Expanded View Figures

Figure EV1. Level of expression and polar distribution of Septins and Borgs in HSCs.

A–G m-RNA expression levels of *Septin1, 2, 6, 7, 8, 9,* and *11* in young LT-HSCs, aged LT-HSCs and aged LT-HSCs treated with CASIN 5 μM normalized to GAPDH and shown relative to respective septins in young LT-HSCs, at least three biological replicates ($n = 3$). ****$P < 0.0001$, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

H–J Expression levels of *Borg2*, *Borg3*, and *Borg4* in young, aged, and aged LT-HSCs treated with CASIN 5 μM normalized to GAPDH and shown relative to respective borgs in young LT-HSCs. At least three biological replicates ($n = 3$) are shown. Error bars indicate mean ± SEM; ***$P < 0.001$, ****$P < 0.0001$.

K Percentage of cells polarized for Septin2 and Septin6 in young, aged, and aged LT-HSCs treated with CASIN 5 μM. Each sample cell was analyzed and scored for Septin-2 and Septin6 polarity distribution. Two biological repeats ($n = 2$) with at least 20 cells per repeat are shown. Error bars indicate mean ± SD.

L, M Representative images of Septin2, Septin6 (red), and tubulin (green) distribution in young, aged, and aged LT-HSCs treated with 5 μM CASIN by immunofluorescence microscopy. Nuclei were stained with DAPI (blue); scale bar = 5 μm.

N Representative images of the distribution of Cdc42-GTP (orange), Borg4 (red), and Septin7 (green) in young and aged LT-HSCs (by immunofluorescence microscopy). Nuclei were stained with DAPI (blue); scale bar = 5 μm.

O Percentage of cells polarized for Cdc42-GTP, Borg4, and Septin7 in young or aged LT-HSCs, $n = 3$. Error bars indicate mean ± SD, *$P < 0.05$ (two-way ANOVA).

P Quantification of the co-localization of Cdc42-GTP, Borg4 or Septin7 proteins in LT-HSCs. Error bars indicate mean ± SD.

