

**One Tissue, Two Fates: Different Roles of Megagametophyte Cells  
during Scots Pine Embryogenesis**

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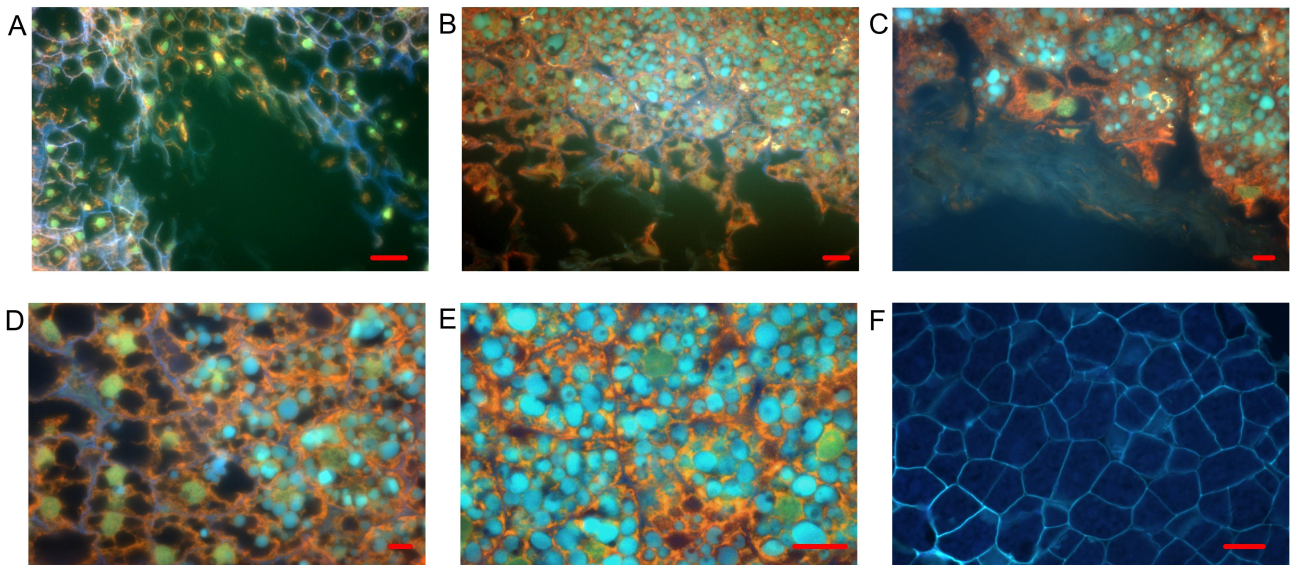
**Supplementary Data**

**Supplemental Table S1.** PCR primers for the amplification of the cDNA fragments of the putative Scots pine *RAD51*, *KU80*, DNA ligase (*LIG*), metacaspase (*MCA*) and Tat-D nuclease (*TAT-D*).

<b>Gene</b>	<b>Sequence of the upstream PCR primer (5'→3')</b>	<b>Sequence of the downstream PCR primer (5'→3')</b>	<b>PCR product size</b>
<i>RAD51</i>	CCAGCCAGCTACATGCTCAA	GTCACCACAACCTGCCACACC	532
<i>KU80</i>	TCAAATGGCTCGAAATAAGGA	GCTCCAGGTCACCTCTGAAT	677
<i>LIG</i>	AACATATGAGCCGGCAAAGC	TTGGGCCCCGATACATTTCTG	508
<i>MCA</i>	CAATGACTAGGAAGGCAGTGC	TGGATGTTCTAATCACATATAAATGG	1267
<i>TAT-D</i>	CTGGTGACTGGTGGATCTCT	TTTAAACATTGCAGTCATCTCCA	819

**Supplemental Table S2.** PCR primers for the real-time PCR amplification of the putative Scots pine *RAD51*, *KU80*, DNA ligase (*LIG*), metacaspase (*MCA*), Tat-D nuclease (*TAT-D*), actin (*ACT*), ubiquitin (*UBI*) and glyceraldehyde-3- phosphate dehydrogenase (*GAPDH*) genes.

