Supplemental material to:
Gang Yin, Eudean D. Garces, Junhao Yang, Juan Zhang, Cuong Tran, Alexander R. Steiner, et al. Aglycosylated antibodies and antibody fragments produced in a scalable in vitro transcription-translation system. MABS 4(2); DOI: 10.4161/mabs.4.2.19202
www.landesbioscience.com/journals/mabs/article/19202

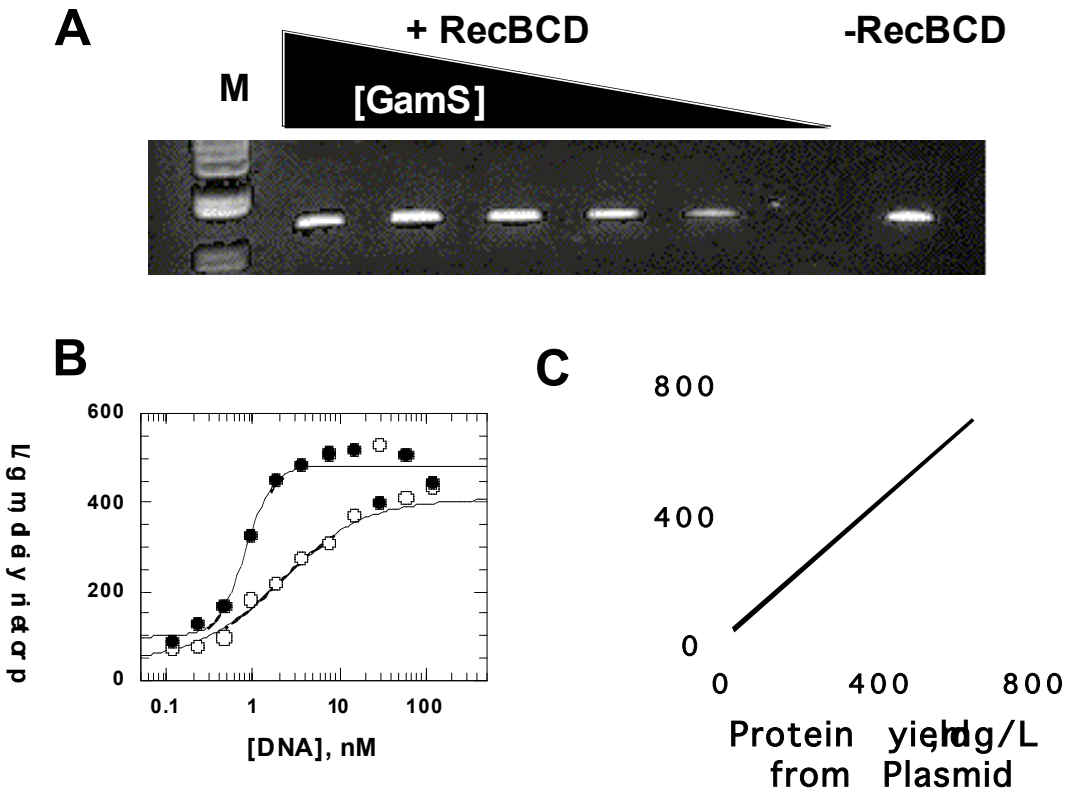
Supplemental Material for:

Aglycosylated Antibodies and Antibody Fragments Produced in a Scalable *in vitro* Transcription-Translation System

