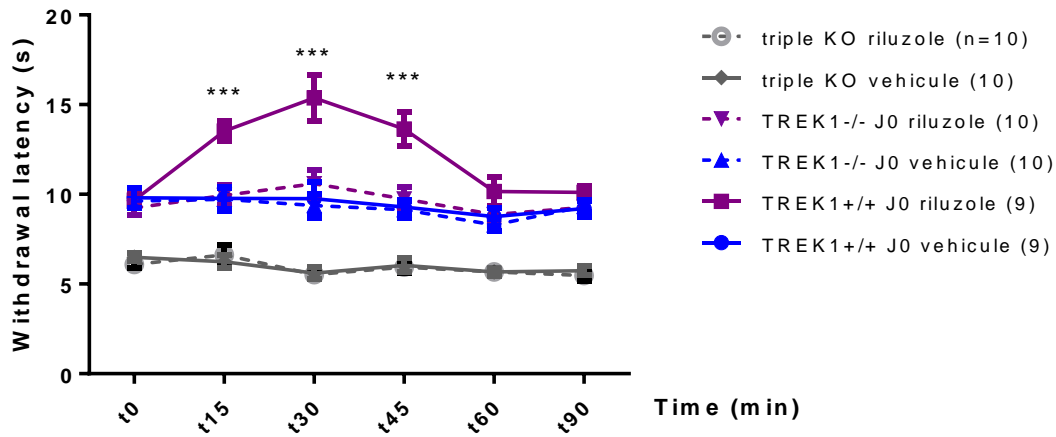


## Supplemental data

### RESEARCH ARTICLE:

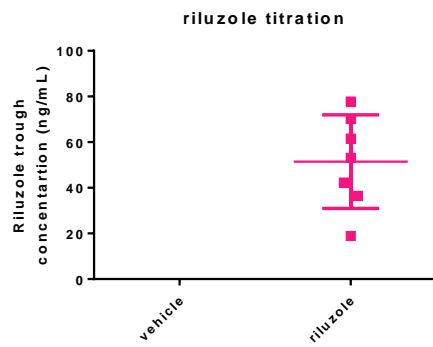
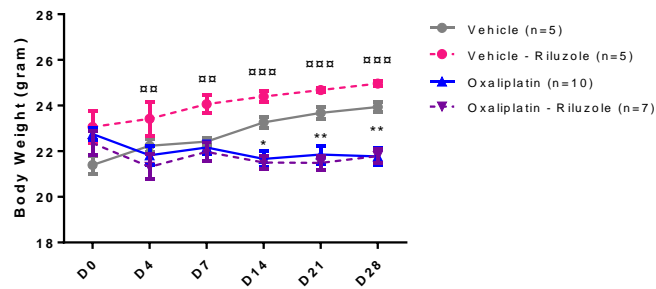
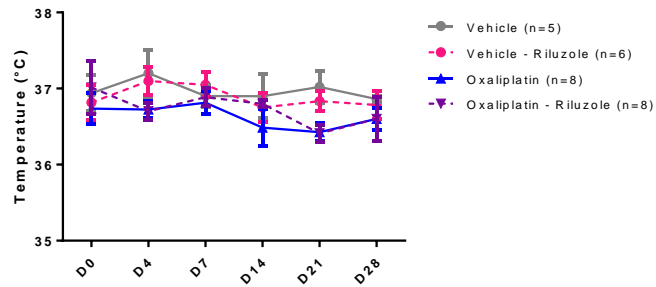
Title: Targeting the TREK-1 potassium channel via riluzole to eliminate the neuropathic and psychopathic effects of oxaliplatin

