Electronic Supplementary Material (ESI) for Biomaterials Science.

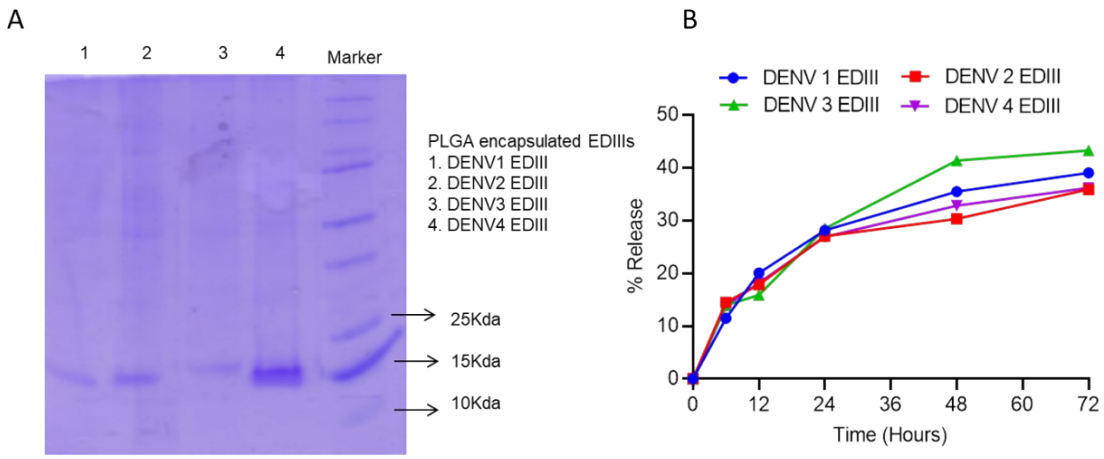

Fig S1. **SDS PAGE shows loading of EDIII in PLGA nanoparticle**. A) Loading of EDIII in PLGA nanoparticle analyzed by SDS-PAGE. Lane 1, 2, 3, and 4 represents the encapsulated EDIII antigen from DENV1, DENV2, DENV3, AND DENV4 respectively B) Antigen release profile under acid condition (pH=5) which emulates the pH of endolysosomal compartment.

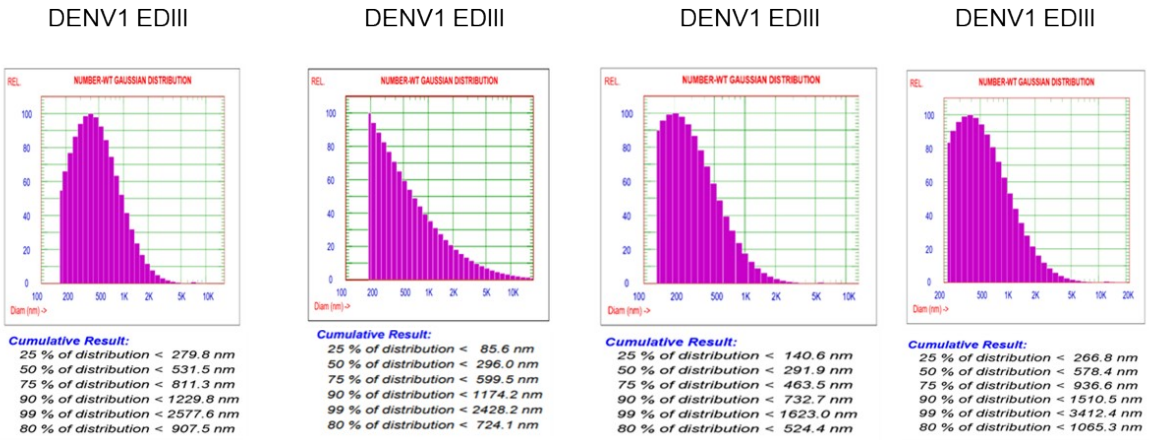

Fig S2. Dynamic Light Scattering (DLS) shows size distribution of EDIII encapsulated PLGA nanoparticles. DLS of antigen loaded nanoparticle indicate that size of PLGA nanoparticles ranges from 200nm to <~2µm

