

Data analysis summary

Figure	Description	n number	Outliers (data points)	Passed normality test ?	Test	F	p	Post-hoc test	versus
1a	RNE28 dose-effect in HEK-293 cells	6 TREK-1 at 0.1 µM 6 TREK-1 at 1 µM 5 TREK-1 at 10 µM 6 TREK-1 at 20 µM 7 TREK-1 at 50 µM 7 TREK-1 at 100 µM 5 TREK-1 at 200 µM 10 TREK-2 at 100 µM 6 TRAAK at 100 µM	0 (58)	N/A	N/A	N/A	N/A	N/A	N/A
1b left	Analgesia induced by RNE28 in naive mice - time-course	5 vehicle p.o. 8 RNE28 15 mg/kg p.o.	2 (140)	N/A	two-way ANOVA	Interaction 4.133 Time 8.888 Treatment 29.76	Interaction <0.0001 Time <0.0001 Treatment <0.0001	Dunnett's	Vehicle
1b right	Analgesia induced by RNE28 in naive mice - AUC	7 RNE28 30 mg/kg p.o. 8 RNE28 60 mg/kg p.o.	0 (28)	no	Kruskal-Wallis	N/A	0.0002	Dunn's	Vehicle
1c left	Analgesia induced by RNE28 in carrageena-injected mice - time-course	5 vehicle p.o. 7 RNE28 15 mg/kg p.o.	0 (135)	N/A	two-way ANOVA	Interaction 6.685 Time 18.52 Treatment 40.93	Interaction <0.0001 Time <0.0001 Treatment <0.0001	Dunnett's	Vehicle
1c right	Analgesia induced by RNE28 in carrageena-injected mice - AUC	7 RNE28 30 mg/kg p.o. 8 RNE28 60 mg/kg p.o.	0 (27)	no	Kruskal-Wallis	N/A	0.0002	Dunn's	Vehicle
1d	RNE28 dose effect curves in naive and carrageenan-injected mice	5 vehicle p.o. - naive 5 vehicle p.o. - carrageenan 8 RNE28 15 mg/kg p.o. - naive 7 RNE28 15 mg/kg p.o. - carrageenan 7 RNE28 30 mg/kg p.o. - naive 7 RNE28 30 mg/kg p.o. - carrageenan 8 RNE28 60 mg/kg p.o. - naive 8 RNE28 60 mg/kg p.o. - carrageenan	0 (55)	N/A	Comparison of fits: extra sum-of-square F test	3.166	0.0220	N/A	N/A
					two-way ANOVA	Interaction 1.211 Model 7.314 Dose 25.25	Interaction 0.3161 Model 0.0095 Dose <0.0001	Sidak's	Naive
2a left	Analgesic effect of RNE28 on mechanical pain in a mouse model of post-operative pain - Time-course	10 vehicle p.o. 10 morphine 3 mg/kg p.o.	2 (240)	N/A	two-way ANOVA	Interaction 2.132 Time 25.82 Treatment 10.45	Interaction 0.0096 Time <0.0001 Treatment <0.0001	Dunnett's	Vehicle

Figure	Description	n number	Outliers (data points)	Passed normality test ?	Test	F	p	Post-hoc test	versus
2a right	Analgesic effect of RNE28 on mechanical pain in a mouse model of post-operative pain - AUC	10 RNE28 15 mg/kg p.o. 10 RNE28 30 mg/kg p.o.	0 (40)	yes	one-way ANOVA	8.185	0.0003	Dunnett's	Vehicle
2b left	Analgesic effect of RNE28 on thermal pain in a mouse model of post-operative pain - Time-course	8 vehicle p.o. 8 morphine 3 mg/kg p.o. 8 RNE28 15 mg/kg p.o. 8 RNE28 30 mg/kg p.o.	1 (224)	N/A	two-way ANOVA	Interaction 2.769 Time 22.50 Treatment 25.99	Interaction 0.0003 Time <0.0001 Treatment <0.0001	Dunnett's	Vehicle
2b right	Analgesic effect of RNE28 on thermal pain in a mouse model of post-operative pain - AUC		0 (32)	yes	one-way ANOVA	2.953	0.0497	Dunnett's	Vehicle
2c left	Analgesic effect of RNE28 on mechanical pain in a rat model of inflammatory pain - Time-course	8 vehicle p.o. 8 morphine 5 mg/kg p.o. 8 RNE28 100 mg/kg p.o. 8 RNE28 200 mg/kg p.o.	1 (256)	N/A	two-way ANOVA	Interaction 7.529 Time 126.9 Treatment 89.29	Interaction <0.0001 Time <0.0001 Treatment <0.0001	Dunnett's	Vehicle
2c right	Analgesic effect of RNE28 on mechanical pain in a rat model of inflammatory pain - AUC		0 (32)	yes	one-way ANOVA	17.89	<0.0001	Dunnett's	Vehicle
2d left	Analgesic effect of RNE28 on mechanical pain in a rat model of neuropathic pain - Time-course	7 vehicle p.o. 9 gabapentin 100 mg/kg p.o. 10 RNE28 100 mg/kg p.o. 10 RNE28 200 mg/kg p.o.	4 (288)	N/A	two-way ANOVA	Interaction 0.9037 Time 64.92 Treatment 9.897	Interaction 0.5866 Time <0.0001 Treatment <0.0001	Dunnett's	Vehicle
2d right	Analgesic effect of RNE28 on mechanical pain in a rat model of neuropathic pain - AUC		0 (36)	no	Kruskal-Wallis	N/A	0.0215	Dunn's	Vehicle
3a left	Analgesia induced by RNE28 in WT and TREK-1 ^{-/-} mice - time course	9 WT 8 TREK-1 ^{-/-}	1 (85)	N/A	two-way ANOVA	Interaction 2.649 Time 5.844 Genotype 13.20	Interaction 0.0399 Time 0.0004 Genotype 0.0005	Sidak's	WT

