SUPPLEMENTARY METHODS

Mobilization assays

Recombinant human G-CSF (100 mg/kg/day intraperitoneally, for six days; Neupogen, Amgen, Thousand Oaks, CA) and AMD-3100 (5 mg/kg, one dose; Sigma, St. Louis, MO) were administered. PB CFU-C counts were determined at different time points.

Bromodeoxyuridine (BrdU) incorporation in HM cells in vivo

As previously described[1]. BM cells were pre-stained with antibodies against CD45, Ter119, Cadherin-11 and Cxcl12.

Retroviral transduction of BM stromal cells

Stromal cells from different animals were transduced with either a SFβ91-IRES-RFP (mock) or a SFβ91-rat Cx43-IRES-RFP (reintroduced Cx43) at a multiplicity of infection of 1, as previously described[2]. Transduction efficiency was less than 10% in all cases. Red fluorescent cells were sorted (FACS Aria II), cultured for up to 2 passages further and analyzed.

Calcein dye transfer

Stromal cell dye transfer to analyze cell-to-cell coupling was performed as previously described[3].

Immunoblots

Western blot analysis of Cx43 expression[4] and Cxcl12 expression analysis of primary BM cells and isolated/expanded BM stromal cells[5], were performed as previously described.

Q-RT-PCR

Quantitation of mRNA expression levels of Cx43, Cxcl12, sclerostin and GAPDH were analyzed as previously described[6,7].

Statistical analysis

Statistical significance was assessed by Student t-test or Mann-Whitney U-test for independent samples. Kaplan-Meyer analysis of 30-day survival of transplanted mice was statistically compared through a log P rank-test. Statistically significant differences were considered at $p<0.05$.

REFERENCES

1. Sengupta A, Duran A, Ishikawa E, et al. Atypical protein kinase C (aPKC{zeta} and aPKC{lambda}) is dispensable for mammalian hematopoietic stem cell activity and blood formation. *Proc Natl Acad Sci U S A*. 2011;108(24):9957-9962.

2. Yamada Y, Rothenberg ME, Lee AW, et al. The FIP1L1-PDGFRA fusion gene cooperates with IL-5 to induce murine hypereosinophilic syndrome (HES)/chronic eosinophilic leukemia (CEL)-like disease. *Blood*. 2006;107(10):4071-4079.

3. Cancelas JA, Koevoet WL, de Koning AE, Mayen AE, Rombouts EJ, Ploemacher RE. Connexin-43 gap junctions are involved in multiconnexin-expressing stromal support of hemopoietic progenitors and stem cells. *Blood*. 2000;96(2):498-505.

4. Presley CA, Lee AW, Kastl B, et al. Bone marrow connexin-43 expression is critical for hematopoietic regeneration after chemotherapy. *Cell Commun Adhes*. 2005;12(5-6):307-317.

5. De La Luz Sierra M, Yang F, Narazaki M, et al. Differential processing of stromal-derived factor-1alpha and stromal-derived factor-1beta explains functional diversity. *Blood*. 2004;103(7):2452-2459.

6. Doring B, Shynlova O, Tsui P, et al. Ablation of connexin43 in uterine smooth muscle cells of the mouse causes delayed parturition. *J Cell Sci*. 2006;119(Pt 9):1715-1722.

