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Supplemental information

An activity-based functional test for identifying homologous recombination deficiencies across cancer types in real time

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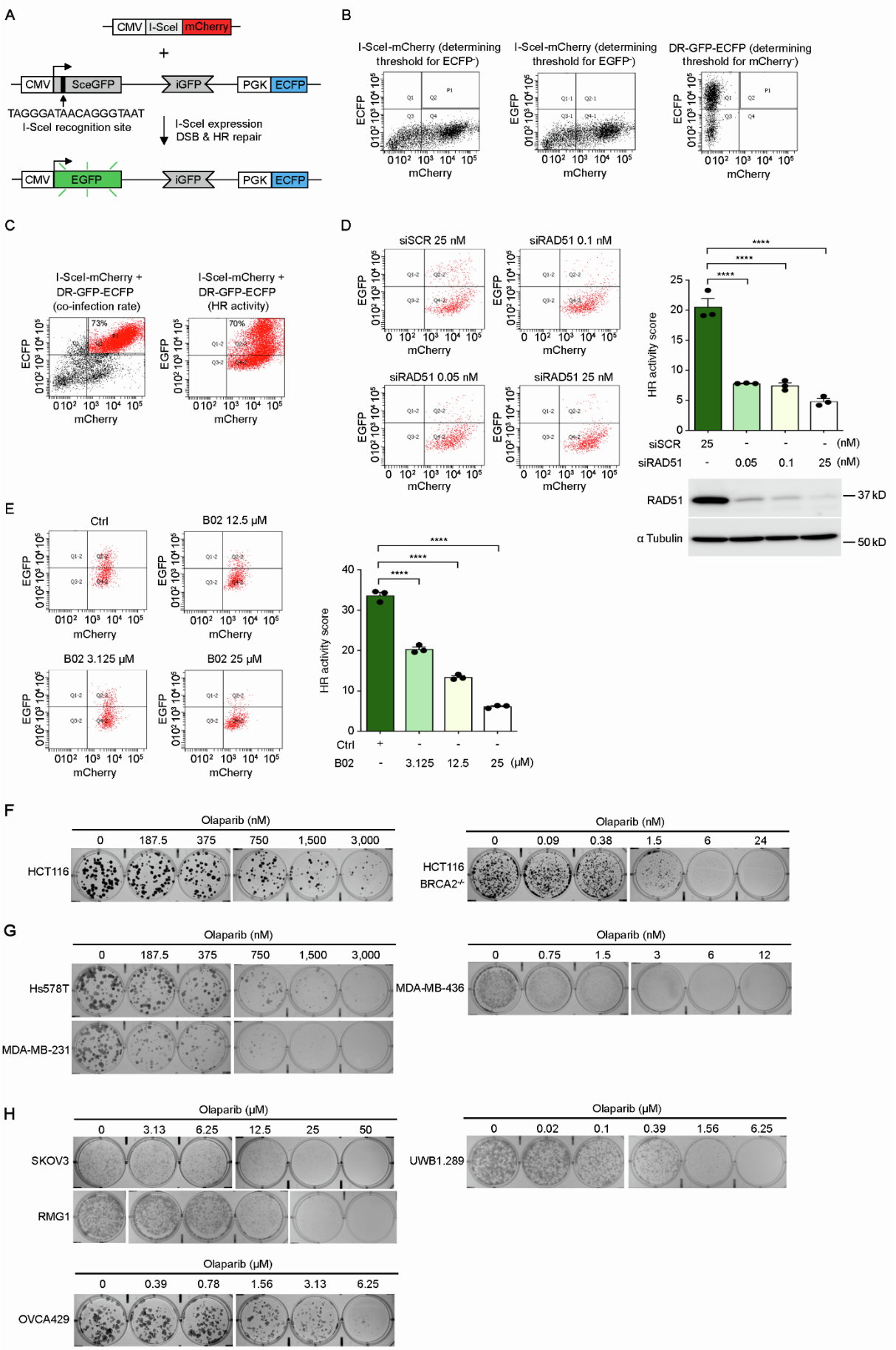
A
CMV I-SceI mCherry
+
CMV SceGFP
iGFP
PGK ECFP
TAGGGATAACAGGGTAAT
I-SceI recognition site
I-SceI expression
DSB & HR repair
CMV EGFP
iGFP
PGK ECFP
B
I-SceI-mCherry (determining threshold for ECFP-)
I-SceI-mCherry (determining threshold for EGFP-)
DR-GFP-ECFP (determining threshold for mCherry-)
ECFP
EGFP
mCherry
C
I-SceI-mCherry + DR-GFP-ECFP (co-infection rate)
I-SceI-mCherry + DR-GFP-ECFP (HR activity)
73%
70%
D
siSCR 25 nM
siRAD51 0.1 nM
siRAD51 0.05 nM
siRAD51 25 nM
HR activity score
siSCR 25 - - - (nM)
siRAD51 - 0.05 0.1 25 (nM)
RAD51
α Tubulin
37 kD
50 kD
E
Ctrl
B02 12.5 μM
B02 3.125 μM
B02 25 μM
HR activity score
Ctrl + - - -
B02 - 3.125 12.5 25 (μM)
F
Olaparib (nM)
0 187.5 375 750 1,500 3,000
HCT116
0 0.09 0.38 1.5 6 24
HCT116 BRCA2-/-
G
Olaparib (nM)
0 187.5 375 750 1,500 3,000
Hs578T
MDA-MB-231
0 0.75 1.5 3 6 12
MDA-MB-436
H
Olaparib (μM)
0 3.13 6.25 12.5 25 50
SKOV3
RMG1
0 0.02 0.1 0.39 1.56 6.25
UWB1.289
Olaparib (μM)
0 0.39 0.78 1.56 3.13 6.25
OVCA429

