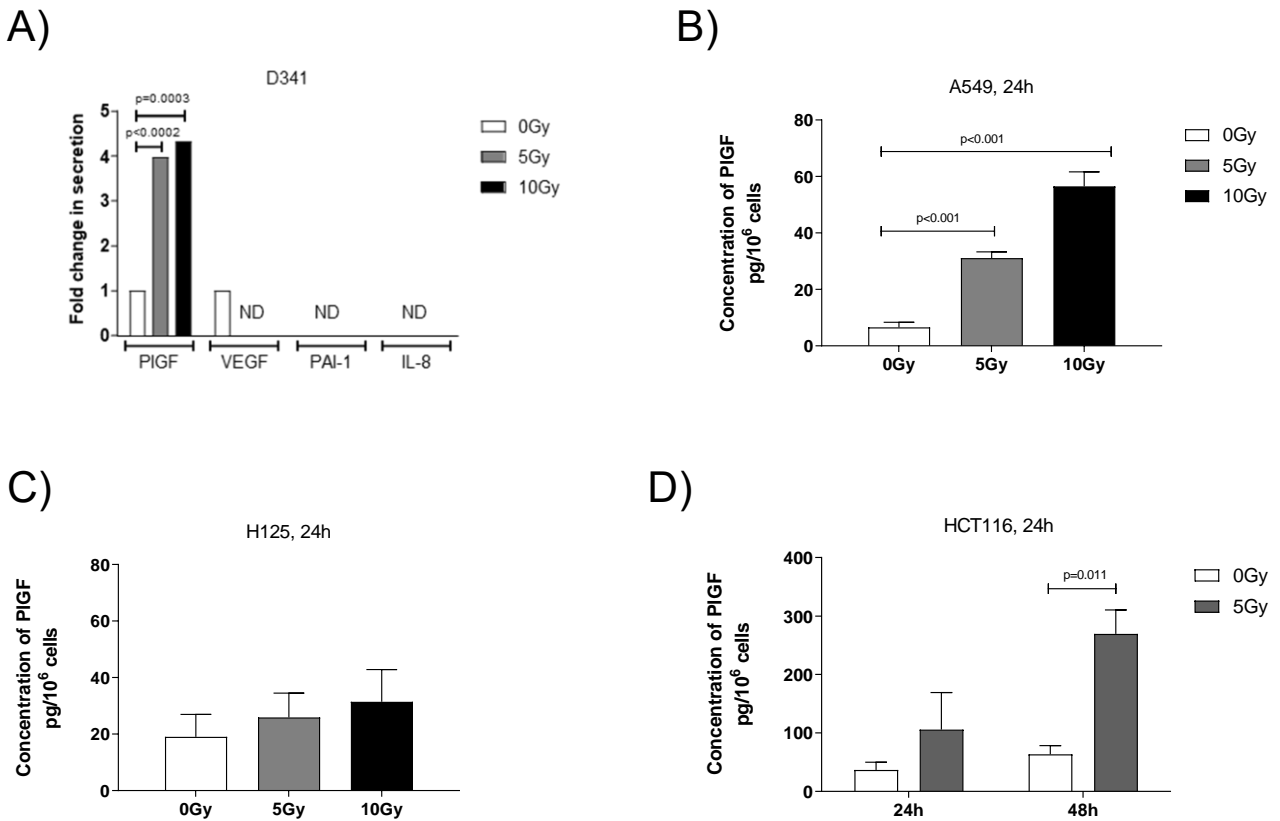
