

Supplementary Information

Chitinases and Imaginal disc growth factors organize the extracellular matrix formation at barrier tissues in insects

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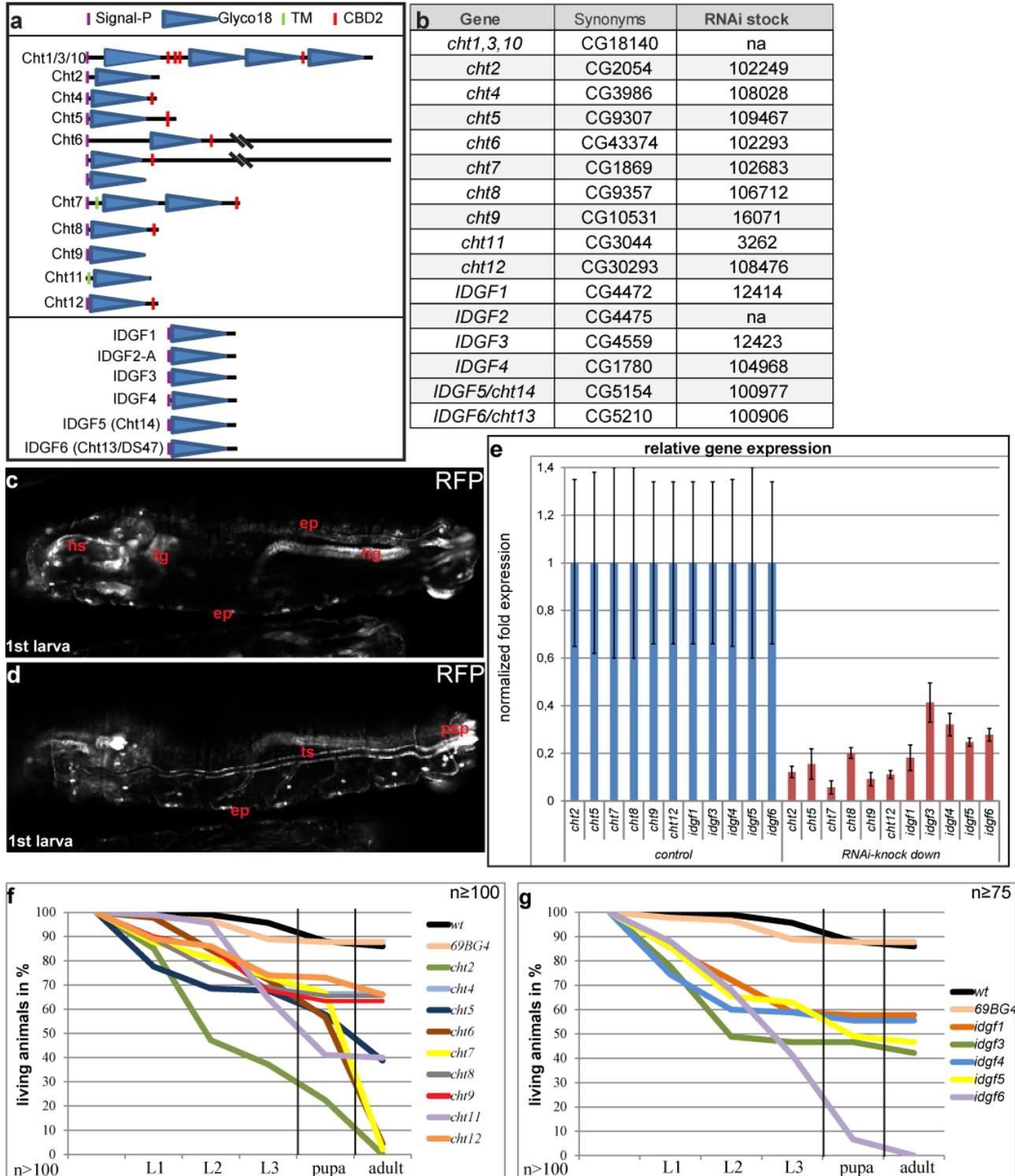
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Supplementary Figures and Legends

