SUPPLEMENTARY TABLE

construct	Expression	N
hβ1 ABCD regions		
hβ1F	3.9 ± 3.8	(109)
hβ1F A	0.6 ± 1.0	(3)
hβ1F B	3.7 ± 2.8	(3)
hβ1F C	3.7 ± 2.8	(7)
hβ1F AB	1.4 ± 1.9	(4)
hβ1F ABC	2.3	(1)
hβ1F ABCD	3.3 ± 10.2	(11)
Mode 1		
hβ1F* (D37C, R41N)	78.2 ± 17.5	(6)
X2F hβ3/hβ1* (D37C, R41N)	89.2 ± 27.3	(3)
hβ1F (R41N)	2.9 ± 1.1	(3)
hβ1F (K68Q)	5.0 ± 4.8	(3)
hβ1F (K68L)	4.6	(1)
hβ1F (D37A, K68Q)	46.1	(1)
hβ1myc (K68L)	11.9 ± 20.6	(3)
hβ1myc (K68Q)	0 ± 0	(2)
hβ1F (D37A, E165N)	56.6 ± 11.9	(4)
hβ1F (T161E)	5.4 ± 7.8	(4)
hβ1F (T161A)	2.4 ± 1.4	(4)
hβ1F (K197A)	1.4 ± 1.5	(3)
hβ1F (K197E)	0.7 ± 0.0	(3)
hβ1F (K197N)	0.1 ± 0.1	(3)
hβ1F (T161A, K197N)	6.4 ± 2.7	(4)
hβ1F (T161E, K197N)	2.6 ± 0.6	(2)
Mode 2		
X1F hβ1/hβ3	8.8 ± 4.0	(5)
X1F hβ1 (E5D, S7G)/hβ3	9.8 ± 5.8	(2)
X1F hβ1 (T3V, E5D, S7G)/hβ3	0.5 ± 0.5	(3)
X2F hβ3/hβ1*	16.0 ± 6.1	(3)
X2F hβ3/hβ1 ABCD	10.6 ± 5.1	(3)
X1F hβ1 (S73A)/hβ3	8.7 ± 5.7	(3)
X1F hβ1 (S66Y, K68R, S73A)/hβ3	35.8 ± 16.1	(6)
hβ1F (S66Y, K68R, S73A)	0.9 ± 1.5	(3)
hβ1F (V44I, A45V, S66Y, K68R, N169R, E172D)	6.0 ± 6.4	(12)
hβ1F (V44I, S66Y, K68R, S73A, N169R)	3.5 ± 9.1	(5)
hβ1F (V44I, S66Y, K68R, N169R)	2.9 ± 2.6	(3)
hβ1F (V44I, K68R, N169R)	2.1 ± 1.9	(3)
hβ1F (V44I, S66Y, N169R, E172D)	2.1	(1)
hβ1F (V44I, S66Y, K68R, N169R, E172D)	1.2 ± 0.7	(4)
hβ1F*(N169R)	0.2 ± 0.2	(2)
Mode 3		
hβ1F (V44I, A45V, D190E, Y191H, K192R, M193L)	13.6 ± 5.0	(8)
hβ1F (V44I) C	9.4 ± 4.1	(4)

Supplementary Table. Additional homomeric assembly data describing the ABCD regions of loop 9 and 10 and modes 1 and 2. Data are expressed as in Table I. The Table predominantly presenta results where little or no effect was observed.

