

## **Supplementary Information**

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

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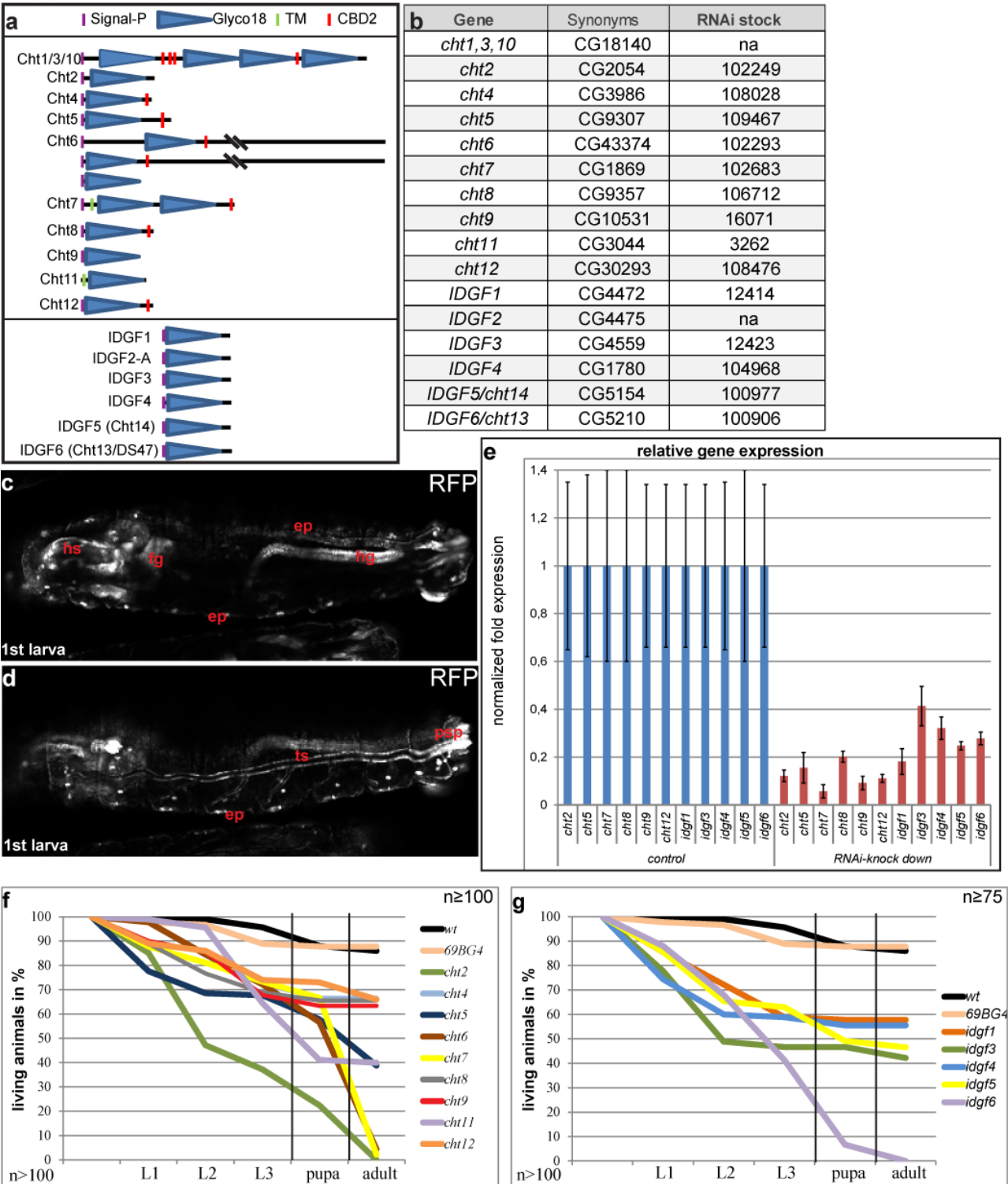
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# Supplementary Figures and Legends

