Supplemental Methods.

λ-phosphatase treatment of ovaries or cells

P0.5 ovary pretreated with indicated doses of calyculin A (CA) for two hours and irradiated with 0.45Gy, 4.5Gy were lysed in 35μl NP40 lysis buffer (20mM Tris HCl, pH8.0, 137mM NaCl, 10% glycerol, 1% Triton X-100, 2mM EDTA, 1 mM DTT, 10 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin). After lysis, 10X λ–phosphatase buffer (50mM Tris-HCl, 0.1mM Na2EDTA, 5mM DTT and 0.01% Brij35 MnCl2) was added to a final 1X concentration and lysates were also supplemented with 2mM MnCl2. λ–phosphatase (8000U) was added or omitted in negative controls. Total reaction volume of 40μl was incubated for 30°C for 15min. The reaction was stopped by adding 5X SDS sample buffer to final 1X concentration and heating at 95°C for 5 min. before SDS-PAGE analysis.

Supplemental Figures

FIG. S1. The full array of oocytes from a representative section of P0.5 ovary showing relative levels of TAp63α expression and γ-H2AX foci. (A) TAp63α expression in P0.5 vs. P5 ovary sections shown in Fig. 1A. TAp63α expression level is more heterogeneous and overall lower in P0.5 oocytes than in P5 oocytes. (B) All oocytes in (A) are numbered and arrayed according to the level of TAp63α expression, and the corresponding number of γ-H2AX foci in each oocyte are shown. p63 (red), γ-H2AX (green), DAPI (blue) and overlay (ovl) of all three stains are shown. γ-H2AX foci detected using Image J are marked by crosses, which were counted and plotted as shown in Fig. 1B. See also Fig. S2. Scale bar, 50mm

FIG S2. Enlarged view of γ-H2AX foci in non-irradiated P0.5 oocytes. (A) Same section as shown in Fig. 1A (P0.5) and Fig.S1, except only the green channel showing γ-H2AX staining is shown here. Oocytes numbered according to the level of TAp63α expression, as described in Fig. 1A and S1. Oocytes labeled with yellow numbers are magnified in (B). (B) Enlarged view of P0.5 ovary section shown in (A) with arrows pointing to representative oocytes magnified in lower panels. Foci detected using Image J are displayed with crosses. The full array of oocytes are shown in Fig. S1B.

FIG. S3. P0.5 oocytes survive normally lethal doses of IR treatment. (A) Representative ovary sections of P7 mice that were either not irradiated (left panels) or irradiated with 0.45Gy at P0.5 (middle panels) or at P5 (right panels). Lower panels are magnified image of boxed region in upper panels. Red arrows, primordial follicle oocytes; blue arrowheads, growing oocytes in primary or secondary follicles. (B) Percent oocyte survival in ovary of P0.5 or P5 mice irradiated with 0.45Gy IR and examined at P7. Up to 40% of P0.5 primordial follicle oocytes may survive. This set of newborn mice were examined immediately after birth, which showed a higher level of survival compared to newborn mice that were born before dusk and analyzed later during the day (Fig. 2B).

FIG. S4. Western blot showing the level of IR-induced TAp63α phosphorylation in P2 ovary lysates irradiated with 4.5Gy and examined two hours after treatment. Phosphorylation shift is induced (double arrowhead). However, a fraction remains incompletely shifted (single arrowhead). Arrow, unshifted TAp63α in non-irradiated ovary.

FIG S5. The level of IR-induced TAp63α phosphorylation in newborn oocytes upon high dose 20 Gy IR treatment in vitro vs in vivo and comparison with P5 oocytes irradiated with lower dose of 4.5Gy IR. All ovaries come from mice of the same litter. IR-induced TAp63α phosphorylation is induced only partially after 20Gy IR in P0.5 oocytes whether

irradiated *in vivo* (by exposing mice to whole body irradiation), or *in vitro* (by dissecting ovaries first and irradiating them *ex vivo* in culture) compared to P5 mice irradiated with 4.5Gy IR.

FIG S6. IR-induced TAp63α phosphorylation is blocked by ATM inhibitor, KU55933, but not by DNA-PKcs inhibitor, NU7441. (A) Immunoblot showing the level of IR-induced ATM Ser1981 phosphorylation (upper portion of blot) and TAp63α phosphorylation shift (lower portion of blot) in DMSO- or KU55933-pretreated and 4.5Gy-irradiated P5 ovaries examined two hours after IR treatment. Left panel, light immunoblot exposure to film. Right panel, dark exposure. Arrow, non-shifted TAp63 α; single arrowhead, low shift TAp63α; double arrowheads, high shift TAp63α. (B) Immunoblot showing the level of IR-induced ATM Ser1981 phosphorylation (upper panel) and TAp63α phosphorylation shift (middle panel) in KU55933- or NU7441-pretreated and 4.5Gy-irradiated 293T cells transduced with TAp63α lentivirus two hours after IR treatment. Tubulin, loading control (lower panel). (C) IR-induced ATM Ser1981 phosphorylation and TAp63α phosphorylation induction in P5 ovaries pretreated with KU55933 or NU7441 at indicated doses. Ovaries examined two hours after IR treatment. Tubulin, loading control.

