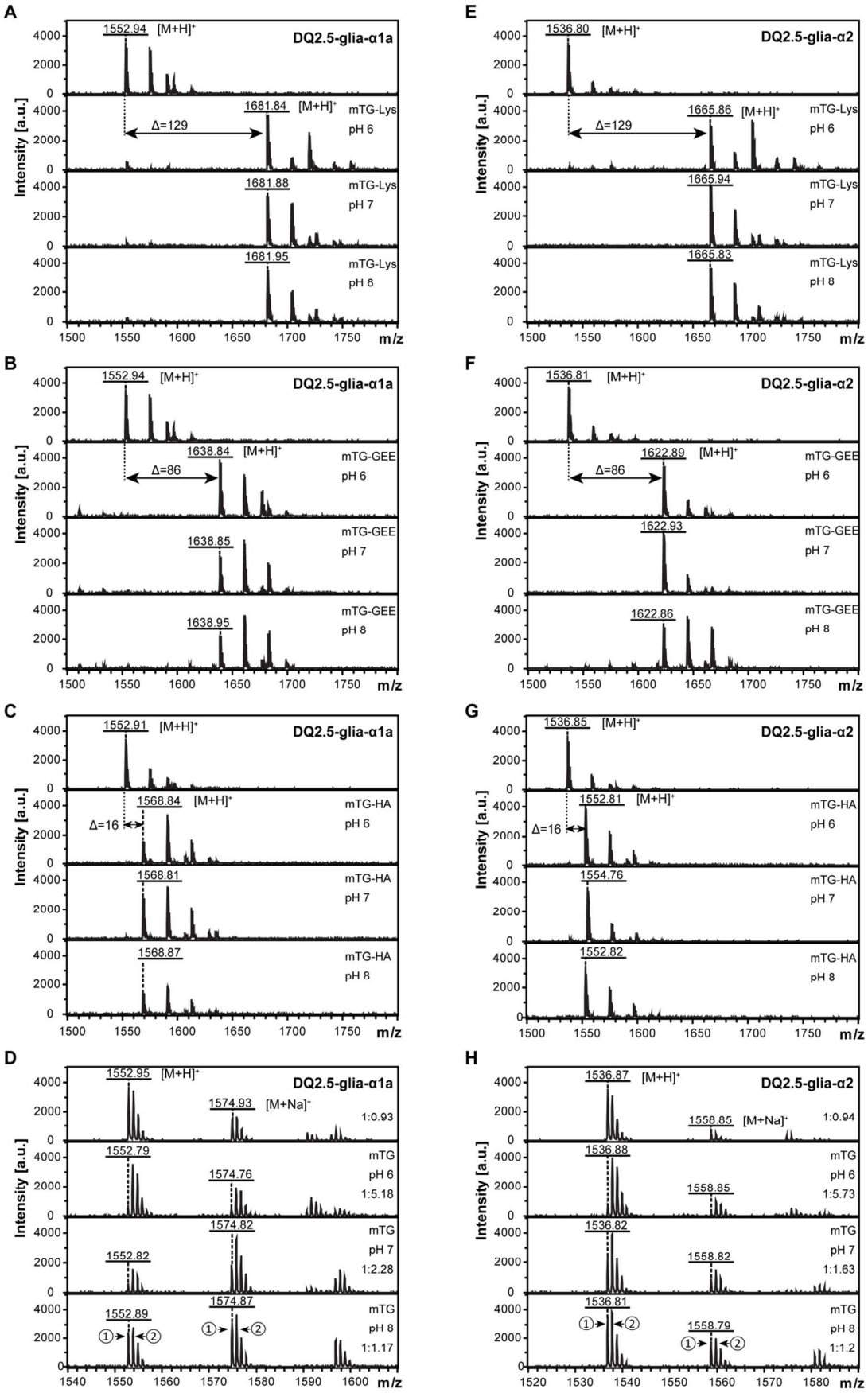
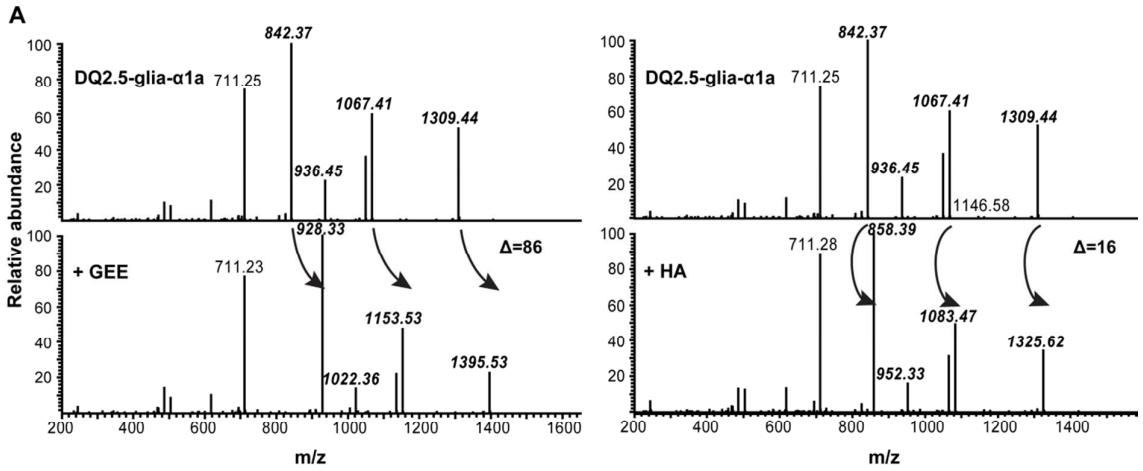
Supplementary Table 1. HLA-DQ2.5-glia-α1/α2-specificT-cell clones and their TCR gene usage

