"New Biology" operates SDRT in Tumor Cure

The Dual Target Model

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Geneva
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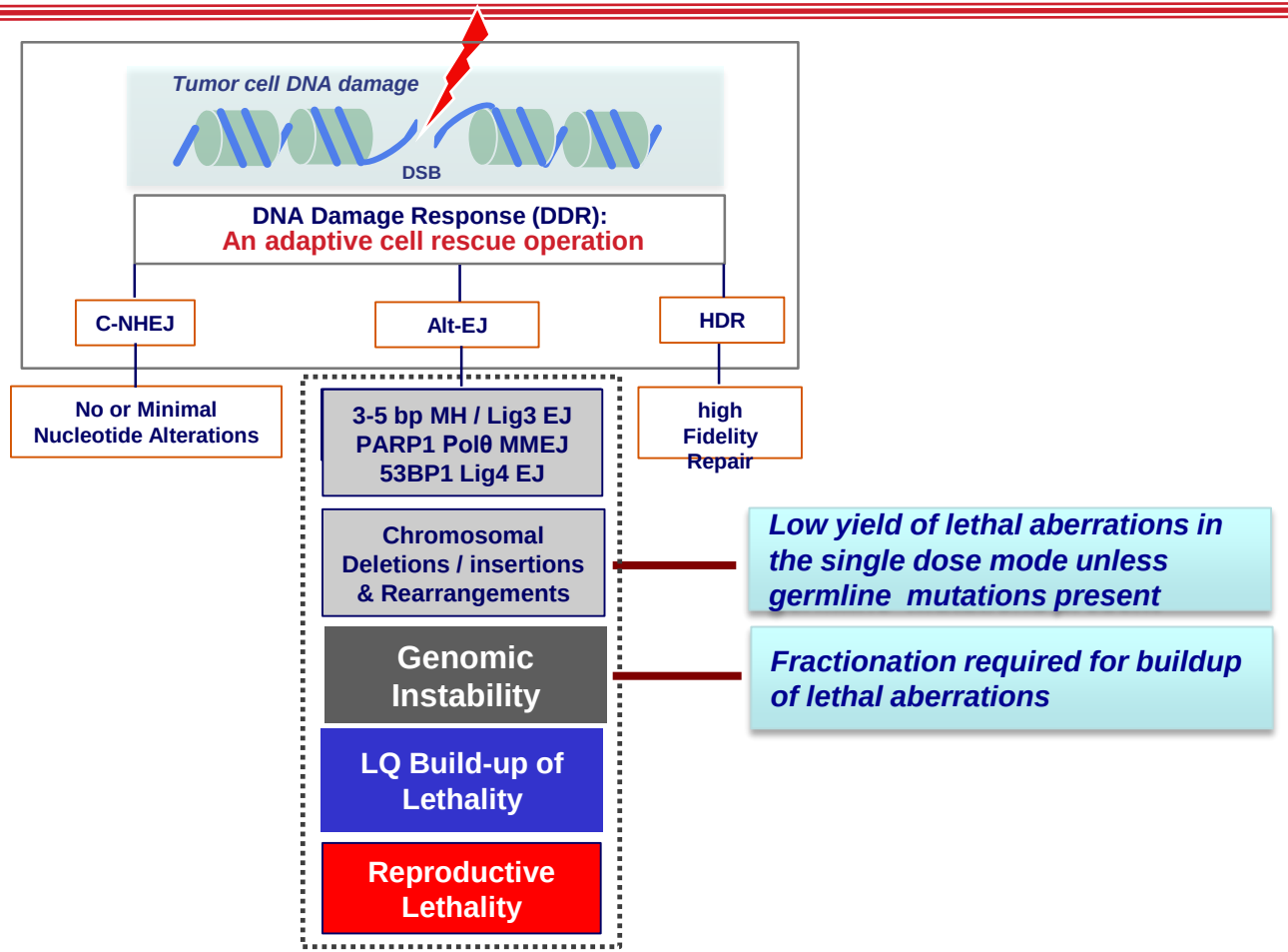
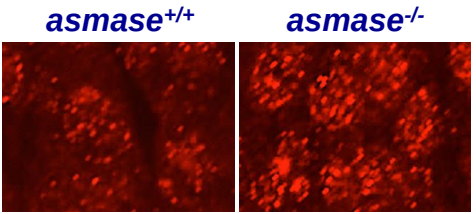
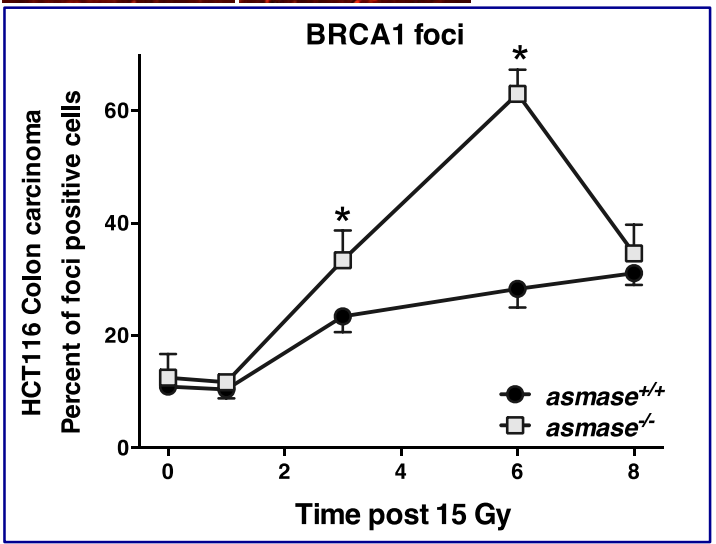
The Single Target Model: A cell autonomous adaptive response to DSB



