Ninagawa et al., http://www.jcb.org/cgi/content/full/jcb.201504109/DC1

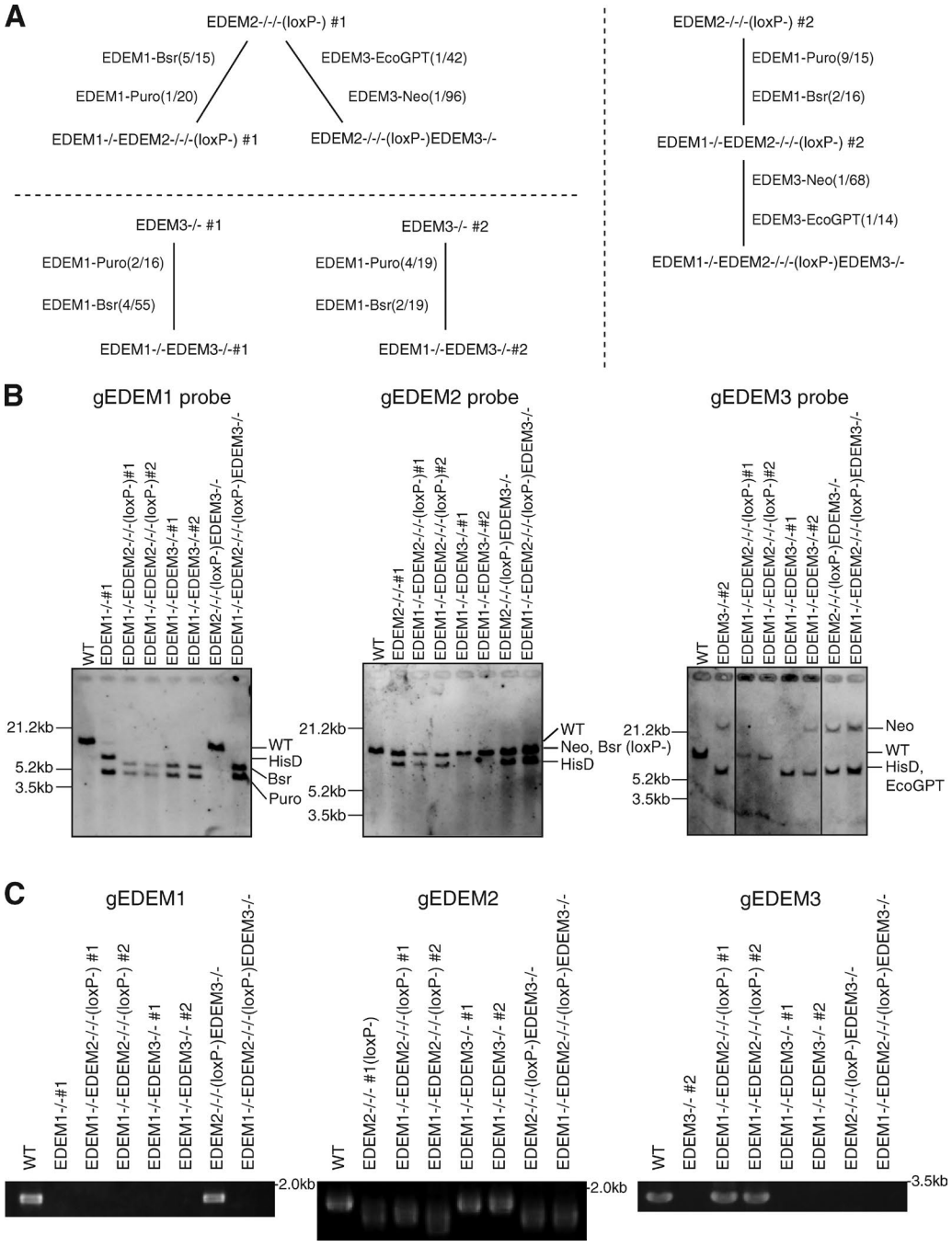

Figure S1. **Generation of DT40 cells deficient in two or three of gEDEM1/2/3.** (A) Strategy for obtaining gEDEM1/2, gEDEM2/3, and gEDEM1/3 double KO cells as well as gEDEM-TKO cells. The right number in the parentheses states the number of clones analyzed by Southern blot hybridization and the left number states the number of clones in which the focused locus was correctly targeted. (B) Southern blot hybridization of genomic DNA isolated from parental DT40 cells (+/+) and cells of various genotypes using the 3′ probe to confirm recombination of the gEDEM1/2/3 genes. (C) RT-PCR to amplify cDNA corresponding to gEDEM1/2/3 mRNA using total RNA prepared from DT40 cells of various genotypes.

