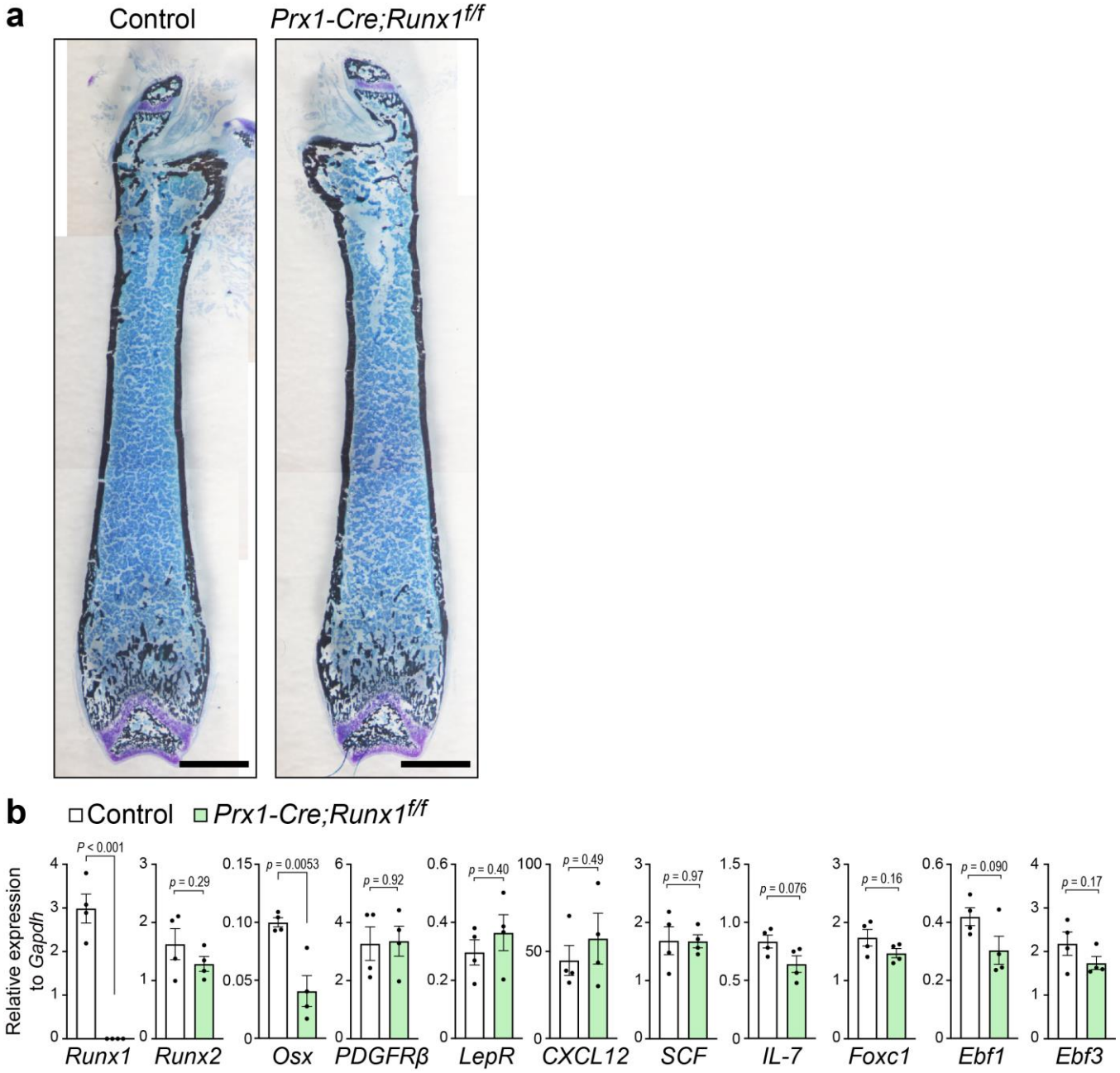
Supplemental Information.



