**Supporting Information**

# Shifting the pH Optima of (*R*)-Selective Transaminases by Protein Engineering

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**Table S1**. List of Primers.

| Primer | Sequence (5' to 3') |
|---|---|
| ATA-Afu-E49Q Fw | CAGGGTTTTATGCATAGTGATCTGACC |
| ATA-Afu-E49Q Rv | ATCCAGCAGCGGGATGC |
| ATA-Afu-T123K Fw | AAAGGTGTTCGTGGTTCTAAACCGG |
| ATA-Afu-T123K Rv | CAGACCACGGGTAACAATAACTTCC |
| ATA-Afu-G127E Fw | ACCGGTGTTCGTGAATCTAAACCGGAAG |
| ATA-Afu-T123E Rv | CAGACCACGGGTAACAATAACTTCC |
| ATA-Gze-Q49E Fw | GAAGGCTTTATGCATGGTGATCTG |
| ATA-Gze-Q49E Rv | ATCCATCAGCGGAATGCG |
| ATA-Gze-K123T Fw | CCGGTTCGCGAAGCAAAACCGGGTG |
| ATA-Gze-K123T Rv | CGTCAGGCCACGCGTAACGATC |
| ATA-Gze-E127G Fw | CCGGTTCGCGGTGCAAAACCGGGTG |
| ATA-Gze-P127G Rv | TTTCAGGCCACGCGTAACGATC |
| ATA-Ate-Q51E Fw | GAAGGTTTTATGCATAGCGATC |
| ATA-Ate-Q51E Rv | ATCCAGCAGCGGAATAC |
| ATA-Ate-K125T Fw | ACCGGTGTTCGTGGCACCC |
| ATA-Ate-K125T Rv | CAGACCACGGGTAACAATCAG |
| ATA-Ate-G129E Fw | GAAACCCGTCCGGAAGATATCGTG |
| ATA-Ate-G129E Rv | ACGAACACCTTTCAGACCACGG |

**Table S2.** Detailed specific activity data

| Variants | pH, specific activity[a] [U mg$^{-1}$] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | pH = 6 | pH = 6.5 | pH = 7 | pH = 7.5 | pH = 8 | pH = 8.5 | pH = 9 | pH = 9.5 |
| ATA-Afu | 1.158 ± 0.010 | 2.247 ± 0.018 | 3.167 ± 0.042 | 3.588 ± 0.077 | 3.960 ± 0.128 | 4.032 ± 0.061 | 3.238 ± 0.044 | 2.240 ± 0.085 |
| ATA-Afu-E49Q | 0.736 ± 0.026 | 2.190 ± 0.039 | 4.827 ± 0.061 | 6.923 ± 0.228 | 6.078 ± 0.306 | 3.596 ± 0.016 | 2.199 ± 0.119 | 0.900 ± 0.030 |
| ATA-Afu-T123K | 1.644 ± 0.030 | 3.198 ± 0.025 | 4.784 ± 0.032 | 5.359 ± 0.025 | 5.553 ± 0.079 | 5.770 ± 0.131 | 4.584 ± 0.079 | 3.231 ± 0.094 |
| ATA-Afu-G127E | 0.472 ± 0.016 | 0.802 ± 0.017 | 1.133 ± 0.031 | 1.246 ± 0.039 | 1.274 ± 0.069 | 1.294 ± 0.024 | 1.097 ± 0.012 | 0.847 ± 0.025 |
| ATA-Afu-E49Q/T123K | 0.562 ± 0.027 | 1.508 ± 0.015 | 3.224 ± 0.109 | 4.468 ± 0.072 | 3.655 ± 0.163 | 2.788 ± 0.100 | 1.455 ± 0.114 | 0.692 ± 0.008 |
