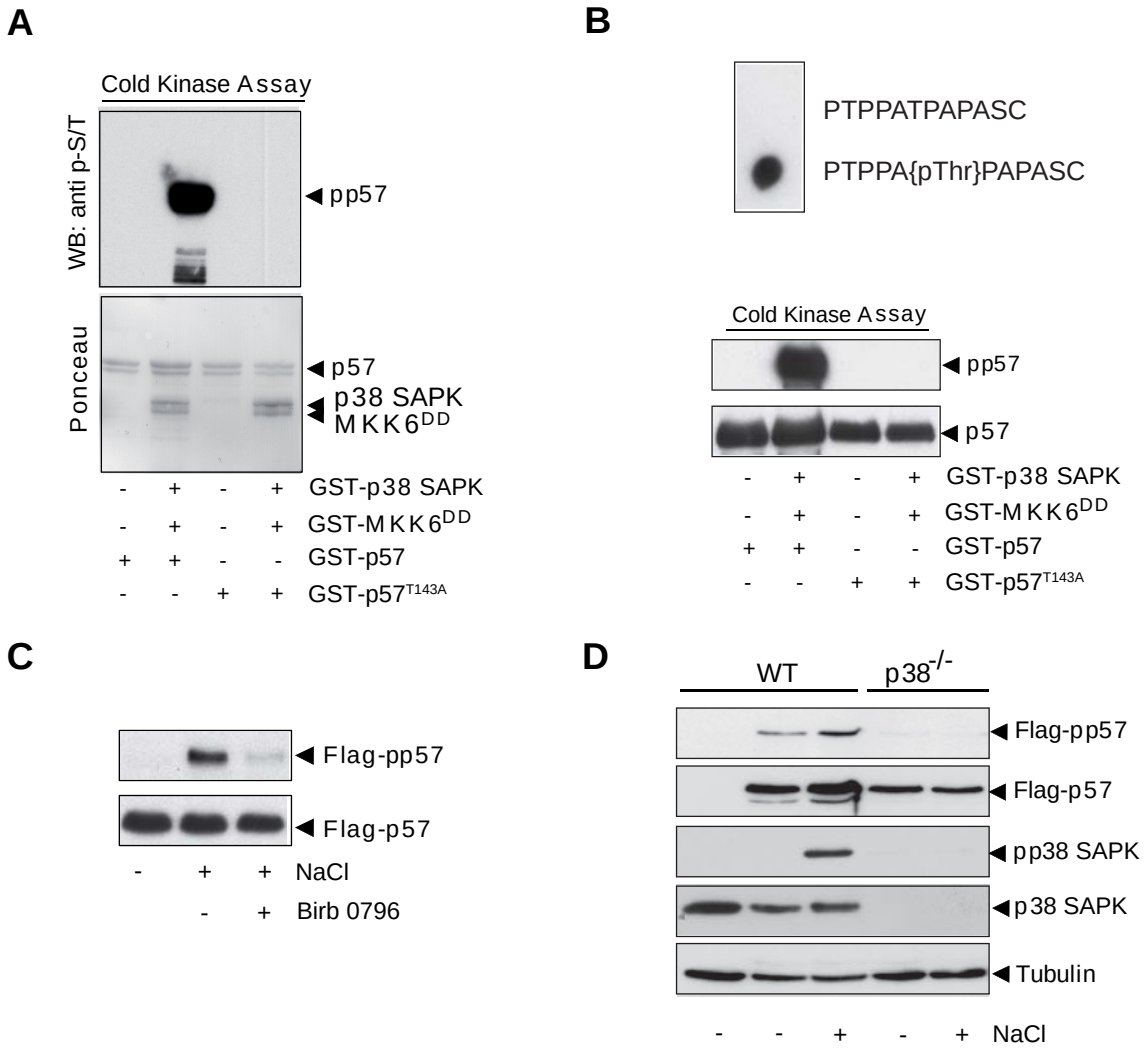
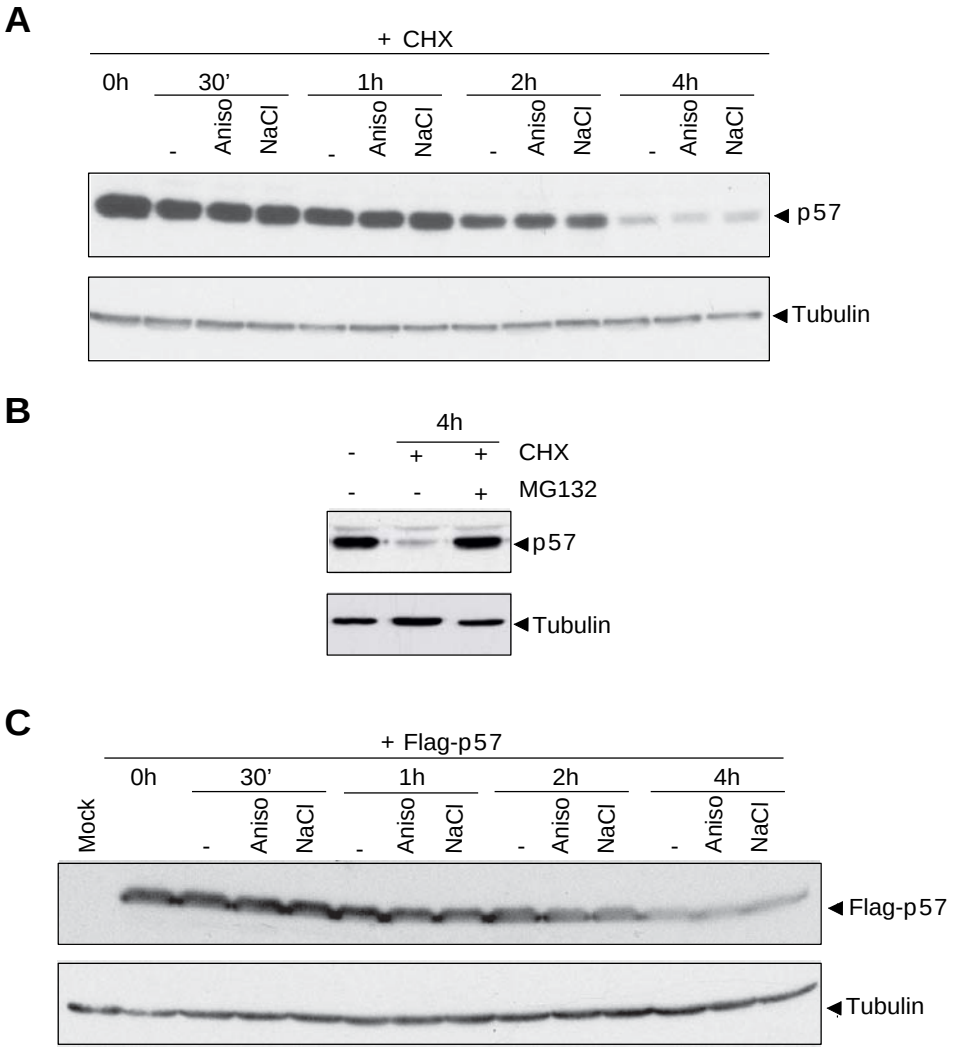
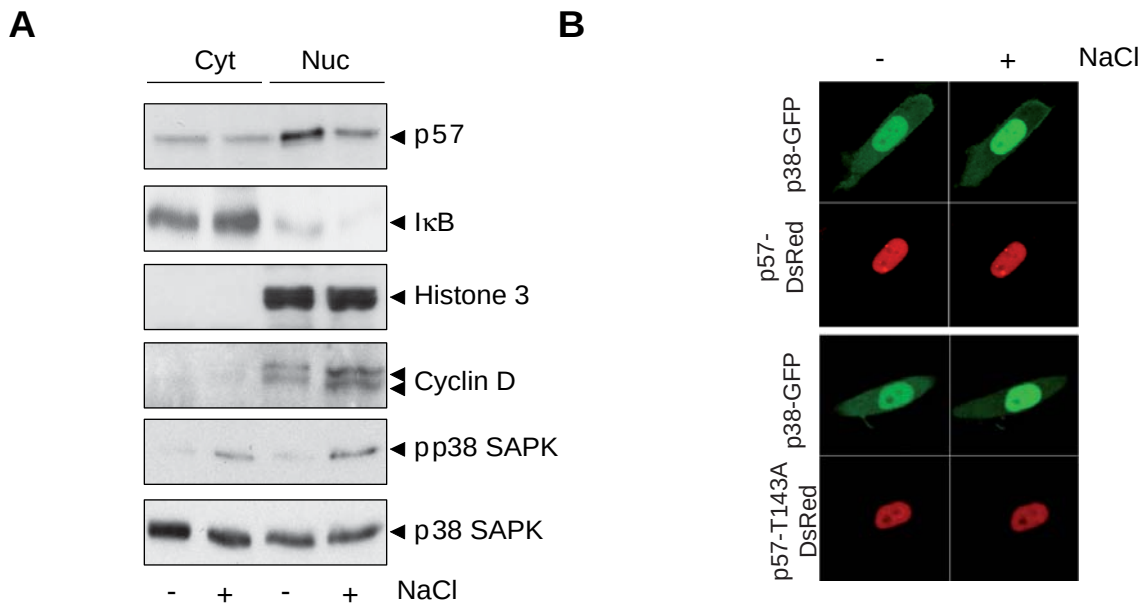


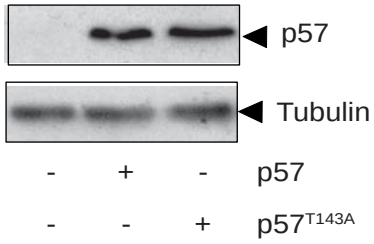
Supplemental Figure S1. p38 SAPK phosphorylates p57 *in vitro* and *in vivo*. (A) GST-p57 and GST-p57^{T143A} were assayed *in vitro* with GST-p38 SAPK in the presence of cold ATP and analyzed by western blot. A generic anti-phosphoserine/threonine antibody specifically recognized phosphorylated p57 at T143. (B) A phosphopeptide surrounding T143 was used to generate phospho-specific p57 antibodies. The purified antibodies recognized the phosphopeptide but not the non-phosphorylated peptide (upper panel). GST-p57 and GST-p57^{T143A} were assayed *in vitro* with GST-p38 SAPK in the presence of cold ATP and analyzed by western blot with the anti-pp57^{T143} antibody (lower panel). (C) HeLa cells were transfected with Flag-p57. 