**Supplemental Materials and Methods**

I**mmunohistochemical (IHC) analysis of NAPRT expression in primary ovarian cancer**

All the surgical specimens were routinely fixed, processed, embedded in paraffin and stored. All of the histological slides were reviewed by a pathologist expert in gynecologic pathology. For tissue micro-array (TMA) preparation, a total of five, 4 mm-wide tissue cores were obtained in each case from the ovarian mass. The tumor cores were obtained from three different paraffin blocks that were considered to be representative of the whole carcinoma. Areas of necrosis or with poor fixation were avoided. As a recipient block we used 6x5 Matrices of Tissue-tek ® Quick-Ray tm  (Sakura Finetek USA, Inc.) according to the manufacturer’s instructions. Both TMA blocks were cut with microtome to obtain 3 µm-thick histological slides, which were mounted on positively charged Superfrost Plus® Slides and submitted for immunohistochemistry (IHC). For IHC NAPRT detection we used an automatic immunostainer, Benchmark XT (Ventana Medical Systems SA, Strasbourg, France). TMA slides were probed with an anti-NAPRT antibody [CL0665, ab211529 (from Abcam)] at a 1:100 dilution. Antigen-detection was done by incubation with citrate buffer (pH 6) at 90°C for 30 minutes, subsequent incubation in primary antibody for 1 h at 37°C followed by the addition of the polymeric detection system Ventana Medical System Ultraview Universal DAB Detection Kit. Slides were counterstained with modified Gill’s hematoxylin and mounted in Eukitt. As a positive control we used a 2% agar pre-embedded OVCAR 5 cell culture block, which was routinely fixed, processed, embedded in paraffin and cut at the microtome. For each of the tumor cores stained for NAPRT we semiquantitatively evaluated both the intensity and the pattern of the staining. Specifically, we evaluated the intensity of the staining as follow: 0: no stain; 1+: weak stain; 2+ moderate stain; 3+: strong stain. We calculated the relative percentage of each staining intensity class (%) evaluating at least 300 cells for each tumor core. For each core we calculated NAPRT Histological Score (H-score) using the following formula Hscore= 3\*(%3+)+2\*(%2+)+1\*(%1+), thus obtaining a continuous variable, ranging from 0 (no staining) to 300 (corresponding to a condition in which all the tumor cells strongly stain for NAPRT).

**Cloning of NAPRT-EGFP and of wild type NAPRT**

Total cDNA from OVCAR-5 cell line was obtained by standard procedures. NAPRT coding sequence was amplified by PCR using the following primers: Forward, AATTGAATTCATGGCGGCGGAGCAGGAC, reverse, AATTACCGGTACGGGGGACTGCCCCGCAC, containing the EcoRI and AgeI restriction sites, respectively. The PCR product was cloned into the pCMV-EGFP vector upstream the EGFP coding sequence by standard procedures. pCMV-NAPRT-EGFP vector was amplified and purified from *E. coli* DH5α. NAPRT-EGFP coding sequence was amplified from pCMV-NAPRT-EGFP by PCR using the following primers: Forward, AATTGAATTCATGGCGGCGGAGCAGGAC, reverse, AATTGTCGACTTACTTGTACAGCTCGTCCA, containing the EcoRI and SalI restriction sites, respectively. The PCR product was cloned into the pBABEpuro plasmid (pBP, from Addgene, Cambrige, MA, USA) vector by standard procedures to generate a pBP-NAPRT-EGFP plasmid. To generate a wild type pBP-NAPRT, NAPRT coding sequence was amplified by PCR from the pBP-NAPRT-EGFP plasmid using the following primers: Forward, AATTGAATTCATGGCGGCGGAGCAGGAC, reverse, AATTGTCGACTCAGGGGGACTGCCCCGCAC, containing the EcoRI and SalI restriction sites, respectively. The PCR product was cloned into the pBp plasmid vector by standard procedures to generate a pBP-NAPRT plasmid. Transduction of MIA PaCa2, OVCAR-5 and Capan-1 cells was done as described elsewhere ([1](#_ENREF_1)).

**Spectrophotometric enzymes assay, oxygen consumption measures and bioluminescent luciferase ATP assay**

Glycolytic enzymes were assayed at room temperature on cellular homogenate, as described in ([2](#_ENREF_2)). ATP and AMP intracellular concentration was evaluated spectrophotometrically as described elsewhere ([2](#_ENREF_2)). O2 consumption was measured as described previously ([2](#_ENREF_2), [3](#_ENREF_3)). The activity the Fo-F1 ATP synthase was determined as described in ([2](#_ENREF_2), [4](#_ENREF_4)). The oxidative phosphorylation efficiency (P/O ratio) was calculated as the ratio between the concentration of the produced ATP and the amount of consumed oxygen. When the oxygen consumption is completely devoted to energy production, the P/O ratio should be approximately 2.5 and 1.5 after pyruvate + malate or succinate addition, respectively.

**2-hydroxynicotinate sodium salt (2-HNANa) preparation**

Equimolar quantities of 2-HNA and sodium hydroxide were incubated overnight with gentle swirling in a glass tube with an excess of acetone at room temperature. The day after, the mixture was lyophilized and washed two times with diethyl ether to remove any trace of acetone that could compromise water solubility.

**Animal experiments**

In the subcutaneous xenografts models (OVCAR-5, OVCAR-8, Capan-1 and MIA PaCa2 cells), tumor size was measured with a caliper twice a week and tumor volume was calculated using the formula: tumor volume (mm3) = (w2 × W) x π/6, where w (minor side) and W (the major side) are in mm. At the end of treatment, mice were sacrificed and tumor masses were excised, photographed and weighted.

In the model generated by intraperitoneal (i.p.) injection of OVCAR-5 cells, six- to eight-week-old BALB/c athymic mice (nu+/nu+) were injected i.p. with 4×106 OVCAR-5 cells engineered with either the scr-shRNA or with the NAPRT-sh2, or with 4×106 plain OVCAR-5. Three days after tumor inoculation, mice were randomly assigned to be treated with FK866 (20 mg/kg i.p. twice a day, for four consecutive days/week) with or without 2-HNANa (200 mg/kg i.p. once a day, for four consecutive days/week) or with vehicle DMSO for a total of three weeks. Mice were sacrificed at the appearance of neoplastic ascites, jaundice, or in the presence of any other sign mandating euthanasia.

Adequate study power (>0.80) of the animal experiments was verified by PS Power and Sample Size Program (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize).

**Supplementary References**

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