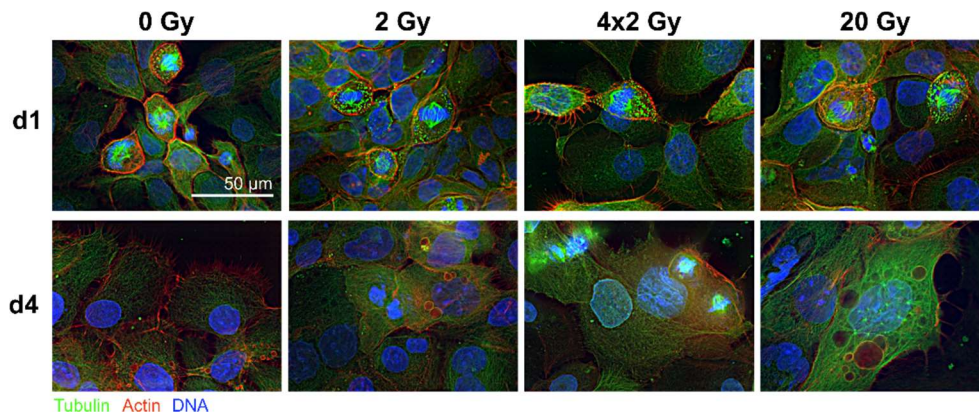
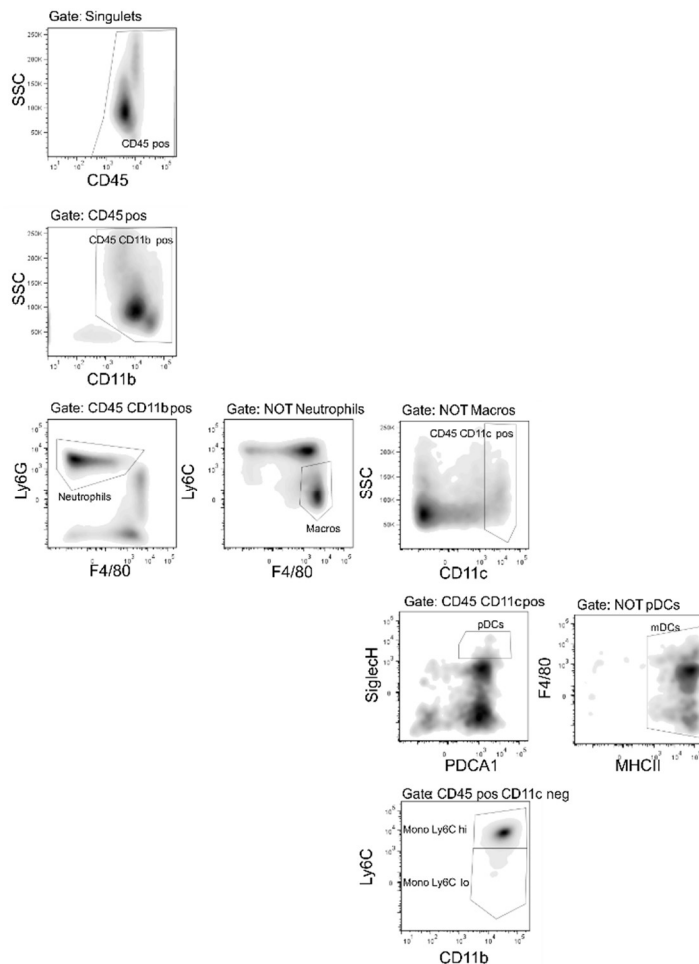