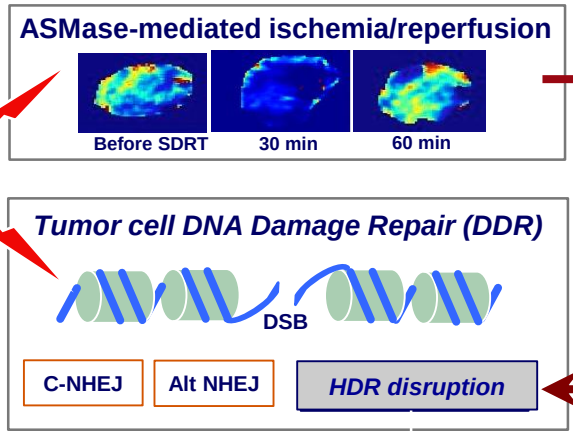
The SDRT Dual Target Model:

Microvascular Dysfunction Couples Tumor Cell DDR to Synthetically Effect Tumor Lethality





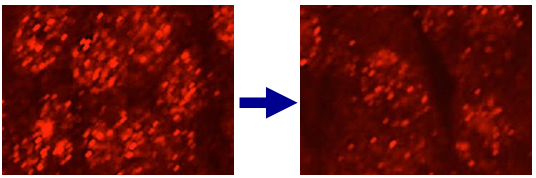
High dose range
(12-24Gy)



The SDRT Dual Target Model:

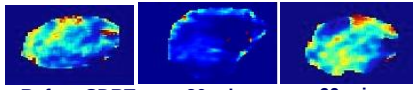
Microvascular Dysfunction Couples Tumor Cell DDR to Synthetically Effect Tumor Lethality

I/R ⊖ I/R ⊕



High dose range (12-24Gy)

ASMase-mediated ischemia/reperfusion

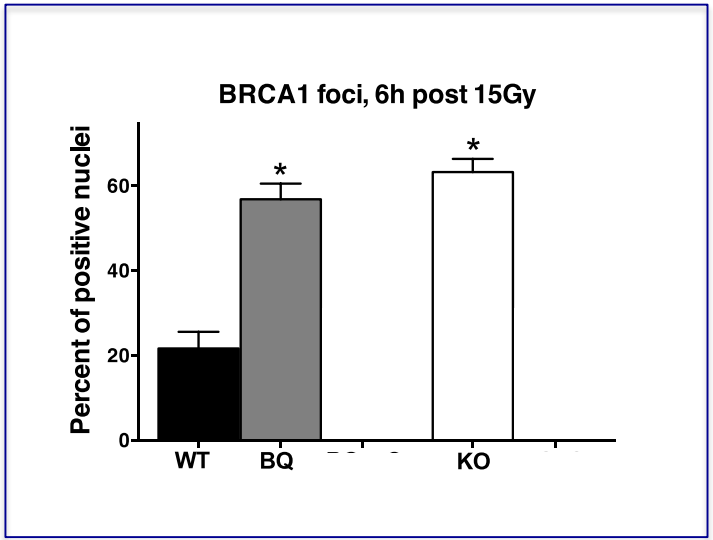


asmase$^{-/-}$(KO)
BQ-123

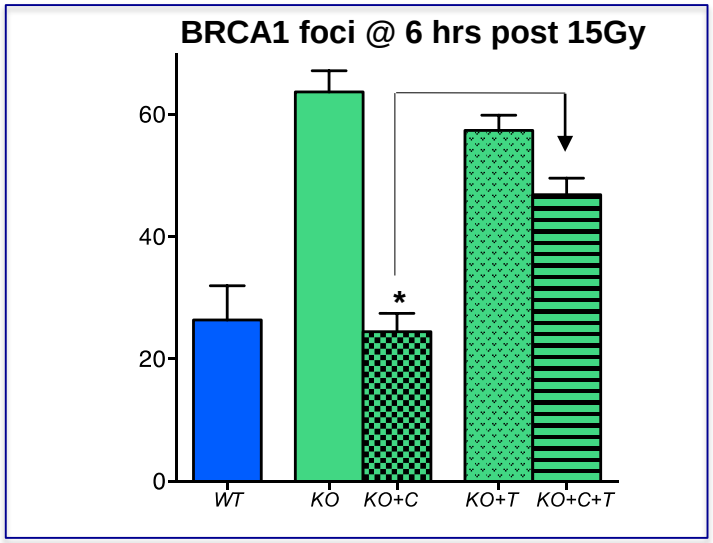
Tumor cell DNA Damage Repair (DDR)

