**Supplemental Digital Content**

**Table 1** Mean (standard deviation) for mechanical withdrawal threshold in the preliminary experiment

(g, n=4 for each group)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Baseline | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 |
| Vehicle+saline | 4.67  (0.77) | 4.00  (0.77) | 6.67  (1.96) | 7.00  (0.86) | 4.00  (1.63) | 4.90  (2.74) | 4.17  (1.14) | 4.23  (1.94) | 4.33  (1.16) |
| AM1241+saline | 5.50  (1.14) | 4.84  (0.84) | 6.67  (2.11) | 5.50  (2.27) | 7.17  (2.58) | 5.17  (2.85) | 6.83  (2.69) | 4.84  (1.84) | 3.95  (1.37) |
| AM630+saline | 3.83  (0.64) | 5.50  (1.48) | 2.78  (0.97) | 4.40  (2.09) | 5.12  (2.77) | 4.90  (2.74) | 6.00  (1.96) | 5.45  (2.29) | 4.50  (1.75) |
| Vehicle+morphine | 5.50  (1.00) | 60.00  (0.00) | 47.75  (14.22) | 39.67  (5.13) | 29.83  (6.88) | 23.42  (5.17) | 15.33  (3.07) | 11.84  (4.60) | 9.42  (1.77) |
| AM1241+morphine | 5.67  (1.16) | 60.00  (0.00) | 57.17  (5.67) | 48.67  (9.25) | 44.92  (7.50) | 34.50  (10.85) | 29.83  (6.88) | 28.92  (7.75) | 17.75  (3.51) |
| AM630+AM1241  +morphine | 4.67  (1.22) | 60.00  (0.00) | 54.34  (6.54) | 37.92  (7.51) | 27.92  (3.84) | 20.67  (6.16) | 15.83  (4.72) | 10.92  (4.07) | 8.83  (1.14) |

**Table 2** Mean (standard deviation) for thermal withdrawal latency in the preliminary experiment

(s, n=4 for each group)

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Baseline | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 | Day 8 |
| Vehicle+saline | 13.32  (5.66) | 12.96  (3.56) | 10.91  (2.91) | 12.33  (4.13) | 10.04  (3.30) | 10.16  (2.13) | 10.30  (2.04) | 9.30  (1.95) | 10.46  (3.39) |
| AM1241+saline | 10.71  (4.43) | 16.84  (2.49) | 13.61  (4.24) | 9.01  (3.07) | 8.98  (2.87) | 11.61  (6.89) | 9.80  (0.89) | 7.98  (2.38) | 12.68  (4.21) |
| AM630+saline | 10.56  (1.62) | 12.10  (3.03) | 9.89  (0.89) | 11.23  (2.28) | 12.51  (2.81) | 13.24  (4.01) | 10.40  (3.42) | 9.53  (2.56) | 11.03  (4.82) |
| Vehicle+morphine | 15.20  (8.30) | 28.25  (2.14) | 23.65  (7.37) | 25.78  (3.48) | 24.34  (4.25) | 19.32  (2.21) | 14.25  (3.70) | 16.12  (2.85) | 11.96  (4.14) |
| AM1241+morphine | 14.38  (0.94) | 27.21  (5.58) | 26.81  (3.15) | 27.78  (2.54) | 25.91  (3.27) | 25.23  (2.22) | 27.06  (2.20) | 23.73  (4.05) | 18.59  (6.39) |
| AM630+AM1241  +morphine | 12.57  (3.40) | 29.44  (1.13) | 27.23  (3.52) | 25.51  (4.22) | 25.86  (3.01) | 22.37  (1.40) | 15.04  (2.36) | 14.10  (3.03) | 12.20  (4.49) |

**Table 3** Mean (standard deviation) for mu opioid receptor protein expression in the preliminary

experiment (n=3 for each group)

|  |  |  |
| --- | --- | --- |
| Group | Spinal cord | DRG |
| Vehicle+saline | 35.2 (4.43) | 35.12 (5.42) |
| AM1241+saline | 35.98 (4.43) | 34.92 (5.75) |
| AM630+saline | 34.35 (5.39) | 32.45 (3.39) |
| Vehicle+morphine | 23.47 (4.96) | 22.57 (6.00) |
| AM1241+morphine | 42.20 (4.78) | 38.26 (7.22) |
| AM630+AM1241+morphine | 24.20 (5.00) | 24.21 (5.49) |

After the behavioral tests on day 8, spinal lumbar L3/L4/L5 segments and DRG ipsilateral to the tumor cell injections were harvested for detecting protein expression.

