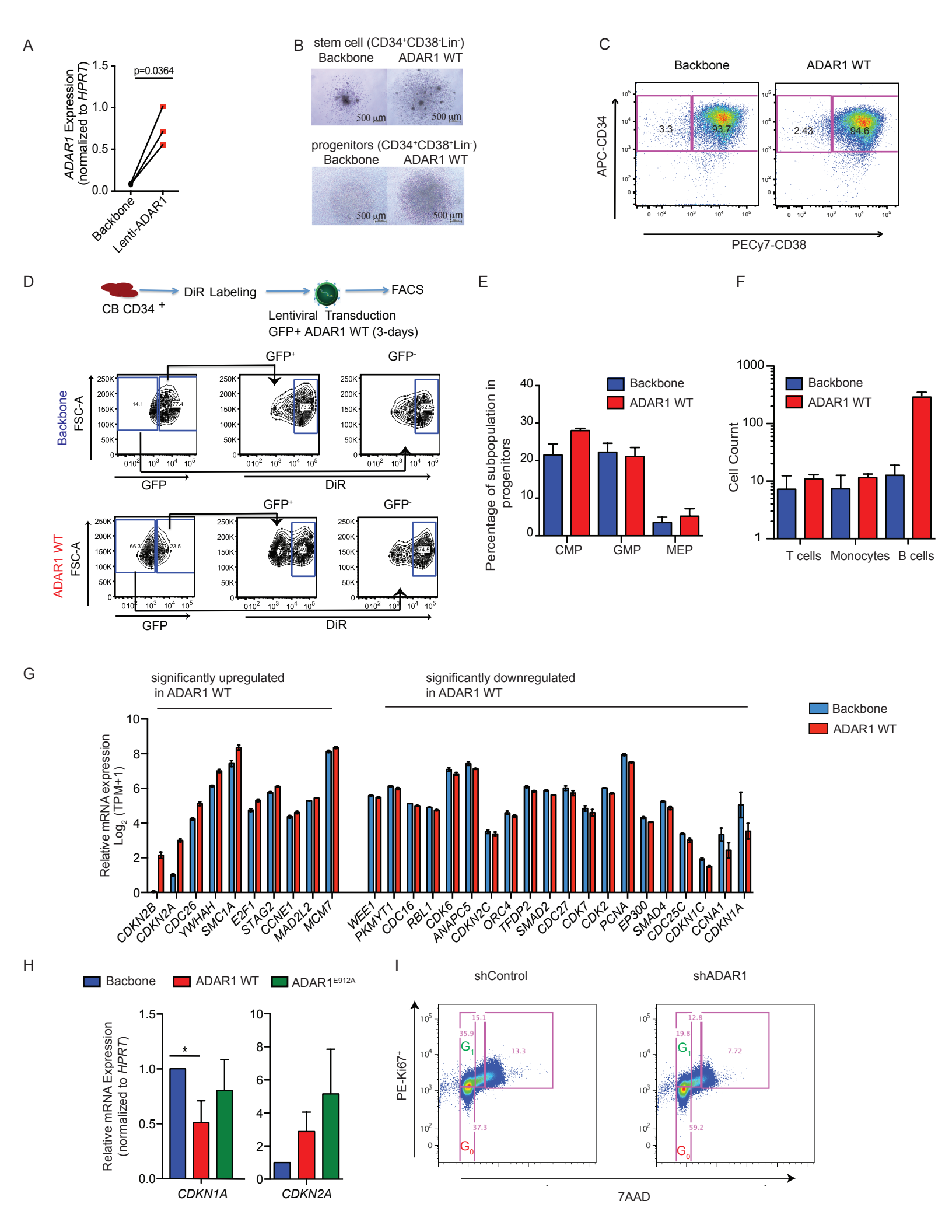
A
ADAR1 Expression (normalized to HPRT)
p=0.0364
Backbone
Lenti-ADAR1
B
stem cell (CD34+CD38-Lin-)
Backbone
ADAR1 WT
500 μm
progenitors (CD34+CD38+Lin-)
C
Backbone
ADAR1 WT
APC-CD34
PECy7-CD38
3.3
93.7
2.43
94.6
D
CB CD34 +
DiR Labeling
FACS
Lentiviral Transduction
GFP+ ADAR1 WT (3-days)
GFP+
GFP-
Backbone
ADAR1 WT
FSC-A
GFP
DiR
E
Percentage of subpopulation in progenitors
CMP
GMP
MEP
F
Cell Count
T cells
Monocytes
B cells
G
significantly upregulated in ADAR1 WT
significantly downregulated in ADAR1 WT
Relative mRNA expression Log2 (TPM+1)
CDKN2B
CDKN2A
CDC26
YWHAH
SMC1A
E2F1
STAG2
CCNE1
MAD2L2
MCM7
WEE1
PKMYT1
CDC16
RBL1
CDK6
ANAPC5
CDKN2C
ORC4
TFDP2
SMAD2
CDC27
CDK7
CDK2
PCNA
EP300
SMAD4
CDC25C
CDKN1C
CCNA1
CDKN1A
H
Bacbone
ADAR1 WT
ADAR1E912A
Relative mRNA Expression (normalized to HPRT)
*
CDKN1A
CDKN2A
I
shControl
shADAR1
PE-Ki67+
7AAD
G1
G0
15.1
35.9
13.3
37.3
12.8
19.8
7.72
59.2