7. Watkins M, Grimston SK, Norris JY, et al. Osteoblast connexin43 modulates skeletal architecture by regulating both arms of bone remodeling. *Mol Biol Cell*. 2011;22(8):1240-1251.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Cx43 expression in osteolineage cells of WT and Cx43-deficient mice. A) Representative examples of longitudinal sections of femurs from Colα1-Cre; Rosa-loxP-Stop-loxP-LacZ reporter mice stained with X-Gal. β-galactosidase-positive cells are sparcely distributed in cortical bone in osteocytes (top and middle parts) and in discrete areas near to endosteal space representing osteoblasts and different marow stromal cell populations (botton part in panel A, arrows). (B) Quantitative RT-PCR for Cx43 expression in primary BM $CD45^-/Ter119^-/Cadherin\text{-}11^+$ and $CD45^-/Ter119^-/Cadherin\text{-}11^-$ populations (n=pool of 3 mice per group). (C) Immunoblot of Cx43 expression in pools of CFU-F from BM of WT and OB/P Cx43-deficient mice (n=2 per group). (D) Representative example of genomic PCR performed in clonal CFU-F derived from four WT or two OB/P Cx43 deficient independent mice to determine Cx43 excision (flox-out). (E) Quantitative RT-PCR of Cx43 expression in stromal cell lines derived from WT BM (FBMD-1 and from Colα1;WT mice) and OB/P Cx43-deficient BM. (F) Immunoblot for Cx43 protein expression in the same stromal cell lines as in E.