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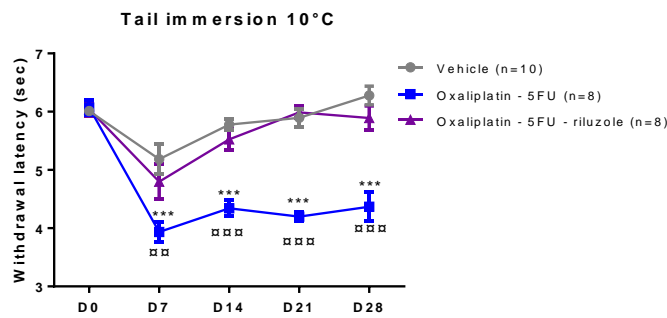
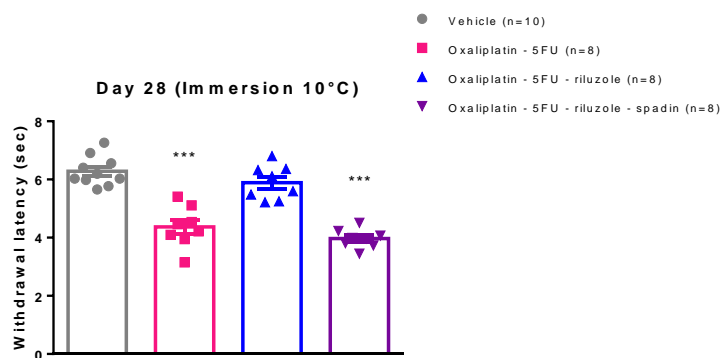


## Suppl. Data 1

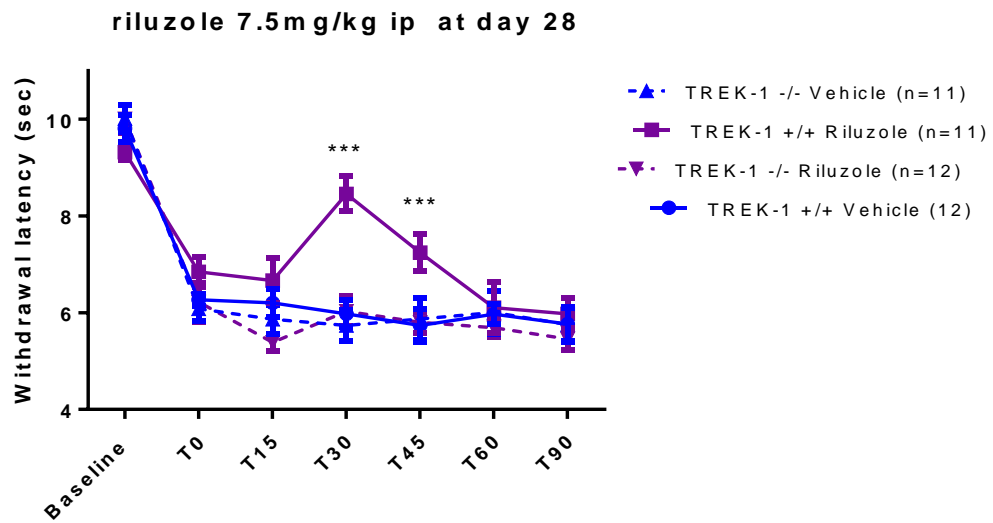
Supplementary data 1: Riluzole causes an analgesic effect that involves TREK-1 channel action in naive mice. After baseline latency recordings, kinetics of the antinociceptive effect of riluzole (7.5 mg/kg, i.p.) were assessed in mice deleted or not for TREK-1 or triple TREK-1/TREK-2/TRAAK channels using the tail immersion test (10°C). Mice were injected either with riluzole (7.5 mg/kg, i.p.) or saline (NaCl 0.9%). Pre- and post- (15, 30, 45, 60 and 90 min after) treatment withdrawal latencies were measured. Data are represented as the time-course of withdrawal latencies (n=9-10 per group). Statistical analysis was performed using a two-way repeated measure analysis of variance (RM ANOVA), detailed in Table1, and a Bonferroni post hoc test; \*\*\*,  $p < 0.001$ , TREK-1<sup>+/+</sup> vehicle *versus* TREK-1<sup>+/+</sup> riluzole.

