Figure Legend

Figure 1. Primary siRNA lethality screen in myeloma cells.
(A) 13,980 distinct siRNA targeting ~7,000 genes were individually transfected into KMS11 human multiple myeloma cells, alongside 3,000 replicate controls, in a single-siRNA-per-well format, in duplicate high-throughput studies; the results of one screen are shown. Cell viability was determined at 96 hours by ATP assay. siRNA-induced changes in viability (B-score) are shown in units of standard deviation from the median of non-inhibitory siRNA, and are plotted in the order in which siRNA were screened. Negative controls for non-specific toxicity included cells treated with transfection reagent alone or transfected with one of three unique non-targeted siRNA; replicate positive controls for transfection efficiency were included at regular intervals throughout the screen reflecting cells treated with an siRNA targeting ubiquitin B. (B) Results of control wells, in raw luminescence units (RLU), demonstrating consistently high transfection efficiency throughout screening (~95%, when assessed by the stringent measure of cell death due to siRNA-induced ubiquitin B silencing) and minimal non specific cytotoxicity associated with transfection conditions. (C) Results of genome-scale siRNA screens in myeloma cells were highly reproducible, with significant correlation between duplicate studies. (D) Cumulative distribution of the proportion of siRNA inducing a loss of viability (black solid line), compared with the standard normal cumulative distribution (grey dashed line), verifying a significantly greater number of lethal siRNA results than can be attributed to random Gaussian effects. The proportion of lethal hits among the subgroup of siRNA (red solid line) whose paired siRNA (targeting the same gene) induced loss of viability exceeds the overall siRNA hit rate, confirming the presence of true gene hits, with enrichment of concordant siRNA hits over the rate expected due to chance. (E-F) Estimated false discovery rates for candidate survival genes associated with 1/2 or 2/2 lethal siRNA respectively, as a function of threshold viability for gene hit selection. False discovery was

determined by two independent methods (black vs. red lines), as described in the text.

Figure 2. Confirmatory siRNA study of candidate myeloma cell survival genes.
(A) Two hundred top-ranked candidate survival genes from fig. 1 were re-tested in a secondary study using 800 siRNA (4 independent siRNA per gene) and KMS11 cells; the effects on viability (Z-score) of 220 siRNA targeting 55 top-ranked survival genes are shown and compared with the effects of negative control non-targeting (NT) siRNA (blue) and positive control ubiquitin B (UBB) siRNA (red). (B) The confirmatory siRNA screen was highly reproducible, with significant correlation between duplicate studies. (C) When compared to the primary siRNA screen (fig 1D), in the confirmatory screen there was marked enrichment in the proportion of lethal siRNA and in the proportion of concordant results from siRNA pairs targeting the same gene; confirming enrichment of true gene hits over false positive hits. (D) False discovery rates for critical KMS11 myeloma cell survival genes. The individual FDR for non-redundant survival genes identified in KMS11 myeloma cells are shown, as a function of the loss of viability (in Z-score standard deviations) induced by up to 4 siRNA directed against them.

Figure 3. Comparative vulnerability of top-ranked survival genes
(A-C) Effect on viability of siRNA directed against top-ranked KMS11 myeloma survival genes in (A) 8226 myeloma cells and (B) JJN3 myeloma cells, and in (C) 293 embryonic kidney and (D) A549 lung carcinoma cells. Cells were transfected with four unique siRNA per genes in separate wells, using conditions optimized for each cell line. Viability was assessed at 96 hours and is shown in units of standard deviations from control siRNA. (E) Comparative vulnerability of target genes in myeloma and non-myeloma cells, plotted as the ratio of average effect on viability in KMS11, 8226 and JJN3 myeloma cells versus the average effect on viability in 293 and A549 non myeloma cells, for each of four siRNA targeting the gene. RNAi Z-scores were scaled to a uniform range for each cell line, using

positive and negative control results. To avoid artificial inflation of ratios due to small denominators reflecting no viability effects, RNAi with non-significant effect on viability with Z-score>-1 were standardized to a score of -1.

Figure 4. Expression of selected siRNA-identified survival genes, in primary myeloma tumor cells and across an atlas of primary human tissues

Histograms show the relative expression of myeloma survival genes: MCL1, CDK11, TNK2 and WBSCR22; and of controls CD138 and β-actin; in primary human somatic tissues (102 arrays), primary multiple myeloma tumor cells (115 arrays) and monoclonal gammopathy of uncertain significance (MGUS) and in normal human plasma cells (NPC) (data from SymAtlas, Novartis Research Foundation and from Mayo Clinic; GEO accession no. GSE 6477). Array-based gene expression data was normalized per chip (sample) to the median signal intensity of expressed genes (genes with present detection call and/or MAS5 signal intensity>200); a value of 1.0 thus represents the median expression intensity of genes within each sample type whose expression can be reliably detected.

Figure 5. Molecular networks enriched for myeloma survival genes.

Networks are derived from Ingenuity pathway analysis of top-ranked survival genes; network members identified by siRNA screening shown in red; not all network members were included in the screen. (A) Transcriptional regulation and mRNA splicing. (B) Ubiquitination, de-ubiquitination and proteasomal degradation of essential regulators of cellular proliferation and survival. (c) Mitosis control and centromere function.