Supporting Information.

Modulation of N-Methyl-N-Nitrosourea Mutagenesis in Mouse Embryo Fibroblasts Derived from the *gpt* Delta Mouse by an Inhibitor of the O^6-Methylguanine Methyltransferase, MGMT

Pennapa Thongararm,[†‡] Bogdan I. Fedeles,[†] Sakunchai Khumsubdee,[†‡]
Amanda L. Armijo,[†] Lina Kim,[†] Apinya Thiantanawat,[‡] Jeerawan Promvijit,[‡] Panida Navasumrit,[‡] Mathuros Ruchirawat,[‡] Robert G. Croy,[†] and John M. Essigmann[†*]

[†] Departments of Biological Engineering and Chemistry, and Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

[‡] Chulabhorn Graduate Institute, Bangkok 10210, Thailand

[*] Address correspondence to J.M. Essigmann at jessig@mit.edu

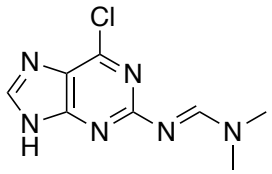
Contents:

1. Synthesis of 2-Amino-6-Methoxy-d_3-9H-Purine.

1.a. General procedures: All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions unless otherwise stated. All glassware was dried in an oven at 110 °C for minimum 6 h prior to use. Dry solvents were obtained by passing the previously degassed solvents through activated alumina columns. Reagents were purchased at a high commercial quality (typically 97% or higher) and used without further purification, unless otherwise stated. High field NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Chemical shifts of ^{1}H and ^{13}C spectra were referenced to the NMR solvents.

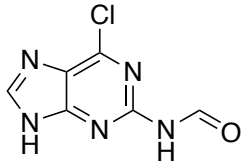
1.b. Procedure for synthesis of 2-amino-6-methoxy-9H-purine.

1.b.i. Procedure for the synthesis of (*E*)-*N*'-(6-chloro-9H-purin-2-yl)-*N*,*N*-dimethylformimidamide.[1]



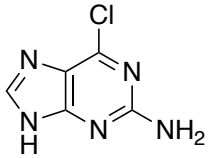
Guanine (6 g, 39.7 mmol) was dissolved in 1,2-dichloroethane (50 mL) and DMF (17.9 mL). $POCl_3$ (10 mL) was added dropwise to the solution and the resulting mixture was heated to 80 °C and stirred for 6 h. Upon the completion, organic solvents were evaporated under reduced pressure and the reaction mixture was poured into 150 mL of ice-cold water. $NaHCO_3$ was added portion-wise to the mixture to neutralize to pH 7, and then the aqueous solution was stirred for another 30 min. The solid was allowed to precipitate and filtered. The solid was washed with an excess amount of water to yield a pale yellow solid as the pure product 5.4 g (61%). All of the characterization data were compared with and consistent with literature reports.

1.b.ii. Procedure for the synthesis of N-(6-chloro-9H-purin-2-yl) formamide.[1]



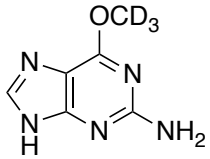
(*E*)-*N*'-(6-chloro-9H-purin-2-yl)-*N*,*N*-dimethylformimidamide (5.4 g, 24 mmol) was dissolved in 12% (v/v) AcOH and the mixture was stirred at 70 °C for 4 h. Upon the completion, the resulting precipitate was filtered and washed with an excess amount of water. The solid was dried in a vacuum oven at 60 °C to give a yellow solid as a product with 3.4 g yield (72%). All characterization data were compared with literature reports.

1.b.iv. Procedure for the synthesis of 2-amino-6-chloro-9H-purine.[1]



N-(6-Chloro-9H-purin-2-yl) formamide (3.4 g, 17 mmol) was dissolved in 10% (w/w) aqueous NaOH solution and stirred at room temperature for 3 h. The mixture was neutralized with conc. HCl (36%) and the resulting solid was filtered and dried in a vacuum oven at 60 °C to yield a pale yellow solid as a pure product. Yield: 2.2 g, (75%). All the characterization data were compared with literature reports.

