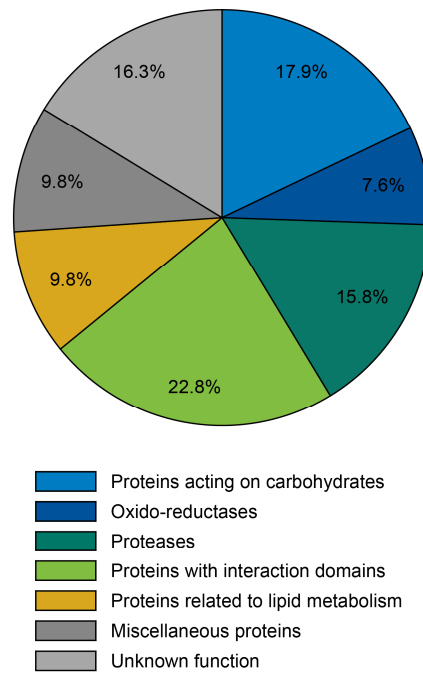


**Supplementary Figure 1: Purification of PCO\_GH28-1 and -3 and heat-dependent fragmentation of PCO\_GH28-1.** A) SDS-PAGE and Coomassie staining of exemplary metal ion affinity purification of PCO\_GH28-1 and -3 via the His<sub>6</sub> tags. Their expected sizes are indicated with an arrow (closed arrowhead). Medium (M), dialyzed medium (MD), precipitated protein (P), flow-through (FT), wash (W) and elution fractions (E0 – E3). Additional bands in the purification of PCO\_GH28-1 (arrows with open arrowheads, 1 – 8) were analyzed by MS/MS (see Supplementary Table 2) and identified as fragments of PCO\_GH28-1. B) PCO\_GH28-1 was treated with increasing temperatures during sample preparation for SDS-PAGE and detected on the western blot with an anti-V5 antibody.

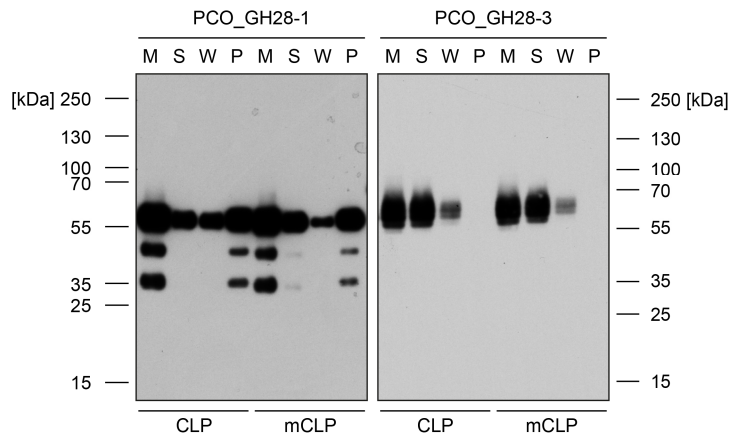




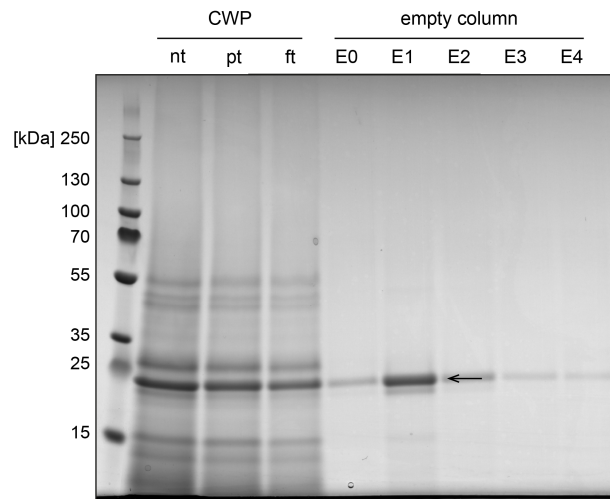




**Supplementary Figure 3: Predicted functional classes from *B. rapa* ssp. *pekinensis* cell wall proteins (CWPs).** The annotation of proteins is based on the presence of functional domains predicted by InterPro, GO Term and BLAST bioinformatics analysis. Analysis includes only proteins that possess a signal peptide for the secretory pathway according to SignalP and were predicted to be localized extracellularly by DeepLoc analysis. For a full list of identified proteins, see Supplementary Table 4.



