Supplementary Data

Table S1. Cloning primers used in this study

Name	Sequence (5' end to 3' end)
$ITEP_A$-F	CGTGCTGCCGGGTGTTGGCGGTGTGTTACCAGGCGTCGGGGGTG TGCTGCCGGGCGTTGGTGGTGTCTTGCCTGGCGTAGGAGG
$ITEP_A$-R	TCCTACGCCAGGCAAGACACCACCAACGCCCGGCAGCACACCCCC GACGCCTGGTAACACACCGCCAACACCCGGCAGCACGCC
$ITEP_B$-F	CGCGGGTGTGCCGGGCGGCGCCGGTGTTCCAGGGGGCGCGGGT GTGCCGGGAGGCGCAGGTGTCCCTGGGGGCGCTGGTGTACCGG GAGG
$ITEP_B$-R	TCCCGGTACACCAGCGCCCCCAGGGACACCTGCGCCTCCCGGCA CACCCGCGCCCCCTGGAACACCGGCGCCGCCCGGCACACCCGCG CC
$(G_1C)_4$-F	CTGTGGTTGCGGCTGCGGGTGTGG
$(G_1C)_4$-R	ACACCCGCAGCCGCAACCACAGCC
$(G_8C)_4$-F	CGGTGGAGGTGGGTGTGGTGGCGGCGGAGGTGGCGGTGGCTGC GGTGGTGGCGGCGGGGGCGGCGGTTGCGGCGGCGGTGGCGGTG GGGGAGGATGTGGTGGGGGTGG
$(G_8C)_4$-R	ACCCCCACCACATCCTCCCCCACCGCCACCGCCGCCGCAACCGC CGCCCCCGCCGCCACCACCGCAGCCACCGCCACCTCCGCCGCCA CCACACCCACCTCCACCGCC
pOVA-F	GGAGAGTATAATCAACTTTGAAAAACTGACTGAAAGCATCATAAATT TCGAAAAGCTGACCGG
pOVA-R	GGTCAGCTTTTCGAAATTTATGATGCTTTCAGTCAGTTTTTCAAAGT TGATTATACTCTCCCC

