Microirradiation for single strand break repair

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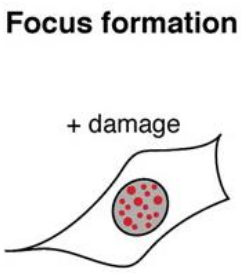
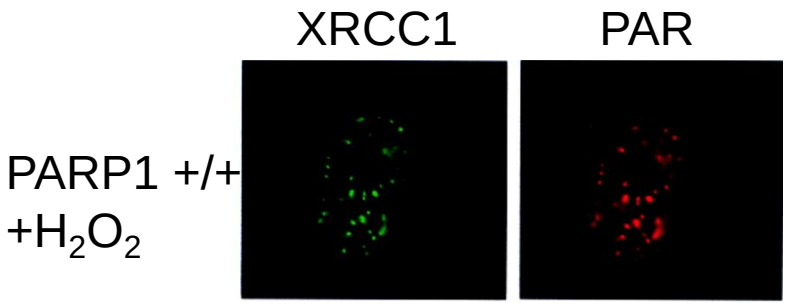
4/18/17

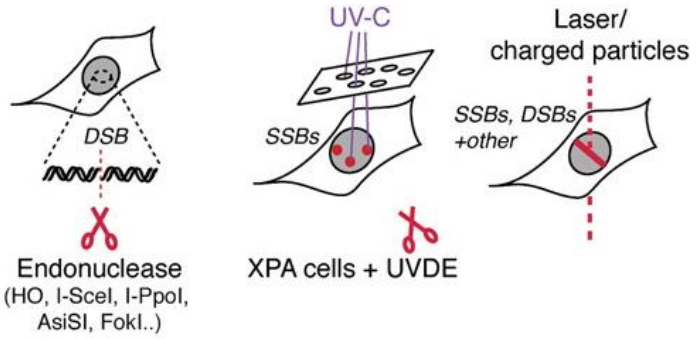
Creating sites of DNA damage

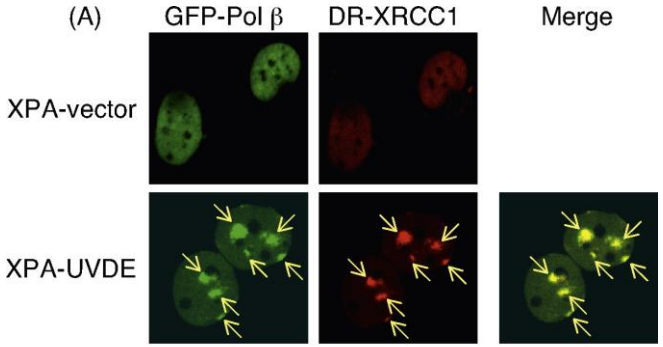




El-Khamisy et al. *Nucl. Acids Res.* 2003

Localized sites of DNA damage

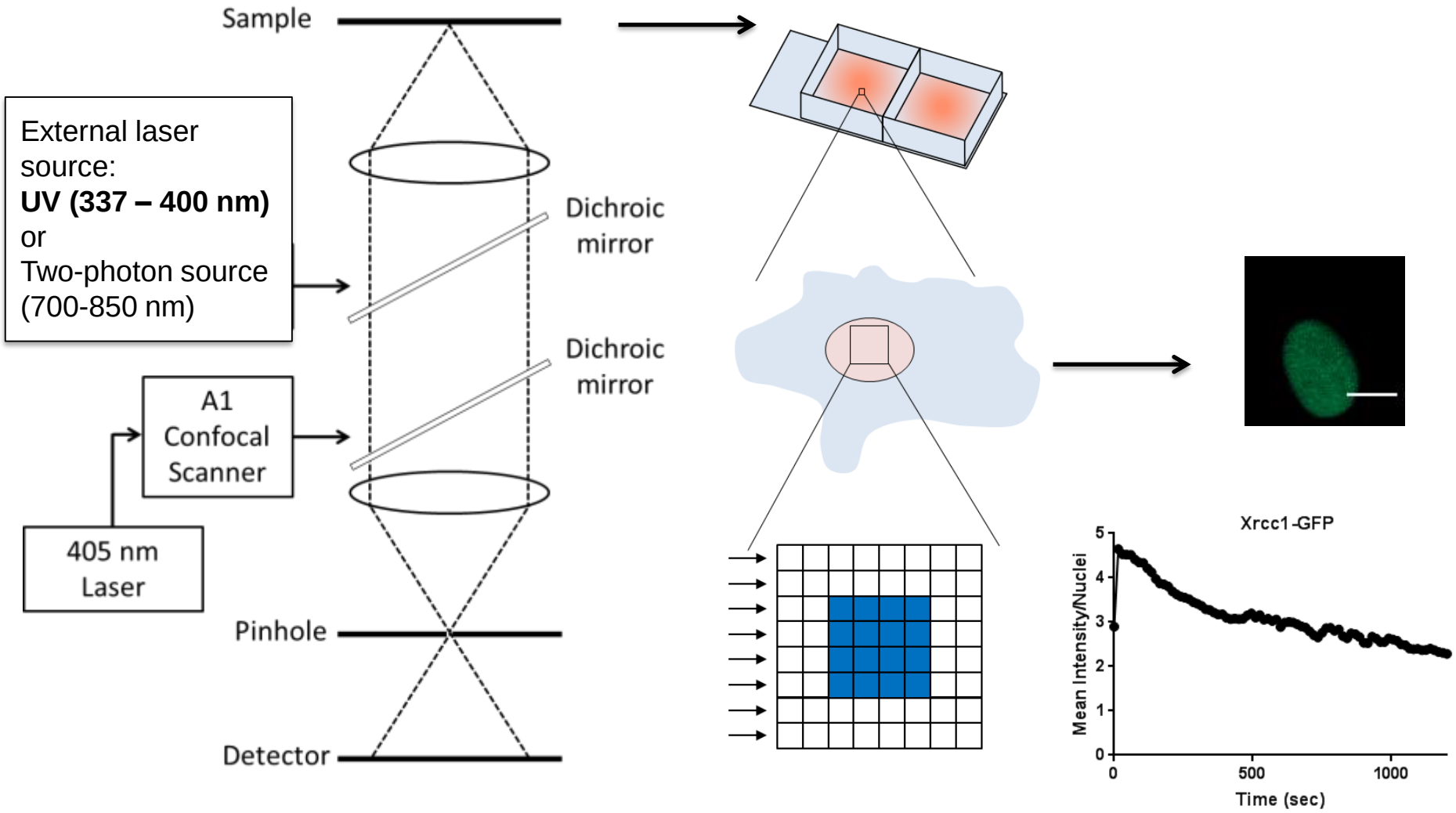




Adapted from Polo and Jackson *Genes Dev.* 2011

Agagoshi et al. *DNA Repair* 2010

Laser microirradiation



What can a microirradiation experiment reveal?

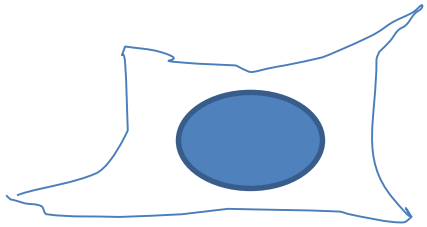
1. Determine if a protein responds to induced damage site (screening for novel interactants or critical protein domains)
2. Peak recruitment time of proteins to site of induced damage
3. Residence time of the protein at the site of damage
4. Co-localization of proteins at site of DNA damage
5. Time courses for repair

Information you get out is only as good as the rigor used to characterize the system.

1. **Types of damage induced**
2. Cellular background used
3. Fluorescent proteins vs. endogenous proteins

Designing your microirradiation experiment

Wavelength



Cell Line

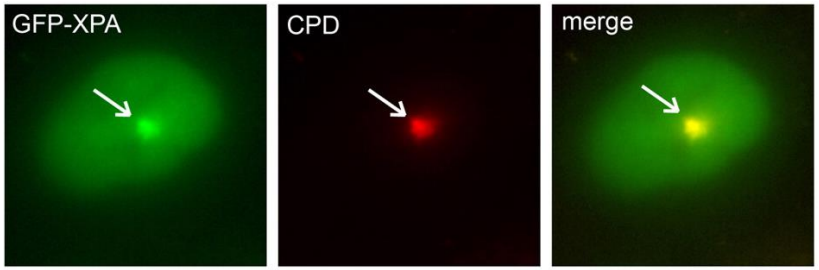
Favorite DNA repair protein

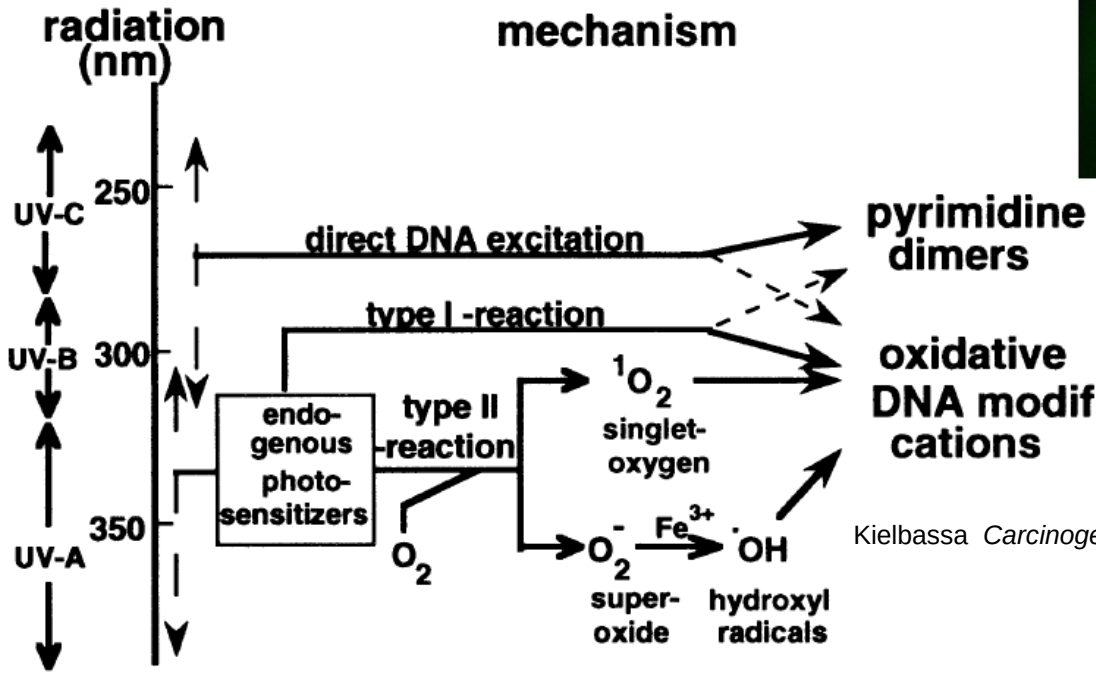
1. Induce a specific type of DNA damage
   A. Power
   B. Time

2. Monitor the response of repair proteins to that site of damage
   A. Live cell
   B. Immunofluorescence

UV and near UV Wavelengths

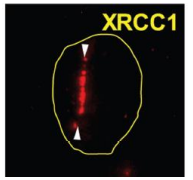
266 nm irradiation (Dinant *J. Cell Sci.* 2007)

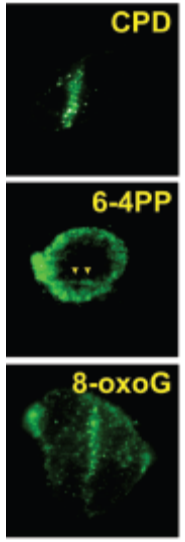




Kielbassa *Carcinogenesis* 1997

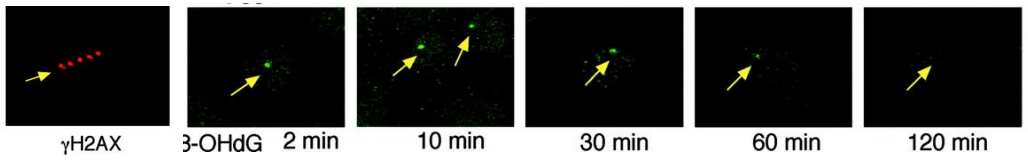
337 nm irradiation
(Kong *NAR* 2009)



