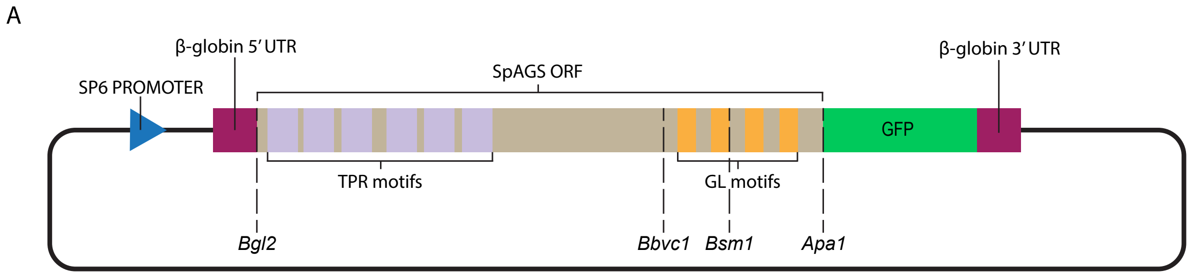
Supplementary Figures and Legends

Supplemental Figure S1



S. purpuratus AGS Protein Sequence

TPR1 TPR2
MQSEASCMELALEGERLCKAGNCREGVRYLEAAVEVGTDDLKTLSAIYSQLGNAYFYLEDYGKALTYHKHDL

117aa, before here removed in 1F
TPR3
TLATSIGDRLGEAKASGNIGNTLKVLGKFDEAICCCQRHLDISRELSEKVGEGRALYNLGNVYHAKGKHAGRS

TPR4
GHQDPGDFPQEVTDCLKQAIQFYEANLAIVRELGDRAAQGRACGNLGNTHYLLGNFETAIQFHTERLAIAKE

222aa, before here removed in 2F
TPR5 TPR6
FGDKAAERRAYSNLGNACVFMVQFEVAAKYYKKSLHIARQLGELAMEAQACYSLGNTYTLLREYEKAVEYHS

301aa, before here removed in 3F
RHMEIAQQLNDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVTAKMNLQDLQTLFG

ISTADLSEVSTTVQTPLQESKGARPRRRRSMENLELVAMTPEKKTEIVQVPKRKIRPGSKLKLKNGGESKDKQS

BbvC1 RE site
GL1 (removed in Δ1)
HKDSKESVSSAKNSVPAPSKAKSQTSARKGGEPIQMLDDADNFFEALSRFQSNRMDEQRCSFGRLQKKLAD

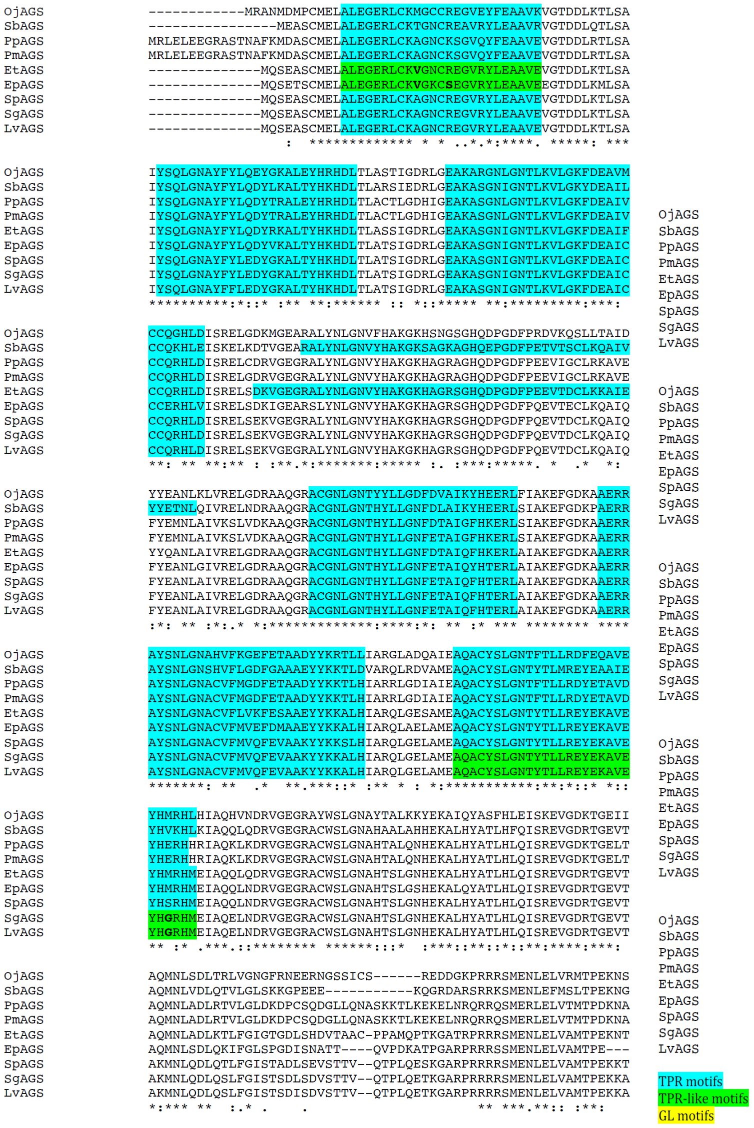
Bsm1 RE site
GL2 (removed in Δ2) GL3 (removed in Δ3)
EEGNGLPEKEELLDEIAKLQGSRLNEQRAFSVKRLPGLPGLRANEDVVGKLLAKGERAEPDDDFFEMIIRCQG

GL4 (removed in Δ4)
ARIEDQRSTLPIQAPAPTVPDEDFFSLIQRIQSKRIEEQRSIAPWEKGSGTSCVCFYDYDTSRTEGTACCVYSRP

659aa
VQESHL

Supplemental Figure 1. **Dissection of SpAGS motifs. A**, The construct design of SpAGS-GFP, including the restriction enzyme sites used to prepare SpAGS mutants. **B**, The protein sequence of SpAGS. Predicted domains are labeled based on NCBI blast results, and the sequence portions deleted for each N-terminal construct are marked. The sequences for each GL motif used for deletion or swapping are indicated in orange. The internal restriction enzyme sites for *BbvCI* and *BsmI* are shown in green.

Supplemental Figure 2 Echinoderm AGS sequence alignment



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OjAGS                 ------------MRANMDMPCMELALEGERLCKMGCCREGVEYFEAAVKVGTDDLKTLSA
SbAGS                 ----------------MEASCMELALEGERLCKTGNCREAVRYLEAAVRVGTDDLQTLSA
PpAGS                 MRLELEEGRASTNAFKMDASCMELALEGERLCKAGNCKSGVQYFEAAVEVGTDDLRTLSA
PmAGS                 MRLELEEGRASTNAFKMDASCMELALEGERLCKAGNCKSGVQYFEAAVEVGTDDLRTLSA
EtAGS                 --------------MQSEASCMELALEGERLCKVGNCREGVRYLEAAVEVGTDDLKTLSA
EpAGS                 --------------MQSETSCMELALEGERLCKVGKCSEGVRYLEAAVEEGTDDLKMLSA
SpAGS                 --------------MQSEASCMELALEGERLCKAGNCREGVRYLEAAVEVGTDDLKTLSA
SgAGS                 --------------MQSEASCMELALEGERLCKAGNCREGVRYLEAAVEVGTDDLKTLSA
LvAGS                 --------------MQSEASCMELALEGERLCKAGNCREGVRYLEAAVEVGTDDLKTLSA
                                       :  ************* * * ..*.*:****. *****: ***

