

Figure S1. Flow cytometric analysis of erastin2 treatment.

(A-C) HT1080 cells incubated with complete media, erastin2 (0.15 μ M), and erastin2 (0.15 μ M) + ferrostatin (1 μ M), respectively. (D-F) H460 cells incubated with complete media, erastin2 (0.15 μ M), and erastin2 (0.15 μ M) + ferrostatin (1 μ M), respectively. (G-I) C6 cells incubated with complete media, erastin2 (0.15 μ M), and erastin2 (0.15 μ M) + f (1 μ M), respectively. All samples treated for 5 h, co-incubated with BODIPY 581/591 C11 (1.5 mM), and stained with DAPI (0.4 mg/mL).

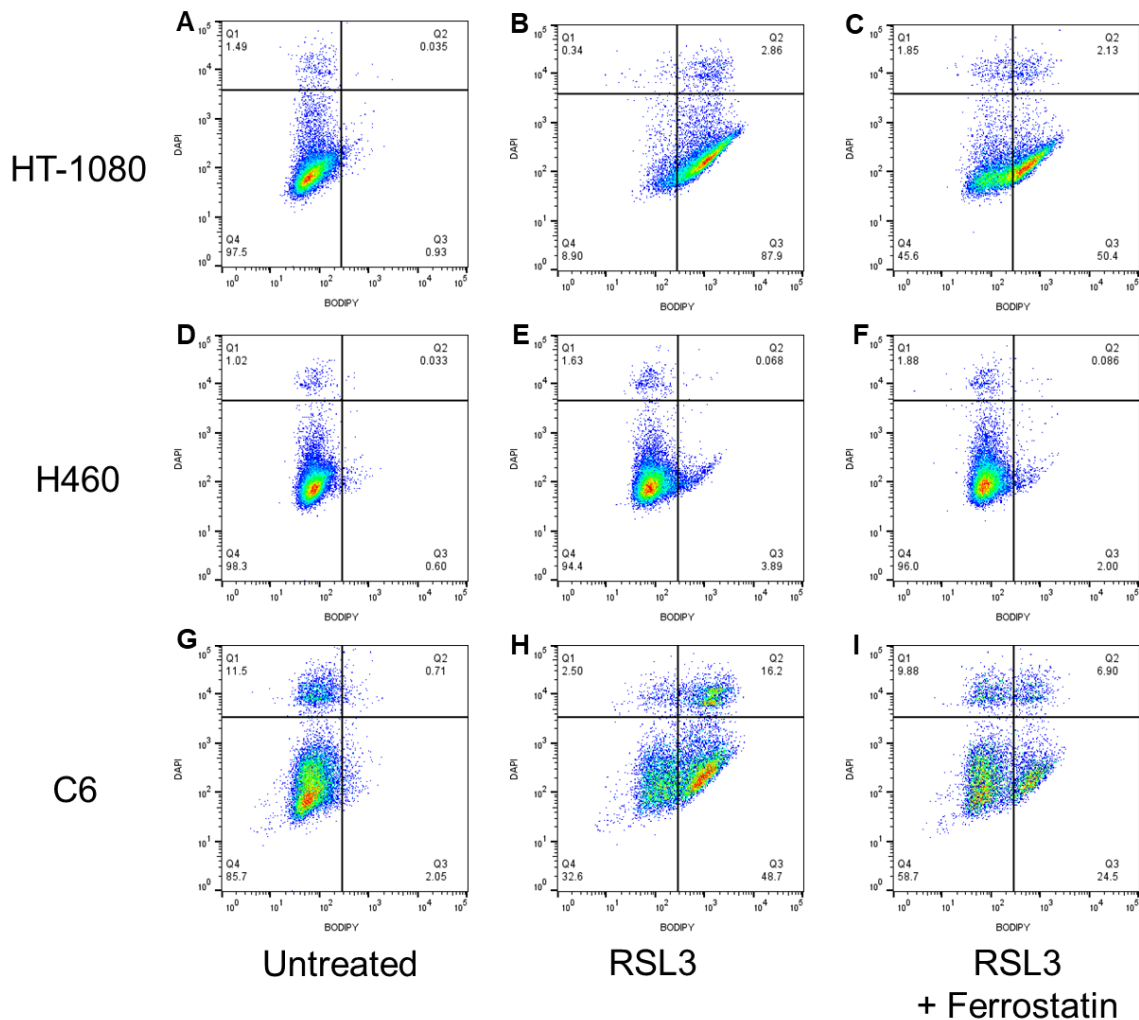


Figure S2. Flow cytometric analysis of RSL3 treatment.

