Supplemental Information

A New DREADD Facilitates the Multiplexed Chemogenetic Interrogation of Behavior

Inventory of Supplemental Information

1- Supplementary Figure S1 and legend;.

2- Supplementary Figure S2 and legend;

3- Supplementary Figure S3 and legend;

4- Supplementary Figure S4 and legend;

5- Supplementary Table S1 and legend;

6- Supplementary Table S2 and legend;

7- References for Supplementary Table S1

Supplemental Figures and Tables

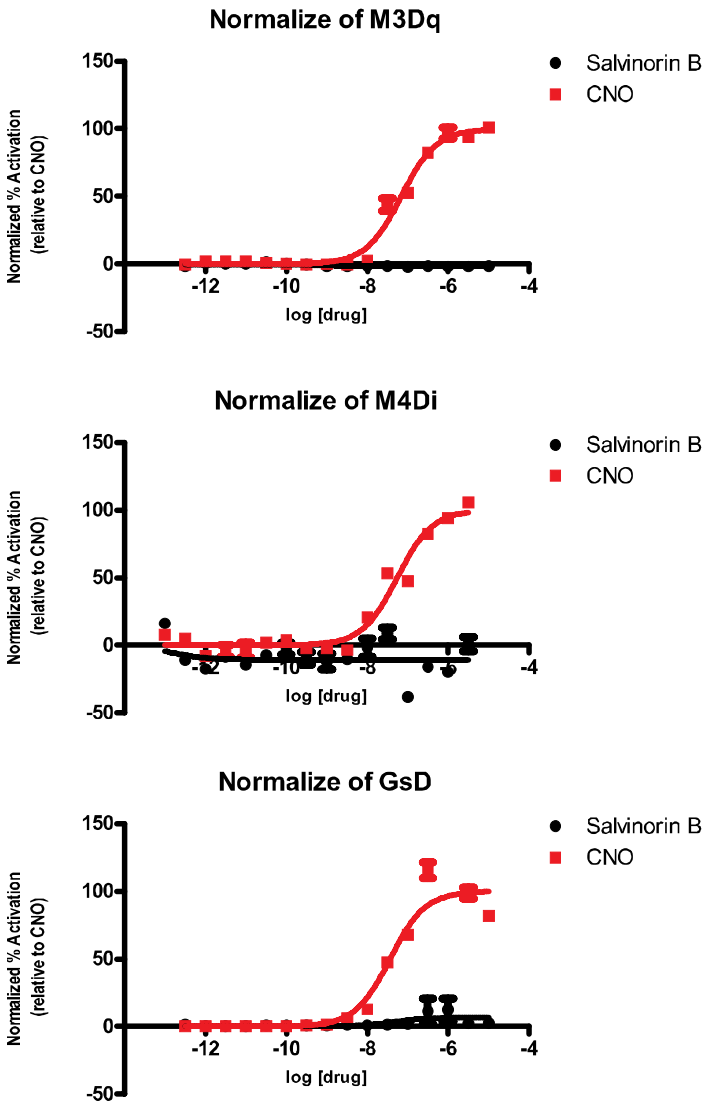

Figure S1A: Salvinorin B is inactive at muscarinic DREADDs, Related to Figure 1. For these studies, concentration response curves were performed with CNO or Salvinorin B at hM3Dq, hM4Di and GsD. Curves represent best-fits as defined by GraphPad Prizm.

