# Supplemental Materials

***Molecular Biology of the Cell***

Wang et al.

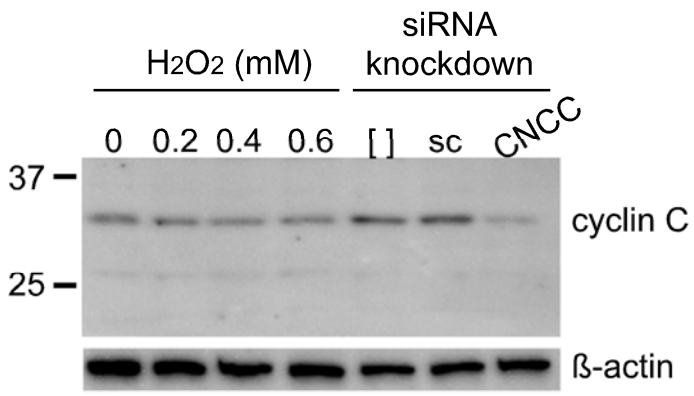



Figure S1. Cyclin C stability is not affected by stress exposure. Cyclin C levels in stressed MEF cultures were determined in soluble extracts (50 µg) by Western blot analysis before and following 6hr treatment with the indicated $H_2O_2$ concentrations. Confirmation of the cyclin C specific signal was accomplished using siRNA knockdown assays. Cyclin C levels in extracts prepared from MEF cultures transfected with either no ([ ]), scrambled (sc) or CCNC specific siRNAs. Molecular weight markers (kDa) are given on the left. ß-actin levels were determined as a loading control.