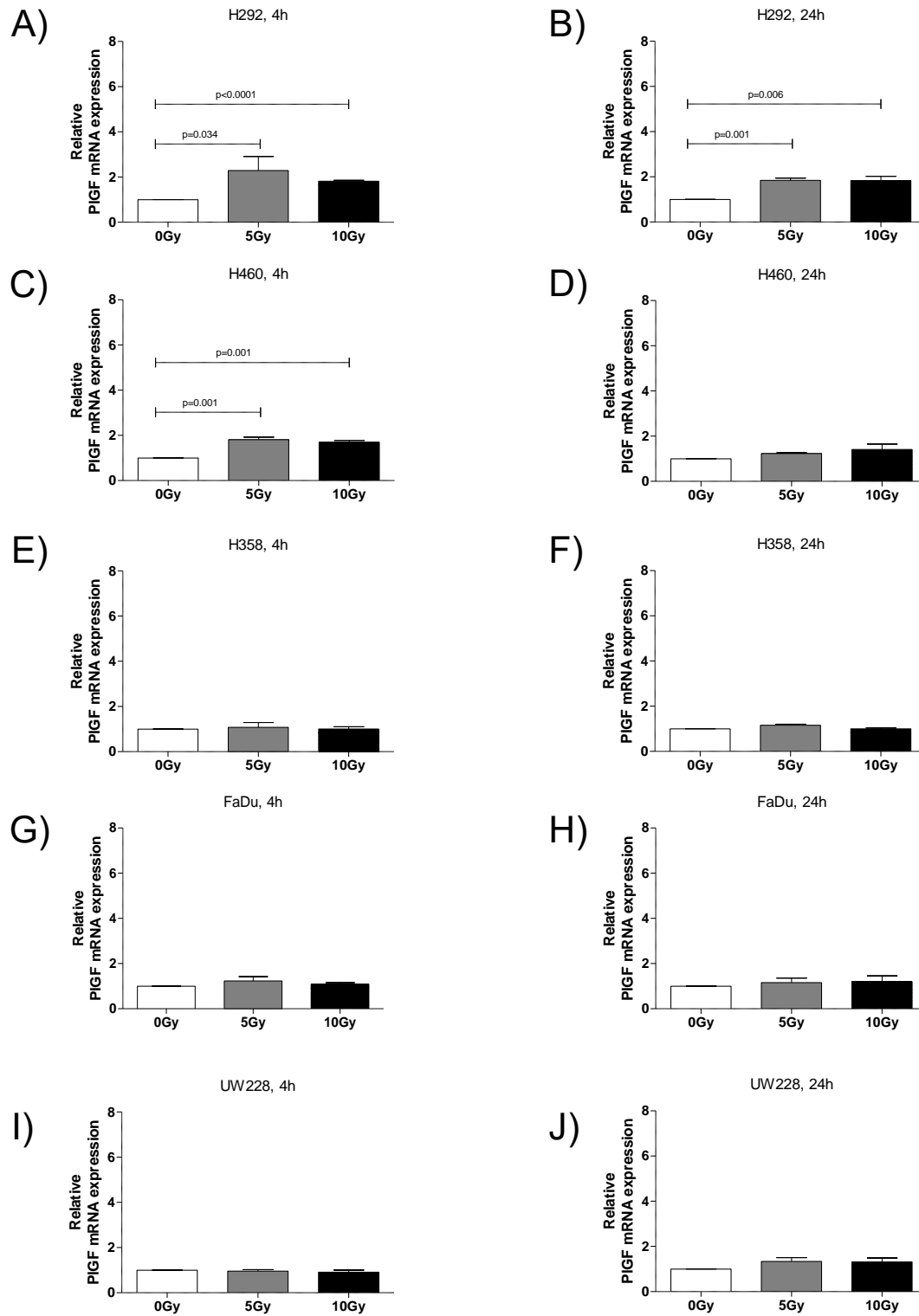


