Supplementary Materials

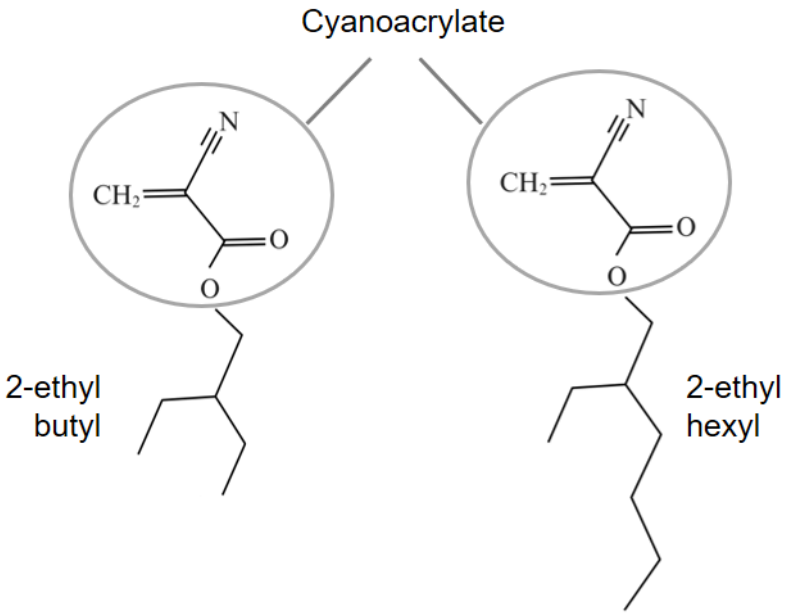

Figure S1: PEBCA and PEHCA precursors (EBCA and EHCA) with their alkyl side chains.

