubated as described in Methods with varying concentrations of [^{3}H]SR141716A. Data are mean % stimulation ± SEM (n = 8).

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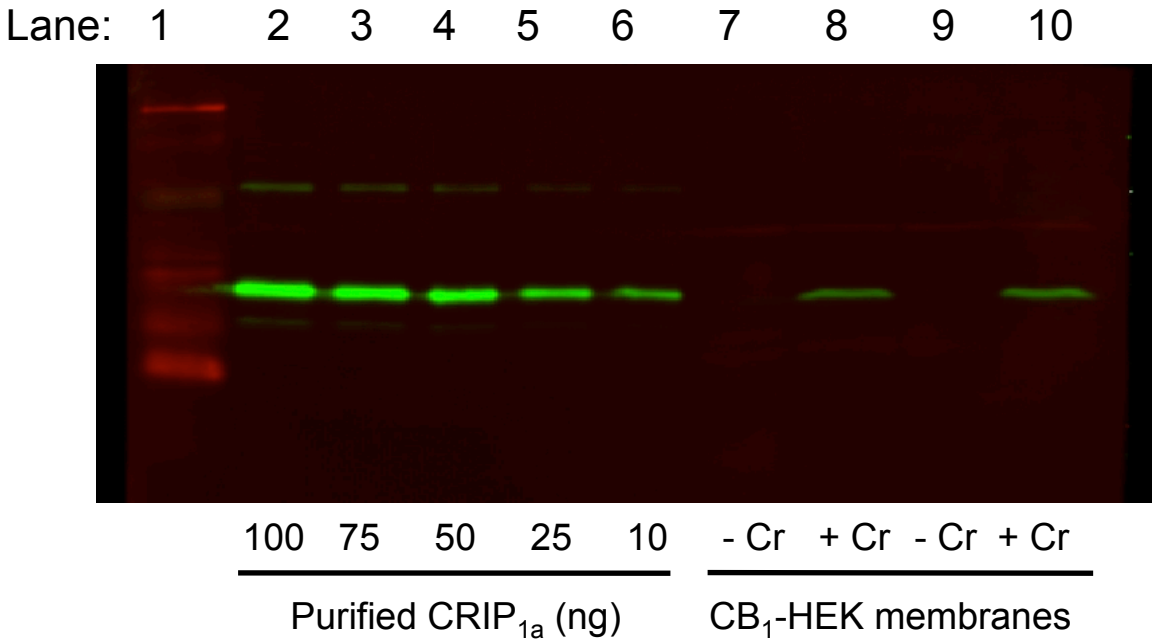

Supplemental Figure 2. Representative quantitative immunoblot of $CRIP_{1a}$ protein. Lane 1: molecular weight marker. Lanes 2 - 6: purified $CRIP_{1a}$. Lanes 7 and 8: membranes from CB_1-HEK cells without transfected $CRIP_{1a}$ (- Cr). Lanes 8 and 10: membranes from CB_1-HEK cells with stably transfected $CRIP_{1a}$ (+ Cr).

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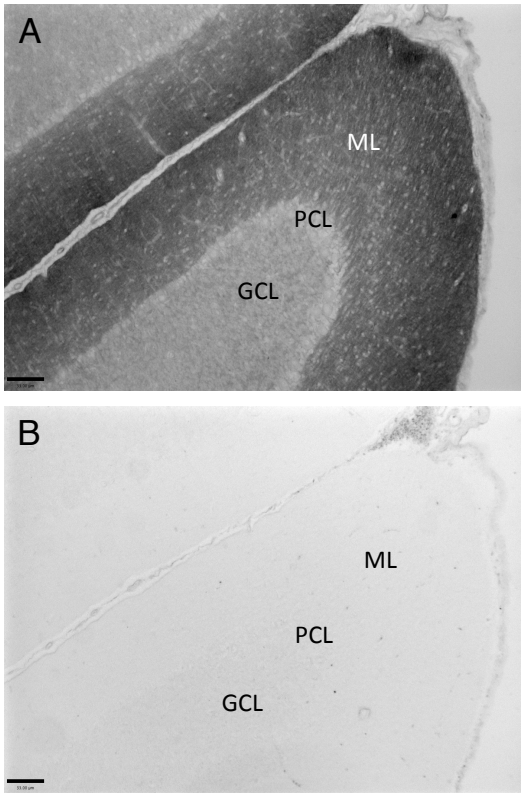