OjAGS                 IYSQLGNAYFYLQEYGKALEYHRHDLTLASTIGDRLGEAKARGNLGNTLKVLGKFDEAVM
SbAGS                 IYSQLGNAYFYLQDYLKALTYHKHDLTLARSIEDRLGEAKASGNIGNTLKVLGKYDEAIL
PpAGS                 IYSQLGNAYFYLQDYTRALEYHRHDLTLACTLGDHIGEAKASGNLGNTLKVLGKFDEAIV
PmAGS                 IYSQLGNAYFYLQDYTRALEYHRHDLTLACTLGDHIGEAKASGNLGNTLKVLGKFDEAIV
EtAGS                 IYSQLGNAYFYLQDYRKALTYHKHDLTLASSIGDRLGEAKASGNIGNTLKVLGKFDEAIF
EpAGS                 IYSQLGNAYFYLQDYVKALTYHKHDLTLATSIGDRLGEAKASGNIGNTLKVLGKFDEAIC
SpAGS                 IYSQLGNAYFYLEDYGKALTYHKHDLTLATSIGDRLGEAKASGNIGNTLKVLGKFDEAIC
SgAGS                 IYSQLGNAYFYLEDYGKALTYHKHDLTLATSIGDRLGEAKASGNIGNTLKVLGKFDEAIC
LvAGS                 IYSQLGNAYFFLEDYGKALTYHKHDLTLATSIGDRLGEAKASGNIGNTLKVLGKFDEAIC
                      **********:*::* :** **:****** :: *::***** **:*********:***:

OjAGS                 CCQGHLDISRELGDKMGEARALYNLGNVFHAKGKHSNGSGHQDPGDFPRDVKQSLLTAID
SbAGS                 CCQKHLEISKELKDTVGEARALYNLGNVYHAKGKSAGKAGHQEPGDFPETVTSCLKQAIV
PpAGS                 CCQRHLDISRELCDRVGEGRALYNLGNVYHAKGKHAGRAGHQDPGDFPEEVIGCLRKAVE
PmAGS                 CCQRHLDISRELGDRVGEGRALYNLGNVYHAKGKHAGRAGHQDPGDFPEEVIGCLRKAVE
EtAGS                 CCQRHLDISRELSDKVGEGRALYNLGNVYHAKGKHAGRSGHQDPGDFPEEVTDCLKKAIE
EpAGS                 CCERHLVISRELSDKIGEARSLYNLGNVYHAKGKHAGRSGHQDPGDFPQEVTECLKQAIQ
SpAGS                 CCQRHLDISRELSEKVGEGRALYNLGNVYHAKGKHAGRSGHQDPGDFPQEVTDCLKQAIQ
SgAGS                 CCQRHLDISRELSEKVGEGRALYNLGNVYHAKGKHAGRSGHQDPGDFPQEVTDCLKQAIQ
LvAGS                 CCQRHLDISRELSEKVGEGRALYNLGNVYHAKGKHAGRSGHQDPGDFPQEVTDCLKQAIQ
                      **: ** **:** : :**.*:*******:***** :. :***:*****. *  .*  *:

OjAGS                 YYEANLKLVRELGDRAAQGRACGNLGNTYYLLGDFDVAIKYHEERLFIAKEFGDKAAERR
SbAGS                 YYETNLQIVRELNDRAAQGRACGNLGNTHYLLGNFDLAIKYHEERLSIAKEFGDKPAERR
PpAGS                 FYEMNLAIVKSLVDKAAQGRACGNLGNTHYLLGNFDTAIGFHKERLSIAKEFGDKAAERR
PmAGS                 FYEMNLAIVKSLVDKAAQGRACGNLGNTHYLLGNFETAIGFHKERLSIAKEFGDKAAERR
EtAGS                 YYQANLAIVRELGDRAAQGRACGNLGNTHYLLGNFDTAIQFHKERLAIAKEFGDKAAERR
EpAGS                 FYEANLGIVRELGDRAAQGRACGNLGNTHYLLGNFETAIQYHTERLAIAKEFGDKAAERR
SpAGS                 FYEANLAIVRELGDRAAQGRACGNLGNTHYLLGNFETAIQFHTERLAIAKEFGDKAAERR
SgAGS                 FYEANLAIVRELGDRAAQGRACGNLGNTHYLLGNFETAIQFHTERLAIAKEFGDKAAERR
LvAGS                 FYEANLAIVRELGDRAAQGRACGNLGNTHYLLGNFETAIQFHTERLAIAKEFGDKAAERR
                      :*: ** :*:.* *:*************:****:*: ** :* *** ******** ****

OjAGS                 AYSNLGNAHVFKGEFETAADYYKRTLLIARGLADQAIEAQACYSLGNTFTLLRDFEQAVE
SbAGS                 AYSNLGNSHVFLGDFGAAAEYYKKTLDVARQLRDVAMEAQACYSLGNTYTLMREYEAAIE
PpAGS                 AYSNLGNACVFMGDFETAADYYKKTLHIARRLGDIAIEAQACYSLGNTFTLLRDYETAVD
PmAGS                 AYSNLGNACVFMGDFETAADYYKKTLHIARRLGDIAIEAQACYSLGNTFTLLRDYETAVD
EtAGS                 AYSNLGNACVFLVKFESAAEYYKKALHIARQLGESAMEAQACYSLGNTYTLLREYEKAVE
EpAGS                 AYSNLGNACVFMVEFDMAAEYYKKALHIARQLAELAMEAQACYSLGNTYTLLREYEKAVE
SpAGS                 AYSNLGNACVFMVQFEVAAKYYKKSLHIARQLGELAMEAQACYSLGNTYTLLREYEKAVE
SgAGS                 AYSNLGNACVFMVQFEVAAKYYKKALHIARQLGELAMEAQACYSLGNTYTLLREYEKAVE
LvAGS                 AYSNLGNACVFMVQFEVAAKYYKKALHIARQLGELAMEAQACYSLGNTYTLLREYEKAVE
                      *******: **  .*  **.***::* :** * : *:***********:**:*::* *::

OjAGS                 YHMRHLHIAQHVNDRVGEGRAYWSLGNAYTALKKYEKAIQYASFHLEISKEVGDKTGEII
SbAGS                 YHVKHLKIAQQLQDRVGEGRACWSLGNAHAALAHHEKALHYATLHFQISREVGDRTGEVT
PpAGS                 YHERHHRIAQKLKDRVGEGRACWSLGNAHTALQNHEKALHYATLHLQISREVGDKTGELT
PmAGS                 YHERHHRIAQKLKDRVGEGRACWSLGNAHTALQNHEKALHYATLHLQISREVGDKTGELT
EtAGS                 YHMRHMEIAQQLQDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVT
EpAGS                 YHMRHMEIAQQLNDRVGEGRACWSLGNAHTSLGSHEKALQYATLHLQISREVGDRTGEVT
SpAGS                 YHSRHMEIAQQLNDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVT
SgAGS                 YHGRHMEIAQELNDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVT
LvAGS                 YHGRHMEIAQELNDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVT
                      ** :* .***.::******** ******:::*  :***::**::*::**:****:***:

OjAGS                 AQMNLSDLTRLVGNGFRNEERNGSSICS------REDDGKPRRRSMENLELVRMTPEKNS
SbAGS                 AQMNLVDLQTVLGLSKKGPEEE-----------KQGRDARSRRKSMENLEFMSLTPEKNG
PpAGS                 AQMNLADLRTVLGLDKDPCSQDGLLQNASKKTLKEKELNRQRRQSMERLELVTMTPDKNA
PmAGS                 AQMNLADLRTVLGLDKDPCSQDGLLQNASKKTLKEKELNRQRRQSMERLELVTMTPDKNA
EtAGS                 AQMNLADLKTLFGIGTGDLSHDVTAAC-PPAMQPTKGATRPRRRSMENLELVAMTPEKNT
EpAGS                 AQMNLSDLQKIFGLSPGDISNATT----QVPDKATPGARPRRRSSMENLELVAMTPE---
SpAGS                 AKMNLQDLQTLFGISTADLSEVSTTV--QTPLQESKGARPRRRRSMENLELVAMTPEKKT
SgAGS                 AKMNLQDLQSLFGISTSDLSDVSTTV--QTPLQETKGARPRRRRSMENLELVAMTPEKKA
LvAGS                 AKMNLQDLQSLFGISTSDISDVSTTV--QTPLQETKGARPRRRRSMENLELVAMTPEKKA
                      *:*** **  :.* .    .                     ** ***.**:: :**:

