Supplementary materials

Mother–fetus immune cross-talk coordinates “extrinsic”/”intrinsic” embryo gene expression noise and growth stability

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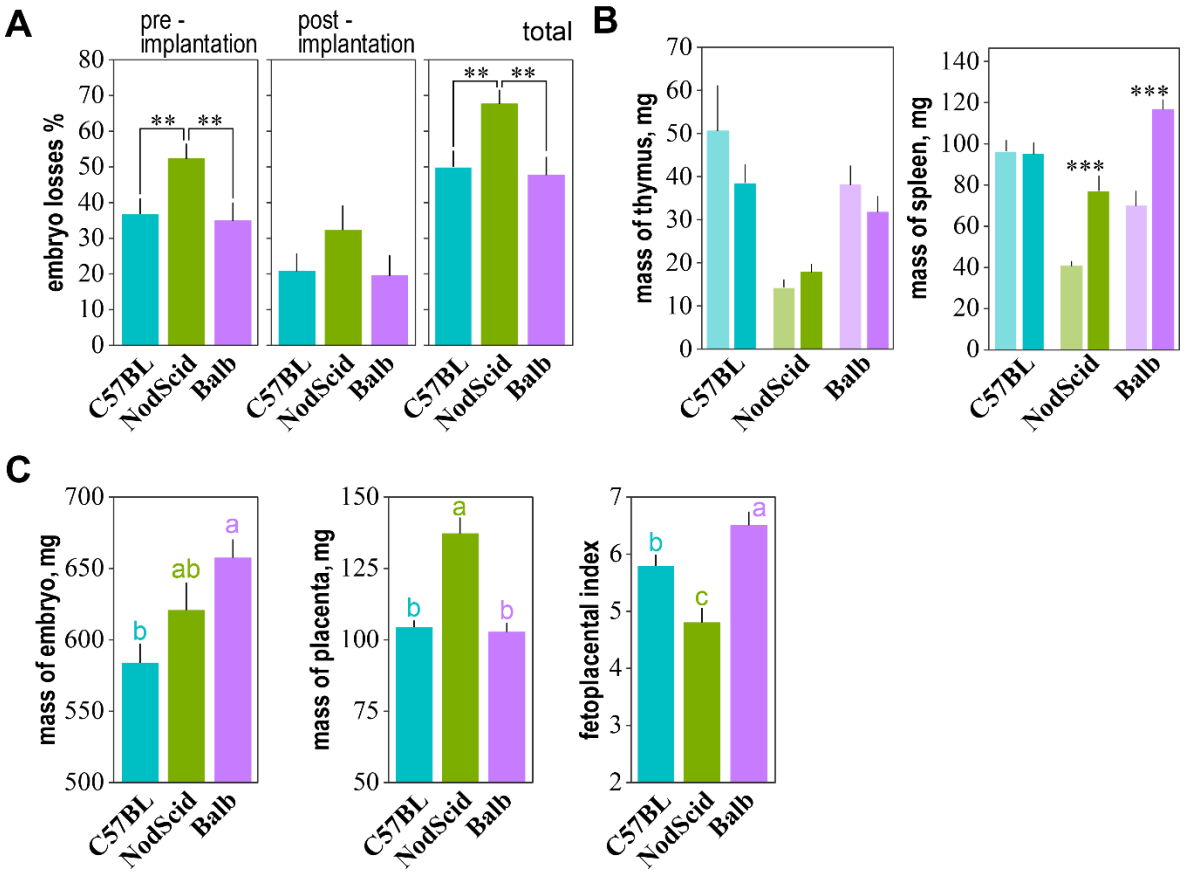

Figure S1. Characteristics of C57BL/6J fetuses and surrogate mothers' immunocompetent organs response.

A) Pre-, post-implantation, and total embryo losses in C57BL/6J, NOD-SCID, and BALB/c surrogate mothers. ** - Student's t-test, $p<0.01$.

B) Females' thymus and spleen mass in non-pregnant females (light-colored bars) and in pregnant (day 16.5 of surrogate pregnancy) females (dark-colored bars). *** - $p<0.001$, t-test; the number of non-pregnant/pregnant females: C57BL/6J (m = 3/13), NOD-SCID (m = 5/16) and BALB/c (m = 5/13).

