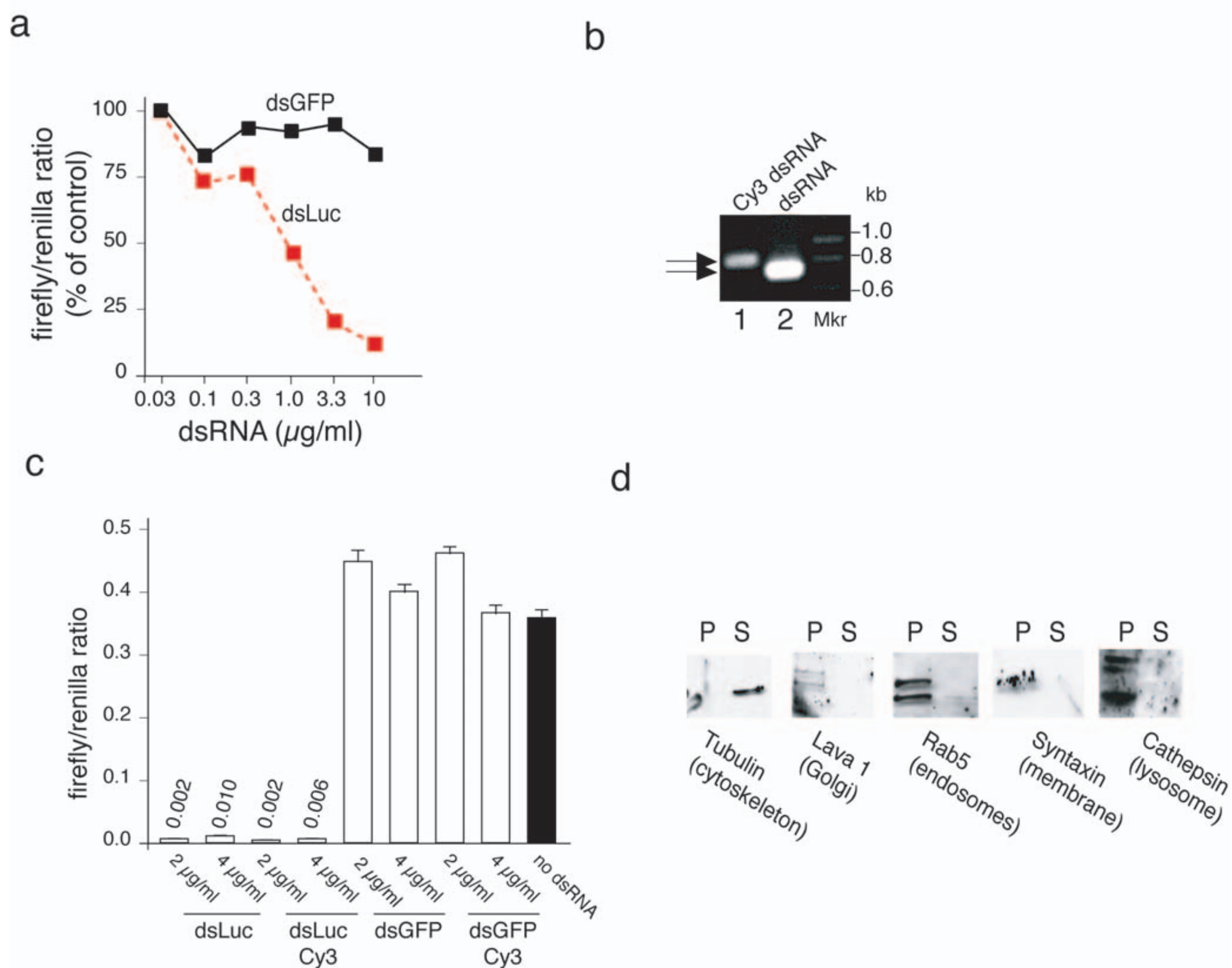

Figure S1 **(a)** Concentration dependent RNAi in *Drosophila* S2 cells. S2 cells were transfected with expression plasmids encoding firefly and *Renilla* luciferase. Different concentrations of specific dsRNA targeting firefly luciferase was added to the culture supernatant at one day after transfection. Luciferase activity was monitored 48 hours later. Firefly over *Renilla* luciferase ratios were expressed as percentage of control transfection without dsRNA treatment. **(b)** Cy3 labeled dsRNA is taken up by *Drosophila* S2 cells and functional in RNAi. Analysis of unlabeled and Cy3-labeled dsRNA on agarose gel. Reduced mobility of Cy3-labeled dsRNA indicates that all dsRNA molecules in the preparation are labeled. **(c)** Functionality of Cy3-labeled dsRNA. S2 cells were transfected with plasmids encoding firefly and *Renilla* luciferase. 