<b>Gene</b>	<b>Sequence of the upstream primer (5'→3')</b>	<b>Sequence of the downstream primer (5'→3')</b>	<b>PCR product size</b>
<i>RAD51</i>	TATGGGGAATTTCTGAACAGG	GTTCCCTCGGCATCAATAAA	119
<i>KU80</i>	GAATGGCTCCAGGTGATTTT	AGGCGTTTATTTCCCTTGCT	94
<i>LIG</i>	GCATTAGCCCTGTTTCATCGT	CGCTTGGTCTGGATTCTTGT	113
<i>MCA</i>	CGGGAGAGGACATGCTAAAA	CTGGCCTAATTTTCCCAACA	107
<i>TAT-D</i>	TGGATGTTTCCTTAAAGACAGTGG	TCTCACAGTATGGAGCGTCTG	95
<i>ACT</i>	GGACAGGTCATTACCGTTGG	GATACCCGCTGCTTCCATT	90
<i>UBI</i>	GAAGGAGCAGTGGAGTCCTG	CAATTTTCAGGGACGAGAGGA	104
<i>GAPDH</i>	CTGGTGTCTTCACCCGACAAA	GGTGCTCATTAAACCCCAACA	120



**Supplemental Figure S1.** Acridine orange stained Scots pine megagametophyte tissue during embryogenesis. During all the developmental stages, the megagametophyte cells in ESR broke down with the release of cell debris and nucleic acids into the corrosion cavity, whereas the inner part of the megagametophyte tissue stayed alive with no symptoms of PCD or necrosis. (A) ESR at the developmental stage of early embryogeny. (B) and (C) ESR during the late embryogeny. (D) and (E) Inner part of the megagametophyte tissue at the developmental stage of early and late embryogeny, respectively. (F) Negative control with no acridine orange staining. Bars = 10  $\mu\text{m}$  (C, D), 20  $\mu\text{m}$  (E), 40  $\mu\text{m}$  (F) and 50  $\mu\text{m}$  (A).