Figure S1. The activity-based functional test reflects real-time HR activity. Related to Figure 1.

(A) Schematic of the fluorescence-based functional assay to quantify HR activity.

(B and C) Gating strategies for analyzing cellular HR activity. The endonuclease I-SceI was fused with the red fluorescent protein mCherry. The HR reporter cassette, DR-GFP, was labeled with the cyan fluorescent protein ECFP. An analysis of the representative ovarian cancer cell line SKOV3 by flow cytometry is shown here. (B) The fluorescence threshold for ECFP-negative (left) or EGFP-negative cells (middle) was determined by infection with the adenovirus encoding I-SceI-mCherry alone. Infection with adenovirus containing the DNA sequence of DR-GFP-ECFP defined the fluorescence threshold for mCherry-negative cells (right). (C) The ECFP-positive and mCherry-positive cells simultaneously infected with two types of adenoviruses were gated as the P1 population (left). The proportion of EGFP-positive cells in the P1 population was quantified as reflecting HR activity (right).

(D) Osteosarcoma U2OS cells were transfected with the indicated concentration of scrambled siRNA (SCR) or RAD51 siRNA for 24 h. The cells were harvested for analyses 72 h after infection with adenoviruses to quantify the HR activity scores. The level of RAD51 and tubulin was examined by immunoblotting.

(E) Cervical carcinoma HeLa cells were treated with the indicated concentration of B02 for 4 h, followed by infection with adenoviruses for 72 h. The HR activity scores were quantified by flow cytometry.

(F-H) Representative images of cells stained with crystal violet 12 days after treatment with olaparib. (F) Paired colorectal carcinoma HCT116 cells. (G) Triple-negative breast cancer cell lines. (H) Ovarian cancer cell lines.

****$P < 0.0001$. Data are the mean ± s.e.m. from three independent experiments (n = 3 biological replicates). Statistical analysis was performed by one-way ANOVA with Tukey's post hoc test.