24 hours after transfection, the cells were soaked in medium containing unlabeled or Cy3-labeled luciferase specific dsRNA with the indicated concentrations, or in non-specific dsRNA (targeting GFP). Cy3-labeled dsRNA is functional in RNAi, although it is less efficient than unlabeled dsRNA in suppressing luciferase activity. Average firefly/*Renilla* ratio is indicated on top of each bar. The asterisk indicate $P<0.01$ respect to no dsRNA control. **(d)** Characterization of pellet and supernatant fraction after ultracentrifugation of S2 cell lysates at 100.000xg. Western blot analysis of pellet and supernatant indicate an enrichment of membranes, endocytic vesicles and golgi complex in the pellet (P) and the presence of a cytoskeletal marker in supernatant (S) fraction.

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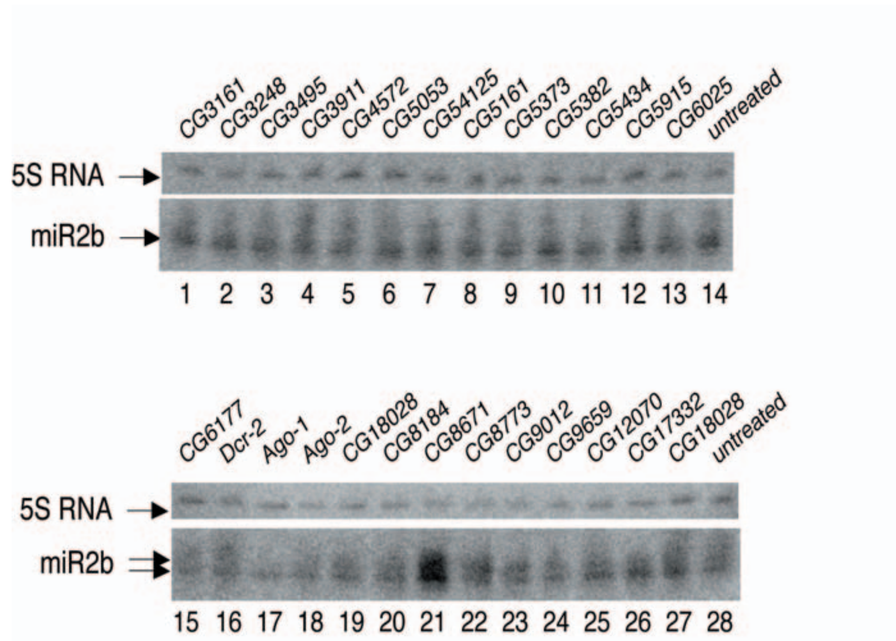