OjAGS                 SEKPNKDQVDGKPKGARVKE---KSSKEKLVHAGSNNSIGANKATDPGQVTV------NL
SbAGS                 ATAIQPVAKPLKPV---NKNKKKTSSKEKLSRKGSDTSSDSGGSAGSNK---AESN----
PpAGS                 AAATANGK-PKQSTGAKFKRK--GSNKEKLSRKSSNTSSTSSSSGDSAACVRTENVNIQV
PmAGS                 AAATANGK-PKQSAGAKFKRK--GSNKEKLSRKSSNTSSTSSSSGESAACLRTENVNIQV
EtAGS                 DSA-VPNG-KLKPTGSKSKRRNGKE---KLSRKGSNTSTGSSNSSSSAPS----QASTQA
EpAGS                 ------------------------------------------------------------
SpAGS                 EIVQVPKR-KIRP-GSKLKLKNGGESKDKQSHKDSKESVSSAKNSAPAPS----KAKSQT
SgAGS                 EINQVPKR-KIRP-GSKIKLKNGGESKDKQSLKHSKEPVSSAKNSAPAPS----KAKTQT
LvAGS                 EINQVPKR-KIRP-GSKIKLKNGGESKDKQSHKHSKEPVSSAKNSAPAPS----KAKTRT

OjAGS                 ---KPPRKYSSPDFDDSFFDLLSRFQGNRMDEQRMSLPKSNSL-----DKCDTSDEGKEE
SbAGS                 -GVKLNGSQLDEDDQNDFFDALSRFQEKRINDQRVSFGLDD-----------TDAANKED
PpAGS                 SP-QQRSSPEFMDDDDSFFDVLSRFQGKRMDEQRCSFNRMQDKQRERENDEDRDYPMKEE
PmAGS                 SP-QQRSSPEFMDDDDSFFDVLSRFQGKRMDEQRCSFNRMQDKQRERENDEDRDYPMKEE
EtAGS                 SSQKGGEPQEMLDDADNFFEALSRFQSNRMDEQRCSFGRMQRKLQE--DEEANGLPEKEE
EpAGS                 ---KGGEPTQMLDDADNFFEALSRFQSNRMDEQRCSFGRLTKKLD---DDEENGLPEKEL
SpAGS                 SARKGGEPIQMLDDADNFFEALSRFQSNRMDEQRCSFGRLQKKLA---DEEGNGLPEKEE
SgAGS                 SARKGGEPTQMLDDADNFFEALSRFQSNRMDEQRCSFGRLQKKLA---DEEGNGLPEKEE
LvAGS                 SARKGGEPTQMLDDADNFFEALSRFQSNRMDEQRCSFGRLQKKLA---DEEGNGLPEKEE
                         :        *  :.**: ***** :*:::** *:                .   **

OjAGS                 LMNSIAKFQGKRMNEQRMHV----ASLPGLRGKDEELLSKLK--EASSVPDDDFFDMLIR
SbAGS                 LLDEIAKVQGNRLNEQRANV----ELLPGLR-GHREVVDRLLASGDGAVPDDDFFEMLMR
PpAGS                 LMNQIASFQRSRLNEQRSSI----CNLPGLKTNNEEVLGQLLQKGNQGVPDDDFFDMLMR
PmAGS                 LMNQIASFQRSRLNEQRSSI----CNLPGLKTNNEEVLGQLLQKGNQGVPDDDFFDMLMR
EtAGS                 LLDEIAKLQGSRLNEQRAFSAQRLPGLPGLR-ANENVLGKLLAKGDLAVPDDDFFEMLMR
EpAGS                 LLDEIAKLQGSRLNEQRVSVAERLPGLPGLR-ANDKVLGTLLAKGERAVPDDDFFEMLMR
SpAGS                 LLDEIAKLQGSRLNEQRAFSVKRLPGLPGLR-ANEDVVGKLLAKGERAEPDDDFFEMIIR
SgAGS                 LLDEIAKLQGSRLNEQRASSAQRLPGLPGLR-ANEDVVGKLLAKGDRVEPDDDFFEMIIR
LvAGS                 LLDEIAKLQGSRLNEQRASSAQRLPGLPGLR-ANEDVVGKLLAKGDRAEPDDDFFEMIIR
                      *::.**..* .*:****         ****:  . .::. *        ******:*::*

OjAGS                 CQGTRIEDQRTDAPERVRAPTVPDEDFFSLISRVQSQRLEEQRCDLPESLSRDMITDEK*
SbAGS                 CQGARMEDQRSSLPSSRPAPTVPDEDFFGLIQRIQSRRIEEQRTDAPWERPTKTRES*--
PpAGS                 CQGSRINDQRSEPPVLQPAPTVPDEDFFALIQRVQSKRMDAQRSDKGPQDQTK*------
PmAGS                 CQGSRINDQRSEPPVLQPAPTVPDEDFFALIQRVQSKRMDAQRSDKGPQEQTK*------
EtAGS                 CQGARIEDQRSTLPMPTPAPTVPDEDFFTLIQRIQSRRIEEQRTIPPWEKGPKPSDK*--
EpAGS                 CQGARIEDQRSSLPVAPPAPTVPDEDFFSLIQRIQSKRIEEQRMIAPWEKD--ASCMCFY
SpAGS                 CQGARIEDQRSTLPIQAPAPTVPDEDFFSLIQRIQSKRIEEQRSIAPWEKGSGTSCVCFY
SgAGS                 CQGARIEDQRSTLPIQVPAPTVPDEDFFSLIQRIQSKRIEEQRTIPPWEKGSGTSCVCFY
LvAGS                 CQGARIEDQRSTLPIQVPAPTVPDEDFFSLIQRIQSKRIEEQRTIPPWEKGSGTSCVCFY
                      ***:*::***:  *    ********** **.*:**:*:: **      .

