SUPPLEMENTARY FIGURES AND FIGURE LEGEND

Figure S1. (Linked to Figure 1)

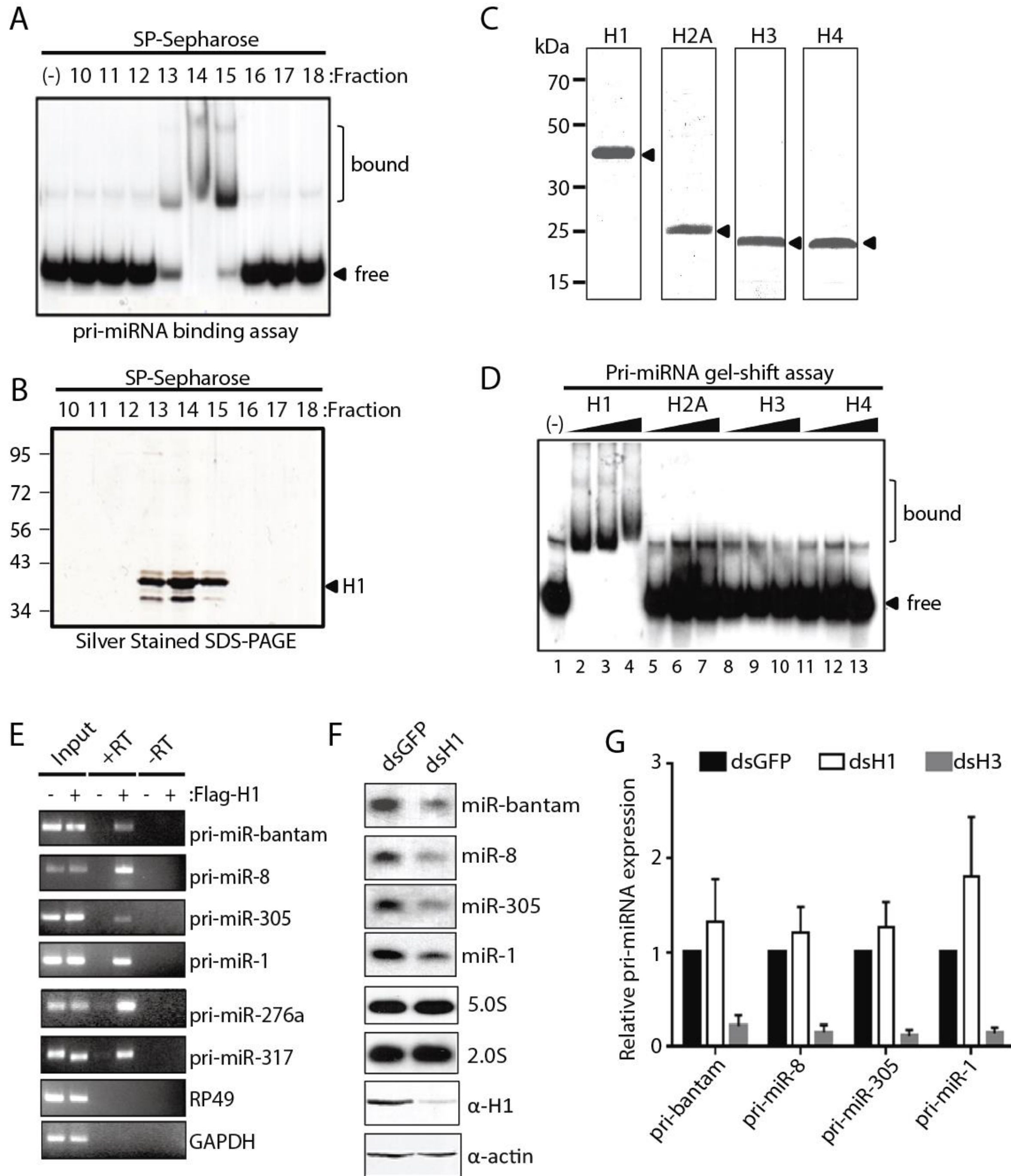

Figure S1 (Linked to Figure 1)

(A-B) Purification of histone H1 through a three-step chromatographic procedure (Nuclear S100 → SP-Sepharose → Q-Sepharose → SP-Sepharose). Following the final column, individual fractions were assayed for pri-miRNA binding activity (A) or resolved by SDS-polyacrylamide gel (PAGE) followed by silver staining (B).

(C) A Coomassie-stained SDS-PAGE showing purified His_6-tagged H1, H2A, H3, and H4 recombinant proteins.

(D) Native gel-shift assays were performed by incubating radiolabeled pri-miRNA in buffer alone (lane 1) or with 50, 100 and 200 nM of recombinant fly H1 (lanes 2-4), H2A (lanes 5-7), H3 (lanes 8-10) and H4 (lanes 11-13). Arrow and bracket mark the positions of free and H1-bound pri-miRNA, respectively.

(E) Forty-eight hours after transfection of S2 cells with Flag- H1 construct, RNA immunoprecipitation (RIP) assays were performed using anti-Flag beads followed by RNA extraction and semi-quantitative RT-PCR to detect the H1-associated endogenous pri-miRNAs, but not abundant RP49 or GAPDH pre-mRNA.

(F) Following dsRNA-mediated knockdown of H1 expression in S2 cells, Northern blotting was performed to measure the levels of mature miR-bantam, miR-8, miR-305, and miR-1. 5.0S and 2.0S rRNA were used as a loading controls. Western blotting was conducted to detect H1 and Actin proteins.

(G) Quantitative analysis of pri-miRNA expression by real time RT-qPCR in GFP, H1 and H3 dsRNA-treated S2 cells (triplicate experiments, data presented as Mean ± SD).

Figure S2 (Linked to Figure 1)

A

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H1.0     24 DH KYSDMIVAAIQAEKNRAGSSRQSIQKYIKSHYK--VGENADSQIKLSIKRLVTTGVLKQT---KGVGASGSFRLA 96
H1.1     39 AG SVSELIVQAASSSKERGGVSLAALKKALAAAGY--DVEKNNSRIKLGIKSLVSKGTLVQT---KGTGASGSFKLN 111
H1.2     36 SG  VSELITKAVAASKERSGVSLAALKKALAAAGY--DVEKNNSRIKLGLKSLVSKGTLVQT---KGTGASGSFKLN 108
H1.3     37 SG  VSELITKAVAASKERSGVSLAALKKALAAAGY--DVEKNNSRIKLGLKSLVSKGTLVQT---KGTGASGSFKLN 109
H1.4     36 SG  VSELITKAVAASKERSGVSLAALKKALAAAGY--DVEKNNSRIKLGLKSLVSKGTLVQT---KGTGASGSFKLN 108
H1.5     39 TG  VSELITKAVAASKERNGLSLAALKKALAAGGY--DVEKNNSRIKLGLKSLVSKGTLVQT---KGTGASGSFKLN 111
H1X      44 QPGKYSQLVVETIRRLGERNGSSLAKIYTEAKKVPW-FDQQNGRTYLKYSIKALVQNDTLLQV---KGTGANGSFKLN 117
H1t      40 PNLSVSKLITEALSVSQERVGMSLVALKKALAAAGY--DVEKNNSRIKLSLKSLVNKGILVQT---RGTGASGSFKLS 112
H1T2     56 SVLRVSQLVLQAISTH---KGLTLAALKKELRNAGY----EVRRKSGRHEAPRGQAKATLLRV---SGSDAAGYFRVW 123
HILS1   113 QK STSKVILRAVADKGTCKYVSLATLKKAVSTTGY--DMARNAYHFKRVLKGLVDKGSA------------GSFTLG 176
H1oo     51 RH  VLRMVLEALQAGEQRRGTSVAAIKLYILHKYPTVDVLRFKYLLKQALATGMRRGLLARPLNSKARGATGSFKLV 128
HP1BP3  157 PR KMDAILTEAIKACFQKSGASVVAIRKYIIHKYPSLELERRGYLLKQALKRELNRGVIKQV---KGKGASGSFVVV 231
HP1BP3  255 PQVKLEDVLPLAFTRLCEPKEASYSLIRKYVSQYYPKLRVDIRPQLLKNALQRAVERGQLEQI---TGKGASGTFQLK 329
HP1BP3  338 GGSLMEYAILSAIAAMNEPKT#STTALKKYVLENHPGTNSNYQMHLLKKTLQK#EKNG!MEQI---SGKGFSGTFQLC 412
fly H1   45 SH  TQQMVDASIKNLKERGGSSLLAIKKYITATYK-CDAQKLAPFIKKYLKSAVVNGKLIQT---KGKGASGSFKLS 118
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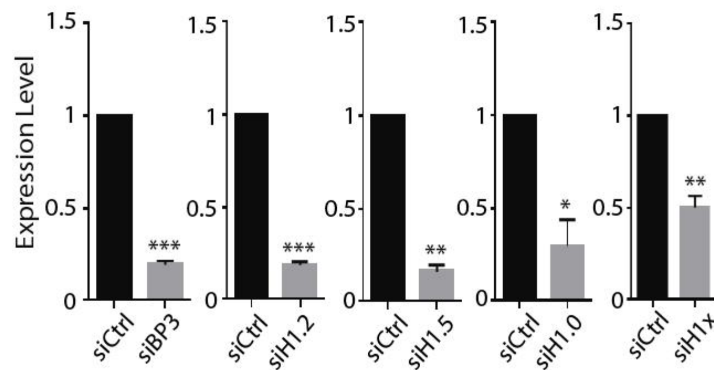