Supplementary Fig. S1

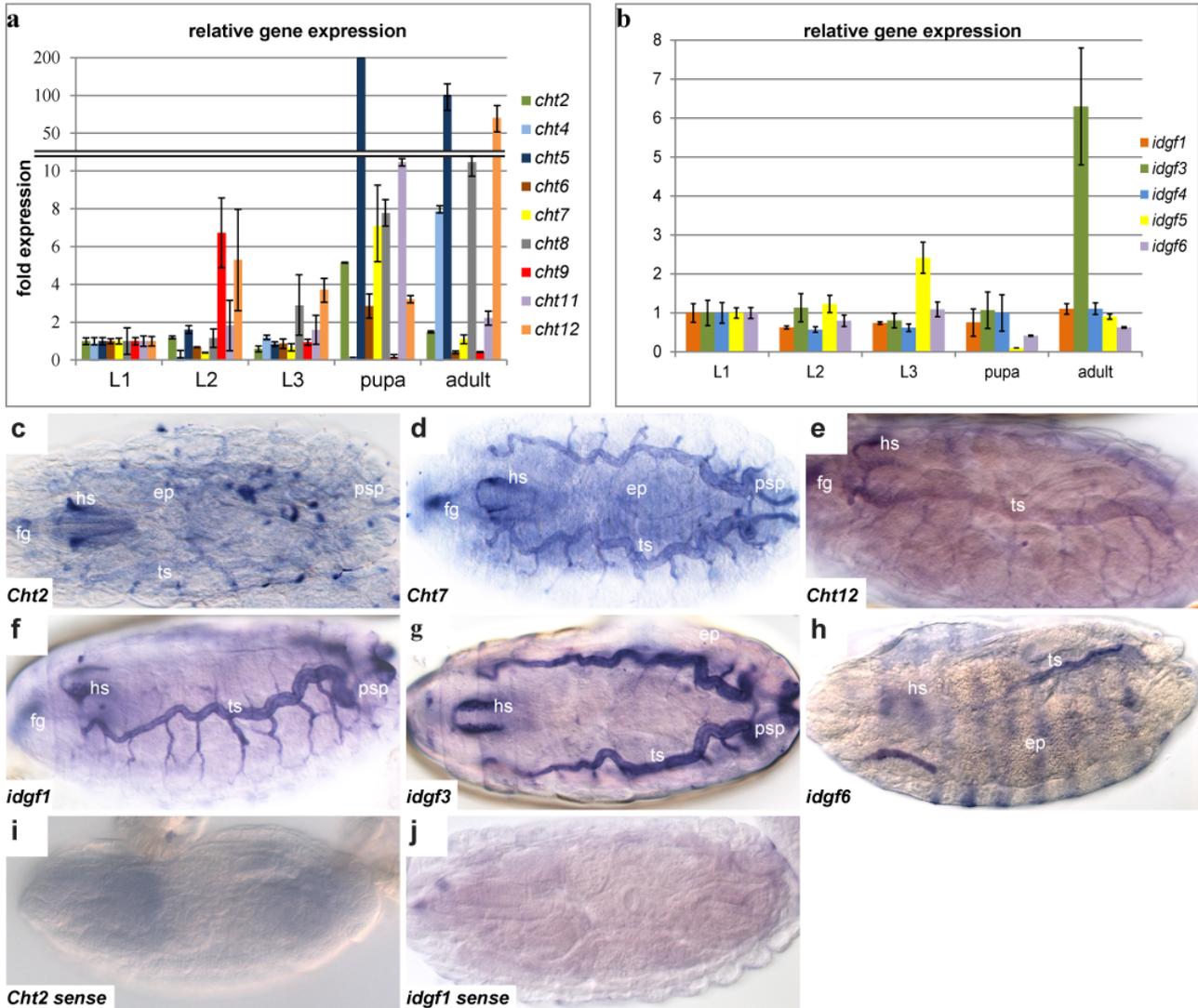


The *Drosophila* family18 members, used RNAi stocks and knockdown expression effects.

(a) Schematic drawings of the glycosylhydrolase family18 indicate the Glyco18 domains (blue), N-terminal Signal-Peptides (Signal-P, purple), transmembrane domains (TM, green) and chitin-binding domains (CBD2, red). The *Drosophila* family18 contains ten genes encoding the Chitinase (Cht) and six genes encoding the Imaginal disks growth factor (Idgf) proteins. The diagram illustrates that Chts contain individual and Idgfs common domain arrangements. Cht1, 3 and 10 are

encoded by a single gene. For *Cht6* different protein variants were predicted. Domains were annotated with the SMART (EMBL) database and the CBS Server (CBS: <http://www.cbs.dtu.dk/>) (SignalP 4.1). **(b)** The table summarizes the *Drosophila family18 cht* and *idgf* genes. The FlyBase gene accession numbers and used Vienna RNAi stocks are provided. With the exception of *Cht1/3/10* gene and the *idgf2* gene, RNAi knockdown lines were used for all other genes. However both genes were investigated previously in *Tribolium castaneum*. In particular the *Cht1/3/10* gene showed severe cuticle molting defects throughout development¹. These data supplement our knockdown screen. It is of note that the knockdown strategy with Vienna RNAi stocks resulted in efficient reduction of transcript levels. **(c,d)** The *69B-Gal4* drives embryonic and larval RFP expression in cuticle forming epithelia such as the epidermis (ep), head skeleton (hs), parts of the digestive system (foregut, fg; hindgut, hg), posterior spiracles (psp) and tracheal system (ts). **(e)** The quantitative RT-PCR analysis compares relative expression levels of *Chts* between *wt* and knockdown first instar larvae. Progeny of crosses from individual UAS-RNAi lines with *69B-Gal4* which showed severe epidermal cuticle defects (Figures 1,2) and reduction of expression levels upon *Cht2*, *Cht5*, *Cht7*, *Cht9* and *Cht12* as well as *idgf1*, *idgf3*, *idgf4*, *idgf5*, *idgf6* knockdown. Standard deviation bars are indicated. The analysis shows fold expression levels normalized to *wt* (1 = 100%). **(f,g)** Lethality of animals upon UAS-RNAi-mediated knockdown of *Cht* (f) and *idgf* (g) genes using the *69B-Gal4* driver line. Individual knockdown animals (n≥100) were monitored for twelve days after egg laying, which is an adequate time period to analyze embryogenesis, larval stages, pupal development and early adulthood. In summary, this assay showed lethality during larval and pupal stages for individual knockdown of all tested *Drosophila Chts/idgfs*, with variability in the phenotypical severity, while *wt* and *69B-Gal4* control animals survived until adulthood. The highest lethality was caused by the *Cht2* and *idgf6* knockdowns during larval stages.

