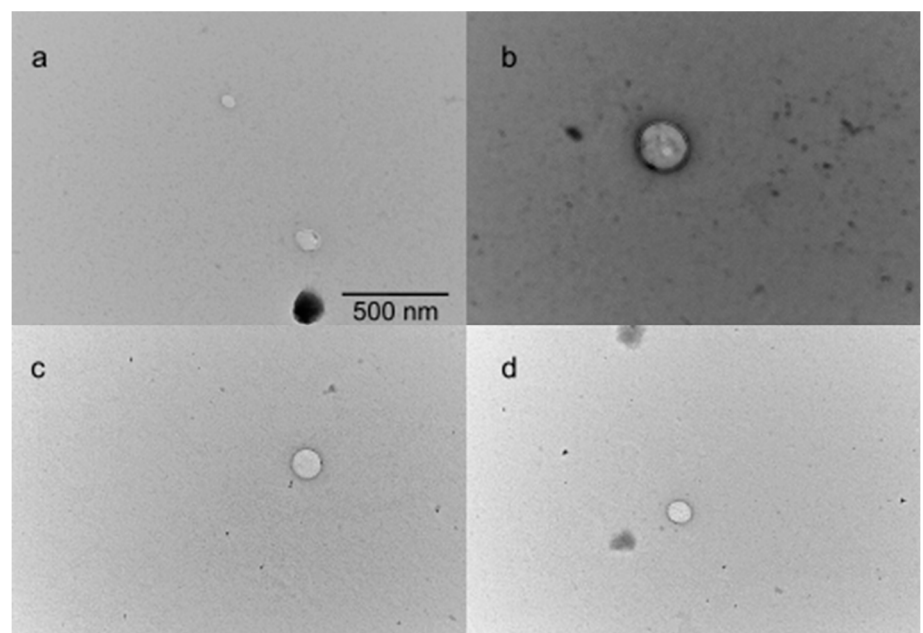


# Low dose rate radiation induced secretion of TGF- $\beta$ 3 together with an activator in small extracellular vesicles modifies low dose hyper-radiosensitivity through ALK1 binding

## 1. Supplementary figures

Marker	1	2	Marker	1	2	Marker	1	Marker	1	2
CD9	■	■	VCP	■	■	HSPA1A	■	HIST2H4A	■	■
HSPA8	■	■	TPI1	■	■	GNAI2	■	GNB1	■	■
PDCD6IP	■	■	PPIA	■	■	ANXA1	■	UBA1	■	■
GAPDH	■	■	MSN	■	■	RHOA	■	THBS1	■	■
ACTB	■	■	CFL1	■	■	MFGE8	■	RAN	■	■
ANXA2	■	■	PRDX1	■	■	PRDX2	■	RAB5A	■	■
CD63	■	■	PFN1	■	■	GDI2	■	PTGFRN	■	■
SDCBP	■	■	RAP1B	■	■	EHD4	■	CCT5	■	■
ENO1	■	■	ITGB1	■	■	ACTN4	■	CCT3	■	■
HSP90AA1	■	■	HSPA5	■	■	YWHAB	■	BSG	■	■
TSG101	■	■	SLC3A2	■	■	RAB7A	■	AHCY	■	■
PKM	■	■	HIST1H4A	■	■	LDHB	■	RAB5B	■	■
LDHA	■	■	GNB2	■	■	GNAS	■	RAB1A	■	■
EEF1A1	■	■	ATP1A1	■	■	TFRC	■	LAMP2	■	■
YWHAZ	■	■	YWHAQ	■	■	RAB5C	■	ITGA6	■	■
PGK1	■	■	FLOT1	■	■	ARF1	■	HIST1H4B	■	■
EEF2	■	■	FLNA	■	■	ANXA6	■	GSN	■	■
ALDOA	■	■	CLIC1	■	■	ANXA11	■	FN1	■	■
HSP90AB1	■	■	CDC42	■	■	ACTG1	■	YWHAH	■	■
ANXA5	■	■	CCT2	■	■	KPNB1	■	TUBA1A	■	■
FASN	■	■	A2M	■	■	EZR	■	TKT	■	■
YWHAE	■	■	YWHAQ	■	■	ANXA4	■	TCP1	■	■
CLTC	■	■	TUBA1B	■	■	ACLY	■	STOM	■	■
CD81	■	■	RAC1	■	■	TUBA1C	■	SLC16A1	■	■
ALB	■	■	LGALS3BP	■	■	RAB14	■	RAB8A	■	■

