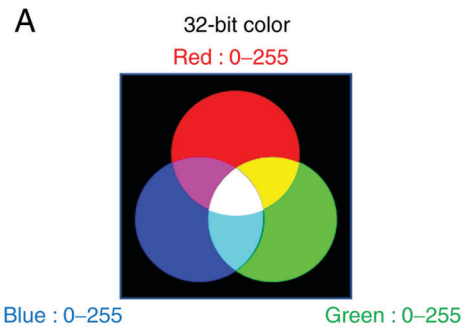
Figure S1. 32-bit colour images. (A) All colours in the 32-bit colour images consisted of red, blue and green ranging from 0-255 signal intensity. (B) Colour examples of DAB (PD-L1), nucleus and background. DAB, 3,3'-Diaminobenzidine, tetrahydrochloride; PD-L1, programmed cell death-1 ligands.



B

		Red		Blue	Green
DAB (PD-L1)		255	>	120	160
Nucleus		80	<	240	70
Background		255	=	255	255

Figure S2. Quantification method of PD-L1 expression and Ku80 positivity using ImageJ and QuPath. (A) Original images of PD-L1 immunohistochemistry obtained by microscopy. The original images which were represented in 32-bit were split into red, blue and green channels using ImageJ. The blue channel signal was subtracted from the red channel signal in each pixel to highlight the PD-L1 signal. The mean signal intensity of the three areas in the tumour tissue area was quantified. (B) Original images of Ku80 immunohistochemistry were obtained via light microscopy. The tumour tissue area was targeted to detect the Ku80-positive cells. The Ku80-positive cells are displayed in red and the Ku80-negative cells are displayed in blue. The Ku80 positivity was quantified using QuPath. Scale bar, 50 μm. PD-L1, programmed cell death-1 ligands.

