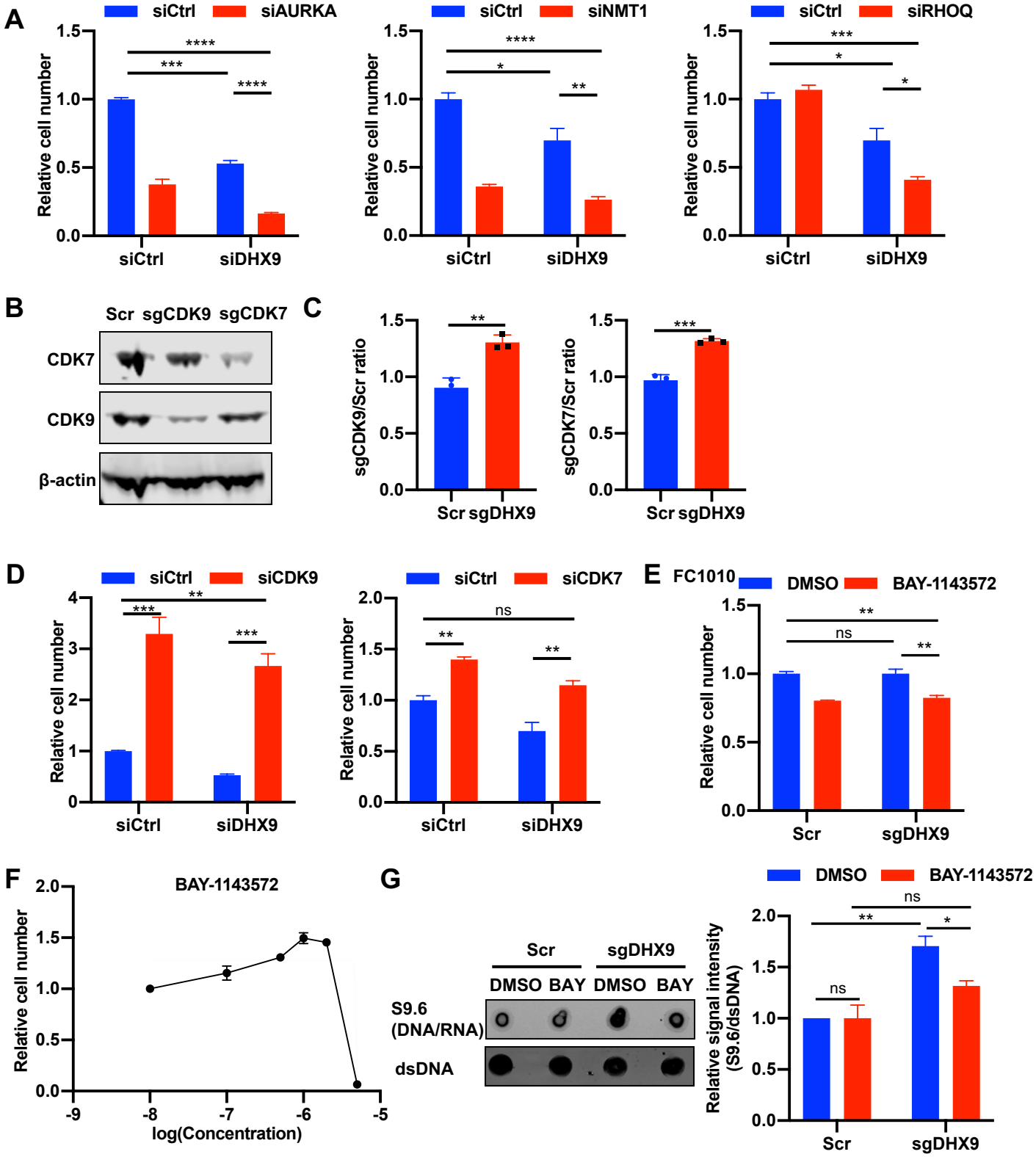
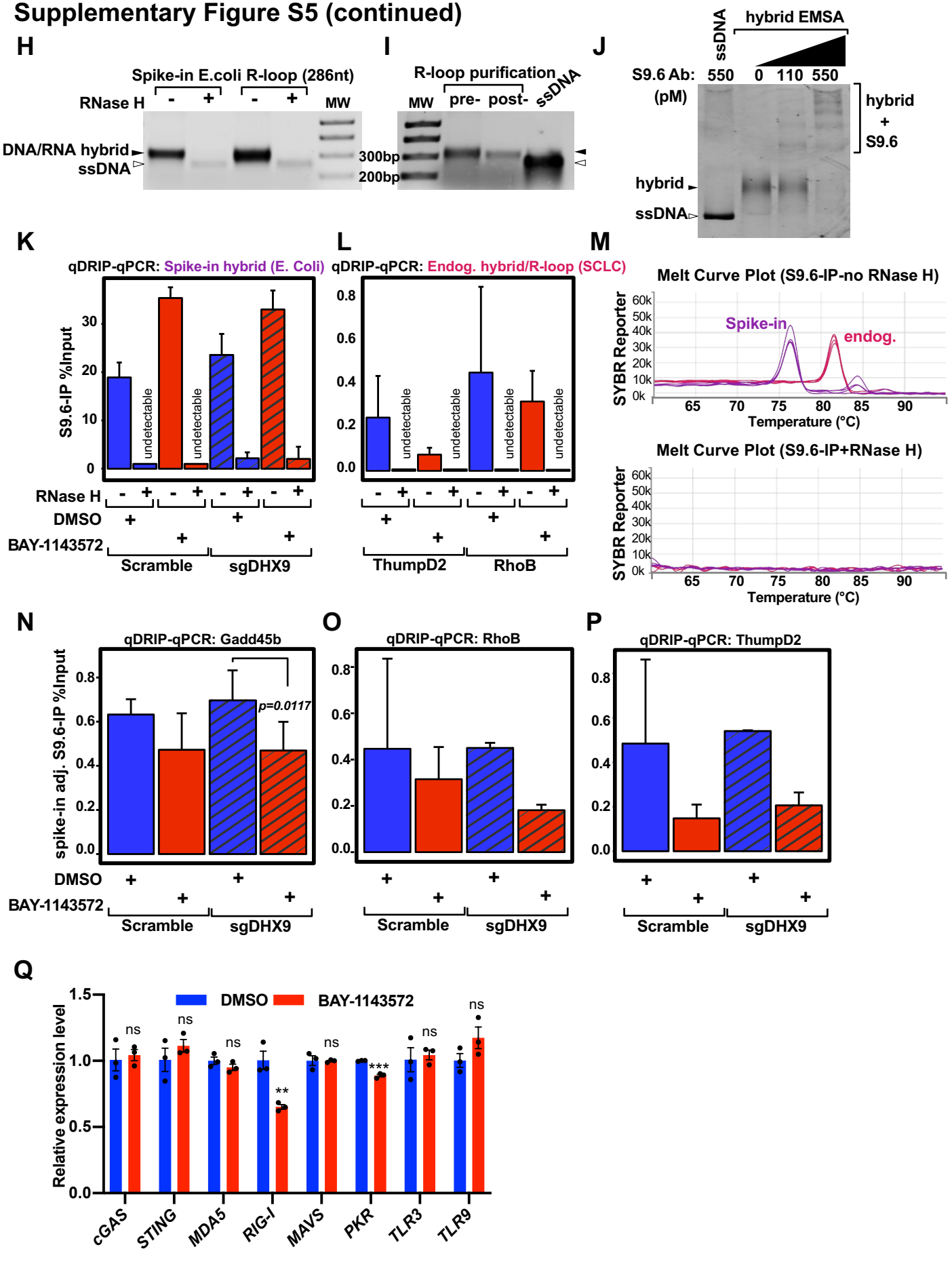
Supplementary Figure S5



Supplementary Figure S5 (continued)



Supplementary Figure S5.

