In-vivo localisation of human acetylcholinesterase-derived species in a β-sheet conformation at the core of Alzheimer's disease senile plaques

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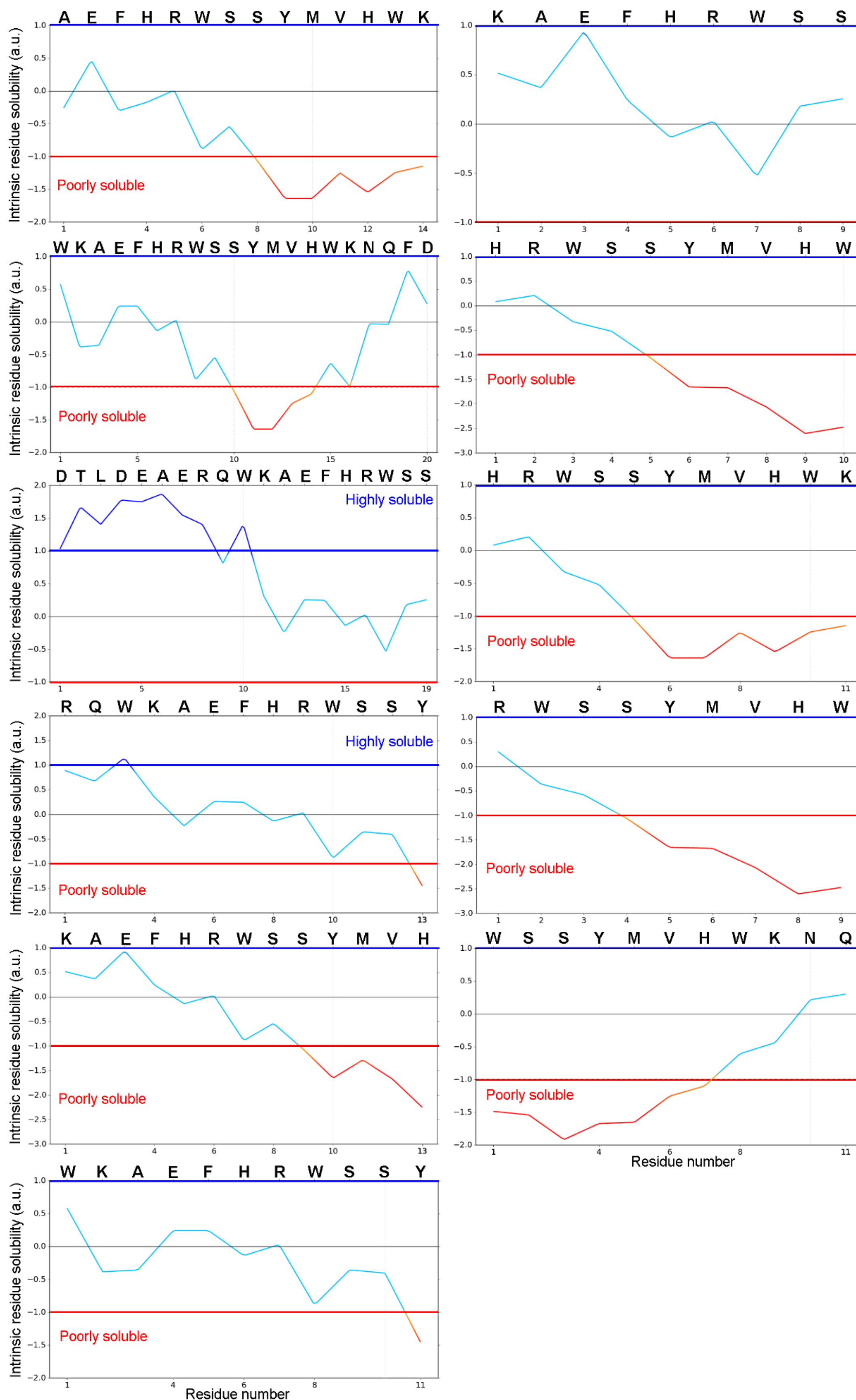
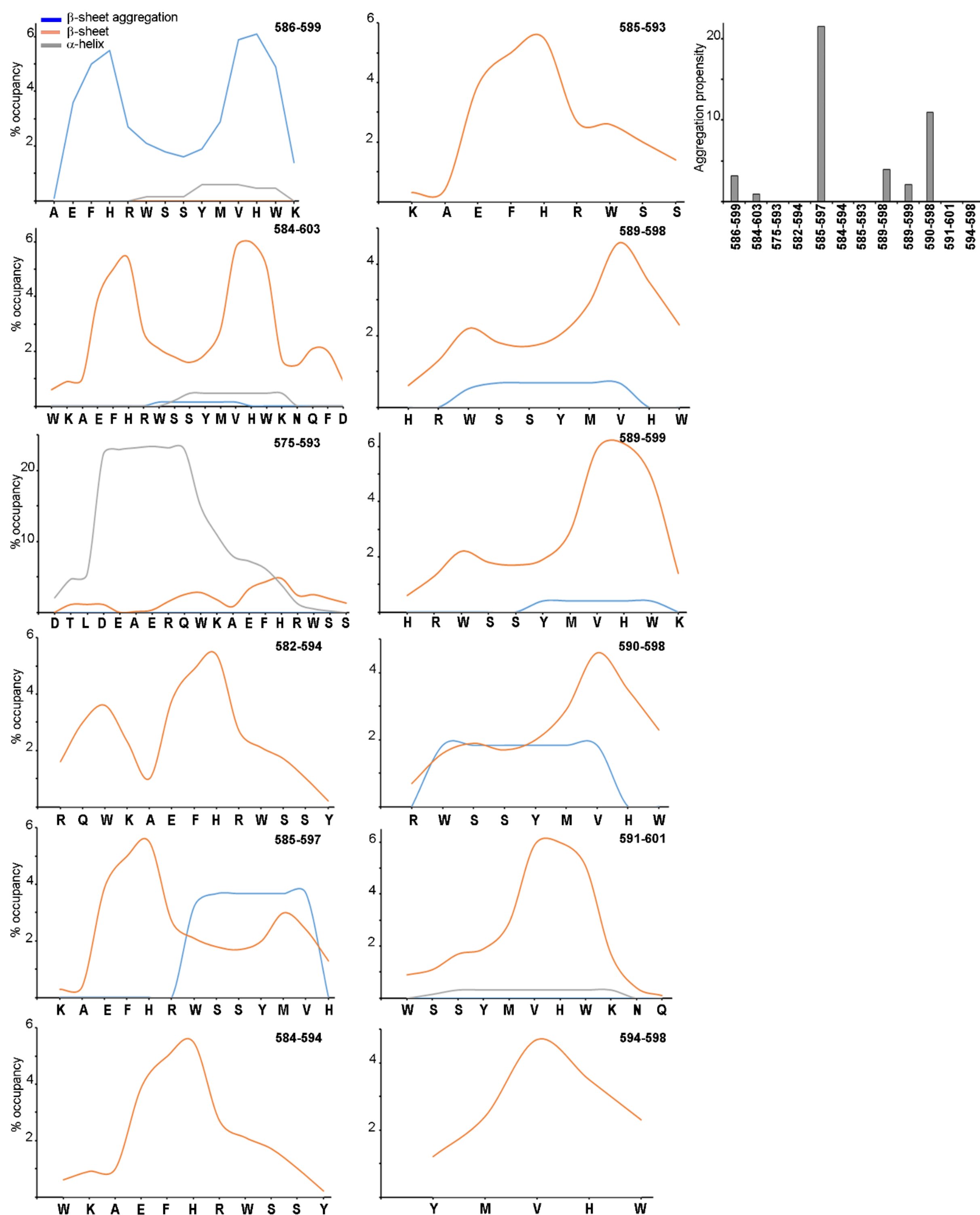
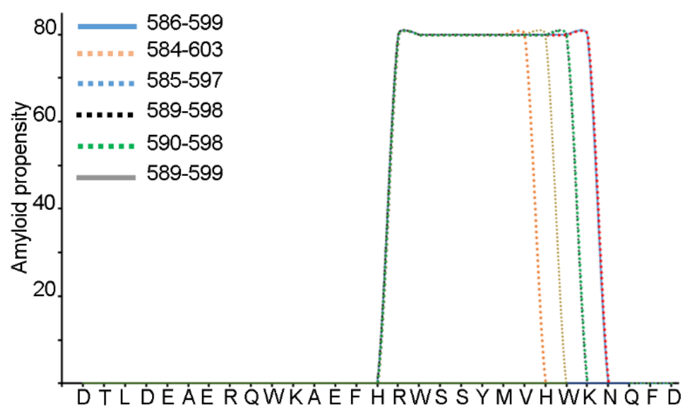
SUPPORTING INFORMATION