OjAGS                 -------------------------
SbAGS                 -------------------------
PpAGS                 -------------------------
PmAGS                 -------------------------
EtAGS                 -------------------------
EpAGS                 SYESSTIHGSACCVYRRPIQQTSI*
SpAGS                 DYDTSRTEGTACCVYSRPVQESHL*
SgAGS                 DYDTSRTEGTACCVYIRPVQESHL*
LvAGS                 DYDTSHAEGTACCVYIRPVQESHL*
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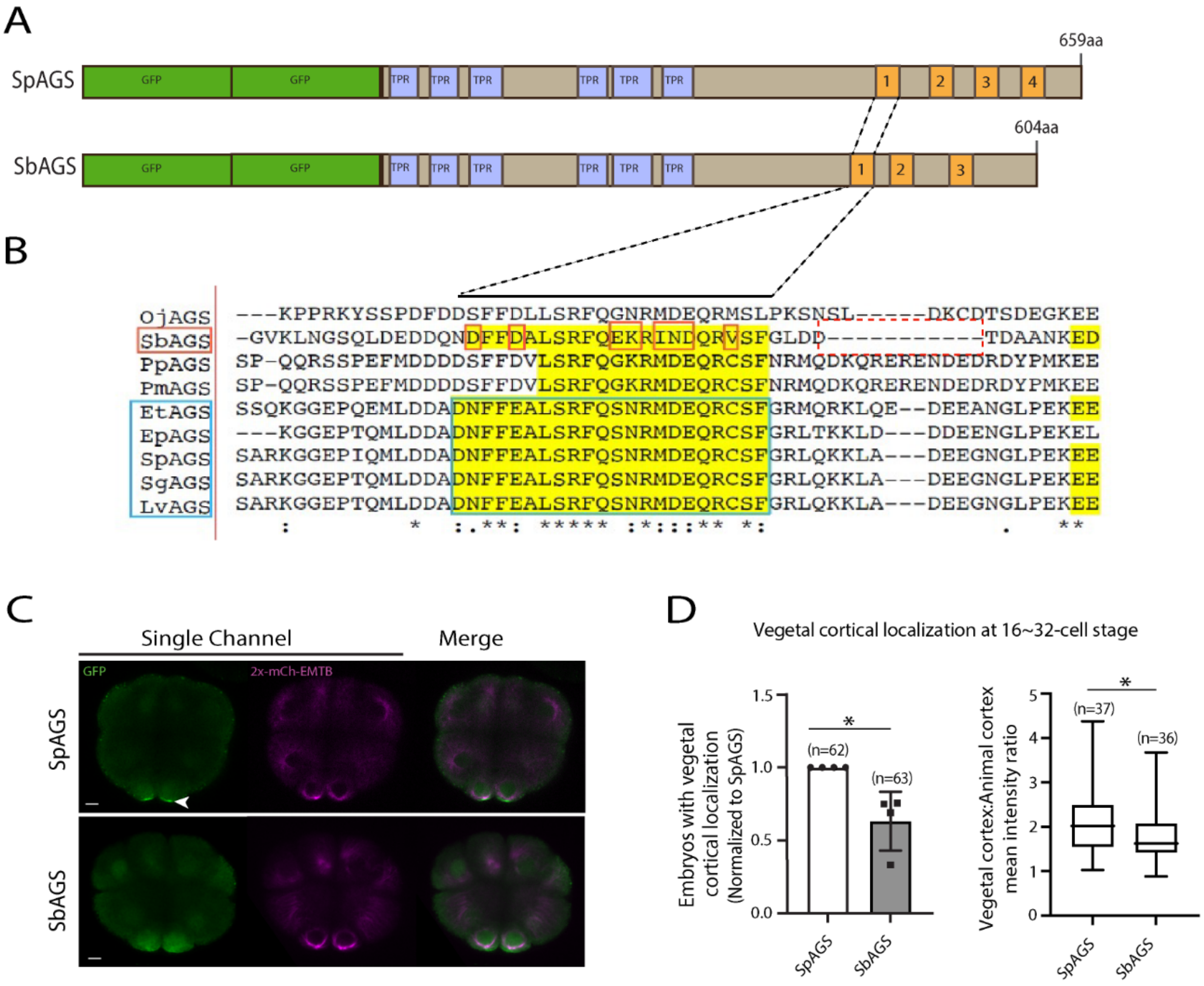
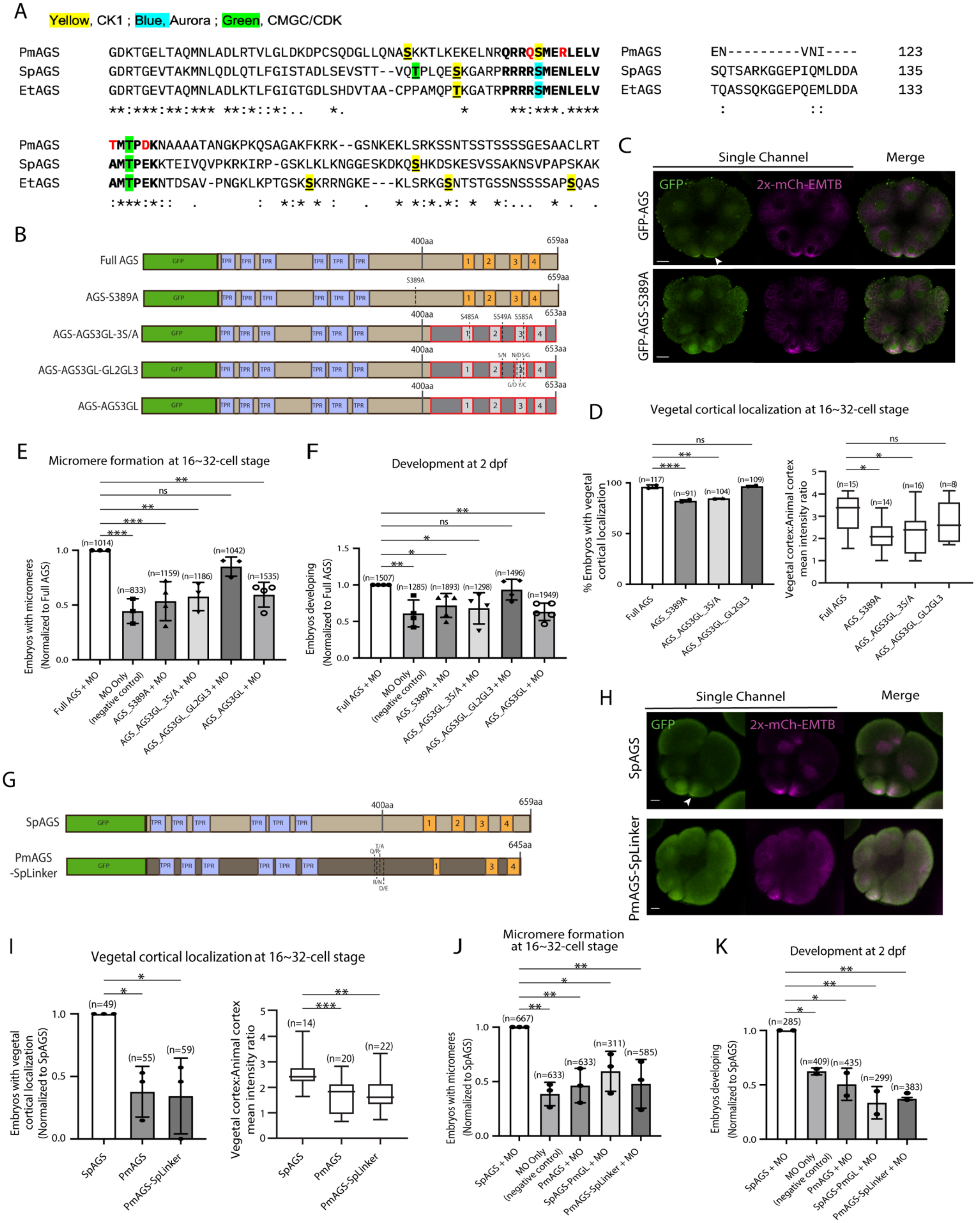
Supplemental Figure 2. Echinoderm AGS sequence alignment. All AGS are similar in the N-terminus with the predicted conserved TPR motifs (blue) but are highly variable in the C-terminus with the predicted GL motifs (yellow).

Supplemental Figure 3



Supplemental Figure 3. SbAGS does not fully localize at the vegetal cortex. A, Design of 2XGFP-AGS constructs that contain AGS orthologs from two different species tested in this study, namely, *S. purpuratus* (Sp; sea urchin), and *S. briareus* (Sb; sea cucumber). TPR motifs are marked in blue, and GL motifs are in orange. **B¸** Alignment of GL1 motif sequences among echinoderms. **C,** Single Z-slice confocal images of sea urchin (Sp) or sea cucumber (Sb) embryos at 16-cell stage showing localization of 2x-GFP-AGS. Embryos were injected with 0.2-0.3µg/µl stock of GFP-AGS mRNA and 0.25µg/µl stock of 2x-mCherry-EMTB mRNA. The white arrowhead indicates vegetal cortical localization of GFP-AGS. **D,** Left graph, the number of embryos with vegetal cortical localization of 2x-GFP-AGS in 16~32-cell embryos was scored and normalized to that of the control group (SpAGS). Right graph, the ratio of the vegetal cortex-to-animal cortex mean intensity. Statistical analysis was performed against the control (SpAGS) by *t*-test. n indicates the total number of embryos scored. * represents p-value < 0.05. Each experiment was performed at least two independent times. Error bars represent standard error. Scale bars=10µm.

