Supplementary Information

- Supplementary Figures and Legends

Supplementary Figures

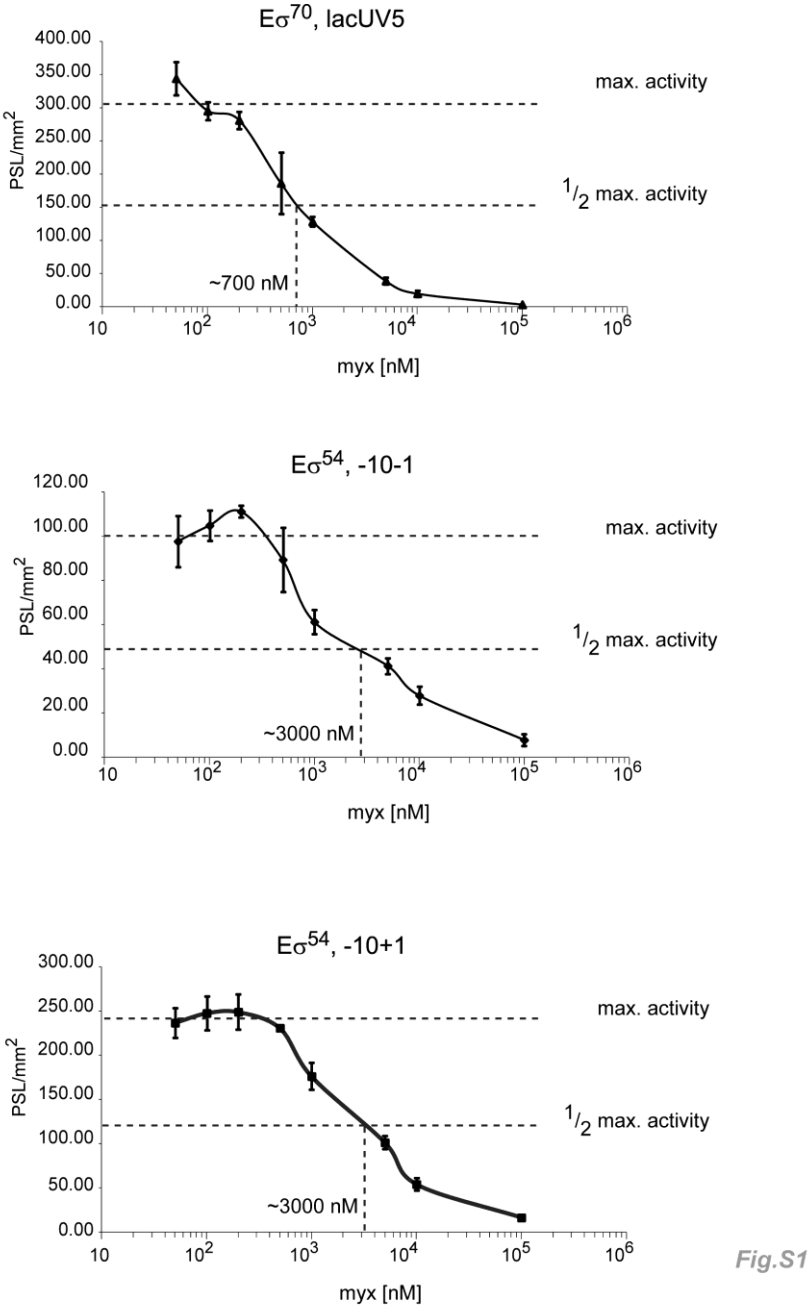

Fig.S1

Fig. S1: Myxopyronin titrations.

$E\sigma^{54}$ holoenzymes are less sensitive to myxopyronin than $E\sigma^{70}$ holoenzymes. In spRNA assays measuring the accumulation of a radiolabelled tetramer, the activity of wt $E\sigma^{54}$ is reduced by 50% in the presence of ~3µM myxopyronin (both with the -10-1/WT and the -10+1/WT template) whereas wt $E\sigma^{70}$ is reduced to the same extent at concentrations of ~700nM myxopyronin. The -10-1/WT and the -10+1/WT templates used represent the *nifH* promoter with a mismatch from nucleotide -10 to -1 (or +1, respectively) to mimic the transcription bubble (detailed information about both templates in main text).

