THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

doi: 10.1387/ijdb.113440sm

SUPPLEMENTARY MATERIAL

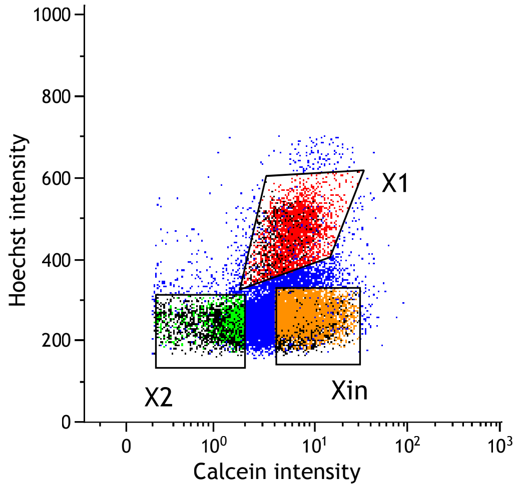
corresponding to:

Heterogeneity of planarian stem cells in the S/G2/M phase

SÖREN MORITZ, FRANZISKA STÖCKLE, CLAUDIA ORTMEIER, HENNING SCHMITZ,
GUSTAVO RODRÍGUEZ-ESTEBAN, GÖRAN KEY and LUCA GENTILE*

***Address correspondence to:** Luca Gentile. Planarian Stem Cell Laboratory, Max Planck Institute for Molecular Biomedicine, von-Esmarch-Str. 54, 48149 Münster, Germany. Tel: +49-251-8346-870. Fax: +49-251-8346-900. e-mail: luca.gentile@mpi-muenster.mpg.de

The corresponding full paper is available at: http://dx.doi.org/10.1387/ijdb.113440sm



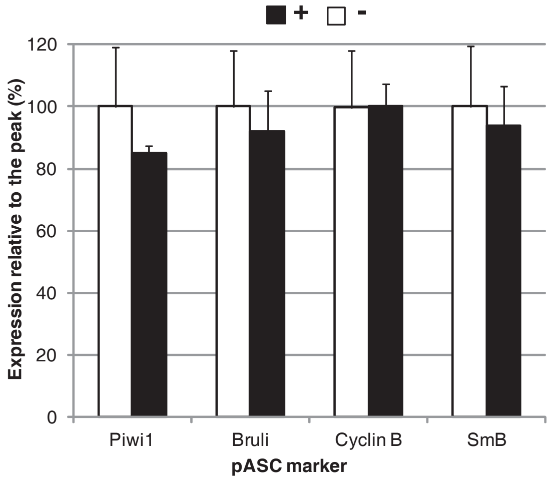


Fig. S1 (Left). X1$^{6\text{-}9.2+}$ cells are not in a specific phase of the cell cycle. *Total planarian cells were stained for FACS analysis, live-immunostained with antibody 6-9.2 and gated according to the intensity of the immunostaining, as described. In the dot plot shown, 6-9.2$^+$ cells (red, green and yellow, in the foreground) and 6-9.2$^-$ cells (black, in the background) are mapped back to their respective FACS populations.*

Fig. S2 (Right). Expression of the stem cell markers Piwi1, Bruli, Cyclin B and SmB. *The expression level of the stem cell markers tested did not differ between X1$^{6\text{-}9.2+}$ and X1$^{6\text{-}9.2-}$ cells. "+" = 6-9.2 cells; "-" = 6-9.2 cells; data are presented as Mean ± SEM of 2 independent experiments.*

