Supplemental Material

Supplemental Figure Legends

Supp Fig. 1 Prodrug 5FURGal does not affect cell cycle. a Schematic representation of the experimental design. **b**. Representative images of human IMR90 fibroblasts in either proliferative or doxorubicin-induced senescent state cultured in the absence (0 μM) or in the presence (25 μM) of EdU. (Scale bar = 1000 μm). **c**. Quantification of IMR90 fibroblasts EdU+ in either proliferative or doxorubicin-induced senescent state cultured in the absence (0 μM) or in the presence (25 μM) of EdU.

Supp Fig. 2 Systemic conversion of prodrug 5FURGal to parent drug 5FU is minimal in mouse. a. CD1 mice received IV injections of 5FU (5 mg/Kg) and mean plasma concentration of 5FU is measured over time. AUC_{last} = 375 hr.ng/mL
b. CD1 mice received IV injections of 5FUR-Gal (40 mg/Kg) and mean plasma concentration of 5FU and 5FUR-Gal is measured over time. The fraction of 5FUR-Gal converted to 5FU is 2%. AUC_{last} = 7.5 hr.ng/mL (dose normalized to 5 mg/kg equiv. 5FU).

Supp Fig. 3 Prodrug 5FURGal prevents chemotoxicity of Doxorubicin in vivo. a. Representative images of histological sections of livers isolated from young mice that received either: 1) Doxorubicin (Doxo) followed by saline treatment; or 2) Doxo followed by 5FURGal treatment. Colorimetric staining in blue shows expression of

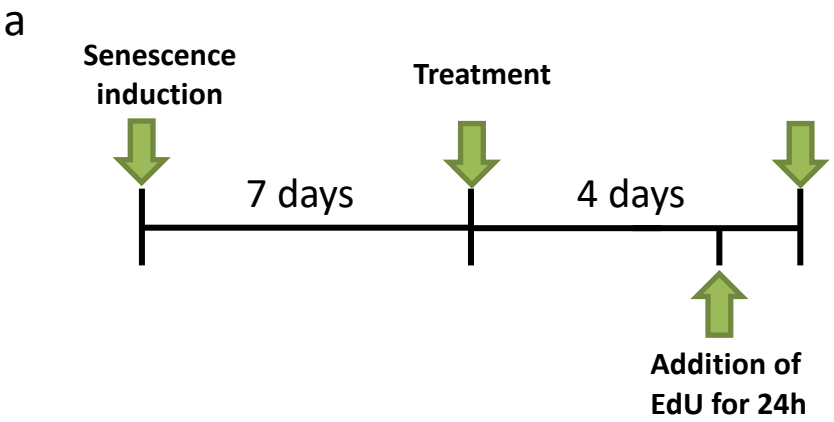
SA-β-Gal. Scale bar = 500 μm). **b**. Quantification of SA-β-Gal positive cells in liver sections of young mice that received either: 1) Saline; 2) Doxorubicin (Doxo) followed by saline treatment; or 3) Doxo followed by 5FURGal treatment. **c**. Quantification of body weight of young mice that received either: 1) Saline; 2) Doxorubicin (Doxo) followed by saline treatment; or 3) Doxo followed by 5FURGal treatment. For statistical analysis, t-tests were used. *P<0.01, **P<0.001, ***P<0.0001, ****P<0.00001 (n = 5).