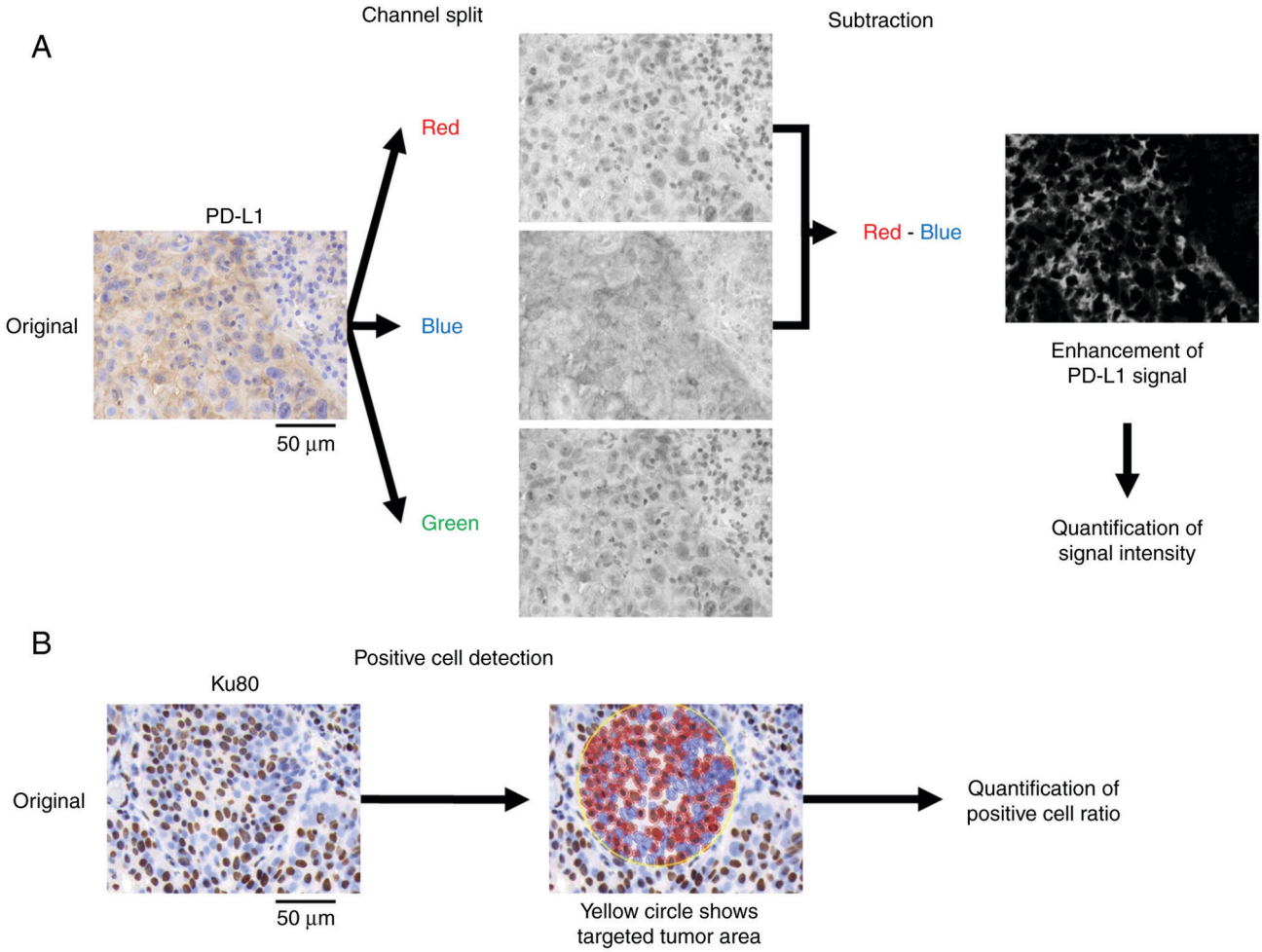


Figure S3. Correlation between Ku80 positivity and cell surface PD-L1 expression. Box and whisker plots present correlation between Ku80 positivity and (A) pre-RT PD-L1 expression, (B) 10 Gy-RT PD-L1 expression and (C) alteration of PD-L1 expression comparing pre-RT and 10 Gy-RT. PD-L1, programmed cell death-1 ligands; RT, radiotherapy.

