SUPPLEMENTAL INFORMATION

Iron homeostasis regulates the activity of the microRNA pathway through poly(C)-binding protein 2

By

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Supplemental Experimental Procedures

Cells, viruses, antibodies, and reagents. 293FT cells, NIH3T3 cells, shLuciferase cells, shVector control cells, shGAPDH cells, shHtt cells, and RNAi-293-EGFP cells co-expressing EGFP and shEGFP were grown and maintained as described previously (Shan et al., 2008).

Antibodies were obtained from the following sources: monoclonal anti-MYC (46-0603, Invitrogen), polyclonal anti-human Dicer (3363S, Cell signaling) for Western blot, poly clonal anti-Dicer (NBP1-06520, Novus Biologicals) for immunoprecipitation, polyclonal anti-PCBP2 (ARP40568-T100, Aviva Systems Biology) for Western blot, monoclonal anti-PCBP2 (H00005094-M07, Abnova) for immunoprecipitation, polyclonal anti-GFP (A-6455, Invitrogen), monoclonal anti-HA (2367, Cell Signaling), monoclonal anti-human GAPDH (437000, Invitrogen), monoclonal anti-human Spectrin (sc-48382, Santa Cruz), and monoclonal anti-FLAG (F3165, Sigma).

Lipofectamine 2000 was purchased from Invitrogen. The reagents for SYBR Green or TaqMan gene expression-based real-time PCR were from Applied Biosystems. All the chemicals were purchased from Sigma. The following siRNA along with their corresponding scramble controls were used:

IRP1 siRNA (SI02779952, Qiagen)
IRP1 siRNA (SASI_HS01_00138944, Sigma)
IRP2 siRNA (SI00060123, Qiagen)
IRP2 siRNA (SASI_Hs01_00127700, Sigma)
PCBP1 siRNA (SI00301105, Qiagen)
PCBP1 siRNA (SASI_Hs01_00034329, Sigma)
PCBP2 siRNA (SI00678615, Qiagen)
PCBP2 siRNA (SASI_Hs02_00319506, Sigma)

PP1A siRNA (AM4616, Ambion)
LMNA siRNA (AM4619, Ambion)
PP1A shRNA (TRCN0000002455, Sigma)
LMNA shRNA (TRCN0000262764, Sigma)
Ferritin siRNA (SI00300251, Qiagen)
Ferritin siRNA (SASI_Hs01_00112824, Sigma)
TFR1 siRNA (SI02780715, Qiagen)
TFR1 siRNA (SASI_Hs02_00330586, Sigma)
Ferroportin siRNA (SASI_HS02_00345861, Sigma)
Ferroportin siRNA (SASI_Hs01_00224698, Sigma)

Small-molecule screen. RNAi-293-EGFP cells were infected with Lenti-RFP (Lentigen) and then subcloned to generate stable RNAi-293-EGFP/RFP reporter cells. Small molecule libraries, including both The Spectrum Collection and The Structural Diversity Set from NCI, were used for the chemical screen with RNAi-293-EGFP/RFP cells as described previously (Shan et al., 2008).

Luciferase assay. Transfections were performed as described previously and each sample was transfected in triplicate (Shan et al., 2008). Transfections were done in a final volume of 600 μl. Luciferase assays were performed using the Dual-Luciferase Reporter Assay System (Promega) and the Luminometer TD-20/20 (Turner Designs).

PAGE purification of mature and precursor forms of microRNAs. 10 μg of total RNA was heated at 65°C for 5 min and back to ice for 1 more min. The small RNA ladder (Illumina) was loaded onto the 15% PAGE supplemented with 7 M urea denatured gel, which has been pre-run for 30 min. The gel ran for 1 h at 200V, followed by recovery of the band of gel corresponding to 20-30–nt (for the mature form of siRNA or microRNAs) and 60-90–nt bands (for short hairpin or precursor of microRNAs) in the

ladder lane. The recovered gel bands were crushed and eluted with 500 µl of 0.3 M NaCl by rotating the tubes at room temperature for 4 h. The elutes and gel debris were transferred to the top of a Spin X cellulose acetate filter (Sigma) and spun down at maximum speed for 2 min. Precipitations were carried out with 1 µl of glycogen and 500 µl of isopropanol for at least 2 h at -80ºC. The precipitated small RNAs were spun down at 14000 rpm for 25 min at 4ºC, and the pellets were washed with 75% ethanol.

Reverse transcription. For the mature miRNA TaqMan assay, the 15-µl reverse transcription reaction consisted of 1 µg of total RNA isolated with TRIZOL (Invitrogen), 1x of specific RT primer, 5 U MultiScribe Reverse transcription, and 0.5 mM of each dNTP, 1× reverse transcription buffer, 4 U RNase inhibitor, and nuclease-free water. Reverse transcription reactions were incubated at 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min, and then stored at 4°C for TaqMan assays.

For miScript precursor assays (Qiagen), the cDNAs were synthesized with the miScript reverse transcription kit. In brief, The 20-µl reverse transcription reactions consisted of 1 µg of total RNA isolated with TRIZOL, 1× miScript RT buffer, 1×miScript Primer, 1 µl miScript Reverse Transcriptase Mix, and nuclease-free water, and incubated at 37°C for 60 min, 95°C for 5 min.

For assays of miRNA precursors and shRNA against GFP and luciferase, polyadenylation and reverse transcription were performed using the Ncode miRNA First-Strand cDNA Synthesis kit (Invitrogen) per the manufacturer's instructions.

Quantitative RT-PCR. The 10-µl TaqMan real-time PCR reaction consisted of 1× TaqMan Universal PCR Master Mix, 1× TaqMan miRNA assay, 3 µl of cDNA, and nuclease-free water. Each TaqMan assay was carried out in triplicate for each sample. Reactions were run with the standard 7500 default cycling protocol, with reactions incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min. Relative quantities of RNAs were determined using the ΔΔCt method as provided by the manufacturer.

Mature miRNAs expression levels were determined using individual TaqMan miRNA real-time PCR assays (Applied Biosystems) per the manufacturer's instructions. A RNU48 TaqMan assay served as the endogenous control.

