Supplementary Materials

The hypoxic tumor microenvironment *in vivo* selects the tumor cells with increased survival against genotoxic stresses.

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Supplementary Fig. 1. Association of the EGFP$^+$ tumor cells with hypoxia in xenografts. (A) Localization of the EGFP$^+$ tumor cells in the perinecrotic zones in a xenograft. The outer edge of the xenograft tumor is to the right. The necrotizing region is to the left of the broken line. (B) Cell surface expression of the hypoxia marker carbonic anhydrase 9 (CA) in the freshly *ex vivo* isolated tumor cells by FACS analysis with *in vitro* hypoxia (1% O_2, 16 hr)-treated parental cells as the positive control.

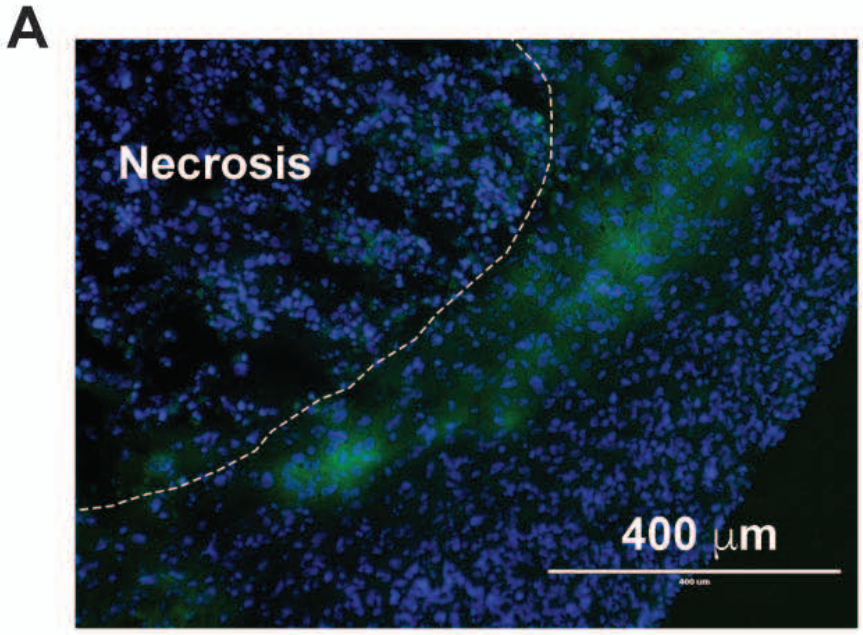
Supplementary Fig. 2. Increased survival of the hypoxic (EGFP$^+$) tumor cells after either in vivo or in vitro γ-irradiation. (A) Xenograft tumors were γ-irradiated at 15 Gy. The hypoxic (EGFP$^+$) and non-hypoxic (EGFP$^-$) tumor cells were sorted and plated at 1 x 10^5 cells/well in 6-well plates immediately for the clonogenic survival assay. (B) The ex vivo tumor cells maintained under the ambient condition for <5 passages (approximately 14 days) and were γ-irradiated at 15 Gy. The irradiated cells were plated at 1 x 10^5 cells/well in 6-well plates for the clonogenic survival assay. After 12 days of culture, surviving tumor cells were stained with Crystal Violet and examined under microscope. Representative images of cells are shown. Bar: 1,000 μm.

