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Supplemental information

Comparing the efficacy of γ- and electron-irradiation of PBMCs to promote secretion of paracrine, regenerative factors

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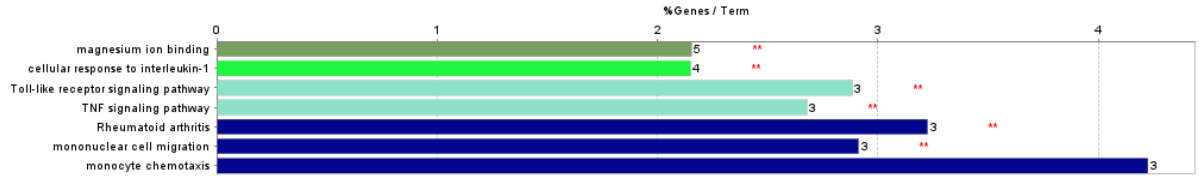
Supplemental Figures

Figure S1. Gene enrichment analysis of genes differentially expressed between γ- and electron irradiation.

A



B

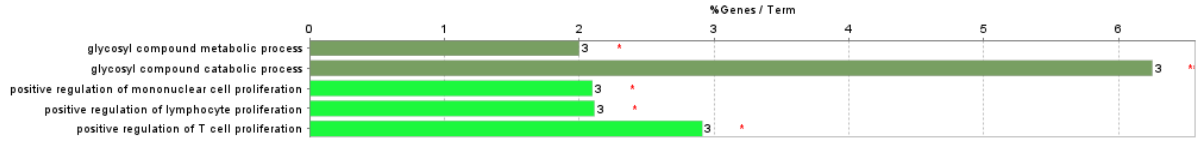


Figure S1. Gene ontologies (GO) associated with genes expressed in (A) γ-irradiated PBMCs and (B) electron-irradiated PBMCs. Numbers next to bars indicate absolute number of genes involved per term. Numbers on x-axes indicate relative amount of genes enriched per term. Asterisks indicate statistical significance. Color codes indicate families of GO terms.

Figure S2. DNA damage response of electron- and γ-irradiated human PBMCs.

