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Supplemental Information

Quantitative PET Reporter Gene Imaging with [^{11}C]Trimethoprim

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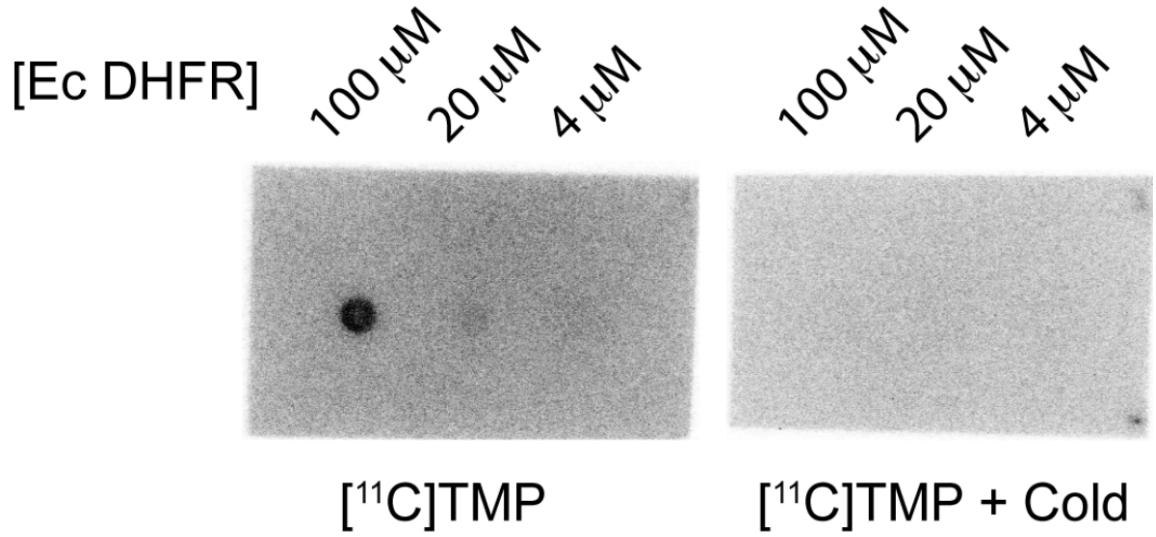

Fig. S1. Dot blot showing various concentrations (100-4 μM) of recombinant *Ec* DHFR spotted on to a nitrocellulose membrane, dried for 1h, blocked, and then incubated in 5% milk with [^{11}C]TMP with or without cold TMP (10 μM) for 30 minutes. Representative blot is shown for an experiment in biological duplicate.

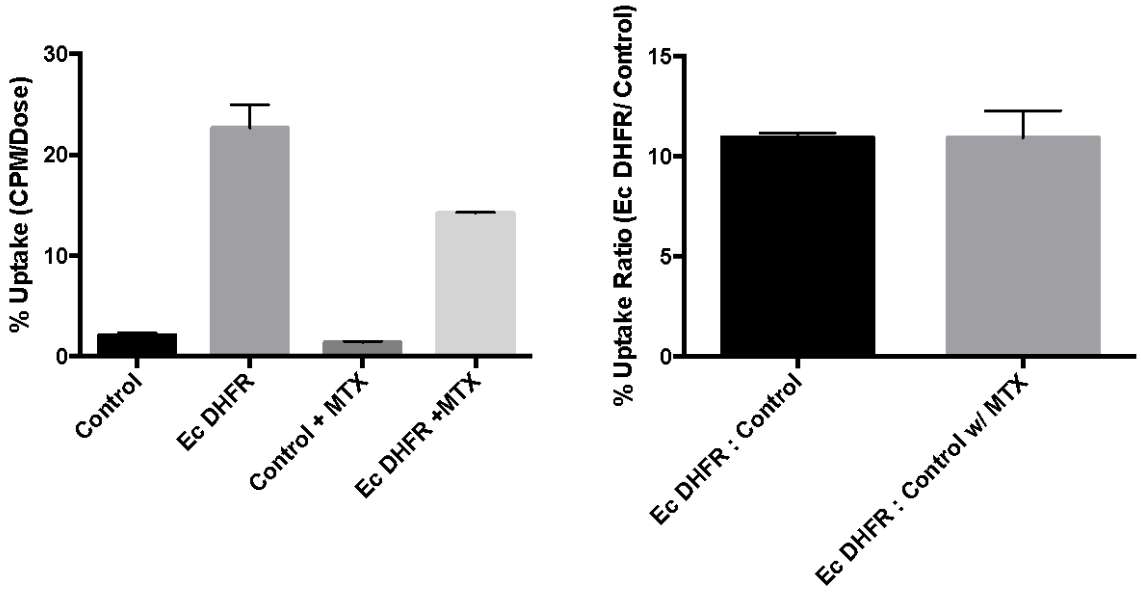

Fig. S2. HEK293 cell uptake study was performed with Methotrexate cotreatment. Confluent control or *Ec* DHFR cells were trypsinized, incubated with [^{11}C]TMP with and without co-treatment with Methotrexate (10 μM, MTX). Cells were washed twice with PBS and then measured for uptake with a gamma counter. Fold uptake is shown in the right graph. Error bars represent standard deviation (n=3).