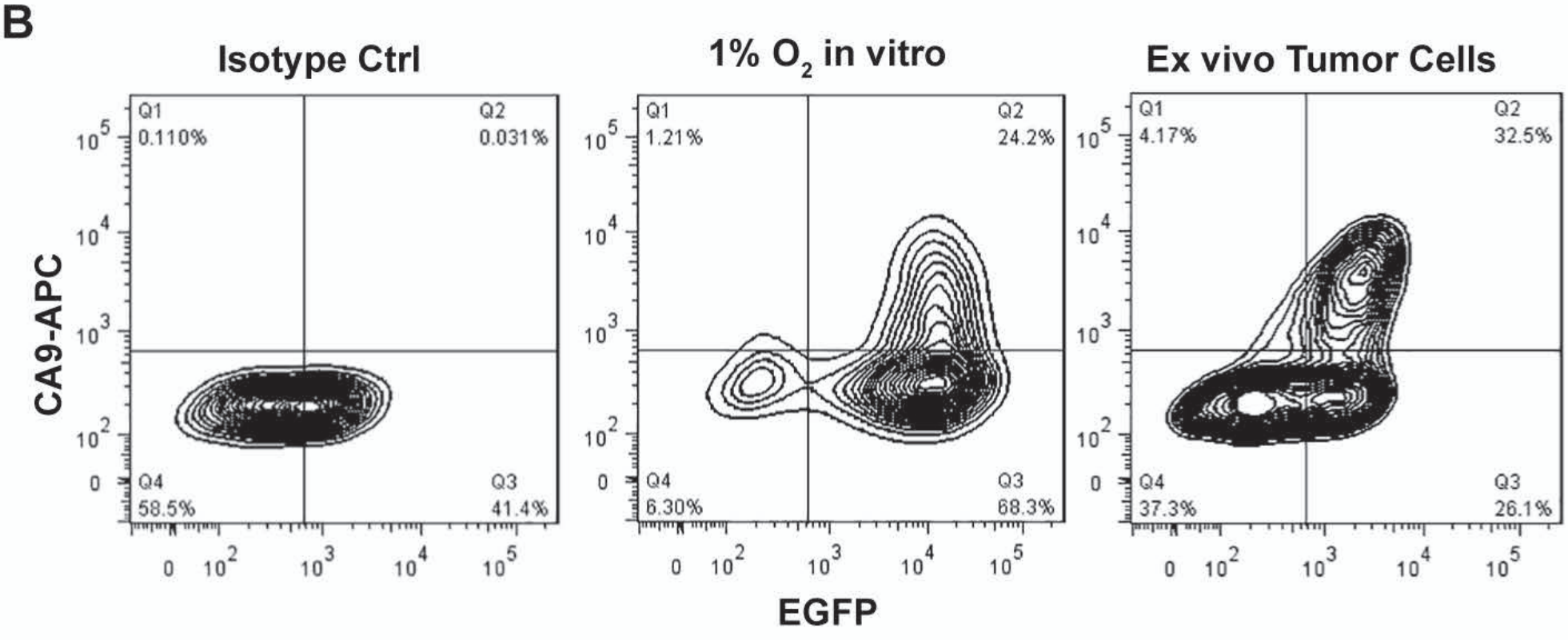
Supplementary Fig. 3. The ex vivo hypoxic tumor cells maintain enhanced clonogenic survival and DNA damage repair potential in response to γ-irradiation under the

conventional culture conditions. The hypoxic ($EGFP^+$) and non-hypoxic ($EGFP^-$) tumor cells were sorted from xenograft tumors and cultured under the ambient condition for <5 passages (approximately 14 days) before γ-irradiation, as described in Figure 3A. The *ex vivo* tumor cells were γ-irradiated at 7 Gy and then cultured under the ambient condition for 24 or 48 hr before they were fixed for detection of nuclear foci by immunofluorescence with anti-53BP1 (A) or anti-γH2AX antibodies (B). Cells with >6 nuclear foci were scored in ten (n=10) randomly selected microscopic fields except n=8 for $53BP1^+$ $EGFP^+$ cells at 48 hr (A) and n=9 for $\gamma H2AX^+$ $EGFP^-$ cells at 48 hr (B).

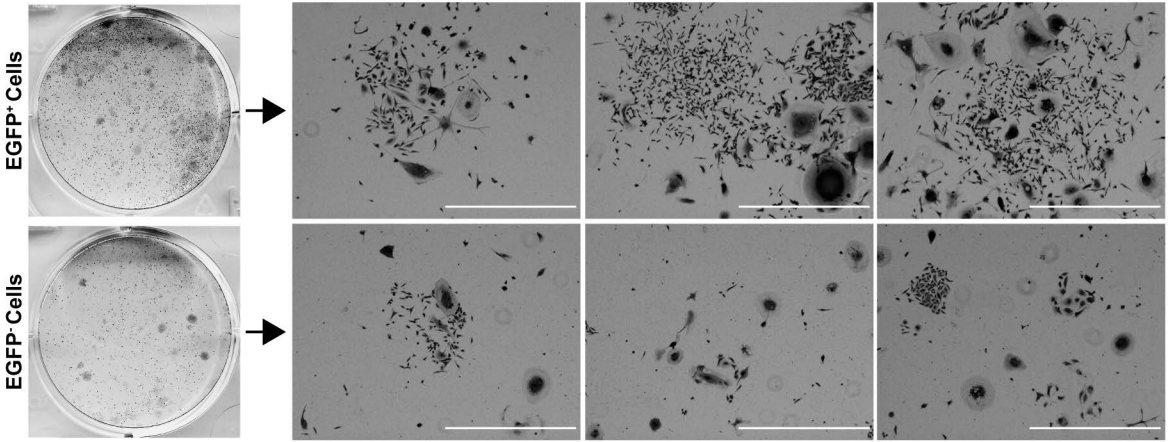
Supplementary Fig. 4. CHKi inhibits activation of the CHK pathway. The hypoxic ($EGFP^+$) and non-hypoxic ($EGFP^-$) tumor cells were treated with 20 μg/ml bleomycin in the presence or absence of the checkpoint kinase (CHK) inhibitor AZD7762 (20 nM). Serine-15 (S15) phosphorylation of p53 tumor suppressor served as a readout of CHK activation. Robust p-p53-S15 was observed in the $EGFP^+$ cells only (lane 3), which was suppressed by AZD7762 (lane 2).