Figure S1, related to Figure 1. ADAR1 overexpression and knockdown in normal HSPC.

(A) Expression of lenti-ADAR1 WT in normal cord blood $CD34^+$ cells as measured by RT-qPCR (n=3). (B) Representative bright-field (BF) microscopy showing normal cord blood stem cells and progenitors transduced with lentiviral vector backbone or human ADAR1 WT lentivirus. (C) Experimental design and examples of FACS gates of DiR tracing of cord blood cells. (D) Flow analysis of stem and progenitor cells in backbone or ADAR1 WT transduced cord blood $CD34^+$ cells (n=3). (E) Percentage of sub-population of progenitors including CMP, GMP, and MEP in backbone or ADAR1 WT transduced cord blood $CD34^+$ HSPC (n=3). (F) Cell number of T cell, B cell and monocytes differentiated from cord blood $CD34^+$ HSPC transduced with backbone or ADAR1 WT (n=3). (G) Significantly differentially expressed transcripts of KEGG Cell Cycle Pathway in ADAR1 WT-transduced cord blood $CD34^+$ HSPC versus lentiviral vector control by RNA-seq analysis (n=3). The TPM gene expression value was transformed to $Log_2(TPM+1)$. (H) Examination of *CDKN1A* and *CDKN2A* mRNA expression by RT-qPCR in cord blood $CD34^+$ HSPC transduced with backbone, ADAR1 WT, or $ADAR1^{E912A}$ (n=4). (I) Representative cell cycle flow cytometry plot of normal cord blood $CD34^+$ HSPC transduced with shControl or shADAR1 measured by Ki-67 and 7AAD levels. All graphs show mean with SEM and statistical analysis was calculated using the Student's t-test. *$p<0.05$.

