SUPPORTING INFORMATION

Identification of two segments of the γ subunit of ATP synthase responsible for the different affinities of the catalytic nucleotide binding sites

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Table S1

Conservation of Residues in the γ5-15 Segment

Species	Position γ5 γ15
Bos taurus	D I T R R L K S I K N
Gallus gallus	D I T R R L K S I K N
Danio rerio (zebrafish)	D I T I R L K S I K N
Drosophila melanogaster	M I S I R L K S V K N
Saccharomyces cerevisiae	E V E M R L K S I K N
Neurospora crassa	E I E T R L K S I R N
Arabidopsis thaliana	E L R E R I D S V K N
Spinacia oleracea	E L R D R I G S V K N
Escherichia coli	E I R S K I A S V Q N
Clostridium acetobutylicum	I I K R R I K S I T N
Vibrio alginolyticus	E I R N K I G S V K S
Bacillus subtilis	D I K S R I T S T K K
Geobacillus stearothermophilus	D I K T R I N A T K K
Bacillus sp. strain TA2.A1	E I K R R I R S V K N
Wolinella succinogenes	E I R K K I T S V K N
Thermotoga maritima	Q I K R K I N A T Q S
Synechococcus elongatus	A I R D R I K S V R N
Consensus	**h p + h s b p p**

The table lists the amino acids found in selected species in the γ5-15 segment which is responsible for determination of the affinity of the catalytic sites. Residue numbers refer to the *G. stearothermophilus* enzyme. Consensus annotation: **b**, β-branched residue; **h**, hydrophobic residue; **p**, polar residue; **s**, small residue; **+**, positively charged residue.

Table S2

Conservation of Residues in the γ256-265 Segment

Species	Position γ256	γ265
Bos taurus	DKLTLTFNRT	
Gallus gallus	DKLTLTFNRT	
Danio rerio	DKLTLTFNRT	
Drosophila melanogaster	DKLTLTFNRT	
Saccharomyces cerevisiae	NRYSILYNRT	
Neurospora crassa	SKYQILFNRT	
Arabidopsis thaliana	KSLSMVYNRA	
Spinacia oleracea	KTLSINYNRA	
Escherichia coli	KELQLVYNKA	
Clostridium acetobutylicum	DALNIKYNRI	
Vibrio alginolyticus	DDLELVYNKA	
Bacillus subtilis	DSLSLSYNRA	
Geobacillus stearothermophilus	RTLTLSYNRA	
Bacillus sp. strain TA2.A1	ETLTLQFNRA	
Wolinella succinogenes	KSLTIAYNKA	
Thermotoga maritima	RELTLAYNKA	
Synechococcus elongatus	GQLTLVYNKA	
Consensus	**hph a**N**+**	

The table lists the amino acids found in selected species in the γ256-265 segment which is responsible for determination of the affinity of the catalytic sites. Residue numbers refer to the *G. stearothermophilus* enzyme. Consensus annotation: **a**, aromatic residue; **h**, hydrophobic residue; **p**, polar residue; **+**, positively charged residue.

