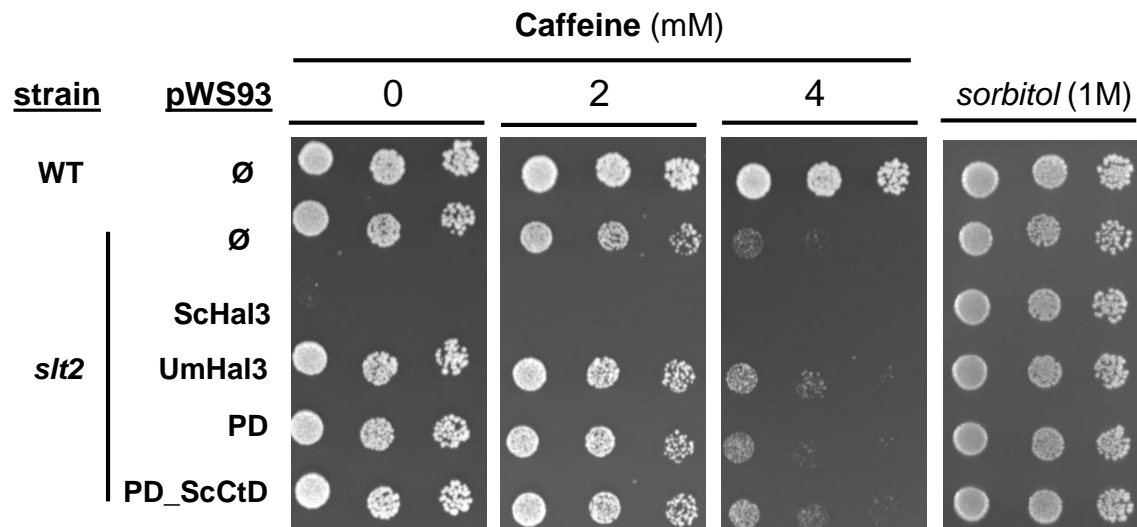
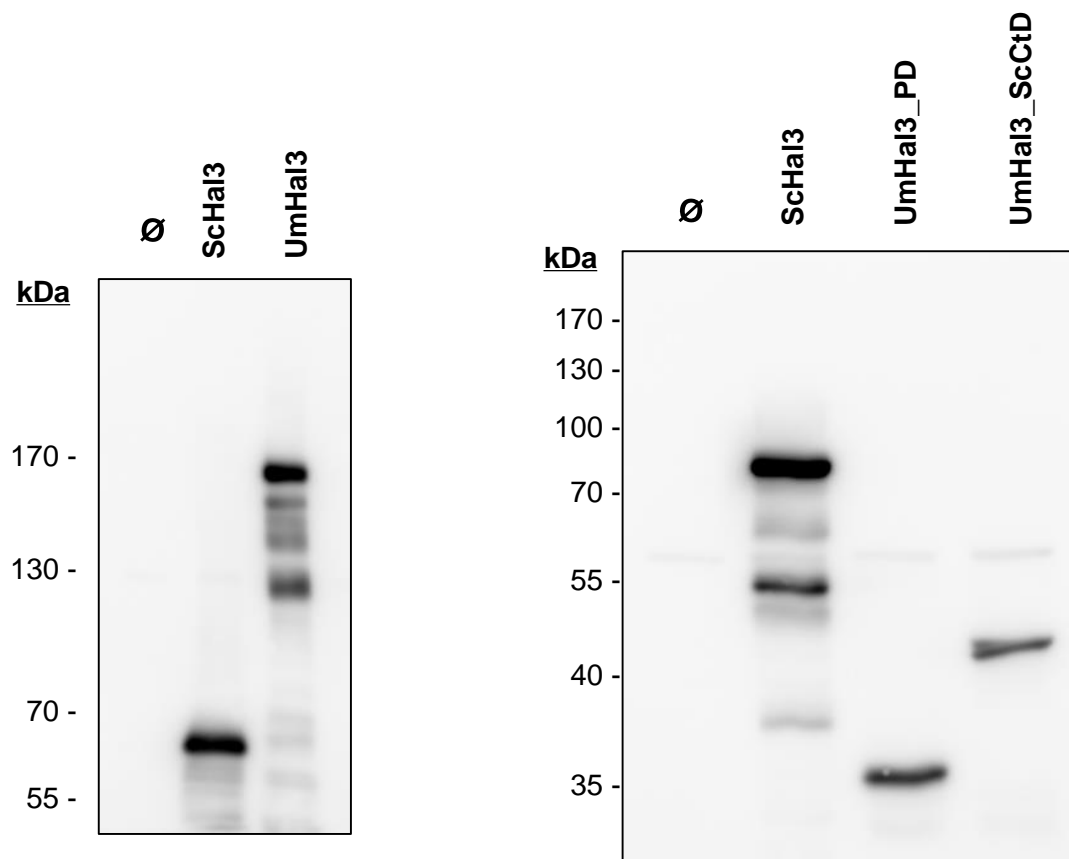


