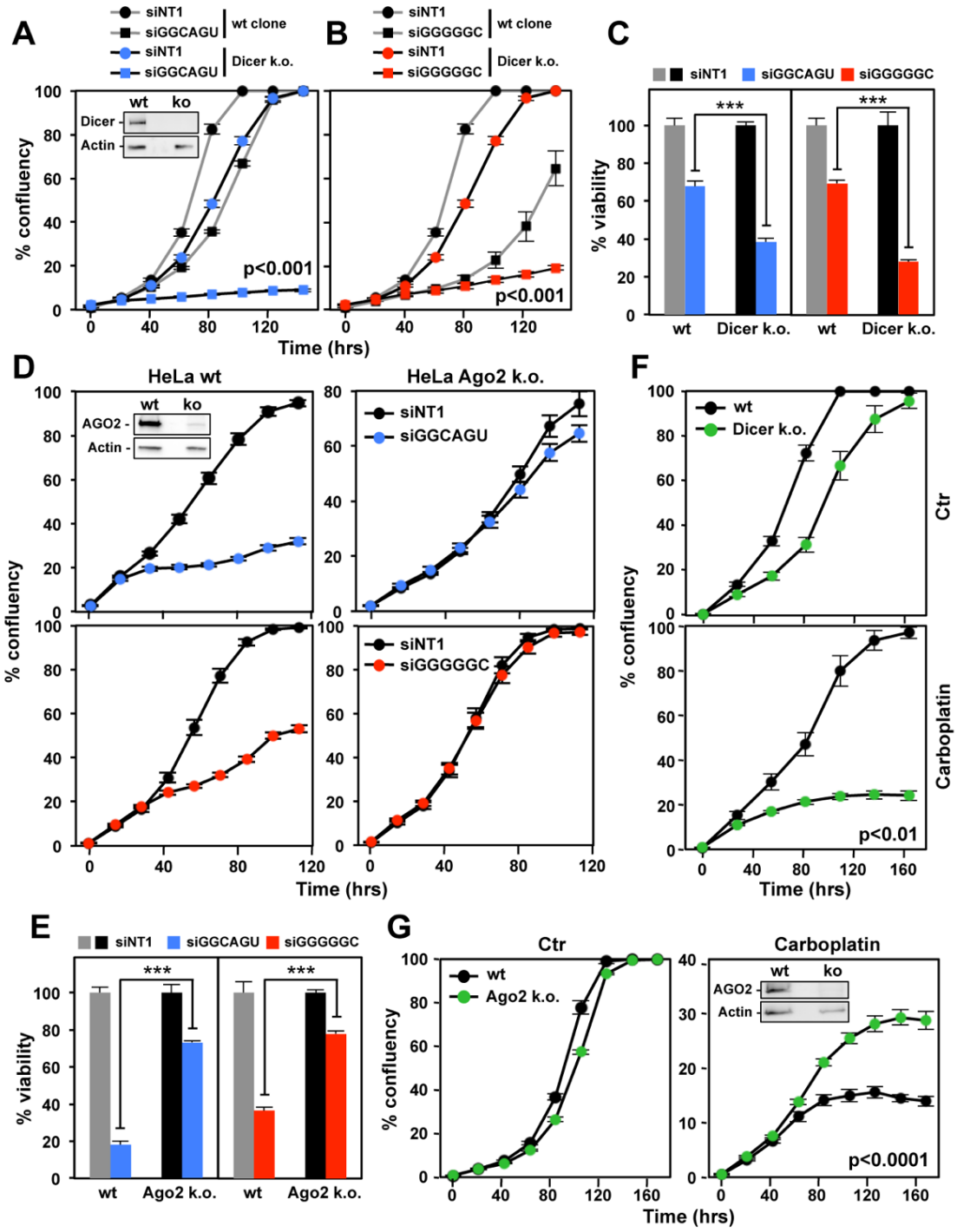
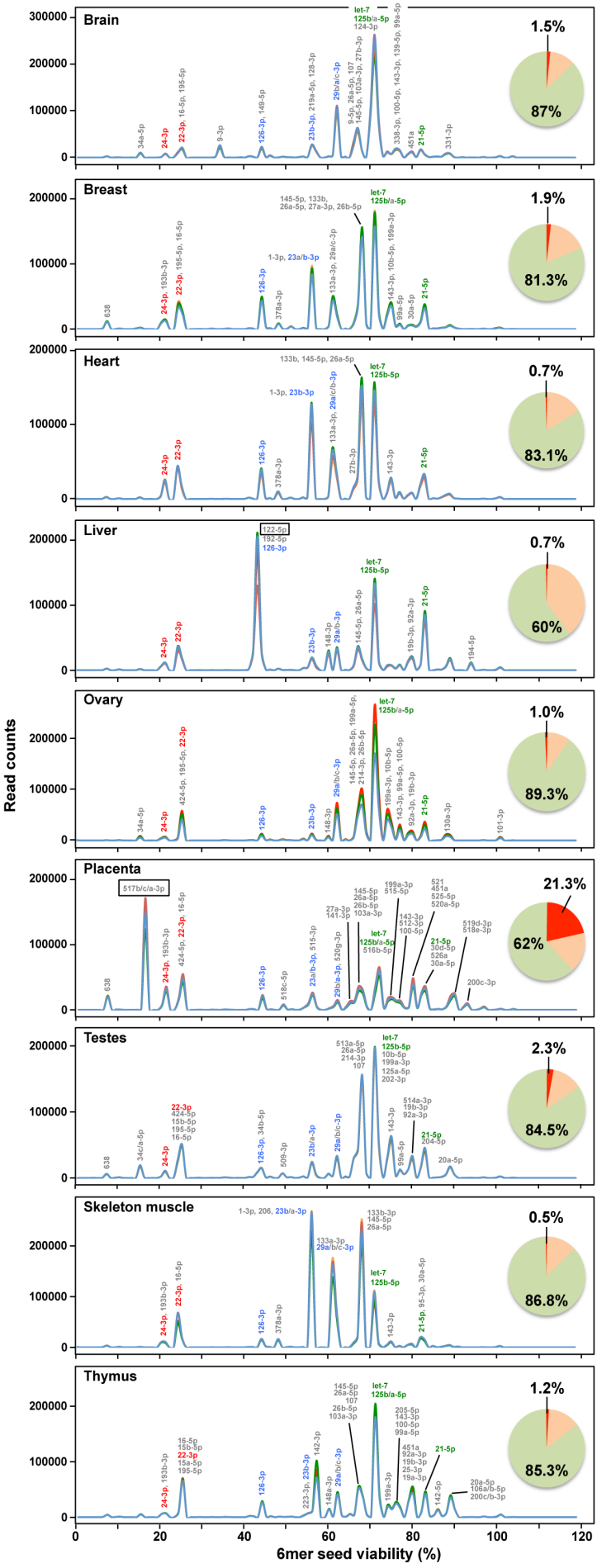
Supplemental Figures

The ratio of toxic-to-nontoxic microRNAs predicts platinum sensitivity in ovarian cancer

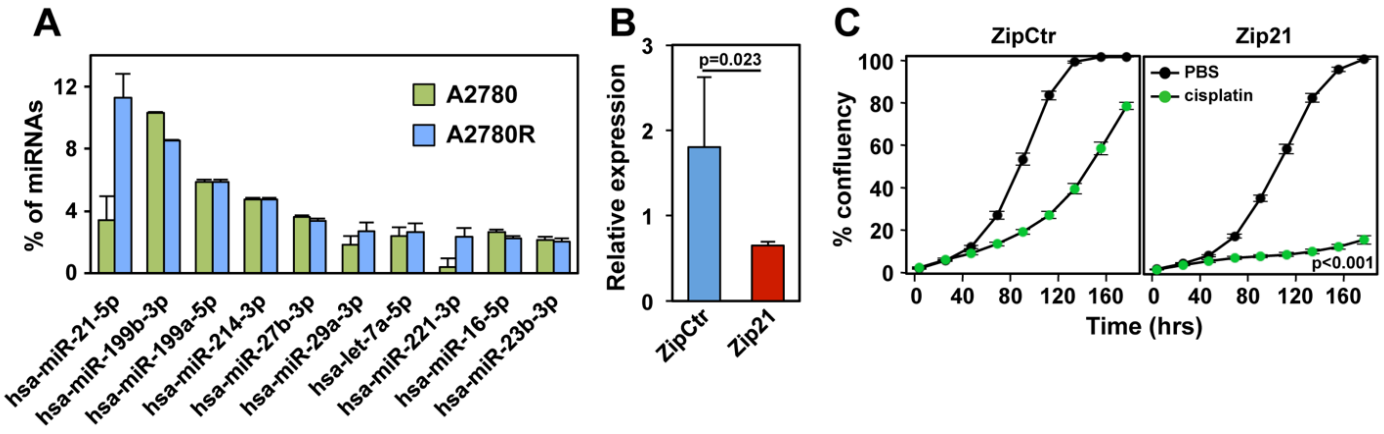
Monal Patel, Yinu Wang, Elizabeth T. Bartom, Rohin Dhir, Kenneth P. Nephew, Daniela Matei, Andrea E. Murmann, Ernst Lengyel, and Marcus E. Peter



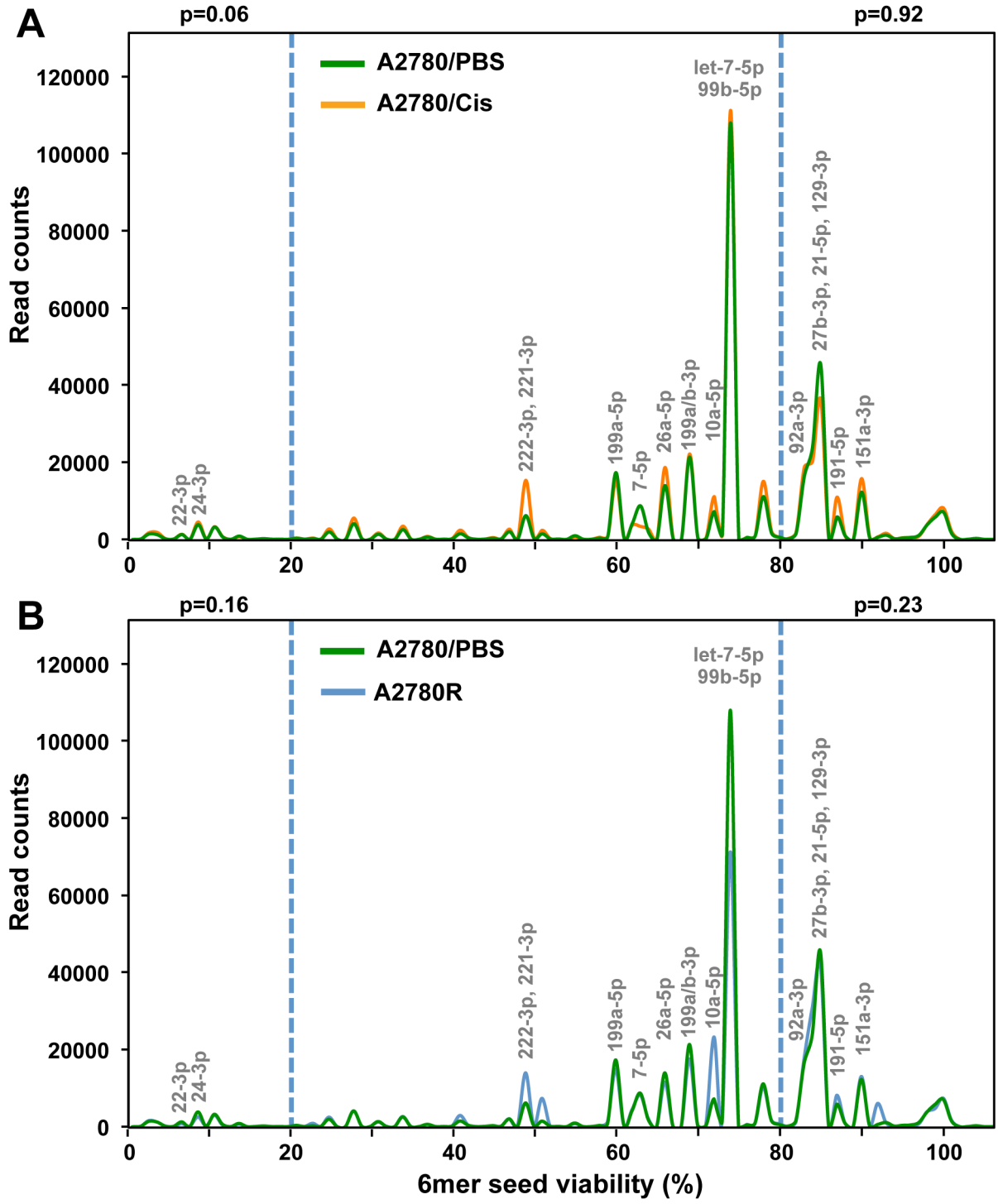
Supplemental Figure S1. Cell death induced by both toxic 6mer containing siRNAs and carboplatin involves RNAi. **A** and **B,** Confluency over time of 293T cells (wt or Dicer k.o.) transfected with 10 nM of siNT1, siGGCAGU or siGGGGGC. Insert: Western blot of Dicer in wt and k.o. cells. **C,** Viability of the cells in A and B 96 hrs after transfection with 10 nM of the siRNAs. **D,** Confluency over time of HeLa cells (wt or Ago2 k.o.) transfected with 10 nM of siNT1, siGGCAGU or siGGGGGC. Insert: Western blot of Ago2 in wt and k.o. cells. **E,** Viability of the 293T (wt or Ago2 k.o.) cells 96 hrs after transfection with 10 nM of the siRNAs. **F,** Confluency over time of 293T (wt or Dicer k.o.) treated with 3 μg/ml carboplatin. **G,** Confluency over time of 293T (wt or Ago2 k.o.) treated with 10 μg/ml carboplatin. Insert: Western blot of Ago2 in wt and k.o. cells. Each data point (A, B, D, F & G) represents mean ± SE of at least three replicates. Each bar (C, E) represents ± SD of three replicates. P-values were calculated using binomial distribution tests (A, B, F), ANOVA (G), or student's T-test (C, E). *** p<0.0001.