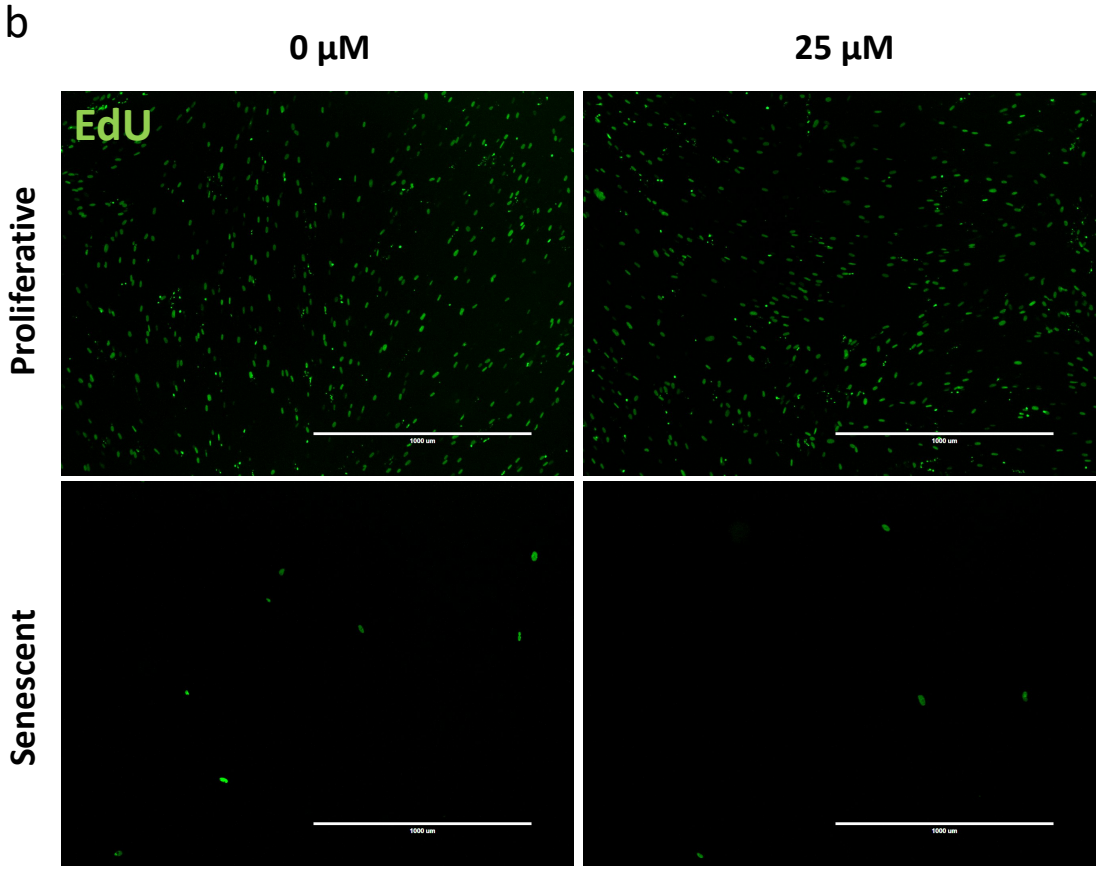
Supp Fig. 4. 5FURGal improves spontaneous activity in geriatric mice . a. Quantification of volunteer running activity measured with computer controlled wheels located in the cage where mice were housed individually. Total distance is quantified for geriatric mice: 1) before initiating treatments; 2) three weeks of treatments (Day 21); 3) 5 weeks after treatments (day 35).

