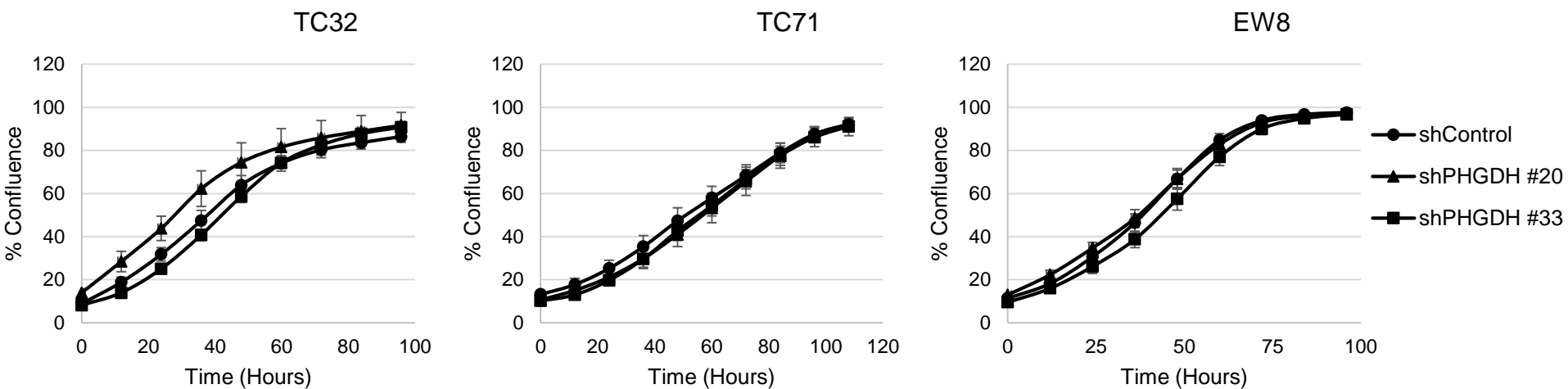


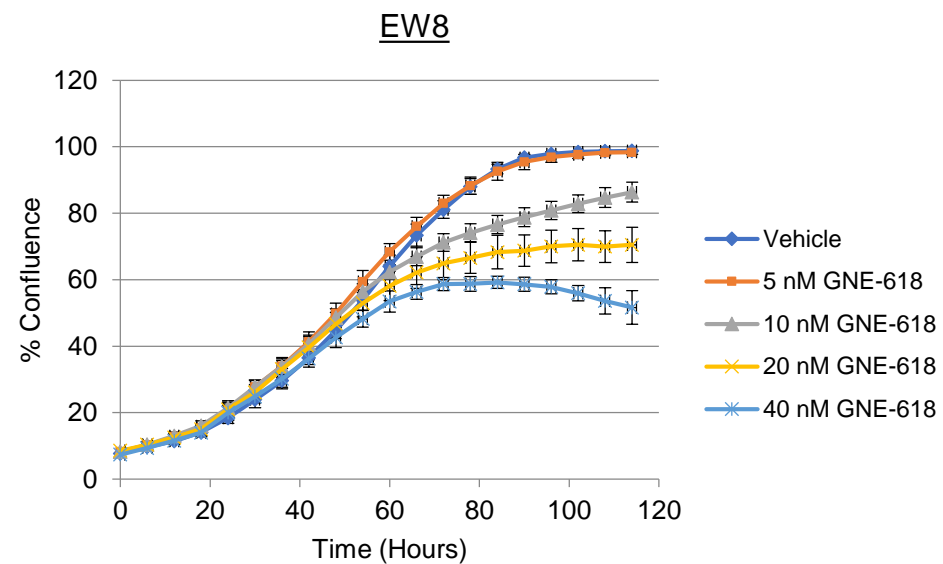
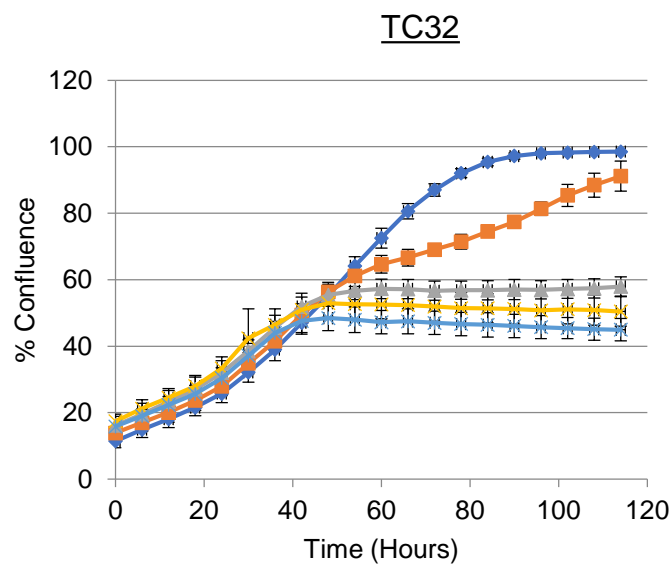
Supplementary Figure S1. PHGDH is highly expressed in Ewing sarcoma cell lines. Immunoblot analysis of PHGDH expression in Ewing sarcoma cell lines. The breast cancer cell lines MDA-MB-231 and MDA-MB-468 serve as negative and positive controls, respectively. Actin was used as a loading control.