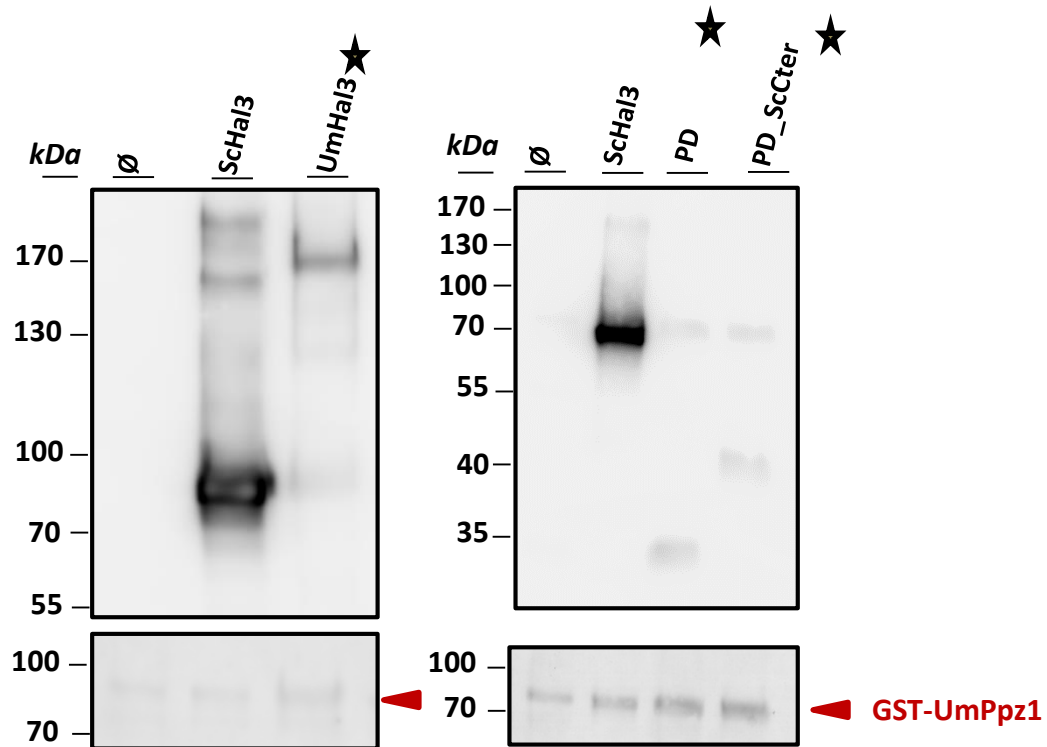
Supplementary Figure S1. Wild type (BY4741) and its *ppz1* derivative were transformed with the indicated plasmids (YEpl81-based) expressing a C-terminally HA-tagged version of the indicated proteins. Cells were spotted on the indicated SD (lacking leucine) plates and grown for four days.