Supplemental Figures and Tables



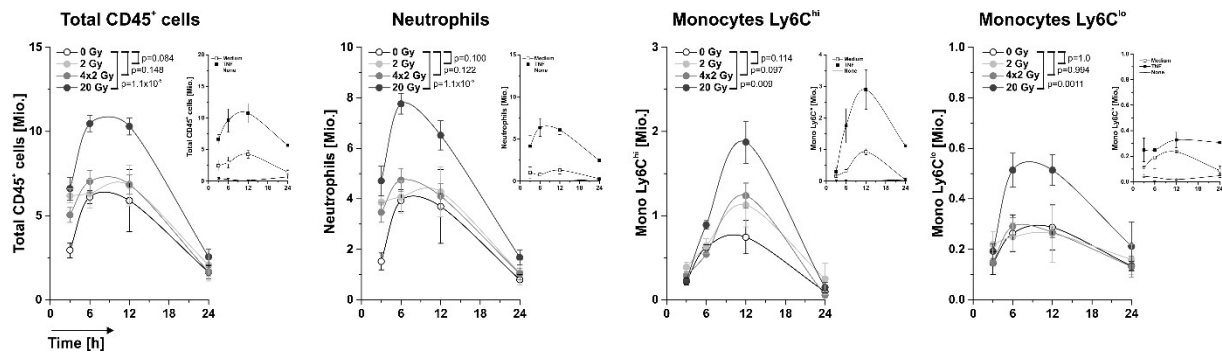
Supplemental Figure 1: Immunofluorescence of irradiated HCC1937 cells.

Representative immunofluorescence photographs of HCC1937 breast cancer cells on day 1 and day 4 after irradiation at the indicated doses. 63x magnification, scale bar 50 μm .



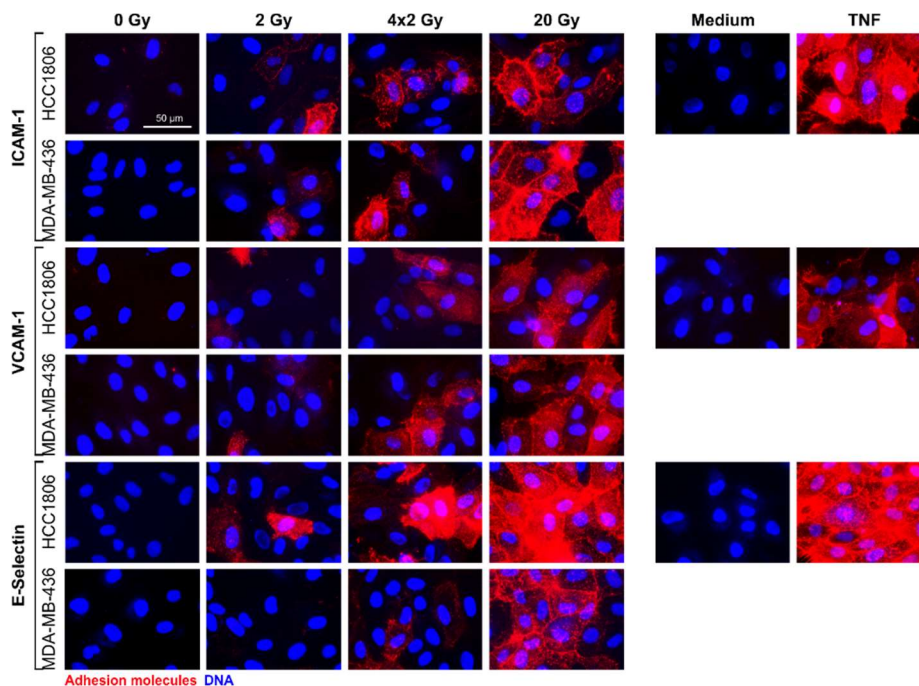
Supplemental Figure 2: Gating strategy for the identification of air pouch-infiltrating leukocyte subsets.

Among all CD45⁺ leukocytes (after doublet discrimination), myeloid cells were identified by CD11b expression. Among the CD11b⁺ cells, neutrophils (Ly6G⁺F4/80⁻) and macrophages (Ly6C⁺F4/80⁺) were sequentially extracted. On the basis of CD11c expression, DCs were discriminated from monocytes. CD11c⁺ DCs were subclassified into pDCs (Siglec-H⁺PDCA1⁺) and mDCs (F4/80⁺MHCII⁺). Monocyte subsets (CD11c⁻) were identified by their Ly6C expression into Ly6C^{hi} and Ly6C^{low} monocytes.



