FIGURE S2. (A) Degradation of WT and D255E Vangl1 proteins expressed in MDCK cells. Stably transfected MDCK cells expressing either GFP-Vangl1 WT or D255E were grown to confluence and treated with cycloheximide (20 μg/ml) for 4 hrs, either in the absence or presence of the proteasome inhibitor MG132 (5 μg/ml) or the lysosome inhibitor chloroquine (100 μM). Cell lysates (50 μg) were subjected to gel electrophoresis and immunoblotting for Vangl1 using the anti-c-Myc antibody. Tubulin was used as an internal loading control. The rapid degradation of GFP-Vangl1 D255E was prevented by MG132, but not by chloroquine, indicating that the D255E protein is mainly degraded by proteasome. These data are representative of two such experiments performed. (B) ER retention of GFP-Vangl1 D255E does not induce UPR. Confluent non-transfected MDCK cells or stably transfected MDCK cells expressing GFP-Vangl1 WT and D255E were lysed, and western blots performed on cell lysates (50μg) using the anti-c-Myc antibody to detect Vangl1, and antibodies directed against UPR markers (anti-BiP, anti-calnexin and anti-PDI). Tubulin was used as an internal loading control. The expression pattern of the UPR markers is identical in all cells, indicating that the ER stress response is not induced by the accumulation of D255E in the ER. These data are representative of two such experiments performed.

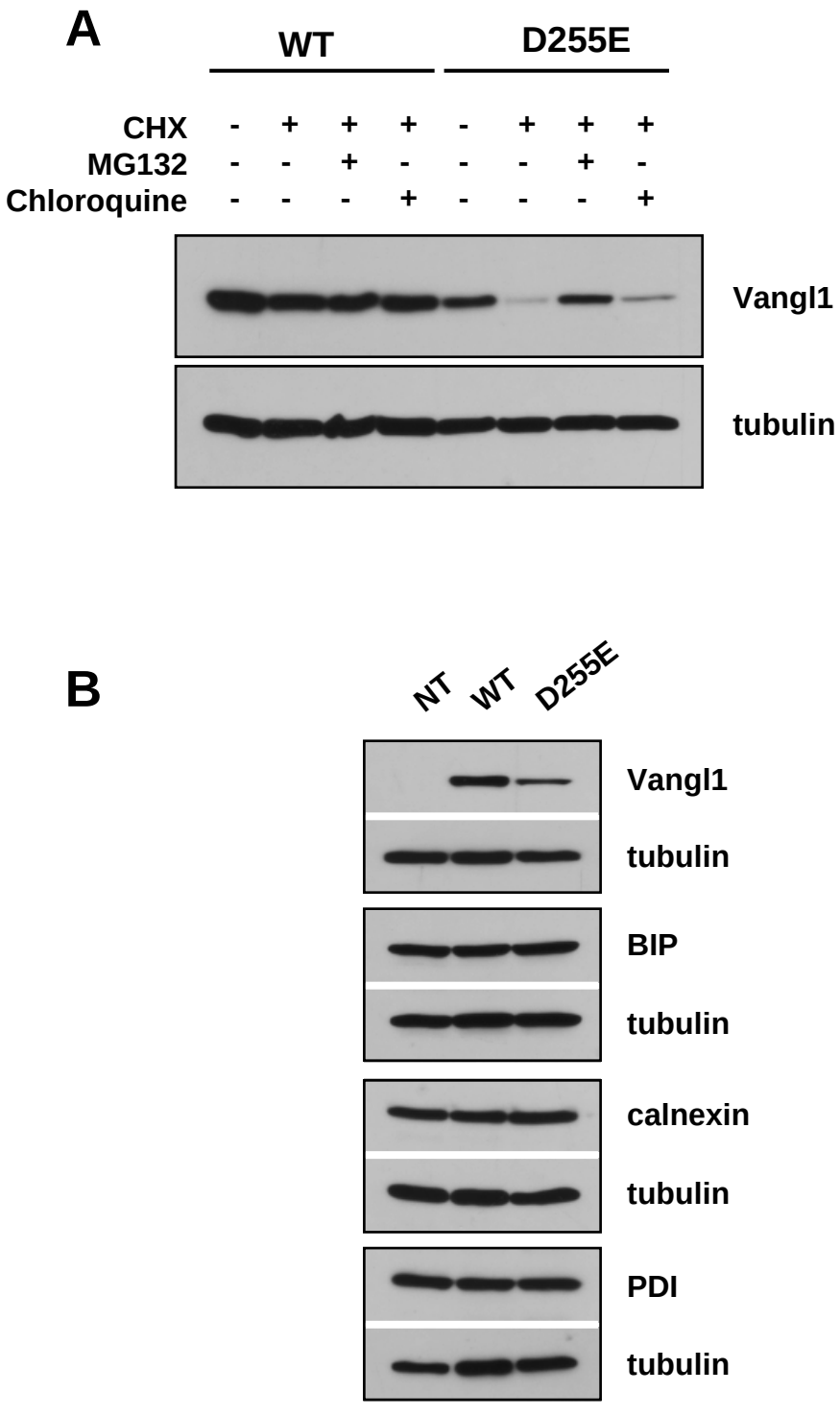
A
WT
D255E
CHX - + + + - + + +
MG132 - - + - - - + -
Chloroquine - - - + - - - +
Vangl1
tubulin
B
NT
WT
D255E
Vangl1
tubulin
BIP
tubulin
calnexin
tubulin
PDI
tubulin

Figure S2