Supplementary Information S1 (figure) | **Identification of reversed forks in vivo by psoralen crosslinking coupled to transmission electron microscopy.**

The analysis of replication intermediates by transmission electron microscopy, performed in cells in which DNA replication intermediates were stabilized by psoralen-crosslinking, is currently the most reliable approach for investigating replication fork reversal in vivo\(^1\). In order to obtain reliable data from this method, high sample quality is paramount, as suboptimal sample quality will increase the risk of mistaking accidental overlap of linear DNA molecules for reversed forks. Samples should therefore strictly meet the following requirements and criteria in order to reliably enable the identification of reversed forks: (i) Low sample density. A low density of DNA molecules will reduce the probability of accidental filament crossing. (ii) High sample quality and high magnification. A fine and homogenous granularity in the background of the visualized molecules is required to inspect the architecture of any junction, which is the key criterion for the assignment of a 4-way junction as a reversed fork (see REF. 1 for technical details). High magnification images (200-250kx; see blow-ups in the figure) have proven very useful in the assignment of DNA junctions. (iii) Junction appearance. If the junction is opened up and its individual strands are easily distinguishable (see the figure, part a, and panel d in Box 1), its assignment as a reversed fork is relatively straightforward. However, in most junctions the four participating strands are collapsed (see the figure, part b) and additional parameters are required for their assignment as reversed forks. In contrast to accidental overlaps of DNA strands, actual junctions have a “flat” appearance with all arms detectable in the same focal plane. (iv) Directionality of the junction arms. DNA helices in accidental overlaps are often aligned along only two axes; this is rarely seen in reversed forks. (v) Contour length symmetry of replicated duplexes. Reversed forks are expected to show symmetric length of the daughter duplexes (D1 and D2, see the
figure, part c) after restriction digestion, as the closest restriction site should be equidistant from the fork on both duplexes. The parental duplex (P) and regressed arm (R) can instead have any length and are usually identified by close inspection of the junction (see blow-ups).

Reference: