LETTER TO THE EDITOR

# m$^6$A reader Ythdf3 protects hematopoietic stem cell integrity under stress by promoting the translation of *Foxm1* and *Asxl1* transcripts

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**Fig. S1**

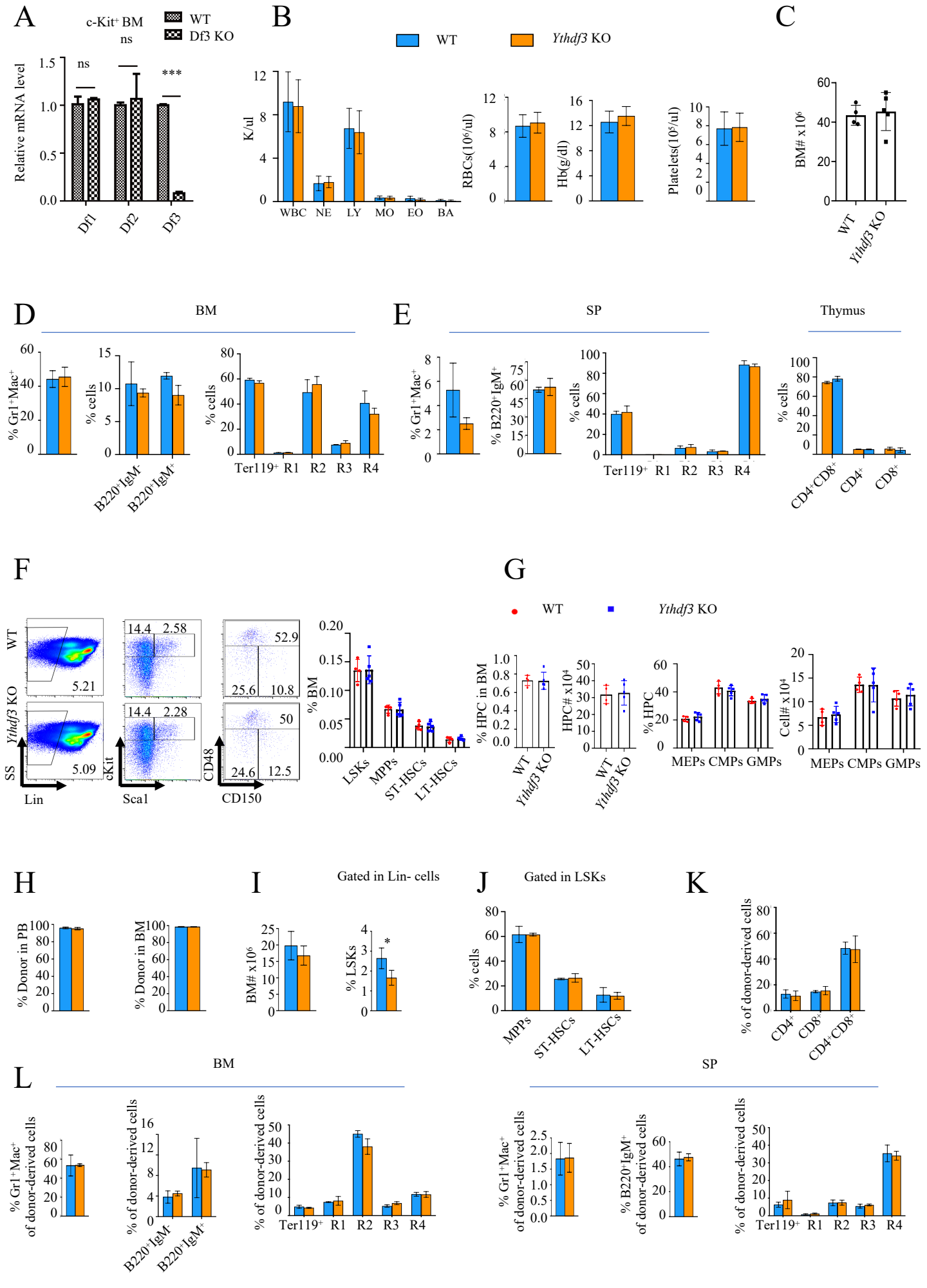
A
c-Kit+ BM
ns
WT
Df3 KO
Relative mRNA level
ns
***
Df1
Df2
Df3
B
WT
Ythdf3 KO
K/ul
WBC
NE
LY
MO
EO
BA
RBCs(10⁶/ul)
Hb(g/dl)
Platelets(10⁵/ul)
C
BM# x10⁶
WT
Ythdf3 KO
D
BM
% Gr1+Mac+
% cells
B220+IgM-
B220+IgM+
Ter119+ R1 R2 R3 R4
E
SP
Thymus
% B220+IgM+
CD4+CD8+
CD4+
CD8+
F
WT
Ythdf3 KO
5.21
5.09
14.4 2.58
14.4 2.28
52.9
25.6 10.8
50
24.6 12.5
SS
Lin
cKit
Sca1
CD48
CD150
% BM
LSKs
MPPs
ST-HSCs
LT-HSCs
G
WT
Ythdf3 KO
% HPC in BM
HPC# x10⁴
% HPC
Cell# x10⁴
MEPs CMPs GMPs
H
% Donor in PB
% Donor in BM
I
Gated in Lin- cells
BM# x10⁶
% LSKs
J
Gated in LSKs
% cells
MPPs
ST-HSCs
LT-HSCs
K
% of donor-derived cells
CD4+
CD8+
CD4+CD8+
L
BM
SP
% Gr1+Mac+ of donor-derived cells
% of donor-derived cells
B220+IgM-
B220+IgM+
Ter119+ R1 R2 R3 R4
% B220+IgM+ of donor-derived cells

