Nesprins comprise a family of multi-isomeric scaffolding proteins that bind to lamin A/C, emerin and SUN1/2 at the nuclear envelope (NE) to form the linker of nucleoskeleton and cytoskeleton (LINC) complex. Mutations in nesprin -1 and -2 contribute to Emery–Dreifuss muscular dystrophy and dilated cardiomyopathy (DCM). We have identified 7 patients harbouring three novel nesprin-1 mutations (R434Q, S566C, N591K) in the C-terminus of nesprin-1α, an evolutionally conserved region containing the lamin and emerin binding domains, following mutation screening in Syne-1 and -2 genes in 218 DCM patients and 210 healthy controls. To explore roles of nesprin-1 in pathogenesis of DCM, overexpression of GFP-nesprin wildtype and mutants was performed. This induced significantly increased numbers of convoluted nuclei in the cells transfected with the S566C construct although nesprin-1alpha was present at the NE in all transfected cells. Immunofluorescence demonstrated lamin A/C was mislocalised by the S566C and SUN2 was mislocalised by all mutants, but emerin was mislocalised by both WT and mutants. Furthermore, GST pull-down showed all nesprin-1 mutants had significantly reduced binding affinity to lamin A, but not emerin. The data suggest nesprin-1 plays an important role in maintaining LINC complex integrity. Future work will investigate if the mutants disrupt the LINC, uncoupling the NE from the cytoskeleton and leading to aberrant activation of mechanotransduction signalling and/or perturbing C2C12 differentiation; processes that may underlie the mechanism of cardiomyopathy.