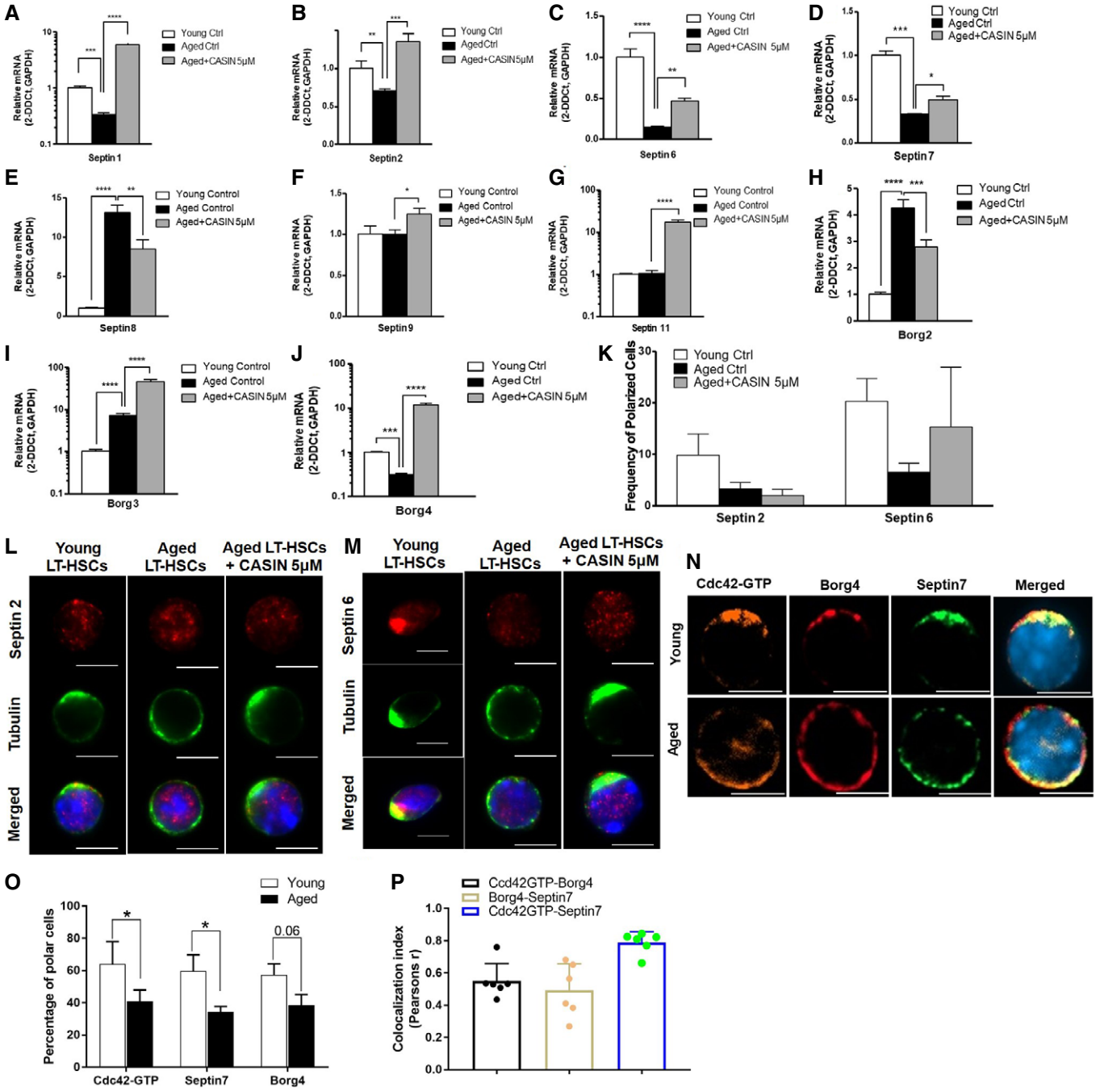

Figure EV1.

Figure EV2. Expression pattern of septins in hematopoiesis.

A Expression of septins in LT-HSCs. $n = 4$ data points, error bars indicate mean ± SD.

B–M Expression of *Septins* in stem cells and hematopoiesis. The x-axis indicates stem and progenitor cells names; the y-axis indicates Log_2 expression values.

Data and graphs from blood spot (https://servers.binf.ku.dk/bloodspot/).

