Nicotine prevents and reverses paclitaxel-induced mechanical allodynia in a mouse model of CIPN

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Supplemental Figure 1. Acute administration of nicotine at doses of 0.3, 0.6, and 0.9 mg/kg i.p. does not affect mechanical threshold in vehicle-treated mice. BL, baseline. n = 8 per group; data expressed as mean ± SEM.

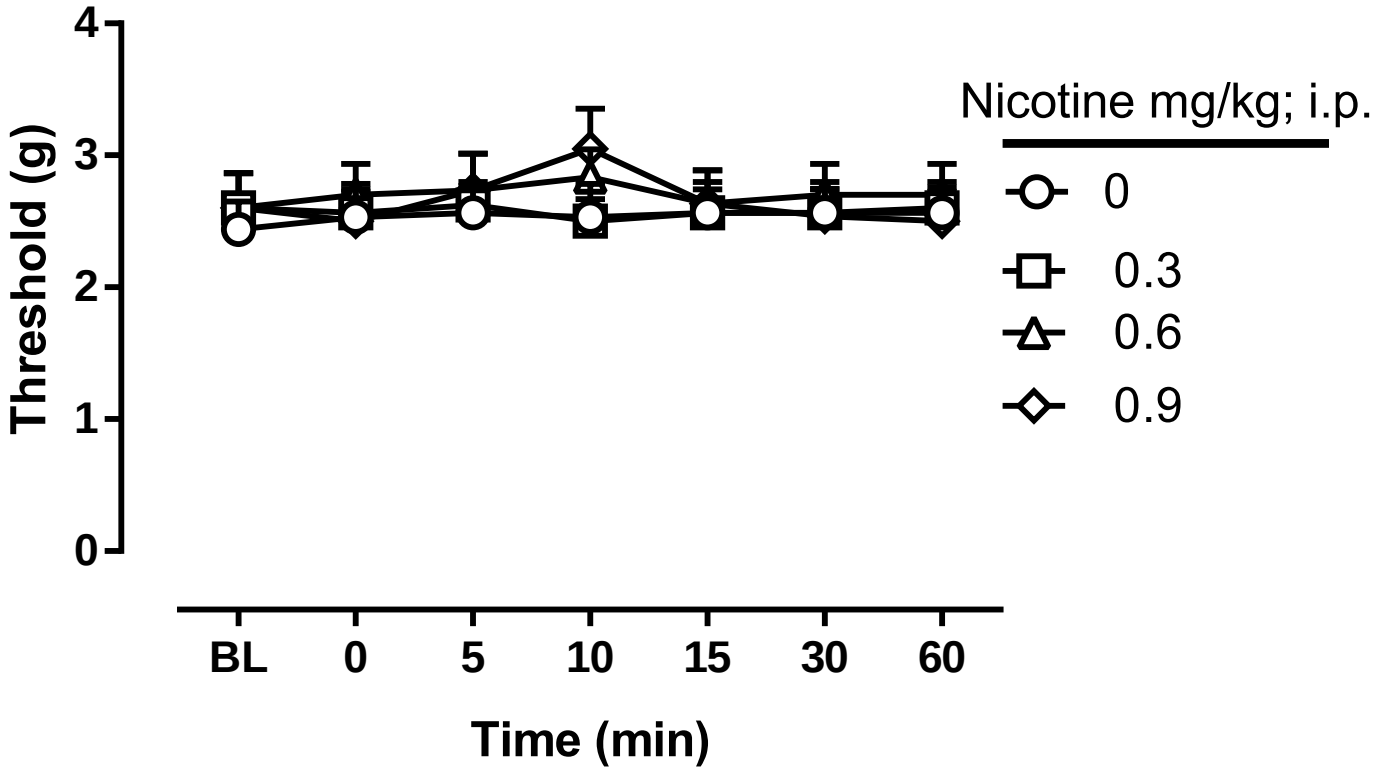
Supplemental Figure 2. Nicotine at doses of 6 and 12 mg/kg/day do not prevent paclitaxel-induced mechanical allodynia. Arrows indicate vehicle/paclitaxel injections on days 0, 2, 4, and 6. Minipumps with nicotine, 6 mg/kg/day (A) or 12 mg/kg/day (B), were implanted s.c. in the mouse, starting 2 days before the vehicle/paclitaxel treatment cycle and ending on day 5. Baseline measurements were taken at BL before saline/nicotine minipump implantation and on day 0 before paclitaxel/vehicle administration. ***$P < 0.001$ Pac-treated mice compared to Veh-treated mice. BL, baseline; Veh, vehicle; Sal, saline; Nic, nicotine; Pac, paclitaxel. n = 6 per group; data expressed as mean ± SEM. *Statistical analysis*: Comparisons were made between chemotherapy treatments (paclitaxel or vehicle) in nicotine- or saline-treated mice by day for both 6 and 12 mg/kg/day doses of nicotine in a 2 x 2 x 8 Mixed Factor ANOVA. The 6 mg/kg [F (7, 140) = 0.139, P = 0.995] and 12 mg/kg [F (7, 140) = 0.054, P = 1.000] nicotine 3-way interactions were not significant. However, both 6 mg/kg [F (7, 140) = 58.597, $P < 0.001$] and 12 mg/kg [F (7, 140) = 42.647, $P < 0.001$] nicotine produced a significant day by chemotherapy drug interaction, where

