Supplement 4: Additional Material and Methods

Protein expression plasmids

pGEX-4T1-polι [492-715] (pAR208) (Kannouche *et al*, 2002) and pET16b-PCNA (pAV38)(Vidal *et al*, 2004) have been described previously. To generate the pGEX-4T1-polι [492-715] harboring the P692R mutation (pBP112), the 0.7 kb *Eco*RI-*Sal*I fragment from pGBKT7-polι [P692R] (pBP102) was cloned into pGEX-4T1 (Amersham Biosciences, Piscataway, NJ). Plasmid pBP114, expressing full-length GST-polι [P692R] was constructed by subcloning an *Nco*I-*Pst*I fragment of pBP102 into the baculovirus expression vector, pJM299 (Tissier *et al*, 2000).

To generate GFP-fusion proteins for *in situ* fluorescent studies, *Eco*RI and *Bam*HI fragments of pAVR76 and pAR116 were cloned into pECFP-polι digested with *Eco*RI and *Bam*HI. These constructs contain more 3'-UTR than the parental pECFP-polι (Kannouche *et al*, 2002). The wild-type and P692R ORFs were then cloned into the *Xho*I and *Bam*HI sites of pEGFP-C1 (Clontech).

pGEX-4T1-polη [581-713] (pWC9) was constructed by amplifying the 3' end of *POLH* using PCR primers EtaC1 and EtaC2 and cloning the amplicon into the *Eco*RI and *Sma*I sites of pGEX-4T1. The polη-H654A mutation (with a silent *Nsi*I restriction site) was generated by site-directed mutagenesis using primers PolHH654A1 and PolHH654A2 and cloned into pESC-Leu polη. The region was then subcloned as a *Bsg*I fragment into pFastbac XPV-His (pBP161), or as a *Kpn*I-*Xho*I fragment to generate pGEX-4T1-polη [581-713] H654A (pBP162).