FIG S7. Determining levels of ATM activation in P0.5 and P5 oocytes is confounded by

follicle cells(A) Immunofluorescence of 4.5Gy- or 0.45Gy-irradiated P5 ovary section two hours after IR treatment stained for γ-H2AX. γ-H2AX foci formation is strongly induced in oocytes than surrounding follicle cells, suggesting DNA damage response in oocytes could be predominant in the ovary. Red boxed region magnified at right. Yellow arrowhead, oocyte; blue arrowheads, follicle cells). (B) Immunoblot analysis showing the levels of activated ATM using phospho-specific Ser1981 mAb and TAp63α phosphorylation shift in lysates of ovary from irradiated P0.5 and P5 mice. One ovary loaded per lane. The level of activated ATM appeared more weakly induced in P0.5 ovaries than P5 ovaries. However, though the number of oocytes in the newborn P0.5 vs. P5 ovaries are not considerably different (3), folliculogenesis has not yet begun in the P0.5 ovary. Thereby, P0.5 ovary has fewer follicle cells, and thus, fewer total cells than the P5 ovary. (C) Loading two-fold more P0.5 ovary lysate than P5 ovary lysate normalized tubulin levels. The level of activated ATM signal appeared no different between irradiated P0.5 and P5 ovaries. (D) The level of IR-induced phospho-ATM activation (1st row blots), TAp63α phosphorylation shift (2nd row) γ-H2AX (3rd row) and tubulin (4th row) in P0.5 vs. P5 ovary lysates. No detectable changes in γ-H2AX levels were found following 0.45Gy IR when analyzed by Western blot despite detectable induction of γ-H2AX foci in oocytes when examined by immunofluorescence (Fig. S5C, Fig. 5A). Thus, changes in oocyte activity appear masked by an follicle cells that outnumber oocytes. Likewise, activated ATM levels shown in irradiated P0.5 and P5 ovary

lysates (upper panels) may represent ATM activation in follicle cells rather than oocytes. (E) The level of IR-induced ATM Ser1981 phosphorylation and TAp63α phosphorylation after ovaries are depleted of primordial follicle oocytes. P5 ovaries were either not irradiated (lanes 1 and 2) or irradiated with 0.45Gy IR (lanes 3 to 5) to eliminate primordial follicle oocytes. At P8, control ovaries not depleted of oocytes were treated with either 0Gy or 0.45Gy, confirming the presence of TAp63α expression and IR-induced TAp63α phosphorylation (middle panel) two hours after IR treatment(lanes 1 to 2). When P5 were depleted of primordial follicle oocytes by 0.45Gy IR pretreatment (shown by loss of TAp63α expression at P8) and irradiated a second time with 0.45Gy or 4.5Gy at P8 and examined two hours later, significant levels of activated ATM appeared (lanes 4 and 5), which indicated activated ATM signal on immunoblots of total ovary lysates is largely contributed by follicle cells rather than oocytes.

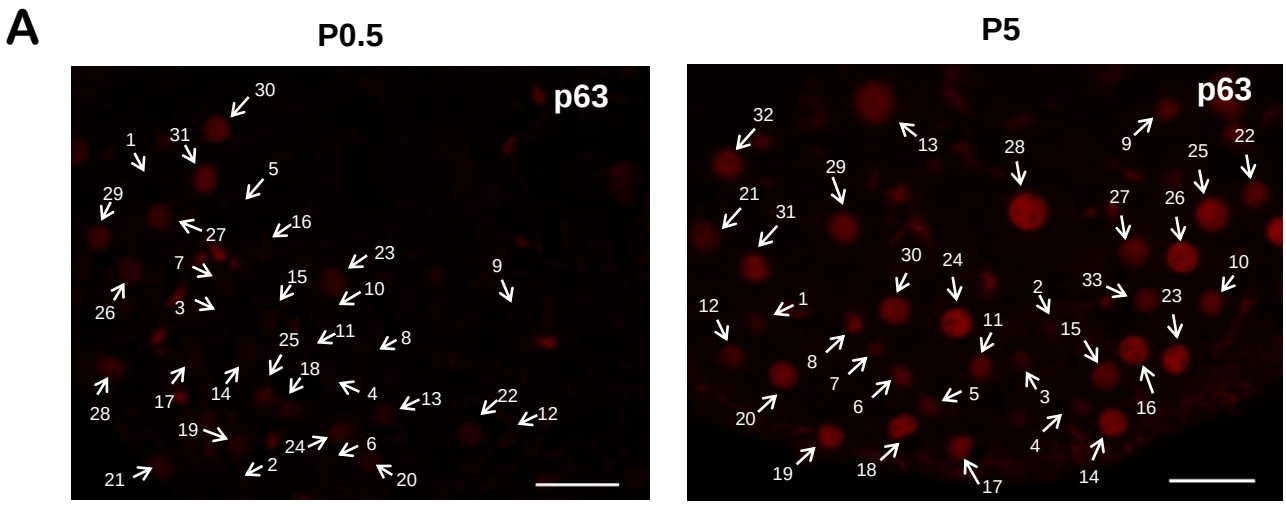
FIG S8. Dot plots showing the number of γ-H2AX foci before and after 0.45Gy IR treatment in P0.5 oocytes lacking (P0.5, p63-) or having (P0.5, p63+) TAp63α expression or P5 oocytes, which all express TAp63α. The mean number of foci under each condition are shown by black bars (also arrows). Each dot represents γ-H2AX foci counts in one oocyte under the different conditions. Some of the dots are heavily congested and overlapping, and thereby appear masked [i.e. P5 p63 (+), 0Gy). Error bars, ±SEM. *** $p<0.001$

(Mann-Whitney Test). P0.5 p63(-) 0Gy, 4 mice, 40 oocytes; P0.5 p63(-) 0.45Gy, 4 mice, 97 oocytes; P0.5 p63(+) 0Gy, 4 mice, 87 oocytes; P0.5 p63 (+) 0.45Gy, 2 mice, 95 oocytes; P5 p63 (+) 0Gy, 2 mice, 212 oocytes; P5 p63+ 0.45Gy, 2 mice, 113 oocytes.

