Supplementary Methods

Ovary extract preparation and sucrose gradient fractionation

Ovaries from 100 females, fed on yeast for 4 days, were dissected in ice-cold PBS. Extra PBS was removed from the tube and ovaries were snap frozen in liquid nitrogen and stored at -80°C. To prepare ovary extracts, approximately 400 µl of frozen ovaries were homogenized on ice in 1 ml of polysome buffer (PB) containing 250 mM NaCl, 25 mM magnesium acetate, 50 mM Tris HCl, pH 7.5, 2.5 mg/ml heparin, 2 mM DTT, 0.2% Triton, 1 mg/ml cycloheximide, 1x EDTA-free Complete protease inhibitor (Roche) and 50 U/ml RNasin (Promega). Homogenates were cleared by centrifugation at 4°C for 10 minutes at 16000 x g and the supernatant was collected for density centrifugation. For EDTA treatment, the supernatant was treated with 50 mM EDTA on ice for 5 minutes before it was layered onto the sucrose gradient.

After measuring the A_{254} ultraviolet (u.v.) absorbance of the cleared supernatant, 700 µl of ovary extracts containing 25 OD units was layered onto an 11 ml 10-50% sucrose gradient containing 250 mM NaCl, 25 mM magnesium acetate and 50 mM Tris HCl, pH7.5. The EDTA-treated supernatant was layered onto a gradient containing 10 mM EDTA in place of magnesium acetate. Gradients were centrifuged in a Beckman SW41 Ti rotor at 36000 rpm for 3.5 hours at 4°C. After centrifugation, thirteen equivalent volume fractions (900 µl each, fractions 1 to 13) were manually collected from each gradient. Since the gradient pellet has been shown to contain very large polysomes (Clark et al., 2000), we solubilized it with 900 µl PB and treated it as an equivalent fraction (fraction 14). In total, 14 fractions were collected per gradient. In addition, 850 µl PB was added into 50 µl of the previously saved supernatant to make the lysate sample (L).

Ovary lysate preparation and immunoprecipitation

Ovaries from 50 females, fed on yeast for 4 days, were dissected in ice-cold PBS. Extra PBS was removed from the tube and ovaries were homogenized on ice in 150 µl of immunoprecipation buffer (IB) containing 150 mM NaCl, 50 mM Tris HCl, pH 7.5, 1 mM EDTA, pH8.0, 0.4% NP-40, 0.1% Triton, 1x EDTA-free Complete protease inhibitor (Roche), 1x PhosSTOP phosphatase inhibitor (Roche) and 40 U/ml SUPERase• In™ RNase Inhibitor (Ambion). Homogenates were cleared by centrifugation at 4°C for 10 minutes at 16000 x g and after three repeats 350 µl of supernatant was collected for IP. 100 µl of ovary lysate was added onto 200 µl of IP mix containing pre-cleared antibody-bound Protein G Sepharose 4 Fast Flow beads (GE Healthcare) and incubated for 8 hours at 4°C. Beads were rinsed 4x in cold IB before RNA extraction. RNA extracted from 10 µl of ovary lysate was used as input.

List of PCR primers

The following PCR primers were used to amplify the genomic DNA at the *eIF1A* locus and sequence the *eIF1A* coding sequence: 5' PCR primer for *eIF1A* genomic region 1, ACATGTTATAATTTAAGATTCAATCGCT; 3' PCR primer for *eIF1A* genomic region 1, AGCCACCTTAGAGAGGGGC; 5' PCR primer for *eIF1A* genomic region 2, CAGAATTACTCACAACACAAAAGAAAC; 3' PCR primer for *eIF1A* genomic region 2, CGCTAGCTAACCTCACATCTCG; 2nd forward sequencing primer for *eIF1A* genomic region 2, GCAGATAACAATGTTTATTTACGGATT; 2nd reverse sequencing primer for *eIF1A* genomic region 2, TTATTAGTTTATGTTTCGCAATGTTCC.

The following PCR primers and UPL probes were used to perform the qPCR analysis: *grk* 5' primer, CCAATGATGCAAATCCCATT; *grk* 3' primer, GCGTATGCTCTCGGAGAAGT; UPL probe ID, #152 (cat. no. 04694384001, Roche). *αTub67C* 5' primer, CACCCAATTTTAGTCCAGCAA; *αTub67C* 3' primer, CAGGTACAGCTCCCAGCAG;

UPL probe ID, #58 (cat. no. 04688554001, Roche). *RpL32* 5' primer, CGGATCGATATGCTAAGCTGT; *RpL32* 3' primer, CGACGCACTCTGTTGTCG; UPL probe ID, #117 (cat. no. 04693515001, Roche).

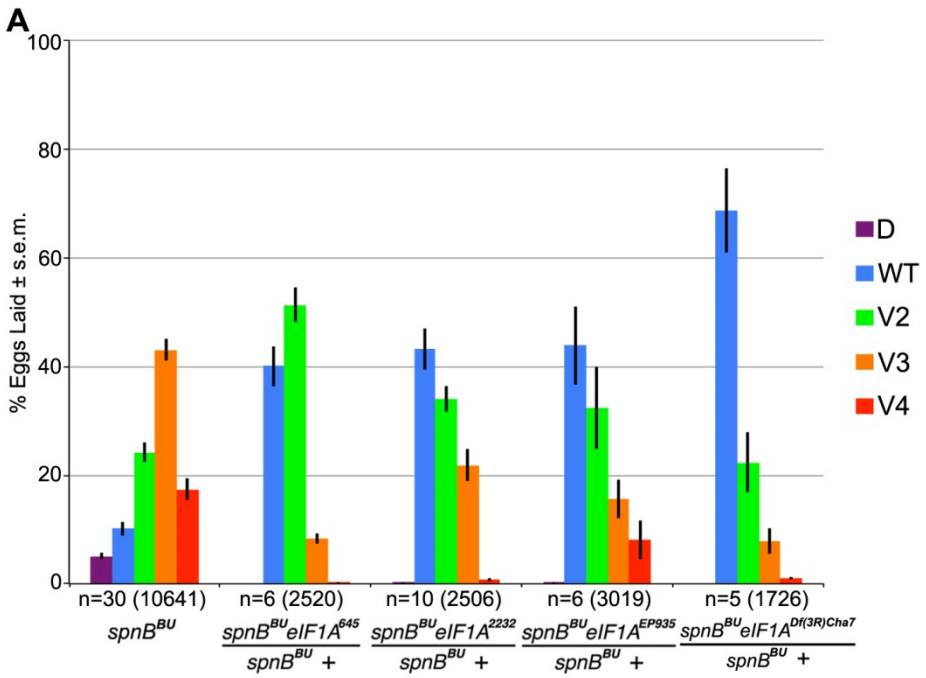

Fig. S1. Additional eggshell phenotype analyses. (**A**) Suppression of the DV patterning defects by other *eIF1A* alleles in *spnB*^{BU} mutants. Percentages of eggs showing different categories of the eggshell phenotype were plotted for each genotype. Error bars represent the standard error between the indicated numbers (n) of independent experiments. Numbers in the parentheses indicate the total number of eggs counted per genotype.

