Supplementary Information

Partial proteasomal degradation of Lola triggers the male-to-female switch of a dimorphic courtship circuit

Sato et al.
Supplementary Figure 1: The fru and lola loci.

a, The fru locus and exon (box)-intron (thin line) organization of isoform types A, E, and B with different zinc-finger motifs and isoform type D without a zinc-finger motif. The types A, E and B in our study correspond to isoforms A, B and C of other research groups, respectively. The P-insertions (triangles), promoters (P1-P4) and exons (1-11) are indicated. The regions containing epitopes for the anti-Fru antibodies are indicated. b, Schematic representation of the genomic organization of the lola locus. The entire locus consists of 32 exons. Transcription starts at either of the 5’ variable exons (exons 1-4), and 3’ variable exons (exons 9-32) are alternatively spliced to the constant exons (exons 5-8), generating variants encoding 20 Lola isoforms with distinct C-terminal domains containing zinc finger motifs. The protein coding regions recognized by the anti-Lola antibodies are indicated. The exon-intron organization and possible splicing patterns are drawn based on Goeke et al.\textsuperscript{1} and Ohsako et al.\textsuperscript{2} for lola and Billeter et al.\textsuperscript{3} and Ito et al.\textsuperscript{4} for fru.
Supplementary Figure 2: Phenotypes associated with heterologous and endogenous Fru expression.

a-g. The eye structure defects induced by fru+ overexpression and its modification by lola gene dosage. a, Strategy to recover fru modifiers using the GeneSearch (GS) system. b, An eye-antennal disc with ectopic FruB expression. c-g, The compound eye of a control fly carrying only GMR-GAL4 (c), flies in which GMR-GAL4 was used to overexpress fru+ type B alone (d) or together with GS2169 (e), that of lola heterozygotes without (f) or with fru+-type B overexpression as driven by GMR-GAL4 (g) The scale bar shown in panel c applies to panels c-g. h, Neuroblast clones of the sexually dimorphic mAL neurons in the female (upper panel) and male (lower panel) brain. The ipsilateral neurite is present only in the male (circled with a dotted line). Scale bars: 100 μm (left-hand side) and 10 μm (right-hand side) in (c-g); 50 μm in (h).
**Supplementary Figure 3: Lola expression detected by an anti-Lola-Exon 29 antibody.**

Western blot analysis of lysates prepared from *lola* homozygous (left-hand side lane) and heterozygous (right-hand side lane) embryos as probed by the anti-Lola-exon 29 antibody, demonstrating that this antibody specifically recognizes Lola29M. α-Tubulin served as a loading control. Source data are provided as a Source Data file.
Supplementary Figure 4: Lola29M is coexpressed with FruM in the wandering-stage larval CNS.

**a-c**, CNS cells in a wandering-stage larva labeled by the combination of UAS-ChRFP and insc-GAL4 (a) are immunopositive for the anti-Deadpan (Dpn) antibody (b), indicating that insc-GAL4 serves as a neuroblast marker. A merged image is shown in (c). 

**d-I**, Immunostaining of CNS cells from wild-type larvae at the wandering stages with anti-FruMale, anti-Propero (Pros), and anti-Lola-exon 29 antibodies. Pros is a marker for ganglion mother cells (GMCs) differentiating into neurons and not expressed in neuroblasts. Neuroblasts are indicated with arrows and differentiating GMCs and neurons are shown with arrowheads. FruM was detectable in some GMCs and neurons (g-i) but not in neuroblasts. The anti-Lola-exon 29 antibody that specifically recognizes Lola29M/F labeled some of the differentiating GMCs and neurons (j-l) but none of the neuroblasts (d-f). Scale bar: 10 µm.
Supplementary Figure 5: No sex difference in promoter usage for transcripts containing the sequence from exon 29.

