

SUPPORTING INFORMATION

1.1 Characterisation of NAs and NACDPs through Zeta analyser, EDAX, SDS-Page

The synthesized NA and NACDPs were analysed for zeta potential, EDAX and SDS page to determine the surface charge, zeta potential and protein band profile. The results of zeta analysis revealed the presence of negative charge in NA and NACDPs (Fig. S1a). The EDAX characterization exhibited the presence of elements like N and O elements in NA, whereas the NACDPs exhibited C, K, Na, Mg, O, S K, P K and Cl predominantly (Fig. S1 b). Moreover the, the SDS page revealed the presence and integrity of cellular proteins with various molecular weights ranging from 11 to 240 kDa (Fig. S1 c).

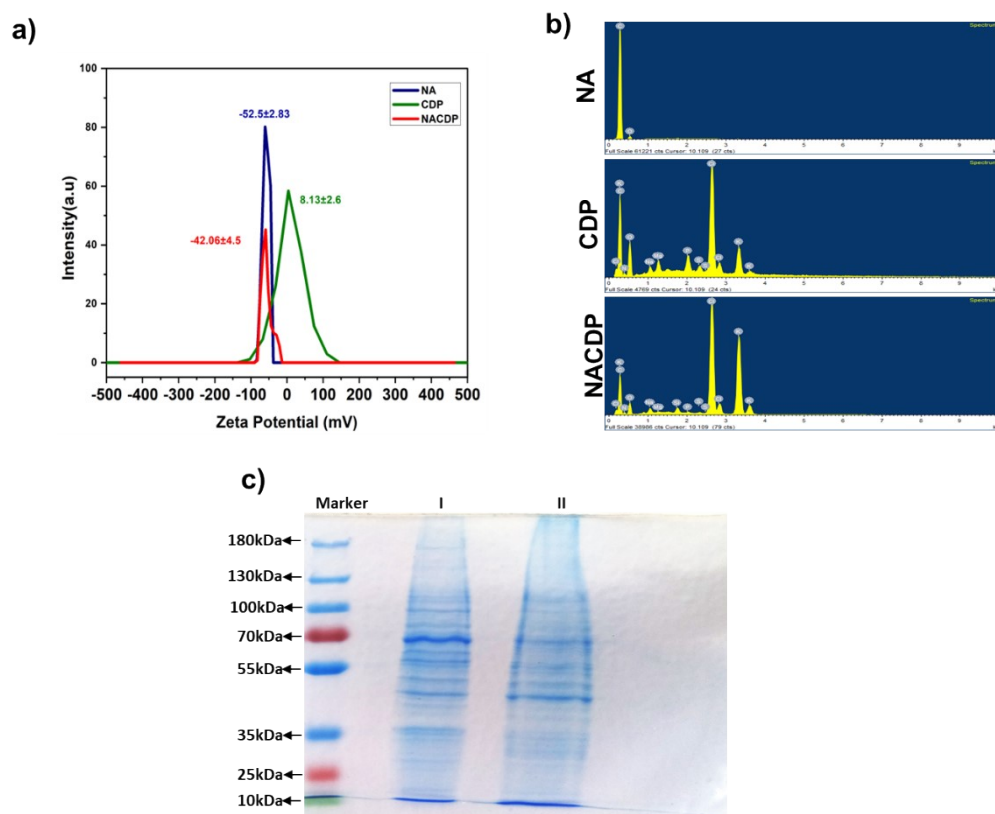
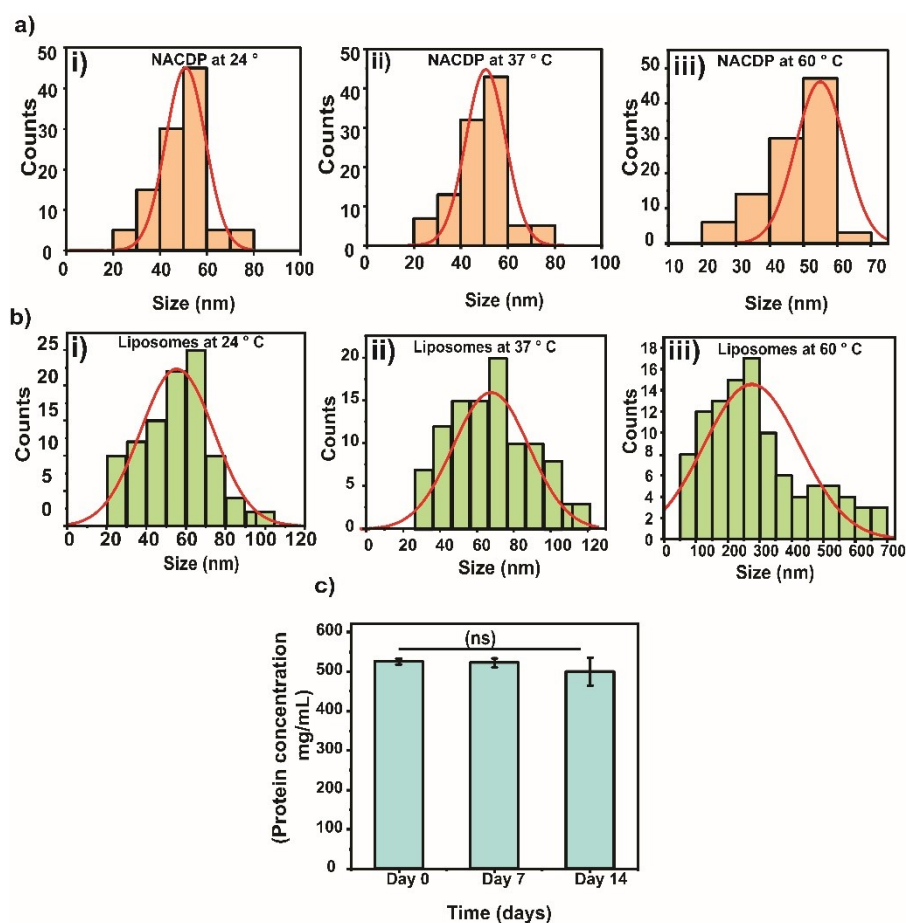


