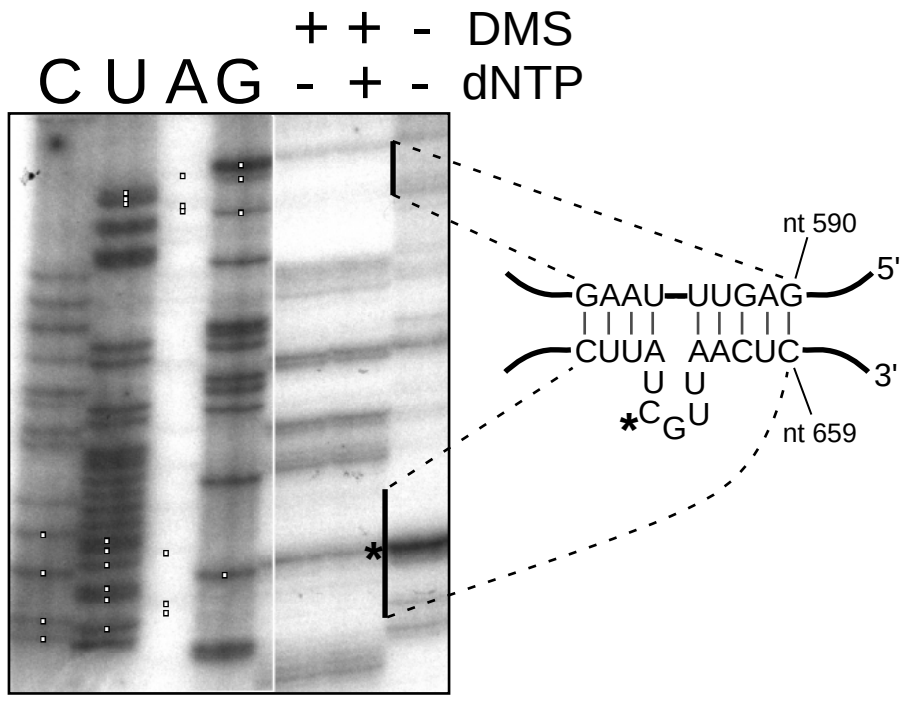
LEGEND TO SUPPLEMENTARY FIGURES

Supplementary Fig 1 DMS accessibility of the Est1p binding region of the telomerase RNA. C651 (asterisk) in the bulge also shows a strong pause in the reverse transcriptase reaction of unmethylated RNA. Termination at this position may therefore reflect an *in vivo* modified RNA base.

