

**Supplementary Information**  
**Histidine catabolism is a major determinant of methotrexate sensitivity**

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### **Source Data\_1**

Sheet “barcoded cell lines” contains the list of genomically-barcoded cell lines alongside with their individual barcodes, presented in Fig. 1a and Extended Data Fig. 1a-c.

Sheet “Counts\_sequencing” contains the sequencing raw data of the results presented in Extended Data Fig. 1b.

Sheet “CRISPR score” contains the CRISPR scores for all genes that passed quality control in the CRISPR/Cas9-based screen presented in Fig. 1b, Extended Data Fig. 1d,e.

Sheet “Screen rpm” contains reads per million (rpm) per sgRNA used in the CRISPR/Cas9-based screen presented in Fig. 1b, Extended Data Fig. 1d,e.

Sheet “Survival MTX” contains the raw data of the survival assays that generated the EC90 presented in Fig. 1f.

### **Source Data\_2**

This file contains the source data for Fig. 2 and Extended Data Fig. 2, 3

### **Source Data\_3**

Sheet “Survival MTX” contains the raw data of the survival assays that generated the EC90 presented in Fig. 3a.

Sheet “metabolite profiling” contains the source data for Fig. 3b-d and Extended Data Fig. 4 c-f.

Sheet “cell lines survival” contains the raw data for the survival assays that generated the EC90 presented in Fig. 3g.

Sheet “HAL KO survival” contains the raw data for the survival assays that generated the EC90 presented in Fig. 3h.

Sheet “ALL patients contains the raw data for Fig. 3i and Extended Data Fig. 5 b-d.

### **Source Data\_4**

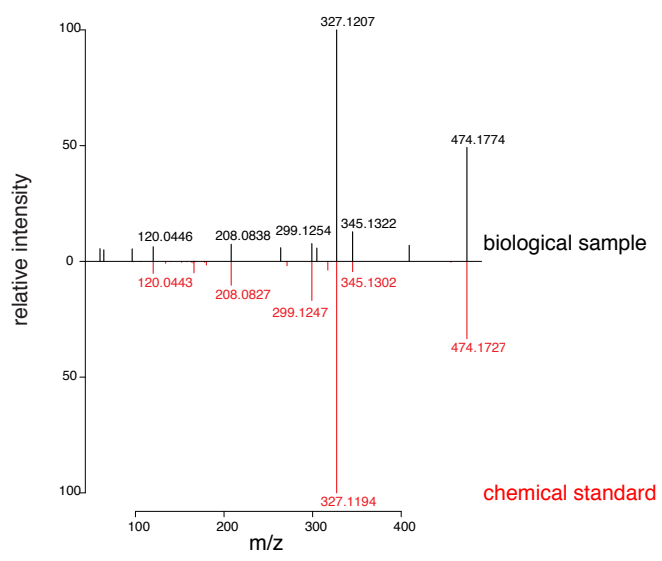
This file contains the source data for Fig. 4 and Extended Data Fig. 8-9

### **Source Data\_5**

This file contains the source data for Extended Data Fig. 10.