**Supplementary Figure 4: Binding of *P. cochleariae* PG family members PCO\_GH28-1 and -3 to pectic substrates.** Culture medium containing PCO\_GH28-1 and -3 (M) was incubated with cross-linked pectin (CLP) and methylated CLP (mCLP), respectively. Centrifugation separated the pectin pellet and associated proteins (P) from the supernatant (S). Unbound proteins were removed from the pellet by washing (W). The volumes applied to the western blot were chosen in such a way that the cumulative signals of S, W and P correspond to the initially used amount of M. The PG family members were detected in a western blot with an anti-V5 antibody. The additional bands below the PCO\_GH28-1 signal in the western blot were analyzed by LC-MS/MS and assigned to fragments of the PG, which occur as an artefact of the sample boiling for SDS-PAGE (Supplementary Figure 1, Supplementary Table 2).



**Supplementary Figure 5: SDS-PAGE of pull-down assay of *B. rapa* ssp. *pekinensis* CWPs with an empty column (negative control).** Non-treated (nt) CWP extracts were pre-treated (pt) with column resin to reduce unspecific binding to the column and then passed over columns without immobilized protein. Proteins not interacting with the column material were in the flow-through (ft), while interacting proteins were eluted from the column analogous to the PG family member columns. Elution fraction E1 was analyzed by LC-MS/MS. For detailed information on interacting LRR and total proteins, see Supplementary Table 6. E0 – 4: elution fractions 1 – 4.

# A

	10	20	30	40	50	60																						
BrPGIP1_BRA_Chifu-401-42	MG	KTT	ILL	LL	FALL	LL	TT	SL	SK	DL	CH	KD	DK	NT	LL	KK	AM	ND	PY	TI	I	SW	DP	KD	DC	CT	WY	
BrPGIP_PGIP1.1_BRA_Cantonner_Witkrop	MG	KTT	ILL	LL	FALL	LL	TT	SL	SK	DL	CH	KD	DK	NT	LL	KK	AM	ND	PY	---	---	SW	DP	KD	DC	CT	WY	
BrPGIP3_BRA_Chifu-401-42	ME	GK	TT	ILL	LL	FALL	LL	TT	SL	SK	DL	CH	KD	DK	NT	LL	KK	AM	ND	PY	TI	I	SW	DP	KD	DC	CT	WY
BrPGIP7_BRA_Chifu-401-42	MD	KTT	ILL	LL	FALL	LL	TT	SL	SK	DL	CH	KD	DK	NT	LL	KK	AM	ND	PY	TI	I	SW	DP	KD	DC	CT	WY	
BnPGIP14_BNA	MG	KTT	ILL	LL	FALL	LL	TT	SL	SK	DL	CH	KD	DK	NT	LL	KK	AM	ND	PY	TI	I	SW	DP	KD	DC	CT	WY	

