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Supplemental Information

Regulation of Glycolysis by Pdk Functions as a Metabolic Checkpoint for Cell Cycle Quiescence in Hematopoietic Stem Cells

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Inventory of Supplemental Information

Figure S1

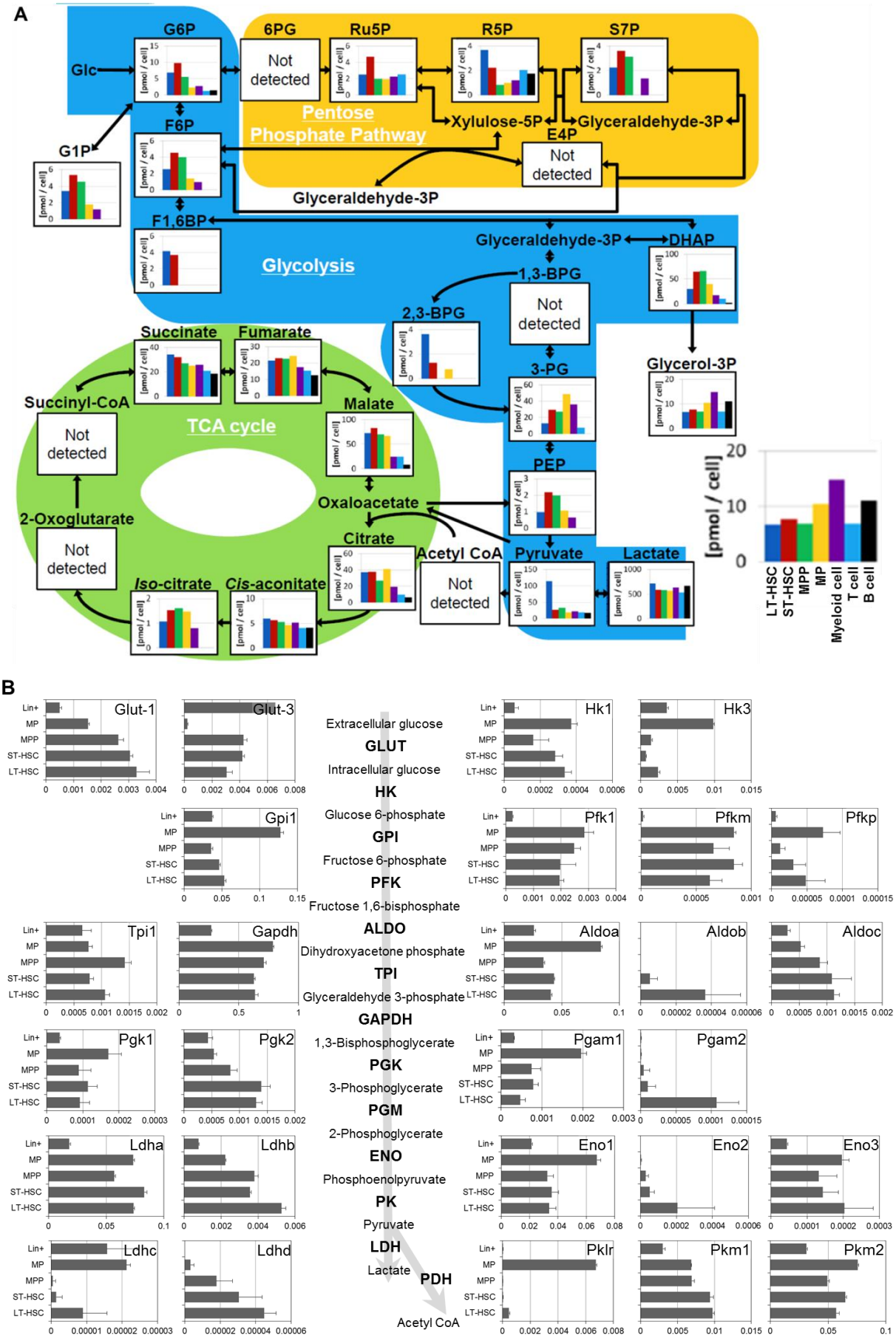
A
Glc
G6P
6PG
Not detected
Ru5P
R5P
S7P
Pentose Phosphate Pathway
Xylulose-5P
Glyceraldehyde-3P
E4P
Not detected
F6P
G1P
F1,6BP
Glycolysis
DHAP
1,3-BPG
Not detected
2,3-BPG
3-PG
Glycerol-3P
PEP
Succinate
Fumarate
Malate
Succinyl-CoA
Not detected
TCA cycle
Oxaloacetate
2-Oxoglutarate
Not detected
Citrate
Acetyl CoA
Not detected
Pyruvate
Lactate
Iso-citrate
Cis-aconitate
[pmol / cell]
LT-HSC
ST-HSC
MPP
MP
Myeloid cell
T cell
B cell
B
Extracellular glucose
GLUT
Intracellular glucose
HK
Glucose 6-phosphate
GPI
Fructose 6-phosphate
PFK
Fructose 1,6-bisphosphate
ALDO
Dihydroxyacetone phosphate
TPI
Glyceraldehyde 3-phosphate
GAPDH
1,3-Bisphosphoglycerate
PGK
3-Phosphoglycerate
PGM
2-Phosphoglycerate
ENO
Phosphoenolpyruvate
PK
Pyruvate
LDH
Lactate
PDH
Acetyl CoA
Glut-1
Glut-3
Hk1
Hk3
Gpi1
Pfk1
Pfkm
Pfkp
Tpi1
Gapdh
Aldoa
Aldob
Aldoc
Pgk1
Pgk2
Pgam1
Pgam2
Ldha
Ldhb
Eno1
Eno2
Eno3
Ldhc
Ldhd
Pklr
Pkm1
Pkm2
Lin+
MP
MPP
ST-HSC
LT-HSC

