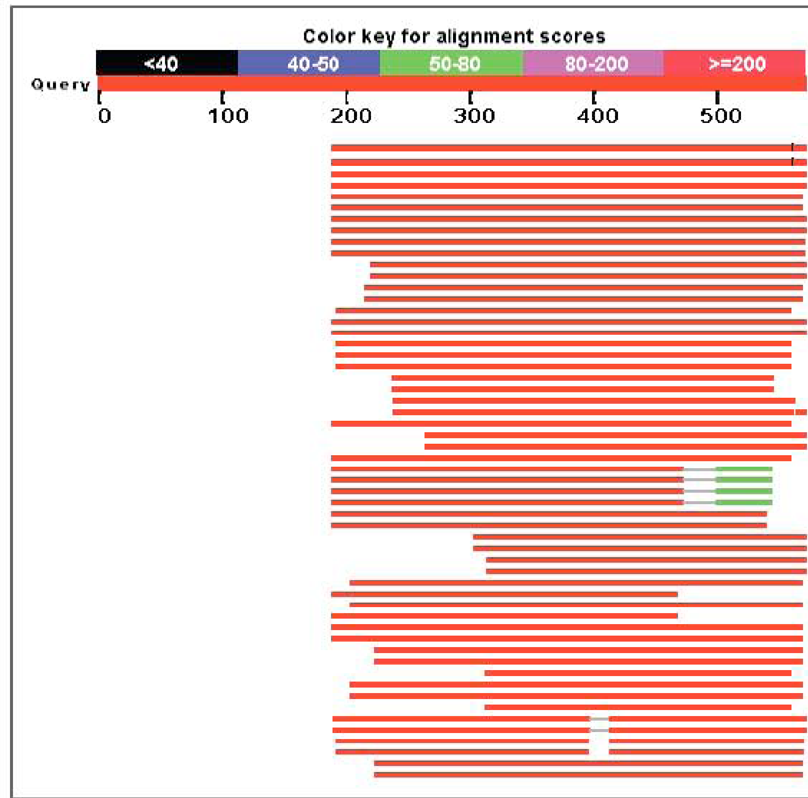
A

Distribution of 120 Blast Hits on the Query Sequence



B Blast search results of random 10 genomic sequences

Locus # (first 400bp of each locus was blasted)		# of hits (over 90% similarity for 300bp)
0	ArsI sequence	120
1	NW_001424536.1	4
2	NW_001424636.1	2
3	NW_001424736.1	2
4	NW_001424937.1	8
5	NW_001462824.1	22
6	NW_001344698.1	2
7	NW_001344798.1	2
8	NW_001344898.1	2
9	NW_001344998.1	2
10	NW_001462446.1	2

Fig. S1. A BLAST result of *H. pulcherrimus* ArsI sequence against the *S. purpuratus* genome. (**A**) 574 bp of *H. pulcherrimus* ArsI sequence was blasted at the NCBI *S. purpuratus* nucleotide BLAST database. Approximately 400 bp of the ArsI sequence from *H. pulcherrimus* is highly conserved throughout the *S. purpuratus* genome. (**B**) To test the significance of the ArsI sequence conservation within the genome, ten genomic sites were randomly selected from the *S. purpuratus* genome and the first 400 bp of each locus was used to perform a BLAST search in the *S. purpuratus* genome: NW_001424536.1, NW_001424636.1, NW_001424736.1, NW_001424937.1, NW_001462824.1, NW_001344698.1, NW_001344898.1, NW_001344998.1, NW_001462446.1 and NW_001344798.1, and the number of hits that demonstrated over 90% identity for 300 bp was recorded. Ten of 10 sequences showed only two to 22 hits that are conserved in sequence for a length of 300 bp, even though 7.5% of the *S. purpuratus* genome is considered to contain repeat sequences (see 'Repetitive Sequences of the *S. purpuratus* Genome' http://www.spbase.org/SpBase/resources/repeats.php). We conclude that the 120 ArsI sites reflect select ArsI functional elements that are non-randomly functioning throughout the genome.

