SI Figures

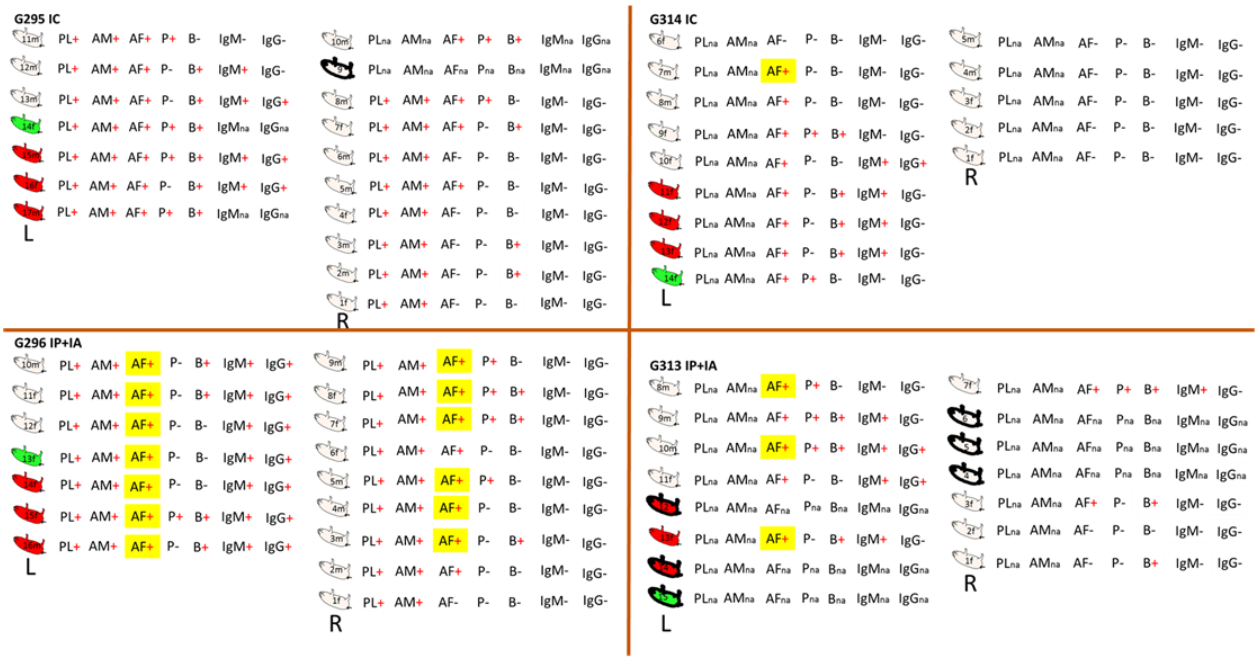


Fig. S1. Schematic representation of uteri with fetuses from experimental gilts. In total, we tested 24 and 62 fetuses from two control (G270 C and G312 C, not shown) and four experimental (G295 IC, G296 IP+IA, G313 IP+IA, and G314 IC) gilts (a gilt is a pig pregnant for the first time) respectively. Four fetuses close to the tip of the left uterine horn (L) were inoculated. R – the tip of the right uterine horn. Fetuses were inoculated with ZIKV (colored red) or mock-inoculated with media (colored green) at 50 gestational days (gd) (duration of gestation in pigs is 114-115 days). Animals were sampled 28 days later. Fetuses demarcated in bold are mummies. In G295 IC and G314 IC, fetuses were inoculated intracerebrally (IC) with 4 $\log_{10}$ $TCID_{50}$ of ZIKV. In G296 IP+IA and G313 IP+IA, fetuses were inoculated intraperitoneally (IP) + intra-amniotic (IA) with 5 $\log_{10}$ $TCID_{50}$ of ZIKV. In each of two control gilts, two fetuses were mock-inoculated with virus-free media IC and two IP+IA (not shown). These fetuses were also inoculated at 50 gd and sampled 28 days later. Numbers and letters (f - female and m - male) represent fetal identification number and gender. Numbers also indicate a sampling order. Placenta (PL), amniotic membranes (AM),

298 amniotic fluid (AF), fetal plasma (P) and brain (B) were tested by the ZIKV-specific RT-PCR.
299 Data for the brain represent combined results from the cerebrum and cerebellum obtained by virus-
300 specific RT-PCR and *in situ* hybridization. The results are "-" negative or "+" positive. Samples
301 were also tested for infectious ZIKV on Vero E6 cells. The infectious virus was identified in
302 highlighted AF. Serological results (IgM – IgM antibodies; IgG – IgG antibodies), are "-" negative
303 or "+" positive. na: samples are not available.

