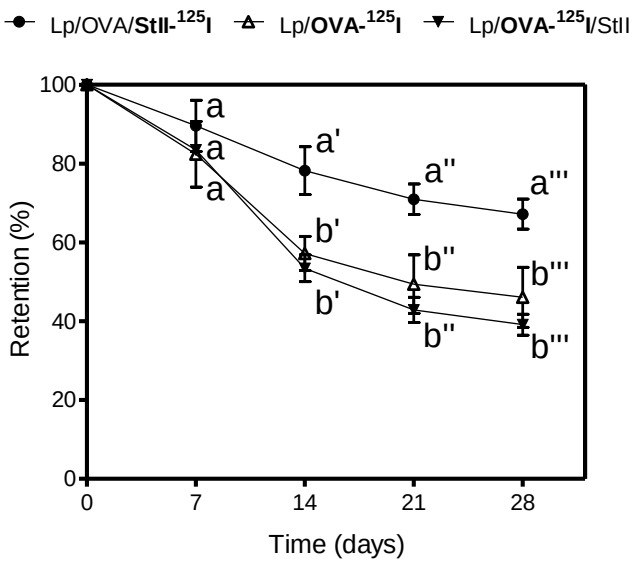
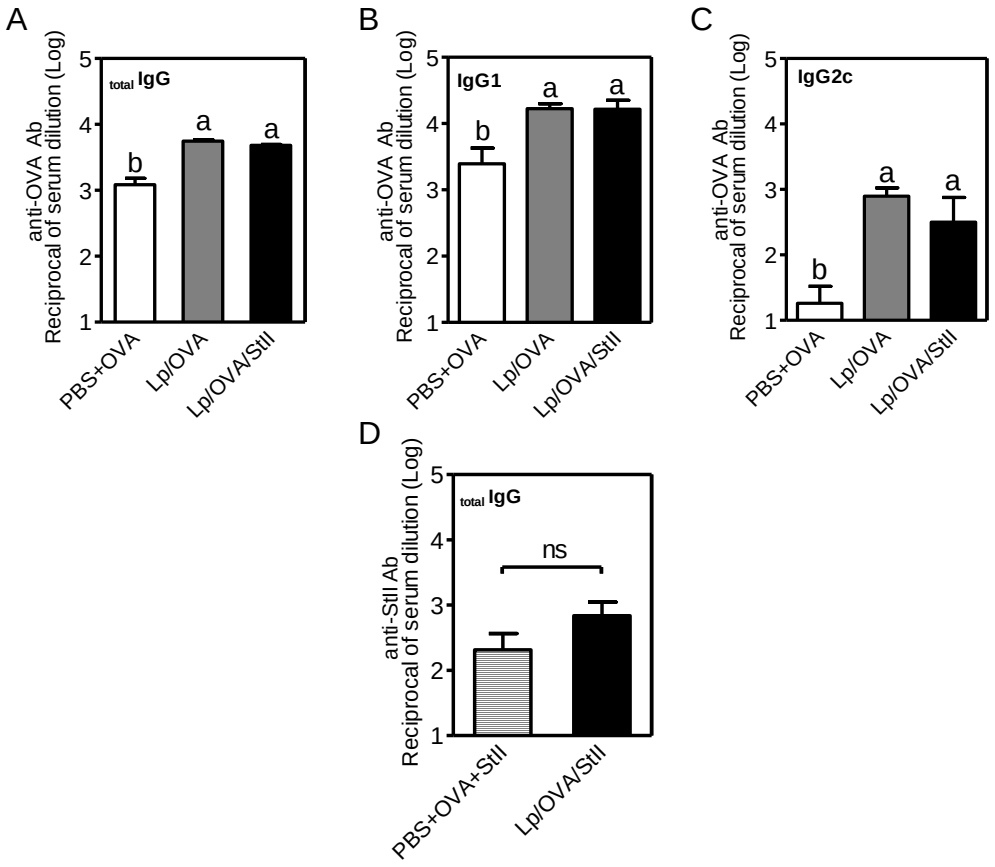
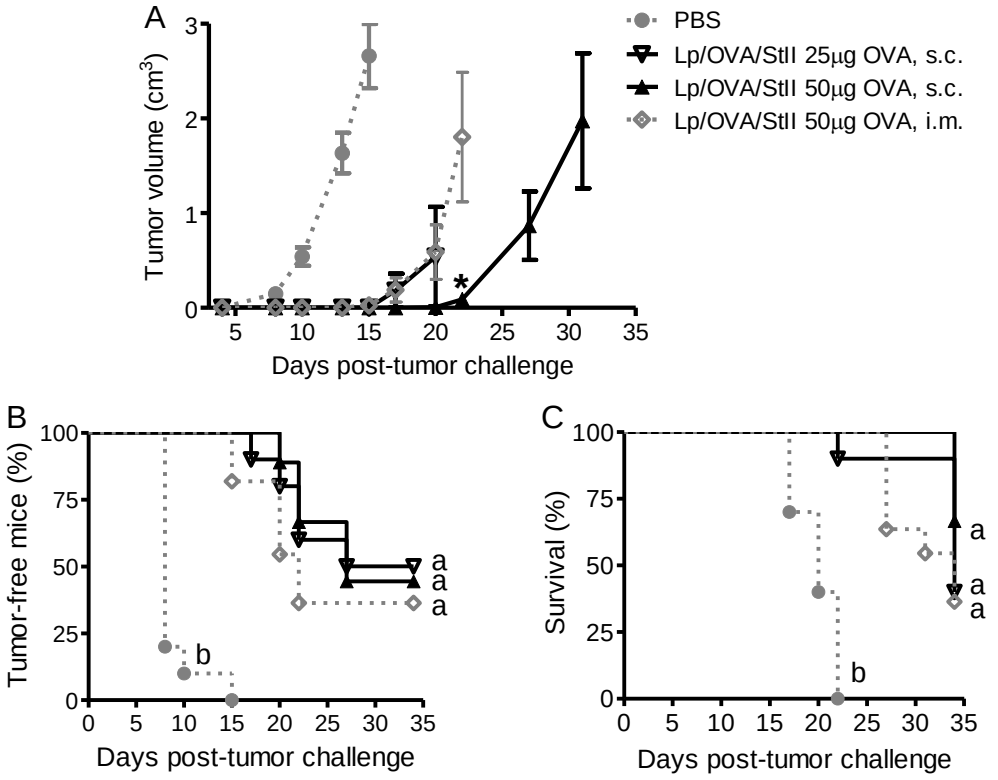