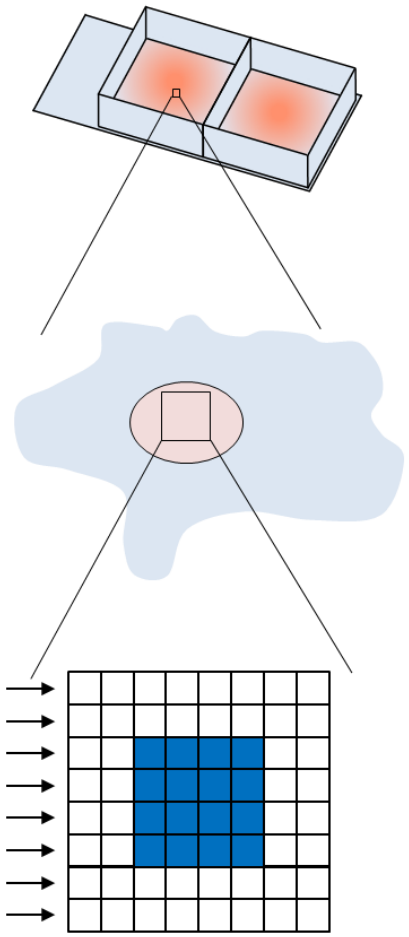


405 nm irradiation (Hanssen-Bauer *EMM* 2011)

365 nm irradiation (Lan *PNAS* 2004)



Inducing a specific mixture of breaks

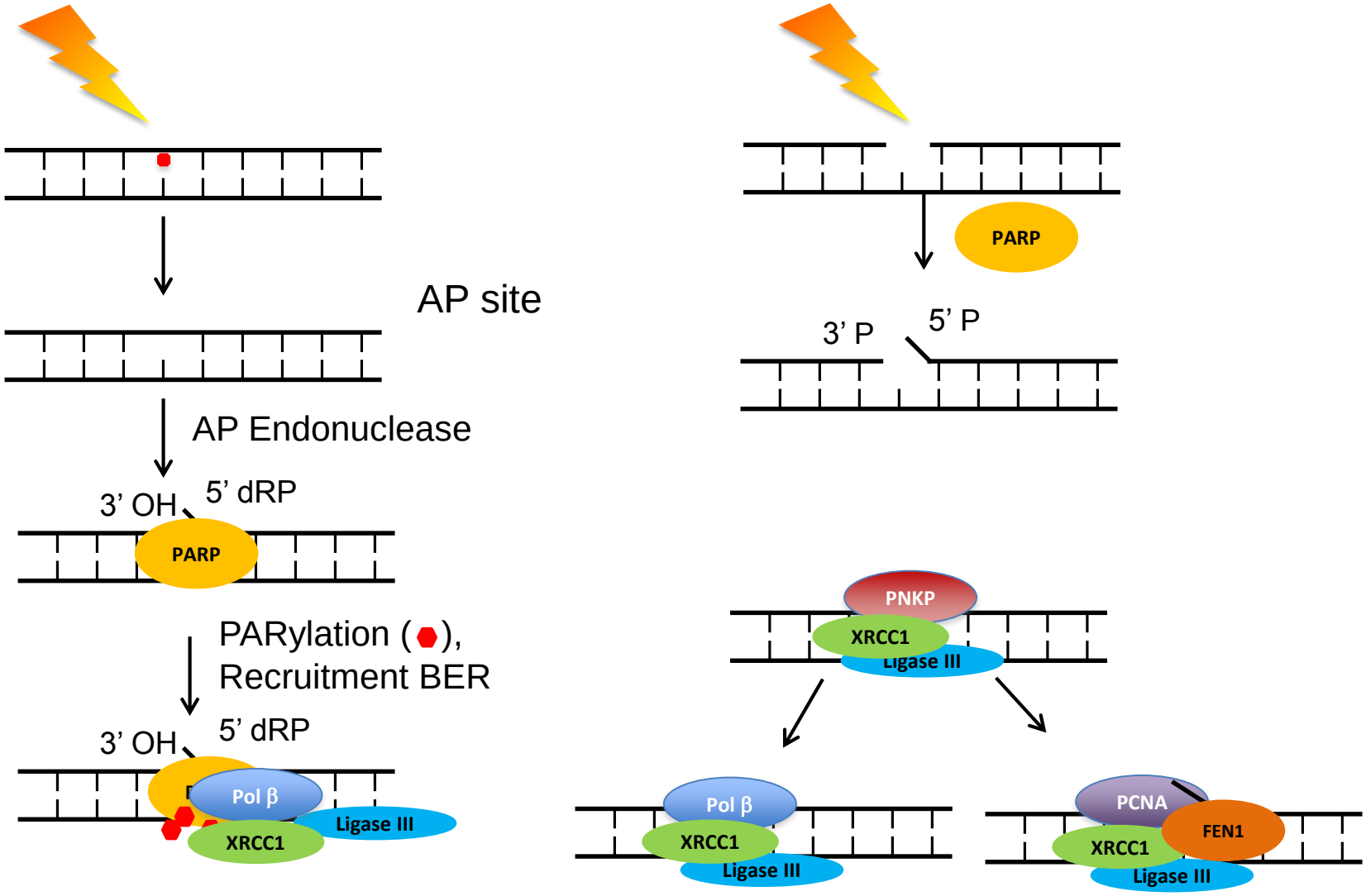


1. Laser power- ideally the amount of energy that passes through the objective into the sample

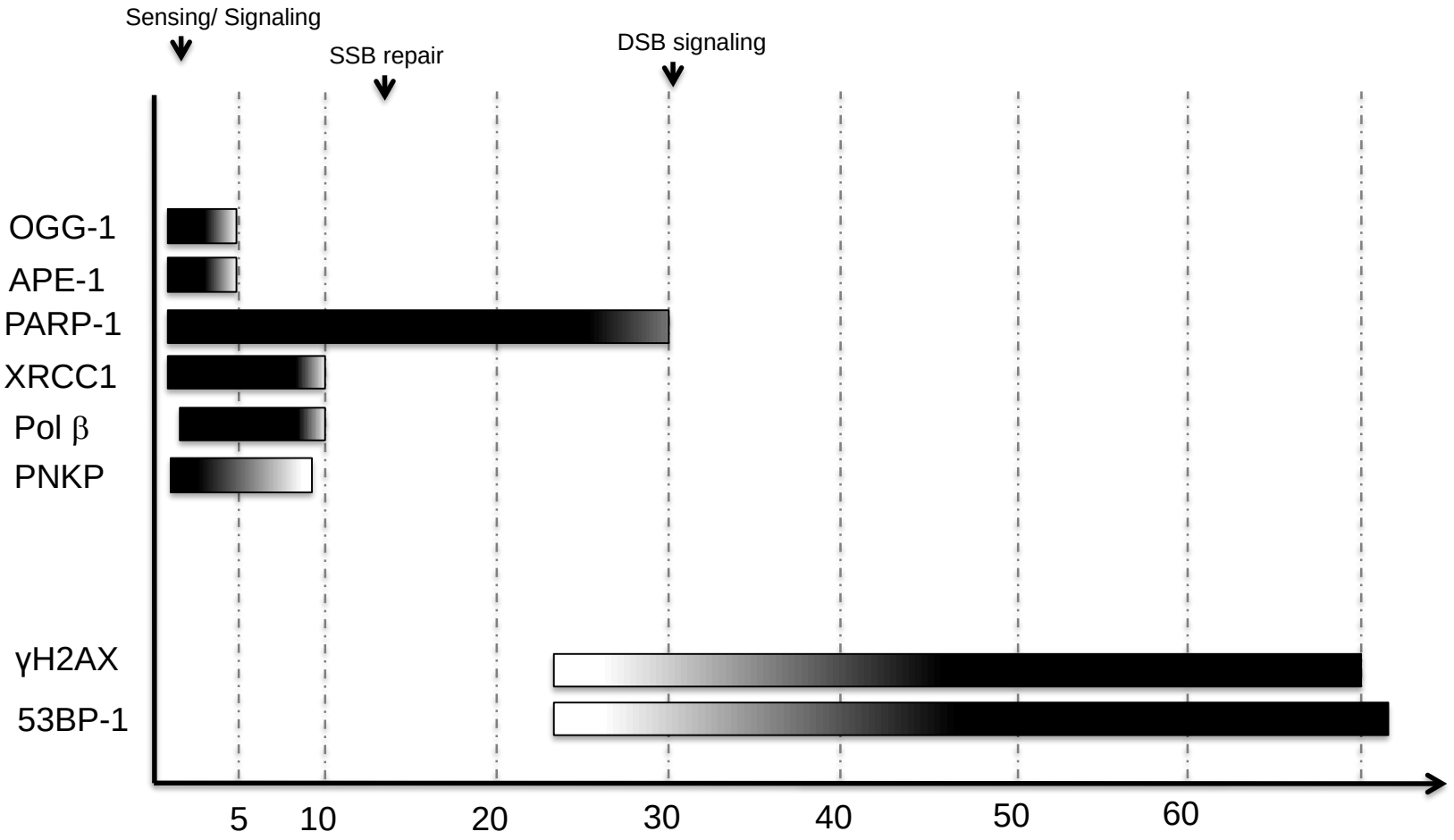
2. Time- the duration the laser spends on the defined ROI (pixel dwell time, frame rate, iterations)

Wavelength	Power Reported	Damage Characterization
337 nm	~0.08 µJ	8-oxodG, 6,4PPs, CPDs γH2AX positive
364 nm	~ 0.17 µJ	γH2AX negative
365 nm (low power)	~ 0.19 µJ	γH2AX negative
365 nm (high power)	~ 0.49µJ	γH2AX positive 8-oxodG
405 nm	~7.5 µW ~16-800 µW	Low power, γH2AX negative 8-oxodG and γH2AX positive

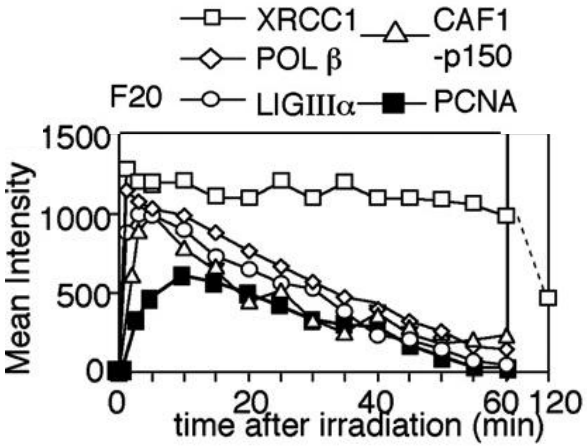
BER or SSBR


AP site
AP Endonuclease
3' OH
5' dRP
PARP
PARylation (●),
Recruitment BER
3' OH
5' dRP
Pol β
Ligase III
XRCC1
PARP
3' P
5' P
PNKP
XRCC1
Ligase III
Pol β
XRCC1
Ligase III
PCNA
FEN1
XRCC1
Ligase III

