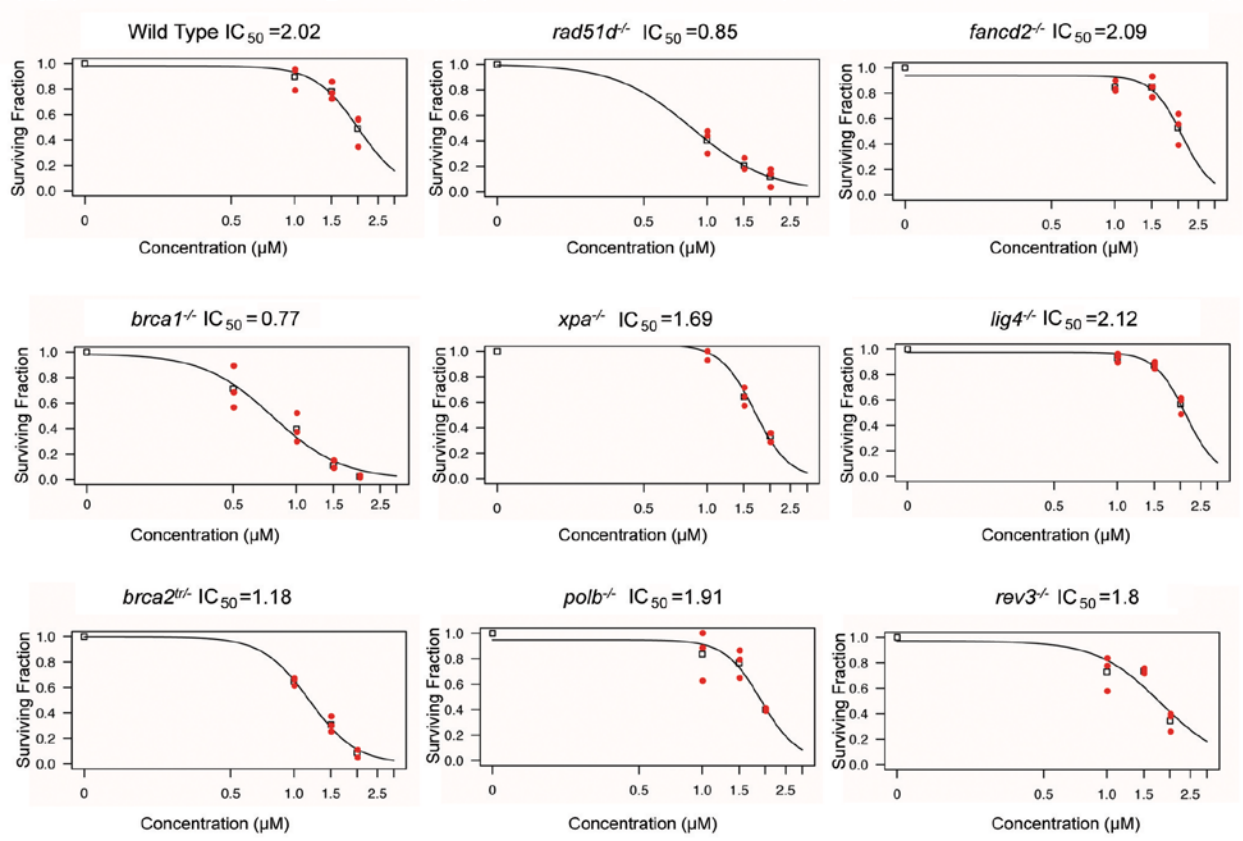
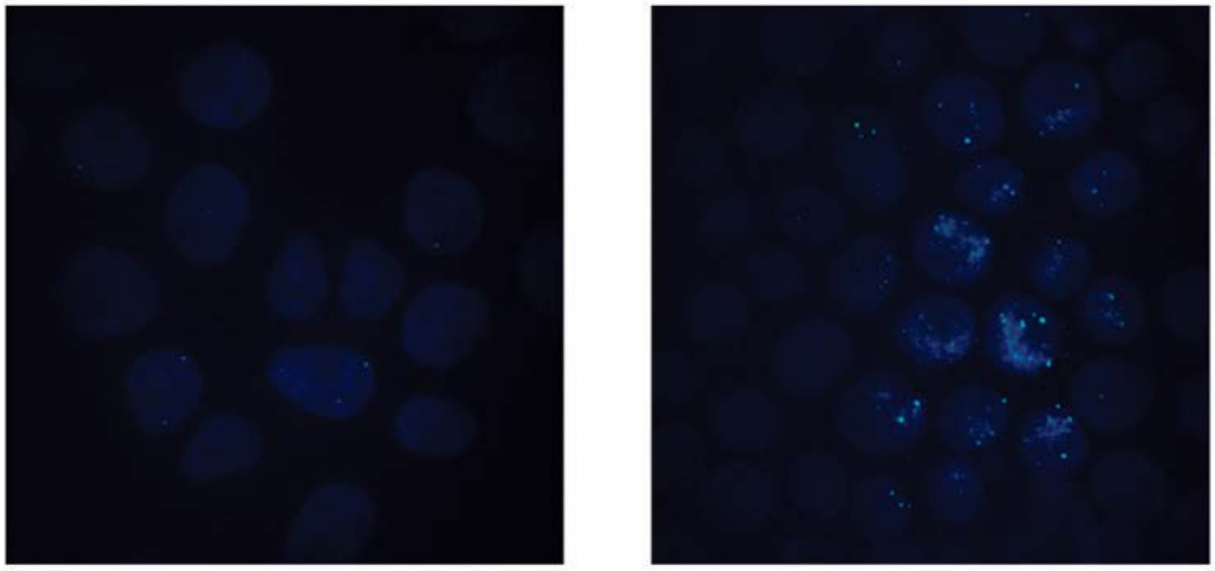
Impact of DNA repair pathways on the cytotoxicity of piperlongumine in chicken DT40 cell-lines.

