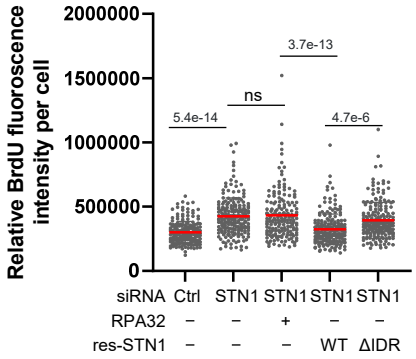
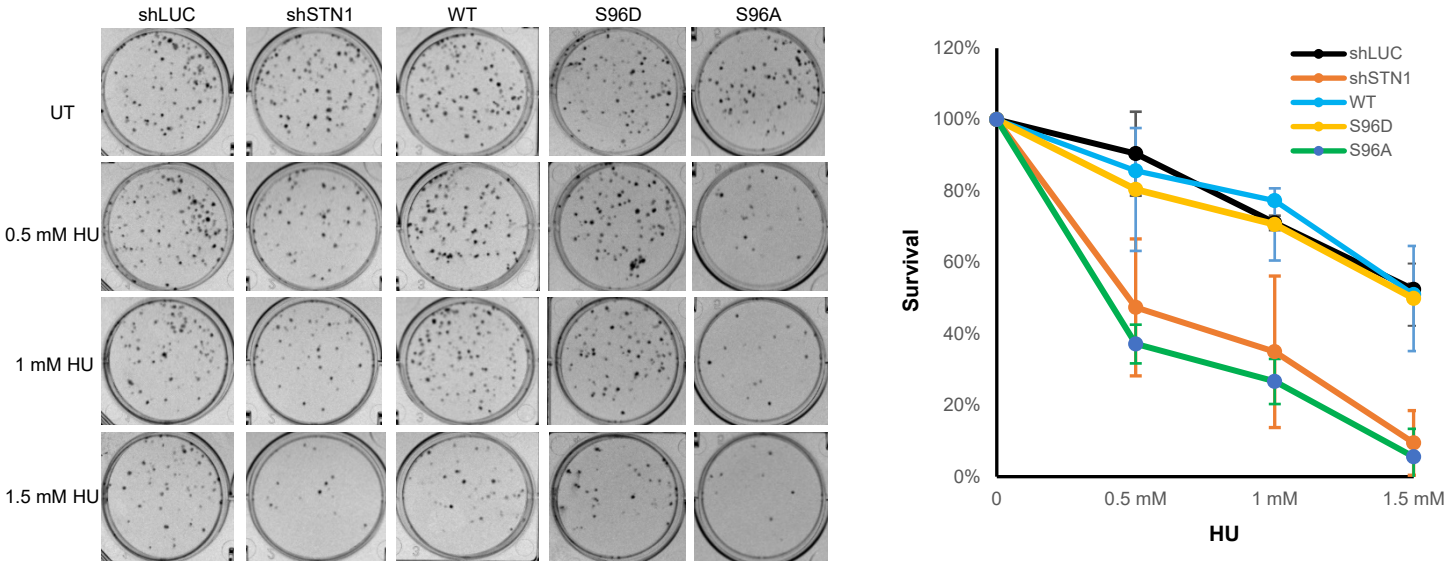
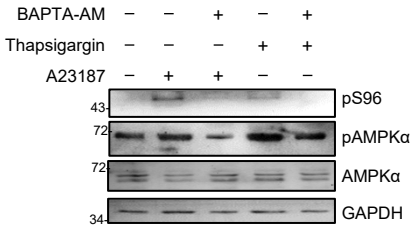