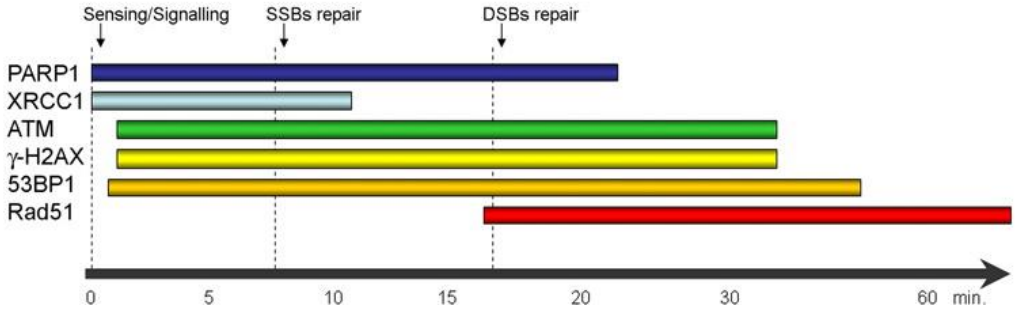
Timeline of Repair Events



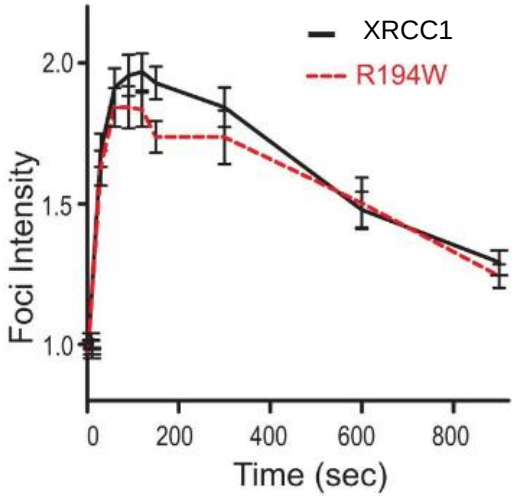
Characterization considerations



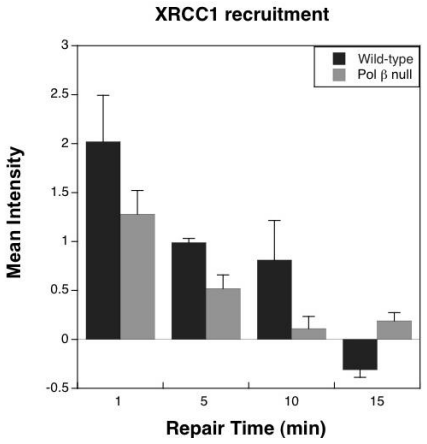
Lan *PNAS* 2004

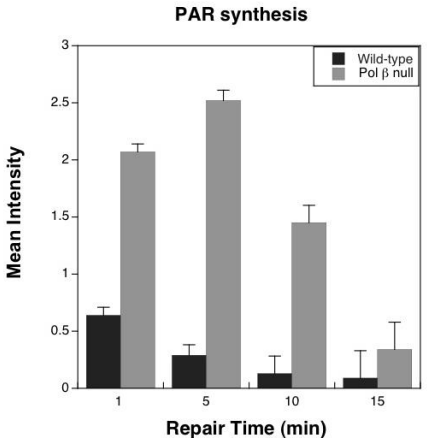


Haince J et al. *J. Biol. Chem.* 2008



Hanssen-Bauer *DNA Repair* 2012

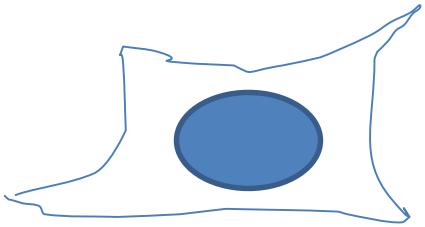




Gassman *PLoS* 2012

Tale of two wavelengths, 355 and 405 nm

355 nm
405 nm



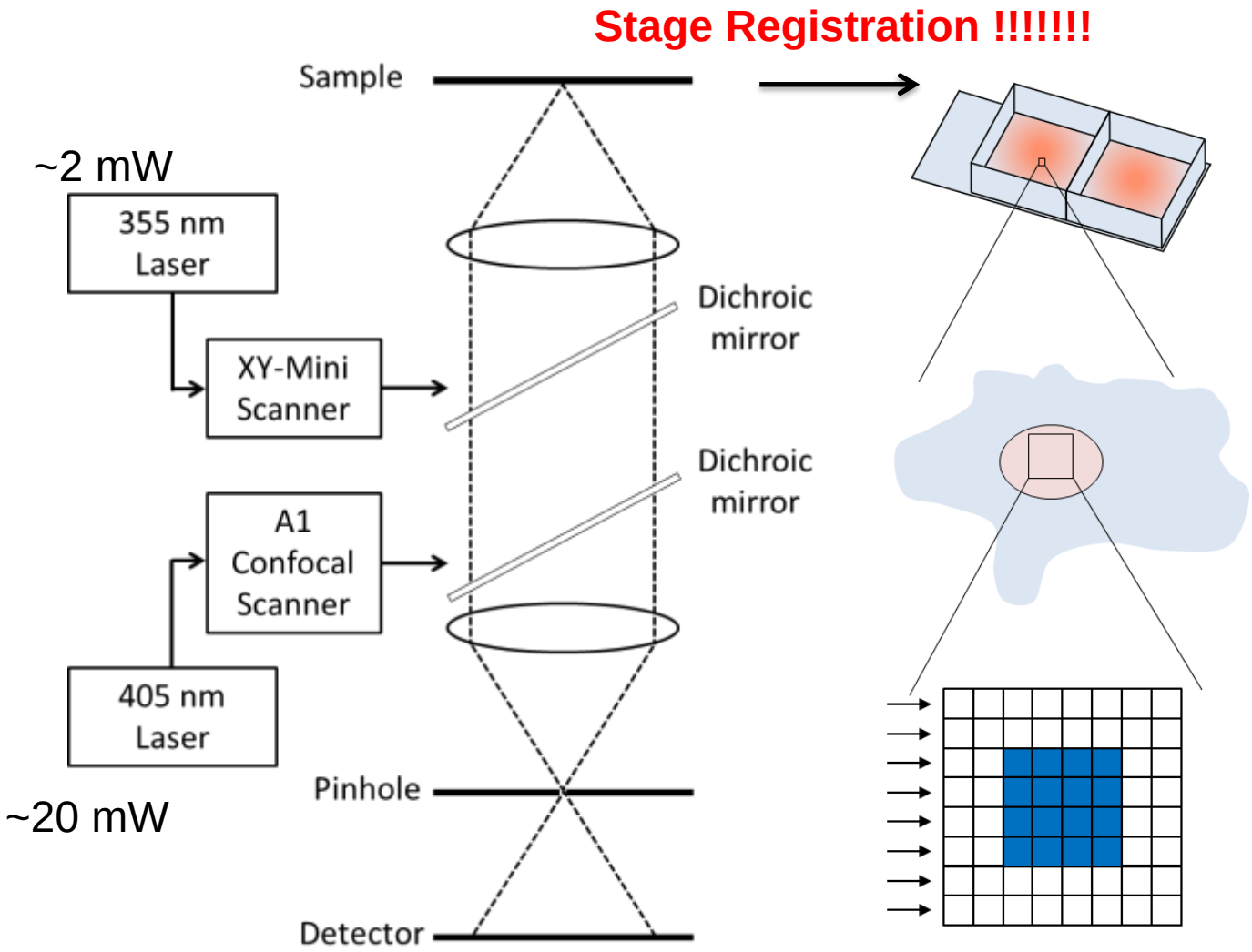
XRCC1-GFP

CHO-K1 (XRCC1 proficient)
MEF Xrcc1$^{-/-}$ p53$^{-/-}$

1. Induce single strand breaks or base lesions without a sensitizer

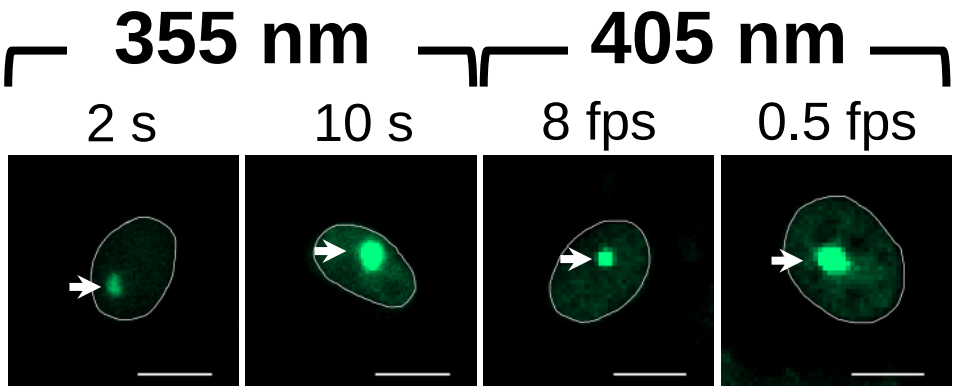
2. Monitor the response of repair proteins to that site of damage
   A. Live cell
   B. Immunofluorescence

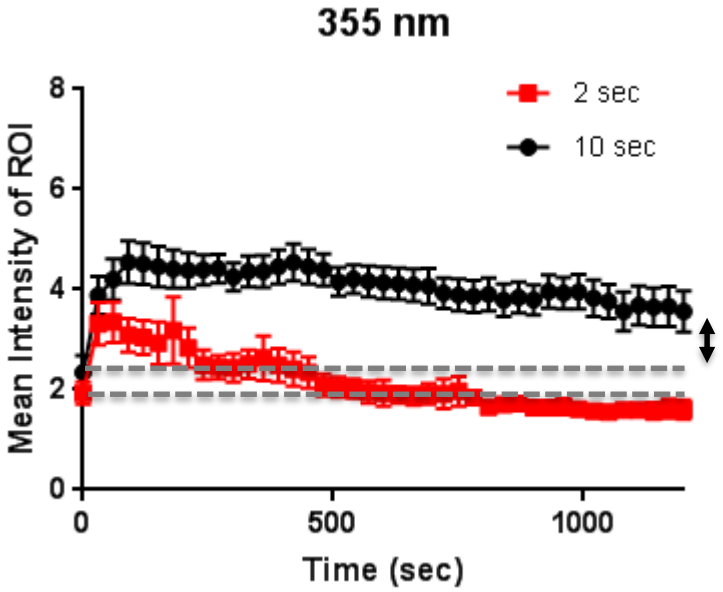
Laser microirradiation

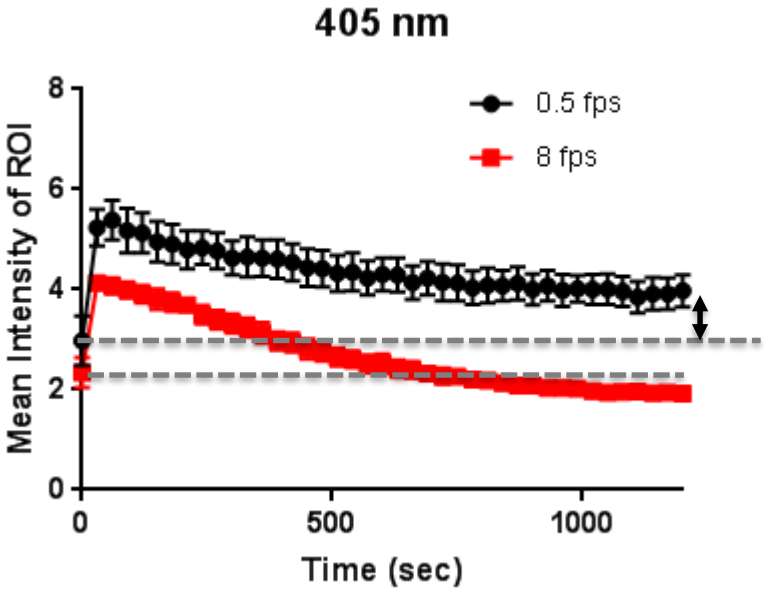


Recruitment of XRCC1

CHO XRCC1-GFP
15 sec post damage







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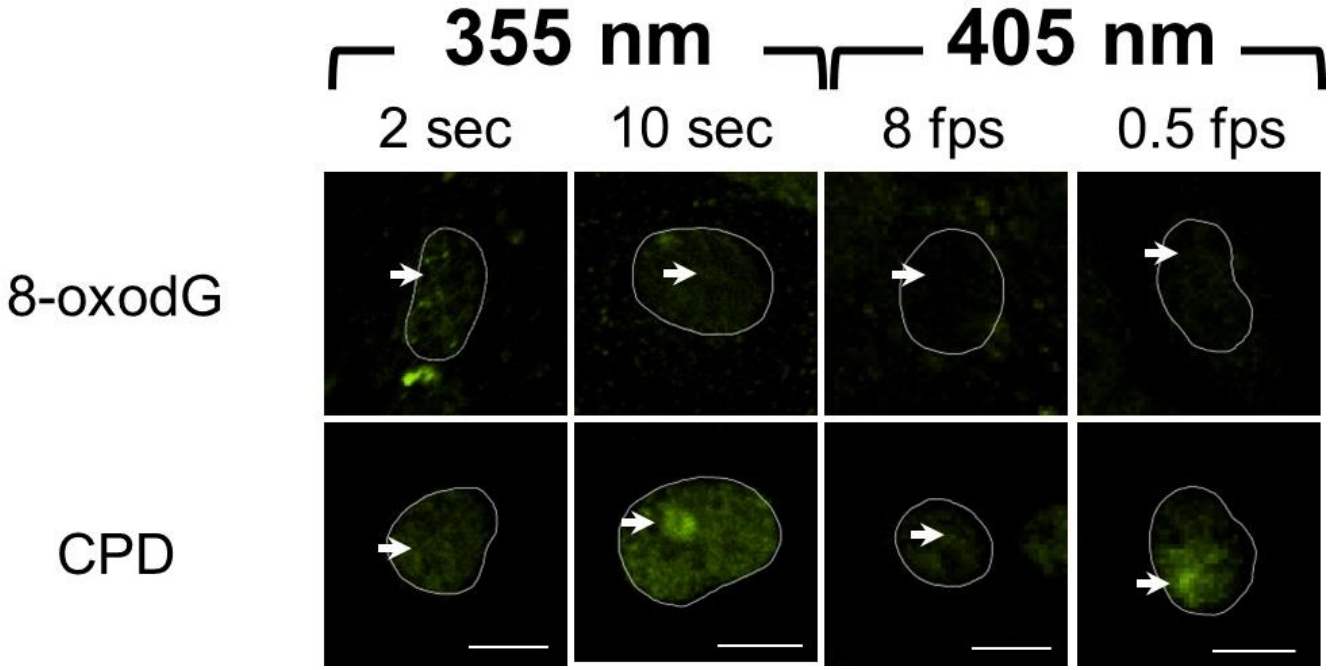
Complex break mixture at high powers