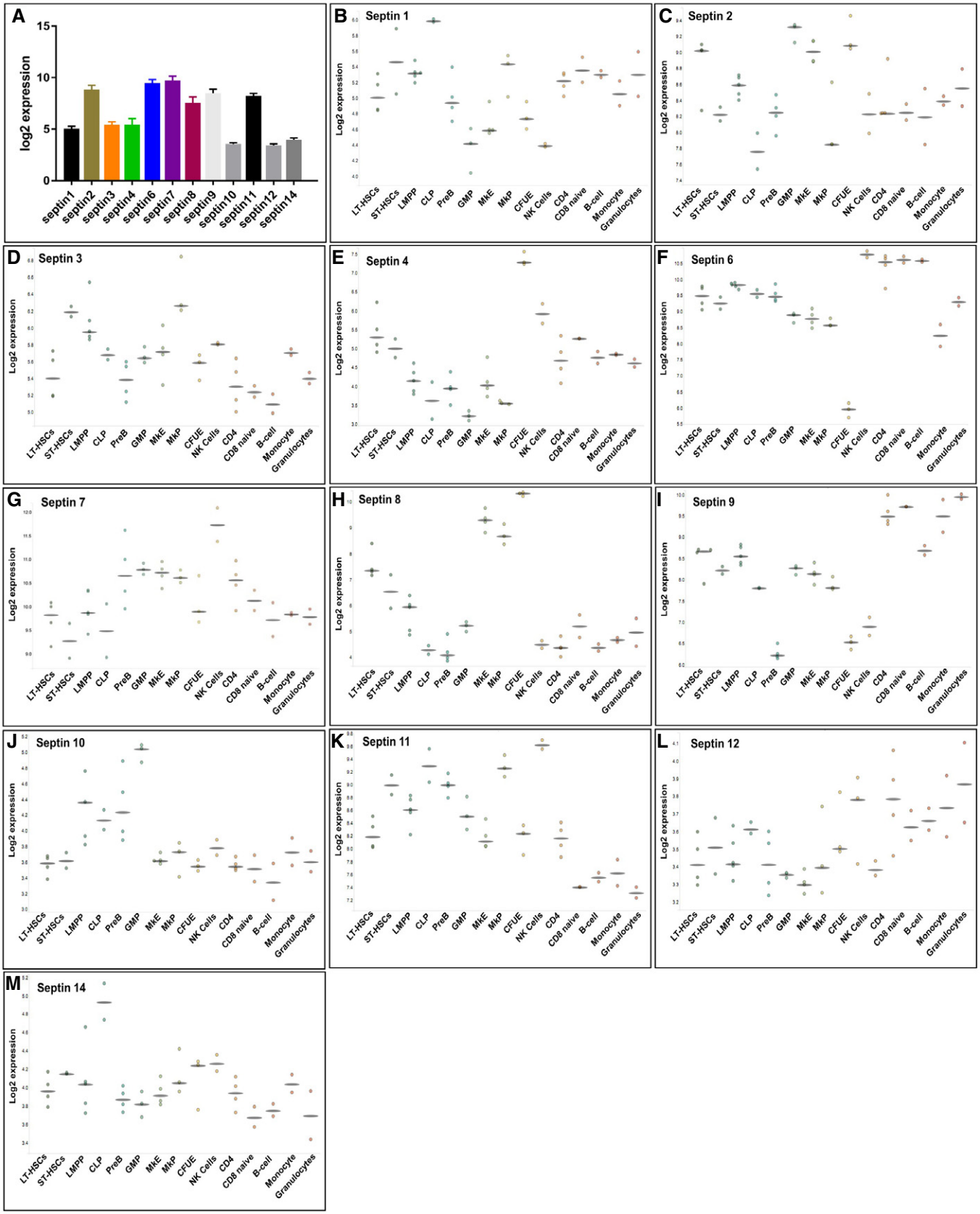

Figure EV2.

Figure EV3. Expression pattern of Cdc42eps (Borgs) in hematopoiesis.

A Expression of *Cdc42eps (Borgs)* in LT-HSCs. $n = 4$ data points, error bars indicate mean ± SD.

B–F Expression of *Cdc42eps (Borgs)* in stem cells and hematopoiesis. The x-axis indicates stem and progenitor cells names; the y-axis indicates Log_2 expression values. (G) Schematic representation of the proximity ligation assay reaction for Cdc42-Borg4 and Borg4-Septin7 interactions.

H Representative confocal 3D image of LT-HSC from Cdc42 KO mice showing Cdc42-Borg4 interaction as a control. Nuclei were stained with DAPI; scale bar = 5 μm.

Data and graphs from blood spot (https://servers.binf.ku.dk/bloodspot/).