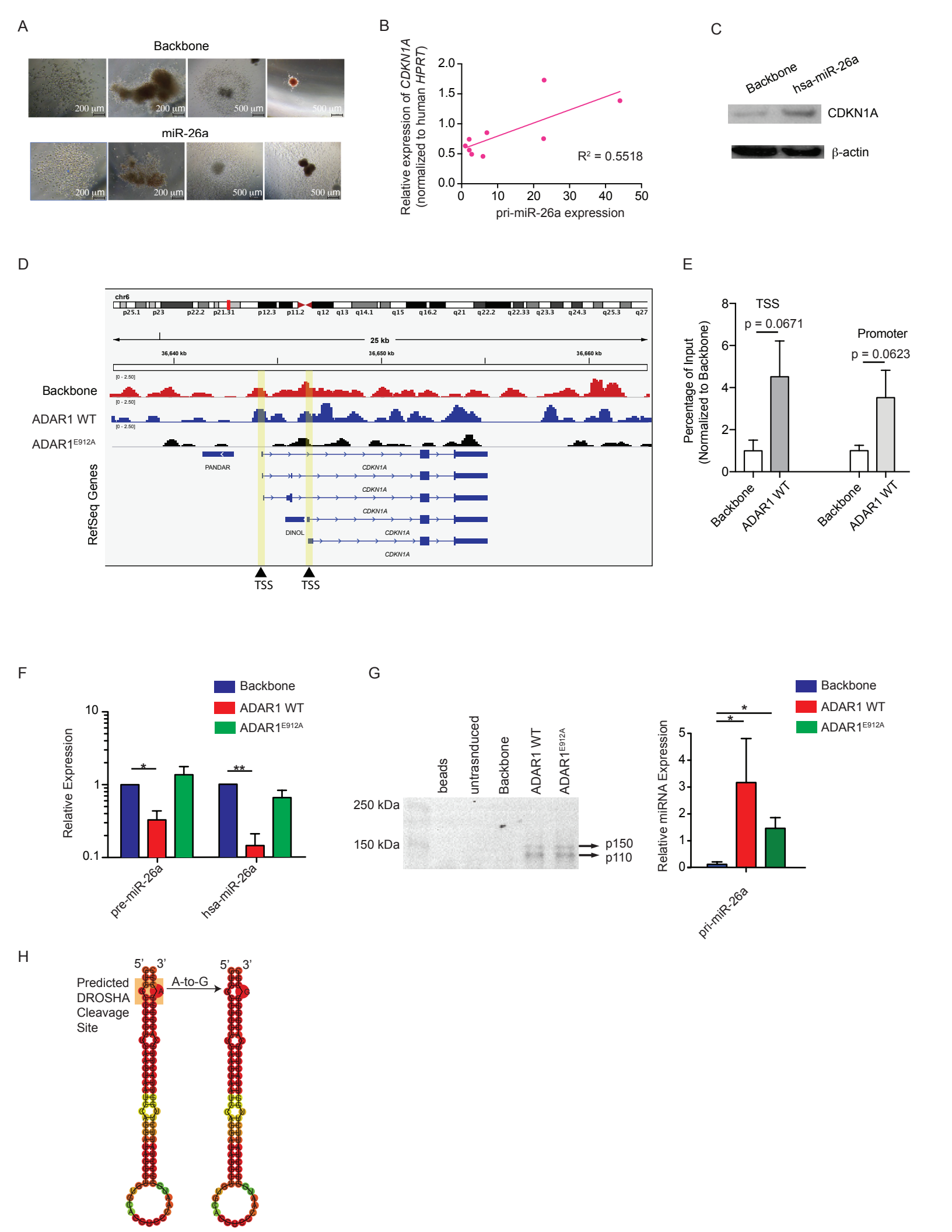
A
Backbone
200 µm
200 µm
500 µm
500 µm
miR-26a
200 µm
200 µm
500 µm
500 µm
B
Relative expression of CDKN1A (normalized to human HPRT)
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
pri-miR-26a expression
R² = 0.5518
C
Backbone
hsa-miR-26a
CDKN1A
β-actin
D
chr6
p25.1 p23 p22.2 p21.31 p12.3 p11.2 q12 q13 q14.1 q15 q16.2 q21 q22.2 q22.33 q23.3 q24.3 q25.3 q27
25 kb
36,640 kb
36,650 kb
36,660 kb
Backbone
ADAR1 WT
ADAR1E912A
RefSeq Genes
PANDAR
CDKN1A
DINOL
TSS
TSS
E
Percentage of Input (Normalized to Backbone)
8
6
4
2
0
TSS
p = 0.0671
Promoter
p = 0.0623
Backbone
ADAR1 WT
Backbone
ADAR1 WT
F
Backbone
ADAR1 WT
ADAR1E912A
Relative Expression
10
1
0.1
*
**
pre-miR-26a
hsa-miR-26a
G
beads
untrasnduced
Backbone
ADAR1 WT
ADAR1E912A
250 kDa
150 kDa
p150
p110
Backbone
ADAR1 WT
ADAR1E912A
Relative miRNA Expression
5
4
3
2
1
0
*
*
pri-miR-26a
H
5’ 3’
5’ 3’
Predicted DROSHA Cleavage Site
A-to-G

