

# Radiation response of human cardiac endothelial cells reveals a central role of the cGAS-STING pathway in the development of inflammation

Jos Philipp <sup>1</sup>, Ronan Le Gleut <sup>2</sup>, Christine von Törne <sup>3</sup>, Prabal Subedi <sup>1,4</sup>, Omid Azimzadeh <sup>1</sup>, Michael J Atkinson<sup>1,5</sup> and Soile Tapio <sup>1,\*</sup>

<sup>1</sup> Institute for Radiation Biology, Helmholtz Center Munich, German Research Center for Environmental Health GmbH, 85764 Neuherberg, Germany; jos.philipp@rub.de, atkinson@helmholtz-muenchen.de, soile.tapio@helmholtz-muenchen.de

<sup>2</sup> Institute of Computational Biology, Helmholtz Center Munich, German Research Center for Environmental Health GmbH, 85764 Neuherberg, Germany; ronan.legleut@helmholtz-muenchen.de

<sup>3</sup> Research Unit Protein Science, Helmholtz Center Munich, German Research Center for Environmental Health GmbH, 85764 Neuherberg, Germany; vontornerne@helmholtz-muenchen.de

<sup>4</sup> Department of Radiation Protection and Health, Federal Office for Radiation Protection, BfS, Neuherberg, Germany; psubedi@bfs.de

<sup>5</sup> Chair of Radiation Biology, Technical University of Munich, Munich, Germany

\* Correspondence: soile.tapio@helmholtz-muenchen.de; Tel.: +4989 3187 3445 (S. T.)

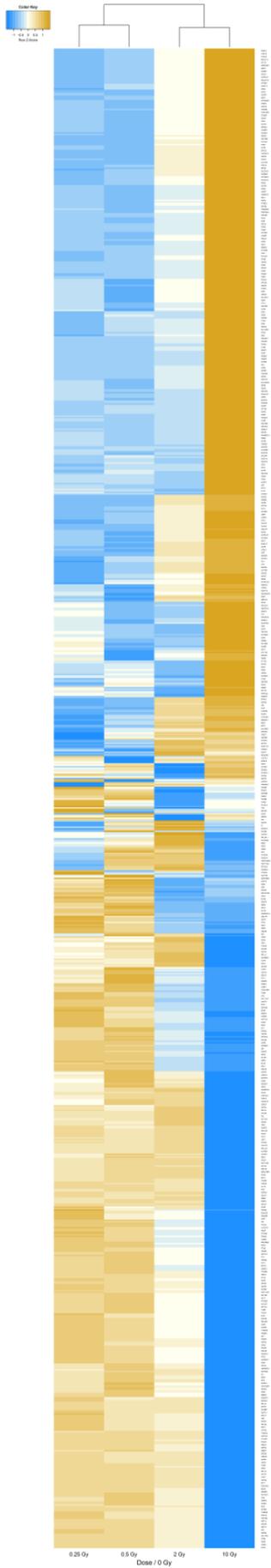


Figure S1. Heat map of all significantly deregulated (1022) proteins in HCECest2 cell line 1 week after different radiation doses. The samples irradiated with doses of 0.25 or 0.5 Gy show a similar expression pattern with less or no fold change (faint color). The samples irradiated with 10 Gy are well separated in up- and downregulated proteins showing a high fold change of deregulation (bright color). Generally, the protein expression at 10 Gy shows an inverse trend in comparison with the expression changes in the lowest dose groups. The expression changes at the dose of 2 Gy is intermediate sometimes resembling those of the lower doses and sometimes those of the 10 Gy dose. The blue color represents downregulation and the yellow upregulation.

