# The basal translation rate of authentic HIV-1 RNA is regulated by 5'UTR nt-pairings at junction of R and U5

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**Supplementary figure legends**

**Figure S1. Flowchart of process to measure translation rate.**

De novo translation activity of mRNA templates was measured by the incorporation of radiolabeled amino acids into nascent polypeptides at 3 consecutive intervals. Gag and beta-Catenin were collected by IP and analyzed by SDS-PAGE. Radiolabeled protein was quantified by phosphorimaging. Drawing made by Ioana Boeras and Kathleen Boris-Lawrie.

**Figure S2. Entire gel of the metabolic labeling assay in Fig. 2**. SDS-PAGE and phosphorimager analysis of $^{35}$S-labeled immunoprecipitated Gag polypeptides (Gag-Pol, Gag precursor, and processed capsid). Beta-Catenin was co-precipitated with exception of the SubB U103C sample, as designated by asterisk. M indicates the lane containing the molecular weight markers (KDa).

**Figure S3. Six representative ribosomal RNA profiles.** Continuous sucrose gradients of cytoplasmic extracts of HEK293 cells were prepared and distribution of ribosomal RNA was monitored at 254nm.

**Figure S4. Monomer-dimer equilibrium assay of WT and U103C 5'UTRs.**

In vitro transcribed RNAs corresponding to HIV 5'UTR (+1 to 356) were incubated in physiologic ionic strength buffer to equilibrium and visualized by native agarose electrophoresis.

**Table S1. Rate of amino acid incorporation to Gag polypeptide is sensitive to U103 substitution in monomer-prone 5'UTR mutants [a].**

| Molecular clone [Predominant conformer] [b] | Gag fold difference relative to WT (Average ± standard error) | p-value | beta-Catenin fold difference relative to WT (Average ± standard error) | p-value |
|---|---|---|---|---|
| WT [Dimer] | 1.0 | Baseline | 1.0 | Baseline |
| SubC [Dimer] | 1.1 ± 0.2 | 0.9319 | 2.8 ± 0.9 | 0.0096 |
| SubA [Monomer] | 0.7 ± 0.2 | 0.5592 | 0.8 ± 0.2 | 0.9439 |
| SubB [Monomer] | 0.8 ± 0.2 | 0.7829 | 1.1 ± 0.3 | 0.9861 |
| SubA U103G [Monomer] | 3.1 ± 0.6 | 0.0001 | 1.9 ± 0.6 | 0.1715 |
| SubB U103C [Monomer] | 2.6 ± 1.0 | 0.0541 | NA | NA |

[a] Average fold difference (± standard error) of de novo Gag and beta-Catenin synthesis over the three time points for each molecular clone relative to WT. Results of three to ten independent experiments were back-transformed from the linear mixed model and standard error was computed by the delta method; p-values adjusted for multiple comparisons by Dunnett's method.

[b] Predominant conformation observed in monomer:dimer equilibrium assay (Figure 1).