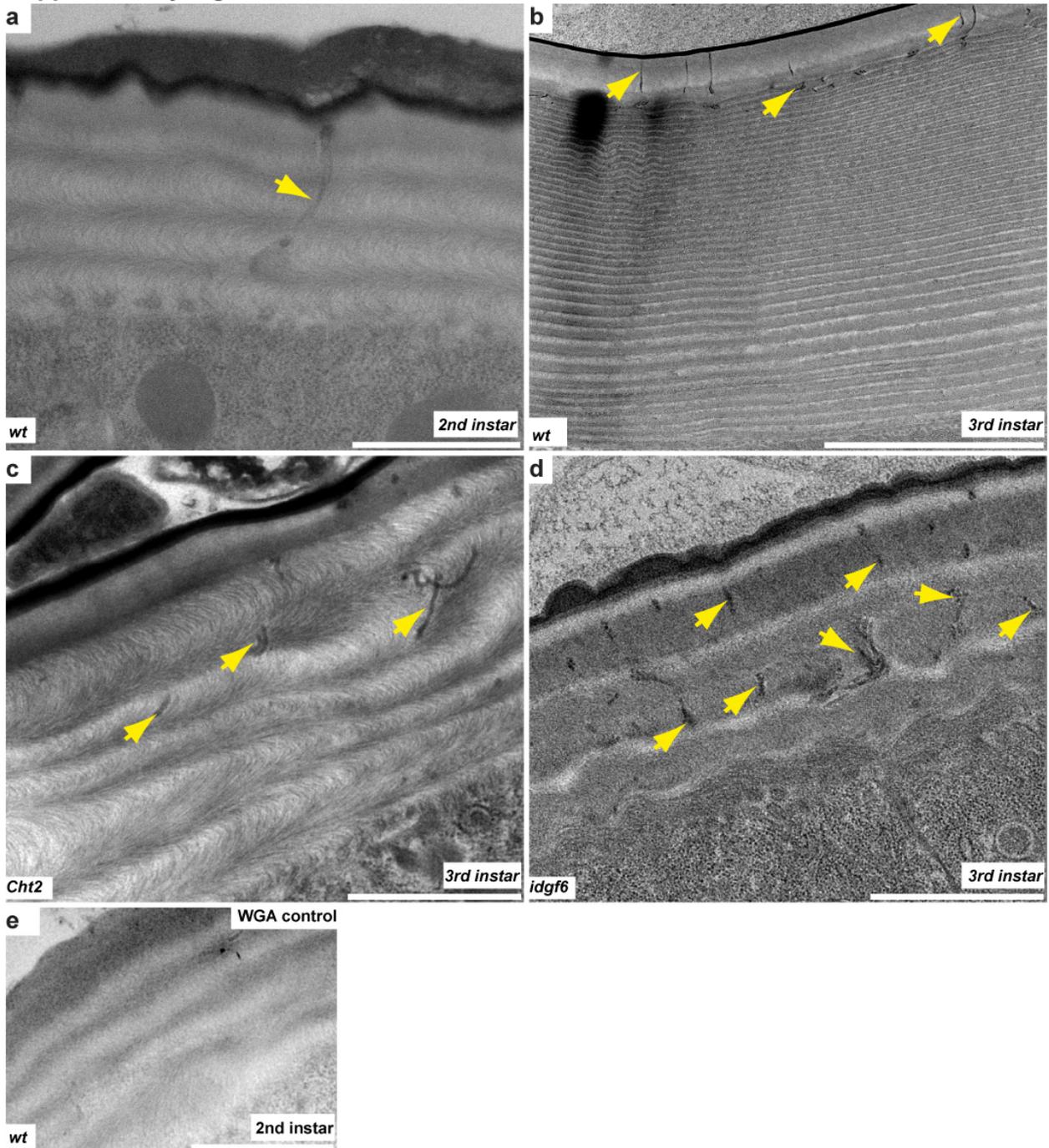
Supplementary Fig. S2



Spatial-temporal gene expression profiles of *Cht* and *idgf* genes.

(a, b) Transcription levels of *chitinase* (a) and *idgf* (b) genes relative to first instar larvae. Most *Cht* genes are constantly expressed throughout larval stages. The *Cht9* gene is up-regulated at second instar, which matches its specific requirement for molting into third instar. The *Cht12* gene is more strongly expressed during larval stages and mutants showed only larval molting defects. The *Cht2,5,6,7* genes show strong up-regulation during pupal stages reflecting their involvement in pupal molting. Note that *Cht5* expression was > 323 fold upregulated in pupae. It is of note that *Cht8* and *Cht11* gene expressions are enriched during pupal development, but pupal molting defects were not observed. The *Cht11* gene product was discussed to be associated with mitochondrial functions for identification of critical infections². The function of *Cht8* remains unknown. *Chts5,8,12* and *idgf3* are specifically enriched in adult flies, and may take over their enzymatic function later on after development. (c-j) Stage 17 whole mount embryos were stained by *in situ* hybridization as previously described³ with DIG-labeled RNA antisense (c-h) and sense (i,j) probes. The *Cht2*, *Cht7*, *Cht12*, *idgf1*, *idgf3*, *idgf6* genes show mRNA expression in the cuticle producing organs, the mouth hooks (mh), the epidermis (ep), the tracheal system (ts) and the posterior spiracles (psp). Little expression is also detected in the foregut (fg). In contrast, the negative controls, the sense probes, did not detect expression.

