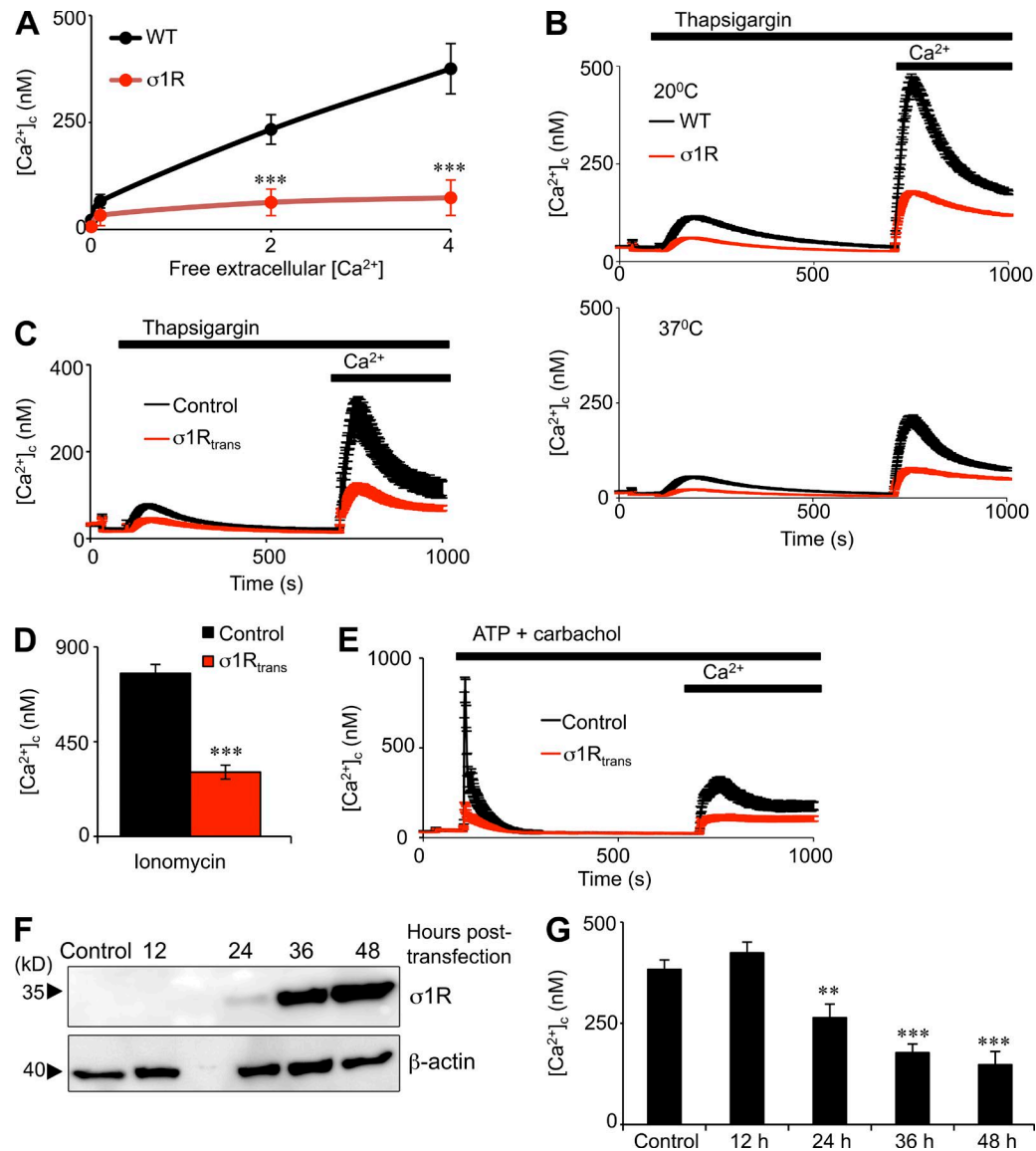
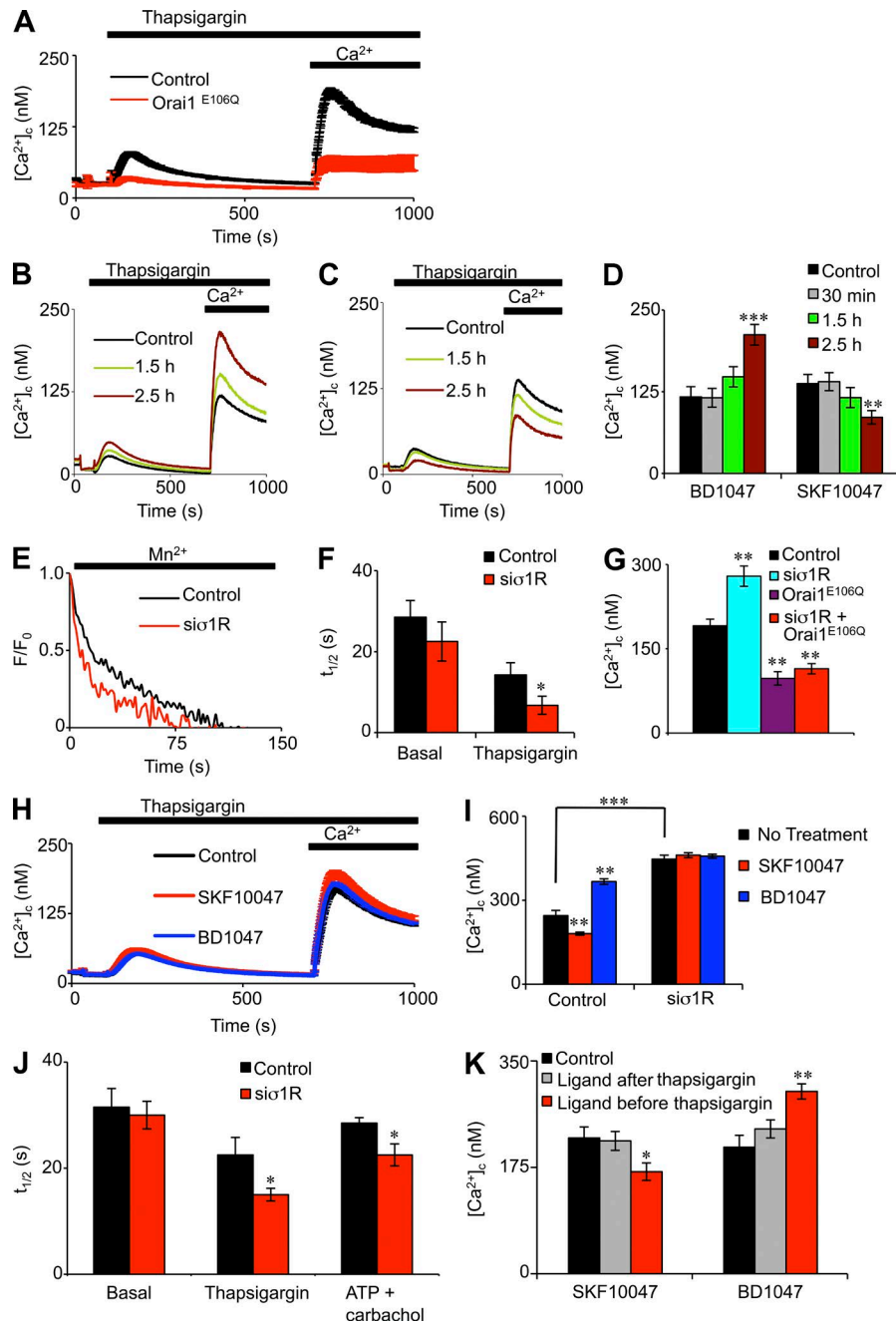


Figure S1. **Key features of  $\sigma 1R$ .**  $\sigma 1R$  comprises 223 residues. It is expressed predominantly in ER membranes, where it adopts the topology shown with two transmembrane domains, a short cytosolic loop, and luminal N and C termini. The N terminus includes an ER retention signal. Residues important for ligand recognition are shown by the green oval.  $\sigma 1Rs$  reside, along with some  $IP_3Rs$ , within MAMs, regions of close contact between the ER and mitochondria that allow  $Ca^{2+}$  and lipid exchanges between them (Hayashi and Su, 2004, 2007).  $IP_3Rs$  associated with  $\sigma 1Rs$  in MAMs are protected from degradation and can thereby deliver sustained physiological  $Ca^{2+}$  signals to mitochondria (Hayashi and Su, 2007). Within MAMs,  $\sigma 1Rs$  also associate with the luminal  $Ca^{2+}$ -binding protein and chaperone, BiP. The interaction between  $Ca^{2+}$ -BiP and  $\sigma 1R$  is disrupted by loss of  $Ca^{2+}$  from the ER or by  $\sigma 1R$  agonists. This frees  $\sigma 1Rs$  to move within ER membranes and establish contacts with different membranes, notably the PM (Hayashi and Su, 2007). The present study demonstrates that STIM1 can then deliver  $\sigma 1Rs$  to specific PM domains.