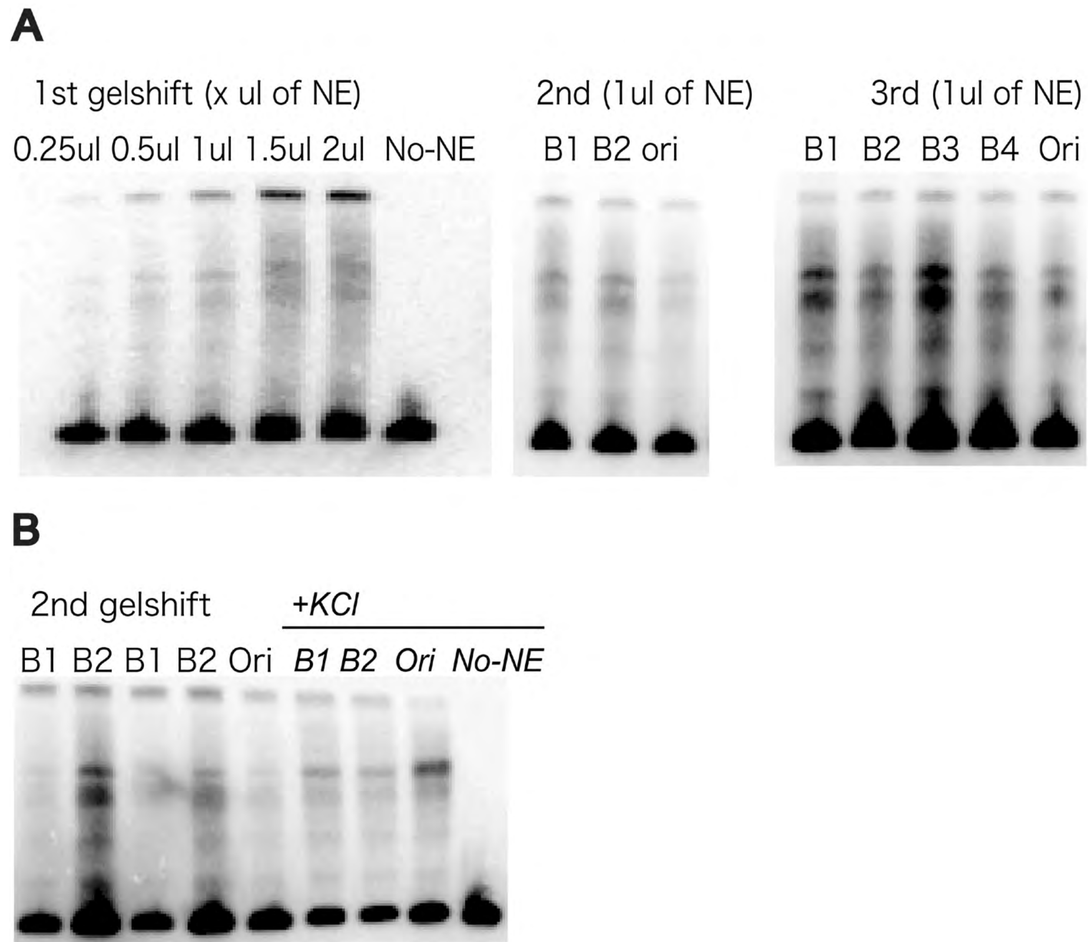


Fig. S2. Mega-shift assay results. (**A**) Raw data of sequential Mega-shift assays. For the first gel shift, various amounts of nuclear extract (NE) were used, yet only 3 μg of NE was used for the second and the third round of gel shifts. Either the top band (B1) or the second from top band (B2) was isolated to extract and amplify probe pools for the next round of gel shift. Probe pools extracted from the bands demonstrated higher signal in shifted bands of the next round of gel shift, suggesting an enrichment of probe pools binding onto each shifted band. (**B**) Protein binding specificity was tested under the condition of high salt (0.15 M KCl).

A

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1st Amino Acid Sequence
  File Name         : SpISWI-protein
  Sequence Size     : 1019

2nd Amino Acid Sequence
  File Name         : Drosophila ISWI verC-antibody
  Sequence Size     : 1027

  Unit Size to compare = 2
  Pick up Location     = 1


[71.7% / 996 aa]
     INT/OPT.Score : <   2159/  3887 >

     1' MADLEERVDTPSPLPPAALKLKIPPKAGGSGVGEEYDEKLEKDRSCRFDFLLQQTEIFTH
                                   ........ ..*.*.*.* *** ******.*******
     1"  MSKTDTAAVEATEENSNETTSDAATSSSGEKEAEFDNKIEADRSRRFDFLLKQTEIFTH

    61' FMSTTSAMKSPTSPLKIKPGRPKMKRDEKQKLSAVGDHRHRKTEEQEDEELLTESRKATS
        **  *.. ****.*    **** *  ..*.*  ..*.********..******.*.  .**.
    60" FM--TNSAKSPTKP----KGRPK-KIKDKDKEKDVADHRHRKTEQEEDEELLAED-SATK