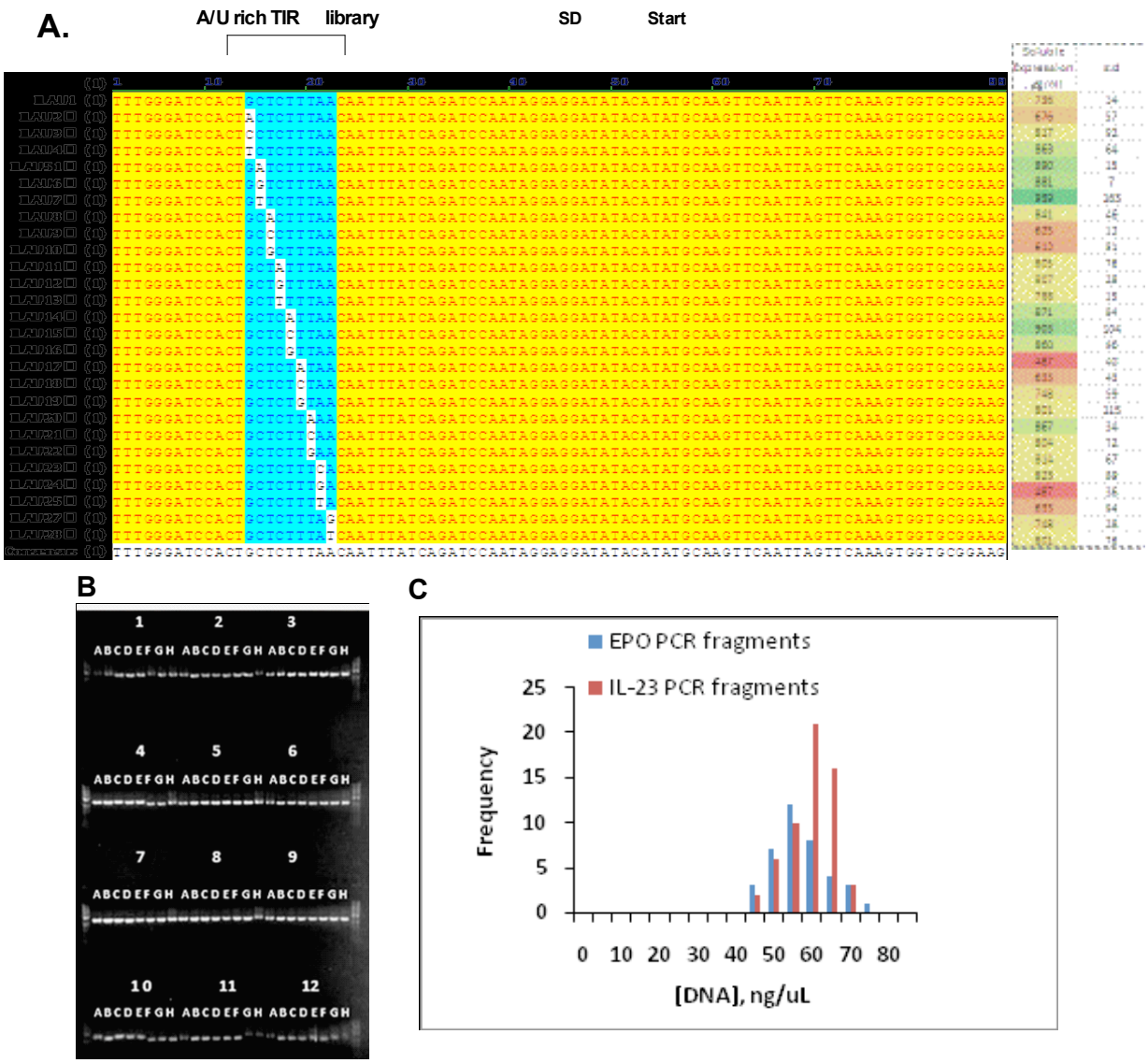
Gang Yin, Eudean D. Garces, Junhao Yang, Juan Zhang, Cuong Tran, Alexander R. Steiner, Christine Roos, Sunil Bajad, Susan Hudak, Kalyani Penta, James Zawada, Sonia Pollitt, & Christopher J. Murray*

*Correspondence should be addressed to Christopher J. Murray cmurray@sutrobio.com.