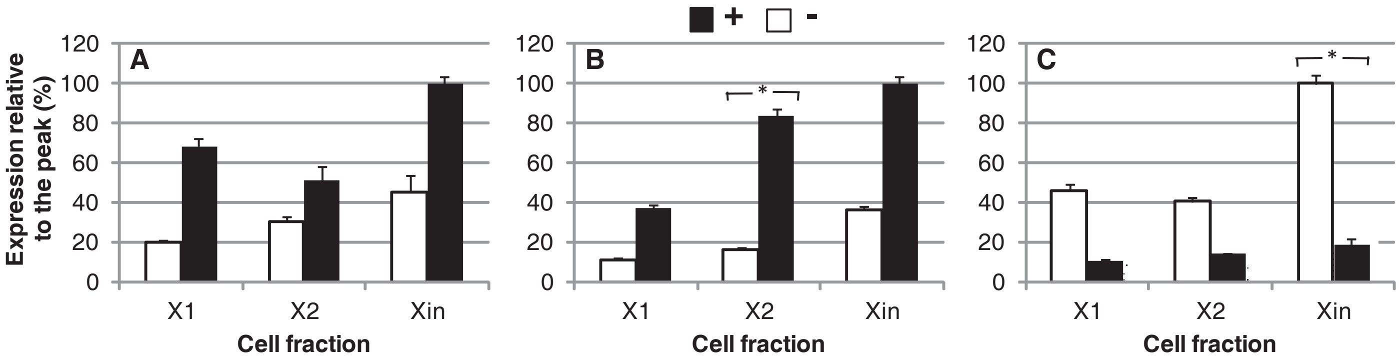


Fig. S3. Expression of the late progeny marker Cyp1a1 positive and negative subfractions of planarian cell FACS populations immuno-stained with 6-9.2, 8-22.2 or 7-22.2 supernatants. *The expression of Cyp1a1 was assessed in the subfractions of the X1, X2 and Xin populations found positive and negative after live-immunostaining with 6-9.2* **(A)**, *8-22.2* **(B)** *or 7-22.2* **(C)**. *"+" = mAb$^+$ cells; "-" = mAb$^-$ cells; * = $p \leq 0.05$; data are presented as Mean ± SEM of 3 independent experiments; peak expression is set to 100%.*

