Supporting Information

Biohybrid Vaccines for Improved Treatment of Aggressive Melanoma with Checkpoint Inhibitor

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In vitro mechanism of APC activation mediated by the nanovaccine components

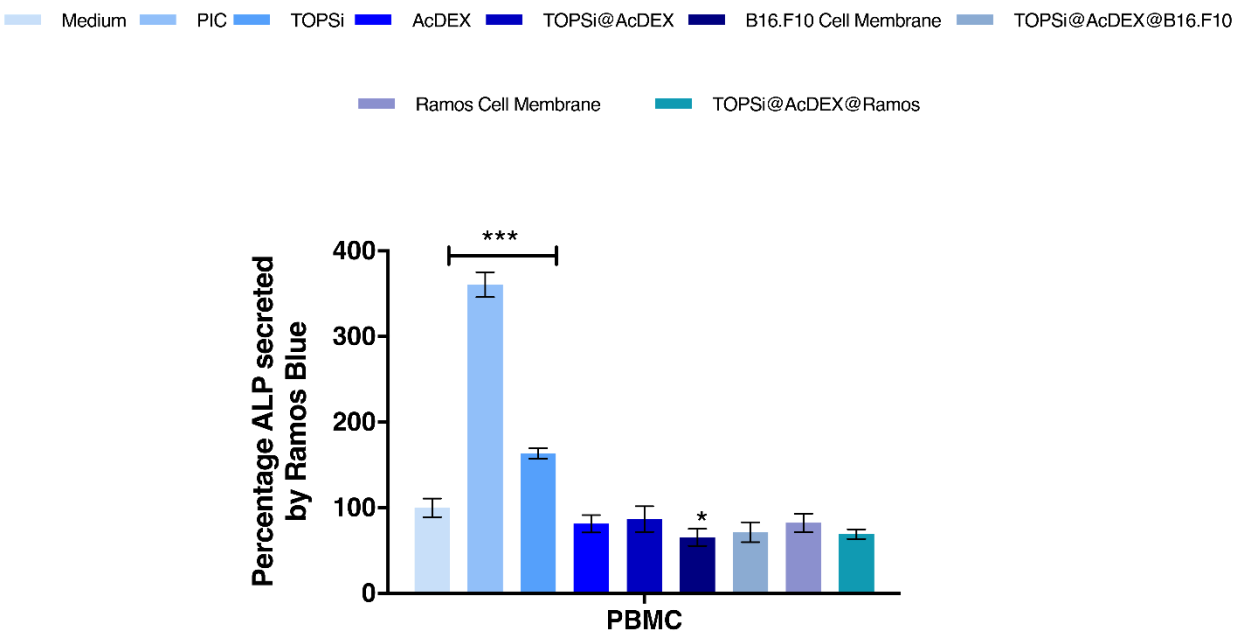

Figure S1. Percentage of alkaline phosphatase secreted by Ramos Blue cells after 48 h incubation with medium derived from 72 h of culture of PBMCs in presence of the different samples. Poly I:C (PIC) represented the positive control, while PBMC incubated in RPMI 10% FBS represented the negative one. The results are presented as mean ± S.D. (n = 3) and were analyzed by one-way ANOVA, followed by Bonferroni's post-test. The levels of significance were set at the probabilities of *p<0.05 and ***p<0.001.

The mechanism of activation of antigen presenting cells was determined by evaluating the indirect secretion of TNF-α, by peripheral blood monocytes stimulated with the nanovaccine and its different components. In particular, as shown in **Figure S1**, only the internal core of TOPSi is inducing the secretion of TNF-α, while this effect is lost when the particles are coated first with the polymeric layer and then with the cell membrane. These results are in agreement with our previous studies reported elsewhere.[1,2]

