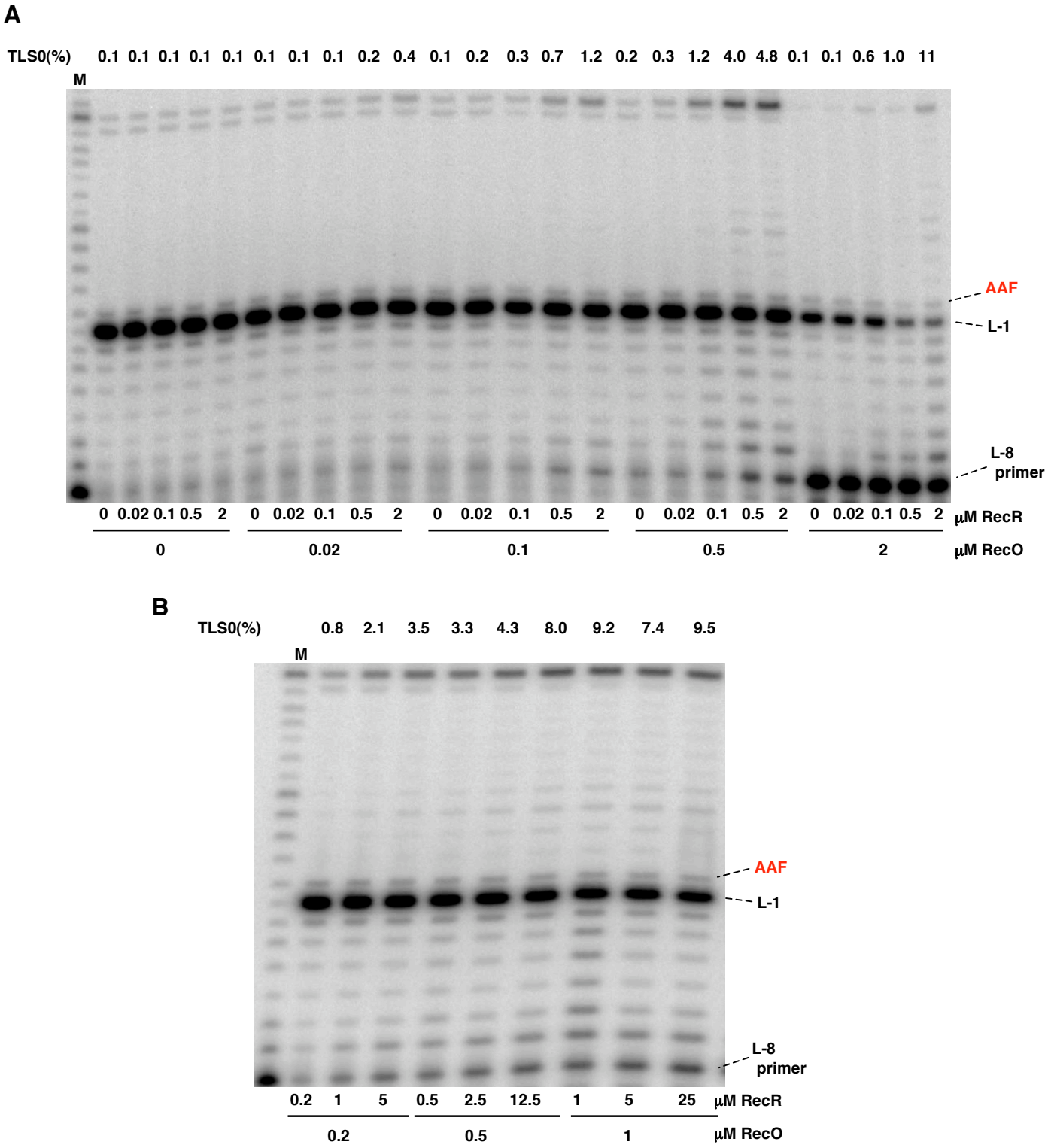
Supplementary information

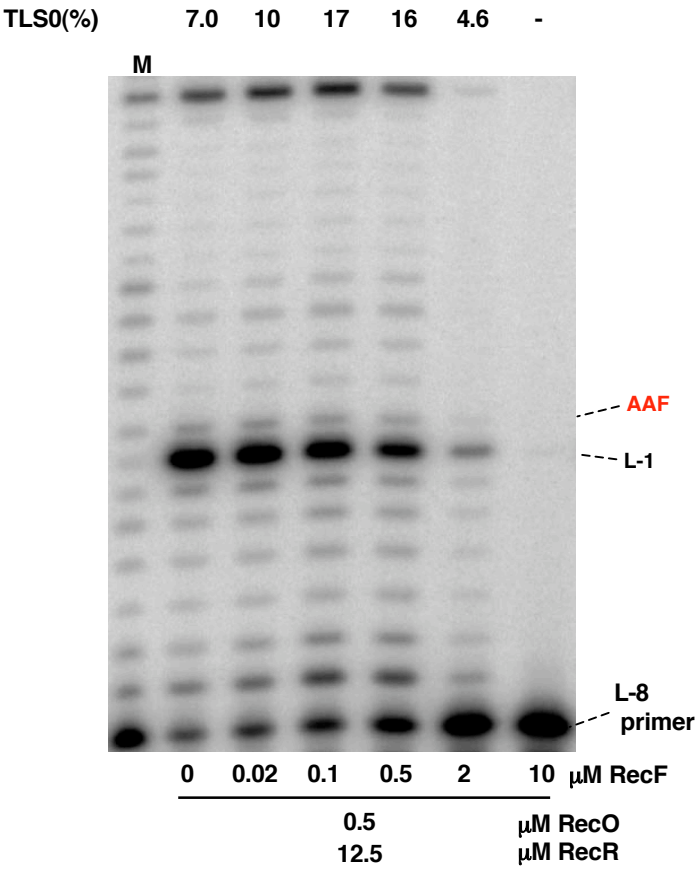
Materials and methods

A standard assay condition: The assay was performed as follows if not indicated in legends of supplemental figures: 300 nM SSB (as a tetramer), 50 nM β (as a dimer), 2 μM RecA, and indicated concentrations of RecFOR were mixed followed by addition of 2 nM template DNA containing a single G-AAF adduct primed with L-8 primer. The mixture incubated for 10 min at 30 °C, followed by the addition of 62.7 nM Pol III*. After 3 min, 100 nM Pol V was mixed and incubated for 10 min at 30 °C. Thereafter, the products were digested by *Eco*R I and analyzed on a sequencing gel.



