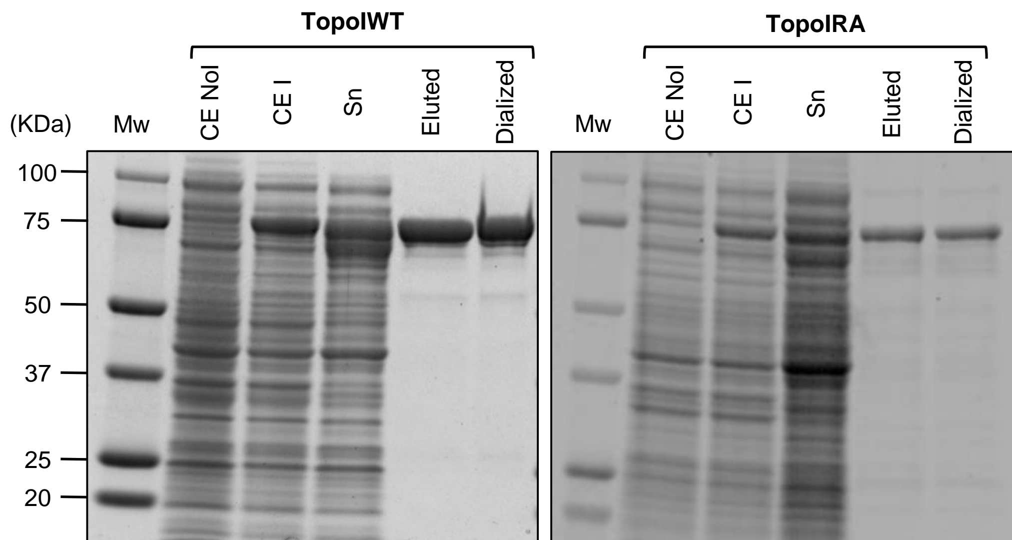

Figure S1. Purification of enzymes. Cultures of *E. coli* M15 (pREP4)/ pQE- topAWT or *E. coli* XL1-Blue/ pQE-topARA were grown in LB medium, induced with IPTG, processed and analyzed as described under Material and Methods. CE, crude extract, either induced (CEI) or not induced (CENo I) with IPTG; Sn, lysate applied to a Ni-NTA column; Eluted, column fraction eluted with 100 mM imidazole; Dialyzed, purified protein after dialysis to remove imidazole. The sizes of protein markers (Mw) are indicated to the left.

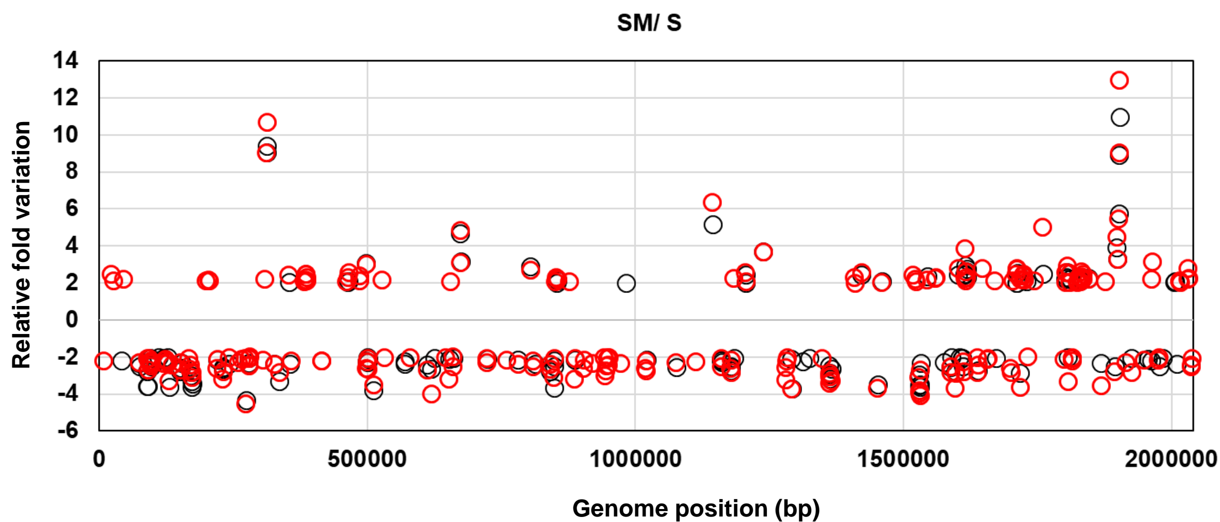

Figure S2. Transcriptomic response in strain Δ*topA*P_{Zn}*topA* carrying pLS1ROM*topAWT* (black symbols) or pLS1ROM*topARA* (red symbols) under overproduction of TopoI enzymes. Fold change and localization of DEGs. The relative fold variation of each gene is represented against the location of 5′ end of each open reading frame in the *S. pneumoniae* R6 chromosome (bases 1 to 2038615). A fold change ≥ 2 (absolute value) and a P-value-adjusted ≤ 0.01 were considered.