Figure S2. Spleen and BM cellularity are severely diminished in secondary recipients transplanted with WT BM from OB/P Cx43-deficient HM. LK and LSK content in circulation after transplant is not impaired in myeloablated OB/P Cx43-deficient mice. Firm adhesion of HSC/P to irradiated WT and Cx43-deficient stromal cells is not impaired. (A) Micrographs of spleen sections on day +71 after secondary transplantation of WT HSC/P residing in either WT or OB/P Cx43-deficient microenvironment (hematoxylin/eosin staining, O.M x10). Notice the recovery of follicular architecture after transplantation (left panel) while there is a practical absence of follicles

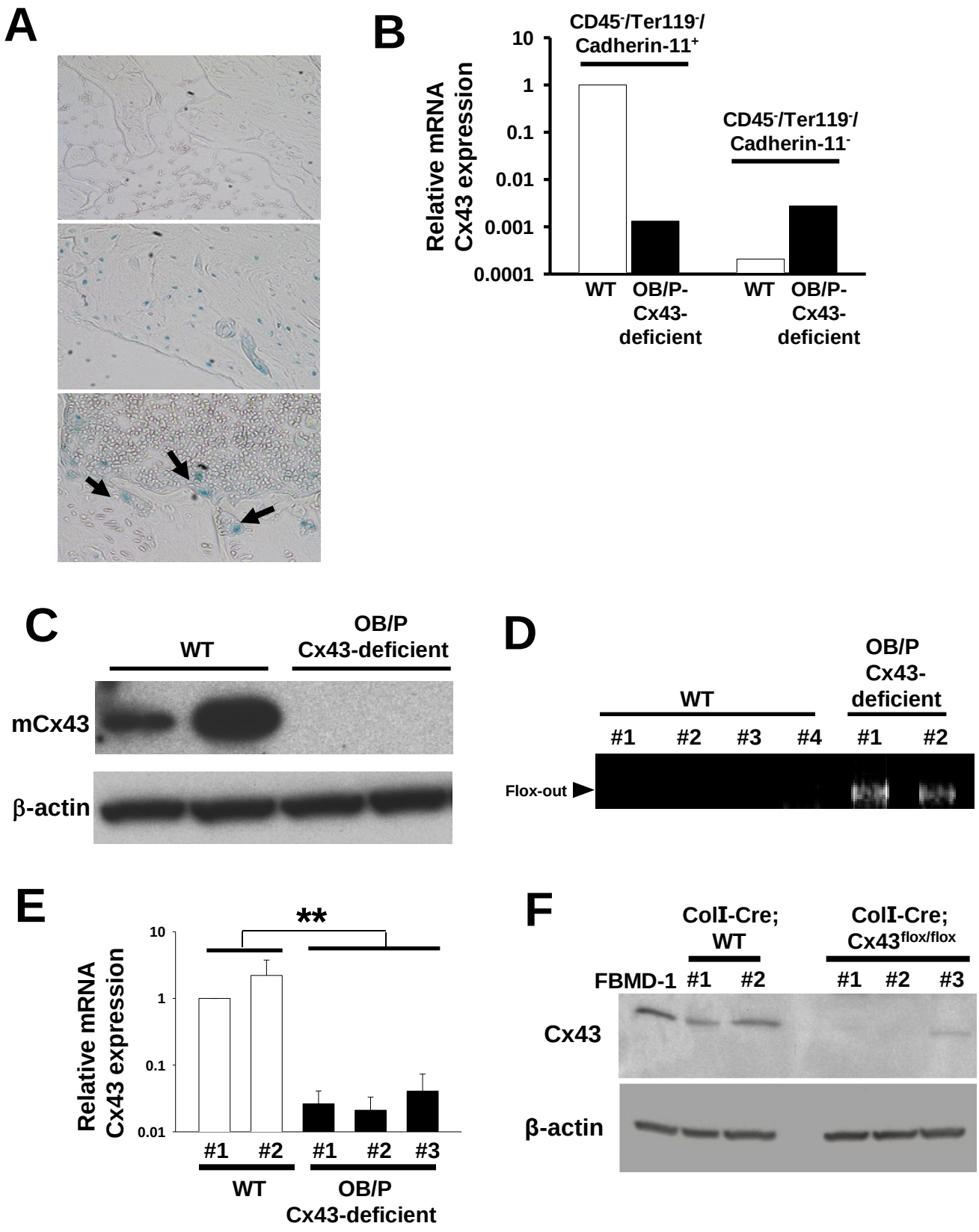
in the spleen of mice transplanted with WT HSC/P residing in an OB/P Cx43-deficient microenvironment (right panel). (B) BM lineage cell populations of secondary recipients (day +71) which had been transplanted with chimeric BM from primary recipients with WT CD45.1+ hematopoiesis and either WT or OB/P Cx43-deficient HM. (C) Irradiated WT or OB/P Cx43-deficient mice were transplanted with WT BM-EGFP^{+} cells. Percentage of green LK+S- and LSK populations in the PB at 16 hours after transplant (D) Adhesion of CFU-C to control (empty bars) or Cx43-deficient (solid bars) stromal cell lines. Each BM stromal cell line was macrophage-depleted and independently generated. Data experiments are shown as mean ± SEM. N=3 different experiments. (**$p < 0.01$).

