Supplemental figures:

Sup. Fig. 1. Cell cycle analysis of duplicate TUT7 KO clone.

A. WT cells (left panel) and TUT7 KO2 cells (right panel) were synchronized by double thymidine block as in Fig. 3. Cells were harvested at hourly intervals 5-8 hrs after release from the block. Cells were labeled with EdU for 30 minutes before harvesting, and cells in S-phase detected by EdU staining. The TUT7 KO2 cells show a cell cycle distribution similar to that of the wild-type cells as they progress through S-phase into G2. The amount of DNA (DAPI staining) is indicated on the X-axis and DNA replication is shown on the Y-axis. Approximately 5% of cells in each population did not enter S-phase as judged by the small number of cells in the lower left that did not synthesize DNA.

B. Total cell RNA was prepared from cells in G2 phase. END-Seq libraries specific for histone mRNAs were prepared and analyzed. The distribution of histone mRNA degradation intermediates and the amount of intermediates containing U-tails was determined as described,.

Sup. Fig. 2. Cell cycle analysis of duplicate 3'hExo KO clone.

A. Wild-type cells (left panel) and 3'hExo KO cells (right panel) were synchronized by double thymidine block as in Fig. 3. Cells were harvested at hourly intervals 3, 6 or 9hrs after release from the block. Cells were labeled with EdU for 30 minutes before harvesting, and cells in S-phase detected by EdU staining.

B. Cells in S-phase (3 hrs after release from the double thymidine block) were treated with HU and total cell RNA isolated before HU treatment and 20 and 40 minutes after HU treatment. RNA was resolved on a 6% polyacrylamide-7M urea gel and analyzed by Northern blotting using a Histone H2a probe and 7SK RNA probe as a control.

C. Mitosis is complete in the wild-type cells about 10 hrs after release from the double thymindine block as judged by flow cytometry (Fig.X), and by 12 hrs after release from the double thymidine block in the 3' hExo cells. Histone mRNA levels were analyzed in the 3' hExo KO cells at the indicated times, and the amount of histone H2a mRNA determined by Northern blotting using 7SK RNA as a control. As shown in Fig. 4, the histone mRNA prior to mitosis in 3'hEXO KO2 and at the same time in 3'hExo KO1.

Suo. Fig. 3. 3'hExo exit from S-phase and entry into mitosis is delayed by 2 hrs.

A and B. Wild type (panel A) or 3'hExo KO2 cells were synchronized in parallel by double thymidine block and samples collected for flow cytometry for 14 hrs after release to allow cells to complete mitosis. About 50% of the wild-type cells have completed mitosis at 10 hrs (G1 and S-phase cells that have reentered S-phase) while 50% of the 3'hExo cells have not completed mitosis until 12 hrs.

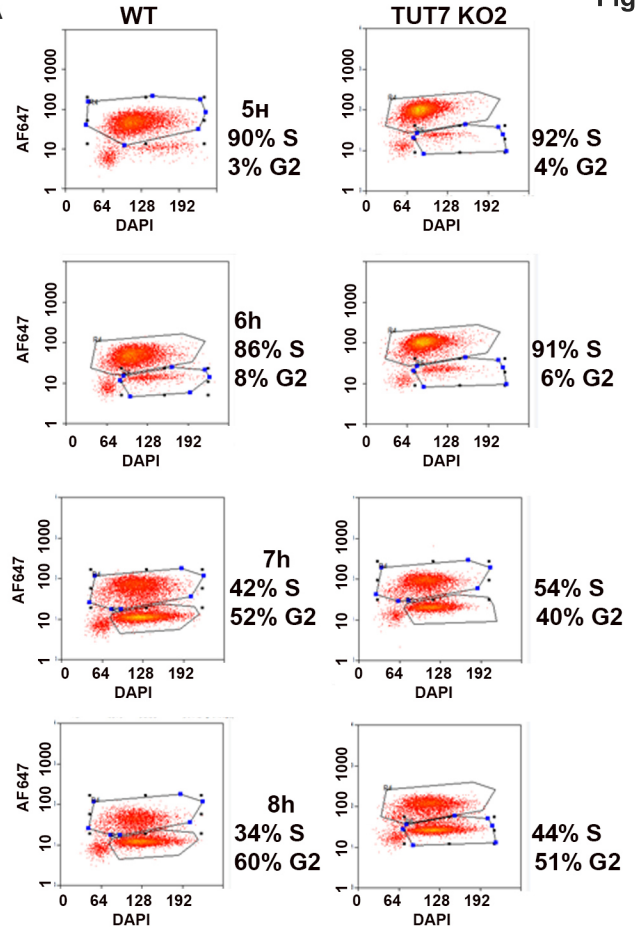
Sup. Fig. 4. Selection of 3'hExo and TUT7 KO cell lines expressing either ectopic 3'hExo or TUT7.

