

Figure S1. Marshall *et al.*

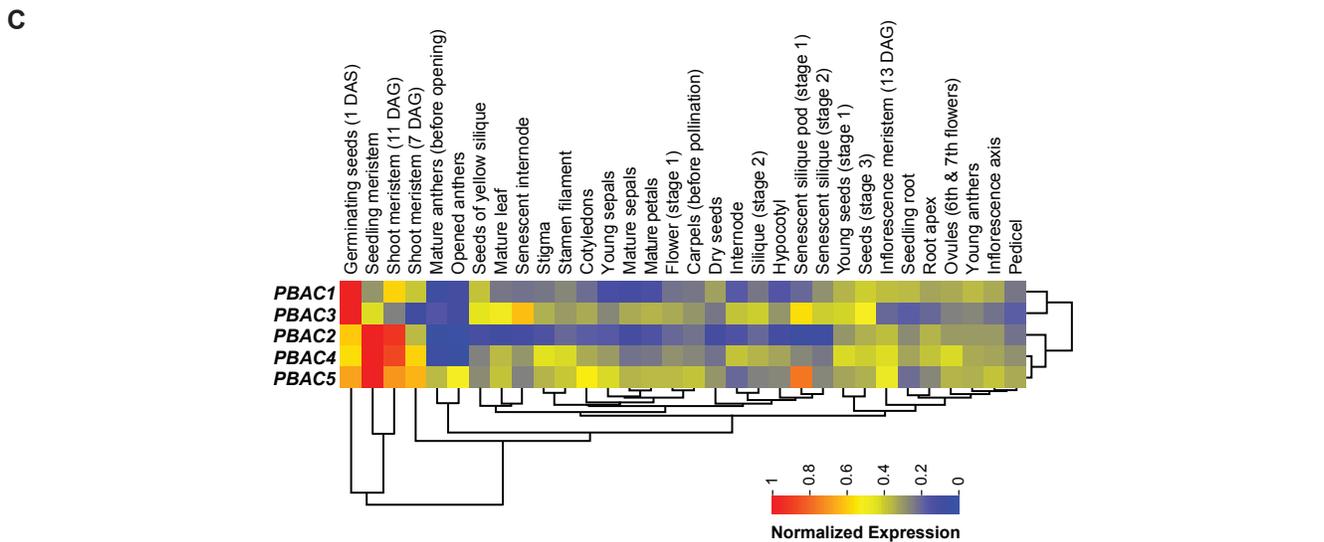
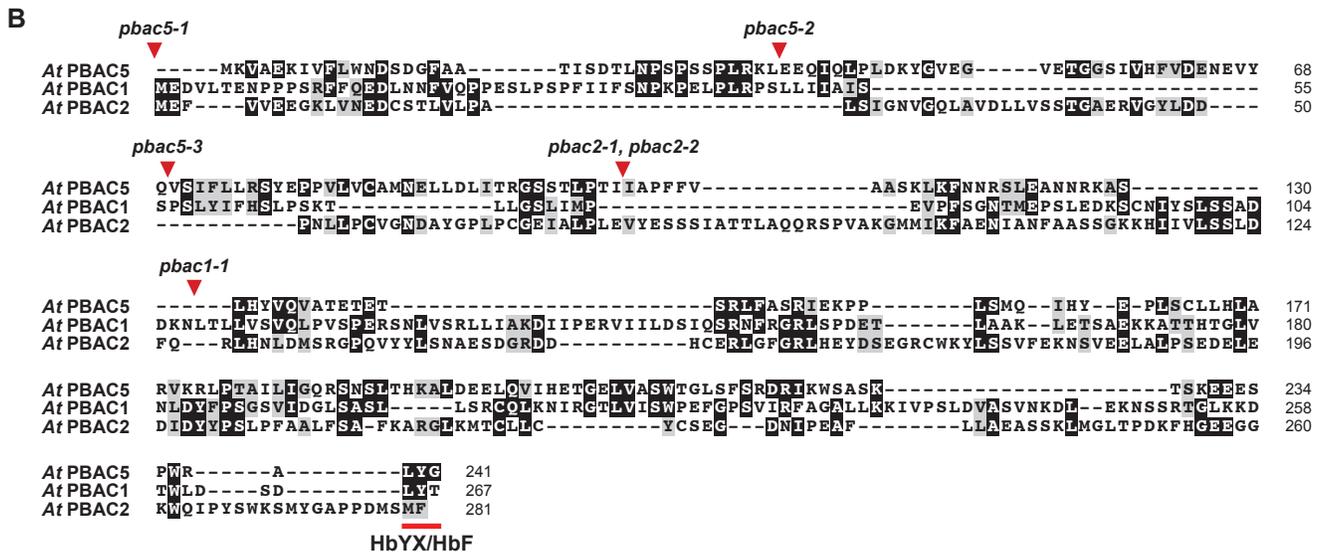
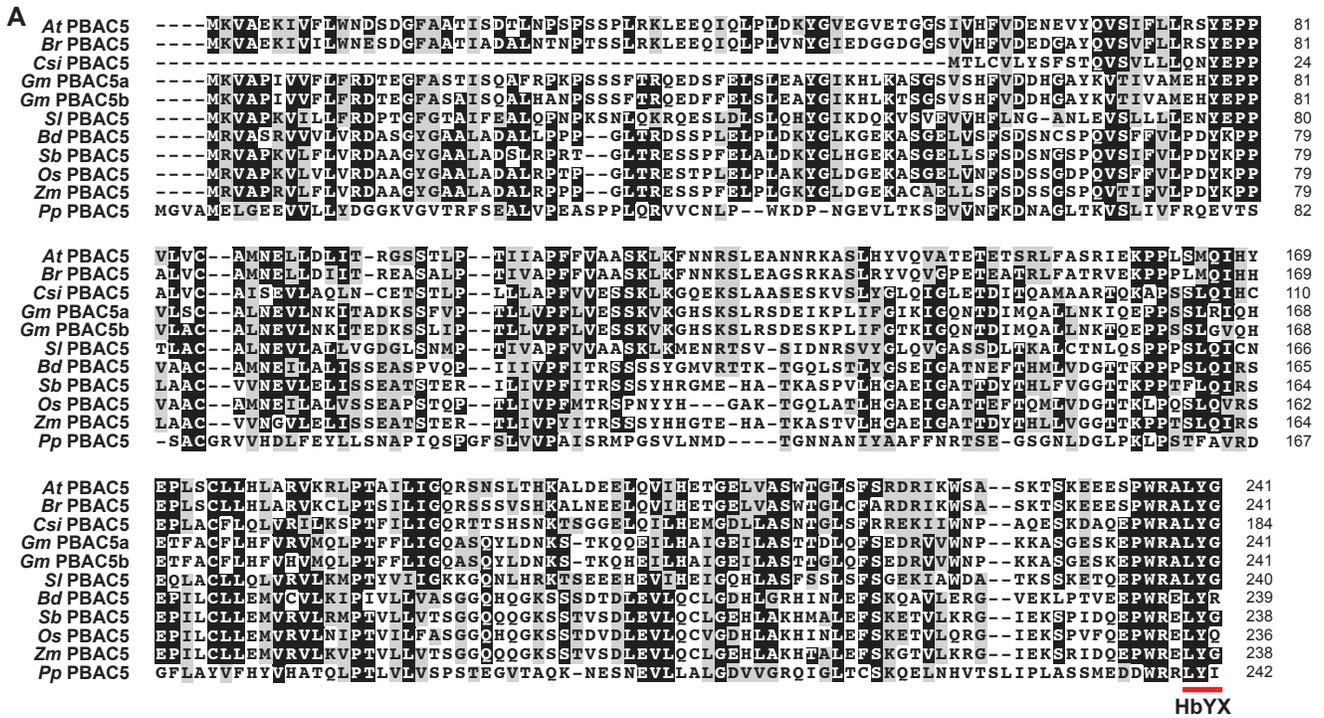


Figure S1. Amino acid sequence comparisons of the PBAC1, PBAC2 and PBAC5 chaperones. (A) Sequence alignment of PBAC5 proteins from a variety of plant species. Identical (55% threshold value) and similar amino acids are shown with black and grey backgrounds, respectively. The position of the C-terminal HbYX motif is indicated by the red line. Amino acid numbers are given on the right. (B) Sequence alignment of the *Arabidopsis* PBAC1, PBAC2 and PBAC5 proteins. The alignment was performed and is annotated as in (A). The positions of the various T-DNA insertions characterized in Fig. 4 are indicated by the red arrowheads. Species abbreviations are given in the Materials and Methods. Accession numbers for all proteins analyzed in this Figure are listed in Table S1. (C) Tissue-dependent expression patterns of the *Arabidopsis* PBAC-type proteasome assembly chaperones. Shown is a heat map displaying relative transcript abundances for the indicated chaperones in 32 manually curated tissues. mRNA levels were obtained from the Transcriptome Variation Analysis database and hierarchically clustered by both tissue distribution and expression patterns. All read counts for each gene were normalized by the “mean-of-ratios” method, with the maximum value set at 1.

