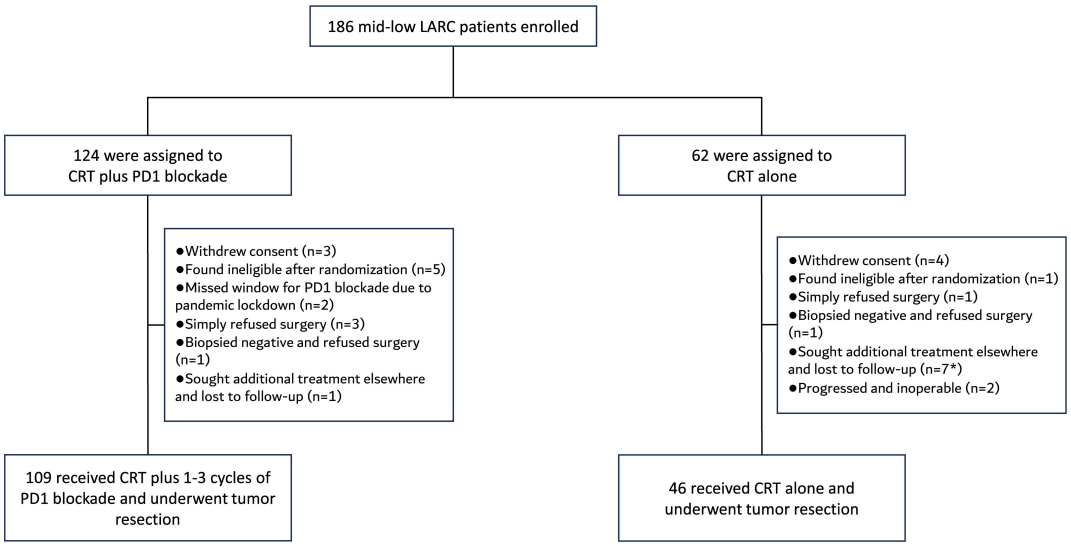

Fig.S1 Detailed workflow for the post-hoc analysis of the randomized POLARSTAR trial