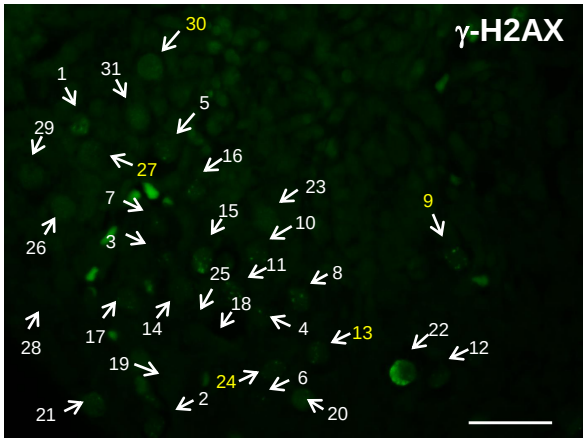
FIG S9. Immoblots showing the level of IR-induced TAp63α phosphorylation in P0.5 oocytes with Ser/Thr phosphatase inhibitor, Calyculin A (CA), pretreatment and inhibitors of ATM or DNA-PKcs kinases. Ovaries of mice numbered in each immunoblot come from one litter. Brackets mark contralateral ovaries from each mice. Double arrowhead, high shift phosphorylation; triple arrowhead, superhigh shift phosphorylation. Tubulin, loading control. (A) IR induced TAp63α high and super high shift bands are blocked by 150μM KU55933 pretreatment, suggesting shifts are ATM-mediated modifications. Note IR-induced TAp63α shift is similarly deficient in newborn littermates, but particularly deficient in mouse #3 probably due to developmental delay (see Fig. 6C and text). (B) IR-induced TAp63α high (double arrowhead) and super high shift (triple arrowheads) TAp63α bands (lane 7) induced in P0.5 oocytes are sensitive to λ-phosphatase treatment (lane 8), suggesting they are phosphorylation modifications.

FIG S10. Non-irradiated CA-pretreated ovaries showed faint TAp63α band shift in P0.5 ovary lysate (lane 2, triple arrowheads), suggesting low level activation possibly by intrinsic

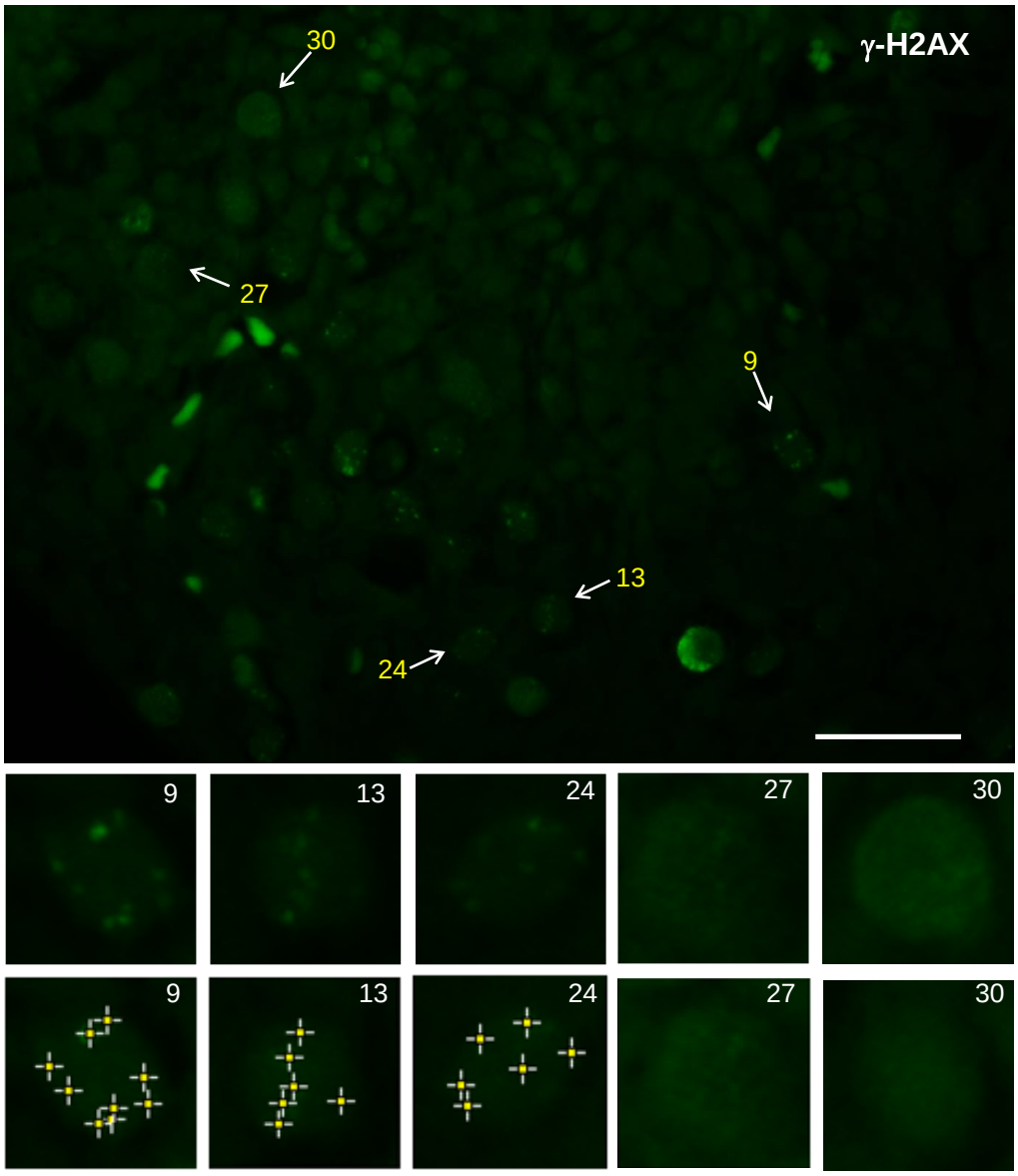
meiotic DNA DSBs.