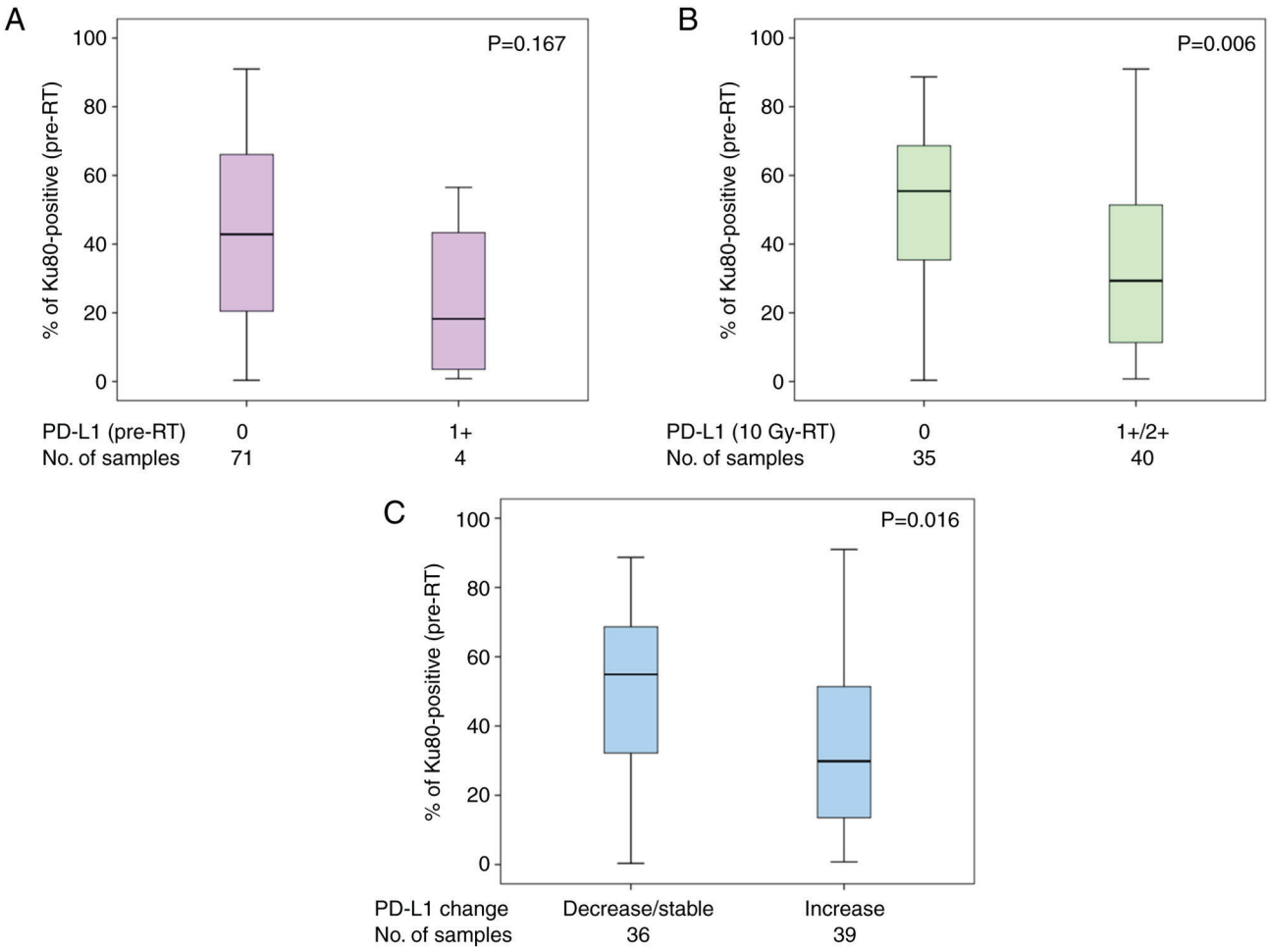


Figure S4. Survival curves of patients with cervical cancer according to Ku80 positivity before RT. Survival curves showing (A) overall survival, (B) progression-free survival and (C) local control after RT using the Kaplan-Meier method. Patients were classified into two groups based on the average value of Ku80 positivity before RT. The P-values indicate the results of the log-rank test. RT, radiotherapy.

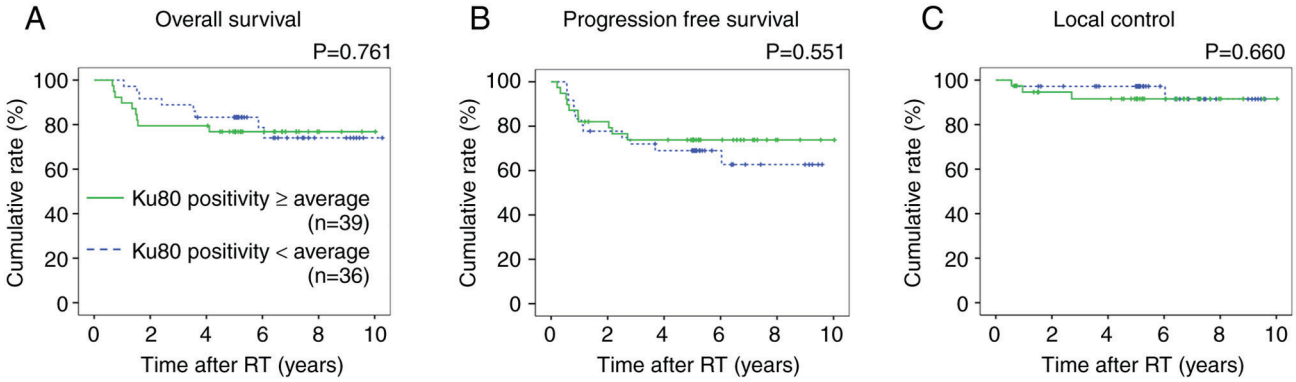


Figure S5. The uncropped blot of Fig. 4A. si, short interfering; cont, control.

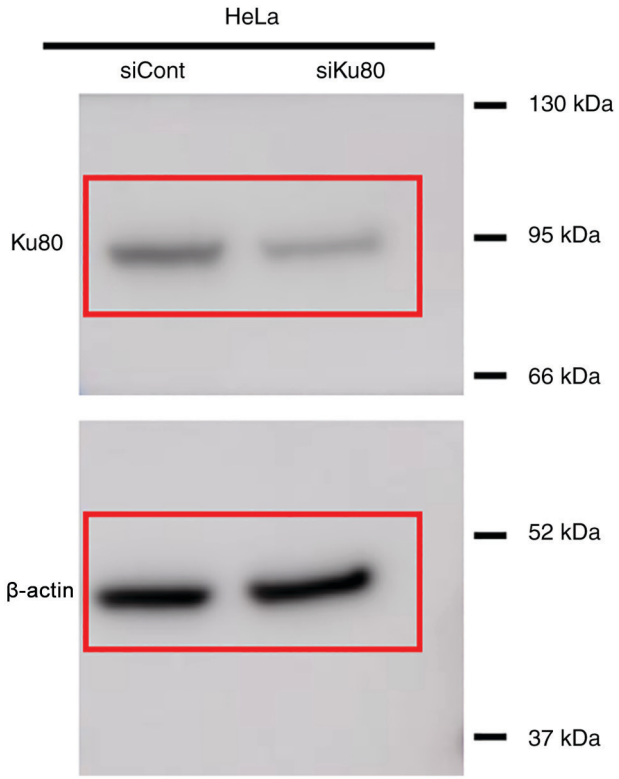


Figure S6. Continued.

