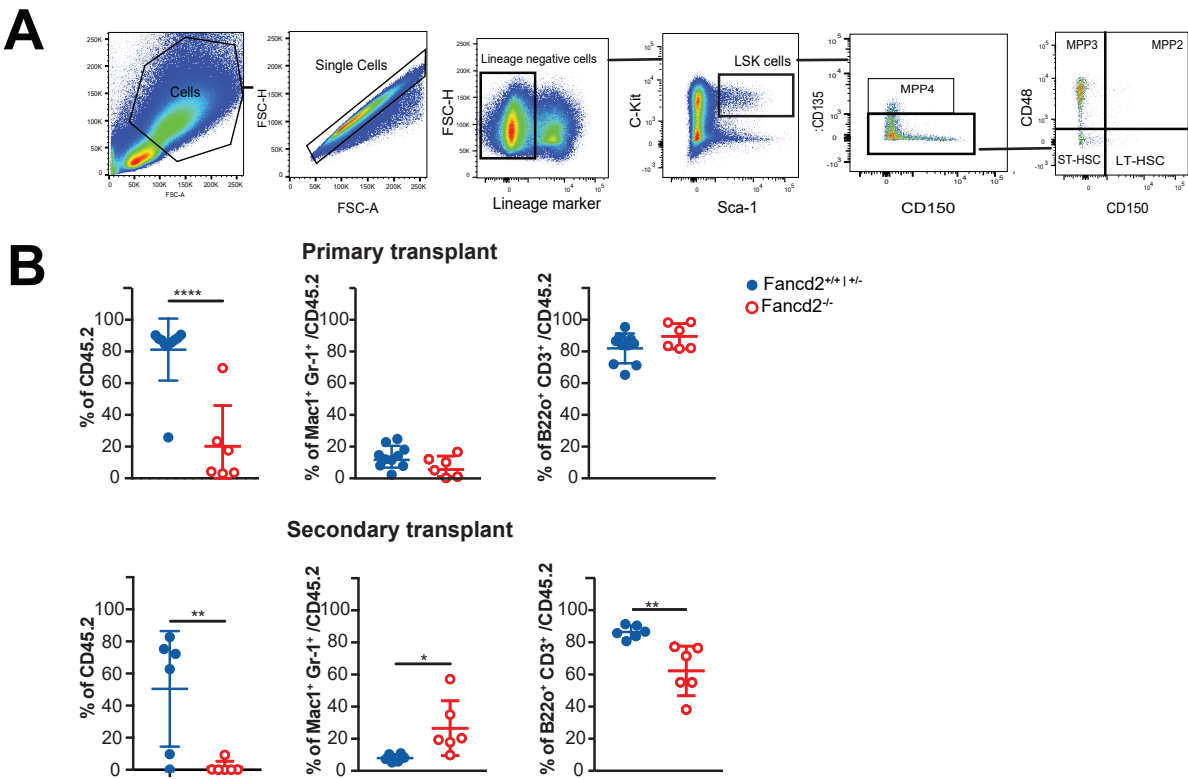
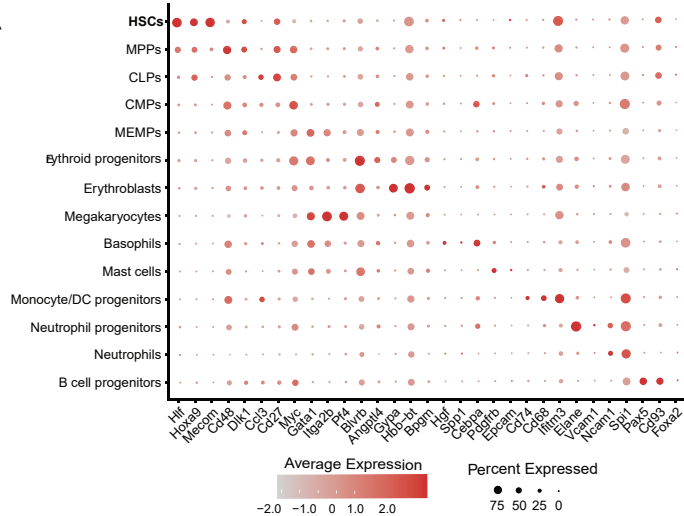