**Figure S1. Characterization of *Ythdf3* wildtype and KO primary and recipient mice.**

**A** Transcripts of Ythdf1 and Ythdf2 were not affected by *Ythdf3* knockout.
c-Kit$^+$ mouse bone marrow cells were isolated from wild-type and Ythdf3 knockout mice and RT-PCR analyses were performed to determine the transcription levels of Ythdf1/2/3. ***, P<0.005 and "ns" denotes no significant difference.

**B-C** Analysis of hematological parameters of primary *Ythdf3* wildtype and KO mice.
**B** Absolute number of white blood cells(WBC), neutrophils(NE), lymphocytes(LY), monocytes(MO), eosinophils(EO) and basophils(BA), as well as red blood cells(RBC) and platelets, and concentration of hemoglobin(Hb), in peripheral blood of 8-week-old *Ythdf3* WT and KO mice, n=5. **C** Number of BM cells in *Ythdf3* WT and KO mice, n=5.

**D-E** Flow cytometric analysis of mature cell lineages from primary *Ythdf3* wildtype and KO mice. Proportion of myeloid (Gr1$^+$Mac$^+$), Immature B cells (B220$^+$ IgM$^-$), mature B cells (B220$^+$ IgM$^+$) and erythroblasts in BM (D). Proportion of myeloid (Gr1$^+$Mac$^+$), mature B cells (B220$^+$ IgM$^+$) and erythroblasts in spleen (E) from primary *Ythdf3* WT and KO mice. Flow cytometric analysis of T cells (E) isolated from thymus from *Ythdf3* WT and KO mice. All the data was obtained from primary *Ythdf3* WT and KO mice, n=3-5.

**F** Characterization of hematopoietic stem/progenitor cell compartments in primary *Ythdf3* wildtype and KO mice. Gating strategy (left panel) and flow cytometric analysis of percentage of LSKs, MPPs, ST-HSCs and LT-HSCs (right panel) from 8-week-old WT and *Ythdf3* KO mice. MPP: Lin$^-$c-Kit$^+$Sca1$^+$CD48$^+$; ST-HSC: Lin$^-$c-Kit$^+$Sca1$^+$CD48$^-$CD150$^-$ , LT-HSC: Lin$^-$c-Kit$^+$Sca1$^+$CD48$^-$CD150$^+$ . n=4-5.

**G** Analysis of subpopulations of myeloid progenitors in primary *Ythdf3* wildtype and KO mice. The frequency and absolute number of HPC (left two panels). The frequency and absolute number of CMPs, GMPs and MEPs in HPC population in BM (right two panels) from 8-week-old WT and *Ythdf*3 KO mice , n = 4-5.

**H** The percentage of WT and *Ythdf3* KO donor-derived cells (CD45.2$^+$) in periphery blood and BM cells from primary recipient mice, n=4-5.

