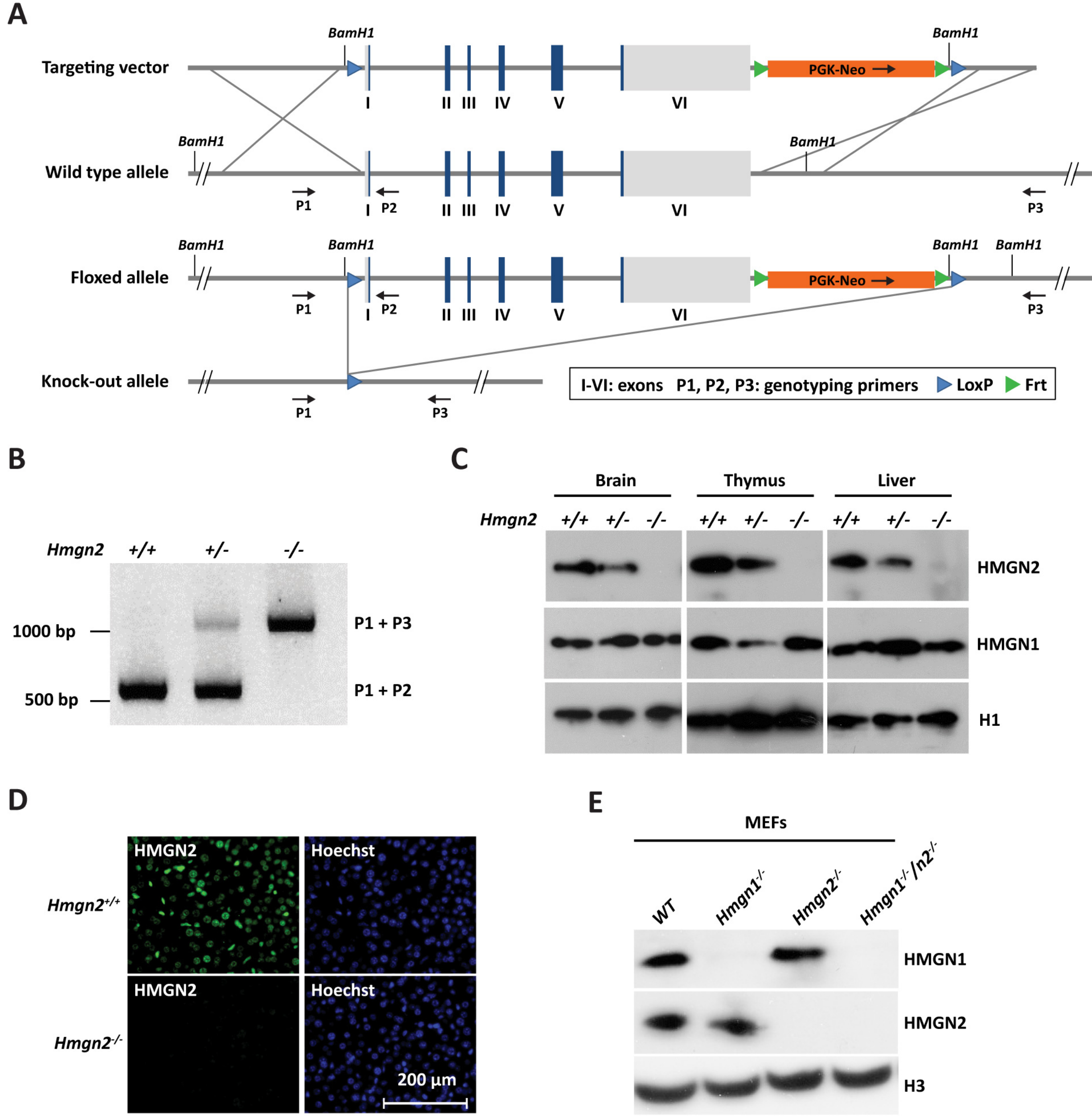
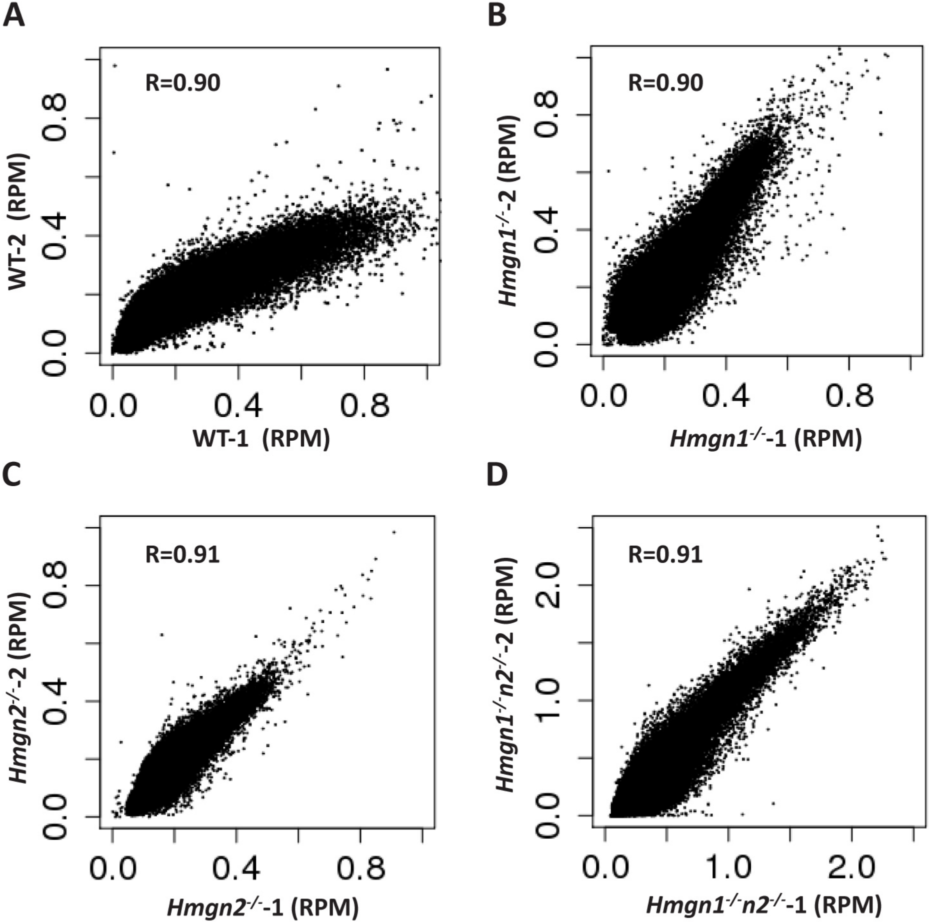
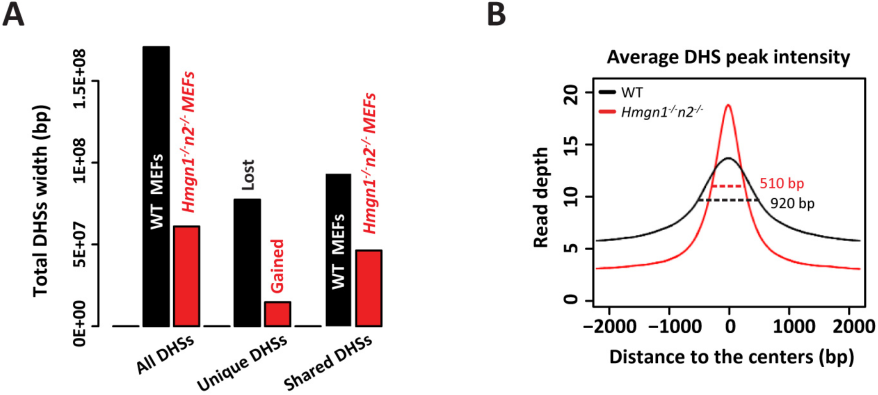


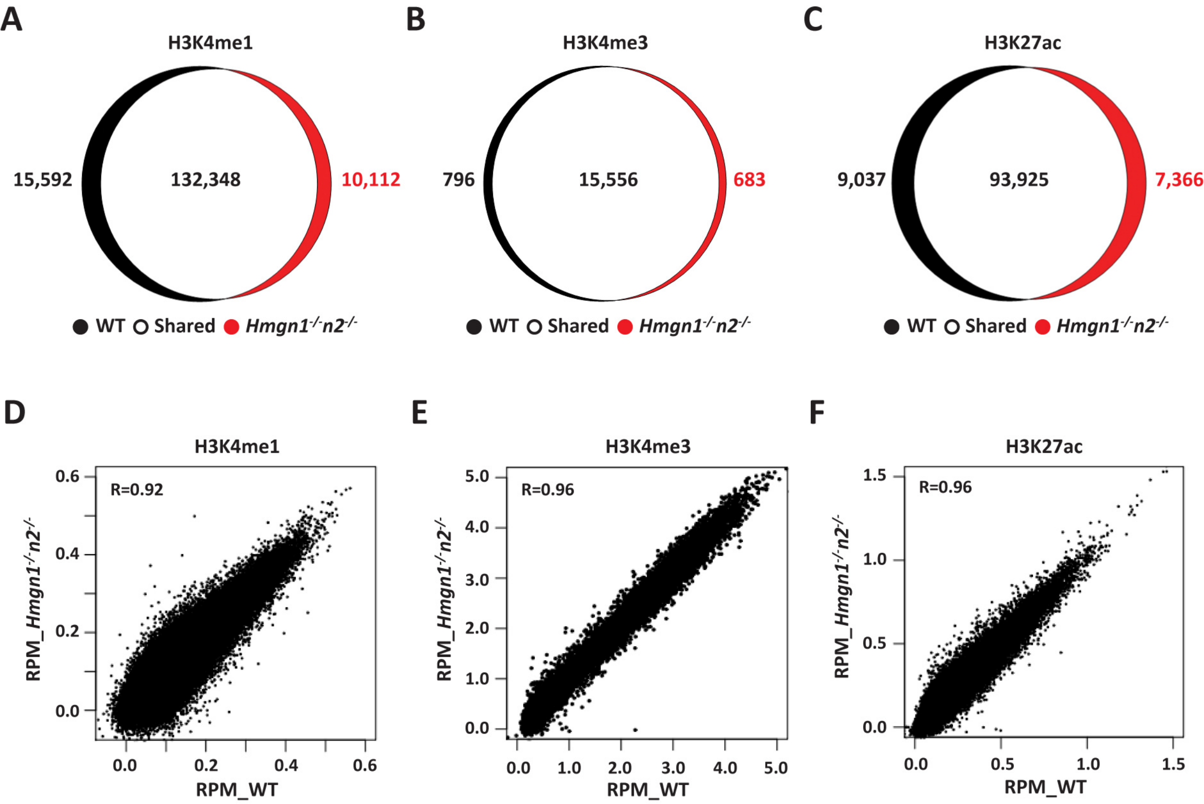
Deng *et al* Figure S1: Generation of *Hmgn2*$^{-/-}$ mice and *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs. (A) The targeting strategy for generation of *Hmgn2*$^{-/-}$ mice. **(B)** PCR genotyping for *Hmgn2*$^{-/-}$ mice. **(C)** Western blots showing loss of HMGN2 protein in various tissues. **(D)** Immunostaining showing loss of HMGN2 proteins in liver. **(E)** Western blots showing loss of HMGN1 and HMGN2 proteins in *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs.



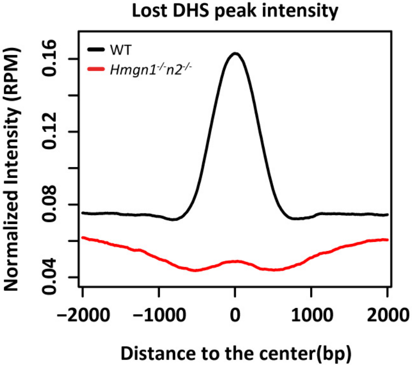
Deng *et al* Figure S2. The reproducibility of DHS-seq assay between biological replicates in MEFs. (A-D) The comparison of DHSs intensity between 2 independent MEF clones with each genotype. R indicates Pearson correlation coefficient.



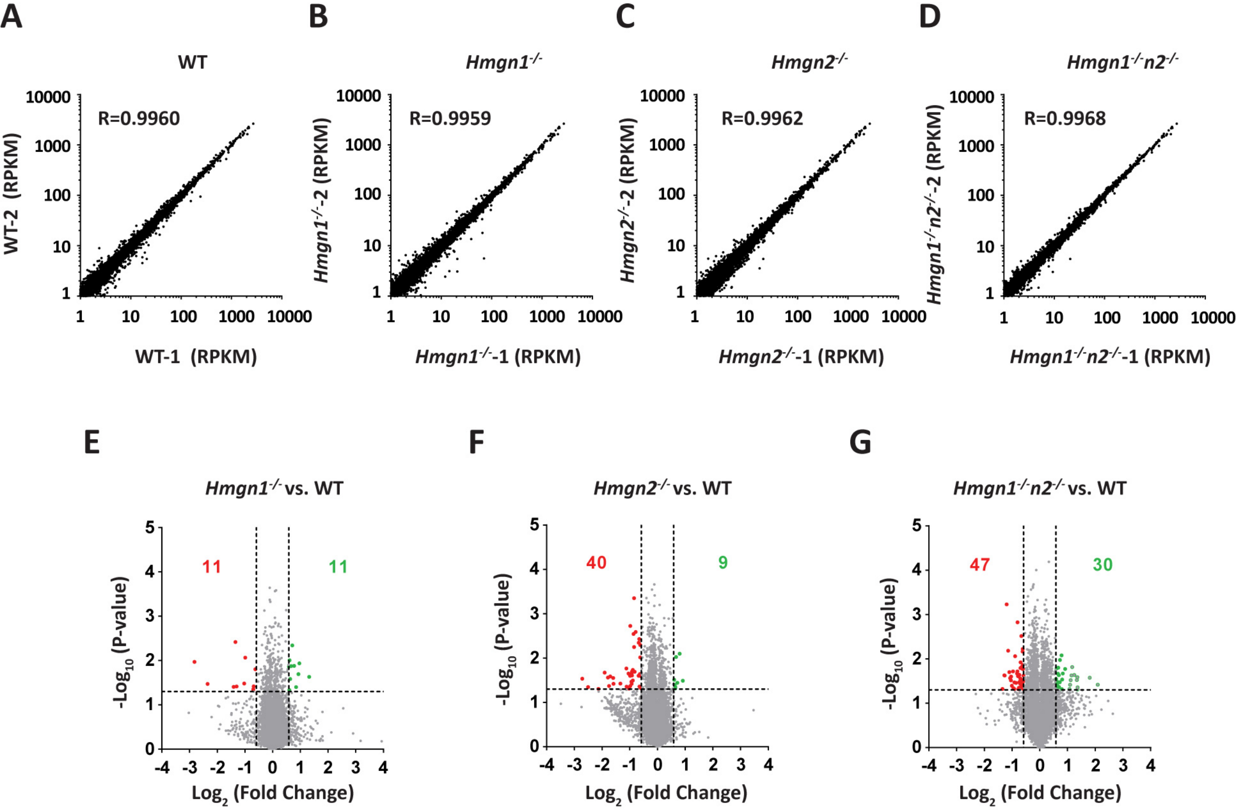
Deng *et al* Figure S3. Reduced DNase I sensitivity in *Hmgn1$^{-/-}$n2$^{-/-}$* MEFs. (A) The total base pairs contained in either all, unique, or shared DHSs of WT and *Hmgn1$^{-/-}$n2$^{-/-}$* MEFs. **(B)** Average read depths of all DHS peaks in WT and *Hmgn1$^{-/-}$n2$^{-/-}$* MEFs. Peak widths calculated at half height for WT and *Hmgn1$^{-/-}$n2$^{-/-}$* MEFs are 920 bp and 510 bp, respectively.



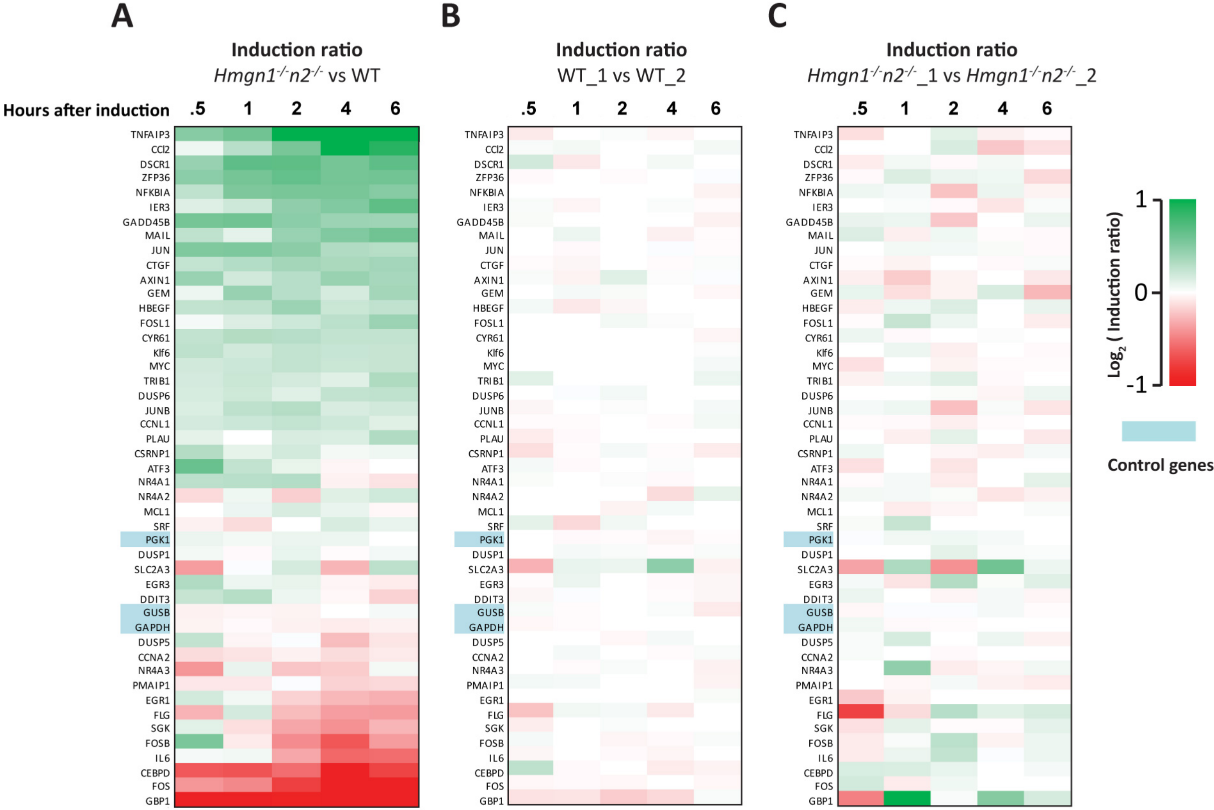
Deng et al Figure S4: Loss of HMGN1 and HMGN2 did not affect histone modification levels. (A-C) The number of peaks identified for H3K4me1, H3K4me3 and H3K27ac by ChIP-seq in WT and *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs. **(D-E)** The correlation of normalized intensity of H3K4me1, H3K4me3, and H3K27ac between WT and *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs. R indicates Pearson correlation coefficient.



Deng *et al* Figure S5. Normalized DHS intensity in WT and *Hmgn1$^{-/-}$n2$^{-/-}$* MEFs at the "lost" DHS sites: peaks detected in WT but not in *Hmgn1$^{-/-}$n2$^{-/-}$* MEFs.



Deng *et al* Figure S6. Transcriptome analysis (RNA-seq) in MEFs lacking HMGN1 or HMGN2 or both. (A-D) The comparison of transcriptome between 2 independent MEF clones with each genotype. R indicates Pearson correlation coefficient. **(E-G)** The significantly up (Green) or down (red) regulated genes in *Hmgn1*$^{-/-}$, *Hmgn2*$^{-/-}$ or *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs.



Deng *et al* Figure S7. Altered immediate early (IE) gene expression in *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs. Heat maps showing the induction fold ratios of IE genes between **(A)** WT and *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs, **(B)** 2 biological replicates of WT MEFs, and **(C)** 2 biological replicates of *Hmgn1*$^{-/-}$*n2*$^{-/-}$ MEFs. The order of the gene array in (B) and (C) was preserved as obtained from hierarchical clustering of the induction fold ratios between *Hmgn1*$^{-/-}$*n2*$^{-/-}$ and WT (A).