Figure S1

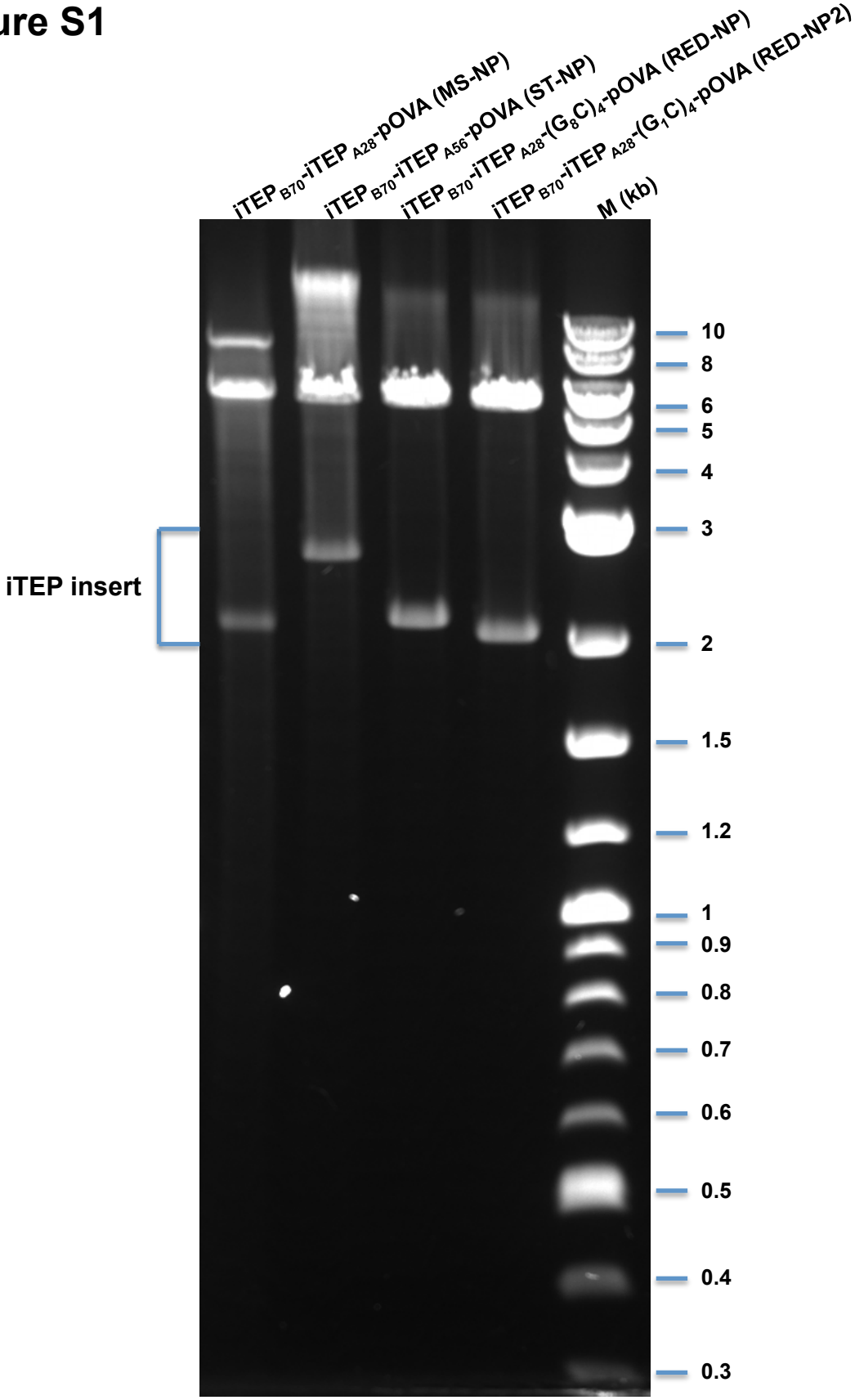

Figure S1. The cloned genes of iTEP-vaccine fusions on agarose gel after they were cleaved from pET25b(+) vector by XbaI and BamHI. The sizes of these genes agree with the expected residue numbers of the fusions.