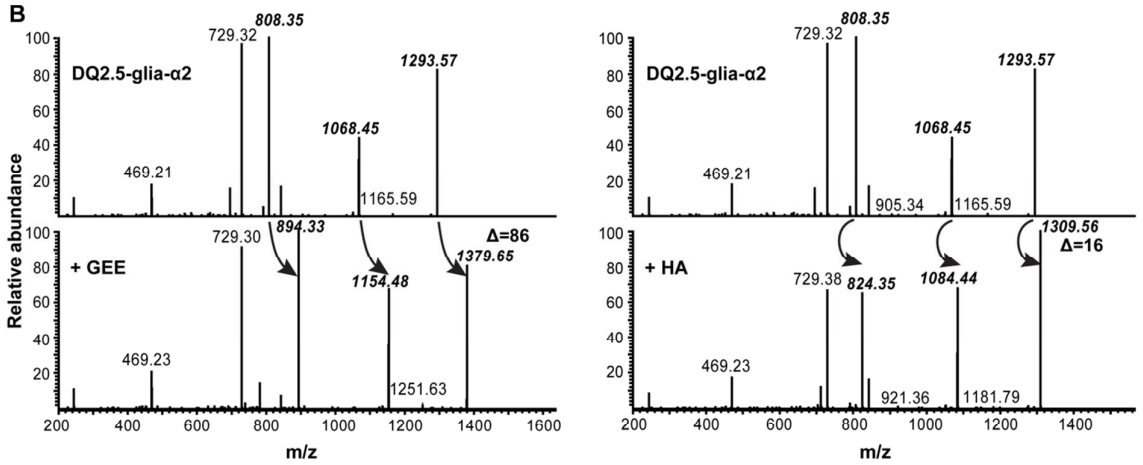
DQ2.5-glia-α1	TRAV	TRAJ	CDR3 α	TRBV	TRBJ	TRBD	CDR3 β
S2	4*01	4*01	CLVGDGGSFSGGYNKLIF	20-1*01	2-5*01	2*01	CSAGVGGQETQYF
L5107	24*01	53*01	CAFIGGSNYKLTF	20-1*01	2-2*01	1*01	CSAREPDNTGELFF
L6	21*01	57*01	CAVTLGGGSEKLVF	5-5*01	1-1*01	1*01	CASSFSPAGSEAFF
L10	8-3*01	44*01	CAVGWEDGTASKLTF	20-1*01	2-3*01	2*02	CSASRWRSTDTQYF
DQ2.5-glia-α2							
S16	26-1*01	32*01	CIVWGGATNKLIF	7-2*01	2-3*01		CASSVRSTDTQYF
D1	8-3*01	42*01	CAVGRGGSQGNLIF	4-2*01	2-1*01	1*01	CASSQYQSLVRGNNEQFF
101136	26-1*01	20*01	CIVHNDYKLSF	7-2*01	2-3*01	1*01	CASSIRSTDTQYF
L3-13	14/DV4*03	40*01	CAMSVLSGTYKYIF	4-1*01	2-5*01	1*01	CASSHVDRGGETQYF