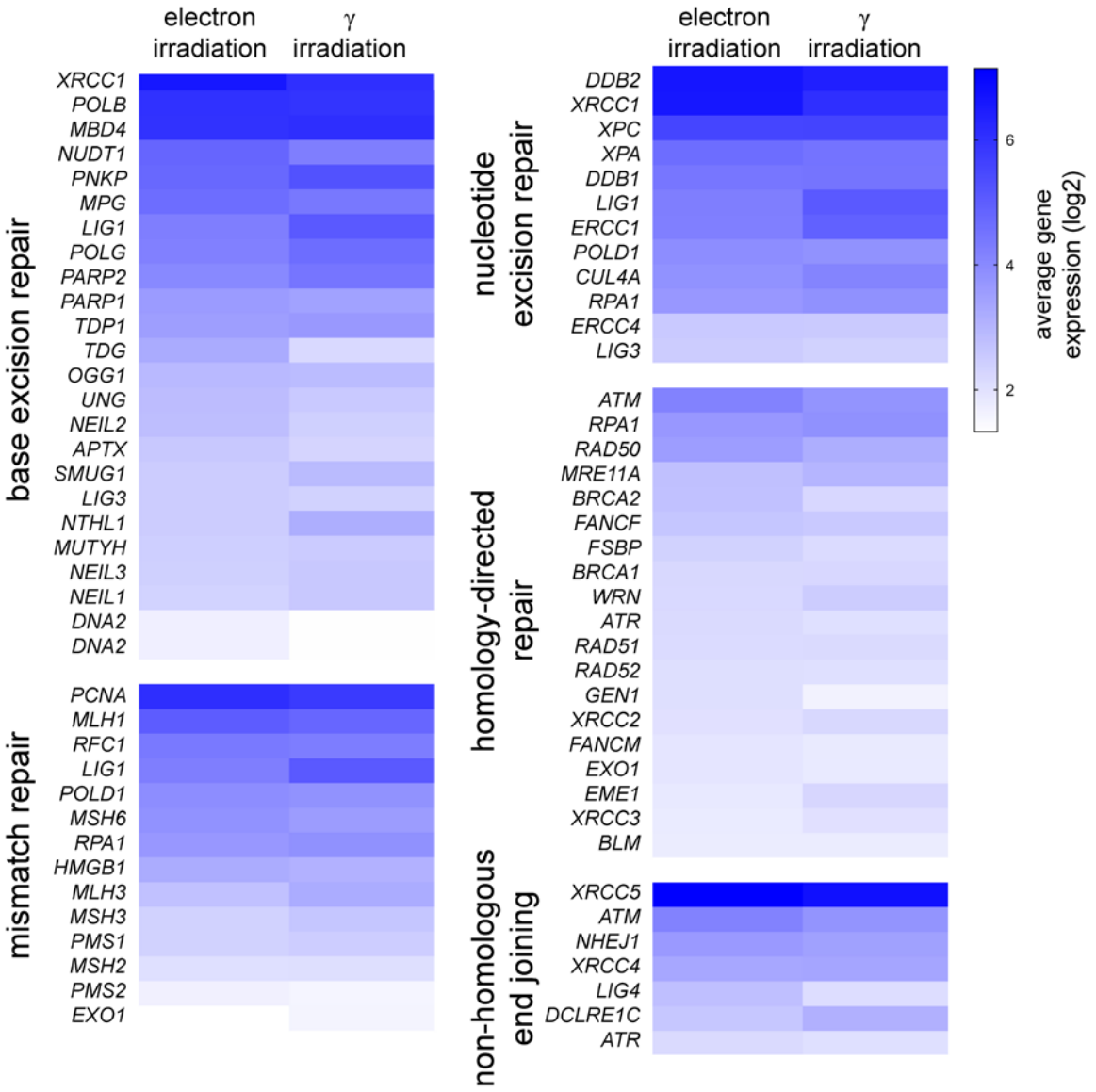
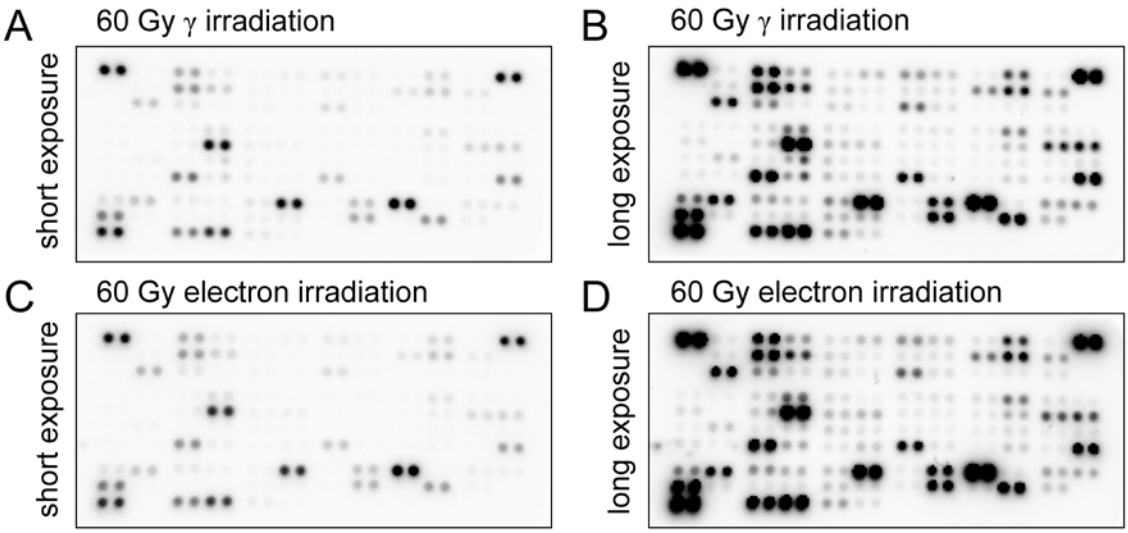


Figure S2. Expression values of genes associated with DNA damage repair. Colors indicate log2-transformed gene expression values as indicated in the color legend. Genes implicated in different processes of DNA damage repair were analyzed.

Figure S3. Semi-quantitative protein analysis of secretomes obtained from γ- and electron-irradiated PBMCs.