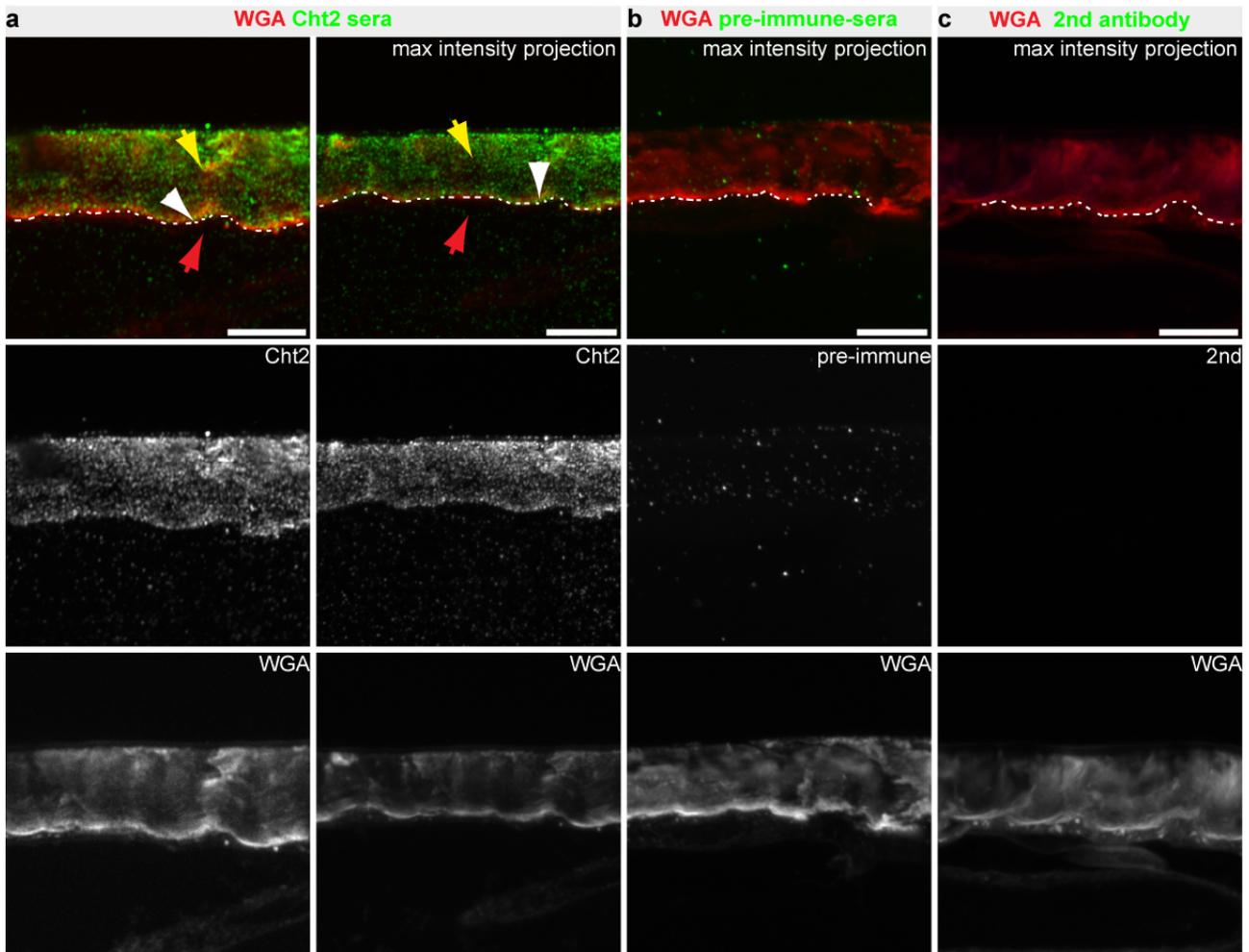
Supplementary Fig. S3



Epidermal cuticle formation in second and third instar larvae.

Ultrastructure analysis of epidermal cuticle in third instar larvae. **(a,b)** In *wt* epidermis the cuticle contains pore-canal-like structures (arrows). The second instar (a) shows canal-like structures running across the procuticle. The third instar (b) shows pore-canal-like structures only in the epicuticle and the outermost two chitin-lamellae of the procuticle. **(c)** In *Cht2* knockdown larvae, pore-canal-like structures appeared in the procuticle but did not cross the lamellae. **(d)** In the *idgf6* knockdown epidermis, pore-canal-like structures (dashes) are distributed throughout the degraded procuticle. **(e)** Negative control for WGA labeling shown in Fig. 5f-f". Second instar larvae after immunogold-labeling but without using WGA. Scale bars represent 5 μm in B and 1 μm in a,c,d,e.

Supplementary Fig. S4



Cht2 is localized within the larval cuticle.