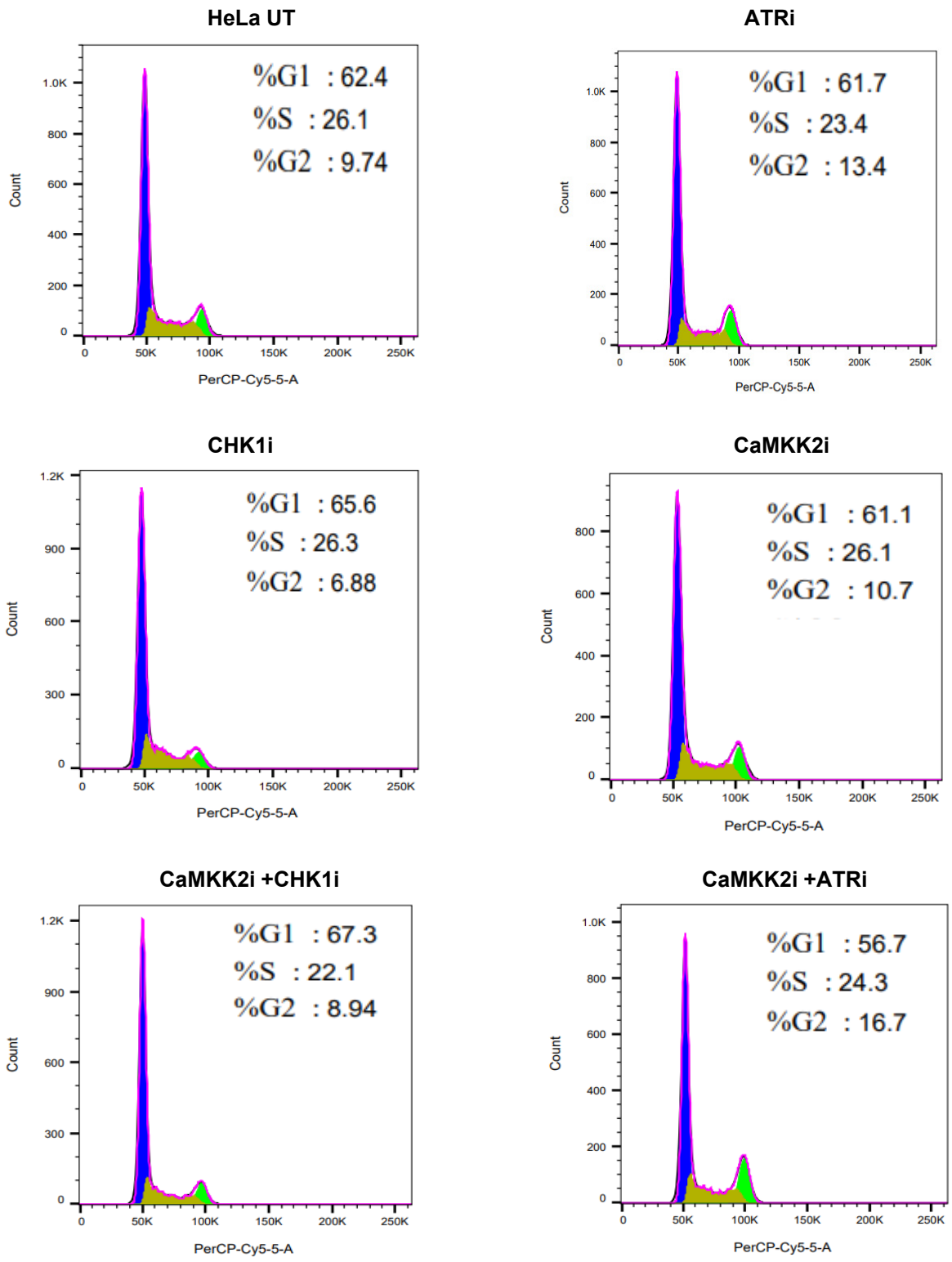

**Supplementary Fig. 1. Non-denaturing BrdU analysis.** Cells used in Figure 1B were cultured in the presence of 10 μM BrdU for 48 hrs, followed by 4 mM HU treatment for 3 hrs. The cells were then stained with anti-BrdU antibody under non-denaturing conditions to detect ssDNA. Statistical significance was determined by one-way ANOVA. N= Approximately 200 cells were counted for each sample in each experiment. Source data are provided as a Source Data file.



