JEM

Supplemental material

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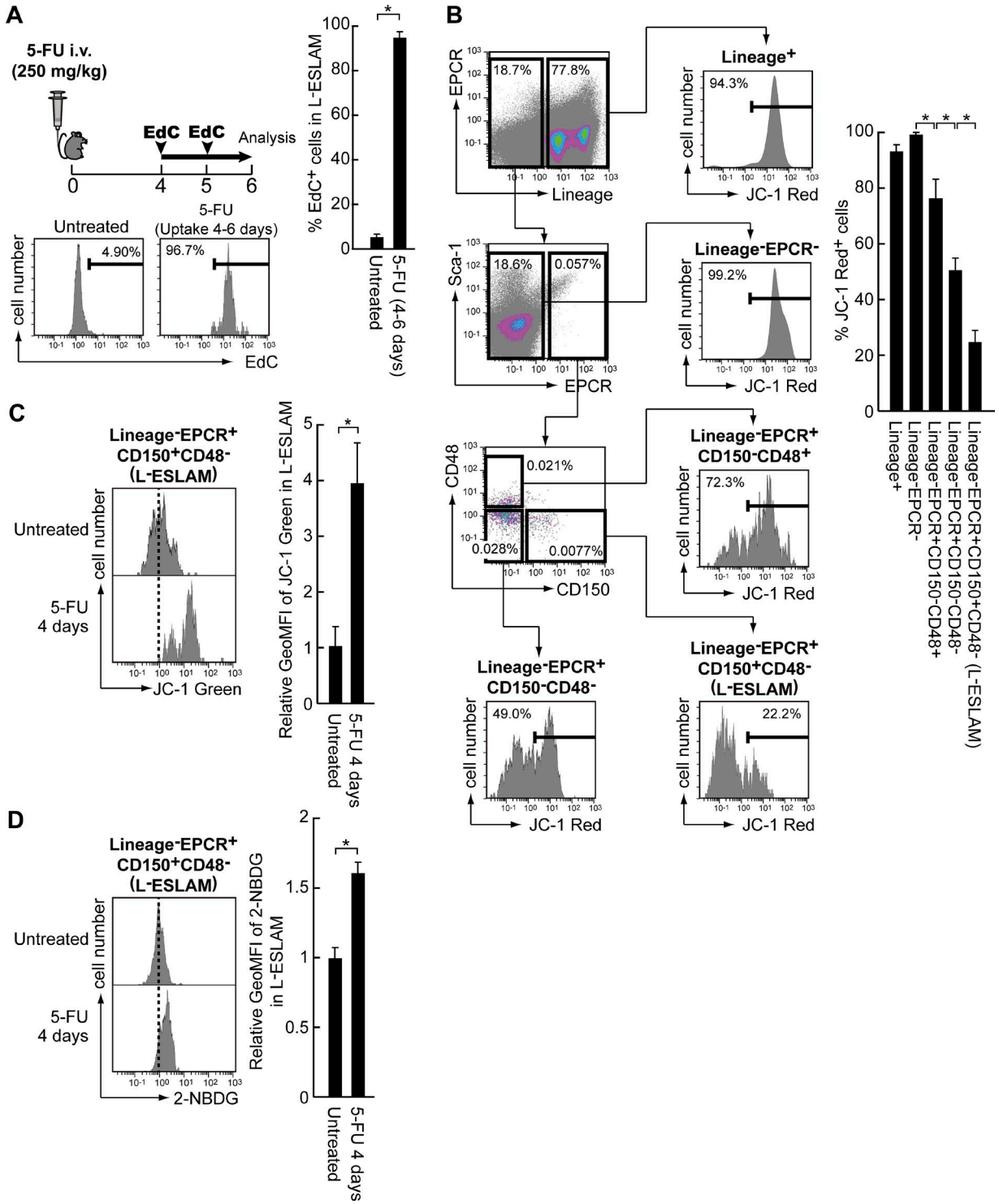

