Table S1. Melting temperatures (T_m) and sequences of forward and reverse primers used of construction of HvTrxh2 mutants

Mutation	T_m	Forward primer	Reverse primer
E86R	78.6	CCATTGCTGAGCAATTCAGTGTACGTGCCATGCCAACGTTCCTG	CAGGAACGTTGGCATGGCACGTACACTGAATTGCTCAGCAATGG
M88A	78.6	GAGCAATTCAGTGTCGAGGCTGCACCAACGTTCCTGTTCATGAAG	CTTCATGAACAGGAACGTTG GTGCAGCCTCGACACTGAATTGCTC
M88G	78.2	GCAATTCAGTGTCGAGGCCGGCCCAACGTTCCTG	CAGGAACGTTGGGCCGGCCTCGACACTGAATTGC
M88L	78.8	GAGCAATTCAGTGTCGAGGCTCTTCCAACGTTCCTGTTCATG	CATGAACAGGAACGTTGGAAGAGCCTCGACACTGAATTGCTC
M88P	78.5	GCAATTCAGTGTCGAGGCTCCTCCAACGTTCCTGTTCATGAAGG	CCTTCATGAACAGGAACGTTGGAGGAGCCTCGACACTGAATTGC
A106G	78.7	GGACAGGGTTGTCGGTGGTATCAAGGAGGAACTG	CAGTTCCTCCTTGATACCACCGACAACCCTGTCC
A106P	78.25	GACAGGGTTGTCGGGCCCATCAAGGAGGAACTGAC	GTCAGTTCCTCCTTGATGGGCCCGACAACCCTGTC
A106S	78.8	GACAGGGTTGTCGGATCCATCAAGGAGGAACTGAC	GTCAGTTCCTCCTTGATGGATCCGACAACCCTGTC
A106Y	78.6	GGACAGGGTTGTCGGTTATATCAAGGAGGAACTGACCGC	GCGGTCAGTTCCTCCTTGATATAACCGACAACCCTGTCC

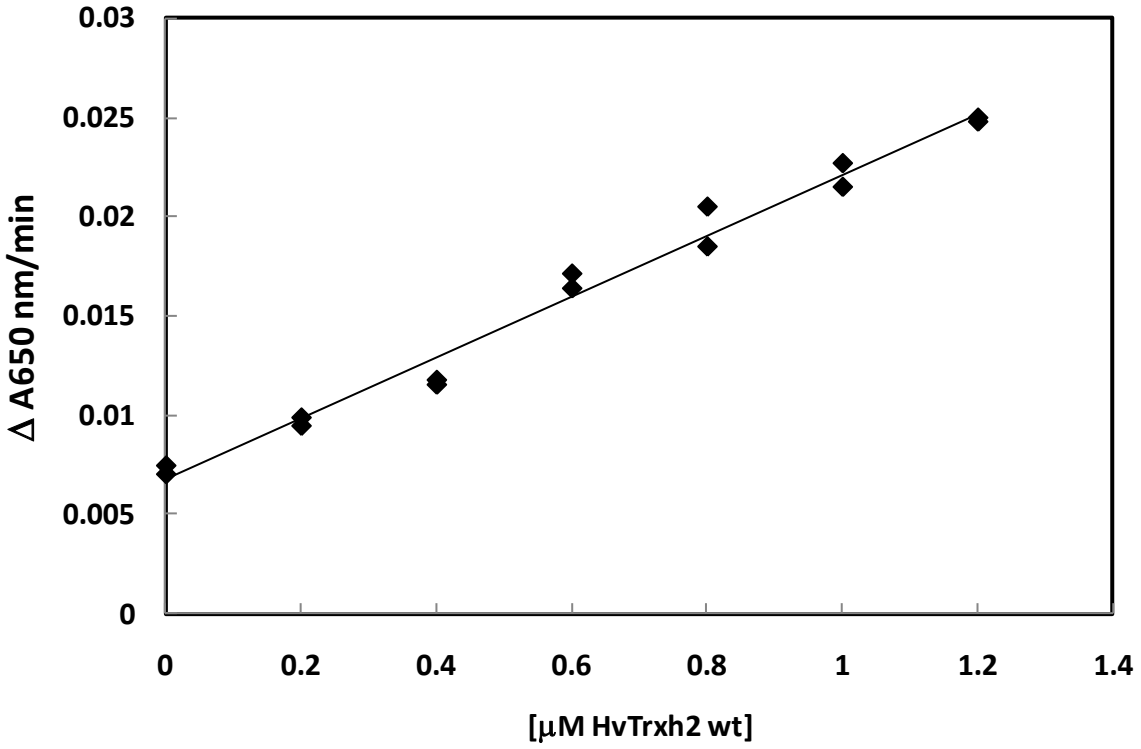

Figure S1. Reduction of disulfide bonds in bovine insulin by HvTrxh2 wt (0.2−1.0 μM) was assayed as described (see Experimental Procedures). Turbidity was monitored as absorbance at 650 nm and the rates of absorbance change at 650 nm per minute in the interval between 0.1 and 0.2 absorbance units were determined.