Supplementary Figures

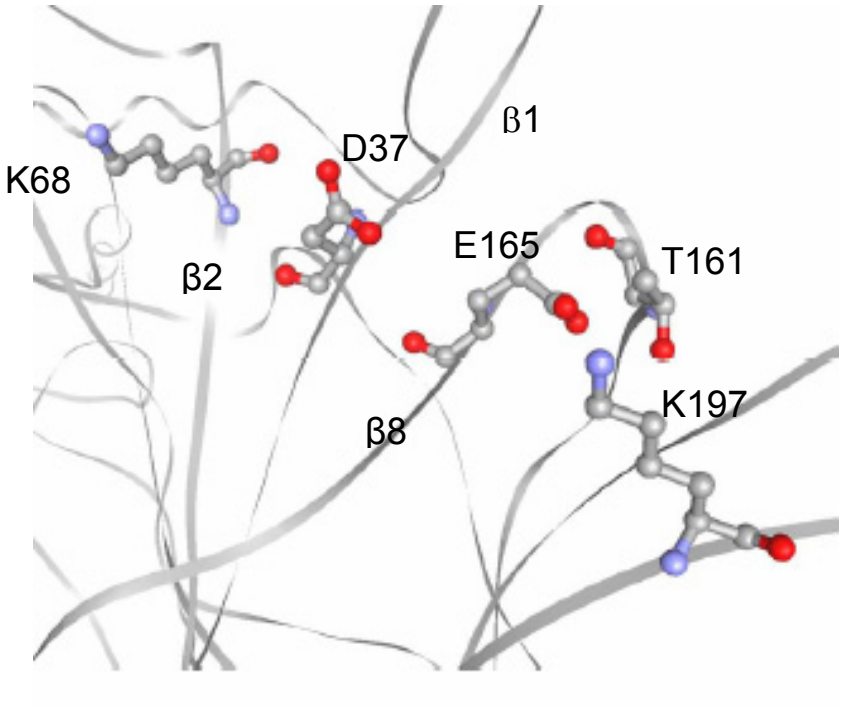
Supplementary Figure 1. Modeling of mode 1 assembly.
D37 is shown between K68 and E165 on flanking β sheets β2 and β8. Mutating E165 to N resulted in expression while K68R did not. Modeling further identified residues near E165, however mutations to T161 and K197 individually or together did not result in assembly.

Supplementary Figure 2. Modeling of mode 3 assembly.
A45V of the subunit contributing the minus side of the interface is shown in relationship to residue F105 and A135 of the plus subunit at the interface. Panel (A) illustrates hβ1 wild type while panel (B) shows A45V.

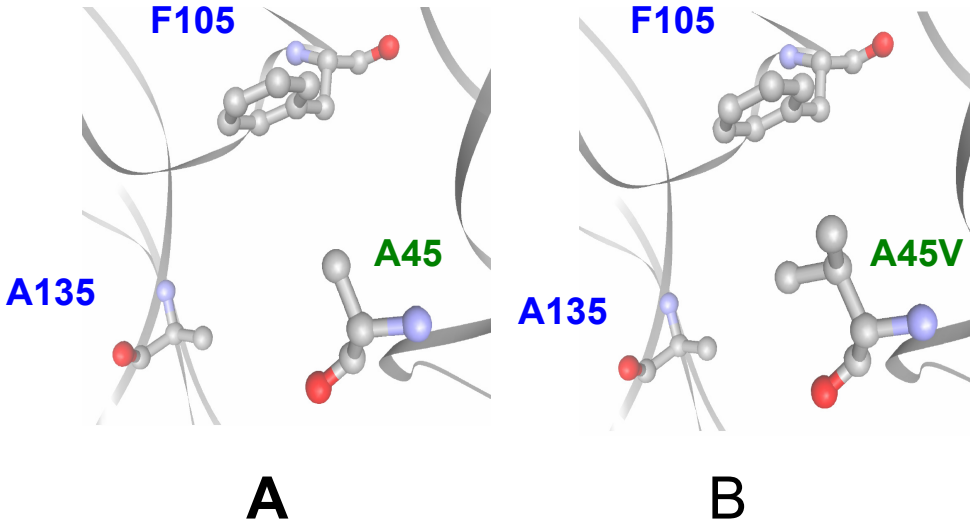
Supplementary Figure 3. Summary of loop and β sheet interactions at the plus and minus interfaces of adjacent subunits.
Each panel shows a map for one construct; the name and relative expression of the construct are shown at the top of each panel. In each panel, the plus face (left) and minus face (right) are shown for a homomeric pair of subunits. The regions from each subunit which can contribute to interactions across the interface are shown from top to bottom on the respective sides, with b1 indicating β sheet 1 and l2 indicating loop 2. When the name of the region is *italicized*, it indicates that the region differs from that of wild type β3, while when the region is in **bold type** it indicates that the region has been mutated in the construct.

