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Supplemental Material to:

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Framework selection can influence pharmacokinetics of a humanized therapeutic antibody through differences in molecule charge

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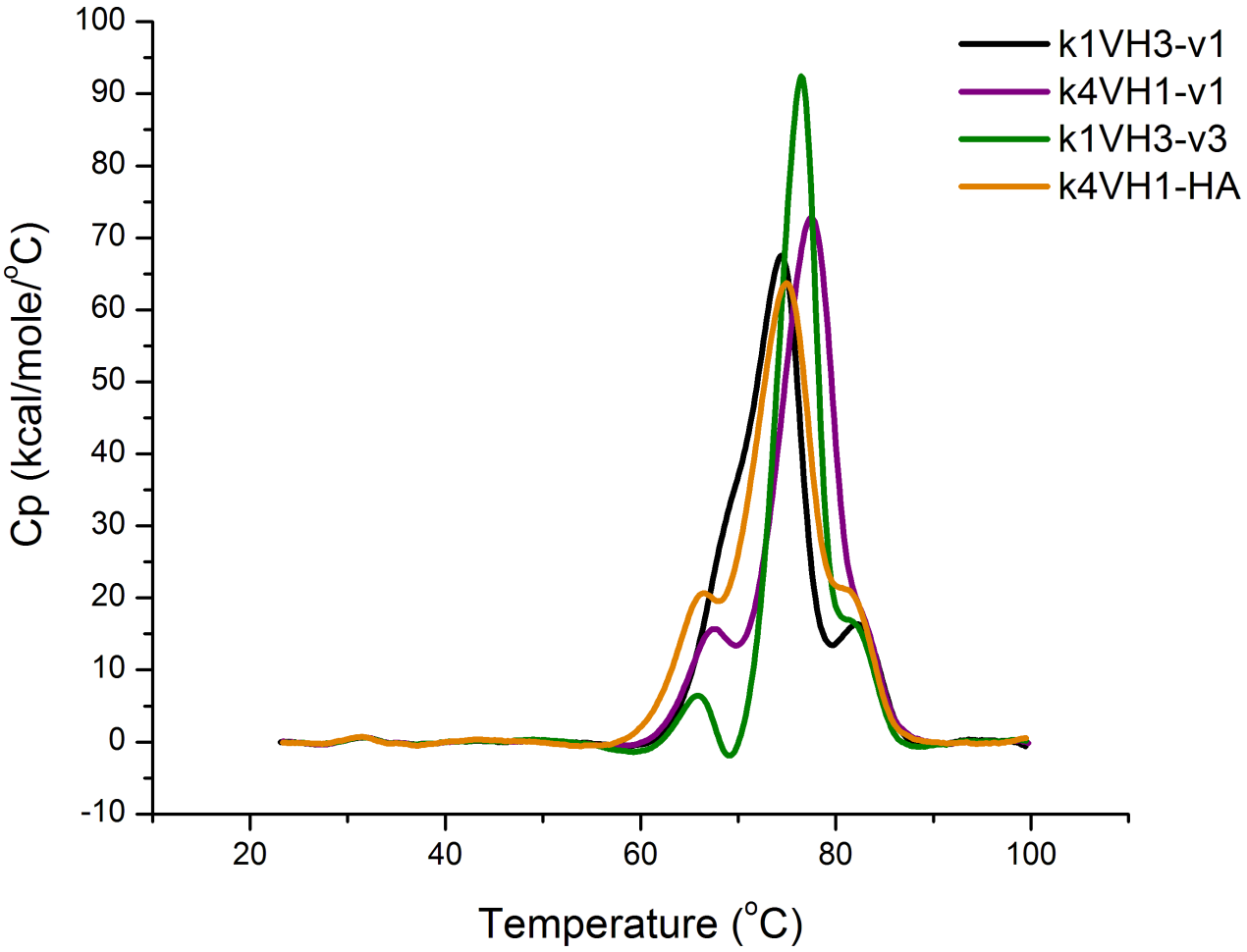
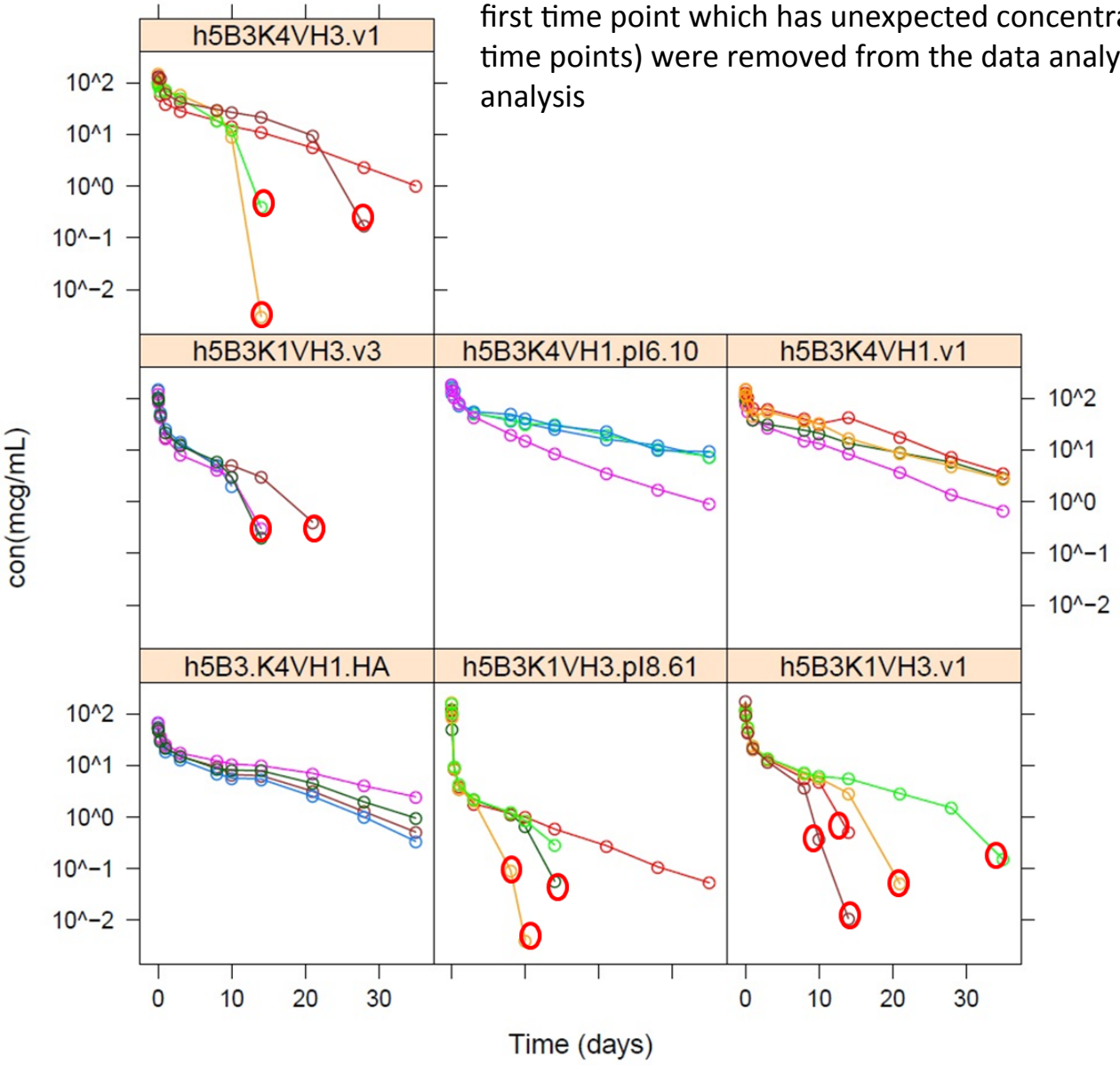
Supplemental Figure 1. DSC of 1 mg/mL solutions (10 mM NaAc pH 5, 150 mM NaCl) of humanized 5B3 variants



