SUPPLEMENTARY MATERIAL

Calculations for the model outlining contributions of the retrospective quality control to the fidelity of translation. During elongation, when an error is committed, the now mismatched translating RNCs will partition between three basic outcomes: 1) premature termination at a sense codon with RF2/RF3 (estimated by Fig. 2 to occur < 2% of the time), 2) selection of the cognate tRNA and peptidyl transfer (estimated in Fig. 3b to occur 10-30% of the time), and 3) selection of another mismatched tRNA and peptidyl transfer (estimated in Fig. 3b to occur 70-90% of the time). The consequence of this most probable event (3) is the generation of a ribosome complex with mismatches in both the E and P sites. As we show in Fig. 4 and Supplementary Fig. 12, these complexes are estimated to partition with high probability (~5-50%) to abortive termination, while ~50-95% of the population will dominantly partition such that another error in tRNA selection occurs. For these estimates, we use our measured K_m values for RF2 and tRNA on singly mismatched complexes (Supplementary Fig 4 and data not shown) of 1-2 μM, and *in vivo* concentration estimates of RF2 and individual tRNAs as essentially equivalent (5-20 μM). These equivalent values of K_m and cellular concentrations simplified the partitioning between release and PT (determined by multiplying the second order rate constants by the concentration) into the relative k_{cat} of the two processes (0.07-2 s^{-1} for release on a doubly mismatched complex, and 1-2 s^{-1} for PT when full competitor tRNA is added).

Supplementary figures legends

Figure S1: Estimate of misincorporation rate of *in vitro* translation mix. a, Autoradiograph of an electrophoretic TLC resolving the products of a 5 minute PT reaction between an initiation complex (with f-[^{35}S]-Met-tRNA^{fMet} programmed in the P site with an mRNA coding for f-Met-Phe, AUG-UUC) and EF-Tu ternary complex containing the indicated aminoacylated tRNA (Phe and Leu indicate pure Phe-tRNA^{Phe} and Leu-tRNA$_5^{Leu}$ tRNAs respectively, while mix indicates total *E. coli* tRNA purchased from Roche aminoacylated with full complement of twenty amino acids) in buffers A to D (Materials and Methods). We note that PT reactions with Leu-tRNA^{Leu} yielded a secondary product that migrates to the same spot as the dipeptide fMet-Phe presumably due to contamination in the tRNA stock. Miscoding by tRNA^{Leu} is evidently the most common error in reactions containing aminoacyl-tRNA mixture. **b,** The error rate was estimated by quantifying the area beneath and above each of the reactions with the total tRNA relative to the corresponding area in the Phe only reaction. The quantitated value represents the average of two experiments.

Figure S2: Efficient decoding of the Asn codon AAU by Lys-tRNA^{Lys}. Dipeptidyl RNCs programmed with the indicated messages were prepared from ICs and were reacted with Lys-tRNA^{Lys}. The reaction mixture was resolved by electrophoretic TLC following hydrolysis with KOH.

Figure S3: Rate of release is strongly affected by buffer condition and methylation status of RF2. Release rate constants for the dipepitdyl tRNA RNCs MKX and MYX (programmed with the mRNAs AUG-AAA-UGA and AUG-UAC-UGA, respectively) under the indicated conditions. Similar relative increases are observed with the two different complexes as a function of buffer conditions and the source of RF2, suggesting that these conditions have general effects on release that are not complex specific. (oe) indicates over-expressed RF2, while (ce) denotes a chromosomally-expressed (more heavily methylated) preparation.

Figure S4: RF2 releases a mismatched complex (MNF) with an efficiency that is >300 fold higher than the matched one (MKF). a, Release time courses with the indicated RNCs were carried out at varying concentrations of RF2. Rates were determined, plotted against the enzyme concentration ([RF2]), and fit to a hyperbola (MNF) or a straight line (MKF). Error bars indicate the error associated with the fit for each time-course data. **b,** Bar graph representing the measured second order rate constant (k_{cat}/K_m) for the mismatched complex, or the inferred value (slope of the line) for the matched complex. Error bars are obtained from the non-linear regression in (a).

Figure S5: The prescribed sense codon occupies the A site in the mismatched complex. Toe-printing experiment examined by PAGE for the MKI and MNI complexes. Initiation complexes (Init.) contain only f-Met-tRNA^{fMet} in the P site whereas elongated complexes (Elong.) are formed following the addition of Lys-tRNA^{Lys} ternary complex and EF-G and movement by 3 nts along the mRNA template. Red dots represent accurately initiated and elongated toeprint, with individual steps between shown with black dots.

Figure S6: Release of mismatched complexes cannot be attributed to peptidyl-tRNA drop-off followed by peptidyl hydrolase-mediated hydrolysis. a, Indicated complexes prepared with [^{32}P]-labeled Lys-tRNA^{Lys} were incubated with excess deacylated, unlabeled tRNA^{Lys} and the off-rate of the dipeptidyl tRNA was followed as a function of time using a nitrocellulose filter-binding assay. Bars represent rates obtained from single exponential decay fits to the data with the error bars representing the error obtained from the non-linear regression of the data. The measured rates are much lower than those observed for RF2-mediated release. **b,** Peptidyl hydrolase only catalyzes hydrolysis of the dipeptidyl tRNA in the sample that has been treated with EDTA to disassociate the ribosome complex (lane 5). Lack of hydrolysis of the dipeptidyl tRNA in the EDTA treated sample with no PTH indicates that there is no visible contamination of included components with PTH (lane 3).

