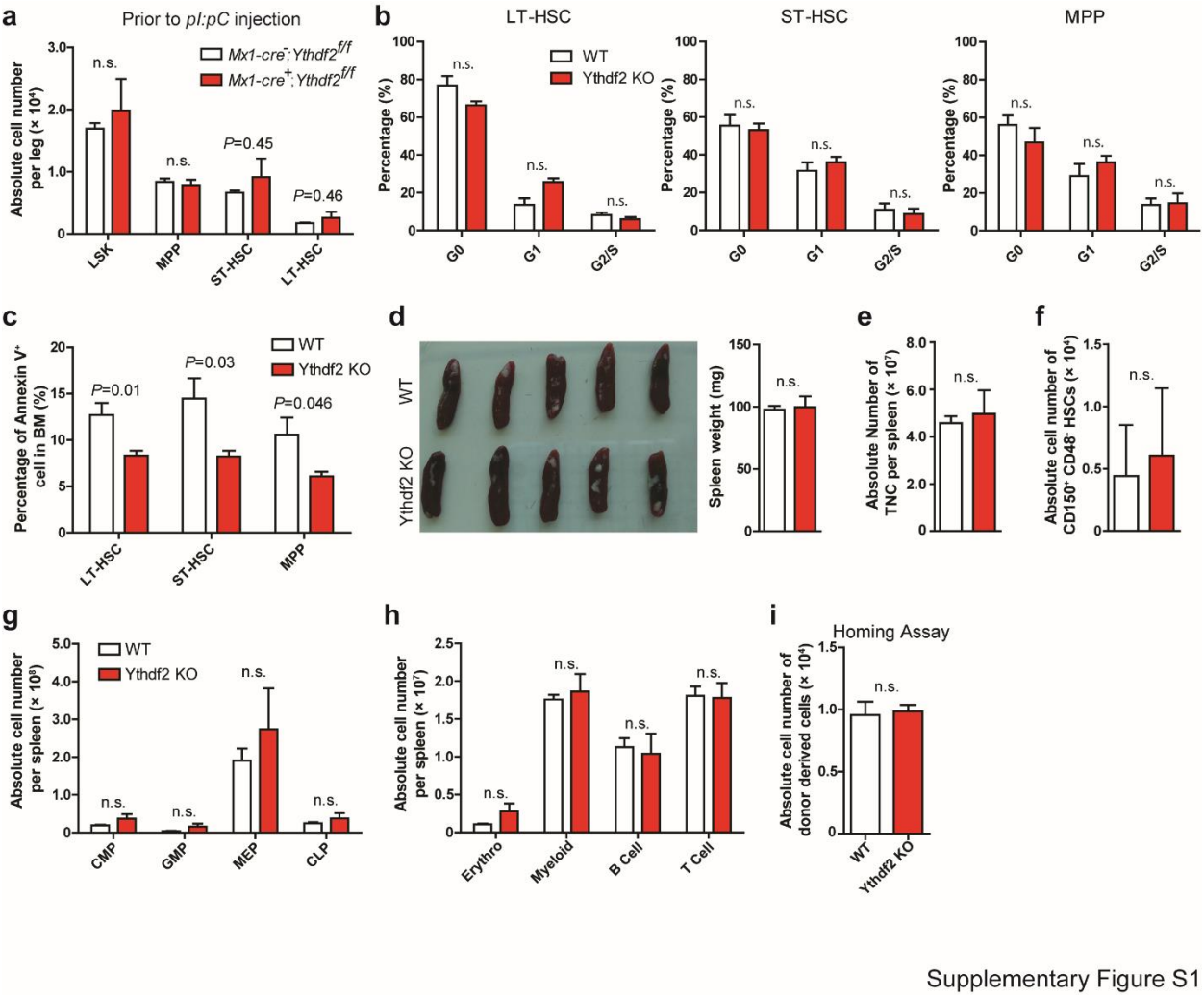


Figure S1 *Ythdf2* KO HSCs show no signs of lineage bias or differences in quiescence and homing ability but exhibit lower apoptotic rate.

(**a**) Absolute cell number of HSPCs in BM from *Mx1-cre*$^{-}$*;Ythdf2*$^{f/f}$ and *Mx1-cre*$^{+}$*;Ythdf2*$^{f/f}$ mice without pI:pC injection (n=3 per group). (**b**) Cell cycle analysis of HSPCs in *wt* (n = 3) and *Ythdf2* KO (n = 4) mice. (**c**) Apoptosis analysis of BM HSPCs in *wt* and *Ythdf2* KO mice (n = 5 for each group). (**d**) Images and the weight of spleens from *wt* and *Ythdf2* KO mice. (**e** to **h**) Absolute number of TNC (**e**), LSK CD48^{-} CD150^{+} HSCs (**f**), committed progenitors (**g**) and lineage cells (**h**) in the spleen of *wt* (n = 3) and *Ythdf2* KO (n = 4) mice. (**i**) Homing ability of *wt* and *Ythdf2* KO cells was determined by transplanting 1×10^{6} CFDA SE-labelled BM cells into lethally irradiated mice. 18 hours later, BM was analyzed for homed events (n = 6 mice per group). Data shown as mean ± s.e.m. Unpaired t-test. n.s., nonsignificant.

