FIGURE S1. SDS PAGE analysis of purified proteins used in this study. Gel was stained with Coomassie Brilliant Blue R250.
FIGURE S2. Interaction between RECQ5 and RAD51 in vivo is not mediated through DNA. 293T cells were transfected with a vector for (His)$_6$-Xpress tagged RECQ5 or with the empty vector. Cell extracts (800 µg of protein) were incubated with or without DNase I (20 U) at 25°C for 30 minutes before addition of Ni-NTA beads (25 µl). Bound proteins were separated by SDS-PAGE and analyzed by Western blotting using Omni-probe (upper panel; (His)$_6$-Xpress-RECQ5) and anti-RAD51 antibodies (lower panel). IN, Input.
ATPase activity of RECQ5 mutants. ATPase activity of RECQ5 variants was determined by colorimetric estimation of the amount of inorganic phosphate (P_i) released by ATP hydrolysis. Reactions were carried out in buffer R at 37°C for 30 minutes and contained 20 nM RECQ5, 25 µg/ml M13ssDNA and 2 mM ATP. Reactions were stopped by addition of EDTA and the amount of inorganic phosphate (P_i) was determined by malachite green assay. Briefly, 10 µl of terminated reactions were added to 30 µl of 0.1 M EDTA (pH 8) in a 96-well microplate followed by addition of 100 µl of a freshly prepared 3:1 mixture of water / 5.72% (w/v) ammonium molybdate in 6 M HCl and 50 µl of 0.0812% (w/v) malachite green in water. The mixtures were incubated for five minutes before measurement of absorbance at 620 nm in a SpectraMax M5 reader (Molecular Devices). The concentration of P_i released by ATP hydrolysis was determined from a calibration curve derived from solutions of known P_i concentration (KH₂PO₄).