E

A1, A2 reference spots	A3, A4 adiponectin	A5, A6 apolipoprotein A-I	A7, A8 angiogenin	A9, A10 angiopoietin-1	A11, A12 angiopoietin-2	A13, A14 BAFF	A15, A16 BDNF	A17, A18 complement C5/C5a	A19, A20 CD14	A21, A22 CD30	A23, A24 reference spots
	B3, B4 CD40 ligan	B5, B6 chitinase 3-like 1	B7, B8 complement factor D	B9, B10 C-reactive protein	B11, B12 cripto-1	B13, B14 cystatin C	B15, B16 Dkk-1	B17, B18 DPPIV	B19, B20 EGF	B21, B22 basigin	
	C3, C4 ENA-78	C5, C6 endoglin	C7, C8 Fas ligand	C9, C10 FGF basic	C11, C12 FGF-7	C13, C14 FGF-19	C15, C16 Flt-3 ligand	C17, C18 G-CSF	C19, C20 GDF-15	C21, C22 GM-CSF	
D1, D2 GROalpha	D3, D4 growth hormone	D5, D6 HGF	D7, D8 ICAM-1	D9, D10 IFN-gamma	D11, D12 IGFBP-2	D13, D14 IGFBP-3	D15, D16 IL-1alpha	D17, D18 IL-1beta	D19, D20 IL-1ra	D21, D22 IL-2	D23, D24 IL-3
E1, E2 IL-4	E3, E4 IL-5	E5, E6 IL-6	E7, E8 IL-8	E9, E10 IL-10	E11, E12 IL-11	E13, E14 IL-12p70	E15, E16 IL-13	E17, E18 IL-15	E19, E20 IL-16	E21, E22 IL-17A	E23, E24 IL-18Bpa
F1, F2 IL-19	F3, F4 IL-22	F5, F6 IL-23	F7, F8 IL-24	F9, F10 IL-27	F11, F12 IL-31	F13, F14 IL-32	F15, F16 IL-33	F17, F18 IL-34	F19, F20 IP-10	F21, F22 I-TAC	F23, F24 kallikrein 3
G1, G2 leptin	G3, G4 LIF	G5, G6 lipocalin-2	G7, G8 MCP-1	G9, G10 MCP-3	G11, G12 M-CSF	G13, G14 MIF	G15, G16 MIG	G17, G18 MIP-1alpha/beta	G19, G20 MIP-3apha	G21, G22 MIP-3beta	G23, G24 MMP9
H1, H2 myeloperoxidase	H3, H4 osteopontin	H5, H6 PDGF-AA	H7, H8 PDGF-AB/BB	H9, H10 pentraxin 3	H11, H12 PF4	H13, H14 RAGE	H15, H16 RANTES	H17, H18 RBP-4	H19, H20 relaxin-2	H21, H22 resistin	H23, H24 SDF-1alpha
I1, I2 serpin E1	I3, I4 SHBG	I5, I6 ST2	I7, I8 TARC	I9, I10 TFF3	I11, I12 TIR	I13, I14 TGF-alpha	I15, I16 thrombospondin-1	I17, I18 TNF-alpha	I19, I20 uPAR	I21, I22 VEGF	
J1, J2 reference spots		J5, J6 vitamin D BP	J7, J8 CD31	J9, J10 TIM-3	J11, J12 VCAM-1						J23, J24 negative controls

Figure S3. Proteome profiler of secretomes obtained from γ- and electron-irradiated PBMCs. We used a commercially available kit (Human XL Cytokine Array Kit Proteome Profiler, R&D Systems) to analyze cytokines present in secretomes. For (A & B) γ- and (C & D) electron irradiation, short and long exposures of chemiluminescent signals are shown. For short and long exposures, images were acquired after 30 seconds and 600 seconds exposure, respectively. Each dot represents one analyte spotted in duplicates. (E) Coordinates of all analytes present on each membrane. Dots are marked from 1 to 24 from left to right and from A to J from top to bottom.

Figure S4. Calibration data of the linear accelerator.

The electron beam was calibrated to deliver 1 Gy/ 100 Monitor units at the depth of maximum dose using a source to surface distance of 100 cm and a 10 cm x 10 cm applicator. For these experiments, a 20 cm x 20 cm applicator was used and the Linac output was corrected for the applicator-specific output factor of 1.024. Regular, machine-specific quality assurance guaranteed a symmetry and a flatness of the lateral dose profile of less than 2% and 5%, respectively. A lateral dose profile of the employed electron beam is depicted in figure S4.