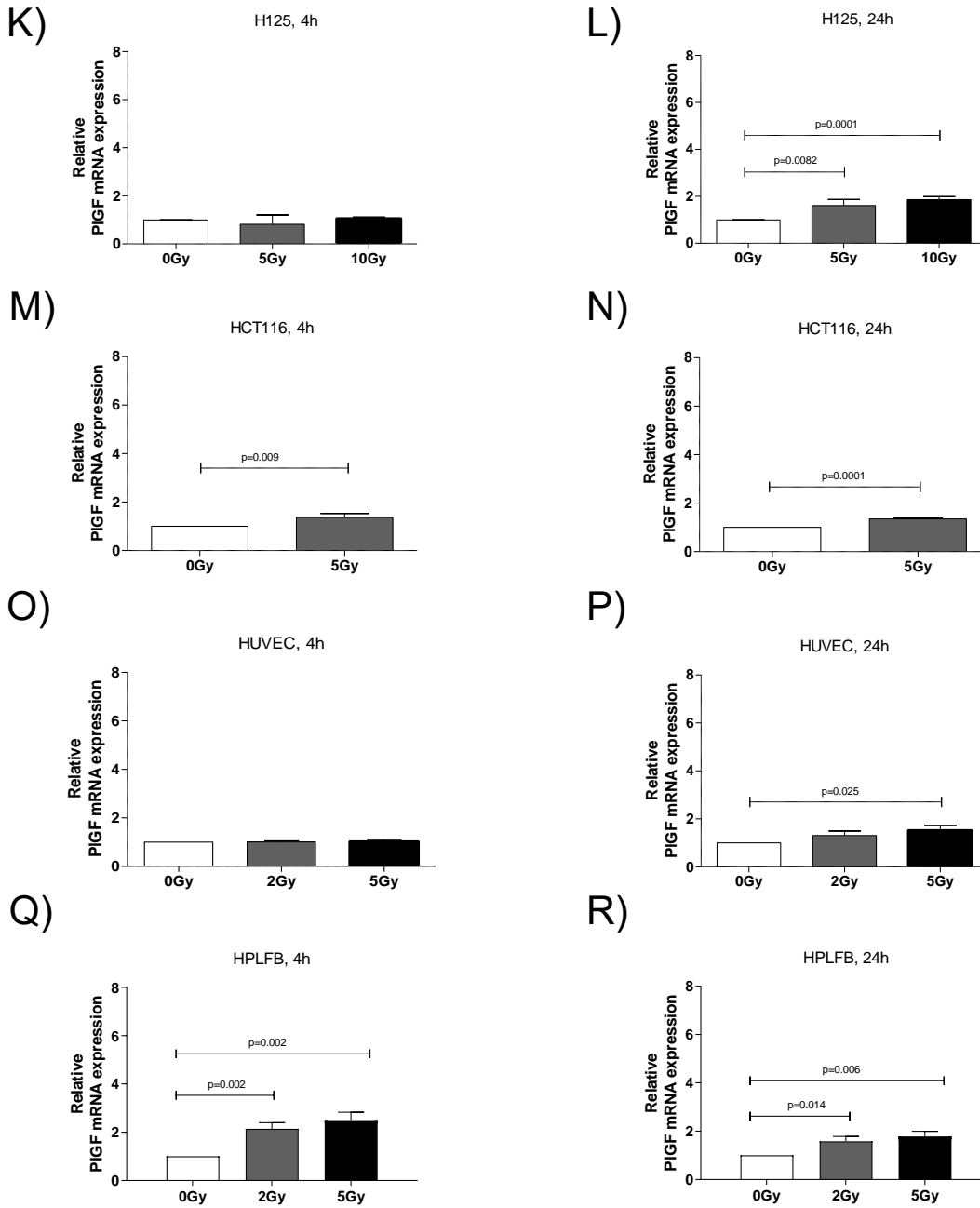
## Supplementary Figure S1



**Supplementary Figure S1. PIGF secretion is increased in multiple cell lines after irradiation.** A, IR-induced secretion of different cytokines from D341 medulloblastoma cells 24 hours after irradiation (ND: not detectable) B –D, Irradiation-induced PIGF-secretion was investigated in tumor cell lines derived from lung adenocarcinoma (A549 and H125) and colon carcinoma (HCT116). IR-enhanced PIGF-secretion was identified in the conditioned media (CM) by ELISA in a time- and IR-dose-dependent manner. Bar graphs represent PIGF concentrations normalized to viable cell count  $\pm$  SEM. Students t-test was used for statistical analysis.