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          10          20          30          40          50          60
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
P. sylvestris -----
O. sativa      MSTSA AAAA- AAEQQQEE ---GEHGPFPIEQ LQASGIAALDVKKLKD SGLYTVESVAYT
L. esculentum --MEQQHRNQKSMQDQND EIEDVQHGFPPVEQLQASGIAALDVKKLKDAGLCTVESV VYA
Z. mays        MSSAAQQQQKAAA AEQEE ---VEHGPFPIEQ LQASGIAALDVKKLKD SGLHTVEAVAYT
P. nigra       --MEQQRNQKAVNQQHEDHEEVQHGFPPVEQLQASGIA SLDVKKLKDAGLCTVESVAFS
A. thaliana    MTTMEQRRNQNAVQQDD --EETQHGFPPVEQLQAAGIASVDVKKLRDAGLCTVEGVAYT
Clustal Consensus
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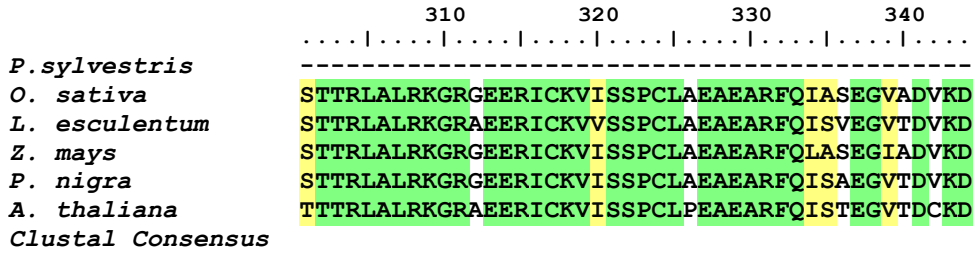
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          70          80          90          100         110         120
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
P. sylvestris -----SQLHAQRLEIIQISSGSRELDKLLLEG
O. sativa      PRKDLLQIKGISEAKVDKIVEAASKLVPLGFTSASQLHAQRLEIIQVTTGSRELDKILDG
L. esculentum  PRKELLQIKGISEAKVDKIEAASKLVPLGFTSASQLHAQRLEIIQITSGSKELDKILEG
Z. mays        PRKDLLQIKGISEAKADKIEAASKIVPLGFTSASQLHAQRLEIIQVTTGSRELDKILEG
P. nigra       PRKELLQIKGISEAKVDKIEAASKLVPLGFTSASQLHAQRQEI IQITSGSRELDKILEG
A. thaliana    PRKDLLQIKGISDAKVDKIVEAASKLVPLGFTSASQLHAQRQEI IQITSGSRELDKIVLEG
Clustal Consensus ***** *****: :*:*****: :*
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          130         140         150         160         170         180
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
P. sylvestris  GVETGSITEIYGEFRTGKTQLCHTLCVTCQLPLDQGGGEGKALFIDAEGTFRPQRLLQIA
O. sativa      GIETGSITEIYGEFRSGKTQLCHTLCVTCQLPLDQGGGEGKALYIDAEGTFRPQRLLQIA
L. esculentum  GIETGSITEIYGEFRSGKTQLCHTLCVTCQLPLDQGGGEGKAMYIDAEGTFRPQRLLQIA