A
DQ2.5-glia-α1a
1552.94 [M+H]+
1681.84 [M+H]+
Δ=129
mTG-Lys pH 6
1681.88
mTG-Lys pH 7
1681.95
mTG-Lys pH 8
B
DQ2.5-glia-α1a
1552.94 [M+H]+
1638.84 [M+H]+
Δ=86
mTG-GEE pH 6
1638.85
mTG-GEE pH 7
1638.95
mTG-GEE pH 8
C
DQ2.5-glia-α1a
1552.91 [M+H]+
1568.84 [M+H]+
Δ=16
mTG-HA pH 6
1568.81
mTG-HA pH 7
1568.87
mTG-HA pH 8
D
DQ2.5-glia-α1a
1552.95 [M+H]+
1574.93 [M+Na]+
1:0.93
1552.79
1574.76
mTG pH 6 1:5.18
1574.82
1552.82
mTG pH 7 1:2.28
1552.89
1574.87
mTG pH 8 1:1.17
E
DQ2.5-glia-α2
1536.80 [M+H]+
1665.86 [M+H]+
Δ=129
mTG-Lys pH 6
1665.94
mTG-Lys pH 7
1665.83
mTG-Lys pH 8
F
DQ2.5-glia-α2
1536.81 [M+H]+
1622.89 [M+H]+
Δ=86
mTG-GEE pH 6
1622.93
mTG-GEE pH 7
1622.86
mTG-GEE pH 8
G
DQ2.5-glia-α2
1536.85 [M+H]+
1552.81 [M+H]+
Δ=16
mTG-HA pH 6
1554.76
mTG-HA pH 7
1552.82
mTG-HA pH 8
H
DQ2.5-glia-α2
1536.87 [M+H]+
1558.85 [M+Na]+
1:0.94
1536.88
mTG pH 6 1:5.73
1536.82
1558.82
mTG pH 7 1:1.63
1536.81
1558.79
mTG pH 8 1:1.2
Intensity [a.u.]
m/z

