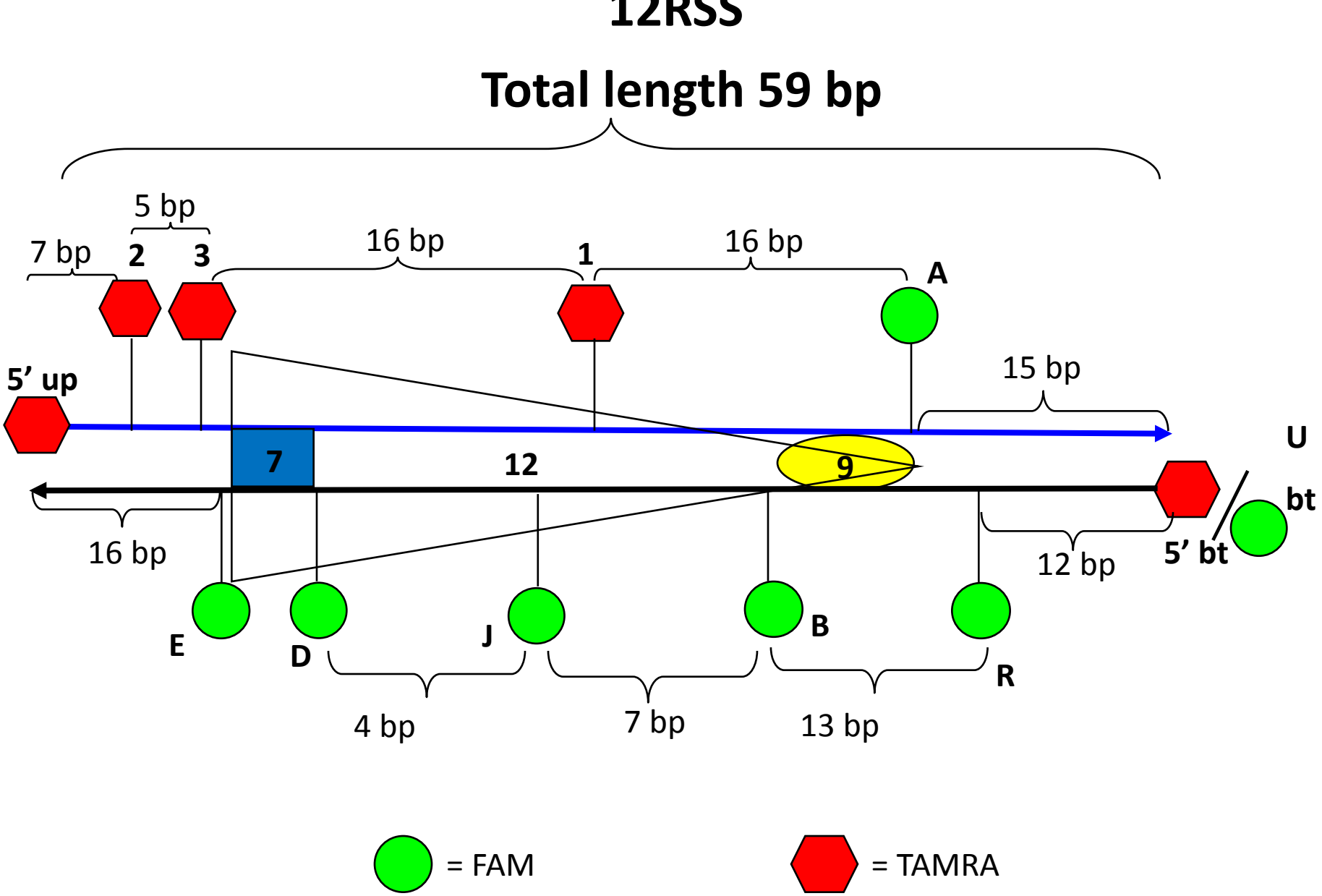
12RSS
Total length 59 bp
5 bp
7 bp
2
3
16 bp
1
16 bp
A
5' up
15 bp
U
7
12
9
bt
16 bp
12 bp
5' bt
E
D
J
B
R
4 bp
7 bp
13 bp
= FAM
= TAMRA