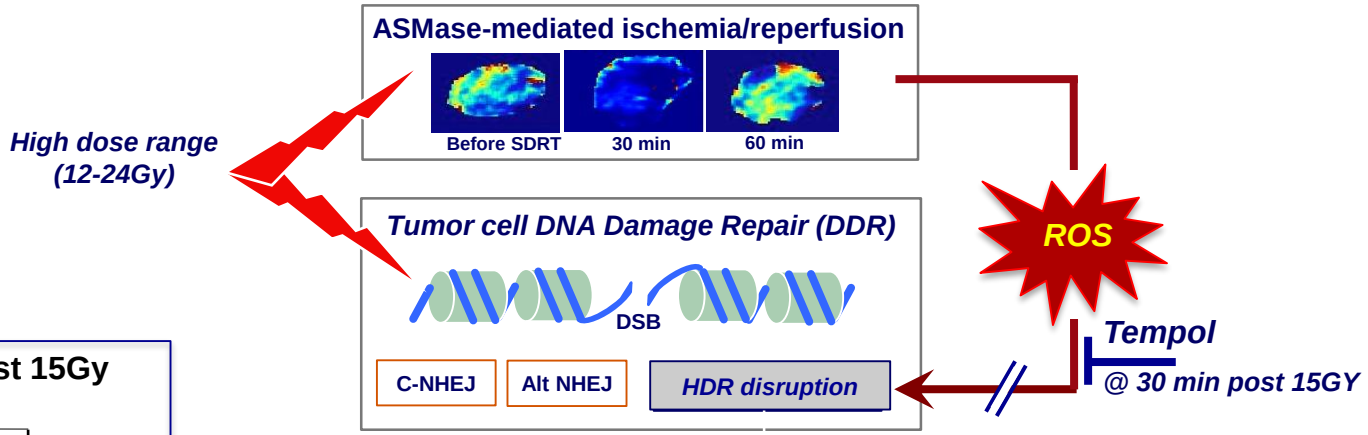
C-NHEJ | Alt NHEJ | *HDR disruption*

ROS

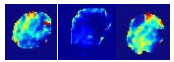
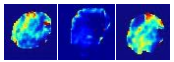
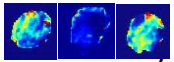


Crosstalk between microenvironmental I/R and tumor cell DDR impairs HDR

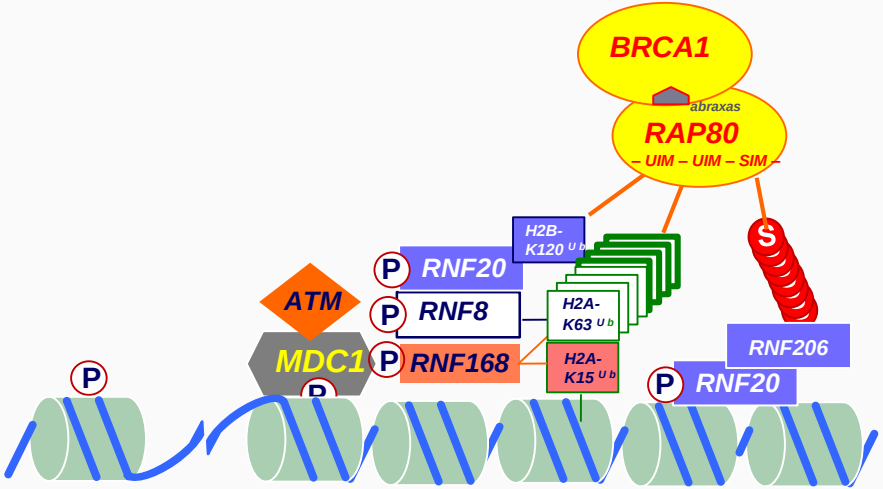


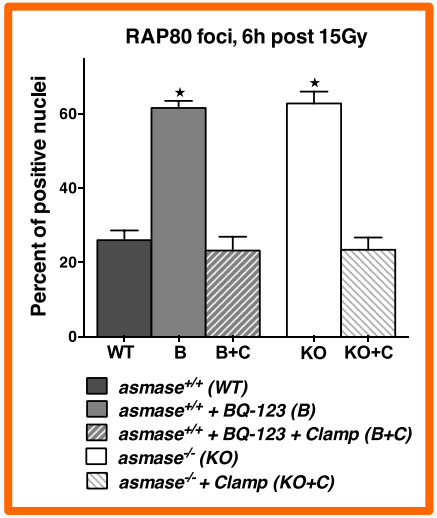


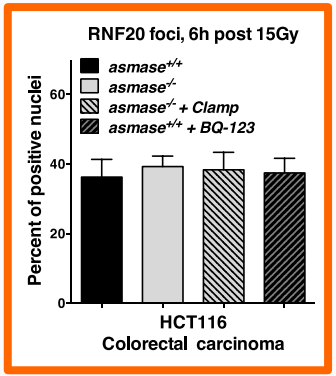
ROS induced by SDRT-I/R Represses IRIF Download of Multiple HDR Mediators