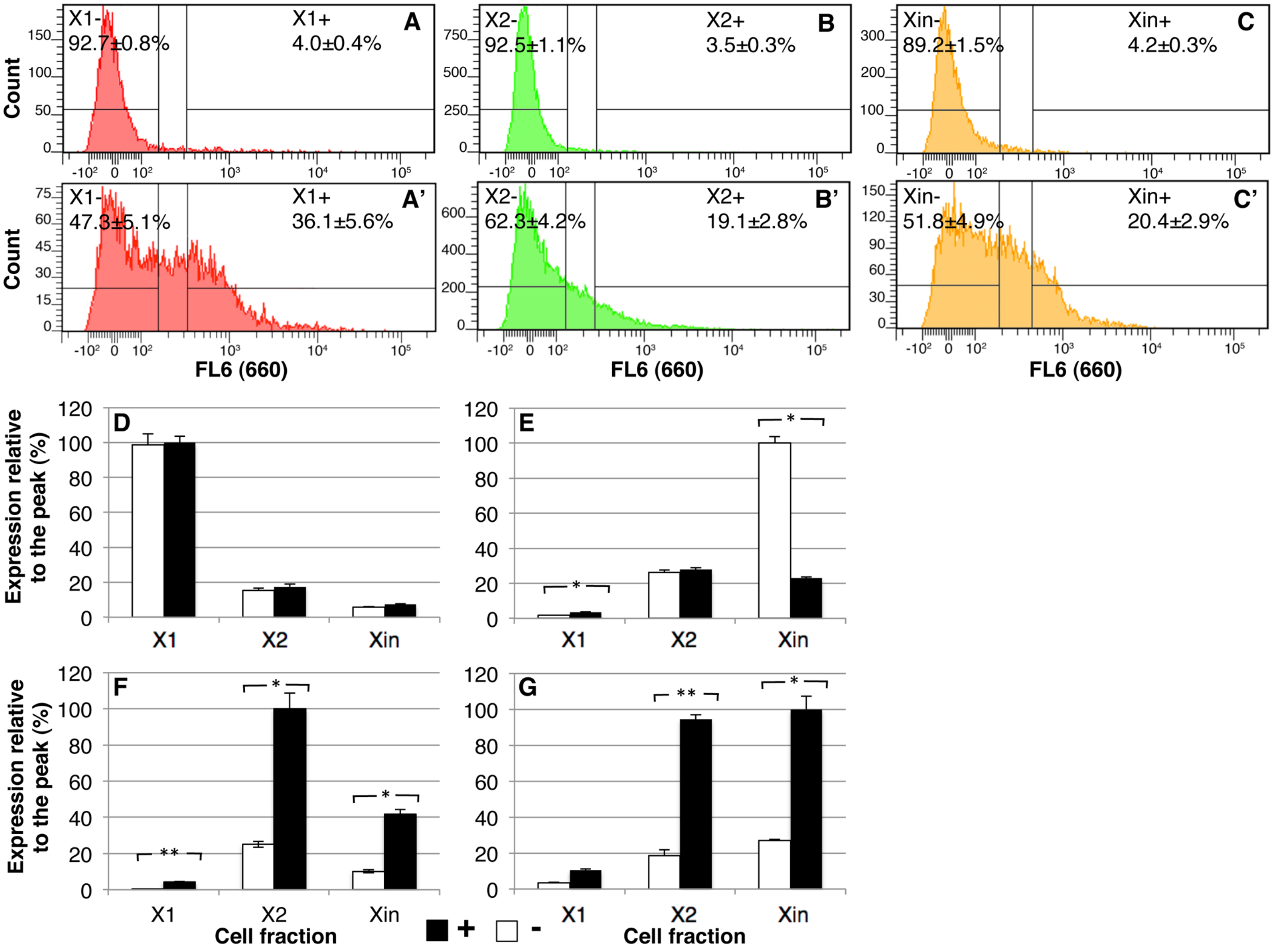


Fig. S4. Correlation between 8-22.2 immunostaining and the expression profile of early and late progeny markers. *Flow cytometric analysis of live planarian cells stained with Hoechst 33342 and Calcein AM and immunostained with either generic mouse IgGs* **(A-C)** *or supernatant from clone 8-22.2* **(A'-C')**. *About one third of the X1* **(A')** *and one fifth of the X2* **(B')** *and Xin* **(C')** *cells were found positive for the 8-22.2 immunostaining. After sorting, positive and negative sub-fractions of the X1, X2 and Xin populations were lysed and processed for qPCR analysis. The expression of the planarian stem cell marker Piwi1* **(D)**, *the muscle cell marker Myhc* **(E)**, *the category-2 marker NB.32.1g* **(F)** *and the category-3 marker Agat1* **(G)** *(Eisenhoffer* et al., *2008) are shown. The difference in NB.32.1g expression between $X1^{8\text{-}22.2-}$ and $X1^{8\text{-}22.2+}$ cells is about 15-fold. "+" = $8\text{-}22.2^{+}$ cells; "-" = $8\text{-}22.2^{-}$ cells; * = $p \leq 0.05$; ** = $p \leq 0.0001$. FACS data are presented as Mean ± SEM of 5 independent experiments; qPCR data are presented as Mean ± SEM of 3 independent experiments; peak expression is arbitrarily set to 100%.*

