

Alteration of DNA Repair Activity in Hypoxic Brain Tumour Underlies Treatment Resistance

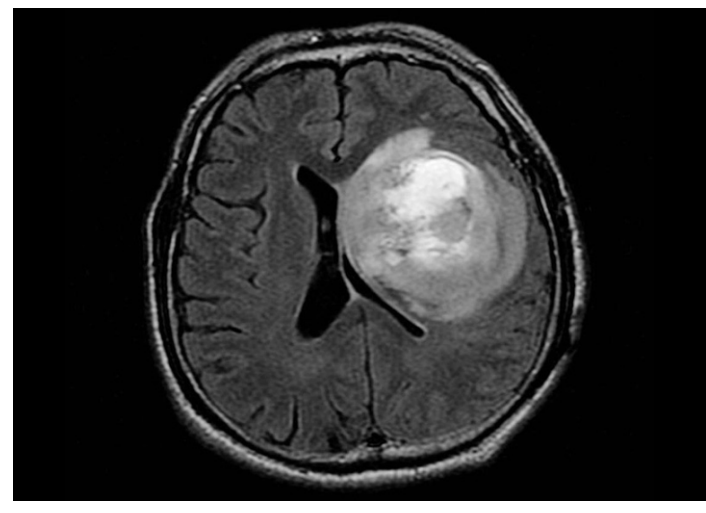


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Hypoxic Brain Tumours

Glioblastoma (GBM) is the most common type of adult brain tumour with 30% 2 year survival rate after treatment. Medulloblastoma (MB) is a common paediatric tumour primarily occurring before the age of 10.

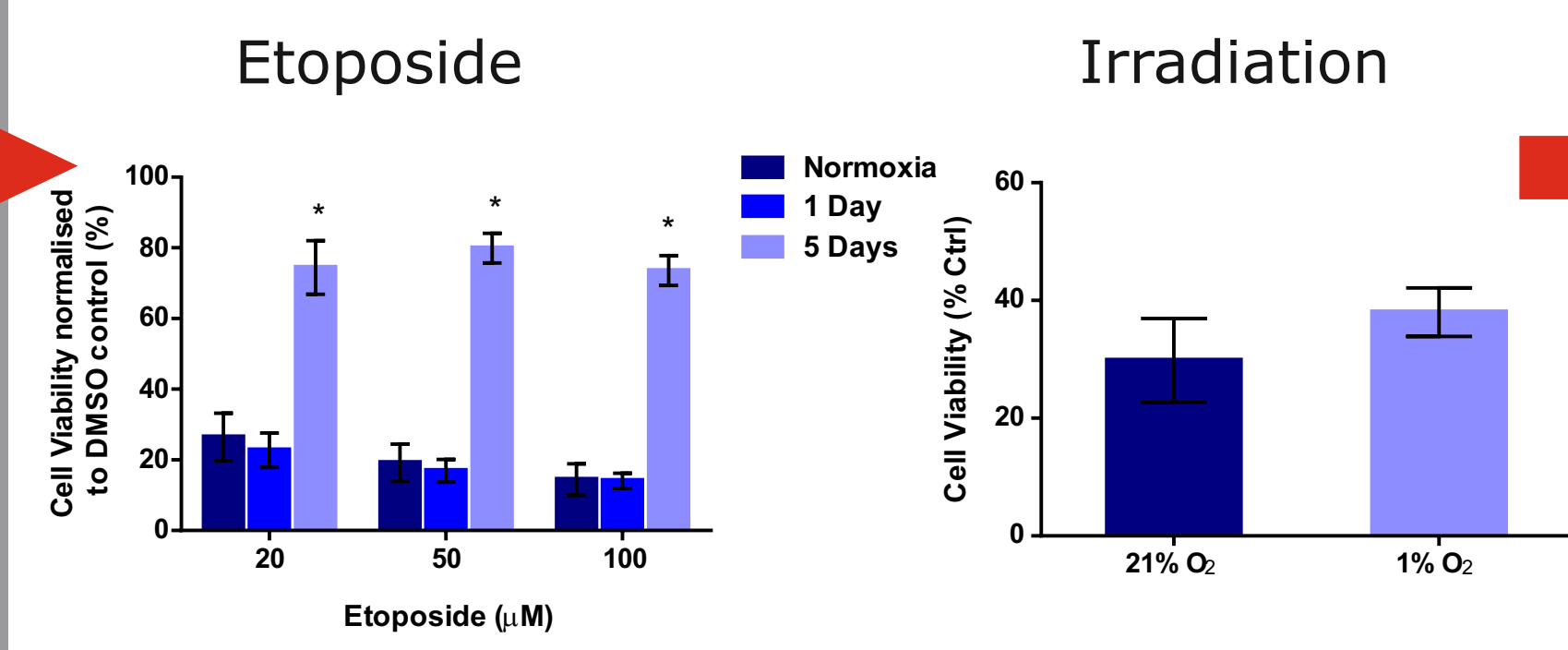


Glioblastoma (Adult)



