Supplemental Information

Interaction of Substrates with γ-Secretase at the Level of Individual Transmembrane Helices—A Methodological Approach

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Table S1. Sequences and frames of all TMDs used in manual testting (approach 1).

APP (A4_human)	NMR[1]	...SNK**GAIIGLMVGGVVIATVIVITLVMLKK**KQY...[1]
	frame[2] 0	...GAIIGLMVGGVVIATVIVITLVML
	frame 1	..LGAIIGLMVGGVVIATVIVITLVM.
	frame 2	.LLGAIIGLMVGGVVIATVIVITLV..
	frame 3	LLLGAIIGLMVGGVVIATVIVITL...
Notch 1 (NOTC1_human)	NMR[1]	...PAQ**LHFMYVAAAAFVLLFFVGCGVLLS**RKRR...[1]
	frame 0	...FMYVAAAAFVLLFFVGCGVLL
	frame 1	..LFMYVAAAAFVLLFFVGCGVL.
	frame 2	.LLFMYVAAAAFVLLFFVGCGV..
	frame 3	LLLFMYVAAAAFVLLFFVGCG...
ErbB4 (ERBB4_human)	NMR[1]	...HART**PLIAAGVIGGLFILVIVGLTFAVYVRR**KSI...[1]
	frame 0	...LIAAGVIGGLFILVIVGLTFAVYV
	frame 1	..LLIAAGVIGGLFILVIVGLTFAVY.
	frame 2	.LLLIAAGVIGGLFILVIVGLTFAV..
	frame 3	LLLLIAAGVIGGLFILVIVGLTFA...
N-Cadherin (CADH2_human)	predicted[3]	...LGTGA**IIAILLCIIILLILVLMFVVW**MKRRD...[3]
	frame 0	...GAIIAILLCIIILLILVLMFVVW
	frame 1	..LGAIIAILLCIIILLILVLMFVV.
	frame 2	.LLGAIIAILLCIIILLILVLMFV..
	frame 3	LLLGAIIAILLCIIILLILVLMF...
Integrin β1 (ITB1_human)	predicted[3]	...PTGPD**IIPIVAGVVAGIVLIGLALLLIW**KLLMI...[3]
	frame 0	...IIPIVAGVVAGIVLIGLALLLIW
	frame 1	..LIIPIVAGVVAGIVLIGLALLLI.
	frame 2	.LLIIPIVAGVVAGIVLIGLALLL..
	frame 3	LLLIIPIVAGVVAGIVLIGLALL...
Nicastrin [4]	frame 0	...LITLTVGFGILIFSLIVTY
	frame 1	..LLITLTVGFGILIFSLIVT.
	frame 2	.LLLITLTVGFGILIFSLIV..
	frame 3	LLLLITLTVGFGILIFSLI...
PEN-2 [4]	frame 0	...AVGFLFWVIVLTSWITIFQIY
	frame 1	..LAVGFLFWVIVLTSWITIFQI.
	frame 2	.LLAVGFLFWVIVLTSWITIFQ..
	frame 3	LLLAVGFLFWVIVLTSWITIF...
PS1 TMD1 [4]	frame 0	HVIMLFVPVTLCMVVVVATI...
	frame 1	.VIMLFVPVTLCMVVVVATIL..
	frame 2	..IMLFVPVTLCMVVVVATILL.
	frame 3	...MLFVPVTLCMVVVVATILLL
PS1 TMD2 [4]	frame 0	...ILNAAIMISVIVVMTILLVVLY
	frame 1	..LILNAAIMISVIVVMTILLVVL.
	frame 2	.LLILNAAIMISVIVVMTILLVV..

	frame 3	LLLILNAAIMISVIVVMTILLV...
PS1 TMD3[4 5]	frame 0	VIHAWLIISSLLLLFFFSFIYLG…
	frame 1	.IHAWLIISSLLLLFFFSFIYLGL..
	frame 2	..HAWLIISSLLLLFFFSFIYLGLL.
	frame 3	...AWLIISSLLLLFFFSFIYLGLLL
PS1 TMD4 [4]	frame 0	...YITVALLIWNFGVVGMISIHW
	frame 1	..LYITVALLIWNFGVVGMISIH.
	frame 2	.LLYITVALLIWNFGVVGMISI..
	frame 3	LLLYITVALLIWNFGVVGMIS...
PS1 TMD5 [4]	frame 0	LRLQQAYLIMISALMALVFI...
	frame 1	.RLQQAYLIMISALMALVFIL..
	frame 2	..LQQAYLIMISALMALVFILL.
	frame 3	...QQAYLIMISALMALVFILLL
PS1 TMD6 [4]	frame 0	...WTAWLILAVISVYDLVAVLCP
	frame 1	..LWTAWLILAVISVYDLVAVLC.
	frame 2	.LLWTAWLILAVISVYDLVAVL..
	frame 3	LLLWTAWLILAVISVYDLVAV...
PS1 TMD7 D385L/K395L [4,6]	frame 0	LGLGLFIFYSVLVGLASA...
	frame 1	.GLGLFIFYSVLVGLASAL..
	frame 2	..LGLFIFYSVLVGLASALL.
	frame 3	...GLFIFYSVLVGLASALLL
PS1 TMD8 [4]	frame 0	...NTTIACFVAILIGLCLTLLLLA
	frame 1	..LNTTIACFVAILIGLCLTLLLL.
	frame 2	.LLNTTIACFVAILIGLCLTLLL..
	frame 3	LLLNTTIACFVAILIGLCLTLL...
PS1 TMD9 D450L [4, 5,6]	frame 0	PALPISITFGLVFYFATLYLV...
	frame 1	.ALPISITFGLVFYFATLYLVL..
	frame 2	..LPISITFGLVFYFATLYLVLL.
	frame 3	...PISITFGLVFYFATLYLVLLL
GpA		LIIFGVMAGVIGTILLISY
GpA G83I [8]		LIIFGVMAIVIGTILLISY
EmrE TMD4		LPAIIGMMLICAGVLIINL
EmrE TMD4 G90V/G97V [8]		LPAIIVMMLICAVVLIINL
L20		LLLLLLLLLLLLLLLLLLL

[1] TMDs where NMR spectroscopy had previously identified the helical parts, as given in bold face (APP, pdb 6HYF; Notch1, pdb 8OR5; ErbB4, pdb 2LCX). In general, juxtamembrane domains were omitted to prevent the potential interference of their polar residues with membrane insertion and/or transmembrane topology. An invariant cytoplasmically located Lys or Arg residue acts as stop-transfer signal in N_{out} and N_{in} constructs.

[2] Frames 0 – 3 were tested for heteromerization. The stepwise insertion of up to three additional residues at a given N-terminus concurrent with the stepwise deletion of three residues at the C-terminus rotates potential TMD-TMD interfaces by up to 3 × 100°, i.e., almost a full helix turn, relative to the soluble domains in the hybrid BLa proteins.

[3] TMDs where the helical parts had been predicted from the sequences, as annotated in UniProtKB.

[4] TMDs from γ–secretase subunits, as identified in the cryo-EM structure (pdb 5fn2 {Bai, 2015 #7886}) except for the exceptionally long PS1 TMD2 where the sequence shown here corresponds to the length yielding the maximal LD_{50} (see: Fig. 2).

[5] PS1 TMD3 is truncated right before E184 as not to interfere with membrane integration.

[6] Residues marked in red indicate point mutations of ionizable residues that were necessary to integrate the TMD into the membrane.

[7] TMD9 is truncated after V453 where a helix kink occurs in the structure.

[8] Residues marked in red indicate point mutations at the respective helix-helix interfaces reducing the affinities of self-interaction.

Table S2. Sequences and frames of all TMDs used in the library screening approach (approach 2).

A4_HUMAN	frame 0	...GAIIGLMVGGVVIATVIVITLVML
	frame 1	..LGAIIGLMVGGVVIATVIVITLVM.
	frame 2	.LLGAIIGLMVGGVVIATVIVITLV..
	frame 3	LLLGAIIGLMVGGVVIATVIVITL...
ANPRC_HUMAN	frame 0	...SAVTGIVVGALLGAGLLMAFYFF
	frame 1	..LSAVTGIVVGALLGAGLLMAFYF.
	frame 2	.LLSAVTGIVVGALLGAGLLMAFY..
	frame 3	LLLSAVTGIVVGALLGAGLLMAF...
APLP1_HUMAN	frame 0	...AVSGLLIMGAGGGSLIVLSMLLL
	frame 1	..LAVSGLLIMGAGGGSLIVLSMLL.
	frame 2	.LLAVSGLLIMGAGGGSLIVLSML..
	frame 3	LLLAVSGLLIMGAGGGSLIVLSM...
APLP2_HUMAN	frame 0	...ALIGLLVIAVAIATVIVISLVML
	frame 1	..LALIGLLVIAVAIATVIVISLVM.
	frame 2	.LLALIGLLVIAVAIATVIVISLV..
	frame 3	LLLALIGLLVIAVAIATVIVISL...
CADH2_HUMAN	frame 0	...GAIIAILLCIIILLILVLMFVVW
	frame 1	..LGAIIAILLCIIILLILVLMFVV.
	frame 2	.LLGAIIAILLCIIILLILVLMFV..
	frame 3	LLLGAIIAILLCIIILLILVLMF...
CD44_HUMAN	frame 0	...WLIILASLLALALILAVCIAV
	frame 1	..LWLIILASLLALALILAVCIA.
	frame 2	.LLWLIILASLLALALILAVCI..
	frame 3	LLLWLIILASLLALALILAVC...
CSF1R_HUMAN	frame 0	...VVVACMSIMALLLLLLLLLLY
	frame 1	..LVVVACMSIMALLLLLLLLL.
	frame 2	.LLVVVACMSIMALLLLLLLL..
	frame 3	LLLVVVACMSIMALLLLLLL...
CSTN1_HUMAN	frame 0	...ATVVIVVCVSFLVFMIILGVF
	frame 1	..LATVVIVVCVSFLVFMIILGV.
	frame 2	.LLATVVIVVCVSFLVFMIILG..
	frame 3	LLLATVVIVVCVSFLVFMIIL...
CSTN2_HUMAN	frame 0	...IATVVIIISVCMLVFVVAMGV
	frame 1	..LIATVVIIISVCMLVFVVAMG.
	frame 2	.LLIATVVIIISVCMLVFVVAM..
	frame 3	LLLIATVVIIISVCMLVFVVA...
DCC_HUMAN	frame 0	...LLVIIVVTVGVITVLVVVIVAVI
	frame 1	..LLLVIIVVTVGVITVLVVVIVAV.
	frame 2	.LLLLVIIVVTVGVITVLVVVIVA..
	frame 3	LLLLLVIIVVTVGVITVLVVVIV...