a, Flow diagram for the steps of 5’ and 3’ RACE experiments. b, Primer design for 5’ and 3’ RACE experiments to obtain full length cDNAs for exon 29-containing transcripts. c, 5’ and 3’ RACE PCRs with female-derived RNAs each yielded cDNAs that encoded sequences identical to the male transcripts. d, Full-length cDNAs contained either exon 2 or exon 3 at the 5’-most segment, indicating that the exon 29-containing primary RNAs are transcribed by two different promoters immediately upstream of exon 2 and exon 3, respectively, in both female and males. Source data are provided as a Source Data file.
Supplementary Figure 6: Effectiveness of *lola* knockdown with RNAi evaluated by western blotting. 
*GMR-GAL4*-driven expression of *lola-COM RNAi* and *lola-exon 29 RNAi* reduced the amount of Lola proteins. Comparisons of a blot probed with the anti-LolaCOM antibody (left-hand panel) and that with anti-Lola-exon 29 antibody (right-hand panel) indicated the specific knockdown of Lola29M by *lola-exon 29 RNAi*. Source data are provided as a Source Data file.
Supplementary Figure 7: S2 cells transfected with a full-length *lola29m* construct produce both Lola29M and Lola29F-like proteins. Western blotting of lysates from S2 cells transfected with a vector encoding Lola29M decorated with the N-terminal HA-tag and the C-terminal V5-tag. Whereas an anti-V5 antibody detected two bands, Lola29M and Lola29F-like (right-hand side panel), an anti-HA antibody detected only Lola29M (left-hand side panel), suggesting that Lola29F-like may be an N-terminally truncated derivative of Lola29M. Source data are provided as a Source Data file.
Supplementary Figure 8: Lola29M is K48-polyubiquitinated.

Western blotting of K48-polyubiquitinated proteins in immunoprecipitates of transfected S2 cells. The anti-Ub-K48 antibody was used as a probe in the western blotting. The proteins were immunoprecipitated with the anti-FLAG antibody that recognizes Lola29M::3xFLAG, which is overexpressed in S2 cells alone or together with FruBM. 0 (-) or 1 (+) μg of pMT-lola29m-3xFLAG and 0 (-), 1 (+) or 3 (++) μg of pMT-frubm were cotransfected into S2 cells (indicated above the gel). Source data are provided as a Source Data file.
Supplementary Figure 9: Lola29M promotes the male-specific ipsilateral neurite formation in mAL neurons.

mAL neuroblast clones induced by MARCM in fru\textsuperscript{NP21} heterozygous females. Overexpression of truncation-resistant Lola29M[K41R] induced the ipsilateral neurite in some females (b cf. a), whereas overexpression of Lola29F-like (Lola29M[Δ1-300]) did not (c). d, Quantification of the effects of overexpression of Lola29M[K41R] and Lola29F-like (Lola29M[Δ1-300]) on the proportion of flies with the ipsilateral neurite. *: P<0.05 by the Fisher’s exact probability test. Scale bar: 50 μm.
Supplementary Figure 10: Diminished Robo1 immunoreactivity of the larval CNS by Lola29M[K41R] overexpression.

The brain-VNC complexes from female third instar larvae with (b, c, e, f) or without (a, c, d, f) Lola29M[K41R] overexpression were subjected to the double immunostaining for Robo1 (a-c) and Lola29M/F (d-f). The control and test samples were processed in the same tubes at the same time. Scale bars: 100 μm in (a, b, d, e) and 200 μm in (c, f).
Supplementary Figure 11: Precocious wing switching during male courtship induced by the deletion of DR1 within the robo1 promoter.

a. Examples of ethograms of a wild-type (CS) male and a robo1Δ4/robo1Δ4 mutant male. The time elapsed since the start of observation is shown on the top. The period during which the fly displayed precocious wing switching (orange bar), wing extension (magenta bar), or any courtship actions (green bar) is indicated. Vertical lines above the magenta bars indicate the time at which the fly switched the wing to be extended from the left wing to the right wing (right) and vice versa (left).
b. The wing usage pattern in courtship compared among the indicated genotypes. The larger wing switching index indicates more-frequent switching of the right and left wings during courtship. The numbers of flies examined are indicated in parentheses below the abscissa. The box plot shows median and 10th, 25th, 75th, and 90th percentiles. The statistical differences among the datasets were evaluated by the Kruskal-Wallis analysis of variance followed by Steel’s nonparametric multiple comparisons. *p < 0.05.
Supplementary Figure 12: Cul1 expression in mAL neurons.