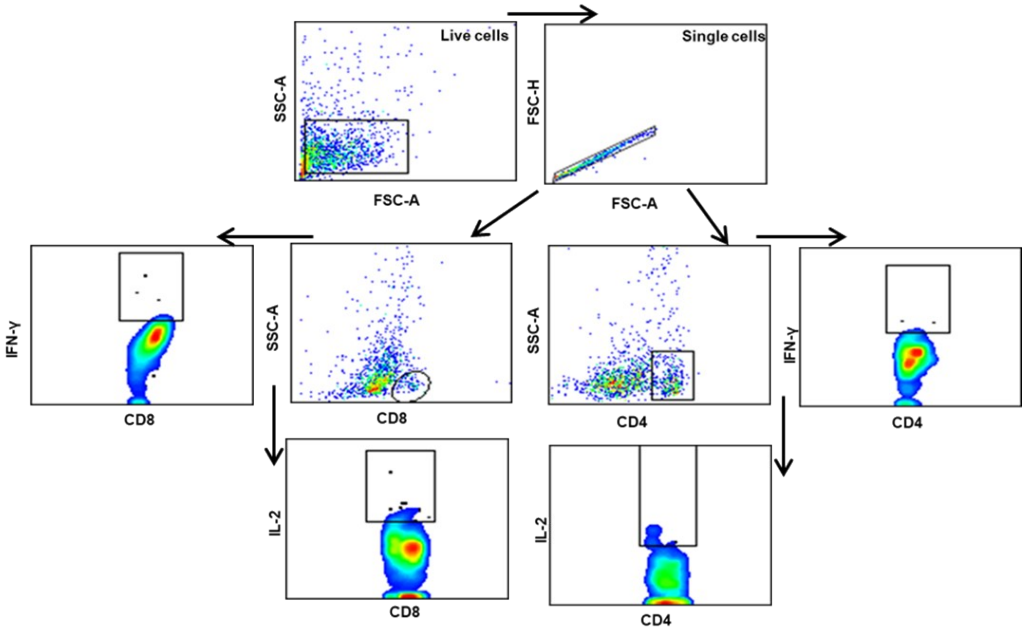

Fig S3. Gating strategy of IL-2 and IFN-γ expressing CD4+ and CD8+ T cell from peripheral blood mononuclear cells (PBMCs).

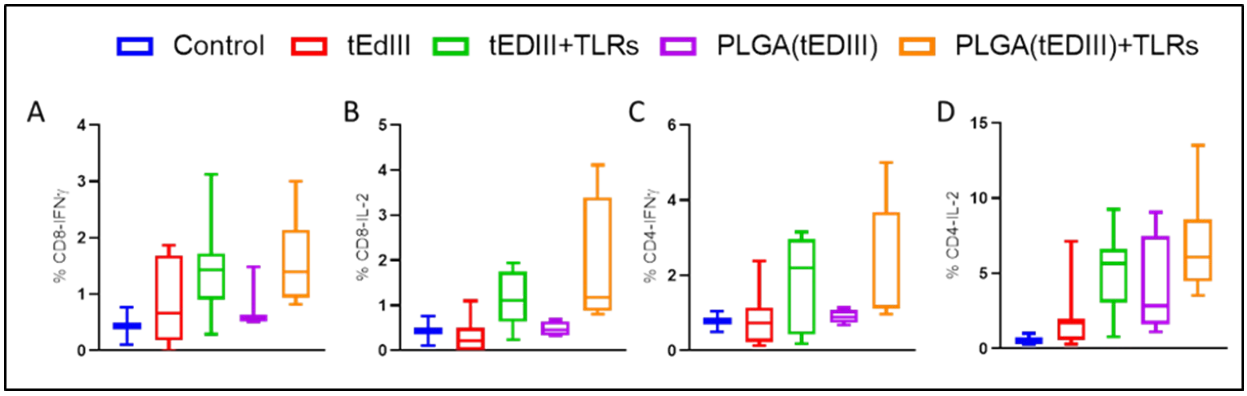

Fig S4. **PLGA nanoparticle loaded with tetravalent EDIII along with TLRs elicits potent antigen-specific T cell response in PBMCs of the mice**. Flow cytometry analysis of antigen-specific IFN-γ producing CD8+ (A), CD4+ (C) T cell and IL2 producing CD8+ (B), CD4+ (D) T cell in PBMCs. Box plot represents the average percentage of double-positive cells and represents the data from two independent experiments, each with n=5 mice per treatment group per experiment.