Figure S7: Release of dipeptidyl tRNA from mismatched ribosome complex (MNF) is paromomycin sensitive. Autoradiograph of electrophoretic TLC demonstrating that paromomycin inhibits release of dipeptidyl tRNA from the mismatched RNC MNF.

Figure S8: Wobble base-pairing is best tolerated at the third position of the P-site codon. k_{hyd} with saturating RF2 and RF3 for dipeptidyl-RNCs with either U:U or G:U (wobble) mispairings (mRNA:tRNA) in the P site at the 1st, 2nd or 3rd codon positions. Error bars represent the error obtained from the non-linear regression of the data

Figure S9: Two-dimensional TLC resolving the products of a PT reaction between the matched MKF (a) or mismatched MNF (b) complex with total aminoacylated tRNA.

Figure S10: Mismatched complex reacts more readily with near-cognate tRNAs. Autoradiograph of one-dimensional electrophoretic TLC resolving products of PT reaction between matched (MKF) and mismatched (MNF) RNCs and indicated pure aminoacylated tRNA (Phe, Val, Tyr, Leu). Note that Phe-tRNA^{Phe} is cognate for the two complexes (MKF and MNF) and the remaining three tRNAs are near cognate (1st, 2nd, and 3rd position).

Figure S11: Relative promiscuous release activity on matched and mismatched RNCs is unaffected by buffer or RF2 source. Rate constants for the indicated complexes in buffer A with over-expressed RF2 (oe RF2), or in buffer D with over-expressed or chromosomally-expressed RF2 (ce RF2). Error bars represent the error obtained from the non-linear regression of the data

Figure S12: Abortive termination is no longer triggered once errors have progressed out of the E site of the ribosome. a, Schematic representation of tetrapetidyl RNCs used in this assay; cognate (MKKKF), out of the E site error (M(UAA)KKF), and P-site mismatch (MKK(UAA)F). **b,** Autoradiograph of electrophoretic TLC showing that

RF2/RF3 promotes premature release only on the P-site-mismatched complex but not the other two (reaction was carried out for 10 minutes).

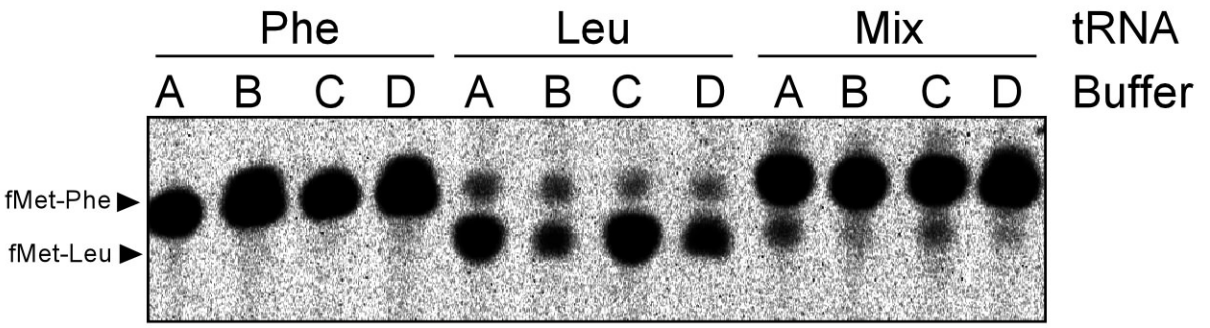
Figure S13: Homopolymeric complexes containing a mismatch in the E site undergo a frameshift. Toe-printing experiment examined by PAGE for the MKKF **a,** or MEEP **b,** series of complexes. Initiation complexes (Init.) contain only f-Met-tRNA^{fMet} in the P site, whereas elongated complexes (Elong.) result from the addition of ternary complex (Glu-tRNA^{Glu} in **a** or Lys-tRNA^{Lys} in **b**) and EF-G. Note in this case, because of the expected addition of two amino acids, the anticipated shift in the toeprint is 6 nts (bottom red circle) relative to the initiation toe-print (top red circle).

Figure S14: A complex containing mismatches in the P and E sites partitions equally between premature release or peptidyl transfer. The indicated tripeptidyl-tRNA RNCs, containing fMetLysLys-tRNA^{Lys} in the P site and Phe codon in the A site, were incubated with an S100 extract containing 120 μM charged total tRNA for 5 minutes, and resolved by electrophoretic TLC.

Figure S15: A single miscoding event results in an overall drop in yield of full-length peptide in buffer D. **a,** Mock *in vivo* experiment recapitulates predictions of model in buffer D. The indicated series of mRNAs (MKX through MKIFHKX for the matched series and MNX through MNIFHKX for the mismatched series) were used in complete translation reactions to observe the consequences of competition between tRNAs and RFs for peptide synthesis. Peptides initiated with the cognate dipeptide MK from the matched mRNA series are assigned the color black (MK-matched), peptides initiated with the cognate MN from the mismatched mRNA series are assigned the color blue (MN-matched), while peptides resulting from an incorrect decoding by Lys-tRNALys on the Asn (N) codon are assigned the color red (MN-mismatched). **b,** Yield was quantified as the fractional radioactivity in each product band relative to the whole lane. Note that in buffer D, initial miscoding by Lys-tRNA^{Lys} is minimized relative to buffer A.