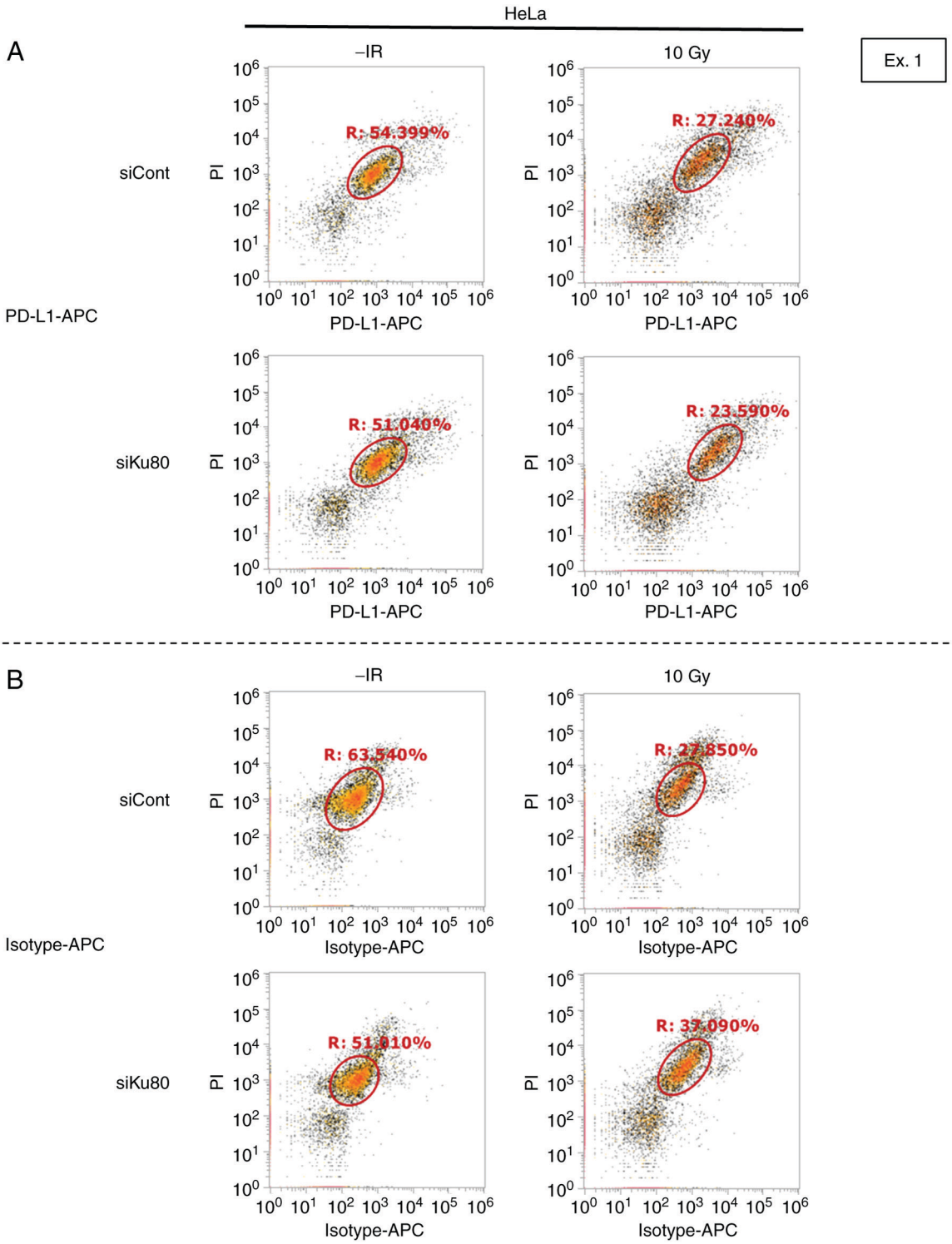


Figure S6. Continued.

