Supplementary Information
for

Advanced Analyses of Kinetic Stabilities of IgGs modified by Mutations and Glycosylation

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Figure S1

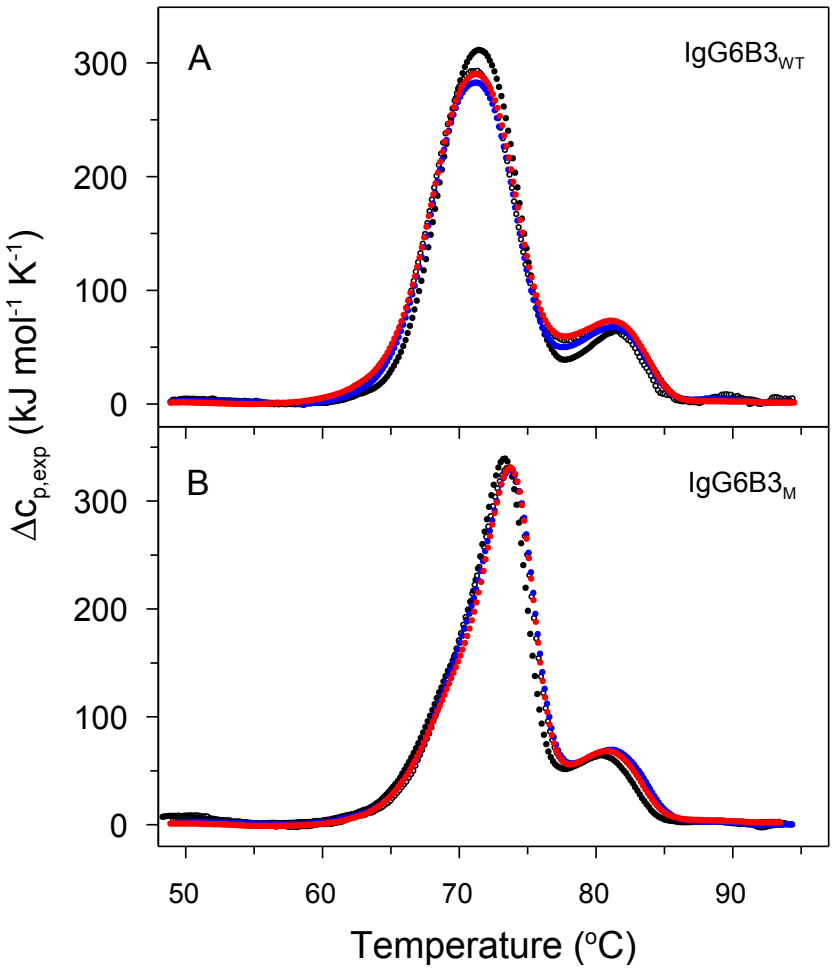

Figure S1. Thermal transitions of $IgG6B3_{WT}$ (A) and $IgG6B3_{M}$ (B) as a function of protein concentration monitored by DSC. Protein concentrations were 0.25 (black), 0.5 (white), 1.0 (blue) and 2.5 (red) mg/ml. Measurements were performed at scan rate 1.0 K/min. In all cases, DSC experiments were performed in PBS buffer, pH 7.4.

