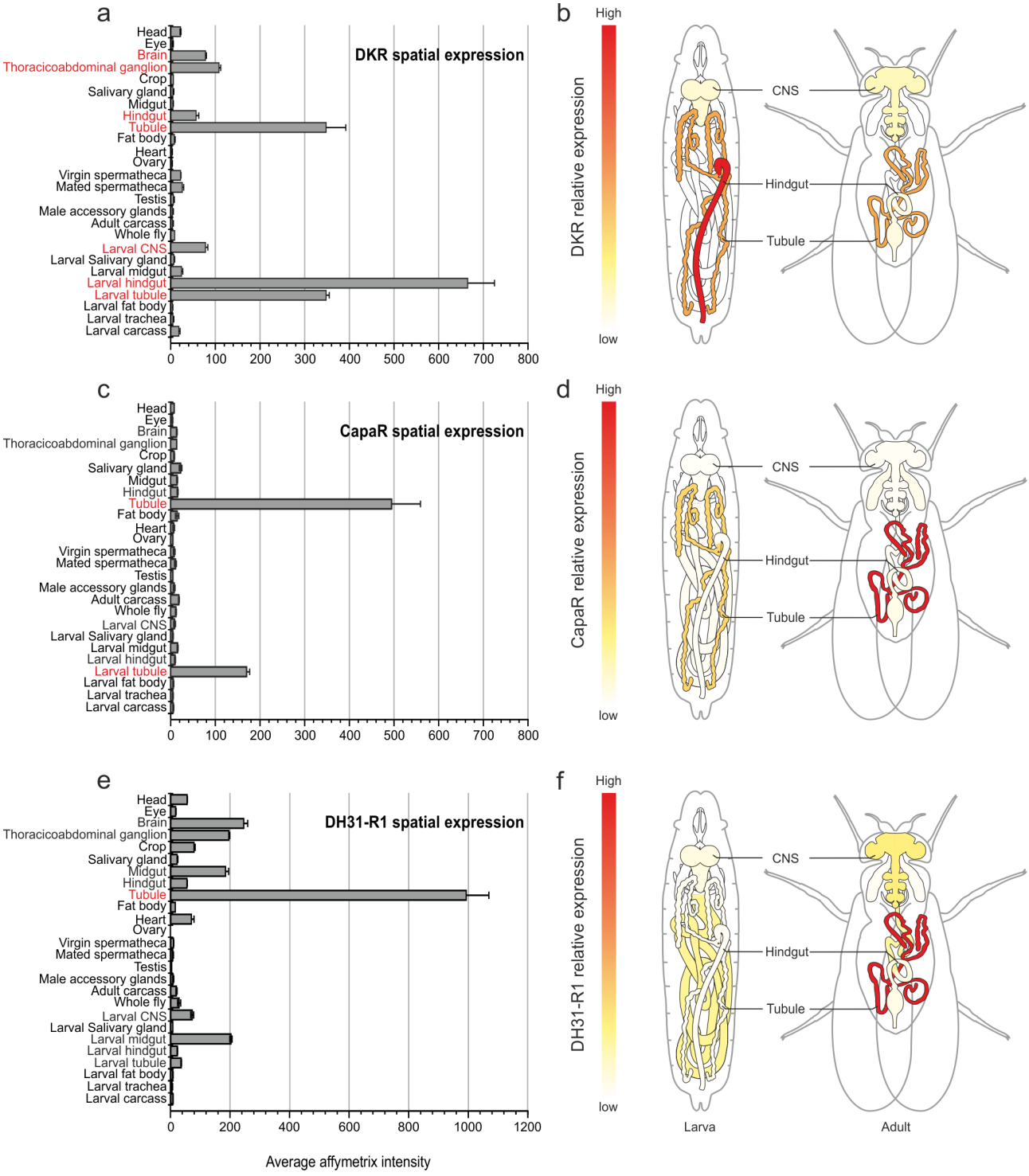
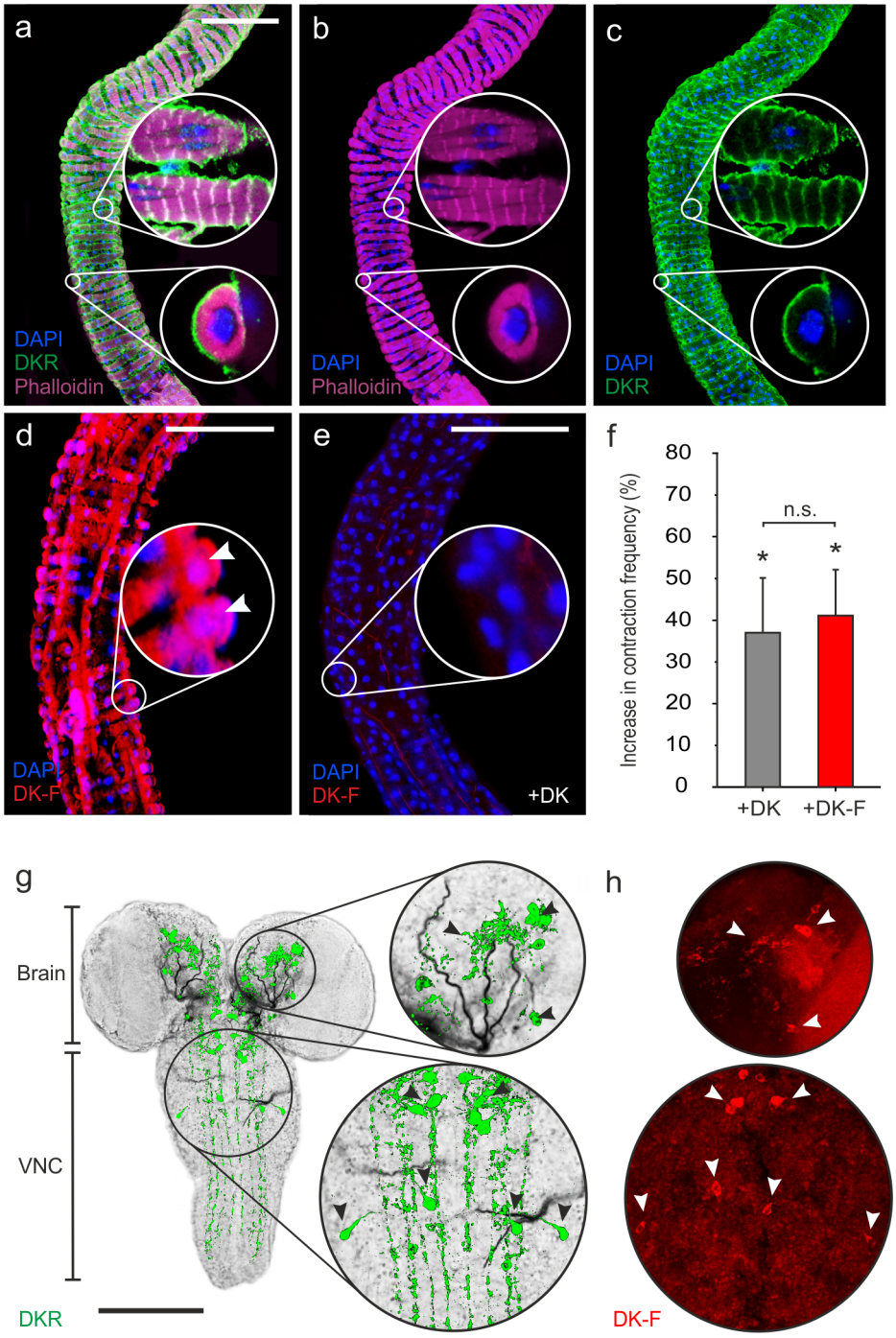
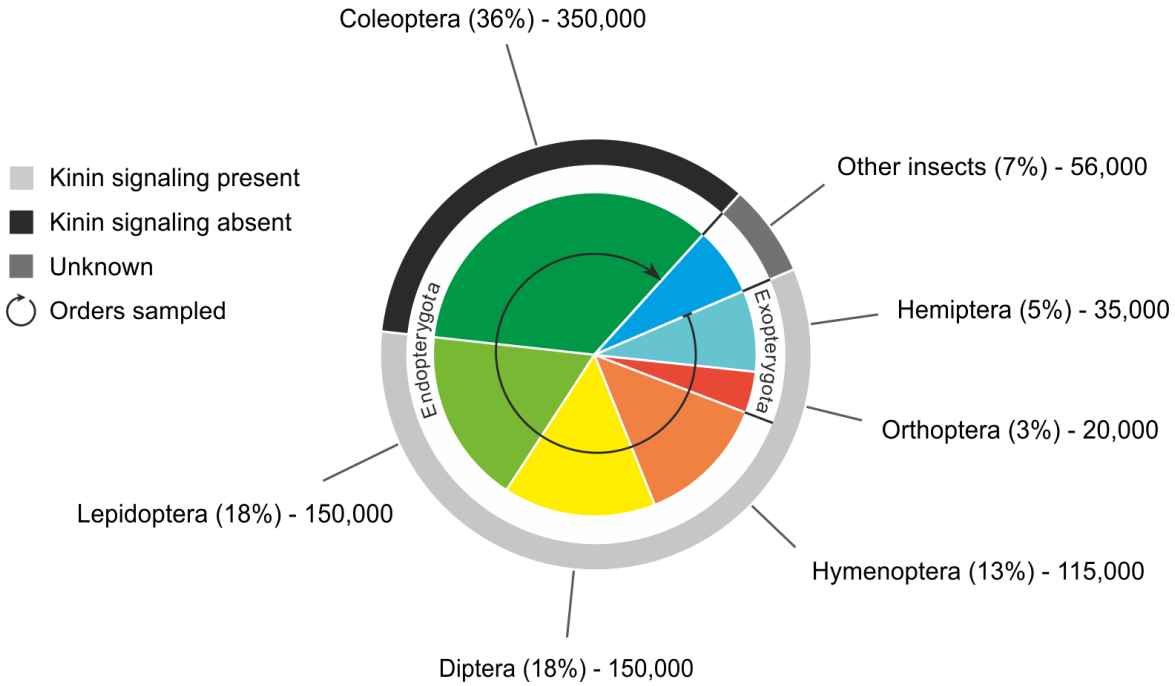