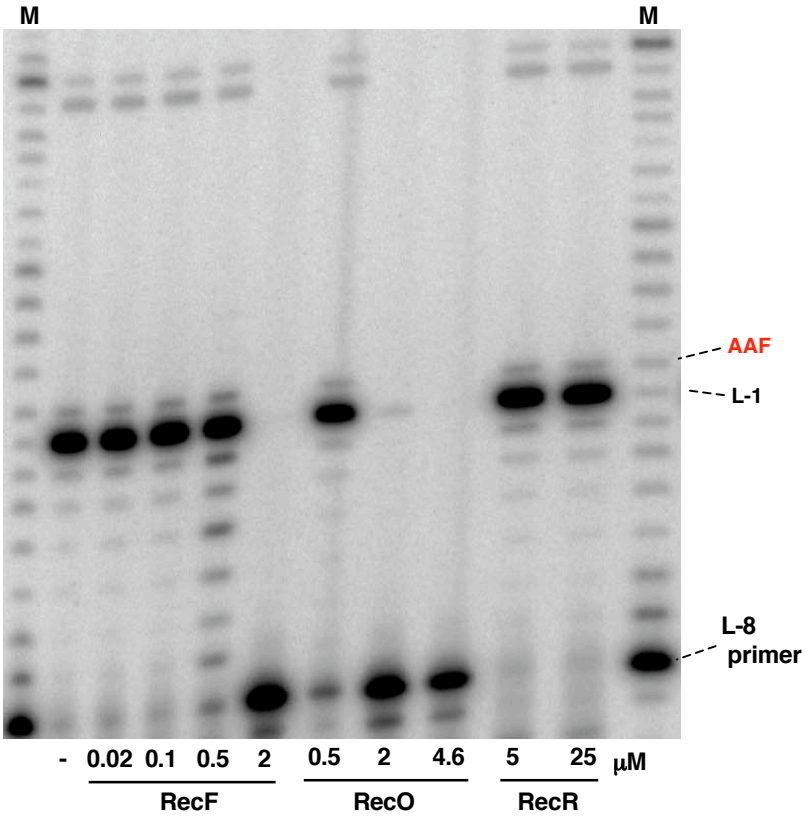
Supplementary Figure S1: RecOR proteins modulate Pol V-mediated TLS. In preliminary experiments, in the presence of RecOR, we could not detect further stimulation by RecF of Pol V-mediated TLS activity. We thus decided to determine optimal conditions for the RecOR combination. (A) Both RecO and RecR are required for stimulation of the TLS activity; 2 μM of RecO inhibits polymerase activity of Pol III. The optimal conditions involve a combination of RecO (0.5 μM) and RecR (0.5 to 2 μM). (B) Refinement of optimal RecO and RecR concentrations. Lane M is a DNA size marker.

