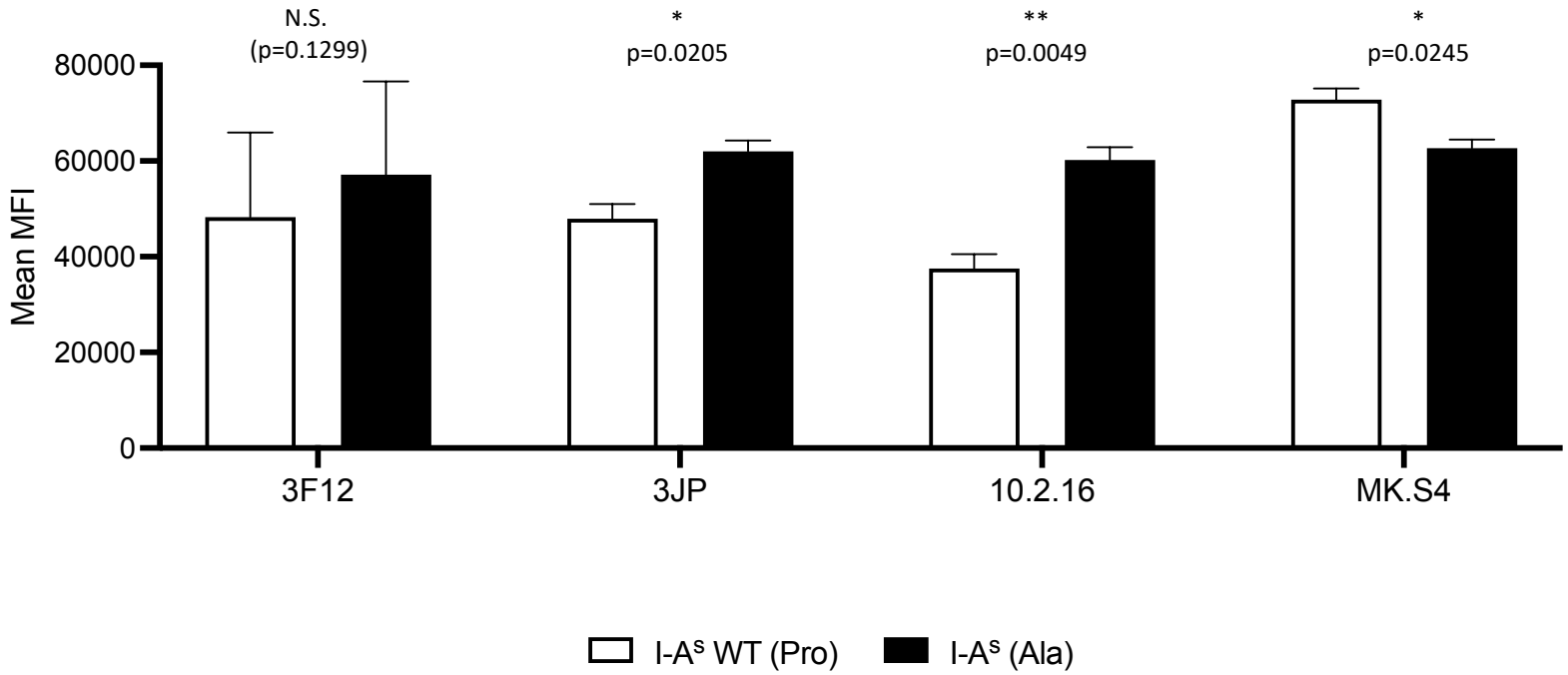
A.

B.

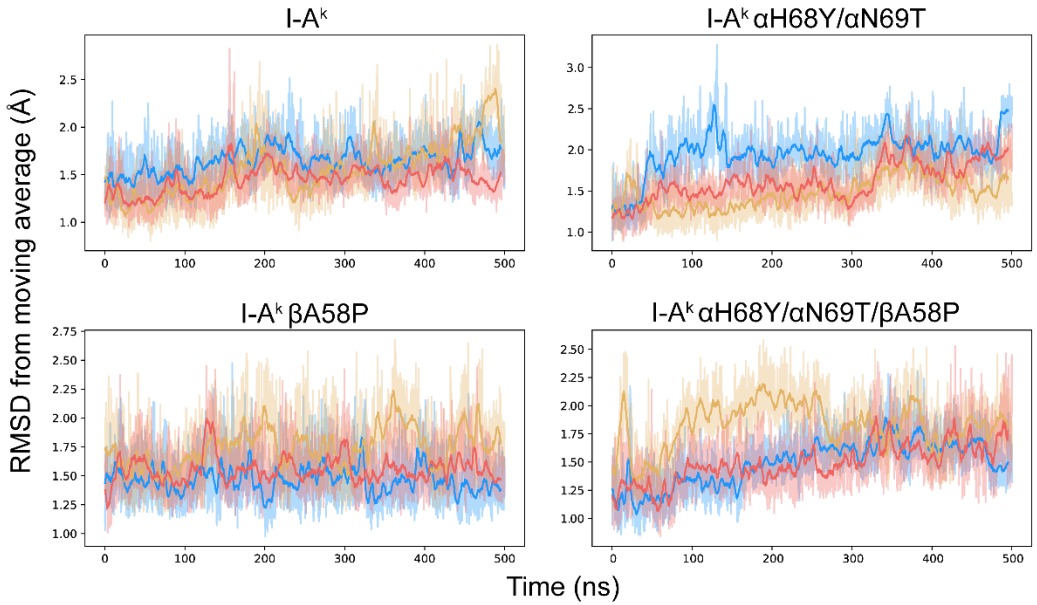
```
  C CAG TTC AAG GGC GAG TGC TAC TTC ACC AAC GGG ACG CAG CGC ATA CGA TCT GTG GAC
     Q   F   K   G   E   C   Y   F   T   N   G   T   Q   R   I   R   S   V   D

AGA TAC ATC TAC AAC CGG GAG GAG TAC CTG CGC TTC GAC AGC GAC GTG GGC GAG TAC CGC
 R   Y   I   Y   N   R   E   E   Y   L   R   F   D   S   D   V   G   E   Y   R

GCG GTG ACC GAG CTG GGG CGG CCA GAC CCC GAG TAC TAC AAT AAG CAG TAC CTG GAG CAA
 A   V   T   E   L   G   R   P   D   P   E   Y   Y   N   K   Q   Y   L   E   Q

ACG CGG GCC GAG CTG GAC ACG GTG TGC AGA CAC AAC TAC GAG GGG GTG GAG A
 T   R   A   E   L   D   T   V   C   R   H   N   Y   E   G   V   E
```

