

Gene expression profiles at different stages for formation of pearl sac and pearl in the pearl oyster *Pinctada fucata*

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Table S1. Statistical analysis of transcriptome sequencing data for each sample

Sample Name	Clean Reads	Clean bases	Read length (bp)	Q20 (%)	GC content	Quantified Reads ($\times 10^6$)	Quantification ratio (%)
Before_A	17,164,384	1,716,438,400	100	98.32	43.20%	16.73	67.75
Before_B	10,026,656	1,002,665,600	100	98.20	43.32%	10.15	68.77
Before_C	12,059,064	1,205,906,400	100	98.24	42.67%	12.18	68.50
0h_A	9,811,942	981,194,200	100	98.17	44.64%	9.74	68.34
0h_B	9,412,514	941,251,400	100	98.22	45.18%	9.27	68.14
0h_C	10,398,038	1,039,803,800	100	98.40	44.86%	11.02	70.54
24h_A1	10,355,918	1,035,591,800	100	98.20	43.00%	10.24	68.09
24h_A2	7,867,464	786,746,400	100	98.44	43.25%	8.08	71.73
24h_B1	8,870,314	887,031,400	100	98.61	42.38%	8.73	72.15
24h_B2	7,403,436	740,343,600	100	98.63	43.34%	22.58	67.36
24h_C1	23,143,452	2,314,345,200	100	98.23	40.50%	9.53	71.33
24h_C2	12,869,554	1,286,955,400	100	98.05	41.23%	13.01	68.30
48h_A1	7,112,274	711,227,400	100	98.62	42.38%	7.39	69.98
48h_A2	6,976,730	697,673,000	100	98.50	41.03%	9.48	68.07
48h_B1	9,891,620	989,162,000	100	98.42	40.95%	6.99	68.82
48h_B2	9,625,184	962,518,400	100	98.35	42.74%	9.19	69.26
48h_C1	9,008,866	900,886,600	100	98.33	41.43%	9.82	68.42
48h_C2	5,665,372	566,537,200	100	98.69	42.34%	5.97	70.70
1w_A1	48,419,298	4,841,929,800	100	95.58	41.15%	40.27	61.44
1w_A2	23,539,310	2,353,931,000	100	96.39	41.38%	21.27	64.77
1w_B1	36,485,718	3,648,571,800	100	96.32	41.45%	32.36	64.06
1w_B2	17,106,708	1,710,670,800	100	95.67	44.09%	14.51	64.41
1w_C1	5,467,236	546,723,600	100	97.90	50.37%	3.83	64.10
1w_C2	30,277,428	3,027,742,800	100	96.51	41.59%	27.64	64.71
2w_A1	34,149,422	3,414,942,200	100	96.46	41.18%	28.76	62.37
2w_A2	69,600,066	6,960,006,600	100	96.03	40.42%	61.38	63.54
2w_B1	32,927,364	3,292,736,400	100	97.05	41.53%	29.5	64.44
2w_B2	26,223,104	2,622,310,400	100	96.49	40.58%	22.49	63.11
2w_C1	38,238,402	3,823,840,200	100	96.50	42.09%	31.83	62.69
2w_C2	55,393,672	5,539,367,200	100	95.13	41.81%	45.3	62.51
1m_A1	45,558,068	4,555,806,800	100	95.67	40.62%	39.64	62.91
1m_A2	26,248,864	2,624,886,400	100	97.31	41.29%	22.59	63.00
1m_B1	25,934,080	2,593,408,000	100	96.55	40.29%	21.51	61.10
1m_B2	32,196,990	3,219,699,000	100	96.97	44.01%	25.97	62.89
1m_C1	25,541,512	2,554,151,200	100	96.38	40.22%	19.85	58.60
1m_C2	32,021,402	3,202,140,200	100	96.35	40.85%	26.87	61.33
3m_A1	23,359,108	2,335,910,800	100	95.64	40.84%	19.25	60.50
3m_A2	24,171,408	2,417,140,800	100	96.33	42.24%	18.64	58.99
3m_B1	30,337,036	3,033,703,600	100	96.66	41.62%	23.83	60.16
3m_B2	11,595,252	1,159,525,200	100	96.56	40.22%	8.99	58.99
3m_C1	1,231,312	123,131,200	100	97.30	39.04%	1.2	66.43
3m_C2	22,436,394	2,243,639,400	100	96.39	40.34%	17.84	59.91
Cell_A	7,382,306	738,230,600	100	98.34	44.65%	7.1	68.67
Cell_B	5,825,620	582,562,000	100	98.26	44.03%	5.37	66.89
Cell_C	6,019,878	601,987,800	100	98.48	45.08%	5.88	69.79