Figure S2

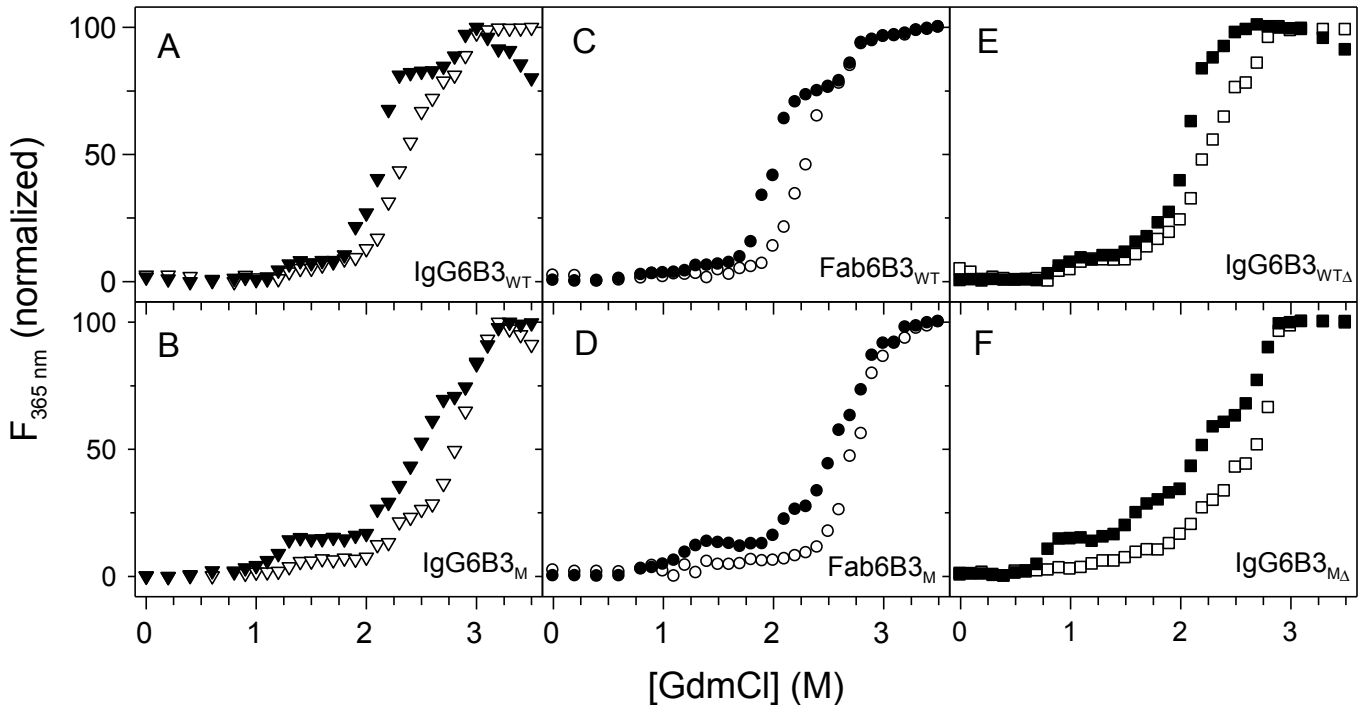

Figure S2. Isothermal transitions of $IgG6B3_{WT}$ (A), $IgG6B3_{M}$ (B), $Fab6B3_{WT}$ (C), $IgG6B3_{M}$ (D), $IgG6B3_{WT\Delta}$ and $IgG6B3_{M\Delta}$ as a function of time monitored by intrinsic tryptophan fluorescence. Samples were incubated at room temperature for either 1 day (white symbols) or 7 days (black symbols). All experiments were performed in PBS buffer, pH 7.4.

