

Supporting Information: Methods S1

Supporting Materials and Methods

Construction of plasmids

To generate EGFP fused dPiwi expression vector, the Gateway[®] LR reaction between the dHP1a-pENTR11 and dPiwi-pENTR11, and pie2GW, pnVW or pcCW [1, 2] was performed using LR Clonase[™] Enzyme Mix (Invitrogen) according to the manufacturer's protocols recommended in manufacturer's manual. The resulting plasmids were named pEGFP-dPiwi, pCC-dHP1a and pNV-dPiwi.

To generate N-terminal fusion DEST vector, pIE2-TetR, for TetR-based I2H baits, *tetR* was amplified from pENTR11-NLS/TetR-Giant [2] by PCR using primers, TetRF and TeRR (Table S1). The PCR product was digested by *SacI* and inserted into a *StuI-SacI* site of pie2FW [1]. The resulting plasmid was named pIE2-TetR. The Gateway[®] LR reaction between the entry vectors, BmAgo3-pENTR11 and Siwi-pENTR11, and the DEST vector, pIE2-TetR, was performed using LR Clonase[™] Enzyme Mix (Invitrogen) according to the manufacturer's protocols recommended in manufacturer's manual. The resulting plasmids were pTetR-Siwi and pTetR-Ago3, which were used as bait expression vector for TetR-based I2H system together with prey expression vector and the tetO-based reporter, p9×tetO-IE2mini(L)-Luc [2].

The DNA sequence corresponding to N-terminal or C-terminal region of Piwi proteins were generated by inverse PCR from each entry clone, Ago3-pENTR11, Siwi-pENTR11 and dPiwi-pENTR11, using phosphorylated primers; for N-terminal

region coding entry clone, pENTR3F as the forward primer and the reverse primer for each gene, Ago3NR, SiwiNR and dPiwiNR; and, for C-terminal region coding entry clone, pENTR5R as the reverse primer and the reverse primer for each gene, Ago3CF, SiwiCF and dPiwiCF. The resulting plasmids were named Ago3NT-pENTR11, SiwiNT-pENTR11, dPiwiNT-pENTR11, Ago3CT-pENTR11, SiwiCT-pENTR11 and dPiwiCT-pENTR11, respectively. The Gateway[®] LR reaction between these entry vectors and the DEST vector, pIE2-DBD, was performed using LR Clonase[™] Enzyme Mix (Invitrogen) according to the manufacturer's protocols recommended in manufacturer's manual. The resulting plasmids were pDBD-Ago3NT, pDBD-SiwiNT, pDBD-dPiwiNT, pDBD-Ago3CT, pDBD-SiwiCT and pDBD-dPiwiCT, which were used as bait for I2H assay.

Preparation of double-stranded RNA

Double-stranded RNA (dsRNA) for *in vivo* RNAi reactions was prepared using the following procedures. For the dsRNA template, BmArmi-574, BmSpnE-533, BmTud-565, BmVLG-406 and BmYb-587 fragments were amplified using primer pairs: BmArmi574F and BmArmi574R; BmSpnE533F and BmSpnE533R; BmTud565F and BmTud565R; BmVLG406F and BmVLG406R; and BmYb587F and BmYb587R, respectively (Table S1), from BmN4 cDNA library and were inserted into a *StuI* site of pLits [3].

The templates for *in vitro* transcription were synthesized via PCR on the templates containing the BmArmi-574, BmSpnE-533, BmTud-565, BmVLG-406 and

BmYb-587 fragments using the litT7 primer (Table S1). The amplified DNA templates amplified were extracted with phenol/chloroform, precipitated with ethanol, and dissolved in H₂O. Bi-directional transcription reactions were performed in the buffer (40 mM Tris-HCl, pH 7.5, 6 mM MgCl₂, 10 mM DTT, 10 mM NaCl, 2 mM spermidine, 2 mM NTPs, 8 units of RNase inhibitor, and 20 units of T7 RNA polymerase), and incubated for 2 h at 37°C. The RNA products were purified and dissolved in 100 mM HEPES, pH7.0, incubated for 5 min at 94°C, and left to stand at room temperature for 30 min to allow the annealing of the two RNA strands, which are used as dsRNA solutions.

References

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