Supplementary Table 1. A key resource table

REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Antibodies		
Anti-SpAGS	Voronina & Wessel, 2006	N/A
Anti-Gαi	Santa Cruz Biotech	# sc-56536
Anti-β-catenin	Yazaki et al., 2015	N/A
Anti-SpInsc	This article	N/A
Anti-SpNuMA	Poon et al., 2019	N/A
Anti-β-actin (8H10D10)	Cell Signaling Technology	# 3700S
Anti-Flag	Millipore-Sigma	# F1804
Alexa 488-conjugated goat anti-mouse IgG	Cell Signaling Technology	# 4412
Alexa 555-conjugated goat anti-mouse IgG	Cell Signaling Technology	# 4409
HRP-conjugated anti-Protein A antibody	Abcam	# ab7245
HRP-conjugated goat anti-mouse IgG	Cell Signaling Technology	# 7076
Anti-Digoxigenin-AP, Fab fragments	Roche	# 11093274910
Chemicals		
Hoechst 33342	Thermo Fisher Scientific	# 62249
DTT	Millipore-Sigma	# 10197777001
Tris buffered saline, with tween (TBST)	Millipore-Sigma	# T9039
Tris-MOPS-SDS Running Buffer	Genscript	# M00138
Transfer buffer powder	Genscript	# M00139
DIG RNA labeling mix	Roche	#11277073910
Critical Commercial Assays		
mMESSAGE mMACHINE SP6 Transcription Kit	Thermo Fisher Scientific	# AM1340
MEGAscript SP6 Transcription kit	Thermo Fisher Scientific	# AM1330
MEGAscript T7 Transcription kit	Thermo Fisher Scientific	# AM1333
In-Fusion HD Cloning	Clonetech	# 639648

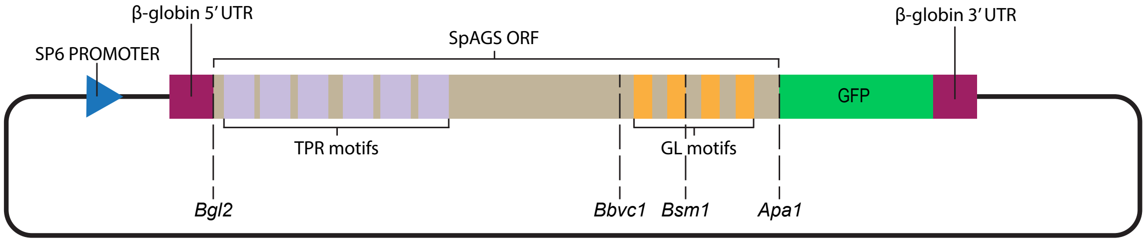
Duolink® In Situ Red Starter Kit Mouse/Rabbit	Millipore-Sigma	# DUO92101
Recombinant DNA		
Plasmid: SpAGS-GFP	Poon et al., 2019	N/A
Plasmids: SpAGS-dC-term-GFP, SpAGS-dGL1/2/3/4-GFP, SpAGS1111/2222/2134/4234-GFP	This article	N/A
Plasmids: SpAGS-dN-term-GFP	This article	N/A
Plasmid: SpAGS-mCherry	Poon et al., 2019	N/A
Plasmid: GFP-SpAGS, GFP-EtAGS, GFP-PmAGS	This article	N/A
Plasmid: GFP-SpAGS4444/GL1GL2/LGNGL/DmGL/EtGL/AGS3GL/PmGL/S389A/AGS3GL-3S/A/AGS3GL-GL2GL3	This article	N/A
Plasmid: GFP-PmAGS-SpLinker	This article	N/A
Plasmid: GFP-SpDlg/PmDlg	This article	N/A
Plasmid: GFP-SpInsc/EtInsc/PmInsc	This article	N/A
Plasmid: GFP-NuMA	This article	N/A
Plasmid: mCherry-NuMA	This article	N/A
Plasmid: GFP-Par3	This article	N/A
Plasmid: Vasa-GFP	Yajima & Wessel, 2011	N/A
Plasmid: Vasa-mCherry	Uchida & Yajima, 2018	N/A
Plasmid : 3xFlag-GFP-SpAGS/SpDlg/SpNuMA, 3xFlag-Vasa-GFP	This article	N/A
Plasmid: 2x-mCherry-EMTB	Addgene	# 26742
Software and Algorithms		
Echinoderm protein sequences	EchinoBase	http://www.echinobase.org/Echinobase/
Protein motif search	NCBI blast	https://blast.ncbi.nlm.nih.gov/Blast.cgi
Protein sequence alignment	Clustal Omega	https://www.ebi.ac.uk/Tools/msa/clustalo/
Quantitative analysis	Image J	https://imagej.nih.gov/ij/
Statistical analysis	Graphpad PRISM 8	https://www.graphpad.com/scientific-software/prism/
Morpholino antisense oligos (Gene Tools: http://www.gene-tools.com/)		
NCBI reference sequence	**Name**	**Sequence**
NM_001040405.1	SpAGS	GGCCCGTTTCACAAAGCCTTTGTTT
Primers for ISH probes		
NCBI reference sequence / Source	**Name**	**Sequence**