(A-C) HT1080 cells incubated with complete media, RSL3 (0.3 μ M), and RSL3 (0.3 μ M) + ferrostatin (1 μ M), respectively. (D-F) H460 cells incubated with complete media, RSL3 (0.3 μ M), and RSL3 (0.3 μ M) + ferrostatin (1 μ M), respectively. (G-I) C6 cells incubated with complete media, RSL3 (0.3 μ M), and RSL3 (0.3 μ M) + ferrostatin (1 μ M), respectively. All samples treated for 2.5 h, co-incubated with BODIPY 581/591 C11 (1.5 mM), and stained with DAPI (0.4 mg/mL).

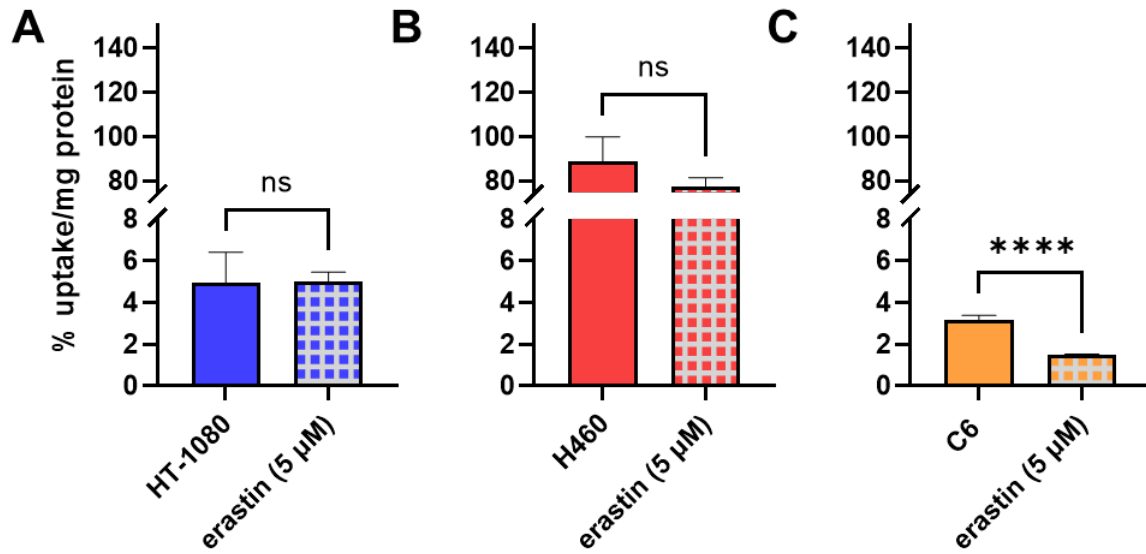


Figure S3. Radiotracer uptake in the presence of erastin.

[¹⁸F]hGTS13 uptake of untreated (A) HT-1080, (B) H460, and (C) C6, and the same cell lines treated with Erastin (5 μM) for 5 h (n = 4). 1-way analysis of variance (ANOVA), multiple comparisons of means with Bonferroni correction.