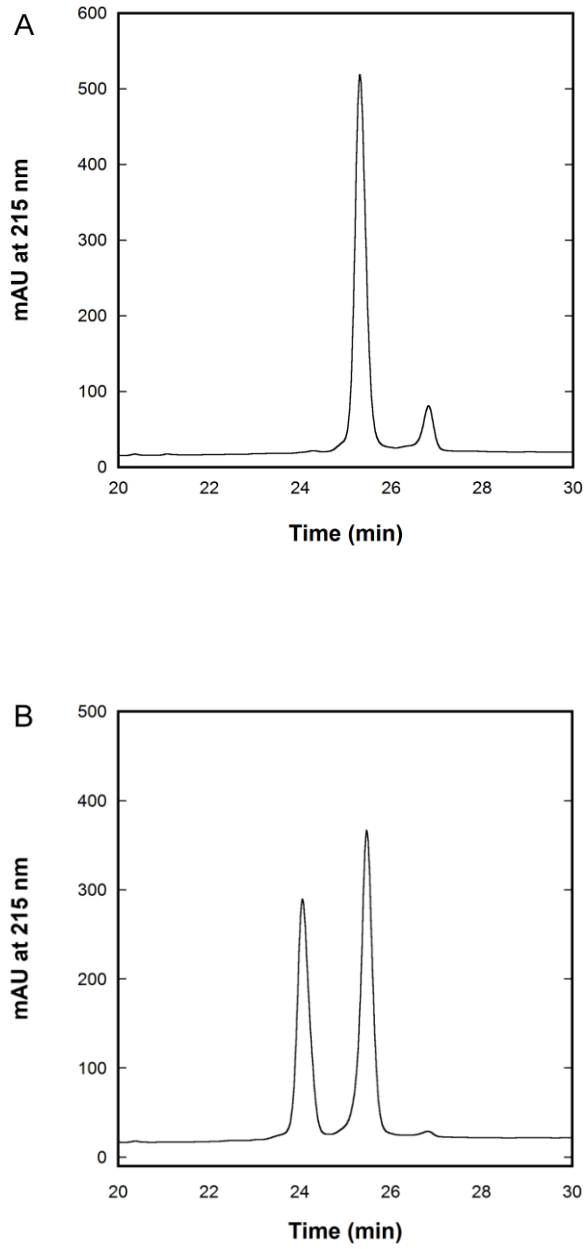

Figure S2. Chromatograms of HvTrxh2 wt (A) and M88P (B) subjected to a 4 min incubation with DTT (100 μM) and separation on a C18 column. Reduced proteins elute earlier than oxidized and the retention times of wt (A) and M88P (B) are not identical. At the 4-min time point, the wt protein is reduced to ca 90 % whereas the mutant is only reduced to a level of ca 45 %.

