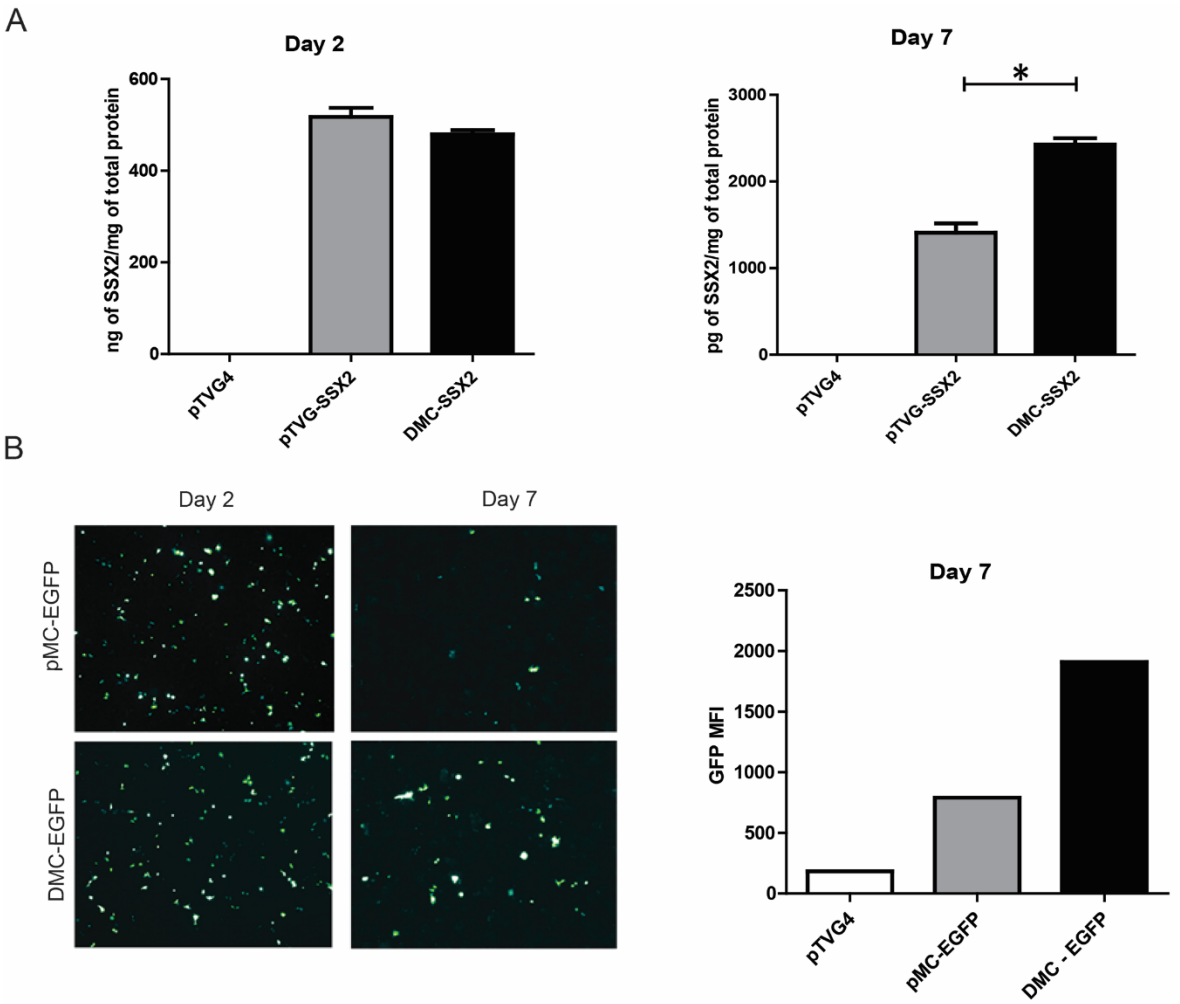
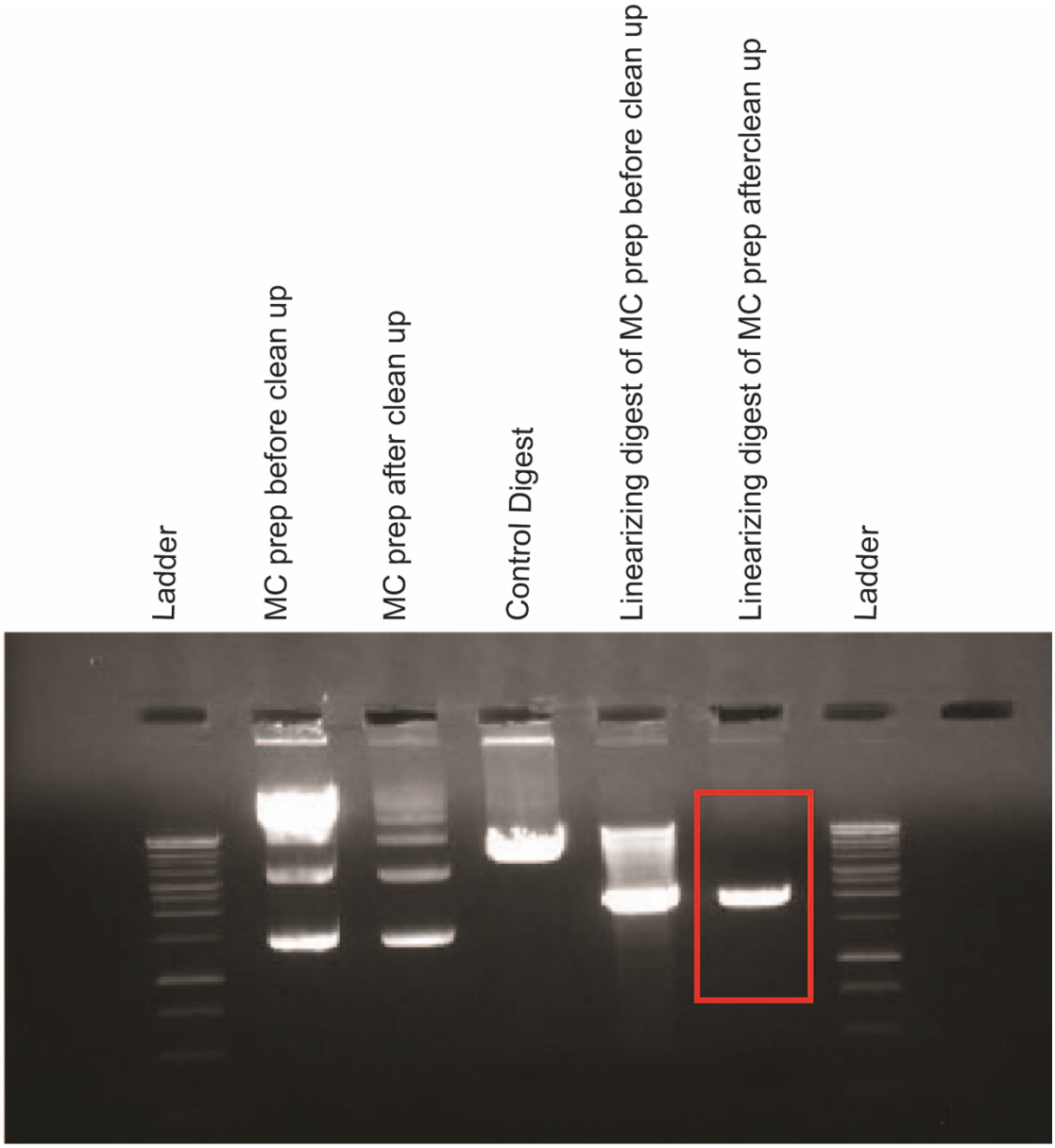
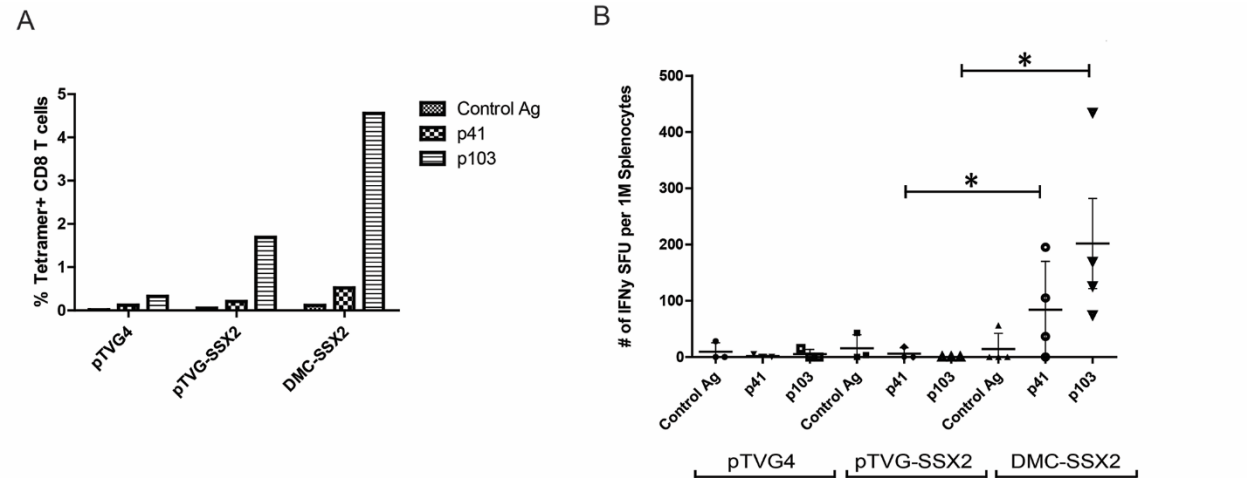


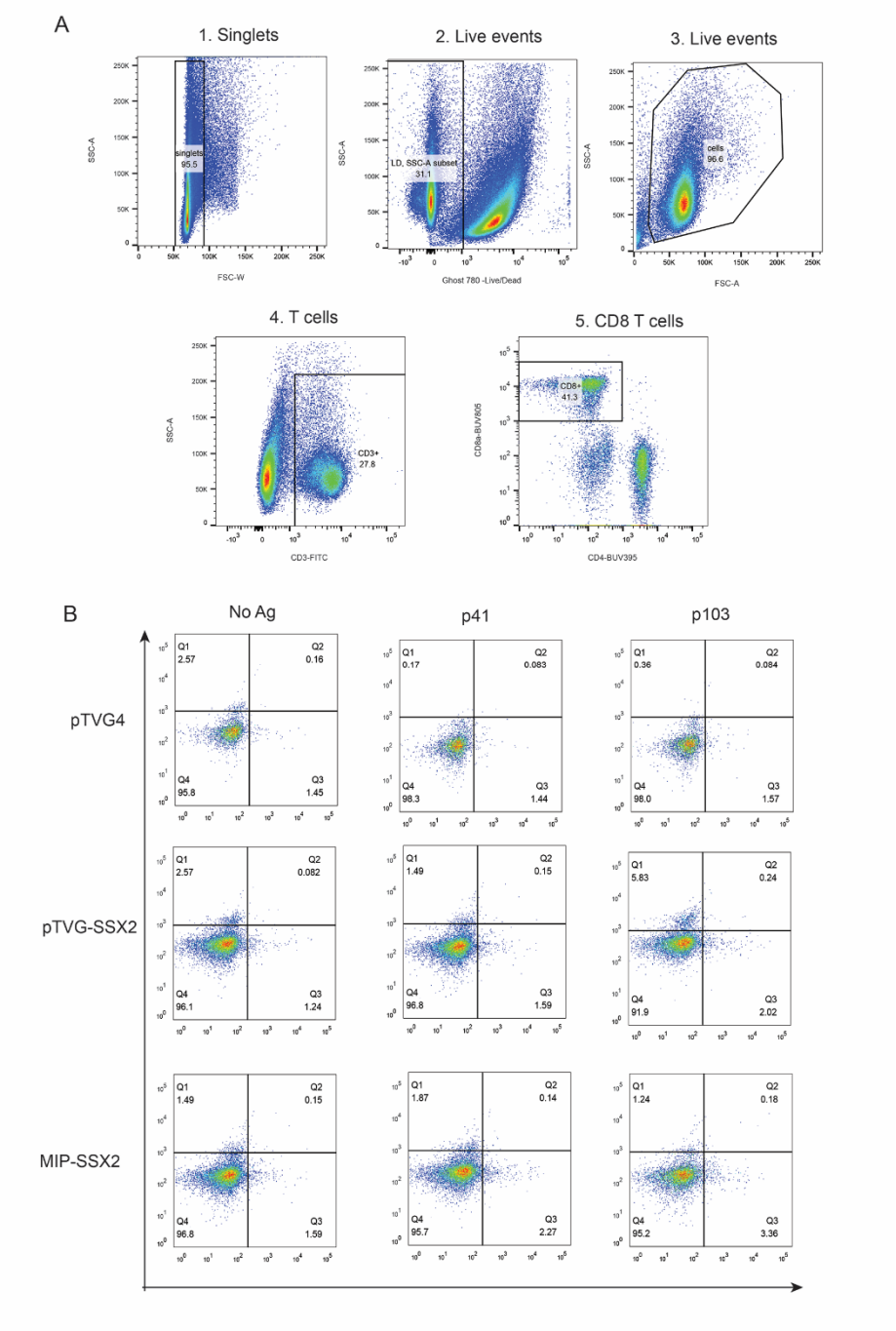
Supplementary Figure 1. Minicircle constructs result in sustained transgene expression *in vitro.* A) LNCaP cells were transiently transfected (n=3 experimental replicates) with equimolar amounts of pTVG4 (2µg), pTVG-SSX2 (2µg), or