Zaher & Green, Supplementary Figure 1

a)



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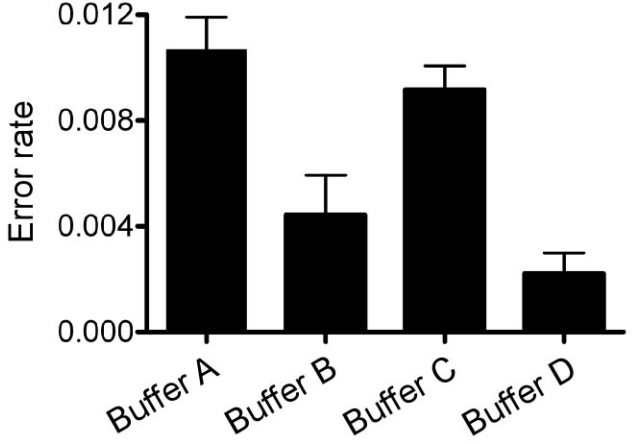
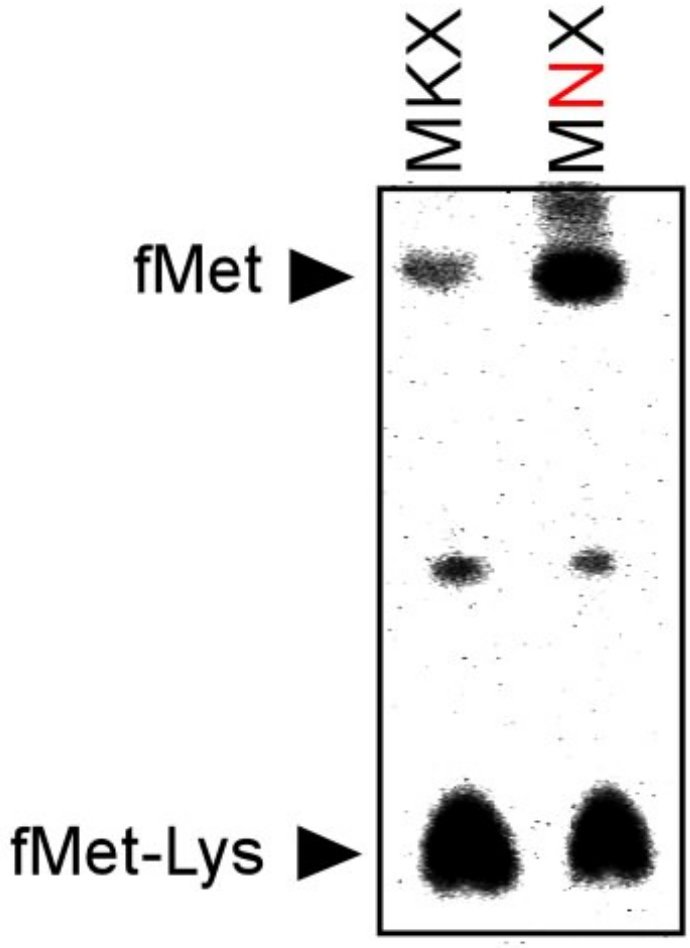
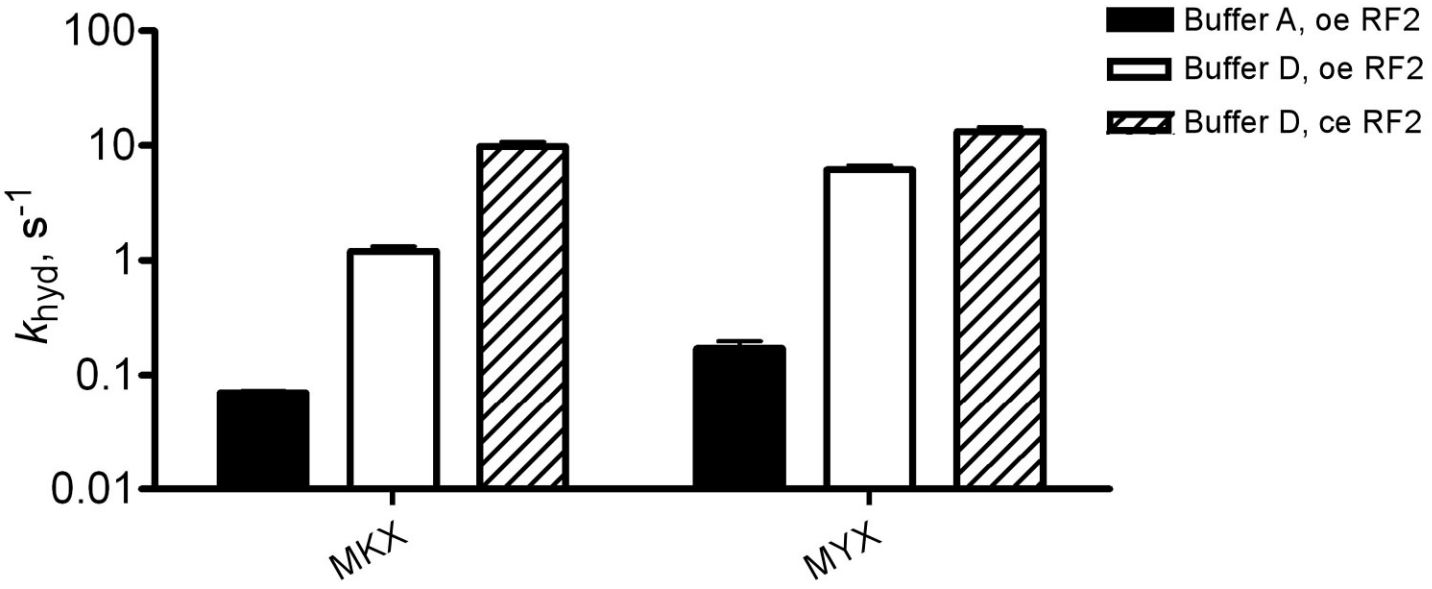