Fig. S1

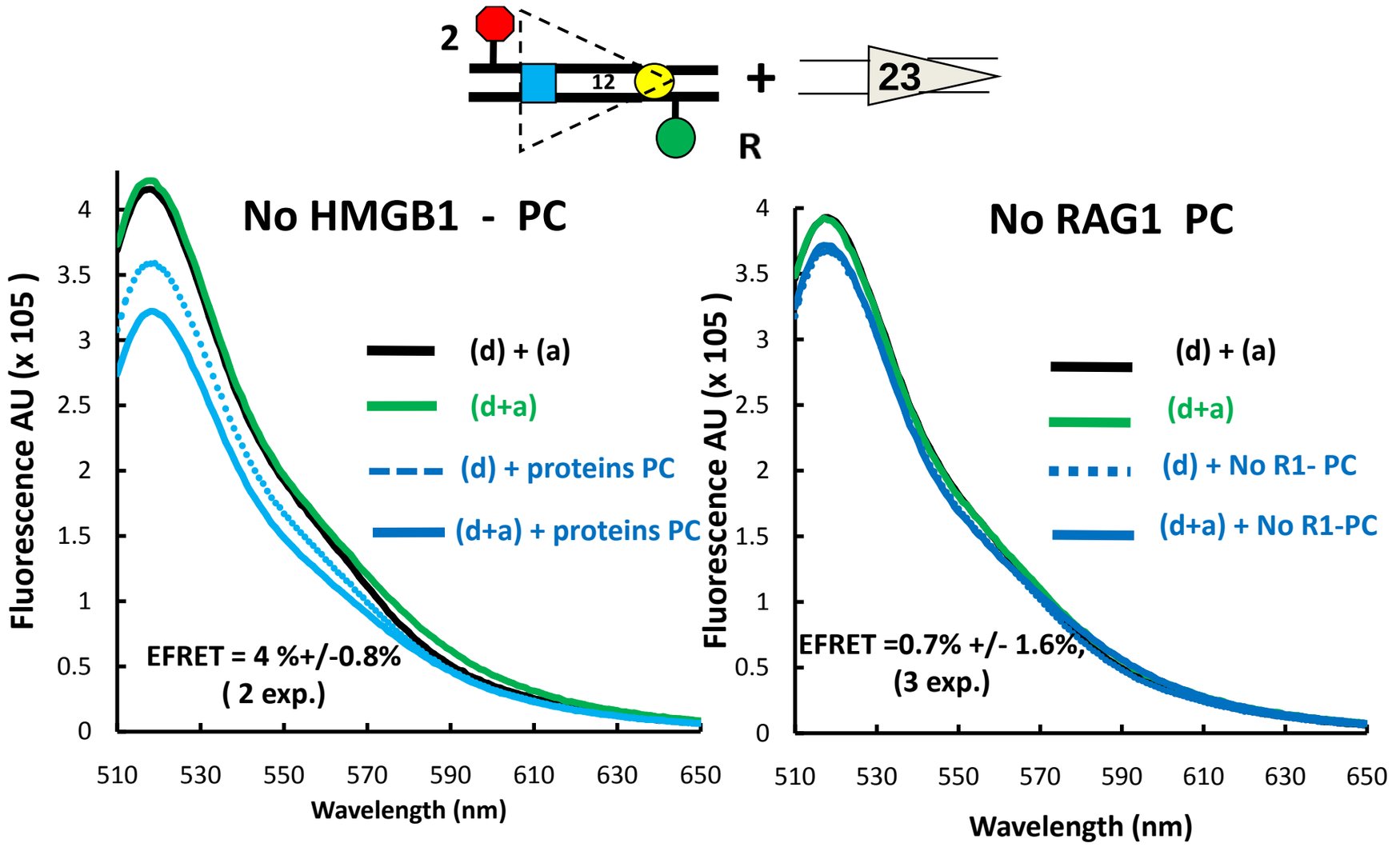
2
12
+
23
R
No HMGB1 - PC
Fluorescence AU (x 105)
(d) + (a)
(d+a)
(d) + proteins PC
(d+a) + proteins PC
EFRET = 4 %+/-0.8%
(2 exp.)
Wavelength (nm)
No RAG1 PC
Fluorescence AU (x 105)
(d) + (a)
(d+a)
(d) + No R1- PC
(d+a) + No R1-PC
EFRET =0.7% +/- 1.6%,
(3 exp.)
Wavelength (nm)