Figure S2, related to Figure 3. MiR-26a regulated normal HSPC cell cycle transit and self-renewal.

(A) Representative pictures of cord blood HSPC colonies. (B) Pearson's correlation of pri-miR-26a and *CDKN1A* expression as measured by RT-qPCR in HEK293T cells transduced with hsa-miR-26a lentivirus (n=9 experimental repeats). (C) Western blot analysis of CDKN1A protein expression HEK293T cells transduced with hsa-miR-26a lentivirus. (D) Representative H3K27me3 CHIP-sequencing plot in cord blood $CD34^+$ HSPC transduced with backbone, ADAR1 WT or $ADAR1^{E912A}$. The transcription start sites (TSS) were highlighted. (E) H3K27me3 CHIP-qPCR of *CDKN1A* TSS and promoter regions in HEK293T cells transduced with backbone or ADAR1 WT (n=7-8 experimental replicates). (F) Expression of pre- and mature miR-26a was measured by RT-qPCR in K562 transduced with backbone, ADAR1 WT, or $ADAR1^{E912A}$ (n=3 experimental triplicate). (G) Crosslinking RNA Immunoprecipitation (CLIP) with an ADAR1 antibody followed by RT-qPCR analysis of pri-miR-26a in K562 cells stably expressing backbone, ADAR1 WT, or $ADAR1^{E912A}$ (n=3 experimental triplicate). (H) ViennaRNA-predicted secondary structure of pri-miR-26a with either adenosine or inosine (guanosine) at the predicted DGCR8/DROSHA cleavage site (highlighted in orange). All graphs show mean with SEM and statistical analysis was calculated using the Student's t-test. *$p<0.05$, **$p<0.005$.

