SUPPLEMENTARY INFORMATION

The depletion of the "don't-eat-me" signal CD47 increases radiosensitivity through phenotypical attenuation of cancer stem cell and EMT properties in Oral squamous cell carcinoma cells

Disclosed by Shin Pai, MD[1,2], Oluwaseun Adebayo Bamodu, MD., PhD[3,4], Yen-Kuang Lin, PhD[5], Chun-Shu Lin, MD[1,6], Pei-Yi Chu, MD., PhD[7], Ming-Hsien Chien, PhD[1], Liang-Shun Wang, MD., PhD[1,3,8], Hsiao M, PhD[9], Chi-Tai Yeh, PhD[1,3,4*], Jo-Ting Tsai, MD., PhD[1, 10,11*]

Authors' affiliation: [1]Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei City, Taiwan; [2]Department of Oral & Maxillofacial Surgery, Saint Martin de Parres Hospital, Chaiyi City, Taiwan; [3]Department of Medical Research and Education, Taipei Medical University - Shuang Ho Hospital, New Taipei City, Taiwan; [4]Department of Hematology and Oncology, Cancer Center, Taipei Medical University - Shuang Ho Hospital, New Taipei City, Taiwan; [5] Biostatistics Center, Taipei Medical University, Taipei, Taiwan, ROC; [6]Department of Radiation Oncology, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; [7]Department of Pathology, Faculty of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan; [8]Department of Thoracic Surgery, Taipei Medical University - Shuang Ho Hospital, New Taipei City, Taiwan; [9]Genomics Research Center, Academia Sinica, Taipei, Taiwan; [10]Department of Radiology, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan; [11]Department of Radiology, Taipei Medical University - Shuang Ho Hospital, New Taipei City, Taiwan.

*Corresponding author(s):

Chi-Tai Yeh, PhD., Department of Medical Research and Education, Taipei Medical University - Shuang Ho Hospital, New Taipei City 23561, Taiwan; Tel: +886-2-2490088 ext. 8881, Fax: +886-2-2248-0900, E-mail: ctyeh@s.tmu.edu.tw

