





Figure S1: EWSR1::FLI1 repressed gene targets defined using EWS cell lines exhibit a broader range of expression in EWS primary tumors. (A) qRT-PCR-based validation of the silencing of *EWSR1::FLI1* (48 hours) in the indicated cell lines (mean \pm SEM from three independent experiments; p-values determined using unpaired t-test). **(B)** Gene ontology analysis of the significantly up-regulated (1107) or down-regulated (1111) genes observed following the silencing of *EWSR1::FLI1* in three EWS cell lines (TC-32, TC-71, and A673). Results are shown for those GO terms that appeared in both the Metascape and GSEA databases (<https://metascape.org/gp/index.html> - /main/step1 and <https://www.gsea-msigdb.org/gsea/msigdb/>) plotting the p values from the Metascape analysis. **(C)** RNA-seq analysis of transcription factors genes that demonstrated a significant decrease in expression following the silencing of *EWSR1::FLI1* in TC-32, TC-71, or A673 (three biological replicates; fold change < -1.5, FDR < 0.05). **(D)** Comparative expression of transcription factor genes in EWS tumor samples (n=79) and EWS cell lines (n=42) (19) that exhibited significant differences (p-values determined using an unpaired t-test with Welch's correction). **(E)** qRT-PCR analysis of *EWSR1::FLI1* (SK-N-MC) or *EWSR1::ERG* (TC-106) and a subset of transcription factor genes in SK-N-MC and TC-106 cells, respectively, transfected with the indicated siRNAs (mean \pm SEM from three independent experiments; p-values determined using multiple unpaired tests). **(F)** Immunoblots of whole-cell lysates prepared from SK-N-MC cells transfected with the indicated siRNA (48 hours post-transfection) and analyzed using the antibodies against the indicated proteins.