Figure S1. **Cell division, mitochondrial statuses and the potential for glucose uptake of HSCs before and after 5-FU administration. (A)** 5-FU–treated mice (250 mg/kg, i.v.) were subjected to EdC administration twice (150 mg/kg/shot i.p.) at indicated schedule. After 48 h from first EdC administration, EdC$^+$ cells within Lineage$^-$EPCR$^+$CD150$^+$CD48$^-$ (L$^-$ESLAM) HSCs were detected by flow cytometry. Numbers in histograms represent the frequency of gated cells within L$^-$ESLAM cells. The graph depicts the frequency of EdC$^+$ cells. (n = 4, two independent experiments). **(B)** $\Delta\Psi_m$ in indicated fractions was examined by JC-1 staining. Numbers in dot plots or histograms represent the frequency of gated cells within total BM cells or indicated fractions, respectively. The graph depicts the frequency of JC-1 Red$^+$ cells within indicated fractions. (n = 4, two independent experiments). **(C)** After 5-FU treatment, mitochondrial mass in L$^-$ESLAM HSCs was examined using JC-1 Green fluorescence, which reflects the height of mitochondrial mass. Histograms show the fluorescent intensity of JC-1 Green. The graph depicts geometric mean fluorescence intensity (GeoMFI) relative to the value of HSCs derived from untreated mice. (n = 4, two independent experiments). **(D)** L$^-$ESLAM HSCs derived from untreated or 5-FU–treated mice were cultured with 2-NBDG, and subsequently the uptake of 2-NBDG was determined by a flow cytometry. The graph depicts geometric mean fluorescence intensity (GeoMFI) relative to the value of HSCs derived from untreated mice. Data are presented as means ± SD (n = 4, two independent experiments; *, P < 0.01).

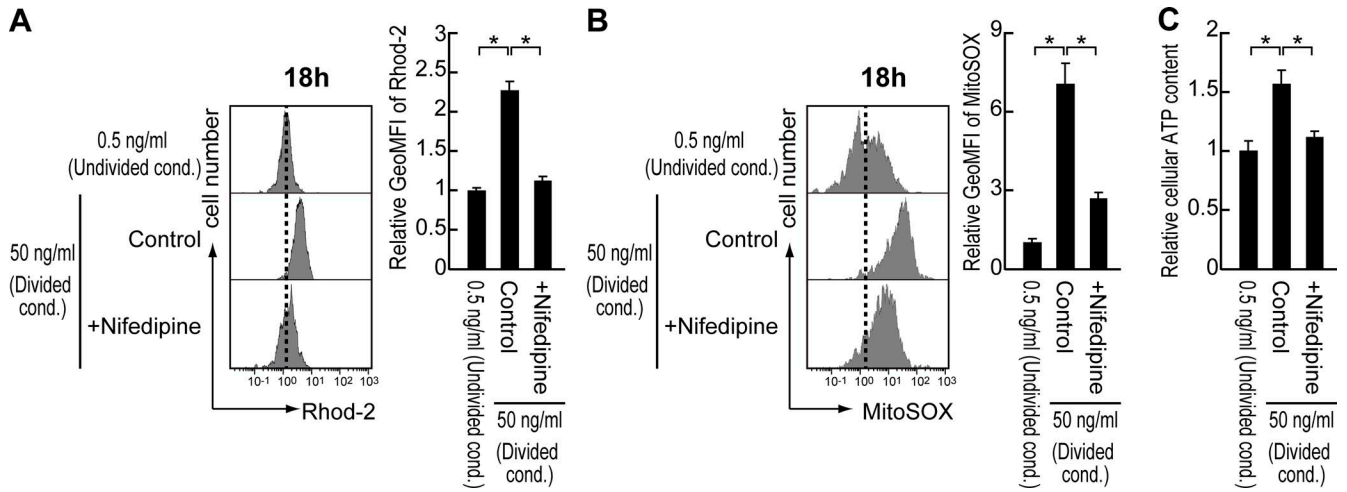

Figure S2. **Nifedipine suppressed mitochondrial functions. (A and B)** After 18 h culture under divided conditions (50 ng/ml SCF and TPO) in the absence (Control) or presence of 60 µM Nifedipine (+Nifedipine), mitochondrial Ca^{2+} or superoxide level of culture HSCs were examined using Rhod-2 or MitoSOX. Graphs depict geometric mean fluorescence intensity (GeoMFI) relative to the value of cells treated with SCF and TPO at 0.5 ng/ml (undivided conditions; *n* = 4, two independent experiments). **(C)** After the culture under divided conditions (50 ng/ml SCF and TPO) for 18 h in the absence (Control) or presence of 60 µM Nifedipine (+Nifedipine), ATP content in L^-ESLAM fractions of BM was examined by luminescence-based assay. The graph depicts intracellular ATP content relative to the value of cells treated with SCF and TPO at 0.5 ng/ml (undivided conditions). Data are expressed as the mean ± SD (*n* = 4, two independent experiments; *, P < 0.01; **, P < 0.05).