A female brain doubly stained for GFP (a, c, d, f; green) and Cul1 (b, c, e, f; magenta) is shown at lower (a-c) and higher (d-f) magnification (scale bars indicate 50 µm for a-c and 5 µm for d-f). GFP expression was targeted to mAL neurons by the intersection of 9-189-GAL4 and fru<sup>FLP</sup> (arrowheads).
Supplementary Figure 13: Lola29M-FruBM interactions depend on the BTB domain of each. The antibody that recognizes the C-terminal V5 tag of Lola29M-V5 (anti-V5) precipitated intact FruBM (a) but not BTB-deleted FruBM (FruBM\textsuperscript{ΔBTB}; b) in lysates from S2 cells cotransfected with constructs encoding the respective proteins. Note that the lack of the BTB domain of FruBM resulted in the production of Lola29F-like, which was not detected when both FruBM and Lola29M were structurally intact and had the BTB-domain. Source data are provided as a Source Data file.
Supplementary Figure 14: Western Blot analysis of FruBM in S2 cell lysates.
FruBM protein was detected only when the cells were transfected with a FruBM-encoding sequence. α-tubulin served as an internal control. Source data are provided as a Source Data file.
| Accession | Description | Score | Coverage | # Peptides | # AAs | MW [kDa] |
|-----------|-------------|-------|----------|------------|-------|----------|
| Q9V5M3    | Longitudinals lacking protein, isoforms N/O/W/X/Y OS=Drosophila melanogaster GN=lola PE=1 SV=3 - [LOLA6_DROME] | 3246.00 | 25.40 | 19 | 878 | 96.1 |
| P02828    | Heat shock protein B3 OS=Drosophila melanogaster GN=Hsp83 PE=1 SV=1 - [HSBP3_DROME] | 1464.02 | 55.23 | 39 | 717 | 81.8 |
| P52034    | ATP-dependent 6-phosphofructokinase OS=Drosophila melanogaster GN=Prk PE=2 SV=2 - [PKFA_DROME] | 1193.64 | 51.40 | 31 | 788 | 86.6 |
| Q9VHP0    | ATP-dependent RNA helicase bel OS=Drosophila melanogaster GN=bel PE=1 SV=1 - [DDX3_DROME] | 1182.89 | 60.03 | 37 | 798 | 85.0 |
| Q99322    | Myosin heavy chain, non-muscle OS=Drosophila melanogaster GN=zip PE=1 SV=2 - [MYSN_DROME] | 980.80 | 18.08 | 28 | 2057 | 236.5 |
| P29894    | Heat shock 70 kDa protein cognate 3 OS=Drosophila melanogaster GN=Hsc70-3 PE=2 SV=2 - [HSP70_DROME] | 813.14 | 38.57 | 22 | 656 | 72.2 |
| P54351    | Vesicle-fusing ATPase 2 OS=Drosophila melanogaster GN=Na+2 PE=2 SV=2 - [NSF2_DROME] | 703.21 | 52.39 | 31 | 752 | 83.4 |
| P11147    | Heat shock 70 kDa protein cognate 4 OS=Drosophila melanogaster GN=Hsc70-4 PE=1 SV=3 - [HSP70D_DROME] | 689.32 | 41.32 | 26 | 651 | 71.1 |
| Q9NFU0    | Fragile X mental retardation syndrome-related protein 1 OS=Drosophila melanogaster GN=Fmr1 PE=1 SV=1 - [FMR1_DROME] | 639.87 | 38.16 | 19 | 694 | 76.0 |
| Q7KN90    | Cysteine--tRNA ligase, cytoplasmic OS=Drosophila melanogaster GN=Aats-cys PE=1 SV=1 - [SYCC_DROME] | 627.24 | 60.19 | 32 | 741 | 84.2 |
| P10987    | Actin-SC OS=Drosophila melanogaster OS=Act5C PE=1 SV=4 - [ACT1_DROME] | 565.54 | 60.11 | 16 | 376 | 41.8 |
| Q9XYU0    | DNA replication licensing factor Mcm7 OS=Drosophila melanogaster GN=Mcm7 PE=1 SV=1 - [MCM7_DROME] | 507.45 | 33.75 | 23 | 720 | 81.2 |
| Q0E5M0    | Eukaryotic translation initiation factor 3 subunit B OS=Drosophila melanogaster GN=eIF3-S9 PE=1 SV=1 - [EIF3B_DROME] | 453.38 | 30.87 | 16 | 690 | 80.4 |
| Q9VHR8    | Dipeptidyl peptidase 3 OS=Drosophila melanogaster GN=DppIII PE=2 SV=2 - [DPP3_DROME] | 449.97 | 27.74 | 21 | 786 | 89.1 |
