Supporting Information

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SI Materials and Methods

Generation of Hematopoietic-Specific Cx43-Deficient Mice. Animals were bred and housed in the Association for Assessment and Accreditation of Laboratory Animal Care-accredited animal facility of Cincinnati Children's Hospital Medical Center or purchased from Jackson Laboratories. All of the experiments were carried out under approved animal protocols by the Cincinnati Children's Hospital Institutional Animal Committee in conformance with all relevant regulatory standards for animal care. All of the animals were backcrossed for a minimum of five generations into C57BL/6 background. Cx43 gene inactivation in the hematopoietic compartment was performed using Vav1-Cre/ $Cx43^{flox/flox}$ mice (hematopoietic-H-Cx43-deficient) in which the Cre expression protein was induced by the promoter Vav1 that is expressed in HSC/P (1–3). In each experiment, mice were i.v. injected with 150 mg/kg body weight 5-FU (Sigma) on day 0. BM was harvested on day 4 postadministration from normal and treated mice. BM cells were obtained by crunching murine femora and tibiae. Single BM was obtained by filtration through a 40-μm filter. The cells were laid on top of Histopaque-1083 (Sigma) and centrifuged at 450 × *g* for 30 min at room temperature to obtain low-density BM (LDBM) cells.

In Vivo Drug Administration and Peripheral Blood Analysis. The 5-FU (150 mg/kg; one dose) (APP Pharmaceuticals) was administered intravenously. NAC (100 mg/kg; Sigma) or PBS (control) were administered i.p. Mice were bled on the indicated days. Peripheral blood samples were collected by retroorbital bleeding. Automated total cell count (Drew Scientific) was performed.

HSC Competitive Repopulation Assay. Adult recipient mice were lethally irradiated with a ^{137}Cs γ-irradiator, as described previously (4, 5). For competitive repopulation experiments, 3×10^6 $CD45.2^+$ BM nucleated cells (BMNCs) were mixed with 3×10^6 $CD45.1^+$ BMNCs and were transplanted into lethally irradiated CD45.1+ $B6.SJL^{Ptprca\ Pep3b/BoyJ}$ recipient mice. Short- and long-term HSC engraftment was analyzed for contribution of $CD45.2^+$ and lineage markers by flow cytometry.

Cell Sorting of HSCs and HSC/P Cells. For sorting of BM HSCs from unchallenged animals, LSK/$CD34^-$ (HSC) or LSK/$CD34^+$ (MPP) cells were sorted. LDBM cells were stained with lineage markers [CD45R/B220, Gr-1, CD4, CD8a, CD3e, Ter119, and CD11b (Mac1, M1/70) and c-kit (2B8) and Sca-1/Ly6A/E (D7), CD34 (RAM3.4); Becton Dickinson]. For sorting of HSCs from BM of 5-FU-treated mice, $lineage^-/CD41^-/CD48^-/CD150^+$ were sorted. LDBM cells were stained with CD45R (B220; clone RA3-6B2), Gr-1 (Ly-6G and Ly-6C; clone RB6-8C5), CD4 (L3T4; clone RM4-5), CD8a (Ly-2; clone 53-6.7), CD3e (clone 145-2C11), Ter119 (Ly-76), CD41 (MWReg30), and CD48 (HM48-1) from BD and anti-CD150 (mShad150) from eBioscience. HSC/P were identified as LSK cells and sorted.

Proliferation, Cell Cycle, and Survival Assays. HSC proliferation and survival in vivo were determined as described previously (6). Briefly, for in vivo BrdU proliferation analysis, mice received single i.p. injections of BrdU (300 μg). Forty-five minutes later, LDBM cells were stained with CD45R/B220, Gr-1, CD4, CD8a, CD3e, Ter119, CD41, CD48 and CD150. The cells were then fixed and stained with anti-BrdU antibody (Cytofix/Cytoperm Kit; BD Biosciences) according to the manufacturer's instructions. For in vivo Ki67 proliferation analysis, LDBM cells were prepared and stained for surface markers. The cells were then fixed with cold 70% (vol/vol) ethanol and stained with anti-Ki67 antibody (BD Biosciences) according to the instructions of the manufacturer. For in vivo pyronin Y/7-AAD proliferation analysis, BM cells were prepared and stained for surface markers. The cells were then fixed and stained with 20 μg/mL 7-AAD and 1.5 μg/mL pyronin Y. For in vivo survival analysis, following with staining of surface markers, LDBM cells were fixed and stained with anti-Caspase-3 antibody (Cytofix/Cytoperm Kit; BD Biosciences). The cells were then fixed and stained with anti-BrdU antibody (Cytofix/Cytoperm Kit; BD Biosciences) according to the instructions of the manufacturer. For in vivo Ki67 proliferation analysis, LDBM cells were prepared and stained for surface markers. The cells were then fixed with cold 70% (vol/vol) ethanol and stained with anti-Ki67 antibody (BD Biosciences) according to the instructions of the manufacturer. For in vivo pyronin Y/7-AAD proliferation analysis, BM cells were prepared and stained for surface markers. The cells were then fixed and stained with 7-AAD and pyronin Y. For in vivo survival analysis, following with staining of surface markers, LDBM cells were fixed and stained with anti-Caspase-3 antibody (Cytofix/Cytoperm Kit; BD Biosciences). Flow cytometric analysis was performed on gated $lineage^-/CD41^-/CD48^-/CD150^+$ events.

