Supporting Information for:

Comparative Biochemical Studies of Disease-Associated Human Dicer Mutations on Processing of a pre-microRNA and snoRNA

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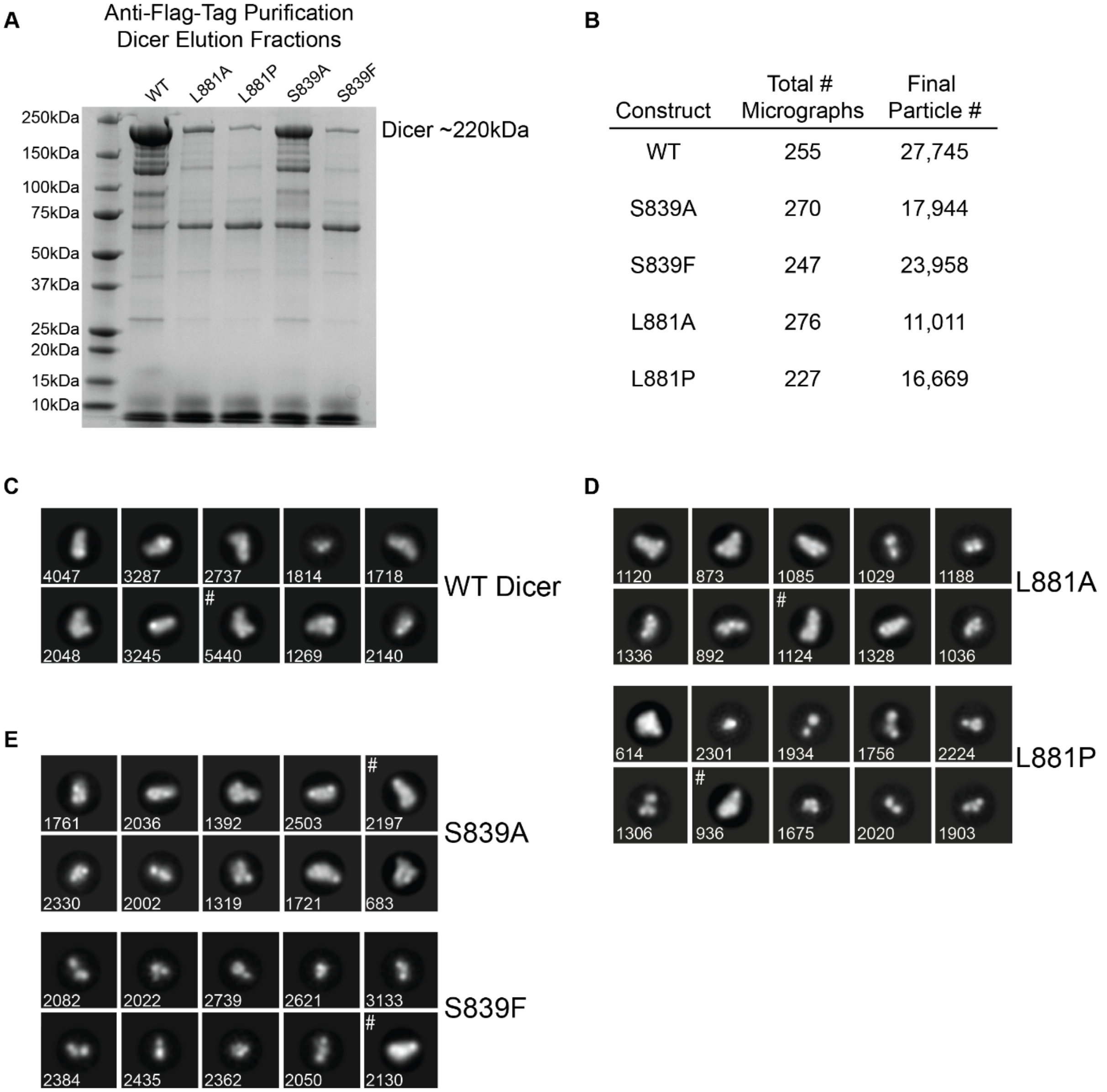