Table S5. Genes significantly ($P < 0.05$) enriched in epithelial cell proliferation and differentiation related GO terms

GO term	Up-regulated DEGs	Down-regulated DEGs
negative regulation of morphogenesis of an epithelium	<i>SULF1</i>	<i>SULF1, LRP6</i>
columnar/cuboidal epithelial cell differentiation	<i>JAG1, RFX3, STRC</i>	<i>JAG1, FGFR2, SAV1, RAC1</i>
glandular epithelial cell maturation	<i>RFX3</i>	<i>RFX3</i>
columnar/cuboidal epithelial cell development	<i>RFX3, STRC</i>	-
columnar/cuboidal epithelial cell maturation	<i>RFX3</i>	<i>RFX3</i>
neuro-epithelial cell differentiation	<i>STRC, JAG1</i>	<i>JAG1, RAC1</i>
negative regulation of epithelial cell proliferation	<i>TSC2, FBXW7, PTPRK, SULF1, MTSSI</i>	<i>PTPRK, TSC2, FBXW7, MTSSI, SULF1, PTPRM, LRP6, SAV1, XDH</i>
epithelial cell differentiation	<i>EHF, TGM1, RFX3, STRC, JAG1, MTSSI, MYO1E</i>	-
regulation of epithelial tube formation	<i>PTK7, SFRP5, FZD1, LRP6</i>	-
epithelium development	<i>RFX3, DMD, RGMA, PTK7, MAF, MEF2A, SFRP5, TGM1, FZD1, GRHL2, TEAD1, PRKDC</i>	<i>TSC2, EHF, RUNX1, JAG1, RAB10, FGFR2, EP300, ENAH, CASP8, CDC42, MTSSI, LRP4, MMP12, LRP6, NRP1, ILK, IPMK, PAK1, SAV1, CLIC4, RAC1, MYO1E, EGFR, TBX1, LBX1</i>
neuronal stem cell division	<i>FGFR1, LRP6</i>	<i>RAB10, FGFR2, LRP6</i>
somatic stem cell division	<i>FGFR1, LRP6</i>	<i>LRP6, FGFR2, ASPM, DOCK7</i>
morphogenesis of an epithelium	<i>CASP8, CDC42, RSPO2, MTSSI</i>	<i>TBX1, RAB10, CASP8</i>
epithelial tube morphogenesis	<i>CASP8, RSPO2, MTSSI</i>	<i>TBX1, CASP8</i>
epithelial tube formation	<i>CASP8</i>	<i>CASP8</i>
morphogenesis of an epithelial fold	<i>EGFR, AR</i>	<i>EGFR</i>
regulation of morphogenesis of an epithelium	<i>SFRP5, WNT2B, LRP6, AR, SULF1, ESR1, RAC1</i>	<i>FGFR2, SULF1, LRP6, RAC1</i>
negative regulation of epidermal cell differentiation	<i>MSX2, DLL1, HES1</i>	-
regulation of epithelial cell proliferation	<i>LAMC1, PTPRK, EGFR</i>	<i>PTPRK, TSC2, FBXW7, FGFR2, MTSSI, SULF1, PTPRM, MMP12, LRP6, NRP1, SAV1, XDH, EGFR, TBX1</i>
positive regulation of epithelial cell proliferation	<i>LAMC1, EGFR</i>	<i>LAMC1, EGFR</i>
connective tissue development	<i>MATN1, SULF1, SPG20, LRP6</i>	<i>MATN1, SULF1, SPG20, LRP6</i>

Table S5. (continued)