**Table 4** Mean (standard deviation) for mu opioid receptor mRNA expression in the preliminary

experiment (n=3 for each group)

|  |  |  |
| --- | --- | --- |
| Group | Spinal cord | DRG |
| Vehicle+saline | 0.32 (0.08) | 0.0086 (0.0016) |
| AM1241+saline | 0.35 (0.14) | 0.0097 (0.0016) |
| AM630+saline | 0.30 (0.09) | 0.0085 (0.0010) |
| Vehicle+morphine | 0.14 (0.08) | 0.0067 (0.0004) |
| AM1241+morphine | 0.52 (0.13) | 0.0092 (0.0006) |
| AM630+AM1241+morphine | 0.13 (0.09) | 0.0067 (0.0008) |

After the behavioral tests on day 8, spinal lumbar L3/L4/L5 segments and DRG ipsilateral to the tumor cell injections were harvested for detecting protein expression.

**Table 5** Results of Student-*t* test for multiple comparisons of mechanical withdrawal threshold at each time point

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Baseline | |  | Day 1 | |  | Day 2 | |  | Day 3 | |  | Day 4 | |  | Day 5 | |  | Day 6 | |  | Day 7 | |  | Day 8 | |
| MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value |
| AM1241+saline *vs.* vehicle+saline | -0.76  (-3.47, 1.95) | 0.4294 |  | 0.91  (-1.76, 3.58) | 0.3391 |  | 1.53  (-1.43, 4.48) | 0.1545 |  | -1.02  (-3.52, 1.49) | 0.2575 |  | 1.85  (-1.02, 4.74) | 0.0767 |  | 0.83  (-2.31, 3.98) | 0.4553 |  | 1.34  (-1.49, 4.16) | 0.1905 |  | 0.44  (-2.12, 2.99) | 0.6383 |  | 1.31  (-1.06, 3.69) | 0.1286 |
| AM630+saline *vs.* vehicle+saline | -0.78  (-2.84, 1.29) | 0.2951 |  | 0.22  (-2.36, 2.80) | 0.8088 |  | -0.42  (-3.25, 2.40) | 0.6714 |  | -1.43  (-4.01, 1.14) | 0.1261 |  | 0.05  (-2.46, 2.57) | 0.9523 |  | -0.91  (-4.04, 2.23) | 0.4158 |  | -0.45  (-3.33, 2.44) | 0.6621 |  | 0.22  (-2.59, 3.03) | 0.8228 |  | -0.06  (-2.06, 1.93) | 0.9285 |
| Vehicle+morphine *vs.* vehicle+saline | -0.12  (-2.88, 2.63) | 0.8982 |  | 53.48  (49.47, 57.49) | <0.0001\* |  | 48.67  (37.58,59.76) | <0.0001\* |  | 35.17  (26.39, 43.95) | <0.0001\* |  | 28.75  (20.52, 36.98) | <0.0001\* |  | 23.13  (16.03, 30.23) | <0.0001\* |  | 15.04  (7.95, 22.12) | <0.0001\* |  | 9.33  (4.52, 14.13) | <0.0001\* |  | 4.93  (2.90, 6.96) | <0.0001\* |
| AM1241+morphine *vs.* vehicle+saline | -1.60  (-4.29, 1.09) | 0.1043 |  | 53.48  (49.47, 57.49) | <0.0001\* |  | 51.60  (45.68, 57.52) | <0.0001\* |  | 43.35  (34.23, 52.47) | <0.0001\* |  | 35.48  (26.65, 44.32) | <0.0001\* |  | 30.91  (20.09, 41.74) | <0.0001\* |  | 24.48  (17.26, 31.70) | <0.0001\* |  | 22.45  (15.45, 29.45) | <0.0001\* |  | 13.34  (8.89, 17.78) | <0.0001\* |
| AM630+AM1241+morphine *vs.* vehicle +saline | -1.60  (-3.60, 0.41) | 0.0342 |  | 54.74  (52.43, 57.05) | <0.0001\* |  | 52.86  (47.82, 57.90) | <0.0001\* |  | 33.13  (23.87, 42.39) | <0.0001\* |  | 27.08  (18.80, 35.35) | <0.0001\* |  | 19.83  (11.35, 28.31) | <0.0001\* |  | 13.93  (5.68, 22.17) | <0.0001\* |  | 9.22  (3.04, 15.39) | <0.0001\* |  | 6.34  (3.29, 9.38) | <0.0001\* |
| AM1241+morphine *vs.*  vehicle +morphine | -1.47  (-4.66, 1.71) | 0.1994 |  | 0.00  (-4.88, 4.88) | 1.0000 |  | 2.93  (-8.15, 14.01) | 0.4568 |  | 8.19  (-3.01, 19.38) | 0.0496 |  | 6.74  (-3.95, 17.42) | 0.0863 |  | 7.78  (-3.66, 19.22) | 0.0658 |  | 9.44  (0.55, 18.34) | 0.0068 |  | 13.12  (5.68, 20.56) | <0.0001\* |  | 8.41  (4.31, 12.50) | <0.0001\* |
| AM630+AM1241+morphine *vs*. vehicle +morphine | -1.47  (-4.11, 1.16) | 0.1249 |  | 1.26  (-2.63, 5.15) | 0.3207 |  | 4.19  (-7.17, 15.54) | 0.2859 |  | -2.04  (-13.32, 9.25) | 0.6100 |  | -1.67  (-12.00, 8.65) | 0.6465 |  | -3.30  (-13.05, 6.46) | 0.3438 |  | -1.11  (-10.69, 8.67) | 0.7425 |  | -0.11  (-6.95, 6.72) | 0.9629 |  | 1.41  (-1.52, 4.33) | 0.1841 |

