SUPPLEMENTARY FIGURES AND LEGENDS

Figure S1

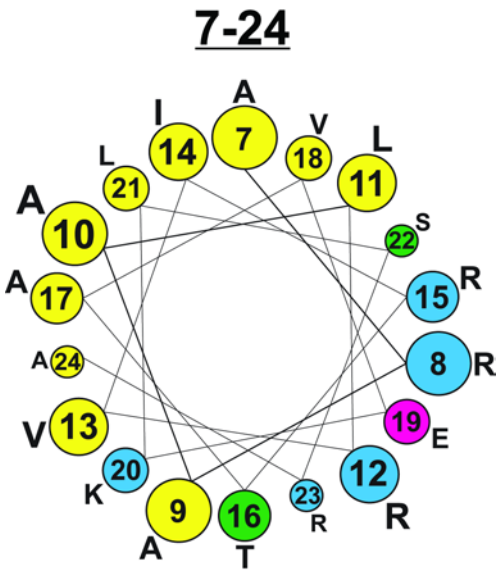

Fig. S1. Helical wheel analysis of the N-terminal amino acid sequence of 2'-PDE. Helical wheel representation of residues 7-24 of 2'-PDE showing the amphipathic nature of the region. The chemical properties of each residue are shown by yellow (hydrophobic residues), blue (basic residues), green (hydrophilic residues) and purple (acidic residues).

Figure S2

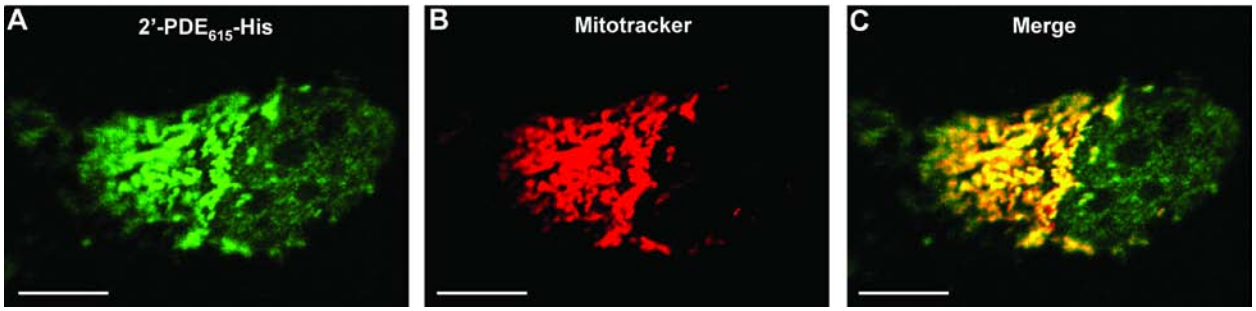

Fig. S2. 2'-PDE co-localizes with mitochondria in HEK293 cells. (A)-(C). Immuno-fluorescence microscopy images of a HEK293 cell transiently expressing the 2'-PDE_{615}-His protein. 36 hours post-transfection, cells were probed with an anti-His-tag antibody (Rockland) and stained with a FITC-conjugated secondary antibody (green). Mitchondria were visualized by treating live cells with Mitotracker (red). Images are merged in panel C. Scale bars; 10 µm.

Figure S3

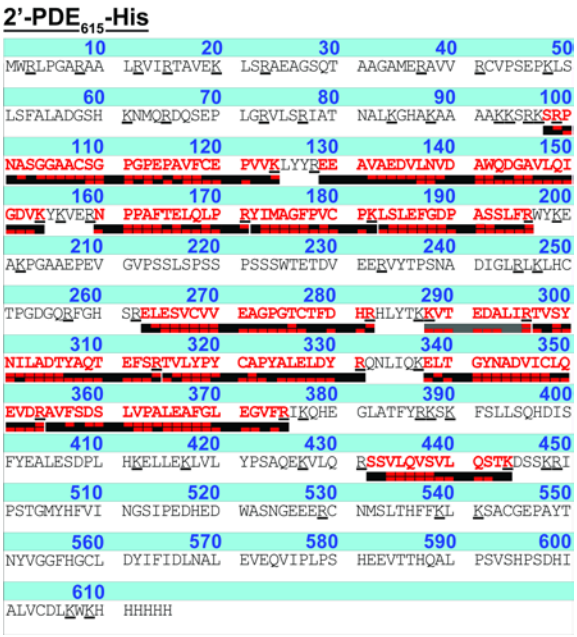

Fig. S3. Identification of 2'-PDE in the 2'-PDE$_{615}$-His-specific mitochondrial preparation from COS-7 cells. The presence of 2'-PDE was determined by LC-MS/MS of tryptic peptides derived from all proteins in the mitochondrial sample. In the listed protein sequence identified peptides are in red, and dark boxes underneath show the detected y-ions (upper red boxes) and b-ions (lower red boxes). Arg(R)/Lys(K) residues are underscored to indicate tryptic clevage sites.

