

Low dose rate radiation induced secretion of TGF- β 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) [1], 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.

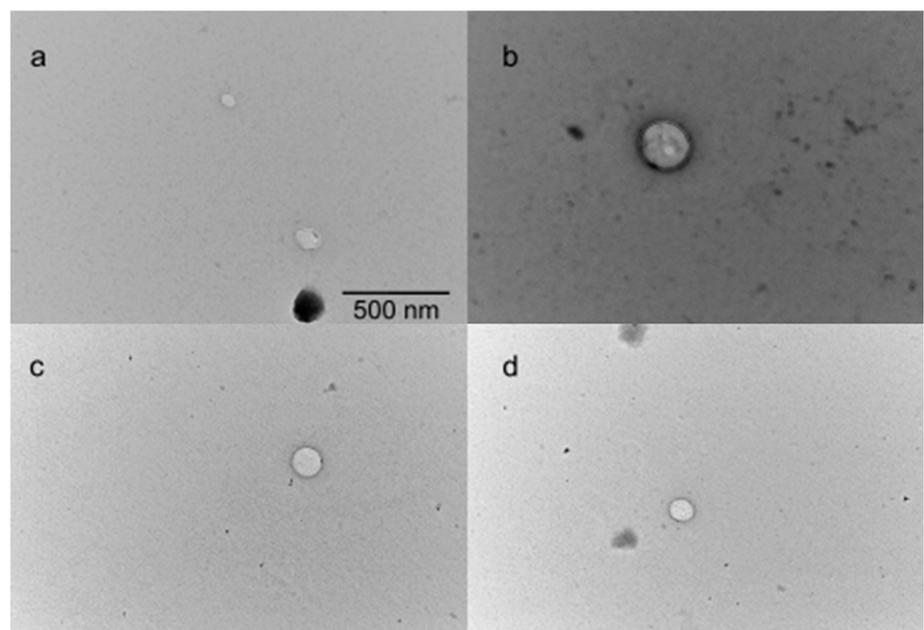


Figure S2. Transmission electron microscope (TEM) images of extracellular vesicles isolated from LDR primed (a,b) and unirradiated (c,d) T-47D cells. The shape and size of the isolated particles are consistent with that of sEVs.

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10      20      30      40      50
MMHMLQRALV VLALLNFATV SLSLSTCTTL DFGHIKKKRV 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
SFDVTDVIRE WLLRFESNLG LEISIHCPCH TFQPNGDILE NIHEVMEIKF
260     270     280     290     300
KGVNEDDHG RGDLGRLKKQ KDHNPHLIL MMIPPHRLDN PGQGGQRKRR
310     320     330     340     350
ALDTNYCFRN LEENCVRPL YIDFRDDLGM KAVHEPFGY ANFCSGPCPY
360     370     380     390     400
LRSADTTHST VGLYNTLNP EASASPCCVP QLEPLTILY YVGRFPFVEQ
410
LSRMVVKSCK CS

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Figure S3. Peptide sequence of TGF- β 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.

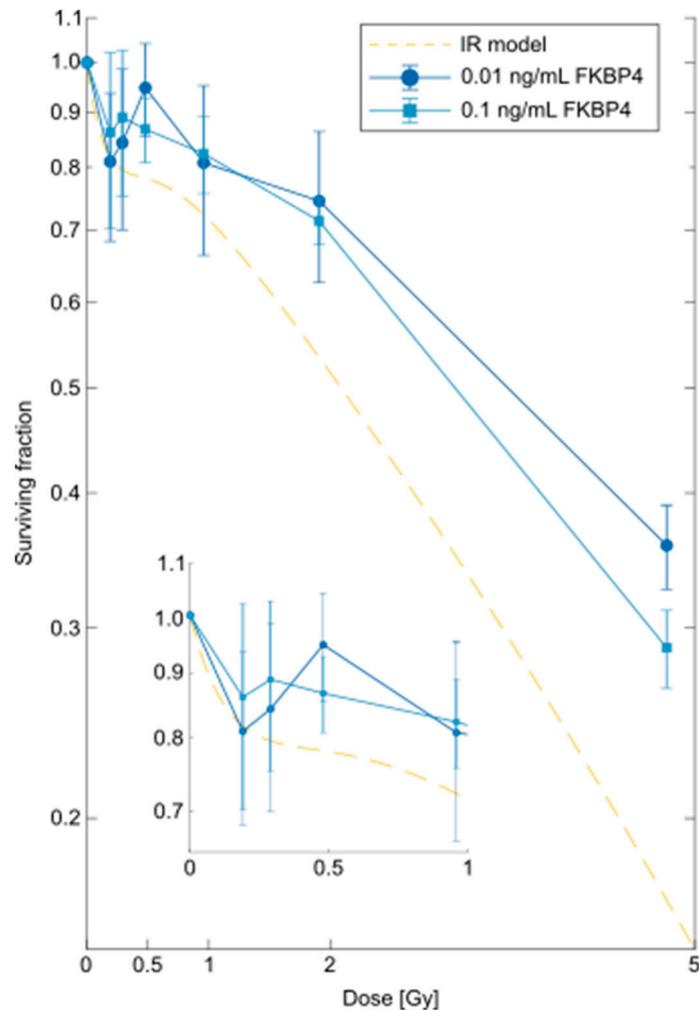


Figure S4. Survival of T-47D cells after pretreatment with Peptidyl-prolyl cis-trans isomerase FKBP4 (FKBP4). Addition of 0.01 ng/mL (●) or 0.1 ng/mL (■) recombinant FKBP4 to the medium of T-47D cells 48 hours before challenge γ -irradiation did not remove hyper-radiosensitivity (HRS) from the cells. IR model= induced repair model^[2]-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>.