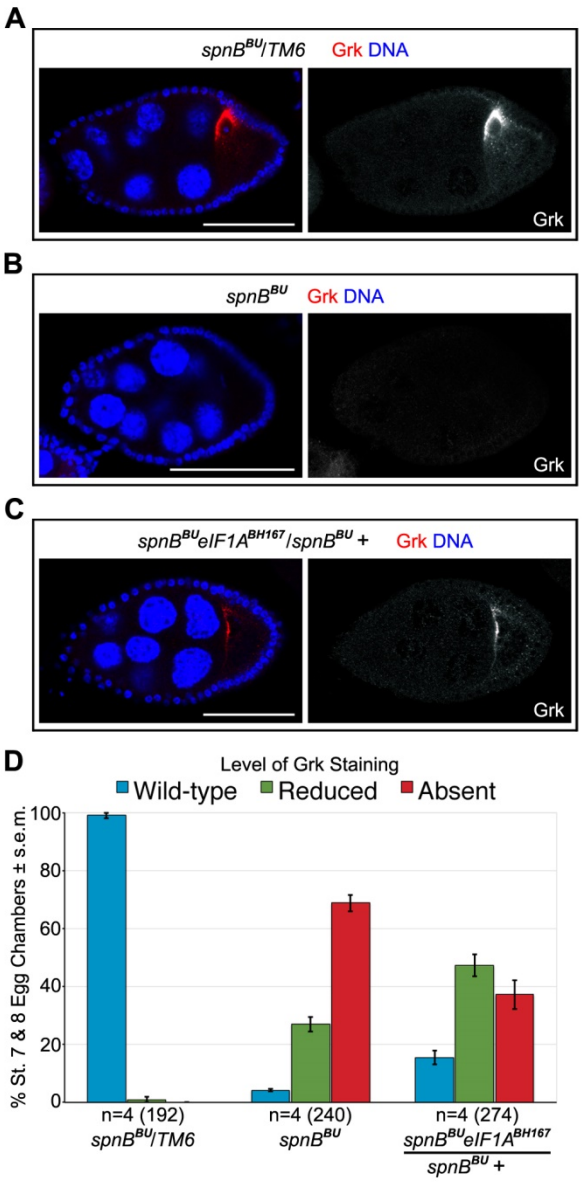

Fig. S2. The *eIF1A*^{BH167} mutation restores the level of Grk protein accumulation in the *spnB* mutant egg chambers during the early stages of mid-oogenesis. (**A-C**) Stage 7/8 egg chambers with Grk stained in red in and DNA in blue. Scale bars: 50 µm. (**D**) Percentage of stage 7 and 8 egg chambers showing different levels of Grk staining in the oocyte. Error bars represent the standard error between the indicated numbers (n) of independent experiments. Numbers in parentheses indicate the total number of egg chambers examined per genotype.

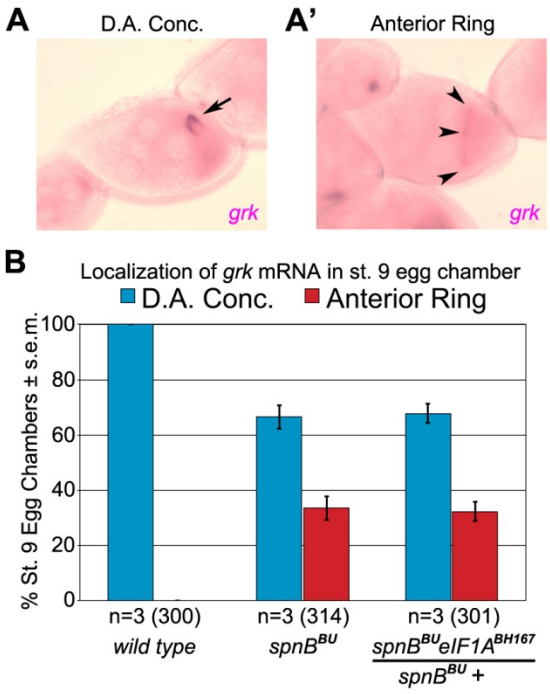

Fig. S3. *eIF1A*^{BH167} heterozygosity does not suppress the *grk* mRNA localization defects in *spnB* mutants. (**A**) RNA *in situ* images showing either dorsoanterior concentration (D.A. Conc., arrow) or anterior ring (arrowheads) localization of *grk* mRNA in stage 9 *spnB*^{BU}*eIF1A*^{BH167} egg chambers. (**B**) Percentage of stage 9 egg chambers with *grk* mRNA either concentrated at the DA corner (D.A. Conc.) or localized in a ring on the anterior margin of the oocyte (Anterior Ring). Error bars represent the standard error between three independent experiments. The numbers in parentheses indicate the total number of egg chambers examined per genotype.