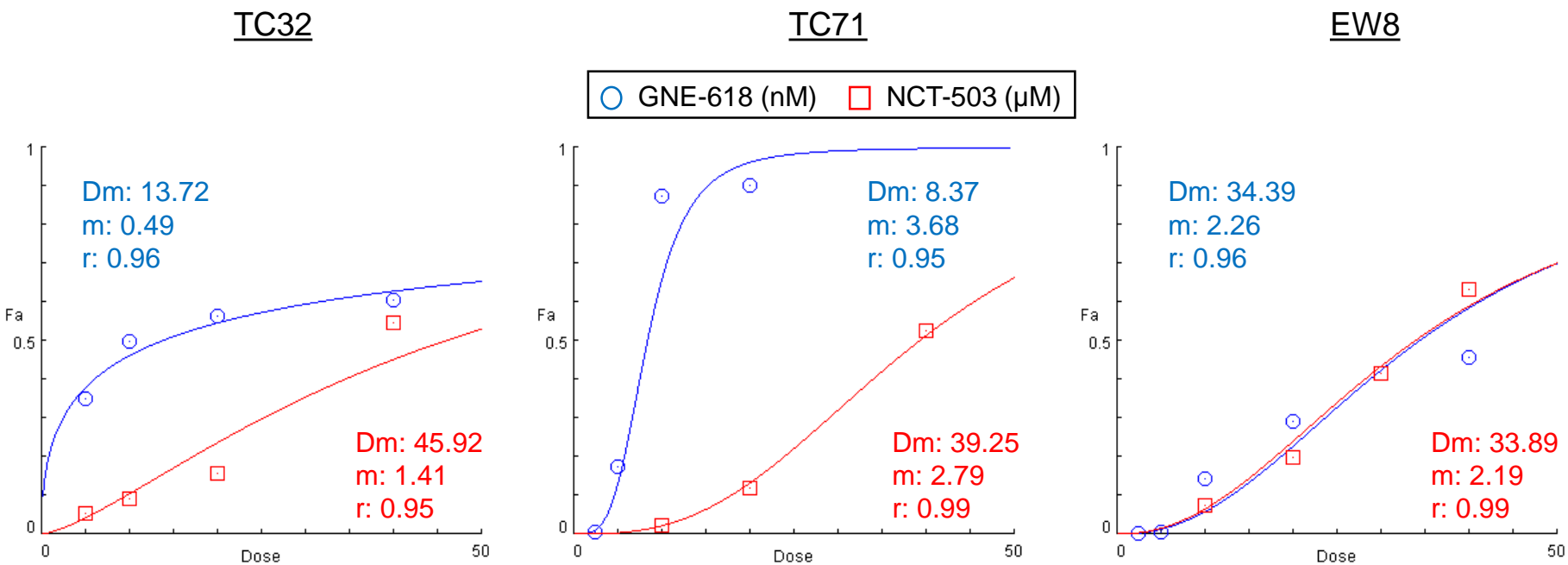
Supplementary Figure S2. Measurement of PHGDH protein expression. Immunoblot analysis of PHGDH in the specified cell lines expressing lentiviral control shRNA (shControl) or no shRNA (Parental). Actin was used as a loading control.