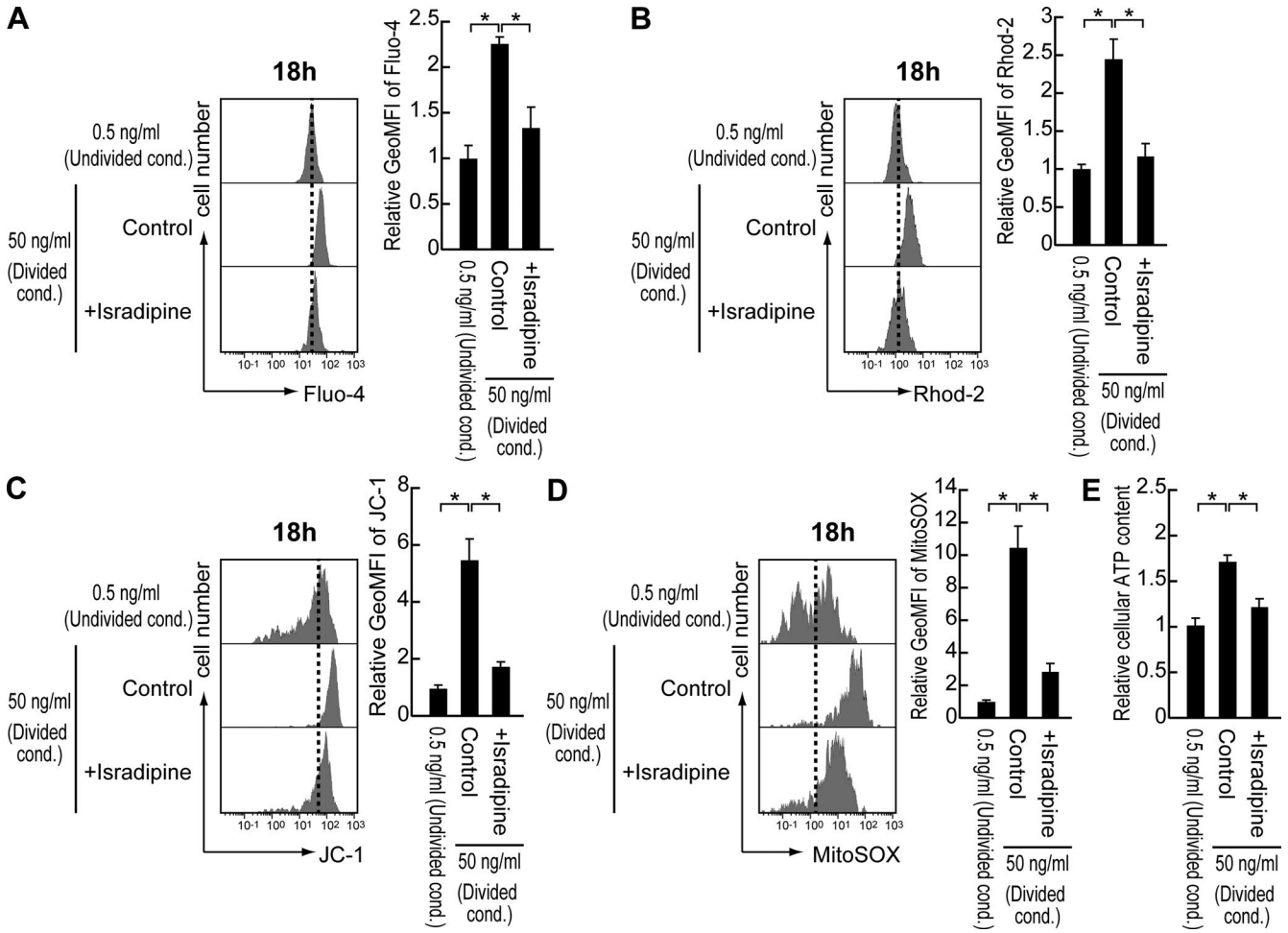

Figure S3. **Isradipine, alternative blocker for LTCCs, also suppressed Ca^{2+}–mitochondria pathway. (A–D)** After the culture under divided conditions (50 ng/ml SCF and TPO) for 18 h in the absence (Control) or presence of 50 µM Isradipine (+Isradipine), $\Delta\Psi_m$ (A), intracellular Ca^{2+} (B), mitochondrial Ca^{2+} (C), or mitochondrial superoxide level (D) of culture HSCs were examined by JC-1, Fluo-4, Rhod-2, or MitoSOX staining. Graphs depict geometric mean fluorescence intensity (GeoMFI) relative to the value of cells treated with SCF and TPO at 0.5 ng/ml (undivided conditions; $n = 4$, two independent experiments). **(E)** After 18 h culture under divided conditions (50 ng/ml SCF and TPO) in the absence (Control) or presence of 50 µM Isradipine (+Isradipine), ATP content in cultured