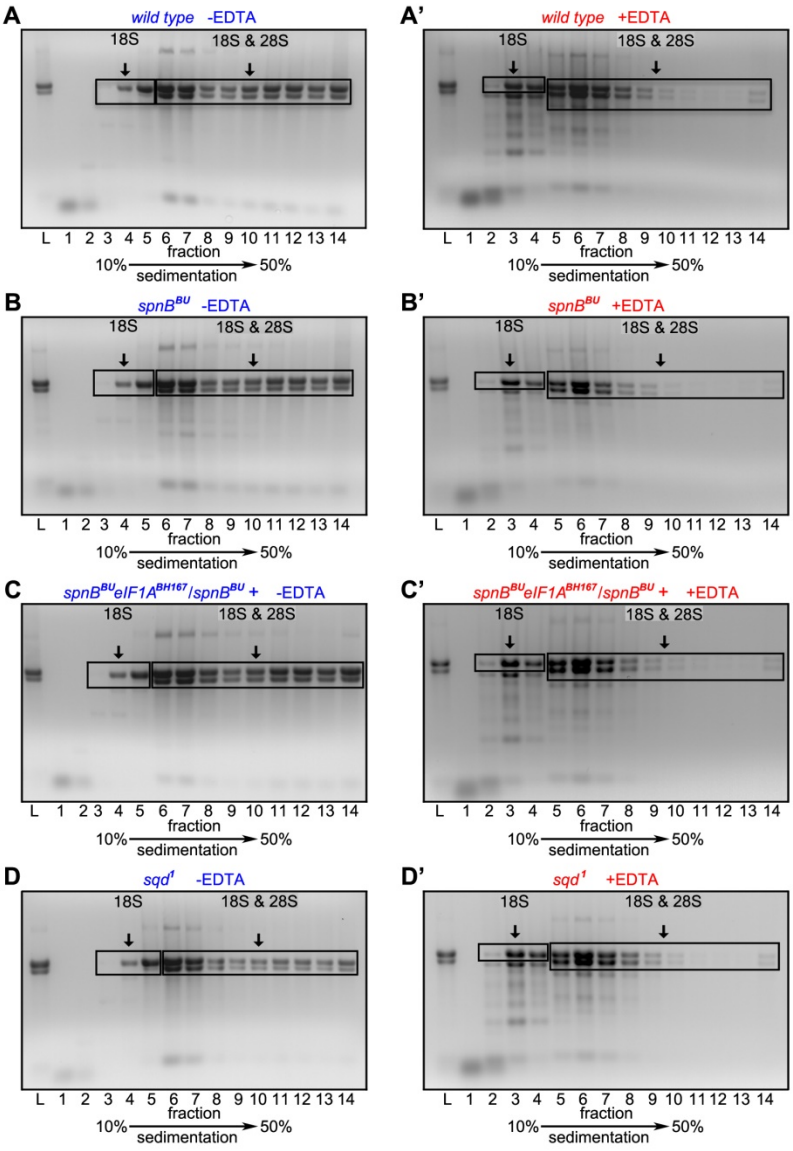

Fig. S4. Distribution of rRNA in the fraction samples. Ethidium bromide staining of agarose gels containing RNA extracted from individual fractions of the wild-type (**A**), *spnB*^{BU} (**B**), *spnB*^{BU}*eIF1A*^{BH167} (**C**), and *sqd*^{1} (**D**) gradients without (**A**, **B**, **C**, **D**) or with (**A'**, **B'**, **C'**, **D'**) EDTA treatment. Representative gel images are shown. Fractions containing 18S rRNA and 28S rRNA are indicated. Polysomes were completely disrupted by EDTA, as shown by the prominent reduction of 18S and 28S rRNA staining in the heavy fractions (fractions 10 to 14) of the EDTA-treated gradients.

