Supplemental Figures and Tables

Identification of chemosensitivity nodes for vinblastine through small interfering RNA high- throughput screens.

Carolyn A. Kitchens, Peter R. McDonald, Tong Ying Shun, Ian F. Pollack and John S. Lazo

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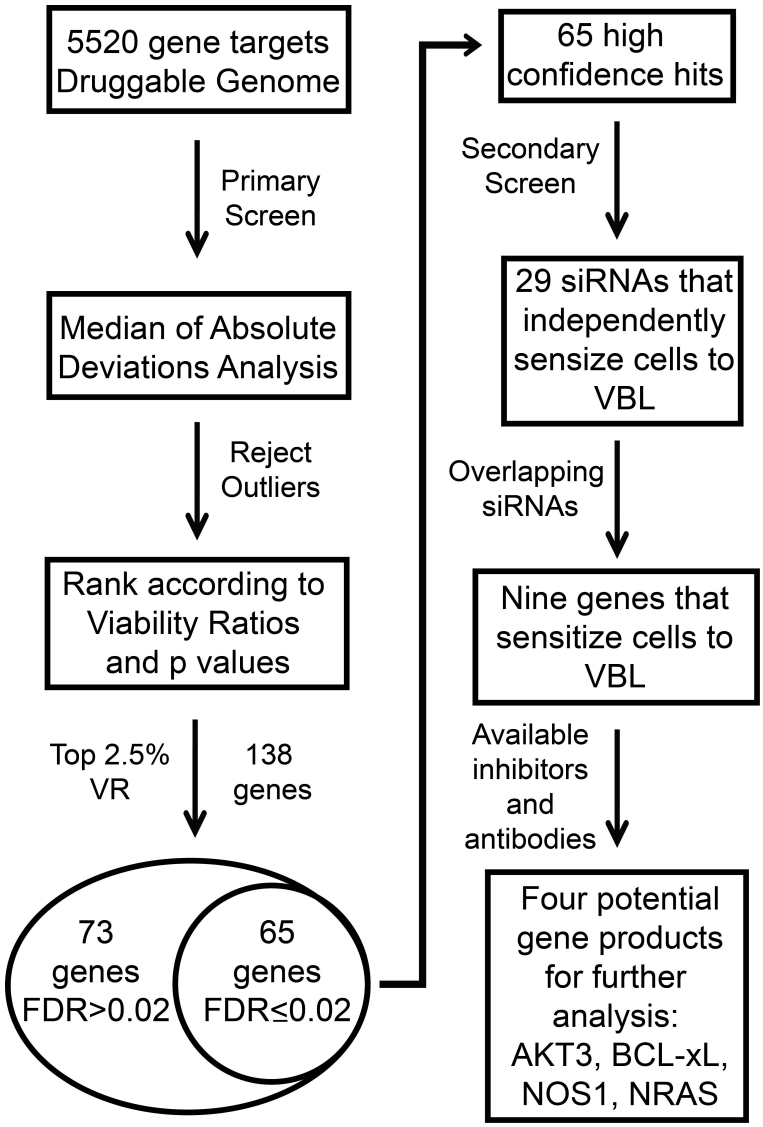
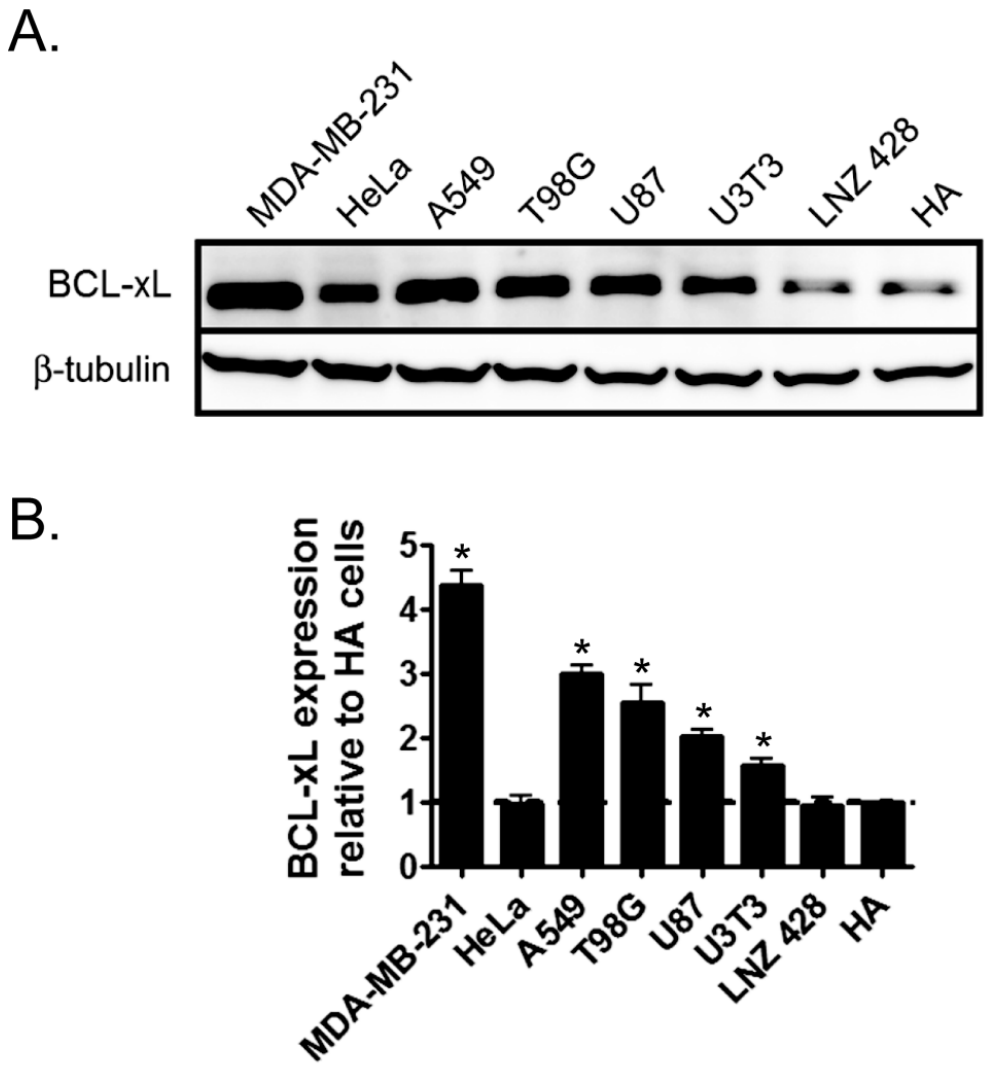