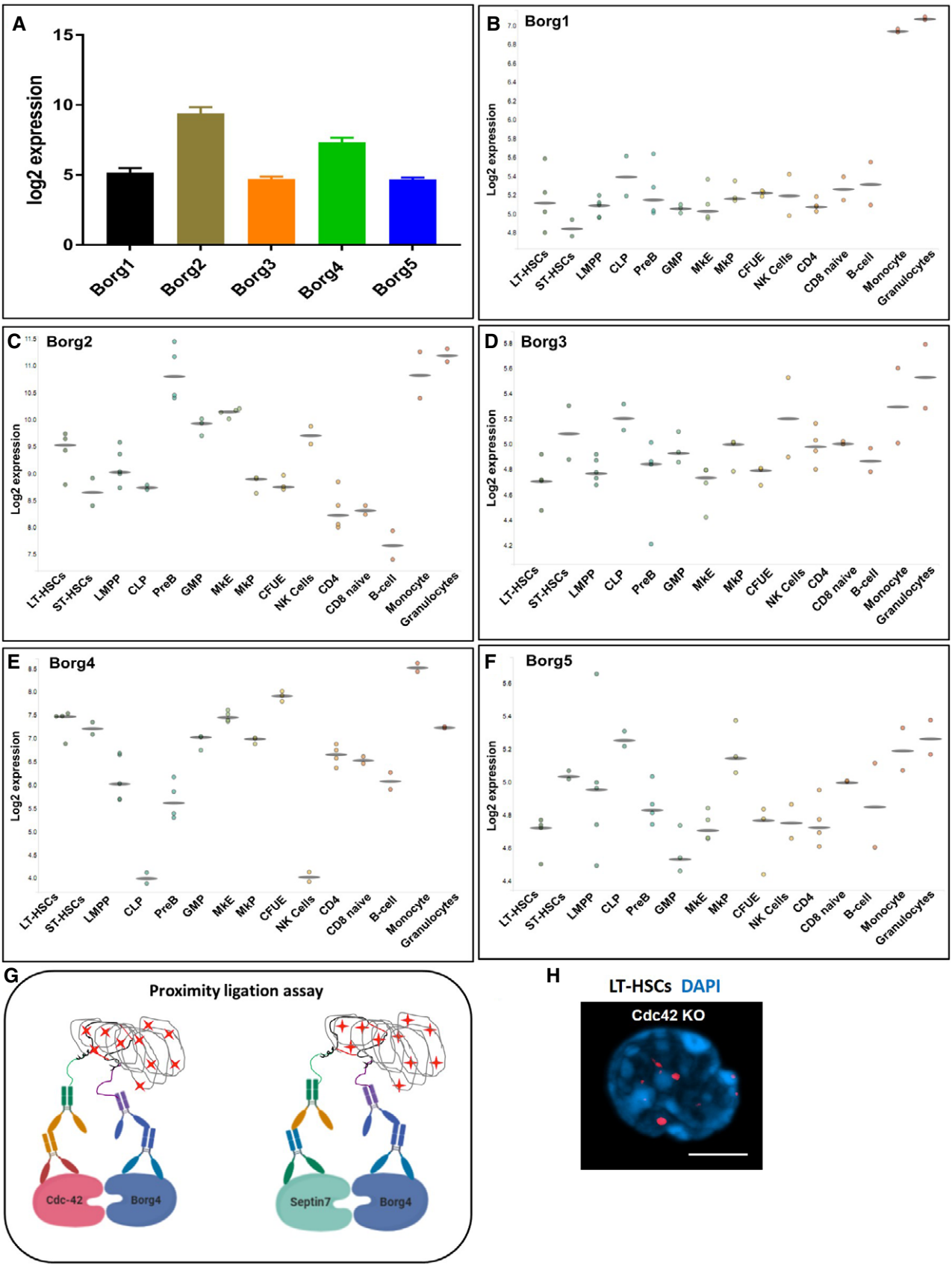

Figure EV3.

Figure EV4. Distribution of polarity proteins in *Borg4*$^{\Delta/\Delta}$ or *Septin7*$^{\Delta/\Delta}$ HSCs.

A QRT-PCR analysis of *Borg4* mRNA in low-density bone marrow cells isolated from *Borg4*$^{fl/fl}$ and *Borg4*$^{\Delta/\Delta}$ mice (n = 3, three biological replicates). ****$P < 0.0001$. Unpaired t-test, error bars indicate mean with SD.