Supplementary Fig. S1

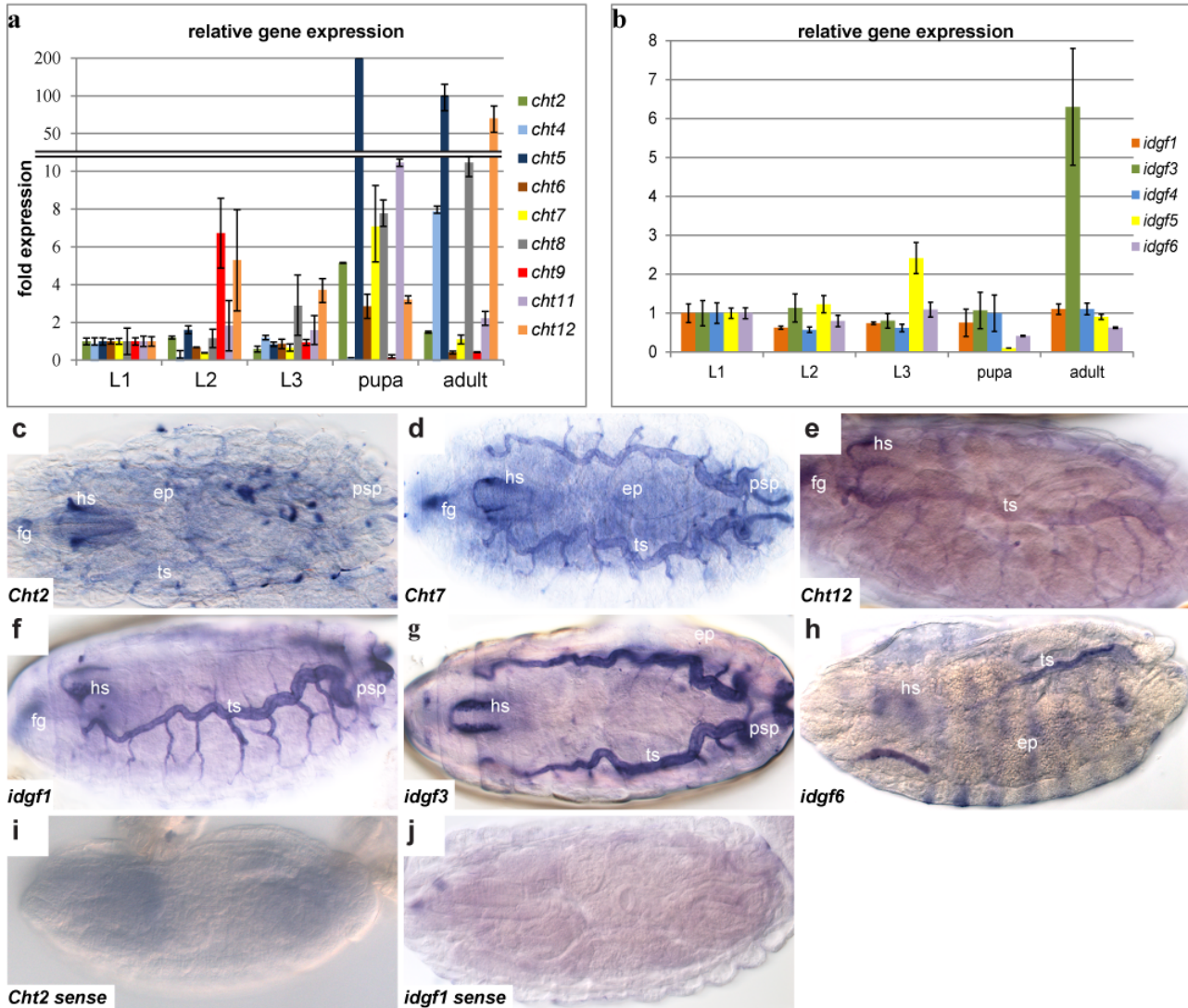


**The *Drosophila* family18 members, used RNAi stocks and knockdown expression effects.**

(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 development <sup>1</sup>. 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