A
P0.5
p63
P5
p63
B
ovI
p63
DAPI
γ-H2AX
γ-H2AX

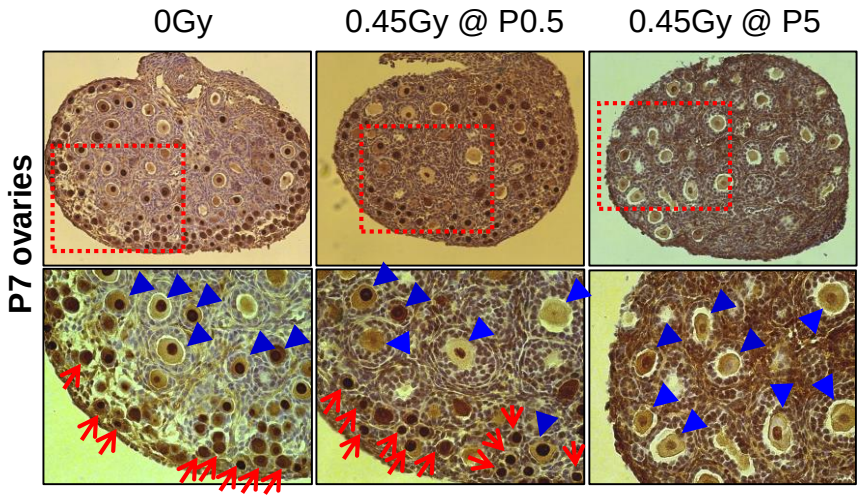
A


P0.5, 0Gy
γ-H2AX
30
1
31
5
29
27
16
23
7
15
9
26
3
10
25
11
8
18
28
17
14
4
13
22
12
19
24
6
21
2
20