Supplementary Figure 1. Primary and secondary siRNA HTS overview. A HTS targeting 5,520 gene products with 16,560 siRNAs was undertaken to identify VBL sensitizers in T98G GBM cells. Outliers from the HTS were determined and rejected by MAD analysis. Genes were ranked according to their viability ratio (VR) and the top 2.5% VRs (138 genes) with FDR≤0.02 were selected as hits, resulting in 65 genes that sensitize cells to VBL (1.2% hit rate). Nine of these genes confirmed in a secondary assay, which was limited to gene products with commercially available antibodies and inhibitors, resulting in four gene products (AKT3, BCL-xL, NOS1 and NRAS) as potential targets for novel chemotherapy combinations with VBL.

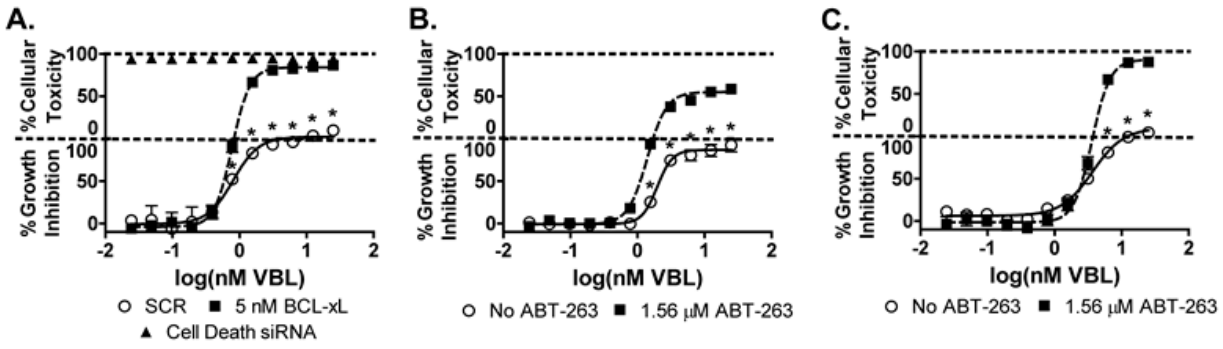
Supplementary Table 1. Viability ratios, p-values and FDRs from the 65 high confidence gene products that sensitized cells to VBL as indicated by the primary siRNA screen.