Lentiviral Cx43 Transduction and Rescue Experiment. The lentiviral vector pCDH-MCS-EF1-copGFP carrying the sequence of Cx43 was used to produce the virus. The empty vector was used as negative control. Sorted LSK cells were plated onto retronectin (Takara Bio)-coated plates and transduced with lentiviral supernatant at a multiplicity of infection of ∼20, supplemented with 100 ng/mL murine stem cell factor (Peprotech) SCF and 50 ng/mL thrombopoietin, and submitted to two rounds of transduction. For the rescue experiment through reexpression of Cx43, 5×10^3 transduced WT or H-Cx43-deficient LSK cells (transduced with empty vector or Cx43-containing vectors; with transduction efficiencies of 69.2 ± 1.0%, 44.07 ± 3.5%, 76.2 ± 1%, 41.42 ± 2.3% for WT+Mock, WT+Cx43, Cx43-deficient+Mock, and Cx43-deficient+Cx43, respectively; $n = 2$ independent experiments) were mixed with 0.5×10^6 $CD45.1^+$ BMNCs and transplanted into each lethally irradiated $CD45.1^+$ $B6.SJL^{Ptprca\ Pep3b/BoyJ}$ recipient mouse. After 4 wk, transplanted mice were i.v. injected with 5-FU and bled on the indicated days. Hematopoietic recovery of $CD45.2^+/GFP^+/CD11b^+$ cell counts in peripheral blood was determined by flow cytometry and cell counts. For the rescue experiment of ROS transfer, GFP-positive HSC/P cells were sorted after transduction and used for the experiment as described below.

Generation of H-Cx43-Deficient Chimeric Mice and HM Cx43-Deficient Mice. Confirmatory experiments on hematopoietic chimeras of Vav1-Cre;WT or Vav1-Cre;$Cx43^{flox/flox}$ BM cells were performed as follows. A total of 3×10^6 BMNCs from Vav1-Cre;WT or Vav1-Cre;$Cx43^{flox/flox}$ were transplanted into lethally irradiated $CD45.1^+$ $B6.SJL^{Ptprca\ Pep3b/BoyJ}$ mice. For experiments on HM H-Cx43-deficient mice, Mx1-Cre;WT or Mx1-Cre;$Cx43^{flox/flox}$ mice received 300 μg of polyI:C (Amersham/Pharmacia) every other day for 16 d i.p. One week after the last polyI:C injection, mice were bled to analyze Cx43 genomic deletion as reported previously by our group (7, 8). BM Cx43-deficient mice were lethally irradiated, and 3×10^6 WT $CD45.1^+$ $B6.SJL^{Ptprca\ Pep3b/BoyJ}$ BM cells were transplanted i.v. to generate HM Cx43-deficient mice.

Eight weeks posttransplantation, mice were challenged with 5-FU as aforementioned.

ROS, Mitochondrial Mass, and Mitochondrial Membrane Potential Analyses. LDBM cells were stained for surface markers (lineage makers/CD41/CD48/CD150). Cells were incubated with 5 mM DCF-DA (20-70-dichlorofluorescein diacetate; Molecular Probes), 50 μM DHE (Molecular Probes) for 30 min at 37 °C or with 5 μM MitoSOX Red mitochondrial superoxide indicator (Molecular Probes) for 10 min at 37 °C, followed by flow cytometric analysis. Mitochondrial mass and membrane potential were determined by MitoTracker Green FM (Molecular Probes) and MitoTrackerRed FM (Molecular Probes), respectively.