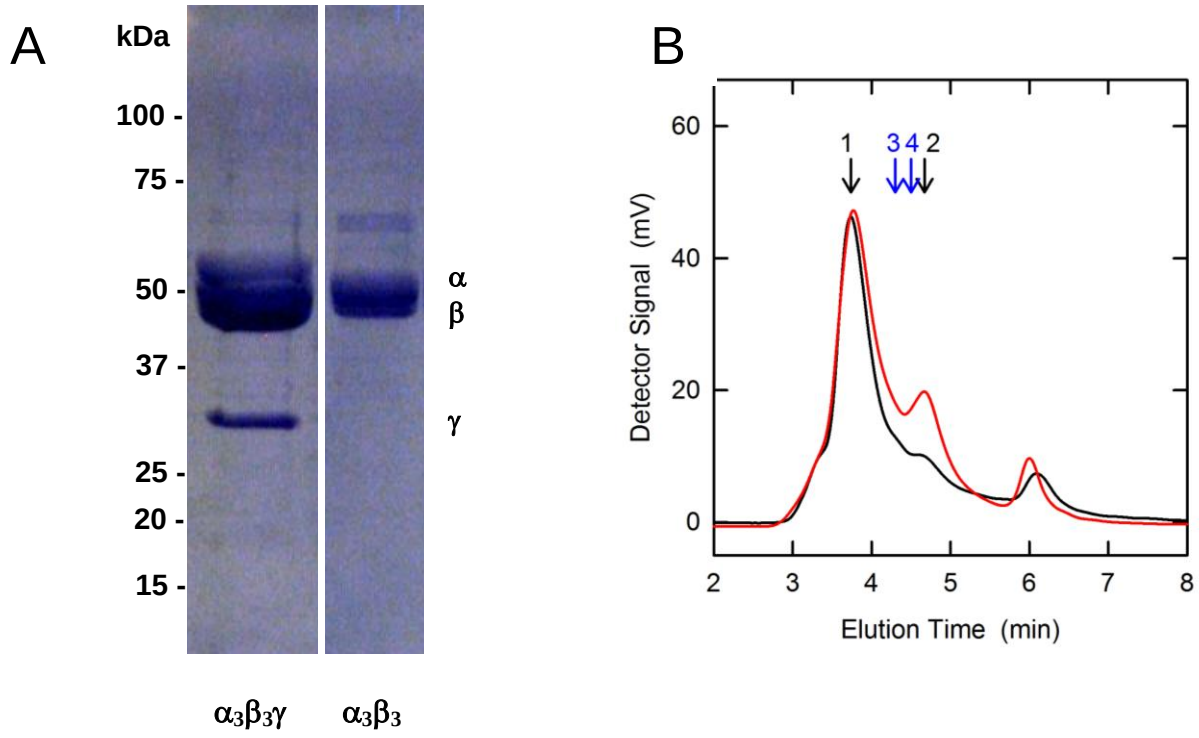

Fig. S1. **Purity and long-term stability of the enzyme preparations.** (A) SDS-PAGE of $\alpha_3\beta_3\gamma$ (left) and $\alpha_3\beta_3$ (right). The gel was run within a week of preparation of the enzymes. The positions of marker protein bands are indicated. (B) Size exclusion chromatography of $\alpha_3\beta_3\gamma$ (black) and $\alpha_3\beta_3$ (red) of samples stored for more than four years as ammonium sulfate precipitate at 4 ˚C. UV absorbance at 280 nm was recorded as signal. Traces represent the average of two runs. Peak 1 at 3.7 min corresponds to $\alpha_3\beta_3\gamma$ or $\alpha_3\beta_3$ (352 kDa and 320 kDa, respectively), peak 2 at 4.7 min to isolated α or β subunits (55 kDa and 52 kDa, respectively). The blue arrows (3 and 4) indicate the elution times (4.3 and 4.5 min) for lactate dehydrogenase (137 kDa) and bovine serum albumin (66 kDa), respectively.