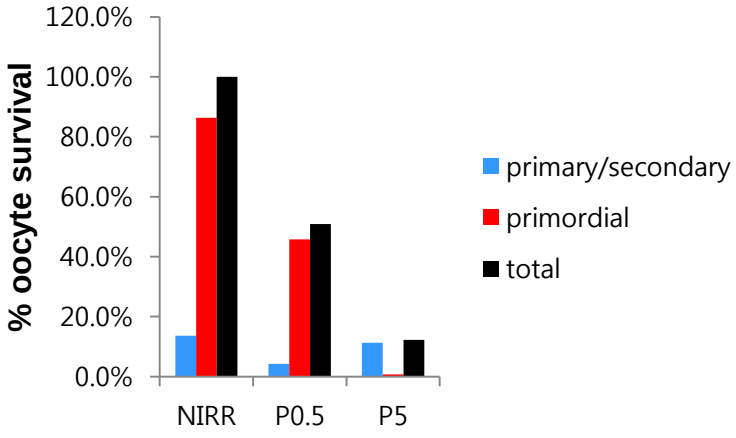
B

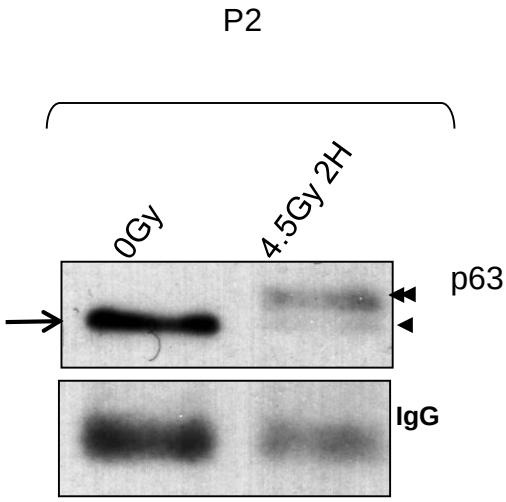

γ-H2AX
30
27
9
13
24
9
13
24
27
30
9
13
24
27
30

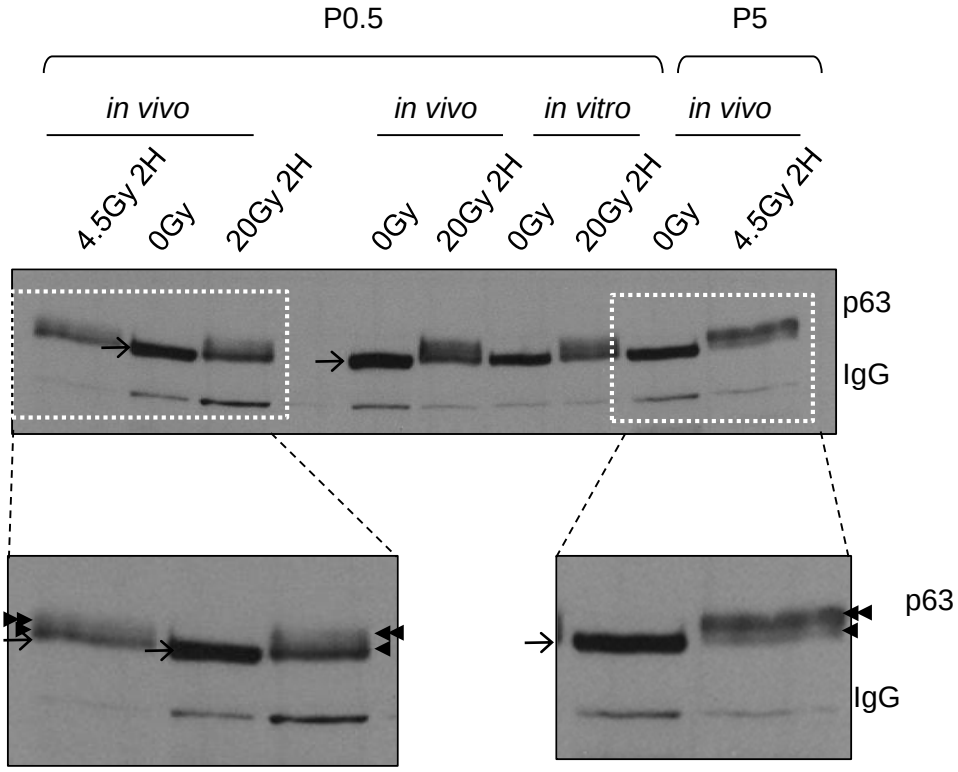
A

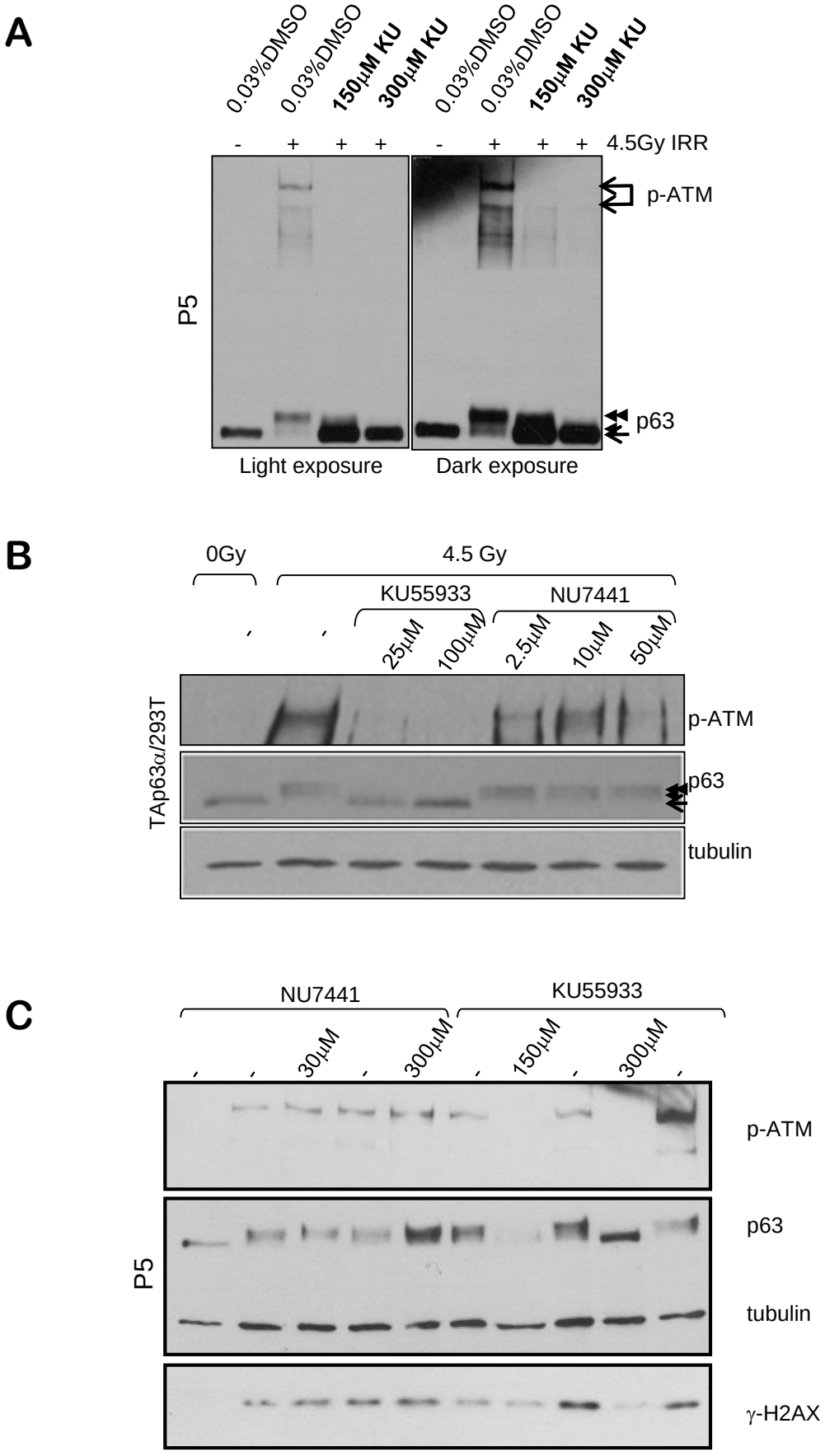

0Gy