### Inflammatory proteins

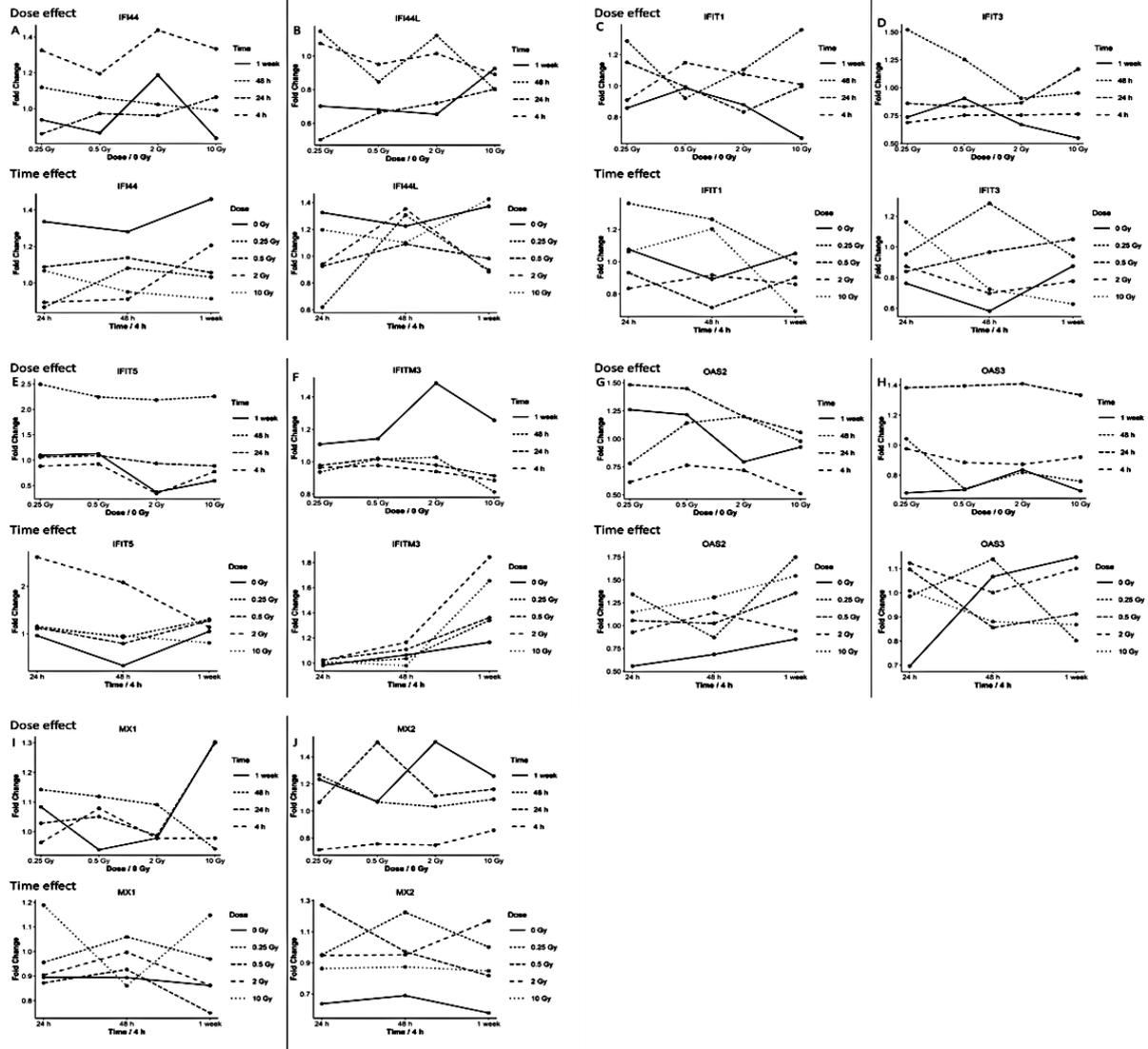


Figure S2. Expression of inflammatory proteins as a function of radiation dose or time. Proteins of the oligoadenylate synthetase (OAS) family, Interferon-induced GTP-binding proteins MX1 and MX2, and proteins of the IFIT family that are known to be induced in response to the type I Interferon-related activation did not show significant dose- or time-dependent expression pattern changes.