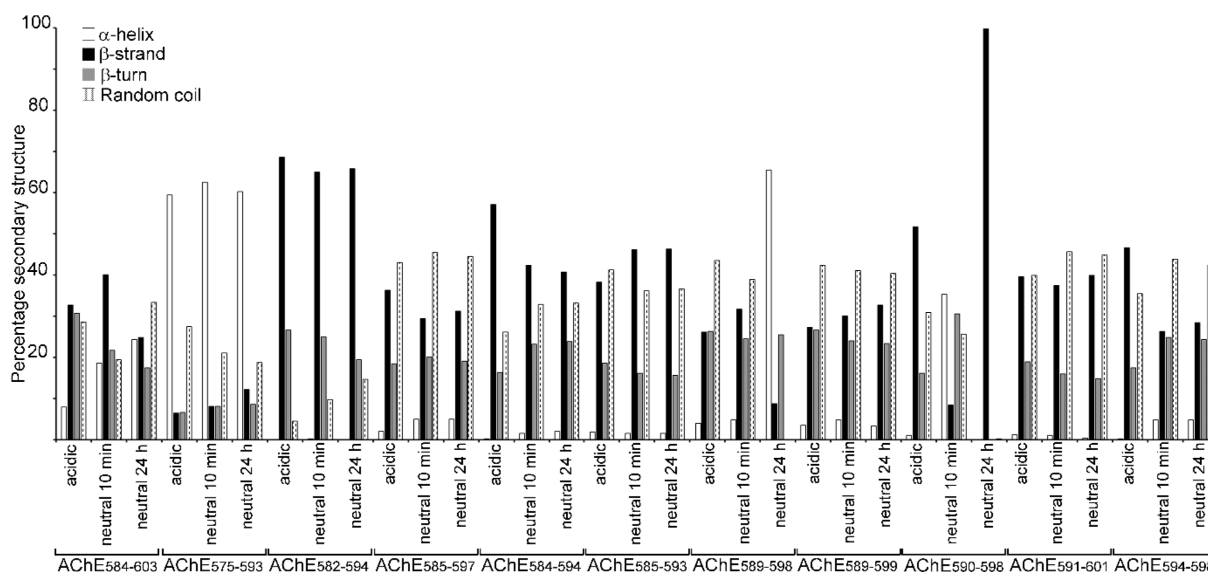
Supplementary Fig. S1. Solubility and generic aggregation propensity of the T40-IDE synthetic peptides. The CamSol algorithm developed by Sormanni *et al*. was used. Solubility and aggregation propensities are presented numerically with values below -1 (below the red line) indicating poor solubility, and values above 1 (above the blue line) indicating high solubility. a.u.: arbitrary units.