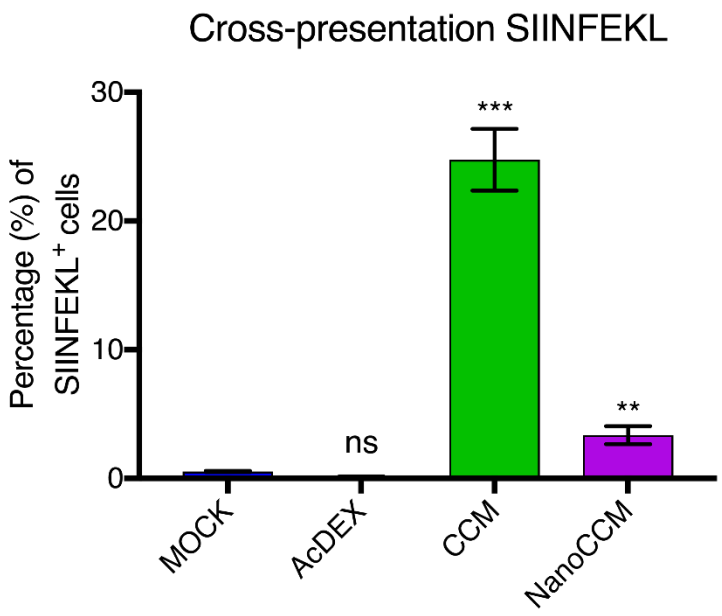

Figure S2. Percentage of immunogenic chicken ovalbumin specific peptide SIINFEKL positive JAWS-II after incubation with 10% a-MEM, 100 mg/mL TOPSi@AcDEX particles (AcDEX), extruded membrane vesicles derived from B16.OVA cells (CCM), and the final system (TOPSi@AcDEX@CCM, Nano CCM) wrapped in cell membranes derived from B16.OVA cells. The results are presented as mean±s.d. (n = 3). The data were analyzed using a Student's t-test and the levels of significance were set at the probabilities of $^{**}p<0.01$ and $^{***}p<0.001$.

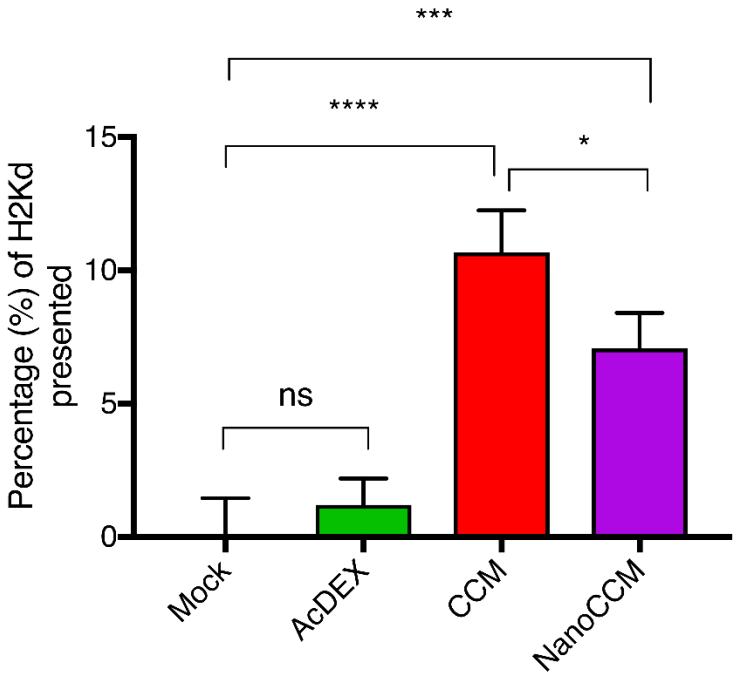

Figure S3. Percentage of H-2Kd presented by the cells incubated with 10% a-MEM, 100 mg/mL TOPSi@AcDEX particles (AcDEX), extruded membrane vesicles derived from 4T1 cells (CCM), and the final system (TOPSi@AcDEX@CCM, Nano CCM) wrapped in cell membranes derived from 4T1 cells. The results are presented as mean±s.d. (n = 3). The data were analyzed by one-way ANOVA followed by Tukey's post test and the levels of significance were set at the probabilities of *p<0.05, ***p<0.001, and ****p<0.0001.

