Supplementary Tables

Supplementary Table 1

Estimated copy numbers of individual miRNA per cell

microRNA	Tissue	Average copies per cell
mir-122	mouse liver	21,000
	rat liver	26,000
	monkey liver	31,000
mir-16	mouse liver	1,400
mir-26b	mouse liver	930
let-7a	mouse liver	780

The average copy numbers of indicated miRNAs per liver cell were deduced by measuring miRNA copies in 10 ng of purified total liver RNA and assuming that each cell contains 20 pg of RNA. The copy numbers of the miRNAs in total RNAs were determined using respective Taqman miRNA assay kits and synthetic miRNA oligonucleotides as the standard. The numbers shown are the average from 4 individual animals. The quantifications for mir-122 and mir-16 in mouse liver RNA were performed twice.

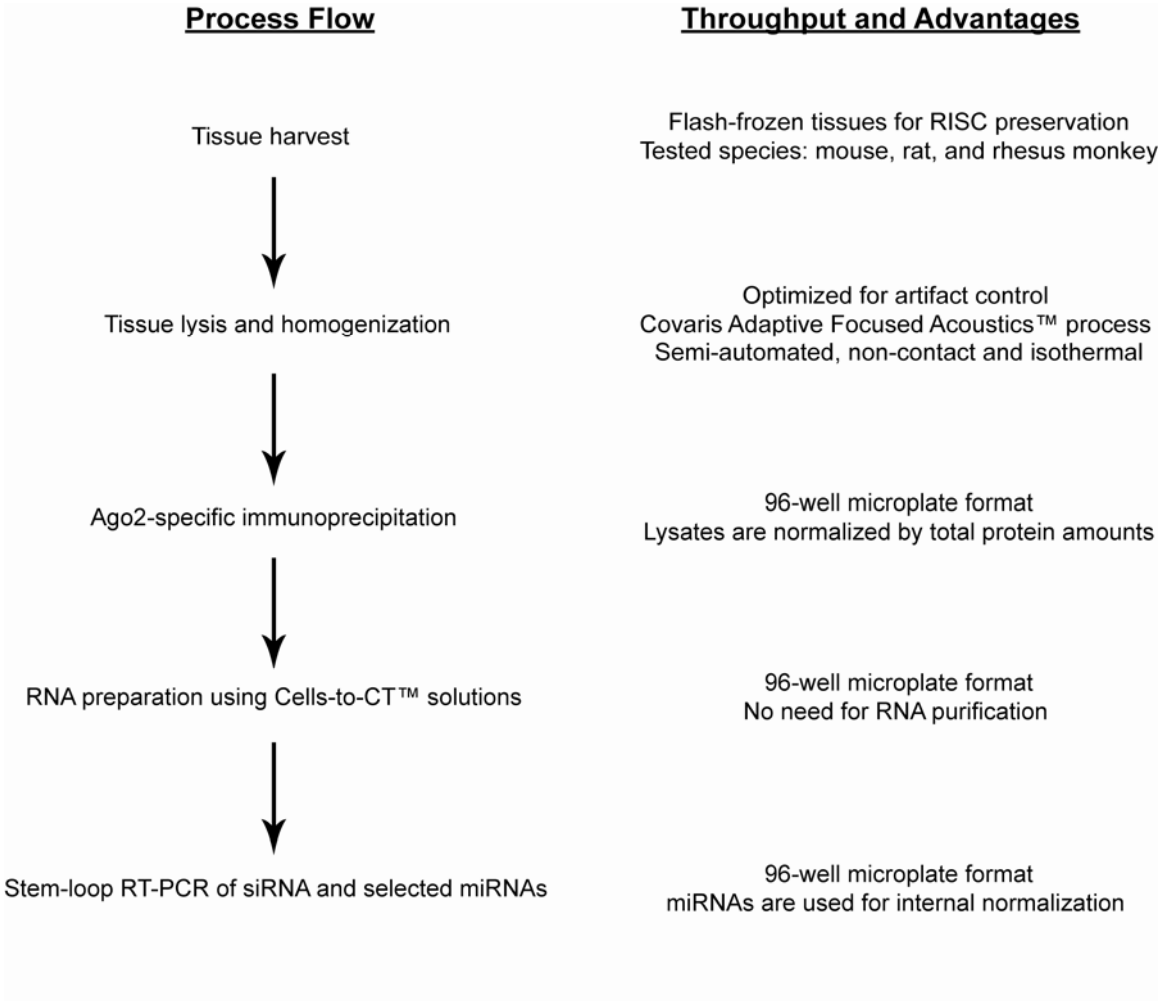
Supplementary Table 2

Copy numbers of siRNA guide strand per liver cell for selected time/dose points

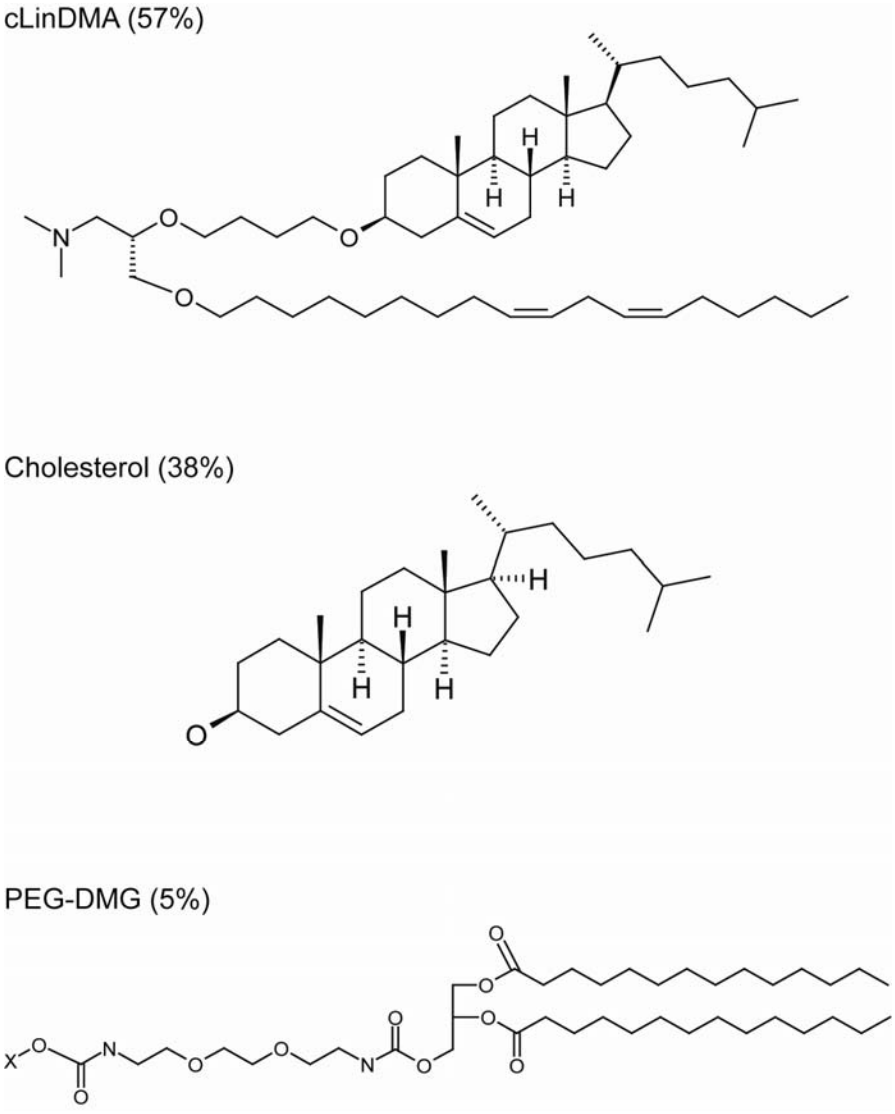
	Day 1, 3.6 mg/kg	Day 14, 1.2 mg/kg	Day 21, 3.6 mg/kg
a: Average ratio			
Guide/mir-122	0.86	0.011	0.024
Guide/mir-16	5.7	0.065	0.16
Guide/mir-26b	20	0.26	0.53
Guide/let-7a	22	0.35	0.73
b: Copy numbers			
From mir-122	18,000	230	500