The levels of processed siGFP (GAACUUCAGGGUCAGCUUGC) and siLuciferase (UUAAUCAGAGACUUCAGGCGG) were detected using custom TaqMan Small RNA assays (Applied Biosystems).

Metal substitution assay. RNAi-293-EGFP/RFP cells grown on regular DMEM supplemented with 10% heat-inactivated FBS were seeded into 96-well plates with FBS-free media overnight before metals were added. Metals, including iron, copper, zinc, nickel, maganese, and cobalt, were tested.

DNA constructs and transfection. FLAG-Dicer expression vector was described previously (Paroo et al., 2009). Full-length PCBP1 and PCBP2 cDNAs were cloned into pcDNA3 vectors containing MYC or HA tag. Full-length PCBP1 and PCBP2 cDNAs were also clone into pET-28a (+) to produce recombinant proteins.

Transfections were conducted in 293FT cells unless otherwise indicated with Lipofectamine 2000 (Invitrogen) per the manufacturer's instructions. The combinations of the plasmids include MYC-PCBP2/FLAG-Dicer, MYC-PCBP1/FLAG-Dicer, and MYC-PCBP12/HA-PCBP2. At 24 h post-transfections, the cells were split and treated under different conditions for another 36 h. The cells were collected for immunoprecipitation experiments. The lysis buffer contains 10 mM Hepes pH 7.4, 150 mM NaCl, 0.5% Triton X-100, and 1x protease inhibitor cocktail tablets (Roche).

Immunoprecipitation and Western blot. The cell lysates were prepared using lysis buffer (10 mM Hepes pH 7.4, 150 mM NaCl, 30 mM EDTA) containing 0.5% Triton X-100 and protease inhibitor cocktail (Roche). Lysis was carried out in ice-cold lysis buffer for 10 min on ice followed by sonication. Lysates were treated with RNase for 30 mins before immunoprecipitation was performed. Protein A agarose beads were used for IP as previously described (Shan et al., 2008). For Western blot, protein samples were separated on SDS-PAGE gels and then transferred to PVDF membranes (Millipore). Membranes were processed following the HyGLO QuickSpray Western blotting protocol (Denville).

MicroRNA profiling. Profiling of mature miRNA expression was performed using Applied Biosystems' TaqMan microRNA assays with 48-plex reverse transcription and individual TaqMan microRNA real-time PCR assays according to protocols provided by the vendor (Lao et al., 2006; Livak and Schmittgen, 2001; Szulwach et al.). Briefly, 8 pools of 48 reverse transcription primers each were used in 20-µl reactions consisting of: 20 ng total RNA, 1X TaqMan miRNA Reverse Transcription Primer Pool, 0.5 mM of each dNTP, 10.0 U/µl MultiScribe Reverse Transcriptase, 1X Reverse Transcription Buffer, 0.25 U/µl RNase Inhibitor, and nuclease-free water. The reactions were incubated at 16°C for 30 min, 42°C for 30 min, and 85°C for 5 min. Reactions were diluted 1:10 with nuclease-free water for use in the TaqMan real-time PCR reactions. Individual TaqMan microRNA real-time PCR reactions for profiling experiments were performed on an Applied Biosystems 7900HT SDS in a 384-well format running treated and control in parallel for each cDNA pool generated in the reverse transcription step. PCR reactions were carried out in triplicate for each sample and each miRNA. The 10-µl reactions consisted of 1X TaqMan Universal Master Mix, No AmpErase UNG, 1X TaqMan miRNA assay mix, 0.8 µl of 1:10 diluted cDNA, and nuclease-free water. All TaqMan PCR reactions were prepared and aliquoted using a custom method on a Beckman Coulter Biomek FX automated pipettor. PCR reaction conditions were run under the Standard protocol without the 50°C incubation using version 2.3 of the SDS software, with reactions incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, and 60°C for 1 min. Relative quantities (RQ) of miRNA were determined using the ΔΔCt method (Livak and Schmittgen, 2001). RQ values and the associated error were determined using Applied Biosystems SDS v1.2 RQ manager to calculate mean RQ and

RQ min/max values based on a 95% confidence interval. All paired samples were incorporated into a single analysis within the SDS v1.2 RQ manager to obtain the reported values. Reverse transcription primer pool-specific endogenous controls were chosen based on miRNA with the least variable expression among all samples tested. All relative quantity calculations were calibrated to mock-treated samples.

miRNA Northern blot

15 µl of 2X gel loading buffer mixed with 15 µg of total RNA in 15 ul of RNase-free water isolated from the cells treated w/o 2,2-Dipyridal @ 50 µM for 48 hr and was heated @ 95C for 2 min. The heated treated total RNA was then immediately transferred onto ice for at least 2 min before separation on the pre-run 15% PAGE TBE urea gel for 1hr @200V. The semi-dry blotting was conducted to transfer the total RNA from the gel to the positively charged Hybond Nitrocellulose membrane @ 150 V for 1hr at room temperature. Then the membrane was UV-crosslinked at 120mJ for 30 seconds. The pre-hybridization was carried out in the buffer containing 6X of SSC, 10X of Denhardt's, and 0.2% SDS at 65C for at least 1 hr. The RNA oligo primers complementary to the mature miRNAs were used for probe labeling with T4-Polynucleotide kinase (T4 PNK) from NEB (Cat# M0201L) in 25 µl of reaction containing 2.5 µl of 10X T4 PNK buffer, 2.5 µl of 10uM of oligo primer, 10 µl of ^{32}P-γATP at 10µci, and 1 µl of T4-PNK for 30 min at 37C followed by 70C for 10 min to kill the T4-PNK activity. After pre-hybridization, the labeled probe was added to the 5ml of hybridization buffer containing 6X of SSC, 5X of Denhardt's, and 0.2% SDS for hybridization at room temperature for 20 hr. After hybridization, 3X wash with wash buffer containing 6X of SSC and 0.2% SDS at room

temperature and 5 min for each wash followed by wash at 42C for two times and 20 min for each wash. The washed membrane was exposed to PhosphorImagerfor scanning.