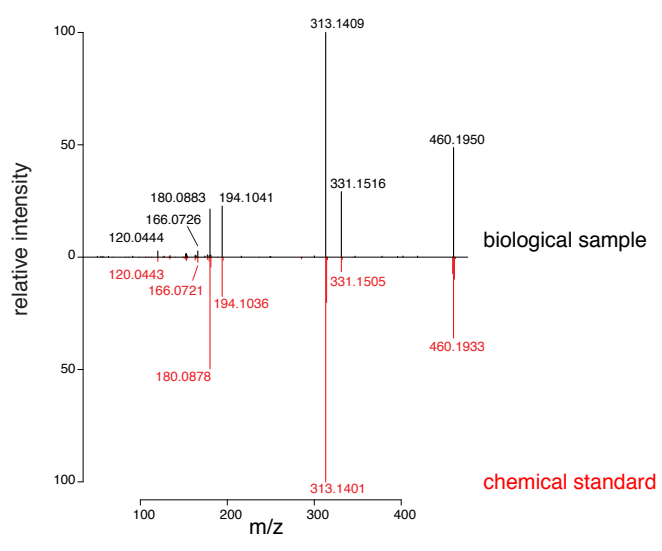
# Supplementary Figure 1

## 5-formyl THF



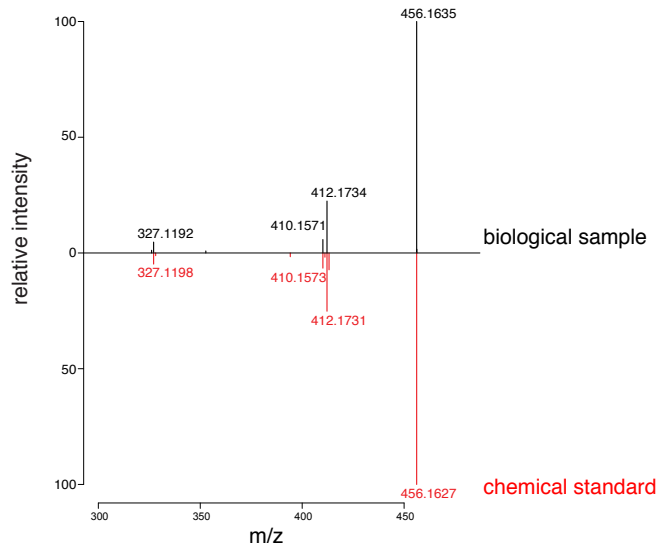
theoretical <i>m/z</i>	proposed chemical formula	proposed structure
120.0444	C <sub>7</sub> H <sub>6</sub> NO <sup>+</sup>	
208.0829	C <sub>8</sub> H <sub>10</sub> N <sub>5</sub> O <sub>2</sub> <sup>+</sup>	
299.1251	C <sub>14</sub> H <sub>15</sub> N <sub>6</sub> O <sub>2</sub> <sup>+</sup>	
327.1200	C <sub>15</sub> H <sub>15</sub> N <sub>6</sub> O <sub>3</sub> <sup>+</sup>	
345.1306	C <sub>15</sub> H <sub>17</sub> N <sub>6</sub> O <sub>4</sub> <sup>+</sup>	
474.1732 (parental)	C <sub>20</sub> H <sub>24</sub> N <sub>7</sub> O <sub>7</sub> <sup>+</sup>	

## 5-methyl THF



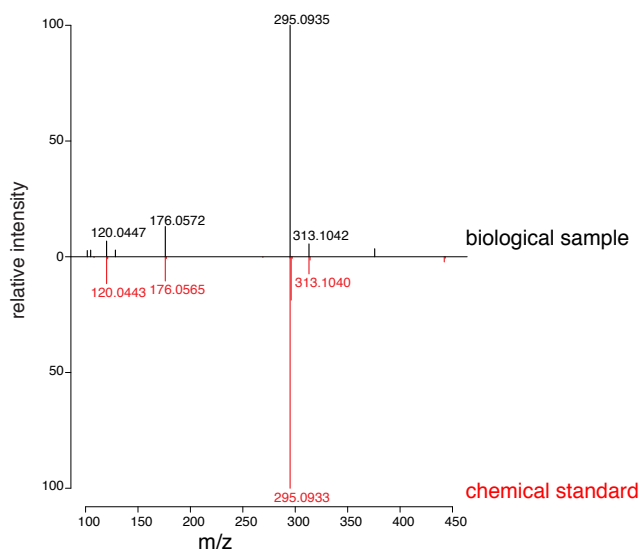
theoretical <i>m/z</i>	proposed chemical formula	proposed structure
120.0444	C <sub>7</sub> H <sub>6</sub> NO <sup>+</sup>	
166.0723	C <sub>8</sub> H <sub>8</sub> N <sub>5</sub> O <sup>+</sup>	
180.0880	C <sub>7</sub> H <sub>10</sub> N <sub>5</sub> O <sup>+</sup>	
194.1036	C <sub>8</sub> H <sub>12</sub> N <sub>5</sub> O <sup>+</sup>	
313.1408	C <sub>15</sub> H <sub>17</sub> N <sub>6</sub> O <sub>2</sub> <sup>+</sup>	
331.1513	C <sub>15</sub> H <sub>19</sub> N <sub>6</sub> O <sub>3</sub> <sup>+</sup>	
460.1939 (parental)	C <sub>20</sub> H <sub>26</sub> N <sub>7</sub> O <sub>6</sub> <sup>+</sup>	

