AMINO ACIDS – SUPPLEMENTARY FILE:

Isopeptidase activity of human transglutaminase 2: disconnection from transamidation and characterization by kinetic parameters

Robert Kiraly1, Kiruphagaran Tangaraju1, Zsófia Nagy1, Russell Collighan3, Zoltán Nemes1, Martin Griffin3# and László Fésüs1,2#*

1Department of Biochemistry and Molecular Biology, 2MTA-DE Stem cell, Apoptosis and Genomics Research Group of Hungarian Academy of Sciences, Faculty of Medicine, University of Debrecen, Egyetem tér 1., Debrecen, Hungary H-4012; Phone: +36 52 416-432; Fax: +36 52 314-989; E-mail: fesus@med.unideb.hu
3School of Life and Health Sciences, Aston University, Birmingham, United Kingdom

#These authors contributed equally to this study
*Corresponding author

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Fig. S1 Ca^{2+}-dependence of isopeptidase (a) and transamidase (b) activities of the TG2-I and TG2-T mutants, respectively, in comparison with WT TG2 using kinetic activity assays. EC_{50} values based on the dose-response curves: Isopeptidase activity: TG2 WT EC_{50}=0.44; TG2-I EC_{50}=1.04; Transamidase activity: TG2 WT EC_{50}=0.1; TG2-T EC_{50}=0.96. Data are presented as means with ±SD from two separate experiments done in triplicate.
Optimal pH values of mutant TG2 enzymes for isopeptidase and transamidase activity

Fig. S2 Dependence of isopeptidase and transamidase reaction rate on different pH. Kinetic MOPS buffer based isopeptidase (a) and Tris-HCl buffer based transamidase (b) assays were used to measure the effect of different pH values on isopeptidase and transamidase activities with addition of 10 or 0.83 μg wild type or mutant TG2 enzymes, respectively. Comparison of isopeptidase activities using MOPS and Tris-HCl based buffers (c). Data are presented as means with ±SD from two separate experiments done in duplicate.
**Table S1** Functional effect of nucleotides on isopeptidase and transamidase activities of mutant and WT TG2 enzymes. Table shows the dose responses for GTPγS or GTP normalized to percent of activity in the absence of nucleotide. Different concentrations of the nucleotides were used in the presence of 1 or 10 mM CaCl₂. Isopeptidase or kinetic transamidase assays contained 10 or 0.83 μg of protein, respectively. Data are presented as means with ±SD from two separate experiments done in triplicate. The data analysis was done by GraphPad Prism 5.
Fig. S3 Graphical comparison of the inhibitory effect of nucleotides on activities of mutant and WT TG2. The data are listed in the Table S1. (a) Dose response for GTP and GTPγS normalized to percent of isopeptidase activity in the absence of nucleotides. Different concentrations of GTP or GTPγS were used in the presence of 1 mM CaCl₂ and 10 μg of protein. (b) Dose response for GTPγS normalized to percent of kinetic transamidase activity in the absence of nucleotide. Different concentration of GTPγS was used in the presence of 1 mM CaCl₂ and 0.5 μg of enzymes.