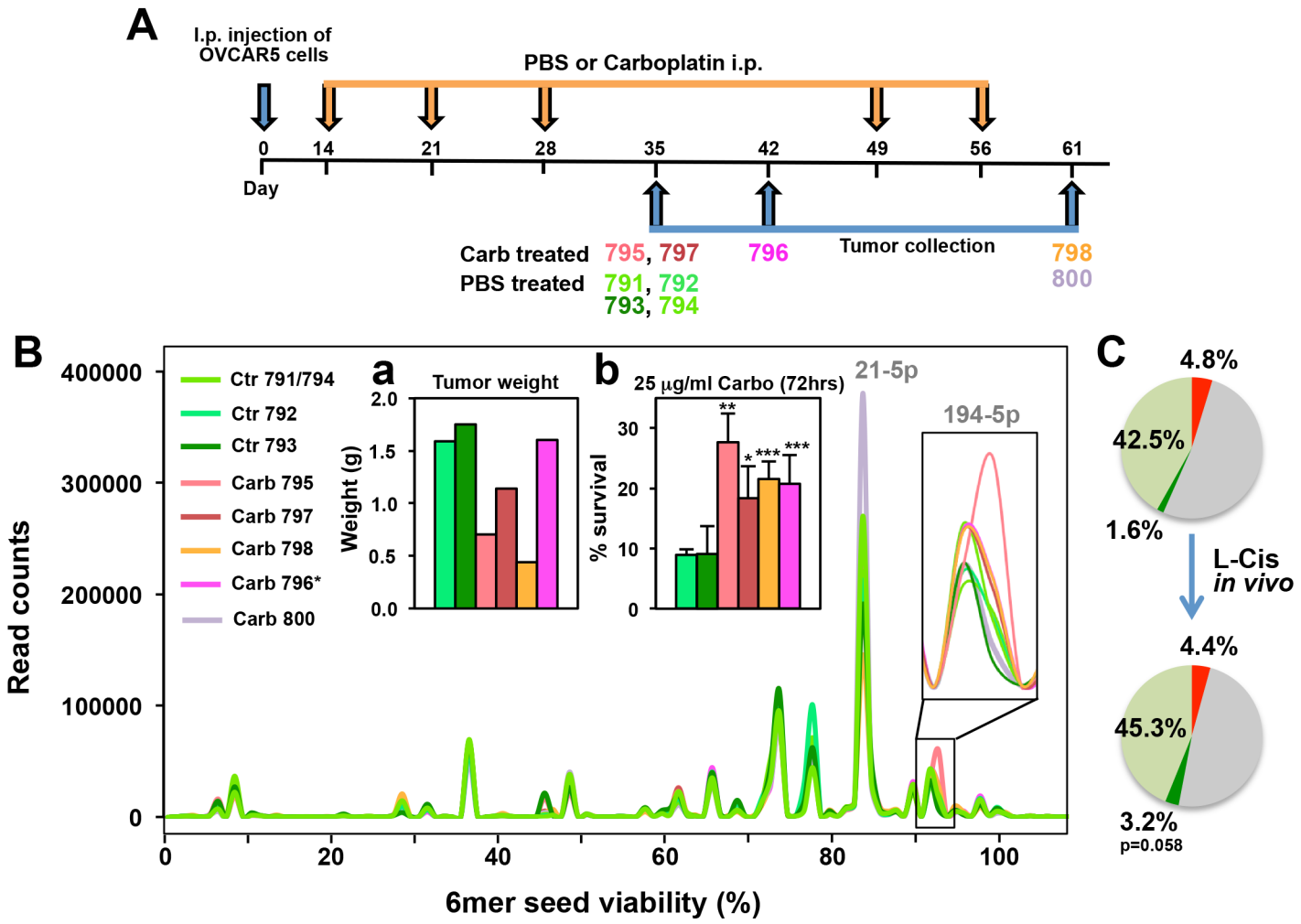
Supplemental Figure S2. Most tissues express predominantly nontoxic miRNAs**.** miRNA Seed Tox graphs across 9 different tissues. In each case the cumulative read numbers are on the Y axis and the predicted toxicity on the X axis. Toxicity (shown as % viability) was the average of three human cell lines determined in a screen of all 4096 6mers in a neutral siRNA backbone (1). miRNAs significantly expressed in all tissues are labeled in color (red = highly toxic, blue = intermediately toxic, and green = nontoxic). When a peak is labeled with multiple miRNAs, the most abundant one is listed first. Pie chart insert: Abundance of miRNAs with seeds of the following predicted viabilities: red: <20%; corral: >=20 <50%; green: >50%. Data were normalized to 1 million reads and all miRNAs were labeled that have more than 5000 normalized average reads across five (for brain, breast, heart, liver, ovary, placenta, and skeleton muscle) or four (for testes and thymus) individual samples, respectively. Some established tissue specific miRNAs are boxed. GSE11879 was the source of the smRNA Seq data analyzed.