Figure S1, related to Figure 1

Metabolomic profiling of central carbon metabolism in HSCs and their progeny.

A, Quantification of metabolites in central carbon metabolism based on CE-TOFMS analysis. Bar graphs for independent metabolites plotted in the central carbon metabolism map are (from left to right): long-term (LT)-hematopoietic stem cells (HSCs) ($CD34^-Flt3^-$ LSK cells; blue bars), short-term (ST)-HSCs ($CD34^+Flt3^-$ LSK cells; red bars), multipotent progenitors (MPPs) ($CD34^+Flt3^+$ LSK cells; green bars), myeloid progenitors (MPs; Lin^- c-Kit^+ $Sca\text{-}1^-$ cells; yellow bars), Gr-1/$Mac\text{-}1^+$ myeloid cells (purple bars), CD4/$CD8^+$ T cells (sky blue bars), and $B220^+$ B lymphocytes (black bars). Metabolites not detected in any fraction are indicated as "Not detected" in the metabolic map. Data is representative of two independent experiments.

B, Expression levels of glycolytic enzymes in various hematopoietic compartments. Relative mRNA expression of glycolytic enzymes in wild-type BM fractions (LT-HSC, ST-HSC, MPP, MP, or Lin^+). Expression levels are normalized to ß-actin expression (mean ± SD, n=4).

