OMTON, Volume *32*

Supplemental information

Prime and pull of T cell responses against cancer-exogenous antigens is effective against CPI-resistant tumors

Fulvia Troise, Guido Leoni, Emanuele Sasso, Mariarosaria Del Sorbo, Marialuisa Esposito, Giuseppina Romano, Simona Allocca, Guendalina Froechlich, Gabriella Cotugno, Stefania Capone, Antonella Folgori, Elisa Scarselli, Anna Morena D'Alise, and Alfredo Nicosia

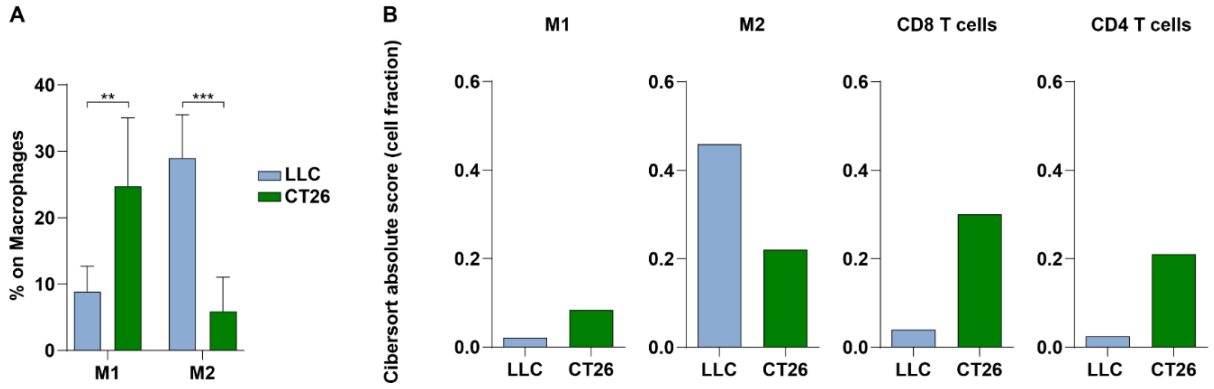

Figure S1: Characterization of tumor microenvironment of LLC and CT 26 tumors. A) LLC and CT26 cells were injected in mice; after 7 days, tumors were collected and analyzed by Flow Cytometry. Graph bars represent the frequency of MHCII+ CD206- M1 Macrophages and CD206+ MHCII- M2 Macrophages. Data are presented as mean values + SEM; n ≥ 3 mice, two-way ANOVA. B) Transcriptome data of LLC and CT26 tumors (n=3) were deconvoluted using CIBERSORTx analysis. Y-axis indicates the fraction of each immune cell type estimated on a reference gene signature of immunologically relevant genes, as described in the methods.