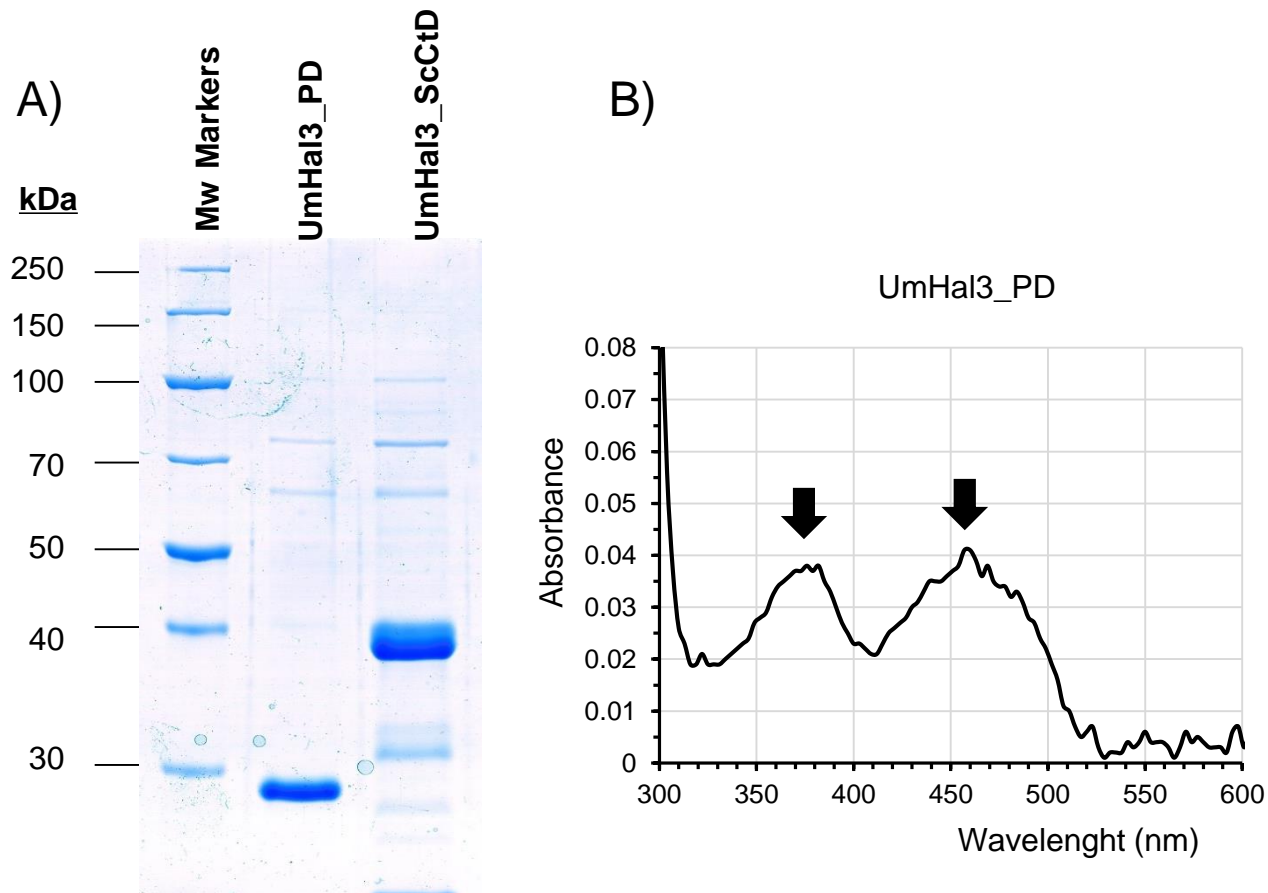
Supplementary Figure S2. Expression of UmHal3 variants in the *S. cerevisiae slt2* background. Cells were spotted on the indicated SD (lacking uracil) plates and grown for 3 days. PD, UmHal3_PD; PD_ScCtD, UmHal3_PD_ScCter.