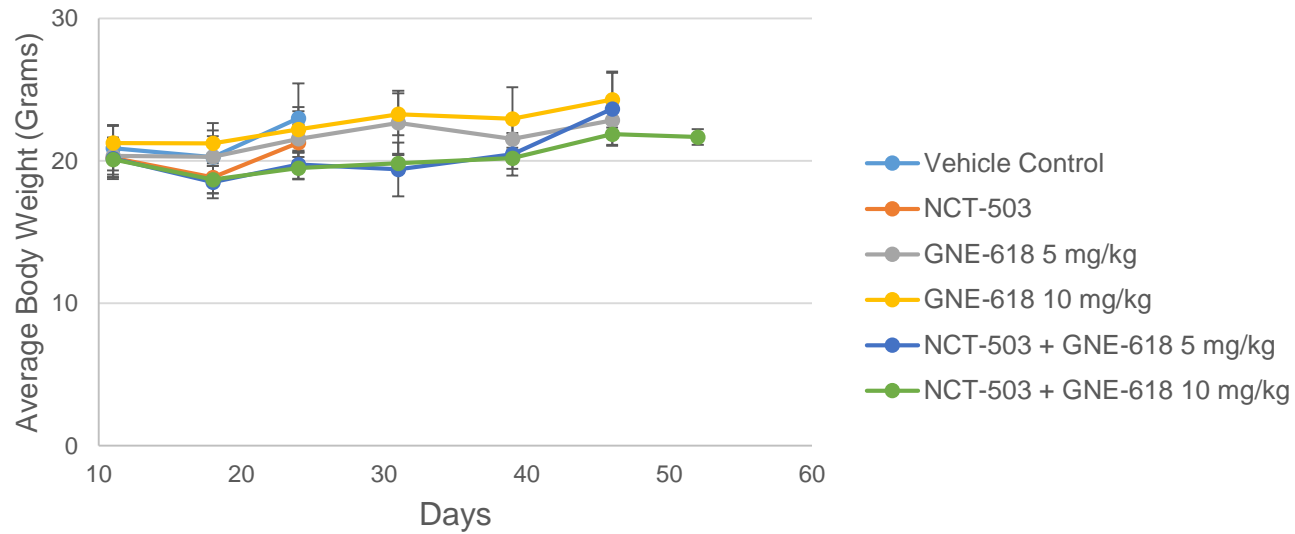
Supplementary Figure S3. PHGDH knockdown and ES proliferation *in vitro*. Cellular proliferation was monitored in an Incucyte FLR. The indicated cell lines were assayed in RPMI 1640 media supplemented with 10% dialyzed FBS. Data represent the mean \pm SD of a representative experiment.



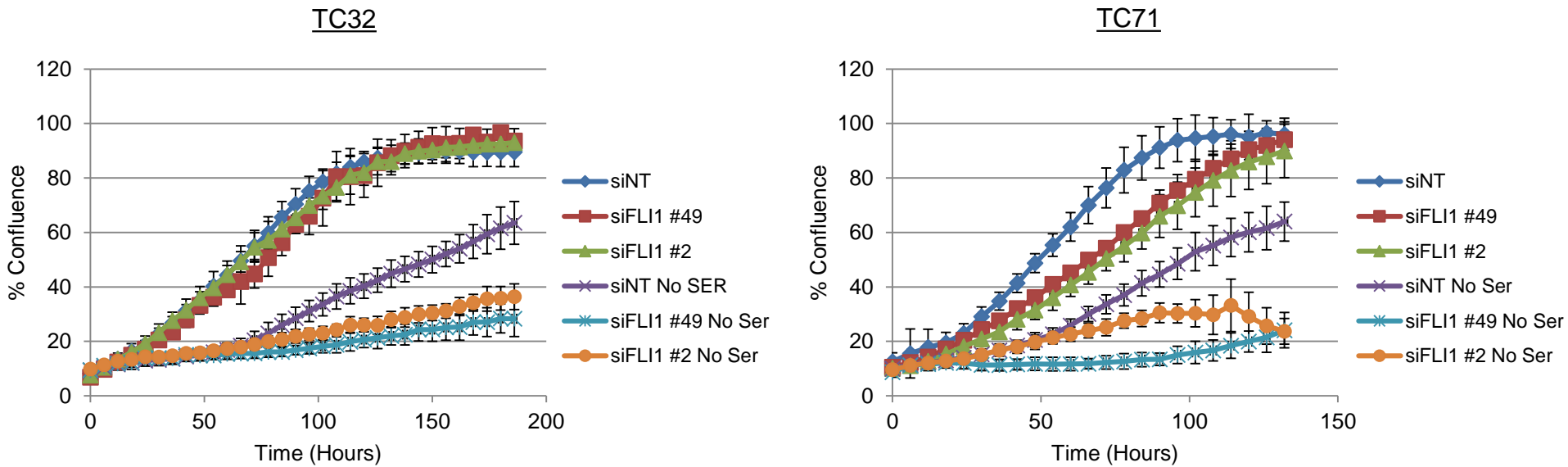
Supplementary Figure S4. Effect of GNE-618 on ES cellular proliferation. Cellular proliferation in response to the indicated concentrations of GNE-618 was monitored in an Incucyte FLR. Data represent the mean \pm SD of a representative experiment.



