Additional file – Supplementary Results: Methotrexate Effects (HIB)

Pairwise comparisons of vehicle vs. methotrexate (MTX) treated groups within each housing system (Type IV conventional cage, TRCC, RCC) at Day 29 and Day 57, restricted to Hematology, Immunology and Body weight gain. Two-sided Welch's t-test, $\alpha = 0.05$. Raw and Benjamini-Hochberg (BH) adjusted p-values are reported.

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Table SR1 – Conventional cage vehicle vs. MTX – Day 29

Domain	Parameter	Conventional cage vehicle	Conventional cage MTX	Difference [95% CI]	p-value (raw)	p-value (BH)
Hematology	Basophils	0.05 +/- 0.02 (n=15)	0.04 +/- 0.02 (n=14)	0.02 [0.00, 0.03]	0.013	0.060
Hematology	Monocytes	0.17 +/- 0.04 (n=15)	0.12 +/- 0.04 (n=14)	0.04 [0.01, 0.07]	0.007	0.060
Hematology	White blood cells	7.04 +/- 1.09 (n=15)	5.92 +/- 1.17 (n=14)	1.12 [0.26, 1.99]	0.013	0.060
Hematology	Reticulocytes	186.45 +/- 31.49 (n=15)	139.34 +/- 64.37 (n=14)	47.11 [7.23, 87.00]	0.023	0.069
Hematology	Lymphocytes	5.48 +/- 1.01 (n=15)	4.67 +/- 0.97 (n=14)	0.81 [0.06, 1.57]	0.036	0.082
Hematology	Haemoglobin	15.92 +/- 0.50 (n=15)	15.06 +/- 1.63 (n=14)	0.86 [-0.11, 1.82]	0.079	0.130
Hematology	Neutrophils	1.20 +/- 0.32 (n=15)	0.98 +/- 0.36 (n=14)	0.23 [-0.04, 0.49]	0.087	0.130
Hematology	Platelets	817.93 +/- 97.12 (n=15)	1087.79 +/- 567.73 (n=14)	-269.85 [-600.35, 60.65]	0.102	0.141
Hematology	Eosinophils	0.13 +/- 0.04 (n=15)	0.10 +/- 0.04 (n=14)	0.03 [-0.01, 0.06]	0.126	0.152
Hematology	Haematocrit	45.90 +/- 1.51 (n=15)	44.07 +/- 4.98 (n=14)	1.83 [-1.12, 4.78]	0.206	0.232
Hematology	Red blood cells	8.60 +/- 0.38 (n=15)	8.33 +/- 0.94 (n=14)	0.27 [-0.30, 0.84]	0.332	0.352
Immunology	IgA	32266.95 +/- 7199.22 (n=10)	22524.90 +/- 7325.04 (n=10)	9742.05 [2918.43, 16565.67]	0.008	0.060
Immunology	NK Cells	0.19 +/- 0.06 (n=10)	0.25 +/- 0.06 (n=10)	-0.06 [-0.12, -0.01]	0.033	0.082
Immunology	T Cells	2.80 +/- 0.56 (n=10)	2.32 +/- 0.53 (n=10)	0.48 [-0.03, 0.99]	0.063	0.113
Immunology	TC Cells	0.79 +/- 0.21 (n=10)	0.62 +/- 0.16 (n=10)	0.17 [-0.01, 0.35]	0.057	0.113
Immunology	TH Cells	1.96 +/- 0.30 (n=10)	1.70 +/- 0.40 (n=10)	0.25 [-0.08, 0.59]	0.127	0.152
Immunology	B Cells	1.87 +/- 0.62 (n=10)	1.70 +/- 0.53 (n=10)	0.17 [-0.37, 0.71]	0.519	0.519
Other	Body weight gain	67.98 +/- 16.86 (n=15)	54.74 +/- 11.44 (n=14)	13.24 [2.28, 24.21]	0.020	0.069