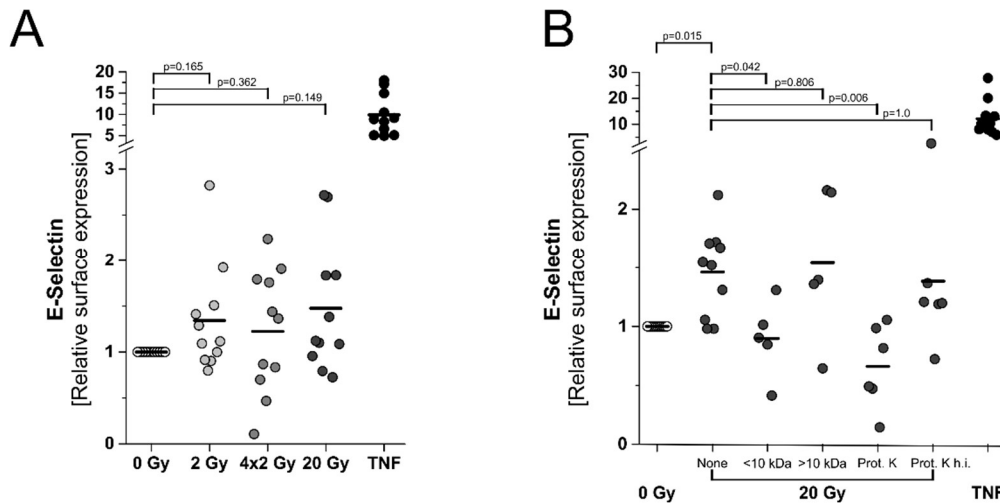
Supplemental Figure 3: Dynamics of leukocyte recruitment in the air pouch model with supernatants of irradiated HCC1806 cells.

Air pouch lavages were collected at the indicated time points after injection, and leukocyte subsets were analyzed by flow cytometry as in Figure 2. Total cell numbers per pouch are shown (n= 4-6 animals for supernatants, n= 2 animals for the control stimuli medium or 50 ng/ml TNF). Means $\pm$ SEM are depicted, and *p*-values were calculated by two-way ANOVA with Bonferroni-Holm correction.



Supplemental Figure 4: Upregulation of adhesion molecule surface expression on endothelial cells in response to supernatants of irradiated breast cancer cells.

HUVECs were incubated for 4 h with supernatants of irradiated HCC1806 or MDA-MB-436 cells, respectively. Medium and TNF (50 ng/ml) served as controls. Adhesion molecule surface expression was detected by native immunofluorescence staining as in Figure 3A. 63x magnification, scale bar 50 μ m.



Supplemental Figure 5: Upregulation of E-selectin surface expression is stimulated by protein DAMPs released from irradiated breast cancer cells.

(A) HUVECs were stimulated for 4 h with supernatants of irradiated HCC1937 cells, and E-selectin surface expression was stained by immunofluorescence and quantified by fluorometric measurement as in Figure 3B. Results of 11 independent experiments are shown, and p -values were determined by unpaired Student's t -tests with Bonferroni-Holm correction. (B) Biochemical characterization of the responsible molecular entities was carried out as in Figure 3C. Results of 5-10 independent experiments are depicted, and group comparisons were performed by unpaired Student's t -tests with Bonferroni-Holm correction.

