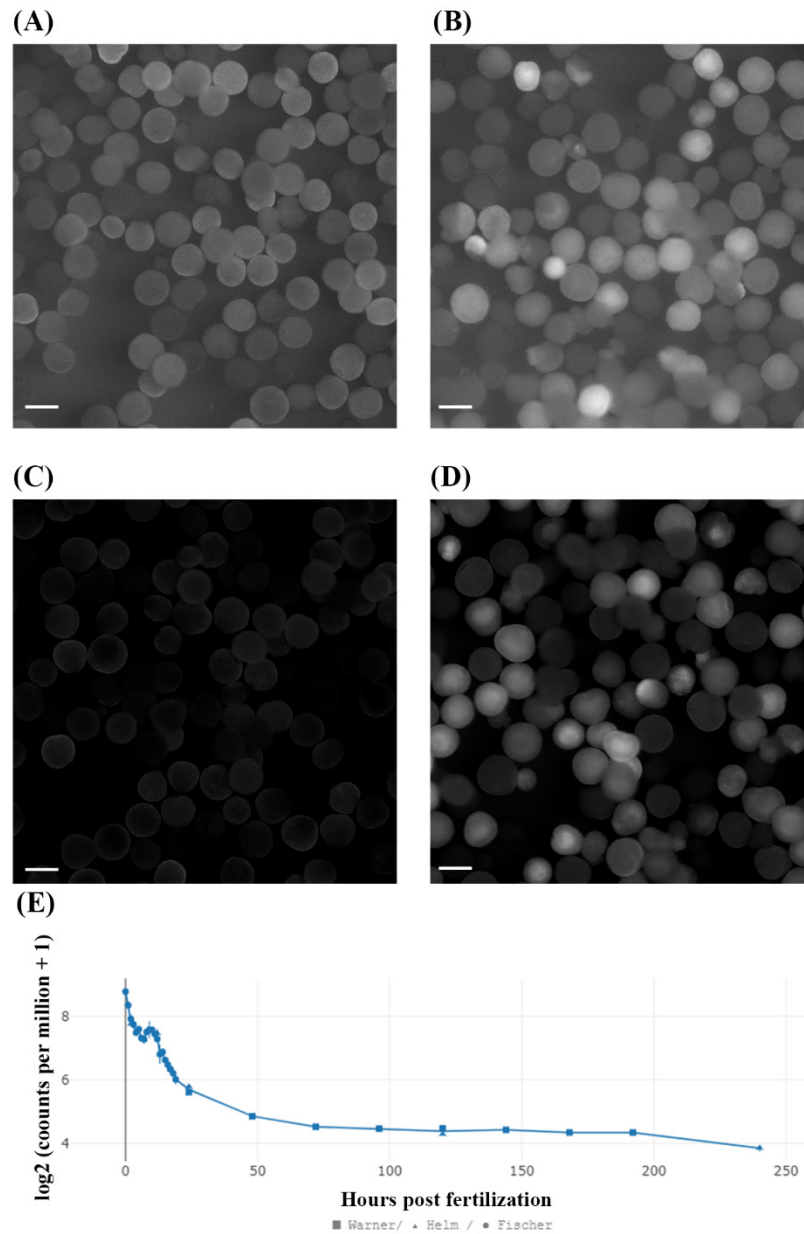


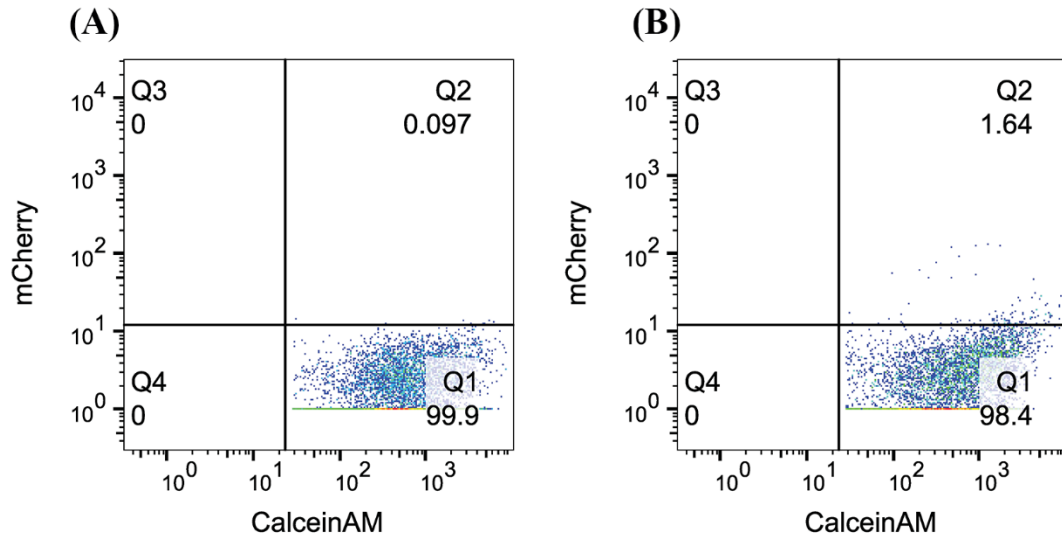
This file contains supplementary figures S1-S4

Supplementary figure S1: *TBP* Promoter sequence (2907 bp) spanned by *PacI* and *AscI* restriction sites:

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TTTATATCATACAGGGAGTAAAAATCCTCCCAGAACCATAATAAAACAAATTTTCAGTGATATTGTTGTGGCAGGTA
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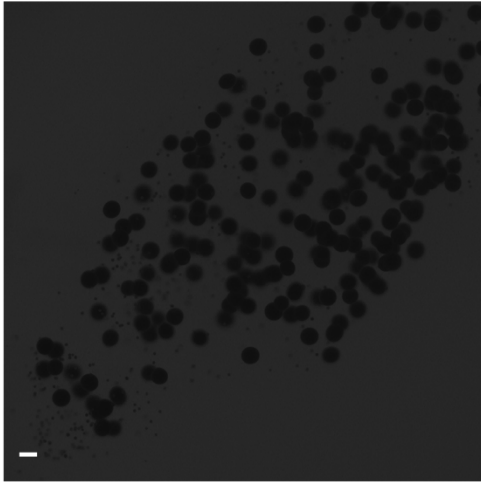


Supplementary figure S2. Early expression of the TBP:mCherry transgene and native TBP gene. TBP:mCherry embryos express mCherry as early as 30 hours post fertilization. **(A)** WT embryos 30 hours after fertilization. **(B)** F₂ WT/TBP:mCherry embryos 30 hours after fertilization. **(C)** WT embryos 40 hours after fertilization. **(D)** F₂ WT/TBP:mCherry embryos 40 hours after fertilization. Red fluorescence is shown in