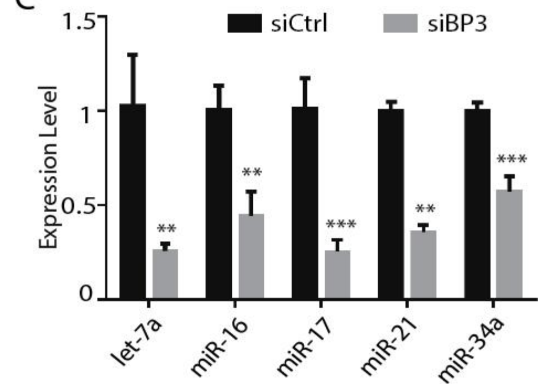

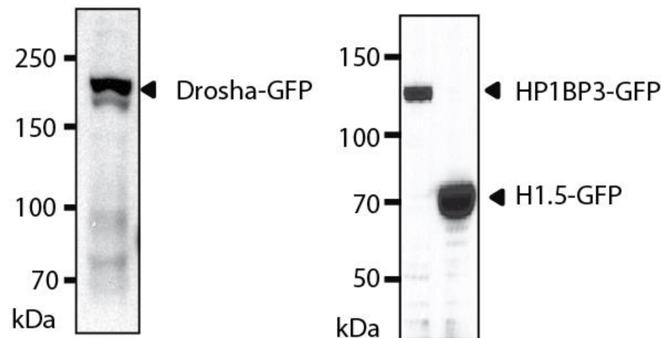

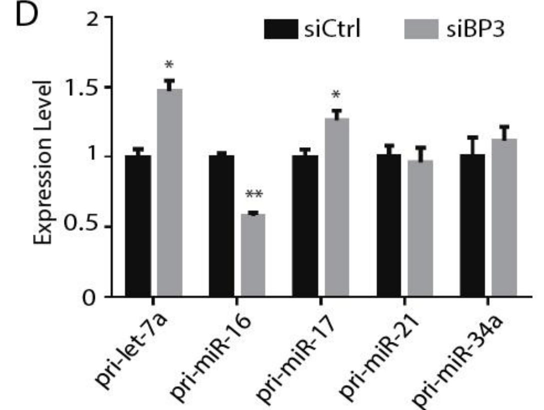

F

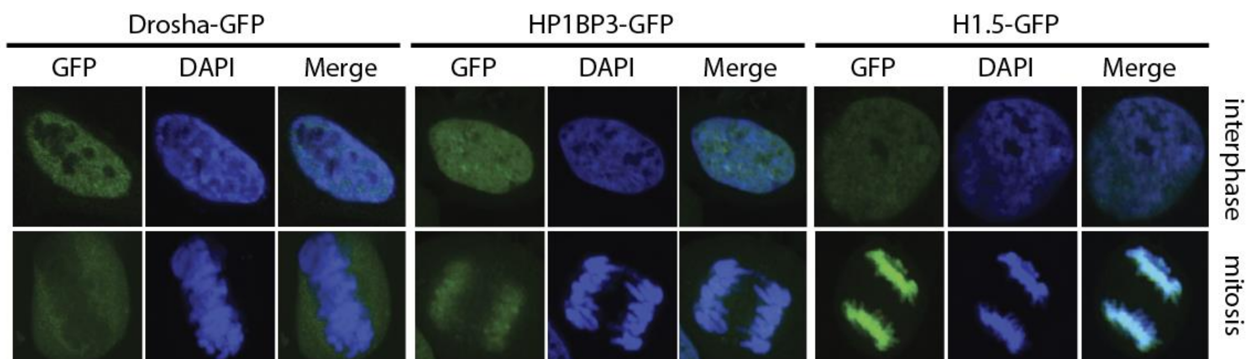

Figure S2 (Linked to Figure 1)

(A) A multi-sequence alignment of the central globular domains (GD) of 11 human H1 variants, fly H1, and the three GDs of human HP1BP3.

(B) Following siRNA-mediated knockdown in HeLa cells, the level of HP1BP3, H1.0, H1.2, H1.5, or H1x mRNA was respectively measured by real time RT-qPCR. Paired t-test was used for statistical analysis (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

(C, D) Following siRNA-mediated knockdown of HP1BP3 in U2OS cells, the levels of miRNA (C) and pri-miRNA (D) were measured by Taqman qPCR, respectively (triplicate samples, data shown as mean ± SD). Paired t-test was used for statistical analysis (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

(E) Western blotting was performed using anti-GFP antibody to detect the expression of Drosha-GFP, HP1BP3-GFP and H1.5-GFP in different BAC-transgenic HeLa cells.

(F) Confocal microscopy images showing the localization of Drosha-GFP, HP1BP3-GFP and H1.5–GFP (green) in the interphase or mitosis phase of BAC transgenic HeLa cells. DNA was stained with DAPI (blue).