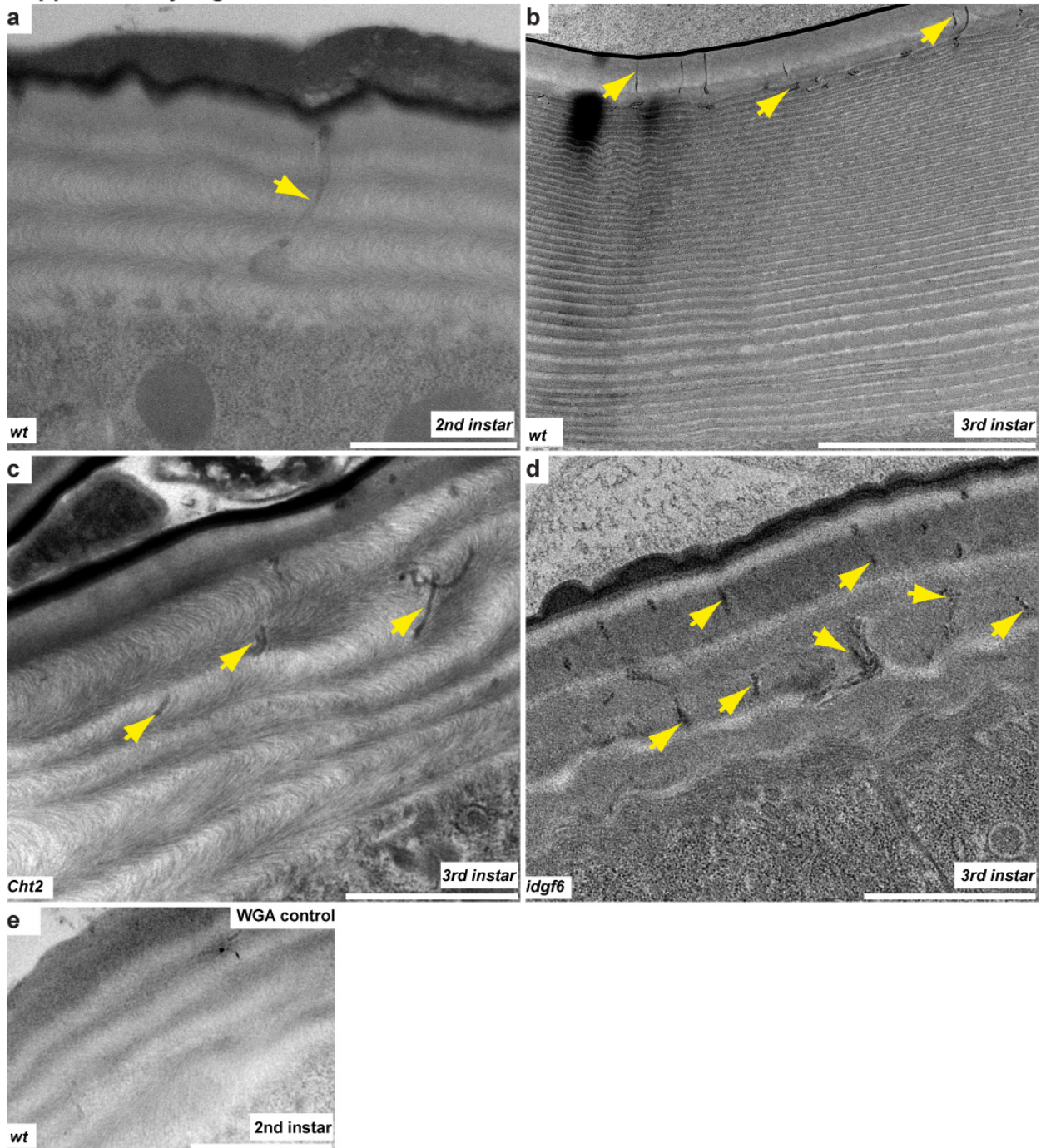


### Spatial-temporal gene expression profiles of *Cht* and *idgf* genes.

(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 infections<sup>2</sup>. 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 described<sup>3</sup> 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.



