Enhancing Clinical Translation of Analgesics for Neuropathic Pain: A Systematic Review and Meta-Analysis on the Role of Non-Evoked Pain Assessment in Preclinical Trials

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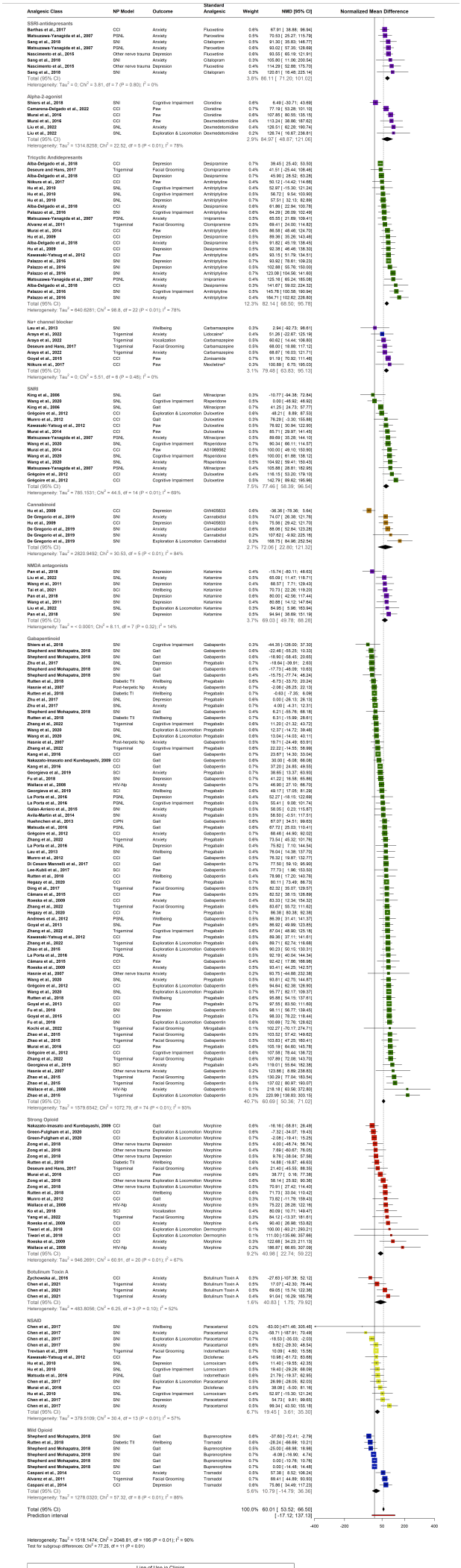
Supplementary Table 1. Search strategy.

Database	
Medline	(("neuropathic pain*"[tw] OR "nerve pain*"[tw] OR "Neurodynia*"[tw] OR "neuropathy"[tw] OR "neuropathies"[tw] OR "nerve injuries"[tw] OR "nerve injury"[tw] OR "nerve damage"[tw] OR "nerve trauma"[tw] OR "nerve trauma*"[tw] OR "Peripheral Nerve Injuries"[Mesh] OR "Nervous System/injuries"[Mesh] OR "Neuralgia"[Mesh] OR "Neuralgia*"[tw] OR Polyneuropathies[Mesh] OR Polyneuropathies [tiab] OR Polyneuropathy [tiab] OR neurodynia [tiab] OR Peripheral Nerve atrophy [tiab] OR axonopathy [tiab] OR axonopathies [tiab] OR myelinopathy [tiab] OR myelinopathies [tiab] OR neuralgic pain [tiab] OR neuralgic pains [tiab] OR “spinal nerve ligation” OR “chronic constriction injury” OR “partial sciatic nerve ligation” OR “spared nerve injury” OR “postherpetic neuralgia” OR “chemotherapy induced peripheral neuropathy” OR “diabetic peripheral neuropathy” OR "trigeminal neuralgia") AND ("non-evoked pain" OR "spontaneous pain" OR "nociceptive behaviour*" OR "pain-related behaviour*" OR "pain-related behavior*" OR "pain at rest" OR "pain in the absence of stimulation" OR "painful behaviour" OR "painful behavior")) AND ("animal experimentation"[MeSH Terms] OR "models, animal"[MeSH Terms] OR "invertebrates"[MeSH Terms] OR "Animals"[Mesh:noexp] OR "animal population groups"[MeSH Terms] OR "chordata"[MeSH Terms:noexp] OR "chordata, nonvertebrate"[MeSH Terms] OR "vertebrates"[MeSH Terms:noexp] OR "amphibians"[MeSH Terms] OR "birds"[MeSH Terms] OR "fishes"[MeSH Terms] OR "reptiles"[MeSH Terms] OR "mammals"[MeSH Terms:noexp] OR "primates"[MeSH Terms:noexp] OR "artiodactyla"[MeSH Terms] OR "carnivora"[MeSH Terms] OR "cetacea"[MeSH Terms] OR "chiroptera"[MeSH Terms] OR "elephants"[MeSH Terms] OR "hyraxes"[MeSH Terms] OR "insectivora"[MeSH Terms] OR "lagomorpha"[MeSH Terms] OR "marsupialia"[MeSH Terms] OR "monotremata"[MeSH Terms] OR "perissodactyla"[MeSH Terms] OR "rodentia"[MeSH Terms] OR "scandentia"[MeSH Terms] OR "sirenia"[MeSH Terms] OR "xenarthra"[MeSH Terms] OR "haplorhini"[MeSH Terms:noexp] OR "strepsirhini"[MeSH Terms] OR "platyrrhini"[MeSH Terms] OR "tarsii"[MeSH Terms] OR "catarrhini"[MeSH Terms:noexp] OR "cercopithecidae"[MeSH Terms] OR "hylobatidae"[MeSH Terms] OR "hominidae"[MeSH Terms:noexp] OR "gorilla gorilla"[MeSH Terms])
Scopus	TITLE-ABS-KEY ((("neuropathic pain*" OR "nerve pain*" OR "Neurodynia*" OR "neuropathy" OR "neuropathies" OR "nerve injuries" OR "nerve injury" OR "nerve damage" OR "nerve trauma" OR "nerve trauma*" OR "Peripheral Nerve Injuries") AND ("non-evoked pain" OR "spontaneous pain" OR "nociceptive behaviour*" OR "pain-related behaviour*" OR "pain-related behavior*" OR "pain at rest" OR "pain in the absence of stimulation" OR "painful behaviour" OR "painful behavior") AND (animals OR animal OR mice OR mus OR mouse OR murine OR woodmouse OR rats OR rat OR murinae OR muridae OR cottonrat OR cottonrats OR hamster OR hamsters OR cricetinae OR rodentia OR rodent OR rodents)))