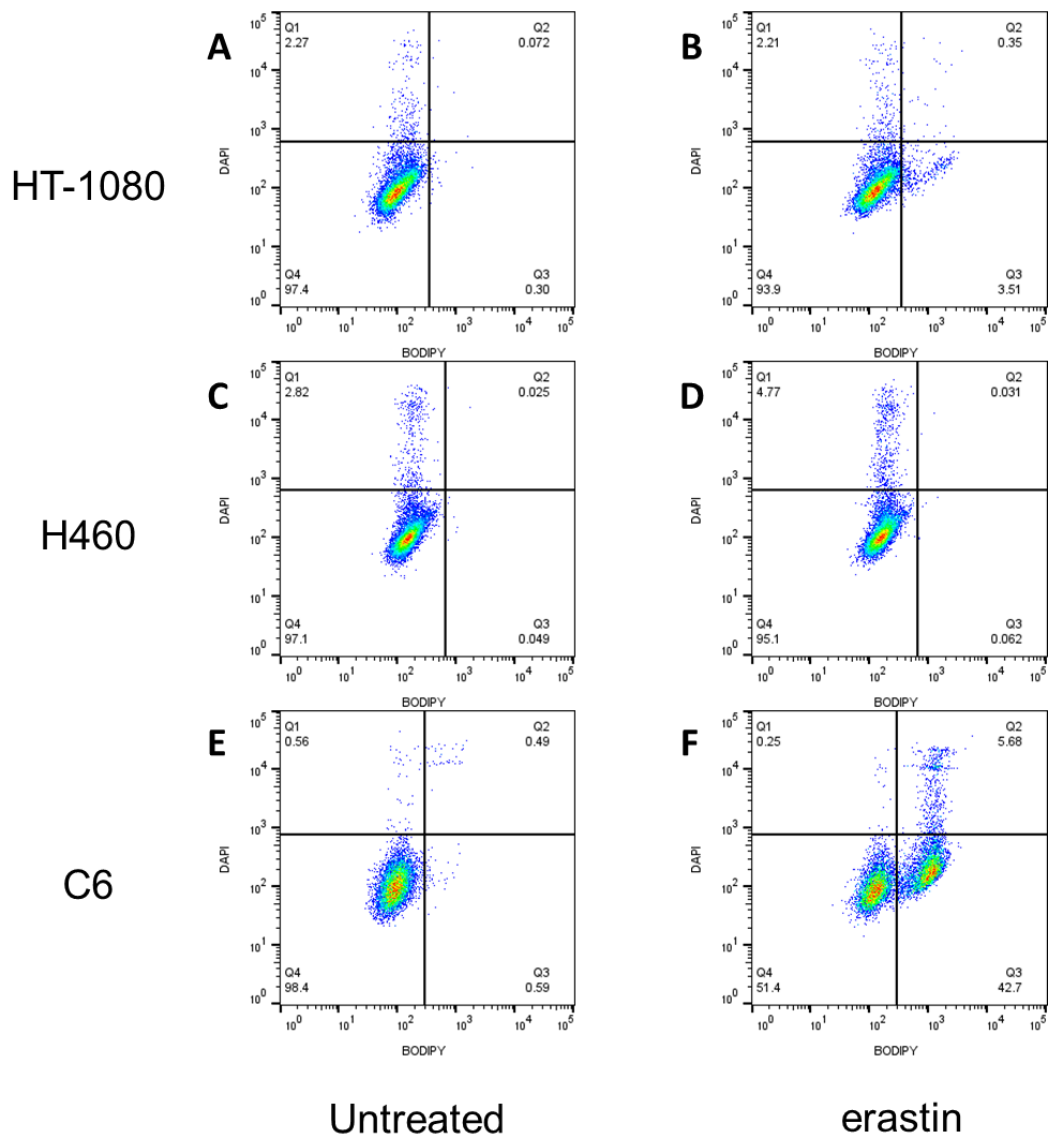


Figure S4. Flow cytometric analysis of erastin treatment.