C) C57BL/6J embryo weight, placenta weight, and fetoplacental index on day 16.5 of surrogate pregnancy. Letters indicate significant statistical differences at $p < 0.05$, LSD-test; the number of embryos by surrogate mother group: C57BL/6J (n = 61), NOD-SCID (n = 46) and BALB/c (n = 49).

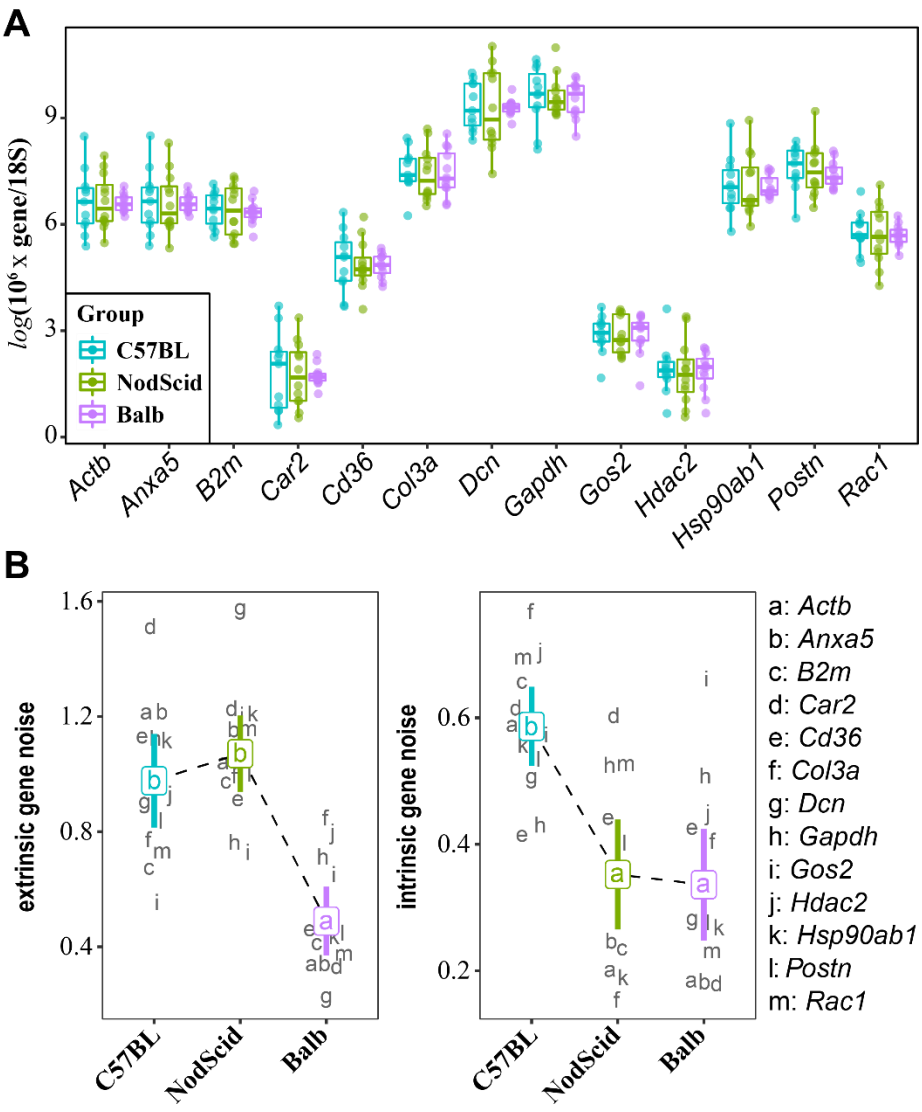

Figure S2. The impact of mother-fetus MHC-mediated interactions on gene-wise "extrinsic"/"intrinsic" fluctuations in left/right forelimbs of C57BL/6J embryos.

A) Left/right mean RT-qPCR $\Delta Ct = log(10^6\, g/18S)$ values for each gene measured on day 16.5 of gestation in forelimbs of C57BL/6J embryos, developing in C57BL/6J, NOD-SCID, and BALB/c surrogate mothers. ANOVA and pair-wise comparisons revealed no significant differences between surrogate mother groups' ΔCts of selected genes.