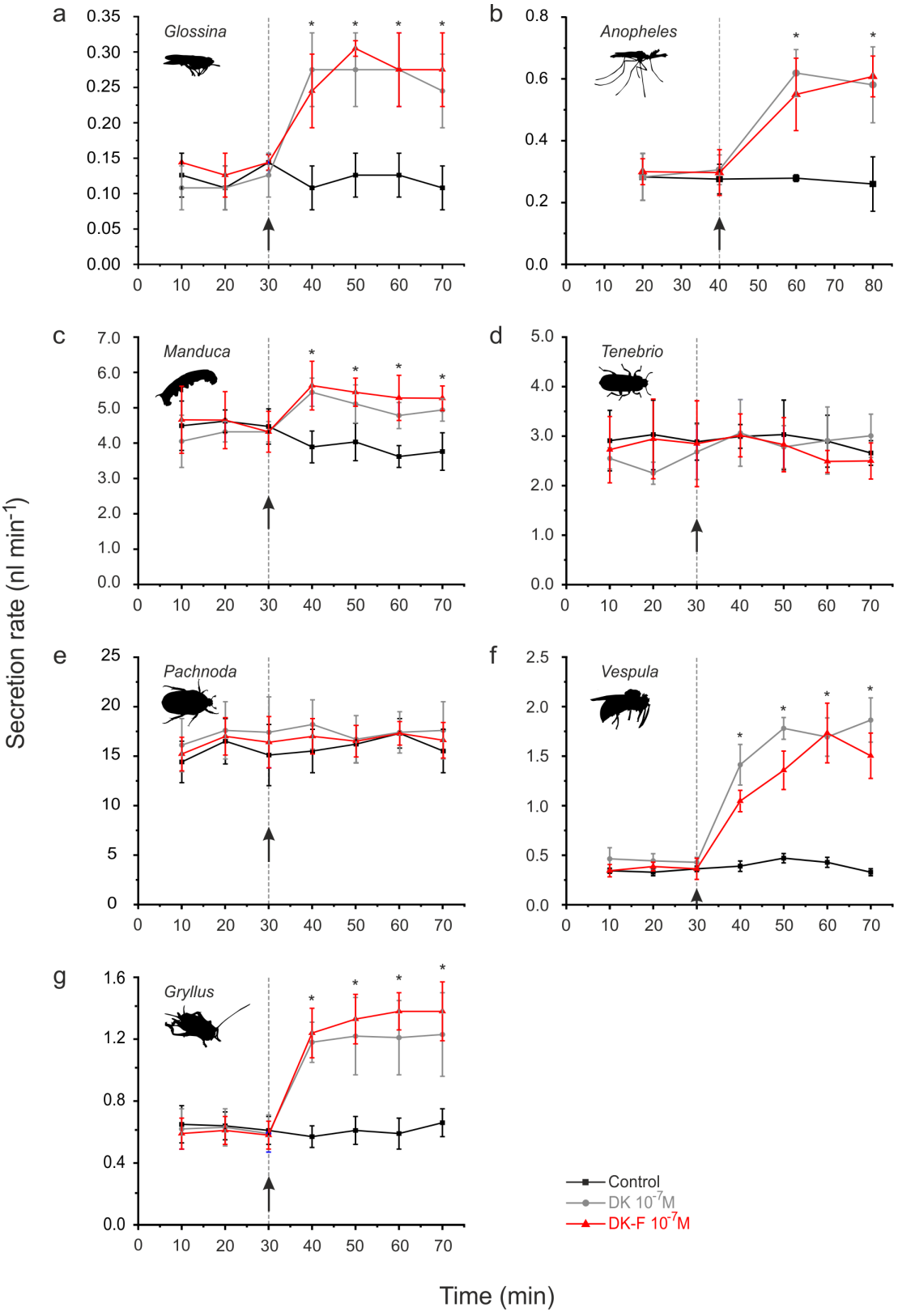
Supplementary Figure 1 | Tissue and developmental specific expression profile of the *Drosophila* kinin (DKR), capa (CapaR) and DH_{31} (DH31-R1) receptors. (**a**, **c**, **e**) Mean normalized Affymetrix signal ± s.e.m. (n=4 tissue samples) showing the DKR, CapaR and DH31-R1 spatial expression pattern across major tissues from both larval and adult *Drosophila* (flyatlas.org[1, 2]). Tissue names in red indicate tissues in which the receptors are most highly enriched compared to whole fly. (**b**, **d**, **f**) Overview of *Drosophila* anatomy with superimposed heat maps of the spatial expression pattern of the receptors, highlighting the tissues in which the highest levels of the receptors are expressed. Based on these heat maps, the CNS, hindgut and MTs of both larva and adult *Drosophila* were identified as candidate tissues for experimental validation of our *ex vivo* receptor-binding assay (DK-F and DKR interaction), while adult tubules were subsequently used for demonstrating specific binding of CapaR and DH31-R1 with their respective fluorescent ligands.