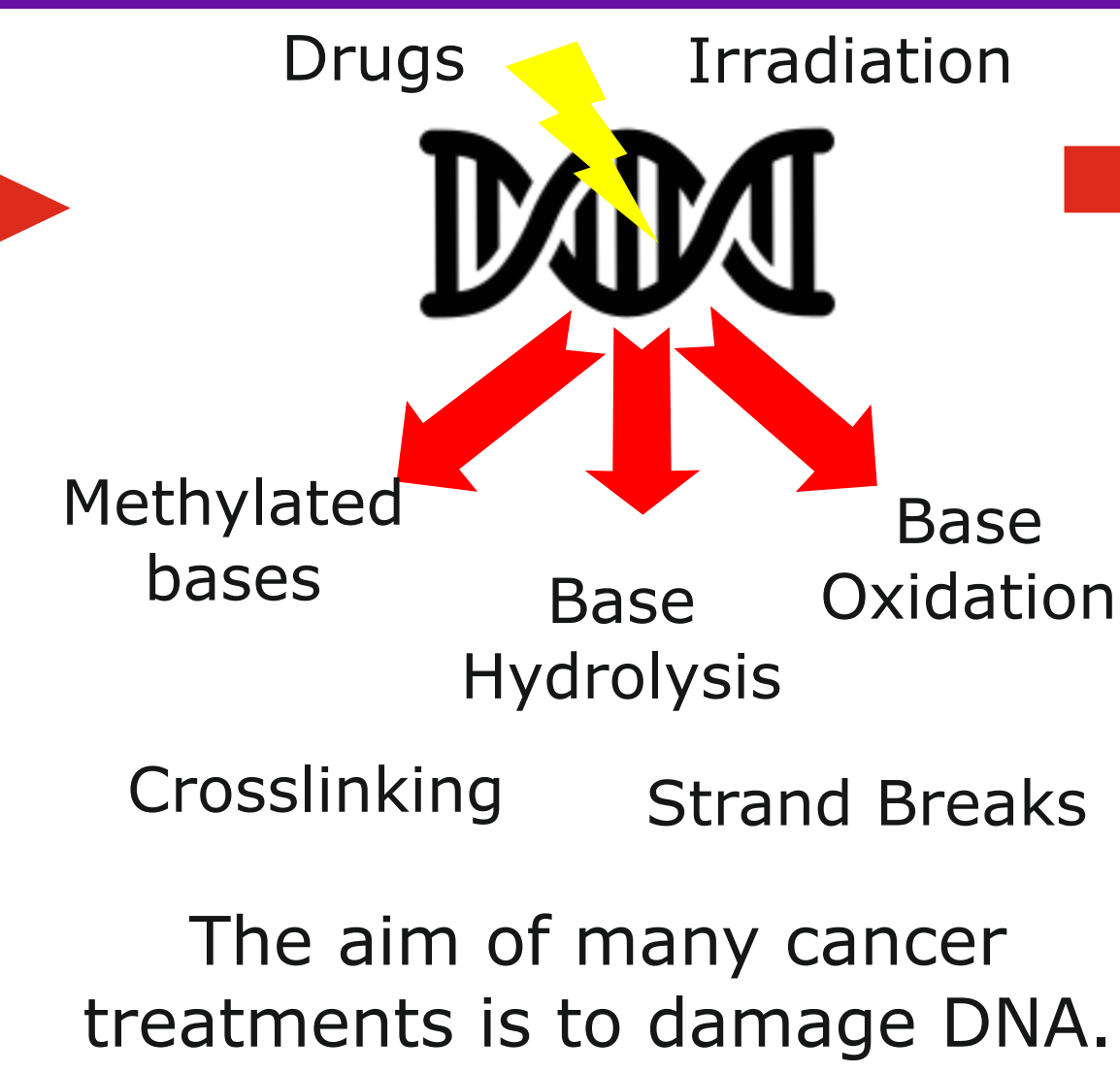
Medulloblastoma (Paediatric)

Hypoxia Causes Treatment Resistance



Hypoxia **reduces sensitivity** of D283-MED (MB) cells to DNA damaging agents such as Etoposide and X-ray Irradiation.

