Supplementary Materials and Methods

Table S1. Primers used for qPCR

Gene	Forward	Reverse
Fanca	TCCTTGTCAGCGAGATCATG	CCTGAAGAAGTGGATGGTAAT
Fancc	CTTACGGTGCTCCATGTCTTG	CTGAGCAGCATCAGGAGACGG
Wnt5a	CACTTAGGGGTTGTTCTCTGA	ATATCAGGCACCATTAAACCA
Gapdh	TCAATGAAGGGGTCGTTGAT	CGTCCCGTAGACAAAATGGT

Supplementary figures

Figure legend:

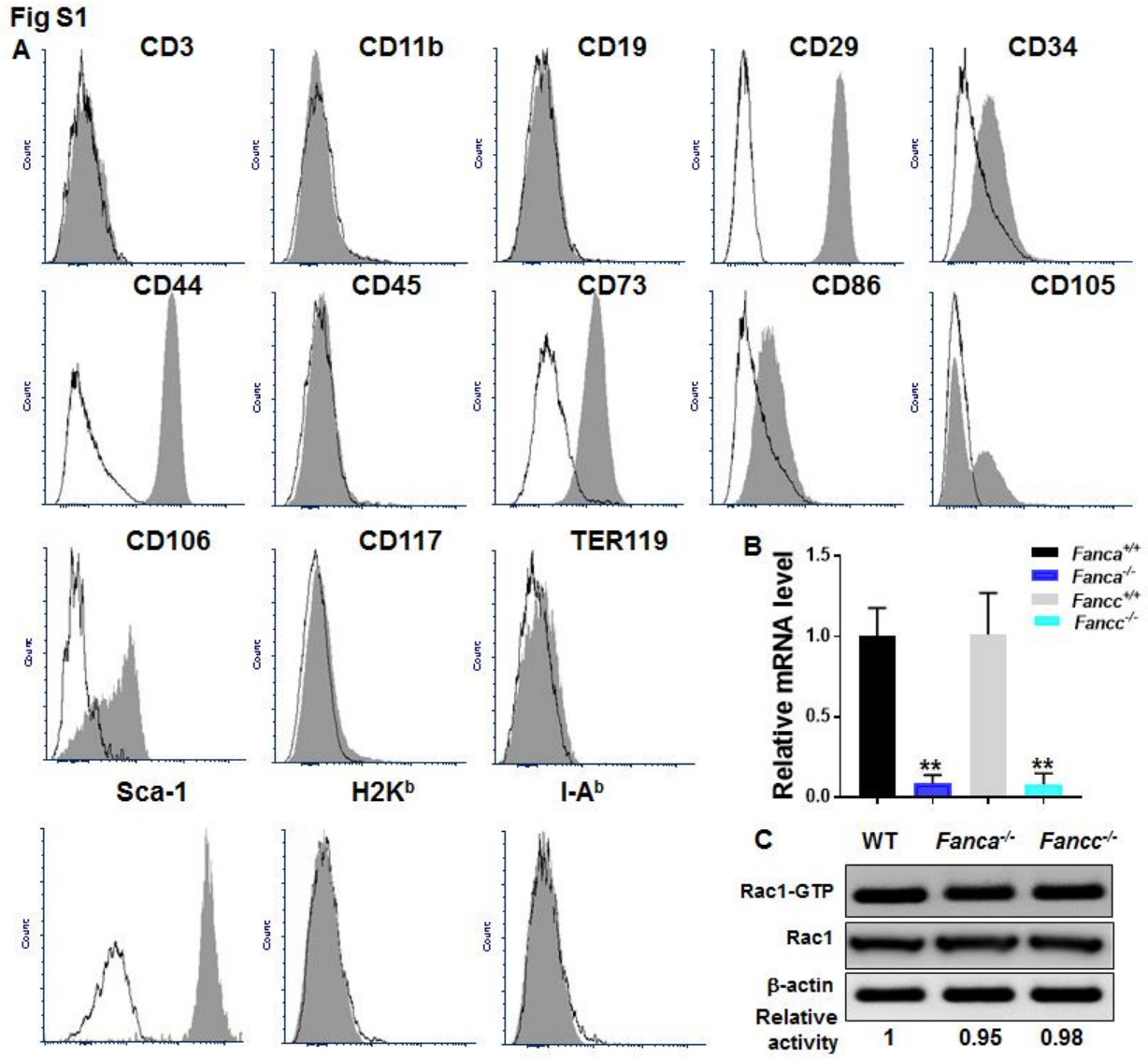


Fig S1. Representative flow plots of phenotypic Mesenchymal stromal cells (**MSC) staining.** (A) Representative flow plots of phenotypic MSC staining. Whole bone marrow cells (WBMCs) isolated from wild-type (WT), *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ mice were cultured in 10 ml of MSC medium. Plastic adherent cells were passaged 3 times then subjected to Flow cytometry analysis using indicated antibodies against MSC surface markers. (B) mRNA

