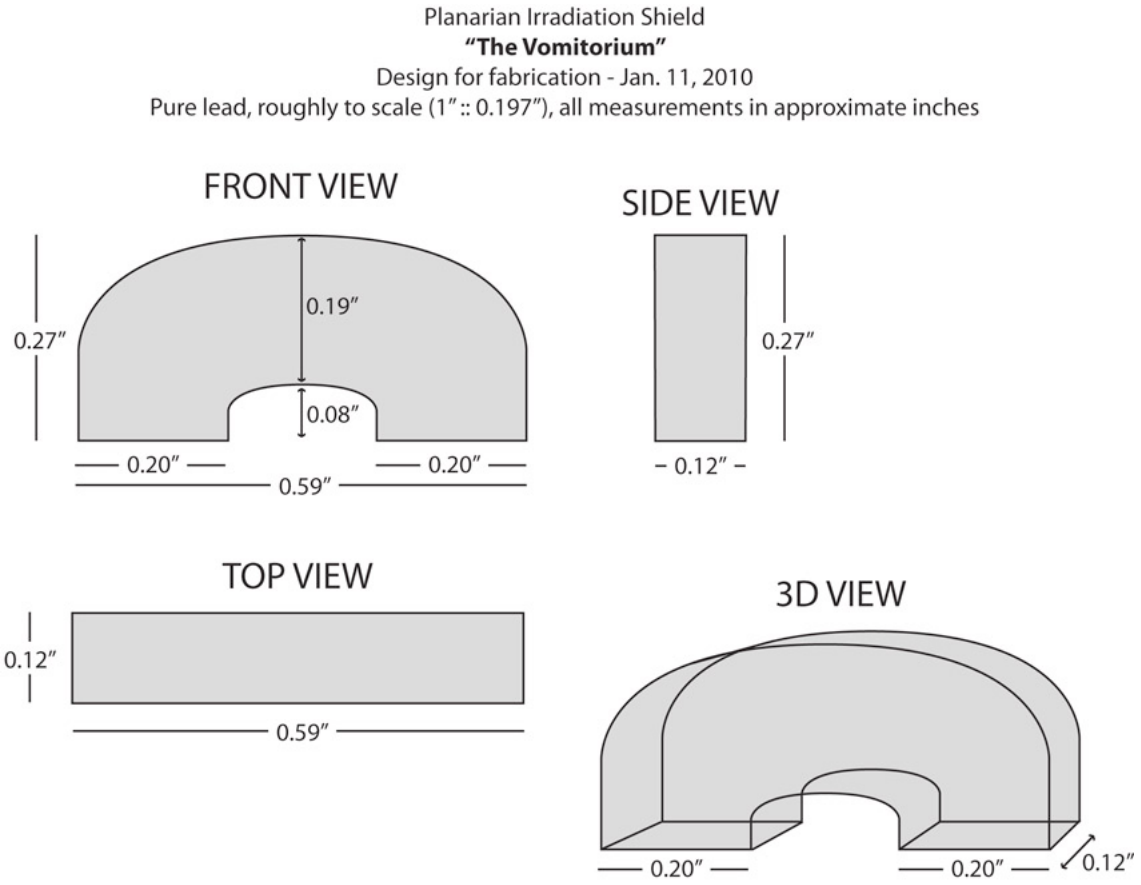

Fig. S1. Design and geometry of the irradiation shield. Four views (front, side, top and 3D rendered) of The Vomitorium, or arch shaped, lead shield with included measurements in inches. The shields are free standing. The design was submitted as shown for fabrication out of pure lead and 30 identical units were produced. Many considerations were taken into account during the shield design. Owing to the highly penetrating nature of gamma rays and therefore the unreasonable thickness of lead that would be required to effectively shield any portion of the animal (>6 cm for 99% theoretical attenuation), we instead used x-ray irradiation for the ablation of planarian stem cells, an effective and well used technique (Dubois, 1949; Steele and Lange, 1976; Hayashi et al., 2006). Re-analysis of published empirical values for the transmittance of 325 kV x-ray beams through various thicknesses of lead (Miller and Kennedy, 1955) revealed that 6 mm thick lead would allow for ~99% hypothetical shielding and 4.5 mm would allow for ~97.5%. Thus, we designed a 4.8 mm thick arch shaped shield, which was custom manufactured (Alpha Systems Corp., Bluffdale, UT, USA) for use in a 320 kV X-RAD 320 Biological Irradiator (PXi Precision X-Ray, North Branford, CT, USA).

