

Supplemental Materials

Molecular Biology of the Cell

Araki et al.

Figure S1

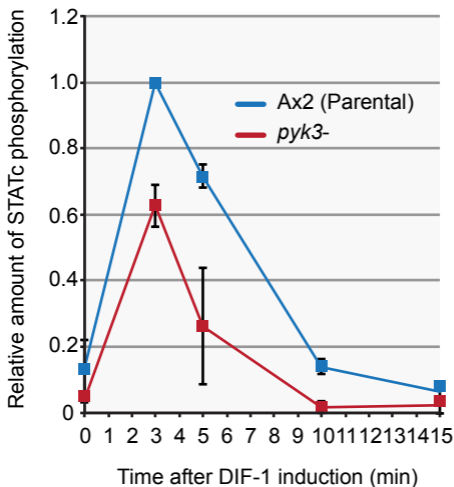
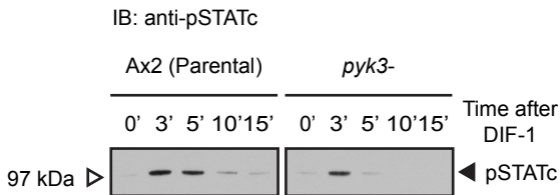
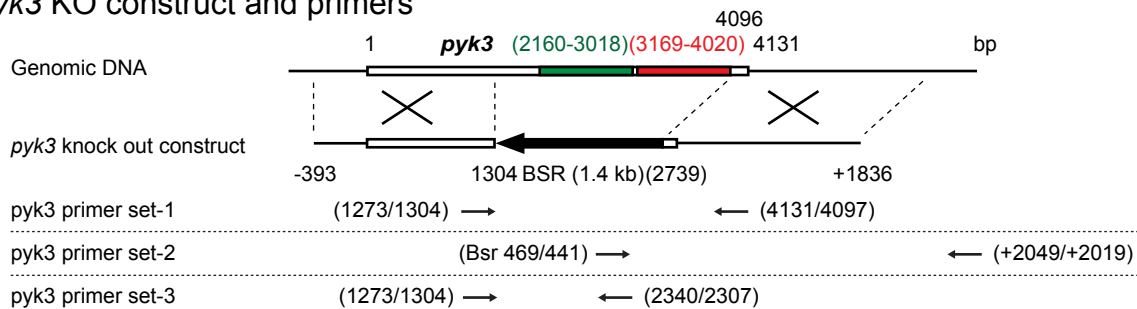
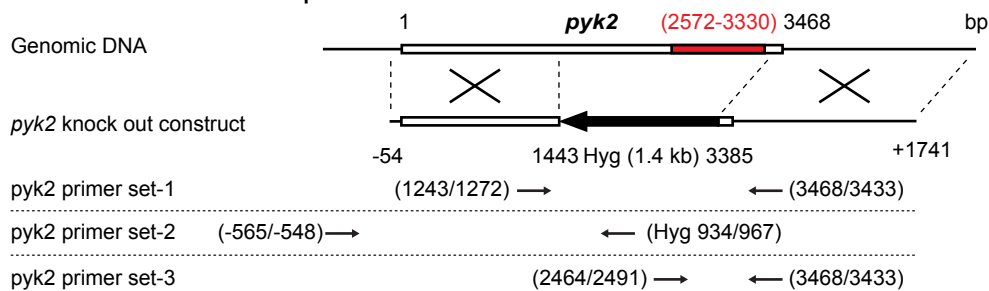