Figure S3 (Linked to Figure 2)

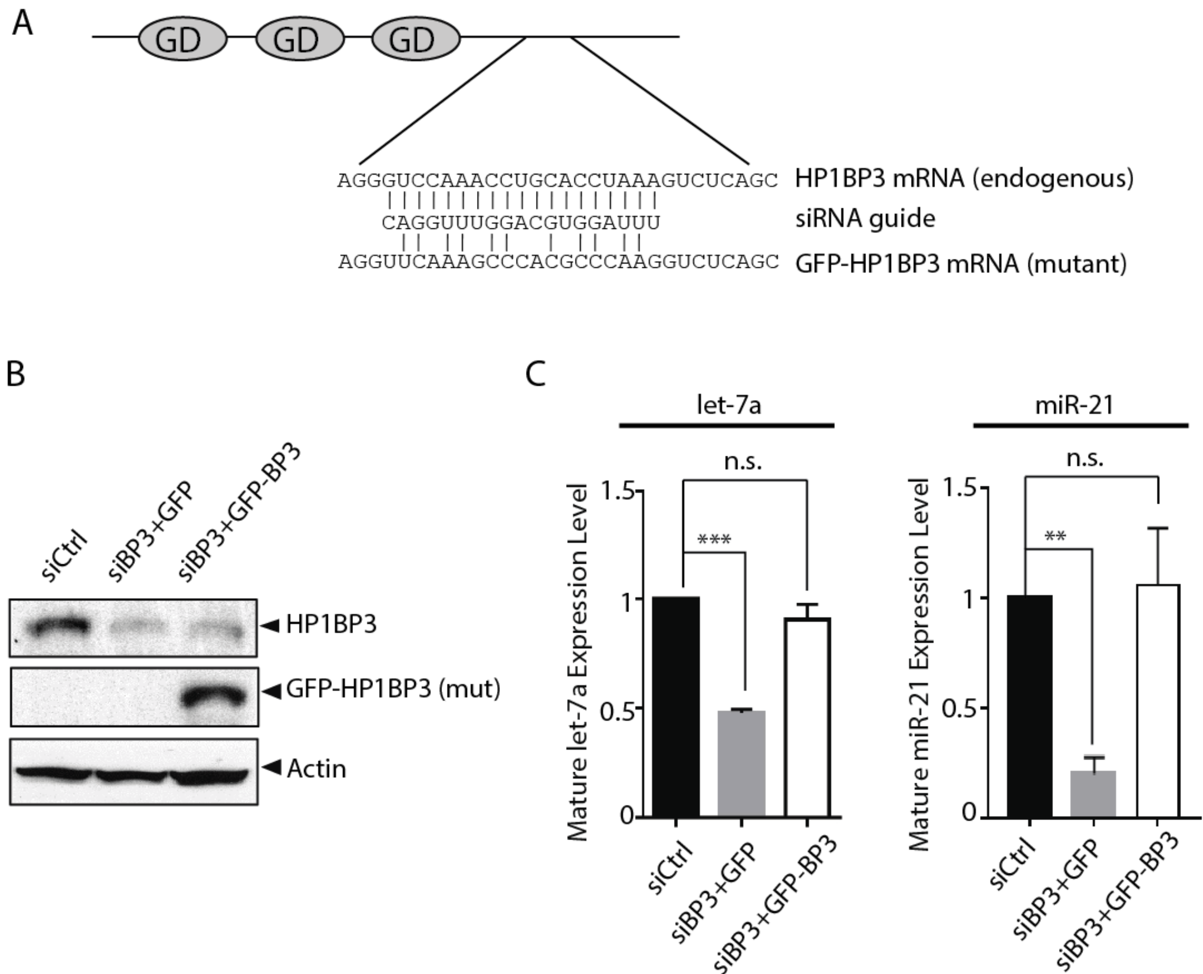

Figure S3 (Linked to Figure 2)

(A) A schematic diagram of base pairing between the siRNA guide RNA with wild-type or mutant (mut) HP1BP3 mRNA sequence.

(B, C) HeLa cells were co-transfected with control (siCtrl) or HP1BP3 siRNA (siBP3) together with GFP or HP1BP3-GFP (mut) construct. After seventy-two hours post transfection, Western blotting was performed to detect Actin, HP1BP3 and HP1BP3-GFP proteins (B). The levels of mature miRNAs (let-7a and miR-21) were measured by Taqman qPCR (triplicate samples, data shown as mean ± SD). Paired t-test was used for statistical analysis (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

Figure S4 (Linked to Figure 3)

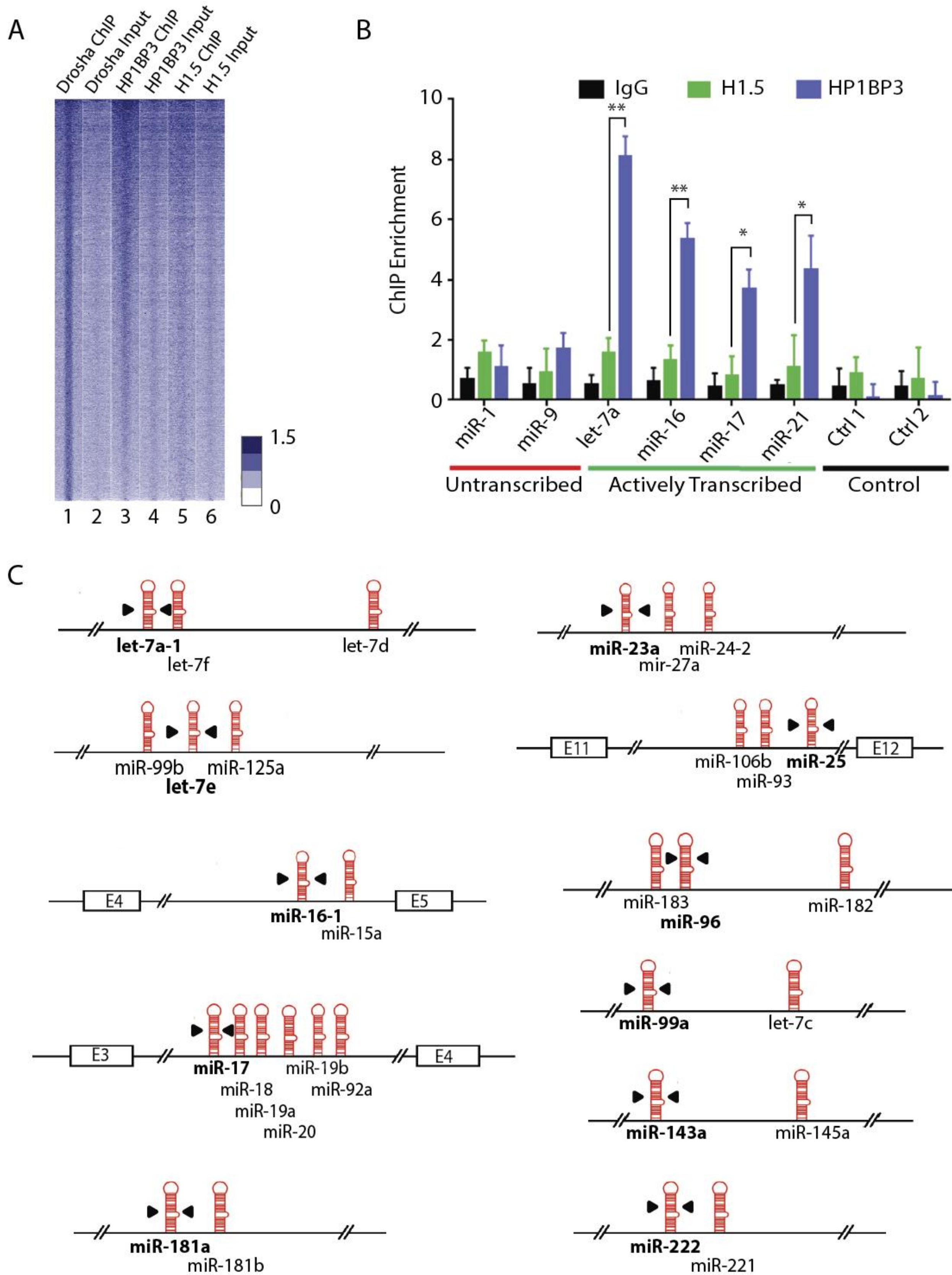

Figure S4 (Linked to Figure 3)

(A) A global view of the ChIP-Seq read density heatmap of Drosha-GFP islands throughout the genome. *X*-axis represents the distance to island center. In *Y*-axis, each row in the heatmap represents one island.