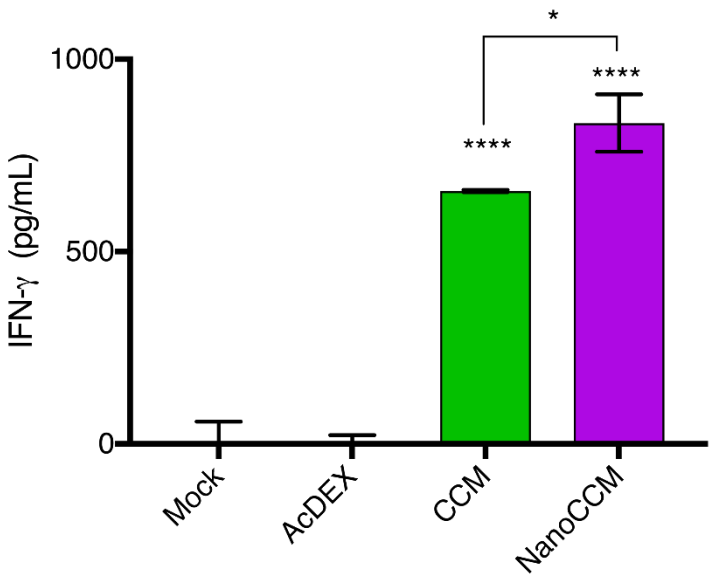

Figure S4. IFN-γ secreted by OT-I cells co-cultured with JAWS-II cells pulsed with 10% a-MEM, 100 mg/mL TOPSi@AcDEX particles (AcDEX), extruded membrane vesicles derived from B16.OVA cells (CCM), and the final system (TOPSi@AcDEX@CCM, Nano CCM) wrapped in cell membranes derived from B16.OVA cells. The results are presented as mean±s.d. (n = 3). The data were analyzed by one-way ANOVA followed by Tukey's post test and the levels of significance were set at the probabilities of *p<0.05 and ****p<0.0001.

Tumor volume average curves in B16.F10 model

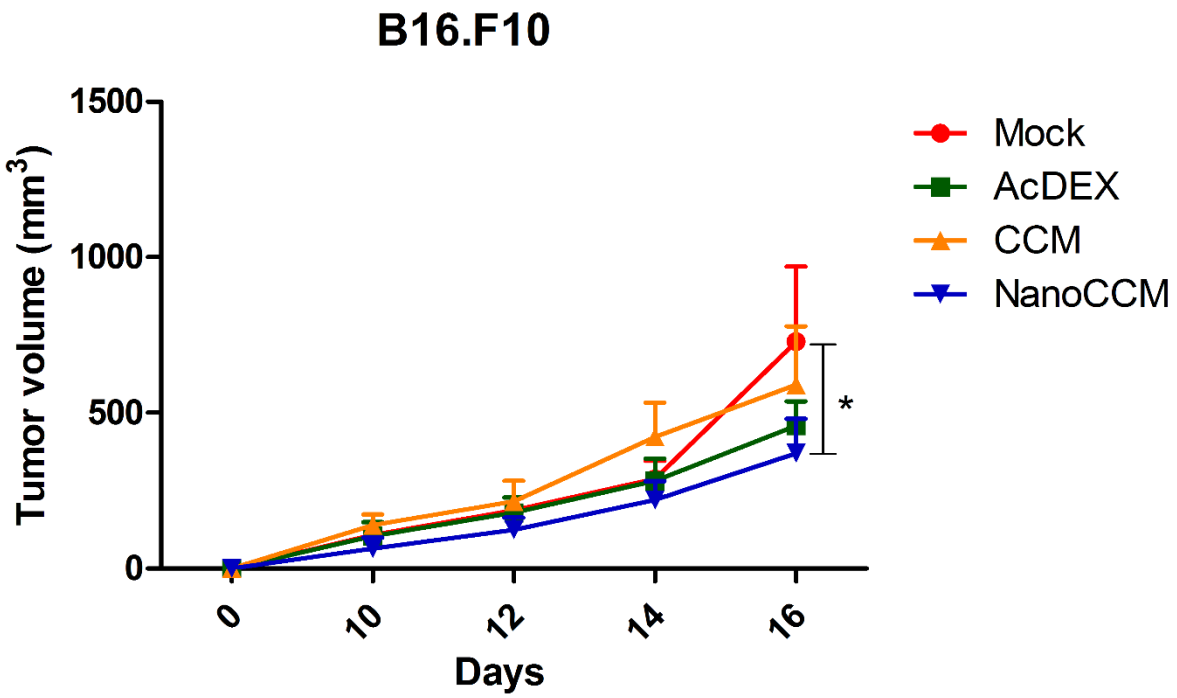

Figure S5. Mean B16.F10 tumor volume (mm^3) in mice treated with mock, adjuvant core alone (AcDEX), cancer cell membrane (CCM), and multistage nanovaccine (NanoCCM) (data taken from **Figure 2** in the manuscript). The results are presented as mean ± SEM ($n \geq 6$). The data were analyzed with two-way ANOVA, followed by Bonferroni's post test and the levels of significance were set at probability of $*p<0.05$.

