- 1 Supplementary Figure 1: Study design. In phase I of the study, the safety of three different dosing
- 2 regimens of Poly-ICLC in three cohorts of 3 patients each was assessed. In Cohort 1, 3 patients
- 3 received subcutaneous vaccinations of 100 µg NY-ESO-1 protein with 1.1mL Montanide and
- 4 0.35mg Poly-ICLC. No DLT was observed. Three patients enrolled in Cohort 2 received 100μg
- 5 NY-ESO-1 with 1.1mL Montanide and 0.7mg Poly-ICLC. Three patients in Cohort 3 received 100
- 6 μg NY-ESO-1 with 1.1 mL Montanide and 1.4 mg Poly-ICLC. No DLT was observed. The 3
- 7 Cohort cycles were repeated every 3 weeks for a total of 4 cycles. Phase II was done using 1.4mg
- 8 Poly-ICLC as the established dose. In phase II, patients were randomized in a blinded fashion to
- 9 receive a s.c. injection of 100 μg NY-ESO-1 protein and 1.4 mg of Poly-ICLC (Arm A; n=12),
- and emulsified with 1.4 mg of Montanide (Arm B; n=13).
- 11 Supplementary Figure 2: Evolution of antibody titers against NY-ESO-1. A. Evolution of
- 12 antibody titers against NY-ESO-1 protein over the treatment for each individual patient within
- phase 1 of the trial (n=3 in cohort 1, 2, and 3). B. Antibody titers against NY-ESO-1 for each
- individual patient within the phase 2 of the study (n=12 and n=10 in arm A and B respectively).
- 15 C. Individual mapping to specific regions of the NY-ESO-1 protein using 20 mer overlapping
- peptides covering the entire protein. Individual patients in arm A and B are shown.
- 17 **Supplementary Figure 3:** T-cell responses from patients within arm A and B throughout NY-
- 18 ESO-1 vaccination. CD4<sup>+</sup> and CD8<sup>+</sup> T cells were isolated from PBMCs of patients and then
- stimulated in vitro with NY-ESO-1 overlapping peptides. NY-ESO-1-specific T cell responses
- were evaluated by ICS at day 14 (CD8<sup>+</sup> T; bottom panel) and day 21 (CD4<sup>+</sup> T; upper panel).
- Quantification of CD4<sup>+</sup> and CD8<sup>+</sup> TNF-  $\alpha$ <sup>+</sup> and IL-2<sup>+</sup> production by intracellular cytokine staining
- 22 at different time points over the vaccination treatment. Arm A (black), n=12 and Arm B (red), n=8.
- 23 **Supplementary Table 1**: Overlapping peptides covering the entire NY-ESO-1 protein.