(B) ChIP analysis comparing chromatin binding of H1.5-GFP and HP1BP3-GFP at the stem-loop regions of several inactive (miR-1, miR-9) and actively transcribed miRNA loci (let-7a, miR-16, miR-17, miR-21). Paired t-test was used for statistical analysis (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

(C) A schematic diagram of the eleven polycistronic miRNA genes that are expressed in HeLa cells. The chosen miRNAs that we examined are in bold.

Figure S5 (Linked to Figure 4)

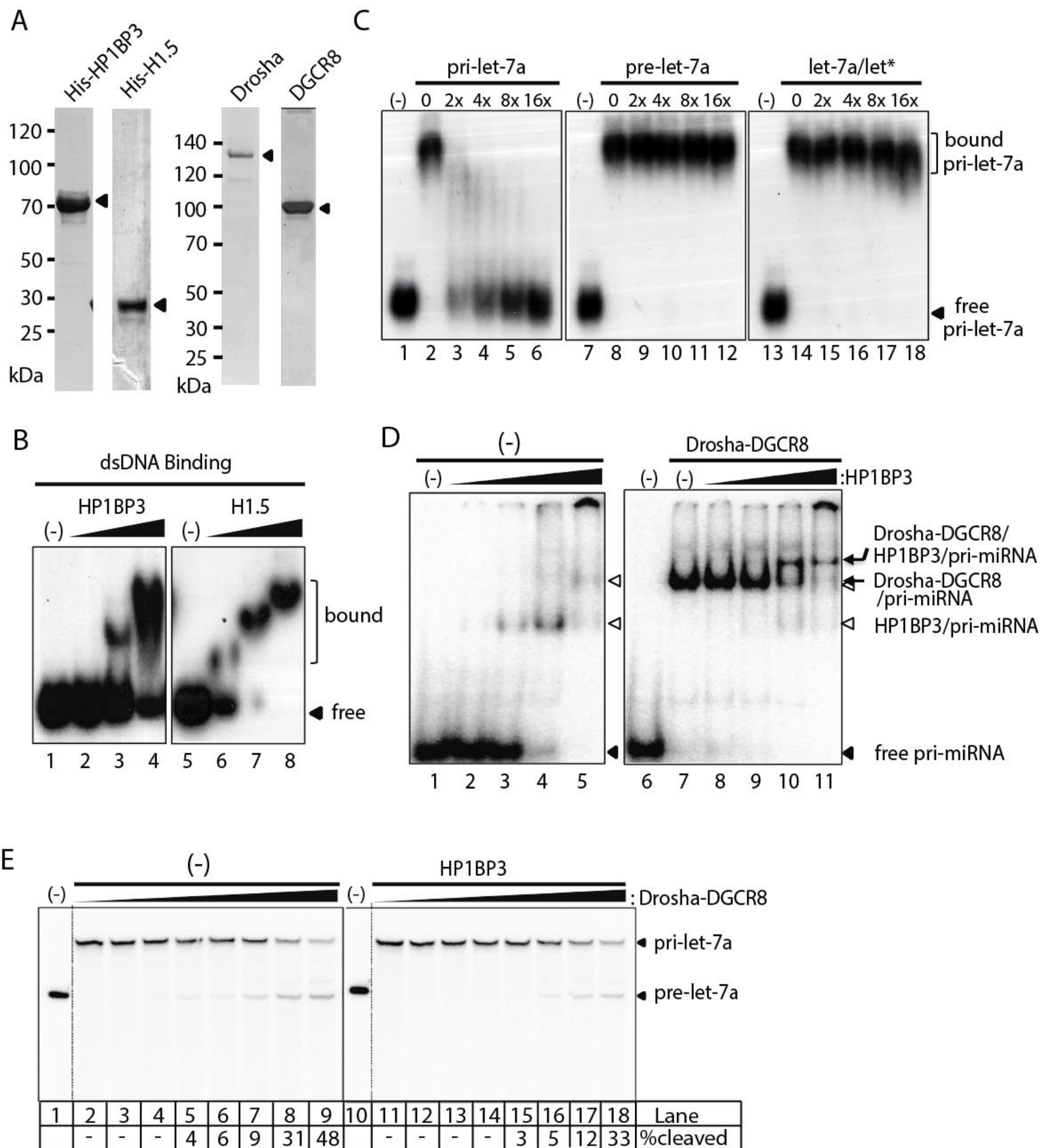

Figure S5 (Linked to Figure 4)

(A) Coomassie-stained SDS-PAGE showing purified His_6-HP1BP3, His_6-H1.5 (His_6-tag was cleaved and removed), and truncated Drosha (aa 398 – 1374) and DGCR8 (aa 276 – 751) recombinant proteins.

(B) Native gel-shift assays were performed by incubating 5' radiolabeled pri-let-7a dsDNA in buffer alone (lane 1, 5) or with increasing concentration of recombinant HP1BP3 (lanes 2-4) and H1.5 (lanes 6-8) proteins.

(C) Native gel-shift assays were performed by using excess non-radiolabeled pri-let-7a, pre-let-7a, or duplex let-7a RNA to compete with binding of radiolabeled pri-let-7a RNA by recombinant HP1BP3 (133nM).

(D) Native gel-shift assays were performed by incubating radiolabeled pri-let-7a in buffer alone (lanes 1 and 6) or increasing concentration (65nM, 125nM, 250nM, 500nM) of recombinant HP1BP3 in the absence (lanes 2-5) or presence (lanes 7-11) of recombinant Drosha-DGCR8 complex (25nM).

(E) The pri-miRNA processing assays were performed by incubating radiolabeled pri-let-7a RNA with increasing concentration of recombinant Drosha-DGCR8 complex in the absence (lanes 1-9) and presence (lanes 10-18) of recombinant HP1BP3. Synthetic pre-let-7a was used as a marker (lanes 1 and 10). The pri-miRNA processing activity was measured by the ratio of pre-let-7a/(pri-let-7a+ pre-let-7a) as listed below each lane.

Figure S6 (Linked to Figure 5)

