Supplementary Figures and Legends

Supplementary Figure 1. Cardiac-specific Bmi1 expression in cardiogenesis and adulthood

(a) qRT-PCR analysis of wild-type mice for total cardiac expression of Ezh2 and Bmi1 mRNA in embryos (E) at 10.5 and 18.5 dpc, newborns at postnatal day 2 (P2), 10-week-old adults, and aged adults (83-week-old). Results are standardized to β-actin and are expressed as the fold-change of the indicated subpopulations compared with the E10.5 population. Data are means ±SD (n=12, * p<0.05; Student’s t-test).

(b) Representative western blots (WB) showing the expression of Bmi1, Ezh2, p16INK4a (p16), αMHC, and β-actin in heart cells from wild-type newborns at postnatal day 2 (P2) and aged adults (43-week-old).

(c) Representative western blots showing Bmi1 and p16INK4a (p16) expression in heart samples from wild-type mice subjected to TAC or chronic infusion with ISO (n = 4
mice per group), and from representative human patients with healthy hearts (NF; n = 8) and with heart failure (HF; n = 8).

**d** qRT-PCR detection of Bmi1 mRNA expression in in non-failing and failing human hearts.

**e** Representative western blots showing the protein levels of Bmi1, H3K9me3, and β-actin in total heart cells from 22-week-old Bmi1<sup>fl</sup>;NkxCre and Bmi1<sup>f/+</sup> mice.

**f** qRT-PCR detection of mRNA expression of the indicated genes in heart samples from Bmi1<sup>fl</sup>;NkxCre and Bmi1<sup>f/+</sup> control mice (means ±SD; n=10, * p<0.05; Student’s t-test).

**g** Myocyte cross-sectorial area in Bmi1<sup>fl</sup>;NkxCre and Bmi1<sup>f/+</sup> hearts (means ±SD; n=9, * p<0.05; Student’s t-test).

**h** Lung weight in 27-week-old Bmi1<sup>fl</sup>;NkxCre and Bmi1<sup>f/+</sup> control mice (means ±SD; n=10, * p<0.05; Student’s t-test).
Supplementary Figure 2. Adult Bmi1 deficiency causes dilated cardiomyopathy

(a) qRT-PCR analysis of Bmi1, p16INK4a, ARF, Ezh2, p21, and p53 mRNA expression in total heart cells from Bmi1\(^{-/-}\);αMHC Cre (Bmi1-KO) mice. Data are standardized to β-actin levels, and are expressed relative to Bmi1\(^{+/+}\) (Control) mice (means ±SD; n=12, ** p<0.001, * p<0.05; Student’s t-test).

(b) Heart-to-body-weight (HW/BW) ratio in 12-week-old Bmi1\(^{+/+}\);αMHC Cre mice and Bmi1\(^{+/+}\) controls (means ±SD; n=12, * p<0.05; Student’s t-test).

(c) Echocardiographic measurements and physiological parameters in 7-week- and 6-month-old Bmi1\(^{+/+}\);αMHC Cre heterozygotes and Bmi1\(^{+/+}\) controls mice. IVSd, diastolic interventricle septal wall thickness; LVDd, diastolic left ventricle internal dimension; LVDs, systolic left ventricle internal dimension; LVPWd, diastolic left ventricle posterior wall thickness; FS, fractional shortening of left ventricle dimension; EF, ejection fraction; LVmass, left ventricle mass. Data are means ±SD; n=12, ** p<0.001, * p<0.05; Student’s t-test).

(d) Heart-to-body-weight (HW/BW) ratio in 6-month-old Bmi1\(^{+/+}\);αMHC Cre heterozygotes and Bmi1\(^{+/+}\) control mice (means ±SD; n=10, * p<0.05; Student’s t-test).
(e) Representative Masson's trichrome staining to detect fibrosis (bars, 40 μm) and WGA staining of left ventricle to outline cardiomyocytes (bars, 10 μm) in 6-month-old Bmi1^{f+};αMHCCre heterozygotes and Bmi1^{f+} control mice. 

(f) Kaplan-Meier survival curve for Bmi1^{f+};αMHCCre heterozygotes and Bmi1^{f+} littermate mice (means ±SD; ** p<0.001; Student’s t-test).
Supplementary Figure 3. Effect of Tgf-β inhibition in DCM mice

(a) Representative western blots (WB) showing the protein levels of Bmi1, H3K9me3, and β-actin in total heart cells from doxycycline-induced 12-week-old iBmi1^{fl/fl};MLC2^{+/+} (iBmi1^{fl};MLC2) mice and iBmi1^{fl/fl};MLC2^{+/+} (iBmi1^{fl}) control littermates.