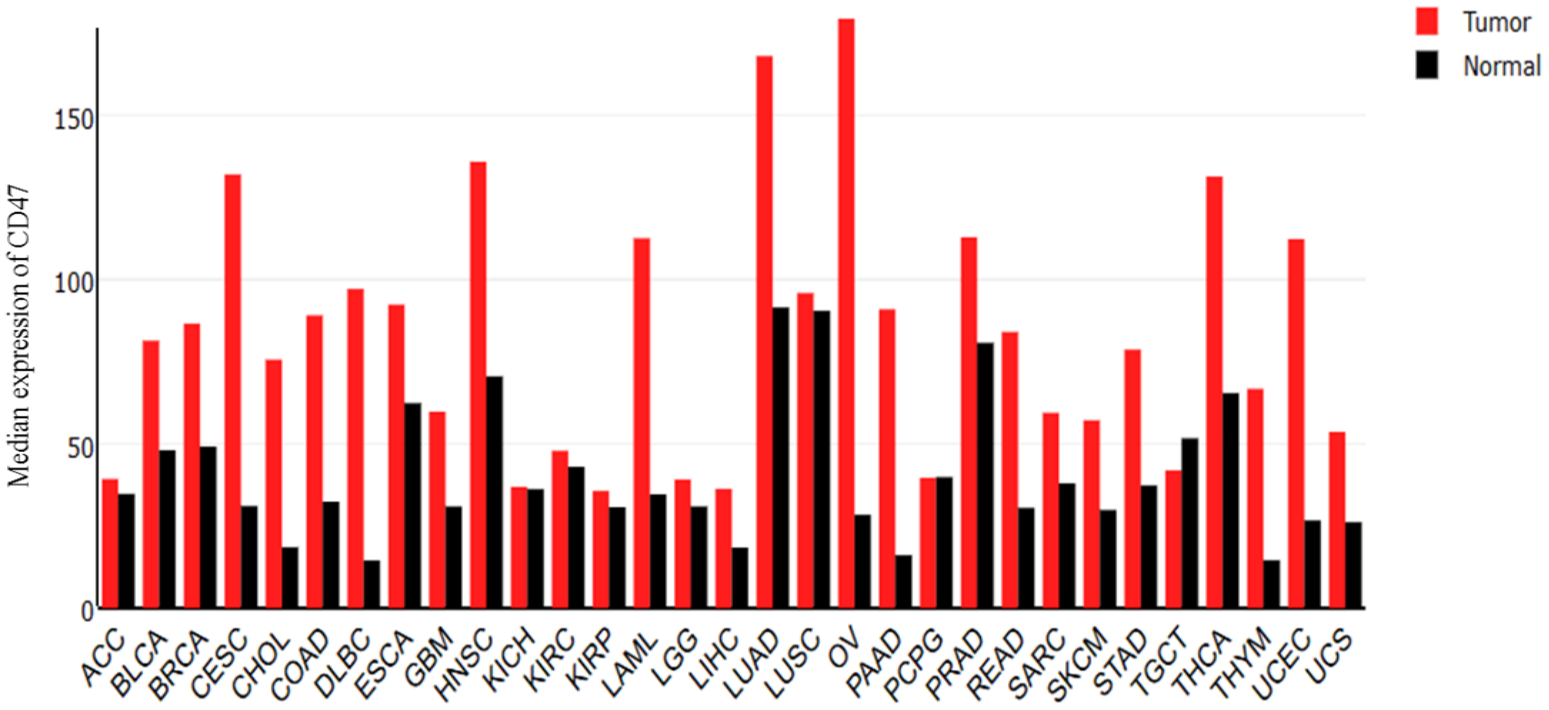
Jo-Ting Tsai, MD., PhD., Department of Radiation Oncology, Cancer Center, Taipei Medical University - Shuang Ho Hospital, New Taipei City 23561, Taiwan; Tel: +886-2-2490088 ext. 8885, Fax: +886-2-2248-0900, E-mail: 10576@s.tmu.edu.tw

*Title Page (with author names and affiliations)

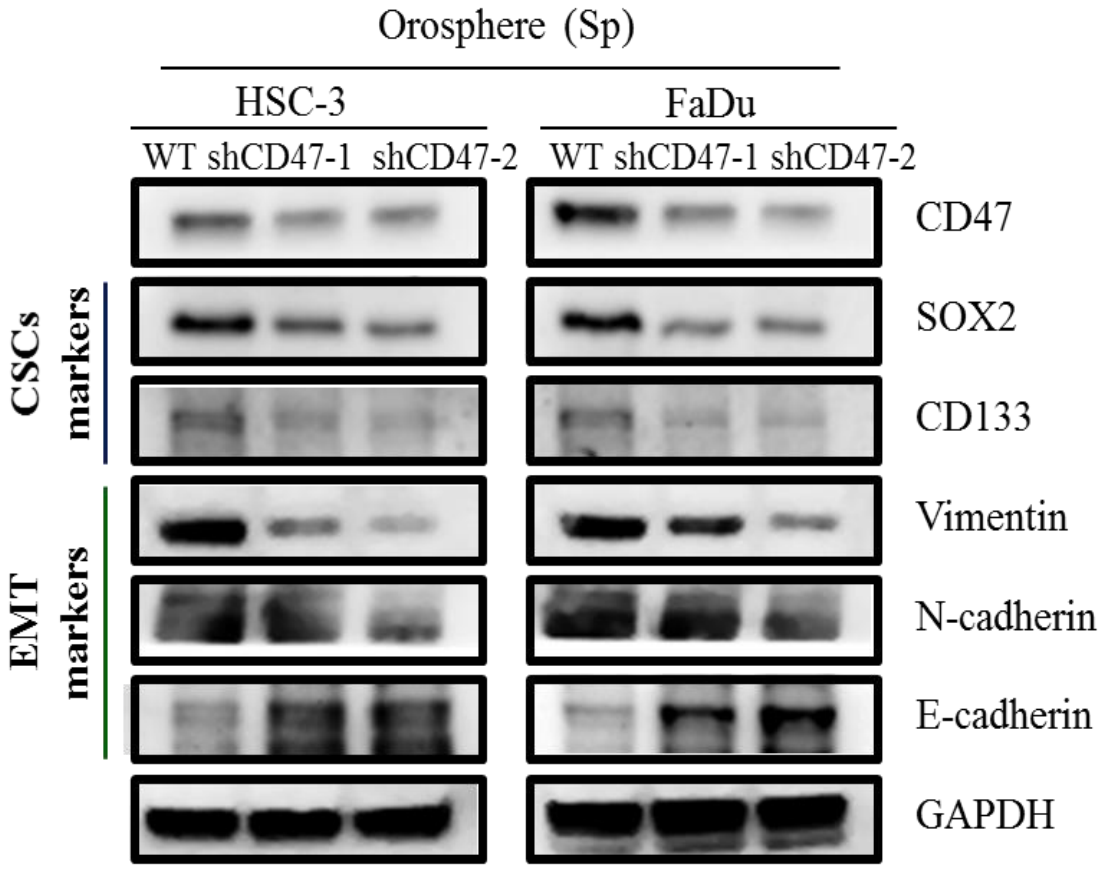
Working title: Targeting the CD47-CSCs-EMT loop enhances radiosensitivity in OSCC

