Temme et al. Supplemental Fig. 1

A

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  1  MSAPRVGGVLALGATALGAFYLGTHVERERQHNGSTSGLPRLPGLPTFGT  50

 51  VSAASLIPAQENNVSLTATPSRIGQIMKYGFPGLDHVRSHSDYVLSYDRR  100

101  NRVPHWVFEHLTAESVAKNDAVDRSKCDFKQDESIHPFFRSQNTDYRRSG  150

151  YDRGHMAAAGNHRLHQKHCDETFYLSNMAPQVGQGFNRDAWNTLEAHVRR  200

201  LTKTYSNVYVCTGPLYLPHKEDDGKSYVKYEVIGANTVAVPTHFYKVIVG  250

251  ESADHKLHMESYVMPNQVISNDTPISVFQVPPESVERSAGLLFFDQINRK  300

301  QLTTINGKKV
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B

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1   MSKRKAEDTQSDKMATAEKVAQNDYTIGLV--DPVKDYQKLIETRVQVDEIVDDDV endoGI 1
192       TTPGELSADDAAALSGEFEATLTKENPLEEYRTLMKRFVLTKIIVPDSV endoGI 2

55  TKENFDRTAAAARDVIWRLLFDEAGTSQSNTEKASQLLEEYRGDACFYDPTPYNEW endoGI 1
241 HQASVKKIAAAAREIIWKLLFDGTPSAE-DQNKAAELLQEYKGDAGFYGPDDYNSW endoGI 2

111 IVKLRDEVLKKELLDFWRDVLVKKQLGPCWSRDSDLFDSDDTPPLEFYAHAGCTAP endoGI 1
296 IFNLRDEVLTKELLDFWRDKMVKMELGPSCARDSDYYDNEDPLPFEFYEKAGCKAP endoGI 2