Figure S3. Reintroduction of Cx43 expression in OB/P-Cre-Cx43$^{flox/flox}$ and Mx1-Cre-Cx43$^{flox/flox}$ mice and migration assays. (A) Cx43 protein expression in WT (one example), three mock-transduced OB/P Cx43-deficient and three Cx43-transduced OB/P Cx43-deficient stromal explants. (B) Trans-stromal migration of mock-transduced, OB/P Cx43-deficient and Cx43-transduced OB/P Cx43-deficient stromal cells. (C) Immunoblot of Cx43 and Cx45 in macrophage-depleted stromal cells expanded from Mx1-Cre;WT and Mx1-Cre;Cx43$^{flox/flox}$ poly I:C treated mice. (D) Similar to A, expression of Cx43 in WT (one example), Cx43-deficient (Mx1-Cre driven, an example is presented), mock-transduced, Cx43-deficient (Mx1-Cre driven) and Cx43 reintroduced (on Cx43-deficient stromal cells; two different derived explants are presented). Data of trans-stromal migration are shown as mean ± SEM. N=4 independent experiments.

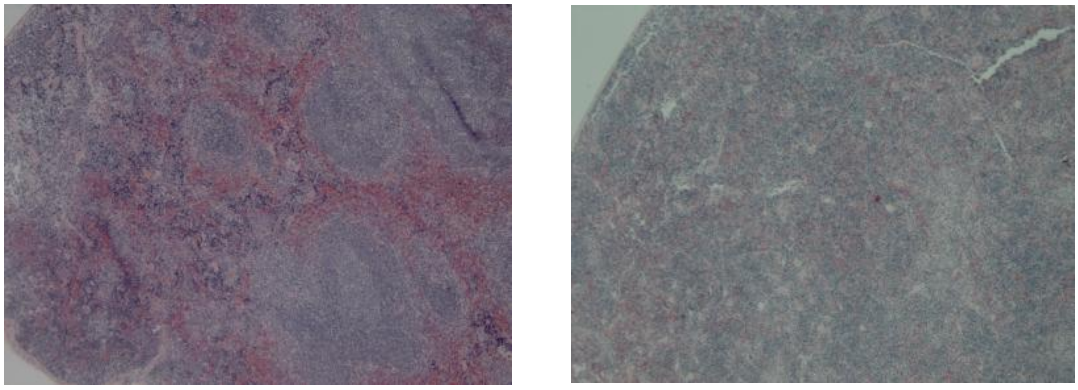
Figure S4. Calcein dye transfer of Cx43-deficient stromal cells. (A-C) Examples of metabolic coupling analysis of stromal cells (WT, Cx43-deficient or Cx43-reintroduced, derived from Mx1-Cre; Cx43$^{flox/flox}$ mice) as assessed by calcein dye transfer. A minimum

of 100 events was analyzed. (D) Metabolic coupling assessed by dye transfer was restored by reintroduction of Cx43 expression (grey bar). Absence of dye transfer of Cx43-deficient stromal cells is shown as 0. Data are shown as mean ± SEM. N=4 independent experiments. (*** $p<0.001$).

Figure S5. Homing and trans-stromal migration are not impaired in absence of irradiation conditioning of the Cx43-deficient HM. Cxcl12 expression and secretion are increased in OB/P Cx43 deficient BM. Spleen CFU-C content is not impaired in conditional Cx43-deficient mice. (A) Basal content of spleen CFU-C in WT and OB/P Cx43-deficient mice (p=N.S.). (B) Homing of UbiquitinC-EGFP transgenic HSC/P (Lin-/ckit+/Sca-1+ and Lin-/c-kit+) in non-myeloablated OB/P-Cx43-deficient mice (p=N.S.). (C) Trans-stromal migration of WT HSC/P through non-irradiated WT or Cx43-deficient stromal cells (p=N.S.). (D-E) Cxcl12 expression and secretion in Cx43-deficient stromal cell lines. Stromal CXCL12 expression (D) and extracellular content determined by ELISA (E) in WT and OB/P Cx43-deficient cell lines. Each lane in the immunoblot represents lysates from macrophage-depleted BM stromal cell explants from independent mice. Data represent two and three independently developed stromal cell explants, analyzed per triplicate. Data experiments are shown as mean ± SEM. N=3 different experiments. (** $p < 0.01$).


A
B
CD45-/Ter119-/
Cadherin-11+
CD45-/Ter119-/
Cadherin-11-
Relative mRNA
Cx43 expression
10
1
0.1
0.01
0.001
0.0001
WT
OB/P-
Cx43-
deficient
WT
OB/P-
Cx43-
deficient
C
WT
OB/P
Cx43-deficient
mCx43
β-actin
D
WT
OB/P
Cx43-
deficient
#1
#2
#3
#4
#1
#2
Flox-out
E
**
Relative mRNA
Cx43 expression
10
1
0.1
0.01
#1
#2
#1
#2
#3
WT
OB/P
Cx43-deficient
F
ColI-Cre;
WT
ColI-Cre;
Cx43flox/flox
FBMD-1
#1
#2
#1
#2
#3
Cx43
β-actin