**Figure S2.  $\sigma$ 1Rs inhibit SOCE-mediated  $\text{Ca}^{2+}$  signals.** (A) Experiments similar to those shown in Fig. 1 D were used to measure thapsigargin-evoked SOCE after restoration of different extracellular  $\text{Ca}^{2+}$  concentrations to wild-type (WT) HEK or HEK- $\sigma$ 1R cells. Results show peak increases in  $[\text{Ca}^{2+}]_i$  after restoration of extracellular  $\text{Ca}^{2+}$  (concentrations refer to the free  $[\text{Ca}^{2+}]$ ; mean  $\pm$  SEM;  $n = 3$  with six replicates in each experiment). (B)  $\text{Ca}^{2+}$  signals evoked by thapsigargin and then restoration of extracellular  $\text{Ca}^{2+}$  at 20°C or 37°C. Results show mean  $\pm$  SD from six replicates. (C) Populations of fluo 4-loaded HEK cells transiently expressing  $\sigma$ 1R-V5 ( $\sigma$ 1R<sub>trans</sub>) or mock transfected (control) were stimulated with 5  $\mu\text{M}$  thapsigargin in  $\text{Ca}^{2+}$ -free HBS before addition of 4 mM extracellular  $\text{Ca}^{2+}$ . Results show the mean  $\pm$  SD of six replicates. (D) Experiments similar to those shown in Fig. 1 (G and H) were used to assess the size of the intracellular  $\text{Ca}^{2+}$  stores in HEK cells transiently expressing  $\sigma$ 1R-V5. 5  $\mu\text{M}$  ionomycin was added to cells in  $\text{Ca}^{2+}$ -free HBS, and the peak increases in  $[\text{Ca}^{2+}]_i$  were recorded (mean  $\pm$  SD from six replicates). (E) Populations of fluo 4-loaded HEK- $\sigma$ 1R<sub>trans</sub> or mock-transfected (control) cells were stimulated with 100  $\mu\text{M}$  ATP and 100  $\mu\text{M}$  carbachol in  $\text{Ca}^{2+}$ -free HBS before addition of 4 mM extracellular  $\text{Ca}^{2+}$ . Results show mean of six replicates. (F) Immunoblot showing  $\sigma$ 1R expression at the indicated times after transfection. (G) Peak increases in  $[\text{Ca}^{2+}]_i$  evoked by SOCE recorded at the indicated times after transient transfection of HEK cells with  $\sigma$ 1R. Results show mean  $\pm$  SD from six replicates. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ , relative to control (Student's  $t$  test in A and D and ANOVA followed by Tukey's posthoc analysis in G). The results show that transient expression of  $\sigma$ 1Rs has similar effects to stable expression, namely inhibition of SOCE and a reduction in the  $\text{Ca}^{2+}$  content of the intracellular stores.