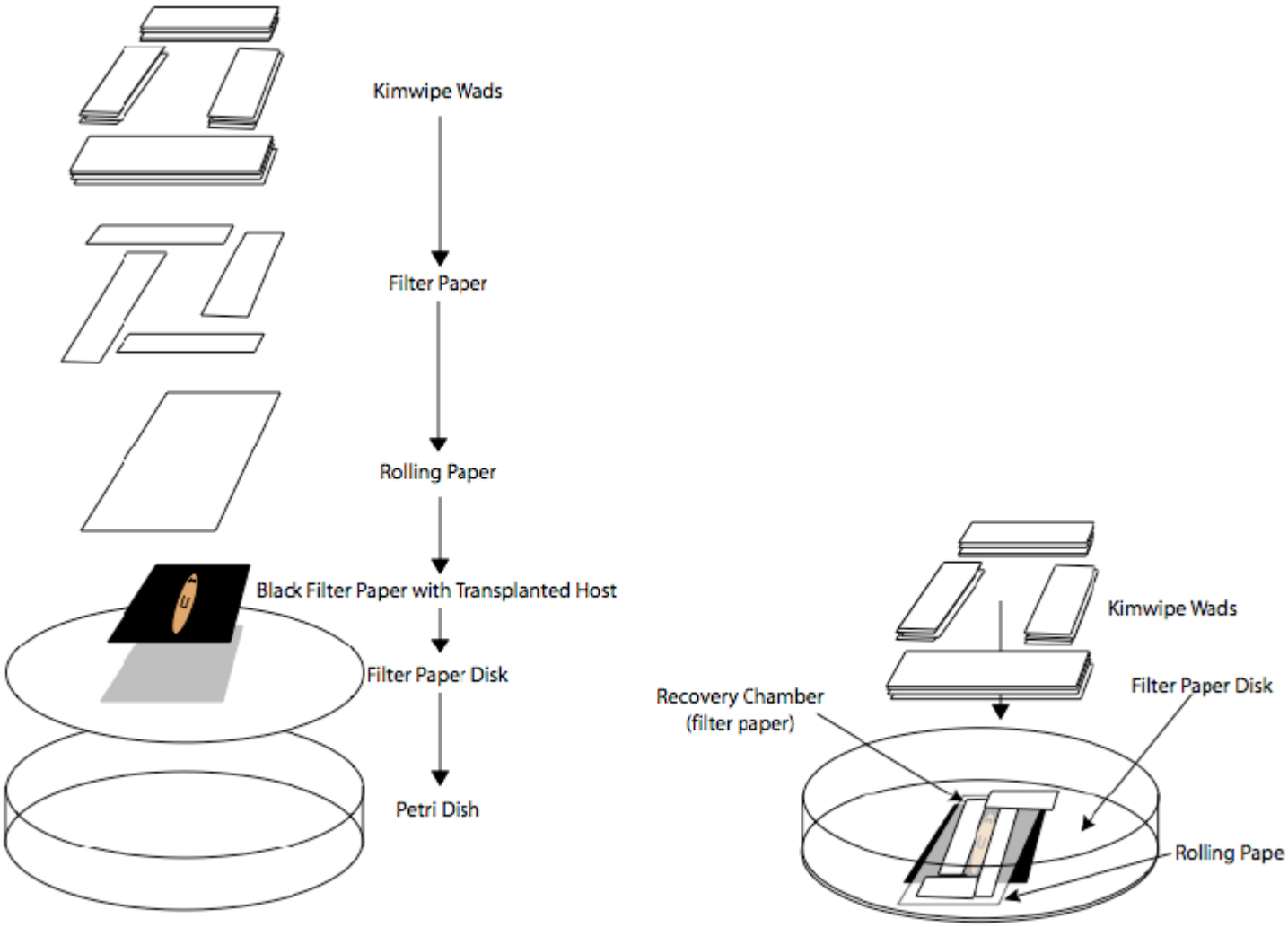

Fig. S2. Building the transplantation recovery chamber. A petri dish was lined with a Holtfreter's solution-soaked filter paper disk (Whatman #2). The operated planarian resting on black filter paper (Schleicher & Schuell) was transferred onto the petri dish. Cigarette rolling paper (Zig-Zag Original) was wetted with Holtfreter's solution and placed on top of the planarian. The covered planarian was encased with rectangles of soaked filter paper (Whatman #3) and four-ply wads of soaked Kimwipe were laid on top of the filter paper rectangles. A properly built recovery chamber maintains hydration and holds the animal motionless facilitating adherence of the graft to the host.