Figure S2

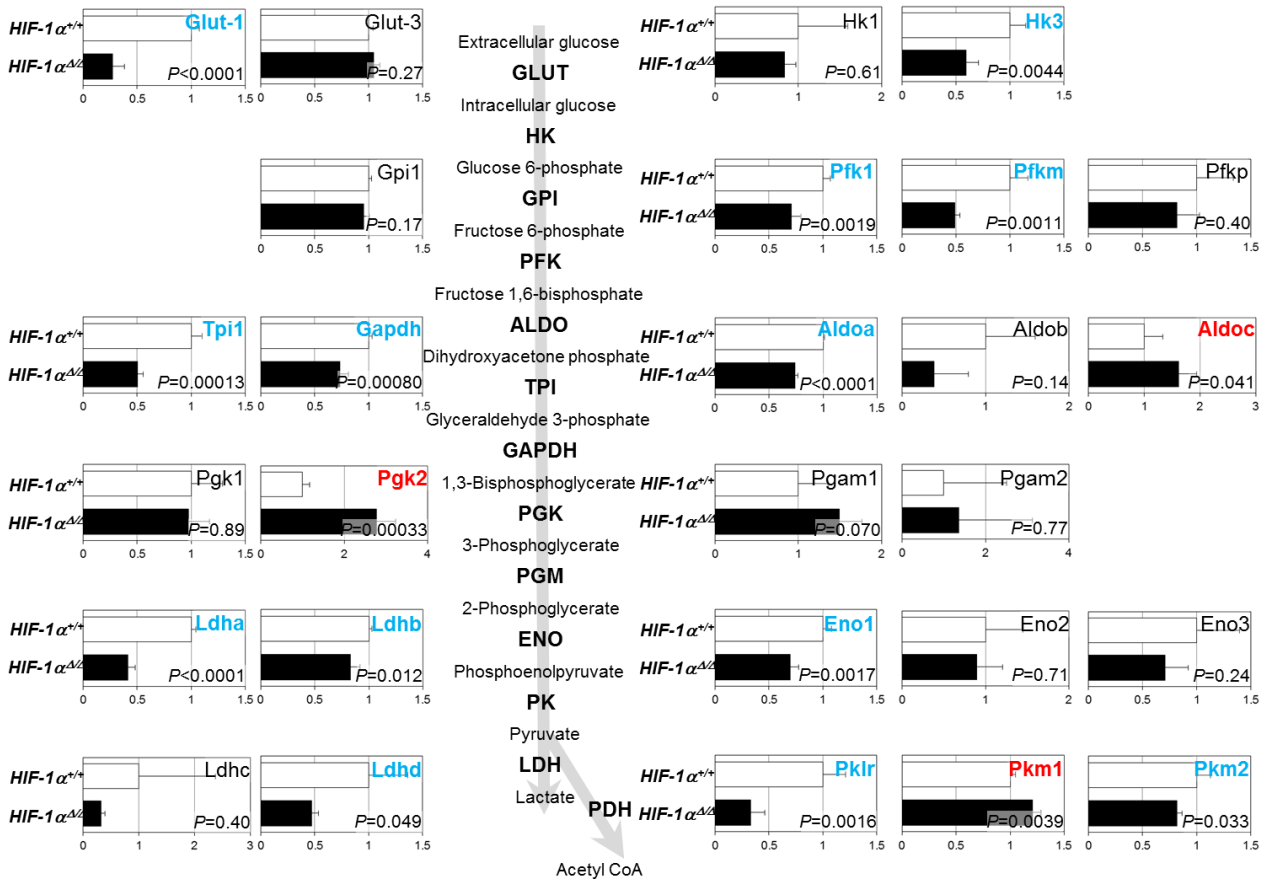

Figure S2, related to Figure 2

Expression levels of glycolytic enzymes in HIF-1α$^{\Delta/\Delta}$ LT-HSCs

Relative mRNA expression of glycolytic enzymes in CD34$^-$ Flt-3$^-$ LSK samples from HIF-1α$^{+/+}$ (open bars) or HIF-1α$^{\Delta/\Delta}$ (closed bars) mice. Each value is normalized to ß-actin expression and expressed as fold-induction compared to levels (set to 1) detected in HIF-1α$^{+/+}$ CD34$^-$ Flt-3$^-$ LSK samples (open bars) (mean ± SD, n=4). Genes significantly downregulated or upregulated ($P < 0.05$) in HIF-1α$^{\Delta/\Delta}$ compared to HIF-1α$^{+/+}$ samples are shown in blue or red, respectively.

Figure S3

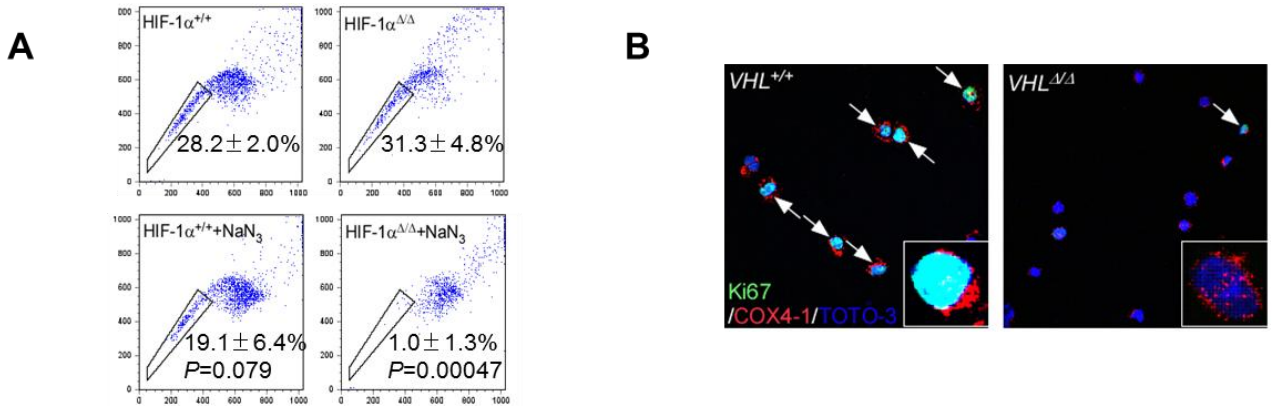

Figure S3, related to Figure 3

Metabolic alterations in HIF-$\alpha^{\Delta/\Delta}$ or VHL$^{\Delta/\Delta}$ primitive hematopoietic cells