48 hours post-transfection cells were treated with 100 mM NaCl for 30' in the presence or the absence of the specific p38 SAPK inhibitor Birb 0796. Birb 0796 was added to a final concentration of 0.5 μM 2 hours prior to NaCl addition. (D) Wild type and p38$^{-/-}$ MEFs were transfected with Flag-p57. 48 hours post-transfection cells were treated with 100 mM NaCl for 30'. Cell lysates from C and D were then analysed by western blot with anti-pp57^{T143}, anti-p57, anti pp38 SAPK, anti-p38 SAPK and anti-tubulin antibodies. Representative western blots are shown.



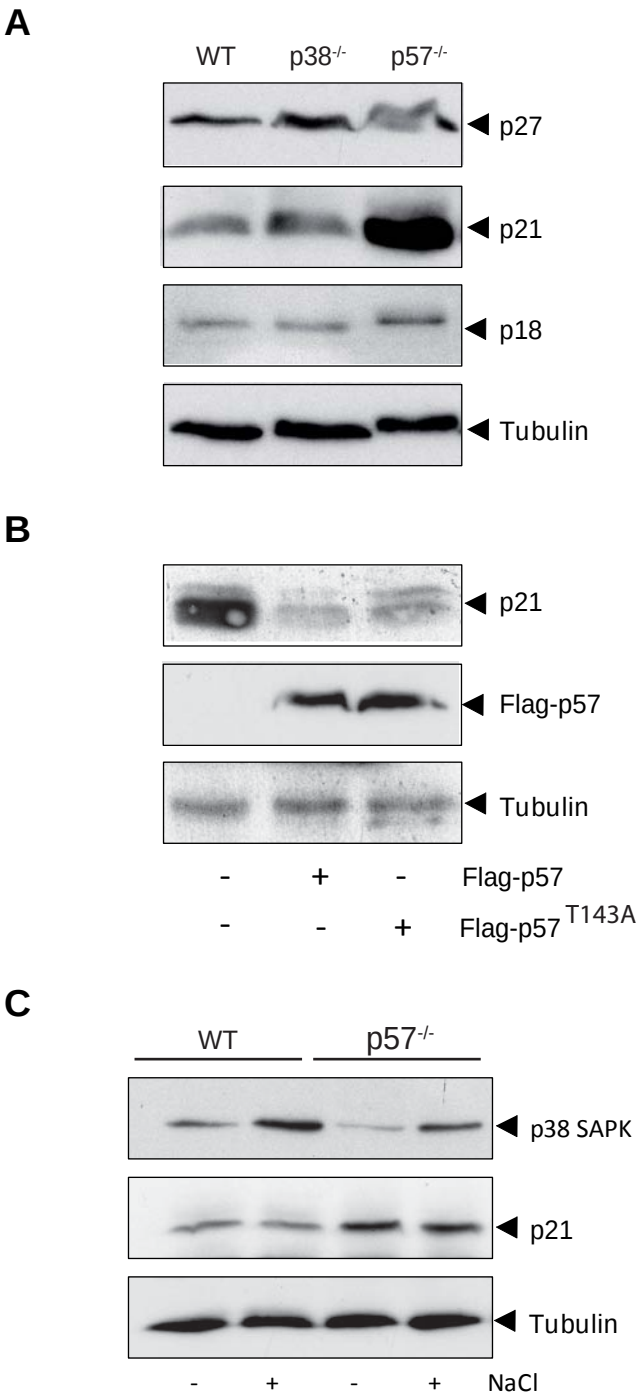
Supplemental Figure S2. p57 protein stability upon stress. (A) HeLa cells were cultured overnight in the presence of 100 nM dexhamethasone to induce the endogenous expression of p57. The protein synthesis inhibitor cycloheximide was added