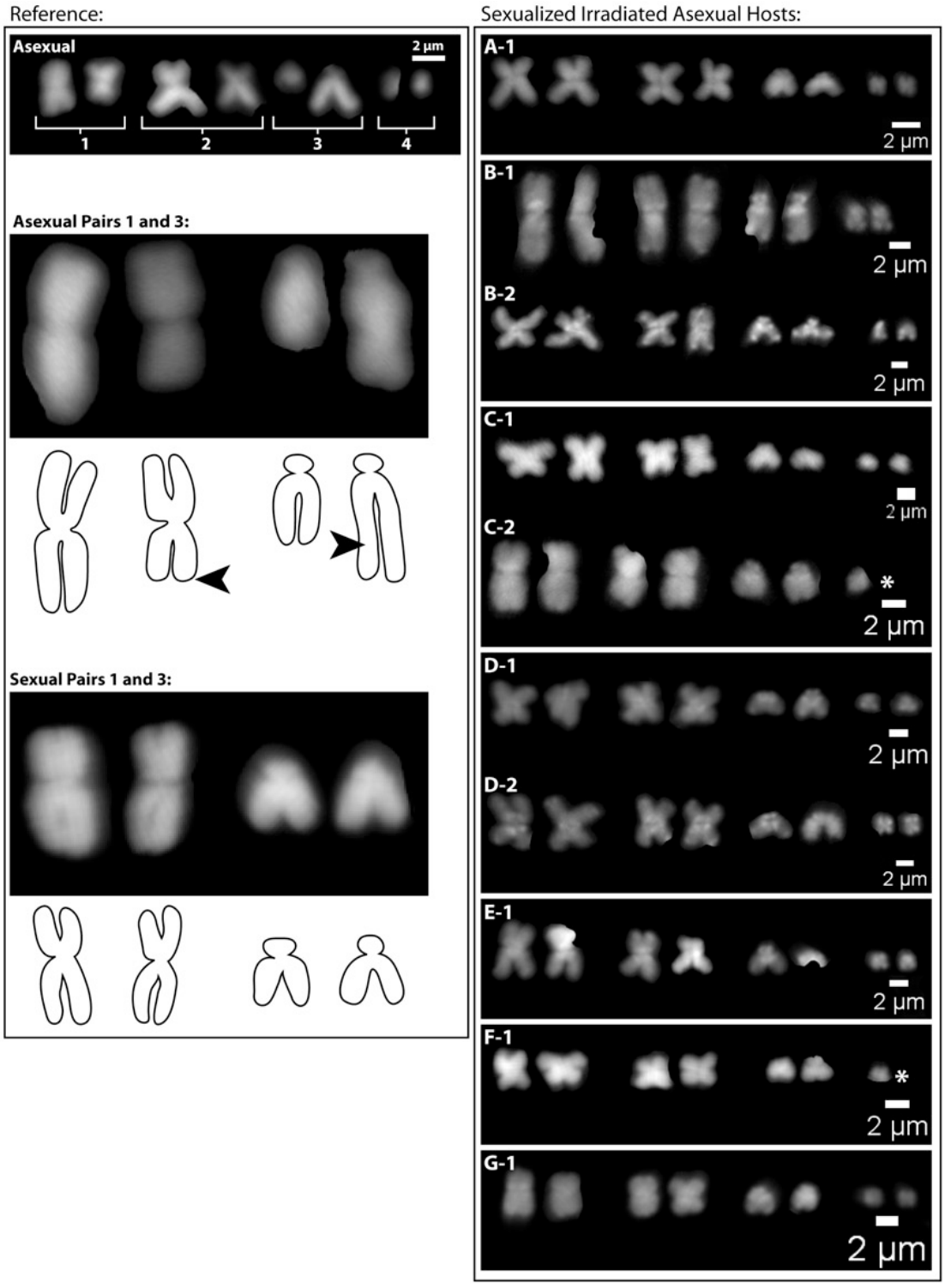

Fig. S3. Asexual host animals sexualized by irradiation and transplantation of healthy sexual host tissue eventually display the sexual karyotype. Reference asexual and sexual karyotypes are shown on the left, focusing on the first and third pair of chromosomes which were used for identification and consist of two identical sets in the sexual animal and four different chromosomes in the asexual owing to a large translocation (indicated by arrowheads) (Newmark and Sánchez Alvarado, 2002). The karyotypes of all remaining sexualized animals (7 of 9, two animals were lost to inadvertent desiccation) are shown on the right (A-G). Karyotypes of all individual animals were consistent with the sexual reference (each letter represents an individual animal and numbers represent extra replicates). Occasionally, because of their small size, the second chromosome of the fourth pair could not be found (asterisk), but this chromosome was not essential for identification. These data indicate that the primary, if not only, cell type present in these animals was derived from the sexual graft.