Expression and purification of recombinant PCBP1 and PCBP2 protein in *E. coli*. Plasmid pET28a (+)-PCBP-2 was transformed into BL21 (DE3) competent cells. A single colony was inoculated into 20 mL of LB supplemented with 30 μg/mL kanamycin and incubated overnight for 14 h at 37°C. A 10-mL portion of overnight culture was transferred into 1000 mL LB supplemented with 30 μg/mL and grown at 37°C. Protein expression was induced with 0.5 mM isopropyl-β-o-thioglaactopyranoside (IPTG) at optical density of 0.6, and continued to grow for 18-20 h at 16°C. Cells were harvested, frozen in liquid nitrogen, and stored at -80°C before use. Cells were resuspended in 30 mL of buffer **A** (20 mM sodium hydrogen phosphate, 450 mM NaCl, 10 mM imidazole pH = 7.4) and lysed by sonication. Phenylmethylsulfonyl fluoride (PMSF) was added to a concentration of 1 mM before sonication. After centrifugation for 30 min at 12000 rpm, cell lysate was then loaded into a Ni-NTA column pre-equilibrated with buffer **A**, washed with 10–15 column volume of buffer A and 7% buffer B, and eluted with a linear gradient of 0 to 100% buffer **B** (20 mM sodium hydrogen phosphate, 450 mM NaCl, 500 mM imidazole pH 7.4). The purity and identity of protein was determined with 12% SDS-PAGE. Fractions containing PCBP-2 were collected and concentrated. PCBP2 was further purified by gel filtration chromatography using sephadex G-100 column pre-equilibrated with 20 mM Tris-HCl pH 8.0, 400 mM NaCl, 10% glycerol. It was necessary to maintain a NaCl concentration over 400 mM during purification steps to avoid the co-purification of DNA with protein. PCBP1 was purified similarly.

RNA filter binding assay. *Let-7* precursor RNA oligos were described previously and synthesized by Dharmacon, which is a synthetic *Drosophila* pre-*let-7* bearing the characteristic end structure created by *Drosha* processing of pri-miRNA (5′-UGAGGUAGUAGGUUGUAUAGUAGUAAUUACACAUCAUACUAUACAAUGUGCUAGCUUUCU-3′) (Forstemann et al., 2005). Pre-let-7 with loop region deleted (pre-let-7-deletion (5′-UGAGGUAGUAGGUUGUAUAGUAUACUAUACAAUGUGCUAGCUUUCU-3′) along with human miR-30a precursor (5'-GCGACUGUAAACAUCCUCGACUGGAAGCUGUGAAGCCACAGAUGGGCUUUCAGUCGGAUGUUUGCAGCUGC-3') were synthesized by Dharmacon. They were radioactively labeled at the 5' end with ^{32}P-g-ATP using T4 polynucleotide kinase (New England Biolabs). The labeled precursor was allowed to refold by heating at 95°C for 2 min, followed by incubation at 37°C for 1 h. An RNA filter binding assay was performed as described previously using PCBP2 (Shan et al., 2008). In each reaction, 200 nM of PCBP2 was used for the binding reaction at 30°C for 30 min, followed by filtration and scintillation counting.

In vitro miRNA precursor processing assay. The above-labeled *Let-7* precursor was incubated with Dicer or immunoprecipitated Dicer with PCBP1 or PCBP2, in the buffer as described previously for 1 h (Forstemann et al., 2005). The previously described recombinant Dicer was used (Paroo et al., 2009). The reaction was terminated and subjected to phenol/chloroform extraction and ethanol precipitation. Then the samples were separated on a 15% TBE urea gel, transferred and UV cross-linked to nylon

membrane, which was then exposed for PhosphorImager scanning. Processed precursors were quantified using Kodak MI software. Three independent experiments were performed and used to calculate mean and SD.

Statistical methods. We used single factor ANOVA analysis to show significant differences between control and metal chelator treatment. We performed post-hoc *t*-test (two-samples assuming equal variances) to determine significance, and indicated *P* value. For all the presented data except noted otherwise, we repeated at least three times and triplicate samples were analyzed each time.

References:

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SUPPLEMENTAL FIGURE LEGENDS

Figure S1. **Metal chelator enhances the gene knockdown by shRNAs. A.** Chemical structures of four small molecules with iron chelating activity identified via chemical screen are shown. **B.** Left: The 293FT cells stably expressing human huntingtin 120Q were transfected with shRNA against Htt and treated with 2,2'-dipirydyl or DMSO for 48 hr before detection of the Htt mRNA levels using quantitative RT-PCR. Metal chelator has no effect on Htt expression in shVC control cells. Values are mean ± SD for triplicate samples. *: $P < 0.001$. Right: The shVC and shLuciferase cells were treated with DMSO or 2,2'-dipirydyl for 48 hr. Luciferase activity for each condition was measured. Metal chelator has no effect on luciferase activity in shVC control cells. Values are mean ± SD for triplicate samples. *: $P < 0.001$. **C.** Iron chelator enhances shRNA-mediated RNAi and promotes miRNA processing in hepatic cells. Iron chelator enhances shRNA-mediated RNAi. Knockdown of each mRNA was graphed as the percentage of mock-treated samples in the presence or absence of metal chelators (2,2'-dipyridyl) as well as its isoform 4,4- bipyridyl. Values are mean ± SD for triplicate samples. *: $P < 0.001$. **D.** Iron chelator promotes the processing of miR-125b and miR-203 in HepG2 cells. Values are mean ± SD for triplicate samples. *: $P < 0.001$.

