Supplementary Material for "Development of an efficient enzyme production and structure-based discovery platform for BACE1 inhibitors for the treatment of Alzheimer's disease"

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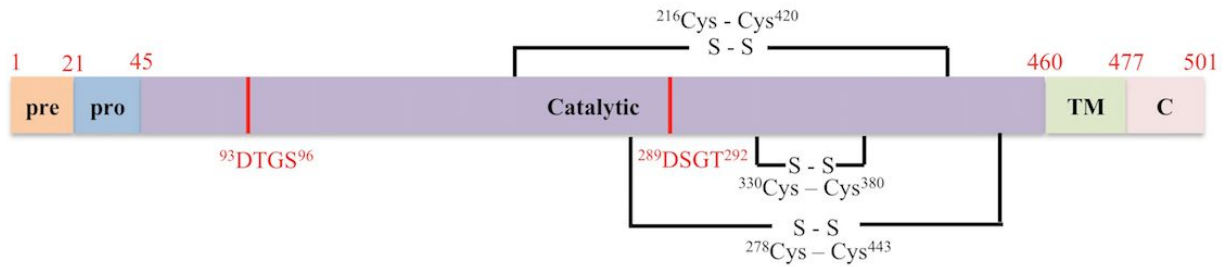
Figure S1. Protein domain scheme of BACE1. BACE1 encodes a full-length protein of 501 amino acids. Boxes indicate the locations of each domains (TM : trans-membrane domain, C : cytosolic domain). Disulfide bonds are indicated by connectors between the respective cysteine residues and the catalytic motifs DTGS and DSGT are highlighted in red.
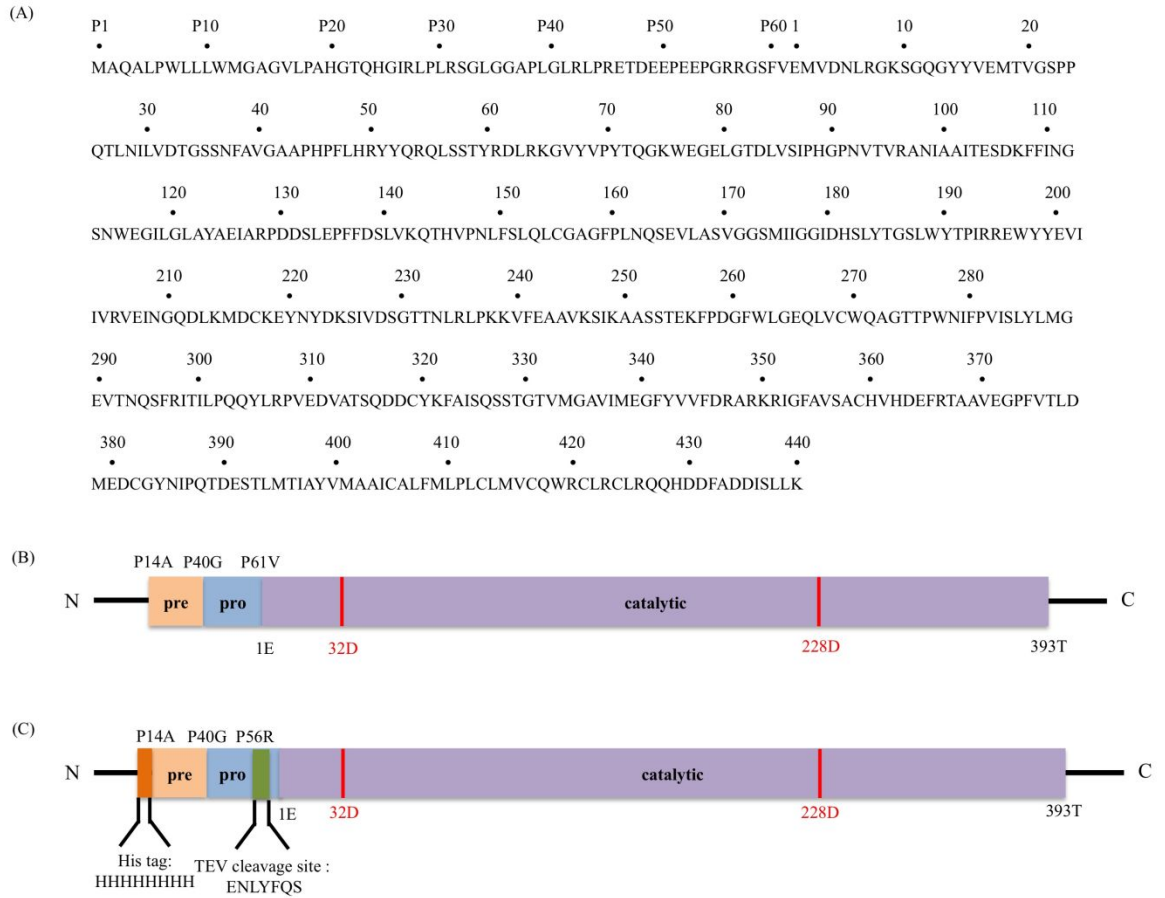
(A)