Figure S2

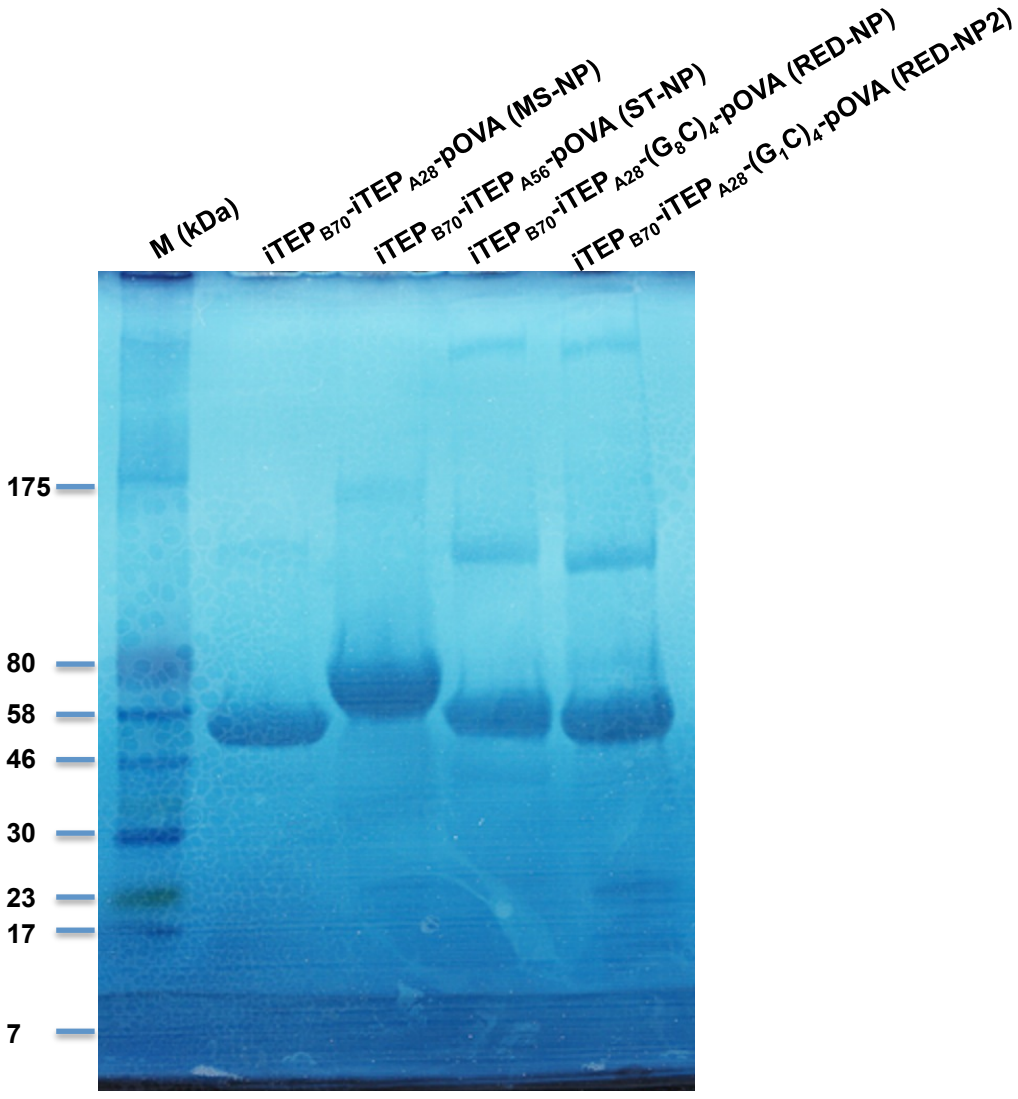

Figure S2. A SDS-PAGE analysis confirming molecular weights and purity of iTEP-vaccine fusions.

Figure S3

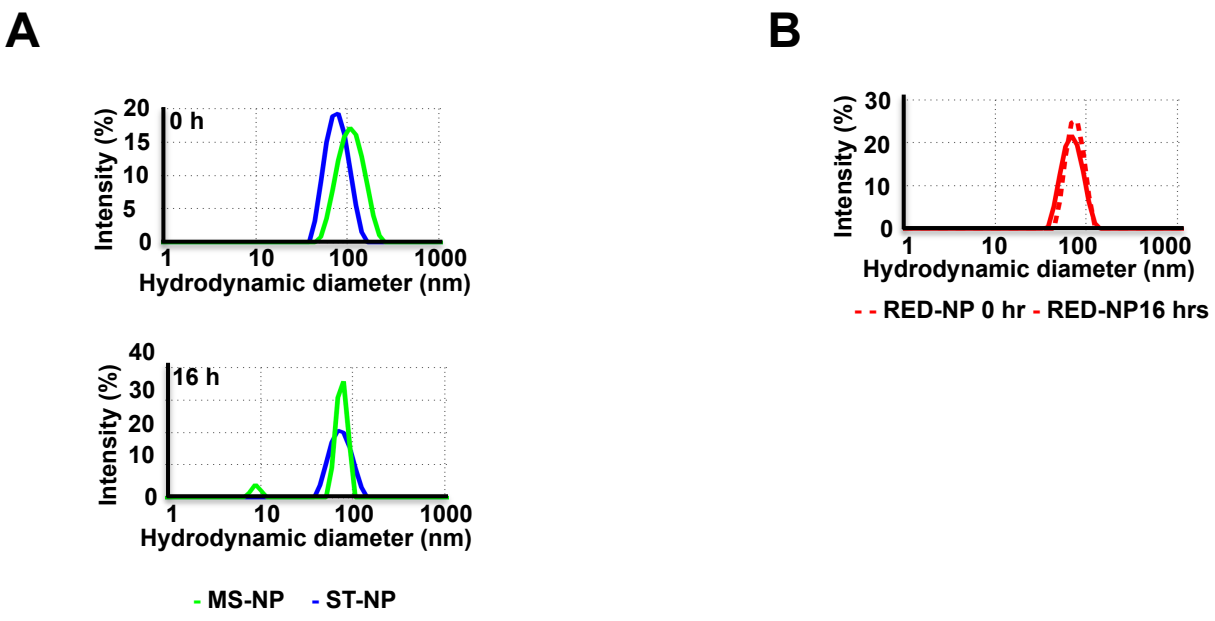

Figure S3. A. Hydrodynamic diameters by intensity of MS-NP and ST-NP. The data were collected by DLS before (0 h) and after (16 h) the NPs were incubated at 37°C for 16 h. The green line represents size distribution of MS-NP. The blue line is for ST-NP. Before the incubation, the diameters for MS-NP and ST-NP were 111.90±35.02 nm and 78.56±21.60nm, respectively. After the incubation, ST-NP had a diameter of 74.45±18.99 nm; the MS-NP had two peaks: 75.47±9.75 nm (92.2%) and 8.86±0.90 nm (7.8%). **B**. Hydrodynamic diameters by intensity of RED-NP were 75.55±15.32 nm and 70.99 ±17.52 nm before and after 16-h incubation, respectively. The red broken line and the red solid line represent size distribution for RED-NP before and after the incubation, respectively.

