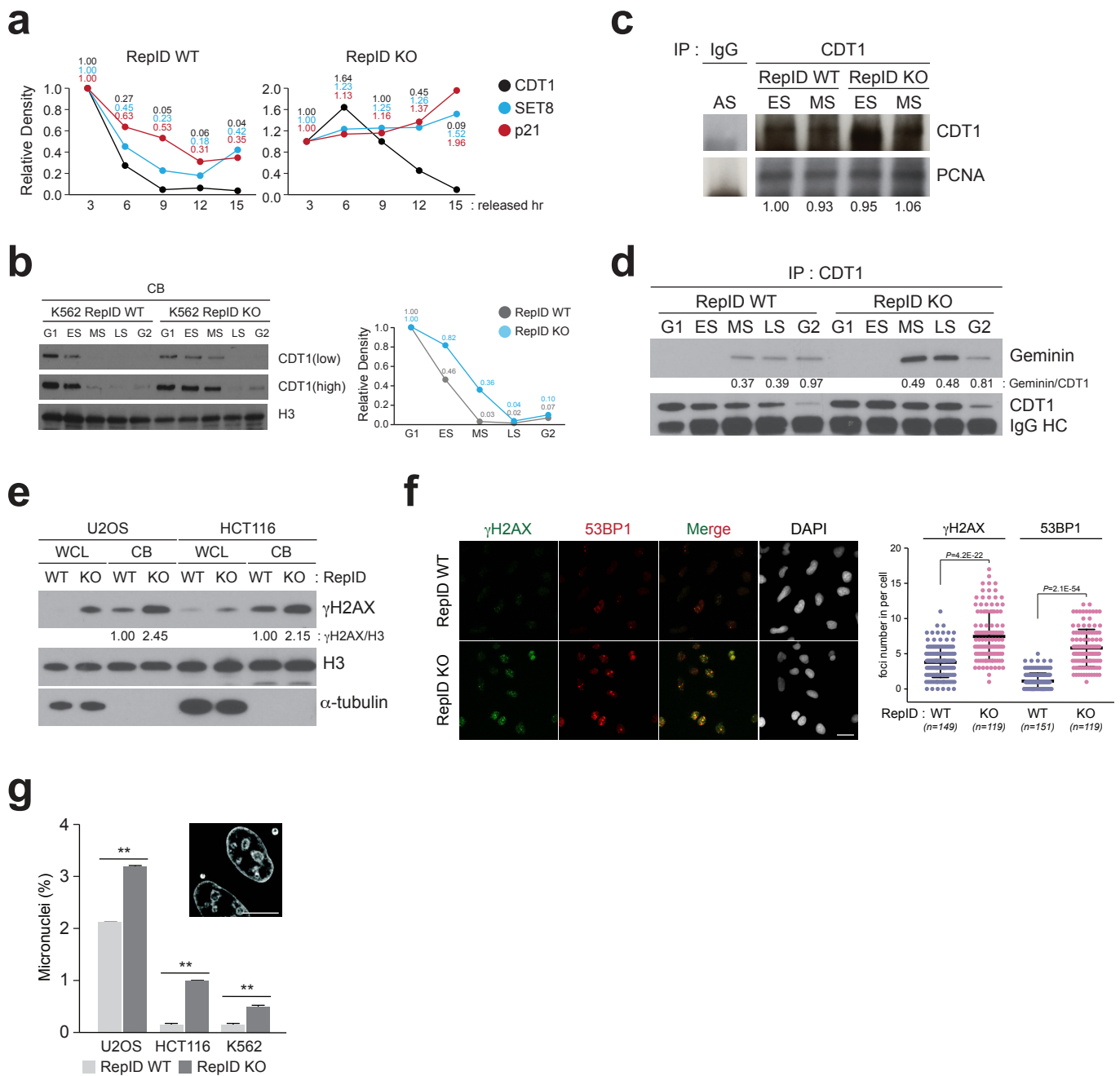
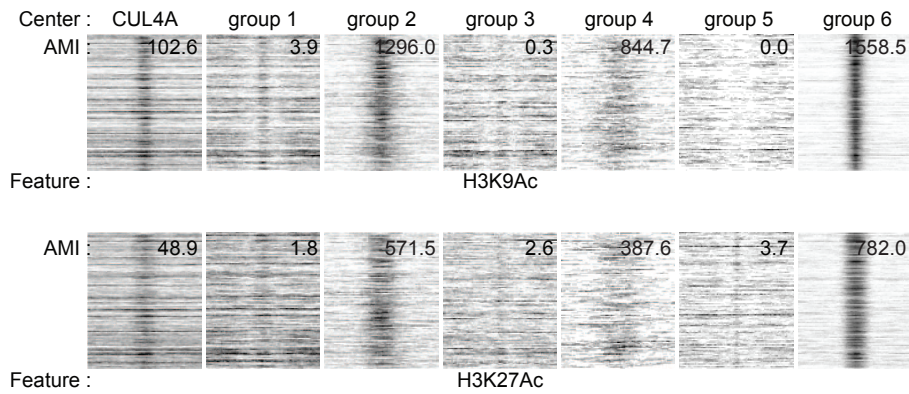
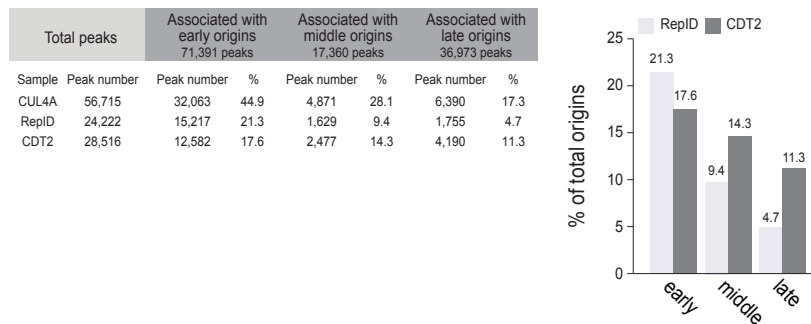