Figure S3

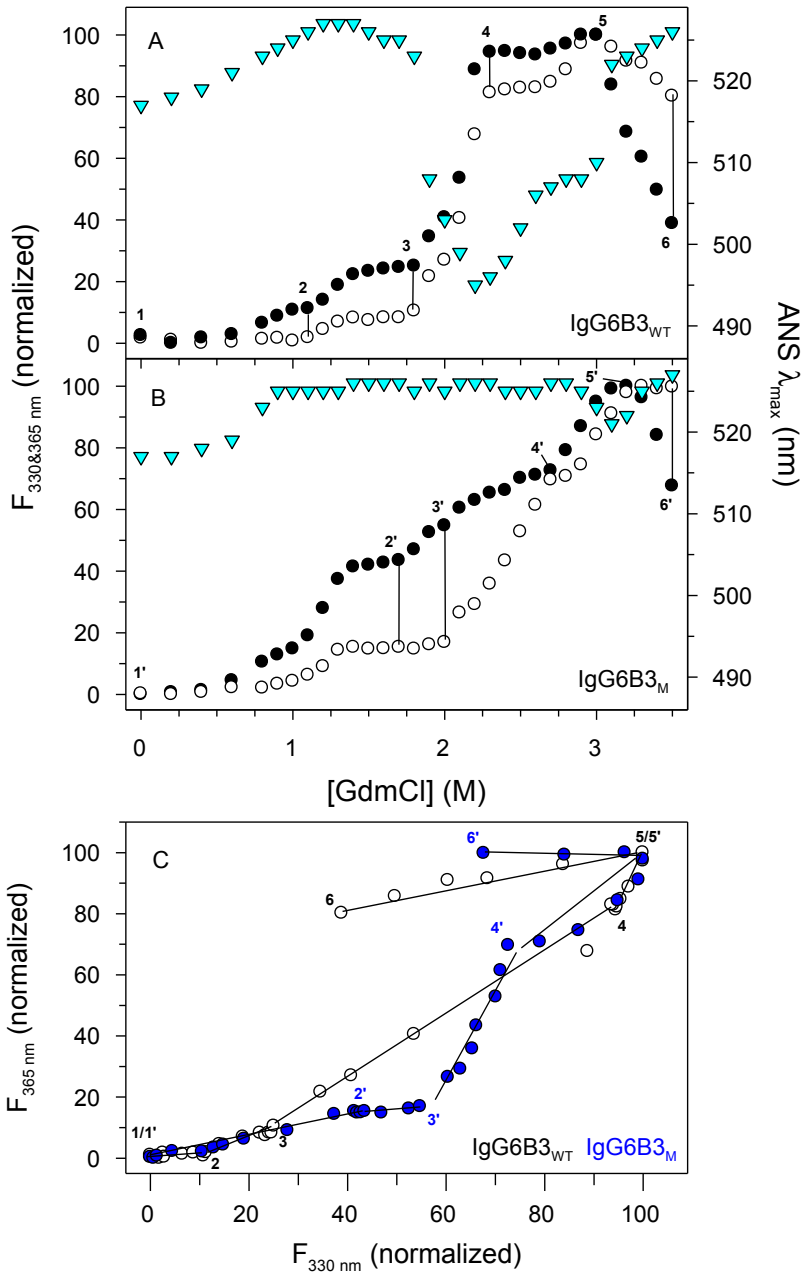

Figure S3. Isothermal denaturation of IgG6B3$_{WT}$ (A) and IgG6B3$_{M}$ (B) monitored by intrinsic tryptophan fluorescence at 330 nm (white circles), 365 nm (black circles) and ANS fluorescence (triangles). Numbers at the curves indicate positions of intermediate states derived from the phase diagram analysis (C). Phase diagram method analysis of isothermal denaturation of IgG6B3$_{WT}$ (white circles) and IgG6B3$_{M}$ (blue circles) monitored by fluorescence emission intensities at 330 and 365 nm (upon excitation at 295 nm). The phase diagrams indicate the presence of six intermediates in isothermal unfolding of both IgG6B3s. All experiments were performed in PBS buffer, pH 7.4.