1 min post-irradiation

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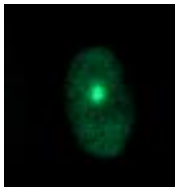
Comparing XRCC1 recruitment

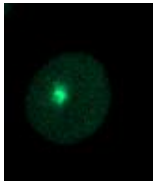
~ 19 µJ

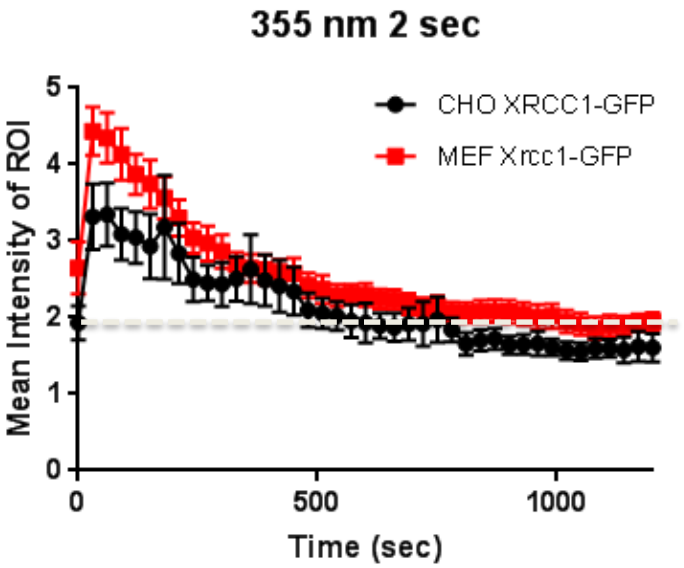
355 nm
2 sec

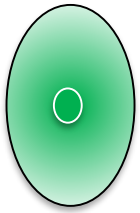
CHO
XRCC1-GFP



MEF
Xrcc1-GFP







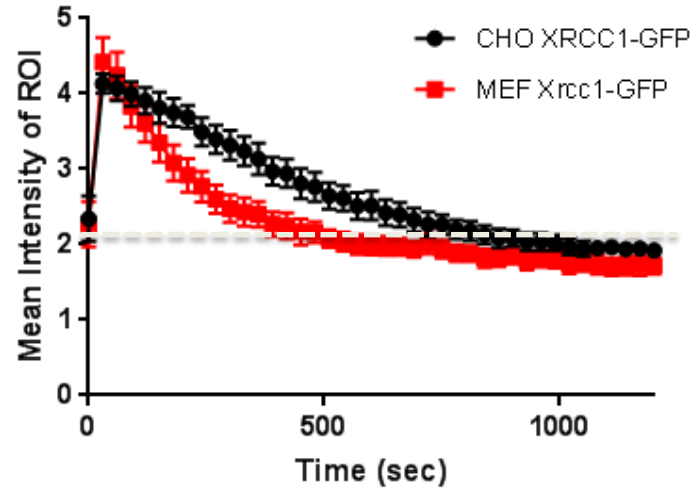
Intensity = $\frac{\text{Focus}}{\text{Nucleus}}$

1.5 mW

405 nm
8 fps

CHO
XRCC1-GFP

MEF
Xrcc1-GFP



15 s post damage

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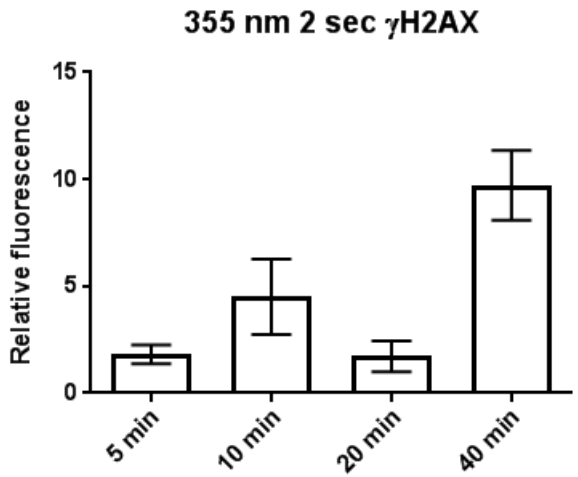
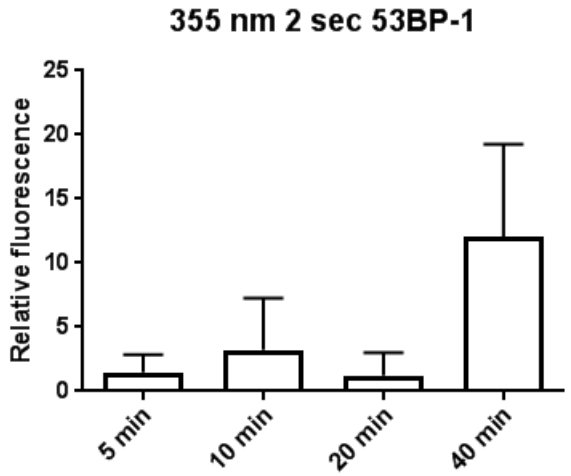
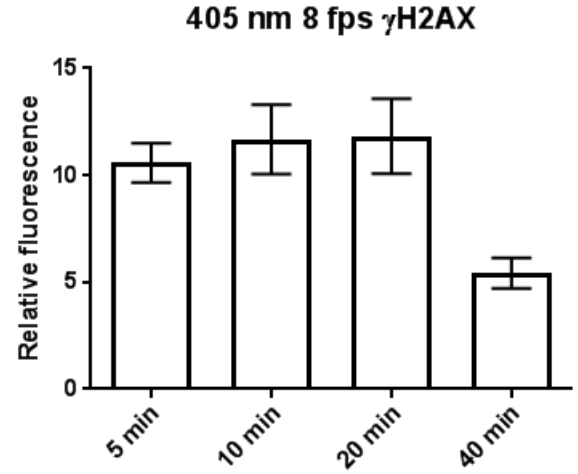
Better separation in between SSB and DSB with 355 nm

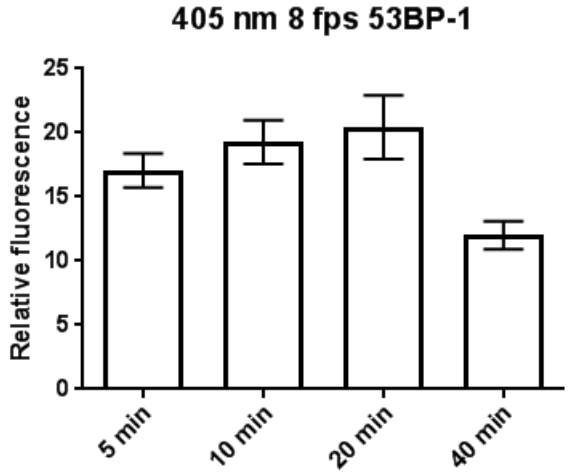
CHO





CHO





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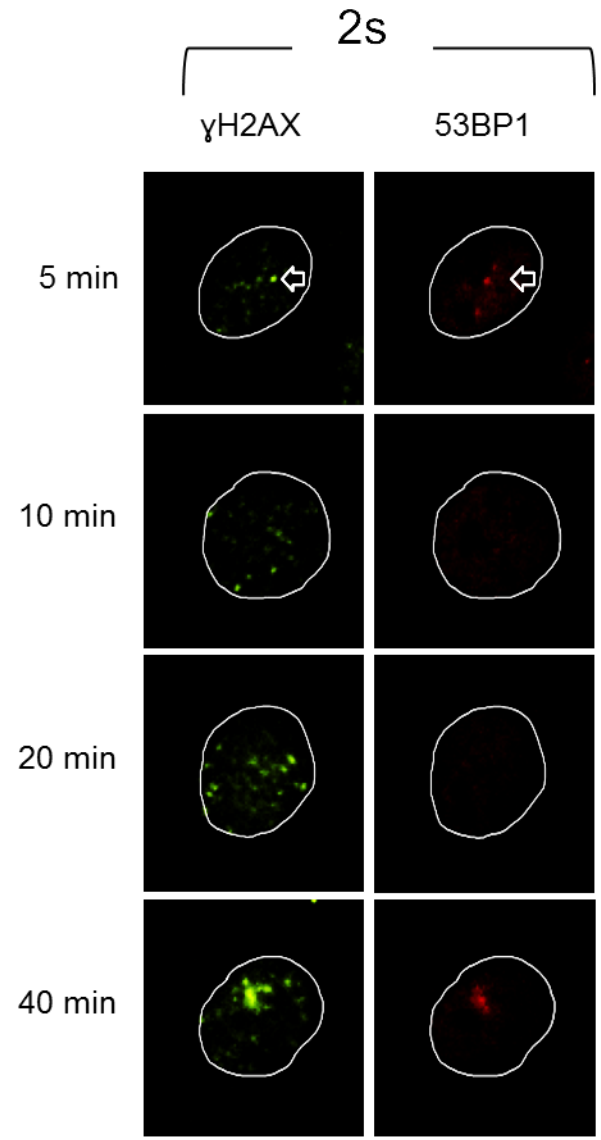
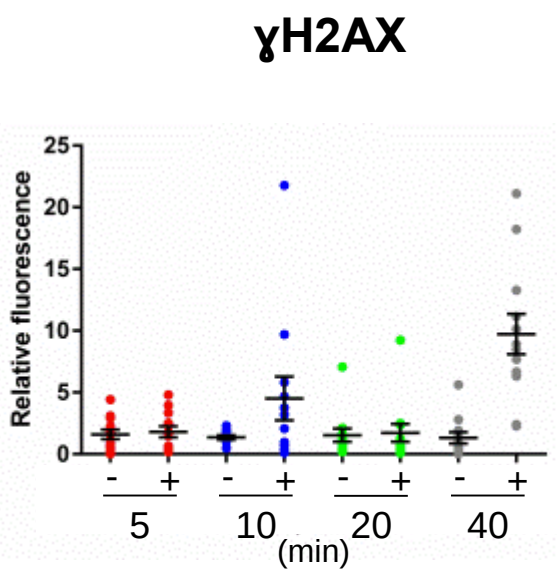
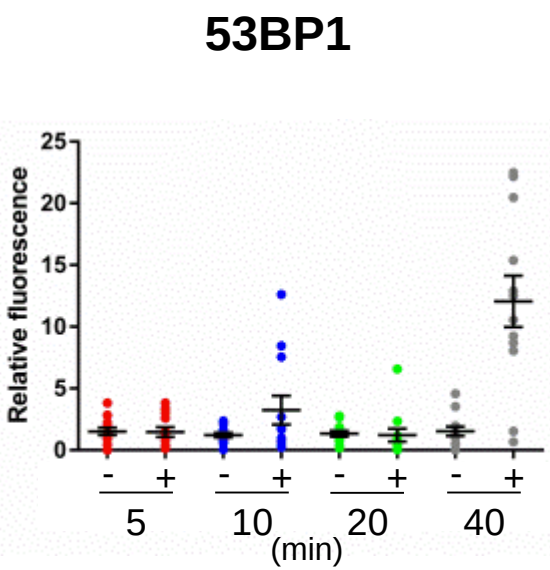
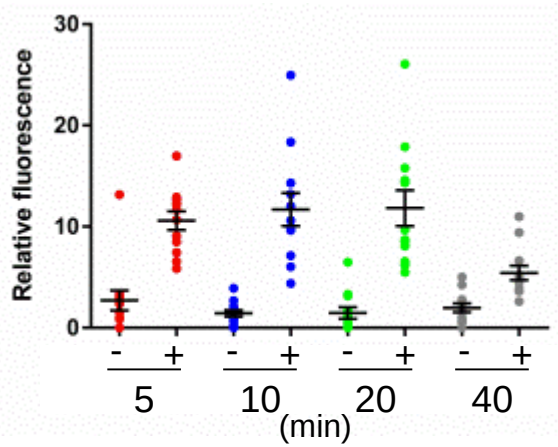
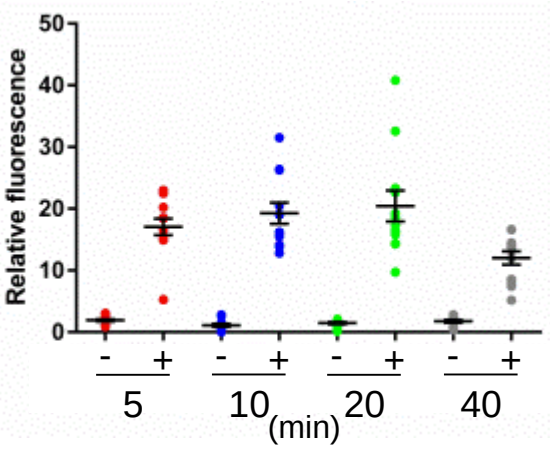
Uniformity of DSB