Fig. S2

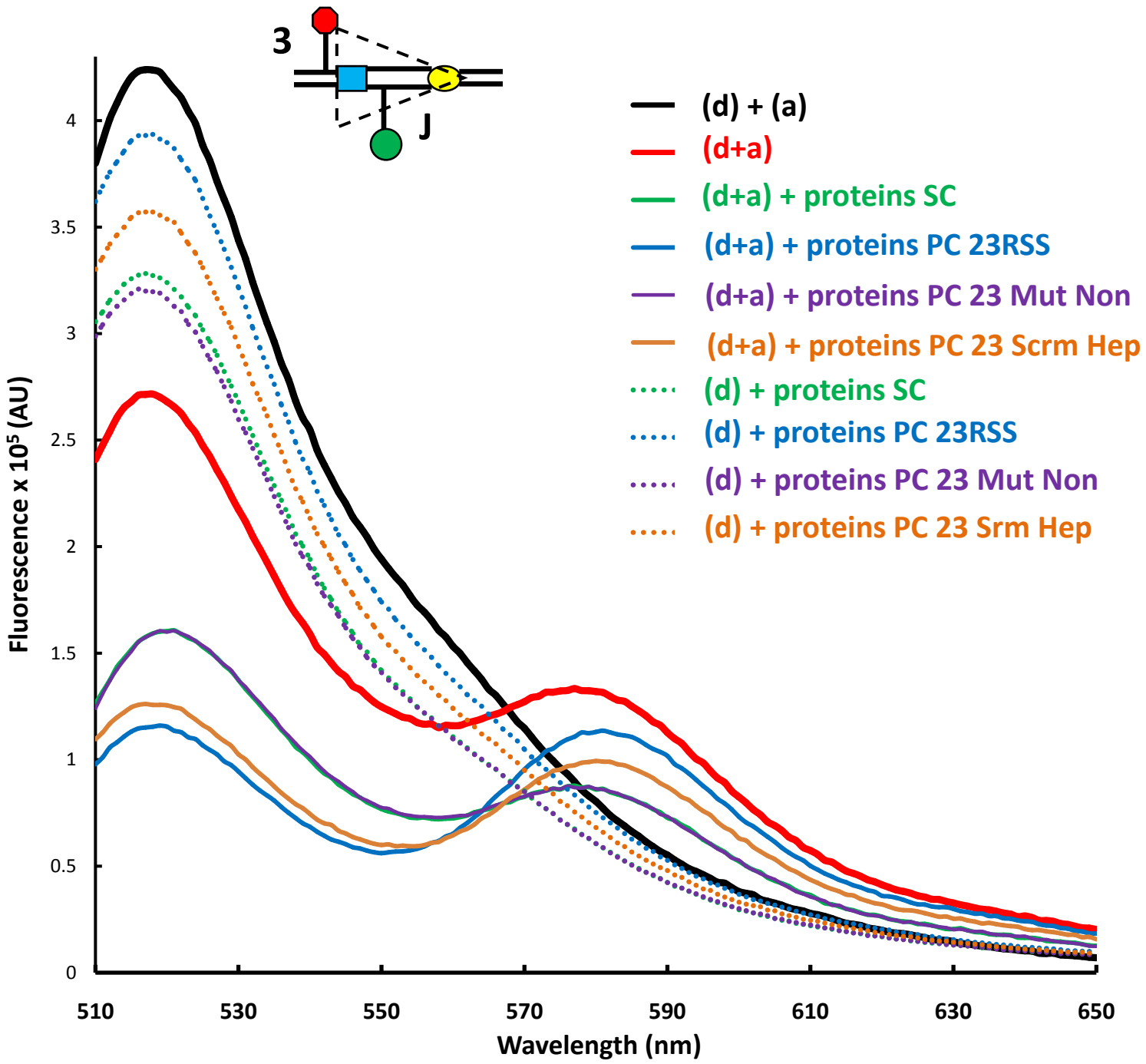
3
J
(d) + (a)
(d+a)
(d+a) + proteins SC
(d+a) + proteins PC 23RSS
(d+a) + proteins PC 23 Mut Non
(d+a) + proteins PC 23 Scrm Hep
(d) + proteins SC
(d) + proteins PC 23RSS
(d) + proteins PC 23 Mut Non
(d) + proteins PC 23 Srm Hep
Fluorescence x 10⁵ (AU)
0
0.5
1
1.5
2
2.5
3
3.5
4
510
530
550
570
590
610
630
650
Wavelength (nm)