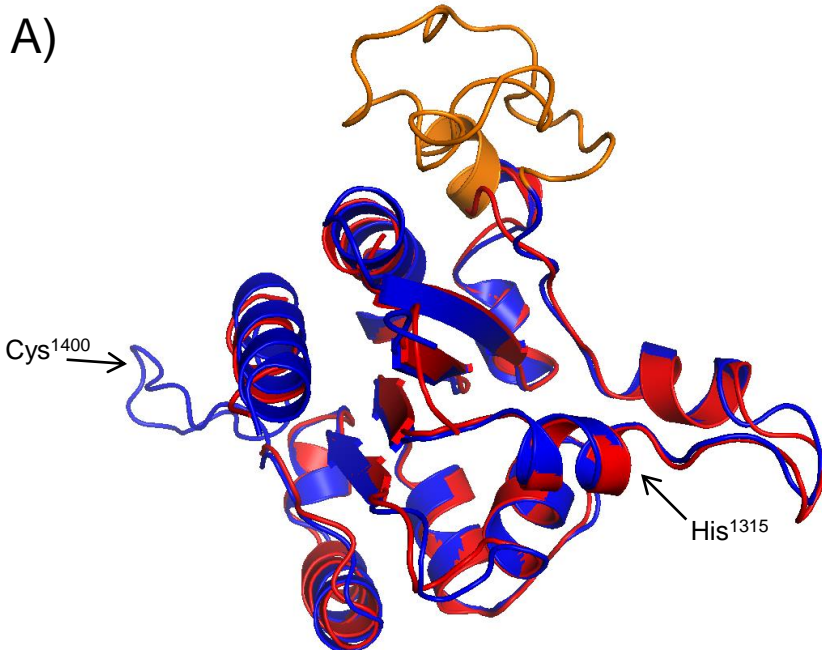
Supplementary Figure S3. Immunoblot analysis of protein extracts of IM21 cells expressing HA-tagged versions of the diverse ScHal3 and UmHal3 proteins used in the binding assays. Forty μg of protein extracts were run on 6% (left panel) or 10% (right panel) SDS-PAGE and transferred to membranes for immunoblot analysis using anti-HA antibodies as indicated in Materials & Methods. Signals were integrated and normalized in order to calculate the amount of extract to be used in the binding assays to ensure equivalent amounts of prey proteins.



Supplementary Figure S4. The *in vitro* binding experiment was as in Figure 5A, except that beads containing 8 μg of recombinant GST-UmPpz1 were employed and that, in the lanes marked with an asterisk, 50 μl of the samples (instead of 25 μl) were loaded in the gel.



Supplementary Figure S5. A) Coomassie blue staining of a SDS-PAGE analysis of recombinant Umhal3_PD and UmHal3_PD_ScCtD after digestion with PreScission protease. B) UV-visible scan of recombinant UmHal3_PD showing the two peaks characteristic of an oxidized flavin.



B)

