Summary

In one aspect, there is provided a method for producing at least two different monospecific Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399, and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439,

said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different Ig-like molecules from the culture. In another aspect, there is provided a method for producing at least two different monospecific Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439, said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different Ig-like molecules from the culture. In another aspect, there is provided a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and d) A fourth nucleic acid encoding a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. In another aspect, there is provided a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and d) A fourth nucleic acid

encoding a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. In another aspect, there is provided a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, and c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. In another aspect, there is provided a method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an

interface, said method comprising providing in said cell a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture. In a further aspect, there is provided a mixture of at least two different monospecific Ig-like molecules obtainable by the methods according to the present invention. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399, and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid

position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368. In a further aspect, there is provided a recombinant host cell comprising one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351. In a further aspect, there is provided a pharmaceutical composition comprising the at least two different monospecific Ig-like molecules of any one of the present invention, and a pharmaceutically acceptable carrier. In a further aspect, there is provided a method for making a host cell for production of at least two different monospecific Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and positively charged amino acid residue at amino acid position 399, and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439. In a further aspect, there is provided a method for making a host cell for

production of at least two different monospecific Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439. In a further aspect, there is provided a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain. In a further aspect, there is provided a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E)

residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B. In a further aspect, there is provided a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368. In a further aspect, there is provided a method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351. In yet a further aspect, there is provided a culture of recombinant host cells of the present invention, or of recombinant host cells obtained by the method according to the present invention, producing at least two different monospecific Ig-like molecules.

Claims

1. A method for producing at least two different monospecific Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and

b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,

and wherein said second CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439,

said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different Ig-like molecules from the culture.

2. A method for producing at least two different monospecific Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) a first nucleic acid encoding a first CH3 domain-comprising polypeptide chain, and

b) a second nucleic acid encoding a second CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439,
said method further comprising the step of culturing said host cell and allowing for expression of said nucleic acids and harvesting said at least two different Ig-like molecules from the culture.

3. The method of claim 1 or 2, wherein said first CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 392 and a lysine (K) residue at amino acid position 399 and an aspartic acid (D) residue at amino acid position 409.

4. The method of any one of claims 1-3, wherein said second CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 356 and a lysine (K) residue at amino acid position 357 and an aspartic acid (D) residue at amino acid position 439 and an aspartic acid (D) residue at amino acid position 370.

5. The method of any one of claims 1-4, wherein the formation of bispecific Ig-like molecules is reduced to below 5%.

6. The method of any one of claims 1-5, wherein the formation of bispecific Ig-like molecules is reduced to below 3%.

7. A method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell
a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain,
b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain,
c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and
d) A fourth nucleic acid encoding a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an

aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture.

8. A method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain,

b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain,

c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and

d) A fourth nucleic acid encoding a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture.

9. A method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain,

b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain, and

c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an

aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture.

10. A method for producing at least two different Ig-like molecules from a single host cell, wherein each of said two Ig-like molecules comprises two CH3 domains that are capable of forming an interface, said method comprising providing in said cell

a) A first nucleic acid encoding a first CH3 domain-comprising polypeptide chain,

b) A second nucleic acid encoding a second CH3 domain-comprising polypeptide chain,

c) A third nucleic acid encoding a third CH3 domain-comprising polypeptide chain, and

wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, said method further comprising the step of culturing said host cell and allowing for expression of said at least four nucleic acids and harvesting said at least two different Ig-like molecules from the culture.

11. The method of any one of claims 7-10, wherein said second CH3 domain-comprising polypeptide chain further comprises a glutamic acid (E) residue or an aspartic acid (D) residue at amino acid position 349.

12. The method of any one of claims 1-11, further comprising providing said host cell with a nucleic acid encoding a common light chain.

13. The method of any one of claims 1-12, wherein each of the encoded CH3 domain-comprising polypeptide chains further comprises a variable region recognizing an epitope.

14. The method of claim 13, wherein the epitope recognized by the variable region of the second encoded CH3 domain-comprising polypeptide chain is different from the epitope recognized by the variable region of the first encoded CH3 domain-comprising polypeptide chain.

15. The method of any one of claims 7-14, wherein each of the variable regions of the third or fourth encoded CH3 domain-comprising polypeptide chains recognizes a different epitope.

16. The method of any one of claims 7-8 or 11-15, wherein the variable regions of the first and the second encoded CH3-domain comprising polypeptide chains recognize different epitopes, whereas the variable regions of the third and the fourth encoded CH3-domain comprising polypeptide chains recognize the same epitopes.

17. The method of claim 16 wherein the epitope recognized by the variable regions of the third and fourth encoded CH3 domain comprising polypeptide chain is the same as the epitope recognized by the variable region of the first or the second encoded CH3-domain comprising polypeptide chain.

18. The method of claim 16 wherein the epitope recognized by the variable regions of the third and fourth encoded CH3 domain comprising polypeptide chain is different from the epitope recognized by the variable region of the first or the second CH3-domain comprising polypeptide chain.

19. The method of any one of claims 7-8 or 11-15, wherein the variable regions of the first and the second encoded CH3-domain comprising polypeptide chains recognize the same epitope, whereas the variable regions of the third and the fourth encoded CH3-domain comprising polypeptide chains recognize a second epitope which differs from the epitope recognized by said first and second variable regions.

20. The method of any one of claims 13-19, wherein the epitopes are located on the same target.

21. The method of claim 20, wherein the target is soluble.

22. The method of claim 20, wherein the target is membrane-bound.

23. The method of any one of claims 13-19, wherein the epitopes are located on different targets.

24. The method of claim 23, wherein the different targets are expressed on the same cells.

25. The method of claim 23, wherein the different targets are expressed on different cells.

26. The method of claim 23, wherein the different targets are soluble.

27. The method of claim 23, wherein one target is soluble whereas the second target is membrane bound.

28. The method of any one of claims 13-23, wherein at least one of said epitopes is located on a tumor cell.

29. The method of any one of claims 13-28, wherein at least one of said epitopes is located on an effector cell.

30. The method of claim 29, wherein said effector cell is an NK cell, a T cell, a B cell, a monocyte, a macrophage, a dendritic cell or a neutrophilic granulocyte.

