

## Article

# Nicotine Neurotoxicity Involves Low Wnt1 Signaling in Spinal Locomotor Networks of the Postnatal Rodent Spinal Cord

Jaspreet Kaur<sup>1,2,\*</sup>, Graciela L. Mazzone<sup>2,3,\*</sup>, Jorge B. Aquino<sup>3</sup>, and Andrea Nistri<sup>2</sup>

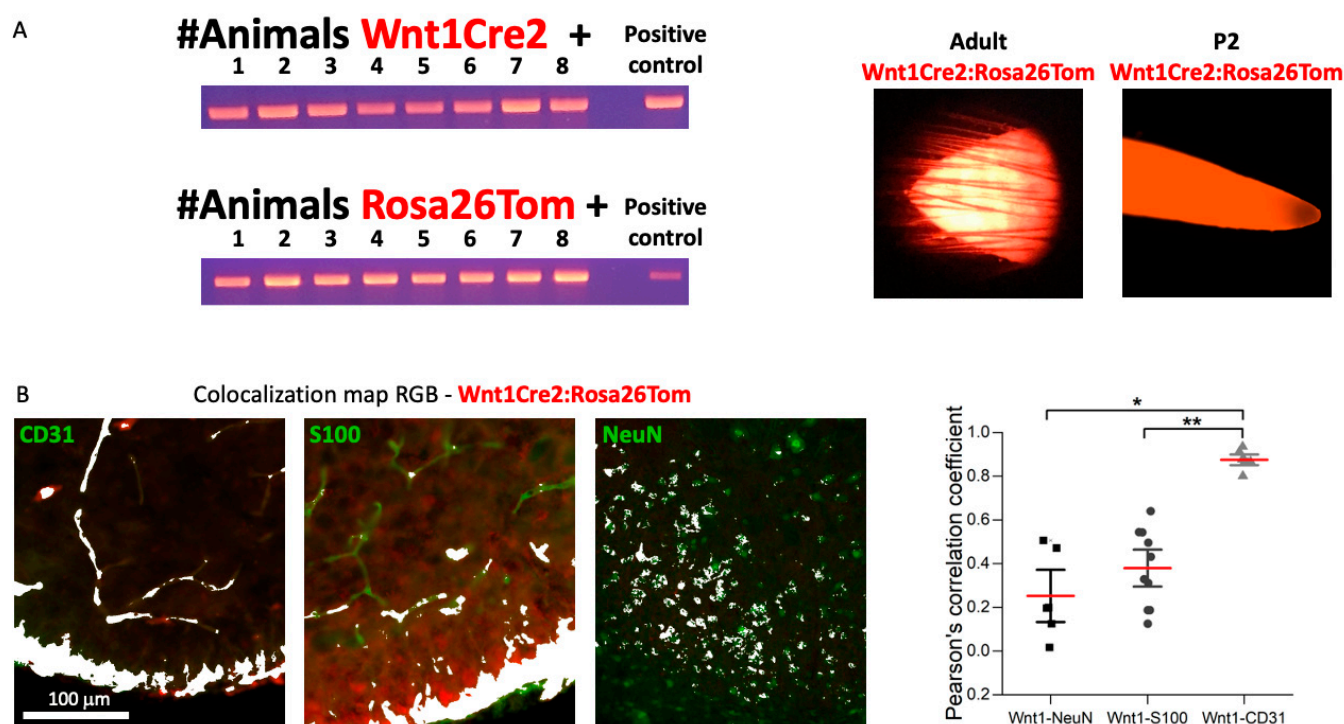
<sup>1</sup> Faculty of Health and Medical Sciences, Department of Neuroscience, University of Copenhagen, Blegdamsvej 3, DK-2200 KBH N, Denmark

<sup>2</sup> Department of Neuroscience, International School for Advanced Studies (SISSA), 34136 Trieste, Italy; nistri@sisssa.it