Z. mays        GIETGSITEIYGEFRSGKTQLCHTLCVTCQLPLDQGGGEGKALYIDAEGTFRPQRLLQIA
P. nigra       GVETGSITEMYGEFRSGKTQLCHTLCVTCQLPLDQGGGEGKAMYIDAEGTFRPQRLLQIA
A. thaliana    GIETGSITELYGEFRSGKTQLCHTLCVTCQLPMDQGGGEGKAMYIDAEGTFRPQRLLQIA
Clustal Consensus *:*****: ***** *****: *****: :*****:*****
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          190         200         210         220         230         240
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
P. sylvestris  ERFGLNGADVLENVAYARAYNTDHSRLLLEAASMMIETRFALMIVDSATS LYRTDFIGR
O. sativa      DRFGLNGADVLENVAYARAYNTDHSRLLLEAASMMIETRFALMIVDSATALYRTDFSGR
L. esculentum  DRYGLNGPDVLENVAYARAYNTDHSRLLLEAASMMVETRFALMIVDSATALYRTDFSGR
Z. mays        DRFGLNGADVLENVAYARAYNTDHSRLLLEAASMMIETRFALMVVDSATALYRTDFSGR
P. nigra       DRFGLNGADVLENVAYARAYNTDHSRLLLEAASMMVETRFALMIVDSATALYRTDFSGR
A. thaliana    DRFGLNGADVLENVAYARAYNTDHSRLLLEAASMMIETRFALLIVDSATALYRTDFSGR
Clustal Consensus *:***: *****:***** *****: :*****:***** *
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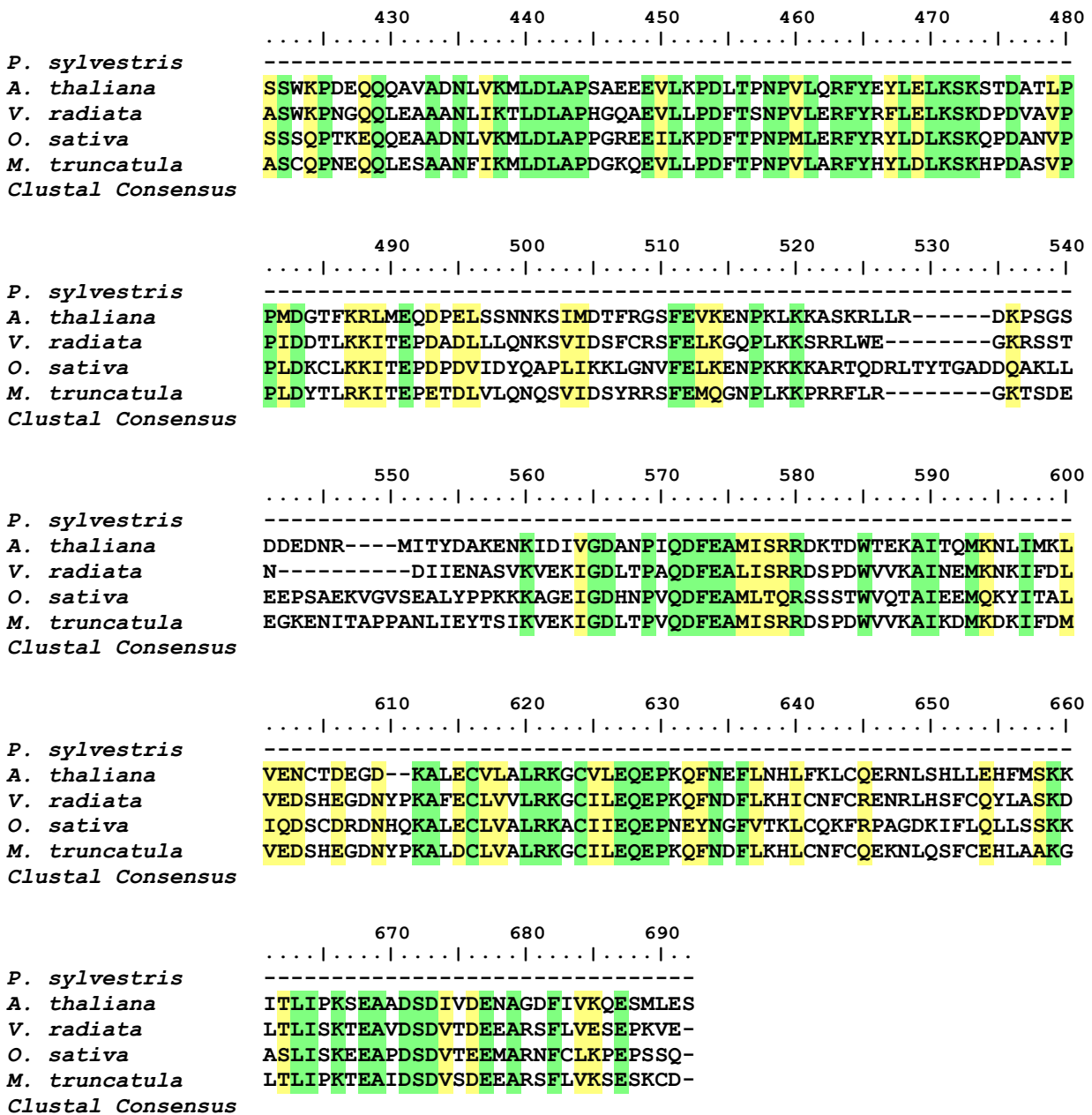
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          250         260         270         280         290         300
.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|.....|
P. sylvestris  GELSARQMHLAKFLRS LQKLADEFGVAVVV-----
O. sativa      GELSARQMHLAKFLRS LQKLADEFGVAVVITNQVVAQVDGSAMFAGPQIKPIG GNIMAHA
L. esculentum  GELSARQMHLAKFLRS LQKLADEFGVAVVITNQVVAQVDGSAMFAGPQIKPIG GNIMAHA
Z. mays        GELSARQMHLAKFLRS LQKLADEFGVAVVITNQVVAQVDGSAMFAGPQFKPIG GNIMAHA
P. nigra       GELSARQMHLAKFLRS LQKLADEFGVAVVITNQVVAQVDGSAIFAGPQIKPIG GNIMAHA
A. thaliana    GELSARQMHLAKFLRS LQKLADEFGVAVVITNQVVAQVDGSAIFAGPQFKPIG GNIMAHA
Clustal Consensus *****:*****:*****:
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Rad51