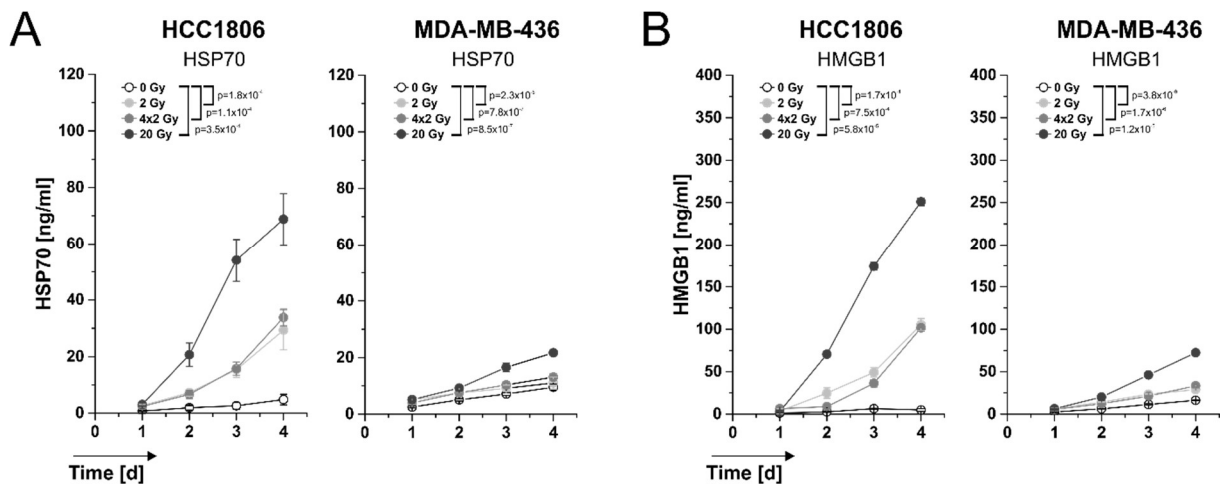
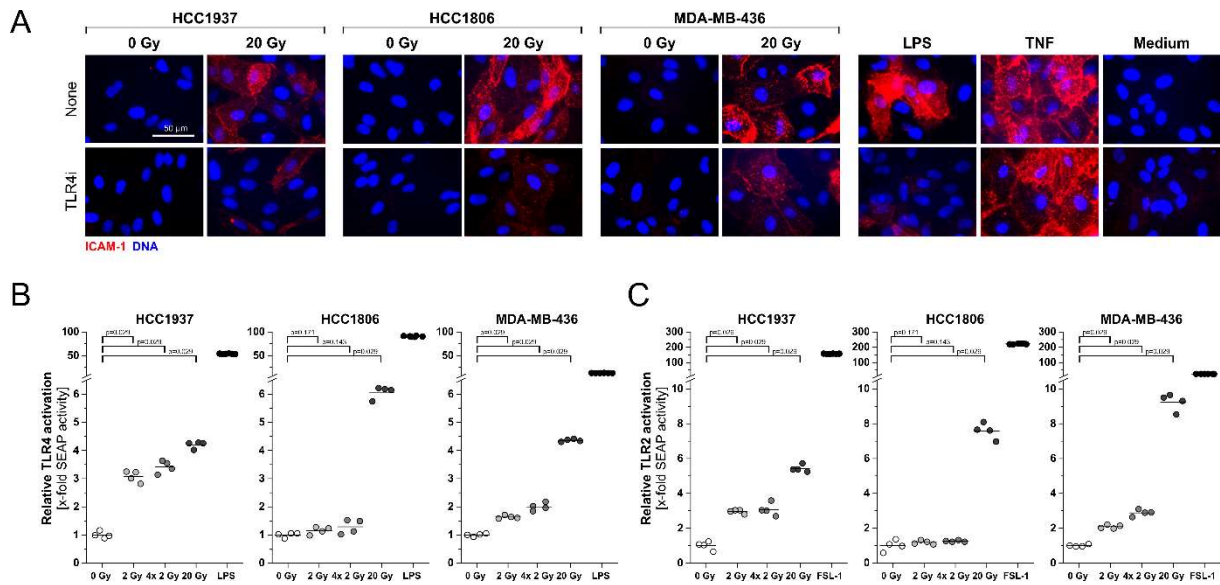


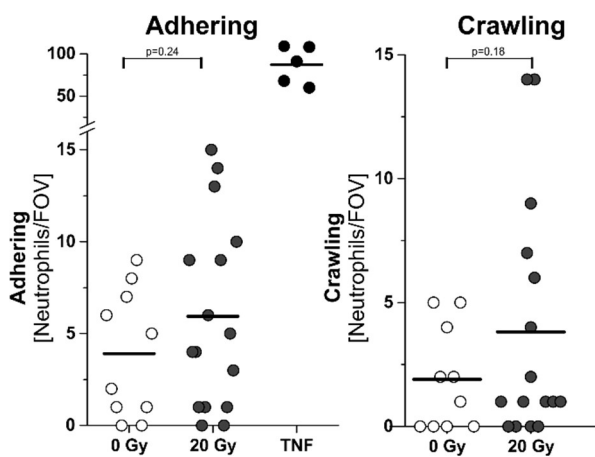
Figure 6: HSP70 and HMGB1 are time- and dose-dependently released by irradiated HCC1806 and MDA-MB-436 cells.