Supplementary Figure 1. Effect of pH on transamidation and deamidation of DQ 2.5-glia-α1a and DQ 2.5-glia-α2 peptides. (A) Effect of pH on DQ 2.5-glia-α1a reaction induced by mTG in presence of Lys. (B) Effect of pH on DQ 2.5-glia-α1a reaction induced by mTG in presence of GEE. (C) Effect of pH on DQ 2.5-glia-α1a reaction induced by mTG in presence of Lys. (D) Effect of pH on DQ 2.5-glia-α1a reaction induced by mTG without acyl-acceptor. (E) Effect of pH on DQ 2.5-glia-α2 reaction induced by mTG in presence of Lys. (F) Effect of pH on DQ 2.5-glia-α2 reaction induced by mTG in presence of GEE. (G) Effect of pH on DQ 2.5-glia-α2 reaction induced by mTG in presence of Lys. (H) Effect of pH on DQ 2.5-glia-α2 reaction induced by mTG without acyl-acceptor. All of presented molecular mass is monoisotopic mass. Peak area ratio in (D) and (H) is peak1: peak2 (peak1= ① + ① and peak 2= ② + ②). The deamidation of Q→E would be present a 1 Dalton shift, namely peak ① shifted to peak ②. Regarding DQ 2.5-glia-α1a peptide (supplementary Figure 1D), the area ratio between peak 1 (peak ①+ peak ①) and peak 2 (peak ②+ peak ②) was 1:5.18 (pH 6), whereas that was 1:2.28 (pH 7), 1:1.17 (pH 8) and 1:0.93 (native peptide). Simultaneously, for the DQ 2.5-glia-α2 peptide (supplementary Figure 1H), the area ratio between peak 1 and peak 2 increased in pace with the ascent of pH, which was 1:5.73 (pH 6), 1:1.63 (pH 7) and 1:1.2 (pH 8). The intensity ratio between peak 1 and peak 2 was similar to the area ration between them (data not shown).



b-HA $[M+H]^+$	114.09	242.15	339.20	486.27	583.32	711.38	808.44	***952.49***	1065.57	1162.63	***1325.69***	1422.74	-
b-GEE $[M+H]^+$	114.09	242.15	339.20	486.27	583.32	711.38	808.44	***1022.53***	1135.61	1232.67	***1395.73***	1492.78	-
b $[M+H]^+$	114.09	242.15	339.20	486.27	583.32	711.38	808.44	***936.49***	1049.58	1146.63	***1309.69***	1406.70	-
	1	2	3	4	5	6	7	8	9	10	11	12	13
	L	Q	P	F	P	Q	P	**Q**	L	P	Y	P	Q
	13	12	11	10	9	8	7	6	5	4	3	2	1
y $[M+H]^+$	-	1439.73	1311.67	1214.62	***1067.55***	970.50	***842.44***	745.39	617.33	504.25	407.19	244.13	147.08
y-GEE $[M+H]^+$	-	1525.77	1397.71	1300.66	***1153.59***	1056.54	***928.48***	831.42	617.33	504.25	407.19	244.13	147.08
y-HA $[M+H]^+$	-	1455.73	1327.67	1230.62	***1083.55***	986.49	***858.44***	761.38	617.33	504.25	407.19	244.13	147.08

