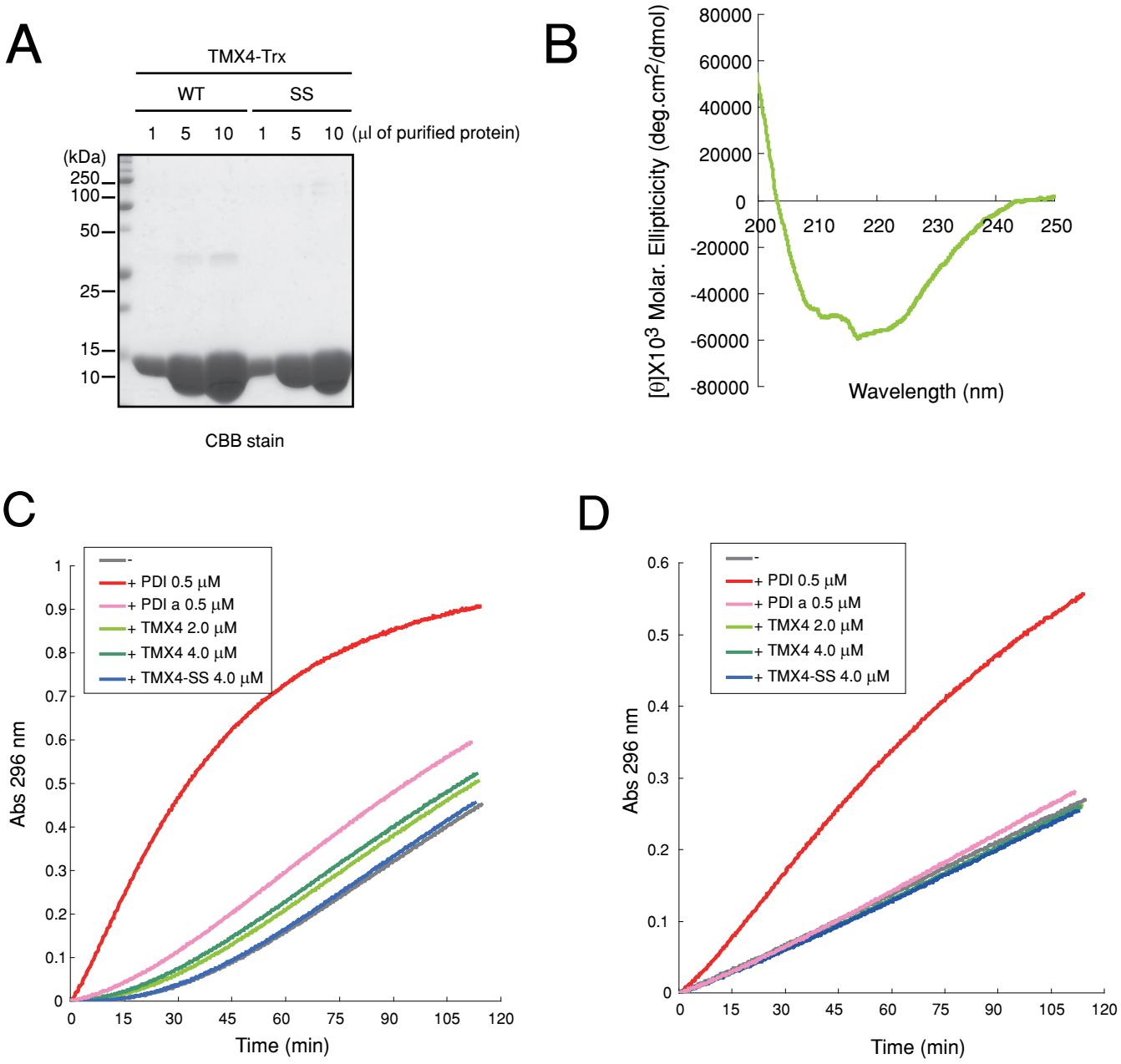
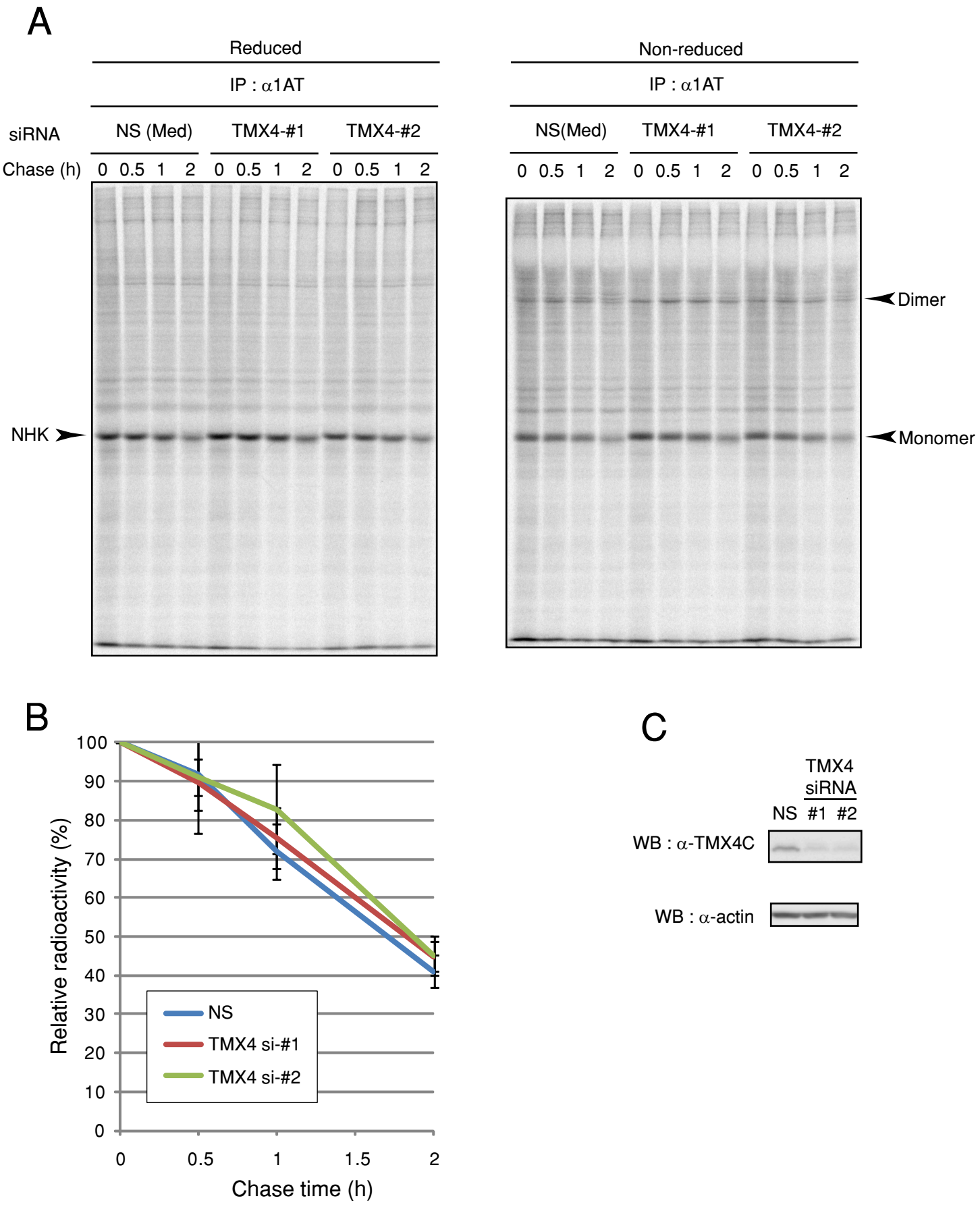
Supplemental information