## 5,10-methenyl THF



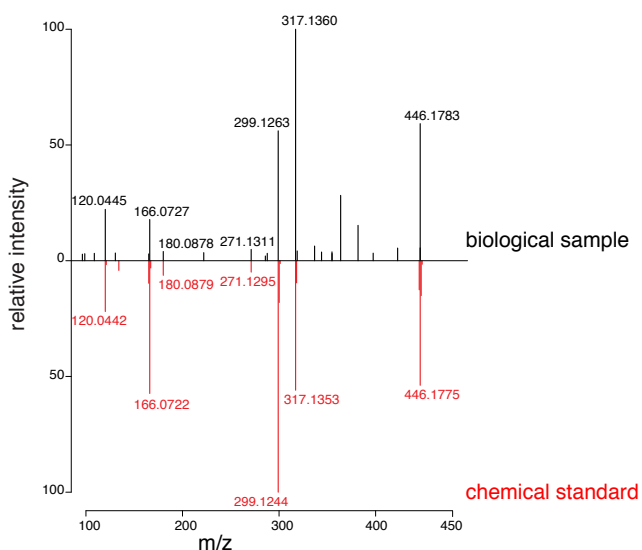
theoretical <i>m/z</i>	proposed chemical formula	proposed structure
327.1200	C <sub>15</sub> H <sub>15</sub> N <sub>6</sub> O <sub>3</sub> <sup>+</sup>	
412.1728	C <sub>19</sub> H <sub>22</sub> N <sub>7</sub> O <sub>4</sub> <sup>+</sup>	
456.1626 (parental)	C <sub>20</sub> H <sub>22</sub> N <sub>7</sub> O <sub>6</sub> <sup>+</sup>	

## folate



theoretical $m/z$	proposed chemical formula	proposed structure
120.0444	$C_7H_8NO^+$	
176.0567	$C_7H_8N_5O^+$	
295.0938	$C_{14}H_{11}N_6O_2^+$	
313.1044	$C_{14}H_{13}N_6O_3^+$	
442.1470 (parental)	$C_{19}H_{20}N_7O_6^+$	

## THF



theoretical $m/z$	proposed chemical formula	proposed structure
120.0444	$C_7H_8NO^+$	
166.0723	$C_6H_8N_5O^+$	
180.0880	$C_7H_{10}N_5O^+$	
271.1302	$C_{13}H_{15}N_6O^+$	
299.1251	$C_{14}H_{15}N_6O_2^+$	
317.1357	$C_{14}H_{17}N_6O_3^+$	
446.1783 (parental)	$C_{19}H_{24}N_7O_6^+$	

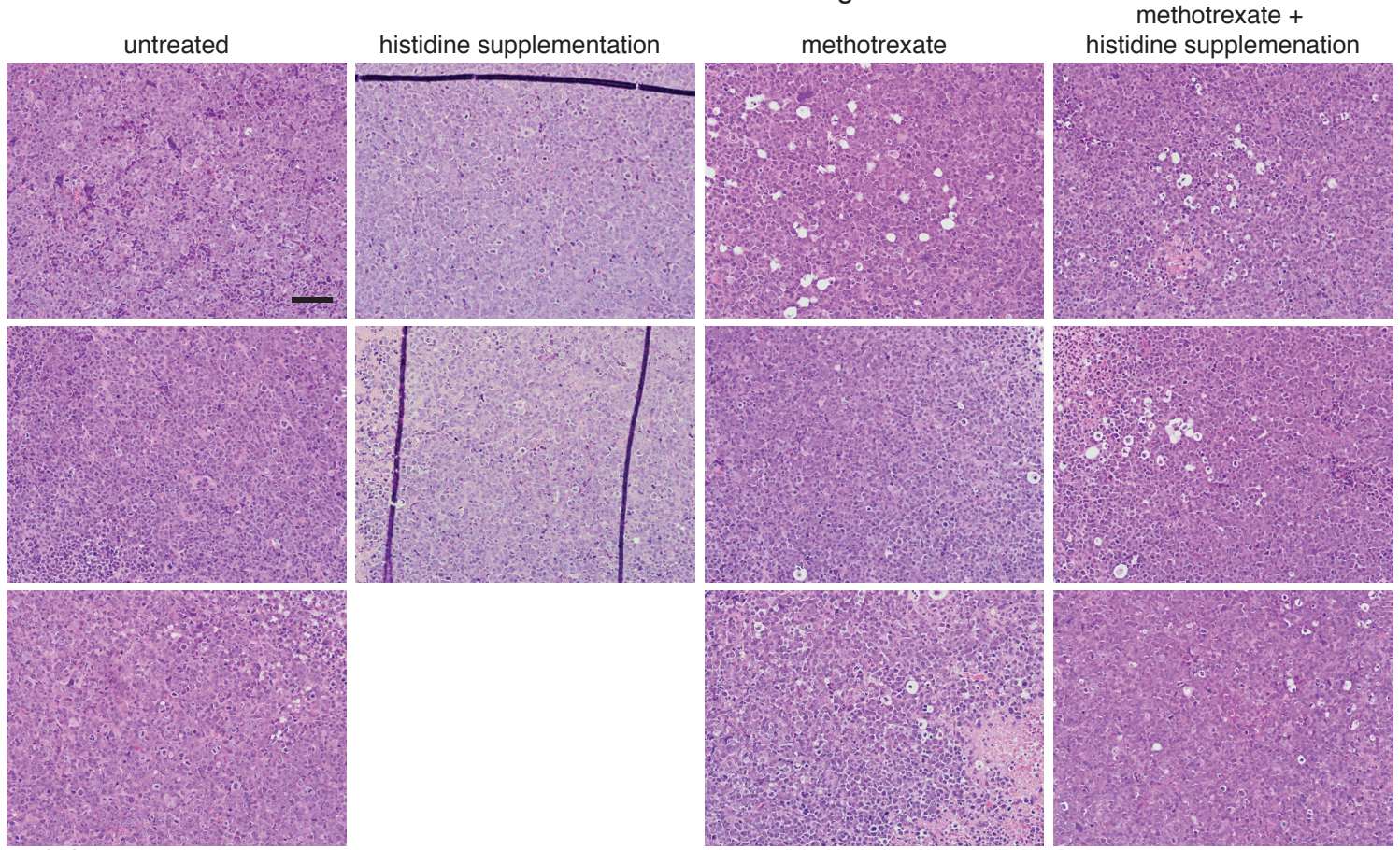
### Supplementary Figure 1.