Figure S2

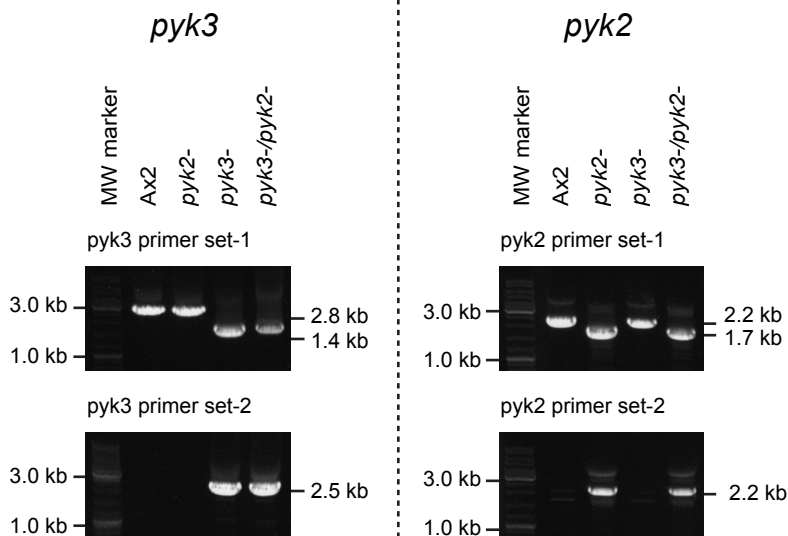
***pyk3* KO construct and primers**



***pyk2* KO construct and primers**



Genomic PCR



RT-PCR

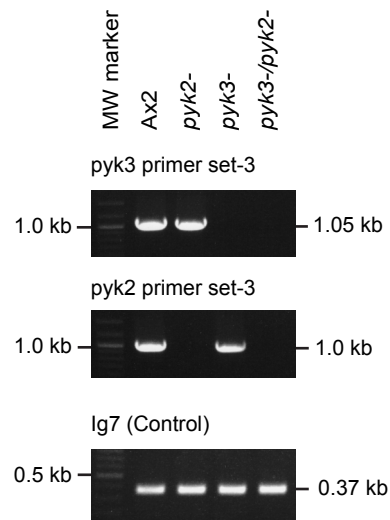
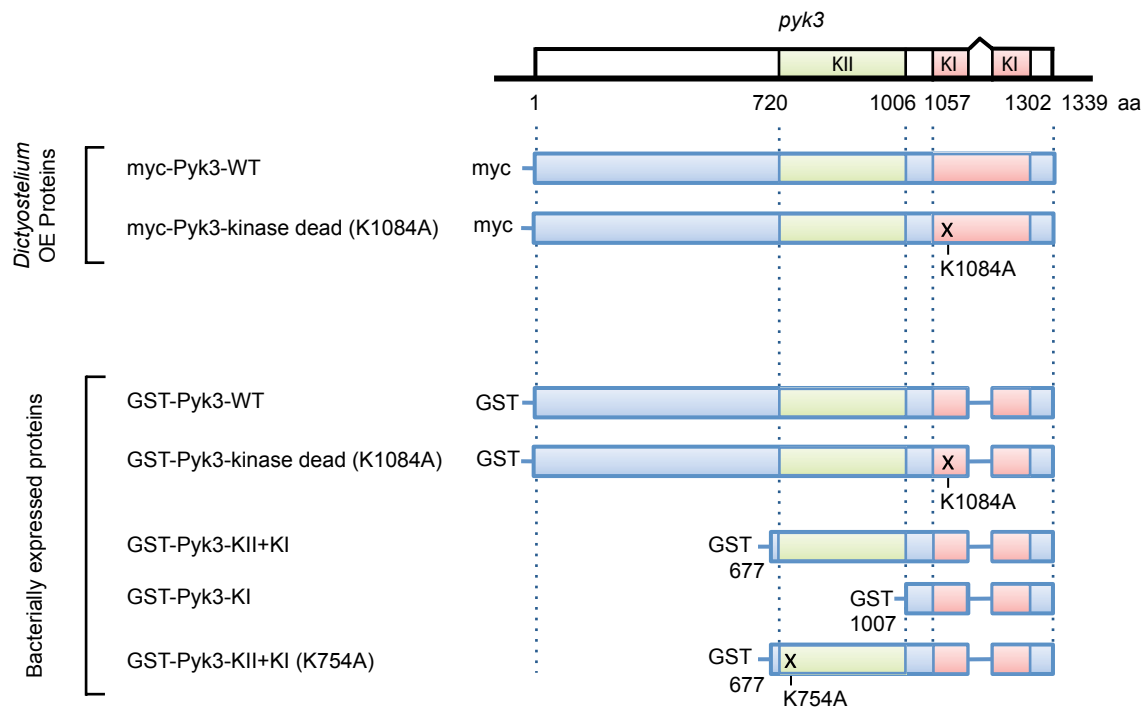