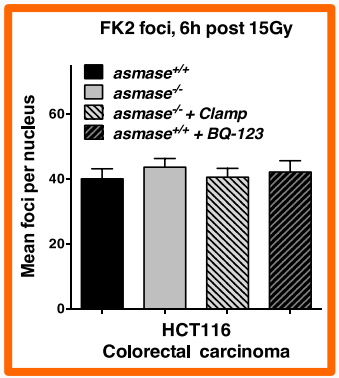
	 *asmase*$^{+/+}$ (WT)	I/R ⊖ *asmase*$^{+/+}$ + BQ-123	 *asmase*$^{+/+}$ +BQ-123+Clamp	I/R ⊖ *asmase*$^{-/-}$ (KO)	 *asmase*$^{-/-}$ + Clamp
RAP80	26 (± 2.6)	61.6 (± 1.9) ★	23.2 (± 3.7)	62.8 (± 3.2) ★★	23.4 (± 3.3)
BRCA1	21.7 (± 3.9)	56.9 (± 3.7) ★	24.1 (± 1.7)	63.3 (± 3.1) ★★	24.5 (± 3.0)
RPA32	24.8 (± 2.9)	37.6 (± 2.3) ★	22.8 (± 2.6)	39.7 (± 3.7) ★★	27.9 (± 1.7)
RAD51	17.2 (± 1.8)	43 (± 3.0) ★	22.5 (± 2.5)	42.2 (± 9.1) ★★	21 (± 4.7)

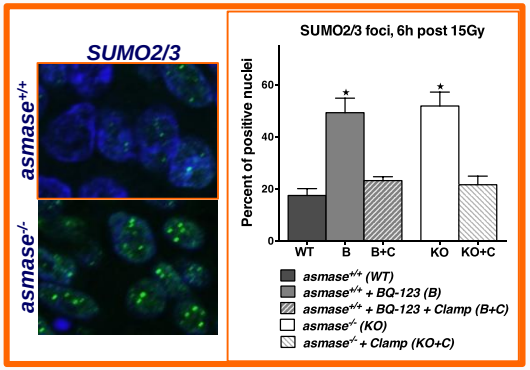
I/R-mediated SUMO2/3 dysfunction aborts RAP80 / BRCA1 recruitment to IRIF







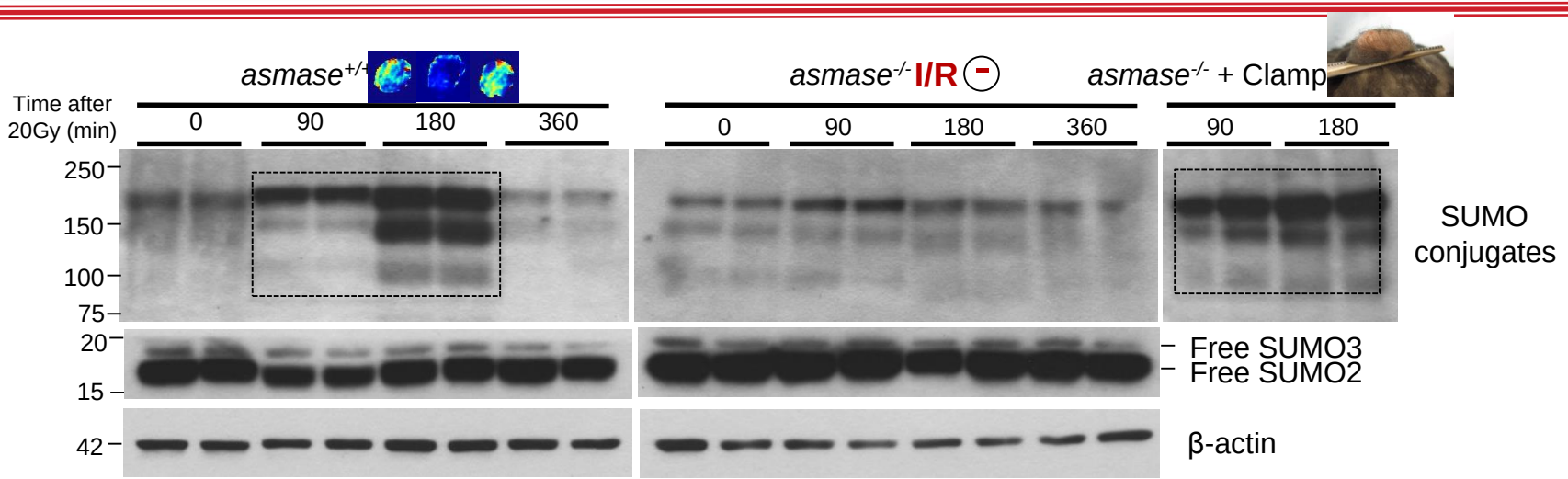


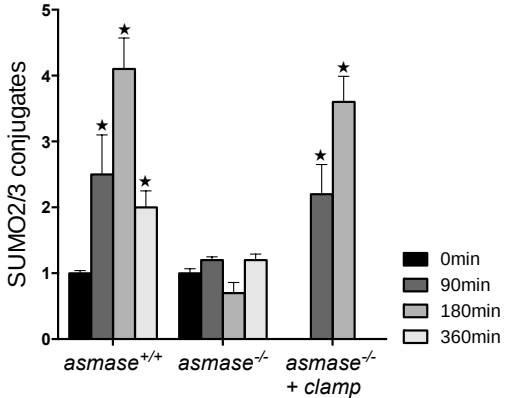


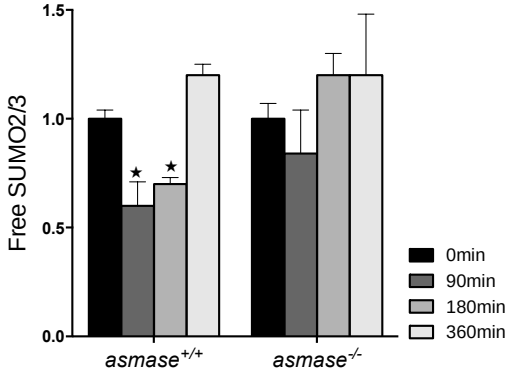
What is SUMO?