**Figure S3. Time course of the effects of  $\sigma$ 1R ligands on SOCE.** (A) Populations of fluo 4-loaded CHO cells transiently transfected with Orai1<sup>E106Q</sup> or mock transfected were stimulated with 5  $\mu$ M thapsigargin in  $\text{Ca}^{2+}$ -free HBS before restoration of 4 mM extracellular  $\text{Ca}^{2+}$ . Results show mean  $\pm$  SD of six replicates. (B and C) HEK- $\sigma$ 1R cells were pretreated with 10  $\mu$ M BD1047 (B) or 25  $\mu$ M (+)SKF10047 (C) for the indicated times, with the final 1.5 h at 20°C (during dye loading) and the preceding 1 h (for the 2.5-h incubation) at 37°C.  $\text{Ca}^{2+}$  signals evoked by thapsigargin in  $\text{Ca}^{2+}$ -free HBS and then restoration of extracellular  $\text{Ca}^{2+}$  were recorded after the preincubations. (D) Summary results show peak increases in  $[\text{Ca}^{2+}]_i$  evoked by SOCE. (E) Experiments similar to those shown in Fig. 2 C were used to measure quenching of fura 2 fluorescence in CHO cells treated with siRNA to  $\sigma$ 1R or with control plasmid. Fura 2-loaded cells were treated with thapsigargin (5  $\mu$ M for 10 min) in nominally  $\text{Ca}^{2+}$ -free HBS before addition of 5 mM  $\text{MnCl}_2$ . Results show normalized fluorescence intensity ( $F/F_0$ ) for six replicates. (F) Summary results show half-times ( $t_{1/2}$ ) for fluorescence quenching before (basal) and after thapsigargin treatment. The results demonstrate that loss of  $\sigma$ 1Rs in CHO cells increases unidirectional entry of  $\text{Mn}^{2+}$  through the SOCE pathway. (G) Summary results show peak increases in  $[\text{Ca}^{2+}]_i$  evoked by SOCE after thapsigargin treatment (1  $\mu$ M) in CHO cells transfected with si $\sigma$ 1R, Orai1<sup>E106Q</sup>, or both. (H) Typical traces show the effects of pretreatment with ligands of  $\sigma$ 1R (+)SKF10047, 25  $\mu$ M; BD1047, 10  $\mu$ M) on thapsigargin-evoked  $\text{Ca}^{2+}$  signals in HEK- $\sigma$ 1R cells treated with siRNA to  $\sigma$ 1R. Fluo 4-loaded cells were stimulated with 5  $\mu$ M thapsigargin in  $\text{Ca}^{2+}$ -free HBS before restoration of 4 mM extracellular  $\text{Ca}^{2+}$ . (I) Summary results show peak increases in  $[\text{Ca}^{2+}]_i$  evoked by 5  $\mu$ M ionomycin. (J) Summary results show half-times ( $t_{1/2}$ ) for fluorescence quenching before (basal) and after treatment with thapsigargin (5  $\mu$ M for 10 min) or carbachol with ATP (100  $\mu$ M of each for 3.5 min). The results demonstrate that the effects of  $\sigma$ 1R ligands on thapsigargin-evoked  $\text{Ca}^{2+}$  release and SOCE are abolished after treatment with siRNA for  $\sigma$ 1R. (K) Fluo 4-loaded HEK- $\sigma$ 1R cells were incubated with 25  $\mu$ M (+)SKF10047 or 10  $\mu$ M BD1047 for 2 h in  $\text{Ca}^{2+}$ -free HBS at 20°C. Thapsigargin (1  $\mu$ M for 10 min) was added before or after addition of the  $\sigma$ 1R ligands, and the increase in  $[\text{Ca}^{2+}]_i$  evoked by SOCE was measured after restoration of extracellular  $\text{Ca}^{2+}$ . The results show that the ligands have less effect on SOCE when added after store depletion. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ , relative to control (ANOVA followed by Tukey's posthoc analysis for D, G, and I-K; Student's  $t$  test for F and for comparison of no treatment conditions in I). (D and F-K) Results are mean  $\pm$  SEM ( $n = 3$ ).