GO term	Up-regulated DEGs	Down-regulated DEGs
epithelial cell-cell adhesion	-	<i>CDC42</i>
epidermis morphogenesis	-	<i>RUNX1, FGFR2, CDC42</i>
epidermal cell differentiation	-	<i>JAG1, CDC42, SAV1, CLIC4, RAC1</i>
epidermis development	-	<i>RUNX1, JAG1, FGFR2, CDC42, LRP4, SAV1, CLIC4, RAC1, EGFR</i>
epithelial cell development	-	<i>FGFR2, CDC42, CLIC4, RAC1, MYO1E</i>
epithelial cell migration	-	<i>MYH9, NRP1</i>
morphogenesis of an epithelial sheet	-	<i>JAG1, MMP12, LRP6</i>
epithelial-mesenchymal cell signaling	-	<i>CDC42</i>
epithelial cilium movement	-	<i>RFX3</i>
epithelial cell maturation	-	<i>RFX3</i>
glandular epithelial cell development	-	<i>RFX3</i>
mammary gland epithelial cell proliferation	-	<i>STAT6, ESR1, MED1</i>
hematopoietic stem cell differentiation	-	<i>XRCC5, ACE, TAL1, SRF, ERCC2</i>
regulation of epithelial cell differentiation	-	<i>XDH, SMO, DMBT1, PROM1, SOX2, APC, PAX2, VDR, NODAL, MED1, CAV1, RFX3, XDH, EZH2, KEAP1</i>
stem cell population maintenance	-	<i>SMC3, RIF1, SOX2, APC, PCMI, SKI, MED27, RTF1, SMC1A, NODAL, MED21, VPS72, GATA2, SRRT, LEO1, SRRT, FGFR3</i>
positive regulation of epithelial cell differentiation	-	<i>DMBT1, PROM1, SOX2, APC, PAX2, VDR, MED1, RFX3</i>
regulation of stem cell population maintenance	-	<i>SMO, CNOT1, PAX2, CNOT2, TAL1, NODAL, CNOT3</i>
neuronal stem cell population maintenance	-	<i>SOX2, PCMI, SRRT</i>
morphogenesis of a branching epithelium	-	<i>TCF21, LRP6, SALL1, SLIT2, PKD1, FGFR2, MET, FOXD3, LAMA1, SOX2, DLG1, WNT4, PAX2, VDR, ESR1, TBX20, IHH, GRB2, RBM15, MED1, MKS1, FOXA2, SRF</i>

Table S6. Gene symbol with full gene name

Gene symbol	Full gene name
<i>ACE</i>	Angiotensin-converting enzyme
<i>APC</i>	Adenomatous polyposis coli protein
<i>AR</i>	Androgen receptor
<i>ASPM</i>	Abnormal spindle-like microcephaly-associated protein
<i>CASP8</i>	Caspase-8 subunit p10
<i>CAV1</i>	Caveolin-1
<i>CDC42</i>	Cell division control protein 42 homolog
<i>CLIC4</i>	Chloride intracellular channel protein 4
<i>CNOT1</i>	CCR4-NOT transcription complex subunit 1
<i>CNOT2</i>	CCR4-NOT transcription complex subunit 2
<i>CNOT3</i>	CCR4-NOT transcription complex subunit 3
<i>DLG1</i>	Disks large homolog 1
<i>DLL1</i>	Delta-like protein 1
<i>DMBT1</i>	Deleted in malignant brain tumors 1 protein
<i>DMD</i>	Dystrophin
<i>DOCK7</i>	Dedicator of cytokinesis protein 7
<i>EGFR</i>	Epidermal growth factor receptor
<i>EHF</i>	ETS homologous factor
<i>ENAH</i>	Protein enabled homolog
<i>EP300</i>	Histone acetyltransferase p300
<i>ERCC2</i>	TFIIH basal transcription factor complex helicase XPD subunit
<i>ESR1</i>	Estrogen receptor
<i>EZH2</i>	Histone-lysine N-methyltransferase EZH2
<i>FBXW7</i>	F-box/WD repeat-containing protein 7
<i>FGFR1</i>	Fibroblast growth factor receptor 1
<i>FGFR2</i>	Fibroblast growth factor receptor 2
<i>FGFR3</i>	Fibroblast growth factor receptor 3
<i>FOXA2</i>	Hepatocyte nuclear factor 3-beta
<i>FOXD3</i>	Forkhead box protein D3
<i>FZD1</i>	Frizzled-1
<i>GATA2</i>	Endothelial transcription factor GATA-2
<i>GRB2</i>	Growth factor receptor-bound protein 2
<i>GRHL2</i>	Grainyhead-like protein 2 homolog
<i>HES1</i>	Transcription factor HES-1
<i>IHH</i>	Indian hedgehog protein
<i>ILK</i>	Integrin-linked protein kinase
<i>IPMK</i>	Inositol polyphosphate multikinase
<i>JAG1</i>	Protein jagged-1
<i>KEAP1</i>	Kelch-like ECH-associated protein 1
<i>LAMA1</i>	Laminin subunit alpha-1
<i>LAMC1</i>	Laminin subunit gamma-1

