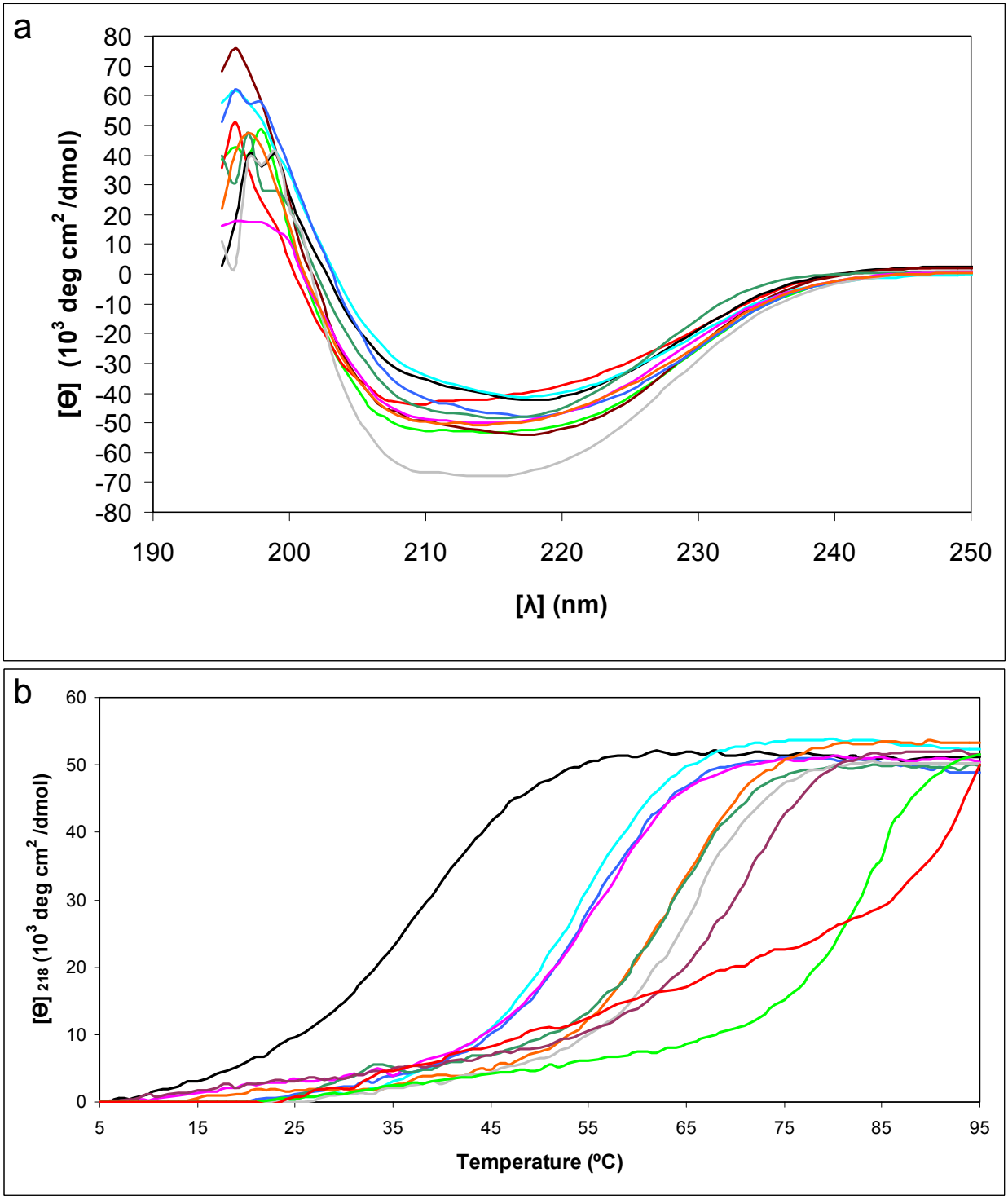

Supplementary Figure. Far UV Circular Dichroism Scans and Thermal Unfolding Curves for Gβ1-WT and Mutant Variants. (a) The CD spectra of the nine variants are nearly identical to that of the wild-type protein. ■MonB-WT, ■MonB-A45V, ■Gβ1-Y45A, ■Gβ1-W43A, ■Gβ1-W43Y, ■MonB-ORDES, ■Gβ1-W43V, ■MonB-A45Y, ■Gβ1-WT, ■MonA (data not normalized). **(b)** Thermal denaturation of all variants monitored by CD at 218 nm (normalized as described in Supplementary Methods).