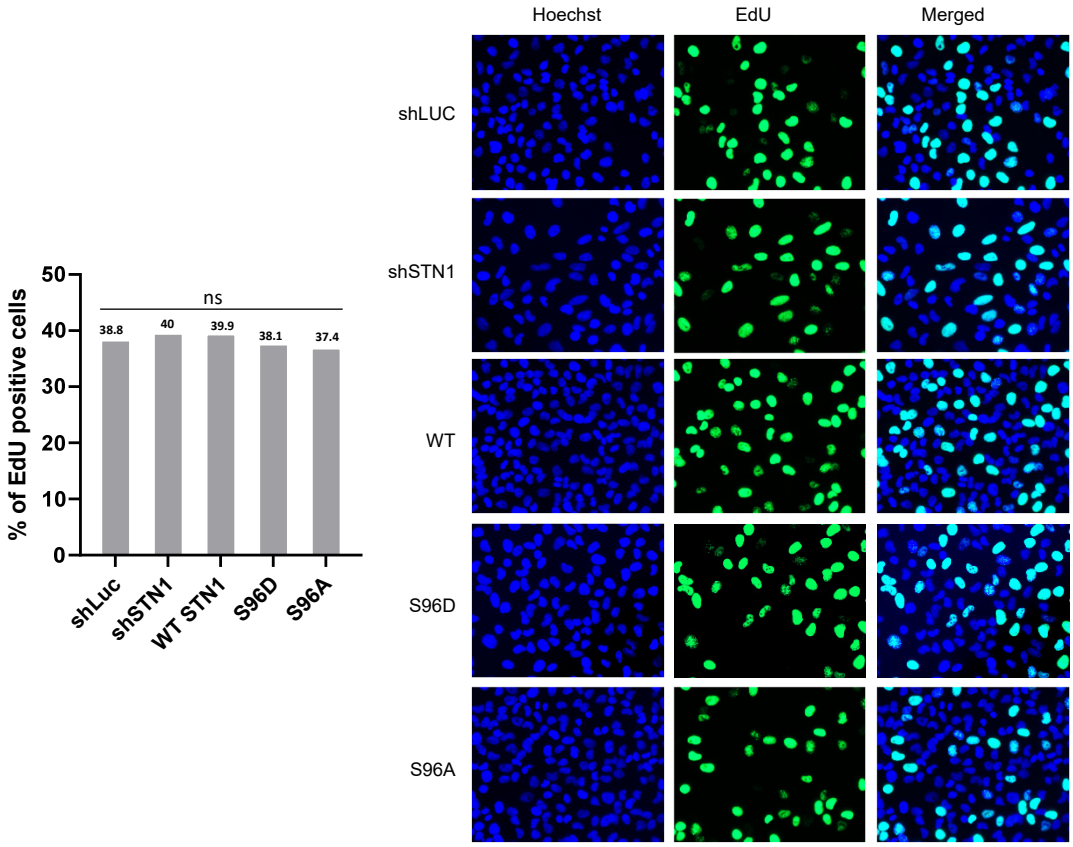

**Supplementary Fig. 2. Colony formation assay of HeLa cells expressing WT-STN1, S96D, or S96A with endogenous STN1 depletion**. These cells were treated with 0.5 mM, 1 mM, 1.5 mM of HU for 10 hrs after seeding. Cells were then allowed to grow and form colonies. Colonies were stained and counted. Results are means from two independent experiments. Error bars: SD. Source data are provided as a Source Data file.



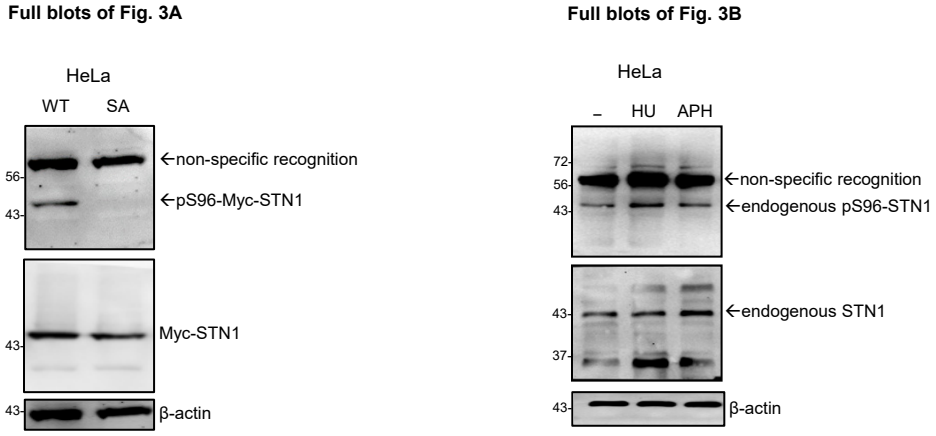

**Supplementary Fig. 3. BAPTA-AM treatment impairs S96 phosphorylation after calcium ionophore A23187 and thapsigargin treatment.** HeLa cells were pre-treated with BAPTA-AM (50 μM, 30 min) to chelate calcium and then treated with calcium ionophore (A23187, 2 μM, 1 h) or thapsigargin (1 μM, 1 h). STN1 pS96 phosphorylation induced by calcium ionophore and thapsigargin was reduced by BAPTA-AM. Reduction of pAMPKα indicates inhibition of the CaMKK2 signaling. Source data are provided as a Source Data file.