1.b.v. Procedure for the synthesis of 2-amino-6-methoxy-d_3-9H-purine.[2]



To a solution of anhydrous MeOH-d_4 (10 mL) under argon, freshly cut sodium wire (1 g, 44 mmol) was added portion-wise. The mixture was stirred until the sodium wire was fully dissolved

and 2-amino-6-chloro-9H-purine (1 g, 5.8 mmol) was added. The mixture was stirred and refluxed for 3 days. Upon the completion, the mixture was cooled to room temperature and the solid precipitate was filtered. The filtrate was neutralized with glacial acetic acid and evaporated under reduce pressure until dry. The resulting solid was recrystallized from water to yield 2-amino-6-methoxy-d_3-9H-purine as a white solid (0.8 g, 4.9 mmol) in 85 % yield. (This compound could be purified by Prep HPLC using 5% acetonitrile/H_2O with 0.1% TFA). All the characterization data were compared with literature reports.

Literature cited for synthesis section:

(1) Reddy, P. L., Khan, S. I., Ponnan, P., Tripathi, M., and Rawat, D. S. (2017) Design, synthesis and evaluation of 4-aminoquinoline-purine hybrids as potential antiplasmodial agents. *European Journal of Medicinal Chemistry 126*, 675-686.

(2) Hulpia, F., Balzarini, J., Schols, D., Andrei, G., Snoeck, R., and Van Calenbergh, S. (2016) Exploring the purine core of 3'-C-ethynyladenosine (EAdo) in search of novel nucleoside therapeutics. *Bioorg Med Chem Lett 26*, 1970-1972.