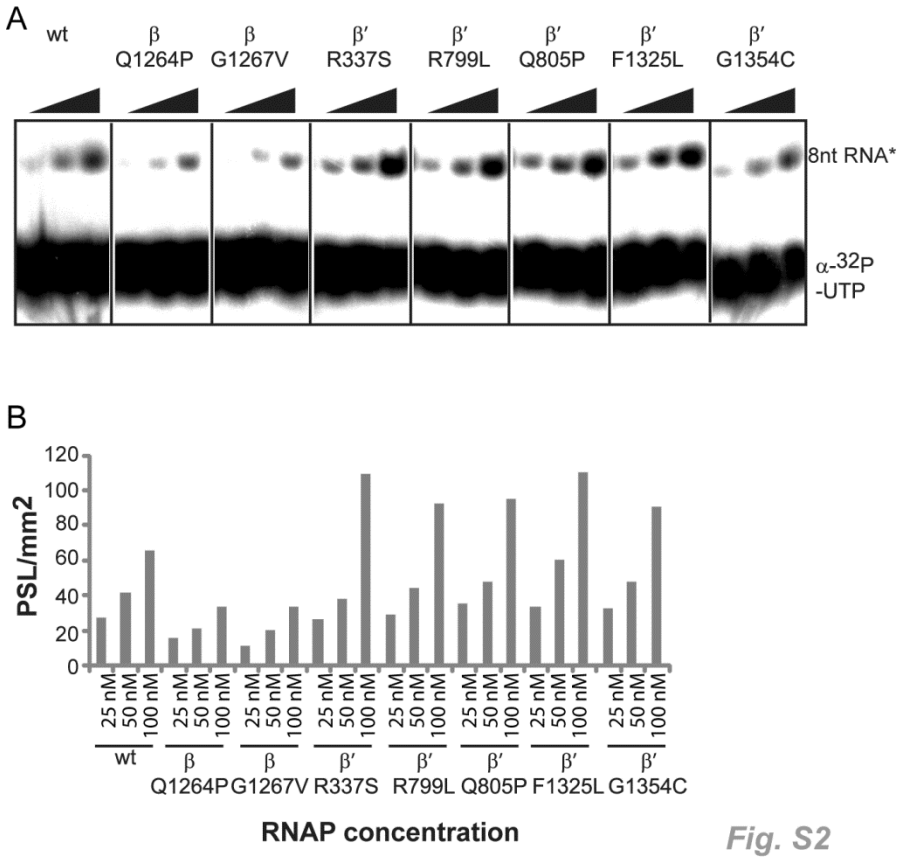

Fig. S2: Minimal scaffold assay – titrations.

A: Minimal scaffold assay showing titrations of the RNAP variants. The accumulation of the 8nt RNA was evaluated is indicated. The 8 nt RNA signal was normalised with respect to the total signal.

B: Titration experiment showing linear responses in the accumulation of the 8nt RNA in proportion to the RNAP concentration.

