

A cathepsin F-like peptidase involved in barley grain protein mobilization, HvPap-1, is modulated by its own propeptide and by cystatins

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Figure S1. Comparison of the amino acid sequences of the cathepsin-F like cysteine proteases used in the phylogenetic tree. The alignment was generated using the MUSCLE program. The specific ERFNAQ motif for cathepsin-F like proteases is marked. At, *Arabidopsis thaliana*; Pt, *Populus trichocarpa*; Rc, *Ricinus communis*; Os, *Oryza sativa*; Sb, *Sorghum bicolor*; Bd, *Brachypodium distachyon*; Hv, *Hordeum vulgare*; Sm, *Selaginella moellendorffii*.

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SbPap-3      -----MASQLAVLLI LLLPLAGA-----STDDGF---IRQVTDGR-RSPAGAG
BdPap-3      -----MTARLLLLL LALVYATFFR--AAAGASGDDV---IRQVTDNGAFAARRFP
OsPap-22     -----MAAAPARLVVL VLVAVVV---VVGDDGDAGV---IRQVTDGGYWP-----
HvPap-3      -----MAARLVSL LLL LALVWVA--HGAGVSAEDV---IRQVTDSGHGAGH---
AtPap-26     MVA-----KALAQLITCI I L FCHVVA-----SVEDLT---IRQVTADNRRIAPNL-
PtPap-25     -----MKNGL-----
RcPap-3      MIQERPTALIFTVI PLAILAFTTLT LTT SATSGDATLQDPT---ILQVTD D P S V T L S N R K
SmPap-8      -----MSSSVVDN-GDRS-----
SbPap-2      -----MARLLLLLLAFLFSTAALCSPDASAAEDPL---IEQVVGGA-GDDE---
BdPap-1      --MDH-----RLVAPL L L L L V G L L L S P A A --ATATAGDEDPL---IRQVVGGA-DPLD---
HvPap-1      --MDH-----RLVAPL L L L L V G L L L S P A P --ATAAGDEEPL---IRQVVGGA-DPLD---
SbPap-1      -----MAHRV L L L L S L A A W A V A -----AAVDTE D P L ---IRQV V P G G - D D N E ---
OsPap-41     -----MDH R L L L L G L L L S P A V A --AASVFGEEEP L ---IRQV V G G G - D D N E ---
OsPap-17     --MARHR--RLAAAA L L L L L S S A A L S S S A E G E E D P L ---IEQV V G G G - E E E D ---
BdPap-2      --MARL-----RRLPIVVAAV L L L S G V A A -----LSSPVEDPL---IEQV V G G D - E K N E ---
HvPap-2      --MARLRLRL R L L L L V A A V L L L H H P A L --S S A T E G L E D P L ---IEQV V G G D - A E N E ---
AtPap25      -----MD---RVVFFFLIAAT L L A G S L G - S T V I S G E V T D G F V N P I R Q V V P E E - N D E ---
AtPap-24     MDYHL-----RVLFSVSL I F V F V S V S V C -----GDEDVL---IRQVVD E T - E P -----
AtPap-23     --MDRL-----KLYFSV F V L S F F I V S V S S ---SDVMDGDDLV---IRQV V G G A - E P -----
RcPap-1      MVMAV-----RPSFFV I S S I L F V S A V T A --E T L T T D G E D P L ---IRQV T D G Q D E S S A N P -
PtPap-15     --MSL-----NLSL L V I L S L L F T S A V H A -----ETLNGDDPL---IREVVDGQDASSS---
PtPap-14     --MSL-----NLSL F L I L S L F F I S A I S A -----E T F N G D D S L ---IRQV V E G Q D E S S S ---
RcPap-2      --ME-----RSCFLS L I V E A F L S S S I L - F T A T S D E L D D P L ---IRQV V P D V - E D -----
PtPap-21     --ME-----RLP L L F L L L L T A L S S T L A - S T V S S D L D D P L ---IRQV V S E G - E D -----
PtPap-17     --ME-----RPSL L A L L L L L T L L C S A V A - S T V S S N D L D D P L ---IRQV V S D G - E D -----