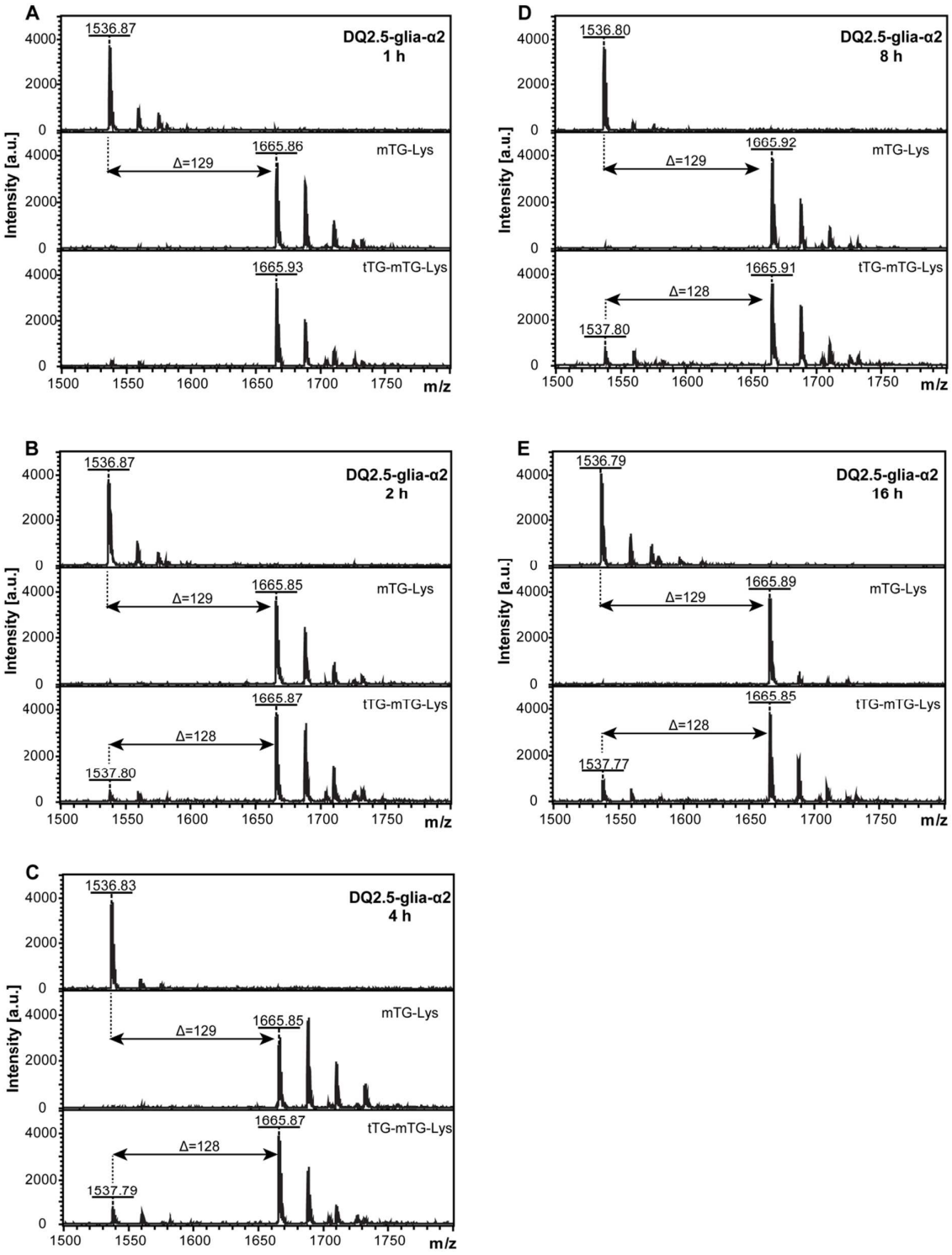


b-HA $[M+H]^+$	98.06	245.13	342.18	470.24	567.29	711.35	***824.43***	921.48	***1084.55***	1181.60	***1309.66***	1406.71	-
b-GEE $[M+H]^+$	98.06	245.13	342.18	470.24	567.29	781.39	***894.47***	991.52	***1154.59***	1251.64	***1379.70***	1476.75	-
b $[M+H]^+$	98.06	245.13	342.18	470.24	567.29	695.35	***808.44***	905.49	***1068.55***	1165.60	***1293.66***	1390.72	-
	1	2	3	4	5	6	7	8	9	10	11	12	13
	P	F	P	Q	P	**Q**	L	P	Y	P	Q	P	Q
	13	12	11	10	9	8	7	6	5	4	3	2	1
y $[M+H]^+$	-	1439.73	1292.66	1195.61	1067.55	970.50	842.44	729.36	632.30	469.24	372.19	244.13	147.08
y-GEE $[M+H]^+$	-	1525.77	1378.70	1281.65	1153.59	1056.54	842.44	729.36	632.30	469.24	372.19	244.13	147.08
y-HA $[M+H]^+$	-	1455.73	1308.66	1211.61	1083.55	986.49	842.44	729.36	632.30	469.24	372.19	244.13	147.08

Supplementary Figure 2. Identification of transamidated sites induced by mTG using LTQ-FT Ultra.

