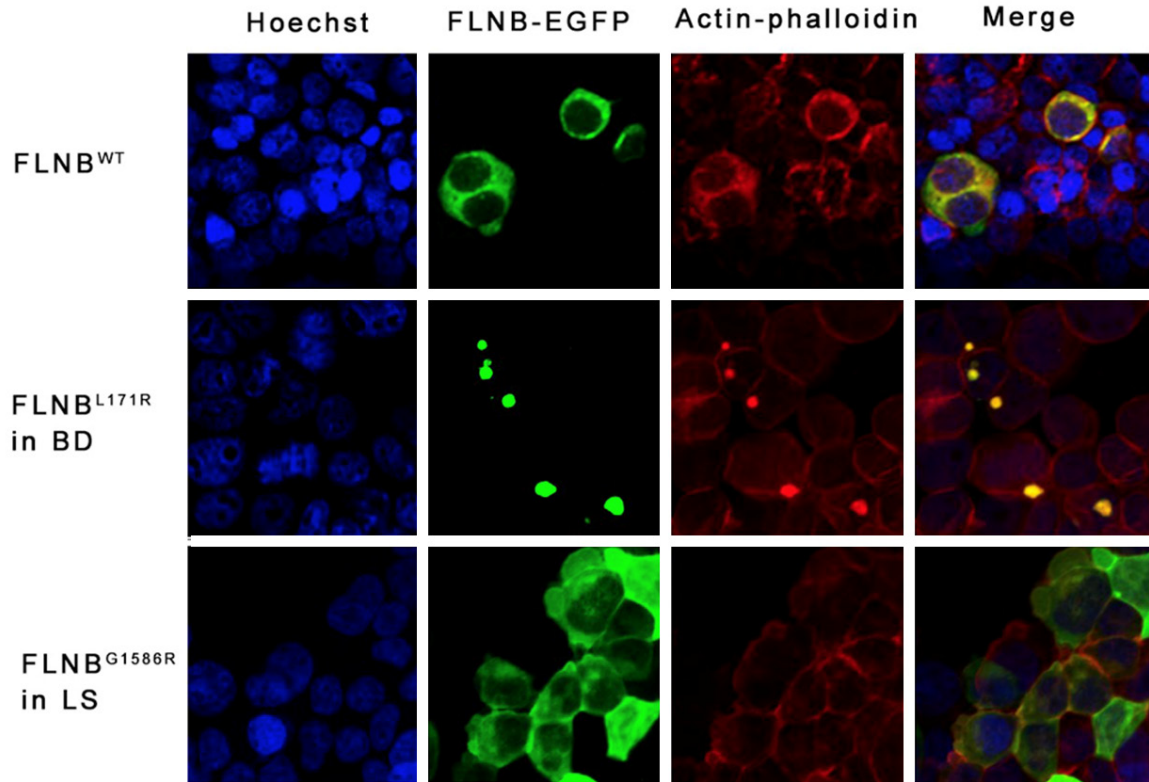
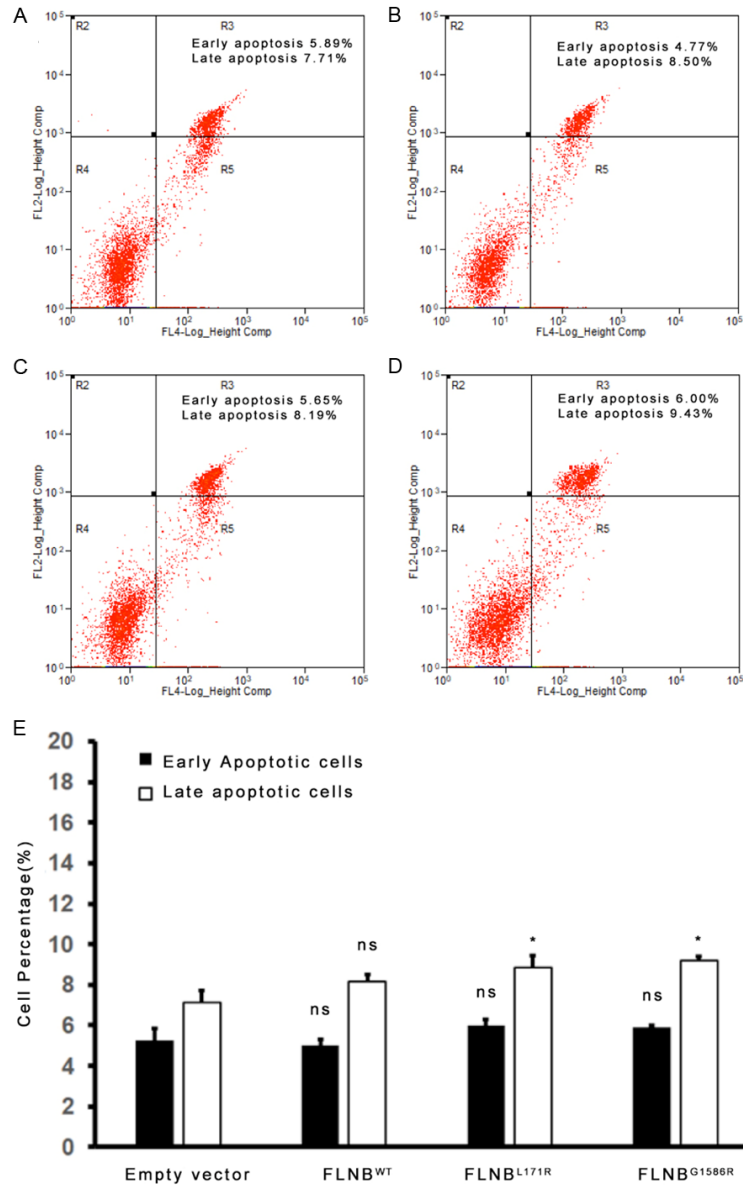