0.45Gy @ P0.5
0.45Gy @ P5
P7 ovaries

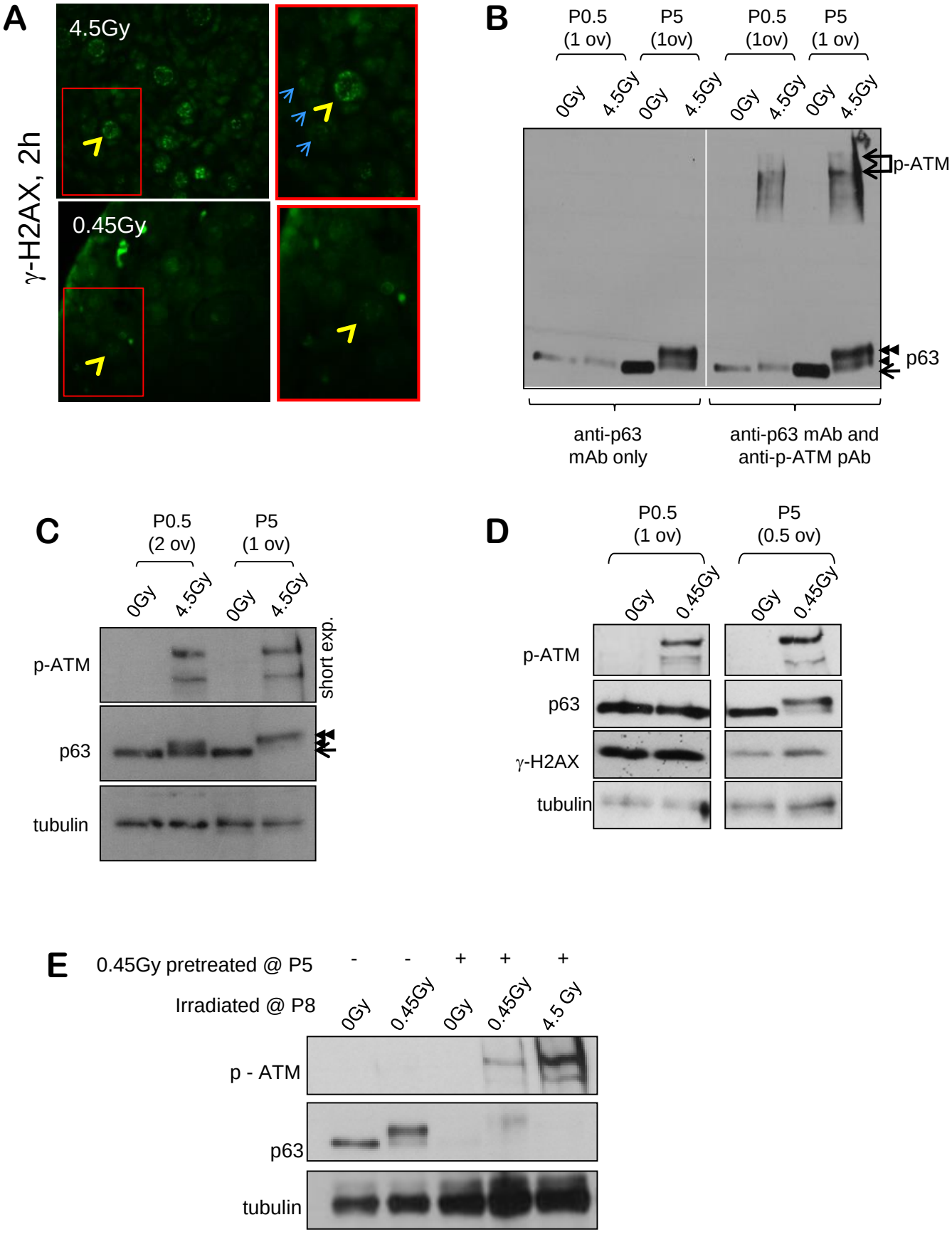
B

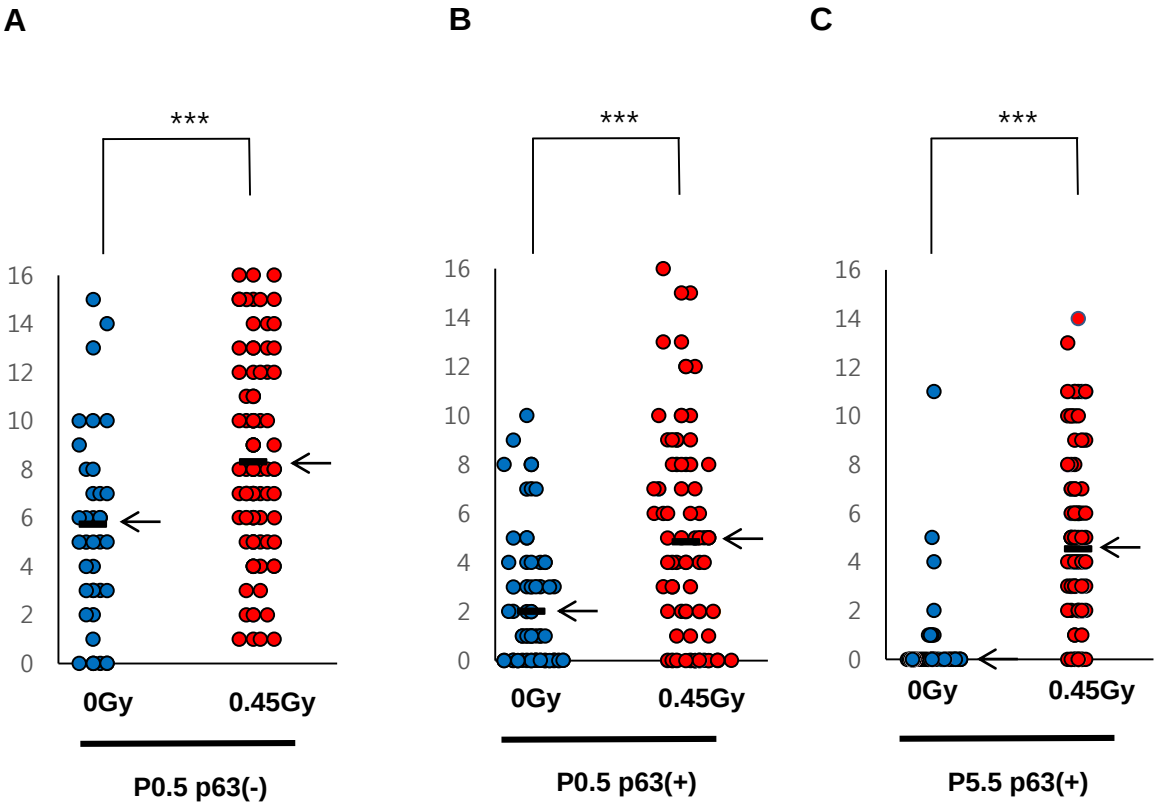

% oocyte survival
120.0%
100.0%
80.0%
60.0%
40.0%
20.0%
0.0%
NIRR
P0.5
P5
primary/secondary
primordial
total