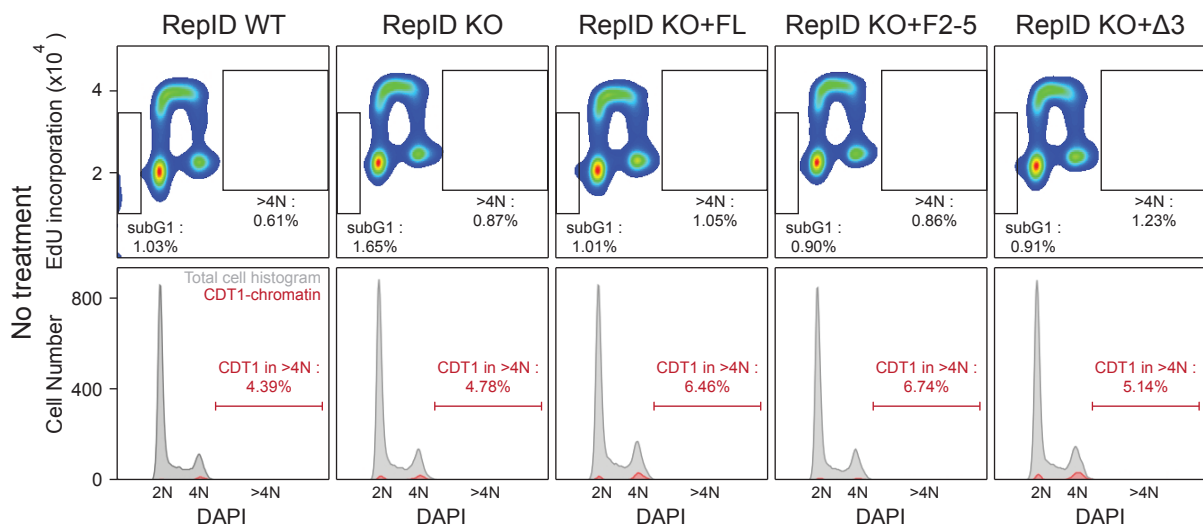
Supplementary Figure 1 (a) Schematic representing RepID KO generation by the CRISPR-Cas9 gene editing system in K562, HCT116 and U2OS. Target sequences are indicated in exon 5 for K562 cells and in exon 8 for U2OS and HCT116 cells. **(b)** U2OS WT cells were transfected with siRNA-DDB2 or siRNA-CDT2 and immunoblot analysis was performed to measure levels of chromatin-bound CUL4. RepID WT and RepID KO cell lysates were used as positive and negative controls. Asterisks indicate DDB2 or CDT2 signals. Histone H3 and α -tubulin were used as markers for cell fractionation. **(c)** Endogenous binding between RepID and CUL4. Chromatin fractions from U2OS RepID WT and RepID KO cells were immunoprecipitated using CUL4A or CUL4B antibodies and co-precipitated RepID was analyzed by immunoblotting. **(d)** In vitro binding between RepID and CRL4 using tagged purified proteins. **(e)** RepID WT and KO K562 cells were fractionated into cell cycle phases by elutriation and chromatin-bound CUL4B was analyzed by immunoblotting. ES, early S; MS, middle S; LS, late S. **(f)** Chromatin fractions from elutriated K562 RepID WT cells were immunoprecipitated using CUL4A antibody and co-precipitated RepID was detected by immunoblotting. **(g)** Endogenous chromatin-bound CUL4A detected by CUL4A-specific antibodies (ab92554) in U2OS RepID WT and KO cells after pre-extraction of soluble proteins (Color threshold: minimum 200, maximum 65,535). Scale bar indicates 25 μ m.