Web of Science	AB=(("neuropathic pain*" OR "nerve pain*" OR "Neurodynia*" OR "neuropathy" OR "neuropathies" OR "nerve injuries" OR "nerve injury" OR "nerve damage" OR "nerve trauma" OR "nerve trauma*" OR "Peripheral Nerve Injuries") AND ("non-evoked pain" OR "spontaneous pain" OR "nociceptive behaviour*" OR "pain-related behaviour*" OR "pain-related behavior*" OR "pain at rest" OR "pain in the absence of stimulation" OR "painful behaviour" OR "painful behavior") AND (animals OR animal OR mice OR mus OR mouse OR murine OR woodmouse OR rats OR rat OR murinae OR muridae OR cottonrat OR cottonrats OR hamster OR hamsters OR cricetinae OR rodentia OR rodent OR rodents))

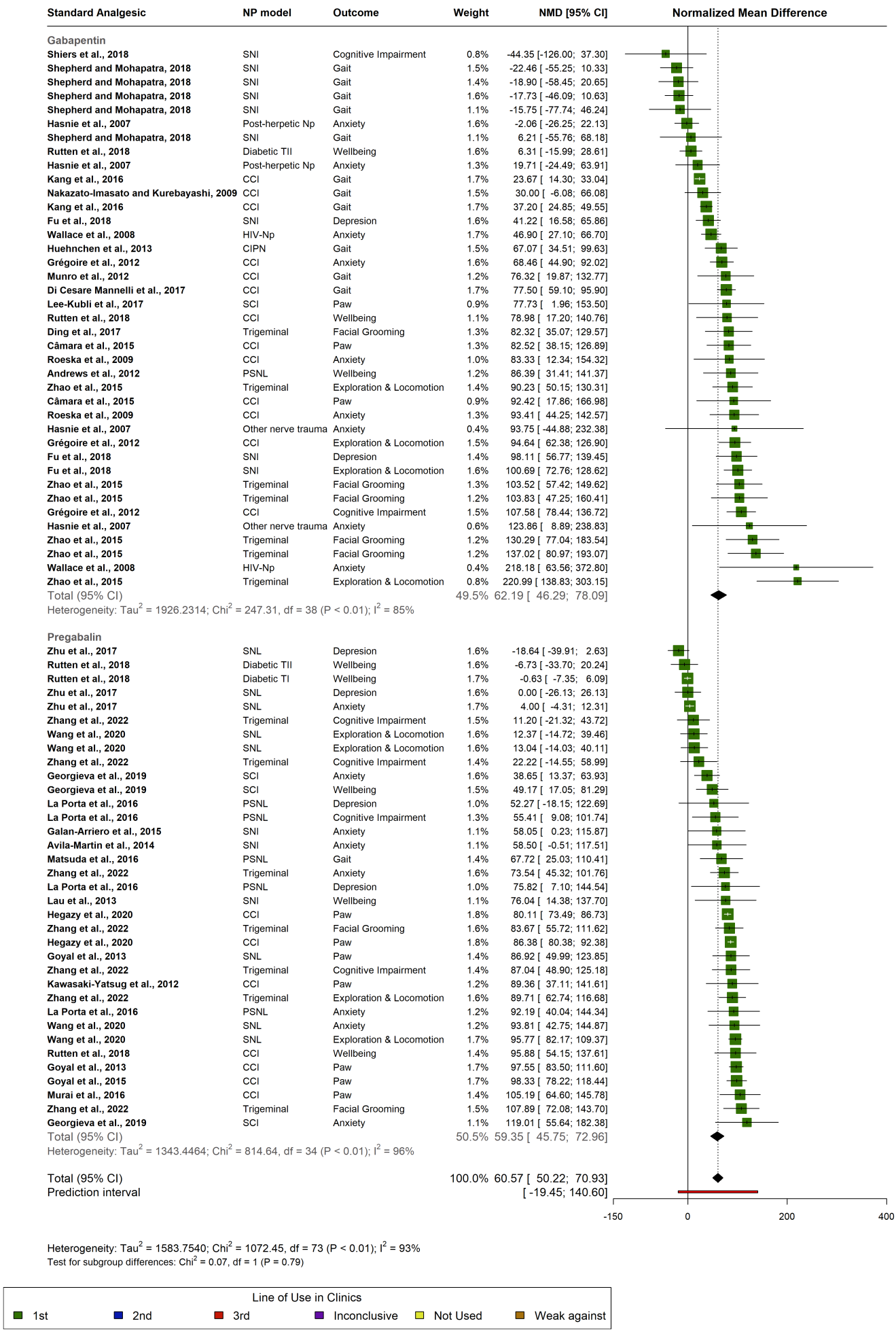
Supplementary Table 2. Analgesics used in the pre-clinical studies analyzed, grouped by corresponding drug class. *Indicates articles eligible for meta-analysis (which exclude those that evaluated only Conditioned Place Preference, CPP, the number of such articles is indicated in parentheses).

