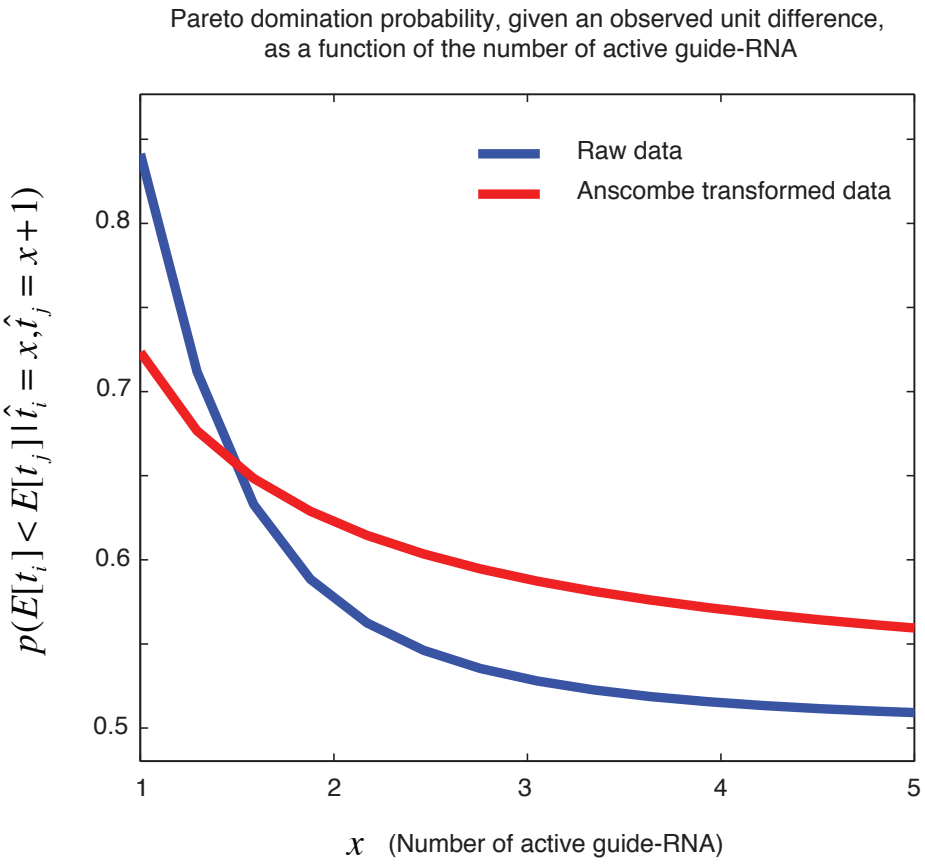
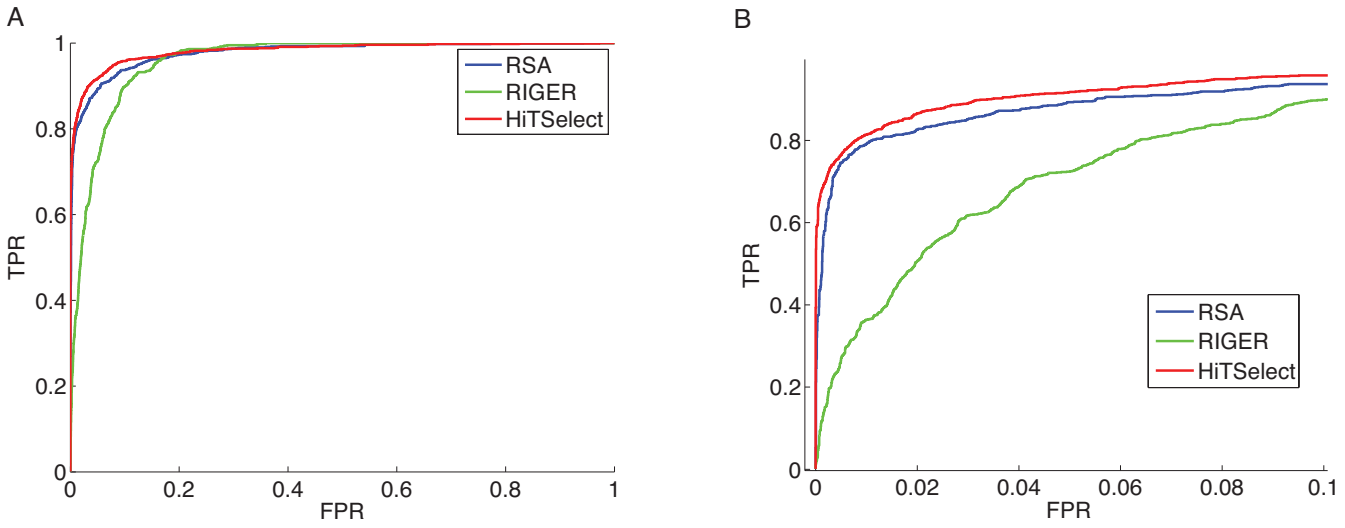
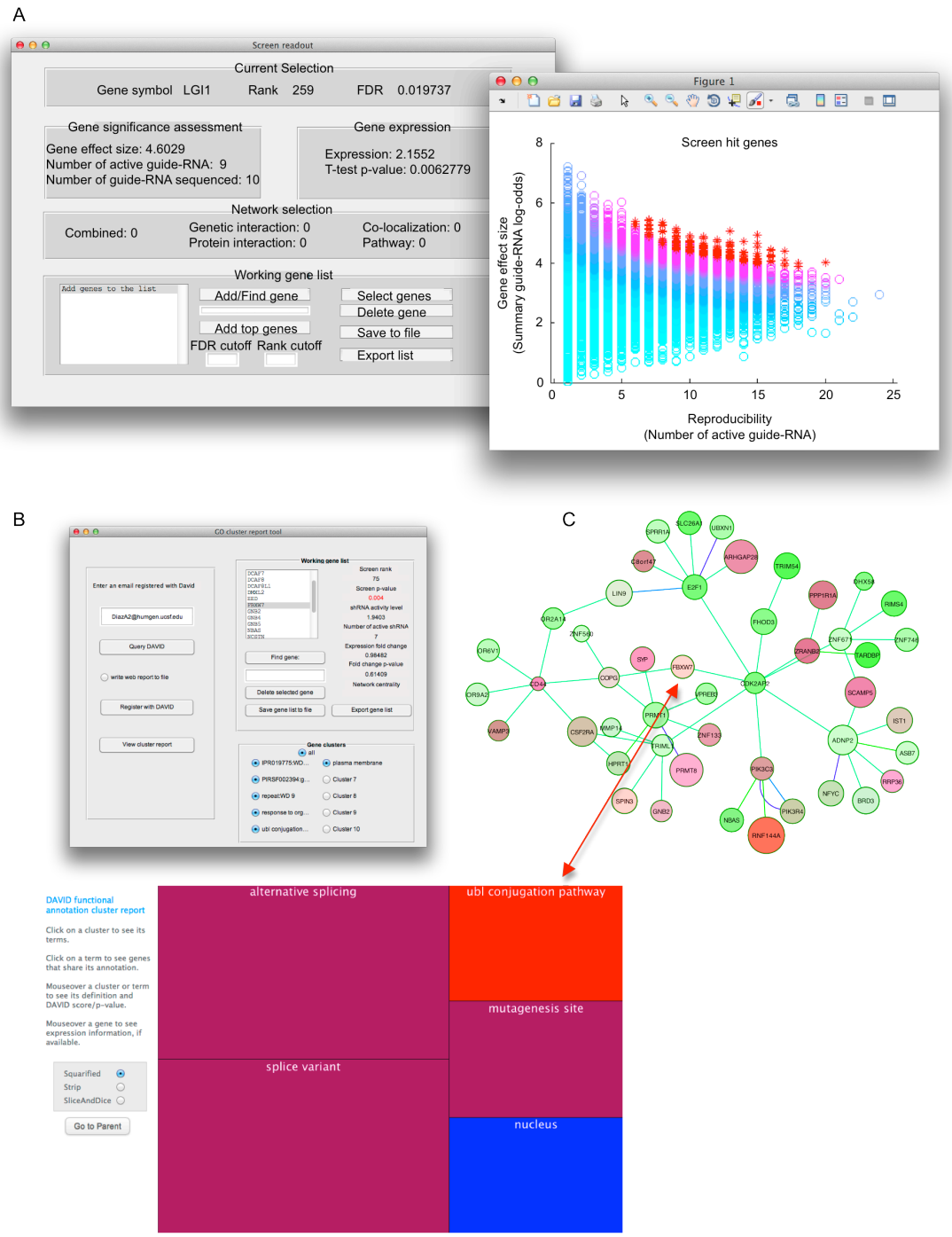


Supplementary Figure S1: HiTSelect analysis of CRISPR screens for mediators of chimeric anthrax and diphtheria toxin intoxication.

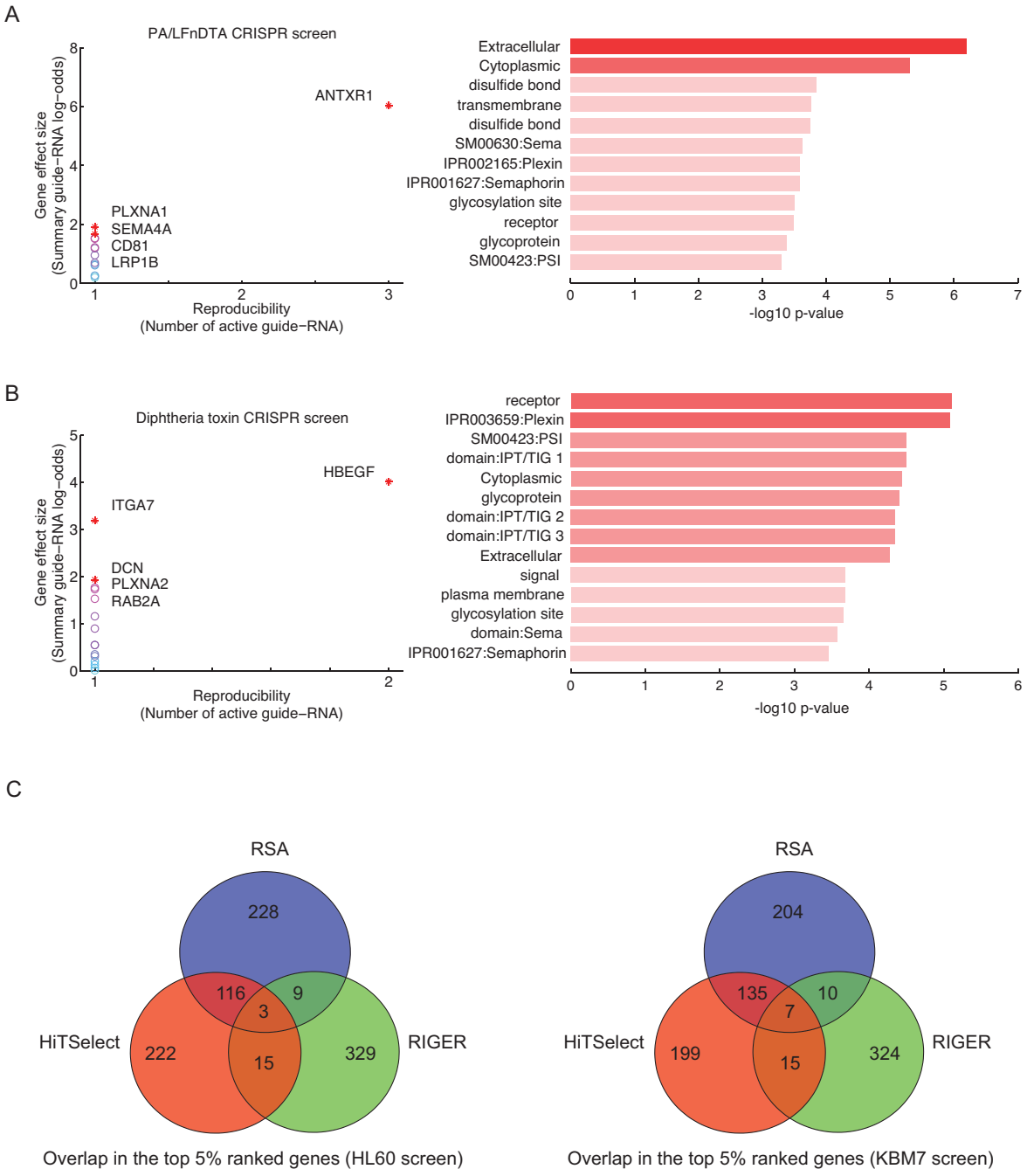
Anscombe's transform ($x \rightarrow 2\sqrt{x+3/8}$) stabilizes variance in sampling the number of active guide-RNA.



Supplementary Figure S2: Receiver Operator Characteristics (ROC) for simulated data.
For 20,000 genes, we generated a Poisson random variable with mean 5 to simulate the number of guide-RNA sequenced for each gene. We chose 5, because it is approximately equal to the median number of guide-RNA sequenced per gene across all screens that we have considered. Then, for