| ATA-Gze | 0.633 ± 0.026 | 2.406 ± 0.036 | 3.622 ± 0.009 | 2.663 ± 0.060 | 1.510 ± 0.033 | 0.976 ± 0.024 | 0.501 ± 0.020 | 0.287 ± 0.015 |
| ATA-Gze-Q49E | 0.279 ± 0.010 | 0.713 ± 0.002 | 1.305 ± 0.008 | 1.610 ± 0.008 | 1.638 ± 0.037 | 1.599 ± 0.008 | 1.295 ± 0.026 | 0.801 ± 0.016 |
| ATA-Gze-E127G | 0.458 ± 0.001 | 1.303 ± 0.018 | 2.780 ± 0.058 | 3.141 ± 0.036 | 1.655 ± 0.094 | 1.012 ± 0.019 | 0.332 ± 0.036 | 0.227 ± 0.013 |
| ATA-Gze-K123T | 0.852 ± 0.003 | 2.514 ± 0.173 | 4.630 ± 0.026 | 3.789 ± 0.185 | 1.831 ± 0.142 | 0.957 ± 0.036 | 0.482 ± 0.034 | 0.241 ± 0.004 |
| ATA-Ate | 0.196 ± 0.005 | 1.140 ± 0.013 | 2.540 ± 0.050 | 3.067 ± 0.021 | 2.273 ± 0.076 | 1.665 ± 0.013 | 0.856 ± 0.015 | 0.467 ± 0.031 |
| ATA-Ate-Q51E | 0.147 ± 0.026 | 0.548 ± 0.012 | 0.902 ± 0.007 | 0.967 ± 0.003 | 1.062 ± 0.083 | 1.273 ± 0.080 | 1.018 ± 0.015 | 0.821 ± 0.020 |
| ATA-Ate-K125T | 0.178 ± 0.008 | 1.088 ± 0.023 | 2.470 ± 0.020 | 2.827 ± 0.051 | 2.027 ± 0.079 | 1.567 ± 0.017 | 0.808 ± 0.011 | 0.425 ± 0.027 |
| ATA-Ate-G129E | 0.181 ± 0.006 | 1.002 ± 0.009 | 1.992 ± 0.012 | 2.173 ± 0.044 | 1.583 ± 0.033 | 1.077 ± 0.005 | 0.485 ± 0.012 | 0.332 ± 0.012 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| ATA-Ate-Q51E/K125T | 0.185 ± 0.004 | 0.680 ± 0.001 | 1.131 ± 0.003 | 1.349 ± 0.016 | 1.353 ± 0.016 | 1.357 ± 0.015 | 1.156 ± 0.017 | 0.891 ± 0.014 |
| ATA-Afu-H53L | 0.023 ± 0.002 | 0.022 ± 0.001 | 0.022 ± 0.001 | 0.023 ± 0.002 | 0.018 ± 0.001 | 0.017 ± 0.001 | 0.015 ± 0.001 | 0.028 ± 0.001 |
| ATA-Afu-H53F | 0.011 ± 0.001 | 0.005 ± 0.003 | 0.006 ± 0.001 | 0.005 ± 0.001 | 0.007 ± 0.001 | 0.005 ± 0.003 | 0.001 ± 0.002 | 0.002 ± 0.001 |
| ATA-Afu-E49Q/H53L | 0.237 ± 0.007 | 0.336 ± 0.015 | 0.363 ± 0.017 | 0.367 ± 0.005 | 0.374 ± 0.006 | 0.406 ± 0.029 | 0.373 ± 0.016 | 0.334 ± 0.005 |
| ATA-Gze-H53L | 0.078 ± 0.001 | 0.148 ± 0.002 | 0.181 ± 0.002 | 0.305 ± 0.002 | 0.282 ± 0.007 | 0.184 ± 0.003 | 0.119 ± 0.003 | 0.070 ± 0.001 |
| ATA-Ate-H55L | 0.112 ± 0.003 | 0.334 ± 0.007 | 0.376 ± 0.002 | 0.379 ± 0.001 | 0.390 ± 0.004 | 0.384 ± 0.003 | 0.372 ± 0.001 | 0.337 ± 0.013 |