Immunofluorescent detection of Cht2 in the cuticle of wt third instar larvae by using Cht2 antibody serum and an anti-rabbit Cy-3 secondary antibody. The chitin-matrix and the apical surfaces were visualized by WGA (red). **(a,b)** Co-labeling studies (n=7 larvae) were performed with the Cht2 antibody serum (rabbit, green) shown as single image (a) or as confocal Z-stack projection (b). **(c,d)** As negative controls (n=7 each) the pre-immune serum (c) and the Cy-3 secondary antibody alone were used (d). Our preliminary data show representative Cht2 staining (a,b) within the cuticle (yellow arrow) and at the apical cell surface where the assembly zone is expected (white arrows). The epidermal cells underneath are indicated by the red arrows. The negative controls reveal no or very little signal in the cuticle (c, d). For comparison, all confocal settings were adjusted to similar settings (laser power, available gain functions, off set, pinhole size). Anti-Cht2 antibody was generated in rabbits (Pineda, Germany) against the peptide WDPQTSQVLAKSERN (amino acid 335 to 349). The peptide sequence is specific to all predicted Cht2 protein variants (see FlyBase) and did not show any cross-reactivity to other *Drosophila* proteins as tested by FlyBase and NCBI Blast search engines with stringent and less stringent matrices. For stainings the unpurified Cht2 antibody serum was used in a 1:1000 dilution. Contrast and brightness of images of original data were not changed afterwards. White dashes mark the apical cell surface. Scale bars indicate 10 μ m.

Supplementary Fig. S5

a

<i>Aedes aegypti</i> (Aa)	<i>Drosophila melanogaster</i> (Dm)	
DmCht2 39	HDKVVCVSTWAVYRPEQAGAYAIENFDNLCNTHVYAFAGLDITQAAIKSLDPWQDLKE	98
	H +VVVVCY+S+HWAYR G++++NFDNLCNTHVYAFAGLDITQAAIKSLDPWQDLK+	
AaCht2 8	HGRVVCVYISSWAVYRSGAGSFLNFDNLCNTHVYAFAGLDITQAAIKSLDPWQDLKE	67
DmCht2 99	EYKGGYERMTGLKRSHPLKVSALGAGWNEGSANYSITLVANNLLRGRFVQVSSPIRKY	158
	YG GGYEK+ L + + + + G W N E G S Y S L A + R F + + F I R + Y	
AaCht2 68	NYLGGYKLVN-----LMSMGWNEGSVYKSLAASQQRQVFAENALFIRRY	119
DmCht2 159	NFDGLDLWEYPTQRKGPADRENFVLLTKELREEFDEHGLLTSAGKSKVIDEAYDV	218
	FDGLDLWEYPTQR G+P DRENFV L +EL + + F + + L L L T S I A + V I D A Y D +	
AaCht2 120	FGDGLDLWEYPTQRGGEPDRENDFVQLVRELSQKFKNNLLSTIEAKQYVIDAAYDI	179
DmCht2 219	RQISRYLDYLHIMCYDHYGSDWRVRYNAPLTAAPADPLSVKFSIDYLLKGLAPPEKLV	278
	+ + + Y L D L H + C Y D Y G R + + + + G N A P L T D + + V + I + + L L G A P P E K L V	
AaCht2 180	ENLTKYLDLHVKCYDHYGSDWRVRYNAPLTAAPADPLSVKFSIDYLLKGLAPPEKLV	237
DmCht2 279	GLPFYGRF--KTLSGFLNDVSEGVGFKGPTREDGLFYNEICQTLNSQTSQWREWD	336
	GLPF+GRF + + + D + E F G P Y T D G + + G Y N E I C L + + S W + W D	
AaCht2 238	GLPFGRFIFDIADSIYFRIGDPAE--TSFSGPYTNDIGYGYNEICTELNAKSSFWNQAWD	296