Note: \* *P* value is lower than the Bonferroni-corrected threshold of 0.0014 (0.01/7). MD = mean difference; CI = confidence interval.

**Table 6** Results of paired Student-*t* test for multiple comparisons of mechanical withdrawal threshold at vehicle+morphine group and AM1241+morphine group

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Day 2 | |  | Day 3 | |  | Day 4 | |  | Day 5 | |  | Day 6 | |  | Day 7 | |  | Day 8 | |
| MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value |
| Vehicle+morphine | -4.19  (-15.03, 6.66) | 0.2412 |  | -15.74  (-25.74, -5.74) | <0.001\* |  | -23.92  (-31.37, -16.47) | <0.0001\* |  | -29.26  (-34.97, -23.55) | <0.0001\* |  | -36.59  (-43.37, -29.72) | <0.0001\* |  | -43.28  (-48.14, -38.37) | <0.0001\* |  | -48.56  (-54.42, -42.69) | <0.0001\* |
| AM1241+morphine | -2.52  (-10.25, 5.22) | 0.3180 |  | -8.81  (-18.24, 0.61) | 0.0141 |  | -18.44  (-27.23, -9.65) | <0.0001\* |  | -22.74  (-35.89, -9.58) | <0.0001\* |  | -28.41  (-37.72, -19.09) | <0.0001\* |  | -31.40  (-39.12, -23.67) | <0.0001\* |  | -41.41  (-46.16, -36.66) | <0.0001\* |

Note: the corresponding days compared with day 1. \* *P* value is lower than the Bonferroni-corrected threshold of 0.0014 (0.01/7). MD = mean difference; CI = confidence interval.