XM_011663478.2	SpAlx1	F: GGATATTTTCTCGACCGGGATC R: CGAGTAACCGTTCATCATCCCC
NM_214574.3	SpBlimp1b	F: ATGGGGTGCAACGACAACGCCGTG R: CTATGATTTGTTCGTACGATTGAG
NM_214519.1	SpEndo16	F: GCAGAGTTCAACAGAATCGAC R: GCCAGTAGACGTAGCAGAAG
XM_030976919.1	SpEts1	F: TCAATCATGGCGTCTATGCACTG R: ACAGCTGCAGGGATAACAGG
NM_001079542.1	SpFoxA	F: ATGGCCAATAGTGCCATGATCTCG R: TCACATTGCATGGTTTGCTTG
XM_003731512.3	SpFoxQ2	F: ATGACTTTATTCAGCATTGACAAC R: TAGCAGGATCCTACAGAAGACCAG
NM_214610.3	SpSm50	F: ATGAAGGGAGTTTTGTTTATTGTGGCTAGTC R: GTTATGCCAACGCGTCTGCCTCTTGAAGC
XM_786173.5	SpTbr1	F: CCACCGCTGCACCAGACGAC R: CTGCCGGCTGGCGCCAATTGCG
NM_214667.1	SpWnt8	F: ATGGATGTTTTTACGGAATTTGTTCG R: CTACAGCCTCGATCCAACGGGCTG

