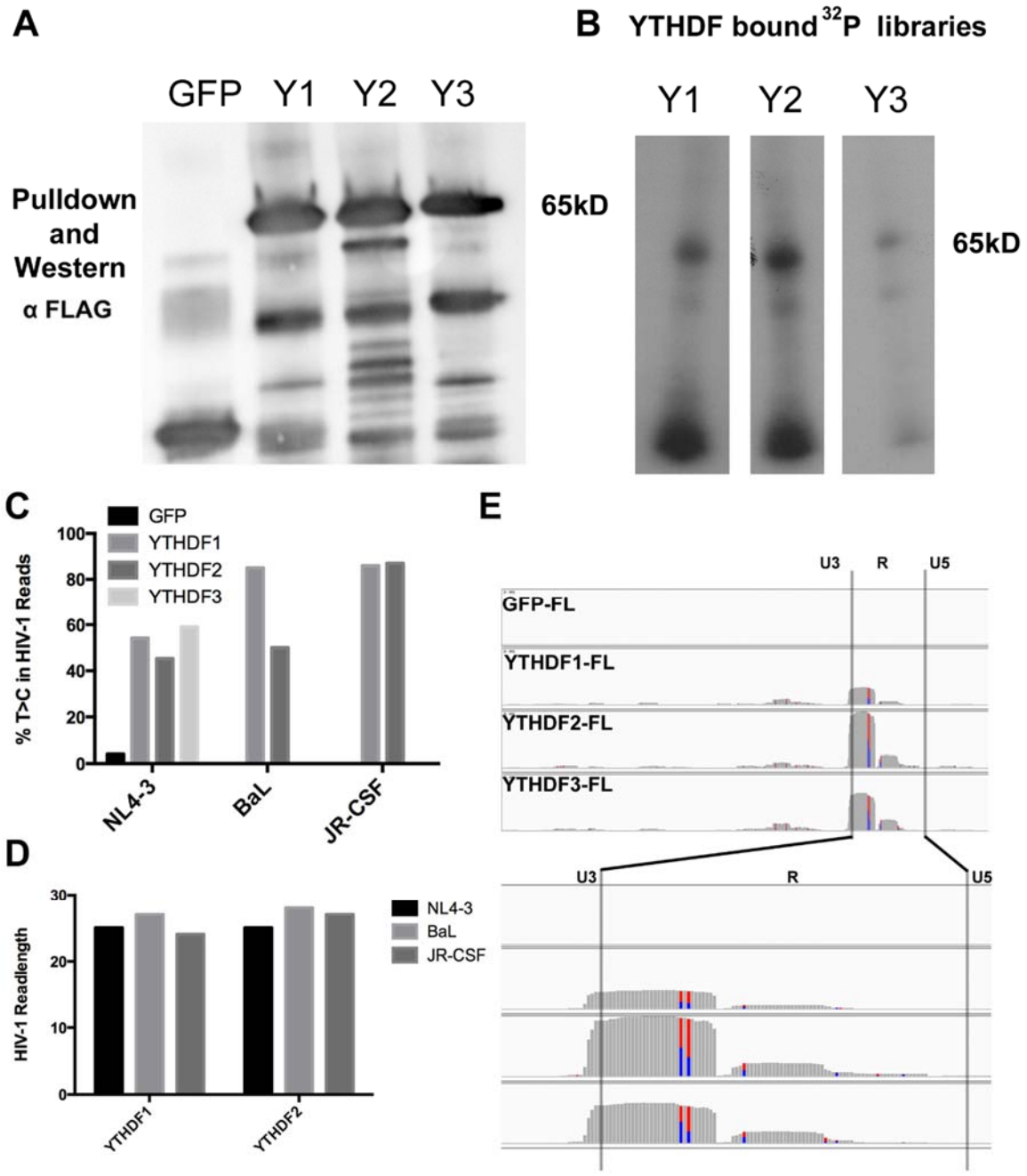

Figure S1, related to Figures 1 and 2. PAR-CLIP analysis of YTHDF protein binding to the HIV-1 genome.

(A) Western blot analysis of a FLAG-specific immunoprecipitation of lysates of 293T cells expressing FLAG-GFP, FLAG-YTHDF1 (Y1), FLAG-Y2 or FLAG-Y3. The YTHDF proteins are ~65 kD in size.

