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SUPPLEMENTARY MATERIAL

corresponding to:

The natural compound sanguinarine perturbs the regenerative capabilities of planarians

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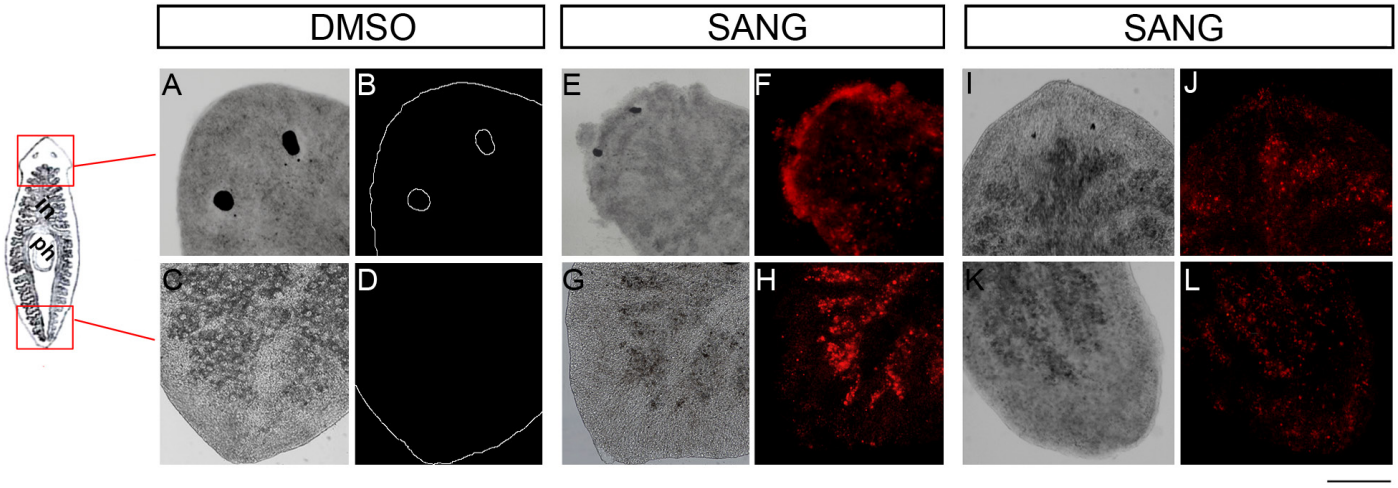
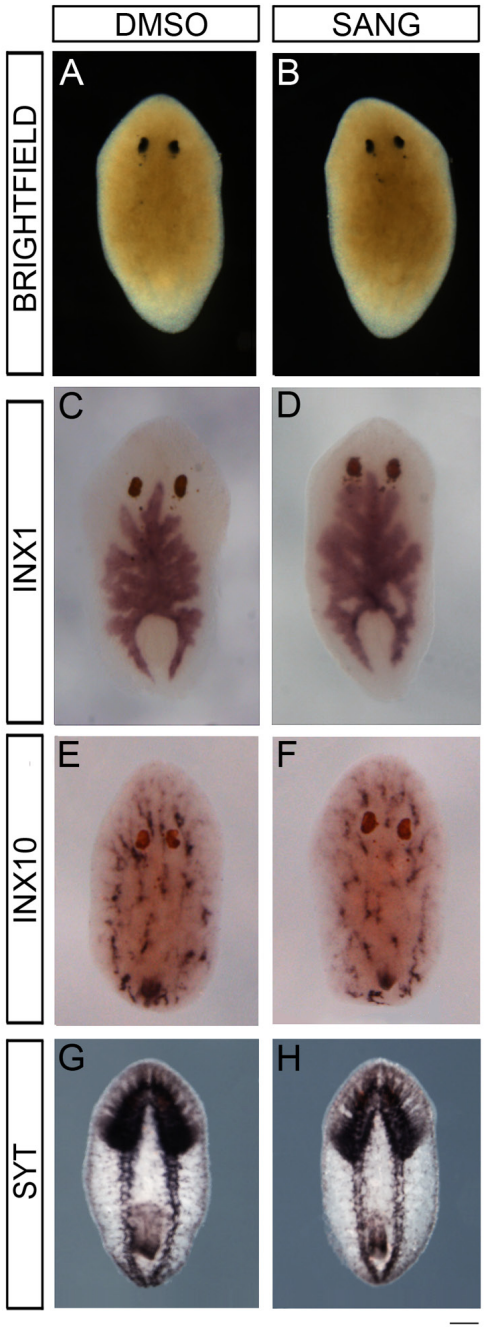