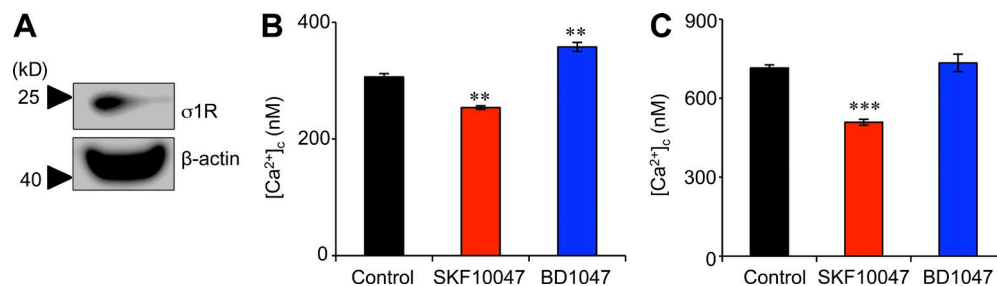


Figure S4. **Effects of ligands of  $\sigma$ 1R on SOCE and intracellular  $\text{Ca}^{2+}$  stores in MDA-MB-231 breast cancer cells.** (A) Immunoblot showing detection of  $\sigma$ 1R in a lysate of MDA-MB-231 cells. (B) Populations of fluo 4-loaded MDA-MB-231 cells were pretreated with 25  $\mu\text{M}$  (+)SKF10047 or 10  $\mu\text{M}$  BD1047 before addition of 5  $\mu\text{M}$  thapsigargin in  $\text{Ca}^{2+}$ -free HBS and then measurement of SOCE after restoration of 4 mM extracellular  $\text{Ca}^{2+}$  after 10 min. Results show the peak  $[\text{Ca}^{2+}]_i$  evoked by the addition of extracellular  $\text{Ca}^{2+}$ . (C) The  $\text{Ca}^{2+}$  content of the intracellular stores was assessed after the same pretreatments with  $\sigma$ 1R ligands by addition of 5  $\mu\text{M}$  ionomycin in  $\text{Ca}^{2+}$ -free HBS. Results show the peak increases in  $[\text{Ca}^{2+}]_i$  evoked by ionomycin. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ , relative to control treatment (ANOVA followed by Tukey's posthoc analysis). Results are mean  $\pm$  SEM ( $n = 3$ ).

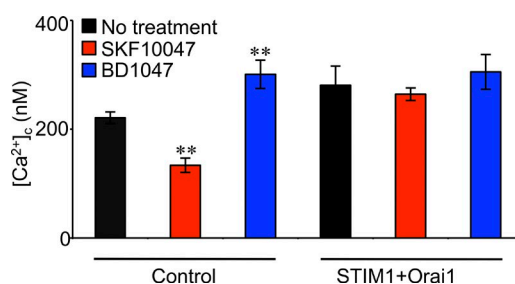


Figure S5. **Effects of ligands of  $\sigma$ 1R on SOCE in cells overexpressing STIM1 and Orai1.** Populations of fluo 4-loaded HEK- $\sigma$ 1R cells were either mock transfected (control) or transfected with STIM1 and Orai1 before pretreatment with 25  $\mu\text{M}$  (+)SKF10047 or 10  $\mu\text{M}$  BD1047. SOCE was then measured by addition of extracellular  $\text{Ca}^{2+}$  to cells treated with thapsigargin in  $\text{Ca}^{2+}$ -free HBS. Results (mean  $\pm$  SD; six replicates) show the peak  $[\text{Ca}^{2+}]_i$  evoked by addition of extracellular  $\text{Ca}^{2+}$ . \*\*,  $P < 0.01$ , relative to no treatment, using ANOVA followed by Tukey's posthoc analysis.