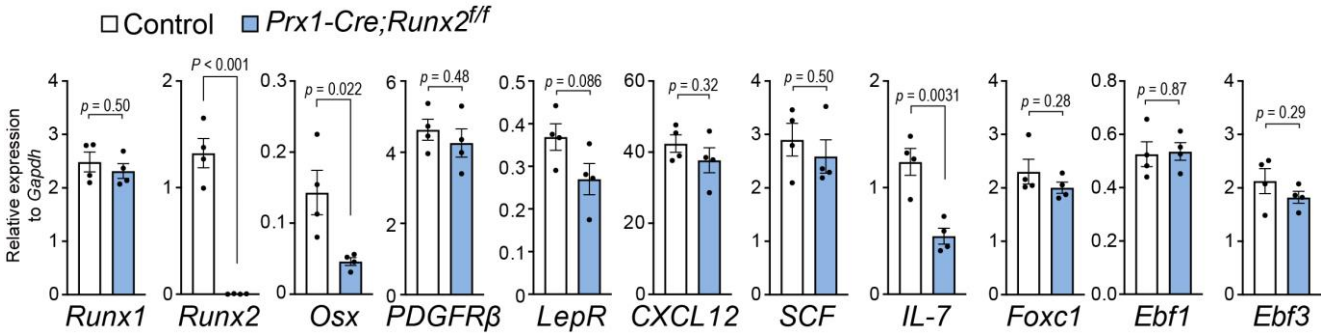
Supplementary Figure 1. Runx1 is dispensable for the development of HSC niches

a,**b** Bone marrow from 3-week-old control and *Prx1-Cre;Runx1*$^{f/f}$ mice was analyzed.

a von Kossa and toluidine blue staining of femurs. Bars: 1 mm.

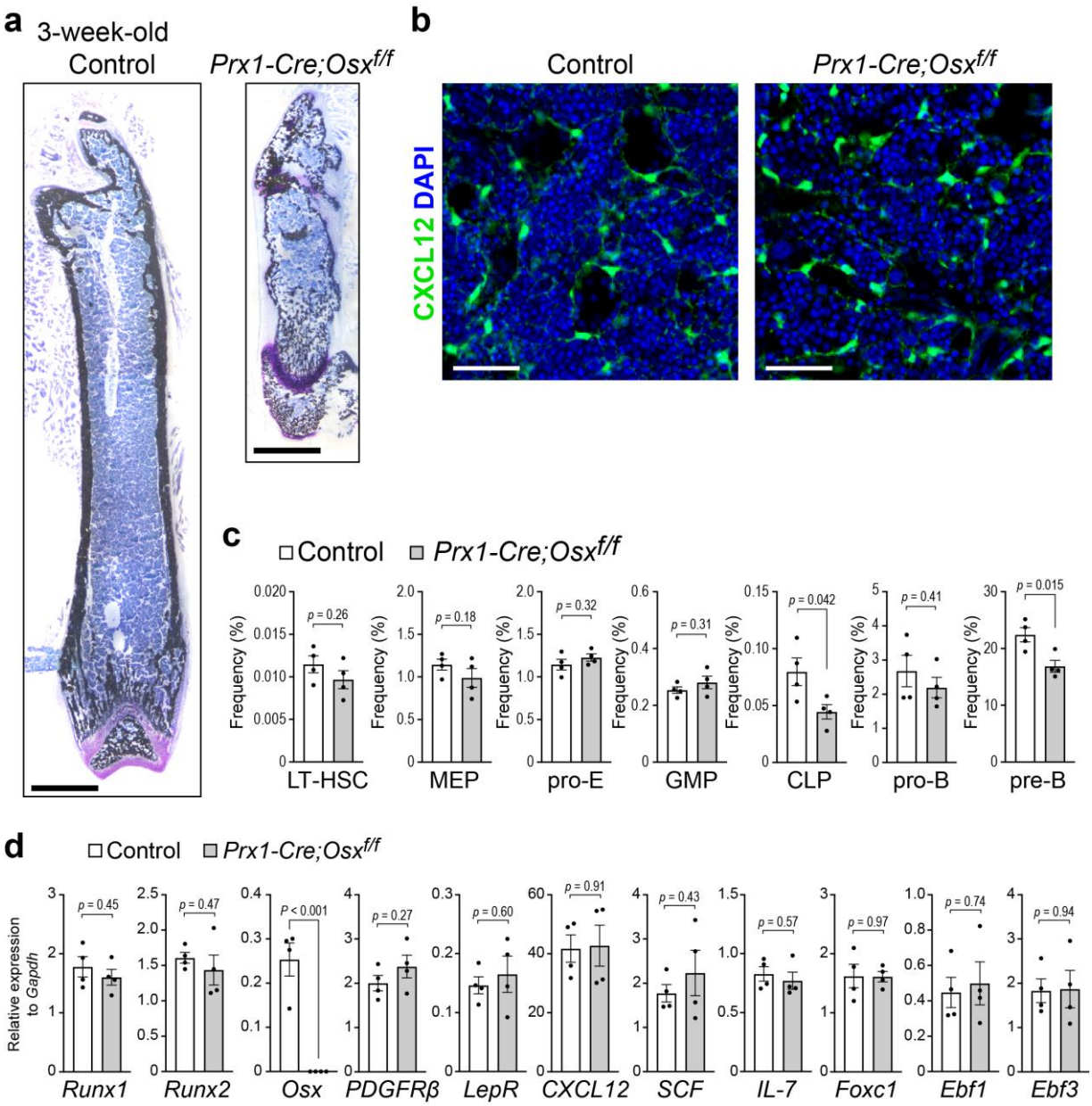
b Relative mRNA expression levels of *Runx1*, *Runx2*, *Osx*, *PDGFRβ*, *LepR*, *CXCL12*, *SCF*, *IL-7*, *Foxc1*, *Ebf1*, and *Ebf3* in sorted CAR cells (n = 4 mice per group).

