**Supplementary Table 1** Primers used in RT-qPCR experiments

**Supplementary Table 2** Percent changes in transcript expression of U251 tumors dosed with 1 according to *Schedule D* (n=8 per condition).

**Supplementary Figure 1. Whole transcriptome analysis of U251 cells dosed with DFO and 1.** A) Design of the RNA-sequencing experiment. U251 cells were dosed with PBS or **1** (1 M) for 48 hours, and then with either PBS vehicle, or DFO (300 M) for 16 hours. Gene expression changes for each sample were then obtained by normalizing a polyamide-treated sample with the appropriate non-treated control – cells treated with PBS and **1** were normalized to cells treated with PBS only (PBS vs **1**), and cells treated with both **1** and DFO were normalized to cells treated with DFO (DFO vs DFO+**1**). B) U251 cells were treated with **1** (1 M) for 48 hours and then treated with either DFO (300 M) or vehicle control for 16 hours. Subsequently, their mRNA was extracted and measured by RNA-sequencing. Clustering was performed comparing the differences in Log2 fold gene expression changes of cells treated with DFO (compared to non-induced state), and DFO with compounds **1** and **3** (1 M, compared to cells dosed with DFO only). C) Number of genes down, and upregulated by **1** and DFO. The Py-Im polyamide **1** inhibited upregulation of 153 DFO-induced genes (17%). D) Fewer genes were affected by compound **3**, indicating its lower potency at this dose. E) A subset of genes was selected for genes that were affected by both **DFO** and **1** (p<0.05). Among those genes, nearly all gene expression changes (96%, 2557 out of 2661) induced by DFO were reversed by **1**. The average magnitude of changes for both **1** and DFO were similar (1.7- and 1.9-fold).

# **Supplementary Figure 2. Pharmacokinetics, tissue distribution of and nuclear uptake of compounds 1-3 in- vivo.** A) GBM39 tumor bearing mice were injected I.P. with a 6.8mg/kg of C-14 radiolabeled polyamide **2**. The tissues were harvested 24-hours post-injection, weighed, dissolved and quantified by scintillation. The absolute scintillation counts are normalized to organs’ weight. B) GBM39 tumor bearing mice were injected I.P. with 6.8mg/kg of **2** (*Schedule A, intraperitoneal*) labeled with a radioactive C-14, harvested and quantified 24 hours after the last injection. C) Compound **3** was injected S.C. into interscapular area at 5 μM/kg (*Schedule A*) and tissues were harvested 24 hours after the last injection. The compound **3** showed nuclear uptake in GBM39 xenograft sections. D) Compound **3** shows nuclear staining consistent with distribution of chromatin in the nucleus. E) Uptake of **3** into the tissues showing nuclear staining. The tissues were harvested 24 hours after last injection fixed with 10% NBF, co-stained with DAPI and imaged. The left panel shows FITC channel, showing nuclear uptake of **3** and right panel shows a DAPI co-stain showing chromatin. Error bars denote 95% CI.

# **Supplementary Figure 3** Py-Im polyamide **1** single-dose escalation study of toxicity. A) The C57BL6 mice were injected with **1** subcutaneously and their weights were measured on days 3, 5, 7 and 10 (n=3 per condition). The maximum weight loss has been observed for **1** dosed at 10 mg/kg reaching an average of 3% (+/- 1.4) below the original mice weight. The weight loss for the other groups was below 1% throughout the study. Arrow denotes an injection. B) A single injection of **1** was administered at doses 1-10 mg/kg in C57BL6 mice. After 10 days, blood was drown retroorbitally, serum was cleared by centrifugation and samples submitted for analysis of serum toxicity markers. No significant changes in any of the tested markers were observed after injection of **1**. Error bars and uncertainty values denote 95% CI.

**Supplementary Figure 4 Py-Im polyamide 1 shows nuclear uptake and attenuates tumor growth in GBM39 xenografts.** A) Schedules for dosing the compound **1** used in the study. Arrows denote injections. B) Final masses and growth curve of s.c. GBM39 xenografts derived from animals subjected to *Schedule A* (n=11, per condition). C) Final masses and growth curve of s.c. GBM39 xenografts (*Schedule B*, n=6 per condition). Arrows denote injections, error bars denote 95% CI for growth curves, and minimum-maximum for final mass plots.

**Supplementary Figure 5** **Mouse weight loss during treatment with Py-Im polyamide 1.** A) Mice bearing U251 xenografts were injected with 6.8 mg/kg of **1** on the 1st, 3rd and 5thday of treatment (*Schedule A*). The weight was recorded and normalized to the initial weight for vehicle (n=7) and polyamide treated (n=8) mice. B) Analogous weight measurement or mice bearing GBM39 xenografts, treated with **1** according to schedule A (n=11, per condition). C) Weight measurements of mice with U251 tumors, treated with Py-Im polyamide **1** over 4 weeks (*Schedule B*) and of mice bearing GBM39 xenografts (D), treated with **1** according to *Schedule C*. Error bars denote 95% CI.

**Supplementary Figure 6 Polyamide 1 reduces microvessel density of GBM39**. A) GBM39 tumors treated with **1** according to *Schedule A* show 1.4-fold reduction of MVD at the endpoint (p<0.0015; n=11 per condition). B) Similarly, when treated according to *Schedule C*, the same level of reduction was observed (p<0.01; n=6 per condition). Error bars denote minimum and maximum.

**Supplementary Figure 7 In vivo Effects of treatment with 1 are consistent between GBM39 and U251 xenografts. A**) Non-necrotic region exhibited lower numbers of HIF-1a positive pixels (B) in comparison to perinecrotic region. However, we did not find increased levels of HIF-1a in treated samples, despite decreased microvessel density. C) The lack of HIF-1a induction in xenografts is unlikely to be caused by HIF-1a protein degradation as **1** did not affect HIF-1a levels in U251 cells in tissue culture. D) TUNEL staining for U251 xenograft sections, treated according to *Schedule C*, showing perinecrotic apoptosis. Positive pixel count was used for 5 fields of view (20x magnification). To account for background in some sections, only strong positive (SP) pixels were counted. ImageScope positive pixel count thresholds were tuned using positive TUNEL cells in non-necrotic regions without significant background. E) TUNEL staining in non-necrotic regions of the same tumors (6 FOV per tumor counted). F) TUNEL staining for GBM39 xenograft sections (non-necrotic due to smaller size, subjected to treatment with **1** according to *Schedule A*, n=11 for vehicle and n=8 for polyamide treated group, at least 3 FOV per tumor) shows no increase in apoptosis upon treatment with **1.** G) For both U251 and GBM39 xenografts, we found an association between presence of necrosis and distance from the nearest CD31+ microvessel closely matching distances obtained for U251 xenografts. For GBM39 tumors (n=6 per condition), 329 measurements were performed and 474 measurements were done for U251 tumors (n=10 per condition). Error bars for all panels except C) denote minimum and maximum. Error bars in the panel C) denote 95% CI.

**Supplementary Figure** **8** **HIF-1a accumulation and necrosis.** A) Nuclear density (a proxy measure for micronecroses) is lower in U251 tumors groups treated with **1**. GBM39 tumors treated with **1** according to *Schedule A* showed increased presence of areas positive in HIF-1a (B-D), which often contained necrotic center (E). Further analysis shows that necrosis occurs in regions that do not contain blood vessels (F). Numbers in boxes denote distance between edge of necrotic regions and the nearest microvessel (m). Error bars for all panels except denote minimum and maximum.