Supplemental Figure 3. $CRIP_{1a}$-immunoreactivity in the rat cerebellar cortex is abolished by pre-absorption of 077.2 $CRIP_{1a}$ rabbit antiserum with antigen peptide. A. $CRIP_{1a}$ immunoreactivity in the molecular layer (ML) and granule cell layer (GCL) of the cerebellar cortex; the Purkinje cell layer (PCL) contains little or no immunostaining. B. $CRIP_{1a}$-immunostaining as seen in (A) is abolished by pre-absorption of the $CRIP_{1a}$ antiserum with 20 μM of the antigen peptide, KPNETRSLMWVNKESFL (C-terminal region of $CRIP_{1a}$). Scale bar = 33 μm.

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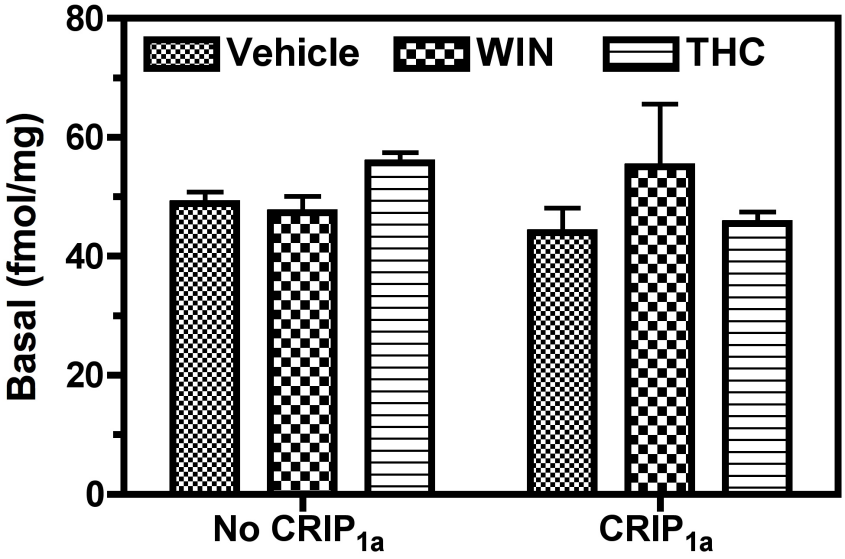

Supplemental Figure 4. Ligand pretreatment of CB_1-HEK and CB_1-HEK-$CRIP_{1a}$ cells does not alter basal [^{35}S]GTPγS binding. Cells were pretreated for 4 hr with 10 μM WIN55,212-2, 6 μM THC or vehicle. Membranes were incubated as described in Methods with 100 mM NaCl, 10 μM GDP, 0.1 nM [^{35}S] GTPγS. Data are mean [^{35}S]GTPγS binding (fmol/mg) ± SEM (n = 4).