(% dose/organ)

	2 min (N=4)	15 min (N=4)	45 min (N=4)
Blood	4.59 ± 1.08	1.86 ± 0.41	0.81 ± 0.23
Heart	0.60 ± 0.19	0.16 ± 0.03	0.03 ± 0.00
Muscle	7.26 ± 1.09	7.57 ± 1.58	8.48 ± 3.54
Lung	1.17 ± 0.27	0.41 ± 0.07	0.14 ± 0.04
Kidney	11.8 ± 2.73	2.71 ± 0.42	0.59 ± 0.09
Pancreas	0.48 ± 0.27	0.42 ± 0.14	0.09 ± 0.02
Spleen	n/a	0.28 ± 0.05	0.08 ± 0.04
Liver	18.5 ± 2.84	5.30 ± 0.82	2.33 ± 0.58
Skin	2.68 ± 0.42	2.97 ± 0.49	2.85 ± 1.46
Brain	0.14 ± 0.03	0.12 ± 0.05	0.06 ± 0.03
Bone	4.65 ± 1.04	3.30 ± 0.60	3.01 ± 1.09
Stomach	0.93 ± 0.18	0.92 ± 0.23	0.44 ± 0.17
Gut	7.80 ± 1.24	6.53 ± 1.30	3.64 ± 1.44

(% dose/gram)

	2 min (N=4)	15 min (N=4)	45 min (N=4)
Blood	3.39 ± 0.81	1.28 ± 0.29	0.59 ± 0.16
Heart	6.76 ± 2.37	1.69 ± 0.41	0.35 ± 0.01
Muscle	0.94 ± 0.16	0.91 ± 0.20	1.07 ± 0.42
Lung	5.84 ± 1.51	2.14 ± 0.45	0.84 ± 0.23
Kidney	46.6 ± 9.10	10.6 ± 2.03	2.37 ± 0.35
Pancreas	5.25 ± 2.48	4.28 ± 1.44	1.01 ± 0.24
Spleen	n/a	2.72 ± 0.66	0.89 ± 0.40
Liver	19.8 ± 4.75	5.55 ± 0.97	2.68 ± 0.72
Skin	0.93 ± 0.18	0.95 ± 0.18	0.98 ± 0.54
Brain	0.35 ± 0.08	0.28 ± 0.11	0.16 ± 0.09
Bone	1.72 ± 0.42	1.13 ± 0.22	1.13 ± 0.16
Stomach	2.55 ± 0.65	3.04 ± 0.56	1.82 ± 0.72
Gut	5.74 ± 1.38	4.37 ± 0.74	2.68 ± 1.02

Fig. S3. Biodistribution report of C-11 TMP in female balb/c mice. 83 µCi of tracer was injected IV. Mice were sacrificed 2, 15 and 45 minutes post-injection. Gut includes large intestine, small intestine and colon. Mice were under anesthesia for the duration of the experiment and did not urinate limiting evaluation of the bladder.

Organ	Total (mSv)
Brain	0.06
Gut	0.05
Stomach	0.18
Heart	0.52
Kidneys	1.16
Liver	0.87
Lungs	0.22
Muscle	0.10
Pancreas	0.50
Bone	0.16
Total Body	0.13

Fig. S4. Estimated Human Dosimetry. Biodistribution data from female balb/c mice were used to estimate human dosimetry after [^{11}C]TMP 10 mCi in an adult female human model that was predefined in OLINDA/EXM 1.1. Kinetic data from time points (2, 15, and 45 min) were fitted as percent-injected dose/organ over time. By fitting the kinetic data using %ID/organ we assume the [^{11}C]TMP distribution would be relative to human and thus did not apply a scaling factor accounting for organ weight to subject total body weight between mouse and human.