(b) Quantification of myocyte cross-sectional area in nontransgenic and Bmi1 transgenic mice subjected to TAC surgery or sham (mean ± s.d. n = 5 mice per group).

(c-e) Left ventricular wall thickness (c), fractional shortening (d), and of myocyte cross-sectional area (e) in 12-week-old Bmi1^{fl/fl},p16^{INK4a-/-}, Bmi1^{fl/fl};αMHC-Cre and Bmi1^{fl};αMHC-Cre;p16^{-/-} mice (means ± s.d. n = 8 mice per group; * p<0.05).

(f) Blood chimerism was analyzed in parabiotic pairs as indicated by measuring the frequency of donor-derived blood cells from one partner (CD45.1+) to the other (means ± SD; n=6; * p<0.05).
### Supplementary Table 1

**Primer sequences used for genotyping**

|                      | Fw LOXP1 Bmi1          | Rv LOXP1 Bmi1          |
|----------------------|-------------------------|-------------------------|
| *cko-Bmi^{fl/fl}* mice | AGAGAATCCAGCTGTCCAGTG   | CCTGGACATCACAATAGGACA   |

|                      | Fw Tg alfa Mhc Cre Ert 2 | Rv Tg alfa Mhc Cre Ert 2 |
|----------------------|---------------------------|---------------------------|
| *α-MHC^{TM}-Cre^{tg/+}* mice | AGGTGGACCTGATCATGGAG     | ATACCGGAGATCATGCAAGC      |
|                       |                           |                           |
| Fw Int Pos Control    | GTAGGTGGAAATCTAGCATCATCC |

|                      | Fw 5 Arm 170pb            |                           |
|----------------------|---------------------------|---------------------------|
| *MLC2-rRTA^{tg/+}* mice | 5'-GGAGGGGAGTGGTCAATACC  |                           |
|                       |                           |                           |
| Fw Int Pos Control    | GTAGGTGGAAATCTAGCATCATCC |

|                      | Fw MHC                    |                           |
|----------------------|---------------------------|---------------------------|
| *p16^{INK4a/-}* mice | GCGGTCTGGCAGTAAAAACTATC   |                           |
|                       |                           |                           |
| Fw Int Pos Control    | GTAGGTGGAAATCTAGCATCATCC |

|                      | Fw MHC                    |                           |
|----------------------|---------------------------|---------------------------|
| *α-MHC-Cre^{tg/+}* mice | GCGGTCTGGCAGTAAAAACTATC   |                           |
|                       |                           |                           |
| Fw Int Pos Control    | GTAGGTGGAAATCTAGCATCATCC |

|                      |                           |                           |
| *Nkx2.5-Cre mice*    |                           |                           |

|                      | NKX 2,5 ANTISENSE          |                           |
|----------------------|---------------------------|---------------------------|
|                       | GCGCACTCACTTTAATGGAAGAG   |                           |
|                       |                           |                           |
|                       | GCCCTGTCCCTCAGATTTCACACC  |                           |
|                       | GATGACTCTGGTCAGAGATACCTG  |                           |
| Primer and probe sequences used in qRT-PCR |
|------------------------------------------|
| Fw Bmi1        | ACCTGCTGCTGGCCCCTTC      |
| Rv Bmi1        | GACTCCTTGATGAAGGTGCCC     |
| Fw Ezh2        | GCCACACCTCGGAATTTTCTTC   |
| Rv Ezh2        | CAGAGCACCCTGGGAGCTGCTG    |
| Fw mBMI1 int   | CAGCAATGACTGTGATGC        |
| Rv mBMI1 int   | CTCCAGCATTCTGCTAGTC       |
| Fw p16 E1      | CGAAGCTTCTTCTGGTGATCC     |
| Rv p16 E2      | TTTGACAGAAGAGCCTGCTAC     |
| Fw1 mp15 qPCR  | AGATCCCACGCCCTGAACCG      |
| Rv1 mp15 qPCR  | TGTCTTTCAGCCAAGTGCTACC   |
| Fw ARF         | TTGGAGGTTCTGGTTTGTC       |
| Rv ARF         | TTCTTTGCGGCGGAGAG         |
| Fw Myh7        | CAACACCAGCCACCCTCTAT      |
| Rv Myh7        | GAGAAGGTGTTGGTCTCGTG      |
| Fw Tgfb        | TACCCCTGGAAGTCTGCTTG      |
| Rv Tgfb        | AACTGCCAGGGATGGAAAAAT     |
| FW E1 p53      | CCGCACACTTCTCAGCAGCGG    |
| RV E2 p53      | GCTTTCAGTACATTTTGGCCC    |
| FW p21         | CCACATCAAACGCTTGGGG      |
| RV p21         | GGAGGACAGAGACAAGGGGC     |
| FW ANP         | CAGGCGTCCAGGCTCCCTGC     |
| RV ANP         | CATCAATTACCTGCCCTACC     |
| FW BNP         | CAAGGCTTGGAGGTACATTGGG   |
| RV BNP         | TCCCTCGGGAAACGGGAGCCC    |
| FW bMHC        | CCTGCCCACGGGTGAGTCG      |
| RV bMHC        | CGCGCTTCTGCATCAGTGACG    |
| FW aMHC        | GCCCTTGCACTTCCACCGG      |
| RV aMHC        | GCCCTTGCACTTCCACCGG      |
qPCR primers used for ChIP analysis