Figure S4

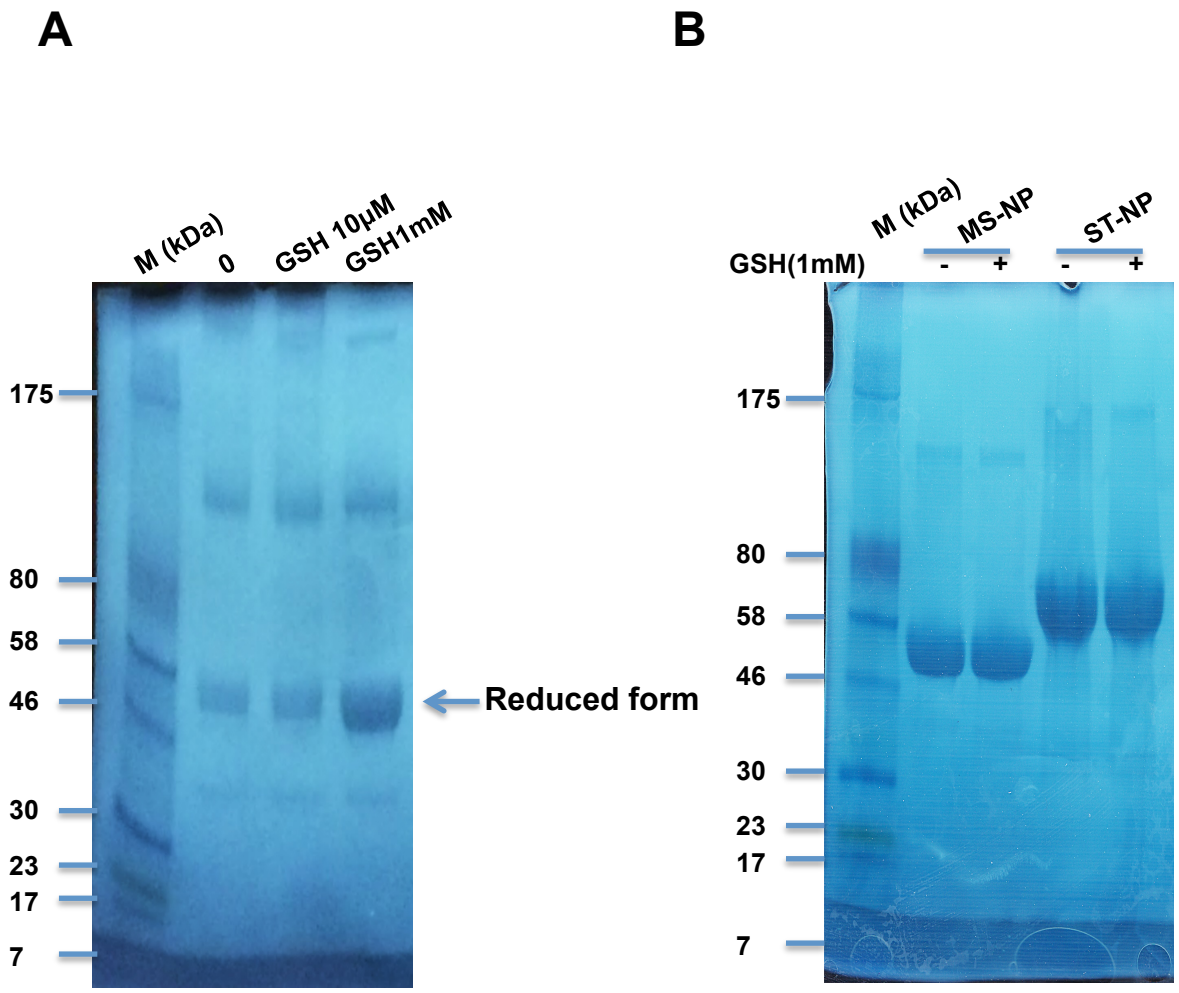

Figure S4. SDS-PAGE analyses of RED-NP (**A**) as well as MS-NP and ST-NP (**B**) after these NPs were treated with different concentrations of GSH overnight. 1 mM but not 10μM of GSH reduced disulfide bonds inside RED-NP. A large fraction of polymers of the RED-NP fusion ($iTEP_{B70}$-$iTEP_{A28}$-$(G_8C)_4$-pOVA) became monomers due to the treatment of 1 mM GSH, showing as a 50-kDa band on the gel. In contrast, GSH had no effect on polymerization status of the fusions of MS-NP and ST-NP. Non-reducing gels and 25 μg of each fusions were used for the analysis.