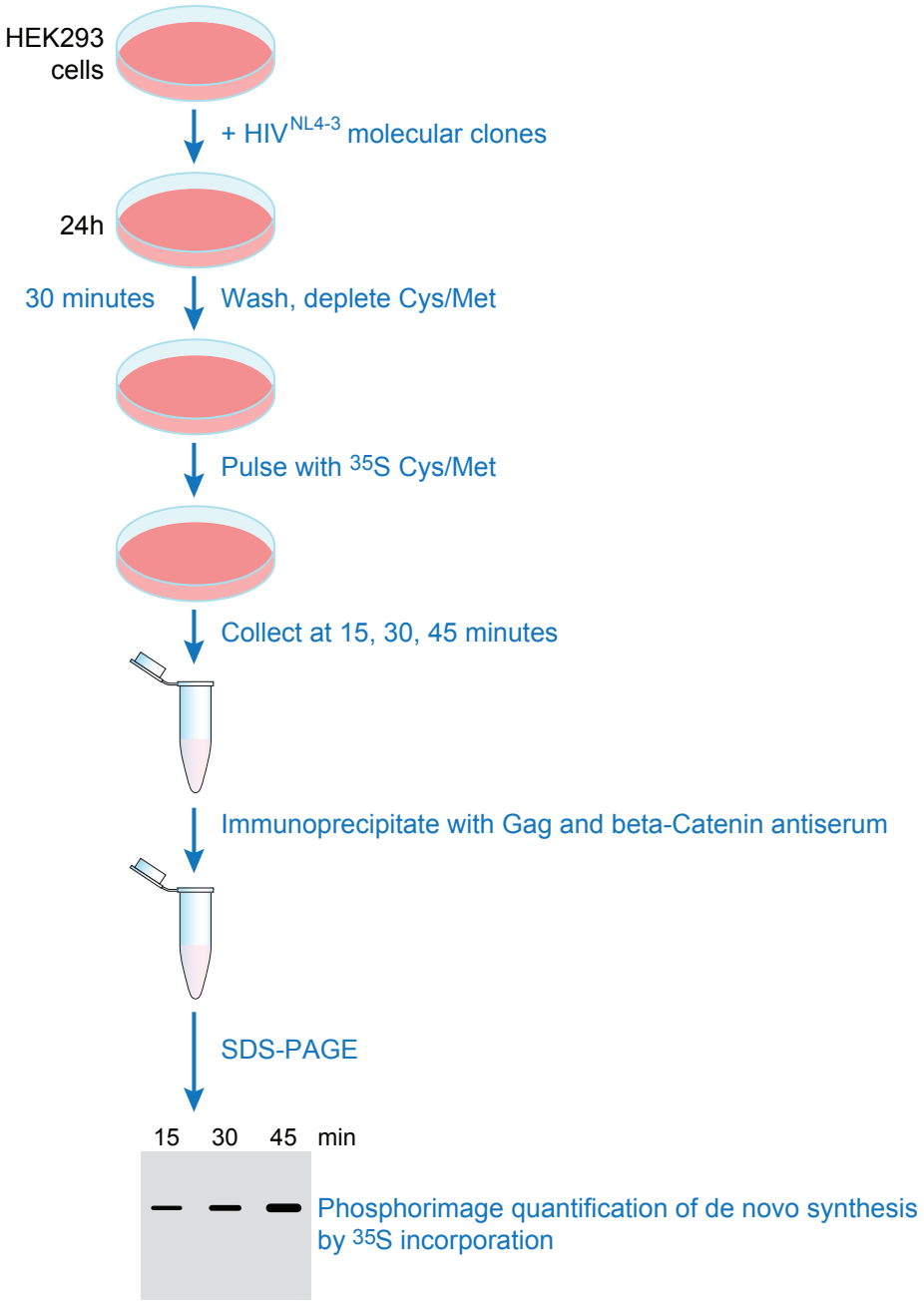
HEK293
cells
+ HIV NL4-3 molecular clones
24h
30 minutes
Wash, deplete Cys/Met
Pulse with 35S Cys/Met
Collect at 15, 30, 45 minutes
Immunoprecipitate with Gag and beta-Catenin antiserum
SDS-PAGE
15 30 45 min
Phosphorimage quantification of de novo synthesis
by 35S incorporation

Figure S1

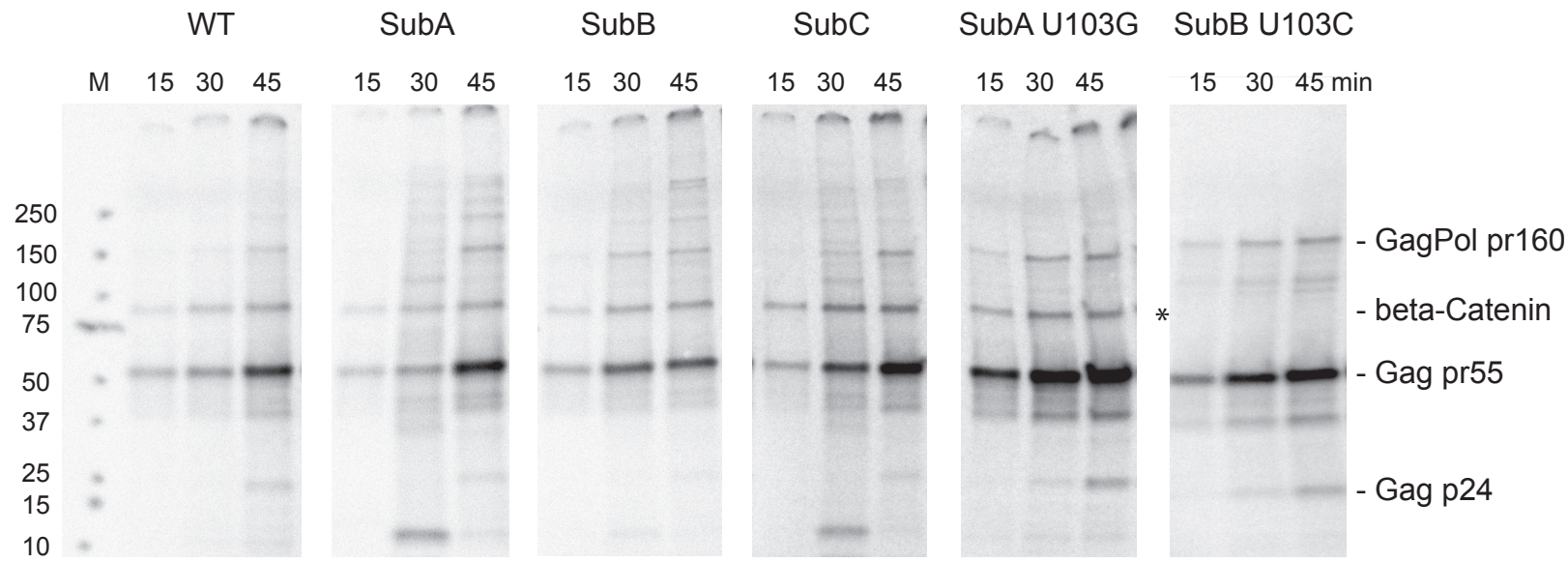
WT
SubA
SubB
SubC
SubA U103G
SubB U103C
M 15 30 45 15 30 45 15 30 45 15 30 45 15 30 45 15 30 45 min
250
150
100
75
50
37
25
15
10
*
- GagPol pr160
- beta-Catenin
- Gag pr55
- Gag p24

