Dimerization of p12, the fourth subunit of human DNA polymerase δ, is a pre-requisite for its interaction with PCNA

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Running title: p12 the smallest subunit of hPolδ

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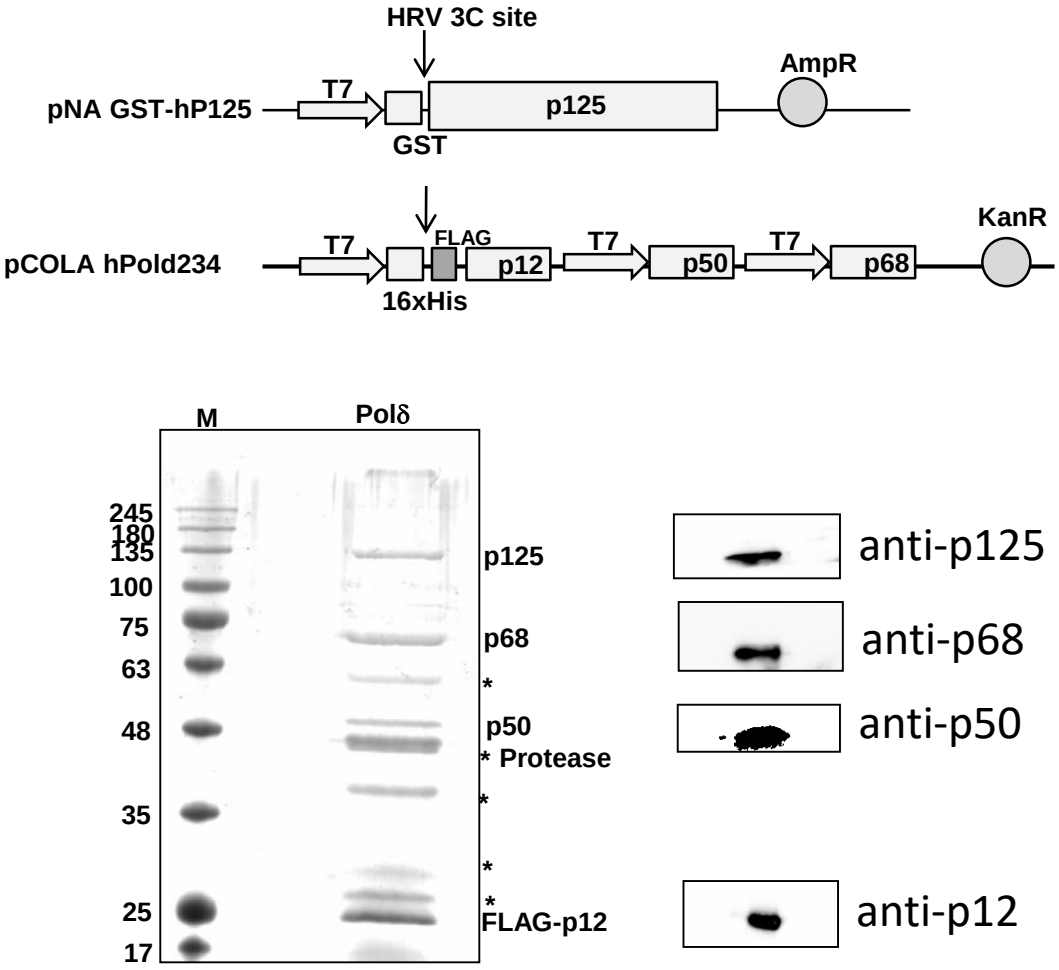
Supplementary Fig. 1: GST-affinity purification of hPolδ holoenzyme was carried out by using bacterial expression constructs harboring GST-p125 and a triple expressing plasmid containing p12, p50 and p68. Both p125 and p12 possess protease site. Cleaved p12 retains FLAG–tag and thus it migrates ~22Kda position. * Bacterial chaperon and other non-specific proteins. Stochiometry of FLAG-p12 is higher than any other subunit. Indicated bands were also authenticated by western blot analysis.

Supplementary Fig. 2: p12 RKR (1-MGRKRLITDSYPVK-14) and PIP (92-GDPRFQCSLWHLYPL-106) motif harboring domains were used for peptide structure prediction by using PEP-FOLD3 server (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/). The models were validated by SAVES and Ramachandran plot. RKR-motif forms a stable α-helix, whereas PIP motif forms a 3_{10} helix.

Supplementary Fig. 3: p12 proteins were purified to near homogeneity from bacterial cells by using GST-affinity column chromatography, GST-tag was cleaved off by PreScission protease, and analyzed by SDS containing polyacrylamide gel electrophoresis (PAGE). Lane 1, MW; Lane 2, CA; Lane 3 wild type; Lane 4 R3A, K4A, R5A and Lane 5 L104A, Y105A p12 proteins.