Table S1. Summary of sequencing results

A. DNase-seq data

Sample ID	# Reads	% Align (PF)	% Mismatch Rate (PF)	% >=Q30 bases (PF)	% unique reads
WT_1	84,301,522	86.76	0.52	93.34	96.30
WT_2	98,744,222	84.97	0.51	93.75	89.94
Hmgn1$^{-/-}$_1	79,620,656	83.94	0.86	90.58	88.65
Hmgn1$^{-/-}$_2	62,673,782	84.13	0.87	91.30	89.18
Hmgn2$^{-/-}$_1	87,734,440	83.49	0.71	91.81	90.00
Hmgn2$^{-/-}$_2	82,498,808	83.25	0.69	92.73	90.66
Hmgn1$^{-/-}$*n2*$^{-/-}$_1	71,739,638	68.51	2.31	81.11	89.22
Hmgn1$^{-/-}$*n2*$^{-/-}$_2	74,113,682	68.19	2.37	81.16	86.57

B. ChIP-seq data

Genotypes	Histone marks	# Reads	% Align (PF)	% Mismatch Rate (PF)	% >=Q30 bases (PF)	% unique reads
WT	H3K4me1	100670054	92.99	0.28	91.21	87.29
	H3K4me3	67064272	91.97	0.35	89.11	27.15
	H3K27ac	90,340,242	93.57	0.28	95.02	89.80
	input	111,292,572	78.24	0.55	96.07	86.99
Hmgn1$^{-/-}$*n2*$^{-/-}$	H3K4me1	84598176	92.34	0.33	91.2	81.69
	H3K4me3	90032064	92.19	0.44	88.76	15.81
	H3K27ac	86,260,526	93.40	0.29	94.94	87.49
	input	78,142,334	78.47	0.56	95.82	81.11

C. MNase-seq data

Sample ID	# Reads	% Align (PF)	% Mismatch Rate (PF)	% >=Q30 bases (PF)	% unique reads
WT_1_Extensive	45,232,694	55.23	1.49	90.22	81.73
WT_1_Limited	105,460,950	58.03	1.39	90.81	79.62
WT_2_Extensive	40,785,338	67.34	1.06	89.76	89.60
WT_2_Limited	105,738,078	68.34	1.05	90.29	85.96
Hmgn1$^{-/-}$*n2*$^{-/-}$_1_Extensive	117,927,050	55.74	1.49	89.50	78.01
Hmgn1$^{-/-}$*n2*$^{-/-}$_1_Limited	61,846,742	63.76	1.22	90.57	84.55
Hmgn1$^{-/-}$*n2*$^{-/-}$_2_Extensive	137,673,830	60.04	1.30	89.28	78.99
Hmgn1$^{-/-}$*n2*$^{-/-}$_2_Limited	45,696,614	62.70	1.21	90.71	85.52

D. RNA-seq data

Sample ID	# Reads	% Align (PF)	% Mismatch Rate (PF)	% >=Q30 bases (PF)	% unique reads
WT_1	142,436,416	81.45	0.28	94.59	70.26
WT_2	129,445,668	81.62	0.28	94.54	72.28
Hmgn1$^{-/-}$_1	136,165,262	82.01	0.30	94.06	67.27
Hmgn1$^{-/-}$_2	152,345,120	82.64	0.30	94.10	68.19
Hmgn2$^{-/-}$_1	134,489,208	84.48	0.31	92.96	67.96
Hmgn2$^{-/-}$_2	198,318,106	83.60	0.31	92.77	65.25
Hmgn1$^{-/-}$*n2*$^{-/-}$_1	145,437,314	82.47	0.27	94.85	72.50
Hmgn1$^{-/-}$*n2*$^{-/-}$_2	114,930,390	82.30	0.28	94.91	74.33

Table S2: Primer sequences used in the study

q-PCR Primers for DNase I accessibility

Region	Primer sequences	Size	Chromosome
Control	F: CACGTGAATGTTGGCTTTCCAT R: ACCTTCTTCTCAGCACCTCATG	176 bp	1
Wrb H3K4me3	F: GTCCCTGTCCTCGTTTGTAAGT R: GATCTGTGAGAAGCTCGGAGG	117 bp	16
Wrb H3K4me1	F: TGAAGGTAAGTTGACAGCCCTC R: TGGATGAGCAGTAACACAGCAT	195 bp	16
Ier3 H3K4me3	F: ATTTTCACCTTCGACCCTCTCC R: TGTTACCTACAAGTTCCAGGGC	167 bp	17
Ier3 H3K4me1	F: TTGGAAGGTTCTTTGTGACCCA R: ACATTCCCCAGTTCAGAAAGCA	165 bp	17
Hdgf H3K4me3	F: ACTCTTATACCGCCACCAACAG R: CATGTCCCAACCCACGTATTTG	115 bp	3
Hdgf H3K4me1	F: AACCCTACCTGATGCCTCTACT R: ACAGAATCTAAGCTGTGTGGCA	214 bp	3
Atp6 H3K4me3	F: CGGAGCCTTGCTTGCGTA R: AGCGCTCTTTCTACAACCCC	130 bp	11
Atp6 H3K4me1	F: CCTGTACTCTCCAGGTTCCG R: AAGTCCGATTCCCATGGTCC	135 bp	11
Ccl2 H3K4me3	F: CAATAAAAGGCTGCCTCAGAGC R: AATGGGGTGATATGCTGGGAAG	234 bp	11
Ccl2 H3K4me1	F: GTAGCTGCTCTTTGAGTCTGGT R: ATTCACAGTGGCTTGGCTATGA	147 bp	11
Gem H3K4me3	F: TAAGTTAGGAAGGCAGCCAGTC R: TTAGAAACCCCCATCAGAAGCG	110 bp	4
Gem H3K4me1	F: AAGAAGTCAGGAAGCAGGAAGG R: CCAGCTTTGCCAAGAAAACAGA	142 bp	4

q-PCR Primers for Heat Shock:

Gene	Primer sequences	Size
Hsp70 (1)	F: AGCCTTCCAGAAGCAGAGC R: GTCGGTGAAGGCCACGTA	165
Hsp70 (2)	F: CGAGGAGGTGGATTAGAGG R: CACAAAGAACTCAACAGTCTCC	389
β-actin	F: TCCTCCTGAGCGCAAGTACTCT R: GCTGATCCACATCTGCTGGAA	82
GAPDH	F: AATGTGTCCGTCGTGGATCTGA R: GATGCCTGCTTCACCACCTTCT	83

Table S3. Genes affected by loss of HMGNs in MEFs.

A. Genes disregulated in *Hmgn1*$^{-/-}$ MEFs

Gene Symbol	Fold Change (*Hmgn1*$^{-/-}$ vs WT)	p-value
Bhlhe22	2.50	0.0233
Rarres1	1.95	0.0115
Nipsnap1	1.91	0.0202
Spint2	1.80	0.0398
Slc1a5	1.71	0.0132
Galnt16	1.63	0.0045
Slc27a6	1.59	0.0133
Ptgis	1.55	0.0260
Plcl1	1.53	0.0099
Phospho1	1.52	0.0463
Gdf15	1.50	0.0137
Lrrc25	-1.54	0.0157
Sema3f	-1.61	0.0381
Gpatch11	-1.62	0.0461
Dusp27	-1.62	0.0445
Cd300lb	-1.98	0.0086
Hmgn1	-2.04	0.0330
Evi2b	-2.46	0.0385
Gm7367	-2.54	0.0038
Pf4	-2.65	0.0393
Cadm1	-5.09	0.0337
AF251705	-7.08	0.0107

B. Genes disregulated in *Hmgn2*$^{-/-}$ MEFs

Gene Symbol	Fold Change (*Hmgn2*$^{-/-}$ vs WT)	p-value
Slc7a3	1.89	0.0324
Spata7	1.74	0.0080
2700086A05Rik	1.64	0.0361
Hipk2	1.59	0.0094
Hoxa6	1.57	0.0451
Kcnj15	1.56	0.0484
Ppm1l	1.54	0.0403
Nmrk1	1.52	0.0308
Bmp5	1.51	0.0312
Pdpn	-1.51	0.0257
1700007L15Rik	-1.51	0.0476
Atp5j2	-1.53	0.0245
Ndufb2	-1.53	0.0051
Etv4	-1.54	0.0096
Cmah	-1.55	0.0229
Lyn	-1.57	0.0438
1110046J04Rik	-1.58	0.0037
Arhgap27	-1.58	0.0240
5730416F02Rik	-1.60	0.0045
Ppbp	-1.60	0.0251
Kcnab2	-1.72	0.0026
Pdgfb	-1.77	0.0203
Ndufaf3	-1.79	0.0004
2610507I01Rik	-1.79	0.0056
Hoxd8	-1.82	0.0029
Hhex	-1.85	0.0186
Tuba8	-1.85	0.0321
Galnt3	-1.89	0.0200
Rps6	-1.89	0.0246
Cxcr4	-1.91	0.0219
Cadm1	-1.91	0.0380
Ccl2	-1.92	0.0341
Slc25a45	-1.97	0.0019
AA467197	-1.98	0.0429
Cxcl16	-1.98	0.0251
Clec16a	-2.04	0.0488
Spon2	-2.07	0.0223
Gm20257	-2.17	0.0440
Mbp	-2.18	0.0172
Tceanc2	-2.53	0.0376
Lair1	-3.01	0.0379
Pld4	-3.02	0.0282
Lce1h	-3.26	0.0259
Cd33	-3.35	0.0426
1810011H11Rik	-3.45	0.0275
Ms4a6c	-3.75	0.0213
P2ry13	-4.38	0.0495
Ifi27l2a	-5.67	0.0450
Pcsk9	-6.56	0.0292

C. Genes disregulated in *Hmgnn1*$^{-/-}$ *2*$^{-/-}$ MEFs

Gene Symbol	Fold Change (*Hmgn1*$^{-/-}$ *n2*$^{-/-}$ vs WT)	p-value
Gm1045	4.27	0.0383
2700086A05Rik	3.50	0.0266
Slc16a2	2.58	0.0437
Stmn2	2.57	0.0489
Hoxa11os	2.56	0.0258
Osr2	2.53	0.0300
Wnt10b	2.38	0.0230
Gjb5	2.25	0.0153
Efna1	2.21	0.0236
Aoc3	2.17	0.0303
LOC100504608	2.14	0.0385
Emilin2	2.10	0.0478
Igdcc4	1.89	0.0169
Pigb	1.78	0.0214
Tnfsf15	1.75	0.0293
Ramp3	1.74	0.0481
6330403K07Rik	1.73	0.0083
Folr2	1.66	0.0106
Lama4	1.65	0.0336
Pex11a	1.62	0.0430
2610305D13Rik	1.61	0.0233
Sh3rf3	1.59	0.0158
Syt11	1.58	0.0216
Myl12b	1.58	0.0427
Dgat2	1.55	0.0392
Plekhg5	1.54	0.0328
Rpl36	1.52	0.0164
Ptch1	1.52	0.0327
Tiam1	1.50	0.0284
Acot1	1.50	0.0285
Slc7a7	-1.51	0.0238
Epas1	-1.52	0.0473
Tle4	-1.52	0.0318
Rab4a	-1.52	0.0168
Anxa1	-1.52	0.0137
Il2rg	-1.53	0.0343
Dok5	-1.53	0.0060
Sash3	-1.53	0.0327
Tdrkh	-1.53	0.0068
Fcgr1	-1.54	0.0169
Mboat2	-1.56	0.0458
Twist2	-1.56	0.0150
Rac2	-1.58	0.0368
Gmnn	-1.58	0.0031
Ccl12	-1.59	0.0391
Ndufb8	-1.59	0.0294
Ptprc	-1.61	0.0120
Cntf	-1.62	0.0308
Fkbp3	-1.62	0.0161
Sytl2	-1.63	0.0277
AI662270	-1.63	0.0362
Pld4	-1.65	0.0349
1810011H11Rik	-1.65	0.0445
Rtp4	-1.66	0.0332
Gm8773	-1.67	0.0230
Rem1	-1.69	0.0449
Pcdh1	-1.71	0.0483
Gm6644	-1.72	0.0245
2610507I01Rik	-1.74	0.0015
Hist1h2be	-1.75	0.0192

Fcgr4	-1.76	0.0214
Cybb	-1.81	0.0348
Cd59a	-1.85	0.0087
LOC547349	-1.89	0.0339
S100a3	-1.89	0.0478
Sema3e	-1.93	0.0457
Clec16a	-1.95	0.0191
Tppp3	-2.01	0.0403
Pde2a	-2.05	0.0250
Map3k7cl	-2.06	0.0305
Cdh10	-2.10	0.0272
Cd52	-2.14	0.0199
Hmgn1	-2.21	0.0066
1110059M19Rik	-2.29	0.0006
Pgf	-2.41	0.0238
Ntf3	-2.43	0.0234
Apoe	-2.54	0.0475

Supplementary Table S4. Immediate early genes analyzed (44 immediate-early genes and 3 controls).

Accession #	Gene symbol
NM_010217	CTGF
NM_001139509	NR4A2
X56790.1	CYR61
NM_013642.3	DUSP1
NM_008036.2	FOSB
NM_010234.2	FOS
NM_010444.2	NR4A1
NM_031168.1	IL6
NM_015743.2	NR4A3
NM_007913.5	EGR1
NM_011756.4	ZFP36
NM_018781.2	EGR3
NM_008416.3	JUNB
NM_007498.3	ATF3
AF260711	DSCR1
NM_001085390.1	DUSP5
NM_010276.4	GEM
NM_011401.4	SLC2A3
NM_008873.3	PLAU
NM_010907.2	NFKBIA
NM_133662.2	IER3
AF205855.1	SGK
NM_011803.2	Klf6
NM_008655.1	GADD45B
AB020974.1	MAIL
AF500171.1	FLG
AF063886.1	MCL1
EF494422.1	GBP1
NM_010591.2	JUN
NM_007837.3	DDIT3
NM_001166402.1	TNFAIP3
NM_007679.4	CEBPD
NM_019937.3	CCNL1
NM_010415.2	HBEGF
NM_144549.4	TRIB1
NM_009828.2	CCNA2
NM_010235.2	FOSL1

NM_001177352.1	MYC
NM_002982	CCL2
NM_026268.3	DUSP6
NM_003131	SRF
NM_153287.3	CSRNP1
NM_021127	PMAIP1
NM_001159598.1	AXIN1
NM_008828.3	PGK1
NM_008084.2	GAPDH
NM_010368	GUSB

Table S5. Biological processes affected by loss of HMGN variants

Tissues	Genotype*	Biological process	Genes in category	P - value (-log10)	Relative expression (ranksum) ▨ up ☐ down			
					Mouse 1	Mouse 2	Mouse 3	Mouse 4
Brain	*Hmgn1* $^{-/-}$	post-translational protein modification	39	3.808	1.415	2.871	2.531	2.124
		response to drug	172	3.218	1.536	1.577	2.434	1.813
		histone H3-K4 methylation	13	3.050	1.798	0.345	1.93	0.743
		negative regulation of interleukin-2 production	16	3.057	-1.934	-2.718	-0.486	-0.482
	Hmgn2 $^{-/-}$	protein ubiquitination during ubiquitin-dependent protein catabolic process	62	3.761	7.455	-0.925	0.334	-0.152
		protein ubiquitination	217	3.146	10.254	0.15	-0.303	-1.091
		muscle development	67	5.424	-0.55	-1.699	-3.016	-2.516
		translational elongation	117	4.336	-16.609	1.792	-0.089	0.457
	Hmgn1 $^{-/-}$ *n2* $^{-/-}$	cell adhesion	623	6.109	4.042	5.226	1.164	2.415
		protein amino acid phosphorylation	726	4.542	0.249	3.425	4.66	3.365
		phosphorylation	658	3.821	0.447	3.345	1.475	4.671
Liver	*Hmgn1* $^{-/-}$	ATP catabolic process	351	4.921	4.199	1.952	3.565	1.347
		protein ubiquitination	217	4.630	0.869	2.114	5.743	1.652
		neurite morphogenesis	46	3.436	0.108	0.508	2.209	3.45
		sterol biosynthetic process	34	4.883	-0.846	-3.641	-0.583	-6.712
		steroid biosynthetic process	74	4.684	-1.505	-3.827	-0.42	-5.816
		response to protein stimulus	65	4.116	-1.753	-3.546	-3.565	-3.448
	Hmgn2 $^{-/-}$	ion transport	741	9.862	2.629	7.826	1.846	3.823
		cell differentiation	696	9.839	1.792	4.962	5.94	4.224
		positive regulation of transcription from RNA polymerase II promoter	643	7.196	2.546	2.085	2.465	4.054
	Hmgn1 $^{-/-}$ *n2* $^{-/-}$	ion transport	741	13.037	5.553	4.606	5.211	3.966
		transmembrane transport	701	10.549	4.763	3.246	3.997	3.66
		cell differentiation	696	8.119	3.262	3.807	4.347	2.352
		positive regulation of myoblast differentiation	14	3.978	-1.354	-1.731	-4.326	-0.708
		defense response to protozoan	23	3.901	-1.228	-3.546	-2.935	-0.309
		pentose-phosphate shunt	14	3.560	-0.925	-0.676	-2.102	-2.278
Spleen	*Hmgn1* $^{-/-}$	cell adhesion	623	5.172	-0.995	6.673	3.681	5.528
		anterior/posterior pattern formation	154	3.262	2.128	1.641	1.814	1.728
		negative regulation of Wnt receptor pathway	54	3.025	0.421	2.93	1.496	2.542
		tryptophan catabolic process to kynurenine	11	3.388	-1.464	-1.915	-1.581	-3.271
		positive regulation of muscle cell differentiation	46	3.152	-0.698	-1.815	-3.257	-2.468
	Hmgn2 $^{-/-}$	mitosis	260	3.568	-0.509	0.717	4.234	0.735
		ER-associated protein catabolic process	32	3.022	0.432	0.66	1.513	4.162
		post-translational protein modification	39	3.019	2.503	1.272	2.008	0.547
		neutrophil chemotaxis	31	4.041	-2.625	-2.076	-3.946	-1.766
		positive regulation of mitochondrial depolarization	14	3.503	-0.291	-1.522	-2.528	-2.525
		negative regulation of MAPKKK cascade	26	3.320	-0.704	-1.715	-2.28	-1.221
	Hmgn1 $^{-/-}$ *n2* $^{-/-}$	translational elongation	117	9.170	6.704	8.008	8.703	6.761
		translation	309	7.109	3.557	3.724	7.352	7.473
		protein amino acid phosphorylation	726	8.813	-3.228	-3.892	-6.876	-4.194
		Rac protein signal transduction	27	6.336	-2.34	-3.928	-6.201	-2.076
		I-kappaB kinase/NF-kappaB cascade	37	5.807	-1.416	-1.893	-5.304	-2.681
Thymus	*Hmgn1* $^{-/-}$	triacylglycerol biosynthetic process	46	4.978	3.699	3.951	0.544	0.064
		cell differentiation	696	4.405	1.513	5.155	2.499	1.712
		glutathione metabolic process	40	4.266	3.902	4.761	-0.085	1.367
		ubiquitin-dependent protein catabolic process	175	3.049	-2.973	-5.587	-0.006	-2.227
	Hmgn2 $^{-/-}$	protein amino acid phosphorylation	726	12.694	3.631	6.711	6.441	10.867
		cell cycle	647	11.962	8.666	5.587	6.562	5.454
		dephosphorylation	293	9.275	5.027	2.891	4.911	4.703
		regulation of caspase activity	28	5.071	-4.934	-2.625	-4.414	-0.77
		ribosomal small subunit biogenesis and assembly	12	3.234	-1.043	-1.341	-2.6	-1.855
		antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	10	3.138	-2.723	-0.612	-5.463	-0.861
	Hmgn1 $^{-/-}$ *n2* $^{-/-}$	chromatin modification	275	14.366	7.155	5.998	3.187	9.25
		phosphorylation	658	10.778	8.441	7.733	0.557	6.288
		response to DNA damage stimulus	423	10.132	6.465	7.038	1.207	5.219
		antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	10	8.110	-4.536	-5.74	-6.327	-2.114

immune response	185	6.430	-2.745	-8.32	-3.69	-0.544
antigen processing and presentation of exogenous peptide antigen via MHC class II	23	5.810	-2.376	-5.24	-5.303	-3.233

***see methods**

Deng et al Supplemental Table S6

Primary Screen HMGN1/2 KO mouse line, German Mouse Clinic (http://www.mouseclinic.de)

	Age (weeks)	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Screens	**Methods**															
Behavior	Open Field			X												
	Acoustic startle response & PPI				X											
Neurology	Modified SHIRPA, grip strength			X												
	Rotarod				X											
Clinical Chemistry	Clinical Chemistry after fasting					X										
Nociception	Hot Plate						X									
Dysmorphology	Anatomical observation						X									
Allergy	Transepidermal water loss (TEWL)						X									
Energy Metabolism	Indirect calorimetry, NMR							X								
Clinical Chemistry	IpGTT								X							
Cardiovascular	Awake ECG/Echocardiography									X						
Eye	Scheimpflug imaging, OCT, LIB, drum										X					
Clinical Chemistry	Clinical Chemical analysis, hematology											X				
Immunology	FACS analysis of PBCs											X				
Allergy	BIOPLEX ELISA (IG concentration)											X				
Neurology	ABR (Auditory brain stem response)												X			
Dysmorphology	x-ray, DEXA												X			
Energy Metabolism	NMR													X		
Clinical Chemistry	Clinical Chemical analysis, hematology															X
Lung Function, optional	Lung function measurements															X
Molecular Phenotyping	Expression profiling															X
Pathology	Macro & microscopic analysis															X

Energy Metabolism

- Body composition analysis, week 13 and 19

Non-invasive qNMR scans (Bruker MiniSpec LF 50, Bruker Biospin, Ettlingen Germany) to determine fat and lean content in mice at two time points.

- Indirect Calorimetry, week 13

Method for determining energy metabolism from the gas exchange during 21 hours (TSE Phenomaster, TSE Systems GmbH, Bad Homburg, Germany).

Variables:

- oxygen consumption VO_2 (ml O_2*h^{-1}*animal*$^{-1}$)

- carbon dioxide production VCO_2 (ml CO_2*h^{-1}*animal*$^{-1}$)

- respiratory exchange ratio RER (VCO_2/ VO_2)

- heat production HP (mW*animal*$^{-1}$)

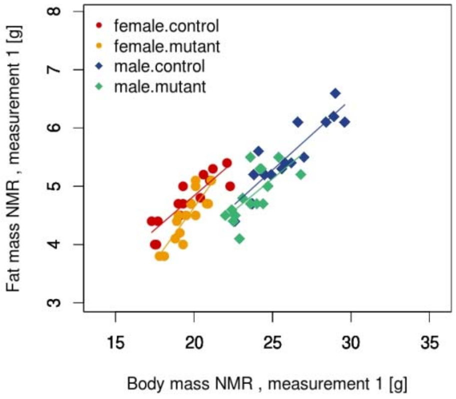
- *ad libitum* food consumption (g)

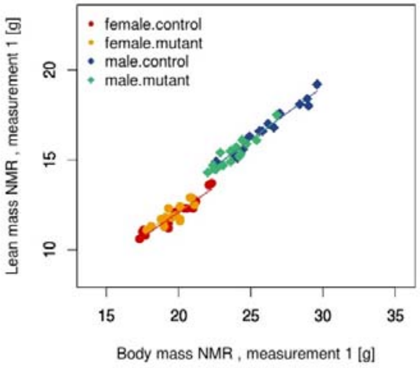
- locomotor activity (distance (cm)and rearing (counts))

- body weight (g)

Energy Metabolism
Body composition analysis - I

	female		male		Linear model			
	control	mutant	control	mutant	sex	genotype	body mass	sex~genotype
	n=15	n=15	n=15	n=15				
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value	p-value
Body mass	19.6 ± 1.6	19.6 ± 1	26 ± 2.2	23.8 ± 1.3	< 0.001	**0.006**	NA	**0.009**
Fat mass	4.7 ± 0.4	4.5 ± 0.4	5.5 ± 0.6	4.9 ± 0.4	< 0.001	**0.015**	< 0.001	0.318
Lean mass	11.9 ± 1	11.9 ± 0.6	16.7 ± 1.3	15.4 ± 0.8	< 0.001	0.922	< 0.001	0.348

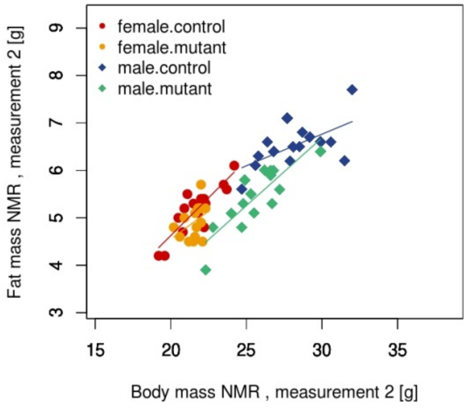


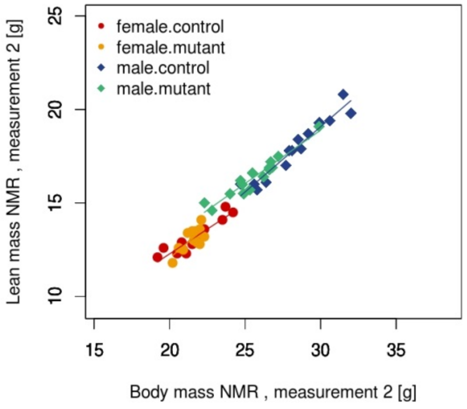


Energy Metabolism

Body composition analysis - II

	female		male		Linear model			
	control	mutant	control	mutant	sex	genotype	body mass	sex~genotype
	n=15	n=15	n=15	n=15				
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value	p-value
Body mass	21.7 ± 1.4	21.6 ± 0.6	28.2 ± 2.2	25.6 ± 1.9	< 0.001	**0.002**	NA	**0.005**
Fat mass	5.2 ± 0.5	4.9 ± 0.4	6.5 ± 0.5	5.4 ± 0.6	0.183	**0.001**	< 0.001	0.143
Lean mass	13.2 ± 0.8	13.1 ± 0.6	17.8 ± 1.6	16.4 ± 1.1	0.001	0.297	< 0.001	0.518