Cancer Treatment and DNA Repair



Research Question

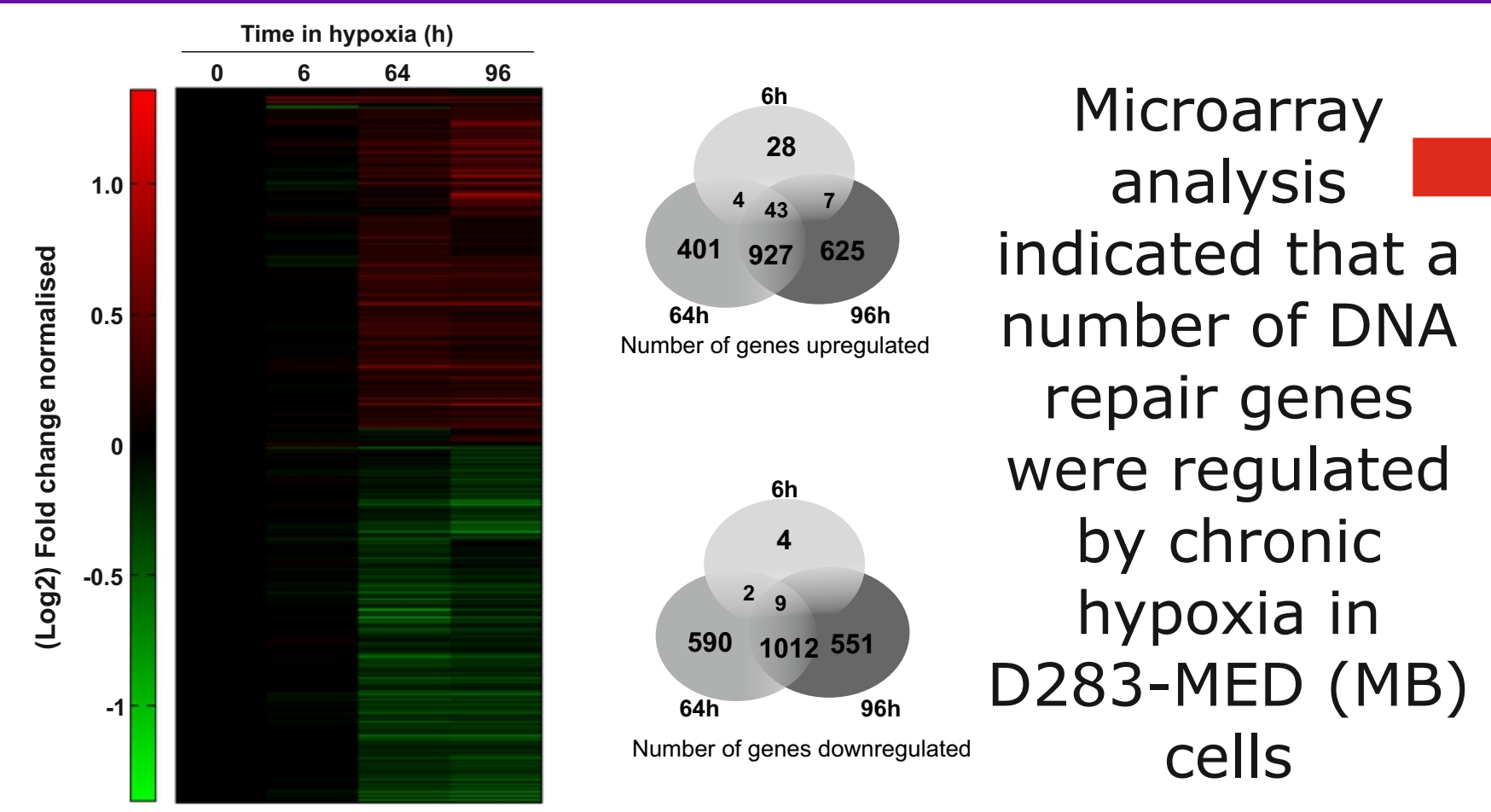
How does hypoxia influence DNA repair mechanisms in brain tumours?

