



Supplementary Information for

The vitellogenin receptor functionality of the migratory locust depends on its phosphorylation by juvenile hormone

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Other supplementary materials for this manuscript include the following:

Datasets S1

Fig. S1.

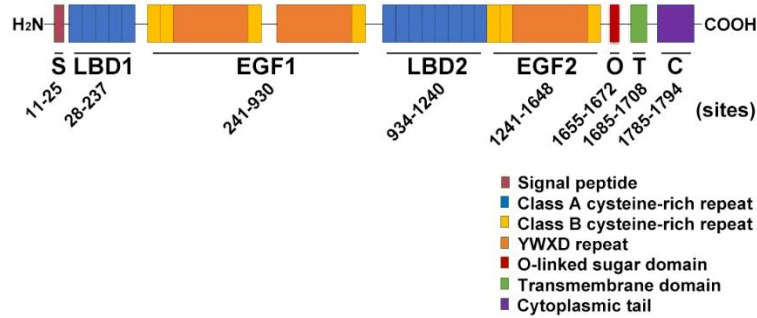


Figure S1. *Locusta migratoria* VgR domain prediction. VgR domains were predicted by using SMART website (<http://smart.embl-heidelberg.de/>) and ExPASy TMHMM 2.0 (<http://www.cbs.dtu.dk/services/TMHMM-2.0/>) with the full length amino acid sequence of VgR (GenBank: QCX35737). S, signal peptide. LBD, ligand binding domain. EGF, epidermal growth factor precursor like domain. O, O-linked sugar domain. T, transmembrane domain. C, cytoplasmic domain.

Fig. S2.

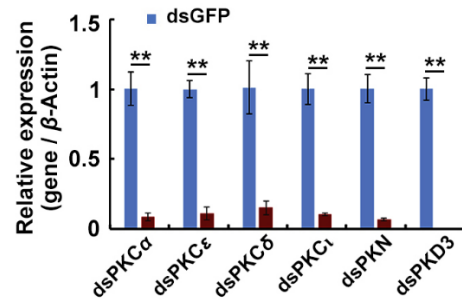


Figure S2. Knockdown efficiency of PKC isoforms in the ovaries of adult female locusts on day 4. PKC α (GenBank: MN793141), PKC ϵ (GenBank: MN793143), PKC δ (GenBank: MN793140), PKC ι (GenBank: MN793142), PKN (GenBank: MN793144) and PKD3 (GenBank: MN793145). **indicates significant difference at $P < 0.01$. n = 15

Fig. S3.

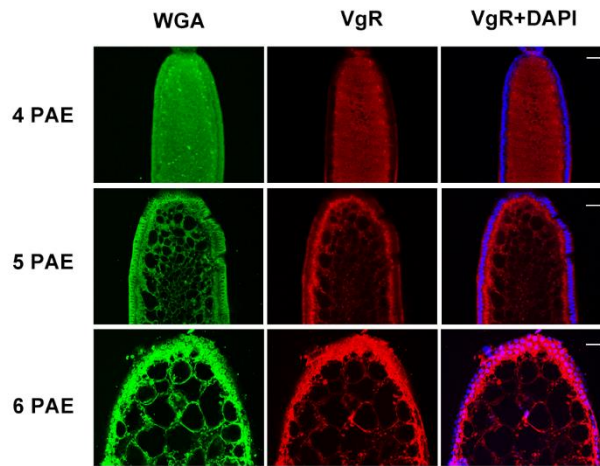


Figure S3. Subcellular localization of VgR in the primary oocytes of adult female locusts at 4-6 days post adult eclosion (PAE). Green, Alexa Fluor 488-WGA for membrane staining. Red, VgR staining by anti-VgR antibody. Blue, DAPI staining for nuclei. Scale bars, 50 μ m.

Fig. S4.

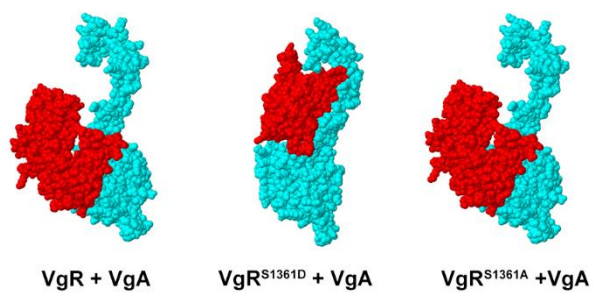


Figure S4. VgR phosphorylation at Ser¹³⁶¹ and its binding to Vg. The 3D structural prediction was computed by a Fast Rotational DOCKing method (<http://frodock.chaconlab.org/>). Red, VgA. Blue indicates VgR, VgR^{S1361D} or VgR^{S1361A}.

Fig. S5.

