**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure S1. Nampt inhibitor effects in glioblastoma tumorsphere cells. A,** NAMPT inhibitor effect on NAD+ levels in *MYC/MYCN* amplified (left panel) and *MYC/MYCN* non-amplified GBM (right panel) tumorsphere lines. *, $P<0.05$ comparison between DMSO (24 hr) and FK866 (12.5 nM, 24hr). **, $P<0.05$ comparison between DMSO (24 hr) and GMX1778 (12.5 nM, 24 hr). **B,** Cell viability of GBM tumorsphere lines and normal human astrocytes (NHA) after treatment with FK866 (12.5nM, white bars) and GMX1778 (12.5nM, black bars) measured by ATP-based CellTiter-Glo assay at indicated time. DMSO treated cells were used as control at each time points.

**Supplementary Figure S2. Characterization of Nampt inhibitor effects in *MYC/MYCN* driven glioblastoma cells. A,** Relative NAD+ change after Nampt inhibitor treatment in *GFP*- or *MYC*-transduced U87 cells. Cells were treated with DMSO (24 hr), FK866 (25 nM, 24 hr), or GMX1778 (25 nM, 24 hr). *, $P<0.05$ for difference between DMSO versus FK866. **, $P<0.05$ for difference between DMSO versus GMX1778. **B and C,** Effects of exogenous supplementation of nicotinamide mononucleotide (NMN) (**B**) or nicotinic acid (NA) (**C**) on FK866 (12.5 nM, 72 hrs)-mediated cytotoxicity of GBM tumorspheres. *, $P<0.05$ for difference between exogenous NMN versus FK866 alone. Bars, SE.

**Supplementary Figure S3. Characterization of NAD+-synthesis pathways in glioblastoma cells. A**, Western blot analysis of NAMPT and Naprt1 expression in GBM tumorspheres. Actin, loading control. **B and C,** Western blot analysis of NAMPT and Naprt1 expression in *control* shRNA- and *MYC* shRNA-transduced MGG4 cells (B) and parental, *GFP*-, or *MYC*-transduced U87 cells (**C**). MGG18 and 23, positive control for Naprt1. Actin, loading control.

**Supplementary Figure S4. Nampt inhibitors induce apoptosis and disrupt intracellular metabolism pathways in *MYC/MYCN* amplified glioblastoma cells. A,** Activation of caspase-3/7 in MGG4 and MGG8 cells after 24 hr incubation with FK866 (12.5 nM) and GMX1778 (12.5 nM) compared to DMSO treated controls. Bars, SE; *, $P<0.05$. **B**, Western blot analysis of the AMPK/mTOR signaling pathways and cleaved PARP in MGG4 (left panels) and MGG8 cells (right panels) treated with DMSO, FK866 (12.5 nM) or GMX1778 (12.5 nM) for 48 hrs. Actin and Vinculin, loading controls. **C**, Western blot analysis of the mTOR signaling pathways and cleaved PARP in MGG85 cells (*MYC*/*MYCN* non-amplified). Cells were treated with DMSO, FK866 (12.5 nM) or GMX1778 (12.5 nM) for 96 hrs. Actin, loading controls. **D**, MGG4 (*MYC* amplified, upper panel), MGG8 (*MYCN* amplified, middle panel), and MGG85 (*MYC*/*MYCN* non-amplified) were treated with DMSO, FK866 (12.5 nM) or GMX1778 (12.5 nM) for 72 hr. Cells were stained with Ki-67 (red). DAPI (blue, nuclear staining). Scale bars, 100μm**.**

**Supplementary Figure S5. Fluorescent in situ hybridization (FISH) for *MYC* and *MYCN* in standard cancer cell lines.** Representative microscopic FISH images are shown. *MYC*, *MYCN* and centromere control (Cen) probes in green, red, or pink as indicated in each panels; Bars, 20 μm.

**Supplementary Figure S6. Effect of Nampt inhibitors in Myc-driven standard cancer cell lines. A,** CellTiter-Glo cell viability assay after 72 hr treatment of cell lines with (colored lines) and without (black lines) genetic alterations of *MYC*/*MYCN* with FK866 (left panel) and GMX1778 (right panel). **B,** Western blot analysis of c-Myc expression in *MYC* shRNA and *control* shRNA transduced H1975 cells. Vinculin, loading control. **C,** Baseline intracellular NAD+ levels of *control* shRNA and *MYC* shRNA transduced H1975 cells. **D,** Relative cell viability of

*control* shRNA and *MYC* shRNA transduced H1975 cells after treatment with FK866 (left panel), GMX1778 (right panel) or DMSO for 72 hrs. *, *P*<0.05.

**Supplementary Figure S7. *In vivo* efficacy of Nampt inhibitor in *MYC* amplified H1975 xenografts. A,** Effect of GMX1778 on growth of established subcutaneous H1975 xenograft tumors. Plot depicts tumor volume changes over time, with values being normalized to tumor volumes at Day 0 when treatment began. Each point represents the average of tumor volumes in a group treated with GMX1778 (250mg/kg, red line, n=7) or vehicle (blue line, n=6) by oral gavage once a week. *, *P*<0.05 comparison between GMX1778 and vehicle. **B, C,** Image (B) and weights (C) of excised subcutaneous H1975 tumors in vehicle- and GMX1778-treated mice at the end of the study shown in **A** (Day 16). Scale bar, 10 mm. *, *P*<0.05. **D,** Hematoxylin and eosin staining (upper panels) and TUNEL immunohistochemistry (lower panels) of vehicle- (left panels) and GMX1778 (right panels)-treated H1975 subcutaneous tumors. Scale bars, 100 μm. Insets show magnification of representative fields. **E,** NAD+ quantitation in vehicle- and GMX1778-treated H1975 tumors. Bars, SE; *, *P*<0.05.