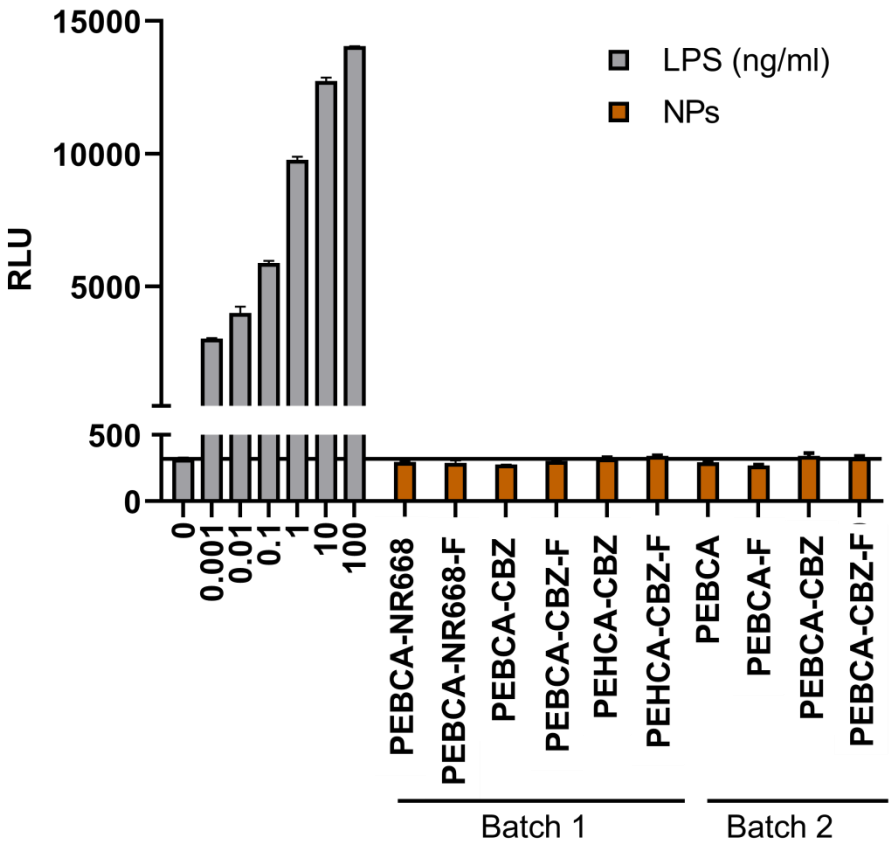

Figure S2: Activation of NF-κB by increasing LPS concentrations**.** NF-κB activation in HEK293 cells transfected with an NF-κB luciferase reporter plasmid and plasmids encoding LPS-receptor components (TLR4, CD14, MD-2) is shown. Cells were exposed to LPS (0–100 ng/mL) or different NP formulations (1 µg/mL). Luciferase activity was measured 20 h after induction. Results show mean and SD from one out of two independent experiments, carried out in duplicates. Batch 1 represents NP batches shown in Figure 1B. Batch 2 represents NP batches used in Figure 7A, and they have similar size and zetapotential as the corresponding NPs included in Figure 1B .

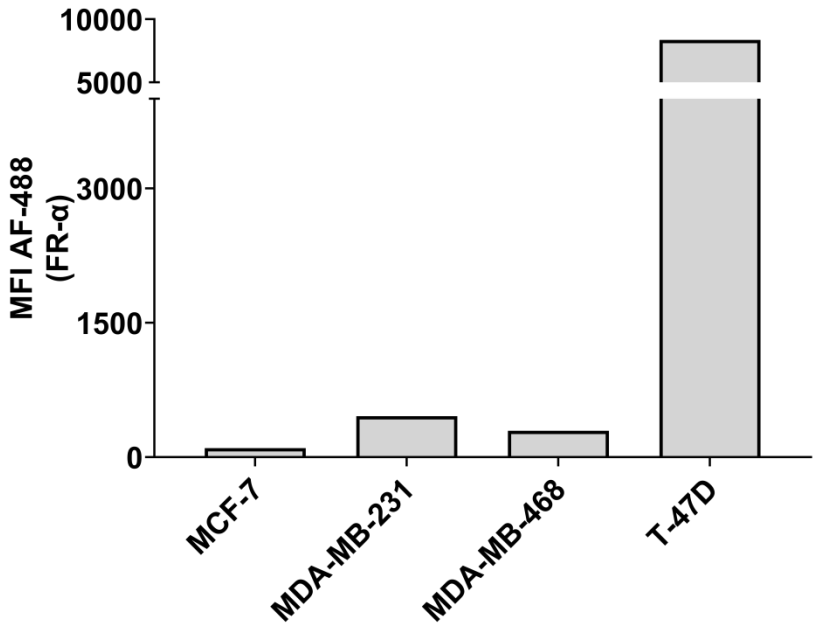

Figure S3: Surface expression of FolR in different breast cancer cell lines, assessed by flow cytometry. Cells were stained with primary antibody against human FOLR1 (1:250), followed by staining with AF488 conjugated secondary antibody (1:500). Cells were then washed twice with PBS with 0.1% BSA and analyzed by flow cytometry (excited by 488 nm blue laser using BD LSRII flow cytometer). Values represent average of duplicate samples, which are so similar that deviations cannot be shown.