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SbPap-3      ALG L L P E - A Q F A A F V R R H G R R Y S - G P E E Y A R R L R V F T A N L A R L A A H Q A L D P T A R H G V T P F
BdPap-3      S P G L L P E - A K F A A F V R R H G K E Y S G G A E E Y A R R L R V F T A N L A R L A A H Q A L D P G A R H G V T P F
OsPap-22     - P G L L P E - A Q F A A F V R R H G R E Y S - G P E E Y A R R L R V F T A N L A R L A A H Q A L D P T A R H G V T P F
HvPap-3      - P G L L P E - A Q F A A F V R R H G K E Y S - G P E E Y A R R L R V F T A N V A R L A A H Q A L D P G A R H G V T P F
AtPap-26     - L G T H T E - S K F R L F M S D Y G K N Y S - T R E E Y I H R L G I F A K N V L K A A E H Q A L D P S A V H G V T Q F
PtPap-25     - N L L G T E - E K F K M F I K E H N K E Y A - T R E E Y V H R F G I F G K N L I R A V E H Q A L D P T A I H G V T P F
RcPap-3      F L G T N T E - E N F K M F M I K Y D K E Y D - T R E E Y M H R L G V F K N L I R A A E H Q A L D P T A V H G I T P F
SmPap-8      - A L L D V E - T H F K S F I A R F G K A Y A - T A E E H A H R L K V F T A N L V R A V S H Q A L D P S A V H G I T Q F
SbPap-2      - L E L N A E - A H F A S F V R R F G K T Y R - D A E E H A H R L S V F T A N L R R A A R R H Q A L D P T A V H G V T K F
BdPap-1      - N D L E L S - S H F T S F V Q R F G K T Y K - D A E E H A H R L S V F T A N L R R A A R R H Q A L D P S A E H G I T K F
HvPap-1      - N D L E L D - S Q F V G F V Q R F G K T Y R - D A E E H A H R L S V F T A N L R R A A R R H Q A L D P S A E H G V T K F
SbPap-1      - L E L N A E - S H F L S F V Q R F G K S Y K - D A E E H A Y R L S I F T A N L R R A A R R H Q A L D P S A E H G V T K F
OsPap-41     - L E L N A E - R H F A S F V Q R F G K S Y R - D A E E H A Y R L S V F T A N L R R A A R R H Q A L D P S A E H G V T K F
OsPap-17     - A Q L D A E - A H F A S F E R R F G R T Y R - D A G E R A Y R M S V F T A N L R R A A R R H Q A L D P T A T H G V T K F
BdPap-2      - L E L N A E - A H F A S F V Q R F N K S Y R - D A D E H A H R L S V F T A N L R R A A R R H Q A L D P S A V H G V T K F
HvPap-2      - L E L N A E - A H F A S F V R R F G K S Y R - D A D E H E H R L S V F T A N L R R A A R R H Q A L D P S A V H G I T K F
AtPap25     - Q L L N A E - H H F T L F K S K Y E K T Y A - T Q V E H D Y R F R V F T A N L R R A A R R M Q A L D P S A V H G V T Q F
AtPap-24     - K V L S S E - D H F T L F G K K F G K V Y G - S I E E H Y Y R F S V F T A N L L R A A R R H Q A L D P S A R H G V T Q F
AtPap-23     - Q V L T S E - D H F S L F K R K F G K V Y A - S N E E H D Y R F S V F T A N L R R A A R R H Q A L D P S A T H G V T Q F
RcPap-1      - N L L G A E - H H F S L F Y G K K F K T Y A - S Q E E H D Y R F K I F T A N L R R A A R R H Q A L D P T A T H G V T Q F
PtPap-15     - N L L S A E Q H H F S L F K S K F K S Y G - S Q E E H D Y R F S V F T A N L R R A A R R H Q A L D P T A S H G V T Q F
PtPap-14     - N L L T A E Q H H F S L F K R K F K S Y L - S Q E E H D Y R F S V F T A N L R R A A R R H Q A L D P T A S H G V T Q F
RcPap-2      - Y L L S A Q - H H F T A F K A K F G K N Y A - T Q E E H D Y R F K V F T A N L R R A A R R H Q A L D P S A V H G V T K F
PtPap-21     - H L L N A E - H H F T T F K S K F G K N Y A - T Q E E H D Y R F S V F T A N L L R A A R R H Q A L D P T A A H G V T K F
PtPap-17     - D L L N A E - H H F T S F K S K F G K T Y A - T Q E E H D Y R F G V F T A N L R R A A R R H Q A L D P T A A H G I T K F

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SbPap-3      S D L T R E E F E A R L T G V - R A G A G G D V Q R L -----V M S G A P A A P P A S Q E E V S R L P A S F D W R D