**A****B****C****Suppl. Data 2**

Supplementary data 2: Blood concentration assessment of riluzole (A) and mice monitoring during chronic oxaliplatin-induced neuropathy development (B-C). (A) Riluzole trough concentration obtained at day 28 (51.5 +/- 7.8 ng/mL), while it was undetectable in the vehicle group (detection limit 5ng/mL), n = 7 per group. Mice body weight (B) and temperature (C) were monitored weekly until day 28. Oxaliplatin treatment slowed down the normal growth of the animals. However, neither oxaliplatin nor riluzole led to significant animal weight loss (B) or to temperature changes (C) along the experiment. Values are mean ± SEM (n= 5-10 per group). Statistical analysis was performed using a two-way repeated measure analysis of variance (RM ANOVA), detailed in Table 1, and a Bonferroni post hoc test. \*,  $p < 0.05$ , \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.001$ , vehicle versus oxaliplatin; α,  $p < 0.05$ , αα,  $p < 0.01$ , ααα,  $p < 0.001$ , oxaliplatin versus oxaliplatin + riluzole.

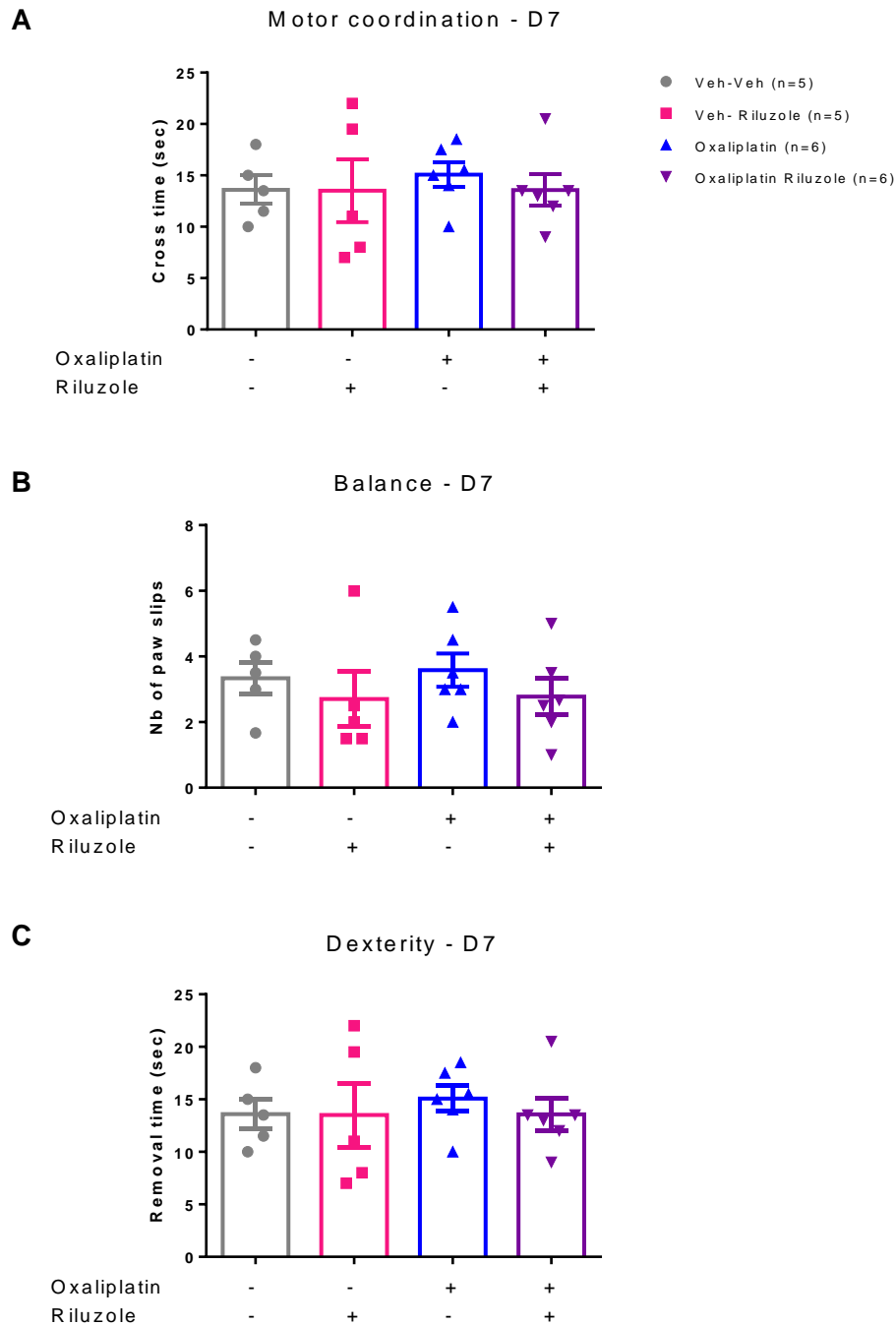
**A****B****Suppl. Data 3**

Supplementary data 3: Spadine significantly prevented riluzole-induced analgesic effect in oxaliplatin + 5FU-treated C57Bl6/JRj mice. **(A)** Thermal cold pain symptom was assessed using the tail immersion test in mice co-administered with oxaliplatin (2 mg/kg) and 5-FU (15mg/k) by i.v. route twice a week for 4 weeks, with or without riluzole treatment (60 µg/mL in the drinking water). Values are mean ± SEM (n=8-10 per