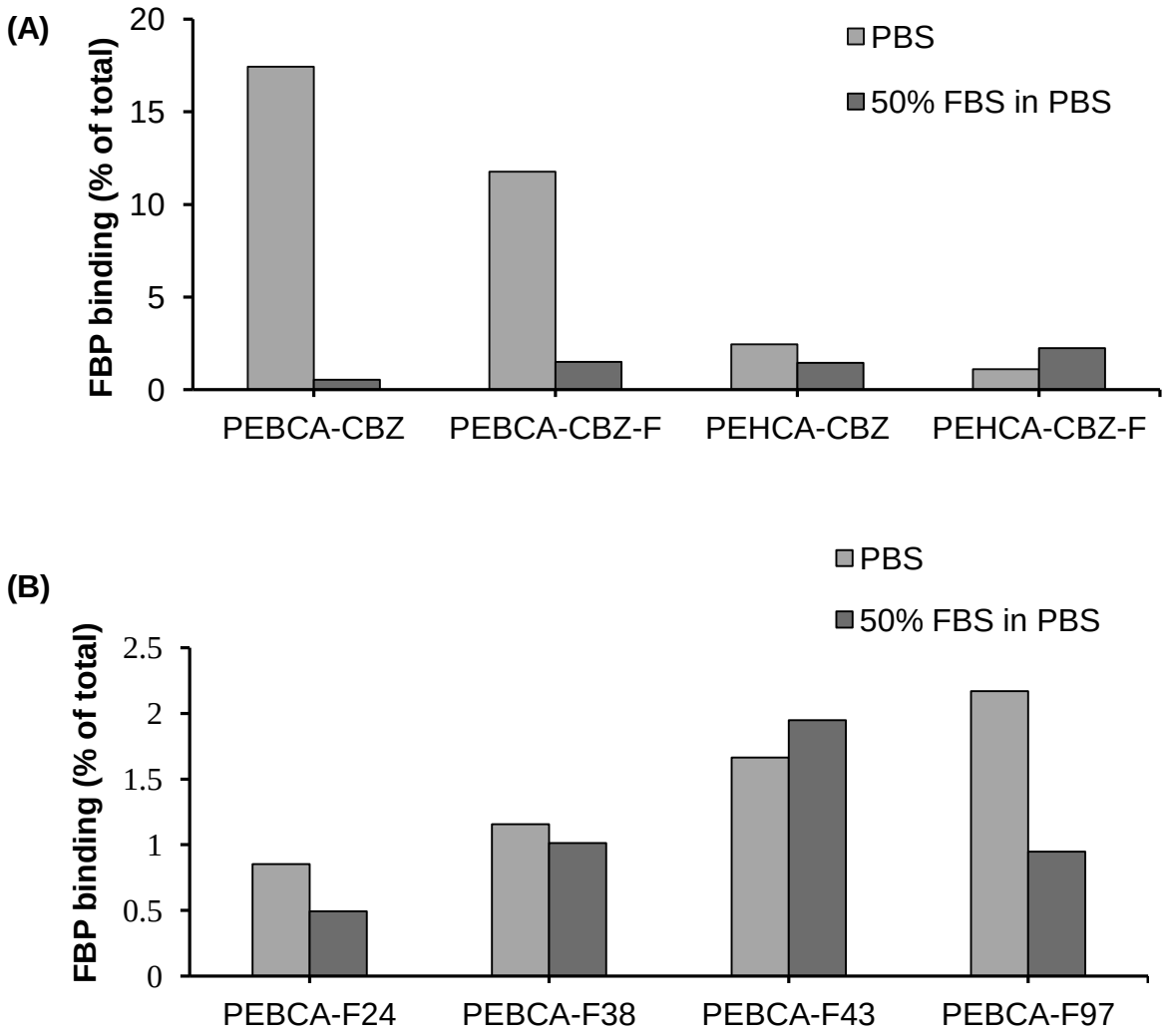

Figure S4: Binding studies of folate conjugated NP variants to radioactive folate binding protein. ^{125}I-labeled bovine FBP (30 nM) was incubated with folate conjugated or unconjugated variants of **(A)** CBZ-loaded PEBCA/PEHCA NPs and **(B)** NR668-loaded PEBCA NPs as mentioned in Figure 5B. All incubations include duplicate samples of NPs (1 mg/mL, 200 µL) and were performed in PBS or in 50% FBS in PBS at 37 °C, for 2 h. PACA NPs were pelleted by high speed centrifugation (15,000 rpm, 30 min), washed two times with PBS and then the radioactivity of [^{125}I]-FBP in NP fraction and supernatant was measured using a gamma counter. NPs used in (A) have similar properties as shown in Figure 1B. NPs used in (B) are the same as shown in Figure 5B. Values represent average of duplicate samples, which are so similar that deviations cannot be shown.