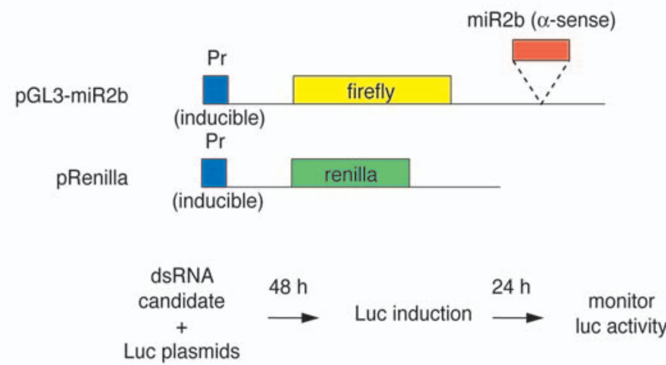

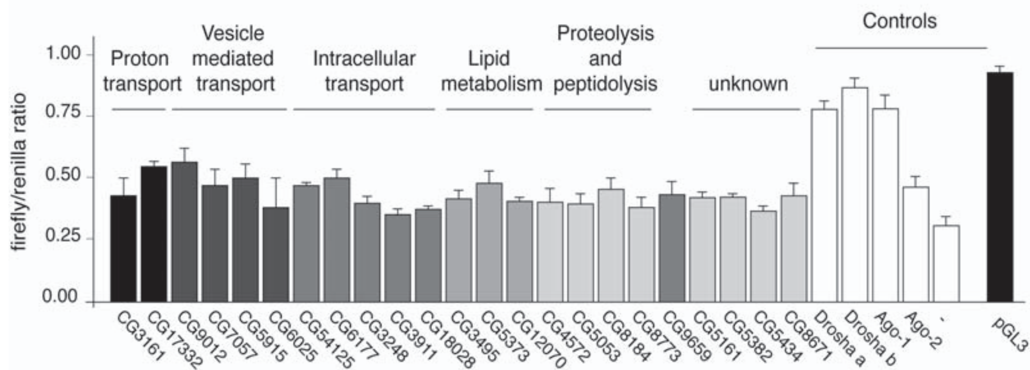

Figure S2 Genes identified in screen for novel components in the RNAi pathway do not affect miRNA biogenesis. **(a)** Detection of miR2b in cells treated with dsRNA targeting the indicated genes for 4 days. Mature miRNA and 5S rRNA are indicated by arrows. None of the candidate RNAi genes affect miRNA biogenesis. **(b)** Assay for miRNA-mediated RNA silencing. Schematic representation of genes encoding *Renilla* and firefly luciferase. The firefly luciferase mRNA contains two copies of a sequence that is perfectly complementary to mature miR2b inserted at the 3′UTR. S2 cells were co-transfected with dsRNA specific for candidate gene and the expression plasmids. Forty eight hours after transfection luciferase expression was induced by adding $CuSO_4$ to the culture supernatant and luciferase activity was monitored after another 24 hour incubation. Controls include treatment of cells with dsRNA targeting genes known to be involved in the miRNA pathway, *Drosha* (two different, non-overlapping dsRNA preparations) and *Argonaute 1*. The bar labeled pGL3 indicates expression of firefly luciferase from a control plasmid without miR2b complementary sequences. The asterisk indicate $P<0.01$ with respect to pGL3 control.