A, Flow cytometric analysis of the effects of 20mM NaN_3 treatment on ATP-transporter-mediated dye exclusion from HIF-1$\alpha^{+/+}$ or HIF-1$\alpha^{\Delta/\Delta}$ LSK cells. The enclosed region in the FACS plots represents the mean percentage of the LSK-gated Side Population (mean ± SD, n=3).

B, Immunocytochemical staining of Ki67 (green), COX4-1 (red), and TOTO-3 (blue) in VHL$^{+/+}$ or VHL$^{\Delta/\Delta}$ LSK cells. Arrows indicate cells positive for both green and red signals in the nucleus. Insets show representative cells from each LSK sample.

Figure S4

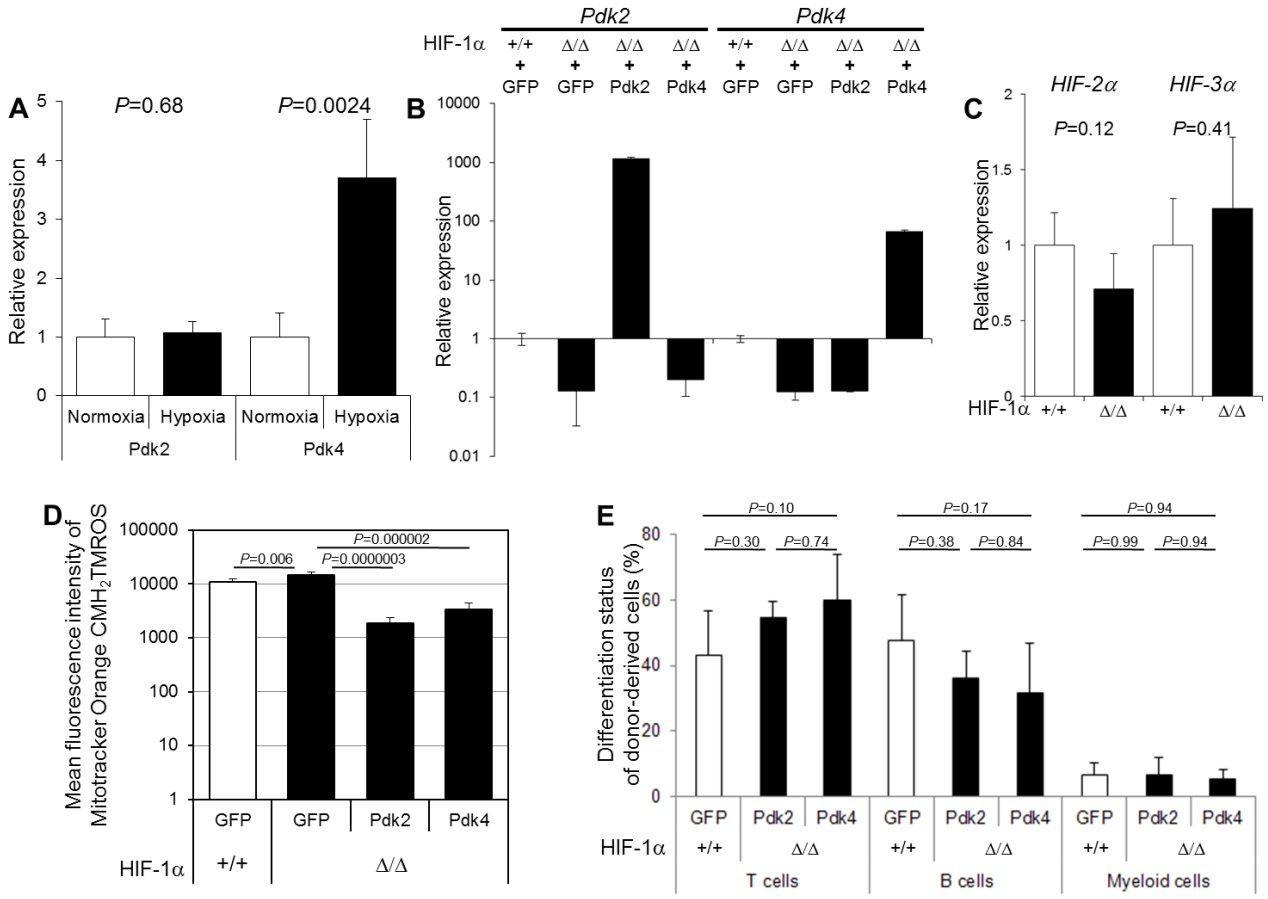

Figure S4, related to Figure 4

Suppression of ROS production and efficient multilineage differentiation by Pdks in HIF-1α$^{\Delta/\Delta}$ HSCs

A, Relative levels of Pdk2 or Pdk4 mRNAs in LT-HSCs (CD34$^-$ Flt-3$^-$ LSK cells) exposed to normoxia (20%O_2) or hypoxia (1%O_2) for 14 hours (n=4). Each value is normalized to ß-actin expression and expressed as fold-induction compared to levels (set to 1) detected in normoxia-treated LT-HSC samples (mean ± SD, n=4). .