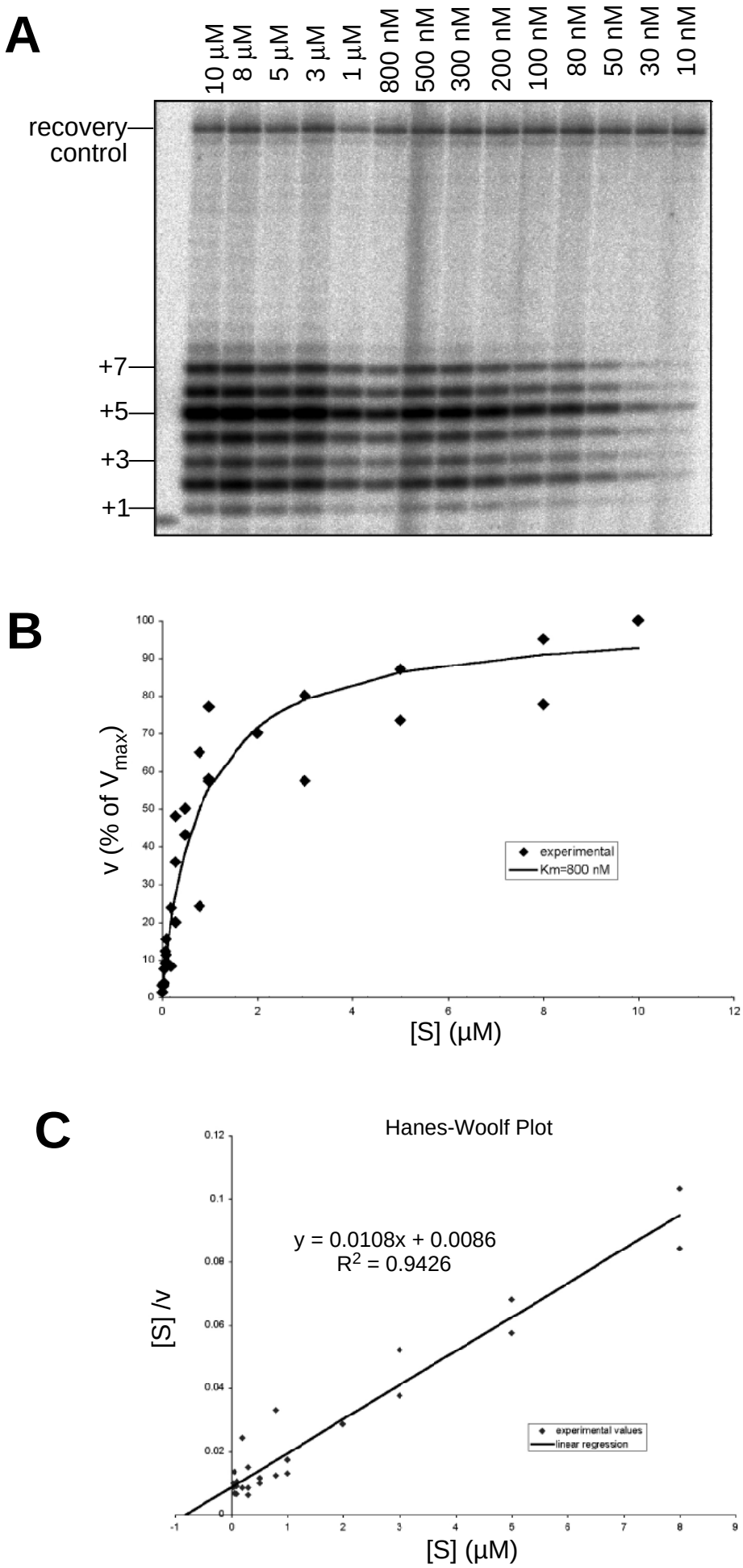
Supplementary Fig 2 Determination of the Km with DNA substrate primer $^{5'}$GTGTGTGTGGG$^{3'}$ for the yeast telomerase preparation used in this study. (**A**) Telomerase assay and primer substrate titration. One of three assays is shown. The primer concentrations are indicated on the top and the length of the reaction products on the side. The reactions were incubated for 60 seconds at 30 °C, which was still in the linear range of product accumulation at the highest substrate concentration used. Thus, the amount of product detected was proportional to the reaction velocity. (**B**) Primer extension plotted as a function of substrate concentration. Data from three independent experiments were pooled and expressed as % of the maximal measured velocity in each experiment. The experimental data fit well to the shown theoretical curve for a Km with 800 nM. (**C**) Hanes-Woolf Plot for determination of K_M. The Hanes-Woolf plot uses a derivation of the Michaelis–Menton equation to $[S]/v = [S]/V_{max} + K_M/V_{max}$. (**C**) In a diagram of [S]/v as a function of [S], the intercept with the horizontal axis equals $-K_M$. This plot will not exaggerate errors in velocity determination at low substrate concentrations, unlike the Lineweaver-Burk plot of 1/v against 1/[S].

The free energy of RNA/DNA hybrid formation was calculated using the HyTher program (http://ozone2.chem.wayne.edu/). The free energy calculated for a 7 bp hybrid

of the sequence $^{5'}$GTGTGGG$^{3'}$ was: ΔG= -8.32 kcal/mol, K_D=0.99 μM under calculation conditions that were set to the best estimation of the reaction conditions (30°C, 65 mM monovalent cation, 1.5 mM Mg^{2+}, 10 nM telomerase RNA (lowest possible value) and 1 μM DNA substrate oligonucleotide), without considering the influence of spermidine (present at 1 mM in the telomerase reaction) on the free energy of hybridization. Spermidine aids the formation of duplex nucleic acids by shielding the negative charges of the phosphate backbone. We therefore consider the calculations of the ΔG and K_D values an overestimate. Given the limitations of this calculation and the experimental errors, the measured K_M of 0.8 μM is consistent with a seven to eight bp RNA/DNA hybrid.



Suppl. Figure 1, Förstemann and Lingner



Suppl. Figure 2, Förstemann and Lingner