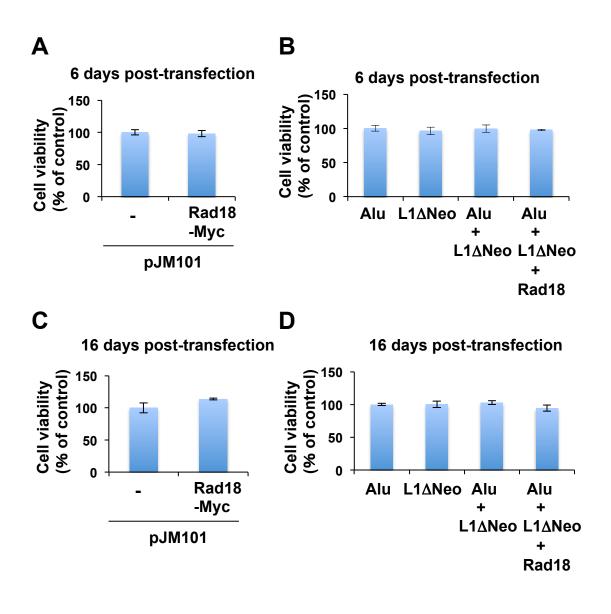
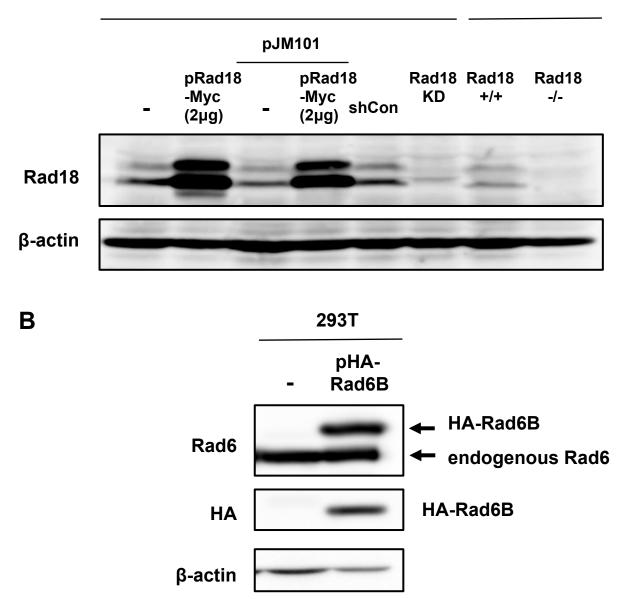
Title: DNA Repair Protein Rad18 restricts LINE-1 mobility

Authors: Yasuo Ariumi, Koudai Kawano, Mariko Yasuda-Inoue, Misao Kuroki, Hiroyuki Fukuda, Rokeya Siddiqui, Priscilla Turelli & Satoshi Tateishi



Supplementary Fig. S1

Effect of Rad18 on cell growth and viability. (**A**, **B**) HeLa cells $(2 \times 10^5 \text{ cells})$ were cotransfected with 2 µg of pJM101 and/or 2 µg of pRad18-Myc (**A**), or 2 µg of pAlu pA +neoTET, 2 µg of pJM101 L1-RP Δ neo and/or 2 µg of pRad18-Myc (**B**). Next day, the cells $(5 \times 10^3 \text{ cells/well})$ were plated onto 96-well plates and cultured for 5 days. (**C**, **D**) HeLa cells $(2 \times 10^5 \text{ cells})$ were co-transfected with 2 µg of pJM101 and/or 2 µg of pRad18-Myc (**C**), or 2 µg of pAlu pA+neoTET, 2 µg of pJM101 L1-RP Δ neo and/ or 2 µg of pRad18-Myc (**D**). After the cells were cultured for 15 days, the cells $(5 \times 10^3 \text{ cells/well})$ were plated onto 96-well plates and cultured for 1day. The cells $(5 \times 10^3 \text{ cells/well})$ were plated onto 96-well plates and cultured for 1day. The cells were subjected to the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) assay according to the manufacturer's instructions (Cell Proliferation Kit I; Roche). The absorbance was read using a microplate reader (xMark microplate spectrophotometer; Bio-rad) at 550 nm with a reference wavelength of 640 nm. The results shown are means from three independent experiments. Error bars indicate standard deviations.



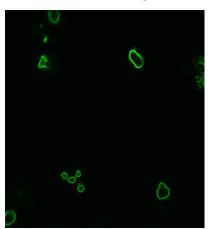
Supplementary Fig. S2

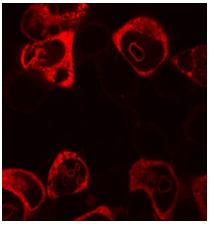
- (A) The levels of Rad18 protein expression were analyzed by Western blotting using the cell lysates of 293T cells, 293T cells trasfeced with 2 μg of pRad18-Myc, 293T cells cotransfected with 2 μg of pJM101 and/or 2 μg of pRad18-Myc, Rad18-knockdown 293T (Rad18KD) or the control cells (shCon), Rad18^{+/+} or Rad18^{-/-} HCT116 cell. The results of Western blot analysis with anti-Rad18 or anti-β-actin antibody are shown.
- (B) The levels of endogenous Rad6 and exogenous HA-Rad6B protein expression were analyzed by Western blotting using the cell lysates of 293T cells trasfeced with 2 μg of pcDNA3-HA or pcDNA3-HA-Rad6B. The results of Western blot analysis with anti-Rad6, anti-HA, or anti-β-actin antibody are shown.

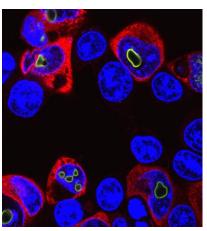
Rad18-Myc

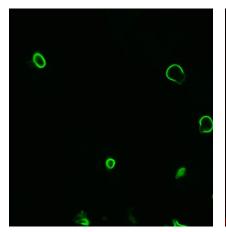
HA-ORF1p

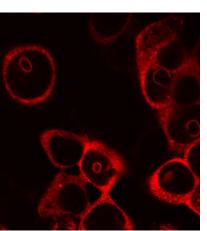
Merged

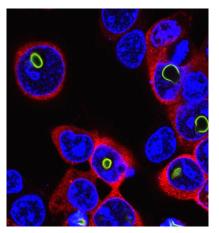


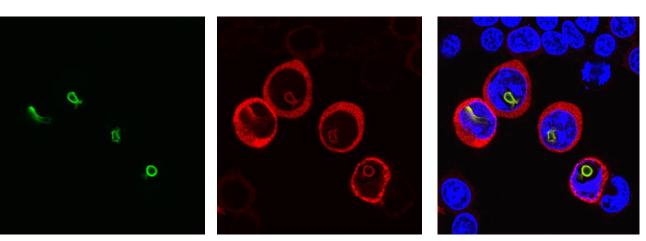












Supplementary Fig. S3

Subcellular localization of Rad18 and L1 ORF1p. 293T cells (2×10⁴ cells/well) cotransfected with 100 ng of Myc-tagged Rad18-expressing plasmid and 100 ng of pcDNA3-HA-L1 ORF1 were examined by confocal laser scanning microscopy. Cells were stained with anti-HA (HA-7) and anti-Myc-tag mAb-Alexa Fluor 488 antibodies and then visualized with Alexa Fluor 594 (L1 ORF1p) or Alexa Fluor 488 (Rad18). Nuclei were stained with DAPI.