Supplementary Figures and Legends

Supplemental Figure S1

A



B

S. purpuratus AGS Protein Sequence

MQSEASCMELALEGERLCKAGNCREGVRYLEAAVEVGTDDLKTLSAIYSQLGNAYFYLEDYGKALTYHKHDL

TLATSIGDRLGEAKASGNIGNTLKVLGKFDEAICCCQRHLDISRELSEKVGEGRALYNLGNVYHAKGKHAGRS

GHQDPGDFPQEVTDCLKQAIQFYEANLAIVRELGDRAAQGRACGNLGNTHYLLGNFETAIQFHTERLAIAKE

FGDKAAERRAYSNLGNACVFMVQFEVAAKYYKKSLHIARQLGELAMEAQACYSLGNTYTLLREYEKAVEYHS

RHMEIAQQLNDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVTAKMNLQDLQTLFG

ISTADLSEVSTTVQTPLQESKGARPRRRRSMENLELVAMTPEKKTEIVQVPKRKIRPGSKLKLKNGGESKDKQS

HKDSKESVSSAKNSVPAPSKAKSQTSARKGGEPIQMLDDADNFFEALSRFQSNRMDEQRCSFGRLQKKLAD

EEGNGLPEKEELLDEIAKLQGSRLNEQRAFSVKRLPGLPGLRANEDVVGKLLAKGERAEPDDDFFEMIIRCQG

ARIEDQRSTLPIQAPAPTVPDEDFFSLIQRIQSKRIEEQRSIAPWEKGSGTSCVCFYDYDTSRTEGTACCVYSRP

VQESHL

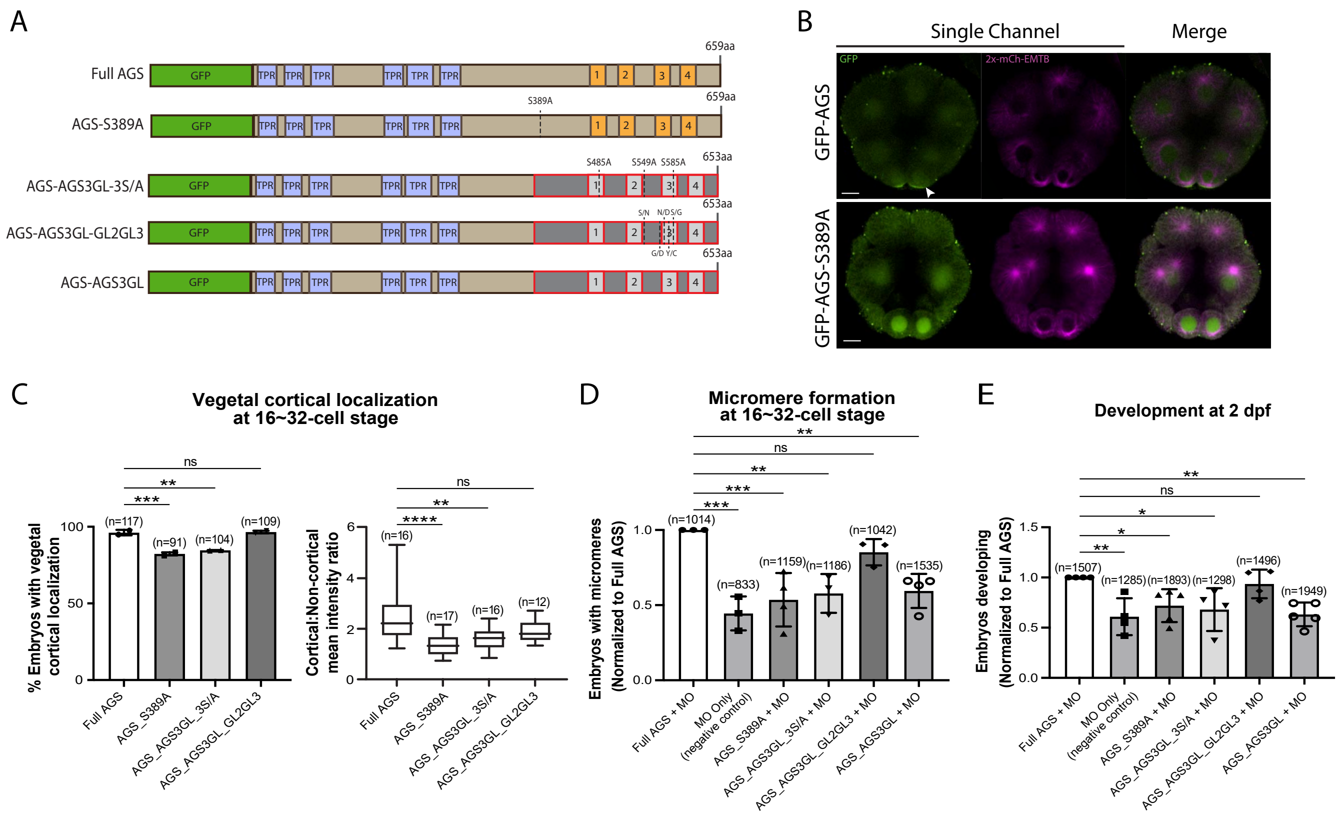
Supplemental Figure 1. **Dissection of SpAGS motifs. A**, The construct design of SpAGS-GFP, including the restriction enzyme sites used to prepare SpAGS mutants. **B**, The protein sequence of SpAGS. Predicted domains are labeled based on NCBI blast results, and the sequence portions deleted for each 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

```
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*
```