A and B. 3'hExo KO cells were infected with lentifirus containing a TET inducible 3' hExo gene (panel A) or a TET inducible catalytically dead 3'hExo gene (panel B). Eight cloned cell lines containing the wild-type 3'hExo ectopic gene (panel A) and seven cloned cells containing the catalytically dead 3'hExo gene (panel B) were tested for 3'hExo expression in the absence of tetracycline by Western blotting using b-actin as a control. WT cells are shown in lane 1, the 3'hXo KO cells in lane 2 and the cloned cell lines in the remaining lanes.

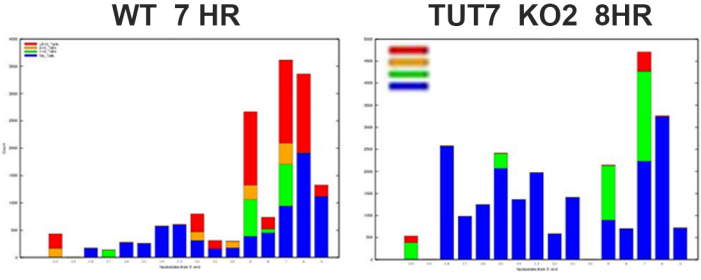
C. and D. A cell line that did not express 3'hExo(Panel C) or catalytically dead 3'hExo (panel D) in the absence of tetracycline. The cells were treated with varying concentrations (2 ng/ml, 20 ng/ml, 200 ng/ml and 2 μg/ml) and the level of 3'hExo Panel D) or catalytically dead 3'hExo expression (panel D) determined by Western blotting using b-actin as a control. 200 ng/ml was selected as the concentration of tetracycline for use in subsequent experiments.

E. 7 cells lines infected with the lentivirus containing at TUT7 gene driven by the EF2 promoter were analyzed by Western blotting for expression of TUT7, 3'hExo and b-actin. The filter was cut and the top portion containing proteins > 100 kDa including the TUT7 protein) and bottom portion (containing proteins <100 kDa including 3'hEXo and b-actin) were incubated with the appropriate antibodies. Lane 1 is WT cells, lane 2 is the TUT7 KO cells and next 7 lanes are the cloned cell lines selected. T7R4 was selected as the cell line that expressed TUT7 at similar levels to the WT cells.

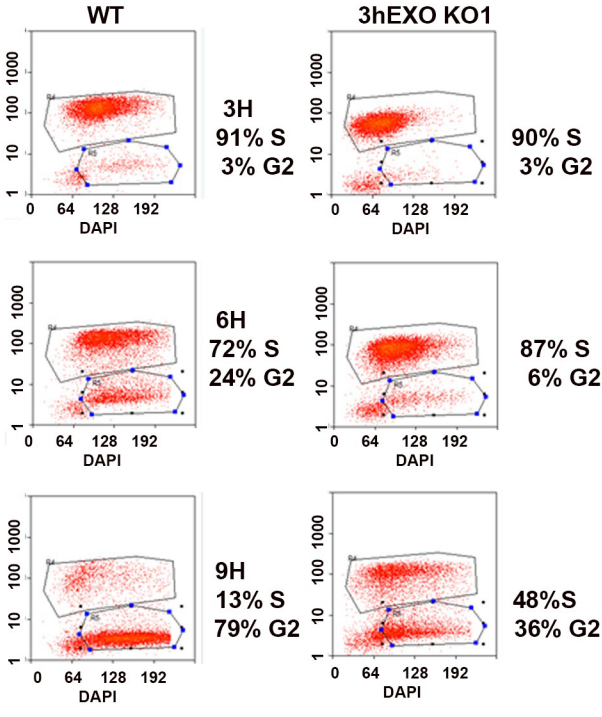
A


WT
TUT7 KO2
AF647
DAPI
5H
90% S
3% G2
92% S
4% G2
6h
86% S
8% G2
91% S
6% G2
7h
42% S
52% G2
54% S
40% G2
8h
34% S
60% G2
44% S
51% G2