Figure S2. **Cytosolic iron is involved in the modulation of the miRNA pathway.** **A.** Quantification of total Fe level in HEK293 cells and HepG2 cells

treated with DMSO, 4,4'-bipyridyl and 2,2'-dipyridyl. **B.** Quantification of heme in HEK293 cells treated with DMSO and succinylacetone (SA). Values are mean ± SD for triplicate samples. *: $P < 0.001$. **C.** Heme has no effect on shRNA-mediated RNAi knockdown efficiency. The EGFP RNAi reporter cells seeded in FBS-free media were treated with succinylacetone (SA) for 24 h to deplete intracellular heme. The SA-treated cells were then supplemented with heme in a range of doses from 0.1-5 μM. The relative GFP/RFP ratio is shown. **D.** Knockdown of the heavy chain subunit of ferritin could reduce the knockdown efficiency of shRNA-mediated RNAi. The cells stably overexpressing shLuciferase were transfected with Luciferase reporter construct along with siNC (control siRNA) or siFerritin. The cells were analyzed at 48 h post transfection. Values are mean ± SD for triplicate samples. *: $P < 0.001$. **E.** Knockdown of transferrin receptor 1 (TFR1) could enhance the knockdown efficiency of shRNA-mediated RNAi. The cells stably overexpressing shLuciferase were transfected with luciferase reporter construct along with siNC (control siRNA) or siTfr1. The cells were analyzed at 48 h post-transfection. Values are mean ± SD for triplicate samples. *: $P < 0.001$. **F.** Western blots show the knockdown efficiency with each specific siRNA with GAPDH as a loading control. **G.** Level of ferroportin, an iron exporter, could modulate the activity of RNAi pathway. The cells stably overexpressing shLuciferase were transfected with Luciferase reporter construct along with pCDNA vector alone, pCDNA-

Ferroportin or siRNAs against ferroportin. The cells were analyzed at 48 h post transfection. Values are mean±SD for triplicate samples. *: $P < 0.001$.

Figure S3. **Iron chelator promotes the processing of shRNAs and microRNAs. A.** Quantitative RT-PCR was used to measure the levels of processed siRNA and shRNA in the cells expressing shRNA against luciferase. 5S was used as loading control. Values are mean ± SD for triplicate samples. *: $P < 0.001$. **B-E.** Quantitative RT-PCR was used to measure the levels of endogenous pri-, pre- and mature forms of miR-96, miR-182, miR-200b and miR-30a in mock and iron chelator treated HEK-293 cells. The relative expression levels as determined by ΔΔCt analyses are shown. Values are mean ± SD for triplicate samples. *: $P < 0.001$.

Figure S4. **Cytosolic iron modulates the processing of pre-miRNAs. A.** Detection of pre- and mature miRNAs by Northern blot. Northern blots were performed using the RNA oligo primers complementary to the mature miRNAs. 5S was used as loading control. **B.** Iron chelator treatment could enhance miRNA-mediated gene regulation. HEK293 cells expressing miR-137 were transfected with psiCHECK-2-Ezh2-3'UTR in the presence or absence of iron chelator as described previously (Szulwach et al, 2010). Iron chelator could enhance the processing of miR-137 (top) and miR-137-mediated suppression on Ezh2 3'-UTR reporter gene expression (bottom). **C.** Iron chelator treatment could enhance mi125a-mediated

regulation on endogenous p53 protein. Western blot was used to determine the protein level of p53 in mock- and iron chelator-treated HEK293 cells expressing the primary transcript of miR-125a. GAPDH was used a loading control. **D.** Heme has no effect on the miRNA processing in vivo. The cells stably overexpressing the primary transcript of miR-125a seeded in FBS-free media were treated with different concentrations of succinylacetone (SA) for 24 h to deplete intracellular heme. The levels of mature, pre- and pri-miR-125a were determined by real-time RT-PCR.

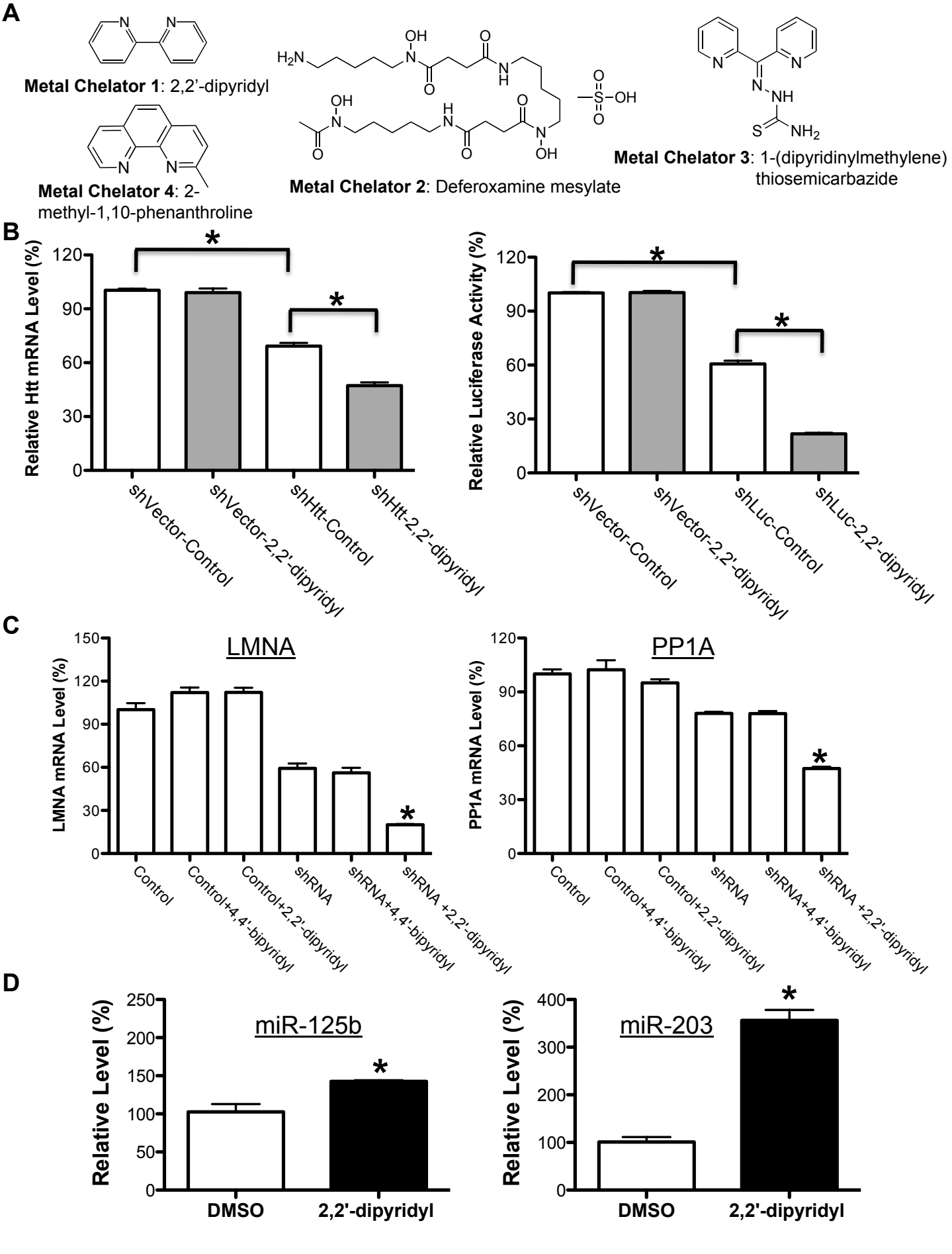
Figure S5. **PCBP2 is associated with Dicer, and involved in the RNAi-enhancing activity mediated by iron chelator. A.** PCBP1 is not associated with Dicer. PCBP1 could not be coimmunoprecipitated with Dicer in the presence or absence of iron chelators. Similar coimmunoprecipitation experiments were performed using MYC-PCBP1 and FLAG-Dicer expression vectors as described in Figure 4A. **B.** Western blots show the knockdown efficiency with each specific siRNA with GAPDH as a loading control. **C.** Knockdown of PCBP2 inhibits the shRNA-mediated RNAi. Left: The GFP mRNA levels in the RNAi reporter cells that were transfected with the siRNAs against PCBP2 were determined by quantitative RT-PCR. *: $P < 0.001$. Right: The cells expressing the shRNA against luciferase were transfected with siRNA against PCBP2 or control siRNA (siNC) for 48 hr and then collected for luciferase assay. *: $P < 0.001$. **D.** The knockdown efficiency of PCBP2 by siRNA against

