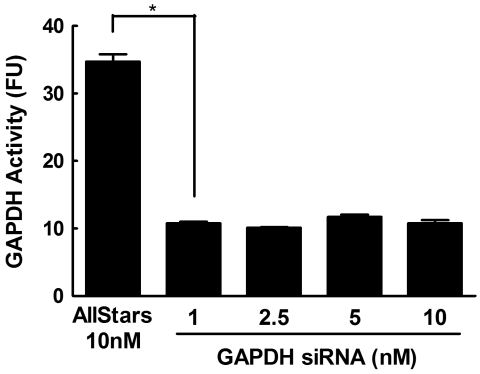

Figure S1. Optimization of reverse-transfection protocols using GAPDH siRNAs. MDA-MB-231 cells were seeded in 96-well plates at a density of 5000 cells per well, and reverse transfected with the indicated concentrations of siRNAs. The cells were lysed three days later, and assayed for GAPDH activity using a fluorescence-based assay. Even the lowest concentrations of siRNAs (1nM) produced a ~70% reduction in GAPDH activity, compared to cells transfected with 10nM negative control siRNA ("AllStars"). The data are representative of three independent experiments and "*" indicate $p \leq 0.05$.

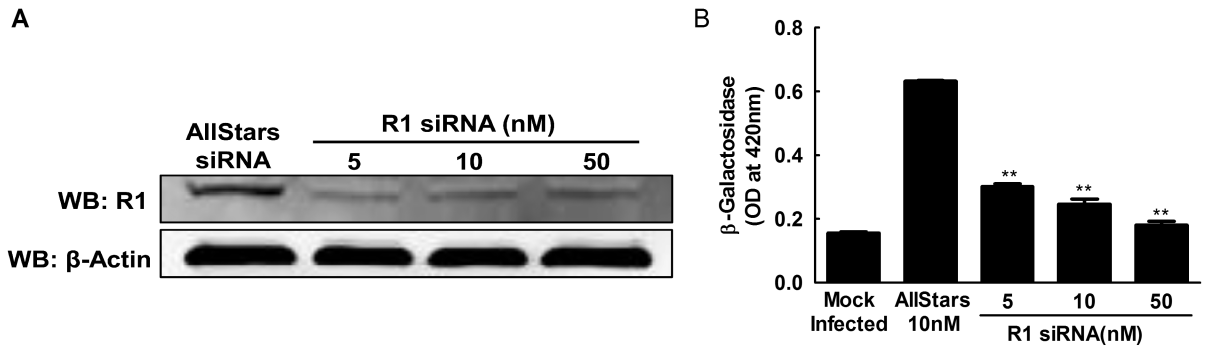

Figure S2. Validation of an internal positive control using siRNAs targeting cellular ribonucleotide reductase. MDA-MB-231 cells were seeded in 96-well plates at a density of 5000 cells per well, and reverse transfected with the indicated concentrations of siRNAs targeting the cellular ribonucleotide reductase large subunit (R1). The cells were cultured for three days, infected with MYXV for 48h, and assayed for R1 protein by western blotting (A) and for virus-encoded β-galactosidase activity (B). The siRNAs reduced the level of β-galactosidase activity by 50-60% and this was correlated with a reduction in the levels of R1 protein. Significance is indicated relative to the AllStars controls: (**, $p \leq 0.001$).