31. The method of claims 29 or 30, wherein said epitope is located on a CD3, CD16, CD25, CD28, CD64, CD89, NKG2D or a NKp46 molecule.

32. A mixture of at least two different monospecific Ig-like molecules obtainable by the methods according to any one of claims 1-31.

33. A mixture according to claim 32, wherein said at least two different monospecific Ig-like molecules bind to different epitopes on the same antigen and/or to different epitopes on different antigens.

34. A recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,
and wherein said second CH3 domain-comprising polypeptide chain comprises:
- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439.

35. A recombinant host cell comprising one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439.

36. A recombinant host cell according to claim 34 or 35, wherein said first CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 392 and a lysine (K) residue at amino acid position 399 and an aspartic acid (D) residue at amino acid position 409.

37. A recombinant host cell according to any one of claims 34-36, wherein said second CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 356 and a lysine (K) residue at amino acid position 357 and an aspartic acid (D) residue at amino acid position 439 and an aspartic acid (D) residue at amino acid position 370.

38. A recombinant host cell comprising one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at

amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain.

39. A recombinant host cell comprising one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B.

40. A recombinant host cell comprising one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368.

41. A recombinant host cell comprising one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351.

42. A recombinant host cell according to any one of claims 38-41, wherein each of the CH3 domain-comprising polypeptide chains further comprises a variable region recognizing an epitope, and wherein each of the variable regions of the 3 or 4 CH3 domain-comprising polypeptide chains recognizes a different epitope.

43. A recombinant host cell according to any one of claims 38-42, wherein said second CH3 domain-comprising polypeptide chain further comprises a glutamic acid (E) residue or an aspartic acid (D) residue at amino acid position 349.

44. A recombinant host cell according to any one of claims 34-43, wherein said host cell further comprises a nucleic acid encoding a common light chain.

45. A pharmaceutical composition comprising the at least two different monospecific Ig-like molecules of any one of claims 32-33, and a pharmaceutically acceptable carrier.

46. A pharmaceutical composition according to claim 45, wherein said at least two different monospecific Ig-like molecules have been produced by recombinant host cells according to any one of claims 34-44.

47. A method for making a host cell for production of at least two different monospecific Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain,
wherein said first CH3 domain-comprising polypeptide chain comprises negatively charged amino acid residues at amino acid positions 392 and 409 and positively charged amino acid residue at amino acid position 399,
and wherein said second CH3 domain-comprising polypeptide chain comprises:
- either a wildtype CH3 domain, or
- positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439.

48. A method for making a host cell for production of at least two different monospecific Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first and a second CH3 domain-comprising polypeptide chain,

wherein said first CH3 domain-comprising polypeptide chain comprises:

- either a wildtype CH3 domain, or
- negatively charged amino acid residues at amino acid positions 392 and 409 and a positively charged amino acid residue at amino acid position 399,

and wherein said second CH3 domain-comprising polypeptide chain comprises positively charged amino acid residues at amino acid positions 356 and 357 and negatively charged amino acid residues at amino acid positions 370 and 439.

49. A method according to claim 47 or 48, wherein said first CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 392 and a lysine (K) residue at amino acid position 399 and an aspartic acid (D) residue at amino acid position 409.

50. A method according to any one of claims 47-49, wherein said second CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 356 and a lysine (K) residue at amino acid position 357 and an aspartic acid (D) residue at amino acid position 439 and an aspartic acid (D) residue at amino acid position 370.

51. A method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third and fourth CH3 domain-comprising polypeptide chains comprise a wild type CH3 domain.

52. A method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second, a third and a fourth CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic

acid (E) residue at amino acid position 368, and wherein said third and/or said fourth CH3 domain-comprising polypeptide chain comprises one or more amino acid substitutions selected from Table B.

53. A method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368.

54. A method for making a host cell for production of at least two different Ig-like molecules, the method comprising the step of introducing into said host cell one or more nucleic acids encoding at least a first, a second and a third CH3 domain-comprising polypeptide chain, wherein said first CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351, and wherein said second CH3 domain-comprising polypeptide chain comprises an aspartic acid (D) residue at amino acid position 351 and a glutamic acid (E) residue at amino acid position 368, and wherein said third CH3 domain-comprising polypeptide chain comprises a lysine (K) residue at amino acid position 366 and a lysine (K) residue at amino acid position 351.

55. A method according to any one of claims 51-54, wherein each of the CH3 domain-comprising polypeptide chains further comprises a variable region recognizing an epitope, and wherein each of the variable regions of the third or fourth CH3 domain-comprising polypeptide chains recognizes a different epitope.

56. A method according to any one of claims 51-55, wherein said second CH3 domain-comprising polypeptide chain further comprises a glutamic acid (E) residue or an aspartic acid (D) residue at amino acid position 349.

57. A method according to any one of claims 47-56, further comprising the step of introducing into said host cell a nucleic acid encoding a common light chain.

58. A culture of recombinant host cells of any one of claims 34-44, or of recombinant host cells obtained by the method according to any one of claims 47-57, producing at least two different monospecific Ig-like molecules.

59. The method according to any one of claims 1-31 or 47-57, or the mixture according to claim 32 or 33, or the recombinant host cell according to any one of claims 34-44, or the pharmaceutical composition according to claim 45 or 46, or the culture according to claim 58, wherein said Ig-like molecules are antibodies.