Table S1. **Ligands targeting  $\sigma$ 1R**

Antagonists	Agonists
<b>BD1047</b>	Cocaine
Haloperidol	Dimemorfan
Metaphit	Donepezil
NE100	DTG
Rimcazole	Fluvoxamine
	Ibogaïne
	Methamphetamine
	<i>N,N</i> -dimethyltryptamine (DMT)
	Panamesine
	PPBP
	PCC
<b>Selective for <math>\sigma</math>1R versus <math>\sigma</math>2R</b>	Igmesine
	MR22
	(+)-Pentazocine
	PRE-084
	SA4503
	<b>(+)-SKF10047</b>
<b>Endogenous neurosteroids</b>	
Progesterone	Dehydroepiandrosterone (DHEA)
	Pregnenolone

This list is incomplete, but it illustrates the diversity of drugs that interact with  $\sigma$ 1Rs, the overlap with drugs that are important in the clinic or as drugs of abuse, and drugs used in the present study (bold). The  $\sigma$ 1R is probably unrelated to  $\sigma$ 2R, although the two have overlapping pharmacology. Drug actions: cocaine, inhibitor of catecholamine uptake (Schwartz et al., 2010) and drug of abuse. Dimemorfan, centrally acting cough suppressant (Shin et al., 2005). Donepezil (Aricept), reversible inhibitor of acetylcholinesterase used to treat Alzheimer's disease (Ramakrishnan et al., 2014). DMT, hallucinogen and possible endogenous ligand of  $\sigma$ 1Rs (Fontanilla et al., 2009). Fluvoxamine, selective serotonin reuptake inhibitor (SSRI) used to treat depression (Hindmarch and Hashimoto, 2010). Many other SSRIs are also agonists of  $\sigma$ 1Rs. Haloperidol (Dozic and Serenace), dopamine D2 receptor antagonist used to treat schizophrenia and other psychotic disorders (Maurice and Su, 2009). Ibogaïne, psychoactive natural product. Potential utility in treatment of drug craving (Mach et al., 1995). Igmesine, possible utility in treatment of depression (Kulkarni and Dhir, 2009). Methamphetamine (crystal meth and ice), stimulates release of catecholamines and blocks their reuptake (Nguyen et al., 2005) and is a drug of abuse. Panamesine, possible utility in treatment of schizophrenia (Kulkarni and Dhir, 2009). Pentazocine, opioid analgesic. Closely related to SKF10047 (Hayashi and Su, 2005). Rimcazole, adverse effects prevented use as a potential anticancer therapy (Happy et al., 2015). BB1047, *N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2,2-(dimethylamino)ethylamine; DTG, 1,3-di-*o*-tolylguanidone; Igmesine, (*R*)-(+)-*N*-cyclopropylmethyl- $\alpha$ -ethyl-*N*-methyl- $\alpha$ -[2E]-3-phenyl-2-propenyl]benzenemethanamine hydrochloride; MR22, (-)-methyl (1*S*,2*R*)-2-[[1-adamantyl(methylamino)methyl]-1-phenylcyclopropanecarboxylate; NE100, 4-methoxy-3-(2-phenylethoxy)-*N,N*-dipropylbenzeneethanamine hydrochloride; PPBP, 4-phenyl-1-(4-phenylbutyl)-piperidine maleate; PCC, (*S*\*,*R*\*)-2-[[4-hydroxy-4-phenyl-1-piperidinyl)methyl]-1-[4-methylphenyl]-cyclopropanecarboxylic acid methyl ester; PRE-084, 2-[4-morpholinethyl] 1-phenylcyclohexanecarboxylate hydrochloride; SA 4503, 1-[2-[3,4-dimethoxyphenyl]ethyl]-4-[3-phenylpropyl]-piperazine dihydrochloride; (+)-SKF10047, [2*S*-(2 $\alpha$ ,6 $\alpha$ ,11*R*\*)]-1,2,3,4,5,6-hexahydro-6,11-dimethyl-3-(2-propenyl)-2,6-methano-3-benzazocin-8-ol hydrochloride.

