<u>Supporting Information 1:</u> List of primers

PGs	cloning into pIB/V5-His-TOPO	pPICZA_FpPG_EcoRI_F	attagaattcGATCCCTGCTCCGTGACTG
		pIB_FpPG_TOPO_R	GCTGGGGCAAGTGTTGGTAG
	cloning into pMIB/V5-His B	pMIB_AnPGII_HindIII_F	taataagcttaGACAGCTGCACGTTCACC
		pMIB_AnPGII_SacII_R	attaccgcggACAAGAGGCCACCGAAGG
	cloning into pPICZα A	pPICZA_FpPG_EcoRI_F	attagaattcGATCCCTGCTCCGTGACTG
		pPICZA_FpPG_XbaI_R	taattctagattGCTGGGGCAAGTGTTGGTAG
		pPICZA_AnPGII_EcoRI_F	taatgaattcGACAGCTGCACGTTCACCAC
		pPICZA_AnPGII_NotI_R	attagcggccgcTCAATGGTGATGGTGATGATG
PGIPs	cloning into pPICZα A	pPICZA_PvPGIP2_EcoRI_F	attagaattcGAGCTATGCAACCCACAAGAC
		pPICZA_PvPGIP2_XbaI_R	taattctagattCTCCTCTTCCTGCCTGCACT
		pPICZA_BrPGIP3_PmlI_F	taatcacgtggAAAGATCTCTGTCACAAAGATGAC
		pPICZA_BrPGIP3_NotI_R	taatgcggccgcCTTGCAACTCTGAAGAGGTGCATCAC
	cloning into pKLAC2	pKLAC_BrPGIP3_NdeI_F	attacatatgAAAGATCTCTGTCACAAAGATGAC
		pKLAC_His,myc_BamHI_R	attaggatccttaatgatgatgatgATGATGATGATGATGATGGTCG
	cloning into pMIB/V5_GPI	pMIB_GPI_PvPGIP2_SphI_F	attagcatgctaGAGCTATGCAACCCACAAGAC
		pMIB_GPI_BrPGIP3_SphI_F	taatgcatgctaAAAGATCTCTGTCACAAAGATGAC
		pMIB_GPI_His,myc_BamHI_R	attaggatccATGATGATGATGATGATGGTCGACG
pMIB/V5_GPI generation		GPI-HarAPN_BamHI_F	taatggatccCCTACGACCACAACTACAGAAGC
		GPI-HarAPN_NotI_STOP_R	attagcggccgcTTAAGCCATATTAACAACGAGAGTCACG

Upper case letters represent GOI ORF sequence in the primer, lower case restriction sites and spacer nucleotides.

Supporting Information 2: Full sequences of *B. rapa* ssp. *pekinensis* BrPGIP3 variants

BrPGIP3 ORF:

atggagggtaagacaacgacactgctcttgctcttgttcgctctcctcctcacgacatctttatccAAAGATCTC
TGTCACAAAGATGACAAGAACACCCTCCTCAAGATCAAGAAAGCCATGAACGACCCTTACACCATCATCTCCTGG
GACCCCAAGGACGACTGCTGCACCTGGTACGCCGTTGAGTGCGGCAACGCATCTATTAACCACCGCGTCACTTCT
CTAGACATATCAAACGACGACGTCTCCACTCAGATCCCACCTGAAGTCGGCGACTTGCCTTACCTAGAATATCTC
ATATTCCACAAACTCCCTAACCTCACCGGTGAAATCCCACCCACCATCACCAAGCTCAAGTATCTCCGTTATCTC
TGGCTCAGCTGGAACAACCTGAGCGGCCCGGTTCCTGAATTTCTGAGTCAGCTCAAGAATCTAGAGTACATTAAC
CTTTCCTTCAATAAGCTCTCCGGTTCGATACCCGGTTCACTCTCTTTGTTACCTAAACTCGAGTTTCTTGAACTC
AGCAGGAACAAGCTTACAGGTTCGATACCAGAGTCATTTGGATCGTTTAAAGGAGTGGTATATGCTCTTTACCTA
TCGCACAACCAGCTGTCCGGTTCTATACCGAAATCATTAGGCAACCTGGACATTAACCAGATTGATCTTTCCCGG
AACAAGCTCGAAGGTGATGCGTCGATGTTGTTTGGAGCCAAAAAGACAACACAGCACATTGACCTATCAAGAAAC
ATGTTCCAGTTCAATATCTCCAAGGTTAAGGTAGCTAAAACAGTTAATTTCTTGGACTTGAACCACAACAGCCTC
ACAGGGAGTATCCCGGTTCAATGGACCCAACTTGATCTTCAGACTTTCAATGTAAGCTATAATAGACTGTGTGGA
CGCATCCCCCAGGGAGGGGACCTTCAGAGATTTGATGCTTATGCGTATTTACACAACAAGTGTTTGTGTGATGCA
CCTCTTCAGAGTTGCAAGTGA