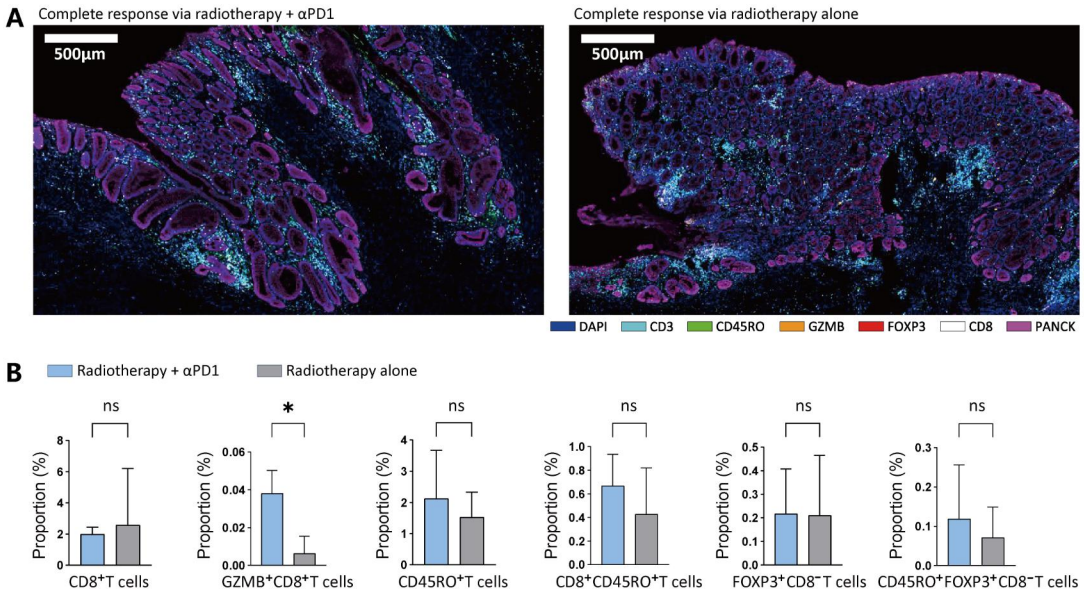

Fig.S2 Significantly more $GZMB^{+}CD8^{+}$ T lymphocytes reside in the scar tissues of pCR patients (former tumor bed, where tumors had completely regressed) resulting from radiotherapy plus PD1 blockade than in those resulting from radiotherapy alone. A) Representative multiplex immunohistochemical images of the tumor-bed scar tissues of the pCR patients after the two different interventions. B) Inter-group comparison of the abundance of T cell subsets in pCR patients' tumor-bed scar tissues.