DNER_HUMAN	frame 0	...IIIGALCVAFILMLIILIVGI
	frame 1	..LIIIGALCVAFILMLIILIVG.
	frame 2	.LLIIIGALCVAFILMLIILIV..
	frame 3	LLLIIIGALCVAFILMLIILI...
DSG2_HUMAN	frame 0	...PAAIALMILAFLLLLLVPLLLLM
	frame 1	..LPAAIALMILAFLLLLLVPLLLL.
	frame 2	.LLPAAIALMILAFLLLLLVPLLL..
	frame 3	LLLPAAIALMILAFLLLLLVPLL...
ERBB4_HUMAN	frame 0	...LIAAGVIGGLFILVIVGLTFAVYV
	frame 1	..LLIAAGVIGGLFILVIVGLTFAVY.
	frame 2	.LLLIAAGVIGGLFILVIVGLTFAV..
	frame 3	LLLLIAAGVIGGLFILVIVGLTFA...
GLPA_HUMAN	frame 0	...ITLIIFGVMAGVIGTILLISYGI
	frame 1	..LITLIIFGVMAGVIGTILLISYG.
	frame 2	.LLITLIIFGVMAGVIGTILLISY..
	frame 3	LLLITLIIFGVMAGVIGTILLIS...
HLAA_HUMAN	frame 0	...VGIIAGLVLLGAVITGAVVAAVMW
	frame 1	..LVGIIAGLVLLGAVITGAVVAAVM.
	frame 2	.LLVGIIAGLVLLGAVITGAVVAAV..
	frame 3	LLLVGIIAGLVLLGAVITGAVVAA...
ITB1_HUMAN	frame 0	...IIPIVAGVVAGIVLIGLALLLIW
	frame 1	..LIIPIVAGVVAGIVLIGLALLLI.
	frame 2	.LLIIPIVAGVVAGIVLIGLALLL..
	frame 3	LLLIIPIVAGVVAGIVLIGLALL...
JAG2_HUMAN	frame 0	...GLLVPVLCGAFSVLWLACVVL
	frame 1	..LGLLVPVLCGAFSVLWLACVV.
	frame 2	.LLGLLVPVLCGAFSVLWLACV..
	frame 3	LLLGLLVPVLCGAFSVLWLAC...
LDLR_HUMAN	frame 0	...ALSIVLPIVLLVFLCLGVFLLW
	frame 1	..LALSIVLPIVLLVFLCLGVFLL.
	frame 2	.LLALSIVLPIVLLVFLCLGVFL..
	frame 3	LLLALSIVLPIVLLVFLCLGVF...
NOTC1_HUMAN	frame 0	...FMYVAAAAFVLLFFVGCGVLL
	frame 1	..LFMYVAAAAFVLLFFVGCGVL.
	frame 2	.LLFMYVAAAAFVLLFFVGCGV..
	frame 3	LLLFMYVAAAAFVLLFFVGCG...
NOTC2_HUMAN	frame 0	...LLYLLAVAVVIILFIILLGVI
	frame 1	..LLLYLLAVAVVIILFIILLGV.
	frame 2	.LLLLYLLAVAVVIILFIILLG..
	frame 3	LLLLLYLLAVAVVIILFIILL...
NOTC3_HUMAN	frame 0	...LLPLLVAGAVLLLVILVLGVMVA
	frame 1	..LLLPLLVAGAVLLLVILVLGVMV.

	frame 2	.LLLLPLLVAGAVLLLVILVLGVM..
	frame 3	LLLLLPLLVAGAVLLLVILVLGV...
NOTC4_HUMAN	frame 0	...VLCSPVAGVILLALGALLVLQLI
	frame 1	..LVLCSPVAGVILLALGALLVLQL.
	frame 2	.LLVLCSPVAGVILLALGALLVLQ..
	frame 3	LLLVLCSPVAGVILLALGALLVL...
PTPRT_HUMAN	frame 0	...MAGVIAGLLMFIIILLGVMLTI
	frame 1	..LMAGVIAGLLMFIIILLGVMLT.
	frame 2	.LLMAGVIAGLLMFIIILLGVML..
	frame 3	LLLMAGVIAGLLMFIIILLGVM...
PXDC2_HUMAN	frame 0	...GLIIGILILVLIVATAILVTV
	frame 1	..LGLIIGILILVLIVATAILVT.
	frame 2	.LLGLIIGILILVLIVATAILV..
	frame 3	LLLGLIIGILILVLIVATAIL...
SDC1_HUMAN	frame 0	...GVIAGGLVGLIFAVCLVGFML
	frame 1	..LGVIAGGLVGLIFAVCLVGFM.
	frame 2	.LLGVIAGGLVGLIFAVCLVGF..
	frame 3	LLLGVIAGGLVGLIFAVCLVG...
SDC2_HUMAN	frame 0	...VLAAVIAGGVIGFLFAIFLILLLV
	frame 1	..LVLAAVIAGGVIGFLFAIFLILLL.
	frame 2	.LLVLAAVIAGGVIGFLFAIFLILL..
	frame 3	LLLVLAAVIAGGVIGFLFAIFLIL...
TNR17_HUMAN	frame 0	...ILWTCLGLSLIISLAVFVLMFLL
	frame 1	..LILWTCLGLSLIISLAVFVLMFL.
	frame 2	.LLILWTCLGLSLIISLAVFVLMF..
	frame 3	LLLILWTCLGLSLIISLAVFVLM...
TYOBP_HUMAN	frame 0	...GVLAGIVMGDLVLTVLIALAV
	frame 1	..LGVLAGIVMGDLVLTVLIALA.
	frame 2	.LLGVLAGIVMGDLVLTVLIAL..
	frame 3	LLLGVLAGIVMGDLVLTVLIA...
PS1 TMD2	cryo-EM[1]	...VGQRALHSILNAAIMISVIVVMTILLVVLY
	frame 3	LLLVGQRALHSILNAAIMISVI..........
	frame 2	.LLVGQRALHSILNAAIMISVIV.........
	frame 1	..LVGQRALHSILNAAIMISVIVV........
	frame 0	...VGQRALHSILNAAIMISVIVVM.......
	frame -1	....GQRALHSILNAAIMISVIVVMT.......
	frame -2	.....QRALHSILNAAIMISVIVVMTI......
	frame -3	......RALHSILNAAIMISVIVVMTIL.....
	frame -4	.......ALHSILNAAIMISVIVVMTILL....
	frame -5	........LHSILNAAIMISVIVVMTILLV...
	frame -6	.........HSILNAAIMISVIVVMTILLVV..
	frame -7	..........SILNAAIMISVIVVMTILLVVL.

frame -8	..........ILNAAIMISVIVVMTILLVVLY
GpA	LIIFGVMAGVIGTILLISY
GpA G83I 8	LIIFGVMAIVIGTILLISY
L20	LLLLLLLLLLLLLLLLLLL

[1] TMD2 from presenilin 1, as identified in the cryo-EM structure (pdb 5fn2 {Bai, 2015 #7886}).

Table S3. DNA oligonucleotide sequences used in qPCR and NGS.

GpA wt control primer pair	
qPCR_NBLa_fwd[1]	CAAACGACGAGCGTGACACC
qPCR_GpA_wt_rev	GAATGGTGCCAATCACGCC
GpA G83I control primer pair	
qPCR_NBLa_fwd[1]	CAAACGACGAGCGTGACACC
qPCR_GpA_G83I_rev	AGCAGAATGGTGCCAATCACAAT
Full plasmid quantification control primer pair	
qPCR_plasmid_control_fwd	CGAGAAGCAGGCCATTATCG
qPCR_plasmid_control_fwd	GATGGTCGTCATCTACCTGC
Oligonucleotides used for NGS amplicon generation	
NBLa_NGS_fwd	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGTAGCAATGGCAACAACG[2]
NBLa_NGS_rev	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATCCGAGTAACCAGAATCGGAACAA[2]
Substrate library amplification	
[BIOTIN]BLa_Library_fwd	[BIOTIN]GTTCTGCTAATCGAGCTAGC
[BIOTIN]BLa_Library_rev	[BIOTIN]TGCCAGTTTGTGGATCCC

[1] Shared oligonucleotide for both controls

[2] underlined sequences are priming regions within the NBLa-plasmid; 5' parts are Illumina universal sequencing adapters