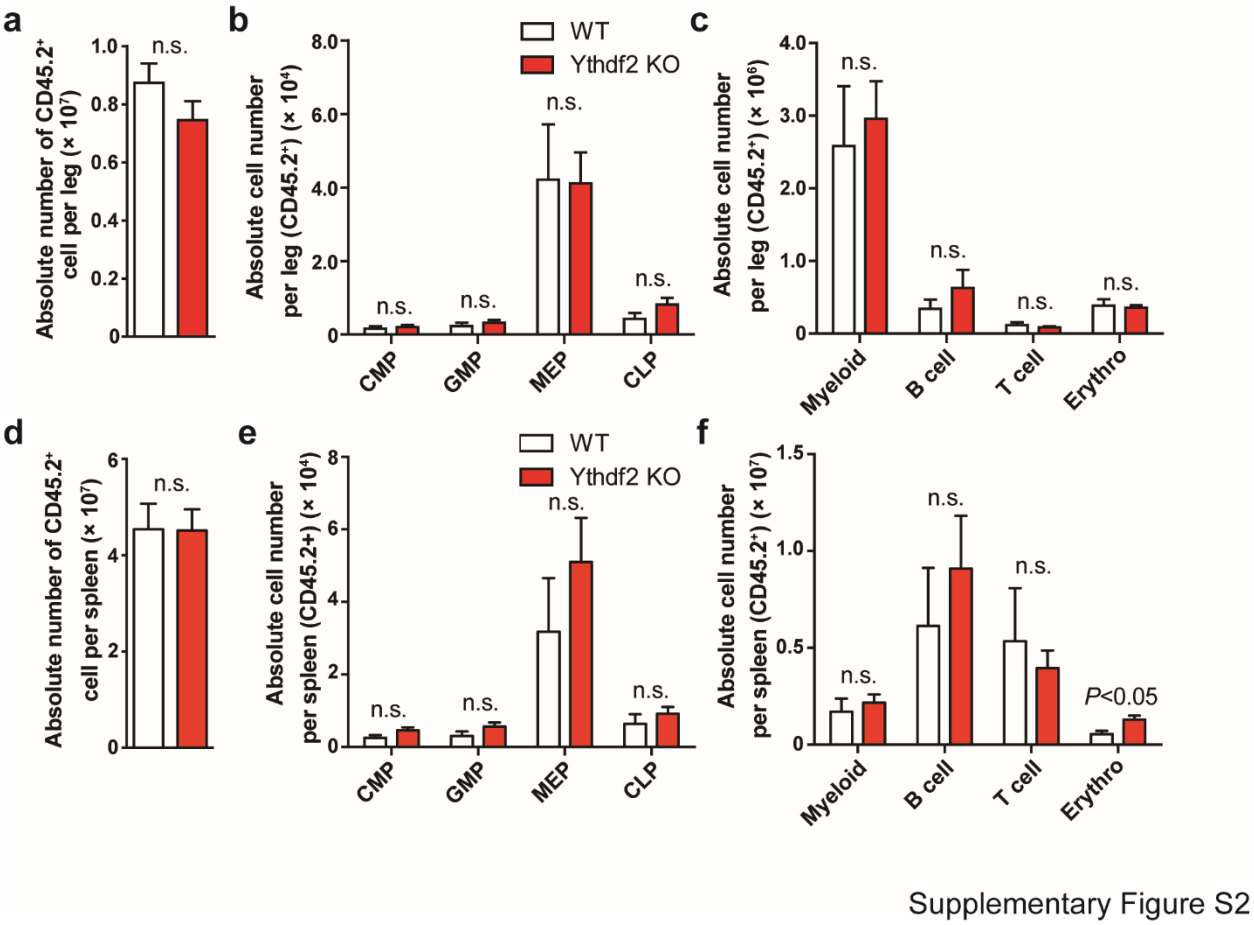
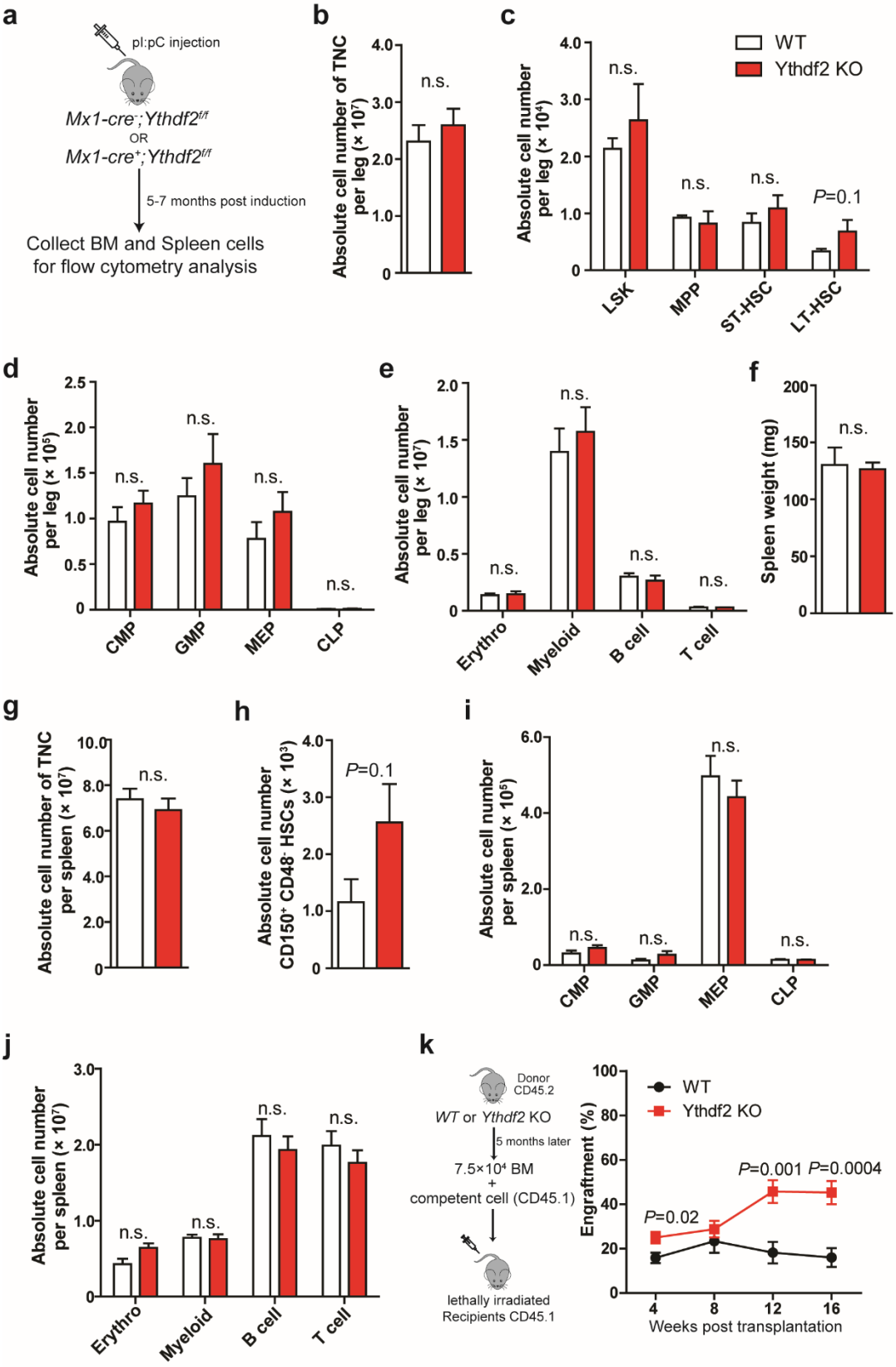