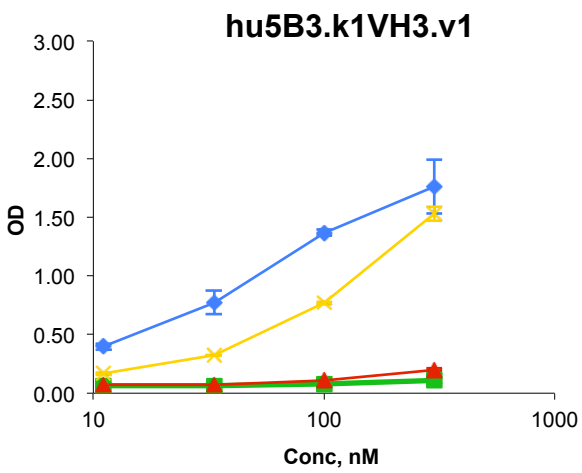
Supplemental Figure 2 Singe dose IV individual pharmacokinetics of 5B3 framework variants in SD rats

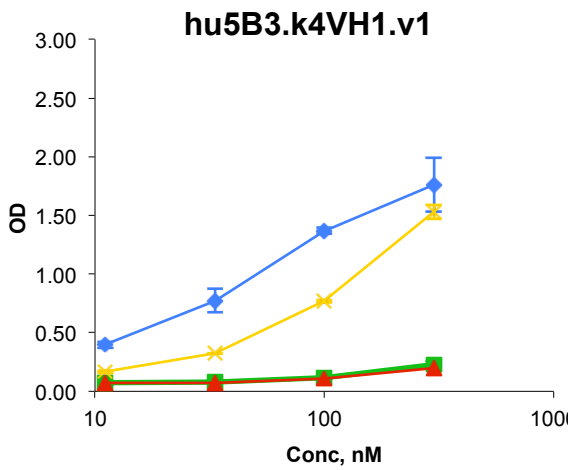
notes: The time points (red circle) that showed potential ATA impacts (i.e. the first time point which has unexpected concentration drop and the following time points) were removed from the data analysis in the two-compartmental analysis