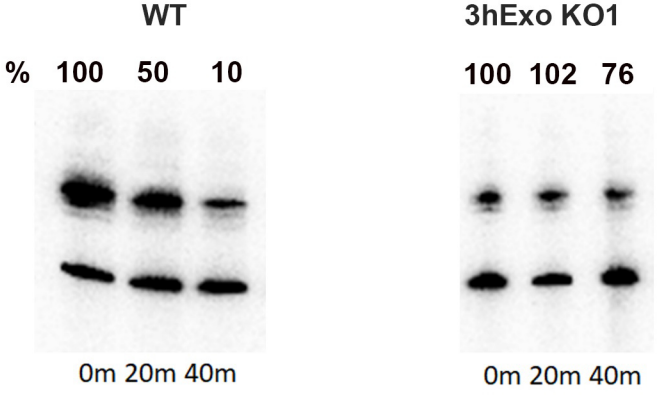
B


WT 7 HR
TUT7 KO2 8HR

A


WT
3hEXO KO1
3H
91% S
3% G2
90% S
3% G2
6H
72% S
24% G2
87% S
6% G2
9H
13% S
79% G2
48%S
36% G2
DAPI

B


WT
3hExo KO1
% 100 50 10
100 102 76
0m 20m 40m
0m 20m 40m

C

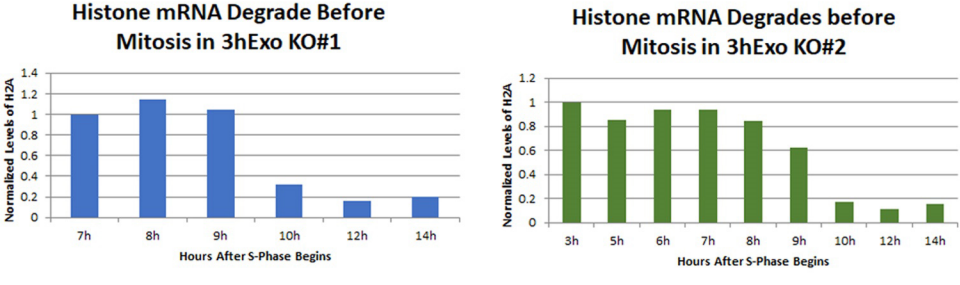
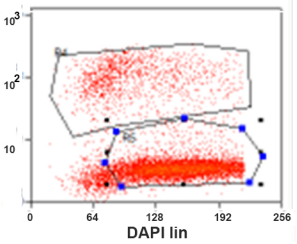

Histone mRNA Degrade Before Mitosis in 3hExo KO#1
Normalized Levels of H2A
Hours After S-Phase Begins
Histone mRNA Degrades before Mitosis in 3hExo KO#2
Normalized Levels of H2A
Hours After S-Phase Begins