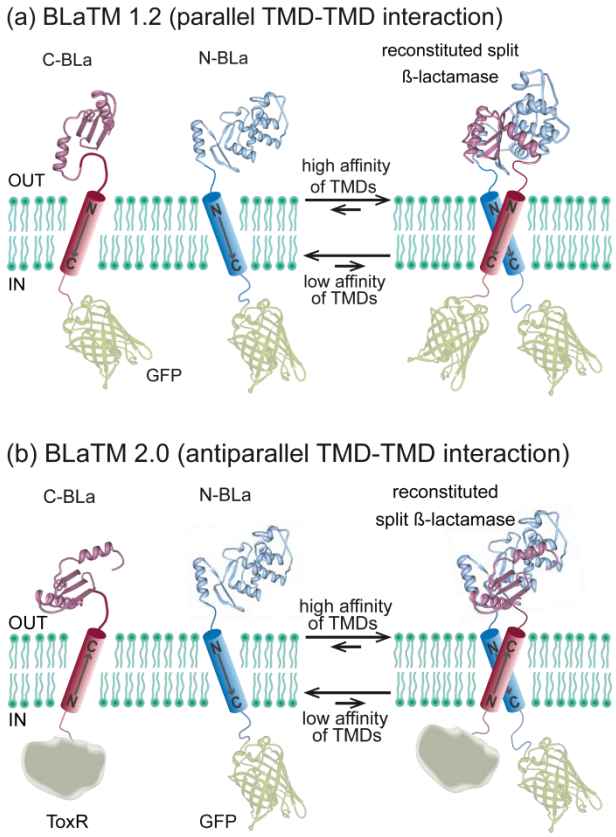
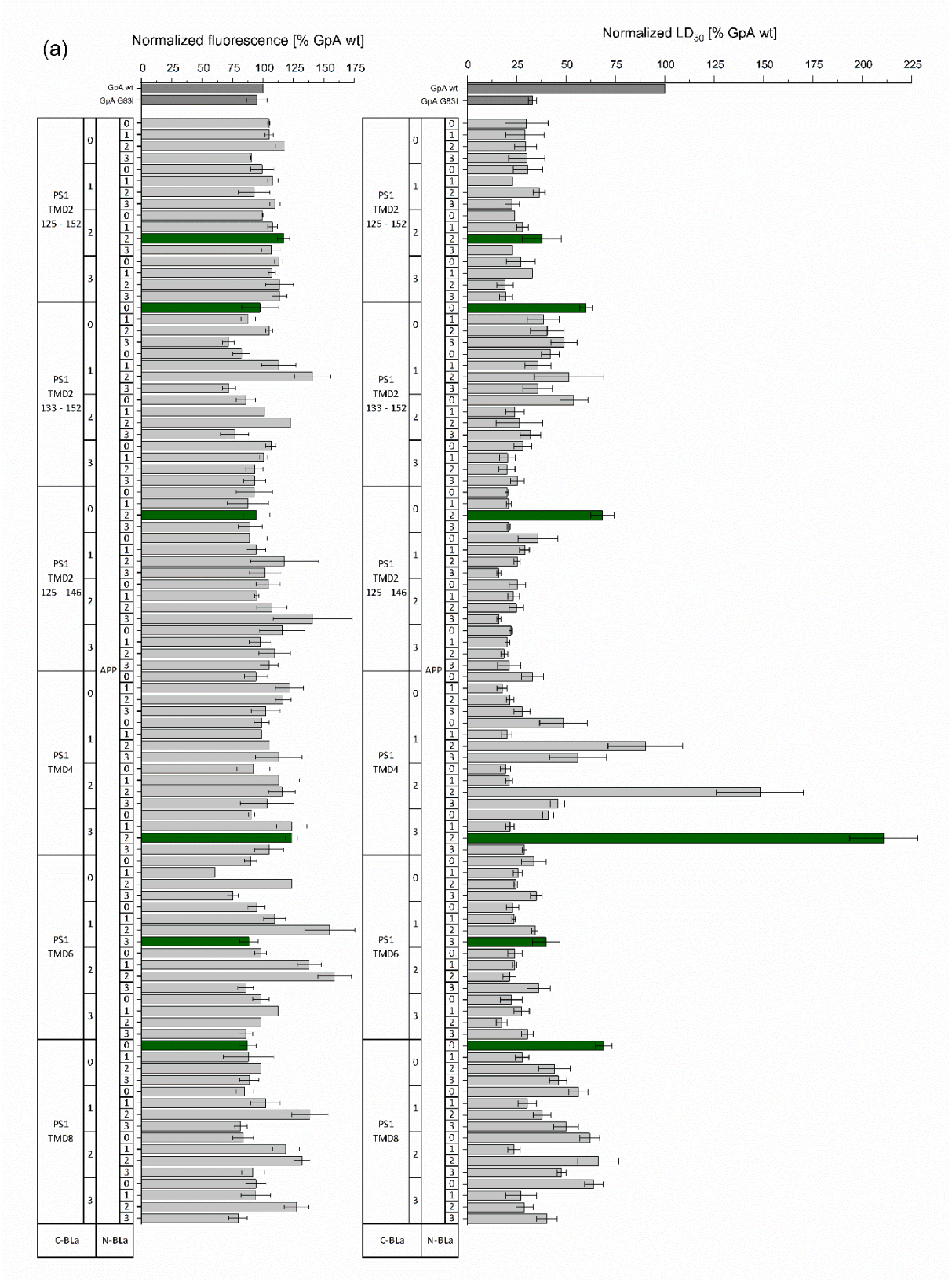
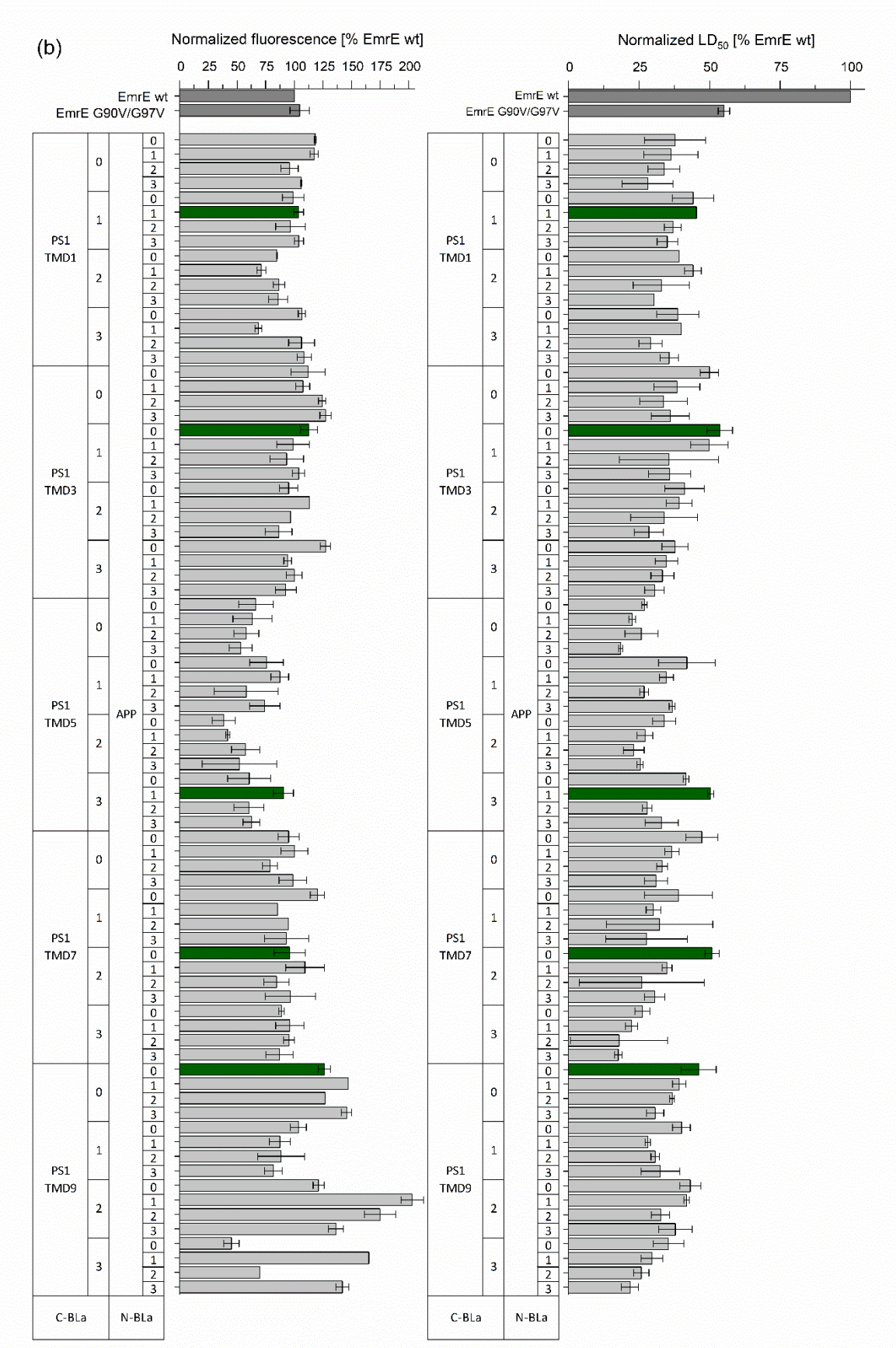
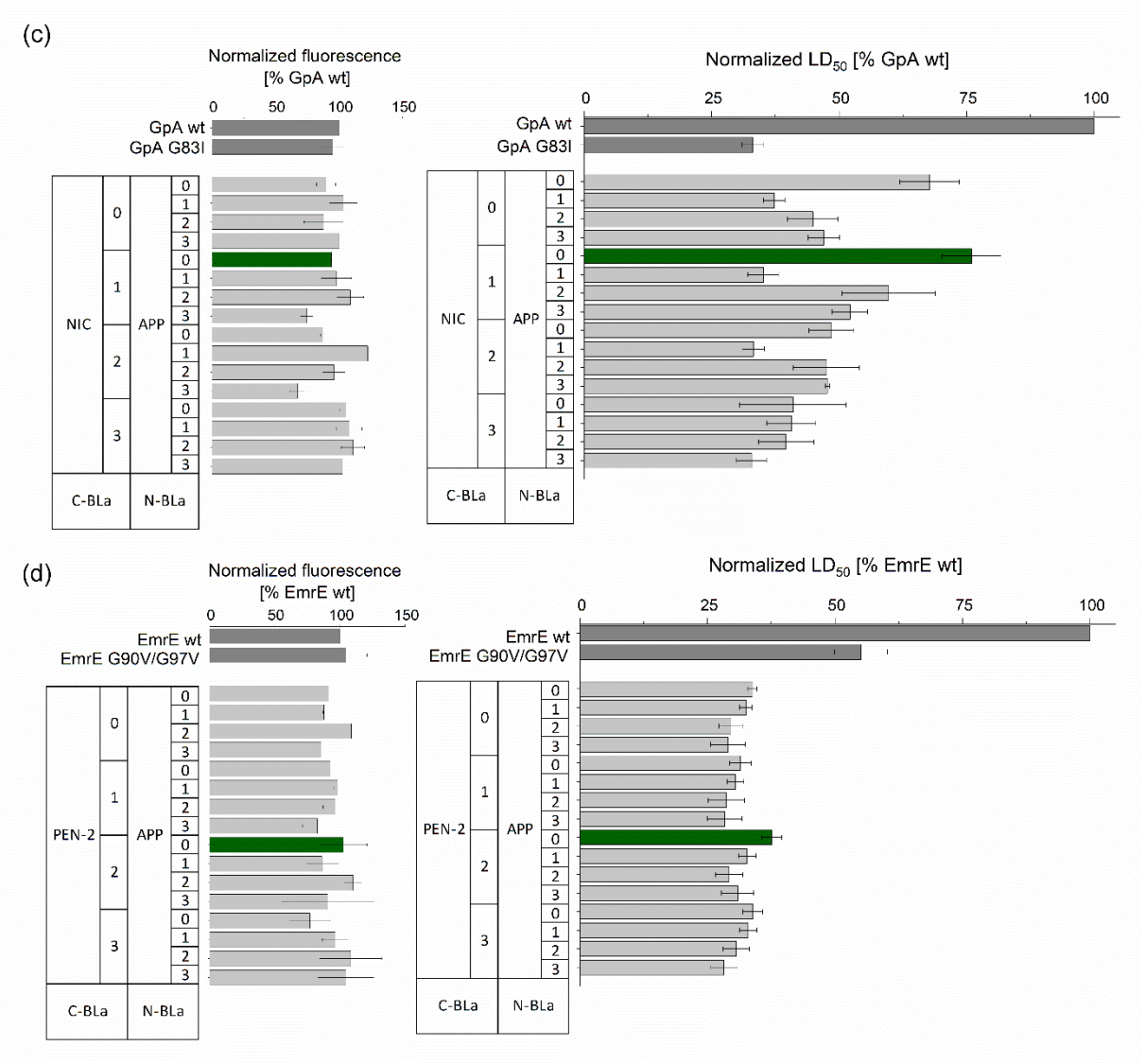
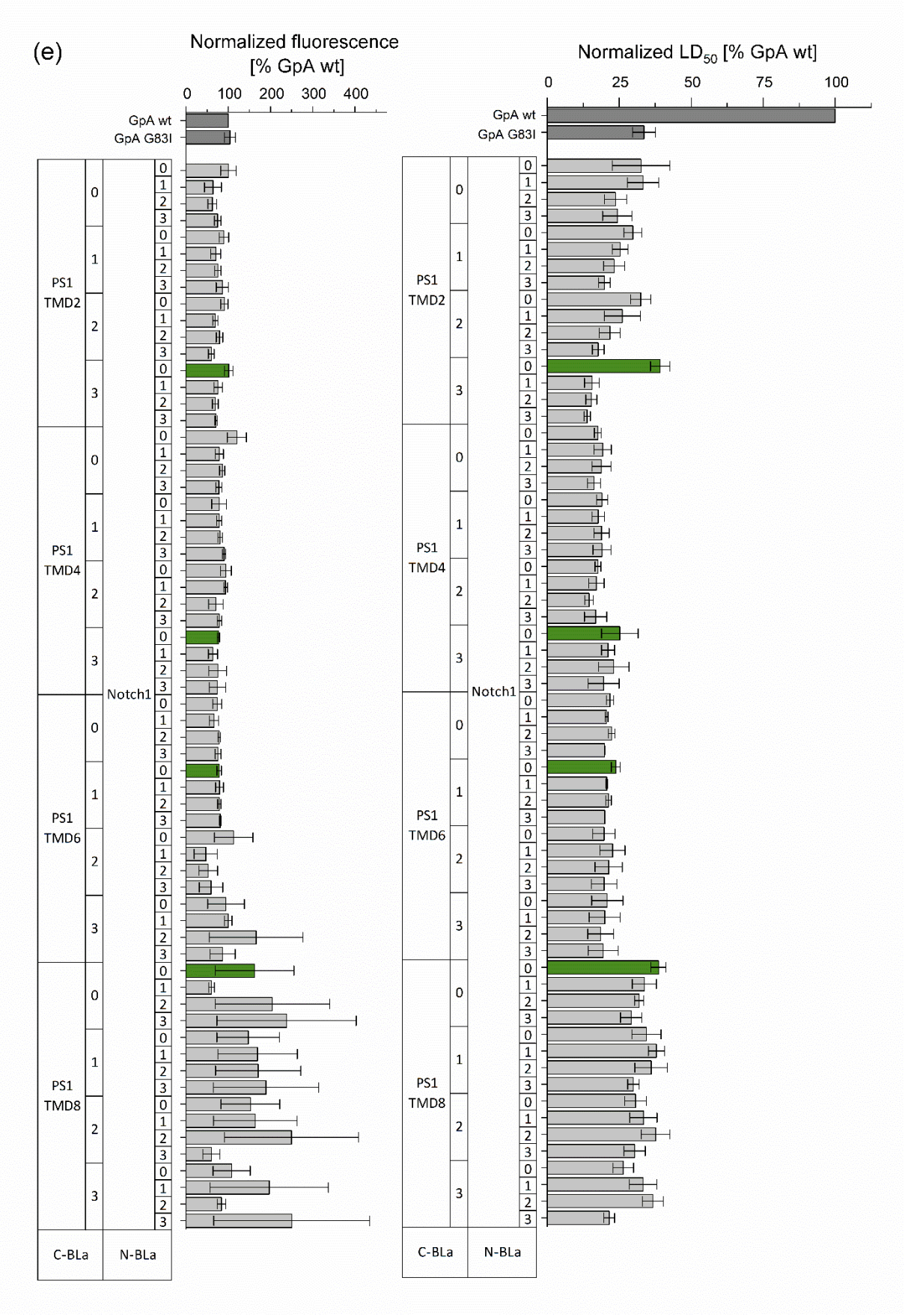


