Supporting Information

Xue et al. 10.1073/pnas.1412686111

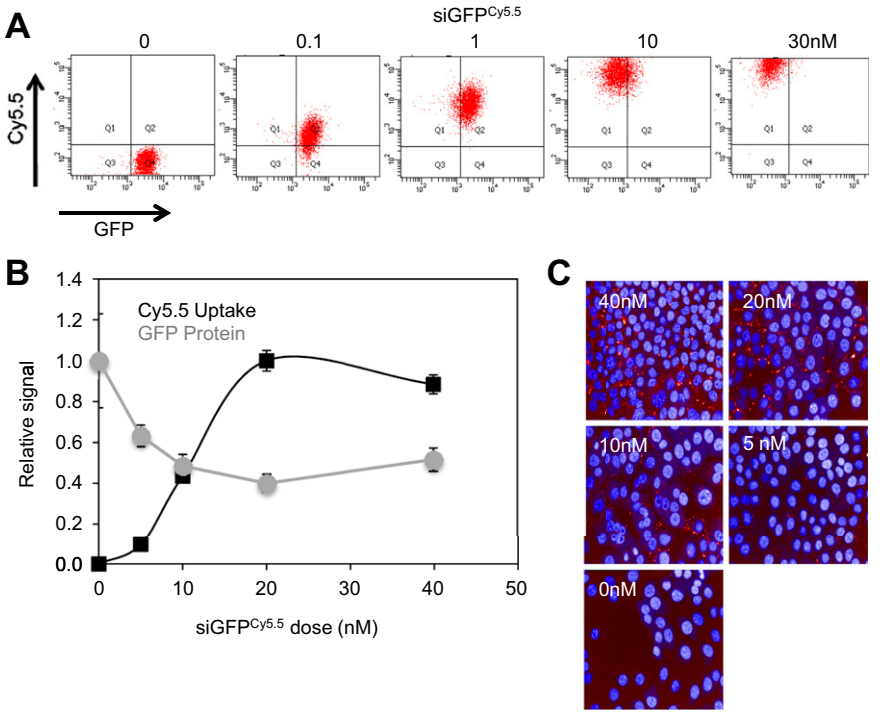


Fig. S1. 7C1 compound nanoparticles efficiently deliver siRNAs to murine lung adenocarcinoma cells in vitro. (*A*) FACS analysis of nanoparticle uptake and GFP knockdown. KP (*Kras*$^{LSL\text{-}G12D/wt}$;*p53*$^{flox/flox}$) cells expressing GFP were incubated with 7C1-small interfering GFP (siGFP; Cy5.5-labeled) at the indicated concentration for 48 h and analyzed by FACS. (*B*) Imaging-based quantification of nanoparticle uptake and GFP knockdown. KP cells expressing GFP were incubated with 7C1-siGFP at the indicated concentration for 48 h and imaged by fluorescent microscopy. The GFP signal at 0 nM and Cy5.5 signal at 20 nM were set to 1. (*C*) Representative images from *B*. Cy5.5 is shown in red, and DAPI is shown in blue. (Magnification: 200×.)