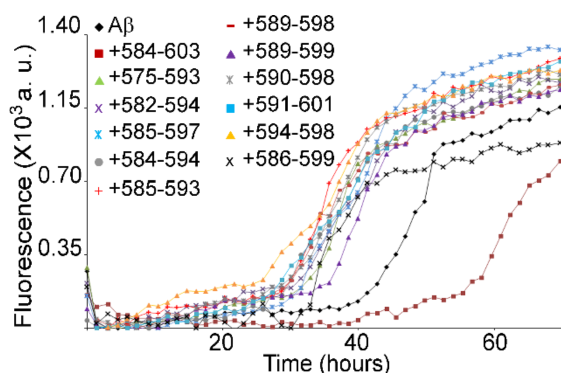
Supplementary Fig. S2. Propensity of the T40-IDE synthetic peptides for β-sheet conformation (orange), α-helix conformation (grey), β-sheet aggregation (blue), and aggregation propensity (right panel). The TANGO algorithm was used.



Supplementary Fig. S3. Amyloid propensity of the T40-IDE synthetic peptides using the WALTZ algorithm.



Supplementary Fig. S4. Secondary structure of T40-IDE peptides. Far UV spectra (250 to 190 nm) of 100 µM T40-IDE peptides were collected at acidic pH and after 10 min or 24 h of pH neutralisation (50 mM NaH_2PO_4, pH 7.2). The percentage of secondary structures were determined from the CD spectra using CONTINLL from the CDPro package.



Supplementary Fig. S5. T40-IDE peptide seeds act as heterologous seeds to promote Aβ fibrilisation. 15 µM Aβ was incubated with 165 µM ThT in PBS, with or without 2 µM T40-IDE peptide seeds, and changes in ThT emission were monitored. Zoom in of the lag phase. a.u.: arbitrary units.