	70	80	90	100	110	120																																																						
BrPGIP1_BRA_Chifu-401-42	S	V	E	C	G	N	A	---	N	R	V	T	S	L	D	L	S	D	D	V	S	A	Q	I	P	P	E	V	G	D	L	P	Y	L	Q	Y	L	T	F	R	K	L	P	N	L	T	G	E	I	P	P	T	I	A	K	L	K			
BrPGIP_PGIP1.1_BRA_Cantonner_Witkrop	S	V	E	C	G	N	A	---	N	R	V	T	S	L	D	L	S	D	D	V	S	A	Q	I	P	P	E	V	G	D	L	P	Y	L	Q	Y	L	T	F	R	K	L	P	N	L	T	G	E	I	P	P	T	I	A	K	L	K			
BrPGIP3_BRA_Chifu-401-42	A	V	E	C	G	N	A	S	I	N	H	R	V	T	S	L	D	L	S	N	D	D	V	S	A	Q	I	P	P	E	V	G	D	L	P	Y	L	E	Y	L	E	F	E	K	L	P	N	L	T	G	E	I	P	P	T	I	K	L	K	
BrPGIP7_BRA_Chifu-401-42	A	V	E	C	G	N	A	T	I	N	H	R	V	T	S	L	D	L	S	N	D	D	V	S	A	Q	I	P	P	E	V	G	D	L	P	Y	L	E	Y	L	E	F	E	K	L	P	N	L	T	G	E	I	P	P	T	I	A	K	L	K
BnPGIP14_BNA	S	V	E	C	G	N	A	---	N	R	V	T	S	L	D	L	S	D	D	V	S	A	Q	I	P	P	E	V	G	D	L	P	Y	L	Q	Y	L	T	F	R	K	L	P	N	L	T	G	E	I	P	P	T	I	A	K	L	K			

	130	140	150	160	170	180																																																	
BrPGIP1_BRA_Chifu-401-42	Y	L	K	S	L	W	S	N	S	L	T	G	P	V	E	F	L	S	Q	L	K	N	L	E	I	N	L	S	F	N	K	L	S	G	S	I	P	G	S	L	S	L	P	K	L	D	F	L	E	L	S	R	N	K	L
BrPGIP_PGIP1.1_BRA_Cantonner_Witkrop	Y	L	K	S	L	W	S	N	S	L	T	G	P	V	E	F	L	S	Q	L	K	N	L	E	I	N	L	S	F	N	K	L	S	G	S	I	P	G	S	L	S	L	P	K	L	D	F	L	E	L	S	R	N	K	L
BrPGIP3_BRA_Chifu-401-42	Y	R	I	N	L	W	S	N	S	L	T	G	P	V	E	F	L	S	Q	L	K	N	L	E	I	N	L	S	F	N	K	L	S	G	S	I	P	G	S	L	S	L	P	K	L	D	F	L	E	L	S	R	N	K	L
BrPGIP7_BRA_Chifu-401-42	Y	I	R	N	L	W	S	N	S	L	T	G	P	V	E	F	L	S	Q	L	K	N	L	E	I	N	L	S	F	N	K	L	S	G	S	I	P	G	S	L	S	L	P	K	L	D	F	L	E	L	S	R	N	K	L
BnPGIP14_BNA	Y	L	K	S	L	W	S	N	S	L	T	G	P	V	E	F	L	S	Q	L	K	N	L	E	I	N	L	S	F	N	K	L	S	G	S	I	P	G	S	L	S	L	P	K	L	D	F	L	E	L	S	R	N	K	L

	190	200	210	220	230	240																																																					
BrPGIP1_BRA_Chifu-401-42	T	G	P	I	P	E	S	F	G	S	F	K	R	T	V	Y	G	I	L	S	H	N	Q	L	S	G	S	I	P	K	S	L	G	N	L	D	I	N	Q	I	D	L	S	R	N	K	L	E	G	D	A	S	M	L	F	G	V	K	K
BrPGIP_PGIP1.1_BRA_Cantonner_Witkrop	T	G	P	I	P	E	S	F	G	S	F	K	R	V	Y	G	I	L	S	H	N	Q	L	S	G	S	I	P	K	S	L	G	N	L	D	I	N	Q	I	D	L	S	R	N	K	L	E	G	D	A	S	M	L	F	G	T	K	K	
BrPGIP3_BRA_Chifu-401-42	T	G	S	I	P	E	S	F	G	S	F	K	G	V	Y	A	L	E	L	S	H	N	Q	L	S	G	S	I	P	K	S	L	G	N	L	D	I	N	Q	I	D	L	S	R	N	K	L	E	G	D	A	S	M	L	F	G	A	K	K
BrPGIP7_BRA_Chifu-401-42	T	G	S	I	P	E	S	F	G	S	F	K	G	V	Y	A	L	E	L	S	H	N	Q	L	S	G	S	I	P	K	S	L	G	N	L	D	I	N	Q	I	D	L	S	R	N	K	L	E	G	D	A	S	M	L	F	G	A	C	K
BnPGIP14_BNA	T	G	P	I	P	E	S	F	G	S	F	K	R	T	V	Y	G	I	L	S	H	N	Q	L	S	G	S	I	P	K	S	L	G	N	L	D	I	N	Q	I	D	L	S	R	N	K	L	E	G	D	A	S	M	L	F	G	A	C	K