Construct	Total # Micrographs	Final Particle #
WT	255	27,745
S839A	270	17,944
S839F	247	23,958
L881A	276	11,011
L881P	227	16,669

Figure S1. Purification and negative stain EM analysis of recombinant WT and mutant hsDicer (A) SDS-Page analysis of eluted fractions following anti-Flag tag purification of WT and mutant Dicer proteins. Band corresponding to WT and mutant Dicer (~220 kDa) is labeled. (B) Table outlining the total number of micrographs collected and final number of particles picked for negative stain analysis of WT and mutant Dicer. (C–E) 2D class averages from negative stain EM of analysis of (C) WT, (D) L881A and L881P mutants, and (E) S839A and S389F mutants. 2D class averages shown in Figure S2 labeled with #. The number of particles in each class average is shown in the bottom left corner. Side length of box, 37 nm.

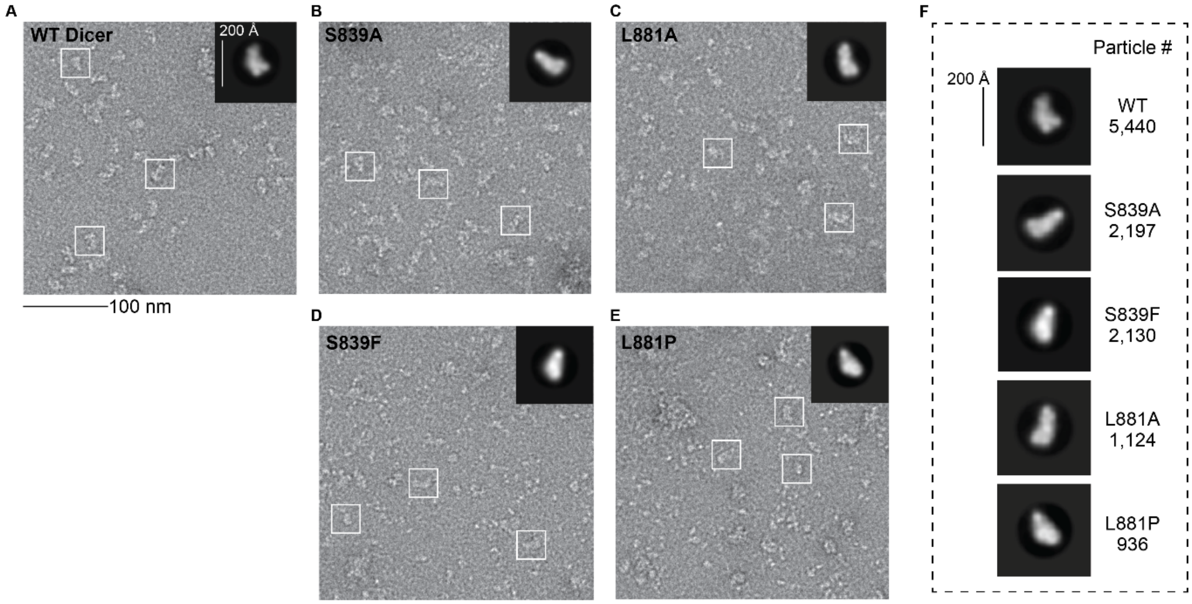


Figure S2. Negative stain EM analysis of recombinant WT and mutant Dicer. A representative negative stain micrograph and corresponding 2D class average are shown for (A) WT Dicer, (B) S839A, (C) L881A, (D) S839F, and (E) L881P. Examples of particles are outlined by a white dashed box. Scale bars shown in A the same for B–E. (F) Total number of particles contributing to the 2D class averages for WT or mutant Dicer.

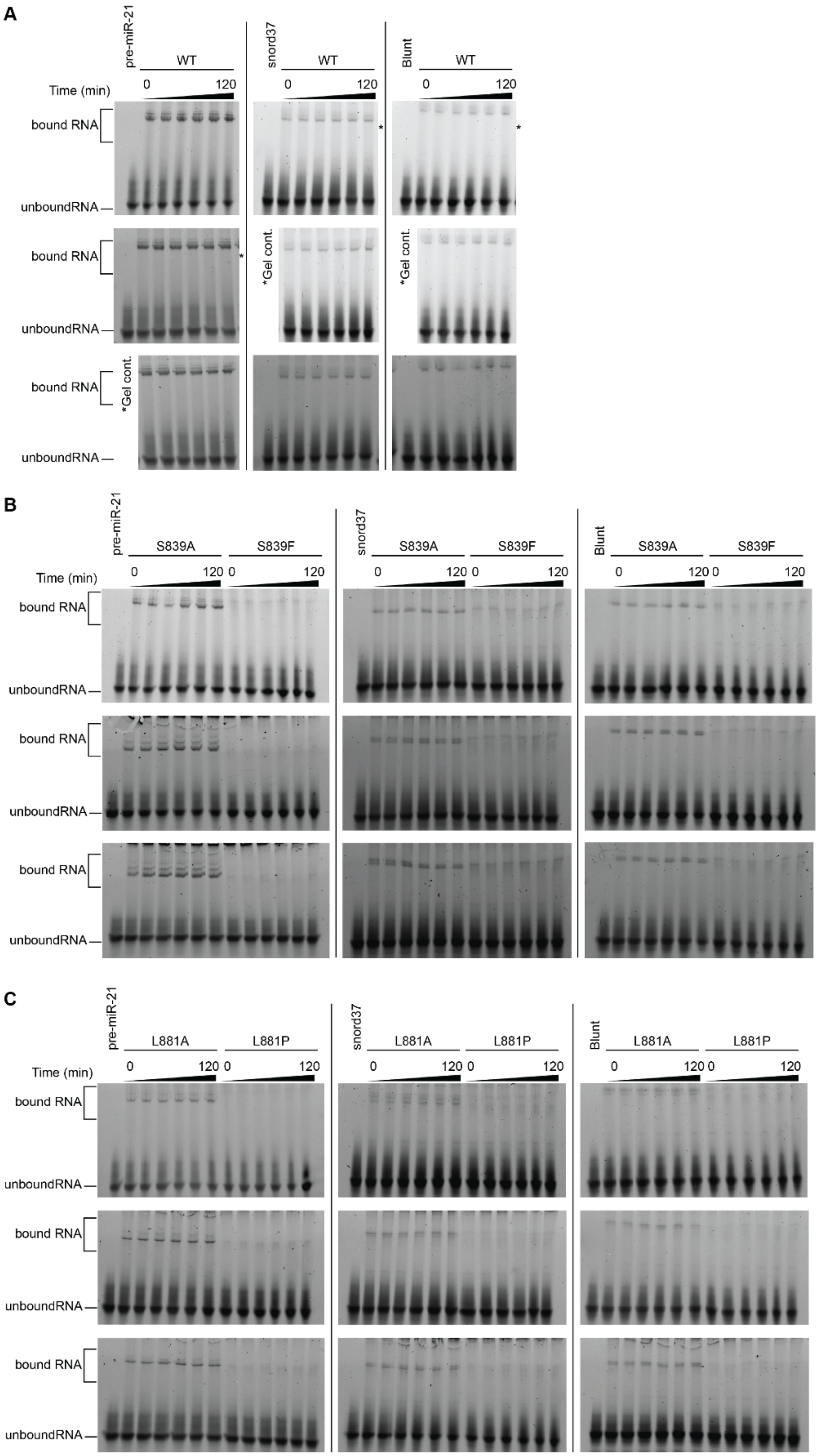


Figure S3. Replicates for EMSA binding assays in the presence of pre-miR-21, snord37 or Blunt-end pre-miR-21 (Blunt) for (A) WT Dicer, (B) S839 mutants and (C) L881 mutants. (* signifies that experiment was ran on the same gel)