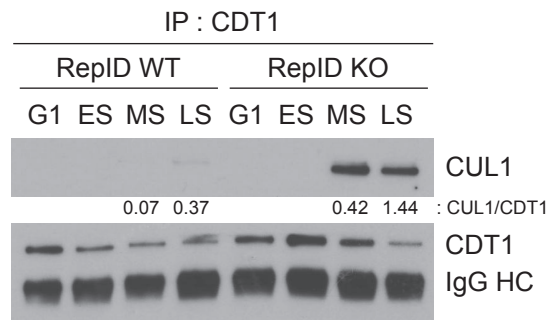
Supplementary Figure 2 (a) Quantification of CDT1, SET8 and p21 from nocodazole arrested, mitotic RepID WT and RepID KO HCT116 cells released in fresh medium and collected every 3 hours. Relative densities of western blot protein signals were normalized using the 3 hr time point (data related to Fig. 2e). **(b)** Chromatin-bound CDT1 levels in elutriated WT and KO K562 cells. Graph showing the relative CDT1 densities normalized using CDT1 signals from G1 is shown on the right. **(c)** Fractionated RepID WT and KO K562 cells were used for CDT1 immunoprecipitation in chromatin-bound fractions of ES and MS. Co-precipitated PCNA was analyzed by immunoblotting and the ratios PCNA/CDT1 are indicated. Asynchronized cells and rabbit-IgG were used as controls. **(d)** CDT1 immunoprecipitation using soluble nuclear and chromatin-bound extracts from fractionated RepID WT and KO K562 cells. Co-precipitated geminin was analyzed using immunoblotting and the ratios Geminin/CDT1 calculated. **(e)** γ H2AX levels on chromatin in RepID WT or KO cells. **(f)** γ H2AX and 53BP1 foci per cell in U2OS RepID WT and KO cells (Color threshold: minimum 200, maximum 40,000). Scale bar indicates 20 μ m. **(g)** Percentage of cells with micronuclei in RepID WT and RepID KO cells in HCT116, U2OS and K562. Ten thousand cells were counted per sample. A representative image of micronuclei is shown. Bar graphs = standard errors. **P < 0.01.

a**b**

Supplementary Figure 3 (a) Heat map depicting the colocalization between the acetylated histone H3 (H3K9Ac or H3K27Ac) and the ChIP-seq peaks of CUL4A and the peaks of the six subgroups described in Fig. 3b. **(b)** Extent of colocalization between early, mid and late replication origins with ChIP-seq peaks from CUL4A, RepID and CDT2 (table, left panel; histogram, right panel).



Supplementary Figure 4 Untreated WT, RepID KO, or RepID KO+RepID fragment-expressing U2OS cells were labelled with EdU for 30 min and analyzed by FACS. Percentages of re-replicating and SubG1 cells are indicated. Chromatin-bound CDT1 distribution throughout the cell cycle was analyzed by FACS using an antibody directed against CDT1 after pre-extraction of soluble nuclear proteins. Total cell histogram and CDT1-bound chromatin histogram are shown, and percentages of chromatin-bound CDT1 in re-replicating cells are indicated in red (bottom panel). This figure is the control for the Fig. 4d and 5g.



Supplementary Figure 5 Interactions between CDT1 and CUL1 are increased in RepID KO cells during middle and late S phase. Soluble nuclear and chromatin-bound fractions from K562 WT and KO RepID cells were used for CDT1 immunoprecipitation. Co-precipitated CDT1 and CUL1 were analyzed by immunoblotting. Numbers represent ratios for CUL1 and CDT1.