No.	Target	Dilution	Catalog	kDa
1	CD47	1:500	CD47 (B6H12) Mouse mAb SC-12730	47
2	GAPDH	1:500	GAPDH (0411K) Mouse mAb SC-27724	37
3	SOX2	1:1000	SOX2 Rabbit mAb #3579T	35
4	OCT4	1:1000	Oct-4 (C30A3) Rabbit mAb #2840	45
5	CD133	1:1000	CD133 Rabbit mAb 18470-1-AP	120
6	Vimentin	1:1000	VimentinRabbit mAb ab137321	57
7	Slug	1:1000	Slug (C19G7) Rabbit mAb #9585	30
8	Snail	1:1000	Snail Rabbit mAb #3879P	29
9	N-cadherin	1:500	N-cadherin Rabbit mAb #4061	140
10	E-Cadherin	1:500	E-Cadherin (24E10) Rabbit mAb #3195	135

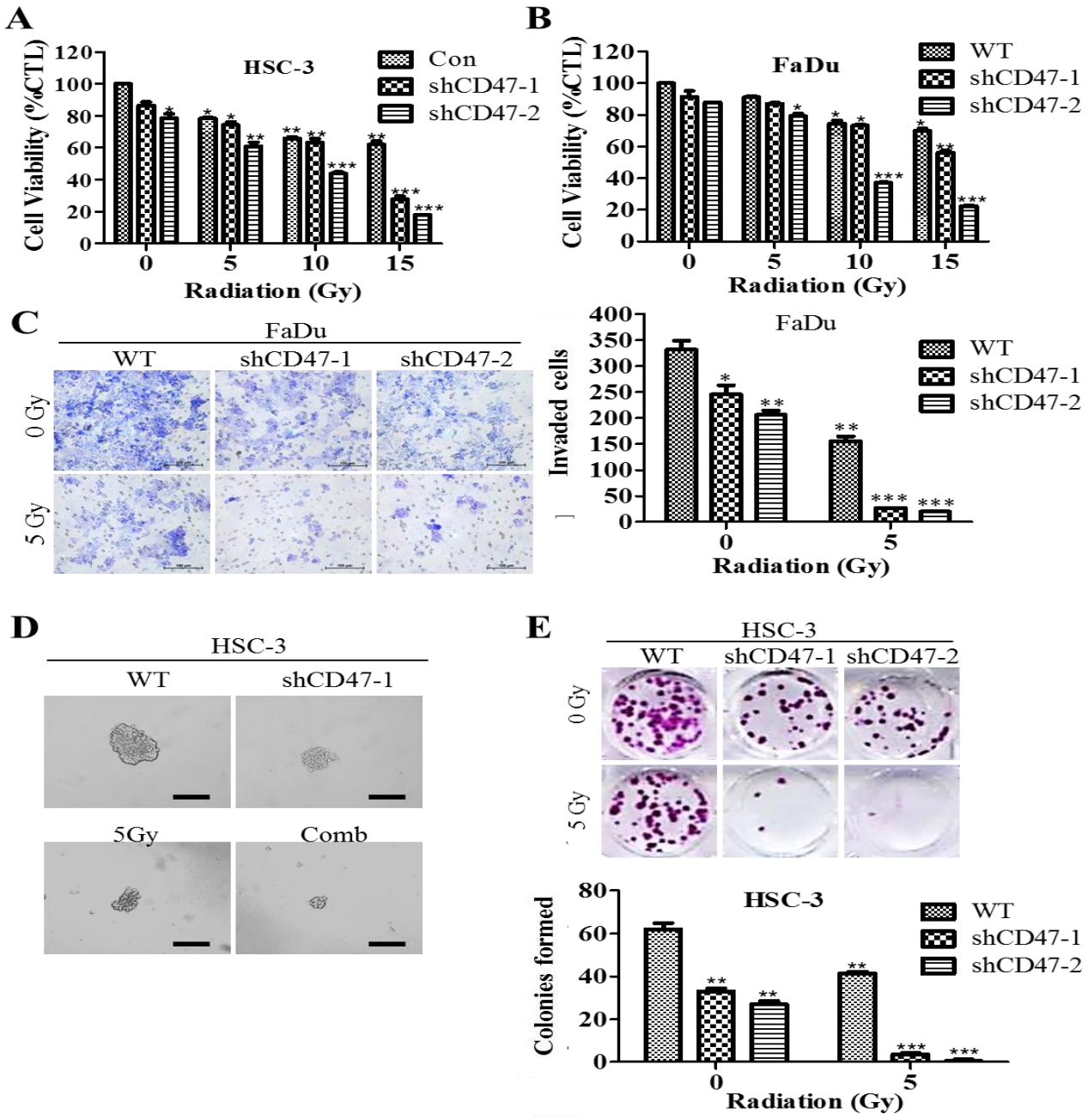
Supplementary Table S1. Western blot antibodies sheet.



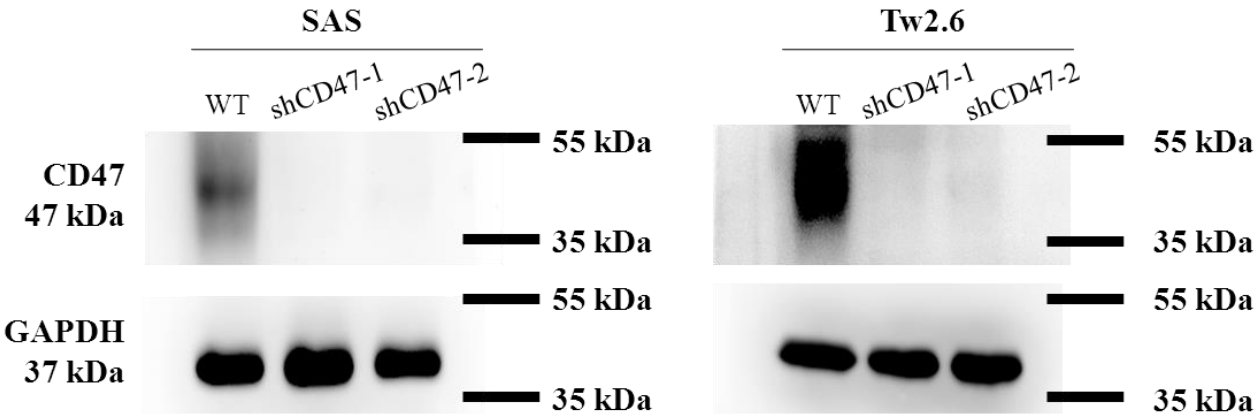
Supplementary Figure S1. CD47 is aberrantly expressed in human oral squamous cell carcinoma. Bar plot of the gene expression profile across all paired tumor samples and normal tissues. The bar height represents the median expression of indicated tumor type or normal tissue.



