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Supplementary Materials: Synthesis and Characterization of Mannosylated Formulations to Deliver a Minicircle DNA Vaccine

Ana Sofia Serra [1], Dalinda Eusébio [1], Ana Raquel Neves [1], Himanshu Bhatt [2], Swati Biswas [2], Diana Costa [1,*] and Ângela Sousa [1,*]

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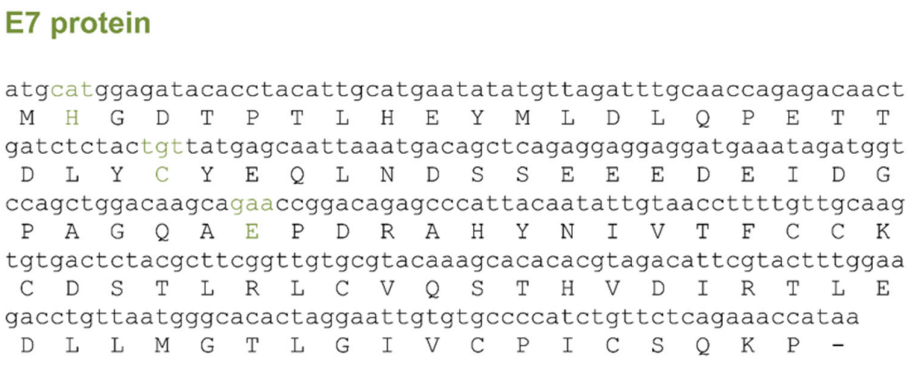
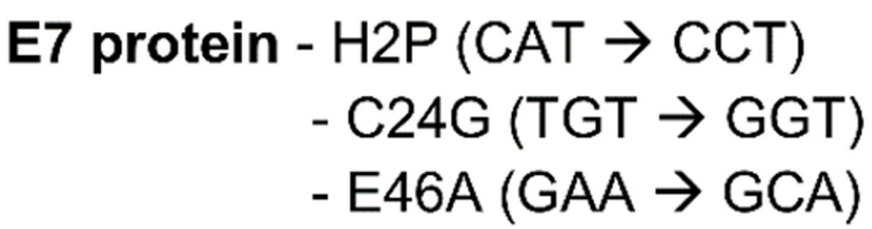


(**A**)



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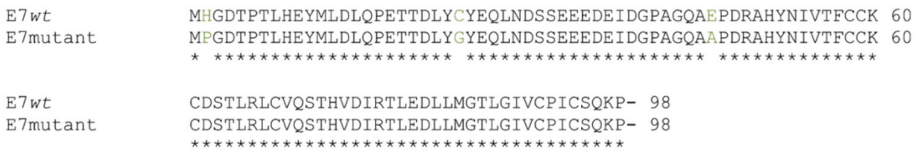
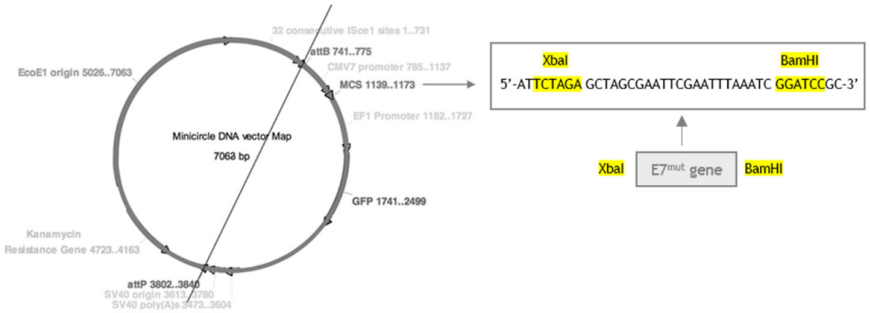


(**C**)



(**D**)

Figure S1. Schematic representation of three mutations int the HPV E7 gene and cloning of mutated gene in the parental plasmid vector. (**A**) E7 gene sequence from HPV wildtype; (**B**) Identification of three mutations to perform in the HPV E7 wildtype gene; (**C**) Sequencing of E7 mutant gene; (**D**) cloning of E7 mutant gene in the parental plasmid vector.

