CLAIMS

WHAT IS CLAIMED IS:

1. A humanized antibody comprising a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising a charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain, and a light chain variable domain comprising a charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain.

2. The humanized antibody of claim 1, wherein the charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain is a positively charged amino acid, and the charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain is a negatively charged amino acid.

3. The humanized antibody of claim 1 or 2, wherein the positively charged amino acid is a basic amino acid and the negatively charged amino acid is an acidic amino acid.

4. The humanized antibody of any one of claims 1-3, wherein the humanized antibody further comprises a substituted phenylalanine derivative at an amino acid position corresponding to Chothia canonical amino acid position 91 of a human IgG1 heavy chain.

5. The humanized antibody of claim 4, wherein the substituted phenylalanine derivative at an amino acid position corresponding to Chothia canonical amino acid position 91 of a human IgG1 heavy chain forms a hydrogen bond with at least one of: an amino acid at an amino acid position corresponding to Chothia canonical amino acid position 38 of a human IgG1 light chain, and an amino acid at an amino acid position corresponding to Chothia canonical amino acid position 39 of a human IgG1 heavy chain.

6. The humanized antibody of claim 4 or 5, wherein the substituted phenylalanine derivative comprises a hydroxy-substituted phenyl group.

7. The humanized antibody of claim 5 or 6, wherein the amino acid at an amino acid position corresponding to Chothia canonical amino acid position 38 of a human IgG1 light chain is a glutamine, and/or wherein the amino acid at an amino acid position corresponding to Chothia canonical amino acid position 39 of a human IgG1 heavy chain is a glutamine.

8. The humanized antibody of any one of claims 1-7, wherein the humanized antibody further comprises at least one of following:

a tryptophan at an amino acid position corresponding to Chothia canonical amino acid position 103 of a human IgG1 heavy chain;

a phenylalanine at an amino acid position corresponding to Chothia canonical amino acid position 100C of a human IgG1 heavy chain; and

a leucine substituted with an alanine at an amino acid position corresponding to Chothia canonical amino acid position 78 of a human IgG1 heavy chain.

9. The humanized antibody of any one of claims 1-8, wherein the humanized antibody further comprises at least one of following:

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 49 of a human IgG1 heavy chain; and

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 74 of a human IgG1 heavy chain.

10. The humanized antibody of any one of claims 1-8, wherein the humanized antibody further comprises at least one of following:

a serine at an amino acid position corresponding to Chothia canonical amino acid position 49 of a human IgG1 heavy chain; and

a serine at an amino acid position corresponding to Chothia canonical amino acid position 74 of a human IgG1 heavy chain.

11. The humanized antibody of any one of claims 1-10, wherein the humanized antibody further comprises at least one of the following:

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain;

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 17 of a human IgG1 light chain;

a tyrosine at an amino acid position corresponding to Chothia canonical amino acid position 36 of a human IgG1 light chain;

an arginine at an amino acid position corresponding to Chothia canonical amino acid position 39 of a human IgG1 light chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 92 of a human IgG1 light chain; and

a valine at an amino acid position corresponding to Chothia canonical amino acid position 104 of a human IgG1 light chain.

12. The humanized antibody of any one of claims 1-11, wherein the humanized antibody further comprises a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain.

13. A humanized antibody comprising a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising a substituted phenylalanine derivative at an amino acid position corresponding to Chothia canonical amino acid position 91 of a human IgG1 heavy chain.

14. The humanized antibody of claim 13, wherein the humanized antibody further comprises a polar uncharged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 39 of a human IgG1 heavy chain, and a light chain variable domain comprising a polar uncharged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 38 of a human IgG1 light chain.

15. The humanized antibody of claim 14, wherein the substituted phenylalanine derivative comprises a hydroxy-substituted phenyl group.

16. The humanized antibody of any one of claims 13-15, wherein the humanized antibody further comprises a positively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain.

17. The humanized antibody of any one of claims 13-16, wherein the humanized antibody further comprises a negatively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain.

18. The humanized antibody of any one of claims 13-17, wherein the humanized antibody further comprises at least one of following:

a tryptophan at an amino acid position corresponding to Chothia canonical amino acid position 103 of a human IgG1 heavy chain;

a phenylalanine at an amino acid position corresponding to Chothia canonical amino acid position 100C of a human IgG1 heavy chain; and

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 78 of a human IgG1 heavy chain.

19. The humanized antibody of any one of claims 13-18, wherein the humanized antibody further comprises at least one of following:

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 49 of a human IgG1 heavy chain; and

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 74 of a human IgG1 heavy chain.

20. The humanized antibody of any one of claims 13-19, wherein the humanized antibody further comprises at least one of the following:

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain;

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 17 of a human IgG1 light chain;

a tyrosine at an amino acid position corresponding to Chothia canonical amino acid position 36 of a human IgG1 light chain;

an arginine at an amino acid position corresponding to Chothia canonical amino acid position 39 of a human IgG1 light chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 92 of a human IgG1 light chain; and

a valine at an amino acid position corresponding to Chothia canonical amino acid position 104 of a human IgG1 light chain.

21. A humanized antibody comprising a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising a positively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain, and a light chain variable domain comprising at least one of the following:

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain;

an alanine residue at an amino acid position corresponding to Chothia canonical amino acid position 92 of a human IgG1 light chain; and

a negatively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain.