Figure S2 Transplantation recipient mice of *Ythdf2* KO BM display no lineage changes or defects 16 weeks post transplantation.

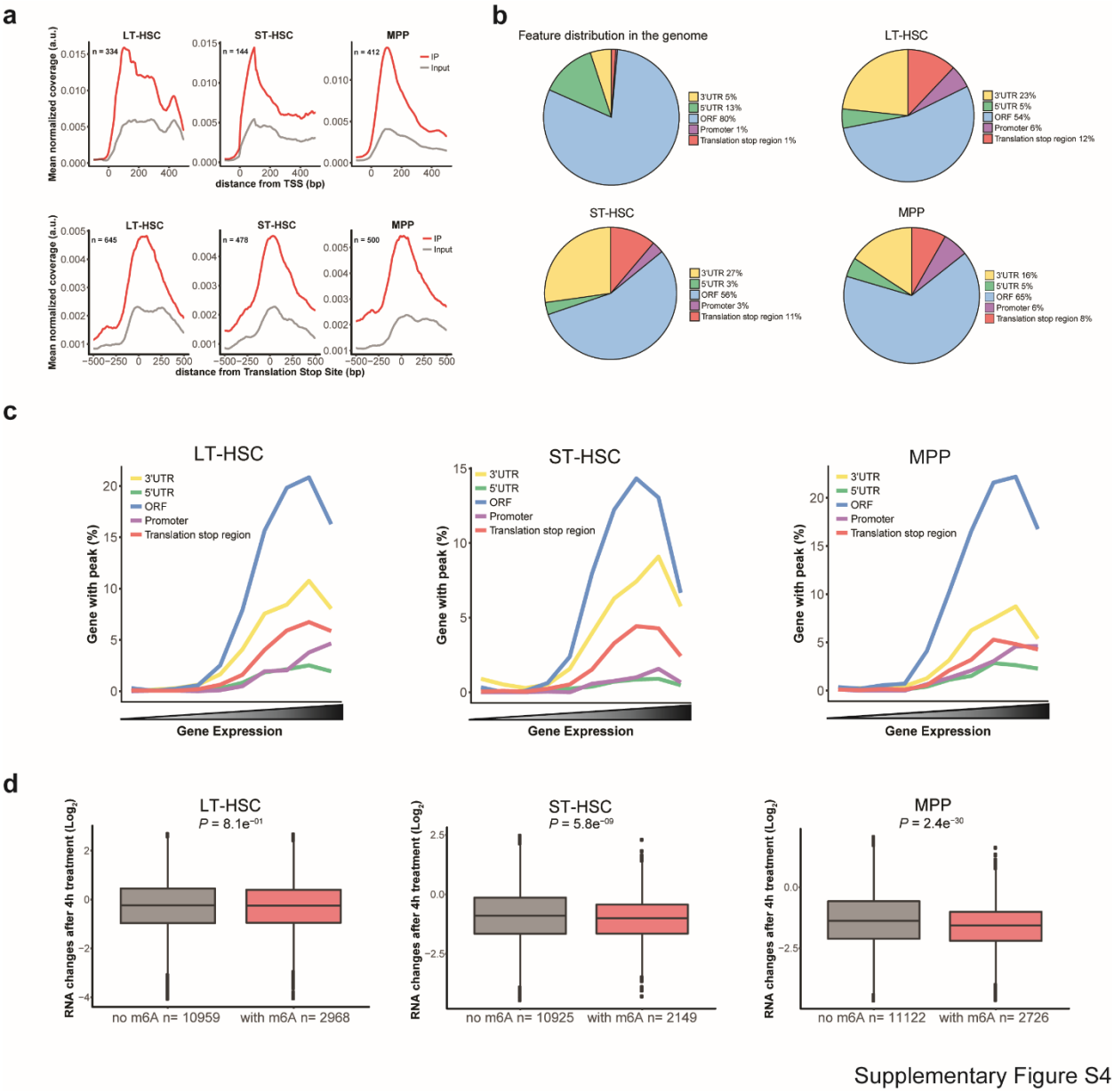
(**a** to **c**) Absolute cell number of donor derived (CD45.2$^+$) TNC (**a**), committed progenitors (**b**) and lineage cells (**c**) in the BM from secondary 200K transplantation recipient mice at 16 weeks after secondary transplantation (n= 7-10 for each group). (**d** to **f**) Absolute cell number of donor derived (CD45.2$^+$) TNC (**d**), committed progenitors (**e**) and lineage cells (**f**) in the spleen from secondary 200K transplantation recipient mice at 16 weeks after secondary transplantation (n= 7-10 for each group). Data shown as mean ± s.e.m. Unpaired t-test. n.s., nonsignificant.