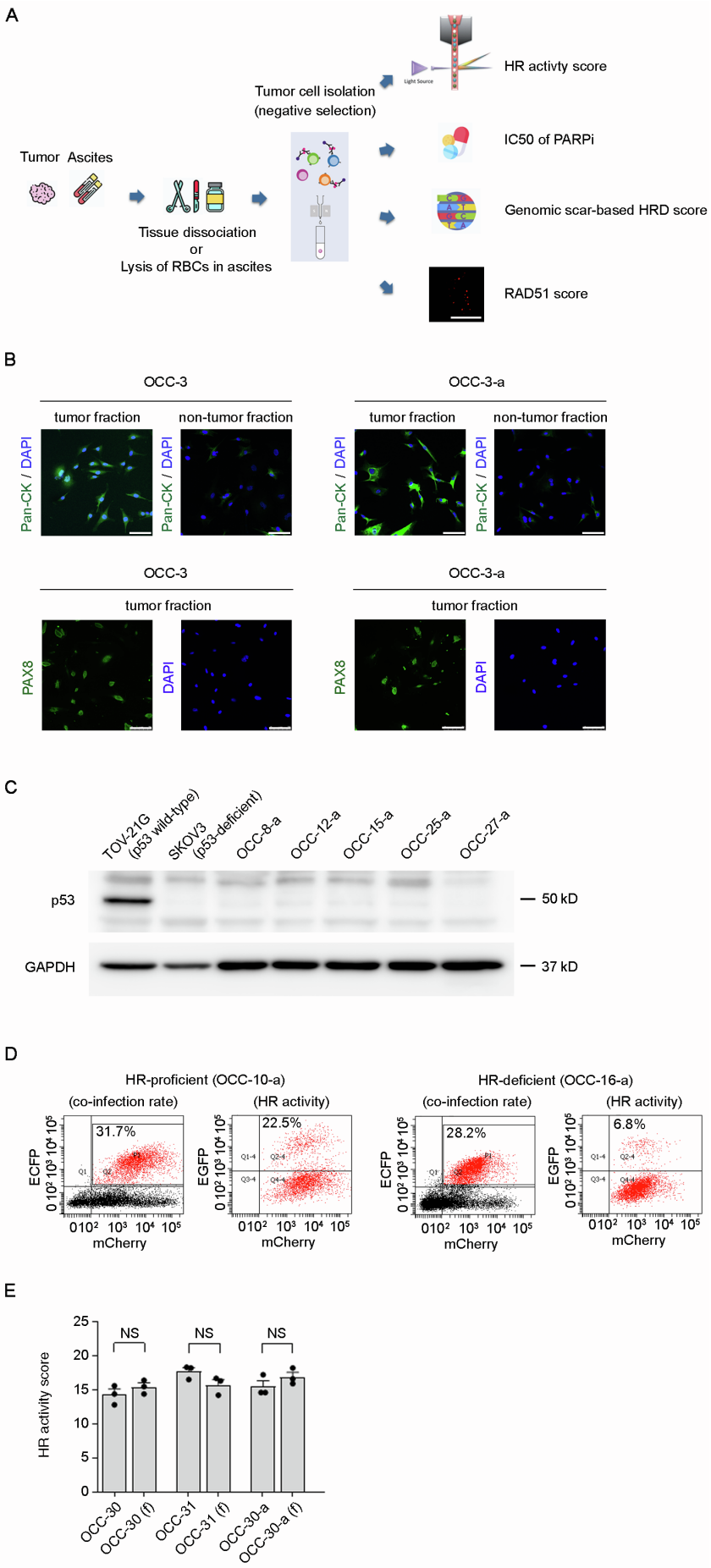
A
Tumor
Ascites
Tissue dissociation
or
Lysis of RBCs in ascites
Tumor cell isolation
(negative selection)
HR activty score
IC50 of PARPi
Genomic scar-based HRD score
RAD51 score
B
OCC-3
OCC-3-a
tumor fraction
non-tumor fraction
Pan-CK / DAPI
PAX8
DAPI
C
TOV-21G (p53 wild-type)
SKOV3 (p53-deficient)
OCC-8-a
OCC-12-a
OCC-15-a
OCC-25-a
OCC-27-a
p53
GAPDH
50 kD
37 kD
D
HR-proficient (OCC-10-a)
HR-deficient (OCC-16-a)
(co-infection rate)
(HR activity)
31.7%
22.5%
28.2%
6.8%
ECFP
EGFP
mCherry
E
HR activity score
NS
OCC-30
OCC-30 (f)
OCC-31
OCC-31 (f)
OCC-30-a
OCC-30-a (f)

Figure S2. Successful application of the activity-based assay to clinical samples. Related to Figure 2.

(A) Schematic workflow for the clinical test. Primary ovarian cancer cells were isolated from clinical samples and then processed, before being analyzed for their HR activity score, cell sensitivity to a PARPi, genomic scar-based HRD score, and RAD51 score. Red blood cells (RBCs) in the ascites samples were lysed with ammonium chloride solution before undergoing downstream isolation and analysis. Scale bar, 15 μm.

(B) Representative images of immunofluorescent stained primary ovarian cancer cells. Pan-CK antibody recognizes multiple forms of cytokeratin, an epithelial cell marker. DNA was counterstained with 4',6-diamidino-2-phenylindole (DAPI). Strong expression of PAX8, an ovarian cancer-associated transcription factor, was confirmed by immunostaining. Scale bar, 75 μm. The primary ovarian cancer cells OCC-3 and OCC-3-a were isolated from an individual patient's tumor tissue and ascites, respectively.

(C) The level of p53 and GAPDH was examined by immunoblotting.

