Real-time PCR analysis

Total RNA was extracted from each sample using TRIzol reagent (Invitrogen). RNA was treated with RNase-free DNase for 20 min (Ambion, Austin, TX) at room temperature before reverse transcription with Superscript II RT (Invitrogen, Carlsbad, CA). For PCR array experiments, an RT^2 Profile Custom PCR Array was used to simultaneously examine the mRNA levels of 48 genes, including three "housekeeping genes" in 96-well plates according to the protocol of the manufacturer (SuperArray Bioscience). Real-time PCR was performed on an MX30000P Stratagene machine with SYBR Green PCR Master Mix (Superarray). PCR conditions consisted of a 10 min hot start at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 min at 60°C. The average threshold cycle for each gene was determined from triplicate reactions and three independent experiments. Values were exported to a template Excel file for analysis. Analyses of the raw data were done through the Superarray Data Analysis Web Portal. $P<0.05$ was considered to indicate statistical significance.

Alkaline phosphatase staining

mES cells were cultured with 2-ME and LIF for 3 days to obtain spheroidal colonies. Cells were washed with PBS (BioWhittaker, Walkersville, MD) and fixed with 4% paraformaldehyde (Sigma) in PBS for 5 min. After washing, Cells were stained with an Alkaline Phosphatase Staining Kit (Chemicon, Temucula, CA) at room temperature for 15 min in the dark. Cells were washed with PBS again, and colonies were visualized using an Olympus Microscope.

Flow cytometry analysis of Oct-4, CD9 and KLF-4

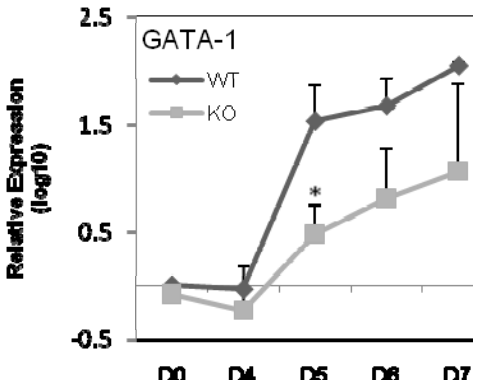
An aliquot of 1×10^6 cells was washed in ice-cold PBS containing 2% FCS serum (PBS-2% FCS) and incubated with anti-mouse CD16/CD32 receptor monoclonal antibody at 1 µg/100 µl (BD Biosciences) to block non-specific binding of immunoglobulins to the mouse FcIII/II receptors. Cells to be stained for Oct-4 and KLF-4 were fixed with 100 µl of IC fixation buffer (eBioscience) for 30 min at room temperature, washed one time with PBS-2% FCS, permeabilized with permeabilization buffer (eBioscience), and incubated with a 1:100 dilution rabbit-mouse Oct3/4 polyclonal antibody (Chemicon) or rabbit-mouse KLF-4 polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min at room temperature. Cells were washed two times with 1 ml of PBS-2% FCS followed by staining with 1:100 dilution of FITC: goat anti-rabbit IgG antibody (Santa Cruz Biotechnology). Finally, the cells were washed two times with PBS-2% FCS and resuspended in PBS-2% FCS for analysis on a FACScan flow cytometer (Becton Dickinson). Cells analyzed for CD9 were incubated first with a 1:100 dilution of anti-mouse CD9 (BD Pharmingen) for 40 min at 4°C, washed two times with ice-cold PBS-2% FCS, and incubated with a 1:500 dilution of FITC: goat-anti rat IgG antibody (Santa Cruz Biotechnology).

	NUCLEATED CELLULARITY (BONE MARROW)					
	5 WEEKS OLD			>12 MONTHS		
	MEAN	STE	P	MEAN	STE	P
WT (+/+)	15.4	1.0	CONTROL	20.4	2.4	CONTROL
HET(+/-)	15.3	3.0	0.490	14.4	4.4	0.230
KO (-/-)	ND	ND	ND	15.1	2.7	0.105

Table S1. Nucleated cellularity/femur for WT, SIRT1$^{+/+}$ and SIRT1$^{-/-}$ mice of 5 weeks and over a year old

ND= not done. Data are the average of 3-5 mice/group.

A



B

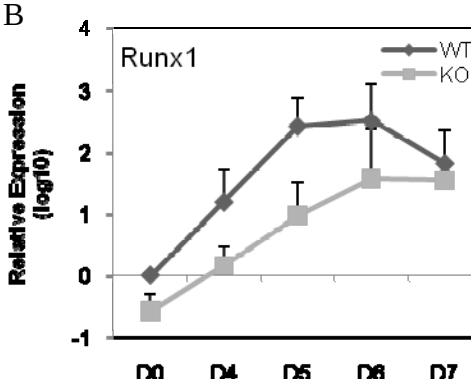
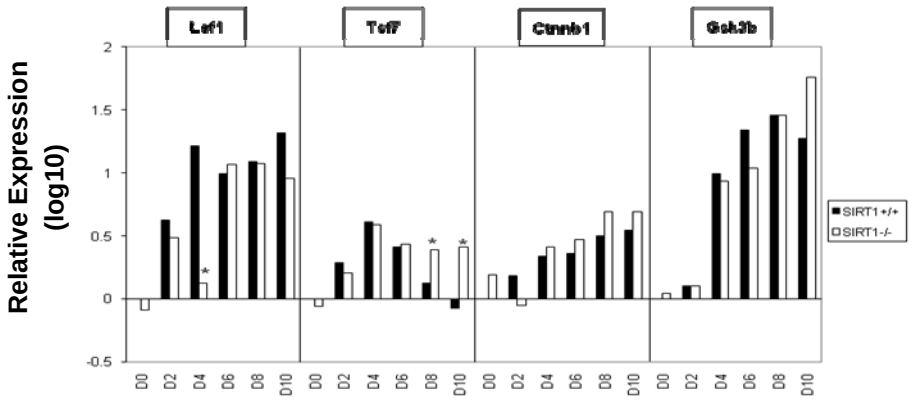


Figure S1. qRT-PCR analysis of mRNA levels of genes (A) Gata-1 and Runx-1 in day 0, day 4-7 SIRT1$^{+/+}$ and SIRT1$^{-/-}$ EBs

Data are relative to SIRT1$^{+/+}$ D0 control and mRNA levels of each gene were compared between SIRT1$^{+/+}$ and SIRT1−/− EBs at each time point. Results were the average of three independent experiments, each performed in triplicate. *P<0.05.