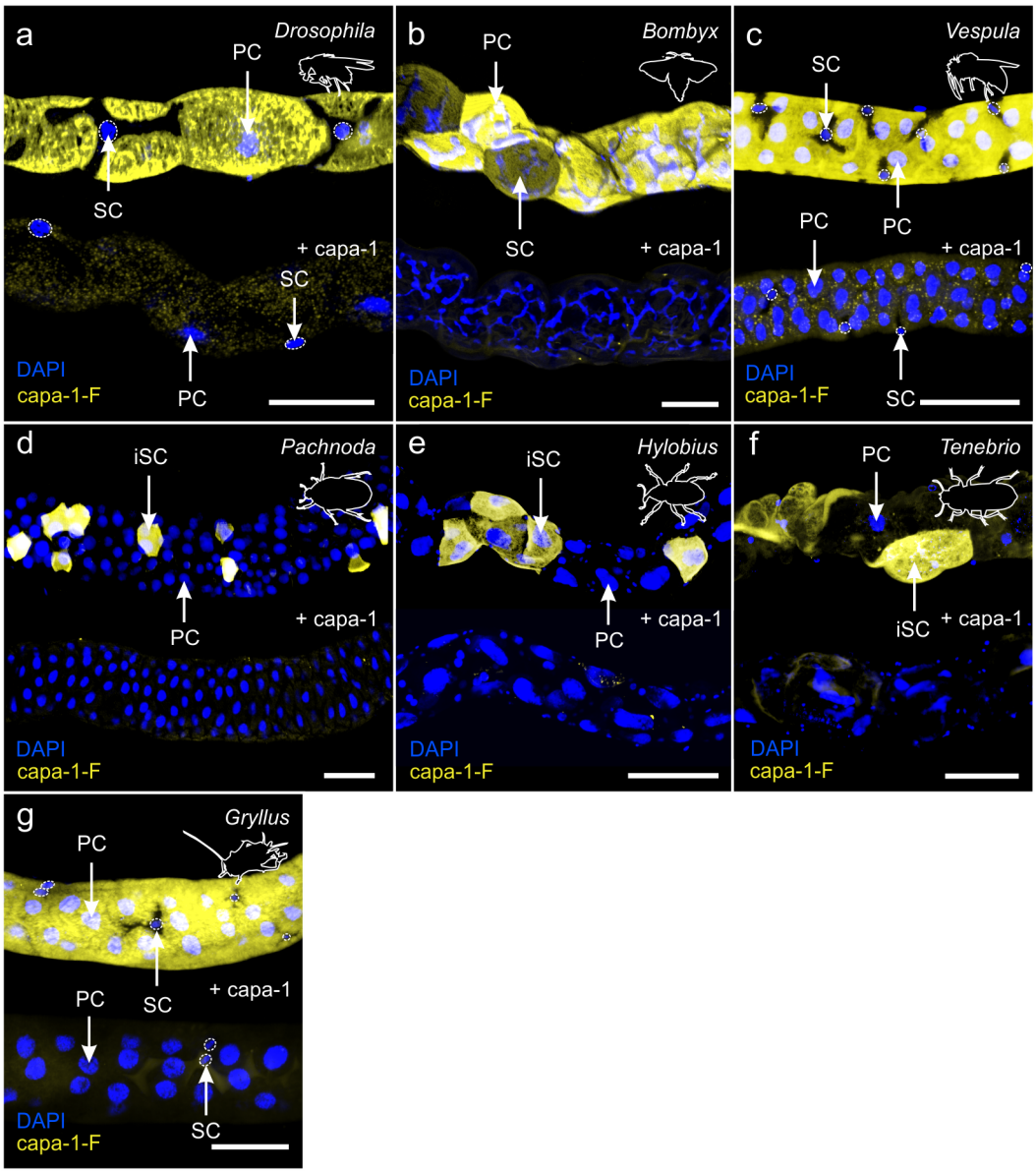
Supplementary Figure 2 | DK-F reports DKR localization in hindgut and CNS of *Drosophila*. (**a-c**) Immunolocalization of DKR suggested that the receptor is highly expressed on circular muscle cells, but not epithelial cells, of the adult *Drosophila* hindgut. Maximum projection of confocal z-series with inserts showing single focal plane images of selected regions. (**d**) Application of DK-F (10^{-7} M) confirmed that the receptor localized to the surface off circular muscle cells of the adult hindgut. (**e**) Competitive inhibition with the unlabeled peptide (DK, 10^{-5} M) almost fully abolished the fluorescent signal demonstrating specificity of the ligand-receptor interaction. (**d-e**) Maximum projections of confocal z-series with inserts showing magnifications of selected regions. (**f**) Myotropic action of DK-F and DK (10^{-7} M) on the hindgut of adult *Drosophila*. Both peptides significantly increased (*; $P<0.05$, paired-samples *t*-test) the frequency of hindgut contractions with respect to basal conditions. The increase in contraction frequency was not significantly different (n.s.; $P>0.05$, unpaired two-sample *t*-test) between the two treatments. Values are expressed as mean ± s.e.m. ($n=6$). (**g**) Immunolocalization of DKR in CNS of 3rd instar larvae. (**h**) Corresponding regions following DK-F (10^{-7} M) application showed partial staining of major regions. (**g-h**) Maximum projections of confocal z-series with inserts showing magnifications of selected regions. Scale bars: 100µm.