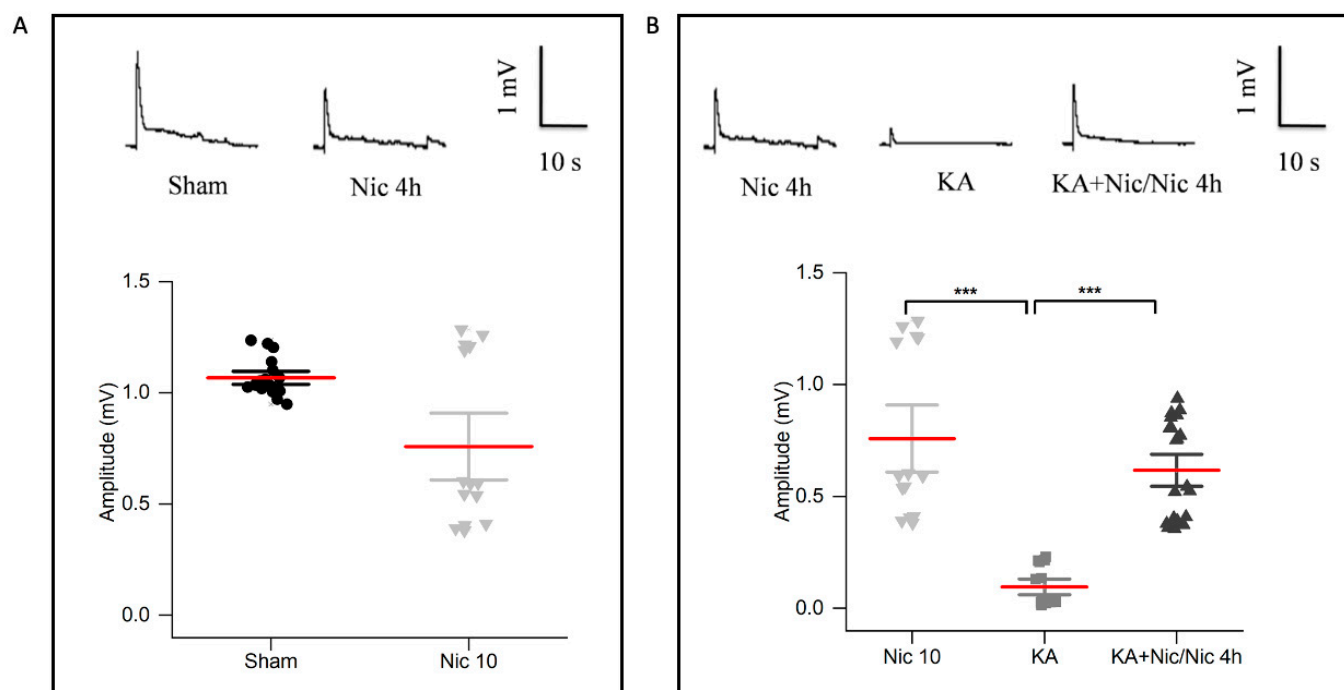
<sup>3</sup> Instituto de Investigaciones en Medicina Traslacional (IIMT), CONICET-Universidad Austral, Av. Pte. Perón 1500, Pilar, B1629AHJ Buenos Aires, Argentina; jaquino@austral.edu.ar

\* Correspondence: jaspreet.kaur@sund.ku.dk (J.K.); GMazzone@austral.edu.ar (G.L.M.); Tel.: +45-5260-1502 (J.K.); +54-23-0438-7425 (G.L.M.)