Table S6. (continued)

Gene symbol	Full gene name
<i>LBX1</i>	Transcription factor LBX1
<i>LEO1</i>	RNA polymerase-associated protein LEO1
<i>LRP4</i>	Low-density lipoprotein receptor-related protein 4
<i>LRP6</i>	Low-density lipoprotein receptor-related protein 6
<i>MAF</i>	Transcription factor Maf
<i>MATN1</i>	Cartilage matrix protein
<i>MED1</i>	Mediator of RNA polymerase II transcription subunit 1
<i>MED21</i>	Mediator of RNA polymerase II transcription subunit 21
<i>MED27</i>	Mediator of RNA polymerase II transcription subunit 27
<i>MEF2A</i>	Myocyte-specific enhancer factor 2A
<i>MET</i>	Hepatocyte growth factor receptor
<i>MKS1</i>	Meckel syndrome type 1 protein
<i>MMP12</i>	Macrophage metalloelastase
<i>MSX2</i>	Homeobox protein MSX-2
<i>MTSS1</i>	Metastasis suppressor protein 1
<i>MYH9</i>	Myosin-9
<i>MYO1E</i>	Unconventional myosin-Ie
<i>NODAL</i>	Nodal homolog
<i>NRP1</i>	Neuropilin-1/Protein kinase C-binding protein NELL1
<i>PAK1</i>	Serine/threonine-protein kinase PAK 1
<i>PAX2</i>	Paired box protein Pax-2
<i>PCM1</i>	Methyl-CpG-binding domain protein 1
<i>PEX13</i>	Peroxisomal membrane protein PEX13
<i>PKD1</i>	Polycystin-1
<i>PRKDC</i>	DNA-dependent protein kinase catalytic subunit
<i>PROM1</i>	Prominin-1
<i>PTK7</i>	Inactive tyrosine-protein kinase 7
<i>PTPRK</i>	Receptor-type tyrosine-protein phosphatase kappa
<i>PTPRM</i>	Receptor-type tyrosine-protein phosphatase mu
<i>RAB10</i>	Ras-related protein Rab-10
<i>RAC1</i>	Ras-related C3 botulinum toxin substrate 1
<i>RBM15</i>	Putative RNA-binding protein 15
<i>RFX3</i>	Transcription factor RFX3
<i>RGMA</i>	Repulsive guidance molecule A
<i>RIF1</i>	Insulin-like peptide INSL6/Telomere-associated protein RIF1
<i>RSPO2</i>	R-spondin-2
<i>RTF1</i>	RNA polymerase-associated protein RTF1 homolog
<i>RUNX1</i>	Runt-related transcription factor 1
<i>SALL1</i>	Sal-like protein 1
<i>SAVI</i>	Protein salvador homolog 1

Table S6. (continued)

Gene symbol	Full gene name
<i>SFRP5</i>	Secreted frizzled-related protein 5
<i>SKI</i>	Ski oncogene
<i>SLIT2</i>	Slit homolog 2 protein
<i>SMC1A</i>	Structural maintenance of chromosomes protein 1A
<i>SMC3</i>	Structural maintenance of chromosomes protein 3
<i>SMO</i>	Smoothed homolog
<i>SOX2</i>	Transcription factor SOX-2
<i>SPG20</i>	Spartin
<i>SRF</i>	Serum response factor
<i>SRRT</i>	Serrate RNA effector molecule homolog
<i>STAT6</i>	Signal transducer and activator of transcription 6
<i>STRC</i>	Stereocilin
<i>SULF1</i>	Extracellular sulfatase Sulf-1
<i>TAL1</i>	T-cell acute lymphocytic leukemia protein 1
<i>TBX1</i>	T-box transcription factor TBX1
<i>TBX20</i>	T-box transcription factor TBX20
<i>TCF21</i>	Transcription factor 21
<i>TEAD1</i>	Transcriptional enhancer factor TEF-1
<i>TGM1</i>	Protein-glutamine gamma-glutamyltransferase K
<i>TSC2</i>	Tuberin
<i>VDR</i>	Vitamin D3 receptor
<i>VPS72</i>	Vacuolar protein sorting-associated protein 72 homolog
<i>WNT2B</i>	Protein Wnt-2b
<i>WNT4</i>	Protein Wnt-4
<i>XDH</i>	Xanthine dehydrogenase/oxidase
<i>XRCC5</i>	X-ray repair cross-complementing protein 5