Tgfb3
FW1 PROM  TTTAATTTCCCTTGTAGACAGCC
RV1 PROM  TGTGCTAGCTTTTCAGTGCCGG
FW2 PROM  TTCCCTTGTAGACAGCCTTCC
RV2 PROM  ACCTAAATCGGATAGCAACC
FW3 PROM  GAGACCTTCTGAACCTTGCCGG
RV3 PROM  GGAAGAGAGGTGATCGGGG
FW4 PROM  CGAAGAAAGCAACAGAAGCCC
RV4 PROM  CTTAGGAGACCGCAGTCCG

CyclinA2
FW1 PROM  AACCTTCACAGAGTGACCAC
RV1 PROM  CAGAAAATCTAAACTCTTGACTACC
FW2 PROM  CCTGACCTGCTGCAAAATGGC
RV2 PROM  CATCTAGCTACACATCATAGGG
FW3 PROM  AATCAAGGCTTTTGGGAGGTCC
RV3 PROM  AGAATCTTCAACAGCTCTGCC
FW4 PROM  AACACAGAACTGCTCTCTCC
RV4 PROM  GGAATGATTTTGGATAGACTTCCG

IL1
FW1 PROM  TGCAGAAAGCCTGTGGTTTGC
RV1 PROM  TTTGGACCTCATAAACAACC
FW2 PROM  TTTCCCTACAGCGCTGCGGG
RV2 PROM  TTTCCTTACACAGCCTGGGG
FW3 PROM  AGAGATTCAGTGGCTGCCAGC
RV3 PROM  AGAATCTTCAACAGCTCTGCC
FW4 PROM  CAGGTGTCTTTTGATACGGCC
RV4 PROM  CCATAACAATGAAAAACCTTGACC

Gbe1
FW1 PROM  ATCCGCGCTGCTCTGCTGCC
RV1 PROM  CCTGCTAATCCAAACTCTTGAGCC
FW2 PROM  CCAATGTCTCTAAGAATCTCC
RV2 PROM  TCAGTGCTGCTGCAGACCAT
FW3 PROM  TTTTGTGCTGAAATTTGCTG
RV3 PROM  TGGTACTGCTTGTGCTGCC
FW4 PROM  ATCATGAAATCTTGACACC
RV4 PROM  AAATAATCATTTGGGTGGAGGG

Gmnn
FW1 PROM  GCTCGCACCTACAAAGCCCG
RV1 PROM  TGTCAAACAAAGTGAGGGCCAGG
FW2 PROM  ACTTGGCTCCTTGCTCCCC
RV2 PROM  GGCTGACTCAGATGACTGGG
FW3 PROM  GCAGGTGAGTCACAGCACCG
RV3 PROM  TTGACTAGGATCCGCCTGCC
| FW4 PROM | TCTTTCTTCCCTGAAATCTGGC |
| RV4 PROM | TCTTCACCTTCTCTGCTCTCC |
| P16INK4a | FW1 PROM TGATAAAAACGTAGTTAGAAAGGC |
| RV1 PROM | GAAATTTATGACAGCTTTATTC |
| FW2 PROM | AACCATTTCAGCTTGTAGAGG |
| RV2 PROM | CAGAGAGATGTGGTGTGATAGC |
| FW3 PROM | AGCTACTGACCTAGAATG |
| RV3 PROM | CTAAGAAATGTAGATTTTGGGC |
| FW4 PROM | CATACAAAGGATCCCTGCC |
| RV4 PROM | TCACAGAATGCCTAGAAGGCC |