**I-L** Flow cytometric analysis of bone marrow cells from primary *Ythdf3* wildtype and KO BM recipient mice. Total bone marrow cell numbers from primary *Ythdf3* WT and KO recipient mice(I) Proportion of LSKs,*, P=0.057 (I) MPPs, ST-HSCs and LT-HSCs (J)from primary *Ythdf3* WT and KO recipient mice. L-K Proportion of myeloid, B cells and erythroblasts in BM and spleen (L) from primary *Ythdf3* WT and KO recipient mice. Flow cytometric analysis of T cells (K) isolated from thymus from indicated mice. All the data was obtained from primary *Ythdf3* WT and KO recipient mice, n=3-5.

Fig. S2

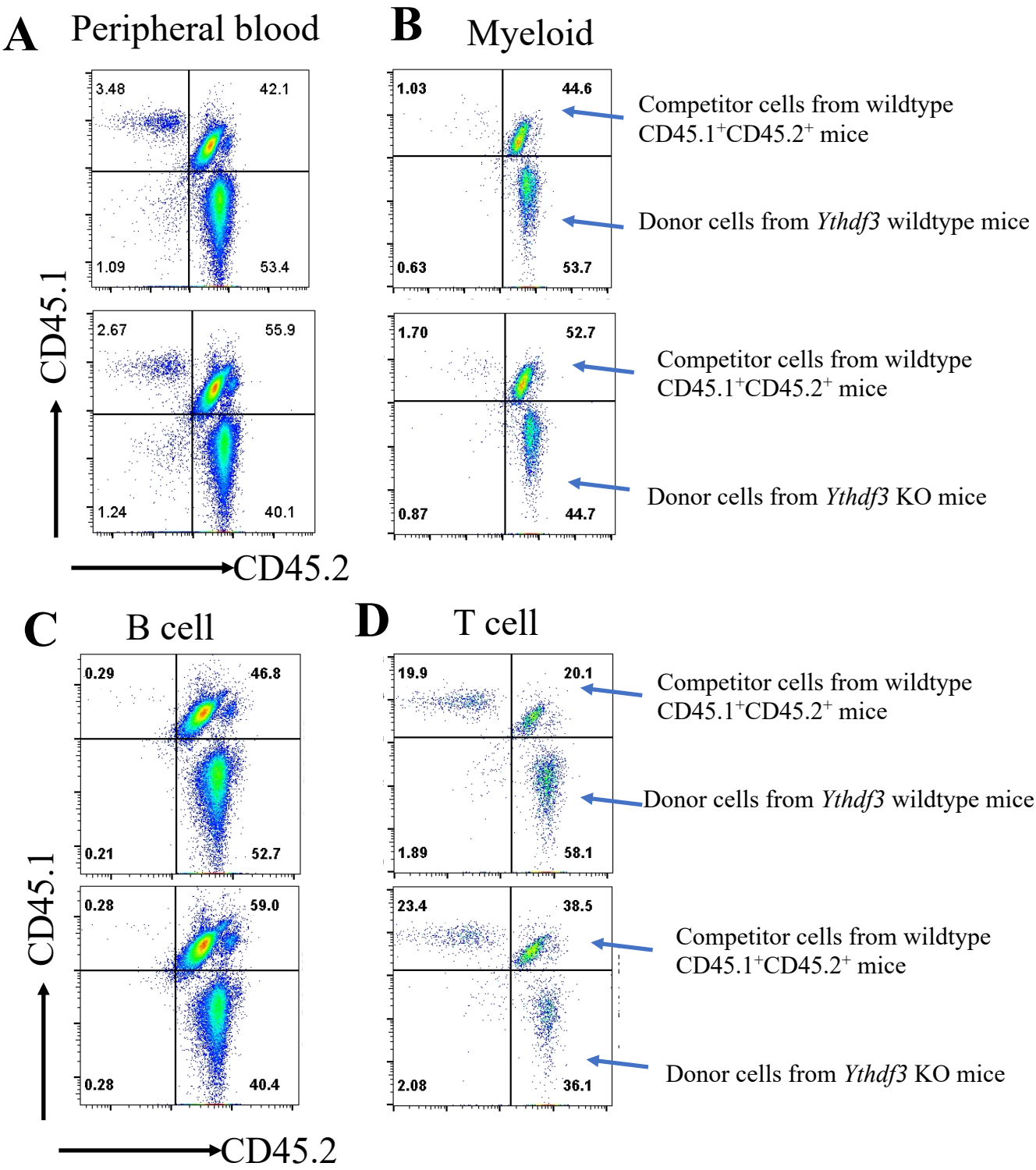


**Figure S2. Representative flow cytometric diagrams showing the frequency of competitor wildtype cells and donor cells from Ythdf3 wildtype and knockout mice.** The results were shown in total blood cells (A), Mac-1$^+$ myeloid cells (B), B220$^+$ B-cells (C) and CD3e$^+$ T cells in peripheral blood from recipient mice one month after primary transplantation.

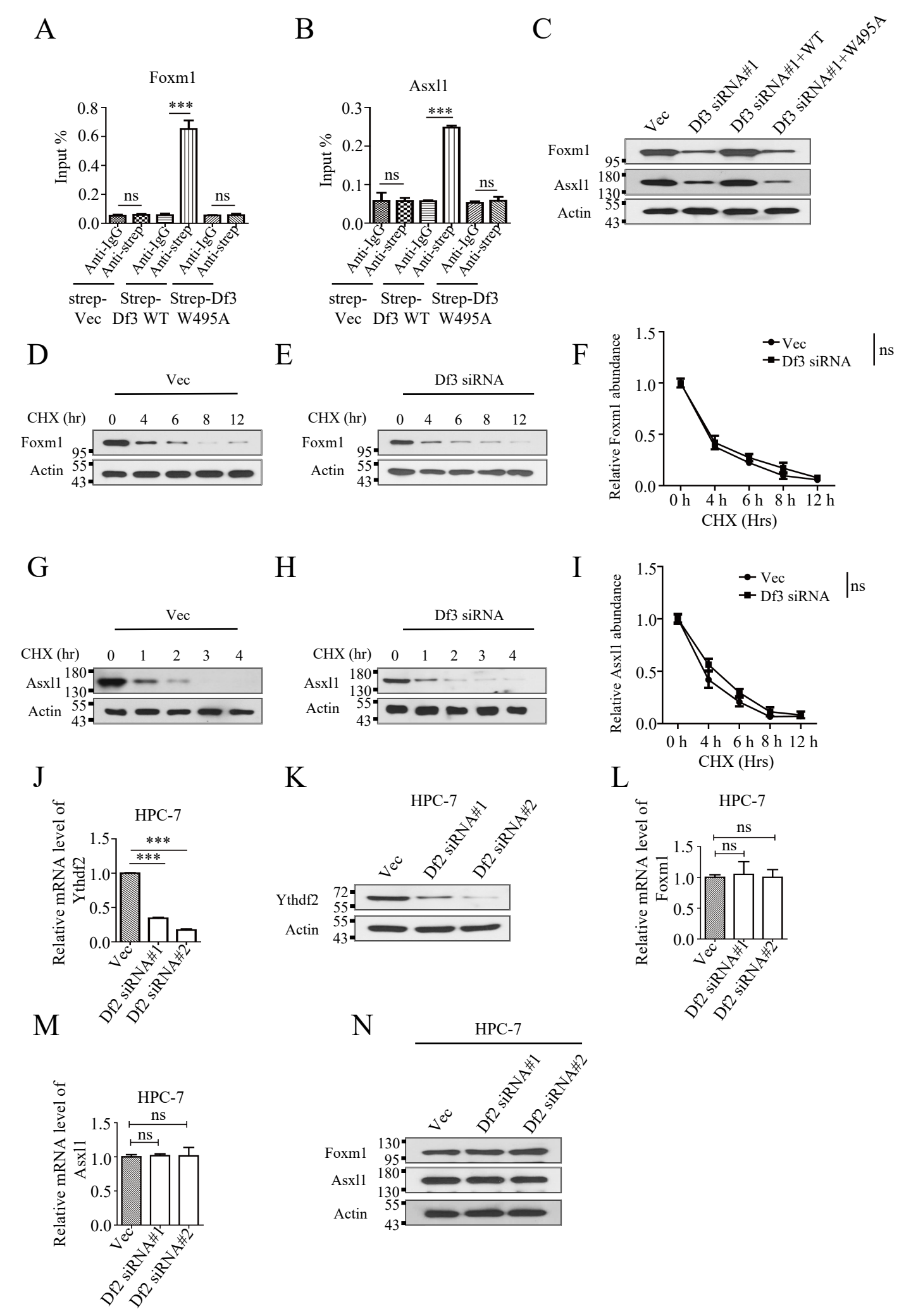
A
Foxm1
Input %
0.8
0.6
0.4
0.2
0.0
***
ns
ns
Anti-IgG