We use a Don Whitley Hypoxystation to mimic tumour hypoxia in tumour cell lines



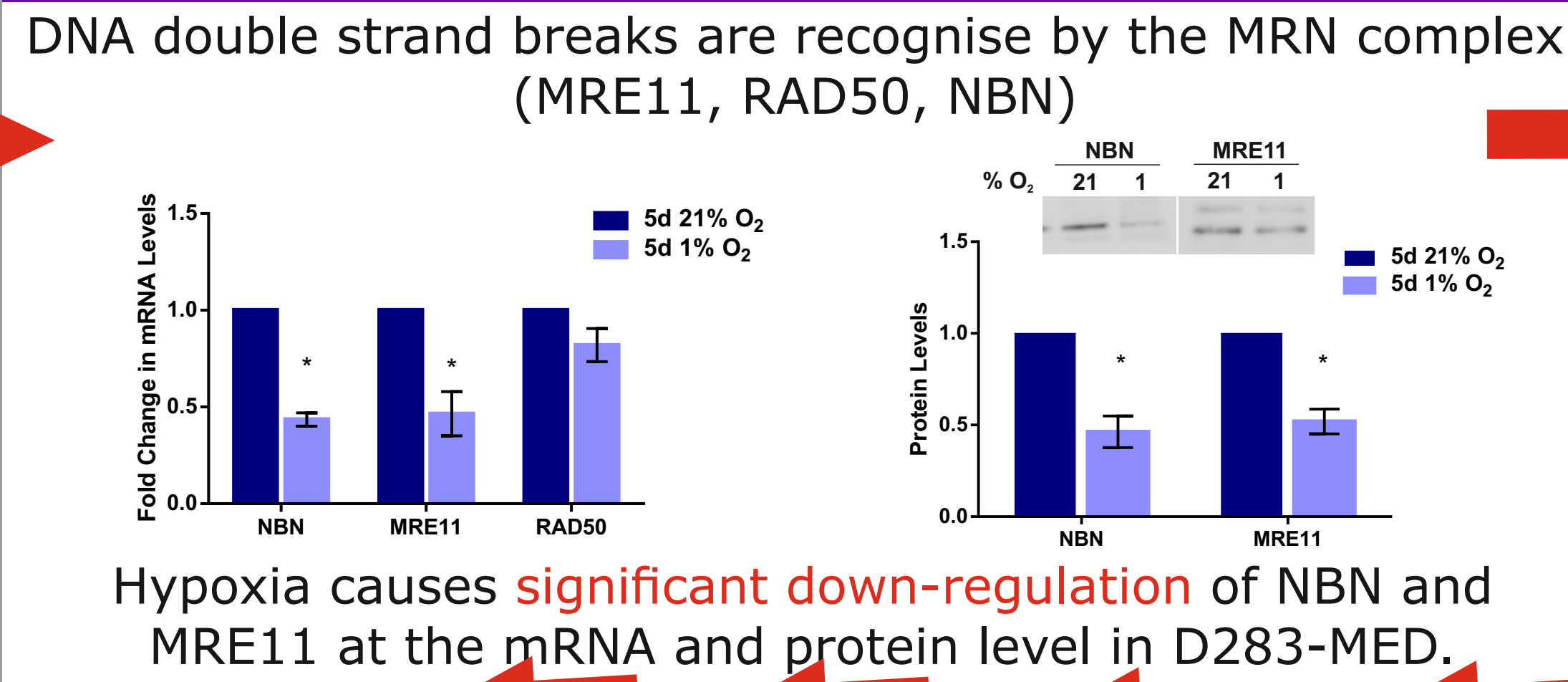
Mechanism 1: DNA break recognition complex is down-regulated...