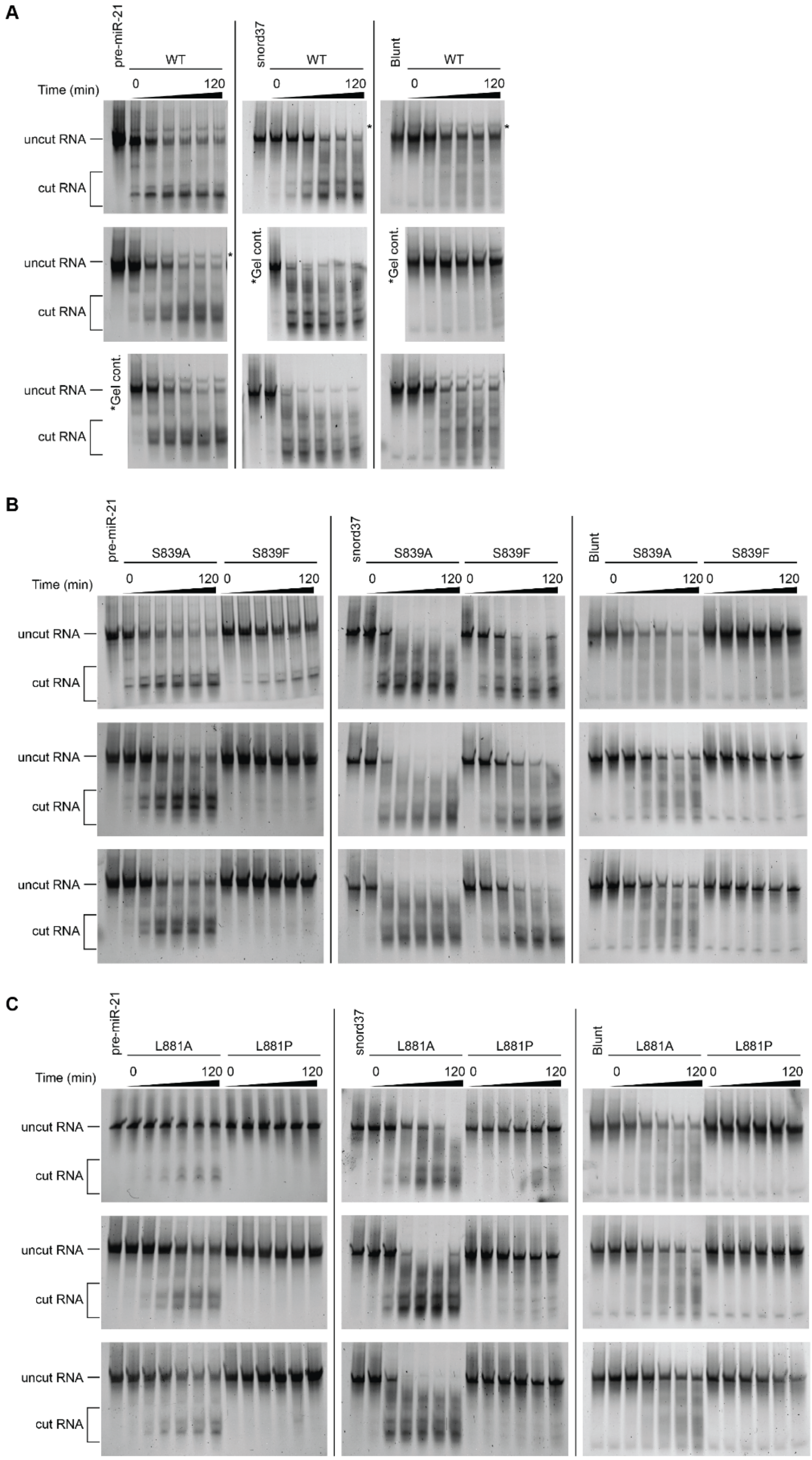