Figure S5

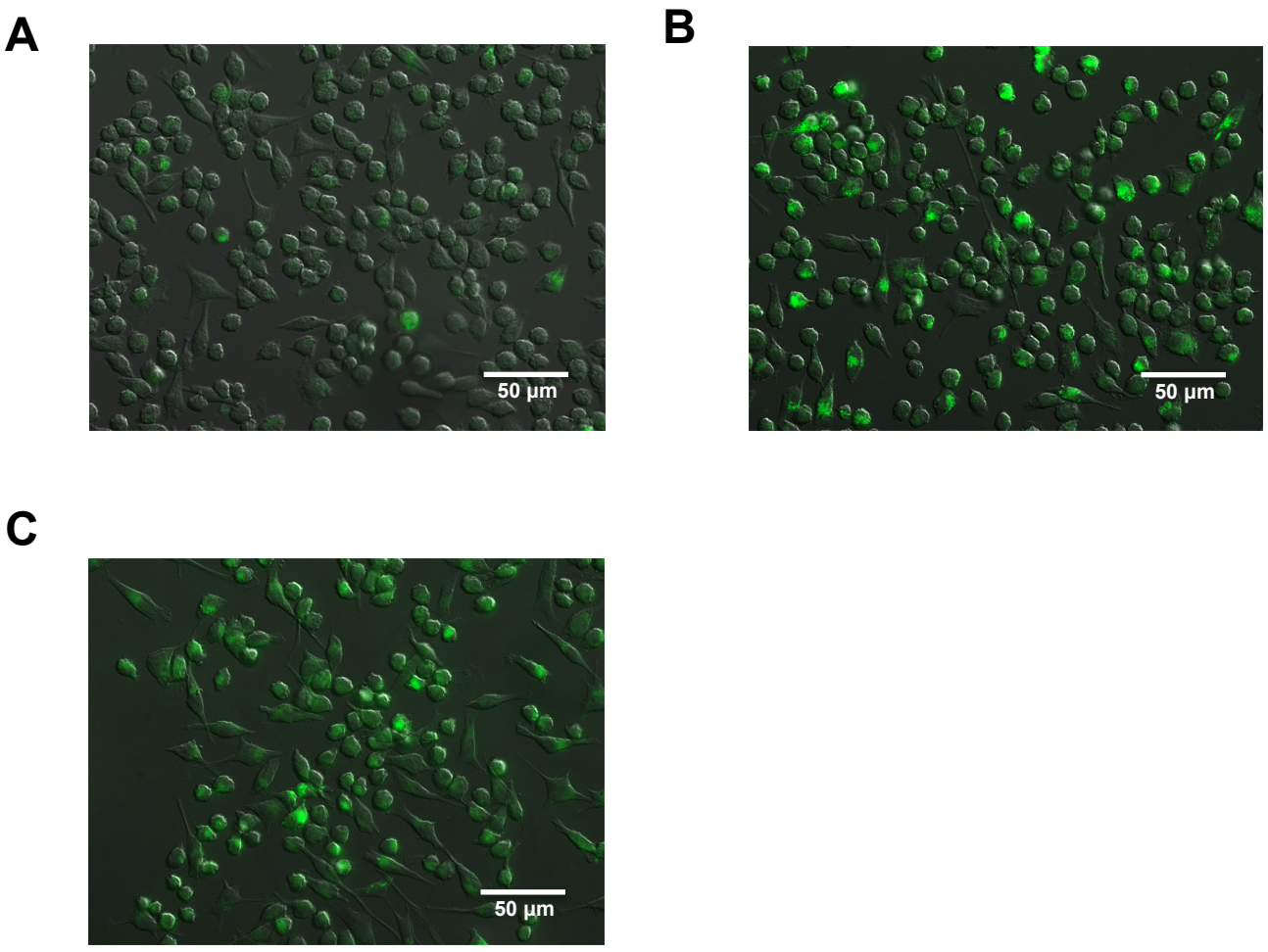

Figure S5. Fluorescence microscopy of MS-NP (**A**), ST-NP (**B**) and RED-NP (**C**) that were internalized by DC2.4 cells. Alexa-488 labelled NPs were incubated with DCs for 1 h before imaging.