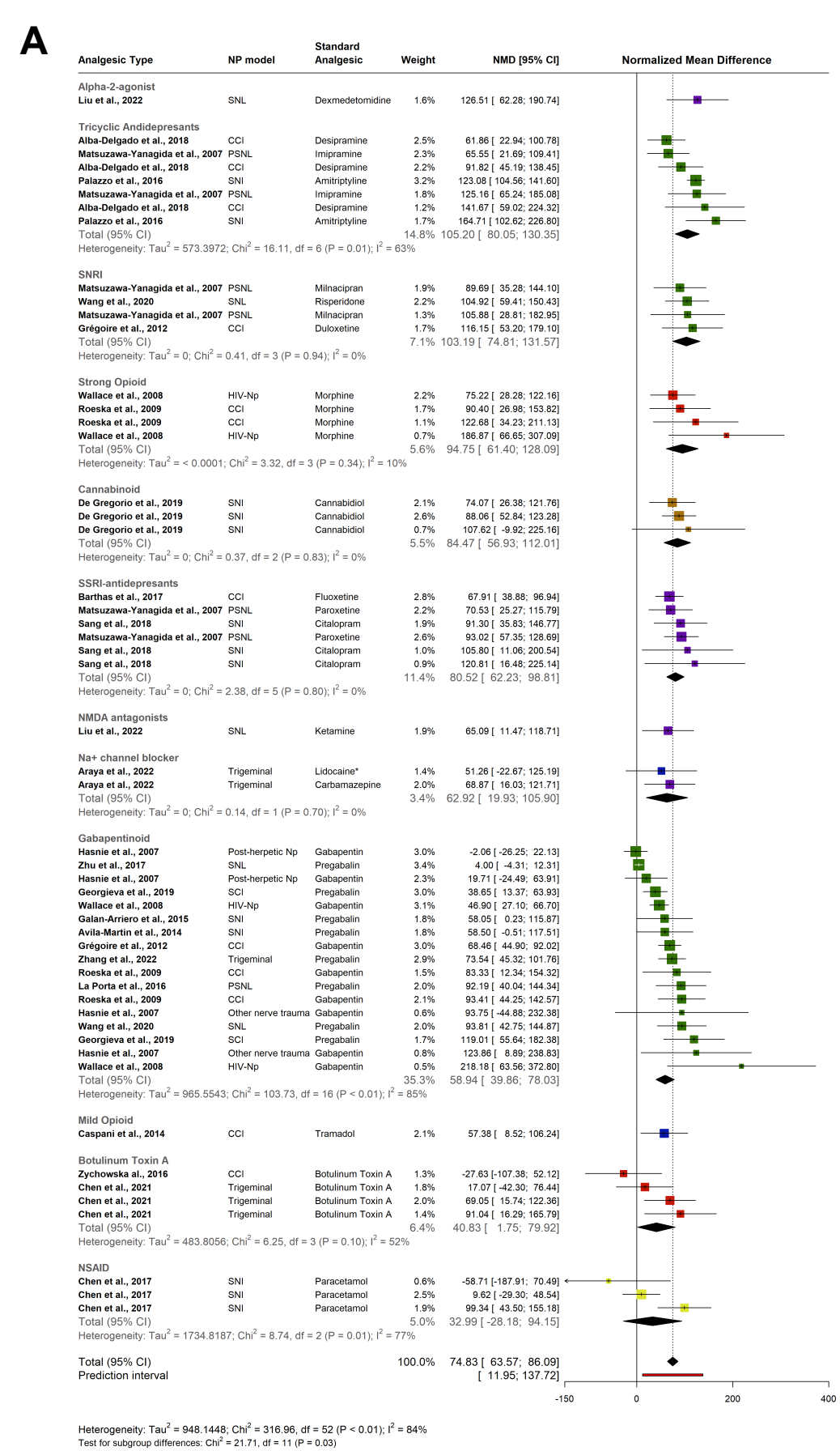
Line of use	Drug Class	Analgesic	nº articles* (CPP)
1st	Gabapentinoid	Gabapentin	20
		Pregabalin	15 (2)
		Mirogabalin	1
	Tricyclic Andidepresants	Amitriptyline	5
		Clomipramine	2
		Desipramine	2
		Imipramine	1
	SNRI	Duloxetine	4
		Milnacipran	1
		Risperidone	1
		AS1069562	1
		Reboxetine	(2)
2nd	Mild Opioid	Tramadol	3
		Buprenorphine	1
3rd	Botulinum Toxin A	Botulinum Toxin A	2
	Strong Opioids	Morphine	11 (2)
		Dermorphin	1
		DAMGO	(1)
Inconclusive	Alpha-2-agonist	Clonidine	3 (12)
		Dexmedetomidine	2
	Na+ channel blocker	Carbamazepine	3
		Lidocaine	1 (7)
		Mexiletine	1
		Zonisamide	1
		Bupivacaine	(1)
	NMDA-antagonist	Ketamine	4
		MK-801	(1)
	SSRI-antidepresants	Fluoxetine	2
		Citalopram	1
		Paroxetine	1
Weak against	Cannabinoid	Cannabidiol	1
Not used	NSAID	Diclofenac	2
		Indomethacin	2
		Lornoxicam	1
		Milnacipran	1
		Paracetamol	1
		GW405833	1



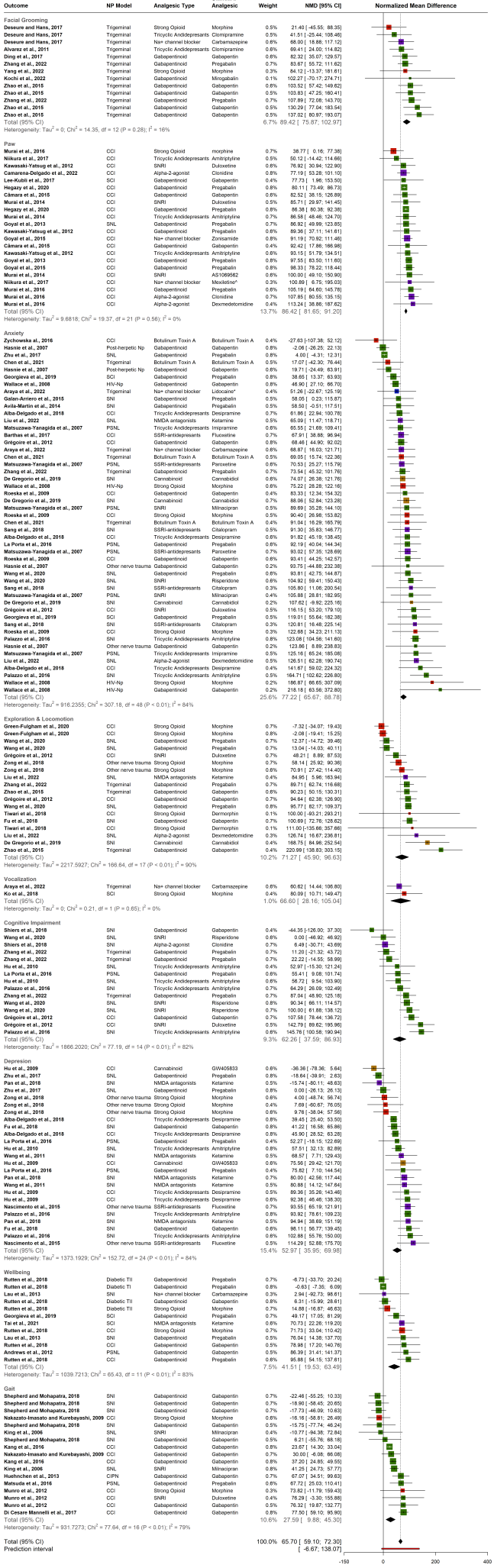
Supplementary Figure 1. Forest plot shows the effect size of the analgesic efficacy stratified by analgesic class. For each study, the neuropathic model (NP model), behavioral outcome and the analgesic used is also noted. Abbreviations: CCI, chronic constriction injury; CINP, chemotherapy induced neuropathic pain; PSNL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation. *Lidocaine was locally applied. ^ considered as "strong against" in the clinical setting