Supplementary Figure S3

Figure S3 *Ythdf2* KO has long-term effect on mouse HSC expansion *in vivo* without inducing lineage bias.

(**a**) BM and spleen collected from *wt* and *Ythdf2* KO mice were analyzed by flow cytometry at 5-7 months post pI:pC inductions. (**b** to **e**) Absolute cell number of TNC (**b**), HSPCs (**c**), committed progenitors (**d**) and lineage cells (**e**) in the BM of *wt* and *Ythdf2* KO mice at 5-7 months post induction. (n = 4-7 mice per group). (**f**) The weight of spleens from *wt* and *Ythdf2* KO mice at 5-7 months post induction. (n = 4-7 mice per group). (**g** to **j**) Absolute cell number of TNC (**g**), LSK $CD48^{-}$ $CD150^{+}$ HSCs (**h**), committed progenitors (**i**) and lineage cells (**j**) in the spleen of *wt* and *Ythdf2* KO mice at 5-7 months post induction. (n = 4-7 mice per group). (**k**) 5 months post pI:pC injection, 75k WBM from *wt* and *Ythdf2* KO mice were transplanted with 200K rescue cells into lethally irradiated recipients. Peripheral blood from transplantation recipients were analyzed every 4 weeks post transplantation to determine the donor derived engraftment (n = 10 for each group). Data shown as mean ± s.e.m. Unpaired t-test. n.s., nonsignificant.



Supplementary Figure S4

Figure S4 Molecular characterization of m^{6}A modification in mouse HSPCs.
(**a**) Metagene profiles depicting sequence coverage in windows surrounding the TSS (up) and stop codon (down). Coverage of m^{6}A IP and control (input) fragments indicated in red and grey, respectively. (**b**) Pie chart presenting the fraction of m^{6}A peaks in each of five transcript segments. (**c**) Fraction of genes in mouse HPSCs with m^{6}A peaks in each of the segments as a function of expression level. (**d**) m^{6}A-tagged and non-m^{6}A-tagged mRNA degradation rates as determined by analysis of the expression level at 0 hour and 4 hours post actinomycin D treatment in HSPCs.