DNA Repair Genes are Regulated in Chronic Not Acute Hypoxia



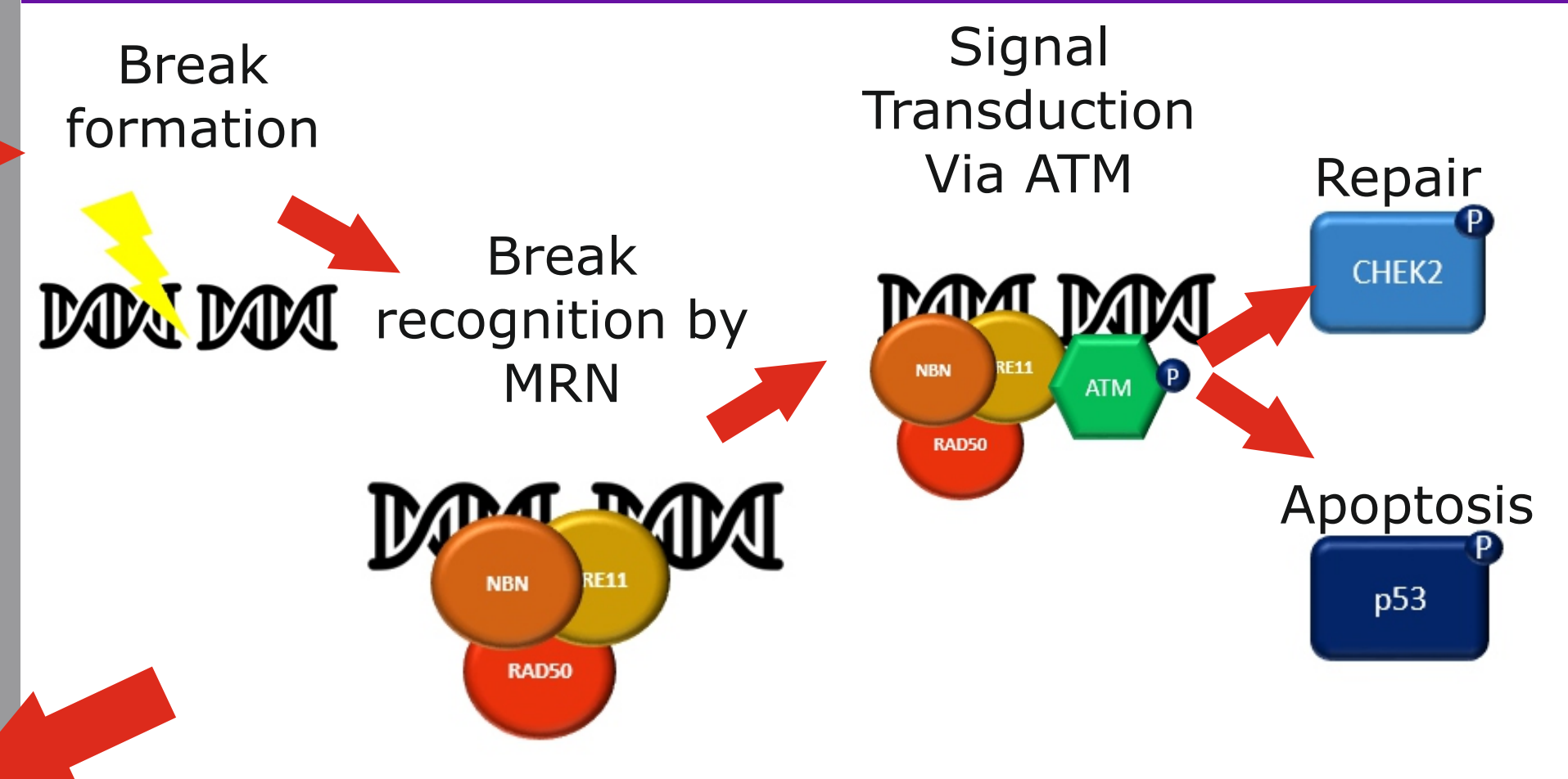
Microarray analysis indicated that a number of DNA repair genes were regulated by chronic hypoxia in D283-MED (MB) cells

