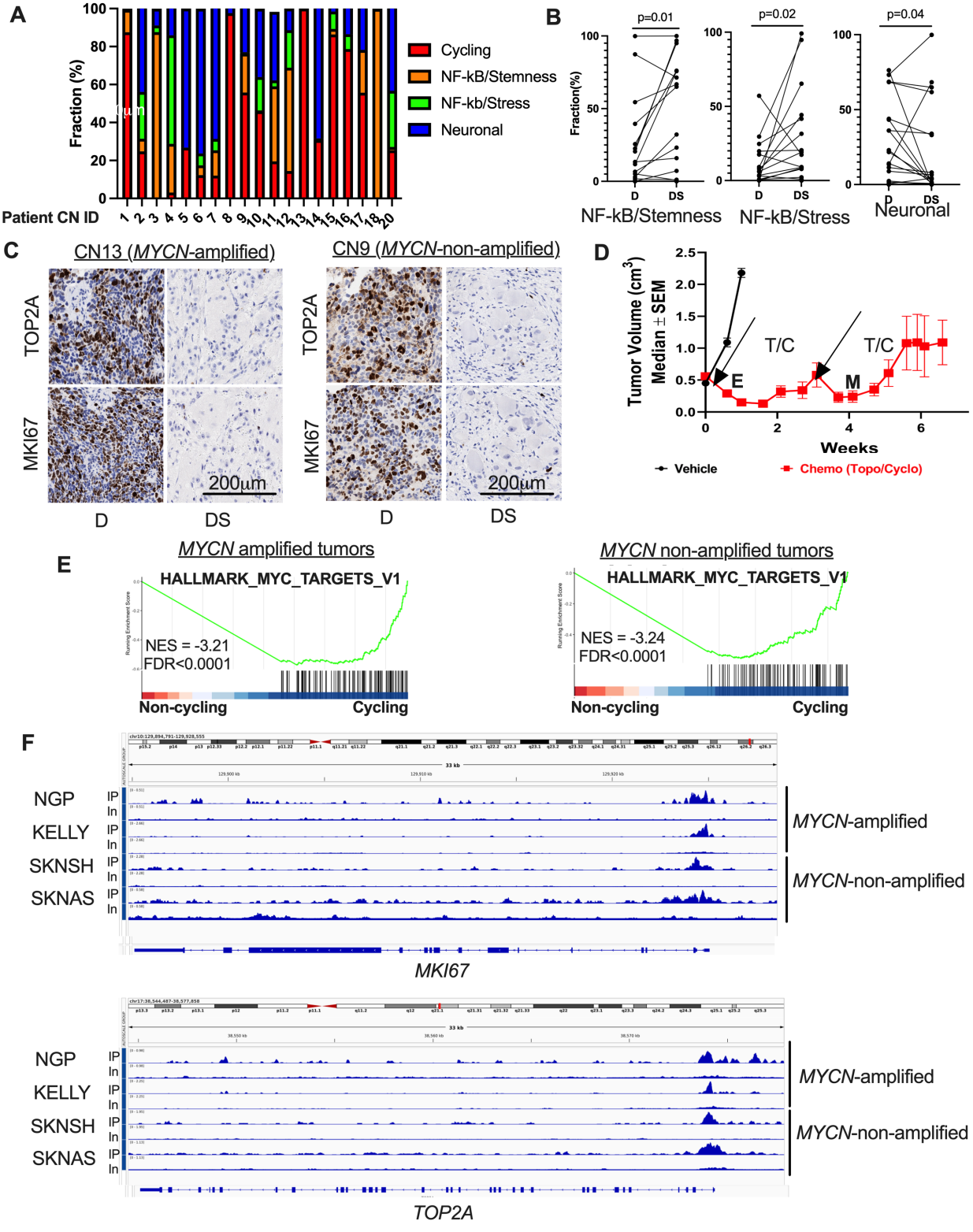
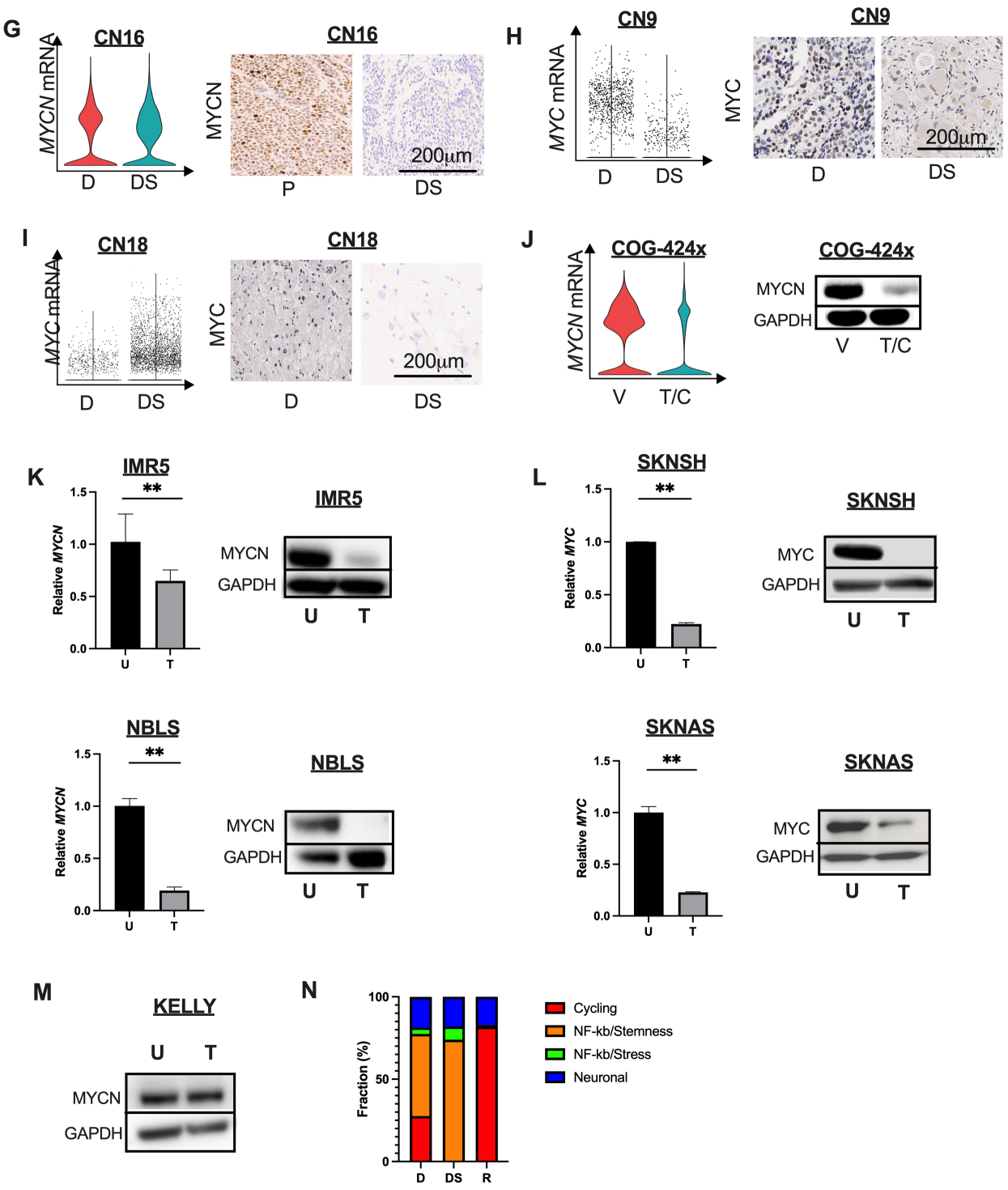
Supplementary Figure S4





Supplementary Figure S4. A. Bar plots of persister cell subtypes decomposition of the malignant compartment of 20 diagnostic specimens matched to the definitive surgery specimens of Figure

2H (Diagnostic CN19 has malignant cells while definitive surgery CN19 does not). **B.** Connected pairs plots of NFκB/Stemness, NFκB/Stress and Neuronal persister cell subtypes percentage at diagnosis (D) and definitive surgery (DS). **C.** IHC staining for *TOP2A* and *MKI67*, two cycling persister cell markers, in a *MYCN* amplified tumor of CN13 and *MYCN* nonamplified tumor of patient CN9. **D.** Tumor volume as a function of time for a PDX model (COG-424x) of a diagnostic tumor with *MYCN* amplification treated with two cycles of topotecan and cyclophosphamide in 3 weeks intervals. E – Enrollment, M- Maximal shrinkage, T- topotecan, C- cyclophosphamide. **E.** Gene set enrichment analysis (GSEA) of genes differentially expressed between non-cycling and cycling persister cells in *MYCN* amplified tumors (left) and *MYCN* non amplified tumors (right). **F.** Integrative Genome Viewer (IGV) screenshots of ChIP-seq dataset (45) for *MYCN* and *MYC* at the promoter regions of *TOP2A* and *MKI67* in *MYCN* amplified (NGP and KELLY) and non-amplified (SKNSH and SKNAS) neuroblastoma cell lines. IP – Immunoprecipitation for *MYCN/MYC*, IN – Input. **G.