Figure S4

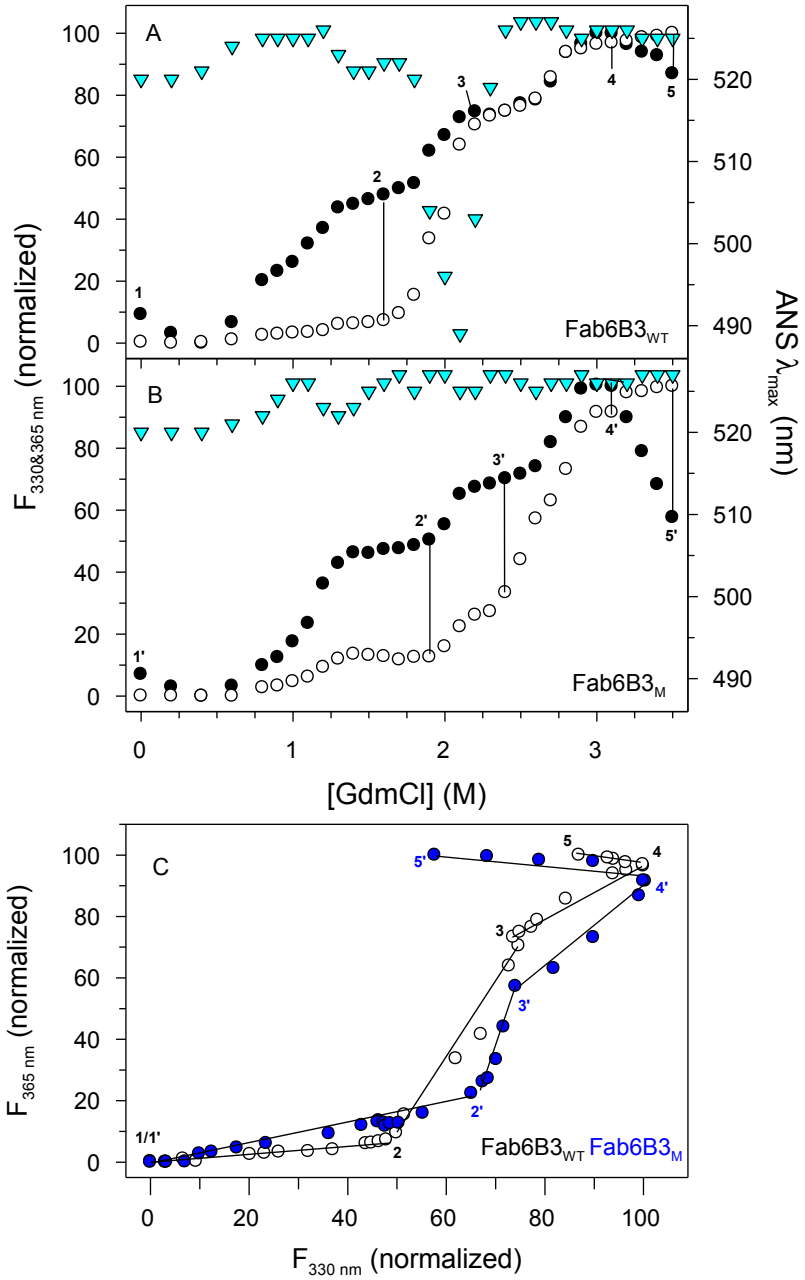

Figure S4. Isothermal denaturation of Fab6B3$_{WT}$ (A) and Fab6B3$_{M}$ (B) monitored by intrinsic tryptophan fluorescence at 330 nm (white circles), 365 nm (black circles) and ANS fluorescence (triangles). Numbers at the curves indicate positions of intermediate states detected from the phase diagram analysis (C). Phase diagram method analysis of isothermal denaturation of Fab6B3$_{WT}$ (white circles) and Fab6B3$_{M}$ (blue circles) monitored by fluorescence emission intensities at 330 and 365 nm (upon excitation at 295 nm). The phase diagrams indicate the presence of five intermediates in isothermal unfolding of both Fab6B3s. All experiments were performed in PBS buffer, pH 7.4.