paclitaxel-treated mice demonstrated lower threshold compared to vehicle-treated mice on days 1, 3, 5, 7, 14, and 21 when compared to both baseline and day 0 responding ($P < 0.001$) as evaluated by Sidak post hoc tests.

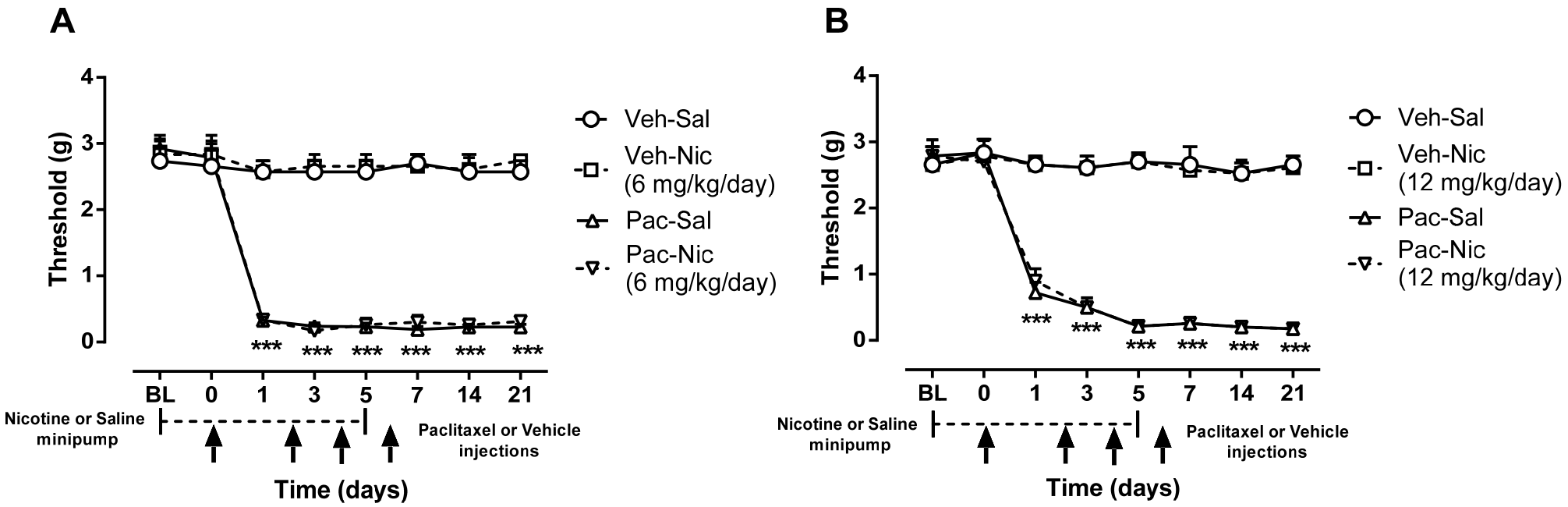
Supplemental Figure 3. Nicotine fails to enhance viable lung cancer cell number. NSCLC cells (A549, H460), Lewis lung carcinoma (LLC) cells, and human primary lung tumor cells (T1) were treated with nicotine for 24 h. Viability was determined with an MTT/MTS colorimetric assay. A one-way ANOVA followed by the Bonferroni post hoc test revealed no significant differences ($P > 0.05$) between control (0.0 μM) and any nicotine-treated cells within each cell line. Data are expressed as mean + SEM of two independent experiments.