$AChE_{586-599}$ -AEFHRWSSYMVHWK

Phage clone 1 -AQFHRIQ--

Phage clone 2 -AQYHRTA--

Phage clone 3 -AQFHRVP--

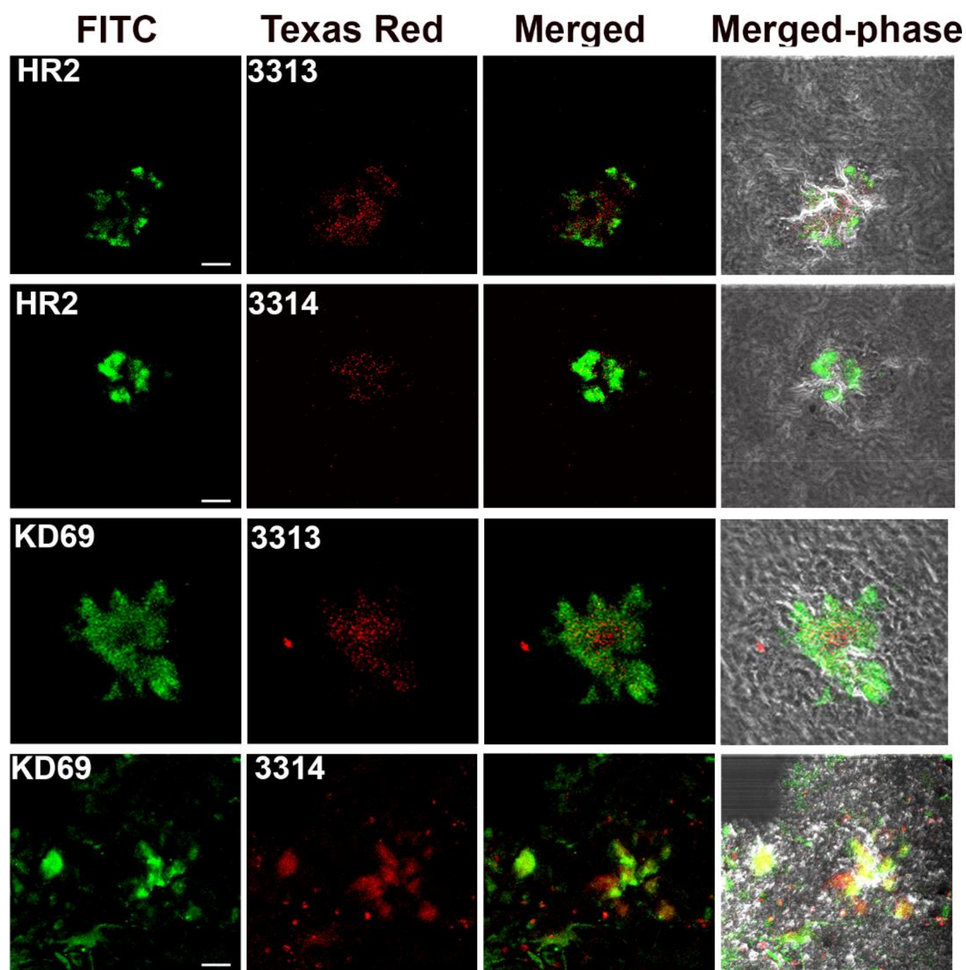
Phage clone 4 -AGFHRID--

Phage clone 5 -AGFHRHP--

Phage clone 6 -AAFHRSD--

Phage clone 7 -AAFHRLS--

Supplementary Fig. S6. Epitope specificity of monoclonal antibody 105A by phage display. ClustalW2 aligned amino acid sequences from phage clones binding to 105A antibody. Phage clones from selection against 105A antibody were picked at random and sequenced. Significant amino acid homology is seen with phage peptides sharing three or more amino acid sequences with $AChE_{586-599}$ at the appropriate residue position (residues 1, 3, 4 and 5). Hydrophobic residues are in red, acidic in blue, basic in pink and hydrophilic-neutral in green.



Supplementary Fig. S7. Overlap of the labelling by 3313 and 3314 with globular AChE-S (HR2) and with the α-helical T40 (KD69) in the brain of hAChE-S/hAPPswe double transgenic mice. Frozen brain sections (12 μm) from double transgenic mice were double labelled with the Elite ABC M.O.M. kit or Vectastain Elite ABC rabbit IgG kit using a biotinylated anti-mouse or anti-rabbit secondary antibody and FITC or Texas red conjugated avidin (Vector). The scale bar represents 10 μm. Shown is one of the z slices from a z stack.

Supplementary experimental procedures

The Mab 105A was used as a target for selecting seven residue phage clones in order to determine its specific epitope. After the fourth round of panning on 105A Mab, ten phage plaques were picked at random from each population and the amino acid sequence at the N-terminus of their pIII proteins determined via conventional means. After four rounds of selection a consensus sequence for phage selected on 105A Mab is clearly visible from the clones sequenced (Fig. S2). Alignment of $AChE_{589\text{-}599}$ with the peptides selected for binding to 105A using the ClustalW2 alignment software shows distinct homology in residues 1, 3, 4 and 5, with the second residue having a tendency to be glutamine or alanine rather than the native glutamic acid.

To select phage clones recognising 105A epitope, 1.5 ml of 100 μg/ml 105A Mab in 0.1 M $NaHCO_3$ pH 8.6 was applied to a 60 mm Petri dish and left overnight at 4ºC in a humidified container to bind to the plate. The dish was then blocked with 0.1 M $NaHCO_3$ pH 8.6, 5 mg/ml BSA, 0.02% NaN_3 for 1.5h at 4ºC, washed six times with 0.1 % Tween-20 in TBS ($TBST_{20}$), before 2 x 10^{11} phage stock diluted in 1 ml $TBST_{20}$ was added to the dish (on a rocker at room temperature for 1 hour). Unbound phage were removed by washing 10 times, and bound phage removed with 1 ml 0.2 M Glycine-HCl pH 2.2 before being added to 150 μl 1M Tris-HCl pH 9.1 to neutralise the solution. In the subsequent three rounds of panning, the process was repeated but with the use of 0.5% Tween-20 in the TBS washes.

