

**Two benthic diatoms, *Nanofrustulum shiloi* and *Striatella unipunctata*,
encapsulated in alginate beads, influence the reproductive efficiency of
*Paracentrotus lividus***

Francesca Glaviano^{1,#}, Nadia Ruocco^{1,#}, Emanuele Somma^{1,#}, Giuseppe De Rosa², Virginia Campani², Pasquale Ametrano¹, Maria Costantini^{1,*}, Valerio Zupo^{1,*}

¹*Department of Marine Biotechnology, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy*

³*Department of Pharmacy, University of Naples Federico II, Naples, Italy*

#These authors contributed equally to this work

*Authors to whom correspondence should be addressed: maria.costantini@szn.it, vzupo@szn.it, Tel.: +39-081-583-3315; Fax: +39-081-764-1355

Figure S1. a. Images of *S. unipunctata* under the optical microscope (a) and SEM (b-c). The diatom shows a big chloroplast in the middle of the cell (a), a frustule with rectangular shape and truncated corners (b), a mucilaginous stalk (b), and characteristic ornamental striae on the surface of each valve (c). Scale bars= 15 μm (a), 10 μm (b), 1 μm (c).

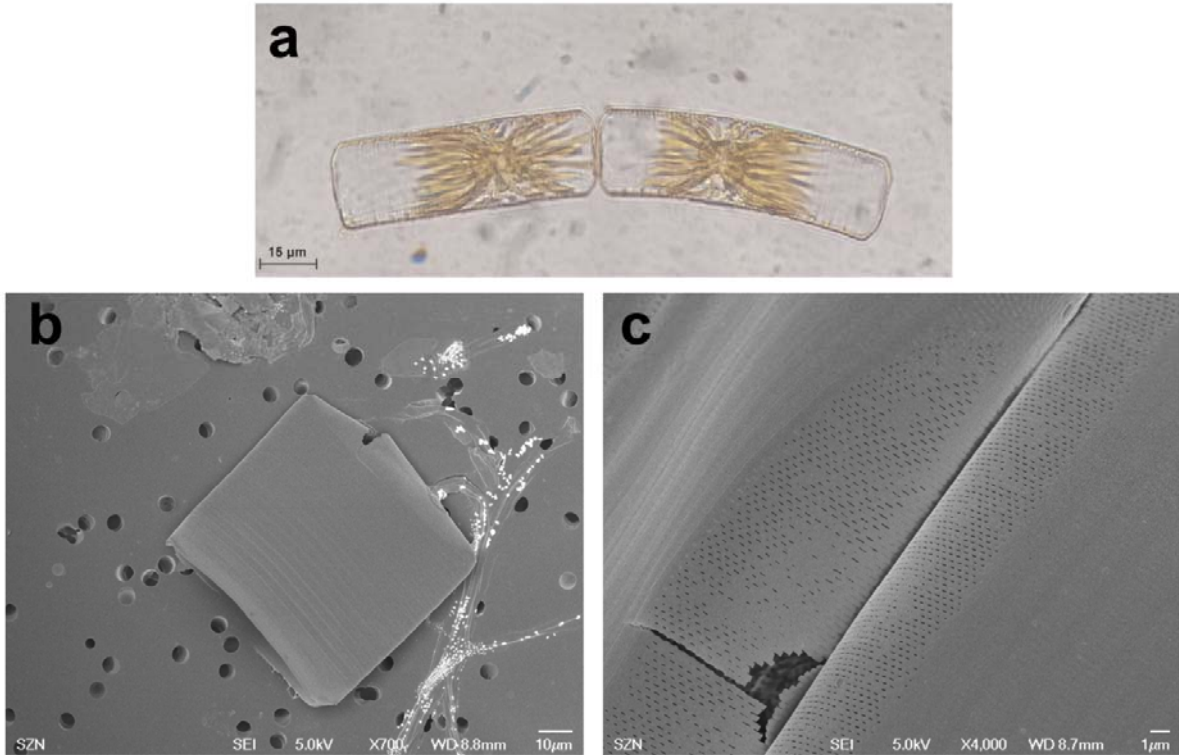
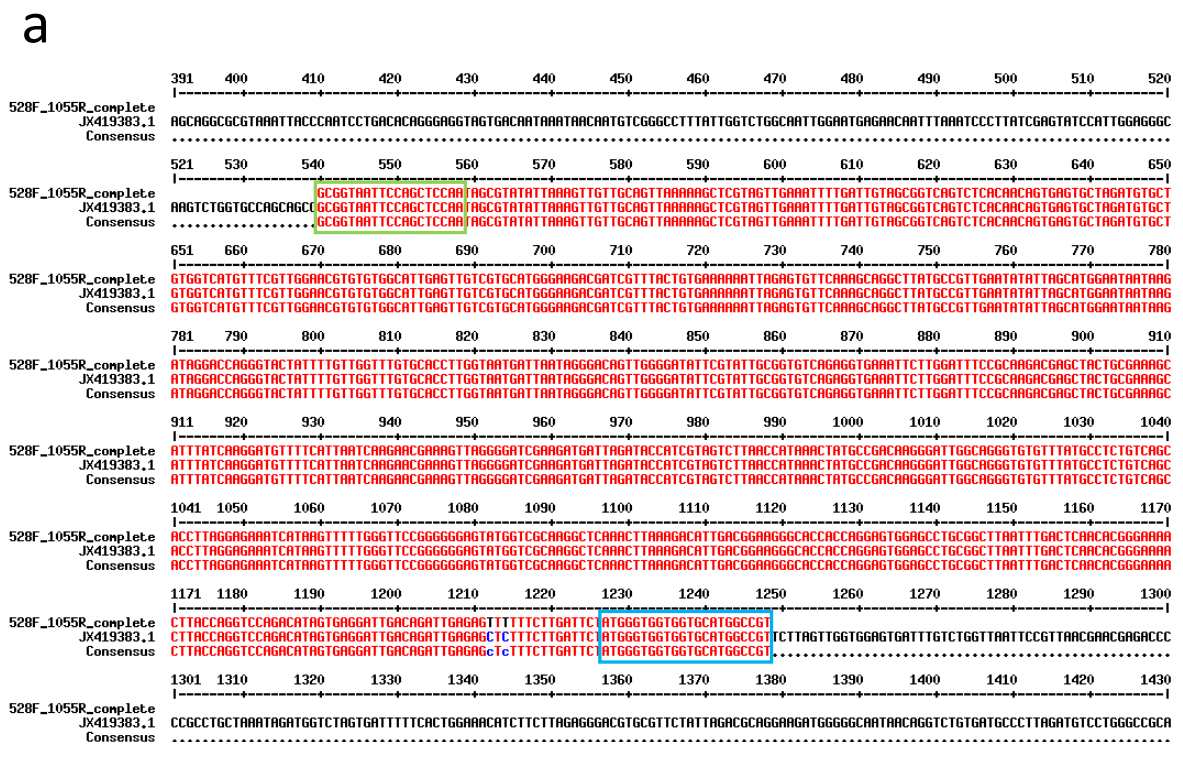