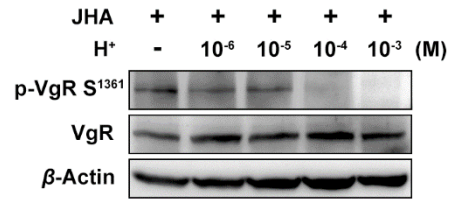


Figure S5. Acidification-induced VgR dephosphorylation. Protein extracted from Sf9 cells transfected with the recombinant pIEx-4-VgR-RFP-His vector and treated with methoprene (JHA) for 30 min. HCl application at a final concentration of 10⁻⁶ to 10⁻³ M for 30 min. p-VgR S¹³⁶¹, phospho-VgR (Ser¹³⁶¹) antibody. VgR, VgR antibody.

Table S1. Differential phosphoric sites and proteins in the ovaries of adult female locusts on day 0-5

Compared groups	Regulation type	Fold change >1.2	Fold change >1.3	Fold change >1.5	Fold change >2
S3/S0	up-regulated	590(446)	426(337)	201(171)	56(51)
	down-regulated	547(372)	381(273)	202(155)	53(45)
S4/S0	up-regulated	730(497)	567(403)	318(238)	107(86)
	down-regulated	606(403)	417(299)	222(167)	75(63)
S4/S3	up-regulated	282(218)	191(140)	95(69)	25(15)
	down-regulated	212(186)	117(109)	39(38)	3(3)
S5/S0	up-regulated	840(557)	755(509)	557(392)	262(182)
	down-regulated	719(488)	641(446)	457(332)	172(152)
S5/S3	up-regulated	635(432)	553(379)	409(282)	171(118)
	down-regulated	565(419)	457(350)	245(207)	62(57)
S5/S4	up-regulated	481(331)	403(281)	264(193)	105(64)
	down-regulated	470(353)	358(283)	154(136)	21(17)

S0, S3, S4 and S5 represent adult female locusts at 0, 3, 4, 5 days PAE, respectively. Numbers and numbers in parentheses indicate the phosphorylation sites and proteins, respectively, identified by quantitative phosphoproteomic analysis. Filtered at $P < 0.05$. The original data are summarized in an Excel file and deposited as a dataset.

Table S2. Protein phosphorylation related to endocytosis and recycling

Protein	Function	Phosphorylation site	Kinase
VgR		SLGS(p)VDVVYK	PKC
ARFGEF		SYPLEWALDDDAGS(p)DECSLPAK	CKII
ARFGAP		KS(p)TGSLTEVTVPQLLPK	PKA
PIP4K	Regulator	GGGDRPWGPLSSPPDS(p)PHAALMR	CDK5
PIP5k		ASGGDGPSS(p)PAAAFIER	CDK5
PLD		LVDLGSVS(p)QTLATR	PKC
Cbl		RQNS(p)VEENLK	PKA
Endophilin		VNS(p)EDVDLTTPATSPITDTK	CKII
ARP		KFQS(p)LDR	PKC
Hsc70		GRLS(p)KEEIER	CKII
NEDD4-1		SRPPS(p)FR	PKC
NEDD4-2	Clathrin-dependent endocytosis	RQDS(p)EWEVLETGR	PKA
Eps15		DPFGCDPFALQSPSPGPPRPES(p)PSPALPPKK	CDK5
SMURF		VDPVGREDS(p)PSGSSR	GSK3
dynamamin		NDWLVSGLENPRLS(p)PPS(p)PGGPR	CDK5
EPN2		AVS(p)PHQIGTSTGAVPR	CDK5
AP-2		ESS(p)ILAVLK	CKI
N-WASP		EFIY(p)DFISR	unsp
WASH		VAPILS(p)PTEMPK	PKA
SNX6	Early endosome	KDS(p)LDDRDILSGDNR	PKA
SNX12		RYS(p)DFEWLR	PKA
VPS37		ELLASLSAAS(p)LDDLRR	CaMKII
VPS35		LESPEs(p)DGPSYEGLA	CKI
MVB12			QASGEES(p)PHEYER
Hrs	Late endosome	QYS(p)PPPLMDNSPSQGEEEDPELAR	CDK5
Alix		AS(p)FFQDFVTK	PKC
CHMP7		GNTSLSFEPEIS(p)DDEDSEYKDHEK	CKII
ZFYVE		INSLESQLS(p)FEK	CKI
Rab11-FIP		VGQIAGEADPGVIS(p)EDEDEFTFDDLCHK	CKII
RABEP	Recycling	SGRDS(p)PILAPSVMLSAVTK	CDK5
PKCi		DLANFPPEFTDEPVHLT(p)PDDTR	unsp
Par6		KGES(p)LEELNGYGTYKPR	PKA
RBSN		LLGLSS(p)PTEGQR	unsp

Phosphorylated proteins and peptides were identified by phosphoproteomic and LC-MS/MS analysis. Kinase-substrate interactions were predicted by NetPhos 3.1 software (<http://www.cbs.dtu.dk/services/NetPhos/>). Amino acids followed by (p) indicate the phosphorylation sites. PKC, protein kinase C. CK, casein kinase. PKA, protein kinase A. CDK5, Cyclin dependent kinase 5. GSK3, Glycogen synthase kinase 3. unsp, unspecific kinase. CaMKII, Ca²⁺/calmodulin-dependent protein kinase II.