# Supplementary Figure 1

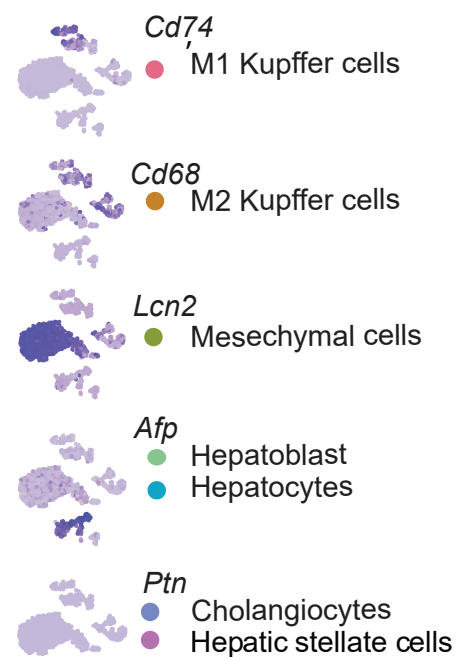


# Supplementary Figure 2

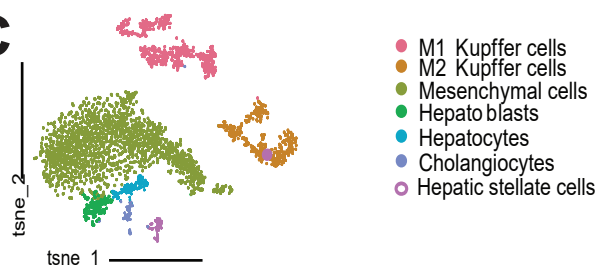
**A**



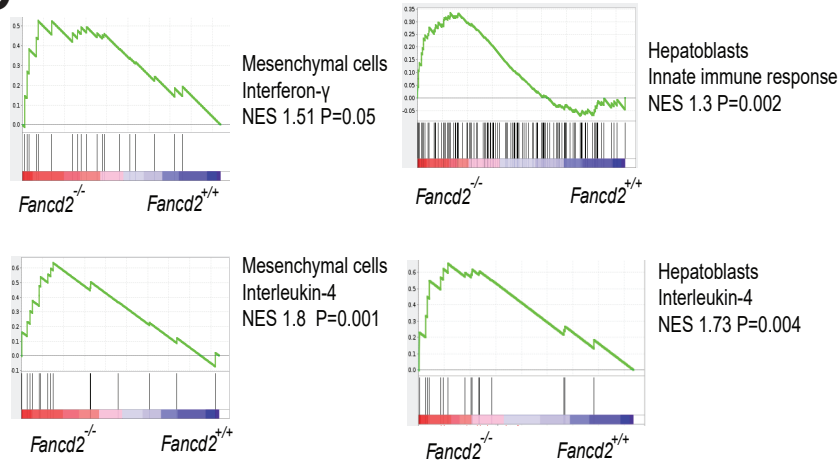
**B**



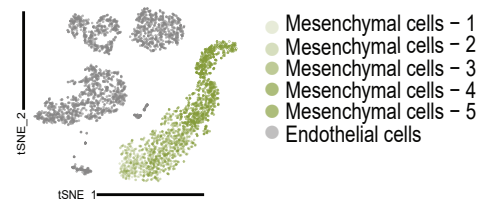
**C**



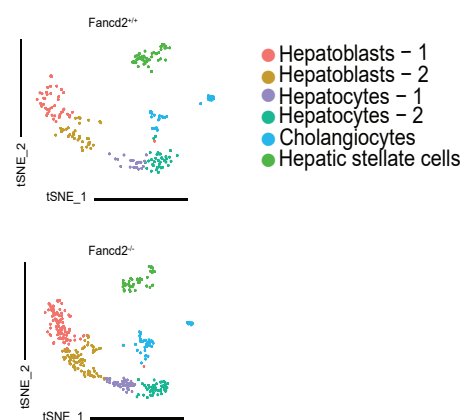
**D**



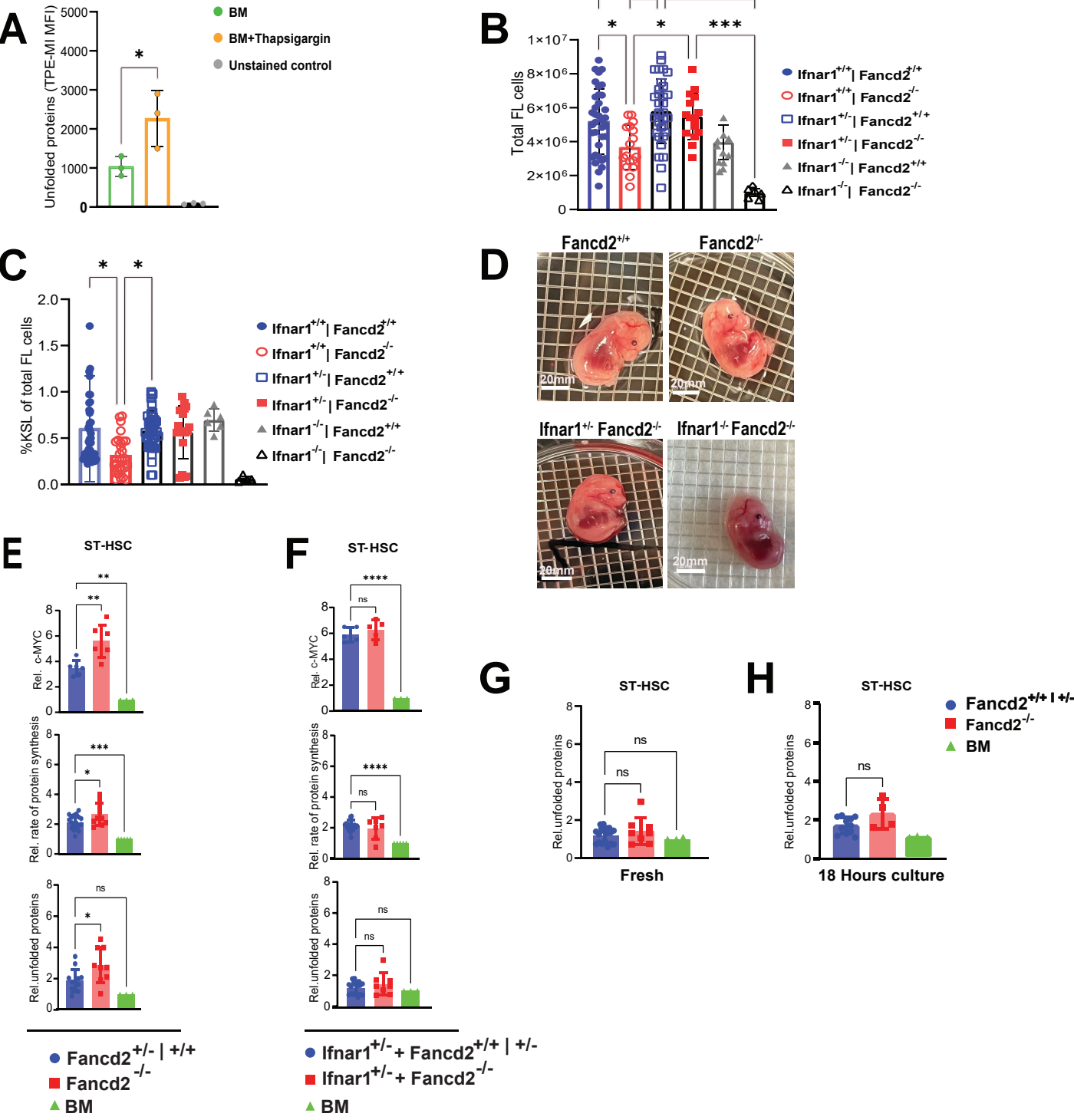
**E**



**F**

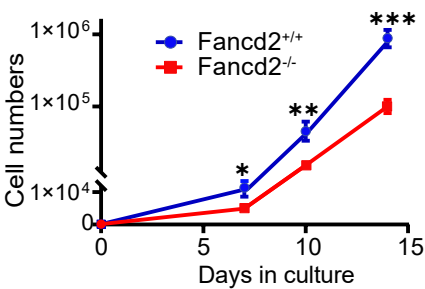


# Supplementary Figure 3

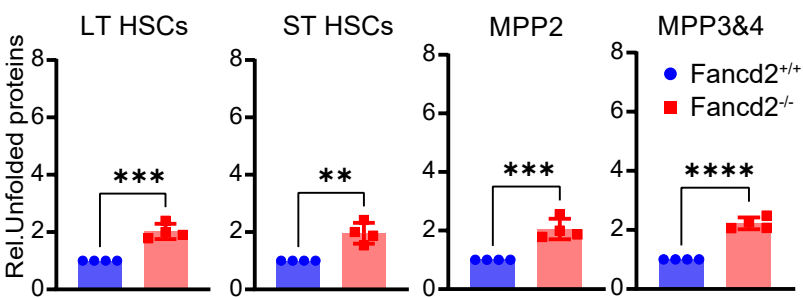


# Supplementary Figure 4

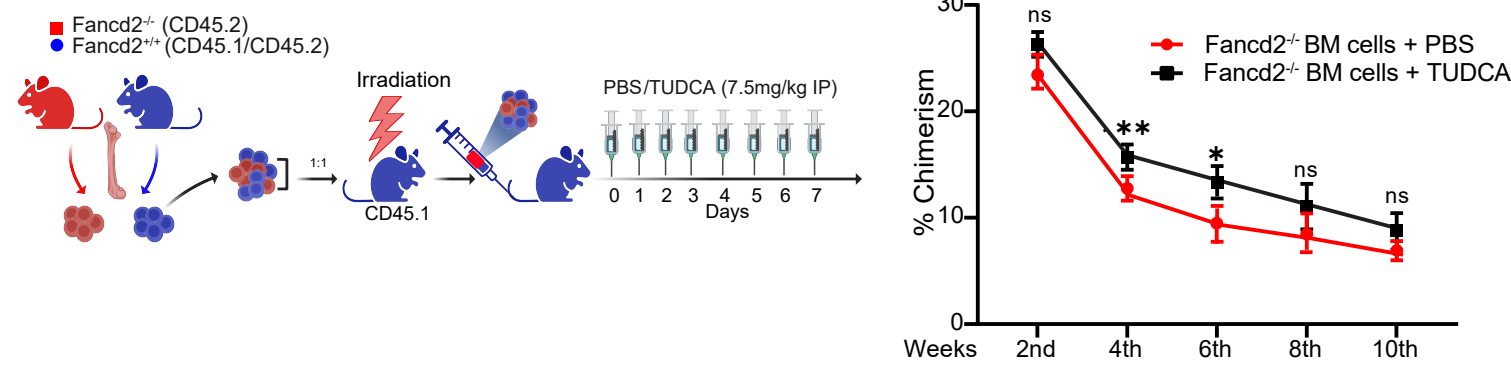
**A**



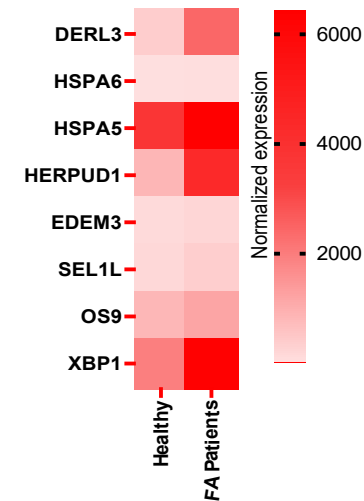
**B**



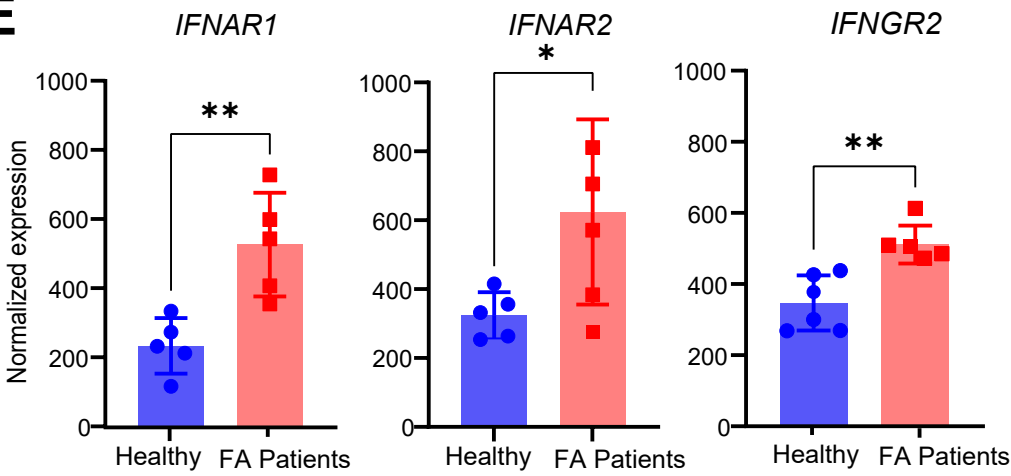
**C**



**D**



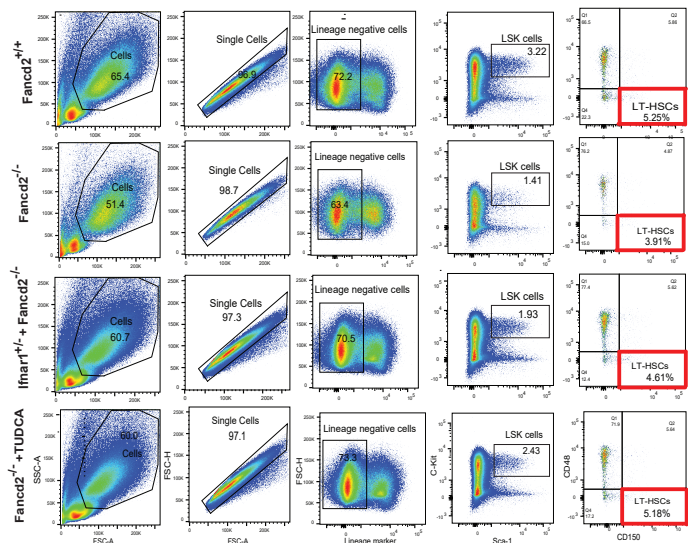
**E**



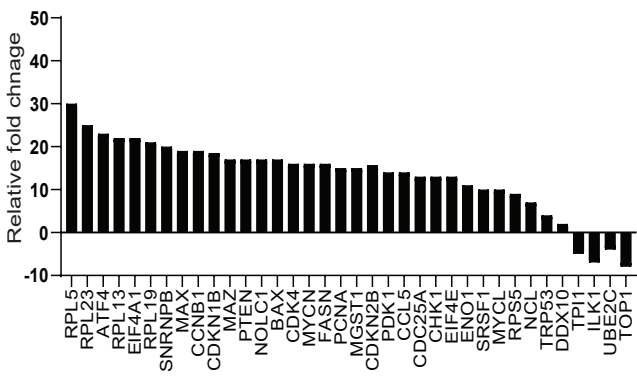


Supplementary Figure 5