- ***SUMO (Small Ubiquitin-like Modifier) is a ubiquitous regulator of post-translational proteins modification***
- ***SUMO conjugates to acceptor ε-amino lysine on target consensus motif to turn on target function***
- ***SUMO2/3 conjugation is mandatory for foci download and coordinated activation of the HDR cluster***
- ***Oxidative stress induces an evolutionarily-preserved adaptive SUMO Stress Response (SSR) to protect cells against ROS-induced proteotoxic damage***

The pan-sumoylation hyperactive SSR encounters resource deficiency of free SUMO2/3







SSR-mediated SUMO2/3 dysfunction confers global loss-of-function HRR

	asmase$^{+/+}$ (WT)	*asmase*$^{+/+}$ + BQ-123 (B) I/R ⊖	*asmase*$^{+/+}$ +BQ-123+Clamp (B+C)	*asmase*$^{-/-}$ (KO) I/R ⊖	*asmase*$^{-/-}$ + Clamp (KO+C)
SUMO 2/3	17.5 (± 2.6)	49.3 (± 5.6) ★	23.2 (± 1.5)	51.9 (± 5.3) ★★	21.6 (± 3.3)
RNF4	25.2 (± 6.5)	57 (± 6.5) ★	29.5 (± 2.9)	62.3 (± 7.9) ★★	26 (± 4.6)
RAP80	26 (± 2.6)	61.6 (± 1.9) ★	23.2 (± 3.7)	62.8 (± 3.2) ★★	23.4 (± 3.3)
BRCA1	21.7 (± 3.9)	56.9 (± 3.7) ★	24.1 (± 1.7)	63.3 (± 3.1) ★★	24.5 (± 3.0)
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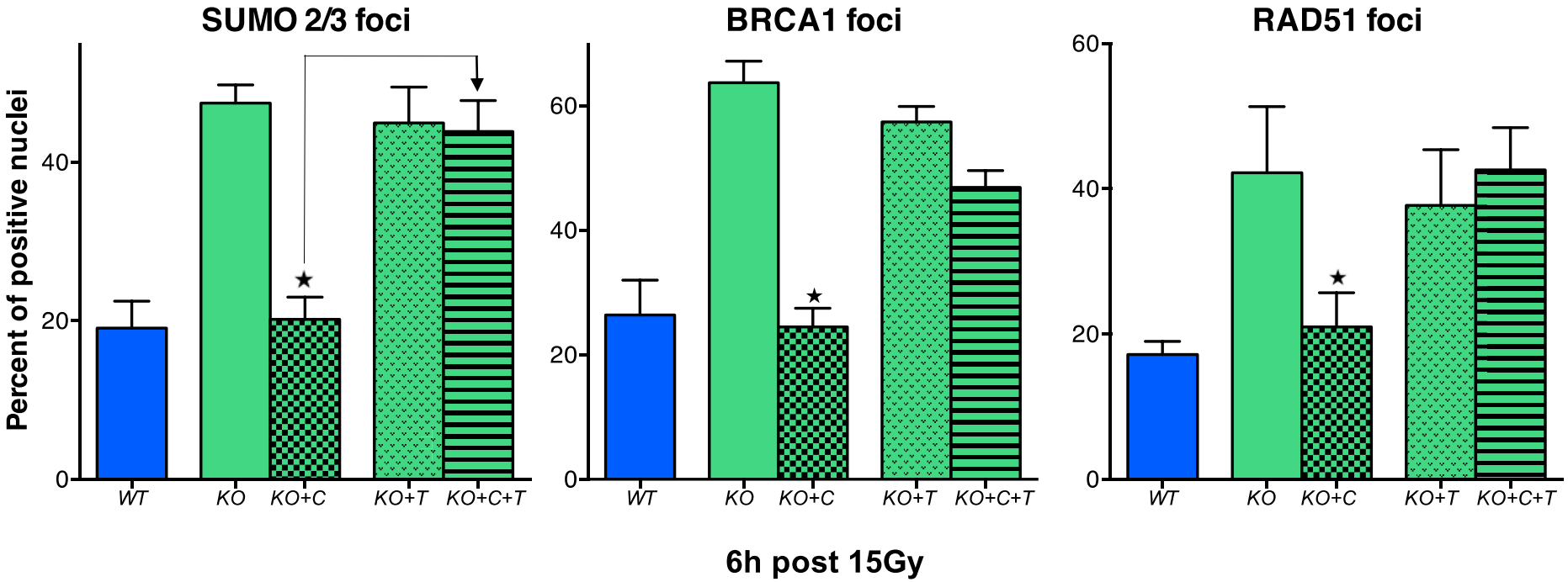
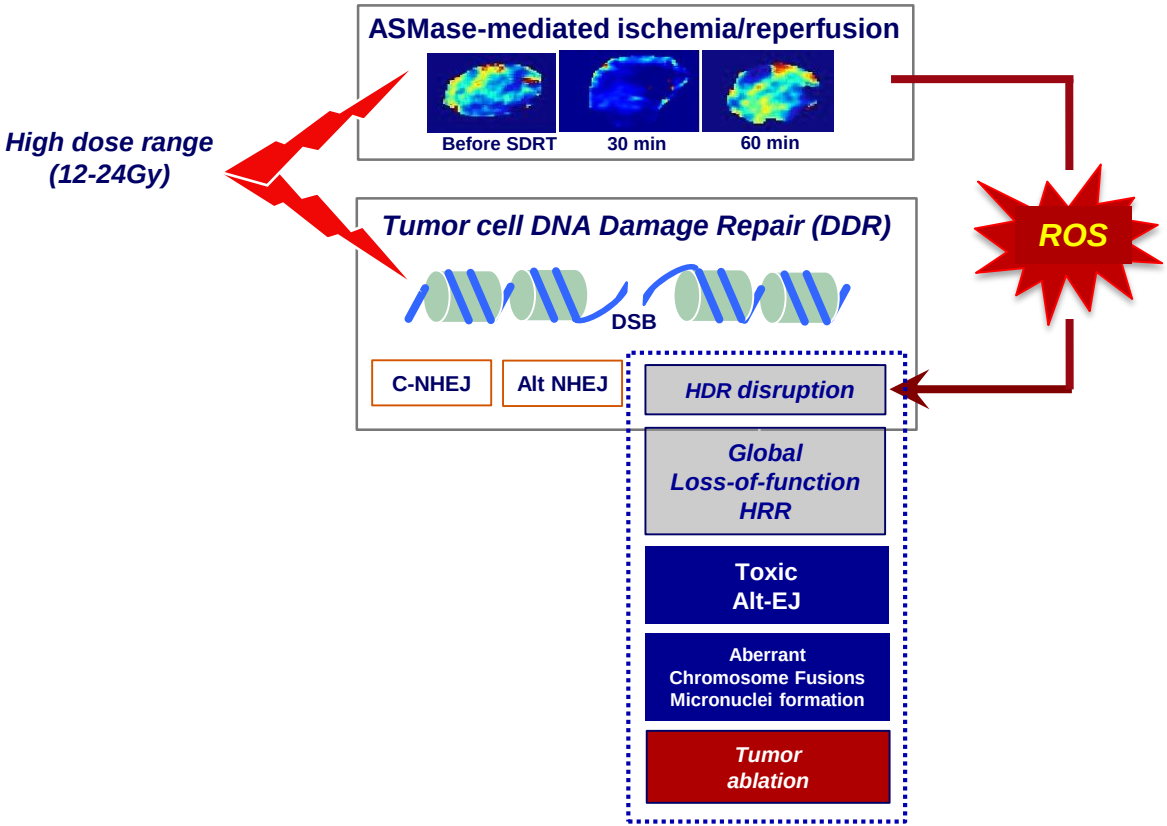
RAD51	17.2 (± 1.8)	43 (± 3.0) ★	22.5 (± 2.5)	42.2 (± 9.1) ★★	21 (± 4.7)