Figure S1: (a) Zeta potential analysis of NA, CDP and NACDP and (b) EDAX analysis of NA, CDP and NACDPs and (c) Qualitative analysis of protein through SDS-PAGE (Lane I: Total cell-derived protein; Lane II: Protein loaded nanoarcheosomes)

1.2 Stability analysis of NACDP through DLS and protein estimation

Stability analyses for the size determination of NACDP and liposomes loaded with CDP were performed through DLS analysis at various temperatures. The NACDP samples at 24 °C, 37 °C, and 60 °C revealed a particle size of 51 ± 1.6 nm, 51 ± 1.5 nm, and 55 ± 0.3 nm (Fig. S2 a). Whereas, the size of conventional liposomes loaded with CDP exhibited 55.5 ± 2.3 , 71.4 ± 3.5 , and 274 ± 3.7 (Fig. S2 b). We observed that liposomes loaded with CDP exhibited colloidal instability with peak broadening, and the average size of the particles increased with an increase in temperature. Subsequently, to study whether NA can prevent CDP denaturation, stability analysis was carried out at room temperature on various days (0, 7, and 14 days). The protein concentration of NACDP at 0th, 7th and 14th day was found to be 525 ± 7.5 , 522 ± 11.9 , and

500 ± 35 mg/mL, respectively. The results revealed that there was no significant difference in



the protein concentration (Fig. S2 c)

Element	App con	Intensity	Weight%	Weight%	Atomic weight %
C K	2006.14	1.8270	88.33	0.19	90.98
O K	40.99	0.2827	11.67	0.19	9.02
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O K	40.99	0.2827	11.67	0.19	9.02
Total	100.00				

Table S1: List of elements present in synthesised nanoarchaeosomes (NA)

Figure S2: (a) DLS analysis of NACDPs (i) at 24 °C (ii) at 37 °C (iii) at 60 °C; (b) DLS analysis of liposomal loaded proteins and (i) at 24 °C (ii) at 37 °C (iii) at 60 °C; (c) Quantitative analysis of NACDPs through Bradford assay. The values indicate the mean ± S.D. (ns represents no significant difference between NACDP samples).

Element	App con	Intensity	Weight%	Weight%	Atomic weight %
C K	442.46	0.3663	49.00	0.33	64.01
N K	13.61	0.0605	9.12	0.37	10.22
O K	93.50	0.2723	13.93	0.17	13.66
Na K	14.71	0.8320	0.72	0.02	0.49
Mg K	1.57	0.7726	0.08	0.01	0.05
Si K	12.05	0.9640	0.51	0.01	0.28
P K	3.40	1.3906	0.10	0.01	0.05
S K	2.16	1.0280	0.09	0.01	0.04
Cl K	298.97	0.8750	13.86	0.10	6.14
K K	310.94	1.0015	12.60	0.09	5.05
Total	100				

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Table S2: List of elements present in the cell-derived proteins

Table S3: List of elements present in the nanoarchaeosomes bounded with cell-derived proteins

1.3 The quantification of total leucocyte counts from peripheral mononuclear blood

Among total lymphocytes gated in flow cytometry analysis ($67\% \pm 13.15$), the subsets were identified with individual cell surface markers. The results revealed that the monocyte cells positive for the high CD14 marker expression was $11.6\% \pm 2.26\%$, and the cells positive for the CD3 marker (lymphocytes) and CD20 marker (B cells) were found to be $47.09 \pm 3.2\%$ and $3.13 \pm 0.17\%$ respectively and there was a significant increase in the internalization of NACDP than NA by the lymphocyte subsets.