Fig. S3

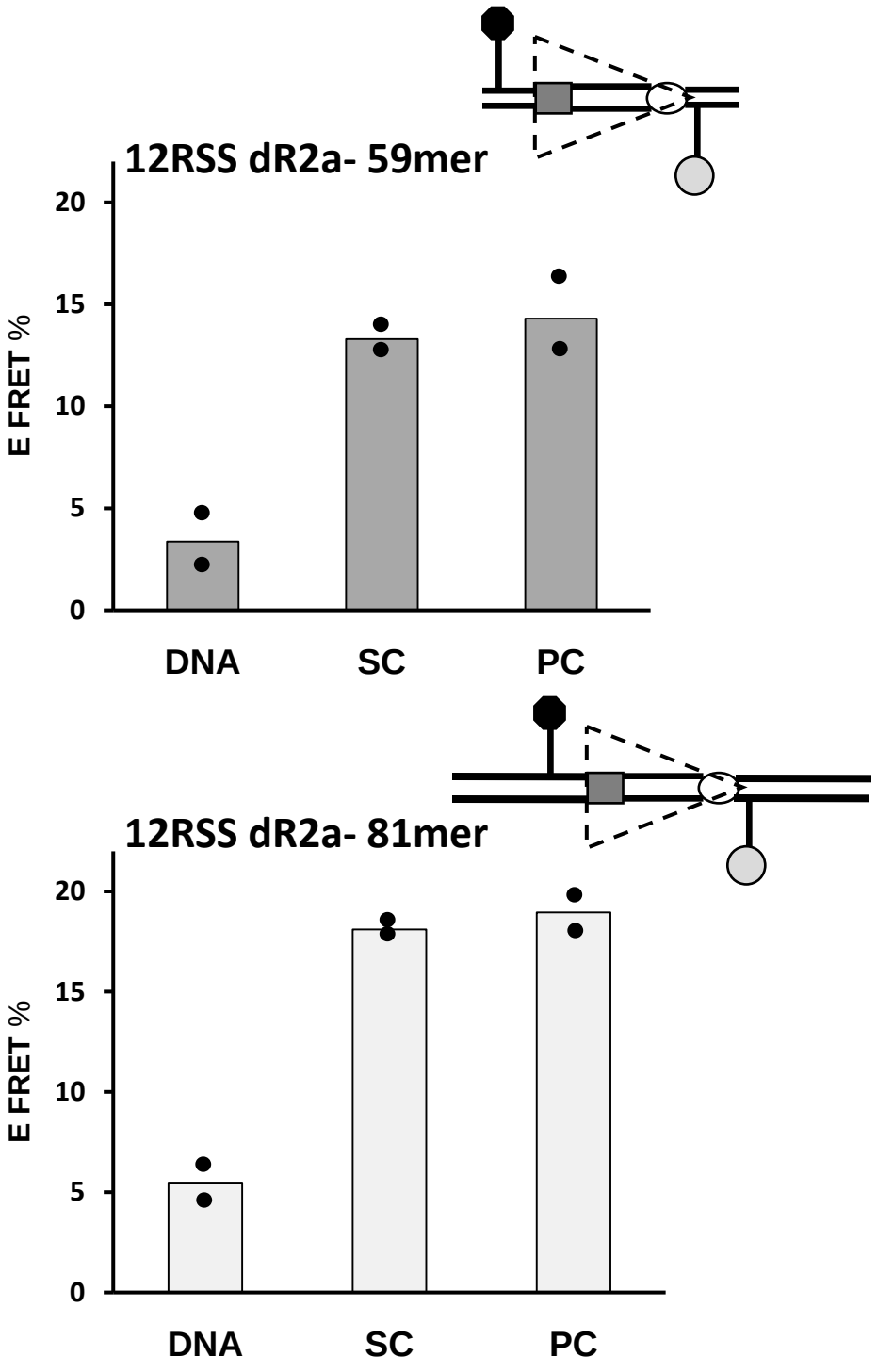
12RSS dR2a- 59mer
E FRET %
20
15
10
5
0
DNA
SC
PC
12RSS dR2a- 81mer
E FRET %
20
15
10
5
0
DNA
SC
PC

Fig. S4

The architecture of the 12RSS in V(D)J recombination signal and synaptic complexes

Mihai Ciubotaru[1,2], Marius D. Surleac[3], Lauren Ann Metskas[4], Peter Koo[5], Elizabeth Rhoades[4], Andrei J. Petrescu[3] and David G. Schatz[1,4,6]*

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Figures 1-4, and Supplementary Data sets 1 and 2.

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Location of the fluorophore-labeling positions on the 12RSS used in this study. Donor labeling positions [green] are designated with a letter, and acceptor labeling positions [red] are designated with a number, while labels at the 5' ends of the two DNA strands are indicated as 5' up for the top strand and 5' bt for the bottom strand.

Figure S2. Additional controls for the FRET analysis of the $12RSS_dR2_a$ substrate under PC conditions except with the omission of (A) HMGB1 or (B) RAG1. Data are displayed as in Figure 3(A) with the traces from the protein containing samples shown in blue and the donor only control as a dashed line.

Figure S3. FRET analysis of the $12RSS_dJ3_a$ under conditions supporting formation of the 12SC (green traces), standard PC with consensus 23RSS partner (blue traces), PC with srambled nonamer 23 RSS (lilac traces), and PC with a scrambled heptamer 23RSS (orange traces). Traces are represented using the same type of symbols as in Figure 2.

Figure S4. Energy transfer occurs with similar efficiencies for reactions performed with a 59-mer or an 81-mer $12RSS_dR2_a$ substrate. Bars represent the average energy transfer efficiency from two independent measurements, with dots indicating each experimental value and the content of the reaction indicated below each bar. The free DNA, SC, and 23RSS partner (PC) reactions were performed as with the $12RSS_dR2_a$ substrate in Fig. 2B and 2D. Data for the 59-mer and 81-mer$12RSS_dR2_a$substrate are shown in dark and light shaded bars.