Supplementary Fig. 5. CHKi does not affect the clonogenic growth of the parental cells that are never exposed to in vivo tumor microenvironment. (A) The parental MDA-MB-231-HRE-EGFP cells were treated with the checkpoint kinase (CHK) inhibitor AZD7762 (20 nM) under ambient culture condition (normoxia) or at 1% O_2 (hypoxia) for 24 hr and then were plated at 1,000 cells per well in 6-well plates for clonogenic growth. (B)The parental cells were first treated as in (A) before they were γ-irradiated at 5 Gy. The irradiated cells were immediately plated at 6,000 cells per well in 6-well plates for clonogenic growth.

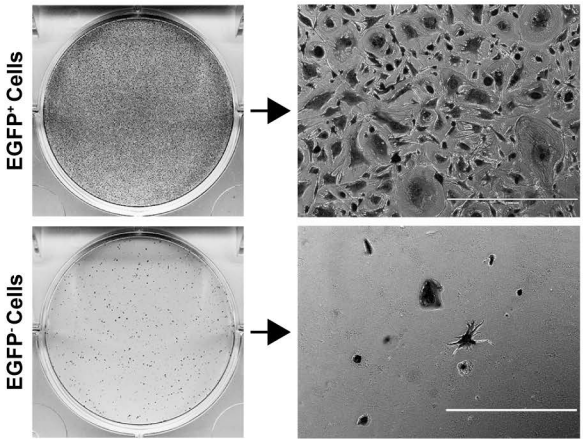

A
Necrosis
400 μm

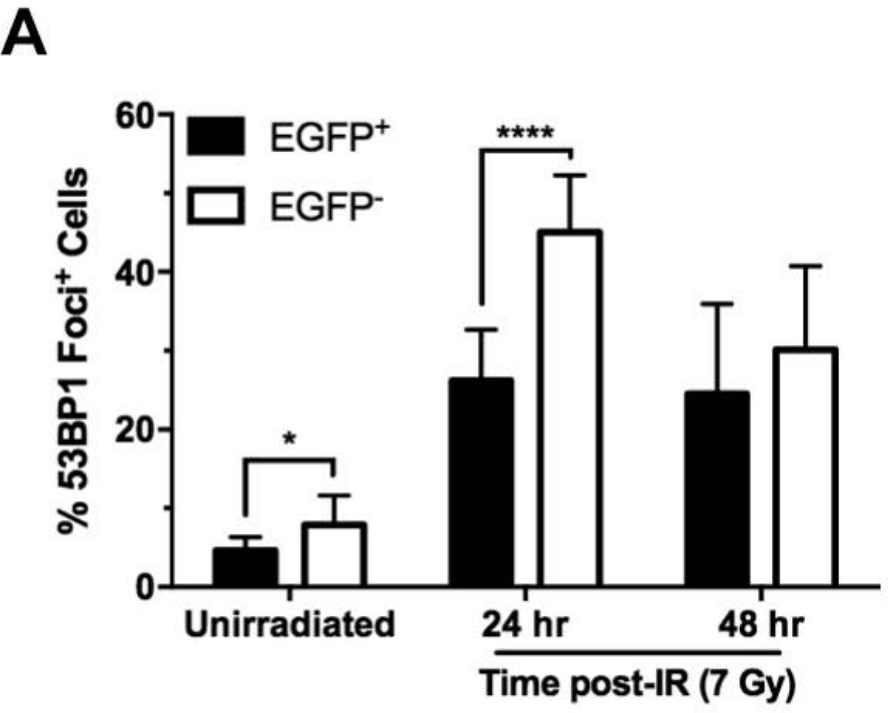

B
Isotype Ctrl
1% O2 in vitro
Ex vivo Tumor Cells
CA9-APC
EGFP
Q1
0.110%
Q2
0.031%
Q4
58.5%
Q3
41.4%
Q1
1.21%
Q2
24.2%
Q4
6.30%
Q3
68.3%
Q1
4.17%
Q2
32.5%
Q4
37.3%
Q3
26.1%