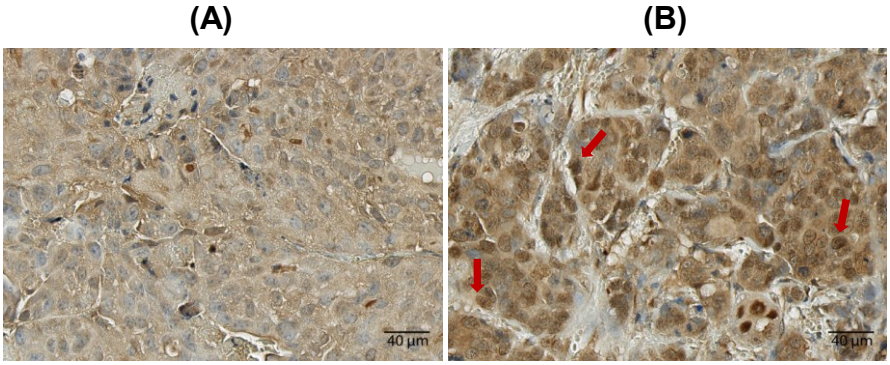

Figure S5: Immunostaining showing FolR expression in PDX tumors. Tissue sections were processed and stained with the primary FOLR1 antibody, which was then detected using DAB chromagen (brown). Counterstaining was performed using hematoxylin before imaging the slides using Olympus VS200 Slide Scanner (20X objective). The two panels represent **(A)** MAS98.12 and **(B)** HBCx39. The red arrows in (B) point to areas with a large amount of FolR expression.

