mRNA polyplexes with post-conjugated GALA peptides efficiently target, transfect and activate antigen presenting cells

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Supporting Information

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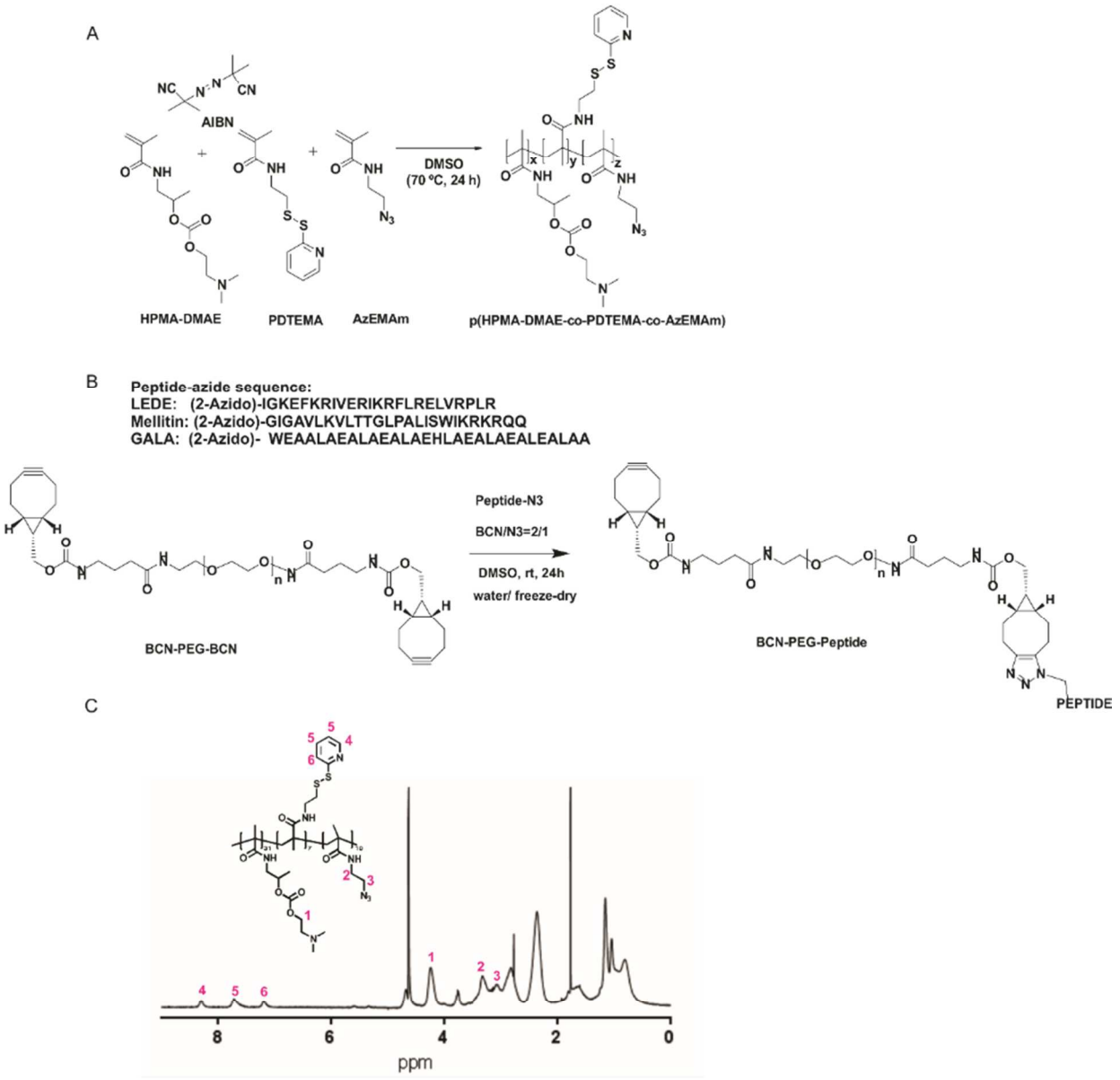

Fig. S1. Synthesis of random copolymers (A) p(HPMA-DMAE-co-PDTEMA-co-AzEMAm) (abbreviated as pHDPA), (B) BCN-PEG-peptide and (C) ^{1}H NMR spectrum of pHDPA in D_2O.

