

Supplementary Figure Legend.

Supplementary Figure 1. a) Representative images showed that BMI1 (green) is expressed in the nucleus (DAPI). The mesenchymal marker VIMENTIN (Red) was co-stained. Scale bar represents 100 μm . b) Surface markers for MSCs. Negative markers (CD14, CD34 and CD45) and positive markers (CD73, CD90 and CD105) for MSCs were evaluated by flow cytometry (Grey filled). IgG was used as a control (white). n=4. (c) Images from Alizarin Red S staining for osteoblasts. (d) Alkaline phosphatase activity ($P=0.003$, n=3), (e) RUNX2 ($P=0.037$, n=3) and BSP ($P=0.044$, n=3) mRNA levels in differentiation. (f) Images from Oil Red O staining for adipogenesis. (g) Nile Red⁺ cells ($P=0.0018$, n=3) (h) PPAR γ ($P=0.009$, n=4) and FABP4 ($P=0.041$, n=4) mRNA levels in differentiation. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0005$ Data are shown as mean \pm SEM.

Supplementary Figure 2. Loss of p16INK4A partially rescues the proliferative defect of BMI1 knockdown MSCs. (a) mRNA expression of cell-cycle dependent kinase - and inhibitor - related genes in double knockdown MSCs, determined by qRT-PCR. (b) MTT cell growth assay shows cell proliferation differences between shRNAs targeting BMI1 and overexpression of BMI1 MSCs and only shp16 samples rescued cell proliferation close to SCRAMBLE. (c) Colony-forming unit-fibroblast (CFU-F) assay following knockdown and overexpression of BMI1 showed knockdown of p16 in BMI1 knockdown MSCs cannot form colonies. (d) MSCs require BMI1 for survival. Annexin V⁺ cells increase over *in vitro* culture at BMI1 loss MSCs. shBMI1+shp27 at day 9, $P=0,007$, n=3. Overexpression of BMI1 reduces apoptosis. BMI1 at day 6, $P=0.029$, n=3, at day 9, $P=0.009$, n=3. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0005$. Data are shown as mean \pm SEM.

Supplementary Figure 3. Gene expression of Imprinted Gene Network (IGN) (a) mRNA expression of IGN in SCRAMBLE, shBMI1 and BMI1 overexpressing samples, normalized as in Fig 1b, were evaluated. GRB10 ($P=0.0064$), H19 ($P=0.017$), and IGF2 ($P=0.017$) were significantly upregulated by

shBMI1. Other IGNs such as p57 (2.08 fold, $P=0.26$), MEG3 (2.3 fold, $P=0.36$), NDN (2.75 fold, $P=0.12$), PEG3 (2.13 fold, $P=0.39$) and PLAGL1 (1.72 fold, $P=0.32$) were upregulated. n=4. (b) IGN expression levels, normalized as in Fig 1b, in osteogenesis were evaluated. GRB10 ($P=0.026$), IGF2 ($P=0.036$), NDN ($P=0.003$) and PLAGL1 ($P=0.029$) were significantly upregulated upon shBMI1 in osteogenesis. Other IGN expression; p57 (15.9 fold, $P=0.16$), DLK1 (5.29 fold, $P=0.15$), MEG3 (7.62 fold, $P=0.13$), MEST (4.8 fold, $P=0.08$) and PEG3 (3.67 fold, $P=0.14$) were upregulated. n=4. (c) IGN expression in adipogenesis is shown; DLK1 ($P=0.007$), H19 ($P=0.023$), IGF2 ($P=0.031$), MEST ($P=0.007$) and NDN ($P=0.46$) were downregulated upon shBMI1 samples in adipogenesis. n=4. *: $P<0.05$, **: $P<0.01$, ***: $P<0.001$, ****: $P<0.0005$ Data shown as mean \pm SEM.

Supplementary Table Legend

Supplementary Table 1. p-value for rescue of cell growth for shBMI MSCs. p-value was calculated in comparison of shBMI1 at specific days. Pink cells show $P<0.05$. Yellow cells represent $P <0.01$. Green cells indicate $P <0.001$. n=3.

Supplementary Table 2. Nucleotide sequences of shRNAs for vector construction, real-time PCR, and overexpression.

Supplementary Table 3. List of antibodies.

Supplementary Table 1. . p-value for rescue of cell growth for shBMI MSCs							
SCRAMBLE	+	-	-	-	-	-	-
shBMI1	-	+	+	+	+	+	+
shRNA for maternal IGN	-	+	-	+	-	-	+
shRNA for paternal IGN	-	-	+	+	-	-	+
shp16	-	-	-	-	+	-	+
shE4F1	-	-	-	-	-	+	+
Day 2	0.175522425	0.608623944	0.048159932	0.866608295	0.584661169	0.697244954	0.836873283
Day 4	0.029136504	0.000253619	0.001409902	2.92316E-05	5.72962E-05	0.00220207	0.001087322
Day 6	0.002041459	0.000581329	0.000159512	0.005777951	2.69353E-05	0.001463788	0.001742609
Day 8	1.6379E-05	1.65681E-06	0.001674481	2.00508E-05	1.59179E-05	1.51406E-06	1.5491E-05
Day 10	3.72337E-05	0.001032103	0.103084782	0.0013955	6.93694E-06	9.25735E-05	0.000136218
		p<0.05					
		p<0.01					
		p<0.001					