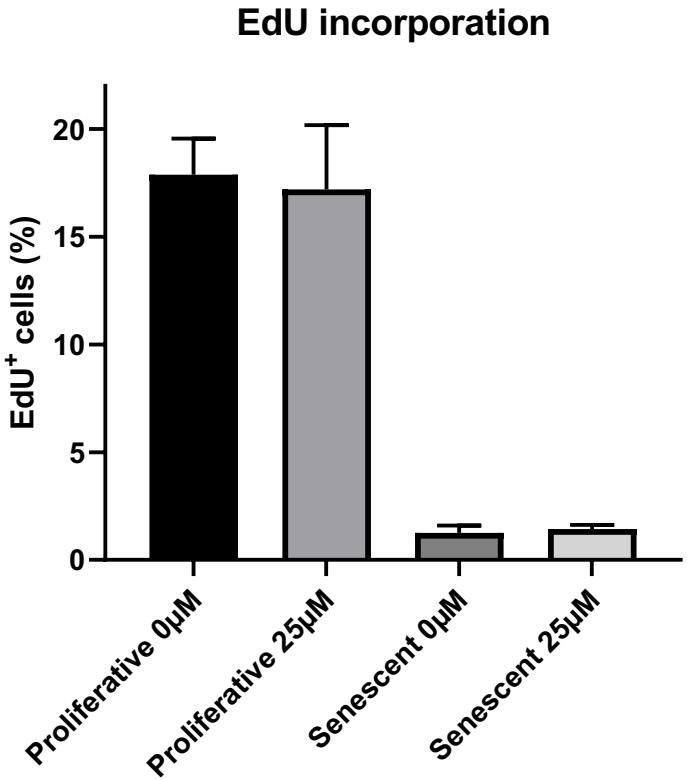
Supp Fig. 5 5FURGal improves cognitive functions in geriatric mice. Geriatric mice were assessed for their spatial working cognitive functions using a Y maze test. Mice were assessed before and after treatments with either (**a**) saline or (**b**) 5FURGal. For statistical analysis, paired t-tests were used. *P<0.01, **P<0.001, ***P<0.0001, ****P<0.00001.

Supp Fig. 6 5FURGal Shows no overt toxicity in Geriatric Mice. Geriatric mice received 5 injections (indicated by arrowheads) of either saline or 5FURGal over the course of three weeks. **a)** Body weight (individual data points and mean plotted) and **b**) viability was assessed from day 0 to day 21 . All mice were then sacrificed at day 21 and: **c**) spleen weight was measured (individual data points, mean and standard

error are plotted; dashed green line indicate average spleen weight in young mice); **d**. blood was isolated and tested on an hematology analyzer via a multi parameter complete blood count panel (individual data points, mean and standard error are plotted; White blood cells (WBC), Red blood cells (RBC), Hemoglobin (HGB), Mean Corpuscular Hemoglobin (MCH), Platelets (PLT), Mean Platelet Volume (MPV), Lymphocytes (LYM, LY), Neutrophils (NEU, NE), Eosinophils (EOS, EO), Monocytes (MON, MO), Basophils (BAS, BA), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Platelet Distribution Width (PDW), Red Cell Distribution Width (RDW), Platelet Hematocrit (PCT)).