Supplemental Figure S1

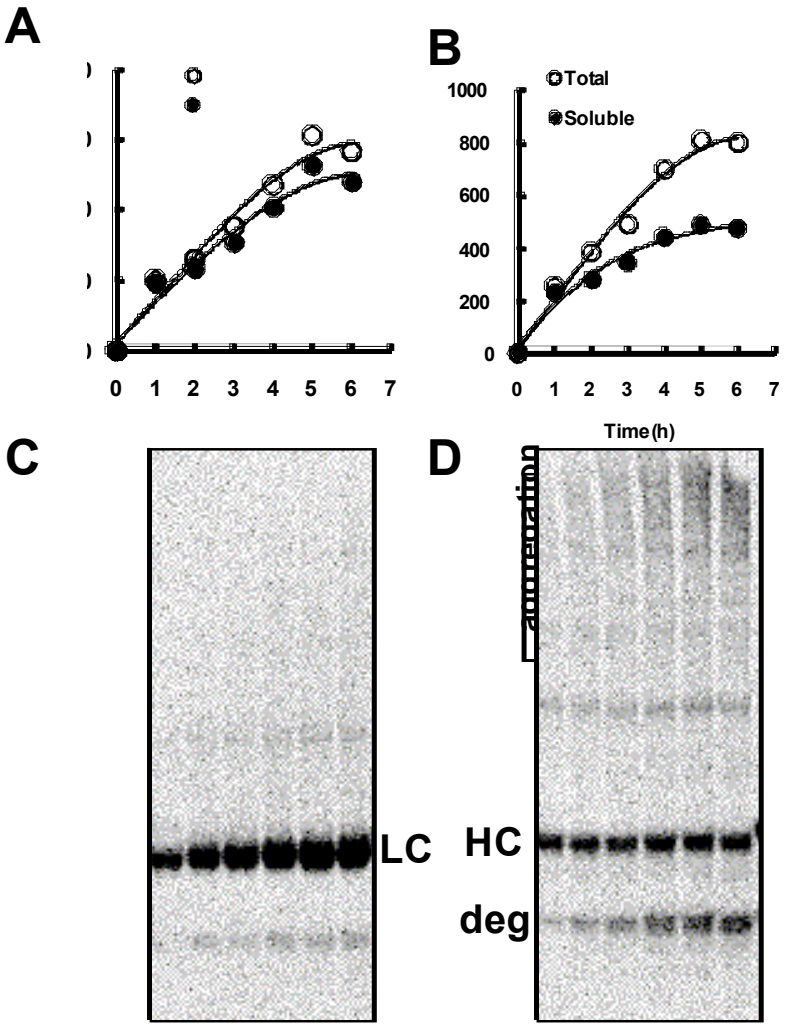


Supplemental Figure S1. Proteins are produced from linear and plasmid DNA templates with similar efficiencies. **(A)**. GamS protein protects linear DNA templates from degradation by RecBCD. A fifty µl mixture containing 23 nM 1500 bp linear DNA template, 10 U RecBCD enzyme (EPICENTRE), and 1 mM ATP in the presence of decreasing concentrations of GamS protein, was incubated at 37 °C for 1 hour. 10 µl from each reaction was analyzed by 1% agarose gel electrophoresis. **(B)** Yield of total scFv-Fc fusion protein as a function of linear (○) and plasmid (●) DNA concentrations.. Proteins were produced at 30 uL scale for 5 hrs, and for linear template, in the presence of 5 uM GamS protein. **(C)** Comparison of protein yields of several proteins transcribed from linear or plasmid DNA templates for the same gene.



Supplemental Figure S2. Translation initiation region (TIR) profiling using 200-mer double stranded DNA templates. (A) Sequence alignment of 5' UTR TIR library sequences for expression optimization of anti-IL23 scFv. Only the first 88 nucleotides of the 200 bp gene synthesized fragments are shown. Soluble expression yields from assembled PCR templates are shown (B) 96-well agarose gel electrophoresis of assembly PCR fragments showing single band purity. (C) Distribution of PCR fragment concentrations after DNA clean-up using 96-well purification kit (Invitrogen, Carlsbad, CA), determined using the Picogreen Assay (Invitrogen, Carlsbad, CA).