at a final concentration of 50 μg/ml 30' prior to the addition of the p38 SAPK activators anisomycin (25 ng/ml) and NaCl (100 mM). Cell lysates were collected at the indicated times and analyzed by western blot with anti-p57 and anti-tubulin antibodies. (B) p57 is actively degraded by the proteosome. The addition of 10 μM MG132 10' prior to the addition of cycloheximide prevents p57 degradation. HeLa cell lysates were analyzed by western blot with anti-p57 and anti-tubulin antibodies. (C) HeLa cells were transfected with Flag-p57 for 48 hours. 50 μg/ml of ciclohexamide was added 30' prior to the addition of anisomycin (25 ng/ml) and NaCl (100 mM). Cell lysates were collected at the indicated times and analyzed by western blot with anti-flag and anti-tubulin antibodies. Representative western blots are shown.



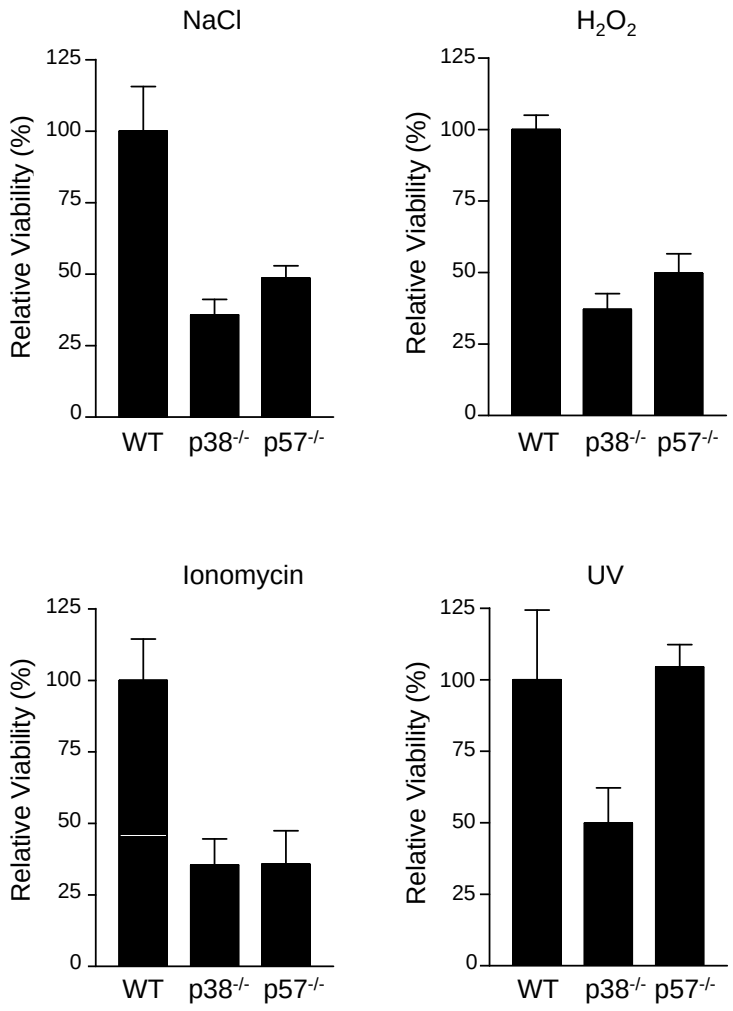
Supplemental Figure S3. p38 SAPK and p57 co-localize at the same sub-cellular compartment. (A) HeLa cells were treated with 100 mM for 30'. Cytosolic and nuclear cell extracts were them analysed by western blot. The endogenous protein levels of p57, IkBα, Histone H3, Cyclin D, pp38 SAPK and total p38 SAPK are shown. (B) HeLa cells were co-transfected with p38-GFP along with DsRed-p57 or Ds-Red-p57^{T143A}. Time-lapse microscopy was performed to follow up the intracellular localization of both proteins *in vivo* upon the addition of 100 mM NaCl. Expressed p38 SAPK was found in both cytosol and the nucleus at basal state and concentrated at the nucleus upon osmostress. Expressed wt p57 and p57^{T143A} were mainly localized at the nuclear compartment throughout the experiment. Representative pictures in the absence (-) and after the addition of 100 mM NaCl for 45' (+) are shown.