Tumor volume average curves in B16.OVA model

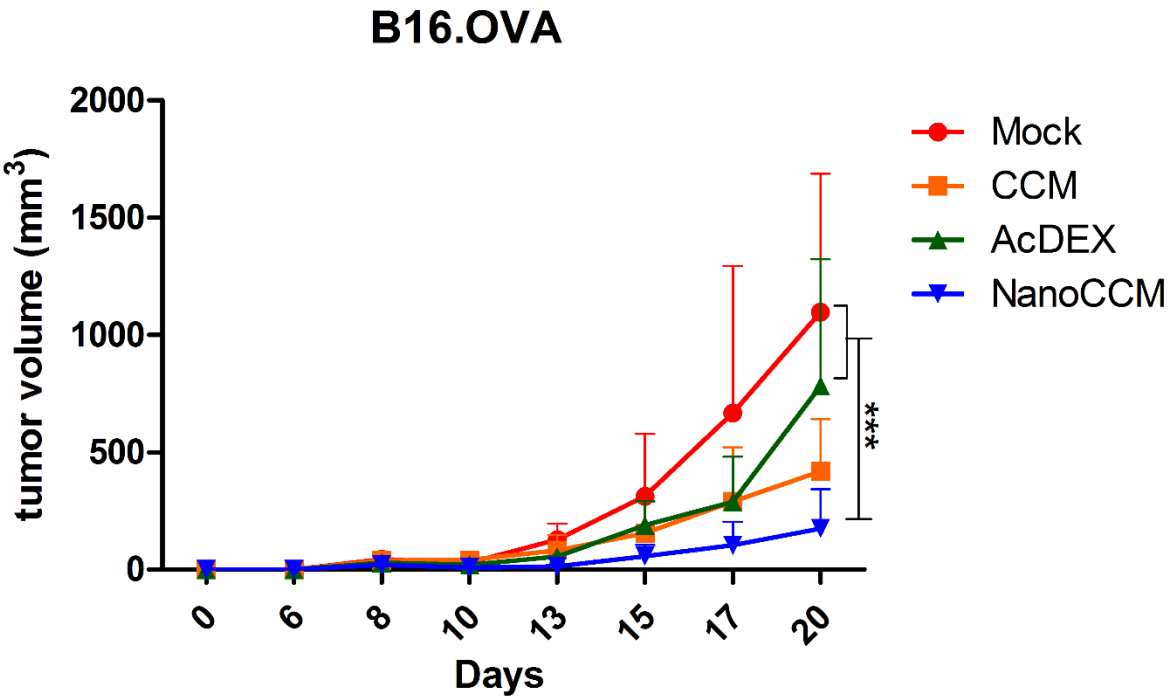

Figure S3. Mean B16.OVA tumor volume (mm^3) in mice treated with mock, adjuvant core alone (AcDEX), cancer cell membrane (CCM), and multistage nanovaccine (NanoCCM) (data taken from **Figure 4** in the manuscript). The results are presented as mean ± SEM ($n \geq 5$). The data were analyzed with two-way ANOVA, followed by Bonferroni's post test and the levels of significance were set at the probability of ***$p<0.001$.

References

1 Shahbazi, M. A.; Fernandez, T. D.; Mäkilä, E.; Le Guevel, X.; Mayorga, C.; Kaasalainen, M. H.; Salonen, J. J.; Hirvonen, J. T.; Santos, H. A., Surface Chemistry Dependent Immunostimulative Potential of Porous Silicon Nanoplatforms. *Biomaterials* **2014**, *35*, 9224-9235.

2 Fontana, F.; Shahbazi, M. A.; Liu, D.; Zhang, H.; Mäkilä, E.; Salonen, J.; Hirvonen, J. T.; Santos, H. A., Multistaged Nanovaccines Based on Porous Silicon@Acetalated dextran@ Cancer Cell Membrane for Cancer Immunotherapy. *Adv Mater* **2017**, *29*, 1603239.