Table SR2 – TRCC vehicle vs. MTX – Day 29

Domain	Parameter	TRCC vehicle	TRCC MTX	Difference [95% CI]	p-value (raw)	p-value (BH)
Hematology	Reticulocytes	189.75 +/- 26.08 (n=15)	132.40 +/- 60.78 (n=15)	57.35 [21.61, 93.10]	0.003	0.027
Hematology	Platelets	816.27 +/- 96.43 (n=15)	1085.93 +/- 411.94 (n=15)	-269.67 [-501.81, -37.52]	0.026	0.092
Hematology	Haematocrit	45.87 +/- 1.39 (n=15)	43.63 +/- 4.49 (n=15)	2.24 [-0.33, 4.81]	0.083	0.166
Hematology	Monocytes	0.16 +/- 0.05 (n=15)	0.13 +/- 0.04 (n=15)	0.03 [-0.00, 0.07]	0.075	0.166
Hematology	Haemoglobin	15.55 +/- 0.48 (n=15)	14.87 +/- 1.46 (n=15)	0.67 [-0.16, 1.51]	0.108	0.195
Hematology	Lymphocytes	5.02 +/- 1.13 (n=15)	4.51 +/- 1.11 (n=15)	0.51 [-0.33, 1.35]	0.225	0.289
Hematology	Red blood cells	8.55 +/- 0.31 (n=15)	8.18 +/- 0.99 (n=15)	0.37 [-0.19, 0.94]	0.182	0.289
Hematology	White blood cells	6.50 +/- 1.34 (n=15)	5.88 +/- 1.23 (n=15)	0.62 [-0.34, 1.58]	0.197	0.289
Hematology	Basophils	0.04 +/- 0.01 (n=15)	0.04 +/- 0.03 (n=15)	0.01 [-0.01, 0.02]	0.370	0.399
Hematology	Eosinophils	0.10 +/- 0.03 (n=15)	0.11 +/- 0.04 (n=15)	-0.01 [-0.04, 0.01]	0.377	0.399
Hematology	Neutrophils	1.17 +/- 0.28 (n=15)	1.08 +/- 0.50 (n=15)	0.09 [-0.22, 0.40]	0.546	0.546
Immunology	IgA	33957.40 +/- 9127.94 (n=10)	19555.00 +/- 5733.58 (n=10)	14402.40 [7142.97, 21661.83]	<0.001	0.013
Immunology	NK Cells	0.19 +/- 0.05 (n=10)	0.27 +/- 0.06 (n=10)	-0.08 [-0.14, -0.03]	0.005	0.027
Immunology	TH Cells	1.85 +/- 0.46 (n=10)	1.41 +/- 0.41 (n=10)	0.44 [0.04, 0.85]	0.034	0.103
Immunology	T Cells	2.46 +/- 0.57 (n=10)	1.96 +/- 0.60 (n=10)	0.50 [-0.05, 1.05]	0.071	0.166
Immunology	B Cells	1.93 +/- 0.71 (n=10)	1.58 +/- 0.51 (n=10)	0.35 [-0.23, 0.93]	0.221	0.289
Immunology	TC Cells	0.66 +/- 0.21 (n=10)	0.55 +/- 0.20 (n=10)	0.11 [-0.08, 0.31]	0.242	0.291
Other	Body weight gain	74.57 +/- 19.73 (n=15)	57.87 +/- 14.92 (n=15)	16.70 [3.57, 29.83]	0.015	0.066