Tempol abolishes SSR loss-of-function SUMO2/3 and HRR



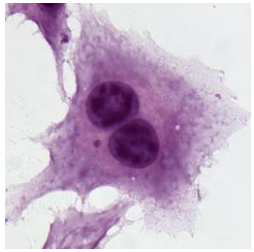
SDRT Operates A Dual target Model

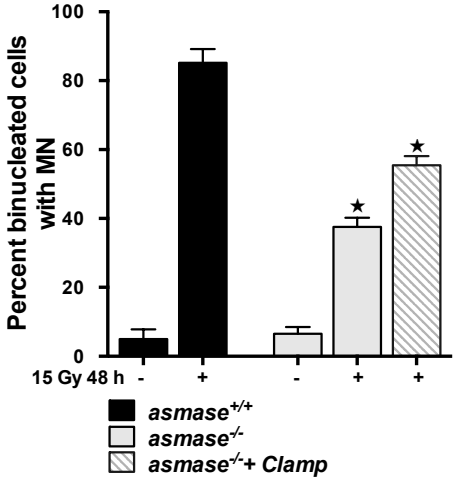
Microvascular dysfunction synthetically couples tumor cell DDR to affect tumor cell lethality



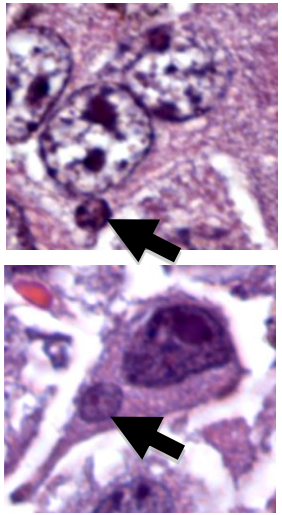
ROS mediate generation of lethal chromosomal aberrations at first mitotic cycle after SDRT