Comparative analysis of FLNB missense mutated disease spectrum



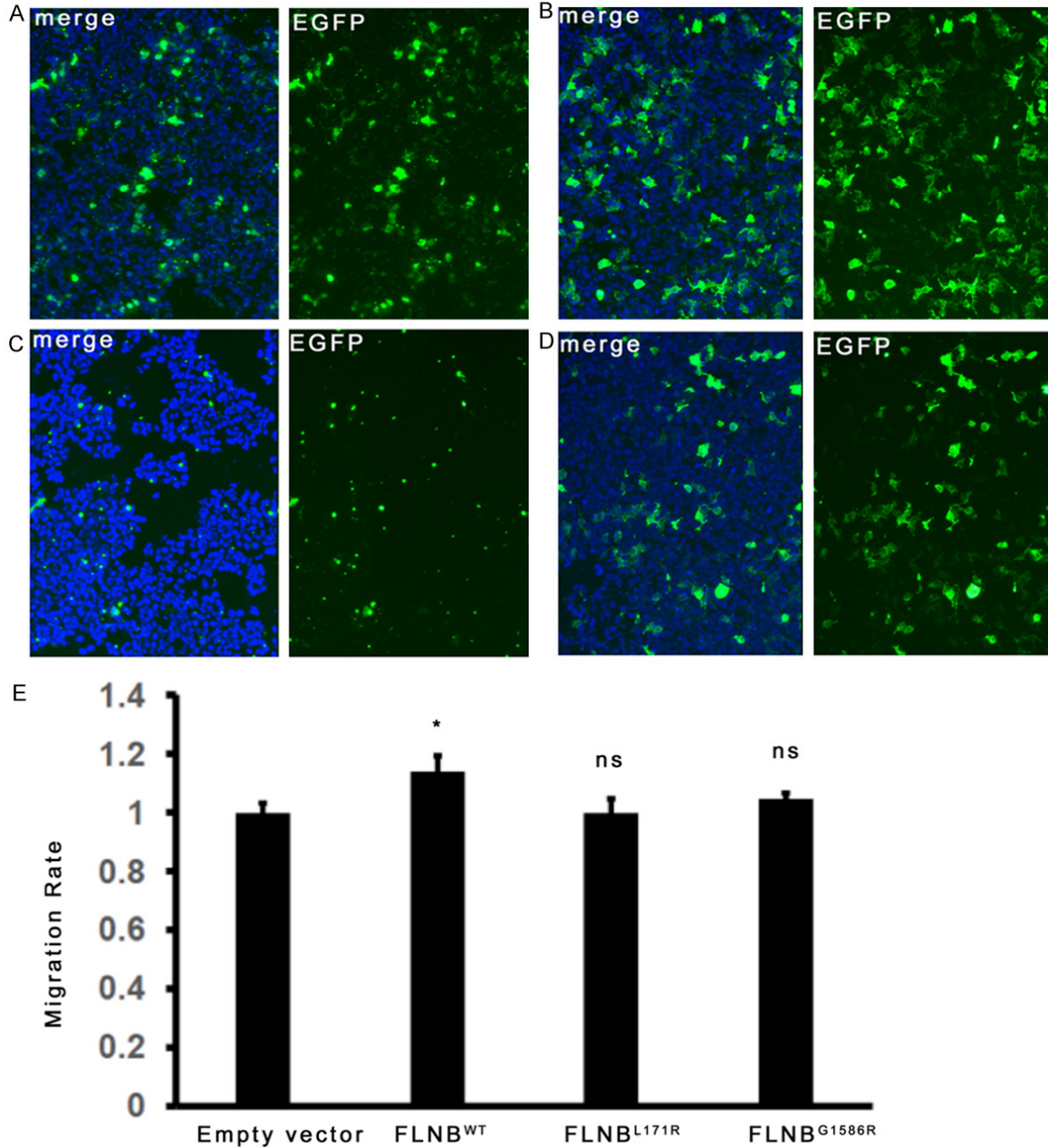
Supplementary Figure 1. *FLNB*, *FLNB*^{G1586R} and *FLNB*^{L171R} were transfected into HEK293 cells. Accumulation of FLNB-actin in HEK293 cells was visualized with confocal immunofluorescence microscopy. Nuclei were marked with Hoechst dye. In HEK293 cells expressing *FLNB*^{WT} and *FLNB*^{G1586R} (a mutation associated with LS), FLNB (EGFP marked) was evenly distributed within the cytoplasm demonstrating a fine meshwork, consistently with the expression of actin cytoskeleton (phalloidin marked). HEK293 cells expressing *FLNB*^{G1586R} demonstrated no obvious changes in cell shape. However, in HEK293 cells expressing *FLNB*^{L171R} (a mutation associated with BD), there showed a globular accumulation of FLNB. WT: wild type; BD: boomerang dysplasia; LS: Larsen syndrome.