Anti-strep
Anti-IgG
Anti-strep
Anti-IgG
Anti-strep
strep-Vec
Strep-Df3 WT
Strep-Df3 W495A
B
Asxl1
Input %
0.3
0.2
0.1
0.0
***
ns
ns
Anti-IgG
Anti-strep
Anti-IgG
Anti-strep
Anti-IgG
Anti-strep
strep-Vec
Strep-Df3 WT
Strep-Df3 W495A
C
Vec
Df3 siRNA#1
Df3 siRNA#1+WT
Df3 siRNA#1+W495A
Foxm1
Asxl1
Actin
95
180
130
55
43
D
Vec
CHX (hr) 0 4 6 8 12
Foxm1
Actin
95
55
43
E
Df3 siRNA
CHX (hr) 0 4 6 8 12
Foxm1
Actin
95
55
43
F
Relative Foxm1 abundance
1.5
1.0
0.5
0.0
Vec
Df3 siRNA
ns
0 h 4 h 6 h 8 h 12 h
CHX (Hrs)
G
Vec
CHX (hr) 0 1 2 3 4
Asxl1
Actin
180
130
55
43
H
Df3 siRNA
CHX (hr) 0 1 2 3 4
Asxl1
Actin
180
130
55
43
I
Relative Asxl1 abundance
1.5
1.0
0.5
0.0
Vec
Df3 siRNA
ns
0 h 4 h 6 h 8 h 12 h
CHX (Hrs)
J
HPC-7
Relative mRNA level of Ythdf2
1.5
1.0
0.5
0.0
***
***
Vec
Df2 siRNA#1
Df2 siRNA#2
K
HPC-7
Vec
Df2 siRNA#1
Df2 siRNA#2
Ythdf2
Actin
72
55
55
43
L
HPC-7
Relative mRNA level of Foxm1
1.5
1.0
0.5
0.0
ns
ns
Vec
Df2 siRNA#1
Df2 siRNA#2
M
HPC-7
Relative mRNA level of Asxl1
1.5
1.0
0.5
0.0
ns
ns
Vec
Df2 siRNA#1
Df2 siRNA#2
N
HPC-7
Vec
Df2 siRNA#1
Df2 siRNA#2
Foxm1
Asxl1
Actin
130
95
180
130
55
43