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BdPap-3 SDLTPEEFQARLTGLQQQ-----GTNN-----NMPAAARATAEELATLPASFDWRA
OsPap-22 SDLTREEFEARLTGL-AADVGGDDVRRR-----PMPAAAPATEEEVSGLPASFDWRD
HvPap-3 SDLTREEFEARLTGL-VG-----AGDV-----LRSARMPAAAPATEEEVAALPASFDWRD
AtPap-26 SDLTEEFKRMVYTGADV-----GSSR-----GGTVGAEAPMVEVDGLPEDFDWRE
PtPap-25 MDLTEEFERMYAGV-LG-----GGTV-----PVEK-GSVSFMDSAGLPDSFDWRE
RcPap-3 MDLTEEFERMYTGV-VG-----GGAV-----GAEVGTATSFLETAGLPSSFDWRK
SmPap-8 SDLTEEFKQQFLGL-RV-----PSRL-----REA-NKAPVLPDNDLPEDFDWRE
SbPap-2 SDLTPAEFRRTQFLGL-RR-----SRSSGQDLLKG--SGSA-HEAPVLPDGLPTDFDWRE
BdPap-1 SDLTPAEFRRTFLGL-KT-----SRRS---FLREI-GGSA-HDAPVLPDGLPDDFDWRD
HvPap-1 SDLTPAEFRRTYLGL-KT-----TRRS---FLREM-AGSA-HDAPVLPDGLPEDFDWRD
SbPap-1 SDLTPAEFRRTYLGL-RK-----SRRS---LLREL-GKSA-NEAPVLPDGLPDDFDWRD
OsPap-41 SDLTPAEFRRAYLGL-RT-----SRRS---FLRGL-GGSA-HEAPVLPDGLPDDFDWRD
OsPap-17 SDLTPGEFRDRFLGL-----RRP---SLEGLVGGEP-HEAPVLPDGLPDDFDWRE
BdPap-2 SDLTPDEFDRFLGL-RK-----YRRS---FLKGL-SGSA-HDAPALPTDGLPTEFDWRE
HvPap-2 SDLTPDEFRRFLGL-RK-----SRRS---FLKGI-SGSA-HDAPALPTDGLPTEFDWRE
AtPap25 SDLTPKEFRKFLGLKRR-----GFRL-----PTDT-QTAPILPTSDLPTEFDWRE
AtPap-24 SDLTRSEFRKHLGV-KG-----GFKL-----PKDA-NQAPILPTQNLPEFDWRD
AtPap-23 SDLTRSEFRKHLGV-RS-----GFKL-----PKDA-NKAPILPTENLPEDFDWRD
RcPap-1 SDLTHSEFRRQFLGL-RR-----LRL-----PKDA-NEAPMLPTNDLPADFDWRE
PtPap-15 SDLTPAEFRKQVLGL-RR-----LRL-----PKDA-NEAPILPTSDLPEDFDWRD
PtPap-14 SDLTPAEFRKQVLGL-RK-----LRL-----PKDA-NTAPILPTNDLPEDFDWRE
RcPap-2 SDLTPREFRRQYLGL-KK-----LRL-----PADA-HEAPVLPDGLPEDFDWRD
PtPap-21 SDLTPKEFRQLLGL-KR-----RLRL-----PTDA-NKAPILPTGDLPTDFDWRD
PtPap-17 SDLTPKEFRRQFLGL-KR-----WLRL-----PTDA-NKAPILPTDLPDPTDWRD
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SbPap-3 KGAVTVKMQGACGSCWAFSTTGAVEGANFLATGKLLLELSEQQLVDCDHTCSAVAQNECN
BdPap-3 KGAVTEVKMQGACGSCWAFSTTGAVEGAHFVATGKLLNLSEQQLVDCDHTCDAVAKNECD
OsPap-22 RGAVTDVKMQGACGSCWAFSTTGAVEGANFLATGNLLDLSEQQLVDCDHTCDAEAKTECD
HvPap-3 KGAVTDVKMQGACGSCWAFSTTGAVEGANFVATGKLLDLSEQQLVDCDHTCDAVAKTECN
AtPap-26 KGGVTEVKMQGACGSCWAFSTTGAVEGAHFVSTGKLLSLSEQQLVDCDQACDFKDKKACD
PtPap-25 KGAVTDVKIQGSCGSCWAFSTTGAVEGANFIATGKLLNLSEQQLVDCDRVCDKTKASC
RcPap-3 KGAVTDVKMQGACGSCWAFSTTGAVEGANFIATGKLLNLSEQQLVDCDRVCDIKEKTACD
SmPap-8 HGAVTEVKMQGACGSCWAFSTTGAVEGAHFLETGKLLSLSEQQLVDCDHSCTDVKVSCD
SbPap-2 HGAVGPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
BdPap-1 HGAVGPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
HvPap-1 HGAVGPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
SbPap-1 HGAVTPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
OsPap-41 HGAVGPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
OsPap-17 HGAVGPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
BdPap-2 HGAVGPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
HvPap-2 HGAVGPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
AtPap25 QGAVTPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
AtPap-24 RGAVTPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
AtPap-23 HGAVTPVKDQGSCGSCWAFSTGSAIEXXXXXXXXXXXXXXXXXXXXXXXXXXCDPSEPRSCD
RcPap-1 KGAVTAVKMQGACGSCWAFSTTGAVEGANFLATGKLVSLSEQQLVDCDHECDPAEAGACD
PtPap-15 KGAVGPIKMQGACGSCWAFSTTGAVEGANFLATGKLVSLSEQQLVDCDHECDPEEFGSCD
PtPap-14 KGAVGPVKDQGSCGSCWAFSTTGAVEGANFLATGKLVSLSEQQLVDCDHECDPEEFGSCD
RcPap-2 HGAVTAVKMQGACGSCWAFSTTGAVEGANFLATGKLVSLSEQQLVDCDHECDPEEYAGACD
PtPap-21 HGAVTSVKDQGSCGSCWAFSTTGAVEGANFLATGKLVSLSEQQLVDCDHECDPEEYAGACD
PtPap-17 HGAVTEVKDQGSCGSCWAFSTTGAVEGANFLATGKLVSLSEQQLVDCDHECDPEEYAGACD
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SbPap-3 NGCAGGLMTNAYAYLMSGGGLMEQRAYPYTGAP-GPCRFDPAKAAVRVANFTAVPA----
BdPap-3 SGCAGGLMTNAYAYLIRAGGLMEQAAYPYTGAP-GTCRFDANKVAVRVTSFTAVFP----
OsPap-22 SGCAGGLMTNAYAYLMSGGGLMEQSAYPYTGAP-GTCRFDANRVAVRVANFTVVAPPGN
HvPap-3 SGCAGGLMTNAYAYLMSGGGLMEQAAYPYTGAP-GPCRFDGKAVAVRVANFTAVPL----
AtPap-26 NGCAGGLMTNAYAYLMEAGGLEEERSYPYTGKR-GHCKFDPEKAVRVNFTTIPL----
PtPap-25 DGCAGGLMTNAYAYLIEAGGLEESSYPYTGKS-GECKFDPEKIAVKVANFTSIAP----
RcPap-3 DGCAGGLMTNAYAYLIEAGGLEDEISYPYTGKP-GKCKFDEKKIAVRVNFVFTSIFI----
SmPap-8 AGCAGGLMTNAYAYLMSGGGLMEQRAYPYTGAP-GPCRFDPAKAAVRVANFTAVPA----
SbPap-2 SGCAGGLMTTAFSYLQKVGGLEREEDYPYNGRD-STCKFDKSKIIVAVQNFVSVV----
BdPap-1 AGCAGGLMTTAFSYLQKVGGLEREEDYPYNGRD-STCKFDKSKIIVAVQNFVSVV----
HvPap-1 AGCAGGLMTTAFSYLQKVGGLEREEDYPYNGRD-STCKFDKSKIAASVQNFVSVV----
SbPap-1 SGCAGGLMTTAFSYLQKVGGLEREEDYPYNGRD-STCKFDKSKIIVAVQNFVSVV----
OsPap-41 AGCAGGLMTTAFSYLQKVGGLEREEDYPYNGRD-STCKFDKSKIIVAVQNFVSVV----
OsPap-17 SGCAGGLMTTAFSYLQKVGGLEREEDYPYNGRD-STCKFDKSKIIVAVQNFVSVV----