   121' VITQFEESPKYIKNGEMRDYQVRGLNWLISLYEHGINGILADEMGLGKTLQTISLLGYMK
         *  .*..** ***.*******.*****.*****.***********************.*
   112" EIFRFDASPAYIKSGEMRDYQIRGLNWMISLYENGINGILADEMGLGKTLQTISLLGYLK

   181' HYRHIPSPHLIICPKSTLANWMAECERWCPSLRAVCLIGNQDQRSAFIRDVMMPGEWDVC
        *... ..**..* *****.**..*  ..************.** *..*****.********
   172" HFKNQAGPHIVIVPKSTLQNWVNEFKKWCPSLRAVCLIGDQDTRNTFIRDVLMPGEWDVC

   241' ITSYEMAIREKSVFKKFNWRYLVIDEAHRIKNEKSKLSEIVREFKTTNRLLLTGTPLQNN
        .***** *********************************.*****.****.********
   232" VTSYEMCIREKSVFKKFNWRYLVIDEAHRIKNEKSKLSEILREFKTANRLLITGTPLQNN

   301' LHELWALLNFLLPDVFNSSEDFDAWFSTQDCLGDNSLVTRLHAVLRPFLLRRLKSEVEKA
        ***********************.**.*..****..*.*******.********.****
   292" LHELWALLNFLLPDVFNSSEDFDEWFNTNTCLGDDALITRLHAVLKPFLLRRLKAEVEKR

   361' LLPKKETKMYVGMSIMQREWYTKILMKDIDVVNGAGKSDKMRLMNILMHLRKCGNHPYLF
        * **** *..**.* ***.****.*.***********  .**** ****.****.******
   352" LKPKKEMKIFVGLSKMQRDWYTKVLLKDIDVVNGAGKVEKMRLQNILMQLRKCTNHPYLF

   421' DGAEPGPPYTTDKHLVENSGKMSVLDKLLPKLKEQGSRVLIFSQMTRLLDILEDYCVWRG
        ************.*** *****..********.**************.******** **.
   412" DGAEPGPPYTTDTHLVYNSGKMAILDKLLPKLQEQGSRVLIFSQMTRMLDILEDYCHWRN

   481' HNYCRLDGQTPHAERQESINNFNMPDSEKFVFLLSTRAGGLGINLATADIVVLYDSDWNP
        .***********..*.  *..***  .*.**.*.****************.*..*******
   472" YNYCRLDGQTPHEDRNRQIQEFNMDNSAKFLFMLSTRAGGLGINLATADVVIIYDSDWNP

   541' QVDLQAMDRAHRIGQKKQVHVFRFISENTVEERIVERAEMKLRLDNIVIQQGRLVDS-NL
        *.*****************.***.*.*.****.******.*****..*** *****.  .
   532" QMDLQAMDRAHRIGQKKQVRVFRLITESTVEEKIVERAEVKLRLDKMVIQGGRLVDNRSN

   600' KLDKDQALEMIRHGANFIFSSKDSTVTDEDIDLILQKTEQKTTKFNEKLNKMGESGLRNF
        .*.**. *..** *** .****....******.**...*.**.. .. *...***.**.*
   592" QLNKDEMLNIIRFGANQVFSSKETDITDEDIDVILERGEAKTAEQKAALDSLGESSLRTF

   660' SMD----APSKSIFSFEGEDYKEKHKQTGI-NWIEPPKRERKANYAVDAYFREALRVSEP
        .**    *  ..*.. *****..**.*  ...  ****************************
   652" TMDTNGEAGTSSVYQFEGEDWREKQKLNALGNWIEPPKRERKANYAVDAYFREALRVSEP

   715' KAPKAPRPPKQPNVQDFQFFPPRLFELLDKEIYAYRKGIGYRVPLNPDV-SDASKVRKEE
        ************ ****************.***  .**..**.** *...  ***.**..**
   712" KAPKAPRPPKQPIVQDFQFFPPRLFELLDQEIYYFRKTVGYKVPKNTELGSDATKVQREE

   774' QRRIDDSCQLDEAEIQEKESLLTQGFTDWNKRDFNQFVKACEKFGRDDVESICRDVEGKP
        **.**.. .*.*.******.**.****.*.*******.** **.****...*  .*****.
   772" QRKIDEAEPLTEEEIQEKENLLSQGFTAWTKRDFNQFIKANEKYGRDDIDNIAKDVEGKT

   834' QEEVREYLNVFWERCNELQDVDRIMAQIERGESKIQRKIGIKKALDAKMARYRAPFHQLR
        .*** **  .******.****..***.******.****...******.**.**********
   832" PEEVIEYNAVFWERCTELQDIERIMGQIERGEGKIQRRLSIKKALDQKMSRYRAPFHQLR