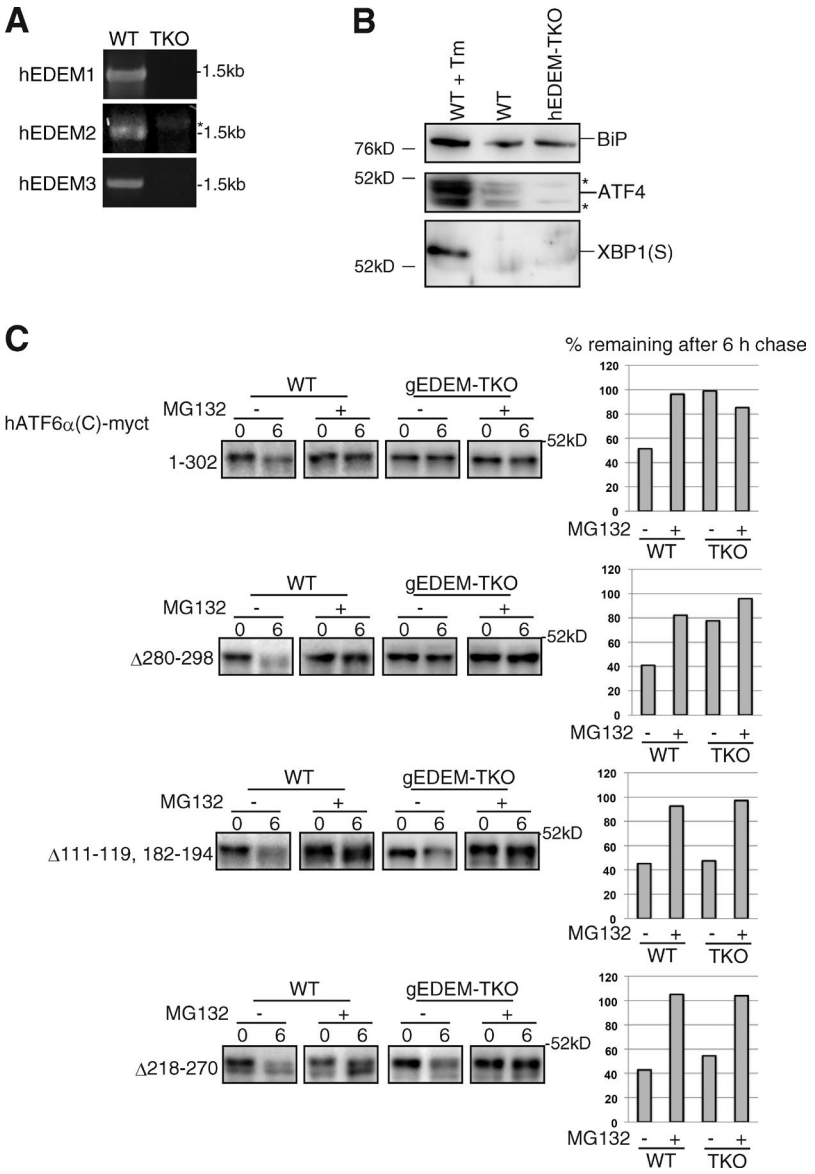

Figure S2. **Generation and characterization of hEDEM-TKO cells and effect of MG132 on the stability of hATF6α(C) derivatives in DT40 cells.** (A) Genomic PCR to confirm homologous recombination in hEDEM-TKO cells. The asterisk denotes a nonspecific band. (B) Immunoblotting of cell lysates prepared from tunicamycin (Tm, 2 µg/ml, 8 h)-untreated or -treated WT HCT116 cells and from hEDEM-TKO cells using anti-KDEL (for BiP), anti-ATF4, and anti-XBP1. XBP1(S) and asterisks denote the spliced form of XBP1 and nonspecific bands, respectively. (C) WT and gEDEM-TKO cells expressing one of the four hATF6α(C) derivatives by transfection were untreated (–) or treated (+) with 30 µM MG132. 1 h later, cycloheximide chase experiments were performed for 6 h without removing MG132. This experiment was completed once.