Fig. 1a

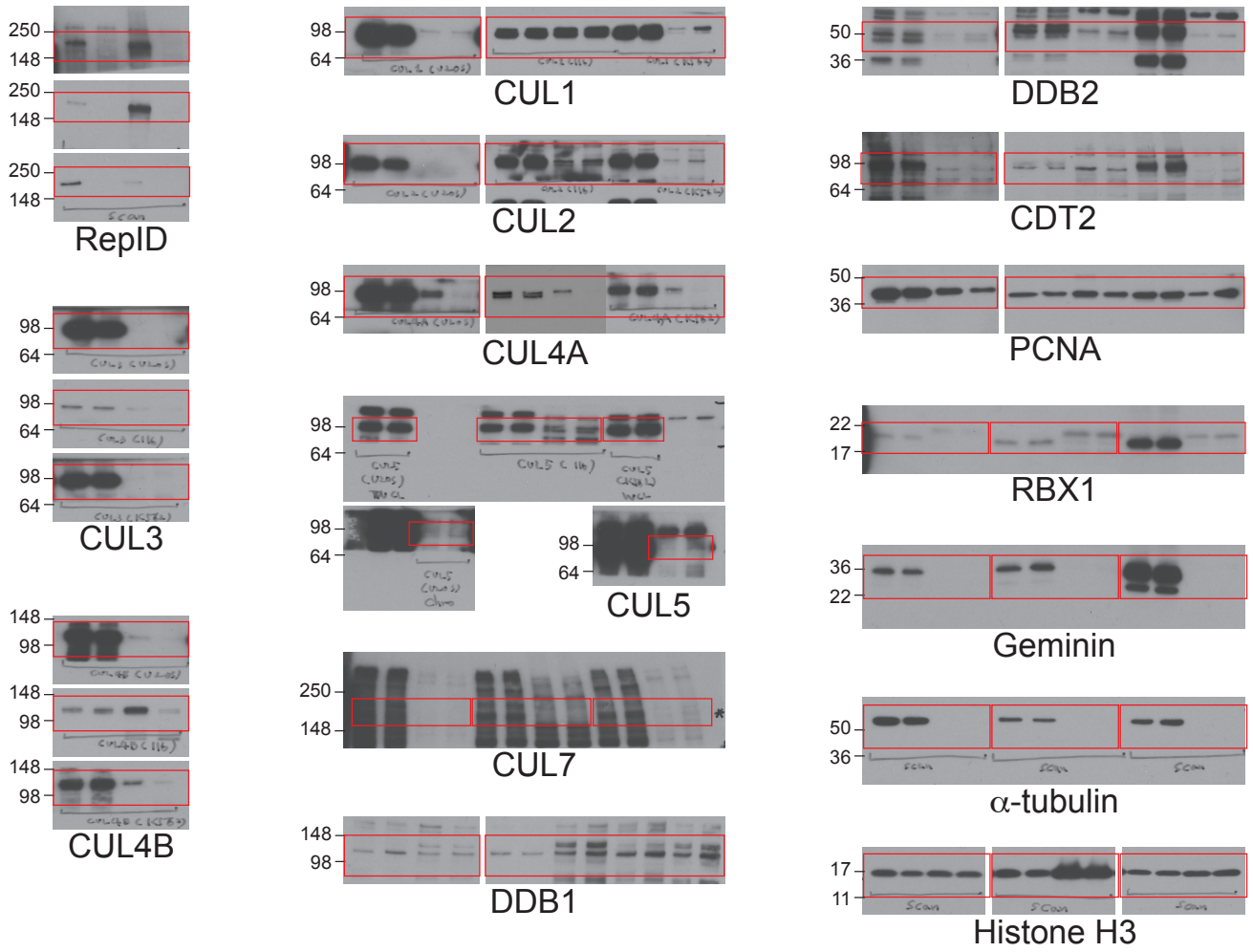


Fig. 1d

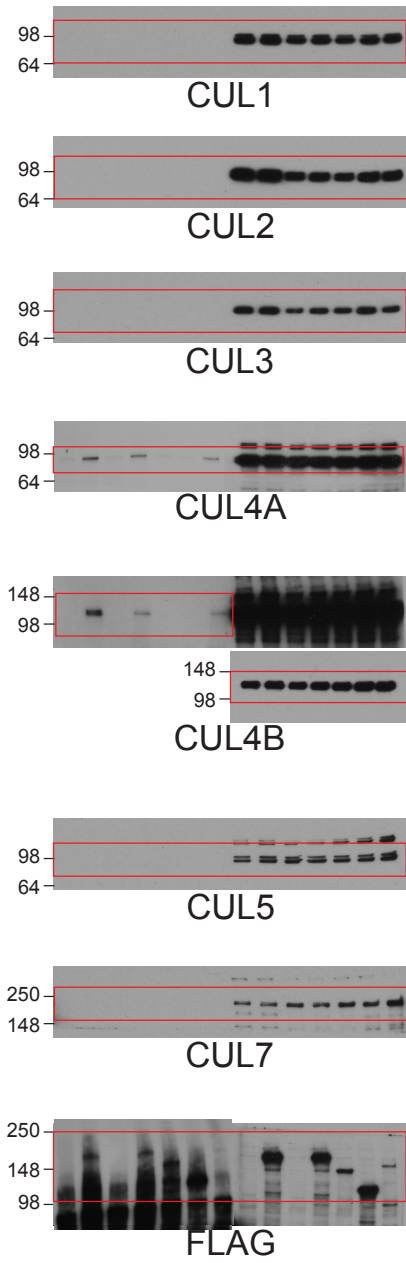


Fig. 1e

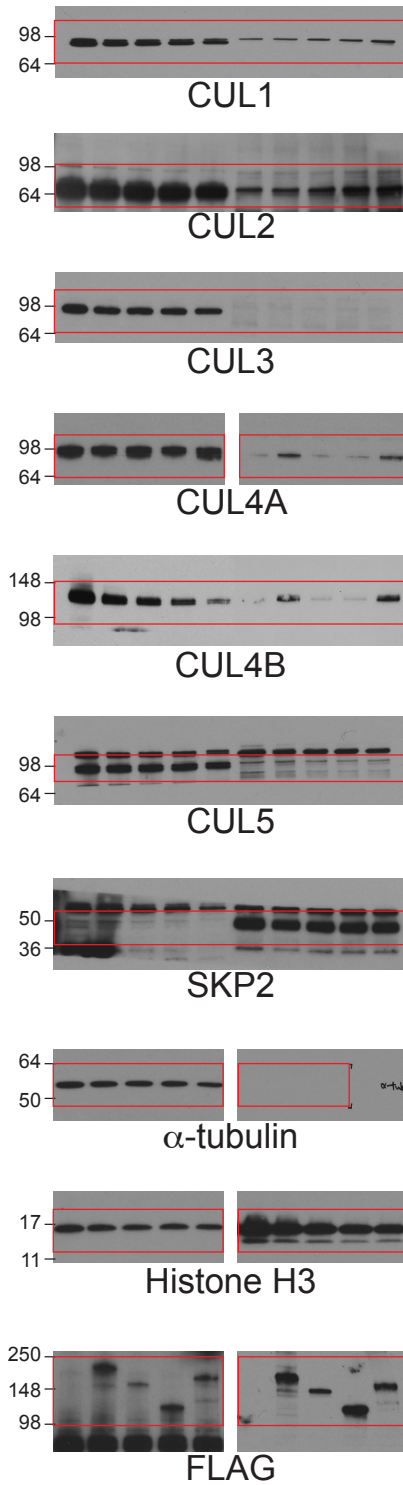