Figure S3



KII (pseudokinase domain): 720-1006 aa
KI (TKL domain): 1057-1302 aa

Figure S4B

Alignment of pseudokinase domains

Pyk3 KII Dd/720-1016 720 **PI**----EFK**EIKLL**EKLESNSK**S**SN**IQ**W**Q**I-----**Y**YK**ST** 749
 JAK2 JH2 Hs/539-816 539 **KI**----RN**EDL**IF**NES**L**G**Q**GT**FT--K**IF**K**G**V**RR**--E**VG**D--**Y**-----**G**OL**H**ET 576
 JAK1 JH2 Hs/578-856 578 **IL**----**KKDL**V**Q**GE**HL**GR**G**TR**T**--H**I**Y**S**T**LM**--D**Y**K**D**--D-----E**G**--T**S**E**KK**I 617
 JAK3 JH2 Hs/515-788 515 **KI**----**P**AD**SL**EW**H**EN**L**GHGS**F**T--K**I**Y**R**GC**R**H--E**V**VD-----**G**E**A**R**K**T 551
 Tyk2 JH2Hs/583-877 583 **RV**----D**Q**KE**IT**QL**S**HL**G**Q**G**TR**T**--N**V**Y**E**GR**LR**--V**E**GS**G**D**E**EE**G**K**M**DD**E**D**L**V**---**PG--R**D**R**G**Q**E**L 637
 JAK JH2 Ci/534-822 534 **VL**----K**ER**D**Y**TF**H**CH**L**GS**R**FT--D**V**N**L**H**V**HR--N**N**-----**P**D**L** 565
 Hopscotch JH2 Dm/548-846 548 **PK**N**L**K**A**K**K**T**D**L**Q**L**S**E**A**EL**Q**R**R**N**E**--Q**I**F**N**E**R**T**D**L**Q**W**Y****P**D--S---I**S**L**S**D**D**G**M**F**T**M**R**G**D**W**I**Q**S**E**V**K**D**V 610

ATP binding (K754 in Pyk3 KII)

*
 Pyk3 KII Dd/720-1016 750 **QL**V**L**K--**Q**PK**D**Q**S**DK**N**IE**K**R**K**Q**L**F**N**G**S**N**V**S**G**S**N**N**S**G**S**S**G**G**N**N**H**N**H**H-----**C**N**N**S**G**N**S**E**V**I**P**S 809
 JAK2 JH2 Hs/539-816 578 **EV**L**L**K--**V**L-----**D**K**A**H**R**N-----**Y**S**E**S**F**F--**E**--**A**A**S** 599
 JAK1 JH2 Hs/578-856 618 **KV**I**L**K--**V**L-----**D**P**S**H**R**D-----I**S**L**A**F**F**--**E**--**A**A**S** 640
 JAK3 JH2 Hs/515-788 552 **EV**L**L**K--**V**M-----**D**K**A**H**K**N-----**C**M**E**S**F**L--**E**--**A**A**S** 574
 Tyk2 JH2Hs/583-877 636 **RV**V**L**K--**V**L-----**D**P**S**H**D**-----I**A**L**A**F**Y**--**E**--**T**A**S** 660
 JAK JH2 Ci/534-822 566 **L**M**S**Q**V**L**K**Q**V**F-----**D**M**N**Q**R**H**D**L**G**Q**K**Q**V**I**D**E**S**F**Q**--**E**--**S**I**S** 596
 Hopscotch JH2 Dm/548-846 611 **S**V**T**M**K**--**M**L-----**K**S--**D**E**G**N-----**F**M--**E**F**F**--**R**--**L**A**Q** 631