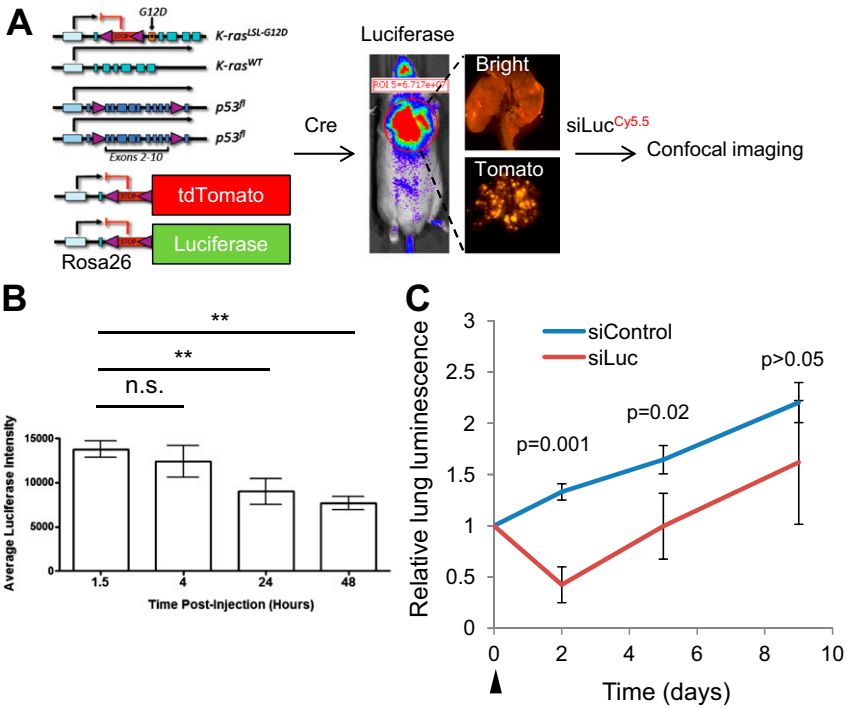


Fig. S2. 7C1 compound nanoparticles carrying luciferase siRNA efficiently knock down luciferase in murine lung adenocarcinoma in vivo. (*A*) Scheme of the *Kras*$^{LSL\text{-}G12D/wt}$;*p53*$^{flox/flox}$;R26$^{LSL\text{-}Luciferase/LSL\text{-}tdTomato}$ mice. Whole-body bioluminescence imaging shows luciferase signal in the lung at 10 wk after Adeno-Cre inhalation. An explanted lung shows Tomato-positive lung tumors. Tumor-bearing mice were injected with a single dose of 1.5 mg/kg of 7C1–siRNA-targeting luciferase (siLuc$^{Cy5.5}$). Lung tumors were harvested at the indicated time points for luciferase immunostaining and determination of natural fluorescence of Tomato. (*B*) Quantification of luciferase immunofluorescence signal in Fig. 1*E*. Error bars are SEM (n = 18, 13, 16, and 7 tumors analyzed). n.s., not significant. $^{**}P < 0.01$. (*C*) Quantification of luminescence by whole-body luciferase imaging. Error bars are SD (n = 3 mice). Arrowheads indicate 1.5 mg/kg of 7C1-siRNA i.v. injections.

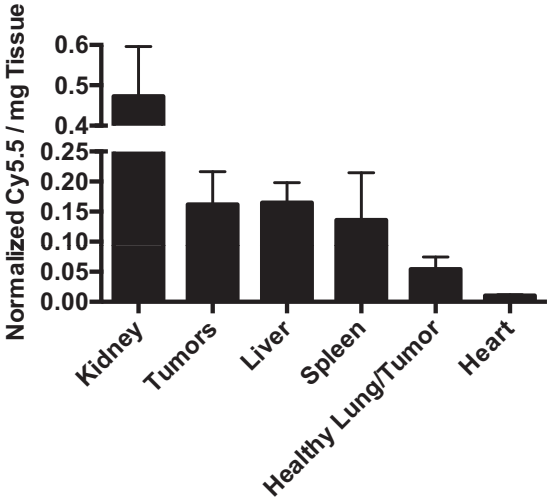


Fig. S3. Biodistribution of 7C1 nanoparticles. KP mice harboring lung tumors were injected with a single dose of PBS or 1.5 mg/kg of 7C1 nanoparticles carrying Cy5.5-labeled siRNA (three mice per group). Cy5.5 signal in microdissected lung tumors and organs was quantified using an Xenogen imager at 3.5 h after injection. Error bars are SD (n = 3). Tumors denote autochthonous lung tumors.

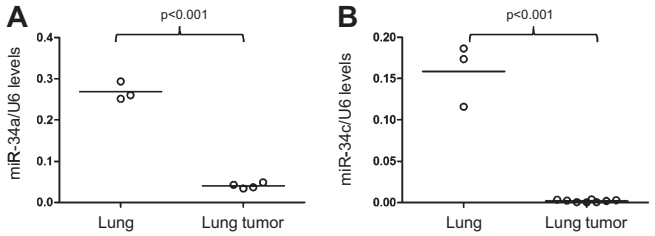


Fig. S4. miR-34a and miR-34c are relatively underexpressed in KP lung tumors compared with normal lung. Expression levels of mature forms of miR-34a (*A*) and miR-34c (*B*) were quantified by RT-quantitative (Q)-PCR and normalized to U6 noncoding RNA in normal lung and KP lung tumors. Each dot represents an individual RNA sample. Lines are mean values.