levels of *Fanca* or *Fancc* in MSCs. RNA were extracted from the cells described in (A) followed by qPCR analysis for *Fanca* or *Fancc* mRNA. Using the primers listed in Table S1. Samples were normalized to the level of *GAPDH* mRNA. (C) Level of Rac1-GTP is similar in *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ MSCs. MSCs isolated WT, *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ mice were cultured in MSC medium and passaged for 3 times. Whole cell lysates (WCL) was then extracted and subjected to Western blotting using antibodies against Cdc42. The level of active, GTP-bound Cdc42, total Cdc42 and β-actin were determined. The relative levels of active Cdc42 are indicated below each pull-down blot.

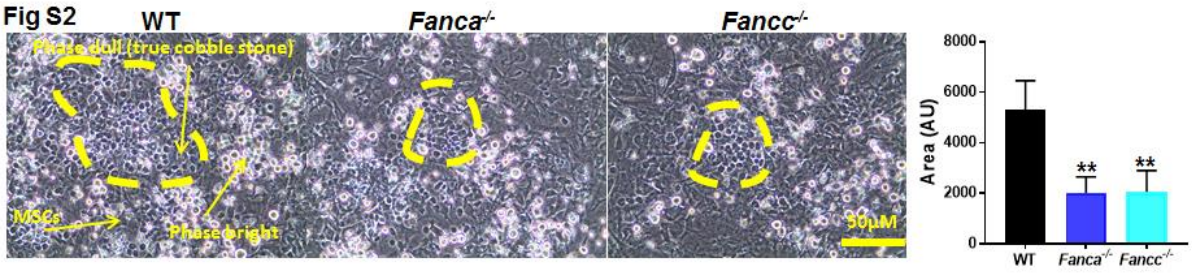
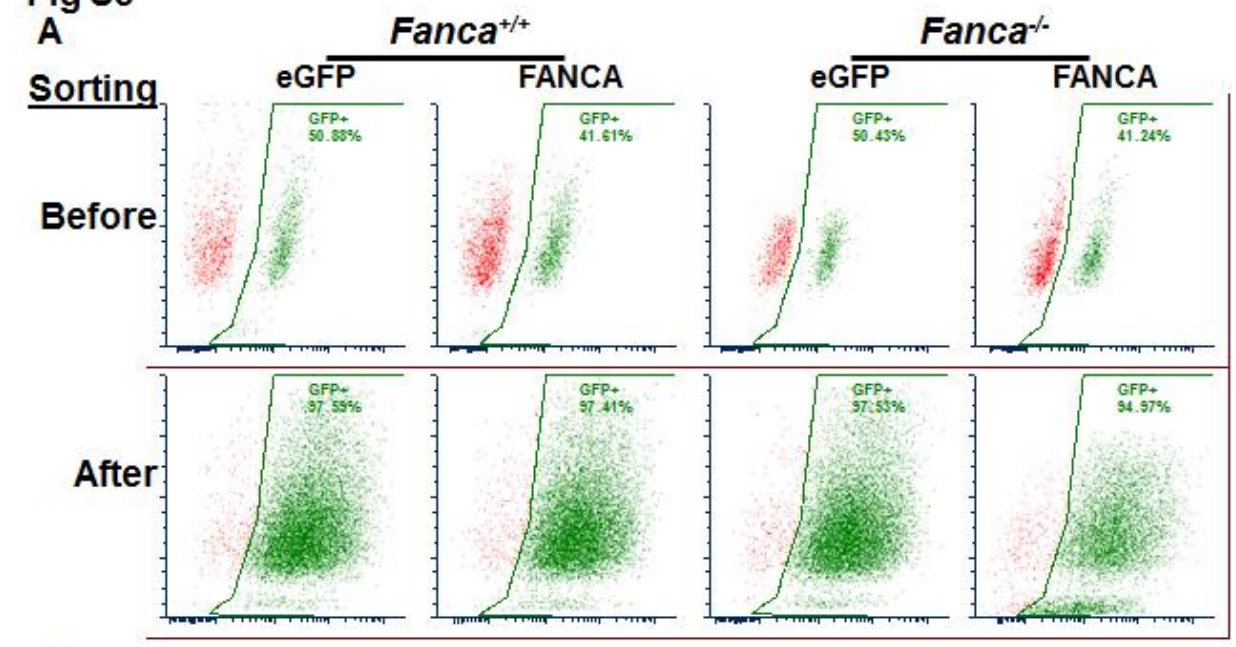
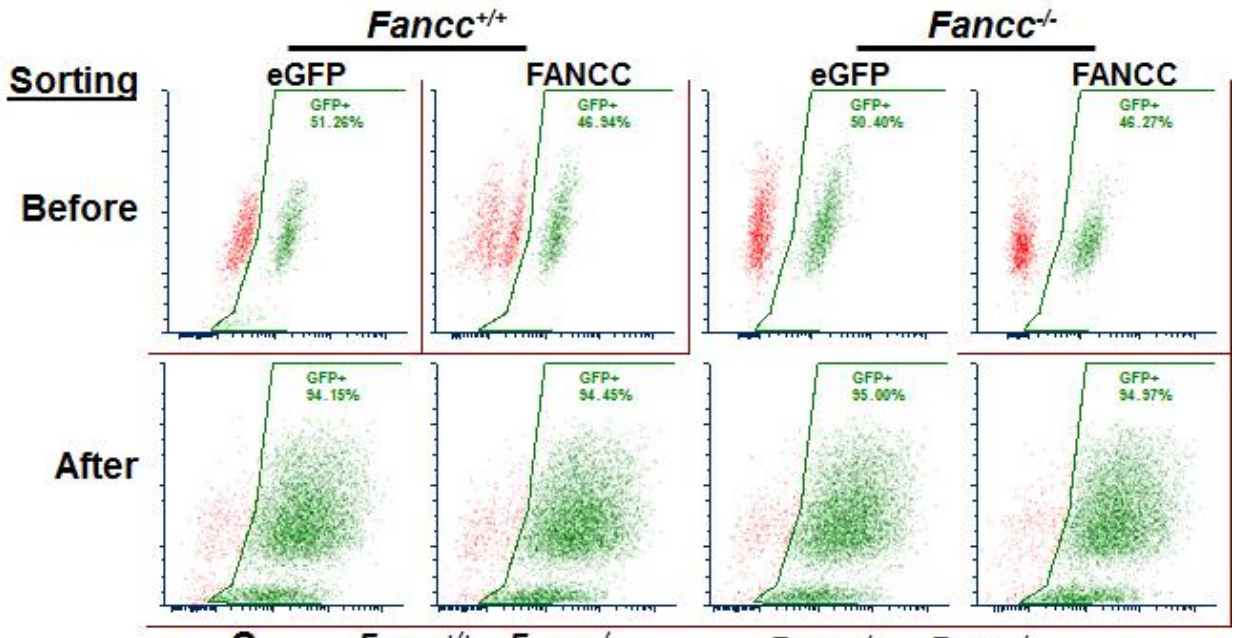