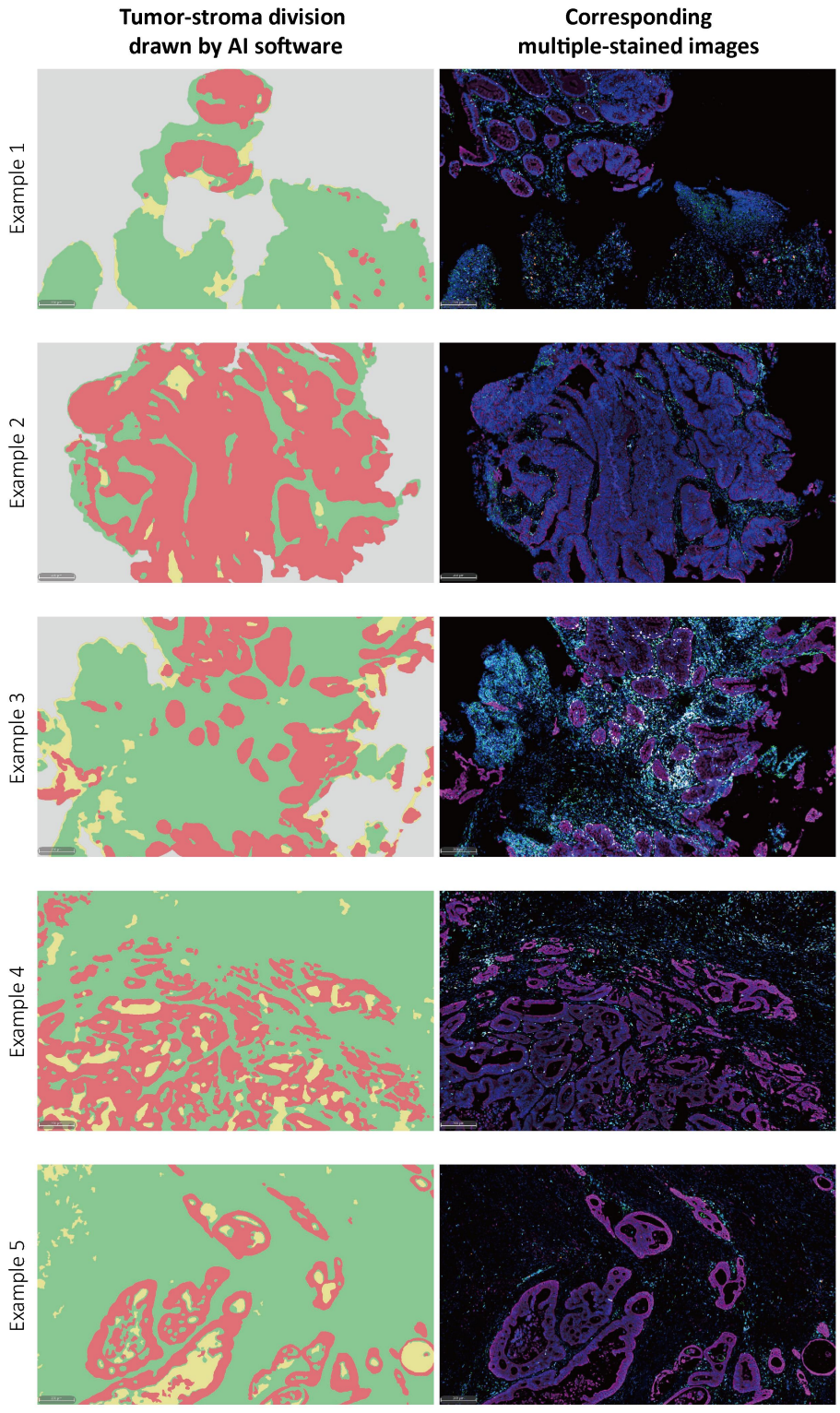

Fig.S3 Examples of the tumor epithelium-stroma division drawn by the AI function of HALO software. Left: AI-drawn tumor-stroma division based on HE staining images. Red zone indicates tumor epithelium area, green zone indicates tumor stroma area, yellow zone indicates blank area (no cells), grey zone indicates un-analyzed area; Right: matched multiplex immunohistochemical images

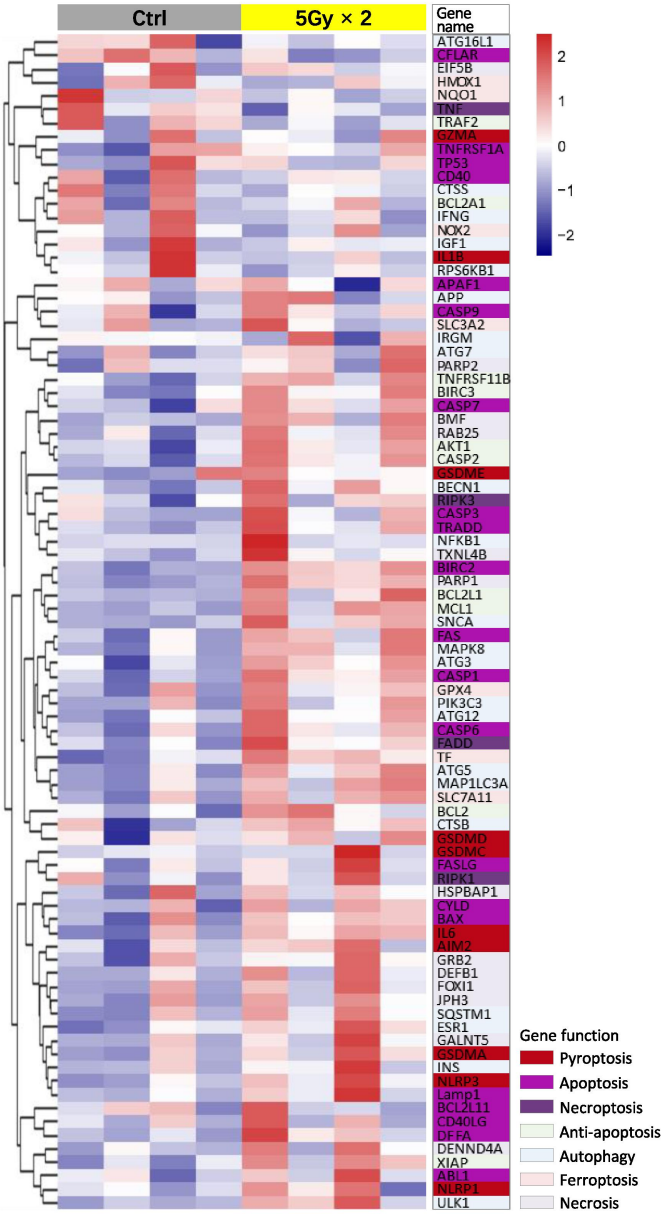

Fig.S4 mRNA expression of cell-death-related genes in mouse tumor tissues. Unirradiated (n=4) and 5×2Gy X-ray irradiated (n=4) mouse tumor tissues (displayed in Figure 3b) were used for the extraction of total RNA. Mouse tumor tissues, instead of cell lines, were used for this comparison of mRNA expression between treatment and control so as to better simulate a real-world therapeutical scenario