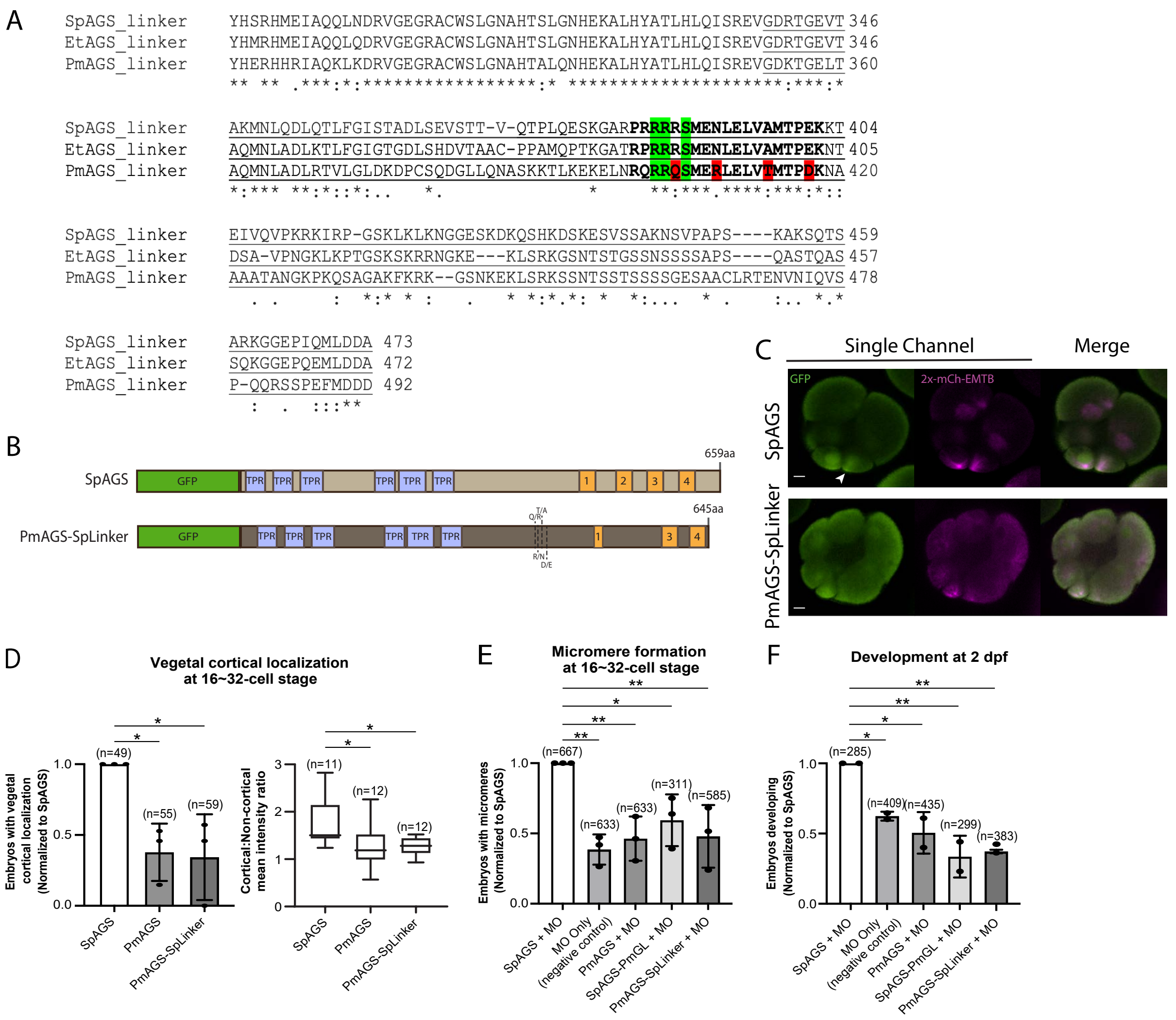
Supplemental Figure 2. Echinoderm AGS sequence alignment. All AGS are similar in the N-terminus with the predicted conserved TPR motifs (blue) but are highly variable in the C-terminus with the predicted GL motifs (yellow).

Supplemental Figure 3



Supplemental Figure 3. The linker domain and GL2-GL3 region are important for AGS localization and function. A, 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 shows the SpAGS portion and the red and grey boxes show the non-sea urchin (non-SpAGS) C-terminal sequence introduced. The dotted lines represent single amino acid mutations. **B¸** 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. **C,** % of the embryos with vegetal cortical localization of GFP-AGS mutants (left) and the ratio of the cortical-to-non-cortical mean intensity (right) in 16~32-cell embryos. Statistical analysis was performed against Full AGS by One-Way ANOVA. **D-E,** Embryos were injected with 0.15µg/µl stock of GFP-AGS mRNAs and 0.75mM SpAGS MO. The number of embryos making micromeres (D) and developing to gastrula or pluteus stage (E) were scored and normalized to that of the Full AGS. 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, *** p-value < 0.001, and **** p-value <0.0001. 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

```
SpAGS_linker    YHSRHMEIAQQLNDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVT 346
EtAGS_linker    YHMRHMEIAQQLQDRVGEGRACWSLGNAHTSLGNHEKALHYATLHLQISREVGDRTGEVT 346
PmAGS_linker    YHERHHRIAQKLKDRVGEGRACWSLGNAHTALQNHEKALHYATLHLQISREVGDKTGELT 360
                ** ** .***:*:*****************:* *********************:***:*

SpAGS_linker    AKMNLQDLQTLFGISTADLSEVSTT-V-QTPLQESKGARPRRRRSMENLELVAMTPEKKT 404
EtAGS_linker    AQMNLADLKTLFGIGTGDLSHDVTAAC-PPAMQPTKGATRPRRRSMENLELVAMTPEKNT 405
PmAGS_linker    AQMNLADLRTVLGLDKDPCSQDGLLQNASKKTLKEKELNRQRRQSMERLELVTMTPDKNA 420
                *:*** **:*::*:..    *.                 *     **:***.****:***:*::

SpAGS_linker    EIVQVPKRKIRP-GSKLKLKNGGESKDKQSHKDSKESVSSAKNSVPAPS----KAKSQTS 459
EtAGS_linker    DSA-VPNGKLKPTGSKSKRRNGKE---KLSRKGSNTSTGSSNSSSSAPS----QASTQAS 457
PmAGS_linker    AAATANGKPKQSAGAKFKRK--GSNKEKLSRKSSNTSSTSSSSGESAACLRTENVNIQVS 478
                  . .     :  *:* * :    .    * *:*.*: *  *:...   * .    :.. *.*

SpAGS_linker    ARKGGEPIQMLDDA 473
EtAGS_linker    SQKGGEPQEMLDDA 472
PmAGS_linker    P-QQRSSPEFMDDD 492
                  :  .  :::**
```