ROS Transfer. LSK cells (1×10^4 cells/sample) were treated with or without 5 μM LY83583 (6-anilino-5,8-quinolinequinone; Sigma-Aldrich) for 16 h at 37 °C and stained with 5 μM carboxyfluorescein diacetate, succinimidyl ester (CFSE) (Molecular Probes). FBMD-1 stromal cells (1×10^3 cells/sample) were plated into a 96-well plate and were incubated with 10 mM NAC for 16 h and stained with 50 μM DHE. Cells were washed in PBS. After that, LSK cells were cocultured with FBMD-1 stromal cells in NAC-containing medium in each of 96 wells for 3 h at 37 °C, followed by flow cytometric analysis.

Analysis of MAPK or p38 Signaling Pathway. LDBM cells were stained for surface markers (lineage makers/CD41/CD48/CD150). The cells were then fixed with cold methanol and stained with anti-phospho ERK antibody, anti-phospho p38, or anti-isotype IgG antibody (BD Biosciences), followed by flow cytometric analysis.

Immunofluorescence Analysis. For Cx43, Cx45, p53, and p16 staining, HSCs were seeded on poly-lysine-coated glass coverslips and fixed with 4% paraformaldehyde in PBS for 15 min at room temperature and gently washed with PBS. Cells were permeabilized with 0.2% Triton X-100 (Sigma) in PBS for 20 min and blocked with 5% goat serum plus 1% BSA in PBS for 1 h at room temperature. Incubations with primary and secondary antibodies were performed overnight at 4 °C. The primary antibodies used were anti-Cx43 (rabbit polyclonal; 1:100) and anti-Cx45 (rabbit polyclonal; 1:100), both from Invitrogen; anti-phospho-p53 antibody (1:100) and anti-p53 antibody (1:100) (both from Cell Signaling); and anti-p16 antibody (1:50) (from Santa Cruz). Coverslips were mounted with Pro-Long Gold Antifade Reagent (Invitrogen). Immunofluorescence samples were analyzed using confocal microscopy (Zeiss). A total of 30–50 individual HSCs were analyzed per sample. The intensity of the fluorescence was analyzed and quantified using computer-imaging software (Axiovision; Carl Zeiss). For Cx staining, freshly sorted HSCs were seeded on fibronectin-coated glass coverslips. After 1 h of incubation at 37 °C (5% CO_2, 3% O_2), HSCs were fixed with 4% paraformaldehyde in PBS for 15 min at 4 °C and gently washed with PBS. Cells were permeabilized with 0.2% Triton X-100 in PBS for 20 min and blocked with 10% donkey serum (Sigma) for 30 min. Incubations with primary and secondary antibodies were performed overnight at 4 °C. The primary antibodies used were anti-Cx43 antibody (1:70) (Invitrogen) and anti-Cx45 antibody (1:100) (Chemicon). Immunofluorescence samples were analyzed using fluorescence microscopy (AxioObserver Z1 equipped with a 63× phase contrast objective and appropriate fluorescence filters; Zeiss). A total of 20 individual HSCs were analyzed per sample.

Genomic PCR Analysis. Genomic PCR analysis was performed as described previously by our group (7, 8).

RT-PCR Analysis. Total RNA was isolated from sorted HSCs using RNeasy Micro Kit (Qiagen). Isolated RNA was reverse-transcribed with Multiscribe Reverse Transcriptase (Applied Biosystems), followed by amplification of cDNA using TaqMan Universal PCR Master Mix (Applied Biosystems). GAPDH was used as an internal control.

Microarray Analysis. Total RNA from sorted LSK cells was isolated using the RNeasy Micro Kit (Qiagen). The cDNA was then hybridized according to the protocol of the manufacturer to the Affymetrix GeneChip Mouse Exon 1.0 ST Array. The RNA quality and quantity assessment, probe preparation, labeling, hybridization, and image scan were carried out in the Cincinnati Children's Hospital Medical Center Affymetrix Core using standard procedures. Hybridization data were sequentially subjected to normalization, transformation, filtration, functional classification, and pathway analysis. Data analysis was performed with Genespring GX11 (Agilent Technologies). Genes that were differentially expressed in Cx43-deficient LSK (5-FU) ($n = 3$) compared with WT LSK (5-FU) ($n = 3$) cells with fold change by 1.5 ($P < 0.05$) were analyzed in Ingenuity Pathways Analysis (Ingenuity Systems) to group into a specific pathway. The top signaling pathways were considered to be overly represented when unpaired Student t test P value was ≤0.05. GJ genes were also compared for significant changes ($P < 0.05$).

Statistical Analysis. Statistical significance was assessed by Student t test or ANOVA test for independent samples. Statistically significant differences were considered at $P < 0.05$.