(D) The HR activity of primary ovarian cancer cells was quantified by analyzing the percentage of EGFP-positive cells in the gated P1 population (ECFP-positive and mCherry-positive cells). Two representative cancer cases are shown here.

(E) The activity-based functional assay was applied to freshly processed clinical samples (OCC-30, OCC-31, and OCC-30-a) and frozen clinical samples (OCC-30 (f), OCC-31 (f), and OCC-30-a (f)). The primary ovarian cancer cells derived from the patient's tumor tissue have been labeled in the form OCC-X, whereas those derived from the patients' ascites have been labeled as OCC-X-a. NS, not significant ($P > 0.05$). Data are the mean ± s.e.m. from three independent experiments (n = 3 biological replicates). Statistical analysis was performed by unpaired two-tailed Student's *t*-test.

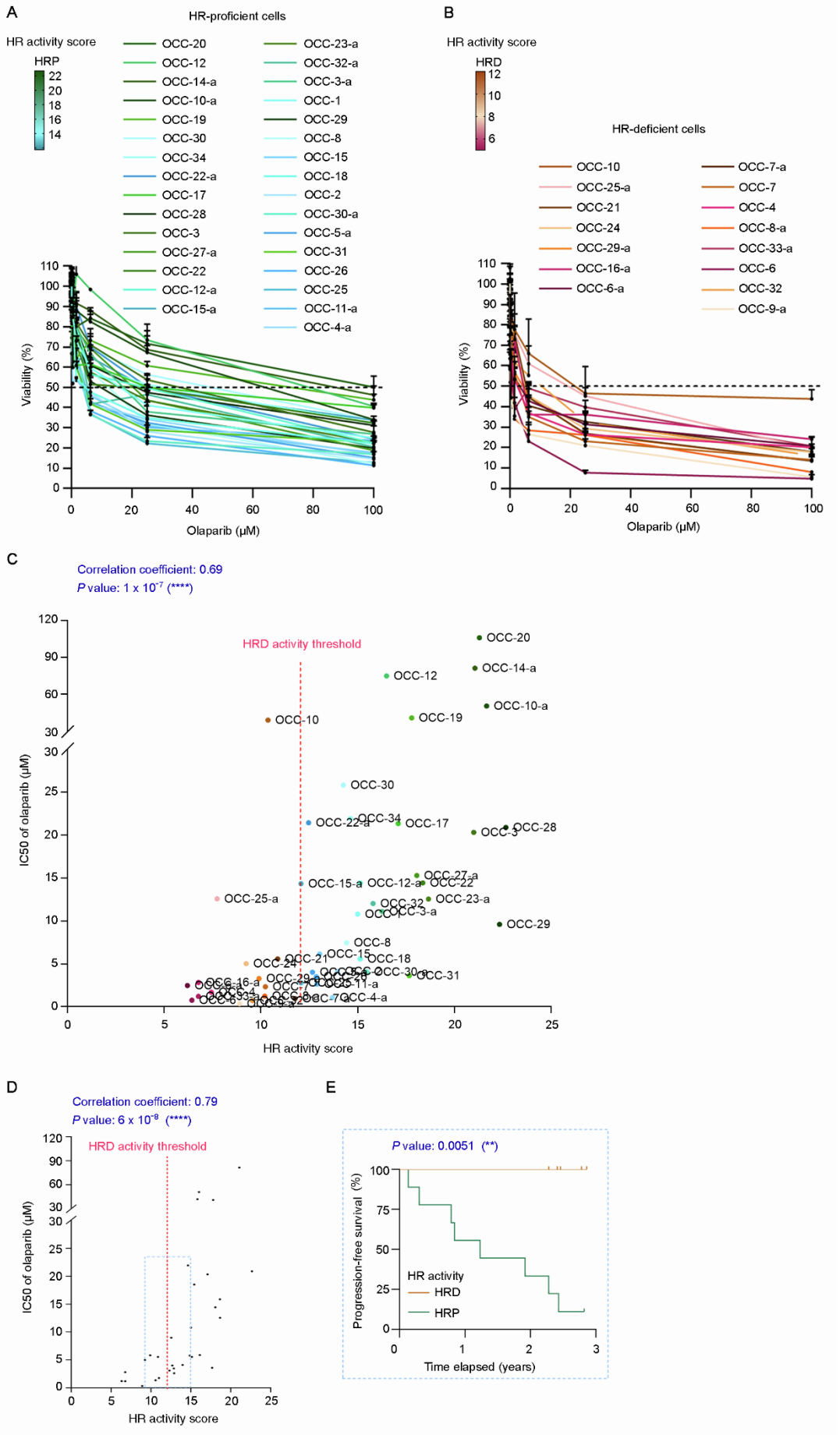
A
HR-proficient cells
HR activity score
HRP
22
20
18
16
14
OCC-20
OCC-12
OCC-14-a
OCC-10-a
OCC-19
OCC-30
OCC-34
OCC-22-a
OCC-17
OCC-28
OCC-3
OCC-27-a
OCC-22
OCC-12-a
OCC-15-a
OCC-23-a
OCC-32-a
OCC-3-a
OCC-1
OCC-29
OCC-8
OCC-15
OCC-18
OCC-2
OCC-30-a
OCC-5-a
OCC-31
OCC-26
OCC-25
OCC-11-a
OCC-4-a
Viability (%)
Olaparib (μM)
B
HR activity score
HRD
12
10
8
6
HR-deficient cells
OCC-10
OCC-25-a
OCC-21
OCC-24
OCC-29-a
OCC-16-a
OCC-6-a
OCC-7-a
OCC-7
OCC-4
OCC-8-a
OCC-33-a
OCC-6
OCC-32
OCC-9-a
Viability (%)
Olaparib (μM)
C
Correlation coefficient: 0.69
P value: 1 x 10-7 (****)
HRD activity threshold
IC50 of olaparib (μM)
HR activity score
D
Correlation coefficient: 0.79
P value: 6 x 10-8 (****)
HRD activity threshold
IC50 of olaparib (μM)
HR activity score
E
P value: 0.0051 (**)
Progression-free survival (%)
HR activity
HRD
HRP
Time elapsed (years)