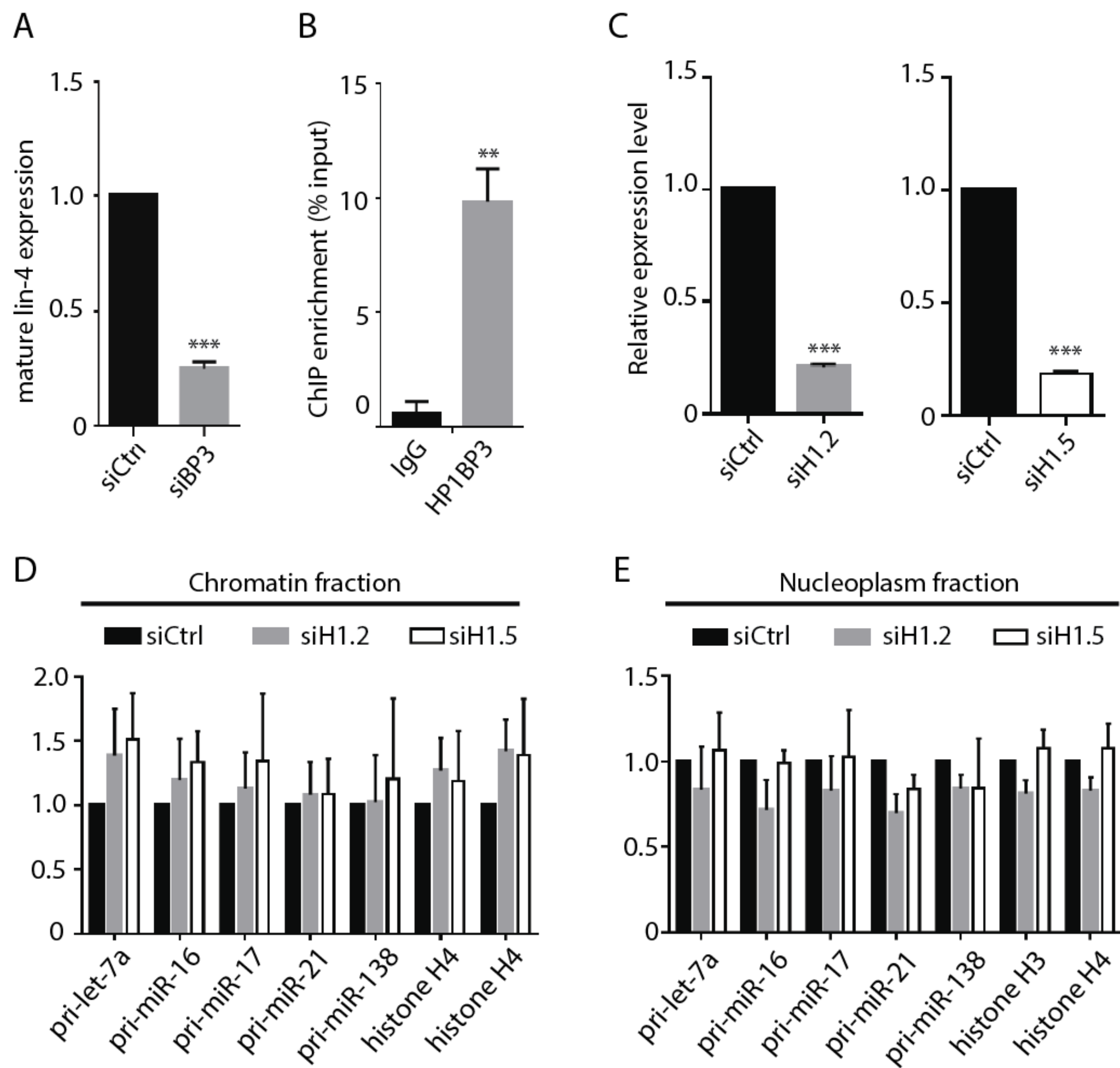

Figure S6 (Linked to Figure 5)

(A) Quantitative analysis of mature lin-4 expression from transfected Pri-lin-4 plasmid between the control and HP1BP3-depleted Hela cells by Taqman qPCR (triplicate samples, data shown as mean ± SD). Paired t-test was used for statistical analysis (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

(B) ChIP analysis showing that HP1BP3-GFP binds to the transfected Pri-lin4 plasmid DNA in the BAC transgenic HeLa cells (triplicate samples, data shown as mean ± SD). Paired t-test was used for statistical analysis (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

(C) Following siRNA-mediated knockdown in HeLa cells, the mRNA levels of H1.2 and H1.5 mRNA levels were measured respectively by real time RT-qPCR. Paired t-test was used for statistical analysis (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).

(D, E) Taqman qPCR was performed to measure the relative abundance of endogenous pri-miRNA and histone H4 transcripts in the chromatin (D) and nucleoplasmic (E) fractions of the control, H1.2-depleted and H1.5-depleted HeLa cells (triplet samples, data present as mean ± SD). 18S rRNA was used as a loading control.

SUPPLEMENTARY TABLES

Table S1 (Related to Figure 2). miRNA downregulated after BP3 knockdown, numbers shown in counts per million (cpm).

Gene ID	siCTRL_1	siCTRL_2	siBP3_1	siBP3_2	Gene ID	siCTRL_1	siCTRL_2	siBP3_1	siBP3_2
mir-31	481.5	384.8	27.35	21.29	mir-27a	1.535	0.524	0	0.458
let-7a-3	161.7	188.8	16.86	20.37	mir-181d	1.535	1.049	0	0
mir-21	160.2	194.5	15.83	24.72	mir-23b	1.535	3.146	0.411	0
mir-99b	103.6	87.56	4.935	6.18	mir-101-1	1.279	0.524	0	0.458
mir-30a	98.25	125.8	16.24	11.67	mir-92b	1.279	1.049	0	0.458
mir-182	82.13	72.88	6.168	5.493	mir-139	1.279	0	0	0
mir-23a	73.94	68.69	11.72	13.05	mir-145	1.279	1.049	0.206	0.229
let-7i	50.92	54.53	11.1	13.5	mir-27b	1.279	2.622	0	0
let-7a-2	36.08	46.66	2.467	3.433	mir-452	1.279	0.524	0.206	0.458
let-7b	32.75	45.09	2.879	5.493	mir-210	1.023	0	0	0
mir-143	25.33	36.7	0.411	1.602	mir-103a-2	1.023	0.524	0.206	0
mir-92a-1	24.82	27.26	4.318	5.722	mir-301b	1.023	0.524	0.206	0
mir-20a	23.28	15.73	2.056	2.518	mir-26a-1	1.023	1.573	0.411	0.229
let-7f-1	23.28	23.59	2.262	4.349	mir-138-1	1.023	2.097	0	0.229
mir-92a-2	23.03	18.88	3.495	4.349	mir-551b	1.023	0	0	0
let-7c	22	39.32	0.617	0.915	mir-29a	1.023	2.097	0.411	0.458
mir-30d	19.7	17.83	1.85	1.373	mir-2278	1.023	0	0	0
mir-181a-2	19.45	18.88	1.85	2.975	mir-32	1.023	0.524	0	0
mir-33a	18.68	10.49	1.028	0.687	mir-503	1.023	1.049	0	0
let-7a-1	16.12	16.25	2.467	1.602	mir-34a	0.768	0	0.206	0.229
mir-224	14.07	4.195	0.822	0.458	mir-146b	0.768	1.049	0	0
mir-93	13.82	19.4	0.411	2.06	mir-1908	0.768	0.524	0	0
mir-222	11.77	22.02	2.262	3.433	mir-125b-1	0.768	0	0.206	0
mir-30c-2	10.75	13.11	1.028	1.373	mir-19a	0.768	0.524	0	0
mir-181b-1	9.978	8.913	0.617	0.229	mir-365a	0.768	0	0	0.229
mir-16-1	9.211	18.88	3.29	4.806	mir-296	0.768	0.524	0.411	0.229
mir-17	9.211	4.195	1.234	1.602	mir-125b-2	0.768	2.097	0	0
mir-16-2	8.955	15.73	1.234	2.289	mir-339	0.768	0.524	0	0
mir-30c-1	8.699	17.3	1.645	3.433	mir-196b	0.768	0	0	0
mir-125a	7.932	21.5	1.234	1.373	mir-335	0.768	0	0.206	0
mir-98	7.932	5.243	0.617	0.458	mir-532	0.768	0.524	0	0
mir-186	7.42	13.63	0	0.687	mir-361	0.768	0	0	0
let-7e	7.42	7.34	0.617	0.687	mir-345	0.512	3.146	0	0