Mirror plots showing experimental MS2 spectra from a synthetic chemical standard for each folate species described in this paper (red), aligned with the corresponding folate species from a biological sample (black). The adjacent table contains the proposed structure, proposed chemical formula, and corresponding theoretical  $m/z$  for each of the major fragments annotated in the mirror plot. Mirror plots were created using R and structures were identified based on the known parental structure (HMDB [www.hmdb.ca](http://www.hmdb.ca)), using a combination of ChemDraw (Perkin Elmer, Waltham, MA), CFM:ID<sup>35</sup> ([cfmid.wishartlab.com](http://cfmid.wishartlab.com)), METLIN ([www.metlin.scripps.edu](http://www.metlin.scripps.edu)) and Sirius<sup>36,37</sup> (version 3.5.1; University of Jena, Germany).

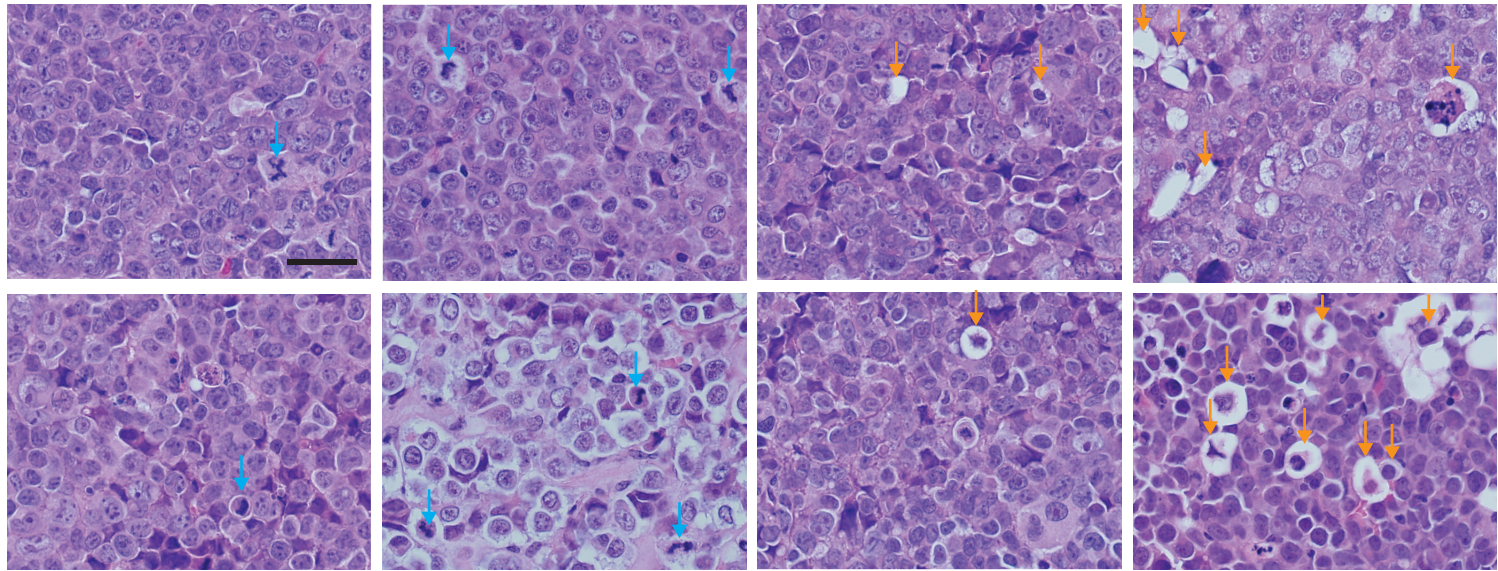


# Supplementary Figure 2

## HEL cell-derived tumor xenografts



scale bar = 50  $\mu$ m



scale bar = 20  $\mu$ m

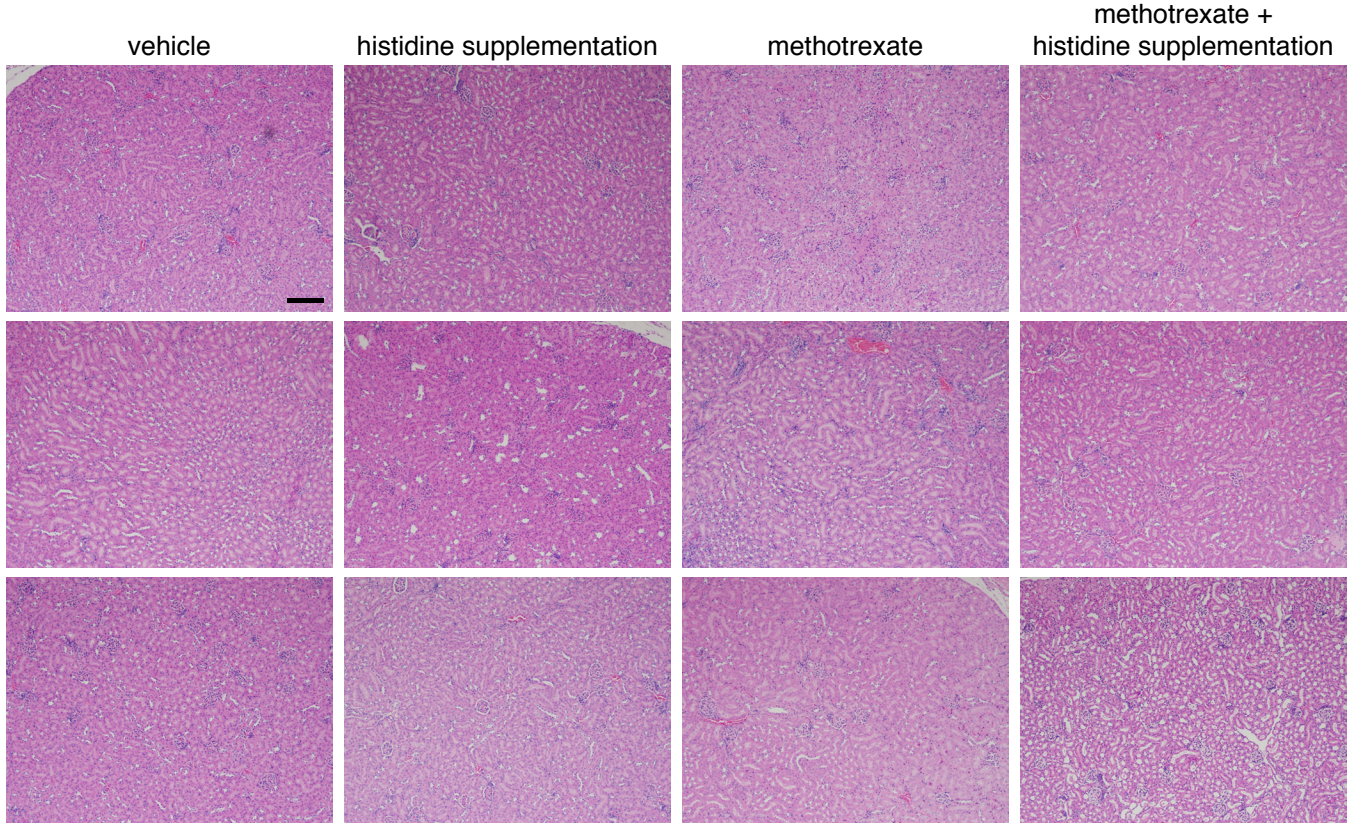
→ mitotic cells  
→ apoptotic or necrotic cells

**Supplementary Figure 2.** Additional images of H&E analyses of HEL cell-derived tumor sections from mice presented in Fig.4.