Fig S2. Phase contrast micrographs of differentiating clones after two-week co-culture. (A) Phase contract micrographs of differentiating clones after two-week co-culture. Maturing hematopoietic cells appear as small refractive (phase bright) cells on the interface of stromal cells and the supernatant. The phase dull cells are cobblestone cells, which are covered by the adherent stromal layer. The scale bar represents 100μM. (B) Quantification of cobblestone area. Area was measured in pixels using ImageJ and plotted as arbitrary units (AU). ** $p<0.01$.

Fig S3


A
Fanca+/+
Fanca-/-
Sorting
eGFP
FANCA
eGFP
FANCA
Before
GFP+ 50.88%
GFP+ 41.61%
GFP+ 50.43%
GFP+ 41.24%
After
GFP+ 97.59%
GFP+ 97.41%
GFP+ 97.53%
GFP+ 94.97%


B
Fancc+/+
Fancc-/-
Sorting
eGFP
FANCC
eGFP
FANCC
Before
GFP+ 51.26%
GFP+ 46.94%
GFP+ 50.40%
GFP+ 46.27%
After
GFP+ 94.15%
GFP+ 94.45%
GFP+ 95.00%
GFP+ 94.97%

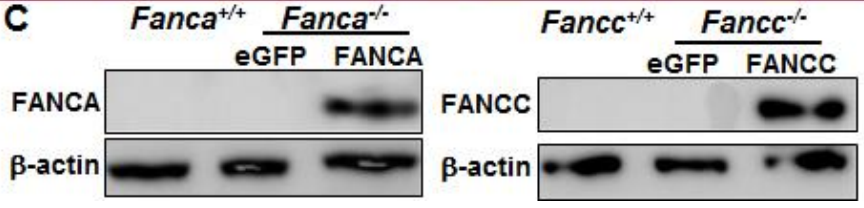

C
Fanca+/+
Fanca-/-
eGFP
FANCA
FANCA
β-actin
Fancc+/+
Fancc-/-
eGFP
FANCC
FANCC
β-actin

Fig 3. Transduction efficiency of MSC cells. 70% confluent MSCs isolated from wild-type (WT), *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ mice were transduced with retroviral vector expressing eGFP, eGFP-FANCA (A) or eGFP-FANCC (B). Flow plots of before and after cell sorting for GFP were shown. (C) Western blot analysis of FANCA and FANCC proteins in complemented MSCs. Whole cell lysates from sorted cells described in (A) and (B) were subjected to immunoblotting using antibodies against FANCA, FANCC or β-actin.

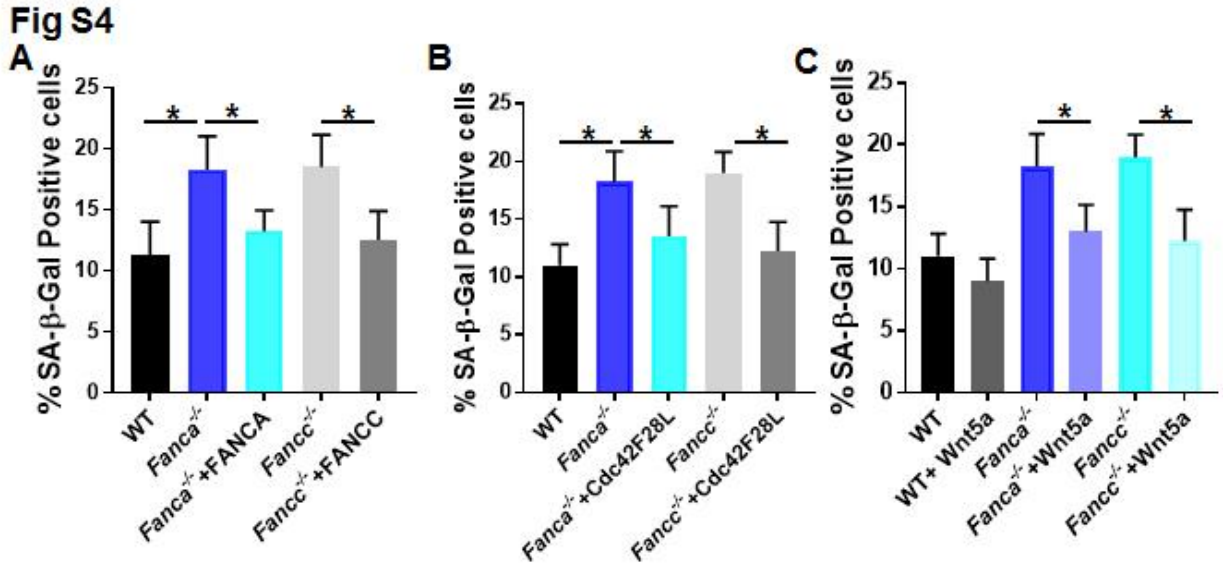


Fig S4. Gene correction, ectopic expression of an active Cdc42 mutant or Wnt5a pretreatment reverses senescent phenotype of FA MSCs. (A) Gene correction of FA deficiency reduces the numbers of *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ MSCs stained positive for SA-β-gal. Sorted cells described in Fig S3 were subjected to SA-β-gal staining. (B) Ectopic expression of the active Cdc42 mutant reverses senescent phenotype of *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ MSCs. MSCs isolated from WT, *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ mice were transduced with retroviral vector expressing the constitutively active Cdc42 mutant (Cdc42F28L). Sorted GFP^{+} MSC cells were subjected to SA-β-gal staining. (C) Wnt5a treatment reduces

senescence of *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ MSCs. MSCs isolated from WT, *Fanca*$^{-/-}$ or *Fancc*$^{-/-}$ mice were treated with Wnt5a followed by SA-β-gal staining. Percentages of the cells stained positive for SA-β-gal were quantified by counting a total of 100 cells in random fields per well. *$p<0.05$.