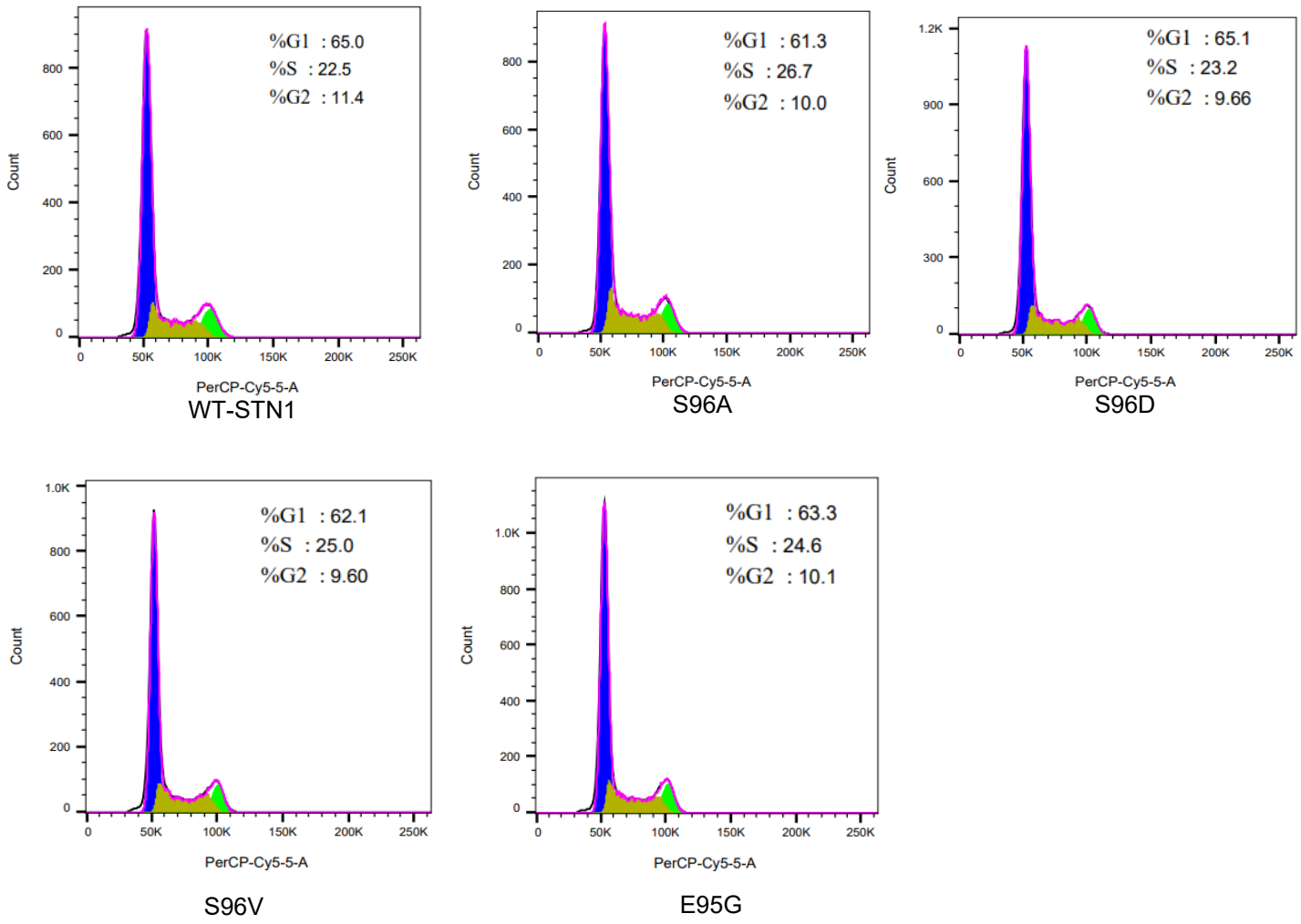

**Supplementary Fig. 4. Cell cycle analysis after inhibition of ATR, CHK1, CaMKK2, alone or in combination.** Cell cycle analysis by propidium iodide staining of HeLa cells after inhibition of ATR, CHK1, CaMKK2, alone or in combination using flow cytometry.