Supplemental Figure 4 SpAGS, EtAGS and PmAGS linker domain sequence alignment



A

Yellow, CK1 ; Blue, Aurora ; Green, CMGC/CDK

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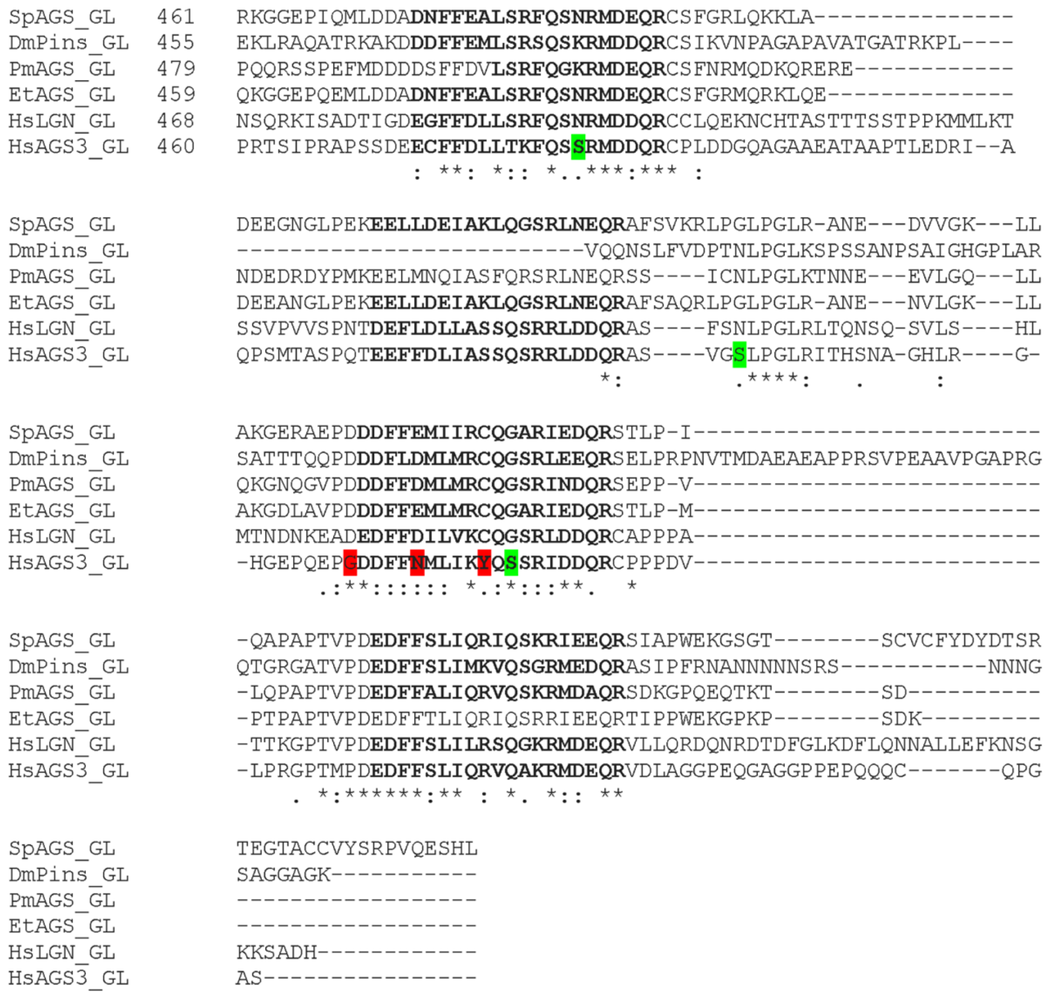
PmAGS      GDKTGELTAQMNLADLRTVLGLDKDPCSQDGLLQNASKKTLKEKELNRQRRQSMERLELV  PmAGS      EN---------VNI----     123
SpAGS      GDRTGEVTAKMNLQDLQTLFGISTADLSEVSTT--VQTPLQESKGARPRRRRSMENLELV  SpAGS      SQTSARKGGEPIQMLDDA     135
EtAGS      GDRTGEVTAQMNLADLKTLFGIGTGDLSHDVTAA-CPPAMQPTKGATRPRRRSMENLELV  EtAGS      TQASSQKGGEPQEMLDDA     133
           **:***:**:*** **:*::*:..    *.              *     **:***.****              :          ::

PmAGS      TMTPDKNAAAATANGKPKQSAGAKFKRK--GSNKEKLSRKSSNTSSTSSSSGESAACLRT
SpAGS      AMTPEKKTEIVQVPKRKIRP-GSKLKLKNGGESKDKQSHKDSKESVSSAKNSVPAPSKAK
EtAGS      AMTPEKNTDSAV-PNGKLKPTGSKSKRRNGKE---KLSRKGSNTSTGSSNSSSSAPSQAS
           :***:*::   .        :  *:* * :    .   * *:*.*: *  *:...  * .  .
```

Supplemental Figure 4. The linker domain and GL2-GL3 regions are important for AGS localization and function. A, Alignment of linker domain between echinoderms including sea urchin (SpAGS), pencil urchin (EtAGS), and sea star (PmAGS). Bold letters represent the conserved core linker domain. The yellow, blue, or green highlights indicate the CK1, Aurora, or CMGC/CDK phosphorylation sites predicted by GPS 6.0. The red letters indicate PmAGS amino acids mutated to those of SpAGS to construct the PmAGS-SpLinker mutant. **B,** Design of GFP-AGS mutant constructs tested in this study. TPR motifs are marked in blue, and GL motifs are in orange. The brown section indicates the SpAGS sequence, and the red and grey boxes show the non-sea urchin (non-SpAGS) introduced at the C-terminus. The dotted lines represent single amino acid mutations. **C¸** Single z-slice confocal images of sea urchin embryos at 8~16-cell stage showing localization of GFP-AGS-S389A mutant. Embryos were injected with 0.3µg/µl stock of GFP-AGS mRNA and 0.25µg/µl stock of 2x-mCherry-EMTB mRNA. The white arrowhead indicates vegetal cortical localization of GFP-AGS. **D,** % of the embryos with vegetal cortical localization of GFP-AGS mutants (left) and the ratio of the vegetal cortex-to-animal cortex mean intensity (right) in 16~32-cell embryos. Statistical analysis was performed against Full AGS by One-Way ANOVA. **E-F,** Embryos were injected with 0.15µg/µL stock of GFP-AGS mRNAs and 0.75mM SpAGS MO. The number of embryos forming micromeres (E) and developing to gastrula or pluteus stage (F) were scored, and each of which was then normalized to that of the Full AGS. Statistical analysis was performed by One-Way ANOVA. **G,** Design of GFP-AGS constructs tested in this study from *S. purpuratus* (Sp) and *P. miniata* (Pm). TPR motifs are marked in blue, and GL motifs are in orange. The dotted lines represent single amino acid mutations. **H¸** Single Z-slice confocal images of sea urchin embryos at 8~16-cell stage showing localization of GFP-AGS. Embryos were injected with 0.3µg/µl stock of GFP-AGS mRNAs and 0.25µg/µl stock of 2x-mCherry-EMTB mRNA. The white arrowhead indicates vegetal cortical localization of GFP-AGS. **I,** The number of embryos with vegetal cortical localization of GFP-AGS mutants in 16~32-cell embryos was scored and normalized to that of the SpAGS (left graph). Right graph shows the ratio of the vegetal cortex-to-animal cortex mean intensity. Statistical analysis was performed against SpAGS by One-Way ANOVA. **J-K,** Embryos were injected with 0.3µg/µl stock of GFP-AGS mRNAs and 0.75mM SpAGS MO. The number of embryos making micromeres (J) and developing to gastrula or pluteus stage (K) were scored and normalized to that of the SpAGS. Statistical analysis was performed by One-Way ANOVA. n indicates the total number of embryos scored. * represents p-value<0.05, ** p-value < 0.01, and *** p-value < 0.001. Each experiment was performed at least two independent times. Error bars represent standard error. Scale bars=10µm.

Supplemental Figure S5   GoLoco Domain sequence alignment



