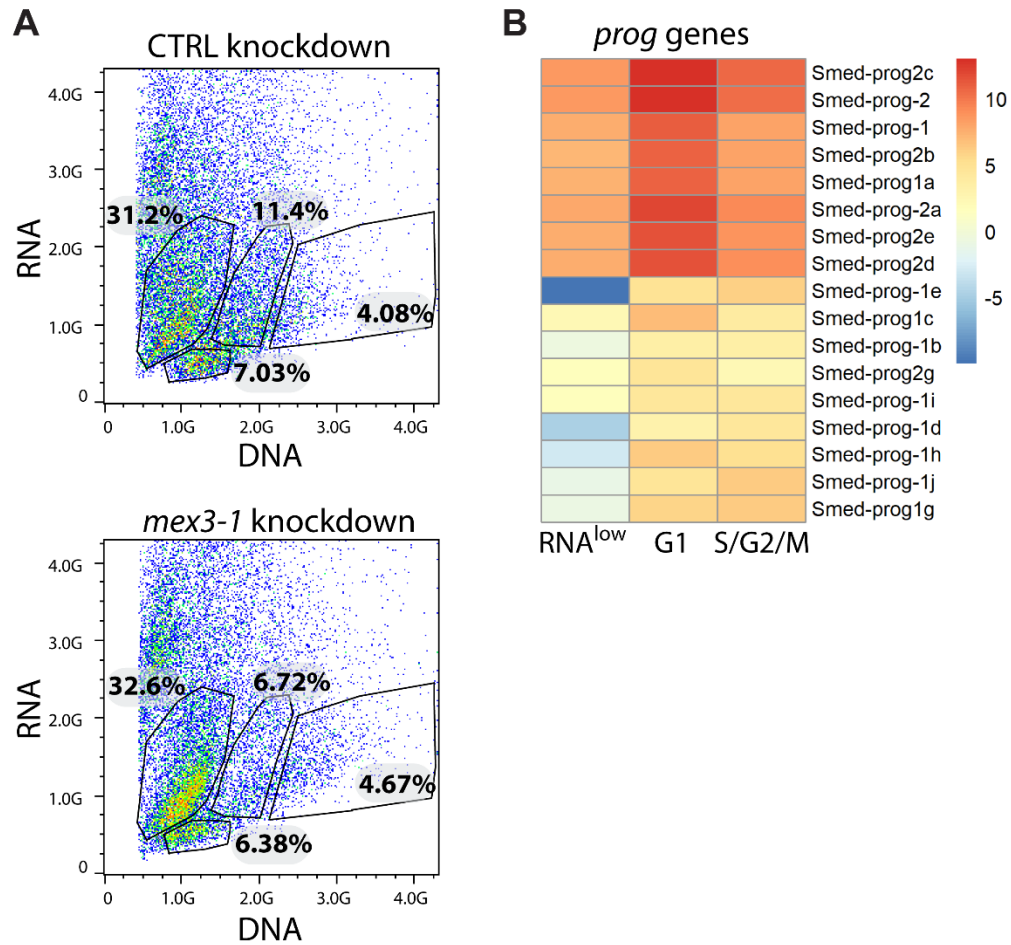


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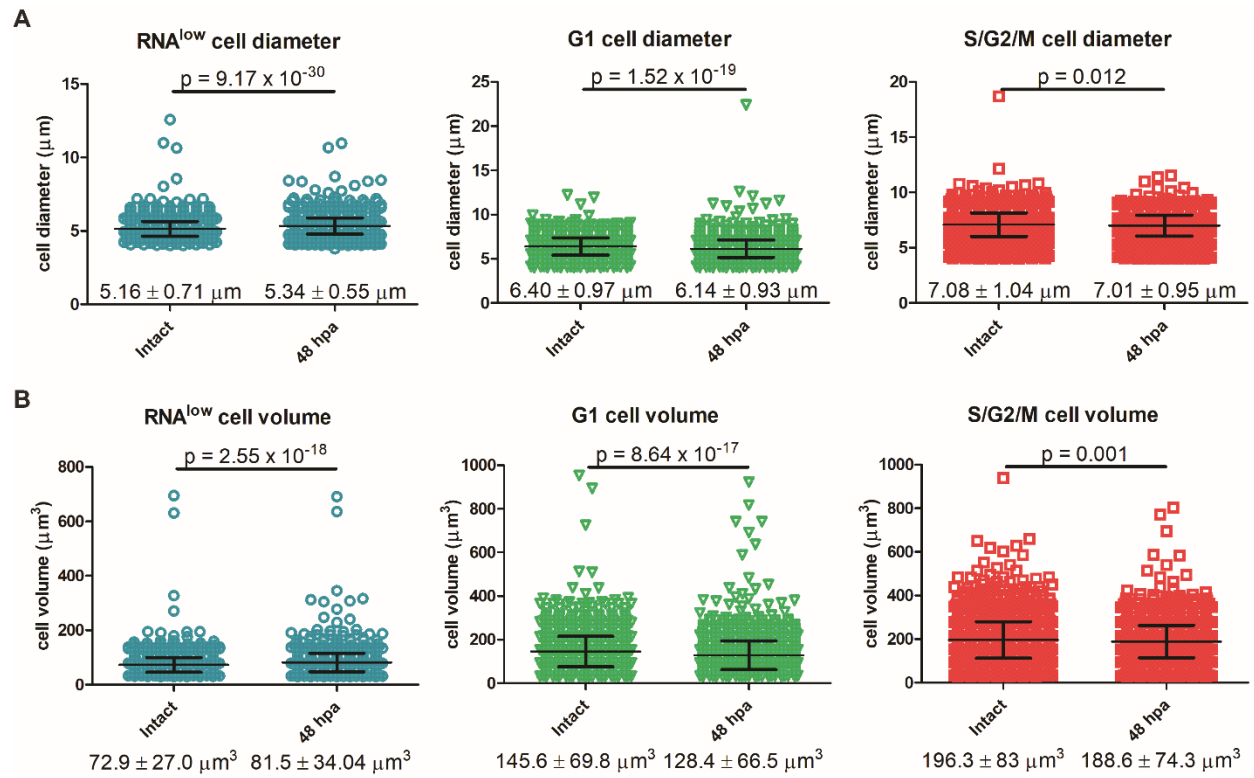


**Appendix Figure S1: The RNA<sup>low</sup> gate is unlikely to include early progeny cells.**

A FACS plots of dissociated cells from control or *mex3-1* KD animals. Percentages indicate the proportion of events within each gate. Plots are representative of n = 2 independent experiments.

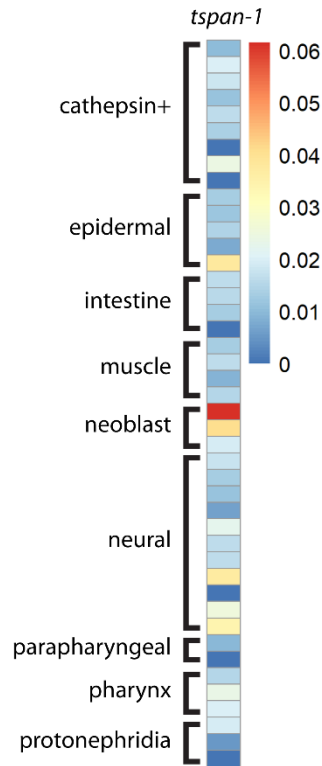
B Expression of *prog*-family genes in bulk RNA-seq data of RNA<sup>low</sup>, G1 and S/G2/M cells.

Heatmap scale, log<sub>2</sub> normalized expression.