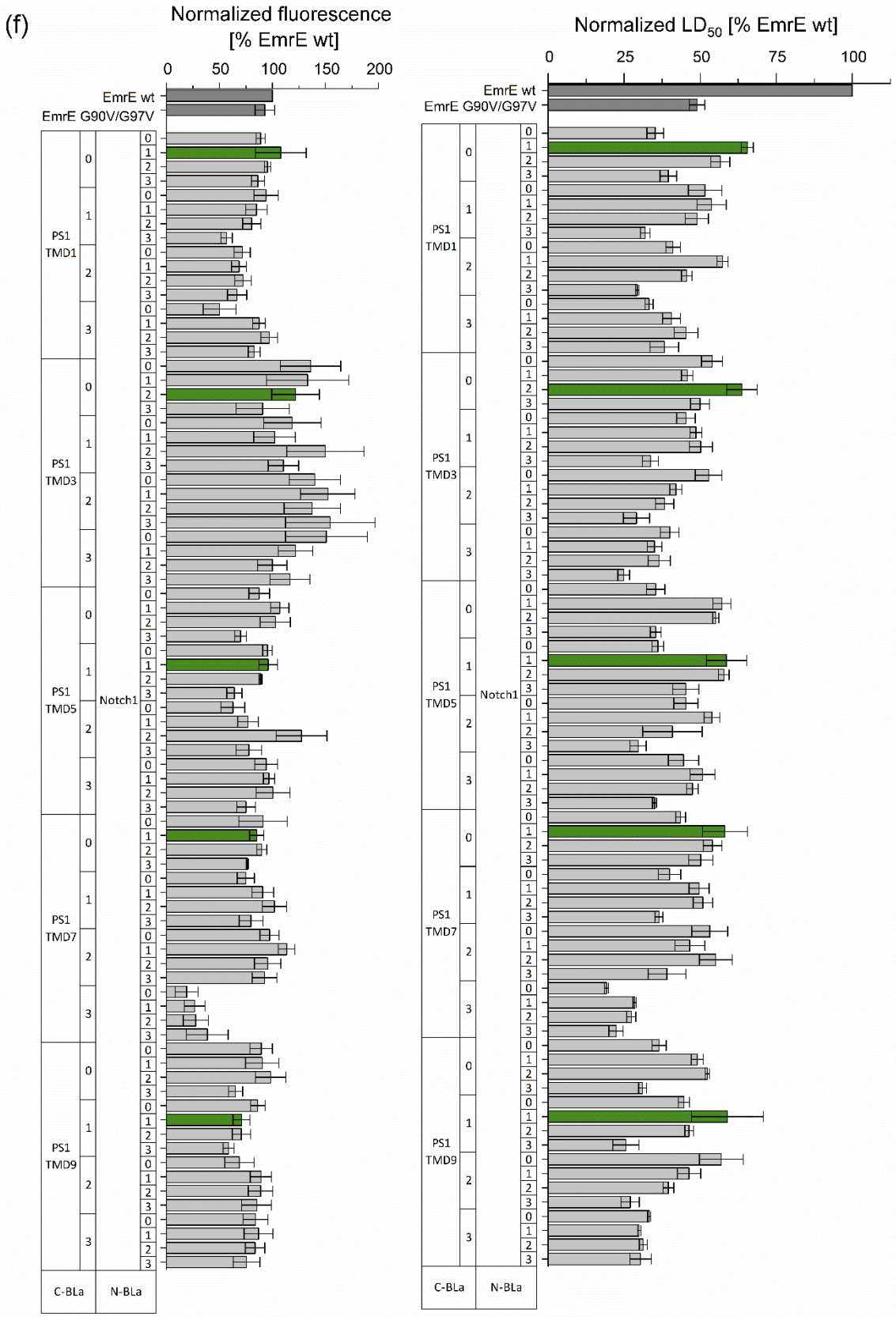
Figure S1. Design of the BLaTM system. **(a)** Scheme of BLaTM 1.2 used to study parallel TMD interactions (modified after Fig. 1 in 17). **(b)** Scheme of BLaTM 2.0 used to study antiparallel interactions. Reproduced, with permission, from ref. {Julius, 2017 #8260}.

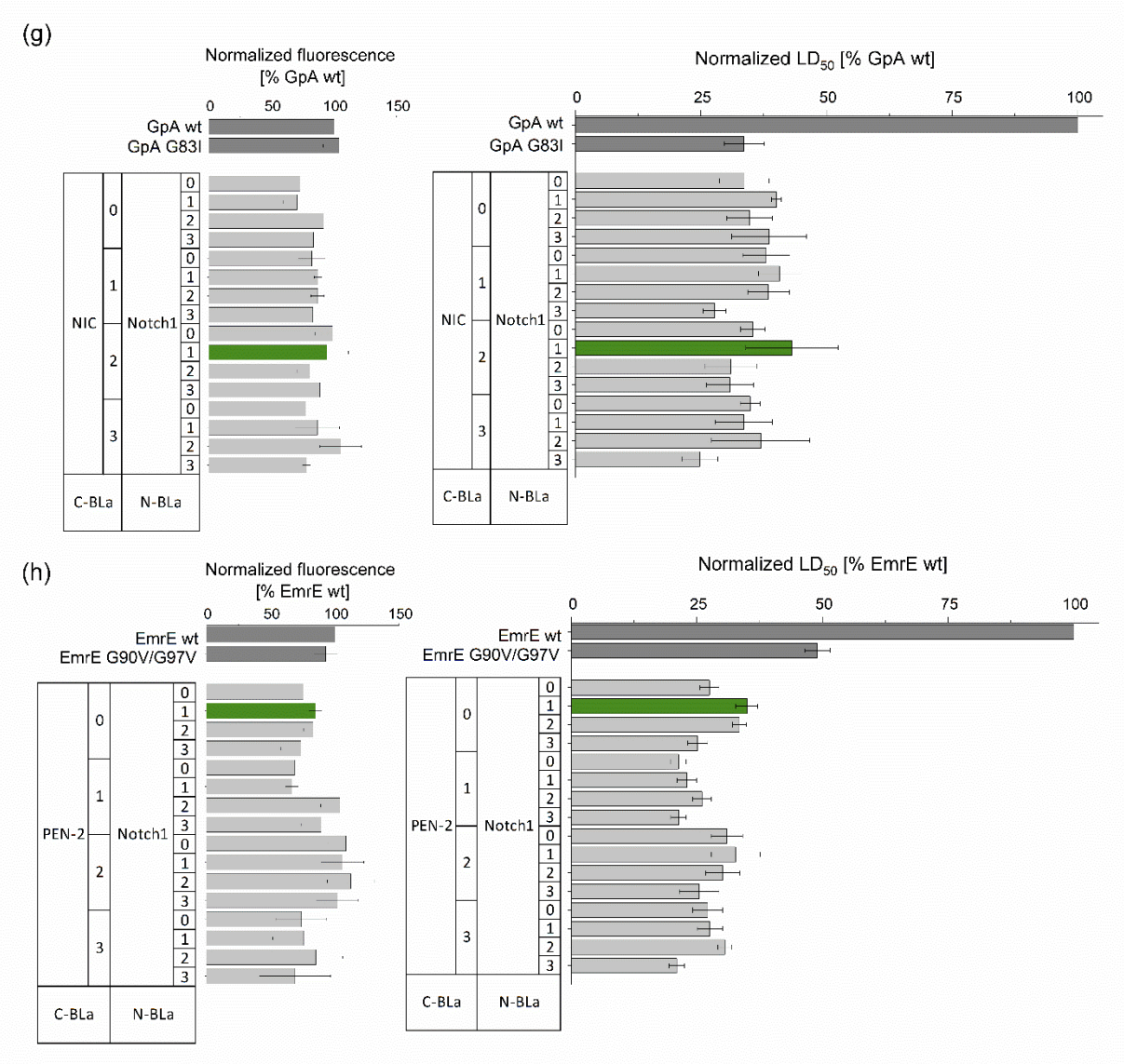

(a)
Normalized fluorescence [% GpA wt]
Normalized LD50 [% GpA wt]
GpA wt
GpA G83I
PS1 TMD2 125 - 152
PS1 TMD2 133 - 152
PS1 TMD2 125 - 146
PS1 TMD4
PS1 TMD6
PS1 TMD8
APP
C-BLa
N-BLa

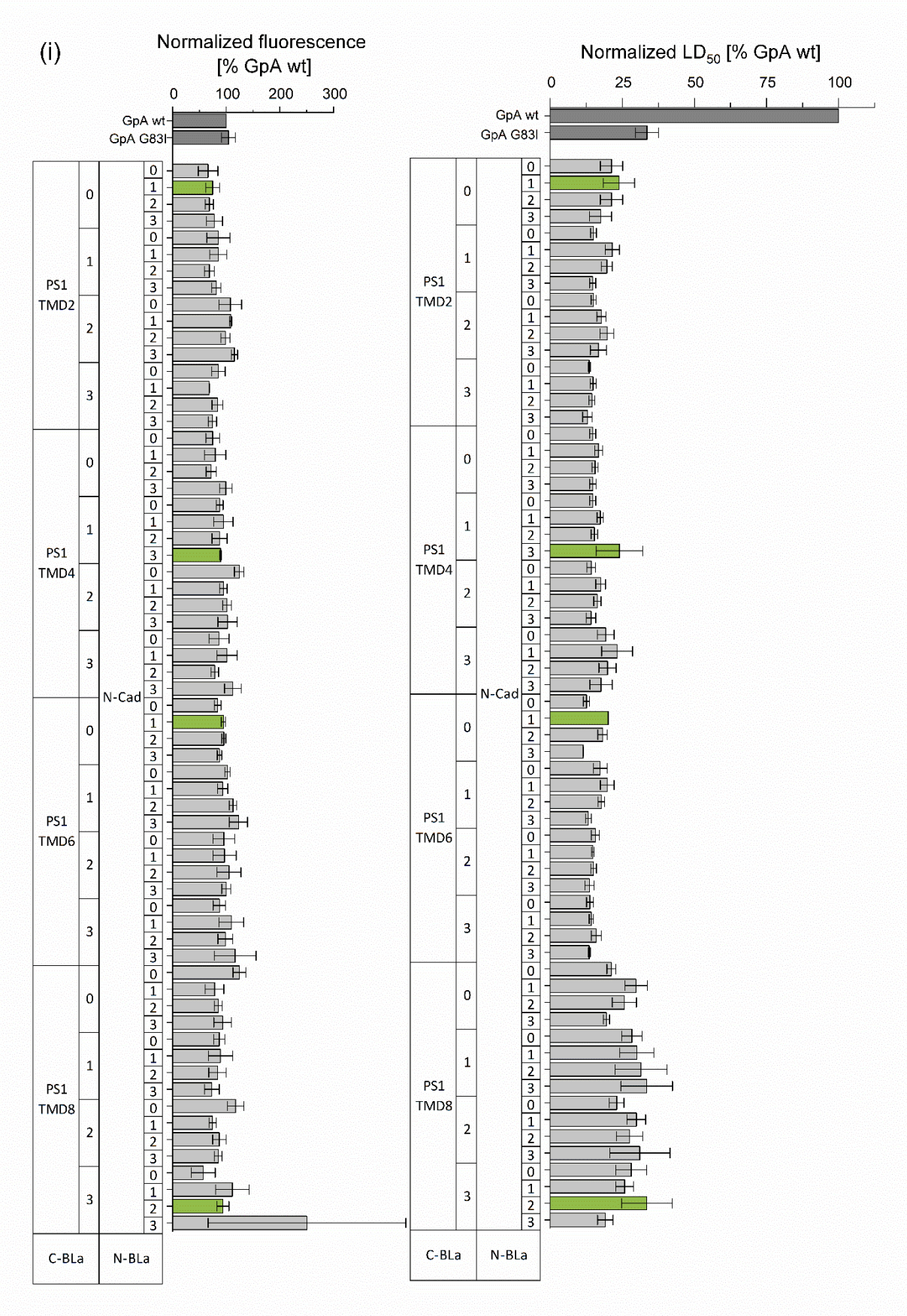

(b)
Normalized fluorescence [% EmrE wt]
0 25 50 75 100 125 150 175 200
EmrE wt
EmrE G90V/G97V
Normalized LD50 [% EmrE wt]
0 25 50 75 100
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
APP
C-BLa
N-BLa