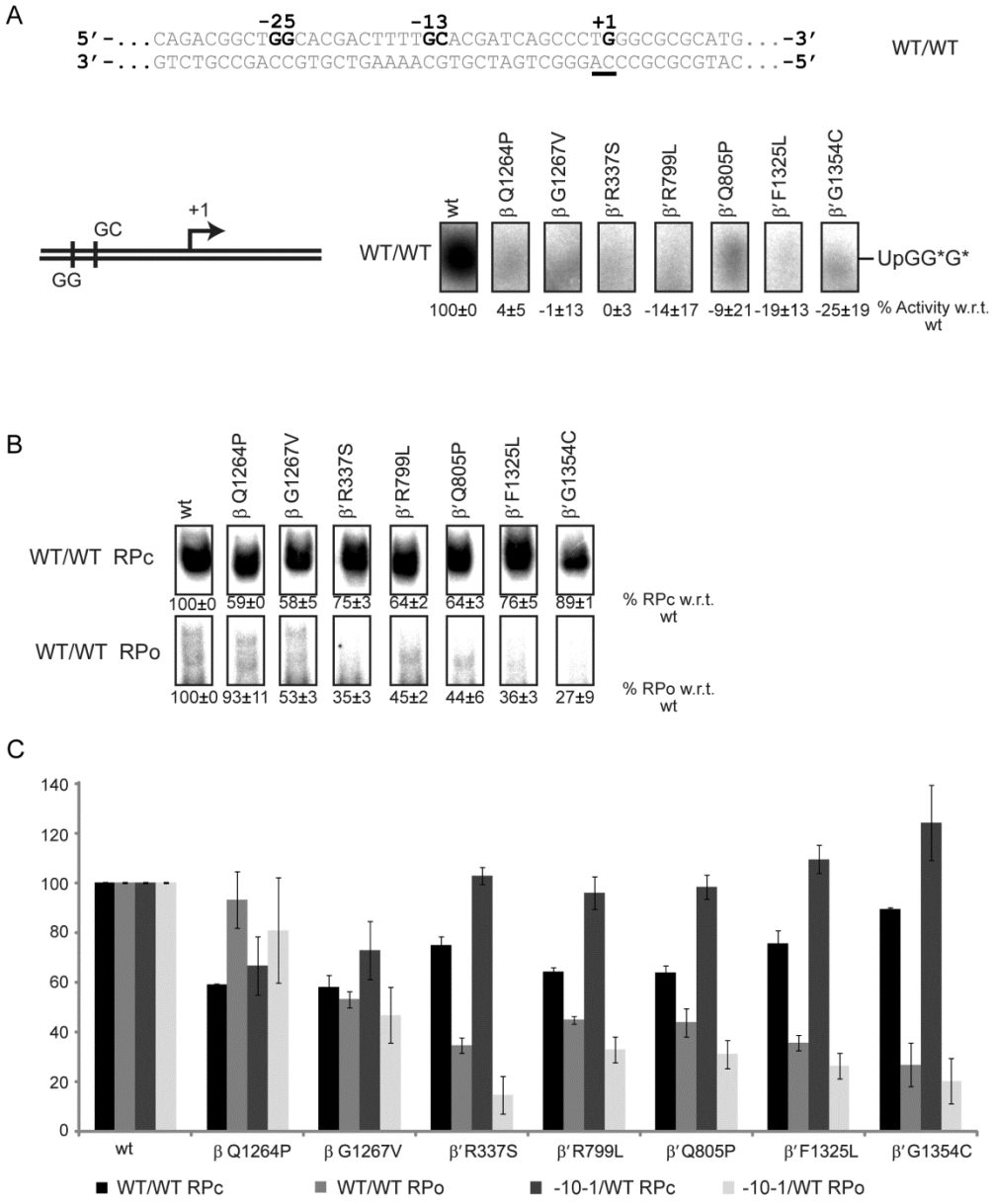

Fig. S3

Fig. S3: Promoter-directed transcription using a homoduplex probe.

The switch mutants are impaired in promoter-directed transcription when the WT/WT fully base paired template is used. The overall signals obtained from this template are substantially weaker than those obtained from the -10-1/WT template.

A: spRNA assay using the WT/WT template

B: RPc and RPo formation

C: RPc and RPo formation using the WT/WT template in comparison to the -10-1/WT template.