All error bars represent SD of the mean. Statistical significances were calculated using the two-tailed unpaired Student's *t*-test. Source data are provided as a Source Data file.



Supplementary Figure 2. HSC niches are formed in mice lacking Runx2

Relative mRNA expression levels of *Runx1*, *Runx2*, *Osx*, *PDGFRβ*, *LepR*, *CXCL12*, *SCF*, *IL-7*, *Foxc1*, *Ebf1*, and *Ebf3* in sorted CAR cells from 3-week-old control and *Prx1-Cre;Runx2*$^{f/f}$*;CXCL12-GFP* mice (n = 4 mice per group). All error bars represent SD of the mean. Statistical significances were calculated using the two-tailed unpaired Student's *t*-test. Source data are provided as a Source Data file.



Supplementary Figure 3. HSC niches are formed in mice lacking Osterix

a-d Bone marrow from 3-week-old control and *Prx1-Cre;Osx*$^{f/f}$*;CXCL12-GFP* mice was analyzed.

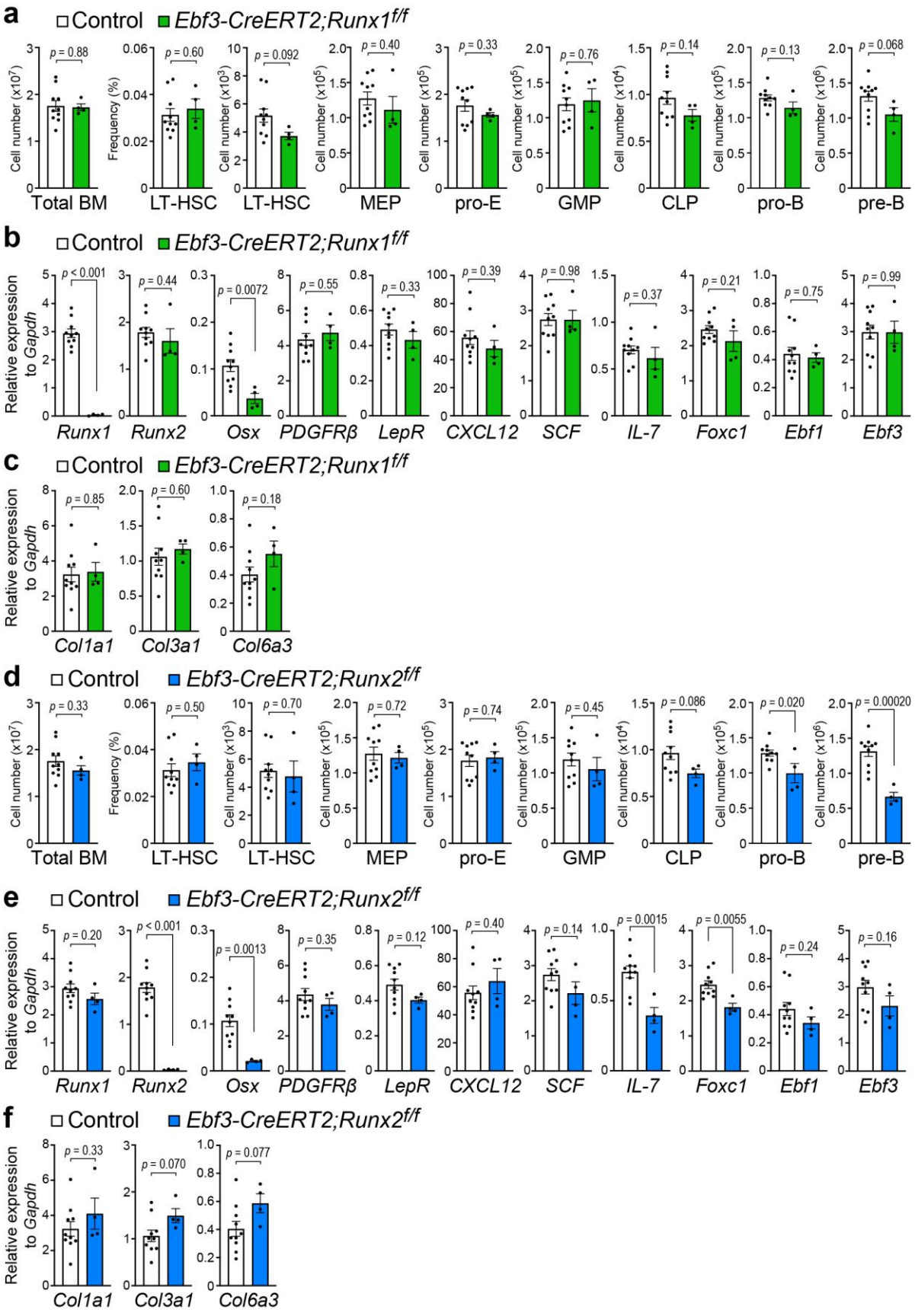
a von Kossa and toluidine blue staining of femurs. Bars: 1 mm.

b Confocal microscopy images showing expression of CXCL12-GFP. Bars: 100 µm.

c Frequencies of LT-HSCs, MEPs, pro-E, GMPs, CLPs, pro-B cells, and pre-B cells (n = 4 mice per group).

d Relative mRNA expression levels of *Runx1*, *Runx2*, *Osx*, *PDGFRβ*, *LepR*, *CXCL12*, *SCF*, *IL-7*, *Foxc1*, *Ebf1*, and *Ebf3* in sorted CAR cells (n = 4 mice per group).

All error bars represent SD of the mean. Statistical significances were calculated using the two-tailed unpaired Student's *t*-test. Source data are provided as a Source Data file.