(A,B) HT1080 cells incubated with complete media, and erastin (10 μ M), respectively, for 5 h. (C,D) H460 cells incubated with complete media, and erastin (10 μ M), respectively, for 5 h. (E,F) C6 cells incubated with complete media, and erastin (10 μ M), respectively, for 5 h. All samples co-incubated with BODIPY 581/591 C11 (1.5 mM), and stained with DAPI (0.4 mg/mL).

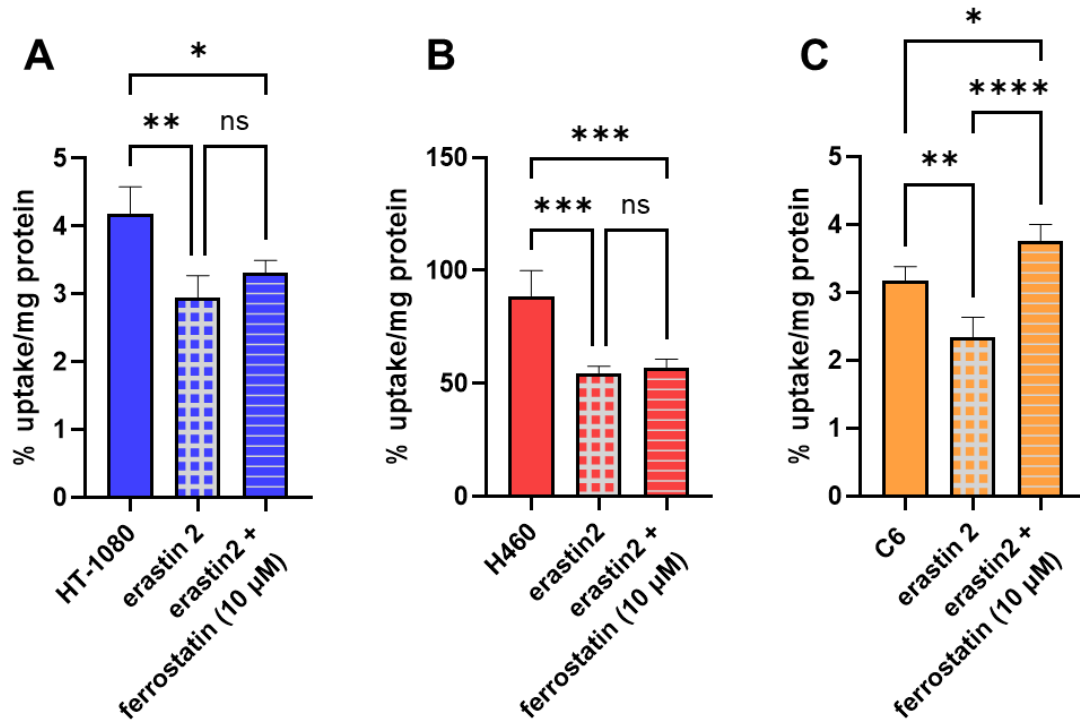


Figure S5. Radiotracer uptake in the presence of erastin2 and Increased ferrostatin.

[¹⁸F]hGTS13 uptake of (A) HT-1080, (B) H460, and (C) C6 in complete media, erastin2 (0.15 μ M), and erastin2 (0.15 μ M) + ferrostatin (10 μ M) (n = 4). 1-way analysis of variance (ANOVA), multiple comparisons of means with Bonferroni correction.

