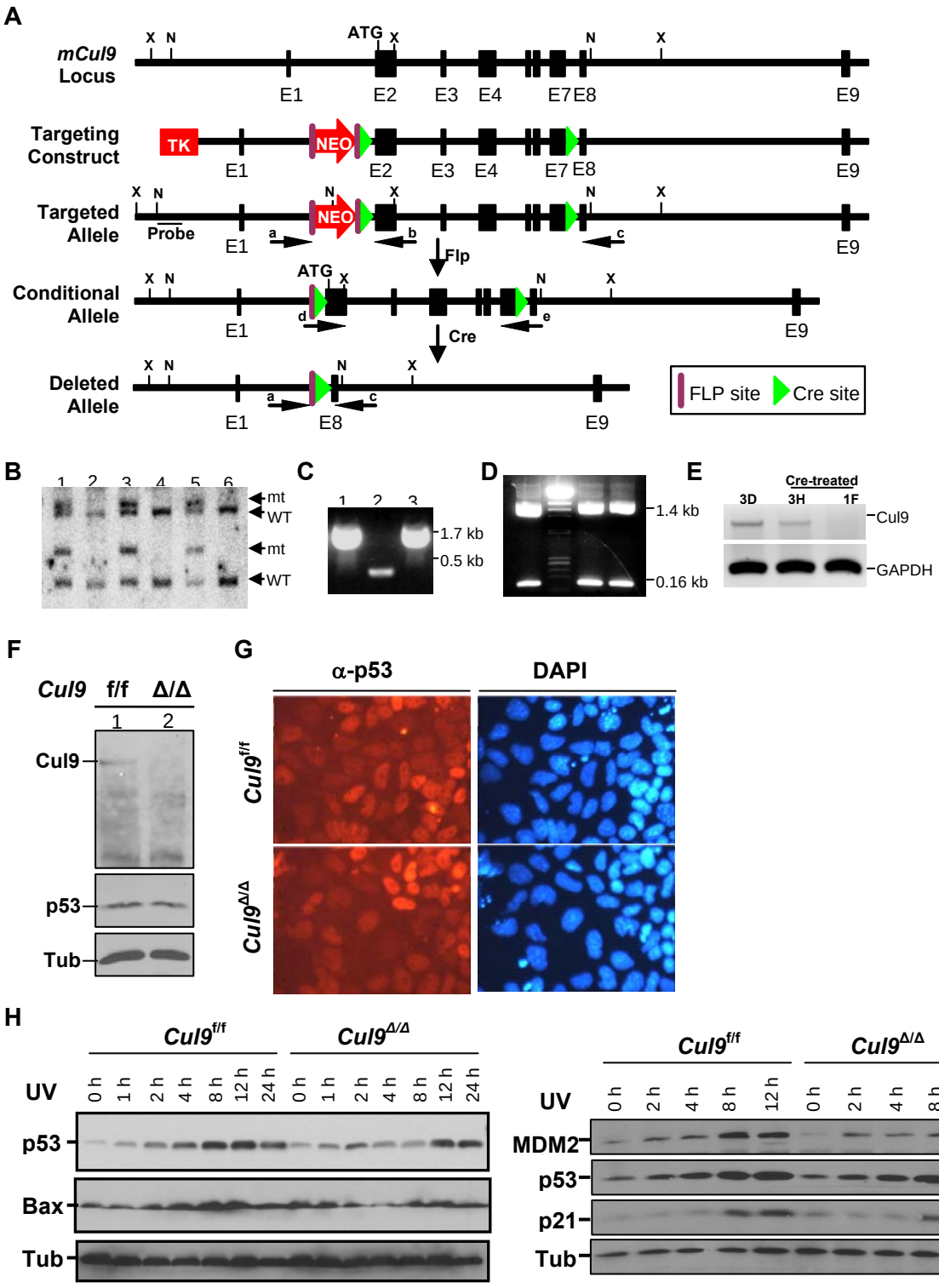
Pei et al. Supplementary Figure S2



Supplementary Figure S2. Generation and characterization of *Cul9* mutant mouse and ES cells
A, Schematic presentation of targeting strategy for generating *Cul9* null ES cells. A 17.8 kb fragment of mouse genomic DNA, spanning from the promoter region to exon 9, was amplified by PCR from mouse ES cell genomic DNA and verified by DNA sequencing. Lox P sites were inserted at the 5' and 3' ends of the region to be deleted. To select for homologous recombination, a neomycin resistance gene, flanked by frt

sites, was inserted immediately upstream of the 5' lox P deletion site, and a thymidine kinase negative selection marker was inserted upstream of exon 1.
B, Isolation of *Cul9*$^{NeoFlox/+}$ heterozygous ES clones. The linearized targeting construct was electroporated into ES cells, G418 and ganciclovir doubly-resistant clones were screened for homologous recombination by Southern blotting. Genomic DNA from ES cells was digested by Xho1 and hybridized with the probe.
C, Isolation of *Cul9*$^{Flox/+}$ (*Cul9*$^{F/+}$) heterozygous ES clones. *Cul9*$^{NeoFlox/+}$ ES cells were transfected with an expression vector encoding Flp recombinase, neo-removed *Cul9*$^{F/+}$ heterozygous ES clones were screened and isolated by PCR with the primers a and b. Note that 0.5 kb-fragment shows the DNA amplified from neo-removed *Cul9*^{F} allele, while 1.7 kb-fragment shows the DNA amplified from *Cul9*^{Neoflox} allele.
D, Generation of *Cul9*$^{F/NeoFlox}$ and *Cul9*$^{-/-}$ ES clones. *Cul9*$^{F/+}$ ES cells were retargeted with the same targeting construct, followed by another round of G418 and ganciclovir double-selection and screening for homologous recombination by PCR and Southern blotting. *Cul9*$^{F/NeoFlox}$ ES clones were expanded and transfected with CMV-Cre vector or infected with retroviral Cre. Deletion of exons 2-7 from both alleles was confirmed by genomic PCR using primers a and c. Note that 0.16 kb-fragment shows the DNA amplified from neo-removed and exons 2-7 deleted *Cul9* allele, while 1.4 kb-fragment shows the DNA amplified from neo-unremoved but exons 2-7 deleted *Cul9*^{Neo} allele.
E, Reduced or loss of *Cul9* gene expression was determined by RT-PCR. RNA was isolated from *Cul9*$^{F/F}$ ES cells and analyzed by RT-PCR with primers d and e. Note that Cre-mediated deletion was incomplete in Clone3H, resulting in a reduction, but not complete loss, of *Cul9* expression.
F, p53 protein level is unchanged in the absence of *Cul9*. Western blot analysis of steady state p53 levels in total protein lysates of *Cul9*$^{F/F}$ and *Cul9*$^{-/-}$ ES cells using antibodies as indicated.
G, Loss of *Cul9* in ES cells did not change p53 localization. Subcellular localization of endogenous p53 in *Cul9*$^{F/F}$ and *Cul9*$^{-/-}$ ES was analyzed by indirect immunofluorescence.
H, Cell extracts were prepared from *Cul9*$^{F/F}$ and *Cul9*$^{-/-}$ ES cells at the different time points after exposure to 50 J/m^2 UV irradiation. The expression of individual proteins was determined by Western blot.