PCR primers were designed which flank the random oligonucleotide site on the phage DNA and incorporate *Xba*I restriction site (absent from the random insert sequence and from the 50 bp flanking that region) at both ends of this sequence:

M13 3' *Xba*I Forwards CCTTTAGTGGTACCTTTCTAGACTCAC

M13 3' *Xba*I Reverse CAGTTTCGGCCGAATCTAGACC

PCR amplification of tag sequences was performed, on 5 x 10^5 phage genomes per 100 μl PCR, with Cloned *Pfu* DNA Polymerase, 0.2 mM of each dNTP, and 3.2 pmoles of each primer. The amplified tag sequences were then electrophoresed on 1% agarose gels to separate tags from template DNA and primers, and then excised from the gel and purified (QIAquick gel extraction). Restriction digestion of PCR products was performed and digested tags were purified (QIAquick Nucleotide Removal). 15 μl purified digested tags were concatenated using 2 units of T4 DNA ligase for 30 minutes at 16ºC and the ligation reaction terminated by addition of 1:6 volume of loading buffer. Concatemers were separated according to size by electrophoresis on 0.8% agarose gel, bands corresponding to 500-900 base pairs were excised from the gel, purified and ethanol precipitated.

The pZErO-1 plasmid was chosen due to its high cloning efficiency, antibiotic resistance to zeocin (to select for transformed bacteria) and the presence of a lethal gene *ccdB* fused to *lacZα* (used to select for insertion of the foreign DNA). 2 μg of pZErO-1 plasmid DNA was digested using *Xba*I for 30 minutes at 37ºC, and the enzyme heat inactivated (65ºC for 10 minutes). The digested vector DNA was then purified (QIAquick PCR purification) and precipitated in 0.1x volume of 3M sodium acetate, 2.5x absolute ethanol and 0.02x mussel glycogen. Concatenated tags were precipitated in 0.25x volume of 10 M ammonium acetate, 5x volume ethanol and 0.01x mussel glycogen. Both pZErO-1 and concatenated tags were precipitated for ≥ 60 minutes at -20º before being centrifuged at 17,900 g for 60 minutes at 4ºC. The soluble phase of each was removed and the pellet washed 2x with 70% ethanol to remove remaining salt and the pellets allowed to dry. Pellets of concatemer DNA were suspended in 6 μl buffer EB, whilst pellets of digested vector were dissolved in 60 μl Buffer EB.

Concatemer DNA (≥ 500 ng) was cloned into 2 μg of pZErO-1 vectors with 20 units of T4 DNA ligase at 16ºC for 16 hours. Cloned concatemers were purified by phenol:chloroform:isoamyl alcohol extraction, 25:24:1, followed by centrifugation at 17,900 g for 5 minutes, the aqueous phase was removed and added to 5x volume of ethanol, 0.5x volume of 10 M ammonium acetate and 0.01x mussel glycogen. This was placed on dry ice for 10 minutes before centrifugation at 17,900x g for 60 minutes at 4ºC. The supernatant was removed and the pellet washed twice with 2x washes with 500 μl 70% ethanol and allowed to dry before suspending in 10 μl of distilled water.

One Shot® TOP10 cells were transformed with 5 μl of the cloned concatemers thawed (30 minutes on ice, and then heat shock for 30 second at 42ºC). 250 μl of S.O.C. medium was then added and the mixture placed on its side on an orbital shaker rotating at 225 r.p.m at 37ºC for 60 minutes, and then 50 to 150 μl plated out onto low salt Luria-Bertani (LB) agar plates containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl, 7.5% agar and 50 μg/ml zeocin. The bacteria were grown at 37ºC for ≥ 16 hours. Overnight

colonies of transformed One Shot® TOP10 cells were selected at random, inoculated into low salt LB broth containing 50 µg/ml zeocin and placed on an orbital shaker rotating at 225 r.p.m at 37ºC overnight. Plasmid DNA was purified using QIAprep Spin Miniprep Kits. The DNA was then sequenced using M13 (-40) Forward primer (GCCAGGGTTTTCCCAGTCACGA).