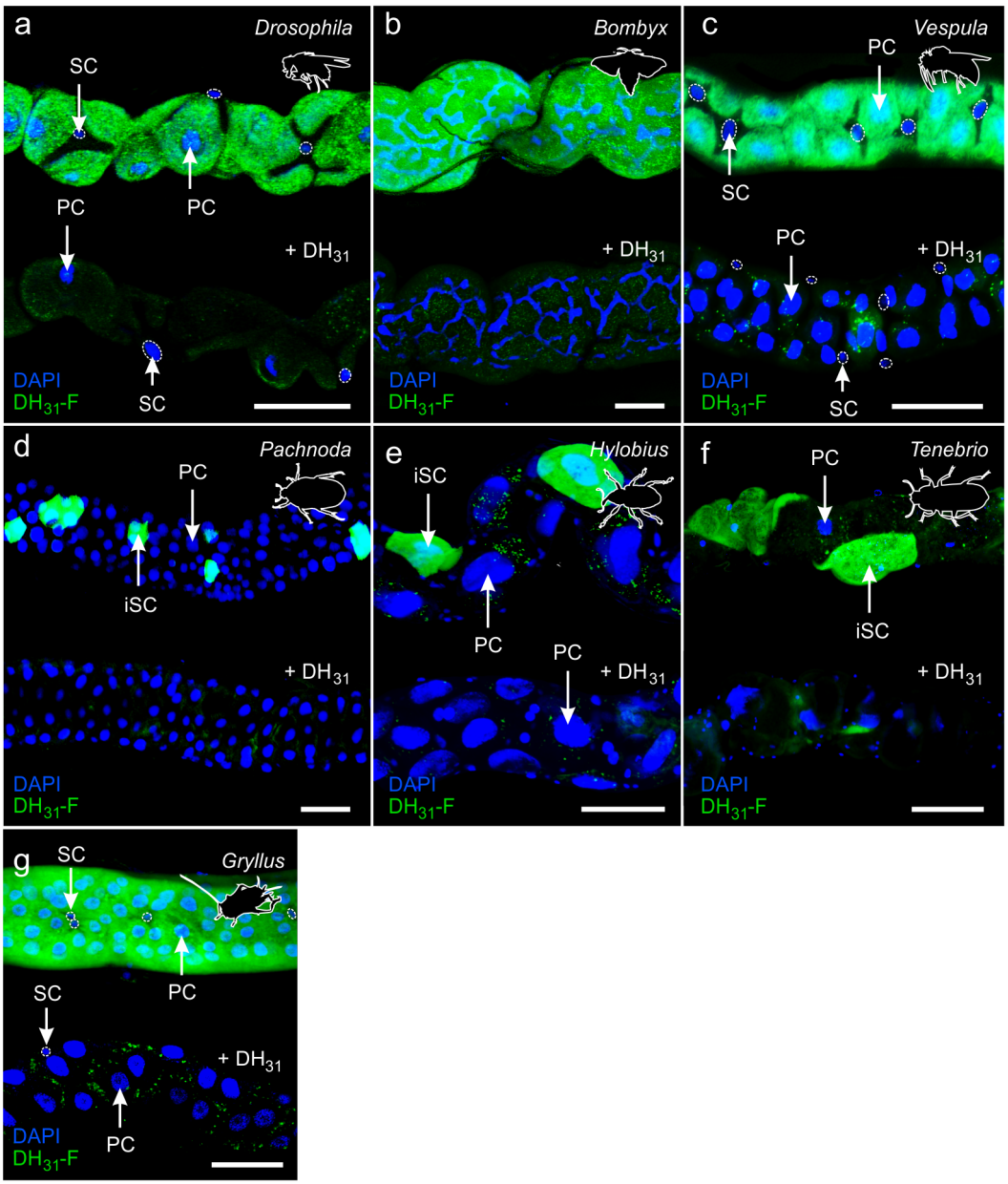
Supplementary Figure 3 | Insect biodiversity and the absence/presence of kinin signaling systems. Pie chart illustrating the relative biodiversity of each insect Order represented by our study (out of the total number of insect species described: modified from[3]), if they are grouped with the ancestral exopterygotes or derived endopterygotes, and whether they possess kinin-signaling systems. Our strategic, compact sampling thus covers approximately 93% of insect biodiversity.