**Table 7** Results of Student-*t* test for multiple comparisons of thermal withdrawal latency at each time point

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Baseline | |  | Day 1 | |  | Day 2 | |  | Day 3 | |  | Day 4 | |  | Day 5 | |  | Day 6 | |  | Day 7 | |  | Day 8 | |
| MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value |
| AM1241+saline *vs.* vehicle+saline | -0.17  (-5.88, 5.53) | 0.9314 |  | 2.52  (-1.77, 6.80) | 0.1079 |  | 2.37  (-1.56, 6.30) | 0.0994 |  | 1.43  (-3.56, 6.41) | 0.4208 |  | 2.12  (-1.64, 5.87) | 0.1224 |  | 2.89  (-2.70, 8.49) | 0.1496 |  | 1.48  (-1.69, 4.65) | 0.1960 |  | 2.72  (-1.15, 6.58) | 0.0584 |  | 2.50  (-1.37, 6.37) | 0.0798 |
| AM630+saline *vs.* vehicle+saline | -1.32  (-6.57, 3.94) | 0.4810 |  | -1.02  (-6.43, 4.39) | 0.5932 |  | -0.127  (-3.99, 3.74) | 0.9257 |  | -0.34  (-5.01, 4.32) | 0.8357 |  | 2.02  (-1.68, 5.73) | 0.1335 |  | 0.80  (-3.77, 5.36) | 0.6215 |  | 1.71  (-0.97, 4.39) | 0.0828 |  | 0.24  (-2.79, 3.27) | 0.8221 |  | 1.98  (-1.11, 5.04) | 0.0822 |
| Vehicle+morphine *vs.* vehicle+saline | 0.99  (-5.44, 7.41) | 0.6637 |  | 13.70  (9.83, 17.58) | <0.0001\* |  | 14.05  (8.57, 19.52) | <0.0001\* |  | 12.29  (7.33, 17.26) | <0.0001\* |  | 13.87  (9.05, 18.70) | <0.0001\* |  | 10.24  (3.66, 16.83) | <0.0001\* |  | 6.02  (1.51, 10.53) | <0.001\* |  | 5.72  (1.81, 9.63) | <0.001\* |  | 1.94  (-1.60, 5.47) | 0.1328 |
| AM1241+morphine *vs.* vehicle+saline | -2.01  (-7.22, 3.20) | 0.2804 |  | 14.20  (9.46, 18.93) | <0.0001\* |  | 16.48  (12.89, 20.06) | <0.0001\* |  | 14.95  (10.60, 19.29) | <0.0001\* |  | 15.53  (12.13, 18.94) | <0.0001\* |  | 15.30  (11.54, 19.05) | <0.0001\* |  | 17.06  (14.41, 19.72) | <0.0001\* |  | 15.63  (10.80, 20.46) | <0.0001\* |  | 12.07  (8.95, 15.19) | <0.0001\* |
| AM630+AM1241+morphine *vs.* vehicle +saline | -1.00  (-5.83, 3.83) | 0.5584 |  | 15.57  (11.76, 19.37) | <0.0001\* |  | 17.21  (13.53, 20.88) | <0.0001\* |  | 12.05  (7.30, 16.80) | <0.0001\* |  | 13.12  (9.46, 16.78) | <0.0001\* |  | 11.44  (7.44, 15.43) | <0.0001\* |  | 6.88  (4.35, 9.41) | <0.0001\* |  | 5.50  (2.76, 8.24) | <0.0001\* |  | 3.24  (0.57, 5.91) | 0.0026 |
| AM1241+morphine *vs.*  vehicle +morphine | -3.00  (-8.81, 2.80) | 0.1541 |  | 0.49  (-3.14, 4.13) | 0.7009 |  | 2.43  (-3.02, 7.89) | 0.2016 |  | 2.65  (-1.08, 6.39) | 0.0520 |  | 1.66  (-3.18, 6.50) | 0.3372 |  | 5.05  (-1.34, 11.44) | 0.0325 |  | 11.05  (6.46, 15.63) | <0.0001\* |  | 9.91  (4.42, 15.39) | <0.0001\* |  | 10.13  (6.39, 13.88) | <0.0001\* |
| AM630+AM1241+morphine *vs*. vehicle +morphine | -1.99  (-7.69, 3.72) | 0.3141 |  | 1.86  (0.16, 3.56) | 0.0057 |  | 3.16  (-2.33, 8.65) | 0.1069 |  | -0.24  (-4.55, 4.07) | 0.8734 |  | -0.75  (-5.78, 4.27) | 0.6713 |  | 1.19  (-5.27, 7.66) | 0.5868 |  | 0.86  (-3.68, 5.40) | 0.5772 |  | -0.22  (-4.00, 3.55) | 0.8675 |  | 1.31  (-2.07, 4.68) | 0.2810 |

Note: \* *P* value is lower than the Bonferroni-corrected threshold of 0.0014 (0.01/7). MD = mean difference; CI = confidence interval.

