Foot-and-mouth disease (FMD) continues to be a significant threat to the health and economic value of livestock species. This acute infection is caused by the highly contagious FMD virus (FMDV), which infects cloven-hoofed animals including large and small ruminants and swine. Current vaccine strategies are all directed toward the induction of neutralizing antibody responses. However, the role of cytotoxic T lymphocytes (CTLs) has not received a great deal of attention, in part because of the technical difficulties associated with establishing a reliable assay of cell killing for this highly cytopathic virus. Here, we have used recombinant human adenovirus vectors as a means of delivering FMDV antigens in a T cell-directed vaccine in pigs. We tested the hypothesis that impaired processing of the FMDV capsid would enhance cytolytic activity, presumably by targeting all proteins for degradation and effectively increasing the class I MHC/FMDV peptide concentration for stimulation of a CTL response. We compared such a T cell targeting vaccine with the parental vaccine, previously shown to effectively induce a neutralizing antibody response. Our results show induction of FMDV-specific CD8(+) CTL killing of MHC matched target cells in an antigen specific manner. Further, we confirm these results by MHC tetramer staining. This work presents the first demonstration of FMDV specific, CTL killing and confirmation by MHC tetramer staining in response to vaccination against FMDV.