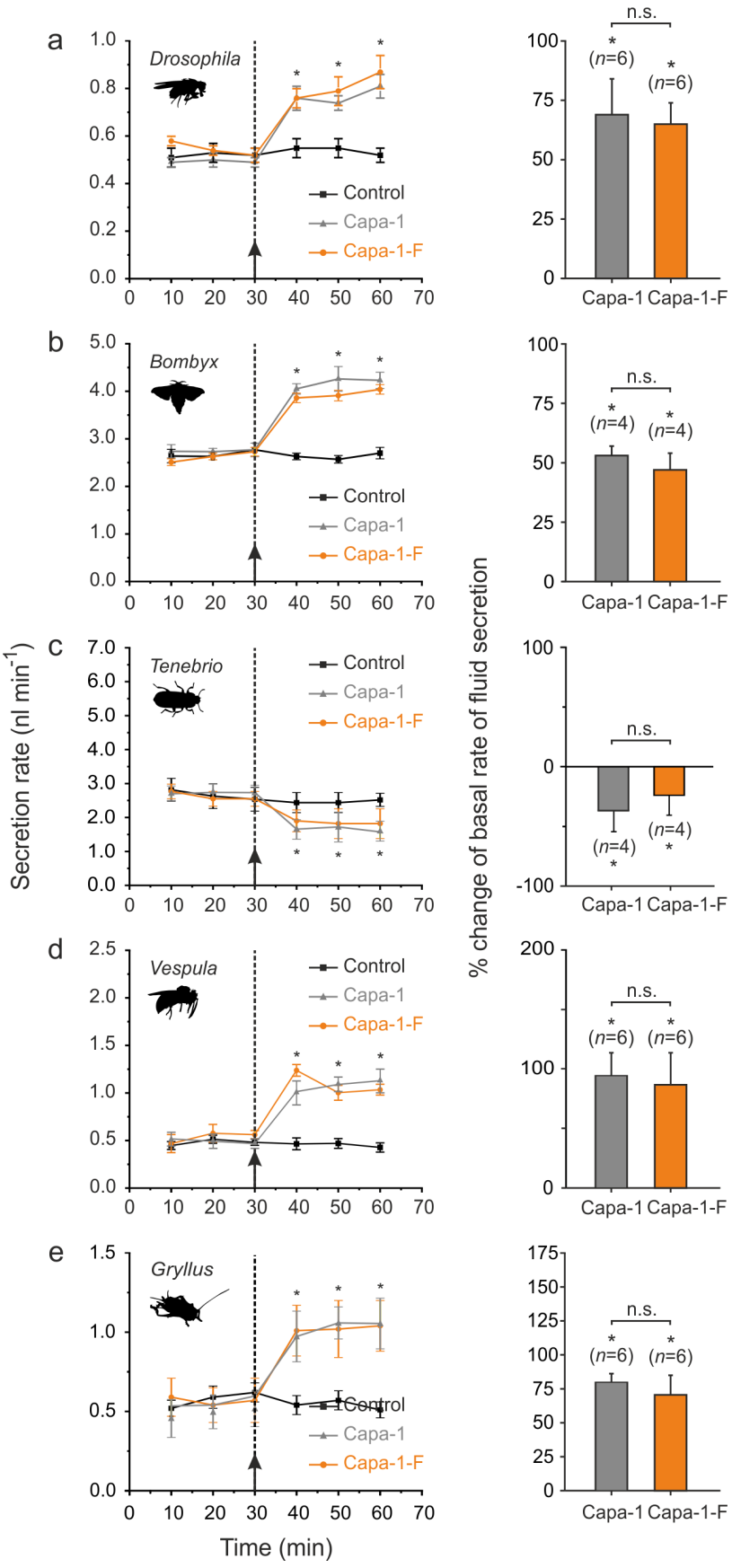
Supplementary Figure 4 | DK-F is biologically active. Fluid secretion assays with DK-F and DK on MTs from (**a**) *Glossina morsitans* (*n*=3), (**b**) *Anopheles gambiae* (*n*=4), (**c**) *Manduca sexta* (*n*=4), (**d**) *Tenebrio molitor* (*n*=4), (**e**) *Pachnoda marginata* (*n*=4) (**f**) *Vespula vulgaris* (*n*=6) and (**g**) *Gryllus assimilis* (*n*=6). Black arrow indicates time of peptide application. Values are expressed as mean ± s.e.m. Significant difference from basal rates of fluid secretion (i.e. immediately prior to peptide application) was tested using a paired samples *t*-test with a significance level of $P < 0.05$ (*).