B, Relative levels of Pdk2 or Pdk4 mRNAs in LT-HSCs (CD34$^-$ Flt-3$^-$ LSK cells) from HIF-1α$^{+/+}$ or HIF-1α$^{\Delta/\Delta}$ mice transduced with GFP, Pdk2 or Pdk4 retroviruses (n=4). Each value is normalized to ß-actin expression and expressed as fold-induction compared to levels (set to 1) detected in GFP-transduced HIF-1α$^{+/+}$ LT-HSC samples (mean ± SD, n=4). .

C, Relative levels of HIF-2α or HIF-3α mRNAs in LT-HSCs (CD34$^-$ Flt-3$^-$ LSK cells) from HIF-1α$^{+/+}$ or HIF-1α$^{\Delta/\Delta}$ mice (n=4). Each value is normalized to ß-actin expression and expressed as fold-induction compared to levels (set to 1) detected in HIF-1α$^{+/+}$ LT-HSC samples (mean ± SD, n=4). .

D, Mitochondrial ROS detection by Mitotracker Orange CMH_2TMROS in LSK cells transduced with GFP, Pdk2 or Pdk4 retroviruses (n=5). Data are presented as the mean fluorescence intensity ± SEM.

E, Differentiation status (CD4/$CD8^+$ T cells, $B220^+$ B cells, or Mac-1/$Gr\text{-}1^+$ myeloid cells) of donor-derived ($Ly5.2^+$) PB cells in primary BMT recipients of GFP virus-transduced HIF-$1\alpha^{+/+}$ LSK cells, GFP virus-transduced HIF-$1\alpha^{\Delta/\Delta}$ LSK cells, Pdk2 virus-transduced HIF-$1\alpha^{\Delta/\Delta}$ LSK cells, or Pdk4 virus-transduced HIF-$1\alpha^{\Delta/\Delta}$ LSK cells (mean ± SD, n=5).