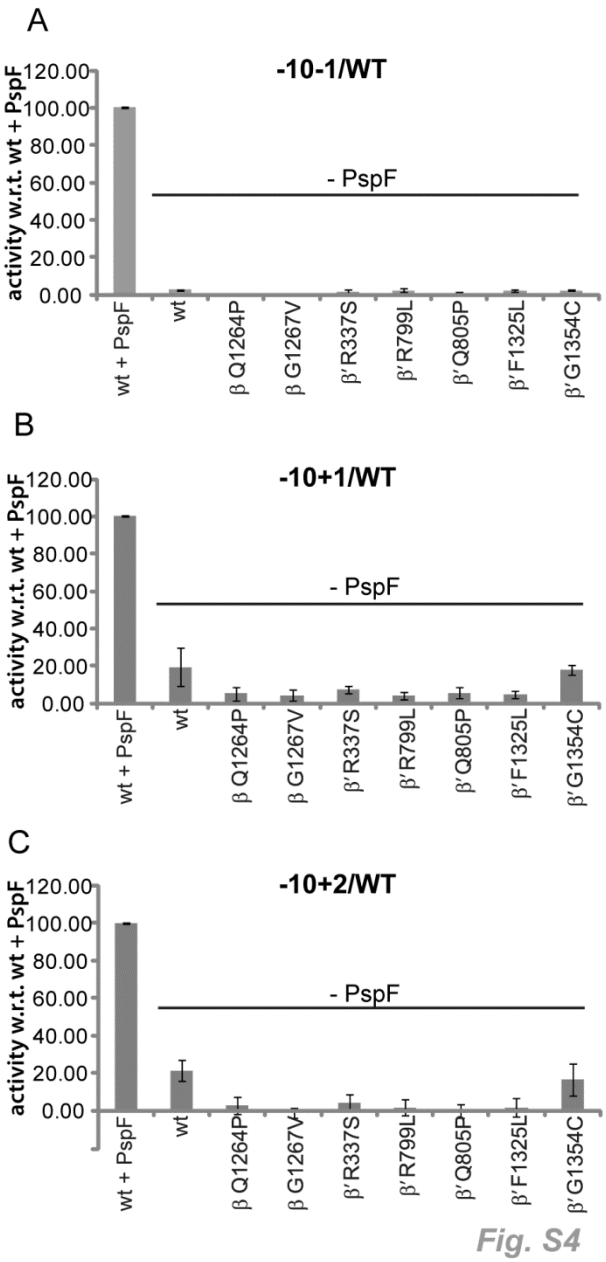

Fig. S4: Activator dependence.

The activator and its ATPase are still required to support transcription from templates where the start site is premelted. A relatively low level of transcription activity is observed in the absence of the activator with all three templates.

