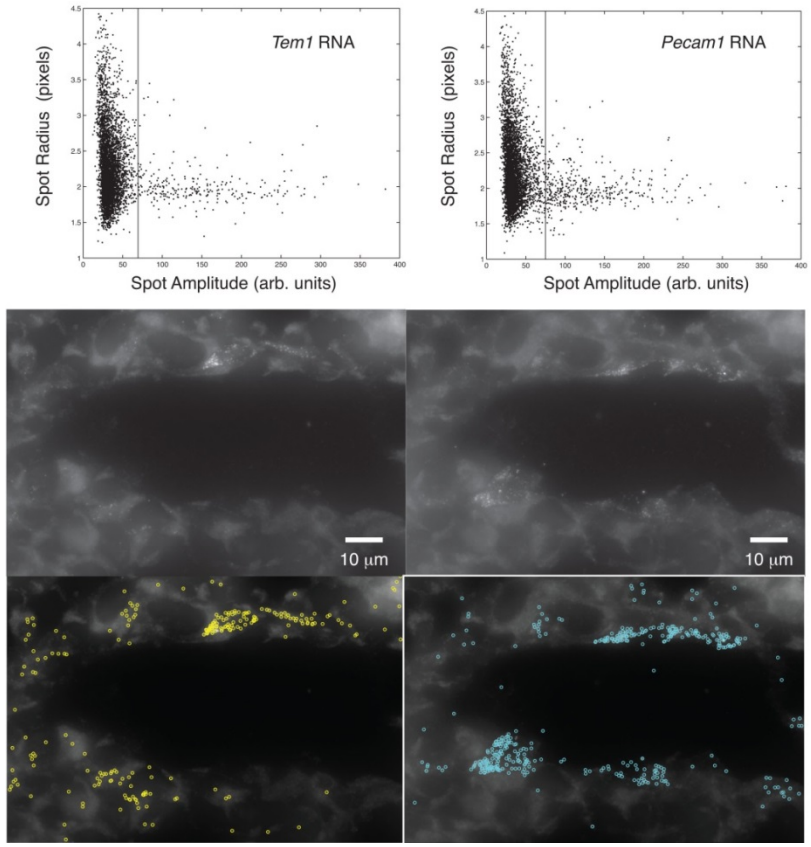
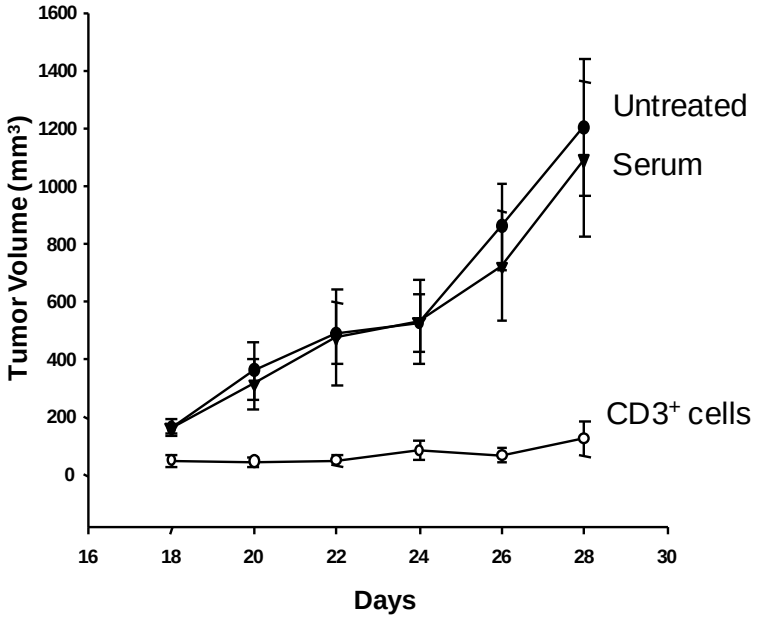
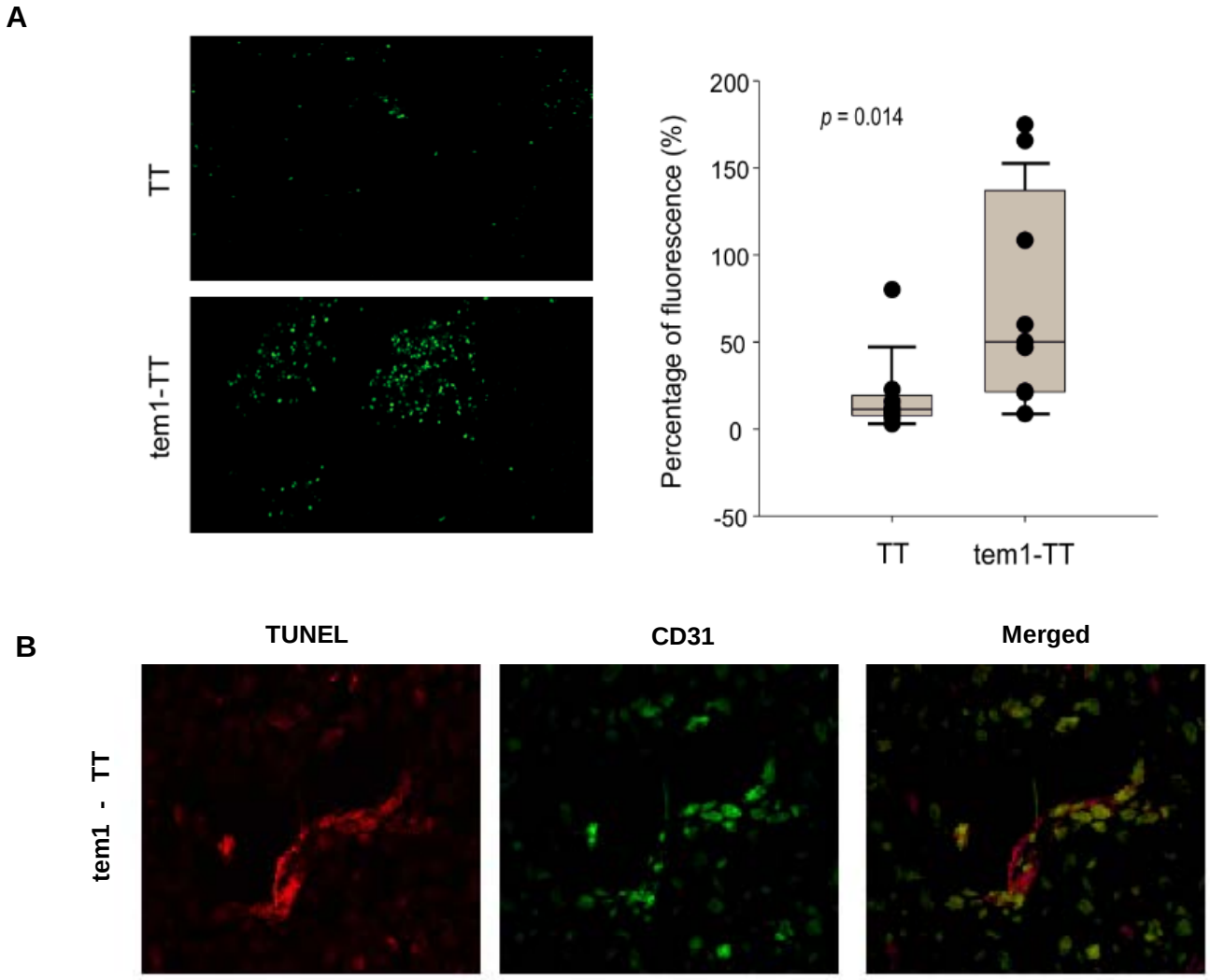


Supplementary Figure 1. RNA-FISH signal for Tem1 and CD31 (PECAM1) mRNA. To distinguish individual RNA molecules from background probe binding, images were filtered to isolate spot-like features, and these spots were individually fitted to determine their amplitudes and radii. Plots of extracted amplitudes and radii of all candidate spots for Tem1 and CD31 (PECAM1) mRNA (top left and right, respectively). True RNA spots have a distinct spot radius of approximately 2 pixels, which corresponds to nearly the diffraction limit, and spot amplitude that is clearly above background. The vertical line was used as a threshold to assign spots as RNA molecules to the right of the line. Maximum merge projection of unfiltered images (middle images). The bottom images are the same as the middle images but with the computationally identified RNA spots overlaid.