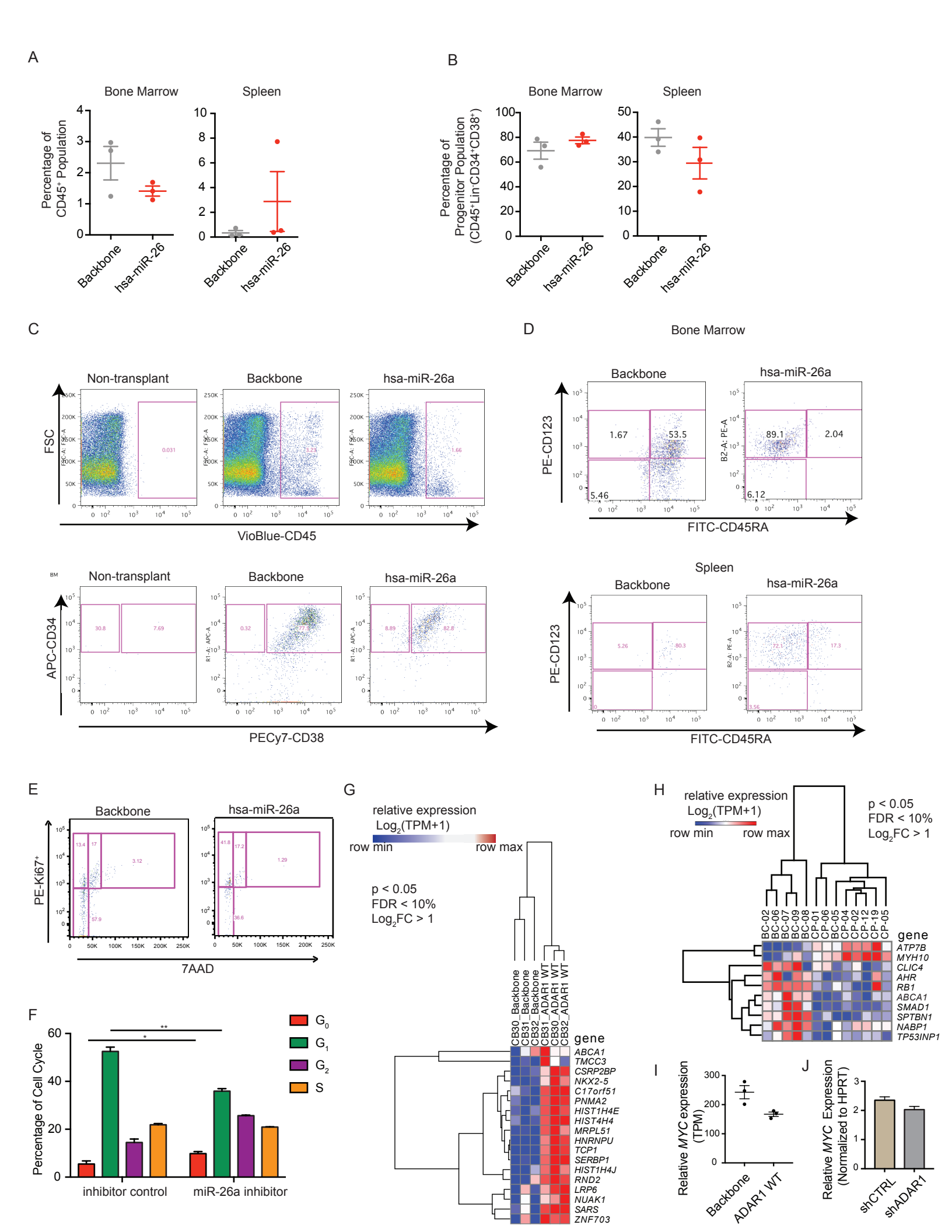
A
Bone Marrow
Spleen
Percentage of CD45+ Population
Backbone
hsa-miR-26
B
Bone Marrow
Spleen
Percentage of Progenitor Population (CD45+Lin-CD34+CD38+)
Backbone
hsa-miR-26
C
Non-transplant
Backbone
hsa-miR-26a
FSC
VioBlue-CD45
APC-CD34
PECy7-CD38
D
Bone Marrow
Backbone
hsa-miR-26a
PE-CD123
FITC-CD45RA
1.67
53.5
5.46
89.1
2.04
6.12
Spleen
5.26
80.3
72.1
17.3
E
Backbone
hsa-miR-26a
PE-Ki67+
7AAD
13.4
17
3.12
57.9
41.8
17.2
1.29
36.6
F
Percentage of Cell Cycle
inhibitor control
miR-26a inhibitor
G0
G1
G2
S
**
*
G
relative expression
Log2(TPM+1)
row min
row max
p < 0.05
FDR < 10%
Log2FC > 1
CB30_Backbone
CB31_Backbone
CB32_Backbone
CB31_ADAR1 WT
CB30_ADAR1 WT
CB32_ADAR1 WT
gene
ABCA1
TMCC3
CSRP2BP
NKX2-5
C17orf51
PNMA2
HIST1H4E
HIST4H4
MRPL51
HNRNPU
TCP1
SERBP1
HIST1H4J
RND2
LRP6
NUAK1
SARS
ZNF703
H
relative expression
Log2(TPM+1)
row min
row max
p < 0.05
FDR < 10%
Log2FC > 1
BC-02
BC-06
BC-07
BC-09
BC-08
CP-01
CP-06
BC-05
CP-04
CP-02
CP-12
CP-19
CP-05
gene
ATP7B
MYH10
CLIC4
AHR
RB1
ABCA1
SMAD1
SPTBN1
NABP1
TP53INP1
I
Relative MYC expression (TPM)
Backbone
ADAR1 WT
J
Relative MYC Expression (Normalized to HPRT)
shCTRL
shADAR1

Figure S3, related to Figure 4. Differential effect of miR-26a overexpression on normal HSPC and CML progenitors.