Down-regulation of the Double Strand Break Recognition Complex by hypoxia

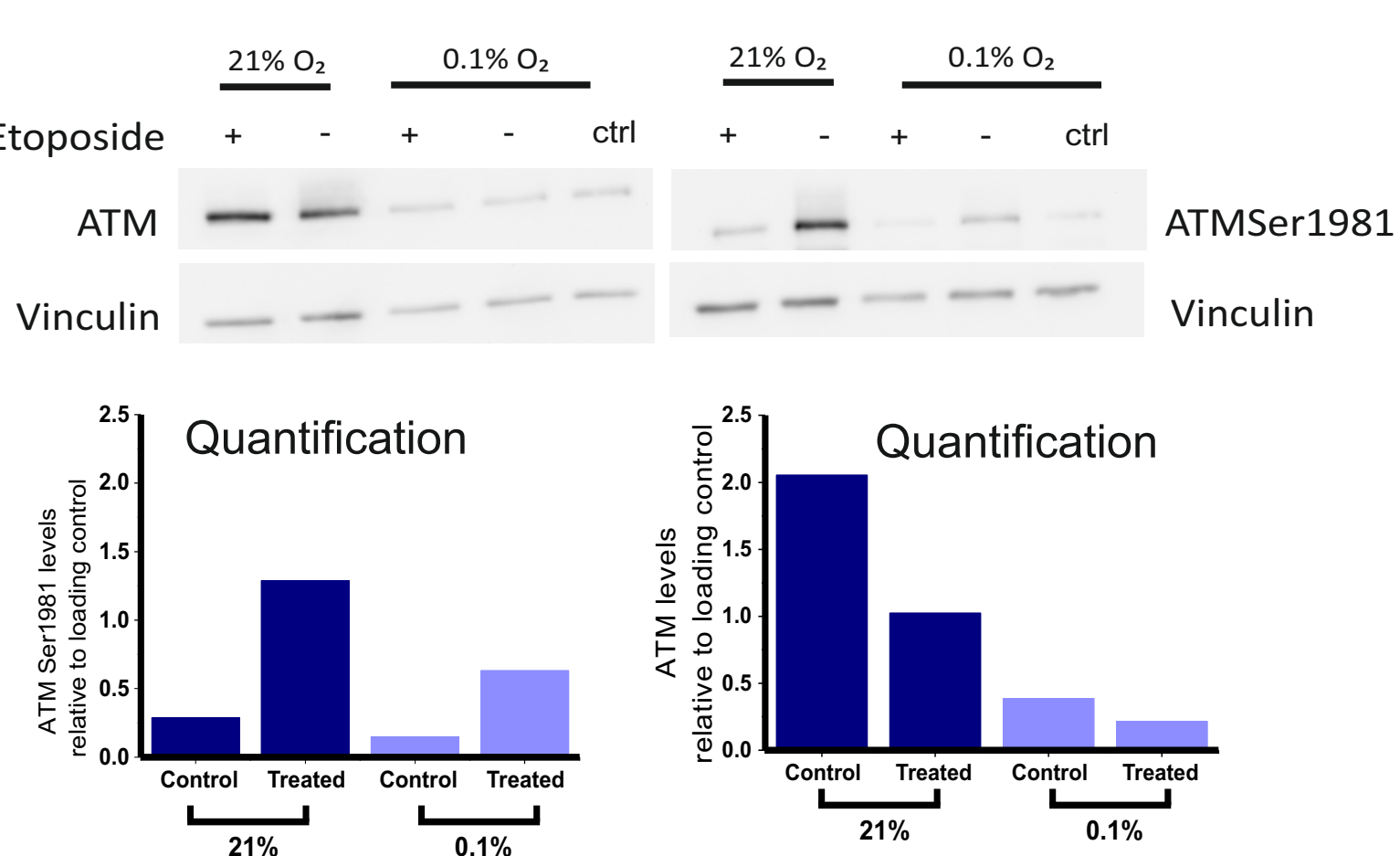


Hypoxia causes **significant down-regulation** of NBN and MRE11 at the mRNA and protein level in D283-MED.

How are Double Strand Breaks Detected and Repaired?