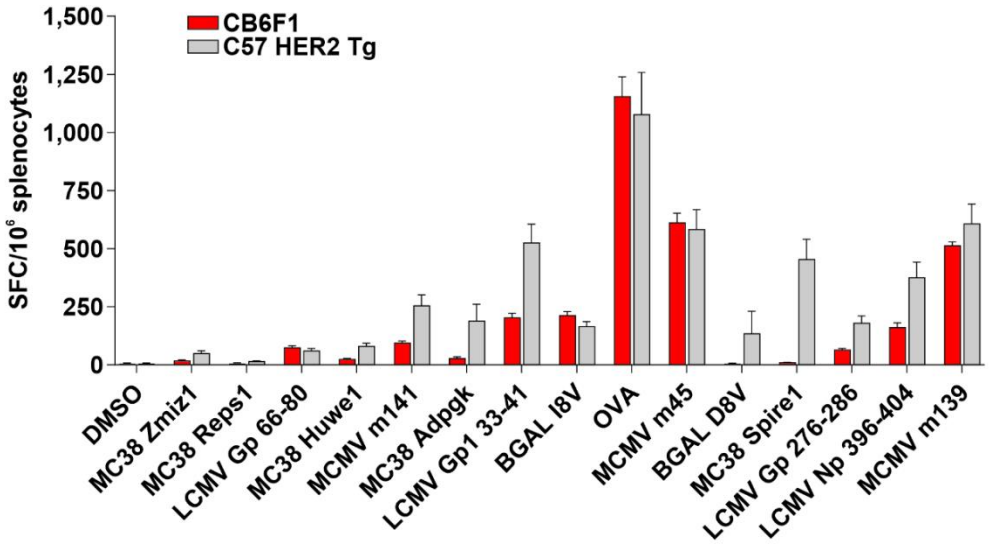

Figure S2: **Immunogenicity Ad5-CAP1 evaluated in CB6F1 and Her2 C57 transgenic mice used for the efficacy experiments.** Mice were injected i.m. with $5x10^8$ vp of Ad5-CAP1 at d0. 28 days after, distinct groups of vaccinated mice were sacrificed for spleen removal. Antigen specific T-cell responses were measured by IFN-γ ELISpot on splenocytes.

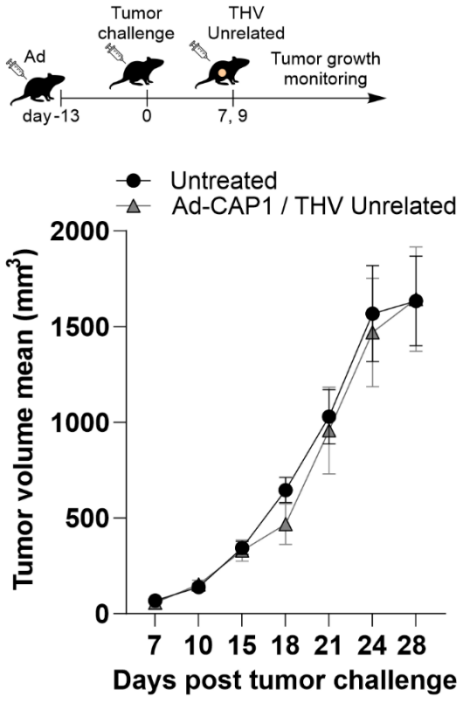

Figure S3: **THV-unrelated vector has no effect on LLC tumor growth in a setting of therapeutic treatment.** Mice were i.m. injected with Ad-CAP1 at $5x10^8$ vp, 13 days before HER2-LLC cells inoculum (s.c., d0). THV-encoding CAP1 unrelated protein was injected at d7 and d9 with $2{,}5x10^8$ PFU upon mice randomization according to tumor volume (60-90 mm^3). Untreated tumors were used as negative control**.**

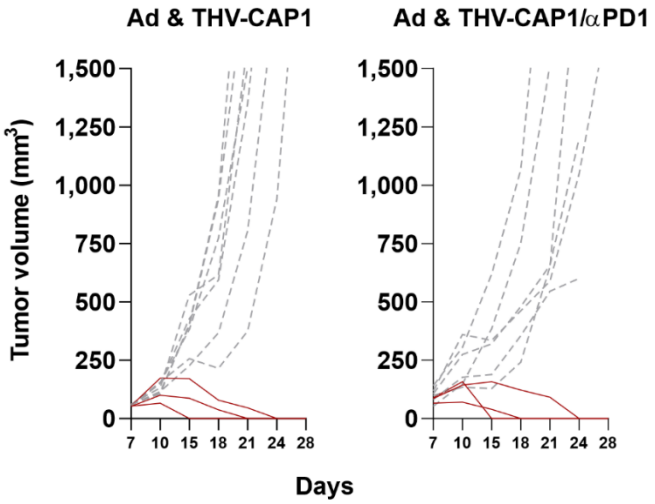

Figure S4: **Efficacy in therapeutic setting of Ad-CAP1 and THV-CAP1 with or without anti-PD1.** The therapeutic effect of Ad5-CAP1 and THV-CAP1 combination therapy was evaluated in HER2-LLC-derived established tumors. Ad5-CAP1 was injected with $5x10^8$ vp, 2 weeks days before HER2-LLC cells inoculum (d0). THV-CAP1 was given i.t. at d7 and d9 with $2,5x10^8$ PFU upon mice randomization according to tumor volume (tumor volume average: 60-90 mm^3). Anti-PD1 was given starting from day 7, twice per week until day 20 (i.p.).