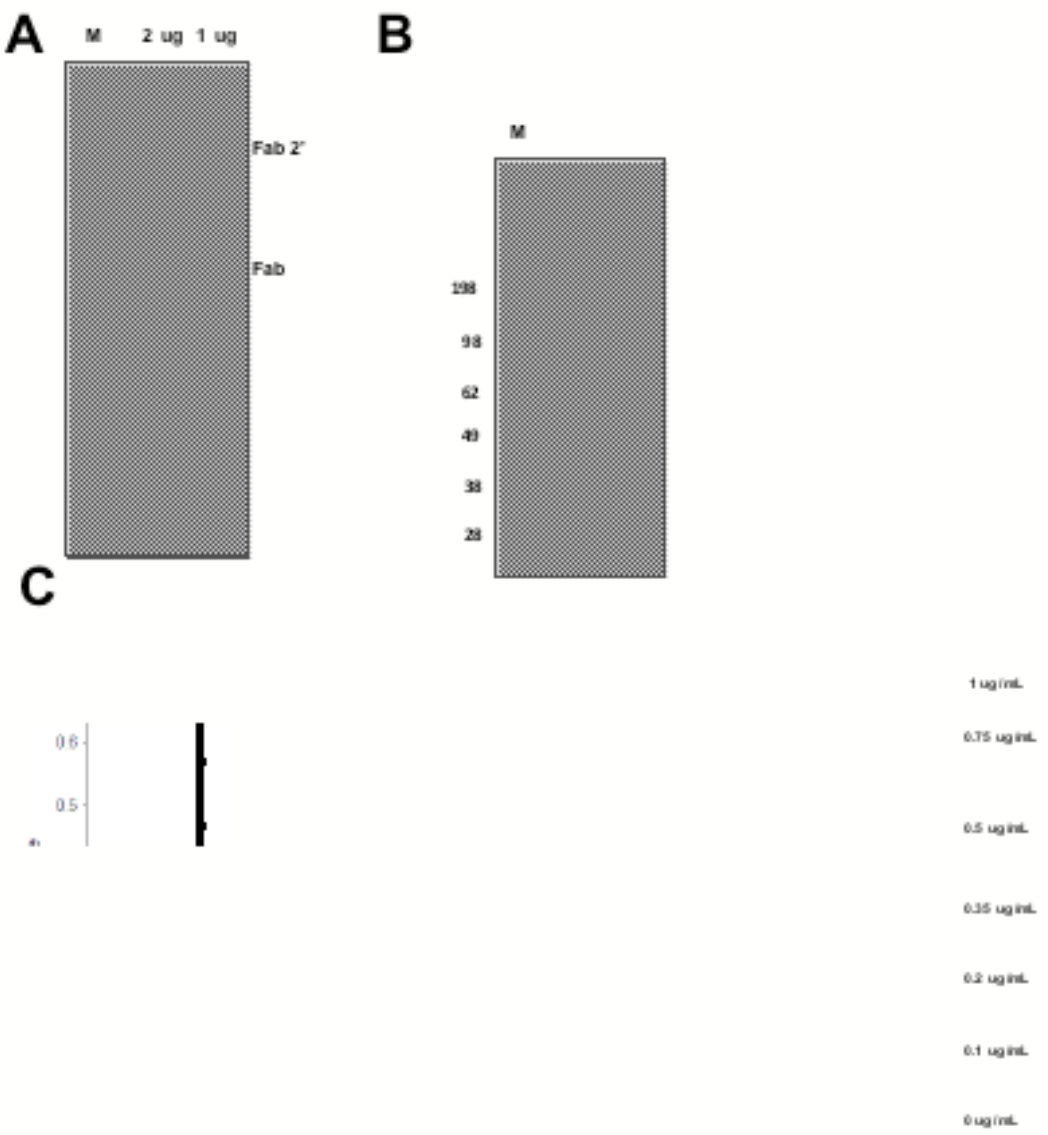
Supplemental Figure S3



Supplemental Figure S3. (A), (B) Time course for total (○) and soluble (●) expression of anti-hIL-13α1R Fab light chain (LC) and heavy chain (HC) based on ^{14}C-Leucine incorporation as described in the **Materials and Methods**. (C) (D) Corresponding non-reducing SDS-PAGE autoradiography of ^{14}C-Leucine incorporated products. Extensive high MW aggregates are observed for Fab HC expression along