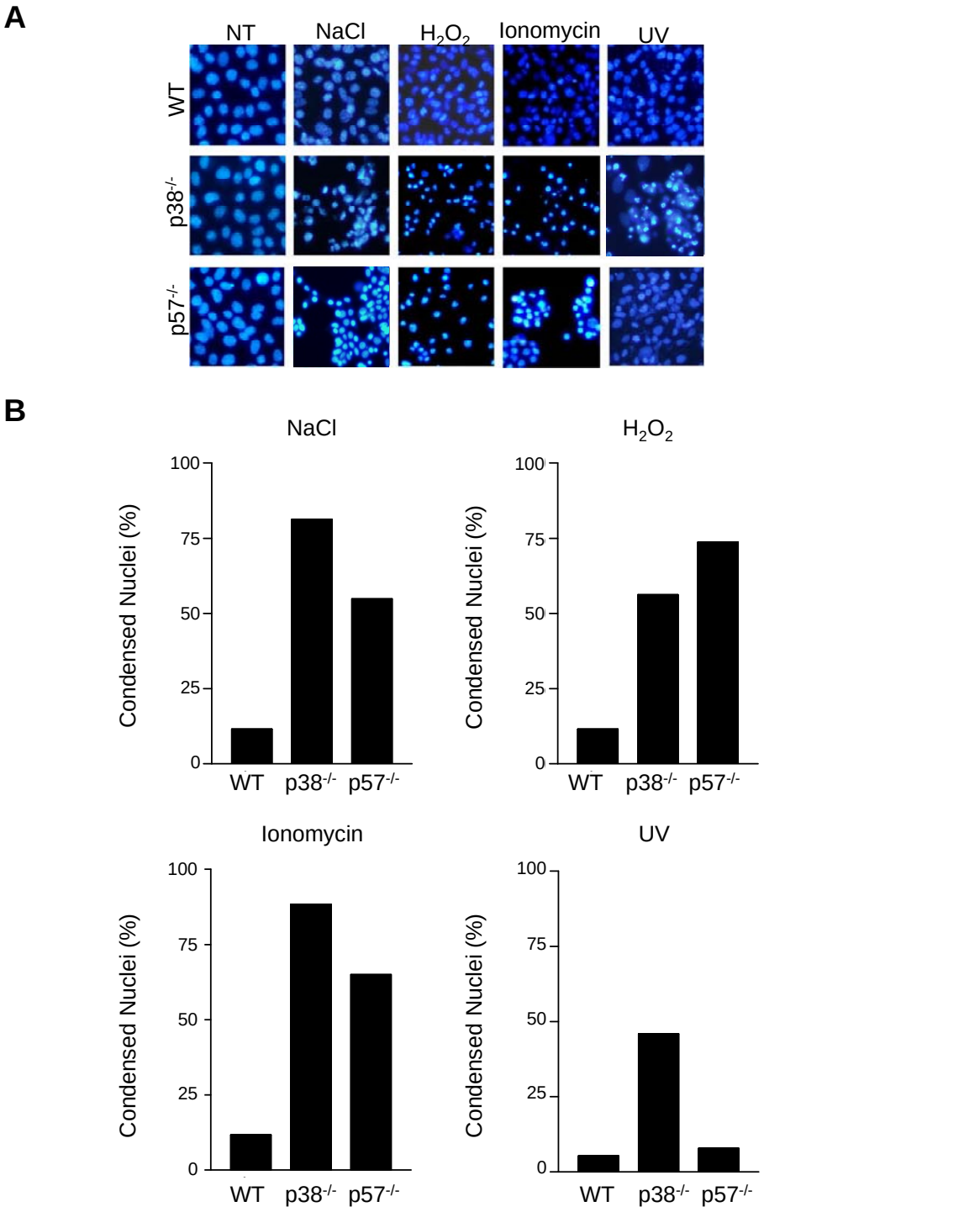
Supplemental Figure S4. p57 ectopic expression in infected $p57^{-/-}$ MEFs. $p57^{-/-}$ MEFs were infected with lentiviruses carrying an empty vector, a wild type p57 or $p57^{T143A}$. p57 expression on $p57^{-/-}$ infected cells was detected by western blot using an anti-p57 antibody.