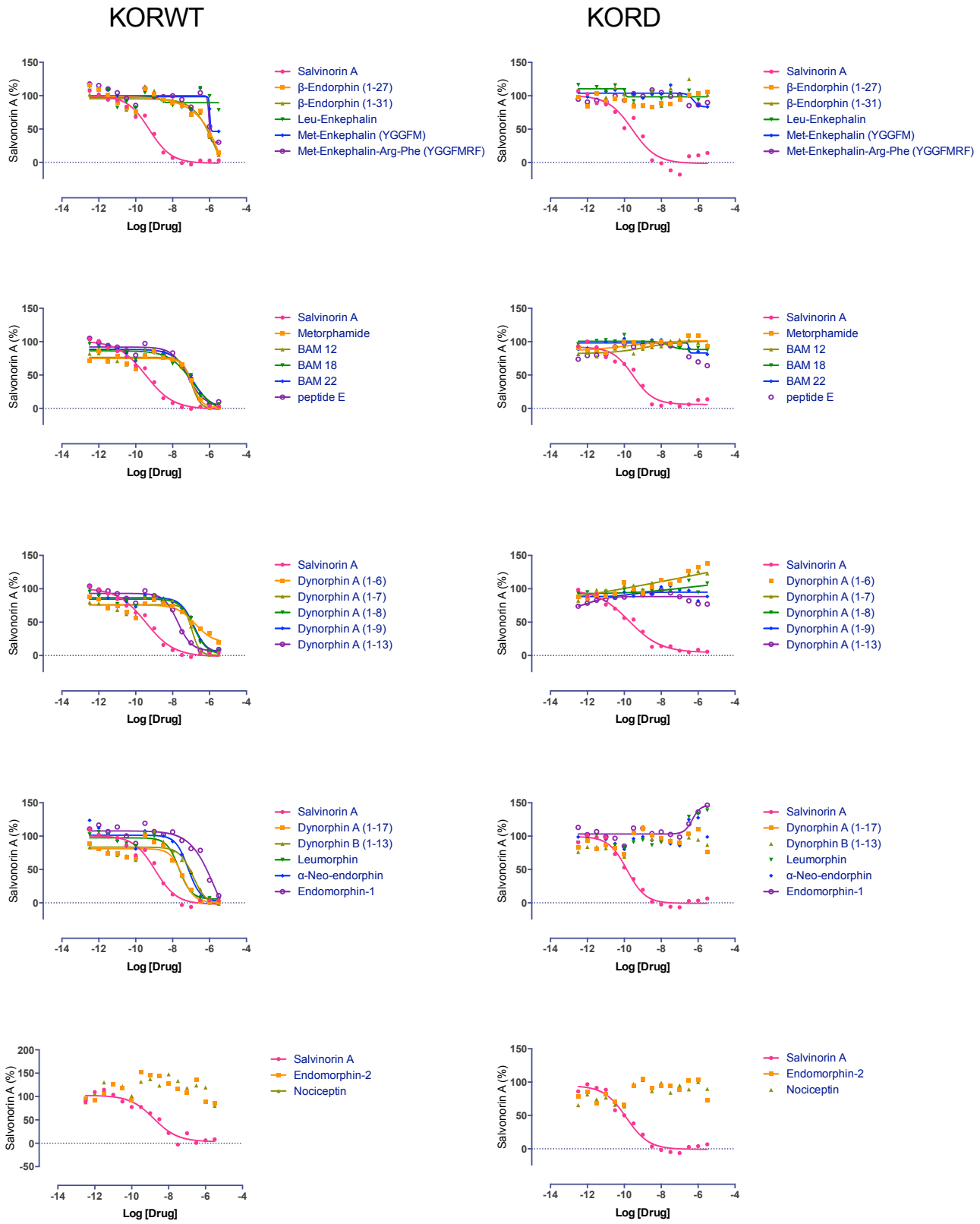

Figure S1B: Opioid peptides are inactive at KORD, Related to Figure 1 and Table 1. For these studies concentration-response studies were performed at WT and KORD. Data represent mean of three separate experiments; EC_{50} and E_{MAX} values for these experiments are in Table 1.

