SUPPLEMENTARY MATERIALS for Quinlan et al., "Direct expression and validation of phage-selected peptide variants in mammalian cells"

Supplementary Fig. 1. The generation of a soft-randomized library using pDQ1. A, Primers used to generate a soft-randomized library of CD4mim variants based on a template of CD4mim4. Regions used for priming and complementary to the STII* signal peptide (Primer 1) or the second splice donor (Primer 2) are boxed. Bold red nucleotides indicate hand-mixes at an 85:5:5:5 ratio favoring the original template nucleotide. S indicates a 50:50 ratio of G:C, used for wobble positions when an amino acid is encoded by four or more distinct codons. Amino acids in bold black are soft-randomized, whereas gray amino acids are held fixed. **B,** Library synthesis. The primers shown in A were phosphorylated and used to amplify a linearized pDQ vector. The 5.6 kB PCR product was digested with Dpn I to eliminate the original vector, and the full-length product was isolated and ligated. Colors correspond to elements of text Fig. 1.

Supplementary Fig. 2. The development of CD4mim6. The sequence of CD4mim4, the template for a second soft-randomized library, is shown along with a representative panel of outputs from this library panned against a clade B (ADA) and clade C (ZM651) isolate. Outputs by immunoprecipitation (for example Fig. 2C), flow cytometry (e.g., Fig. 3A) and neutralization studies (e.g. Figs. 2D and 3C) identified best performers whose elements were recombined. The resulting composite variants were reassayed, and an optimal variant, in this case, CD4mim6 was identified. As shown CD4mim6 combines elements of CD4mim5, the best output from this screen, and two additional good outputs.

A

Primer 1 (reverse complement)