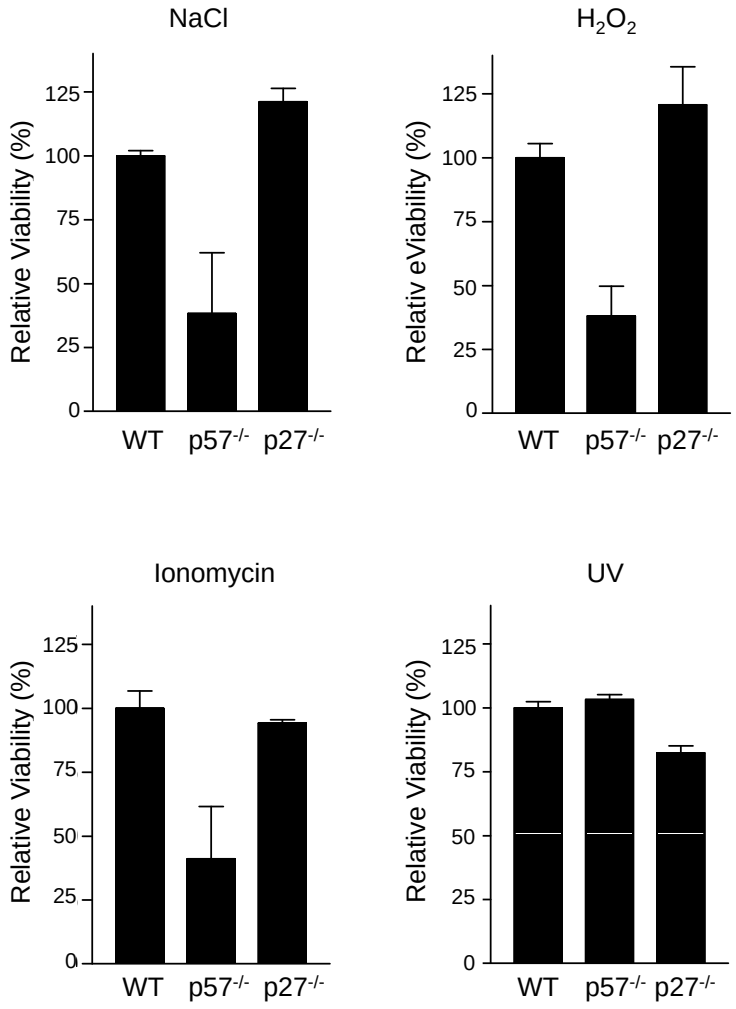
Supplemental Figure S5. CDKi protein levels in MEFs cells. (A) Wild type, $p38^{-/-}$ and $p57^{-/-}$ MEFs lysates were analyzed by western blot with anti-tubulin, anti-p21, anti-p27 and anti-p18 antibodies. (B) $p57^{-/-}$ MEFs were transfected with wild type Flag-p57 or Flag-$p57^{T143A}$ for 48 hours. Cell lysates were then analyzed by western blot with anti-tubulin, anti-p21 and anti-p57 antibodies. (C) Wild type and $p57^{-/-}$ MEFs were treated with 100 mM for 60'. Cell lysates were analyzed by western blot with anti-pp38 SAPK, anti-p21 and anti-tubulin antibodies. Representative western blots are shown.