167 FAASLKVRAALEEQASLDQDGPATP                               endoGI 1
352 FEGPVNDD                                                 endoGI 2
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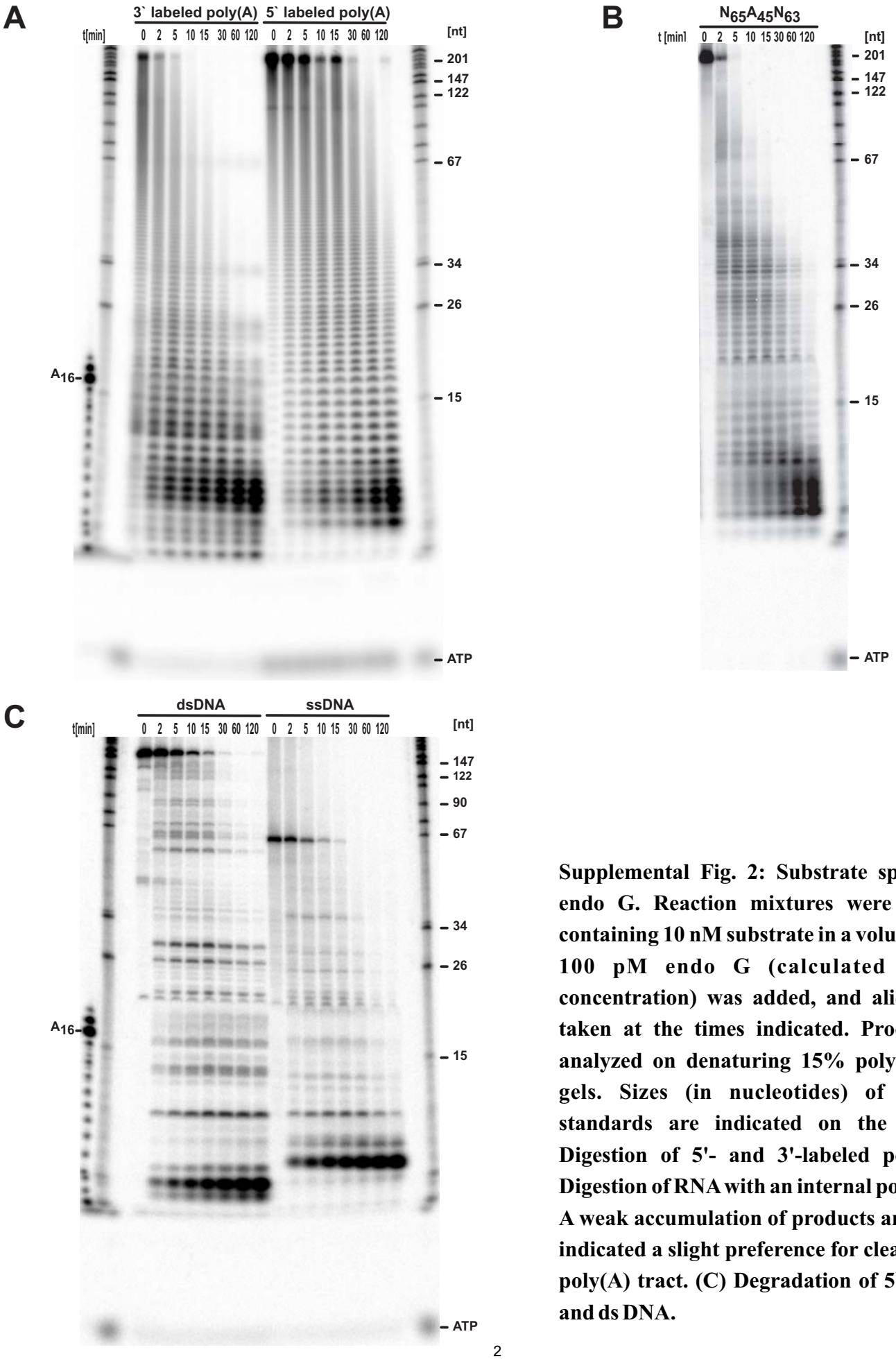
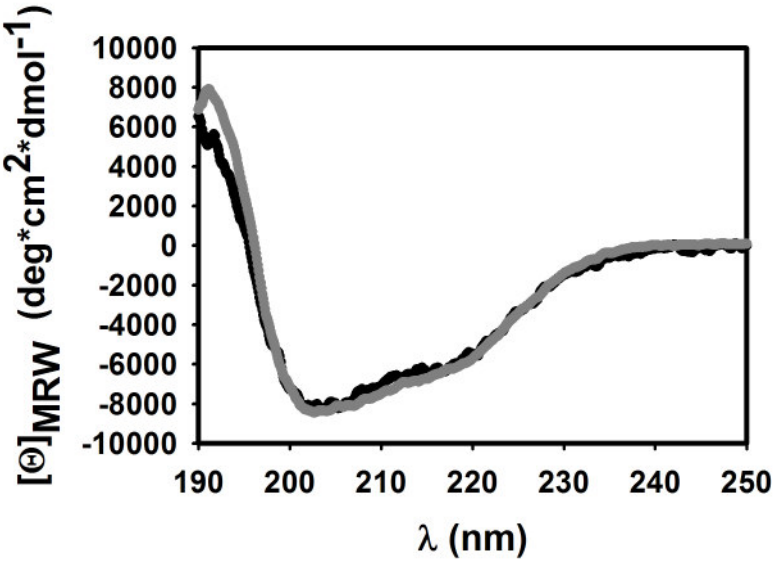
Supplemental Fig.1: (A) Amino acid sequence of endoG with mitochondrial targeting sequence in gray, the active site residues underlined and Asp 187 boxed in grey. (B) Endo GI sequence and tandem repeat structure. The two halves of the endo GI sequence are aligned with each other as indicated by the amino acid numbering. Identical residues are marked in color.