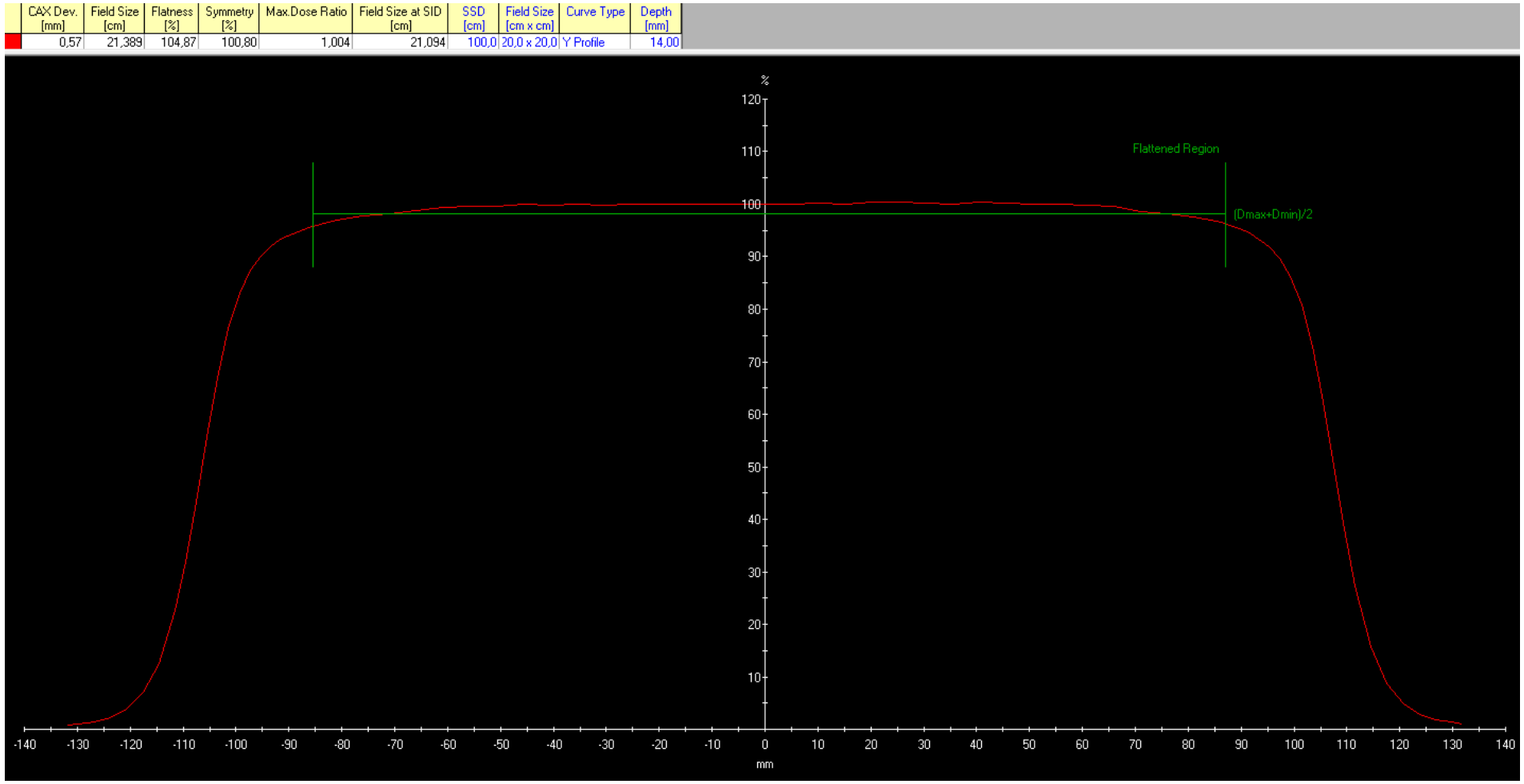


Figure S4. A lateral dose profile of a 6 MeV electron beam.

Figure S5. RNA quality control.

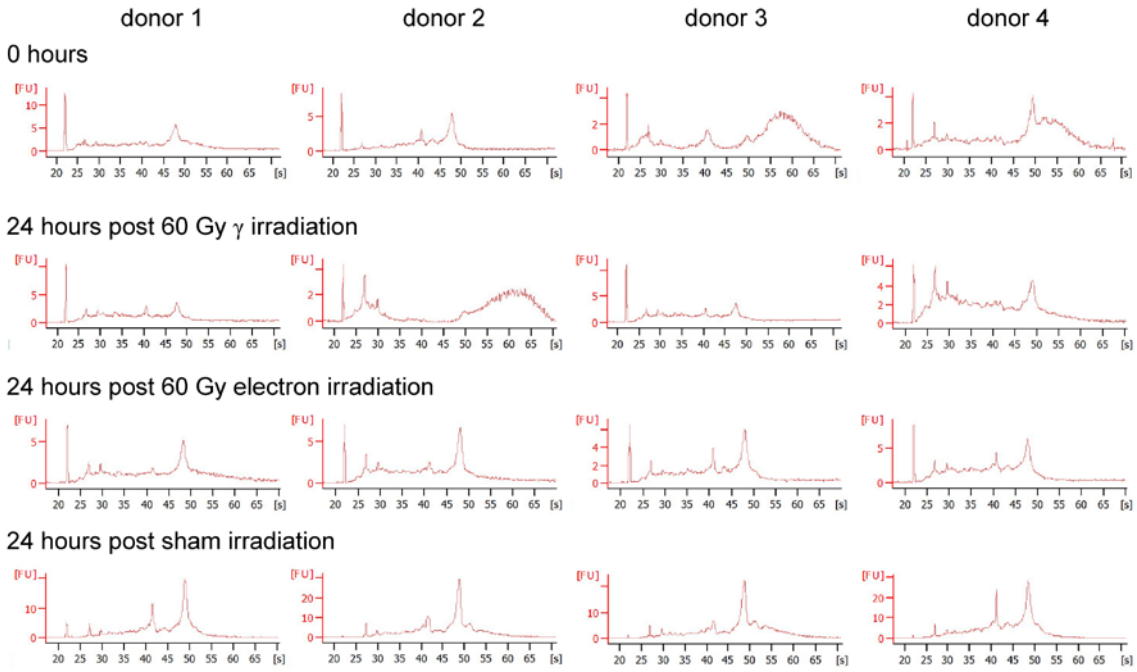


Figure S5. RNA quality of electron- and γ-irradiated human PBMCs of 4 donors. RNA quality was assessed by Agilent 2100 Bioanalyzer.

Figure S6. Quality control of EV-derived small RNAs.