Upper case letters represent cloned part of ORF, lower case letters represent native signal peptide for extracellular secretion. Underlined stop codon TGA was not amplified to add a myc and His_6 epitope from pPICZα A (Thermo Fischer Scientific GmbH). BrPGIP3 was expressed in *K. lactis* using pKLAC2 (New England Biolabs GmbH).

BrPGIP3 amino acid sequence:

EAEARRAHMKDLCHKDDKNTLLKIKKAMNDPYTIISWDPKDDCCTWYAVECGNASINHRVTSLDISNDDVSTQIP
PEVGDLPYLEYLIFHKLPNLTGEIPPTITKLKYLRYLWLSWNNLSGPVPEFLSQLKNLEYINLSFNKLSGSIPGS
LSLLPKLEFLELSRNKLTGSIPESFGSFKGVVYALYLSHNQLSGSIPKSLGNLDINQIDLSRNKLEGDASMLFGA
KKTTQHIDLSRNMFQFNISKVKVAKTVNFLDLNHNSLTGSIPVQWTQLDLQTFNVSYNRLCGRIPQGGDLQRFDA
YAYLHNKCLCDAPLQSCK

BrPGIP3_GPI amino acid sequence (immature, pre-processed protein):

MKFLVNVALVFMVVYISYIYAGMLKDLCHKDDKNTLLKIKKAMNDPYTIISWDPKDDCCTWYAVECGNASINHRV
TSLDISNDDVSTQIPPEVGDLPYLEYLIFHKLPNLTGEIPPTITKLKYLRYLWLSWNNLSGPVPEFLSQLKNLEY
INLSFNKLSGSIPGSLSLLPKLEFLELSRNKLTGSIPESFGSFKGVVYALYLSHNQLSGSIPKSLGNLDINQIDL
SRNKLEGDASMLFGAKKTTQHIDLSRNMFQFNISKVKVAKTVNFLDLNHNSLTGSIPVQWTQLDLQTFNVSYNRL
CGRIPQGGDLQRFDAYAYLHNKCLCDAPLQSCKAAASFLEQKLISEEDLNSAVDHHHHHHGSPTTTTTEATTTPV
PGSANIATLSIVTMIVTLVVNMA

BrPGIP3_GPI amino acid sequence (mature protein):

GMLKDLCHKDDKNTLLKIKKAMNDPYTIISWDPKDDCCTWYAVECGNASINHRVTSLDISNDDVSTQIPPEVGDL
PYLEYLIFHKLPNLTGEIPPTITKLKYLRYLWLSWNNLSGPVPEFLSQLKNLEYINLSFNKLSGSIPGSLSLLPK
LEFLELSRNKLTGSIPESFGSFKGVVYALYLSHNQLSGSIPKSLGNLDINQIDLSRNKLEGDASMLFGAKKTTQH
IDLSRNMFQFNISKVKVAKTVNFLDLNHNSLTGSIPVQWTQLDLQTFNVSYNRLCGRIPQGGDLQRFDAYAYLHN
KCLCDAPLQSCKAAASFLEQKLISEEDLNSAVDHHHHHHGSPTTTTTEATTTPVPGSA-(GPI anchor)

