Supplementary data figure legends

Supplemental Figure 1. FACS analysis of DNA content indicates cell line variability and complex relationships between mitotic arrest and cell death. Non-synchronized cells were treated with 500nM EMD534085 for 8, 16, 24, 48, 72 and 96h, and processed for PI-labeling and flow cytometry. A, B) HT29 and MCF7 both arrest strongly, but HT29 die and MCF7 do not. By 8h post-drug, 4N arrest was already increased. By 24h, 89.4% of HT29 and 72.4% of MCF7 progressed from 2N to 4N (mitotic or post-mitotic). After 24h, the 4N HT29 population decreased to 38.9% at 96h, while this population remained high, dropping only to 61.0% for MCF7. C) HeLa, HT29, U-2 OS, and RPE1 had significantly accumulated 4N of >60% at 16 and 24h, but HL60 reach a maximum of only 45% at 16h. The 4N HeLa, HT29 and U-2 OS population decreased continuously to 96h and the HL60 4N dropped dramatically to only 4% by 72h. MCF7 and RPE1, despite strong 4N accumulation, showed the least loss of 4N cells, which remained high until 96h. D) HL60 showed increased sub-2N as soon as 8h and the sub-2N increased together with the 4N indicating a short mitotic arrest and close relationship between arrest and death, but, all other cell lines showed strong 4N arrest at 16h, but no significant death, indicating prolonged mitotic arrest and/or slippage before death. At 24h in drug, the HeLa sub-2N population had increased to 4.5%, while all other cell lines showed little change. At 96h, HT29 and HeLa showed 18.9 and 13.8% sub-2N, while RPE1 and MCF7 showed only 8.1 and 3.9% sub-2N, respectively. RPE1 and MCF7 sub-2N showed little or no change after 48h, indicating cell cycle arrest and no additional growth or death.

Supplemental Figure 2. Kaplan-Meier cell survival is intrinsic to the cell line and not influenced by the K5I used. A) Overall survival is plotted for EMD534085 (500nM), stlc (1.0 μM) and Kinesin-5 siRNA treated cells. All cells that arrested are included in the analysis. Despite subtle differences, the curves indicate that differential inhibition of Kinesin-5 results in the same survival response. EMD534085 n = 355, stlc n = 230, Kinesin-5 siRNA n = 130.

Supplemental Figure 3. Mitotic arrest time distributions are unique between different cell lines. A-C) All arrest times for HeLa, HT29 and MCF7 were plotted as histograms with 4h bins. HeLa and HT29 have very similar mean arrest times of 31.7 and 30.4 hours and HeLa show a broader distribution than HT29. MCF7 cells have a mean arrest of 12.0h and an asymmetrical distribution. A'-C') Arrest time distributions for only cells that undergo arrest, slippage, and death. The mean arrest times were 32.4, 30.9 and 17.2h for HeLa, HT29 and MCF7, respectively. For HT29 and HeLa note the loss of cells from earlier bins. Only MCF7 cells show an arrest time for this population that is significantly different than the mean arrest time for all cells that arrested (C); TTEST = <0.001. n = number of cells in the population.

Supplemental Figure 4. Mitotic arrest time distributions for U-2 OS, N/TERT-1 and HL60 treated with EMD534085. A) U-2 OS cells have a single population, with a tight distribution about the mean of 12.9h. A') U-2 OS that slip and die showed no significant change in their mean arrest time or distribution. B) N/TERT-1 have a single, tight distribution, with a mean arrest of only 8.5h. C) HL60 have a single, very tight

distribution, with a mean arrest of only 4.6h. Analysis of arrest, slip, die could not be completed for N/TERT-1 or HL60 as this population is exceedingly rare (6/185 cells for N/TERT-1 and 5/215 for HL60).