B PCR amplification of genomic DNA from lineage negative cells of *Borg4*$^{fl/fl}$ and *Borg4*$^{\Delta/\Delta}$ Here, we used two sets of primers to distinguish *Borg4*$^{fl/fl}$ (with primer a + b: 580 bp) and *Borg4*$^{\Delta/\Delta}$ (primer a + c: 490 bp). For the amplification of the cre allele (375 bp), another set of primers was used.

C Immunofluorescence staining of Septin7(red) in LT-HSCs of *Septin7*$^{fl/fl}$ and *Septin7*$^{\Delta/\Delta}$ mice. Scale bar = 5 μm.

D Septin7 knock-out confirmation by PCR amplification of genomic DNA in lineage negative cells using primers P1, P2, and P3.

E, F Representative images of H4K16ac (red) and tubulin (green) distribution in *Borg4*$^{fl/fl}$ and *Borg4*$^{\Delta/\Delta}$; *Septin7*$^{fl/fl}$ and *Septin7*$^{\Delta/\Delta}$ LT-HSCs by immunofluorescence microscopy. Nuclei were stained with DAPI (blue), scale bar = 5 μm.

G Representative images of Septin2 (cyan) and Septin6 (orange) distribution in *Borg4*$^{fl/fl}$ and *Borg4*$^{\Delta/\Delta}$. Scale bar = 5 μm.

H, I Percentage of cells polarized for H4K16ac in *Borg4*$^{fl/fl}$ and *Borg4*$^{\Delta/\Delta}$; *Septin7*$^{fl/fl}$ or *Septin7*$^{\Delta/\Delta}$ LT-HSCs. At least three biological replicates (n = 3) are shown. Error bars indicate mean ± SD, *$P < 0.05$, unpaired t-test.

J Percentage of cells polarized for Septin2 and Septin6 in *Borg4*$^{fl/fl}$ and *Borg4*$^{\Delta/\Delta}$ LT-HSCs. At least three biological replicates (n = 3) are shown. Error bars indicate mean ± SD.

K Representative images of Crumbs3 (purple) distribution in *Borg4*$^{fl/fl}$ or *Borg4*$^{\Delta/\Delta}$ LT-HSCs by immunofluorescence microscopy. Nuclei were stained with DAPI (blue), scale bar = 5 μm.

L Percentage of cells polarized for Crumbs3 in *Borg4*$^{fl/fl}$ or *Borg4*$^{\Delta/\Delta}$ LT-HSCs. At least, three biological replicates (n = 3) are shown. Error bars indicate mean ± SD.