The structure at the interaction is indicated by the following notations: *** with yellow shading indicates regions like β3 in the wild type subunit; ~ with no shading regions different from β3; ++ with green shading regions mutated to be like β3; and xx with blue shading regions of β3 mutated to reduce expression. Panel A1 also shows the locations of residues and regions examined in this study, for example 45 indicates residue 45 and A the A region. This figure summarizes the data suggesting that β sheets 1 and 2, and loops 9 and 10 contribute strongly to assembly.

The top row (A1-A4) shows wild-type β1 and β2 subunits, then constructs which reduced expression of β3 or enhanced expression of β2. The bottom row shows constructs which enhanced expression of β1, including examples of mode 2 (B1, B2) and mode 3 (B3, B4). Note that mode 1 assembly is not shown in this figure.



Supplementary Figure 1.



Supplementary Figure 2


A1
hβ1
4%
I1 ~ a1
I1 *** b3
I2 ~ I9 A, B
I4 *** b5
I5 ~ b1 44, 45
I5 *** b5
I5 *** b6
I7 ~ b1 44, 45
I7 ~ I9 A, B
I8 ~ a1
I8 ~ b2 66, 68
I8 *** b3
I8 *** b5
I8 *** b6
D I10 ~ I9 A, B
(Plus face) (Minus face)
A2
mβ2
6%
I1 ~ a1
I1 *** b3
I2 ~ I9
I4 *** b5
I5 *** b1
I5 *** b5
I5 *** b6
I7 *** b1
I7 ~ I9
I8 ~ a1
I8 ~ b2
I8 *** b3
I8 *** b5
I8 *** b6
I10 ~ I9
A3
hβ3 (V45A, DTNK)
13%
I1 *** a1
I1 *** b3
I2 xx I9
I4 *** b5
I5 xx b1
I5 *** b5
I5 *** b6
I7 xx b1
I7 xx I9
I8 *** a1
I8 *** b2
I8 *** b3
I8 *** b5
I8 *** b6
I10 xx I9
A4
mβ2 GKER
20%
I1 ~ a1
I1 *** b3
I2 ++ I9
I4 *** b5
I5 *** b1
I5 *** b5
I5 *** b6
I7 *** b1
I7 ++ I9
I8 ~ a1
I8 ~ b2
I8 *** b3
I8 *** b5
I8 *** b6
I10 ++ I9
B1
X3 hβ1 (S66Y, K68R, S73A) / hβ3
36%
I1 ~ a1
I1 *** b3
I2 ++ I9
I4 *** b5
I5 ~ b1
I5 *** b5
I5 *** b6
I7 ~ b1
I7 ++ I9
I8 ~ a1
I8 ~ b2
I8 *** b3
I8 *** b5
I8 *** b6
I10 ++ I9
B2
hβ1* AB
61%
I1 ~ a1
I1 *** b3
I2 ++ I9
I4 *** b5
I5 ++ b1
I5 *** b5
I5 *** b6
I7 ++ b1
I7 ++ I9
I8 ~ a1
I8 ++ b2
I8 *** b3
I8 *** b5
I8 *** b6
I10 ++ I9
B3
X3 hβ1 (V44I, A45V) / hβ3
52%
I1 ~ a1
I1 *** b3
I2 ++ I9
I4 *** b5
I5 ++ b1
I5 *** b5
I5 *** b6
I7 ++ b1
I7 ++ I9
I8 ~ a1
I8 ~ b2
I8 *** b3
I8 *** b5
I8 *** b6
I10 ++ I9
B4
hβ1 (A45V, K196R)
43%
I1 ~ a1
I1 *** b3
I2 ~ I9
I4 *** b5
I5 ++ b1
I5 *** b5
I5 *** b6
I7 ++ b1
I7 ~ I9
I8 ~ a1
I8 ~ b2
I8 *** b3
I8 *** b5
I8 *** b6
I10 ++ I9