Table SR3 – RCC vehicle vs. MTX – Day 29

Domain	Parameter	RCC vehicle	RCC MTX	Difference [95% CI]	p-value (raw)	p-value (BH)
Hematology	Basophils	0.08 +/- 0.04 (n=15)	0.04 +/- 0.02 (n=15)	0.05 [0.02, 0.07]	<0.001	0.003
Hematology	Platelets	829.87 +/- 59.50 (n=15)	1208.60 +/- 379.07 (n=15)	-378.73 [-590.29, -167.17]	0.002	0.007
Hematology	Haemoglobin	15.86 +/- 0.65 (n=15)	14.18 +/- 2.30 (n=15)	1.68 [0.37, 2.99]	0.015	0.034
Hematology	Reticulocytes	180.73 +/- 30.16 (n=15)	112.69 +/- 97.09 (n=15)	68.04 [12.57, 123.51]	0.019	0.038
Hematology	Haematocrit	45.91 +/- 2.07 (n=15)	41.10 +/- 7.07 (n=15)	4.81 [0.78, 8.83]	0.022	0.040
Hematology	Monocytes	0.16 +/- 0.06 (n=15)	0.12 +/- 0.06 (n=15)	0.05 [-0.00, 0.09]	0.053	0.079
Hematology	White blood cells	7.16 +/- 0.96 (n=15)	6.24 +/- 1.45 (n=15)	0.92 [-0.01, 1.84]	0.052	0.079
Hematology	Red blood cells	8.63 +/- 0.48 (n=15)	7.89 +/- 1.37 (n=15)	0.74 [-0.05, 1.53]	0.063	0.088
Hematology	Lymphocytes	5.93 +/- 0.91 (n=15)	5.23 +/- 1.18 (n=15)	0.70 [-0.10, 1.49]	0.082	0.099
Hematology	Neutrophils	0.85 +/- 0.24 (n=15)	0.70 +/- 0.30 (n=15)	0.15 [-0.06, 0.35]	0.151	0.168
Hematology	Eosinophils	0.12 +/- 0.03 (n=15)	0.12 +/- 0.05 (n=15)	-0.01 [-0.04, 0.03]	0.736	0.736
Immunology	IgA	33535.10 +/- 8894.44 (n=10)	15549.30 +/- 5432.21 (n=10)	17985.80 [10956.71, 25014.89]	<0.001	0.001
Immunology	TC Cells	0.87 +/- 0.22 (n=10)	0.47 +/- 0.23 (n=10)	0.40 [0.19, 0.61]	<0.001	0.005
Immunology	T Cells	2.94 +/- 0.72 (n=10)	1.64 +/- 0.88 (n=10)	1.31 [0.55, 2.06]	0.002	0.007
Immunology	TH Cells	2.07 +/- 0.51 (n=10)	1.13 +/- 0.70 (n=10)	0.94 [0.36, 1.52]	0.003	0.009
Immunology	NK Cells	0.17 +/- 0.05 (n=10)	0.23 +/- 0.09 (n=10)	-0.06 [-0.13, 0.01]	0.074	0.096
Immunology	B Cells	2.30 +/- 0.48 (n=10)	1.91 +/- 0.68 (n=10)	0.39 [-0.17, 0.95]	0.158	0.168
Other	Body weight gain	76.15 +/- 19.59 (n=15)	54.80 +/- 16.81 (n=15)	21.35 [7.69, 35.02]	0.003	0.009

Table SR4 – Conventional cage vehicle vs. MTX – Day 57

Domain	Parameter	Conventional cage vehicle	Conventional cage MTX	Difference [95% CI]	p-value (raw)	p-value (BH)
Hematology	Haematocrit	48.04 +/- 1.22 (n=5)	49.98 +/- 1.18 (n=4)	-1.94 [-3.86, -0.01]	0.049	0.878
Hematology	Basophils	0.02 +/- 0.01 (n=5)	0.02 +/- 0.01 (n=4)	0.01 [-0.01, 0.02]	0.336	0.950
Hematology	Eosinophils	0.09 +/- 0.04 (n=5)	0.07 +/- 0.03 (n=4)	0.02 [-0.04, 0.08]	0.413	0.950
Hematology	Haemoglobin	16.26 +/- 0.68 (n=5)	16.60 +/- 0.50 (n=4)	-0.34 [-1.27, 0.59]	0.414	0.950
Hematology	Monocytes	0.08 +/- 0.02 (n=5)	0.10 +/- 0.04 (n=4)	-0.02 [-0.07, 0.04]	0.477	0.950
Hematology	Neutrophils	1.01 +/- 0.22 (n=5)	1.11 +/- 0.36 (n=4)	-0.10 [-0.64, 0.43]	0.631	0.950
Hematology	Red blood cells	9.08 +/- 0.29 (n=5)	8.96 +/- 0.46 (n=4)	0.11 [-0.57, 0.79]	0.686	0.950
Hematology	Reticulocytes	175.30 +/- 25.99 (n=5)	166.50 +/- 14.37 (n=4)	8.80 [-24.14, 41.74]	0.542	0.950
Hematology	Lymphocytes	3.74 +/- 0.73 (n=5)	3.81 +/- 1.15 (n=4)	-0.07 [-1.78, 1.64]	0.920	0.954
Hematology	Platelets	795.60 +/- 120.45 (n=5)	799.75 +/- 89.92 (n=4)	-4.15 [-170.11, 161.81]	0.954	0.954
Hematology	White blood cells	4.97 +/- 0.69 (n=5)	5.13 +/- 1.25 (n=4)	-0.17 [-2.03, 1.70]	0.822	0.954
Immunology	B Cells	1.31 +/- 0.31 (n=5)	1.52 +/- 0.58 (n=4)	-0.21 [-1.07, 0.66]	0.555	0.950
Immunology	NK Cells	0.17 +/- 0.06 (n=5)	0.14 +/- 0.04 (n=4)	0.04 [-0.04, 0.12]	0.316	0.950
Immunology	T Cells	2.20 +/- 0.67 (n=5)	1.87 +/- 0.36 (n=4)	0.33 [-0.52, 1.17]	0.384	0.950
Immunology	TH Cells	1.62 +/- 0.51 (n=5)	1.31 +/- 0.14 (n=4)	0.30 [-0.32, 0.92]	0.263	0.950
Immunology	TC Cells	0.53 +/- 0.19 (n=5)	0.55 +/- 0.15 (n=4)	-0.02 [-0.28, 0.24]	0.851	0.954
Immunology	IgA	27932.80 +/- 4227.35 (n=5)	26510.50 +/- 8560.40 (n=4)	1422.30 [-11367.62, 14212.22]	0.776	0.954
Other	Body weight gain	100.16 +/- 25.72 (n=5)	93.50 +/- 21.29 (n=4)	6.66 [-30.43, 43.75]	0.684	0.950