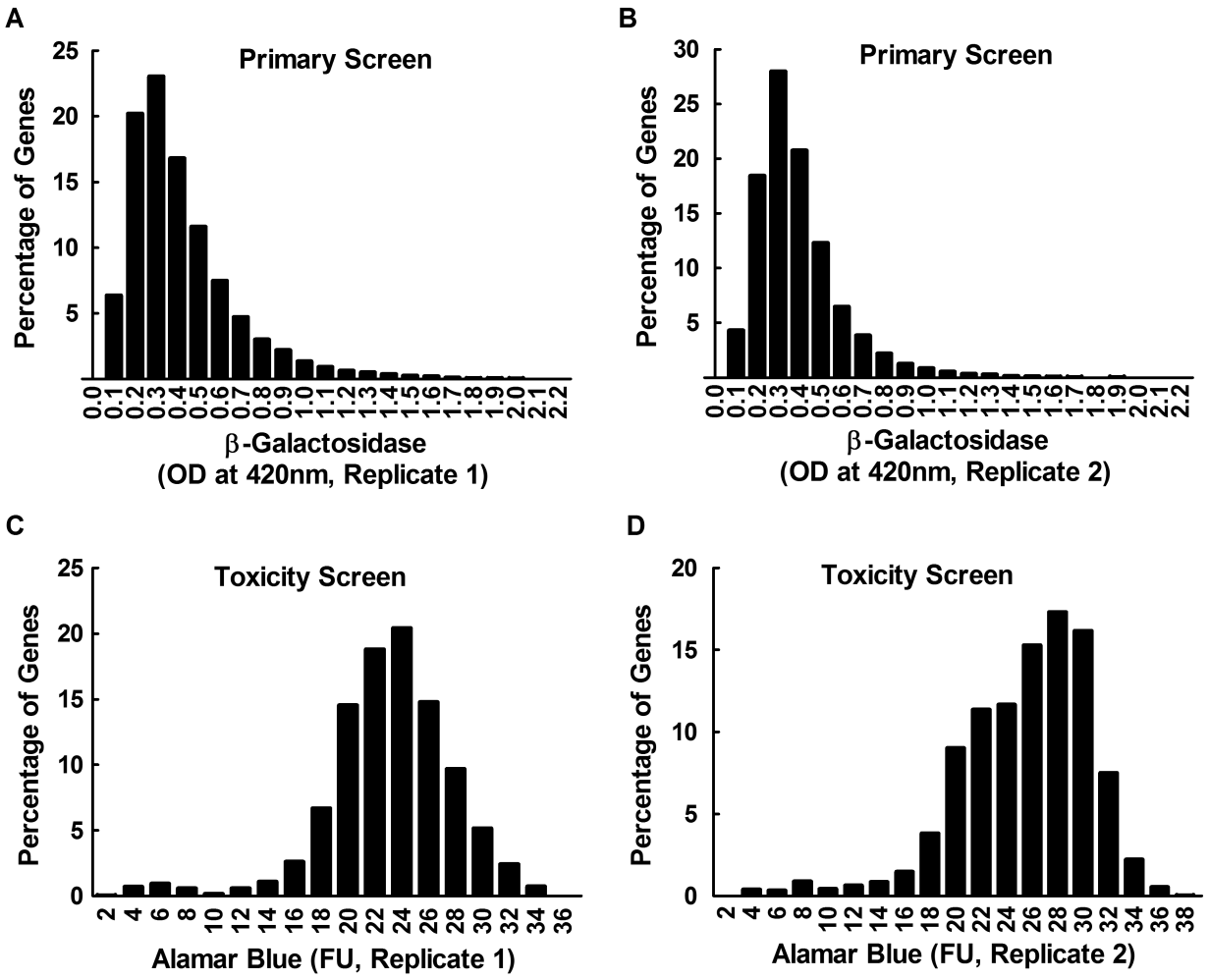

Figure S3. Data frequency distribution plots. The virus-encoded β-galactosidase optical density/absorbance measurements, acquired as replicates in the whole genome primary screen, were binned into 0.1 intervals and the centers of the intervals were plotted against the percentage of genes falling into each bin (panels A and B). Both replicates produced positively skewed distribution curves with the medians being ~15% less that the means. In contrast, the Alamar blue toxicity screen showed no strong evidence of a data skew. In this case the data were binned into 2 fluorescent unit intervals and the centers of the intervals were again plotted against the percentage of genes that fall in each bin (panels C and D). The mean and median values were essentially identical in both replicates.