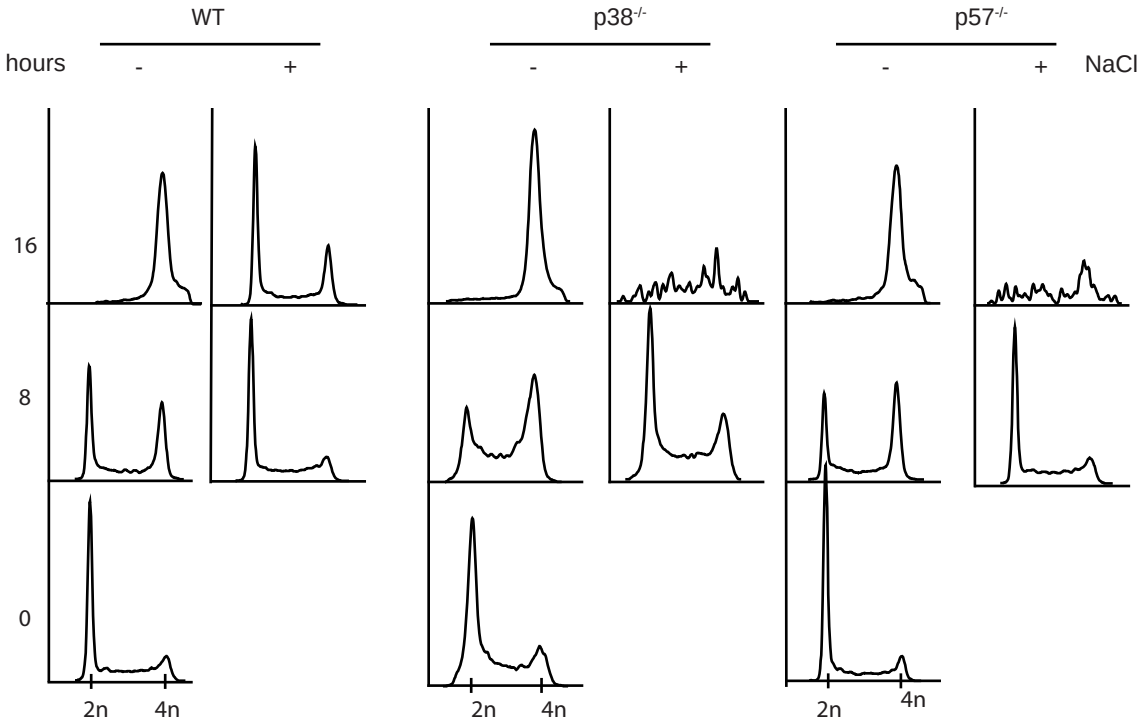
Supplemental Figure S6. MTT Cell viability assay in MEFs cells upon stress. Wild type, $p38^{-/-}$ and $p57^{-/-}$ MEFs were treated with 200 mM NaCl, 600 μM H_2O_2, 7.5 mM ionomycin and 5 mJ of UV for 24 hours. After the treatments an MTT assay was carried out to assess cell viability. The graph represents the average and SEM of three independent experiments.



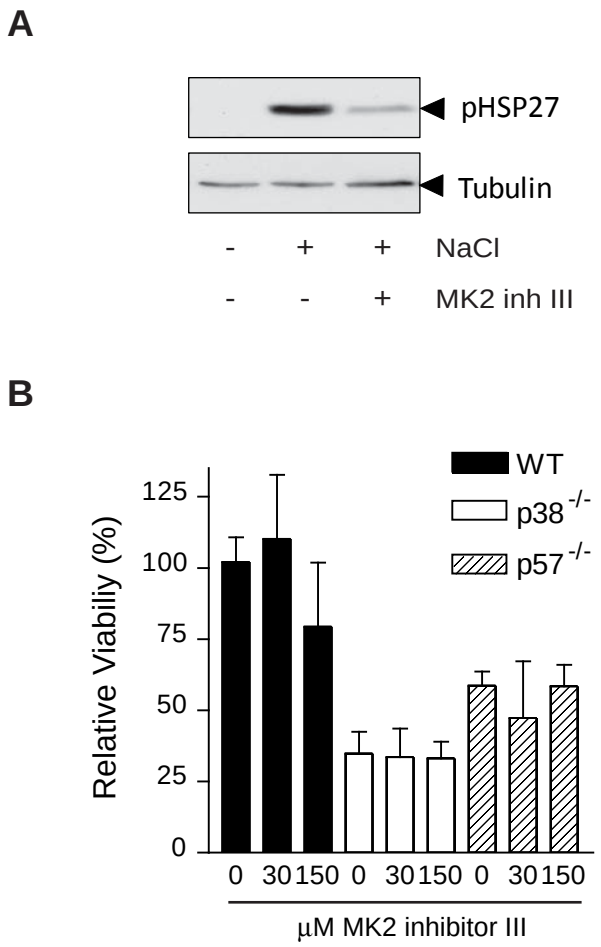
Supplemental Figure S7. DNA condensation in MEFs cells upon stress. (A) Wild type, $p38^{-/-}$ and $p57^{-/-}$ MEFs were treated with 200 mM NaCl, 600 μM H_2O_2, 7.5 mM ionomycin and 5 mJ of UV for 24 hours. Nuclear DNA was stained with Hoechst 3342. Pictures were taken using an inverted Olympus CKX 41 microscope and the Olympus Cell^D imaging software. Representative pictures are shown. (B) The percentage of condensed nuclei upon the treatments described in (A) is plotted. Between 250 and 500 nuclei were counted.