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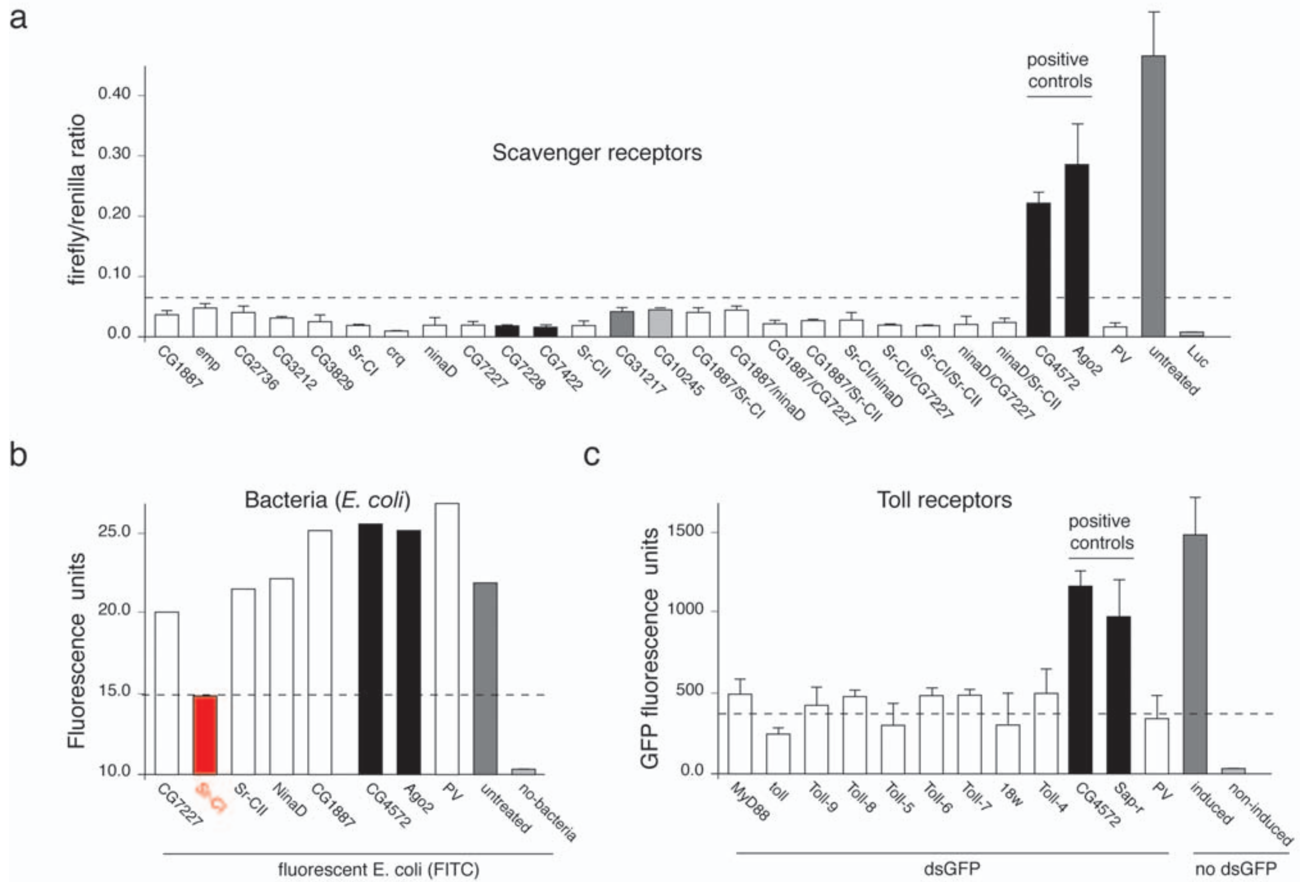

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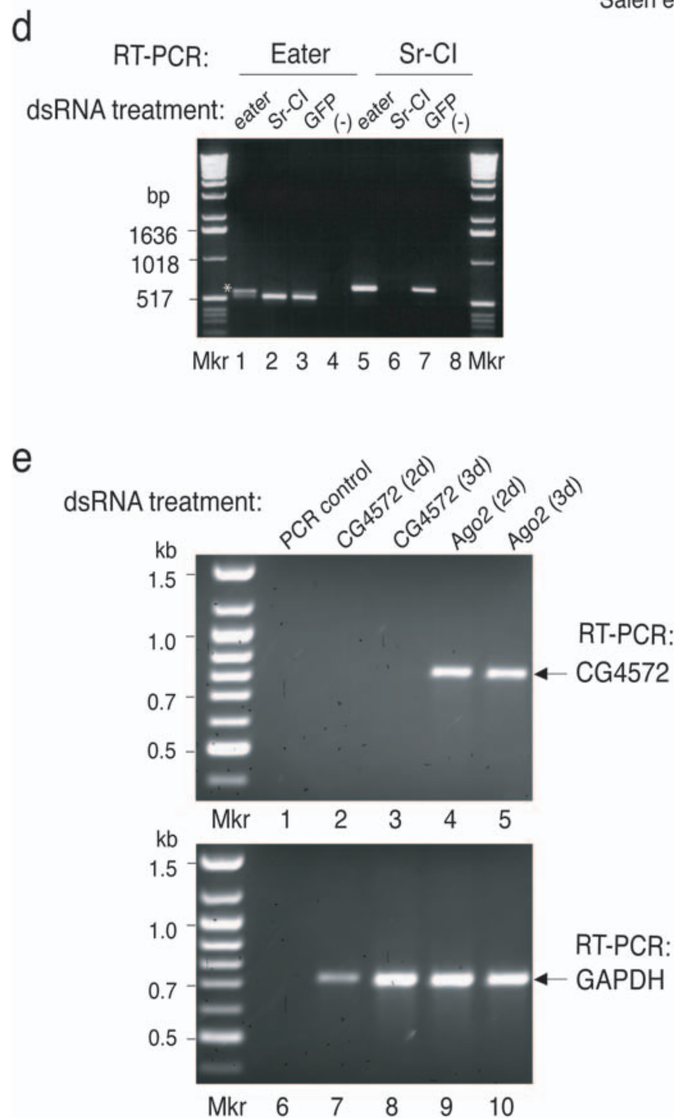

