

Figure S3 Pulse Field Gel Electrophoresis (PFGE) was performed on cells disrupted for the error prone bypass factor rev3A in combination with esa1-L254P. Cells were arrested in  $\alpha$ -factor for 2 hours followed by release into YPAD media containing bromodeoxyuridine (BrdU; 400µg/ml) and either nocodazole (7.5µg/ml) for 2.5 hours or 0.01% MMS for 1 hour. Following MMS treatment, cells were released into YPAD + BrdU and samples were collected at the indicated time points. Pulse Field Gel Electrophoresis (PFGE) was performed, followed by a Southern transfer to nitrocellulose, and blotted with  $\alpha$ -BrdU antibodies in wild type (JC604), esa1-L254P (JC3060), rev3 $\Delta$  (JC2978) and esa1-L254P /  $rev3\Delta$  (JC3053) - (A) pictures of one representative gel for wild type and the double mutant demonstrates that in the absence of MMS, replication and chromosome integrity is indistinguishable between wild type and the double mutant (150\* on gel). (B) The cell cycle stage was monitored by flow cytometry. (C) The BrdU signal at the 0 min (blue) and 60 min (green) time points were quantified by Image J with the migration distance of chromosomes vs the intensity of BrdU plotted, giving a measure of newly synthesized chromosomes during one round of DNA replication. All data shown here follows the same protocols as in Figs. 2 and 3.