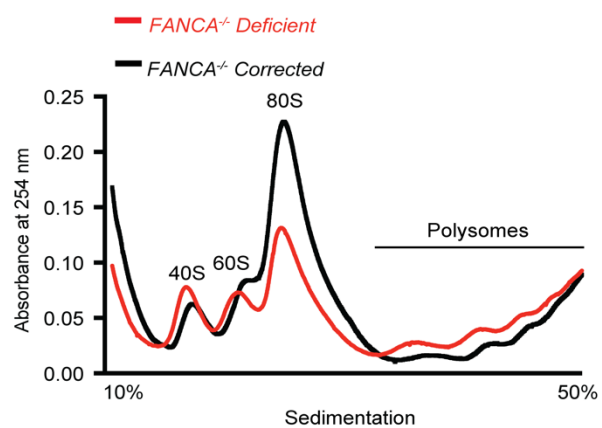


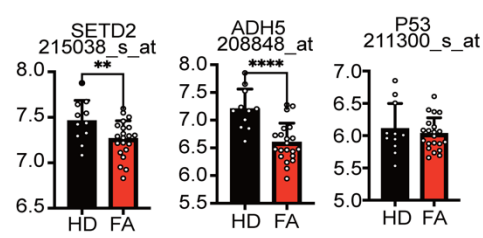
Supplemental Fig. 1



1003 **Supplemental Figure 1. *FANCA* deficiency reduces levels of the 60S subunit and**
1004 **mature 80S ribosomes.** Cytosolic extracts were prepared from 20×10^6 cells from
1005 HSC72 FA-A pairs and sedimented by centrifugation on 10-50% sucrose gradients.
1006 Peaks corresponding to the 40S and 60S ribosomal subunits, 80S mono-ribosomes and
1007 ribosomes in polysome fractions were detected.

1008

Supplemental Fig. 2



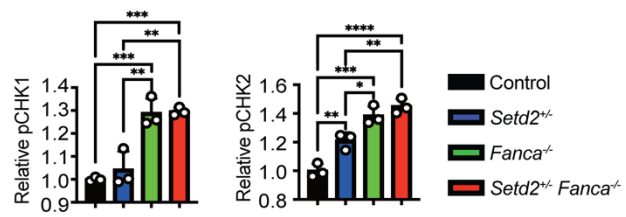
Supplemental Figure 2. Gene expression of FA patient low-density bone marrow cells.

Relative expression levels of SETD2, ADH5, and TP53 in healthy donors (HD, n = 11) and Fanconi anemia patients (FA, n = 21) were analyzed using normalized microarray data (GSE16334). Expression values are presented as log2-transformed intensities.

Data are shown as mean $\pm$ SEM. Statistical significance was determined by one-tailed

Student's t test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, and $****p < 0.0001$.

Supplemental Fig. 3



1019 **Supplemental Figure 3. Replication stress were accumulated in Setd2^{+/-} Fanca^{-/-}**
1020 **HSCs.**