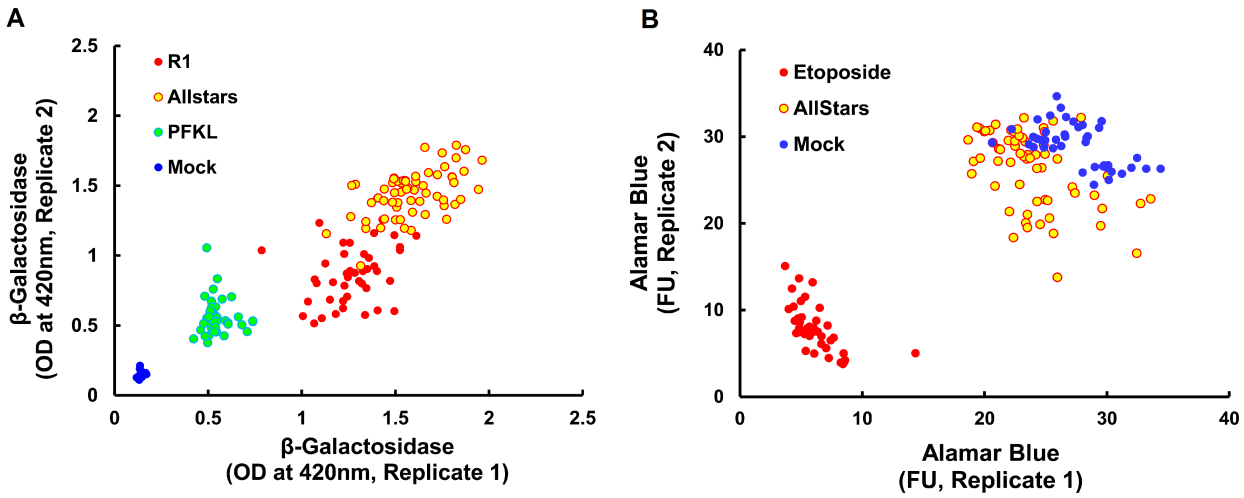

Figure S4. Assay reproducibility and discriminatory capacity. Panel A. Each of the plates used in the validation screen included extra wells containing siRNAs targeting the cellular ribonucleotide reductase (R1) and PFKL genes, as well as the AllStars control siRNA. The virus was also omitted from some wells to provide a blank for the β-galactosidase assays. The scatterplot illustrates the manner in which these replicate datapoints cluster across the multiple assay plates and shows that the screening method can readily discriminate between siRNAs that have no (AllStars), moderate (R1), or strongly (PFKL) inhibitory effects. Panel B. Each of the plates in the toxicity screen included extra wells where the cells were either mock treated, treated with a poisonous topoisomerase inhibitor (20 μM etoposide), or transfected with AllStars siRNA. The Alamar blue fluorescence assay readily discriminates between wells containing cells killed by etoposide *versus* wells containing AllStars transfected or mock-treated cells.

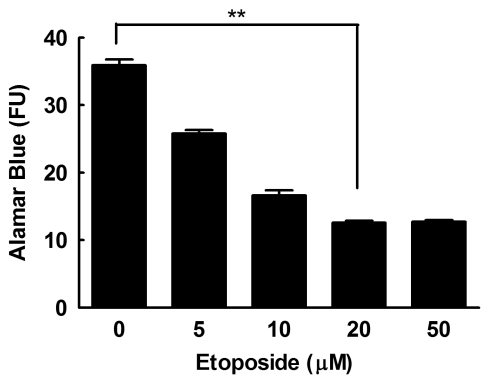

Figure S5. Titration of etoposide in MDA-MB-231 cells. MDA-MB-231 cells were plated overnight in 96-well dishes (5000 cells/well) and then cultured in media containing 0-50μM etoposide for three more days. An Alamar blue cell viability assay showed that a 70% reduction in fluorescence (maximal killing) can be achieved using 20μM drug.