1. Daria D, et al. (2008) The retinoblastoma tumor suppressor is a critical intrinsic regulator for hematopoietic stem and progenitor cells under stress. *Blood* 111:1894–1902.
2. Ghiaur G, et al. (2008) Rac1 is essential for intraembryonic hematopoiesis and for the initial seeding of fetal liver with definitive hematopoietic progenitor cells. *Blood* 111: 3313–3321.
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5. Cancelas JA, et al. (2005) Rac GTPases differentially integrate signals regulating hematopoietic stem cell localization. *Nat Med* 11:886–891.
6. Sengupta A, Arnett J, Dunn S, Williams DA, Cancelas JA (2010) Rac2 GTPase deficiency depletes BCR-ABL+ leukemic stem cells and progenitors in vivo. *Blood* 116: 81–84.
7. Presley CA, et al. (2005) Bone marrow connexin-43 expression is critical for hematopoietic regeneration after chemotherapy. *Cell Commun Adhes* 12:307–317.
8. Presley CA, Cancelas JA (2007) Isolation of high-quality genomic DNA from murine bone marrow samples using the ChargeSwitch gDNA Blood kit with a modified protocol. *Quest* 4:11–16.

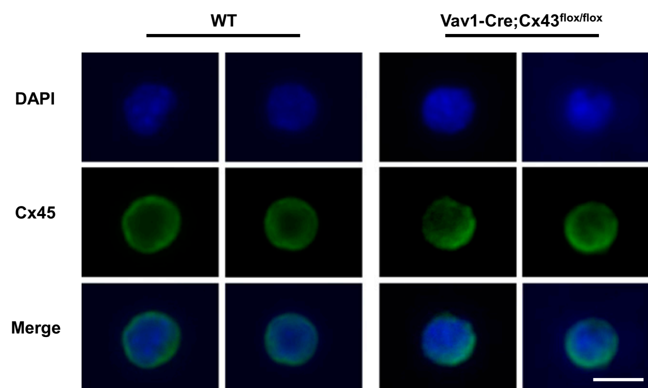

Fig. S1. Cx45 expression is not altered in H-Cx43-deficient HSCs. Immunofluorescence pictures showing Cx45 (green) along with DAPI (blue) in WT or H-Cx43-deficient HSCs. Cx45 is detected around the cell membrane both in WT HSCs and H-Cx43-deficient HSCs (n = 20 HSCs from individual mouse; n = 3 mice per group). (Scale bar, 5 μm.)

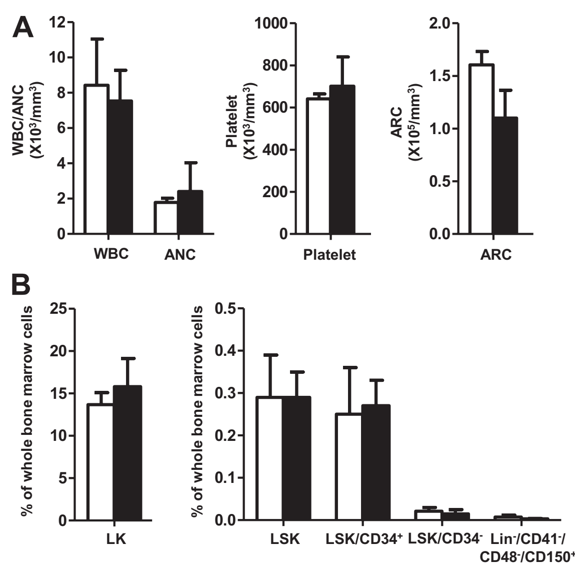

Fig. S2. Lineage cells in peripheral blood and HSC/P content in BM are not altered in H-Cx43-deficient mice. Peripheral blood count of WT (open bars) or H-Cx43-deficient mice (solid bars) is shown. (*A*) Whole blood count (WBC), absolute neutrophil counts (ANC), platelet counts and absolute reticulocyte count (ARC) (n = 3 mice per group). (*B*) The content of LK, LSK, LSK/CD34$^+$, LSK/CD34$^-$, and Lin$^-$/CD41$^-$/CD48$^-$/CD150$^+$ in BM were measured by FACS analysis (n = 11 mice per group).