ELISAs for HSP70 (A) and HMGB1 (B) were performed with supernatants of irradiated HCC1806 and MDA-MB-436 cells as in Figure 3D. Means $\pm$ SD of three independent experiments (HSP70) or results from one representative experiment performed in triplicates (HMGB1) are shown. Group comparisons were calculated by two-way ANOVA followed by Bonferroni-Holm correction.



Supplemental Figure 7: TLR4 is activated by supernatants of irradiated breast cancer cells and contributes to upregulation of adhesion molecule surface expression on endothelial cells.

(A) ICAM-1 surface expression. HUVECs were incubated for 4 h with supernatants of irradiated breast cancer cells in the presence or absence of the TLR4 antagonist RS-LPS (TLR4i, 10 µg/ml). Medium, LPS (100 ng/ml), and TNF (50 ng/ml) served as controls. ICAM-1 surface expression was detected by native immunofluorescence staining as in Figure 3A. 63x magnification, scale bar 50 µm. (B, C) TLR activation. Activation of TLR4 (B) and TLR2 (C) was measured in HEK-Blue™ hTLR reporter cells upon exposure to supernatants of irradiated breast cancer cells for 7 h. LPS (10 ng/ml) and FSL-1 (10 ng/ml) were used as positive control stimuli. Results from one representative experiment performed in quadruplicates are shown. Group comparisons were performed by two-way exact Wilcoxon rank test.



Supplemental Figure 8: Functional analysis of endothelial cell activation in a flow chamber setup.

HUVECs were seeded into microflow chambers overnight and subsequently stimulated with supernatants of 20 Gy- or non-irradiated HCC1937 cells for 4 h, respectively. 50 ng/ml TNF were used as positive control. Primary human neutrophils were perfused through the chambers at 0.2 dyn/cm², and numbers of adherent and crawling neutrophils on the HUVEC layer were counted over a time period of 10 min. Results of 10-16 independent flow chambers are depicted, and group comparison was performed by unpaired Student's *t*-test.

Supplemental Table 1: Mouse qRT-PCR primer pairs.

Target	Primer	Sequence
CXCL1	Forward	CAAACCGAAGTCATAGCCACACT
	Reverse	TACTTGGGGACACCTTTTAGCATC
CXCL2	Forward	GCCACTCTCAAGGGCGGT
	Reverse	TCAGTTAGCCTTGCCTTTGTTCA
CXCL3	Forward	ATGGTCAAGAAGTTTGCCTCAAC
	Reverse	GGACTTGCCGCTCTTCAGTATC
IL-1 β	Forward	TGACAGTGATGAGAATGACCTGTTC
	Reverse	AGGTTTGAAGCAGCCCTTC
IL-6	Forward	GAAATCGTGGAATGAGAAAAGAGTT
	Reverse	GTGCATCATCGTTGTTTCATACAATC
L-Selectin	Forward	CACTGCTCTGTTGTGACTTCCTGA
	Reverse	GGTAAGTCCAACAGTGAGTTCCATG
VCAM-1	Forward	AACTACAAGTCTACATCTCTCCCAGGAA
	Reverse	GTCACAGCACCAACCCTCTTGA
18S rRNA	Forward	CGGCTACCACATCCAAGGAA
	Reverse	AGCTGGAATTACCGCGGC
δ -ALAS	Forward	ATCATCCCTGTGCGGGTTG
	Reverse	TAATTGATGGCCTGGACGTAGATATT
PECAM-1	Forward	GTCACCGTGCAGGAGTCCTT
	Reverse	AATGTGCAGCTGGTCCCCT
β -Actin	Forward	CGCCACCAGTTCGCCAT
	Reverse	ACGACCAGCGCAGCGATA
α -Tubulin	Forward	AGGATTCGCAAGCTGGCTG
	Reverse	AAAGCTGTGGAAAACCAAGAAGC

Supplemental Table 2: Human qRT-PCR primer pairs.