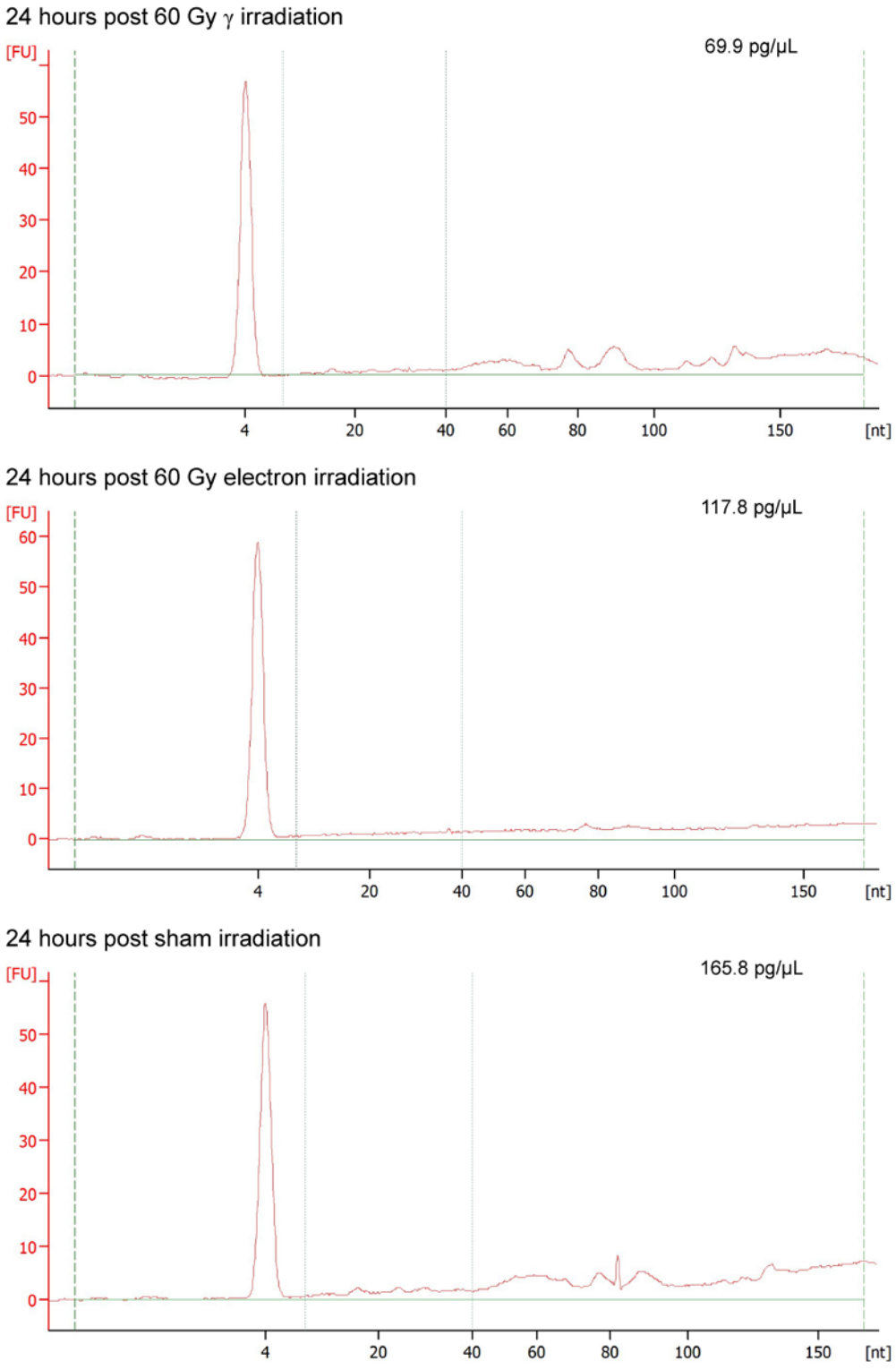


Figure S6. Quality of small RNAs present in PBMC-derived EVs. A pool of n = 6 donors was analysed. RNA quality was assessed by Agilent 2100 Bioanalyzer. Numbers indicate RNA concentrations.

Supplemental Tables

Table S1. MicroRNA species present in the EVs of γ- and electron-irradiated PBMCs. Numbers indicate UMIs.

