SUPPLEMENTARY MATERIAL

Co-expressed Cyclin D variants cooperate to regulate proliferation of germline nuclei in a syncytium.

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Table S1. Cyclin accession numbers

Gene	Accession number
Human Cyclin D1a	NP_444284
Mouse Cyclin D2	NP_033959
Mouse Cyclin D2SV	AK007904
O .dioica Cyclin Db α	CBZ41121
O .dioica Cyclin Db β	CBZ41122
O .dioica Cyclin Db Y	CBZ41123
O .dioica Cyclin Db δ	CBZ41124

The human Cyclin D1b protein sequence was obtained from Lu et al [1]

Table S2. Primer sequences for qPCR

Target Transcript	Primer Name	Primer Sequence (5' to 3')
Cyclin Da	CCQ73F	ACATGGAGCTGCTGATCCTGTTGA
	CCQ74R	ACATCAGCGTACGTAGAAGCGTGA
Cyclin Db	CCQ385F	AGAGATCAAACGCGCTTATTGGAG
	CCQ386R	CTGCGCTGGATCTTTGAGACTTTG
Cyclin Db γ/δ	CCQ77F	GTCTCAAAGATCCAGCGCAGGTAA
	CCQ78R	AAACAAGGCACGTGAGCGCATAAG
Cyclin Db α/γ	CCQ344F	ACATGCTGGAGGAGCTTCTTCCTT
	CC220R	CCTGGAAGAGTTGAACAGGACTG
Cyclin Dc	CCQ69F	TGACCACACAACTACAGCATCCGA
	CCQ70R	CTGCAGTAACAACAGCAGGCGAAA
Cyclin Dd	CCQ87F	TCCTGTCTATATCTGGCCGCGAAA
	CCQ88R	GGGCTTGGCAGAAGAGAAGAATGA
EF1β	CCQ43F	AGGTCATCCCTGAACTTAACGGCA
	CCQ44R	GGCAGATTTGATGGCAGCGTTGAT

Table S3. Primer sequences for cloning.

Construct	Primer	Primer Sequence (5' to 3')
Cyclin Dbβ ORF	Dbβ_OF	GATATCTAATACGACTCACTATAGGGAGAGCCACCATGGAAGCTACACACGAATTCA
	Dbβ_OR	CTCCAGATTACTGTAATGGCCCGAACATGGATCCGCTGCC
Cyclin Dbβ UTR	Dbβ_UF	TGTACAAGTAGAACGGCTTTTTATCCTGATTTTTAGAA
	Dbβ_UR	TTTTTGAAATAAATAAATAGAATACATTTTTTAAACGCCCGGG
CKIa ORF	CKIa_OF	GATATCTAATACGACTCACTATAGGGAGAGCCACCATGAAATCGGGCGGGAATTCG
	CKIa_OR	GGCAGCGGATCCATGGTGGCCGACGAGCGA
CKIa UTR	CKIa_UF	TGTACAAGTAGAACTTTTGGCAGCTCCATATTATATTGTACTGCTGTTCTAC
	CKIa_UR	TCGCATATGCGTTATGCGATTTTAACCCTTCCCGGG