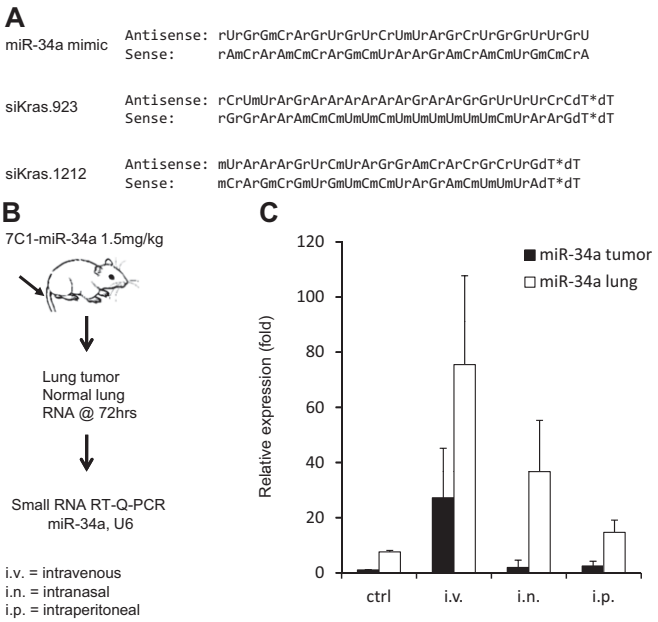


Fig. S5. Comparing miR-34a delivery efficiency using three injection methods. (*A*) Sequences of miR-34a mimic and small interfering Kras (siKras; 5′–3′). mA, mG, mC, and mU are 2′O-methyl RNA; rA, rG, rC, and rU are RNA. dT*, phosphorothioated DNA. (*B*) Experiment design. (*C*) miR-34a levels in KP lung tumors or normal lung were quantified by RT-Q-PCR and normalized to U6 noncoding RNA. Error bars are SD (n = 4 tumors). The miR-34a level in control siRNA (ctrl)-treated tumors is set to 1. The highest miR-34a level in lung tumors is delivered by i.v. injection.

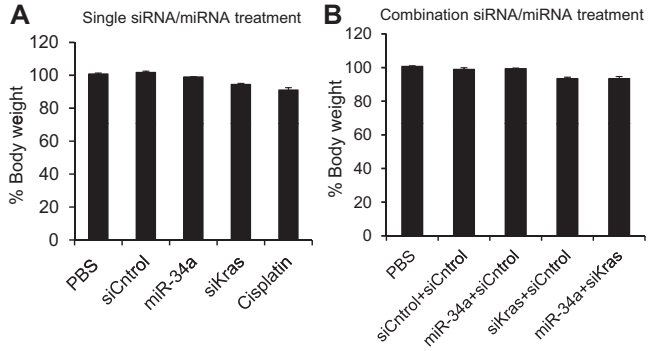


Fig. S6. Body weight in mice dosed with 7C1 nanoparticles carrying single siRNA/microRNA (miRNA) or combinations. (*A*) 7C1-small interfering control (siCntrol), 7C1-siKras, or 7C1–miR-34a was injected at a dose of 1.5 mg/kg every other day for four doses. Cisplatin treatment was given at a therapeutic dose (7 mg/kg). Body weight was measured 7 d after injection compared with preinjection. (*B*) 7C1 combination siRNA/miRNA was dosed at 2 mg/kg every other day for four doses. Error bars are SD (n = 3 mice).