Additional file Figure:

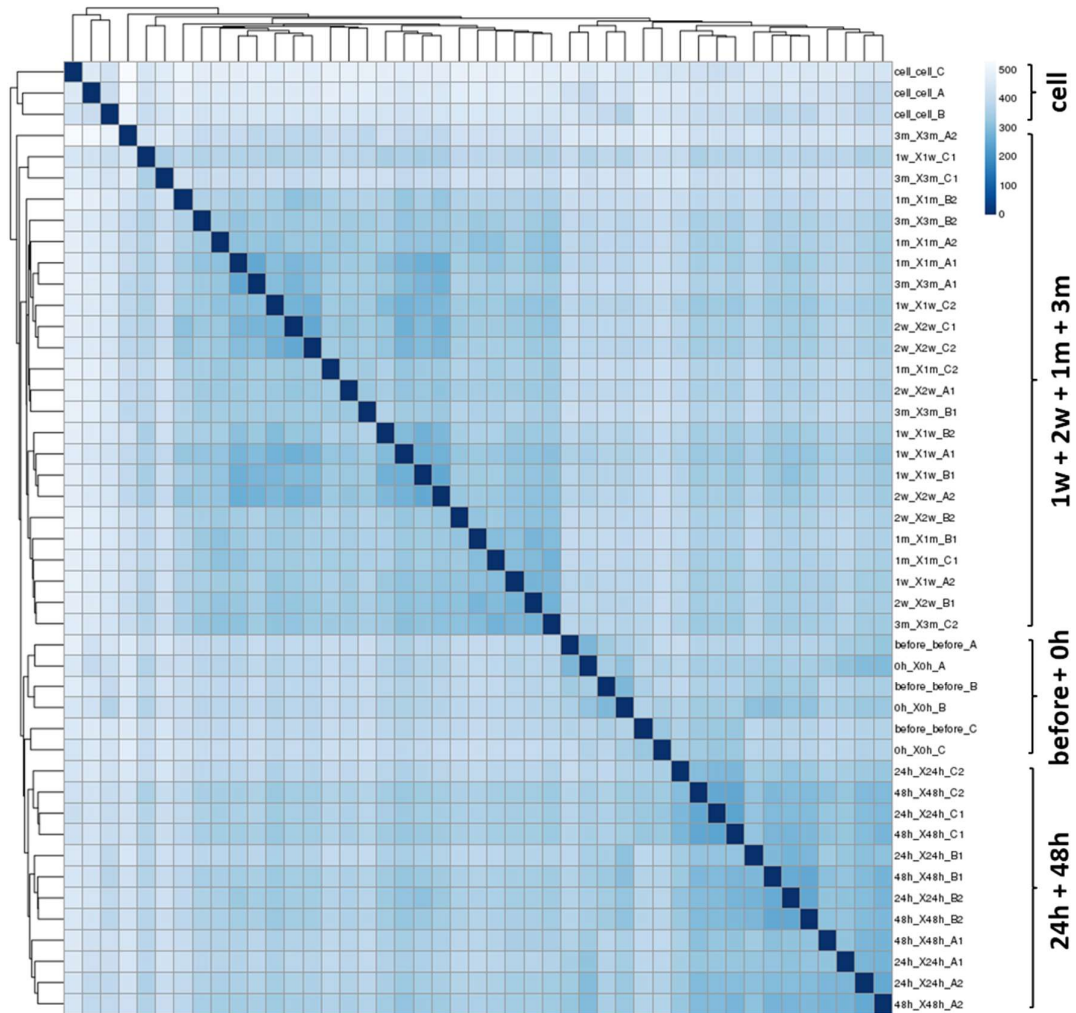


Figure S1. Heat map demonstrating whole gene expression profile at different stages of pearl grafting. Hierarchical clustering divided all the samples into four groups (cell, before + 0 h, 24 h + 48 h and 1 w + 2 w + 1 m + 3 m) as indicated on figure. Colour scale indicates the differences in expression.