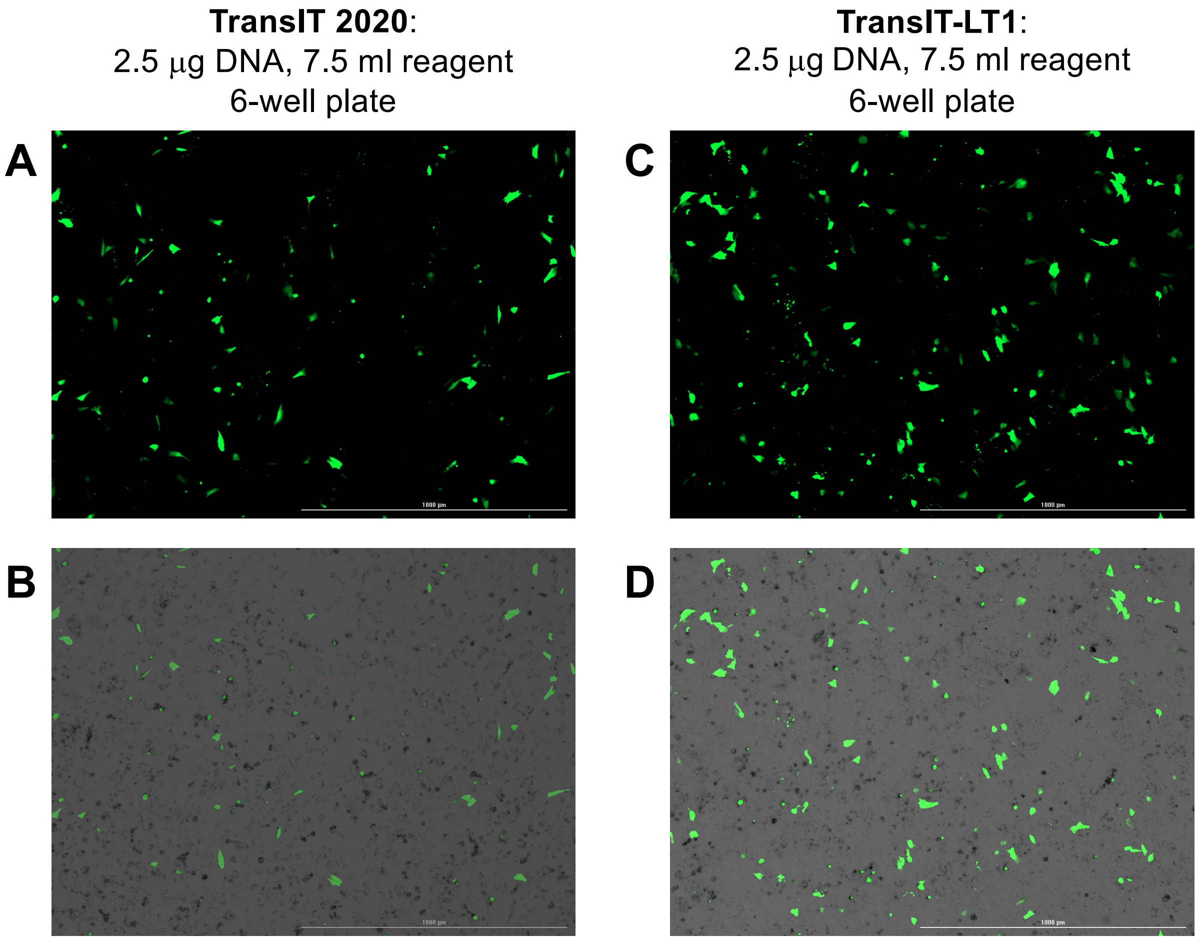


