## Supplementary Figure Legends

**Supplementary Figure S1: Loss of membrane motion precedes loss of innate fluorescence in MM fluorescent cell line.** The cell line RMPI-8226 was stably transfected with the fluorescent protein dsRed2. Upon death, cells stop producing the protein, which can degrade inside the cytoplasm and/or diffuse to media upon cell membrane bursting. The figure above depicts four examples of these cells exposed to 50μM melphalan for 24h. The first column depicts the initial time point, when all cells are alive. The red fluorescent channel is superimposed to the transmitted light, while the motion detection algorithm pseudo-colors them as green. The second column shows the moment of loss of membrane motion, while the third column shows the moment of loss of red fluorescence. The last column represents the last time point, when Calcein AM was added (pseudo-colored in blue), to identify live cells.

**Supplementary Figure S2: Loss of membrane motion is an early event in cell death, preceding loss of membrane integrity.** Above are four examples of H929/S cells exposed to a stable drug gradient of Melphalan, from 50 to 10-uM. At the beginning of the experiment, all live cells are pseudo-colored in green from motion detection. The moment of loss of membrane motion is marked by the disappearance of green in the image. There is a variable delay between loss of membrane motion and acquisition of red fluorescence, which is a combination of loss of membrane integrity and binding of EthD-1 to DNA.

**Supplementary Figure S3: The duration of melphalan toxicity post-withdrawal.** 8226/LR5 (top) and NCI-H929 (bottom) cells were exposed to a melphalan gradient with highest concentration of 20μM. Melphalan's half-life is approximately 1-2h in media. At highest concentration, NCI-H929 cells begin to die after 24h, while 8226/LR5 viability begins to decline at 36h. Both cell lines continue dying for over a week. This delayed toxicity of melphalan was thus considered in the computational model for clinical response (**Equation 2**).

**Supplementary Figure S4: The duration of bortezomib activity post-withdrawal.** NCI-H929 cells in two microfluidic chambers were exposed to a bortezomib gradient for 24h. After 24h, the media from both chambers were replaced. Drug was refreshed in the first chamber (top), while drug was removed from the second (bottom, P, for pulsed exposure). As shown in the 65h-study, upon removal of bortezomib, cell death stops and cells resume growth. Same assay could be used to study lingering effects of experimental drugs with short half-lives.

**Supplementary Figure S5: *In vitro* melphalan chemosensitivity of three MM patients.** Patient 14 was a newly diagnosed/high-risk patient, patient 12 was a relapsed/standard-risk patient previously treated with high-dose melphalan and bone marrow transplantation. Patient 11 was a smoldering/standard-risk patient. In all three assays,

cells were seeded in single culture in 3D collagen matrix with patient plasma-supplemented medium, as described in methods. (Top, left) After 24h continuous exposure to a stable melphalan gradient ranging from 0 to 50μM, cells from the three patients responded at different rates, with patient 12 being the most resistant, and patient 11 the most sensitive. While the EC50 for 24h (Top, right) was ~4μM for patients 12 and 14, and ~1μM for patient 11, the EC20, for example, was significantly higher in the relapsed patient: 50μM for patient 12, 10μM for patient 14, and ~3μM for patient 11.

**Supplementary Figure S6: Computational modeling of *in vivo* response to therapy.** By parameterizing **Equation 3** with the dose response constants obtained from NCI-H929 cells exposed to a bortezomib gradient (**Figure 2**), it was possible to simulate how a bi-weekly treatment of a subcutaneous SCID mouse model would affect tumor growth. Pearson's correlation "r value" was 0.9869 (P=0.0003) between actual measurements (T Ishii et al., 2012) and simulated tumor burden under a 1mg/kg bi-weekly bortezomib regimen, which leads to a stable concentration of ~0.4nM bortezomib in plasma (Williamson et al., 2009). Using the same computational model, it was possible to test hypothetical treatments, such as, for instance, a regimen with holidays where the double of the amount of bortezomib would be administered every other week (0.8nM w/ holidays).

**Supplementary Figure S7: Computational modeling of clinical response to therapy.** The parameters of bortezomib chemosensitivity obtained *in vitro* from four patients (**Figure 7**) were used in **Equation 3** to simulate how these patients would have responded to a single agent bortezomib hypothetical treatment. The regimen simulated was 1.3mg/m$^2$ doses on days 1, 4, 8, and 11, leading to a stable plasma concentration of ~1nM (Reece et al., 2010).