[a]Initial activity measured using the acetophenone assay (for (*R*)-PEA and pyruvate). One unit (U) was defined as the formation of 1 µmol acetophenone per minute. All measurements were performed in triplicates.

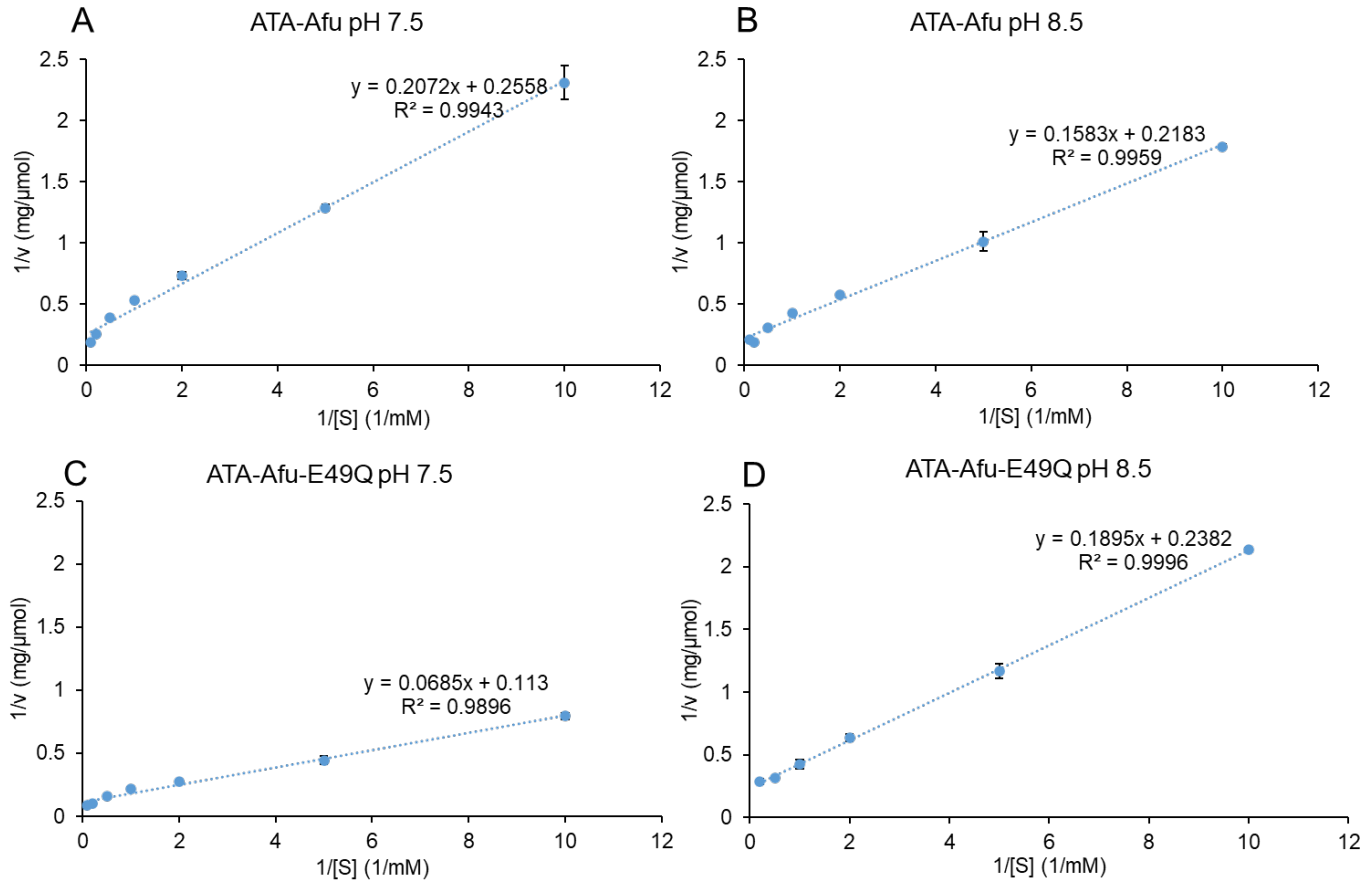


**Figure S1**. Lineweaker-Burk linearization plots of ATA-Afu and ATA-Afu-E49Q at pH 7.5 and 8.5. The kinetic parameters $k_{cat}$ and $K_m$ were calculated accordingly. All measurements were performed in triplicates.

**Table S3.** Kinetic parameters of the reactions catalyzed by ATA-Afu and ATA-Afu-E49Q

| pH | Enzyme | $K_m$ [mM] | $k_{cat}$ [$s^{-1}$] | $k_{cat}/K_m$ [mM $s^{-1}$] |
|---|---|---|---|---|
| 7.5 | ATA-Afu | 0.81 ± 0.07 | 4.91 ± 0.19 | 6.06 |
| 8.5 | ATA-Afu | 0.73 ± 0.02 | 5.77 ± 0.13 | 7.90 |
| 7.5 | ATA-Afu-E49Q | 0.61 ± 0.04 | 11.11 ± 0.43 | 18.21 |
| 8.5 | ATA-Afu-E49Q | 0.80 ± 0.03 | 5.27 ± 0.19 | 6.59 |