(B) After crosslinking of 4SU residues to bound proteins, the YTHDF proteins were immunoprecipitated and RNase treated before labeling of protein bound RNA oligonucleotides using γ-^{32}P-ATP. This gel shows that this results in a readily detectable radiolabeled protein band at the predicted ~65 kD size for all three YTHDF proteins.

(C) This bar graph shows the percent of HIV-1-specific reads that contain T-to-C mutations, characteristics of a 4SU crosslink, in the PAR-CLIP libraries obtained from 293T cells expressing GFP, YTHDF1, YTHDF2 or YTHDF3 after infection with the indicated HIV-1 isolates.

(D) This bar graph shows the mean read length for the PAR-CLIP libraries obtained in 293T cells infected with the indicated HIV-1 isolates. These data derive from Fig. 2.

(E) Fine mapping of PAR-CLIP reads that map to the NL4-3 TAR element for YTHDF1, 2 and 3. As may be observed, many of these extend into U3, thus demonstrating that the 3' LTR R element is m^6A modified.

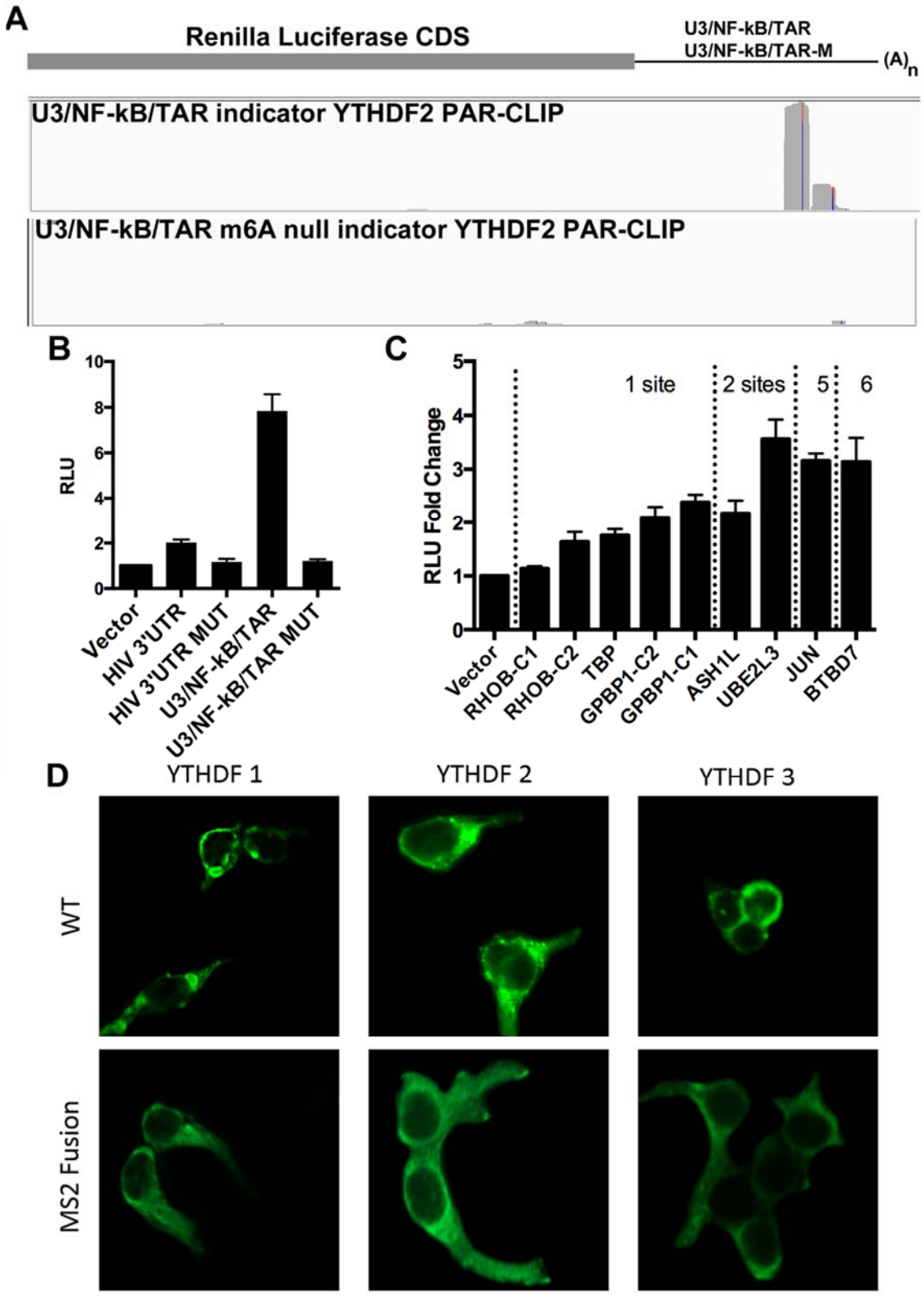
A
Renilla Luciferase CDS
U3/NF-kB/TAR
U3/NF-kB/TAR-M
(A)n
U3/NF-kB/TAR indicator YTHDF2 PAR-CLIP
U3/NF-kB/TAR m6A null indicator YTHDF2 PAR-CLIP
B
RLU
Vector
HIV 3'UTR
HIV 3'UTR MUT
U3/NF-kB/TAR
U3/NF-kB/TAR MUT
C
RLU Fold Change
1 site
2 sites
5
6
Vector
RHOB-C1
RHOB-C2
TBP
GPBP1-C2
GPBP1-C1
ASH1L
UBE2L3
JUN
BTBD7
D
YTHDF 1
YTHDF 2
YTHDF 3
WT
MS2 Fusion