Supplementary Figure 2. Forest plot shows the effect size of the analgesic efficacy of gabapentinoids. For each study, the neuropathic model (NP model) and the behavioral outcome is also noted. Abbreviations: CCI, chronic constriction injury; CINP, chemotherapy-induced neuropathic pain; PSNL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation

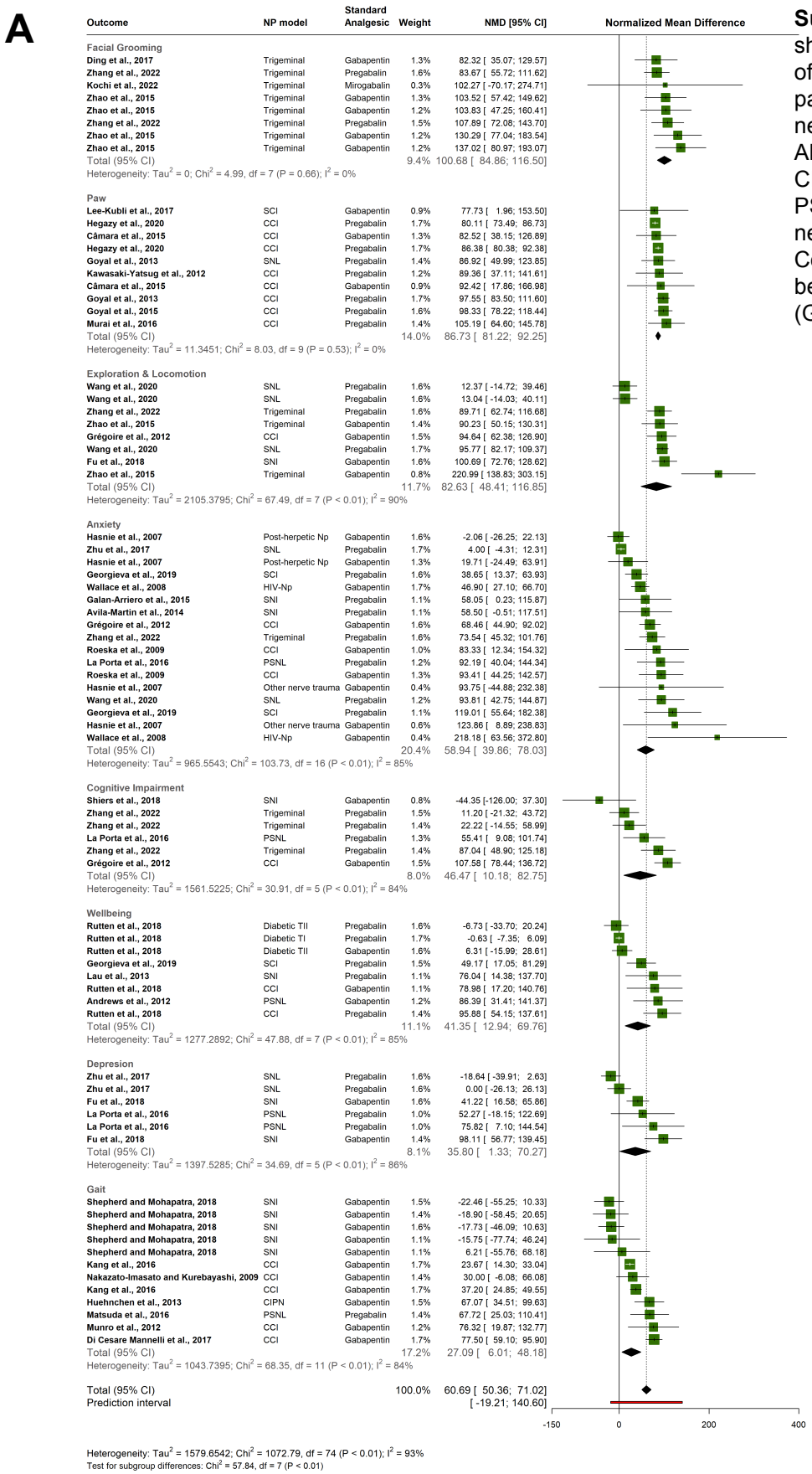




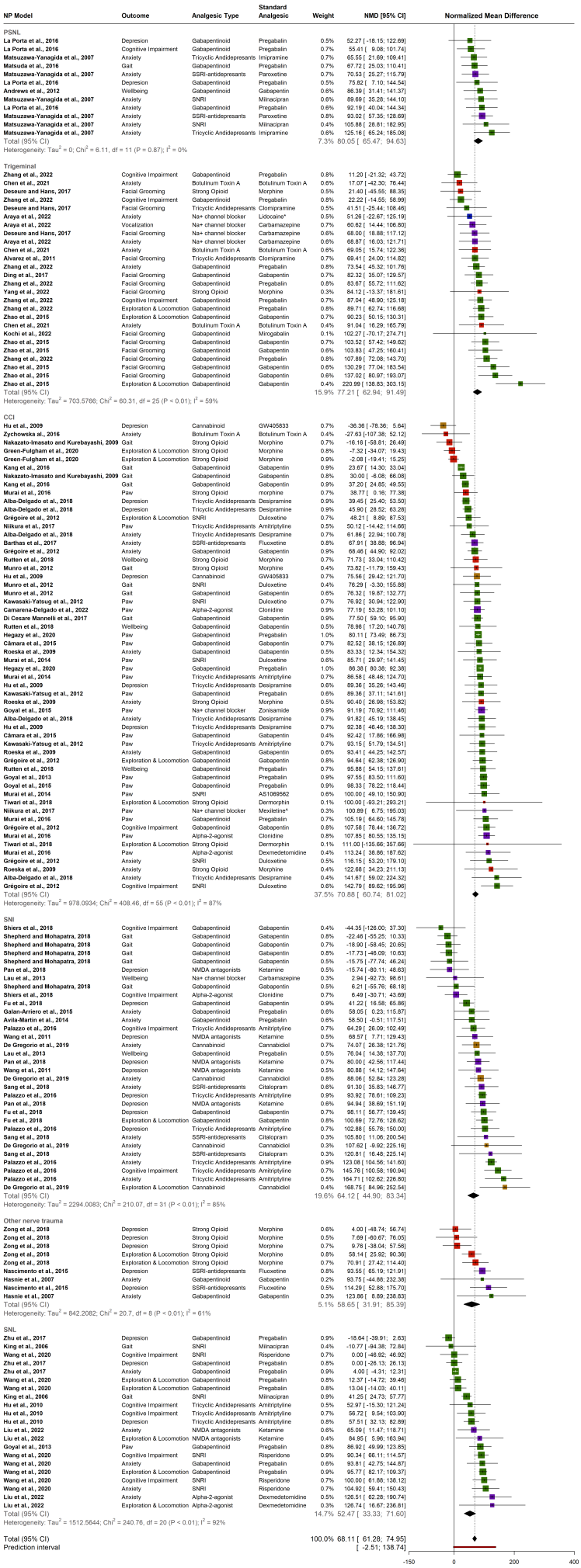
Supplementary Figure 3. (A) Forest plot shows the effect size of the analgesic efficacy in the anxiety outcome stratified by different analgesic types. For each study, the neuropathic pain model is also noted. Abbreviations: CCI, chronic constriction injury; CINP, chemotherapy-induced neuropathic pain; PSNL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation.**(B)** Comparison of the analgesic effect size between all outomes (All) and Anxiety (Axn). Only drugs that were tested at least in three studies are compared. Significant differences were only seen for the group of strong opiod (t-test).