Figure S2. Marshall *et al.*

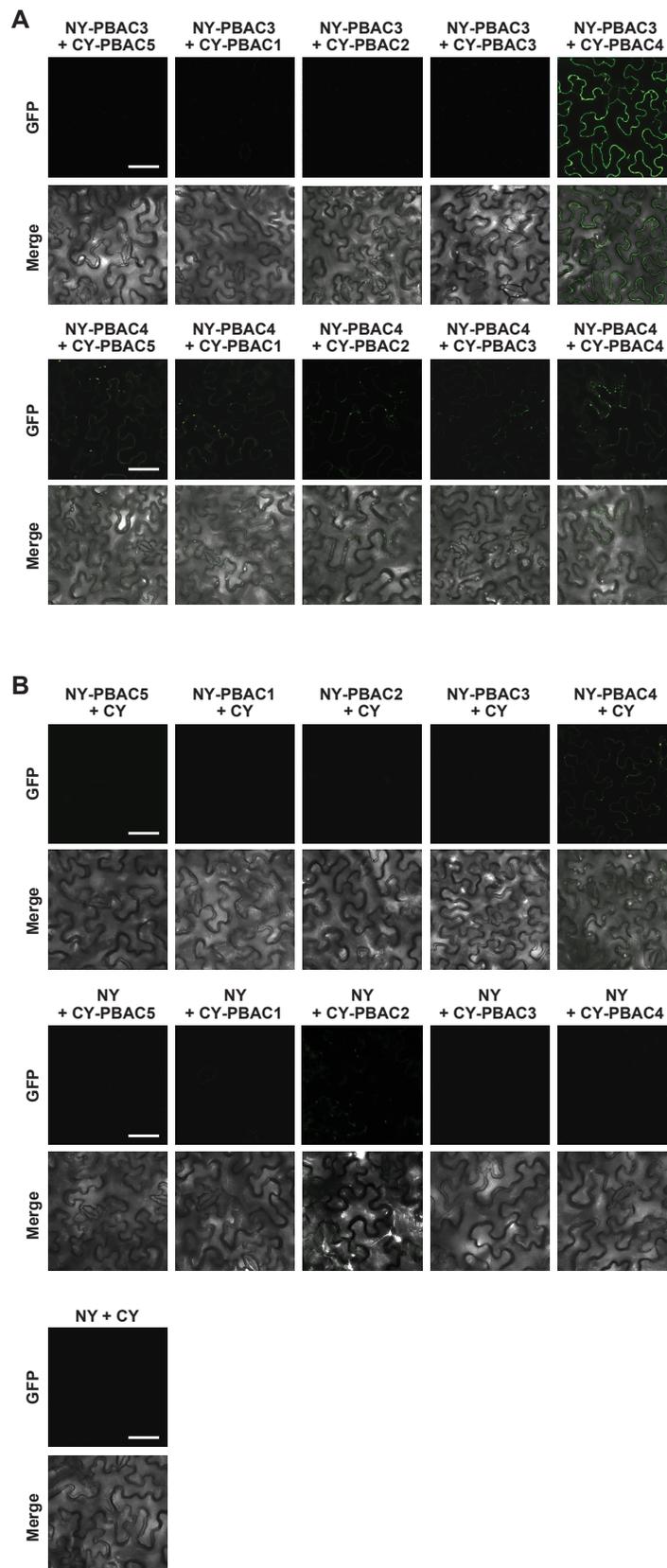


Figure S2. Control bimolecular fluorescence complementation (BiFC) assays involving PBAC1-5.

(A) Pairwise expression of the PBAC3 and PBAC4 chaperones with themselves and PBAC1, PBAC2 and PBAC5 indicates that PBAC3 and PBAC4 interact *in planta*. (B) Pairwise expression of the PBAC1-5 chaperones fused to the N-terminal (NY) and C-terminal (CY) halves of YFP by themselves indicate that only the NY-PBAC4 construct produces a fluorescence signal due to auto-activation. *Nicotiana benthamiana* leaf epidermal cells were co-infiltrated with the indicated plasmid combinations, and fluorescence signals were detected by confocal fluorescence microscopy 36 h after infiltration. Shown are the fluorescence images alone or merged with their companion bright field images. Scale bar = 20 μm .

Figure S3. Marshall *et al.*

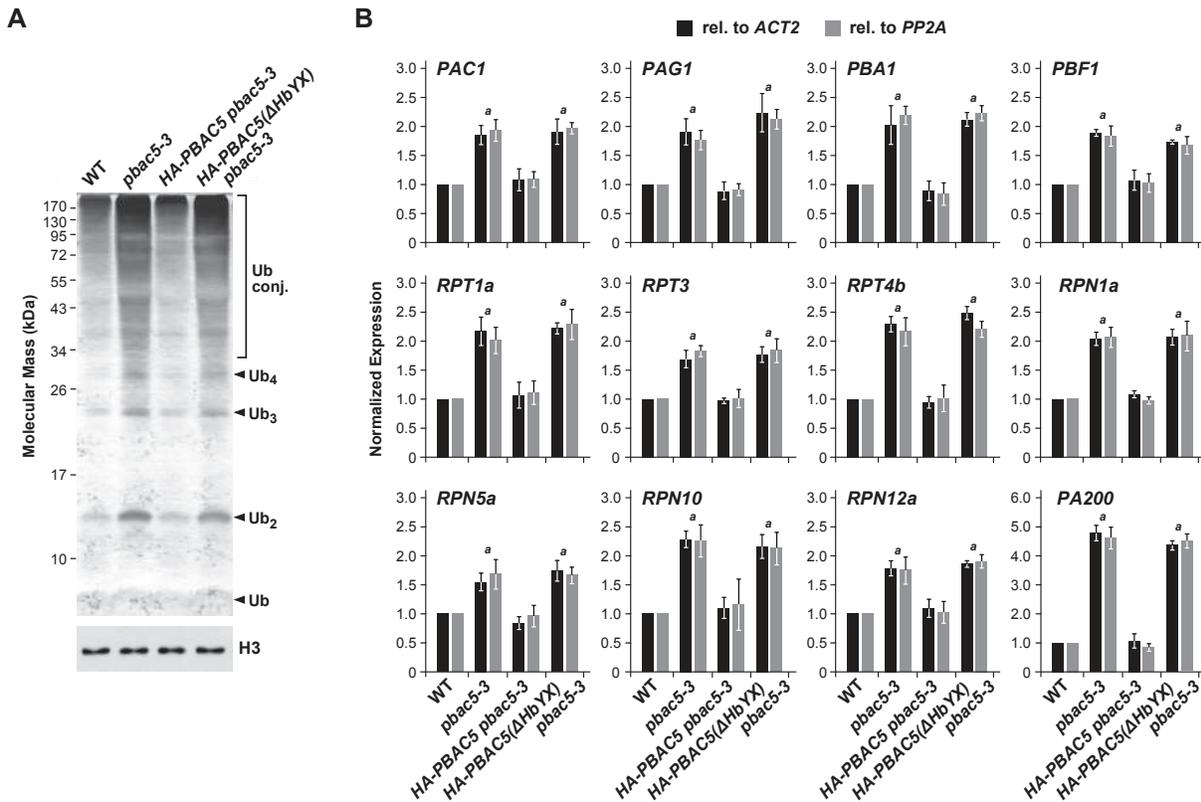


Figure S3. Elevated levels of ubiquitin conjugates and mRNA transcripts encoding 26S proteasome subunits and PA200 in *pbac5-3* seedlings could be rescued with full-length HA-PBAC5. The *pbac5-3* mutant was complemented with a gene encoding full-length HA-PBAC5 or HA-PBAC5(ΔHbYX) missing the C-terminal HbYX motif, as described in Fig. 4. (A) Seedlings lacking PBAC5 hyperaccumulate ubiquitin conjugates. Total protein extracts from 7-day-old WT, *pbac5-3*, HA-PBAC5 *pbac5-3*, or HA-PBAC5(ΔHbYX) *pbac5-3* seedlings were subjected to SDS-PAGE followed by immunoblot analysis with anti-ubiquitin antibodies. Ubiquitin conjugates and poly-ubiquitin chains of various lengths are indicated by the bracket and arrowheads, respectively. Immunodetection of histone H3 was used to confirm near equal protein loading. (B) Seedlings lacking PBAC5 hyperaccumulate transcripts encoding various components of the 26S proteasome. Total RNA was extracted from 7-day-old seedlings of the indicated genotypes, and converted to first-strand cDNA. Relative transcript abundance of the CP α -subunits *PAC1* and *PAG1*, the CP β -subunits *PBA1* and *PBF1*, the RP subunits *RPT1a*, *RPT3*, *RPT4b*, *RPN1a*, *RPN5a*, *RPN10* and *RPN12a*, and the accessory factor *PA200* was determined by quantitative real-time (qRT)-PCR, using the *ACT2* and *PP2A* genes as internal reference standards. All data points were normalized to WT seedlings. The bars represent mean (\pm SD) from three independent biological replicates, each with three technical replicates. The letters identify values that were significantly different from the WT, as determined by one-way ANOVA followed by Tukey's post hoc test (p -value < 0.05).