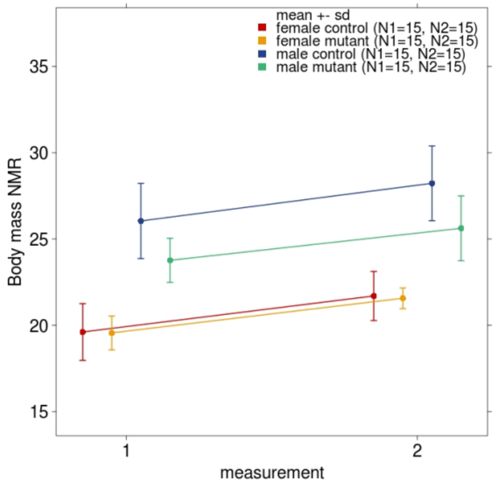


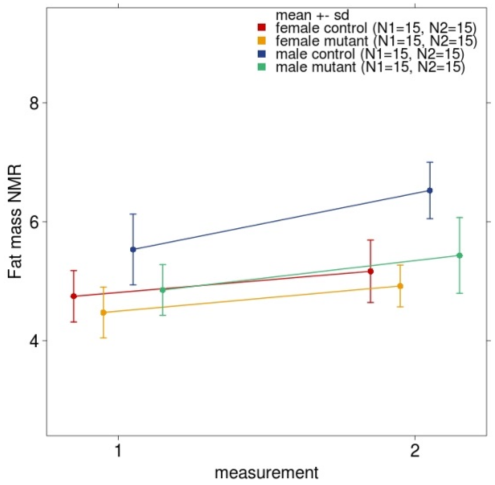


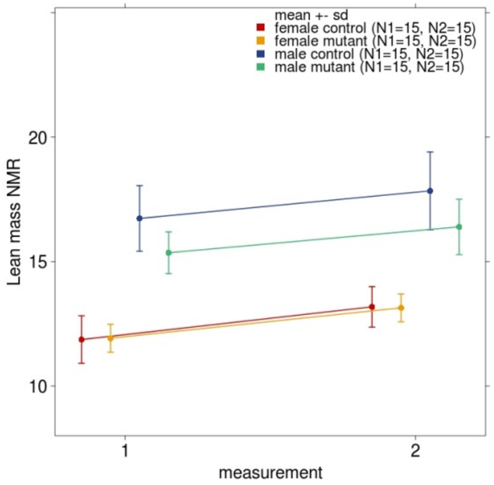
Energy Metabolism

Body composition analysis

	Linear mixed effects model				
	sex	genotype	Measurement	body mass	genotype:measurement
	p-value	p-value	p-value	p-value	p-value
Body mass	< 0.001	**0.004**	< 0.001	NA	0.29
Fat mass	< 0.001	**< 0.001**	0.091	< 0.001	0.205
Lean mass	< 0.001	0.517	0.598	< 0.001	0.689





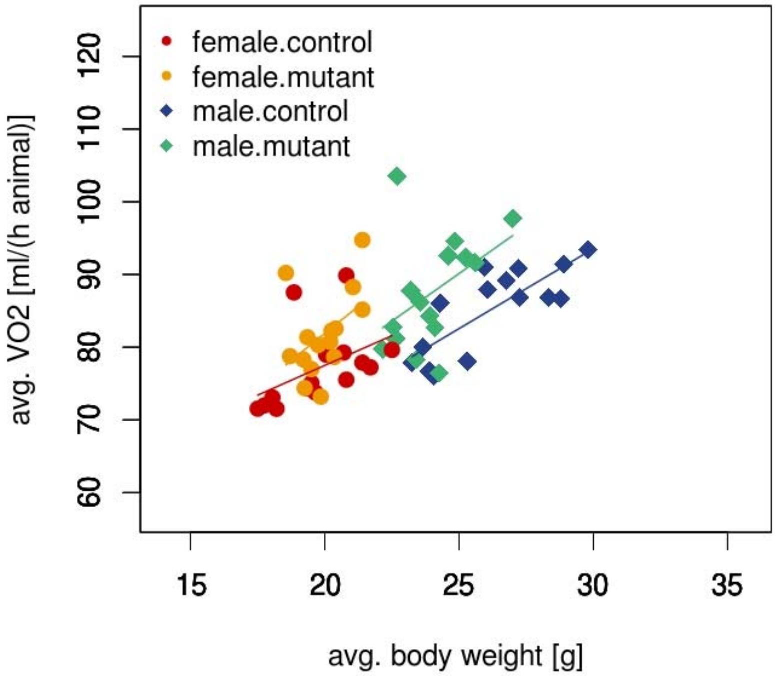


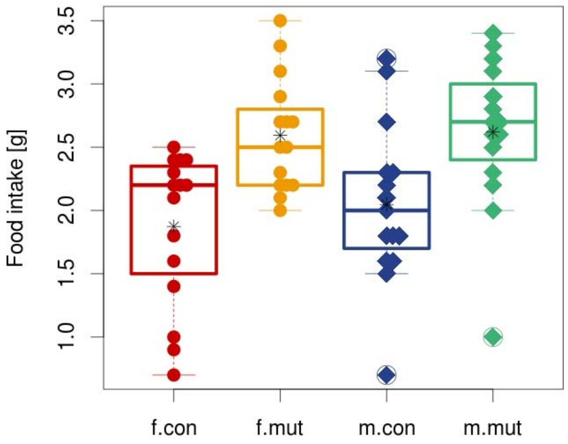
Energy Metabolism

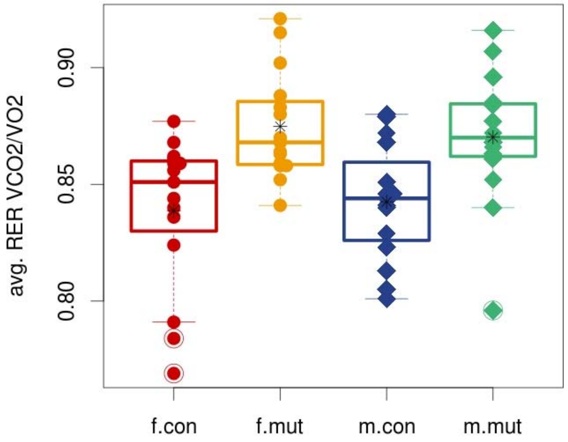
Indirect calorimetry

	female		**male**		**Linear model**	**Linear model**	**Linear model**	**Linear model**
	control	mutant	control	mutant	sex	genotype	body mass	sex:genotype
	n=15	n=15	n=15	n=15				
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value	p-value
avg. mass [g]	19.8 ± 1.5	19.9 ± 0.9	26.2 ± 2.1	24 ± 1.3	< 0.001	**0.011**	NA	0.004
Food intake [g]	1.9 ± 0.6	2.6 ± 0.5	2 ± 0.6	2.6 ± 0.6	0.058	**< 0.001**	0.011	0.614
avg. VO2 [ml/(h animal)]	77.15 ± 5.46	81.73 ± 5.86	85.26 ± 5.92	87.46 ± 7.75	0.106	**< 0.001**	< 0.001	0.334
min. VO2 [ml/(h animal)]	48.27 ± 6.81	52.93 ± 6.39	59.13 ± 4.24	56.87 ± 7.06	0.05	**0.009**	< 0.001	0.696
max. VO2 [ml/(h animal)]	112.87 ± 8.79	110.4 ± 7.21	121.33 ± 8.11	121.67 ± 10.57	0.721	0.59	0.003	0.083
avg. RER [VCO2/VO2]	0.84 ± 0.03	0.88 ± 0.02	0.84 ± 0.03	0.87 ± 0.03	0.956	**< 0.001**	NA	0.56
avg. distance [cm]	4086 ± 1156	4716 ± 4027	3103 ± 888	4685 ± 5327	0.568	0.215	NA	0.592
avg. rearing [counts]	188 ± 84	168 ± 72	125 ± 45	118 ± 47	0.001	0.424	NA	0.685

Energy Metabolism
Indirect calorimetry







Energy Metabolism

Summary of results

Body mass was significantly decreased in male mutants. Fat content in male was significantly decreased even when adjusted for the body mass difference.

During the indirect calorimetry trial energy expenditure (daily energy expenditure and resting metabolic rate) were significantly increased in mutants (hypermetabolism). Mutants were also hyperphagic and showed increased RER.

Eye, week 16

- Eye morphology

- Scheimpflug imaging

- OCT

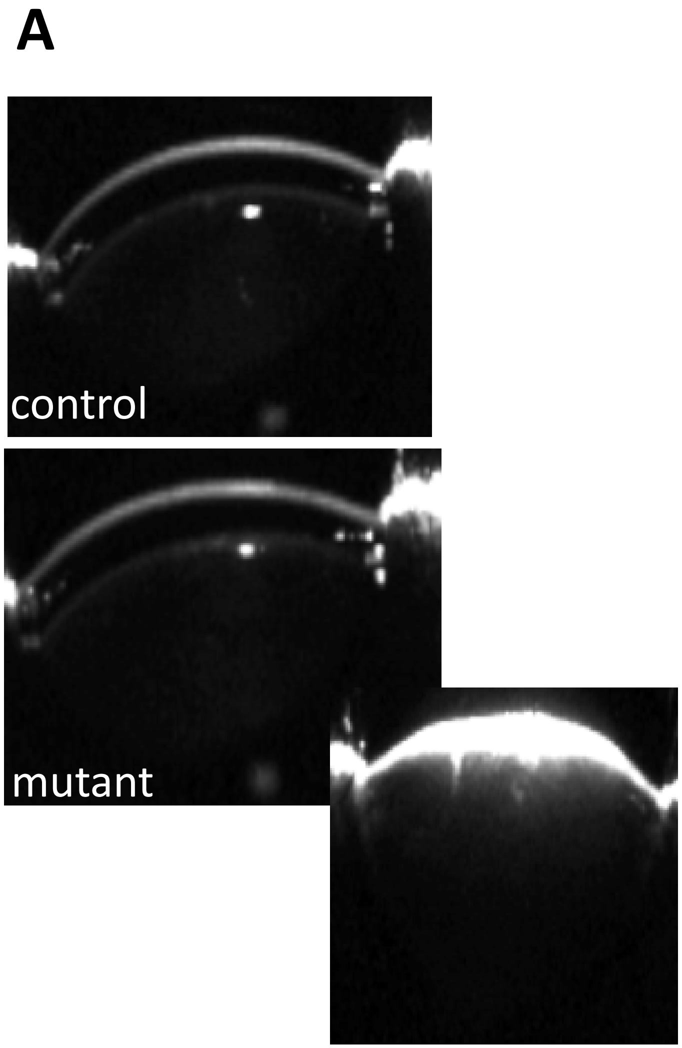
- Eye size

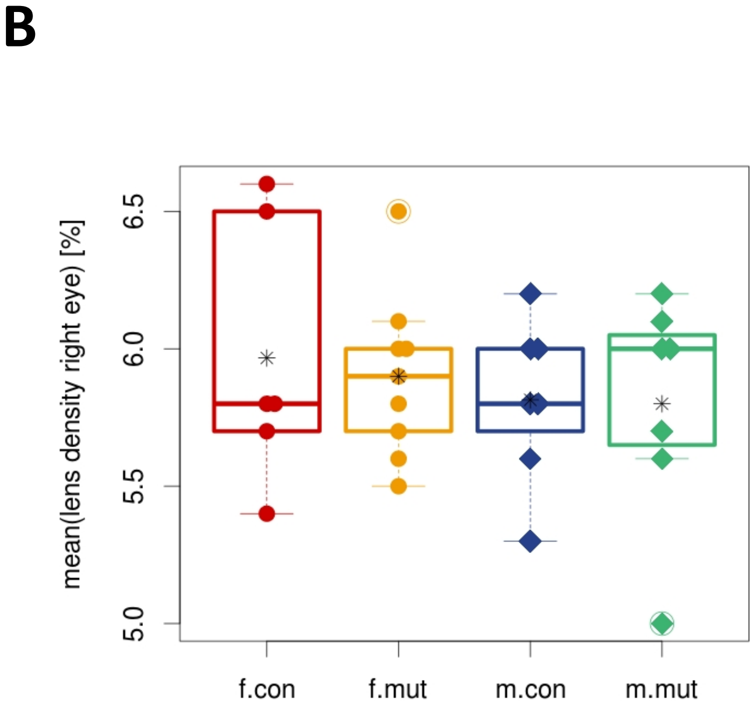
- LIB

- Eye functionality

- drum

Eye morphology, Scheimpflug imaging

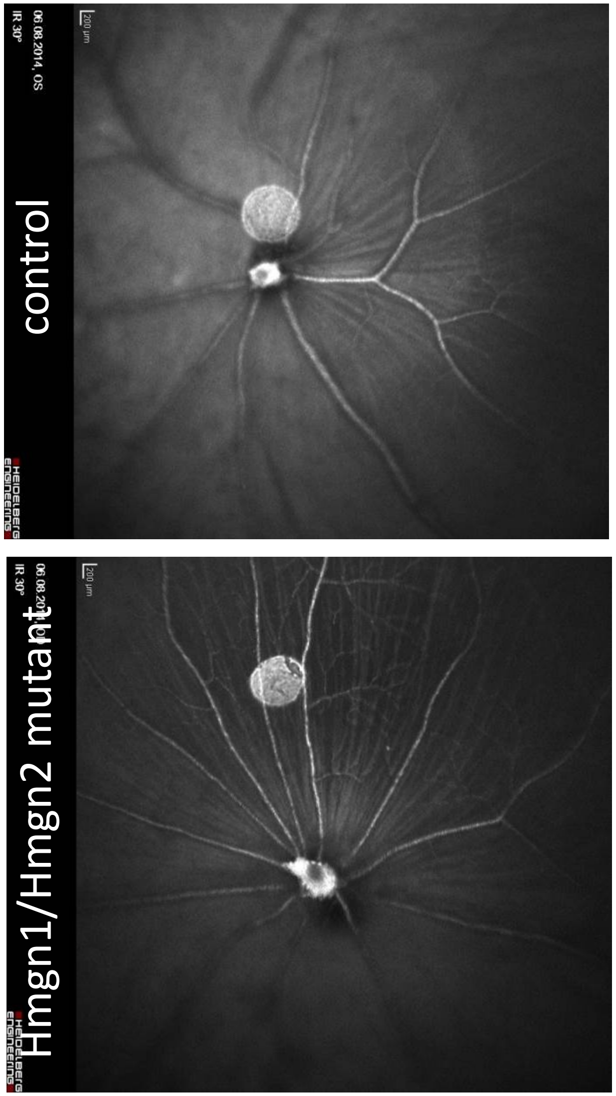




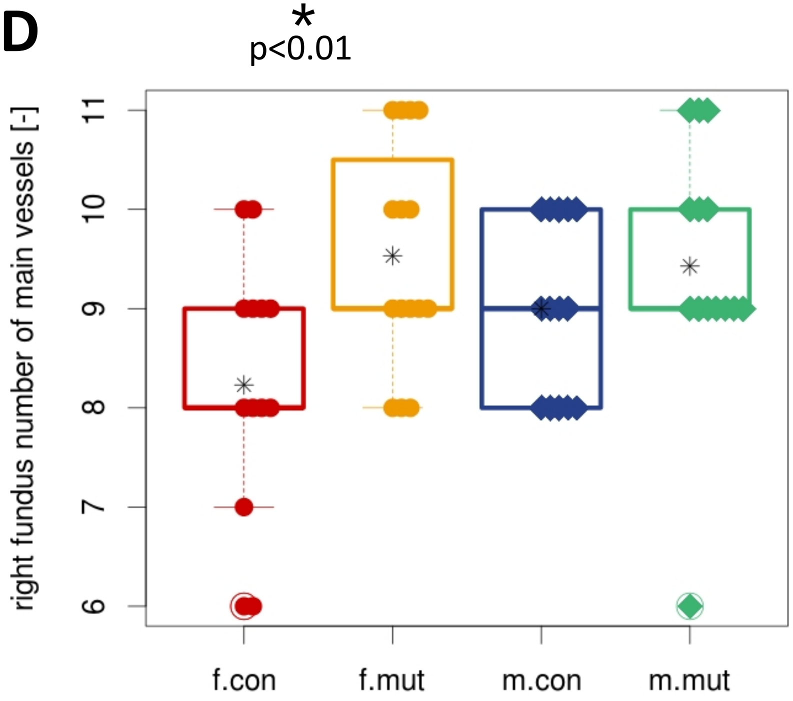
No effect on the lens transparancy.

Eye morphology, Optical Coherence Tomography

C



D

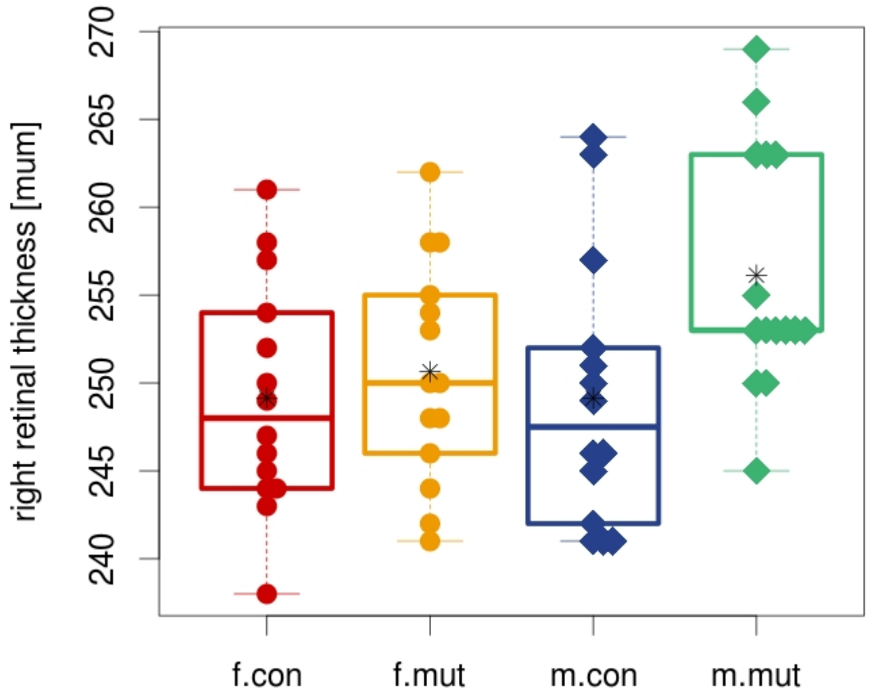


Tendency to increased number of the main blood vessels.

Eye morphology, Optical Coherence Tomography

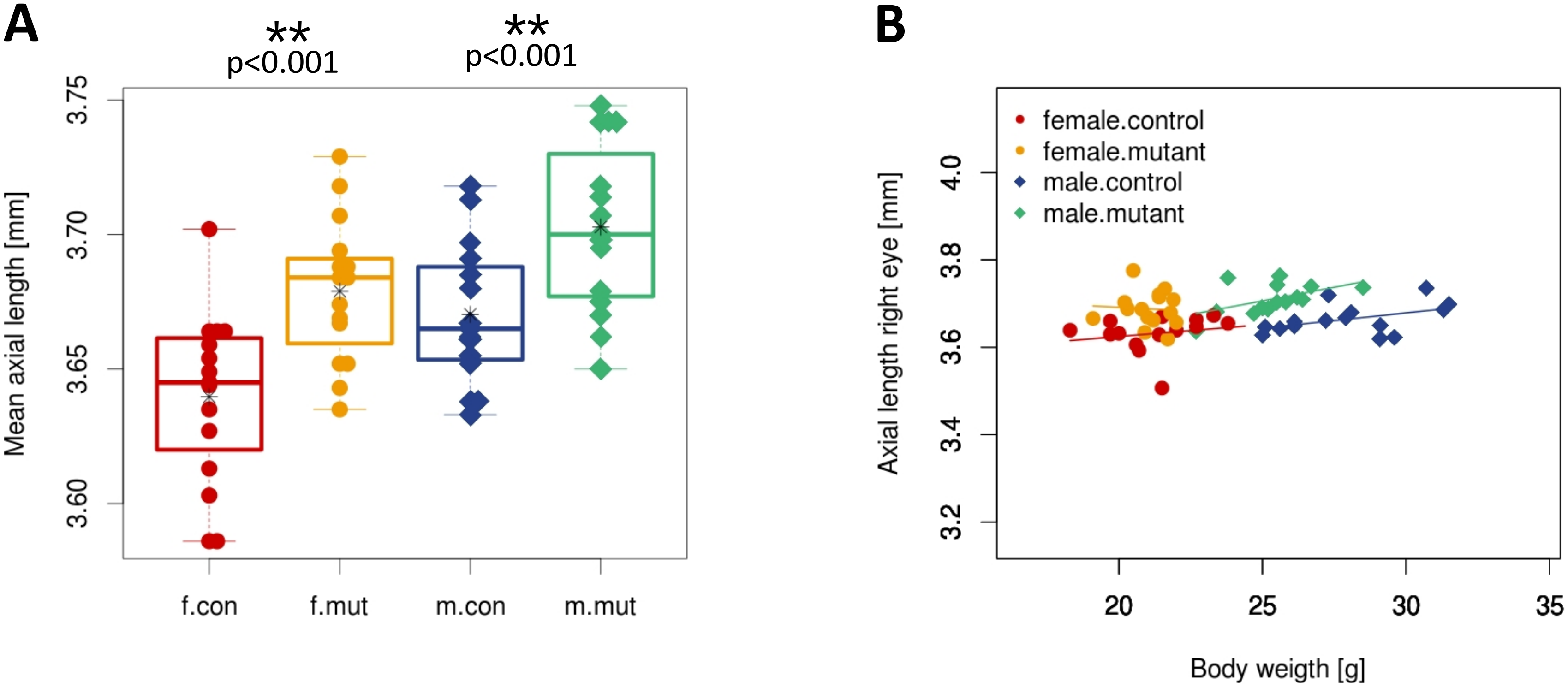
E

F



No effect on the retinal layers and the thickness of it.