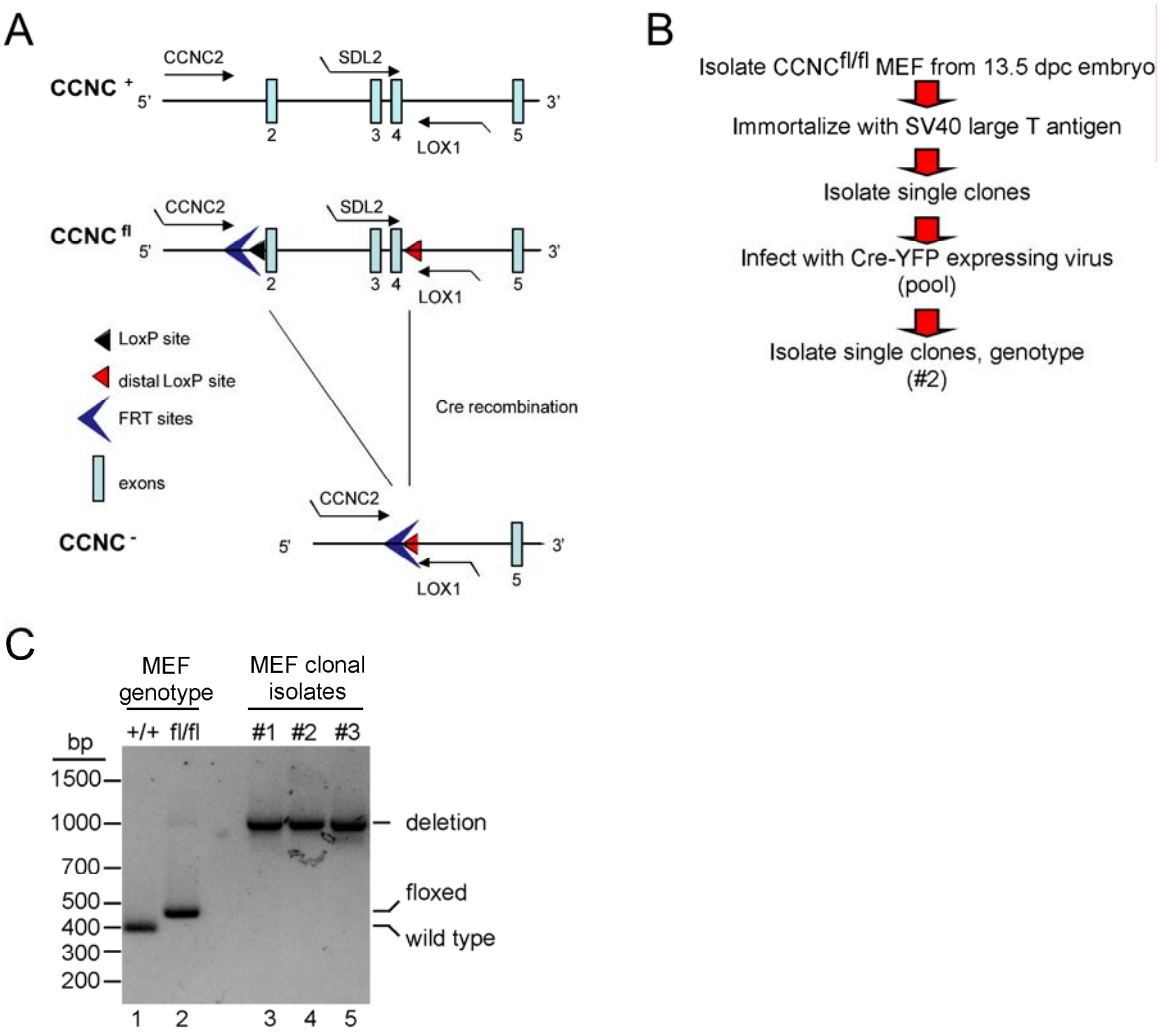
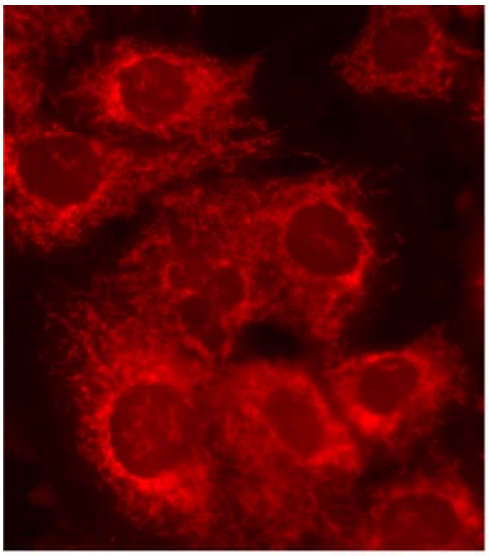
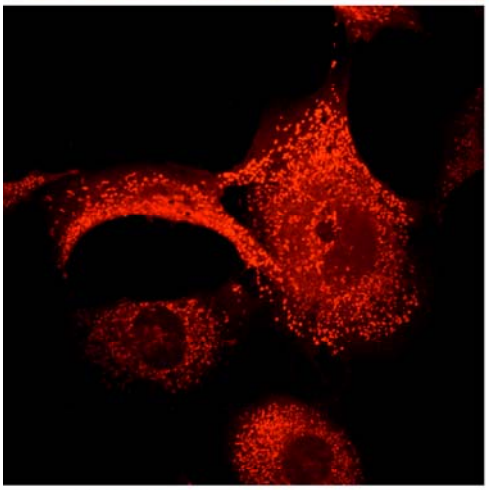



Figure S2. Generation of CCNC knockout Mouse Embryonic Fibroblast ($CCNC^{-/-}$ MEF) cells. (A) Schematic representation of CCNC conditional knockout strategy. The locations of three primers (CCNC2, SDL2 and LOX1), loxP sites and FRT recognition sequences are indicated on a portion of the CCNC intron/exon structure. The genotype was determined using a multiplexed primer system incorporating the three indicated primers. In brief, deletion of intron 2-4 by Cre recombination will shorten the distance between the CCNC2 and LOX1 primersproducing a 1kbp PCR product. The wild type and floxed alleles were identified with product size differences of the SDL1 and LOX1 primer amplifications. The longer CCNC2, LOX1 amplification product was not observed

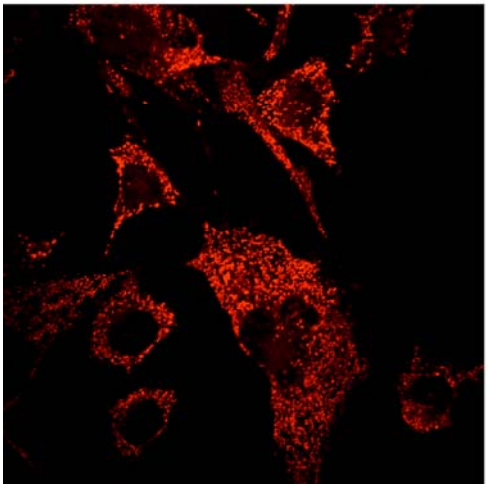
in these assays as the extension time was set to one minute. (B) Flowchart for generating $CCNC^{-/-}$ MEF cells. $CCNC^{+/+}$ and $CCNC^{fl/fl}$ MEF cells were isolated from 13.5dpc embryos (see (C), lanes 1 and 2) and immortalized by transfecting with SV40 T antigen. The cells were then infected with retrovirus expressing YFP fused Cre recombinase. The YFP positive cells were sorted by flow cytometry to generate the Cre expressing “pool”. Single clones were then obtained by dilution subcloning. Three individual clones isolated through this approach (#1-3) were analyzed further (see (C), lanes 3-5). The immortalized $CCNC^{+/+}$ cells were used as controls for the experiments described in the text.