each guide-RNA, we randomly assigned to it a log-odds ratio, drawn from a Gaussian mixture model with 3 components. The means of the 3 components were chosen to be -4, 0, and 4. The standard deviation was chosen to be 1 for all 3 components. The mean value 4 is close to the median summary log-odds ratio over all screen hits in the screens considered. The first component represents guide-RNAs enriched in the control population, the second component represents guide-RNAs with no enrichment, and the third component represents guide-RNAs enriched in the treatment population. To *a priori* designate screen hits, we used the mixing weights (0.025, 0.075, 0.9) for 1000 genes and (0.9, 0.075, 0.025) for another set of 1000 genes. To simulate 18,000 genes that should have no enrichment, we used the mixing weights (.1, .8, .1). (A) We processed this simulated screen readout with HiTSelect, RSA and RIGER. HiTSelect had the highest area under the curve (AUC) in its Receiver Operator Characteristic: HiTSelect AUC 0.9818, RSA AUC 0.9778, RIGER AUC 0.9602. (B) This difference is most pronounced below the 10% significance level, under which researchers typically choose their hit threshold.



Supplementary Figure S3: HiTSelect contains modules for screen hit selection and downstream analysis. HiTSelect provides modules to identify significant genes in large primary screens and to interface with powerful functional annotation, gene interaction analysis, and visualization software. (A) HiTSelect's hit selection and visualization module allows one to browse and search screen hits and screen hit meta-data. (B) HiTSelect's functional annotation module allows the user to automatically generate interactive graphical reports based on DAVID functional analysis. (C) HiTSelect's Genemania and Cytoscape interfaces facilitate the identification and visualization of screen hit genetic, physical, and pathway interactions.



Supplementary Figure S4: Comparative analysis of CRISPR screens.
(A-B) HiTSelect gene ranking algorithm identifies anthrax toxin receptor (ANTXR1) and diphtheria toxin receptor (HBEGF) as the most significant genes in the anthrax and diphtheria screens respectively. (C) Overlaps between the gene sets ranked as top 5% by RSA, RIGER, and HiTSelect in the CRISPR screens of Wang *et al*..