DmCht2 337	PQTSQVLAKSERNVFTQIEINVVYDSSRSIANKVLFAMSKRLAGVMVMSVDTDFLGNCK	396
	P+ S+V+A+ + T+ + YDS+RSIA KV +A+ K L G+M S+D+D+D GNC	
AaCht2 297	PEASEVIARMQDGSSTK--VIVDYSRSIAAKVRYAVQKNLRGLMASSIDTDFDNGCN	353
DmCht2 397	LEDETYEDFQKVTAAKRRSSQNYPLLRITINEATMLAVDEL	436
	+E+T+ DF P + LLRTN A + A DE+	
AaCht2 354	AEEETFDVDFNKLRTFPPIQGFKLLRITINNAVAEAEI	393
<i>Pediculus humanus corporis</i> (Ph) <i>Drosophila melanogaster</i>		
DmCht2 33	ARTGPHLHDKVVCVSTWAVYRPEQAGAYAIENFDNLCNTHVYAFAGLDITQAAIKSLDP	92
	R P+HDK VVCY+ TW+VYR +G+ IEN D NLCTH+VYAF GL+ T + + S+DP	
PhCht2 5	TRLKHPIHDKVVCVSTWAVYRPEQAGAYAIENFDNLCNTHVYAFAGLDITQAAIKSLDP	64
DmCht2 93	WQDLKEEYKGGYERMTGLKRSHPLKVSALGAGWNEGSANYSITLVANNLLRGRFVQVSS	152
	+ DL+E YGRK + + M T L K + P + L K V + L A + G W N E G S A N Y S + R + F + V	
PhCht2 65	YYDLEENYKGGYERMTGLKRSHPLKVSALGAGWNEGSANYSITLVANNLLRGRFVQVSS	124
DmCht2 158	SFIRKYNFDGLDLWEYPTQRKGPADRENFVLLTKELREEFDEHGLLTSAGKSKVI	212
	+ + K Y N F D G D L W E + P R G + P D + N F L L K E L + E + L + L T + A + G A + I	
PhCht2 125	EYTYKYNFDGLDLWEYPTQRKGPADRENFVLLTKELREEFDEHGLLTSAGKSKVI	184
DmCht2 218	DEAYDVRQISRYLDYLHIMCYDHYGSDWRVRYNAPLTAAPADPLSVKFSIDYLLKGLAP	272
	+ A Y D V + I S + H D L H M C Y D Y H G W D + V G N A P L T + D L + + S I + L L + L G A P	
PhCht2 185	NTAYDVRQISRYLDYLHIMCYDHYGSDWRVRYNAPLTAAPADPLSVKFSIDYLLKGLAP	242
DmCht2 278	PEKLVMLGPFYGRF--KTLSGFLNDVSEGVGFKGPTREDGLFYNEICQTLNSQTSQ	330
	P K L V + G + P Y G F T K + + + + G G + G + T + + G F G Y N E I C L	
PhCht2 243	PHKLVGIPAYGHTFLTKVNTFRMGTPTTGGPGEVFTKQQGFGYNEICTELNKDDEK	302
DmCht2 331	WTREWDPTQTSQVLAKSERNVFTQIEINVVYDSSRSIANKVLFAMSKRLAGVMVMSVDTDD	390
	W + + W D + + S A + + + V + D + + S I K V + + + G M + W S + D T D D	
PhCht2 303	WSKHWDDESSPIYAVNGN-----HVIAFDDEKSIGEVNGLKYGKGVFMHWSIDTDD	355
DmCht2 391	FLGNCKLEDETYEDFQKVTAAKRRSSQNYPLLRITINEATMLAVDELAVEPQDDSE	447
	F G + C K K S + Q + + P L L R A A + + + D D	
PhCht2 356	FHGD-----DKERLKVESAQDKYTFPLLR----AVHTAIQQYK----QDDN	397
DmCht2 448	NEIPHGSADRKNAGASMV--SLGLVAVFMLL	479
	N+IP D + + V S + T A F + + +	
PhCht2 398	NDIPEVGNIDIDDKENNVVPSQSSITANFVVI	431
DmCht2	(AAF47562.1; see also flybase: http://flybase.org/reports/FBgn0022702.html)	
	1 mclrsrlsge apqlwlllll astasslwas vaartgplhd kvvvcyvwsc avyrpeqgay	
	61 aienfdpnic thvvyafagl ditqgaikl dpwgdikeey gkygyekmtg lkrshphlky	
	121 slaiggnwne sanystlvannllrgrfvqk vssfirkyfnf dgllddweyp tqrgkypadr	
	181 enfvlltkel reedehgll ltsaigaskk videaydvrg isryldylhi mcydyhgsawd	
	241 rrvygnaplt apadpplsvk fsidylkllg appeklvmgl pfygrtftkl asgfndvse	
	301 gvygkpytr edglfyneic qctlnsqtsq wtrewdpqts qvlaksernv ftqieinvvty	
	361 dssrsiankv lfamskrlag vmvmsvdtdd flgnckldeed tyedfkgkva aprkssqny	
	421 llrtineatm lavdelave pqpddsenei phgsiadrkn agasmvslgl gtvavfmilh	
	481 rlaq	