BdPap-2 AGCNGGLMTTAFSYLAKAGGLETEKDYFPYTGRRG-GACKFDKSKIAAQVKNFSTVAV----
HvPap-2 AGCNGGLMTTAFSYLAKAGGLETEKDYFPYTGRRN-SACKFDKSKIAAQVKNFSTVAI----
AtPap25 GCCSGGLMNNAFEYALKAGGLMKEEDYFPYTGDRDHTACKFDKSKIVASVSNFVSVSS----
AtPap-24 SGCNGGLMNSAFEYTLKAGGLMREKDYFPYTGTDGGSCKLDRSKIIVASVSNFVSVSI----
AtPap-23 SGCNGGLMNSAFEYTLKAGGLMKEEDYFPYTGKDGKTCCKLDKSKIIVASVSNFVSVISI----
RcPap-1 SGCNGGLMNSAFEYTLKAGGLMREEDYFPYTGTDRGACQFDKTKIAAKVANFVSVSL----
PtPap-15 SGCNGGLMNSAFEYTLKAGGLMREEDYFPYTGTDRGACKFDKSKVAAARVANFVSVSL----
PtPap-14 SGCNGGLMNSAFEYTLKAGGLMREEDYFPYTGMDRGACKFDKSKVAAAGVANFSAVSL----
RcPap-2 SGCNGGLMNTNAFEYILKAGGLEREEDYFPYTGSDRGPKFERAKIAASVMNFVSVSV----
PtPap-21 GCCSGGLMNNAFEYALKAGGLEREKDYFPYTGMDRGACKFEKSKVAAASVSNFVSVSL----
PtPap-17 GCCDGLMNNAFEYALKAGGLEREADYFPYTGTDGGTCKFDKSKVVASVSNFVSVSI----
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SbPap-3 --GDEAQIRAAALVRRGPLAVGLNAAFMQTYVGGVSCPLLCPRAMVNHGV---LLVGYGAR
BdPap-3 --DDEDQIRASLVRAGPLAVGLNAAFMQTYLGGVSCPLLCPRKLI NHGV---LLVGYGAR
OsPap-22 DGDGDAQMRAALVRHGPLAVGLNAAFMQTYVGGVSCPLVCPRAWVNHGV---LLVGYGER
HvPap-3 ---DEDQMRAALVRRGPLAVGLNAAFMQTYVGGVSCPLICPRAMVNHGV---LLVGYGAR
AtPap-26 ---DENQIAANLVRHGPLAVGLNAVFMQTYIGGVSCPLICSKRWVNHGV---LLVGYGSK
PtPap-25 ---DENQIAANLVHGGPLAIGLNAIFMQTYIGGVSCPLICGKRWVNHGV---LLVGYGAR
RcPap-3 ---DENQIAAHLVHGGPLAIGLNAVFMQTYIGGVSCPLICGKRWVNHGV---LLVGYGAK
SmPap-8 ---DEDQIAANLVKHGPLAIGINAVFMQTYIGGVSCPIICSKHHI DHGV---LLVGYGAK
SbPap-2 ---NEDQIAANLVKHGPLAIGINAVFMQTYIGGVSCPYICGRH-LDHGV---LLVGYGSA
BdPap-1 ---DEEQIAANLVKHGPLAIGINAAFMQTYIGGVSCPYICGRS-LDHGV---LLVGYGAS
HvPap-1 ---DEEQIAANLVKYGPLAIGINAAFMQTYIGGVSCPYICGRH-LDHGV---LLVGYGAS
SbPap-1 ---DEGQIAANLIKHGPLAIGINAAFMQTYIGGVSCPYICGRT-LDHGV---LLVGYGAA
OsPap-41 ---DEDQIAANLVKHGPLAIGINAAFMQTYIGGVSCPYICGRH-LDHGV---LLVGYGAS
OsPap-17 ---NEDQIAANLVKHGPLAIGAINAAFMQTYIGGVSCPFICGRH-LDHGV---LLVGYGSA
BdPap-2 ---DEDQIAANLVKHGPLAIGINAVFMQTYIGGVSCPFICGRH-LDHGV---LLVGYGSA
HvPap-2 ---DEDQIAANLVKHGPLAIGINAVFMQTYIGGVSCPYICGRH-LDHGVPGWLMI SWLRT
AtPap25 ---DEDQIAANLVQHGPLAIGAINAMMMQTYIGGVSCPYVCSKS-QDHGV---LLVGFSS
AtPap-24 ---NEDQIAANLIKNGPLAVAINAAFMQTYIGGVSCPYICSRRLNHGV---LLVGYGSA
AtPap-23 ---DEEQIAANLVKNGPLAVAINAGFMQTYIGGVSCPYICTRR-LNHGV---LLVGYGAA
RcPap-1 ---DEDQIAANLVKNGPLAVAINAVFMQTYIGGVSCPYICSKR-LDHGV---LLVGYGSA