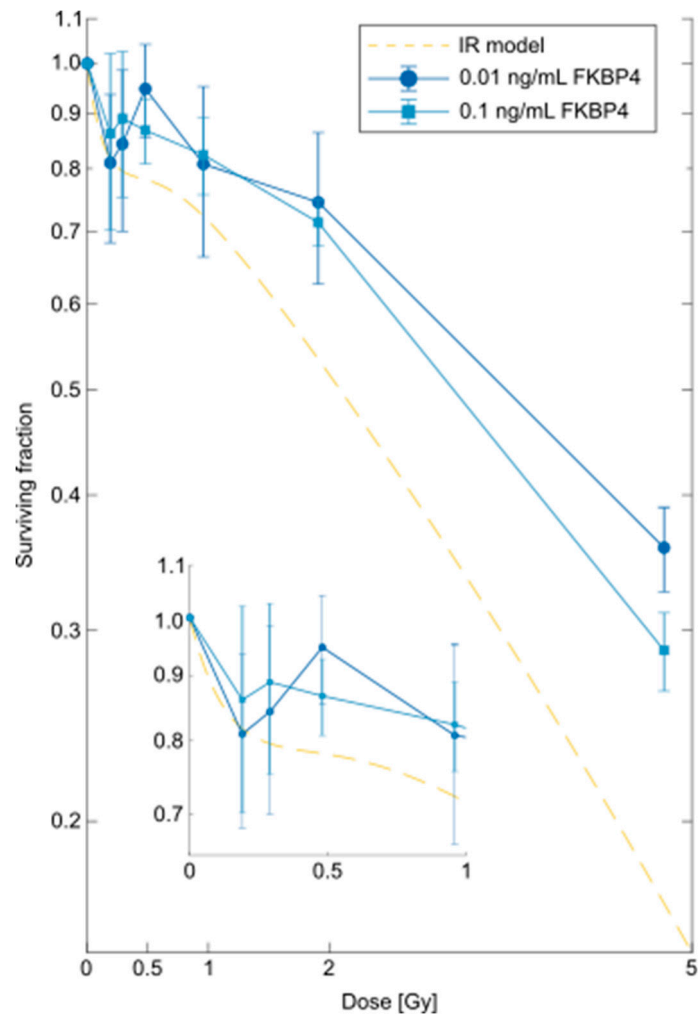
Figure S1. List of the 100 proteins most frequently detected in small extracellular vesicles (sEVs) <sup>[1]</sup>, and their detection in two separate experiments (each with three biological replicates) of sEVs from low dose rate (LDR) irradiated and unirradiated T-47D cells: ■ – detected, □ - not detected.



**Figure S2. Transmission electron microscope (TEM) images of extracellular vesicles isolated from LDR primed (a,b) and unirradiated (c,d) T-47D cells. The shape and size of the isolated particles are consistent with that of sEVs.**

10	20	30	40	50
MMQHLQRALV	VLALLNFATV	SLSLSTCTTL	DFGHIKKRV	EAIRGQILSK
60	70	80	90	100
LRLTSPPEPT	VMTHVPYQVL	ALYNSTRELL	EEMHGEREEG	CTQENTESEY
110	120	130	140	150
YAKEIHKFDM	IQGLAEHNEL	AVCPKGITSK	VFRFNVSSVE	KNRTNLFRAE
160	170	180	190	200
FRVLRVPNPS	SKRNEQRIEL	FQILRPDEHI	AKQRYIGGKN	LPTRGTAEWL
210	220	230	240	250
SFDVTDTVRE	WLLRFESNLG	LEISINCPCH	TFQPNGDILE	NIHEVMEIKF
260	270	280	290	300
KGVDNEDDHG	RGDLGRLKKQ	KDHHNPHLIL	MMIPPHRLDN	PGQGGQRKKR
310	320	330	340	350
ALDTNYCFRN	LEENCCVRPL	YIDFRDILGW	KAVHEPFGYY	ANFCSGPCPY
360	370	380	390	400
LRSADTTHST	VLGLYNTLNP	EASASPCVP	QDLEPLTILY	YVGRFPFVEQ
410				
LSNMVVK	SCK	CS		

**Figure S3.** Peptide sequence of TGF- $\beta$ 3 (blue) with the latency-associated peptide (LAP) (red). Rectangles mark the peptide sequences that were detected in tandem mass spectrometry (MS/MS) analysis of sEVs from LDR primed and unirradiated cells, in either of two separate experiments , each with three biological replicates.



**Figure S4. Survival of T-47D cells after pretreatment with Peptidyl-prolyl cis-trans isomerase FKBP4 (FKBP4).** Addition of 0.01 ng/mL (●) or 0.1 ng/mL (■) recombinant FKBP4 to the medium of T-47D cells 48 hours before challenge  $\gamma$ -irradiation did not remove hyper-radiosensitivity (HRS) from the cells. IR model= induced repair model<sup>[2]</sup>-fit for untreated T-47D cells. Surviving fractions are given as error-weighted averages of three separate experiments, each with five biological replicates. Error bars: standard error of the mean. Note that the surviving fractions were calculated relative to the plating efficiency of control cells, which were also exposed to the pretreatments.

- [1] Keerthikumar, S.; Chisanga, D.; Ariyaratne, D.; Al Saffar, H.; Anand, S.; Zhao, K.; Samuel, M.; Pathan, M.; Jois, M.; Chilamkurti, N.; et al. ExoCarta: A Web-Based Comendium of Exosomal Cargo. *J Mol Biol*, **2017**, 428 (4), 688–692. <https://doi.org/10.1016/j.jmb.2015.09.019>.
- [2] Marples, B.; Joiner, M. C. The Response of Chinese Hamster V79 Cells to Low Radiation Doses: Evidence of Enhanced Sensitivity of the Whole Cell Population. *Radiat. Res.*, **1993**, 133 (1), 41–51. <https://doi.org/10.2307/3578255>.