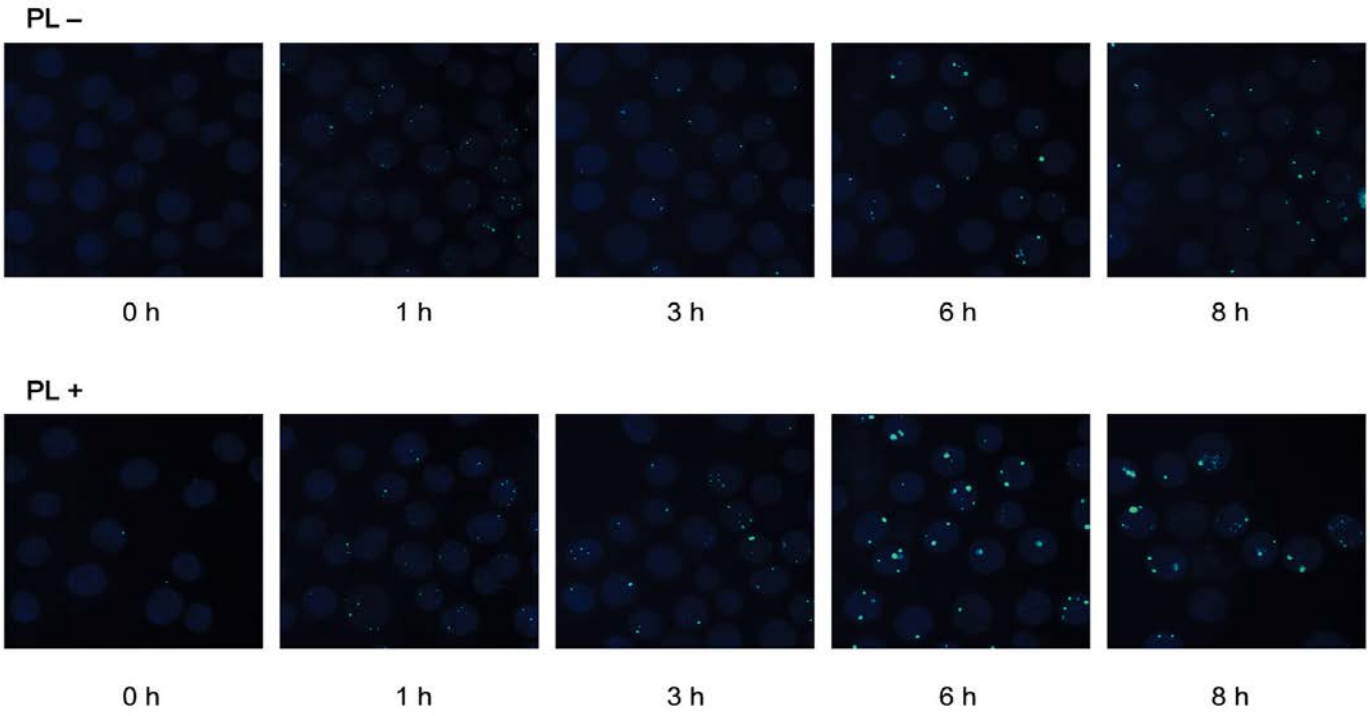


Supplementary Figure S1. *Dose response curves to piperlongumine in representative DNA repair deficient DT40 cell-lines.*

Each cell-line was exposed to the indicated concentrations of piperlongumine and the surviving fractions were calculated. The red dots represent surviving fraction at each concentration of piperlongumine and the open squares represent mean values at each concentration. The dose response curves (survival curves) were obtained with a three- parameter logistic curve using package dose response curve in R.



Supplementary Figure S2. *Induction of Rad51-foci formation by piperlongumine*. Wild type DT40 cells were treated with 2 μM of piperlongumine (PL) for 24 hr. Representative images of control cells (left panel) and PL-treated cells (right panel) were shown.



Supplementary Figure S3. *Suppression of homologous recombination by piperlongumine*.

Wild type DT40 cells were pre-treated with 1 μM of piperlongumine (PL) for 1 hr. After removing piperlongumine, the cells were irradiated with γ-ray at 2 Gy. Foci-formations of Rad51 were examined at the indicated time points after the