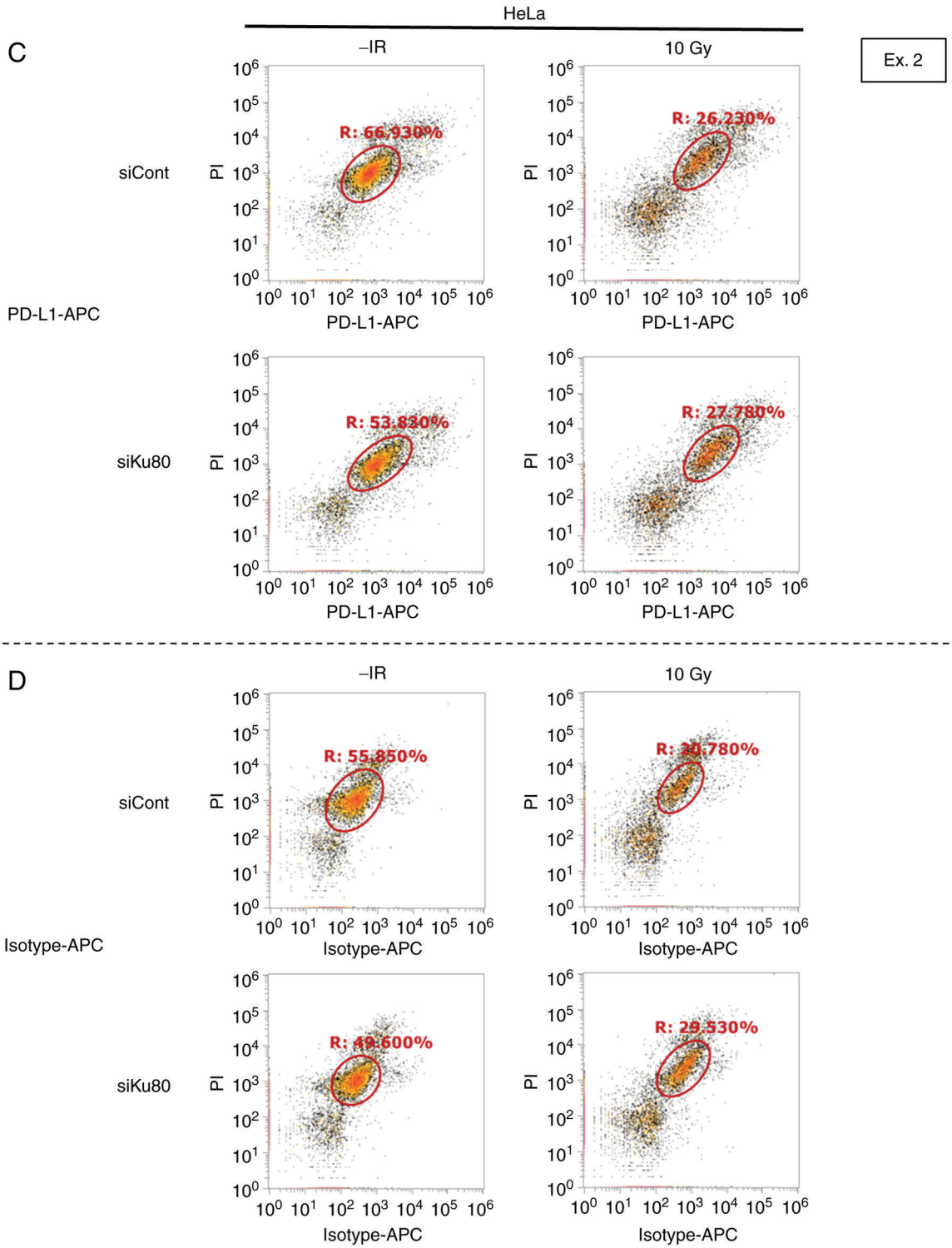


Figure S6. Original plots with population hierarchies of flow cytometry of PD-L1 expression after 10 Gy irradiation of HeLa cells. Cells were stained with PI + PD-L1-APC or isotype-APC to examine PD-L1 expression in live cells. (A) Experiment 1, PD-L1 APC, (B) experiment 1, isotype APC; (C) experiment 2, PD-L1 APC, (D) experiment 2, isotype APC; (E) experiment 3, PD-L1 APC and (F) experiment 3, isotype APC. PD-L1 intensities in live cells gated by ellipses, which excluded dead cells (PI-positive) and debris (PI-negative), were analysed in Fig. 4B and C. PI, propidium iodide; si, short interfering; cont, control; PD-L1, programmed cell death-1 ligands.