	250	260	270	280	290	300																																																					
BrPGIP1_BRA_Chifu-401-42	T	T	W	H	I	D	L	S	R	N	M	F	Q	F	I	S	K	V	K	V	A	K	T	V	N	F	L	D	L	N	H	N	G	L	T	G	S	I	P	Q	W	T	Q	L	D	L	Q	T	F	N	V	S	Y	N	R	L	C	G	
BrPGIP_PGIP1.1_BRA_Cantonner_Witkrop	T	T	W	H	I	D	L	S	R	N	M	F	Q	F	I	S	K	V	K	V	A	K	T	V	N	F	L	D	L	N	H	N	S	L	T	G	S	I	P	Q	W	T	Q	L	D	L	Q	T	F	N	V	S	Y	N	R	L	C	G	
BrPGIP3_BRA_Chifu-401-42	T	T	Q	H	I	D	L	S	R	N	M	F	Q	F	I	S	K	V	K	V	A	K	T	V	N	F	L	D	L	N	H	N	S	L	T	G	S	I	P	Q	W	T	Q	L	D	L	Q	T	F	N	V	S	Y	N	R	L	C	G	
BrPGIP7_BRA_Chifu-401-42	T	T	H	N	I	D	L	S	R	N	M	F	Q	F	I	S	K	V	K	V	A	K	T	V	N	F	L	D	L	N	H	N	G	L	T	G	T	I	F	I	Q	W	T	Q	L	D	L	Q	T	F	N	V	S	Y	N	R	L	C	G
BnPGIP14_BNA	T	T	H	N	I	D	L	S	R	N	M	F	Q	F	I	S	K	V	K	V	A	K	T	V	N	F	L	D	L	N	H	N	G	L	T	G	T	I	F	I	Q	W	T	Q	L	D	L	Q	T	F	N	V	S	Y	N	R	L	C	G

	310	320	330	340																																						
BrPGIP1_BRA_Chifu-401-42	R	I	P	Q	G	D	L	Q	R	F	D	A	Y	A	L	H	N	K	L	C	G	A	P	L	S	O	N	V	K	I	Q	A	T	D	L	Y	L	N	L	P	S	E
BrPGIP_PGIP1.1_BRA_Cantonner_Witkrop	R	I	P	Q	G	D	L	Q	R	F	D	A	Y	A	L	H	N	K	L	C	G	A	P	L	S	O	N	V	K	I	Q	A	T	D	L	Y	L	N	L	P	S	E
BrPGIP3_BRA_Chifu-401-42	R	I	P	Q	G	D	L	Q	R	F	D	A	Y	A	L	H	N	K	L	C	G	A	P	L	Q	S	C	K														
BrPGIP7_BRA_Chifu-401-42	R	I	P	Q	G	D	L	Q	R	F	D	A	Y	A	L	H	N	K	L	C	G	A	P	L	Q	S	C	K														
BnPGIP14_BNA	R	I	P	Q	G	D	L	Q	R	F	D	A	Y	A	L	H	N	K	L	C	G	A	P	L	Q	S	C	K														



**B**

BrPGIP-likel/Bra-FLOR1\_BRA\_Chifu-401-42  
 BrPGIP\_likel\_BRA\_Cantonner\_Witkrop  
 PREDICTED:PG\_inhibitor\_1-like\_BOL