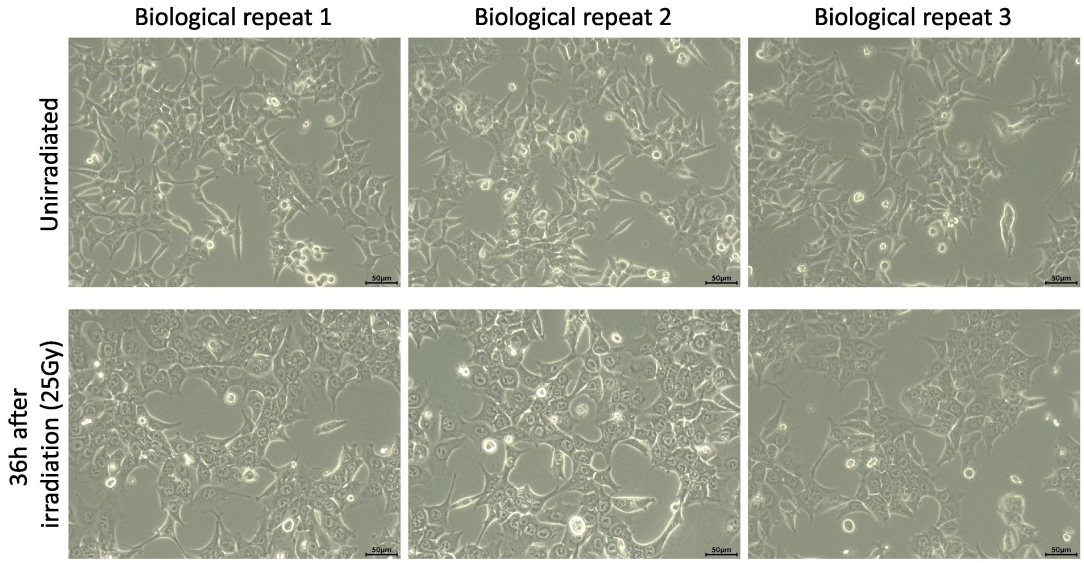

Fig.S5 Tumor cells swell substantially after clinical-dose X-ray irradiation. Upper: unirradiated HCT116 cells; Lower: HCT116 cells 36 hours after 25Gy irradiation