a
Control
Ebf3-CreERT2;Runx1f/f
Cell number (x10^7)
p = 0.88
Total BM
Frequency (%)
p = 0.60
LT-HSC
Cell number (x10^3)
p = 0.092
LT-HSC
Cell number (x10^5)
p = 0.40
MEP
Cell number (x10^5)
p = 0.33
pro-E
Cell number (x10^5)
p = 0.76
GMP
Cell number (x10^4)
p = 0.14
CLP
Cell number (x10^5)
p = 0.13
pro-B
Cell number (x10^6)
p = 0.068
pre-B
b
Control
Ebf3-CreERT2;Runx1f/f
Relative expression to Gapdh
p < 0.001
Runx1
p = 0.44
Runx2
p = 0.0072
Osx
p = 0.55
PDGFRβ
p = 0.33
LepR
p = 0.39
CXCL12
p = 0.98
SCF
p = 0.37
IL-7
p = 0.21
Foxc1
p = 0.75
Ebf1
p = 0.99
Ebf3
c
Control
Ebf3-CreERT2;Runx1f/f
Relative expression to Gapdh
p = 0.85
Col1a1
p = 0.60
Col3a1
p = 0.18
Col6a3
d
Control
Ebf3-CreERT2;Runx2f/f
Cell number (x10^7)
p = 0.33
Total BM
Frequency (%)
p = 0.50
LT-HSC
Cell number (x10^3)
p = 0.70
LT-HSC
Cell number (x10^5)
p = 0.72
MEP
Cell number (x10^5)
p = 0.74
pro-E
Cell number (x10^5)
p = 0.45
GMP
Cell number (x10^4)
p = 0.086
CLP
Cell number (x10^5)
p = 0.020
pro-B
Cell number (x10^6)
p = 0.00020
pre-B
e
Control
Ebf3-CreERT2;Runx2f/f
Relative expression to Gapdh
p = 0.20
Runx1
p < 0.001
Runx2
p = 0.0013
Osx
p = 0.35
PDGFRβ
p = 0.12
LepR
p = 0.40
CXCL12
p = 0.14
SCF
p = 0.0015
IL-7
p = 0.0055
Foxc1
p = 0.24
Ebf1
p = 0.16
Ebf3
f
Control
Ebf3-CreERT2;Runx2f/f
Relative expression to Gapdh
p = 0.33
Col1a1
p = 0.070
Col3a1
p = 0.077
Col6a3