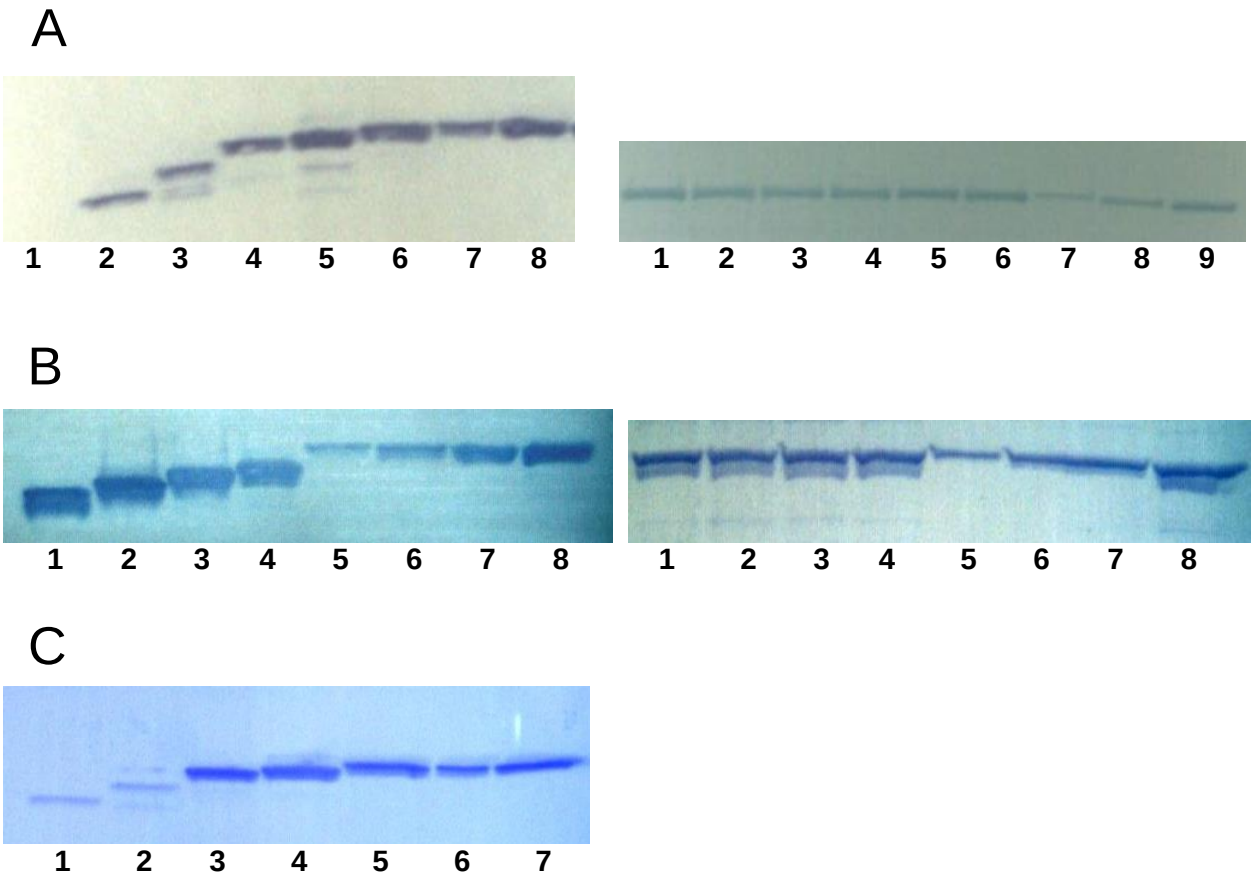

Fig. S2. **γ content of the truncation mutants.** Western blots with a primary antibody against the globular portion of γ are shown. Figs. A and B also show a control experiment with an anti-β antibody (right-hand panel). (A) $\alpha_3\beta_3$ and N-terminal truncations. Left panel: anti-γ antibody. 5 μg protein was loaded per lane, except for lane 7 (2.5 μg). Lane 1, $\alpha_3\beta_3$; lane 2, γΔN45; lane 3, γΔN29, lane 4, γΔN13; lane 5, γΔN9; lane 6, γΔN4; lanes 7 and 8, wild-type $\alpha_3\beta_3\gamma$. Right panel; anti-β antibody. 5 μg protein was loaded per lane, except for lanes 7 (1.25 μg) and 8 (2.5 μg). Lane 1, $\alpha_3\beta_3$; lane 2, γΔN45; lane 3, γΔN29, lane 4, γΔN13; lane 5, γΔN9; lane 6, γΔN4; lanes 7 to 9, wild-type $\alpha_3\beta_3\gamma$. (B) C-terminal truncations. Left panel: anti-γ antibody; right panel: anti-β antibody. 20 μg protein was loaded per lane, except for lanes 5 (2.5 μg), 6 (5 μg), and 7 (10 μg). Lane 1, γΔC36; lane 2, γΔC27; lane 3, γΔC20; lane 4, γΔC14; lanes 5 to 8, wild-type $\alpha_3\beta_3\gamma$. (C) N-terminal truncations after fluorescence binding assay and Centricon (see RESULTS). 5 μg protein was loaded per lane, except for lane 6 (2.5 μg). Lane 1, γΔN45; lane 2, γΔN29, lane 3, γΔN13; lane 4, γΔN9; lane 5, γΔN4; lanes 6 and 7, wild-type $\alpha_3\beta_3\gamma$. The blue tinge in some of the blots is due to the filter setting used to take the picture.

