Claims

1. A polypeptide with transpositional activity comprising a Sleeping Beauty (SB) transposase having at least 80% amino acid identity to SEQ ID NO: 18 and having a substitution in amino acid position 124, wherein the amino acid in position 124 is selected from the group consisting of C, D, N, E, G, L, M, F, S, T, Y, V, A and R,

   wherein the polypeptide has an enhanced transpositional activity compared to an otherwise identical transposase not comprising said substitution in position 124.

2. The polypeptide of claim 1, wherein the transposase comprises an amino acid sequence of SEQ ID NO: 19 or having 1, 2, 3 or 4 amino acids difference compared to SEQ ID NO: 19, wherein the transposase preferably has a Q124C substitution.

3. The polypeptide of any of the preceding claims, wherein the amino acid in position 124 is C.

4. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is G.

5. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is D.

6. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is N.

7. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is E.

8. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is L.

9. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is M.

10. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is F.

11. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is S.

12. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is T.

13. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is Y.

14. The polypeptide of any of claims 1-2, wherein the amino acid in position 124 is V.

15. The polypeptide of any of the preceding claims, further comprising a substitution in any of positions 187, 247 and/or 248,

    wherein, optionally,

    a) the amino acid in said substituted position 187 is A, N, C, Q, G, I, L, M, S, V, W, K, R, E, P, T and S, preferably, V;

    b) the amino acid in said substituted position 247 is R, C, A or S; preferably, R, and/or

    c) the amino acid in said substituted position 248 is R, S, V, I or C, preferably, R.

16. The polypeptide of any of the preceding claims, further comprising at least one of the following substitutions or groups of substitutions:

(1) K14R, K13D, K13A, K30R, K33A, T83A, I100L, R115H, R143L, R147E, A205K/H207V/K208R/D210E, H207V/K208R/D210E, R214D/K215A/E216V/N217Q; M243Q, E267D, T314N, and/or G317E;
(2) K14R//R214D/K215A/E216V/N217Q;
(3) K33A/R115H/R214D/K215A/E216V/N217Q/M243H;
(4) K14R/K30R/A205K/H207V/K208R/D210E/R214D/K215A/E216V/N217Q/M243H;
(5) K13D/K33A/T83N/H207V/K208R/D210E/M243Q;
(6) K13A/K33A/R214D/K215A/E216V/N217Q;
(7) K33A/T83N/R214D/K215NE216V/N217Q//G317E;
(8) K14R/T83A/M243Q;
(9) K14R/T83A/I100L/M243Q;
(10) K14R/T83A/R143L/M243Q;
(11) K14R/T83A/R147E/M243Q;
(12) K14R/T83A/M243Q/E267D;
(13) K14R/T83A/M243Q/T314N;
(14) K14R/K30R/I110L/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q/M243H;
(15) K14R/K30R/R143L/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q//M243H;
(16) K14R/K30R/R147E/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q/M243H;
(17) K14R/K30R/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q//M243H/E267D;
(18) K14R/K30R/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q/M243H/T314N;
(19) K14R/K30R/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q//M243H/G317E;
(20) K14R/K33A/R115H/R214D/K215A/E216V/N217Q/M243H;
(21) K14R/K30R/R147E/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q//M243H/T314N;
(22) K14R/K30R/R143U/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q//M243H/E267D;
(23) K14R/K30R/R143L/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q//M243H/T314N;
(24) K14R/K30R/R143L/A205K/H207V/K208R/D210E/R214D/K215A/ E216V/N217Q//M243H/G317E;
(25) K14R/K33A/R115H/R143L/R214D/K215A/E216V/N217Q/M243H;
(26) K14R/K33A/R115H/R147E//R214D/K215A/E216V/N217Q/M243H;
(27) K14R/K33A/R115H/R214D/K215A/E216V/N217Q//M243H/E267D;
(28) K14R/K33A/R115H/R214D/K215A/E216V/N217Q//M243H/T314N;
(29) K14R/K33A/R115H/R214D/K215A/E216V/N217Q//M243H/G317E;

(30) K14R/T83A/M243Q/G317E; or
(31) K13A/K33A/T83N/R214D/K215A/E216V/N217Q.

17. A nucleic acid encoding a polypeptide according to any of the preceding claims.

18. The nucleic acid of claim 17 that is mRNA, a minicircle or a plasmid, wherein, optionally, if the nucleic acid is not mRNA, said nucleic acid sequence is operably linked to at least one transcription control unit, preferably, a promotor active in a human cell.

19. A cell comprising the nucleic acid of any of claims 17-18 and/or the polypeptide of any of claims 1-16.

20. The cell of claim 19, wherein the cell is a human cell selected from the group comprising a pluripotent stem cell or a human lymphocyte, preferably, a human T lymphocyte.

21. A kit comprising

a) the polypeptide of any of claims 1-16, the nucleic acid of any of claims 17-18 and/or the cell of any of claims 19-20; and

b) a nucleic acid comprising a cargo nucleic acid flanked by terminal inverted repeats (TIR) of a transposon capable of being mobilized by said transposase.

22. A pharmaceutical composition comprising the polypeptide of any of claims 1-16, the nucleic acid of any of claims 17-18; the cell of any of claims 19-20, and/or the kit of claim 21 and, optionally, a pharmaceutically acceptable carrier and/or excipient.

23. The polypeptide of any of claims 1-16, the nucleic acid of any of claims 17-18, the cell of any of claims 19-20, the kit of claim 21, and/or the pharmaceutical composition of claim 22, for use in therapy.

24. Use of the polypeptide of any of claims 1-16, the nucleic acid of any of claims 17-18, the cell of any of claims 19-20, the kit of claim 21, and/or the pharmaceutical composition of claim 22, in the manufacture of a medicament for use in adoptive T cell therapy or gene therapy <u>of a metabolic or immunological disease or for treatment of cancer</u>.

25. Use of the polypeptide of any of claims 1-16, the nucleic acid of any of claims 17-18, the cell of any of claims 19-20 or the kit of claim 21 for introducing an exogenous nucleic acid into the genome of a cell, wherein the use is an *in vitro* use or an *ex vivo* use.

26. A *in vitro* method or an *ex vivo* method of preparing a cell with an exogenous nucleic acid integrated into the genome of the cell, comprising steps of

a) providing an isolated cell;
b) providing to the cell a polypeptide of any of claims 1-16; and
c) providing to the cell a nucleic acid comprising the exogenous nucleic acid flanked by terminal inverted repeats (TIR) of a transposon capable of being mobilized by said transposase;

wherein, optionally, the polypeptide is provided to the cell by introducing the nucleic acid of any of claims 17-18 into the cell.