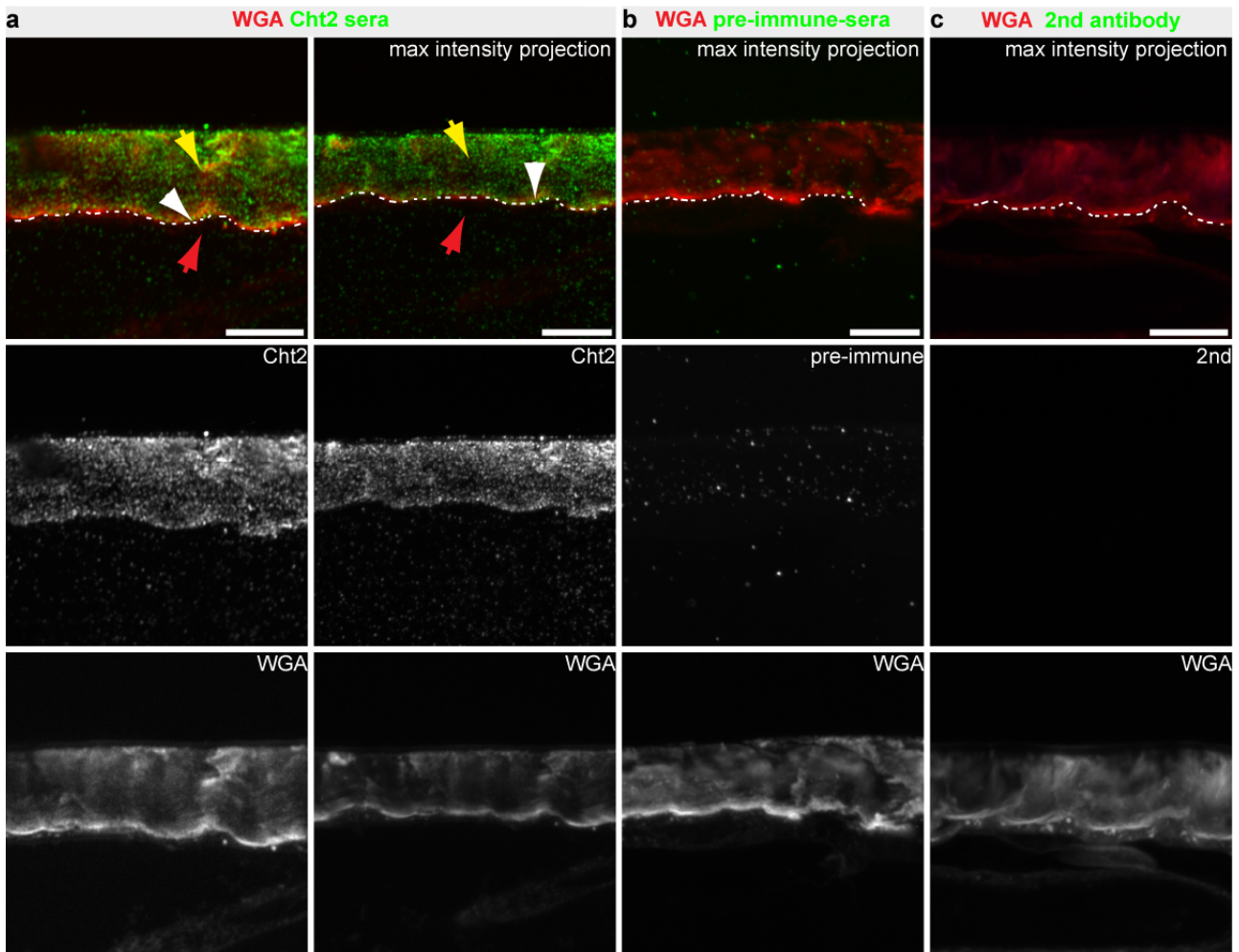
Supplementary Fig. S3



### 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  $\mu$ m in B and 1  $\mu$ m 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  $\mu$ m.