## References

- Fontanilla, D., M. Johannessen, A.R. Hajipour, N.V. Cozzi, M.B. Jackson, and A.E. Ruoho. 2009. The hallucinogen *N,N*-dimethyltryptamine (DMT) is an endogenous sigma-1 receptor regulator. *Science*. 323:934–937. <http://dx.doi.org/10.1126/science.1166127>
- Happy, M., J. Dejoie, C.K. Zajac, B. Cortez, K. Chakraborty, J. Aderemi, and M. Sauane. 2015. Sigma 1 Receptor antagonist potentiates the anti-cancer effect of p53 by regulating ER stress, ROS production, Bax levels, and caspase-3 activation. *Biochem. Biophys. Res. Commun.* 456:683–688. <http://dx.doi.org/10.1016/j.bbrc.2014.12.029>
- Hayashi, T., and T.-P. Su. 2004. Sigma-1 receptors at galactosylceramide-enriched lipid microdomains regulate oligodendrocyte differentiation. *Proc. Natl. Acad. Sci. USA*. 101:14949–14954. <http://dx.doi.org/10.1073/pnas.0402890101>
- Hayashi, T., and T.-P. Su. 2005. The potential role of sigma-1 receptors in lipid transport and lipid raft reconstitution in the brain: implication for drug abuse. *Life Sci*. 77:1612–1624. <http://dx.doi.org/10.1016/j.lfs.2005.05.009>
- Hayashi, T., and T.-P. Su. 2007. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca<sup>2+</sup> signaling and cell survival. *Cell*. 131:596–610. <http://dx.doi.org/10.1016/j.cell.2007.08.036>
- Hindmarch, I., and K. Hashimoto. 2010. Cognition and depression: the effects of fluvoxamine, a sigma-1 receptor agonist, reconsidered. *Hum. Psychopharmacol.* 25:193–200. <http://dx.doi.org/10.1002/hup.1106>
- Kulkarni, S.K., and A. Dhir. 2009.  $\sigma$ -1 receptors in major depression and anxiety. *Expert Rev. Neurother.* 9:1021–1034. <http://dx.doi.org/10.1586/ern.09.40>
- Mach, R.H., C.R. Smith, and S.R. Childers. 1995. Ibogaïne possesses a selective affinity for sigma 2 receptors. *Life Sci*. 57:PL57–PL62. [http://dx.doi.org/10.1016/0024-3205\(95\)00301-L](http://dx.doi.org/10.1016/0024-3205(95)00301-L)
- Maurice, T., and T.-P. Su. 2009. The pharmacology of sigma-1 receptors. *Pharmacol. Ther.* 124:195–206. <http://dx.doi.org/10.1016/j.pharmthera.2009.07.001>
- Nguyen, E.C., K.A. McCracken, Y. Liu, B. Pouw, and R.R. Matsumoto. 2005. Involvement of sigma ( $\sigma$ ) receptors in the acute actions of methamphetamine: receptor binding and behavioral studies. *Neuropharmacology*. 49:638–645. <http://dx.doi.org/10.1016/j.neuropharm.2005.04.016>
- Ramakrishnan, N.K., A.K.D. Visser, M. Schepers, G. Luurtsema, C.J. Nyakas, P.H. Elsinga, K. Ishiwata, R.A.J.O. Dierckx, and A. van Waarde. 2014. Dose-dependent sigma-1 receptor occupancy by donepezil in rat brain can be assessed with <sup>11</sup>C-SA4503 and microPET. *Psychopharmacology (Berl.)*. 231:3997–4006. <http://dx.doi.org/10.1007/s00213-014-3533-2>
- Schwartz, B.G., S. Rezkalla, and R.A. Kloner. 2010. Cardiovascular effects of cocaine. *Circulation*. 122:2558–2569. <http://dx.doi.org/10.1161/CIRCULATIONAHA.110.940569>
- Shin, E.J., S.Y. Nah, W.K. Kim, K.H. Ko, W.K. Jho, Y.K. Lim, J.Y. Cha, C.F. Chen, and H.C. Kim. 2005. The dextromethorphan analog dimemorfan attenuates kainate-induced seizures via  $\sigma_1$  receptor activation: comparison with the effects of dextromethorphan. *Br. J. Pharmacol.* 144:908–918. <http://dx.doi.org/10.1038/sj.bjp.0705998>