Subdomain II

Pyk3 KII Dd/720-1016 810 **K**Y**T**M**I**Q**H**K**N**L**L**V**G**W**C**--**G**D--S**I**F**E**S**F**K**G**M**N**S**L**H**D**L**H**R**D**G**L**K**I**--**D**M**A**L**F**I**K**I**S**K**D**I**A**S**V**M**G**L**L**H 872
 JAK2 JH2 Hs/539-816 600 **M**S**K**L**S**H**K**H**L**V**L**N**Y**G**V**C**V**C**G**D**E**N**I**L**V**O**E**F**V**K**F**G**S**L**D**T**Y**L**K**K**N**K**N**C**I**--**N**I**L**W**K**L**E**V**A**K**Q**L**A**W**A**M**H**F**L**E 665
 JAK1 JH2 Hs/578-856 641 **M**M**R**Q**V**S**H**K**H**I**V**I**Y**L**G**V**C**V**R**D**V**E**N**I**M**V**E**E**F**V**E**G**G**P**L**D**L**F**M**H**R**K**S**D**V**L--**T**P**W**K**F**K**V**A**K**Q**L**A**S**A**S****S****Y**L**E** 706
 JAK3 JH2 Hs/515-788 575 **L**M**S**Q**V**S**Y**R**H**L**V**L**L**H**G**V**C**M**A**G**D**--S**T**M**V**O**E**F**V**H**L**G**A**I**D**M**Y**L**R**K**R**G**H**L**V**--**P**A**S**W**K**L**Q**V**V**Q**L**A**L**A**N****Y**L**E** 639
 Tyk2 JH2Hs/583-877 661 **L**M**S**Q**V**S**H**T**H**L**A**F**V**H**G**V**C**V**R**G**P**E**N**I**M**V**T**E**Y**V**E**H**G**L**D**V**W**L**R**R**E**R**G**H**V**--**P**M**A**W**K**M**V**V**A**Q**L**A**S**A**S****S****Y**L**E** 726
 JAK JH2 Ci/534-822 597 **L**L**I**C**L**K**N**V**H**I**V**K**Q**M**G**T**M**G--**T**--**Q**--I**G**L**E**V**A**P**F**R**S**T**I**N**Y**L**Q**L**A**G**N**E**O**G**P**W**S**W**F**L**Y**A**L**W**L**T**H**A**C**N**Y**L**E** 660
 Hopscotch JH2 Dm/548-846 632 **T**W**S**L**I**Q**S**P**Q**F**L**K**L**Y**G**L**T**L**A**D**P**--**Y**T**M**V**M**E**Y**S**R**Y**G**P**L**N**K**F**L**H**S**M**P**N--**V**--**T**L**H**C**L**L**D**L**M**H**G**L**V**R**G**M**H**Y**L**E 695

