Blockade of spinal dopamine D1/D2 receptor heteromers by *levo*-Corydalmine suppressed calcium signaling cascade in spinal neurons to alleviate bone cancer pain in rats

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Additional file 1: Fig. s1. *l*-CDL shows micromolar affinity to D1DR with half

maximal inhibitory concentration (IC50) of 0.20 μM.

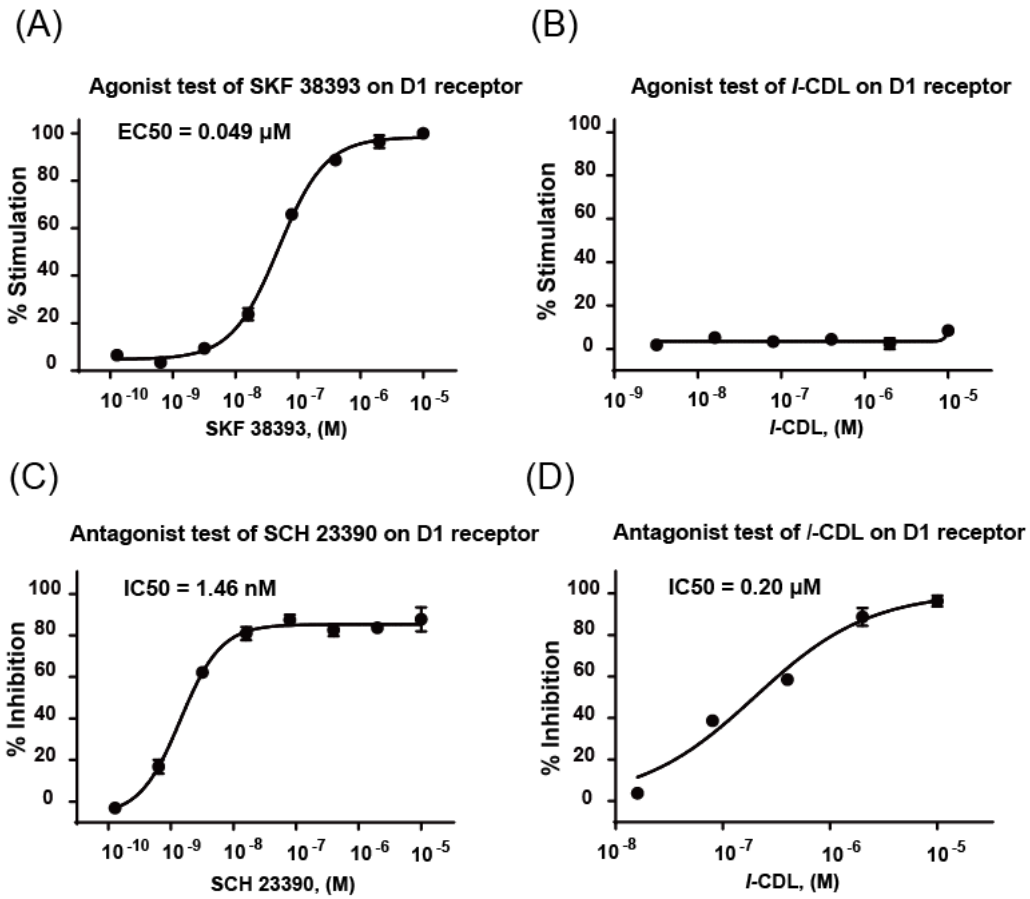

FIGURE s1 *l*-CDL shows micromolar affinity to D1DR with half maximal inhibitory concentration (IC50) of 0.20 μM. *l*-CDL could inhibit D1DR agonist SKF 38393 increased cAMP in D1/CHO-K1 cell lines with IC50 of 0.20 μM (Figure s1D), while it could not activate D1DR to increase cAMP (Figure s1B), indicating that *l*-CDL exhibited micromolar affinities to D1DR. The D1DR agonist SKF 38393 and D1DR antagonist SCH-23390 were used as positive control, SKF 38393 could increase cAMP in D1/CHO-K1 cell lines was half maximal effective concentration (EC50) of 0.049 μM ((Figure s1A), and D1DR antagonist SCH 23390 inhibited dopamine increased cAMP in D1/CHO-K1 cell lines with an IC50 of 1.46 nM (Figure s1C).

Additional experimental procedures

Cell culture

The D1/CHO-K1 cell lines were cultured in Ham's F12 containing 10% FBS supplement and 200 μg/mL Zeocin were added. The D1/CHO-K1 cell lines were plated onto 384 wells microplate with a density of 1.5×10^4 cells/well (20 μL/well). Cells were incubated at 37 ℃ with 5% CO_2 and 95% humidity for 18 hours.

cAMP analysis

The cAMP concentration was determined by cAMP direct immunoassay kit (Cisbio Bioassays). The The D1/CHO-K1 cells were planted onto a 384-well microplate with a density of 3000cell/5 μL. And 5 μL of the (2 ×) agonist or compound solutions were added to corresponding well to incubate for 30 min. Then the cells were incubated with 10 μL detection reagent for 1 h. The signals were detected at 665 nm and 620 nm. For agonist tests, *l*-CDL (2 ×) was added, for antagonist tests, *l*-CDL (1 ×) and agonist (1 ×) were added simultaneously.