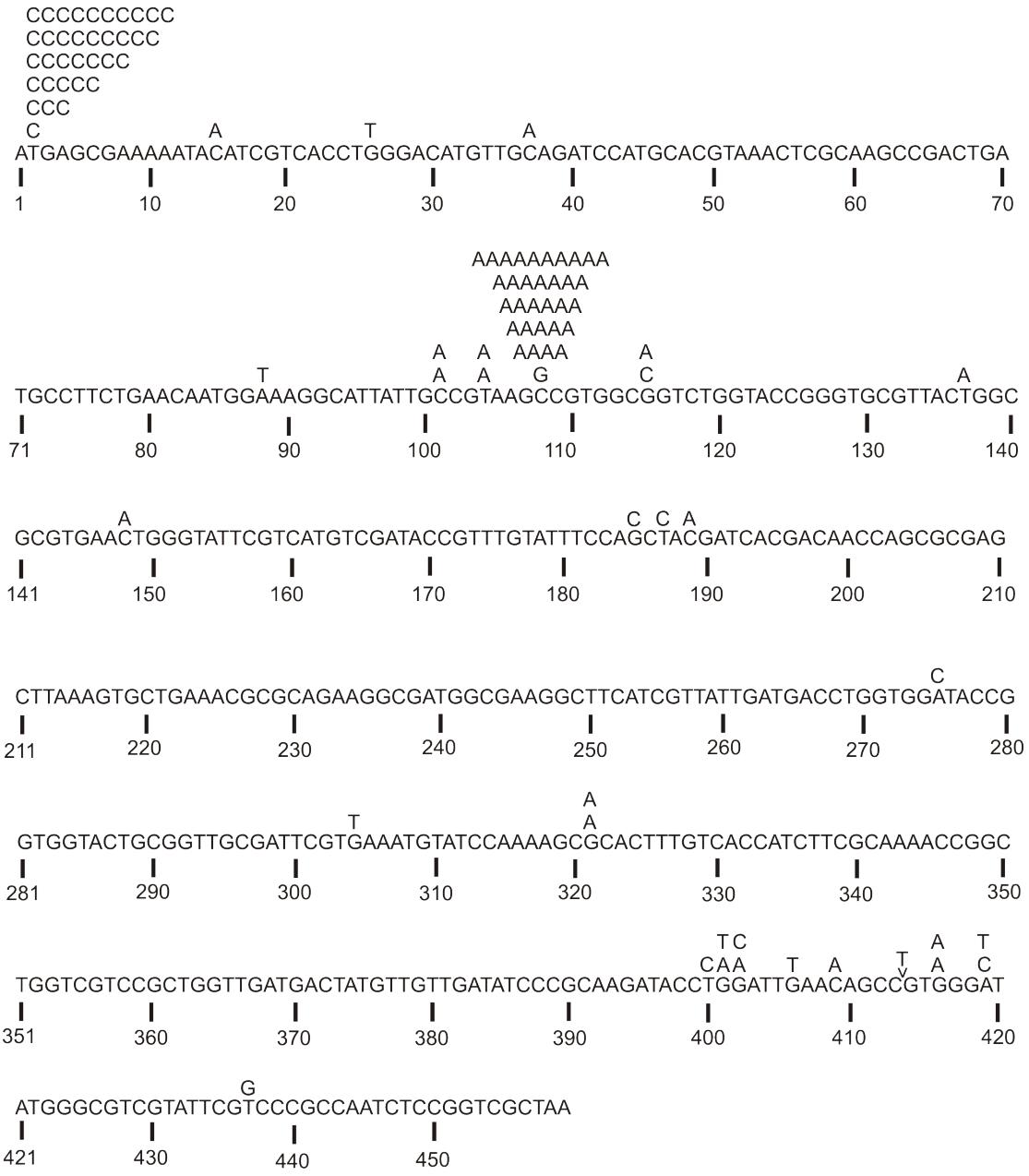


Figure S1. Control mutational spectrum (MNU-0) in the 459 bp *gpt* gene pf MEFs derived from the *gpt* delta C57BL/6J mouse. Only the transcribed strand is portrayed. See text for details.