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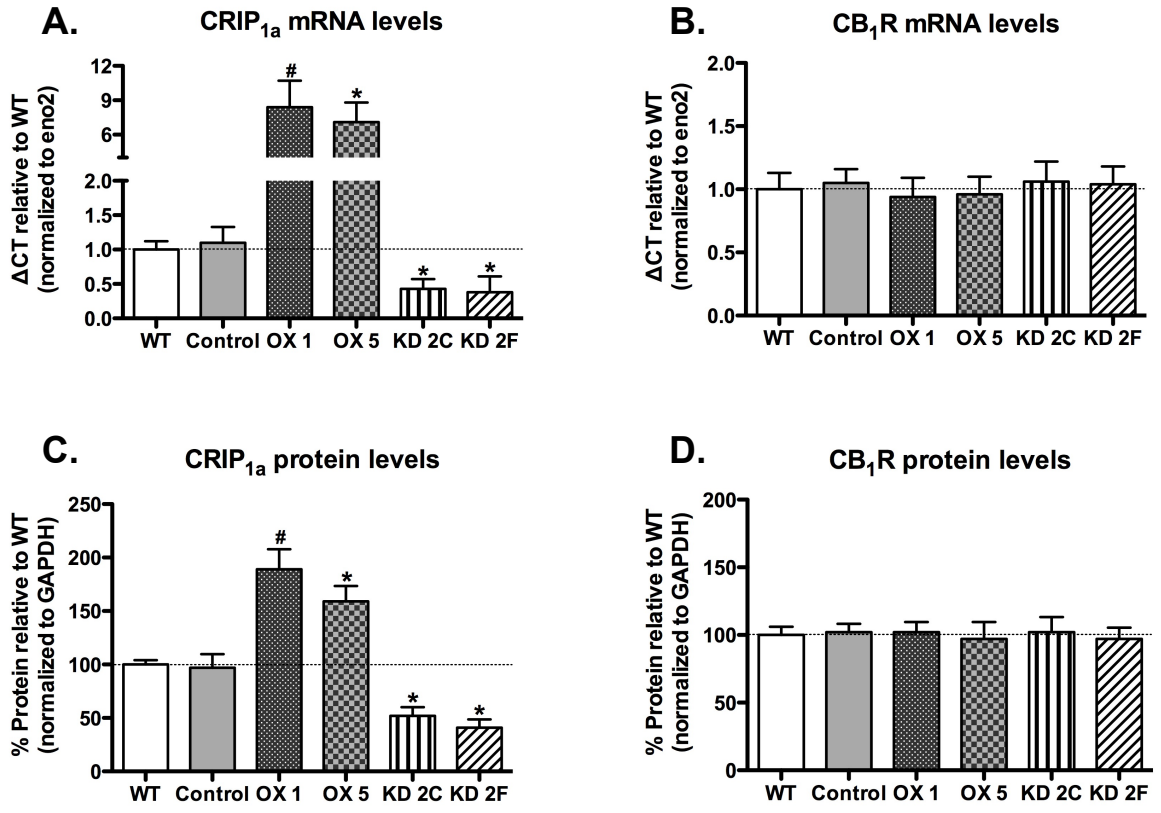

Supplemental Figure 5. Effects of stable $CRIP_{1a}$ over-expression and knockdown on $CRIP_{1a}$ and CB_1R mRNA and protein levels. ***A*** and ***B***, Quantification of $CRIP_{1a}$ (***A***) and CB_1R (***B***) transcript levels in N18TG2 WT (WT), N18TG2 empty vector (Control), and stable $CRIP_{1a}$ over-expression and knockdown clones. qPCR data were normalized to eno2, and relative mRNA abundance was expressed as the difference in CT values, relative to WT (expressed as 1). ***C*** and ***D***, Western blot quantification of $CRIP_{1a}$ and CB_1R protein expression in WT, Control, and stable $CRIP_{1a}$ over-expression and knockdown clones. Immunoreactive band densities for $CRIP_{1a}$ (***C***) and CB_1R (***D***) were normalized to GAPDH and expressed relative to WT cells (represented as 100%). qPCR and Western blotting were performed as described in material and methods. qPCR and Western blot data were calculated from three independent experiments, and are expressed as the mean ± S.E.M. $^{*}p<0.05$, $^{\#}p<0.01$ indicates significantly different from WT cells using Student's *t*-test.

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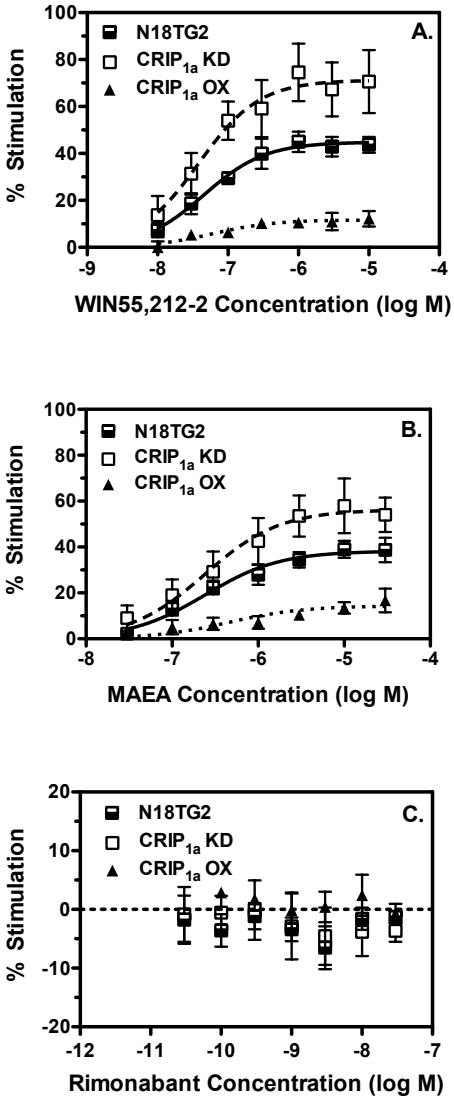