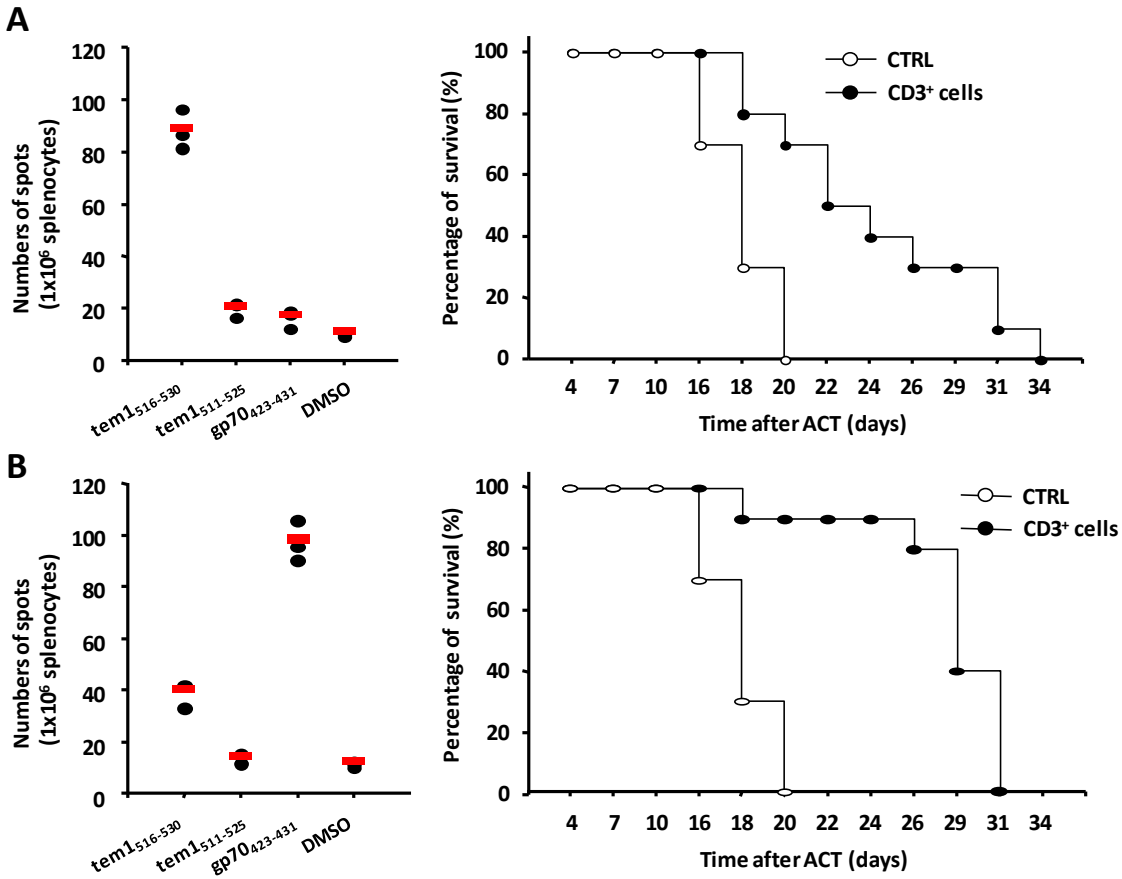
Supplementary Figure 2. Therapeutic efficacy of CD3$^+$ T cells by adoptive cell transfer (ACT) from tem1-TT vaccinated mice into TC1-tumor bearing mice. C57BL/6 were vaccinated three times, after a week from the last vaccination mice were sacrificed and splenocytes used to magnetically isolate the lymphocyte population (CD3$^+$ T cells). Isolated lymphocytes or serum were injected into separate TC1 tumor-bearing mice (challenged the day before the ACT), sub-lethally irradiated (500 rads) three days before the ACT, i.v. or i.p., respectively. Serum was administrated to tumor-bearing mice twice a week for a total of six injections. The log rank statistic for the survival curves is greater that would be expected by chance; there is a statistically significant difference between serum vs. CD3$^+$ cells survival curves ($p < 0.05$).