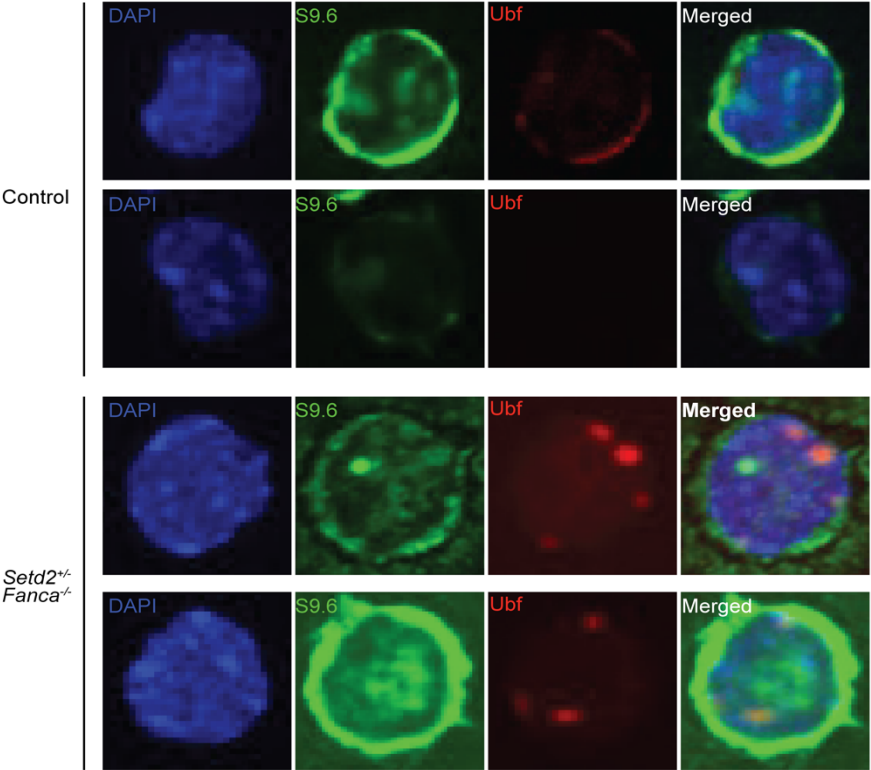
1021 The levels of pCHK1 and pCHK2 in HSC from Control (black, n=3), *Setd2*^{+/-} (Blue, n=3),
1022 *Fanca*^{-/-} (Green, n=3) and *Setd2*^{+/-} *Fanca*^{-/-} (Red, n=3) mice. Data are shown as mean ±
1023 SEM. Statistical significance was determined by one-way ANOVA with Tukey's multiple-
1024 comparison test. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

1025

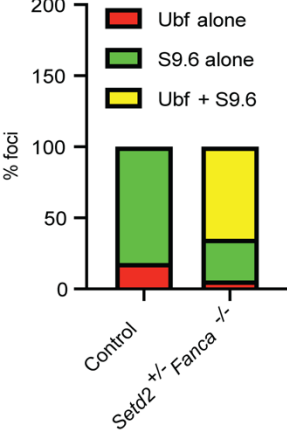
1026

Supplemental Fig. 4

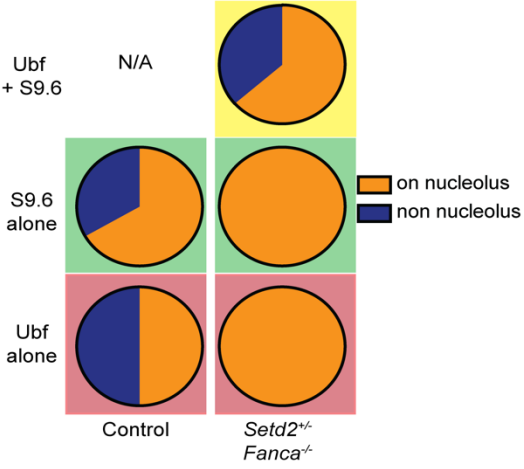
A.



B.



C.



1028 **Supplemental Figure 4. R-loops accumulate at ribosome-related loci in nucleoli in**
1029 ***Setd2*^{+/-} *Fanca*^{-/-} HSCs.**

