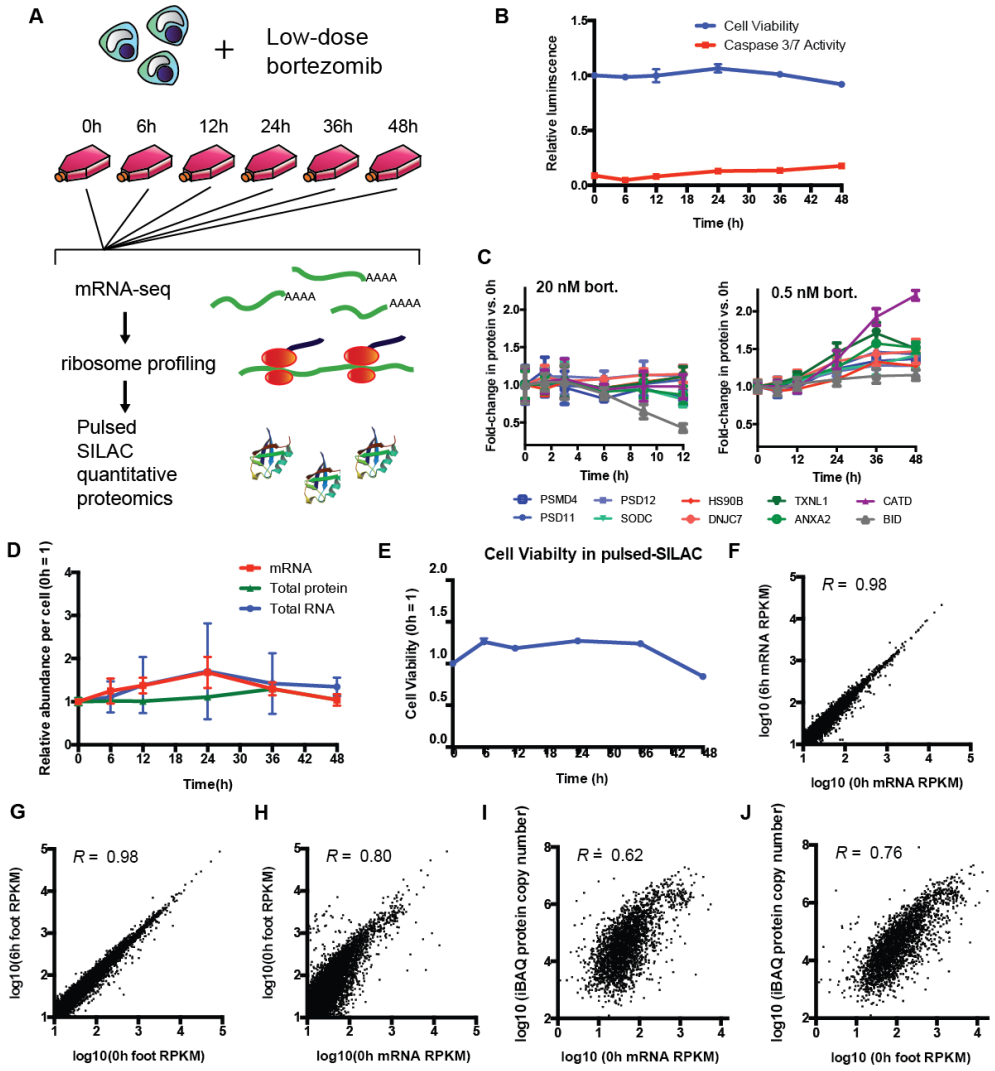

Figure S1. Related to Figure 1. Biochemical changes after bortezomib treatment and comparison of baseline deep sequencing and proteomic data. A. Experimental design in MM1.S myeloma cells to monitor protein synthesis under low-dose bortezomib treatment. **B.** Under 0.5 nM bortezomib there is little change in cell viability nor significant evidence of caspase activation. Values measured in duplicate +/- S.D. Normalized to 0h = 1 for cell viability (measured with Promega CellTiter-Glo assay), 0h = 0.1 for caspase 3/7 activity (measured by Promega Caspase 3/7-Glo assay). **C.** Selected Reaction Monitoring (SRM) proteomic assays on total protein demonstrate measurable increases in many proteins under low-dose (0.5 nM) bortezomib treatment that cannot be detected under rapid translational shutdown after high-dose (20 nM) bortezomib. Values +/- S.D. measured in technical duplicate. **D.** Total RNA, mRNA, and protein per cell show little change during the time course after low-dose bortezomib treatment. Values +/- S.D. measured in biological quadruplicate and normalized to cell viability data at each time point to determine concentration per cell. **E.** Low-dose bortezomib treatment with heavy SILAC media pulse also reflects similar response to drug at level of cell viability as compared to non-SILAC media (B). Values +/- S.D. measured in technical duplicate. **F-H.** Correlation between normalized read density (in RPKM) across 5642 transcripts with a minimum RPKM = 10 mapping to each gene, per the Ribomap algorithm (STAR Methods), comparing 0h and 6h mRNA-seq datasets (**F**), 0h and 6h ribosome footprint datasets (**G**), and 0h mRNA and 0h ribosome footprint datasets (**H**). **I-J**. Correlation between baseline mRNA-seq read density (**I**) and ribosome footprint read density (**J**) with estimated protein copy number per cell, as determined by averaged iBAQ analysis of duplicates (STAR Methods).