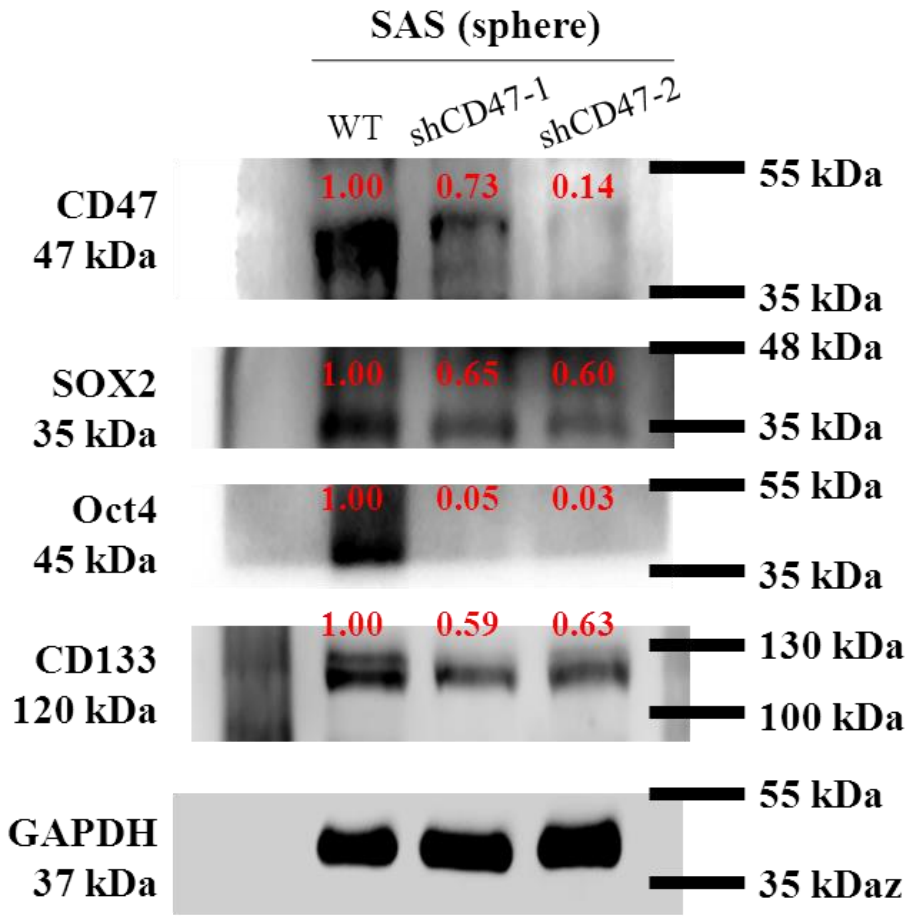
Supplementary Figure S2. CD47 modulates the cancer stem cell-like and metastatic phenotypes of oral squamous cell carcinoma cells. The inhibitory effect of shCD47 on the expression level of CD47, Sox2, CD133, vimentin, N-cadherin, and E-cadherin proteins in HSC-3 and FaDu cells as demonstrated by western blot analyses. GAPDH served as loading control.