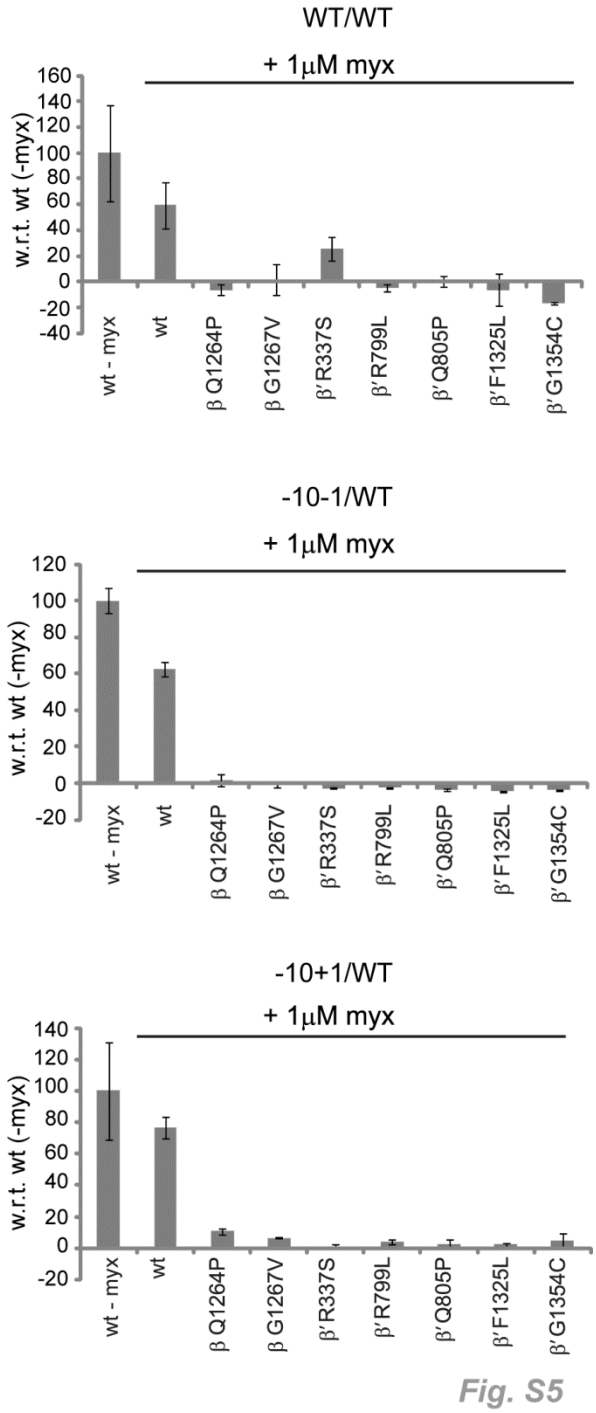

Fig. S5: Myxopyronin inhibition.

1 μM myxopyronin inhibits the switch domain variants on both the WT/WT and the -10-1/WT template and the start site melting requirement is not bypassed.

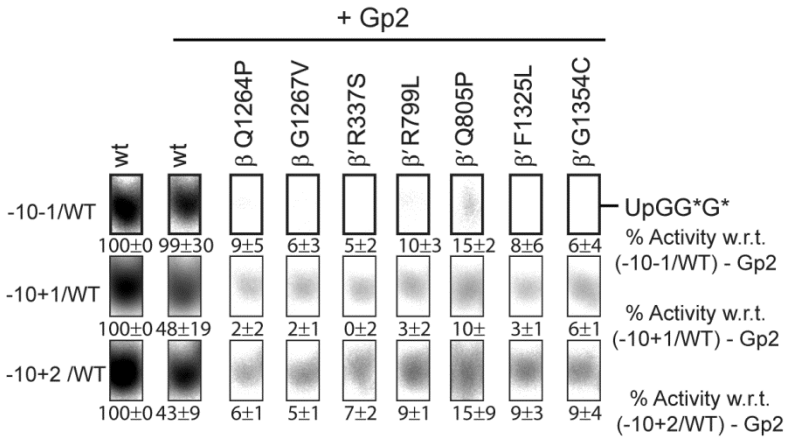

Fig. S6

Fig.S 6: Gp2 inhibition.

If start site melting is slow or lost, Gp2 inhibits $E\sigma^{54}$ more efficiently.

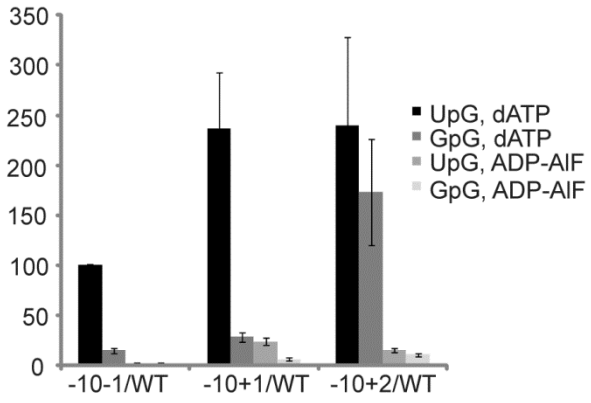

Fig. S7

Fig. S7: Template comparison.

Comparison of transcription initiation from the RPo or the RPi with UpG or GpG as priming dinucleotides, data are from three different templates.