Supplemental Figure Legends

Figure S1: Mutation pattern remains unchanged upon miR-155 over-expression in vivo. Mutants were isolated, PCR amplified, sequenced, and analyzed for transition/transversion or insertion/deletion mutations. Results are reported as a percentage of total mutants sequenced.

Figure S2: Validation of DSB repair assays

Matched pair control cell lines with known DSB repair phenotypes: xrs6 and xrs6 + Ku80, DLD1 and DLD1 BRCA2 knockout, PEO1 and PEO1 C4-2 BRCA2 complemented, were analyzed for (A) NHEJ pathway activity and (B) HR pathway activity using luciferase based DSB reporters. (C) Western bot analysis of FOXO3a following pre-miR-155 transfection and confirmation of siRNA targets. (D) Effect on miR-155, siBRCA2, siRAD51 and siXRCC4 on the U2OS DR-GFP assay for HR. (E) Mutagenic end-joining analysis using the U2OS DR-EJ cells. MMEJ analysis by host cell reactivation of pEJ2 comparing (F) xrs6 cells to xrs6 + Ku80, (G) 155 KI MEFs to wild type controls, and (H) BICKO MEFs.

Figure S3: Confirmation of polymerase delta mRNA down-regulation

qRT-PCR analysis of polymerase delta subunit mRNA levels in AV16 155 and AV16 NTC cells

Figure S4: NHEJ activity is unchanged upon FOXO3a knockdown in AV16 cells

NHEJ pathway activity in AV16 cells after siRNA knockdown of FOXO3a

Figure S1

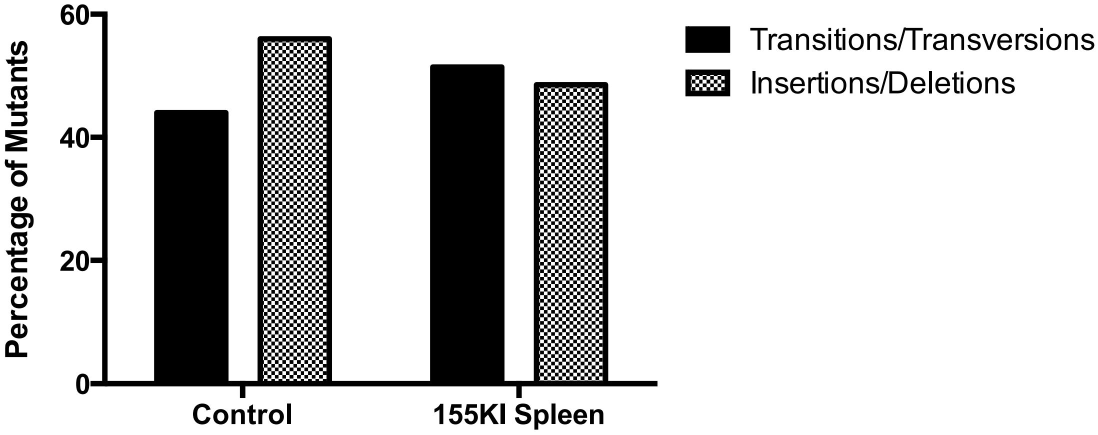

Percentage of Mutants
60
40
20
0
Control
155KI Spleen
Transitions/Transversions
Insertions/Deletions