Figure S2

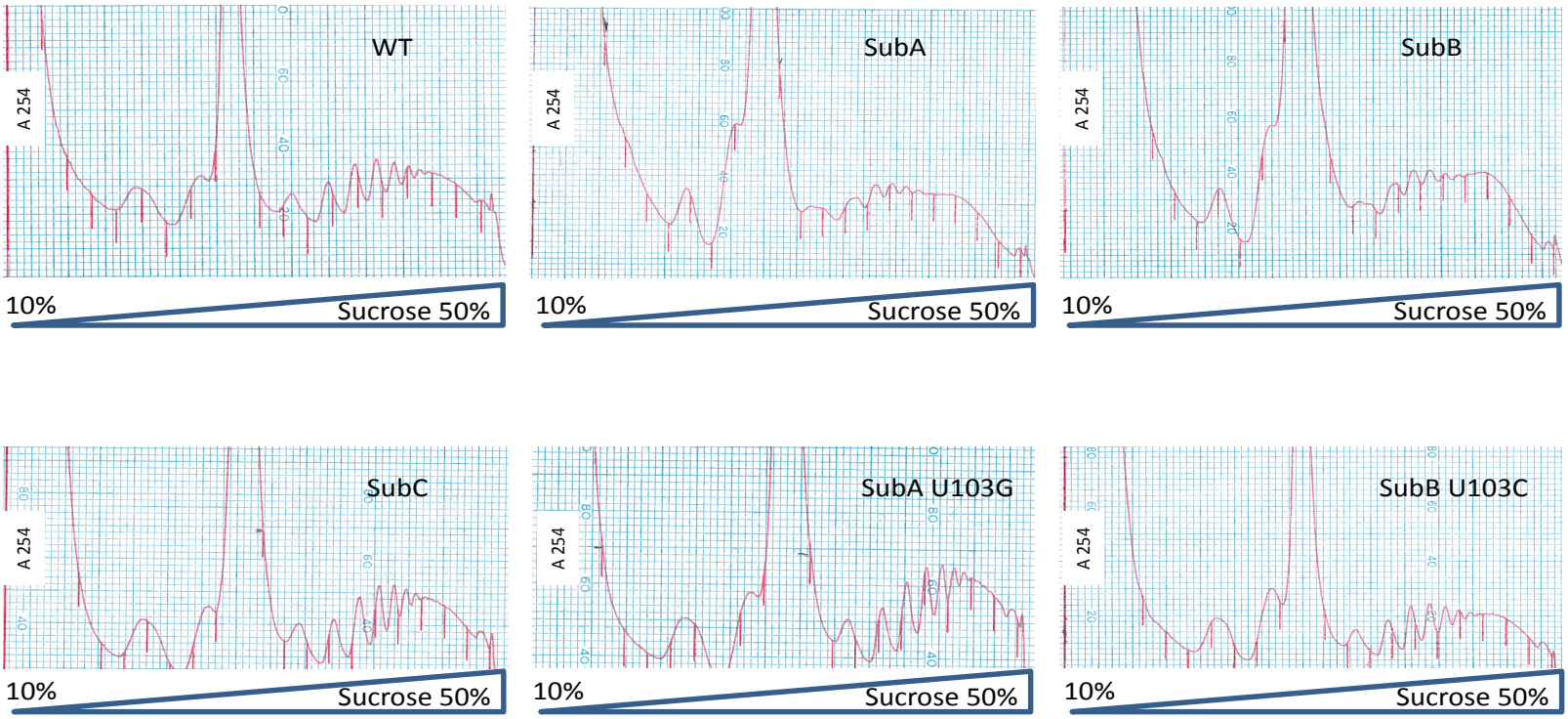


Figure S3

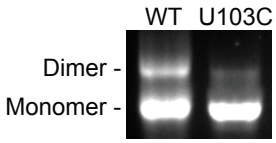


Figure S4