A, Relative cell number of H82 cells, transfected with the indicated siRNAs, targeting genes depleted in the CRISPR screen. Luminescence of CellTiter-Glo was detected on Day 4 after seeding (n = 3). **B**, Immunoblot (IB) of the indicated proteins in H82 cells (Scramble, sgCDK9 and sgCDK7). **C**, The ratios of sgCDK9/Scr and sgCDK7/Scr cell number were compared in the background of Scramble and sgDHX9. Luminescence of CellTiter-Glo was detected on Day 4 after seeding (n = 3). **D**, Relative cell number of H82 cells, transfected with the indicated siRNAs, targeting genes enriched in the CRISPR screen. Luminescence of CellTiter-Glo was detected on Day 4 after seeding (n = 3). **E**, Relative cell number of Scramble and sgDHX9 FC1010 cells, treated with DMSO or 0.5 μM BAY-1143572. Luminescence of CellTiter-Glo was detected on Day 4 after seeding (n = 3). **F**, Relative cell number of H82 cells, treated with 0.01, 0.1, 0.5, 1.0, 2.0, 5.0 μM BAY-1143572. DMSO-treated control was used as a reference. Luminescence of CellTiter-Glo was detected on Day 4 after seeding (n = 3). **G**, Representative dot blots of DNA/RNA hybrids (S9.6) of Scramble and sgDHX9 H82 cells treated with DMSO or 0.5 μM BAY-1143572 (left) and quantifications from 3 biological replicates (right). dsDNA was used as a reference. **H,** Two independent E. coli synthetic R-loop reconstitutions as the spike-in reference for qDRIP. Agarose gel electrophoresis showing the R-loop species (solid triangle) are sensitive to RNase H digestion, leaving a 286nt single strand DNA (ssDNA, open triangle) as shown by M.W (DNA molecular weight ladder). **I,** Reconstituted E. coli R-loop fraction "pre-" was gel-purified and visualized as "post-", in comparison with an ssDNA reference to show the recovery percentage (~around 40%) and the product integrity. **J,** R-loop EMSA (Electrophoretic Mobility Shift Assay) incubating synthetic E. coli R-loop (550 pM) with the indicated concentration of antibody raised against R-loop (S9.6 Ab, 0/110/550 pM). S9.6-bound R-loop signal indicated as "R-loop+S9.6". ssDNA, as a negative control, showed only background EMSA signal near the gel well. **K,** Synthetic spike-in recovery across four qDRIP experiments (Scramble or sgDHX9, treated with DMSO or the CDK9 inhibitor - BAY1143572), quantitated as a percentage value of IPed DNA divided by Input DNA per quantitative PCR (qPCR); RNase H treatment showed undetectable or depleted signal. **L,** Endogenous (Endog.) R-loop recovery from H82 small cell lung cancer (SCLC) at two genes: ThumpD2 and RhoB. All RNase H treated samples showed undetectable signals. **M,** Melting Curve analyses of qDRIP-qPCR amplicons (the spike-in and an endogenous R-loop), either with no RNase H (upper panel) or with RNase H (lower panel). **N-P,** qDRIP-qPCR signal at Gadd45b, RhoB, and ThumpD2 genes across the four indicated experimental conditions (n= 2). **Q**, qRT-PCR analysis of the indicated genes comparing H196 cells with the indicated sgRNA vectors (n = 3). 36B4 gene was used as a reference. DNA fiber assay of Scramble and sgDHX9 H82 cells treated with DMSO or 0.5 μM BAY-1143572.

Data represent mean ± SEM. ns, not significant; *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 by unpaired Student's t test (C and H), paired t-test (N), two-way ANOVA followed by Tukey's multiple comparisons test (A, D, E and G).