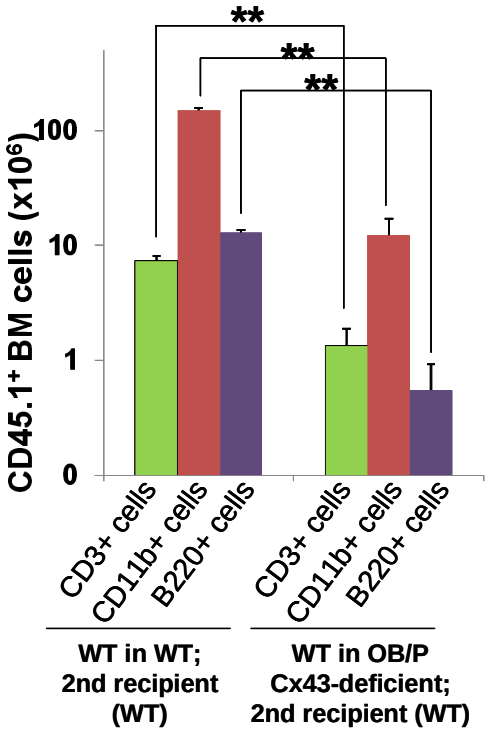
A



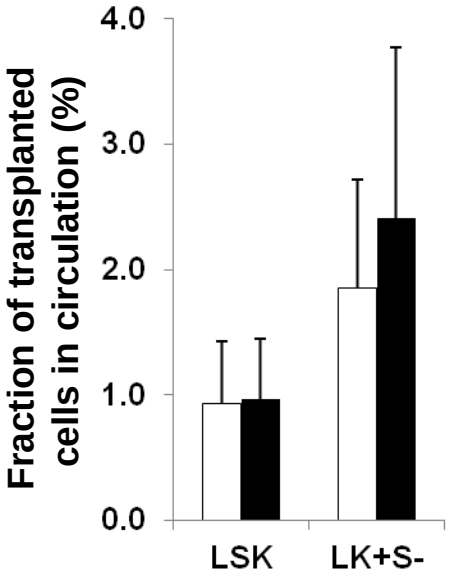
WT in WT; 2nd recipient (WT)

WT in OB/P Cx43-deficient; 2nd recipient (WT)