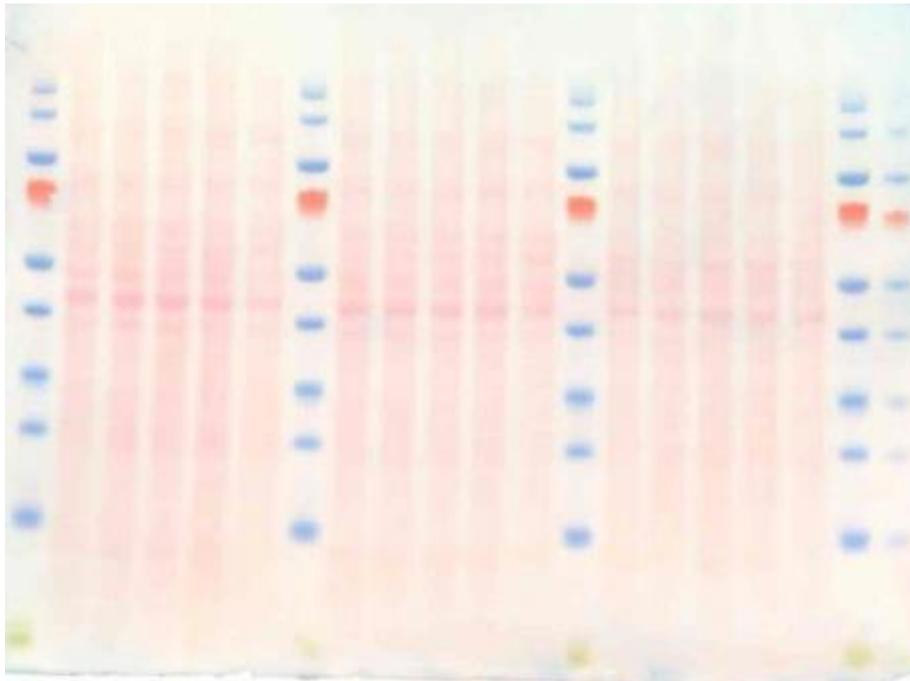


Figure S3. Ponceau staining as the loading control of the immunoblot analysis for Figure 4.

