Supplementary Figure Legends

Supplementary figure 1. Specificity controls for BG4 and TMPyP4. a. DNAse I treatment of 42MGBA cells ameliorates BG4 G-quadruplex antibody staining. b,c. Treatment with 50 µM TMPyP4 (c) vs. DMSO (b) for 24 hours in 42MBGA-TMZres increases discrete punctate staining of BG4 and increased surface plot height. Nuclear surface plot of signal intensity depicted on the right was generated by FIJI. d. RNAse H treatment of 42MBGA-TMZres cells ameliorates S9.6 R-loop antibody staining.

Supplementary figure 2. **Characterization of a novel CLK inhibitor (GW807982X, cdc2-like kinase inhibitor (CLK2i)).** a. Commercial kinase binding and activity assays were performed at Nanosyn (data included are percent inhibition at 100 nM and 1 µM CLK2i; assays were conducted at [ATP] = K_m^{app} of each kinase), DiscoverX (data included are K_D values), and Reaction Biology Corporation (data expressed are IC_{50} values of kinase inhibition; assays were conducted at 10 µM ATP). Differential scanning fluorimetry assays were conducted at the SGC The blue box denotes closely related family members of CLK2 within the CMGC family. Gray cells indicate that the assay was unavailable, and "nt" denotes that the compound was not tested in these assays. b. NanoBRET assay(Vasta et al., 2018) of CLK2i (CAF-022) with multiple CLK family members in live cells. c. NanoBRET IC_{50} values determined for CLK1, 2, and 4. d. Western blot of pSR changes over a time-course of CLK2i treatment in 42MGBA-TMZres cells. e. Densitometry quantification of maximal pSRSF5 hypophosphorylation in d.

Supplementary figure 3. Controls for EWSR1 IF, aggregation, and STED imaging. a. 42MBGA-TMZres cells stained with the mouse mAb (sc, 48404) and DAPI. b. rabbit mAb in 42MGBA, 42MBGA-72 hr 100µM TMZ treated, 42MBGA-TMZres, and T98G cells showing higher magnification images of cytoplasmic EWSR1 aggregation in both c. Leica Sp8 confocal, and d. STED images. Higher magnification of nuclear EWSR1staining is shown in both e. Leica Sp8 confocal and f. STED images.

Supplementary figure 4. Controls for EWSR1 colocalization with G4s. Co-stain of the G4 antibody BG4 with EWSR1 in a. 42MBGA, b. 42MBGA-TMZres, c. T98G cells. The enhanced* panels permit visualization of cytoplasmic aggregates. Merged panels show an inset with higher magnification of BG4 and EWSR1 localization.

Supplementary figure 5. Controls for endogenous EWSR1 depletion and nucleo-cytoplasmic trafficking, and ectopic YFP-EWSR1 cytoplasmic aggregation. a. Western blot of EWSR1 knockdown with four different siRNAs. b. FACS analysis of subG1 DNA content following 72 hours of EWSR1 depletion. Cell cycle profile of cells analyzed in b. for c. 42MBGA, d, 42MBGA-TMZres, e. T98G cells; three biological replicates. Treatment with 25nM Leptomycin B (CRM1 inhibitor; LMB) in f. 42MBGA, g 42MBGA-TMZres, and h. T98G cells for 24 hours. Quantification of the phenotype resulting from overexpression of YFP-EWSR1 phenotype in i. 42MBGA-TMZres n=87 cells, and j. T98G n=92 cells. Representative images below for "puncta", "nucleoli", and "ribbon" phenotypes.

Supplementary figure 6. FLAIR-predicted isoforms of a. EWSR1 and d. CLK2 with representative percentage expressed in b. for EWSR1 and c. for CLK2, with cell lines denoted on the x-axis.