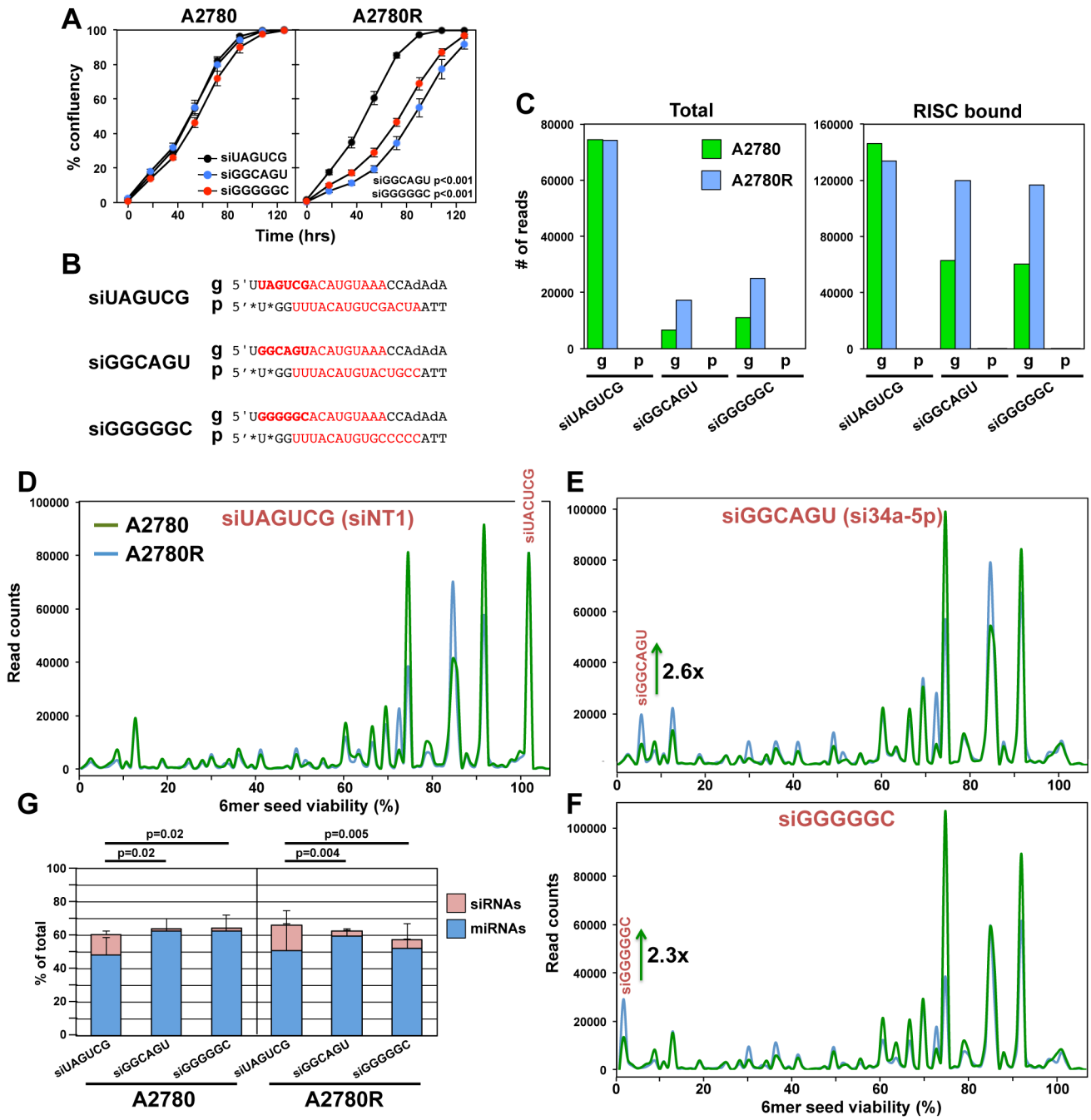
Supplemental Figure S3. miR-21-5p protects A2780R cells from platinum toxicity. **A,** Relative abundance (% of total reads) of the ten most highly expressed miRNAs in A2780 and A2780R cells (ranked according to highest expression in A2780R cells). Shown is the variance between duplicates. **B,** Real time PCR quantification of miR-21-5p in A2780R cells infected with control Zip vector or the miR-21 inhibitory lentivector Zip21. P-value from Student's T-test is shown. **C,** Confluency over time of ZipCtr or Zip21 expressing cells treated with either PBS or 5 μM cisplatin. P-value was calculated using a binomial distribution test. Experiments in B and C were done in triplicates.



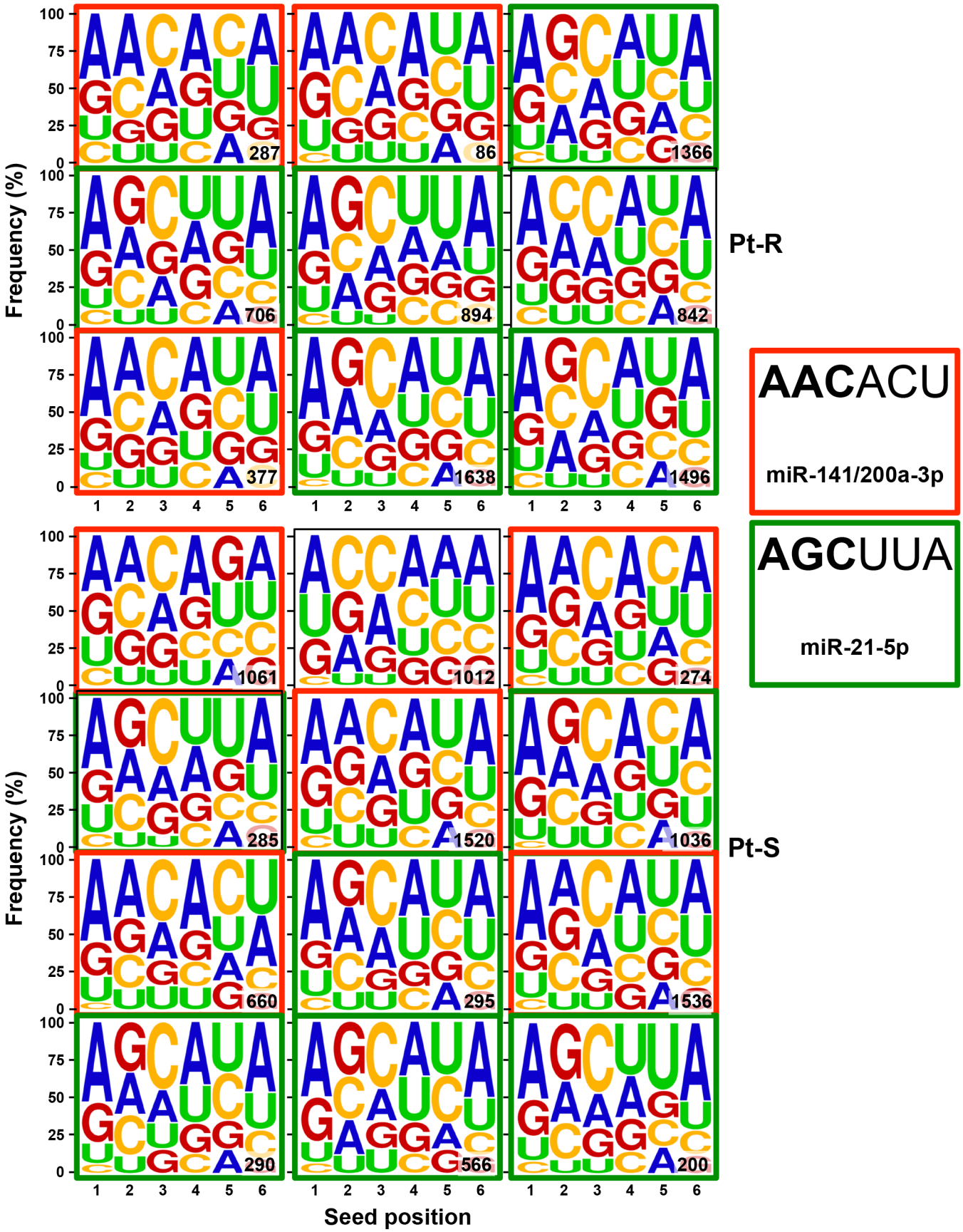
Supplemental Figure S4. Effect of cisplatin treatment on Seed Tox composition of the total miRNA fraction. **A,** Seed Tox graph of all miRNAs in A2780 cells treated with PBS or cisplatin (Cis) for 72 hours. **B,** Seed Tox graph of all miRNAs in A2780 cells or A2780R cells. miRNAs that contribute to peaks with more than 5000 reads are labeled. For each labeled peak miRNAs are listed in the order of abundance. P-values were calculated using pairwise comparisons of all reads in the two different treatments in two groups: reads with predicted viability >80% and <20% (blue stippled lines).



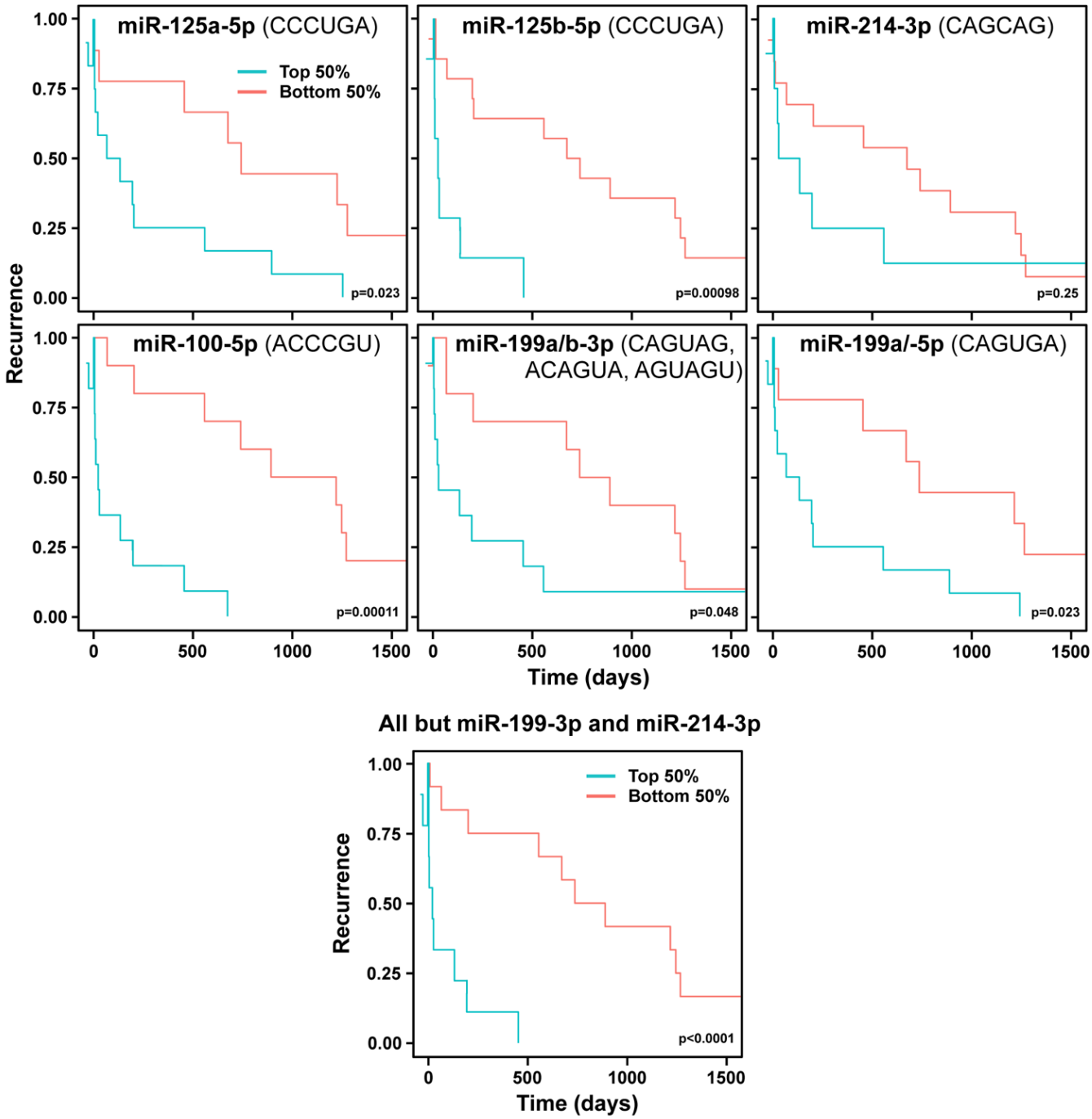
Supplemental Figure S5. Effect of long-term *in vivo* carboplatin treatment on Seed Tox of RISC-bound miRNAs in OVCAR5 cells. **A,** Treatment course. OVCAR5 (2 million) cells were injected i.p. into nude mice. Two weeks after inoculation, mice were grouped and treated i.p. with PBS (control, mice ID: 791, 