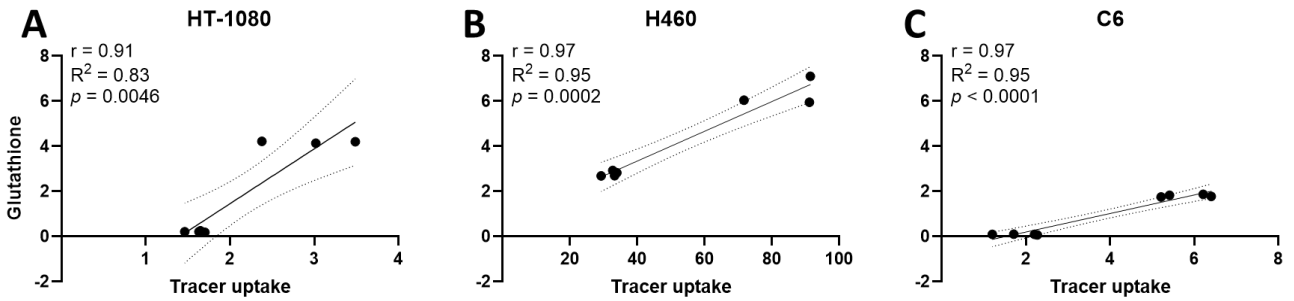


Figure S6. Correlation of radiotracer uptake and glutathione content.

Correlation plots of % radiotracer uptake / mg total protein vs. total intracellular glutathione concentration (μM) of (A) HT-1080, (B) H460, and (C) C6 cell lines in the presence and absence of erastin2 ($0.15 \mu\text{M}$). Simple linear regression using two-tailed Pearson correlation (r). Dotted lines represent 95% confidence interval.

Table S1. Patient demographic information

Sample ID	Age	Gender	Diagnosis	Primary/Recurrent sample
GBM WT-1	53	F	Glioblastoma, IDH1-wild type, Grade IV	Recurrent
GBM WT-2	62	F	Glioblastoma, IDH1-wild type, Grade IV	Recurrent
GBM WT-3	61	M	Glioblastoma, IDH1-wild type, Grade IV	Recurrent
GBM WT-4	61	M	Glioblastoma, IDH1-wild type, Grade IV	Primary
GBM WT-5	72	M	Glioblastoma, IDH1-wild type, Grade IV	Primary
GBM WT-6	69	M	Glioblastoma, IDH1-wild type, Grade IV	Primary
GBM WT-7	76	F	Glioblastoma, IDH1-wild type, Grade IV	Primary
GBM WT-8	79	M	Glioblastoma, IDH1-wild type, Grade IV	Recurrent
GBM WT-9	75	M	Glioblastoma, IDH1-wild type, Grade IV	Primary
GBM WT-10	63	M	Glioblastoma, IDH1-wild type, Grade IV	Primary
GBM WT-11	60	M	Glioblastoma, IDH1-wild type, Grade IV	Primary
GBM WT-12	69	M	Glioblastoma, IDH1-wild type, Grade IV	Recurrent
GBM WT-13	63	F	Glioblastoma, IDH1-wild type, Grade IV	Recurrent
GBM IDH-1	36	M	Glioblastoma, IDH1-mutant, Grade IV	Recurrent
GBM IDH-2	41	M	Glioblastoma, IDH1-mutant, Grade IV	Primary
GBM IDH-3	31	M	Glioblastoma, IDH1-mutant, Grade IV	Recurrent
AA-1	69	M	Anaplastic astrocytoma, IDH1, Grade III	Primary
OG-1	63	F	Anaplastic oligodendroglioma, IDH1-mutant, 1p/19q co-deleted, Grade III	Primary
DA-1	53	F	Diffuse astrocytoma, IDH1-mutant, Grade II	Primary
Brain tissue from autopsy	73	M	Cause of death: Acute fungal pneumonia contributing: Escherischia coli sepsis	

