Supplementary Figure 1.

(A) The region of SIRT1 targeted by the HS6 shRNA. Top sequence shows the wild type sequence in SIRT1 and the one below outlines the six silent mutations in bold that were created in the wild type SIRT1 (verified by sequencing) to render it insensitive to degradation by HS6 shRNA.

(B) Efficiency of SIRT1 shRNA knockdown in BJT cells and expression of the SIRT1-R construct. These four cell lines were used in experiments of Figure 1J and Figure 3D. Lysates were subjected to immunoblotting with an anti-SIRT1 antibody and a beta actin antibody.

(C) Overexpression of control vector (PBP) wild type SIRT1(PBP-SIRT1) and SIRT1H363Y (PBP-SIRT1HY) in Hela cells. Cell lysates were subjected to TRAP analysis as in Figure 1.

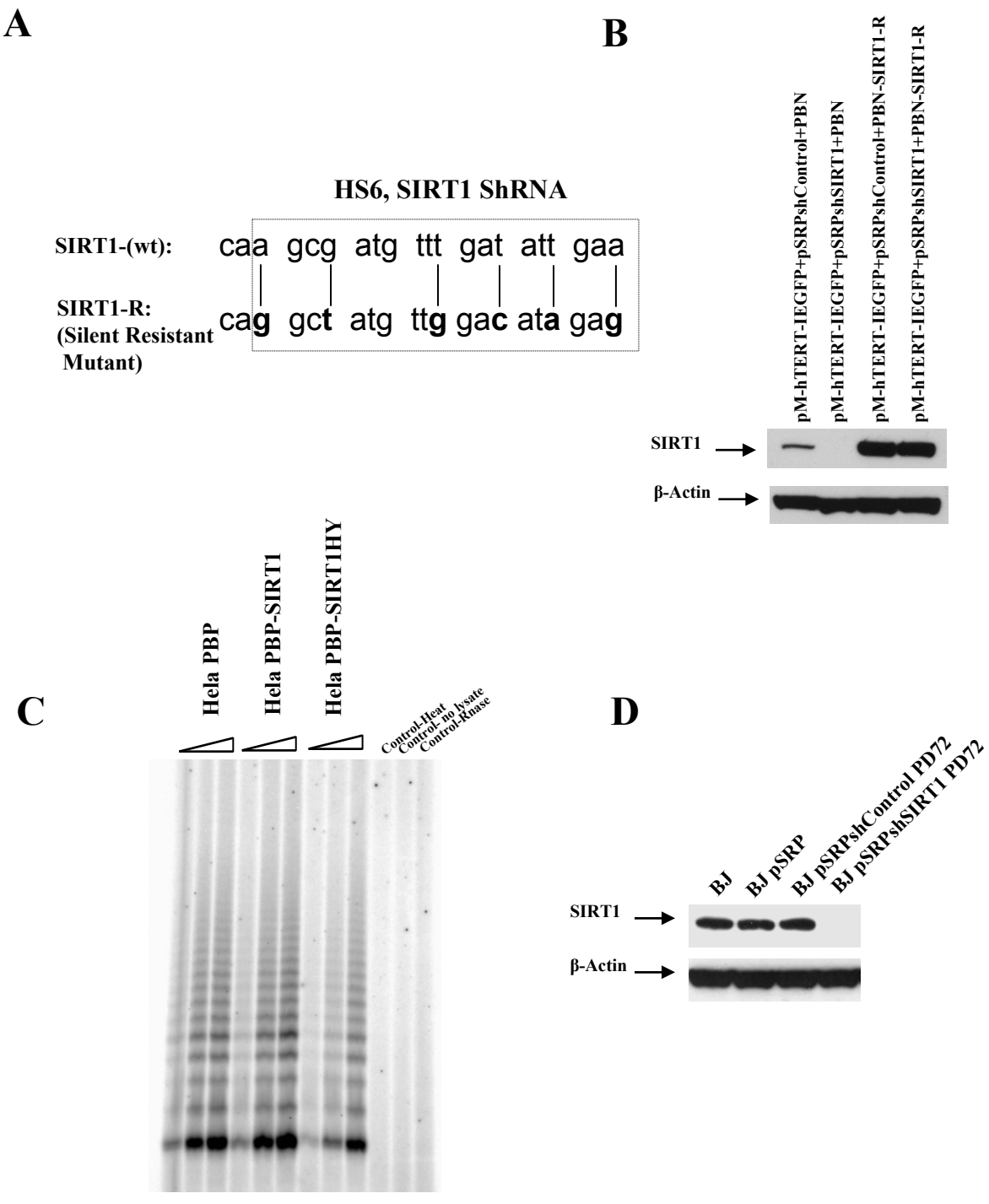
(D) Primary BJ fibroblasts generated were infected with pSRP, pSRP-shControl or pSRPshSIRT1, selected and their lysates were subjected to immunoblotting with an anti-SIRT1 antibody and a β-actin antibody.

Supplementary Figure 2.

(A) Colony growth of HSCs in complete media. HSCs from heterozygote and null mice were stained as described in Materials and Methods, and 100 HSC were sorted individual wells of a 48-well plate containing complete media (x-Vivo 15 serum free media plus cytokine cocktail. Pictures were taken after one week of growth. All donor mice were eight weeks old. (B) Generation of the SIRT1 shRNA resistant mutant SIRT1-R. Mutated

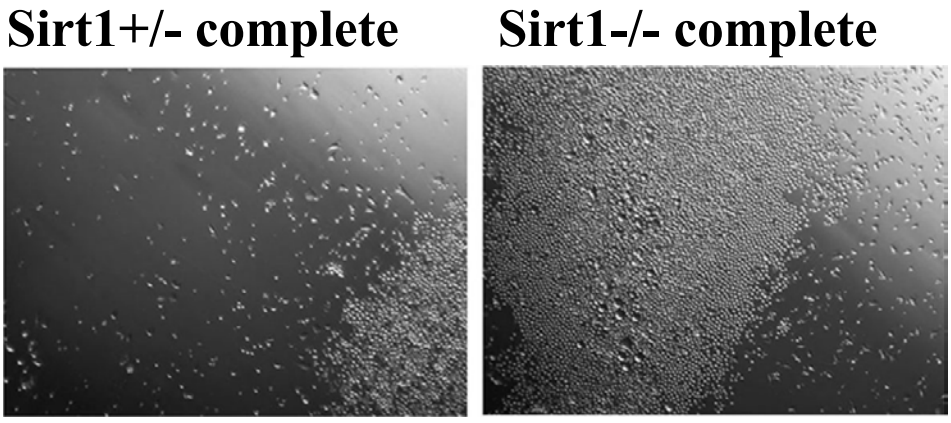
residues that result in silent mutations are shown in bold below. Sequence of the wild type SIRT1 is shown above.

(B) Schematic of the primitive hematopoietic stem cell compartment showing the developmental relationship and cell surface phenotype of hematopoietic stem cells (LT-HSC), and multi-potent progenitor subsets (ST-HSC and MPP^{flk2+}). Self-renewing LT-HSC reside at the top of the hematopoietic hierarchy and give rise to the multi-potent progenitor subsets, which in turn give rise to all mature blood cells by differentiation through a number of intermediate oligo-potent and lineage-restricted progenitors (not shown).

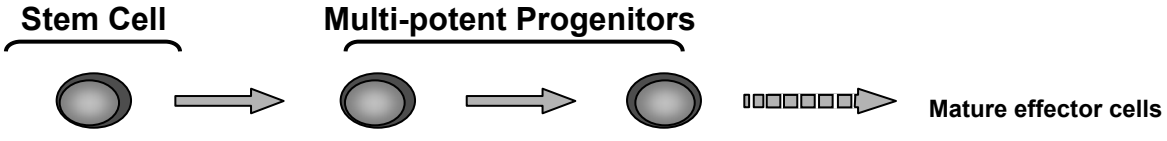


Suppl Fig_1. Narala et al

A



B



Long-term Repopulating Hematopoietic Stem Cell (LT-HSC) $c\text{-}kit^{+}$ $Lineage^{-}$ $Sca\text{-}1^{+}$ $flk2^{-}$ $CD34^{-}$

Short-term Repopulating Hematopoietic Stem Cell (ST-HSC) $c\text{-}kit^{+}$ $Lineage^{-}$ $Sca\text{-}1^{+}$ $flk2^{-}$ $CD34^{+}$

Multi-potent Progenitor (MPP^{flk2+}) $c\text{-}kit^{+}$ $Lineage^{-}$ $Sca\text{-}1^{+}$ $flk2^{+}$ $CD34^{+}$

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