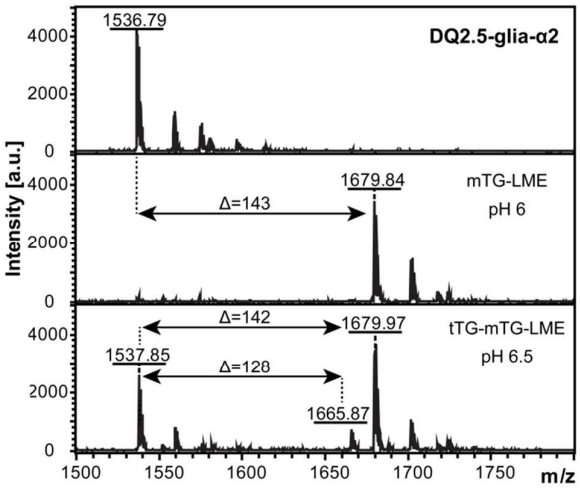
(A) Observed fragment of DQ 2.5-glia-α1a before treatment (upper spectrum), after treatment with

mTG in presence of GEE (left lower spectrum) and HA (right lower spectrum); predicted fragment ion masses in the table. Ions illustrating transamidation of the Q at position 8. (B) Observed fragment of DQ 2.5-glia-α2 before treatment (upper spectrum), after treatment with mTG in presence of GEE (left lower spectrum) and HA (right lower spectrum); predicted fragment ion masses in the table. Ions illustrating transamidation of the Q at position 6. All of presented molecular mass is accurate mass. Arrows indicate the Da shift due to transamidation.

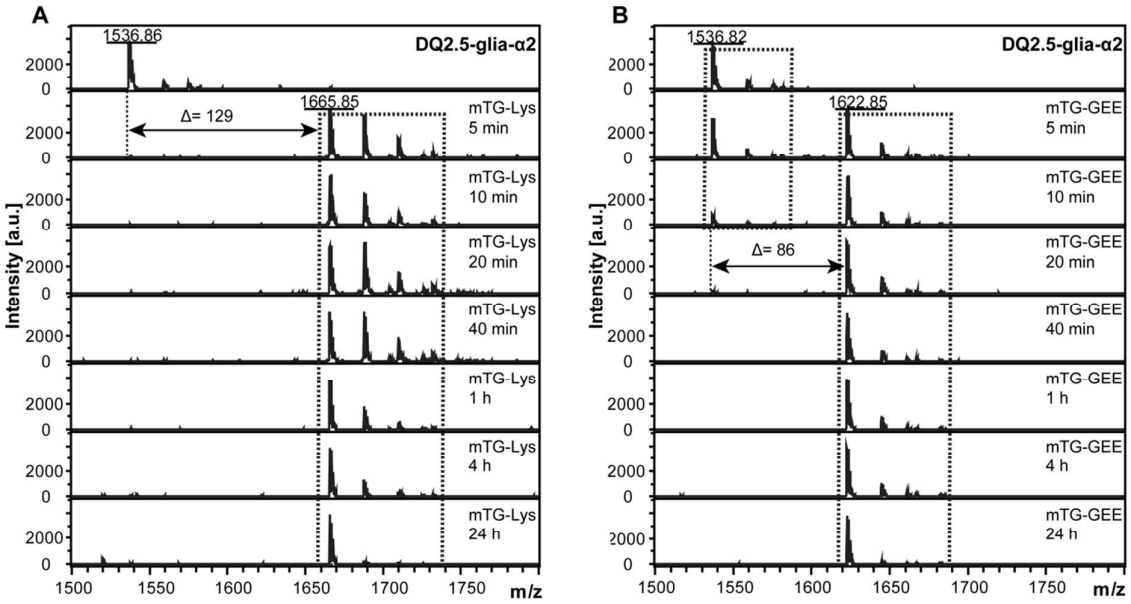


Supplementary Figure 3. Identification of tTG-mediated deamidation of transamidated DQ2.5-glia-α2 by MALDI-TOF. (A) Mass spectrum of treatment for 1 h at 37 °C. DQ2.5-glia-α2 (upper panel), DQ2.5-glia-α2 after mTG transamidation with Lys (mTG-Lys, middle panel, absence of tTG), and