DMC-SSX2 (0.96µg) and SSX2 expression was analyzed on Days 2 and 7 by ELISA. B) DMC-EGFP was prepared by cloning the EGFP gene into the pMC.CMV-MCS-SV40polyA minicircle production vector and prepared as per the manufacturer's instructions. Equimolar amounts of pMC-EGFP - the non-recombined full length plasmid encoding EGFP, and DMC-EGFP were transfected into Cos7 cells (n = 3 experimental replicates) growing under low serum conditions. Representative fluorescence microscopy images of the samples are shown in the left panel. The right panel shows a graphical representation of the EGFP MFI of transfected samples on Day 7.



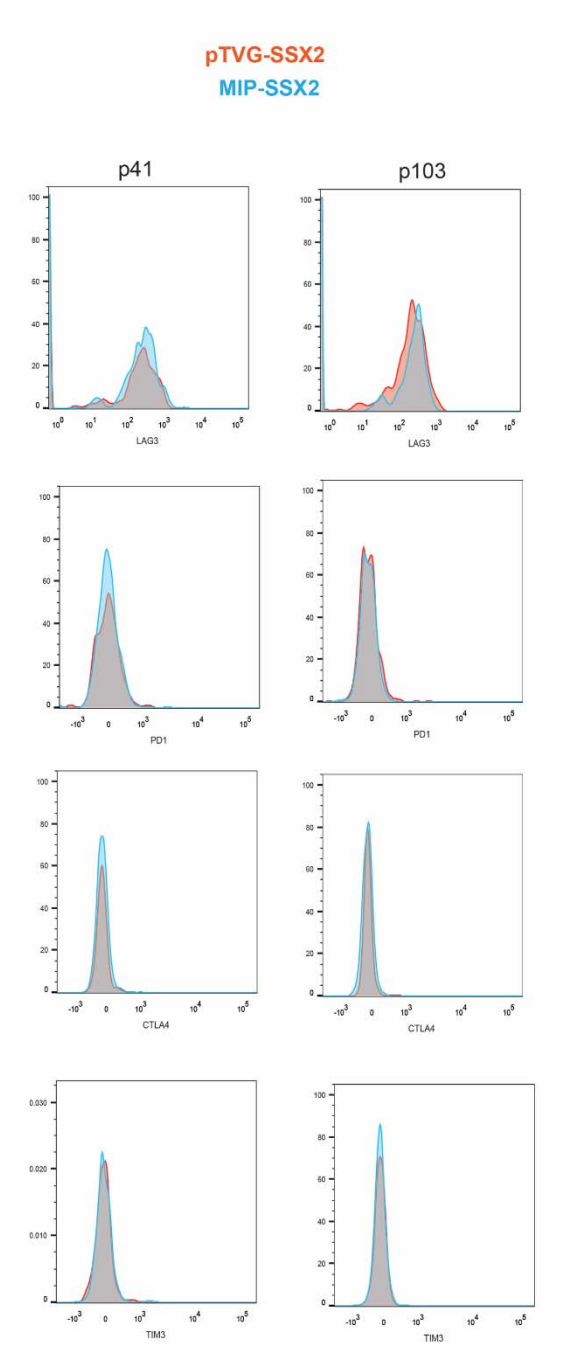
Supplementary Figure 2. DMC-SSX2 plasmid preps can be contaminated with genomic and parental plasmid DNA. The figure is of a representative gel showing DNA obtained from a miniciricle DNA prep (lane 2) based on the ΦC31 integrase sensitive ZYCY10P3S2T *E. coli* production system from System biosciences. Enzymatic clean up consisted of a linearizing digest followed by overnight digestion with a λ-nuclease and Exonuclease-I combination to yield pure minicircle (lane 3) confirmed by a linearizing digest (lane 5).