The copy numbers of siRNA guide strand in a liver cell were calculated via two steps: first the ratios of the guide strand to mir-122 in RISC were determined as described in Fig. 3 and are shown in section ***a***. Then the copy numbers of the guide strand per liver cell (shown in ***b***) were estimated by multiplying the ratios to the copy number of mir-122 in a liver cell, which is 21,000 (Supplementary Table 1). For the copy numbers of siRNA guide stand required for 50% silencing, the mean of the groups of day 14, 1.2 mg/kg and day 21, 3.6 mg/kg was used ((230+500)/2).

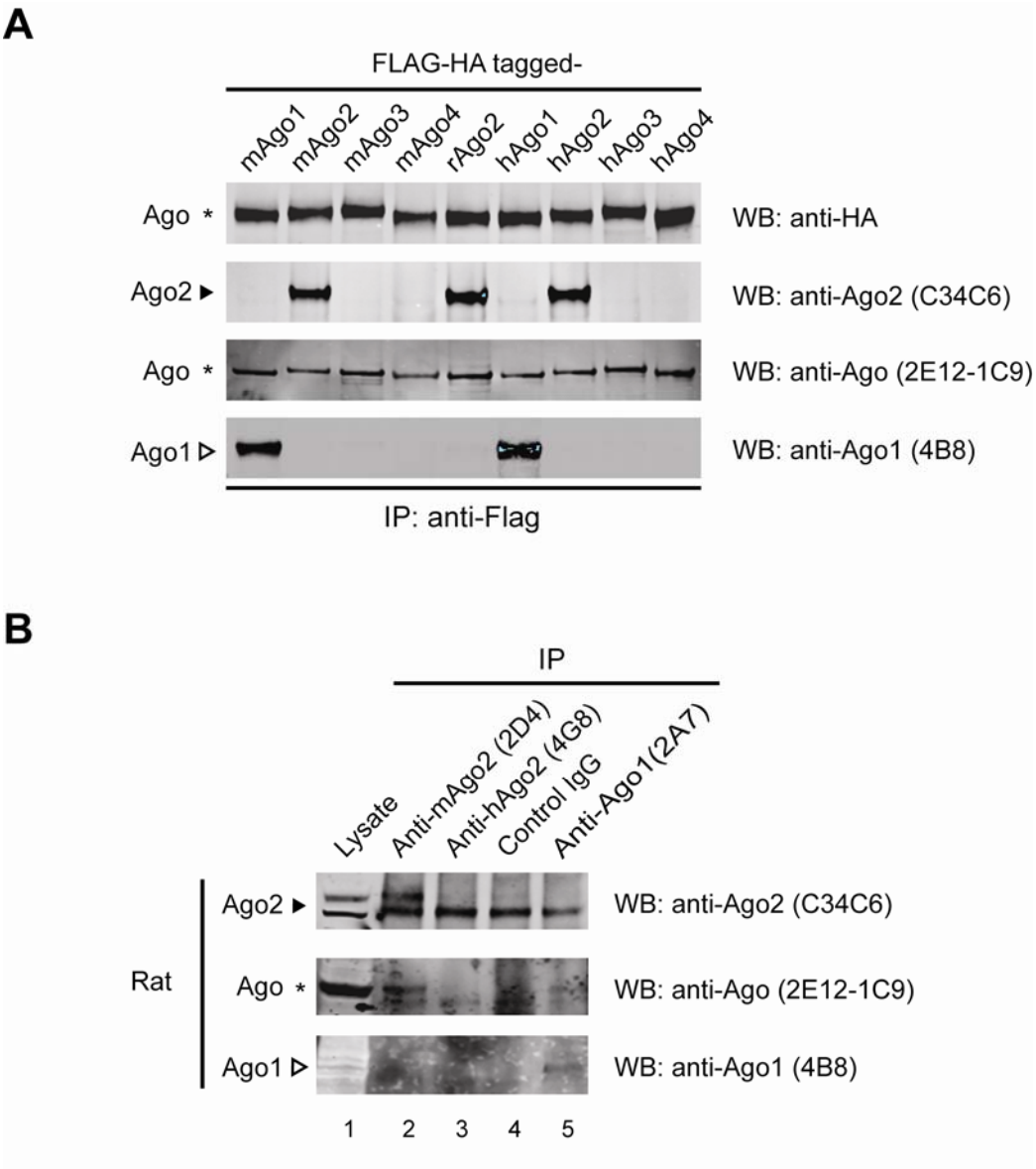
Supplementary Figures



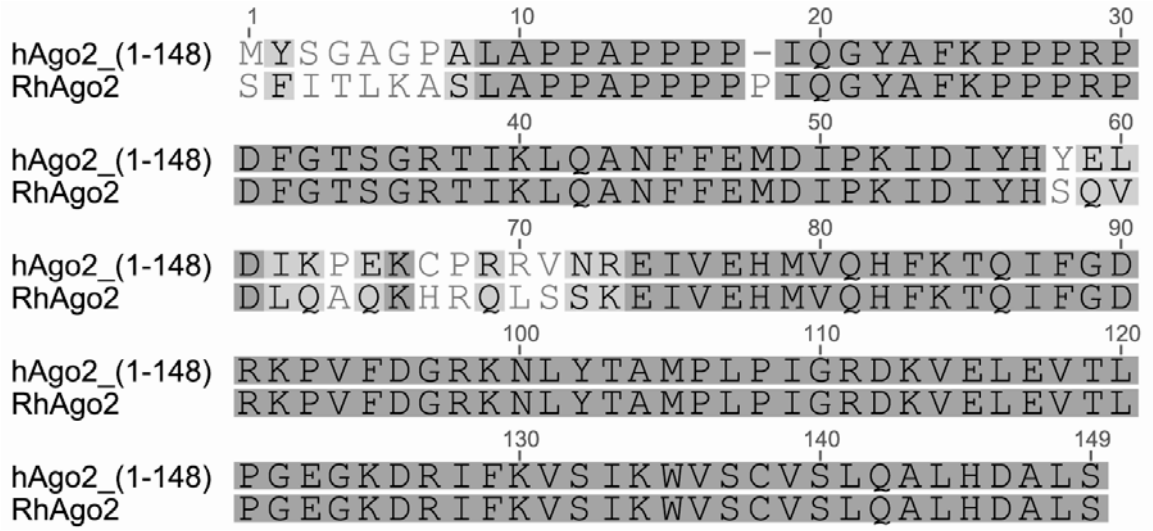
Supplementary Figure 1. Scheme and noteworthy features of the Ago-associated small RNA quantification procedure.