irradiation. The upper panel: Irradiated cells without pre-treatment with PL. The lower panel: Irradiated wild type cells with pre-treatment with PL Representative images were shown.

Supplementary Table S1
DT40 isogenic DNA repair mutant cells used in this study.

Cell line	Function inactivated	References
ku70$^{-/-}$	NHEJ	(Takata et al., 1998)
ligase IV$^{-/-}$	NHEJ	(Adachi et al., 2001)
53bp1$^{-/-}$	Inhibition of HR	(Nakamura et al., 2006)
atm$^{-/-}$	DDR	(Takao et al., 1999)
rad52$^{-/-}$	HR, SSA	(Yamaguchi-Iwai et al., 1998)
xrcc2$^{-/-}$	HR	(Takata et al., 2001)
xrcc3$^{-/-}$	HR	(Takata et al., 2001)
brca1$^{-/-}$	HR	(Martin et al., 2007)
brca2$^{tr/-}$	HR	(Hatanaka et al., 2005)
fancc$^{-/-}$	ICL	(Hirano et al., 2005)
fancd2$^{-/-}$	ICL	(Yamamoto et al., 2005)
polb$^{-/-}$	BER	(Tano et al., 2007)
xpa$^{-/-}$	NER	(Okada et al., 2002)
rev3$^{-/-}$	TLS	(Sonoda et al., 2003)
rad51c$^{-/-}$	HR	(Takata et al., 2001)
rad54$^{-/-}$ *ku80*$^{-/-}$	HR, NHEJ	(Takata et al., 1998)
fen1$^{-/-}$	BER	(Matsuzaki et al., 2002)
53bp1$^{-/-}$*brca1*$^{-/-}$		Unpublished
rad54$^{-/-}$*rad54b*$^{-/-}$	HR	Unpublished

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