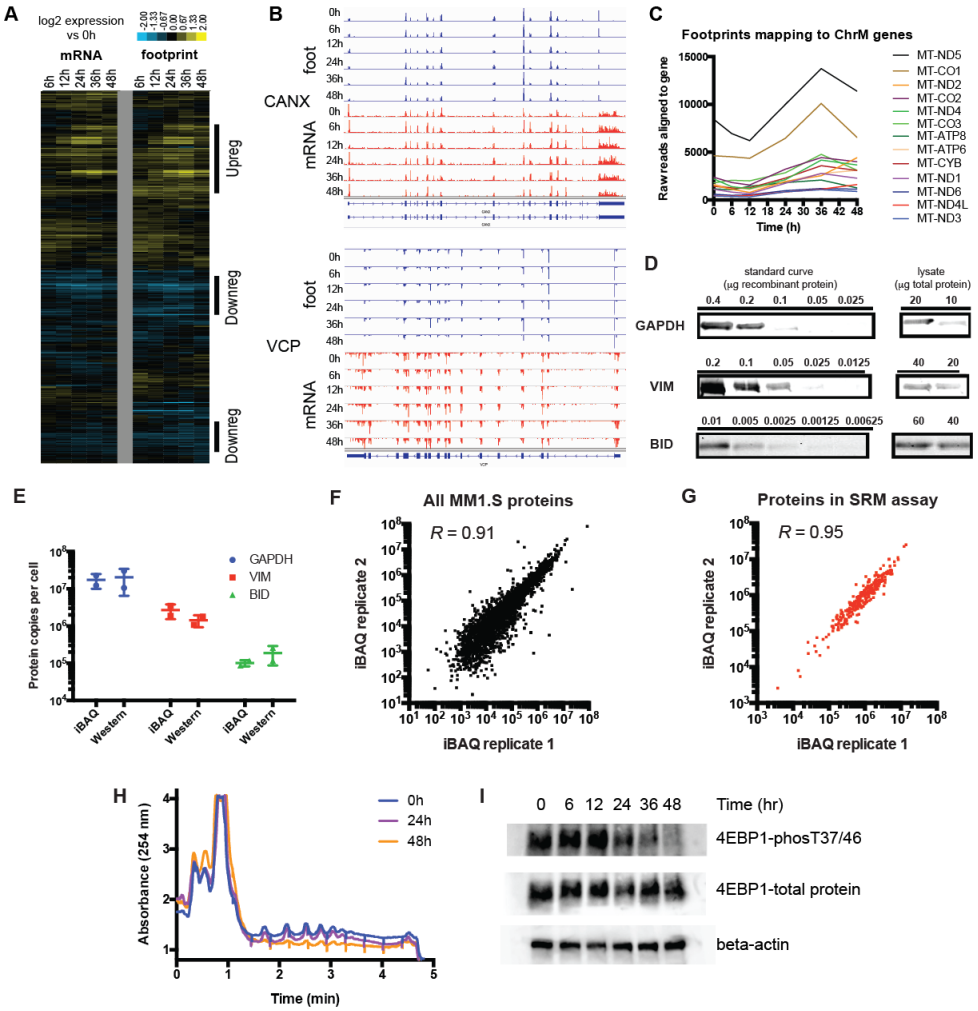

Figure S2. Related to Figures 2 and 3. Changes in global translation across the time course and characteristics of proteins included in SRM assay. A. Heatmap comparing mRNA-seq and ribosome footprint coding sequence read density (in RPKM) across all included transcripts. "Upreg" and "Downreg" clusters used for analysis in Dataset S2. **B.** Raw aligned ribosome footprint and mRNA-seq reads for two example proteins monitored by SRM assay (CANX and VCP). Note that footprint reads only map to coding exons (noted as thick bars in blue track at bottom of each panel; medium bars are untranslated regions; thin bars are intronic or intergenic) whereas mRNA-seq reads also map to 5' and 3' untranslated regions. Direction of reads reflects gene encoding on plus (reads directed up) or minus (reads directed down) strand of genome. Screenshot from Integrative Genomics Viewer. **C.** Ribosome footprint reads across the time course mapping to ChrM. Increased ChrM mapping reads across the time course are consistent with decreased cytosolic ribosomal translation (Iwasaki et al., 2016). **D.** Quantitative Western blot data for three high-abundance example proteins. **E.** Comparison of iBAQ and quantitative Western blot data for independent biological replicates suggests iBAQ provides similar estimates for high-abundance proteins. Error bars show mean +/- S.D. **F.** Baseline protein copy numbers per cell estimated using iBAQ are largely consistent across two biological replicates in MM1.S cells (n = 2704 protein groups with iBAQ estimate in both replicates). **G.** Proteins monitored by SRM assay (n = 272) show high reproducibility in baseline quantification based on iBAQ data. **H.** Polysome profiles obtained by sucrose gradient ultracentrifugation indicate diminishment of translation at the 48h time point after treatment with low-dose bortezomib. **I.** Western blot shows eIF4E Binding Protein-1 (4EBP1) dephosphorylation after low-dose bortezomib treatment, consistent with general translational inhibition.