Figure S4

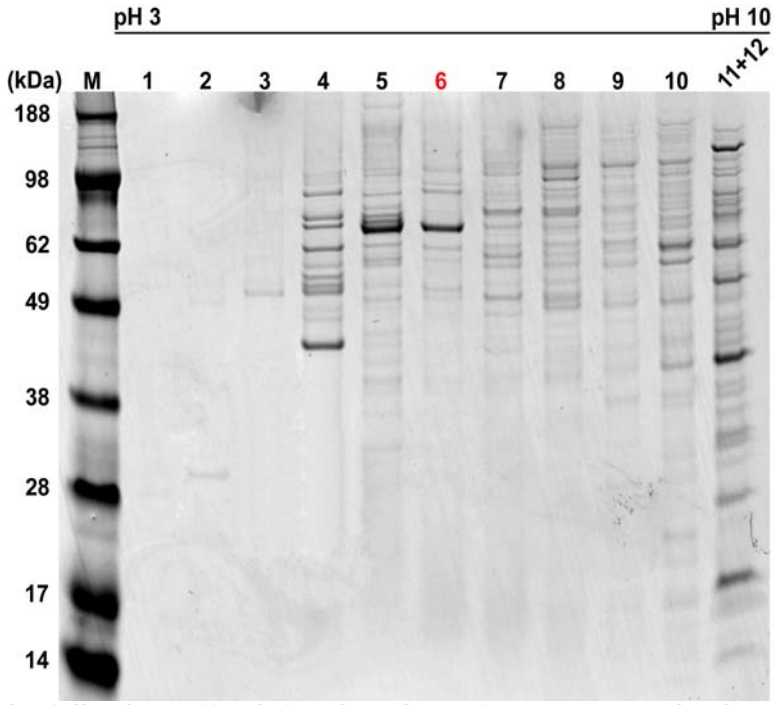

Fig. S4. Gel-analysis following off-gel fractionation of a HEK293 mitochondrial preparation. Subsequent to off-gel isoelectric focusing in the pH interval 3-10, the protein composition within collected samples (1-12) was analyzed by Coomassie blue staining of the gel-electrophoresed fractions. Fraction 6, in which two 2'-PDE-specific peptides was later identified is marked in red. The M lane contains marker proteins of different molecular weights. 20 µl of each protein sample were applied to the gel.