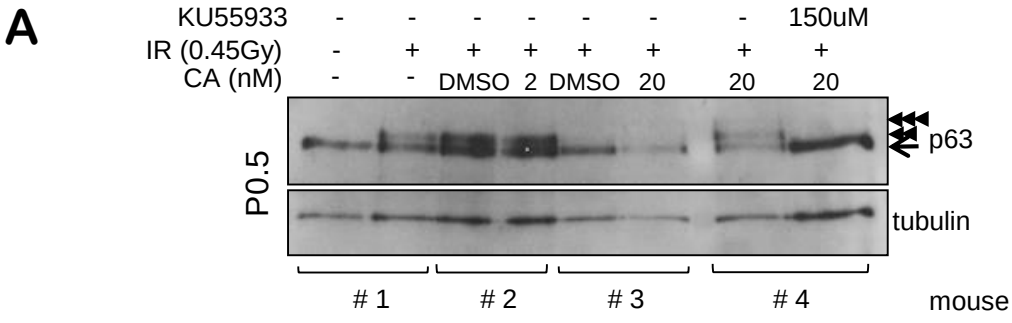

P2
0Gy
4.5Gy 2H
p63
IgG

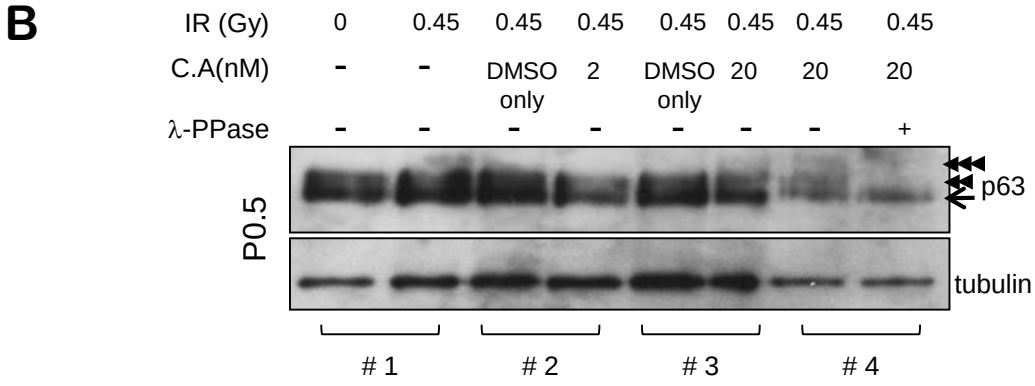

P0.5
P5
in vivo
in vivo
in vitro
in vivo
4.5Gy 2H
0Gy
20Gy 2H
0Gy
20Gy 2H
0Gy
20Gy 2H
0Gy
4.5Gy 2H
p63
IgG
p63
IgG