Figure S2

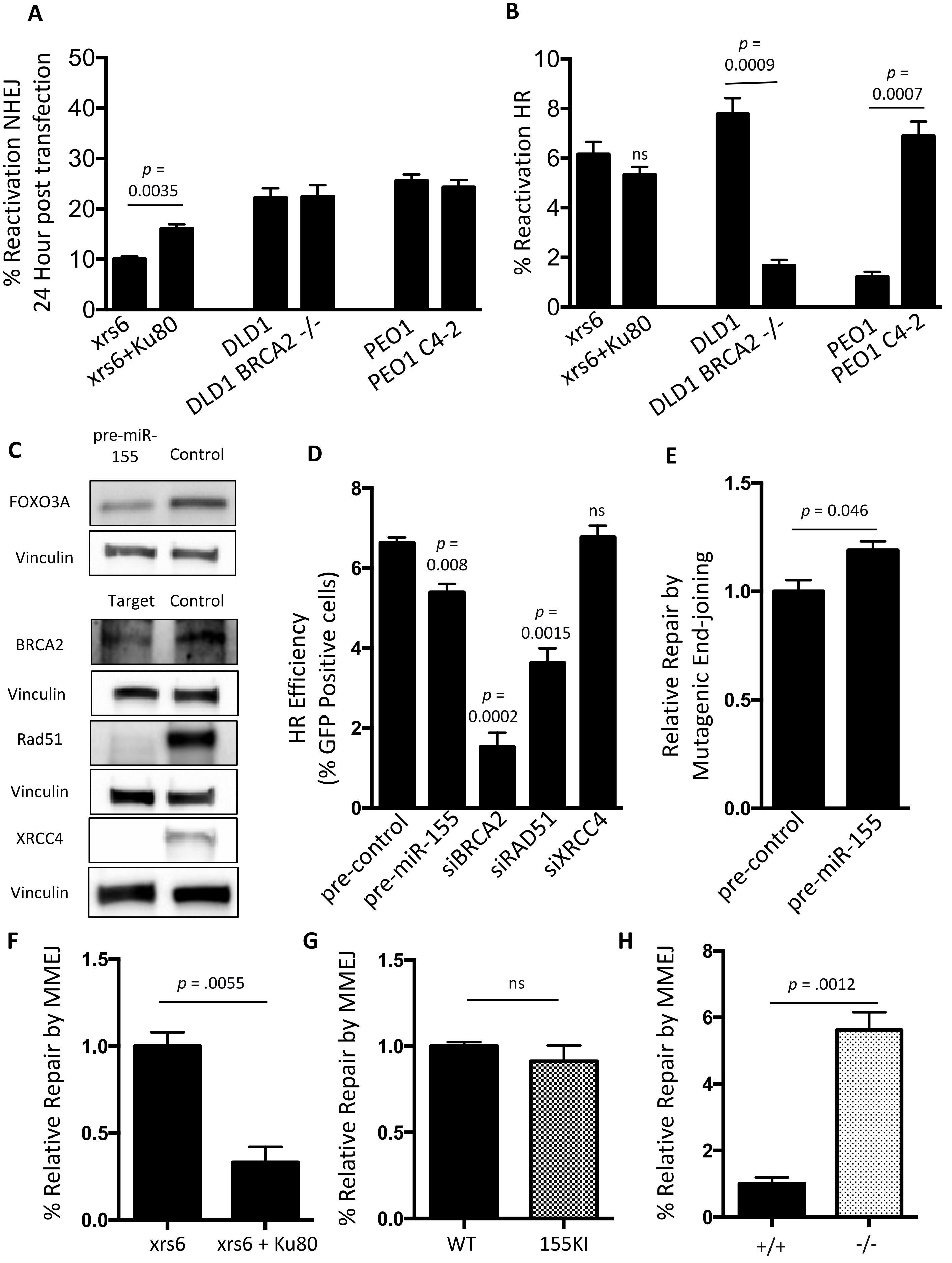
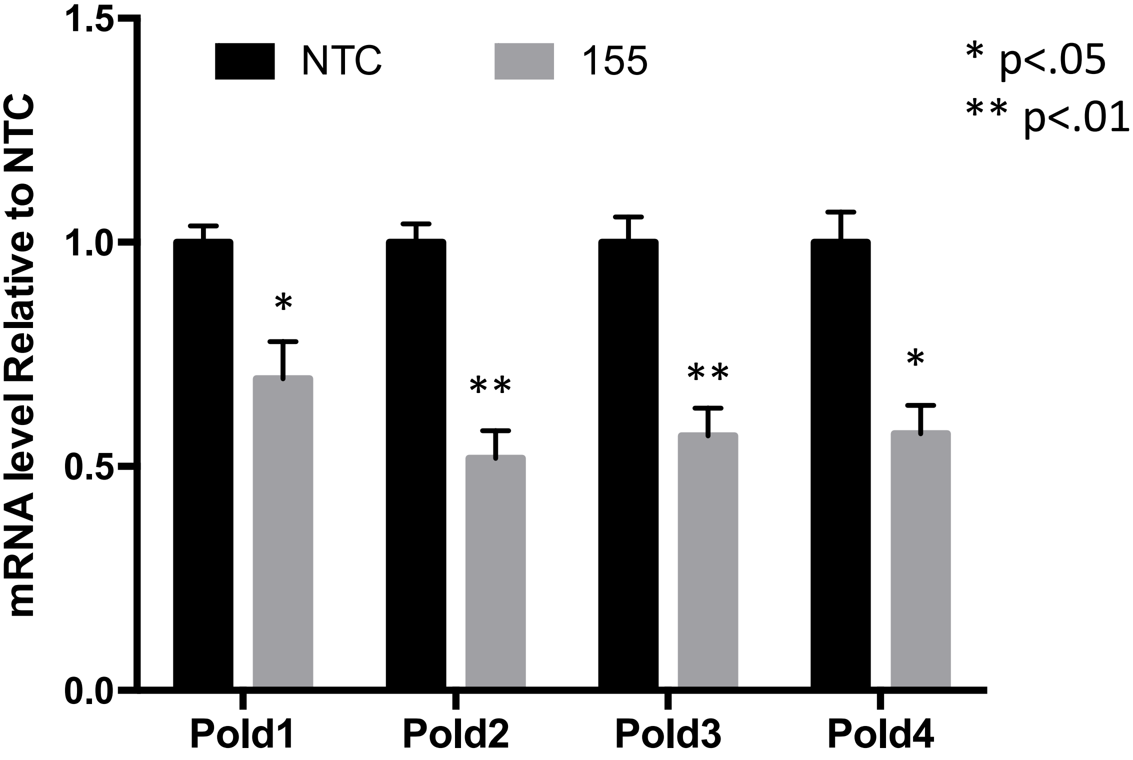