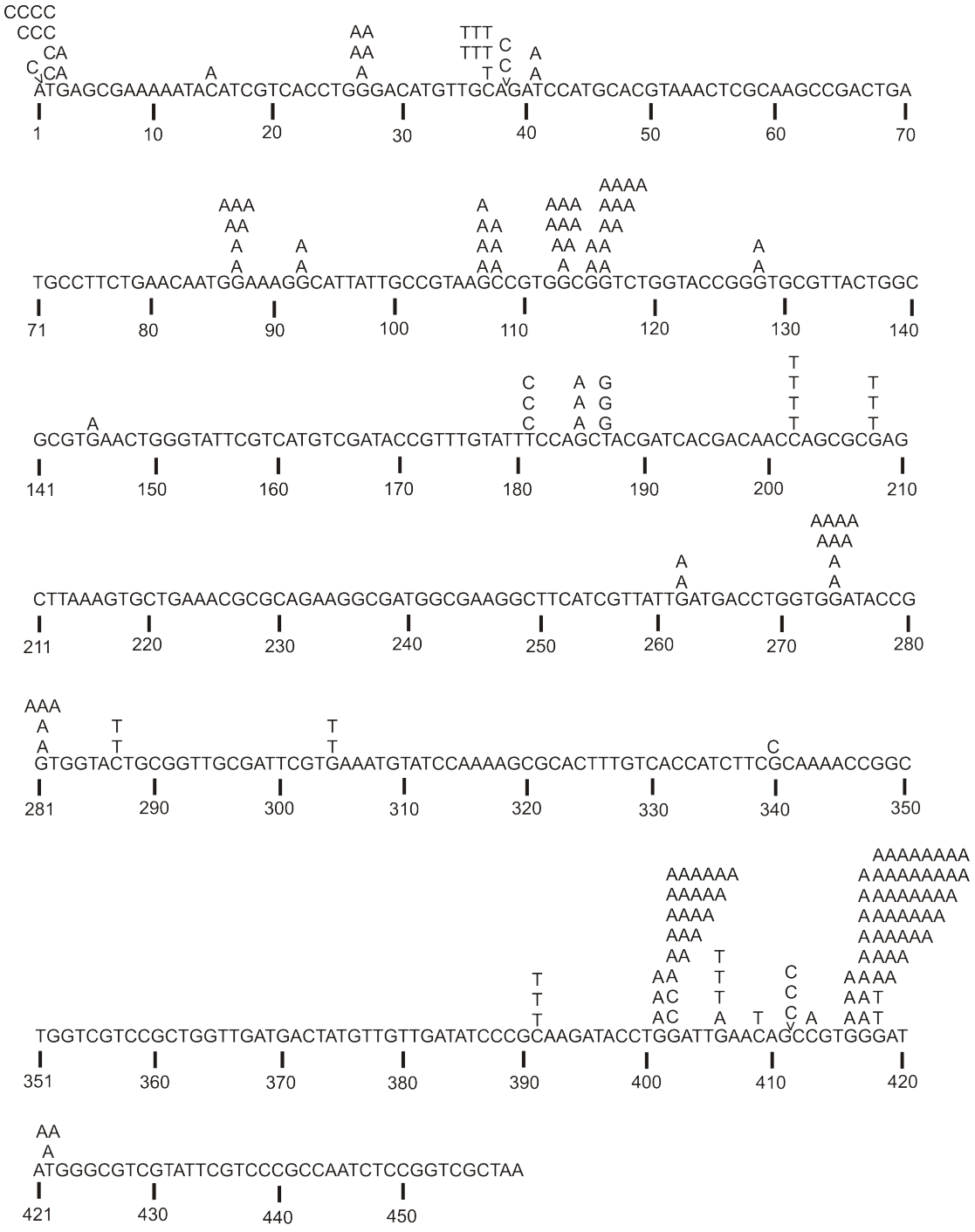


Figure S2. Mutational spectrum of MNU at a dose of 500 μM (MNU-500) in the 459 bp *gpt* gene of MEFs derived from the *gpt* delta C57BL/6J mouse. Only the transcribed strand is portrayed. A "V" in the sequence indicates the presence of an insertion mutation. See text for details.

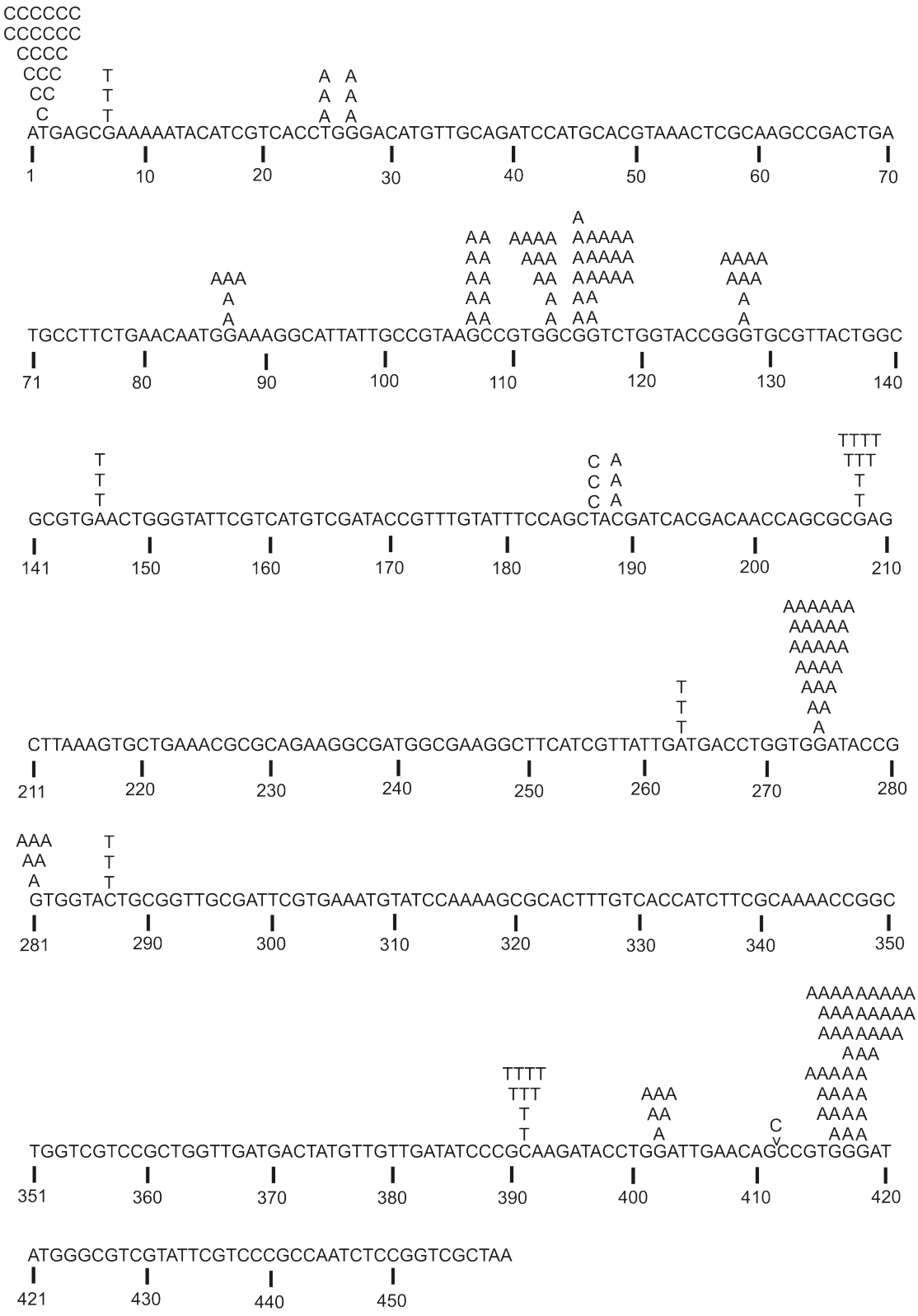


Figure S3. Mutational spectrum of MNU at a dose of 500 μM (MNU-500AA; these cells were co-treated with the MGMT inhibitor, AA-CW236) in the 459 bp *gpt* gene of MEFs derived from

the *gpt* delta C57BL/6J mouse. Only the transcribed strand is portrayed. A "V" in the sequence indicates the presence of an insertion mutation. See text for details.