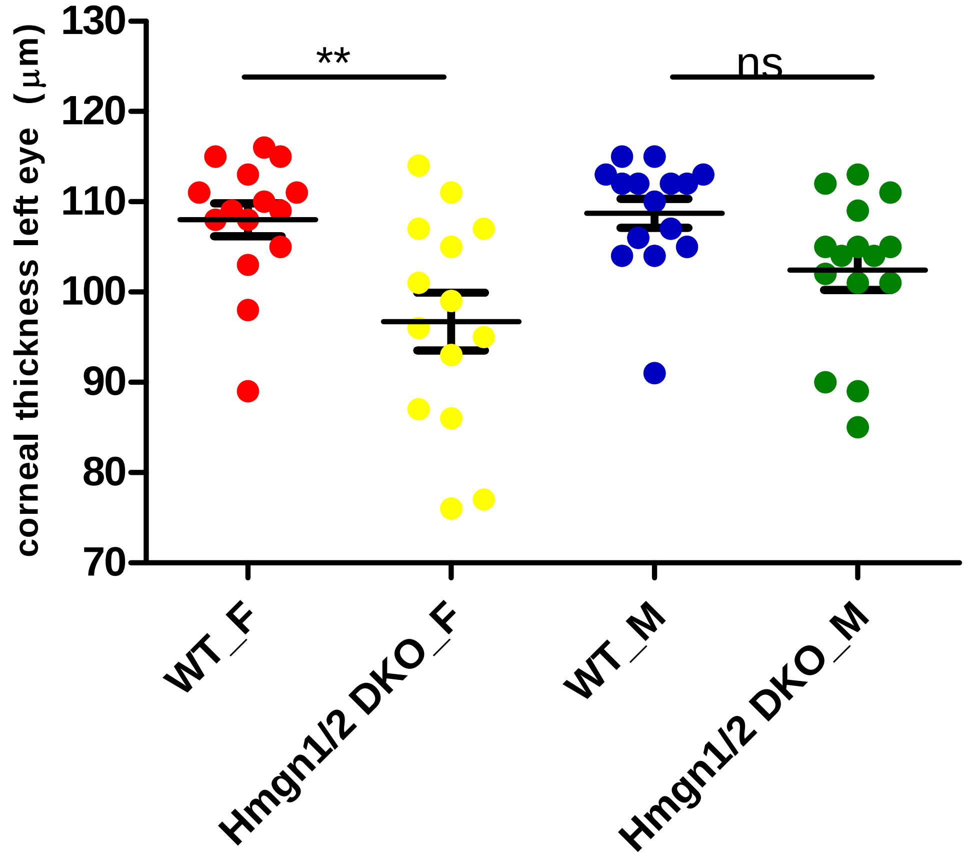
Eye size, Laser Interference Biometry



Increased axial eye lenght in both mutants: females and males (p<0.001).

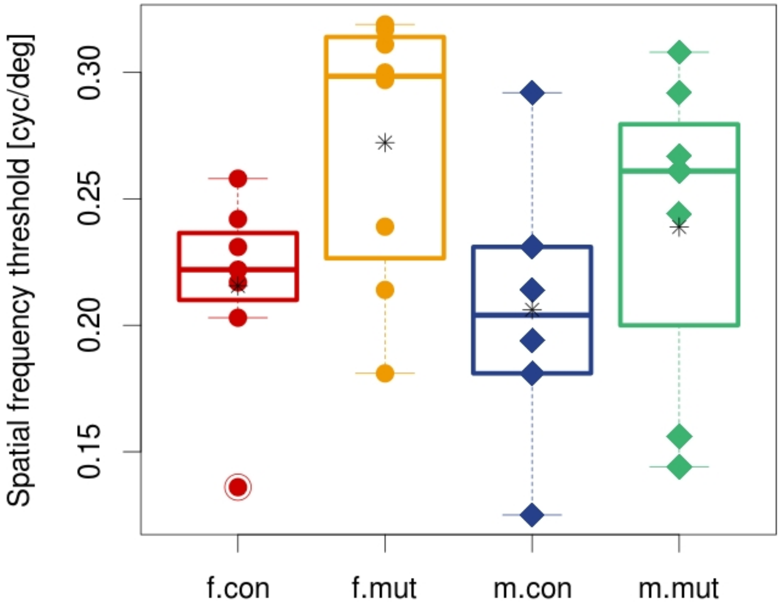
Eye size, Laser Interference Biometry

C



Decreased cornea thickness in mutant females (p<0.001).

Eye functionality, Drum



No effect on the visual acuity.

Eye

Summary of results

We found

- regular anterior eye morphology
- regular retinal layers and thickness
- regular vision

We found *increased* number of the *main blood vessels* in ocular Hmgn1/Hmgn2 fundi.

We further found *increased axial eye sizes* in both females and male mutants, and an significant *decrease in the cornea thickness* of mutant females.

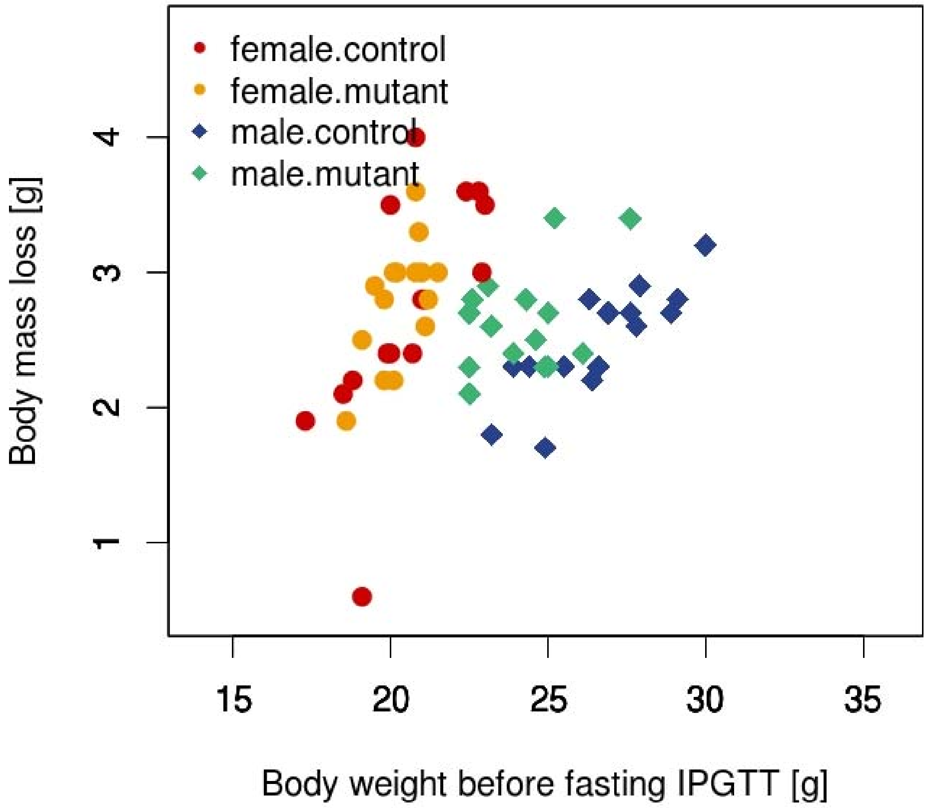
Clinical Chemistry & Hematology

- Clinical Chemistry after fasting, week 11
- IpGTT, week 14
- Clinical Chemical analysis, hematology, week 17

Clinical Chemistry & Hematology

Ip Glucose Tolerance Test (ipGTT)

Results IpGTT: Overnight Body Mass Loss

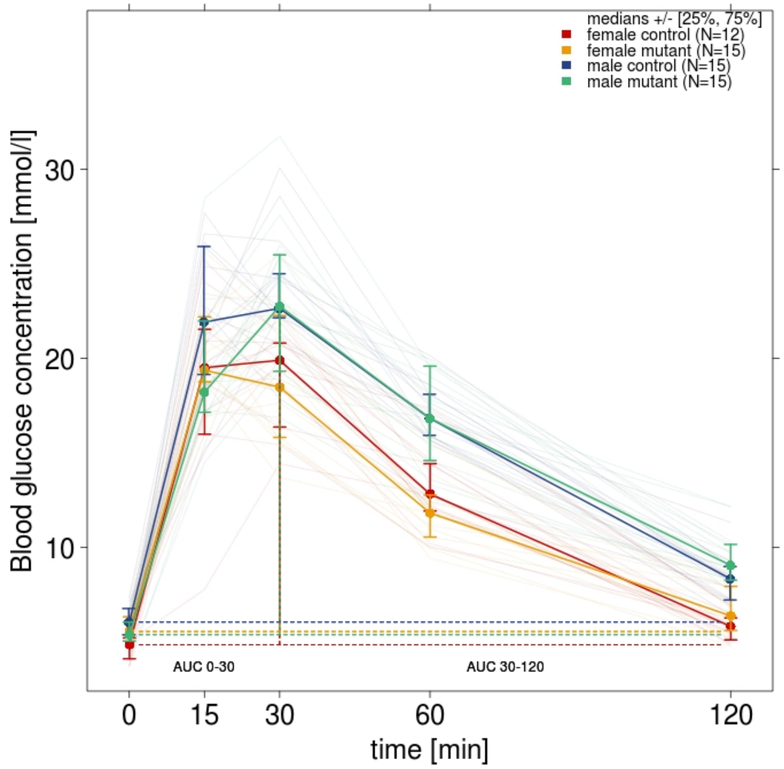


Slightly increased body mass loss due to overnight food deprivation in male mutant mice.

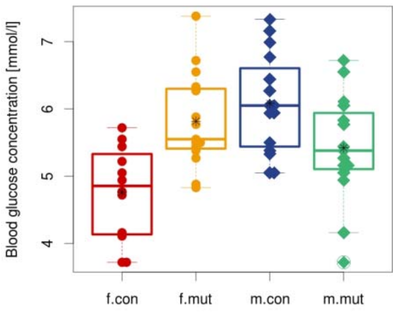
Hmgn1/Hmgn2	Estimate	CI	p-value
(Intercept)	-1.79	[-3.38 , -0.2]	0.03
genotypemutant	0.12	[-0.21 , 0.45]	0.46
sexm	-1.57	[-2.14 , -1]	<0.001
bw_before	0.22	[0.14 , 0.3]	<0.001
genotypemutant:sexm	0.56	[0.07 , 1.06]	0.03

Clinical Chemistry & Hematology

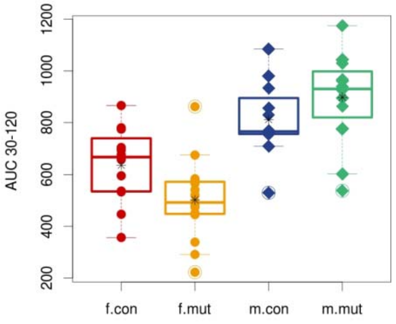
Results IpGTT: Response to Glucose Challange



Glucose T0



AUC 30-120

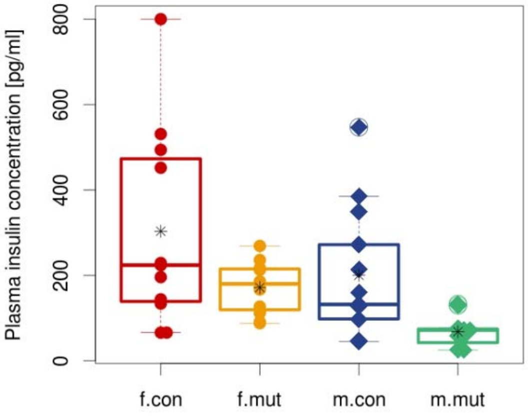


Increased basal fasting glucose levels and decreased AUC 30-120 values in female mutants.

Hmgn1/Hmgn2	**female**		**male**		**Linear model**	**Linear model**	**Linear model**
	control	mutant	control	mutant	genotype	sex	genotype:sex
	n=12	n=15	n=15	n=15			
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value
Glucose (T=0)	4.76 ± 0.69	5.82 ± 0.71	6.08 ± 0.74	5.42 ± 0.81	0.323	0.023	< 0.001
AUC 0-30	322.32 ± 94.54	313.46 ± 53.06	371.64 ± 59.86	338.05 ± 75.1	0.266	0.056	0.515
AUC 30-120	635.4 ± 147.6	501.99 ± 154.39	812.26 ± 137.4	897.76 ± 168.25	0.558	< 0.001	0.009

Clinical Chemistry & Hematology

Results Insulin: Fasting insulin levels


Fasting Insulin

Hmgn1/ Hmgn2 fasting	**female**		**male**		**female**	**male**	**overall**
	control	mutant	control	mutant			
	n = 11	n = 11	n = 13	n = 11			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
Plasma Insulin	224 [139,473]	180 [119.5,215]	132 [98,272]	72 [42.5,74.5]	0.292	0.006	0.013

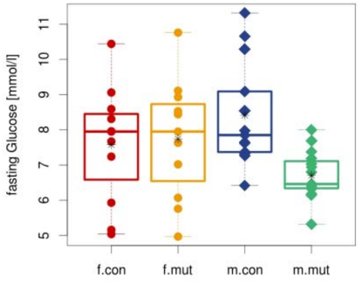
Plasma insulin levels of Hmgn1/Hmgn2 double-KO mice were low in comparison to corresponding controls, due to decreased variance with less animals showing rather elevated levels.

Clinical Chemistry & Hematology

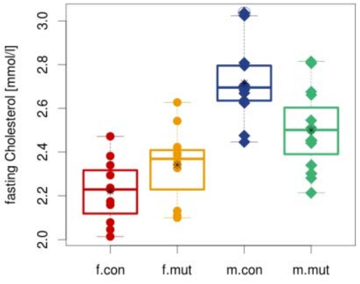
Results Clinical Chemistry – fasting values

Hmgn1/Hmgn2	female		male		Linear model	Linear model	Linear model
	control	mutant	control	mutant	genotype	sex	genotype:sex
	n=15	n=15	n=13	n=15			
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value
fasting Glucose [mmol/l]	7.58 ± 1.65[a]	7.74 ± 1.69[a]	8.41 ± 1.49	6.71 ± 0.68	0.058	0.803	0.023
fasting Cholesterol [mmol/l]	2.221 ± 0.144[a]	2.341 ± 0.17[a]	2.715 ± 0.173	2.5 ± 0.18	0.335	< 0.001	0.001
fasting HDL-cholesterol [mmol/l]	1.427 ± 0.108[a]	1.452 ± 0.109[a]	1.794 ± 0.139	1.657 ± 0.102	0.098	< 0.001	0.017
fasting non-HDL Cholesterol [mmol/l]	0.794 ± 0.072[a]	0.889 ± 0.082[a]	0.922 ± 0.058	0.843 ± 0.09	0.706	0.067	< 0.001
fasting Triglycerides [mmol/l]	0.963 ± 0.204[a]	1.094 ± 0.324[a]	1.28 ± 0.389	1.457 ± 0.387	0.121	0.001	0.815
fasting NEFA [mmol/l]	1.4 ± 0.21[a]	1.54 ± 0.23[a]	1.62 ± 0.21	1.7 ± 0.24	0.086	0.004	0.57
fasting Glycerol [mmol/l]	0.297 ± 0.075[a]	0.336 ± 0.049[a]	0.291 ± 0.043	0.297 ± 0.042	0.142	0.153	0.271

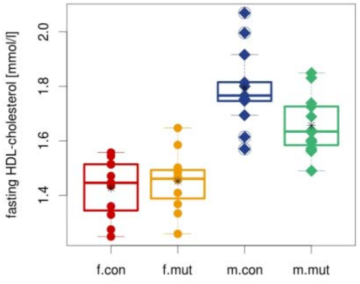
Glucose



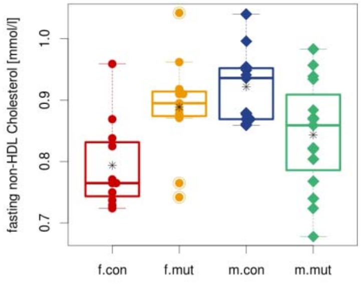
Cholesterol



HDL-Cholesterol



Non-HDL-Chol.



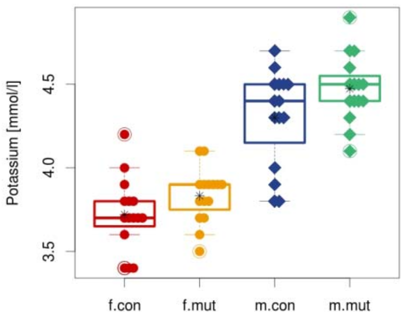
[a] Number not based on the full number of animals
(low body mass in 8 females, welfare issue)

Clinical Chemistry & Hematology

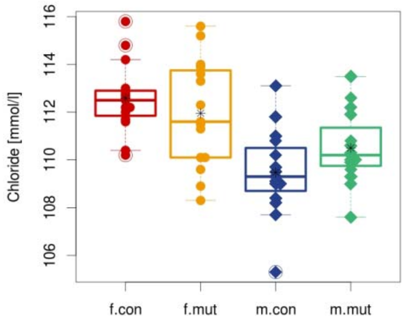
Results Clinical Chemistry – *ad libitum* fed values

Hmgn1/Hmgn2	female		male		Linear model	Linear model	Linear model
	control	mutant	control	mutant	genotype	sex	genotype: sex
	n=15	n=15	n=15	n=15			
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value
Sodium [mmol/l]	149 ± 2	149 ± 2	149 ± 2	150 ± 2	0.423	0.423	0.423
Potassium [mmol/l]	3.7 ± 0.2	3.8 ± 0.2	4.3 ± 0.3	4.5 ± 0.2	0.016	< 0.001	0.606
Chloride [mmol/l]	112.6 ± 1.5	112 ± 2.3	109.5 ± 1.8	110.5 ± 1.5	0.67	< 0.001	0.082
Total protein [g/l]	45.2 ± 2	45.2 ± 1.7	45.3 ± 1.9	45 ± 1.2	0.688	0.87	0.789
Albumin [g/l]	23.5 ± 1	22.6 ± 0.9	22.8 ± 1.2	21.4 ± 0.8	< 0.001	< 0.001	0.302
Creatinine enz. [mumol/l]	10.76 ± 1.74	11.04 ± 2.7	8.72 ± 4.01	10.64 ± 2.16	0.13	0.096	0.259
Urea [mmol/l]	10.37 ± 1.26	10.38 ± 1.64	12.18 ± 1.22	12.85 ± 1.47	0.356	< 0.001	0.361

Potassium

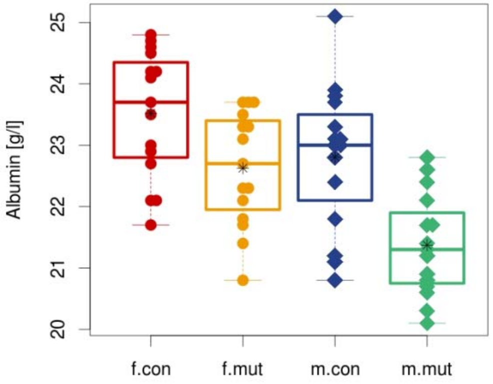


Chloride



Accidental effects due to outlier values.

Albumin



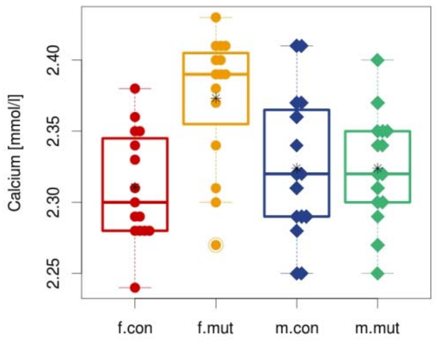
Significantly decreased albumin levels in mutant animals.

Clinical Chemistry & Hematology

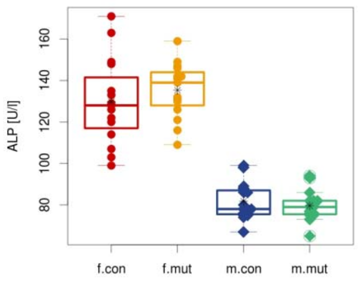
Results Clinical Chemistry – *ad libitum* fed values

Hmgn1/Hmgn2	female		male		Linear model	Linear model	Linear model
	control	mutant	control	mutant	genotype	sex	genotype: sex
	n=15	n=15	n=15	n=15			
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value
Calcium [mmol/l]	2.31 ± 0.04	2.37 ± 0.05	2.32 ± 0.05	2.32 ± 0.04	0.008	0.122	0.008
Inorganic phosphate [mmol/l]	1.86 ± 0.29	1.95 ± 0.29	2.09 ± 0.32	2.04 ± 0.35	0.819	0.053	0.414
ALP [U/l]	130 ± 21	135 ± 13	82 ± 9	80 ± 7	0.641	< 0.001	0.281
Iron [µmol/l]	18.673 ± 2.725	19.731 ± 2.829	17.178 ± 2.119	15.386 ± 1.876	0.56	< 0.001	0.026
UIBC [µmol/l]	30 ± 4.1	28.2 ± 4.6	28.4 ± 6.7	29.4 ± 4.2	0.753	0.888	0.283
TIBC [µmol/l]	48.6 ± 3.6	47.9 ± 3.6	45.6 ± 6.8	44.8 ± 3.4	0.515	0.011	0.985
Calc transf. sat. [%]	38.5 ± 5.6	41.4 ± 6.6	38.4 ± 6.8	34.6 ± 5.1	0.785	0.03	0.036

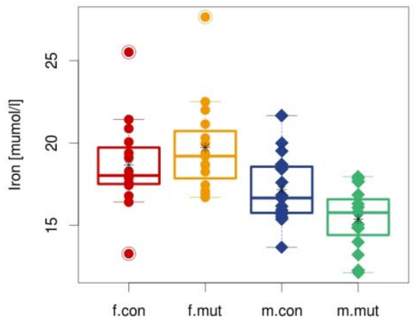
Calcium



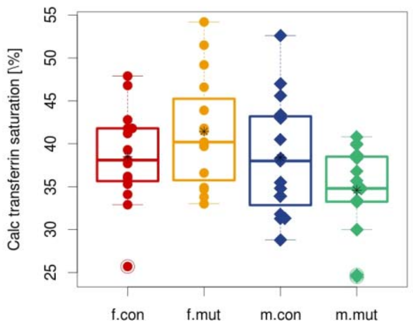
ALP



Iron



Transf. Sat.