Supplemental Figure 1. Amino acid sequence of TMX4. The amino acid sequences of human TMX4 and mouse TMX4 were aligned. The predicted signal sequence is indicated in blue (the gray arrowhead indicates the putative cleavage site). The amino acid sequence of the Trx.like domain is shown in red and the CXXC active site motif is boxed in red. The transmembrane region, N-glycosylation site, cysteine residues, and ER membrane retention signal are highlighted with green, pink, yellow, and blue, respectively.



Supplemental Figure 2. *In vitro* analysis of recombinant TMX4-Trx. A, purification of recombinant TMX4–WT–Trx and TMX4–SS–Trx, in which the cysteines of the CXXC motif are substituted with serines. B, CD spectra of TMX4–WT–Trx. C and D, oxidase and isomerase activity of TMX4–Trx. Oxidase (C) and isomerase (D) activities were measured spectrophotometrically by monitoring (at 296 nm) the RNase A-catalyzed hydrolysis of cCMP. This hydrolysis is concomitant with the enzyme-catalyzed folding of reduced (C) and scrambled (D) RNase A in redox buffer conditions simulating the ER lumen ([GSSG]:[GSH] = 1:3).



Supplemental Figure 3. Effect of endogenous TMX4 knockdown on ERAD. A, HEK293 cells were labeled for 15 min with [^{35}S]–methionine-cysteine at 24 h after NHK transfection (72 h after siRNA transfection) and chased for the indicated times. The metabolically labeled NHK were immunoprecipitated with an α1-antitrypsin antibody. Non–specific siRNA (NS) was used as control. B, Quantification of ERAD as shown in A. Results are means ± SD of three independent experiments. C, knock down efficiency of TMX4 by siRNA at the protein level. Endogenous TMX4 was efficiently knocked down at 72 h after siRNA transfection.