Figure S5

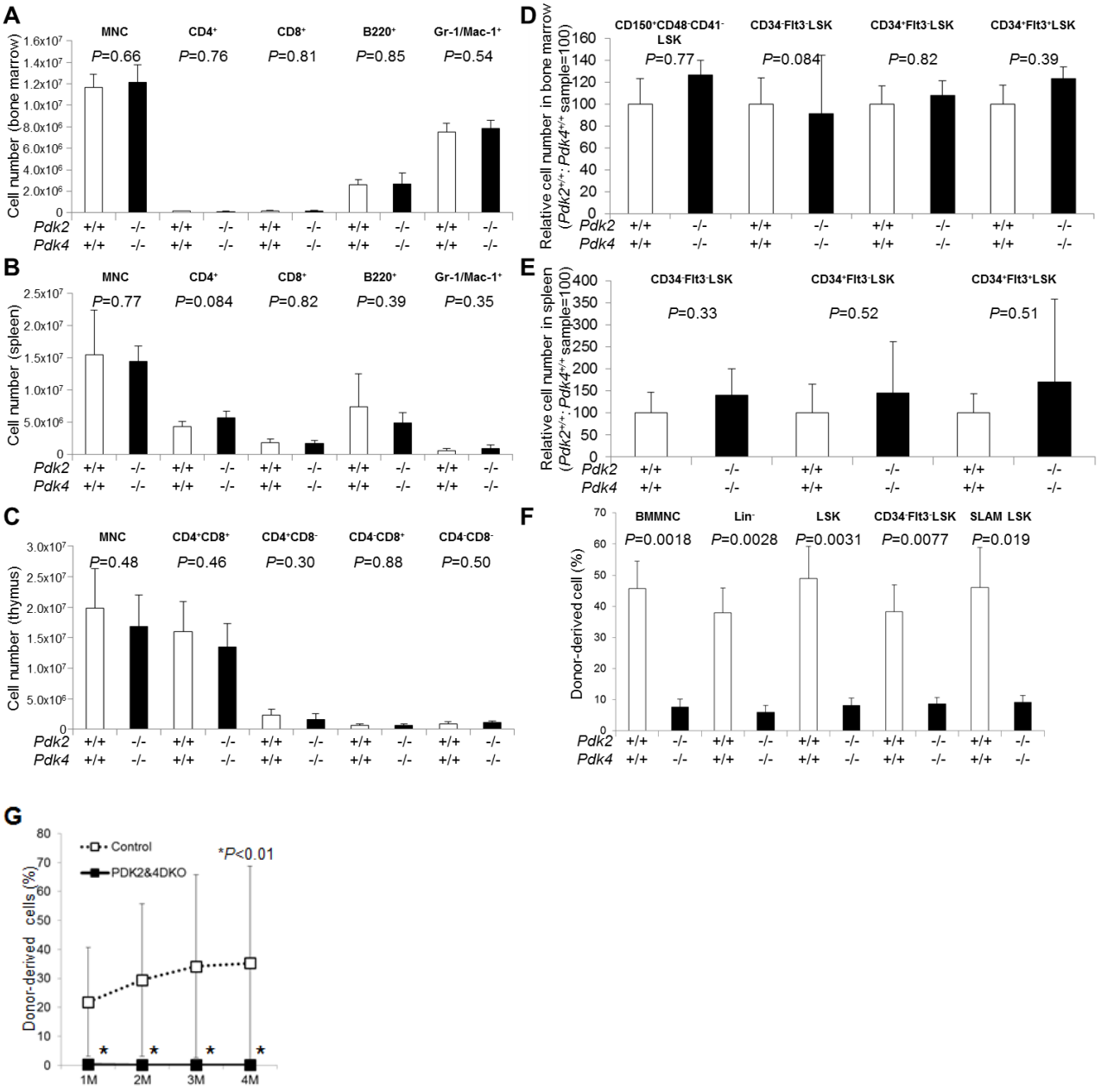

Figure S5, related to Figure 5

Number of differentiated and primitive hematopoietic cells in *Pdk2$^{-/-}$: Pdk4$^{-/-}$* mice

A-C, Number of MNCs or cells from various differentiated cell fractions in BM (**A**), spleen (**B**) or thymus (**C**) of *Pdk2$^{-/-}$: Pdk4$^{-/-}$* mice (mean ± SD, n=4).

D, E, Number of cells in primitive cell fractions including CD34^{-}Flt3^{-} LSK, CD34^{+}Flt3^{-} LSK, or CD34^{+}Flt3^{+} LSK in BM (**D**) or spleen (**E**) of *Pdk2$^{-/-}$: Pdk4$^{-/-}$* mice (mean ± SD, n=4).

F, Donor-derived (Ly5.2+) BM MNC, Lin-, LSK, CD34-Flt3- LSK or SLAM-LSK chimerism in secondary BMT recipients of control (open bars) or *Pdk2*$^{-/-}$*: Pdk4*$^{-/-}$ (closed bars) LT-HSCs 4 months after primary BMT (mean ± SEM, n = 10).

G, PB chimerism in tertiary recipients of BM derived from secondary recipients of control (open boxes) or *Pdk2*$^{-/-}$*: Pdk4*$^{-/-}$ (closed boxes) MNCs, at indicated times after BMT (mean ± SD, n = 10).