control

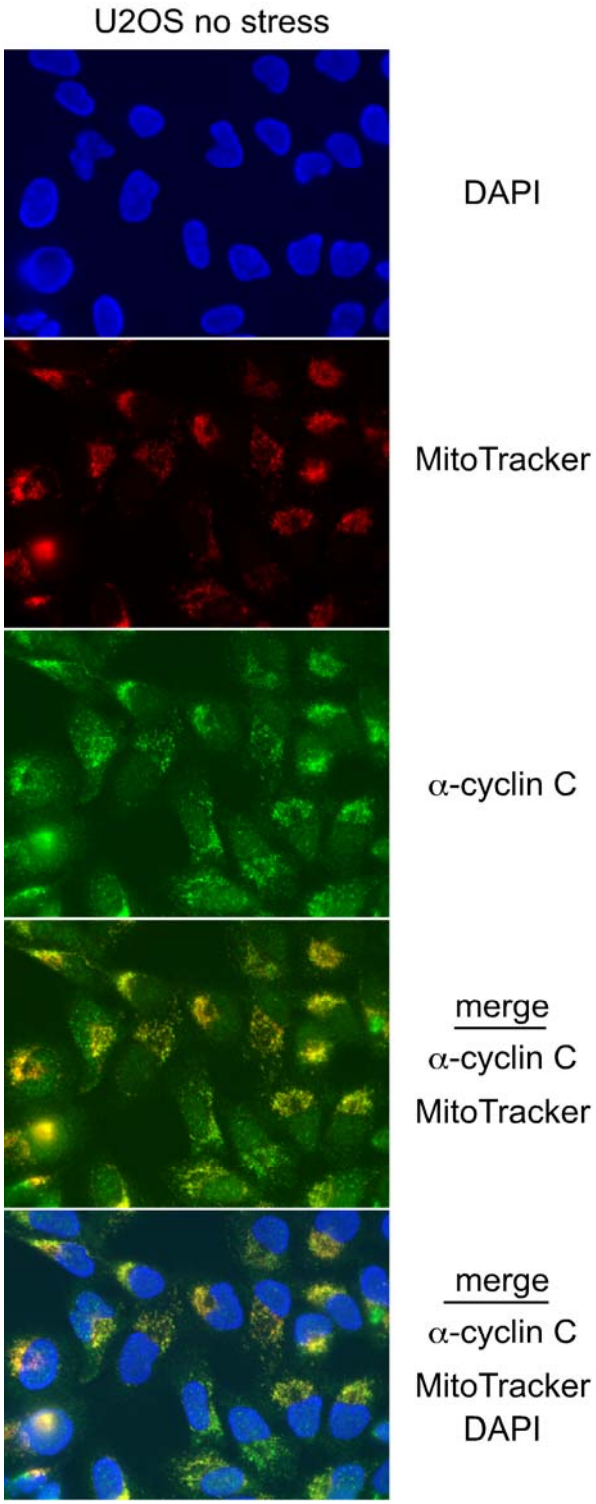


GST-cyclin C



GST-cyclin C

Supplemental Figure 3. Whole field view of permeabilized CCNC$^{-/-}$ MEF cells treated with GST-cyclin C (4 nM) for 18 min. Control cells were incubated with GST alone. Confocal images were taken of live cells in an atmosphere and temperature controlled chamber. These cells were not exposed to the laser other than this timepoint.




Supplemental Figure 4. Whole field view of studies examining the subcellular localization of the nuclei (DAPI), mitochondria (MitoTraker), and cyclin C was determined in unstressed U2OS cells. Merged images of the indicated signals are shown as indicated. Cytoplasmic cyclin C and its co-localization with the mitochondria are indicated by the yellow signal.