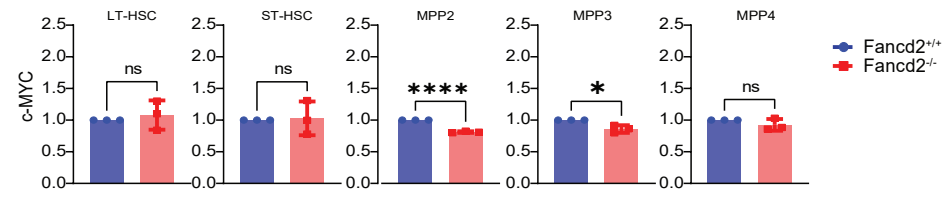
A



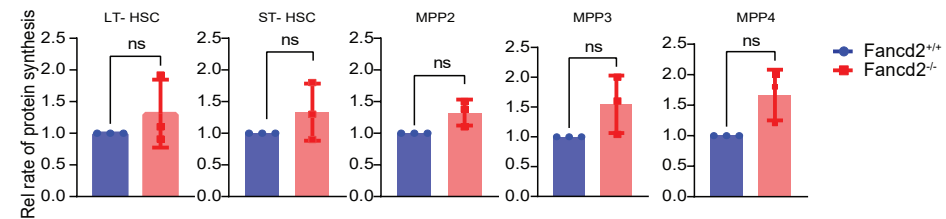
B



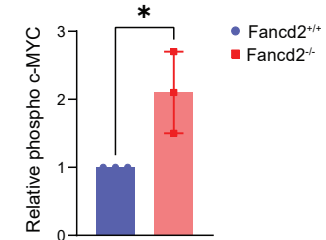
C



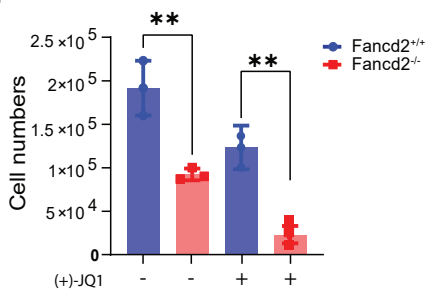
D



E



F



## Kovuru et al., Supplementary Figure Legends

**Supplementary Fig. S1: Flow cytometry gating strategy for HSPCs and transplantation analysis of E12.5 Fetal liver cells. (A)** Representative flow-cytometry gating strategy for HSPC subpopulation analysis with surface staining markers. **(B)** Peripheral blood chimerism after serial transplantation using  $5 \times 10^5$  E12.5 FL  $\text{Fancd2}^{+/+}$  (n=10 mice) and  $\text{Fancd2}^{-/-}$  (n=6 mice). Chimerism of total CD45 (left panel), -myeloid  $\text{Mac1}^{+}\text{Gr1}^{+}$  (mid panel), and -lymphoid  $\text{CD220}^{+}\text{CD3}$  (right panel) populations 9 weeks after primary transplantation (upper panels). Chimerism 9 weeks after secondary transplantation from  $\text{Fancd2}^{+/+}$  (n=6 mice) or  $\text{Fancd2}^{-/-}$  (n=6 mice) donors (lower panels). Welch's t-test for statistical analysis, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ . Source data are provided as a Source Data file.

**Supplementary Fig. S2: Fetal liver niche cell composition and lineage trajectory analysis.**

**(A)** Dot plot showing the expression pattern of marker genes used for identification of hematopoietic subpopulations. **(B)** t-SNE plots illustrating the fetal liver niche cell gene expression pattern of specific marker genes. **(C)** t-SNE plot showing the global fetal liver niche for Kupffer (M1 and M2) cells, mesenchymal cells, hepatoblasts, hepatocytes, cholangiocytes, and hepatic stellate cells. **(D)** GSEA analysis shows  $\text{Fancd2}^{-/-}$  mesenchymal cell enrichment for "Response to Interferon- $\gamma$ " and "Interleukin-4", and  $\text{Fancd2}^{-/-}$  hepatoblast enrichment for "Innate immune response" and "Response to interleukin-4". **(E)** Overlay of  $\text{Fancd2}^{+/+}$  and  $\text{Fancd2}^{-/-}$  mesenchymal and endothelial cell trajectory analyses. **(F)** Differentiation trajectory analysis of  $\text{Fancd2}^{+/+}$  and  $\text{Fancd2}^{-/-}$  hepatoblast. Source data have been deposited in Gene Expression Omnibus (GEO) with accession number: GSE173908. Source data are provided as a Source Data file.