Figure S5

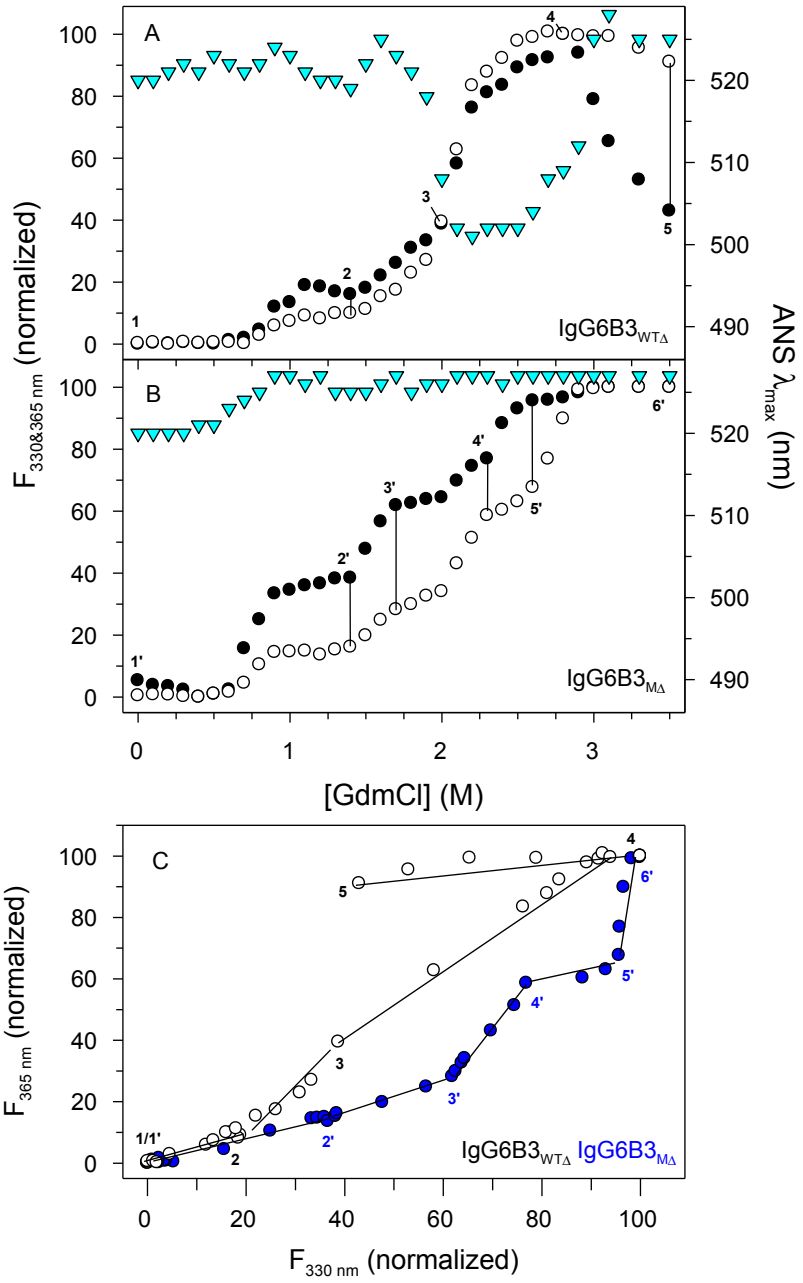

Figure S5. Isothermal denaturation of IgG6B3$_{WT\Delta}$ (A) and IgG6B3$_{M\Delta}$ (B) monitored by intrinsic tryptophan fluorescence at 330 nm (white circles), 365 nm (black circles) and ANS fluorescence (triangles). Numbers at the curves indicate positions of intermediate states detected from the phase diagram analysis (C). Phase diagram method analysis of isothermal denaturation of IgG6B3$_{WT\Delta}$ (white circles) and IgG6B3$_{M\Delta}$ (blue circles) monitored by fluorescence emission intensities at 330 and 365 nm (upon excitation at 295 nm). The phase diagrams indicate the presence of five and six intermediates in isothermal unfolding of IgG6B3$_{WT\Delta}$ and IgG6B3$_{M\Delta}$, respectively. All experiments were performed in PBS buffer, pH 7.4.