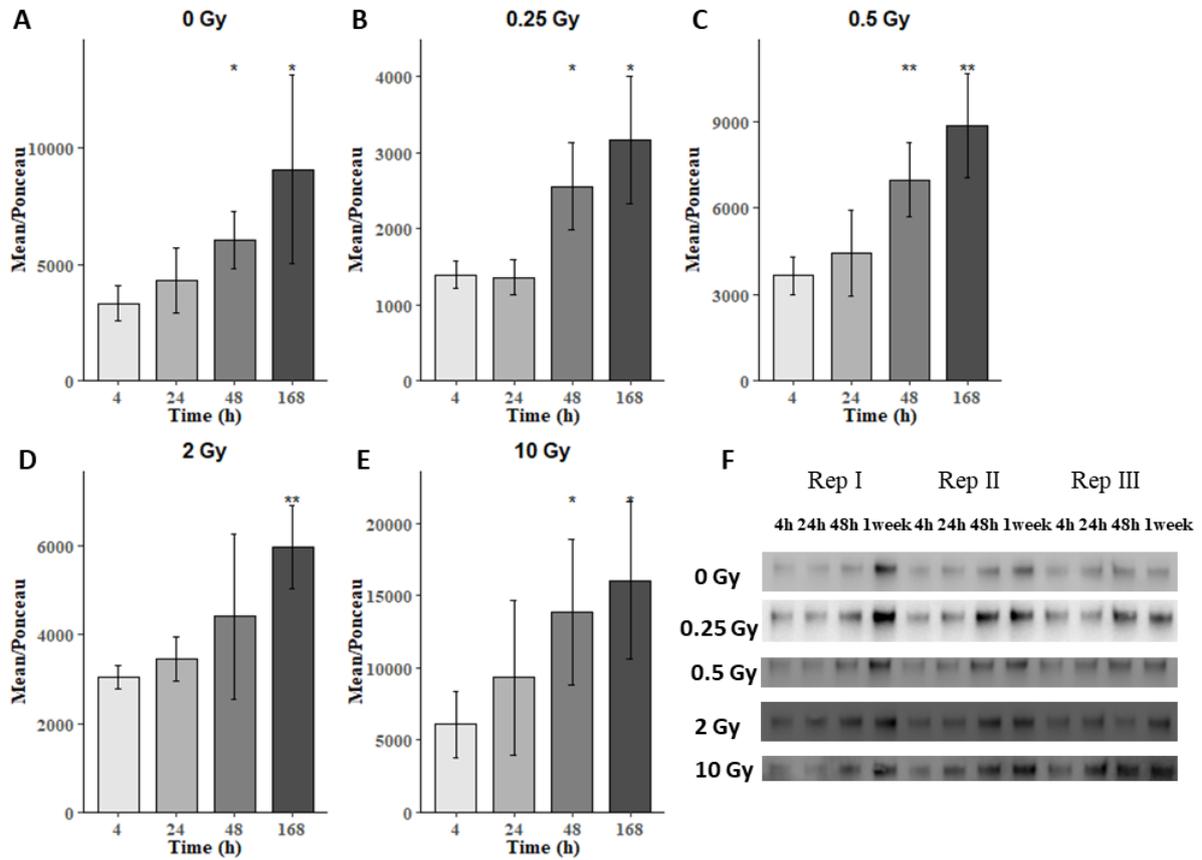


Figure S4. Immunoblot verification of ICAM1 levels as a function of time in HCECest2 cells. The expression of ICAM1 in the sham-irradiated control group (A), group irradiated with 0.25 Gy (B), 0.5 Gy (C), 2.0 Gy (D) or 10.0 Gy (E) at 4 h, 24 h, 48 h or 168 h (1 week) is shown. The bars represent the relative expression after correction for background and normalization to Ponceau. The error bars are calculated as SEM (t test; \*p < 0.05; n = 3). The visualization of protein bands using three biological replicates is shown (F). The Ponceau stainings are shown in Figure S6.

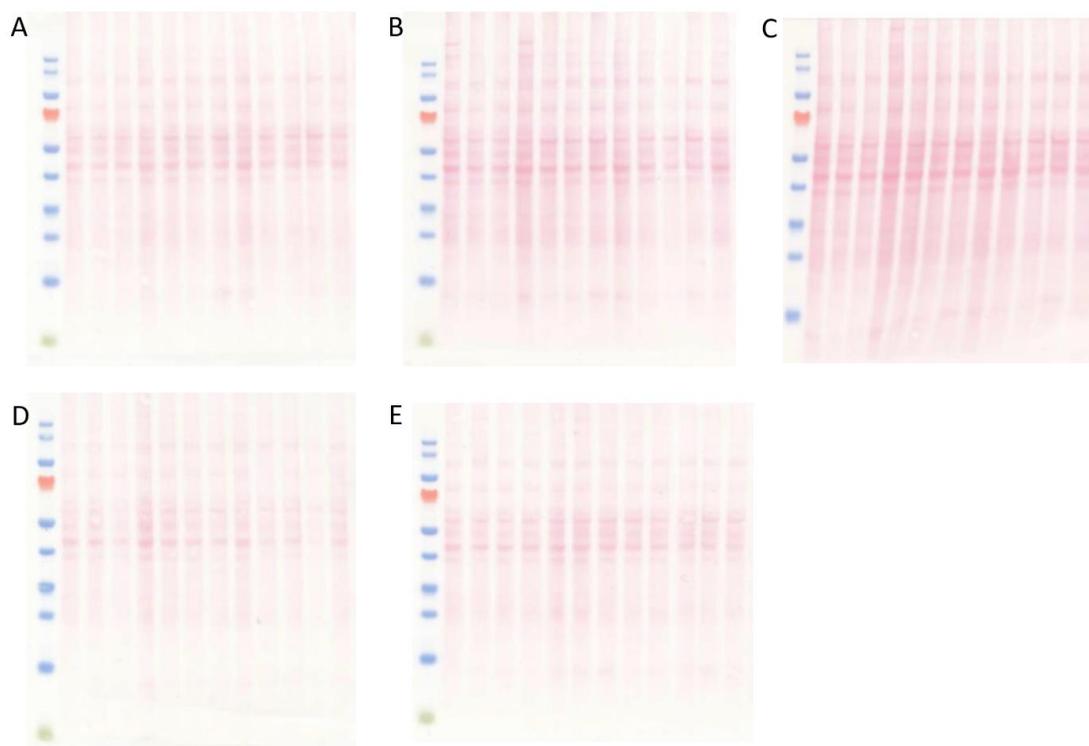


Figure S5. Ponceau stainings as the loading controls for the immunoblot analysis for Figure S4. The radiation doses are 0 Gy (A), 0.25 Gy (B), 0.50 Gy (C), 2.0 Gy (D), and 10.0 Gy (E).