## Supplementary Figure S2

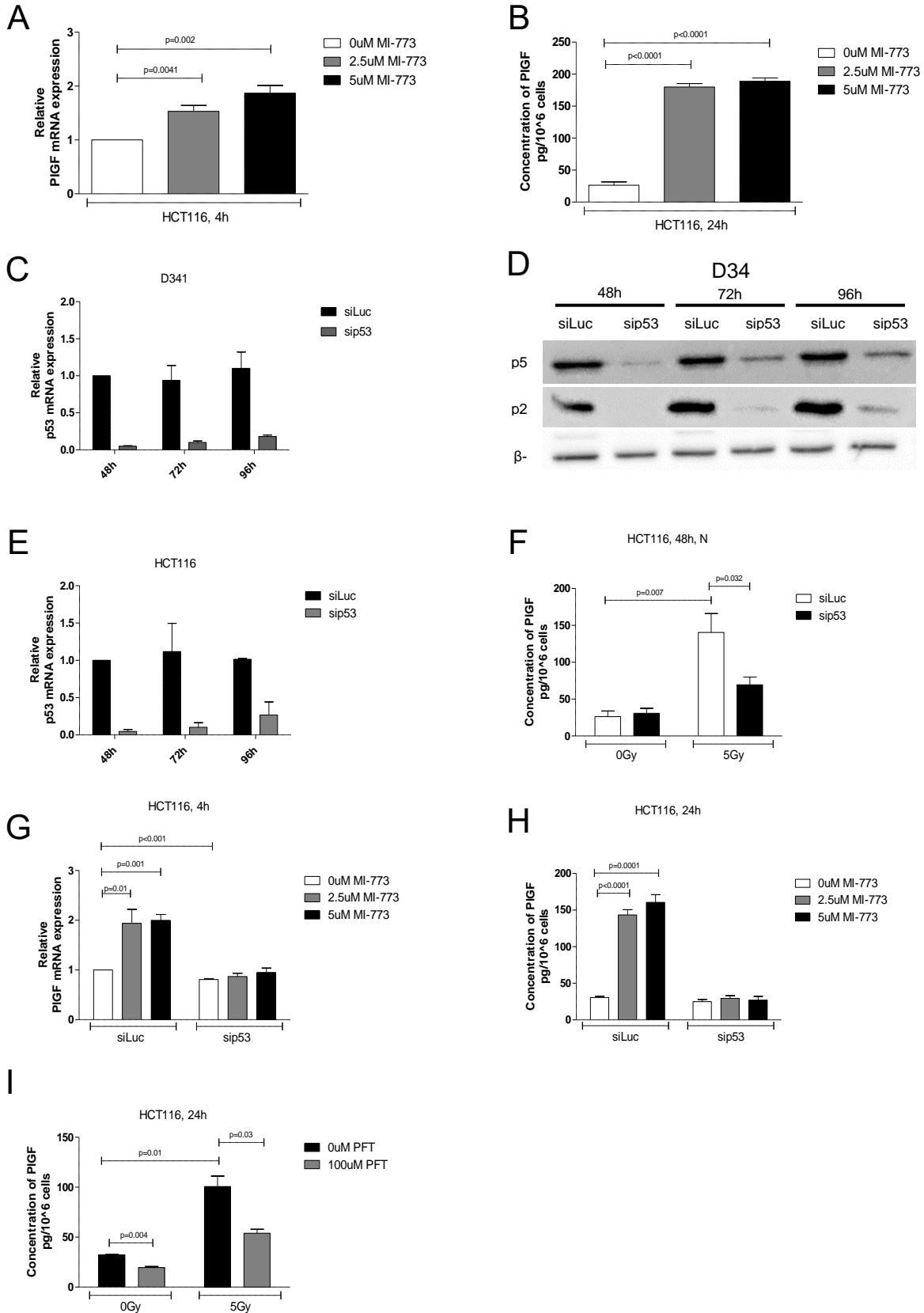




**Supplementary Figure S2. PIGF mRNA expression is increased in response to IR.** A – F, qRT PCR-based relative PIGF mRNA expression was determined in lung adenocarcinoma (H292 and H460) and colon carcinoma (HCT116) cells 4 hours and 24 hours after irradiation with increasing doses of ionizing radiation. G – N, Minimal or no change in relative PIGF mRNA expression in head and neck squamous

carcinoma (FaDu), medulloblastoma (UW228) and lung adenocarcinoma (H125 and H358) cells after irradiation. O-R, Minimal or no change in relative PIGF mRNA expression in human umbilical vein endothelial cells (HUVECs) and increase in relative PIGF mRNA expression with increasing doses of ionizing radiation in human pulmonary lung fibroblasts (HPLFBs). Bar graphs show PIGF mRNA expression relative to GAPDH  $\pm$  SEM. Students t-test was used for statistical analysis.

# Supplementary Figure S3



**Supplementary Figure S3. P53 is a major regulator of PIGF in response to IR. A-B,** MI-773-induced PIGF-expression and secretion were confirmed with p53-wt HCT116 colon carcinoma cells. **C,** Relative p53 mRNA expression was determined 48-96 hours after p53 downregulation in D341 cells. **D,** p53 protein level determined in cell lysates of D341 cells 48-96 hours after sip53 treatment start. As a control and downstream target of p53, p21 levels are represented following sip53 treatment. **E-H,** Relative p53 mRNA expression was determined 48-96 hours after p53 downregulation in HCT116 colon carcinoma cells and corresponding PIGF secretion. Neither expression nor secretion were enhanced by MI-773 in the sip53 pretreated HCT116 cells. **I,** Decrease in PIGF secretion in Pifithrin-pretreated HCT116 cells. Graphs show p53 mRNA expression relative to GAPDH  $\pm$  SEM.