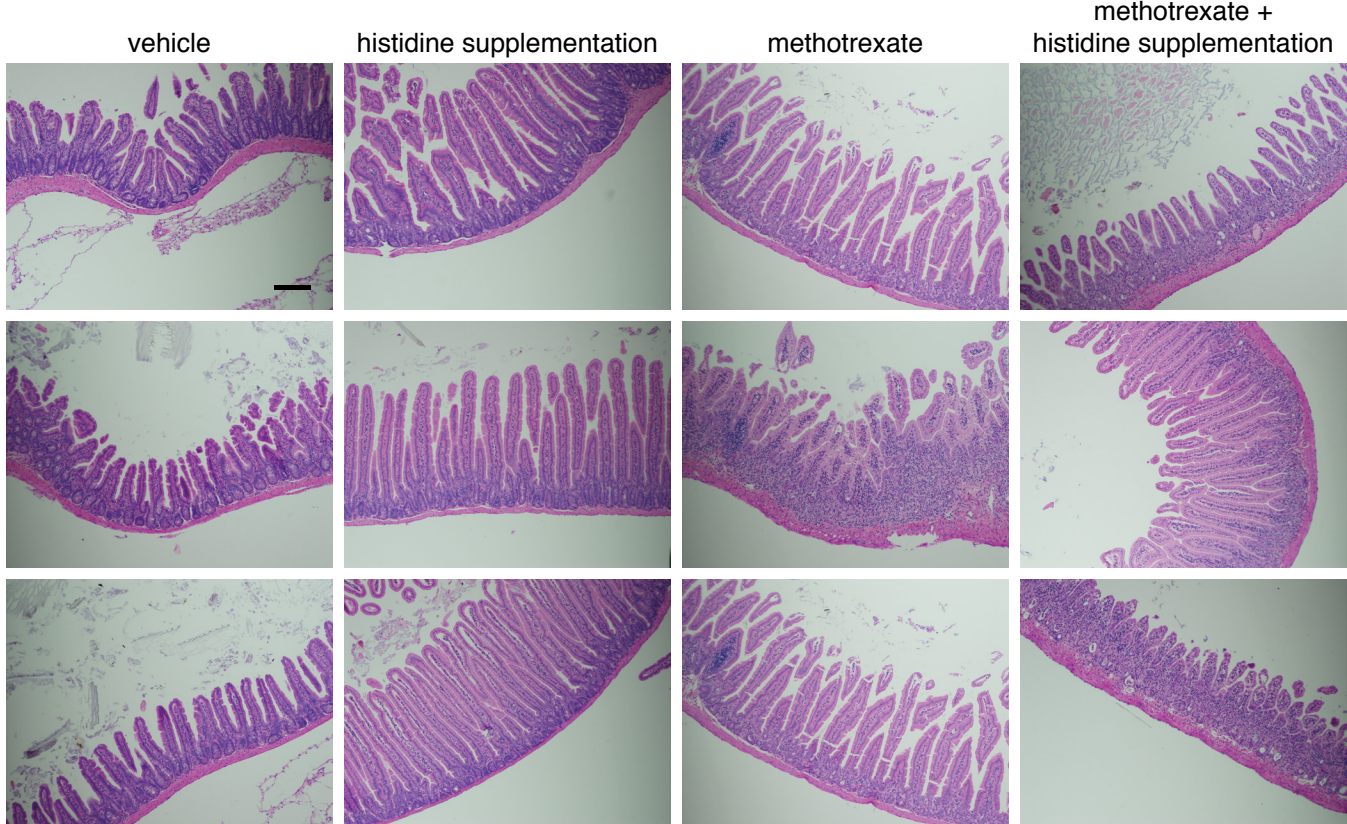


# Supplementary Figure 3

## a Kidneys from mice with HEL cell-derived tumor xenografts - long term regime



## b Intestines from mice with SEM cell-derived tumor xenografts



### Supplementary Figure 3.

- a. H&E analyses of kidneys from mice bearing HEL cell-derived tumors presented in Fig. 4.
- b. H&E analyses of intestines from mice bearing SEM cell-derived tumors presented in figure 4.