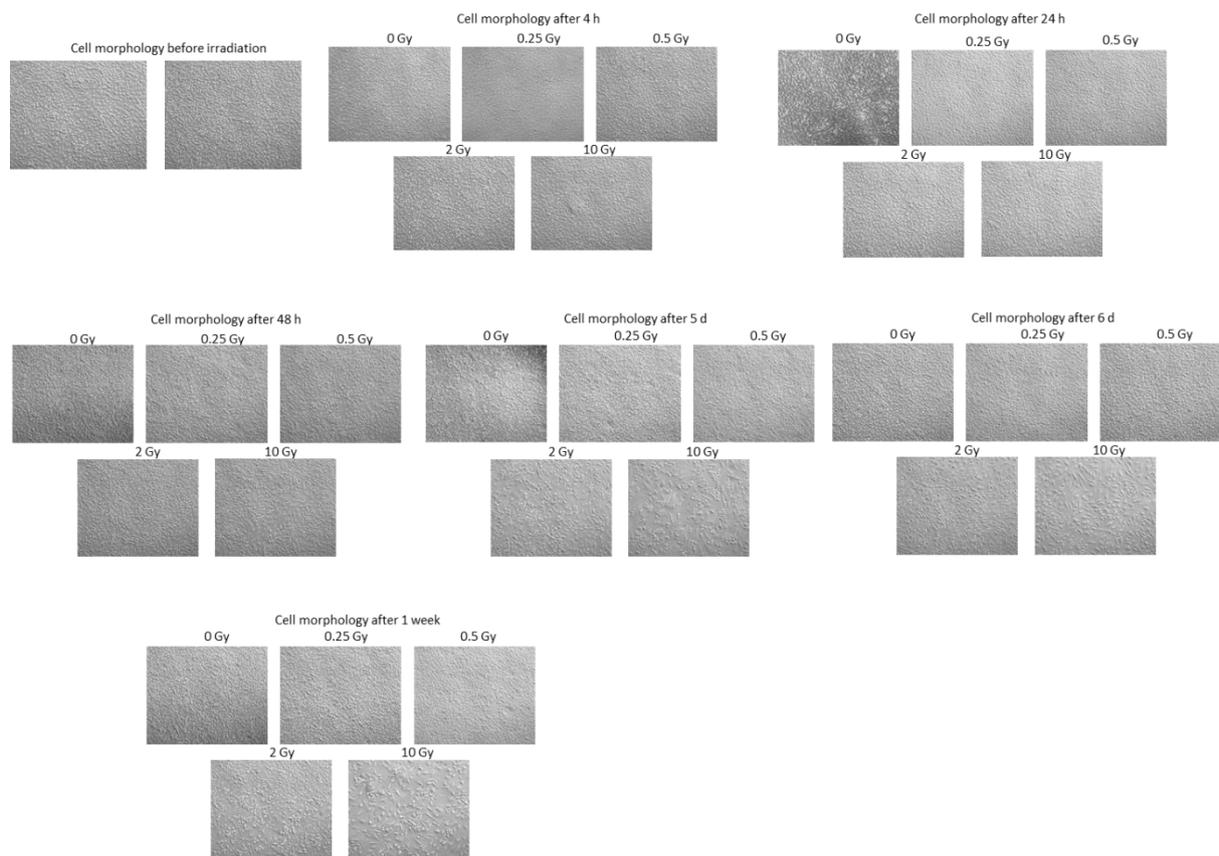


Figure S6. Radiation-induced cell morphology changes of the endothelial cell line HCECest2. The microscopic images were recorded using Keyence BZ-9000 (Keyence Corporation, Germany) with a 4x/0.20 objective before irradiation and at 4 h, 24 h, 48 h, 5 d, 6 d and 1 week post-irradiation. Sham-irradiated and 0.25 Gy or 0.5 Gy irradiated cells did not show any changes in morphology during the observation period of one week. The dose of 10 Gy induced the most marked morphological changes: The cell density started to decrease at the same time as the cell size turned bigger (24 h post-irradiation). In addition, the cobblestone monolayer pattern, typical for endothelial cell cultures and as seen in the sham-irradiated, 0.25 Gy and 0.5 Gy irradiated cells, was lost beginning after 48 h at 10 Gy. At the day five, the cell density of the 2 Gy irradiated cells also started to decrease.