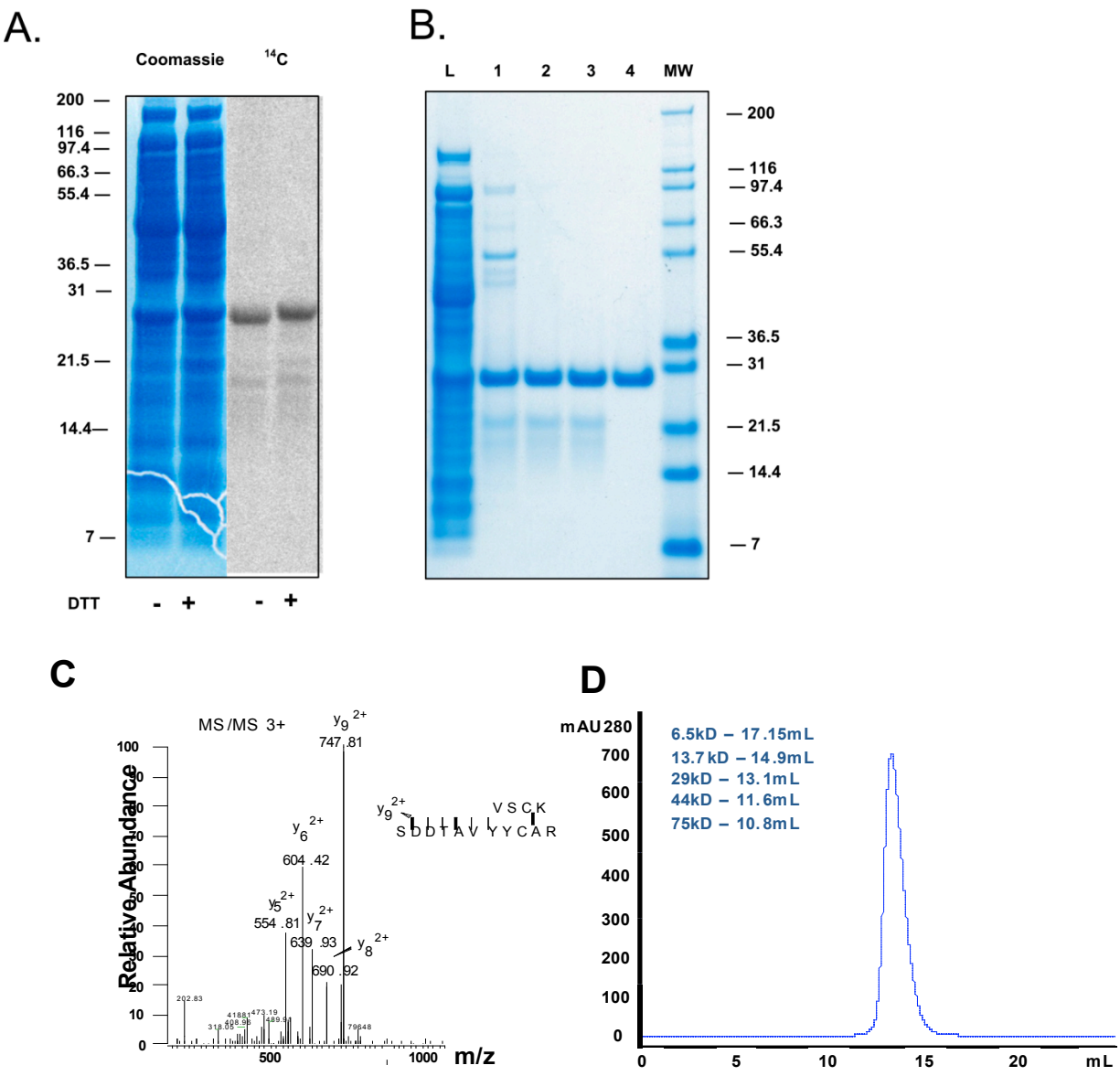
with degradation (deg) products due to proteolysis of incorrectly folded HC.

Supplemental Figure S4



Supplemental Figure S4. Analytical characterization of anti-hIL-13α1R Fab. (A) SDS PAGE (non-reducing) of purified Fab. (B) Western blot analysis of purified Fab with anti-His (HC) and Protein L-HRP conjugate (LC). (C) Quantitation of Fab concentration based on the initial rate of IL-13□1R binding as a function of Fab concentration as measured by biolayer interferometry using a ForteBio Octet 384.