A: Irradiation *in vivo*



B: Irradiation *in vitro*




A
60
40
20
0
EGFP+
EGFP-
% 53BP1 Foci+ Cells
*
****
Unirradiated
24 hr
48 hr
Time post-IR (7 Gy)

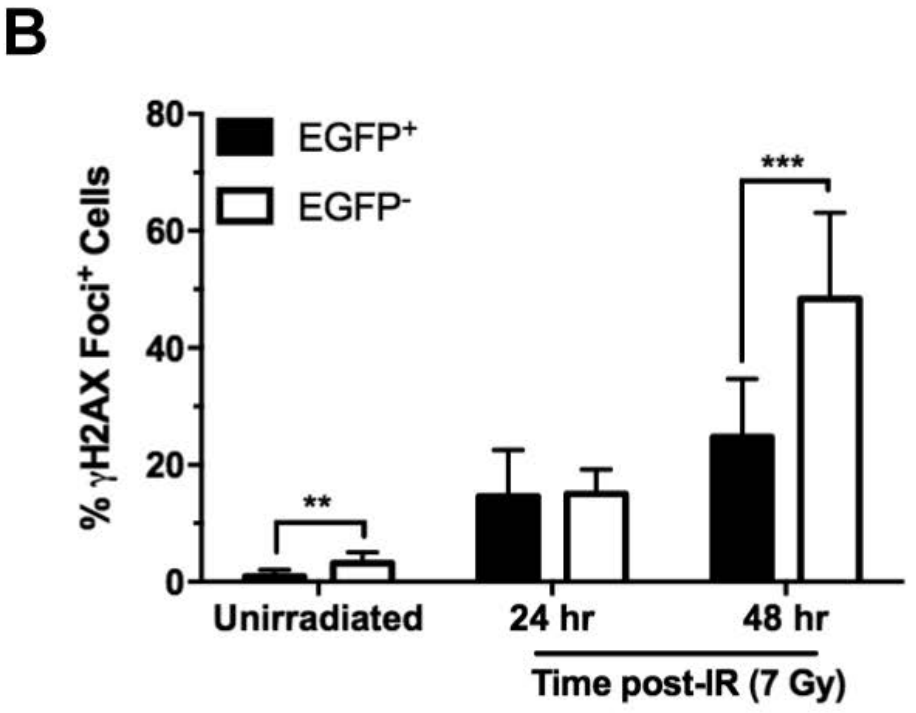
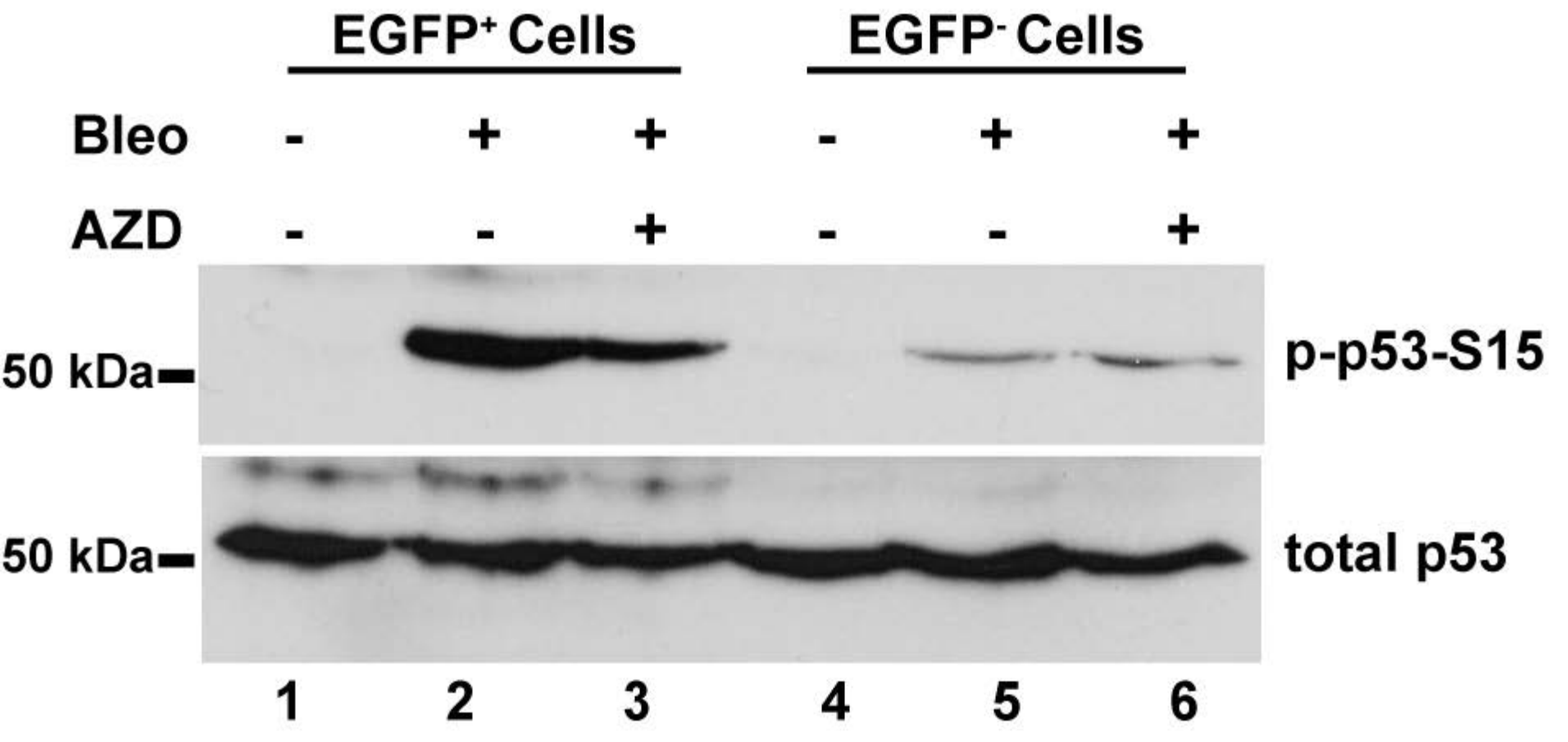
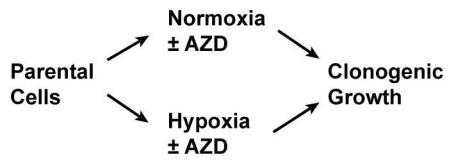
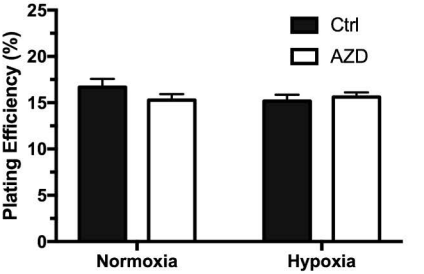