Comparative analysis of FLNB missense mutated disease spectrum



Supplementary Figure 2. Apoptosis of HEK293 cells was analyzed with flow cytometry. Hoechst staining was performed for early apoptosis of cells and Propidium iodide (PI) staining was performed for late apoptosis of cells. There were no significant differences in apoptosis ratio among HEK293 cells expressing empty vector (A), FLNB^{WT} (B), FLNB^{L171R} (a mutation associated with BD) (C) and FLNB^{G1586R} (a mutation associated with LS) (D). (E) Bar plot of percentages of apoptosis cells in different cell systems. Three experimental replicates were performed. Student's t-test was used for the statistical analysis. All values were compared to Empty vector group. ns: not significant; ***: p -value < 0.001; **: p -value < 0.01; *: p -value < 0.05. Error bars indicate SD. WT: wild type; BD: boomerang dysplasia; LS: Larsen syndrome.

Comparative analysis of FLNB missense mutated disease spectrum



Supplementary Figure 3. Transwell assay of HEK293 cells expressing empty vector, FLNB^{WT}, FLNB^{L171R} and FLNB^{G1586R}. The cells that migrated through the membrane and adhered to the lower surface of the membrane were fixed with 4% paraformaldehyde and stained with DAPI. For quantification, cells were counted under a microscope in four random fields. There was no significant difference in cell migration rates among HEK293 cells expressing empty vector (A), FLNB^{WT} (B), FLNB^{L171R} (a mutation associated with BD) (C), and FLNB^{G1586R} (a mutation associated with LS) (D). (E) Bar plot of cell migration rates in different cell systems. Three experimental replicates were performed. Student's t-test was used for the statistical analysis. All values were compared to Empty vector group. ns: not significant; ***: p -value < 0.001; **: p -value < 0.01; *: p -value < 0.05. Error bars indicate SD. WT: wild type; BD: boomerang dysplasia; LS: Larsen syndrome.