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UmHal3      KTRPVQNRRTSYLASQLSRTTATPPRTSSGSLSAVLADEVVAAKPLPASLRSPYAPLS 1200
Hs_PPCDC    -----MEPK----ASCPA 9
AtHal3a     -----MENG--KRDRQDM 11
:
UmHal3      RPPTCDRPLHIVLASTGSAVSVKIPLIVQELLTYANVRVQVIATDNSLHFYDRADIAKLN 1260
Hs_PPCDC    AAPLMERKFHVLVGVGTGSAALKPLLVSKLLDIPGLEVAVVTERAKHFYSPQDI---- 65
AtHal3a     EVNTTPRKPRVLLAASGSVAAIKFGNLCHCFTEWAE--VRAVTKSSSLHFLDKLSLPQ-- 67
          *  : : : : : * : : : : : * : : : : * : : : : :
UmHal3      AASGGDGD EYTVASLAAENQ SASVCGRAASHVRAHLWTNADEWTSFSRIGDPILHIELRR 1320
Hs_PPCDC    -----PVTLYSDADEWEMWKSRSDPVLHIDLRR 93
AtHal3a     -----EVTLYTDEDEWSWNKI GDPVLHIELRR 95
          . * : : * * * . : . * : * : * : * : *
UmHal3      WADMVLIAPCSANTLAKIYGGMCDLLTSFVRALARDTPKWMFPAMNTLMWENVEVTVHV 1380
Hs_PPCDC    WADLLLVA PLDANTLGKVASGICDNL L TCVMRADWDRSKPLLFCPAMNTAMWEHPITAQV 153
AtHal3a     WADVLVIAPLSANTLGIAGGLCDNLLTCIIRAWDYTKPLFVAPAMNTLMWNNPFTERHL 155
          * * : : : * * * * * : * : * : * * : : * * * * * : : * : :
UmHal3      DALRRRGWVVHGFVEKMLA C GDMGTGAMVEWTEL VQTLV--QWAHLVRDESR--- 1430
Hs_PPCDC    DQLKAFGYVEIPCVAKKLV C GDEGLGAMAEVGTIVDKVKEVLFQHSGFQQS---- 204
AtHal3a     LSLDELGITLIPPIKRLA C GDYGN GAMAEP S LIYSTVRLF-WESQAHQQTGGTS 209
          * * . : * * * * * * * * * : : : : : : :

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Supplementary Figure S6. The sequence comprised between residues 1121 to the C-terminus of UmHal3 was submitted to the SWISS-MODEL workspace and a 3D-model generated using the structure of *C. neoformans* Hal3b (PDB ID: 6eoa). The model (deep blue) was aligned with the 3D structure of human PPCDC (PDB ID: 1QZU, red). The catalytic Cys and His are denoted by arrows and the insert described in the text is denoted in deep orange. B) Alignment of the region relevant to the structure in panel A for UmHal3, human PPCDC (Hs_PPCDC) and *A. thaliana* Hal3 (AHal3a). Catalytic regions/residues are in color.

Supplementary Figure S7. Protein sequence of UmHal3 and multiple sequence alignment (Clustal Ω 1.2.4) with ScHal3 and ScCab3. The PD domain is underlined and the residues critical for PPCDC activity are denoted in color.

UmHAL3	MQRTPSDSLDPSTIAASKALDASQSGSDCSSRTTSPRFPIARLEPTDRAEWQSQTVGRSSH	60
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	RRDSQHHPFGDHTAQRIVPTSSSTPSTPTSALPTSRTYHSLNYAPRQRNSMLRPNAASVFQ	120
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	PHSPQSAQQQQAVTTAFTGLALPSGRVSPALPSYSSPLSATSYLRTSPSYPTISLPDAVP	180
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	PRDSSRVRDSVSLQADTAPTSLARMQLQQMQDEARRLGLNEKSAGWLI LEALQAATEGEW	240
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	AAVAELLANGDATLLLPRDPPSVFTSSQLSASFAYDHTIFNAASASSTKPSPSAVPSSA	300
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	TCPDALSILTL SGLRGLSRAPSDQSEPD TLVQAVESQQFQLILQSFVVKTSQSAISNLK	360
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	DASSRQETLEMLAPLPIITSLDKSNCRYPFSLLASNAQFPLPPPLNVRKASSQSQAGGK	420
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	NRARSNSKLNAAAGSRASASFASIFGGSGRERRRQAEAAGTTGSEM LDAQRLSVNATHLDV	480
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	QPFQGANVAQDGLIEESGAQIPSSLGVELRGDLQHTTND EATSIKESTCATTQRRSVSVW	540
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	VVDHLVRRSTVMKMIRKALDTRIKERLLAKSIPESISDVVASFAATYLP PAPS LNDVSAS	600
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	RSGGAERGQASHSNRSRAASPVNVVNPYPYLADPDELSENFQDF FNSIREQLYNFDSSQAE	660
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	YLRADSDTAAAIARNPAPNASLEEQAHQHDIERQLEAVETVLCEEVYDRIFCPVTSRDR	720
ScHal3	-----	0
ScCab3	-----	0
UmHAL3	YHDDALASRIAALNVLGLSLRHLGLVVPSERLQEDEIEATESNALLDGI ERIVQQCGDEL	780
ScHal3	-----	0
ScCab3	-----	0