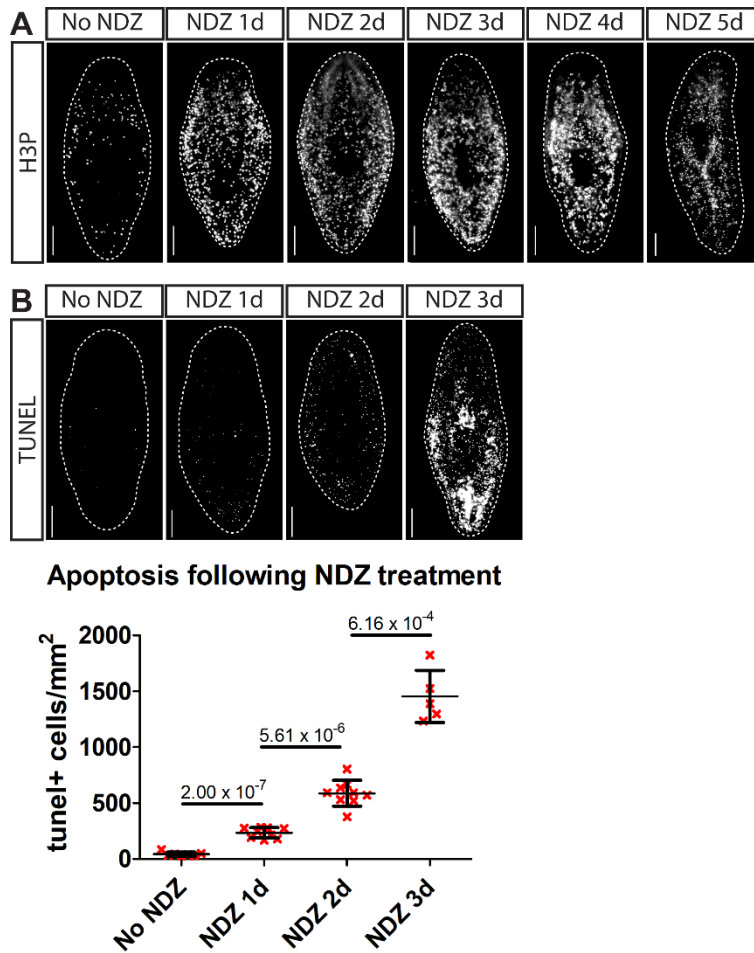
**Appendix Figure S2: Automated cell size measurements during regeneration.**

A-B Automated measurements of cell diameter (A) and volume (B) in RNA<sup>low</sup>, G1 and S/G2/M cells from intact and 48 hpa regenerating animals ( $n \geq 1700$ ). Data presented as mean  $\pm$  s.d. (indicated below data points). Statistical significance was assessed using Welch's t-tests (p values are indicated above data points).



**Appendix Figure S3: *tspan-1* is broadly expressed by scRNAseq.**

Expression levels of *tspan-1* in major cell clusters from a published scRNAseq atlas of planarian tissues (Data ref: Fincher et al., 2018, downloaded from <https://compgen.bio.ub.edu/PlanNET/planexp>).



**Appendix Figure S4: Effect of NDZ treatment on mitosis and cell death.**

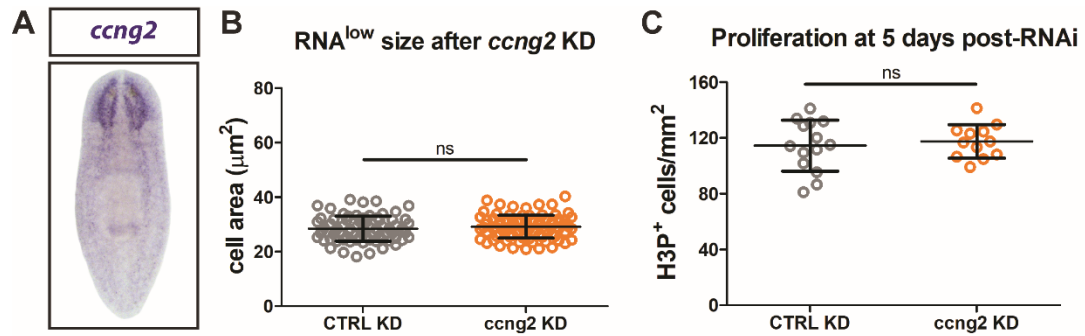
A H3P immunostaining in untreated animals and after 1-5 days of continuous NDZ treatment.

Scale bars = 250  $\mu$ m.