792, 793, 794, n=4), or 25 mg/kg carboplatin (Carbo) (n = 5) as indicated: 3 doses of carboplatin (n=2, mice ID: 795, 797); 3-weekly doses of carboplatin + two-week recovery (n = 1, mice ID: 796); 3-weekly doses of carboplatin, followed by 2-weeks recovery, followed by 2-weeks carboplatin (n = 2, mice ID: 798, 800). **B,** Seed Tox graph of total RISC-bound miRNAs in OVCAR5 tumors isolated at different times after PBS or carboplatin treatment as indicated in A. a, Total tumor weight; b, Viability assay of tumors 24 hours after isolated from mice treated as shown in A. ATP assay was performed 96 hrs after treatment. Student's t-test was performed. Significance shown (Student's t-test) is between a carboplatin treated tumor and either of the two control treated tumors. *** $p<0.0001$, ** $p<0.001$, * $p<0.05$. The area around the miR-194-5p peak is magnified. **C,** Pie charts: Average Seed Tox composition of RISC-bound miRNAs in mice treated with PBS (top) or with carboplatin (bottom). miRNA reads with a predicted 6mer seed viability of <20% are shown in red, >80% in green and 20-80% in grey. Reads of miR-194-5p (part of the green section) are highlighted in darker green. miRNA content of this analysis was 96.1% (average of PBS treated) and 92.6% (carboplatin treated).



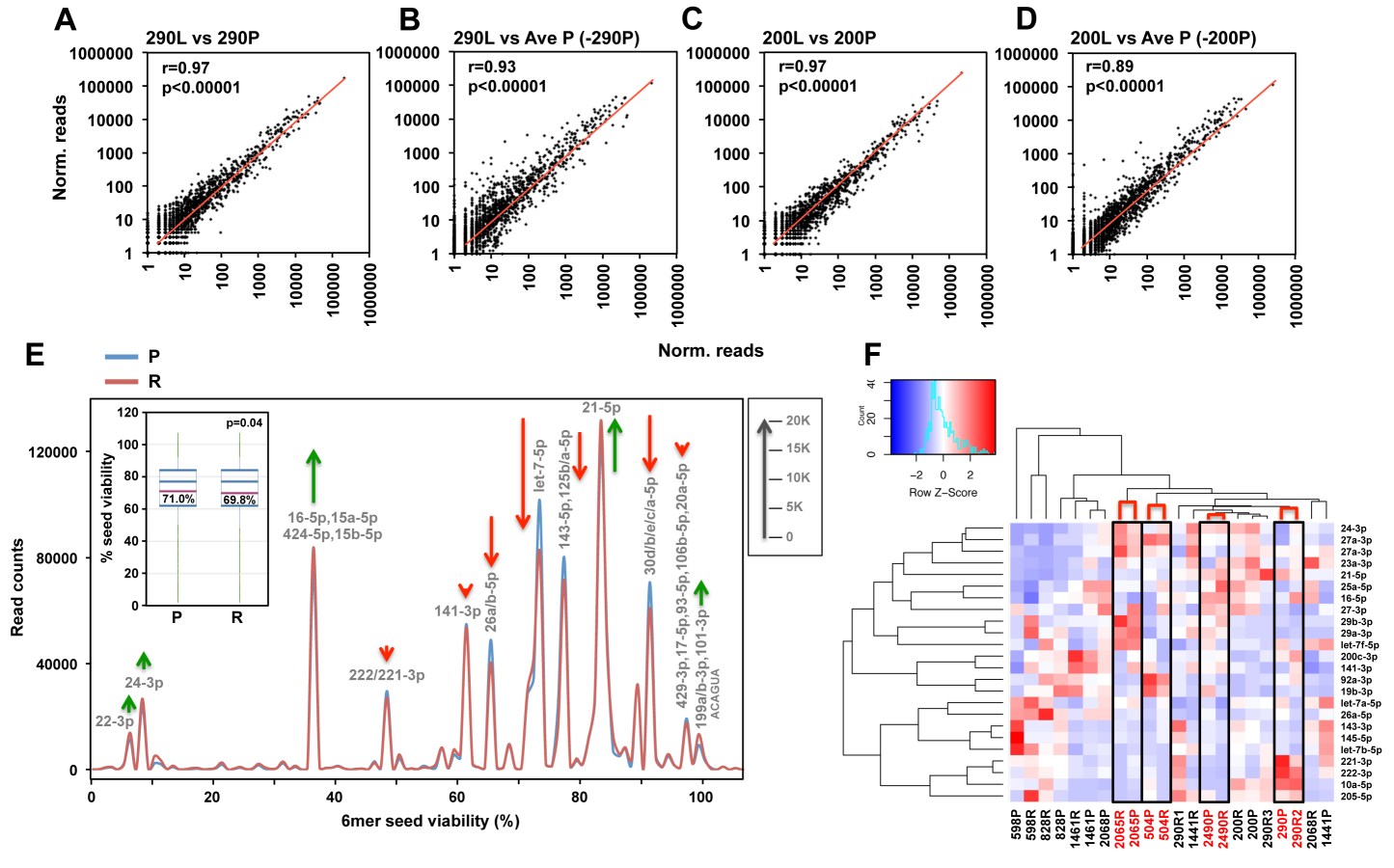
Supplemental Figure S6. Preferential uptake of siRNAs with toxic seeds by Pt-R cells. **A,** Change in confluency of A2780/A2780R cells transfected with 1 nM of an siRNA backbone used in the 4096 siRNA screen containing three different 6mer seeds shown in B. P-values were calculated using ANOVA. **B,** Sequences of the three siRNAs differing in only the 6mer seed (6mers bolded). Both