

Figure S2. Expression of Bcl2 retards DNA replication via reduction of deoxyribouclotide synthesis and RNR activity in normal human bronchial epithelial (BEAS-2B) cells. A. Bcl2 was stably transfected in BEAS-2B cells. Expression levels of Bcl2, hRRM1 and hRRM2 were analyzed by Western blot. B, BEAS-2B cells expressing Bcl2 or vector-only control were pulsed-labeled with 100 µM CldU for 20 min and 100 µM IdU for another 20 min. The labeled cells were processed for DNA combing. Representative pairs of sister replication forks are shown. Red, CldU. Green, IdU. Bar, 5 µm (1 µm = 2.59 kb). C, distribution of fork rate (kb/min) in BEAS-2B cells expressing Bcl2 or vector-only control. The mean±SD for fork rate and number of scores are summarized. The p value was determined by a two-tailed Mann-Whitney test. D, distribution of inter origin distances (kb) are compared in BEAS-2B cells expressing Bcl2 or vector-only control. The mean \pm SD for distances and number of scores are summarized. E. distribution of the degree of asymmetry of bidirectional replication forks was compared in BEAS-2B cells expressing Bcl2 or vector-only control. F, intracellular levels of dNTPs in BEAS-2B cells expressing WT or vector-only control were measured. Error bars represent ±SD of three repeated determinations. G-H, extracts from BEAS-2B cells expressing WT Bcl2 or vector-only control were incubated with ¹⁴C-CDP. The generation of ¹⁴C-dCDP was analyzed by a phosphorImager on TLC. The relative abundance of ¹⁴C-dCDP and ¹⁴C-CDP was quantified with imageQuant software. RNR activity was calculated by a formula as ¹⁴C-dCDP/ (¹⁴C - $CDP+^{14}C-dCDP$). Error bars represent $\pm SD$ of three repeated determinations.