Supplementary Material

Oligonucleotide Sequences

Construction of the Chimeric Construct Plasmid

The gene for RNAP-α was amplified by PCR from the pTRG plasmid and cloned into the pBT vector downstream of λcI via EcoRI/BamHI (oligonucleotides: 5'-GCGTCTG AATTCAT GCAGGGTTCTGTGACAGAGTTTCT-3' and 5'-GAATTATAGATCCGGCCGCCTCTGGTT TCTCTTCTTT-3'). The genes for the Gβ1 variants were sub-cloned into the chimeric vector with the engineered restriction sites NotI/EcoRI (oligonucleotides: 5'-AAGAGGCGGCCGCAT CTACTACTTACAAATTAATCCTTAA-3' and 5'-GGTGGTGATTCCCTTCAGTAACTGTA AAGGTCTTAGT-3').

Creation of the Gβ1 Mutant Variants

Point mutants of Gβ1-WT and MonB were produced using the QuikChange® method with the following oligonucleotides:

ProG W43Y F (5'GACAACGGTGTTGACGGTGAATATACTTACGACGATGCGACTAAG)

ProG W43Y R (5'CTTAGTCGCATCGTCGTAAGTATATTCACCG TCAACACCGTTGTC)

ProG W43V F (5'GACAACGGTGTTGACGGTGAAGTGACTT ACGACGATGCGACTAAG)

ProG W43V R (5'CTTGTCGCATCGTCGTAAGTCACTT CACCGTCAACACCGTTGTC)

ProG W43A F (5'GACAACGGTGTTGACGGTGAAGC GACTTACGACGATGCGACTAAG)

PROG W43A R (5'CTTAGTCGCATCGTCGTAA GTCGCTTCACCGTCAACACCGTTGTC)

MONB A45Y F (5'TTAAGGGTGAATGGA CAGTAGATGAAGCGACCAAGAC)

MONB A45Y R (5’GTCTTGGTCGCTTCATCTAC TGTCCATTCACCCTTAA)

MONB A45V F (5’TTAAGGGTGAATGGACATACGATGAAGCGACCAAGAC)

MONB A45V R (5’GTCTTGGTCGCTTCATCGTATGTCCATT CACCCTTAA).

Total Gene Synthesis via Recurcive PCR

Synthetic DNA oligonucleotides were used for recursive PCR synthesis of the genes for the MonB-ORDES variant and the genes in the randomized library.

1(B1+LIB): (5’GCGGCCGCATCTACCTATAAGCTGATTCTGAATGGCAAGACCCTGAA AGGTGAAACCACGACCGAA)

2(B2): (5’ CCGCTGCATACTGTGCAAATACATCCTTTGCTGTTGCTTTGTCCACTGCTTC GGTCGTGGTTTCACCTTT)

3 (B3+LIB): (5’-TATTTGCACAGTATGCAGCGGATAACGGTGTTAAGGGTGAATGG)

4 (B4): (5’-GGAATTCCCTTCGGTCACGGTGAACGTCTTGGTCGCTTCGTCGAATGTCC ATT CACCCTTAACACCGTT)

5 (B_PRIMER_F) (5’GCGCACTATCGCGGCCGCATCTAC)

6 (B_PRIMER_R) (5’GCGCACGCTCGTGGAATTCCCTTCG)

2’ (B_LIB2): (5’CCGCTGCATACTGTGCAAATACATCXXXTGCTGTTGCXXXGTCCACT GCTTCGGTCGTGGTTTCACCTTT)

4’ (B_LIB4): (5’GGAATTCCCTTCGGTCACGGTGAACGTCTTGGTCGCTTCGTCXXXTG TCCATTCACCCTTAACACCGTT)

Protein Expression and Purification

For protein expression the genes for the Gβ1 variants were sub-cloned into pET-21a (Novagen) using the restriction enzymes NdeI and EcoRI (oligonucleotides: 5'-GTC CGC GGT CAT ATG ACT ACT TAC AAA-3' and 5'-GGC GCA GAT GAA TTC TTA TTC AGT AAC TGT AAA-3') and transformed into BL21(DE3).