mir-181a-1	7.164	6.816	0.206	0.687	mir-138-2	0.512	0.524	0	0
mir-140	7.164	2.622	0.411	0.229	mir-195	0.512	0	0	0
mir-221	7.164	8.389	1.439	1.144	mir-301a	0.512	0.524	0.206	0.458
let-7f-2	6.908	11.53	1.234	1.602	mir-181c	0.512	1.573	0	0
mir-19b-1	6.652	6.292	0.822	0.915	mir-149	0.512	0	0.206	0
mir-22	6.652	17.83	0.411	2.06	mir-378a	0.512	3.146	0.411	0.687
mir-100	6.396	10.49	0.206	1.602	mir-340	0.512	0	0	0
mir-24-2	6.396	2.622	0.617	0.915	mir-219a-1	0.512	0.524	0.206	0
mir-25	6.396	6.292	0.617	0.458	mir-30b	0.512	1.573	0.411	0
mir-374a	6.141	8.389	0	0	mir-7-1	0.512	0	0	0
mir-28	5.629	3.146	0	0.229	mir-362	0.512	0	0	0
let-7d	5.373	6.292	0	0.229	mir-148b	0.256	1.049	0	0
mir-181b-2	5.117	10.49	0.411	0.915	mir-331	0.256	1.049	0	0
let-7g	4.861	13.63	0.206	0.915	mir-190a	0.256	1.573	0	0
mir-106b	4.861	8.389	0.822	0.458	mir-152	0.256	1.573	0	0
mir-196a-2	4.35	1.573	0	0.458	mir-7-3	0.256	0.524	0	0
mir-26b	4.35	4.195	0.617	0.687	mir-330	0.256	1.049	0	0
mir-155	4.35	4.195	0.206	0.229	mir-2355	0.256	0.524	0	0
mir-671	4.35	4.719	0.411	0.687	mir-99a	0.256	1.049	0	0
mir-151a	4.094	3.67	0	0.915	mir-15b	0.256	0.524	0.206	0
mir-324	3.582	2.622	0.206	0.229	mir-103a-1	0.256	1.573	0	0
mir-101-2	3.582	0.524	0.206	0	mir-126	0.256	1.049	0	0
mir-425	3.326	1.049	0.206	0.458	mir-652	0.256	0.524	0.206	0
mir-455	3.326	2.622	0.411	0.458	mir-19b-2	0.256	0.524	0	0
mir-196a-1	3.07	1.049	0	0	mir-505	0.256	0.524	0	0
mir-574	3.07	0.524	0.411	0.458	mir-137	0	1.573	0	0
mir-766	3.07	1.573	0	0.229	mir-197	0	0.524	0	0
mir-193a	2.814	3.146	1.028	0.458	mir-130a	0	2.097	0	0.229
mir-15a	2.559	2.097	0.411	0.458	mir-200c	0	0.524	0	0
mir-183	2.559	2.097	0.206	0	mir-615	0	1.049	0	0
mir-744	2.303	0.524	0	0	mir-132	0	1.573	0.411	0
mir-30e	2.047	3.146	0.411	0.687	mir-769	0	1.049	0	0
mir-26a-2	2.047	3.146	0.411	0.687	mir-128-1	0	0.524	0	0
mir-10a	2.047	1.049	0.206	0	mir-130b	0	0.524	0	0
mir-18a	1.791	1.049	0.206	0.229	mir-3619	0	0.524	0	0
mir-191	1.791	2.097	0.822	0.229	mir-582	0	1.049	0	0.229
mir-501	1.791	2.622	0	0	mir-589	0	0.524	0.206	0
mir-660	1.791	1.049	0	0	mir-148a	0	0.524	0	0
mir-1307	1.535	3.146	0	0.687	mir-96	0	1.049	0	0
mir-193b	1.535	2.622	0.822	0.458	mir-29b-1	0	0.524	0	0.458

Table S2 (Related to Figure 3). List of expressed miRNA loci co-localized with Drosha and HP1BP3.

Gene ID	Drosha	HP1BP3	Fold Change	Method
mir-92b	+	-	0.19663	CS
mir-26b	+	-	0.15256	CS
mir-191	+	+	0.27038	CS
mir-425	+	+	0.15163	CS
mir-219a-1	+	+	0.19846	CS
mir-151a	+	-	0.11792	CS
mir-4664	+	+	0.80361	CS
mir-365a	+	-	0.29817	CS
mir-23a	+	+	0.17364	both
mir-24-2	+	+	0.16992	both
mir-27a	+	+	0.22226	both
mir-181c	+	+	*	CS
mir-181d	+	+	*	CS
let-7e	+	+	0.08831	both
mir-99b	+	+	0.05814	CS
mir-125a	+	+	0.08858	CS
mir-1249	+	+	*	CS
mir-3619	+	+	*	CS
let-7a	+	+	0.12571	IC
let-7i	+	+	0.23336	IC
mir-16-1	+	+	0.28825	IC
mir-17	+	+	0.21154	IC
mir-21	+	+	0.11433	IC
mir-22	+	+	0.10095	IC
mir-25	+	+	0.08469	IC
mir-30b	+	+	0.19726	IC
mir-31	+	+	0.05613	IC
mir-96	+	+	*	IC
mir-99a	+	+	*	IC
mir-143	+	+	0.03245	IC
mir-181a	+	+	0.12953	IC
mir-193	+	+	0.24928	IC
mir-222	+	+	0.16853	IC
mir-574	-	+	0.24174	CS
mir-378a	-	+	0.30015	CS
mir-589	-	+	0.39215	CS

mir-196b	-	+	*	CS
mir-106b	+	+	0.96615	both
mir-93	+	+	0.07439	both
mir-126	-	+	*	CS
mir-1908	-	+	*	CS
mir-615	-	+	*	CS
mir-18a	+	+	0.15301	both
mir-20a	+	+	0.11724	both
mir-19a	+	+	*	both
mir-19b-1	+	+	0.13426	both
mir-92a-1	+	+	0.19276	both
mir-342	-	+	*	CS
mir-324	-	+	0.07004	CS
mir-769	-	+	*	CS
let-7f	+	+	0.14101	both
mir-15	+	+	0.18664	both
mir-183	+	+	0.04416	both
mir-182	+	+	0.07523	both
let-7c	+	+	0.02499	both
mir-181b	+	+	0.04477	both
mir-221	+	+	0.16612	both

a. * - No miRNA was detected in either siBP3_1 or siBP3_2 samples of RNA-seq
b. CS – ChiP-seq analysis. IC – individual ChIP analysis. Both – CS and IC analysis

Table S3 (Related to Experimental Procedures). Primer set for generation of Bac-transgenic cell line.

Drosha	5’- agagcatcaagagagagagccagatgagactgaagacatcaagaaagattatgatattccaactactgca-3’ 5’- cagtcacagttactgagcaagtaaatactccacacttgcatgccctccttcagaagaactcgtcaagaag-3’
HP1BP3	5-’gggcaagggcaaatccaccatgaagaagtctttcagagtgaaaaaggattatgatattccaactactgca-3’ 5’-gaaaataagattttgaatttcatcatgataccctttttttcctataaaattcagaagaactcgtcaagaag-3’
H1.5	5’- aaaacctaaagctgcaaaggccaagaaggcggctgccaaaaagaaggattatgatattccaactactgca-3’ 5’- tctgaaaagagcctttggggctttgttgcggttttcacacgccagcttctcagaagaactcgtcaagaag-3’

Table S4 (Related to Experimental Procedures). Primer sets for ChIP targeting pri-miRNA transcription loci.