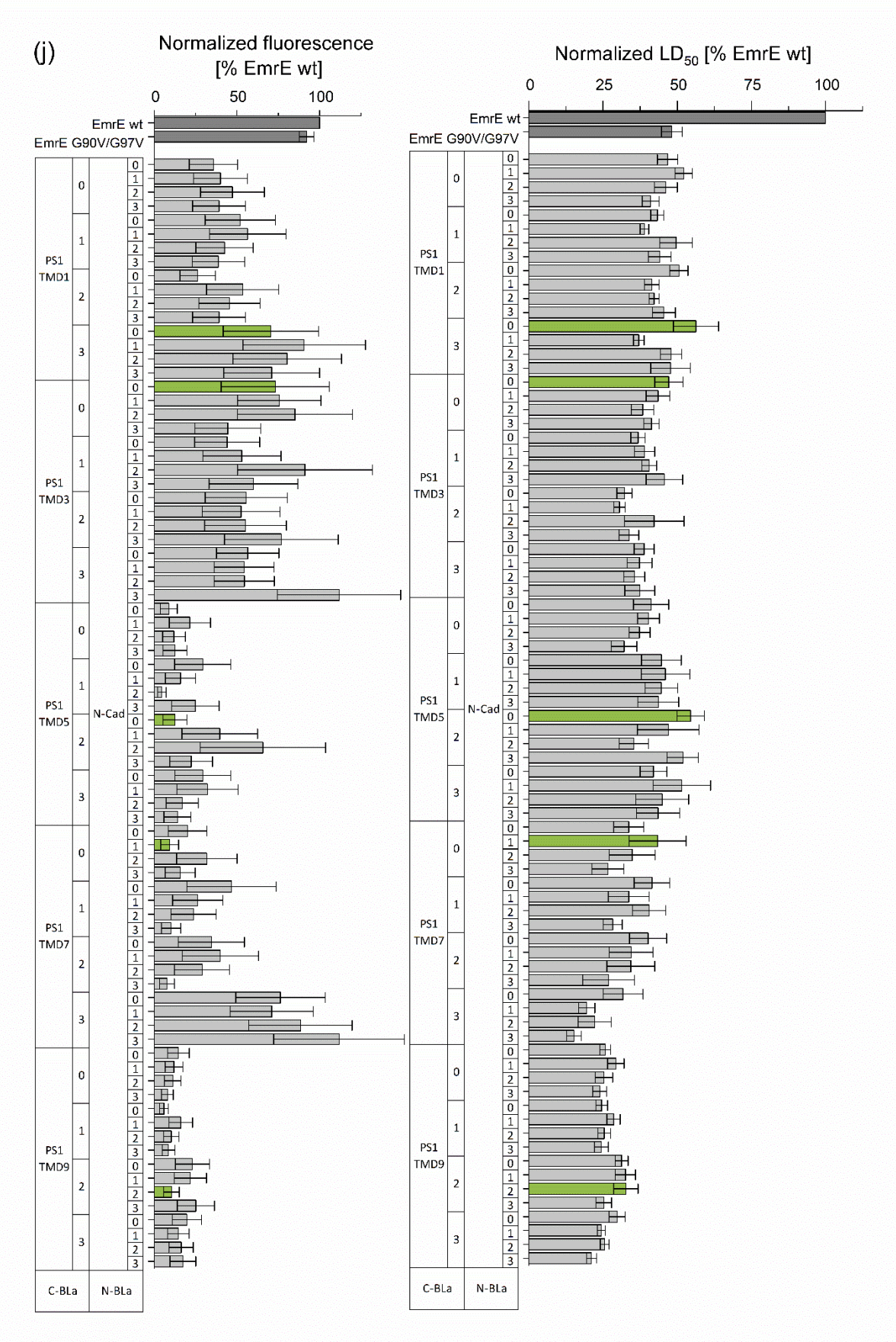

(c)
Normalized fluorescence
[% GpA wt]
0
50
100
150
GpA wt
GpA G83I
NIC
APP
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
(d)
Normalized fluorescence
[% EmrE wt]
EmrE wt
EmrE G90V/G97V
PEN-2
Normalized LD50 [% EmrE wt]

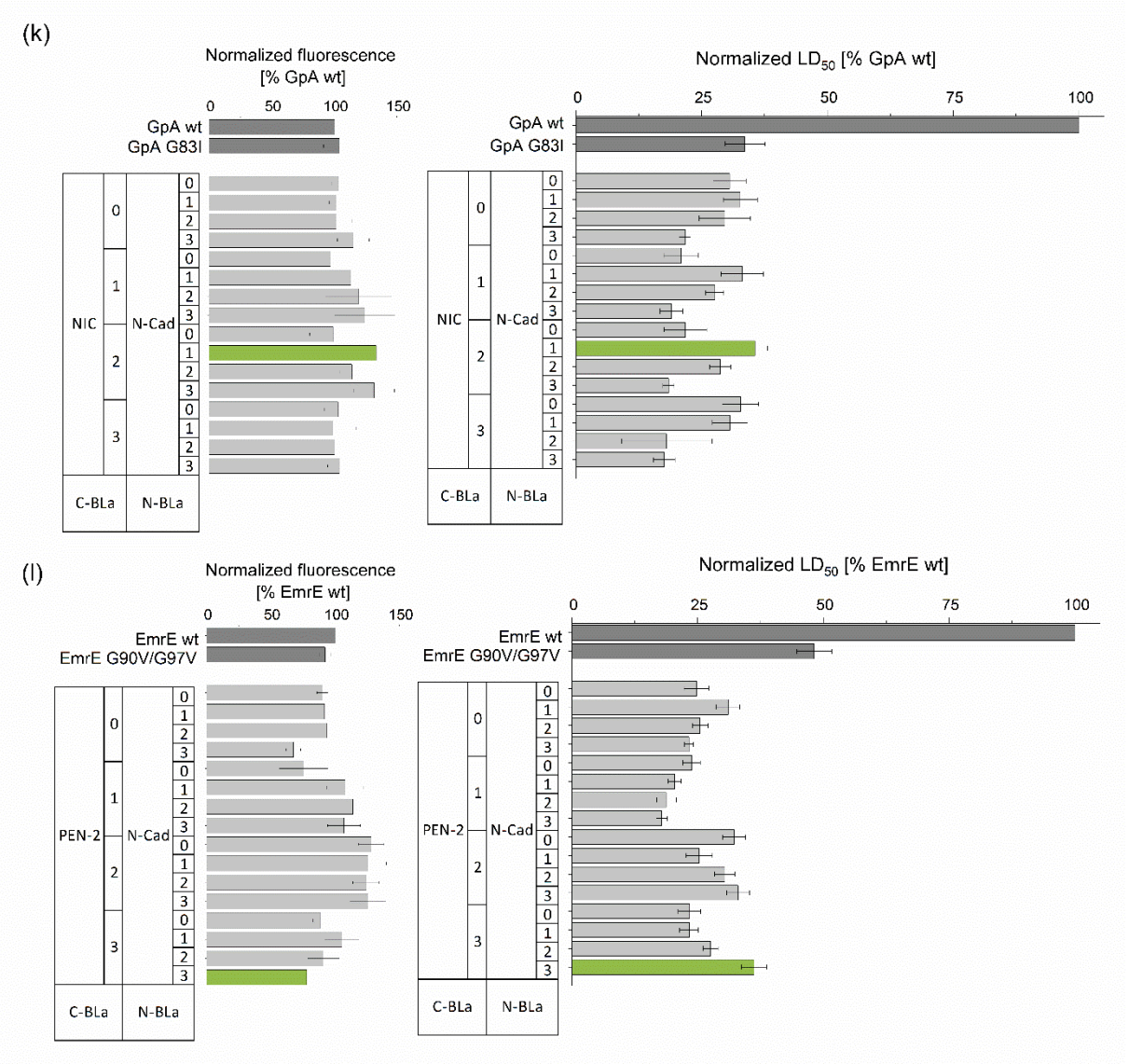

(e)
Normalized fluorescence [% GpA wt]
0
100
200
300
400
GpA wt
GpA G83I
PS1 TMD2
PS1 TMD4
PS1 TMD6
PS1 TMD8
Notch1
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
GpA wt
GpA G83I
PS1 TMD2
PS1 TMD4
PS1 TMD6
PS1 TMD8
Notch1
C-BLa
N-BLa

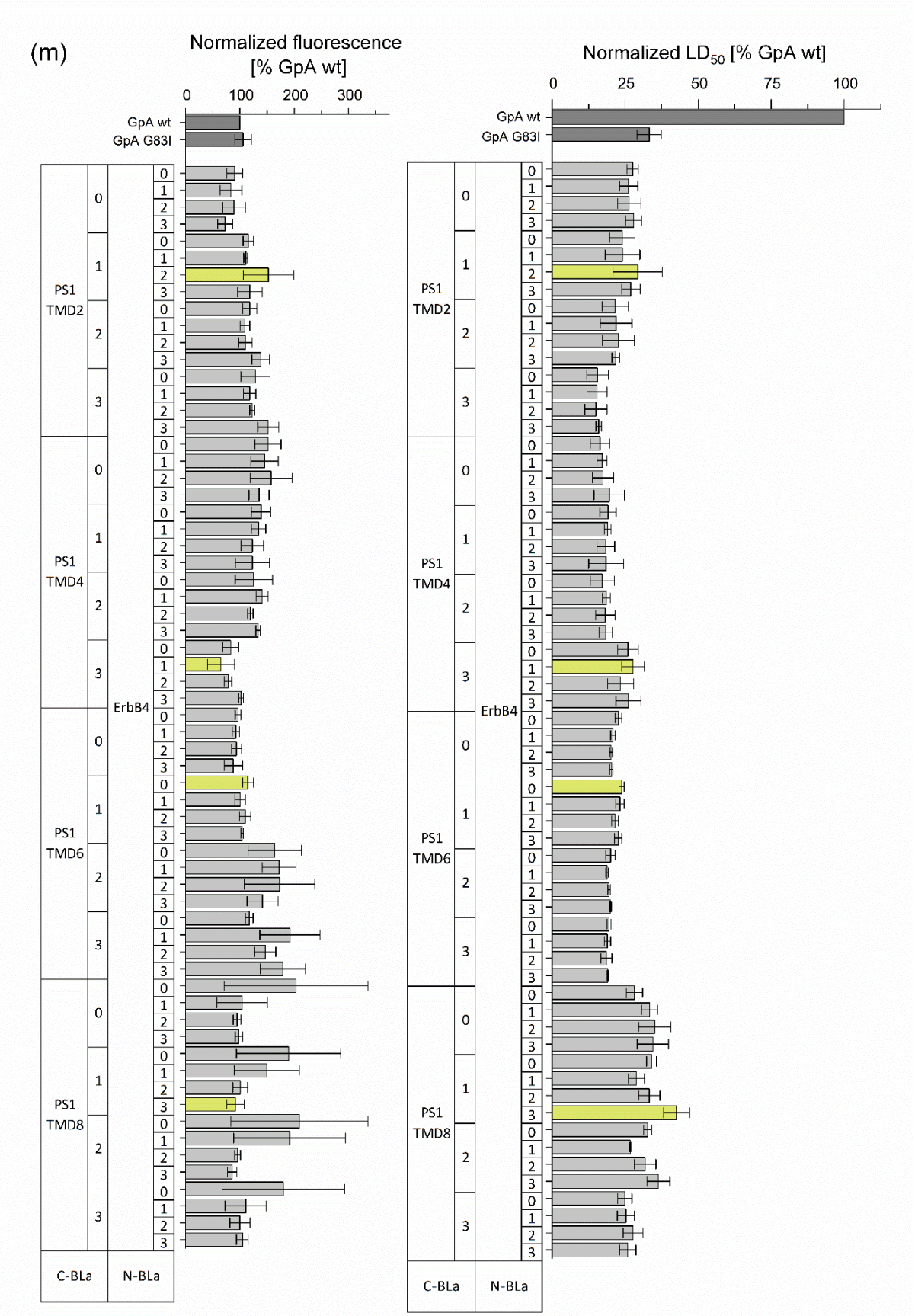

(f)
Normalized fluorescence [% EmrE wt]
0
50
100
150
200
Normalized LD50 [% EmrE wt]
0
25
50
75
100
EmrE wt
EmrE G90V/G97V
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
Notch1
C-BLa
N-BLa