## Supplementary Figure S4

A)

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wt 200 GTGGTACCCTTCCAGGAAGTGTGGGGCCGCAGCTACTGCCGGGCGCTGGAGAGGCTGGTG 259
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260 GACGTCGTGTCCGAGTACCCCAGCGAGGTGGAGCACATGTTTCAGCCCATCCTGTGTCTCC 319
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61   GACGTCGTGTCCGAGTACCCCAGCGAGGTGGAGCACATGTTTCAGCCCATCCTGTGTCTCC 120

320 CTGCTGCGCTGCACCGGCTGCTGCGGGCGATGAGAATCTGCACTGTGTGCCGGTGGAGACG 379
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121 CTGCTGCGCTGCACCGGCTGCTGCGGGCGATGAGAATCTGCACTGTGTGCCGGTGGAGACG 180

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241 TAGAAGGTACCTGGAGAGGGAAGAAGATAGGCCAGGT 278

wt 214 GGAAGTGTGGGGCCGCAGCTACTGCCGGGCGCTGGAGAGGCTGGTGGACGTCGTGTCCGA 273
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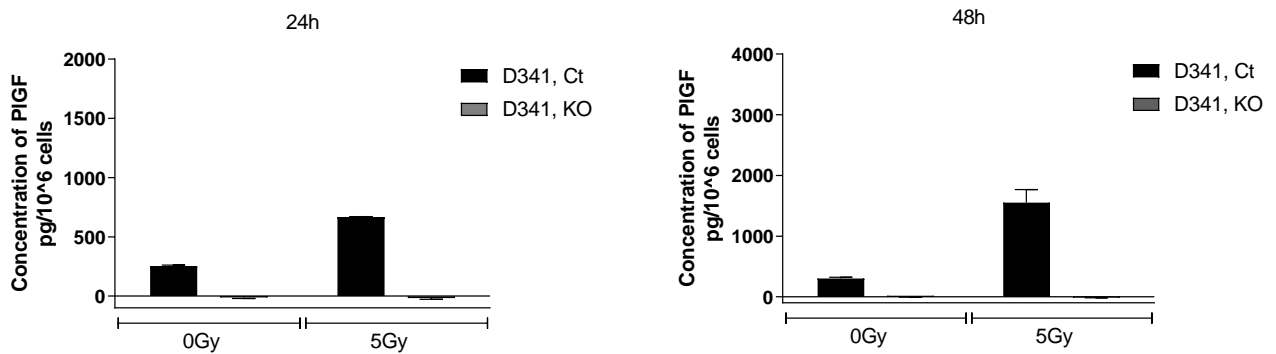
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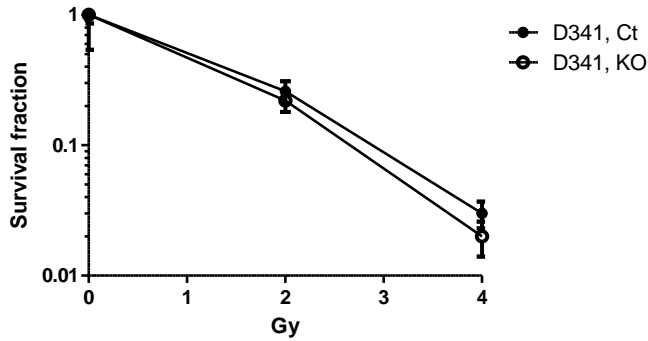
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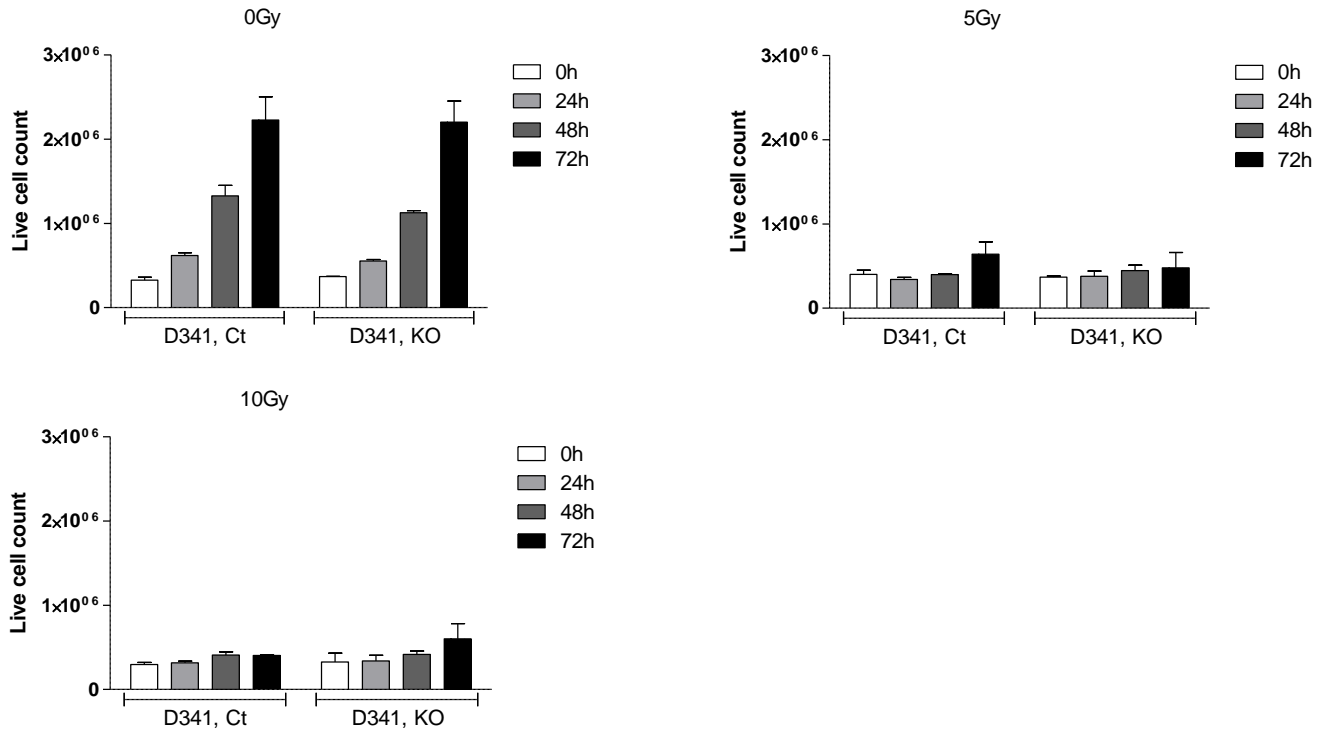
B)