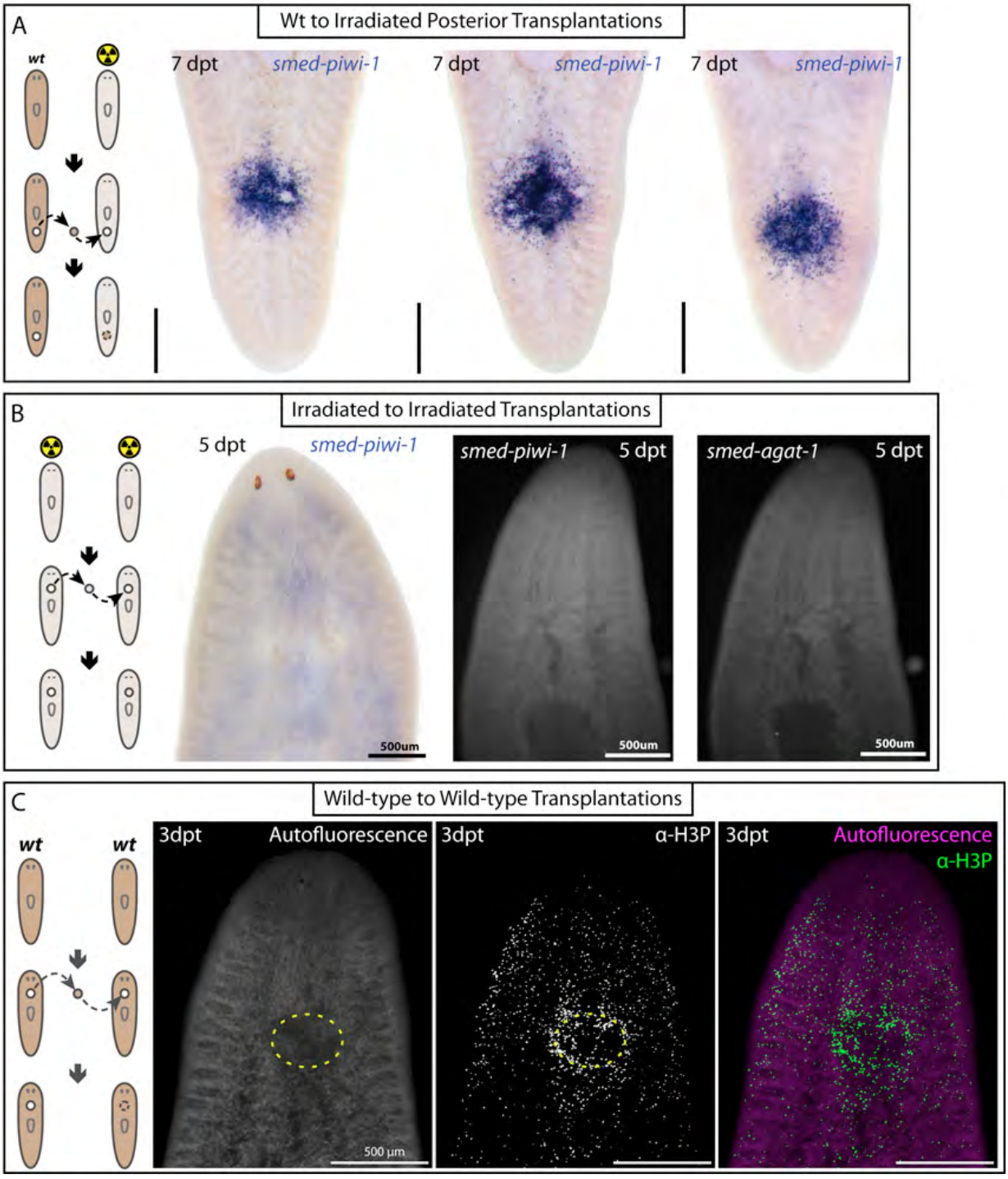

Fig. S4. Transplantation control experiments. (**A**) Wild-type to irradiated transplantations, from which posterior tissue was taken from a wild-type donor and grafted into the posterior of a lethally irradiated host (diagram), was carried out as for anterior transplantations (Fig. 2). Animals were then WISH labelled for the stem cell marker *Smed-piwi-1* 7 days post transplantation (dpt) and the grafts within the tails (n=3) were imaged (top panel). The observed stem cell positions are comparable with anterior grafts (compare with Fig. 3E). (**B**) Irradiated to irradiated anterior transplantations were performed (diagram) and the animals were labelled by WISH for *Smed-piwi-1* and the late progeny marker *Smed-agat-1* at 5 dpt. A lack of any staining for either colorimetric or fluorescent detection of the stem cell marker or the progeny marker was observed. (**C**) To determine whether the simple act of tissue transplantation was initiating a mitotic wound response similar to those described by others (Salo and Baguñà, 1986; Wenemoser and Reddien, 2010), we grafted wild-type tissue into a wild-type host using our described transplantation procedure (diagram) and immunostained for anti-phosphohistone H3 (anti-H3P) at 3 dpt. Autofluorescence was used to identify the boundary between the graft and host tissue (dashed yellow ellipse). A qualitatively obvious local increase in mitotic activity was observed proximal to the graft as compared with the level of mitosis in the rest of the animal (merged image).