## Supplementary Fig. S5

a

<i>Aedes aegypti</i> (Aa)	<i>Drosophila melanogaster</i> (Dm)		
DmCht2 39 HDKVVCYVSTWAVYRPEQAYAIENFDNLC <th>THVYAFAGLDITQAAIKSLDPWQDLKE</th> 98	THVYAFAGLDITQAAIKSLDPWQDLKE		
H +VVVCY+ <b>S</b> +WAVYR G++++NFDNLC <th>THVY+<b>A</b>+AGLD+ +</th> IKSLD WQDLK+	THVY+ <b>A</b> +AGLD+ +		
AaCht2 8 HGRVVCYVSISSWAVYRSGAGSFLNFDNLC <th>THVYAVAGLDVERNTIKSLDRWQDLKD</th> 67	THVYAVAGLDVERNTIKSLDRWQDLKD		
DmCht2 99 EYKGKGYEKMGLKRSHPHLKVSALGGWNEGSANYSTLVANNLLRGRFVKVQSSFIRKY 158			
YG GGYEK+ L + +++GWNES YS L A+ R F + +FIR+Y			
AaCht2 68 NYGLGGYELVNL-----LMISMGWNEGSVYKSLAASQQRQVFAENALFIRRY 119			
DmCht2 159 NFDGLDLWEYPTQRKGKPADRENFVLLTKELREEFDEHGLLLTSAIGASKVIDEAYDV 218			
FDGLDLWEYPTQR G+P DRENFV L +EL ++F ++ LLLTS I A + VID AYD+			
AaCht2 120 GFDGLDLWEYPTQRGGEENDRENFVQLVRELSQKFKNNLLTSTIEAKQYVIDAAYDI 179			
DmCht2 219 RQISRYLDYLHIMCYDYHGSWDRVRGYNAPLTAAPADPLSVKFSIDYLLKLGAPPEKLV 278			
+++YLD LH+ CYDY G W++++G+NAPLT D+ +V+E+I+LL LGAPP KLV+			
AaCht2 180 ENLTKYLDLHVKCYDYRGVNIWQKIFGNAPLT--GDEVHNVETIEHLLALGAPPKNLVL 237			
DmCht2 279 GLPFYGRIF--KTLASGLNDVSEGCGFKGPTREDGLFYNEICQTLNQTSQWTREWD 336			
GLPF+GRIF ++ + D +E F GPYT DG+GYNEIC L+ ++S + WD			
AaCht2 238 GLPFHGRIFDIADFIYTRIGDPAE--TSFGSPYTNIDGYGYNEICTELNAKSSFFWQAWD 296			
DmCht2 337 PQTSQLAKSERNVFTQINVTYDSSRSIANKVLFAMSKRLAGVMVMSVDTDDFLGN <b>C</b> 396			
P+ S+V+A+ + T+ V+ YDS+RSIA KV +A+ K L G+M S+D+DDF GNC			
AaCht2 297 PEASEVIARMQDGSKTK---VIVYDTSRIAIAKVYAVQKNLRCGLMASSIDTDDFNG <b>CN</b> 353			
DmCht2 397 <b>L</b> DEITYED <b>FQ</b> KVTAAPKRSSQNY <b>PL</b> LRTINEAT <b>ML</b> AV <b>DEL</b> 436			
+E+T+ DF P + LLRTIN A + A DE+			
AaCht2 354 <b>A</b> EEET <b>FV</b> DFDK <b>NK</b> LRT <b>PP</b> PI <b>Q</b> G <b>K</b> FK <b>L</b> LRTIN <b>NA</b> VA <b>AE</b> DE <b>I</b> 393			
<i>Pediculus humanus corporis</i> (Ph) <i>Drosophila melanogaster</i>			
DmCht2 33 ARTGPHLHKVVVCYVSTWAVYRPEQAYAIENFDNLC <th>THVYAFAGLDITQAAIKSLDP</th> 92	THVYAFAGLDITQAAIKSLDP		
R P+HDK VVCY+ TW+VYRP +G+ IEN D NLCTH+VYAF GL+ T ++S+DP			
PhCht2 5 TRKPHDKHVVVCYVSTWAVYRPEQAYAIENFDNLC <th>THVYAFVGLNATTNLT</th> LRSIDP 64	THVYAFVGLNATTNLT		
DmCht2 93 WQDLKEEYGGKGYEKMGLKRSHPHLKVSALGGWNEGSANYSTLVANNLLRGRFVKVS 152			
+ DL+E YRGK ++KMT LK +P+LKV+LA+GWNESGANYS + R +F+ V			
PhCht2 65 YYDLNEYKSGKSFYKQTQKLVKLVNLTAVGGWNEGSANYSNALVFNRRRFINV 124			
DmCht2 153 SFIRKYNFDGLDLWEYPTQRKGKPADRENFVLLTKELREEFDEHGLLLTSAIGASKV 212			
++ KYNFDG DLWE+P R G+P D+NF LL KEL++E + L+LT+A+GA+ I			
PhCht2 125 EYTYKYNFDGLDLWEYPTQRAAGRPEDKQNFALLVKEQLGKKNLLTALGAANT 184			
DmCht2 213 DEAYDVQRISRYLDYLHIMCYDYHGSWDRVRGYNAPLTAAPADPLSVKFSIDYLLKLGAP 272			
+ AYDV +IS+LD LH MCYDYHG WD+ VG NAPLT + D L ++ SI +LL+LGAP			
PhCht2 185 NTAYDVFEISKHLDLHFMCYDYHGSWDRVKGANAPLT--SKDSLDESITHLGLAP 242			
DmCht2 273 PEKLVMLGPFYGRIF--KTLASGLNDVSEGCGFKGPTREDGLFYNEICQTLNQTS 330			
P KLV+G+P YG TF K + + + G G +G +T++ GF GYNEIC L			
PhCht2 243 PHKLVGPIYAGHTFLLTKVNVNPKMTPIGTGPGFVFTKQCGFYGYNEICTELKDD 302			
DmCht2 331 WIREWDPQTSQLAKSERNVFTQINVTYDSSRSIANKVLFAMSKRLAGVMVMSVDTDD 390			
W++ WD ++S A + +V+ +D +SI KV + ++ G M+W+S+D+DD			
PhCht2 303 WSKHWDDESSTPIAYVNGN-----HVIAFDDEKSIGEVNLLGKYKGGFMIWSIDTDD 355			
DmCht2 391 FLGNCK <b>L</b> DEITYED <b>FQ</b> KVTAAPKRSSQNY-- <b>Y</b> PLLR <b>T</b> INEAT <b>ML</b> AV <b>DEL</b> AV <b>PE</b> Q <b>PD</b> SE 447			
F G+C K K S+Q+ +PLLR A A + + + DD			
PhCht2 356 FHGDC-----DKERLKVESQADKY <b>TF</b> LLR-----AV <b>TA</b> I <b>Q</b> Y <b>K</b> L-----Q <b>DD</b> DN 397			
DmCht2 448 <b>NE</b> IP <b>W</b> GS <b>I</b> ADR <b>K</b> NAG <b>S</b> MY-- <b>SL</b> GL <b>V</b> TA <b>V</b> FM <b>L</b> 479			
N+IP D + + V S +TA F+++			
PhCht2 398 <b>N</b> D <b>I</b> PE <b>V</b> GT <b>N</b> D <b>I</b> DD <b>K</b> EN <b>N</b> V <b>S</b> P <b>S</b> Q <b>S</b> I <b>T</b> AN <b>F</b> V <b>I</b> 431			
DmCht2 (AAF47562.1; see also flybase: <a href="http://flybase.org/reports/FGbn0022702.html">http://flybase.org/reports/FGbn0022702.html</a> )			
1 mtlrsrlsge apqlwlllll astasslwas vaartgplnd kvvvcyvstw avyrpeggav			
61 aienfdpnlc thvryafaglt ditqaiksl dpwgdikeey gkggyekmtg lkrshphlk			
121 slaiggwneq sanystlvann llrgrfvkv vssfirkynd dgllddweyp tqrgkqpadr			
181 enfvlltkel reefdehgl ltsaigaskk videaydvrg isryldylhi mcydyhgsawd			
241 rrvygnaplt apadpplsvk fsisdyllkkg appekvlmgl pfygrtftkl asgflndvae			
301 gvgfkgpytr edglfynei cqtlnsqtsq wtrewdpqts qvylaksernv ftqeinvvty			
361 dssrsiankv lfamskrlag vmvmsvdtdd flgnckided tyedfgkvtv apkrssqny			
421 llrtineatm lavdelavep pqpddsenel phgsiadrn agasmvslgl gvtavfmilh			
481 rlaq			