group). Statistical analysis was performed using a two-way repeated measure analysis of variance (RM ANOVA), detailed in Table 1, and a Bonferroni post hoc test; \*\*\*,  $p < 0.001$ , versus the vehicle group; \*\*,  $p < 0.01$ , \*\*\*,  $p < 0.0001$  versus the oxaliplatin + 5-FU group. **(B)** At day28, oxaliplatin + 5-FU + riluzole treated animals received vehicle or spadin (1 mg/kg, s.c.). Animal receiving neither oxaliplatin nor 5-FU or riluzole served as control. Values are mean ± SEM (n=8-10 per group). Statistical analysis was performed using a one-way analysis of variance (ANOVA), detailed in Table 1, and a Bonferroni post hoc test; \*\*\*,  $p < 0.001$ , versus the vehicle group and the FOLFOX + riluzole group.



## Suppl. data 4

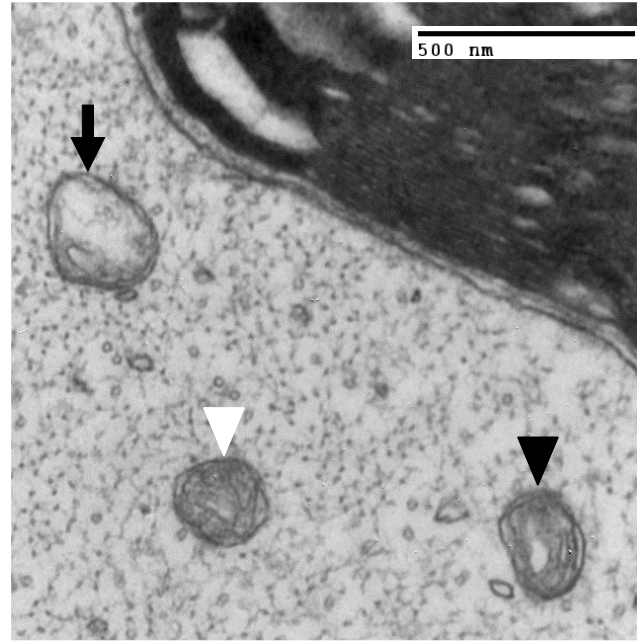
Supplementary data 4: Riluzole exerts an analgesic effect in oxaliplatin-treated TREK-1<sup>+/+</sup>, but not in TREK-1<sup>-/-</sup>, mice in the presence of a fully developed allodynia (day 28). Thermal cold pain symptom was assessed using the tail immersion test in mice administered with oxaliplatin (6 mg/kg, i.p.) or vehicle (Glucose 5%) twice a week for 4 weeks. Riluzole (7.5 mg/kg, i.p.) was administered at day 28. Values are mean  $\pm$  SEM (n=11-12 per group). Statistical analysis was performed using a two-way repeated measure analysis of variance (RM ANOVA), detailed in Table 1, and a Bonferroni post hoc test; \*\*\*,  $p < 0.001$ , TREK-1<sup>+/+</sup> vehicle group versus TREK-1<sup>+/+</sup> riluzole group.