Figure S2. Alignments of *S. unipunctata* to the four annotated 18S rRNA sequences found in BLASTn search with accession numbers JX419383.1 (a), AB430609.1 (b), AF525666.1 (c) and HQ912643.1 (d). Froward and reverse primers were highlighted by a green and sky blue rectangle, respectively.



b

528F_1055R_complete
AB430609.1
Consensus

391 400 410 420 430 440 450 460 470 480 490 500 510 520
CCAGGGAGGCGAGCGGCGGTAARATACCCAACTCGACACAGGGAGGTAGTGACAAATAATACAAATGTCGGGCCCTTATTGGCTGGCAATTGGAAATGAGAACAAATTAATCCCTATTCGAGTATC

528F_1055R_complete
AB430609.1
Consensus

521 530 540 550 560 570 580 590 600 610 620 630 640 650
CATTGGAGGGCAGCTGGTCCAGCAGCGCGGTAATCCAGCTCCAAAGCGTATATTAAGTGTGTCAGTTAAAAGCTCGTAGTTGAARATTTGATTTAGCGGTCAGCTCCACACAGTGGTG
CGGTAATCCAGCTCCAAAGCGTATATTAAGTGTGTCAGTTAAAAGCTCGTAGTTGAARATTTGATTTAGCGGTCAGCTCCACACAGTGGTG

528F_1055R_complete
AB430609.1
Consensus

651 660 670 680 690 700 710 720 730 740 750 760 770 780
CTAGATGCTGTGGTCATGTTTCGTTGGACAGCTGTGGCATTGAGTTGTCGTCATGGGAGACGATCGTTTACTGTGAAAAATTAGAGTGTTCARAGCAGGCTTATGCCGTTGAATATATAGCAT
CTAGATGCTGTGGTCATGTTTCGTTGGACAGCTGTGGCATTGAGTTGTCGTCATGGGAGACGATCGTTTACTGTGAAAAATTAGAGTGTTCARAGCAGGCTTATGCCGTTGAATATATAGCAT