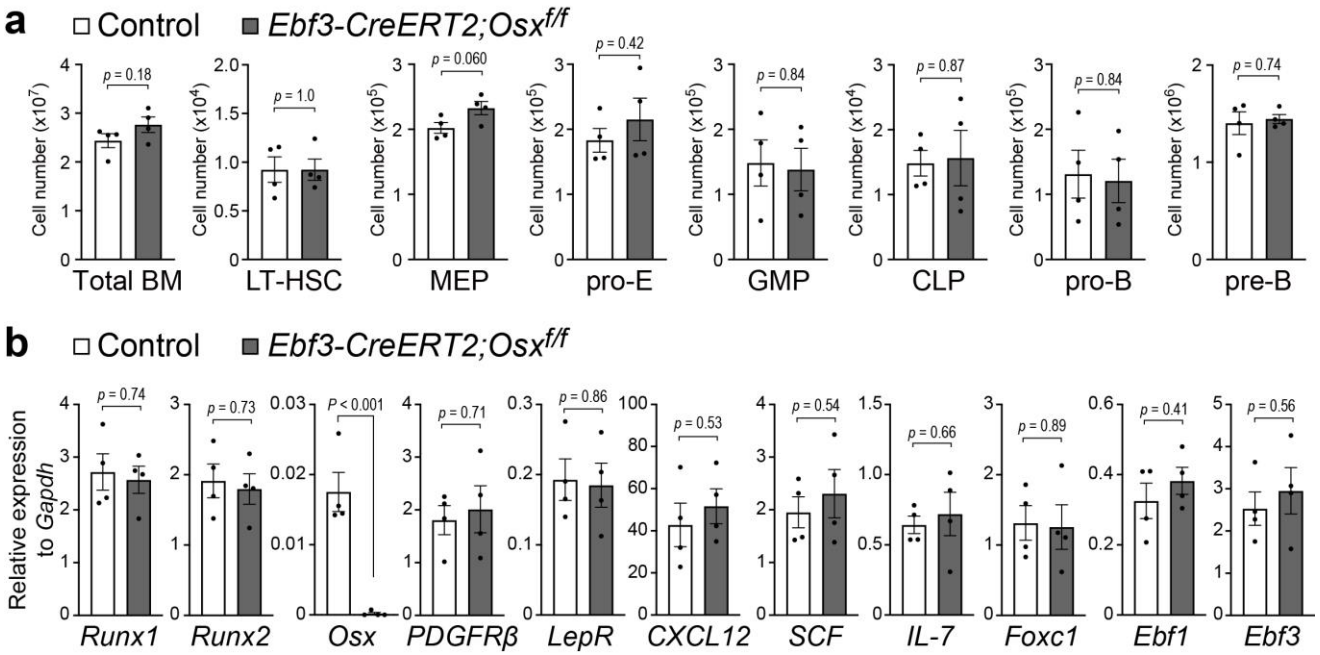
Supplementary Figure 4. Runx1 and Runx2 are dispensable for the maintenance of HSC niches

a-f Bone marrow from 20- to 24-week-old tamoxifen-treated control (n = 10 mice), *Ebf3-CreERT2;Runx1*$^{f/f}$ (n = 4 mice) (**a-c**), or *Ebf3-CreERT2;Runx2*$^{f/f}$ (n = 4 mice) (**d-f**) mice was analyzed.