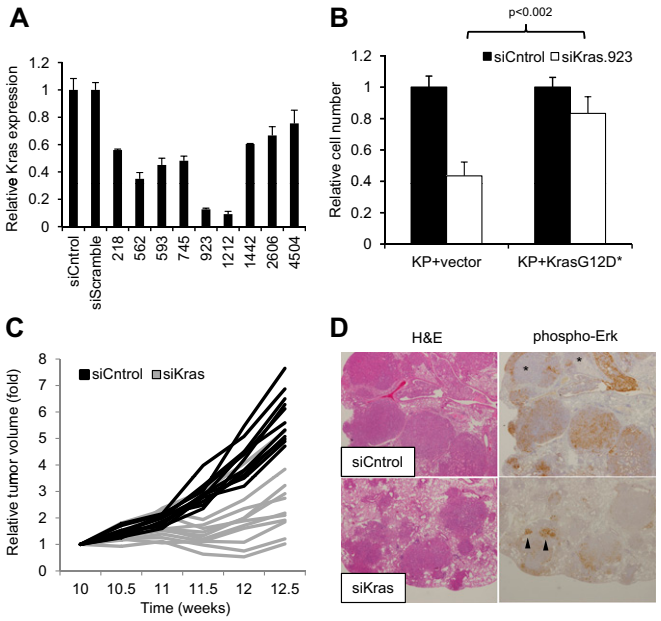


Fig. S7. Screening effective siRNA targeting mouse Kras. (*A*) KP cells were transfected with indicated Kras siRNAs or a control siRNA (siCntrol) at 10 nM. Kras expression levels were measured by RT-Q-PCR at 48 h. siRNA numbers indicate positions in the Kras mRNA. Error bars are SD ($n = 3$). siScramble, scrambled siRNA. (*B*) siKras effect was rescued by a nonsilencible Kras cDNA ($Kras^{G12D}$*) as in Fig. 3*B*. KP cells stably expressing vector or $Kras^{G12D*}$ were incubated with 7C1 carrying siCntrol or siKras. Cell numbers were quantified by a CellTiter-Glo assay (Promega) at 72 h. (*C*) Individual lung tumor volume curve from Fig. 3*D*. (*D*) Representative low-magnification view of Fig. 3*E*. (Magnification: 20×.) Twelve weeks after tumor initiations, mice were injected with two doses of 1.5 mg/kg of 7C1-siRNA every other day and harvested 72 h later. *Grade 2 tumors with low phospho-Erk (pErk) in the siCntrol group. Arrowheads denote tumors retaining high pErk in the siKras group.

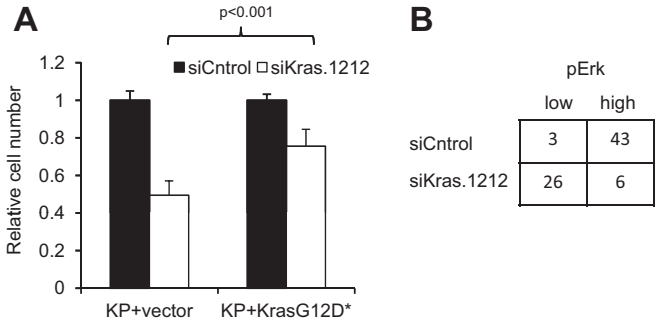
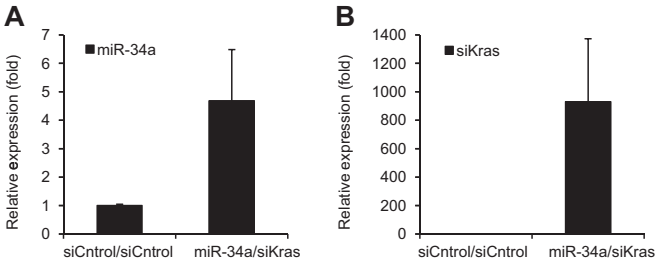


Fig. S8. 7C1 nanoparticles deliver siKras.1212 in the KP model. (*A*) Effect of siKras.1212 on cell number was rescued by a nonsilencible Kras cDNA (KrasG12D*). KP cells stably expressing vector or $Kras^{G12D*}$ were incubated with 7C1 carrying control siRNA or siKras. Cell numbers were quantified by a CellTiter-Glo assay at 72 h. (*B*) Quantification of pErk low- and high-grade lung tumors. $P < 10^{-12}$.



C

	siCntrol/siCntrol			miR-34a/siKras		
Tumor ID	T1	T2	T3	T1	T2	T3
miR-34a	29.1	29.0	26.8	26.1	25.2	26.2
siKras	39.0	40.0	37.2	29.1	27.9	28.9
U6	24.2	24.2	22.6	23.3	22.5	24.4

Fig. S9. Detecting *miR-34a* mimic and siKras in lung tumors dosed with 7C1 nanoparticles simultaneously complexed to siKras and miR-34a. Mice were dosed with 7C1 complexed with miR-34a/siKras (2 mg/kg) or 7C1 complexed with siCntrol. Small RNA in tumors was quantified by Q-PCR using Taqman probes specific to mature miR-34a (*A*) or siKras (*B*). Error bars are SD ($n = 3$ tumors). (*C*) Q-PCR cycle threshold (Ct) numbers (maximum = 40 cycles) of miR-34a, siKras, and U6. The miR-34a probe measures both endogenous miR-34a and delivered miR-34a mimic. The high Ct number of siKras in the siCntrol/siCntrol group indicates lack of endogenous siKras in control tumors. T1–T3 denote three individual lung tumors.