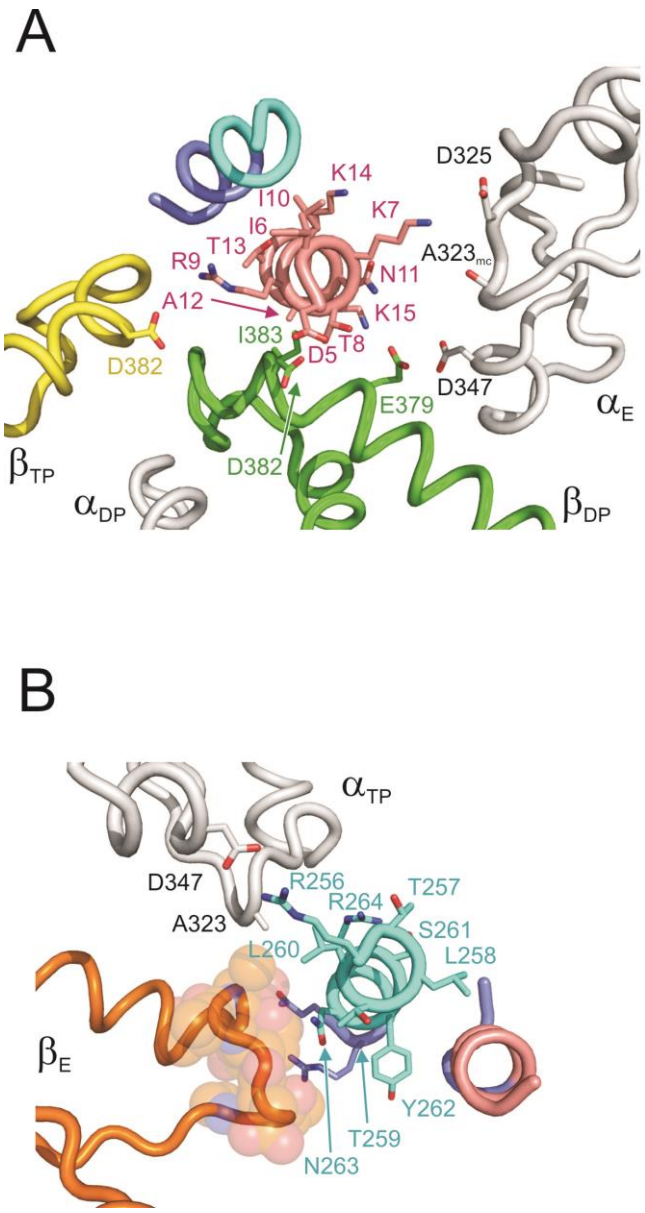

Fig. S3. **Possible interactions of the segments γ5-15 and γ256-265 with α and β.** (A) γ5-15. γ5-15 is shown in pink; subunits α_E and α_{DP} are colored light grey, β_{DP} green, and β_{TP} yellow. Amino acids in α_E, β_{DP} and β_{TP} that might make contact with side chains of γ5-15 are shown in "stick" representation and labeled (subscript "mc" indicates main chain atoms). The portion of the C-terminal helix of γ running antiparallel to segment γ5-15 is shown in cyan (γ256-265) and purple. (B) γ256-265. γ256-265 is shown in cyan, α_{TP} in light grey, and β_E in orange; residue γA265 is hidden in this view. Amino acids in α_{TP} that might make contact with side chains of γ256-265 are shown in "stick" representation and labeled. The most intensive contacts between the C-terminal helix of γ and the "catch loop" of β_E (shown in transparent "spacefill") are formed by the two residues immediately downstream of the identified segment, γR266 and γQ267 (shown in purple and unlabeled). The portion of the C-terminal helix of γ running antiparallel to segment γ256-265 is shown in pink (γ5-15) and purple.