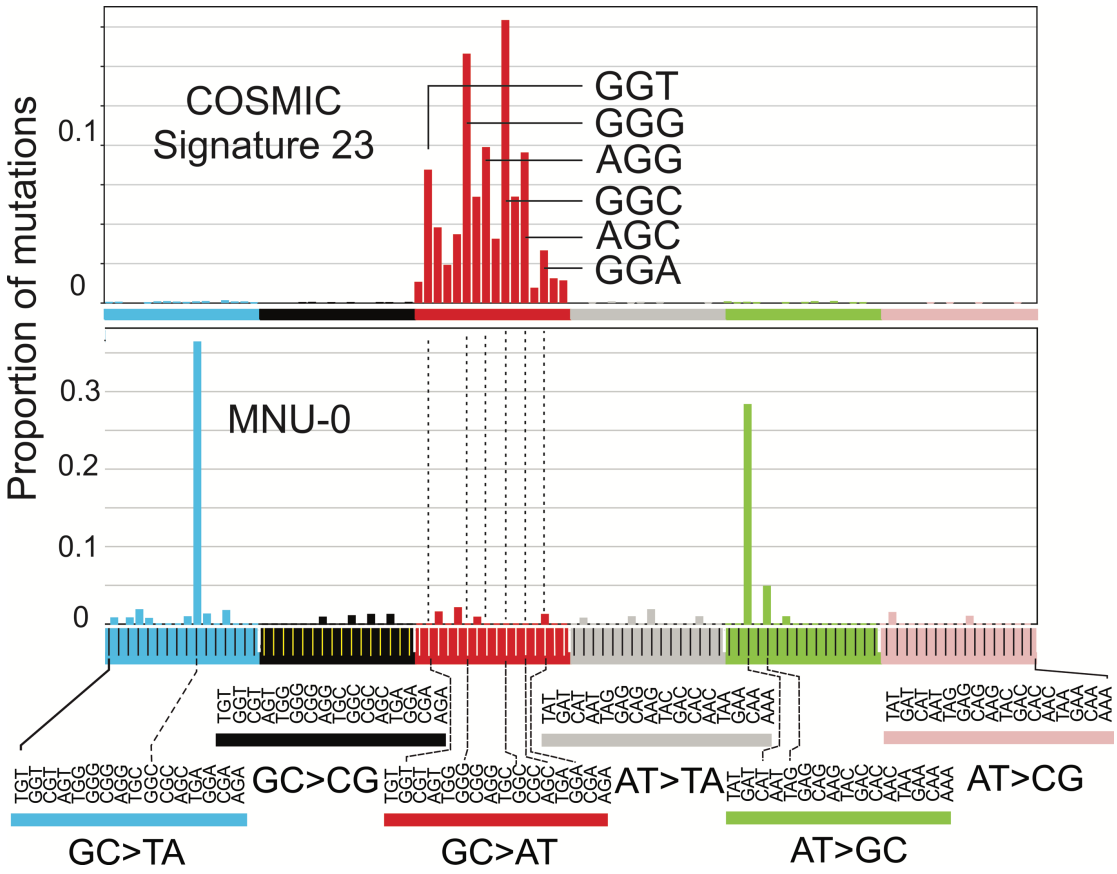


Figure S4. Top: COSMIC mutational signature 23, transformed to reflect equal trinucleotide probabilities (see Experimental Procedures). Bottom: Mutational spectrum of MNU-0. In both cases, mutation distributions were determined in all 96 possible 3-base contexts (3'-NXN-5', where X is the position of the mutation and N and N are the 5' and 3' flanking bases).