B
80
60
40
20
0
EGFP+
EGFP-
% γH2AX Foci+ Cells
**
***
Unirradiated
24 hr
48 hr
Time post-IR (7 Gy)

Fig. s4

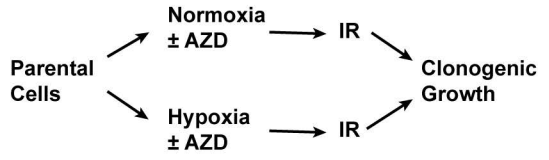

EGFP+ Cells
EGFP- Cells
Bleo - + + - + +
AZD - - + - - +
50 kDa
p-p53-S15
50 kDa
total p53
1 2 3 4 5 6

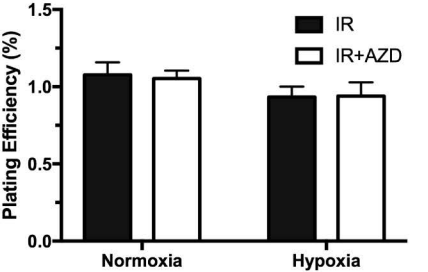
A


Parental Cells
Normoxia ± AZD
Hypoxia ± AZD
Clonogenic Growth


Plating Efficiency (%)
25
20
15
10
5
0
Ctrl
AZD
Normoxia
Hypoxia

B


Parental Cells
Normoxia ± AZD
Hypoxia ± AZD
IR
IR
Clonogenic Growth


Plating Efficiency (%)
1.5
1.0
0.5
0.0
IR
IR+AZD
Normoxia
Hypoxia