**Supplementary figure legends**

**Supplementary figure 1. Ovarian cancer cell lines used as references.**

**(A-D)** Examples of immunostaining for DNA damage (visualized by γH2Ax, in green) and HR-mediated repair (visualized by RAD51, in magenta) in the HR-proficient OVCAR-3 (A) and COV318 (B), and HR-deficient OVCAR-8 (C) and Kuramochi (D) cells. RAD51 can be detected only in few cells in the HR-deficient cell lines.

**(E-F)** Co-immunostaining for HR-mediated DNA damage repair (RAD51, in magenta) and the cell cycle markers for G1 (Cyclin E2, in orange) and G2 phase (Cyclin A2, in green). Co-localization CyclinA2-RAD51 indicates that cells are performing HR and therefore HR-proficient, as seen for OVCAR-3 (E). In contrast, CyclinA2-positive OVCAR-8 cells fail to form RAD51 foci, thus demonstrating HR-deficiency (F). Scale bar represents 500 µm.

**Supplementary figure 2. Assessing the intra-sample heterogeneity in tumor vs stroma is crucial for accurate quantification of tumor functional HRD.**

**(A-D)** Examples of immunostaining for *bona fide* epithelial cancer cells (visualised by CK7, in green) and stromal cells (visualised by vimentin, in magenta). Note how epithelial cells grow in nests, as opposed to stromal cells. **(E)** Quantification of the epithelial (CK7-positive) fraction in all individual samples included in the study. **(F)** Comparison between “global HR”, calculated as percentage of RAD51-positive/total cells (striped bars), and the HR score, calculated as percentage of CK7-CyclinA2-RAD51 triple-positive/epithelial cells (solid bars, HR-deficient=green, HR-low=yellow, HR-proficient=red). Note how different the two HR quantifications can be in some samples, due to tumor purities.

**Supplementary figure 3. Rationale for exclusion of two patient samples from the study.**

**(A)** Immunostaining for DNA damage (visualized by γH2Ax, in green) and HR-mediated repair (visualized by RAD51, in magenta) in two primary ovarian cancer samples excluded from the study. Without cell cycle or cell-type specific markers, the ascites from patient EOC268 would have been classified as HR-deficient (based on the lack of RAD51 foci, A’), while the ovarian sample from patient EOC116 would be scored as HR-proficient (virtually all cells are RAD51-positive, A”). **(B)** Immunostaining for cellular proliferation (visualized by Ki67, in magenta) reveals that ascites cells from patient EOC268 stop proliferating after DNA damage (visualized by γH2Ax, in green, B’). Non-proliferating cells cannot perform HR-mediated repair. **(C)** Co-immunostaining for epithelial cancer cells (CK7, in green), the G2-phase marker CyclinA2 (in orange) and RAD51 (in magenta) was used to calculate HR scores. Ascites cells from patient EOC268 were CK7-positive, but did not express CyclinA2 and, therefore, could not be scored (C’). In contrast, cells from patient EOC116 co-expressed both CyclinA2 and RAD51, but were CK7-negative (C”). Because we used CK7 as a marker for *bona fide* epithelial ovarian cancer cells, this sample could not be scored and was also excluded from the study. Scale bars, 500 µm.

**Supplementary figure 4. Low HR scores correlate with longer overall survival.**

**(A-D)** Kaplan-Meier plots showing overall survival for HR category (A), surgical strategy (B), FIGO stage (C) and age at diagnosis (D). Of these, only low HR scores correlate with increased overall survival (p=0.0254). P values were calculated in R using a log-rank (Mantel-Cox) test.

**Supplementary figure 5. HR-deficient/-low tumors are characterized by elevated levels of DNA damage and apoptosis, but low proliferation.**

**(A)** Basal HR scores (white bars) and HR scores at 8 and 24 hours post-IR in individual ovarian cancer samples. Note how the score in HR-deficient samples (in green) generally remain constant at both 8 and 24 hours post-IR, while decreases in HR-proficient samples. **(B-C)** IR-induced DNA damage (B) and apoptosis (C) in individual ovarian cancer samples, 8 and 24 hours post-IR. **(D)** Cellular proliferation, detected as Ki67 positivity, in individual ovarian cancer samples, before and 8 hours after IR. Bars with a striped pattern show values for HR-deficient OVCAR-8 (striped, green) and HR-proficient OVCAR-3 (striped, red). n.a. not available.

**Supplementary figure 6. Intra-patient variation in the DNA damage and apoptotic responses to IR.**

**(A)** DNA damage and proliferation for five individual samples from patient EOC415. **(B)** Apoptosis for five individual samples from patient EOC415. Patient overall values are shown as average (striped bars). Scale bars, 500 µm.

**Supplementary figure 7. Mutational signatures in individual ovarian cancer samples.**

**(A)** Overview of the contribution of different mutational signatures in primary ovarian cancer samples. **(B)** Detailed visualization of the mutational signatures contribution in individual samples. Asterisks denote tumors for which a sample from a different anatomical location was used for sequencing.

**Supplementary figure 8. *Ex vivo* functional HR testing can be performed on solid biopsies.**

Comparison of HR detection in FFPE-embedded HR-deficient (B-D) and HR-proficient (F-H) ovarian tumors, and in primary cells derived from the same samples (A and E, respectively). Irradiated biopsies were allowed to recover for 16 hours, prior to routine formaldehyde fixation, paraffin embedding and sectioning. **(B, F)** CK7 and Vimentin were used to identify nests of epithelial cancer cells and stroma, respectively. Dashed lines highlight the tumor borders. Tum, tumor. Str, stroma. **(C, G)** Immunostaining for RAD51 confirms that epithelial cancer cells are HR-deficient in C, and HR-proficient in G, in agreement with what was measured in their derived primary cultures (A and E). DNA damage is higher in epithelial (cancer) than in stromal (normal) cells. Notice the intra-tumoral RAD51 heterogeneity in G. **(D, H)** Immunostaining for cleaved caspase 3 (cCasp3) revealed that epithelial cells are more prone to apoptosis, compared to stromal cells. Scale bars, 200 µm.