HSCs was examined. The graph depicts intracellular ATP content relative to the value of cells treated with SCF and TPO at 0.5 ng/ml (undivided conditions). Data are expressed as the mean ± SD ($n = 4$, two independent experiments; *, $P < 0.01$).

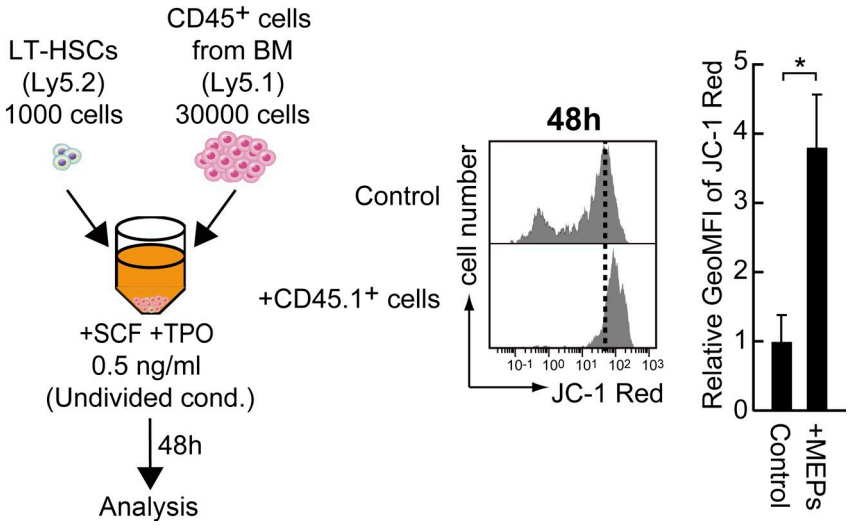

Figure S4. **Mature hematopoietic cells hardly have the potential for the suppression of $\Delta\Psi_m$ in HSCs.** Sorted HSCs (Ly5.2) were cultured with CD45$^+$ cells (Ly5.1) derived from total BM for 48 h, and subsequently $\Delta\Psi_m$ of HSCs was examined by using JC-1 staining. Cells cultured without CD45$^+$ cells serve as control. The graph depicts geometric mean fluorescence intensity (GeoMFI) relative to the value of cells cultured under control conditions. Data are presented as means ± SD (n = 4, two independent experiments; *, P < 0.01).