Figure S4. Marshall *et al.*

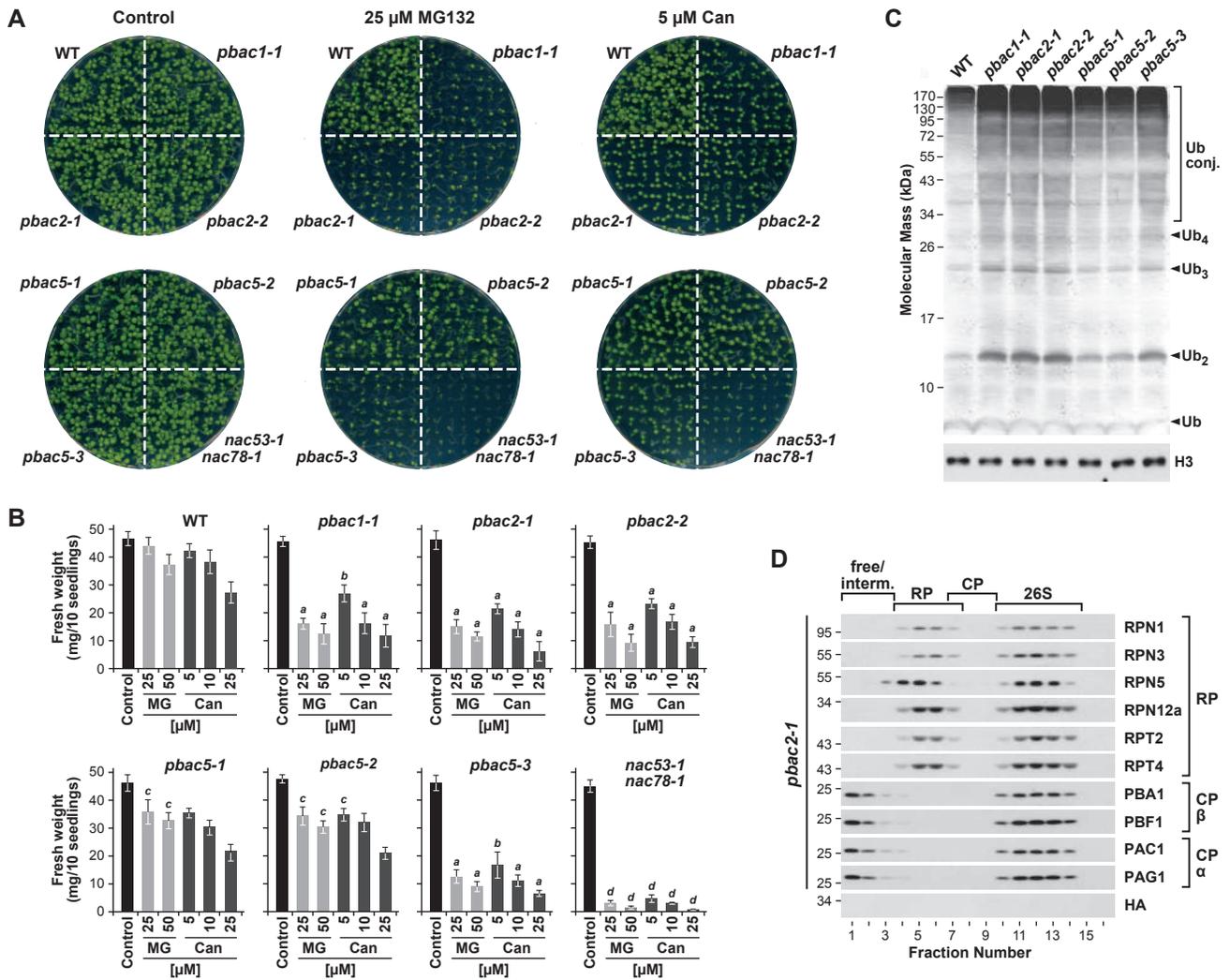


Figure S4. *Arabidopsis* seedlings missing PBAC1, PBAC2 or PBAC5 are hypersensitive to proteotoxic stress and hyperaccumulate ubiquitin conjugates. (A) Homozygous seedlings of the indicated genotypes were grown for 10 days on solid GM medium containing either DMSO (control), 25 μ M MG-132, or 5 μ M canavanine (Can). The *nac53-1 nac78-1* double mutant (Gladman et al., 2016) was used as a positive control. (B) Quantification of seedling sensitivity to various concentrations of MG132 or Can. Shown is the fresh weight of 10 seedlings of the indicated genotypes grown as in (A). Bars represent the mean (\pm SD) from three independent biological replicates. Different letters represent values that were significantly different from one another and the control, as determined by one-way ANOVA followed by the Tukey's post hoc test (p -value < 0.05). (C) Total protein extracts from 7-day-old wild type (WT) and single *pbac1*, *pbac2* or *pbac5* mutant seedlings were subjected to SDS-PAGE followed by immunoblot analysis with anti-ubiquitin antibodies. Ubiquitin conjugates and poly-ubiquitin chains of various lengths are indicated by the bracket and arrowheads, respectively. Immunodetection of histone H3 was used to confirm near equal protein loading. (D) *Arabidopsis* seedlings missing PBAC2 display 26S proteasome assembly defects. Total protein extracts from 10-day-old *pbac2-1* seedlings were subjected to glycerol gradient fractionation, and samples from each fraction were analyzed by SDS-PAGE followed by immunoblot with antibodies against the indicated proteasome subunits. The location of each protein within the 26S complex is indicated on the right. The predicted positions of free proteasome subunits (free), assembly intermediates (interm.), free CP, free RP, and the holo-26S proteasome are indicated by the horizontal brackets. Numbers on the left represent molecular mass markers in kDa. The comparative analysis of wild type and other chaperone mutants can be found in Fig. 6.

Figure S5. Marshall *et al.*

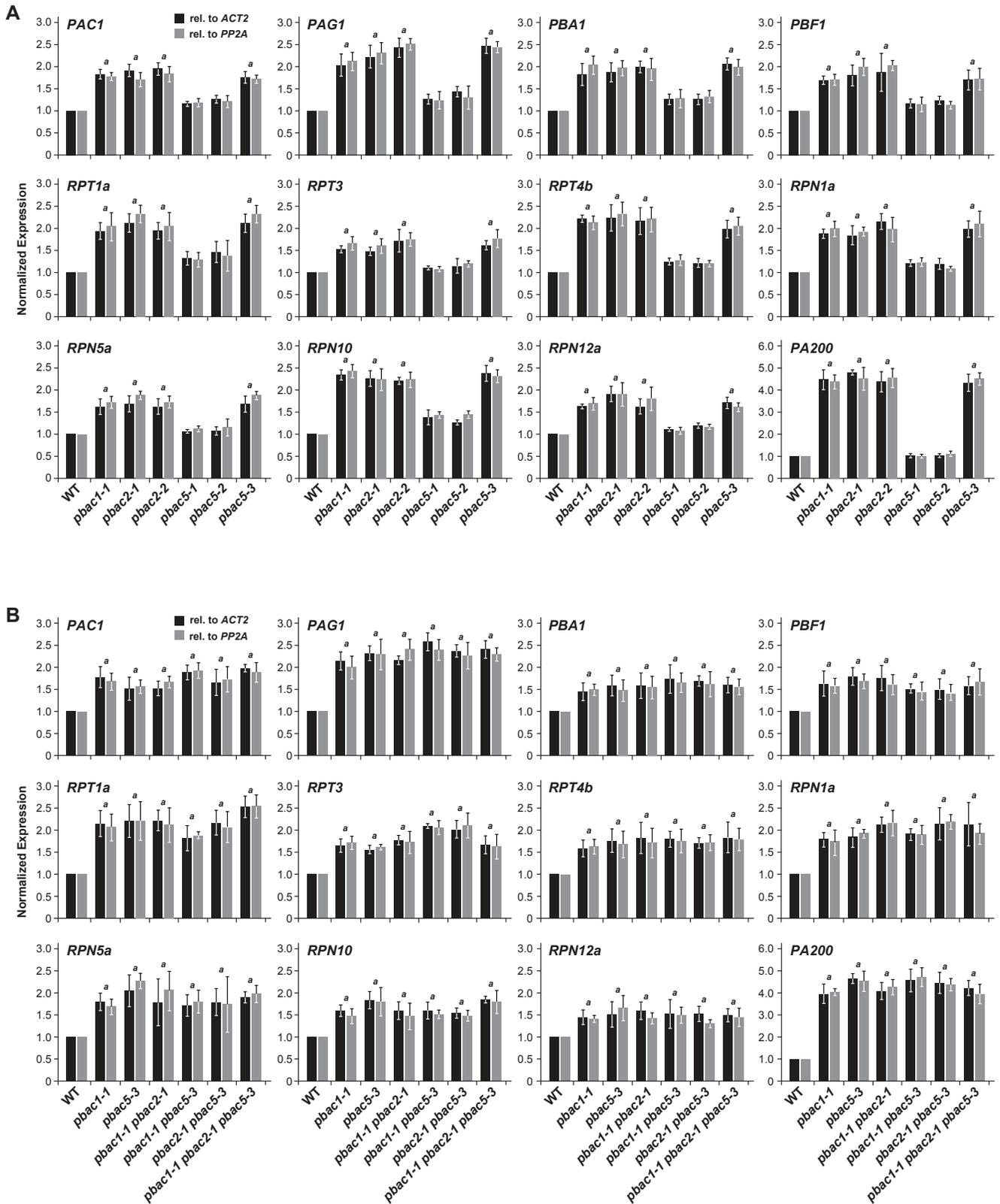


Figure S5. Elevated levels of mRNA transcripts encoding 26S proteasome subunits and PA200 in *pbac1*, *pbac2* and/or *pbac5* mutant seedlings. Total RNA was extracted from 7-day-old single (A) or higher order (B) *pbac1*, *pbac2* and/or *pbac5* mutant seedlings, and converted to first-strand cDNA. Relative transcript abundance of the CP α -subunits *PAC1* and *PAG1*, the CP β -subunits *PBA1* and *PBF1*, the RP subunits *RPT1a*, *RPT3*, *RPT4b*, *RPN1a*, *RPN5a*, *RPN10* and *RPN12a*, and the accessory factor *PA200* was determined by qRT-PCR, using the *ACT2* and *PP2A* genes as internal reference standards. All data points were normalized to WT seedlings. The bars represent mean (\pm SD) from three independent biological replicates, each with three technical replicates. The letters identify values that were significantly different from WT, as determined by one-way ANOVA followed by Tukey's post hoc test (p -value < 0.05).

Figure S6. Marshall et al.