Figure S6

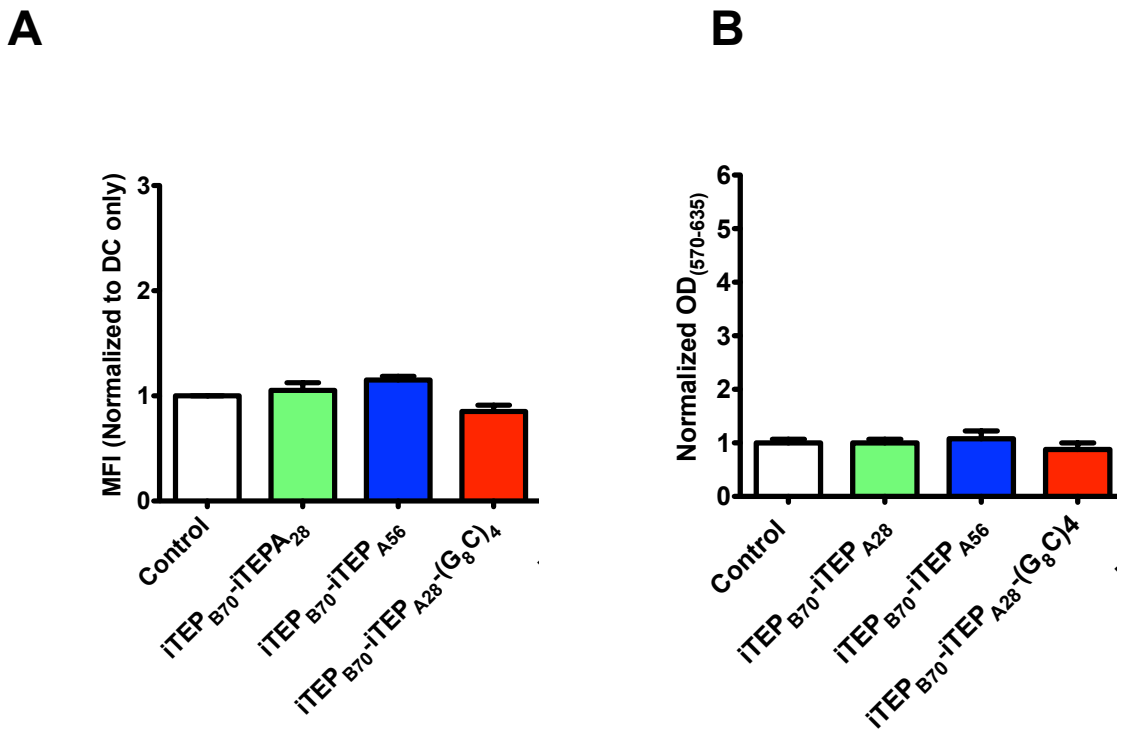

Figure S6. A. Presentation of pOVA by DC 2.4 cells after the cells were incubated with empty iTEP carriers. The data are presented as MFI means ±SD of DC cells in each treatment. Each treatment had three repeats (N=3). **B.** The activation of B3Z cells by DC 2.4 cells which were pre-incubated with empty iTEP carriers. The shown values are mean ODs ± SD of samples of each treatment (N=3).