Supplemental Figure 1. OVA and StII retention (%) by DRVs of DPPC:Cho (1:1). Liposomal suspensions containing OVA(OVA-^{125}I), OVA(OVA-^{125}I) + StII or OVA + StII(StII-^{125}I) were resuspended in PBS and stored at 4°C during 28 days. Liposomes were centrifuged every week to determine protein retention by measuring OVA-^{125}I or St II-^{125}I radioactivity in supernatants and pellets. Each point is the mean (± SD) of 3-6 preparations. Different letters indicate significant differences among formulations according to Dunn's test ($p < 0.05$). Data are from a single experiment representative of three experiments yielding comparable results.

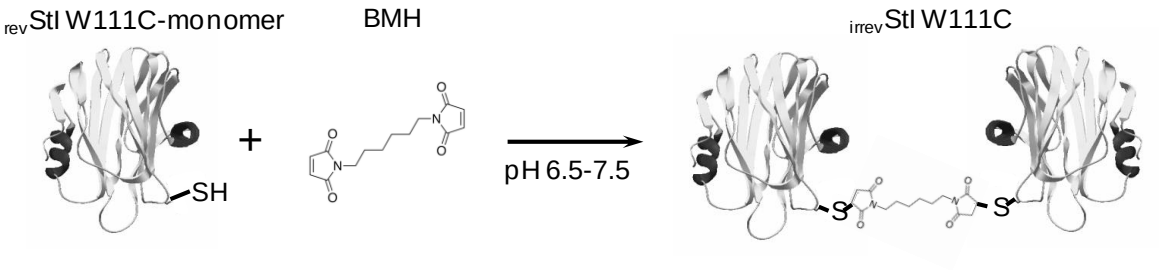


Supplemental Figure 2. Humoral response generated by the DPPC:Cho liposomes containing OVA and StII. C57BL/6 mice were immunized with Lp/OVA (50 μg OVA), Lp/OVA/StII (50 μg OVA and 6.25 μg StII), OVA in PBS (control group), or OVA mixed with StII in PBS (control group) as described in Fig. 4. At day 21, (**A**) OVA-specific total-IgG, (**B**) IgG1, (**C**) IgG2c, and (**D**) StII-specific total-IgG titres were analyzed in the sera of mice. Abs titres are expressed as the logarithm of reciprocal of maximal serum dilution at which the absorbance was twice that of the pre-immune serum. Bar graphs show the mean of five individual mice ± SEM. (A-C) Different letters indicate significant differences among groups according to Tukey's test ($^{a}p > 0.05$, $^{b}p < 0.05$). (D) Not statistical difference was established by two-tailed unpaired t test (ns, $p = 0.148$). Figure shows a representative experiment that was repeated twice with similar results.