Table S2. hGTS13 Toxicity Data

Table 2.1 Hematology

	Day 3		Day 15	
	hGTS13: 31.9 µg/kg Males/Females	Vehicle Control: 0 µg/kg Males/Females	hGTS13: 31.9 µg/kg Males/Females	Vehicle Control: 0 µg/kg Males/Females
Hematocrit	38.0 ± 1.5/38.1 ± 2.0	40.0 ± 1.0/39.0 ± 1.9	41.9 ± 2.4/41.6 ± 1.9	43.0 ± 0.7/40.8 ± 1.1
Hemoglobin	13.4 ± 0.5/13.5 ± 0.7	14.0 ± 0.3/13.7 ± 0.6	14.8 ± 0.9/14.9 ± 0.6	15.2 ± 0.3/14.6 ± 0.3
Mean corpuscular hemoglobin	21.7 ± 0.4/21.3 ± 0.1	21.4 ± 0.6/20.6 ± 0.4	20.8 ± 0.5/21.1 ± 1.3	20.5 ± 0.3/20.6 ± 0.6
Mean corpuscular hemoglobin concentration	35.2 ± 0.2/35.4 ± 0.3	35.1 ± 0.1/35.1 ± 0.4	35.4 ± 0.4/25.9 ± 0.4	35.3 ± 0.3/35.9 ± 0.5
Mean corpuscular volume	61.8 ± 1.1/60.3 ± 0.4	61.2 ± 1.7/58.7 ± 0.9	58.8 ± 1.8/58.8 ± 3.1	58.3 ± 1.1/57.4 ± 1.1
Platelets	1109.0 ± 101.3/1032.6 ± 185.6	974.6 ± 101.1/991.4 ± 389.6	1020.4 ± 52.3/888.8 ± 137.1	986.4 ± 38.8/1061.4 ± 64.6
Mean Platelet volume	6.0 ± 0.2/6.6 ± 0.4	6.3 ± 0.4/6.4 ± 0.4	6.0 ± 0.3/6.4 ± 0.2	5.9 ± 0.2/6.2 ± 0.2
Red blood corpuscles	6.16 ± 0.32/6.32 ± 0.31	6.55 ± 0.32/6.66 ± 0.42	7.13 ± 0.36/7.11 ± 0.67	7.37 ± 0.24/7.12 ± 0.29

Red cell distribution width	17.5 ± 1.7/17.1 ± 1.2	16.4 ± 0.5/16.7 ± 1.8	14.1 ± 0.7/13.3 ± 0.8	14.1 ± 0.8/13.9 ± 0.5
White blood corpuscles	5.35 ± 1.08/3.66 ± 0.34	5.83 ± 0.82/3.54 ± 0.75	8.27 ± 0.6/5.54 ± 1.10	6.99 ± 1.06/4.31 ± 1.29
Neutrophils	0.58 ± 0.24/0.29 ± 0.04	0.68 ± 0.24/0.54 ± 0.40	1.04 ± 0.13/0.63 ± 0.13	0.90 ± 0.16/0.51 ± 0.13
Lymphocytes	4.62 ± 0.91/3.27 ± 0.31	4.96 ± 0.81/2.85 ± 0.53	6.82 ± 0.61/4.68 ± 0.98	5.75 ± 1.24/3.63 ± 1.16
Monocytes	0.11 ± 0.05/0.06 ± 0.03	0.15 ± 0.03/0.10 ± 0.04	0.33 ± 0.06/0.16 ± 0.04	0.27 ± 0.06/0.13 ± 0.05
Eosinophils	0.03 ± 0.01/0.02 ± 0.02	0.04 ± 0.02/0.04 ± 0.02	0.07 ± 0.05/0.07 ± 0.02	0.07 ± 0.01/0.04 ± 0.01
Basophils	0.00 ± 0.00/0.00 ± 0.00	0.00 ± 0.00/0.00 ± 0.00	0.00 ± 0.00/0.00 ± 0.00	0.00 ± 0.00/0.00 ± 0.00
Reticulocyte count	4.12 ± 0.40/3.41 ± 0.50	4.37 ± 0.34/3.32 ± 0.44	3.59 ± 0.49/3.55 ± 0.43	3.62 ± 0.41/3.41 ± 0.33