Target	Primer	Sequence
ICAM-1	Forward	GGAACAACCGGAAGGTGTATGA
	Reverse	GTTCTGGAGTCCAGTACACGGTG
VCAM-1	Forward	ACGCAAACACTTTATGTCAATGTTG
	Reverse	AGCTGCCTGCTCCACAGG
E-Selectin	Forward	CCTACCTGTGAAGCTCCCACTG
	Reverse	AGGAGGGAGAGTCCAGCAGC
P-Selectin	Forward	CCACCAATGTGTGAAGCCATC
	Reverse	TCAGAACAATCCAGGCTGCC
IL-1 α	Forward	GCTTCCTGAGCAATGTGAAATACA
	Reverse	CAAATTTCACTGCTTCATCCAGATT
IL-6	Forward	GGTACATCCTCGACGGCATCT
	Reverse	AGTGCCTCTTTGCTGCTTTCAC
TNF	Forward	TCTTCTCGAACCCCGAGTGA
	Reverse	GGAGCTGCCCCTCAGCTT
CCL1	Forward	GCTTGCTGCTAGCTGGGATGT
	Reverse	CTCCGCAAATGAGAAGCAACA
CCL2	Forward	CAGCAAGTGTCCCAAAGAAGCT
	Reverse	TGGAATCCTGAACCCACTTCTG
CCL5	Forward	CTCTGCGCTCCTGCATCTG
	Reverse	GCGGGCAATGTAGGCAAA
CCL7	Forward	GAGAGCTACAGAAGGACCACCAGT
	Reverse	GGGTCAGCACAGATCTCCTTGT
CCL-8	Forward	GCTGGAGAGCTACACAAGAATCAC
	Reverse	GCCCCGTTTGGTCTTGAA
CCL13	Forward	AGGCTGAAGAGCTATGTGATCACC
	Reverse	CCTTGCCCAGTTTGGTTCTG
CCL14&15	Forward	GCGTCAGCGGATTATGGATTAC
	Reverse	ACGGAATGGCCCCTTTTG
CXCL1	Forward	AAGCTTGCCTCAATCCTGCAT

	Reverse	TGGATTTGTCAGTGTTCAGCATCT
CXCL2	Forward	CGCATCGCCCATGGTTAA
	Reverse	CAGTTGGATTTGCCATTTTTCAG
CXCL3	Forward	CCCATGGTTCAGAAAATCATCG
	Reverse	GTTGGTGCTCCCCTTGTTCA
CXCL8	Forward	TGGCAGCCTTCCTGATTTCT
	Reverse	TGCACTGACATCTAAGTTCTTTAGCA
CXCL10	Forward	TGGCATTCAAGGAGTACCTCTCT
	Reverse	GTAGCAATGATCTCAACACGTGG
CXCL11	Forward	TGTTCAAGGCTTCCCATGT
	Reverse	GAGGCTTTCTCAATATCTGCCACT
CXCL12	Forward	TGCTGGTCCTCGTGCTGAC
	Reverse	TGGCAACATGGCTTTTCGAA
CX3CL1	Forward	ACAGAACCAGGCATCATGCG
	Reverse	CGGGTCGGCACAGAACAG
18S rRNA	Forward	CGGCTACCACATCCAAGGAA
	Reverse	AGCTGGAATTACCGCGGC
δ-ALAS	Forward	TCCACTGCAGCAGTACACTACCA
	Reverse	ACGGAAGCTGTGTGCCATCT
β ₂ -Microglobulin	Forward	TGCTCGCGCTACTCTCTCTTTC
	Reverse	TCTCTGCTGGATGACGTGAGTAAAC