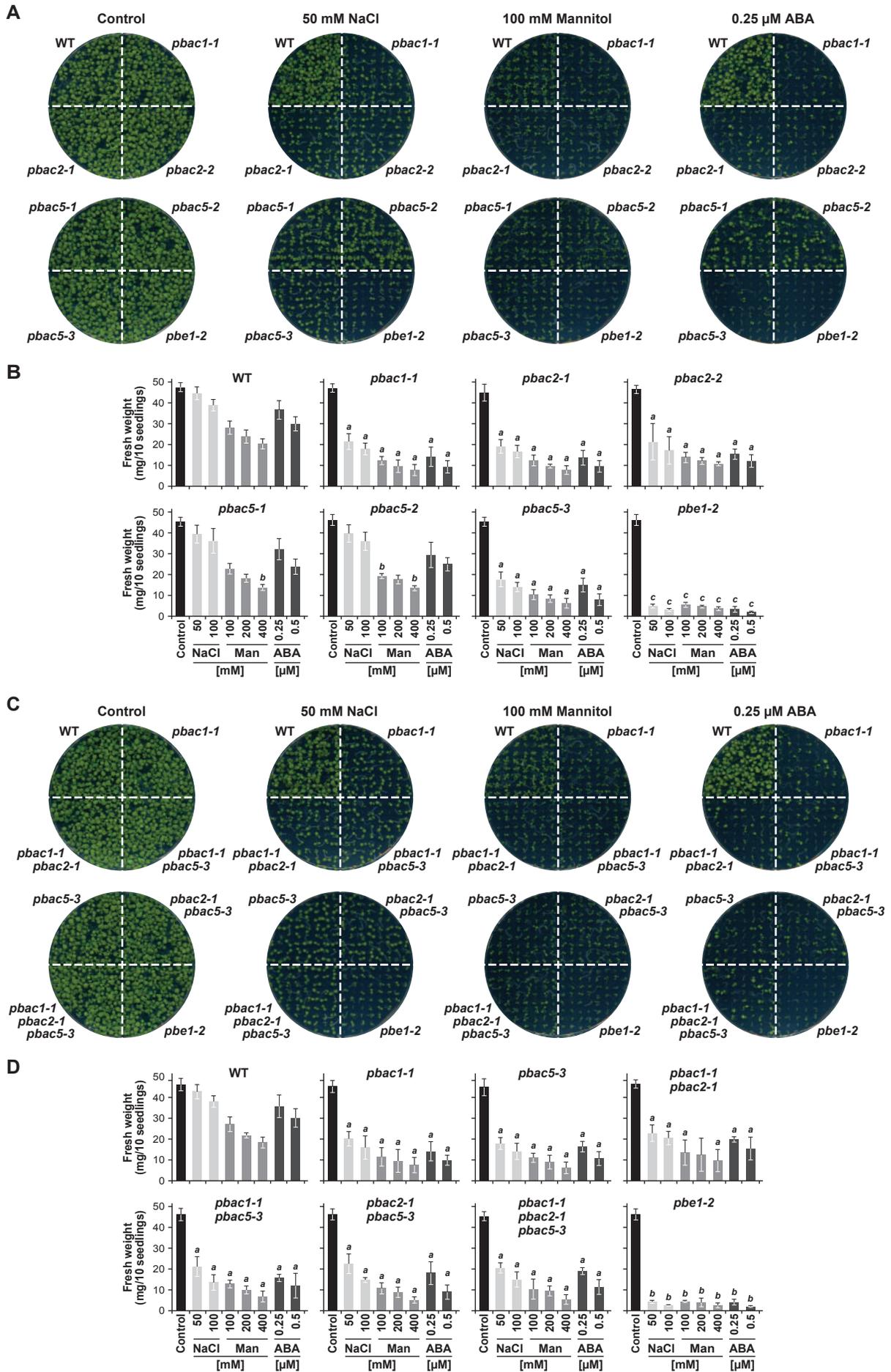


Figure S6. *Arabidopsis* seedlings missing PBAC1, PBAC2 and/or PBAC5 are hypersensitive to salt and drought stress. (A) and (C) Homozygous seedlings of the indicated genotypes were grown for 10 days on solid GM medium containing either methanol (control), 50 mM NaCl, 100 mM mannitol, or 0.25 μ M ABA. The *pbe1-2* mutant (Han et al., 2019) was used as a positive control. (B) and (D) Quantification of seedling sensitivity to various concentrations of NaCl, mannitol or ABA. Shown is the fresh weight of 10 seedlings of the indicated genotypes grown as in (A) and (C). Bars represent the mean (\pm SD) from three independent biological replicates. Different letters represent values that were significantly different from one another and the control, as determined by one-way ANOVA followed by the Tukey's post hoc test (p -value < 0.05).

Figure S7. Marshall *et al.*

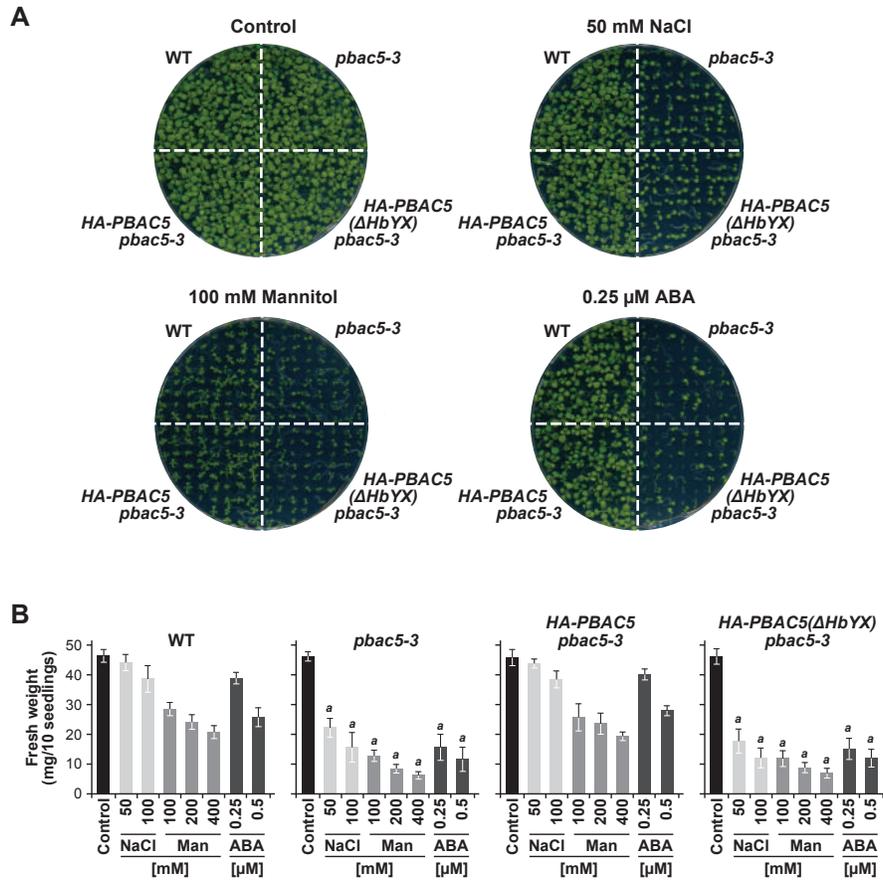


Figure S7. Hypersensitivity of the *pbac5-3* mutant to salt and drought stress could be rescued with full-length HA-PBAC5. The *pbac5-3* mutant was complemented with a gene encoding full-length HA-PBAC5 or HA-PBAC5(Δ HbYX) missing the C-terminal HbYX motif, as described in Fig. 5. (A) Homozygous seedlings of the indicated genotypes were grown for 10 days on solid GM medium containing either methanol (control), 50 mM NaCl or 100 mM mannitol, or 0.25 μ M ABA. The *pbe1-2* mutant (Han *et al.*, 2019) was used as a positive control. (B) Quantification of seedling sensitivity to various concentrations of NaCl, mannitol or ABA. Shown is the fresh weight of 10 seedlings of the indicated genotypes grown as in (A). Bars represent the mean (\pm SD) from three independent biological replicates. Different letters represent values that were significantly different from one another and the control, as determined by one-way ANOVA followed by the Tukey's post hoc test (p -value < 0.05).

Figure S8. Marshall et al.