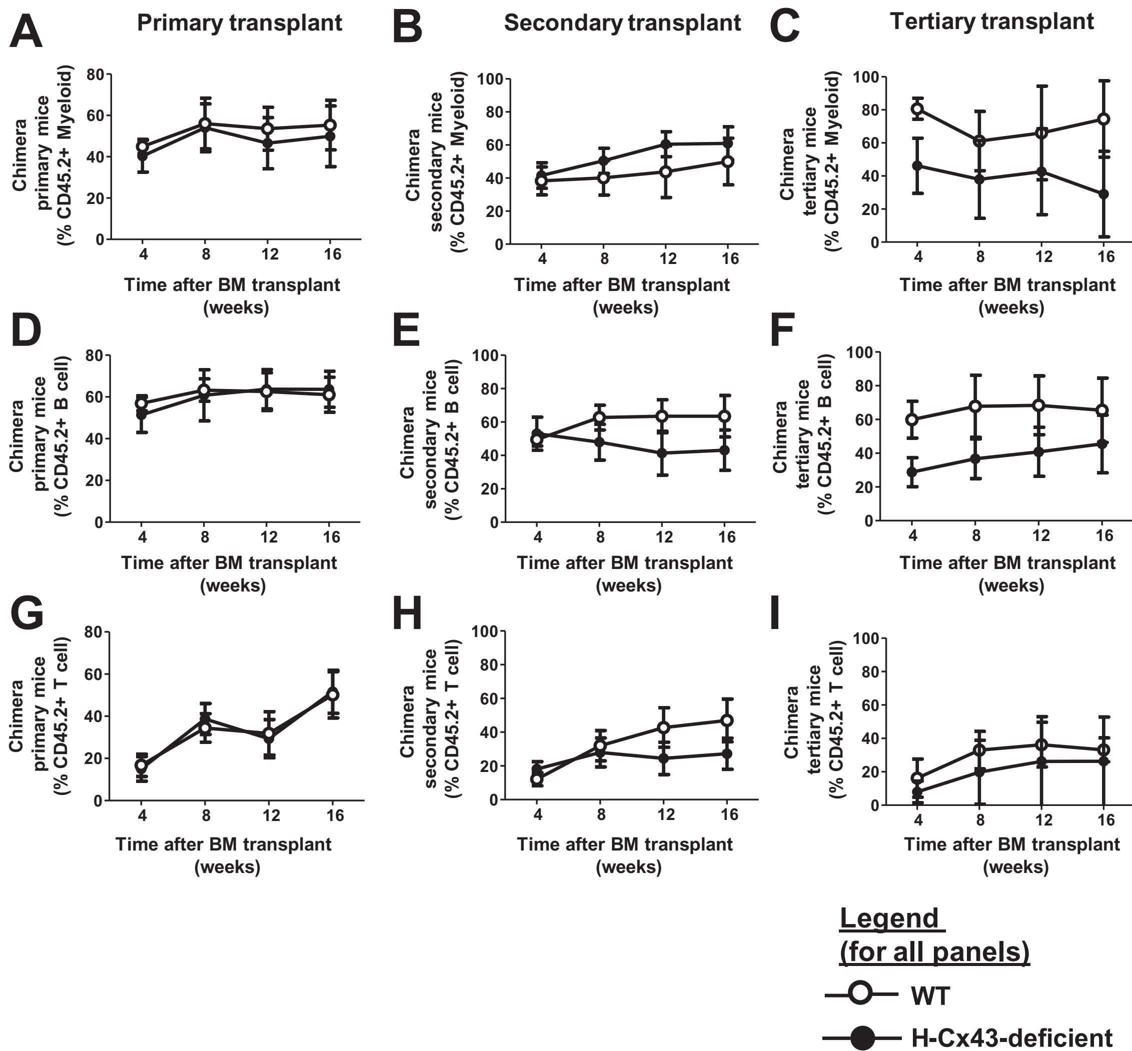

Fig. S3. Peripheral blood lineage cells in long-term competitive repopulation assay of H-Cx43-deficient mice. Lethally irradiated primary recipient $CD45.1^{+}$ mice were transplanted with a mixture of Vav1-Cre; H-Cx43-deficient BM cells ($CD45.2^{+}$) and WT BM ($CD45.1^{+}$) cells (solid circles). Control group was transplanted with a mixture of Vav1-Cre; WT BM cells ($CD45.2^{+}$) and WT BM ($CD45.1^{+}$) cells (open circles). (*A*–*C*) Peripheral blood myeloid ($CD45.2^{+}$/$Mac\text{-}1^{+}$). (*D*–*F*) B cell ($CD45.2^{+}$/$B\text{-}220^{+}$). (*G*–*I*) T cell ($CD45.2^{+}$/$CD3^{+}$) from primary (Fig. 1*G*), secondary (Fig. 1*H*), and tertiary (Fig. 1*I*) transplant recipients (n = 2 independent experiments; n = 8–16 mice per group and experiment).