Figure S4. Replicates for Dicer cleavage assays in the presence of pre-miR-21, snord37 or Blunt-end pre-miR-21 (Blunt) for (A) WT Dicer, (B) S839 mutants and (C) L881 mutants. (* signifies that experiment was ran on the same gel)



TransIT 2020:
2.5 µg DNA, 7.5 ml reagent
6-well plate

TransIT-LT1:
2.5 µg DNA, 7.5 ml reagent
6-well plate

Figure S5. Transfection of Dicer1-/- mouse mesenchymal cells (CRL-3221™). A TurboGFP plasmid was used to identify optimal conditions. Of the transfection reagents tested (Lipofectamine 2000, 300, and LTX; TransIT 2020; and TransIT-LT1), only TransIT-LT1 was found to exhibit suitable transfection efficiency with minimal impact on cell viability. Representative comparative data are shown in this figure. GFP fluorescence and brightfield overlay for TransIT 2020 ((A) and (B)) and TransIT-LT1 ((C) and (D)).

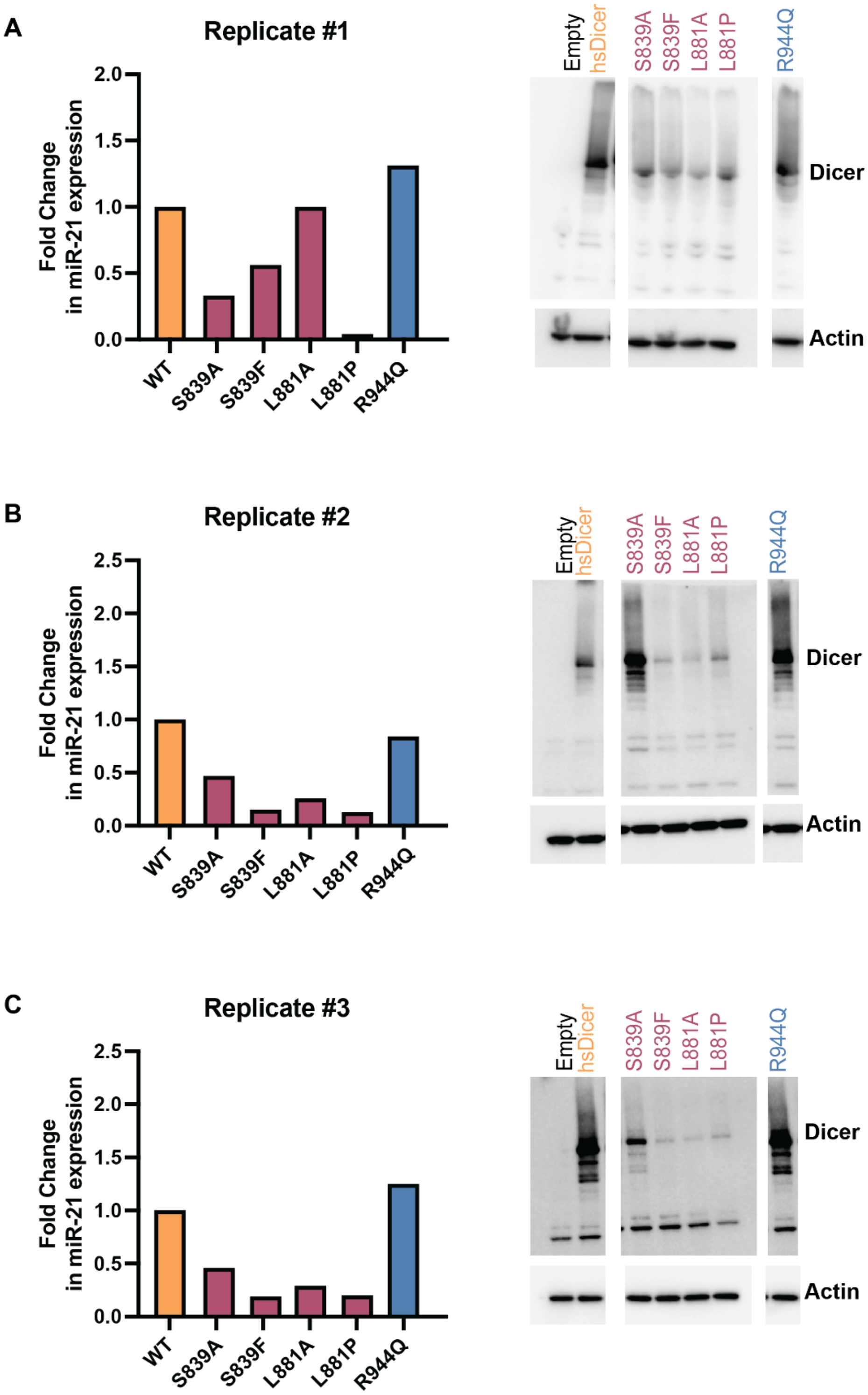


Figure S6. qRT-PCR replicates with corresponding Western blots showing relative protein levels of WT and mutant hsDicer. All sample were run on same gel, but the blots were cut to match the order of the corresponding qRT-PCR data.

Table S1. Gel quantification from EMSAs showing average across 3 independent experiments and the corresponding standard deviation (SDV)

WT Dicer

	pre-miR-21		snord37		Blunt pre-miR-21	
Time (min)	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV
0	0	0.00	0	0.01	0	0.01
10	28	2.61	20	4.29	21	1.39
30	28	1.67	20	2.09	24	1.93