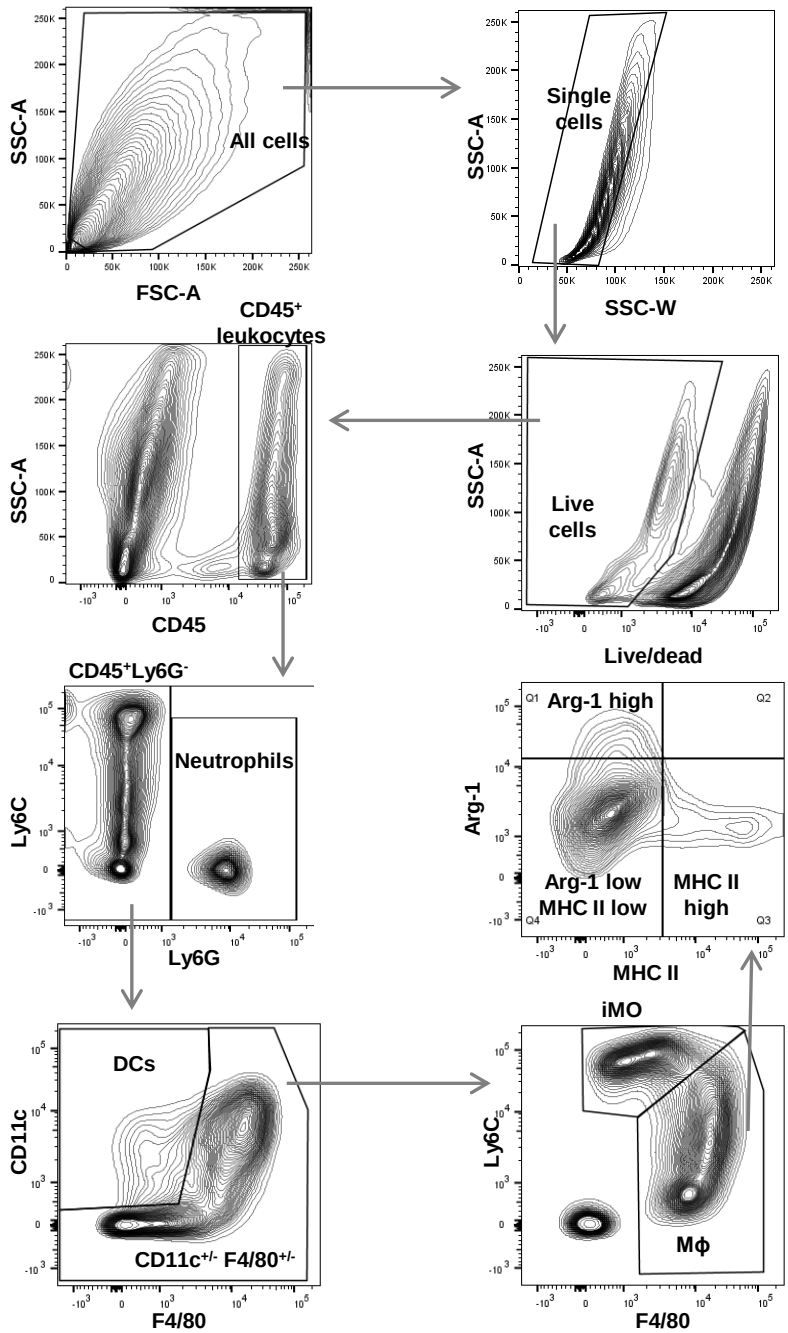

Figure S6: Flow cytometric analysis of tumor samples. Cell populations represented by contour plots and gating strategy used to identify different immune cell subsets using the markers included in the panel. Cell populations are described either by cell type (includes leukocytes, neutrophils, DCs, iMO, Mϕ) or marker status.

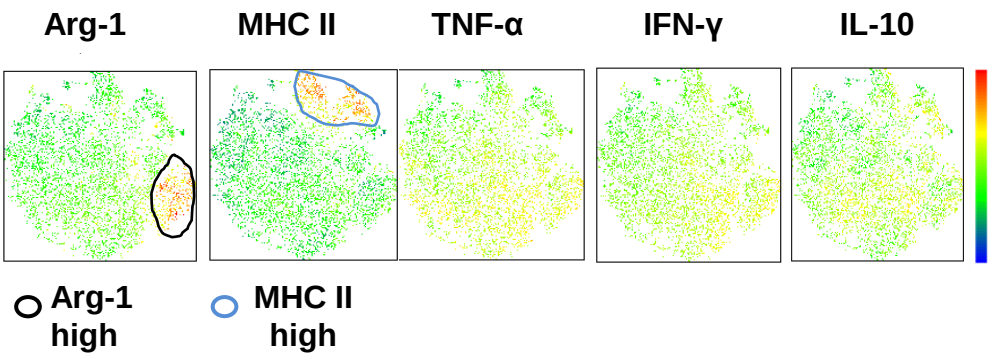

Figure S7: t-sne (t-distributed stochastic neighbor embedding) plots of whole macrophage population showing different macrophage subsets and expression of intracellular cytokines in untreated sample.