22. The humanized antibody of claim 21, wherein the humanized antibody further comprises one or both of:

an alanine residue at an amino acid position corresponding to Chothia canonical amino acid position 49 of a human IgG1 heavy chain; and

an alanine residue at an amino acid position corresponding to Chothia canonical amino acid position 74 of a human IgG1 heavy chain.

23. A humanized antibody comprising a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising a polar uncharged amino acid at an

amino acid position corresponding to Chothia canonical amino acid position 49 of a human IgG1 heavy chain, and a light chain variable domain comprising at least one of the following:

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 92 of a human IgG1 light chain; and

a negatively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain.

24. A humanized antibody comprising a heavy chain variable domain and a light chain variable domain, the heavy chain variable domain comprising a polar uncharged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 74 of a human IgG1 heavy chain, and a light chain variable domain comprising at least one of the following:

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain;

an alanine residue at an amino acid position corresponding to Chothia canonical amino acid position 92 of a human IgG1 light chain; and

a negatively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain.

25. The humanized antibody of any one of claims 1-24, wherein the antibody is conjugated to a therapeutic, cytotoxic, cytostatic, immunomodulatory, neurotrophic, or neuroprotective agent.

26. A pharmaceutical composition comprising the humanized antibody of any one of claims 1-25 and a pharmaceutically acceptable carrier.

27. A nucleic acid encoding the heavy chain variable domain and/or the light chain variable domain of an antibody of any one of claims 1-25.

28. A vector comprising a nucleic acid encoding a heavy chain variable domain and a light chain variable domain operably linked to one or more regulatory sequences to effect expression in a mammalian cell of the humanized antibody of any one of claims 1-25.

29. A host cell comprising the nucleic acid of claim 27.

30. A method of reducing immunogenicity of a parent antibody by introducing amino acid substitution(s) into the parent antibody to produce a modified antibody comprising:

(i) a heavy chain variable domain comprising one or more of:

a positively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 49 of a human IgG1 heavy chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 74 of a human IgG1 heavy chain;

a leucine substituted with an alanine at an amino acid position corresponding to Chothia canonical amino acid position 78 of a human IgG1 heavy chain;

a substituted phenylalanine derivative at an amino acid position corresponding to Chothia canonical amino acid position 91 of a human IgG1 heavy chain;

a tryptophan at an amino acid position corresponding to Chothia canonical amino acid position 103 of a human IgG1 heavy chain; and

a phenylalanine at an amino acid position corresponding to Chothia canonical amino acid position 100C of a human IgG1 heavy chain; and/or

(ii) a light chain variable domain comprising one or more of:

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain;

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 17 of a human IgG1 light chain;

a tyrosine at an amino acid position corresponding to Chothia canonical amino acid position 36 of a human IgG1 light chain;

an arginine at an amino acid position corresponding to Chothia canonical amino acid position 39 of a human IgG1 light chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 92 of a human IgG1 light chain;

a negatively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain; and

a valine at an amino acid position corresponding to Chothia canonical amino acid position 104 of a human IgG1 light chain.

31. A method of increasing thermal stability of an antibody by introducing amino acid substitution(s) into a parent antibody to produce a modified antibody comprising:

(i) a heavy chain variable domain comprising one or more of:

a positively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 49 of a human IgG1 heavy chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 74 of a human IgG1 heavy chain;

a leucine substituted with an alanine at an amino acid position corresponding to Chothia canonical amino acid position 78 of a human IgG1 heavy chain;

a substituted phenylalanine derivative at an amino acid position corresponding to Chothia canonical amino acid position 91 of a human IgG1 heavy chain;

a tryptophan at an amino acid position corresponding to Chothia canonical amino acid position 103 of a human IgG1 heavy chain; and

a phenylalanine at an amino acid position corresponding to Chothia canonical amino acid position 100C of a human IgG1 heavy chain; and/or

(ii) a light chain variable domain comprising one or more of:

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 3 of a human IgG1 light chain;

a glutamine at an amino acid position corresponding to Chothia canonical amino acid position 17 of a human IgG1 light chain;

a tyrosine at an amino acid position corresponding to Chothia canonical amino acid position 36 of a human IgG1 light chain;

an arginine at an amino acid position corresponding to Chothia canonical amino acid position 39 of a human IgG1 light chain;

an alanine at an amino acid position corresponding to Chothia canonical amino acid position 92 of a human IgG1 light chain;

a negatively charged amino acid at an amino acid position corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain; and

a valine at an amino acid position corresponding to Chothia canonical amino acid position 104 of a human IgG1 light chain.

32. A method of reducing immunogenicity of a parent antibody by introducing a first amino acid substitution and a second amino acid substitution into the parent antibody to generate a modified antibody, wherein the first amino acid substitution is at an amino acid corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain; and the second amino acid substitution is at an amino acid corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain; wherein the modified antibody has a reduction in immunogenicity compared to the parent antibody.

33. A method of increasing thermal stability of a parent antibody by introducing a first amino acid substitution and a second amino acid substitution into the parent antibody to generate a modified antibody, wherein the first amino acid substitution is at an amino acid corresponding to Chothia canonical amino acid position 44 of a human IgG1 heavy chain; and the second amino acid substitution is at an amino acid corresponding to Chothia canonical amino acid position 100 of a human IgG1 light chain; wherein the modified antibody has increase in melting temperature of 2°C or greater compared to the parent antibody as measured by differential scanning calorimetry.