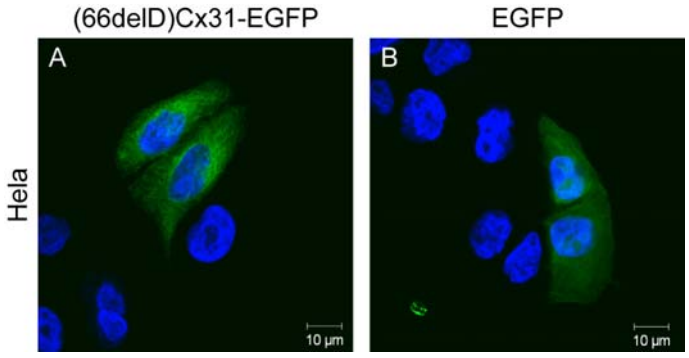
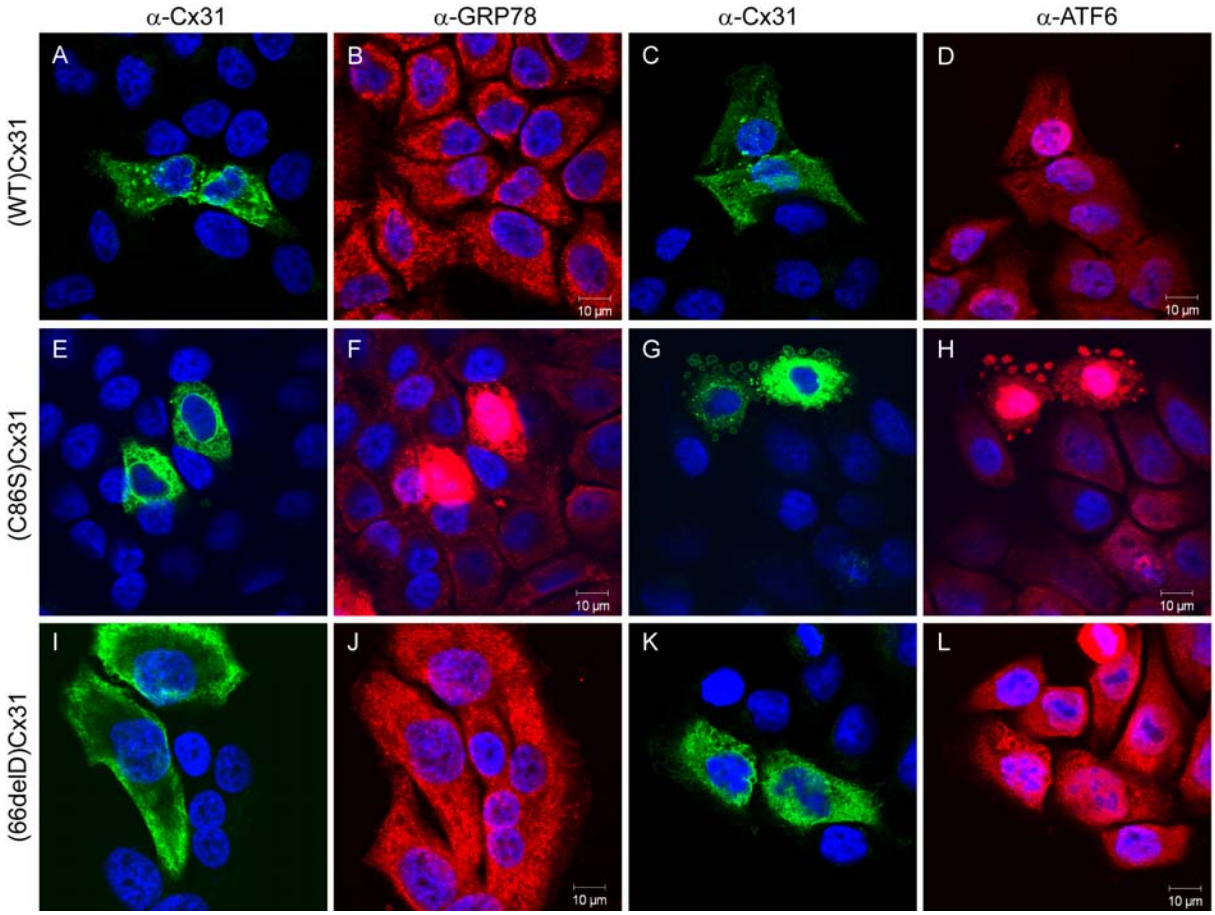
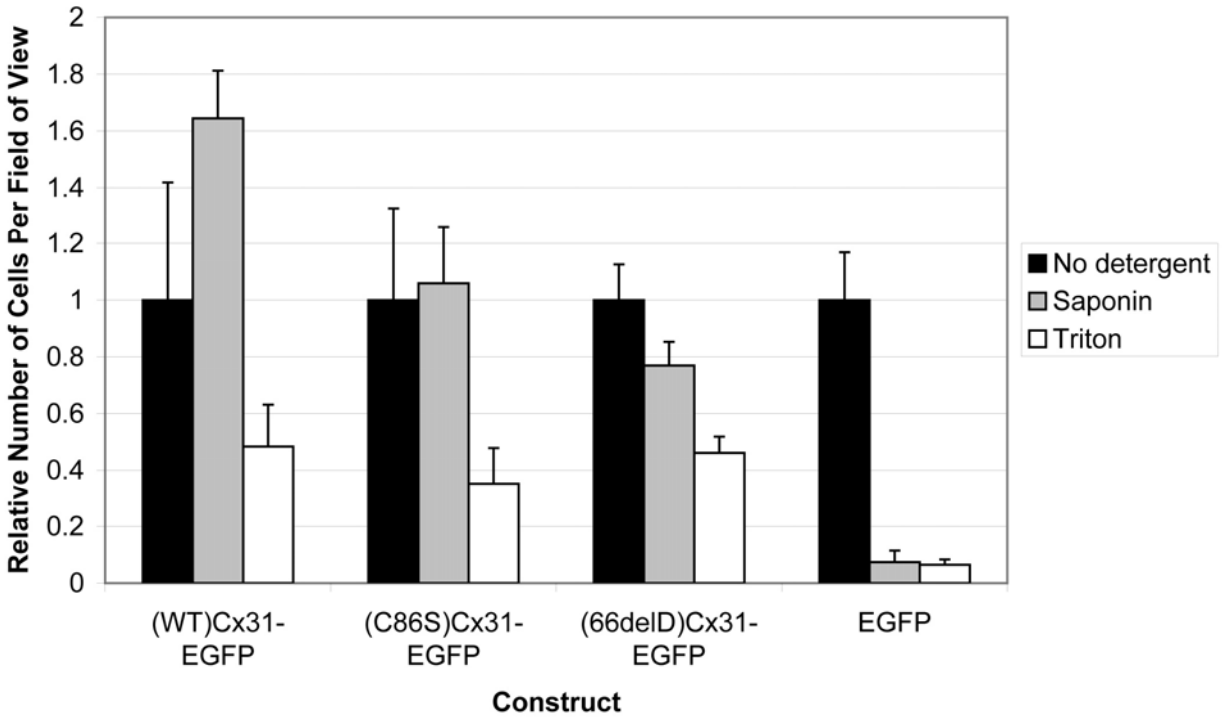


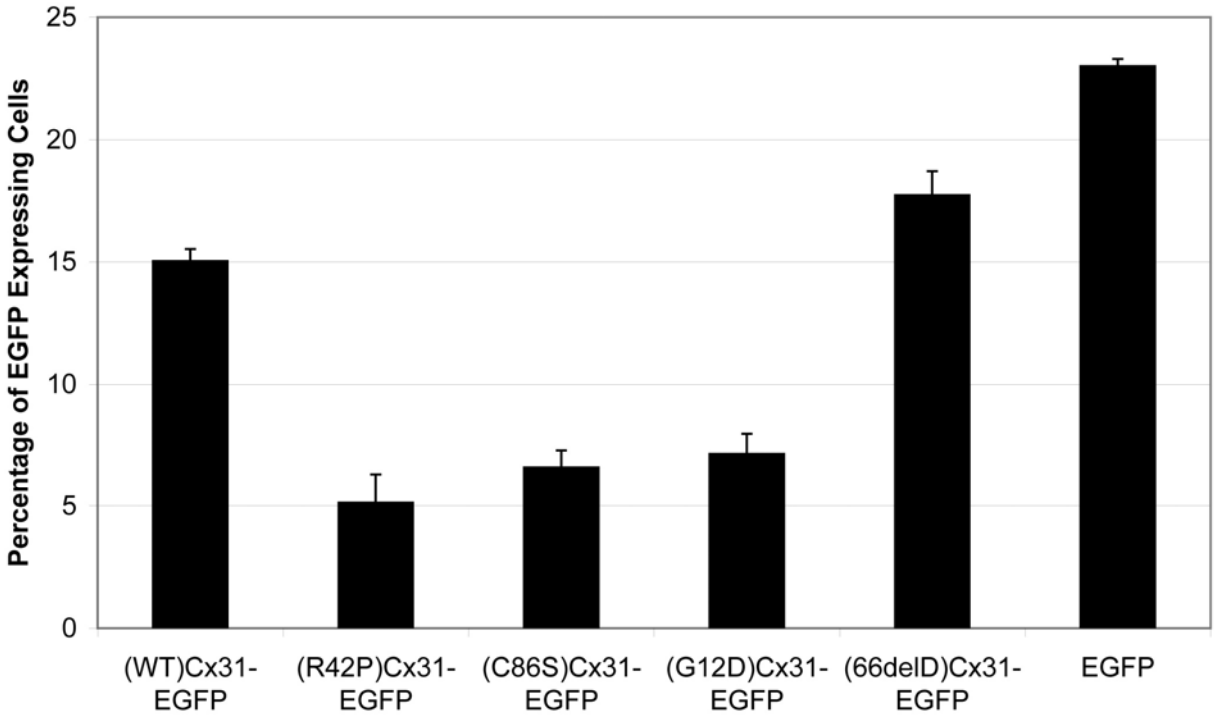
Supplementary Figure 1 – The punctate staining observed in HeLa cells expressing the neuropathy mutant (66delD)Cx31-EGFP (A) is distinct from the diffuse non-punctate localisation of EGFP alone (B). DAPI-stained nuclei are shown in blue.



Supplementary Figure 2 – Non-EGFP-tagged Cx31 protein displays a similar localisation as those tagged with EGFP, and causes a similar upregulation / nuclear translocation of BiP/GRP78 and ATF6. Constructs were generated to express (WT)Cx31, (C86S)Cx31 and (66delD)Cx31 without the EGFP tag. Staining with a commercial Cx31 antibody revealed a typical localisation pattern for the (WT)Cx31 (A and C), (C86S)Cx31 (E and G) and (66delD)Cx31 (I