Figure S3 Knockdown of Pattern-Recognition receptors fails to inhibit RNA silencing **(a)** Knockdown of scanvenger receptors does not impair silencing of luciferase. S2 cells were cotransfected with dsRNA specific for different pattern recognition receptors and firefly and *Renilla* luciferase plasmids. After two days, cells were fed with dsRNA specific for firefly luciferase. Luciferase expression was induced by addition of CuSO4. Data are presented as the ratio between firefly and *Renilla* activity. Poliovirus (PV) specific dsRNA was used as a negative control. Positive controls include two candidates identified in our screen to be involved in RNAi. The asterisk indicate $P<0.01$ with respect to untreated control. **(b)** Knockdown of scavenger receptor *Sr-CI* impairs phagocytosis of bacteria in S2 cells. S2 cells were fed with dsRNA specific for scavenger receptors for three days. Cells were then serum starved for 90 min, incubated with FITC-labelled *E. coli* for 20 min, washed with PBS and fluorescence was measured by flow cytometry. As described previously (M. Ramet *et al.* Immunity 2001 15: 1027-1038), phagocytosis of *E. coli* was inhibited after Sr-CI knockdown. This experiment serves as a positive control for knockdown of Sr-CI in a functional assay. **(c)** Knockdown of Toll receptors fails to inhibit RNA silencing of luciferase expression. GFP:Relish S2 cells were treated with dsRNA specific for toll family members and three days later, cells were soaked in dsRNA targeting GFP. After induction of GFP expression by addition of CuSO4, GFP expression in cytoplasmic extract was monitored in a Tecan Safire plate reader. Poliovirus (PV) specific dsRNA was used as a negative control. Positive controls include two candidates identified in our screen to be involved in RNAi. The asterisk indicate $P<0.01$ with respect to no dsGFP control. **(d)** Scavenger receptors mRNA levels are reduced after RNAi. S2 cells were treated with dsRNA specific for the scavenger receptors Eater, and Sr-CI or for GFP as a negative control. After three days, poly-A RNA was extracted, reverse transcribed and amplified using primers for Eater (lanes 1 to 4) or Sr-CI (lanes 5 to 8). Lane 4 and 8 represent negative controls for PCR in the absence of input cDNA. While Sr-CI mRNA is undetectable after dsRNA treatment (lane 6), Eater mRNA quantity was reduced by 70% (lane 1). The band indicated by an asterisk in lane 1 corresponds to the amplification of genomic DNA contamination in the RNA preparation as denoted by the slower mobility of the band since PCR primers are flanking a 55 bp intron and should yield a larger band than that derived from cDNA. **(e)** dsRNA treatment induce mRNA knockdown within 3 days of treatment. S2 cells were fed with dsRNA specific for CG4572 or Ago2. After 2 or 3 days of treatment, poly-A RNA was extracted, reverse transcribed and amplified using primers for CG4572 (lane 1-5) and for internal control GAPDH (lane 6-10). CG4572, a gene identified in our screen to be involved in RNAi, is undetectable after incubation of S2 cells with dsRNA for as little as two days.

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Table S1 Scavenger receptor-like genes identified in Drosophila melanogaster

Gene ID	Symbol	Gene name
CG4099	Sr-CI	Scavenger receptor class C, type I
CG8856	Sr-CII	Scavenger receptor class C, type II
CG31962	Sr-CIII	Scavenger receptor class C, type III
CG3212	Sr-CIV	Scavenger receptor class C, type IV
CG10345		
CG12789		
CG1887		
CG2736		
CG31217		
CG31741		
CG3829		
CG7422		
CG7000		
CG7227		
CG7228		
CG4280	crq	croquemort
CG2727	emp	epithelial membrane protein
CG31783	ninaD	neither inactivation nor afterpotential D
FBgn0082595	eater	

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