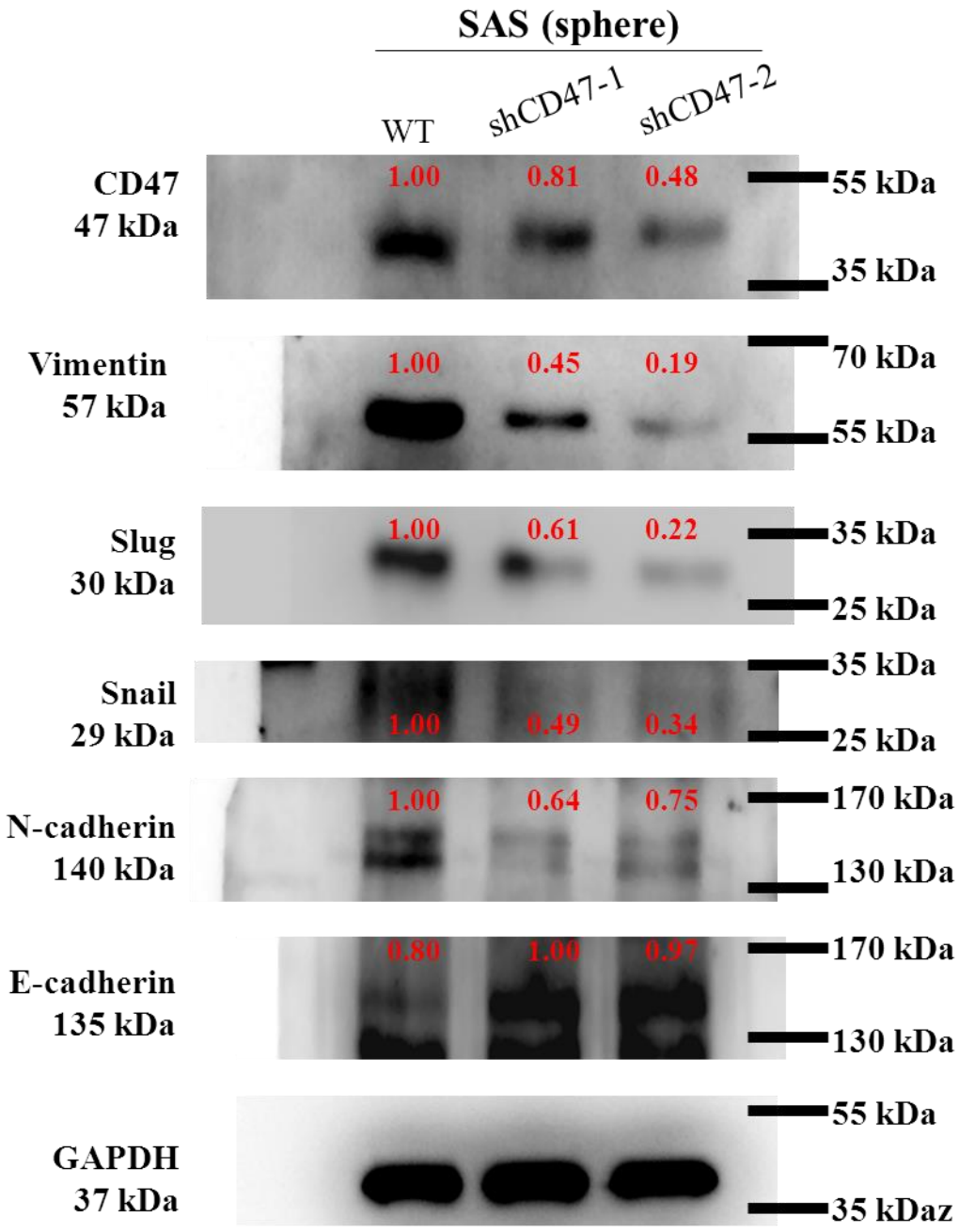
Supplementary Figure S3. Suppression of CD47 expression enhances the sensitivity of OSCC-SCs to radiation therapy. shCD47 with or without 0 Gy - 15 Gy radiation decreased the viability of (A) HSC-3 and (B) FaDu cells dose-dependently. (C) Transwell invasion assay images show reduced invasion in 5 Gy-exposed shCD47 FaDu cells, compared to their WT counterparts. (D) shCD47-transfected HSC-3 cells exposed to 5 Gy yielded smaller tumorspheres compared to their WT, shCD47, or 5 Gy alone counterparts. (E) shCD47-1 or shCD47-2 HSC-3 cells formed fewer colonies when exposed to 5 Gy, compared to the WT alone cells. *p<0.05, **p<0.01, ***p<0.001