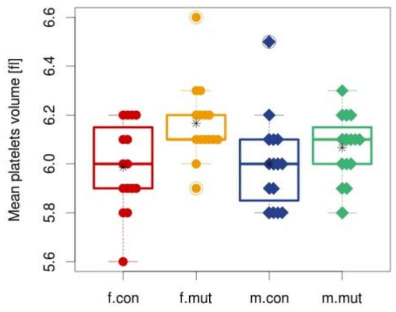


Clinical Chemistry & Hematology

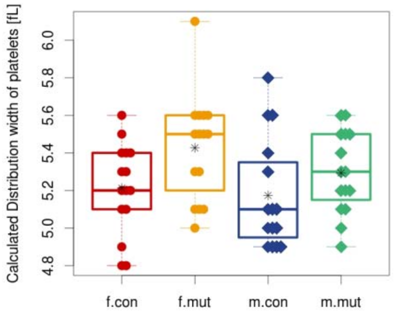
Results Hematology

Hmgn1/Hmgn2	female		male		Linear model	Linear model	Linear model
	control	mutant	control	mutant	genotype	sex	genotype:sex
	n=15	n=15	n=15	n=15			
	mean ± sd	mean ± sd	mean ± sd	mean ± sd	p-value	p-value	p-value
RBC [Mio/mm^3]	10.97 ± 0.47	11.27 ± 0.49	10.97 ± 0.47	11.12 ± 0.3	0.048	0.518	0.533
HGB [g/dl]	17.29 ± 0.86	17.32 ± 0.72	17.07 ± 0.86	16.94 ± 0.59	0.789	0.136	0.688
HCT [%]	55.41 ± 2.32	54.69 ± 2.3	55.55 ± 2.53	54.41 ± 1.8	0.116	0.905	0.711
MCV [fl]	50.53 ± 1.19	48.47 ± 0.74	50.73 ± 0.7	49.07 ± 0.8	< 0.001	0.084	0.382
MCH [pg]	15.76 ± 0.43	15.37 ± 0.49	15.56 ± 0.37	15.21 ± 0.32	0.001	0.094	0.851
MCHC [g/dl]	31.21 ± 0.84	31.69 ± 0.71	30.75 ± 0.75	31.14 ± 0.61	0.025	0.009	0.82
RDW [%]	15.41 ± 0.45	15.24 ± 0.37	15.23 ± 0.31	15.57 ± 0.56	0.442	0.515	0.028
WBC [10^3/mm^3]	7.42 ± 2.24	7.09 ± 1.43	7.95 ± 2.06	7.78 ± 1.58	0.604	0.209	0.874
PLT [10^3/mm^3]	914.6 ± 221.19	959.33 ± 226.8	954.53 ± 90.94	1001.6 ± 91.93	0.303	0.356	0.979
MPV [fl]	5.99 ± 0.18	6.17 ± 0.16	6 ± 0.19	6.07 ± 0.13	0.006	0.32	0.194
PDW [fL]	5.21 ± 0.24	5.43 ± 0.28	5.17 ± 0.3	5.29 ± 0.22	0.017	0.205	0.493
PLCR [%]	1.64 ± 0.54	2.01 ± 0.53	1.74 ± 0.56	2.04 ± 0.54	0.021	0.636	0.813
PCT [%]	0.55 ± 0.14	0.59 ± 0.14	0.57 ± 0.06	0.61 ± 0.05	0.174	0.487	0.923

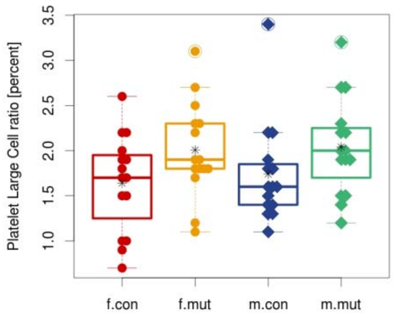
MPV



PDW

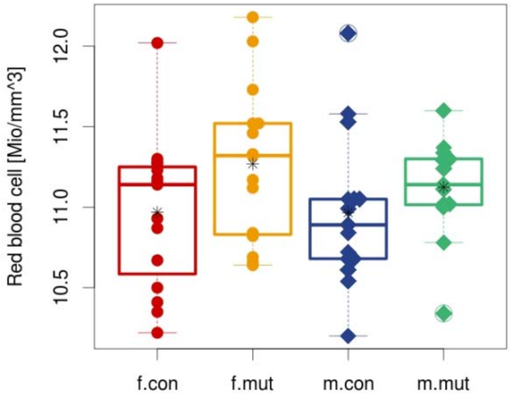


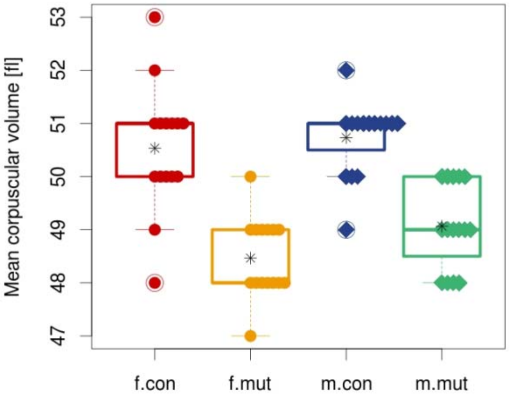
P-LCR



Clinical Chemistry & Hematology

Results Hematology

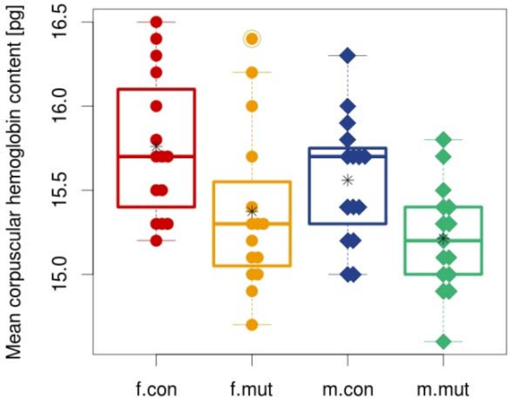

Red blood cell count

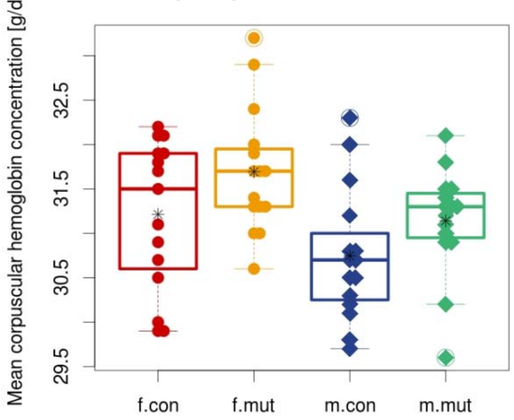

MCV

Microcytic peripheral blood cell count in mutants but without anemia.

Increased cell cycle frequency during erythropoiesis?

Smaller cell size could also be partly due to osmotic effects.


MCH


MCHC

Clinical Chemistry & Hematology

Summary Results

- IpGTT

Increased basal fasting glucose level and trend towards decreased AUC 30-120 values in female mutant mice.

But fasting insulin levels rather decreased compared to controls.

- Clinical Chemistry Results

Fasting: Genotype x sex interaction effects – slightly increased levels in mutant females, but decreased values in mutant males for fasting glucose and cholesterol (HDL and non-HDL).

Fed: Decreased albumin, alpha-amylase and - as a trend – fructosamine (latter could be secondary); Increased cholesterol, triglyceride and calcium level mainly in females.

- Hematology Results

Microcytic blood cell count without anemia

>>> effects on erytropoiesis and/or osmotic effects?

Increased frequency of large platelet

Immunology

FACS analysis of PBCs, week 17

Parameter	findings	comment
Frequencies of main leukocyte subsets	Slightly higher frequencies of T cells, NK cells and NKT cells in male mutants	*Sex-dependent phenotype. The higher frequency of T cells in* ***males*** *correlates with a lower frequency of B cells*
Frequencies of T lymphocyte subsets	• Higher proportion of CD4 single positive • decreased proportion of CD8 single positive T cells in male mutants female mutants are showing the same tendencies. In males further subtle changes in subpopulations	*Especially in* ***males*** *differences between mutants and controls in the frequencies of CD4 and CD8 T cells and Ly6C/CD44 co-expressing CD8 T cell subsets.*

Immunology

Parameter	findings	comment
Frequencies of B lymphocyte subsets	The majority of B cells show a mature naïve phenotype. Lower frequency of $IgD^{+}IgM^{+}CD23^{+}CD21^{-}$ cells within the B cell compartment	In both sexes we found a difference between mutants and controls in the frequency of a tiny population
Frequencies of Monocyte subsets	Lower frequencies of $Ly6C^{high}$ expressing cells and a higher frequency of $Ly6C^{intermediate}$ expressing cells within the monocyte compartment in female mutants. Furthermore, the frequency of CD11c+ $Ly6C^{intermediate}$ expressing cells is decreased in female mutants, and the frequency of CD11c+ $Ly6C^{high}$ expressing cells is decreased in female and male mutants	*Phenotypic changes in female mutants*
Frequenices of NK cell subsets	Lower frequency of $CD11c^{+}$ NK cells in female mutants	The functional role of CD11c expressing NK cells is not elucidated yet.

Frequencies of main leukocyte populations in blood

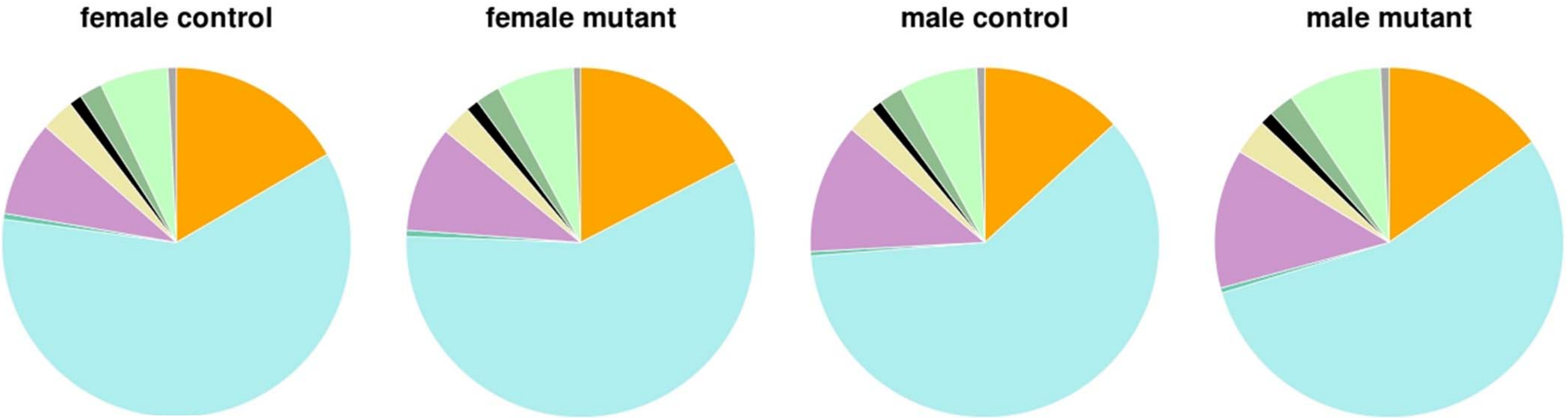
T NKT NK B2 B1 Gr M

Frequencies are referring to all CD45$^+$ cells (all leukocytes)

T cells
NKT cells
NK cells
B2-Bcells (CD5$^-$CD19$^+$)
B1-Bcells (CD5$^+$CD19$^+$),
Granulocytes (Ly6G$^+$CD11b$^+$)
Monocytes (Ly6G$^-$, NK$^-$, CD11b$^+$/Ly6C$^{+/\text{ or }-}$)
Cells not belonging to one of these populations (rest)

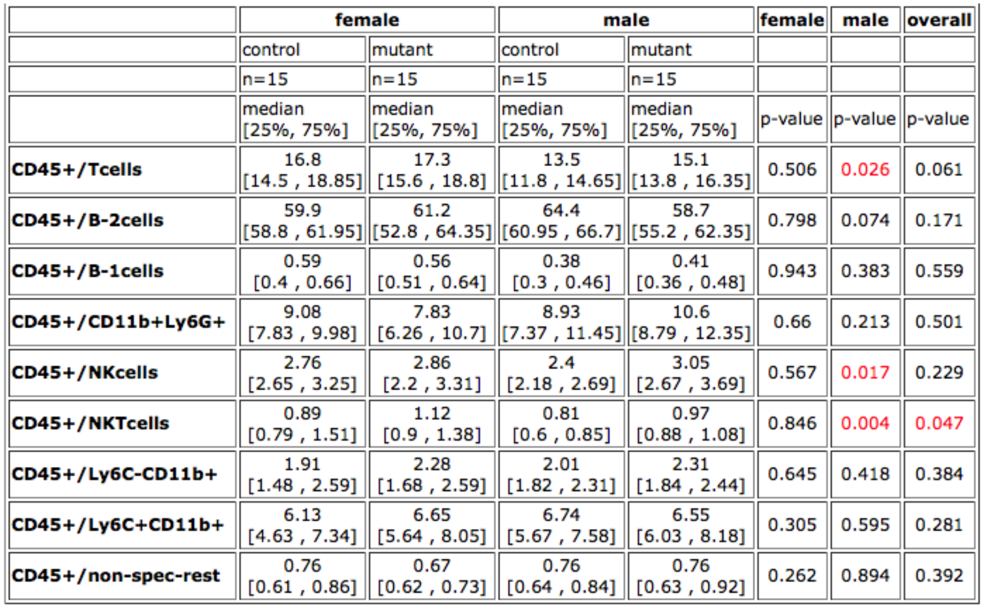
Immunology

Results: frequencies of main leukocyte subsets in peripheral blood [% of all CD45+ cells]



T cells
B2-Bcells
B1-Bcells (CD5$^+$)
Granulocytes
NK cells
NKT cells
Monocytes (Ly6C$^-$)
Monocytes (Ly6C$^+$)
Rest
CD45+/Ly6C-CD11b+
CD45+/Ly6C+CD11b+
CD45+/non-spec-rest

Slightly higher frequencies of T cells, NK cells and NKT cells in male mutants

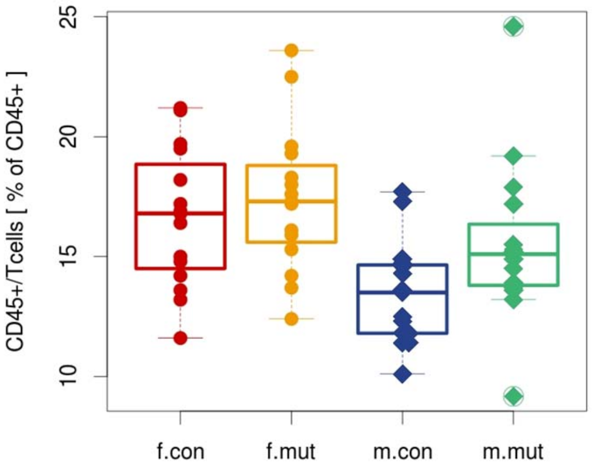


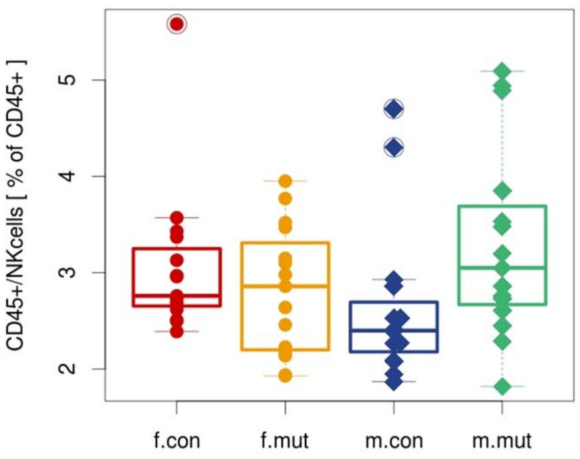
	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
CD45+/Tcells	16.8 [14.5 , 18.85]	17.3 [15.6 , 18.8]	13.5 [11.8 , 14.65]	15.1 [13.8 , 16.35]	0.506	0.026	0.061
CD45+/B-2cells	59.9 [58.8 , 61.95]	61.2 [52.8 , 64.35]	64.4 [60.95 , 66.7]	58.7 [55.2 , 62.35]	0.798	0.074	0.171
CD45+/B-1cells	0.59 [0.4 , 0.66]	0.56 [0.51 , 0.64]	0.38 [0.3 , 0.46]	0.41 [0.36 , 0.48]	0.943	0.383	0.559
CD45+/CD11b+Ly6G+	9.08 [7.83 , 9.98]	7.83 [6.26 , 10.7]	8.93 [7.37 , 11.45]	10.6 [8.79 , 12.35]	0.66	0.213	0.501
CD45+/NKcells	2.76 [2.65 , 3.25]	2.86 [2.2 , 3.31]	2.4 [2.18 , 2.69]	3.05 [2.67 , 3.69]	0.567	0.017	0.229
CD45+/NKTcells	0.89 [0.79 , 1.51]	1.12 [0.9 , 1.38]	0.81 [0.6 , 0.85]	0.97 [0.88 , 1.08]	0.846	0.004	0.047
CD45+/Ly6C-CD11b+	1.91 [1.48 , 2.59]	2.28 [1.68 , 2.59]	2.01 [1.82 , 2.31]	2.31 [1.84 , 2.44]	0.645	0.418	0.384
CD45+/Ly6C+CD11b+	6.13 [4.63 , 7.34]	6.65 [5.64 , 8.05]	6.74 [5.67 , 7.58]	6.55 [6.03 , 8.18]	0.305	0.595	0.281
CD45+/non-spec-rest	0.76 [0.61 , 0.86]	0.67 [0.62 , 0.73]	0.76 [0.64 , 0.84]	0.76 [0.63 , 0.92]	0.262	0.894	0.392

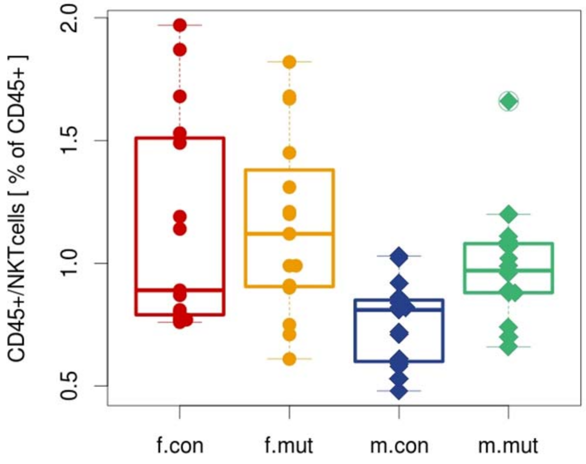
Immunology

Results: frequencies of main leukocyte subsets in peripheral blood [% of all CD45$^+$ cells]

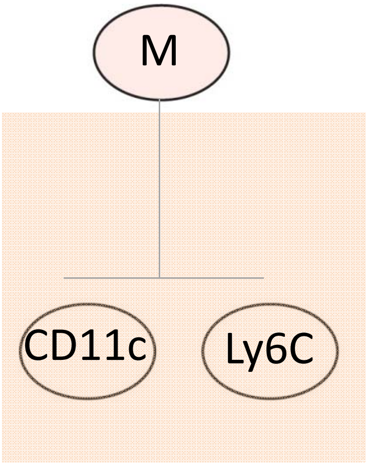
In male mutants higher frequencies of T cells, NK cells, NKT cells







Frequencies of monocyte subpopulations

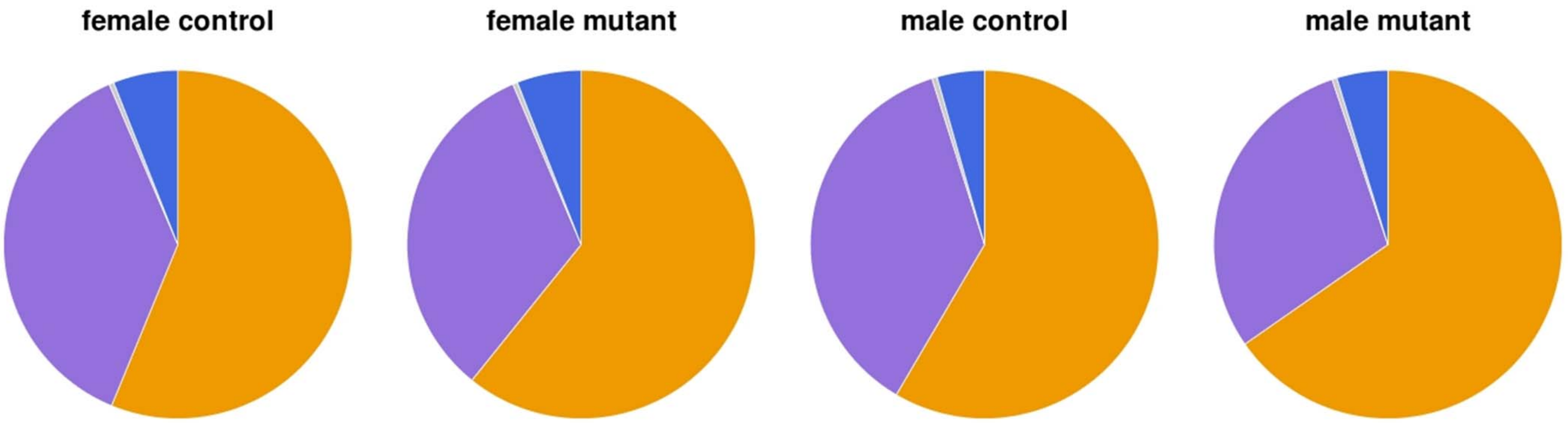


Frequencies are referring to all monocytes
(CD11b$^+$ but no Ly6G$^+$ and not NK cells)

Monocytes can be subdivided according to their CD11c or Ly6C expression

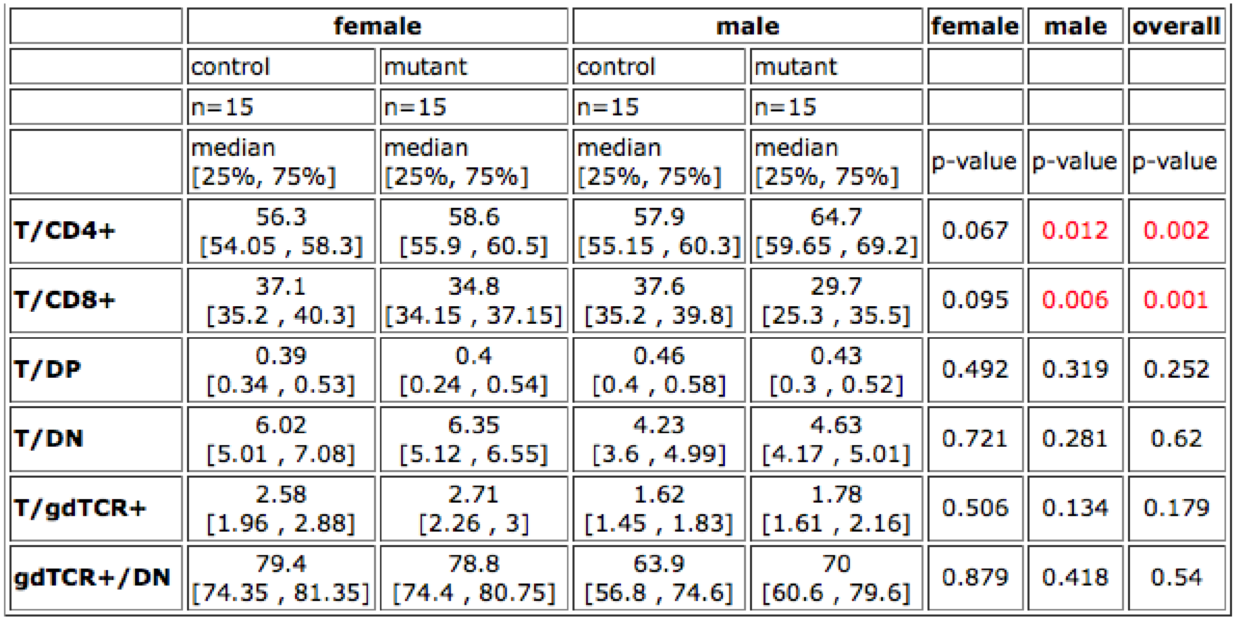
Recent bonemarrow emigrants express high levels of Ly6C
Inflammatory monocytes express intermediate levels of Ly6C
Steady state monocytes express low levels Ly6C