   894' IQYGTNKGKNWTEDEDRFLVCMLHKLGFDKENVYDDLRSGVRQAPQFRFDWFIKSRTAME
        .***.*****.** ********************..**...*..**************.*
   892" LQYGNNKGKNYTEIEDRFLVCMLHKLGFDKENVYEELRAAIRASPQFRFDWFIKSRTALE

   954' LQRRCNTLITLIERENQELEEKEKVDKKKGRGGGKVTGRGSMGTPKTEKRKADDDTDGRG
        **************** ******...***    *.*.. .. ....*  . .. ... .
   952" LQRRCNTLITLIERENIELEEKERAEKKKKAPKGSVSAGSGSASSNTPAPAPQPKASQKR

  1014' RKKNKK

  1012" KSEVVATSSNSKKKKK
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B

Fig. S3. Specificity of ISWI antibody. (**A**) ISWI sequence is highly conserved between *Drosophila* and sea urchin. The highlighted region indicates that used for antibody production in *Drosophila*. (**B**) A nuclear-specific localization of ISWI is observed in every cell during embryogenesis. Scale bars: 50 µm.

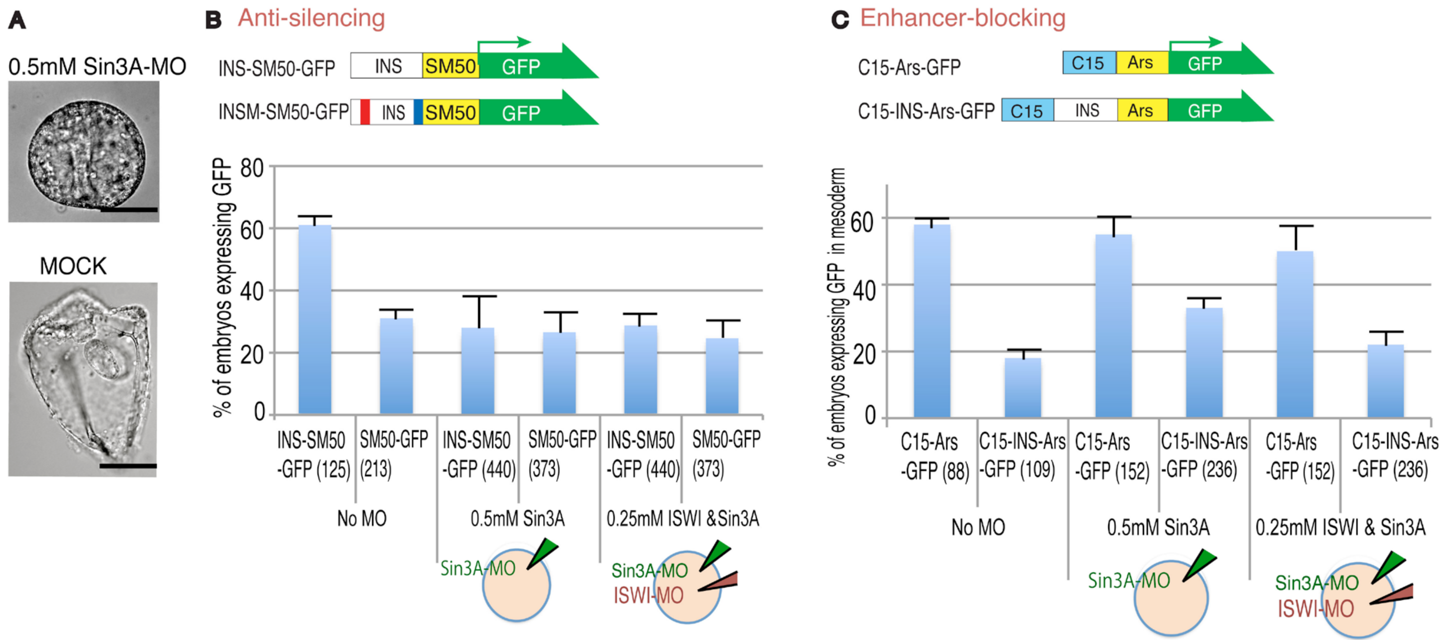

Fig. S4. Functional contribution of Sin3A to ArsI activities. (**A**) Sin3A-MO-injected (0.5 mM) embryos showed developmental defects yet successfully formed three germ layers, a blastocoel, skeleton and cilia. (**B**,**C**) In vivo functional assay for anti-silencing and enhancer-blocking activities of ArsI. Promoter activity of each reporter construct was tested by GFP expression in the presence/absence of Sin3A or Sin3A and ISWI. The total number of embryos injected is indicated in parentheses.