Fig. 1g

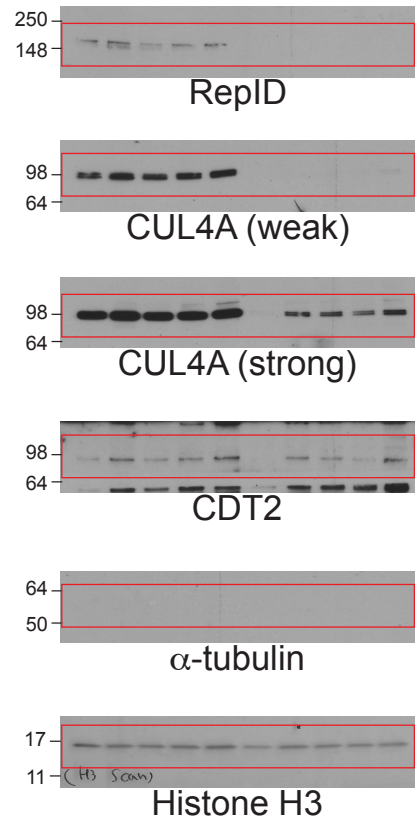


Fig. 2b

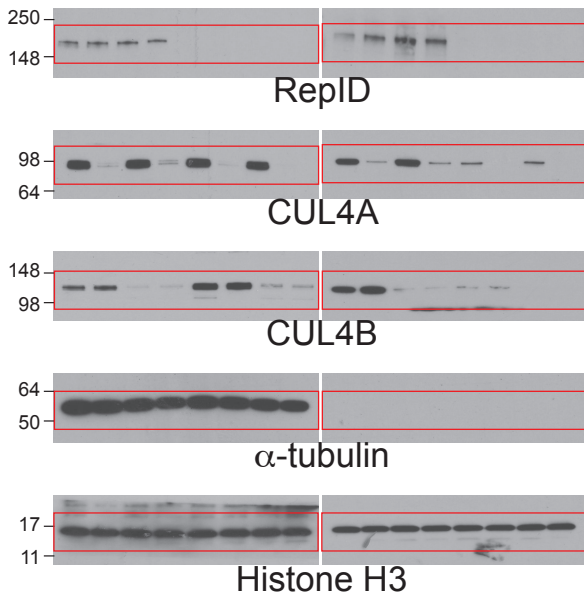


Fig. 2e