Table SR5 – TRCC vehicle vs. MTX – Day 57

Domain	Parameter	TRCC vehicle	TRCC MTX	Difference [95% CI]	p-value (raw)	p-value (BH)
Hematology	Basophils	0.02 +/- 0.01 (n=5)	0.02 +/- 0.01 (n=5)	-0.00 [-0.02, 0.01]	0.636	0.914
Hematology	Eosinophils	0.09 +/- 0.03 (n=5)	0.08 +/- 0.02 (n=5)	0.01 [-0.03, 0.04]	0.695	0.914
Hematology	Haematocrit	47.70 +/- 1.83 (n=5)	48.58 +/- 1.17 (n=5)	-0.88 [-3.19, 1.43]	0.397	0.914
Hematology	Haemoglobin	15.96 +/- 0.39 (n=5)	16.50 +/- 0.51 (n=5)	-0.54 [-1.21, 0.13]	0.100	0.914
Hematology	Monocytes	0.09 +/- 0.01 (n=5)	0.10 +/- 0.02 (n=5)	-0.01 [-0.04, 0.02]	0.351	0.914
Hematology	Neutrophils	1.09 +/- 0.36 (n=5)	1.00 +/- 0.19 (n=5)	0.09 [-0.35, 0.52]	0.648	0.914
Hematology	Platelets	806.40 +/- 76.50 (n=5)	826.60 +/- 55.20 (n=5)	-20.20 [-119.19, 78.79]	0.646	0.914
Hematology	Red blood cells	8.97 +/- 0.49 (n=5)	9.12 +/- 0.50 (n=5)	-0.16 [-0.88, 0.57]	0.632	0.914
Hematology	Reticulocytes	192.02 +/- 16.70 (n=5)	200.28 +/- 12.88 (n=5)	-8.26 [-30.26, 13.74]	0.408	0.914
Hematology	Lymphocytes	3.34 +/- 0.67 (n=5)	3.41 +/- 0.64 (n=5)	-0.07 [-1.02, 0.89]	0.874	0.997
Hematology	White blood cells	4.63 +/- 0.93 (n=5)	4.62 +/- 0.76 (n=5)	0.00 [-1.24, 1.24]	0.997	0.997
Immunology	B Cells	1.55 +/- 0.53 (n=5)	1.41 +/- 0.30 (n=5)	0.14 [-0.52, 0.80]	0.621	0.914
Immunology	TH Cells	1.22 +/- 0.32 (n=5)	1.11 +/- 0.27 (n=5)	0.11 [-0.32, 0.54]	0.563	0.914
Immunology	TC Cells	0.43 +/- 0.15 (n=5)	0.47 +/- 0.20 (n=5)	-0.04 [-0.31, 0.22]	0.711	0.914
Immunology	IgA	37248.80 +/- 5572.42 (n=5)	30766.00 +/- 8121.63 (n=5)	6482.80 [-3908.32, 16873.92]	0.184	0.914
Immunology	NK Cells	0.16 +/- 0.04 (n=5)	0.16 +/- 0.05 (n=5)	0.00 [-0.06, 0.07]	0.927	0.997
Immunology	T Cells	1.64 +/- 0.33 (n=5)	1.63 +/- 0.43 (n=5)	0.01 [-0.56, 0.57]	0.978	0.997
Other	Body weight gain	122.62 +/- 25.47 (n=5)	101.38 +/- 21.75 (n=5)	21.24 [-13.45, 55.93]	0.195	0.914