Supplemental Figure 5. The timing of cell death and the relationship between mitotic arrest and death differ between cell lines. A) Arrest times were compared for those cells that arrest, slip and persist, arrest, slip and die and die from mitosis. For HeLa and HT29, the death in mitosis population shows a significantly different average length of arrest (*, TTESTs <<0.001) and for MCF7 the average arrest time for arrest slip and die is significantly different than arrest, slip, don't die (*, TTEST <<0.001). There were only 9 deaths in mitosis for MCF7 so a TTEST was not performed. B) Only HeLa and HT29 showed significant death inside mitotic arrest, mostly during quartiles 1 and 2. C) The % of cells that died post-slip for all cells arrested for each quartile shows that HeLa and HT29 have no trend for arrest time and death and cells arrested for quartiles 2, 3 and 4 died at a higher frequency post-slip than for quartile 1. U-2 OS showed no trend and MCF7 showed a trend with more post-slip death occurring with longer arrest for quartiles 3 and 4. D) The % of total death (death inside mitosis + death post-slippage) as a function of the total number of cells that died shows for MCF7 >60% of the total death occurs in cells arrested for quartile 4.

Supplemental Figure 6. Distribution of slip to death times for HeLa, HT29 and MCF7 cells. A-C) The distribution of slip to death times were plotted in 4h bins for those cells that slip and die. For HeLa, ~95% died within 24h of slippage. HT29 have a wider distribution that appears bimodal and <50% of cells died within 24h of slippage. For MCF7 ~80% of the cells died within 24h of slippage. Cells were treated with 500nM EMD534085.

Supplemental Figure 7. Analysis of the timing of death and correlation to mitotic arrest time in HeLa, HT29 and MCF7 cells. A- C) Thin lines indicate mean values. Correlograms of hours arrested with hours from slip to death indicate a tight cluster for HeLa, but a horizontally spread cluster for HT29. Three separate groups appeared for MCF7, each with shorter arrest but longer slip to death times. We explored the relationship between mitotic arrest and slip to death times. For HeLa the rc = -0.31, (95%CI -0.51 to -0.10, p = 0.003). For HT29 the rc = -0.36, (95%CI -0.49 to -0.33, p<0.001). For MCF7 the rc = -0.17, (95%CI -0.25 to -0.10, p<0.001). These negative slopes indicate a trend toward longer arrest times resulting in shorter slip to death times, even though the points are not well clustered. rc = regression coefficient. CI = confidence interval. D) Box plots of slip to death times for each arrest quartile indicate that slip to death times are progressively less variable (FTEST and variance test) with longer arrest for HeLa and MCF7; HT29 do not show this relationship. For HeLa and MCF7, average slip to death times for quartile 2-4 arrestors are significantly shorter than for quartile 1 arrestors (TTESTs <0.02). For HT29, average slip to death time is significantly shorter for quartile 4 arrestors, compared to quartiles 1, 2 and 3. All TTEST values on the graph are in comparision to quartile 1. + are statistical outliers. – in boxes are medians. • are means. See supplemental Table 1 for arrest quartile times.

Supplemental Figure 8. Analysis of the timing of death and correlation to mitotic arrest time in HeLa treated with stlc or Kinesin-5 siRNA and U-2 OS cells treated with EMD534085. A-C) The distribution of slippage to death times was plotted for those cells that slip and die. For HeLa stlc, the mean slip to death time is 11.1h and 95% died within 24h of slippage. HeLa treated with Kinesin-5 siRNA have a larger mean slip to death time of 21.8h and 65% of the cells died within 24h of slippage. For U-2 OS treated with 500nM 534085, the mean is 10.8h and 100% of the cells died within 24h of slippage. A'- C') Thin lines indicate mean values. A correlogram of hours arrested with hours from slip to death indicates stlc-treated HeLa are as for EMD534085-treated cells. Kinesin-5 siRNA treated HeLa show a smaller mean arrest time but longer slip to death time than EMD534085 or stlc-treated cells and a less clustered correlation. U-2 OS have very similar arrest and slip to death times, and one symmetrical cluster. The relationship between mitotic arrest time and slip to death time was explored. For HeLa stlc, 84 cells resulted in a rc of -0.27, (95%CI -0.58 to 0.04, $p=0.09$). For HeLa Kinesin-5 siRNA, 99 cells resulted in a rc of -0.24, (95%CI -0.40 to -0.08, $p<0.004$). The U-2 OS cell cluster has a rc of 0.0007 ($n = 34$) indicating no relationship between length of arrest and slip to death time.