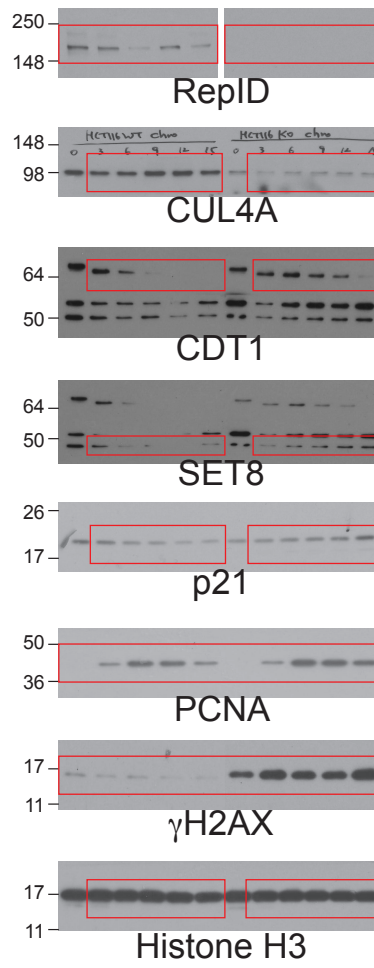


Fig. 4c

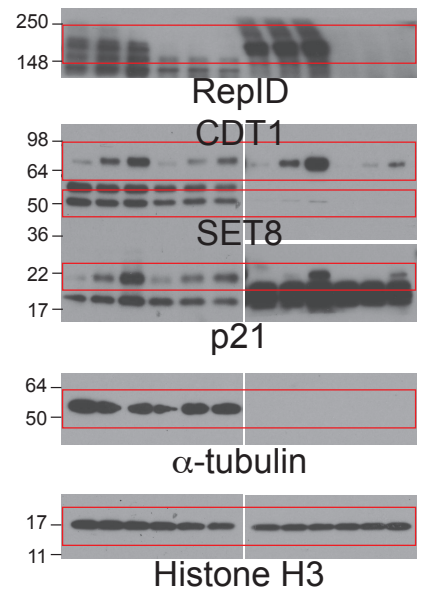


Fig. 4e

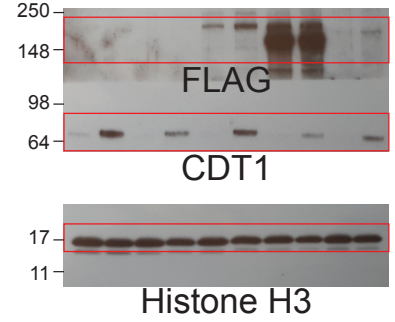