PtPap-15 ---DEDQIAANLVKNGPLAVAINAVFMQTYIGGVSCPYICSRRLDHGV---LLVGYGSA
PtPap-14 ---DEDQIAANLVKNGPLAVAINAVFMQTYIGGVSCPYICSRRLDHGV---LLVGYGSA
RcPap-2 ---DEDQIAANLVQNGPLAVGINAVFMQTYIGGVSCPYICSKR-QDHGV---VLVGYGSA
PtPap-21 ---DEDQIAANLVKHGPLSVAINAVFMQTYIGGVSCPYICSKH-QDHGV---LLVGYGAA
PtPap-17 ---DEDQIAANLVKHGPLSVAINAAFMQTYVGGVSCPYICSKR-QDHGV---LLVGYGSA
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SbPap-3 GFPAALRLGYRFPYWI IKNSWGERWGEQGYRRLCRG---SNVCGVDSMVSAVAVAPVP----
BdPap-3 GLAPLRLGYRFPYWI IKNSWGEKWEQGYRRLCRGARNRNVCGVDSMVSAVAVAL-----
OsPap-22 GFPAALRLGHRFPYWI IKNSWGWKWEQGYRRLCRG---RNVCGVDTMVSAVAVAPPPP---
HvPap-3 GFSALRLGYRFPYWI IKNSWGAQWGEQGYRRLCRG---RNVCGVDSMVSAVAVAP-----
AtPap-26 GFSILRLSNKFPYWI IKNSWGGKWEQGYRRLCRG---HDICGINSMVSAVATQVSS-----
PtPap-25 GYSILRFGYKFPYWI IKNSWGNHWGEQGYRRLCRG---HGMCGMDSMVSAVVTKVA-----
RcPap-3 GFSILRLGYKFPYWI IKNSWGGKWEQGYRRLCRG---YGMCGMDRMVSAVVTQVS-----
SmPap-8 GYAPIRFTEKFPYWI IKNSWGATWGEQGYRRLCRG---HGMCGMNTMVSTVA-----
SbPap-2 GYAPIRFEKFPYWI IKNSWGENWGEQGYRRLCRGPHVQNKCGVDSMVSTVAIHTSSKKE--
BdPap-1 GFAPSRLLKFPYWI IKNSWGENWGEQGYRRLCRGNSVRNCGVDSMVSTVAIAHTSKEE--
HvPap-1 GFAPSRFTEKFPYWI IKNSWGENWGDQGYRRLCRGNSVRNCGVDSMVSTVSATHSSKEE--
SbPap-1 GFAPIRLKDKFPYWI IKNSWGENWGEQGYRRLCRGNSVRNCGVDSMVSTVSAVRTSKE--
OsPap-41 GFAPIRLKDKA YWI IKNSWGENWGEHGYRRLCRGNSVRNCGVDSMVSTVSAIHTSKE--
OsPap-17 GYAPIRFTEKFPYWI IKNSWGENWGEQGYRRLCRGPHDKNCGVDSMVSSVTAIHTSKEE--
BdPap-2 GYAPLRFTEKFPYWI IKNSWGENWGESGYRRLCRGAVHKNCGVDSMVSTVTAIHTSKEE--
HvPap-2 --APL--QGETILDHKELMGRELGRERVLQDLQ---STCQKQVRCRFHGLHGYPHPYL
AtPap25 GYAPIRLKEKFPYWI IKNSWGAMWGEHGYRRLCRG---HNMCGMDTMVSTVAAVTSPK---
AtPap-24 GFSQARLKEKFPYWI IKNSWGESWGEHGYRRLCRG---RNICGVDSLSTVAAVTSS---
AtPap-23 GYAPARFTEKFPYWI IKNSWGETWGEHGYRRLCRG---RNICGVDSMVSTVAAVTSTTAH
RcPap-1 GYAPIRMKKFPYWI IKNSWGENWGESGYRRLCRG---RNICGVDSMVSTVAAVQTASE--
PtPap-15 GYSPVFMKEKFPYWI IKNSWGEKWEHGYRRLCRG---RNVCGVDSMVSTVAAVQTSSQ--
PtPap-14 AYAPVFMKEKFPYWI IKNSWGESWGEHGYRRLCRG---RNICGVDSMVSTVAAVQTNSL--
RcPap-2 GYAPVRLKDKFPYWI IKNSWGENWGEHGYRRLCRG---RNVCGVDAMVSTVAIHTTAR--
PtPap-21 GYAPIRFTEKFPYWI IKNSWGENWGEHGYRRLCRG---RNICGVDSMVSTVAIHTATAQ--
PtPap-17 GYAPIRFTEKFPYWI IKNSWQNWGEHGYRRLCRG---RNICGVDSMVSTVAIHTTAAQ--
: . * : * * . . . * :