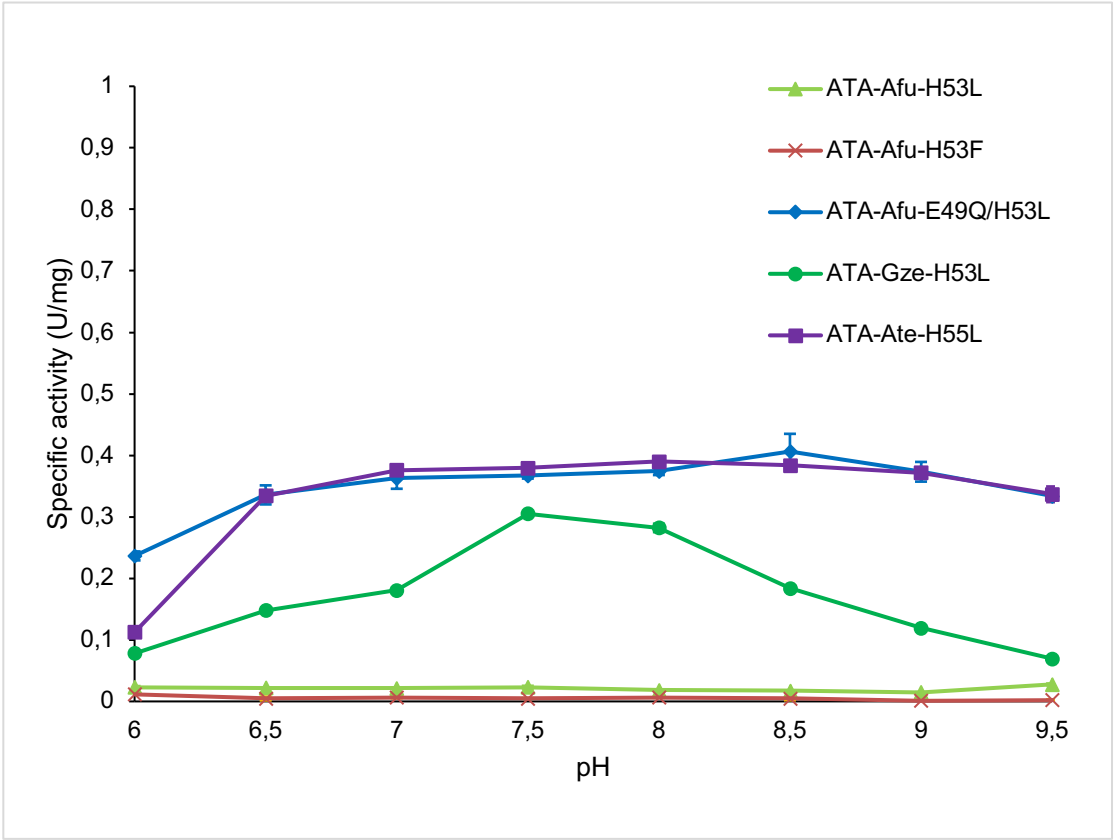


**Figure S2.** pH activity profiles of mutants ATA-Gze-H53L and ATA-Ate-H55L. Data points corresponded to the mean values of three independent experiments.

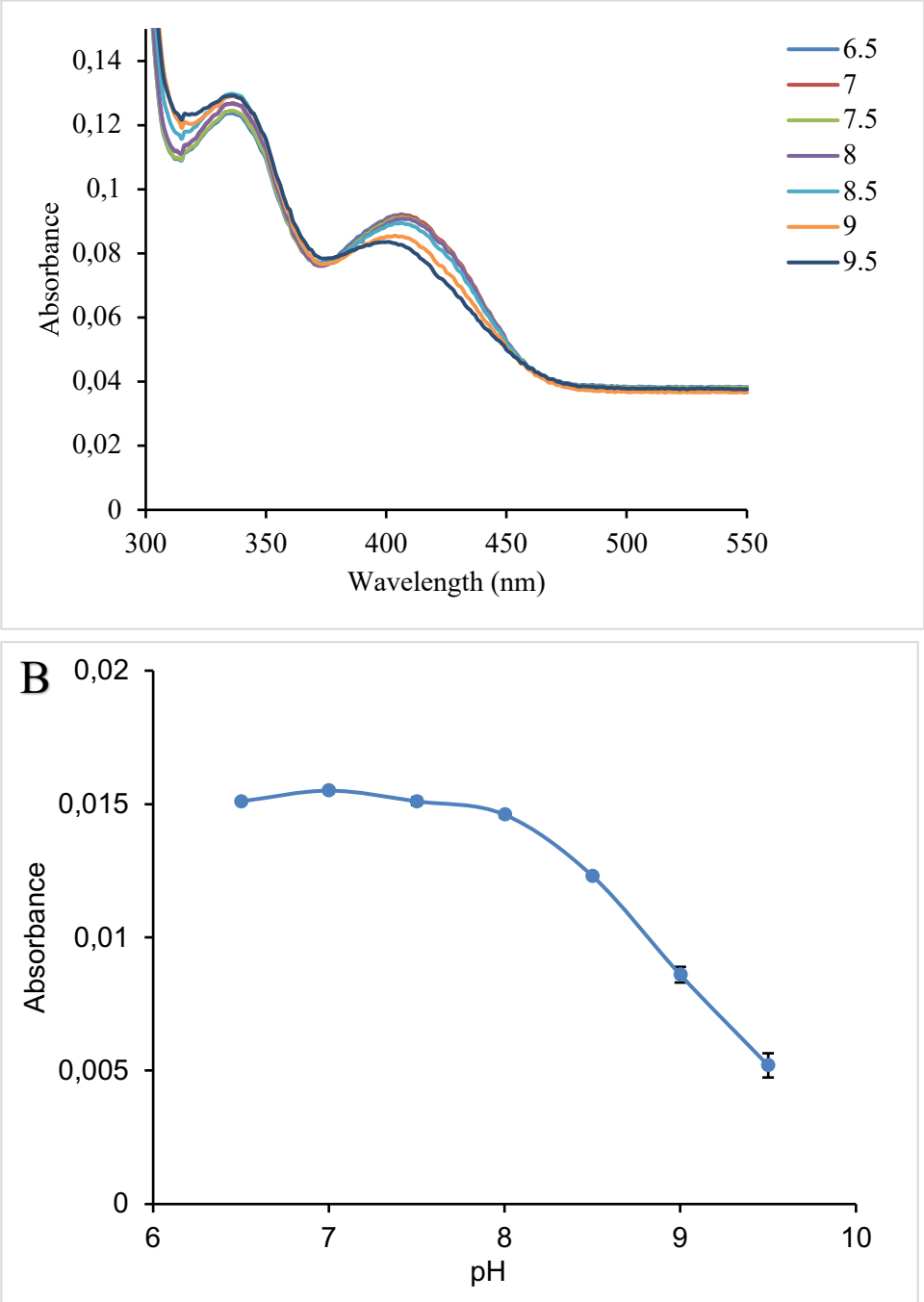


**Figure S3.** pH-dependent spectral changes of the PLP form of ATA-Afu-E49Q. (A) Absorption spectra of ATA-Afu-E49Q in the pH range from 6.5 to 9.5. (B) Absorption changes at 410 nm as a function of the pH. ATA-Afu-E49Q was less stable at pH > 9.5, and thus the data could not be obtained at more alkaline pH values. All measurements were performed in triplicates.