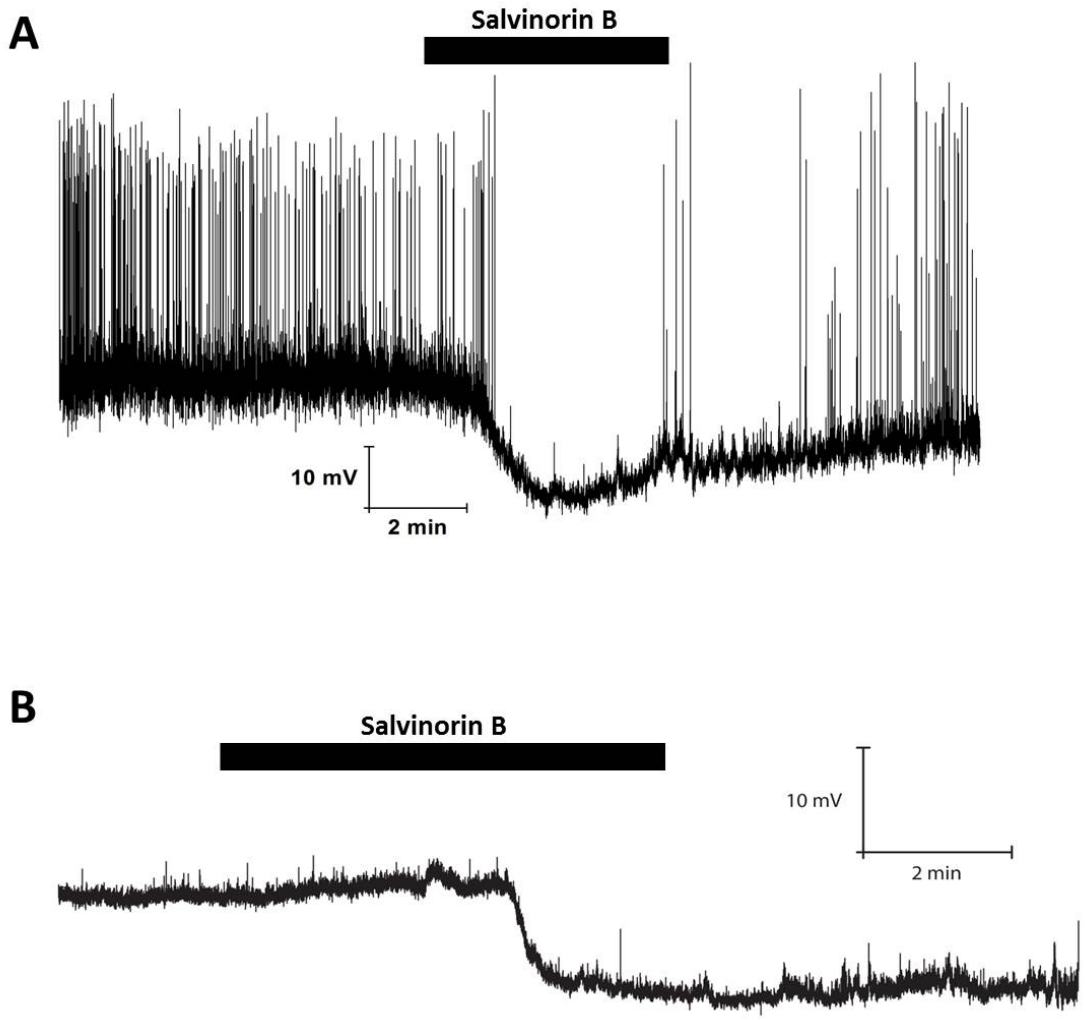

Figure S2: Effects of Salvinorin B-activation of KOR DREADD on PVH^{SIM1} and ARC^{AgRP} neurons, Related to Figure 3. Representative traces of resting membrane potential shift in sated animals following bath application of Salvinorin B. SALB hyperpolarized both (A) PVH^{SIM1} and (B) ARC^{AgRP} neurons.