Figure S6

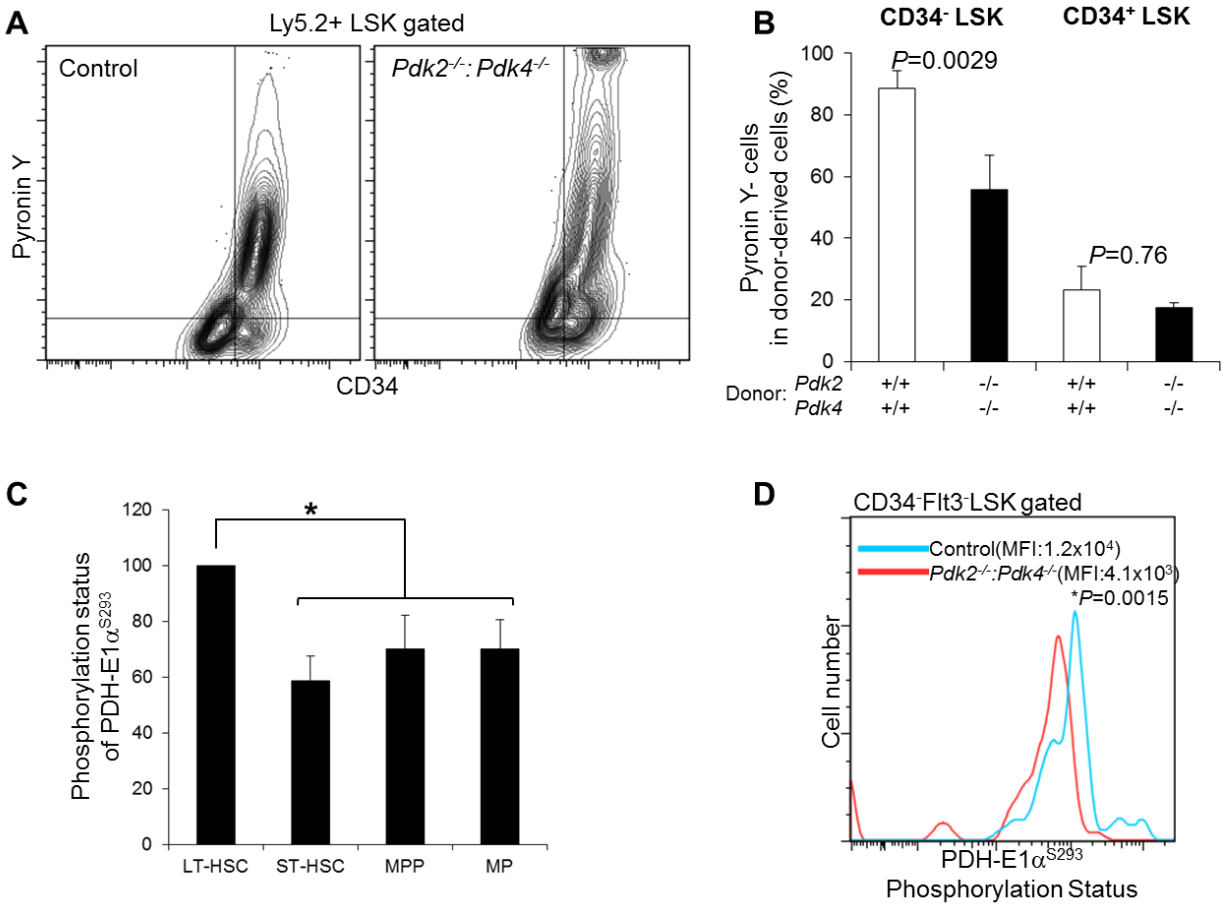

Figure S6, related to Figure 6

***Pdk2*$^{-/-}$*: Pdk4*$^{-/-}$ LT-HSCs lose quiescence in a normal microenvironment**

A, Representative flow cytometric plot of Pyronin Y analysis in the LSK gated fraction of control or *Pdk2*$^{-/-}$*: Pdk4*$^{-/-}$ BM mice.

B, Summary of flow cytometric Pyronin Y analysis of CD34^{-} LSK or CD34^{+} LSK fractions in control or *Pdk2*$^{-/-}$*: Pdk4*$^{-/-}$ BM mice (mean ± SD, n=3).

C, Detection of phosphorylated PDH-E1α^{S293} by intracellular flow cytometry in LT-HSC, ST-HSC, MPP, and MP fractions. Mean fluorescence intensity (MFI) of anti-phosphorylated PDH-E1α^{S293} antibody staining is normalized to MFI detected in the LT-HSC fraction (mean ± SD, n=3, **P*<0.001).