** Left - violin plot of *MYCN* mRNA expression in a MYCN amplified tumor obtained from patient CN16 at diagnosis (D) and definitive surgery (DS), right - IHC staining for *MYCN* of the same tumor at DS. Positive (P) control was used for MYCN staining, since we did not have access to the diagnostic sample of CN16 available for IHC. **H.** Left- violin plots of *MYC* mRNA expression in the *MYCN* non-amplified tumors obtained from patients CN9, right - IHC staining for *MYCN* of the same tumor at D and DS. **I.** Left- violin plots of *MYC* mRNA expression in the *MYCN* non-amplified tumors obtained from patients CN18, right - IHC staining for *MYCN* of the same tumor at D and DS. **J.** Left - violin plots of *MYCN* mRNA expression in PDX model (COG-424x) of a *MYCN* amplified tumor before therapy (V- vehicle) and after two cycles of topotecan and cyclophosphamide (T/C). Right - a western blot of the corresponding MYCN protein level before and after therapy. **K.** Upper panel – left - bar plots of real-time PCR (RT-PCR) for *MYCN* before (U- untreated) and after treatment with Topotecan followed by Etoposide (T- treated) in neuroblastoma cell lines with *MYCN* amplification (IMR5), right - a western blot for MYCN protein before (U) and after (T) therapy. Lower panel – left - bar plots of

real-time PCR (RT-PCR) for *MYCN* before (U- untreated) and after treatment with Topotecan followed by Etoposide (T- treated) in neuroblastoma cell lines with *MYCN* overexpression (NBLS), right - a western blot for MYCN protein before (U) and after (T) therapy. **L.** Upper panel – left - bar plots of real-time PCR (RT-PCR) for *MYC* before (U- untreated) and after treatment with Topotecan followed by Etoposide (T- treated) in neuroblastoma cell lines without *MYCN* amplification but MYC overexpression (SKNSH), right - a western blot for MYC protein before (U) and after (T) therapy. Lower panel – left - bar plots of real-time PCR (RT-PCR) for *MYCN* before (U- untreated) and after treatment with Topotecan followed by Etoposide (T- treated) in neuroblastoma cell lines without *MYCN* amplification but *MYC* overexpression (SKNAS), right - a western blot for MYCN protein before (U) and after (T) therapy. **M.** Western blot for MYCN protein in a cell line with *MYCN* amplification and *TP53* and *ALK* mutation (KELLY). **N.** Bar plot of persister cell subtypes decomposition at diagnosis (D), definitive surgery (DS) and relapse (R) of a tumor from a patient with intermediated risk and high-risk features neuroblastoma. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ns, not significant