miRNA	60 Gy γ irradiation	60 Gy electron irradiation	miRNA	60 Gy γ irradiation	60 Gy electron irradiation	miRNA	60 Gy γ irradiation	60 Gy electron irradiation
hsa-miR-16-5p	4 686	2 373	hsa-miR-146b-5p	73	27	hsa-miR-1469	26	33
hsa-miR-150-5p	3 129	1 630	hsa-miR-4767	72	151	hsa-miR-6865-5p	26	26
hsa-miR-223-3p	2 638	1 364	hsa-miR-24-3p	72	69	hsa-miR-99b-5p	26	
hsa-miR-142-3p	1 766	589	hsa-miR-320b	68	61	hsa-miR-3666	25	70
hsa-miR-423-5p	1 144	980	hsa-miR-663a	65	77	hsa-miR-432-5p	25	
hsa-miR-26a-5p	1 047	400	hsa-miR-4508	65	57	hsa-miR-4516	24	35
hsa-miR-92a-3p	900	415	hsa-miR-339-5p	64	74	hsa-miR-320d	24	29
hsa-miR-342-3p	854	485	hsa-miR-6727-5p	64	43	hsa-miR-940	24	29
hsa-miR-486-5p	770	800	hsa-miR-3135b	62	58	hsa-miR-30c-5p	24	28
hsa-miR-191-5p	717	441	hsa-miR-423-3p	60	24	hsa-miR-2110	24	
hsa-miR-142-5p	616	280	hsa-miR-15b-5p	59	38	hsa-miR-1268a	24	
hsa-miR-26b-5p	546	258	hsa-miR-636	58	39	hsa-miR-222-3p	24	
hsa-miR-3960	423	396	hsa-miR-4787-5p	57	24	hsa-miR-3152-5p	23	51
hsa-miR-29a-3p	289	96	hsa-miR-4800-3p	56	38	hsa-miR-92b-3p	23	26
hsa-miR-146a-5p	284	212	hsa-miR-1246	55	54	hsa-miR-128-2-5p	23	25
hsa-miR-3656	282	178	hsa-miR-6087	55	41	hsa-miR-1181	23	
hsa-miR-30d-5p	281	183	hsa-miR-151a-3p	52	28	hsa-miR-125b-5p	23	
hsa-miR-155-5p	277	90	hsa-miR-28-3p	50		hsa-miR-4322	21	20
hsa-miR-1915-3p	276	281	hsa-miR-4784	48	93	hsa-miR-1307-3p	21	
hsa-miR-140-3p	269	148	hsa-miR-223-5p	47	60	hsa-miR-128-3p	20	
hsa-miR-21-5p	258	106	hsa-miR-221-3p	47	45	hsa-miR-505-5p	20	
hsa-miR-4301	246	74	hsa-miR-16-2-3p	46	47	hsa-miR-106b-3p	20	
hsa-miR-486-3p	245	226	hsa-miR-451a	46	40	hsa-miR-3915		108
hsa-miR-125a-5p	231	198	hsa-miR-7704	46	23	hsa-miR-138-2-3p		67
hsa-miR-103a-3p	206	67	hsa-miR-6089	45	52	hsa-miR-370-3p		62
hsa-miR-25-3p	192	63	hsa-miR-4649-5p	45	25	hsa-miR-3180-5p		47
hsa-miR-4532	188	134	hsa-miR-107	44	43	hsa-miR-212-5p		46
hsa-miR-126-3p	186	226	hsa-miR-3141	41	62	hsa-miR-4730		43
hsa-miR-361-3p	180	45	hsa-miR-4492	41	39	hsa-miR-27a-3p		40
hsa-miR-320a	163	83	hsa-miR-3615	40	47	hsa-miR-6756-3p		37
hsa-miR-320c	134	102	hsa-miR-361-5p	39	24	hsa-miR-518b		33
hsa-miR-23a-3p	133	75	hsa-miR-941	39		hsa-miR-4707-5p		32
hsa-miR-6789-5p	127	155	hsa-miR-6724-5p	38	27	hsa-miR-625-5p		31
hsa-miR-148a-3p	117	64	hsa-miR-199a-3p	37	30	hsa-miR-3200-3p		30
hsa-miR-1343-5p	117	27	hsa-miR-26a-2-3p	35		hsa-miR-1178-3p		29
hsa-miR-425-5p	113	110	hsa-miR-4734	34	30	hsa-miR-1260b		29
hsa-miR-181a-5p	110	65	hsa-miR-4515	34	27	hsa-miR-8072		29
hsa-miR-6752-3p	109	71	hsa-miR-148b-3p	34	20	hsa-miR-8069		28
hsa-miR-93-5p	103	35	hsa-miR-4792	32	61	hsa-miR-6758-3p		27
hsa-miR-6126	93	45	hsa-miR-4632-5p	32	30	hsa-miR-6090		27
hsa-miR-29b-3p	93	38	hsa-miR-143-3p	32		hsa-miR-1294		27
hsa-miR-4488	90	75	hsa-miR-328-3p	29		hsa-miR-4286		26
hsa-miR-103b	82	23	hsa-miR-744-5p	28		hsa-miR-6733-3p		26
hsa-miR-29c-3p	80	36	hsa-miR-181a-2-3p	28		hsa-miR-197-3p		25
hsa-miR-101-3p	78	29	hsa-miR-200c-3p	28		hsa-miR-635		25
hsa-miR-195-5p	77	49	hsa-miR-4785	27	31	hsa-miR-4650-5p		25
hsa-miR-30e-5p	76		hsa-miR-98-5p	27	26	hsa-miR-6805-3p		24
hsa-miR-342-5p	75	53	hsa-miR-6746-3p	27		hsa-miR-4789-3p		24

miRNA	60 Gy γ irradiation	60 Gy electron irradiation
hsa-miR-4661-5p		23
hsa-miR-186-5p		22
hsa-miR-6770-3p		22
hsa-miR-150-3p		21
hsa-miR-6081		21
hsa-miR-6720-3p		21
hsa-miR-6812-5p		21
hsa-miR-3664-5p		21
hsa-miR-218-5p		21
hsa-miR-23b-3p		20
hsa-miR-718		20
hsa-miR-3195		20
hsa-miR-27a-5p		20
hsa-miR-4720-5p		20
hsa-miR-6803-3p		20

Table S2. Functional annotations of the most abundant microRNAs in the EVs of γ- and electron-irradiated PBMCs. Most relevant functions were highlighted in green.