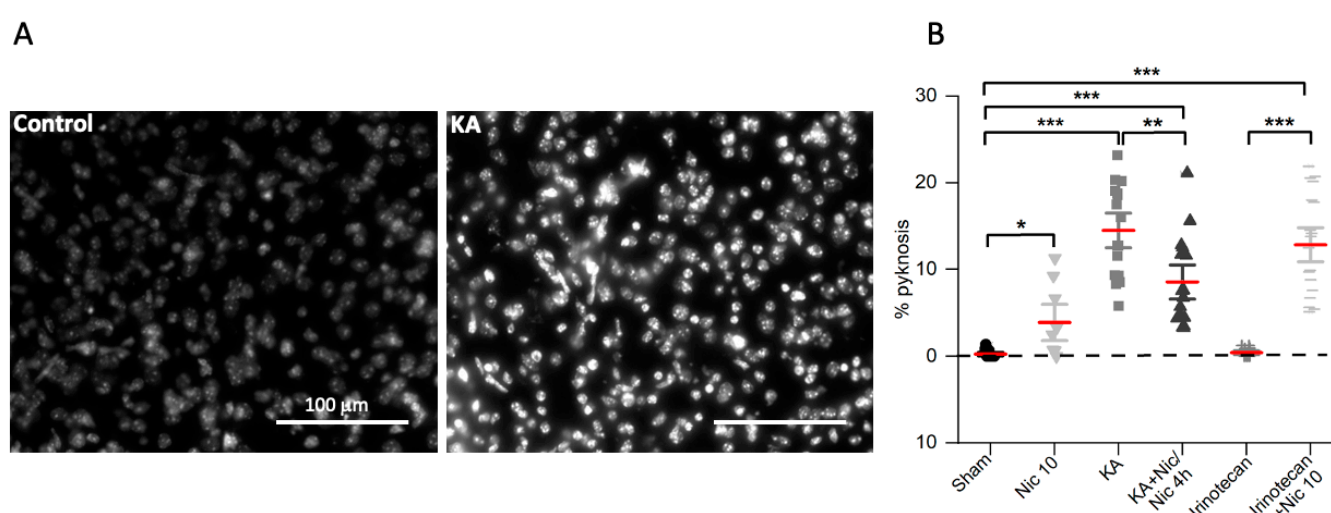
## Supplemental Figure Legends



**Figure S1.** (A) Left: bands showing the expression of Cre and Tomato in Wnt1Cre2:Rosa26Tom mouse tail samples by PCR. Right: examples of Wnt1Cre2:Rosa26Tom tail tips of adult and postnatal (P2) mice. (B) Representative binary versions of images of Wnt1Cre2:Rosa26Tom signal with CD31, S100 or NeuN marker. After applying Costes threshold, pixels with positive signals for both are shown in white. Whisker plot showing a comparison of Pearson's Correlation Coefficient for CD31, S100 or NeuN and Wnt1Cre2:Rosa26Tom:  $0.87 \pm 0.01$ ,  $0.20 \pm 0.07$ ,  $0.41 \pm 0.05$ , respectively (S100 vs CD31:  $**p < 0.004$ ; NeuN vs CD31:  $*p < 0.047$ ; Dunn's Method). Scale bar (100  $\mu$ m) applies to all panels. Note that mean bars in the whisker plots are represented in red.



**Figure S2.** D-DRPs recorded from rat lumbar spinal cord. **(A)** Nic 10 ( $n = 3$ ) application (4 h) did not block the amplitude in comparison to sham ( $n = 4$ ) as indicated in the panel A example and histogram ( $p = 0.106$ ,  $U = 83$ , Mann-Whitney test). **(B)** KA ( $n = 4$ ) application drastically reduced the D-DRP amplitude (Nic 10 vs KA:  $***p \leq 0.001$ ,  $U = 0$ , Mann-Whitney test) which was recovered to normal after Nic 10 co-application ( $n = 5$ ) (Nic 10 vs KA+Nic/Nic 4 h:  $***p \leq 0.001$ ,  $U = 0$ , Mann-Whitney;  $***p \leq 0.001$ ,  $H_{(2)} = 30.156$ , Kruskal-Wallis One Way Analysis of Variance on Ranks test). Note that mean bars in the whisker plots are represented in red.



**Figure S3.** Pyknosis evoked by nicotine, KA or irinotecan in the ventral spinal region of mice. **(A)** Images showing nuclear staining by DAPI in sham and KA treated sections. **(B)** Whisker plot showing % pyknosis evoked by Nic 10 (sham vs Nic 10:  $*p = 0.03$ ,  $t_{8.15} = -2.616$ , Welch's  $t$ -test), KA (sham vs KA:  $***p \leq 0.001$ ,  $t_{15.31} = -10.629$ , Welch's  $t$ -test) or KA+Nic/Nic 4h (KA vs KA+Nic/Nic 4h:  $**p = 0.003$ ,  $t_{29.975} = 3.195$ , Welch's  $t$ -test) treatment. Extensive pyknosis was

evoked by irinotecan+Nic 10 treatment (sham vs Irinotecan+Nic 10:  $***p \leq 0.001$ ,  $t_{18.4} = -9.58$ ; Irinotecan vs Irinotecan+Nic 10:  $***p \leq 0.001$ ,  $t_{18.496} = -9.435$ , Welch's  $t$ -test). Scale bar (100  $\mu\text{m}$ ) applies to all panels. Note that mean bars in the whisker plots are represented in red.