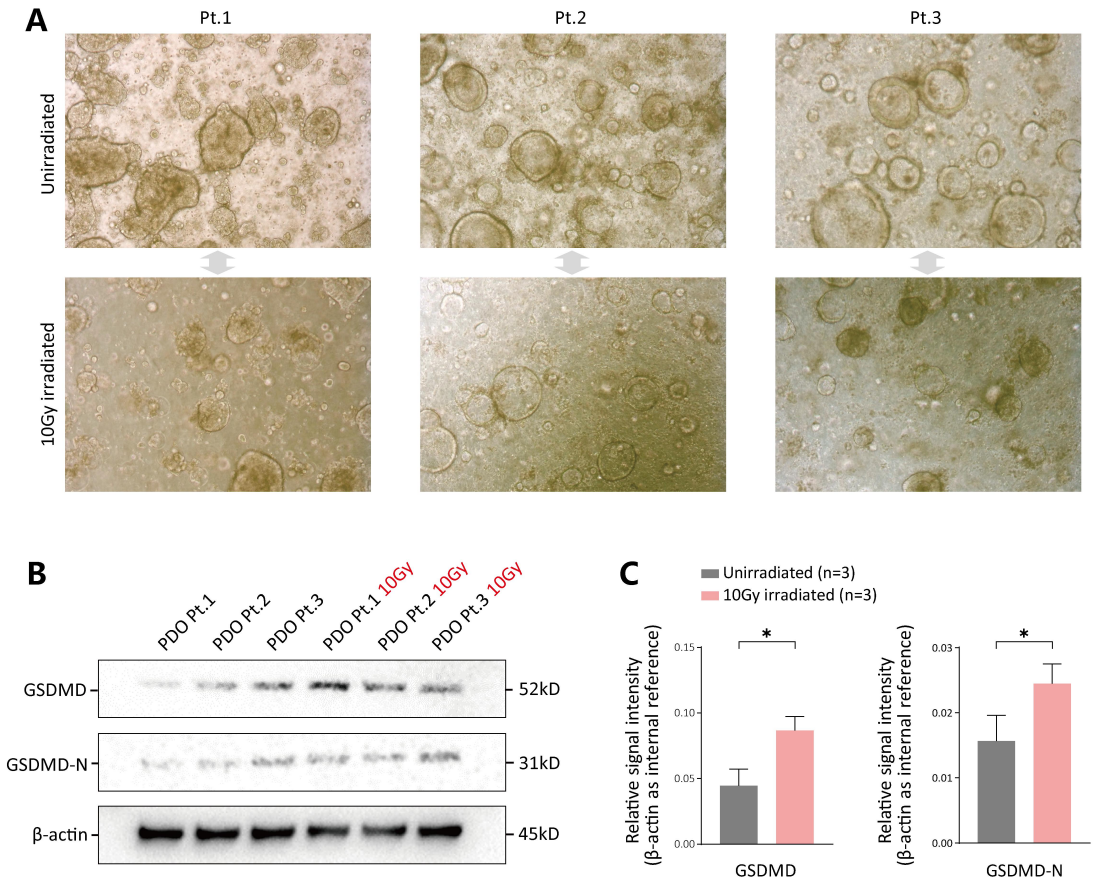

Fig.S6 Clinical-dose X-ray radiation inhibits growth of rectal cancer patient-derived organoids (PDOs) and increases cleavage of GSDMD in the composing tumor cells. A) Tumor organoids derived from treatment-naïve patients with locally advanced rectal cancer. Upper: unirradiated PDOs; Lower: PDOs (same patient, same passage) 36 hours after 10Gy irradiation. B) Westernblot analysis on the expression and cleavage of GSDMD in PDOs. For irradiated PDOs, proteins were extracted at 36 hours post-irradiation, while unirradiated PDOs were processed simultaneously. C) Quantitative analysis of the expression of GSDMD and GSDMD-N