(A and B) Percentage of human $CD45^+$ (A) or progenitor ($CD45^+Lin^-CD34^+CD38^+$) (B) engraftment in bone marrow and spleen of BC CML xenografted mice (n=3 mice per group). (C) Representative flow showing human $CD45^+$ and progenitor engraftment of BC CML cells transduced with backbone or hsa-miR-26a in bone marrow. (D) Representative GMP engraftment (% of parent cells) of BC CML cells transduced with backbone or hsa-miR-26a in bone marrow and spleen. (E) Representative cell cycle flow of engrafted human $CD45^+$ BC CML cells transduced with backbone and hsa-miR-26a in bone marrow. (F) Cell cycle flow analysis of K562 cells transfected with inhibitor control or a miR-26a inhibitor (n=3 experimental triplicate). (G) RNA-seq quantification of differentially expressed genes (Log_2 Fold Change > 1, $p < 0.05$, FDR < 0.10) corresponding to miR-26a targets from miRTarBase in cord blood transduced with lentiviral backbone control or ADAR1 WT (n=3). (H) Differentially expressed miR-26a targets (Log_2 Fold Change > 1, $p < 0.05$, FDR < 0.10) in BC progenitors (n=6) compared to CP counterparts (n=7) by RNA-seq analysis. (I and J) *MYC* expression was determined by RNA-seq in cord blood $CD34^+$ cells overexpressing ADAR1 WT (n=3) (I) or by RT-qPCR in cord blood $CD34^+$ cells with ADAR1 knockdown (n=3) (J). The graph shows mean with SEM and statistical analysis was calculated using the Student's t-test. *$p<0.05$, **$p<0.005$.