528F_1055R_complete
AB430609.1
Consensus

781 790 800 810 820 830 840 850 860 870 880 890 900 910
GGAAATAGATAGGACACAGGCTACTATTTGTTGGTTGTGCACCTTGGTAATGATTAAATAGGACAGTGGGGATATTCGATTCGGGTGTCAGAGGTGAARATCTGGATTTCCGACAGACAGCT
GGAAATAGATAGGACACAGGCTACTATTTGTTGGTTGTGCACCTTGGTAATGATTAAATAGGACAGTGGGGATATTCGATTCGGGTGTCAGAGGTGAARATCTGGATTTCCGACAGACAGCT

528F_1055R_complete
AB430609.1
Consensus

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
ACTGCAAGGCAATTTATCAGGATGTTTCATTATCAGACAGAAAGTTAGGGGATCGAGATGATTAGATACCATCGTAGCTTAAACATAAACTATGCCACAGGGATTGGCAGGGTGTGTTATG
ACTGCAAGGCAATTTATCAGGATGTTTCATTATCAGACAGAAAGTTAGGGGATCGAGATGATTAGATACCATCGTAGCTTAAACATAAACTATGCCACAGGGATTGGCAGGGTGTGTTATG
ACTGCAAGGCAATTTATCAGGATGTTTCATTATCAGACAGAAAGTTAGGGGATCGAGATGATTAGATACCATCGTAGCTTAAACATAAACTATGCCACAGGGATTGGCAGGGTGTGTTATG

528F_1055R_complete
AB430609.1
Consensus

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
CCTCTGTCAGCACCCTAGGAGAAATCATAGTTTTGGGTCGCGGGGGAGTATGGTCGACAGGCTCAAACTTAAGACATTTAGCGAGGGGACACACAGGAGTGGAGCTCGGGCTTAAATGACTCA
CCTCTGTCAGCACCCTAGGAGAAATCATAGTTTTGGGTCGCGGGGGAGTATGGTCGACAGGCTCAAACTTAAGACATTTAGCGAGGGGACACACAGGAGTGGAGCTCGGGCTTAAATGACTCA

528F_1055R_complete
AB430609.1
Consensus

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
ACACGGGAARACTTACAGGTCAGACATAGTGGGATGACAGATTGAGAGTTTCTCTGATTCATGGGTTGGTGCATGGCCGTTCTTAGTTGGTGGAGTATTGCTGGTAAATCCGTTAAC
ACACGGGAARACTTACAGGTCAGACATAGTGGGATGACAGATTGAGAGTCTCTCTGATTCATGGGTTGGTGCATGGCCGTTCTTAGTTGGTGGAGTATTGCTGGTAAATCCGTTAAC
ACACGGGAARACTTACAGGTCAGACATAGTGGGATGACAGATTGAGAGTCTCTCTGATTCATGGGTTGGTGCATGGCCGTTCTTAGTTGGTGGAGTATTGCTGGTAAATCCGTTAAC

528F_1055R_complete
AB430609.1
Consensus

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
GAACGAGACCCCGCTGCTAATAGATGGTCTAGTGTATTTTCTACTGAAACATCTTCTTAGAGGGACGTCGCTTCTATTAGCGCAGGAGGATGGGGCAATACAGGCTGTGTATGCCCTTAGATGT

C

528F_1055R_complete
AF525666.1
Consensus

391 400 410 420 430 440 450 460 470 480 490 500 510 520
GCGTAATATCCCACTCGACACAGGGAGGTAGTGACAAATAATACAAATGTCGGGCCCTTATTGGCTGGCAATTGGAAATGAGAACAAATTAATCCCTATTCGAGTATCCATTGGAGGGCAGCTG

528F_1055R_complete
AF525666.1
Consensus

521 530 540 550 560 570 580 590 600 610 620 630 640 650
GTGCCAGCAGCGCGGTAATCCAGCTCCAAAGCGTATATTAAGTGTGTCAGTTAAAAGCTCGTAGTTGAARATTTGATTTAGCGGTCAGCTCCACACAGTGGTG
CGGTAATCCAGCTCCAAAGCGTATATTAAGTGTGTCAGTTAAAAGCTCGTAGTTGAARATTTGATTTAGCGGTCAGCTCCACACAGTGGTG