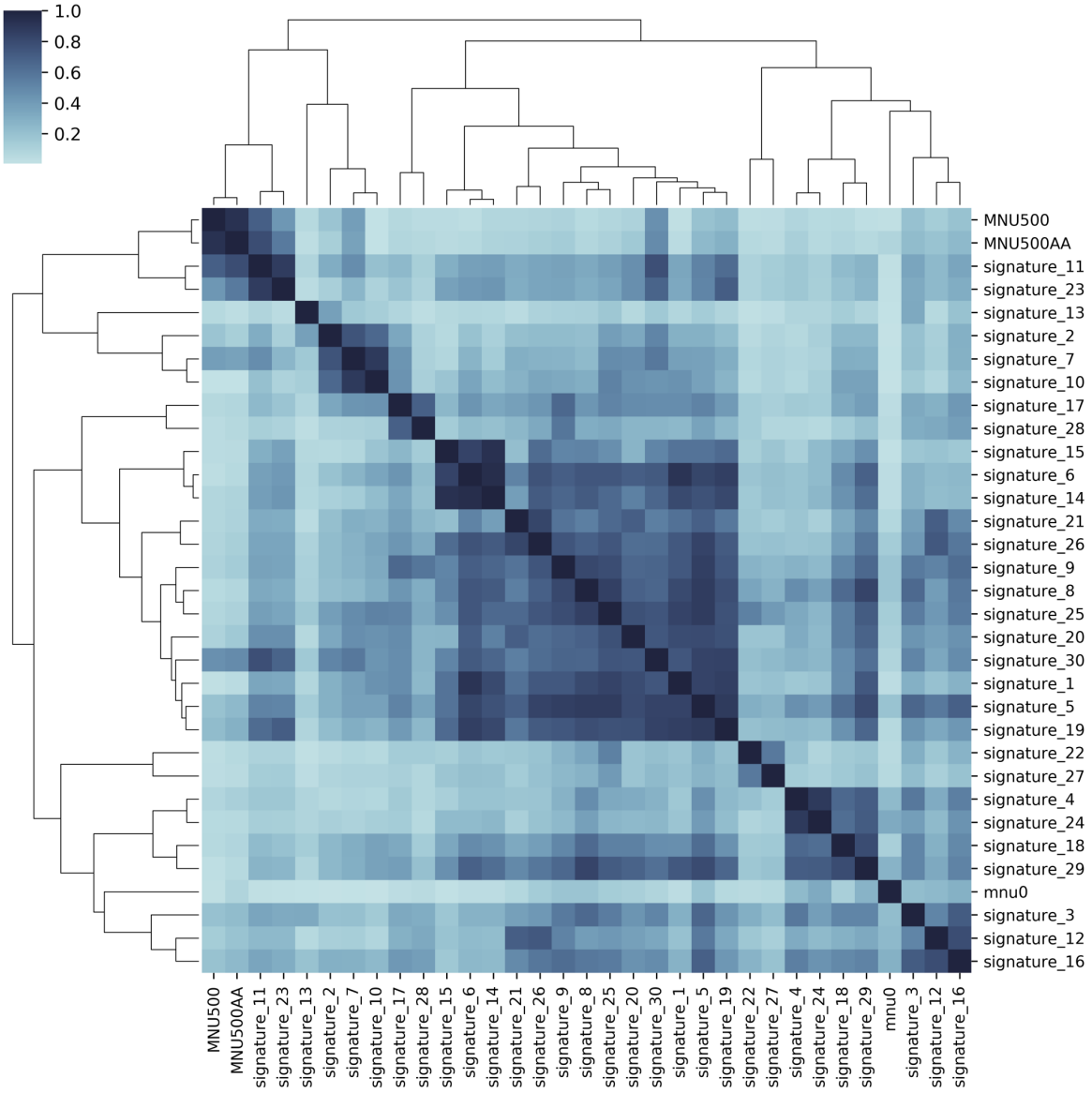


Figure S5. Cosine similarity matrix comparing MNU-treated MEFs with COSMIC mutational signatures derived from human cancers**.** This heat map compares the cosine similarities of MNU at 0 μM (MNU-0; the control), MNU at 500 μM (MNU-500) and MNU+AA-CW236 at 500 μM (MNU-500AA) with the mutational signatures of a range of human cancers (COSMIC mutational signatures v2). The darkness of the shading in each box reflects the cosine similarity of the spectra (or signatures); the darker the shading, the more similar the patterns.. As indicated in the text, mutantional signatures data were obtained from the COSMIC database, and then transformed

(as described in Experimental Procedures) to reflect equal trinucleotide frequency, before the dendrogram and heatmap construction. The mutational background represented in MNU-0 was subtracted from the raw data of MNU-500 and MNU-500AA, to yield the data shown.