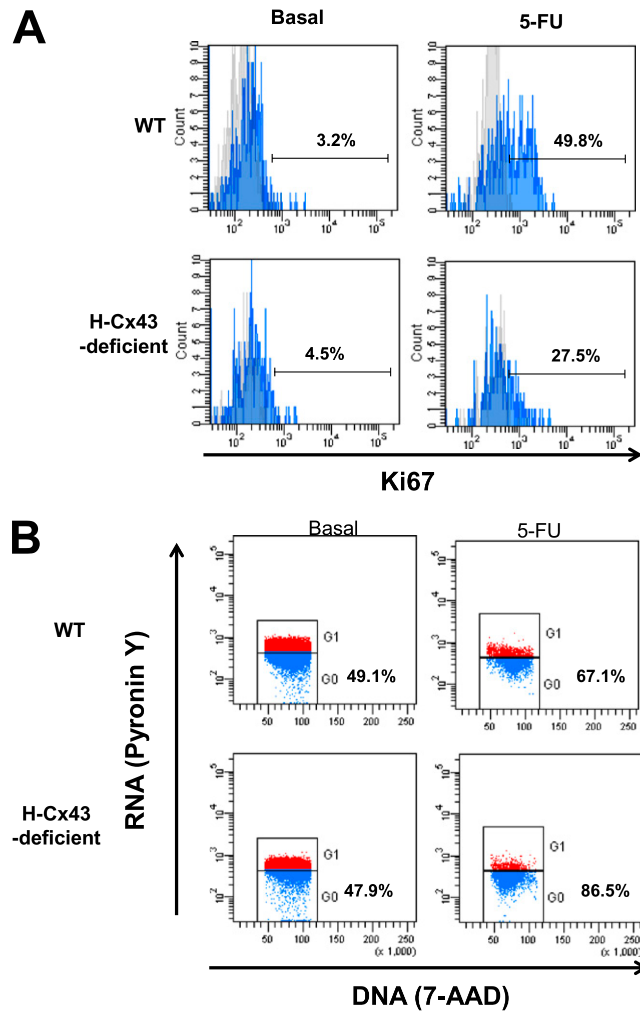

Fig. S4. Impaired cell cycle entry after 5-FU administration in H-Cx43-deficient HSCs. (*A* and *B*) Representative examples of flow cytometric analysis of Fig. 2 *B* and *C*. (*A*) Representative example of Ki67 staining (Fig. 2*B*) on $Lin^{-}/CD41^{-}/CD48^{-}/CD150^{+}$ BM cells. Blue, anti-Ki67 staining; gray, isotype control. (*B*) Representative example of 7-AAD/pyronin Y staining (Fig. 2*C*) on $Lin^{-}/CD41^{-}/CD48^{-}/CD150^{+}$ BM cells.

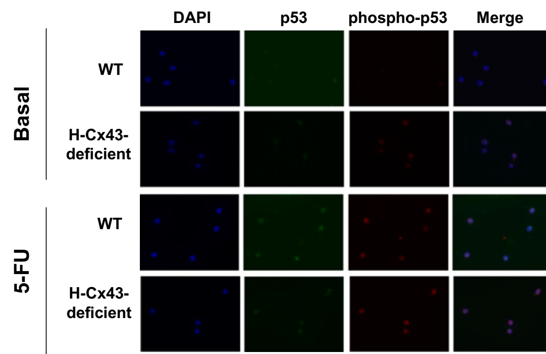

Fig. S5. Immunohistochemistry of p53 and phospho-p53. Representative example of immunofluorescence staining for p53 (green) and phospho-p53 (red) along with DAPI (blue) in WT or H-Cx43-deficient BM HSCs from untreated (basal) and 5-FU (96 h)-treated animals.