| P1 | P10 | P20 | P30 | P40 | P50 | P60 1 | 10 | 20 |

MAQALPWLLLWMGAGVLPAHGTQHGIRLPLRSGLGGAPLGLRLPRETDEEPEEPGRRGSFVEMVDNLRGKSGQGYYVEMTVGSPP

| 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 |

QTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYTQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFING

| 120 | 130 | 140 | 150 | 160 | 170 | 180 | 190 | 200 |

SNWEGILGLAYAEIARPDDSLEPFFDSLVKQTHVPNLFSLQLCGAGFPLNQSEVLASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVI

| 210 | 220 | 230 | 240 | 250 | 260 | 270 | 280 |

IVRVEINGQDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMG

| 290 | 300 | 310 | 320 | 330 | 340 | 350 | 360 | 370 |

EVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIMEGFYVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLD

| 380 | 390 | 400 | 410 | 420 | 430 | 440 |

MEDCGYNIPQTDESTLMTIAYVMAAICALFMLPLCLMVCQWRCLRCLRQQHDDFADDISLLK

Figure S2. (A) The amino acid sequence of BACE1. The expression construct scheme of (B) construct A and (C) construct B. The catalytic dyad (32D and 228D) is shown in red.
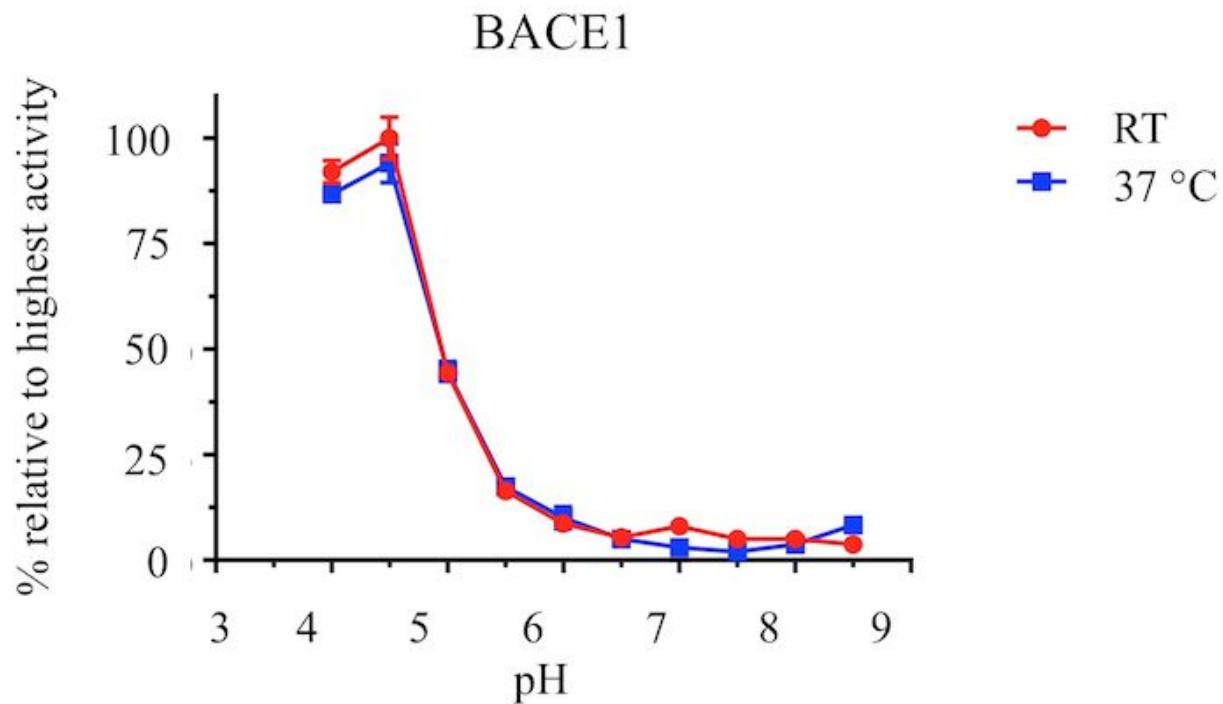
Figure S3. BACE1 activity dependence on pH. The pH range from 4 to 8.5 was tested to determine the pH profile for BACE1 activity. Buffer used are described as following. 0.1M acetic acid was used for pH 4, 4.5, and 5. 0.1M MES was used for pH 5.5, 6, and 6.5. 0.1M HEPES was used for pH 7 and 7.5. 0.1M Tris-Hcl was used for pH 8 and 8.5. Assays were ran at RT (red line) and 37°C (blue line).
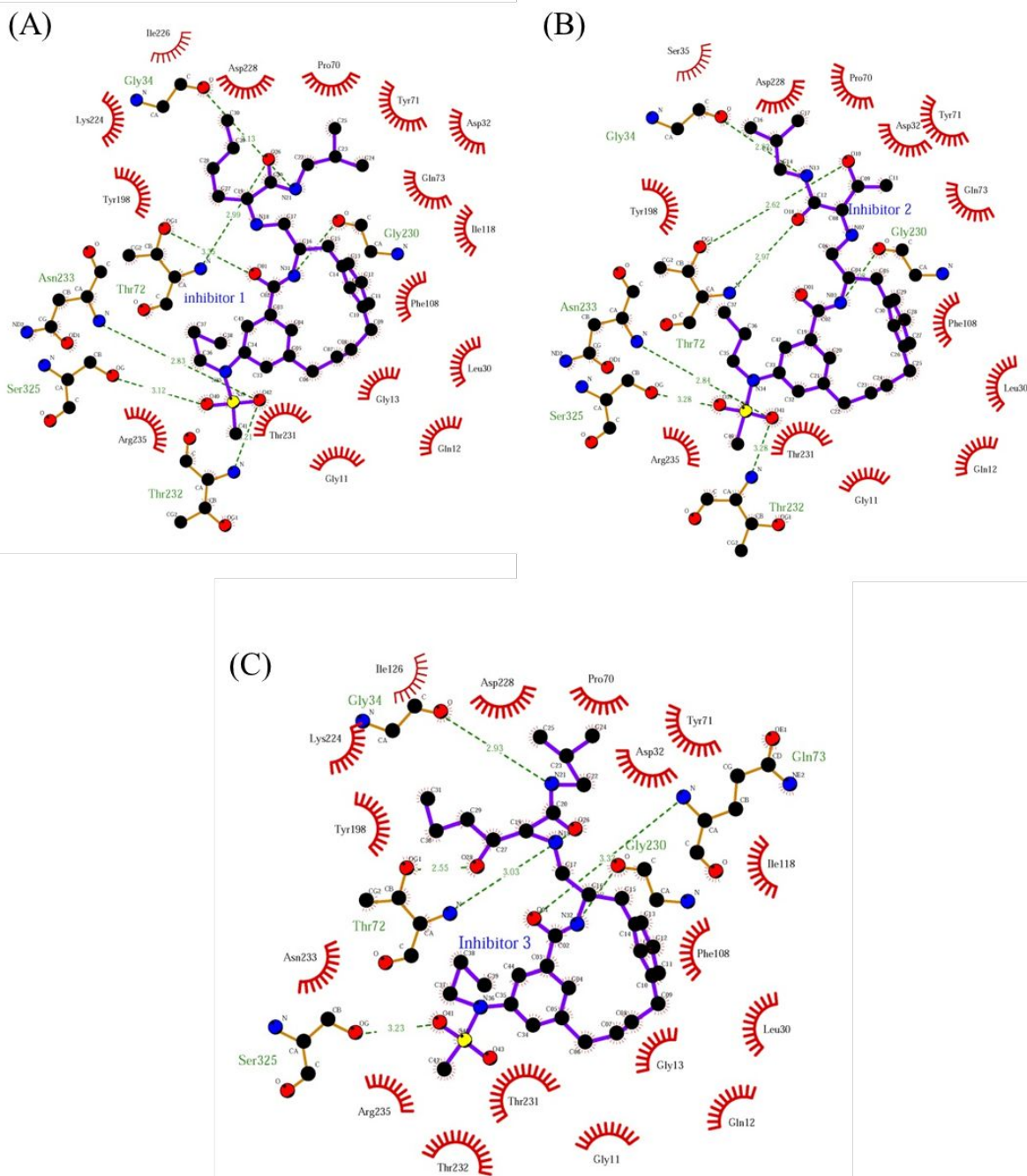
(A)

(B)

(C)

Figure S4. Chemical structure of (A) inhibitor -1, (B) inhibitor -2, and (C) inhibitor 3.