Figure S1



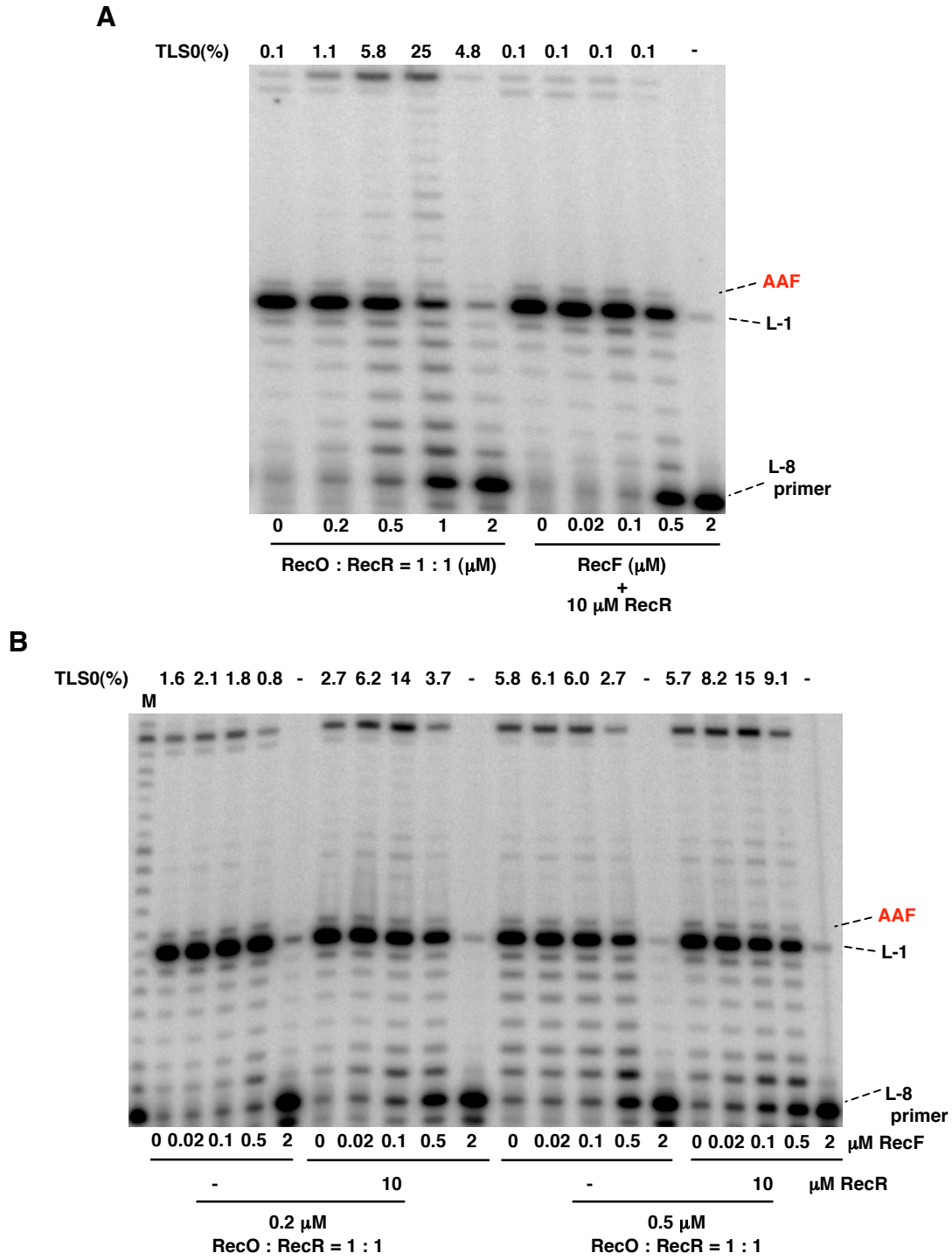
Supplementary Figure S2: RecF modulates Pol V-mediated TLS. In the presence of 0.5 μM RecO and 12.5 μM RecR, RecF up to 0.5 μM stimulates the TLS activity while higher concentration (i.e, 10 μM) almost completely inhibits polymerase activities. Lane M is a DNA size marker.