| O46037    | Vinculin OS=Drosophila melanogaster GN=Vinc PE=1 SV=1 - [VINC_DROME] | 445.56 | 20.29 | 14 | 961 | 83.7 |
| Q9VWV3    | ATP-dependent RNA helicase Ddx1 OS=Drosophila melanogaster GN=Ddx1 PE=2 SV=2 - [DDX1_DROME] | 406.40 | 19.94 | 11 | 727 | 80.8 |
| Q9WWX9    | Centromere/kinetochore protein zw10 OS=Drosophila melanogaster GN=mtn1(1)15 PE=1 SV=2 - [ZW10_DROME] | 401.08 | 30.51 | 15 | 721 | 82.2 |
| Q94S11    | NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondri OS=Drosophila melanogaster GN=ND75 PE=2 SV=3 - [NDU1_DROME] | 399.35 | 23.94 | 11 | 731 | 78.6 |
| Q29114    | Cytochrome--tRNA ligase, cytoplasmic OS=Drosophila melanogaster OS=pseudosubcuape pseudobosueca GN=Aats-cys PE=3 SV=1 - [SYCC_DROPS] | 391.27 | 19.97 | 12 | 741 | 83.7 |
| P13469    | DNA-binding protein module OS=Drosophila melanogaster GN=mod PE=1 SV=2 - [MODU_DROME] | 373.82 | 30.26 | 12 | 542 | 60.3 |
| Q9VRA2    | Molybdenum cofactor sulfatase OS=Drosophila melanogaster GN=mal PE=1 SV=1 - [MCOS_DROME] | 349.64 | 19.33 | 10 | 781 | 88.0 |
| Q9VF8C    | Glycerokin [starch] synthase OS=Drosophila melanogaster GN=GlyS PE=1 SV=2 - [GYS_DROME] | 345.01 | 23.13 | 10 | 709 | 81.7 |
| A12A1X    | Eukaryotic translation initiation factor 3 subunit C OS=Drosophila melanogaster GN=eIF3-S8 PE=1 SV=1 - [EIF3C_DROME] | 330.39 | 22.42 | 17 | 910 | 105.6 |
| P29843    | Heat shock 70 kDa protein cognate 1 OS=Drosophila melanogaster GN=Hsc70-1 PE=1 SV=1 - [HSPIA_DROME] | 327.98 | 13.73 | 6 | 641 | 70.6 |
| Q9VP61    | Acetyl-coenzyme A synthetase OS=Drosophila melanogaster GN=AcCoA5 PE=2 SV=1 - [ACSA_DROME] | 315.81 | 31.49 | 16 | 670 | 75.9 |
| Q960Z0    | Kinesin-like protein Klp10A OS=Drosophila melanogaster GN=Klp10A PE=1 SV=1 - [K10A_DROME] | 313.98 | 22.61 | 14 | 805 | 88.6 |
| P25991    | Protein suppressor of forked OS=Drosophila melanogaster GN=suf(f) PE=1 SV=2 - [SUF_DROME] | 277.35 | 16.99 | 10 | 765 | 88.2 |
| P10981    | Actin-87E OS=Drosophila melanogaster GN=Act87E PE=1 SV=1 - [ACT5_DROME] | 247.80 | 41.76 | 12 | 376 | 41.8 |
| B4HY41    | Elongation factor G, mitochondrial OS=Drosophila melanogaster GN=igo PE=3 SV=1 - [EFM_DROSE] | 236.87 | 22.15 | 12 | 745 | 83.5 |
| Q9VKE2    | Inhibitor of nuclear factor kappa-B kinase subunit beta OS=Drosophila melanogaster GN=iir5 PE=1 SV=2 - [IKKB_DROME] | 229.55 | 13.85 | 9 | 751 | 86.3 |
| Q24311    | Culkin homolog 1 OS=Drosophila melanogaster GN=Cul1 PE=1 SV=2 - [CUL1_DROME] | 209.28 | 20.67 | 15 | 774 | 89.5 |
| P20480    | Protein claret segregational OS=Drosophila melanogaster GN=ncd PE=1 SV=1 - [NCD_DROME] | 206.23 | 23.00 | 13 | 700 | 77.4 |
| Q9W1A2    | N-alpha-acetyltransferase, 35 Na+auxiliary subunit homolog OS=Drosophila melanogaster GN=CG0655 PE=2 SV=1 - [NAAS5_DROME] | 182.93 | 10.97 | 6 | 784 | 89.1 |
| Q9VZI3    | Unc-112-related protein OS=Drosophila melanogaster GN=Ft1 PE=1 SV=1 - [UN112_DROME] | 176.39 | 10.88 | 6 | 708 | 80.4 |
| Q9V8K2    | Exocyst complex component 3 OS=Drosophila melanogaster GN=secb PE=2 SV=2 - [EXOC3_DROME] | 175.79 | 11.38 | 8 | 738 | 86.6 |
| Q7KN62    | Transitional endoplasmic reticulum ATPase 70K OS=Drosophila melanogaster GN=TER94 PE=1 SV=1 - [TERA_DROME] | 175.72 | 15.98 | 9 | 801 | 88.8 |
| Q9VSH4    | Cleavage and polyadenylation specificity factor subunit CG7185 OS=Drosophila melanogaster GN=CG7185 PE=1 SV=2 - [CPSF6_DROME] | 172.77 | 8.90 | 4 | 652 | 71.1 |

