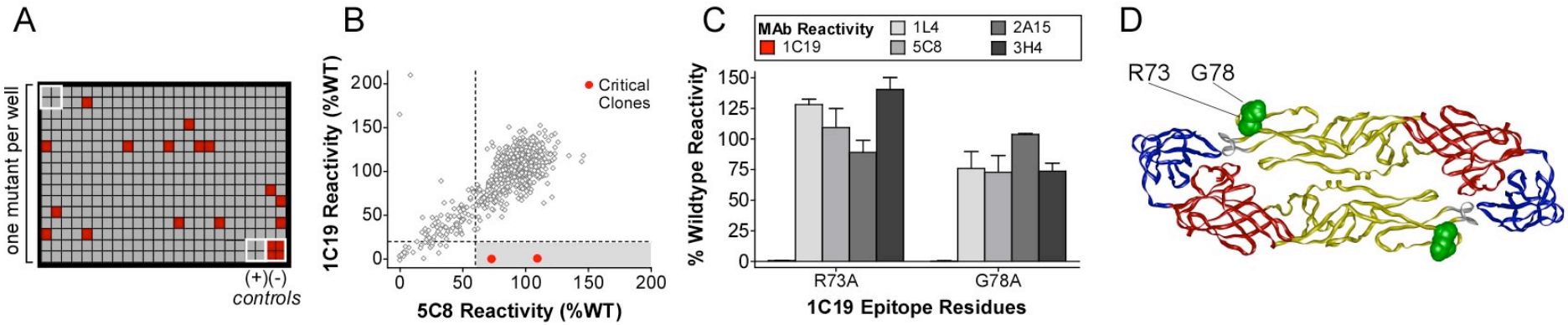

Supplemental Figure 2. Shotgun mutagenesis epitope mapping. (A) A mutation array for DENV4 prM/E protein encompassing prM and E proteins was constructed where each amino acid was mutated individually to alanine. Each well of the mutation array plate contained one defined mutant, expressed in human cells. Eight positive and four negative control wells were included on each plate. **(B)** Mammalian cells expressing mutant DENV4 prm/E proteins were tested for immunoreactivity with each mAb of interest and assessed using the Intellicyt high-throughput flow cytometer (shown for mAb 1C19). Clones with low reactivity for 1C19 relative to wild-type DENV protein, yet high reactivity for other MAbs (5C8 shown) were identified as critical for antibody binding. **(C)** Mutation of two individual residues in the DENV4 library significantly reduced 1C19 binding (red bars) but did not greatly affect binding of other conformation-dependent antibodies (black/gray bars). Bars represent the mean and range of two replicates. **(D)** Critical residues R73 and G78 were mapped onto the protein structure to visualize the 1C19 epitope.