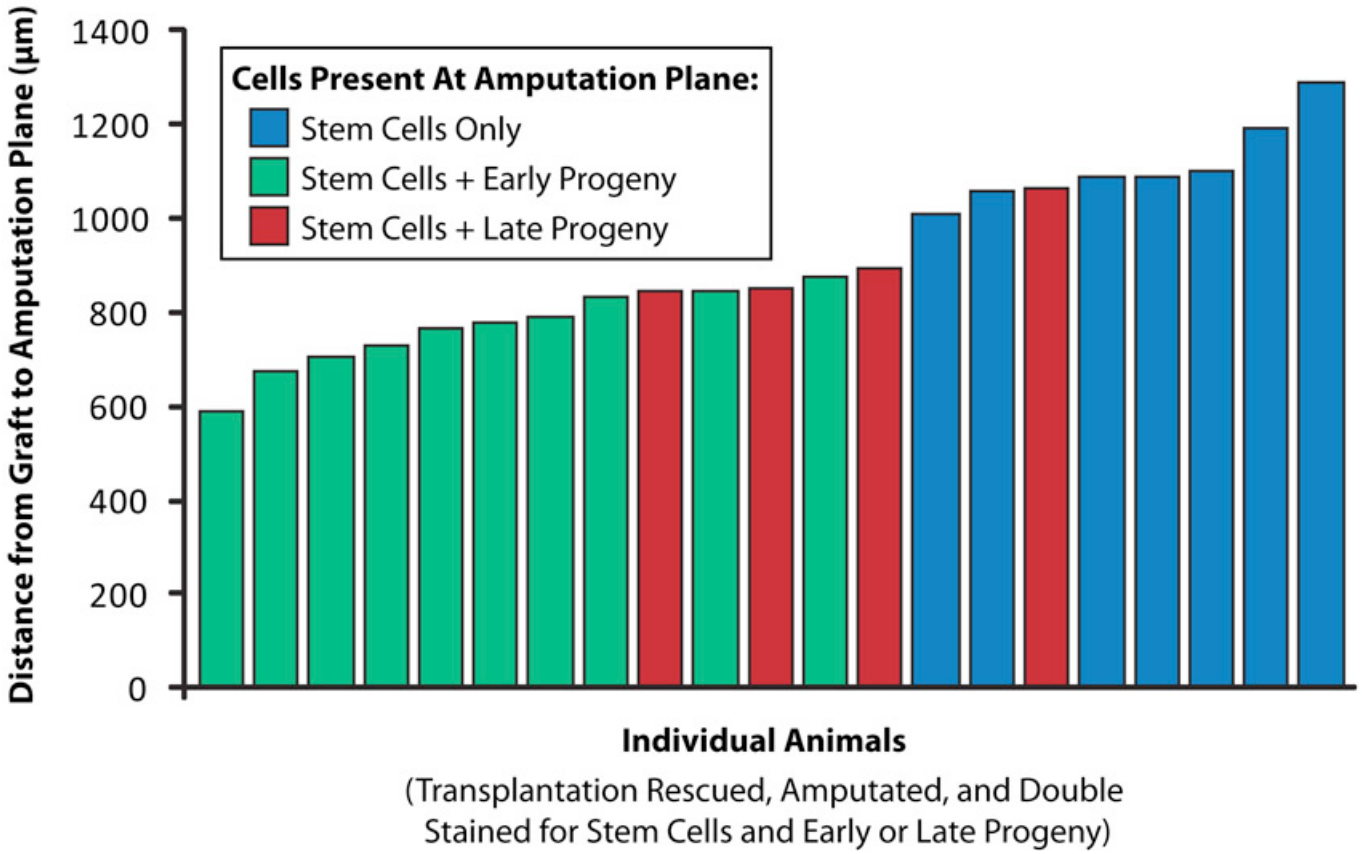

Fig. S5. Stem cells appear at the amputation plane before progeny cells following transplantation, amputation and migration. Double WISH staining for stem cells (*piwi*) and progeny (*prog-1* or *agat-1*) (as shown in Fig. 7A,B) were preformed and the results were pooled into three categories: only stem cells present at the amputation plane, stem cells present with early progeny at the amputation plane and stem cells present with late progeny at the amputation plane. The distance between amputation plane and the graft was then measured for each animal (n=21) and the categorized data were ranked. Animals double stained for stem cells and late progeny, and with graft to amputation plane distances shorter than 800 µm and were not included in this analysis.