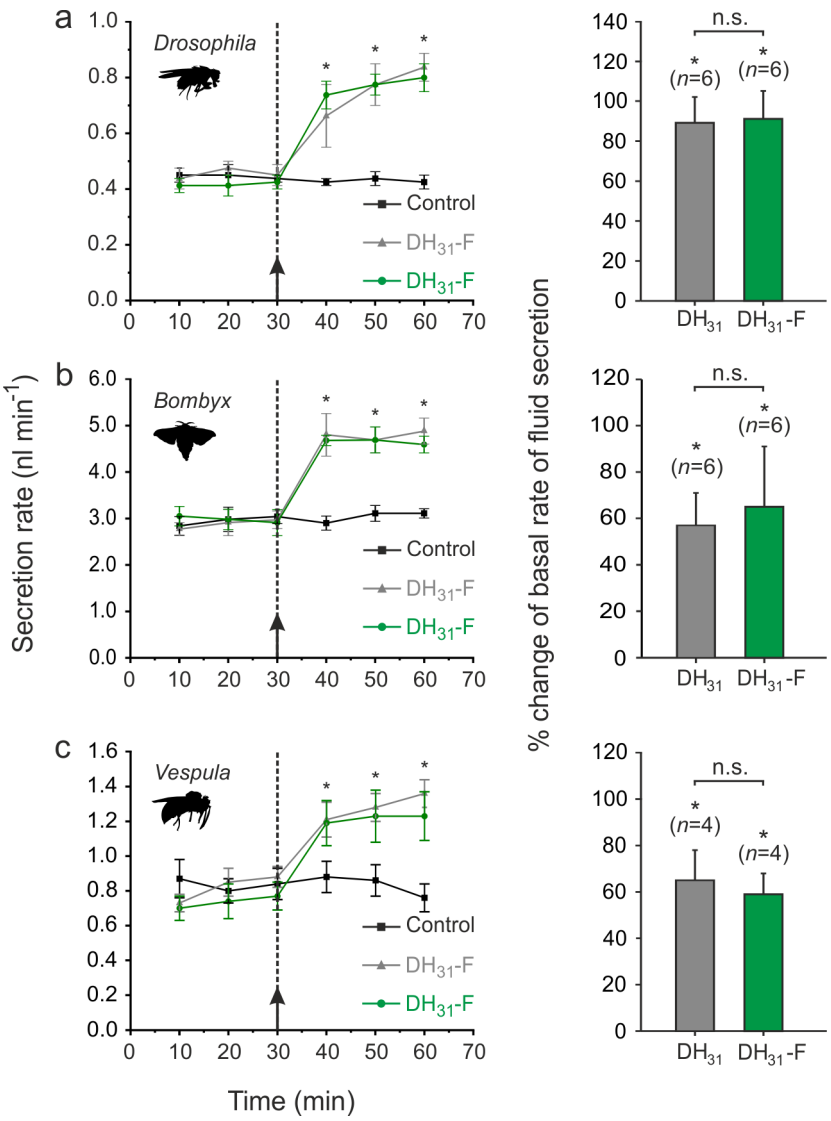
Supplementary Figure 5 | capa-1-F maps capa receptor localization. Application of capa-1-F (10^{-7} M) maps capa receptors to a PC type in endopterygote insects, such as (**a**) *Drosophila melanogaster* (Diptera), (**b**) *Bombyx mori* (Lepidoptera) and (**c**) *Vespula vulgaris* (Hymenoptera). However, capa-1-reactivity was localized to a distinct iSC in tubules of Coleoptera, such as (**d**) *Pachnoda marginata*, (**e**) *Hylobios abietis* or (**f**) *Tenebrio molitor*. In an exopterygote (**g**) *Gryllus assimilis*, capa receptors mapped to PCs; however, the small SCs showed no reactivity to any of the neuropeptides tested. In all cases, excess unlabeled peptide (+ capa-1) reduced the fluorescent signal, thus indicating that the binding was specific. PC, principal cell; SC, secondary cell; iSC, 'inverse' secondary cell type. Scale bars, 25 µm in (**a**) and (**c**), but 50 µm in (**b**), (**d**), (**e**), (**f**) and (**g**).