miRNA	Forward Primer	Reverse Primer
let-7a	TGGGATGAGGTAGTAGGTTGTA	CTATCACGTTAGGAAAGACAGTAGAT
miR-7	CAGCATGGGACATGGGTATAG	GATAAAGTACAGGACAGCCAGTTA
miR-16	CCTTACTTCAGCAGCACAGTTA	TGCCTTAGCAGCACGTAAATA
miR-17	GTGCTTACAGTGCAGGTAG	GAGGCAGCTGTCACCATAAT
miR-21	CACCTTGTCGGGTAGCTTATC	GGATATGGATGGTCAGATGAAAGA
miR-33	CATTTGCTCCAGCGGTTTG	CAAACCGCTGGAGCAAATG
miR-1	CTCTTCTAGACCTTGCCCTAAC	CCAGACATAGCACTACCACAA
miR-9	CAAGGGTGACCATCTGCTAAT	CCTCTGAAAGAAGGTGATCCAG
miR-31	CCTTACTTCCTGTCCTGCTATG	CCTAGGTGTGTCCAAGGAATAG
let-7e	TCCCTGTCTGTCTGTCTGT	CTAGGAGGCCGTATAGTGATCT
miR-96	TGCCGTGGGTTGGAAAG	TCACAACCTGCAGCAGAAA
miR-99a	GATCCGATCTTGTGGTGAAGTG	TGTTGAACGGCACTGTGTATAG
miR-30b	CTGGGAGGTGGATGTTTACTT	AGCCTCTGTATACTATTCTTGCC
miR-181	AACGCTGTCGGTGAGTTT	GAATTCTGAGCACCATGGAGTA
let-7i	CTGGCTGAGGTAGTAGTTTGTG	CAGCACTAGCAAGGCAGTAG
miR-143	CAGTTGGGAGTCTGAGATGAAG	CACTCTGTCCTTCCTGTTATGG
miR-222	GCCAGTGTAGATCCTGTCTTTC	CTGCCCAATAATCTCTCTCAGG
miR-25	ATTGCACTTGTCTCGGTCTG	GACACCCTTGTTCTGGCTTTA
miR-193a	CGAACTCCGAGGATGGGA	CCGAGAACTGGGACTTTGTAG
miR-23a	ACATTGCCAGGGATTTCCA	CCTGCTCACAAGCAGCTAA
miR-22	ATGCCCTGCTCAGATCTTTC	CTTTAGCTGGGTCAGGACATAA

Table S5 (Related to Experimental Procedures). RNA probes used in Northern Blot Analysis

miRNA	Probe Sequence
Anti-let-7a	AACUAUACAACCUACUACCUCA
Anti-miR-16	CGCCAAUAUUUACGUGCUGCUA
Anti-miR-21	UCAACAUCAGUCUGAUAAGCUA
Anti-miR-23a	AAAUCCCAUCCCCAGGAACCCC
Anti-5S	CCGACCCTGCTTAGCTTCCGAGATCA
Anti-miR-1 (fly)	UAUUGAAUGCAAGGAAGCAUGG
Anti-miR-8 (fly)	UCUAAUGCUGCCCGGUAAGAUG
Anti-miR-305 (fly)	CAGAGCACCUGAUGAAGUACAAU
Anti-5S (fly)	CAACACGCGCTGTTCCCAAGCGGT
Anti-Bantam (fly)	AGUCAAACCAAAUCGAAAACCGG

Table S6 (Related to Experimental Procedure). PCR primers used for checking pri-miRNA levels in S2 cells.

	Sequence (5’-3’)
pri-miR-1 F	GTTAGCCGCGTTGTGGAAAATC
Pri-miR-1 R	CATTTCATTACGGTTCTACTTCTG
pri-miR-8 F	AGAACTTTGAGCTTCCTCTGGC
pri-miR-8 R	TTTGGTGCTGCTGCTGCTGTTG
pri-miR-276a F	AAAAGGGAAACGCGCTGCCAAG
pri-miR-276a R	CGTTTGTCCAGCGTTTTCTCATC
pri-miR-305 F	GAAATGCTCGCAGGCGAGTCC
pri-miR-305 R	GTTGAACACTTGTATCGGTCGC
pri-miR-317 F	ACGGTTTGTGTCTCTGCTGAGC
pri-miR-317 R	CTGTGGGGCATTCTCGTTATCC
pri-bantam F	CGCTCAGATGCAGATGTTGTTG
pri-bantam R	TCGACCATCGGAATGTGGAATG
RP49 F	CCAAGCACTTCATCCGCCACCA
RP49 R	GCGGGTGCGCTTGTTCGATCC
GAPDH F	GGCAACTTCTGCGAAACG A
GAPDH R	CGCAGCACCTTGCCATACTT

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Purification of fly histone H1 as a pri-miRNA-binding protein

Drosophila S2 cells were grown in suspension in SFX media (Invitrogen), harvested at ~2 x 10^7 cells/ml, and washed twice by PBS. Cells were incubated in five volumes of hypotonic buffer A (10 mM KOAc, 10 mM HEPES, pH7.4, 2 mM $Mg(OAc)_2$, 5mM DTT) supplemented with fresh proteinase inhibitorz on ice for 20 minutes. Then, cells were broken with a Douncer by 40 strokes and centrifuged at 2,000g at 4 ºC for 30 minutes. The nuclei pellet was washed twice in buffer A followed by centrifugation at 2,000 *g* for 10 min, and was resuspended in five volumes of nuclear extraction buffer (20 mM HEPES, pH 7.4, 10% glycerol, 350 mM NaCl, 0.1% Triton X-100, 1 mM DTT) supplemented with fresh proteinase inhibitors at 4°C for 1 hour. After centrifugation at 20,000 *g* for 30 min at 4°C, the S10 supernatant was further centrifuged at 100,000g for 1 hour at 4ºC to obtain nuclear S100 extract. The purification of histone H1 was carried out using a three-step chromatographic purification procedure (Nuclear S100 → SP-Sepharose → Q-Sepharose → SP-Sepharose) on the ÄKTA FPLC system (GE Healthcare Inc) at 4 ºC. First, the nuclear S100 extract was fractionated by SP-Sepharose column. The pri-miRNA binding activity was measured by native gel-shift assay by incubating individual fractions with radiolabeled pri-miRNA. The fractions with peak activity were dialyzed overnight and fractionated on Q-Sepharose column. The peak activity fractions were dialyzed overnight and fractionated again by SP-Sepharose column. After the final step, individual fractions were examined for the priRNA-binding activity and resolved by SDS-PAGE followed by silver staining. After silver staining, the protein bands that correlated with pri-miRNA-binding activity were excised, digested by trypsin, and identified by Mass Spectrometry analysis.

Native gel-shift assays

Native gel-shift assays were carried out as previously described (Liang et al., 2013), pri-miRNA, pre-miRNA, duplex miRNA and dsDNA (180-nt pri-let-7a DNA sequence) were respectively 5' radiolabeled with [γ-^{32}P] ATP by T4 polynucleotide kinase (PNK) followed by purification by G25 column (Ambion). Typically, recombinant proteins and 5×10^4 cpm radiolabeled RNA/DNA were incubated at 37 °C for 30 min in a 10-µl reaction (100 mM KOAc, 15 mM HEPES pH7.4, 1mM Mg(OAc)2, 2.5 mM EDTA, 2.5 mM DTT, pH7.4). The reaction mixture was resolved by 4.5% native PAGE and exposed to X-ray film.

RNAi knockdown in S2 cells

Different dsRNAs were prepared using Megascript T7 *in vitro* transcription kit (Ambion). Briefly, 1 mL 2 x 10^6 S2 cells were soaked with 30 ug GFP, H1 or H3 dsRNA for 6 hours in 1mL serum-free SFX media (Invitrogen) in 6-well dish, then add 2 ml medium to the cells. After 48 hours, the media was removed and dsRNA treatment was repeated. After another 48 hours, the dsRNA treated S2 cells were harvested for protein extraction followed by Western blotting, or RNA extraction followed by Northern blotting and real-time RT-qPCR.

Mammalian cell culture and RNAi knockdown

HeLa and U2OS cells were cultured at 37 °C in 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin /streptomycin. At 70% confluency, HeLa/U2OS cells were transfected with 20nM of siRNAs (Table S4) by Lipofectamine RNAiMax (Thermofisher Scientic) according to manufacturer's protocol (HP1BP3-GUCCAAACCUGCACCUAAA[dT][dT]; H1.5-GCUAAGAAGAAGGCAACUA [dT][dT]; H1.2-GGUUAAAAAGGCGGGCGGA[dT][dT]; H1.0-CCCGUUGUGAGAGUU UCAG[dT][dT]; H1x-CCUACCUCAAGUAACUCGAU[dT][dT]). A second siRNA transfection was repeated 48 hours later. Transfected cells were harvested after another 48 hours for further analysis, e.g. RT-qPCR, Northern blotting, Western blotting, ChIP, RIP, and chromatin fractionation. Total RNA was extracted using Trizol® reagent (Thermofisher Scientic). The knockdown efficiency of HP1BP3 and H1 variants in Figure 1B was evaluated by RT-qPCR. The knockdown efficiency of HP1BP3 and H1.5 is further examined by Western blotting.