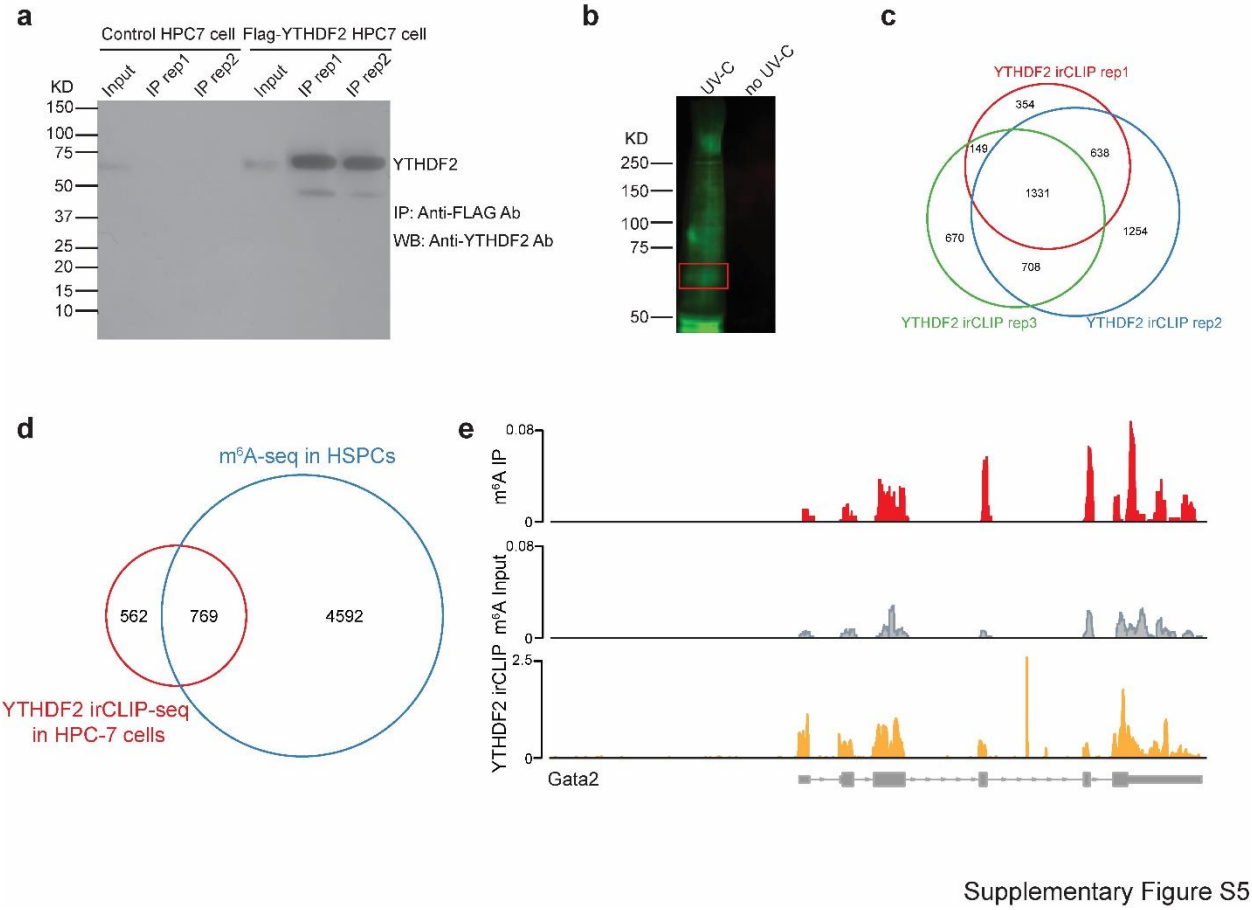
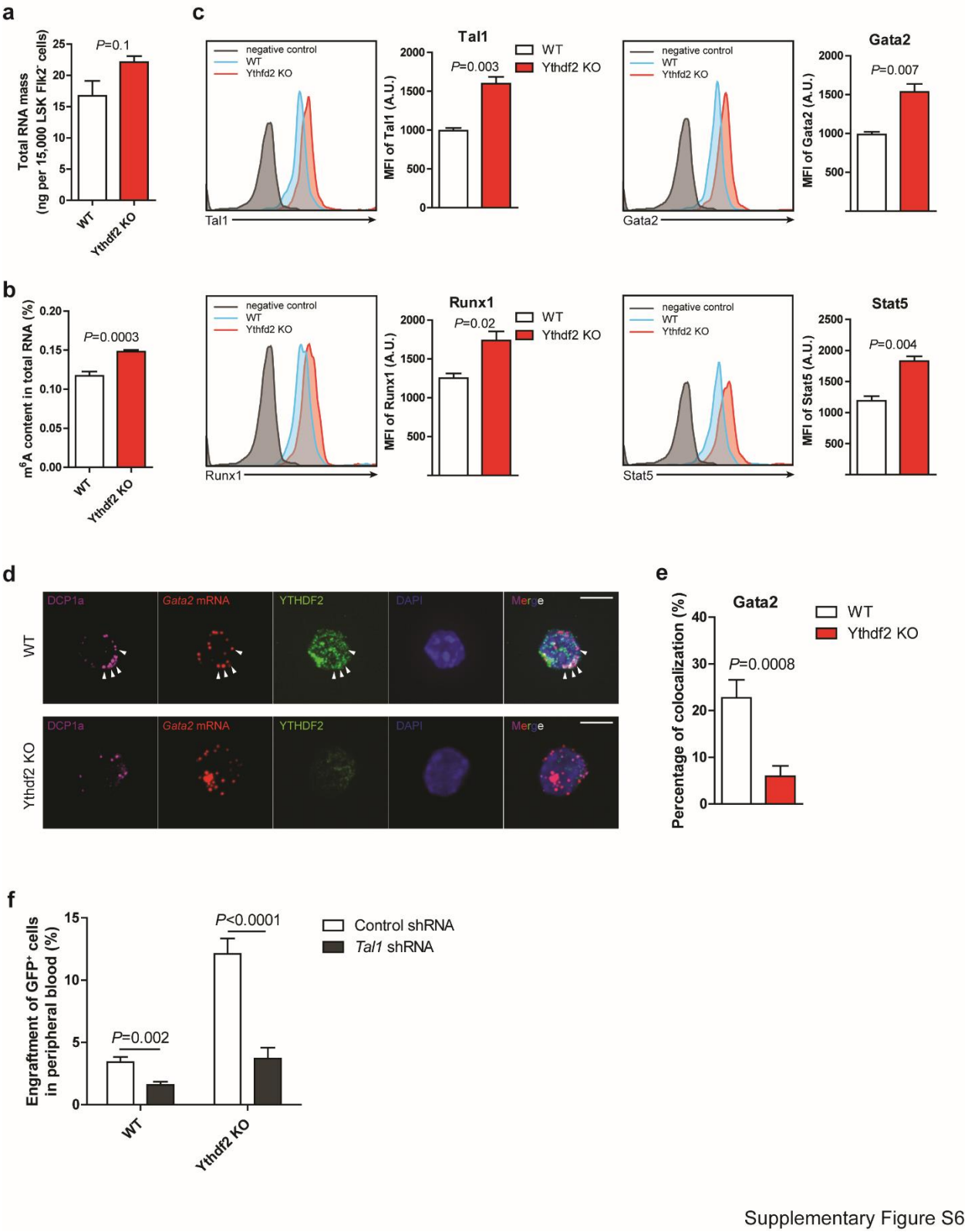