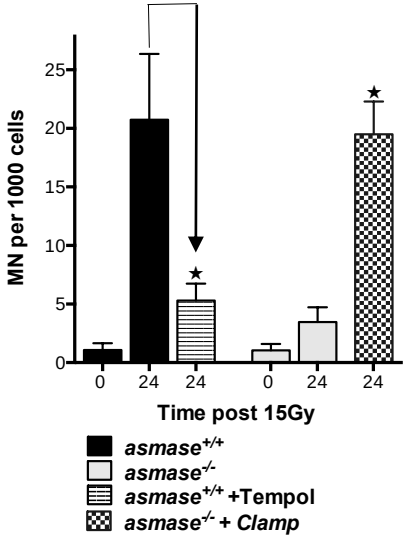
MCA/129 Fibrosarcoma *ex vivo* Cytokinesis-block Assay



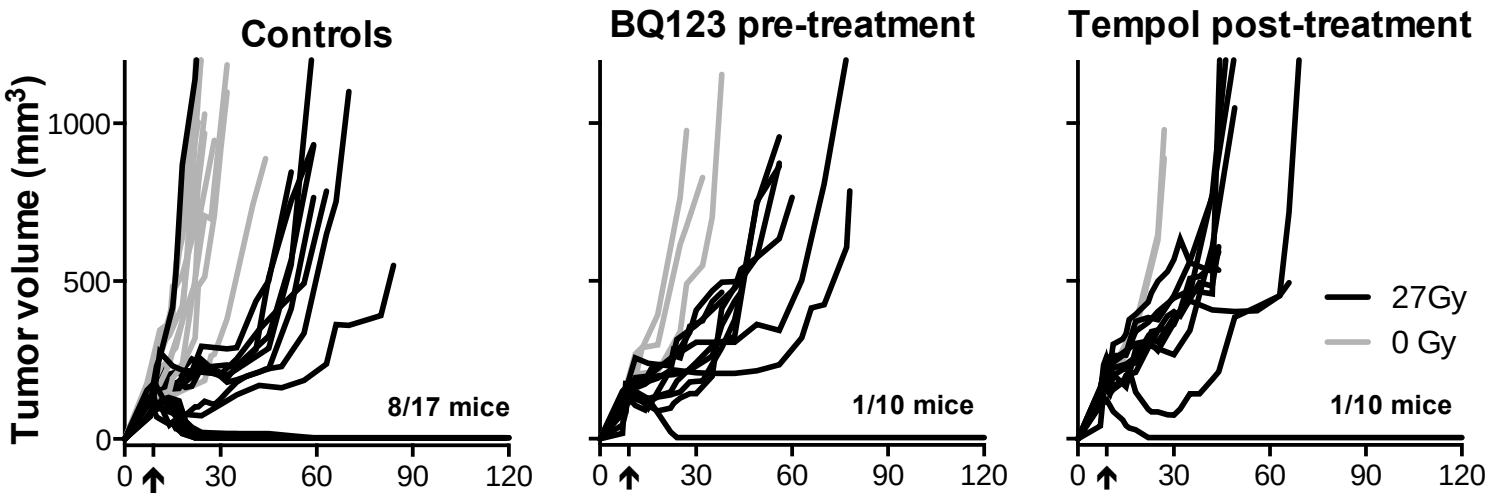


MCA/129 Fibrosarcoma Tumor *in vivo*

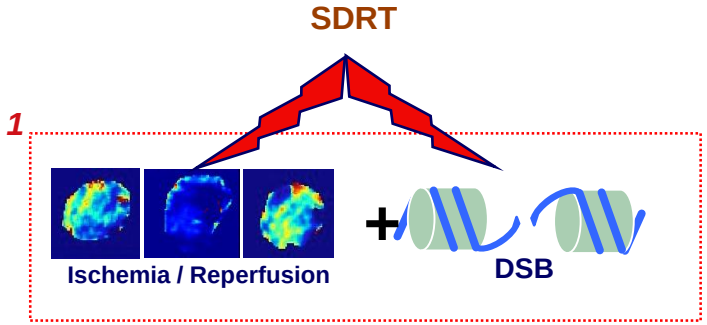




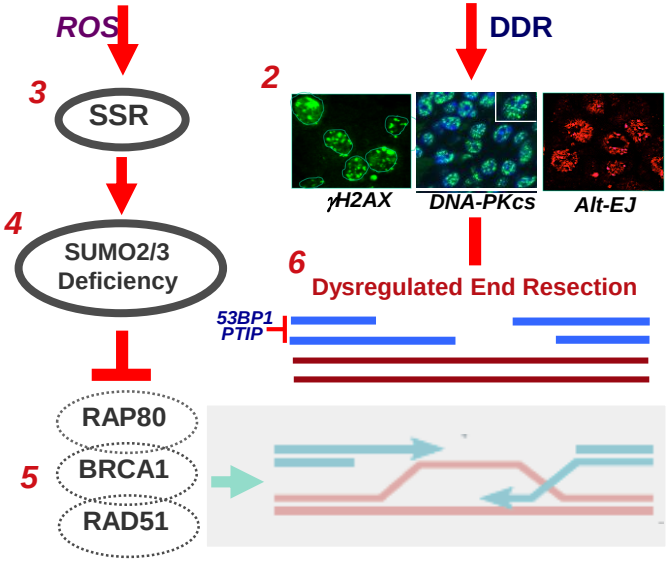
SDRT tumor cure is abolished by I/R inhibition or by ROS scavenging