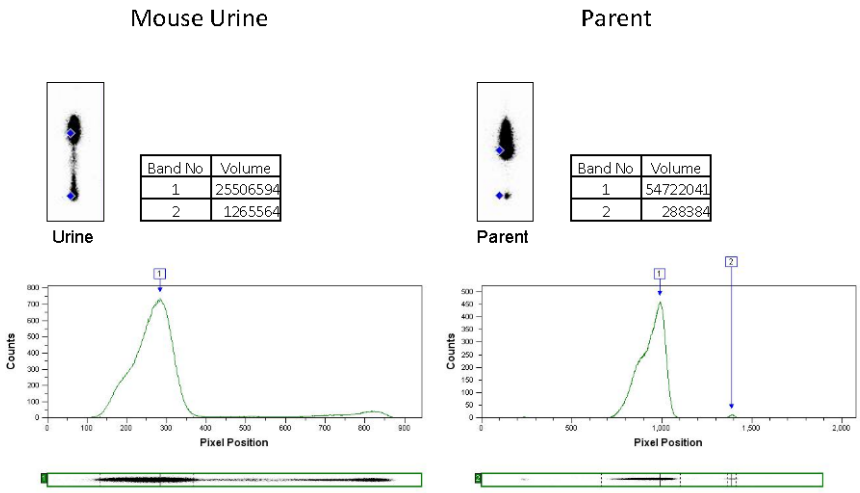

Fig. S5. Thin layer radio-chromatography shows the spot size and relative counts from mouse urine compared to parent [^{11}C]TMP. A small amount of mouse urine and [^{11}C]TMP in saline was dried on a silica plate. The spots were run on with MeOH/CH_2Cl_2 (1:5). The time/length of development for each TLC was slightly different accounting for the difference in the X-axis, but the R_f values are the same for the largest spot from both samples..

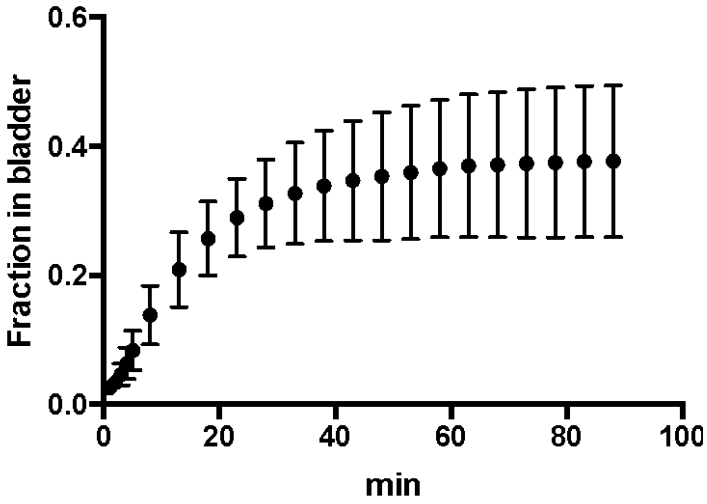

Fig. S6. Percent of radiosignal in the bladder while under anesthesia (no urination) as assessed by measuring the mean signal (Amide) in the bladder * bladder volume divided by the mean signal in the animal * whole animal volume (n=3).

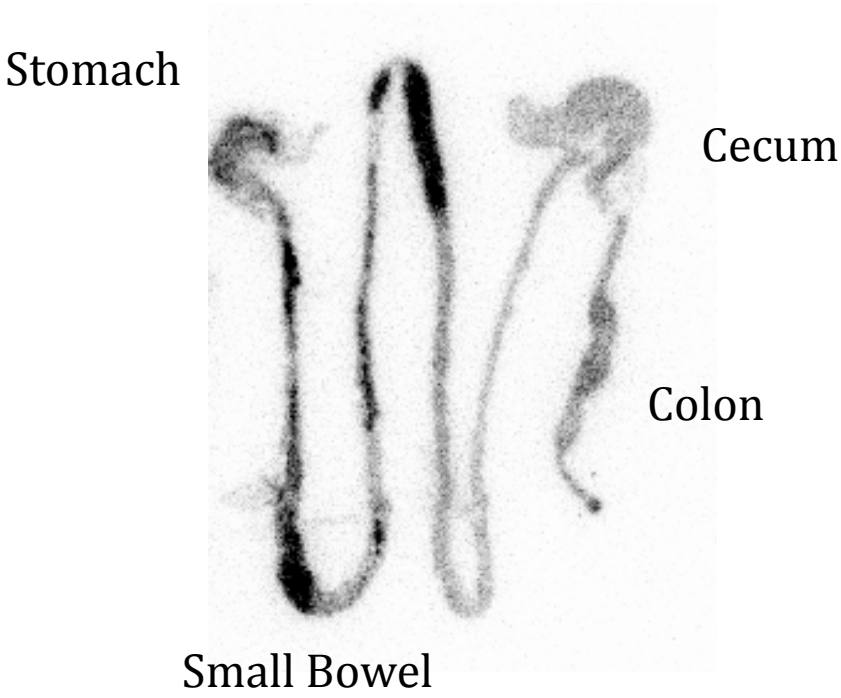

Fig. S7. Representative autoradiography of explanted GI tract from the stomach to the rectum.