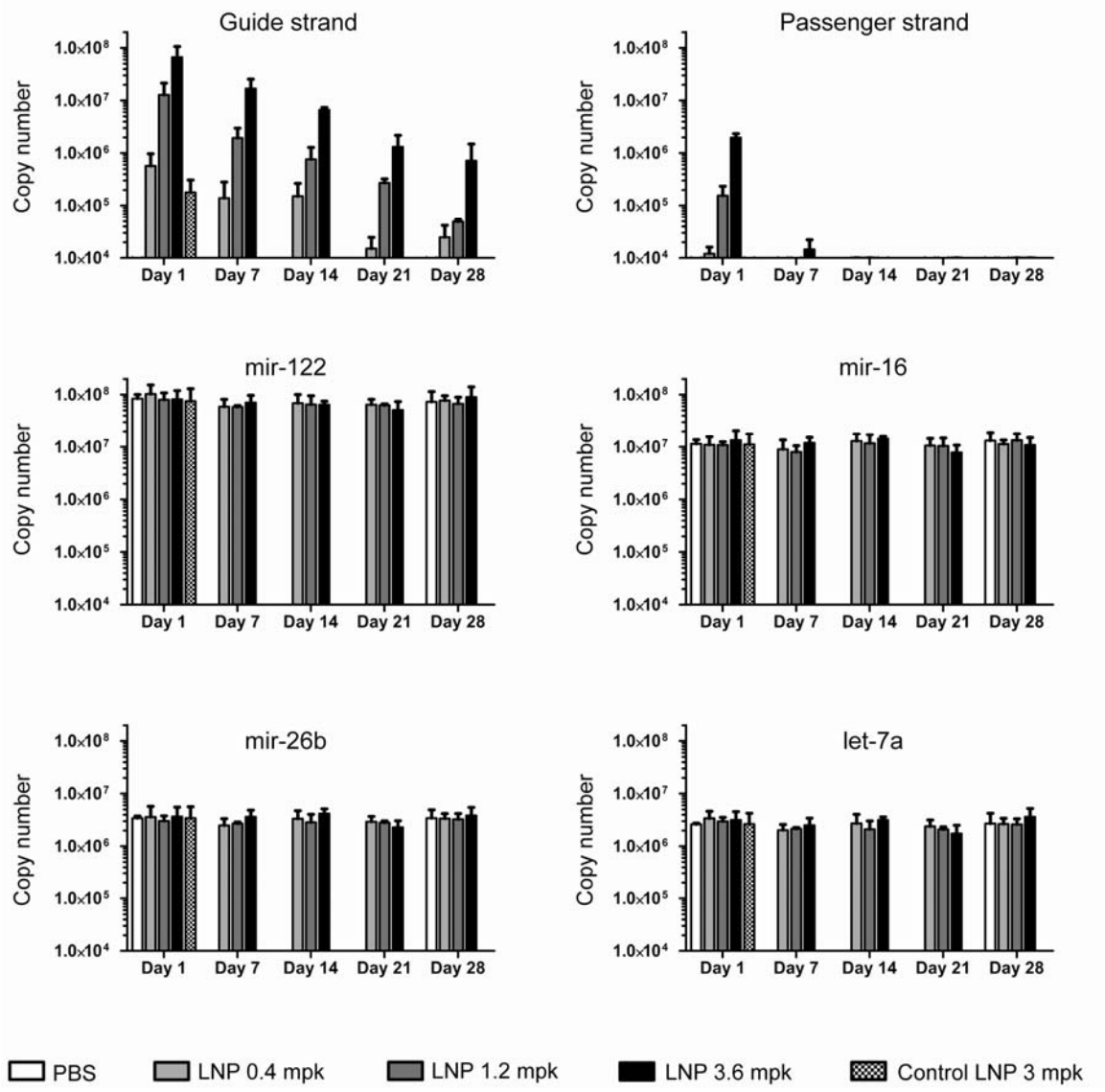
Supplementary Figure 2. Structure and ratio of the lipid components of LNP201b formulation.