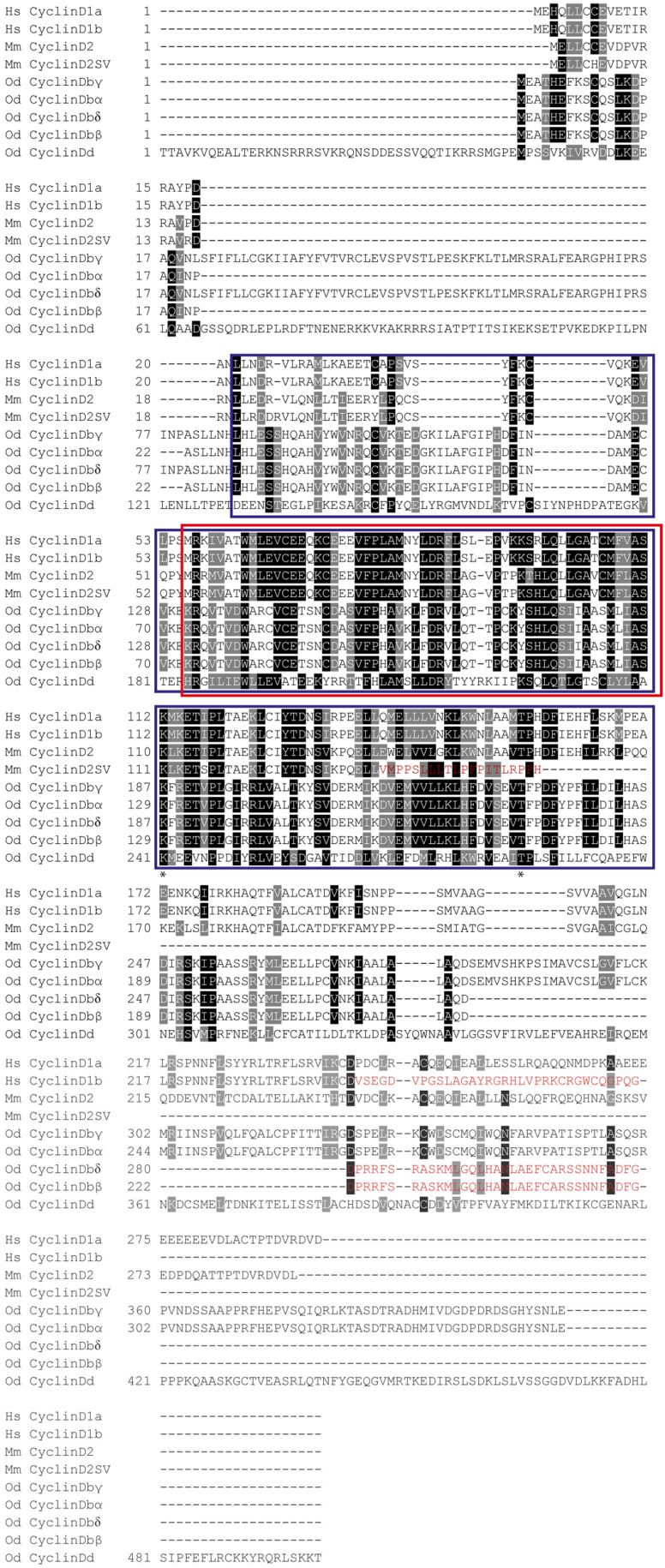
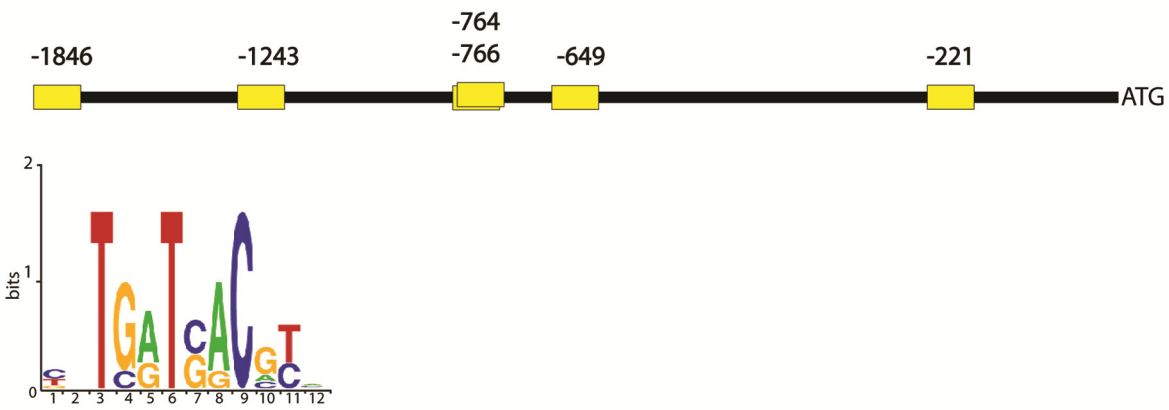


Figure S1. **Multiple sequence alignment of Cyclin Ds and their splice variants.** *O. dioica* Cyclin Dd, and Cyclin Db splice variants aligned with human and mouse Cyclin D splice variants using MUSCLE.[2] Dark shading indicates identical amino acids and grey shading represents similar amino acids. Altered C-terminal end amino acid sequences resulting from alternative splicing are indicated in red. CDK6 (red box) and CKI (p27/p21) interaction regions (blue box) are indicated.[3] Conserved residues for Cyclin Dependent Kinase Activator (CAK) binding on odCyclin D variants are marked with asterisks below the corresponding aligned sequences. Hs, Human; Mm, Mouse; Od, *Oikopleura dioica*.