60	27	2.15	20	2.17	20	4.18
90	27	2.47	20	1.43	23	2.69
120	28	4.58	21	1.86	24	1.24
	30	1.72	22	3.91	26	4.03

S839A

	pre-miR-21		snord37		Blunt pre-miR-21	
Time (min)	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV
0	0	0.12	0	0.20	0	0.02
10	21	6.67	18	1.51	13	5.84
30	26	4.11	17	0.63	14	6.20
60	21	2.84	19	1.52	14	5.58
90	27	4.30	19	3.61	14	5.81
120	25	4.65	16	1.86	15	6.50
	25	4.64	17	3.66	14	6.95

S839F

	pre-miR-21		snord37		Blunt pre-miR-21	
Time (min)	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV
0	0	0.12	0	0.20	0	0.02
10	3	1.67	13	2.11	6	3.33
30	4	1.08	15	0.44	5	2.32
60	5	3.09	15	1.76	7	2.83
90	5	3.25	17	3.40	7	4.89
120	5	2.33	16	4.93	8	4.50
	5	1.86	16	2.11	9	4.28

L881A

	pre-miR-21		snord37		Blunt pre-miR-21	
Time (min)	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV
0	0	0.01	0	0.11	0	0.05
10	9	4.31	22	5.62	14	2.71
30	10	4.64	21	5.58	14	3.77
60	11	4.19	23	5.89	13	4.24
90	12	4.04	20	5.95	14	2.49
120	11	3.51	21	5.13	14	3.72
	12	3.29	22	5.05	13	5.40

L881P

	pre-miR-21		snord37		Blunt pre-miR-21	
Time (min)	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV	Average Percent % Bound n=3	SDV
0	0	0.01	0	0.11	0	0.05
10	3	1.08	7	3.07	6	1.91
30	3	1.10	11	3.07	6	3.20
60	4	1.62	12	0.95	6	1.36
90	5	2.89	10	0.91	8	2.04
120	4	1.06	12	0.11	9	0.86
	4	1.68	11	1.46	9	1.29

Table S2. Gel quantification for cleavage assays showing average across 3 independent experiments and the corresponding standard deviation (SDV).