Supplementary Table 2. Nucleotide sequences of shRNAs for vector construction, real-time PCR, and overexpression

(a) shRNAs primers	
Name	Sequences (5' to 3')
BMI1	F CCGGCCAGACCACTACTGAATATAACTCGAGTTATATTCAGTAGTGGTCTGGTTTTTG R AATTCAAAAACCAGACCACTACTGAATATAACTCGAGTTATATTCAGTAGTGGTCTGG
p16/p14	F CCGGCACTACCGTAAATGTCCATTTCTCGAGAAATGGACATTTACGGTAGTGTITTTGCTAGCG R AATTCGCTAGCAAAAACACTACCGTAAATGTCCATTTCTCGAGAAATGGACATTTACGGTAGTG
p27	F CCGGACGTAAACAGCTCGAATTAAGCTCGAGCTTAATTCGAGCTGTTTACGTTTTTGTAGCG R AATTCGCTAGCAAAAACGTAAACAGCTCGAATTAAGCTCGAGCTTAATTCGAGCTGTTTACGT
p57	F CCGGCCACGCACTAGCTCGGTTATTCTCGAGAATAACCGAGCTAGTGCCTGGTTTTGTAGCG R AATTCGCTAGCAAAAACCACGCACTAGCTCGGTTATTCTCGAGAATAACCGAGCTAGTGCCTGG
NOXA	F CCGGGCTTCTGTTCCAGATGATCTTTCTCGAGAAAGATCATCTGAACAGAAGCTTTTTGTAGCG R AATTCGCTAGCAAAAAGCTTCTGTTCCAGATGATCTTTCTCGAGAAAGATCATCTGAACAGAAGC
E4F1	F CCGGTAGAGACTTCAACCGTGATTCCTCGAGGAATCACGGTGAAGTCTCTATTTTTGTAGCG R AATTCGCTAGCAAAAATAGAGACTTCAACCGTGATTCCTCGAGGAATCACGGTGAAGTCTCTA
EZH2	F CCGGCAACACAAGTCATCCATTAACCTCGAGTTAATGGGATGACTTGTGTTGTTTTGTAGCG R AATTCGCTAGCAAAAACAACAAGTCATCCATTAACCTCGAGTTAATGGGATGACTTGTGTTG
DLK1	F CCGGAGTTCGCGAGACTTACTATACTCGAGTATAGTAAGCTCTGCGGAACTTTTGTAGCG R AATTCGCTAGCAAAAAGTTCGCGAGACTTACTATACTCGAGTATAGTAAGCTCTGCGGAAC
GRB10	F CCGGTTGGACTTAAACGACGATTTCTCGAGAAATCGTCGTTAAGTCCAACTTTTGTAGCG R AATTCGCTAGCAAAAAGTTGGACTTAAACGACGATTTCTCGAGAAATCGTCGTTAAGTCCAAC
PLAGL1	F CCGGCTTTGCTCTGTCTAGCTTAACTCGAGTTAAGCTAGACAGAGCAAAGTTTTGTAGCG R AATTCGCTAGCAAAAACCTTGTCTGTCTAGCTTAACTCGAGTTAAGCTAGACAGAGCAAAG
MEST	F CCGGCTTGAGGTTTCATCGGGTGTCTCGAGATCACCCGATGAAACCTCAAGTTTTGTAGCG R AATTCGCTAGCAAAAACCTTGAGGTTTCATCGGGTGTCTCGAGATCACCCGATGAAACCTCAAG
NDN	F CCGGCGAAATCACCAAGATGCAAATCTCGAGATTTGCATCTTGGTGATTTGTTTTGTAGCG R AATTCGCTAGCAAAAACGAAATCACCAAGATGCAAATCTCGAGATTTGCATCTTGGTGATTTG
PEG3	F CCGGGACACTTGTGTAACGTATATACTCGAGTATATACGTTACACAAGTGTCTTTTTGTAGCG R AATTCGCTAGCAAAAAGACACTTGTGTAACGTATATACTCGAGTATATACGTTACACAAGTGT
IGF2	F CCGGCAGAAGCCCAAAGAGCCAAATCTCGAGATTTGGCTCTTTGGGCTTCTGTTTTGTAGCG R AATTCGCTAGCAAAAACAGAAGCCCAAAGAGCCAAATCTCGAGATTTGGCTCTTTGGGCTCTG
H19	F CCGGCGTCCCTTCTGAATTTAATTTCTCGAGAAATTAATTCAGAAGGGACGTTTTGTAGCG R AATTCGCTAGCAAAAACGTCCTTCTGAATTTAATTTCTCGAGAAATTAATTCAGAAGGGACG
MEG3	F CCGGGGTTGTTGTGAGAATTAATCTCGAGATTTAATCTCACAACAACCTTTTTGTAGCG R AATTCGCTAGCAAAAAGGTTGTTGTGAGAATTAATCTCGAGATTTAATCTCACAACAACCC
CHEK2	F CCGGTGTAAGAAAGTAGCCATAAAGCTCGAGCTTATGGCTACTTTCTTACATTTTTGTAGCG R AATTCGCTAGCAAAAATGTAAGAAAGTAGCCATAAAGCTCGAGCTTATGGCTACTTTCTTACA
PPARG	F CCGGGACAGCAGCTTGGCAATATTTCTCGAGAAATATTGCCAAGTCGCTGTCTTTTTGTAGCG R AATTCGCTAGCAAAAAGACAGCAGCTTGGCAATATTTCTCGAGAAATATTGCCAAGTCGCTGT