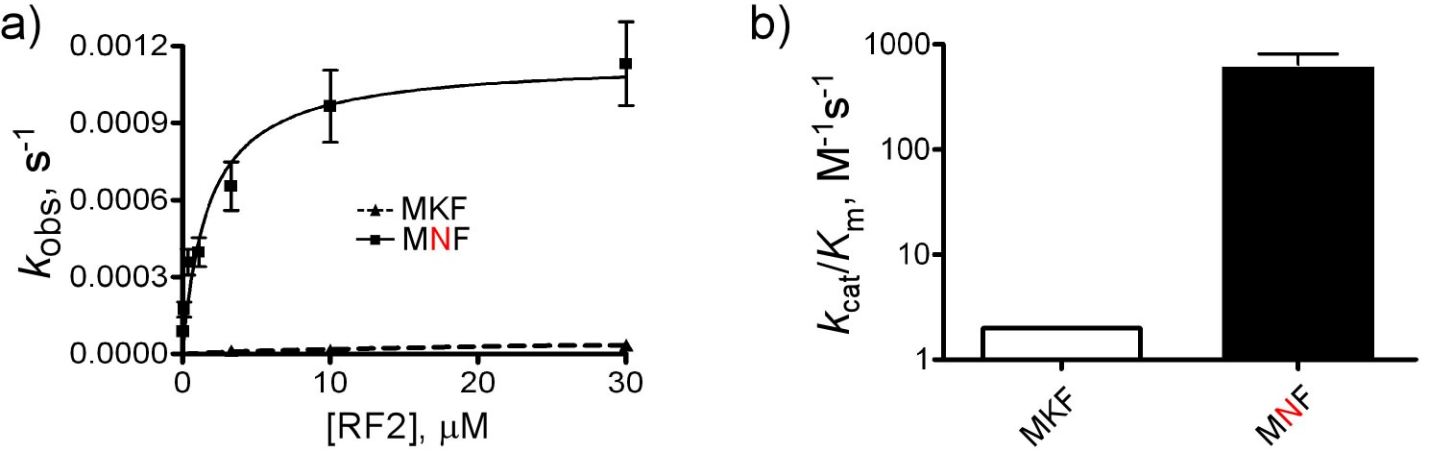
Zaher & Green, Supplementary Figure 2



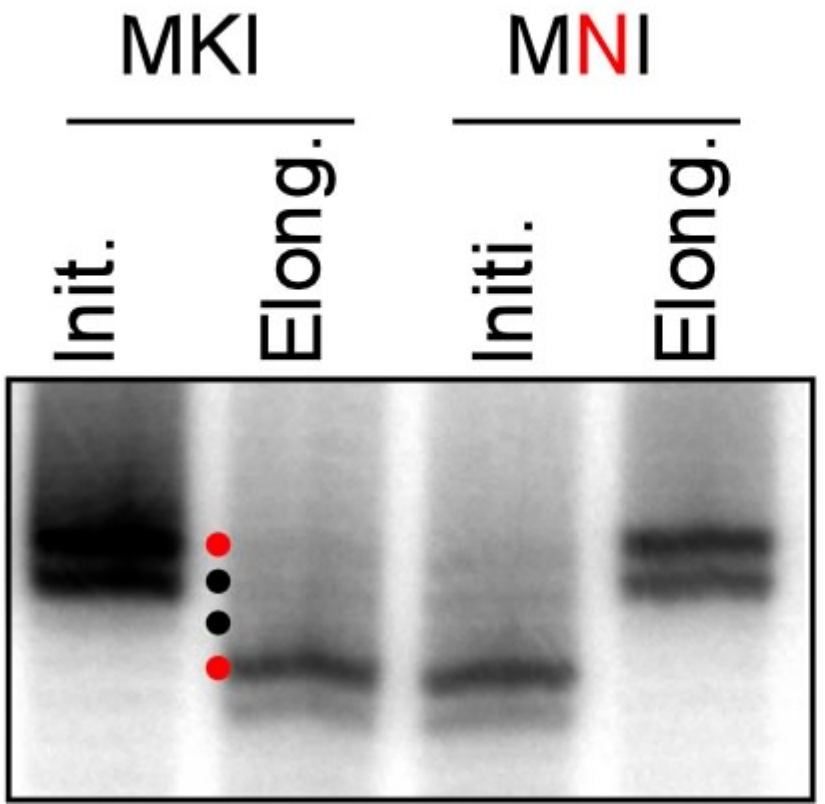
Zaher & Green, Supplementary Figure 3



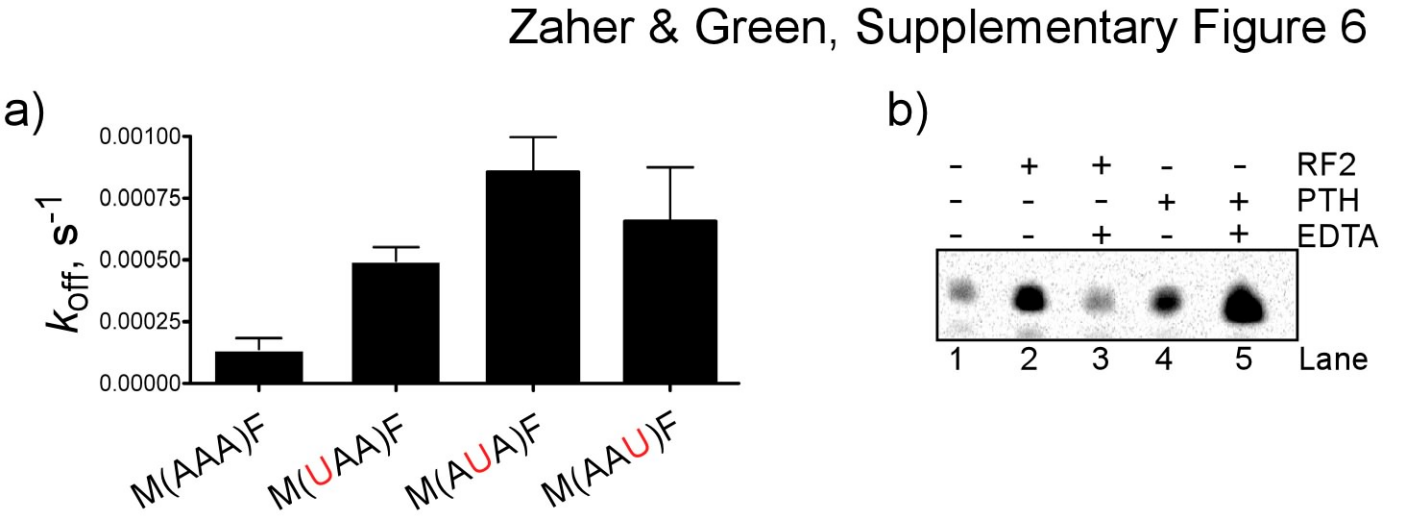
