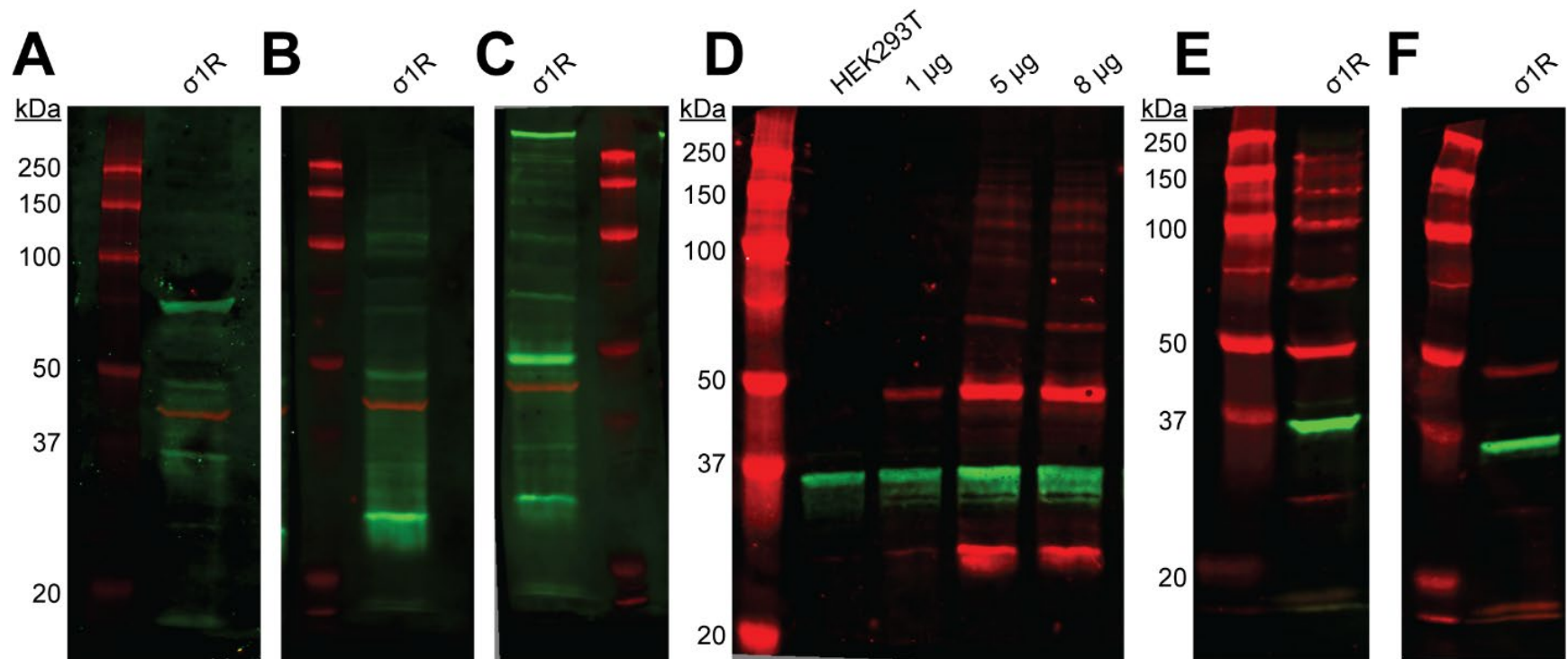


**Supplementary Figure 1.** Immuno-reactivities of commercially available  $\sigma$ 1R antibodies. **A-C.** Green and red bands are visualized by anti- $\sigma$ 1R (**A.** AB 53852, Abcam, **B.** AB 223702, Abcam, **C.** DHJ2E, Cell Signaling) and anti-actin antibodies respectively on 10% SDS-PAGE. **D.** Endogenous  $\sigma$ 1R expressed in WT HEK293T cells or increasing amounts of  $\sigma$ 1R expressed in  $\sigma$ 1R KO cells (1, 5, 8  $\mu$ g) are detected by the anti- $\sigma$ 1R antibody (B-5, Santa Cruz) in red along with GAPDH loading control in green. **E-F.** Immuno-reactivities are diminished over a prolonged storage time (**E.** ~1 month vs. **F.** > 6 months in 4 °C). Each blot image is representative of n > 3 experiments.