Figure S2



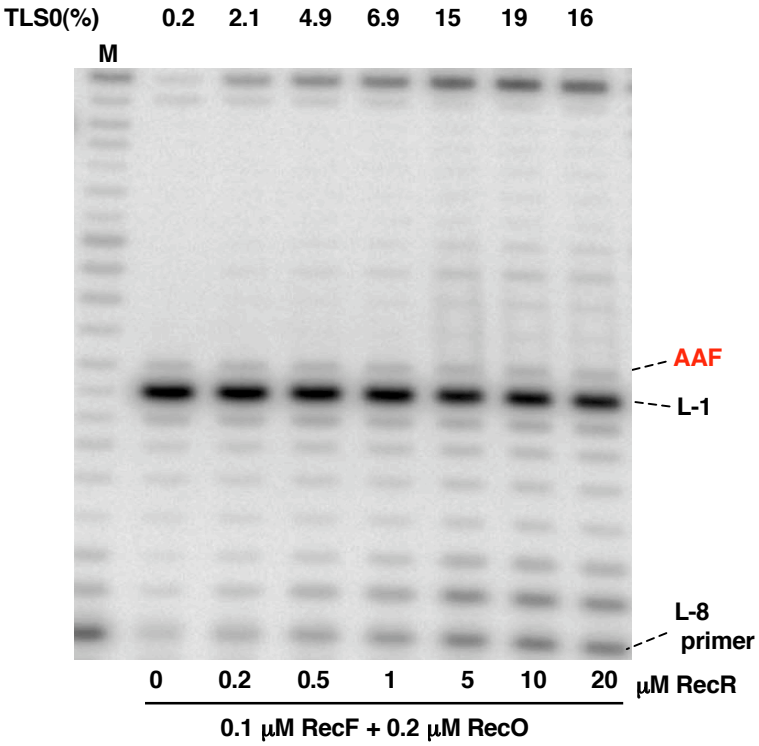
Supplementary Figure S3: High concentrations of either RecF or RecO but not RecR inhibit Pol III activity. 50 nM β (as a dimer) and indicated concentrations of either RecF, RecO, or RecR were mixed followed by addition of 2 nM template DNA containing a single G-AAF adduct primed with L-8 primer. The mixture incubated for 10 min at 30 °C, followed by the addition of 62.7 nM Pol III*. After 13 min, the reactions were terminated. High concentration of either RecF or RecO remarkably inhibits Pol III polymerization activity, however RecR does not show such inhibitory effect. Lane M is a DNA size marker.

Figure S3



Supplementary Figure S4: Determination of optimal concentrations of RecFOR for Pol V-mediated TLS. 2 nM template, 300 nM SSB (as a tetramer), and 50 nM β (as a dimer) were mixed and incubated for 5 min, followed by addition of 2 μM RecA. After 3 min, indicated concentrations of RecFOR were mixed and incubated for 5 min, followed by the addition of 62.7 nM Pol III*. After 3 min, 100 nM Pol V was mixed and incubated for 10 min at 30 °C. (A) RecO and RecR concentrations of either 0.2 or 0.5 μM stimulate TLS to an extent that will allow further titration by RecF in the next stage. Higher concentrations of RecO and RecR (1μM) while triggering TLS efficiently strongly inhibit Pol III activity (due to RecO, see Figure S3). RecF has no stimulatory effect in the presence of RecR. (B) 0.1 μM RecF significantly stimulates the TLS activity in the presence of 0.2 μM RecO and excess amount of RecR (10.2 μM). On the other hand, 0.1 μM RecF moderately stimulates the TLS activity in the presence of 0.5 μM RecO and excess amount of RecR (10.5 μM).

Figure S4



Supplementary Figure S5: Excess amount of RecR is needed to achieve for efficient Pol V-mediated TLS in the presence of RecOR. Experimental condition is as same as Figure S4 except that the concentrations of RecF (0.1 μM) and RecO (0.2 μM) are fixed while RecR is variable. Based on these results, we determined the following optimal conditions: 0.1 μM RecF, 0.2 μM RecO, and 10 μM RecR. Lane M is a DNA size marker.

Figure S5