Figure S7

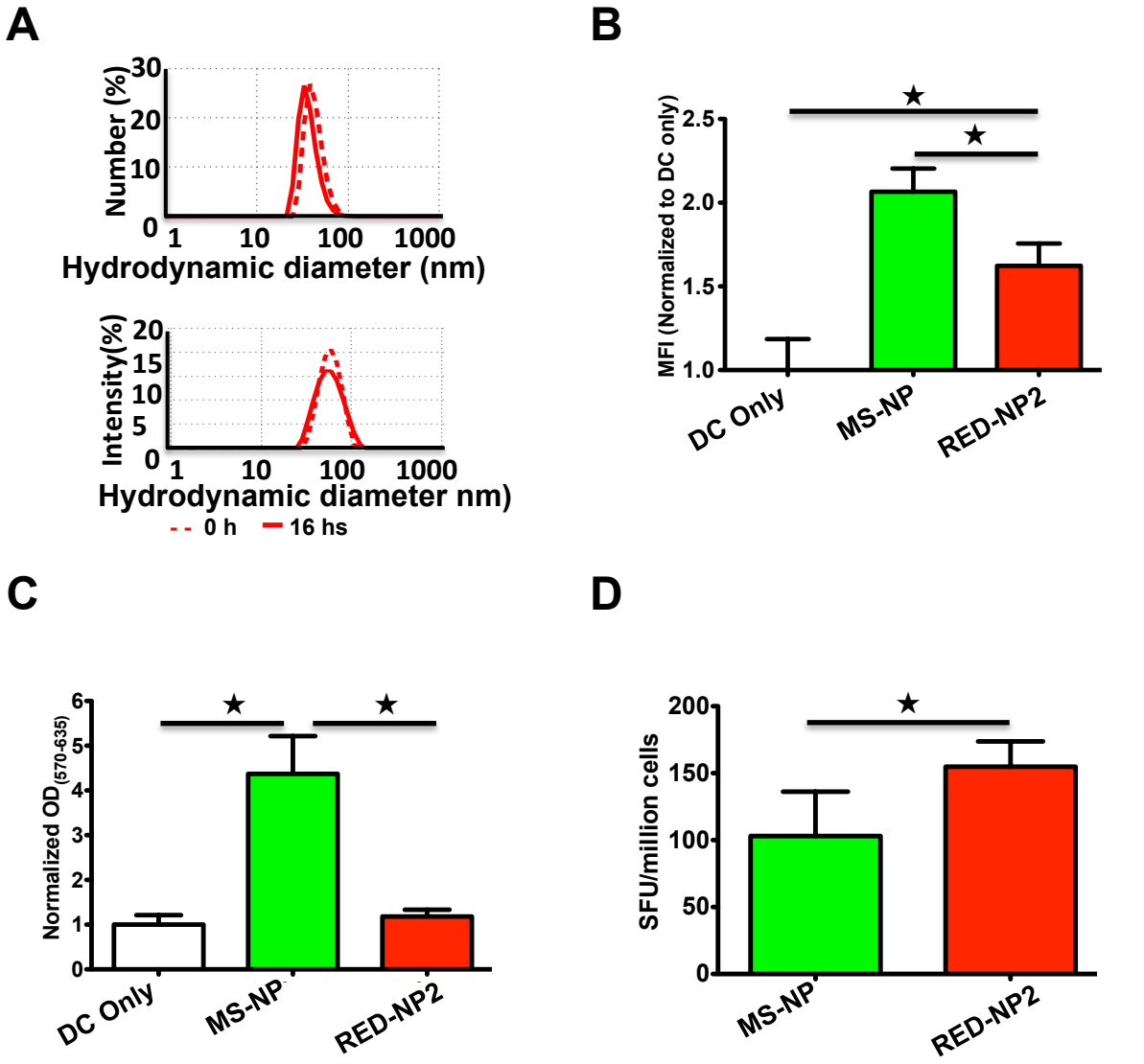

Figure S7. **A**. Hydrodynamic diameter distributions by numbers (upper panel) and by intensity (lower panel) of RED-NP2 before and after 16 h incubations at 37°C. The hydrodynamic diameter values were 42.49±10.23 nm (by number), 59.15±15.32 nm (by intensity), and 89.25 (Z-average) before the incubation; the values were 36.75±9.56 nm (by number), 58.95±19.53 nm (by intensity), and 54.81 (Z-average) after the incubation. **B.** Presentation of pOVA by DC 2.4 cells after the cells were incubated with MS-NP and RED-NP2. The data are presented as MFI means ± SD of DC cells in each treatment. Each treatment had three repeats (N=3). ★ $p < 0.05$ (t-test). The graph represents data collected from three independent experiments. **C**. The activation of B3Z cells by DC 2.4 cells which were pre-incubated with MS-NP and RED-NP2. The shown values are mean ODs ± SD of samples of each treatment (N=3). ★ $p < 0.05$ (t-test). The graph represents data collected from three independent experiments. **D**. Ex vivo analysis of active, SIINFEKL-restricted splenocytes from mice (N=5) immunized with MS-NP and RED-NP2. Data were presented as Spot Forming Units (SFU)/million cells ± SD. ★ $p<0.05$ (t-test)