10 20 30 40 50 60  
 MKLLHLHSIFFTILFISLPSHSSSSNDKNALLQIKKALNNPPLSSWNPQTDFCFTWTG  
 MKLLHLHSIFFTILFISLPSHSSSSNDKNALLQIKKALNNPPLSSWNPQTDFCFTWTG  
 MKLLHLHSIFFTILFISLPSHSSSSNDKNALLQIKKALNNPPLSSWNPQTDFCFTWTG

BrPGIP-likel/Bra-FLOR1\_BRA\_Chifu-401-42  
 BrPGIP\_likel\_BRA\_Cantonner\_Witkrop  
 PREDICTED:PG\_inhibitor\_1-like\_BOL

70 80 90 100 110 120  
 VECTNGRVTALTISSGEISGQIPAQVGDLELRLTDFSYLTHLTGNIPHTITKLYLDLL  
 VECTNGRVTALTISSGEISGQIPAQVGDLELRLTDFSYLTHLTGNIPHTITKLYLDLL  
 VECTNGRVTALTISSGEISGQIPAQVGDLELRLTDFSYLTHLTGNIPHTITKLYLDLL

BrPGIP-likel/Bra-FLOR1\_BRA\_Chifu-401-42  
 BrPGIP\_likel\_BRA\_Cantonner\_Witkrop  
 PREDICTED:PG\_inhibitor\_1-like\_BOL

130 140 150 160 170 180  
 RLKQTNLSGYIPDFISELKSVTFLDLSFNQFTGPIPGSLSQMPKLDAIQINDNKLTGSIP  
 RLKQTNLSGYIPDFISELKSVTFLDLSFNQFTGPIPGSLSQMPKLDAIQINDNKLTGSIP  
 RLKQTNLSGYIPDFISELKSVTFLDLSFNQFTGPIPGSLSQMPKLDALQINDNKLTGSIP

BrPGIP-likel/Bra-FLOR1\_BRA\_Chifu-401-42  
 BrPGIP\_likel\_BRA\_Cantonner\_Witkrop  
 PREDICTED:PG\_inhibitor\_1-like\_BOL

190 200 210 220 230 240  
 NSFSGSVGKVPNLYLFNNKLSGKVPESLSKYDFNAVDLSGNSFTGDGSMFFGRNKTTIRV  
 NSFSGSVGKVPNLYLFNNKLSGKVPESLSKYDFNAVDLSGNSFTGDGSMFFGRNKTTIRV  
 NSFSGSVGKVPNLYLFNNKLSGKVPESLSKYDFNAVDLSGNSFTGDGSMFFGRNKTTIRV

BrPGIP-likel/Bra-FLOR1\_BRA\_Chifu-401-42  
 BrPGIP\_likel\_BRA\_Cantonner\_Witkrop  
 PREDICTED:PG\_inhibitor\_1-like\_BOL

250 260 270 280 290 300  
 DLSRNMFEFDLSKVKFARSIVSLDLSHNKIFGKFPRELNKLRLLEHFNVSYNHLCKGKIPSG  
 DLSRNMFEFDLSKVKFARSIVSLDLSHNKIFGKFPRELNKLRLLEHFNVSYNHLCKGKIPSG  
 DLSRNMFEFDLSKVKFARSIVSLDLSHNKIFGKFPRELNKLRLLEHFNVSYNHLCKGKIPSG