UmHAL3	QRLESAMCRSPQAKLDVLVRAHKIAVEGVAQLPSIKMRDDDTAVEGQEKEQTARPSSGKK	840
ScHal3	-----MT-----AVASTSGKQ	11
ScCab3	-----MTDE-----KVNSDQNMNGKQ	16
	* : .** :	
UmHAL3	GDGSTSA--DLILPILIYSIVSS--NPSHLASNLLYIQRFR--AESLVQGETSYCLVNVQA	895
ScHal3	DADHN-----QSI ECPFRSFG	27
ScCab3	GVNLISSLPTTQVPVSI LTNKERRKSIHDESNFERSDSHEDQSKSNSNRRIYKNDYSTN	76
	
UmHAL3	AVAFI-ENV---DVKDLGLDSNQIGAHLPVHSRDEGHAPRSSTGSSHS LVPSSKIAETSS	951
ScHal3	QKEILLDH-----EDAKGKDSIINS PV-----SGRQSI SPTLSNATTTT	66
ScCab3	LRDFSANLQKQNSERNKDGHEIQINTSMFA-----NTNGQQKRFSPLSAVS--	124
	: : .: .. *: :	
UmHAL3	ATLAMPARIRGRLTQEIGDLGASNKVIT-----GVMGSSISAFSRMMGANASVAEAGVD	1006
ScHal3	-----TKSIMNATGTSGAVVSNTPPEPLKRVPAVTFSDL-----KQQQKQ	106
ScCab3	-----FTVPEVERLPYHRYSISNKP--GKQQQQQ	151
	: : * : :	
UmHAL3	ESLGRKRAKSSASLNAAHSEGAVAPTQADAQRPLRSRTSSSVTPTDDSYLGEQSK-EKEK	1065
ScHal3	DSLTO-----LKNDSERTKSPN--SNP-A---PVSNSI-PGNHAVIPNHTNTRRT	150
ScCab3	EQLQQ-----NQQQEEQQAQLQEONQRA---KQQEEV-K---QIQEQV---QKK	191
	:. * : ..* : : : : .	
UmHAL3	DTVGTPTSTDK-----PSIG-DRLAMLSRLGTSALSSSPSSSLGSGLPSPDS---GLGVSAS	1116
ScHal3	QLSGSPLVNEKDYDPKKKDSALKIVDTMK-----PDKIMATSTPI SR-----	193
ScCab3	QTERQQQLIDEKE-----RIANAIF-----KENTTNDGTDIRKHSVSSGTSNS	233
	: : : :	
UmHAL3	GQSLIASPR---AVSRELPGPPPTKTKTRPVQNRRTSYLASQLSRTTATPPRTSSGS	1172
ScHal3	-----ENNKVTAKAPTSITLRKEDAQDQANNVS-----GQIN-VRSTPEE-----	232
ScCab3	-EDEVSDSPMEKNSIV-HMPGDFIYFN-----PKSNAS-----KPIT-AKAAPLSA---N	277
	: . * : . * : : . . : * *	
UmHAL3	LSAVLADEVVAAAKPLPASLRSPYAPLSRPPTCDRPLHIVLASTGVSASVKIPLIVQELL	1232
ScHal3	--TPVKQSVIPS--IIPKRENSKND-PRLPQDDGKLHVLFGATGSLSVFKIKPMIKKLE	287
ScCab3	NSTHKNKEVIT---APTGPRVPFTE-FFQKEDDKKFHILIGATGVSATIKVPLIIDKLF	332
	: . . * : * . * : * : : : * : : : * *	