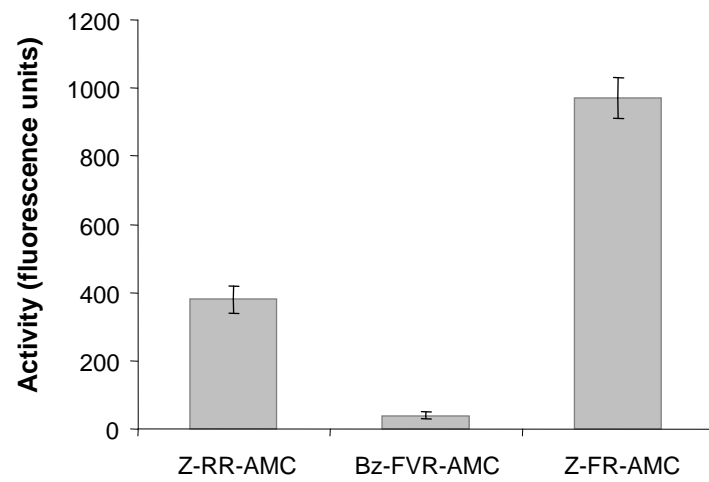


Fig. S2. Enzymatic activity of recombinant HvPap-1 determined using the fluorogenic substrates Z-FR-AMC, Z-RR-AMC and Bz-FVR-AMC, susceptible to degradation by cathepsin L-, B- and H-like peptidases, respectively.

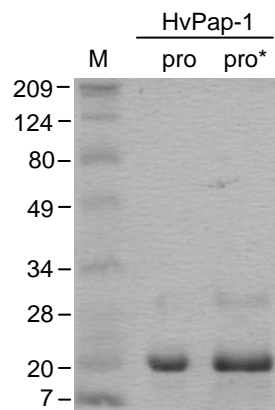


Fig. S3. Purification of recombinant propeptides derived from the HvPap-1 protease. SDS-PAGE of recombinant propeptides HvPap-1pro and HvPap-1pro* purified from *E. coli* cultures. Molecular markers (M) in kDa are indicated. The gel was stained with Coomassie Brilliant Blue R-250.