Supp. Fig. 1. Sanguinarine absorption in planarians. (A,B) *Head of an intact planarian, after 7 days of DMSO treatment (control). (A) Brightfield image. (B) Fluorescence image. No red signal is detected. A schematic of an intact planarian is shown on the right: the intestine (in), including a single anterior and two posterior primary branches from which secondary and tertiary branches diverge. ph: pharynx (the planarian muscular feeding organ). Red boxes show the anterior and posterior region.* **(C,D)** *caudal region of an intact planarian, following 7 days of DMSO treatment: (C) Brightfield image. (D) Fluorescence image. No red signal is detected.* **(E,F)** *Anterior region of an intact planarian after 7 days of sanguinarine treatment. (E) Brightfield image. (F) Fluorescence image. The alkaloid appears widely distributed in the body and is also visualized in the intestine. Slight accumulation of red signal is detected in the head. This region appears in initial regression. Head is the most sensitive region to the effects of the alkaloid. Intact planarians show progressive head regression following sanguinarine treatment.* **(G,H)** *Caudal region of an intact planarian, following 7 days of sanguinarine treatment. (G) Brightfield image. The intestine appeared of different morphology with respect to the control (C), probably due to the toxic effects of alkaloid on this tissue. In fact, while the typical morphology of the gastrodermis can be visualized at this magnification in the control (C), the morphology of this tissue is not more discernible in sanguinarine-treated planarians. (H) Fluorescence image showing accumulation of red signal at the intestinal level.* **(I,J)** *Anterior region of a regenerating planarian after 7 days of sanguinarine treatment (regeneration 7 days); (I) Brightfield image; (J) Fluorescence image.* **(K,L)** *Posterior region of a regenerating planarian after 7 days of sanguinarine treatment (regeneration 7 days); (I) Brightfield image; (J) Fluorescence image. We observed that the distribution of sanguinarine does not differ significantly in regenerating animals. Sanguinarine appears widely distributed in the body and slight accumulation of red signal is visualized in the intestine, but no preferential accumulation can be noted in the regenerating head. All animals were sedated on ice before to be photographed. Control: DMSO 0,015%; SANG: sanguinarine 0.15 μM. Scale bar: 150 μm.*



Supp. Fig. 2. Sanguinarine does not affect posterior regeneration. (A,B) *Representative brightfield images of a control (A) and a sanguinarine-treated planarian (B) amputated at the prepharyngeal level and regenerating a tail after 7 days of treatment (regeneration: 7 days). No anomalous phenotypes were observed following sanguinarine treatment. All sanguinarine-treated animals regenerated normal tails comparable to those of controls (100%).* **(C,D)** *Whole mount* in situ *hybridization with the intestinal marker* Dj-inx1 *in a control (C) and in a sanguinarine-treated planarian (D) after 7 days of treatment (regeneration: 7 days). All sanguinarine-treated head fragments regenerating a tail showed normal morphogenesis of the intestine (100%).* **(E,F)** *Whole mount* in situ *hybridization with the excretory system marker* Dj-inx10 *in a control (E) and in a sanguinarine-treated planarian (F) after 7 days of treatment (regeneration: 7 days). All sanguinarine-treated head fragments regenerating a tail showed an excretory system comparable to that of controls (100%).* **(G,H)** *Whole mount* in situ *hybridization with the pan-neural marker* Dj-syt *in a control (G) and in a sanguinarine-treated planarian (H) after 7 days of treatment. All sanguinarine-treated head fragments (100%) regenerating a tail showed nerve cords comparable to those of controls. For each experiment, 30 animals were used. Control: DMSO 0,015%; SANG: sanguinarine 0.15 μM. Anterior is up. Scale bar: 1 mm.*