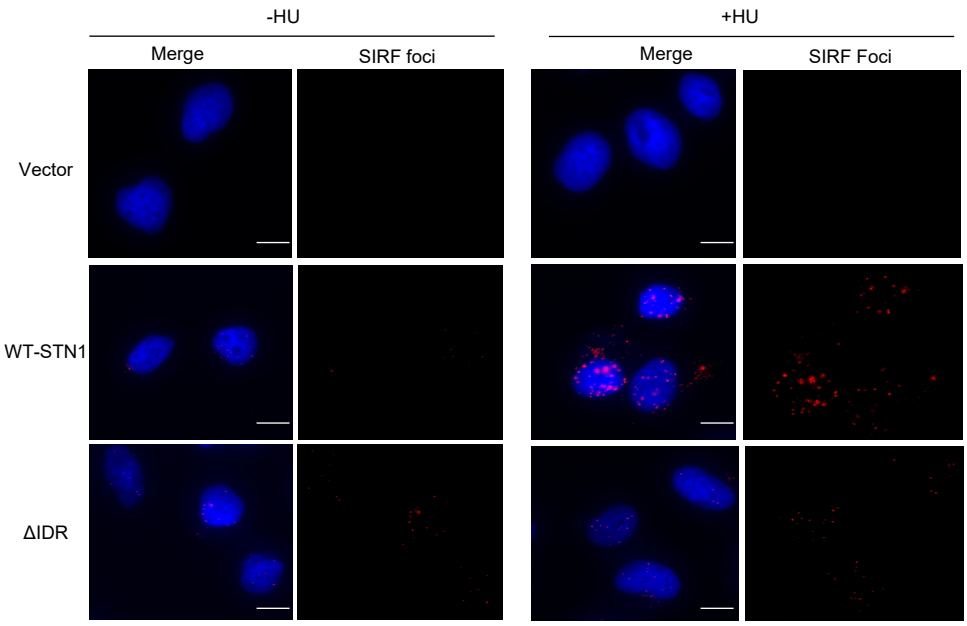

**Supplementary Fig. 5. EdU labeling to check the S phase population in cells.** EdU labeling in HeLa cells expressing Flag-WT-STN1, S96D, and S96A and with concurrent knockdown of endogenous STN1. Cells were labeled with 10 mM EdU for 2 hr prior to staining. Source data are provided as a Source Data file.



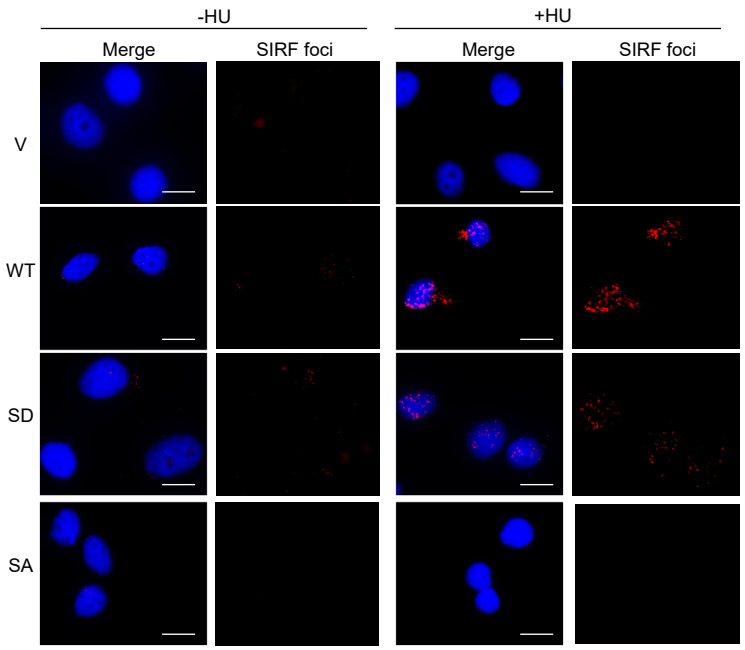

**Supplementary Fig. 6. Full blots of Figure 3A and 3B showing that the anti-pS96 antibody exhibits a strong non-specific recognition of a protein bigger than STN1.** In the Figure 3A blots, whole cell lysates of HeLa cells transiently expressing Myc-WT-STN1 or Myc-S96A were used on SDS-PAGE and pS96 was detected using the anti-pS96 antibody. Anti-Myc antibody was used to detect Myc-STN1. In the Figure 3B blots, HeLa cells were treated with HU (10 mM, 3 h) and anti-pS96 and anti-STN1 antibodies were used to detect pS96 and endogenous STN1 protein, respectively. Source data are provided as a Source Data file.