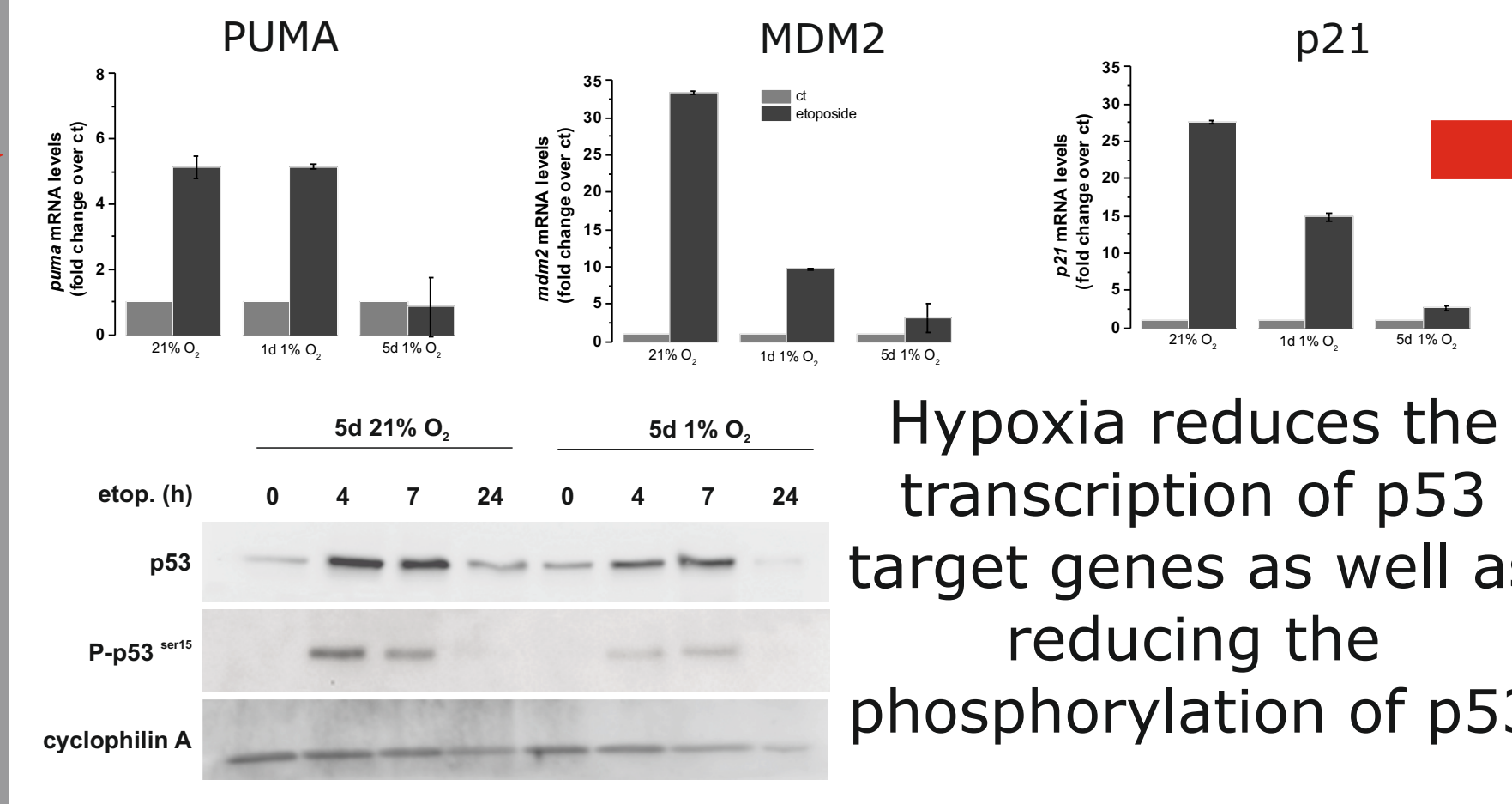


Hypoxia Reduces ATM Activation in MB



Hypoxia **decreases** the signal mediator ATM, and its phosphorylated active form, in hypoxic MB cells

p53 Activity is Dampened in Hypoxia



Hypoxia **reduces** the transcription of p53 target genes as well as reducing the phosphorylation of p53

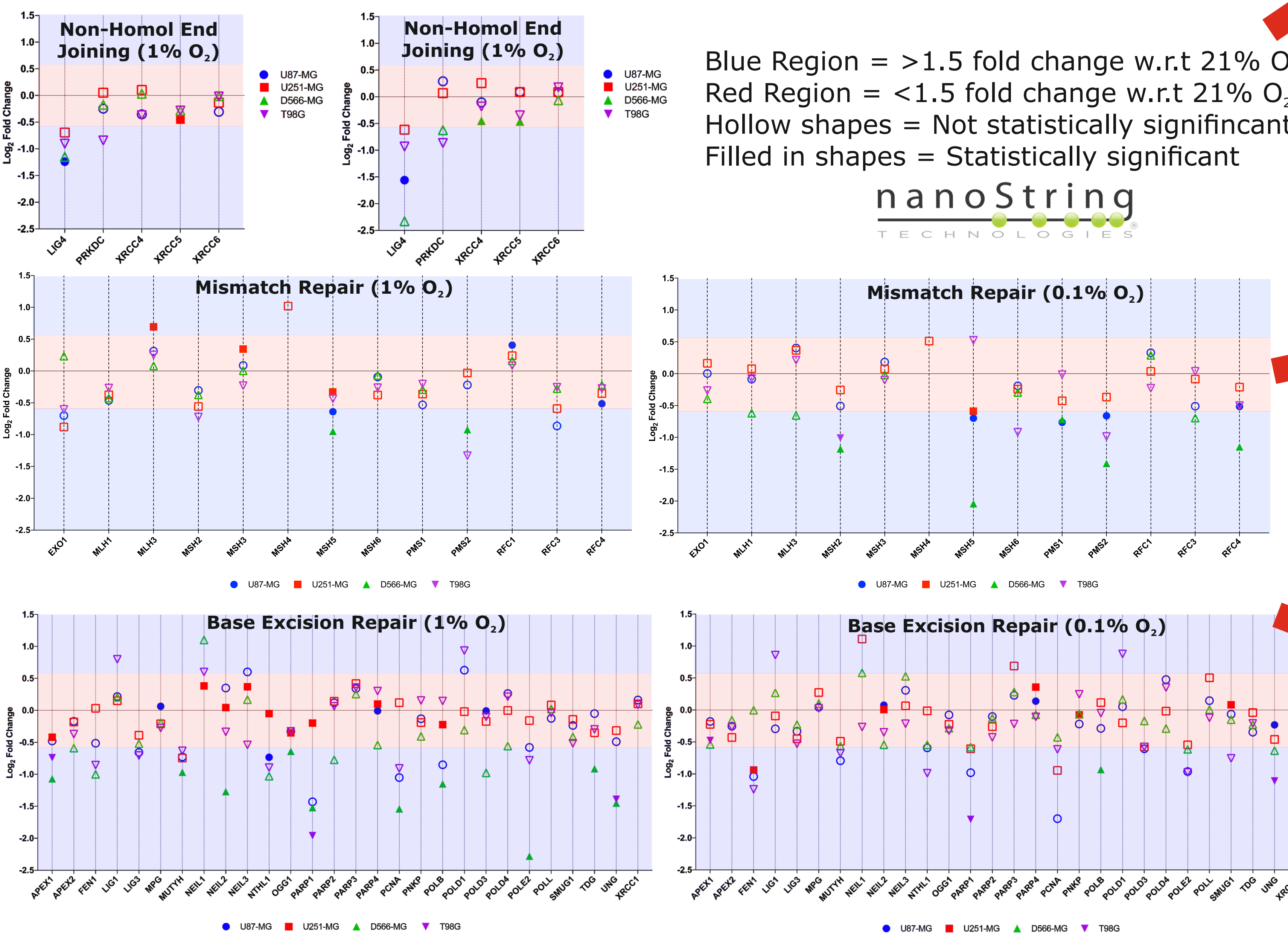
Mechanism 1 Conclusions

Take Home Message: Chronic hypoxia causes a down-regulation of DNA damage sensing proteins NBN and MRE11, as well as reduced activation of ATM. This results in dampening of p53 activity thus cells are more resistant to treatment in hypoxia
However..... Similar mechanisms were not found in all cell lines tested, so further investigation into the effects of hypoxia on DNA repair was undertaken.