**Supplementary Fig. S3: Validation of TPE-MI staining in BM cells and analysis of cell count**

**in  $\text{Ifnar1}^{-/-}$  -  $\text{Fancd2}^{-/-}$  crosses. (A)** TPE-MI fluorescence (unfolded protein levels) increases in BM lineage-negative cells after Thapsigargin-induced endoplasmic reticulum stress conditions compared to untreated cells (n=3, t-test for statistical analysis). **(B)** Combined removal of functional  $\text{Ifnar1}$  and  $\text{Fancd2}$  ( $\text{Ifnar1}^{-/-}$   $\text{Fancd2}^{-/-}$ ) alleles severely affect total FL cell counts.  $\text{Ifnar1}^{+/+}$   $\text{Fancd2}^{+/+}$  (embryo numbers: n=36/8),  $\text{Ifnar1}^{+/+}$   $\text{Fancd2}^{-/-}$  (n=17/8),  $\text{Ifnar1}^{+/-}$   $\text{Fancd2}^{+/+}$  (n=33/),  $\text{Ifnar1}^{+/-}$   $\text{Fancd2}^{-/-}$  (n=15/10),  $\text{Ifnar1}^{-/-}$   $\text{Fancd2}^{+/+}$  (n=10/3) and  $\text{Ifnar1}^{-/-}$   $\text{Fancd2}^{-/-}$  (n=6/3). **(C)** KSL Frequency in FL of

$lfnar1$  and  $Fancd2$  genetic combinations showing. **(D)** Representative images of E14.5  $Fancd2^{+/+}$ ,  $Fancd2^{-/-}$ ,  $lfnar1^{+/+} Fancd2^{-/-}$ , and  $lfnar1^{-/-} Fancd2^{-/-}$  embryos. **(E)**  $Fancd2^{+/+} l^{+/+}$  versus  $Fancd2^{-/-}$  ST-HSCs comparison of: total c-MYC protein analysis (upper panel) between  $Fancd2^{+/+}$  (n=5/3) and  $Fancd2^{-/-}$  (n=6/3), the relative rate of protein synthesis (middle panel) between  $Fancd2^{+/+} l^{+/+}$  (n=5/3) and  $Fancd2^{-/-}$  (n=6/3), and unfolded proteins (bottom panel) between the  $Fancd2^{+/+} l^{+/+}$  (n=18/5) and  $Fancd2^{-/-}$  (n=10/5). **(F)**  $lfnar1^{+/+} Fancd2^{+/+} l^{+/+}$  versus  $lfnar1^{+/+} Fancd2^{-/-}$  ST-HSCs comparison of total c-MYC (upper panel) between  $lfnar1^{+/+} Fancd2^{+/+}$  (n=5/3) and  $lfnar1^{+/+} Fancd2^{-/-}$  (n=5/3), the relative rate of protein synthesis (middle panel) between  $lfnar1^{+/+} Fancd2^{+/+} l^{+/+}$  (n=17/5) and  $lfnar1^{+/+} Fancd2^{-/-}$  (n=7/5), with unfolded proteins in the (bottom panel) comparison between the  $lfnar1^{+/+} Fancd2^{+/+} l^{+/+}$  (n=17/3) vs  $lfnar1^{+/+} Fancd2^{-/-}$  (n=8/3) ST-HSCs. **(G)** Analysis of unfolded proteins from  $Fancd2^{+/+} l^{+/+}$  (n=19/4), and  $Fancd2^{-/-}$  (n=6/4) fetal liver ST-HSCs immediately following recovery from TUDCA-injected pregnant  $Fancd2^{+/+}$  mice. **(H)** ST-HSCs unfolded proteins analysis as in G, but fetal liver samples from TUDCA-injected  $Fancd2^{+/+}$  mice were placed in 18 hours of ex vivo culture in the absence of TUDCA before analysis. One-way ANOVA was used for statistical analysis unless mentioned specifically. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001 and ns: non-significant. Source data are provided as a Source Data file.