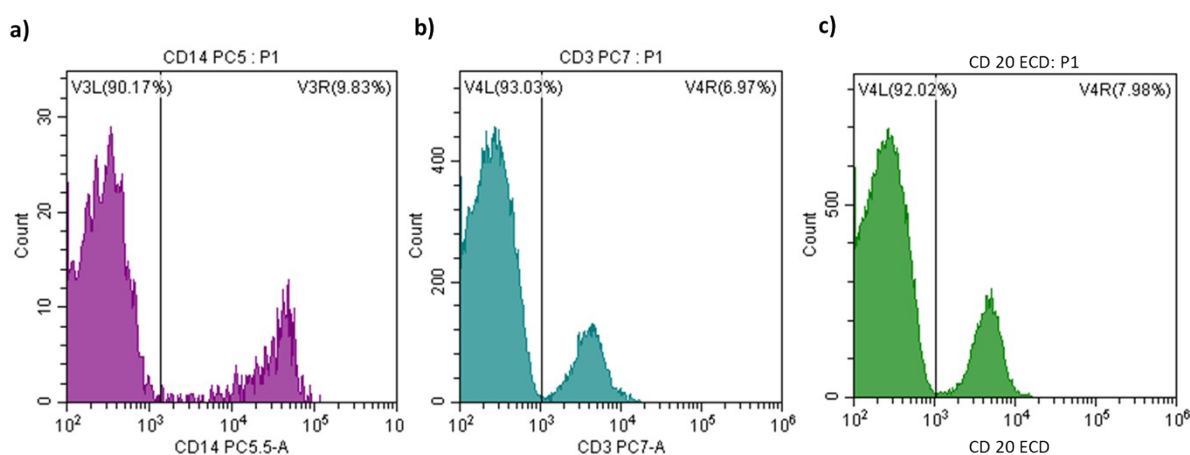


Figure S3: FACS analysis of leucocyte subpopulation in total PBMC. (a) Monocytes; (b) B cells and (c) T cells

1.4 The quantification of proliferation capacity and IFN- γ production of T cells primed with APCs

The proliferation capacity was observed in effector T cells through FACS analysis. Initially, the cell division among PBMCs and into specific T cells analysed using CFSE dye indicated that an active division of cells occurred to be around 55.88 % among the total cell population (Fig. S4 a). The divided populations were further gated for identifying the percentage of CD4(helper T cell) and CD8 (cytotoxic T cell) proliferation. The Fig. S4 b contour plot shows distinct population of divided CD4 and CD8 populations.

The level of IFN- γ production in T cells estimated at the end of 5 days of co-culture with APC resulted in a significant production of IFN- γ in cells co-treated with NACDP when compared to that of cells primed with NA alone. Supp Fig. 4 c & d shows plot for IFN- γ production by CD4 and CD8 cells. in the quadrant gating, the region of double positivity for IFN FITC and cell surface marker (UR-upper right) were used to acquire the percentage positivity.

