**S1:** DNA primers for mutagenesis of Guy’s 13 heavy chain. Complementary forward and reverse primer pairs for generating each mutant are shown, with amino acid translations shown below the nucleotide sequences. Mutated amino acid residues are shown in bold and their positions relative to the protease cleavage site are shown by P2, P1, P1’, P2’ etc. Mutations were designed to incorporate diagnostic restriction enzyme cleavage sites (underlined, and see Table S1).

1. HC1 mutant (Ser113Thr/Ala114Gly).

**VH\_Guys13HC#1.1**

5’-ccactctcacagtctccAcCgGTaaaacgacacccccatc-3’

 T L T V S **T G** K T T P P S ►

 **P1 P1’**

**VH\_Guys13HC#1.2**

5’-gatgggggtgtcgttttACcGgTggagactgtgagagtgg-3’

 ◄ S P P T T K **G T**  S V T L T

 **P1’P1**

2. HC2 mutant (Ser113Val/Ala114His).

**VH\_Guys13HC#2.1**

5’-ccactctcacagtctccGTGCAcaaaacgacacccccatc-3’

 T L T V S **V H** K T T P P S ►

 **P1 P1’**

**VH\_Guys13HC#2.2**

5’-gatgggggtgtcgttttgTGCACggagactgtgagagtgg-3’

 ◄ S P P T T K **H V** S V T L T

 **P1’P1**

3. HC3 mutant (Ser112Thr/Ser113Ala).

**VH\_Guys13HC#3.1**

5’-ggcaccactctcacagtcAcGGcCgccaaaacgacaccccc-3’

 G T T L T V **T A** A K T T P P ►

 **P2 P1**

**VH\_Guys13HC#3.2**

5’-gggggtgtcgttttggcGgCCgTgactgtgagagtggtgcc-3’

 ◄ P P T T K A  **A T** V T L T T G

 **P1 P2**

4. HC4 mutant (Thr110Asn/Val111Ala/Ser112Thr/ Ser113Glu/Ala114Glu).

**VH\_Guys13HC#4.1**

5’-gccaaggcaccactctcaACgCcAccGAagAGaaaacgacacccccatc-3’

 Q G T T L NATEE K T T P P S ►

 **P4 P3 P2 P1 P1’**

**VH\_Guys13HC#4.2**

5’-gatgggggtgtcgttttCTctTCggTgGcGTtgagagtggtgccttggc-3’

 ◄ S P P T T K **E E T A N** L T T G Q

 **P1’P1 P2 P3 P4**