Figure S3. The activity-based assay assesses homologous recombination deficiency in primary ovarian cancer cells. Related to Figures 2 and 3.

(A and B) Primary ovarian cancer cells were treated with olaparib for 4 days, followed by measurement of the IC_{50} of olaparib by alamarBlue cell viability assay. The dashed line represents 50% viability. The heatmaps for color palettes and associated HR activity scores are indicated. (A) The survival curves for HR-proficient cells with HR activity scores >12.

(B) The survival curves for HR-deficient cells with HR activity scores ≤ 12. The primary ovarian cancer cells derived from an individual patient's tumor tissue have been labeled in the form OCC-X, with respective ascites being labeled as OCC-X-a.

(C) HR activity score and PARPi sensitivity of cancer cells derived from tumor tissue or ascites were analyzed separately and then subjected to the Spearman correlation test, assessing the relationship between the two variables.

(D) A Spearman correlation test was used to measure the relationship between the HR activity score and PARPi sensitivity of primary ovarian cancer cells. For patients with multiple clinical samples, HR activity has been calculated as their average. The blue dashed box indicates the overlapping region of HR activity scores from 9 to 15, where the HRD and HRP groups exhibited some overlap in PARPi sensitivity.

(E) Kaplan-Meier analysis for the progression-free survival in 14 patients whose ovarian tumors showed HR activity scores from 9 to 15 in the functional test. The brown line represents patients with HRD tumors, while the green line represents those with HRP tumors. All patients received platinum-based chemotherapy. Statistical analysis of survival benefits was performed by log-rank test.

Data are the mean ± s.e.m. from three independent experiments (n = 3 biological replicates).

Each data point shown in (D) is the mean from at least three independent experiments (n = 3, biological replicates); thirteen of them, which represent patients with multiple clinical samples, are the mean from six independent experiments (n = 6 biological replicates). $^{**}P < 0.01$; $^{****}P < 0.0001$.