B) Estimations of "extrinsic" ($\eta_{ext.} \approx \sqrt{2\lambda_1}$) and "intrinsic" ($\eta_{int.} \approx \sqrt{2\lambda_2}$) gene noise for the selected genes following PCA decomposition of left/right gene expression (ΔCt) matrices for each gene (eq. (9)). Colored boxes indicate means, whiskers - 95% confidence intervals, letters within mean boxes - multiple t-test comparisons with significant differences at FDR < 0.05.

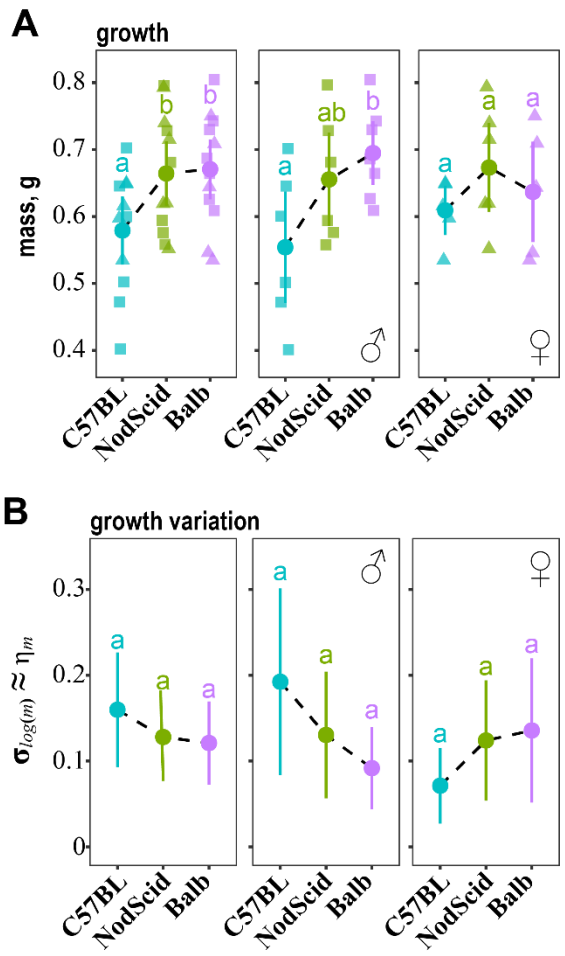

Figure S3. The effect of surrogate mother MHC haplotype on C57BL/6J embryos mass and its fluctuation.

A) Non-adjusted mass of C57BL/6J embryos developed in C57BL/6J, NOD-SCID, and BALB/c surrogate mothers; see Figure 3C.

B) GAMLSS estimations of $\hat{\sigma}_{log(m)} \approx \hat{\eta}_m$ for non-adjusted embryo mass; see Figure 3D.

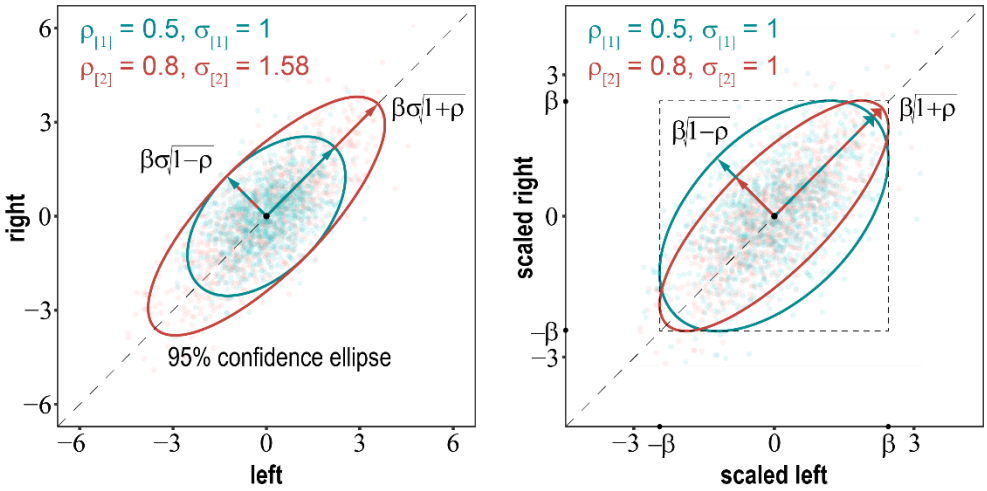

Figure S4. Correlation is not a proper measure of the FA/"intrinsic" variability.