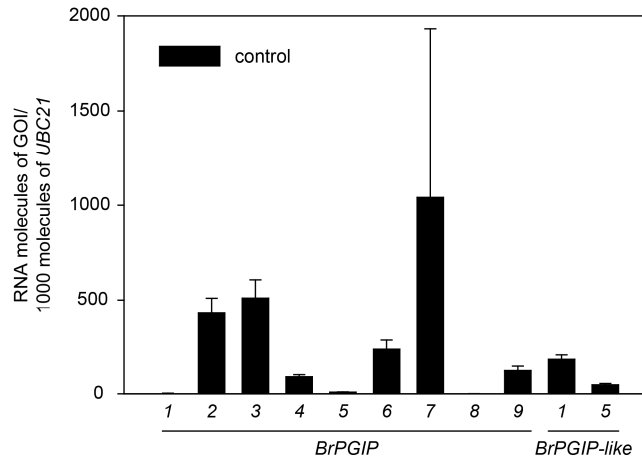
BrPGIP-likel/Bra-FLOR1\_BRA\_Chifu-401-42  
 BrPGIP\_likel\_BRA\_Cantonner\_Witkrop  
 PREDICTED:PG\_inhibitor\_1-like\_BOL

310 320  
 GLLQTFEPSAFSHNLCLCGTPLKAC  
 GLLQTFEPSAFSHNLCLCGTPLKAC  
 GLLQTFEPSAFSHNLCLCGTPLKAC

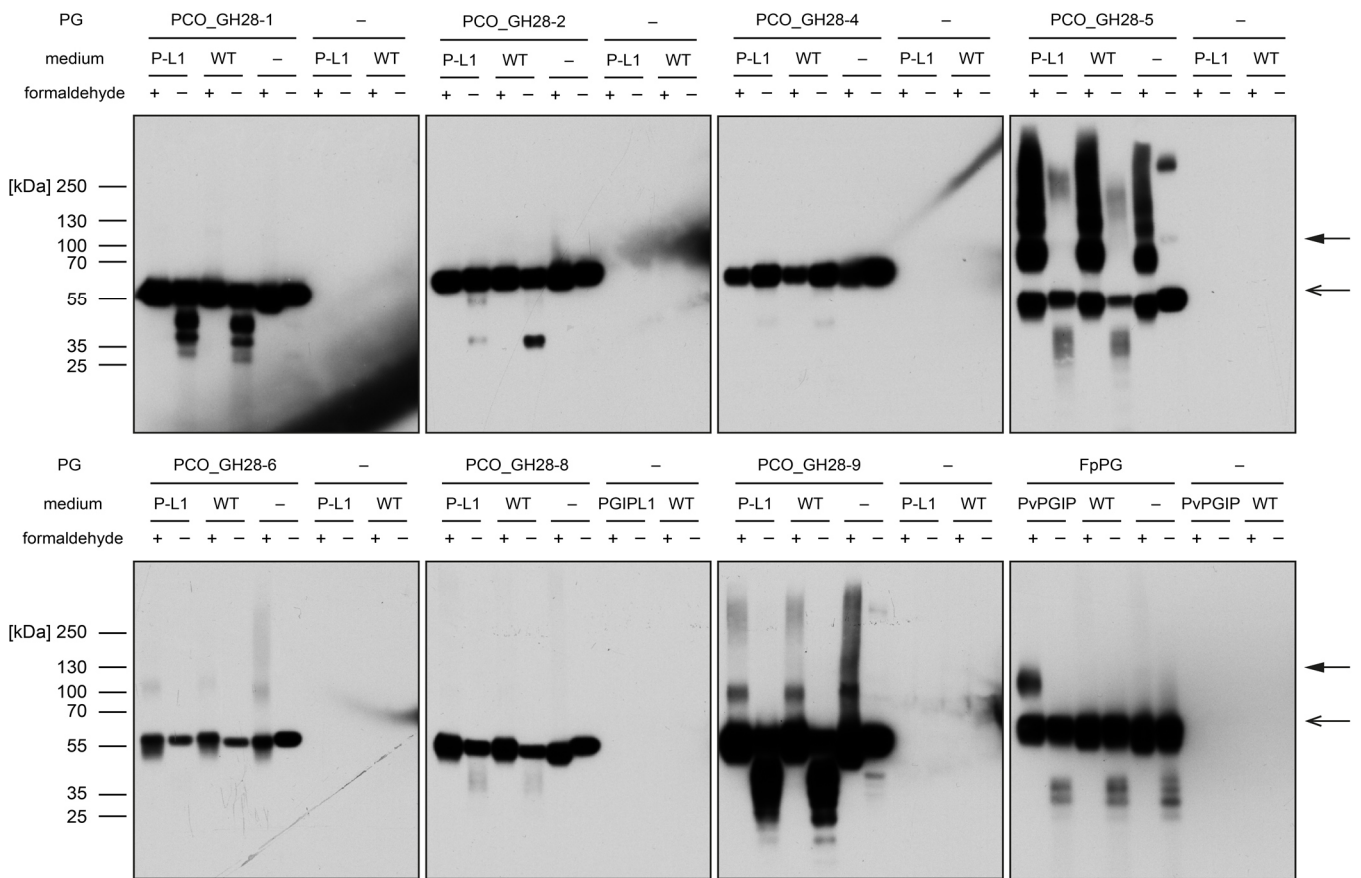
**C**

	10      20      30      40      50      60
BrPGIP-like5/FLOR1_BRA_Chifu-401-42	MKLLHLHSIFFAILFISLFPSSYSCNSVEKNALLQIKKSFNNPRKFSSWNPQTDCCTTWSG
BrPGIP_like5_BRA_Cantonner_Witkrop	MKLLHLHSIFFAILFISLFPSSYSCNSVEKNALLQIKKSFNNPRKFSSWNPQTDCCTTWSG
unnamed_protein_product_BRA_Z1	MKLLHLHSIFFAILFISLFPSSYSCNSVEKNALLQIKKSFNNPRKFSSWNPQTDCCTTWSG
	70      80      90      100      110      120
BrPGIP-like5/FLOR1_BRA_Chifu-401-42	VKCTNGRVTDL <sup>■</sup> SLFSSKLYGQIPDQIGDLELRSLFLTYLPHLTGNI <sup>■</sup> PRITITKLNIDSL
BrPGIP_like5_BRA_Cantonner_Witkrop	VKCTNGRVTDL <sup>■</sup> SLFSSKLYGQIPDQIGDLELRSLFLTYLPHLTGNI <sup>■</sup> PRITITKLNILEYL
unnamed_protein_product_BRA_Z1	VKCTNGRVTDL <sup>■</sup> SLFSSKLYGQIPDQIGDLELRSLFLTYLPHLTGNI <sup>■</sup> PRITITKLNILEYL
	130      140      150      160      170      180
BrPGIP-like5/FLOR1_BRA_Chifu-401-42	ILRHNNLSGPIPDNIGELK <sup>■</sup> KLRFLLDLSYNQFTGPIPGSLSQMPMLETIIVEHNKLTGSIP