Gene Symbol	p-value	FDR	viability ratio	Gene Symbol	p-value	FDR	viability ratio
AACS	0.002	0.004	0.451	PPIH	0.006	0.008	0.426
ADSL	0.001	0.003	0.239	PRDX1	<0.001	0.001	0.486
AKT3	0.001	0.004	0.280	PRDX6	0.008	0.009	0.339
ANKRD30A	0.001	0.002	0.469	PROCR	0.002	0.004	0.179
BCL-xL	<0.001	<0.001	0.330	PSMC1	0.001	0.004	0.485
BGLAP	0.007	0.009	0.427	PTER	0.003	0.006	0.430
BTN3A2	0.002	0.005	0.491	PXN	0.003	0.006	0.443
C1GALT2	0.005	0.007	0.351	RAB1B	0.003	0.006	0.360
CDC42	0.002	0.005	0.400	RAB21	0.004	0.006	0.457
CST6	0.003	0.006	0.391	RAB9A	0.004	0.007	0.450
DKFZp434C1418	0.001	0.003	0.471	SCARF1	0.008	0.009	0.473
DKFZp762F0713	<0.001	<0.001	0.460	SCDR10	<0.001	0.002	0.416
DLGAP2	0.002	0.005	0.497	SCDR9	0.005	0.007	0.402
DNASE2	<0.001	0.001	0.495	SDCCAG10	0.006	0.008	0.491
DYT1	<0.001	<0.001	0.397	SDHA	<0.001	0.001	0.170
FAH	<0.001	0.001	0.464	SERPINA10	<0.001	0.002	0.386
FLJ10858	<0.001	0.001	0.444	SERPINA3	<0.001	0.002	0.431
FTS	0.002	0.004	0.394	SERPINB13	0.007	0.008	0.462
FUK	0.007	0.008	0.434	SERPINB3	0.001	0.002	0.322
GSTA1	0.009	0.009	0.371	SERPINB5	0.005	0.007	0.484