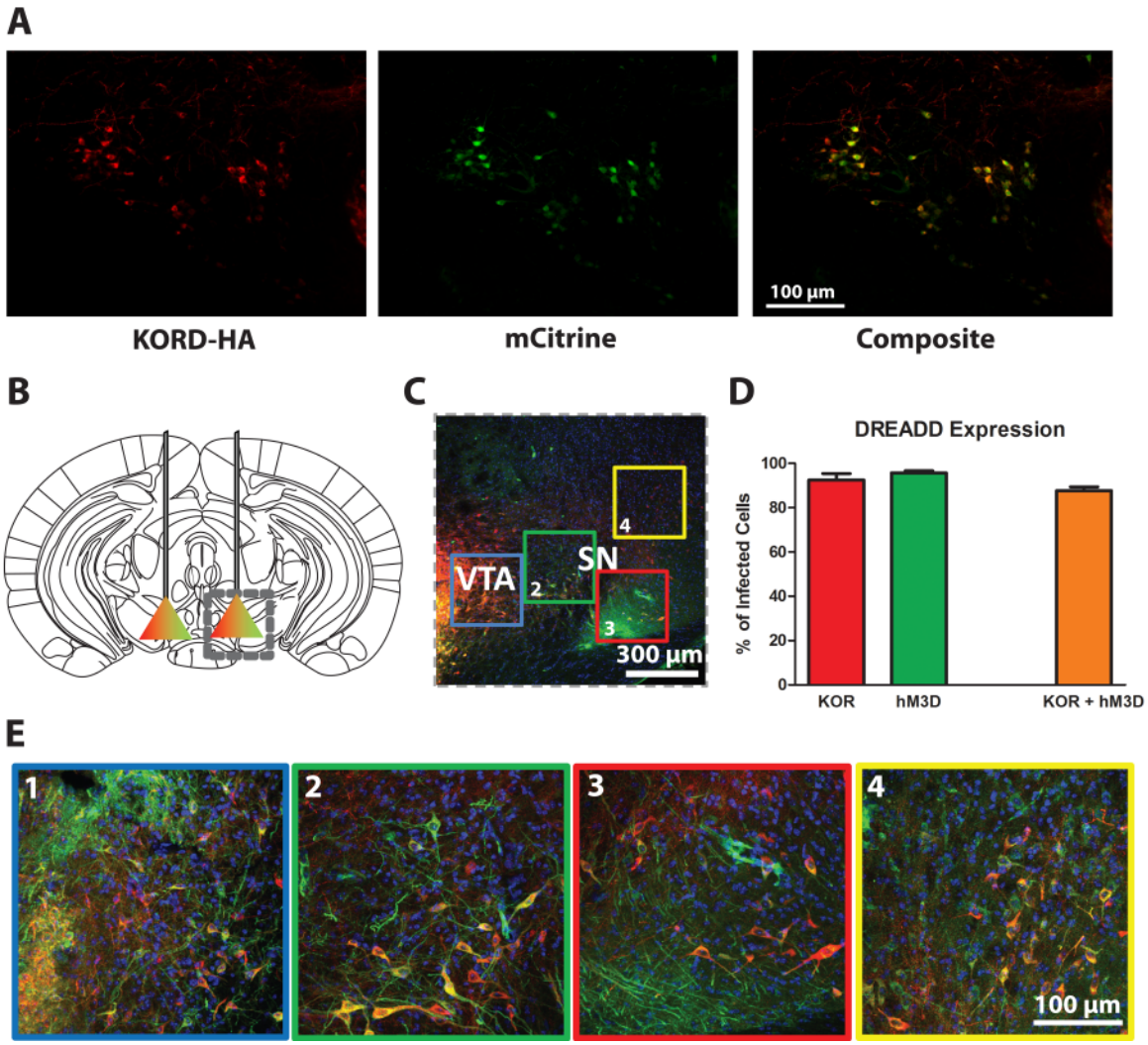

Figure S3: Co-expression of KOR and hM3D DREADD *in vivo*, Related to Figure 4

(A) 20x epiflourescent image of KOR-DREADD infected VTA/SN GABAergic neurons showing co-expression of the HA-tagged KOR DREADD and the cytosolic reporter mCitrine. (B)Localization of VTA/SN injections for multiplex DREADD experiments. Grey-box demarcates example image in panel C. (C) 10x confocal z-projection of VTA/SN as marked in panel B (Red: HA-tagged KOR DREADD. Green: mCherry-tagged hM3D DREADD. (D) Percentage of infect cells expressing HA-tagged KOR-DREADD (92.5 ± 2.9%), mCherry-tagged hM3D DREADD (95.7 ± 1.0%), or both (87.7 ± 1.7%). (E) 40x confocal z-stacks showing areas marked in panel C. Colored border and number refer to location taken from 10x area in panel C.