To show that the correlation could be a misleading measure of the FA, let's assume a trait x under two conditions [1] (green) and [2] (red). Under [1], the correlation between left and right $\rho_{l,r[1]} = 0.5$, and $\sigma_{x_{[1]}} = 1.0$; under [2], $\rho_{l,r[2]} = 0.8$ and $\sigma_{x_{[2]}} = \frac{\sigma_{x_{[1]}}\sqrt{1-\rho_{l,r[1]}}}{\sqrt{1-\rho_{l,r[2]}}} = 1.58$.

Left panel, from the eq. (2), the "extrinsic" component of the DI of $x_{[1]}$ is greater than that of $x_{[2]}$:

$$\mathrm{Var}[\mathrm{E}(x_{[1]}|\,\xi)] \approx \frac{1}{2}\sigma^2_{x_{[1]}}\left(1 + \rho_{l,r[1]}\right) = 0.75, \text{ and } \mathrm{Var}[\mathrm{E}(x_{[2]}|\,\xi)] \approx 2.25.$$

From the eq. (3), the "intrinsic" component of the DI (FA) is equal for $x_{[1]}$ and $x_{[2]}$:

$$\mathrm{E}\left[\mathrm{Var}\left(x_{[1]}|\xi_{[1]}\right)\right] \approx \frac{1}{4}FA_{x_{[1]}} \approx \frac{1}{2}\sigma^2_{x_{[1]}}\left(1 - \rho_{l,r[1]}\right) = 0.25, \text{ and } \mathrm{E}\left[\mathrm{Var}\left(x_{[2]}|\xi_{[2]}\right)\right] \approx 0.25.$$

Ellipses correspond to 95% confidence ellipses, $\beta = \sqrt{\chi^2_{2,\alpha=0.05}} \approx 2.45$.

Right panel, estimation of the FA using only the correlation leads to a conclusion that $FA_{x_{[1]}} > FA_{x_{[2]}}$. The such discrepancy stems from that that correlation is estimated as the covariance of standardized variables. In other words, information on the fluctuation of x (σ^2_x) is dropped off. Rewriting the FA for the standardized trait x: $FA_x \approx 2\left(1 - \rho_{l,r}\right)$ (eq. (2)), it is easy to see that omitting the changes in σ^2_x could result in a misleading conclusion about changes in the FA_x.

Table S1. Primers used for RT-qPCR

Gene	Description	Ensembl	Forward	Reverse
Actb	Actin, beta	ENSMUSG00000029580	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Anxa5	Annexin A5	ENSMUSG00000027712	ATCCTGAACCTGTTGACATCCC	AGTCGTGAGGGCTTCATCATA
B2m	Beta-2 microglobulin	ENSMUSG00000060802	TTCTGGTGCTTGTCTCACTGA	CAGTATGTTCGGCTTCCCATTC
Car2	Carbonic anhydrase 2	ENSMUSG00000027562	CGGATGGATTGGCTGTTTTGG	GCACGCTTCCCCTTTGTTTTA
Cd36	CD36 antigen	ENSMUSG00000002944	ATGGGCTGTGATCGGAACTG	GTCTTCCCAATAAGCATGTCTCC
Col3a1	Collagen, type III, alpha 1	ENSMUSG00000026043	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
Dcn	Decorin	ENSMUSG00000019929	TCTTGGGCTGGACCATTTGAA	CATCGGTAGGGGCACATAGA
Gapdh	Glyceraldehyde-3-phosphate dehydrogenase	ENSMUSG00000057666	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
G0s2	G0/G1 switch gene 2	ENSMUSG00000009633	AAGGAGATGATGGCGCAGAAG	GCTGCACACCGTCTCAACTA
Hdac2	Histone deacetylase 2	ENSMUSG00000019777	TGTGCTTGCCATCCTCGAATT	AGCTTCCTCAACACCATCACC
Hsp90ab1	Heat shock protein 90 alpha, class B member 1	ENSMUSG00000023944	TCAAACAAGGAGATTTTCCTCCG	GCTGTCCAACTTAGAAGGGTC
Postn	Periostin, osteoblast specific factor	ENSMUSG00000027750	GAGGTCTCCAAGGTCACAAAGT	TGTGTCTCCCTGAAGCAGTCT
Rac1	RAS-related C3 botulinum substrate 1	ENSMUSG00000001847	GAGACGGAGCTGTTGGTAAAA	ATAGGCCCAGATTCACTGGTT
Rn18s	18S ribosomal RNA	ENSMUSG00000119584	CTCAACACGGGAAACCTCAC	CGCTCCACCAACTAAGAACG