Generation of recombinant histone proteins

Polyhistidine His_6-tagged *Drosophila* histone H1, H2A, H3 and H4 and human HP1BP3 recombinant proteins were expressed in the pET-28a vector in *E. coli* BL21(DE3) strain as previously described (Liang et al., 2015). The various His_6-tagged recombinant proteins were purified using Ni-NTA agarose (Qiagen) at 4ºC followed by Q-Sepharose chromatography.

Multi-sequence alignment and phylogenetic tree analysis

Linker histone H1 sequences were collected with PSI-BLAST (Altschul et al., 1997) against the UniProtKB/Swiss-Prot (Magrane and Consortium, 2011) database (default cutoffs, 5 iterations to convergence) initiated from a query sequence (gi|6016184, residue range 32-129). The query sequence range includes the H1 globular domain (GD) defined by the conserved domain database (CDD) (Marchler-Bauer et al., 2015) with some additional anchoring sequence on either side. The resulting 145 sequences producing significant alignments were from animals (112), green plants (24), fungi (6), Olisthodiscus (2), and cellular slime mold (1), with human possessing 14 variants. One reported human H1 sequence (PMID: 26474902) encoded by the H1T2 gene was not identified by this method, so we also performed more sensitive sequence searches against the human genome with the same query using the HHPRED server (Hildebrand et al., 2009). HHPRED confidently identified human sequences with probability > 95% that corresponded to the same genes identified using PSI-BLAST, as well as the H1T2 sequence with slightly lower confidence (probability 92.26%). Taxonomic distribution of HP1BP3 sequences was defined with BLAST using the full-length human sequence as a query, restricting identified sequences to those containing tandem G15 domains defined by CDD. Selected sequences corresponding to the GD were aligned using MAFFT (Katoh and Standley, 2014), and the resulting multiple sequence alignment was used to estimate phylogenetic distances (JTT with frequencies option) and build a maximum likelihood tree using local rearrangement of an initial NJDIST tree with the Molphy

package (Adachi and Hasegawa, 1992). Because the H1T2 sequence is more distantly related to the other H1 GDs, we displayed the tree using H1T2 as an outgroup.

Immunostaining and co-immunoprecipitation (IP)

The Drosha-GFP, HP1BP3-GFP and H1.5-GFP BAC transgenic HeLa cells grown on coverslips were fixed with 4% paraformaldehyde in PBS (pH 7.4) for 15 min at 37 °C, and then permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature. After washing, the cells were stained with 1 µg/ml 4′,6-diamidino-2-phenylindole (DAPI) and imaged under the LSM 700 Laser Scanning Microscope (Carl Zeiss). For co-IP studies, cell lysate (containing 500µg of total protein) was pre-cleared using 40µL of protein A/G beads for 2 hours at 4 ºC. When necessary, RNaseA (1µg) was added to cell extract for additional 30 minutes of incubation at room temperature. Pre-cleared HeLa extract was first mixed with 2µg of antibody at 4 ºC overnight, then mixed with 40µL protein A/G plus Argarose (Santa Cruz Biotechnology) resin at 4 ºC for 2 hours. Resin was washed 5 times with IP wash buffer (10mM Tris pH 7.5, 50mM NaCl, 2mM $MgCl_2$, 0.05% NP-40, 0.025% SDS). Protein associated with resin was boiled in SDS-PAGE loading buffer at 100 ºC for 2min, and then analyzed by Western blotting. For IP of Flag-tagged proteins, Anti-Flag (M2) Affinity Gel (Sigma) was used directly instead of Protein A/G resin and antibody.

ChIP-Seq experiments

BAC transgenic cells (primers used to generate BAC transgenic cells listed in table S3) at 80% confluency (~$1x10^7$) were cross-linked with 1% formaldehyde for 10 minutes at 37°C, and quenched with 125 mM glycine at room temperature for 5 minutes. The fixed cells were washed twice with cold PBS, scraped, and transferred into 1 ml PBS containing protease inhibitors (Roche). After centrifugation at 700 g for 4 minutes at 4°C, the cell pellets were resuspended in 100 µl ChIP lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl [pH 8.1] with protease inhibitors) and sonicated at 4°C with a Bioruptor (Diagenode) (30 seconds ON and 30 seconds OFF at highest power for 15 minutes). The sheared chromatin with a fragment length of ~200 – 600 bp) was centrifuged at 20,000 g for 15 minutes at 4°C. 100 µl of the supernatant was used for ChIP or as input. A 1:10 dilution of the solubilized chromatin in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 167 mM NaCl 16.7 mM Tris-HCl [pH 8.1]) was incubated at 4°C overnight with 6 µg/ml of a goat anti-GFP (raised against His-tagged full-length EGFP and affinity-purified with GST-tagged full-length EGFP). Immunoprecipitation was carried out by incubating with 40 µl pre-cleared Protein G Sepharose beads (Amersham Bioscience) for 1 hour at 4°C, followed by five washes for 10 minutes with 1ml of the following buffers: Buffer I (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl [pH 8.1], 150 mM NaCl), Buffer II (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl [pH 8.1], 500 mM NaCl), Buffer III (0.25 M LiCl, 1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl [pH 8.1]); twice with TE buffer [pH 8.0]. Elution from the beads was performed twice with 100 µl ChIP elution buffer (1% SDS, 0.1 M NaHCO3) at room temperature for 15 minutes. Protein-DNA complexes were de-crosslinked by heating at 65°C in 192 mM NaCl for 16 hours. DNA fragments were purified using QiaQuick PCR Purification kit (QIAGEN) and eluted into 30 µl H_2O according to the manufacturer's protocol after treatment with RNase A and Proteinase K. For ChIP-Seq, barcoded

libraries of Drosha-GFP, HP1BP3-GFP and H1.5-GFP ChIP and input DNA were generated with the TruSeq® ChIP Sample Preparation Kit (Illumina), and 50-nt single-end reads were generated with the HiSeq2000 system (Illumina). Sequence reads were aligned to the human reference genome (hg19) using Bowtie (v.0.12.7) (Langmead et al., 2009). Uniquely mapped reads with ≤2 mismatches to the reference sequence were retained for further analysis; for Drosha-GFP we obtained 28,343,999 reads from 28,580,957 input reads, for HP1BP3-GFP-GFP we obtained 26,606,724 reads from 30,200,875 input reads, for H1.5-GFP-GFP we obtained 21,719,813 reads from 31,055,062 input reads. We used SICER algorithm (v1.1) (Zhang et al., 2008) to define genome wide occupied islands for these proteins from ChIP-Seq data. We used a window size of 200bp, a fragment size of 200bp, and a gap size of 600bp parameters to quantify the Drosha, HP1BP3 or H1.5 ChIP signal fold enrichment over input sample in each island. A false discovery rate (FDR) cutoff of 1% was used to select islands for further analysis. For annotation of Drosha or HP1BP3 or H1.5 islands, we used annotate Peaks function in Homer tools (Heinz et al., 2010) to assign Drosha islands relative to their specific positions in the genome. This function takes the Drosha islands coordinates and tag directories as input, and extends each tag by their estimated ChIP-fragment length. We normalized each library to 10 million reads and calculate ChIP fragment coverage represented in per base pair per peak. We used 5000 bp regions flanking the islands center to generate density plots showing Drosha, HP1BP3 and H1.5 ChIP signal across all Drosha islands.

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