Fig. 5a

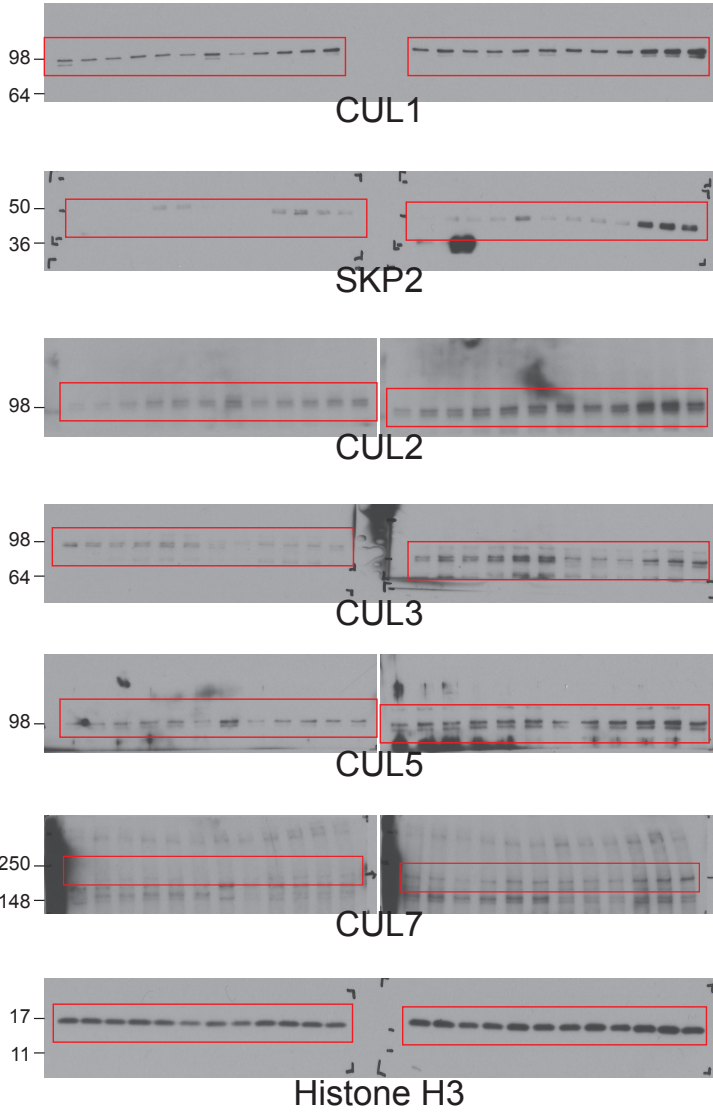
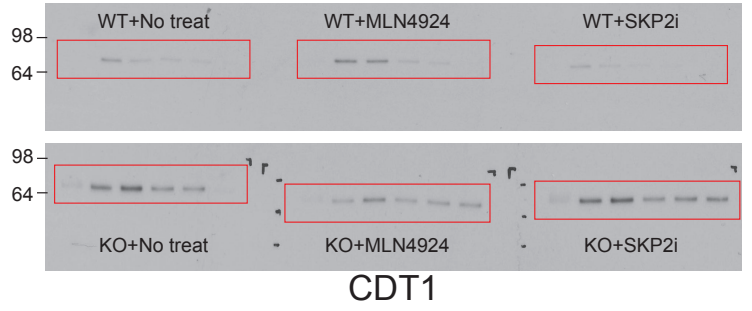
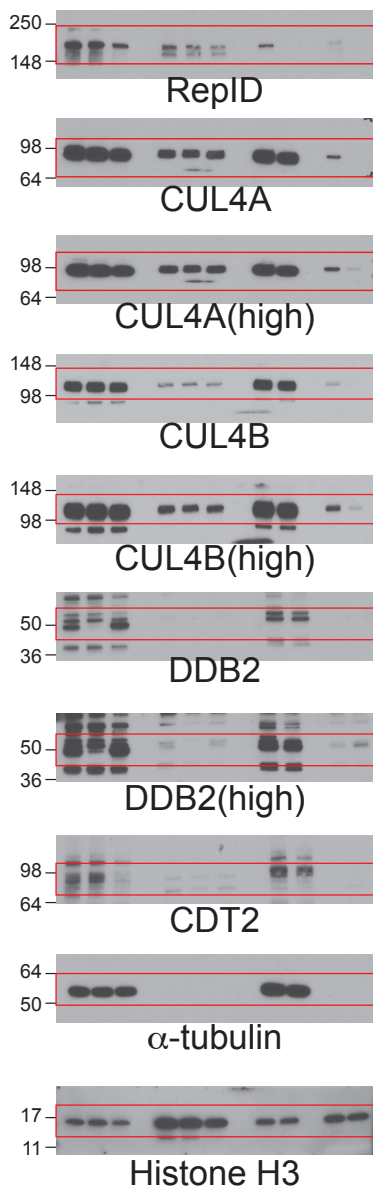