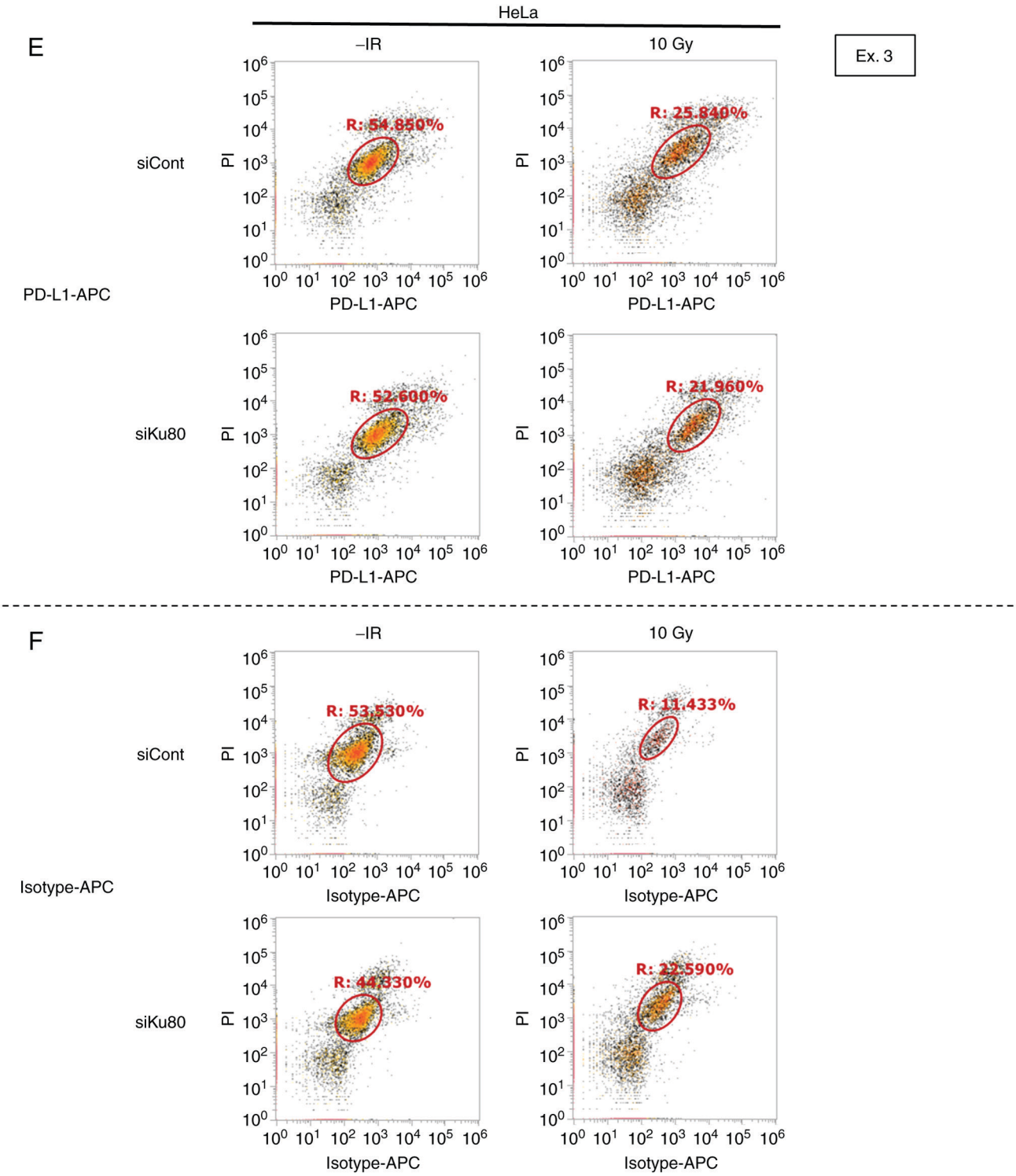


Table SI. List of siRNA used in the present study.

Name	Supplier	Sequence (5'-3') or product name
siKu80	GE Healthcare Dharmacon, Inc.	ON-TARGETplus Mouse XRCC5 (22596) siRNA-SMARTpool (cat. no. L-046264-01-0005)
siControl	Sigma-Aldrich; Merck KGaA	GGGAUACCUAGACGUUCUAdTdT

Si, short interfering; Ku80, ATP-dependent DNA helicase 2 subunit 2.