Figure S5

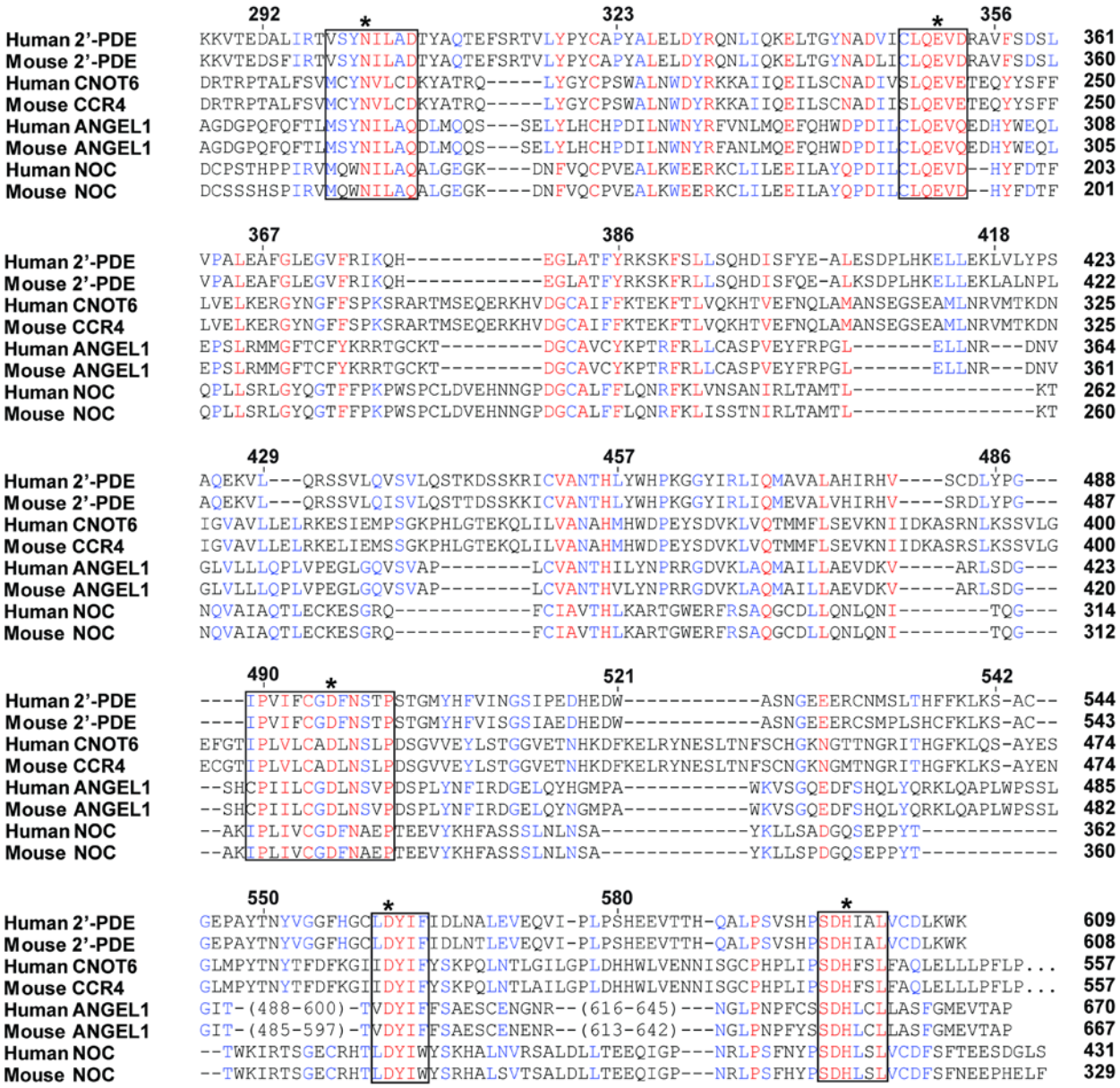

Fig. S5. Sequence alignment of the nuclease domains from known EEP nuclease proteins. Human (*Homo sapiens*) and mouse (*Mus musculus*) 2'-PDE sequences aligned with known deadenylase enzymes belonging to the CCR4, ANGEL, and Nocturnin families. Strictly conserved residues are coloured in red and partly conserved residues in blue. The five critical catalytic residues in the EEP enzymes are conserved amongst all families and marked with asterisks (*) while preserved motifs around these residues are indicated by black boxes. The alignment was done using the MultiAlin multiple alignment tool (74).