C)



D)



**Supplementary Figure S4. Generation of PIGF knockout cell lines using CRISPR/Cas9 system.** A, A non-targeting (Ct) and an exon 3-targeting single guide RNA of the PIGF gene were separately cloned into the Cas9 and EGFP expressing pLentiCRISPR plasmids. Sequencing of the targeted region of exon 3 revealed successful PIGF-gene targeting. Aligned sequences of exon 3 of wildtype (upper sequence) and



transfected cell lines (lower sequence) was compared. Successful knockout is shown by an insertion of a nucleotide in the knockout cell line (red square). **B**, IR-induced PIGF secretion was determined in the supernatants of the cell clones 24 hours and 48 hours after irradiation. **C**, Clonogenicity and **D**, proliferative activity of PIGF-wt and PIGF-ko cell clones in response to irradiation.

**Supplementary Table S1.** Correlation of genetic background to tumor cell lines to early PLGF expression in response to IR.

CELL LINES	SOURCE/DISEASE	KRAS STATUS	P53 STATUS	PLGF EXPRESSION AT 4H	REFERENCES
H460	Lung /carcinoma	Q61H	Wild-type	Increased	den Dunnen JT 2000, Leroy B 2017
H292	Lung/carcinoma	Wild-type	Wild-type	Increased	Lu W 2017, Acunzo M 2017
H358	Lung/carcinoma	G12C	Null	Unchanged	den Dunnen JT 2000, Tung MC 2015
FADU	Head and neck/ squamous cell carcinoma	Wild-type	R248L	Unchanged	Somers KD 1992, Hoa M 2002
DAOY	Cerebellum/Medulloblastoma	Wild-type	C242F	Unchanged	Saylor RL 1991
D341	Cerebellum/Medulloblastoma	Wild-type	Wild-type	Increased	Saylor RL 1991
A549	Lung/carcinoma	G12S	Wild-type	Increased	den Dunnen JT 2000, Leroy B 2017
H125	Lung/carcinoma	Wild-type	Frameshift	Unchanged	Phelps RM 1996, Padanad MS 2016
UW228	Cerebellum/Medulloblastoma	Mutated	T155N	Unchanged	Lacroix 2014
HCT116	Colon carcinoma	G13D	Wild-type	Increased	Liu Y 2005, Alves S 2015