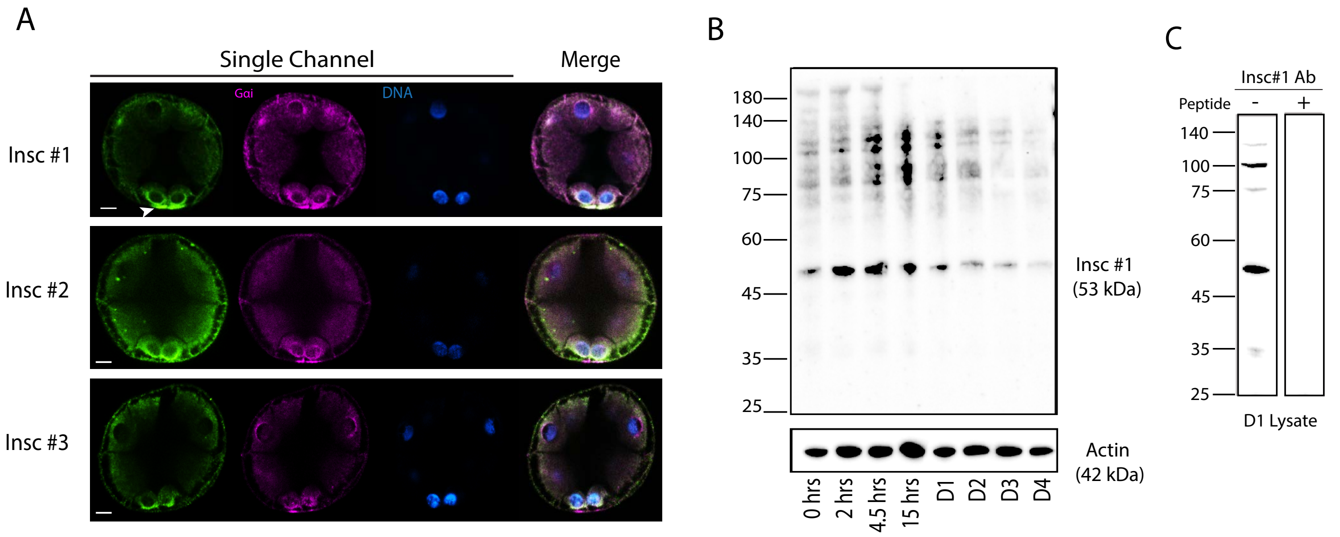
Supplemental Figure 4. The Dlg recognition sequence in the linker domain is important for the AGS function. A, Alignment of linker domain between echinoderms including sea urchin (SpAGS_linker), pencil urchin (EtAGS_linker), and sea star (PmAGS_linker). Bold letters represent the conserved core of the linker domain. The green highlight indicates conserved amino acids in the consensus kinase recognition motif. The red highlight represents amino acid mutated to match SpAGS residues in the PmAGS-SpLinker mutant. **B,** 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. **C**, 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. **D,** 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 cortical-to-non-cortical mean intensity. Statistical analysis was performed against SpAGS by One-Way ANOVA. **E-F,** Embryos