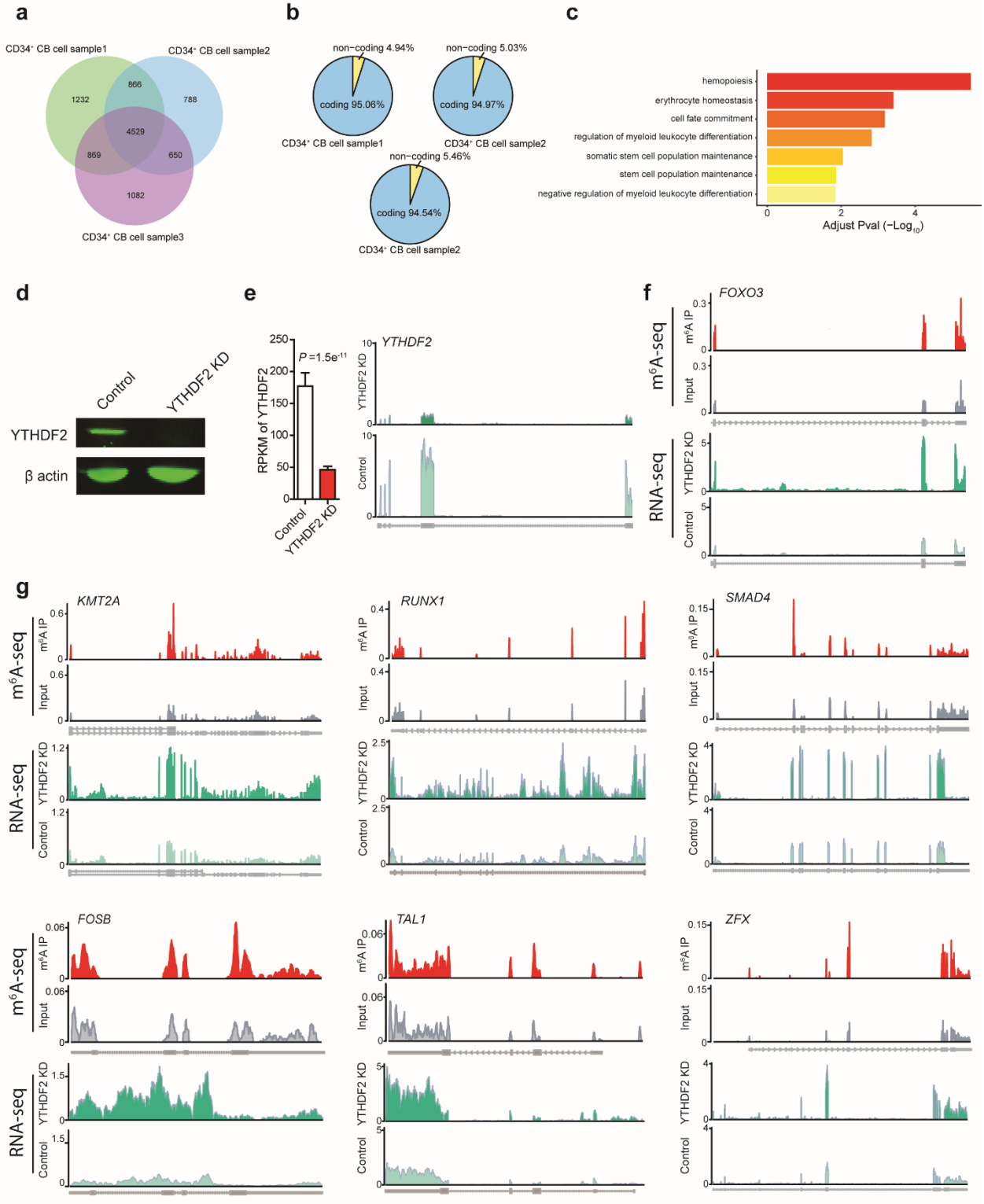
Figure S5 Define Ythdf2 functionality in mouse HSPCs by irCLIP-seq.
(**a**) Immunoprecipitation of Ythdf2 in control or Flag-Ythdf2 overexpressed HPC7 cells. (**b**) irCLIP membrane image showing IR800 labeled RNA-Ythdf2 complex. Red box indicates the RNA-Ythdf2 complex collected for library construction. Samples without UV crosslinking serve as controls. (**c**) Venn diagram showing intersection genes identified in three independent Ythdf2 irCLIP-seq experiments. (**d**) Venn diagram showing overlap of Ythdf2 binding targets and m^6A labeled mRNAs. (**e**) Representative tracks of *Gata2* harboring m^6A peaks and Ythdf2 irCLIP peaks. Coverage of m^6A immunoprecipitation and input fragments indicated in red and grey, respectively. Ythdf2 irCLIP reads highlighted in yellow.