- BrPGIP3
- honeybee melittin secretion signal
- multiple cloning site (MCS) amino acids from cloning GOI in vectors
- myc epitope
- His_6 tag
- amino acids in tag region from pPICZα A
- spacer
- omega site
- GPI anchor transmembrane domain

<u>Supporting Information 3:</u> Full sequence of pMIB/V5_GPI

- pMIB/V5 A (Thermo Fischer Scientific GmbH, Bonn, Germany)
- honeybee melittin secretion signal
- BamHI/NotI restriction site
- remaining MCS (including SphI, HindIII, Asp718I, KpnI, SacI restriction sites)
- spacer
- omega site
- GPI anchor transmembrane domain + Stop (TAA)

CATGATGATAAACAATGTATGGTGCTAATGTTGCTTCAACAACAATTCTGTTGAACTGTGTTTTCATGTTTGCCA
ACAAGCACCTTTATACTCGGTGGCCTCCCCACCACCAACTTTTTTGCACTGCAAAAAAACACGCTTTTGCACGCG
GGCCCATACATAGTACAAACTCTACGTTTCGTAGACTATTTTACATAAATAGTCTACACCGTTGTATACGCTCCA
AATACACTACCACACATTGAACCTTTTTGCAGTGCAAAAAAGTACGTGTCGGCAGTCACGTAGGCCGGCCTTATC
GGGTCGCGTCCTGTCACGTACGAATCACATTATCGGACCGGACGAGTGTTGTCTTATCGTGACAGGACGCCAGCT
TCCTGTGTTGCTAACCGCAGCCGGACGCAACTCCTTATCGGAACAGGACGCGCCTCCATATCAGCCGCGCGTTAT
CTCATGCGCGTGACCGGACACGAGGCGCCCGTCCCGCTTATCGCGCCTATAAATACAGCCCGCAACGATCTGGTA
AACACAGTTGAACAGCATCTGTTCGAATTTAAAGCTACCATGAAATTCTTAGTCAACGTTGCCCTTGTTTTTATG
GTCGTATACATTTCTTACATCTATGCCGGCATGCTAAGCTTGGTACCGAGCTCGGATCCCCTACGACCACAACTA
CAGAAGCTACAACAACACCTGTACCTGGCTCAGCAAACATCGCCACTCTTAGCATCGTCACAATGATCGTGACTC
TCGTTGTTAATATGGCTTAAGCGGCCGCTCGAGTCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCCTCTC
CTCGGTCTCGATTCTACGCGTACCGGTCATCATCACCATCACCATTGAGTTTATCTGACTAAATCTTAGTTTGTA
TTGTCATGTTTTAATACAATATGTTATGTTTAAATATGTTTTTAATAAATTTTATAAAATAATTTCAACTTTTAT
TGTAACAACATTGTCCATTTACACACTCCTTTCAAGCGCGTGGGATCGATGCTCACTCAAAGGCGGTAATACGGT
TATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAA
AGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGA
GGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTC
CGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCT
GTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACC
GCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCA
CTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCT
ACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCTCTT
GATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAG
GATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTT
TGGTCATGCGAAACACGCACGGCGCGCGCACGCAGCTTAGCACAAACGCGTCGTTGCACGCGCCCACCGCTAACC
GCAGGCCAATCGGTCGGCCGGCCTCATATCCGCTCACCAGCCGCGTCCTATCGGGCGCGGCTTCCGCGCCCATTT
TGAATAAATAAACGATAACGCCGTTGGTGGCGTGAGGCATGTAAAAGGTTACATCATTATCTTGTTCGCCATCCG
GTTGGTATAAATAGACGTTCATGTTGGTTTTTGTTTCAGTTGCAAGTTGGCTGCGGCGCGCGCAGCACCTTTGCC
GGGATCTGCCGGGCTGCAGCACGTGTTGACAATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAG
GTGAGGAACTAAACCATGGCCAAGCCTTTGTCTCAAGAAGAATCCACCCTCATTGAAAGAGCAACGGCTACAATC
AACAGCATCCCCATCTCTGAAGACTACAGCGTCGCCAGCGCAGCTCTCTCTAGCGACGGCCGCATCTTCACTGGT
GTCAATGTATATCATTTTACTGGGGGACCTTGCGCAGAACTCGTGGTGCTGGGCACTGCTGCTGCTGCGGCAGCT
GGCAACCTGACTTGTATCGTCGCGATCGGAAATGAGAACAGGGGCATCTTGAGCCCCTGCGGACGGTGCCGACAG
GTTCTTCTCGATCTGCATCCTGGGATCAAAGCCATAGTGAAGGACAGTGATGGACAGCCGACGGCAGTTGGGATT
CGTGAATTGCTGCCCTCTGGTTATGTGTGGGAGGGCTAAGCACTTCGTGGCCGAGGAGCAGGACTGACACGTCCC
GGGAGATCTGCATGTCTACTAAACTCACAAATTAGAGCTTCAATTTAATTATATCAGTTATTACCCATTGAAAAA
GGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTG
CTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGG
ATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTC
TGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGA
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ACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA
CTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCC
TTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGG
GGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATA
GACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTT
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<u>Supporting Information 4:</u> Figures