Supplementary Table 1. List of proteins identified by mass spectrometry in immunoprecipitates that were obtained with an antibody recognizing Lola29M.
| Accession | Description                                      | Score | Coverage | # Peptide | AAs | MW  |
|-----------|--------------------------------------------------|-------|----------|-----------|-----|-----|
| Q9XTM1    | Exocyst complex component OS=Drosophila melanogaster GN=sec10 PE=2 SV=1 - [EXOC5_DROME] | 20.61 | 1.41     | 1         | 710 | 82.0|
| Q27415    | Nucleoplasmin-like protein OS=Drosophila melanogaster GN=Nlp PE=1 SV=1 - [NLP_DROME]    | 20.52 | 7.89     | 1         | 152 | 17.0|

The name of identified proteins (Description, 2nd column) is listed in order from largest to smallest peptide probability score (Score: 3rd column). Accession, accession number in NCBI; Coverage, the percent sequence coverage identified from MS/MS results; # Peptide, number of the identified peptides by LC-MS/MS; AAs, number of amino acids; MW, molecular weight. The S2 cells transfected with a Lola29M[ Δ1-150] expression vector were the source of lysates subjected to immunoprecipitation. The E3 ubiquitin ligase Cullin1 is highlighted in red.
| #  | Name Sequence 5'-3' Plasmid vector | Note                  |
|----|-----------------------------------|-----------------------|
| 1  | fruBM-DBTB F1 TGCGAATTCGGATCC     | pact-FLAG-fruBM[DBTB] and pact-FLAG-fruBM[DZn-finger, DBTB] vectors Paired with FruBM-DBTB R1 |
| 2  | fruBM-DBTB R1 GTCCATGCTCCTTGTCAG  | pact-FLAG-fruBM[DBTB] and pact-FLAG-fruBM[DZn-finger, DBTB] vectors Paired with FruBM-DBTB R2 |
| 3  | fruBM-DBTB F2 CAAGGAGCGATGGAC     | pact-FLAG-fruBM[DBTB] and pact-FLAG-fruBM[DZn-finger, DBTB] vectors Paired with FruBM-DBTB R2 |
| 4  | fruBM-DBTB R2 TCTTCAATGTCGAGCGCTACTTTAATGAGTGAGTTCAGCT | pact-FLAG-fruBM[DBTB] and pact-FLAG-fruBM[DZn-finger, DBTB] vectors Paired with FruBM-DBTB R2 |
| 5  | HA-lola29m(WT) F GGGTGATCCAAATTGCTACCGTAGTGGCCGGATTACGGCATAGCATCAGCAGTTTGGTC (KpnI) pMT-HA-lola29m-V5 vector Paired with HA-lola29m(WT) R |
| 6  | HA-lola29m(WT) R GGGCGGCCGCGTTGCAAGATTCGCTCC (NotI) pMT-HA-lola29m-V5 vector Paired with HA-lola29m(WT) F |
| 7  | COM F GGGATCTAGATCGGGGTACC         | pMT-HA-lola29m-V5 vector Paired with COM R |
| 8  | COM R GAGAAAGGGCTCTCGGCGG         | pMT-HA-lola29m-V5 vector Paired with COM F |
| 9  | K41R F GAGGCCGCGTTTCTCAAGGCCC    | pMT-HA-lola29m-V5 vector Paired with K41R F |
| 10 | K41R R GGGCGGCCGCGTTGCAAGG        | pMT-HA-lola29m-V5 vector Paired with K41R R |
| 11 | K44R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K44R R |
| 12 | K47R R GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 13 | K47R F GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 14 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 15 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 16 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 17 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 18 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 19 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 20 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 21 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 22 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 23 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 24 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 25 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 26 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 27 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 28 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 29 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 30 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 31 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 32 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 33 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 34 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 35 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 36 | K47R F GCACCACCGGCTGAGCC         | pMT-HA-lola29m-V5 vector Paired with K47R F |
| 37 | K47R R GGCGGCGCTGAGCCGCGGGCG      | pMT-HA-lola29m-V5 vector Paired with K47R R |
| 38 | K47R R GTGGGCAGCG       | pMT-HA-lola29m-V5 vector Paired with K47R R |