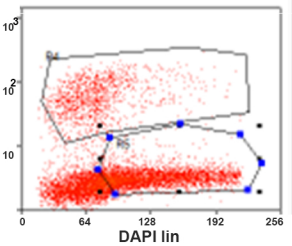
Fig. S3

A WT



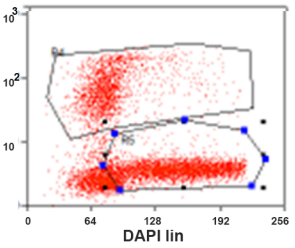
9 hrs

G1 5%
S 13%
G2 80%



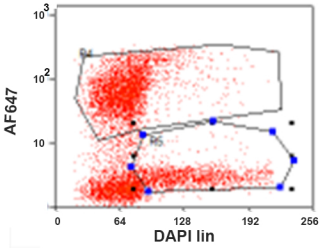
10 hrs

G1 39%
S 14%
G2 42%



12 hrs

G1 38%
S 22%
G2 36%



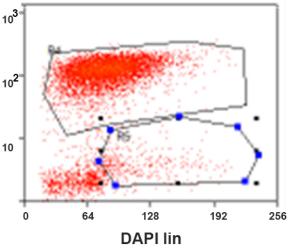
14 hrs

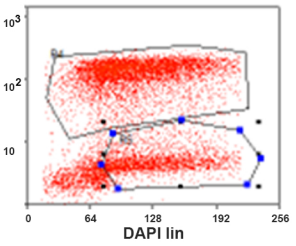
G1 30%
S 51%
G2 15%

B 3'hExo KO2

8 hrs

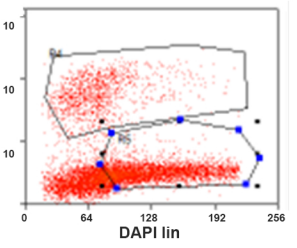
S 85%
G2 4%





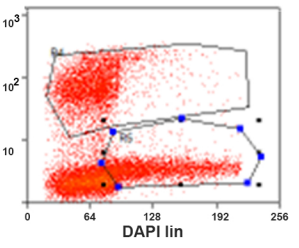
10 hrs

G1 5%
S 61%
G2 24%



12 hrs

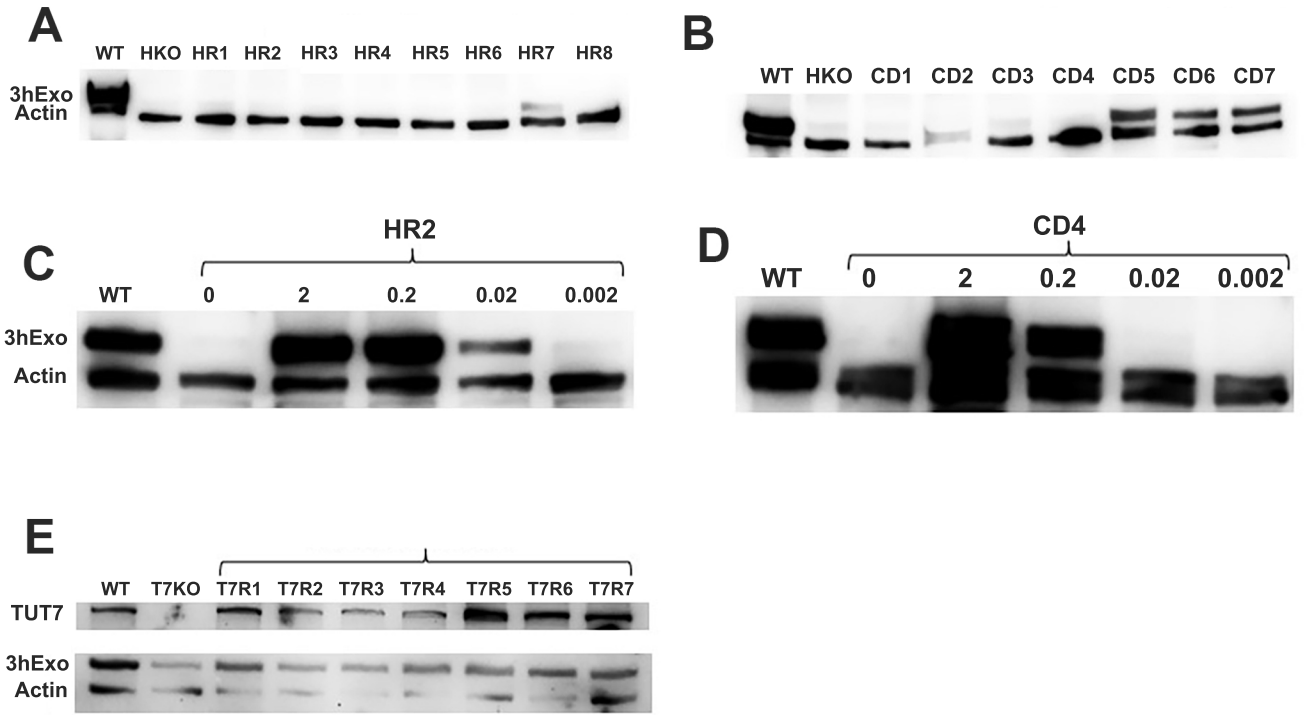
G1 35%
S 14%
G2 48%



14 hrs

G1 54%
S 30%
G2 12%

FIG. S4


A
WT HKO HR1 HR2 HR3 HR4 HR5 HR6 HR7 HR8
3hExo
Actin
B
WT HKO CD1 CD2 CD3 CD4 CD5 CD6 CD7
C
HR2
WT 0 2 0.2 0.02 0.002
3hExo
Actin
D
CD4
WT 0 2 0.2 0.02 0.002
E
WT T7KO T7R1 T7R2 T7R3 T7R4 T7R5 T7R6 T7R7
TUT7
3hExo
Actin