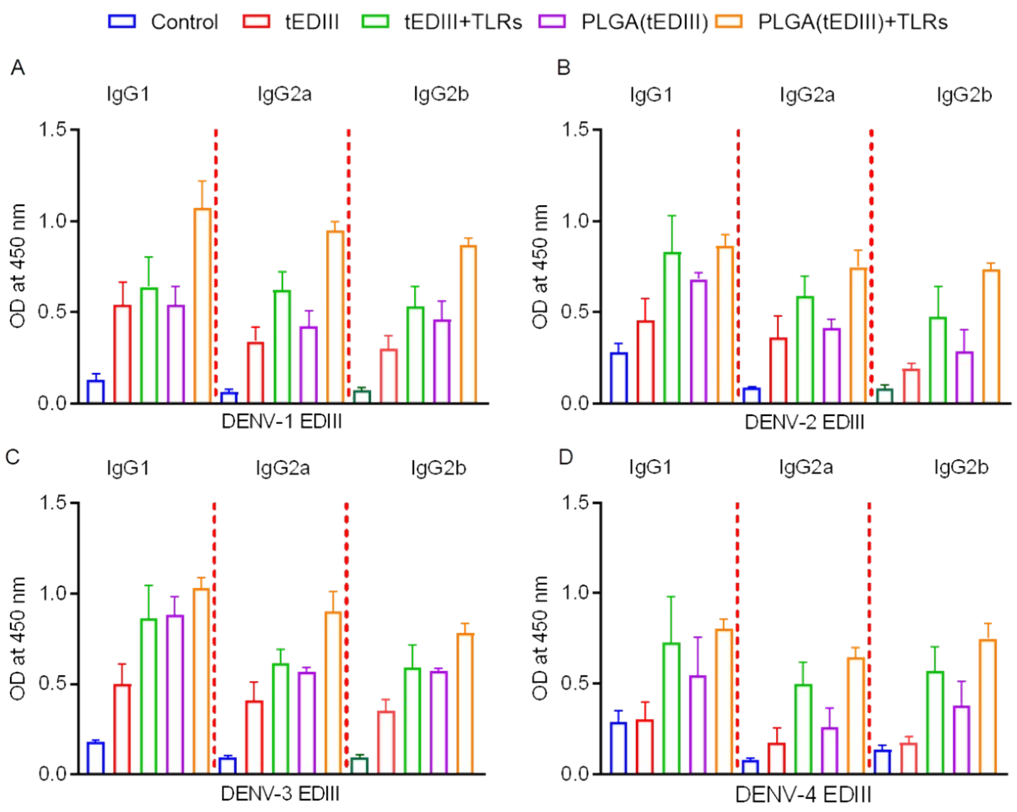

Fig S5: PLGA (tEDIII)+TLRs induces enhanced IgG subclasses as compared to other groups. Antigen specific IgG subclasses in the serum measured by Indirect ELISA for all the serotypes A) DENV 1, B) DENV 2, C) DENV 3 and D) DENV 4. Data are represented as the mean of multiple biological and experimental replicates with a standard error of mean.

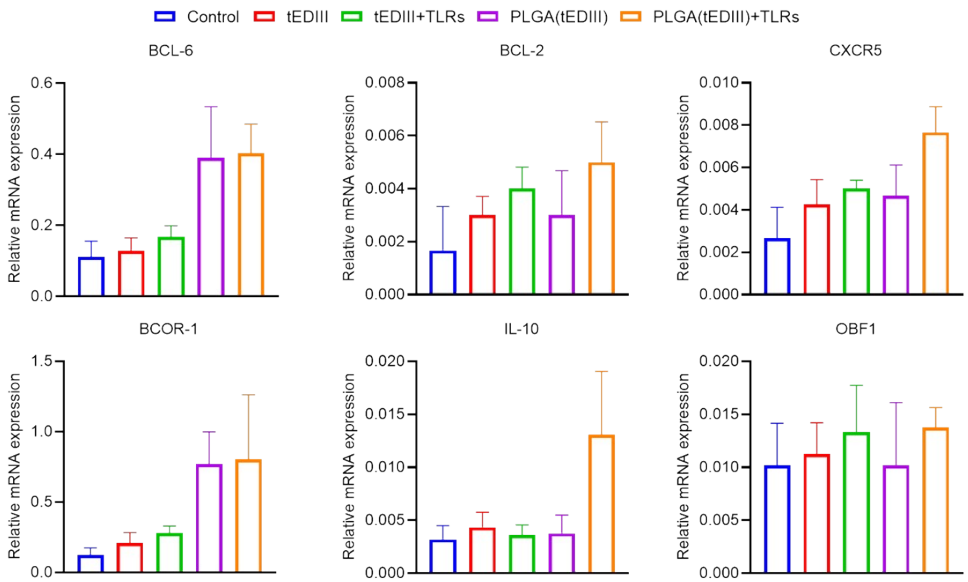

Fig S6. Nano formulation of DENV tEDIII induces upregulation of genes regulating TfH cells and GC B cell maintenance. qRT-PCR analysis of gene expression in the lymph node cells of immunized mice. The data were presented as relative mRNA expression. Data are represented as the mean of multiple biological and experimental replicates with a standard error of mean.