Table S2. The number of implanted and live C57BL/6J embryos in experimental surrogate mother groups (mean ± SE)

Surrogate mother group	n	implanted embryo	live embryos
C57BL/6J	13	5.92 ± 1.05	4.69 ± 1.01
NOD-SCID	16	4.25 ± 0.98	2.88 ± 0.75
BALB/c	13	4.69 ± 1.31	3,77 ± 1.06

Table S3. GAMLSS models of the relationships between the distribution parameters (mean - μ, dispersion - σ) of logarithm-transformed embryo mass ($log(m)$) and individual estimates of pulled "intrinsic" gene noise ($\eta_{int.}$) for C57BL/6J embryos

A) Models for $log(m)$

group	GAMLSS model	estimate ($\hat{\alpha}, \hat{\beta}$) ± *s.e.*	p
all[a]	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.264±0.028**	**< 0.001**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$[b]	**0.777±0.276**	**0.008**
C57BL/6J	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.208±0.024**	**< 0.001**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$	**3.524±1.168**	**0.019**
NOD-SCID	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.255±0.054**	**0.002**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$	0.715±0.594	0.263
BALB/c	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.241±0.046**	**0.001**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$	0.877±0.534	0.139

B) Models for litter-size adjusted $log(m)$

group	GAMLSS model	estimate ($\hat{\alpha}, \hat{\beta}$) ± *s.e.*	p
all	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.196±0.021**	**< 0.001**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$	**0.979±0.247**	**< 0.001**
C57BL/6J	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.144±0.017**	**< 0.001**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$	**3.031±0.667**	**0.003**
NOD-SCID	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.24±0.039**	**< 0.001**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$	**1.309±0.449**	**0.015**
BALB/c	(1) $\mu_{log(m)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.103±0.021**	**0.001**
	(2) $\sigma_{log(m)} \sim \beta_0 + \beta log(\eta_{int.})$	**1.801±0.506**	**0.007**

[a] - all embryos.

[b] – Note that from the Taylor expansion for the moments $\sigma_{log(m)} \approx \frac{\mu_m}{\sigma_m} = \eta_m$. Assuming a normal distribution of $log(m)$: $\sigma_{log(m)} = \sqrt{log(1+\eta_m^2)}$, and for $\eta_m^2 < 1$, $\sigma_{log(m)} \approx \eta_m$.

Table S4. GAMLSS models of the relationships between the distribution parameters (μ, σ) of $log(PLGF)$ (PLGF concentrations, ng/l) and individual estimates of pulled "intrinsic" gene noise ($\eta_{int.}$) for C57BL/6J embryos

group	GAMLSS model	estimate ($\hat{\alpha}, \hat{\beta}$) ± *s.e.*	*p*
all	(1) $\mu_{log(PLGF)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.551±0.081**	**< 0.001**
	(2) $\sigma_{log(PLGF)} \sim \beta_0 + \beta log(\eta_{int.})$	**1.424±0.314**	**< 0.001**
C57BL/6J	(1) $\mu_{log(PLGF)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.555±0.092**	**0.001**
	(2) $\sigma_{log(PLGF)} \sim \beta_0 + \beta log(\eta_{int.})$	1.823±0.896	0.081
NOD-SCID	(1) $\mu_{log(PLGF)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.524±0.105**	**0.001**
	(2) $\sigma_{log(PLGF)} \sim \beta_0 + \beta log(\eta_{int.})$	**2.338±0.555**	**0.003**
BALB/c	(1) $\mu_{log(PLGF)} \sim \alpha_0 + \alpha log(\eta_{int.})$	**-0.353±0.089**	**0.004**
	(2) $\sigma_{log(PLGF)} \sim \beta_0 + \beta log(\eta_{int.})$	0.372±0.53	0.502