b

<i>Aedes aegypti</i> (Aa)	<i>Drosophila melanogaster</i> (Dm)	
DmIdgf6 7 <b>A</b> IV <b>S</b> LC <b>L</b> AS <b>I</b> Q <b>A</b> SK <b>V</b> GA <b>P</b> Q <b>L</b> PK <b>K</b> H <b>L</b> V <b>C</b> YD <b>S</b> AS <b>F</b> Y <b>K</b> EG <b>L</b> GL <b>V</b> I <b>D</b> E <b>L</b> EP <b>A</b> LC <b>D</b> Y <b>L</b> V <b>Y</b> G 66		
A+ ++CL + + G Q K ++CYD+A+F+ EGLK+ + ++E AL FC +LVYG		
AaIdgf6 7 <b>A</b> V <b>T</b> A <b>I</b> CL <b>L</b> GV <b>L</b> AP <b>T</b> GY <b>S</b> Q <b>S</b> T <b>A</b> K-- <b>V</b> LCYD <b>G</b> AN <b>F</b> IE <b>L</b> EG <b>L</b> GV <b>S</b> L <b>D</b> IE <b>A</b> AL <b>P</b> FC <b>T</b> HL <b>V</b> Y <b>G</b> 65		
DmIdgf6 67 YAGIERDSHKAVSLNQQLDLGLGKLYRTVTRLKRKYPNVKILLSVGGDKDIELDKDAKE 126		
YA ++ DS+KAVS N LDLD GKG YR VT LKRK+P +K+LL +GG +		
AaIdgf6 66 YAAVDPDSNKAVERSNANLDLTGKGNRYQVTLKRKFPGLKVLVLLIGGYR-----FSA 118		
DmIdgf6 127 LNKYLELLESPTGRIFRNVTVYSLVKTYGFDGLDVAWQFPKNKPKKVHSGIGSLWKGFK 186		
KYLELLES R F+N+VYS+VK Y FDG+D+AWQFP+NKPKV S G LW GFK		
AaIdgf6 119 ASPKYLELLESGAARITFINSVYSIVKAYDFDGLDVAWQFPQNKPKKVHSSPGKLWHGFK 178		
DmIdgf6 187 KVFSGDSIVDEKSEEHEQFTALLRDVKNAPRPNLLSTTLVLPNVNSSLFYDIPAVVNY 246		
KVF+GDS++DEK+EEH+E+FTALLR++KNAFR D L TVL +VN+S+F DIPA+VNY		
AaIdgf6 179 KVFITGDSVLDEKADEHREFTALLREMKNAFRSDGYQLGITVLSHVNSMFMIDIAIVNY 238		
DmIdgf6 247 LDFVNLGTFDFFTPQRNPEVADYAAPIYELSERNEPFEVNAQVYKWLNNCPASKINVG 306		
LDFVN+ +D TP+RN +AD+ APIYELSER P NV VK WL ++ PASK+ V +		
AaIdgf6 239 LDFVNLAAVDMQTPERNKEADFYAPIYELSERIPGNVGLVWLNSHAPASKLIVSI 298		
DmIdgf6 307 ATYGRPWKLDDSDGTGVPFVKVDKDEA--FVGNTQVPGIYSWPEVALLPNQNNAYLKG 365		
A+ GR WK+T+DSG TGVPP+ V D A P G Q G YSW E CA LPN +N LKG		
AaIdgf6 299 PTHGRGWMTEDSGITGVPP--VADGASPAQTQQEGFYSWAETCAKLPNSMTALKG 356		
DmIdgf6 366 ANAPLIKVDPAKRFSGYAYRAADKGDNGIWSFEDPDTAADKAGYVRTENLGVALFD 425		
A+ PL KV DP KRFGSYA+R D G+G+WW+EDPD+A +KA YV+ + LGG+A+ D		
AaIdgf6 357 ADGPLRKVDGPTKRFSGYAFRLPDSSGEHGMVITYEDPSAGNKAAYKAKGLGGIAND 416		
DmIdgf6 426 LSYDDFRGLCTNEKYPIILRAIKYRL 450		
L++DDFRG C EKYPIIRA KYRL		
<i>Anopheles gambiae</i> (Ag) <i>Drosophila melanogaster</i> (Dm)		
DmIdgf6 1 <b>M</b> I <b>K</b> A <b>L</b> A <b>I</b> V <b>S</b> L <b>C</b> L <b>A</b> S <b>I</b> Q <b>A</b> SK <b>V</b> GA <b>P</b> Q <b>L</b> PK <b>K</b> H <b>L</b> V <b>C</b> YD <b>S</b> AS <b>F</b> Y <b>K</b> EG <b>L</b> GL <b>V</b> I <b>D</b> E <b>L</b> EP <b>A</b> LC <b>D</b> 60		
M K A L L + S+ Q P K ++CYD+A+F+ EGLK+ + +++ AL FC		