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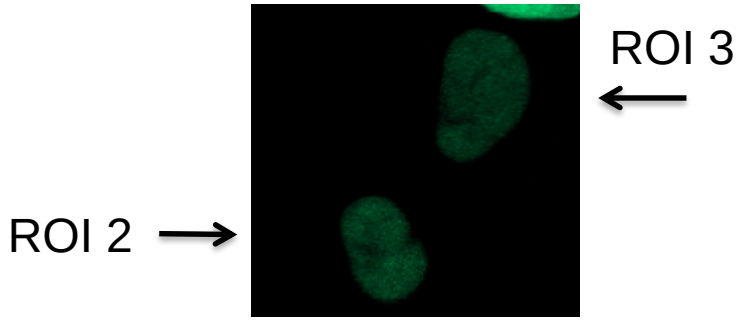
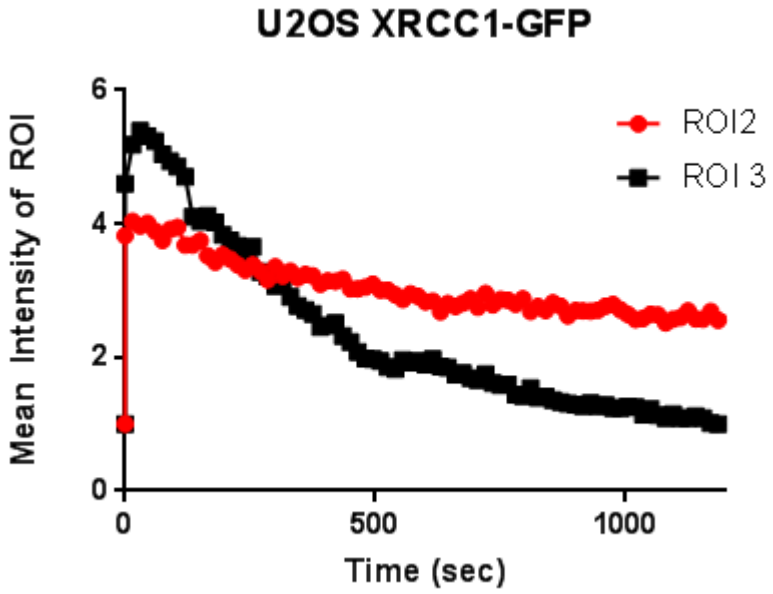
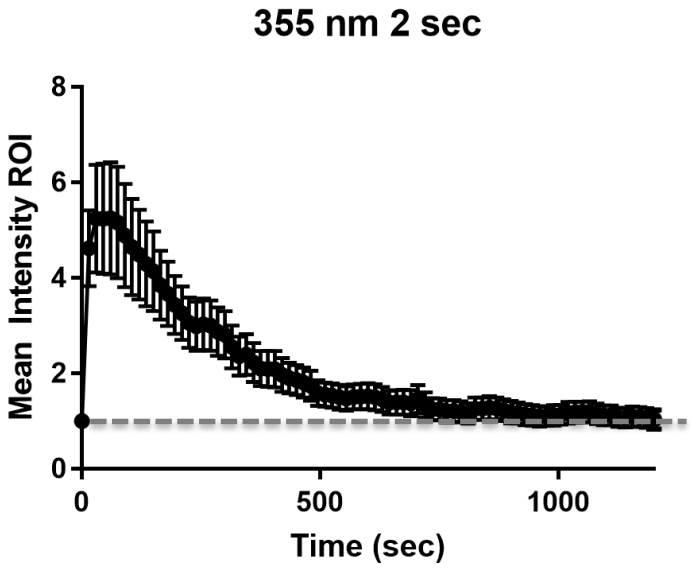
SSBR in U2OS XRCC1-GFP