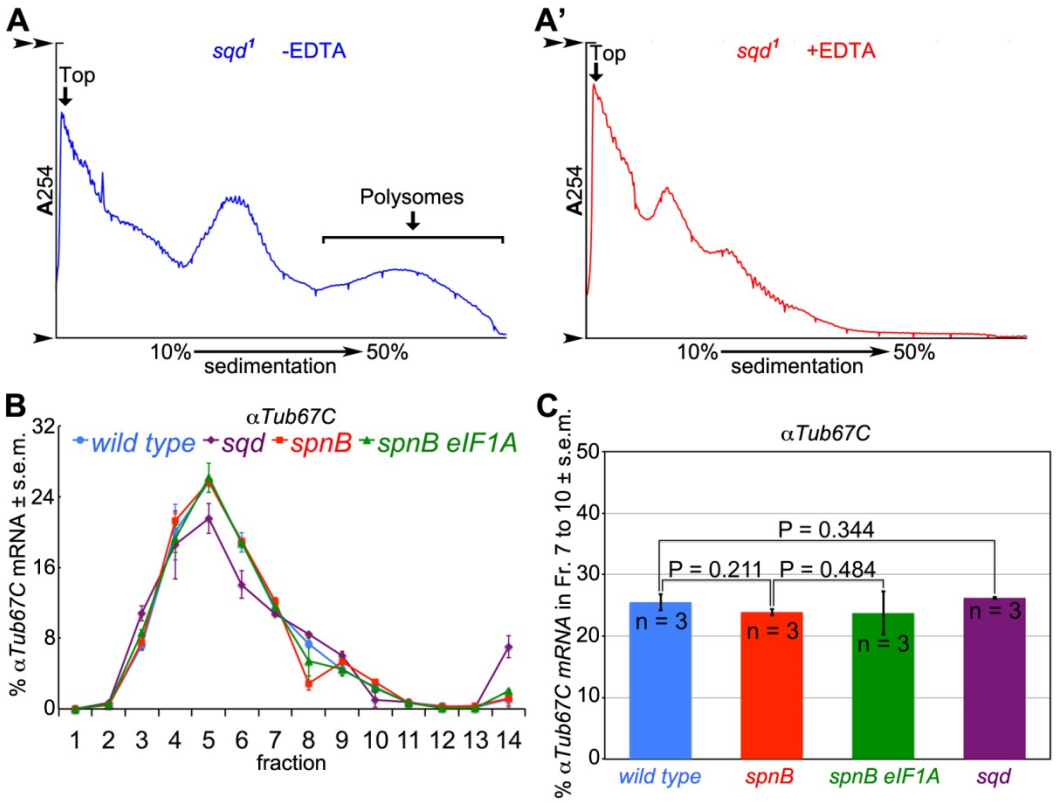

Fig. S5. (**A**) Representative UV absorbance profiles of the *sqd*^{1} gradients without (**A**) or with (**A'**) EDTA treatment. (**B**) The distribution profile of α*Tub67C* mRNA in EDTA-treated wild-type (blue circles, *wild type*), *spnB*^{BU} (red squares, *spnB*), *spnB*^{BU} *eIF1A*^{BH167}(green triangles, *spnB eIF1A*), and *sqd*^{1} (purple diamonds, *sqd*) gradients. Data are plotted as the percentage of total mRNA in the gradient detected in each fraction. (**C**) Comparison of the amount of *αTub67C* mRNA in the heavier intermediate fractions (fractions 7 to 10) of the EDTA-treated gradients. Error bars represent standard error from three independent experiments. P values in the figure were generated by two-sample one-tail heteroscedastic t-tests.