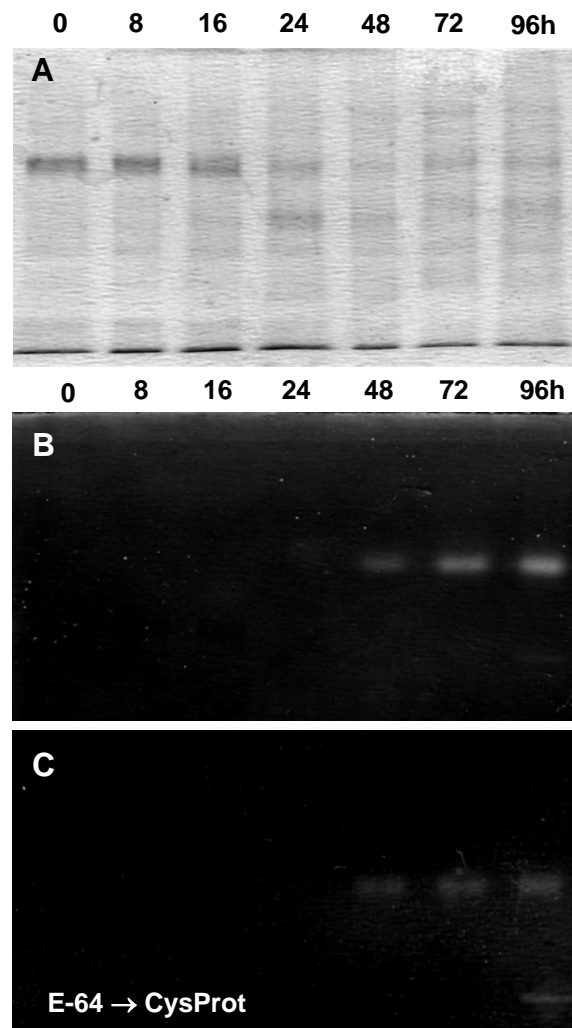


Fig. S4. Protein expression pattern and protease activity of embryos during germination. **A**, Protein pattern of crude extracts of embryo barley grains at different germination times (0-96h) stained with Coomassie Brilliant Blue R-250. **B-C**, Zymograms of the gelatinolytic activity of embryos isolated at different germination times (0-96h). Before electrophoresis, 2 μg of protein samples were incubated in buffer alone or with 100 μM E-64.

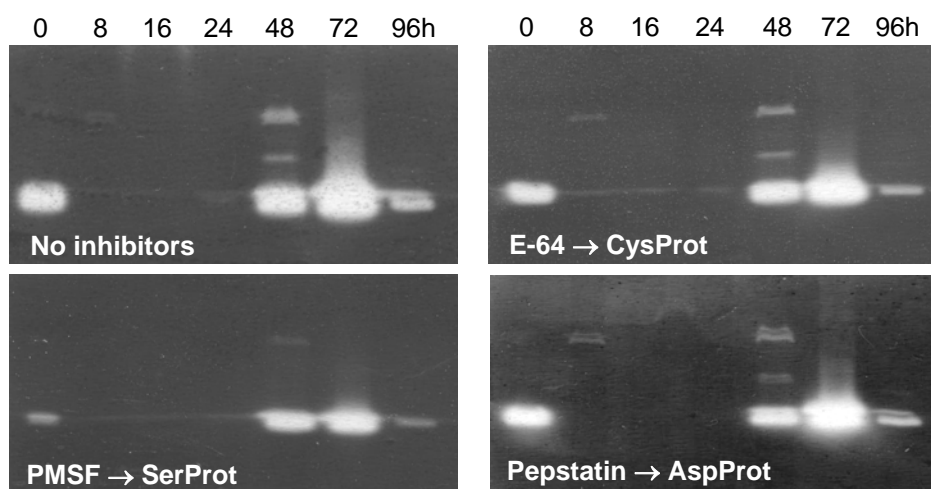


Fig. S5. Zymograms of the gelatinolytic activity of protein extracts from de-embryonated barley grains at different germination times (0-96 h). Before electrophoresis, samples were incubated in buffer alone, 100 μ M E-64, 250 μ M PMSF and 20 μ M pepstatin. CysProt: cysteine-proteases; SerProt: Serine-proteases; AspProt: Aspartic-proteases.

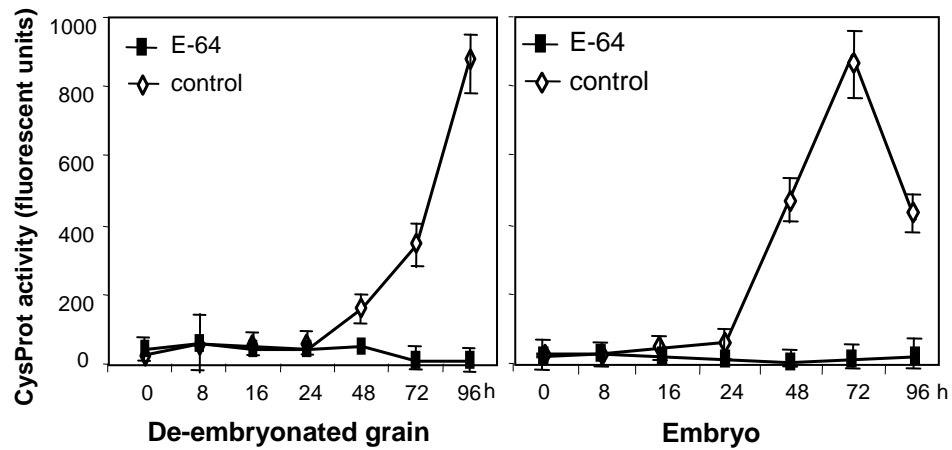


Fig. S6. *In vitro* quantification of CysProt activity in extracts from de-embryonated grain and embryo fractions of barley grain at different germination times. 2 μ g of protein extract were incubated in buffer alone or with 100 μ M of E-64 using Z-FR-AMC as fluorescent substrate. Proteolytic activity was expressed as fluorescent units and represents the mean \pm se of triplicate measurements.