Figure S5. Ligand interaction plot of BACE1 and inhibitors. The binding pocket of BACE1 with (A) inhibitor-1, (B) inhibitor-2, (C) inhibitor -3. The ligands are shown in blue line and the atoms are colored according to the atom types. The polar contacts are indicated by the dash lines with distances shown in angstroms and the interacting residues are represented in orange line. The residues involved in the hydrophobic interactions are labeled and shown in red hashes.

Table S1. Data collection and refinement statistics

	BACE1 in complex with		
Data Collection	**Inhibitor-1**	**Inhibitor-2**	**Inhibitor-3**
X-ray source and detector	LRL-CAT Sector 31 ID-D	LRL-CAT Sector 31 ID-D	LRL-CAT Sector 31 ID-D
Wavelength (Å)	0.9793	0.9793	0.9793
Space Group	$P2_1$	$P2_1$	$P2_1$
Unit Cell dimensions:			
a, b, c (Å)	81.5, 102.4, 99.5	82.1, 102.3, 102.2	82.2, 103.1, 101.5
α, β, γ (°)	105.0	104.6	104.2
Data Processing Statistics	Overall [Last Shell]	Overall [Last Shell]	Overall [Last Shell]
Resolution range (Å)	20-2.35 [2.44-2.35]	98-2.13 [2.21-2.13]	98-2.13 [2.20-2.13]
No. reflection recorded	246,900	334,715	346,163
No. averaged reflections	65,032	90,383	91,867
Average Redundancy	3.8	3.7	3.8
$CC_{1/2}$ (%)	100 [76.8]	99.8 [76.1]	99.5 [78.5]
Rpim[1]	4.0 [37.5]	3.5 [34.4]	4.6 [33.4]
I/σI	13.7 [2.1]	12.7 [2.2]	10.7 [2.4]
% Completeness	99.5 [98.7]	99.2 [99.9]	99.6 [100]
Refinement			
Resolution Range (Å)	20-2.35	98-2.13	98-2.13
No. Reflections in Working Set	63,697	90,320	91,791
No. Reflections in Test Set	3,224	2,000	1,999
R_{work} (%) [2]	18.0	22.2	16.6
R_{free} (%)	21.5	25.8	20.4
Average B-factor (Å²)	35.8	43.2	42.9
RMSD from ideal geometry:			
Bond Lengths (Å)	0.003	0.003	0.009
Bond Angles (degrees)	0.724	0.757	1.117
Ramachandran Plot			
Most Favored (%)	96.75	97.01	96.28
Allowed (%)	3.07	2.9	3.09
Disallowed (%)	0.18	0.09	0.64

[1] $R_{pim} = \sum_{hkl}\sqrt{1/n-1}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where n is the multiplicity.
[2] $R_{work} = \sum_{hkl}||F_{obs}| - |F_{calc}|| / \sum_{hkl}|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively.

Table S2. BACE1 expression systems

Construct	Expression system	Yield (mg/L)	Reference
BACE1-Met^{P1}-Ser^{392} (His$_6$)	CHO-K1	0.75	
BACE1-Met^{P1}-Ser^{392} (myc/His$_6$)	HEK293	0.87	Emmons *et al.* (34)
BACE1-Met^{P1}-Ser^{392} (myc/His$_6$)	CHO-Lec-1	0.4	
(Flag) BACE1-Glu^{P46}-Thr^{393}	HighFive insect cells	3	Bruinzeel *et al.* (35)
BACE1-Ala^{P14}-Thr^{393}	*E.coil*	1	
BACE1-Leu^{P41}-Thr^{393}	*E.coil*	1.2-1.8	
BACE1-Leu^{P43}-Thr^{393}	*E.coil*	2.5-3.5	Sardana *et al.* (12)
BACE1-Arg^{P56}-Thr^{393}	*E.coil*	0.4-0.6	
BACE1-Ala^{P14}-Ser^{392}	*E.coil*	5.5	
BACE1-Arg^{P57}-Ser^{392}(His$_6$)	*E.coil*	7.3	Tomasselli *et al.* (13)
(His$_6$) BACE1-Thr^{P22}-Ser^{392}	*E.coil*	10.3	
BACE1-Ala^{P14}-Thr^{393}	*E.coil*	5.5	This work
(His$_8$)BACE1-Ala^{P14}-Thr^{393}	*E.coil*	16	

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