Figure S2, related to Figure 4. Analysis of the YTHDF protein function.

(A) This PAR-CLIP analysis examines reads obtained from transfected 293T cells that map to the psiCheck2-based indicator construct containing the HIV-1 U3/NF-κB/TAR region used in Fig. 4. As may be observed, we readily detect YTHDF2 binding to the TAR region present in this indicator in the wildtype HIV-1 sequence but we do not observe any YTHDF2 binding to either the HIV-1 or RLuc sequences in the indicator plasmid containing HIV-1 sequences in which the viral m^6A editing sites have been mutated.

(B) These data are identical to the results shown in Fig. 4C except that they are here normalized to the parental psiCheck2 vector lacking any 3'UTR insert.

(C) These data are identical to the results shown in Fig. 4E except that they are here normalized to the parental psiCheck2 vector lacking any 3'UTR insert.

(D) Immunofluorescence analysis of the subcellular location of full-length FLAG-tagged YTHDF proteins or the FLAG-tagged YTHDF-MS2 fusion proteins used in Fig. 4F, showing that all are expressed equivalently and localized to the cytoplasm.

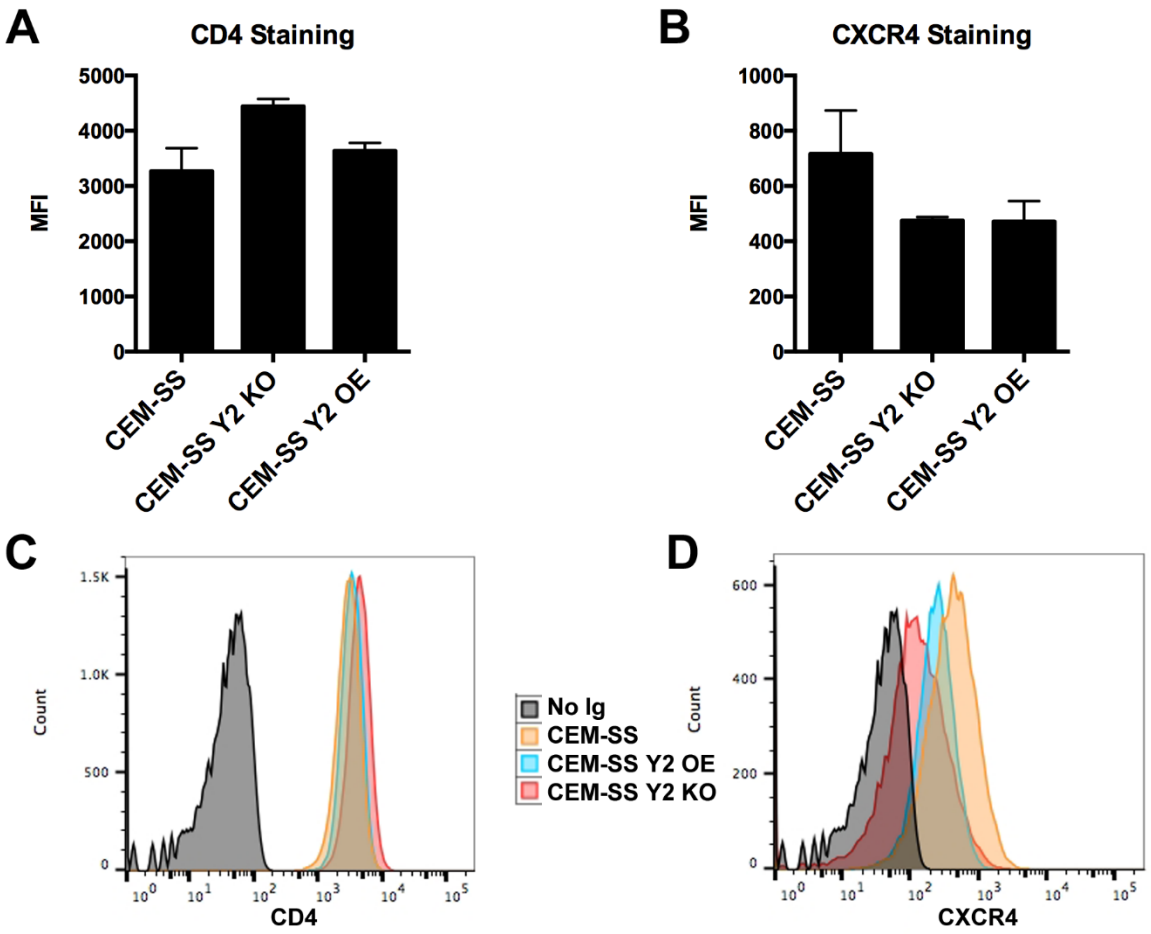

Figure S3, related to Figure 6. Analysis of CD4 and CXCR4 expression on CEM-SS subclones.

(A) Comparison of the level of CD4 cell surface expression on the parental CEM-SS cells and the Y2-KO and Y2-OE subclones analyzed in Fig. 6. Average of three independent experiments with SD indicated.

(B) Similar to (A) except looking at cell surface CXCR4 expression. While both subclones show ~2-fold less cell surface CXCR4 than wildtype cells, they are closely similar to each other.

(C) Representative trace of a cell surface CD4 FACS analysis looking at the parental CEM-SS cell line and the CEM-SS Y2-KO and Y2-OE subclones.

(D) Similar to (C) except looking at cell surface CXCR4.

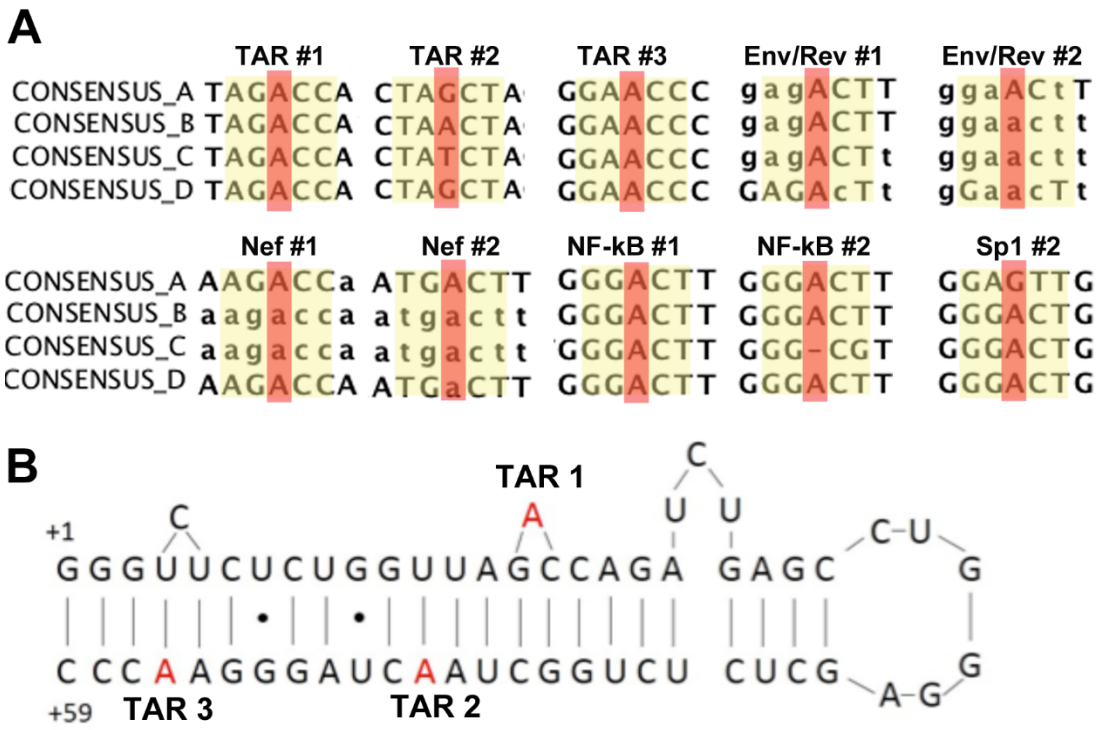

Figure S4, related to Figures 2 and 3. Conservation of HIV-1 m^{6}A editing sites.