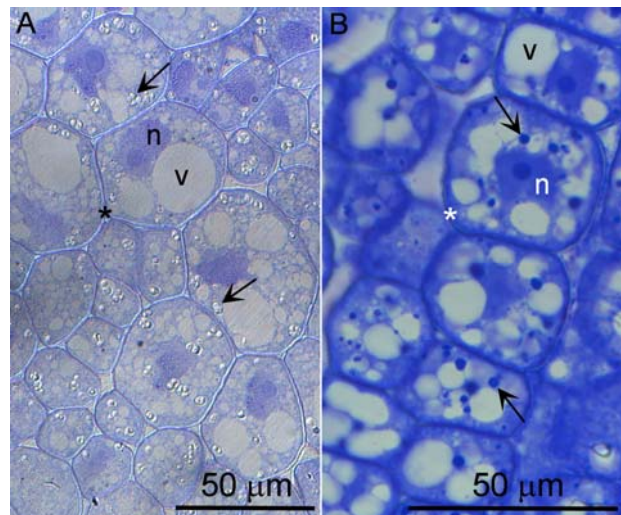


Fig. S7. A, Histochemical characterization of barley embryos 24 hai on 2 μm sections from formaldehyde-fixed and LRwhite-embedded specimens. Toluidine blue O staining reveals a good structural preservation. Purple nuclei (n) and a cytoplasm filled with unstained vacuoles of different sizes can be identified. DIC gives contrast to numerous small refringent structures (arrows) in the cytoplasm and the cell walls (asterisk), as well. B, Coomassie stains protein containing structures within the cell: nuclear (n) chromatin and nucleolus, cytoplasm and abundant small, round cytoplasmic structures (arrows).

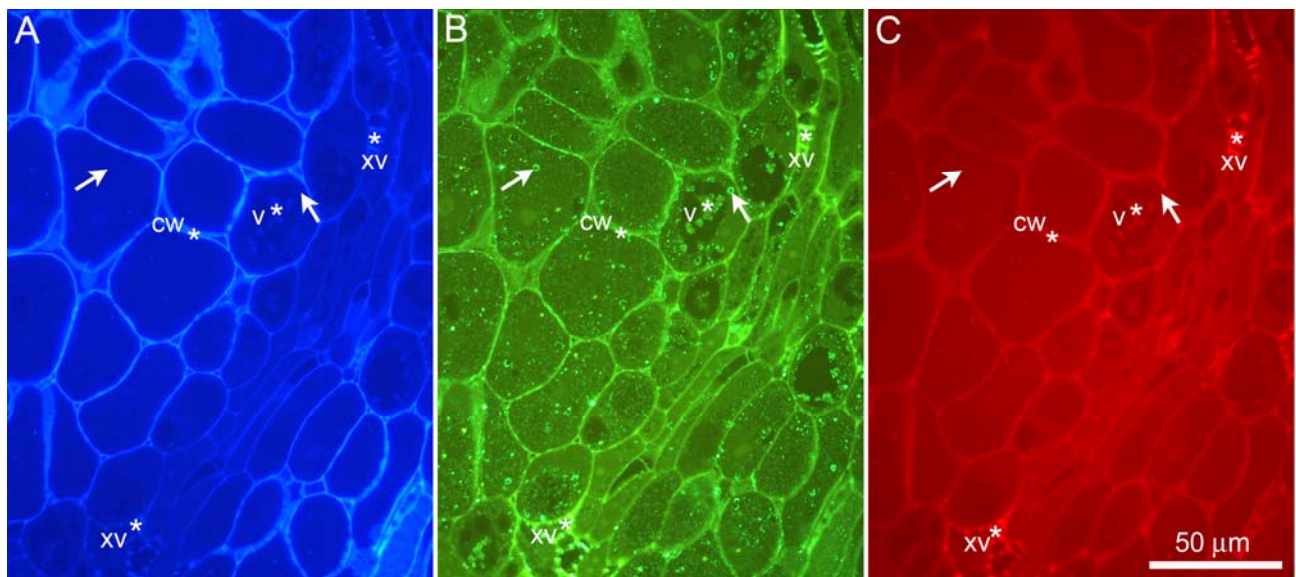


Fig. S8. Autofluorescence control on sections from barley embryos 24 hai
immuno-labelled with the antibody to the protease HvPap-1 and revealed with
Alexa Fluor 488. **A**, section examined under UV light (filter set ex BP 365, FT
395, em LP 397). **B**, the same section as in A under blue light (filter set ex 450-
490, FT 510, em LP 520). **C**, the same section as in A and B under green light
(filter set ex BP 546, FT 580, em LP 590). The precise sub-cellular localization of
the protease HvPap-1 was identified in abundant bright foci dispersed in the
cytoplasm and round-shaped structures (arrows). Other green-fluorescent
components (asterisks) in the cell walls (cw), xylem vessels (xv) and vacuolar
deposits (v) corresponded to autofluorescence background, since these
structures were also seen under UV and green light excitations.