Zaher & Green, Supplementary Figure 4



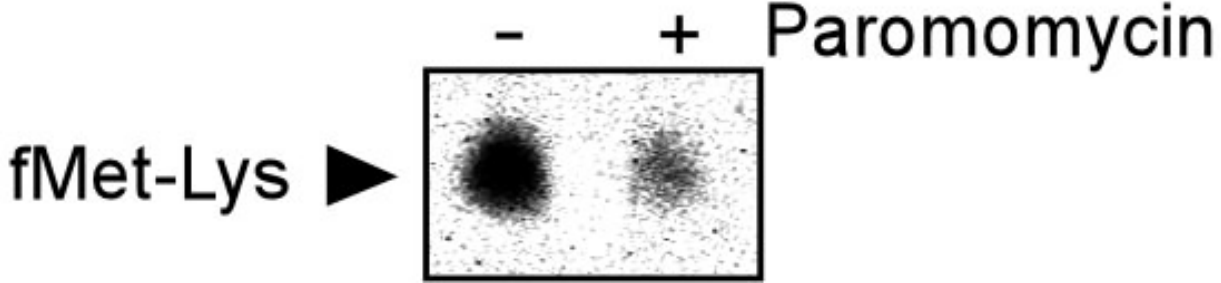
Zaher & Green, Supplementary Figure 5



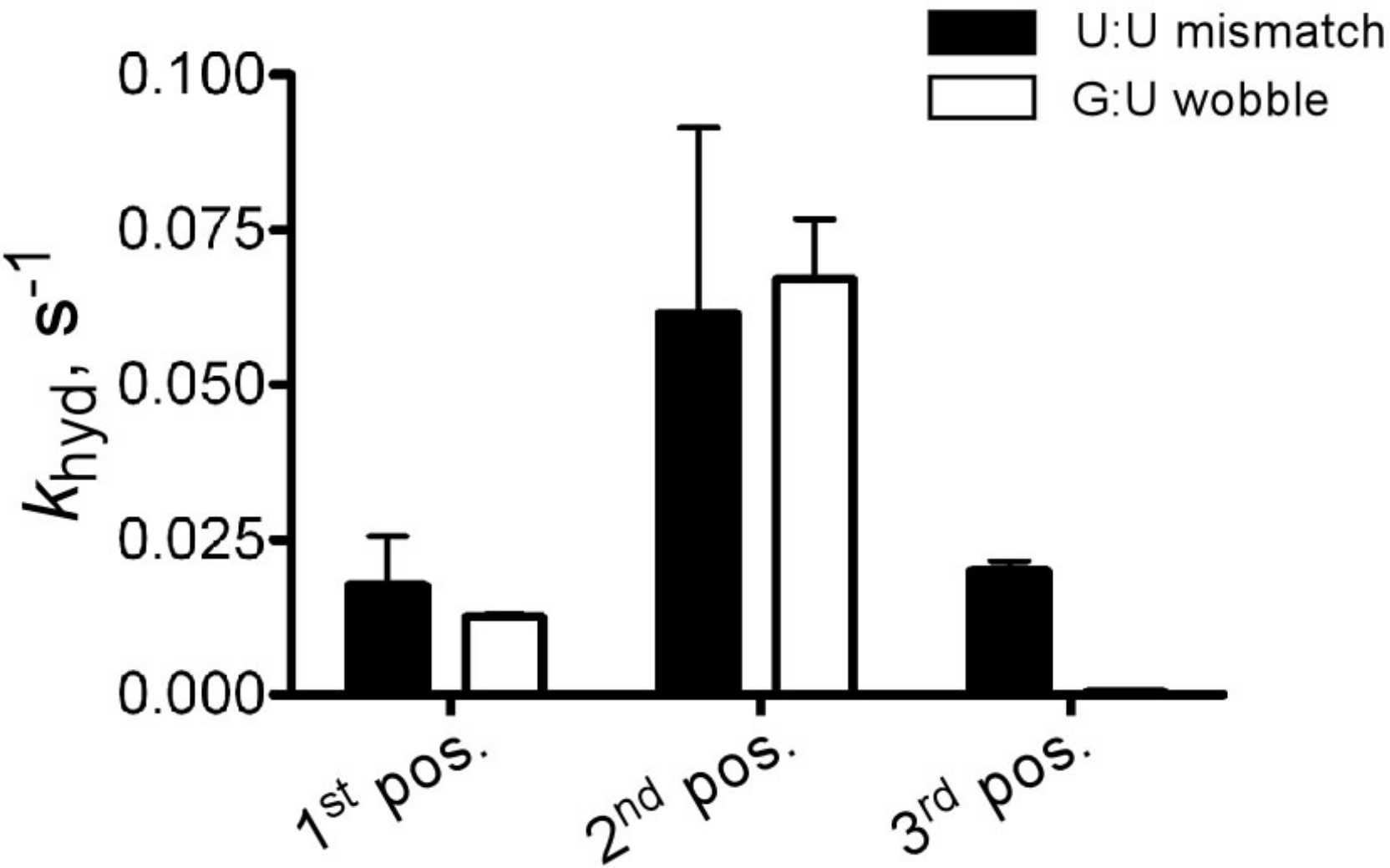
Zaher & Green, Supplementary Figure 6



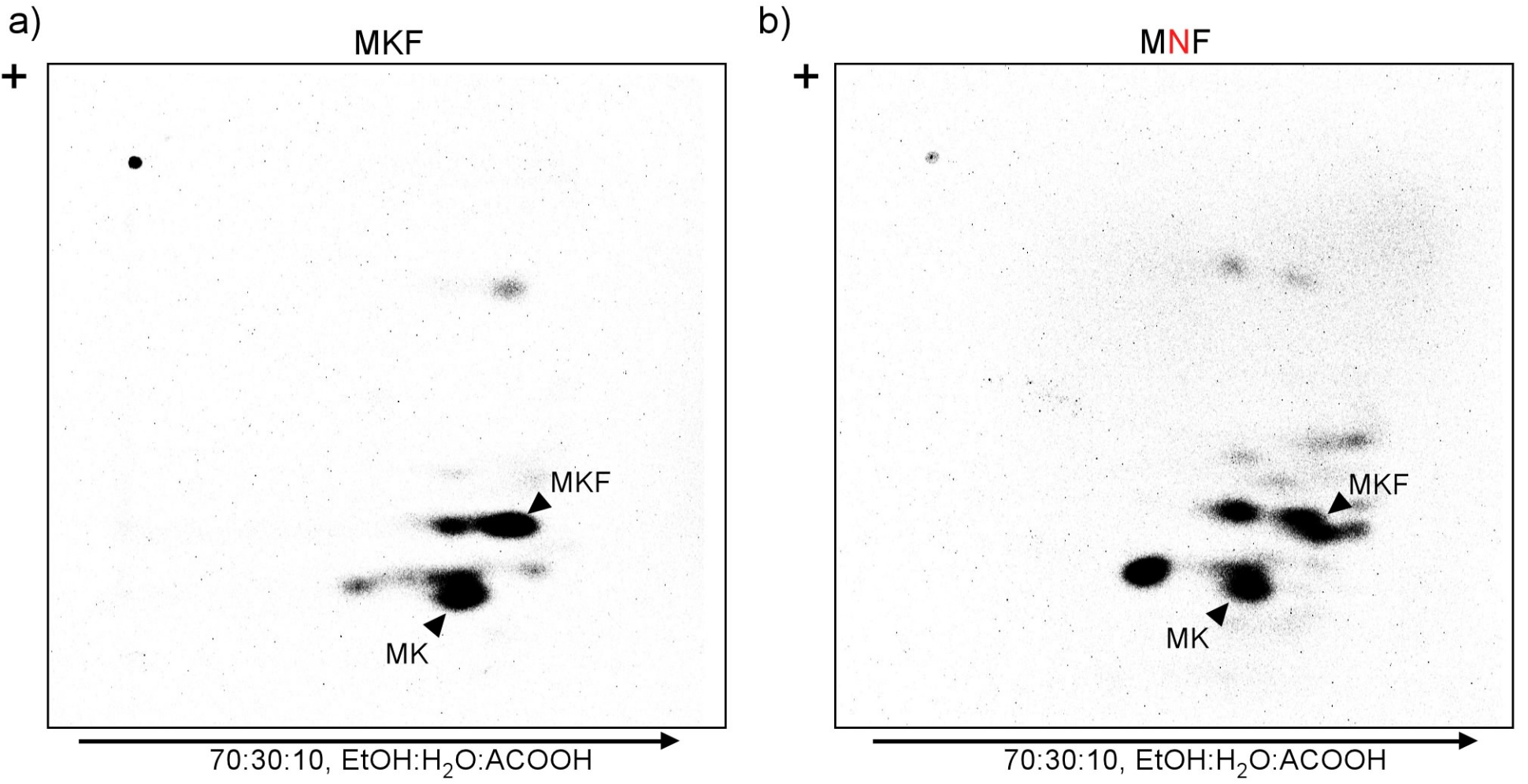