guide (g) and passenger (p) strands are shown 5' -> 3'. The sequences used to search the RNA Seq data (as DNA sequences) for presence of exogenous RNAs is shown in red. **C,** Total normalized numbers of sequences derived from the transfected siRNAs [either guide (g) or passenger (p) strand] identified in the total or RISC-bound sRNA in cells transfected with the indicated siRNA. **D-F,** Seed Tox graph of miRNAs and exogenous siRNAs in total smRNA in A2780/A2780R cells 24 hrs after transfection with either siUAGUCG (siNT1), and the highly toxic siGGCAGU or siGGGGGC. **G,** Percent miRNAs and exogenous siRNAs in total small RNAs of siRNA transfected cells. Student's test p-values are shown on the change in percent of transfected siRNAs.



Supplemental Figure S7. OC patients can be divided into two subgroups. Seed composition plots of all RISC-bound sRNAs in tumors from Pt-R and Pt-S patients. Seeds that are dominated by AAC (present in miR-141/200a-3p) in the first three positions are boxed in red, the ones dominated by AGC (present in miR-21-5p) are boxed in green.



Supplemental Figure S8. Kaplan-Meier analyses of individual miRNAs. Kaplan-Meier analysis of patients with the top and bottom highest RISC content of the indicated miRNA. For miR-199-3p the average of all three isomiRs was used.



Supplemental Figure S9. Analysis of primary tumors and matched recurrences. **A,** Pearson correlation between reads from an Ago pull down analysis of the primary tumor from patient #290 in experiment #1 (short versus long-term survivors) and #2 (primary versus recurrence in long-term survivors). **B,** Pearson correlation between reads from an Ago pull down analysis of patient #290L in experiment #1 versus the average of all patients of experiment #2 minus patient #290P reads. **C,** Pearson correlation between reads from an Ago pull down analysis of the primary tumor from patient #200 in experiment #1 and #2. **D,** Pearson correlation between reads from an Ago pull down analysis of patient #200L in experiment #1 versus the average of all patients of experiment #2 minus patient #200P reads. In all cases all reads were analyzed but only the ones were plotted with an average minimum read count of 1 across all samples. Note, in both cases while the tumors analyzed were from the same patient, the analysis was performed with a different tumor from the same patient. **E,** Seed Tox graph of all RISC-bound reads in primary tumors and matching recurrences. Green arrows, net increase, and red arrows, net decrease of >1000 reads in peak in recurrent tumors, respectively. Insert: Average Seed Tox of all sRNAs in either the primary tumor (P) or recurrences (R). P-value was calculated using a Wilcoxon rank test. **F,** Heat map of an unsupervised hierarchical cluster analysis of the most abundant miRNAs (read average across all samples >10,000) in primary tumors and matching recurrences from 10 patients. Primary tumor and matching recurrence that clustered directly together are boxed. Note, for patient #290 only recurrence R2 clustered directly together with the primary tumor and this pair was therefore used for further analysis. Patients in a subgroup of four highly related primary/recurrent tumor pairs are shown in red.

Supplemental References

1. Gao QQ, Putzbach W, Murmann AE, Chen S, Ambrosini G, Peter JM*, et al.* 6mer seed toxicity in tumor suppressive miRNAs. Nature Comm **2018**;9:4504