white. Scale bar 0.2mm. Eggs in panels A and B were photographed at a four-fold larger digital gain compared to panels C and D. **(E)** TBP expression levels obtained from quantitative RNA-seq data at the NvERTx platform (Warner et al. 2017 Development 145:dev162867).

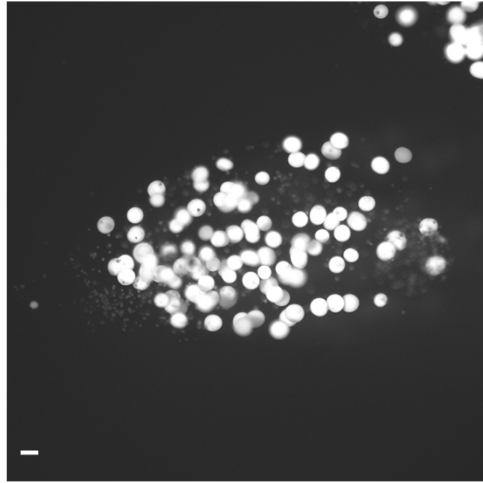


Supplementary figure S3. Representative FACS analyses of WT *N. vectensis* adult individuals. **(A)** FACS analysis of dissociated cells obtained from the physa. **(B)** FACS analysis of dissociated cells obtained from the body column.

(A)



(B)



Supplementary figure S4. Maternal deposition of mCherry. **(A)** unfertilized egg package obtained from wildtype females following induction **(B)** unfertilized egg package exhibiting red fluorescence obtained from TBP:mCherry F₁ females following induction. The pictures were taken using the same exposure parameters. Scale bar 0.2 mm.