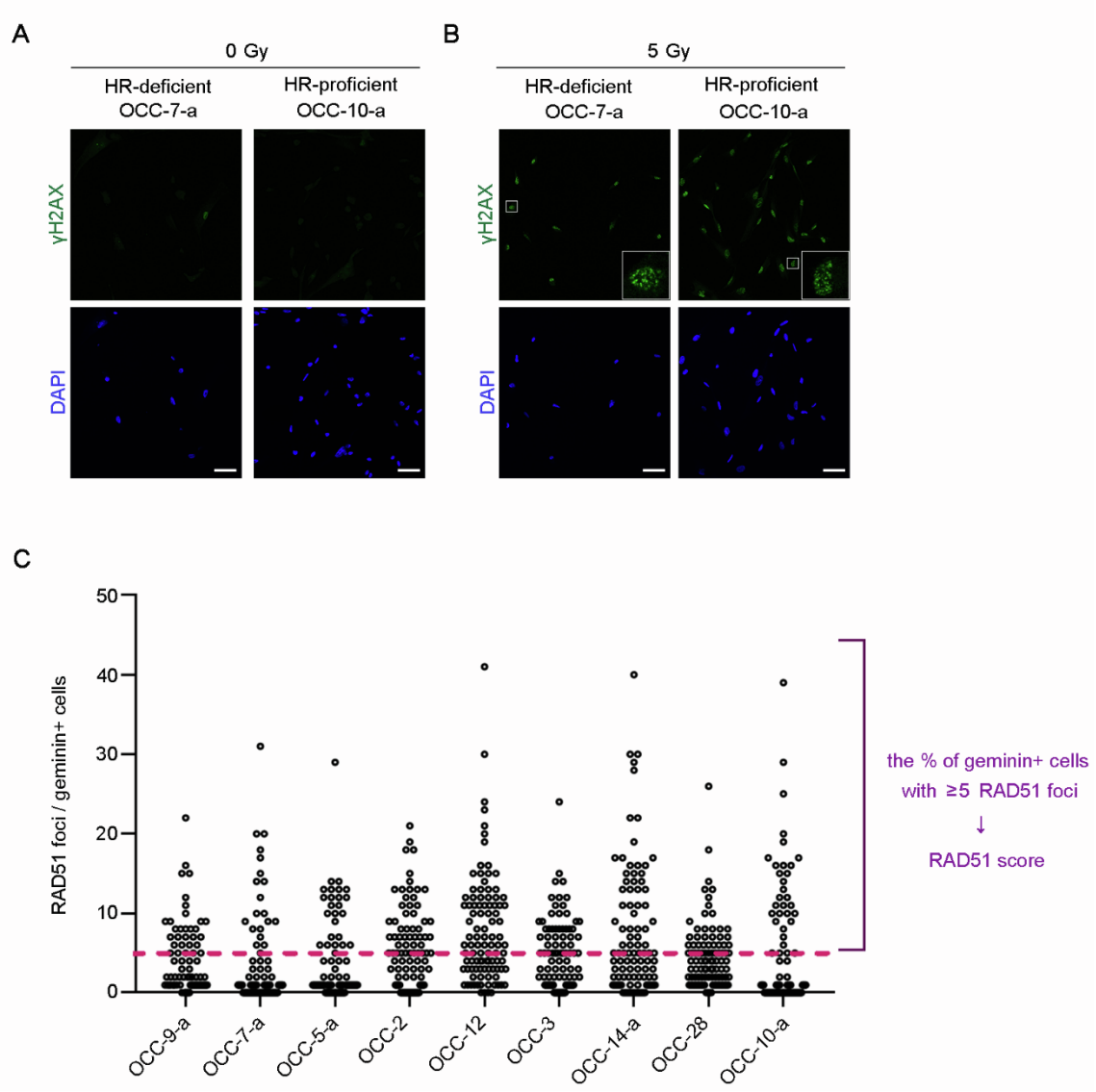

Figure S4. RAD51 foci-based assay in primary ovarian cancer cells. Related to Figure 4.

(A-B) Primary ovarian cancer cells were stained for γH2AX, a marker for DNA double-strand breaks, (A) without or (B) with 5 Gy γ-radiation. Immunofluorescence analysis was performed 2 h after radiation exposure. DNA was counterstained with DAPI. Scale bar, 50 μm.

(C) Primary ovarian cancer cells were co-stained for RAD51 and geminin, a marker of S/G2 cell phases, 2 h after 5 Gy radiation exposure. Each circle represents the number of RAD51 foci from a single geminin-positive cell (n = 100 geminin-positive cells). RAD51 scores were calculated as the percentage of geminin-positive cells with ≥5 RAD51 foci.

Variable	HRD	HRP	*P* value
Age (years)	53.5	52.4	0.836
FIGO Stage			0.155
I	4	3	
II	0	2	
III	2	13	
IV	1	3	
Histologic types			0.620
Serous carcinoma	3	9	
Clear cell carcinoma	4	8	
Endometrioid carcinoma		1	
others		3	
Regimen			0.274
Paclitaxel + Carboplatin + Avastin	5	10	
Paclitaxel + Carboplatin	2	11	

Table S1. Clinical and pathologic characteristics. Related to Figure 3.

HRD: homologous recombination deficiency; HRP: homologous recombination proficiency