PCBP2. Values are mean ± SD for triplicate samples. *: $P < 0.001$. **E.** Knockdown of PCBP2 blocks the processing of miRNA precursor**.** Knockdown of PCBP2 affects the processing of additional miRNAs. The left panels-mature miRNA levels; the middle panels-pre+pri-miRNA levels; and the right panels-pri-miRNA levels. Values are mean ± SD for triplicate samples. *: $P < 0.001$.

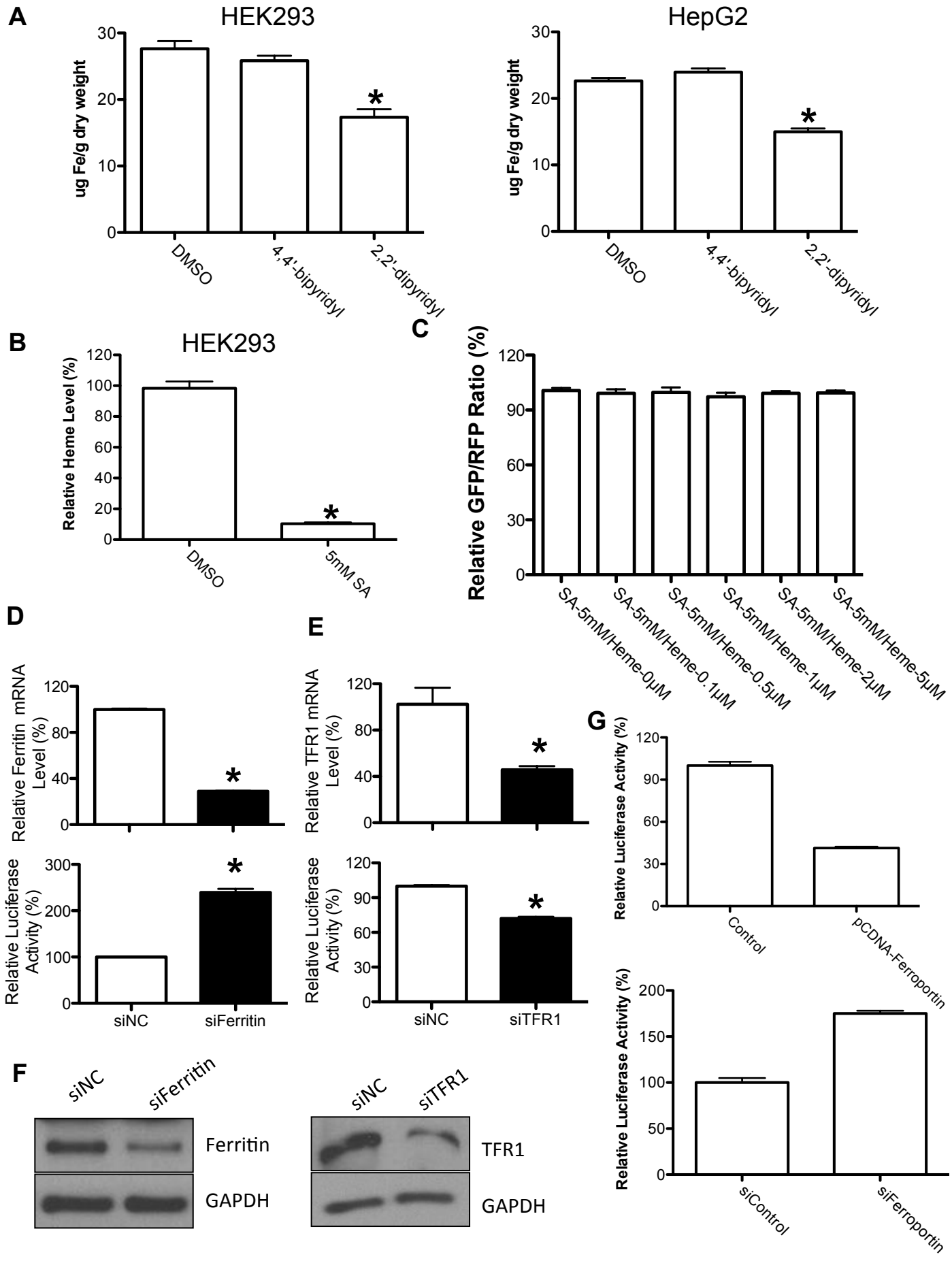
Figure S6. **PCBP2 modulates the Dicer-mediated miRNA processing. A.** Iron chelator increases the miRNA precursor associated with PCBP2 in vivo. The immunoprecipitated complex using anti-MYC antibody as shown in Fig. 5a was used to determine the amount of miR-125a precursor associated with PCBP2. Values are mean ± SD for triplicate samples. **B.** The inhibition of PCBP2-RNA interaction by iron could be abolished by iron chelator. The panel shows that the inhibition of PCBP2-RNA interaction by iron could be abolished by the iron chelators 2,2'-dipyridyl (50 mM), but not by its inactive isomer 4,4'-bipyridyl (50 mM). *: $P < 0.001$ when 2,2'-dipyridyl was compared with control (DMSO) and 4,4'-bipyridyl in the presence of iron. **C.** The loop region of *pre-let-7* is critical for PCBP2-pre-miRNA interaction. Deletion of the loop region (*pre-let-7* deletion) abolished the binding of PCBP2 to *pre-let-7*. RNA filter-binding assay with recombinant PCBP2 protein (200 nM) and same amount of 5'-^{32}P-labeled *let-7* precursor or deletion mutant is shown. Values are mean ± SD for triplicate samples. **D.** PCBP1 could not enhance the processing of