Table S1. Fitting parameters for thermal transitions of IgG6B3 and IgG2C2 and Fab6B3 fragments obtained from fits of experimental data using Eq. 3 and 4, respectively.

Protein	Scan rate (K/min)	$T_{1/2}$ (°C)	ΔH_1 (kJ/mol)	T_2^* (°C)	ΔE_{a2} (kJ/mol)	ΔH_2 (kJ/mol)	T_3^* (°C)	ΔE_{a3} (kJ/mol)	ΔH_3 (kJ/mol)	R^2
IgG6B3$_{WT}$	0.5	69.9 ±0.1	692 ±5	75.8 ±0.1	262 ±4	1723 ±8	85.6 ±0.1	316 ±3	676 ±4	0.9996
	1.0	69.3 ±0.1	656 ±4	75.3 ±0.1	295 ±2	1539 ±6	84.4 ±0.1	390 ±4	471 +3	0.9997
	1.5	70.0 ±0.1	645 ±5	75.7 ±0.1	283 ±3	1776 ±7	84.6 ±0.1	358 ±4	595 ±3	0.9997
IgG6B3$_{M}$	0.5	69.5 ±0.1	623 ±4	75.2 ±0.1	648 ±5	1742 ±9	85.9 ±0.1	307 ±4	698 ±4	0.9990
	1.0	69.6 ±0.1	612 ±4	75.6 ±0.1	510 ±4	1754 ±8	84.6 ±0.1	338 ±5	588 ±4	0.9994
	1.5	70.9 ±0.1	588 ±3	76.1 ±0.1	516 ±4	1711 ±8	85.2 ±0.1	296 ±4	664 ±4	0.9987
Fab6B3$_{WT}$	0.5	*n.a.*	*n.a.*	74.2 ±0.1	496 ±6	1304 ±13	*n.a.*	*n.a.*	*n.a.*	0.9828
	1.0	*n.a.*	*n.a.*	75.1 ±0.1	443 ±4	1273 ±9	*n.a.*	*n.a.*	*n.a.*	0.9892
	1.5	*n.a.*	*n.a.*	75.7 ±0.1	414 ±5	1318 ±13	*n.a.*	*n.a.*	*n.a.*	0.9847
Fab6B3$_{M}$	0.5	*n.a.*	*n.a.*	77.1 ±0.1	689 ±4	1349 ±7	*n.a.*	*n.a.*	*n.a.*	0.9964
	1.0	*n.a.*	*n.a.*	77.6 ±0.1	663 ±5	1335 ±9	*n.a.*	*n.a.*	*n.a.*	0.9936
	1.5	*n.a.*	*n.a.*	78.0 ±0.1	600 ±6	1401 ±12	*n.a.*	*n.a.*	*n.a.*	0.9900
IgG2C2$_{WT}$	0.5	69.4 ±0.2	467 ±7	88.0 ±2.6	113 ±17	761 ±16	88.1 ±0.1	342 ±2	1819 ±14	0.9934
	1.0	69.6 ±0.1	507 ±8	88.3 ±5.4	113 ±29	733 ±73	88.8 ±0.1	295 ±2	1701 ±78	0.9979
	1.5	70.9 ±0.1	598 ±8	88.1 ±2.9	122 ±18	972 ±74	88.4 ±0.1	381 ±7	1680 ±80	0.9972
IgG2C2$_{M}$	0.5	68.9 ±0.1	648 ±3	85.2 ±0.6	325 ±10	756 ±64	89.3 ±0.1	401 ±7	1608 ±64	0.9943
	1.0	69.6 ±0.1	599 ±4	84.4 ±0.2	407 ±8	733 ±39	89.3 ±0.1	425 ±9	1497 ±40	0.9961
	1.5	70.7 ±0.1	620 ±3	85.0 ±0.3	370 ±8	765 ±42	89.9 ±0.1	435 ±7	1643 ±44	0.9912

n.a. – not applicable.