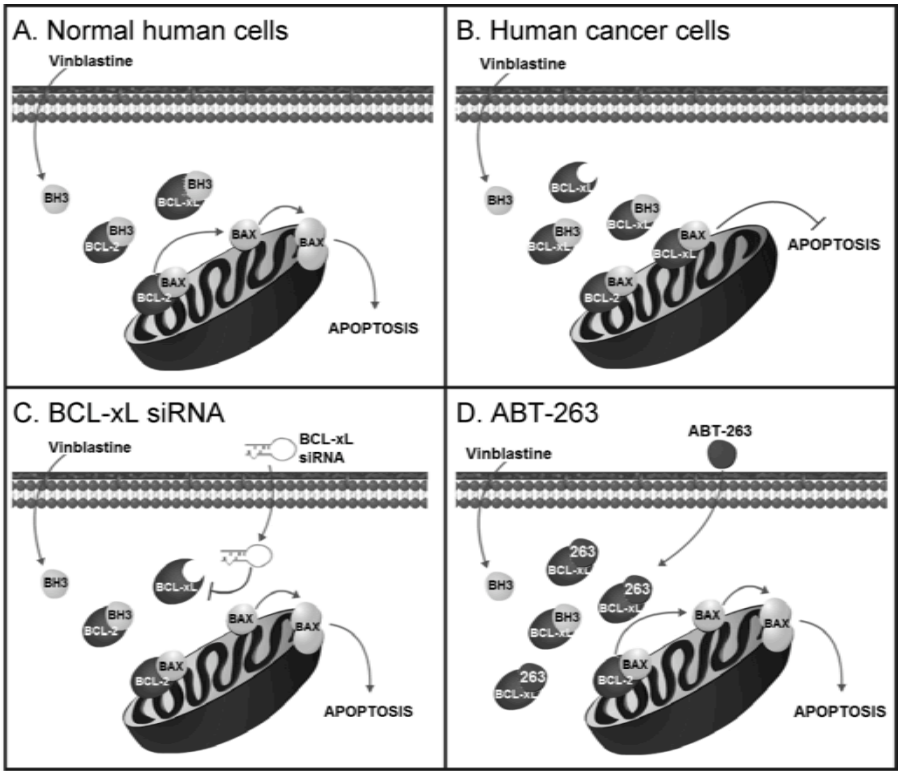
Gene Symbol	p-value	FDR	viability ratio	Gene Symbol	p-value	FDR	viability ratio
GSTZ1	0.001	0.003	0.455	SEZ6L2	0.005	0.007	0.496
GYG	0.003	0.006	0.485	SPINK1	0.001	0.002	0.411
GYS1	0.006	0.007	0.460	SULF2	0.007	0.008	0.490
ITPR3	0.009	0.009	0.399	TGM3	0.001	0.003	0.483
KDELR1	0.008	0.009	0.410	THEA	0.007	0.008	0.441
KIF11	0.009	0.009	0.481	TNFRSF25	0.002	0.005	0.475
LOC136242	0.006	0.008	0.456	TPRA40	<0.001	0.001	0.450
LOC200895	0.002	0.005	0.438	UGT1A9	0.002	0.005	0.498
NIT1	<0.001	0.001	0.402	UGT2B11	0.002	0.005	0.440
NOS1	<0.001	0.003	0.483	UGT2B17	0.001	0.003	0.496
NRAS	0.001	0.003	0.389	UMPS	0.001	0.004	0.380
PMM2	0.005	0.007	0.397	WARS2	0.003	0.006	0.479
POLR1A	0.001	0.004	0.497				



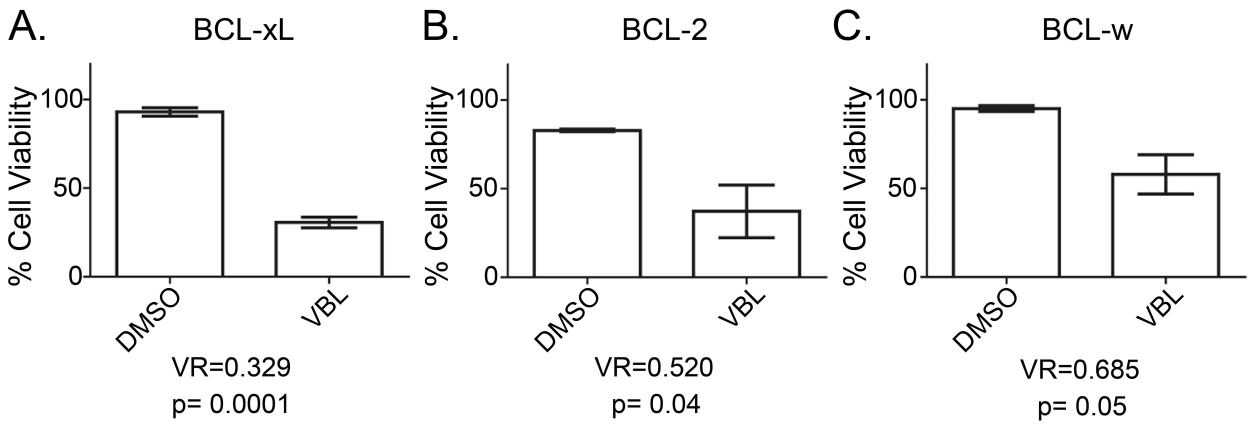
Supplementary Figure 2. Overexpression of BCL-xL in human cancer cells. (A) BCL-xL protein expression was measured by Western blot analysis in various cancer cells including (from left to right) MDA-MB 231 (breast cancer), HeLa (cervical cancer), A549 (non-small cell lung cancer), T98G, U87, U3T3, LNZ 428 (glioblastoma), and HA (human astrocytes). Expression levels were normalized to a β-tubulin loading control. (B) Protein expression levels were quantified by densitometry normalized to the tubulin control and then normalized to the relative expression levels of HA cells. Blot representative of three replicates. Bars equal S.E.M. *p≤0.05