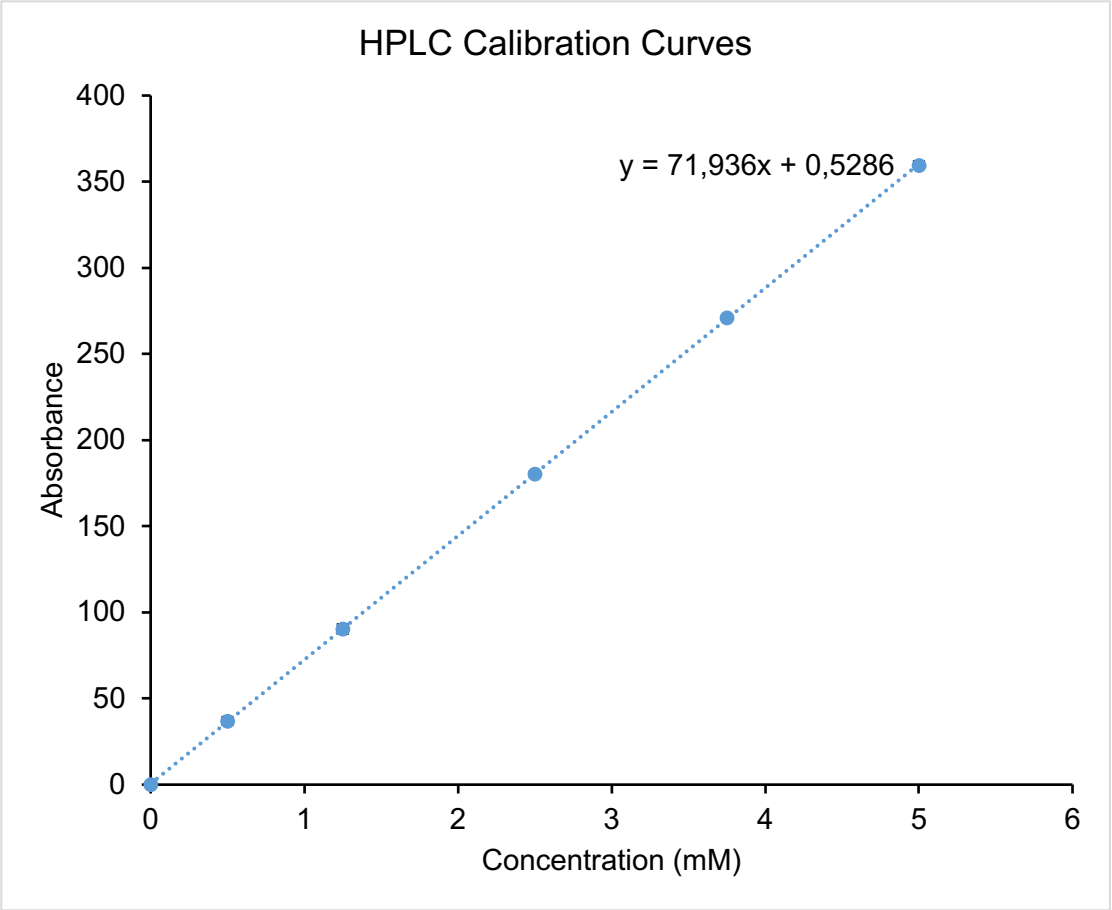


**Figure S4.** HPLC calibration curves of (*R*)-PEA. All measurements were performed in triplicates.

**Table S4. Detailed data of asymmetric synthesis**

| pH | Enzyme | Conversion rates (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 h | 2 h | 4 h | 6 h | 8 h | 10 h | 24 h | 48 h |
| 7.5 | ATA-Afu | 0 ± 0 | 15.56 ± 1.59 | 28.52 ± 1.83 | 37.96 ± 1.54 | 46.30 ± 2.40 | 53.61 ± 1.26 | 86.48 ± 2.65 | 98.06 ± 2.30 |
| 8.5 | ATA-Afu | 0 ± 0 | 20.56 ± 1.59 | 33.33 ± 1.64 | 42.22 ± 1.42 | 50.09 ± 1.73 | 55.93 ± 1.61 | 88.98 ± 2.87 | 98.06 ± 1.49 |
| 7.5 | ATA-Afu-E49Q | 0 ± 0 | 31.02 ± 1.67 | 54.44 ± 1.04 | 64.35 ± 2.45 | 71.30 ± 3.06 | 76.11 ± 2.62 | 98.33 ± 0.60 | 98.98 ± 0.26 |
| 8.5 | ATA-Afu-E49Q | 0 ± 0 | 21.94 ± 0.45 | 39.44 ± 0.68 | 50.93 ± 0.35 | 57.96 ± 1.82 | 63.70 ± 1.54 | 95.83 ± 1.86 | 99.07 ± 1.12 |

Assay conditions for asymmetric synthesis: 10 mL final volume, 50 mM phosphate buffer at pH 7.5 or 8.5, 0.1 mM PLP, 5 mM ketone, 100 mM D,L-alanine, $NAD^+$ (1 mM), GDH (1 U $ml^{-1}$), D-glucose (50 mM) and LDH (3 U $ml^{-1}$), DMSO 5%, 5 mg purified enzyme, 30 °C under stirring. All measurements were performed in triplicates.