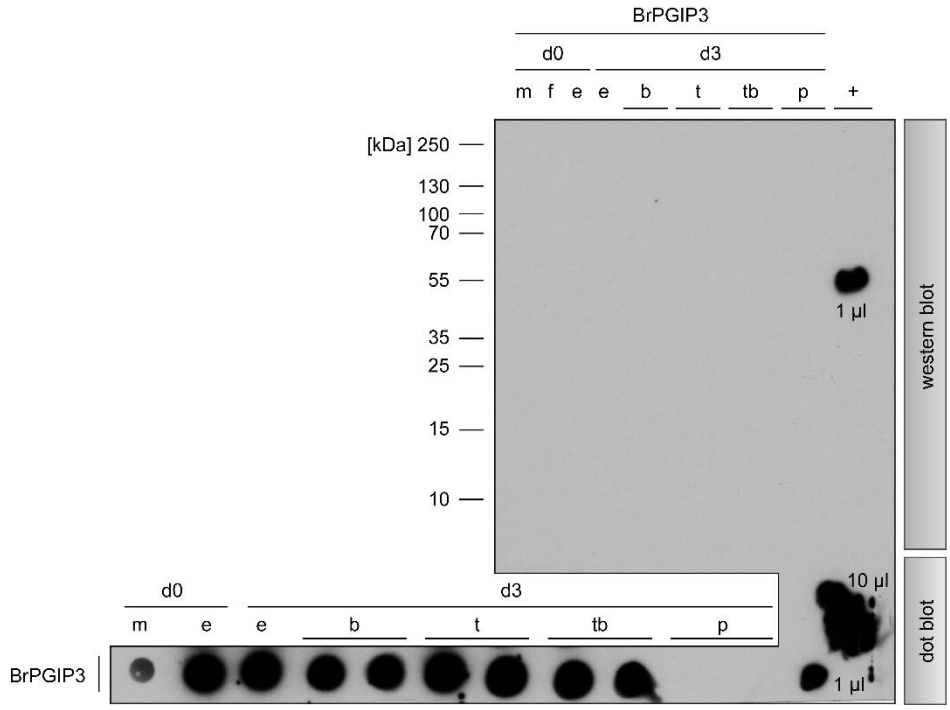

Figure S1: Comparison of signal intensities on western and dot blot indicate an aggregation of the soluble BrPGIP3. Equal volumes of culture medium (m) from yeast expression, flow-through (f) and elution (e) of purification were applied onto a western blot and a dot blot. Samples were taken on the day of harvest and purification (d0) and elution fractions were stored under different conditions at 4°C for 3 days (d3). The elution fractions were supplemented with 1 and 5 µg/µl% BSA (b), 0.001 and 0.1% Tween20 (t), 1 and 5 µg/µl BSA + 0.01% Tween20 (tb) and 0.2 and 0.5% PGA (p). An anti-His_6 antibody detects both BrPGIP3 as well as the positive control PCO_GH28-1 (+). This stable protein results in a band and dot of corresponding size on the western and dot blot (1 µl), when simultaneously developed on the same film.