528F_1055R_complete
AF525666.1
Consensus

651 660 670 680 690 700 710 720 730 740 750 760 770 780
TGTTCGTTGGACAGCTGTGGCATTGAGTTGTCGTCATGGGAGACGATCGTTTACTGTGAAAAATTAGAGTGTTCARAGCAGGCTTATGCCGTTGAATATATAGCATGGAAATAGATAGGAC
TGTTCGTTGGACAGCTGTGGCATTGAGTTGTCGTCATGGGAGACGATCGTTTACTGTGAAAAATTAGAGTGTTCARAGCAGGCTTATGCCGTTGAATATATAGCATGGAAATAGATAGGAC

528F_1055R_complete
AF525666.1
Consensus

781 790 800 810 820 830 840 850 860 870 880 890 900 910
CAGGGTACTATTTGTTGGTTGTGCACCTTGGTAATGATTAAATAGGACAGTGGGGATATTCGATTCGGGTGTCAGAGGTGAARATCTGGATTTCCGACAGACAGCTACTGCGAAGCATTTATC
CAGGGTACTATTTGTTGGTTGTGCACCTTGGTAATGATTAAATAGGACAGTGGGGATATTCGATTCGGGTGTCAGAGGTGAARATCTGGATTTCCGACAGACAGCTACTGCGAAGCATTTATC

528F_1055R_complete
AF525666.1
Consensus

911 920 930 940 950 960 970 980 990 1000 1010 1020 1030 1040
AAGGATGTTTCATTATCAGACAGAAAGTTAGGGGATCGAGATGATTAGATACCATCGTAGCTTAAACATAAACTATGCCACAGGGATTGGCAGGGTGTGTTATGCCCTTGTACACACTTAG
AAGGATGTTTCATTATCAGACAGAAAGTTAGGGGATCGAGATGATTAGATACCATCGTAGCTTAAACATAAACTATGCCACAGGGATTGGCAGGGTGTGTTATGCCCTTGTACACACTTAG
AAGGATGTTTCATTATCAGACAGAAAGTTAGGGGATCGAGATGATTAGATACCATCGTAGCTTAAACATAAACTATGCCACAGGGATTGGCAGGGTGTGTTATGCCCTTGTACACACTTAG

528F_1055R_complete
AF525666.1
Consensus

1041 1050 1060 1070 1080 1090 1100 1110 1120 1130 1140 1150 1160 1170
GAGAAATCATAGTTTTGGGTCGCGGGGGAGTATGGTCGACAGGCTCAAACTTAAGACATTTAGCGAGGGGACACACAGGAGTGGAGCTCGGGCTTAAATGACTCAACAGGGAAACTTACCA
GAGAAATCATAGTTTTGGGTCGCGGGGGAGTATGGTCGACAGGCTCAAACTTAAGACATTTAGCGAGGGGACACACAGGAGTGGAGCTCGGGCTTAAATGACTCAACAGGGAAACTTACCA

528F_1055R_complete
AF525666.1
Consensus

1171 1180 1190 1200 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
GGTCCAGACATAGTGGGATGACAGATTGAGAGTTTCTCTGATTCATGGGTTGGTGCATGGCCGTTCTTAGTTGGTGGAGTATTGCTGGTAAATCCGTTAACGACAGGACAGCCCGCTGCTG
GGTCCAGACATAGTGGGATGACAGATTGAGAGTCTCTCTGATTCATGGGTTGGTGCATGGCCGTTCTTAGTTGGTGGAGTATTGCTGGTAAATCCGTTAACGACAGGACAGCCCGCTGCTG

528F_1055R_complete
AF525666.1
Consensus

1301 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400 1410 1420 1430
TAATAGATGGTCTAGTGTATTTCTACTGAAACATCTTCTTAGAGGGACGTCGCTTCTATTAGCGCAGGAGGATGGGGCAATACAGGCTGTGTATGCCCTTAGATGTCTGGCCGACAGCCGCTA