a,**d** Total hematopoietic cell counts, frequencies of LT-HSCs, and the numbers of LT-HSCs, MEPs, pro-E, GMPs, CLPs, pro-B cells, and pre-B cells in femurs and tibias.

b,**c**,**e**,**f** Relative mRNA expression levels of *Runx1*, *Runx2*, *Osx*, *PDGFRβ*, *LepR*, *CXCL12*, *SCF*, *IL-7*, *Foxc1*, *Ebf1*, and *Ebf3* (**b**,**e**), *Col1a1*, *Col3a1*, and *Col6a3* (**c**,**f**) in sorted CAR cells.

All error bars represent SD of the mean. Statistical significances were calculated using the two-tailed unpaired Student's *t*-test. Source data are provided as a Source Data file.



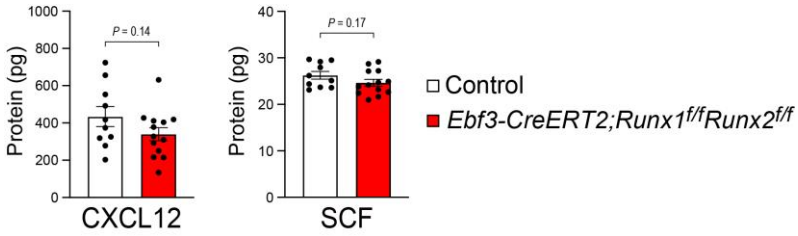
Supplementary Figure 5. Osterix is dispensable for the maintenance of HSC niches

a,**b** Bone marrow from 20- to 24-week-old tamoxifen-treated control and *Ebf3-CreERT2;Osx*$^{f/f}$ mice was analyzed.

a Total hematopoietic cell counts and the numbers of LT-HSCs, MEPs, pro-E, GMPs, CLPs, pro-B cells, and pre-B cells in femurs and tibias (n = 4 mice per group).

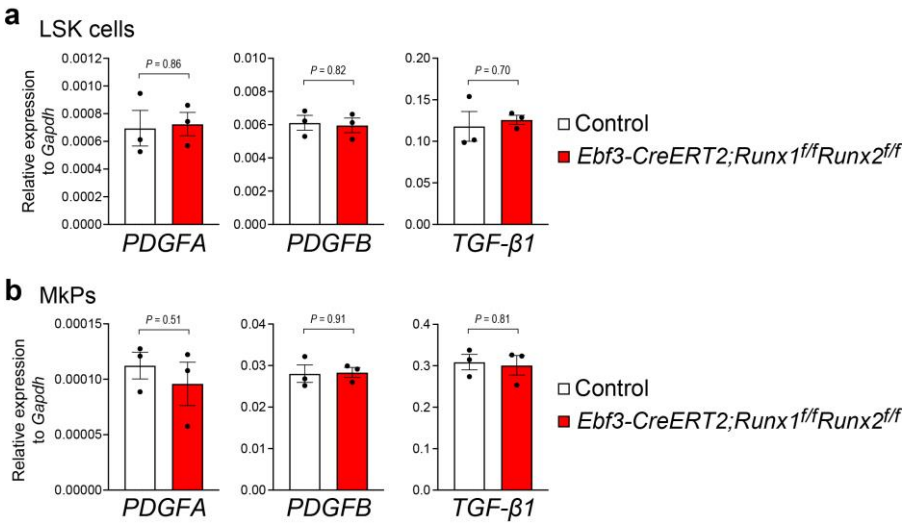
b Relative mRNA expression levels of *Runx1*, *Runx2*, *Osx*, *PDGFRβ*, *LepR*, *CXCL12*, *SCF*, *IL-7*, *Foxc1*, *Ebf1*, and *Ebf3* in sorted CAR cells (n = 4 mice per group).

All error bars represent SD of the mean. Statistical significances were calculated using the two-tailed unpaired Student's *t*-test. Source data are provided as a Source Data file.