DHFR Protein Sequences


```
########################################
# Program: needle
# Rundate: Wed 13 Jul 2016 21:11:11
# Commandline: needle
#    -auto
#    -stdout
#    -asequence emboss_needle-E20160713-211110-0797-67085135-pg.asequence
#    -bsequence emboss_needle-E20160713-211110-0797-67085135-pg.bsequence
#    -datafile EBLOSUM62
#    -gapopen 10.0
#    -gapextend 0.5
#    -endopen 10.0
#    -endextend 0.5
#    -aformat3 pair
#    -sprotein1
#    -sprotein2
# Align_format: pair
# Report_file: stdout
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 190
# Identity:      52/190 (27.4%)
# Similarity:    89/190 (46.8%)
# Gaps:          34/190 (17.9%)
# Score: 169.0
#
#
#=======================================

EMBOSS_001         1 MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRMTTTSSVEGKQNL     50
                     .::.|.|::.:..||....:|| .|..:..:|:|.|....
EMBOSS_001         1 ---MISLIAALAVDRVIGMENAMPW-NLPADLAWFKRNTLNKP-------     39

EMBOSS_001        51 VIMGKKTWFSIPEKNRPLKGRINLVLSRELKEPPQGAHFL-SRSLDDALK     99
                     ||||:.||.||   .|||.||.|::||   .:|..... .:|:|:|:.
EMBOSS_001        40 VIMGRHTWESI---GRPLPGRKNIILS---SQPGTDDRVTWVKSVDEAIA     83

EMBOSS_001       100 LT-EQPELANKVDMVWIVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTF    148
                     .. :.||       :.:::||..||::.:  |...||::|.|..:.|.||.
EMBOSS_001        84 ACGDVPE-------IMVIGGGRVYEQFL--PKAQKLYLTHIDAEVEGDTH    124

EMBOSS_001       149 FPEIDLEKYK-LLPEYPGVLSDVQEEKGIKYKFEVYEKND    187
                     ||:.:.:.:: :..|:    .|...:....|.||:.|:.
EMBOSS_001       125 FPDYEPDDWESVFSEF----HDADAQNSHSYCFEILERR-    159


#---------------------------------------
#---------------------------------------
```

HUMAN
UniProtKB - P00374 (DYR_HUMAN)
MVGSLNCIVA VSQNMGIGKN GDLPWPPLRN EFRYFQRMTT TSSVEGKQNL VIMGKKTWFS IPEKNRPLKG RINLVLSREL KEPPQGAHFL SRSLDDALKL TEQPELANKV DMVWIVGGSS VYKEAMNHPG HLKLFVTRIM QDFESDTFFP EIDLEKYKLL PEYPGVLSDV QEEKGIKYKF EVYEKND

E .coli
UniProtKB - P0ABQ4 (DYR_ECOLI)
MISLIAALAV DRVIGMENAM PWNLPADLAWFKRNTLNKPVIMGRHTWESIGRPLPGRKNI ILSSQPGTDD RVTWVKSVDE AIAACGDVPE IMVIGGGRVYEQFLPKAQKL YLTHIDAEVE GDTHFPDYEP DDWESVFSEF HDADAQNSHSYCFEILERR

TK Protein Sequences

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# Rundate: Tue 27 Sep 2016 20:24:31
# Commandline: needle
#   -auto
#   -stdout
#   -asequence emboss_needle-I20160927-202430-0138-5489320-oy.asequence
#   -bsequence emboss_needle-I20160927-202430-0138-5489320-oy.bsequence
#   -datafile EBLOSUM62
#   -gapopen 10.0
#   -gapextend 0.5
#   -endopen 10.0
#   -endextend 0.5
#   -aformat3 pair
#   -sprotein1
#   -sprotein2
# Align_format: pair
# Report_file: stdout
########################################

#=======================================
#
# Aligned_sequences: 2
# 1: EMBOSS_001
# 2: EMBOSS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 438
# Identity:     48/438 (11.0%)
# Similarity:   76/438 (17.4%)
# Gaps:        266/438 (60.7%)
# Score: 17.0
#
#
#=======================================