BrPGIP_like5_Cantonner_Witkrop	ILRHNNLSGPIPDNIGELK <sup>■</sup> KLRFLLDLSYNQFTGPIPGSLSQMPMLETIIVEHNKLTGSIP
unnamed_protein_product_BRA_Z1	ILRHNNLSGPIPDNIGELK <sup>■</sup> KLRFLLDLSYNQFTGPIPGSLSQMPMLETIIVEHNKLTGSIP
	190      200      210      220      230      240
BrPGIP-like5/FLOR1_BRA_Chifu-401-42	NSFGSFVGEVPNLYLSNNKLSGNIPELSKNDFN <sup>■</sup> GVFLSKNSFTGDGSMFFGRNKSTVSL
BrPGIP_like5_BRA_Cantonner_Witkrop	NSFGSFVGEVPNLYLSNNKLSGNIPELSKNDFN <sup>■</sup> GVFLSKNSFTGDGSMFFGRNKSTVSL
unnamed_protein_product_BRA_Z1	NSFGSFVGEVPNLYLSNNKLSGNIPELSKNDFN <sup>■</sup> GVFLSKNSFTGDGSMFFGRNKSTVSL
	250      260      270      280      290      300
BrPGIP-like5/FLOR1_BRA_Chifu-401-42	DLSRNMF <sup>■</sup> FDLSKVQFARGIVDLDL <sup>■</sup> SHNRIFGNLPRELI <sup>■</sup> ELRGLKLFNVSHNRLCGKIPR
BrPGIP_like5_BRA_Cantonner_Witkrop	DLSRNMF <sup>■</sup> FDLSKVQFARGIVDLDL <sup>■</sup> SHNRIFGNLPRELI <sup>■</sup> ELRGLKLFNVSHNRLCGKIPR
unnamed_protein_product_BRA_Z1	DLSRNMF <sup>■</sup> FDLSKVQFARGIVDLDL <sup>■</sup> SHNRIFGNLPRELI <sup>■</sup> ELRGLKLFNVSHNRLCGKIPR
	310      320
BrPGIP-like5/FLOR1_BRA_Chifu-401-42	GGLLQSFKSYVFAHNRC <sup>■</sup> LCGTPLKAC
BrPGIP_like5_BRA_Cantonner_Witkrop	GGLLQSFKSYVFAHNRC <sup>■</sup> LCGTPLKAC
unnamed_protein_product_BRA_Z1	GGLLQSFKSYVFAHNRC <sup>■</sup> LCGTPLKAC