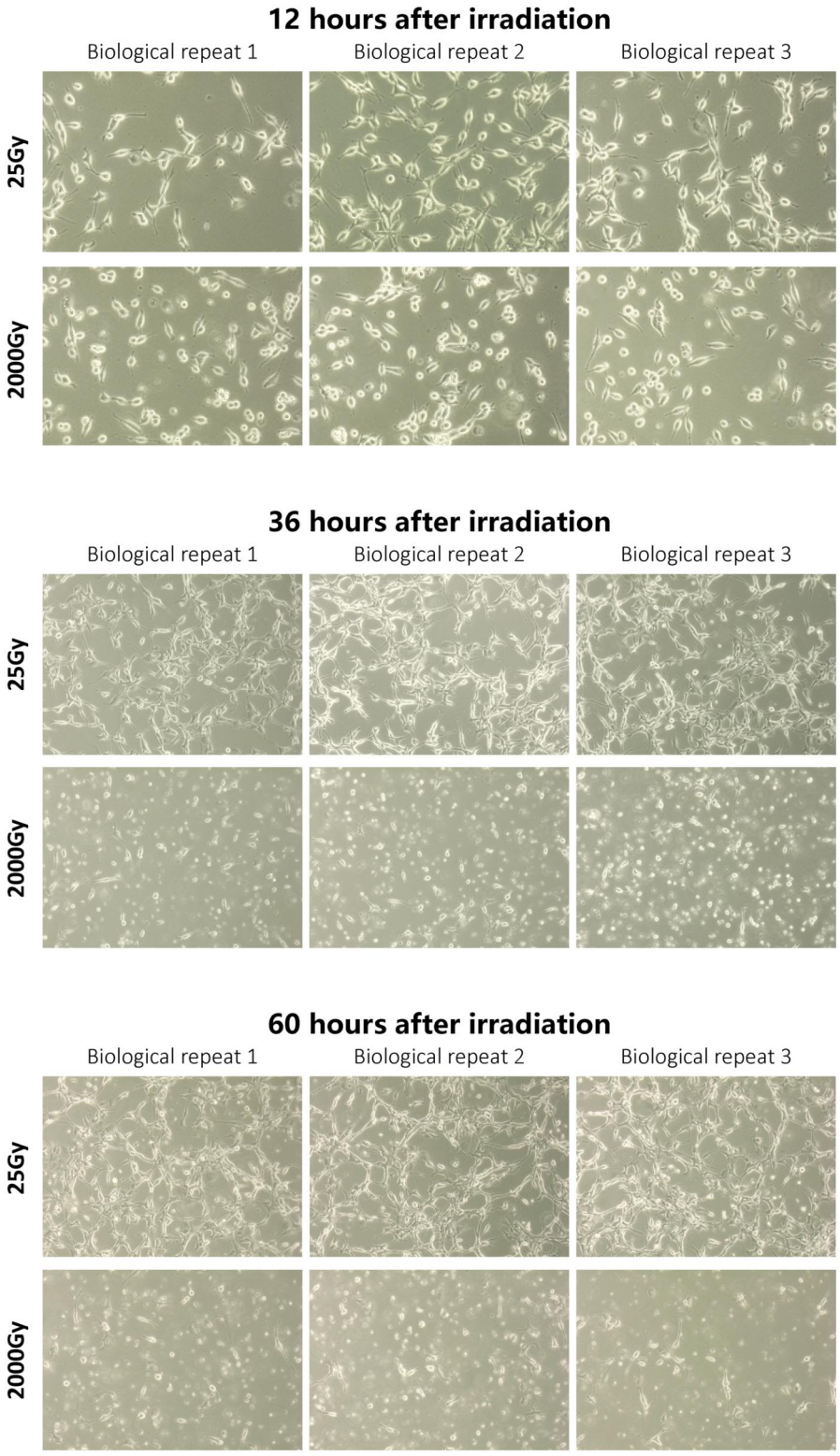

Fig.S7. Sterilization-level irradiation (2000Gy) impact CT26 tumor cells more profoundly than clinical-dose irradiation