At PBAC5 MKV-----AEKIVFLWN^SDGFAATISDTI^NPS^SSSPT-----RKLEE^OI^OL^PLD-- 45
Cp PBAC5 MKV-----APKIFLFRD^PEGFCTAIAESI^NPN^NNSTL-----RRQESFES^STE 45
Rc PBAC5 MKV-----AQNVFLFKD^SDGFASAVANAL^CPS^NNTSF-----HRLEEF^ESL^TLD 45
Bd PBAC5 MRV-----ASRVVFLVRD^AAGYGAALADAL^RPP^G-L-----TRDSSPLE^LPLD 43
Zm PBAC5 MRV-----APRVFLVLRD^AAGYGAALADAL^RPP^G-L-----TRESSPFE^LPLG 43
Pp PBAC5 MGVA-----GEEV^VLDYDGGKVG^VTRFSEAL^VPEASPTL-----QRV--VCN^LEWKDP 49
Gp PBAC5 MYAGR-----SGSPRT----- 12
Pi PBAC5 MPA-----PSL-----ASRVY^AFD^LLSRA^KGHV^LHF^AKFDGAQA^T-----FVP 39
Bf PBAC5 MF-----KHHV^LAY^TDLQA^GSRALL^GTD^E-----HHKYEYRT^LPVP 39
Sa PBAC5 MPPLAT-----TILHNQOD^TMFKIV^SSEAA^TQC-----GPTV^VFHAH^DQACIS^NRALYL^IDAGCK^NSI-----VDEY^TLKDG^SEASIK^PIT 76
Bm PBAC5 MTTAT-----FTLNNEQN^VHL-----HPKVI^LGFP^NCIA^GTRLLNS^IONST^DAKL-----NFDGYEAE^EPI 59
Nc PBAC5 MIKISE-----KL-----NKYVI^LAYC^DVSLF^GPKVAL^TVNTNNS^AVK-----VECP^LLDDK^VKEY^ODKKIE 56
At PBAC1 MEDVLTENP-----PPSRFF^OE^DLNNF^VQPPES^LPS^FI^FSNPK^EL^PL-----RPSLL^II-----A^ISSPS^LY^IFHSL^SSKT^LLGSL^IMP-----EV^EFS 82
Hs PAC1 MAATFFGEV^VKAPCRAGTEDEE-----EEEEGR^RET^PEDREV^RIQ^L-----ARKREV^LIR^RO^TK^TS^L----- 58
Sc Pba1 MLFKQW-----NDL----- 9
At PBAC2 MEFVVE-----EG-KLVNED-----CSTL^VVP^AL^SIG^NV^QLAV^DL^IV^SST^GAER-----VGYL^DDP^NL^LFC^V 57
Hs PAC2 MFPVPCG-----ESAPD^L-----AGFT^LLMP^AV^SV^GNU^QLAM^DL^IIST^LN^MSK-----IGY^FY^TDC^LV^EMV 56
Sc Pba2 MSC-----LV^DPL^VSV^GNI^PQ^LS^IDI^WL^INS^QANEW-----EY^LEAL^SKY^LVE^FV 45

At PBAC5 -----KYC^VEGV^ETGG^SIV^HF-----VDENE^VYO^VS^IFL^LRS^YE^PVP--L 83
Cp PBAC5 -----HYG^IKD^HKA^GNI^LH^F-----IDDD^DIY^RV^SFL^LQ^SY^KP^PI--L 83
Rc PBAC5 -----KYG^IKDL^KAI^GNLI^HF-----VDSG^NYO^VS^VLL^LE^KY^EP^T-L 83
Bd PBAC5 -----KXG^LKGE^KAS^GELV^SF-----SDSN^CSP^OV^SFF^VPD^YK^PVP--A 81
Zm PBAC5 -----KYCLD^GEK^ACAE^LLS^F-----SDSSG^SPO^VT^IFV^LPD^YK^PPL--A 81
Pp PBAC5 NG-----EVLT^KSE^VVNF-----KDNAG^LTK^VS^LI^VF^ROE^VTS 83
Gp PBAC5 -----OATV^ETLL^TAEL^ST^FVO^IEQ^WP-----GAAQ^VSL^VI^NI^AV^EG 29
Pi PBAC5 -----PACV^RCR^PCA^PAE^QE^FO^PL-----GNOA^MAIT^VNT^AE^LO^AS 78
Bf PBAC5 -----EAD^ECE^VPD^LK^RV^THIL^NAH-----QILN^CKY^EEP^SV^VV^VNAV^VGGG 86
Sa PBAC5 -----ADLD^GEE-----RIV^VKN^F-----GVAO^VS^VDC^LLA^HI^RE 111
Bm PBAC5 -----ADLD^GEE-----RIV^VKN^F-----DSLE^TS^VI^VIN^VLP^SDK 90
Nc PBAC5 -----KYN^NE-----LLE^ET^CSN^LEP^IS^EF^OD^E-SD-----FIVIQ^NE^KD^SLIT^TAR^LPE^V 105
At PBAC1 -----GN-----TM^EPS^LED^KSC^NI^YSL^S-----ADD^KN^LTLL^VS^VQ^LEV^S--P 120
Hs PAC1 -----EVS^LE^KY^PCK^FI^IA^IGN^NAV^AFL^SSV^MNS^GV^WEE^VG^CAK^LW^NE^RCT^TDT^HLS^STE^AFC^VF^YH^LKS^NPS^VFL^CQC^SC^YVA^ED 144
Sc Pba1 -----PEPK^HLD^LPE^ISK^NL^QSL-----EVCP^VPK^VE^FFP^DL-----DVPO^YST^AVIT^TK^I 57
At PBAC2 -----GN-----DAY^GPL^CGC^EI^AL^PLEV^Y-----ESS^SAT^TLA^QRS^RV^AK--G 96
Hs PAC2 -----GNN-----PYAT^TEG^NST^EL^SINAE^VY-----SLPS^RK^LV^AQL^RS^IF^IK--Y 97
Sc Pba2 -----GPL^DR^PED^GSD^SL^KD^ADM^KY^SSA^LEV^F-----Y^NKK^RGL^FAI^QRT^ELV^SV^NY 94

At PBAC5 VC--AMNE^LLD^LIT^RG-----SST^LPT^II^APF^VAA^SKL^KF^NR-----SLEAN^RKAS^LH^YVO-----VAT^ET 140
Cp PBAC5 VC--AVNE^VAK^LAGE^I-----PST^MPT^VL^APF^IVE^SKL^KCEN-----SLT^TNS--SSL^VGI^O-----IG^PER 139
Rc PBAC5 VC--AVSE^VTM^VES-----SLS^IPA^LI^VPF^VGM^ASK^LKH^ET-----SATS^NDGR^AS^FY^GVO-----IG^PET 139
Bd PBAC5 AC--AMNE^LLA^ISS^EA-----SPV^OPI^IIV^PIT^RSS^SY^GM^VR-----TTKT^GQL^ST^LMG^SE-----IG^ATN 138
Zm PBAC5 AC--VVNG^VLE^ISS^EA-----TST^ERT^LIV^PY^ITR^SSY^HH^GTE-----HAT^KAS--TVL^HGA^E-----IG^AT 137
Pp PBAC5 ACGR^VVHD^LFE^YLL^SNA^P-----IQSP^GFS^IV^VPA^ISR^MPG^SV^LN^MD-----TGNN^AN^IAA^F-----FN^RTS 141
Gp PBAC5 TOAE^VAAAL^LDM^LTS^AP^GGG^GGG^GAA^AGG^GGG^GSK^AVT^VAG^ML^MQH^VS^QPA^A-----LX^QLO-----LNG^AK 91
Pi PBAC5 QRWEL^ADAL^VSALS^OAD-----VQEL^TIA^AL^HLP^AKE^GGL^N-----V^FY^SG-----LN^GHV 126
Bf PBAC5 VCH^HV^SRL^LDE^CLR^SG-----VEK^ITA^VAAL^HFP^HPG^EL-----L^VETS-----FF^TO 131
Sa PBAC5 CAHV^AME^LDM^FVT-----SQ^{LL}LV^SA^YH^FST^GV^NSS^D-----VHA^AT-----LD^TSL 156
Bm PBAC5 KLLAL^ADAL^VNF^ISS^E-----IQ^TLY^VGA^FDF^TASK^ES-----LH^IAS-----LNT^DA 135
Nc PBAC5 TNALL^SKS^LEL^LFN^VNS-----VES^VI^ISS^QDM^NIP^SDK^K-----TG^YST^SGK 151
At PBAC1 -----ERS^NLV^SRL^IIA^KDI-----IP^ERV^II^DSI^QSR^NFR^GRL^SPD^E-----TLA^AK-----LET^SA 168
Hs PAC1 QYQ^WLE^KV^GSC^PR^KN-----MQ^ITI^LTC^RH^VTD^YK^TSE^TSG^S-----LP^SPF^LRA-----LKT^QN 193
Sc Pba1 MNPL^FPKN^LQ^TS^IGE-----IK^TTL^VK^SSL^PQS^GK^HSW^NYD^EN^FPN^EV^DP^QK^ND^AET^VGF^S-----L^VIS^VST^EG--V^TA 121
At PBAC2 MM^IK^FA^EN^IN^FA^ASS^G-----KK^HI^IV^SSL^DQ^RL^HN^LDM^SR^G-----PO^VY^LLS^NAE^SD^GR^DD^HCE^RL^GF^RL^HE^YD 166
Hs PAC2 KSK^PFE^KL^SW^VK^SSG-----CAR^VI^VSS^SHS^YQ^RND^LQ^LRS^T-----PFR^LL^TPS^MQ^SV^QN^KKS-----LN^WEE 162
Sc Pba2 LNN^FIVE^IT^LP^LSK^YN-----ISE^IC^WDS^LY^AME^DENG^VIV^RP-----QEV^VSL^GE^FY^F--DDE^AEL^LSN^LHL^ND^QE 156