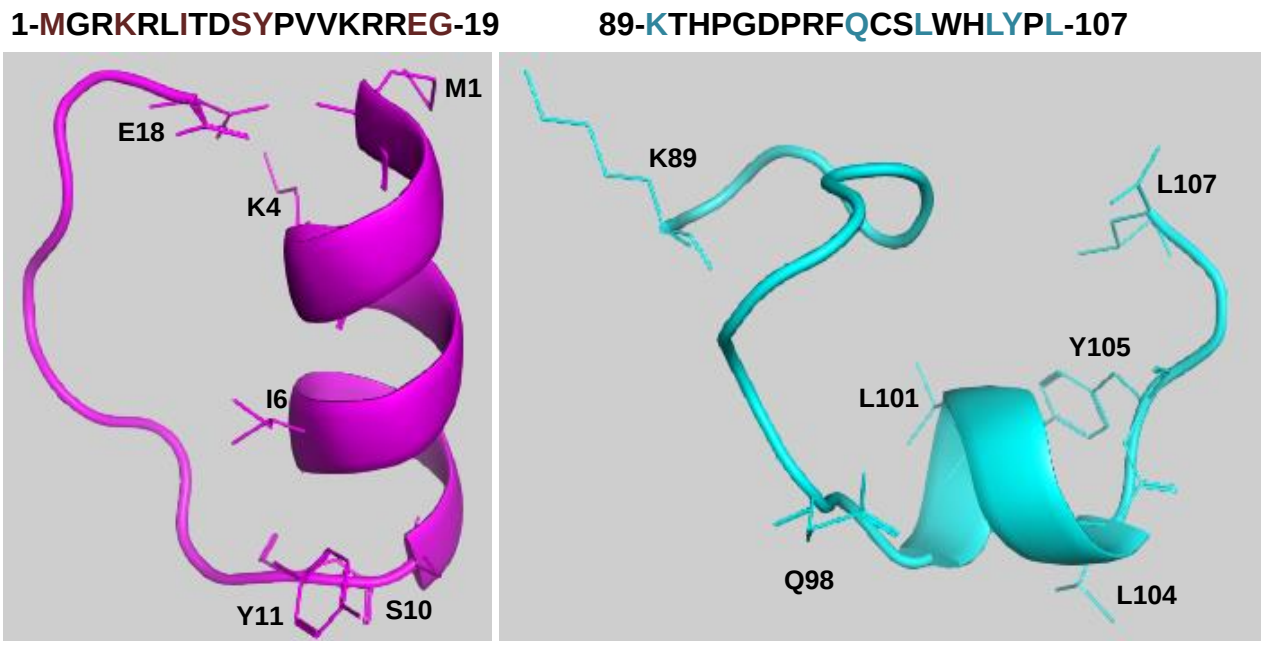
Supplementary table- 1: List of primers used for various orfs amplification with underlined restriction enzymes.

Primer	Primer sequence (5'----3')	Name of the ORF
NAP 239	ccggggatccacatatgttcgaggcgcgcc	hPCNA
NAP 240	ccggggatcctaagatccttcttcatc	hPCNA
NAP 251	ccggggatccgtatgttcgaggcgcgcc	hPCNA
NAP 300	ctacacagctgtactcctgttctggagctccagcttgttcaacatc	hPCNA
NAP 304	gatgttgaacaagctggagctccagaacaggagtacagctgtgtag	hPCNA
NAP 305	ggccggatccctaagatccttcttcatcctcgaggcggcagccaag	hPCNA
NAP 254	ggccggatcctcaggggcccagccccagg	p50
NAP 255	ggcgaattcatgttttctgagcaggctgc	p50
NAP 257	ggccggatcctttatttcctctggaagaagcc	p68
NAP 258	ggcgaattcatggcggaccagctttatctgg	p68
NAP 260	ccggggatccacatatgggccggaagcggctc	p12
NAP 261	ggccggatcctcataggggatagagatgcc	p12
NAP 262	ggcgaattcatgggccggaagcggctc	p12
NAP 265	ggccggatcctcataggggagcggcatgccagagactg	p12
NAP 362	ccggggatccgtatgggcgccgctgcactcatcactgattcc	p12
NAP 373	ggccggatccacatatgggcgccgctgcactcatcactg	p12
NAP 252	ggcgaattcatggatggcaagcggcgg	p125
NAP 248	ggccggatcctcaccaggcctcaggtccaggggtcc	p125
NAP 361	ggccggatccctagcctggaatggtttgaag	Cdm1
NAP 451	ccggggatccacatatgaagaagcgcac	Cdm1
NAP 452	ggccggatccacatatgactactcaagcgaaaaaatcaggg	Cdm1
NAP 448	cggcgaattctatgggccggaagcgg	p12
NAP 450	ccggggatccggtaggggatgatagagatg	p12
NAP 444	ccgggaattctatgctagaaaacaatgc	Polθ
NAP 151	ccgggtcgacggatcccttacacatcaaagtcctttagctctcccc	Polθ