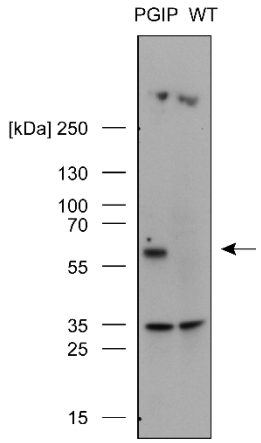

Figure S2: Comparison of membrane preparations from BrPGIP3_GPI-expressing and wild type Sf9 insect cells. Membrane proteins from BrPGIP3_GPI-expressing (PGIP) as well as wild type (WT) Sf9 cells were applied onto a western blot and the proteins were detected with an anti-myc antibody. The arrow indicates the expected size of BrPGIP3_GPI.

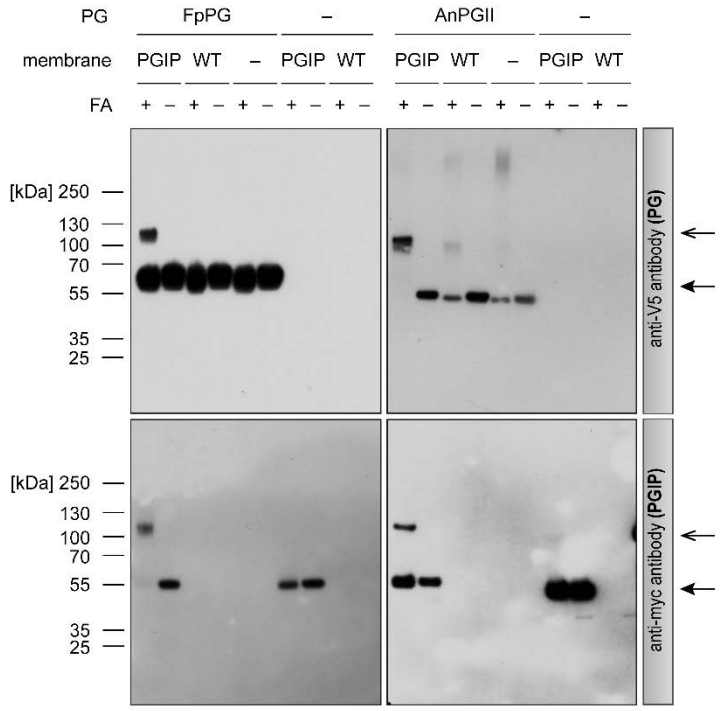

Figure S3: Both fungal PGs, FpPG and AnPGII, specifically interacted with PvPGIP2_GPI. Membrane proteins from wild type (WT) as well as PvPGIP2_GPI-expressing Sf9 cells were incubated with FpPG as well as AnPGII and cross-linked with formaldehyde (FA). The PGs can be detected with an anti-V5 antibody (top) and the PGIP with an anti-myc antibody (bottom). Arrows indicate the expected size of PG or PGIP alone (closed arrowhead) and the PG-PGIP complex (open arrowhead), respectively. Note that the Western blots using the anti-V5 antibody (upper panels) are also used in Figure 3 panels A and C.