A
% Reactivation NHEJ
24 Hour post transfection
50
40
30
20
10
0
p = 0.0035
xrs6
xrs6+Ku80
DLD1
DLD1 BRCA2 -/-
PEO1
PEO1 C4-2
B
% Reactivation HR
10
8
6
4
2
0
ns
p = 0.0009
p = 0.0007
xrs6
xrs6+Ku80
DLD1
DLD1 BRCA2 -/-
PEO1
PEO1 C4-2
C
pre-miR-155
Control
FOXO3A
Vinculin
Target
Control
BRCA2
Vinculin
Rad51
Vinculin
XRCC4
Vinculin
D
HR Efficiency
(% GFP Positive cells)
8
6
4
2
0
p = 0.008
p = 0.0002
p = 0.0015
ns
pre-control
pre-miR-155
siBRCA2
siRAD51
siXRCC4
E
Relative Repair by
Mutagenic End-joining
1.5
1.0
0.5
0.0
p = 0.046
pre-control
pre-miR-155
F
% Relative Repair by MMEJ
1.5
1.0
0.5
0.0
p = .0055
xrs6
xrs6 + Ku80
G
% Relative Repair by MMEJ
1.5
1.0
0.5
0.0
ns
WT
155KI
H
% Relative Repair by MMEJ
8
6
4
2
0
p = .0012
+/+
-/-

Figure S3



mRNA level Relative to NTC
1.5
1.0
0.5
0.0
NTC
155
* p<.05
** p<.01
*
**
**
*
Pold1
Pold2
Pold3
Pold4

Figure S4