b

<i>Aedes aegypti</i> (Aa)	<i>Drosophila melanogaster</i> (Dm)	
DmIdgf6 7	AIVSLCLASIQASKVGAQPLPKKHLVVCYDASAFVKEGLKGLVIDEALQFCDYLVYG	66
	A+ ++CL + + G Q K ++CYD A+F+ EGLK+ + ++ AL FC +LVYG	
AaIdgf6 7	AVTAICLLGLVLLAPTQYSQSTAK-VLCYDGANFLIEGLKGLVIDEALQFCDYLVYG	65
DmIdgf6 67	YAGIERDSHKAVSLNQQLDLGKGLYRTVTRLKRKYPNKILLSVGGDKDIELDKDAKE	126
	YA ++ DS+KAVS N LDDL GKG YR VT LKRK+P +K+LL +GG +	
AaIdgf6 66	YAAVDFPSNKAVSRNANLDDTQGNKYRQVTLKRKFPGLKGLVLLGIGYR-----FSA	118
DmIdgf6 127	LPNKYLELLESPTGRTRFVNTVYSLVKTYGFDGLDVAWQFPKPKPKVHSGIGSLWKGFK	186
	KYLELLES R F+N+VYS+VK Y FDG+D+AWQFP+NKPKV S G LW GFK	
AaIdgf6 119	ASPKYLELLESGAARITFINSVYSIVKAYDFDGLDVAWQFPKPKPKVHSGIGSLWKGFK	178
DmIdgf6 187	KVFSGDSIVDEKSEEHKEQFTALLRDVKNFRPNLLSTVLPVNVNSSLFYDIPAVVNY	246
	KVF+GDS++DEK+EH+E+FTALLR++KNAFR D L TVL +VN+S+F DIPA+VNY	
AaIdgf6 179	KVFTGDSVLEKADREHEFTALLREMKNAFRSDYQGLGITVLSHVNASMDFPAIVNY	238
DmIdgf6 247	LDFVNLGTFDFFTQQRNPEVADYAAPIYELSERNEPEFNVAQVYKYLNNCPASKINVG	306
	LDFVN+ +D TP+RN +AD+ APIYELSER P NV VK WL ++ PASK+ V +	
AaIdgf6 239	LDFVNLAAVDMQTFERNKKEADFYAPIYELSERIPGNVNDGLVWLNSHAPASKLIVSI	298
DmIdgf6 307	ATYGRPWKLDSDGDTGVPVVKVDEA-FVGNQTVPGIYSWPEVCALLPNQNNAYLKG	365
	T+GR WK+T+DSG TGVPV+ V D A P G Q G YSW E CA LEN +N LKG	
AaIdgf6 299	PTHGRGWMTEDSGITGVPPV--VADGASPAQTQQEGFYSWAETCAKLENPSNTALKG	356
DmIdgf6 366	ANAPLIKVDPAKRFSGYAYRAADKGDNGIWSFEDPDTAADKAGYVRTENLGGVALFD	425
	A+ EL KV DP KRFGSYA+R D G+G+WV+EDPD+A +KA YV+ + LGG+A+ D	
AaIdgf6 357	ADGFLKVDGDPTRKRFSGYAFRLPDSSEGHGMWVYEDPDSAGNKAAYKAKGLGAIIND	416
DmIdgf6 426	LSYDDFRGLCTNEKYPILRAIKYRL	450
	L++DDFRG C EKYPIIRA KYRL	
<i>Anopheles gambiae</i> (Ag) <i>Drosophila melanogaster</i> (Dm)		
DmIdgf6 1	MIKALAIIVSLCLASIQASKVGAQPLPKKHLVVCYDASAFVKEGLKGLVIDEALQFC	60
	M K A L L L + S+ Q P K ++CYD A+F+ EGLK+ + ++ AL FC	
AgBRP2 1	MWCKQFAGALLL--VATSYQVQSQPSK-VLCYDAAFLIEGLKGLVIDADALPFC	57
DmIdgf6 61	DYLVYAGYIERDSHKAVSLNQQLDLGKGLYRTVTRLKRKYPNKILLSVGGDKDIEL	120
	+LVYGYAGI+ +++KAVS LDDL GKG YRVT+LK KYP++K+LL +GG K	
AgBRP2 58	THLVYAGYIWEINKAVSRQENLDDTQGNKYRQVTLKRKFPGLKGLVLLGIGYK----	113
DmIdgf6 121	DKDAKELPNKYLELLESPTGRTRFVNTVYSLVKTYGFDGLDVAWQFPKPKPKVHSGIGS	180
	E KYL LLES R F+N+VYSL+KTYGFDG+D WQFP NKPKV S +G	
AgBRP2 114	---FSEPSIKYLLLESGAARITFINSVYSLKTYGFDGVDLEWQFPKPKPKVHSGIGS	170
DmIdgf6 181	LWKGFKKVSFSGDSIVDEKSEEHKEQFTALLRDVKNFRPNLLSTVLPVNVNSSLFYDI	240
	+W GFKKVSFSGDS+DEK+EEH+E+FTALLR++KNAFR D L TVL +VN+S+F DI	
AgBRP2 171	VWKGFKKVSFSGDSVLEKADREHEFTALLRELKNAFRSDYQGLGITVLSHVNVSVFMDI	230
DmIdgf6 241	PAVVNYLDFVNLGTFDFFTQQRNPEVADYAAPIYELSERNEPEFNVAQVYKYLNNCPAS	300
	PA++NYLDFVN+ +D TP RN +AD+AAP+YELS+R P NV QV+ WL NN PAS	
AgBRP2 231	PAIINYLDFVNLAAVDMQTFERNKKEADFYAPIYELSERIPGNVNDGLVWLNSHAPAS	290
DmIdgf6 301	KINVGATYGRPWKLDSDGDTGVPVVKVDEA-FVGNQTVPGIYSWPEVCALLPNQNN	360
	K+ V +T+GR WK+ DSG TGVPV+ P G T Q G YSW EVCA+LEN +N	
AgBRP2 291	KLIVSIPTRGGMWMTEDSGITGVPPVLPADGFSN--GPQTQTEGFSWAETCAKLENPSN	349
DmIdgf6 361	AYLKGANAPLIKVDPAKRFSGYAYRAADKGDNGIWSFEDPDTAADKAGYVRTENLGG	420
	LKGA+APL KV DP KRFGSYA+R D G+G+WVS+EDPDTA +KAGYV+ +NLGG	
AgBRP2 350	TALKGADAPLRKVDGDPTRKRFSGYAFRLPDSNGEHWVWSYEDPDTAGNKAAYKAKLGG	409
DmIdgf6 421	VALFDLSYDDFRGLCTNEKYPILRAIKYRL	450
	+A+ DLSYDDFRG C EK+PIIRA KYRL	
AgBRP2 410	IAINDLSYDDFRGSCAGEKFPILRAIKYRL	439
DmIdgf6, (NP_477081.1; see also flybase: http://flybase.org/reports/FBgn0013763.html)		
	1 miikalaiivs lclasiqask vgapqlpkkh lvcydsasf vkeglglvi delepalqfc	
	61 dylvygyagi erdshkaval ngqldldlgl glytrvtrlk rkykpnkill svvgdkdiel	
	121 dkdakelpnk yleleaptg rtrfvntvys lvkytygfdgl dvawqfknk pkkvhsigis	
	181 lwkgfkvks qdsivdeke ehkeqftall rdvknafpnd nllstvtvlp nvnsalfydi	
	241 pavvnyldfv nlgtfdfft qrnpevadya apiyelsern pefnvaagk ywlrnncpas	
	301 kinvgatyg rpwkldsdsg dtgvpvkvk vdeapvgnt qvqyiswpe vcallpnqn	
	361 aylkganapl ikvdpakrf gsyayraadk kdngiwsf edpdtadka gvytrtenlg	
	421 valfdlsydd frglctneky pilraikyr l	

Cht2 and Idgf6 amino acid sequence analysis shows non-conserved regions.