355 nm 2 sec

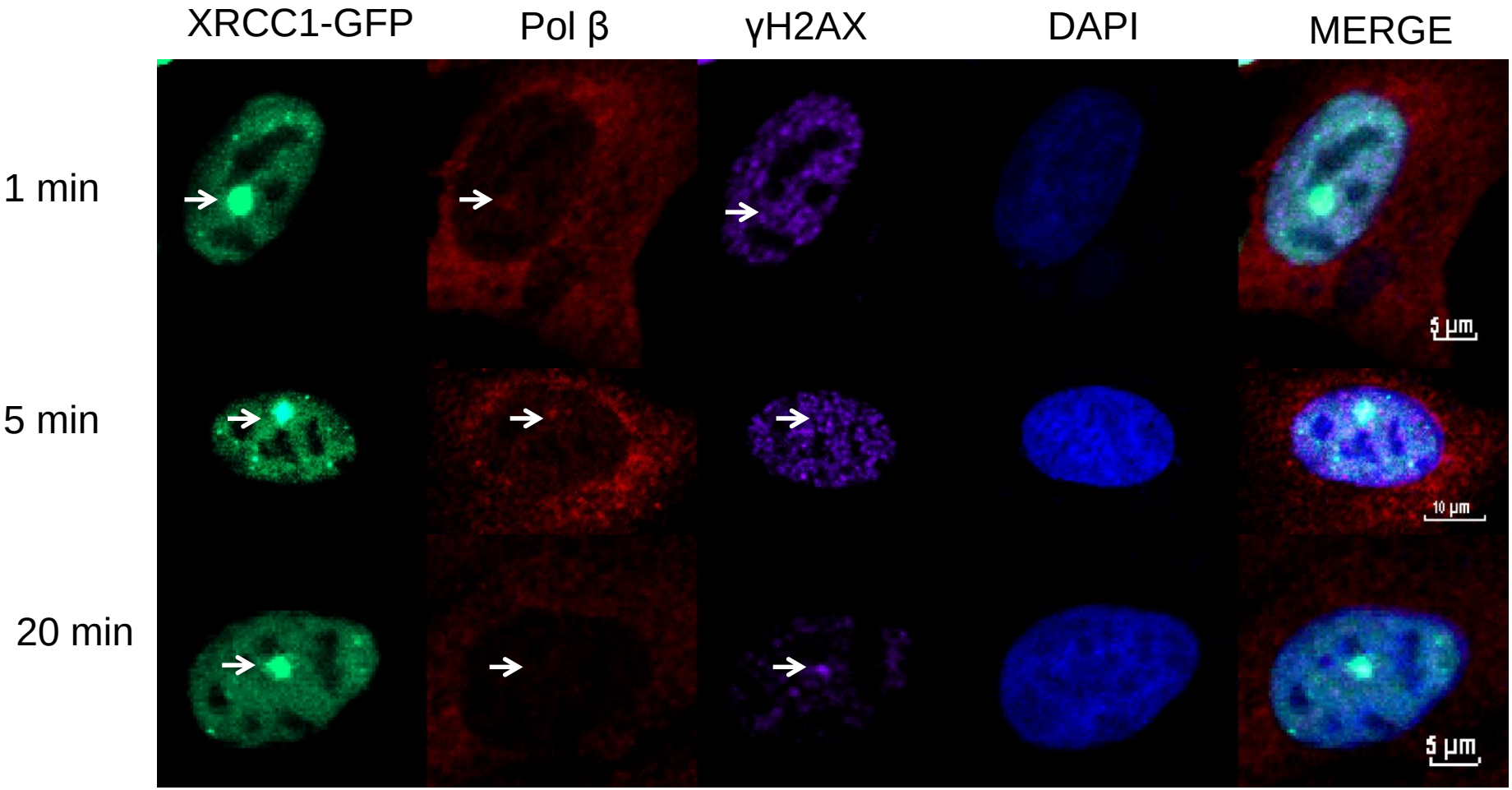




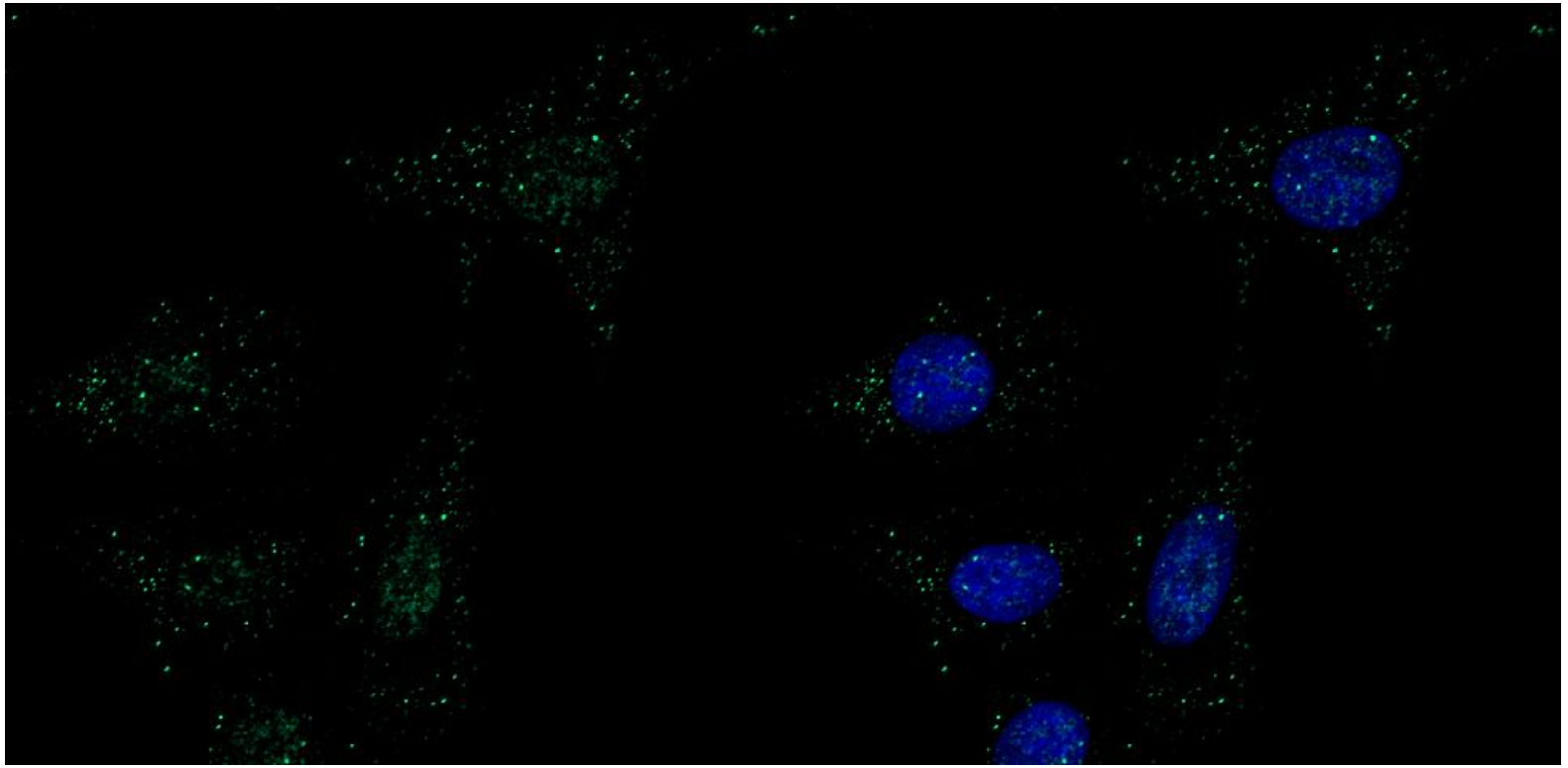


~12.35 min

SSBR in U2OS XRCC1-GFP

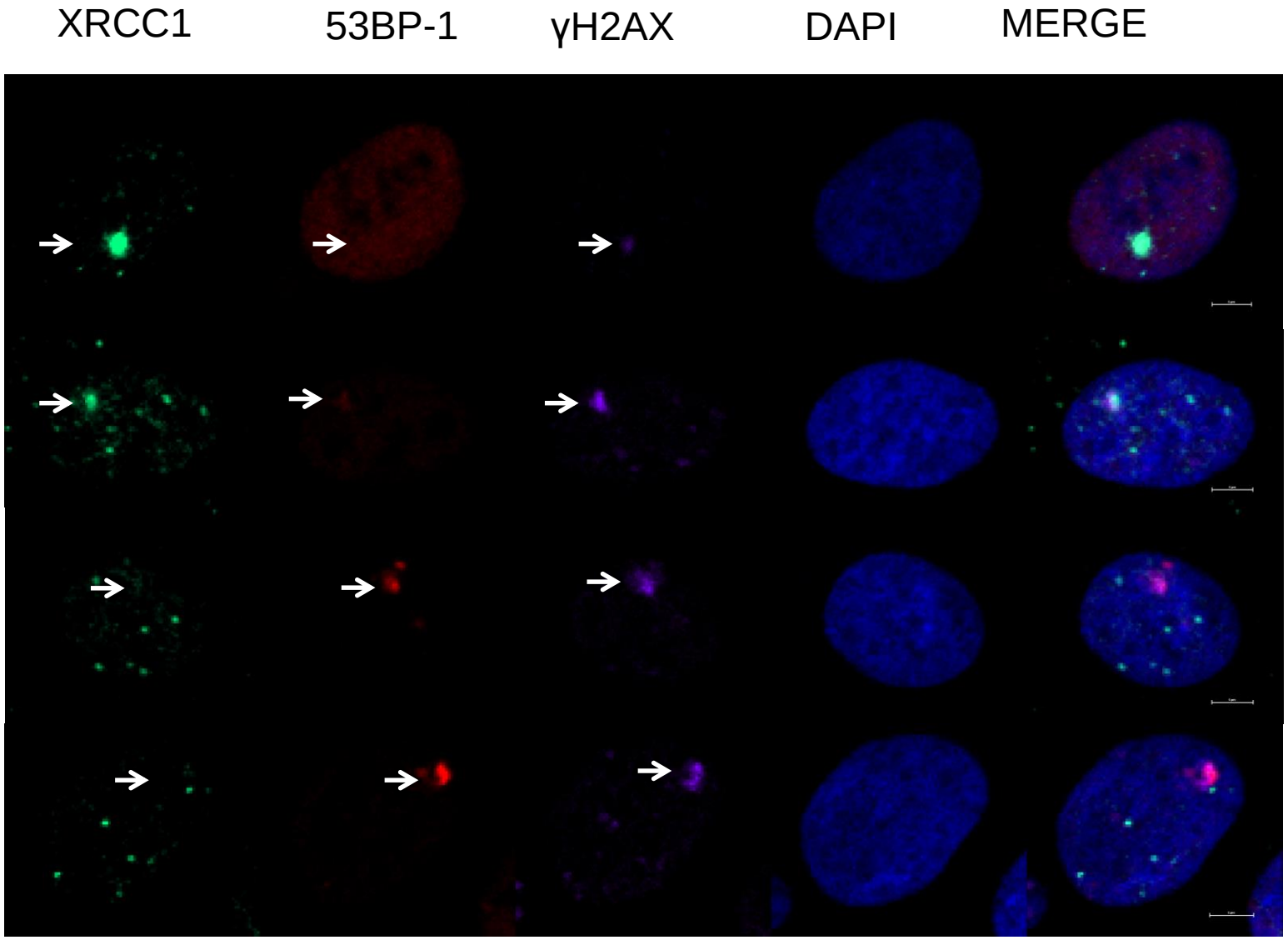


SSBR in U2OS endogenous



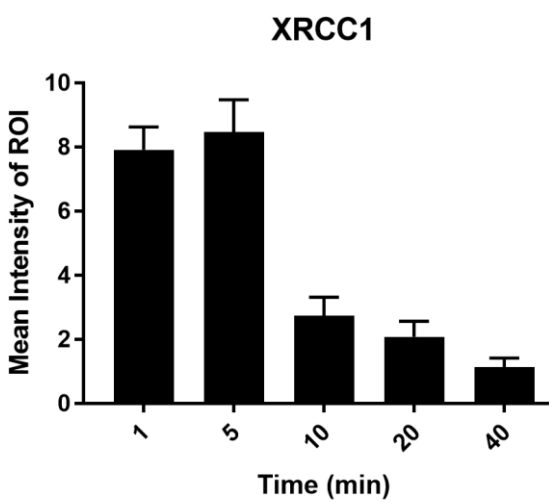
SSBR in U2OS endogenous

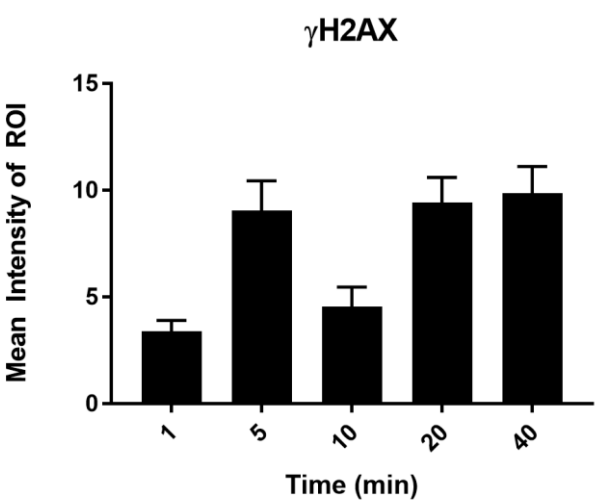
355 nm 2 sec

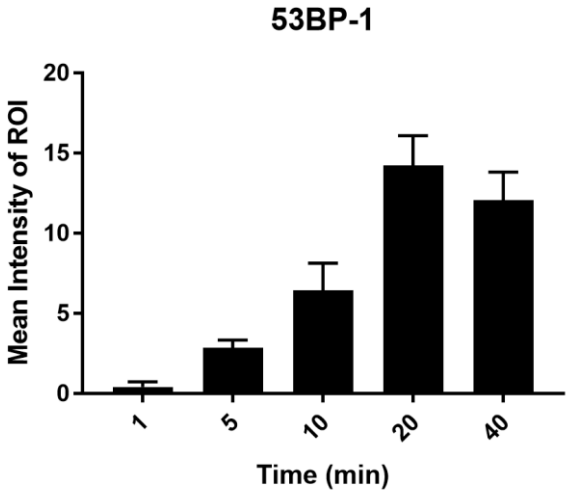


SSBR in U2OS endogenous XRCC1

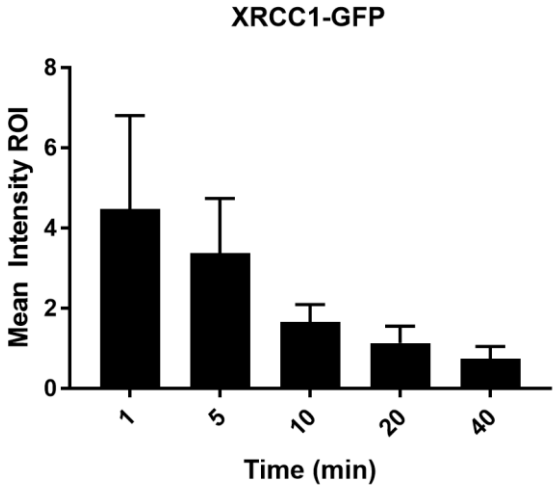
355 nm 2 s

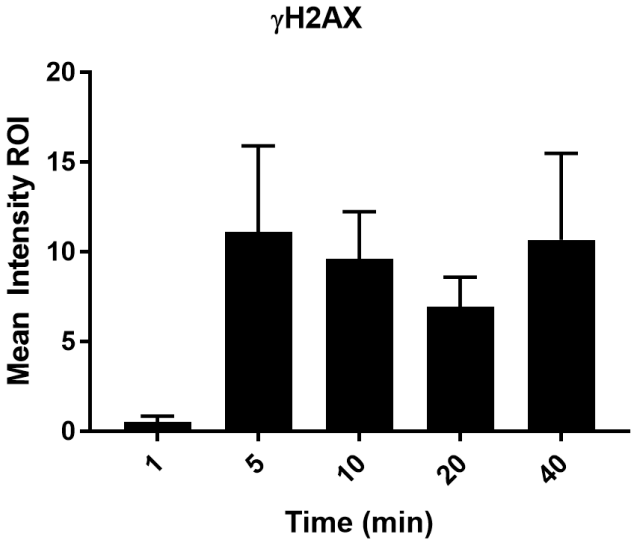


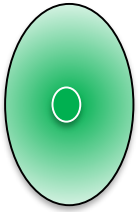




U2OS XRCC1-GFP



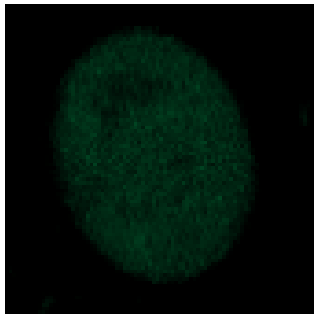


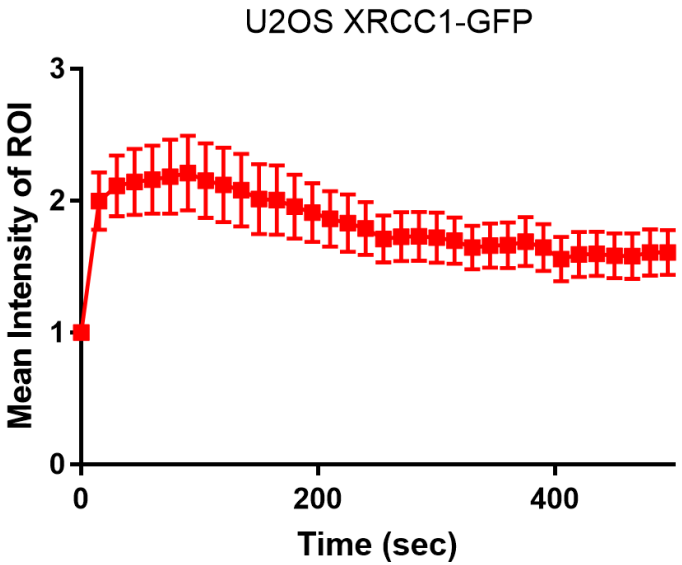


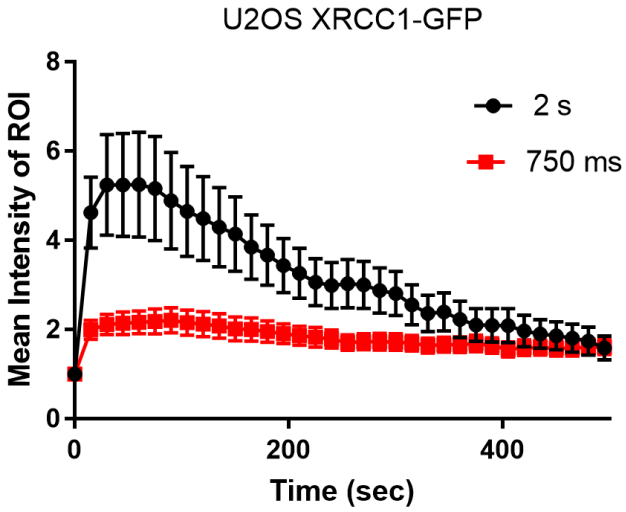
$$\text{Intensity} = \frac{\text{Focus}}{\text{Nucleus}}$$