Supplementary Figure 6 | DH_{31}-F maps receptor localization. Application of DH_{31}-F (10^{-7} M) maps calcitonin-like (DH_{31}) receptors to a PC type in endopterygote insects, such as (**a**) *Drosophila melanogaster* (Diptera), (**b**) *Bombyx mori* (Lepidoptera) and (**c**) *Vespula vulgaris* (Hymenoptera). By contrast, DH_{31}-reactivity colocalized with capa receptors to a distinct cell type in tubules of Coleoptera, such as (**d**) *Pachnoda marginata*, (**e**) *Hylobios abietis* or (**f**) *Tenebrio molitor*. In an exopterygote (**g**) *Gryllus assimilis*, DH_{31} receptors mapped to PCs; however, the small SCs showed no reactivity to any of the neuropeptides tested. In all cases, excess unlabeled peptide (+ DH_{31}) reduced the fluorescent signal, thus indicating that the binding was specific. PC, principal cell; SC, secondary cell; iSC, 'inverse' secondary cell. Scale bars, 25 µm in (**a**) and (**c**), but 50 µm in (**b**), (**d**), (**e**), (**f**) and (**g**).



Supplementary Figure 7 | Capa-1-F is biologically active. Fluid secretion assays with capa-1-F and capa-1 on MTs from (**a**) *Drosophila melanogaster*, (**b**) *Bombyx mori,* (**c**) *Tenebrio molitor*, (**d**) *Vespula vulgaris* and (**e**) *Gryllus assimilis*. Bar graphs indicate the percent change in rate of fluid secretion immediately prior to peptide application compared to 30 min after stimulation. Black arrows indicate time of peptide application. Values are expressed as mean ± s.e.m. Significant difference from basal rates of fluid secretion was tested using a paired samples *t*-test, while an unpaired two-sample *t*-test was used to test differences in secretory response between capa-1-F and capa-1 treatments. In both cases, a significance level of $P<0.05$ (*) was taken as the critical value. n.s., not significant.



Supplementary Figure 8 | DH_{31}-F is biologically active. Fluid secretion assays with DH_{31}-F and DH_{31} on MTs from (**a**) *Drosophila melanogaster*, (**b**) *Bombyx mori* and (**c**) *Vespula vulgaris*. Bar graphs indicate the percent change in rate of fluid secretion immediately prior to peptide application compared to 30 min after stimulation. Black arrows indicate time of peptide application. Values are expressed as mean ± s.e.m. Significant difference from basal rates of fluid secretion was tested using a paired samples *t*-test, while an unpaired two-sample *t*-test was used to test differences in secretory response between DH_{31}-F and DH_{31} treatments. In both cases, a significance level of $P<0.05$ (*) was taken as the critical value. n.s., not significant.

	Species	Number	Sequence	Reference
Diptera	*Drosophila melanogaster*	I	NSVVLGKKQRFHSWGamide	[4]
	Glossina morsitans	-	n.a.	-
	Tipula oleracae	-	n.a.	-
	Anopheles gambiae	I	DTPRYVSKQKFHSWGamide	[5]
		II	NPFHSWGamide	
		III	NTAQVFYPWGamide	
Lepidoptera	*Manduca sexta*	-	n.a.	-
	Helicoverpa zea	I	YFSPWGamide	[6]
		II	VRFSPWGamide	
		III	KVKFSAWGamide	
	Bombyx mori	II	VRFSPWGamide	[7]
		III	KVKFSAWGamide	
Hymenoptera	*Vespula vulgaris*	-	n.a.	-
	Apis mellifera	I	GVFDRWGamide	[8]
		II	FHWIPFNSWGamide	
		III	TKFNPWGamide	
Coleoptera	*Tenebrio molitor*	-	n.a.	-
	Tribolium castaneum	-	n.p.	[9]
	Pachnoda marginata	-	n.a.	-
	Hylobius abietis	-	n.a.	-
Hemiptera	*Philaenus spumarius*	-	n.a.	-
	Acyrthosiphon pisum	I	QKTVFSSWGamide	[10]
		II	QSTYPYGamide	
		III	PAFSSWGamide	
		IV	ASDKHGamide	
		V	PKQTFSSWGamide	
		VI	SSDFFPWGamide	
Dictyoptera	*Leucophaea maderae*	I	DPAFNSWGamide	[11]
		II	DPGFSSWGamide	
		III	DPGFNSWGamide	[12]
		IV	DPGFHSWGamide	
		V	DPGFSSWGamide	[13]
		VI	DPGFHSWGamide	
		VII	DPAFSSWGamide	[14]
		VIII	GADFYSWGamide	