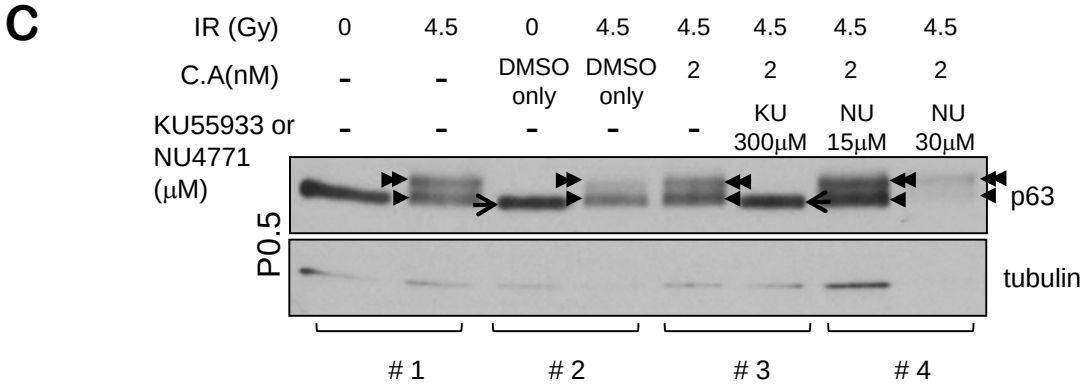

A
0.03%DMSO
0.03%DMSO
150μM KU
300μM KU
0.03%DMSO
0.03%DMSO
150μM KU
300μM KU
- + + + - + + + 4.5Gy IRR
p-ATM
P5
p63
Light exposure
Dark exposure
B
0Gy
4.5 Gy
KU55933
NU7441
- - 25μM 100μM 2.5μM 10μM 50μM
TAp63α/293T
p-ATM
p63
tubulin
C
NU7441
KU55933
- - 30μM - 300μM - 150μM - 300μM -
p-ATM
p63
P5
tubulin
γ-H2AX

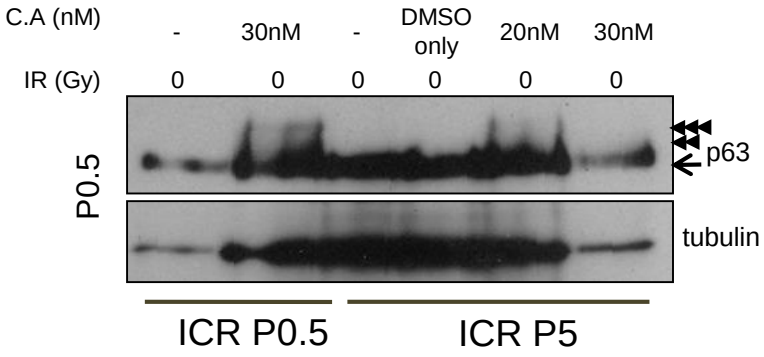

A
4.5Gy
0.45Gy
γ-H2AX, 2h
B
P0.5 (1 ov)
P5 (1ov)
P0.5 (1ov)
P5 (1 ov)
0Gy
4.5Gy
p-ATM
p63
anti-p63 mAb only
anti-p63 mAb and anti-p-ATM pAb
C
P0.5 (2 ov)
P5 (1 ov)
p-ATM
short exp.
p63
tubulin
D
P0.5 (1 ov)
P5 (0.5 ov)
0Gy
0.45Gy
p-ATM
p63
γ-H2AX
tubulin
E
0.45Gy pretreated @ P5
Irradiated @ P8
0Gy
0.45Gy
4.5 Gy
p - ATM
p63
tubulin


A
B
C
***
***
***
16
14
12
10
8
6
4
2
0
0Gy
0.45Gy
P0.5 p63(-)
P0.5 p63(+)
P5.5 p63(+)


A
KU55933 - - - - - - - 150uM
IR (0.45Gy) - + + + + + + +
CA (nM) - - DMSO 2 DMSO 20 20 20
P0.5
p63
tubulin
1
2
3
4
mouse


B
IR (Gy) 0 0.45 0.45 0.45 0.45 0.45 0.45 0.45
C.A(nM) - - DMSO only 2 DMSO only 20 20 20
λ-PPase - - - - - - - +
P0.5
p63
tubulin
1
2
3
4


C
IR (Gy) 0 4.5 0 4.5 4.5 4.5 4.5 4.5
C.A(nM) - - DMSO only DMSO only 2 2 2 2
KU55933 or NU4771 (μM) - - - - - KU 300μM NU 15μM NU 30μM
P0.5
p63
tubulin
1
2
3
4


C.A (nM)
-
30nM
-
DMSO only
20nM
30nM
IR (Gy)
0
0
0
0
0
0
P0.5
p63
tubulin
ICR P0.5
ICR P5