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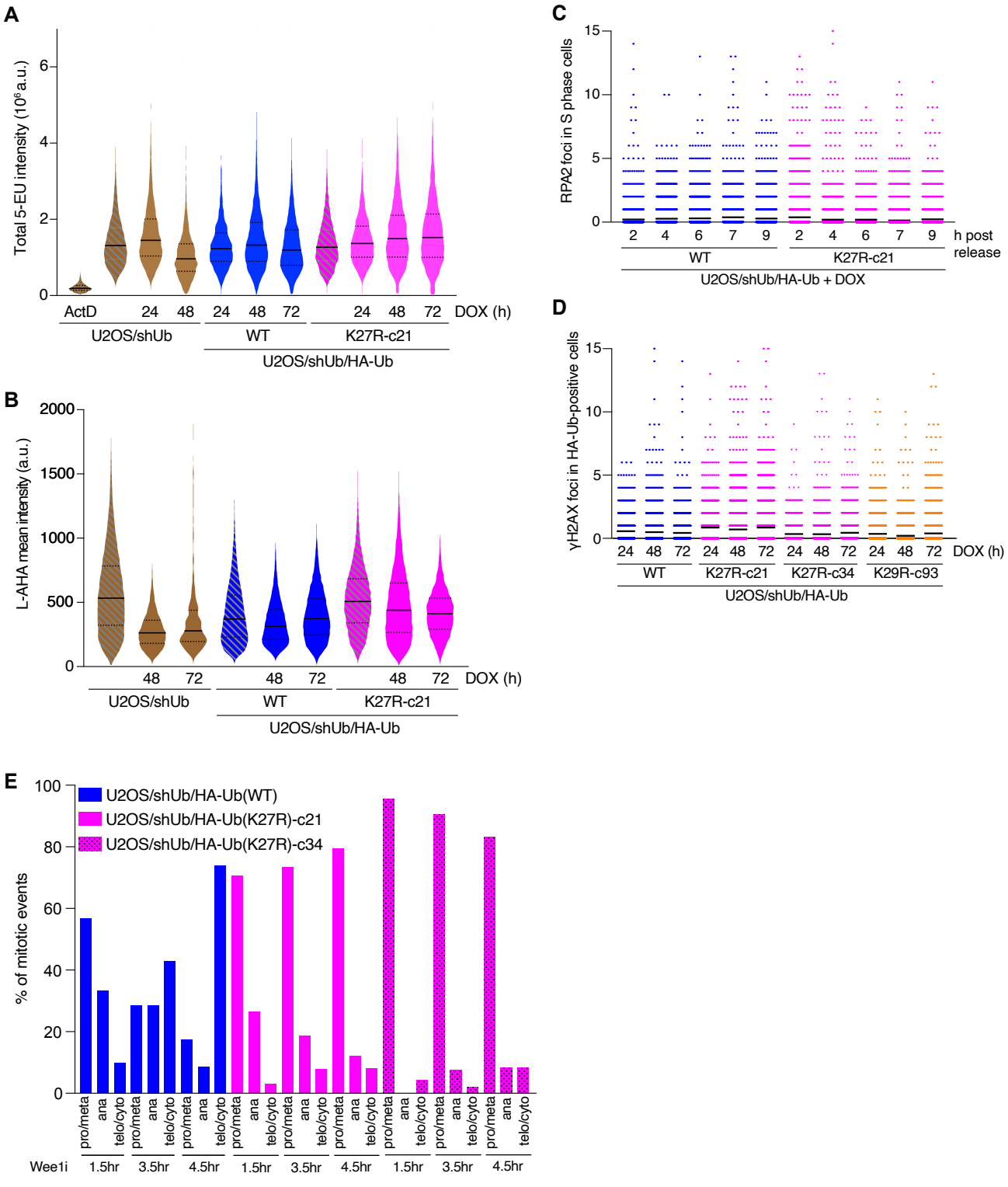
A
Total 5-EU intensity (10⁶a.u.)
ActD
24
48
24
48
72
24
48
72
DOX (h)
U2OS/shUb
WT
K27R-c21
U2OS/shUb/HA-Ub
B
L-AHA mean intensity (a.u.)
48
72
48
72
48
72
DOX (h)
U2OS/shUb
WT
K27R-c21
U2OS/shUb/HA-Ub
C
RPA2 foci in S phase cells
2
4
6
7
9
2
4
6
7
9
h post release
WT
K27R-c21
U2OS/shUb/HA-Ub + DOX
D
γH2AX foci in HA-Ub-positive cells
24
48
72
24
48
72
24
48
72
24
48
72
DOX (h)
WT
K27R-c21
K27R-c34
K29R-c93
U2OS/shUb/HA-Ub
E
U2OS/shUb/HA-Ub(WT)
U2OS/shUb/HA-Ub(K27R)-c21
U2OS/shUb/HA-Ub(K27R)-c34
% of mitotic events
pro/meta
ana
telo/cyto
Wee1i
1.5hr
3.5hr
4.5hr

Appendix Figure S1.
Additional characterization of Ub replacement cell lines
A. Transcriptional activity of U2OS/shUb, U2OS/shUb/HA-Ub(WT) and U2OS/shUb/HA-Ub(K27R) cell lines treated or not with DOX, assayed by QIBC-based analysis of 5-ethynyl uridine (5-EU) incorporation (solid lines, median; dashed lines, quartiles (a.u., arbitrary units); >2000 cells analyzed per condition). The transcriptional inhibitor Actinomycin D (ActD) was used as a negative control. **B**. Translational activity of U2OS/shUb, U2OS/shUb/HA-Ub(WT) and U2OS/shUb/HA-Ub(K27R) cell lines treated or not with DOX, assayed by QIBC-based analysis of incorporation of the methionine analog L-Azidohomoalanine (L-AHA) into new proteins (solid lines, median; dashed lines, quartiles; >5000 cells analyzed per condition). **C**. Quantification of endogenous RPA2 foci in DOX-treated S phase (EdU-positive) U2OS/shUb/HA-Ub cell lines at the indicated time points after release from a double thymidine block (black bars, mean; >2000 S phase cells analyzed per condition). **D**. Quantification of endogenous γH2AX foci of U2OS/shUb/HA-Ub(WT), U2OS/shUb/HA-Ub(K27R) and U2OS/shUb/HA-Ub(K29R) cell lines treated with DOX for the indicated times (black bars, mean; >1500 phase cells analyzed per condition). Cells were gated for Ub replacement based on HA signal positivity. **E**. Live-cell imaging-based quantification of DOX-treated U2OS/shUb/HA-Ub cell lines in different stages of mitosis after treatment with Wee1i for the indicated times.
Data are representative of three (**A,E**) and two (**B,C,D**) independent experiments.