d

```
391 400 410 420 430 440 450 460 470 480 490 500 510 520
528F_1055R_complete
HQ912643.1
Consensus
ATCCARGGAGGGCAGCAGGGCGGTAAATACCCAACTCCGACACAGGGAGGTAGTGACAAATAAATACAAATGTCGGGCCCTTATTGGCTGGCAATGGAAATGAGAACAAATTAATCCCTATCCAGTA
.....
528F_1055R_complete
HQ912643.1
Consensus
TCCATTGGAGGGCAAGTCTGGTCCAGCAGC(GCGGTAATCCAGCTCCAA)TAGCGTATATTAAGTTGTTGCAGTTAAAAGCTCGTAGTTGAARATTTGATTTAGCGGTCAGTCCACACAGTGAG
.....
528F_1055R_complete
HQ912643.1
Consensus
TGCATGATGTGCTGGTCATGTTTCGTTGGACGCTGTGGCATTGAGTTGTCGTCATGGGAGACGATCGTTTACGTGAAAARATAGAGTGTCCAAAGCAGGCTTATGCCGTTGAAATATAGC
.....
528F_1055R_complete
HQ912643.1
Consensus
ATGGAAATAATAGATAGGACCAGGTAATTTTGTGGTTGTCACCTTGGTAATGATTAATAGGACAGTTGGGATATTCGATTGCGGTGTCAGAGTGAAATCTTGGATTTCCGACAGCAG
.....
528F_1055R_complete
HQ912643.1
Consensus
CTACTCGAAGCATTATCAGGATGTTTTTCATTAATCAGAGCAGAGTTAGGGGATCGAGATGATTAGATACCATCGTAGCTTAAACATAAATATGCCACAGGGATTTGCCAGGGTGTGTTA
.....
528F_1055R_complete
HQ912643.1
Consensus
TGCCCTGTGACACCTTAGGAGAAATCATAAGTTTTGGGTTCCGGGGGAGTATGGTCGACAGGCTCAAACTAAGACATTGACGGAGGGACACCAGGAGTGGAGCCTGCGGCTAATTTGACT
.....
528F_1055R_complete
HQ912643.1
Consensus
CACCACGGAAACTTACCAGGTCAGACATAGTGAGGATTGACAGATTGAGATTTTCTTGATTCATGGGTGGTGGTGCATGGCCGTCTTAGTTGGTGGAGTGATTTGCTGGTAAATCCGTTA
.....
528F_1055R_complete
HQ912643.1
Consensus
ACGACAGACCCCGCCTGCTAATAGGTGGTCTAGTGATTTTCACTGGATACATCTTCTAGAGGGACGTGCGTCTATTAGCGCAGGAGATGGGGCAATACAGGCTGTGATGCCCTTAGAT
```

Figure S3. Images of sea urchin embryos under optical microscope. Normal (a) and malformed (b-c) plutei were reported (Zeiss Axiovert135TV microscope, 10×/0.30 magnification/numerical aperture). Scale bar: 50 μm .

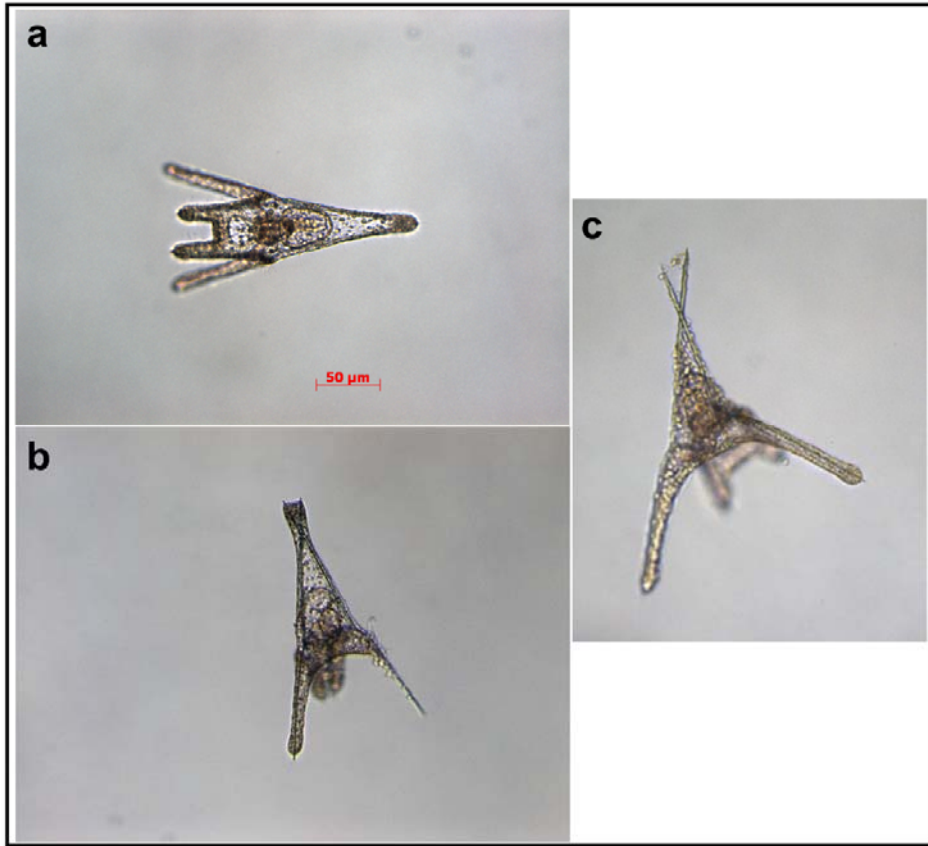


Table S1. Diameter in millimeters (mm) of alginate beads before and after dehydration.