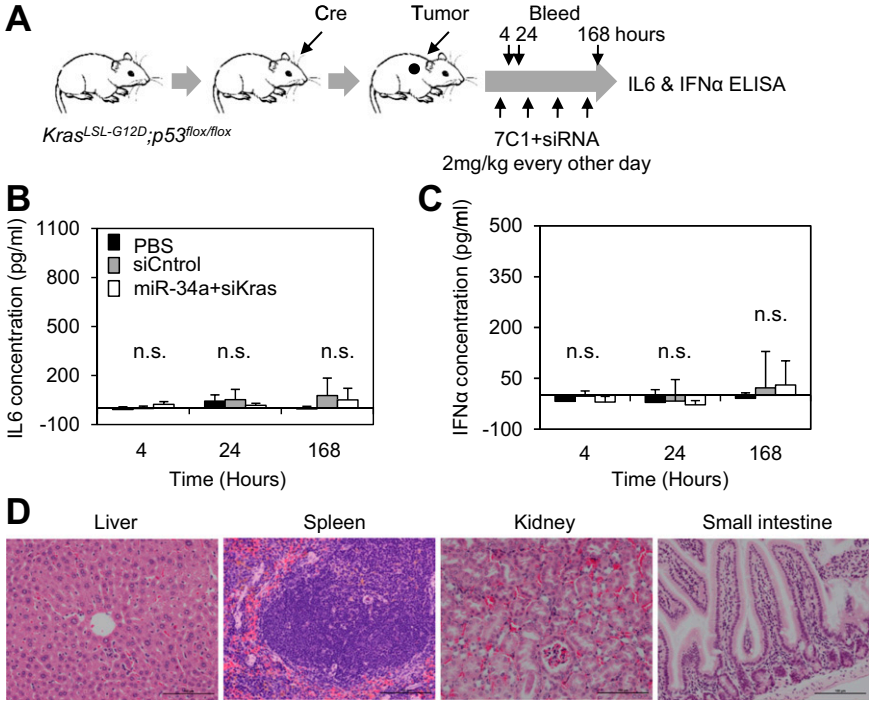


Fig. S10. 7C1 nanoparticles carrying miRNA/siRNA do not induce an immune response in mice. (*A*) Peripheral blood was collected from animals injected with 7C1 complexed to siCntrol or miR-34a/siKras combination at the indicated time points. These time points mimicked the therapeutic regimen (Fig. 4). (*B* and *C*) IL-6 and IFN-α concentrations were measured by ELISA (six mice per group). $P > 0.05$ (n.s.) for 7C1-siRNA vs. PBS comparison at all three time points. In the literature, siRNAs with known immunostimulatory effect induced >500 pg/mL IL-6 and >200 pg/mL IFN-α in the peripheral blood. (*D*) Representative histology in mice treated with the 7C1 miR-34a/siKras combination. (Scale bars: 100μm.)

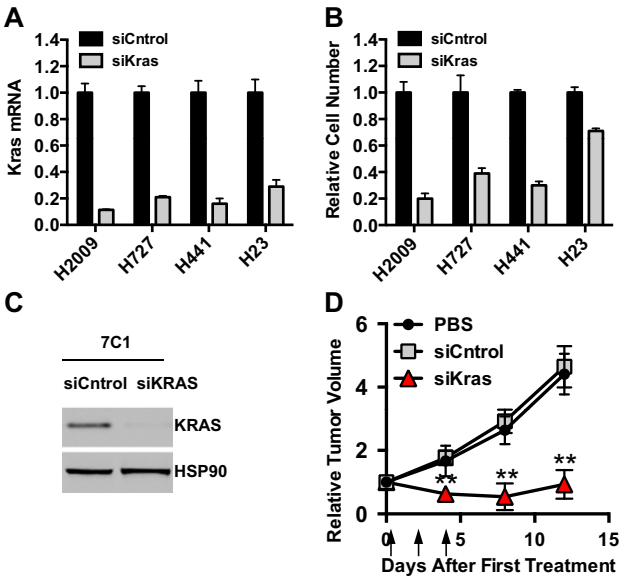


Fig. S11. 7C1 nanoparticles deliver siRNA to human lung adenocarcinoma cells. (*A*) *KRAS* mRNA expression following treatment with 30 nM 7C1-siKRAS in four *KRAS* mutant human lung adenocarcinoma cell lines [H2009 ($KRAS^{G12A}$), H727 ($KRAS^{G12V}$), H441 ($KRAS^{G12V}$), and H23 ($KRAS^{G12C}$)] was measured by RT-Q-PCR. (*B*) Normalized cell number (compared with 7C1.siCntrol, and quantified by CellTiter-Glo assay) 48 h following treatment with 7C1-siKRAS. Error bars are SD ($n = 3$ wells per group). (*C*) Immunoblots of H2009 protein lysates 48 h following incubation with 30 nM 7C1 siRNA. Heat shock protein 90 (HSP90) serves as a loading control. (*D*) Tumor volume, normalized to pretreatment volumes, in nude mice harboring H2009 xenograft tumors after treatment with 1 mg/kg of 7C1 siRNA ($n = 3$ mice per group, $n = 2$ tumors per mouse). Arrows indicate tail vein injections. $^{**}P < 0.01$.