Table SR6 – RCC vehicle vs. MTX – Day 57

Domain	Parameter	RCC vehicle	RCC MTX	Difference [95% CI]	p-value (raw)	p-value (BH)
Hematology	Eosinophils	0.07 +/- 0.03 (n=4)	0.10 +/- 0.04 (n=5)	-0.03 [-0.09, 0.03]	0.262	0.911
Hematology	Haematocrit	46.50 +/- 1.64 (n=4)	47.64 +/- 2.33 (n=5)	-1.14 [-4.27, 1.99]	0.418	0.911
Hematology	Haemoglobin	16.18 +/- 0.38 (n=4)	16.42 +/- 0.52 (n=5)	-0.25 [-0.95, 0.46]	0.439	0.911
Hematology	Monocytes	0.10 +/- 0.02 (n=4)	0.11 +/- 0.04 (n=5)	-0.01 [-0.06, 0.04]	0.557	0.911
Hematology	Platelets	802.50 +/- 93.95 (n=4)	762.80 +/- 72.97 (n=5)	39.70 [-102.62, 182.02]	0.515	0.911
Hematology	Red blood cells	8.62 +/- 0.44 (n=4)	8.86 +/- 0.46 (n=5)	-0.24 [-0.96, 0.48]	0.449	0.911
Hematology	Reticulocytes	160.38 +/- 29.46 (n=4)	173.98 +/- 30.23 (n=5)	-13.61 [-61.39, 34.18]	0.519	0.911
Hematology	Basophils	0.03 +/- 0.02 (n=4)	0.03 +/- 0.01 (n=5)	-0.00 [-0.03, 0.03]	0.965	0.973
Hematology	Lymphocytes	3.87 +/- 0.59 (n=4)	4.02 +/- 0.75 (n=5)	-0.15 [-1.21, 0.91]	0.747	0.973
Hematology	Neutrophils	0.83 +/- 0.30 (n=4)	0.85 +/- 0.28 (n=5)	-0.02 [-0.50, 0.45]	0.916	0.973
Hematology	White blood cells	4.92 +/- 0.56 (n=4)	5.14 +/- 1.03 (n=5)	-0.22 [-1.51, 1.08]	0.701	0.973
Immunology	NK Cells	0.12 +/- 0.02 (n=4)	0.16 +/- 0.06 (n=5)	-0.05 [-0.12, 0.03]	0.161	0.911
Immunology	T Cells	2.16 +/- 0.39 (n=4)	1.95 +/- 0.34 (n=5)	0.21 [-0.39, 0.81]	0.432	0.911
Immunology	TH Cells	1.61 +/- 0.25 (n=4)	1.44 +/- 0.22 (n=5)	0.17 [-0.22, 0.55]	0.328	0.911
Immunology	B Cells	1.54 +/- 0.39 (n=4)	1.53 +/- 0.18 (n=5)	0.01 [-0.58, 0.60]	0.973	0.973
Immunology	TC Cells	0.56 +/- 0.17 (n=4)	0.53 +/- 0.14 (n=5)	0.03 [-0.23, 0.28]	0.808	0.973
Immunology	IgA	37855.00 +/- 7594.25 (n=5)	39443.00 +/- 12769.89 (n=5)	-1588.00 [-17539.95, 14363.95]	0.818	0.973
Other	Body weight gain	115.96 +/- 15.36 (n=5)	79.46 +/- 17.04 (n=5)	36.50 [12.80, 60.20]	0.008	0.136