**Supplemental Figure S2.** Alignment of the predicted partial amino acid sequence of the putative *Pinus sylvestris* Rad51 with *Oryza sativa* Rad51 (BAB85491), *Lycopersicon esculentum* Rad51 homolog (Q40134), *Zea mays* Rad51 homolog (Q67EU8) *Populus nigra* Rad51 homolog (BAF02935) and *Arabidopsis thaliana* Rad51 (NP\_568402). Identical and similar amino acids are highlighted in green and yellow, respectively, using 70 % threshold. The amino acids which are totally conserved in the Rad51 proteins are shown with asterisks.





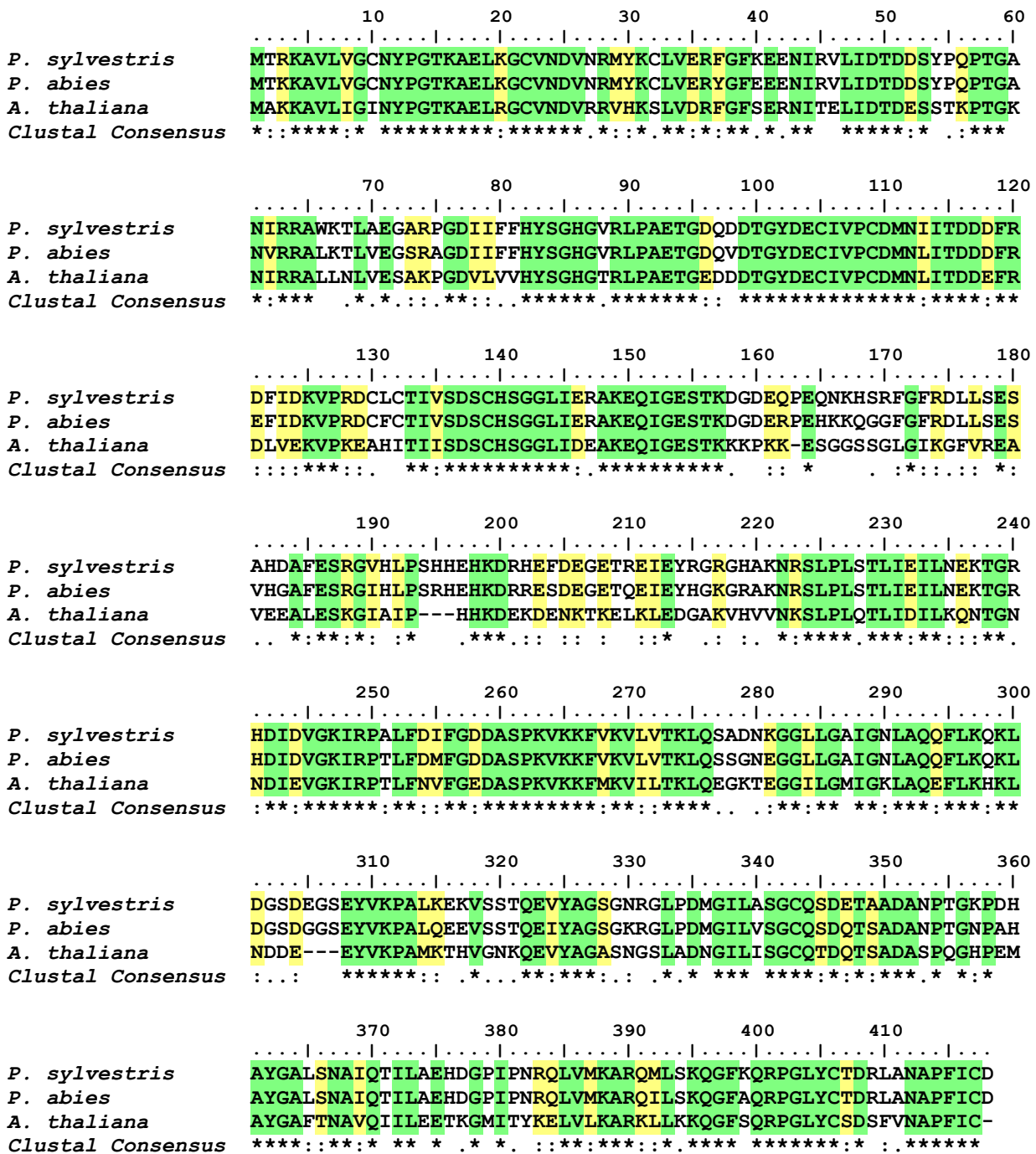
**Supplemental Figure S3.** Alignment of the predicted partial amino acid sequence of the putative *Pinus sylvestris* Ku80 protein with *Arabidopsis thaliana* Ku80 homolog (NP\_564520), *Vigna radiata* Ku80-like protein (AAX43993), *Oryza sativa* putative H-box binding protein, KAP2 (AAS01977) and *Medicago truncatula* KAP2-protein (AAL87543). Identical and similar amino acids are highlighted in green and yellow, respectively, using 70 % threshold. The amino acids which are totally conserved between the Ku80 and KAP2 proteins are shown with asterisks.







Metacaspase



**Supplemental Figure S5.** Alignment of the predicted amino acid sequence of the putative *Pinus sylvestris* metacaspase with the metacaspase type II sequences of *Picea abies* (CAD59226) and *Arabidopsis thaliana* (NP\_178051). Identical and similar amino acids are highlighted in green and yellow, respectively, using 70 % threshold. The amino acids which are totally conserved in the type II metacaspase proteins are shown with asterisks.