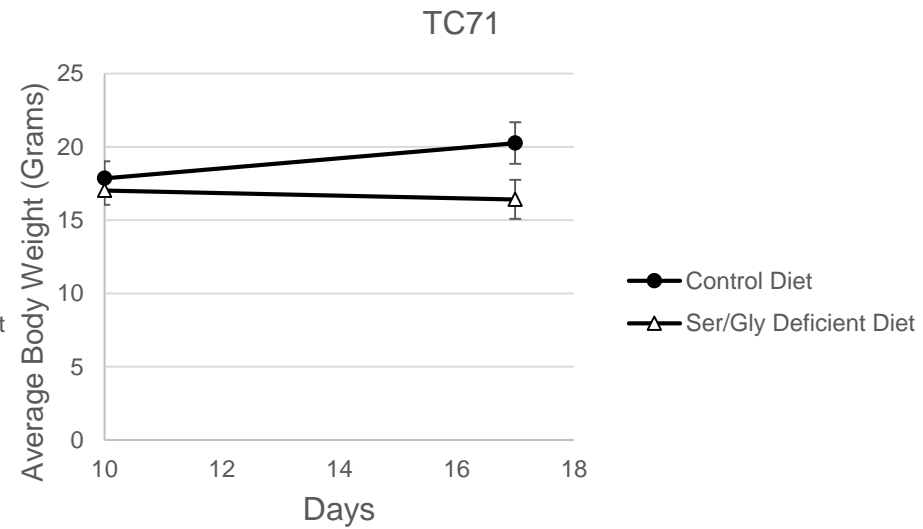
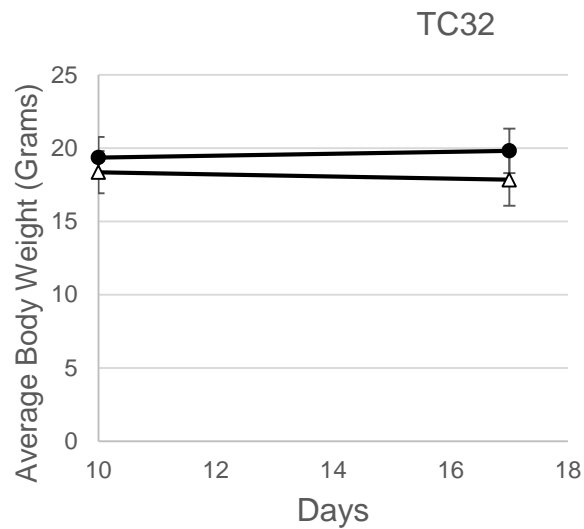
Supplementary Figure S5. Dose-effect Curves for GNE-618 and NCT-503. Dose-effect curves for GNE-618 and NCT-503 were generated from the values in Supplementary Tables S1 and S2 in the indicated Ewing sarcoma cell lines using Compusyn. Fa = fraction affected, Dm = median-effect dose, m = slope of median-effect plot, or shape of dose-effect curve, r = linear correlation coefficient.



Supplementary Figure S6. Mouse body weight is unaffected by inhibitor treatments. Average body weight over time of mice receiving the indicated treatments. Data represent the mean \pm SD.



Supplementary Figure S7. EWS-FLI1 is important for sarcoma cellular proliferation in the absence of serine. Starting the day after transfection with the indicated siRNAs, cellular proliferation was monitored in an Incucyte FLR. Cell lines were assayed in RPMI 1640 media supplemented with 10% dialyzed FBS, with or without serine and glycine (No Ser). Data represent the mean \pm SD of a representative experiment.



Supplementary Figure S8. Mouse body weight is unaffected by serine/glycine deficient diet. Average body weight over time of mice fed a control diet or a serine and glycine deficient diet. Data represent the mean \pm SD.