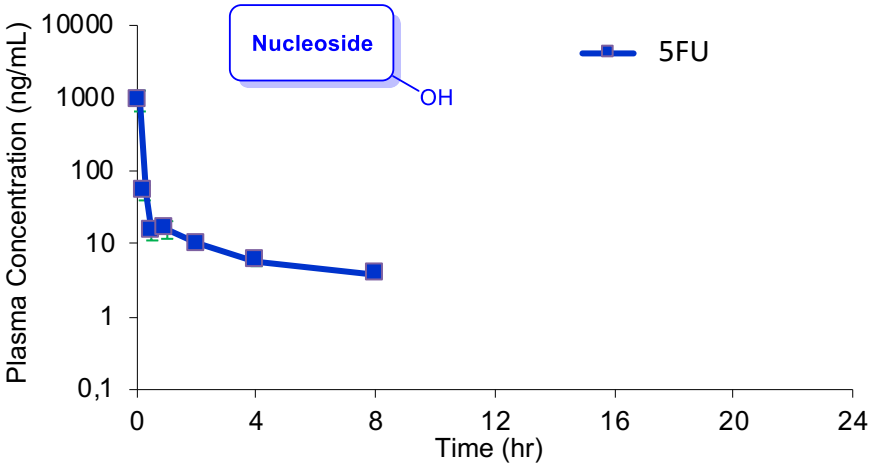

a
Senescence induction
Treatment
7 days
4 days
Addition of EdU for 24h


b
0 µM
25 µM
EdU
Proliferative
Senescent

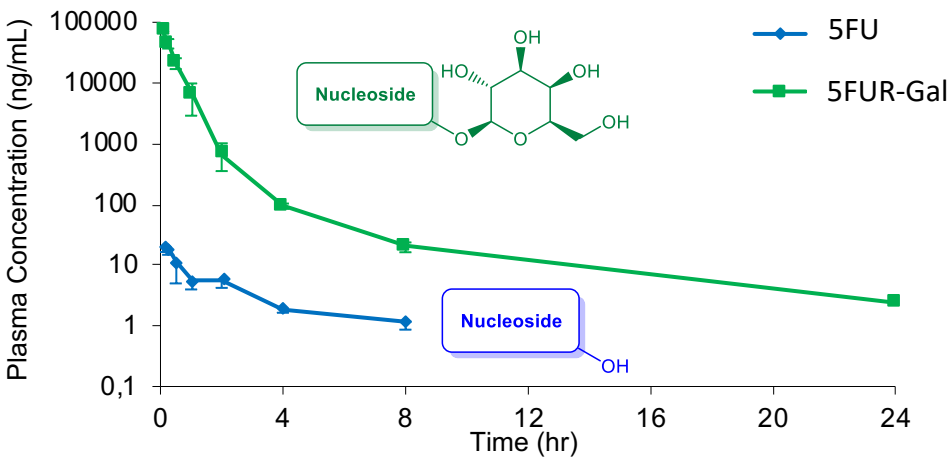

c
EdU incorporation
EdU+ cells (%)
20
15
10
5
0
Proliferative 0µM
Proliferative 25µM
Senescent 0µM
Senescent 25µM

Supp. Fig 1

a

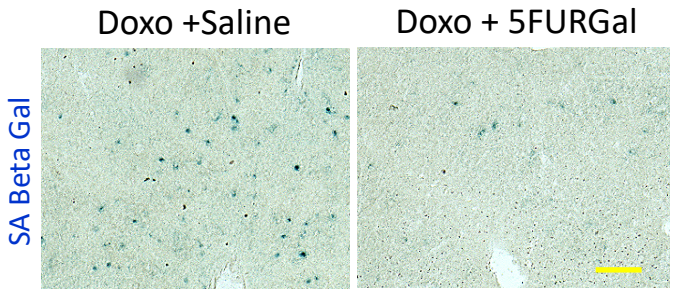

Nucleoside
OH
5FU
Plasma Concentration (ng/mL)
10000
1000
100
10
1
0,1
0
4
8
12
16
20
24
Time (hr)

b

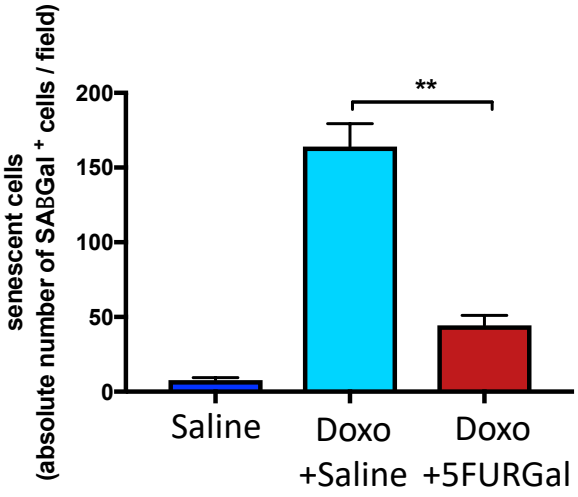

5FU
5FUR-Gal
Nucleoside
OH
HO
OH
OH
OH
O
O
Nucleoside
OH
Plasma Concentration (ng/mL)
100000
10000
1000
100
10
1
0,1
0
4
8
12
16
20
24
Time (hr)