UmHAL3	<u>TY--ANVRVQVIATDNSLHFYDRADIAKLNAASGGDG--DEYTVASLAAENQSASVCGR</u>	1287
ScHal3	<u>EIYGRDRISIQVILTQSATQFFEQRYTKKIKSSEKLNKMSQYESTPATPVTPTPGQCNM</u>	347
ScCab3	<u>KIYGPEKISIQLVTKPAEHFLKG-----</u>	356
	. : : * * * . : * .	
UmHAL3	---AASHVRAHLWTNADEWTSFS-----RIGDPI LH IELRRWADMVLIAPCSANTLAK	1337
ScHal3	AQVVELPPHIQLWTDQDEWDAWK-----QRTDPV LH IELRRWADILVVAPLTANTLSK	400
ScCab3	---LKMSTHVKIWREEDAWVFDVKNKNTSLSLNLILHHELKRWADIFLIAPLSANTLAK	413
	: : * : * * : : * * * * * : : * * * * * : * * * * *	
UmHAL3	<u>IYGGMCDLLTSFVRALARDTPKWMFPAMNTLMWENEVTEVHVDALRRR-GWV-VHGPVE</u>	1395
ScHal3	<u>IALGLCDNLLTSVIRAWNPSYPILLAPSMVSSSTFNSMMTKKQLTIKEEMSVVTVFKPSE</u>	460
ScCab3	<u>LANGICNLLTSVMRDWSPLTPVLIAPAMNTFMYINPMTKKHLTSLVQDYPFIQVLKPVE</u>	473
	: * : * : * * * . * : * : * : . . : * : : : * * *	
UmHAL3	KML-A C GDMGTGAMVEWTELVTQTLVQVAHLVRDESR-----	1430
ScHal3	KVMDINGDIGLGGMMDWNEIVNKIVMKLGGYPKNNEEEDDEDEEEDDEEEDTEDKNE	520
ScCab3	KVL-I C GDIGMGMREWTDIVEIVRRRINEIRKARDEETGDKQEQQEQEGADN-----	526
	* : : * * * * * * * : : : : .	
UmHAL3	----- 1430	
ScHal3	NNDDDDDDDDDDDDDDDD-----DDDDDDDDDEDEDEAETPGIIDKHQ* 562	
ScCab3	-EDDDDEDEDEDEDEDEEALNETASDESNDDEDEDEEEDVK--TEV*---- 571	

Supplementary Figure S8. Sequence of the hybrid protein UmHal3_PD_ScCtD as expressed from plasmid pWS93. The tripe HA epitope is underlined and the acidic tail from ScHal3 is denoted in yellow background.

MYPYDVPDYAGYPYDVPDYAGSYPYDVPDYAEFPLPASLRSPYAPLSRPPTCDRPLHIVLASTGSVASVKIPLIVQE
LLTYANVRVQVIATDNSLHFYDRADIAKLNAASGGDGDEYTVASLAAENQSASVCGRAASHVRAHLWTNADEWTSFS
RIGDPILHIELRRWADMVLIAPCSANTLAKIYGGMCDDLTSFVRALARDTPKWMFPAMNTLMWENEVTEVHVDALR
RRGWVVHGPVEKMLACGDMGTGAMVEWTELVQTLVQWAHLVRDES**RPKNNEEEDDDEDEEEDDDEEEDTEDKNENN**
DDDDDDDDDDDDDDDDDDDDDDDEDEDEAEETPGIIDKHQ*