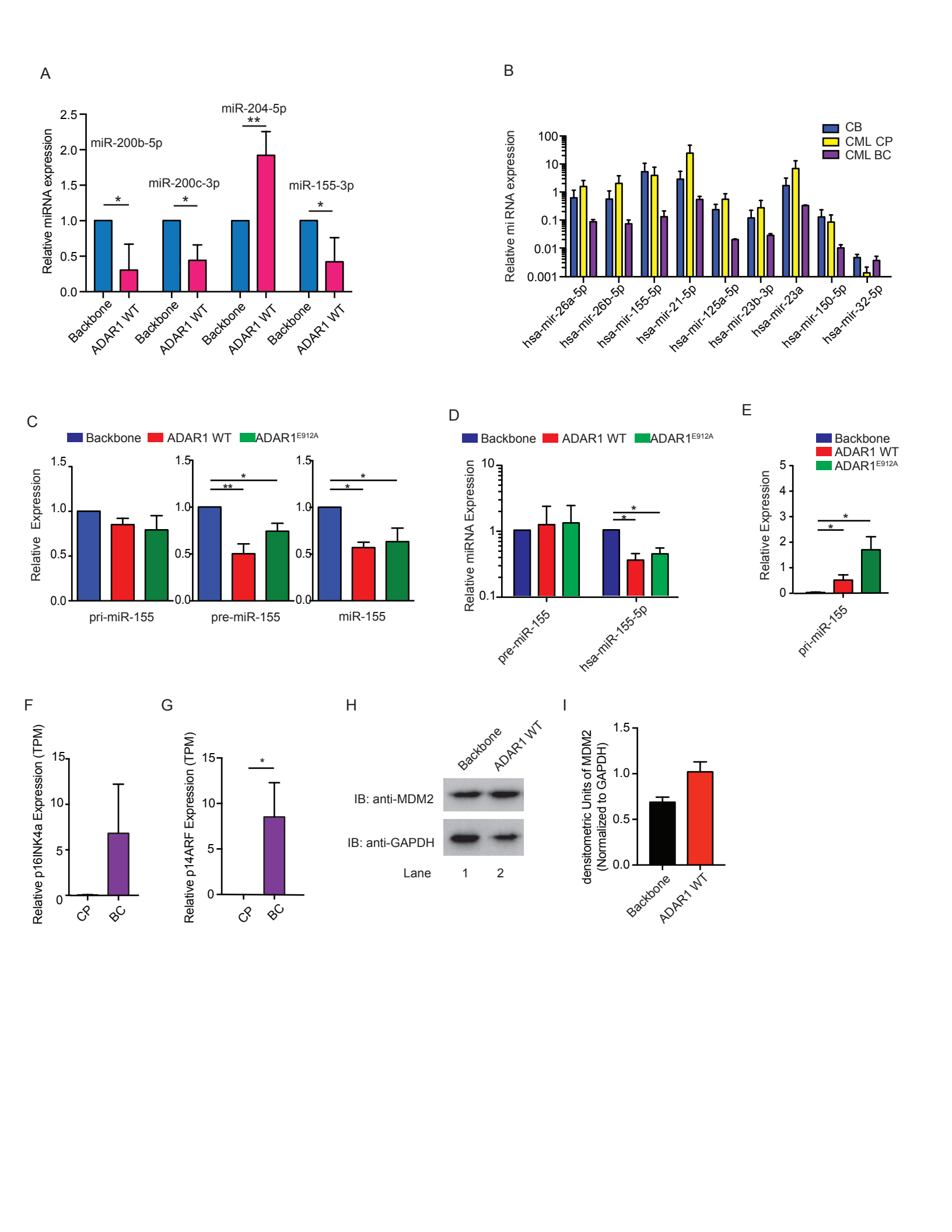
A
Relative miRNA expression
miR-200b-5p
miR-200c-3p
miR-204-5p
miR-155-3p
Backbone
ADAR1 WT
B
Relative mi RNA expression
CB
CML CP
CML BC
hsa-mir-26a-5p
hsa-mir-26b-5p
hsa-mir-155-5p
hsa-mir-21-5p
hsa-mir-125a-5p
hsa-mir-23b-3p
hsa-mir-23a
hsa-mir-150-5p
hsa-mir-32-5p
C
Backbone
ADAR1 WT
ADAR1E912A
Relative Expression
pri-miR-155
pre-miR-155
miR-155
D
Relative miRNA Expression
pre-miR-155
hsa-miR-155-5p
E
Relative Expression
pri-miR-155
F
Relative p16INK4a Expression (TPM)
CP
BC
G
Relative p14ARF Expression (TPM)
H
IB: anti-MDM2
IB: anti-GAPDH
Lane
1
2
I
densitometric Units of MDM2 (Normalized to GAPDH)

Figure S4, related to Figure 5. ADAR1 regulates MDM2 3'UTR targeting and miRNA biogenesis in CML progenitors.