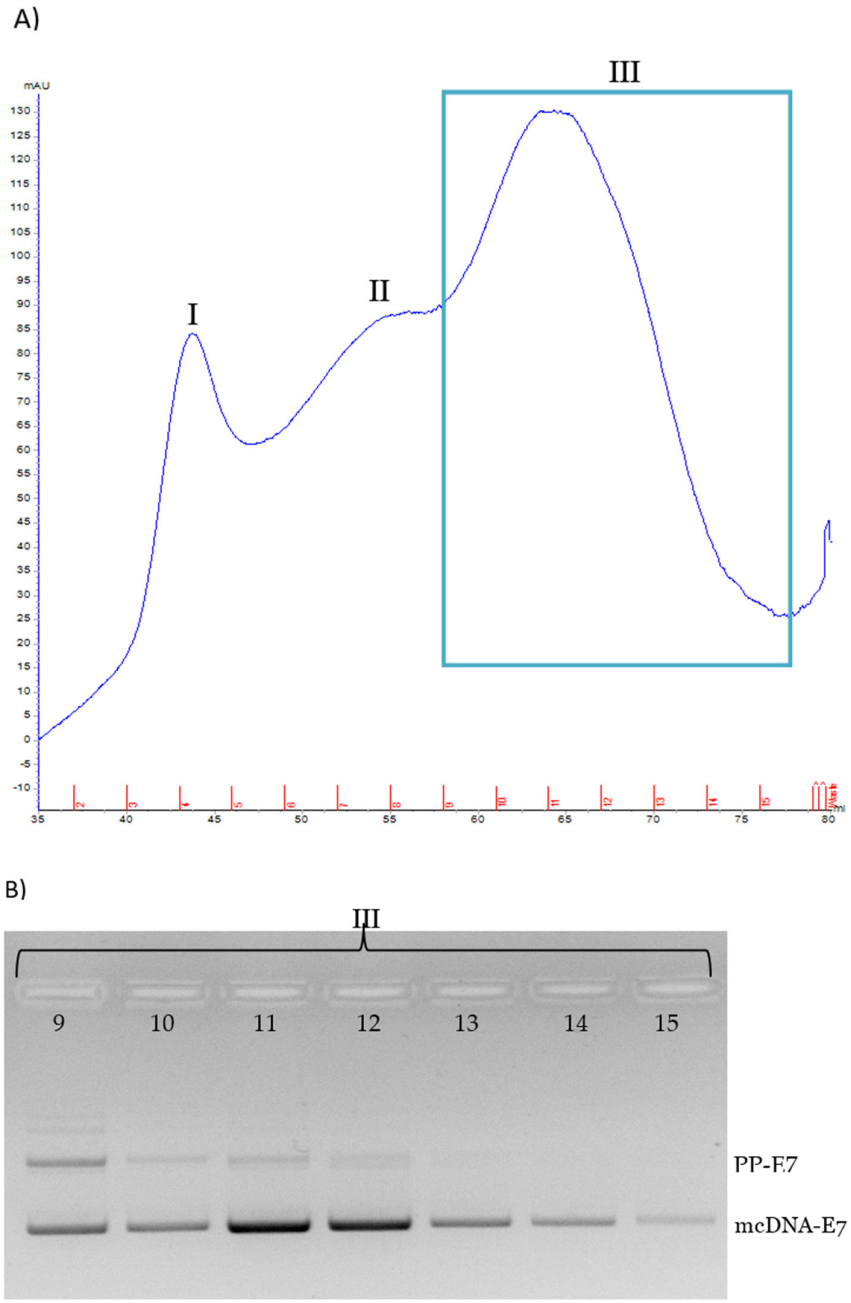


Figure S2. Chromatographic profile of mcDNA isolated by size exclusion chromatography in the Sephacryl SF-1000 column (**A**), using the following conditions, flow-rate of 0.3 mL/min, sample loading of 2 mL and fractionation of 3 mL; and agarose gel electrophoresis of fractions from peak III (**B**).

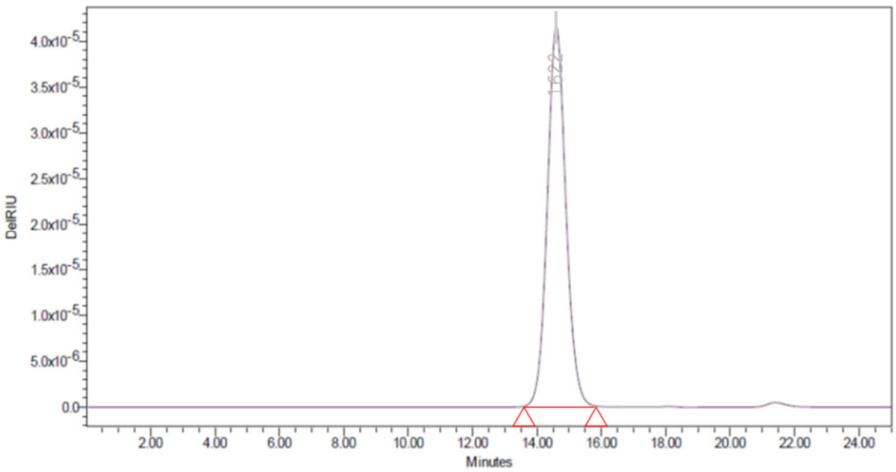


Figure S3. SEC chromatogram of MPITC-R8.