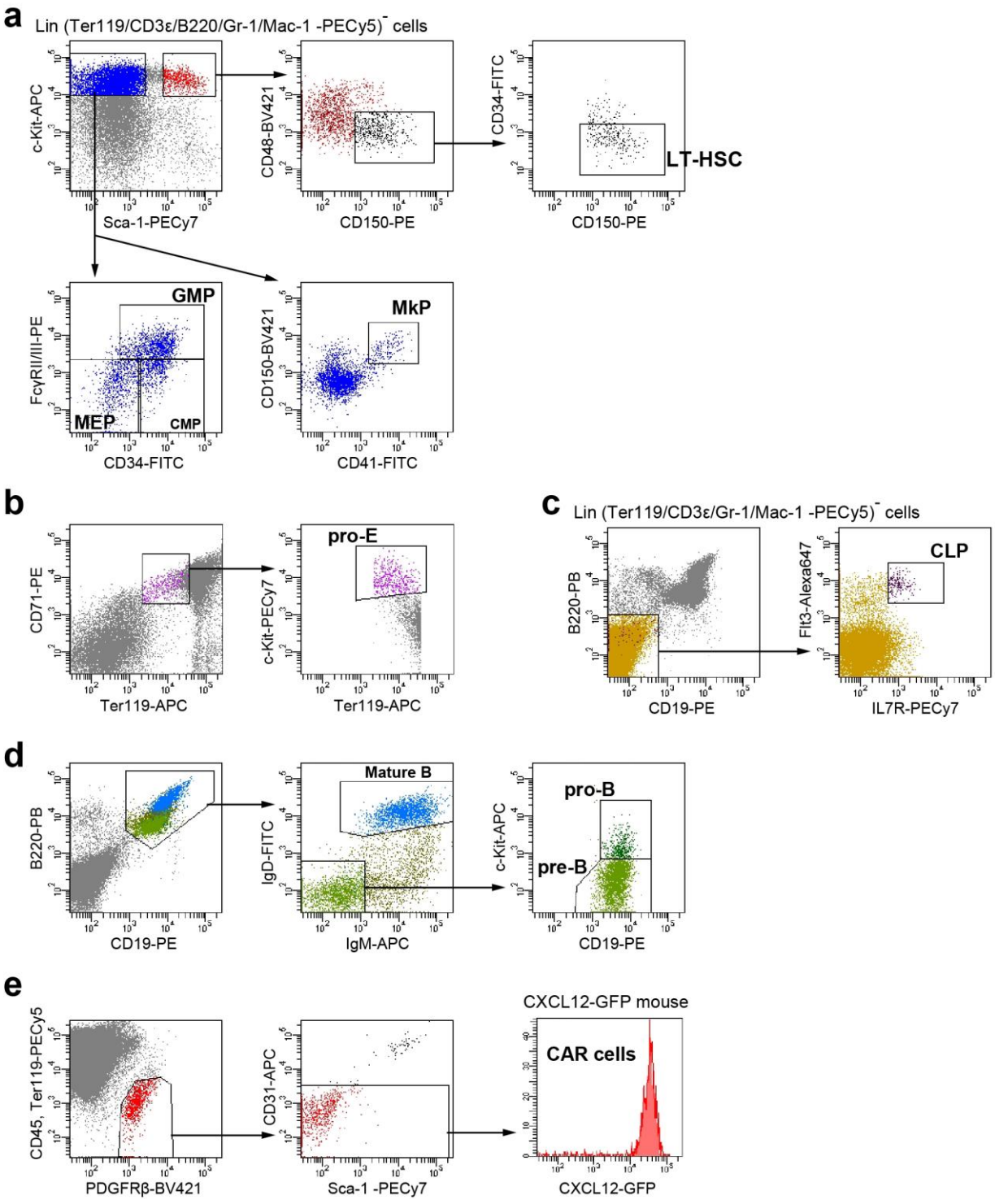
Supplementary Figure 6. Protein expression of CXCL12 and SCF in mice lacking both Runx1 and Runx2 in CAR cells

Humeri from 20- to 24-week-old tamoxifen-treated control (n = 10 mice) and *Ebf3-CreERT2;Runx1*$^{f/f}$*Runx2*$^{f/f}$ mice (n = 13 mice) were analyzed. All error bars represent SD of the mean. Statistical significances were calculated using the two-tailed unpaired Student's *t*-test. Source data are provided as a Source Data file.