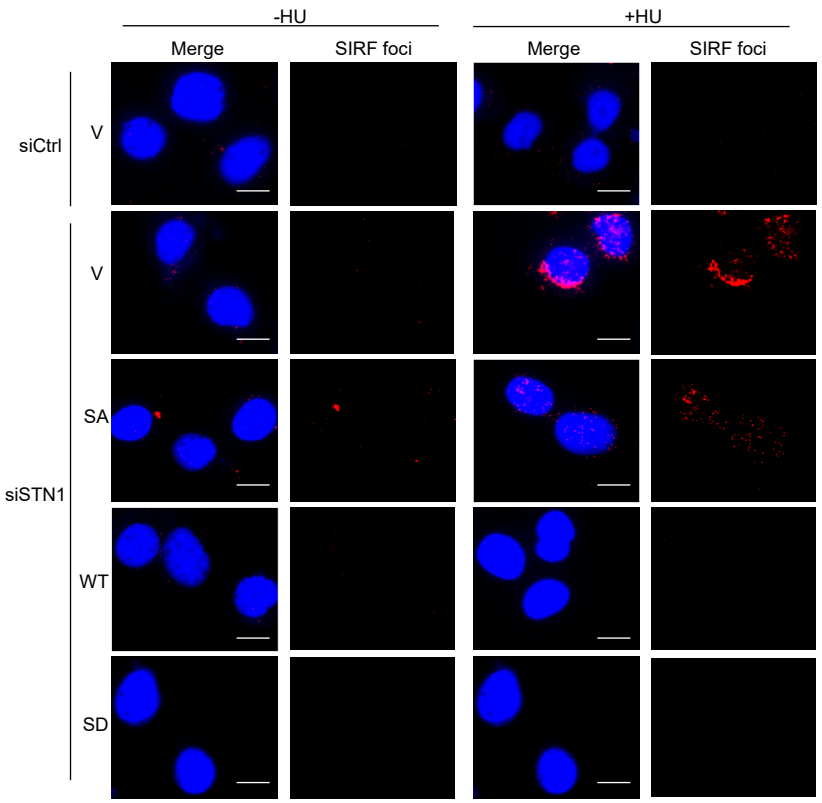

**Supplementary Fig. 7. Cell cycle analysis of HeLa expressing RNAi-resistant Myc-WT-STN1, Myc-S96V, Myc-S96A, Myc-E95G with concurrent depletion of endogenous STN1.**



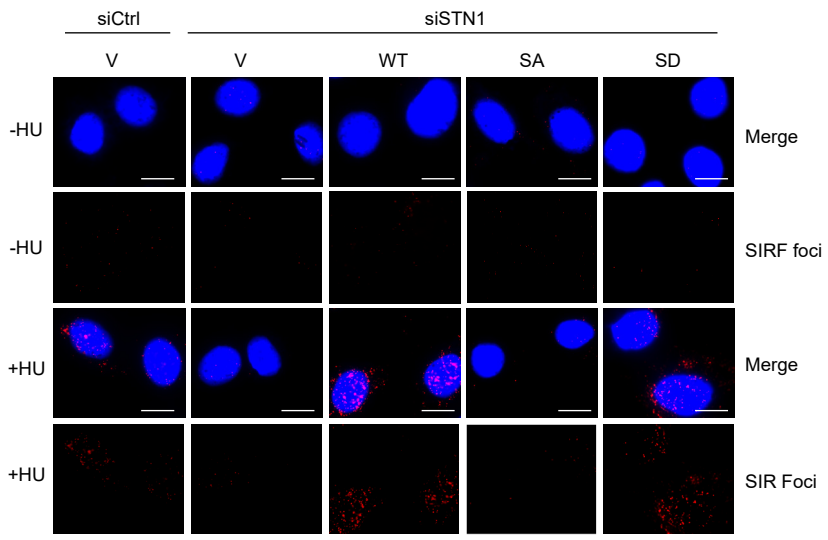

**Supplementary Fig. 8 (related to Fig 1E).** STN1 SIRF with red channel images.




**Supplementary Fig. 9 (related to Fig 2B).** STN1 mutants SIRF with red channel images.




**Supplementary Fig. 10 (related to Fig 2E).** MRE 11 SIRF with red channel images.




**Supplementary Fig. 11 (related to Fig 6A).** Rad51 SIRF with red channel images.