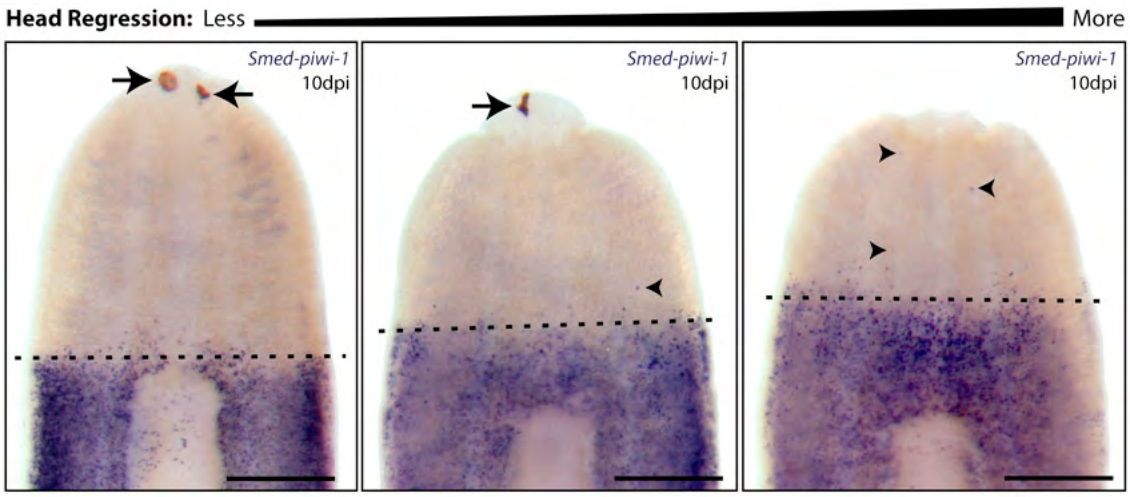

Fig. S6. Head regression in intact partially irradiated animals may trigger stem cell migration akin to wounding. Intact partially irradiated planaria develop anterior head regression at variable rates as a normal consequence of irradiation and tissue turnover. In situ hybridization for the stem cell marker *Smed-piwi-1* reveals diffuse populations of stem cells at the boundaries between unshielded and shielded tissue (dashed lines) and isolated stem cells in the anterior irradiated tissue (arrowheads) in separate animals with different degrees of head regression. Degree of head regression is estimated by the loss of photoreceptors (arrows). Animals were partially irradiated as in Fig. 1 and fixed 10 days post irradiation (dpi). Anterior is up. Scale bars are 500 µm.