Supplementary Table 2. List of primer pairs for constructing plasmid vectors.
Single underlining is used to denote 15 bp overlaps for In-fusion cloning. Double underlining is used to denote restriction enzyme recognition sites. An amino acid replacement (K to R) is highlighted in red. F, forward primer; R, reverse primer.
### Supplementary Table 3. List of primer pairs for 5'/3' RACE experiments (Supplementary Fig. 5).

| #  | Name               | Sequence 5'-3'                                                                 | Note                                                                 |
|----|--------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------|
| 1  | 5' RACE primer (F) | Mixture of oligos: 5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3' (0.4 μM) and 5'-CTAATACGACTCACTATAGGGC-3' (2 μM) | Same as Universal Primer A Mix (UPM) in SMARTer RACE cDNA Amplification Kit (Clontech) Paired with Primer 1 |
| 2  | Primer 1 (R)       | CGTGCTGTCACCTTCATGGCCTCC                                                    | Paired with 3' RACE primer                                           |
| 3  | Primer 2 (F)       | GGAGGCCCATGAAGGTGACCAGCACG                                                  | Paired with 3' RACE primer                                           |
| 4  | 3' RACE primer (R) | Mixture of oligos: 5'-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3' (0.4 μM) and 5'-CTAATACGACTCACTATAGGGC-3' (2 μM) | Same as Universal Primer A Mix (UPM) in SMARTer RACE cDNA Amplification Kit (Clontech) |

F, forward primer; R, reverse primer.
Supplementary Table 4. DNA probes used in EMSA experiments (Figure 4).

|   | Name       | Sequence 5’-3’                                                                 | Note     |
|---|------------|-------------------------------------------------------------------------------|----------|
| 1 | Probe DNA B| CCGGGCGTTGCGCTCTCAAAATTTCACAGACACGACCCACGTCAATTGTGAGGTTTTCGCTGCGCCGTGAA      | 120 bp   |
|   |            | TCACAAAGGAGCAGGAAAATAGTTAATTTCACACAGTTAATTGAG                                 |          |
| 2 | Probe DNA B△DR1 | CCGGGCGTTGCGCTCTCAAAATTTCACAGACACGACCCACGTCAATTGTGAGGTTTTCGCTGCGCCGTGAA | 102 bp   |
|   |            | TCATAGTTAATTTCACACAGTTAATTGAG                                                |          |

Direct repeat 1 (DR1) is highlighted in red.
Supplementary References

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