Figure S8

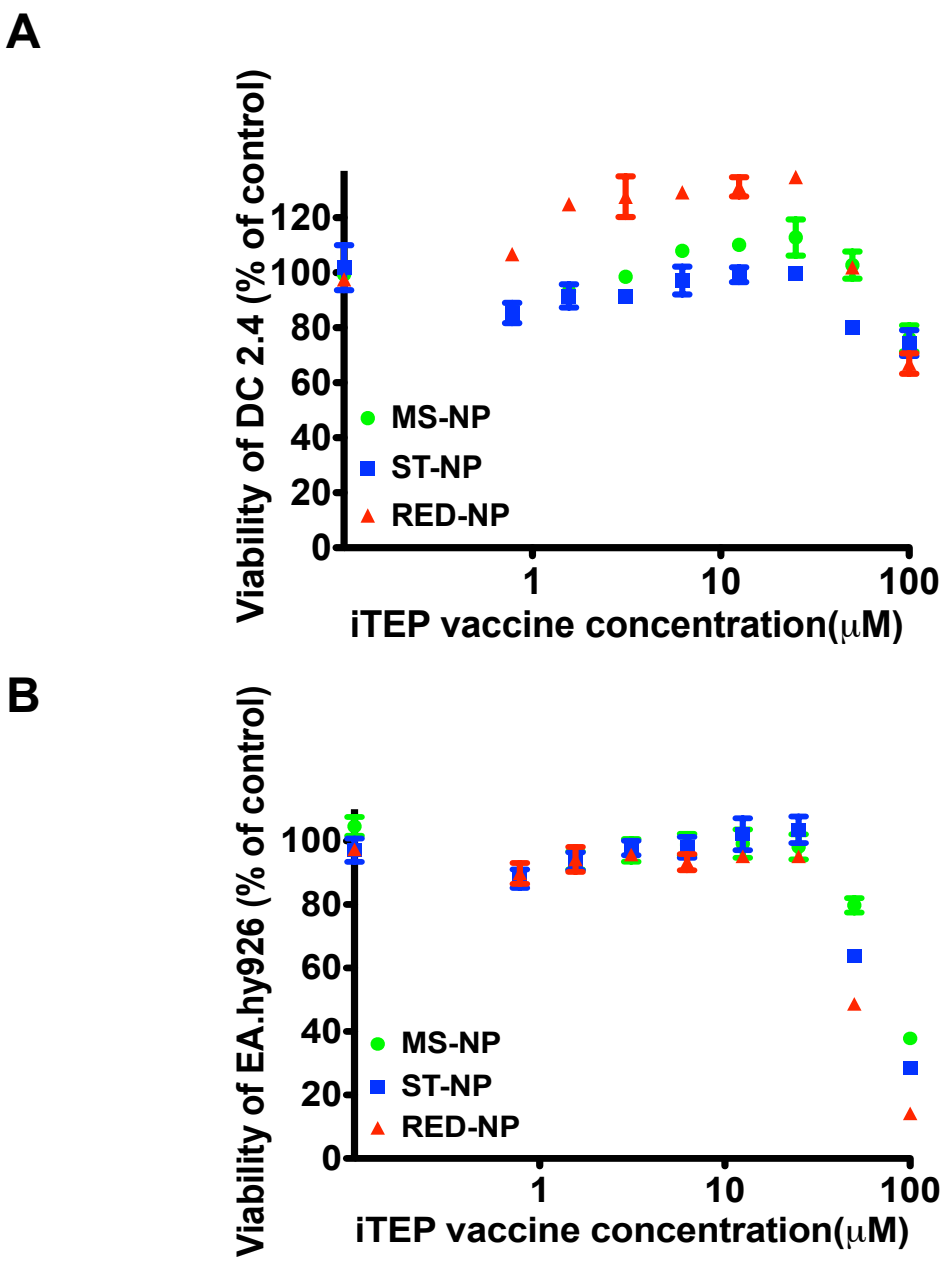

Figure S8. iTEP-vaccine fusions are not cytotoxic. **A.** The viability of DC 2.4 cells after they were treated with various iTEP-vaccine fusions. **B.** The viability of EA.hy926 cells after they were treated with various iTEP-vaccine fusions. Green dots: MS-NP; blue squares: ST-NP; red triangles: RED-NP. The data are presented as mean ± SD. Each of the two graphs represent results from 3 independent experiments.