Supplementary Table 1 | Comparison of the amino acid sequences of the insect kinins reveals a highly conserved C-terminal pentapeptide sequence ($FX_1X_2WGamide$). Residues identical to those of DK are highlighted in black. n.a., not available; n.p., not present.

	Species	Number	Sequence	Reference
Diptera	*Drosophila Melanogaster*	I	GANMGLYAFPRVamide	[15]
		II	ASGLVAFPRVamide	
	Glossina morsitans	-	n.a.	-
	Anopheles gambiae	I	GPTVGLFAFPRVamide	[15]
		II	pQGLVPFPRVamide	
Lepidoptera	*Manduca sexta*	I	DGVLNLYPFPRVamide	[15]
		II	pQLYAFPRVamide	
	Bombyx mori	I	PDGVLNLYPFPRVamide	[15]
		II	QLYAFPRVamide	
Hymenoptera	*Vespula vulgaris*	-	n.a.	-
	Solenopsis invicta	I	SAGLVAYPRIamide	[16]
		II	KSDLFPRLamide	
		III	TFGIIQKPRVamide	
Coleoptera	*Tenebrio molitor*	-	n.a.	-
	Tribolium castaneum	I	NKLASVYALTPSLRVamide	[9]
		II	RIGKMVSFPRIamide	
Hemiptera	*Acyrthosiphon pisum*	I	ESAVAGLIPFPRVamide	[17]
		II	EGLIPFPRIamide	
	Nezera viridula	I	DQLFPFPRVamide	[15]
		II	EQLIPFPRVamide	
Dictyoptera	*Leucophaea maderae*	I	GSSGLIPFGRTamide	[15]
		II	GSSGLISMPRVamide	
		III	GSSGMIPFPRVamide	

Supplementary Table 2 | Comparison of the amino acid sequences of the insect capa neuropeptides reveals a conserved C-terminal tetrapeptide sequence (FPRVamide). Residues identical to those of *Drosophila* capa-1 are highlighted in black. n.a., not available.

	Species	Sequence	Reference
Diptera	*Drosophila Melanogaster*	TVDFGLARGYSGTQEAKHRMGLAAANFAGGPamide	18
	Glossina morsitans	n.a.	-
	Anopheles gambiae	TVDFGLSRGYSGAQEAKHRMAMAVANFAGGPamide	19
Lepidoptera	*Bombyx mori*	AFDLGLGRGYSGALQAKHLMGLAAANFAGGPamide	20
Hymenoptera	*Vespula vulgaris*	n.a.	-
	Apis mellifera	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGPamide	21
	Nasonia vitripennis	GLDLGLNRGFSGSQAAKHLMGLAAANYAGGPamide	22
Coleoptera	*Tenebrio molitor*	n.a.	-
	Tribolium castaneum	GLDLGLGRGFSGSQAAKHLMGLAAANFAGGPamide	9
Hemiptera	*Acyrthosiphon pisum*	GLDLGLSRGYSGTQAAKHLMGMAAANFAGGPamide	17
	Rhodnius prolixus	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP amide	23
Dictyoptera	*Diploptera punctate*	GLDLGLSRGFSGSQAAKHLMGLAAANYAGGP amide	24

Supplementary Table 3 | Comparison of the amino acid sequences of the insect DH_{31} neuropeptides reveals an evolutionary conserved sequence. Residues identical to those of *Drosophila* DH_{31} are highlighted in black. n.a., not available.

mmol l^{-1}	*Drosophila melanogaster*	*Glossina morsitans*	*Anopheles gambiae*	*Manduca sexta*	*Tenebrio molitor*	*Vespula vulgaris*	*Gryllus assimilis*
NaCl	117.5	118	117.5	-	90	40	115
KCl	20	10	-	32	50	40	12
$MgCl_2$	2	2	8.5	1	5	5	4
$CaCl_2$	2	2	2	1	2	14	3.5
$NaHCO_3$	10.2	-	10.2	3	6	6	10
NaH_2PO_4	4.5	5	4.3	2	4	4	4
HEPES	8.6	-	8.6	-	-	-	-
Glycine	-	-	-	-	10	10	2
Proline	-	-	-	-	10	10	5
Serine	-	-	-	-	10	10	-
Histidine	-	-	-	-	10	10	1
Glutamine	-	-	-	-	10	10	2
Glucose	20	20	20	200	50	100	10
Sucrose	-	-	-	-	-	-	78
pH*	6.8	7.0	7.0	6.7	7.0	7.0	7.1
mOsm/kg**	354	300	300	320	390	390	380
Reference	[25]	[26]	[19]	[27]	[28]	[29]	[30]

Supplementary Table 4 | Experimentally optimized salines used for acute dissections and functional assays.

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