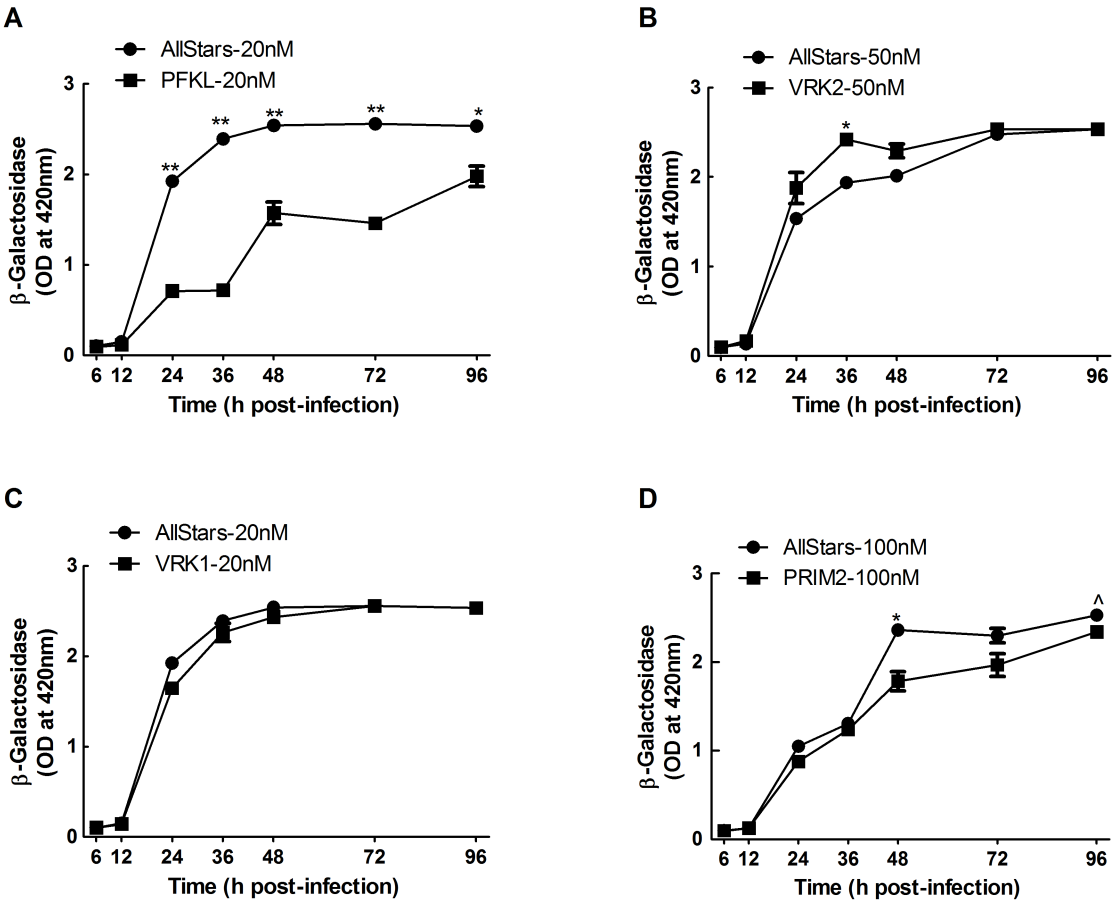

Figure S6. The growth kinetics of vMYX-LacZ in siRNA transfected cells. MDA-MB-231 cells were transfected with the indicated concentrations of siRNAs for 72h. Then the cells were infected with vMYX-LacZ at MOI of 1. At the indicated time points after infection, the cells were lysed and β-galactosidase activity was determined. Cells transfected with PFKL siRNA showed a significant reduction in virus replication starting from 24h post infection (A). VRK2 siRNA transfected cells showed a significant increase in virus replication at 36h post infection compared to AllStars negative control siRNA transfected cells (B). However, subsequent time points showed a reduction in the difference. There was no significant difference in virus replication between VRK1 siRNA and AllStars negative control siRNA transfected cells (C). For PRIM2 siRNA transfected cells, the most significant reduction in virus replication was observed at 48h post infection (D). The data are representative of two independent experiments and the significance is indicated relative to AllStars negative control siRNA transfected cells: (^, $p \leq 0.05$; *, $p \leq 0.01$; **, $p \leq 0.001$).

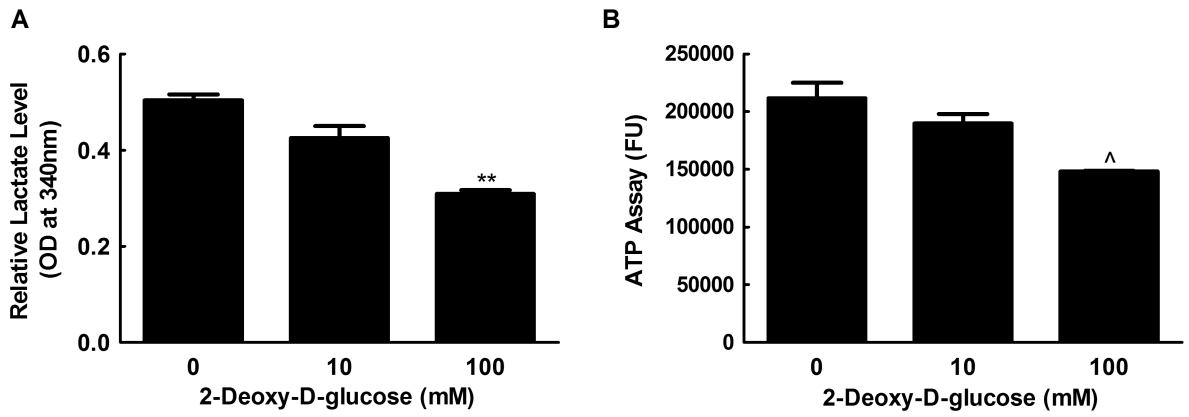

Figure S7. Treating cells with 2-deoxy-D-glucose (2DG) reduces the levels of lactate and ATP. MDA-MB-231 cells were treated for 24h with 0, 10, or 100mM 2DG and the effects on the concentrations of lactate (A) and ATP (B) determined using colorimetric and luminescence assays, respectively. Although 10mM 2DG causes some reduction in the rate of glycolysis, 100mM 2DG is required to significantly reduce the levels of lactate and ATP. The data are representative of two independent experiments and the significance is indicated relative to untreated control cells: (^, $p \leq 0.05$; **, $p \leq 0.001$).