Supp. Fig 2

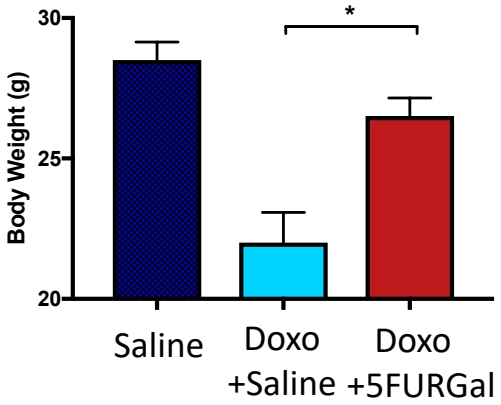
a



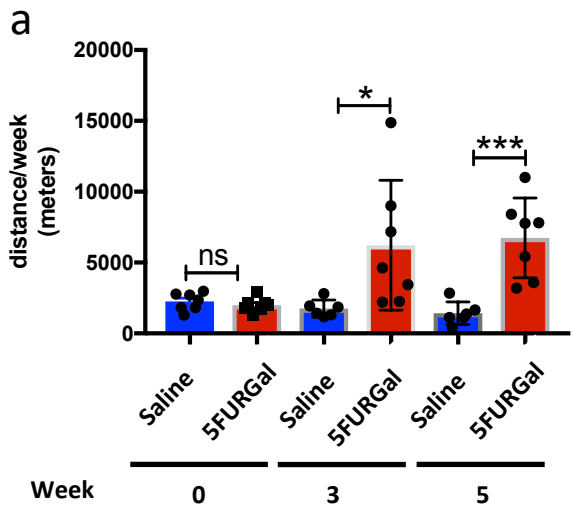
b



c

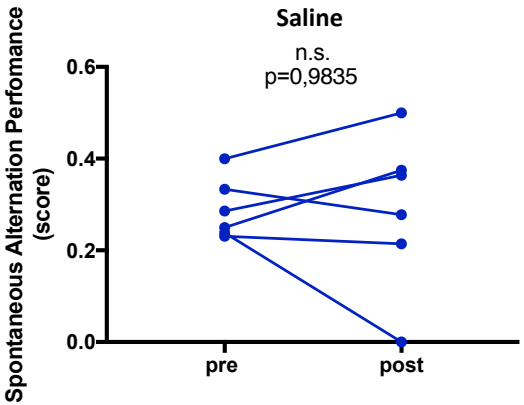


Supp. Fig 3

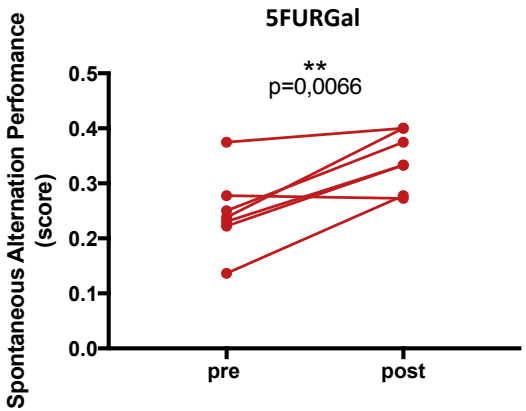

a
distance/week (meters)
20000
15000
10000
5000
0
ns
*
***
Saline
5FURGal
Saline
5FURGal
Saline
5FURGal
Week
0
3
5