Term	Count	Percent	Fold	P-value	Bonferroni	FDR	miRNA
Immune Response	9	0.09782609	10.22570332	3,13E-08	1.57e-5	6.19e-6	hsa-mir-92a-1,hsa-mir-486-1,hsa-mir-16-2,hsa-mir-16-1,hsa-mir-92a-2,hsa-mir-150,hsa-mir-486-2,hsa-mir-342,hsa-mir-223
Latent Virus Replication	5	0.29411765	30.74394464	2,44E-07	1.22e-4	2.71e-5	hsa-mir-223,hsa-mir-92a-1,hsa-mir-26a-1,hsa-mir-26a-2,hsa-mir-92a-2
Hematopoiesis	7	0.12280702	12.8369453	3,65E-07	1.83e-4	3.09e-5	hsa-mir-486-2,hsa-mir-92a-1,hsa-mir-486-1,hsa-mir-223,hsa-mir-142,hsa-mir-92a-2,hsa-mir-150
Cholesterol Metabolism	4	0.5	52.26470588	3,93E-07	1.97e-4	3.17e-5	hsa-mir-486-2,hsa-mir-92a-1,hsa-mir-486-1,hsa-mir-92a-2
Aging	7	0.11111111	11.61437908	7,41E-07	3.71e-4	4.70e-5	hsa-mir-92a-1,hsa-mir-16-1,hsa-mir-16-2,hsa-mir-26a-1,hsa-mir-223,hsa-mir-26a-2,hsa-mir-92a-2
Angiogenesis	7	0.10769231	11.25701357	9,23E-07	4.62e-4	5.46e-5	hsa-mir-486-2,hsa-mir-92a-1,hsa-mir-486-1,hsa-mir-16-1,hsa-mir-16-2,hsa-mir-92a-2,hsa-mir-150
Circadian Rhythm	5	0.22727273	23.75668449	1,01E-06	5.05e-4	5.78e-5	hsa-mir-16-1,hsa-mir-191,hsa-mir-16-2,hsa-mir-26a-1,hsa-mir-26a-2
Ovarian Follicle Development	3	0.75	78.39705882	2,90E-06	1.45e-3	1.23e-4	hsa-mir-26a-2,hsa-mir-26b,hsa-mir-26a-1
Cell Cycle	7	0.08433735	8.81573352	5,02E-06	2.51e-3	1.90e-4	hsa-mir-92a-1,hsa-mir-16-1,hsa-mir-191,hsa-mir-16-2,hsa-mir-223,hsa-mir-150,hsa-mir-92a-2
Toxicity	5	0.13888889	14.51797386	1,33E-05	6.67e-3	4.01e-4	hsa-mir-16-1,hsa-mir-16-2,hsa-mir-26a-1,hsa-mir-26a-2,hsa-mir-150
Neurotoxicity	4	0.2	20.90588235	2,53E-05	0.0127	6.17e-4	hsa-mir-16-2,hsa-mir-92a-1,hsa-mir-92a-2,hsa-mir-16-1
Regulation of Akt Pathway	4	0.15384615	16.08144796	7,55E-05	0.0378	1.58e-3	hsa-mir-92a-1,hsa-mir-26a-1,hsa-mir-26a-2,hsa-mir-92a-2
Hormone-mediated Signaling Pathway	5	0.0862069	9.01115619	1,43E-04	0.0715	2.64e-3	hsa-mir-92a-1,hsa-mir-16-1,hsa-mir-16-2,hsa-mir-223,hsa-mir-92a-2
Tumor Suppressor MiRNAs	5	0.07692308	8.04072398	2,47E-04	0.1238	4.10e-3	hsa-mir-26b,hsa-mir-16-1,hsa-mir-16-2,hsa-mir-26a-1,hsa-mir-26a-2
Onco-MiRNAs	4	0.10810811	11.30047695	3,12E-04	0.1565	4.55e-3	hsa-mir-92a-1,hsa-mir-191,hsa-mir-92a-2,hsa-mir-150
T-Cell Differentiation	3	0.1875	19.59926471	3,78E-04	0.1892	5.32e-3	hsa-mir-16-1,hsa-mir-16-2,hsa-mir-150
Innate Immunity	4	0.0952381	9.95518207	5,14E-04	0.2575	6.52e-3	hsa-mir-26a-1,hsa-mir-223,hsa-mir-142,hsa-mir-26a-2
Cell Proliferation	5	0.0625	6.53308824	6,59E-04	0.3304	8.03e-3	hsa-mir-92a-1,hsa-mir-16-1,hsa-mir-16-2,hsa-mir-92a-2,hsa-mir-150