Abbreviations: PCR, polymerase chain reaction; shRNA, short hairpin RNA

(b) Cloning primers

Name	Sequences (5' to 3')
BMI1	F CAATTGGCTAGCCAAGCAGAAATGCATCGAACAACG R CAATTGTCAACCAGAAGAAGTTGCTGATGACCCATTTACTG

Abbreviations: PCR, polymerase chain reaction; shRNA, short hairpin RNA

(c) qPCR primers	
Name	Sequences (5' to 3')
BMI1	F AAATGCTGGAGAACTGGAAAG R CTGTGGATGAGGAGACTGC
p16	F GAAGGTCCTCAGACATCCCC R CCCTGTAGGACCTTCGGTGAC
p14	F CCCTCGTGCTGATGCTACTG R ACCTGGTCTTCTAGGAAGCGG
p15	F GAGGAGAACAAGGGCATGCCAGTG R GCTTCCAGGAGCTGTCGCACCTTCT
p21	F ACAGCAGAGGAAGACCATGTGGACC R CGTTTTCGACCCTGAGAGTCTCCAG
p57	F CCCATCTAGCTTGCAGTCTCTT R CAGACGGCTCAGGAACCATT
p27	F GGTTAGCGGAGCAATGCG R TCCACAGAACCGGCATTTG
p18	F GGGGACCTAGAGCAACTTACT R GGCAATCTCGGGATTTCCAAG
NOXA	F GAGATGCCTGGGAAGAAGG R ACGTGCACCTCCTGAGAAAA
E4F1	F CCACGCATCTGACCTTGT R CCATCTCACCGTCTCCCAG
BSP	F ATGGCCTGTGCTTTCTCAATG R AGGATAAAAAGTAGGCATGCTT
RUNX2	F CGGAATGCCTCTGCTGTTAT R TTCCCGAGGTCCATCTACTG
OCN	F GACTGTGACGAGTTGGCTGA R CTGGAGAGGAGCAGAACTGG
FABP4	F TGAAAGAAGTAGGAGTGGGCTT R ATCCCCATTCACTGATGATC
PPARG	F TGCAGGTGATCAAGAAGACG R TGGAAGAAGGGAAATGTTGG
IGF2	F TTTGTCCCTCTCCTCTCCA R CAAGGCTCTCTGCCGAACT
MEG3	F GTTTCTGGACTGTGGGCTGT R CAACAGCAACAAAACCTCAGAACATTCA
MEST	F TGACCACATTAGCCACTATCCA R CCTGCTGGCTTCTTCTATACA
NDN	F CTGATGATGTGTGTTGGGGTA R GGCTTTGCTGGTGACTTCTT
PEG3	F ACATTTCTGGTGTGAGGAGTT R AGACCAGGTTCCGGTAATTCT
PLAGL1	F CATATTTGCATGTTAGAAGAATCAGC R TGAGTCAGTTAGGTGAGTGTAGAGAGA
GAPDH	F CTCAGTGTAGCCAGGATGC R ACCACCATGGAGAAGGCTGG

Supplementary Table 3. List of antibodies						
Name	Assay	Antibody	company	Origin	Dilution	Incubation period
IgG Control	FC	559320	BD	mouse IgG	1/10	1 hour, RT
CD14	FC	555398	BD	mouse IgG	1/10	1 hour, RT
CD34	FC	550761	BD	mouse IgG	1/10	1 hour, RT
CD45	FC	555483	BD	mouse IgG	1/10	1 hour, RT
CD73	FC	550257	BD	mouse IgG	1/10	1 hour, RT
CD90	FC	FAB2067P	R&D systems	mouse IgG	1/10	1 hour, RT
CD105	FC	FAB10971P	R&D systems	mouse IgG	1/10	1 hour, RT
BMI1	ICH	05-637	Millipore	mouse IgG	1/50	1 hour, RT
VIMENTIN	ICH	AB1620	Millipore	goat	1/50	1 hour, RT
Hoechst 33342	ICH	H3570	Invitrogen		0.2 µg/mL	10 min, RT
Abbreviations: FC, flow cytometry; ICH, immunocytochemistry; RT, room temperature						