Supplementary Figure S6

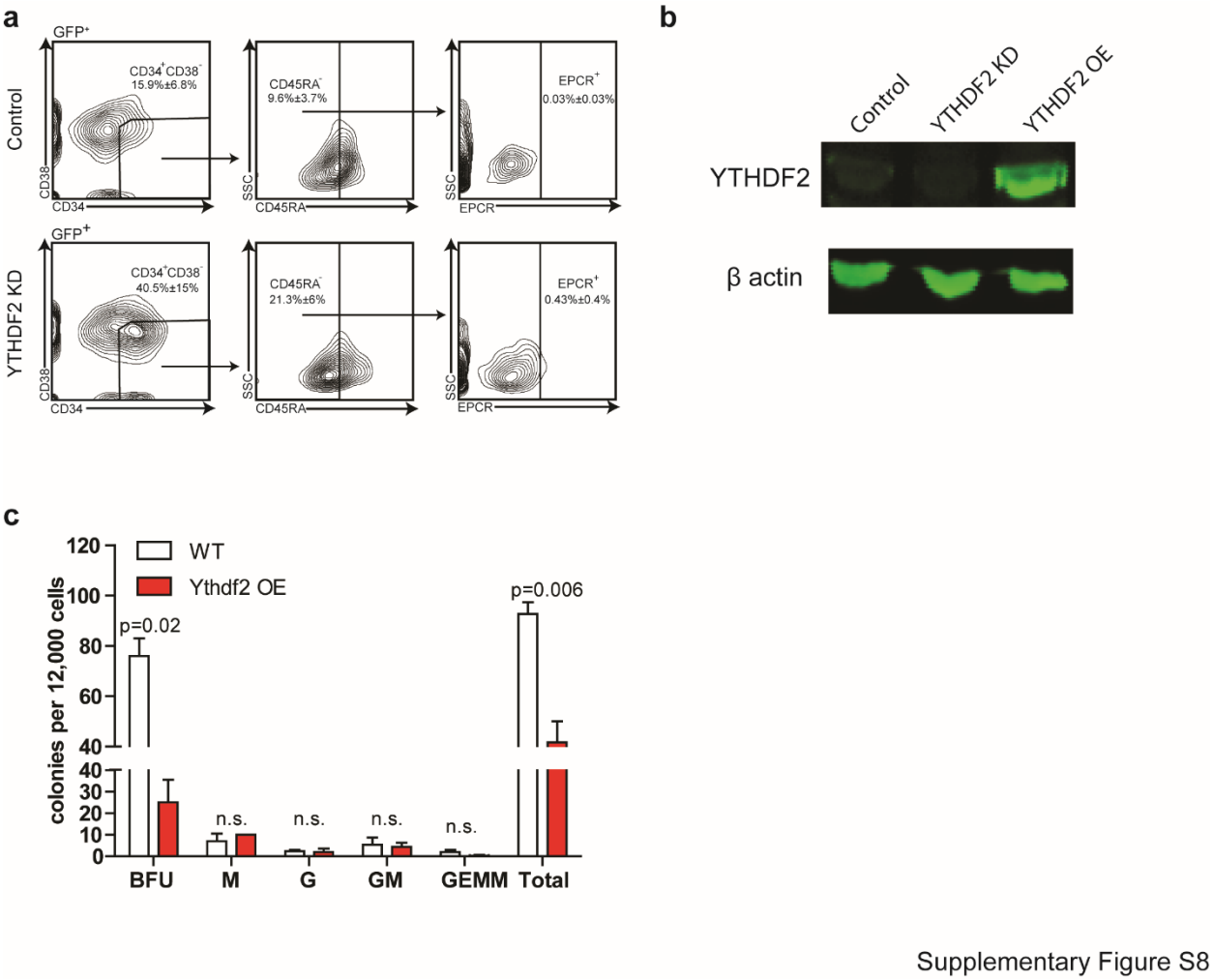
Figure S6 *Ythdf2* KO increases m^{6}A-tagged mRNA expression, contributing to HSC expansion.

(**a**) Total RNA was extracted from 15,000 sorted BM LSK Flk2$^-$ cells. (**b**) Quantification of m^{6}A RNA methylation in *wt* and *Ythdf2* KO Lin$^-$ cells (n = 6). (**c**) Quantification (right) and histogram (left) showing intracellular flow validation of increased expression of TAL1, GATA2, RUNX1 and STAT5 in *Ythdf2* KO LT-HSCs comparing to *wt* LT-HSCs (n = 3 mice per group). (**d**) Fluorescence in situ hybridization of *Gata2* mRNA (red) and fluorescence immunostaining of Dcp1a (P-body marker) (magenta), Ythdf2 (green) in *wt* and *Ythdf2* KO HSPCs. Arrows indicate co-localized staining. Scale bars, 5 μm. (**e**) Quantification of *Gata2* mRNA and DCP1a co-localization in sorted LSK cells from *wt* and *Ythdf2* KO mice. Percentage indicates the average frequency of the *Gata2* mRNA that co-localized with DCP1a over total *Gata2* mRNA level in each LSK cells (n = 12-20). (**f**) Percentage of GFP$^+$ cells in the CD45$^+$ population at 4 weeks post transplantation (n = 10). Data shown as mean ± s.e.m. Unpaired t-test. n.s., nonsignificant.