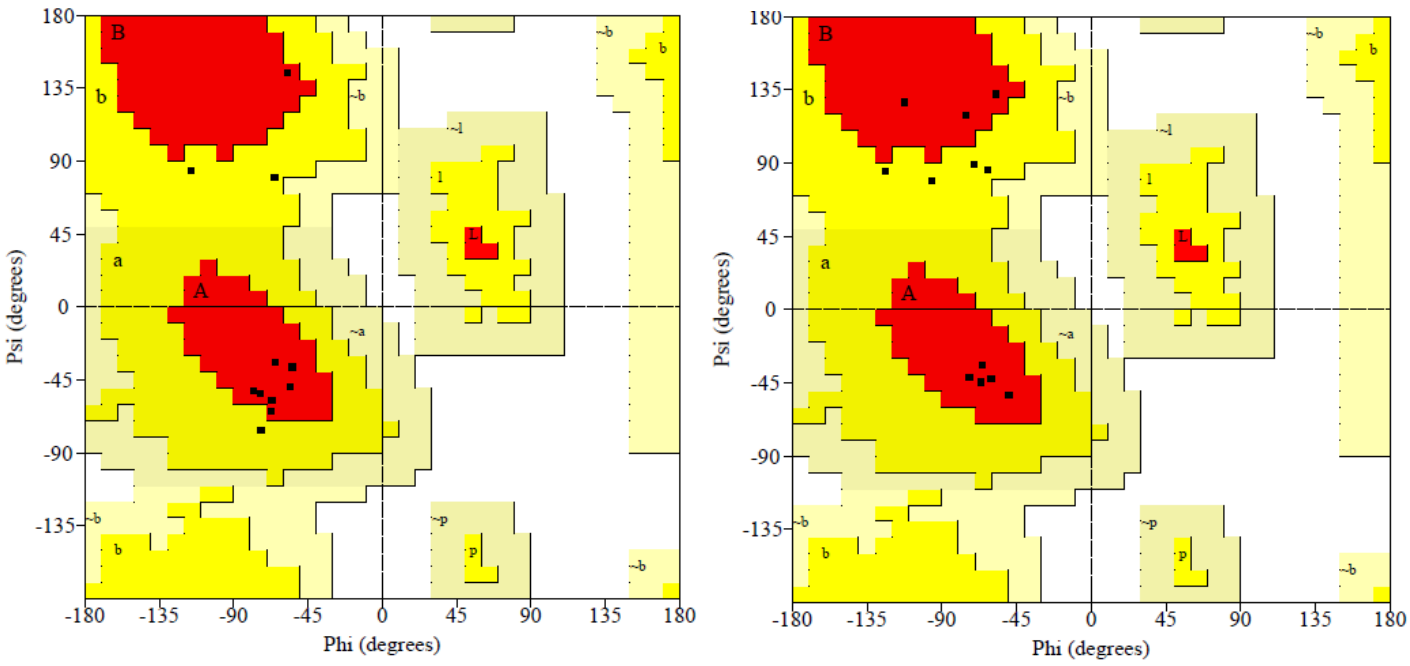


Supp. Figure. 1

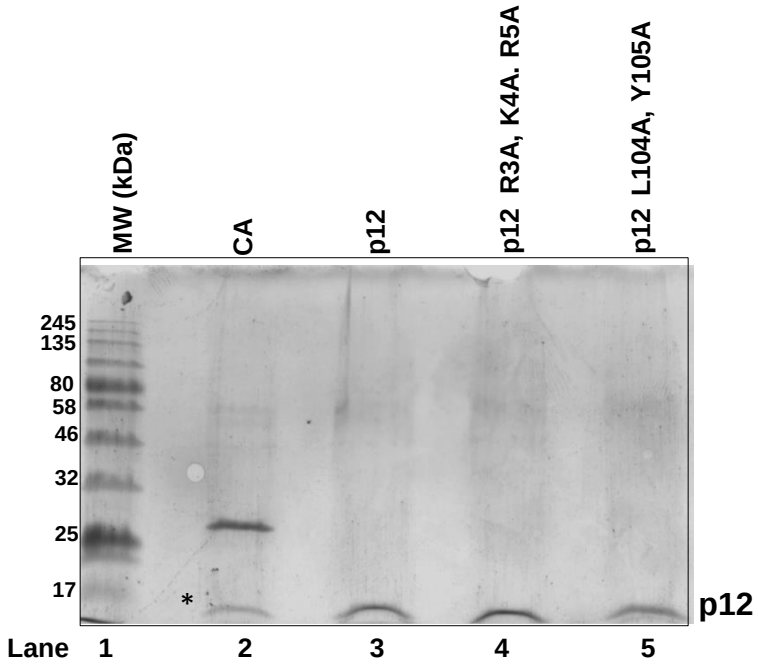
A.



B.



Supp. Figure. 2



Supp. Figure. 3