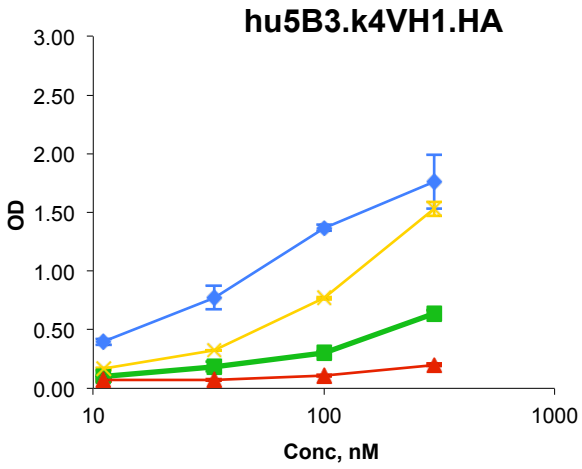


Suppl. Figure 3. Non-specific binding of humanized 5B3 in BV ELISA

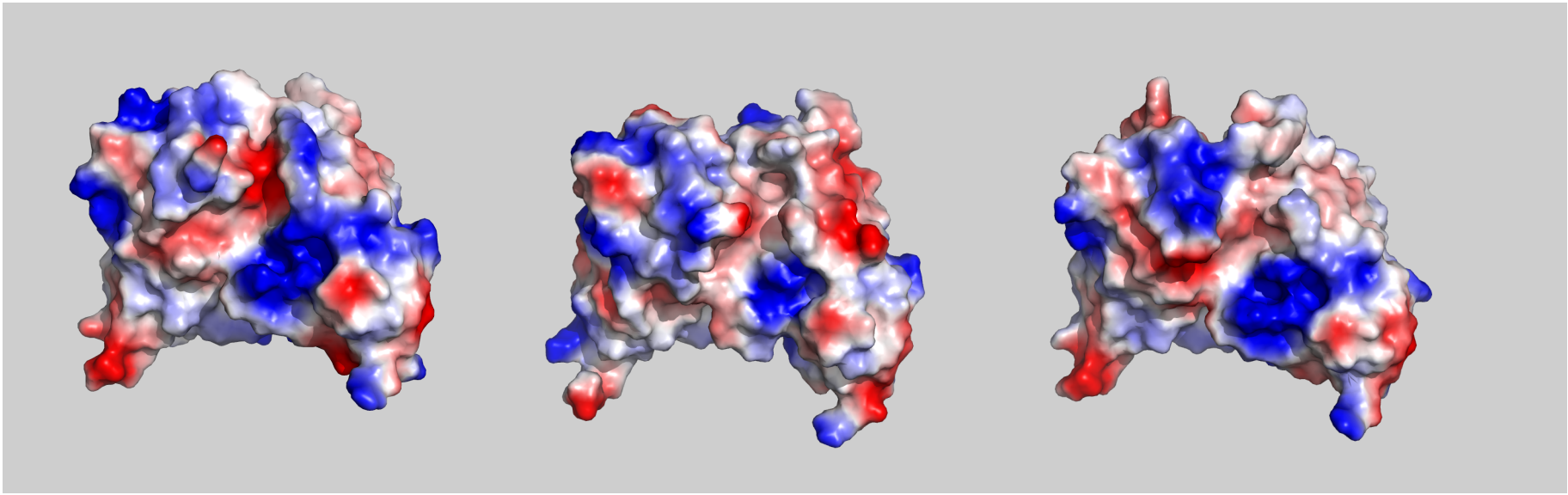
Test article
High binding control
Intermediate binding control
Low binding control