Supplemental Figure 6. Effect of $CRIP_{1a}$ knockdown and over-expression on concentration-effect curves of ligand-modulated [^{35}S]GTPγS binding in N18TG2 neuroblastoma cell membranes. Membranes from wild-type N18TG2 cells or N18TG2 cells with siRNA-mediated knockdown ($CRIP_{1a}$-KD; clone 2F) or overexpression ($CRIP_{1a}$-OX; clone 5) of $CRIP_{1a}$ were incubated as described in Methods with 100 mM NaCl, 20 μM GDP, 0.1 nM [^{35}S] GTPγS and varying concentrations of WIN55,212-2 (WIN), methanandamide (MAEA) or rimonabant (RIM). Data are mean % stimulation ± SEM (n = 4-9).

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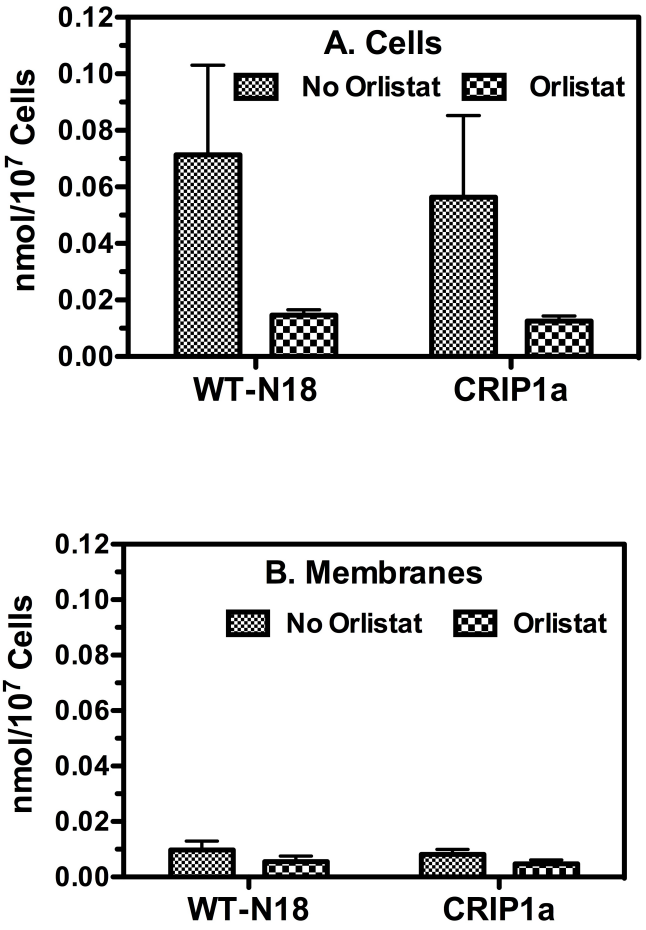

Supplemental Figure 7. $CRIP_{1a}$ over-expression does not affect orlistat-sensitive 2-AG levels in extracts from N18TG2 cells or membranes. N18TG2 cells with and without stable co-transfection of $CRIP_{1a}$ were incubated for 2 hr in 1 μM tetrahydrolipstatin (orlistat). Endocannabinoids were extracted from A) intact cells or B) membrane homogenates (as described in Methods). Data are mean nmol/10^7 cells ± SEM (n = 5). No CRIP, no transfection of $CRIP_{1a}$; $CRIP_{1a}$, stable transfection of $CRIP_{1a.}$