D, Representative histogram comparing phosphorylated PDH-E1α^{S293} levels using intracellular flow cytometry analysis of control versus *Pdk2*$^{-/-}$*: Pdk4*$^{-/-}$ LT-HSCs. Average MFIs are shown (n=3, **P*=0.0015).

Figure S7

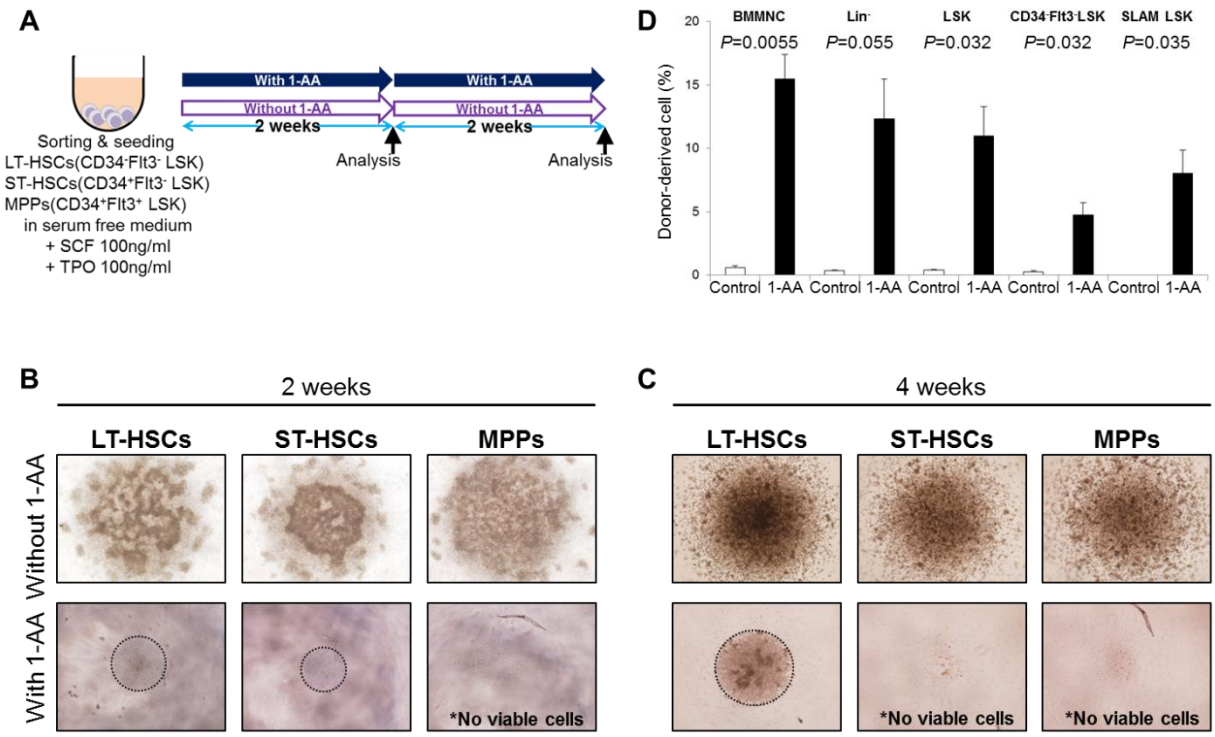

Figure S7, related to Figure 7

Effect of 1-AA on colony formation of LT-HSCs, ST-HSCs or MPPs

A, Design of LT-HSC, ST-HSC, or MPP cultures treated without or with 1-AA for 2 or 4 weeks.

B, Light microscopic analysis of colonies cultured 2 weeks from LT-HSC, ST-HSC, or MPP cultures treated with or without 1-AA.

C, Light microscopic analysis of colonies cultured 4 weeks from LT-HSC, ST-HSC, or MPP cultures treated with or without 1-AA.

D, Donor-derived (Ly5.1+) BM MNC, Lin-, LSK, CD34-Flt3- LSK or SLAM-LSK chimerism in primary BMT recipients of control LT-HSCs (open bars) or LT-HSCs treated with 1-AA for 4 weeks (closed bars) 4 months after BMT (mean ± SEM, n = 4-5).