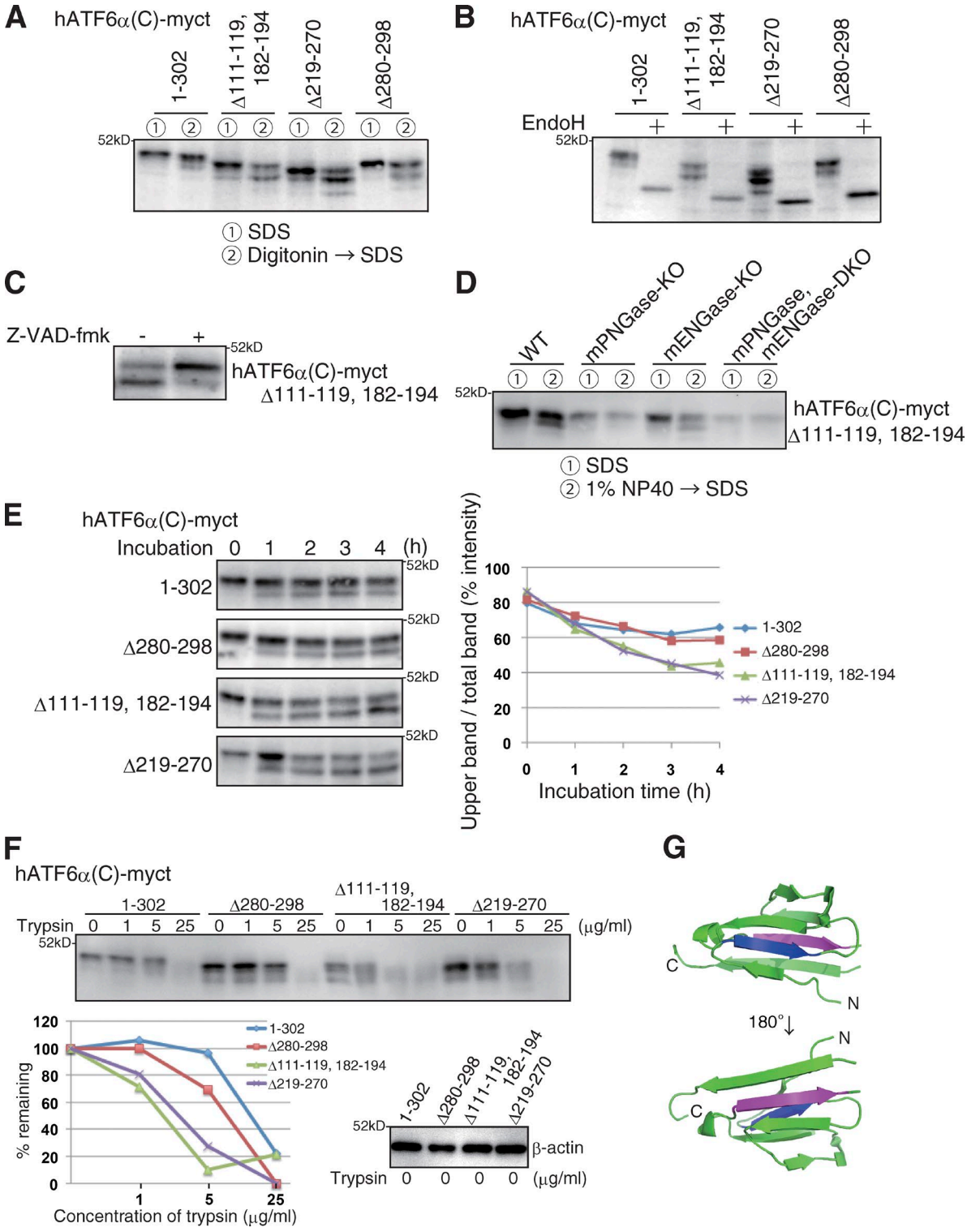

Figure S3. **Analysis of folding status of hATF6α(C) derivatives.** (A) WT DT40 cells expressing one of the four hATF6α(C) derivatives by transfection were (1) lysed directly in SDS sample buffer or (2) lysed with 1% digitonin buffer (containing protease inhibitor cocktail and 10 μM MG132), left on ice for 20 min, and centrifuged at 14,000 rpm for 10 min at 4°C. Cleared lysates were left on ice for 3 h and then mixed with 2× SDS sample buffer. These samples were analyzed by immunoblotting using anti–c-myc. (B) Samples prepared as in A (method 2) were untreated or treated with EndoH, and then analyzed by immunoblotting as in A. (C) WT DT40 cells expressing transfected hATF6α(C)-myctΔ111–119, 182–194 were lysed as in A (method 2) with (+) or without (–) 2 μM Z-VAD-fmk and then analyzed as in A. (D) WT MEF cells, MEF cells deficient in PNGase, MEF cells deficient in ENGase, and MEF cells deficient in both PNGase and ENGase, each expressing transfected hATF6α(C)-myctΔ111–119, 182–194, were analyzed as in A. (E) gEDEM-TKO cells expressing one of the four hATF6α(C) derivatives by transfection were subjected to PNGase sensitivity assay in cell lysates (immunoblotting with anti–c-myc). The data shown are from a single representative experiment out of two repeats. (F) gEDEM-TKO cells expressing one of the four hATF6α(C) derivatives by transfection were subjected to trypsin sensitivity assay in cell lysates (immunoblotting with anti–c-myc). The data shown are from a single representative experiment out of three repeats. (G) Crystal structure of human CD3-δ (PDB accession no. 1XIW). The blue and magenta indicate the second and sixth β-sheet, respectively.