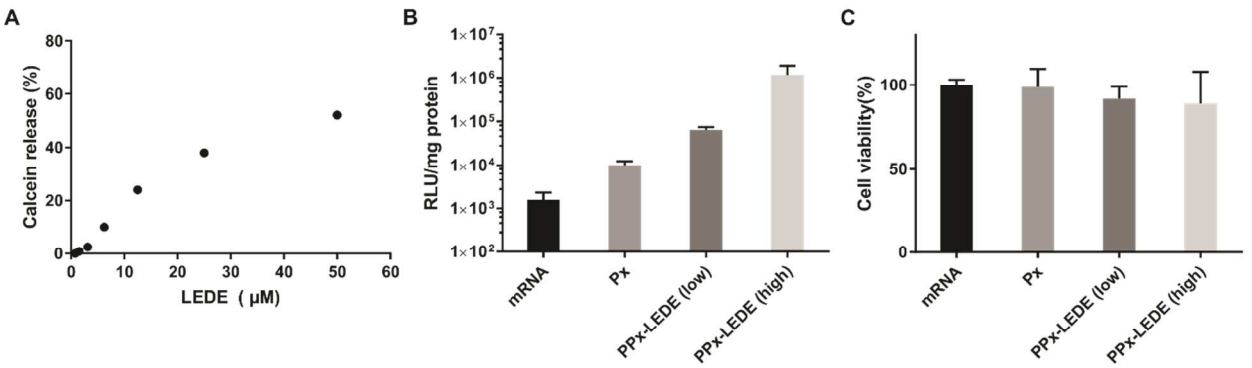

Fig. S2. (A) Membrane activity of LEDE peptide in the calcein leakage assay. Calcein release from target liposomes with self-quenched concentrations of entrapped calcein, was measured after 1 h incubation at pH 7.4. Results are plotted relative to 100% leakage, induced by addition of 0.5% Triton X-100 to the calcein liposomes (EPC:Chol in 2:1 ratio) [81]. Transfection efficiency (B) and cytotoxicity of LEDE modified luc_mRNA polyplexes on NIH3T3 cells in the absence of serum after 24 h with mRNA dose of 250 ng /well in a 96 well plate. Data are the mean ± SD, n=3.

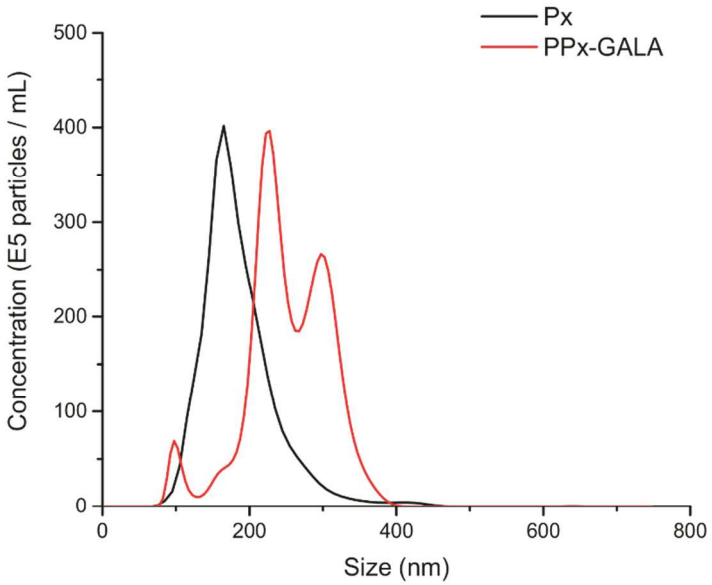

Fig. S3. Size distribution of Px (black) and PPx-GALA (red) mRNA polyplexes after lyophilization and rehydration measured in PBS buffer by Nanosight with a final mRNA concentration of 0.5 μg/mL.