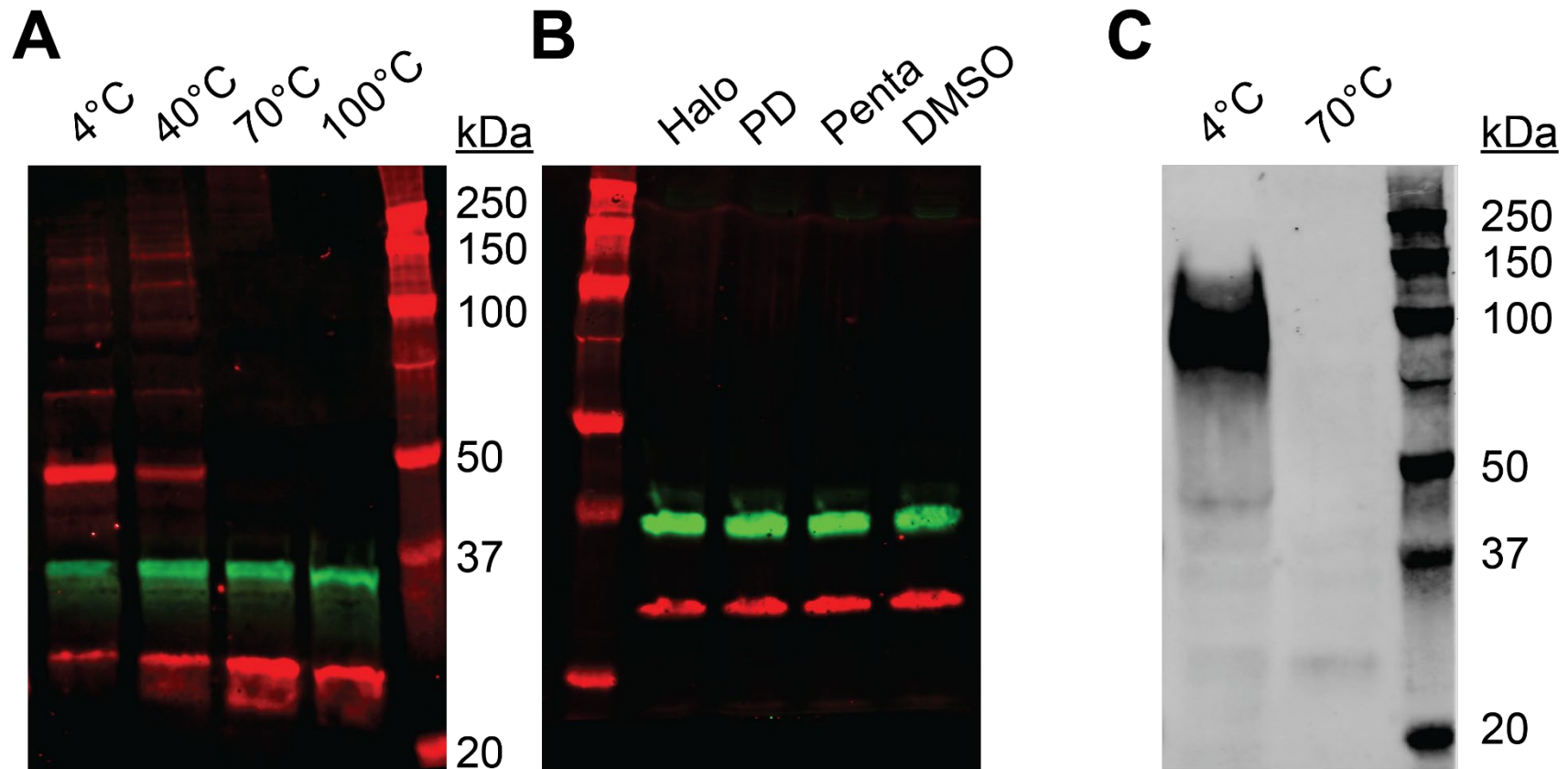


**Supplementary Table 1.**

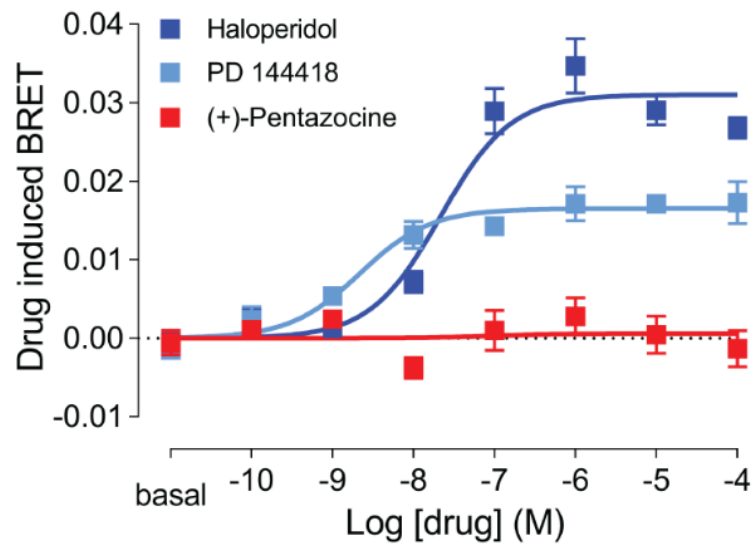
Product information and dilutions of primary and secondary antibodies

	Product ID, Vendor	Epitope (residue numbers)	Dilution
<i>Primary antibody</i>			
$\sigma$ 1R (mouse monoclonal)	B-5, Santa Cruz	136-169	1:1,000
$\sigma$ 1R (rabbit polyclonal)	AB 53852, Abcam	C-terminus	1:625
$\sigma$ 1R (rabbit polyclonal)	AB 223702, Abcam	35-88	1:250
$\sigma$ 1R (rabbit monoclonal)	DHJ2E, Cell Signaling	around 137	1:1,000
GAPDH (rabbit monoclonal)	14C10, Cell Signaling	C-terminus	1:2,000
Actin (mouse monoclonal)	C-2, Santa Cruz	357-375	1:2,000
Myc (mouse monoclonal)	9B11, Cell Signaling	410-419	1:1,000
<i>Secondary antibody</i>			
Donkey $\alpha$ mouse	IRDye 680RD, LI-COR		1:5,000; 1:10,000
Goat $\alpha$ rabbit	IRDye 800CW, LI-COR		1:10,000; 1:5,000

**Supplementary Figure 2.** Protein sample preparation at different temperatures. **A-B.** Sample preparation from HEK 293T cells, red and green bands are visualized by anti- $\sigma$ 1R and anti-GAPDH antibodies respectively on 10% SDS-PAGE. **A.** Samples were prepared in increasing temperatures (4, 40, 70, and 100 °C). **B.** Ligand-treated samples were heated at 70 °C for 15 min (protein standard, haloperidol, PD 144418, (+)-pentazocine, DMSO vehicle). **C.** Samples from mouse brain cortex were prepared in 4 and 70°C, and were visualized by anti- $\sigma$ 1R antibody. Each blot image is representative of  $n > 3$  experiments.



**Supplementary Figure 3.** Drug induced changes of  $\sigma$ 1R homomer BRET. Drug induced BRET between the C-terminally tagged  $\sigma$ 1R-Nluc and  $\sigma$ 1R-Venus in  $\sigma$ 1R KO cells is detected at 60 min for (+)-pentazocine (red), haloperidol (blue), and PD144418 (cyan). Data represents mean $\pm$ S.E.M. (n=5 or more).



**Supplementary Figure 4.** Homologous radioligand competition binding of [<sup>3</sup>H](+)-pentazocine for  $\sigma$ 1R (black),  $\sigma$ 1R-myc (orange),  $\sigma$ 1R-Nluc (red), myc- $\sigma$ 1R (blue), and Nluc- $\sigma$ 1R (purple). Each curve is the average of n = 3 using one-site fitting.