(a) The Cht2 C-terminal (a) and the Idgf6 N-terminal (b) amino acid sequences are not conserved in insects. Alignments compare amino acid sequences of *Drosophila*, mosquitoes and other harmful insects. The amino acids marked in red are less conserved. Non conserved sequences are potential candidate target regions for a species specific insecticide. *Drosophila melanogaster* Cht2 (AAF47562.1); *Pediculus humanus corporis* (body louse, ectoparasite and transmitter of epidemic typhus; XP_002423089.1); *Aedes aegypti* (Cht2, XP_001657537.1; Idgf6, XP_001660748.1) and *Anopheles gambiae* (bacteria responsive protein2 (BRP2; AAS80138.1)). Blast search and alignments by NCBI database.

Supplementary Table 1

Gene	Primer	sequence	efficiency	splice var., #no.
rp49	for	GCTAAGCTGTTCGCACAAATG	100%	
rp49	rev	GTTTCGATCCGTAACCGATGT		
Cht2	for	ACGAGAAAATGACGGGTCTG	98%	detects all, #6
Cht2	rev	TGAAACTGGACACCTGCTTG		
Cht4	for	ATGACAATCCCGAAAGCATC	105%	
Cht4	rev	ACAGACCACGGAAATCATCC		
Cht5	for	CAACAACCACCACAACAACC	94%	detects all, #2
Cht5	rev	ATCTCGTTGGGATCGAACTG		
Cht6	for	ATGCCAGTTTGTCCAAGAG	99%	detects all, #14
Cht6	rev	AACTTGGGCACATCGTAACC		
Cht7	for	TTACGAGGCCATCGACTTTC	91%	detects all, #2
Cht7	rev	CCACTCGCAATTCCTTTAGC		
Cht8	for	TCTGGGCAAGTTCGGTATG	70%	detects all, #2
Cht8	rev	GCGTCCACTATTCTCCTCAAG		
Cht9	for	TCGTGCCAACTTTGTGACTC	90%	detects all, #2
Cht9	rev	CGATTTTCGTAGGATGCACTG		
Cht11	for	CTGCAAGGCGAACTATGTGA	93%	
Cht11	rev	TGGGTTAGCGGAAATACAGG		
Cht12	for	TCGGACTTTATCCACCTGATG	75%	detects all, #2 red= intron
Cht12	rev	CTCCTCCATTTCGTTTCCAG		

The table 1 summarizes used primer sequences and their efficiencies for Chts. The Cht12 forward primer recognizes exon (black) and intron (red) sequences. The table further shows the gene splicing variants and if they are detected by the primer pairs.

Supplementary Table 2

Gene	Primer	sequence	efficiency	splice var., #no.
idgf1	for	CAGCTCCGATTTTCTTCCAG	97%	detects all, #2
idgf1	rev	TCCATGCTCTGCCATAACTG		
idgf3	for	TGGAACACTGGTTGCTTCAG	97%	detects all, #8
idgf3	rev	CCGAATCCTTGCTCATCTTC		
idgf4	for	GAAATTGGACCTGGATCTGG	99%	red, detects all, #6
idgf4	rev	CAGCAGGGTCAGATACTTGTTG		
idgf5	for	ACCAGACATCCGAAATCAGTG	94%	detects all, #2
idgf5	rev	TCGGGTCATATTCCAAGCTC		
idgf6	for	AGCGCAAGTATCCCAATGTC	88%	detects all, #4
idgf6	rev	CGGTATTCACGAAACGAGTG		

The table 2 summarizes used primer sequences and their efficiencies for idgfs. The idgf4 forward primer sequences marked in red detects all predicted splicing variants. The black labeled part detects only 5 variants. The table further indicates the gene splicing variants and if they are recognized by the primer pairs.

Supplementary Table 3

Gene	web link for FlyBase gene card
Cht2	http://flybase.org/reports/FBgn0022702.html
Cht4	http://flybase.org/reports/FBgn0022700.html
Cht5	http://flybase.org/reports/FBgn0038180.html
Cht6	http://flybase.org/reports/FBgn0263132.html
Cht7	http://flybase.org/reports/FBgn0035398.html
Cht8	http://flybase.org/reports/FBgn0034580.html
Cht9	http://flybase.org/reports/FBgn0034582.html
Cht11	http://flybase.org/reports/FBgn0029913.html
Cht12	http://flybase.org/reports/FBgn0050293.html
idgf1	http://flybase.org/reports/FBgn0020416.html
idgf3	http://flybase.org/reports/FBgn0020414.html
idgf4	http://flybase.org/reports/FBgn0026415.html
idgf5	http://flybase.org/reports/FBgn0064237.html
idgf6	http://flybase.org/reports/FBgn0013763.html

Table 3 provides the direct link to FlyBase

Supplementary References

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