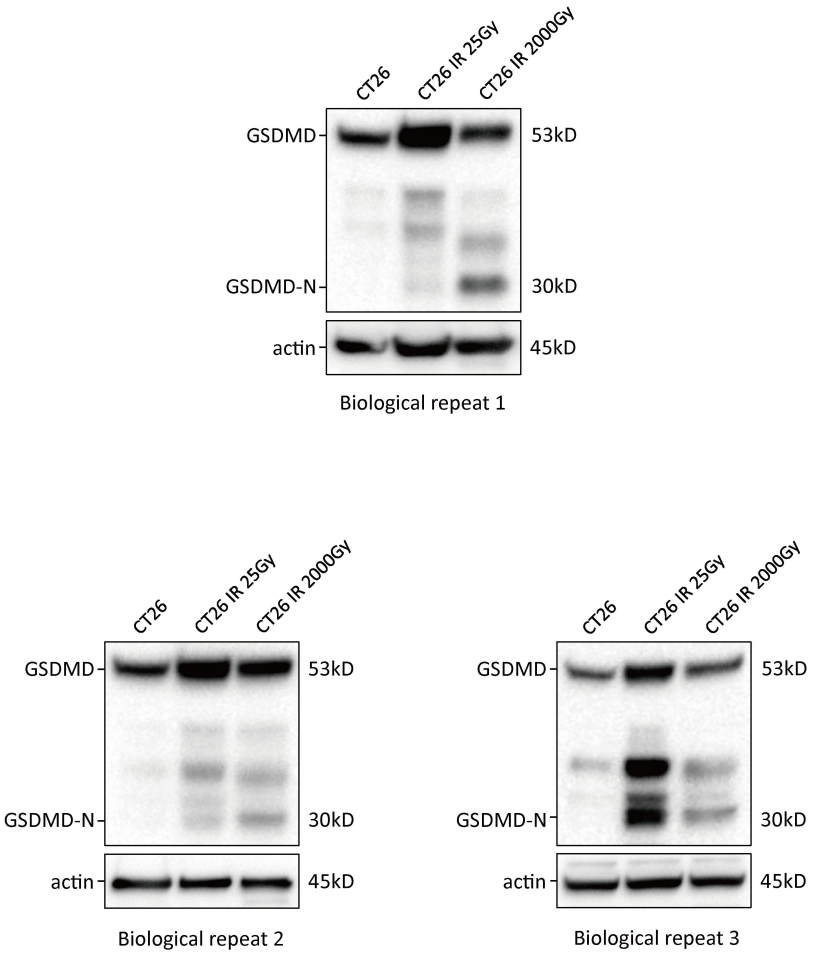

Fig.S8 Sterilization-level irradiation (2000Gy) also elicits pyroptosis of CT26 tumor cells. For these irradiated CT26 tumor cells, proteins were extracted at 12 hours post-irradiation, while unirradiated controls were processed simultaneously

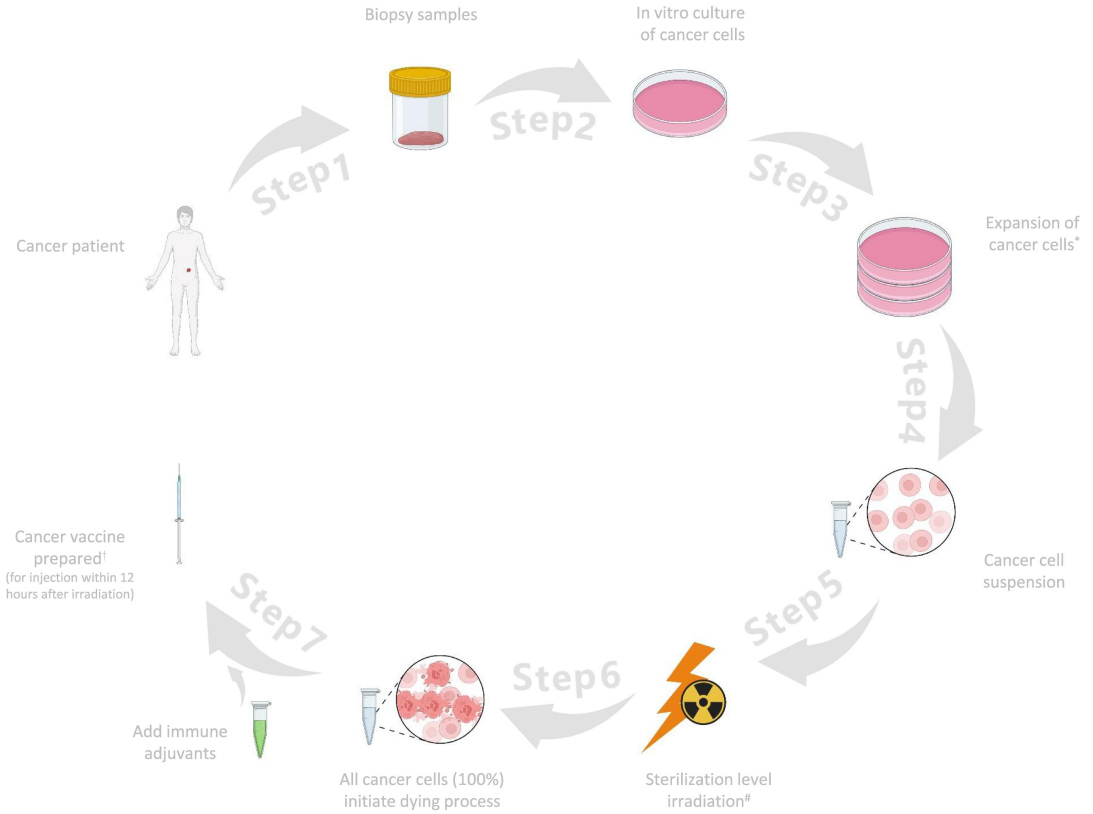

Fig.S9 A brief schema illustrating the potential application of tumor vaccines composed of sterilization-level irradiated autologous tumor cells. *Expansion of cancer cells could be carried out by either 2D or 3D culture, or even omitted if the size of the biopsied tissue is already large enough to produce sufficient tumor cells (e.g. surgically resected samples); #Sterilization-level irradiation requires specialized industrial facilities, instead of bio-medical irradiation device; † Sterilization-level irradiated cancer cells ought to be injected as soon as possible to effectively exploit the antigen-releasing process of dying tumor cells