**Figure S3. Ythdf3 specifically facilitates mRNA translation of *Foxm1* and *Asxl1*.** **A-B** RIP analyses suggesting that only wild-type but not the $m^6A$-binding-deficient mutant (Df3 W495A) associates with transcripts of Foxm1 and Asxl1. **C** Western blot analysis showing that reduced protein levels of Asxl1 and Foxm1 mediated by Ythdf3 knockdown can be rescued by wild-type but not $m^6A$- binding-deficient mutant Ythdf3. **D-I** HPC-7 cells stably expressing vector, or siRNAs against Ythdf3 were treated with or without 50 ug/mL Cycloheximide to time interval as indicated and Western blot analyses were carried out to determine protein levels of Foxm1 and Asxl1.**F** quantitative data for **D** and **E** by Image J software. **I** quantitative data for **G** and **H** by Image J software. **J** RT-qPCR analysis showing the knockdown efficiency of *Ythdf2* in HPC-7 cells. **K** Western blot analysis showing the knockdown efficiency of Ythdf2. **L,M** RT-qPCR analysis showing the effect of *Ythf2* knockdown on transcription level of *Foxm1* (L) and *Asxl1* (M) in HPC-7 cells. **N** Western blot analysis showing protein levels of Foxm1 and Asxl1 in HPC-7 cells stably expressing vector, or siRNAs against *Ythdf2*. ***, $P<0.005$ and "ns" denotes no significant difference.