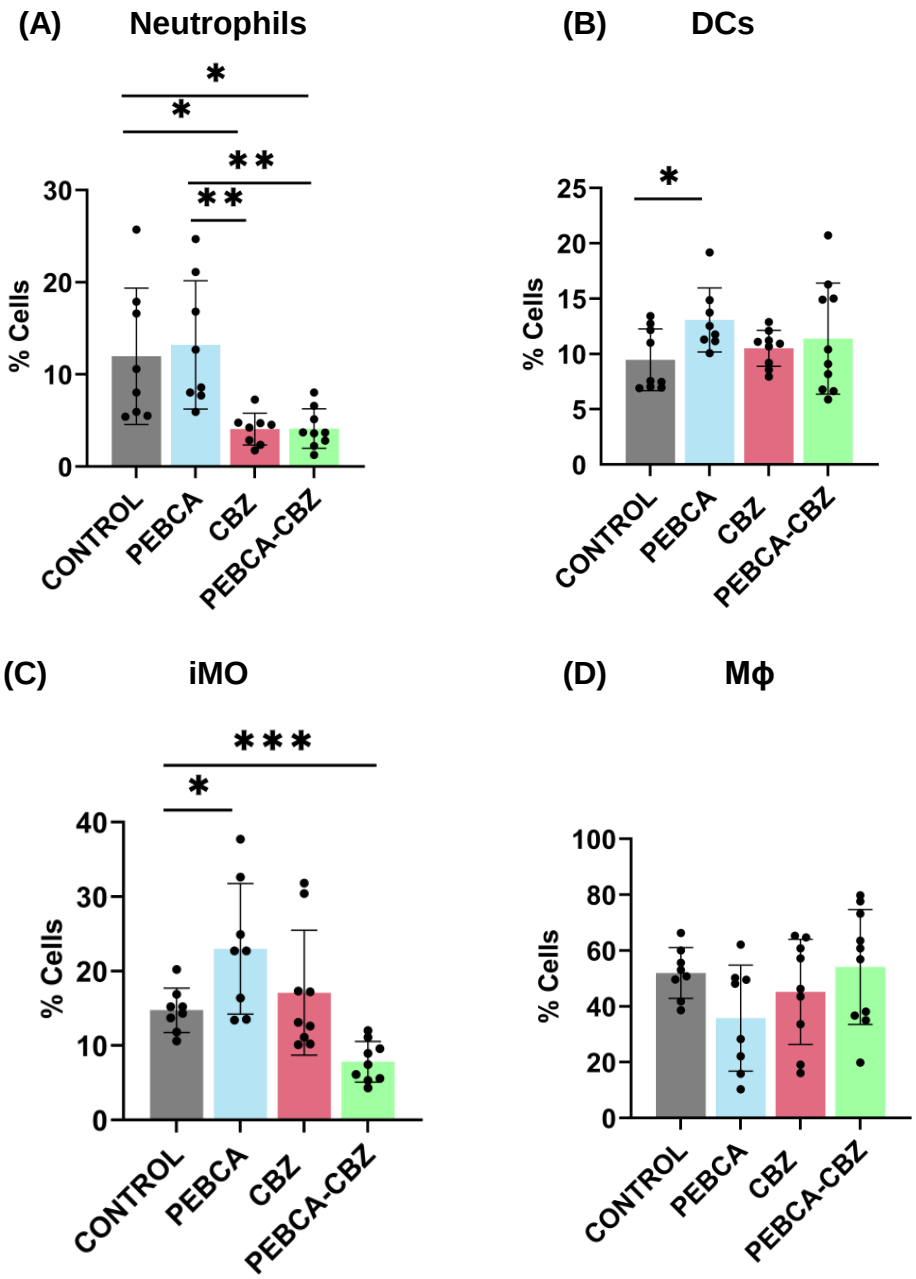

Figure S8: Treatment induced early effects on tumor associated immune cells *in vivo*. Tumors were collected after 96 h of single treatment either with PEBCA-CBZ NPs/free CBZ/empty PEBCA NPs along with untreated controls. Tumors were processed and stained with the same antibody panel as mentioned in Fig 8, and analyzed by multicolor flow cytometry. Data analysis was done by Flowjo. At least $5x10^5$ events were collected from each tumor sample. As shown here, represented are percent of different immune cell subsets in control or treated samples. **(A)** neutrophils, **(B)** DCs, **(C)** iMO and **(D)** Mϕ. All subsets were normalized to total leukocytes. NP batches used for this study are similar to those shown in Fig 1B. * p <0.05, ** p <0.01, *** p <0.001.

Table S1: Panel of antibodies used for multiparametric flow cytometry

Antibody	Marker for	Dilution	Cat. no.	Manufacturer
Set 1: Cell surface staining				
BUV395-CD45	leukocytes	1:400	564279BD	BD
BUV496-F4/80	Mϕ / iMO	1:150	750644	BD
BV605-CD11c	Mϕ / DCs/ iMO	1:200	117334	Biolegend
PE-Cy7-Ly6C	Mϕ / iMO	1:500	560593	BD
APC-Cy7-Ly6G	Neutrophils	1:500	560600	BD
BUV737-MHC II	DCs, Mϕ	1:200	748845	BD
AF700-CD206	M2 Mϕ	1:250	141734	Biolegend
Set 2: Intracellular staining				
BV711-IFN-γ	M1 Mϕ	1:20	564336	BD
AF488-TNF-α	M1 Mϕ	1:170	506315	Biolegend
PE-Dazzle 594-IL-10	M2 Mϕ	1:150	505034	Biolegend
APC-arginase-1	M2 Mϕ	1:30	17-3697 -80	Thermo Fisher Scientific