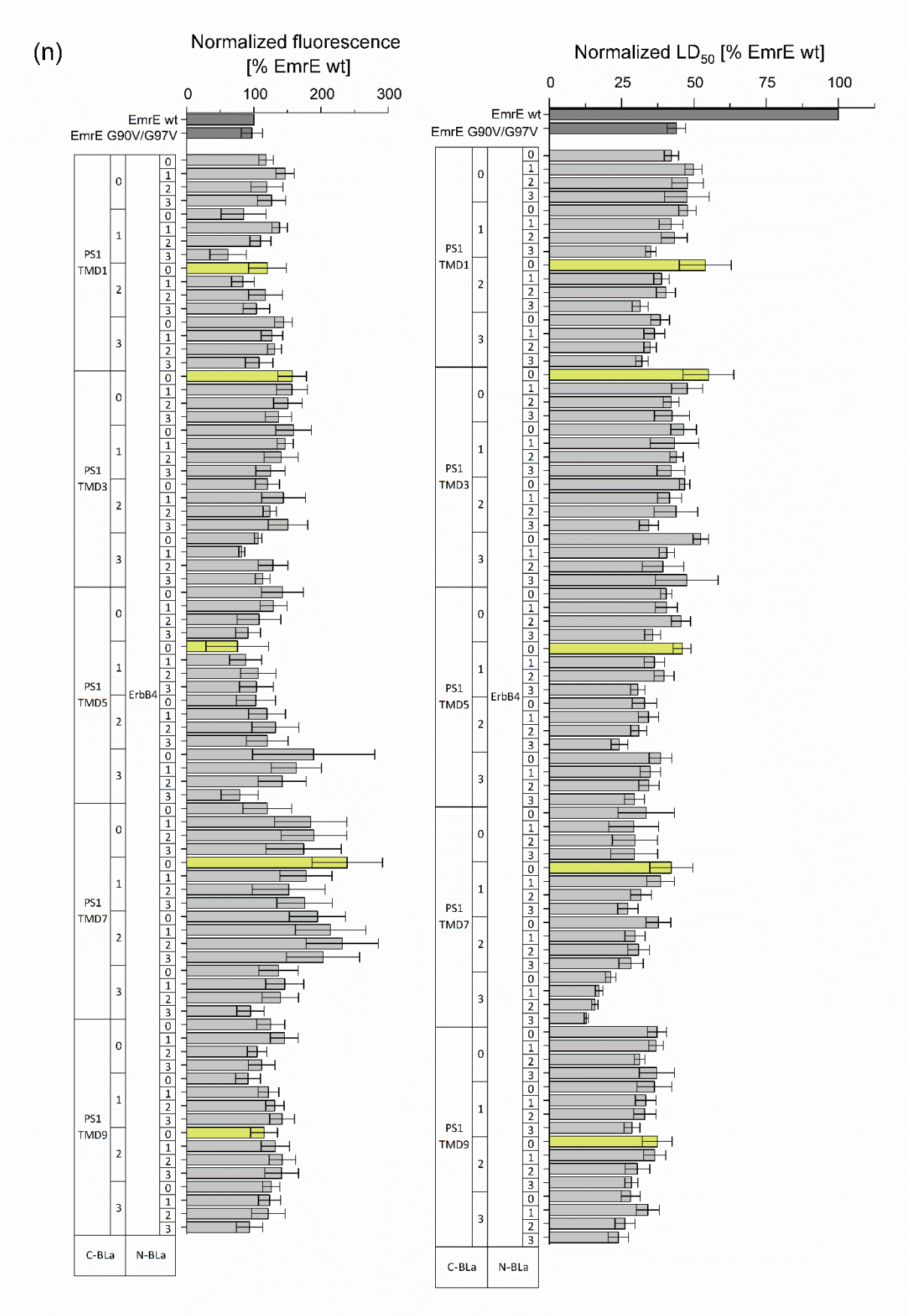

(g)
Normalized fluorescence
[% GpA wt]
0
50
100
150
GpA wt
GpA G83I
NIC
Notch1
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
(h)
Normalized fluorescence
[% EmrE wt]
EmrE wt
EmrE G90V/G97V
PEN-2
Notch1
Normalized LD50 [% EmrE wt]

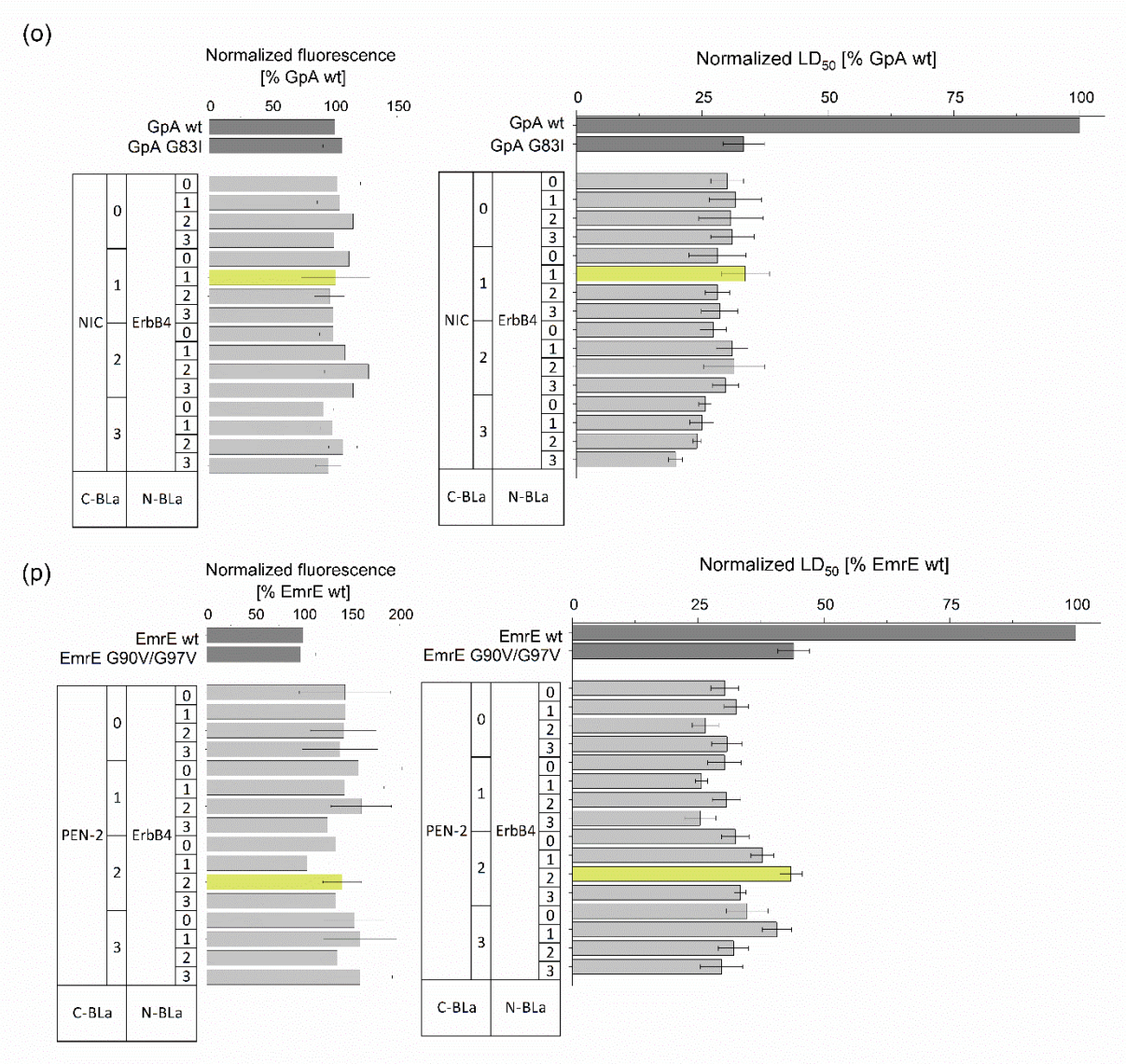

(i)
Normalized fluorescence [% GpA wt]
0
100
200
300
GpA wt
GpA G83I
PS1 TMD2
PS1 TMD4
PS1 TMD6
PS1 TMD8
N-Cad
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
GpA wt
GpA G83I
PS1 TMD2
PS1 TMD4
PS1 TMD6
PS1 TMD8
N-Cad
C-BLa
N-BLa

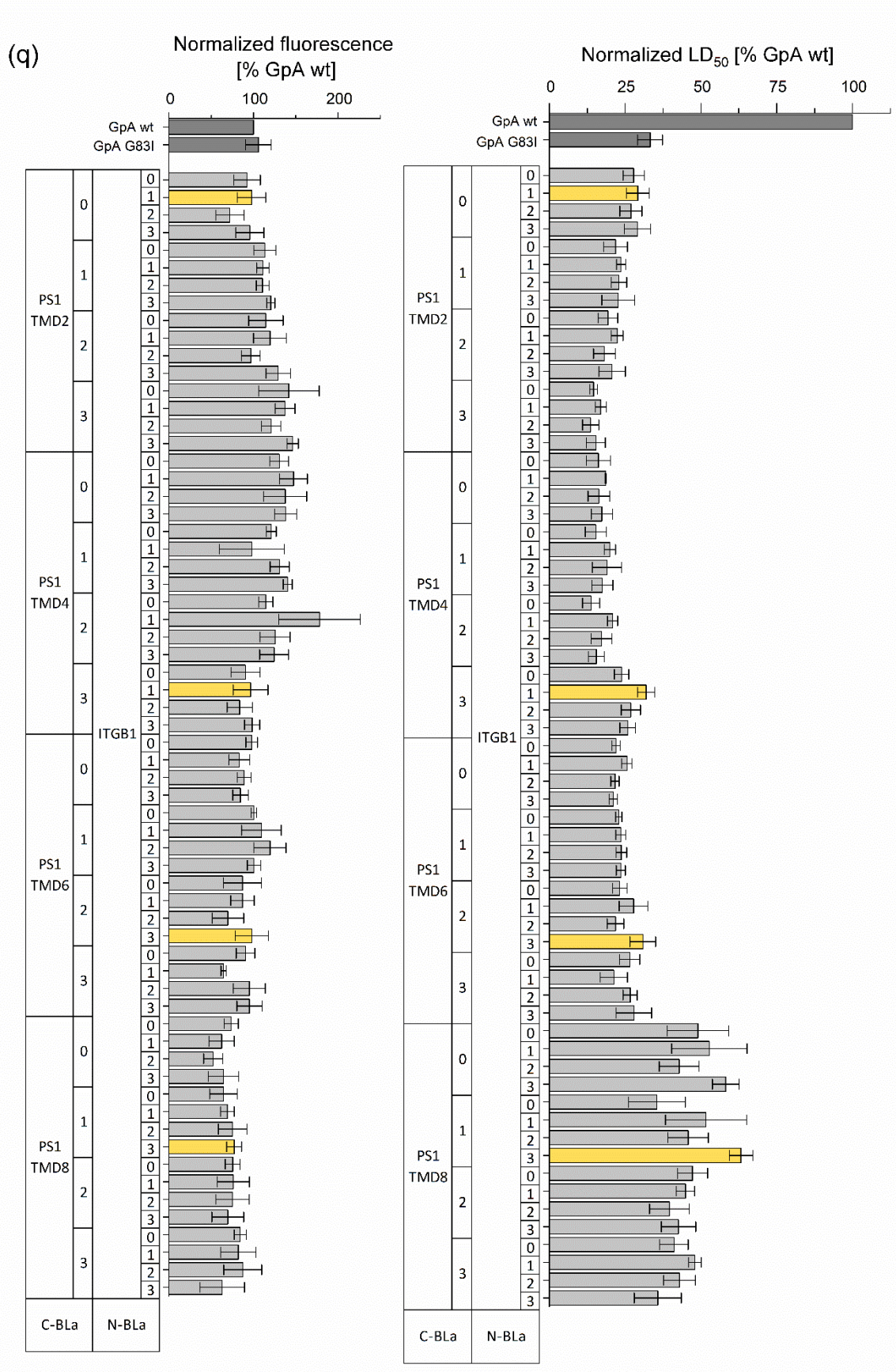

(j)
Normalized fluorescence [% EmrE wt]
0
50
100
EmrE wt
EmrE G90V/G97V
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
N-Cad
C-BLa
N-BLa
Normalized LD50 [% EmrE wt]
0
25
50
75
100
EmrE wt
EmrE G90V/G97V
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
N-Cad
C-BLa
N-BLa