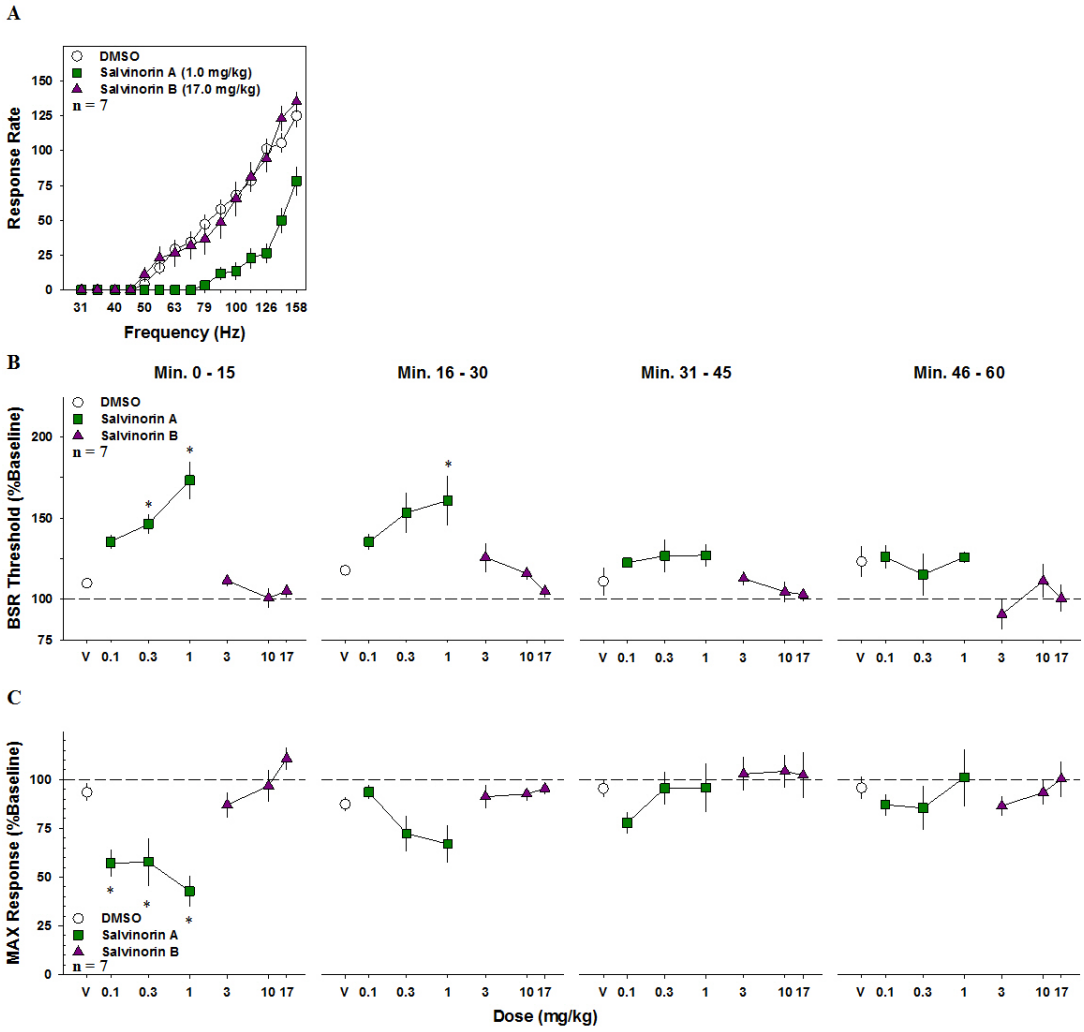

Figure S4. Effects of SALA (0.1 – 1.0 mg/kg, s.c.) and SALB (3.0 – 17.0 mg/kg s.c.) on intracranial self-stimulation of the medial forebrain bundle, Related to Figure 1

(A) Average rate frequency curve following DMSO (white circles), 17.0 mg/kg SALB (purple triangles), and 1.0 mg/kg SALA (green squares). B-C. Effects of SALA and SALB on BSR threshold (B) and maximum operant response rate (C) during 4 consecutive 15-minute post-injection response periods.

Table S1: Salvinorin B is inactive at PDSP tested targets, Related to Table 1. Salvinorin B was initially tested in quadruplicate at 10 μM final concentration using the following human cloned molecular targets except where noted (R=rat brain; L=rat liver; P=PC12 cells; see (Besnard et al., 2012). Where >50% inhibition was measured, K_i determinations were performed as detailed (Besnard et al., 2012).

Receptor	K_i (nM)	Receptor	K_i (nM)
5-HT1A	>10,000	δ-opioid	>10,000
5-HT1B	>10,000	GABA-A (R)	>10,000
5-HT1D	>10,000	H1-histamine	>10,000
5-HT1E	>10,000	H2-histamine	>10,000
5-HT2A	>10,000	H3-histamine	>10,000
5-HT2B	>10,000	H4-histamine	>10,000
5-HT2C	>10,000	Kainate (R)	>10,000
5-HT3	>10,000	M1-muscarinic	>10,000
5-HT5A	>10,000	M2-muscarinic	>10,000
5-HT6	>10,000	M3-muscarinic	>10,000
5-HT7	>10,000	M4-muscarinic	>10,000
α1A-adrenergic	>10,000	M5-muscarinic	>10,000
α1B-adrenergic	>10,000	μ-opioid	>10,000
α1D-adrenergic	>10,000	Norepinephrine transporter	>10,000
α2A-adrenergic	>10,000	NMDA (R)	>10,000
α2B-adrenergic	>10,000	Peripheral benzodiazepine (L)	>10,000
α2C-adrenergic	>10,000	Serotonin transporter	>10,000
AMPA (R)	>10,000	Sigma1 (R)	>10,000
β1-adrenergic	>10,000	Sigma2 (P)	>10,000
β2-adrenergic	>10,000		
β3-adrenergic	>10,000		
Benzodiazepine	>10,000		
Ca++ channel (R)	>10,000		
D1-dopamine	>10,000		
D2-dopamine	>10,000		
D3-dopamine	>10,000		
D4-dopamine	>10,000		
D5-dopamine	>10,000		
Dopamine Transporter	>10,000		

Table S2: KOR-DREADD DNA sequence, Related to Figure 1

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tcctgcttacctgagggacatcgatgggatgaataaaccagtatga

SUPPLEMENTAL REFERENCES

Besnard, J., Ruda, G.F., Setola, V., Abecassis, K., Rodriguiz, R.M., Huang, X.P., Norval, S., Sassano, M.F., Shin, A.I., Webster, L.A.*, et al.* (2012). Automated design of ligands to polypharmacological profiles. Nature *492*, 215-220.