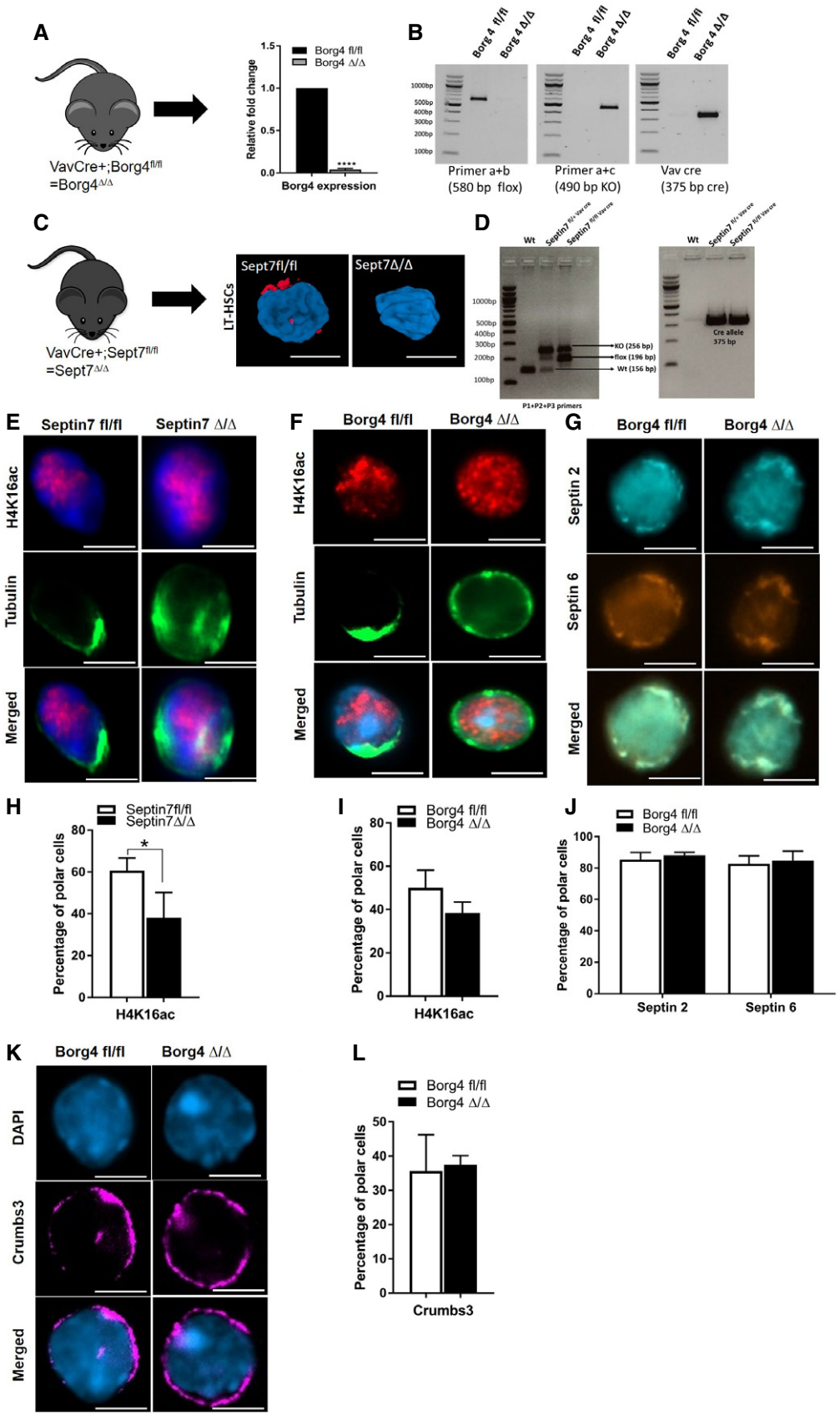

Figure EV4.