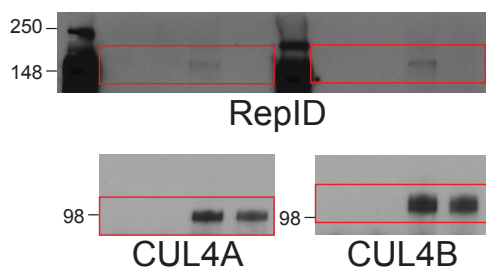
Fig. 5h



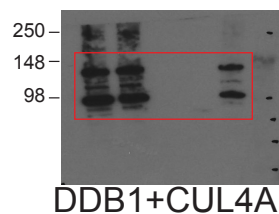
Supplementary Fig. 1b



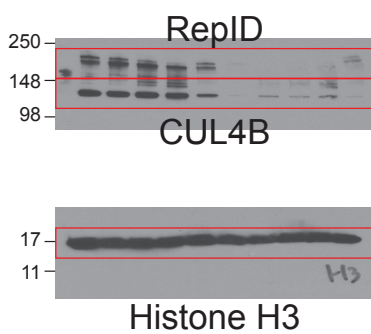
Supplementary Fig. 1c



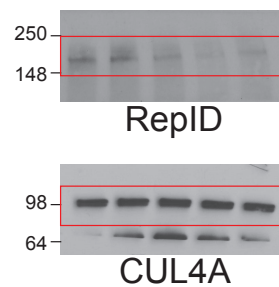
Supplementary Fig. 1d



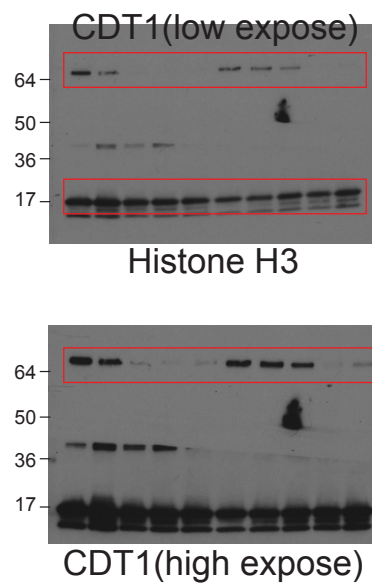
Supplementary Fig. 1e



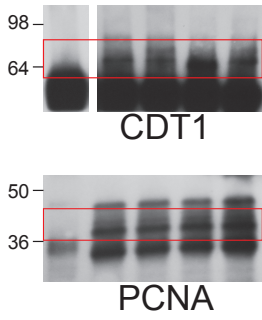
Supplementary Fig. 1f



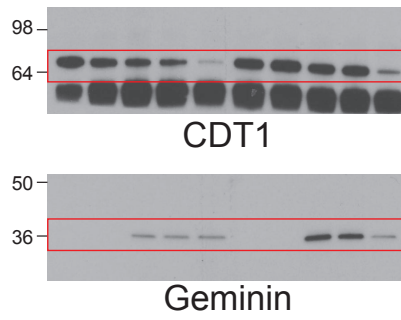
Supplementary Fig. 2b



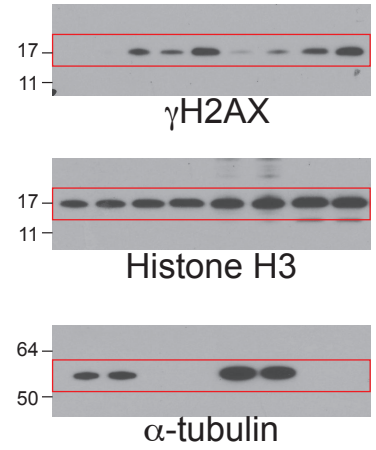
Supplementary Fig. 2c



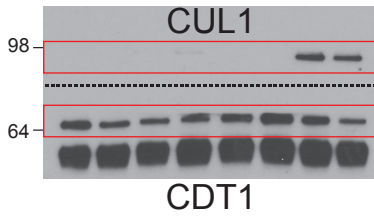
Supplementary Fig. 2d



Supplementary Fig. 2e



Supplementary Fig. 5



Supplementary Figure 6 Unprocessed original scan files for immunoblot analysis.