	Diameter (mm)	
	Hydrated form	Dehydrated form
Alginate beads	3.8 ± 0.2	1.3 ± 0.1
Diatoms/alginate beads	3.7 ± 0.2	1.2 ± 0.1

Table S2. Fold change values of sixty-two genes belonging to stress response, development and differentiation, skeletogenesis and detoxification processes analyzed by RT-qPCR. Up-regulated genes and down-regulated genes were highlighted in red and blue, respectively.

		<i>N. shiloi</i>	<i>S. unipunctata</i>
Stress	<i>ARF1</i>	-2.1	-3.6
	<i>caspase 3/7</i>	-2.5	-3.8
	<i>CASP8</i>	0.6	-4.5
	<i>cytb</i>	1.9	2.3
	<i>ERCC3</i>	-0.4	-3.9
	<i>GRHPR</i>	-1.1	-1.7
	<i>GS</i>	3.4	4.5
	<i>HIF1A</i>	-2.3	-2.6
	<i>hsp56</i>	-2.9	-2.4
	<i>hsp60</i>	2.7	2.9
	<i>hsp70</i>	3.5	4.9
	<i>MTase</i>	-3.7	-2.8
	<i>NF-kB</i>	2.6	3.7
	<i>PARP</i>	2.3	2.3
	<i>p38 MAPK</i>	-0.5	2.2
	<i>p53</i>	-3.6	-4.6
	<i>SDH</i>	-1.9	-2.9
<i>14-3-3 ε</i>	3.9	3.3	
Development/ Differentiation	<i>ADMP2</i>	-0.5	3.2
	<i>Alix</i>	0.6	0.5
	<i>Blimp</i>	1.5	-0.6
	<i>BP10</i>	0.3	0.5
	<i>BRA</i>	2.6	3.5
	<i>DELTA</i>	3.3	4.1
	<i>δ-2-catenin</i>	-3.1	-3.3
	<i>FOXA</i>	-3.5	7.7
	<i>FoxG</i>	3.1	2.0
	<i>Foxo</i>	3.1	3.9
	<i>GFI1</i>	-2.7	-3.4
	<i>GOOS</i>	-6.9	-5.4
	<i>hat</i>	-0.4	-0.7

	<i>H3.3</i>	-2.2	-4.8
	<i>JNK</i>	0.3	-4.9
	<i>KIF19</i>	-1.7	-4.4
	<i>nodal</i>	2.2	-1.5
	<i>NOTCH</i>	1.6	-0.2
	<i>OneCut</i>	3.9	-6.4
	<i>SMAD6</i>	0.9	0.8
	<i>sox9</i>	0.1	2.1
	<i>TAK1</i>	-4.6	9.7
	<i>tcf4</i>	0.7	0.1
	<i>TCF7</i>	-4.1	-5.9
	<i>VEGF</i>	-2.2	1.8
	<i>Wnt5</i>	0.4	2.3
	<i>Wnt6</i>	1.7	2.5
	<i>Wnt8</i>	0.4	3.4
<i>Skeletogenesis</i>	<i>BMP5-7</i>	-2.7	-4.9
	<i>C-jun</i>	1.9	-3.4
	<i>Nec</i>	2.3	3.7
	<i>p16</i>	1.2	2.2
	<i>p19</i>	3.7	4.8
	<i>SM30</i>	-3.4	-5.4
	<i>SM50</i>	0.3	-2.5
	<i>uni</i>	-2.5	-4.7
<i>Detoxification</i>	<i>CAT</i>	3.8	4.1
	<i>MDR1</i>	2.7	4.1
	<i>MT</i>	2.4	0.2
	<i>MT4</i>	3.7	1.2
	<i>MT5</i>	4.7	3.4
	<i>MT6</i>	2.8	1.0
	<i>MT7</i>	2.6	0.2
	<i>MT8</i>	-2.2	2.5