### Tran et al.:

## Assessment of Iron Oxide Nanoparticle Ecotoxicity on Regeneration and Homeostasis in the Replacement Model System *Schmidtea mediterranea*

### **Supplementary Data**

### Supplementary information

### Tab. S1: List of primers and probes used for RT-qPCR

Gene	Primer forward	Probe	Primer reverse
PCNA	GTGATGGTTTTGAGACTTATCGATG	TGTTAGGGAATCATTACTACCAAGCGCC	GTTTCACTTGAATCAGCGGC
SMEDWI1	AGTTCCTGTTCCAACGCATTATG	CTGAACTCGTTGGCAAGA	CTGGAGGAGTAACACCACGATGA
P53	ATCGTCGAGCCTGTTTCATC	TCCGACGACATGCCAACATTGTCT	ATCAAATTCTCCGTTGGGAATAAAG
AGAT1	GGTTGGAAGATTGTGAAGGG	TGTATGAAGGCATGAGTTACAAGTGGC	CCAACCTCTCGCTTTTCA
NB32.1.G	GGCACTCATTTCTCGTTTCTGTATT	TGTCGAGTCGCATTTTAAATCGGCG	GTTCTCGCTGTGTTATTTGTTTACGT
HB19.11.G	CGAATGTCGTTATAGAGCTCG	ACAAGCGTGAATTGAGTGCTGAATGC	GCGCCTCGTCCAATTTT
MYHC	TGAAGAGCGAGCTGATCAAGC	AGCTCGGTATCTGTTAGTC	GCGGATTGATGTCGCAGTTATAG
GAPDH	GAGTTGGAATCAATGGCTTCG	CGCGCAACACCAATCGTCCAATTC	TCAACTGTGCCTTTCTCCAG

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ALTEX 36(4), SUPPLEMENTARY DATA



# Fig. S1: Effects of CTAB-AuNPs in homeostatic planarians

Either CTAB-AuNPs or their solvent alone were added to the planarian medium at a concentration of 0.01 mg/ml. All Planarians treated with CTAB-AuNPs died within 30 minutes following the lysis of the tissues (A). Control animals exposed to solvent alone did not show any toxic effects (B). n = 9 for each condition tested.



### **Fig. S2: Injection of IONs in living planarian** Animal photographed before

Animal photographed before (left) and after (right) the injection of a 1% Trypan blue solution. The black arrow indicates the injection site, which targeted the place where the three branches of the gastrovascular system meet, roughly at the center of the animal. The black arrowheads indicate the gut branches stained with Trypan blue. Scale bar: 0.5 mm.



#### Fig. S3: Effects of low concentration PSNPs in homeostatic or regenerating planarians

regenerating planarians (A) Survival and regeneration rates of animals injected with 0.1 mg/ml and 1 mg/ml PSNPs in the gastrovascular system. (B) The morphology of homeostatic and regenerating planarians incubated with 1 mg/ml PSNPs in planarian artificial medium (PAM). One representative animal/condition is shown; PAM and solvent are the negative controls. n = 9 for each condition tested.



### Fig. S4: Effects of high concentration PSNPs injected in planarians

10 mg/ml PSNPs were injected in the gastrovascular system of homeostatic planarians. All injected animals shoved morphology defects at 12 days post injection. Red arrows indicate the parts affected: photoreceptors (A), entire head (B), tail portion (C-E). n = 9 for each condition tested.



#### Fig. S6: Effects of agglomerated IONs on the cell sub-populations in planarian

(A) Hoechst blue / Hoechst red plot displaying the three cell sub-populations (stem cells, progenitor cells, and differentiated cells; X1, X2, Xin, respectively) defined by DNA content (blue channel) and size (red channel) in wild type animals. (B) Boxplots displaying the percentage of the X1, X2, and Xin sub-populations in animals injected with no IONs – either in planarian artificial medium (0.0 mg/ml IONs) or solvent (S) – or injected with 0.1 or 1.0 mg/ml non-sonicated IONs (n  $\ge$  3). All the measurements were performed 14 dpi. \*:  $p \le 0.05$