Supp. Fig 4

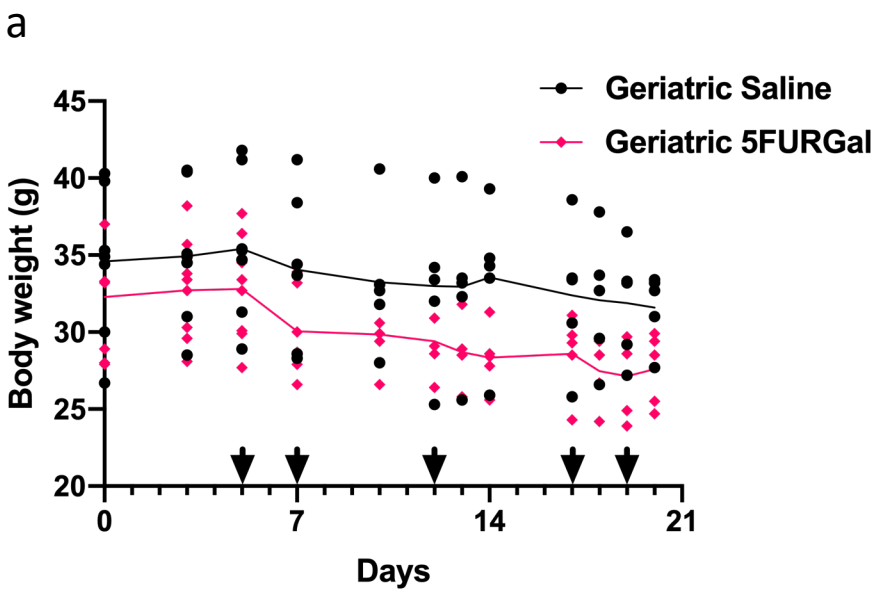
a

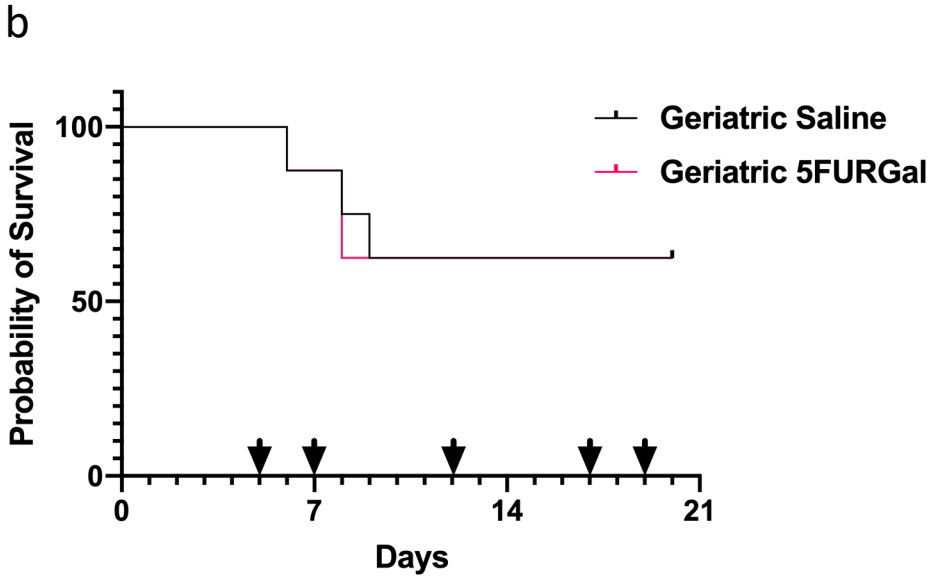

Saline
n.s.
p=0,9835
Spontaneous Alternation Perfomance (score)
0.6
0.4
0.2
0.0
pre
post