Pyk3 KII Dd/720-1016 873 **S**K**D**V**A**H**G**N**L**T**S**R**S**I**Y**L**D**R**F**Q-----**I**V**K**V**S**F**P**K**L**N**A**T**D**L**N**N**P**A**I**---**E**--**P**R**Y**M**A**P**E**M**T**R**M**E--**E**D 926
 JAK2 JH2 Hs/539-816 666 **E**N**T**L**I**R**H**G**N**V**C**A**K**N**I**L**L**I**R**E**E**D**R**K**T**G**N**P**P**F**I**K**L**S**D**P**G**I**S**I**T**V**L**P**K**D**I**L---**Q**E**R**I**P**W**V**P**P**E**C**I**E**--**N**--**P**K 728
 JAK1 JH2 Hs/578-856 707 **D**K**D**L**V**H**G**N**V**C**T**K**N**L**L**L**A**R**E**G**I**D--S**E**C**G**P**F**I**K**L**S**D**P**G**I**P**I**T**V**L**S**R**Q**E**C**--**I**E**R**I**P**W**I**A**P**E**C**V**E**--**D**--**S**K 768
 JAK3 JH2 Hs/515-788 640 **D**K**G**L**P**H**G**N**V**S**A**R**K**V**L**L**A**R**E**G**A**--**D**--**G**S**P**P**F**I**K**L**S**D**P**G**V**S**P**A**V**L**S**L**E**M**L**---**T**D**R**I**P**W**V**A**P**E**C**L**R**--**E**--**A**Q 700
 Tyk2 JH2Hs/583-877 727 **N**K**N**L**V**H**G**N**V**C**G**R**N**I**L**L**A**R**L**G**L**A--**E**G**T**S**P**F**I**K**L**S**D**P**G**V**G**L**G**A**L**S**R**E**R**---**V**E**R**I**P**W**L**A**P**E**C**L**E**--**G**G**A**N 789
 JAK JH2 Ci/534-822 661 **E**M**G**C**A**H**G**N**I**R**G**R**N**V**L**L**C**O**A**S-----**P**O**P**F**V**K**L**G**D**P**G**V**R**I--**C**L**G**R**N**R**V**S**E**P**V**L**P**A**P**W**L**A**P**E**F**C**R**I**D**--**S**E 721
 Hopscotch JH2 Dm/548-846 696 **D**N**K**I**I**H**N**Y**I**R**C**S**N**L**Y**V**T**K**Y**D**P**N--**S**Y**V**L**D**A**K**I**S**D**P**G**Y**P**R**-----**P**Y**R**--**E**S**D**S**P**W**I**P**V**K**Y**Y**R**--**N**--**L**Q 751

Subdomain VIb

Subdomain VII

Subdomain VIII

Pyk3 KII Dd/720-1016 927 --**Q**I**S**-----**C**S**I**D**V**Y**A**A**F**V**L**W**E**A**L**T**S**H**L**E**F**R**K**F**N**D**I**S**V**A**A**K**V**--**A**Y**E**-----**N**L**R**P**K**I**P**T**S**--- 975
 JAK2 JH2 Hs/539-816 729 --**N**L**N**-----**L**A**D**D**K**W**S**F**G**T**T**L**W**E**I**C**S**G**G**D--**K**P**L**S**A**L**D**S**Q**R**K**L**Q**F**Y**-----**E**D**R**H**Q**L**P**A**P**--- 775
 JAK1 JH2 Hs/578-856 769 --**N**L**S**-----**V**A**A**D**K**W**S**F**G**T**T**L**W**E**I**C**Y**N**G**E--**I**P**L**K**D**K**T**L**I**E**K**E--**R**F**Y**-----**E**S**R**C**R**P**V**T**P**--- 815
 JAK3 JH2 Hs/515-788 701 --**T**L**S**-----**L**E**A**D**K**W**G**F**G**A**T**V**V**E**V**S**G**V**T**--**M**P**I**S**A**L**D**P**A**K**K**L**Q**F**Y**-----**E**D**R**Q**Q**L**P**A**P**--- 747
 Tyk2 JH2Hs/583-877 790 --**S**L**S**-----**T**A**M**D**K**W**G**F**G**A**T**L**L**E**I**C**F**D**G**E--**A**P**L**Q**R**S**P**S**E**K**E**--**H**F**Y**-----**Q**R**O**H**R**L**P**E**P**--- 836
 JAK JH2 Ci/534-822 722 --**P**L**S**F**P**T**I**D**G**D**K**W**S****V**A**T**T**V**E**I**C**W**G**V**S**P**E**P**F**N**A**Y**S**L**S**A**E**M**K**E**K**Y**-----**R**T**A**S**V**I**P**I**P**E**I**I 778
 Hopscotch JH2 Dm/548-846 752 **A**K**T**D---**Q**F**A**Q**L**W**A**F**A**T**T**I**V**E**I**F**S**R**C**K--**E**D**L**S**T**L**R**Q**E**Q**L**L--**R**Q**K**N**L**D**G**N**I**L**K**M**L**D**Q**D**I**C**A**P--- 809