miRNA precursor by Dicer. *In vitro* Dicer cleavage assay was performed as described in Figure 6C with recombinant PCBP1. Both the precursor and processed miRNAs are indicated. **E.** Iron has no effect on Dicer activity. *In vitro* Dicer cleavage assay was performed in the presence or absence of iron. Both the precursor and processed miRNAs are indicated. **F.** PCBP2 does not destabilize *pre-let-7*. Different amounts of recombinant PCBP2 protein were incubated with 5'-^{32}P-labeled *let-7* precursor and no mature *let-7* was produced. **G.** PCBP2 enhances the processing of human pre-miR-30a by Dicer. Shown are the averages of relative cleavage activity from three independent experiments. *: $P<$ 0.001. **H.** Mutant PCBP2 is not associated with Dicer. Mutant PCBP2 used in Figure 6E could not be coimmunoprecipitated with Dicer. Similar coimmunoprecipitation experiments were performed using HA-Mutant PCBP2 and FLAG-Dicer expression vectors as described in Figure 4A.

Supplemental Figure 1-Li et al.



Supplemental Figure 2-Li et al.



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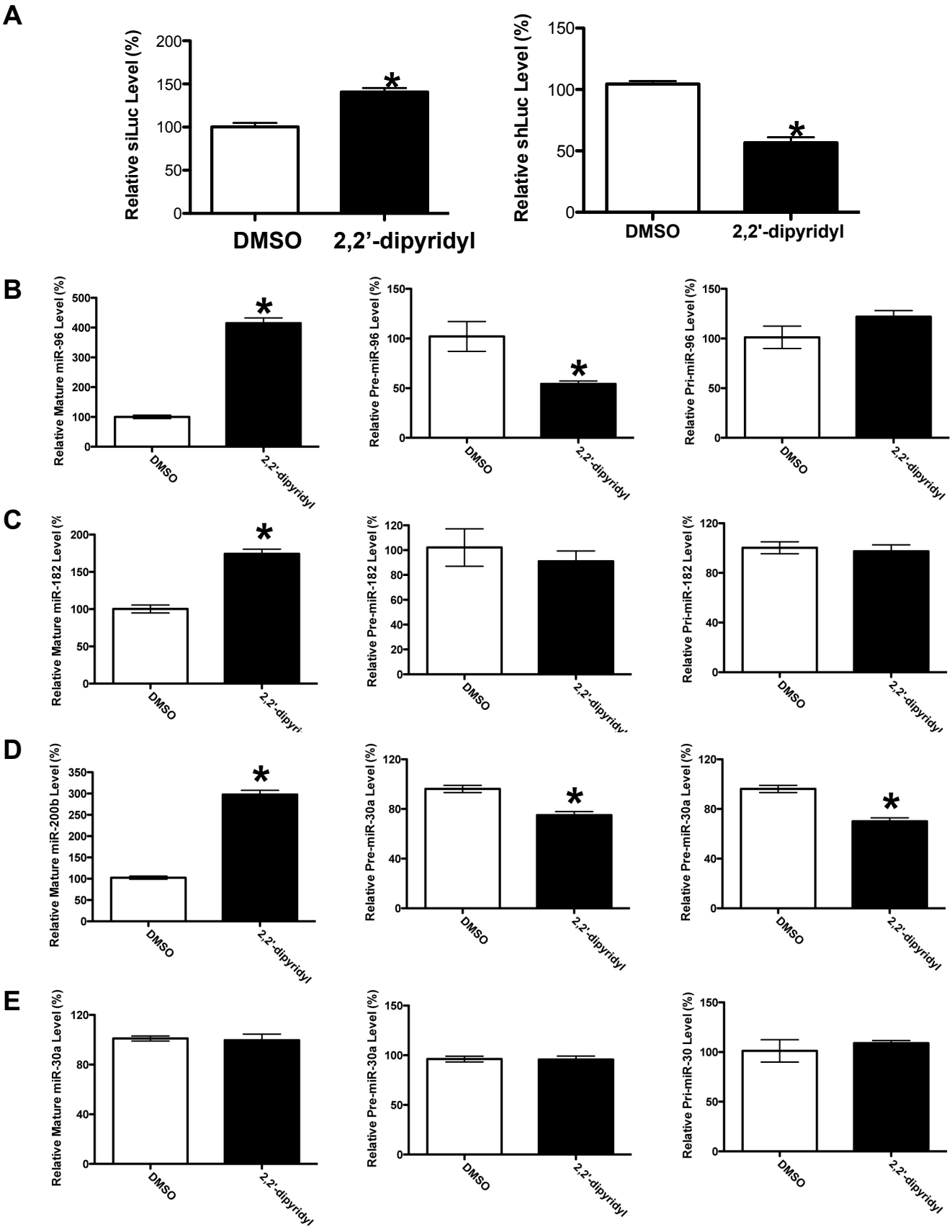
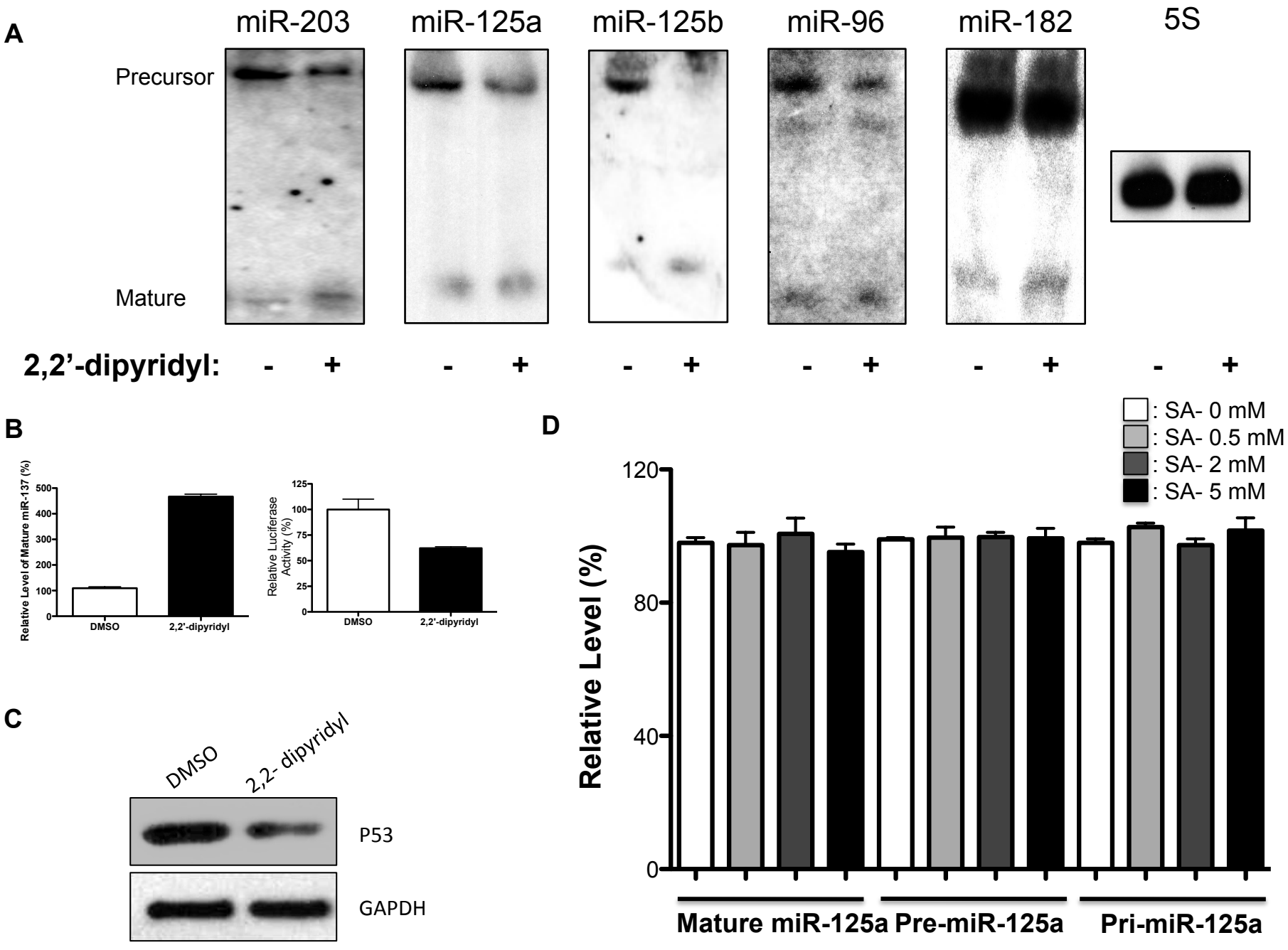


Supplemental Figure 4-Li et al.