**Table S5**. Docking results.

| Rank | Binding energy (kcal/mol) |
|---|---|
| 1 | -5.21 |
| 2 | -4.51 |
| 3 | -4.46 |

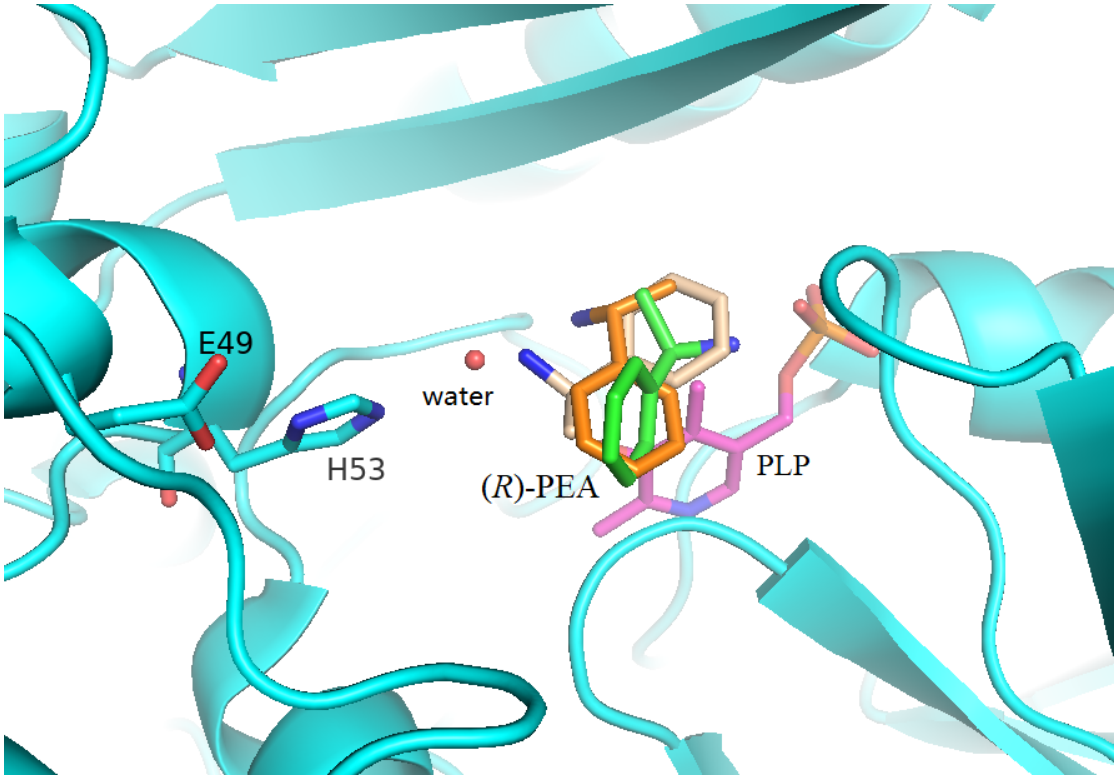


**Figure S5.** The superposed structure of substrate (*R*)-PEA accommodated in the active site of ATA-Afu. The protein is shown in cyan. The carbon atoms of the three docking results of substrate are colored orange, wheat and green, respectively. The figure was created using PyMOL.