Figure	Description	n number	Outliers (data points)	Passed normality test ?	Test	F	p	Post-hoc test	versus
3a right	Analgesia induced by RNE28 in WT and TREK-1 ^{-/-} mice - AUC	9 WT 8 TREK-1 ^{-/-}	0 (17)	yes	unpaired two-tailed t-test	4.2	0.0294	N/A	N/A
3b left	Administration of subcutaneous morphine together with subcutaneous spadine in naive mice - time course	6 Morphine 3 mg/kg + Vehicle s.c. 9 Morphine 3 mg/kg + Spadine 0.1 mg/kg s.c. 9 Morphine 3 mg/kg + Spadine 1 mg/kg s.c. 6 Morphine 3 mg/kg + Spadine 10 mg/kg s.c.	0 (180)	N/A	two-way ANOVA	Interaction 4.478 Time 15.06 Treatment 43.26	Interaction <0.0001 Time <0.0001 Treatment <0.0001	Sidak's	Vehicle
3b right	Administration of subcutaneous morphine together with subcutaneous spadine in naive mice - AUC	6 Morphine 3 mg/kg + Vehicle s.c. 9 Morphine 3 mg/kg + Spadine 0.1 mg/kg s.c. 9 Morphine 3 mg/kg + Spadine 1 mg/kg s.c. 6 Morphine 3 mg/kg + Spadine 10 mg/kg s.c.	0 (30)	no	Kruskal-Wallis	N/A	0.0002	Dunn's	Vehicle
3c left	Administration of intraperitoneal RNE28 together with subcutaneous spadine in naive mice - time course	9 RNE28 30 mg/kg i.p. + Vehicle s.c. 10 RNE28 30 mg/kg i.p. + Spadine 1 mg/kg s.c.	1 (95)	N/A	two-way ANOVA	Interaction 3.704 Time 21.51 Treatment 36.42	Interaction 0.0040 Time <0.0001 Treatment <0.0001	Sidak's	RNE28 + Vehicle
3c right	Administration of intraperitoneal RNE28 together with subcutaneous spadine in naive mice - AUC	9 RNE28 30 mg/kg i.p. + Vehicle s.c. 10 RNE28 30 mg/kg i.p. + Spadine 1 mg/kg s.c.	0 (19)	yes	unpaired two-tailed t-test	2.925	0.0003	N/A	N/A
3d left	Administration of intraperitoneal RNE28 together with intrathecal spadine in naive mice - time course	10 RNE28 30 mg/kg i.p. + Vehicle i.t. 10 RNE28 30 mg/kg i.p. + Spadine 5 pmol i.t.	0 (100)	N/A	two-way ANOVA	Interaction 9.486 Time 17.95 Treatment 54.34	Interaction <0.0001 Time <0.0001 Treatment <0.0001	Sidak's	RNE28 + Vehicle
3d right	Administration of intraperitoneal RNE28 together with intrathecal spadine in naive mice - AUC	10 RNE28 30 mg/kg i.p. + Vehicle i.t. 10 RNE28 30 mg/kg i.p. + Spadine 5 pmol i.t.	0 (20)	yes	unpaired two-tailed t-test	1.878	<0.0001	N/A	N/A
3e left	Administration of intraperitoneal RNE28 together with intracerebroventricular spadine in naive mice - time course	9 RNE28 30 mg/kg i.p. + Vehicle i.c.v. 10 RNE28 30 mg/kg i.p. + Spadine 2 pmol i.c.v.	0 (95)	N/A	two-way ANOVA	Interaction 2.526 Time 20.13 Treatment 28.76	Interaction 0.0341 Time <0.0001 Treatment <0.0001	Sidak's	RNE28 + Vehicle
3e right	Administration of intraperitoneal RNE28 together with intracerebroventricular spadine in naive mice - AUC	9 RNE28 30 mg/kg i.p. + Vehicle i.c.v. 10 RNE28 30 mg/kg i.p. + Spadine 2 pmol i.c.v.	0 (19)	no	unpaired two-tailed Mann-Whitney test	N/A	0.0044	N/A	N/A
3f left	Administration of intraperitoneal RNE28 together with intraplantar spadine in naive mice - time course	9 RNE28 30 mg/kg i.p. + Vehicle i.pl. 10 RNE28 30 mg/kg i.p. + Spadine 20 pmol i.pl.	1 (95)	N/A	two-way ANOVA	Interaction 3.450 Time 15.71 Treatment 8.803	Interaction 0.0064 Time <0.0001 Treatment 0.0038	Sidak's	RNE28 + Vehicle