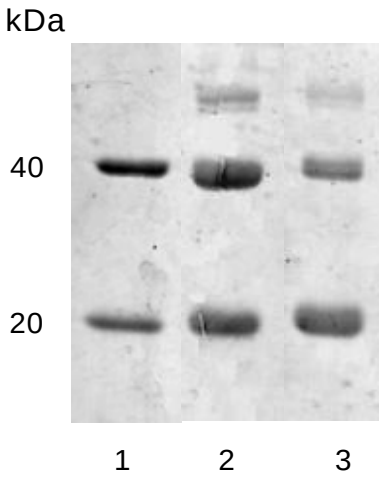


Supplemental Figure 3. Antitumor protection generated by two different doses of OVA co-encapsulated into DPPC:Cho liposomes with StII administered by two alternative routes. C57BL/6 mice (n = 10) were immunized twice at days 0 and 12 with Lp/OVA/StII by two different liposome doses and routes: 1- s.c., 50 μg OVA and 6.25 μg StII, 2- s.c., 25 μg OVA and 3.13 μg StII, and 3- i.m., 50 μg OVA and 6.25 μg StII. Seven days later animals were challenged with 3 x 10^5 cells of E.G7-OVA tumor cells. Animals that received PBS were used as control. (**A**) Temporal courses of the tumor volume increase (mean ± SEM). (**B**) tumor grafting, and (**C**) survival curves of experimental groups. (A) Statistical significance between the tumor volumes of the two groups that survived more time was calculated at day 22 by a two-tailed unpaired t-test (*$p < 0.05$). (B and C) Different letters indicate statistical differences among groups according to Log rank test ($^a p > 0.05$, $^b p < 0.001$). Data are representative of two experiments with similar results.

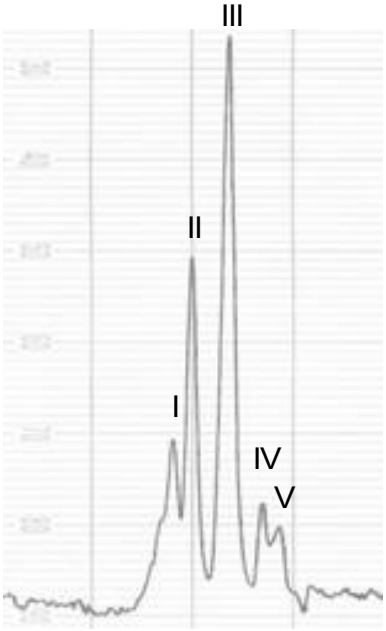
A


rev StI W111C-monomer
BMH
irrev StI W111C
SH
pH 6.5-7.5
S
S

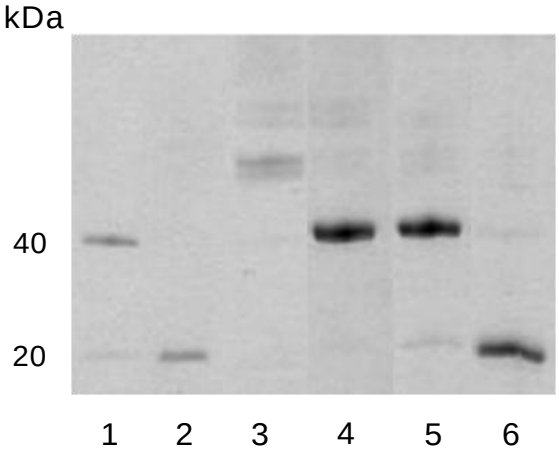
B


kDa
40
20
1
2
3

C


III
II
I
IV
V

D


kDa
40
20
1
2
3
4
5
6

Supplemental Figure 4. Preparation of the irreversibly inactive dimer $_{irrev}$StI W111C from $_{rev}$StI W111C. $_{rev}$StI W111C (a mutant of rStI that forms reversible dimers spontaneusly) was reduced by 0.1 M 2-ME followed by an incubation with the BMH (protein:BMH, 2:1 molar ratio) for 2h at 4°C. $_{irrev}$StI W111C was purified by fast protein liquid chromatography and the conjugation products were analyzed by SDS-PAGE (12.5 %). (**A**) Scheme of the reaction between the sulfhydryl group of Cys 111 of $_{rev}$StI W111C-monomer and BMH. (**B**) SDS-PAGE of the final reaction products obtained by conjugation of $_{rev}$StI W111C-monomer with BMH. Lines: 1- $_{rev}$StI W111C (under non-reducing conditions forming dimer, ~40 kDa, and monomer, ~20 kDa); 2- reaction products; 3- reaction products plus 2-ME. (**C**) Chromatographic separation of the final products followed by absorbance at 280 nm. I, II, III, IV and V indicate the main chromatographic peaks. (**D**) SDS-PAGE analysis of chromatographic peaks. Lines: 1- $_{rev}$StI W111C; 2- $_{rev}$StI W111C plus 2-ME; 3- peak I plus 2-ME; 4- peak II; 5- peak II plus 2-ME; 6- peak III plus 2-ME.