Pyk3 KII Dd/720-1016 976 ---**C**P**L**I**R**K**L**I**N**R**C**W**A**L**P**S**D**R**P**T**F**N**D**I**L**K**L**F**D**H**L**E**G**L**K**L**F**S**S** 1016
 JAK2 JH2 Hs/539-816 776 ---**K**W**A**E**L**A**N**L**I**N**C**M**D**Y**E**P**D**F**R**P**S**F**R**A**I**R**D**L**N**S**L**F**T**P**D**Y**E**L**L** 816
 JAK1 JH2 Hs/578-856 816 ---**S**C**K**E**L**A**D**L**M**T**R**C**M**N**Y**D**P**N**Q**R**P**F**F**R**A**I**M**R**D**I**N**K**L**E**E**Q**N**P**D**I**V** 856
 JAK3 JH2 Hs/515-788 748 ---**K**W**E**L**L**A**L**L**I**Q**C**A**M**A**E**Y**P**V**Q**R**P**S**F**R**A**V**I**R**D**L**N**S**L**I**S**S**D**Y**E**L**L** 788
 Tyk2 JH2Hs/583-877 837 ---**S**C**P**Q**L**A**T**L**T**S**Q**C**L**T**Y**E**P**T**Q**R**P**S**F**R**T**I**L**R**D**L**T**R**L**Q**P**H**N**L**A**D**V** 877
 JAK JH2 Ci/534-822 779 **A**A**N**G**V**G**E**L**G**A**L**L**R**Q**C**W**N**E**Y**P**P**K**R**P**P**F**K**Q**I**L**R**E**L**G**T**V**L**S**S**D**V**I**L**P 822
 Hopscotch JH2 Dm/548-846 810 -----**I**F**E**T**I**M**D**G**W**S**D**E**T**K**R**F**S**H**H**D**I**F**S**R**L**N**I**T**K**A**E**I**L**P**N**Y 846

Supplementary Materials: Figure Legends and Table

Figure S1. Activation of STATc by DIF-1. Parental Ax2 cells and *pyk3*- cells (in Ax2 background) developed in shaking culture for four hours were treated with DIF-1 (Differentiation Inducing Factor-1, Enzo Life Sciences (UK) Ltd., Exeter, UK) at 100 nM for the indicated times. Tyrosine phosphorylation of STATc was assayed by Western transfer using phospho-STATc antibody CP22. The blot was re-probed with total STATc antibody (data not shown). Lower panel shows normalized data from three independent experiments, \pm s.d..

Figure S2. Construction of single and double knock out strains. To generate a *pyk3* null mutant, internal region (1304-4096 bp) containing two kinase domains, pseudokinase KII (2160-3018 bp) and active TKL KI (3169-4020 bp), was replaced with a blasticidin resistant cassette (BSR: 1.4 kbp length with reverse orientation). Sequences from -393 (393 bp upstream of the *pyk3* gene) to +1836 were used for the homologous recombination. After transformation, genomic DNA was extracted with Phenol/Chloroform and analysed with two sets of primers and a TITANIUM Taq PCR Kit (Clontech Laboratories, Inc., Mountain View CA, USA), to check the gene replacement. Total RNA was extracted from four hours starved cells in shaking culture with RNeasy Mini Kit and on-column DNase digestion (QIAGEN GmbH, Hilden, Germany). Expression of *pyk3* was analysed with Primer Set-3 and Superscript III One-Step RT-PCR System with Platinum Taq DNA Polymerase (Life Technologies, Ltd, Paisley, UK). For an RT-PCR control, the constitutively expressed RNA, *Ig7* (625-652 bp for forward primer; 992-966 bp for reverse primer), was used. *pyk3/pyk2* double null (*pyk3*-/*pyk2*-) was created by knocking out *pyk3* gene in *pyk2* null strain (Araki *et al.*, 2012). The knock out of *pyk2* gene is also confirmed by genomic PCR and RT-PCR as described previously (Araki *et al.*, 2012).