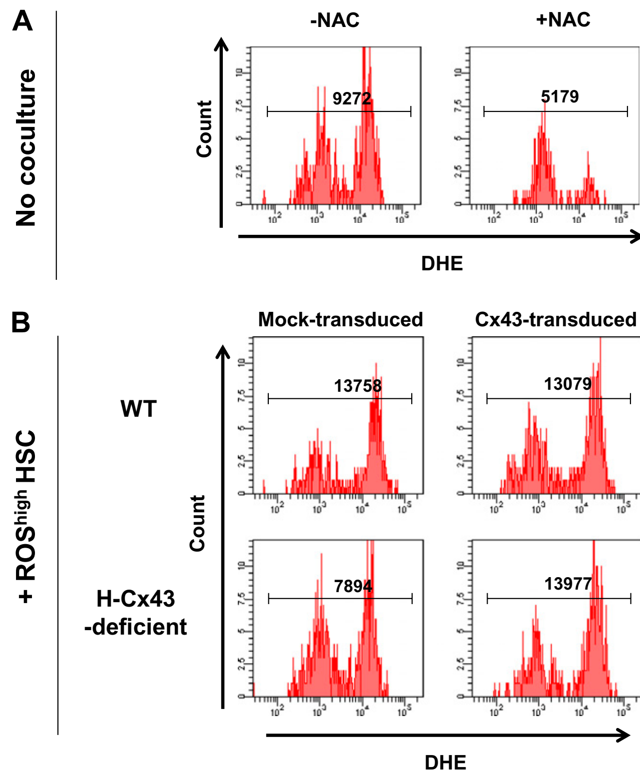

Fig. S6. Lentivirus Cx43 transduction rescues the impaired ROS transfer of H-Cx43-deficient HSCs into stromal cells. Representative examples of flow cytometric analysis of ROS uptake by BM stromal cells after coculture with ROS^{high} HSCs. (*A*) MFI of DHE in FBMD-1 without (*Left*) or with (*Right*) NAC treatment without coculture with HSCs. (*B*) FBMD-1, treated with NAC, was cocultured with WT (upper plots) or H-Cx43-deficient (lower plots) HSCs transduced with empty vector (left plots) or Cx43 vector (right plots). HSCs were treated with LY83583 to induce ROS (ROS^{high} HSCs) before coculture. Note that FBMD-1 is a heterocellular line with two populations with different levels of ROS. Coculture with ROS^{high} HSCs translates into an increase in the frequency of ROS^{high} FBMD-1 cells.

Table S1. Differential expression of gap junction genes ($P < 0.05$)

Symbol	Entrez gene name	Fold change
Gja1	Gap junction α-1; Connexin-43	−6.09
Gja4	Gap Junction α-4; Connexin-50	1.13
Gja8	Gap Junction α-8; Connexin-37	1.17

Transcript profiles of CD150^{+}/CD48^{-}/CD41^{-}/lineage^{-} cells isolated from WT or H-Cx43-deficient mice after 5-FU treatment were analyzed by Affymetrix exon array. Affymetrix hybridization of cDNA was performed in triplicate. The expression of all other connexin genes was found not to be significantly changed in 5-FU-treated Cx43-deficient BM HSCs.

Table S2. Ingenuity top Tox signaling pathway analysis ($P < 0.05$)

Symbol	Entrez gene name	Fold change
Oxidative stress response		
MGST1	microsomal glutathione S-transferase (GST) 1	−2.448
GCLM	glutamate-cysteine ligase, modifier subunit	−1.889
PRDX1	peroxiredoxin 1	−1.866
DNAJB11	DnaJ (Hsp40) homolog, subfamily B, member 11	−1.691
RRAS	related RAS viral (r-ras) oncogene homolog	−1.646
MAFF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	−1.628
MGST2	microsomal GST 2	−1.547
Cyp2j9	cytochrome P450, family 2, subfamily j, polypeptide 9	−1.536
GSTM5	GST μ 5	−1.534
DNAJC1	DnaJ (Hsp40) homolog, subfamily C, member 1	−1.51
EIF2AK3	eukaryotic translation initiation factor 2-α kinase 3	1.552
PRKCB	protein kinase C, β	1.582
SQSTM1	sequestosome 1	1.584
EPHX1	epoxide hydrolase 1, microsomal (xenobiotic)	1.604
HMOX1	heme oxygenase (decycling) 1	1.679
MAP3K7 (includes EG:172842)	mitogen-activated protein kinase kinase kinase 7	1.695
MAP3K1	mitogen-activated protein kinase kinase kinase 1	1.764
NRAS	neuroblastoma RAS viral (v-ras) oncogene homolog	1.834
GSTO1	GST ω 1	1,644
DNAJC13	DnaJ (Hsp40) homolog, subfamily C, member 13	2.962
Glutathione depletion		
MGST1	microsomal GST 1	−2.448
CCNG1	cyclin G1	−1.697
MGST2	microsomal GST 2	−1.547
GSTM5	GST μ 5	−1.534
EPHX1	epoxide hydrolase 1, microsomal (xenobiotic)	1.604
GSTO1	GST ω 1	1.644
G1/S checkpoint		
RB1	retinoblastoma 1	1.696
BTRC	β-transducin repeat containing protein	1.746
CDK6	cyclin-dependent kinase 6	1.707
TGFB1 (includes EG:21803)	transforming growth factor, β 1	2.135
E2F3	E2F transcription factor 3	2.165
G2/M DNA damage checkpoint		
CDK7	cyclin-dependent kinase 7	−1.892
CKS1B	CDC28 protein kinase regulatory subunit 1B	−1.65
Ccnb1	cyclin B1	−1.579
CKS2	CDC28 protein kinase regulatory subunit 2	−1.515
BTRC	β-transducin repeat containing	1.746
Cell death		
NDUFAB1	NADH dehydrogenase (ubiquinone) 1, α/β subcomplex, 1, 8 kDa	−2.776
TMX1	thioredoxin-related transmembrane protein 1	−2.738
TRPS1	trichorhinophalangeal syndrome I	−2.637
SIAH1	seven in absentia homolog 1 (*Drosophila*)	−1.901
CCNI	cyclin I	−1.896
CFLAR	CASP8 and FADD-like apoptosis regulator	−1.771
PTN	pleiotrophin	−1.742
NAMPT	nicotinamide phosphoribosyltransferase	−1.644