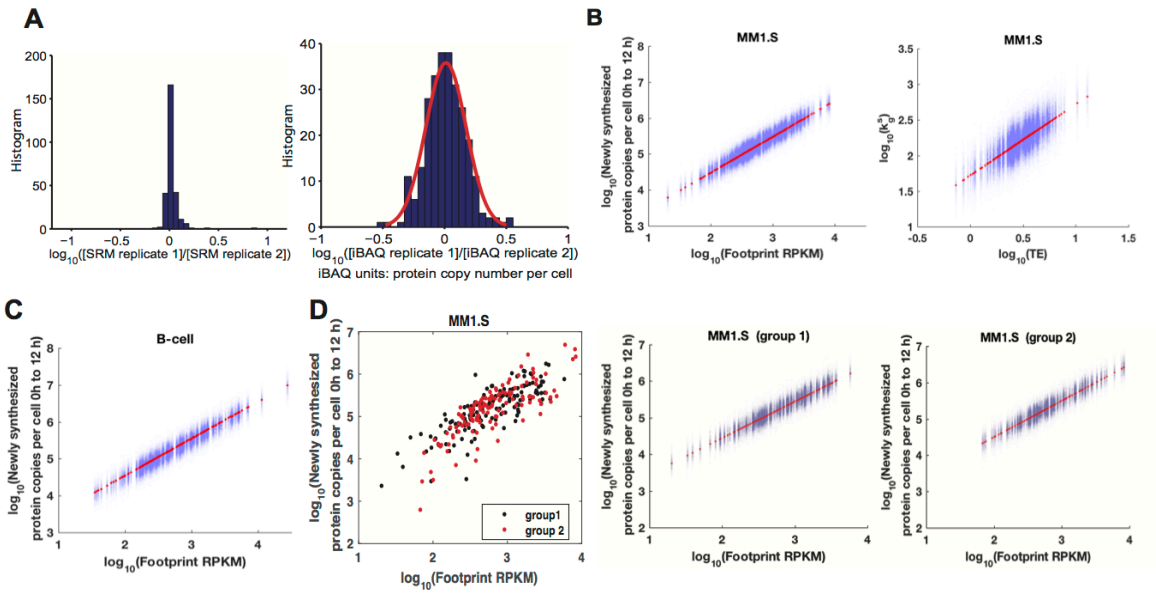

Figure S3. Related to Figures 2 and 4. Error modeling based on iBAQ values and transcript-level isoforms. A. MM1.S data demonstrates strong reproducibility for SRM replicate intensity but increased variance in iBAQ absolute copy number replicates. **B.** A gene specific scaling factor s_g is randomly generated according to the fitted normal distribution of the differences between the log-transformed iBAQ replicates (red curve in **A**, *right*) to simulate the effect of iBAQ noise. These simulated iBAQ values are used to generate point clouds of newly synthesized proteins per cell measured by SRM intensity as in Fig. 2A for MM1.S. Compared to the distributions in Fig. 2A and the distribution of baseline of TE and $k_g^s(t)$ in Fig. 2D, the iBAQ noise explained 36% and 10% of the deviation from the linear regression fits, respectively. **C.** Compared to the distribution in Fig. 4D for B-cells, the iBAQ noise explained 23% of the deviation from the linear regression fits. **D.