Supplemental Figure 4. Nicotine fails to stimulate ovarian cancer cell proliferation. SKOV-3/DDP and OVCAR-3 cells were treated with nicotine for 48 h, then counted via trypan blue exclusion. A one-way ANOVA followed by the Bonferroni post-hoc test revealed no significant differences ($p > 0.05$) between any concentration of nicotine vs. control (0 μM) in each cell line. Data are expressed as mean + SEM of one representative study of two independent experiments.

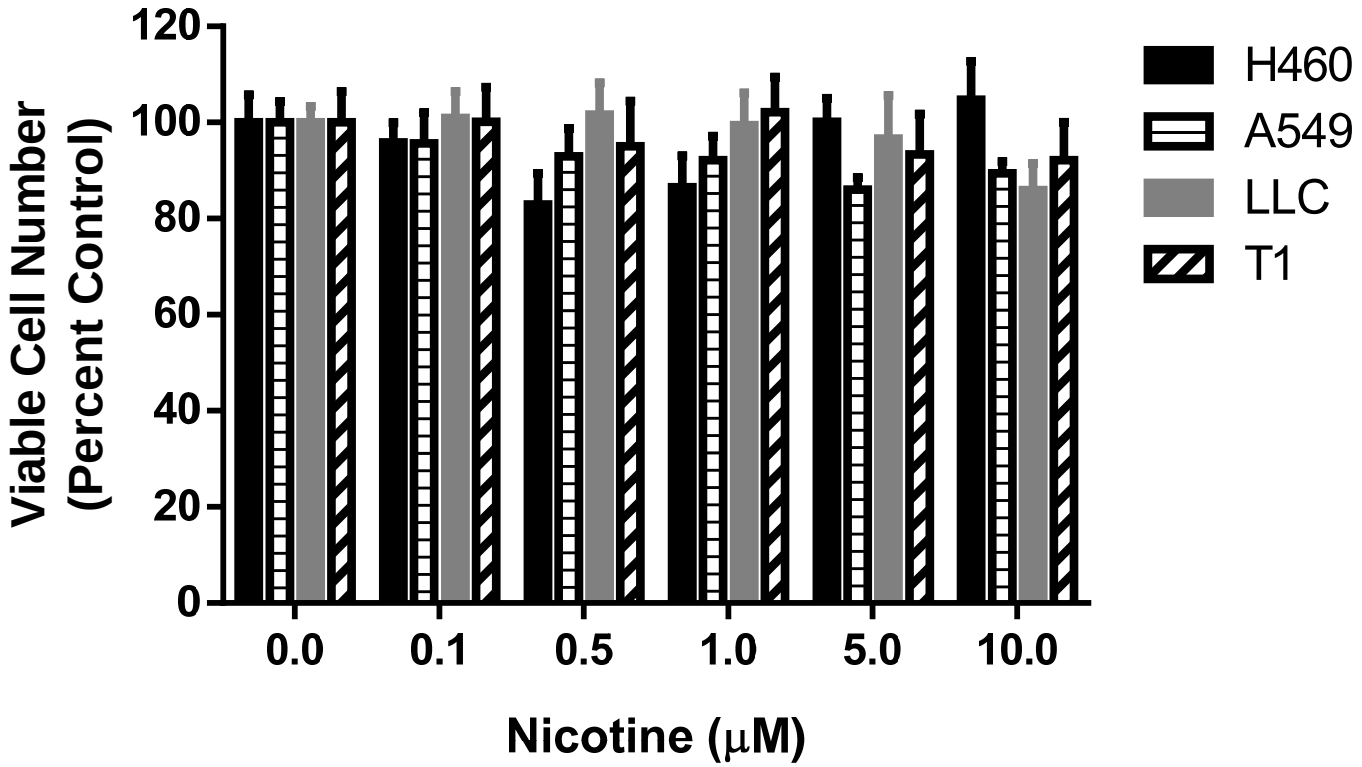
Sup Figure 1


Threshold (g)
4
3
2
1
0
BL
0
5
10
15
30
60
Time (min)
Nicotine mg/kg; i.p.
0
0.3
0.6
0.9