Figure	Description	n number	Outliers (data points)	Passed normality test ?	Test	F	p	Post-hoc test	versus
3f right	Administration of intraperitoneal RNE28 together with intraplantar spadine in naive mice - AUC	9 RNE28 30 mg/kg i.p. + Vehicle i.pl. 10 RNE28 30 mg/kg i.p. + Spadine 20 pmol i.pl.	1 (19)	no	unpaired two-tailed Mann-Whitney test	N/A	0.0028	N/A	N/A
3g	Forced swimming test in RNE28-treated naive mice	9 vehicle p.o. 9 duloxetine 10 mg/kg i.p. 9 RNE29 15 mg/kg p.o. 9 RNE29 30 mg/kg p.o. 9 RNE29 60 mg/kg p.o.	0 (45)	yes	one-way ANOVA	19.54	<0.0001	Dunnett's	Vehicle
3h	Tail suspension test in RNE28-treated naive mice	8 vehicle p.o. 8 duloxetine 10 mg/kg i.p. 8 RNE28 15 mg/kg p.o. 8 RNE28 30 mg/kg p.o. 8 RNE28 60 mg/kg p.o.	0 (40)	no	Kruskal-Wallis	N/A	0.0002	Dunn's	Vehicle
4b	Changes in current density at 60 mV induced by RNE28 on TREK1/TRAAK chimeras	13 TREK-1 ; 6 TRAAK ; 11 Chimera 1 ; 7 Chimera 2 ; 10 Chimera 3 ; 20 Chimera 4 ; 6 Chimera 5 ; 14 Chimera 6 ; 8 Chimera 7	0 (190)	N/A	two-way ANOVA	Interaction 3.652 Construct 3.652 Perfusion 27.65	Interaction 0.0006 Construct 0.0006 Perfusion <0.0001	Sidak's	Baseline
4c	TREK-1 baseline vs RNE28 I/V curve	13 TREK-1	0 (856)	N/A	two-way ANOVA	Interaction 10.87 Voltage 42.96 Perfusion 353.4	Interaction <0.0001 Voltage <0.0001 Perfusion <0.0001	Sidak's	Baseline
4d	TRAAK baseline vs RNE28 I/V curve	6 TRAAK	0 (396)	N/A	two-way ANOVA	Interaction 0.3631 Voltage 45.34 Perfusion 5.406	Interaction 0.9995 Voltage <0.0001 Perfusion 0.0207	Sidak's	Baseline
4e	Chimera 4 baseline vs RNE28 I/V curve	20 chimera 4	6 (1320)	N/A	two-way ANOVA	Interaction 3.819 Voltage 49.65 Perfusion 150.4	Interaction <0.0001 Voltage <0.0001 Perfusion <0.0001	Sidak's	Baseline
4f	Chimera 6 baseline vs RNE28 I/V curve	14 chimera 6	4 (924)	N/A	two-way ANOVA	Interaction 8.194 Voltage 54.65 Perfusion 257.7	Interaction <0.0001 Voltage <0.0001 Perfusion <0.0001	Sidak's	Baseline
5a	Intestinal transit (bead expulsion) in RNE28-treated naive mice	6 vehicle p.o. 6 morphine 3 mg/kg p.o. 6 RNE28 15 mg/kg p.o. 5 RNE28 30 mg/kg p.o. 6 RNE28 60 mg/kg p.o.	0 (29)	no	Kruskal-Wallis	N/A	0.0184	Dunn's	Vehicle
5b	Gastrointestinal transit (methylene blue gavage) in RNE28-treated naive mice	9 vehicle p.o. 9 morphine 3 mg/kg p.o. 9 RNE28 15 mg/kg p.o. 9 RNE28 30 mg/kg p.o. 8 RNE28 60 mg/kg p.o.	0 (44)	yes	one-way ANOVA	10.46	<0.0001	Dunnett's	Vehicle