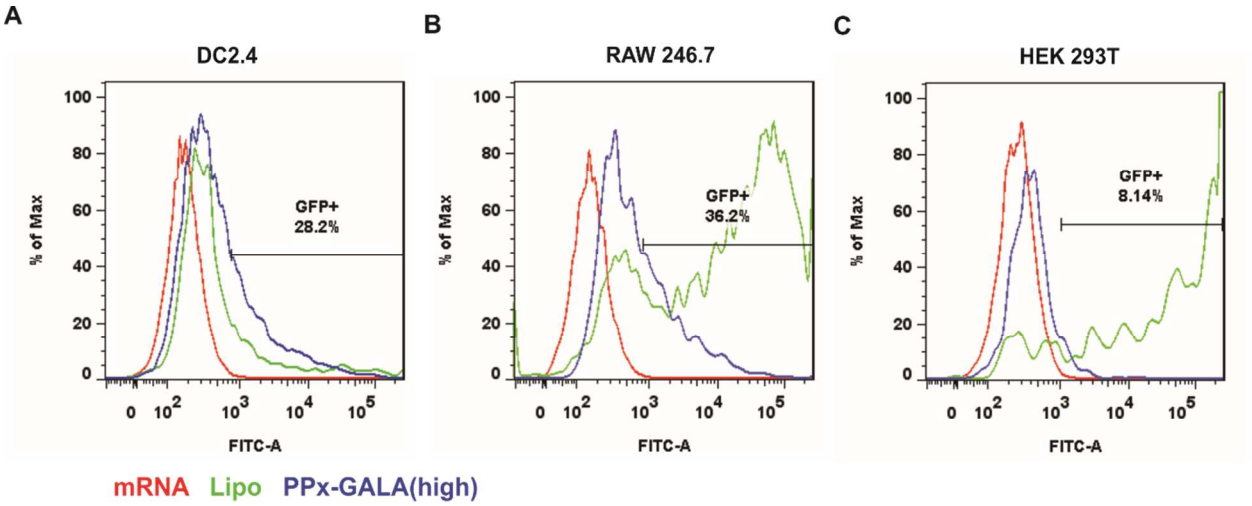

Fig. S4. Representative flow cytometry histograms of EGFP expression in DC2.4 (A), RAW246.7 (B) and HEK 293T(C) cells 24 h after incubation with free EGFP mRNA (red), Lipoplexes (Lipo, green) and PPx-GALA (blue), with a dose of 250 ng/well.