Zaher & Green, Supplementary Figure 7



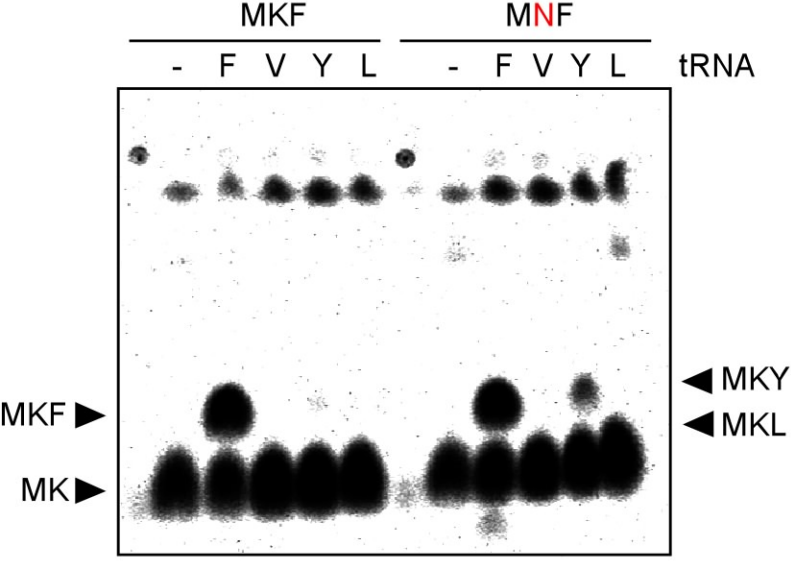
Zaher & Green, Supplementary Figure 8



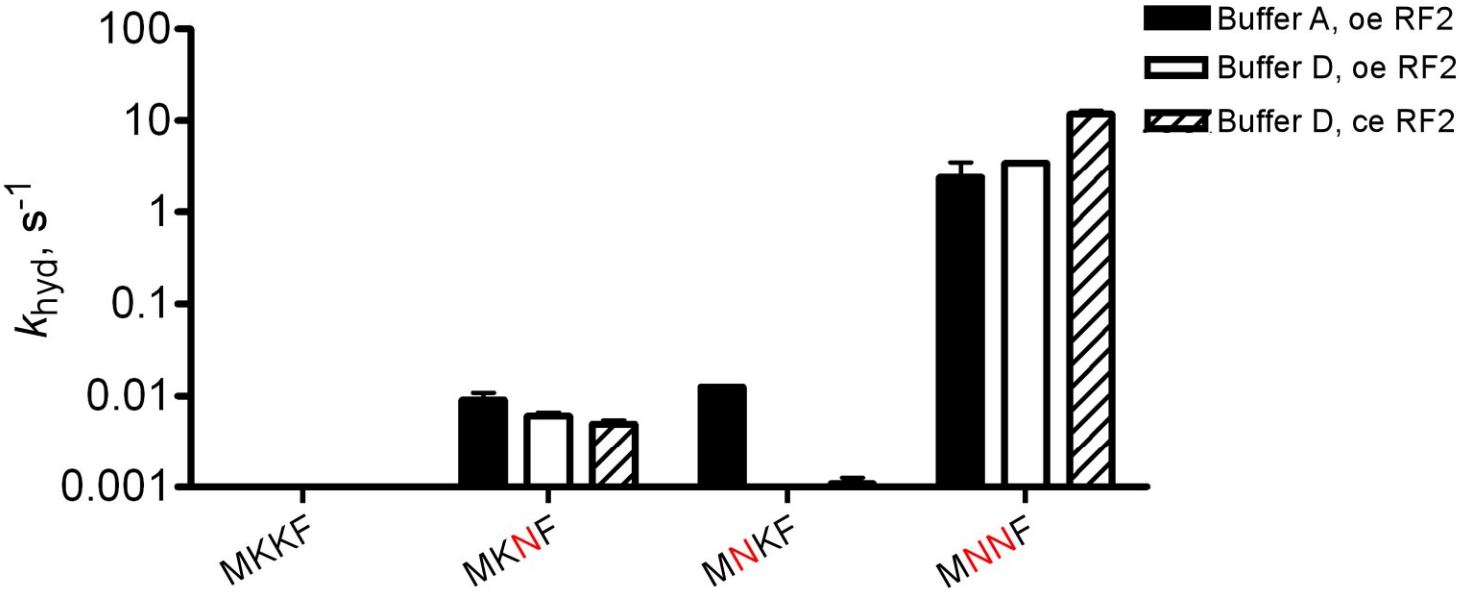
Zaher & Green, Supplementary Figure 9