Supplementary Figure 4. Forest plot shows the effect size of the analgesic efficacy stratified by Outcome. For each study, the neuropathic model (NP model), analgesic class and the analgesic used is also noted. Abbreviations: CCI, chronic constriction injury; CINP, chemotherapy induced neuropathic pain; PSNL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation.

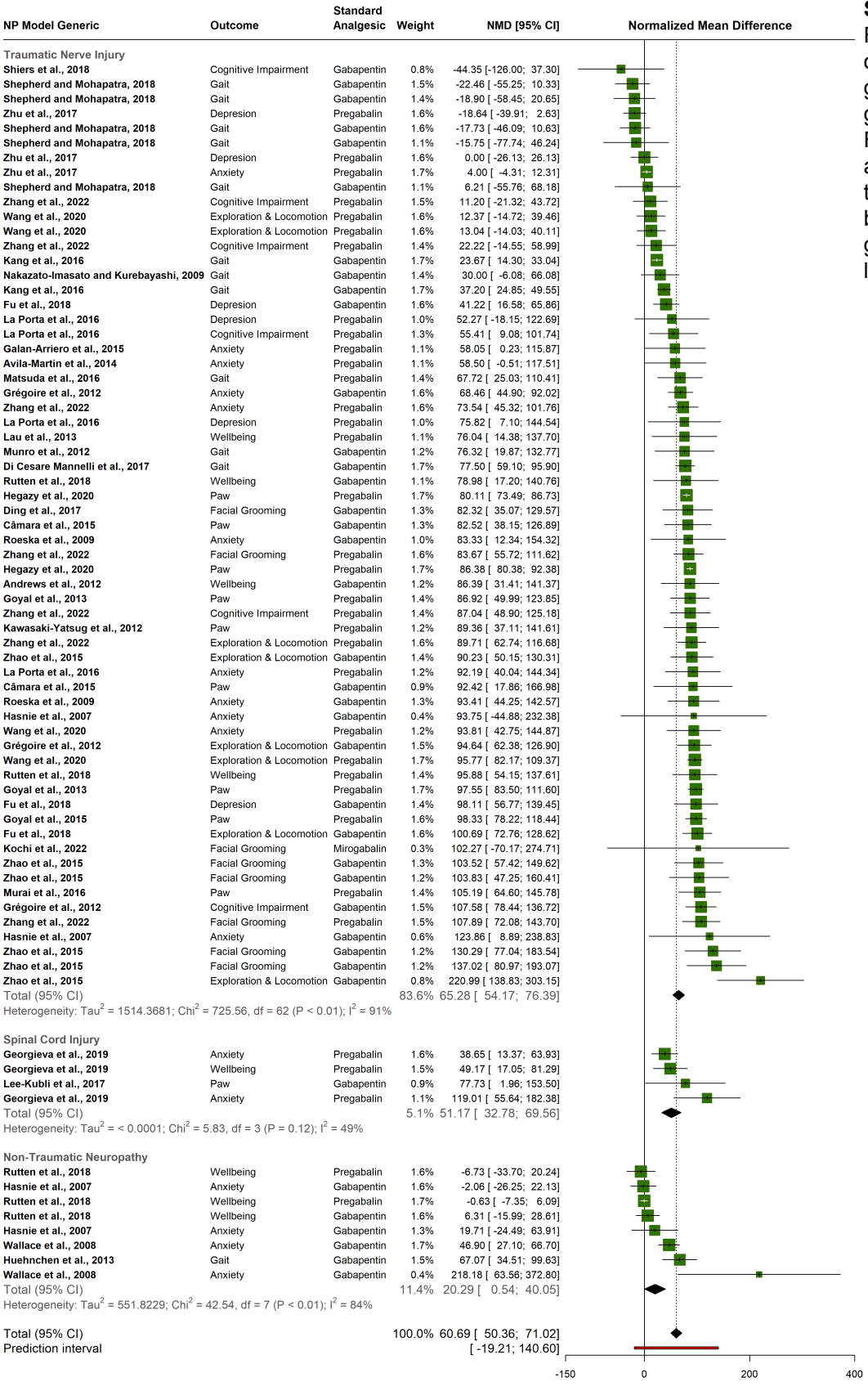


Supplementary Figure 5. (A) Forest plot shows the effect size of the analgesic efficacy of the gabapentinoids stratified by non-evoked pain related outcome. For each study, the neuropathic pain model is also noted. Abbreviations: CCI, chronic constriction injury; CINP, chemotherapy-induced neuropathic pain; PSNL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation.**(B)** Comparison of the analgesic effect size between all drugs (A) and gabapentinoid only (GP). Note the lack of statistical significance.