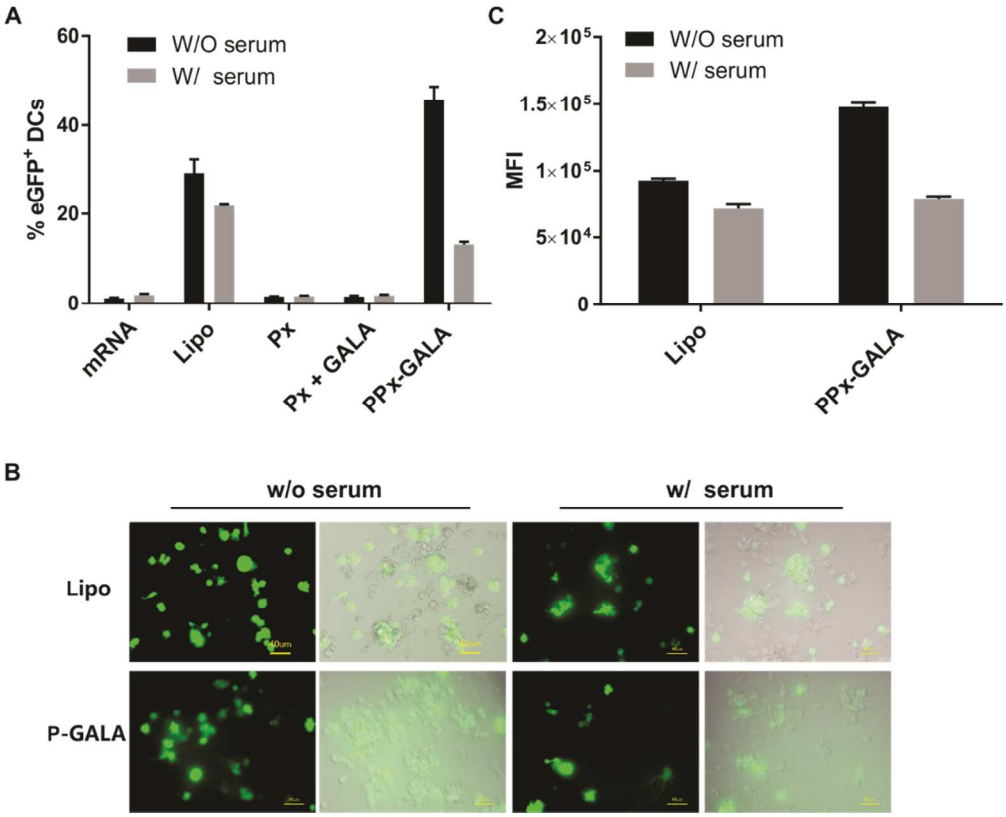

Fig. S5. The influence of serum on the transfection of D1 cells. (A) EGFP expression in D1 cells 24 h after addition of naked mRNA, lipofectamine-complexed mRNA or indicated Px and PPx formulations in the absence (W/O) or presence (W/) of serum as measured by flow cytometry. (B) Epifluorescence microscopy images showing EGFP fluorescence alone and a bright-field overlay of D1 cells. (C) D1 cellular uptake of Cy5-mRNA after 24 h when cells were transfected with (W/) or without (W/O) serum during the first 4 h of incubation. mRNA was added with a dose of 250 ng/well. Data are the mean ± SD, n=3. Size bar represents 40 μm.