**Supplementary Fig. S4: Bile acid (TUDCA) supplementation improves  $Fancd2^{-/-}$  cell peripheral blood chimerism, and gene expression of endoplasmic reticulum-associated degradation (ERAD) genes, unfolded protein response, and interferon receptor genes in publicly available human FA HSPCs dataset.** **(A)** Cell proliferation kinetics comparing  $Fancd2^{+/+}$  and  $Fancd2^{-/-}$  BM LT-HSCs during ex vivo expansion. **(B)** Unfolded protein analysis in ex vivo expanded adult BM  $Fancd2^{+/+}$  (n=3 mice),  $Fancd2^{-/-}$  (n=3 mice) HSPCs subsets. t-test was used for statistical analysis. **(C)** Transient gains in peripheral blood chimerism of  $Fancd2^{-/-}$  cells in recipient mice after competitive transplantation of whole bone marrow  $Fancd2^{+/+}$ ,  $Fancd2^{-/-}$  cells followed by injection of PBS (n=6 mice) or TUDCA (n=6 mice, t-test for statistical analysis). Schematic Figure was created with [BioRender.com](https://BioRender.com) released under a [Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International license](https://creativecommons.org/licenses/by-nc-nd/4.0/). **(D)** Heatmap showing the expression profile of endoplasmic reticulum-associated degradation (ERAD) and unfolded protein response genes in human FA patients HSPCs vs healthy group, data was extracted from GEO data set (GSE157591). **(E)** Increased expression of Interferon- $\alpha$  receptor-1 (IFNAR1), Interferon- $\alpha$  receptor-2 (IFNAR2), and Interferon- $\gamma$  receptor-2 (IFNGR2) in human FA patients HSPCs compared to healthy HSPCs (GSE157591). Data are represented as mean  $\pm$ SD. P-values of 0.01 - 0.05 were considered significant (\*), 0.001 - 0.01 (\*\*) p>0.05 were considered non-significant (ns), t-test for statistical analysis. Source data are provided as a Source Data file.

**Supplementary Fig. S5: Analysis of MYC protein in adult BM cells and MYC target gene expression in fetal liver Fancd2<sup>-/-</sup> HSPCs. (A)** Flow cytometry plots for identification of LT-HSCs from Fancd2<sup>+/+</sup>, Fancd2<sup>-/-</sup>, Ifnar1<sup>+/+</sup> Fancd2<sup>-/-</sup> fetal livers and Fancd2<sup>-/-</sup> fetal livers harvested from TUDCA-injected pregnant mice. **(B)** MYC target profiling in Fancd2<sup>+/+</sup> (n=12/7), Fancd2<sup>-/-</sup> (n=11/7) fetal liver KSL cells using “RT2 MYC profiler” PCR array. Results were normalized to Fancd2<sup>+/+</sup> and plotted as relative mean fold change for each target. **(C)** Relative levels of total MYC protein in age-matched adult BM LT-HSCs and HSPCs between Fancd2<sup>+/+</sup> (n=3 mice) and Fancd2<sup>+/+</sup> (n=3 mice) conditions. **(D)** Rate of protein synthesis by in vivo OPP assay in age-matched Fancd2<sup>+/+</sup> (n=3 mice) and Fancd2<sup>-/-</sup> (n=3 mice) adult BM HSPC subsets, t-test for statistical analysis in Figure panels C and D. \*P<0.01, \*\*P<0.001, \*\*\*P<0.001, \*\*\*\*P<0.0001. **(E)** Phosphorylated c-MYC flow cytometry analysis in day 10 ex vivo expanded (under stress conditions) adult BM HSPCs, Fancd2<sup>+/+</sup> (n=3 mice) and Fancd2<sup>-/-</sup> (n=3 mice), t-test for statistical analysis. **(F)** Analysis of total cell numbers in day 10 ex vivo expanded BM LT-HSCs treated with 250uM (+)-JQ1 on day 7 of culture; Fancd2<sup>+/+</sup> (n=3 mice) and Fancd2<sup>-/-</sup> (n=3 mice), (+)-JQ1 treated Fancd2<sup>+/+</sup> (n=3 mice) and (+)-JQ1 treated Fancd2<sup>-/-</sup> (n=3 mice) t-test was used for statistical analysis, \*\*P<0.001. Source data are provided as a Source Data file.

## Supplementary Table 1

Antibody details with manufacturer's and catalog number details.