Supplemental Figure 3. Concentration dependent cytotoxicity **of VBL with BCL-xL siRNA or ABT-263.** We conducted growth inhibition studies comparing the cell viability (CV) at 48 and 96 h after VBL addition using National Cancer Institute growth inhibition methodology, where growth inhibition equals (1-CV96 h)/(1-CV 48 h). Reduction in CV96 h greater than the DMSO CV at 48 h was defined as cytotoxicity. (A) T98G cells transfected with scrambled (SCR), 5 nM BCL-xL or "Cell Death" siRNA, were treated with increasing VBL concentrations. We found ≥1.56 nM VBL caused a cytostatic effect (100% growth inhibition). In the presence of 5 nM BCL-xL siRNA, there was an additional loss of cell viability, where 100% cellular toxicity was complete lethality. "Cell Death" siRNA was completely lethal in the cells. (B) T98G and (C) A549 cells were treated with increasing VBL concentrations in the presence and absence of 1.56 μM ABT-263. The antimitotic VBL arrested cell growth but the addition of ABT-263 with ABT-263 in T98G and A549 cells resulted in the loss of cell viability consist with cytotoxcity. Each value is the mean of three independent experiments. (○) Cells treated with increasing VBL concentrations. (■) Cells treated with increasing VBL concentrations in the presence of nontoxic BCL-xL siRNA or ABT-263. (▲) T98G cells transfected with "Cell Death" negative control siRNA. Each value is the mean of three independent experiments. Bars equal S.E.M. *p≤0.05



Supplementary Figure 4. Resensitization of cancer cells to VBL by BCL-xL siRNA and ABT-263. (A) Normal human cells have a balance of BCL-2 pro-survival and pro-apoptotic proteins. Upon activation of intrinsic apoptosis, BH3 only proteins compete with BCL-2 pro-apoptotic for binding to the BCL-2 pro-survival proteins (BCL-2, BCL-xL and BCL-w). The pro-apoptotic proteins are then free to translocate to the mitochondria and activate apoptosis. (B) In certain cancer cells, the BCL-2 pro-survival proteins are overexpressed and the balance of pro-survival to pro-apoptotic proteins is disrupted. Upon activation of intrinsic apoptosis by anticancer agents, the overexpressed pro-survival proteins are able to bind the BH3 only proteins, while still suppressing the pro-apoptotic proteins, preventing apoptosis even in the presence of chemotherapy. (C) siRNAs targeting pro-survival proteins decrease the expression of the pro-survival proteins, thereby reestablishing the balance between pro-survival and pro-apoptotic proteins and resensitizing cancer cells to chemotherapeutic agents. (D) BH3 mimetics, such as ABT-263, bind to the overexpressed pro-survival proteins and resensitize cells to other anticancer agents.



Supplementary Figure 5. Less stringent statistical criteria from the primary HTS includes BCL-2 and BCL-w as sensitizers of GBM to VBL. By decreasing the stringency of the siRNA primary screen, all three BCL-2 prosurvival proteins inhibited by ABT-263 would have been identified as sensitizers to VBL. (A) BCL-xL was originally identified as a high confidence VBL sensitizer with a $p \leq 0.01$ and a viability ratio in the top 2.5%. While (B) BCL-2 and (C) BCL-w also had viability ratios in the top 2.5%, they both had $p \leq 0.05$ but $p > 0.01$, which eliminated them as high confidence gene products in from the primary siRNA HTS. If the initial Student's t-test had an α of 0.05 versus 0.01, all three prosurvival proteins would have been identified as high confidence VBL sensitizers from the primary screen.

Supplementary Table 2. siRNA sequences for BCL-xL in secondary analyses.

siRNA name	siRNA ID	sense	antisense
BCL-xL "A"	s1920	AUACUUUUGUGGAACUCUATT	UAGAGUUCCACAAAAGUAUCC
BCL-xL "B"	s1921	GCUGGAGUCAGUUUAGUGATT	UCACUAAACUGACUCCAGCTG
BCL-xL "C"	s1922	GGAACUCUAUGGGAACAAUTT	AUUGUUCCCAUAGAGUUCCAC