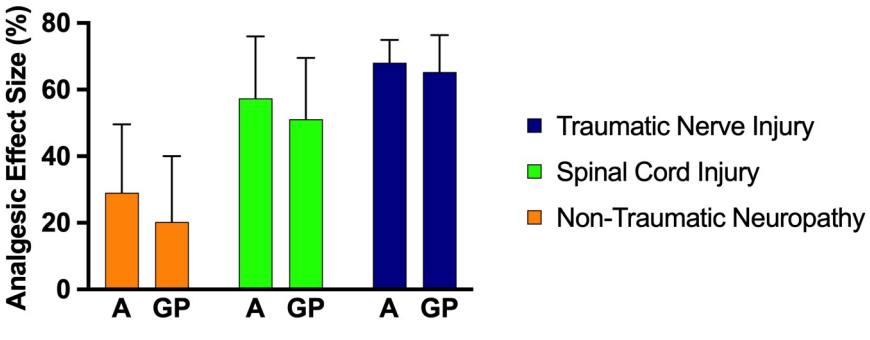
Supplementary Figure 6. Forest plot shows the effect size of the analgesic efficacy stratified by neuropathic pain model (comprising the traumatic nerve injury group only). For each study, the outcome, the analgesic class and the analgesic used is also noted. Abbreviations: CCI, chronic constriction injury; CINP, chemotherapy-induced neuropathic pain; PSNL, partial sciatic nerve ligation; SNI, spared nerve injury; SNL, spinal nerve ligation.

A



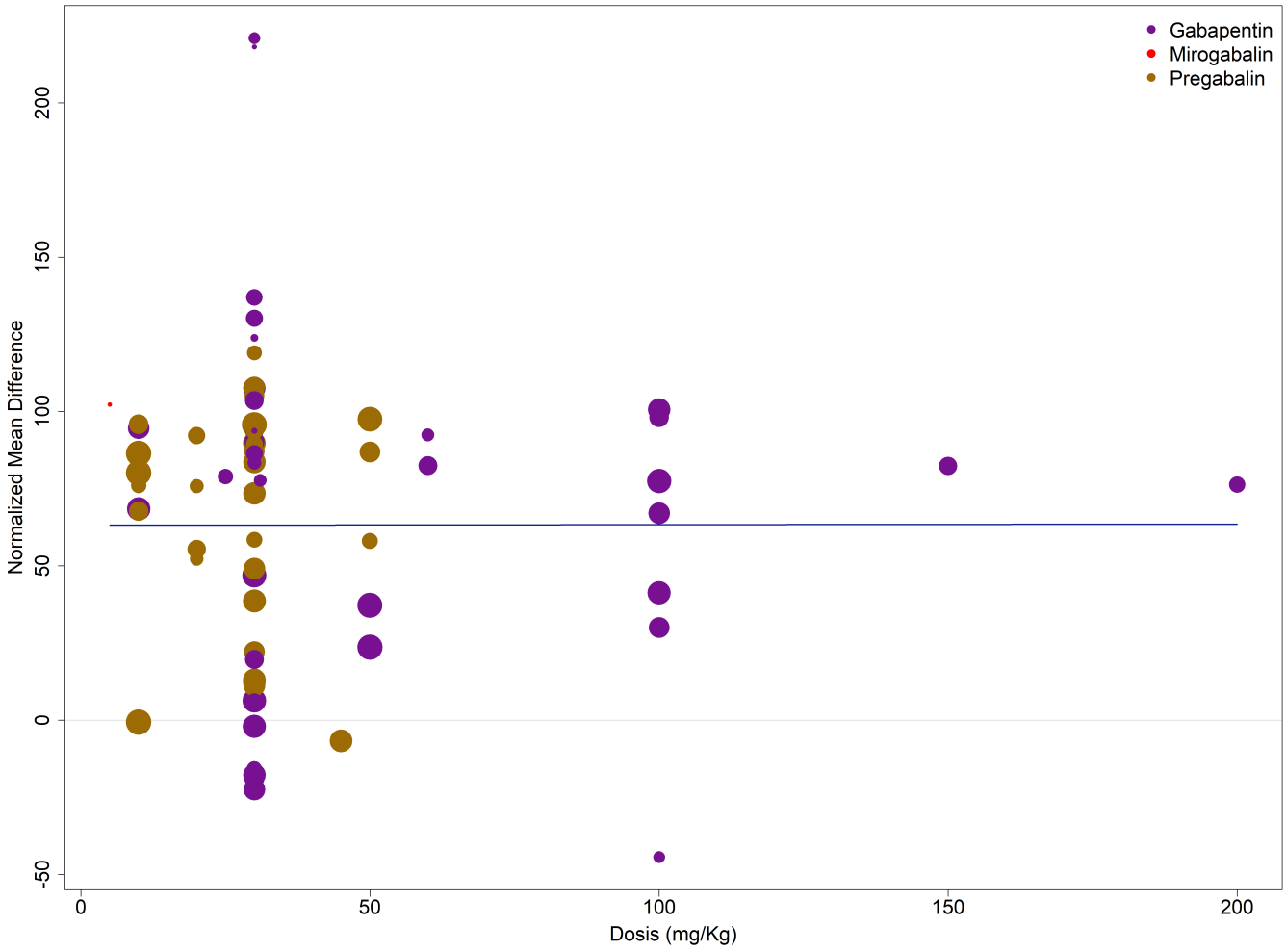
Heterogeneity: Tau² = 1579.6542; Chi² = 1072.79, df = 74 (P < 0.01); I² = 93%
Test for subgroup differences: Chi² = 15.23, df = 2 (P < 0.01)