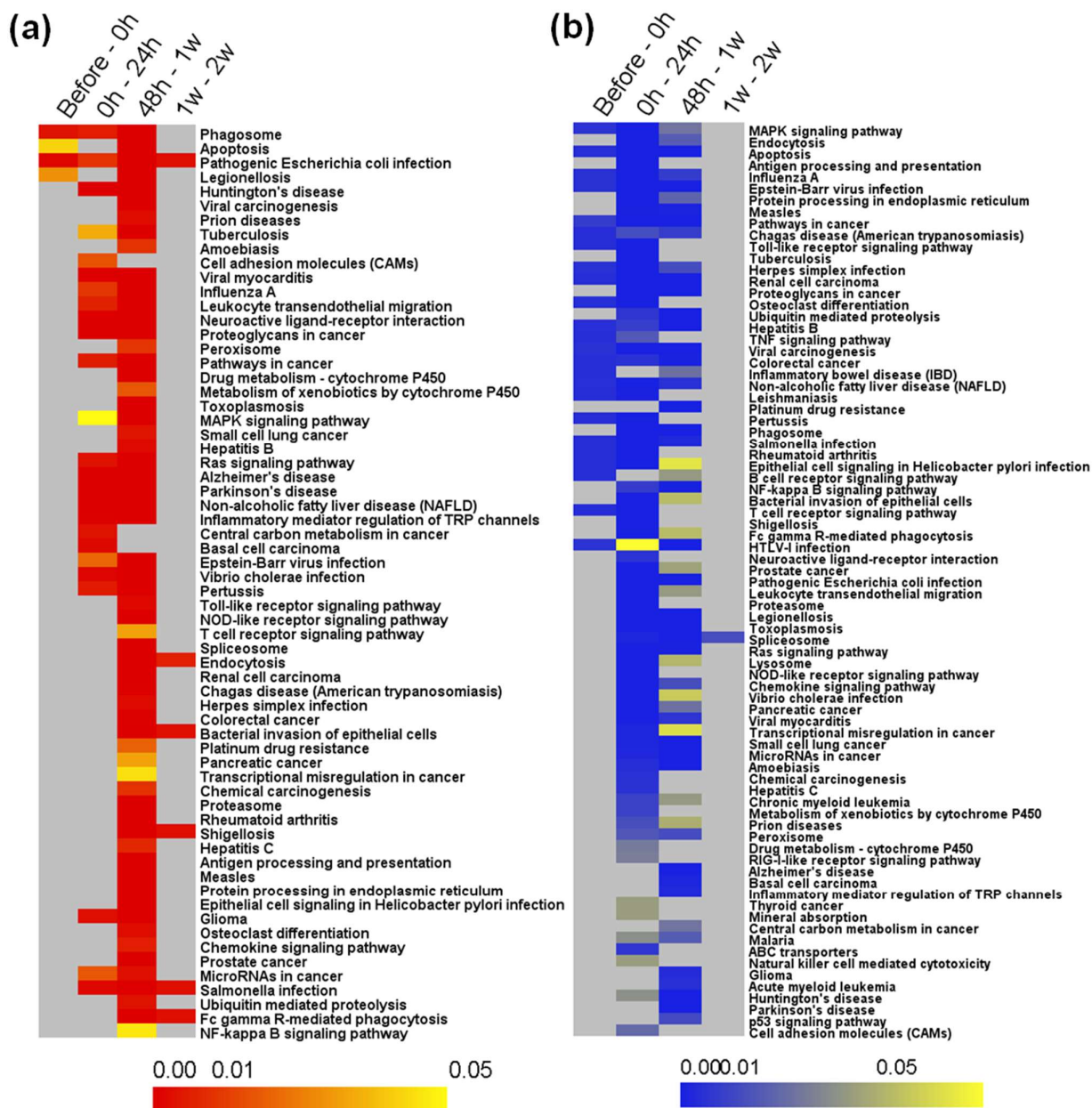


Figure S2. Heat maps of KEGG pathway enrichment analysis for immune related DEGs estimated by sleuth. (a) Up-regulated DEGs. (b) Down-regulated DEGs. Up- or down-regulated DEGs at each time point were submitted to KEGG pathway analysis using Kobas 3.0 web-based software. Columns and rows in the heat maps indicate treatments and enriched pathway terms, respectively. Sample names are displayed above the heat maps. Color scales indicate P values of enrichment tests and gray cells represent an empty value or a value > 0.05 .