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5c	Sedation (infrared actimeter) in RNE28-treated naive mice	9 vehicle p.o. 10 RNE28 15 mg/kg p.o. 10 RNE28 30 mg/kg p.o. 10 RNE28 60 mg/kg p.o.	0 (39)	no	Kruskal-Wallis	N/A	0.1816	Dunn's	Vehicle
5d	Sedation (rotarod) in RNE28-treated naive mice	9 vehicle p.o. 10 RNE28 15 mg/kg p.o. 10 RNE28 30 mg/kg p.o. 10 RNE28 60 mg/kg p.o.	0 (39)	yes	one-way ANOVA	2.275	0.0970	Dunnett's	Vehicle
5e	Respiratory rate of RNE28-treated naive mice	6 vehicle p.o. 6 morphine 50 mg/kg p.o. 6 RNE28 15 mg/kg p.o. 6 RNE28 30 mg/kg p.o. 6 RNE28 60 mg/kg p.o.	0 (180)	N/A	two-way ANOVA	Interaction 0.7013 Time 7.836 Treatment 14.35	Interaction 0.8204 Time <0.0001 Treatment <0.0001	Dunnett's	Vehicle
5f	Conditioned place preference test with chronic RNE28 treatment in naive mice	12 vehicle p.o. 9 morphine 15 mg/kg p.o. 12 RNE28 15 mg/kg p.o. 12 RNE28 30 mg/kg p.o. 12 RNE28 60 mg/kg p.o.	0 (57)	yes	one-way ANOVA	5.63	0.0008	Dunnett's	Vehicle
Supp Fig 2	Analgesic activity of morphine in naive and inflamed animals	10 morphine 3 mg/kg p.o., naive 10 morphine 3 mg/kg p.o., carrageenan	0 (80)	N/A	Comparison of fits: extra sum-of-square F test	12.06	<0.0001	N/A	N/A
Supp Fig 4	RNE28 +/- spadin effect on TREK-1 current density	24 Vehicle + Vehicle 29 RNE28 100 μM + Vehicle 14 RNE28 100 μM + spadin 1 μM 10 Vehicle + spadin 1 μM	0 (77)	yes	one-way ANOVA	3.916	0.0119	Dunnett's	Vehicle + Vehicle
Supp Fig 5	Tolerance to the antinociceptive effect of RNE28	12 morphine 5 mg/kg p.o. 12 RNE28 30 mg/kg p.o.	0 (96)	N/A	two-way ANOVA	Interaction 13.09 Time 24.99 Treatment 5.154	Interaction 0.0002 Time 0.0749 Treatment <0.0001	Dunnett's	Day 0
Supp Fig 6	Naloxone-precipitated withdrawal in RNE28-treated mice	6 morphine 5 mg/kg p.o. 6 RNE28 30 mg/kg p.o.	1 (12)	no	unpaired two-tailed Mann-Whitney test	N/A	0.0043	N/A	N/A

Figure	Description	n number	Outliers (data points)	Passed normality test ?	Test	F	p	Post-hoc test	versus
Supp Fig 6a left	Analgesia induced by riluzole in naive WT and TREK-1 KO mice - time-course	6 WT vehicle 6 TREK-1 KO vehicle 6 WT riluzole 7.5 mg/kg i.p. 6 TREK-1 KO riluzole 7.5 mg/kg i.p.	0 (96)	N/A	two-way ANOVA	Interaction 1.844 Time 2.196 Treatment 13.79	Interaction 0.0509 Time <0.0001 Treatment 0.0045	Sidak's	every other group
Supp Fig 6a right	Analgesia induced by riluzole in naive WT and TREK-1 KO mice - AUC		0 (24)	no	Kruskal-Wallis	N/A	0.0042	Dunn's	every other group
Supp Fig 7b left	Analgesia induced by riluzole in carrageena-injected WT and TREK-2 KO mice - time-course	7 WT vehicle 7 TREK-2 KO vehicle 7 WT riluzole 7.5 mg/kg i.p. 7 TREK-2 KO riluzole 7.5 mg/kg i.p.	0 (112)	N/A	two-way ANOVA	Interaction 4.120 Time 15.90 Treatment 16.18	Interaction <0.0001 Time <0.0001 Treatment <0.0001	Sidak's	every other group
Supp Fig 7b right	Analgesia induced by riluzole in carrageena-injected WT and TREK-2 KO mice - AUC		0 (28)	no	Kruskal-Wallis	N/A	0.0001	Dunn's	every other group