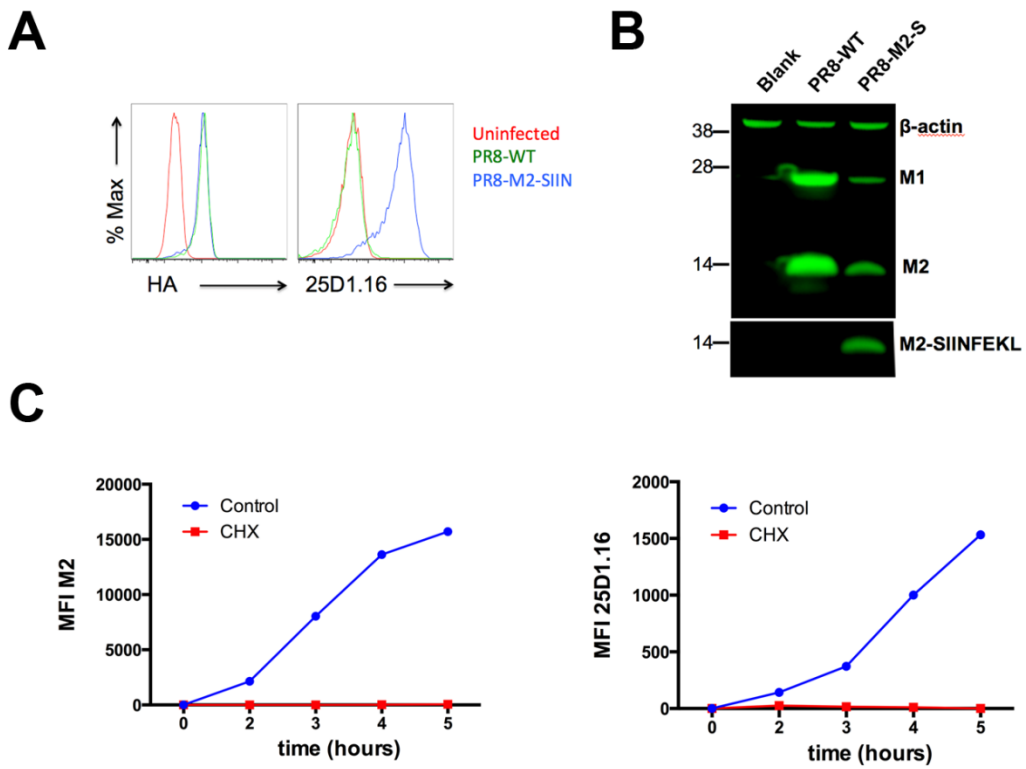

Fig S1. Characterization of PR8-M2-SIIN and antigen presentation after infection. (*A*) Flow cytometry analysis of surface levels of HA (left panel) and K^b-SIIN (right panel) in L-K^b cells after PR8 or PR8-M2-SIIN infection for 12 h. (*B*) Immunoblot of M1, M2, SIINFEKL and β-actin (loading control) present in total cell lysates from L-K^b cells infected with PR8 for 12 h Uninfected cells were used as a blank control. (*C*) L-K^b cells were infected with PR8-M2-SIIN, then CHX (25 μg/ml) or DMSO (control) were added. At indicated times, cell-surface M2 and K^b-SIINFEKL were measured by flow cytometry.

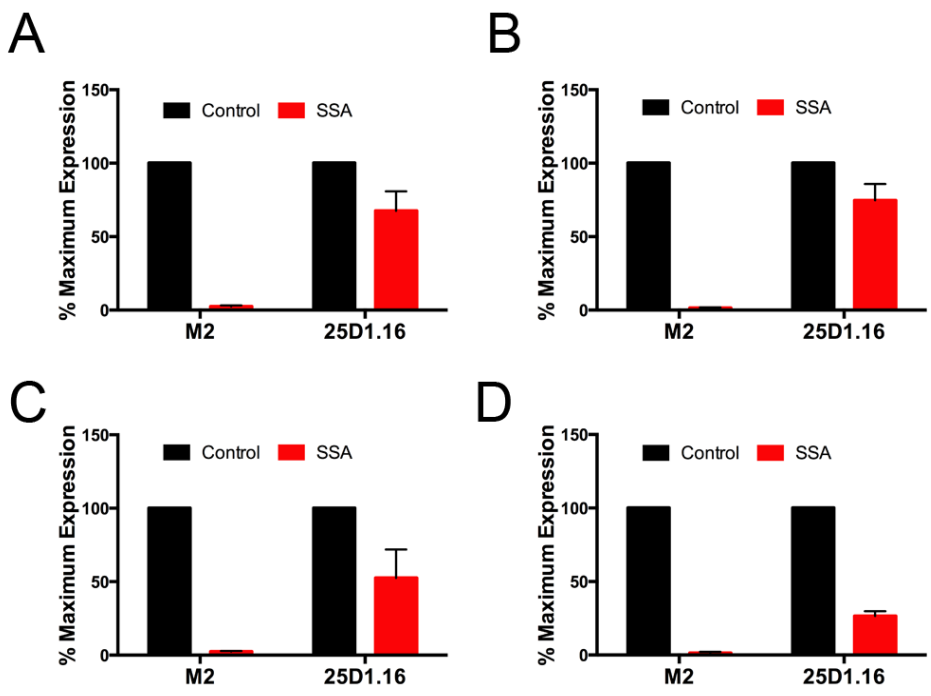

Fig S2. CUG start codon mediated mRNA translation partially contributes to antigen peptide generation after mRNA splicing inhibition. (*A*) L-K^b cells were infected with PR8-M2-M72L-SIIN, then SSA or MeOH (control) was added. After 20 h infection, cell-surface M2 and K^b-SIIN were analyzed by flow cytometry for the indicated times. The relative levels are represented as the percentage of the control. (*B-D*) L-K^b cells were infected with PR8-M2-C888T-SIIN (*B*), PR8-M2-G1001A-SIIN (*C*) or PR8-M2-C888T-G1001A-SIIN (*D*), Cell-surface M2 and K^b-SIIN were analyzed by flow cytometry.

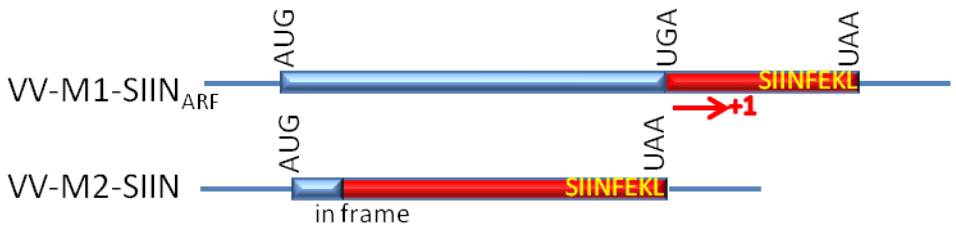

Fig S3. Diagram of constructs expressed in rVVs. Blue represents M1 reading frame, red represents M2 reading frame. Note that in M1-SIIN$_{ARF}$ SIINFEKL is downstream of a stop codon and in the +1 reading frame. As vaccinia virus cannot splice mRNA, the SIINFEKL must be synthesized via non-standard translation.