Table S2. Cont.

Symbol	Entrez gene name	Fold change
SMPD1	sphingomyelin phosphodiesterase 1, acid lysosomal	−1.633
TBK1	TANK-binding kinase 1	−1.595
ADIPOQ	adiponectin, C1Q and collagen domain containing	−1.547
CCNT1	cyclin T1	−1.535
GSTM5	GST μ 5	−1.534
SLC25A6	solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 6	−1.525
FDFT1	farnesyl-diphosphate farnesyltransferase 1	−1.523
HIPK2	homeodomain interacting protein kinase 2	−1.516
PLRG1	pleiotropic regulator 1	−1.513
IRF8	IFN regulatory factor 8	1.51
FADD	Fas (TNFRSF6)-associated via death domain	1.511
MYD88	myeloid differentiation primary response gene (88)	1.514
S100A1 (includes EG:20193)	S100 calcium binding protein A1	1.525
TCF3	transcription factor 3 (E2A Ig enhancer binding factors E12/E47)	1.531
XIAP	X-linked inhibitor of apoptosis	1.543
SHC1 (includes EG:20416)	SHC (Src homology 2 domain containing) transforming protein 1	1.545
PRKCB	protein kinase C, β	1.582
PRMT2	protein arginine methyltransferase 2	1.589
PIM1	pim-1 oncogene	1.609
CHMP5	charged multivesicular body protein 5	1.614
RASSF4	Ras association (RalGDS/AF-6) domain family member 4	1.64
TXNIP	thioredoxin interacting protein	1.668
HMOX1	heme oxygenase (decycling) 1	1.679
STK4	serine/threonine kinase 4	1.688
RB1	retinoblastoma 1	1.696
HSPA1A/HSPA1B	heat shock 70kDa protein 1A/1B	1.742
MAP3K1	mitogen-activated protein kinase kinase kinase 1	1.764
IRF1 (includes EG:16362)	interferon regulatory factor 1	1.787
ATP1A1	ATPase, Na^{+}/K^{+} transporting, alpha 1 polypeptide	1.823
CD2	CD2 molecule	1.83
IL2RA	interleukin 2 receptor, α	1.976
VOPP1	vesicular, overexpressed in cancer, prosurvival protein 1	1.978
TGFB1 (includes EG:21803)	transforming growth factor, β 1	2.135
ADM	adrenomedullin	2.24
BCL2A1	BCL2-related protein A1	2.577
CYBB	cytochrome *b*-245, β polypeptide	2.836
Hypoxia-inducible factor signaling		
UBE2N	ubiquitin-conjugating enzyme E2N	−2.379
EIF2B3	eukaryotic translation initiation factor 2B, subunit 3 γ, 58kDa	1.673
SHC1 (includes EG:20416)	SHC (Src homology 2 domain containing) transforming protein 1	1.545
UBE2D1	ubiquitin-conjugating enzyme E2D 1	1.571
PRKCB	protein kinase C, β	1.582
PLCG1	phospholipase C, γ 1	1.583
UBE2S	ubiquitin-conjugating enzyme E2S	1.589

Transcript profiles of $CD150^{+}/CD48^{-}/CD41^{-}/lineage^{-}$ cells isolated from WT or H-Cx43-deficient mice after 5-FU treatment were analyzed by Affymetrix exon array. Affymetrix hybridization of cDNA was performed by triplicate. Genes that were differentially expressed (<1.5- or >1.5-fold) in H-Cx43-deficient LSK treated with 5-FU compared with WT treated with 5-FU were analyzed in Ingenuity Pathways Analysis (Ingenuity Systems) to group into specific pathways. Selected genes with a differential expression of more or less than 1.5-fold compared with WT controls.