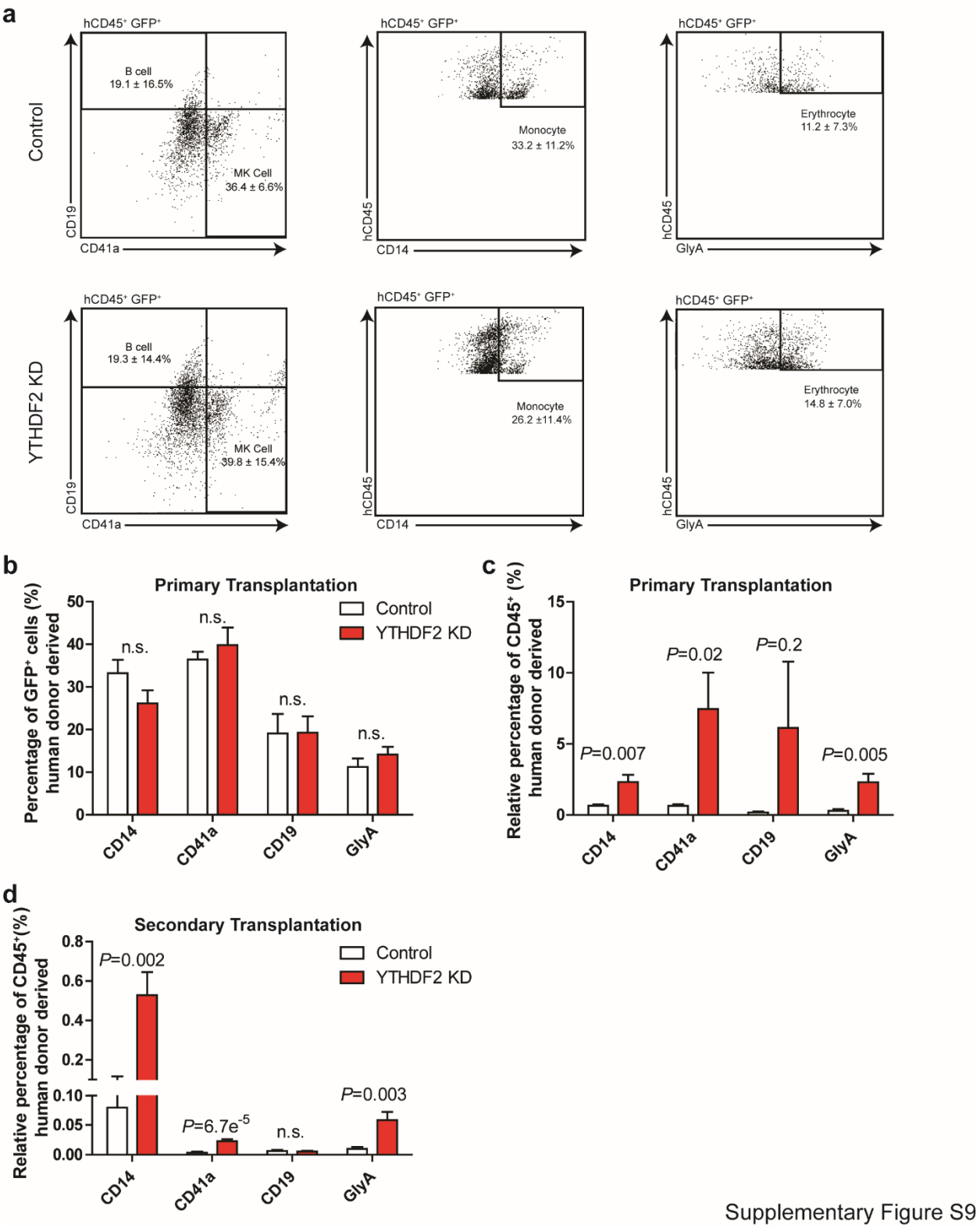
Supplementary Figure S7

Figure S7 YTHDF2 regulates expression of transcription factors related to stem cell self-renewal in human cord blood stem cells.
(**a**) Venn diagram showing intersection genes identified in three independent m^6A-seq experiments, using three independent cord blood samples. (**b**) Percentage of mRNAs and non-coding RNAs containing m^6A peaks. (**c**) GO enrichment analysis of the transcription factors harboring m^6A modifications in hUCB $CD34^+$ cells. (**d**) Western blotting of YTHDF2 (up) and β-Actin (down) in sorted GFP^+ control and *YTHDF2* KD hUCB cells, showing knockdown efficiency of YTHDF2. (**e**) Expression level (left) and representative track plot (right) of YTHDF2 from RNA-seq analysis of control and *YTHDF2* KD hUCB $CD34^+$ cells, showing knockdown efficiency of YTHDF2. (**f** and **g**) Representative track plots of indicated transcription factors harboring m^6A peaks (up) and their representative coverage plots from the RNA-seq analysis (bottom). Adjusted P values are indicated.



Supplementary Figure S8

Figure S8 YTHDF2 regulates hUCB HSC maintenance *ex vivo*.
(**a**) Representative flow plots of GFP$^+$ CD34$^+$ CD38$^-$ CD45RA$^-$ EPCR$^+$ HSCs in control and *YTHDF2* KD hUCB cells post 7 days culture. (**b**) Confirmation of YTHDF2 protein knockdown and overexpression in transduced Hela cells. (**c**) CFU production by YTHDF2 overexpression (OE) and control transduced CD34$^+$ CD38$^-$ CB from day 10 cultures (n = 3 independent human samples). Data shown as mean ± s.e.m. Unpaired t-test. n.s., nonsignificant.



Supplementary Figure S9

Figure S9 YTHDF2 KD in hUCB cells results in HSC expansion without changing lineage output.

(**a**) Representative flow plots of $hCD45^+$ GFP^+ monocyte, megakaryocyte (MK cell), B cell and erythrocyte in primary NSG recipient BM. (**b** and **c**) Percentage of lineage cells in $hCD45^+$ GFP^+ (**b**) and in total $CD45^+$ (**c**) BM cells from primary NSG recipients at 10 weeks post transplantation (n = 13-15). (**d**) Summary of human donor derived lineage chimerisms in total $CD45^+$ BM cells from secondary NSG recipients at 12 weeks post transplantation (n = 6). Data shown as mean ± s.e.m. Unpaired t-test. n.s., nonsignificant.

Table S1. Key transcription factors critical for HSC self-renewal and maintenance are labeled by m^{6}A in HSPCs.

Table S2. YTHDF2 targeted mRNAs from three irCLIP-seq replicates.

Table S3. Genes marked by m^{6}A in human UCB CD34$^+$ HSPCs from individual samples.

Table S4. Percentages of human donor derived chimerism used to calculate CRU.

Table S5. qPCR primers used to verify the expressional levels of transcription factors in *wt* and *Ythdf2* KO HSPCs.