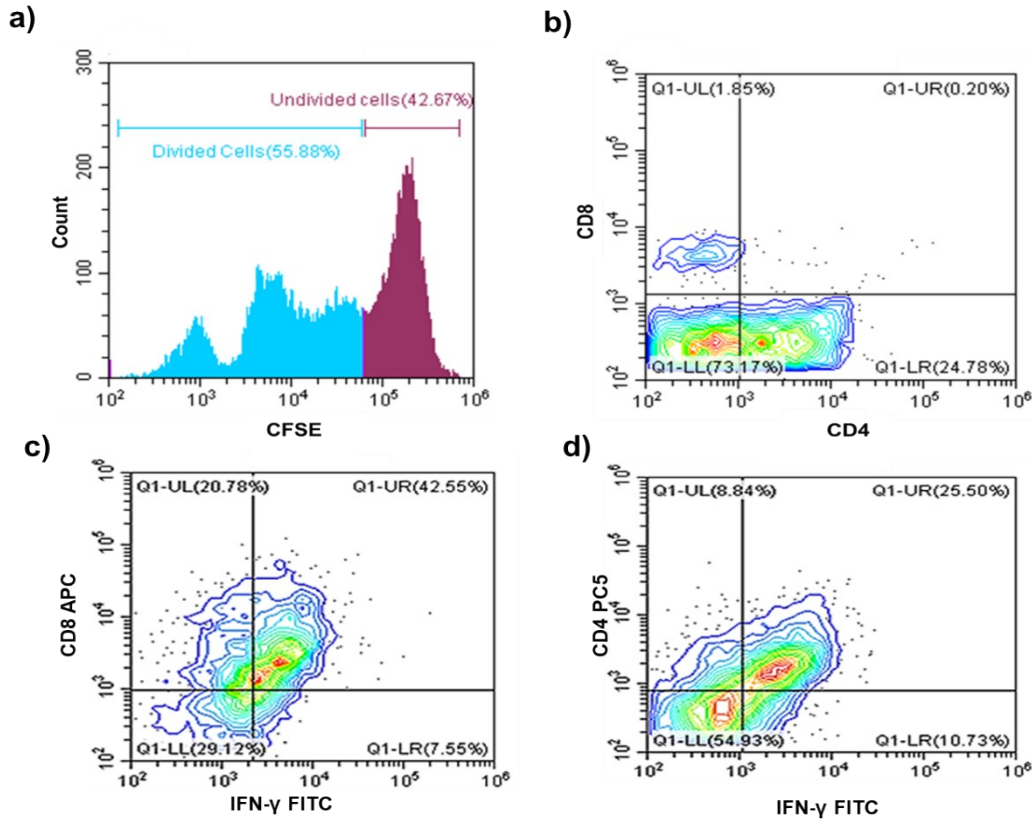


Figure S4: (a) histogram showing the divided and undivided population of cells based on the CFSE dye intensity. (b) contour plot with a quadrant gate showing the distinct population of divided CD4(Q1 LR-Lower right) and CD8 population (Q1 UL-Upper Left). (c) Contour plot with a quadrant gate showing the percentage of double positivity (Q1-UR-Upper Right) for CD8 and IFN γ . (d) Contour plot with a quadrant gate showing the percentage of double positivity (Q1-UR-Upper Right) for CD4 and IFN- γ .

1.5 Evaluation of cytotoxic effect of T cells against SiHa tumor cells

The tumor killing potency of effector T cells primed with APCs were analysed through FACS analysis. The effector T cells generated by NACDP-primed dendritic cells demonstrated a robust cytotoxic effect achieving a higher percentage of 87 ± 1.5 % killing rate at a minimum ratio of 1:20. In comparison, effector T cells stimulated by NACDP-primed macrophages demonstrated a significant, albeit slightly lower, cytotoxic effect of 67.49 ± 3.2 % at the same ratio, as illustrated in Fig. S5.

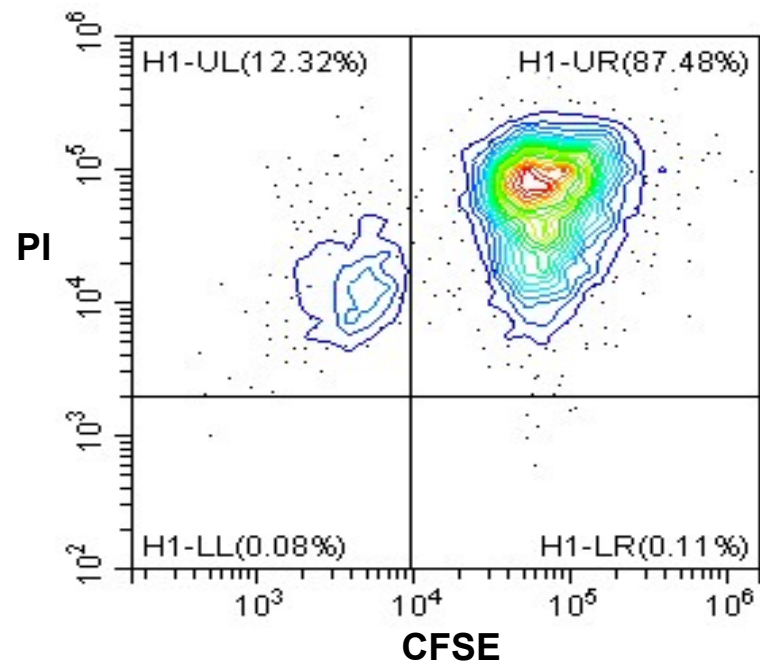


Figure S5: FACS analysis of cytotoxicity of effector T cells against SiHa cells using CFSE and propidium iodide dye. The effector T cells: Tumor cells coculture were analysed for viability by gating CFSE stained tumor cells versus PI stain. The percentage of tumour cell death was derived from double-positive cells (Both CFSE & PI) (H1 UR- Upper Right population of quadrant gate)