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          10          20          30          40          50          60
...|...|...|...|...|...|...|...|...|...|...|...|
P. sylvestris -----TGGSLKESK
P. sitchensis MAK--LKLIDIAANLTDGMFKGIYNGKQYHVSDIAEVLKRAWEAGVERIIVTGGSLKESK
A. thaliana MAS--LKMIDIAVNFETDGMFKGLYHGKNCHVDPDIATVLNRAWSAGVDRIIVTGGSLKESR
O. sativa MASTGVKLIIDIAVNFETDGMFRGIYHGKQCHAADIPAVLARAWAAGVDRIIVTGGSLKESR
H. sapiens MSR--FKFIDIGINLTDPMFRGIYRGVQKHQDDLQDVIGRAVEIGVKKFMITGGNLQDSK
M. musculus MSL--FKFVDIGINLTDPMFRGIYRGVQKHQDDLQDVIERAIQIGVKKFMITGGSLQDSK
C. elegans --MALYELVDIGANLGHPSYQ-----KDLNDVLDRAKQAGLSKIMVTGTSEKISH
E. coli ---MEYRMFDIGVNLTSQFA-----KDRDDVVARAFDAGVNGLLITGTNLRESQ
Clustal Consensus ** . . * :

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          70          80          90          100         110         120
...|...|...|...|...|...|...|...|...|...|...|...|
P. sylvestris EALAI AETDG-RLE FCTVGVHPTRCKE FEESGNPEHYFQELVSLAKEGVERGKVV AIGECG
P. sitchensis EALAI AETDG-RLE FCTVGVHPTRCKE FEESGNPEHYFQELVSLAKEGVOR GKVVAIGECG
A. thaliana EALAI AETDG-RLE FCTVGVHPTRCNE FEESGDPEKHYQALFSLAKEGMQKGKVV AIGECG
O. sativa EALEI AETDG-RLE FCTVGVHPTRCGE FEESGDPEGHFQALLALAKEGIAKGVVA VAVGECG
H. sapiens DALHLAQTNG-MFFSTV GCHPTRCGE FEK-NNPDLYLKE LLNLAEN--NKGKVV AIGECG
M. musculus DALQLAQTND-MFFSTV GCHPTRCDE FEK-GSPDQYLAGLLSLAEN--NKGKVV AIGECG
C. elegans ECADLVEKYPGFLYFTAGVHPHDAKDWND-----GTLEALKKLOEN----PSCVAVGECG
E. coli QAQKLARQYS-SCWSTAGVHPHDS SQWA-----VTEEA IIE LA AQ----PEVVAIGECG
Clustal Consensus :. :. : * * * * . : : : : * : : : * * : * * * *

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          130         140         150         160         170         180
...|...|...|...|...|...|...|...|...|...|...|...|
P. sylvestris LDYDRLQFCPADVQRQYFEKQFELADALRLPMFLHMRAAGKDFCDIIEQNRHRFISGVAH
P. sitchensis LDYDRLQFCPADVQRQYFEKQFELADALRLPMFLHMRAAGKDFCDIIEQNRHRFISGVAH
A. thaliana LDYDRLQFCSDVIQKQYFEKQFELAYATKLPMLFHMRAAAEDFCEIVERNKNRFTGGVAH
O. sativa LDYDRLHFCSVDVQKQYFKKQFELAEAVKLPMLFHMRAAGEDFCEIVSENLYRFPGGVTH
H. sapiens LDFDRLQFCPKDTQLKYFEKQFELSEQTCLPMFLHCRNSHAEFLDIMKRRNRDRCVGGVVH
M. musculus LDFDRLQFCPKDTQLKYFEKQFELSEQTCLPMFLHCRNSHTEFLDIMRRNRDRYVGGVVH
C. elegans LDFNR-NFSPQDVQKEVFAKQVDMAVKLQKPLFIHEREAHEDMVKILTAAGPSLPPAVIH
E. coli LDFNR-NFSTPEEQELAFVAQLRIAAELNMPVFMHCRDAHERFMTLLEPWLDKLPGAVLH
Clustal Consensus * * : * . : * * * . : : . * : * * * : : : : * *