Supplementary Figure 7. The expression of *PDGF*s and *TGF-β1* was unaltered in HSPCs and MkPs from mice lacking both Runx1 and Runx2 in CAR cells

a,**b** Femurs and tibias from 20- to 24-week-old tamoxifen-treated control and *Ebf3-CreERT2;Runx1$^{f/f}$Runx2$^{f/f}$* mice were analyzed (n = 3 mice per group). Relative mRNA expression levels of *PDGFA*, *PDGFB*, and *TGF-β1* in sorted LSK cells (**a**) and MkPs (**b**). All error bars represent SD of the mean. Statistical significances were calculated using the two-tailed unpaired Student's *t*-test. Source data are provided as a Source Data file.



Supplementary Figure 8. FACS Gating strategies for HSPCs and CAR cells

FACS Gating strategies for LT-HSCs, MEPs, GMPs, Mkps (**a**), proerythroblasts (pro-E) (**b**), CLPs (**c**), pro-B cells, pre-B cells (**d**), and CAR cells (**e**).

Gene Name	Sequence (5' - 3')
Mouse	
Gapdh	TCA TGA GCC CTT CCA CAA TG
	GGT GTG AAC CAC GAG AAA TAT GAC
Runx1	GGC AAC GAT GAA AAC TAC TCG GC
	TCT ACC GCT CCG CCC GAC
Runx1b (proximal) specific	ATG GGA ATT TTG CCT CCG GG
	GTG GGC AAC CAG AAT TCA AA
Runx1c (distal) specific	ATA GAA TCC CCC GCC TTC AG
	CTC TCA TGA AGC ACT GTG GA
Runx2	GAC TGT GGT TAC CGT CAT GGC
	GGG GAC CGT CCA CTG TCA C
Osx (Sp7)	ATG GCG TCC TCT CTG CTT G
	TGA AAG GTC AGC GTA TGG CTT
Pdgfrb	CAA CTC ACT AGG GCC GGA G
	GCA CGG AAT TGT CGT CTC AG
Lepr	ATG CCC CAA TTT CAA ACC TG
	GGA ACC TTG AGG CTT CTT GGA
CXCL12	CCA GAG CCA ACG TCA AGC AT
	CAG CCG TGC AAC AAT CTG AA
SCF (Kitl)	GGT AGC TAG TTC TAT CCA TGC GGT
	CCT GTA AGG ACT TTT CTG GAG AGT CT
IL7	TCC TCC ACT GAT CCT TGT TC
	CTT CAA CTT GCG AGC AGC AC
Foxc1	GGT ACA GAG ACT GAC TGG CA
	TTC TAA CCT GCG GAA ATC CAAC
Ebf1	CAC TCG GTG CGG GAA ATG T
	CAC AAA GGC CGT CCT CTC AA
Ebf3	CGA AAG GAC CGC TTT TGT GG
	AGT GAA TGC CGT TGT TGG TTT
Col1a1	CAC CCT CAA GAG CCT GAG TC
	GTT CGG GCT GAT GTA CCA GT
Col3a1	CTG TAA CAT GGA AAC TGG GGA AA
	CCA TAG CTG AAC TGA AAA CCA CC
Col6a3	GAG GTT GGA AAT TGG ACA GGA C
	CCG TAC AGC AGT TAT GGC ATC
Pdpn	GAG GGC TTA ATG AAT CTA CTG GC
	GAC AGT TCC TCT AAG GGA GGC
Pdgfra	CCC TAT CCT GGC ATG ATG GTC
	TGA GGT GGT AGA AGG AGG GTC
Gli1	CCA AGC CAA CTT TAT GTC AGG G
	AGC CCG CTT CTT TGT TAA TTT GA
Ly6a	AGG AGG CAG CAG TTA TTG TGG
	CGT TGA CCT TAG TAC CCA GGA
Pdgfa	TGG CTC GAA GTC AGA TCC ACA
	TTC TCG GGC ACA TGG TTA ATG
Pdgfb	AAG TGT GAG ACA ATA GTG ACC CC
	CAT GGG TGT GCT TAA ACT TTC G
Tgfb1	CTT CAA TAC GTC AGA CAT TCG GG
	GTA ACG CCA GGA ATT GTT GCT A

Supplementary Table 1. The primer pairs used for qRT-PCR