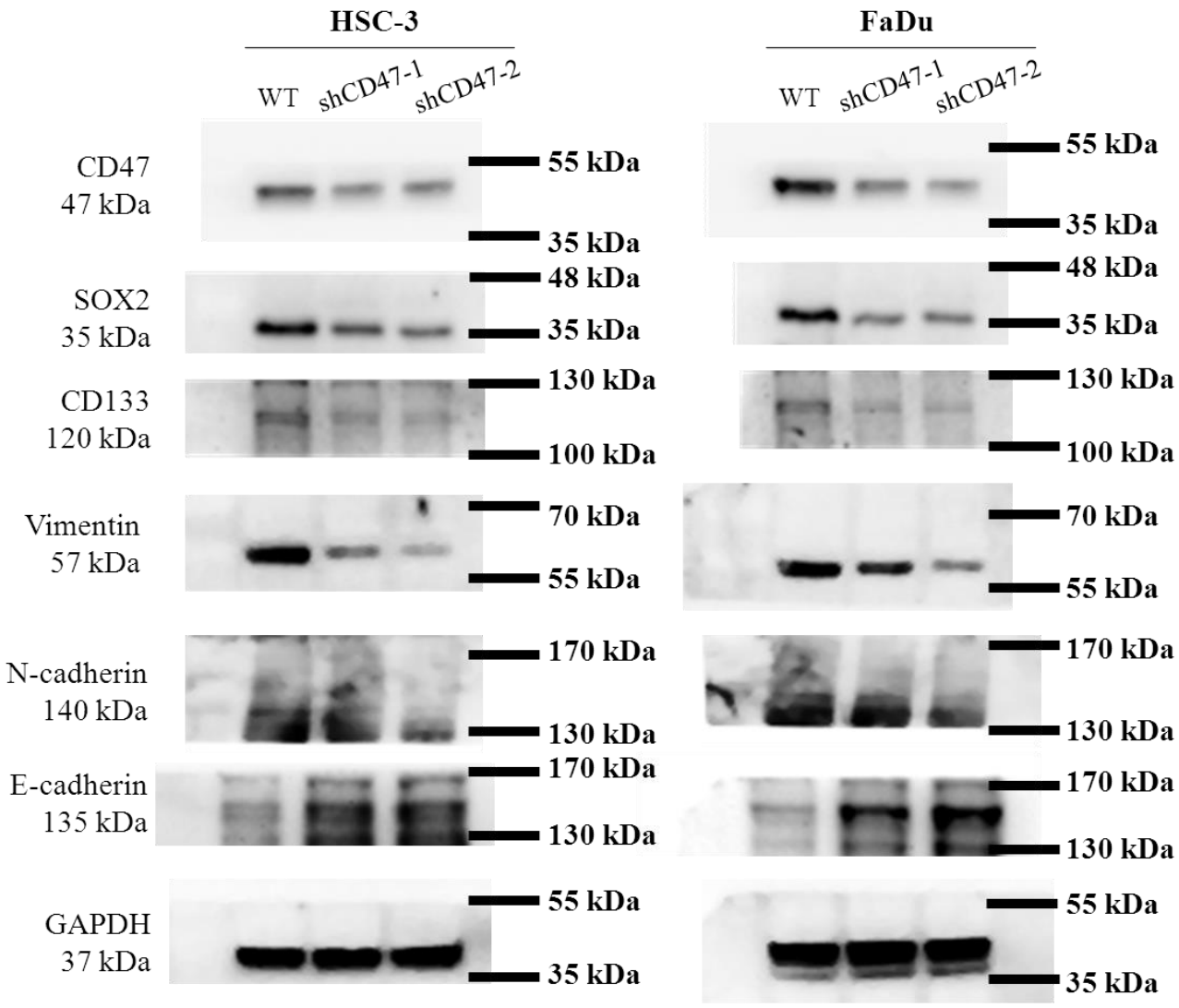
Supplementary Figure S4. Full-size blots of Figure 3B



Supplementary Figure S5. Full-size blots of Figure 3C



Supplementary Figure S6. Full-size blots of Figure 4D



Supplementary Figure S7. Full-size blots of Figure S1