B



C



D

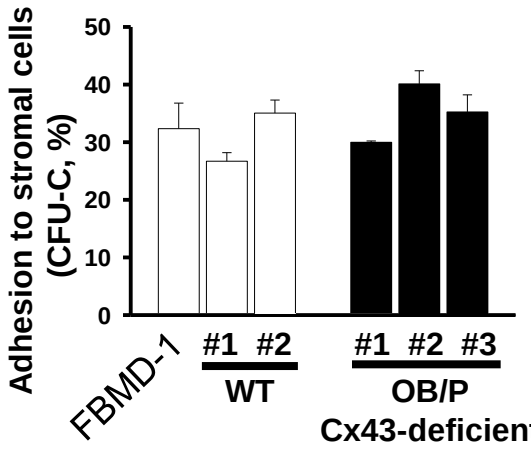
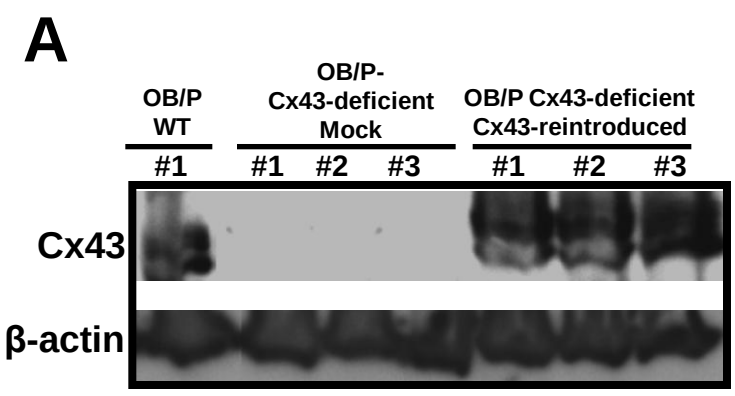
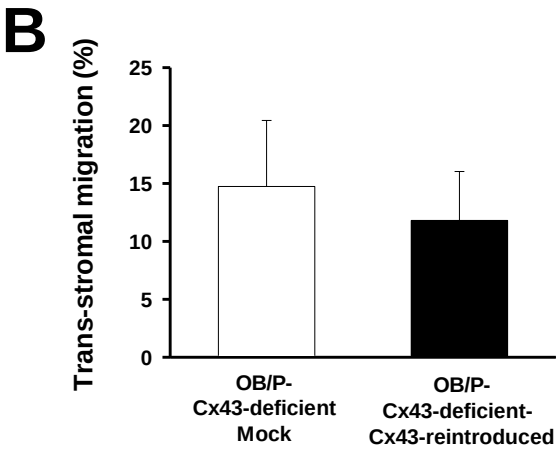
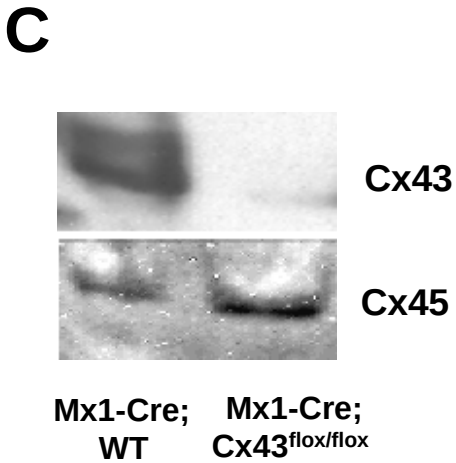
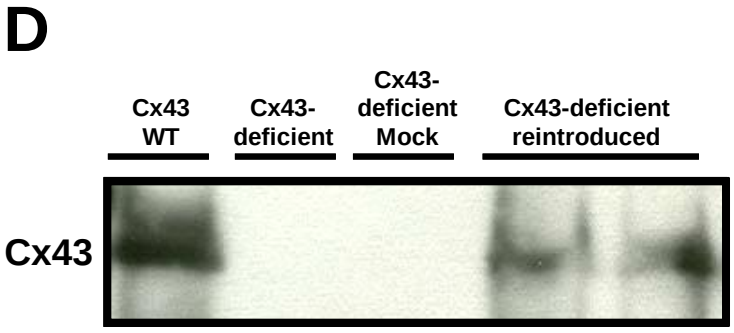