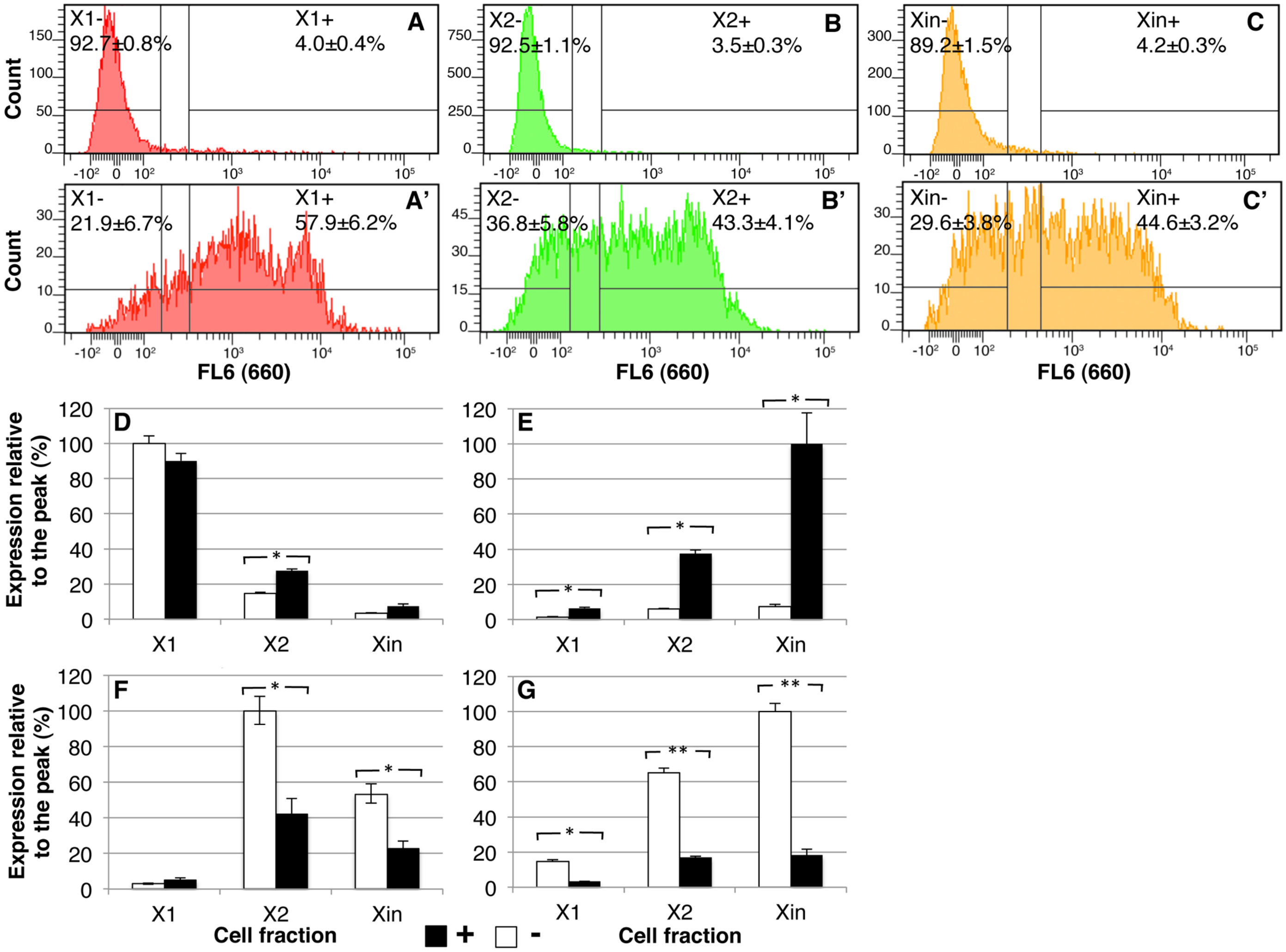


Fig. S5. Correlation between 7-22.2 immunostaining and the expression profile of early and late progeny markers. *Flow cytometric analysis of live planarian cells stained with Hoechst 33342 and Calcein AM and immunostained with either generic mouse IgGs* **(A-C)** *or supernatant from clone 7-22.2* **(A'-C')**. *About two third of the X1* **(A')** *and half of the X2* **(B')** *and Xin* **(C'**) *cells were found positive for the 7-22.2 immunostaining. After sorting, positive and negative sub-fractions of the X1, X2 and Xin populations were lysed and processed for qPCR analysis. The expression of the planarian stem cell marker Piwi1* **(D)**, *the muscle cell marker Myhc* **(E)**, *the category-2 marker NB.32.1g* **(F)** *and the category-3 marker Agat1* **(G)** *(Eisenhoffer* et al., *2008) are shown. "+" = 7-22.2$^+$ cells; "-" = 7-22.2$^-$ cells; * = p≤0.05; ** = p≤0.0001. FACS data are presented as Mean ± SEM of 5 independent experiments; qPCR data are presented as Mean ± SEM of 3 independent experiments; peak expression is arbitrarily set to 100%.*

TABLE S1

qPCR PRIMERS

Gene (symbol)		Primer sequence (5'->3')	Pro
Gapdh	Fw	GCTGTCGGTCAAGTCATT	AAA
	Rev	GGATACATCGGCTACAGGTA	
Agat1 (Gatm)	Fw	GGTTGGAAGATTGTGAAGGG	TGT
	Rev	CCAACCTCTCGCTTTTCA	
NB321g	Fw	CGAATGTCGTTATAGAGCTCG	ACA
	Rev	GCGCCTCGTCCAATTTT	
Piwi1 (Piwil1)	Fw	AGTTCCTGTTCCAACGCATTATG	CTG
	Rev	CTGGAGGAGTAACACCACGATGA	
Cyp1a1	Fw	GTAGTGGAGCTTTTACCGAC	CCC
	Rev	GGTTATTGAAGTTCCCGGAC	
Myhc	Fw	TGAAGAGCGAGCTGATCAAGC	AGC
	Rev	GCGGATTGATGTCGCAGTTATAG	
Bruli (Celf3)	Fw	AACACTCCCGCATTAGGCTTATC	ATA
	Rev	ACCCTTCAGGACCAGTTACAATATTG	
Cyclin B (Ccnb1)	Fw	GCGATGTCCTTGGTCGACAT	TCT
	Rev	AAGTTTCGGCGCATAATGAGAA	
SmB (Snrpb)	Fw	GCCAGCATTTACAACAGTTGCA	CCT
	Rev	GTGGCATACCTGCTAACATTGGA	