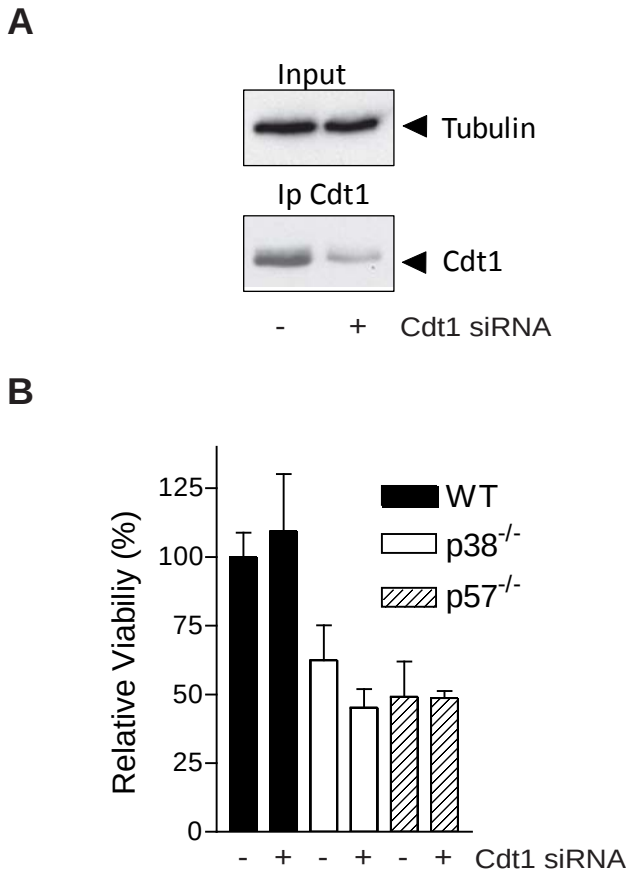
Supplemental Figure S8. Cell viability is not compromised in $p27^{-/-}$ MEFs. Cell viability was assessed in wild type, $p57^{-/-}$ and $p27^{-/-}$ MEFs after the treatment with 200 mM NaCl, 600 μM H_2O_2, 7.5 mM ionomycin and 5 mJ of UV for 24 hours. The graph represents the average and SEM of three independent experiments.



Supplemental Figure S9. Osmostress mediates a G1 cell cycle delay in wild type MEFs. Wild type, $p38^{-/-}$ and $p57^{-/-}$ MEFs were stressed with 200 mM NaCl. One hour later nocodazole was added to trap the cells at the G2/M transition. Cell cycle progression was monitored by FACS by collecting samples at indicated times. Representative DNA profiles are shown.



Supplemental Figure S10. MK2 activity does not contribute to cell viability upon osmostress. (A) HeLa cells were treated with 100 mM NaCl for 60'. The MK2 inhibitor was added 30' prior to osmostress at the final concentration of 150 μM. Cell lysates were analyzed by western blot with anti-pHSP27 and anti-tubulin antibodies. Representative western blots are shown. (B) Wild type, $p38^{-/-}$ and $p57^{-/-}$ MEFs were treated with 200 mM NaCl, for 24 hours. The MK2 inhibitor was added 30' prior to osmostress at the final concentrations of 30 and 150 μM. After the treatments an MTT assay was carried out to assess cell viability. The graph represents the average and SEM of three independent experiments.



Supplemental Figure S11. Cdt1 protein does not contribute to cell viability upon osmostress. (A) Wild type MEFs were transfected with Cdt1 siRNA. Cell lysates were then immunoprecipitated with anti-Cdt1 antibodies and analyzed by western blot with an anti-Cdt1 antibody. Tubulin was used to monitor the input protein levels. (B) Wild type, $p38^{-/-}$ and $p57^{-/-}$ MEFs were transfected with Cdt1 siRNA for 2 days prior to the addition of 200 mM NaCl. Cell viability was assessed by MTT assaty 24 hours after the osmostress. The graph represents the average and SEM of three independent experiments.