Adipogenesis	3	0.15	15.67941176	7,51E-04	0.376	8.95e-3	hsa-mir-26b,hsa-mir-26a-1,hsa-mir-26a-2
Epithelial-to-Mesenchymal Transition	5	0.06024096	6.29695252	7,83E-04	0.3921	9.27e-3	hsa-mir-486-2,hsa-mir-26b,hsa-mir-486-1,hsa-mir-191,hsa-mir-223
Skeletal Muscle Cell Differentiation	3	0.13636364	14.2540107	1,00E-03	0.502	0.0108	hsa-mir-26b,hsa-mir-26a-1,hsa-mir-26a-2
Chemosensitivity Of Tumor Cells	2	0.25	26.13235294	2,33E-03	1	0.0218	hsa-mir-16-1,hsa-mir-16-2
Embryonic Stem Cell Differentiation	3	0.09677419	10.11574953	2,77E-03	1	0.0253	hsa-mir-92a-1,hsa-mir-223,hsa-mir-92a-2
Cell Death	4	0.05128205	5.36048265	5,29E-03	1	0.0437	hsa-mir-92a-1,hsa-mir-16-1,hsa-mir-16-2,hsa-mir-92a-2
Adipocyte Differentiation	3	0.07317073	7.64849354	6,20E-03	1	0.0489	hsa-mir-26b,hsa-mir-92a-1,hsa-mir-92a-2
Lipid Metabolism	3	0.06666667	6.96862745	8,05E-03	1	0.0601	hsa-mir-423,hsa-mir-92a-1,hsa-mir-92a-2
Cholesterol Efflux	2	0.13333333	13.9372549	8,41E-03	1	0.0625	hsa-mir-486-2,hsa-mir-486-1
Chondrocyte Development	2	0.13333333	13.9372549	8,41E-03	1	0.0625	hsa-mir-92a-1,hsa-mir-92a-2
DNA Damage Response	2	0.125	13.06617647	9,56E-03	1	0.069	hsa-mir-16-2,hsa-mir-16-1
Cell Division	2	0.11764706	12.29757785	0,0108	1	0.0757	hsa-mir-16-2,hsa-mir-16-1
Cardiotoxicity	2	0.11764706	12.29757785	0,0108	1	0.0757	hsa-mir-486-2,hsa-mir-486-1
Cell Differentiation	3	0.05357143	5.59978992	0,0147	1	0.0984	hsa-mir-16-1,hsa-mir-16-2,hsa-mir-150
Immune System(Xiao's Cell2010)	2	0.0952381	9.95518207	0,0163	1	0.1074	hsa-mir-223,hsa-mir-150
Cytokine Secretion	1	0.5	52.26470588	0,019	1	0.1209	hsa-mir-150
Endothelial Cell Apoptosis	1	0.5	52.26470588	0,019	1	0.1209	hsa-mir-223
Tumor Cell Radiation Sensitivity	1	0.33333333	34.84313725	0,0284	1	0.1605	hsa-mir-223
Bone Regeneration	2	0.06666667	6.96862745	0,032	1	0.1714	hsa-mir-92a-1,hsa-mir-92a-2
Regulation of Stem Cell	3	0.03797468	3.96947133	0,0367	1	0.1933	hsa-mir-26b,hsa-mir-223,hsa-mir-142

Table S3. Calibration data of the γ-irradiator. Settings as well as calculated and measured values of dosimetry.

irradiator	IBL 437
configured value	30 Gy
irradiation period	336 seconds
mean irradiation temperature	24.3 °C
calculated dose in center	31.56 Gy
measured dose in center	30.90 Gy
deviation	2.1%
dose rate according to calculation	5.63 Gy/min
dose rate according to measurement	5.52 Gy/min

Table S4. Dosimetry results of the γ-irradiator. Values indicate measured values at certain positions within the cylinder at a set value of 30 Gy.

measured doses (Gy)

		distance from rotation axis (mm)				
		0	20	40	50	57
distance from bottom (mm)	280	24.1	27.2	28.2	30.4	32.2
	260	27.9	28.6	30.8	33.8	35.3
	230	31.3	31.6	34.6	36.6	38.8
	150	30.9	31.7	33.2	34.3	37.3
	70	30.2	32.1	33.8	36.8	38
	40	27.2	28.8	32.4	34.4	35.5
	20	26.3	26.9	27.7	31	31.8

For dosimetry, the γ-irradiator was set to emit a dose of 30 Gy in 336 seconds. The deviation from the measured values and the configured value is 2.1 %, which lies within the range of measuring errors. Therefore, no adjustments or corrective measures of the irradiator were required.