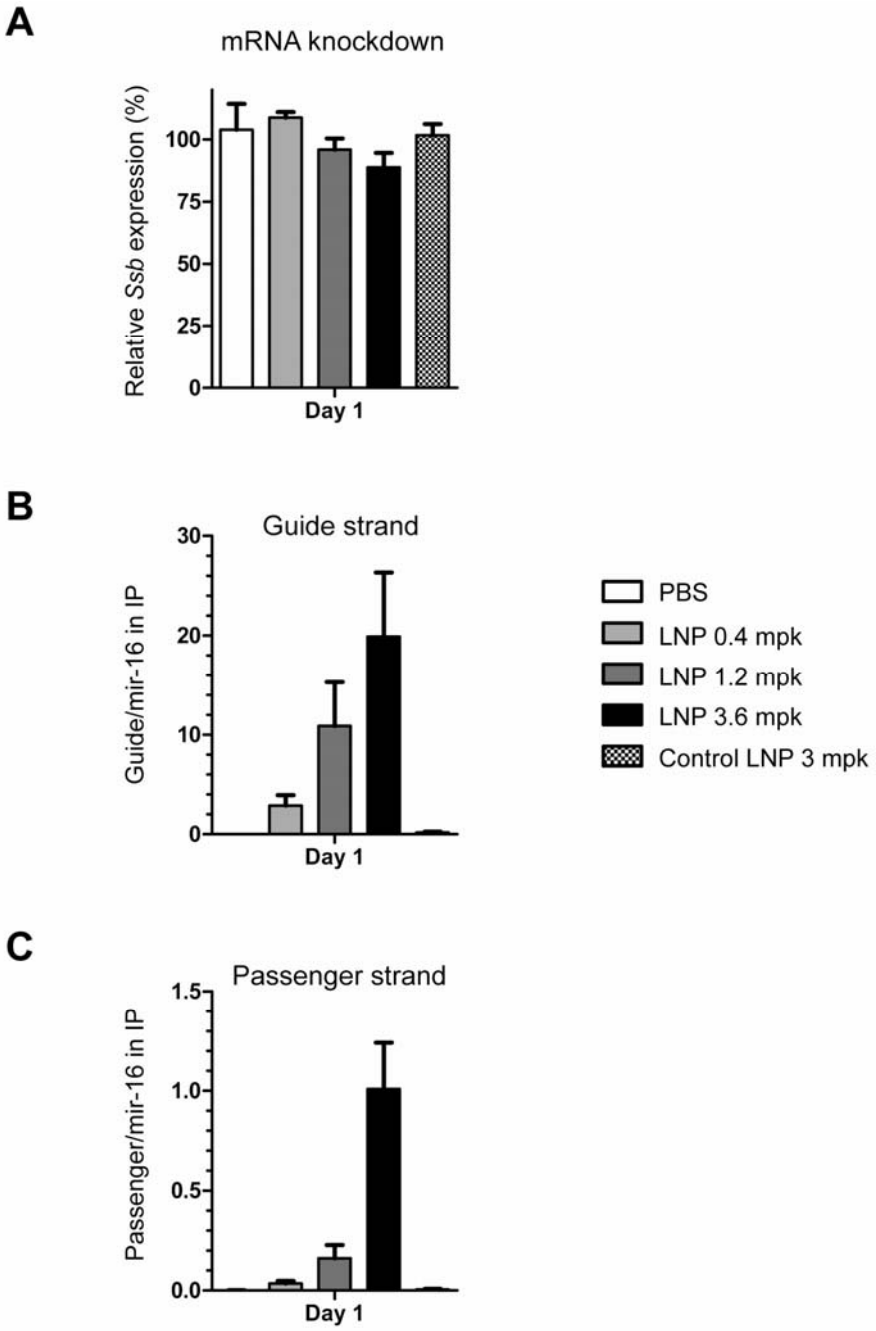
Supplementary Figure 3. Characterization of Ago-specific antibodies. (*A*) Specificity of the antibodies for Western blot analysis of overexpressed Ago proteins. Indicated rodent and human Ago proteins with N-terminal Flag-HA tags were transiently expressed in HEK 293T cells, immunoprecipitated with the anti-Flag antibody and detected in Western blot using indicated antibodies. (*B*) Effectiveness of the indicated antibodies for immunoprecipitation in rat total liver lysates. Lysates (30 µg) were loaded as input. Antibodies used for Western blot after immunoprecipitation are indicated to the *right* of individual blots. Solid arrowhead, hollow arrowhead and asterisk denote Ago2, Ago1, and Ago protein members, respectively.