Table 2.2 Clinical chemistry

	Day 3		Day 15	
	hGTS13: 31.9 µg/kg Males/Females	Vehicle Control: 0 µg/kg Males/Females	hGTS13: 31.9 µg/kg Males/Females	Vehicle Control: 0 µg/kg Males/Females
Glucose	161 ± 0.78/150.3 ± 12.2	161.3 ± 13.3/156.5 ± 7.6	111.4 ± 5.4/114.0 ± 5.4	111.3 ± 9.6/119.7 ± 7.5
Alkaline phosphatase	261.6 ± 44.3/170.4 ± 34.5	303.3 ± 43.2/157.7 ± 17.7	242.1 ± 32.9/153.8 ± 51.6	234.0 ± 40.2/140.5 ± 26.6
Alanine aminotransferase	43.5 ± 9.5/29.3 ± 4.6	42.4 ± 3.0/27.4 ± 2.0	42.4 ± 10.3/33.0 ± 4.9	42.7 ± 4.4/27.3 ± 5.0
Aspartate aminotransferase	73.2 ± 7.4/66.5 ± 4.2	73.1 ± 8.5/59.8 ± 5.1	76.2 ± 15.8/65.0 ± 6.5	81.9 ± 8.3/57.2 ± 11.3
Gamma glutamyl transferase	3.3 ± 1.9/4.1 ± 1.2	2.6 ± 1.6/6.1 ± 1.4	4.6 ± 2.7/6.1 ± 1.7	3.0 ± 1.6/7.2 ± 2.9
Total protein	4.72 ± 0.13/4.94 ± 0.24	4.84 ± 0.15/5.02 ± 0.13	5.3 ± 0.2/5.44 ± 0.3	5.37 ± 0.11/5.52 ± 0.26
Albumin	3.27 ± 0.06/3.50 ± 0.19	3.34 ± 0.15/3.46 ± 0.11	3.54 ± 0.09/3.68 ± 0.13	3.60 ± 0.07/3.68 ± 0.16
Globulin	1.49 ± 0.08/1.43 ± 0.12	1.50 ± 0.06/1.52 ± 0.07	1.76 ± 0.11/1.78 ± 0.17	1.75 ± 0.11/1.82 ± 0.1

Albumin/Globulin ratio	2.20 ± 0.11/2.45 ± 0.25	2.23 ± 0.06/2.27 ± 0.13	2.02 ± 0.07/2.08 ± 0.14	2.05 ± 0.15/2.02 ± 0.08
Total bilirubin	0.1 ± 0.02/0.08 ± 0.01	0.09 ± 0.03/0.01 ± 0.01	0.17 ± 0.02/0.19 ± 0.14	0.18 ± 0.04/0.19 ± 0.06
Total cholesterol	87.9 ± 4.3/89.6 ± 15.4	77.6 ± 5.0/102.9 ± 13.8	79.6 ± 10.4/103.6 ± 20.9	74.8 ± 6.4/96.3 ± 9.4
Triglycerides	41.8 ± 16.1/15.5 ± 7.2	28.9 ± 9.8/24.6 ± 6.6	44.5 ± 11.4/34.5 ± 2.8	37.7 ± 18.0/28.1 ± 6.4
Urea	39.2 ± 4.9/34.3 ± 5.1	38.3 ± 3.9/33.8 ± 8.7	33.5 ± 5.3/40.3 ± 5.9	33.8 ± 4.3/47.8 ± 5.8
Creatinine	0.43 ± 0.1/0.43 ± 0.08	0.43 ± 0.11/0.6 ± 0.16	0.43 ± 0.03/0.55 ± 0.14	0.39 ± 0.09/0.49 ± 0.05
Creatine Kinase	309.0 ± 39.4/217.9 ± 27.4	332.4 ± 53.7/268.1 ± 29.0	258.1 ± 49.5/190.3 ± 29.7	285.0 ± 45.2/165.7 ± 32.1
Sodium	138.4 ± 0.9/137.5 ± 0.9	136.8 ± 1.3/137.5 ± 0.9	137.7 ± 1.3/138.0 ± 0.9	138.7 ± 1.0/137.2 ± 0.6
Potassium	4.57 ± 0.27/4.13 ± 0.2	4.59 ± 0.27/4.39 ± 0.26	4.40 ± 1.4/4.12 ± 0.12	4.37 ± 0.17/4.12 ± 0.17
Calcium	11.0 ± 0.4/11.0 ± 0.2	11.2 ± 0.2/11.0 ± 0.3	11.4 ± 0.2/11.5 ± 0.4	11.5 ± 0.1/11.6 ± 0.3
Chloride	103.0 ± 2.0/102.9 ± 0.9	102.0 ± 1.3/103.6 ± 1.3	104.3 ± 1.0/104.6 ± 0.9	104.1 ± 2.1/104.2 ± 1.4
Phosphorous	7.5 ± 0.3/6.8 ± 0.3	8.3 ± 0.4/7.1 ± 0.6	8.0 ± 0.4/6.6 ± 0.3	7.5 ± 0.3/7.2 ± 0.6