Table S3. Primers used in qRT-PCR, RNAi and vector construction

Primer	Oligonucleotide sequence
qRT-PCR	
VgR-qRT F	5'- acaccctgtactggtcagact -3'
VgR-qRT R	5'- cagcatgcagatgtcactgca -3'
PKC α -qRT F	5'- cggttggaacaagtgg -3'
PKC α -qRT R	5'- tggcttgaaggaggttg -3'
PKC δ -qRT F	5'- ttgctgtacgggtatttc -3'
PKC δ -qRT R	5'- cagggttccacgatgag -3'
PKC ϵ -qRT F	5'- ccaagaatcctgccaac -3'
PKC ϵ -qRT R	5'- cagtgtcccagtcac -3'
PKC ζ -qRT F	5'- ggcatgtgcaaggaagg -3'
PKC ζ -qRT R	5'- gtgccaccaatcaacta -3'
PKD3-qRT F	5'- cgctttatgtatccagc -3'
PKD3-qRT R	5'- ctgcttctccagtctcgt -3'
PKN-qRT F	5'- cagtgcgtatgaatccc -3'
PKN-qRT R	5'- ccctggcaatgatgtctc -3'
β -Actin-qRT F	5'- aattaccattgtaacgagcgatt -3'
β -Actin-qRT R	5'- tgettccatacccaggaatga -3'
RNA interference	
VgR-RNAi F	5'- gcgtaatacgactcactatagggcacaagttaggggaagga -3'
VgR-RNAi R	5'- gcgtaatacgactcactatagggacggatcatacgcaggctc -3'
PKC α -RNAi F	5'- gcgtaatacgactcactatagggctttaaagcagccgacgtt -3'
PKC α -RNAi R	5'- gcgtaatacgactcactatagggctggcctcttcttcagtgt -3'
PKC δ -RNAi F	5'- gcgtaatacgactcactatagggaaagacgttacggaacaccg -3'
PKC δ -RNAi R	5'- gcgtaatacgactcactataggggcactttctgtggcagtga -3'
PKC ϵ -RNAi F	5'- gcgtaatacgactcactatagggagcagtgaggaggtagcaa -3'
PKC ϵ -RNAi R	5'- gcgtaatacgactcactataggggctgcaaggcctagaatagc -3'
PKC ζ -RNAi F	5'- gcgtaatacgactcactatagggagaaggcctgtcactgat -3'
PKC ζ -RNAi R	5'- gcgtaatacgactcactataggggtgccaccaatcaactaa -3'
PKD3-RNAi F	5'- gcgtaatacgactcactataggggttccgtcatgaccacagt -3'
PKD3-RNAi R	5'- gcgtaatacgactcactatagggcggcactgtaaccctgttt -3'
PKN-RNAi F	5'- gcgtaatacgactcactataggggttcacaacaagcctgggat -3'
PKN-RNAi R	5'- gcgtaatacgactcactatagggcttctctcccagcaactg -3'
GFP-RNAi F	5'- gcgtaatacgactcactataggtgggtcccaattctgtggaac -3'
GFP-RNAi R	5'- gcgtaatacgactcactataggtggaagttgacctgatgcc -3'
Recombinant pIEx-4-RFP-His vector construction	
VgR-OE F	5'- tactcaggatcccatgagttgtggaccagggggcagaacga -3'
VgR-OE R	5'- tactcaggatccaggtagaaggcgcactcttgcactttgc -3'
VgR ^{1361m} -OE F	5'- aggtgtcgcgctcactggcgctgtggacgtcgtgtacaacaa -3'
VgR ^{1361m} -OE R	5'- ttgtttgtacacgagtcacagcgcagcagtgagcgcgacacct -3'
PKC ζ -OE F	5'- tggcatcgttaacacgtcaagagctcatgggctgcagccccgtgag -3'
PKC ζ -OE R	5'- gcgccgagatctccaattgggatccgccacgcagctcctccagcgaca -3'
Recombinant pET-32a(+) vector construction	
VgR-exp F	5'- tactcaggatcctcaatcgcagctcttcgagaac -3'
VgR-exp R	5'- tactcactcagtcacacgagtggtcttaattacac -3'
PKC ζ -exp F	5'- tactcaggatccagtgagcaagttgagccatt -3'
PKC ζ -exp R	5'- tactcactcagtcacacagaatgtgaagtggt -3'