Mechanism 2: Down-regulation of multiple repair pathways in GBM...

Investigating DNA Repair Gene Expression in GBM

The expression of 180 DNA repair genes was evaluated in 4x GBM cell lines incubated in 21% O₂, 1% O₂ and 0.1% O₂. A NanoString approach was chosen for this.

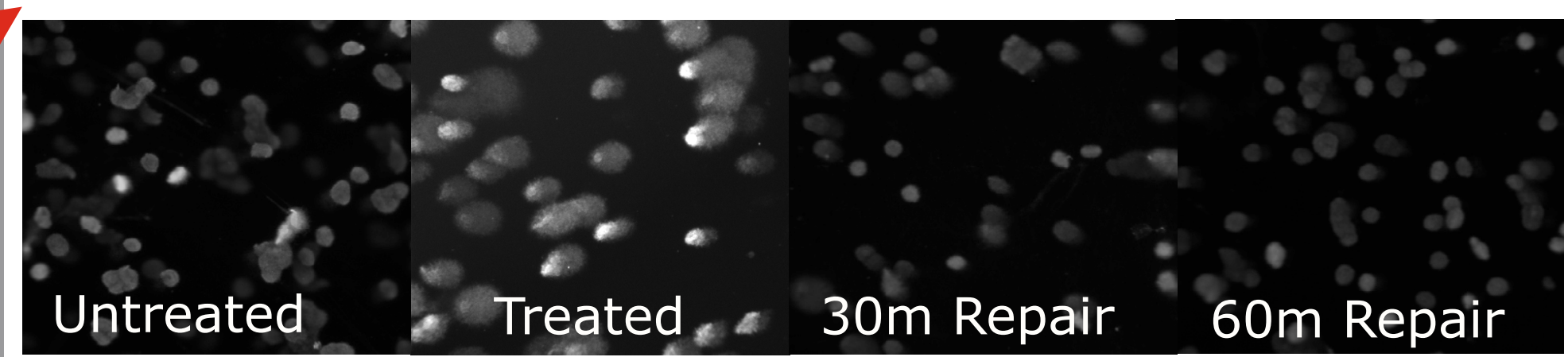


Blue Region = >1.5 fold change w.r.t 21% O₂
Red Region = <1.5 fold change w.r.t 21% O₂
Hollow shapes = Not statistically significant
Filled in shapes = Statistically significant



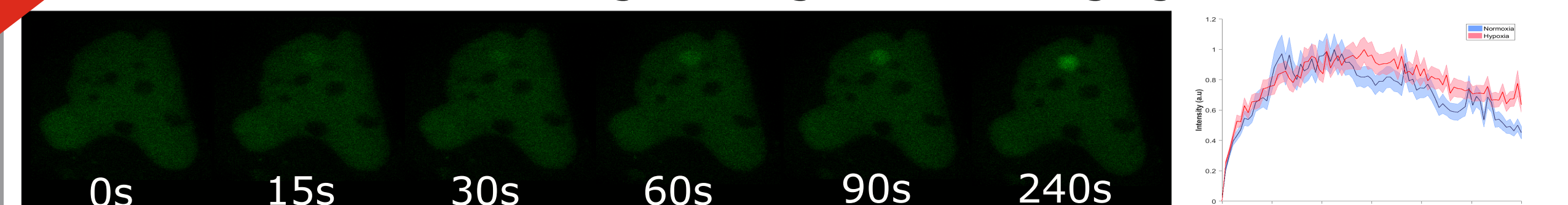
Measuring Repair Kinetics

Using the Comet Assay we can measure the kinetics of repair. We can compare the repair kinetics of cells incubated in normoxia and hypoxia



Measuring kinetics of DNA Repair Protein Recruitment

We have optimised a multi-photon based protocol for the induction of DNA damage during live cell imaging.



We can measure the kinetics of DNA repair protein recruitment and compare rates between normoxia and hypoxia

Mechanism 2 Conclusions

Main Take Home Messages:

- Hypoxia impacts the expression of numerous DNA repair genes from BER, MMR and NHEJ
- Increasing severity of hypoxia strengthens the regulation of DNA repair genes
- We can use advance confocal microscopy with a laser damage approach as well as traditional biochemical methods to study the impact of these changes.

To be continued.....