Immunology



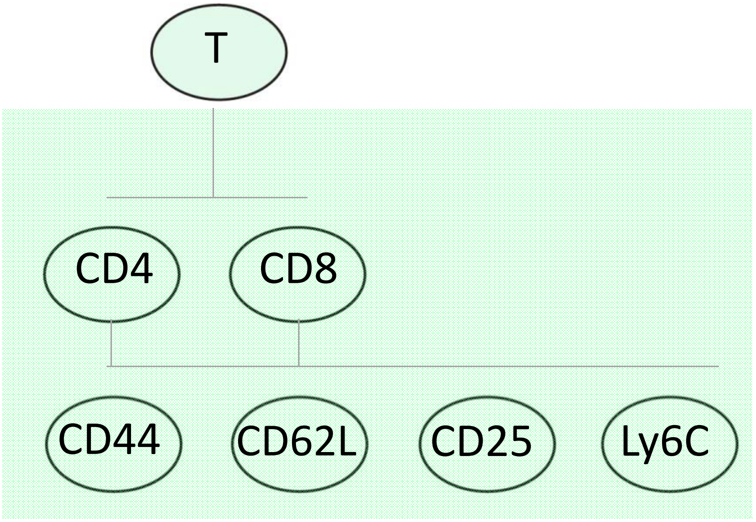
- 🟧 CD4+
- 🟪 CD8+
- ⬜ DP
- 🟦 DN

We found a significantly higher proportion of CD4 $^{single\ positive}$ and a decreased proportions of CD8 $^{single\ positive}$ T cells in male mutants; female mutants are showing the same tendency



	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
T/CD4+	56.3 [54.05 , 58.3]	58.6 [55.9 , 60.5]	57.9 [55.15 , 60.3]	64.7 [59.65 , 69.2]	0.067	0.012	0.002
T/CD8+	37.1 [35.2 , 40.3]	34.8 [34.15 , 37.15]	37.6 [35.2 , 39.8]	29.7 [25.3 , 35.5]	0.095	0.006	0.001
T/DP	0.39 [0.34 , 0.53]	0.4 [0.24 , 0.54]	0.46 [0.4 , 0.58]	0.43 [0.3 , 0.52]	0.492	0.319	0.252
T/DN	6.02 [5.01 , 7.08]	6.35 [5.12 , 6.55]	4.23 [3.6 , 4.99]	4.63 [4.17 , 5.01]	0.721	0.281	0.62
T/gdTCR+	2.58 [1.96 , 2.88]	2.71 [2.26 , 3]	1.62 [1.45 , 1.83]	1.78 [1.61 , 2.16]	0.506	0.134	0.179
gdTCR+/DN	79.4 [74.35 , 81.35]	78.8 [74.4 , 80.75]	63.9 [56.8 , 74.6]	70 [60.6 , 79.6]	0.879	0.418	0.54

T cell subpopulations



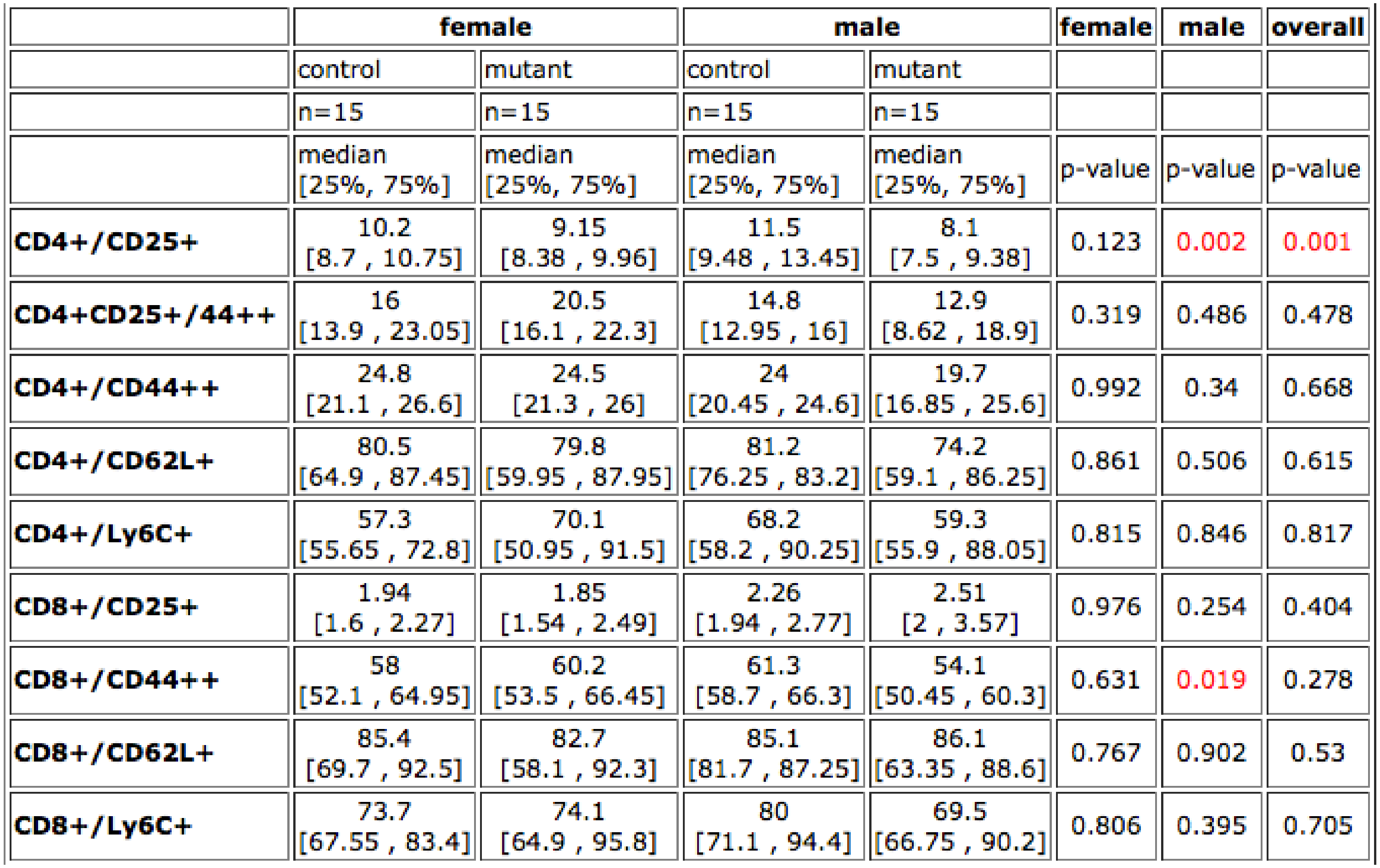
Frequencies are referring to
CD4 *single positive* or
CD8 *single positive* T cells

T cells can be further subdivided according to their expression of the activation marker CD44, the memory marker Ly6C and the homing marker CD62L (L-selectin) and IL-2 receptor CD25

Regulatory T cells (Tregs) are characterized by expression of $CD4^+$ and $CD25^+$

Immunology

Results: frequencies of T cell subsets in peripheral blood, concerning CD44, CD62L, Ly6C - Boolean gating [% of all CD4$^+$ T cells]

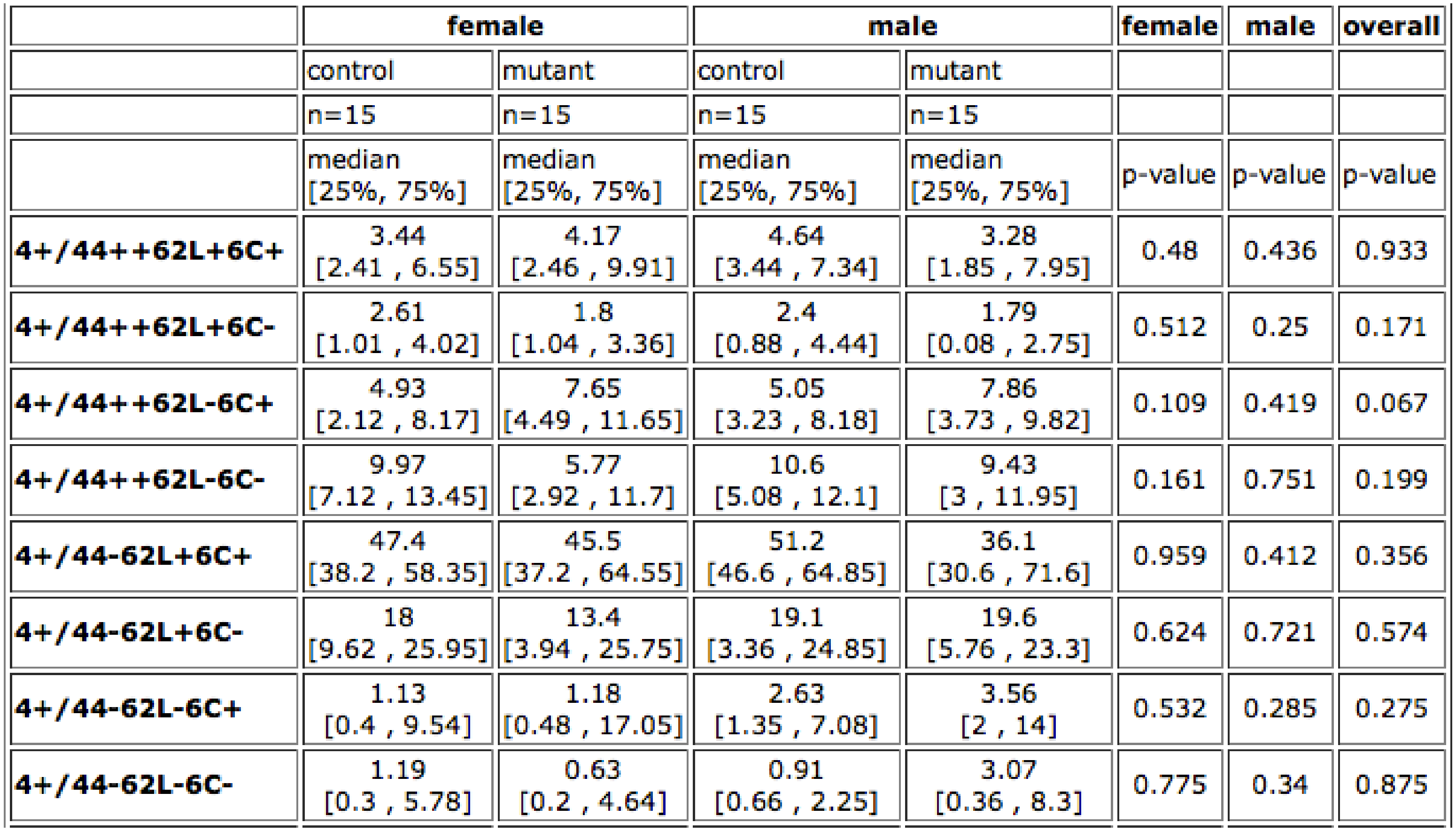


	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
CD4+/CD25+	10.2 [8.7 , 10.75]	9.15 [8.38 , 9.96]	11.5 [9.48 , 13.45]	8.1 [7.5 , 9.38]	0.123	0.002	0.001
CD4+CD25+/44++	16 [13.9 , 23.05]	20.5 [16.1 , 22.3]	14.8 [12.95 , 16]	12.9 [8.62 , 18.9]	0.319	0.486	0.478
CD4+/CD44++	24.8 [21.1 , 26.6]	24.5 [21.3 , 26]	24 [20.45 , 24.6]	19.7 [16.85 , 25.6]	0.992	0.34	0.668
CD4+/CD62L+	80.5 [64.9 , 87.45]	79.8 [59.95 , 87.95]	81.2 [76.25 , 83.2]	74.2 [59.1 , 86.25]	0.861	0.506	0.615
CD4+/Ly6C+	57.3 [55.65 , 72.8]	70.1 [50.95 , 91.5]	68.2 [58.2 , 90.25]	59.3 [55.9 , 88.05]	0.815	0.846	0.817
CD8+/CD25+	1.94 [1.6 , 2.27]	1.85 [1.54 , 2.49]	2.26 [1.94 , 2.77]	2.51 [2 , 3.57]	0.976	0.254	0.404
CD8+/CD44++	58 [52.1 , 64.95]	60.2 [53.5 , 66.45]	61.3 [58.7 , 66.3]	54.1 [50.45 , 60.3]	0.631	0.019	0.278
CD8+/CD62L+	85.4 [69.7 , 92.5]	82.7 [58.1 , 92.3]	85.1 [81.7 , 87.25]	86.1 [63.35 , 88.6]	0.767	0.902	0.53
CD8+/Ly6C+	73.7 [67.55 , 83.4]	74.1 [64.9 , 95.8]	80 [71.1 , 94.4]	69.5 [66.75 , 90.2]	0.806	0.395	0.705

Decreased frequency of CD25 positive cells within the CD4 T cell compartment
Decreased frequency of CD44 positive cells within the CD8 T cell compartment in male mutants

Immunology

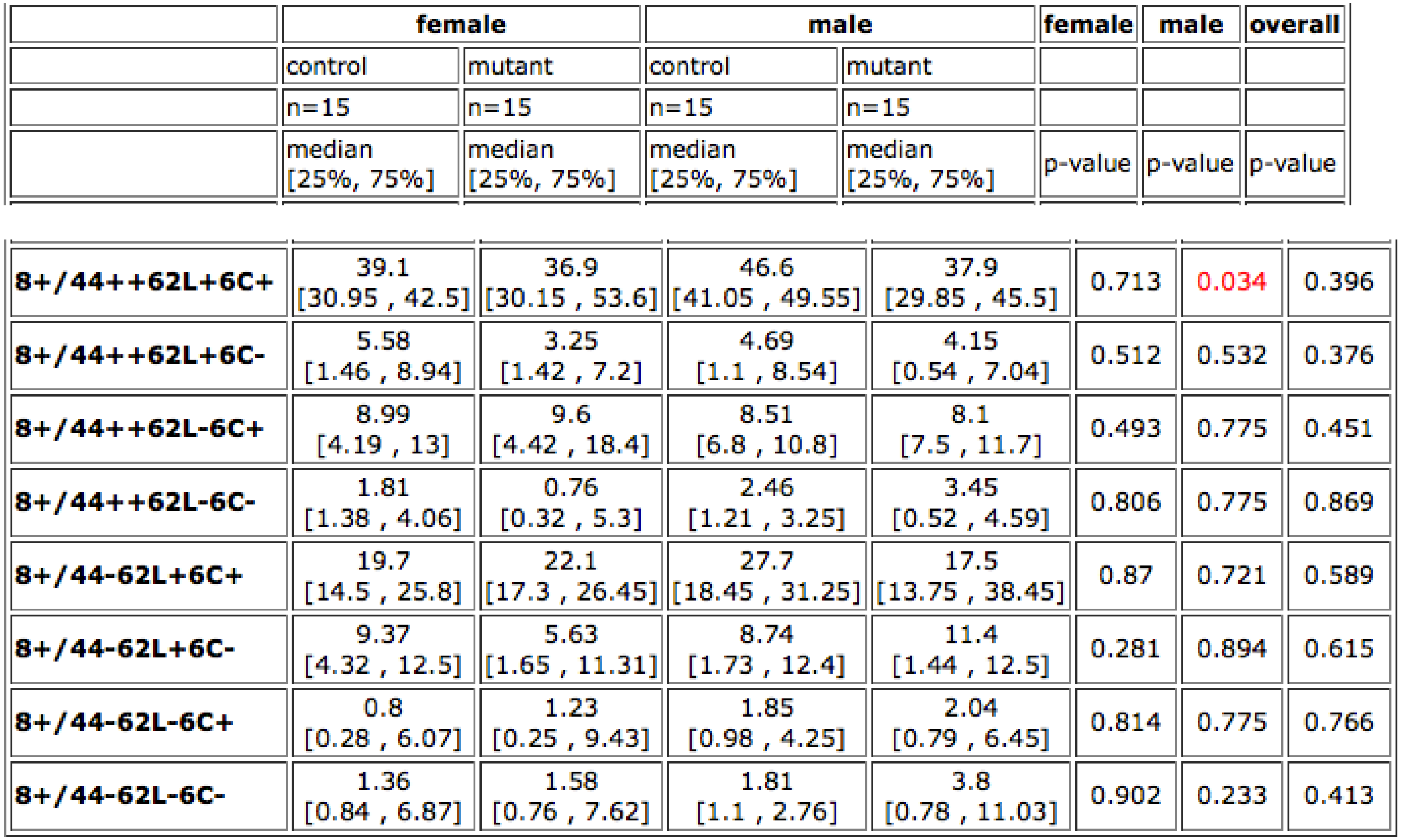
Results: frequencies of T cell subsets in peripheral blood, concerning CD44, CD62L, Ly6C - Boolean gating [% of all CD4^{+} T cells]



	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
4+/44++62L+6C+	3.44 [2.41 , 6.55]	4.17 [2.46 , 9.91]	4.64 [3.44 , 7.34]	3.28 [1.85 , 7.95]	0.48	0.436	0.933
4+/44++62L+6C-	2.61 [1.01 , 4.02]	1.8 [1.04 , 3.36]	2.4 [0.88 , 4.44]	1.79 [0.08 , 2.75]	0.512	0.25	0.171
4+/44++62L-6C+	4.93 [2.12 , 8.17]	7.65 [4.49 , 11.65]	5.05 [3.23 , 8.18]	7.86 [3.73 , 9.82]	0.109	0.419	0.067
4+/44++62L-6C-	9.97 [7.12 , 13.45]	5.77 [2.92 , 11.7]	10.6 [5.08 , 12.1]	9.43 [3 , 11.95]	0.161	0.751	0.199
4+/44-62L+6C+	47.4 [38.2 , 58.35]	45.5 [37.2 , 64.55]	51.2 [46.6 , 64.85]	36.1 [30.6 , 71.6]	0.959	0.412	0.356
4+/44-62L+6C-	18 [9.62 , 25.95]	13.4 [3.94 , 25.75]	19.1 [3.36 , 24.85]	19.6 [5.76 , 23.3]	0.624	0.721	0.574
4+/44-62L-6C+	1.13 [0.4 , 9.54]	1.18 [0.48 , 17.05]	2.63 [1.35 , 7.08]	3.56 [2 , 14]	0.532	0.285	0.275
4+/44-62L-6C-	1.19 [0.3 , 5.78]	0.63 [0.2 , 4.64]	0.91 [0.66 , 2.25]	3.07 [0.36 , 8.3]	0.775	0.34	0.875

Immunology

Results: frequencies of T cell subsets in peripheral blood, concerning CD44, CD62L, Ly6C - Boolean gating [% of all $CD8^+$ T cells]

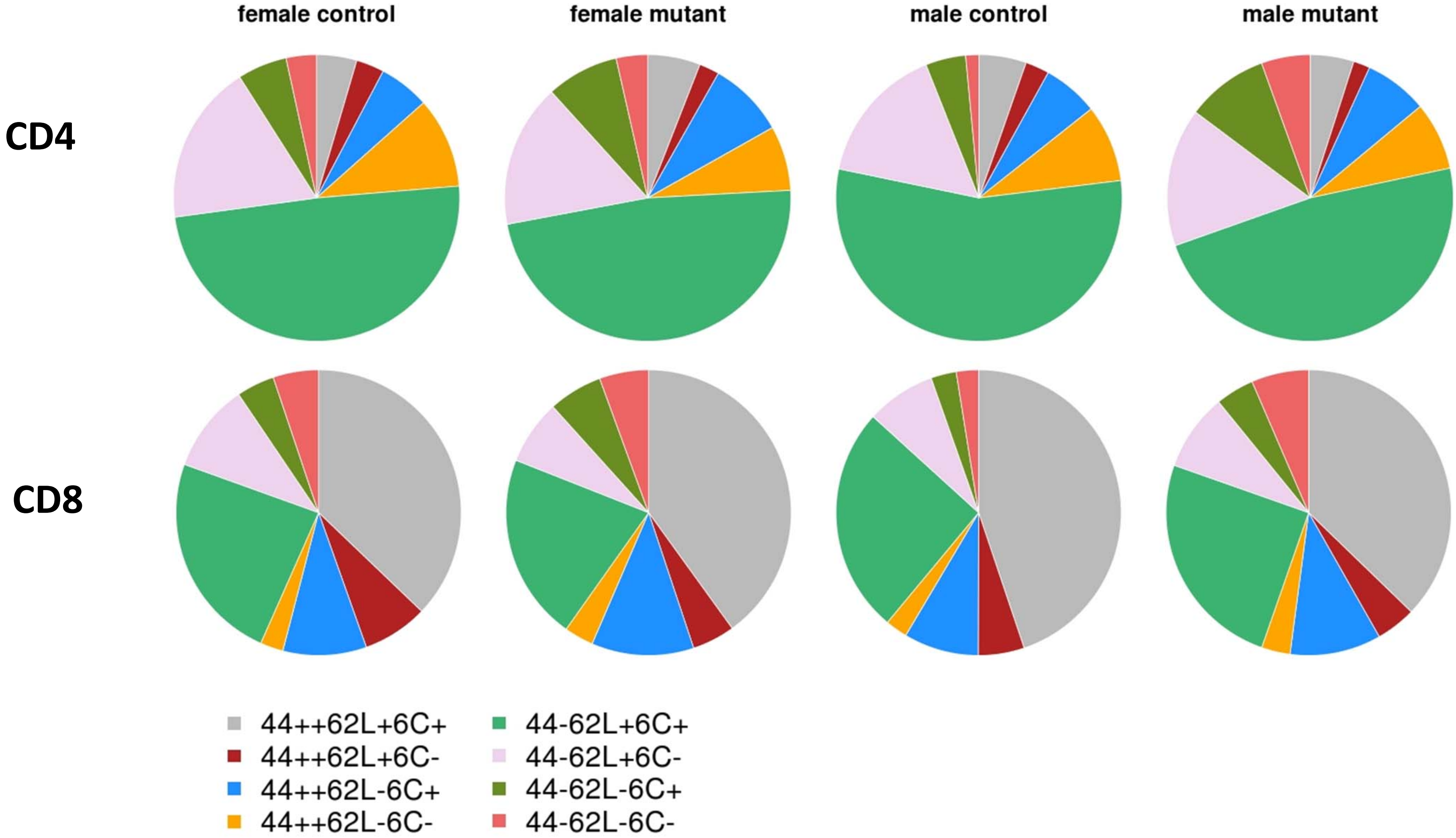


	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
8+/44++62L+6C+	39.1 [30.95 , 42.5]	36.9 [30.15 , 53.6]	46.6 [41.05 , 49.55]	37.9 [29.85 , 45.5]	0.713	0.034	0.396
8+/44++62L+6C-	5.58 [1.46 , 8.94]	3.25 [1.42 , 7.2]	4.69 [1.1 , 8.54]	4.15 [0.54 , 7.04]	0.512	0.532	0.376
8+/44++62L-6C+	8.99 [4.19 , 13]	9.6 [4.42 , 18.4]	8.51 [6.8 , 10.8]	8.1 [7.5 , 11.7]	0.493	0.775	0.451
8+/44++62L-6C-	1.81 [1.38 , 4.06]	0.76 [0.32 , 5.3]	2.46 [1.21 , 3.25]	3.45 [0.52 , 4.59]	0.806	0.775	0.869
8+/44-62L+6C+	19.7 [14.5 , 25.8]	22.1 [17.3 , 26.45]	27.7 [18.45 , 31.25]	17.5 [13.75 , 38.45]	0.87	0.721	0.589
8+/44-62L+6C-	9.37 [4.32 , 12.5]	5.63 [1.65 , 11.31]	8.74 [1.73 , 12.4]	11.4 [1.44 , 12.5]	0.281	0.894	0.615
8+/44-62L-6C+	0.8 [0.28 , 6.07]	1.23 [0.25 , 9.43]	1.85 [0.98 , 4.25]	2.04 [0.79 , 6.45]	0.814	0.775	0.766
8+/44-62L-6C-	1.36 [0.84 , 6.87]	1.58 [0.76 , 7.62]	1.81 [1.1 , 2.76]	3.8 [0.78 , 11.03]	0.902	0.233	0.413