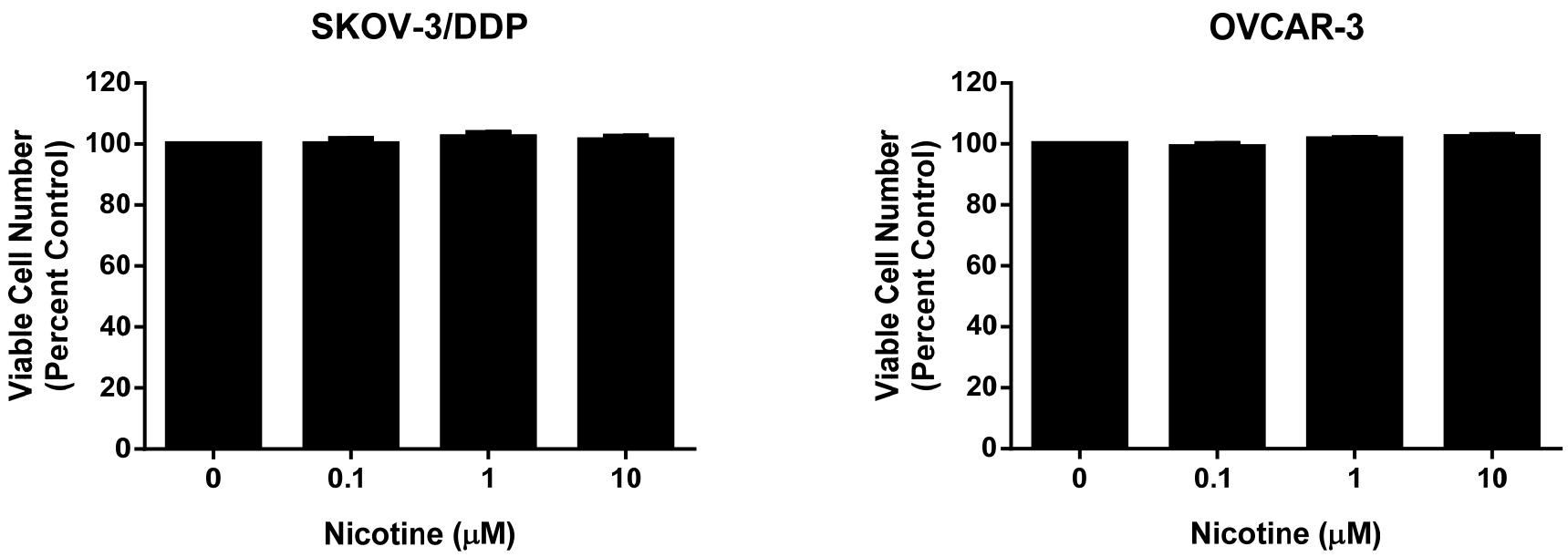
Sup Figure 2


A
Threshold (g)
4
3
2
1
0
***
***
***
***
***
***
BL
0
1
3
5
7
14
21
Nicotine or Saline minipump
Paclitaxel or Vehicle injections
Time (days)
Veh-Sal
Veh-Nic (6 mg/kg/day)
Pac-Sal
Pac-Nic (6 mg/kg/day)
B
Threshold (g)
4
3
2
1
0
***
***
***
***
***
***
BL
0
1
3
5
7
14
21
Nicotine or Saline minipump
Paclitaxel or Vehicle injections
Time (days)
Veh-Sal
Veh-Nic (12 mg/kg/day)
Pac-Sal
Pac-Nic (12 mg/kg/day)

Sup Figure 3


Viable Cell Number
(Percent Control)
120
100
80
60
40
20
0
0.0
0.1
0.5
1.0
5.0
10.0
Nicotine (μM)
H460
A549
LLC
T1

Sup Figure 4


SKOV-3/DDP
Viable Cell Number (Percent Control)
120
100
80
60
40
20
0
0
0.1
1
10
Nicotine (μM)
OVCAR-3
Viable Cell Number (Percent Control)
120
100
80
60
40
20
0
0
0.1
1
10
Nicotine (μM)