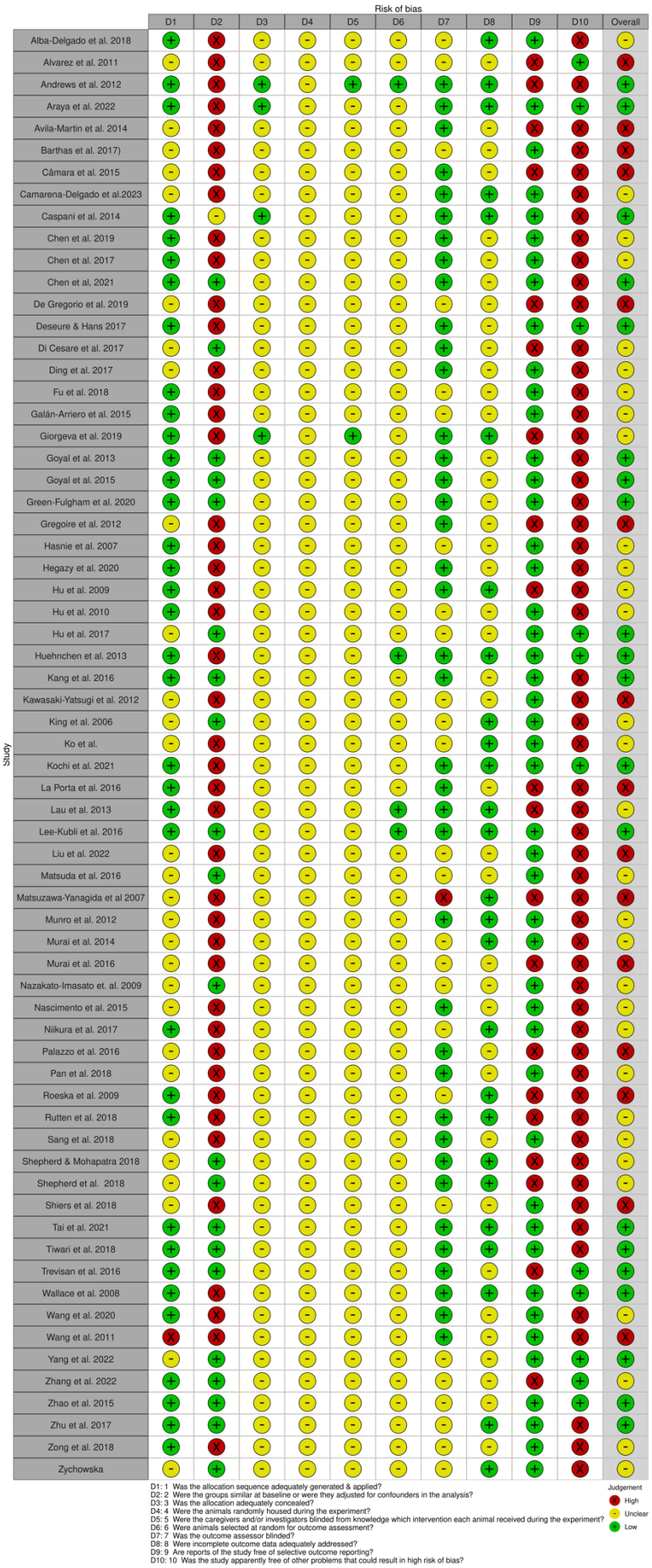
B



Supplementary Figure 7. (A) Forest plot shows the effect size of the analgesic efficacy of the gabapentinoids stratified by generic neuropathic pain model. For each study, the outcome is also noted. **(B)** Comparison of the analgesic effect size between all drugs (A) and gapabentinoids (GB). Note the lack of statistical significance.

Supplementary Figure 8. Meta-regression analysis evaluating the relationship between gabapentinoid dosage and analgesic efficacy. Gabapentinoid dosage (x-axis) and analgesic efficacy (y-axis). Each point represents an individual study, with bubble sizes reflecting the study's weight in the analysis. The solid line indicates the fitted regression line, and the shaded area represents the 95% confidence interval. The analysis was conducted using a mixed-effects model (k = 71 studies).





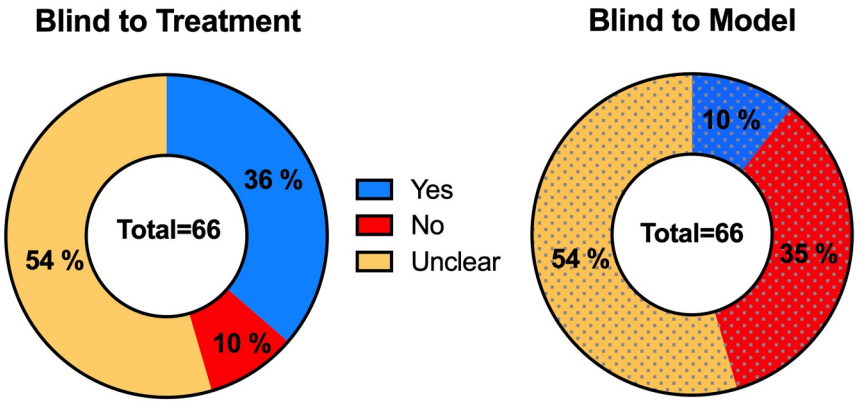
Supplementary Figure 9. Evaluatiom of the risk of bias using SYRCLE's tool. Green circle: low risk of bias; yellow, unclear risk of bias/not applicable to the study design; red circle: high risk of bias

Supplementary Table 3. Blinding conditions in the studies included in the meta-analysis according to the treatment and neuropathy