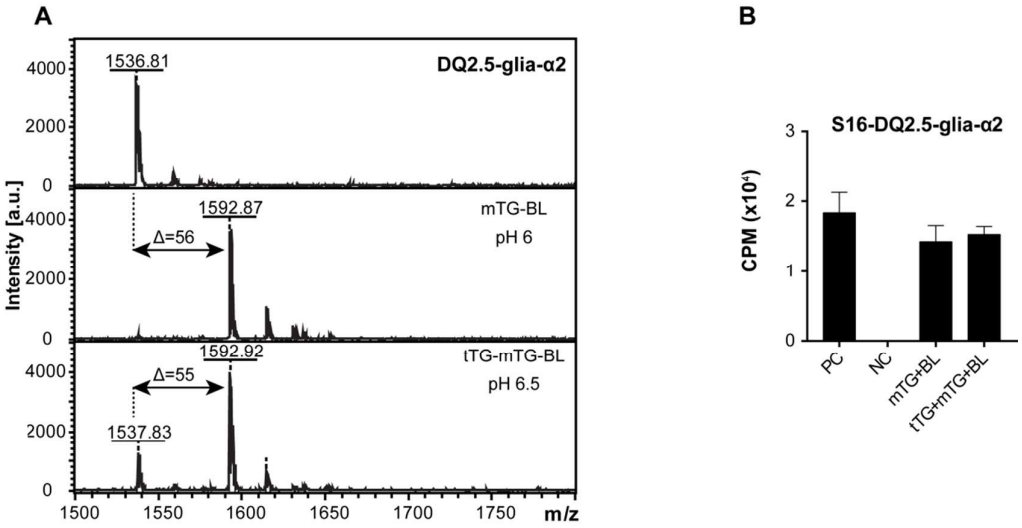
after tTG treatment of Lys transamidated DQ2.5-glia-α2 (tTG-mTG-Lys, lower panel, with tTG). (B) Mass spectrum of treatment for 2 h at 37 °C. (C) Mass spectrum of treatment for 4 h at 37 °C. (D) Mass spectrum of treatment for 8 h at 37 °C. (E) Mass spectrum of treatment for 16 h at 37 °C. Arrows indicate the Da shift.



Supplementary Figure 4. Transamidation of DQ2.5-glia-α2 with LME and effect of tTG treatment of transamidated peptide. Mass spectrum of DQ2.5-glia-α2 (upper panel), DQ2.5-glia-α2 after mTG transamidation with LME (mTG-LME, middle panel), and after tTG treatment of LME transamidated DQ2.5-glia-α2 (tTG-mTG-Lys, lower panel).



Supplementary Figure 5. Time-tracking of mTG transamidation by MALDI-TOF. (A) mTG mediated transamidation in presence of Lys at 5 min, 10 min, 20 min, 40 min, 1 h, 4 h and 24 h. (B) mTG mediated transamidation in presence of GEE at 5 min, 10 min, 20 min, 40 min, 1 h, 4 h and 24 h. All of presented molecular masses are accurate mass. Arrows indicate the Da shift due to transamidation.



Supplementary Figure 6. Transamidation of DQ2.5-glia-α2 with BL and reactivity pattern of glia-α2-specific-T-cell clone to mTG-treated and tTG-mTG treated peptide. (A) Mass spectrum of DQ2.5-glia-α2 (upper panel), DQ2.5-glia-α2 after mTG transamidation with BL (mTG-BL, middle panel), and after tTG treatment of BL transamidated DQ2.5-glia-α2 (tTG-mTG-BL, lower panel). (B) Reactivity pattern of glia-α2-specific T-cell clone S16 to DQ2.5-glia-α2 (left panel), before (NC) and after mTG transamidation with BL (mTG-BL) and after tTG treatment of mTG-BL (tTG-mTG-BL). All of presented molecular mass is accurate mass. Arrows indicate the Da shift.