were injected with 0.3µg/µl stock of GFP-AGS mRNAs and 0.75mM SpAGS MO. The number of embryos making micromeres (E) and developing to gastrula or pluteus stage (F) 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, and ** p-value < 0.01. 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----------------
```

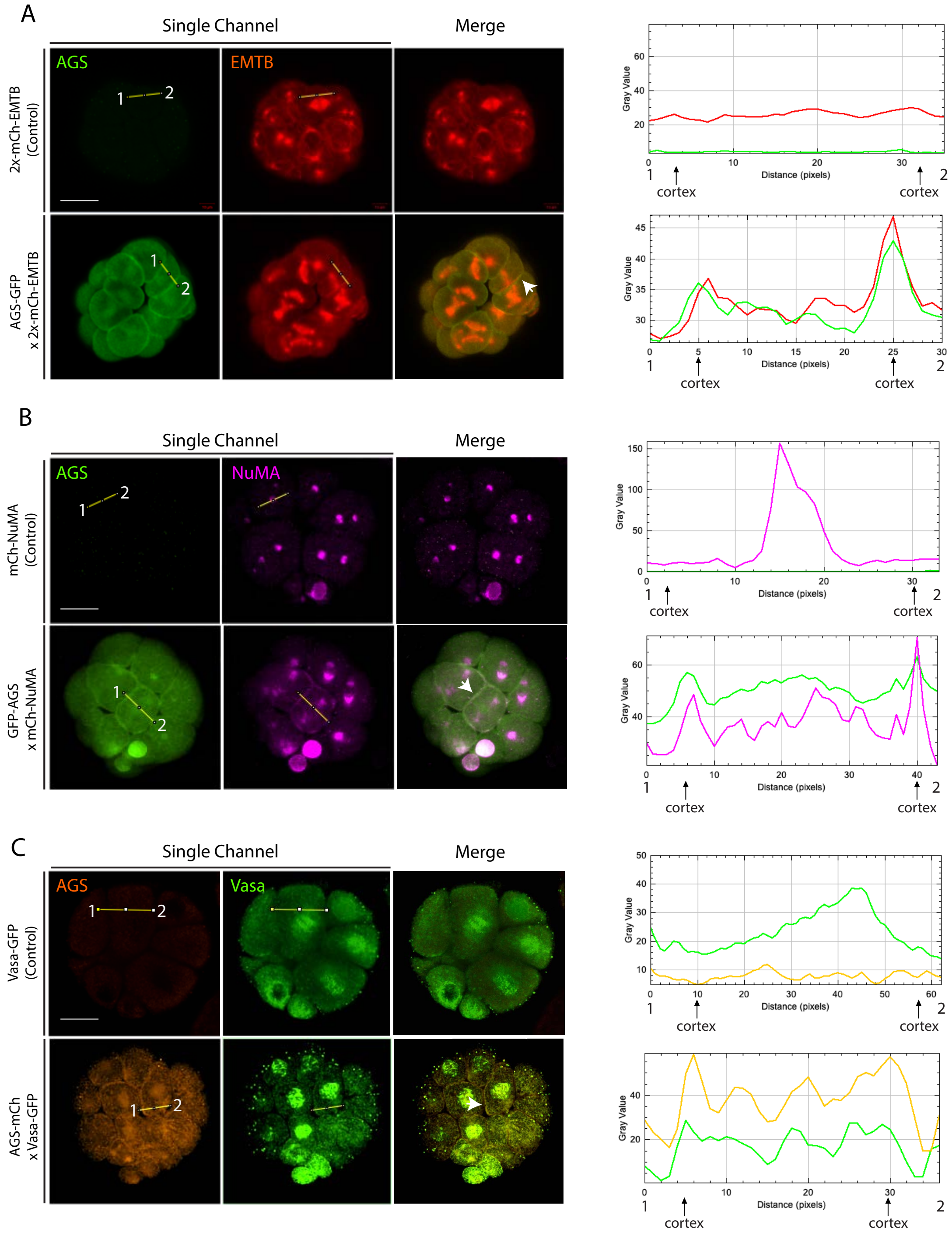
Supplemental Figure 5. Alignment of C-terminus GoLoco domain sequences used for chimeric mutants. Sea urchin *S. purpuratus* (SpAGS_GL), *Drosophila* (DmPins_GL), sea star *P. miniata* (PmAGS_GL), pencil urchin *E. tribuloides* (EtAGS_GL), human *H. sapiens* LGN (HsLGN_GL) and human *H. sapiens* AGS3 (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 GL2 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. Error bars represent standard error. 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.