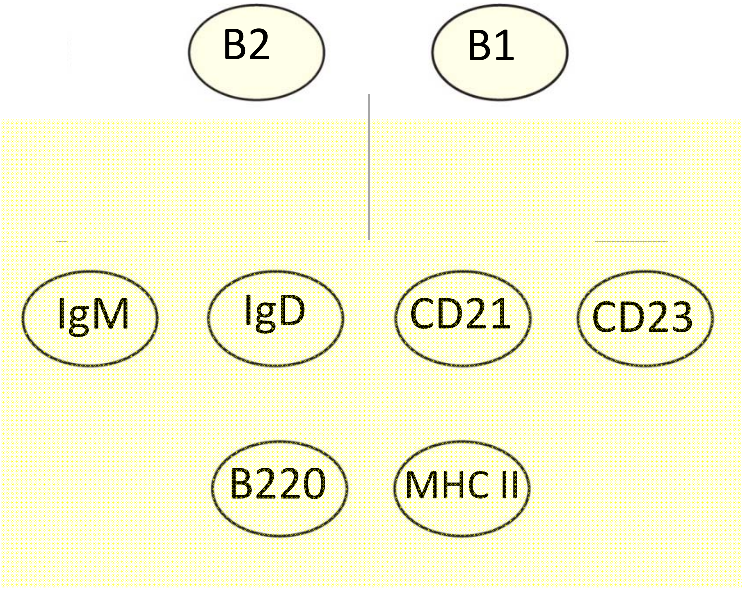
Decreased frequency of Ly6C positive central memory-like cells within the CD8 T cell compartment in male mutants

Immunology

Results: patterns concerning CD44 high, CD62L, Ly6C of CD4 and CD8 T cell subsets in peripheral blood - [CD4$^+$ or CD8$^+$ T cells, corresponding to the values in the tables of the anterior slides]



B cell subpopulations



Frequencies are referring to
all B cells (CD19^{+})
or B-2 B cells *(CD5 negative)*

B cells can be subdivided according to their co-expression of the surface immunoglobulins IgD and IgM and the Fc-receptor CD23, and the complement receptors 1 and 2 (CD21/CD35).

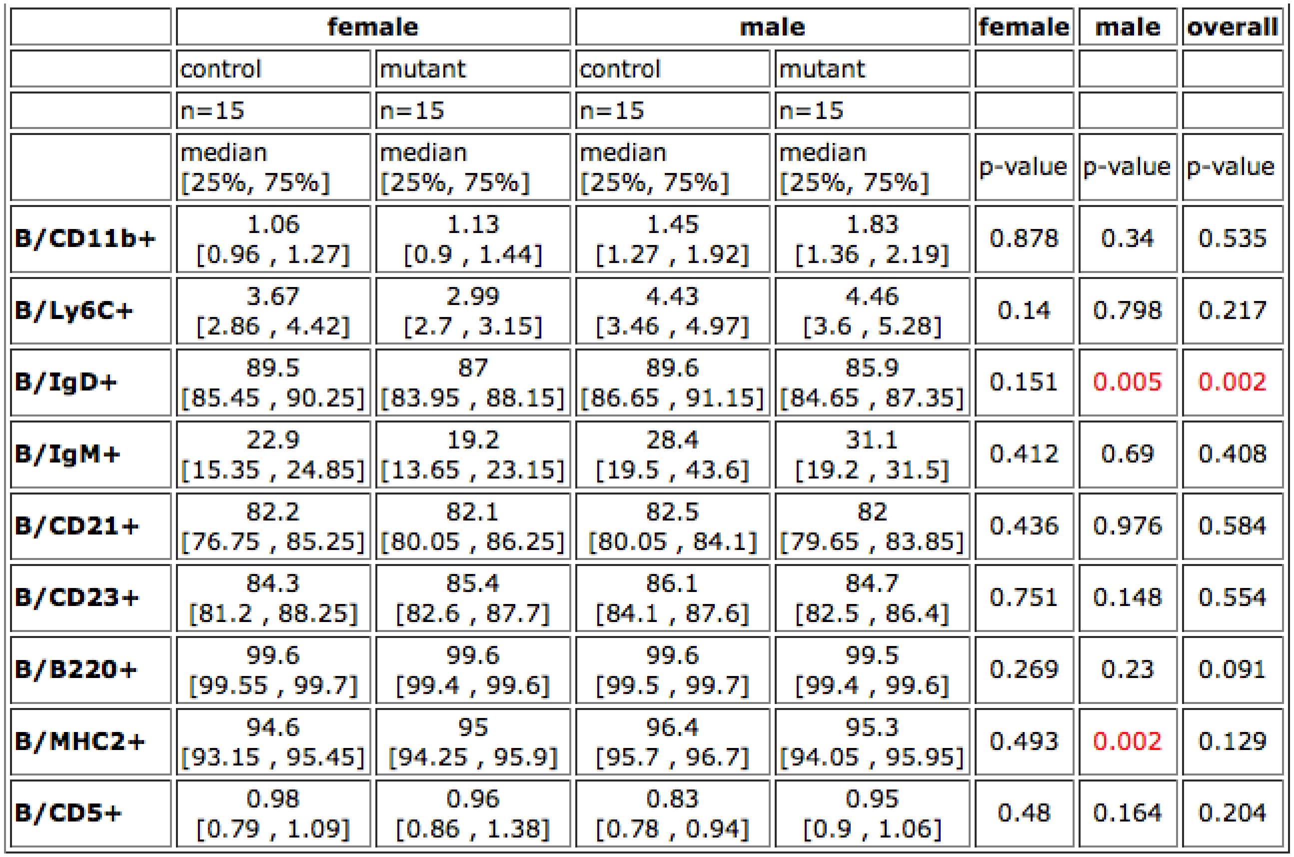
B1-B cells is a very small murine B cell population in blood, and found predominantly in the pleural and peritoneal cavity.

Mature naïve B cells are expected to be IgD^{+}/ IgM$^{low/negative}$ /CD23^{+} /CD21^{+}

Immature B cells have a IgM^{high} / IgD^{low} phenotype and can be further classified according to their CD23 and CD21 expression

Immunology

Results: frequencies of B cell subsets in peripheral blood [% of all B cells]

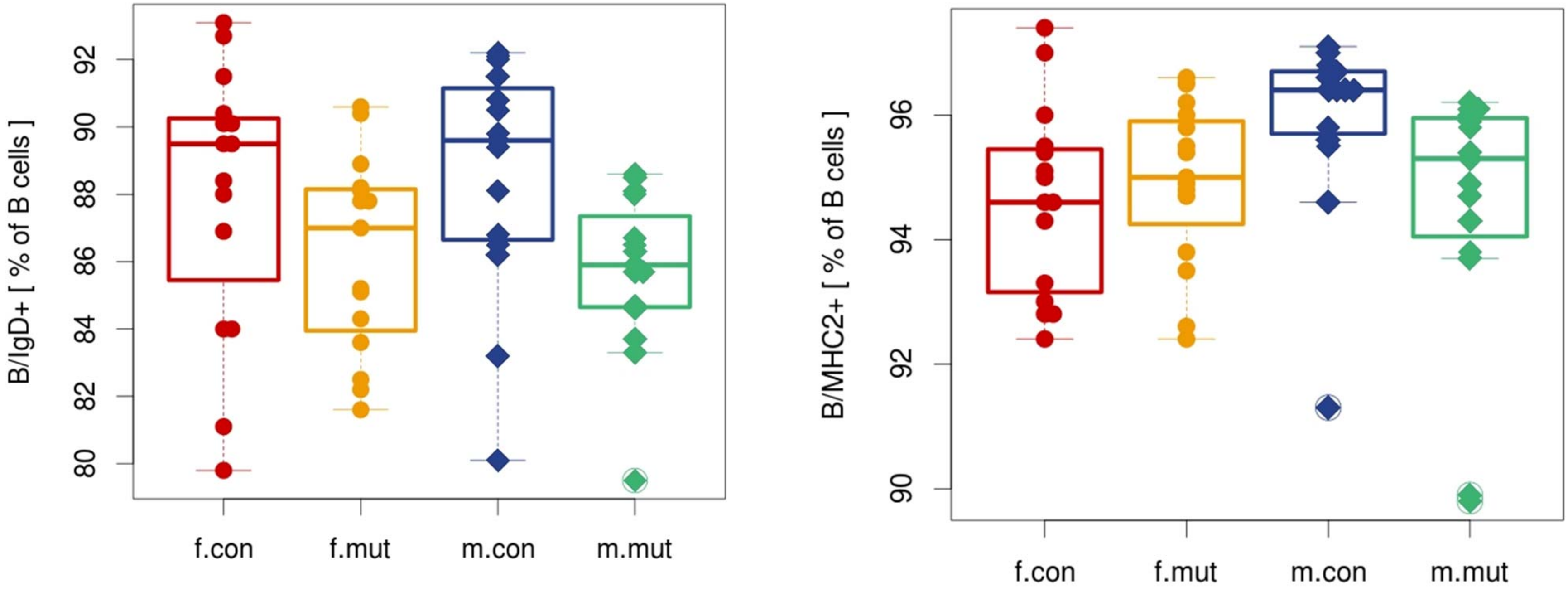


	female		**male**		**female**	**male**	**overall**
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
B/CD11b+	1.06 [0.96 , 1.27]	1.13 [0.9 , 1.44]	1.45 [1.27 , 1.92]	1.83 [1.36 , 2.19]	0.878	0.34	0.535
B/Ly6C+	3.67 [2.86 , 4.42]	2.99 [2.7 , 3.15]	4.43 [3.46 , 4.97]	4.46 [3.6 , 5.28]	0.14	0.798	0.217
B/IgD+	89.5 [85.45 , 90.25]	87 [83.95 , 88.15]	89.6 [86.65 , 91.15]	85.9 [84.65 , 87.35]	0.151	0.005	0.002
B/IgM+	22.9 [15.35 , 24.85]	19.2 [13.65 , 23.15]	28.4 [19.5 , 43.6]	31.1 [19.2 , 31.5]	0.412	0.69	0.408
B/CD21+	82.2 [76.75 , 85.25]	82.1 [80.05 , 86.25]	82.5 [80.05 , 84.1]	82 [79.65 , 83.85]	0.436	0.976	0.584
B/CD23+	84.3 [81.2 , 88.25]	85.4 [82.6 , 87.7]	86.1 [84.1 , 87.6]	84.7 [82.5 , 86.4]	0.751	0.148	0.554
B/B220+	99.6 [99.55 , 99.7]	99.6 [99.4 , 99.6]	99.6 [99.5 , 99.7]	99.5 [99.4 , 99.6]	0.269	0.23	0.091
B/MHC2+	94.6 [93.15 , 95.45]	95 [94.25 , 95.9]	96.4 [95.7 , 96.7]	95.3 [94.05 , 95.95]	0.493	0.002	0.129
B/CD5+	0.98 [0.79 , 1.09]	0.96 [0.86 , 1.38]	0.83 [0.78 , 0.94]	0.95 [0.9 , 1.06]	0.48	0.164	0.204

Slightly decreased frequency of IgD positive and of MHC class II positive cells within the B cell compartment from male mutants

Immunology

Results: frequencies of B cell subsets in peripheral blood [% of all B cells]

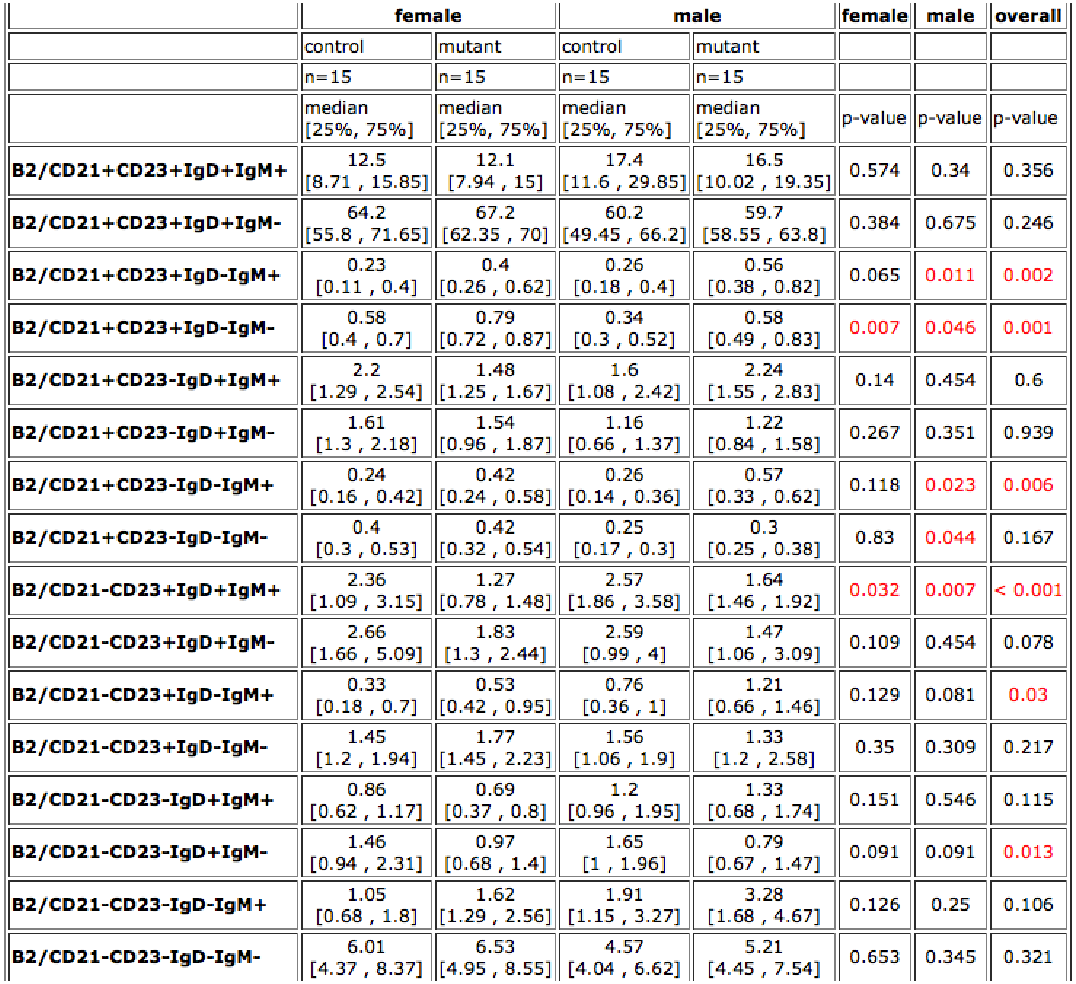


Slightly decreased frequency of IgD positive and of MHC class II positive cells within the B cell compartment from male mutants

Immunology

Results: patterns concerning CD21, CD23, IgD, IgM of CD5^{negative} B cells (B-2 B cells) in peripheral blood [% of all B2-B cells]

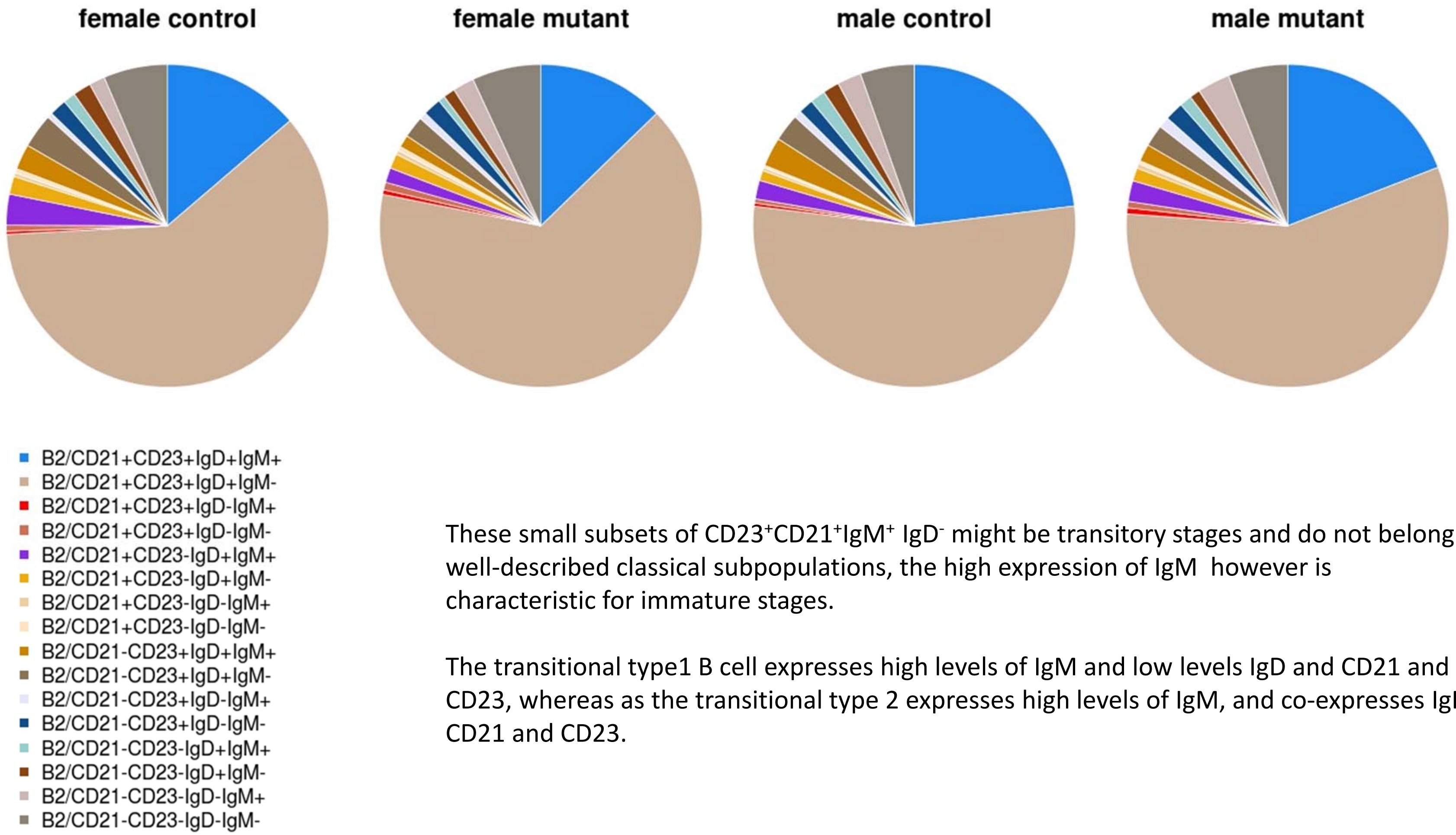
Slightly increased frequencies of cellswith an immature phenotype (IgD low, IgM positive)
and a lower frequency of IgD^{+}IgM^{+}CD23^{+}CD21^{-} cells within the B cell compartment of mutants



	female		**male**		**female**	**male**	**overall**
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
B2/CD21+CD23+IgD+IgM+	12.5 [8.71 , 15.85]	12.1 [7.94 , 15]	17.4 [11.6 , 29.85]	16.5 [10.02 , 19.35]	0.574	0.34	0.356
B2/CD21+CD23+IgD+IgM-	64.2 [55.8 , 71.65]	67.2 [62.35 , 70]	60.2 [49.45 , 66.2]	59.7 [58.55 , 63.8]	0.384	0.675	0.246
B2/CD21+CD23+IgD-IgM+	0.23 [0.11 , 0.4]	0.4 [0.26 , 0.62]	0.26 [0.18 , 0.4]	0.56 [0.38 , 0.82]	0.065	0.011	0.002
B2/CD21+CD23+IgD-IgM-	0.58 [0.4 , 0.7]	0.79 [0.72 , 0.87]	0.34 [0.3 , 0.52]	0.58 [0.49 , 0.83]	0.007	0.046	0.001
B2/CD21+CD23-IgD+IgM+	2.2 [1.29 , 2.54]	1.48 [1.25 , 1.67]	1.6 [1.08 , 2.42]	2.24 [1.55 , 2.83]	0.14	0.454	0.6
B2/CD21+CD23-IgD+IgM-	1.61 [1.3 , 2.18]	1.54 [0.96 , 1.87]	1.16 [0.66 , 1.37]	1.22 [0.84 , 1.58]	0.267	0.351	0.939
B2/CD21+CD23-IgD-IgM+	0.24 [0.16 , 0.42]	0.42 [0.24 , 0.58]	0.26 [0.14 , 0.36]	0.57 [0.33 , 0.62]	0.118	0.023	0.006
B2/CD21+CD23-IgD-IgM-	0.4 [0.3 , 0.53]	0.42 [0.32 , 0.54]	0.25 [0.17 , 0.3]	0.3 [0.25 , 0.38]	0.83	0.044	0.167
B2/CD21-CD23+IgD+IgM+	2.36 [1.09 , 3.15]	1.27 [0.78 , 1.48]	2.57 [1.86 , 3.58]	1.64 [1.46 , 1.92]	0.032	0.007	< 0.001
B2/CD21-CD23+IgD+IgM-	2.66 [1.66 , 5.09]	1.83 [1.3 , 2.44]	2.59 [0.99 , 4]	1.47 [1.06 , 3.09]	0.109	0.454	0.078
B2/CD21-CD23+IgD-IgM+	0.33 [0.18 , 0.7]	0.53 [0.42 , 0.95]	0.76 [0.36 , 1]	1.21 [0.66 , 1.46]	0.129	0.081	0.03
B2/CD21-CD23+IgD-IgM-	1.45 [1.2 , 1.94]	1.77 [1.45 , 2.23]	1.56 [1.06 , 1.9]	1.33 [1.2 , 2.58]	0.35	0.309	0.217
B2/CD21-CD23-IgD+IgM+	0.86 [0.62 , 1.17]	0.69 [0.37 , 0.8]	1.2 [0.96 , 1.95]	1.33 [0.68 , 1.74]	0.151	0.546	0.115
B2/CD21-CD23-IgD+IgM-	1.46 [0.94 , 2.31]	0.97 [0.68 , 1.4]	1.65 [1 , 1.96]	0.79 [0.67 , 1.47]	0.091	0.091	0.013
B2/CD21-CD23-IgD-IgM+	1.05 [0.68 , 1.8]	1.62 [1.29 , 2.56]	1.91 [1.15 , 3.27]	3.28 [1.68 , 4.67]	0.126	0.25	0.106
B2/CD21-CD23-IgD-IgM-	6.01 [4.37 , 8.37]	6.53 [4.95 , 8.55]	4.57 [4.04 , 6.62]	5.21 [4.45 , 7.54]	0.653	0.345	0.321