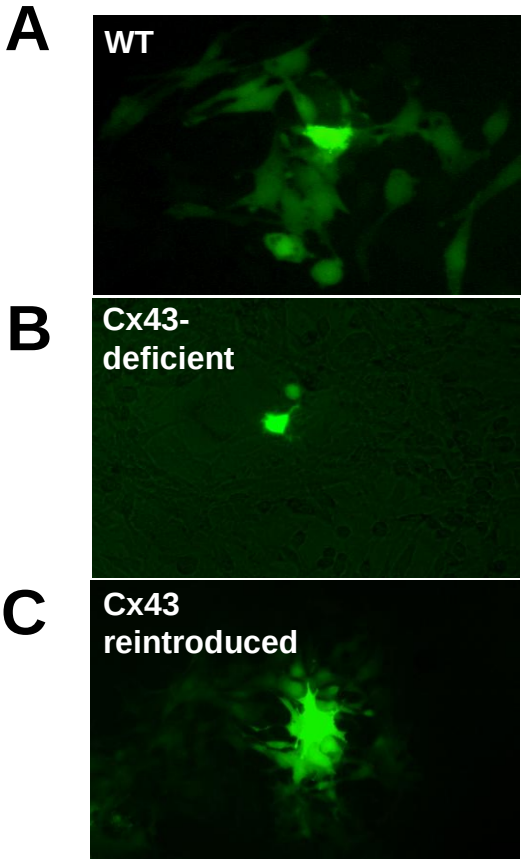
Figure S3

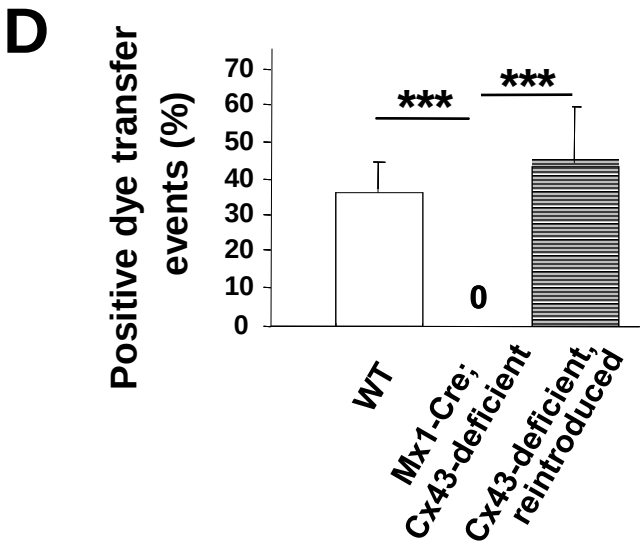

A
OB/P WT
OB/P-Cx43-deficient Mock
OB/P Cx43-deficient Cx43-reintroduced
#1
#1 #2 #3
#1 #2 #3
Cx43
β-actin

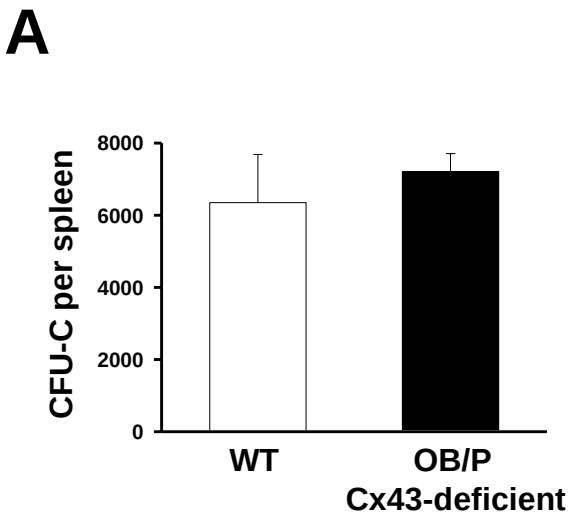

B
Trans-stromal migration (%)
25
20
15
10
5
0
OB/P-Cx43-deficient Mock
OB/P-Cx43-deficient-Cx43-reintroduced