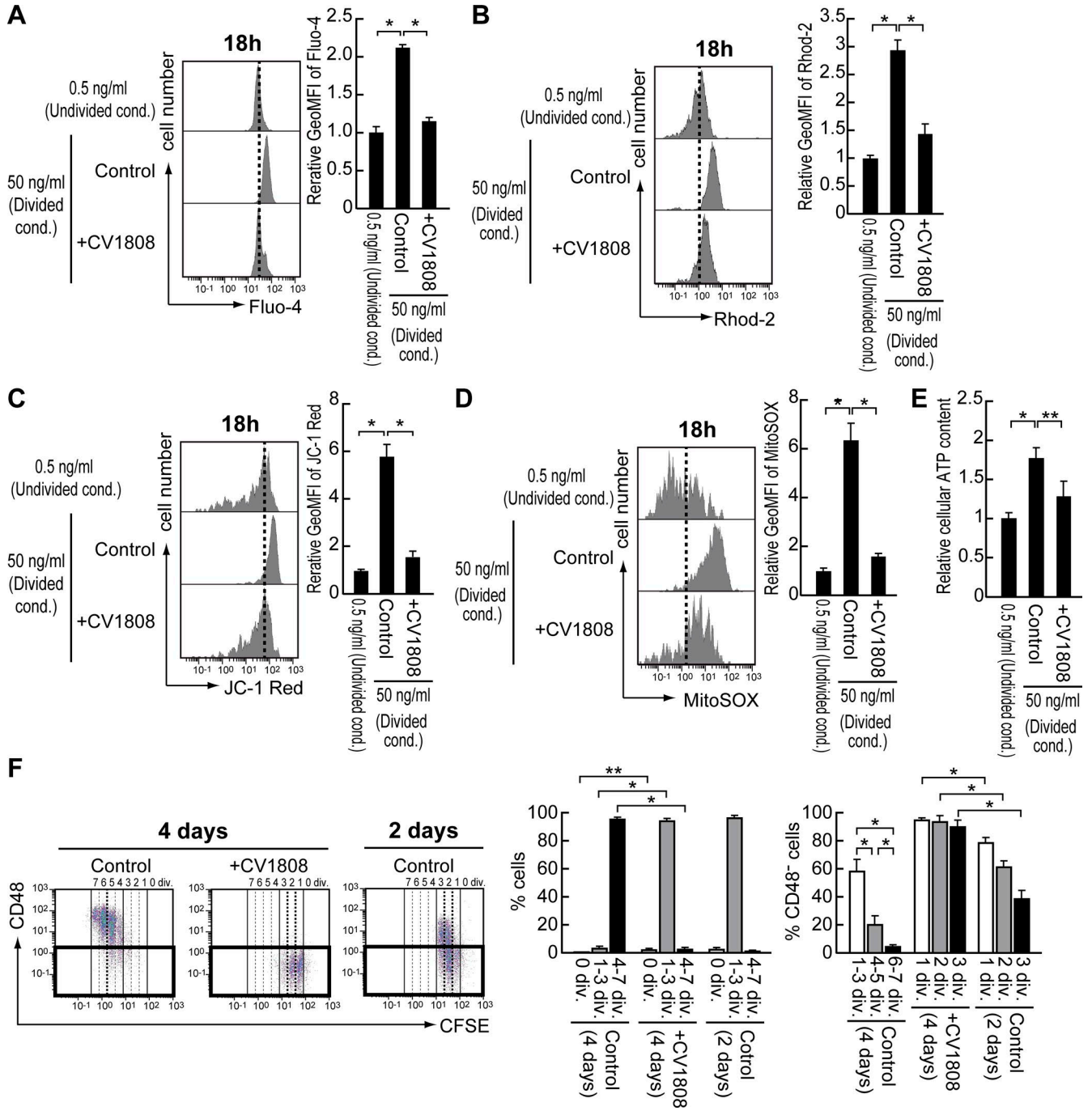

Figure S5. **CV1808, an agonist of adenosine A2 receptors, suppressed Ca^{2+}–mitochondria pathway and prolongs the interval of cell divisions. (A–D)** $\Delta\Psi_m$ (A), intracellular Ca^{2+} (B), mitochondrial Ca^{2+} (C), or mitochondrial superoxide level (D) in HSCs after the culture for 18 h under divided conditions (50 ng/ml SCF and TPO) in the absence (Control) or presence of 50 µM CV1808 (+CV1808). Graphs depict geometric mean fluorescence intensity (GeoMFI) relative to the value of cells treated with SCF and TPO at 0.5 ng/ml (undivided conditions; *n* = 4, two independent experiments). **(E)** ATP content of HSCs cultured for 18 h under conditions as described above. The graph depicts intracellular ATP content relative to the value of cells treated with SCF and TPO at 0.5 ng/ml (undivided conditions; *n* = 4, two independent experiments). **(F)** Cell division number and expressions of CD48 in HSCs cultured under conditions as described above. The gate described by heavy line within each dot plot represents $CD48^-$ fractions. Dot lines separated cells that undergoes indicated number of cell division. Graph depicts the frequency of cells undergoing the indicated number of cell divisions in whole cultured cells (left) or $CD48^-$ cells within cells undergoing the indicated number of cell divisions (right). Data are presented as means ± SD (*n* = 4, four independent experiments; *, P < 0.01; **, P < 0.05).