WT Dicer

	pre-miR-21		snord37	
Time (min)	Average Percent % Clevage n=3	SDV	Average Percent % Clevage n=3	SDV
0	0	0.02	0	0.03
10	15	7.68	6	1.39
30	49	4.15	66	40.23
60	68	3.49	77	30.86
90	77	2.80	92	5.24
120	81	7.01	88	8.98
	82	6.28	89	9.42

S839A

	pre-miR-21		snord37	
Time (min)	Average Percent % Clevage n=3	SDV	Average Percent % Clevage n=3	SDV
0	0	0.04	0	0.10
10	12	9.45	7	5.64
30	40	13.16	84	10.28
60	66	6.74	100	0.27
90	78	5.31	99	0.34
120	80	3.33	99	0.44
	76	2.23	99	0.51

S839F

	pre-miR-21		snord37	
Time (min)	Average Percent % Clevage n=3	SDV	Average Percent % Clevage n=3	SDV
0	0	0.04	0	0.10
10	0	0.20	1	0.81
30	3	3.60	29	6.14
60	8	8.27	61	10.95
90	13	12.09	89	5.22
120	16	17.36	96	3.02
	20	18.56	95	6.20

L881A

	pre-miR-21		snord37	
Time (min)	Average Percent % Clevage n=3	SDV	Average Percent % Clevage n=3	SDV
0	0	0.06	0	0.04
10	3	2.31	4	0.40
30	14	2.29	41	1.16
60	34	6.72	82	0.41
90	56	4.24	95	7.71
120	66	7.16	97	17.56
	68	5.37	98	10.38

L881P

	pre-miR-21		snord37	
Time (min)	Average Percent % Clevage n=3	SDV	Average Percent % Clevage n=3	SDV
0	0	0.06	0	0.04
10	0	0.07	1	3.08
30	0	0.35	1	36.75
60	0	0.09	4	23.20
90	1	1.00	13	7.27
120	3	4.36	24	3.16
	0	0.25	20	2.57

Table S3. Quantification of qRT-PCR from Figure S5 showing relative fold changes in miR-21 expression and corresponding calculations across three independent experiments. Calculations were performed after total RNA was normalized to U6 snRNA and then relative fold changes in miR-21 expression for Dicer mutants were calculated relative to normalized WT Dicer (AVG - Average; SD – Standard Deviation; SEM – Standard Error of Mean).

Dicer Construct	Am_4537 (miR-21)	Am_4548 (miR-21)	Am_4552 (miR-21)	AVG	SD	SEM
WT	1	1	1	1	0	0
S839A	0.33	0.47	0.46	0.42	0.08	0.09
S839F	0.56	0.15	0.19	0.30	0.23	0.16
L881A	1	0.26	0.29	0.52	0.42	0.22
L881P	0.04	0.13	0.2	0.12	0.08	0.09
R944Q	1.31	0.84	1.25	1.13	0.26	0.17

Table S4: Quantification of Western blots from Figure S5 showing relative protein levels and corresponding calculations across three independent experiments. Calculations were performed after WT Dicer was normalized to Actin and then relative protein levels for Dicer mutants were calculated relative to normalized WT Dicer protein levels (AVG - Average; SD – Standard Deviation; SEM – Standard Error of Mean).

Dicer Construct	Am_4537 (Protein level)	Am_4548 (Protein level)	Am_4552 (Protein level)	AVG	SD	SEM
WT	1	1	1	1	0	0
S839A	0.722	1.313	0.272	0.769	0.522	0.240
S839F	0.394	0.145	0.045	0.195	0.179	0.141
L881A	0.372	0.167	0.036	0.192	0.169	0.137
L881P	0.496	0.452	0.072	0.340	0.232	0.160
R944Q	1.313	1.846	1.090	1.417	0.388	0.207