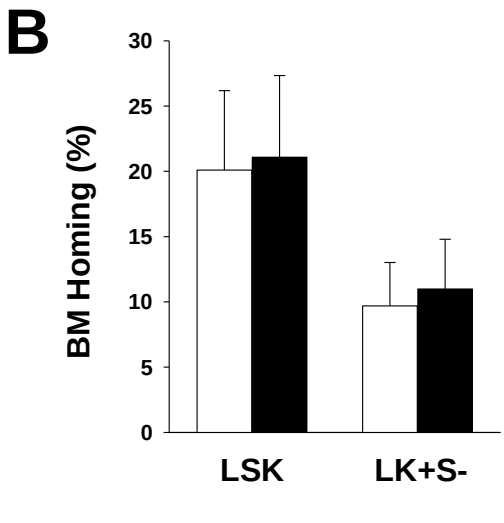

C
Cx43
Cx45
Mx1-Cre; WT
Mx1-Cre; Cx43flox/flox

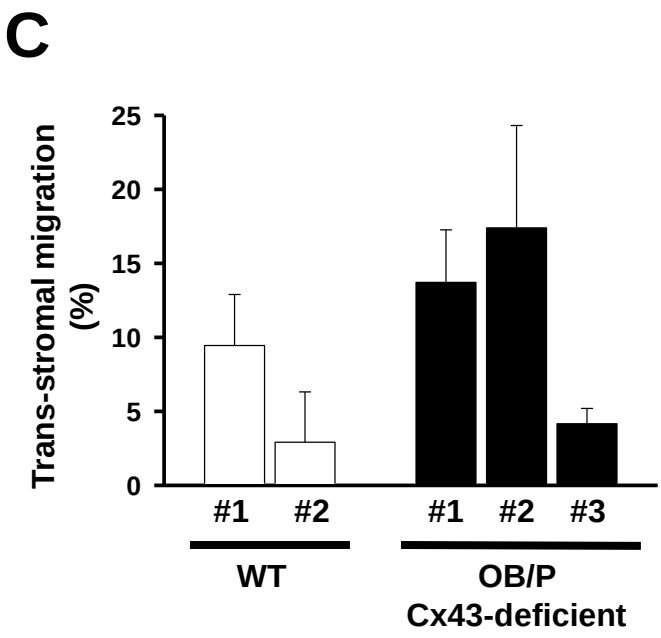

D
Cx43 WT
Cx43-deficient
Cx43-deficient Mock
Cx43-deficient reintroduced
Cx43

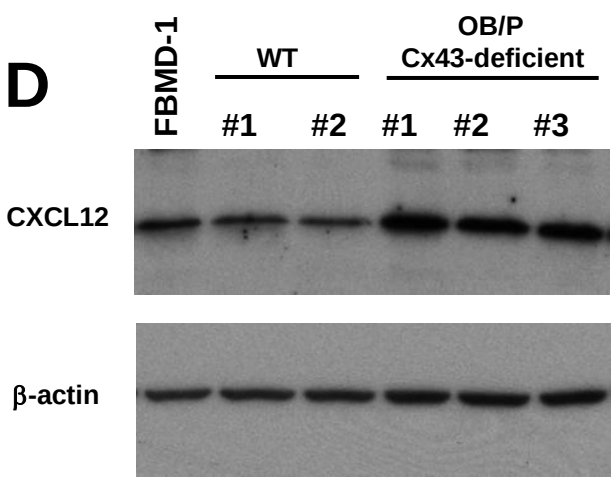

A
WT
B
Cx43-
deficient
C
Cx43
reintroduced

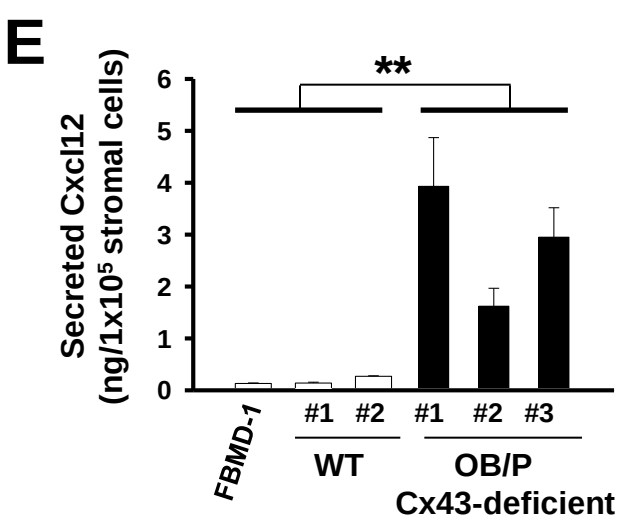

D
Positive dye transfer
events (%)
70
60
50
40
30
20
10
0
***
***
0
WT
Mx1-Cre;
Cx43-deficient
Cx43-deficient;
reintroduced


A
CFU-C per spleen
8000
6000
4000
2000
0
WT
OB/P
Cx43-deficient


B
BM Homing (%)
30
25
20
15
10
5
0
LSK
LK+S-


C
Trans-stromal migration (%)
25
20
15
10
5
0
#1
#2
#1
#2
#3
WT
OB/P
Cx43-deficient


D
FBMD-1
WT
OB/P
Cx43-deficient
#1
#2
#1
#2
#3
CXCL12
β-actin


E
**
Secreted Cxcl12 (ng/1x10⁵ stromal cells)
6
5
4
3
2
1
0
FBMD-1
#1
#2
#1
#2
#3
WT
OB/P
Cx43-deficient