Supplement 4: Additional Material and Methods

**Protein expression plasmids**

pGEX-4T1-pol [492-715] (pAR208) (Kannouche et al, 2002) and pET16b-PCNA (pAV38) (Vidal et al, 2004) have been described previously. To generate the pGEX-4T1-pol [492-715] harboring the P692R mutation (pBP112), the 0.7 kb EcoRI-SalI fragment from pGBK7-pol[P692R] (pBP102) was cloned into pGEX-4T1 (Amersham Biosciences, Piscataway, NJ). Plasmid pBP114, expressing full-length GST-pol[P692R] was constructed by subcloning an Ncol-PstI fragment of pBP102 into the baculovirus expression vector, pJM299 (Tissier et al, 2000).

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

pGEX-4T1-pol[581-713] (pWC9) was constructed by amplifying the 3' end of POLH using PCR primers EtaC1 and EtaC2 and cloning the amplicon into the EcoRI and SmaI sites of pGEX-4T1. The pol[581-713]-H654A mutation (with a silent Nsil restriction site) was generated by site-directed mutagenesis using primers PolHH654A1 and PolHH654A2 and cloned into pESC-Leu pol[581-713]. The region was then subcloned as a BsgI fragment into pFastbac XPV-His (pBP161), or as a KpnI-XhoI fragment to generate pGEX-4T1-pol[581-713] H654A (pBP162).