At PBAC5 ETS^RL^FAS^R-----IEK^PPL^SMO^TH^YE^PT^SC-----L^HL^AR^VK^RL^PT^A-----I^LI^GORS^NS--L^TH 191
Cp PBAC5 DIT^MAM^TTK-----IQ^KPP^SSL^OI^YSE^PLS^C-----FL^QLV^HIL^KL^PT^V-----V^LI^GHS^SQ^S--L^SN 190
Rc PBAC5 DITS^AIV^RR-----TK^KPP^SSL^OI^HFE^PL^AC-----F^LQ^VVR^IL^KL^PT^V-----L^LF^GRL^SDK--A^AG 190
Bd PBAC5 EFT^HML^VDG-----TT^KPP^SSL^OI^RSE^PIL^C-----L^LEM^VC^VL^KI^PT^V-----L^LV^AS^GG^QQ--O^GK 189
Zm PBAC5 DYT^HLL^VGG-----TT^KPP^SSL^OI^RSE^PIL^C-----L^LEM^VR^VL^KL^PT^V-----L^LV^TS^GG^QQ--O^GK 188
Pp PBAC5 EGSG^NLDG-----LP^KLS^SFA^VR^DGL^AY-----V^FHY^VHAT^QL^PT^L-----V^LV^SP^TEG--V^TA 191
Gp PBAC5 PLD^PSL^PQ^L-----V^CSG^TG^APS^AAAAA^RVR^DG^OL^AA-----L^HV^AA^VSG^TPL^A-----C^LL^AF^GH^KPP^AAS 153
Pi PBAC5 EKDA^KLS^L-----AD^PSW^EW^KDP^WLS^A-----F^HL^IK^VEQ^WRS-----H^LL^AK^YK^P--G 172
Bf PBAC5 -----PV-----TSAP^AL^PDD^LOV^SDP^IN^L-----F^LVO^FLL^VDS^LPT^V-----F^LV^VP^GH^KA--TAG 176
Sa PBAC5 PQDM^NISS^F-----PT-----L^PT^SMR^VN^PPL^LGA-----V^NGL^SVE^DL^PT^V-----I^LAT^SSR^RP--N 205
Bm PBAC5 QLQ-----YPT-----L^NTA^APL^NN^LFL^NT-----L^ITL^LOLE^NIS^TQ-----F^LLY^PSK^FF--K 178
Nc PBAC5 -----LM^VEN-----VE^ILD^NN^IPM^NSS^FL^NT-----L^AILL^NIK^NI^PT^I-----Y^LT^CL^GK^KI--N 195
At PBAC1 EKKA^THT^TGL^VN^LD^YFP^SG-----S^VI^DGL^SAS-----L^SRC^QL^KN^IR^GT-----L^VIS^VW^FEP^G-- 220
Hs PAC1 FKDS^ACC^PLE^Q-----PN-----V^HDL^PAA-----V^ISY^CO^VWK^IPA^LLY^LC^YTD^VM^KLD^LIT^VE^AFK^PI--L^ST 256
Sc Pba1 SEGR^CWK^Y-----F^PI-----Y^SFG^KTL^LFS^MEEN^FIS^IS-----PI^FG--NM^IRS^IS^OLA^OF--SP^DI--IV^IGT^SDK^I--A^SM 177
At PBAC2 -----LSS^VFE^KNS^VEEL^APS^EDE^LED^ID^YPS^LPP^A-----A^MFS^AFK^AR^GL^KM^TCL^L-----CY^CSE^GDN^IP 234
Hs PAC2 MEK^SRC-----I^FE-----I^DD^SE^FCI-----RIP^GGG^IT^KT^T-----Y^DES^CSK^EI^QMA^VLL-----K^FV^SE^GDN^IP 215
Sc Pba2 SMV^NN^WL^HF-----T^ET-----SF^QDK^IS^VD^QIF^KI-----F^QIL^NAS^OR^EKA-----L^RSI^KY^CS^LAN^EG^DNS--L^DS 221

At PBAC5 KALD-----EEL^QV^IH^ET^GEL^VA-----SW^TGL^SFS-----RDR^IK^WSA-----SK^TS^KEES^PW^RA-----L^VG 241
Cp PBAC5 KALQ-----EEL^QV^IY^KI^GEL^LA-----SST^GL^HFS-----KEK^VI^WNP-----IK^TSK^DEAP^WRA-----L^VG 240
Rc PBAC5 KELE-----LIF^QIL^SEM^GEL^LA-----S^TMS^LS^FS-----REK^IT^WNP-----AA^NTS^KD^IKE^PW^RA-----L^VG 241
Bd PBAC5 SSSD-----TD^LEV^LQ^CL^GD^HL^G-----RH^IN^LEF^S-----KQ^AV^LER^G-----VE^KL^PT^VEE^PW^RE-----L^VR 239
Zm PBAC5 SSTV-----SD^LEV^LQ^CL^GER^LA-----K^HT^ALE^FS-----K^GT^VL^KR^G-----I^EK^SR^ID^QEP^WRE-----L^VG 238
Pp PBAC5 QKN-----ES^NEV^LLA^LG^DV^VG-----RQ^IGL^TCS-----KQ^ELN^HVT--SL^IP^LASS^MED^DWR-----L^VI 242
Gp PBAC5 LVLP-----DS^AAC^DAL^GAA^VA-----AAL^GLS^YD--AA^ACR^TV^RAS^CK^FV--P^ESA^AGS^DI-----M^VL 206
Pi PBAC5 RDLS-----G^TYEA^VAA^LQ^AAL^QL^F-----TK^DK^VT^VD-----SQ^EV^OR^GL^PRR^L--AM^ET^AST^TG^DD^HL^LT-----L^VH 231
Bf PBAC5 AAT^VAD-----G^SLAN^ISL^QD^TL^T-----S^IT^GL^RS^F-----G^DI^SCS^LLY^KG-----T^NNP^OEV^MVD^L-----L^VM 229
Sa PBAC5 KHT^HS-----DEC^VAI^RK^QAA^VSS-----L^VSE^VT^N-----T^PDR^DSE^IP--T^IE^LT^AAY^KLE^OEA^RT^Q-----M^YO 263
Bm PBAC5 TG^TK-----DD^VOL^LT^SEQ^LT-----Q^IF^GT^VI-----R^ER^LDS^LEM^KS--SE^AG^SSM-----M^VL 225
Nc PBAC5 KDTQ-----L^EON^QK^LM^RN^TLN-----S^TIG^LT^KDI-----S^PK^LND^SLS^QRT-----E^EL-----L^VI 241
At PBAC1 RSLK-----GL^VKN^IPS^TEL^LK^LM-----I^VPS^LDA-----S^VN^RD^LE^KNS--S^RT^GL^KK^DT^WLD^SD-----L^VT 267
Hs PAC1 KVM^TEN^ECT^LQP^PE^TIG^FLS^VL^TQ^LIV^GPS^GKL^KK^LCL^VAP^SEG^PNG^FE^KLS^DMG^SL^DCG^QW^LGF^EPS^RY^SE^EC^YRL^WR^CDS^AA^IGA^OSG^LY^I 276
Sc Pba1 -----EAF^LLA^BASS-----K^LM^GL^TPD--K^FH-----G^EE^GG^KQ^VD^IPS^YSW^KS-----M^YGA--P^PDM^SMF 281
At PBAC2 -----DAL^GLV^EY^NEW^LQ-----I^LK^PL^SDD-----P--T^VS^ASR^WK^IPS^WRL-----L^FG^SGL^PP--A^LF 264
Sc Pba2 QQ^FL^QW^I-----IS^QV^IKN^APP^IV^K-----F^VR^PIS^QG^AY^GM^AD^AR^DK^FV-----D-----L^VN 267

Figure S8. Amino acid sequence comparisons of representative members of the PBAC5 chaperone family from various plants and a variety of fungal, metazoan and oomycete species. Identical (50% threshold value) and similar amino acids are shown with black and grey backgrounds, respectively. Representatives of the PBAC1 and PBAC2 families were included for comparison. The position of the C-terminal HbYX motif is indicated by the red line. Amino acid numbers are given on the right. A phylogenetic tree of the expanded PBAC5 family and its relationship to representatives from the PBAC1 and PBAC2 family in eukaryotes, and the PBAC family in bacteria, can be found in Fig. 8A. Species abbreviations are given in the Materials and Methods. Accession numbers for all proteins analyzed in this Figure are listed in Table S1.

TABLE S1: Accession Numbers and Sequences of Proteins Used in This Study.

[Click here to Download Table S1](#)

TABLE S2: T-DNA Insertion Lines Used in This Study.

Gene name	Locus ^a	Line name	Line ID ^b	Insertion site	Ecotype ^c	Reference
<i>NAC53</i>	At3g10500	<i>nac53-1</i>	SALK_009578	3 rd exon	Col-0	Gladman <i>et al.</i> 2016
<i>NAC78</i>	At5g04410	<i>nac78-1</i>	SALK_025098	2 nd exon	Col-0	Gladman <i>et al.</i> 2016
<i>PBAC1</i>	At3g25545	<i>pbac1-1</i>	SALK_112630	2 nd exon	Col-0	This study
<i>PBAC2</i>	At3g18940	<i>pbac2-1</i>	SAIL_598_E03	2 nd intron	Col-3	This study
		<i>pbac2-2</i>	SAIL_447_D02	2 nd intron	Col-3	This study
<i>PBAC5</i>	At3g07640	<i>pbac5-1</i>	SALK_044042	5'-UTR	Col-0	This study
		<i>pbac5-2</i>	SALK_027892	1 st intron	Col-0	This study
		<i>pbac5-3</i>	GABI_041G01	2 nd intron	Col-0	This study
<i>PBE1</i>	At1g13060	<i>pbe1-2</i>	SALK_092686	7 th exon	Col-0	Han <i>et al.</i> , 2019

^a Locus identifiers are taken from the *Arabidopsis* Information Resource (www.arabidopsis.org).

^b T-DNA collections are described in O'Malley *et al.*, (2015). *Methods Mol. Biol.* 1284, 323 – 342.

^c Lines were backcrossed at least three times to the respective wild type before analysis.

TABLE S3: Transgenic Plant Lines Used in This Study.

Genotype^a	Construct type	Transformation vector^b	Ecotype	Antibiotic resistance	Reference
<i>pbac5-3 UBQ10::HA-PBAC5</i>	Genomic	pMDC99	Col-0	Hygromycin	This study
<i>pbac5-3 UBQ10::HA-PBAC5(ΔHbYX)</i>	Genomic	pMDC99	Col-0	Hygromycin	This study

^a All constructs were transformed into the indicated ecotype via the floral dip method (Clough and Bent, 1998. *Plant J.* 16, 735 – 743).