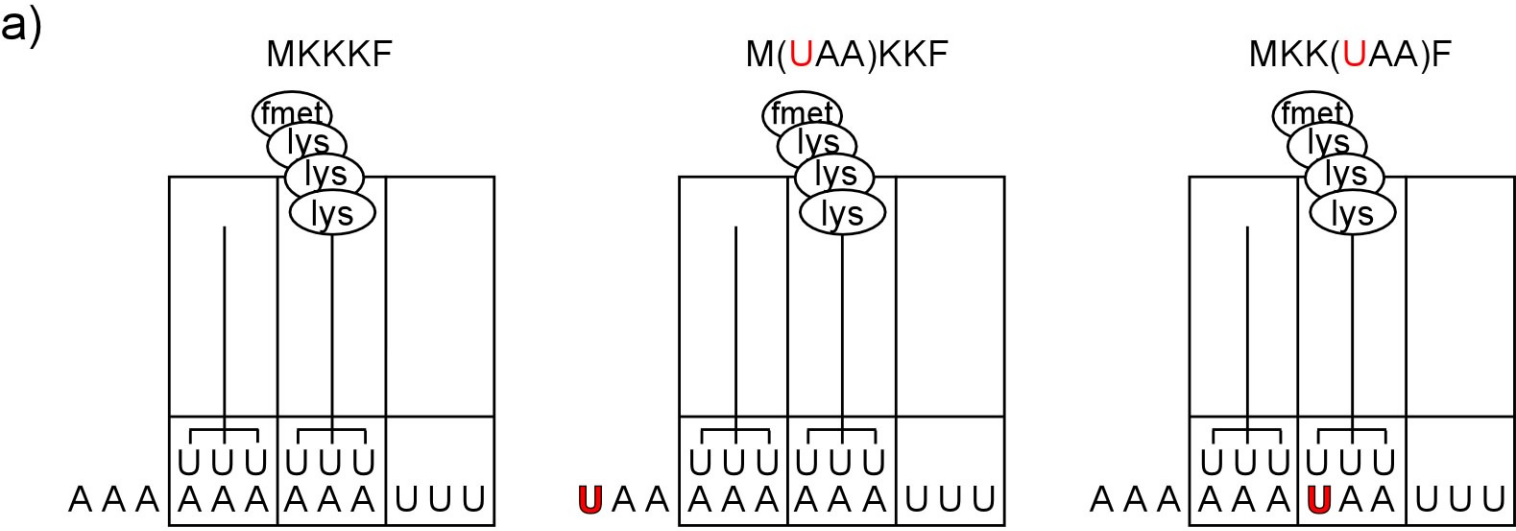
Zaher & Green, Supplementary Figure 10

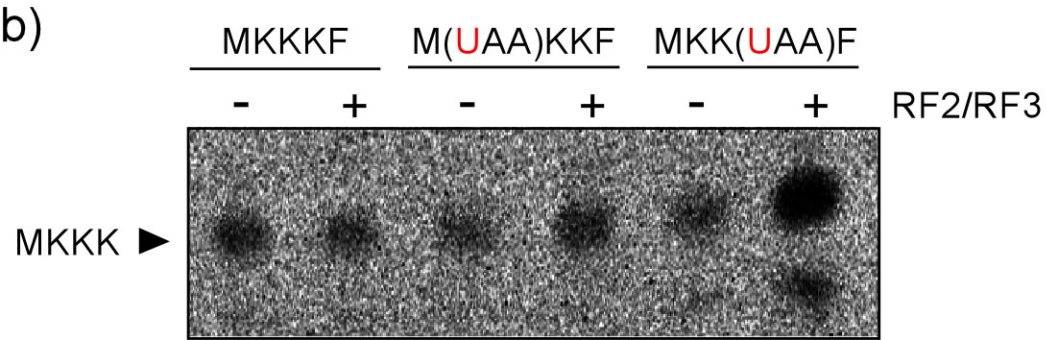


Zaher & Green, Supplementary Figure 11



Zaher & Green, Supplementary Figure 12





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