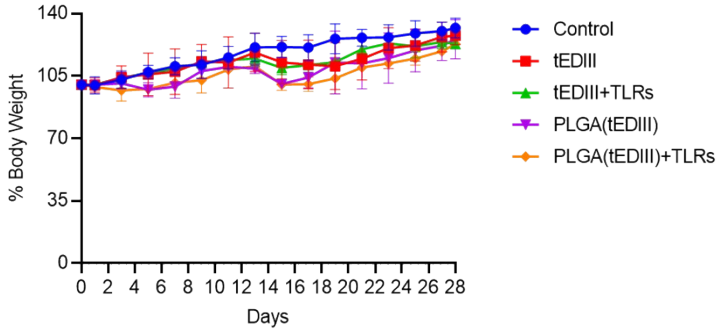

Fig. S7 Body weight of mice from different immunization groups indicates that formulation does not induce apparent toxicity.

Table S1

Formulation	%Encapsulation Efficiency (EE)
D1	**47.77**
D2	**57.55**
D3	**52.2**
D4	**44.34**

Encapsulation efficiency of EDIII antigen from all the four serotypes of DENV

Table S2: Primer List

BCl-6 FP	5' CCGGCACGCTAGTGATGTT 3'
BCl-6 RP	5'TGTCTTATGGGCTCTAAACTGCT 3'
BCL-2 FP	5’ ATGCCTTTGTGGAACTATATGGC 3’
BCL-2 RP	5’ GGTATGCACCCAGAGTGATGC 3’
BCOR1 FP	5’ ATGCCTTTGTGGAACTATATGGC 3’
BCOR1 RP	5’ GGTATGCACCCAGAGTGATGC 3’
CXCR5 FP	5’ TGGCCTTCTACAGTAACAGCA 3’
CXCR5 RP	5’ GCATGAATACCGCCTTAAAGGAC 3’
ICOS FP	5’ TCCAGCAGTTAAAAATGCGATTG 3’
ICOS RP	5’ ATCCTCCACTAAGGTTCCTTTCT 3’
IL-10 FP	5’ AGGATGCACATCAAAAGGCTT 3’
IL-10 RP	5’ GGCCTCGGTTAGGAAGGATAC 3’

ACTIN FP	5’ GTGACGTTGACATCCGTAAAGA 3’
ACTIN RP	5’ GCCGGACTCATCGTACTCC 3’

Table S3: Biochemical parameters of mice immunized with different formulations.

S.No	Name of the Test	Control	tEDIII	tEDIII+TLRs	PLGA(tEDIII)	PLGA(tEDIII) + TLRs
1	Alanine Transaminase (ALT) U/L	29 ±24	43 ±27.4	53 ±28.4	29 ±11.4	84 ±32
2	Albumin/Globulin (A/G)	1.56 ±0.89	1.6 ±1.12	1.8 ±1.04	1.16 ±0.28	1.15 ±0.10
3	**Serum Electrolytes**					
a)	Sodium (mmol/L)	154.33 ±17.38	136 ±5.29	141.33 ±29.16	142.3 3±6.23	146.66 ±19.59
b)	Potassium(mmol/L)	18.4 ±1.21	20.8 ±2.45	22.7 ±13.46	18.6 ±5.30	23.8 ±12.18
c)	Chloride(mmol/L)	107.33 ±13.57	96.33 ±3.78	99.66 ±21	99 ±3.74	94 ±1.41

Biochemical parameters of mice in immunization groups are not showing any significant variation from control group except ALT however it comes under normal range[1] which indicates that vaccine does not induce any visible toxicity after 28th days of immunization.

[1] Silva-Santana, Giorgio, et al. "Clinical hematological and biochemical parameters in Swiss, BALB/c, C57BL/6 and B6D2F1 Mus musculus." *Animal Models and Experimental Medicine* 3.4 (2020): 304-315.