(A) miRNome array-derived expression of miRNAs that were predicted to bind to MDM2 3'UTR region in cord blood $CD34^+$ HSPC overexpressing ADAR1 WT or $ADAR1^{E912A}$ compared with vector control (n=3-4). (B) Relative miRNA expression determined by miRNA qPCR array of 84 miRNAs in cord blood, CML CP, and CML BC $CD34^+$ cells (n=3 per patient group). (C) The expression of pri-, pre- and mature miR-155 transcripts evaluated by RT-qPCR in cord blood $CD34^+$ HSPCs transduced with backbone, ADAR1 WT, or $ADAR1^{E912A}$ (n=3). (D) Expression of pre- and mature miR-155 in K562 cells stably expressing backbone, ADAR1 WT, or $ADAR1^{E912A}$ (n=3 experiment triplicate). (E) Relative expression of pri-miR-155 compared to loading controls in crosslinking RNA Immunoprecipitation (CLIP) with an ADAR1 antibody in K562 stably transduced with backbone, ADAR1 WT, or $ADAR1^{E912A}$. (F and G) The expression of *CDKN2A* transcripts, p16INK4a (F) and p14ARF (G), determined by RNA-seq in progenitor population of normal peripheral blood (NPB), CML CP (n=7), and CML BC (n=6). (H) Representative western blot showing MDM2 protein expression in CP CML $CD34^+$ cells transduced with backbone or ADAR1 WT. (I) Densitometry analysis of MDM2 protein level in CP CML CD34+ cells transduced with backbone or ADAR1 WT (n=2). All graph show mean with SEM and statistical analysis was calculated using the Student's t-test. *$p<0.05$, **$p<0.005$.

Table S4, related to STAR METHODS. CML patient sample information

Patient ID	Diagnosis	Treatment	Gender / Age	Date	WBC count (K/mm3)	% Blast (PB)
CP-01	CML CP	None	M / 60	13-Nov. 2008	189	<5
CP-02	CML CP	None	F / 63	23-May 2008	326	5
CP-04	CML CP	None	M / 44	14-Oct. 2008	306	5.8
CP-05	CML CP	None	M / 26	21-Sep. 2009	231	<1
CP-06	CML CP	None	F / 62	25-Sep. 2009	87.7	<5
CP-12	CML CP	None	N/A	26-Aug. 2009	390	<5
CP-19	CP	None	M / 40	20-Oct. 2010	221	13
BC-02	CML BC	None	M / 34	26-Aug. 2004	241	92
BC-05	CML BC	None	M / 43	8-Dec. 2003	82.4	32
BC-06	CML BC	Hydroxyurea	M / 30	26-Oct. 1993	170	94
BC-07	CML BC	Hydroxyurea	M / 48	29-Oct. 1993	209	86
BC-08	CML BC	Hydroxyurea	M / 53	27-Jul. 2000	98	82.6
BC-09	CML BC	None	M / 65	17-Oct. 1991	72	42
BC-11	CML BC	Hydroxyurea	M / 31	16-Mar. 2006	40.1	79
BC-19	CML BC	Imatinib followed by dasatinib	M / 46	23-Nov. 2007	127	30

Table S5, related to STAR METHODS. List of RT-qPCR primers.

Transcript	FW primer (5'-3')	Rev primer (5'-3')
Lenti-ADAR1	AAA AAG CAG GCT CCA CCA T	AAA AAG CAG GCT CCA CCA T
Total ADAR1	TGC TGC TGA ATT CAA GTT GG	TCG TTC TCC CCA ATC AAG AC
CDKN1A	ATG AAA TTC ACC CCC TTT CC	AGG TGA GGG GAC TCC AAA GT
CDKN2A	ATA TGC CTT CCC CCA CTA CC	CGT GAG TGC TCA CTC CAG AA
MDM2	TTC CCA GCC TAG GTT TCA GA	AAC ACG GAG CTT GAG AGG AA
pri-miR-26a	GCC CAA TGG CAT AGC AAG A	GGC CAG TCA TGC TTA CAG TCA C
pri-miR-155	AGC TTT ATA ACC GCA TGT GCA TAC	CAG ATT TCC CCT TCC TGG TTT
HPRT	TCA GGG ATT TGA ATC ATG TTT GTG	CGA TGT CAA TAG GAC TCC AGA TG
CDKN1A Prom	CTT CTC TGA GCC CCA GTT TCC	GGA TTT GAC GAG TGA GTT GTC TGT
CDKN1A TSS	CGC GAG GAT GCG TGT TC	CAT TCA CCT GCC GCA GAA A