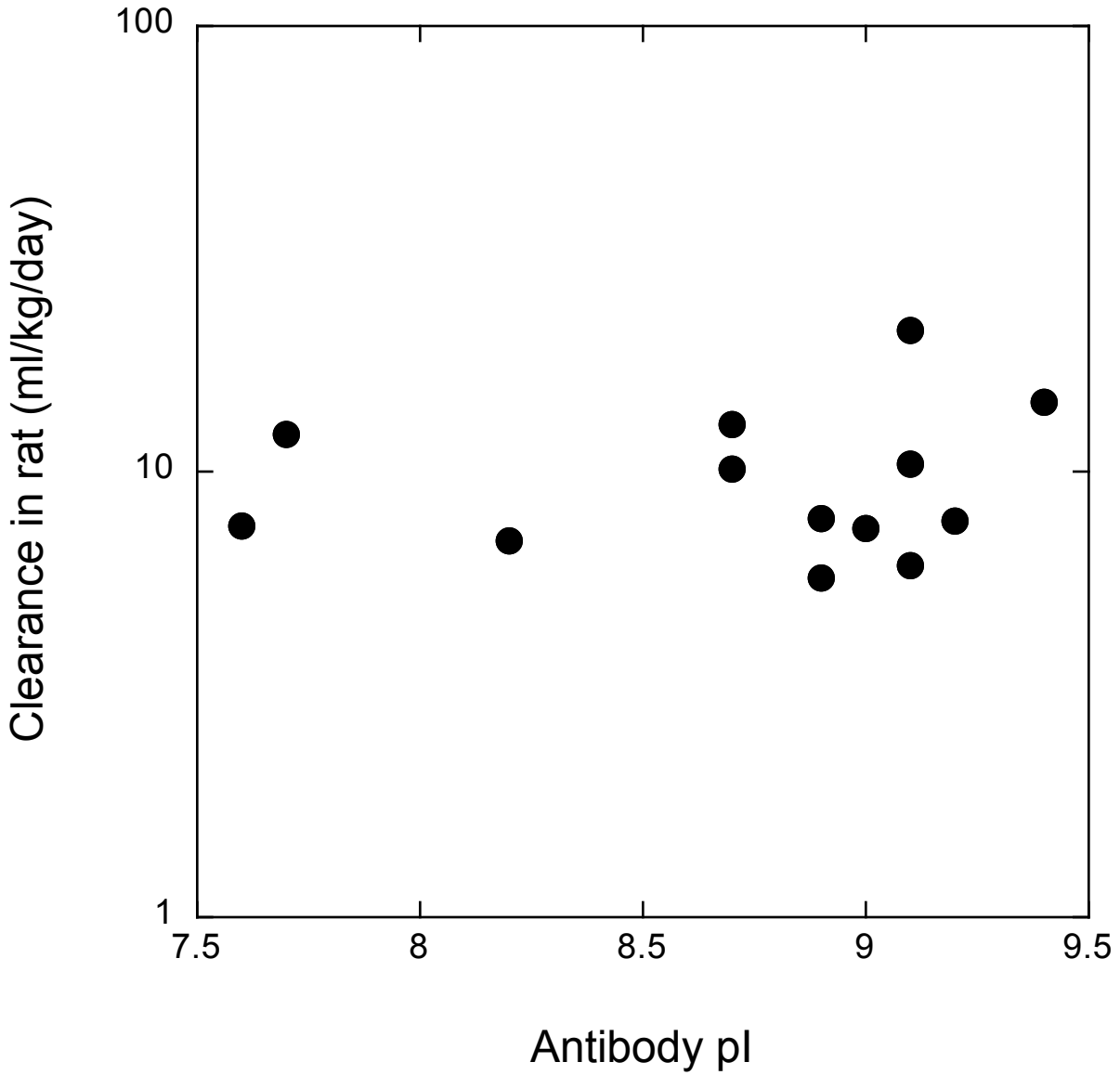




Suppl. Figure 4. Electrostatic surface calculated for hu5B3.k1VH3.v3 (left), hu5B3.k4VH1.v1 (middle), and bevacizumab (right). Surfaces calculated using vacuum electrostatics option of PyMOL (Schrödinger LLC) and x-ray coordinates of hu5B3.k1VH3.v3 (4HS8), bevacizumab (1BJ1), or a model of hu5B3.k4VH1.v1 built using humanized anti-Fas (1IT9) as template. Bound antigen (4HS8, 1BJ1) and constant domains were removed for display. In these renderings, the VL domain is on the right.



Supplemental Figure 5. Clearance in rats for a series of antibodies humanized on κ1, VH3 framework. For these antibodies, rat is a non-binding species or clearance value was determined at a dose that saturates target-dependent clearance



Supplemental Table 1. Mean (± SD) of pharmacokinetic parameter estimates following single intravenous administration of 5 mg/kg humanized 5B3 variants to SD rats

PK Parameter	hu5B3.κ1VH3.v1	hu5B3.κ1VH3.v3	hu5B3.κ4VH1.v1	hu5B3.κ4VH3.v1	hu5B3.κ1VH3. pI 8.61	hu5B3.k4VH1. pI6.10	hu5B3.k4VH1.HA
$AUC_{Days\ 0–10}$ (μg/mL· day)	142 ± 14.5	130±18.0	395±134	397±84.5	37.3 ± 5.00	516 ± 76.5	367± 54.1
C_0 (μg/mL)	143 ± 44.0	146±30.1	126±31.4	125±25.3	170 ± 37.6	178 ± 22.3	159±24.4
Vss* (mL/kg)	170±40.0	173±39.9	88.7 ± 29.4	75.0 ± 25.2	433 ± 199	69.9 ± 5.87	87.7±4.49
$t_{1/2,\beta}$ *(day)	5.01±2.41	4.24±0.95	7.77±1.50	5.77±2.43	3.26±1.98	10.1±2.77	8.35 ± 2.36

Note: $AUC_{Days\ 0–10}$ = Area under the serum concentration–time curve from Time = 0 to Day 10; C_0 = concentration at Time = 0 following an IV bolus dose; Vss= volume of distribution at steady state, $t_{1/2,\beta}$ = beta phase half-life, * obtained by two compartmental analysis and the potential ATA impact time points were removed from data analysis. The results may be confounded by potential ATA