and K) when compared to the EGFP-tagged versions. Co-staining with antibodies against BiP/GRP78 (B, F and J) and ATF6 (D, H and L) revealed the non-tagged version of (C86S)Cx31 caused a nuclear translocation and/or upregulation of these proteins in an identical way to (C86S)Cx31-EGFP. In contrast, similarly to the EGFP-tagged versions, the (WT)Cx31 and (66delD)Cx31 constructs did not exhibit this effect.



Supplementary Figure 3 – An immunofluorescence approach confirms that the (C86S)Cx31-EGFP and (66delD)Cx31-EGFP are saponin insoluble but triton soluble, suggesting they are membrane-bound. Three low-power fields of view were taken of a near-confluent mono-layer of HeLa cells expressing (WT)Cx31-EGFP, (C86S)C31-EGFP, (66delD)Cx31-EGFP or EGFP alone after being subjected to either saponin extraction or triton extraction prior to paraformaldehyde fixation. The number of green transfected cells were counted in each field of view, and compared to the number of cells in a field that had no detergent treatment. The cytosolic EGFP protein was removed upon extraction with saponin. Like (WT)Cx31-EGFP both mutants tested ((C86S)Cx31-EGFP and (66delD)Cx31-EGFP) were saponin insoluble but triton soluble, indicating they were not present in the cytosol but instead were membrane-bound within the cytoplasm.



Supplementary Figure 4 – The percentage of cells expressing the skin disease mutant constructs (R42P, C86S and G12D) tagged to EGFP is significantly less than either the (WT)Cx31-EGFP, the neuropathy mutant (66delD), or EGFP alone. HeLa cells transfected with each Cx31-EGFP construct were quantified for EGFP expression by FACS analysis to determine the transfection efficiency.