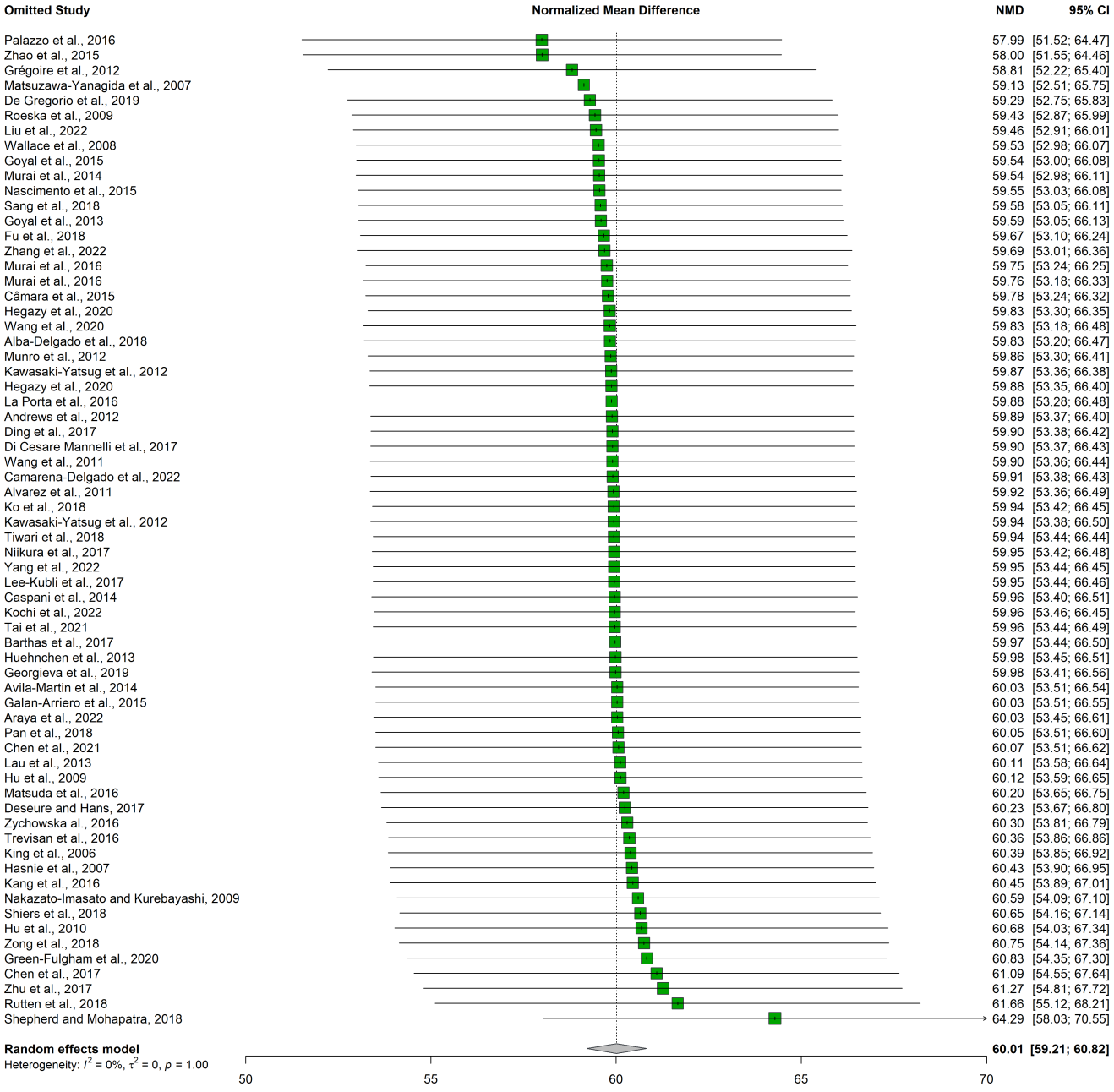
Study	Blind to Treatment	Blind to Neuropathy
Alba-Delgado et al. 2018	Unclear	Unclear
Alvarez et al. 2011	Unclear	Unclear
Andrews et al. 2012	No	Yes
Araya et al. 2022	Yes	No
Avila-Martin et al. 2014	Unclear	Unclear
Barthas et al. 2017	Unclear	Unclear
Câmara et al. 2015	Unclear	Unclear
Camarena-Delgado et al.2023	No	Yes
Caspani et al. 2014	Yes	Yes
Chen et al. 2019	Unclear	Unclear
Chen et al. 2018	Unclear	Unclear
Chen et al, 2021	Unclear	Unclear
De Gregorio et al. 2019	Unclear	Unclear
Deseure & Hans 2017	Unclear	Unclear
Di Cesare et al. 2017	Yes	No
Ding et al. 2017	Yes	No
Fu et al. 2018	Unclear	Unclear
Galán-Arriero et al. 2015	Unclear	Unclear
Giorgeva et al. 2019	Yes	No
Goyal et al. 2013	Unclear	Unclear
Goyal et al. 2015	Yes	No
Green-Fulgham et al. 2020	Unclear	Unclear
Gregoire et al. 2012	Yes	No
Hasnie et al. 2007	Yes	No
Hegazy et al. 2020	Unclear	Unclear
Hu et al. 2009	Yes	No
Hu et al. 2010	Unclear	Unclear
Hu et al. 2017	Unclear	Unclear
Huehnchen et al. 2013	Unclear	Unclear
Kang et al. 2016	Yes	No
Kawasaki-Yatsugi et al. 2012	Unclear	Unclear
King et al. 2006	Unclear	Unclear
Ko et al.	Unclear	Unclear
Kochi et al. 2021	Yes	No
La Porta et al. 2016	Unclear	Unclear
Lau et al. 2013	Yes	No
Lee-Kubli et al. 2016	Yes	No
Liu et al. 2022	Unclear	Unclear
Matsuda et al. 2016	Unclear	Unclear

Matsuzawa-Yanagida et al 2007	No	No
Munro et al. 2012	Yes	No
Murai et al. 2014	Unclear	Unclear
Murai et al. 2016	Unclear	Unclear
Nazakato-Imasato et. al. 2009	Unclear	Unclear
Nascimento et al. 2015	Unclear	Unclear
Niikura et al. 2017	Unclear	Unclear
Palazzo et al. 2016	Yes	No
Pan et al. 2018	Yes	No
Roeska et al. 2009	Yes	Yes
Rutten et al. 2018	No	Yes
Sang et al. 2018	No	Yes
Shepherd & Mohapatra 2018	No	Yes
Shepherd et al. 2018	Yes	No
Shiers et al. 2018	Unclear	Unclear
Tai et al. 2021	Unclear	Unclear
Tiwari et al. 2018	Yes	No
Trevisan et al. 2016	Yes	No
Wallace et al. 2008	Yes	No
Wang et al. 2020	Yes	No
Wang et al. 2011	Yes	No
Yang et al. 2022	Unclear	Unclear
Zhang et al. 2022	Yes	No
Zhao et al. 2015	Unclear	Unclear
Zhu et al. 2017	Unclear	Unclear
Zong et al. 2018	Unclear	Unclear
Zychowska et al., 2016	Unclear	Unclear

Supplementary Figure 10. Blinding conditions in the studies included in the meta-analysis according to the treatment and neuropathic condition of the animal.



Supplementary Figure 11. Forest plot shows the Sensitivity Analysis performed by the leave one-out method of the analyzed studies included in the Meta-Analysis



Supplementary Figure 12. Assessment of publication bias using funnel plots before and after adjustment with the trim-and-fill method. Studies positioned to the right of the pyramid indicate a larger effect size of the intervention, while those on the left show a smaller effect size. The upper panel (A) shows the original funnel plot where each individual study is represented as a blue circle. The lower panel (B) presents the funnel plot adjusted using the trim-and-fill method, which imputes a total of 59 additional studies (red triangles) to correct the asymmetry and estimate the true effect.