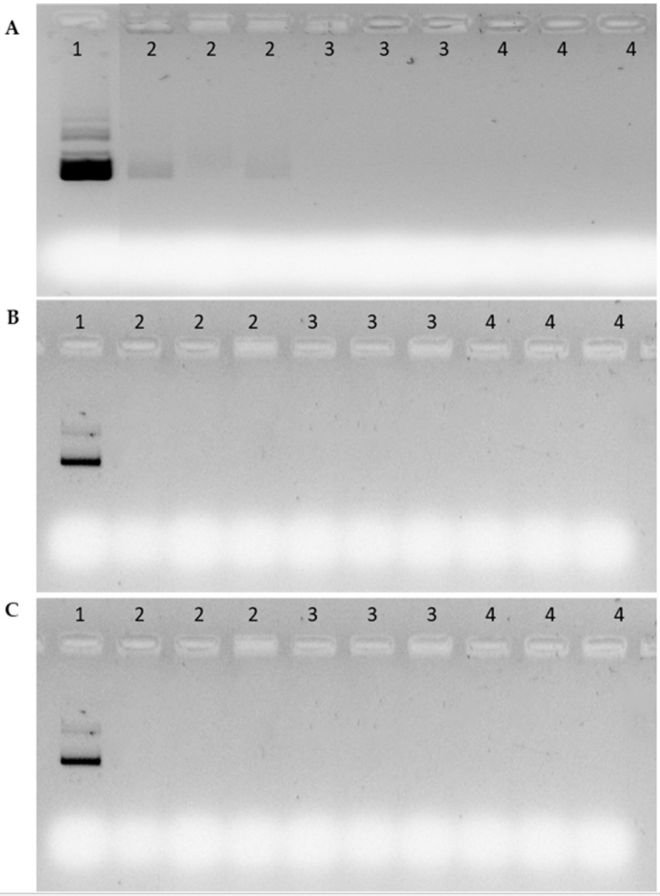


Figure S4. Electrophoretic mobility of supernatants from several formulations studied at various N/P ratios. (**A**) R8-mannose/mcDNA, (**B**) R8-mannose/PEI/mcDNA maintaining PEI N/P ratio at 5 and changing R8 N/P ratios and (**C**) R8-mannose/PEI/mcDNA maintaining PEI N/P ratio at 10 and changing R8 N/P ratios. Image A: lane 1-R8-mannose/mcDNA N/P ratio of 1:1; lane 2-R8-mannose/mcDNA N/P ratio of 1.5:1; lane 3-R8-mannose/mcDNA N/P ratio of 2:1. Image B: lane 1-R8-mannose/PEI/mcDNA N/P ratio of 1:5:1; lane 2-R8-mannose/PEI/mcDNA N/P ratio of 1.5:5:1; lane 3-R8-mannose/PEI/mcDNA N/P ratio of 2:5:1. Image C: lane 1-R8-mannose/PEI/mcDNA N/P ratio of 1:10:1: lane 2-R8-mannose/PEI/mcDNA N/P ratio of 1.5:10:1; lane 3-R8-mannose/PEI/mcDNA N/P ratio of 2:10:1.

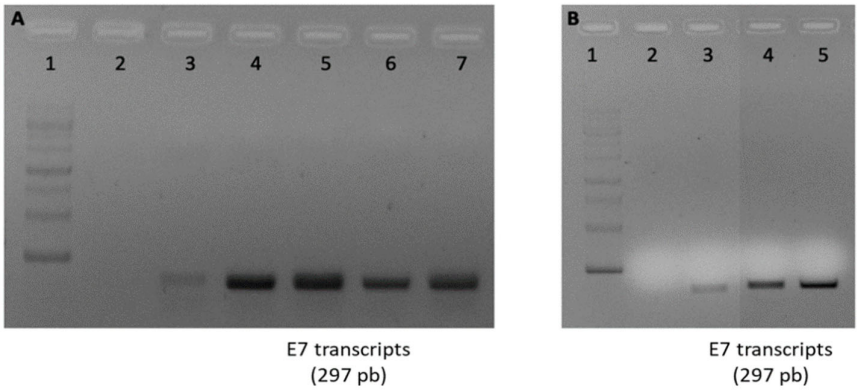


Figure S5. Analysis of RT-PCR products by agarose gel electrophoresis. Evaluation of E7 transcripts in Raw cells (**A**) and Fibro cells (**B**). Lane 1 - DNA molecular weight marker; lane 2-control without cDNA sample; lane 3-non-transfected cells; lane 4-cells transfected by PEI/mcDNA N/P ratio 5:1; lane 5-cells transfected by R8-mannose/PEI/mcDNA N/P ratio 2:5:1; lane 6-cells transfected by PEI/mcDNA N/P ratio 10:1; lane 7-cells transfected by R8-mannose/PEI/mcDNA N/P ratio 2:10:1.

Table S1. The molecular weight of each MPITC-R8 conjugate by SEC analysis.

Conjugates	Observed Relative Molecular Weight (Mw) (Da)	Theoretical Molecular Weight (Da)
MPITC-R8	1622	1643