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Supplementary Figure 1

Immunofluorescence analyses using an anti-myc antibody revealed that myctagged Loqs-PC expressed in S2 cells localized in large foci in the cytoplasm while other isoforms localized evenly in the cytosol. Immunofluorescence was performed by fixing S2 cells with 2% formaldehyde for 15 min. Cells were permeabilized using 0.1% Triton X-100. To visualize myc-tagged Loqs proteins, cells were stained with a monoclonal anti-c-myc antibody (9E10). Alexa Fluor 546-tagged anti-mouse IgG was used as a secondary antibody. Cells were also mounted with SlowFade Gold Antifade Reagents containing DAPI (Invitrogen). All images were collected using a Zeiss LSM510 laser scanning microscope.



Supplementary Figure 2

Loqs-PD depletion causes a severe defect in esiRNA production in S2 cells. (A) A schematic drawing showing the regions in the Loqs coding gene to which the dsRNAs for RNAi, ds-ALL, ds-RB+RC, ds-RC, ds-RC+RD, and ds-RD correspond. (B) Western blot analysis using anti-Loqs antibodies on S2 cell lysates after RNAi treatment shows the efficiency and the specificity of Loqs isoform depletion. ds-EGFP was employed as a negative control. (C) Northern blot analyses using DNA oligo probes specific for bantam miRNA, miR-8 and miR-2b show that depletion of Loqs-PA and Loqs-PB caused a severe defect in miRNA biogenesis. A probe for U6 snRNA was used as a loading control. (D) Northern blot analyses using DNA oligo probes specific for esiRNA-sl-1 and CG18854A-esiRNA show that depletion of Loqs-PD led to a severe defect in esiRNA biogenesis. DM1731-derived esiRNA (esiRNA-1731) was also affected by Loqs-PD RNAi. To detect esiRNA-1731 derived from a retrotransposon, we carried out 1-ethyl-3-[3-dimethylaminopropyl]carbodimide hydrochloride cross-linking reactions for 1 h at 60°C (Pall GS, Hamilton AJ. 2008. *Nat Protoc* **3**: 1077-1084). Probes used for detecting esiRNA-1731#2, 5'-CCG GTT CAT CAC TCT AGG ACG-3'; esiRNA-1731#3, 5'-GGT CGC CAT CTC GTT GCA CCT-3'; esiRNA-1731#4, 5'-TGC TCT GCT CGT TGA TCG GTT-3'; esiRNA-1731#5, 5'-GGC CAC GAT CAT CAT CAT CAC TCT AGG ACG-3'; esiRNA-1731#3, 5'-GGT CGC CAC GAT CAT CAT CAT CAT CAC TCT AGG ACG-3'; esiRNA-1731#3, 5'-GGT CGC CAC GAT CAT CAT CAT CAT CAC TCT AGG ACG-3'; esiRNA-1731#3, 5'-GGT CGC CAC GAT CAT CAT CAT CAT CAC TCT GAT CGT TGA TCG GTT-3'; esiRNA-1731#5, 5'-GGC CAC GAT CAT CAT CAT CAC TCT AGG ACG-3'; esiRNA-1731#3, 5'-GGT CGC CAC GAT CAT GAT CAT CAT CAC TCT GCT CGT TGA TCG GTT-3'; esiRNA-1731#4, 5'-TGC TCT GCT CGT TGA TCG GTT-3'; esiRNA-1731#4, 5'-TGC TCT GCT CGT TGA TCG GTT-3'; esiRNA-1731#4, 5'-TGC TCT GCT CAT CAC TCT GA CAG TGF CAC CAC GAT CAT CAT CAT CAC TCT GAC ATC TTT ACC TGA CAG TAT TA-3'; esiRNA-18854A, 5'-TCA TTT GAT CCA TAG TTT CCC GT-3'.



Supplementary Figure 3

The expression levels of Loqs isoforms vary among tissues. (A) Western blot analysis was performed on S2, testis and ovary lysates using anti-Loqs antibodies. (B) RNA expression from each Loqs isoform was measured by quantitative RT-PCR in S2, testes and ovaries. Total RNAs isolated from S2, testes and ovaries were treated with DNase I and cDNAs were synthesized using Transcriptor Reverse Transcriptase (Roch) and oligo-dT primer. PCR were conducted with LightCycler 480 (Roch) and SYBR Premix Ex Taq (TaKaRa). The primer sequences used are; 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and TGT TCT TCA AGC AAG TCT TTT CGC CC for Loqs-RA; 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-ATT TTC ACT ACT GCG GGG TTC GC-3' for Loqs-RB; 5'-TGC AGG AGA CTC CCA TCG AAT GGT GAG TTG AA-3' for Loqs-RC; 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC AGG AGA CTC CCA TCG ATT CG-3' and 5'-TGC TCG TAA CGA TCG ATG TCT TGA ATG-3' for Loqs-RD.