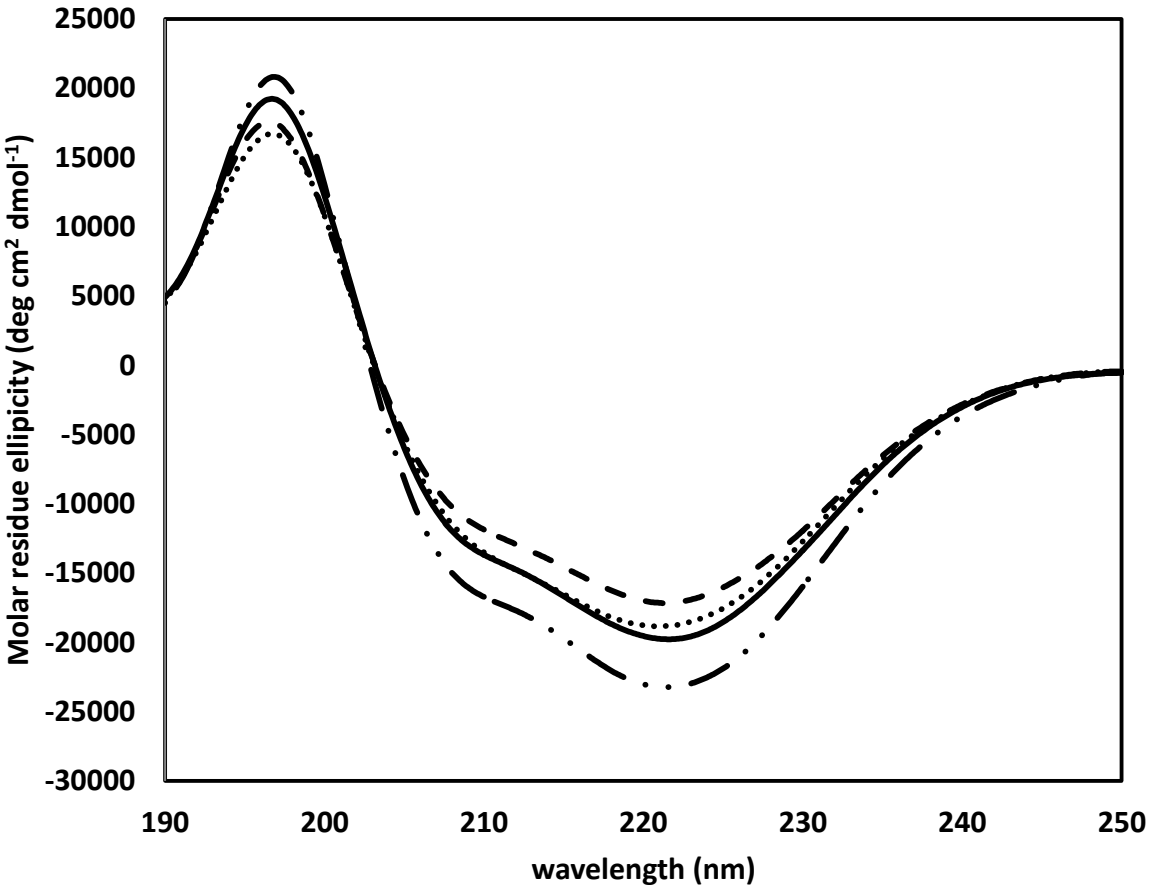

Figure S3. CD spectra of 10 μM HvTrxh2 wt (solid line), M88P (dashed/dotted line), M88G (dotted line) and A106P (dashed line) in 20 mM NaH_2PO_4 pH 7.0 acquired on a Jasco J-600 CD spectrometer at 25°C.

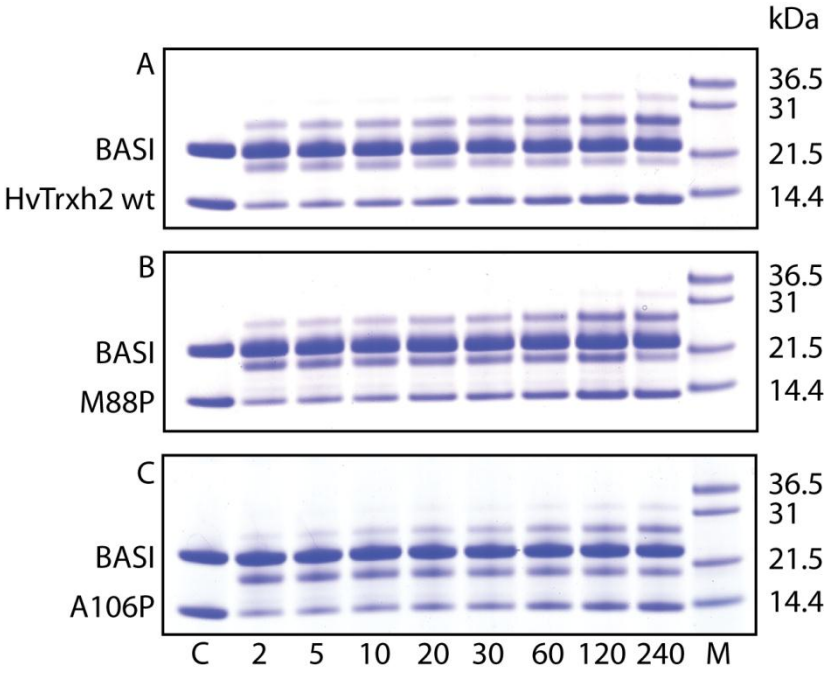

Figure S4. Reduction of BASI by HvTrxh2 wt (**A**), M88P (**B**), and A106P (**C**) visualized by $TMM(PEG)_{12}$ labeling. BASI (20 µM) and reduced Trx (20 µM) were incubated on ice bath in Reaction Buffer. At 2, 5 10, 20, 60, 120 and 240 min, aliquots were removed and reduced disulfides in BASI were modified by $TMM(PEG)_{12}$. Control (C) with non-labeled sample containing BASI and reduced Trx. Molecular weights (kDa) of marker proteins (M) are indicated.