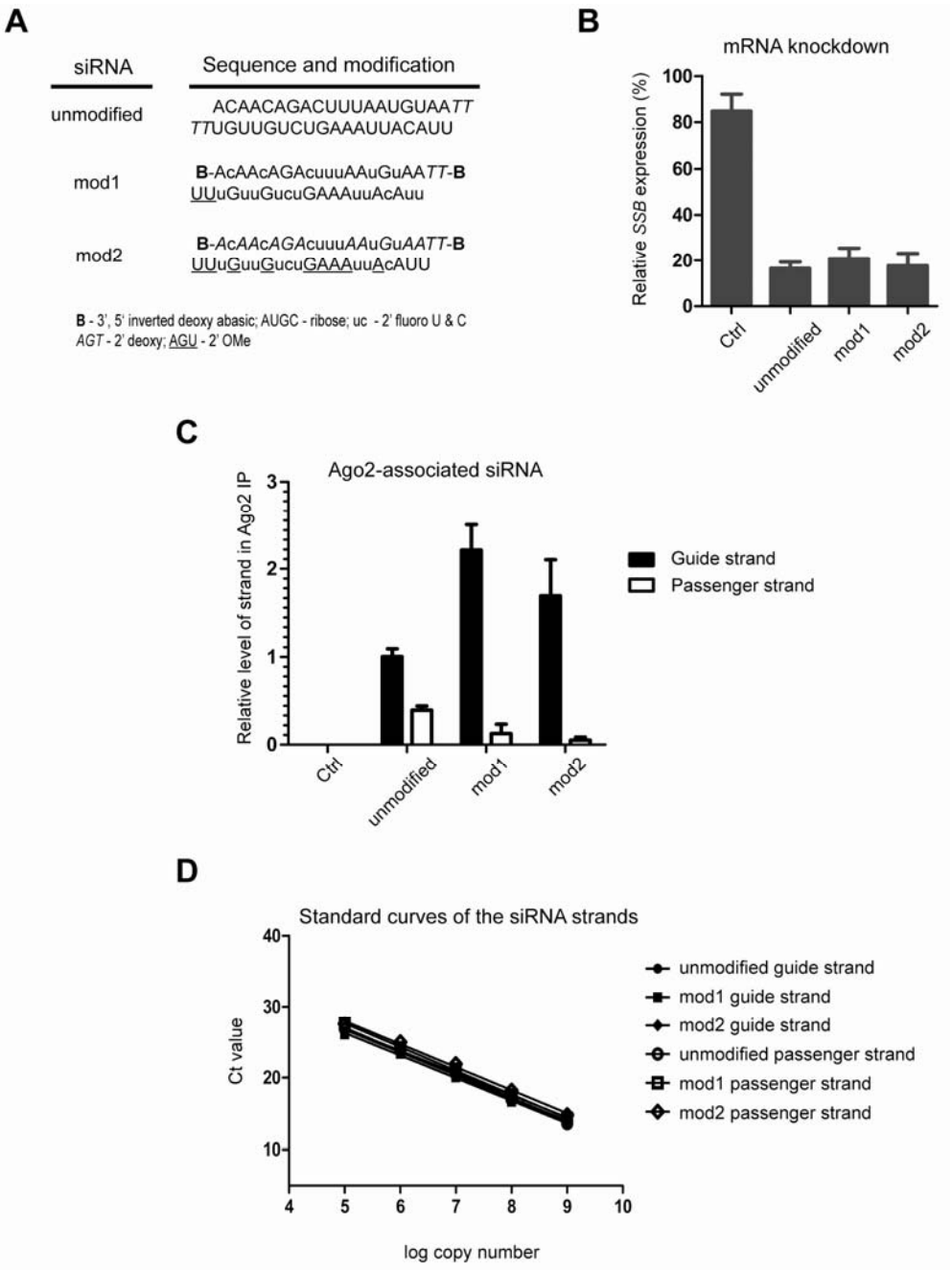
Supplementary Figure 4. Sequence alignment of the N-terminal portion of human Ago2 (hAgo2(1-148)) and a fragment of the putative rhesus monkey Ago2 (RhAgo2) (accession no. XP_001100725). The residues are numbered according to hAgo2. Identical and conserved amino acids are indicated in deep and light shade respectively.