^b Plant transformation vectors are described in Curtis and Grossniklaus (2003). *Plant Physiol.* 133, 462 – 469 and Suttangkakul *et al.* (2011) *Plant Cell* 23, 3761 – 3779.

TABLE S4: Oligonucleotide Primers Used in This Study.

Primer name	Sequence (5' to 3')	Comment
SALK LBa1	TGGTTCACGTAGTGGGCCATCG	For genotyping T-DNA mutants from the SALK collection
SALK LBb1.3	ATTTTGCCGATTCGGAAC	
SAIL LB1	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC	For genotyping T-DNA mutants from the SAIL collection
GABI o8474	ATAATAACGCTGCGGACATCTACATTTT	For genotyping T-DNA mutants from the GABI collection
<i>nac53-1</i> LP	TATGGGTCGTGGCTCAGTAAC	For genotyping the <i>nac53-1</i> allele
<i>nac53-1</i> RP	GATTCTGCTGGTTGCTCAAAG	
<i>nac78-1</i> LP	TCTTTCGCATTTGCGATATTC	For genotyping the <i>nac78-1</i> allele
<i>nac78-1</i> RP	TTCAAGTTCTGGTTTTACCG	
<i>pbac1-1</i> LP	GGGAGTGTAATCGACGGTCT	For genotyping the <i>pbac1-1</i> allele
<i>pbac1-1</i> RP	CCTAGTAAGCATAGAAACATGAAACAA	
<i>pbac2-1/2</i> LP	TATTGGGCTTTAATATGGCCC	For genotyping the <i>pbac2-1</i> and <i>pbac2-2</i> alleles
<i>pbac2-1</i> RP	GTCCCAACACAAAAGCATTG	
<i>pbac2-2</i> RP	CTTCTTTCCACTTGAAGCAGC	
<i>pbac5-1</i> LP	TTGTGACAGAGATCCGCTTG	For genotyping the <i>pbac5-1</i> allele
<i>pbac5-1</i> RP	TCGAGAAAGGACGAGTCGAG	
<i>pbac5-2</i> LP	AAGTATGGCGTTGAAGGTGTG	For genotyping the <i>pbac5-2</i> allele
<i>pbac5-2</i> RP	TCGGCAAATACAATTTAATGAAGA	

<i>pbac5-3</i> LP	ACCACAAGAACTTGGCATTG	For genotyping the <i>pbac5-3</i> allele
<i>pbac5-3</i> RP	TCAGCTCTCACGAAAACCTTC	
<i>pbe1-2</i> LP	GAATGCGATGTGAAATTTTGG	For genotyping the <i>pbe1-2</i> allele
<i>pbe1-2</i> RP	AGAGAATAATCCGCGAGCAAC	
RT <i>PBAC1</i> P1	GCCGAGAAGACTGAAACCAA	For RT-PCR analysis of the <i>PBAC1</i> gene
RT <i>PBAC1</i> P2	GGAGATGGAAGGGATTTCAGG	
RT <i>PBAC1</i> P3	GGATTCCATTCAGAGCCGTA	
RT <i>PBAC1</i> P4	CACACTCGCAACATCCAAAC	
RT <i>PBAC2</i> P5	GAACGCAGGCAGAGATTCTT	For RT-PCR analysis of the <i>PBAC2</i> gene
RT <i>PBAC2</i> P6	AAAGTCGAGCAATCTTCATTCA	
RT <i>PBAC2</i> P7	AGGCTTGGGTTTGGGAAGATT	
RT <i>PBAC2</i> P8	TCAGGAGTCAGACCCATAAGC	
RT <i>PBAC5</i> P9	CAGGATTAACAGATTCGCTCA	For RT-PCR analysis of the <i>PBAC5</i> gene
RT <i>PBAC5</i> P10	AATGATTCCGATGGATTTGC	
RT <i>PBAC5</i> P11	GATACTCCCGCCGGTTTC	
RT <i>PBAC5</i> P12	CGCCACAAAGAAAGGAGCTA	
RT <i>PBAC5</i> P13	GTGTAAACGCCTGCCTACC	
RT <i>PBAC5</i> P14	AGCAGAGTGACGTGGATTTG	
RT <i>PAC1</i> LP	CTCGCCAGAAGGTCGTCTTT	For qRT-PCR analysis of the <i>PAC1</i> gene
RT <i>PAC1</i> RP	ATGGTGCTTGTCCTATCCTG	
RT <i>PAG1</i> LP	CCGTCACCACTTTCTCTCCC	For qRT-PCR analysis of the <i>PAG1</i> gene
RT <i>PAG1</i> RP	CCTGAGCCACCAATACAGGG	

RT <i>PBA1</i> LP	CGCTACTTCCTTCACCAGCA	For qRT-PCR analysis of the <i>PBA1</i> gene
RT <i>PBA1</i> RP	CCTCCAATGGCAAACGGTTG	
RT <i>PBF1</i> LP	TCGCGATTACTCCAAAATCC	For qRT-PCR analysis of the <i>PBF1</i> gene
RT <i>PBF1</i> RP	GGCGTGTTTGAATCCTGTTT	
RT <i>RPT1a</i> LP	CGATTTGGAAATCCGGCGAC	For qRT-PCR analysis of the <i>RPT1a</i> gene
RT <i>RPT1a</i> RP	TGATCTTCTTGGCGAGATCCT	
RT <i>RPT3</i> LP	TCTTCACCGTCACTCCAACG	For qRT-PCR analysis of the <i>RPT3</i> gene
RT <i>RPT3</i> RP	ACTTCCCTATCGGCTCCTGT	
RT <i>RPT4b</i> LP	TGTTCGCAATCAGAGCAGAG	For qRT-PCR analysis of the <i>RPT4b</i> gene
RT <i>RPT4b</i> RP	CAGCTTTCTCACAGCCTTCA	
RT <i>RPN1a</i> LP	TCGATAGCACAAGCACAAGC	For qRT-PCR analysis of the <i>RPN1a</i> gene
RT <i>RPN1a</i> RP	GTTGAAGCTCGGATTTCTGT	
RT <i>RPN5a</i> LP	TCACAAAGCCGCTCTCAGAC	For qRT-PCR analysis of the <i>RPN5a</i> gene
RT <i>RPN5a</i> RP	CCAAAACTAATCCACCAACACTGA	
RT <i>RPN10</i> LP	AACCGCAGCTATCCAGATCG	For qRT-PCR analysis of the <i>RPN10</i> gene
RT <i>RPN10</i> RP	ACATAGCCACTTGACCCCTC	
RT <i>RPN12a</i> LP	TCCTTCATGGAGGGTGCCTA	For qRT-PCR analysis of the <i>RPN12a</i> gene
RT <i>RPN12a</i> RP	ACGGAATCTCTTTGCACGGT	
RT <i>PA200</i> LP	GTTTGTCTCGTCACCCTGCT	For qRT-PCR analysis of the <i>PA200</i> gene
RT <i>PA200</i> RP	GCCACCTGCTCATCCTTAGA	
RT <i>PBAC5</i> LP	CGAAGAGCTTCAGGTGATCC	For qRT-PCR analysis of the <i>PBAC5</i> gene
RT <i>PBAC5</i> RP	AGTGTTGGGCCATGAAAAAG	

RT <i>ACT2</i> LP	GGCATCACACTTTCTACAATGAGC	For qRT-PCR analysis of the <i>ACT2</i> gene
RT <i>ACT2</i> RP	ACCCTCGTAGATTGGCACAG	
RT <i>PP2A</i> LP	AAGCTTGGTGCTCTTTGCAT	For qRT-PCR analysis of the <i>PP2A</i> gene
RT <i>PP2A</i> RP	CCATTACTGGAGCGAGAAGC	
<i>PBAC1</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGG AAGATGTACTTACC	For cloning the full-length untagged <i>PBAC1</i> gene
<i>PBAC1</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTATCATGT ATATAGATCAGA	
<i>PBAC2</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGG AGTTTGTTGTTGAA	For cloning the full-length untagged <i>PBAC2</i> gene
<i>PBAC2</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAATAA CATAGACATATC	
<i>PBAC3</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGG AAAGCTTGGACACTAAT	For cloning the full-length untagged <i>PBAC3</i> gene
<i>PBAC3</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTATTACCA AAGGCGATTCTC	
<i>PBAC4</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGG AAGACTGAATACGAC	For cloning the full-length untagged <i>PBAC4</i> gene
<i>PBAC4</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTATTATGT TGCTCTTACAGG	
<i>PBAC5</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGA AAGTAGCGGAGAAG	For cloning the full-length untagged <i>PBAC5</i> gene
<i>PBAC5</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTATTAGCC ATAAAGAGCACG	
<i>PBAC5 gen</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCAAAA ACAGGATTAACAGATTCGCT	For cloning the full genomic sequence of the <i>PBAC5</i> gene
<i>PBAC5 gen</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTCTG AGAGTTTAAGATTTGGTTGA	
<i>PBAC5 ΔHbYX</i> LP	AAAGTCCGTGGCGTGCTTAATCTTCACAGGTCAG	For deleting the <i>PBAC5</i> HbYX motif by site-