EMBOSS_001       1 MASYPCHQHASAFDQAARSRGHNNRRTALRPRRQQEATEVRPEQKMPTLL    50
                                         .....:||:|
EMBOSS_001       1 ----------------------------------------MSCINLPTVL    10

EMBOSS_001      51 RVYIDGPHGMGKTTTTQLLVALGSRDDIVYVPEPMTYWRVLGASETIANI    100
                  .|....|..|:.|.||        ||..:.....:...
EMBOSS_001      11 -------PGSPSKTRGQIQVILG----------PMFSGKSTELMRRVRRF    43

EMBOSS_001     101 YTTQHRL----------------DQGEISAGDAAVVMTSAQITMGMP-    131
               ...|::.              |:..:|..|.::...||...:|:.
EMBOSS_001      44 QIAQYKCLVIKYAKDTRYSSSFCTHDRNTMEALPACLLRDVAQEALGVAV    93

EMBOSS_001     132 YAVTDAVLAPHI------GGEAGSSHAPPPALTLIFDRHPIAALL-----    170
               ..:.:....|.|     ...||.:: ....||...|.|.|..|:|
EMBOSS_001      94 IGIDEGQFFPDIVEFCEAMANAGKT-VIVAALDGTFQRKPFGAILNLVPL    142

EMBOSS_001     171 -------------CYPAARYLMGSMTPQAVLAFVALIPPTLPGTNIVLGA    207
                           |:...|.|.....|.:.|             .|:|.
EMBOSS_001     143 AESVVKLTAVCMECFREAAYTKRLGTEKEV---------------EVIGG    177

EMBOSS_001     208 LPEDRHIDRL------AKRQ--------------RPGERLDLAMLAAIRRV    238
               ..:.:...:||    |..|            :|||.:.....|.|.:::
EMBOSS_001     178 ADKYHSVCRLCYFKKASGQPAGPDNKENCPVPGKPGEAVAARKLFAPQQI    227

EMBOSS_001     239 YGLLANTVRYLQCGGSWREDWGQLSGTAVPPQGAEPQSNAGPRPHIGDTL    288
                  |||..::
EMBOSS_001     228 ----------LQCSPAN--------------------------------    234

EMBOSS_001     289 FTLFRAPELLAPNGDLYNVFAWALDVLAKRLRSMHVFILDYDQSPAGCRD    338

EMBOSS_001     235 ------------------------------------------------    234

EMBOSS_001     339 ALLQLTSGMVQTHVTTPGSIPTICDLARTFAREMGEAN    376

EMBOSS_001     235 ---------------------------------------    234


#---------------------------------------
#---------------------------------------
```

Human cytosolic TK1
UniProtKB - P04183 (KITH_HUMAN)
MSCINLPTVLPGSPSKTRGQIQVILGPMFSGKSTELMRRVRRFQIAQYKCLVIKYAKDTR
YSSSFCTHDRNTMEALPACLLRDVAQEALGVAVIGIDEGQFFPDIVEFCEAMANAGKTVI
VAALDGTFQRKPFGAILNLVPLAESVVKLTAVCMECFREAAYTKRLGTEKEVEVIGGADK
YHSVCRLCYFKKASGQPAGPDNKENCPVPGKPGEAVAARKLFAPQQILQCSPAN

Human Herpes Virus 1 TK
UniProtKB - P03176 (KITH_HHV11)
MASYPCHQHASAFDQAARSRGHNNRRTALRPRRQQEATEVRPEQKMPTLLRVYIDGPHGM
GKTTTTQLLVALGSRDDIVYVPEPMTYWRVLGASETIANIYTTQHRLDQGEISAGDAAVV
MTSAQITMGMPYAVTDAVLAPHIGGEAGSSHAPPPALTLIFDRHPIAALLCYPAARYLMG
SMTPQAVLAFVALIPPTLPGTNIVLGALPEDRHIDRLAKRQRPGERLDLAMLAAIRRVYG
LLANTVRYLQCGGSWREDWGQLSGTAVPPQGAEPQSNAGPRPHIGDTLFTLFRAPELLAP
NGDLYNVFAWALDVLAKRLRSMHVFILDYDQSPAGCRDALLQLTSGMVQTHVTTPGSIPT
ICDLARTFAREMGEAN

Fig. S8. Sequence alignment of *Ec* DHFR and Human DHFR and thymidine kinase from human herpes virus 1 and human cytosolic thymidine kinase. Alignment algorithm was done using EMBOSS NEEDLE Pairwise Protein Alignment.