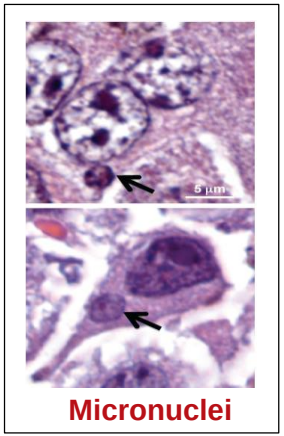


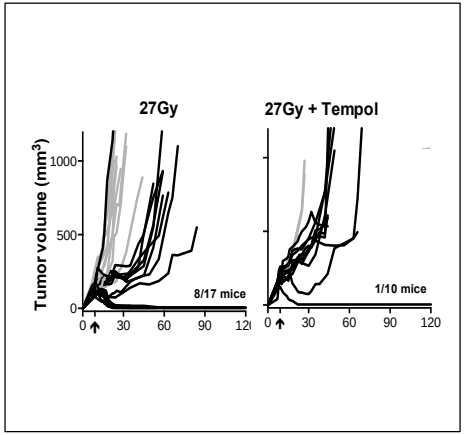
The pathophysiology of ischemia/reperfusion engagement in tumor cure by SDRT



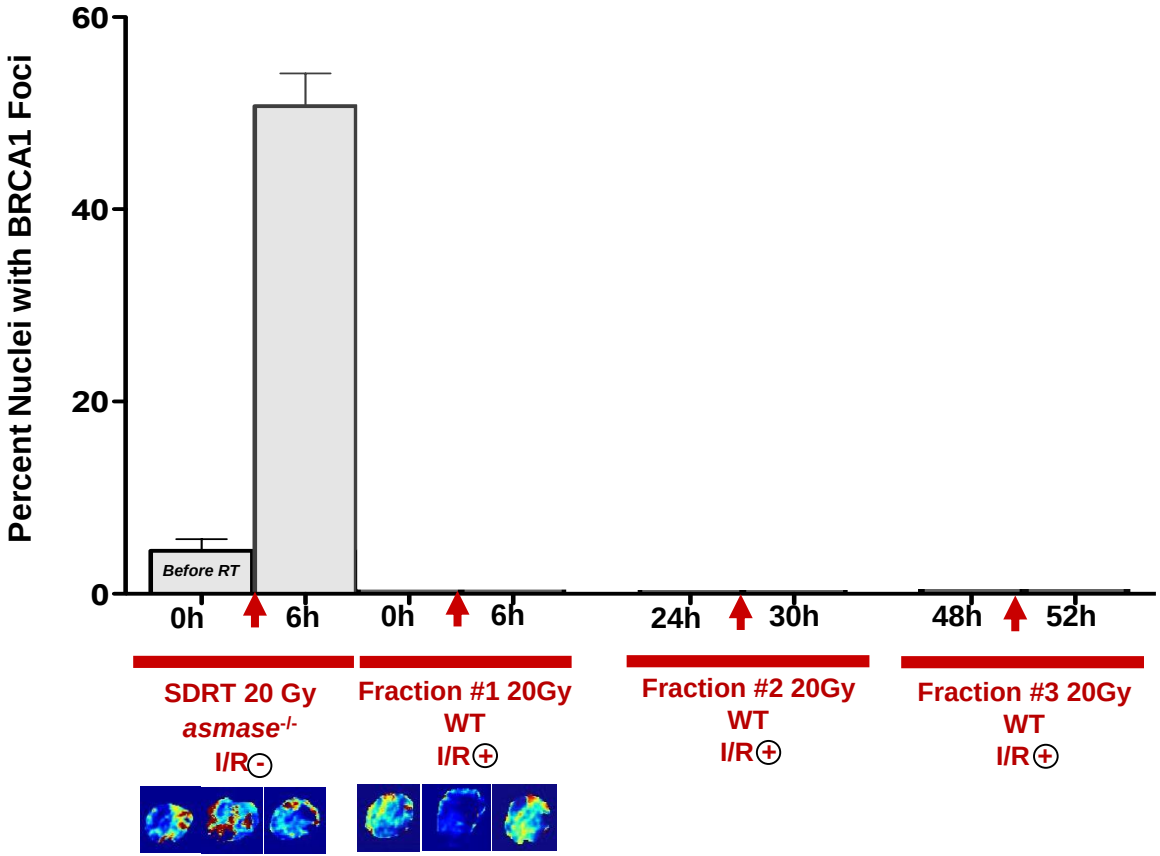
1. *SDRT induces Ischemia/Reperfusion and tumor cell DSBs*
2. *DDR functions of NHEJ and Alt-EJ are not affected*
3. *I/R-induced ROS activate the SUMO Stress Response (SSR) in tumor cells*
4. *SSR/ROS impairs SUMO2/3 availability and function at nuclear foci*
5. *SUMO2/3 dysfunction confers global loss-of-function HRR*
6. *DSB repair is diverted to a highly toxic Alt-EJ pathway*
7. *Significant lethal chromosomal aberrations at first post-SDRT mitosis*
8. *SDRT tumor cure can be abrogated by I/R inhibition or ROS scavenging*







HD-SBRT operates a mixed SDRT / classical fractionated RT mechanism in tumor cure



Contributors to unpublished Data

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