Figure S3. Schematic representation of Pyk3 constructs for expression in *Dictyostelium* cells and for bacterial expression and purification.

Figure S4. Amino acid sequence alignments of C terminal active kinase domains of *Dictyostelium* Pyk2, Pyk3 and metazoan JAK family kinases (A) and of pseudokinase domains of Pyk3 and JAKs (B). TOPALi software v2.5 with the Tcoffee algorithm (The James Hutton Institute, UK; <http://www.topali.org>) was used for the alignment of the two kinase domains. Major kinase subdomains (Hanks and Hunter, 1995) and ATP binding site are also indicated. The following is the list of amino acid sequence information used for the alignment:

Pyk2 <i>D. discoideum</i> :	NCBI Reference Sequence: XP_638253.1
Pyk3 <i>D. discoideum</i> :	NCBI Reference Sequence: XP_636435.1
JAK2 Human:	NCBI Reference Sequence: NP_004963.1
JAK1 Human:	NCBI Reference Sequence: NP_002218.2
JAK3 Human:	GenBank Accession Number: AAC50950.1
Tyk2 Human:	NCBI Reference Sequence: NP_003322.3
Janus kinase <i>C. intestinalis</i> :	NCBI Reference Sequence: NP_001071749.1
Hopscotch <i>D. melanogaster</i> :	NCBI Reference Sequence: NP_511119.2

Table S1 Primer pairs used for quantitative real-time PCR analysis. Oligonucleotide primers were designed on the basis of sequence information and purchased from Metabion Corp. (Munich, Germany).

dictyBase ID	Gene Name	Sequence
DDB_G0293532	<i>dstC</i>	Forward: 5' -CAATTACTTTGTGGCACTCG-3' Reverse: 5' -CCAAATTTGAGGGTTACTGG-3'
DDB_G0282145	<i>ptpC</i>	Forward: 5' -ACCGATATGGGTATTCGTTC-3' Reverse: 5' -TGTTGTGGTGGGAATTTTAG-3'
DDB_G0285321	<i>pyk2</i>	Forward: 5' -CTGTACACACTACTAGTGGTGG-3' Reverse: 5' -CTACCTCTAGGTCTCATATGAG-3'
DDB_G0289001	<i>pyk3</i>	Forward: 5' -TTTTATGGGTGCCTGTATTG-3' Reverse: 5' -TCATCGCTTAAAGTTGTTGC-3'
DDB_G0271916	<i>rtoA</i>	Forward: 5' -GGATCCTCATCTGATGG-3' Reverse: 5' -GAACCAGTGCATCTACTAATATAGCC-3'
DDB_G0290071	<i>sigG</i>	Forward: 5' -CTACCCCTCAAGCGATGCC-3' Reverse: 5' -CTTGATTCTCTTGCTGCATC-3'
DDB_G0277667	NA	Forward: 5' -CGTATACTGGTATTACATTAAG-3' Reverse: 5' -CGAAGCCTTTGAATGATCTTCC-3'

Supplementary Materials: Reference

Hanks SK and Hunter T (1996). Protein kinases 6. The eukaryotic protein kinase superfamily: kinase (catalytic) domain structure and classification. *FASEB J.* **9**, 576-96.