Temme et al. Supplemental Fig. 2



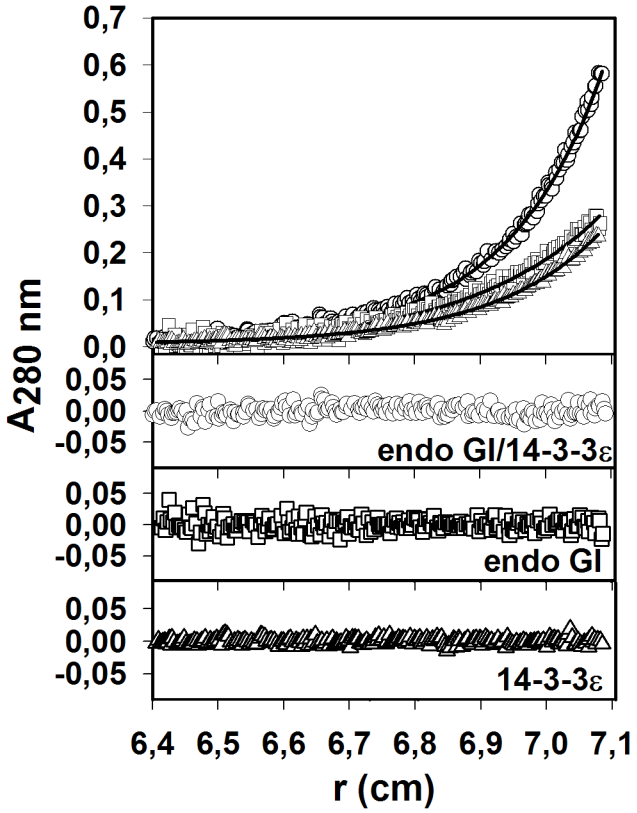
Supplemental Fig. 2: Substrate specificity of endo G. Reaction mixtures were assembled containing 10 nM substrate in a volume of 20 µl. 100 pM endo G (calculated monomer concentration) was added, and aliquots were taken at the times indicated. Products were analyzed on denaturing 15% polyacrylamide gels. Sizes (in nucleotides) of DNA size standards are indicated on the right. (A) Digestion of 5'- and 3'-labeled poly(A). (B) Digestion of RNA with an internal poly(A) tract. A weak accumulation of products around 65 nt indicated a slight preference for cleavage in the poly(A) tract. (C) Degradation of 5`-labeled ss and ds DNA.

Temme et al. Supplemental Fig. 3



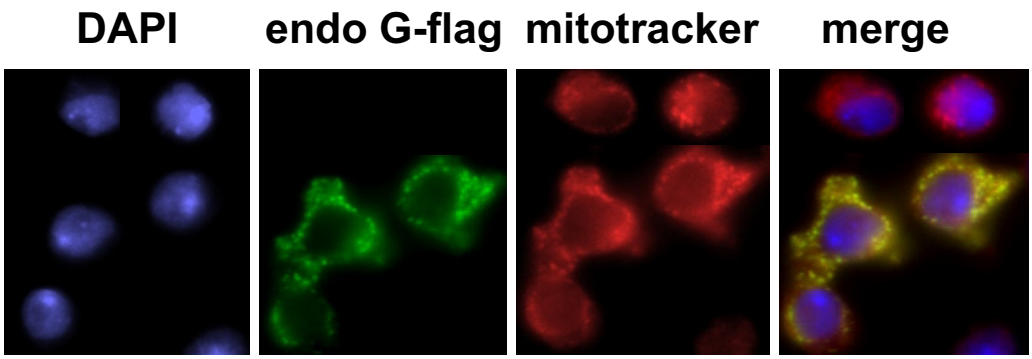
Supplemental Fig. 3: CD spectra of endo G DAGA and endo G N187K. Spectra were recorded at 20°C in a Jasco J710 instrument in a 0.1 mm cuvette. The buffer was 50 mM potassium phosphate, pH 7.5, 100 mM KCl, 10% glycerol. Protein concentrations were 0.45 mg/ml for endo G ΔN53 DAGA (black curve) and 1.0 mg/ml for endo G ΔN55 N187K (grey curve).

Temme et al. Supplemental Fig. 4



Supplemental Fig. 4: Endo GI forms a low affinity complex with 14-3-3 ε. Endo GI at a concentration of 1 µM was incubated with 1 µM dimeric 14-3-3 ε, and complex formation was monitored by analytical ultracentrifugation. The apparent molecular mass was calculated from equilibrium data analysed at 280 nm. As a control, the single proteins were measured at the same protein concentrations. The upper graph shows the experimental data (circle, endoGI/14-3-3 ε; square, endo GI; triangle 14-3-3 ε) and the respective fits (solid lines), the lower panels represent the deviation of the fits to the data. The molecular masses obtained were: endo GI/14-3-3 ε, 89.9 kDa, corresponding to complex formation of > 85 % (theoretical Mr = 101.7 kDa); endo GI, 45.4 kDa; 14-3-3 ε, 68.5 kDa.

Temme et al. Supplemental Fig. 5



Supplemental Fig. 5: Mitochondrial localization of endo G. S2 cells were transiently transfected with an expression construct for endo G DAGA with a C-terminal flag-tag in the pMT/V5-His C vector . 48 hours after transfection, transcription of the endo G plasmid was induced by the addition of 0.5 mM copper sulfate. 24 hours later, cells were exposed to Mitotracker in fresh medium for 30 min and then fixed and stained as indicated. Cells shown here were assembled from two different microscopic images taken from the same sample.