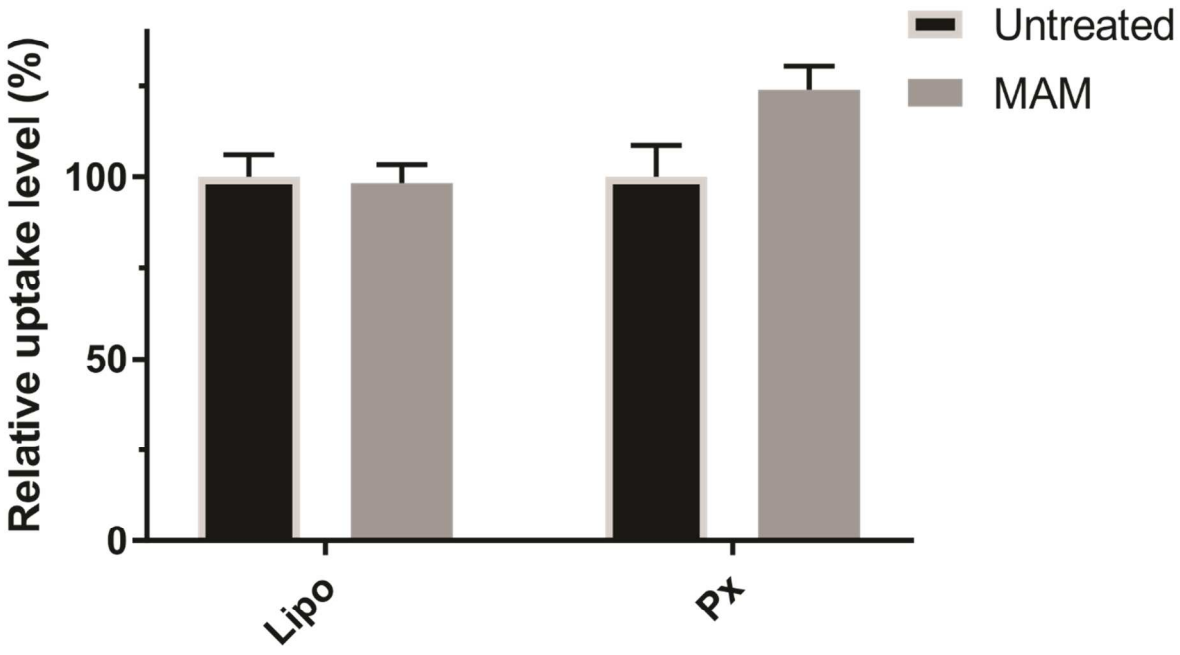

Fig. S6. D1 cellular uptake of Lipoplexes or Px (Cy5-luc_mRNA) after 1 h when cells were preincubated with Maackia amurensis agglutinin (MAM). Data are the mean ± SD, n=3.

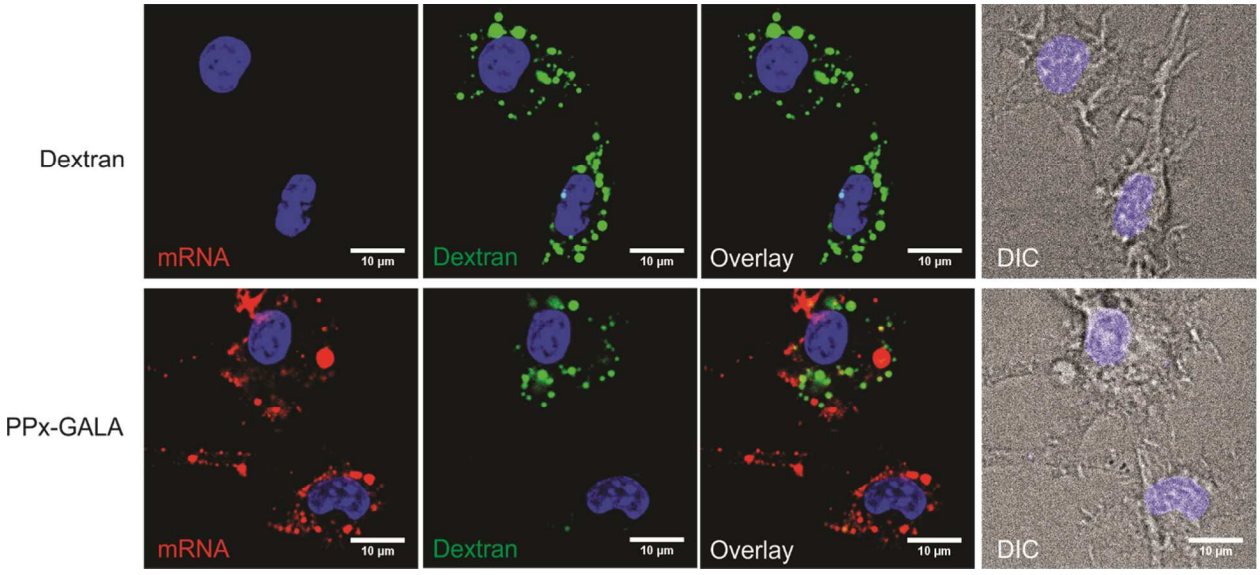

Fig. S7. Localization of Cy5-luc mRNA in D1 cells after co-transfection of PPx-GALA with dextran-FITC (70 kDa, 150 µg/mL) for 3 h.