**Supplementary Figure 6: Alignment of BrPGIPs and BrPGIP-like proteins with homologs from different *B. rapa* ssp. *pekinensis* cultivars and Brassica species.** CWP for the pull-down assay was obtained from the *B. rapa* ssp. *pekinensis* (BRA) cultivar “Cantonner Witkrop,” while sequences for mass spectrometry and bioinformatic analyses were obtained from the NCBI database and originate from the “Chifu-401-42” cultivar. If the Mascot analyses resulted in hits other than from the “Chifu-401-42” sequences, we aligned those together with the sequences from “Cantonner Witkrop.” Matching peptides from the Mascot analyses were marked in blue, amino acid differences were highlighted in black. A) For the hit BnPGIP14 (ABX46560.1) from *B. napus* (BNA), one peptide matched to BrPGIP1 and -1.1, while the second matched BrPGIP3 and -7. B) One amino acid difference between the detected peptide and the BrPGIP-like1 sequences resulted in the identification of the homologous “Predicted: polygalacturonase inhibitor-like1” (XP\_013617122.1) from *B. oleraceae* var. *oleracea* (BOL). C) All peptides match perfectly with the hit “unnamed protein product” (VDC72802.1) from the *B. rapa* cultivar “Z1” and BrPGIP-like5 from “Cantonner Witkrop,” but differ in one amino acid from the BrPGIP-like5/FLOR1 sequence (XP\_009146639) from “Chifu-401-42.” Altogether, we are confident that discrepancies resulted from cultivar-dependent or even individual-dependent amino acid differences and that the proteins we identified were BrPGIP1.1 and -3 as well as BrPGIP-like1 and -like5.



**Supplementary Figure 7: Expression of *BrPGIPs* and *BrPGIP-like proteins* in untreated *B. rapa ssp. pekinensis*.** The expression levels of the *BrPGIPs* and *BrPGIP-like proteins* were quantified by RT-qPCR, and transcript abundance was expressed as RNA molecules of the gene of interest (GOI) per 1000 RNA molecules of the reference gene *UBIQUITIN-CONJUGATING ENZYME 21 (UBC21)*. Error bars indicate the standard error of the mean (SEM).



**Supplementary Figure 8: Interaction assay of PCO\_GH28-1, -2, -4, -5, -6, -8, -9 with BrPGIP-like1-containing culture medium.** Culture medium from BrPGIP-like1-expressing (P-L1) as well as wild-type (WT) yeast was incubated with various *P. cochleariae* PG family members and cross-linked with formaldehyde. The PG family members were detected in a western blot with an anti-V5 antibody. Arrows indicate the expected size of PG family members (open arrowhead) and PG-PGIP complex (closed arrowhead).