Immunology

Results: patterns concerning CD21, CD23, IgD, IgM of CD5^{negative} B cells (B-2 B cells) in peripheral blood [% of all B2-B cells]

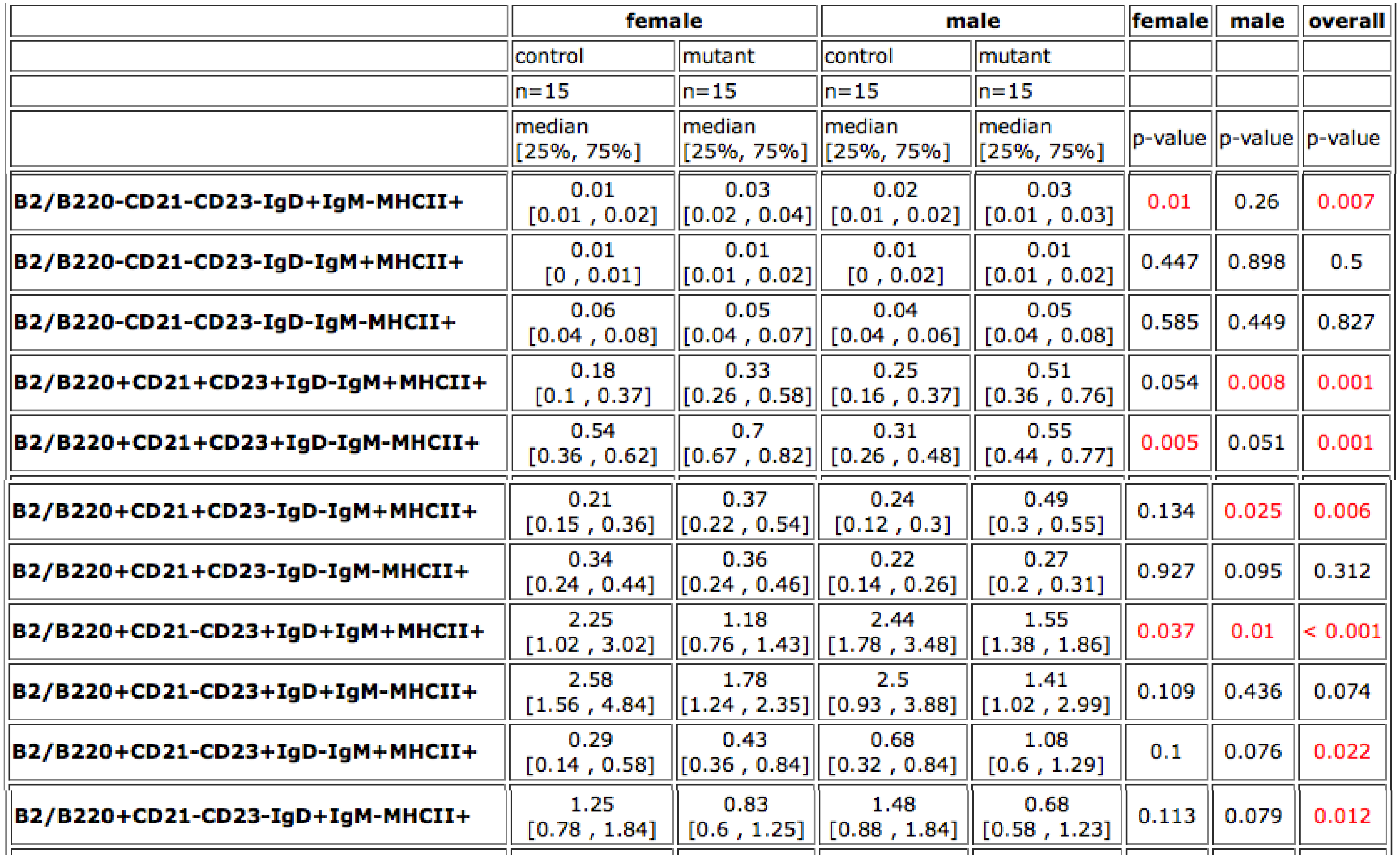


These small subsets of CD23$^+$CD21$^+$IgM$^+$ IgD$^-$ might be transitory stages and do not belong to well-described classical subpopulations, the high expression of IgM however is characteristic for immature stages.

The transitional type1 B cell expresses high levels of IgM and low levels IgD and CD21 and CD23, whereas as the transitional type 2 expresses high levels of IgM, and co-expresses IgD, CD21 and CD23.

Immunology

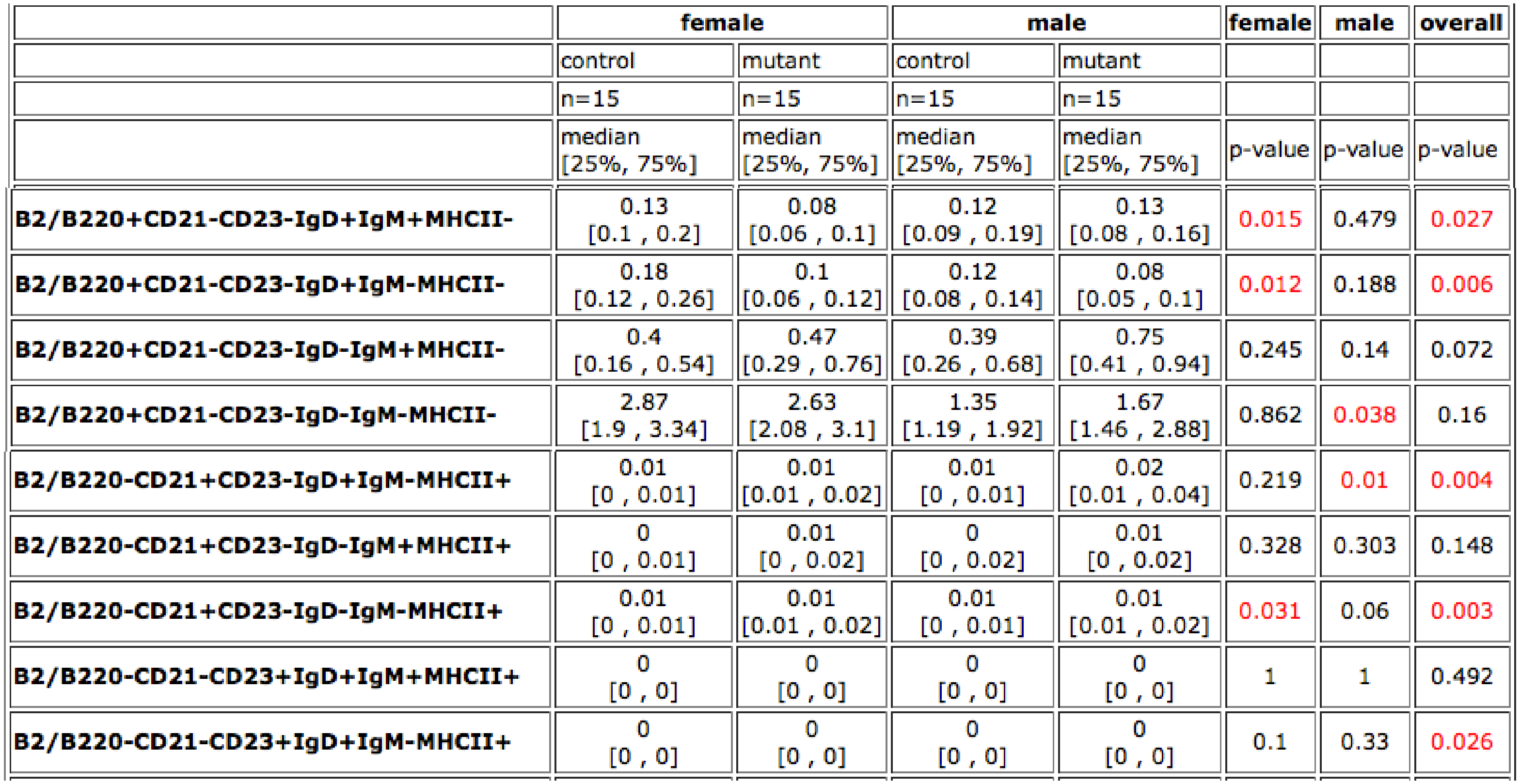
Results: patterns concerning CD21, CD23, IgD, IgM, B220 and MHC class II of CD5^{negative} B cells (B-2 B cells) in peripheral blood [% of all B2-B cells]



	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
B2/B220-CD21-CD23-IgD+IgM-MHCII+	0.01 [0.01 , 0.02]	0.03 [0.02 , 0.04]	0.02 [0.01 , 0.02]	0.03 [0.01 , 0.03]	0.01	0.26	0.007
B2/B220-CD21-CD23-IgD-IgM+MHCII+	0.01 [0 , 0.01]	0.01 [0.01 , 0.02]	0.01 [0 , 0.02]	0.01 [0.01 , 0.02]	0.447	0.898	0.5
B2/B220-CD21-CD23-IgD-IgM-MHCII+	0.06 [0.04 , 0.08]	0.05 [0.04 , 0.07]	0.04 [0.04 , 0.06]	0.05 [0.04 , 0.08]	0.585	0.449	0.827
B2/B220+CD21+CD23+IgD-IgM+MHCII+	0.18 [0.1 , 0.37]	0.33 [0.26 , 0.58]	0.25 [0.16 , 0.37]	0.51 [0.36 , 0.76]	0.054	0.008	0.001
B2/B220+CD21+CD23+IgD-IgM-MHCII+	0.54 [0.36 , 0.62]	0.7 [0.67 , 0.82]	0.31 [0.26 , 0.48]	0.55 [0.44 , 0.77]	0.005	0.051	0.001
B2/B220+CD21+CD23-IgD-IgM+MHCII+	0.21 [0.15 , 0.36]	0.37 [0.22 , 0.54]	0.24 [0.12 , 0.3]	0.49 [0.3 , 0.55]	0.134	0.025	0.006
B2/B220+CD21+CD23-IgD-IgM-MHCII+	0.34 [0.24 , 0.44]	0.36 [0.24 , 0.46]	0.22 [0.14 , 0.26]	0.27 [0.2 , 0.31]	0.927	0.095	0.312
B2/B220+CD21-CD23+IgD+IgM+MHCII+	2.25 [1.02 , 3.02]	1.18 [0.76 , 1.43]	2.44 [1.78 , 3.48]	1.55 [1.38 , 1.86]	0.037	0.01	< 0.001
B2/B220+CD21-CD23+IgD+IgM-MHCII+	2.58 [1.56 , 4.84]	1.78 [1.24 , 2.35]	2.5 [0.93 , 3.88]	1.41 [1.02 , 2.99]	0.109	0.436	0.074
B2/B220+CD21-CD23+IgD-IgM+MHCII+	0.29 [0.14 , 0.58]	0.43 [0.36 , 0.84]	0.68 [0.32 , 0.84]	1.08 [0.6 , 1.29]	0.1	0.076	0.022
B2/B220+CD21-CD23-IgD+IgM-MHCII+	1.25 [0.78 , 1.84]	0.83 [0.6 , 1.25]	1.48 [0.88 , 1.84]	0.68 [0.58 , 1.23]	0.113	0.079	0.012

Immunology

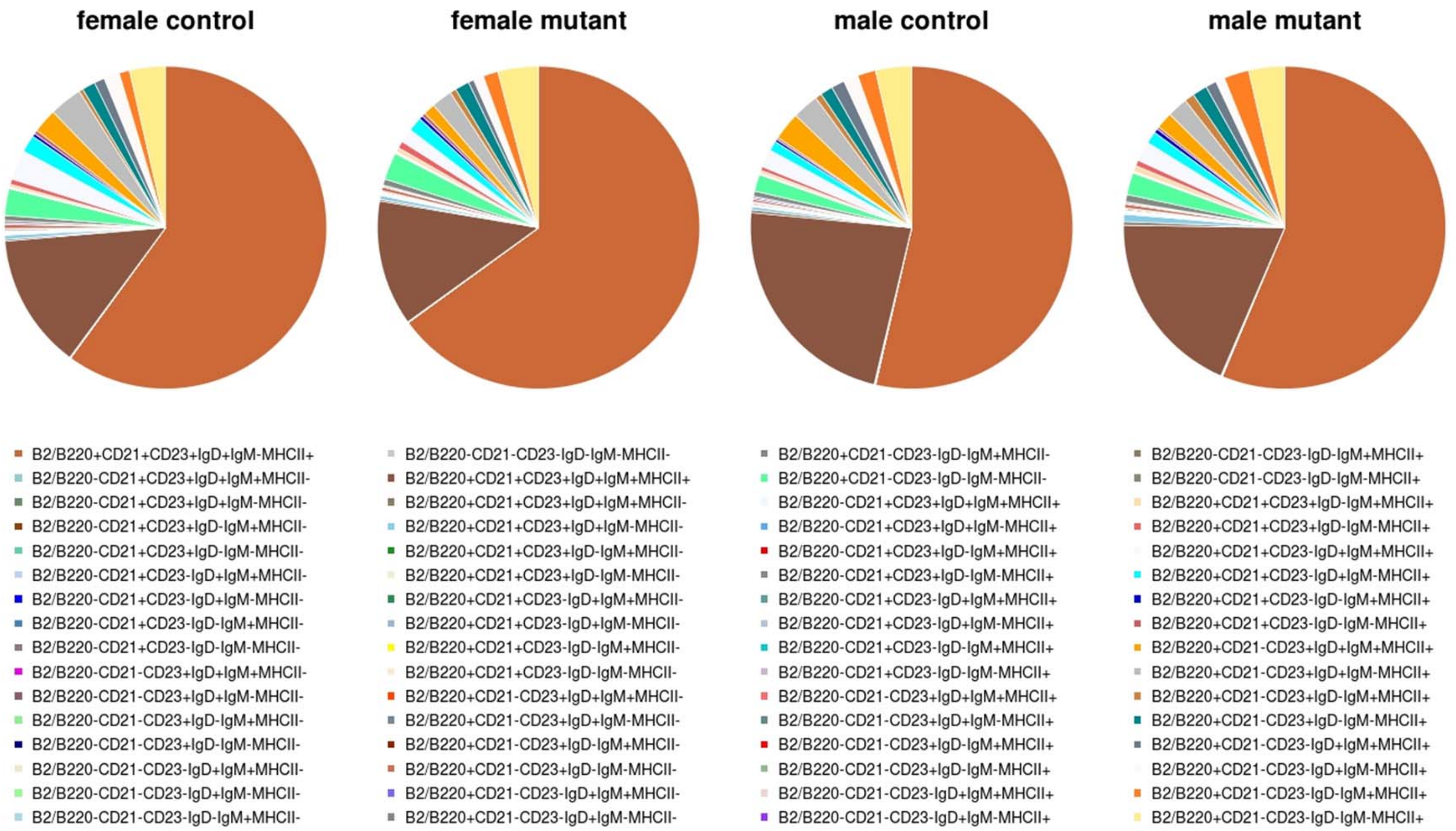
Results: patterns concerning CD21, CD23, IgD, IgM, B220 and MHC class II of $CD5^{negative}$ B cells (B-2 B cells) in peripheral blood [% of all B2-B cells]



	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
B2/B220+CD21-CD23-IgD+IgM+MHCII-	0.13 [0.1 , 0.2]	0.08 [0.06 , 0.1]	0.12 [0.09 , 0.19]	0.13 [0.08 , 0.16]	0.015	0.479	0.027
B2/B220+CD21-CD23-IgD+IgM-MHCII-	0.18 [0.12 , 0.26]	0.1 [0.06 , 0.12]	0.12 [0.08 , 0.14]	0.08 [0.05 , 0.1]	0.012	0.188	0.006
B2/B220+CD21-CD23-IgD-IgM+MHCII-	0.4 [0.16 , 0.54]	0.47 [0.29 , 0.76]	0.39 [0.26 , 0.68]	0.75 [0.41 , 0.94]	0.245	0.14	0.072
B2/B220+CD21-CD23-IgD-IgM-MHCII-	2.87 [1.9 , 3.34]	2.63 [2.08 , 3.1]	1.35 [1.19 , 1.92]	1.67 [1.46 , 2.88]	0.862	0.038	0.16
B2/B220-CD21+CD23-IgD+IgM-MHCII+	0.01 [0 , 0.01]	0.01 [0.01 , 0.02]	0.01 [0 , 0.01]	0.02 [0.01 , 0.04]	0.219	0.01	0.004
B2/B220-CD21+CD23-IgD-IgM+MHCII+	0 [0 , 0.01]	0.01 [0 , 0.02]	0 [0 , 0.02]	0.01 [0 , 0.02]	0.328	0.303	0.148
B2/B220-CD21+CD23-IgD-IgM-MHCII+	0.01 [0 , 0.01]	0.01 [0.01 , 0.02]	0.01 [0 , 0.01]	0.01 [0.01 , 0.02]	0.031	0.06	0.003
B2/B220-CD21-CD23+IgD+IgM+MHCII+	0 [0 , 0]	0 [0 , 0]	0 [0 , 0]	0 [0 , 0]	1	1	0.492
B2/B220-CD21-CD23+IgD+IgM-MHCII+	0 [0 , 0]	0 [0 , 0]	0 [0 , 0]	0 [0 , 0]	0.1	0.33	0.026

Immunology

Results: patterns concerning CD21, CD23, IgD, IgM, B220 and MHC class II of CD5^{negative} B cells (B-2 B cells) in peripheral blood [% of all B2-B cells]



NK cell subpopulations

NK

CD11b CD44 Ly6C CD11c CD62L

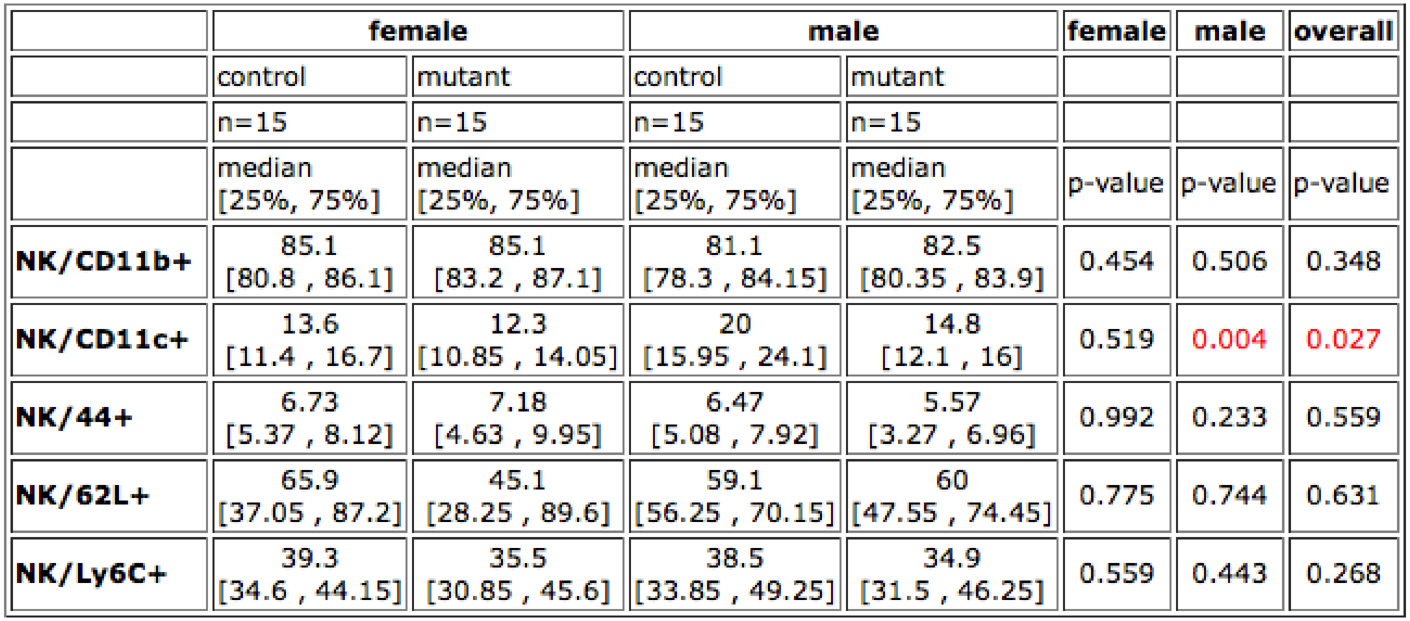
Frequencies are referring to
all NK cells (NK1.1$^+$/NKp46$^+$ CD5$^-$)

NK cells can subdivided according to their expression of the integrin CD11b, the activation marker CD44, the memory marker Ly6C, the homing marker CD62L. A small subset expresses the DC marker CD11c.

Mature NK cells co-express CD11b.

Immunology

Results: frequencies of NK cell subsets in peripheral blood [% of all NK cells]



	female		male		female	male	overall
	control	mutant	control	mutant			
	n=15	n=15	n=15	n=15			
	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	median [25%, 75%]	p-value	p-value	p-value
NK/CD11b+	85.1 [80.8 , 86.1]	85.1 [83.2 , 87.1]	81.1 [78.3 , 84.15]	82.5 [80.35 , 83.9]	0.454	0.506	0.348
NK/CD11c+	13.6 [11.4 , 16.7]	12.3 [10.85 , 14.05]	20 [15.95 , 24.1]	14.8 [12.1 , 16]	0.519	0.004	0.027
NK/44+	6.73 [5.37 , 8.12]	7.18 [4.63 , 9.95]	6.47 [5.08 , 7.92]	5.57 [3.27 , 6.96]	0.992	0.233	0.559
NK/62L+	65.9 [37.05 , 87.2]	45.1 [28.25 , 89.6]	59.1 [56.25 , 70.15]	60 [47.55 , 74.45]	0.775	0.744	0.631
NK/Ly6C+	39.3 [34.6 , 44.15]	35.5 [30.85 , 45.6]	38.5 [33.85 , 49.25]	34.9 [31.5 , 46.25]	0.559	0.443	0.268

Slightly decreased frequency of CD11c positive cells within the NK cell compartment from mutants especially from males.

Immunology

Summary of results

- Our analysis of leukocyte subpopulations in blood revealed differences in the frequencies of several leukocyte subpopulations.

- Most of the found differences were sex-dependent.

- The relationship between the subpopulations is unclear.