AgBRP2 1 <b>M</b> W <b>C</b> K <b>Q</b> F <b>A</b> GA <b>L</b> L <b>L</b> -- <b>V</b> AT <b>S</b> Q <b>V</b> Y <b>S</b> Q <b>Q</b> P <b>S</b> K-- <b>V</b> LCYD <b>A</b> AN <b>F</b> IE <b>L</b> EG <b>L</b> GV <b>S</b> L <b>D</b> IE <b>A</b> AL <b>P</b> FC 57		
DmIdgf6 61 DYLVYGYAGIERDSHKAVSLNQQLDLGLGKLYRTVTRLKRKYPNVKILLSVGGDKDIEL 120		
+LVYGYAGI+ ++KAVS LDLD GKG YRTVT+LK KYP+K+LL +GG K		
AgBRP2 58 THLVYGYAGIDVEINKAVSRQNLDLDTGKGNRYVTLQKSKYPSLKVLLGLGKYK---- 113		
DmIdgf6 121 DKDAKELPNKYLELLESPTGRIFRNVTVYSLVKTYGFDGLDVAWQFPKNKPKKVHSGIGS 180		
E KYL LLES R F+N+VYS+LKGFDG+D+ WQFP NKPKV S +G		
AgBRP2 114 ---FSEPSIKYLLLESGAARITFINSVYSLKTYGFDGLDVAWQFPKNKPKKVHSGIGS 170		
DmIdgf6 181 LNKGFKKVSGDSIVDEKSEEHEQFTALLRDVKNAPRPNLLSTTLVLPNVNSSLFYDI 240		
+W GFKKVFSGDS++DEK+EEH+E+FTALLR++KNAFR D L TVL +VN+S+F DI		
AgBRP2 171 VWHGFKKVFSGDSVLDEKAEHREFTALLRELKNAFRSDGYQLGITVLSHVNSVFMID 230		
DmIdgf6 241 PAVVNYLDFVNLGTFDFFTPQRNPEVADYAAPIYELSERNEPFEVNAQVYKWLNNCPAS 300		
PA+++NYLDFVN+ +D TP RN +AD+AAP+YELS+R P NV QV+ WL NN PAS		
AgBRP2 231 PAIINYLDFVNIAYDQQTPTRNKKEADHAAPLYELSDRVPGNNVGLVQVRLWLTNNAPAS 290		
DmIdgf6 301 KINVGATYGRPWKLDDSDGTGVPFVKVDKDEA--FVGNTQVPGIYSWPEVALLPNQNN 360		
K+ V +T+GR WK+ DSG TGVPP+ P G TQ G YSW EVCA+LPN +N		
AgBRP2 291 KLIVSIPTFGRWKMMNGDSGITGVPPFLPADGFSNP--GPQTQTEGFSWAECAMLPNSN 349		
DmIdgf6 361 AYLKGANAPLIKVDPAKRFSGYAYRAADKGDNGIWSFEDPDTAADKAGYVRTENLG 420		
LKGA+APL KV DP KRFGSYA+R D G+G+WW+EDPD+A +KAGYV+ +NLGG		
AgBRP2 350 TALKGADAPLRKVGDPTRKFGSYAFRLPDSSGEHGMVITYEDPSAGNKAAYKAKGLGG 409		
DmIdgf6 421 VALFDLSYDDFRGLCTNEKYPIILRAIKYRL 450		
+A+ DLSYDDFRG C EK+PIIRA KYRL		
AgBRP2 410 IAINLSYDDFRGSCAGEKFPILRAIKYRL 439		
DmIdgf6, (NP_417081.1; see also flybase: <a href="http://flybase.org/reports/FGbn0013763.html">http://flybase.org/reports/FGbn0013763.html</a> )		
1 <b>m</b> i <b>k</b> a <b>i</b> a <b>i</b> v <b>s</b> <b>l</b> c <b>l</b> a <b>s</b> i <b>q</b> a <b>s</b> k <b>v</b> g <b>a</b> q <b>l</b> p <b>k</b> h <b>l</b> v <b>c</b> y <b>d</b> s <b>a</b> s <b>f</b> <b>v</b> k <b>e</b> g <b>l</b> g <b>k</b> l <b>v</b> i <b>d</b> e <b>l</b> e <b>p</b> a <b>l</b> q <b>c</b>		
61 dylvygyagi erdshkaval ngqldldlgl glyrtvtrlk rkypnkvill svvgdkdiel		
121 dkdakelpnk ylelleaptg rtrfvntvys lvkytygfdgl dvawqfknk pkkvhsgigs		
181 lwgfkfvfs qdsivdekse ehkeqftall rdvknafpnd nllsttvlvp nvnsalfydi		
241 pavvnyldfv nlgtfdfttp grnpevadya apiyelsern pefnvaaqv ywlrnnpcas		
301 kinvgvatygv rpwkltddsg dtgvpvkvdv kdaeppvgnt qvpglyswpe vcallpnqnn		
361 aylkganapl ikvqdpakrf gsyayraadk kgdngiwsf edpdtaadka gyvrtlenlg		
421 valfdlsydd frglctneky pilraikyr l tn		