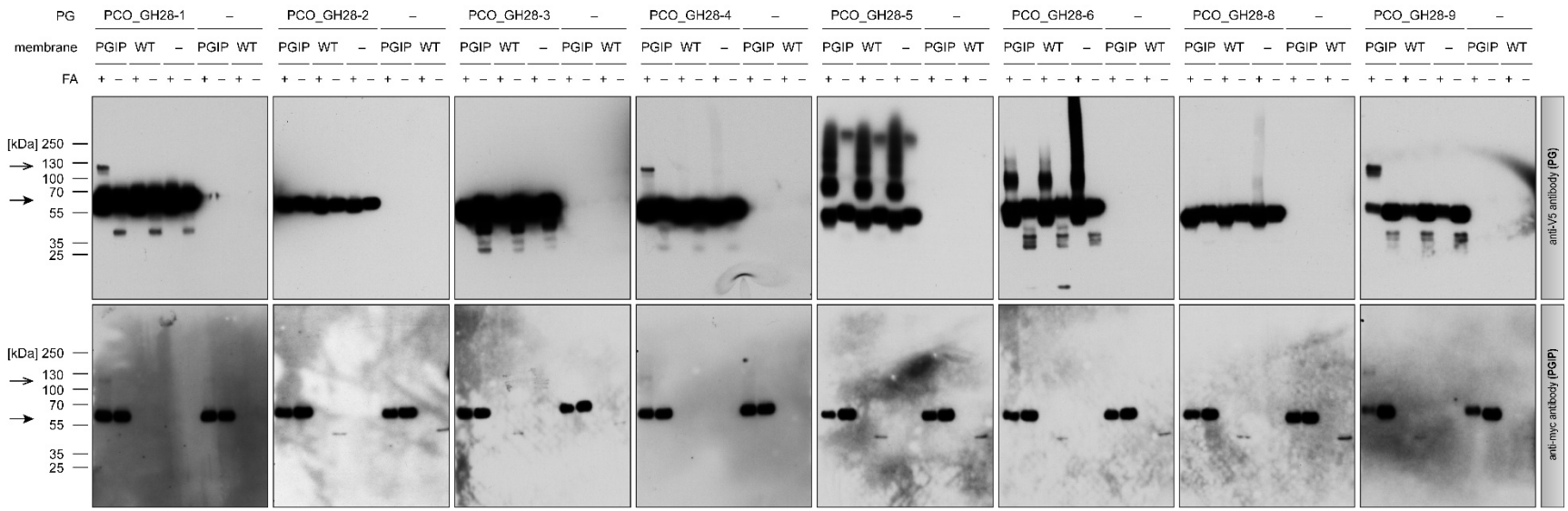

Figure S4: Interaction assay of BrPGIP3_GPI with the beetle PG family members reveals specific interaction of PCO_GH28-1, -4 and -9 with BrPGIP3_GPI. Membrane proteins from BrPGIP3_GPI-expressing as well as wild type (WT) Sf9 cells were incubated with PCO_GH28-1-9 (except PCO_GH28-7) and cross-linked with formaldehyde (FA). The PGs can be detected with an anti-V5 antibody (top) and the PGIP with an anti-myc antibody (bottom). Arrows indicate the expected size of PG or PGIP alone (closed arrowhead) and the PG-PGIP complex (open arrowhead), respectively. Note that the Western blots using the anti-V5 antibody (upper panels) for PCO_GH28-1, PCO_GH28-5 and PCO_GH28-9 are also used in Figure 4 panels A, C and E respectively.

Supporting Information 5: **Table**

Table S1. Inhibitory activity of PvPGIP_GPI and BrPGIP3_GPI on the fungal FpPG and AnPGII as well as the *P. cochleariae* gut content and PGs PCO_GH28-1, -5 and -9.

membrane protein	FpPG		AnPGII		PCO_GH28-1		PCO_GH28-5		PCO_GH28-9		gut content	
[µg]	%	p	%	p	%	p	%	p	%	p	%	p
5			75*	=0.001								
10			83*	≤0.001								
25	-8	n.s.	91*	≤0.001	-3	n.s.	1	n.s.	0	n.s.	-6	n.s.
50	12	n.s.	100*	≤0.001	2	n.s.	-1	n.s.	5	n.s.	13	n.s.
100	25	n.s.			15*	≤0.001	7	n.s.	10*	=0.006	17*	≤0.001
150	58*	≤0.001			20*	≤0.001	18*	=0.015	14*	≤0.001	18*	≤0.001
200	80*	≤0.001			22*	=0.001	13*	=0.043	16*	=0.002	19*	≤0.001
250	83*	≤0.001			38*	≤0.001	13*	=0.002	11	n.s.	18*	≤0.001
300					51*	≤0.001	13	n.s.	22*	≤0.001	33*	≤0.001

The inhibitory activity is displayed as the % reduction of PG activity by PGIP-containing membrane proteins compared to an equal amount of wild type membrane proteins. The corresponding samples (n=3) were compared with a Student's t-test or rank sum test (Mann-Whitney). Significantly different samples are marked with an asterisk. n.s., not significant; empty cells, not tested.