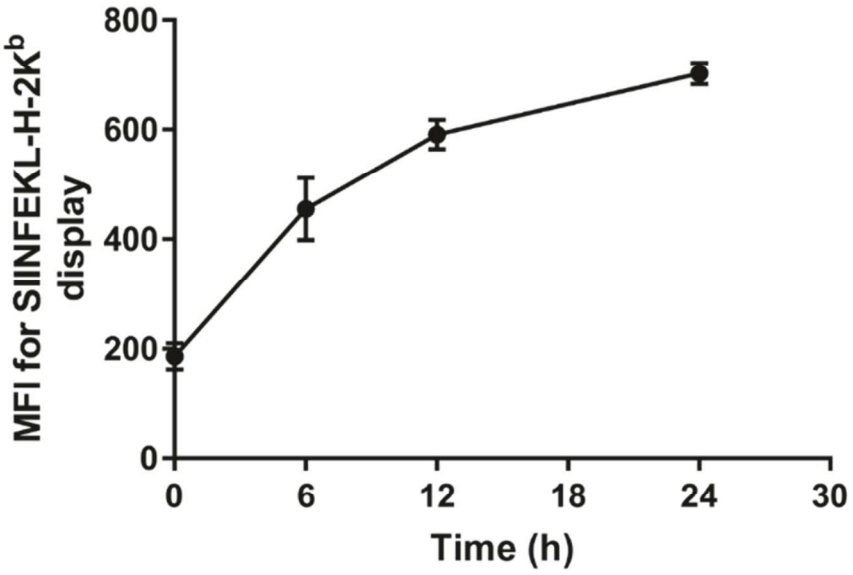

Fig. S8. D1 cells were incubated with PPx-GALA mRNA polyplexes encoding ovalbumin for the indicated times and antigen presentation was quantified by flow cytometry analysis of DCs stained with 25-D1.16 mAb that recognizes SIINFEKL-H-2K^b complexes. Data are the mean ± SD, n=3.

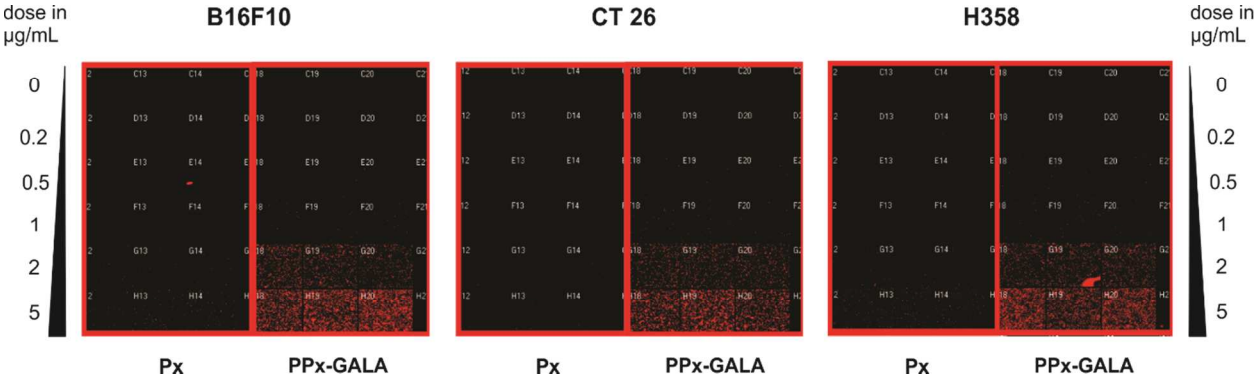

Fig. S9. Cellular uptake image of Px and PPx-GALA (high) formulation in the presence of serum. Tumor cells were plated in 384 well plates and cultured in an incubator. Cells were seeded to result in confluency at the end of the experiment (cell types tested = B16F10 – 4,000/well, CT26 4,000/well, H358 15,000/well. 50 µ media per well). The formulations were added directly to the cells (no media change) in triplicate at indicated concentration at the following day. After 24 h, the cells were fixed (4% PFA) and imaged on the ImageXpress.