**Table 8** Results of paired Student-*t* test for multiple comparisons of thermal withdrawal latency at vehicle+morphine group and AM1241+morphine group

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Group | Day 2 | |  | Day 3 | |  | Day 4 | |  | Day 5 | |  | Day 6 | |  | Day 7 | |  | Day 8 | |
| MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value | MD (99% CI) | *P* value |
| Vehicle+morphine | -2.28  (-6.54, 1.98) | 0.1161 |  | -2.67  (-6.17, 0.84) | 0.0355 |  | -3.51  (-7.96, 0.94) | 0.0305 |  | -6.41  (-12.09, -0.72) | 0.0052 |  | -12.45  (-16.63, -8.28) | <0.0001\* |  | -13.73  (-16.93, -10.52) | <0.0001\* |  | -16.93  (-19.82, -14.04) | <0.0001\* |
| AM1241+morphine | -0.34  (-2.46, 1.78) | 0.6165 |  | -0.51  (-3.39, 2.38) | 0.5826 |  | -2.35  (-5.22, 0.53) | 0.0265 |  | -1.85  (-4.44, 0.74) | 0.0457 |  | -1.90  (-5.71, 1.91) | 0.1393 |  | -4.31  (-9.00, 0.38) | 0.0152 |  | -7.29  (-10.76, -3.82) | <0.0001\* |

Note: the corresponding days compared with day 1. \* *P* value is lower than the Bonferroni-corrected threshold of 0.0014 (0.01/7). MD = mean difference; CI = confidence interval.

**Table 9** Results of Student-*t* test for multiple comparisons of mu opioid receptor protein expression

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | Spinal cord | |  | DRG | |
| MD (99% CI) | *P* value | MD (99% CI) | *P* value |
| AM1241+saline *vs.* vehicle+saline | 0.06 (-0.12, 0.23) | 0.2476 |  | 0.03 (-0.64, 0.70) | 0.8484 |
| AM630+saline *vs.* vehicle+saline | -0.03 (-0.23, 0.17) | 0.6291 |  | -0.11 (-0.74, 0.53) | 0.5236 |
| Vehicle+morphine *vs.* vehicle+saline | -0.25 (-0.47, -0.02) | 0.0058 |  | -0.38 (-0.90, 0.14) | 0.0410 |
| AM1241+morphine *vs.* vehicle+saline | 0.24 (0.04, 0.45) | 0.0037 |  | 0.27 (-0.35, 0.89) | 0.1424 |
| AM630+AM1241+morphine *vs.*  vehicle +saline | -0.22 (-0.39, -0.04) | 0.0031 |  | -0.30 (-0.81, 0.21) | 0.0817 |
| AM1241+morphine *vs.*  vehicle +morphine | 0.49 (0.26, 0.72) | <0.0001\* |  | 0.65 (0.39, 0.91) | <0.0001\* |
| AM630+AM1241+morphine *vs*.  vehicle +morphine | 0.03 (-0.17, 0.23) | 0.6541 |  | 0.08 (-0.13, 0.29) | 0.2427 |

Note: \* *P* value is lower than the Bonferroni-corrected threshold of 0.0014 (0.01/7). MD = mean difference; CI = confidence interval.

**Table 10** Results of Student-*t* test for multiple comparisons of mu opioid receptor mRNA expression in

spinal cord

|  |  |  |
| --- | --- | --- |
| Group | MD (99% CI) | *P* value |
| AM1241+saline *vs.* vehicle+saline | 0.05 (-0.60, 0.72) | 0.7429 |
| AM630+saline *vs.* vehicle+saline | 0.06 (-0.58, 0.69) | 0.7412 |
| Vehicle+morphine *vs.* vehicle+saline | -0.57 (-1.09, -0.47) | 0.0064 |
| AM1241+morphine *vs.* vehicle+saline | 1.09 (0.47, 1.71) | <0.001\* |
| AM630+AM1241+morphine  *vs.* vehicle +saline | -0.55 (-1.07, -0.03) | 0.0072 |
| AM1241+morphine *vs.* vehicle+morphine | 1.65 (1.19, 2.11) | <0.0001\* |
| AM630+AM1241+morphine *vs.*  vehicle +morphine | 0.01 (-0.17, 0.20) | 0.8066 |

Note: \* *P* value is lower than the Bonferroni-corrected threshold of 0.0014 (0.01/7). MD = mean difference; CI = confidence interval. The P value of ANOVA of Mu opioid receptor mRNA expression in the DRG is greater than the significant level of 0.01 (*P*=0.0447), which indicates no statistical significant difference among the six groups. Therefore, no further comparisons between any two groups were performed.