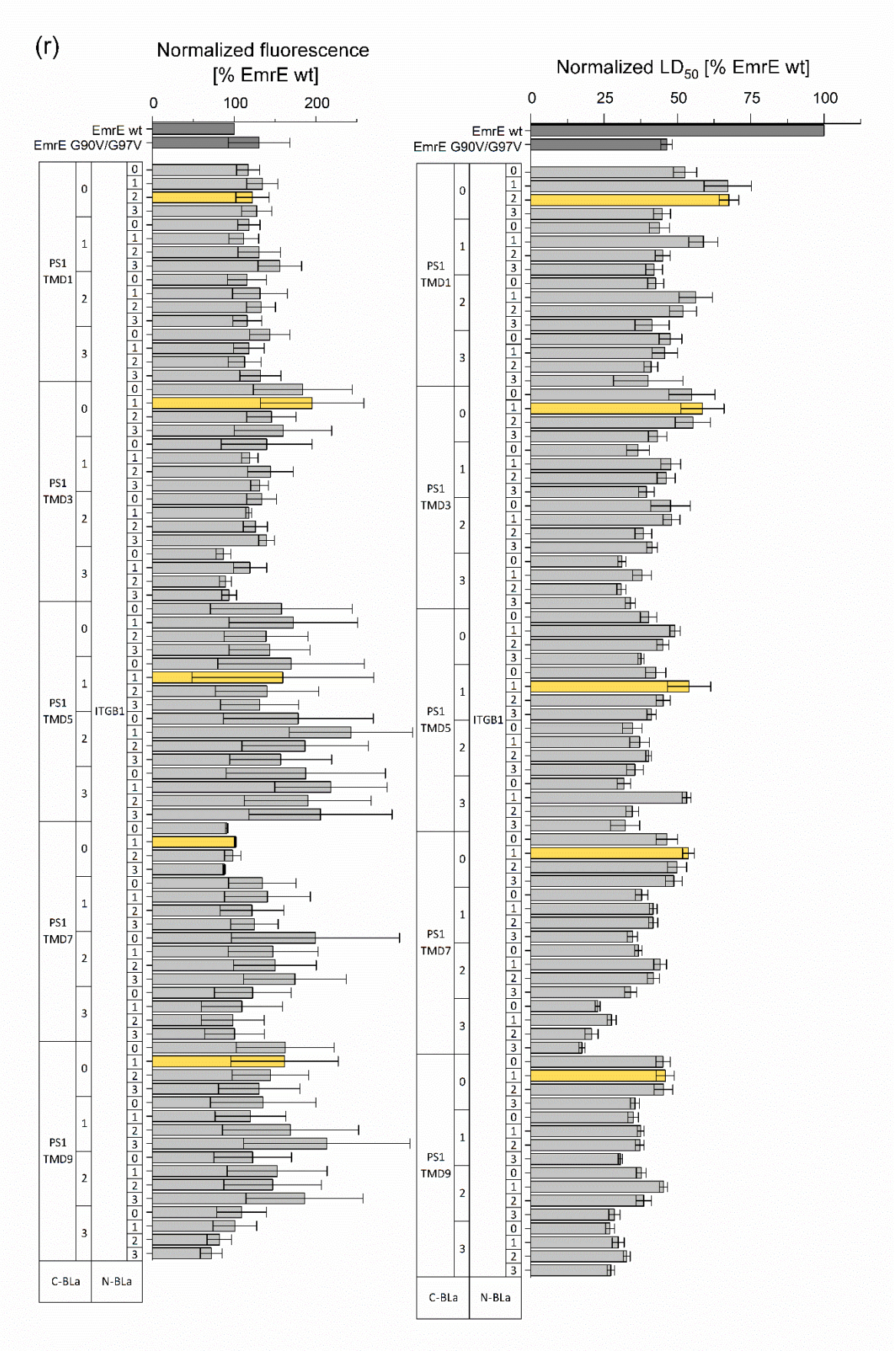

(k)
Normalized fluorescence
[% GpA wt]
0
50
100
150
GpA wt
GpA G83I
NIC
N-Cad
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
(l)
Normalized fluorescence
[% EmrE wt]
EmrE wt
EmrE G90V/G97V
PEN-2
Normalized LD50 [% EmrE wt]


(m)
Normalized fluorescence
[% GpA wt]
0
100
200
300
GpA wt
GpA G83I
PS1
TMD2
PS1
TMD4
PS1
TMD6
PS1
TMD8
ErbB4
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
GpA wt
GpA G83I
PS1
TMD2
PS1
TMD4
PS1
TMD6
PS1
TMD8
ErbB4
C-BLa
N-BLa


(n)
Normalized fluorescence
[% EmrE wt]
0
100
200
300
EmrE wt
EmrE G90V/G97V
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
ErbB4
C-BLa
N-BLa
Normalized LD50 [% EmrE wt]
0
25
50
75
100
EmrE wt
EmrE G90V/G97V
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
ErbB4
C-BLa
N-BLa


(o)
Normalized fluorescence
[% GpA wt]
0
50
100
150
GpA wt
GpA G83I
NIC
ErbB4
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
GpA wt
GpA G83I
NIC
ErbB4
C-BLa
N-BLa
(p)
Normalized fluorescence
[% EmrE wt]
0
50
100
150
200
EmrE wt
EmrE G90V/G97V
PEN-2
ErbB4
C-BLa
N-BLa
Normalized LD50 [% EmrE wt]
0
25
50
75
100
EmrE wt
EmrE G90V/G97V
PEN-2
ErbB4
C-BLa
N-BLa


(q)
Normalized fluorescence [% GpA wt]
0
100
200
GpA wt
GpA G83I
PS1 TMD2
PS1 TMD4
PS1 TMD6
PS1 TMD8
ITGB1
C-BLa
N-BLa
Normalized LD50 [% GpA wt]
0
25
50
75
100
GpA wt
GpA G83I
PS1 TMD2
PS1 TMD4
PS1 TMD6
PS1 TMD8
ITGB1
C-BLa
N-BLa


(r)
Normalized fluorescence
[% EmrE wt]
0
100
200
EmrE wt
EmrE G90V/G97V
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
ITGB1
C-BLa
N-BLa
Normalized LD50 [% EmrE wt]
0
25
50
75
100
EmrE wt
EmrE G90V/G97V
PS1 TMD1
PS1 TMD3
PS1 TMD5
PS1 TMD7
PS1 TMD9
ITGB1
C-BLa
N-BLa

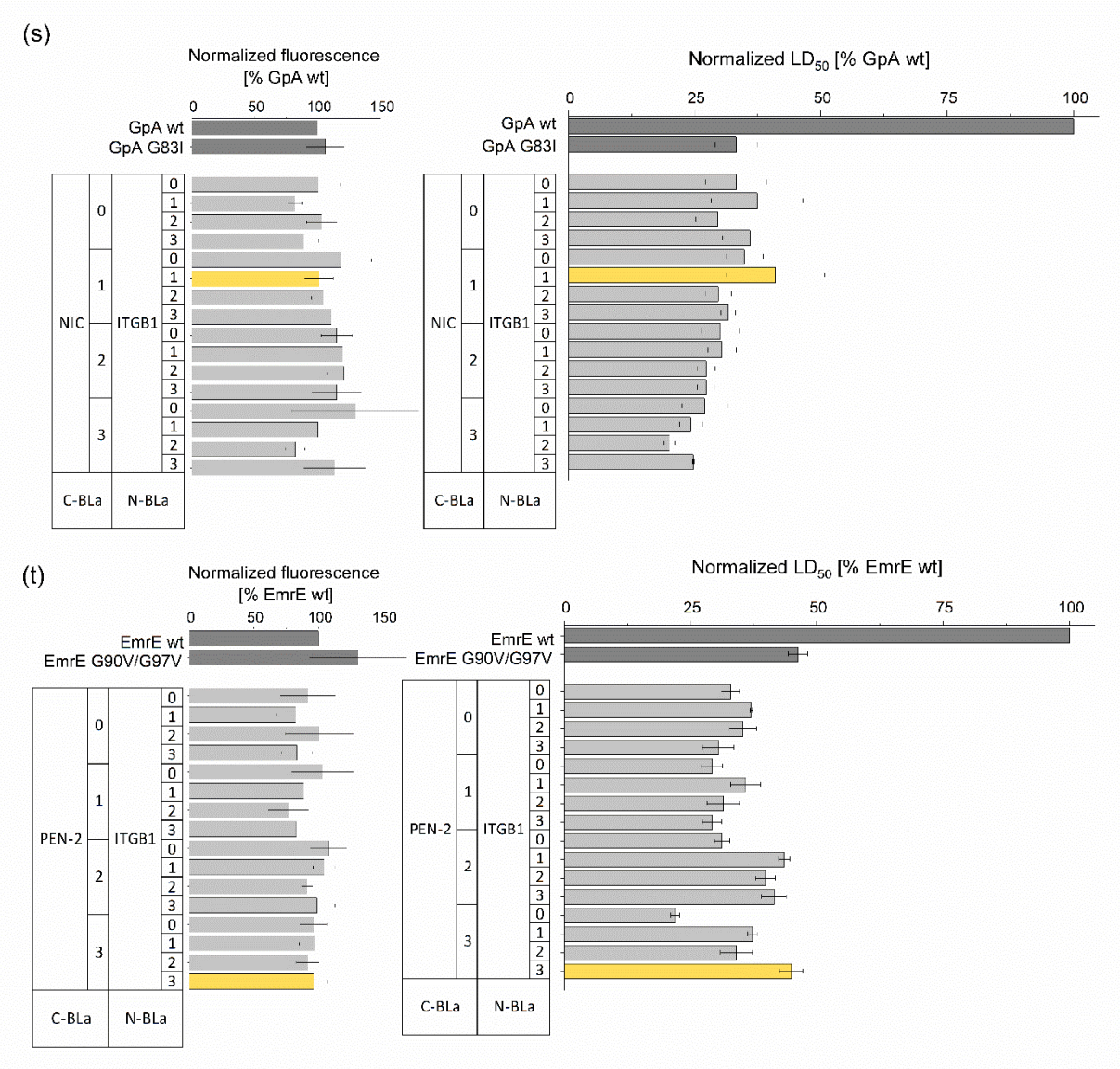


Figure S2. Heterotypic interactions of all investigated pairings of non-/substrate TMDs and γ-secretase TMDs. The strongest interaction in any given pairing is depicted in color and reproduced in Fig. 3 and Fig. 4. The right or upper panels show the LD_{50} values of the tested pairs while the left or lower panels represent the respective GFP expression controls. Parts **(a)** – **(d)** show all pairs of the APP TMD and the TMDs of PS1, nicastrin, and PEN-2. Parts **(e)** – **(h)** show all pairs of the Notch 1 TMD and the TMDs of PS1, nicastrin, and PEN-2. Parts **(i)** – **(l)** show all pairs of the N-cadherin TMD and the TMDs of PS1, nicastrin, and PEN-2. Parts **(m)** – **(p)** show all pairs of the ErbB4 TMD and the TMDs of PS1, nicastrin, and PEN-2. Parts **(q)** – **(t)** show all pairs of the integrin β1 TMD and the TMDs of PS1, nicastrin, and PEN-2. Means ± SEM, n = 3-6. The color code corresponds to that Figs. 3 and 4.

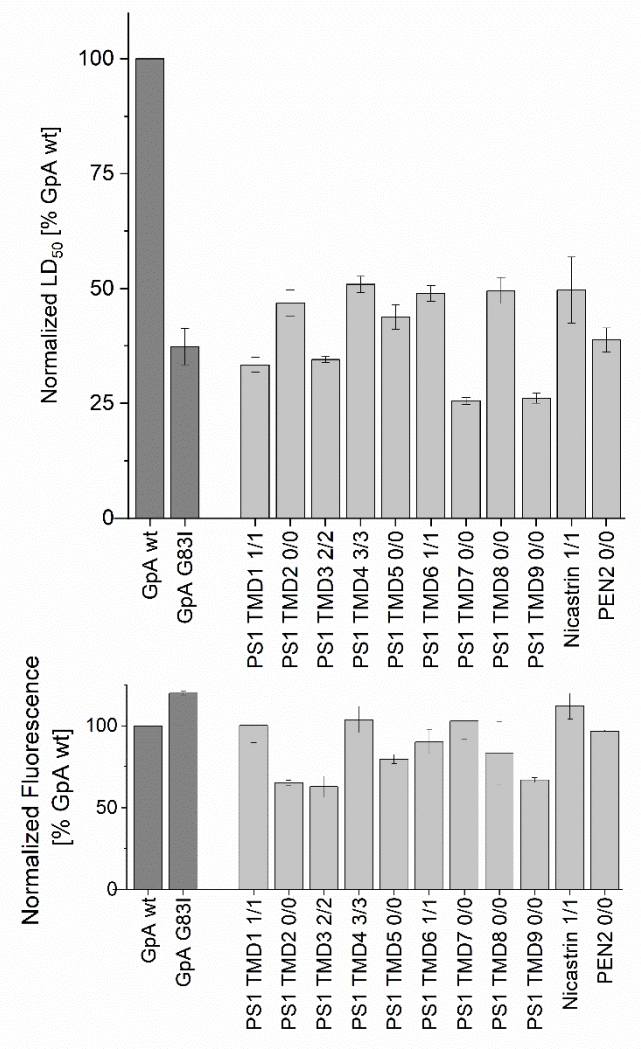


Figure S3. Homodimerization of γ-secretase TMDs. Upper panel: Strength of homotopic interactions of γ-secretase TMDs normalized to the homodimerization signal of GpA, used as reference. Lower panel: GFP expression controls of the respective pairs normalized to the GFP expression of GpA. Note that the frames tested here correspond to those frames yielding the highest heteromerization signals given in Fig. 3. Means ± SEM, n = 3.