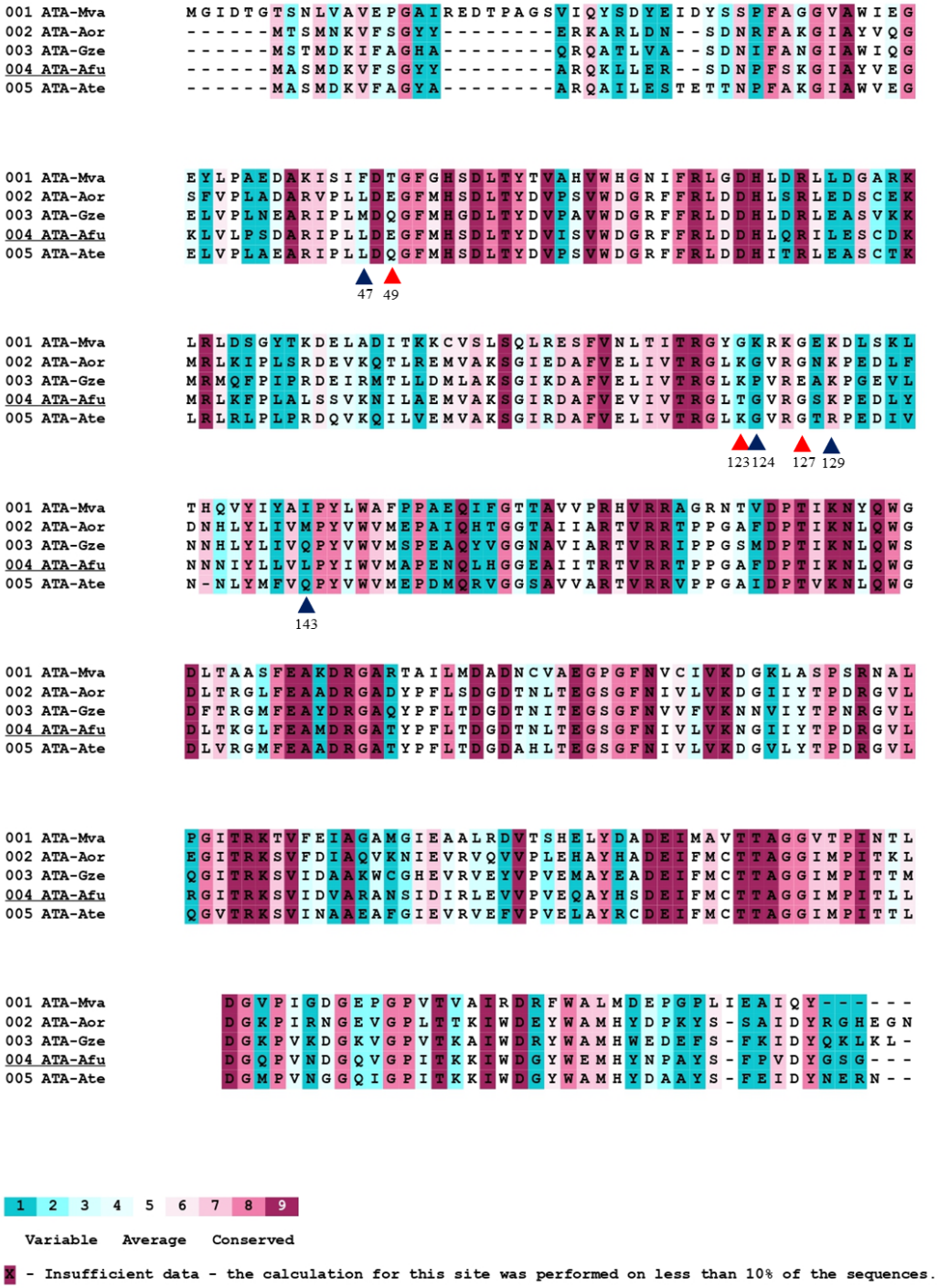


**Figure S6.** Evolutionary conservation alignment of transaminases. The residues in the substrates tunnel area are marked with triangles. Among them, E49, T123 and G127 present in ATA-Afu (red colored triangles) have differently charged side chains compared to the other two ATAs. The alignment tool needs at least five homologous proteins, therefore two homologous transaminases ATA-Mva (Uniprot: Mvan_4516) and ATA-Aor (Uniprot: CNMCM6457_008109) were added to the alignment. The figure was created using the online alignment tools *ConSurf* [46].

## Protein and DNA sequences of the enzymes studied

>Protein sequence of ATA-Afu wildtype (Uniprot: AFUA_7G06900):

MASMDKVFSGYYARQKLLERSDNPFSKGIAYVEGKLVLPSDARIPLLDEGFMHSDLT
YDVISVWDGRFFRLDDHLQRILESCDKMRLKFPLALSSVKNILAEMVAKSGIRDAFVE
VIVTRGLTGVRGSKPEDLYNNNIYLLVLPYIWVMAPENQLHGGEAIITRTVRRTPPGA
FDPTIKNLQWGDLTKGLFEAMDRGATYPFLTDGDTNLTEGSGFNIVLVKNGIIYTPDR
GVLRGITRKSVIDVARANSIDIRLEVVPVEQAYHSDEIFMCTTAGGIMPITLLDGQPVN
DGQVGPITKKIWDGYWEMHYNPAYSFPVDYGSGSGSHHHHHH

>DNA sequence of ATA-Afu wildtype (Uniprot: AFUA_7G06900)