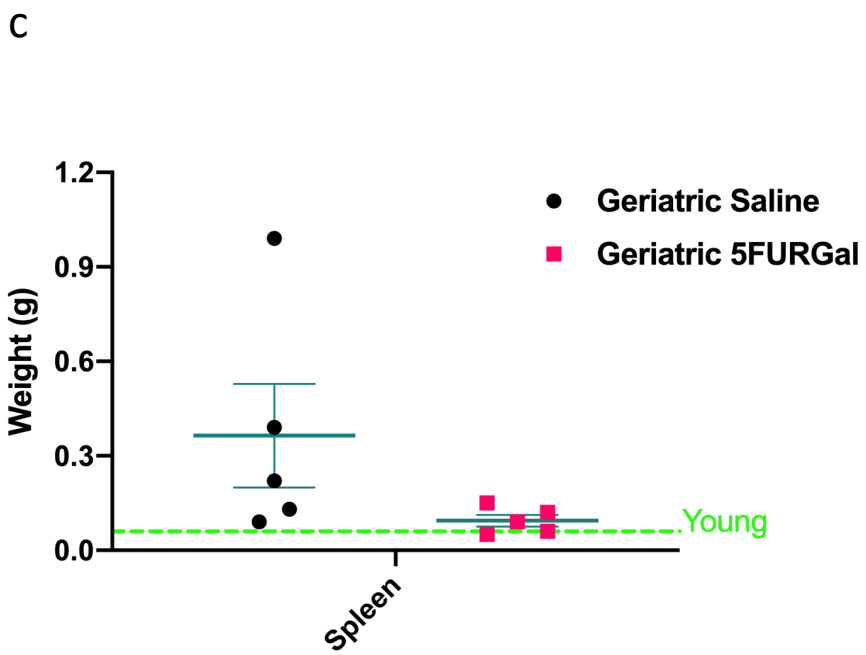
b

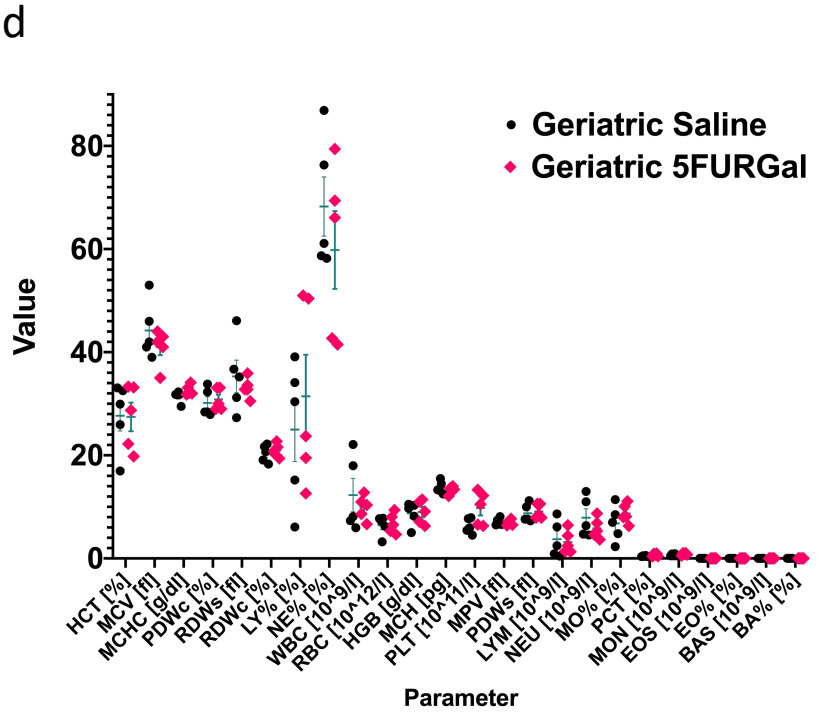

5FURGal
**
p=0,0066
Spontaneous Alternation Perfomance (score)
0.5
0.4
0.3
0.2
0.1
0.0
pre
post

Supp. Fig 5


a
Geriatric Saline
Geriatric 5FURGal
Body weight (g)
45
40
35
30
25
20
0
7
14
21
Days


b
Geriatric Saline
Geriatric 5FURGal
Probability of Survival
100
50
0
0
7
14
21
Days


c
Geriatric Saline
Geriatric 5FURGal
Weight (g)
1.2
0.9
0.6
0.3
0.0
Young
Spleen


d
Geriatric Saline
Geriatric 5FURGal
Value
80
60
40
20
0
HCT [%]
MCV [fl]
MCHC [g/dl]
PDWc [%]
RDWs [fl]
RDWc [%]
LY% [%]
NE% [%]
WBC [10^9/l]
RBC [10^12/l]
HGB [g/dl]
MCH [pg]
PLT [10^11/l]
MPV [fl]
PDWs [fl]
LYM [10^9/l]
NEU [10^9/l]
MO% [%]
PCT [%]
MON [10^9/l]
EOS [10^9/l]
EO% [%]
BAS [10^9/l]
BA% [%]
Parameter

Supp. Fig 6