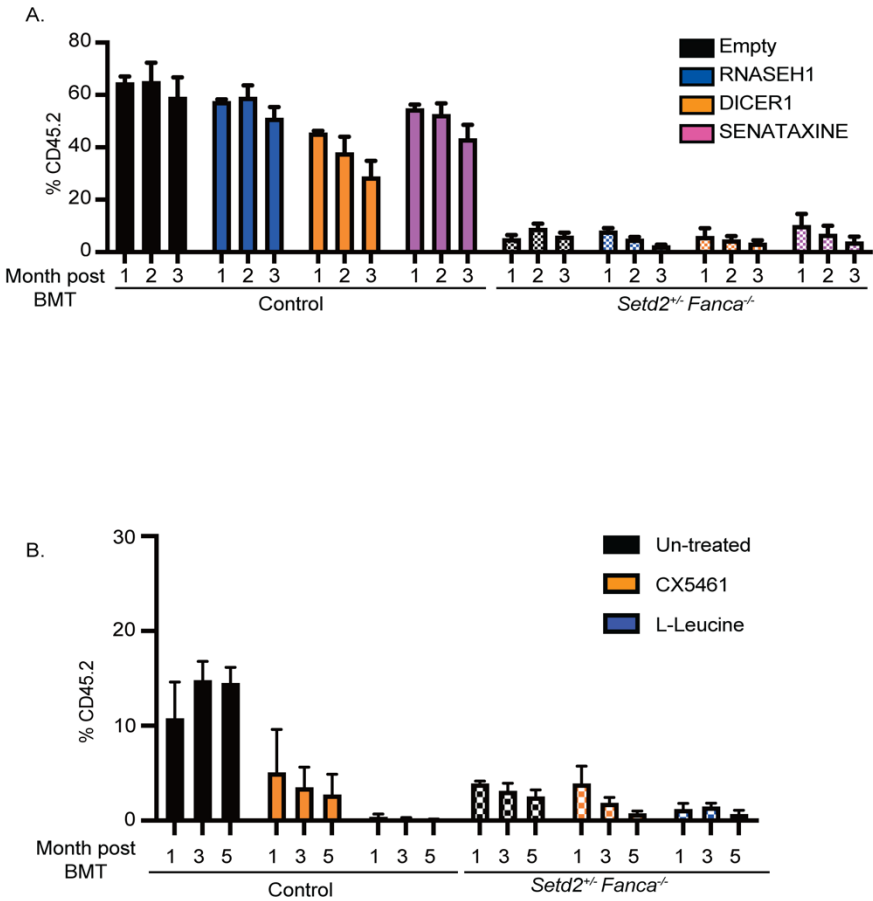
1030 **(A)** Additional image for visualization of colocalization of ribosomal protein and R-loops in
1031 HSCs. UBF (Red, Marker for RNA polymerase I), S9.6 (Green, R-loops), and DAPI (Blue).

1032 **(B)** Frequency of foci for UBF alone (Red), S9.6 alone (Green), and UBF and S9.6
1033 colocalization (Yellow). **(C)** Frequency of foci (UBF alone (Red area), S9.6 alone (Green
1034 area), and UBF and S9.6 colocalization (Yellow area)) location on the nucleolus or not.

1035

1036

Supplemental Fig. 5



Supplemental Figure 5. CX5461 and L-Leucine treatment do not rescue engraftment of *Setd2*^{+/-} *Fancc*^{-/-} HSPCs.

(A) Bone marrow transplantation of Control (no pattern), *Setd2*^{+/-} *Fancc*^{-/-} (pattern), with lentivirus-mediated transduction of Empty, *RNASEH1*, *DICER1*, and *SENATAXINE*. Peripheral bloods (PBs) were analyzed at 1, 2, and 4 months post-transplantation (SD; n = 5 in each condition). (B) Bone marrow transplantation of Control (no pattern), *Setd2*^{+/-} *Fancc*^{-/-} (pattern), with or without treatment with CX5461 (yellow bar) and L-Leucine (Blue bar). PBs were analyzed at 1, 3, and 5 months post-transplantation (SD; n = 3 in each condition). Data are shown as mean $\pm$ SEM. Statistical significance was assessed using **two-way ANOVA** followed by **Tukey's multiple-comparisons test**. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Table 1

	Forward	Reverse
Human		
47S	GCTGACACGCTGCCTCTG	ACGCGCGAGAGAACAGCAG
45S	GAACGGTGGTGTGTCGTT	GCGTCTCGTCTCGTCTCACT
18S	GATGGTAGTCGCCGTGCC	GCCTGCTGCCTTCCTTGG
5.8S	ACTCGGCTCGTGCGTC	GCGACGCTCAGACAGG
28S	AGAGGTAACGGGTGGGTC	GGGGTCGGGAGGAACGG
5S	GGCCATACCACCTGAACGC	CAGCACCCGGTATCCAGG
Mouse		
45S	GTAAACGGCGGGAGTAACATG	GACAGTGGGAATCTCGTTCATC
18S	CACGGACAGGATTGACAGATT	GCCAGAGTCTCGTTCGTTATC
5.8S	ACTCTTAGCGGTGGATCACT	GCGTTCGAAGTGTGATGAT
28S	GTAAACGGCGGGAGTAACATG	GACAGTGGGAATCTCGTTCATC
5S	CCCCGAGAAGACTCAACAC	AGCCAGATGGCCTGATCAC

1050

1051

Table 1. Probes for ribosomal gene detection.