B TUNEL staining and quantification in untreated animals and after 1-3 days of continuous NDZ

treatment. Data presented as mean  $\pm$  s.d. Statistical significance was assessed using Welch's t-

tests (p values are indicated). Scale bars = 250  $\mu$ m. n = 5-10 animals per timepoint.



**Appendix Figure S5: *ccng2* knockdown does not affect RNA<sup>low</sup> neoblast size or homeostatic proliferation.**

A Representative WISH images for *ccng2*.

B RNA<sup>low</sup> neoblast size in intact animals following control or *ccng2* knockdown (n ≥ 75).

C Quantification of H3P immunostaining in intact animals following control or *ccng2* knockdown (n = 12-14).

Data information: Data in B and C are presented as mean ± s.d. Statistical significance was assessed using Welch's t-tests (ns, not significant (p > 0.05)).

## Appendix Table S1: Transcript IDs

This table contains a list of all genes referenced in this manuscript and their transcript IDs corresponding to the transcriptome under NCBI BioProject PRJNA215411.

GENE	TRANSCRIPT ID
atg7	SmedASXL_014971
aurkb	SmedASXL_013325
blm	SmedASXL_017447
cdc45	SmedASXL_011414
chaf1b	SmedASXL_013663
ctdsp1	SmedASXL_018115
cyclin B2	SmedASXL_013627
cyclin C	SmedASXL_011068
cyclin D2	SmedASXL_010420
cyclin G2 (ccng2)	SmedASXL_007037
dicer	SmedASXL_006374
egfr-3	SmedASXL_000685
egr-1	SmedASXL_008042
elav-2	SmedASXL_013243
exo1	SmedASXL_015035
ezh1	SmedASXL_013155
fen1	SmedASXL_006671
foxo3	SmedASXL_012875
fst	SmedASXL_012581
gata123	SmedASXL_006281
gata456	SmedASXL_011703
gins2	SmedASXL_010901
hnf4	SmedASXL_002078
kif20b	SmedASXL_016228
kif23	SmedASXL_017628
lbr	SmedASXL_019163
lkb1	SmedASXL_005843
Lrig-1	SmedASXL_013974
mcm2	SmedASXL_014198
mcm4	SmedASXL_012910
mcm5	SmedASXL_012139
mcm7	SmedASXL_007040
mki67	SmedASXL_018866
msh2	SmedASXL_013478

msi-1	SmedASXL_011563
nasp	SmedASXL_000706
p53	SmedASXL_008454
pcna	SmedASXL_002240
piwi-1	SmedASXL_008653
piwi-2	SmedASXL_018810
piwi-3	SmedASXL_002725
pola1	SmedASXL_018375
prim1	SmedASXL_000760
rad51	SmedASXL_006619
randap1	SmedASXL_010114
rb	SmedASXL_016270
rpa2	SmedASXL_019468
rrm1	SmedASXL_012448
ski-1	SmedASXL_002407
slbp	SmedASXL_008863
smarca2	SmedASXL_017781
smc4	SmedASXL_016045
Smed-prog-1	SmedASXL_011484
Smed-prog1a	SmedASXL_013664
Smed-prog-1b	SmedASXL_008207
Smed-prog1c	SmedASXL_003799
Smed-prog-1d	SmedASXL_005723
Smed-prog-1e	SmedASXL_004473
Smed-prog1g	SmedASXL_003595
Smed-prog-1h	SmedASXL_009962
Smed-prog-1i	SmedASXL_013905
Smed-prog-1j	SmedASXL_002658
Smed-prog-2	SmedASXL_073532
Smed-prog-2a	SmedASXL_014068
Smed-prog2b	SmedASXL_008106
Smed-prog2c	SmedASXL_010535
Smed-prog2d	SmedASXL_010536
Smed-prog2e	SmedASXL_012073
Smed-prog2g	SmedASXL_004274
soxP-1	SmedASXL_013552
soxP-2	SmedASXL_013986
soxP-3	SmedASXL_004704
tef	SmedASXL_011746
thra	SmedASXL_017328
ttk	SmedASXL_015562



tym5	SmedASXL_004854
ubr7	SmedASXL_019427
ung	SmedASXL_005855
zfp-1	SmedASXL_001731
znf-91	SmedASXL_011708