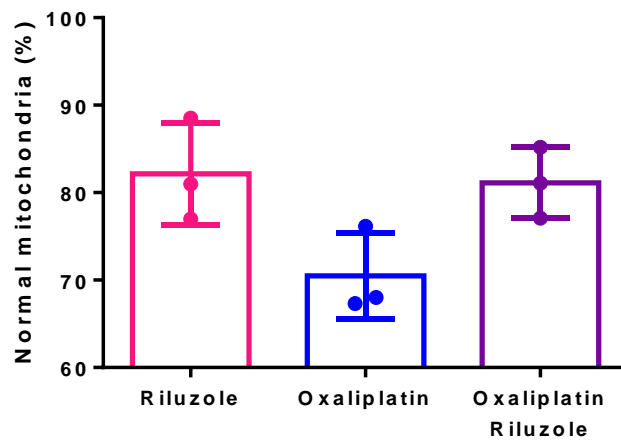
### Suppl. data 5

Supplementary data 5: Sensory-motor abilities are not impaired in early phase of oxaliplatin-induced neuropathy development in C57Bl6/J mice. Fine motor coordination (A), balance (B) and dexterity (C) were assessed using the beam walking and the adhesive removal tests at day 7 after two injections of oxaliplatin (6 mg/kg, i.p.) with or without riluzole treatment (60 µg/mL in the drinking water). Values are mean ± SEM (n=5/6 per group). Statistical analysis was performed using a two-way analysis of variance (ANOVA), detailed in Table 1.

A

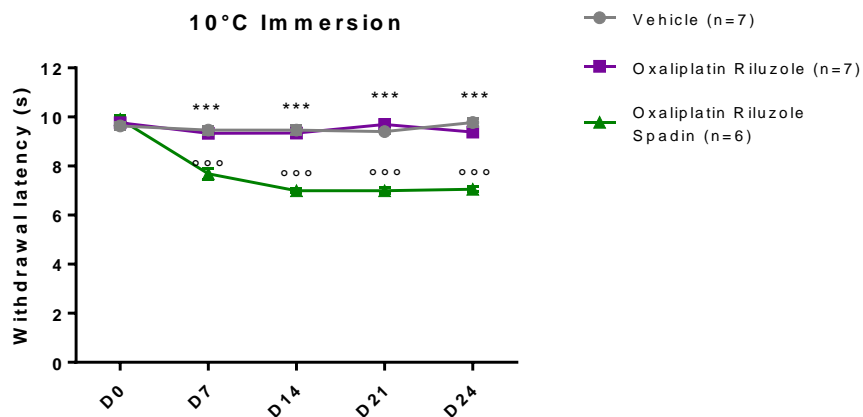


B



### Suppl. data 6

Supplementary data 6: (A) Transmission electron microscopy (TEM) image showing normal and abnormal mitochondria (white arrowhead: normal mitochondria; black arrowhead: vacuolated mitochondria; black arrow: degraded mitochondria) observed from sciatic nerves (scale bar = 500nm). (B) Percentage of normal mitochondria found in sciatic nerves myelinated and amyelinated fibers from the riluzole, oxaliplatin and oxaliplatin + riluzole groups. Values are mean  $\pm$  SEM (n=3 per group). Statistical analysis was performed using a one-way analysis of variance (ANOVA), detailed in Table 1.



## Suppl. data 7

Supplementary data 7: Spadin significantly decreased riluzole-induced analgesic effect in *Apc<sup>Min/+</sup>* mice. Thermal cold pain symptoms were assessed using the tail immersion test once a week after the first injection of oxaliplatin (6 mg/kg, i.p.) with riluzole treatment (60 µg/mL in the drinking water). Spadin (1mg/kg, s.c.) was administrated in one group of oxaliplatin and riluzole-treated mice. Animal receiving neither oxaliplatin, nor riluzole or spadin served as control. Values are mean ± SEM (n = 6-7 per group). Statistical analysis was performed using a two-way repeated measure analysis of variance (RM ANOVA), detailed in Table 1, and a Bonferroni post hoc test; \*\*\*,  $p < 0.001$ , vehicle group *versus* the oxaliplatin-riluzole-spadin, °°°,  $p < 0.001$ , between the oxaliplatin-riluzole group and the oxaliplatin-riluzole-spadin group.