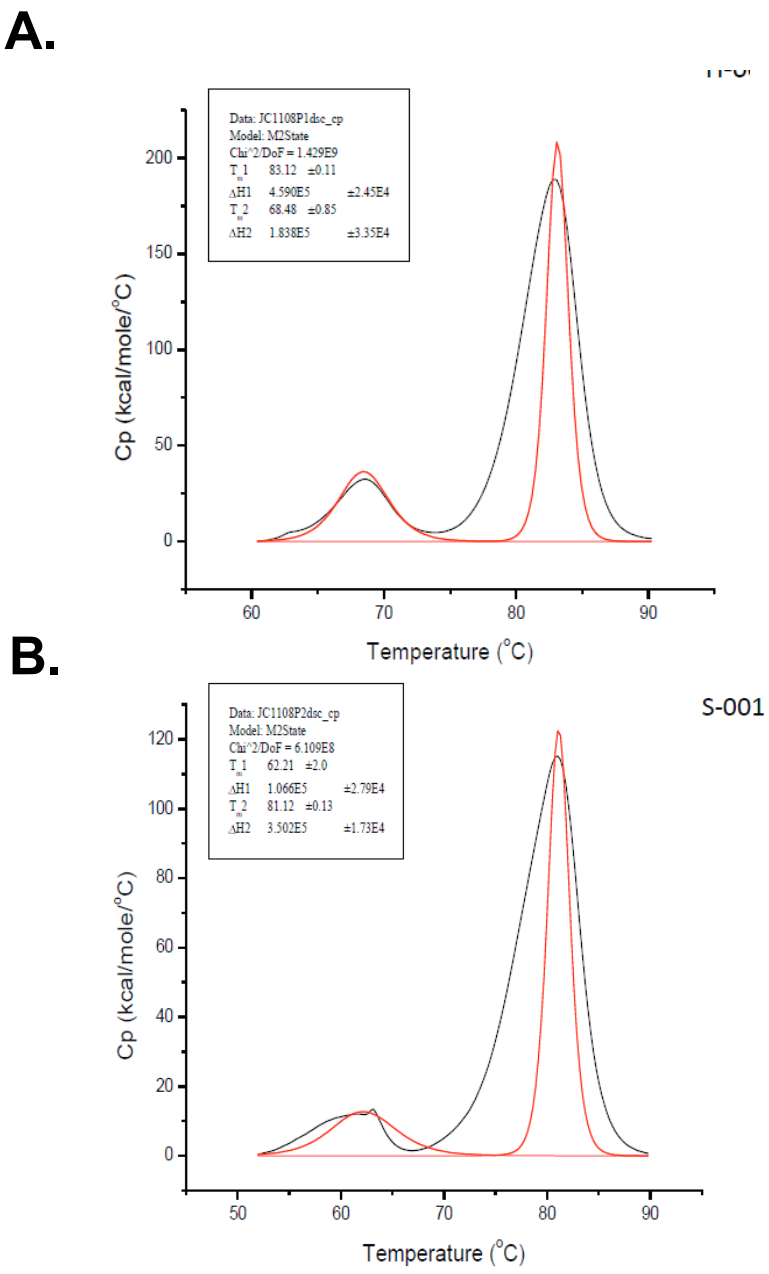
Supplemental Figure S5



Supplementary Figure S5. Purification and characterization of the anti-hIL-23 scFv (A)T7-based transcription of pYD317-anti-hIL-23scFv and cell-free translation for 5 h in mixtures containing [^{14}C]leucine; Samples were incubated with or without DTT, separated by SDS−PAGE, and analyzed by Coomassie staining or autoradiography. (B) SDS-PAGE of anti-hIL-23 scFv purification from a 5 L *in vitro* transcription translation reaction for 10 hrs using the method of Zawada et al. (2011). 1.2 µg of anti-hIL-23 scFv was loaded per lane. Lane L: transcription translation reaction product pool, Lane 1: Cation

exchange capture pool, Lane 2: HIC Pool (*E coli* host cell protein removal), Lane 3: Anion exchange pool (DNA and endotoxin removal), Lane 4: Gel filtration pool (cleavage product removal). (C) Tandem mass spectrum of Glu-C peptides derived from protein L- purified anti-IL-23 scFv. The fragmentation sites for each fragment ion are illustrated above the spectum. The partial sequence of the peptide containing V_H 22C – 96C confirmed the expected disulfide bond is formed. (**D)** Analytical SEC of purified anti-IL-23 scFv.

Supplemental Figure S6



Supplementary Figure S6. Temperature-induced unfolding of (A) Herceptin® and (B) aglycosylated trastuzumab . The data were fit (in red) to a simple two state model for thermal unfolding of two species

corresponding to unfolding the the CH2 domain, followed by irreversible unfolding of Fab and CH3 domains.