```
SpAGS_GL     461   RKGGEPIQMLDDADNFFEALSRFQSNRMDEQRCSFGRLQKKLA---------------
DmPins_GL    455   EKLRAQATRKAKDDDFFEMLSRSQSKRMDDQRCSIKVNPAGAPAVATGATRKPL----
PmAGS_GL     479   PQQRSSPEFMDDDDSFFDVLSRFQGKRMDEQRCSFNRMQDKQRERE------------
EtAGS_GL     459   QKGGEPQEMLDDADNFFEALSRFQSNRMDEQRCSFGRMQRKLQE--------------
HsLGN_GL     468   NSQRKISADTIGDEGFFDLLSRFQSNRMDDQRCCLQEKNCHTASTTTSSTPPKMMLKT
HsAGS3_GL    460   PRTSIPRAPSSDEECFFDLLTKFQSSRMDDQRCPLDDGQAGAAEATAAPTLEDRI--A
                                : **: *:: *..***:*** :

SpAGS_GL           DEEGNGLPEKEELLDEIAKLQGSRLNEQRAFSVKRLPGLPGLR-ANE---DVVGK---LL
DmPins_GL          --------------------------VQQNSLFVDPTNLPGLKSPSSANPSAIGHGPLAR
PmAGS_GL           NDEDRDYPMKEELMNQIASFQRSRLNEQRSS----ICNLPGLKTNNE---EVLGQ---LL
EtAGS_GL           DEEANGLPEKEELLDEIAKLQGSRLNEQRAFSAQRLPGLPGLR-ANE---NVLGK---LL
HsLGN_GL           SSVPVVSPNTDEFLDLLASSQSRRLDDQRAS----FSNLPGLRLTQNSQ-SVLS----HL
HsAGS3_GL          QPSMTASPQTEEFFDLIASSQSRRLDDQRAS----VGSLPGLRITHSNA-GHLR----G-
                                              *:        .****:   .     :

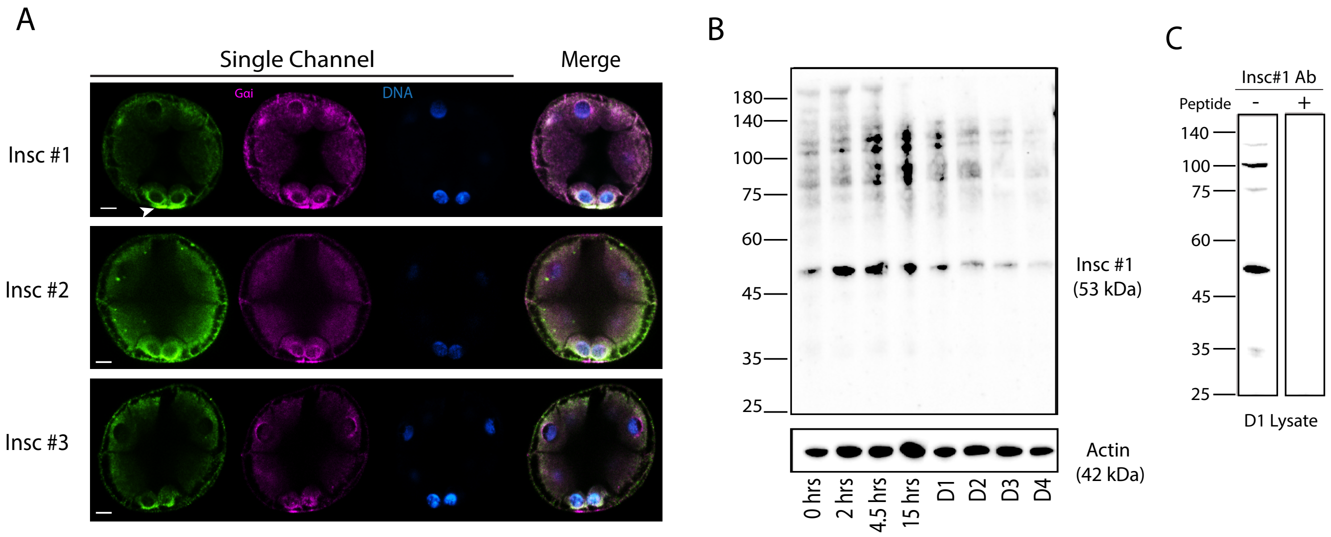
SpAGS_GL           AKGERAEPDDDFFEMIIRCQGARIEDQRSTLP-I--------------------------
DmPins_GL          SATTTQQPDDDFLDMLMRCQGSRLEEQRSELPRPNVTMDAEAEAPPRSVPEAAVPGAPRG
PmAGS_GL           QKGNQGVPDDDFFDMLMRCQGSRINDQRSEPP-V--------------------------
EtAGS_GL           AKGDLAVPDDDFFEMLMRCQGARIEDQRSTLP-M--------------------------
HsLGN_GL           MTNDNKEADEDFFDILVKCQGSRLDDQRCAPPPA--------------------------
HsAGS3_GL          -HGEPQEPGDDFFNMLIKYQSSRIDDQRCPPPDV--------------------------
                         .:**:::::: *.:*:::**.  *

SpAGS_GL           -QAPAPTVPDEDFFSLIQRIQSKRIEEQRSIAPWEKGSGT--------SCVCFYDYDTSR
DmPins_GL          QTGRGATVPDEDFFSLIMKVQSGRMEDQRASIPFRNANNNNNSRS-----------NNNG
PmAGS_GL           -LQPAPTVPDEDFFALIQRVQSKRMDAQRSDKGPQEQTKT--------SD----------
EtAGS_GL           -PTPAPTVPDEDFFTLIQRIQSRRIEEQRTIPPWEKGPKP--------SDK---------
HsLGN_GL           -TTKGPTVPDEDFFSLILRSQGKRMDEQRVLLQRDQNRDTDFGLKDFLQNNALLEFKNSG
HsAGS3_GL          -LPRGPTMPDEDFFSLIQRVQAKRMDEQRVDLAGGPEQGAGGPPEPQQQC-------QPG
                       . *:******:** : *. *:: **