A



B

CGTGATCACGTACGAGCTTTGGTTACACTTGTCCACTCGAGTTTTCCACTTTTTACACTTACACAC
GAAGAAAGACCAAATATTTTTTCGAAAACTGAAATTTAAATTAAATAAGATTCTTAAGCCTGAATC
ACGTTGAAATTGAGGCGAGAAAATATGGTGCAGCGCGCCACTGGAAGGGATTAACACCTCCATATT
CTGGGCAGTCAACTGGAAGAGGTATAATAGTTCAGAGAAATTTTCAAAATTTCTTAAAAATATTGA
CCTCAAATGACCTCAAATGACCCCGAGTGATCTATAGTGACCCGAGATGACCTTAGATTAAAAATC
AGTCCTCTCTGTCACGAAACAAAAGAAAAATGTGAAAATTATACCGAAAATACTCCTTATCAGTGA
ACTTCAAGAGCCCGTAACTTTTTATTAAGGGGGGTGGGGACCTTAACAACTTTGCATCAAAATGAT
CCCGAGAACCTCCTCTACAACTTTAGTATTGAAAATTAGGCTCTGAAGCGGCATGCCGGTCGATTT
TTTAACCGGTTTGTCAAAAGAGTCACGTTTTATGACCTTCCAGCGACCCAAAATATAAAAAAAAGT
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TAAAAAGGCCGAAAATAAAAGCCTAGAAAAAGCTCATTTATTCTCCTCTTTACCTATAAATTATGC
ATAAAATAAAAGTACTTACTGAGAAATTTACAAAAAATTTTAGACCGGTAAACGAGATACCAAATG
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TTTCTATCAAGAATCAACTTGATTGCAAGATCAATCGGCGCAAAAAAGCGCCAATTGTTGATTCTG
TTGAAAATAGGATGCTAAGTGTGAGTGTAAAACAAAAAACCCACTTCCACTTGCAAAATTTGGAGC
GCGCGCCGAGTGCGAGTGTAAATCAAAAATCCACTTCCACTTTCGCATGTGTAAGTGTAAATCGCA
TTTCCACACGAACTTTTGGAATTCTACGTGATCACGCAAGCATTCCACTTACACATGAAAATTTTT
CCACTTTACACTTGCAAAGTGGAGATTTTTTTCGAGTGCACTTTTCCCTCACCCCTGGTTCAAACT
CTATCAATCTCGTGACCTGAATTTGAGTTCGAAAATAAACTCAGGAAATGATTCTGCGCTAATTCG
CAAATCGAAAACCTTTTTCAATTTCTGTCAAAGATAAAATTCGAGTAAAAACTACGAAATTATTTT
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AACACTGTTTTGATTTTATGCGTGAAATTCAGCATTAAAAAAGCGCTGTTGTCTAAAGGTTCAGCT
AAGATGTTTCTCACCCTTTTTCTTTAGACAGGTTTCCGAGGCCAGGCCCTGTCCTGGTTTTCTGTT
CTTAGAAAAAACAACTGCTGAATTCAACAAGTCATGAAAATATGATGGCGCCATTTGTTAGGCACC
TTTGAACGGGATAAAATAAAATAAAGCTGCTTGAGTTAGCGCCTTGTTTCGCTTTTTTTTTGTCTT
TCGAAACTGCGAGCCCTAAAATGGACCCCGCTTATTGATTTTATTATCTTTGGCCTGGAAGTGCAA
ATAGTACTTTTTTAGCTCTCCAAATAAGGCGTGTTTGAAAAAAATATTAACAAAGTTTCTAGTCGC
TGCAAAAAAATG

Figure S2. CREB binding sites upstream of the *Oikopleura dioica cyclin Db* start site. The upstream sequence of *cyclin Db* was extracted from the *O. dioica* genome.[4] Predicted consensus *O. dioica* CREB binding sites in this region were identified using JASPAR (http://jaspar.genereg.net) and the site sequence logo (A) was created in web logo (http://weblogo.berkeley.edu/logo.cgi). These sites are indicated by yellow boxes in (A) and yellow highlighted sequences in (B).

References

1. Lu FM, Gladden AB, Diehl JA. An alternatively spliced cyclin D1 isoform, cyclin D1b, is a nuclear oncogene. Cancer Res 2003; 63:7056-61
2. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 2004; 32:1792-797.
3. Zwicker J, Brüsselbach S, Jooss KU, Sewing A, Behn M, Lucibello FC, et al. Functional domains in cyclin D1: pRb-kinase activity is not essential for transformation. Oncogene 1999; 18:19-25.
4. Denoeud F, Henriet S, Mungpakdee S, Aury JM, Da Silva C, Brinkmann H, et al. Plasticity of animal genome architecture unmasked by rapid evolution of a pelagic tunicate. Science 2010; 330:1381-385.