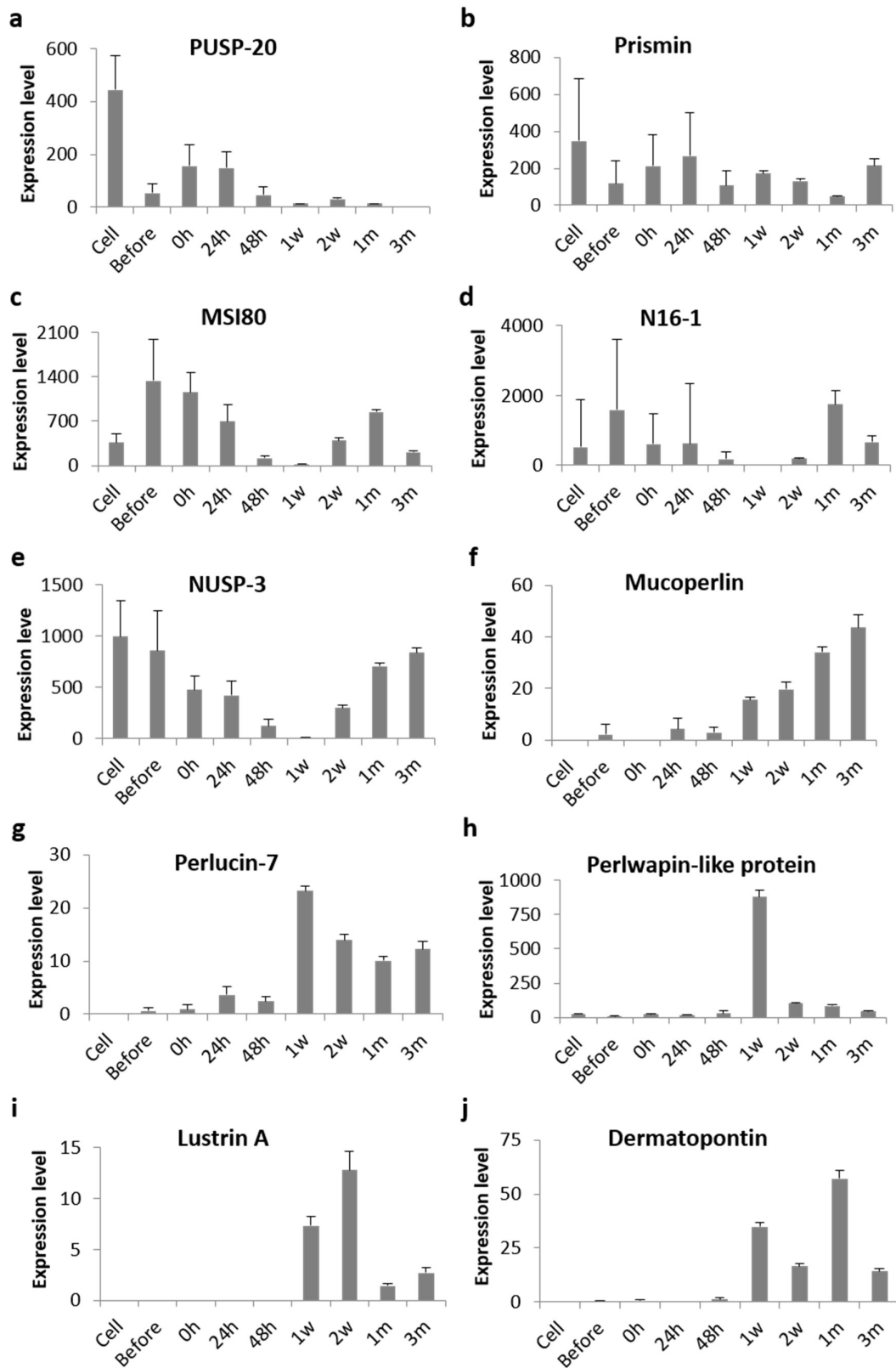


Figure S3. Expression patterns of SMPs involved in prismatic layer (a-b) and nacreous layer formation (c-j) at various time points of pearl sac and pearl development. Expression levels are indicated by adjusted TPM values (transcripts per kilobase million).