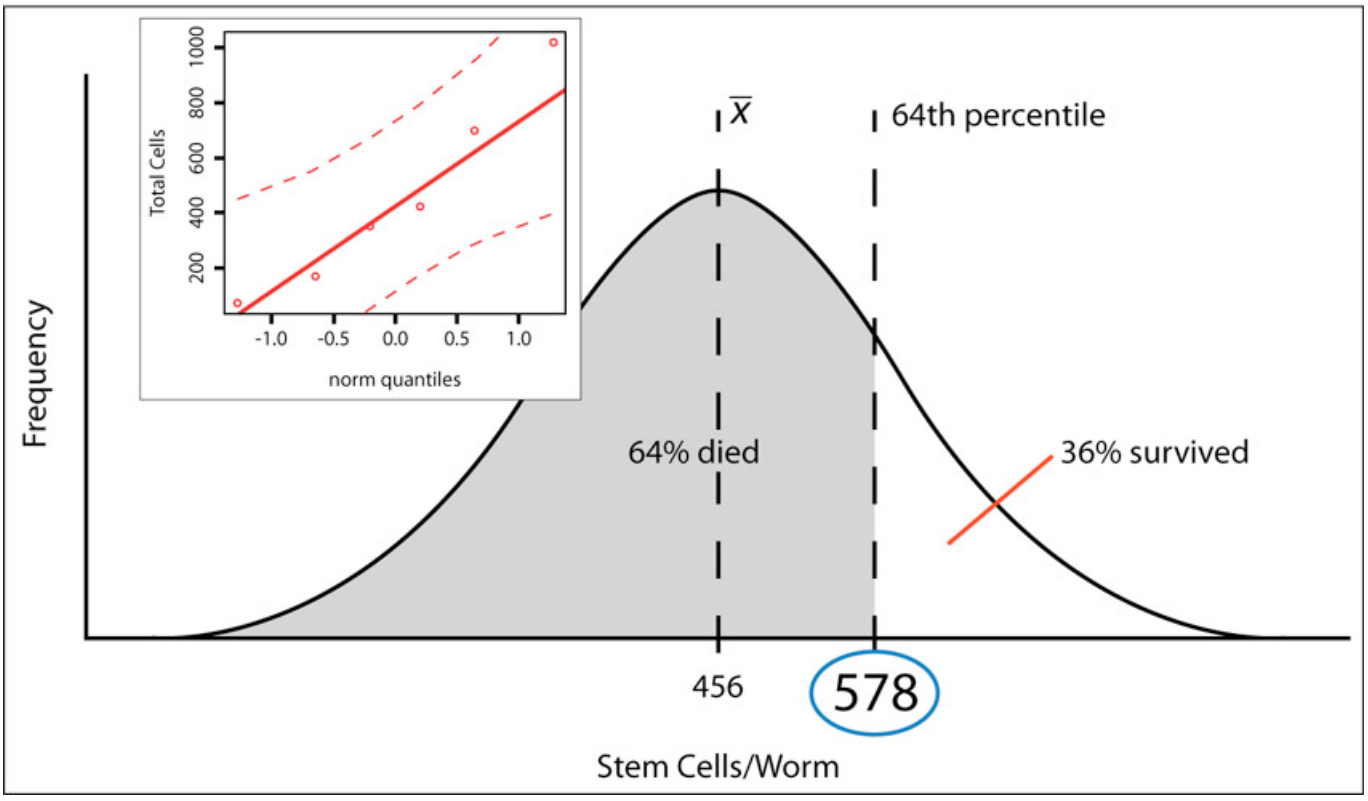

Fig. S7. Estimation of minimal number of serially transplanted SCs needed to rescue a lethally irradiated host. The minimal number of cells needed for rescue was calculated from 36% survival of the population (n=17) from which the counted cohort (n=6) was taken. The mean was calculated (=456 cells) and the measurements were evaluated for normality (inset, P=0.7, Shapiro-Wilk normality test). We estimated the number of cells present in animals at the 64th percentile of the normally distributed population (~578 cells, ~100 cell/mm^2). As migrating SCs are likely self-renewing from transplantation until fixation, we can assume that the true number of transplanted SCs is in fact less than the measured amount (~168 cells, based on average cell cycle of ~21 hours).