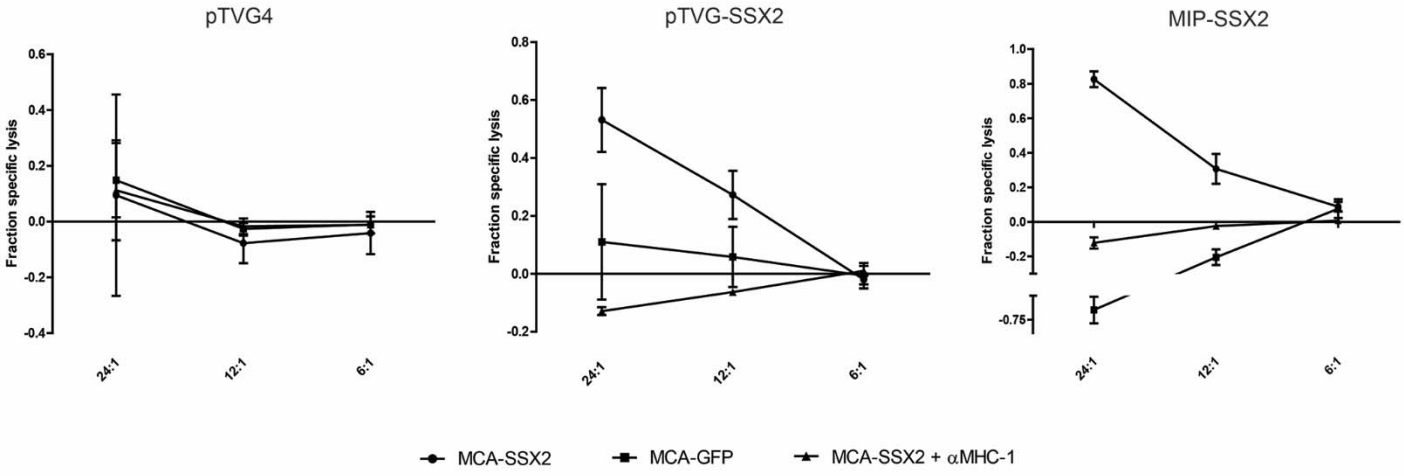
Supplementary Figure 3. DMC-SSX2 immunization elicits greater frequency of antigen-specific CD8 T cells when compared to conventional plasmid. HLA-A2/DRI mice (n= 4-6/group) were administered 3 biweekly intradermal immunizations with either empty vector control (pTVG4), pTVG-SSX2 (100µg), or DMC-SSX2 (48µg). Two weeks after the final immunization, splenocytes from these animals were assayed for SSX2-specific immune responses by tetramer staining (panel A) and ELISPOT (panel B). A) CD8 T cells were pooled from animals in each treatment group and stained for p41 and p103 tetramers. B) Splenocytes from individual animals were stimulated with the different peptides in an ELISPOT assay and the spot forming units (SFU) were counted.



Supplementary Figure 4. Gating strategy and representative flow plots for intracellular cytokine staining assay. A) Representative gating strategy to isolate CD8 T cells for analysis. B) Flow plots representing IFNγ (x-axis) and TNFα (y-axis) secretion by antigen re-stimulated CD8 T cells from a representative animal from each of the treatment groups.



Supplementary Figure 5. Expression of other checkpoint molecules on epitope specific CD8 T cells. From one representative animal are shown flow cytometry histogram overlays of LAG3, PD1, CTLA4, and TIM3 levels on CD8 T cells specific for p41 (left) and p103 (right) as assayed by tetramer staining.



Supplementary Figure 6. ***In vitro*** **cytotoxicity assay of immunized animals.** Splenocytes from animals treated as in Figure 4A were pooled into three replicate wells and re-stimulated with p41 and p103 SSX peptides along with 10U/ml IL2 for one week. Cells were then plated with either a relevant (MCA-SSX2) or irrelevant (MCA-GFP) target at different effector-to-target ratios for 4 hours, and assayed for LDH release. Cytotoxicity to MCA-SSX2 was also assessed in the presence of a HLA-A2 (MHC-I) blocking antibody as indicated.