

**Table S1:** Composition of different Fe diets used in the study set up

<b>Ingredient</b>	<b>Control diet</b>	<b>Fe deficient diet</b>	<b>35 ppm Fe diet</b>	<b>70 ppm Fe diet</b>
	<i>g/kg</i>			
Casein	200	200	200	200
Sucrose	93	100	93	86
Cornstarch	398	398	398	398
Dyetrose	132	132	132	132
L-Cysteine	3	3	3	3
Cellulose	50	50	50	50
Soybean oil	70	70	70	70
t-butylhydroquinone	0.014	0.014	0.014	0.014
Mineral Mix (no Fe)	35	35	35	35
Vitamin Mix	10	10	10	10
Choline bitartrate	2.5	2.5	2.5	2.5
<b>Fe Premix ferric citrate (5 mg Fe / g)</b>	<b>7</b>	<b>-</b>	<b>-</b>	<b>7</b>
<b>Fe Premix ferrous sulphate (5 mg Fe / g)</b>	<b>-</b>	<b>-</b>	<b>7</b>	<b>7</b>

**Table S2:** Faecal microbiota composition of the donor and rats fed diets differing only in Fe concentration at baseline, midpoint and endpoint assessed by quantitative PCR and expressed as log number of 16S rRNA gene copies / g caecum.

Group	Time	total 16S		Firmicutes		<i>Bacteroides</i> spp.		<i>Clostridium</i> Cluster IV		<i>F. prausnitzii</i>		<i>E. hallii</i>		<i>Enterobacteriaceae</i>		<i>Lactobacillus/L. euconostoc/Ped. iococcus</i> spp.	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Donor		12.58		10.95		9.99		10.26		10.08		9.18		7.24		6.43	
control	BL	11.61 <sup>b</sup>	0.06	10.28 <sup>b</sup>	0.06	9.73 <sup>b</sup>	0.05	9.81 <sup>b</sup>	0.06	9.26 <sup>b</sup>	0.07	8.72	0.09	7.19 <sup>b</sup>	0.14	6.28	0.08
	15 weeks	11.74 <sup>b</sup>	0.14	10.33 <sup>b</sup>	0.17	9.88 <sup>b</sup>	0.06	10.77 <sup>a</sup>	0.15	9.68 <sup>ab</sup>	0.09	9.11	0.11	8.13 <sup>a</sup>	0.15	6.16	0.14
	19 weeks	12.21 <sup>a</sup>	0.14	10.87 <sup>a</sup>	0.13	10.34 <sup>a</sup>	0.14	10.61 <sup>a</sup>	0.11	9.90 <sup>a</sup>	0.14	9.32	0.19	8.38 <sup>a</sup>	0.25	6.05	0.08
deficient	BL	11.84	0.08	10.35	0.03	9.84	0.07	9.84 <sup>b</sup>	0.15	9.36	0.14	8.70	0.16	7.28	0.19	6.57	0.25
	15 weeks	12.02	0.09	10.53	0.12	10.12	0.13	10.89 <sup>a</sup>	0.15	10.06	0.39	8.77	0.40	7.66	0.22	6.21	0.16
	19 weeks	12.08	0.15	10.71	0.16	10.29	0.13	10.50 <sup>ab</sup>	0.15	9.71	0.15	8.74	0.22	7.98	0.26	6.08	0.14
35 ppm Fe	BL	12.09	0.11	10.69 <sup>ab</sup>	0.10	10.02	0.09	9.97 <sup>b</sup>	0.14	9.51	0.14	9.19	0.06	7.52	0.15	6.55	0.09
	15 weeks	12.03	0.04	10.47 <sup>b</sup>	0.05	10.36	0.08	10.85 <sup>a</sup>	0.08	9.77	0.08	9.12	0.09	8.14	0.14	6.64	0.35
	19 weeks	12.25	0.08	10.89 <sup>a</sup>	0.09	10.48	0.07	11.12 <sup>a</sup>	0.08	10.00	0.06	9.38	0.09	8.17	0.09	6.41	0.19
70 ppm Fe	BL	11.91	0.06	10.55	0.07	9.96	0.06	10.03 <sup>b</sup>	0.10	9.58	0.09	8.84	0.08	7.20 <sup>b</sup>	0.12	6.74 <sup>a</sup>	0.12
	15 weeks	11.79	0.11	10.26	0.21	9.91	0.12	10.55 <sup>a</sup>	0.13	9.51	0.16	8.74	0.19	7.82 <sup>a</sup>	0.16	6.19 <sup>b</sup>	0.12
	19 weeks	11.90	0.06	10.54	0.11	10.09	0.05	10.68 <sup>a</sup>	0.05	9.57	0.05	8.64	0.13	7.73 <sup>ab</sup>	0.09	6.11 <sup>b</sup>	0.06
Fe excess	BL	11.67	0.28	10.34 <sup>a</sup>	0.23	9.73	0.18	9.98 <sup>b</sup>	0.11	9.52	0.10	9.01	0.11	7.67	0.14	6.53	0.16
	15 weeks	12.02	0.03	10.62 <sup>ab</sup>	0.05	10.27	0.04	10.80 <sup>a</sup>	0.16	10.07	0.20	9.29	0.05	7.94	0.11	6.69	0.28
	19 weeks	12.10	0.12	10.78 <sup>b</sup>	0.14	10.19	0.11	11.01 <sup>a</sup>	0.13	9.81	0.11	9.22	0.12	8.11	0.19	6.58	0.17

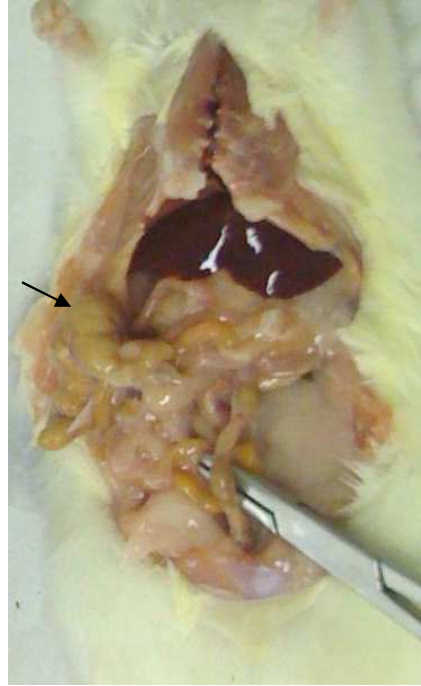
SEM, standard error of the mean; BL, baseline; Fe, iron

<sup>a,b</sup> Mean values within groups and the same bacterial target with unlike superscript letters differ significantly using repeated-measures ANOVA with post-hoc Bonferroni test,  $P < 0.05$

(a)



(b)



**Figure S1: Representative pictures of a control rat (A) and a rat of the Fe deficient group (B) after sacrifice.** Rats of the Fe deficient group had a lighter caecum (indicated by arrows) and a lighter liver colour than control rats.