CGATTGCTACAGGTGCCTACGCA**GATAAGCTSCATTC**STGC**AAGCTSCG**STGC**AATTCSCTSGGSCTSCATGGSAAGCTSGC**SGGCAGC

I A T G A Y A **D K L H S** C **K L R** C **N S L G L H G K L A** G S

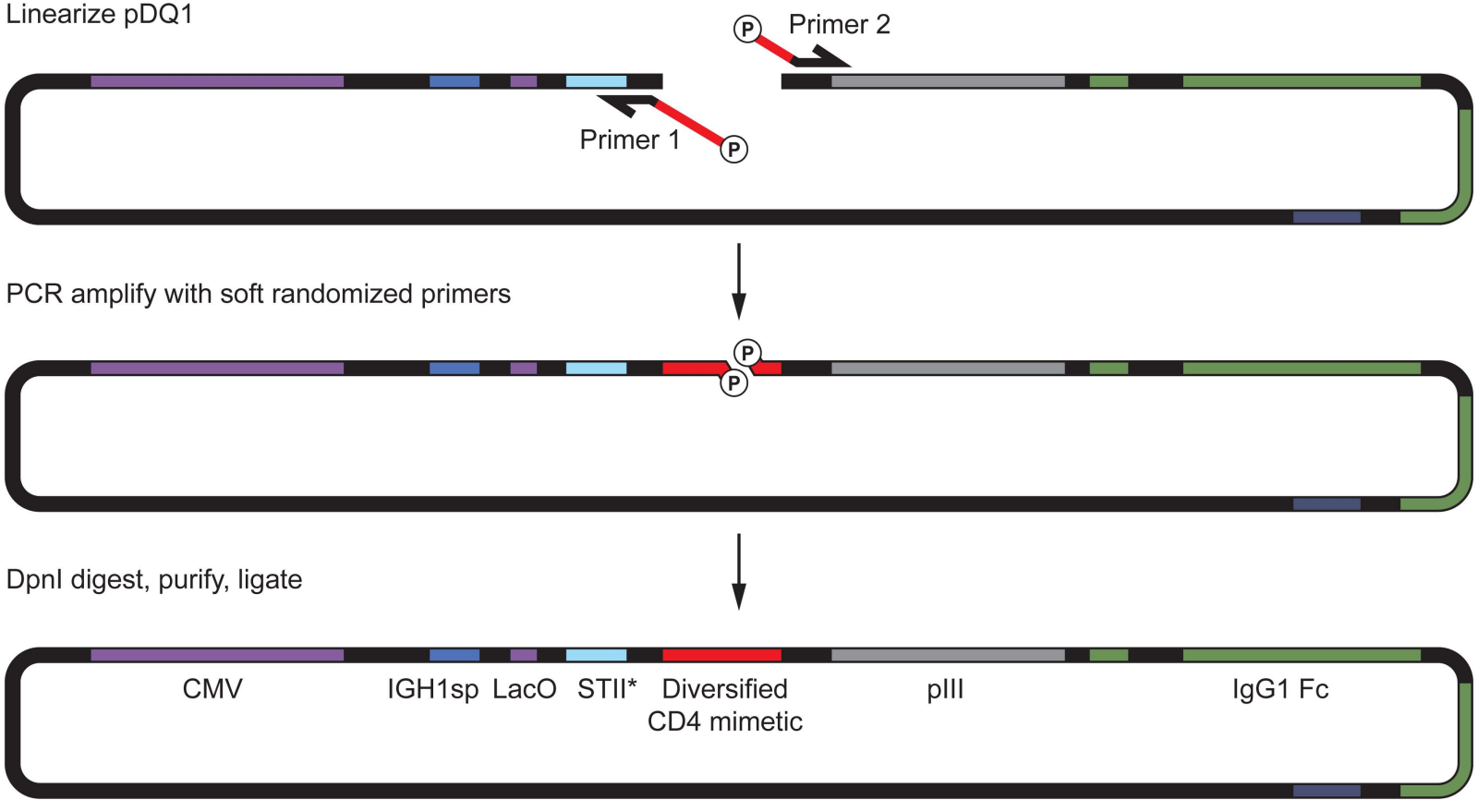
Primer 2

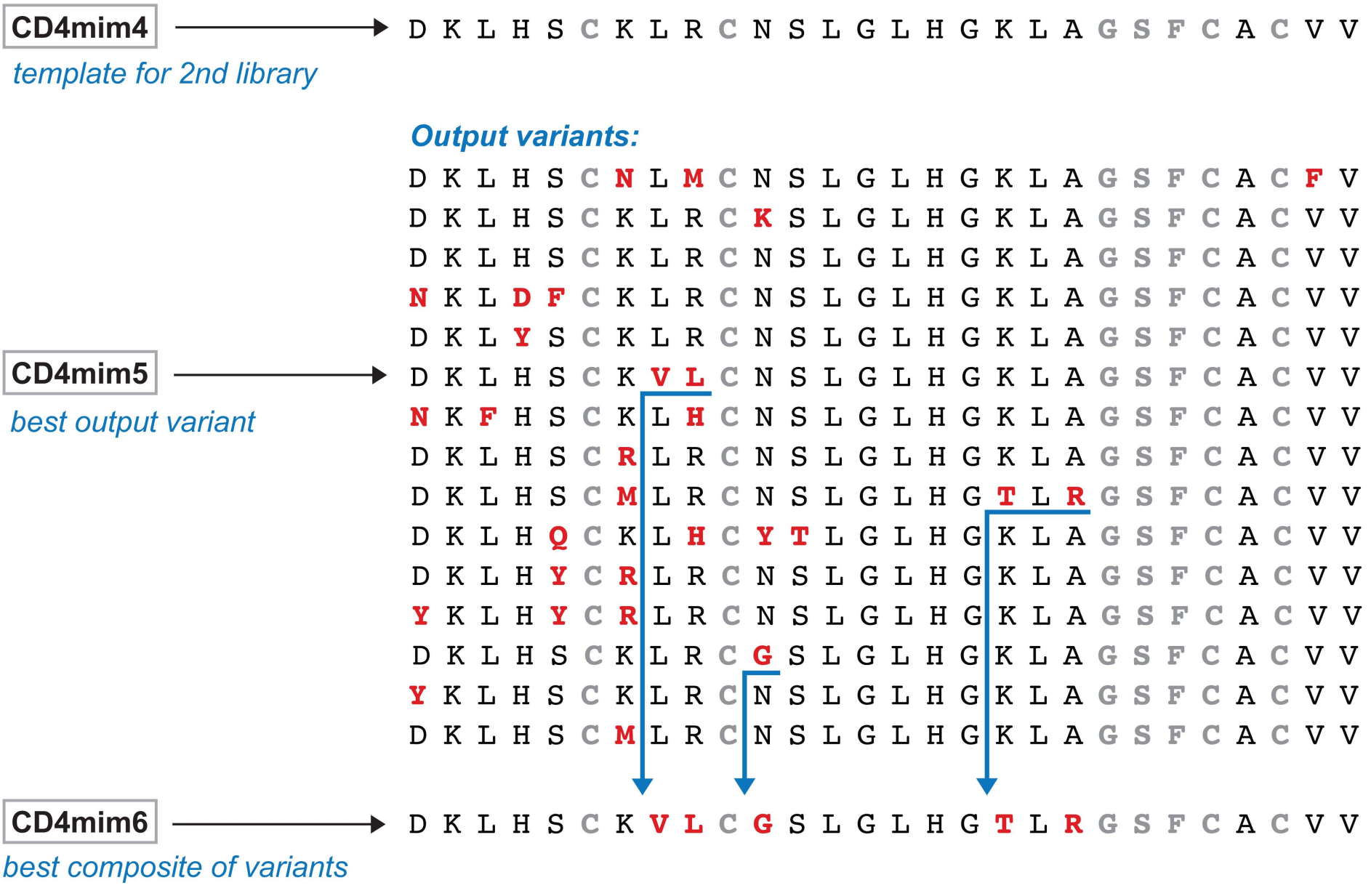
TTCTGC**GC**STGC**GT**S**GT**S GGCGGCGCCGCGGATCCCGAGGGTGA

F C **A** C **V V** G G A A D P E G

G/A/C/T = doped nucleotides at 85:5:5:5 ratio S = 50:50 G:C = Complementary to pDQ1

B




CD4mim4
D K L H S C K L R C N S L G L H G K L A G S F C A C V V
template for 2nd library
Output variants:
D K L H S C N L M C N S L G L H G K L A G S F C A C F V
D K L H S C K L R C K S L G L H G K L A G S F C A C V V
D K L H S C K L R C N S L G L H G K L A G S F C A C V V
N K L D F C K L R C N S L G L H G K L A G S F C A C V V
D K L Y S C K L R C N S L G L H G K L A G S F C A C V V
CD4mim5
D K L H S C K V L C N S L G L H G K L A G S F C A C V V
best output variant
N K F H S C K L H C N S L G L H G K L A G S F C A C V V
D K L H S C R L R C N S L G L H G K L A G S F C A C V V
D K L H S C M L R C N S L G L H G T L R G S F C A C V V
D K L H Q C K L H C Y T L G L H G K L A G S F C A C V V
D K L H Y C R L R C N S L G L H G K L A G S F C A C V V
Y K L H Y C R L R C N S L G L H G K L A G S F C A C V V
D K L H S C K L R C G S L G L H G K L A G S F C A C V V
Y K L H S C K L R C N S L G L H G K L A G S F C A C V V
D K L H S C M L R C N S L G L H G K L A G S F C A C V V
CD4mim6
D K L H S C K V L C G S L G L H G T L R G S F C A C V V
best composite of variants