<i>PBAC5 ΔHbYX</i> RP	CTGACCTGTGAAGATTAAGCACGCCACGGACTTT	directed mutagenesis
KanB	CTGCAGCGAGGAGCCGTAAT	For genotyping all yeast deletion strains
KanC	TGATTTTGATGACGAGCGTAA	
<i>PBA1-A</i>	ATTGAAATCAATTTAAGAAGTTGCG	For genotyping <i>Δpba1</i> deletion strains
<i>PBA1-B</i>	ACTATTATATCAGGGGAGAACTGGG	
<i>PBA1-C</i>	CCCAGTTCTCCCCTGATATAATAGT	
<i>PBA1-D</i>	AAAGAAGGACAAAAAGGAAAAGAAA	
<i>PBA2-A</i>	CCAATGATATAAAGATTTCCAATGC	For genotyping <i>Δpba2</i> deletion strains
<i>PBA2-B</i>	ACTAGGTATTTCGAATCTAACGCCT	
<i>PBA2-C</i>	TCTATATGCAATGGAGGATGAAAAT	
<i>PBA2-D</i>	ACTGCCAAATTCAAAGAGATGTTAC	
<i>doa5-1</i> LP	AGCACATTTTCCCCAGAAGG	For genotyping the <i>doa5-1</i> mutation
<i>doa5-1</i> RP	GGCACCTTCACCGAACCTTA	
<i>HA-PBA1</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGT CCCATACGATGTTCCAGATTACGCTATGCTTTTTTAA ACAATGGAATGACT	For cloning the full-length HA-tagged <i>PBA1</i> gene
<i>HA-PBA1</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATAT ATATAGGCCTGATTGTGCAC	
<i>HA-PBA2</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGT CCCATACGATGTTCCAGATTACGCTATGAGCTGCCT GGTGTTCG	For cloning the full-length HA- or FLAG-tagged <i>PBA2</i> gene
<i>FLAG-PBA2</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGG ACTACAAAGACGATGACGACAAGATGCTTTTTTAAAC AATGGAATGACT	
<i>HA/FLAG-PBA2</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAATT GTATAAATCTACAAATTT	

<i>HA-PBAC1</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGT ACCCATACGATGTTCCAGATTACGCTATGGAAGATG TACTTACCGAGA	For cloning the full-length HA- or FLAG-tagged <i>PBAC1</i> gene
<i>FLAG-PBAC1</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGG ACTACAAAGACGATGACGACAAGATGGAAGATGTA CTTACCGAGA	
<i>HA/FLAG-PBAC1</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCATGT ATATAGATCAGAGTCAAGCC	
<i>HA-PBAC2</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGT ACCCATACGATGTTCCAGATTACGCTATGGAGTTTG TTGTTGAAGAAGGA	For cloning the full-length HA- or myc-tagged <i>PBAC2</i> gene
<i>MYC-PBAC2</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGG AACAAAACTCATCTCAGAAGAGGATCTGATGGAG TTTGTGTTGAAGAAGGA	
<i>HA/MYC-PBAC2</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAAA CATAGACATATCCGGAGGA	
<i>HA-PBAC5</i> LP	GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGT ACCCATACGATGTTCCAGATTACGCTATGAAAGTAG CGGAGAAGATTGT	For cloning the full-length HA-tagged <i>PBAC5</i> gene
<i>HA-PBAC5</i> RP	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGCC ATAAAGAGCACGCCA	
<i>PBA1</i> Y275A LP	TGGTGCACAATCAGGCCTAGCTATATGAGACCCAGC TTTC	For site-directed mutagenesis of the <i>PBA1</i> HbYX motif
<i>PBA1</i> Y275A RP	GAAAGCTGGGTCTCATATAGCTAGGCCTGATTGTGC ACCA	
<i>PBA2</i> Y266A LP	GGCGGATGCAAGAGATAAATTTGTAGATTTAGCCAA TTGAGACCCAGCTTT	For site-directed mutagenesis of the <i>PBA2</i> HbYX motif
<i>PBA2</i> Y266A RP	AAAGCTGGGTCTCAATTGGCTAAATCTACAAATTTA TCTCTTGCATCCGCC	
<i>PBAC1</i> Y266A LP	TACTTGGCTTGACTCTGATCTAGCTACATGAGACCC AGCTTTC	For site-directed mutagenesis of the <i>PBAC1</i> HbYX motif
<i>PBAC1</i> Y266A RP	GAAAGCTGGGTCTCATGTAGCTAGATCAGAGTCAAG CCAAGTA	

<i>PBAC2</i> F281A LP	CTCCTCCGGATATGTCTATGGCTTAAGACCCAGCTTT CTTGT	For site-directed mutagenesis of the <i>PBAC2</i> HbF motif
<i>PBAC2</i> F281A RP	ACAAGAAAGCTGGGTCTTAAGCCATAGACATATCC GGAGGAG	
<i>PBAC5</i> Y240A LP	AAAGTCCGTGGCGTGCTCTTGCTGGCTAAGACCCAG	For site-directed mutagenesis of the <i>PBAC5</i> HbYX motif
<i>PBAC5</i> Y240A RP	CTGGGTCTTAGCCAGCAAGAGCACGCCACGGACTTT	

TABLE S5: *S. cerevisiae* Strains Used in This Study.

Strain name ^a	Genotype ^b	Reference ^c
BY4741	<i>MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i>	Brachmann <i>et al.</i> , 1998
BY4742	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Brachmann <i>et al.</i> , 1998
MaV203	<i>MATa ade2-10 his3Δ200 leu2-3, 2-112 trp1Δ901 can1^R cyh2^R gal4Δ gal80Δ gal1::lacZ lys2::pHIS3(GAL1-UAS)-HIS3 ura3::pSPAL10(GAL1UAS)-URA3</i>	Vidal <i>et al.</i> , 1996
<i>Δpba1</i>	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pba1Δ::kanMX4</i>	Dharmacon
<i>Δpba2</i>	<i>MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pba2Δ::kanMX4</i>	Dharmacon
MHY500	<i>MATa his3Δ200 leu2-3, 2-112 lys2-801 trp1-1 ura3-52 gal2</i>	Chen and Hochstrasser, 1996
MHY794	<i>MATa his3Δ200 leu2-3, 2-112 lys2-801 trp1-1 ura3-52 gal2 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1)]</i>	Chen and Hochstrasser, 1996
RSM386	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22- doa5-1 (TRP1)]</i>	This study
RSM387	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22- doa5-1 (TRP1)]</i>	This study
RSM388	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1)]</i>	This study
RSM389	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22- doa5-1 (TRP1) pGPD1-HA-ScPBA1 (LEU2)]</i>	This study
RSM390	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22- doa5-1 (TRP1) pGPD1-HA-AtPBAC1 (LEU2)]</i>	This study
RSM391	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22- doa5-1 (TRP1) pGPD1-HA-AtPBAC2 (LYS2)]</i>	This study

RSM392	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study
RSM393	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-ScPBA2 (LYS2)]</i>	This study
RSM394	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-AtPBAC1 (LEU2)]</i>	This study
RSM395	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-AtPBAC2 (LYS2)]</i>	This study
RSM396	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study
RSM397	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-ScPBA1 (LEU2) pGPD1-FLAG-ScPBA2 (LYS2)]</i>	This study
RSM398	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1 (LEU2) pGPD1-myc-AtPBAC2 (LYS2)]</i>	This study
RSM399	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1 (LEU2) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study
RSM400	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-myc-AtPBAC2 (LYS2) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study
RSM401	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1 (LEU2) pGPD1-myc-AtPBAC2 (LYS2) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study

RSM402	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-ScPBA1-Y275A (LEU2) pGPD1-FLAG-ScPBA2 (LYS2)]</i>	This study
RSM403	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-ScPBA1 (LEU2) pGPD1-FLAG-ScPBA2-Y266A (LYS2)]</i>	This study
RSM404	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-HA-ScPBA1-Y275A (LEU2) pGPD1-FLAG-ScPBA2-Y266A (LYS2)]</i>	This study
RSM405	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1-Y266A (LEU2) pGPD1-myc-AtPBAC2 (LYS2) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study
RSM406	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1 (LEU2) pGPD1-myc-AtPBAC2-F281A (LYS2) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study
RSM407	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1 (LEU2) pGPD1-myc-AtPBAC2 (LYS2) pGPD1-HA-AtPBAC5-Y240A (URA3)]</i>	This study
RSM408	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1-Y266A (LEU2) pGPD1-myc-AtPBAC2-F281A (LYS2) pGPD1-HA-AtPBAC5 (URA3)]</i>	This study
RSM409	<i>MATa/a his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1-Y266A</i>	This study

	<i>(LEU2) pGPD1-myc-AtPBAC2 (LYS2) pGPD1-HA-AtPBAC5-Y240A (URA3)]</i>	
RSM410	<i>MATa/α his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1 (LEU2) pGPD1-myc-AtPBAC2-F281A (LYS2) pGPD1-HA-AtPBAC5-Y240A (URA3)]</i>	This study
RSM411	<i>MATa/α his3Δ1/Δ200 leu2Δ0/2-3, 2-112 lys2Δ0/2-801 trp1-1 ura3Δ0/3-52 pba1Δ::kanMX4 pba2Δ::kanMX4 doa5Δ1::HIS3 [YC-pLAC22-doa5-1 (TRP1) pGPD1-FLAG-AtPBAC1-Y266A (LEU2) pGPD1-myc-AtPBAC2-F281A (LYS2) pGPD1-HA-AtPBAC5-Y240A (URA3)]</i>	This study

^a Previously described strains are named as in their relevant publication. Strains purchased from the yeast gene knockout collection are named after the gene which has been deleted. Strains generated either by mating or transformation during this study are named after the first author.

^b Following certain crosses, the mating type and the status of some selective markers was unknown. This has been indicated by showing *MATa/α* for the mating type locus, and by omitting the ambiguous selective markers.

^c References are: Brachmann *et al.* (1998) *Yeast* 14, 115 – 132; Chen and Hochstrasser (1996) *Cell* 86, 961 – 972; Vidal *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93, 10315 – 10320.

TABLE S6: Protein Identification and Quantification Data for Proteasome Affinity Purifications, Performed in Proteome Discoverer.

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