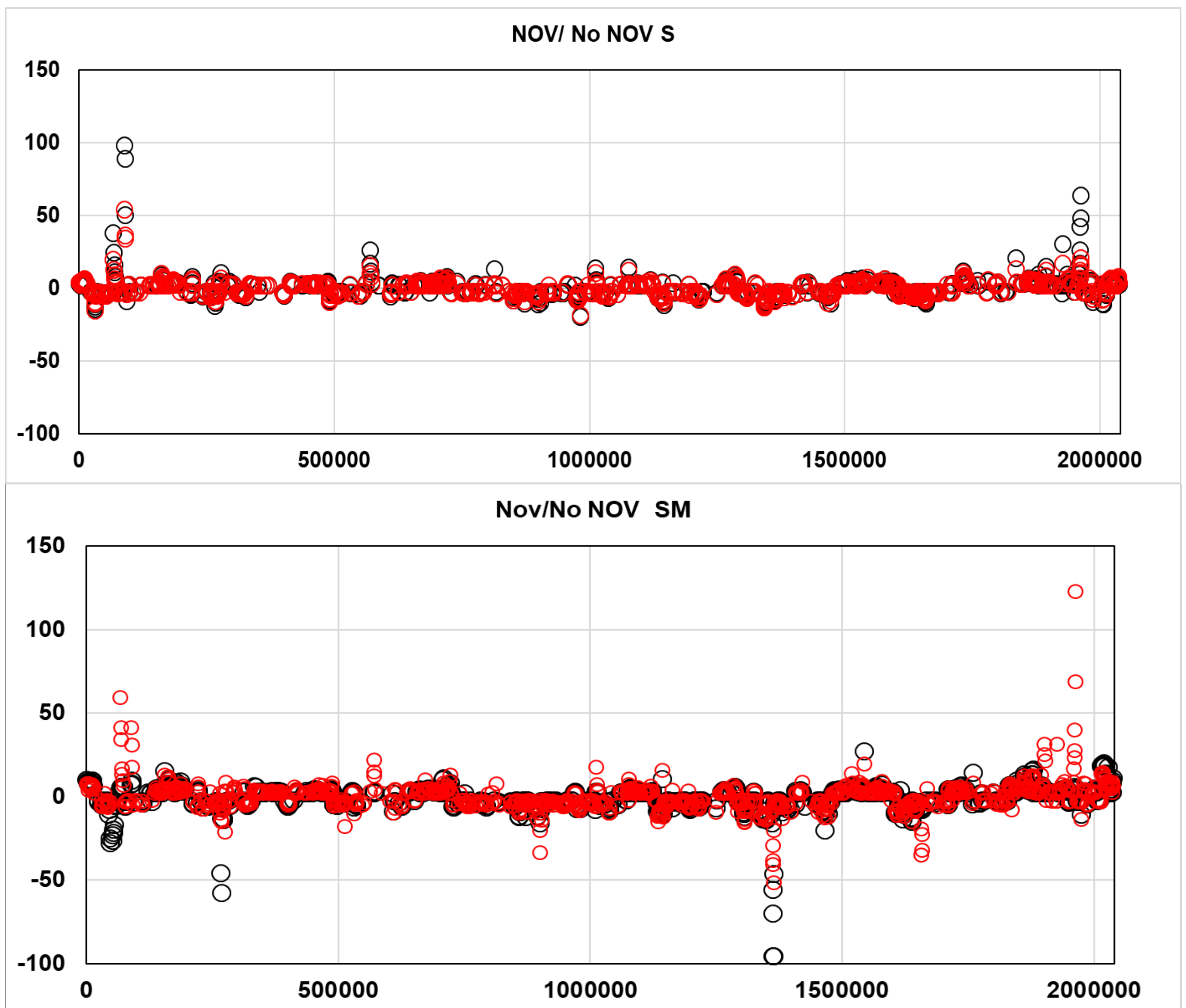

Figure S3. Transcriptomic response to NOV treatment in strain Δ*topA*P_{Zn}*topA* expressing *topAWT* (black symbols) or *topARA* (red symbols). b) Fold change and localization of DEGs in the strain carrying either pLS1ROMtopAWT (black symbols) or pLS1ROMtopARA (red symbols). The relative fold variation of each gene is represented against the location of the 5′ end each open reading frame in *S. pneumoniae* R6. A fold change ≥ 2 (absolute value) and a P-value-adjusted ≤ 0.01 were considered.