SpAGS_GL           TEGTACCVYSRPVQESHL
DmPins_GL          SAGGAGK-----------
PmAGS_GL           ------------------
EtAGS_GL           ------------------
HsLGN_GL           KKSADH------------
HsAGS3_GL          AS----------------
```

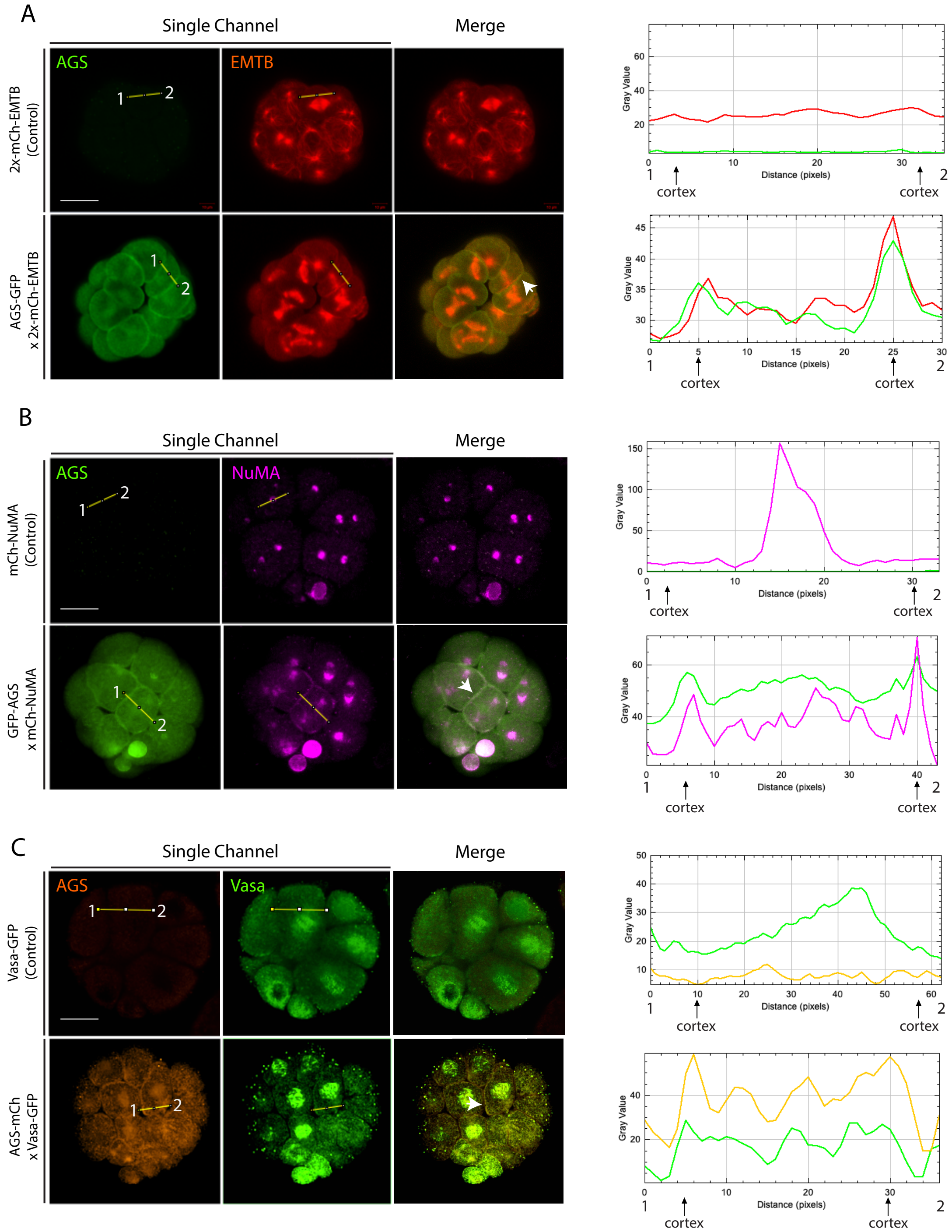
Supplemental Figure 5. Alignment of C-terminus GoLoco domain sequences used for chimeric mutants. Sea urchin *S. purpuratus* (SpAGS_GL), *Drosophila* (DmPins_GL), sea star *P. miniata* (PmAGS_GL), pencil urchin *E. tribuloides* (EtAGS_GL), human *H. sapiens* LGN (HsLGN_GL) and human *H. sapiens* AGS3 (HsAGS3_GL). Bold letters indicate GoLoco motif sequences. The green highlight indicates additional serine amino acid present uniquely in HsAGS3_GL and mutated to Alanine in AGS_AGS3GL_3S/A mutant. The highlighted amino acids between GL2 and GL3 and within GL3 are those mutated to match HsLGN_GL in AGS_AGS3GL_GL2GL3 mutant.

Supplemental Figure 6



Supplemental Figure 6. Insc protein expression during embryonic development. A, Endogenous Insc protein localization by immunofluorescence. Embryos were stained with three Insc antibodies (green) designed for different Insc amino acid sequence sections. Embryos were stained with Gαi antibody (magenta) and Hoechst dye (blue). During the 16-cell stage, all antibodies show signal enriched at the vegetal pole. With #2 and #3 antibodies, some non-specific cortex signals were also observed around the entire embryo. **B**, Insc immunoblot analysis. Embryos were collected at 0, 2, 4.5, 15, 24, 48, 72, and 96 hours post fertilization and subjected to immunoblot with Insc #1 antibody. Actin (42kDa) was used as a loading control. The expected size of Insc is 53kDa. **C**, Peptide competition assay with Insc #1 antibody. The 24-hour lysate was used. Each experiment was performed at least two independent times. Scale bars=10μm.

Supplemental Figure S7



Supplemental Figure 7. SpAGS colocalizes with micromere-specific fate determinants. A, Embryos were co-injected with 2x-mCherry-EMTB (0.5µg/µl stock) mRNA with or without GFP-AGS (0.5µg/µl stock) mRNA. **B,** Embryos were co-injected with mCherry-NuMA (0.15µg/ul) mRNA with or without GFP-AGS (0.5µg/µl stock). **C,** Embryos were co-injected with Vasa-GFP (1µg/µl stock) mRNA with or without AGS-mCherry (0.5µg/µl stock) mRNA. The intensity of each signal, from one cortex to the other, was measured and plotted from point 1 to 2 on the corresponding graph (right) using *ImageJ*. White arrows indicate the cortical colocalization of each construct. All images represent over 50% of the embryos observed (n=30 or larger) per group. Scale bars=20µm.

Supplemental Figure 8 Sea urchin SpDlg and sea star PmDlg sequence alignment

```
SpDlg           ------------------------------------------------------------      0
PmDlg           MNNIEEWEYEDIILNRGGAGLGFSIAGGVDNPHVPDDPSIFITKLIPGGAAAVGGRLRLN      60


SpDlg           ------------------------------MVLRRWPRNPSTGKTLSIELIKGSKGLGFS      30
PmDlg           DVIIRVNETEIHNVPHQNAVDALKKTGSIVNLVVRRRRQRRTGDVIRIKLVKGTKGLGFS      120
                                               :: *  *:  **..: *:*:**:******

SpDlg           IAGGQGNQHVPGDNGIFVTKIIDGGAAEQDGRLQVGDKITAVGDNNLVEVTHEDAVAVLK      90
PmDlg           IAGGCGNQHVAGDNGIFVTKIIDGGAAQMDGNLQIGDKIIAVGNDKLEEVTHENAVAVLK      180
                **** ***** ****************: **.**:**** ***:::* *****:******

SpDlg           ATSNRVLLTVIKNATLLAGTQMAPYSQNDENAQPPDVSNVDMSAANPVYAT---------      141
PmDlg           ATSEVVLLTVIKGAVISYPSPPASI------TSQPDVSTMQPSSPPPPYPEAIVSSVAGV      234
                ***: *******.*.:   :  *        :. ****.:: *:  * *

SpDlg           ---MQPTLMQEPRSSDLQELDGAFGTNVAFGTNVANGANGANGSNGEPPSYNRVTNSQPP      198
PmDlg           SLQSEPAL-QRPPTPPHQEMSNMMNPPLTYHQPPQDTAYGSM---------KKMHVPPSP      284
                    :*:* *.* :   **:.. :.  :::     : * *:          :::     *

SpDlg           NAFNNTPAPKIFIQNDDGIPREPRQVVLNKGATGLGFNIVGGEDGEGIFISFILAGGVAD      258
PmDlg           TANYEGNGVKPYVQNDDDFPREARTVVLNKGATGLGFNIVGGEDGEGIFISFILAGGVAD      344
                .*  :  . * ::****.:*** * ***********************************

SpDlg           LSGALRRGDQILAVNSKDLVNATHEDAALALKGAGQVVTIDAQYKPEEYNCFEAKIQSLR      318
PmDlg           LSGELKRGDQILSVNKKDLRGATHEDAALALKGTGQTVLIEAQYKPEEYNRFEAKIQSLR      404
                *** *:******:**.*** .************:**.* *:********* *********

SpDlg           EEMMQNSVS--PTGSLRTSAKRTLFVRTLFDYDKSRDSGLPSQGLSFDFGDIIHVTNASD      376
PmDlg           EEMMNSSVSSTTTGSLRTSAKRSLYVRALFDYDKTKDSGLPSQGLSFNYGDILHVTNASD      464
                ****:.***   **********:*:**:******::***********::***:*******

SpDlg           DEWWQARHILPNGEEGEIGIIPSKRRVERKEKARLRSVKFSGNKDRNNIQEPAGTVNSKG      436
PmDlg           DEWWQAKQVLPTGEEGEMGVIPSKRRVEKRERARLKSVKFSGRGGS---LESKGSMNEKR      521
                ******:::**.*****:*:********::*:***:******. .     *  *::*.*

SpDlg           KKGFF-RKFKKNRDSDQETSDPEPFTSNASDSESSYRNEEVILSYEAVVQQEIKYTRPVI      495
PmDlg           KKGFFSRKFKKGRESDLDTSDAEHITSNASDSESSYRNDEMILSYEGVVQQEVKYTRPVI      581
                ***** *****.*:** :*** * :*************:*:*****.*****:*******

SpDlg           ILGPGKDRINDDLISEMPDEFGSCVPHTTRARRPHEVDGRDYHFVESREQMEKDIQNHLF      555
PmDlg           ILGSGKDRVNDDLISEMPEKFGSCVPHTTRPRRPHEVDKRDYHFVESREQMEKDIQDHLF      641
                *** ****:*********::********** ******* *****************:***

SpDlg           IEAGQYNENLYGTSVASVKEVSEKGKHCILDVSGNAIKRLQVAQLYPIAIFLKPKSVESI      615
PmDlg           IEAGQYNENLYGTSVASVREVADKGKHCILDVSGNAIRRLQVANLHPIAIFIRPKSVESV      701
                ******************:**::**************:*****:*:*****::******:

SpDlg           VEMNRRVSEDQARKTYERALKLEQEFLEYFTAIILGDTIDDIYQQSKEIIHEQSGPTIWI      675
PmDlg           MEMNRRMSREQAEKTYERAIKLEIEFGEFFTAIISGDTLDEIYYKCKEVIHDQSGSTIWV      761
                :*****:*.:**.******:*** ** *:***** ***:*:** :.**:**:*** ***:

SpDlg           PAKEKL    681
PmDlg           PAKDKL    767
                ***:**
```

PDZ
SH3
GUK

Supplemental Figure 8. Sea urchin (SpDlg) and sea star (PmDlg) sequence alignment. Blue, yellow, and green highlights indicate the PDZ, SH3, and GUK domains, respectively.

Supplemental Figure 9 Sea urchin SpInsc, pencil urchin EtInsc and sea star PmInsc
sequence alignment

```
PmInsc          ----------MNGFNES-ALAGWLADDVIEMHMTDEDSVVRWLQDLRTMTECECMCILQG          49
SpInsc          ----------MSDN----KNTESFP-EKKLMQRCDVDSVQQWLIDIRLMPEQECTTVMET          45
EtInsc          MTCLYGVVRTMYGPQAPVICEEKLPTSTSRFQLTEVRSVQQWLSDLQGMMECECN-ILET          59
                          * .          :  .   ::  :  ** :** *:: * * **  :::

PmInsc          KPIRTSAHDMAKVTSSARDNIYILRQRASSISADFAKLYYKLDSGSWDQVH-WLCLSLTI          108
SpInsc          KPIHNSPLNLENLAATVKESVYRLQSIKDGLFSDLSQIYMCLDLSDQ-------------          92
EtInsc          KPIHNSPLNLENVAAATKENIYRLRNRAENLARELQQLYHKLTSGTYSQRLRPAVLALTL          119
                ***:.*  :: :::::.::.:* *:.  ..:  :: ::*  *  .

PmInsc          RLRTFLHECTSYVPTMPLHLFQRQQTVLEECGRLVKHAESFEELNGTLPPALPTLNVLSS          168
SpInsc          -----------------------------------------------SVK--LFIQSLTT          103
EtInsc          HVRSCLEECNEYCPDF-LHMKEKQQVVIDAMATLVKNFEMYDNSKGCELD--SLIHSLTS          176
                                                      :: *::

PmInsc          LGKAFNDLVHMVQGILIQTVVRSIHESASEYNIKIAINSIHCLSEDDSEIRRLLAKEGAI          228
SpInsc          VAKAIKAYLEMVQGALVRKLVDMVQGAATELNVKFAVLTVHSLAQDGVWLRRLLIQEDTM          163
EtInsc          LGNALNSIRQTVQSALVRKLVMMIQDSASELNVKFAVITAHSLSQDSIWLRRILIQENAM          236
                :.:*::   . **. *::.:*  :: :*:* *:*:*: : *.*::*.  :**:* :*.::

PmInsc          QALLQLCQQASL--NAMKPMALKTVANLCNVAEGRQELERASGVEYLSEILSSPTATESV          286
SpInsc          RALLAICRITTFSIRSIPMTALEIVSILCTEVEGRAELEKVGGVGALSEILASPITLEAV          223
EtInsc          GALFAVCRITTLSIPSIVTTALQAISTLCAEPEGRVELEKIGGLGFLGEILSSPSTSEAV          296
                 **: :*: :::   ::   **: :: **   *** ***: .*:  *.***:** : *:*

PmInsc          KAEAASVIAQITSPNIDNYHHLLGFIEYMEDLLKDLTALSGSASSSSVFVIATSAIANIT          346
SpInsc          KKEAASVLAEITSPDADIFHRMLCFIEHMEDLLRNLTVLCDQTSSPGVFLVATTAIANIT          283
EtInsc          KLEAASVLAEITSPDINSYHRMLCFIEHMEDLLKNLTALCDQTSNPLVFLVSASAIANIT          356
                * *****:*:****: : :*::* ***:*****::**.*...:*.  **:::::******

PmInsc          FMDSMACDCLASFHTARALVKGCNNGMANTLYCKDQVATILANMAVTDQCRKEIMVSGGT          406
SpInsc          FMDAMASDYLAEFQTAEVLIQGYLKGKAPSIYSKHQVITIIANISCSKSCREQIVSSGGM          343
EtInsc          FMDTMSSDFLAMFNTATILIDGYTSGKAQNMYSKDQVVTIIANMSGSEKCREQIIKAGGM          416
                ***:*:.* ** *:**  *:.*  .* * .:*.*.** **:**:: :..**::*: :**

PmInsc          ELLVDFLHTRALVHPSSAK---KGLKGSGLSGGCSSGGKQDVVMSSSCEKVHQKAAIALA          463
SpInsc          NILAELLQGVPSTSRPTSLSRKLSFDGN--------VSLGQMGEESAYEDIYQKAATALG          395
EtInsc          KVLVELLHTRAPFTTVKTNGVN---QRA--------DSKAACGMASTCEHVHQKSAIALG          465
                ::*.::*:        .:       .           .       *: *.::**:* **.

PmInsc          RLARDQDSAELILELDAIPRLVELCCNRRERNDSEPVLVACLAALRKLHNLLGPSVFKSS          523
SpInsc          RLCQDYETCKMAVDLKVVSRLADLCKHARERNHRSDVLVACLAALRRIHSHIGSSPLKEN          455
EtInsc          RLCQDHDTAMMAVDLGVIPRLVDMCRHRRERNDSEPVLVACLAALRKLHSVVGASKFEPK          525
                **.:* ::. : ::* .: **.::* : ****. . **********::*. :* * :: .

PmInsc          DVDQLIKPRLIDSYLMCSATK--ESFV  548
SpInsc          DVEQLIQPRLMESFLHCSTTVRRETFV  482
EtInsc          ALQQLIRPRLIESFVTCSSIGSTETFV  552
                 ::***:***::*:: **:    *:**
```

LBD (LGN-binding domain)

Supplemental Figure 9. Sea urchin (SpInsc), pencil urchin (EtInsc), and sea star (PmInsc) sequence alignment. The blue highlight indicates the LBD domain.