Figure S6

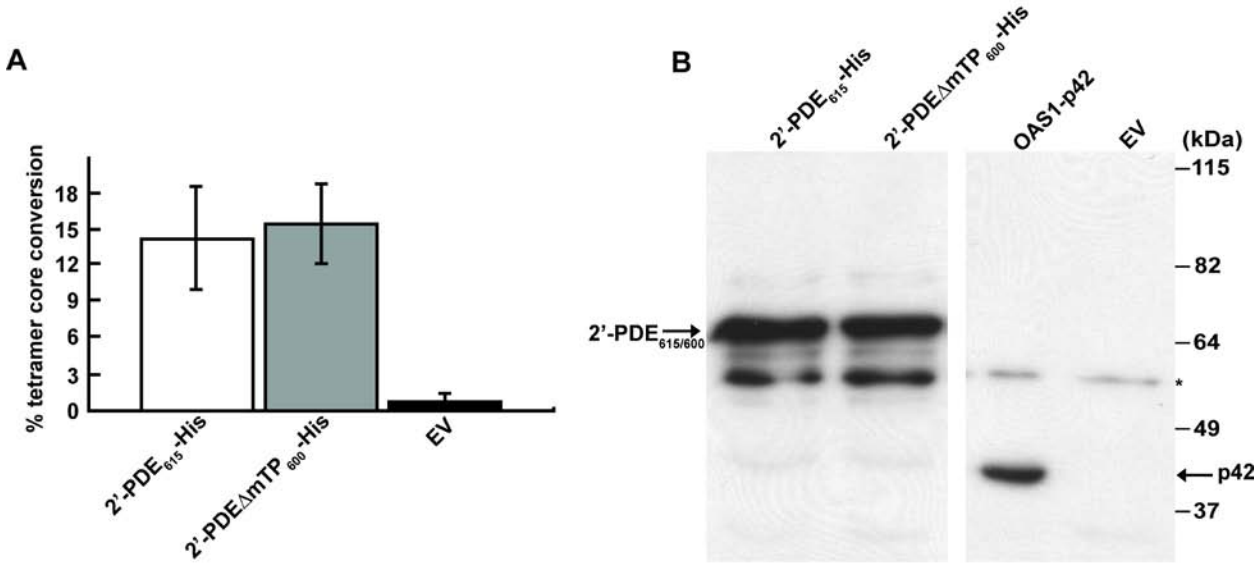

Fig. S6. Deletion of the mTP in 2'-PDE renders enzyme catalysis unchanged. (A). 2'-5' oligoadenylate degradation assay with bacterial lysates expressing 2'-PDE$_{615}$-His or 2'-PDEΔmTP$_{600}$-His using a 2'-5' oligoadenylate tetramer, ApApApA, as substrate. EV (empty vector), a negative control lysate obtained from bacteria transformed with empty pTriEx-3 Neo vector. Reactions were performed in triplicate (mean/s.e.m.) using 10 µg of total protein. (B). Immunoblotting against the 2'-PDE-specific lysates from (A) using an anti-His antibody (Genscript) and 20 µg of total protein. The EV lysate (also from (A)) and a bacterial lysate expressing the OAS1-p42 protein were employed as negative and positive controls, respectively. Arrows mark the position of indicated proteins. The asterisk (*) shows an unspecific band.

Figure S7

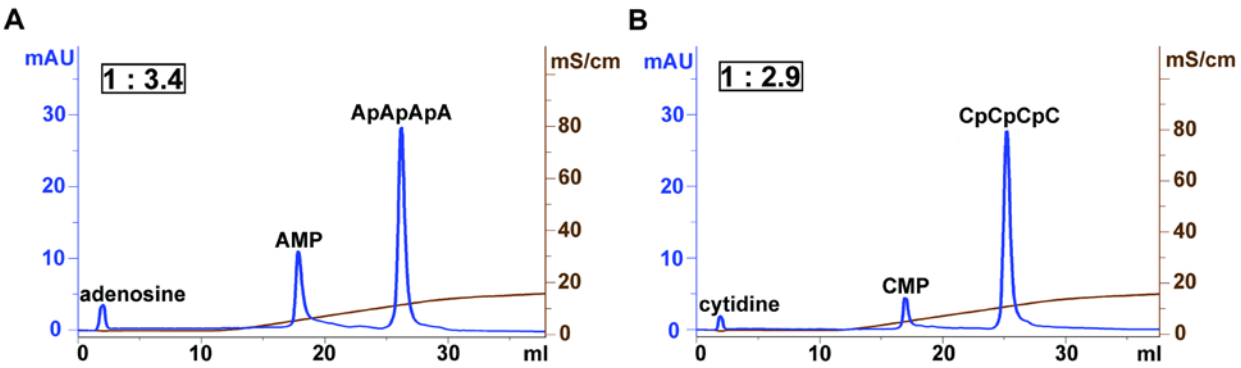

Fig. S7. Chromatographic analyses of the 3'-5' exoribonuclease activity of 2'-PDE. Chromatograms showing the composition of the resulting sample after a 20 minutes incubation of 2'-PDEΔmTP$_{600}$-His with 0.5 mM oligo-A, ApApApA (A), or oligo-C, CpCpCpC (B), substrates. The ratios of ~1:3 between the nucleoside (adenosine/cytidine) and the 5'-monophosphate nucleotide (AMP/CMP) are indicated. Chromatographic elution profiles were obtained by MonoQ Sepharose using 256 nm as the absorbance wavelength. Brown curves represent the experimental salt gradient.