Supplementary Figure_2

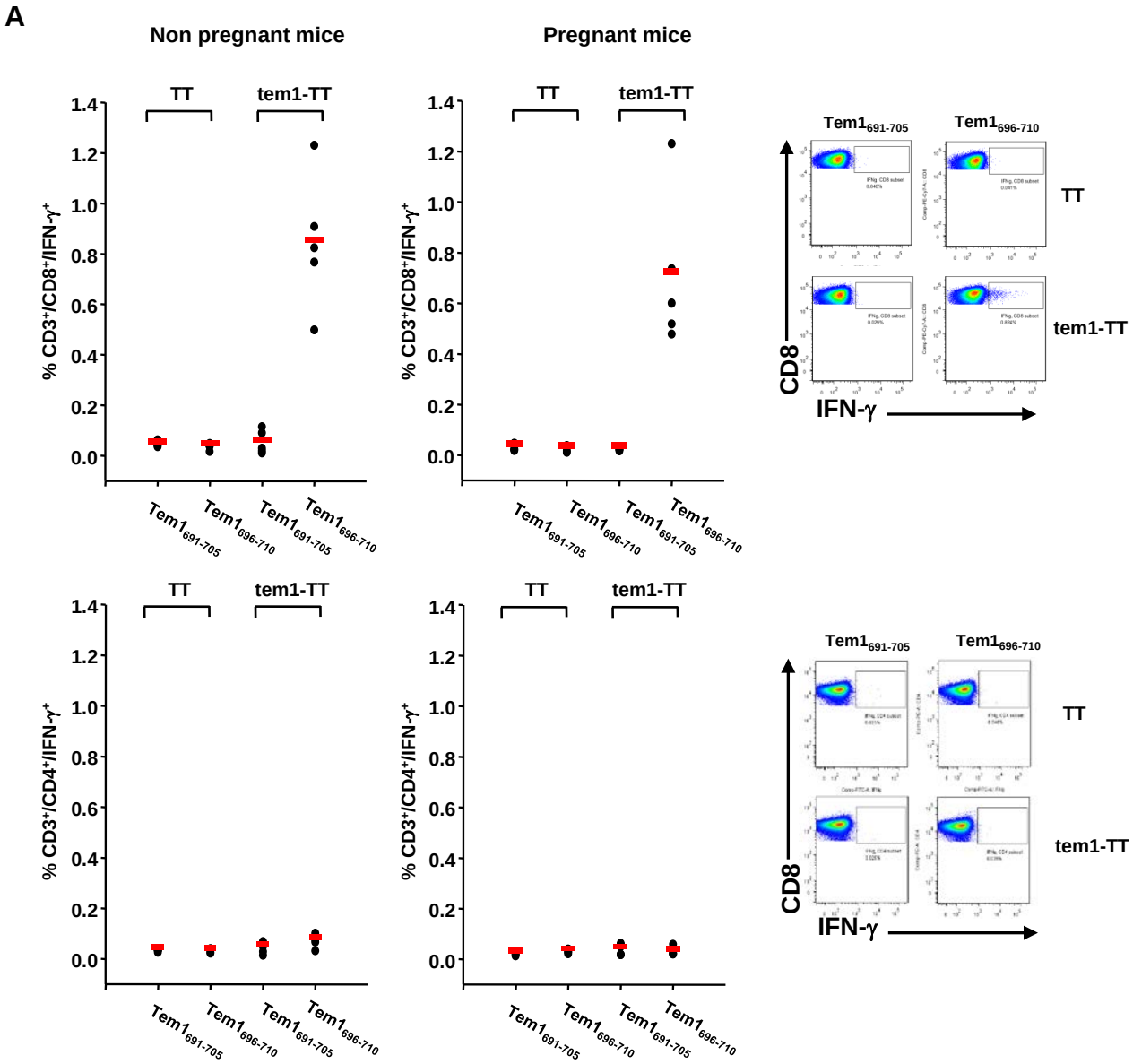


Supplementary Figure 3. Tem1-TT vaccine induces specific ablation of tumor vasculature. **A)** Apoptotic status of CT26 tumors was assessed by TUNEL immunofluorescence in TT and tem1-TT treated mice. Greater numbers of cells were TUNEL positive in CT26 tumors from tem1-TT treated mice (*p* value=0.014). Quantification of fluorescence was normalized, each data point is the mean of positive cells in 3 fields of view (10x) (n=9). **B)** CD31 and TUNEL double-staining of CT26 tumor from tem1-TT-immunized animals. Images identifies CD31 positive endothelial cells (red) and TUNEL positive apoptotic cells (green) within CT26 tumor excised from tem1-TT treated mice. The merged photo shows overlapping yellow signal, demonstrating that CD31 positive (endothelial) cells have a high apoptotic index. Overlapping signal was predominant in CT26 tumors from tem1-TT immunized mice and localized to highly vascular areas. Very few TUNEL positive cells were detected in areas surrounding endothelial cells in CT26 tumor from TT-vaccinated mice (data not shown).

Supplementary Figure_3



Supplementary Figure 4: Adoptive Cell Transfer (ACT) of the lymphocyte population of tem1-TT vaccinated mice. A) BALB/c tumor-free mice were vaccinated three times**,** after a week from the last vaccination mice were sacrificed and splenocytes were tested for their ability to recognize the tem1-specific, AH1-specific and control peptide through ELISpot assay (left panel). Splenocytes were used to magnetically isolate the lymphocyte population ($CD3^+$ T cells). Isolated lymphocytes were injected i.v. in CT26 tumor-bearing mice (challenged the day before the ACT) sub-lethally irradiated (500 rads) three days before the ACT. The log rank statistic for the survival curves is greater that would be expected by chance; there is a statistically significant difference between survival curves ($p<0.001$). **B) CT26 tumor-bearing mice were vaccinated three times,** after a week from the last vaccination, mice were sacrificed and splenocytes were tested for their ability to recognize the tem1-specific, AH1-specific and control peptide through ELISpot assay (left panel). Isolated lymphocytes were injected i.v. in CT26 tumor-bearing mice. The log rank statistic for the survival curves is greater that would be expected by chance; there is a statistically significant difference between survival curves ($p<0.001$).



Supplementary Figure 5: Tem1 specific immune responses are similar in immunized non-pregnant and pregnant mice. C57BL/6 mice were immunized and mated as described in Materials and Methods. Forty-five days after the last immunization, both pregnant and non-pregnant mice were boosted with a single injection of tem1-TT or TT DNA. Two weeks later the mice were sacrificed to assess the immune response against tem1 antigen. Splenocytes of mice vaccinated with tem1-TT vaccine or TT vaccine were tested against the immunodominant $Tem1_{696-710}$ peptide or an irrelevant peptide ($Tem1_{691-705}$) using IFN-γ ICS. $CD3^+/CD8^+$ splenocytes from tem1-TT-vaccinated pregnant and non-pregnant mice recognized $Tem1_{696-710}$ peptide and responded with roughly the same frequency. As expected, there was lack of a immune response against the irrelevant peptide ($Tem1_{691-705}$) and lack of $CD3^+/CD4^+$ T-cell response towards $Tem1_{696-710}$ peptide since it is a $CD8^+$ tem1 specific peptide (shown in Figure 2E).