Normalized to undamaged cells

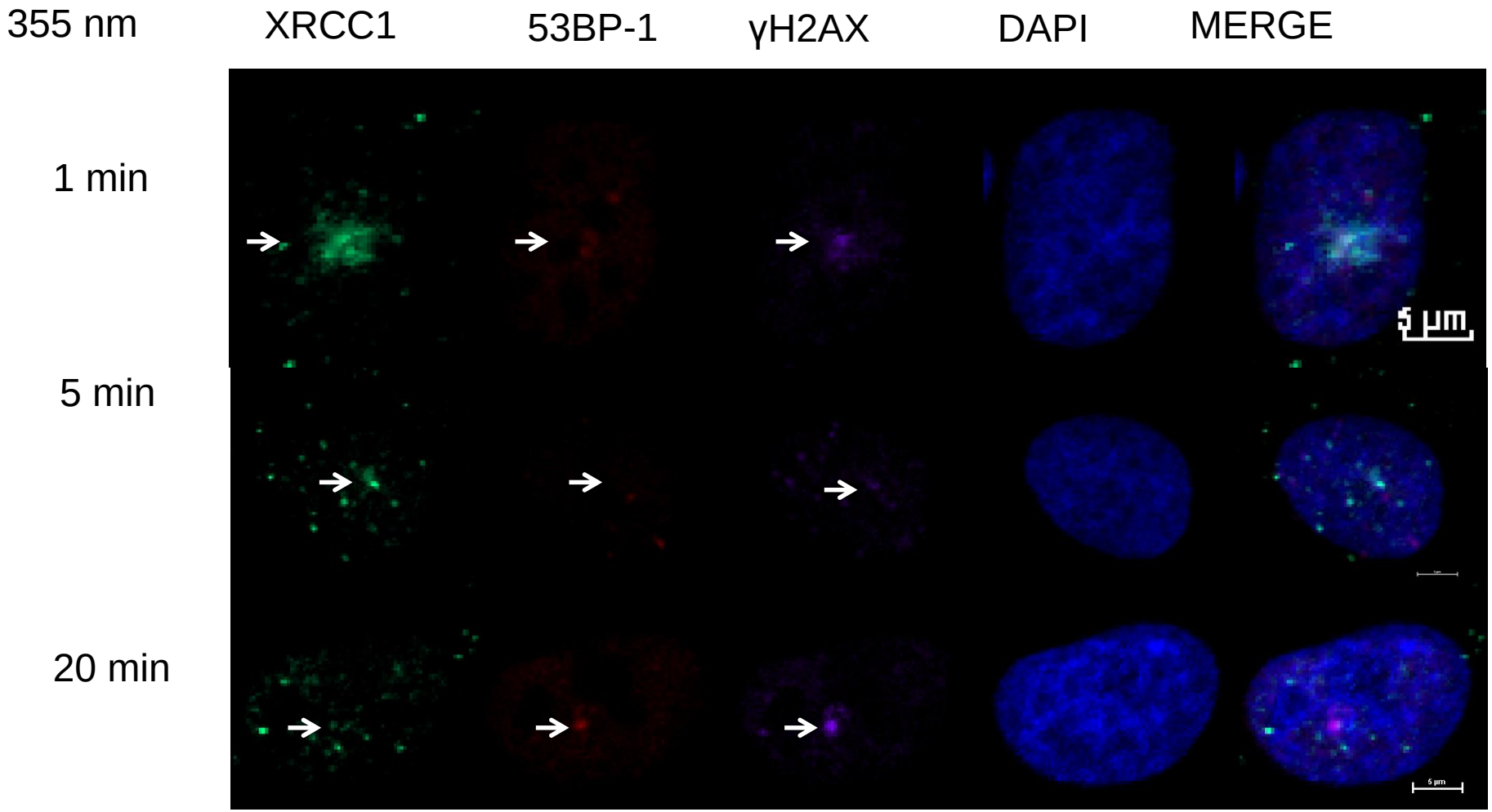
355 nm 750 ms U2OS



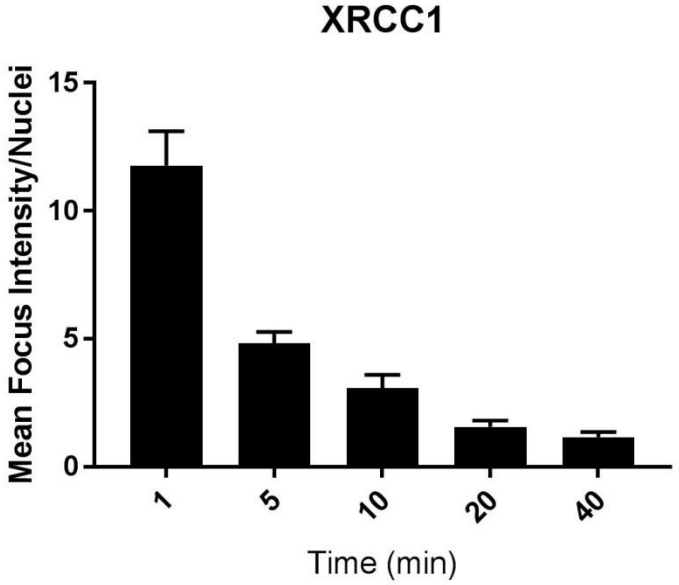


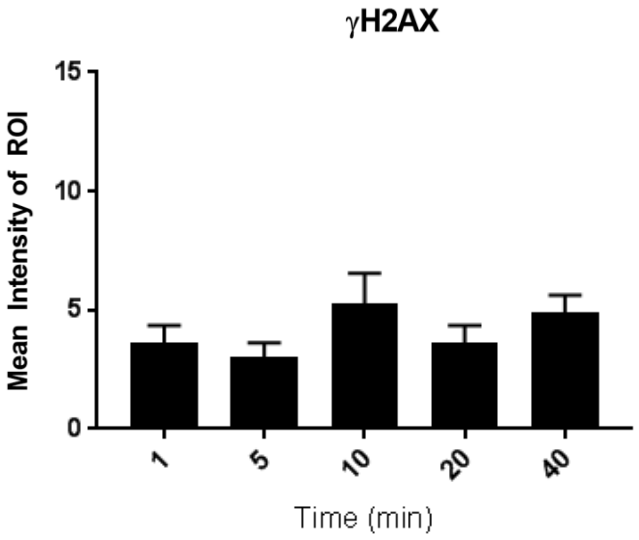


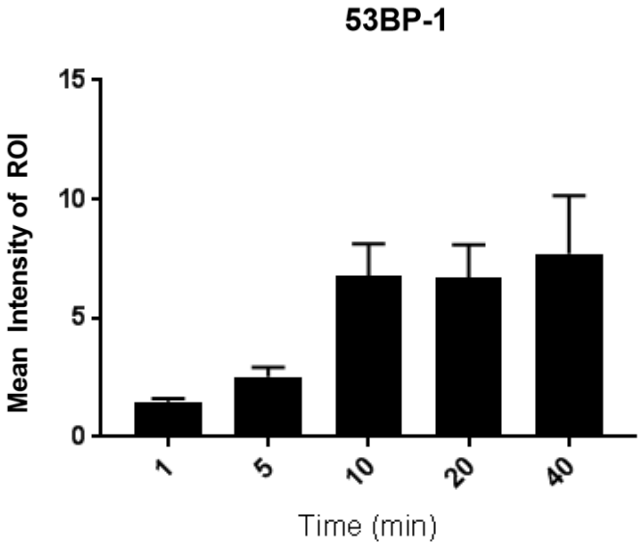
Endogenous XRCC1 750 ms U2OS



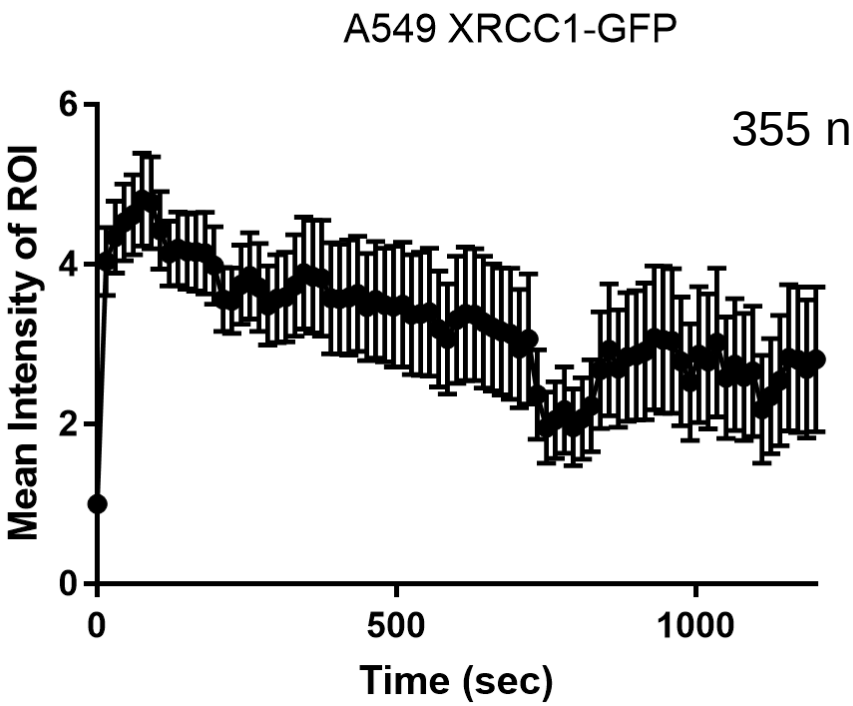
Endogenous XRCC1 750 ms U2OS



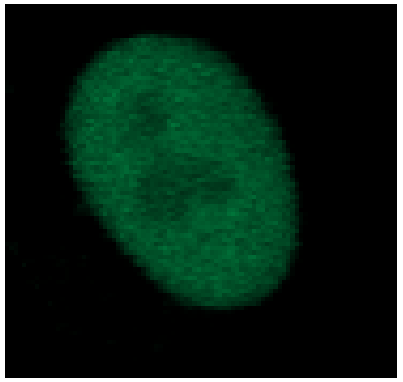




SSBR in A549 XRCC1-GFP

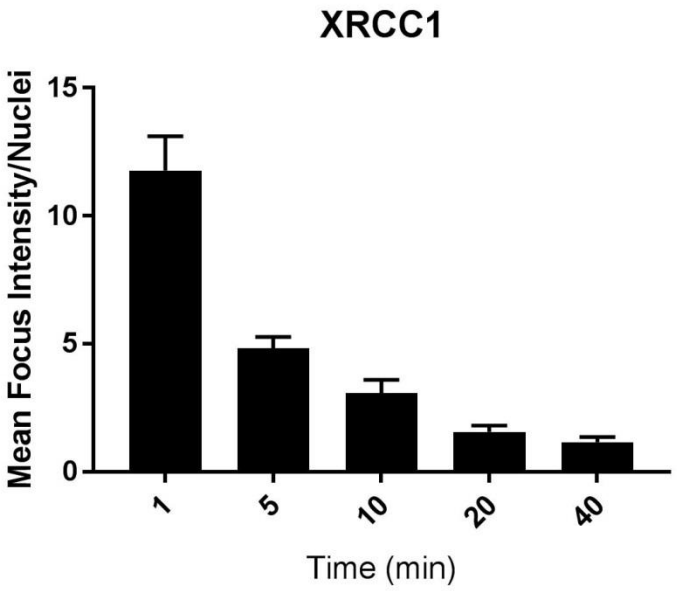


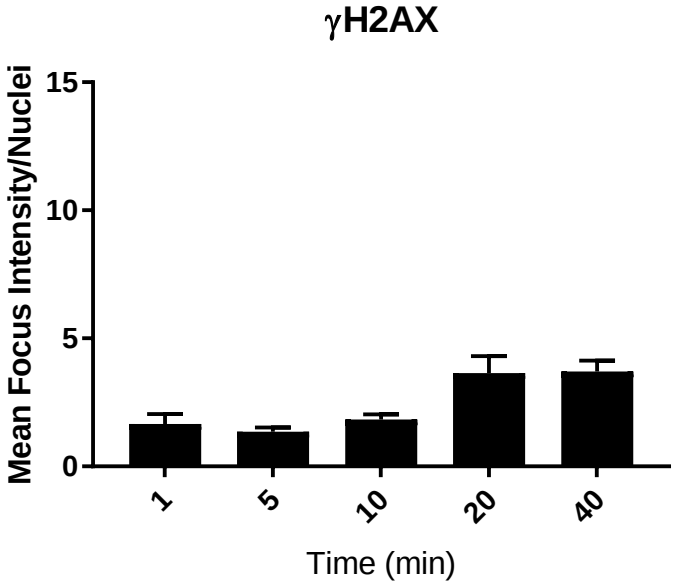
355 nm 2 sec

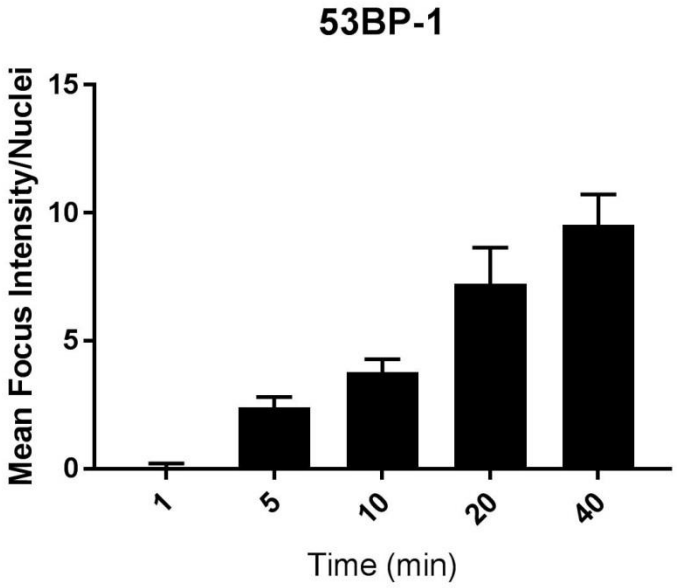


SSBR in A549 endogenous XRCC1

355 nm 2 sec

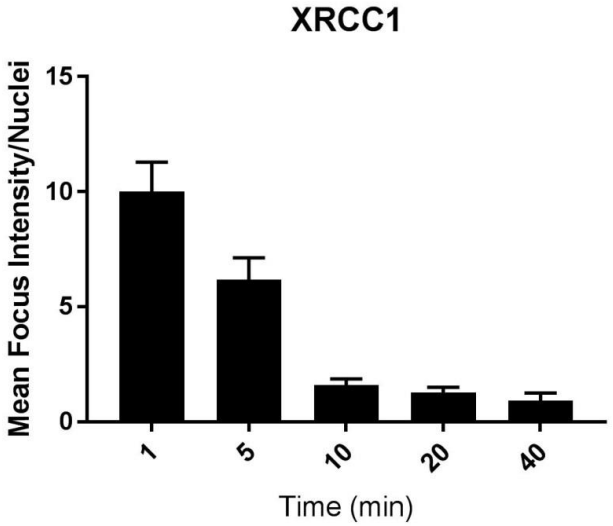


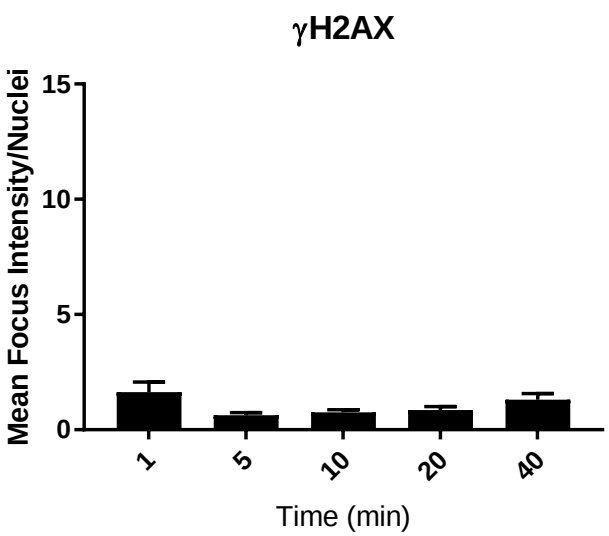


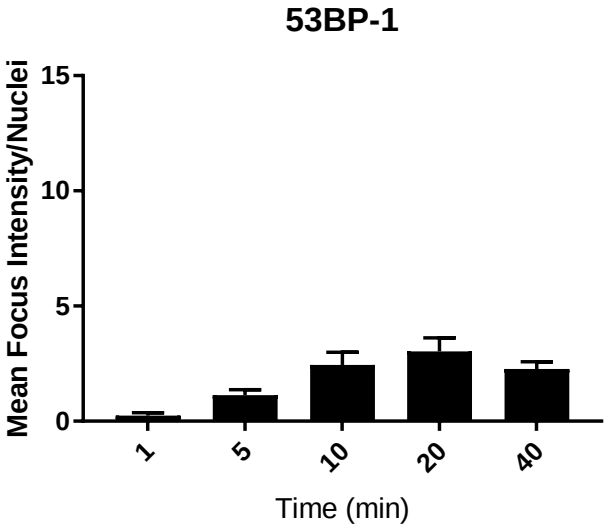


SSBR in A549 endogenous XRCC1

355 nm 750 msec



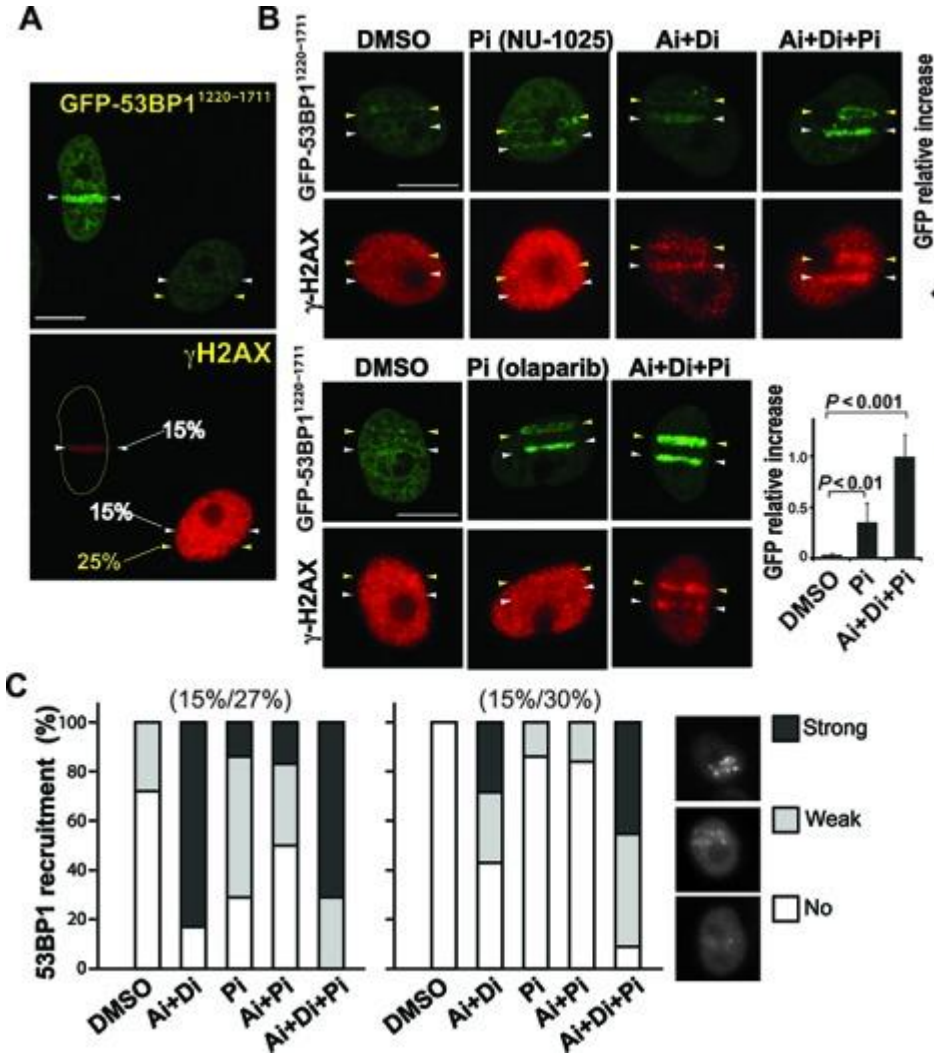


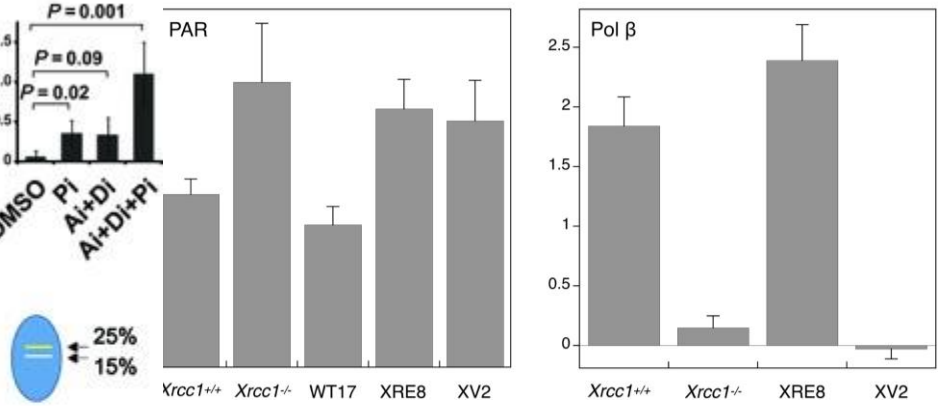


Microenvironment influence response

	Cell Line	XRCC1 response	γH2AX	53BP-1
2 s	CHO-K1 (GFP-XRCC1)	Peaks ~ 1 sec **Resolves ~ 8 min**	10 min (weak) **40 min**	20 min **40 min**
	U2OS (GFP-XRCC1)	Peaks ~ 1 sec **Resolves ~12 min**	**5 min**	
	U2OS	**Resolves ~20 min**	**5 min**	**10 min**
	A549 (GFP-XRCC1)	Peak~ 1 sec **Resolves > 20 min**	**5 min**	
	A549	**Resolves ~ 20 min**	**20-40 min**	**20 min**
750 ms	CHO-K1 (GFP-XRCC1)	**Resolves within 5 min**	40 min (weak)	Not detected
	U2OS	**Resolves ~ 15 min**	**Low levels from 5 min forward**	**10 min**
	A549	**Resolves ~ 10 min**	Not detected	Very low levels > 10 min

DNA damage signaling may impact recruitment and response





PARP activation levels allow to discriminate between repair pathways to adjust damage mixtures.

ATM-DNAPK-PARP signaling can alter the recruitment of repair factors and markers, like 53BP-1

Summary

- Use laser induced DNA damage to monitor recruitment of BER/SSBR proteins
- Inconsistencies in recruitment, timing, and other interactions may be due to differences in strand break mixtures
- **Significant unknowns in how the microenvironment (cell line difference, signaling alterations, germline or somatic mutations) impacts the induction of damage and the resulting repair response**
- **Best practices for damage induction**
  - Multiple markers for repair process of interest and strand breaks
  - Sample damage across a broad window
  - Iterate across multiple cell lines, preferably repair-proficient or wild-type and cancer or repair-deficient cell line
  - Attempt to separate signaling events as much as possible
  - Increase the n of the experiment, whenever possible

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