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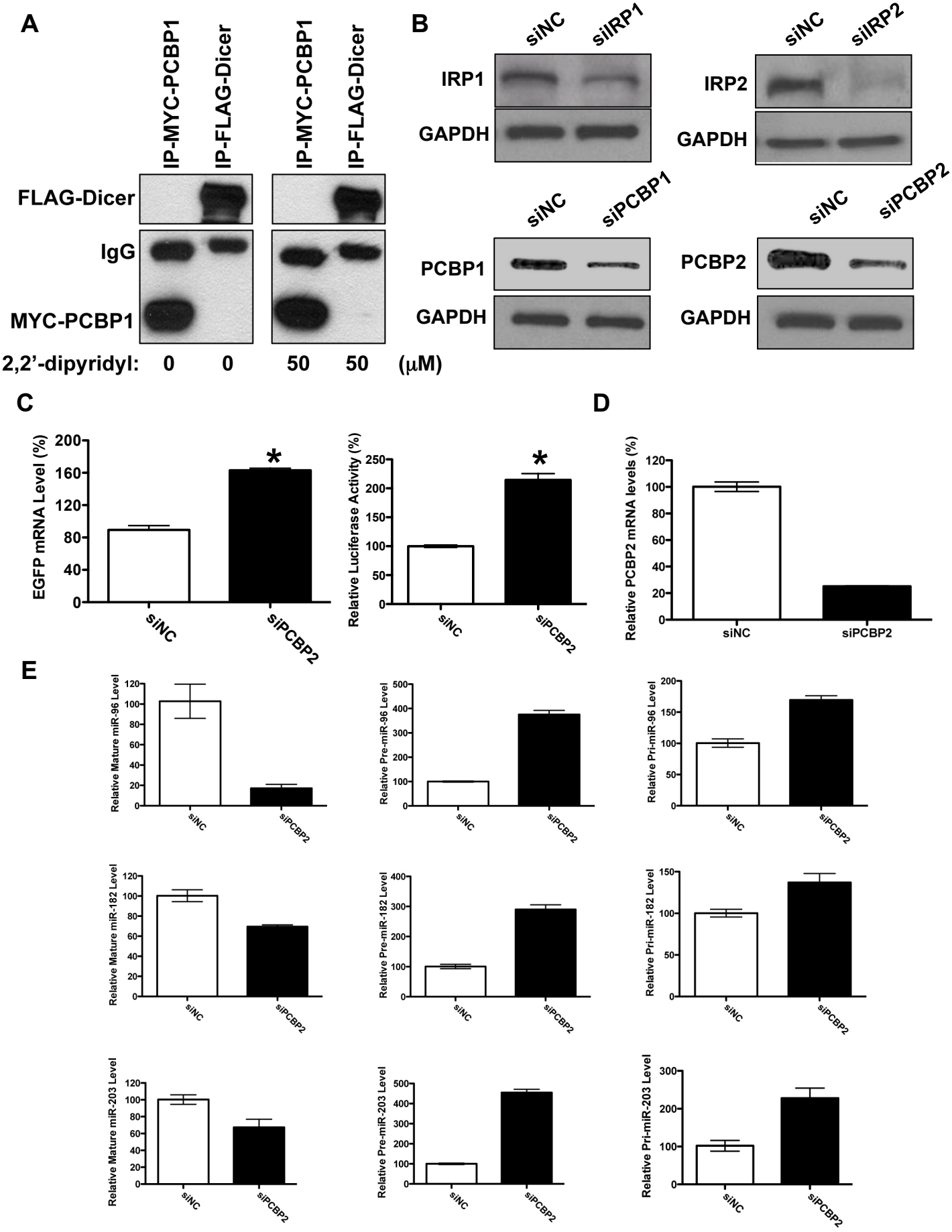
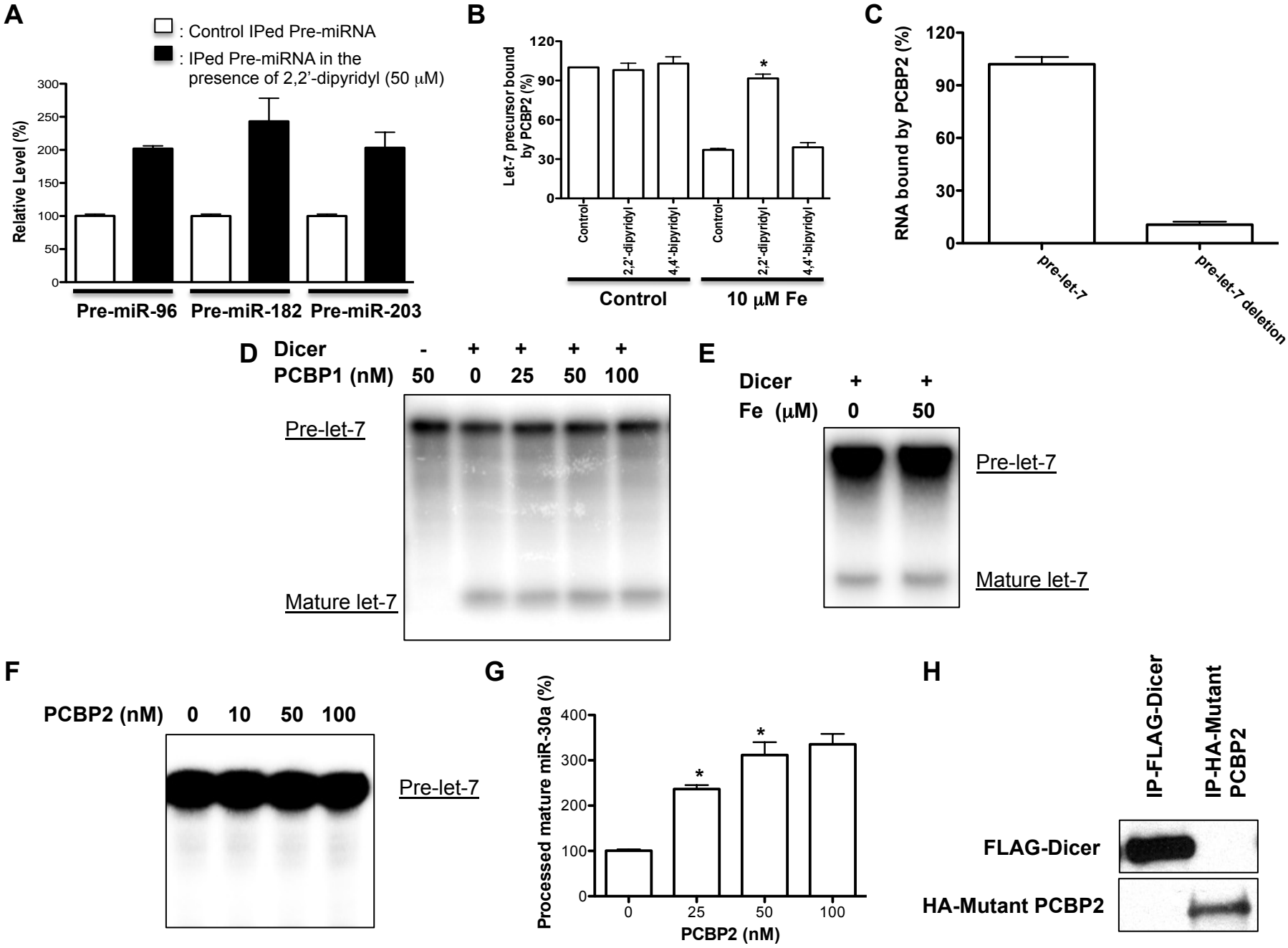


Supplemental Figure 6-Li et al.



Supplemental Table 1-Li et al.

Average Ct for Different MiRNA Precursors Determined by miScript Precursor Assay from Qiagen

MiRNAs-Mature forms are upregulated by iron chelator

Precursor	***Ct***
MiR-125a	26.65
MiR-203	24.5
MiR-96	27.3
miR-182	26.8

MiRNAs-Mature forms remain unchanged with the addition of iron chelator

Precursor	***Ct***
MiR-30a-5p	36.5
MiR-30a-3p	36.5
MiR-15a	35.8
MiR-20	Undetectable

RNAs with Ct over 35 cycles are generally considered low abundance.