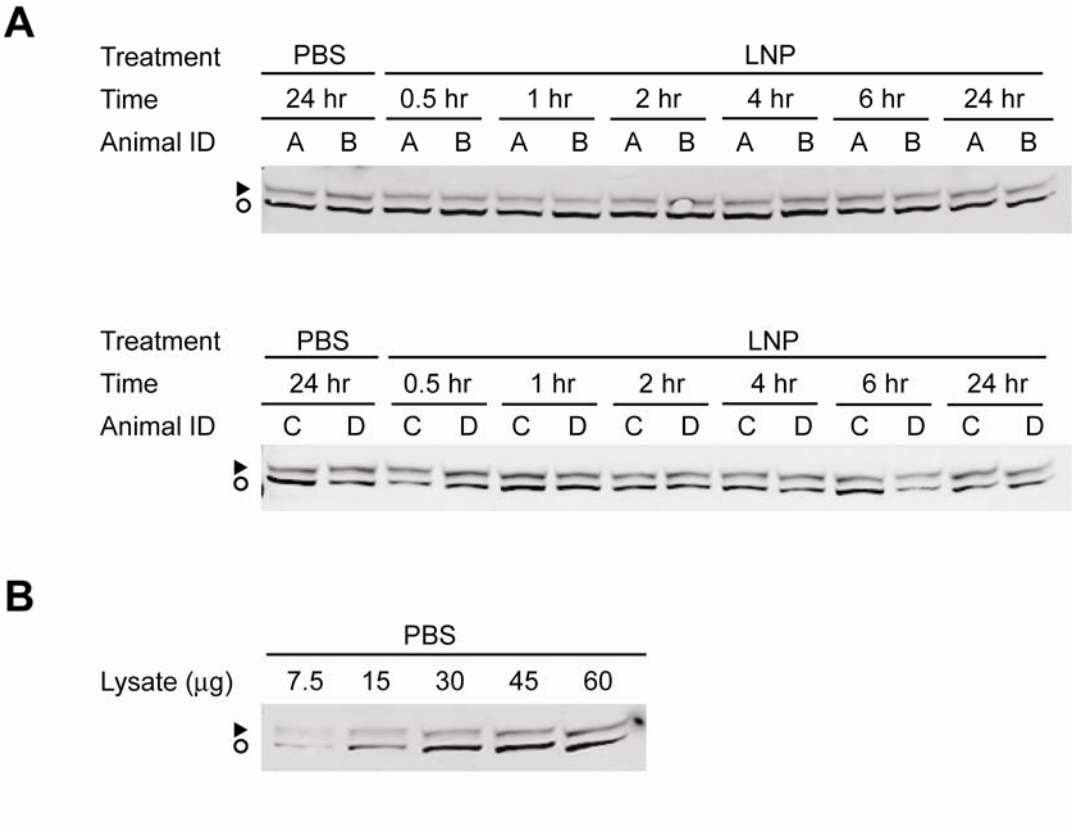
Supplementary Figure 5. Copy numbers of Ssb siRNA guide strand, passenger strand and four representative miRNAs (mir-122, mir-16, mir-26b and let-7a) in Ago2 immunoprecipitates from liver samples of mice that were treated as indicated. Quantification of small RNAs was performed using quantitative stem-loop RT-PCR. Each datum is presented as the group mean ± s.d in logarithm scale (n=4 per group).



Supplementary Figure 6. Silencing effect and Ago2 strand loading in mouse spleen samples (1 day post dosing). (*A*) Effect on *Ssb* mRNA expression (relative to *Ppib*) after a single intravenous administration of PBS or indicated LNP-siRNA. Expression levels are computed as percent values relative to PBS-treated animals, which is designated as 100%. (*B, C*) Levels of Ago2-associated siRNA guide strand (*B*) or passenger strand (*C*) after normalizing their copy numbers to those of co-immunoprecipitated mir-16. Each bar represents the group mean ± s.d (n=4 per group).



Supplementary Figure 7. Chemical modification influences strand selection. (*A*) Sequence and chemical modifications of the siRNA duplexes. (*B*) Silencing efficacy of the indicated siRNA duplexes. Expression levels of *Ssb* relative to *Ppib* were normalized to that of mock transfected cells. The control siRNA was against firefly luciferase. (*C*) Relative amounts of the siRNA strands in RISC, with that of the guide strand from unmodified siRNA designated as 1. The comparability of each strand was confirmed by respective standard curves shown in (*D*). The plotted data are the average from two independent experiments ± s.d.



Supplementary Figure 8. Expression level of mouse Ago2 in liver lysates after LNP-Ssb siRNA treatment. (*A*) Liver lysates (30 µg) of mice with indicated treatments and harvest time points were resolved on 4-12% NuPAGE gels and analyzed by Western blot using anti-Ago2 antibody (C34C6). The triangle denotes mouse Ago2, and the circle is a non-specific band detected by the antibody (see Fig. 1*B*). (*B*) Liver lysates of indicated total protein amounts were separated and analyzed by the Western blot for calibration of the relative signal.