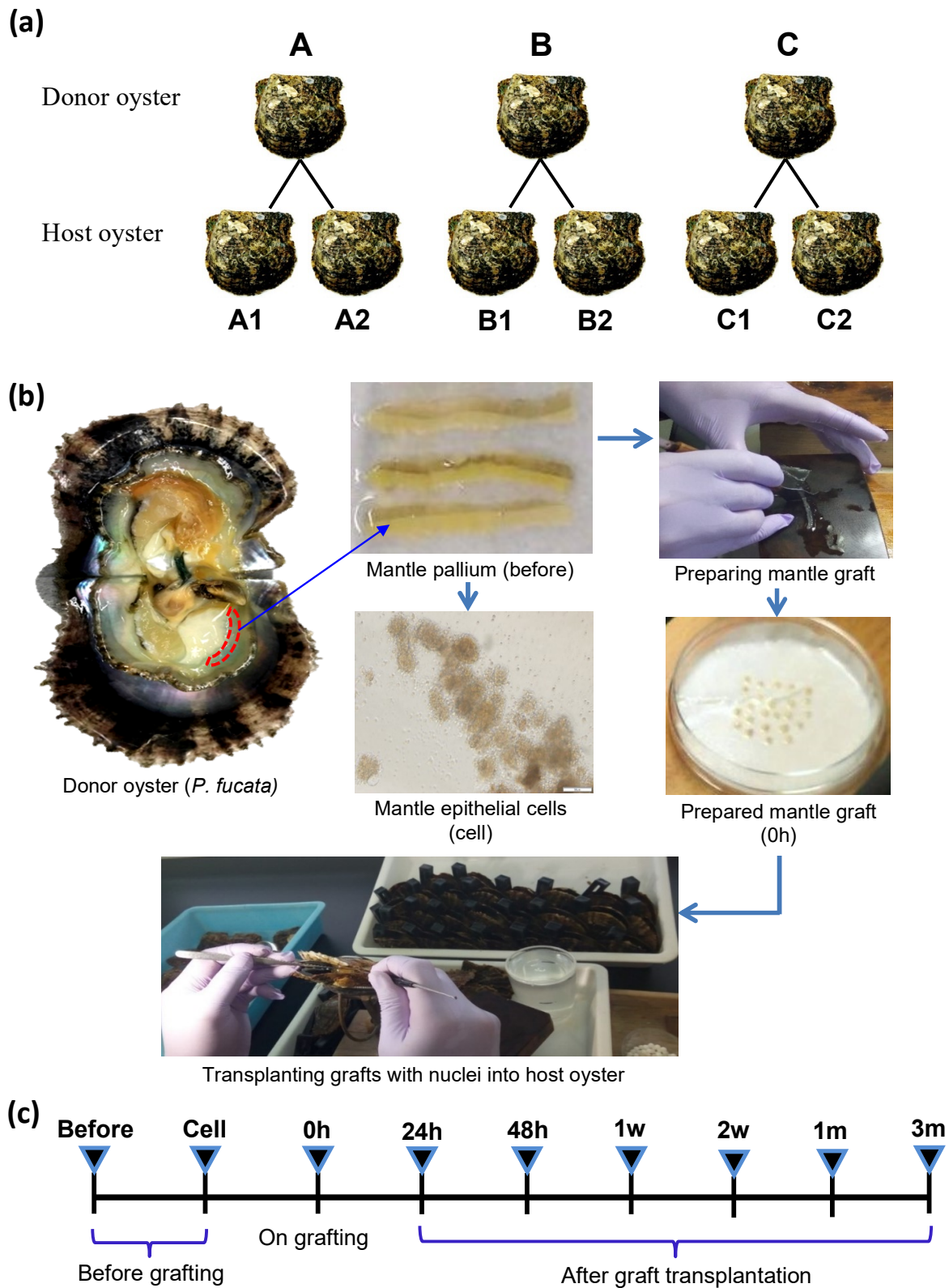


Figure S4. Experimental design for mantle grafting. (a) donor and host oysters used for grafting, (b) grafting process, and (c) sampling schedule. (b) Briefly, a pallial zone of mantle was excised from the pearl oysters and sterilized. The pallium strips were then used to prepare the grafts as well as to collect the epithelial cells, separately.

Additional references:

1. Kintsu H, Okumura T, Negishi L, Ifuku S, Kogure T, Sakuda S, et al. Crystal defects induced by chitin and chitinolytic enzymes in the prismatic layer of *Pinctada fucata*. Biochem Biophys Res Commun. 2017;489:89-95.
2. Wang Q , Yang C, Hao R, Zheng Z, Y Jiao, Du X , et al. Molecular characterization of CHST11 and its potential role in nacre formation in pearl oyster *Pinctada fucata martensii*. Electron J Biotechnol. 2017;28:113-119.