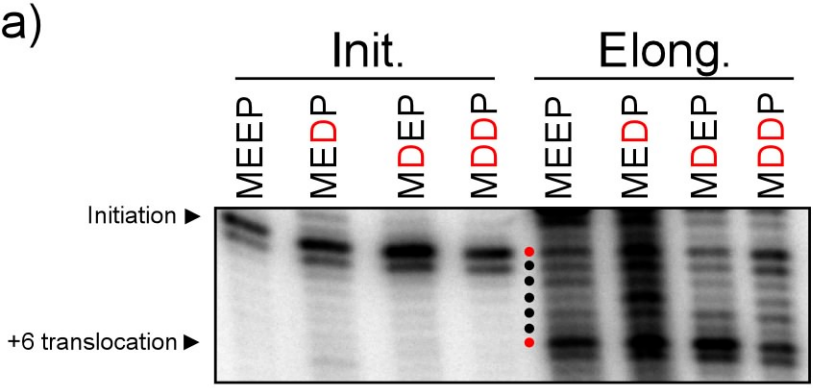
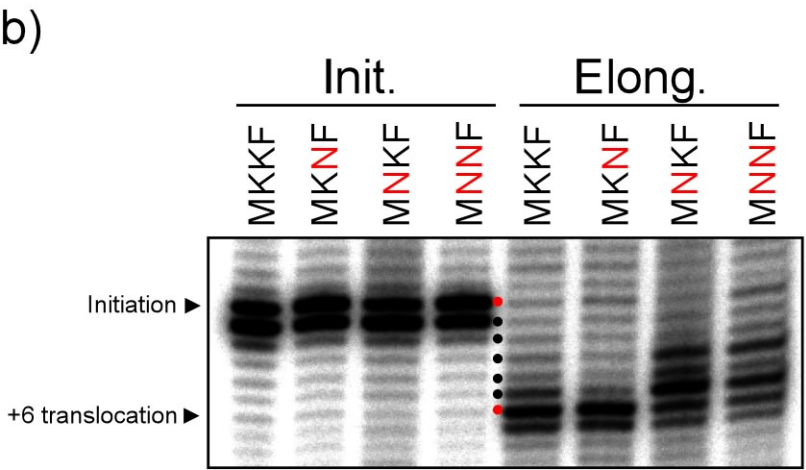
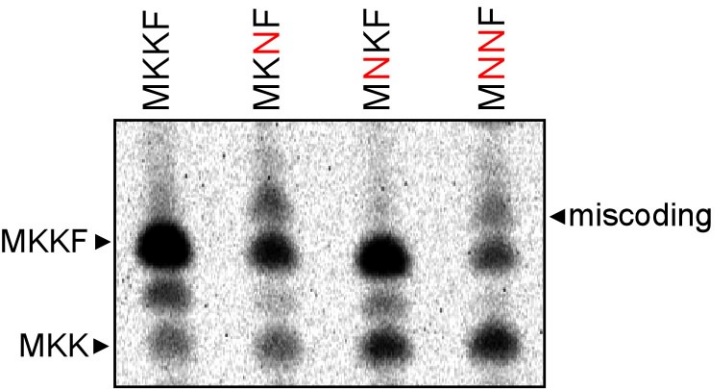




Zaher & Green, Supplementary Figure 14



Zaher & Green, Supplementary Figure 15