**Supplementary Figure S8. NAMPT inhibitors were well tolerated in SCID mice bearing flank or orthotopic xenografts. A,** Body weights of animals bearing MGG8 orthotopic xenografts were measured twice a week during treatment with GMX1778 (250mg/kg, red line, n=7) or vehicle (blue line, n=7) by oral gavage once a week. Arrows indicate the time points when treatment doses were given. **B**, Body weights of animals bearing H1975 subcutaneous flank xenografts were measured three times a week during treatment with GMX1778 (250mg/kg, red line, n=7) or vehicle (blue line, n=6) by oral gavage once a week. Arrowheads indicate the time points when treatment doses were given.

**SUPPLEMENTARY METHODS:**

***$^{13}$C Metabolic flux analysis and measurement of glycolytic intermediates***

Intracellular metabolites were then extracted by the method as follows: The culture medium was carefully removed by aspiration. Cells were washed twice by 5% mannitol solution (10 mL first and then 2 mL) and treated with 800 μL of methanol and left at rest for 30 sec to inactivate enzymes. Next, the cell extract was treated with 550 μL of Milli-Q water containing internal standards (H3304-1002, Human Metabolome Technologies, Inc., Tsuruoka, Japan) and left at rest for another 30 sec. The extract was centrifuged at 2,300×*g* and 4ºC for 5 min and then 900 μL of upper aqueous layer was centrifugally filtered through a Millipore 5-kDa cutoff filter at 9,100×*g* and 4ºC for 240 min to remove proteins. The filtrate was centrifugally concentrated and re-suspended in 50 μL of Milli-Q water.

Metabolite levels were measured by capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) by Human Metabolome Technology Inc. (Tsuruoka, Japan) using an Agilent CE Capillary Electrophoresis System equipped with an Agilent 6210 Time of Flight mass spectrometer, Agilent 1100 isocratic HPLC pump, Agilent G1603A CE-MS adapter kit, and Agilent G1607A CE-ESI-MS sprayer kit (Agilent Technologies, Waldbronn, Germany). The systems were controlled by Agilent G2201AA ChemStation software version B.03.01 for CE (Agilent Technologies, Waldbronn, Germany). The metabolites were analyzed by using a fused silica capillary (50 μm i.d. × 80 cm total length), with commercial electrophoresis buffer (Solution ID: H3301-1001 for cation analysis and H3302-1021 for anion analysis, Human Metabolome Technologies) as the electrolyte. The sample was injected at a pressure of 50 mbar for 10 sec (approximately 10 nL) in cation analysis and 25 sec (approximately 25 nL) in anion analysis. The spectrometer was scanned from m/z 50 to 1,000. Other conditions were as described previously (1-3). Peaks were extracted using automatic integration software MasterHands (Keio

University, Tsuruoka, Japan) in order to obtain peak information including m/z, migration time for CE-TOFMS measurement (MT) and peak area (4). Signal peaks corresponding to adduct ions and other product ions of known metabolites were excluded, and remaining peaks were annotated with putative metabolites from the HMT metabolite database based on their MTs/RTs and m/z values determined by TOFMS. The tolerance range for the peak annotation was configured at ±0.5 min for MT and ±10 ppm for m/z. In addition, peak areas were normalized against those of the internal standards and then the resultant relative area values were further normalized by sample amount.

***Qualitative NAD+ Assessment***

To evaluate qualitative values of NAD+, the NAD/NADH-Glo$^{TM}$ Assay (Promega) was used according to the manufacturer's recommendations. $1x10^5$ cells in 400 µl of PBS were lysed by adding 400 µl of 0.2N NaOH with 1% dodecyltrimethylammonium bromide (Sigma-Aldrich). To measure NAD+, equal volumes of 0.4N HCl were added to lysed cell samples (400 µl), followed by heating at 60C for 15 min. After 10 min incubation at room temperature, 0.5M Trizma base buffer (200 µl) was added. Finally, samples were seeded into 96 well plates and incubated with NAD/NADH-Glo detection reagent for 30 min. Data were compared with DMSO treated cells and expressed as % control.

***IDH1/2 genotyping and MGMT promotor methylation analysis***

Genomic PCR-based sequencing was used to sequence all coding exons of the *IDH1* and *IDH2* genes. PCR products were amplified from genomic DNA templates with Platinum Taq polymerase per manufacturer's protocol using intron-based primers spanning the expressed coding sequences and subsequently Sanger sequenced (Beckman Coulter Genomics). To assess *MGMT* promotor methylation status, methylation-Specific PCR was used. Genomic and bisulfite-modified DNA was extracted using DNeasy

Blood & Tissue and EpiTect Bisulfite kits (Qiagen). Methylation-specific PCR was performed in a two-step approach as described (5).

**SUPPLEMENTARY REFERENCES:**


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