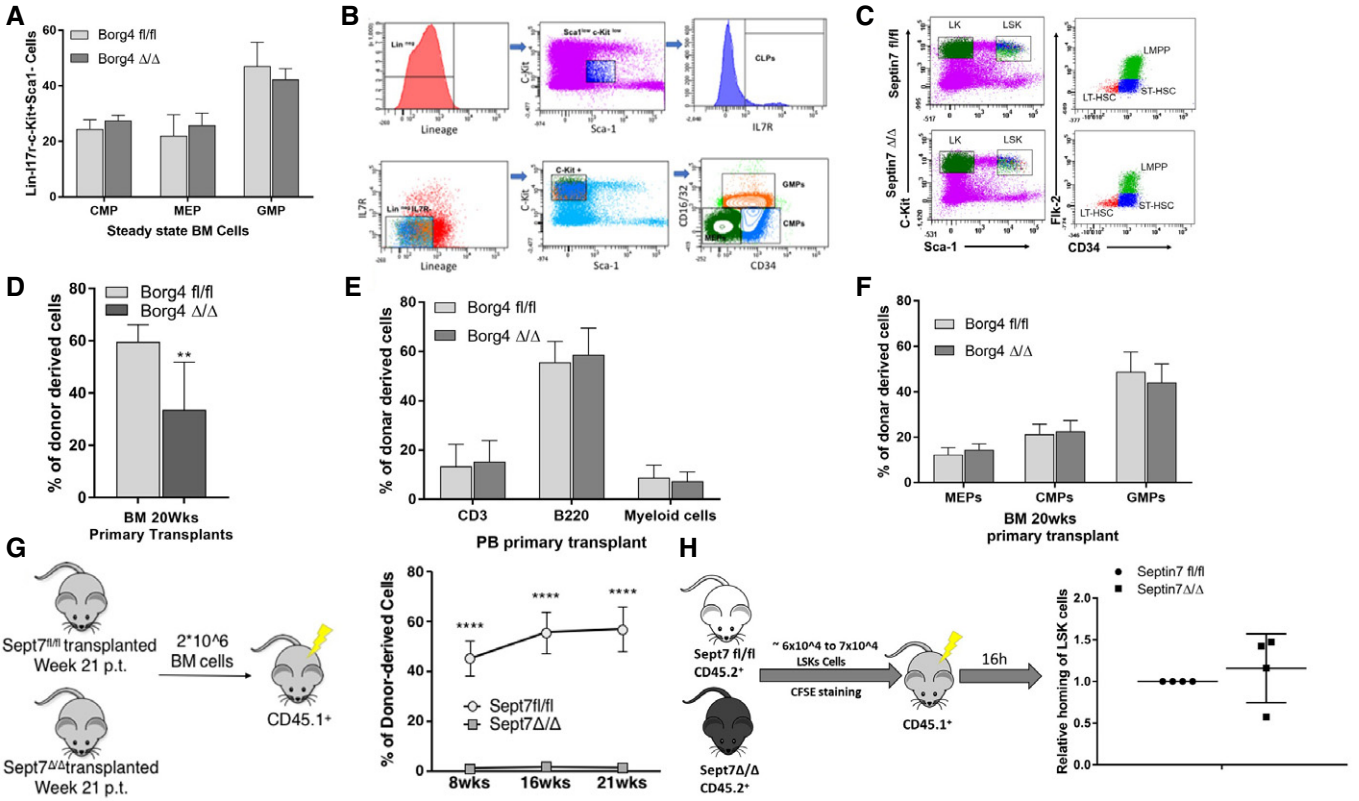

Figure EV5. Impaired function of *Borg4*$^{\Delta/\Delta}$ or *Septin7*$^{\Delta/\Delta}$ HSCs.

A Percentage of MEPs, GMPs, and CMPs among Lin$^-$IL-R7$^-$Ckit+Sca- cells in steady-state BM analysis. Error bars indicate mean ± SD.

B Gating strategy for calculation of percentages of CLPs, GMPs, CMPs, and MEPs.

C Representative FACS dot plots of stem and progenitor cells gating in *Septin7*$^{fl/fl}$ and *Septin7*$^{\Delta/\Delta}$ mice at steady state.

D Frequency of total donor-derived cells in bone marrow after 20 weeks in competitive primary transplant (n = 12, $^{**}P < 0.01$, unpaired t-test, error bars indicate mean with SD).

E Analysis of donor-derived lineage differentiated cells (B cells as B220, CD3 cells as T cells, myeloid cells as Mac-1$^+$ and Gr$^+$) in PB primary transplants. Error bars indicate mean ± SD.

F Quantitative analysis of MEPs, CMPs, and GMPs in primary BM transplant. Error bars indicate mean ± SD.

G Experimental setup for secondary transplantation and percentage of engrafted donor-derived cells in PB 8–21 weeks after secondary transplantation. n = 12, $^{****}P < 0.0001$, Error bars indicate mean ± SD (two-way ANOVA).

H Experimental setup for homing of LSK cells and number of CFSE-positive LSK cells homed to bone marrow in *Septin7*$^{fl/fl}$ and *Septin7*$^{\Delta/\Delta}$ three biological groups (n = 3). Error bars indicate mean ± SD.