** All 272 protein-transcript pairs monitored in MM1.S cells were sorted into two groups. Group 1: One dominant transcript isoform (>80% of RNA-seq read density on a single isoform, per paired-end RNA-seq analysis at www.keatslab.org/data-repository/HMCL66_Transcript_Expression_FPKM.xlsx) (n =145). Group 2: No dominant transcript isoform (n = 127). The iBAQ noise explained 47% and 35% of the deviation from the linear regression fits for group 1 and group 2, respectively.

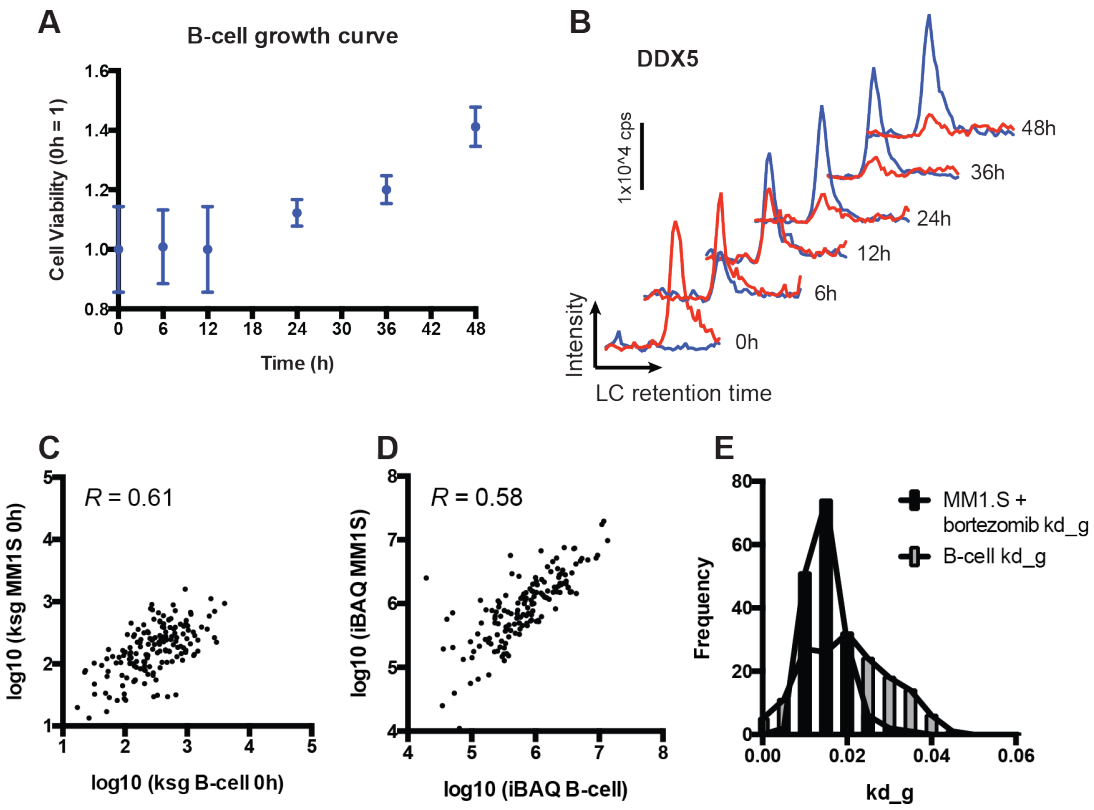

Figure S4. Related to Figure 4. Extension of quantitative model to additional dataset in EBV-immortalized B-cells. **A.** Growth of EBV-immortalized B-cells over the untreated time course (CellTiterGlo reagent; technical triplicate at each time point +/- S.D.). **B.** Example pSILAC targeted proteomic data for peptide from protein DDX5 shows similar kinetics of synthesis and degradation as in MM1.S (Fig. 1A). Blue = newly synthesized across time course, red = degraded from baseline. Similar correlations between (**C**) $k_g^s(0)$ as well as (**D**) iBAQ values in MM1.S and EBV B-cells for the proteins included in both SRM assays. **E.** Comparison of protein degradation rate constants k_g^d for proteins monitored in both cell lines suggests that low-dose bortezomib treatment in MM1.S leads to a decrease in degradation rate for some proteins.