Supplemental Figure 1. Sequence of I-A^s beta alleles from SJL/J and B10.S mice.

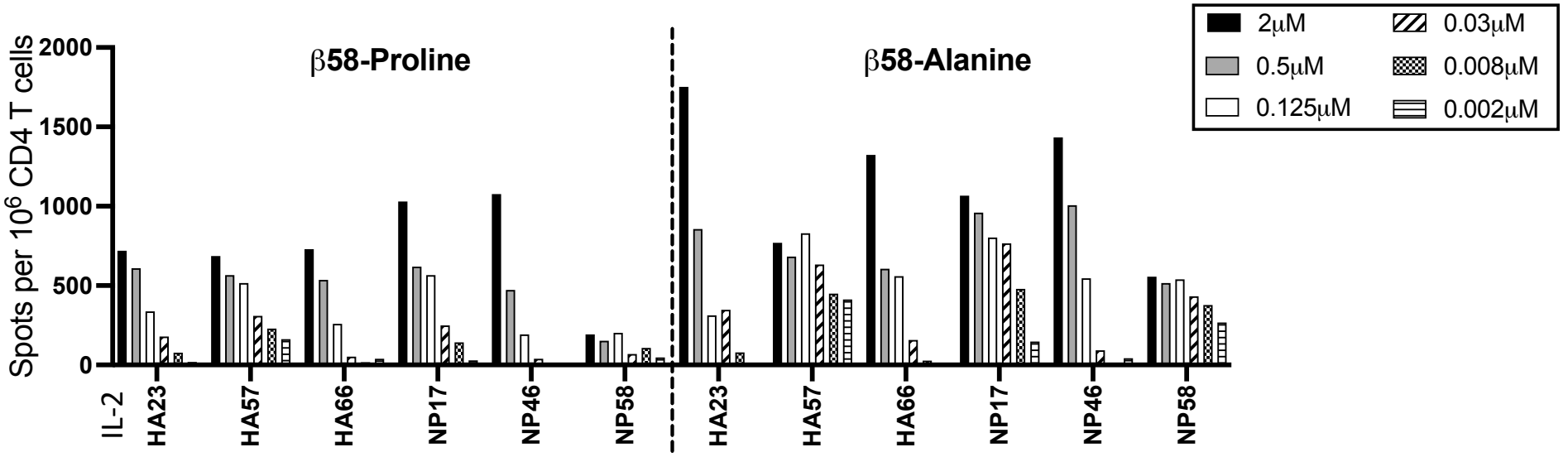
A. Exon 2 encoding the β1 domain is shown as a black arrow. PCR primers (red arrows) were used to amplify the β1 region from B10.A and SJL genomic DNA. Four independent PCR reactions were done for both B10.A and SJL/J to avoid PCR errors. The PCR product was gel purified and sequenced from both ends using internal primers (blue arrows).
B. The IA^s beta chain nucleotide and corresponding protein sequence is shown and the Proline at position 58 is highlighted in red. The nucleotide sequences from B10.S and SJL/J, both purchased from Jackson laboratories were identical.



Supplemental Figure 2. Reactivity of APC expressing either I-A^s (βPro58) or its variant I-A^s (βAla58). The average of three independent experiments with the standard error of the mean is shown for the mAb reactive with both I-A^s MHC class II molecules. An unpaired t-test was used to assess statistical significance.



Supplemental Figure 3 . RMS deviations (RMSD) values calculated from the average structure for the α carbons of I-A^k and each variant simulated, showing results for each of the three simulations (blue, red, orange). Thick bars represent moving averages. The similarities for each simulation demonstrate the reproducibility among the three simulations.



Supplemental Figure 4. CD4 T cell recognition of APC expressing either I-A^s (βPro58) or its variant I-A^s (βAla58). B10.S mice were infected with A/New Caledonia/20/99 H1N1 influenza virus and at day 10 post infection CD4 T cells were isolated from the spleen and tested for reactivity by production of IL-2, with the indicated influenza peptides, previously identified by our laboratory. Graded doses of the indicated peptide were added to the cultures and cytokine producing cells were enumerated by cytokine EliSpot assays. Results are presented as the spots per million CD4 T cells with background subtracted.