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          190         200         210         220         230         240
...|...|...|...|...|...|...|...|...|...|...|...|
P. sylvestris SFTGCAEDRDQLLKFENLFIGINGCSLKTV----ENLQVLAGIPLERMMIETDAPYCEI
P. sitchensis SFTGCAEDRDQLLKFENLFIGINGCSLKTV----ENLQVLAGIPLERMMIETDAPYCEI
A. thaliana SFTGSASDRDKLLSFDKMYLGVNGCSLKTA----ENLEV MKGIPVERMMIETDSPYCDI
O. sativa SFTGTAEDRDKLLSFEKMFINGINGCSLKTS----ENLEVLQGI PAERMMIETDSPYCDI
H. sapiens SFDGTKEAAAALIDL D-LYIGFNGCSLKTE----ANLEVLKSIPSEKLM IETDAPWCGV
M. musculus SFDGTKEAAAALVDLG-LYIGFNGCSLKTE----ANLEVLKSIPSEKLM IETDAPWCGV
C. elegans CFTGTVEAKKYLEMG-FYIGLTGFLWKDRSDNGVQAGLRSGEIP IEKLVLET DAPYMYP
E. coli CFTGTREEMQACVARG-IYIGITGWVCDER--RGLLELRELLPLI PAEKLLIETDAPYLLP
Clustal Consensus . * * : : * * * . : : * * : * * : * * : * * : * *

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          250         260         270         280         290         300
...|...|...|...|...|...|...|...|...|...|...|...|
P. sylvestris RSTHAGAHYIKSVWSSKKKKEKFDPGCTVKNRNEPCFVRQVLEVVAGQRGIEDVGS LAKTL
P. sitchensis RSTHAGARYIKSMWSSKKKKEKYDPGCTVKNRNEPCFVRQVLEVVAGQRGIEDVGI LAKTL
A. thaliana KNTHAGIKFVKSTWPSKKKKEKYDQESLVKGRNEPCLVRQVLEVVAGYKGLGDLN QVSSTL
O. sativa KNTHAGIKFVKSVWPSKKKKEKYPDSTVKGRNEPCLVRQVLEVVAGCKGIADIEGL SKTL
H. sapiens KSTHAGSKYIRTAFTPKKK--WESGHCLKDRNEPCHIIQILEIMSAVR-DEDPLE LANTL
M. musculus KSTHAGSKYINTSFPTKKK--WENGHCLKDRNEPCHIIQILEIMSAVR-EEDPLE LANTL
C. elegans KINDKKIPKEIKSLITPETEALHNF-SSFNRNEPCSLAAVCELVAFA-GRDPKEVAKIT
E. coli RD-----LTPK-----PSSRRNEPAHLPHILQRIAHWR-GEDAAWLAATT
Clustal Consensus : : : * * . : : : : * : :

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Tat-D

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                                310      320      330
                                .....|.....|.....|.....|.....|
P. sylvestris      YANTCRVFFPHDLDTMAGVVLGDDCNI---
P. sitchensis     YANTCRVFFPHDLDTMAGVVLGDDCNI---
A. thaliana       YHNTCRVFFPQDLDSAADALLS-GHETD-
O. sativa         YHNTCRLFFPQDLDSADAQLESGAFIQNS
H. sapiens        YNNTIKVFFPGI-----
M. musculus       YNNTIKVFFS-----
C. elegans        TENAKKVYKLE-----
E. coli           DANVKTLEFGIAF-----
Clustal Consensus  *.  ::
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**Supplemental Figure S6.** Alignment of the predicted partial amino acid sequence of the putative *Pinus sylvestris* Tat-D with *Picea sitchensis* unknown protein (ABK24610), *Arabidopsis thaliana* Tat-D-related deoxyribonuclease family protein (NP\_190807), *Oryza sativa* Tat-D like protein (NP\_001058926), *Homo sapiens* Tat-D like protein (NP\_114415), *Mus musculus* Tat-D DNase (AAH61187), *Caenorhabditis elegans* Tat-D like protein (NP\_504476) and *Escherichia coli* Tat-D DNase (YP\_671915). Identical and similar amino acids are highlighted in green and yellow, respectively, using 70 % threshold. The amino acids which are totally conserved in the Tat-D proteins of the species are shown with asterisks.