Table 2.3 Coagulation analysis

	Day 3		Day 15	
	hGTS13: 31.9 µg/kg Males/Females	Vehicle Control: 0 µg/kg Males/Females	hGTS13: 31.9 µg/kg Males/Females	Vehicle Control: 0 µg/kg Males/Females
Prothrombin time (sec)	16.1 ± 0.6/15.8 ± 0.3	15.5 ± 1.0/15.4 ± 0.5	16.7 ± 1.5/16.0 ± 0.5	17.0 ± 0.6/16.1 ± 1.0
Activated partial thromboplastin time (sec)	14.4 ± 2.2/11.9 ± 1.7	12.9 ± 1.1/13.0 ± 0.7	13.1 ± 1.4/11.3 ± 0.2	14.5 ± 1.8/10.7 ± 1.7

Table 2.4 Urine analysis

	Day 3		Day 15
	hGTS13: 31.9 µg/kg	Vehicle Control: 0 µg/kg	hGTS13: 31.9 µg/kg
	Males/Females	Males/Females	Males/Females
Blood (RBC/µL)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)
Bilirubin (mg/dL)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)
Urobilinogen (mg/dL)	Normal (5/5)/Normal (5/5)	Normal (5/5)/Normal (5/5)	Normal (5/5)/Normal (5/5)
Ketone (mg/dL)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)
Protein (mg/dL)	Negative (5/5)/Negative (5/5)	Negative (1/5), 10 (3/5), 30 (1/5)/Negative (5/5)	10 (2/5), 30 (2/5), 100 (1/5)/Negative (3/5), 10 (1/5), 30 (1/5)
Nitrite	Negative (5/5), Negative (5/5)	Negative (5/5)/Negative (3.5), Positive (2/5)	Negative (5/5)/Negative (5/5)
Glucose (mg/dL)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)	Negative (5/5)/Negative (5/5)
pH	7.5 ± 0.5/6.9 ± 0.65	6.5 ± 0.35/6.6 ± 0.42	6.9 ± 0.65/6.6 ± 0.42
Specific gravity	≤1.005/≤1.005	≤1.005-1.020/≤1.005-1.010	1.010-1.020/1.005-1.020
Leukocytes	10 (5/5)/Negative (5/5)	Negative (3/5), 10 (2/5)/Negative (5/5)	Negative (2/5), 10 (2/5), 7 (1/5)/Negative (5/5)
Volume	7.68 ± 1.85/6.58 ± 2.06	8.06 ± 1.98/4.54 ± 2.02	8.10 ± 6.41/10.10 ± 3.94
Color	Light yellow (5/5)/Light yellow (5/5)	Light yellow (5/5)/Light yellow (5/5)	Light yellow (5/5)/Light yellow (5/5)