Antibody	Manufacturer Catalog No.	Dilution	Usage
B220 APC	Biolegend 103211	1:250	Lineage (Lin)
B220 PE	BD 12-0452-82	1:250	Lineage (Lin)
B220/CD45R FITC	Biolegend 103205	1:100	Lineage (Lin)
CD117 APC	BD 17-1171-82	1:100	c-kit
CD117 BUV395	BD Biosciences 564011	1:100	c-Kit
CD117 BV785	Biolegend 105841	1: 100	c-kit
CD117 PE	Biolegend 105808	1:100	c-kit
CD11b FITC	BD Biosciences 561691	1:100	Lineage (Lin)
CD135 BV421	Biolegend 135313	1:100	
CD150 BV711	Biolegend 115941	1:100	SLAM
CD150 PECy7	Biolegend 115914	1:100	SLAM
CD3 APC	Biolegend 100235	1:250	Lineage (Lin)
CD3 PE	BD 12-0031-82	1:250	Lineage (Lin)
CD3e FITC	Biolegend 100305	1:100	Lineage (Lin)
CD4 APC	Biolegend 100412	1:250	Lineage (lin)
CD4 FITC	Biolegend 100405	1:100	Lineage (Lin)
CD4 PE	BD Biosciences 12-0041-82	1:250	Lineage (Lin)
CD48 AF700	Biolegend 103425	1:100	
CD48 PerCp-Cy5.5	Biolegend 103422	1:100	
CD5 APC	Biolegend 100626	1:250	Lineage (Lin)
CD5 FITC	Biolegend 100605	1:100	Lineage (Lin)
CD5 PE	BD Biosciences 12-0051-82	1:250	Lineage (Lin)
c-MYC	Cell Signaling Technology 5605S	1:1000	
Gr-1 APC	Biolegend 108411	1:250	Lineage (Lin)
Gr-1 FITC	Biolegend 108405	1:100	Lineage (Lin)
Gr-1 PE	BD Biosciences 12-5931-82	1:250	Lineage (Lin)
IgG (H+L) secondary PE	ThermoFisher Scientific P-2771MP	1:100	
IgG-AF488 (anti-mouse)	Thermo Fisher A-21202	1:1000	
IgG-FITC (anti-rabbit)	SANTA CRUZ sc-2012	1:1000	
pChk1 S345	CellSignalling Technology 2348	1:50	
pMcm2 S108	Bethyl IHC-00014	1:500	
pRpa32 S4/S8	Bethyl A300-245A	1:500	
APC/ Sca-1	Biologend 122511	1:100	Sca-1
APC-Cy7/ Sca-1	Biolegend 108125	1:100	
Ter119 APC	Biolegend 116211	1:250	Lineage (Lin)
TER-119 FITC	Biolegend 116205	1:100	Lineage (Lin)
Ter119 PE	BD Biosciences 12-5921-82	1:250	Lineage (Lin)
Tgfbr1-APC	R&D FAB5871A	3:100	

## Supplementary Table 2

Primers and thermocycler conditions used for genotyping and determining the sex of embryos used for single-cell RNA sequencing of murine E13.5 WT and *Fancd2*<sup>(-/-)</sup> fetal livers. Genotyping and SRY primer sequences were received from Dr. Alan D'Andrea and Dr. Sherif Abdelhamad, respectively.

Primer	Sequence (5' to 3')	Function		Thermocycler conditions
FANCD2 2F	CATGCATATAG GAACCCGAAG G	WT (forward)	genotype	95°C for 15'
FANCD2 2R	CAGGACCTTT GGAGAAGCAG	Common (reverse)		(95°C for 30", 60°C for 30", 72°C for 50") x 35
FANCD2 V76F	CTTGCAAAATG GCGTTACTTAA GC	KO (forward)		72° for 10'
Mouse SRY F	GCTGGGATGC AGGTGGAAAA	forward	sex	standard conditions
Mouse SRY R	CCCTCCGATG AGGCTGATATT	reverse		

## Supplementary Table 3

Primer sequences utilized for RT-PCR experiments.

Target	Species	Sequence
IFN-α	Mouse	F- CCTGAGAGAGAAGAAACACAGCC R- TCTGCTCTGACCACYTCCCAG
IL-6	Mouse	F- CCAAGAGGTGAGTGCTTCCC R- CTGTTGTTCACTCTCTCCCT

## Supplementary Table 4

### Commercial assays and reagents

Assay/reagents	Source	Identification
Proteasome-Glo Chymotrypsin- like cell-based assay	Promega	Cat#G8660
MG-132	Adooq- Bioscience	Cas# 133407-82-6
O-Propargyl-Puromycin (OPP)	Medchem Source	Cat#HY-156801CS-6850
Alexa Fluor 555- Conjugated Azide	Life Technologies	Cat #A20012
Click-iT Cell Reaction Buffer Kit	Life Technologies	Cat# C10269
Tetraphenylethene maleimide(TPE-MI)	Dr. Yuning Hong	Custom- Synthesized
Proteostat-Aggresome detection kit	Enzo	Cat#ENZ-51035-K100
RNeasy plus Micro kit	Qiagen	Cat#74034
2-Mercapto ethanol	Sigma	Cat#M3148
Tauroursodeoxycholic acid (TUDCA)	Sigma-Aldrich Inc	Cat#35807-85-3
(+)-JQ1	Med Chem Express USA	HY-13030
SYBR™ Green master mix	Thermo fisher-SCIENTIFIC	Cat#A25742
RT <sup>2</sup> Profiler PCR Array	QIAGEN	Cat# 330231 PAMM-177ZA
KAPA Mouse Genotyping Kit	Roche	Cat# KK7352
Hot Start Taq Master Mix Kit	Qiagen	Cat# 203443
collagenase I	StemCell Technologies	Cat# 07416
RBC lysis buffer	Invitrogen	Cat# 00-4300-54
Propidium Iodide (PI) solution	Miltenyi Biotec	Cat# 130-093-233
KAPA Library Quantification Kit	Roche	Cat# 07960336001
Agilent Tape Station - High Sensitivity D1000 Screen Tape	Agilent Tape Station	Cat# 5067-5584

Agilent Tape Station Reagents	Agilent Tape Station	Cat# 5067-5585
-------------------------------	----------------------	----------------