## 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*, mosquitos 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	GCTAAGCTGTCGCACAAATG	100%	
rp49	rev	GTTCGATCCGTAACCGATGT		
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	CGGTATTACGAAACGAGTG		

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	<a href="http://flybase.org/reports/FBgn0022702.html">http://flybase.org/reports/FBgn0022702.html</a>
Cht4	<a href="http://flybase.org/reports/FBgn0022700.html">http://flybase.org/reports/FBgn0022700.html</a>
Cht5	<a href="http://flybase.org/reports/FBgn0038180.html">http://flybase.org/reports/FBgn0038180.html</a>
Cht6	<a href="http://flybase.org/reports/FBgn0263132.html">http://flybase.org/reports/FBgn0263132.html</a>
Cht7	<a href="http://flybase.org/reports/FBgn0035398.html">http://flybase.org/reports/FBgn0035398.html</a>
Cht8	<a href="http://flybase.org/reports/FBgn0034580.html">http://flybase.org/reports/FBgn0034580.html</a>
Cht9	<a href="http://flybase.org/reports/FBgn0034582.html">http://flybase.org/reports/FBgn0034582.html</a>
Cht11	<a href="http://flybase.org/reports/FBgn0029913.html">http://flybase.org/reports/FBgn0029913.html</a>
Cht12	<a href="http://flybase.org/reports/FBgn0050293.html">http://flybase.org/reports/FBgn0050293.html</a>
idgf1	<a href="http://flybase.org/reports/FBgn0020416.html">http://flybase.org/reports/FBgn0020416.html</a>
idgf3	<a href="http://flybase.org/reports/FBgn0020414.html">http://flybase.org/reports/FBgn0020414.html</a>
idgf4	<a href="http://flybase.org/reports/FBgn0026415.html">http://flybase.org/reports/FBgn0026415.html</a>
idgf5	<a href="http://flybase.org/reports/FBgn0064237.html">http://flybase.org/reports/FBgn0064237.html</a>
idgf6	<a href="http://flybase.org/reports/FBgn0013763.html">http://flybase.org/reports/FBgn0013763.html</a>

Table 3 provides the direct link to FlyBase

## Supplementary References

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