(A) This panel looks at the sequence conservation of the 10 potential m^{6}A editing sites in HIV-1 strain NL4-3 identified in Fig. 3. As may be observed, seven of these 10 sites are highly conserved across HIV-1 isolates in subtypes A, B, C and D.

(B) The location of potential m^{6}A editing sites in the HIV-1 TAR element are indicated in red.

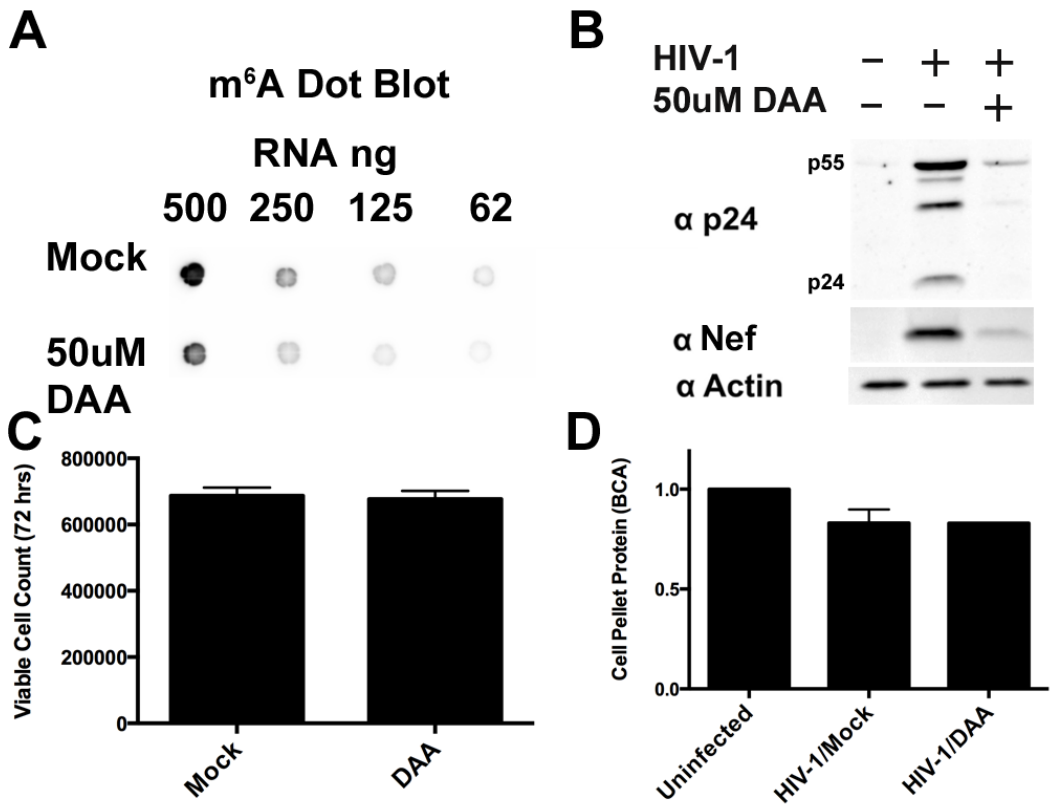

Figure S5, related to Figure 6. Inhibition of HIV-1 replication by the m6A inhibitor DAA.

(A) This panel shows an m^6A dot blot for mock treated or DAA treated CEM-SS cells.

(B) A representative Western blot is shown for HIV-1 NL4-3 infection of mock or DAA treated CEM-SS cells harvested at 72 hpi. The Western shown in (B) is representative of four independent biological experiments.

(C) This bar graph shows the level of viable CEM-SS cells observed in cultures grown in the absence or presence of 50 micromolar DAA for 72 h.

(D) This graph measures the total level of cellular protein, determined by BCA assay, recovered from the cell pellets derived from the cultures analyzed in panel B. Note that the level of protein is similar in the HIV-1 infected cultures in the presence or absence of DAA.