ATGGCATCTATGGATAAAGTTTTTAGTGGTTACTACGCGCGCCAGAAACTGCTGG
AACGTAGTGATAATCCGTTCAGCAAAGGTATTGCCTATGTTGAAGGCAAACTGGT
GCTGCCGAGTGATGCACGCATCCCGCTGCTGGATGAAGGTTTTATGCATAGTGAT
CTGACCTACGATGTTATTAGCGTGTGGGATGGCCGTTTCTTTCGCCTGGATGATCA
CCTGCAGCGCATCCTGGAAAGCTGCGATAAAATGCGTCTGAAATTTCCGCTGGCA
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GATGGCGCCGGAAAATCAGCTGCATGGCGGTGAAGCCATTATTACCCGTACCGTT
CGTCGCACCCCGCCGGGTGCATTTGATCCGACCATTAAAAACCTGCAGTGGGGTG
ATCTGACCAAAGGCCTGTTTGAAGCCATGGATCGTGGTGCAACCTATCCGTTCCT
GACGGATGGCGATACCAATCTGACGGAAGGCTCTGGTTTCAATATCGTTCTGGTG
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AATCTGTTATCGATGTGGCGCGCGCCAACAGTATTGATATCCGTCTGGAAGTGGT
TCCGGTGGAACAGGCGTATCATAGCGATGAAATTTTTATGTGTACCACGGCCGGC
GGTATTATGCCGATCACCCTGCTGGATGGTCAGCCGGTTAATGATGGTCAAGTGG
GCCCGATTACCAAGAAAATTTGGGATGGCTATTGGGAAATGCACTATAACCCGGC
GTACAGCTTCCCGGTGGATTACGGCTCTGGTTCAGGATCCCATCATCATCATCATC
ATTGA

>Protein sequence of ATA-Gze wildtype (Uniprot: FG01766.1)

MSTMDKIFAGHAQRQATLVASDNIFANGIAWIQGELVPLNEARIPLMDQGFMHGDLT
YDVPAVWDGRFFRLDDHLDRLEASVKKMRMQFPIPRDEIRMTLLDMLAKSGIKDAF
VELIVTRGLKPVREAKPGEVLNNHLYLIVQPYVWVMSPEAQYVGGNAVIARTVRRIP
PGSMDPTIKNLQWSDFTRGMFEAYDRGAQYPFLTDGDTNITEGSGFNVVFVKNNVIY
TPNRGVLQGITRKSVIDAAKWCGHEVRVEYVPVEMAYEADEIFMCTTAGGIMPITTM
DGKPVKDGKVGPVTKAIWDRYWAMHWEDEFSFKIDYQKLKLSGSHHHHHH

>DNA sequence of ATA-Gze wildtype (Uniprot: FG01766.1)

ATGAGTACCATGGATAAAATTTTTGCAGGCCATGCGCAGCGTCAGGCAACGCTGG
TTGCGAGCGATAACATCTTCGCCAATGGCATTGCATGGATCCAGGGTGAACTGGT
GCCGCTGAACGAAGCGCGCATTCCGCTGATGGATCAGGGCTTTATGCATGGTGAT
CTGACCTATGATGTGCCGGCGGTTTGGGATGGCCGTTTCTTTCGCCTGGATGATCA
CCTGGATCGTCTGGAAGCCTCTGTGAAGAAAATGCGCATGCAGTTTCCGATTCCG
CGTGATGAAATCCGCATGACCCTGCTGGATATGCTGGCAAAAAGTGGTATTAAAG
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TTCGTCGCATCCCGCCGGGTAGCATGGATCCGACCATCAAAAATCTGCAGTGGTC
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CGGTATTATGCCGATCACCACGATGGATGGTAAACCGGTGAAAGATGGCAAAGT
GGGTCCGGTTACCAAAGCCATTTGGGATCGCTATTGGGCAATGCACTGGGAAGAT
GAATTTAGCTTCAAAATCGATTACCAGAAACTGAAACTGTCAGGATCCCATCATC
ATCATCATCATTGA

>Protein sequence of ATA-Ate wildtype (Uniprot: ATEG_10023)

MASMDKVFAGYAARQAILESTETTNPFAKGIAWVEGELVPLAEARIPLLDQGFMHSD
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VELIVTRGLKGVRGTRPEDIVNNLYMFVQPYVWVMEPDMQRVGGSAVVARTVRRV
PPGAIDPTVKNLQWGDLVRGMFEAADRGATYPFLTDGDAHLTEGSGFNIVLVKDGV
LYTPDRGVLQGVTRKSVINAAEAFGIEVRVEFVPVELAYRCDEIFMCTTAGGIMPITT
LDGMPVNGGQIGPITKKIWDGYWAMHYDAAYSFEIDYNERNSGSHHHHHH

>DNA sequence of ATA-Ate wildtype (Uniprot: ATEG_10023)

ATGGCATCAATGGATAAGGTTTTTGCCGGTTATGCAGCACGTCAGGCAATTCTGGA
AAGCACCGAAACCACCAATCCGTTTGCAAAAGGTATTGCATGGGTTGAAGGTGAA
CTGGTTCCGCTGGCAGAAGCACGTATTCCGCTGCTGGATCAGGGTTTTATGCATAG
CGATCTGACCTATGATGTTCCGAGCGTTTGGGATGGTCGTTTTTTTCGTCTGGATGA
TCATATTACCCGTCTGGAAGCCAGCTGTACCAAACTGCGTCTGCGTCTGCCGCTGC
CTCGTGATCAGGTTAAACAAATTCTGGTTGAAATGGTTGCCAAAAGCGGTATTCGT
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