Table.S1 A summary on the purpose of the application of different cells and irradiation doses

Cells or radiation doses	Experiments	Purpose	Related figure
MC38 cell line	Tumor inoculation on C57BL/6 mice	To form syngeneic, immune competent mouse tumor model. The MC38 cell line was originally isolated and established from a spontaneous colon adenocarcinoma in a C57BL/6 mouse. Thus, it shares an identical major histocompatibility complex (MHC) genotype with all C57BL/6 mice.	Fig.3a
5Gy×2 irradiation	Tumor localized irradiation to mouse tumor model composed of C57BL/6 mice and MC38 cells	To mimic the clinical radiotherapy of rectal cancer. The clinical radiotherapy of rectal cancer, whether long-course (2Gy×25) or short course (5Gy×5), usually consists of repeated doses of 2Gy or 5Gy.	Fig.3a
HCT116 cell line	γ-H2AX immunofluorescence to detect DNA damage, and colony formation assay	Among all three colorectal cancer cell lines (human and mouse) used in this study, HCT116 is a human cell line. It is thus more representative of human biology. The rationale of "radiation causes DNA damage" is already well established. Therefore, repeated experiments on all three cell lines would be redundant.	Fig.4a,b

All three cell lines (MC38, CT26, and HCT116)	Cell viability assay, flow cytometry cell death analysis, and GSDMD detection by WB assay	All three cell lines were used for these experiments so as to find out whether or not there are differences among the cell lines regarding the extent of cell deaths in response to radiation	Fig.4c,d,e,f
25Gy irradiation	γ-H2AX immunofluorescence to detect DNA damage	To mimic the full dose of clinical radiotherapy of rectal cancer. In common practice, the full dose is 25Gy for short-course radiotherapy and 50Gy for long-course radiotherapy.	Fig.4a
5Gy irradiation and 25Gy irradiation	Colony formation assay, and cell viability assay	To mimic the single dose (5Gy) and repeated, full dose (25Gy) of clinical radiotherapy of rectal cancer, respectively.	Fig.4b,c
25Gy irradiation	Flow cytometry cell death analysis, and GSDMD detection by WB assay	To mimic the full dose of clinical radiotherapy of rectal cancer.	Fig.4d,e,f
CT26 cell line	Tumor inoculation on BALB/c mice	To form another syngeneic, immune competent mouse tumor model. The CT26 cell line was originally isolated and developed from a chemically-induced colon carcinoma in a BALB/c mouse. Thus, it shares an identical major histocompatibility complex (MHC) genotype with all BALB/c mice.	Fig.5a,e

2000Gy irradiation	The sterilization level irradiation of CT26 cells	To transform CT26 cells into potential therapeutical vaccines, guaranteeing 100% death initiation for all cells irradiated.	Fig.5a,e
HCT116 cell line	Observation of cell morphology change after clinical dose irradiation	Among all three colorectal cancer cell lines (human and mouse) used in this study, HCT116 is a human cell line. Morphology observation of this cell line can better elucidate the in vivo situation of our interest as to how human tumor reacts to clinical radiotherapy.	Fig.S5
25Gy irradiation	Observation of cell morphology change after clinical dose irradiation	To mimic the full dose of clinical radiotherapy of rectal cancer.	Fig.S5
CT26 cell line	Observation of cell morphology change after clinical dose versus sterilization dose irradiation, and GSDMD detection after clinical dose versus sterilization dose irradiation	The cell line we used to produce the vaccine is CT26. Therefore, we intend to display how this particular cell line react to sterilization dose irradiation.	Fig.S7,S8
25Gy irradiation and 2000Gy irradiation	Observation of cell morphology change after clinical dose versus sterilization dose irradiation, and GSDMD detection after clinical dose versus sterilization dose irradiation	To display and compare how CT26 cells react to clinical dose versus sterilization dose irradiation.	Fig.S7,S8