A



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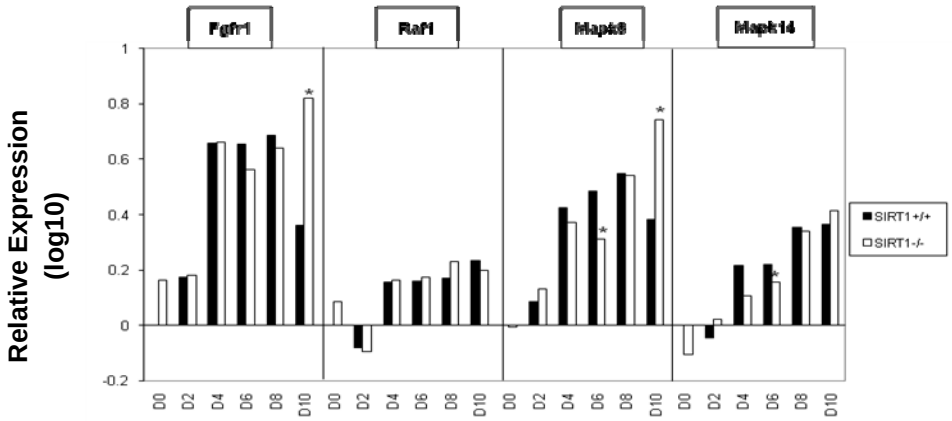

Figure S2
(A) qRT-PCR analysis of mRNA levels of genes (Lef1, Tcf7, Ctnnb1, Gsk3b) involved in Wnt pathways in day 0-10 SIRT1$^{+/+}$ and SIRT1$^{-/-}$ EBs. (B) qRT-PCR analysis of mRNA levels of genes (Fgfr1, Raf1, Mapk8, Mapk14) involved in FGF pathways in day 0-10 SIRT1$^{+/+}$ and SIRT1$^{-/-}$ EBs. Graphs were plotted in logarithmic scale. Data are relative to WT D0 control and mRNA levels of each gene were compared between WT and SIRT1−/− EBs at each time point. Results were the average of three independent experiments, each performed in triplicate. *P<0.05.

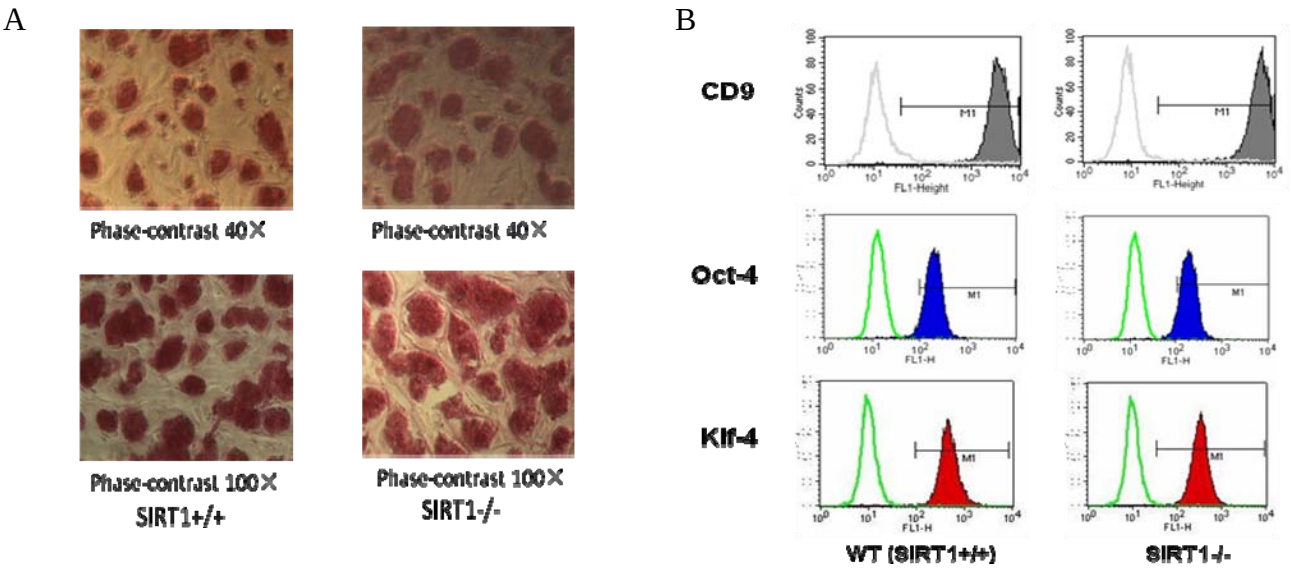

Figure S3
(A) Alkaline phosphatase staining of SIRT1$^{+/+}$ R1 and SIRT1−/− cells when cultured with 2-ME and LIF. (B)Expression of CD9, Oct-4 and KLF-4 were measured by flow cytometry.