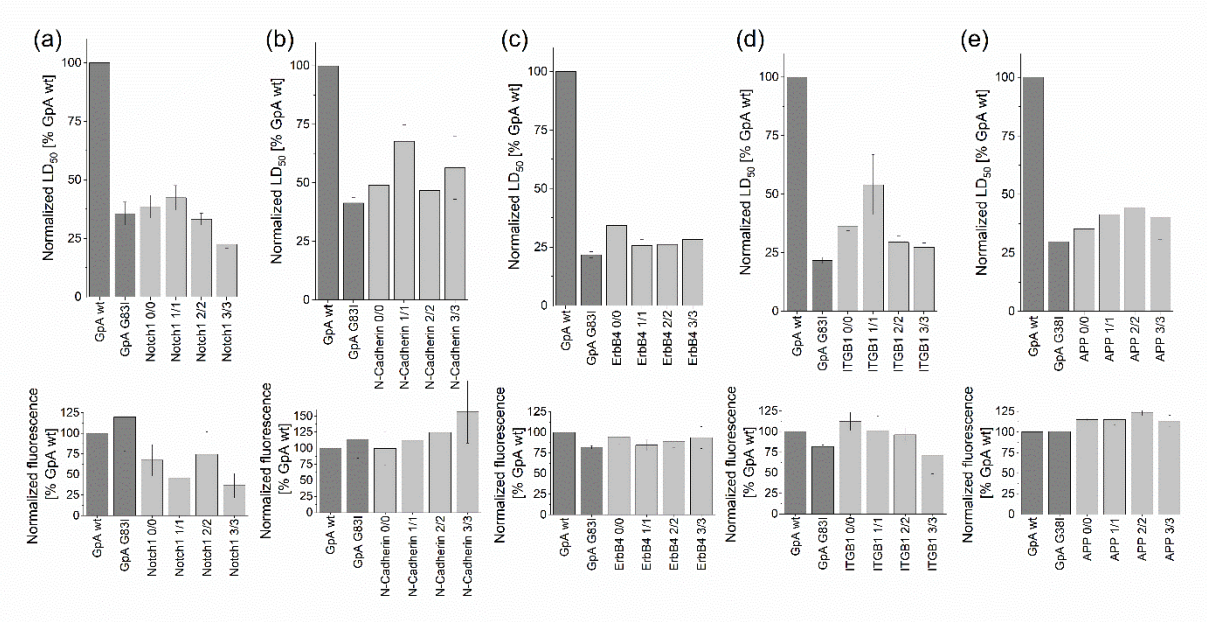


Figure S4. Homodimerization of (non)-substrate TMDs. Upper panels: strength of homotopic interaction of the Notch 1 **(a)**, N-cadherin **(b)**, ErbB4 **(c)**, integrin β1 **(d)**, or APP **(e)** TMD. Lower panels: respective GFP expression controls. All values are normalized to the homodimerization signal or GFP expression of the GpA TMD, respectively. Means ± SEM, n = 3-4.

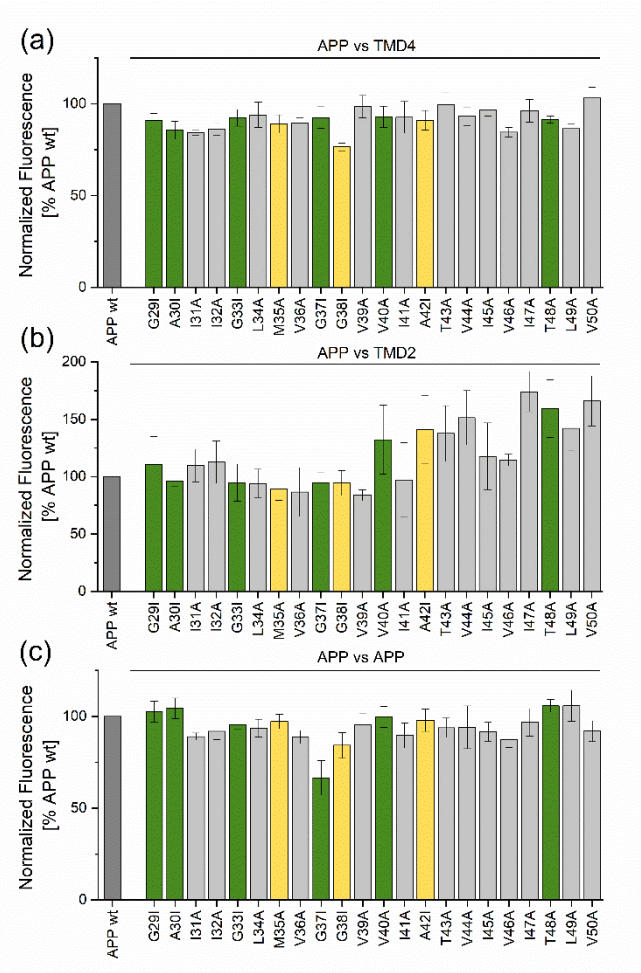


Figure S5. GFP expression controls corresponding to the mutational analysis of the heterotypic or homotypic interactions of the APP TMD. **(a)** GFP expression controls of APP TMD (frame 2) mutants paired with the PS1 TMD4 (frame 3). **(b)** GFP expression controls of APP TMD (frame 2) mutants paired with the PS1 $^{125\text{-}146}$TMD2 (frame 0). **(c)** GFP expression controls of the homodimerization of APP TMD (frame 2) mutants normalized to the signal of wt. Values are normalized to the signal of APP wt in the respective part. Means ± SEM, n = 4-5. The corresponding LD_{50} values are shown in Fig. 5 using the same color coding.

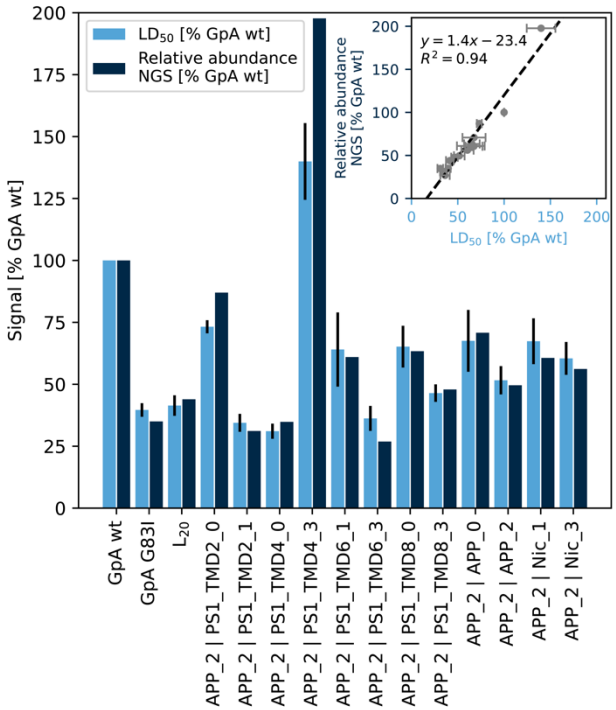


Figure S6. Validation of the library-based BLaTM system. The abundances of pairs of various reference TMD-TMD interactions, as determined by deep sequencing (black bars), are compared to manually measured LD_{50} values (blue bars). All values were normalized to the respective signals obtained with GpA wt. Inset: correlation analysis of both sets of data.

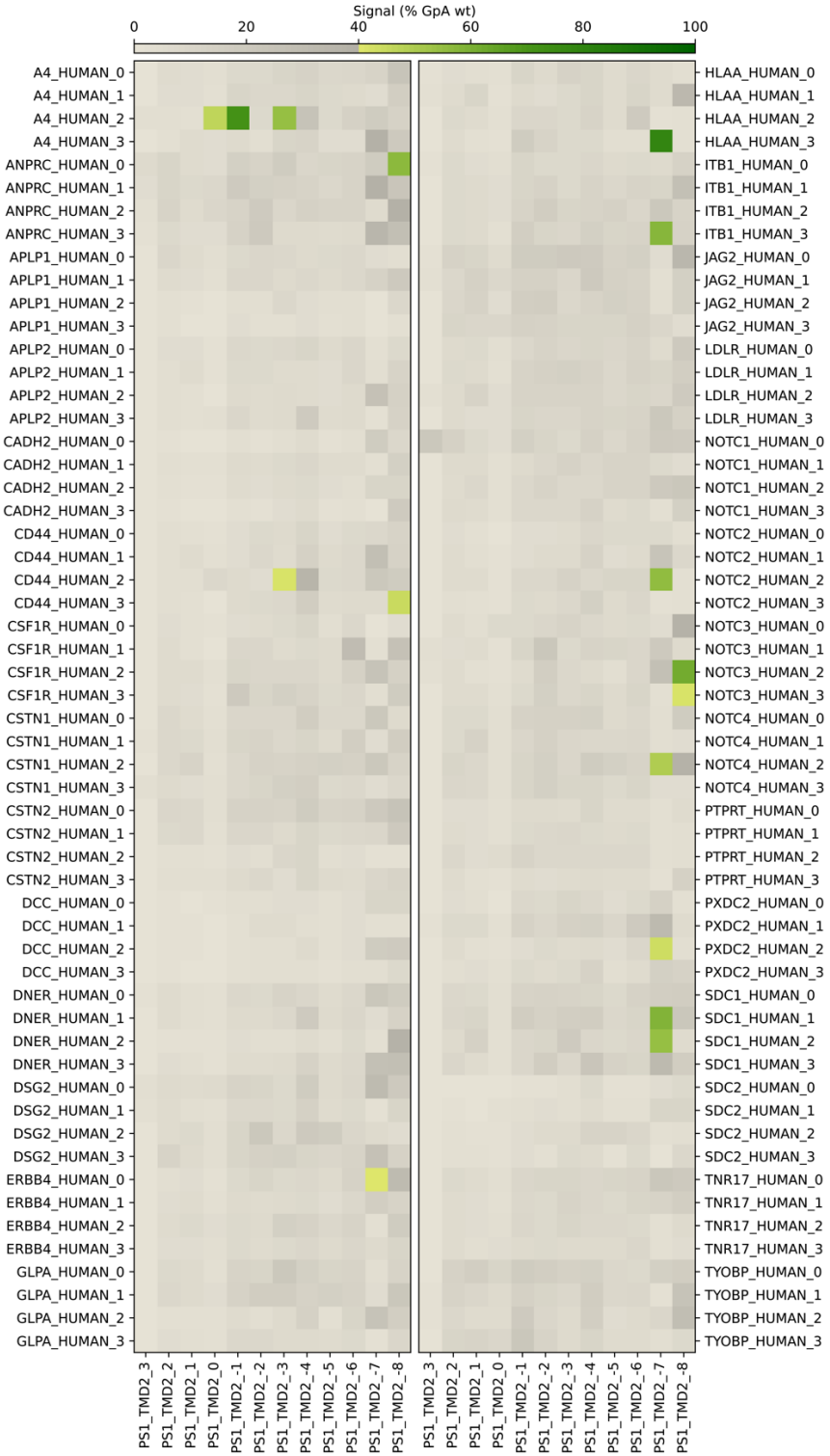


Figure S7. TMD-TMD interactions of various parts of the PS1 TMD2 with substrate TMDs, as examined with the BLaTM library screening system. The heatmap depicts the abundances of pairs obtained with all tested combinations of sequences and frames. Abundances in excess of 40% of the abundance seen with the GpA positive control are depicted in green and were confirmed by manual testing as shown in Fig. 6.

Supplemental reference

1. Bai, X. C.; Yan, C.; Yang, G.; Lu, P.; Ma, D.; Sun, L.; Zhou, R.; Scheres, S. H.; Shi, Y., An atomic structure of human gamma-secretase. *Nature* **2015,** 525, (7568), 212-7.