304

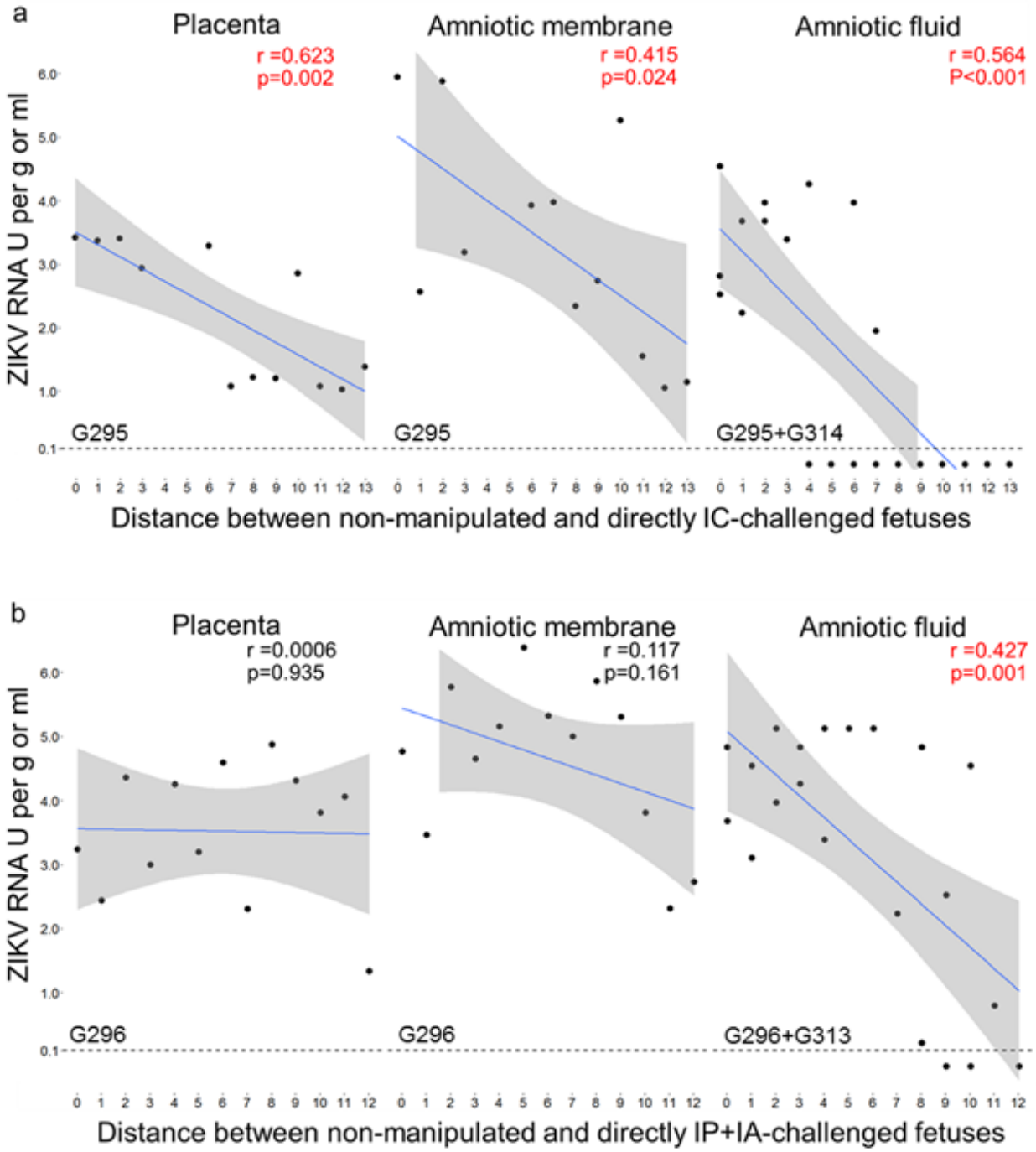


Fig. S2. Linear regression modeling the relationship between ZIKV PCR titers in fetal samples and the distance between directly virus-inoculated and non-manipulated siblings in the uterus. The linear regression models in intracerebrally (IC) (**a**) and intraperitoneally+intraamniotic (IP+IA) (**b**) exposed litters. Only ZIKV titers from trans-infected

siblings are represented. **X-axis:** 0 – a fetus adjacent to a directly challenged sibling; 1 to 13 – one, two, …, and thirteen fetuses between the directly virus-challenged fetus and non-manipulated sibling. **Y-axis:** Relative $\log_{10}$ $TCID_{50}$ values were defined as RNA units (U) and expressed as ZIKV RNA U per gram (g) of tissues or milliliter (ml) of fluids. A blue line is the fitted regression line with a shade representing the 95% confidence region. A dotted line represents the assay detection limit. Dots below the detection limit are negative samples. A multiple linear regression analysis (not shown) modeling the relationship between ZIKV titers, distance between challenged and non-manipulated siblings, and fetal gender, did not show a significant influence of the fetal gender on viral loads in PL ($p=0.570$ for IC litters, $p=0.073$ for IP+IA litters), AM ($p=0.132$ for IC litters, $p=0.875$ for IP+IA litters), and AF ($p=0.104$ for IC litters, $p=0.218$ IP+IA litters), while the distance between siblings precisely matched linear regression analyses and remained significant.

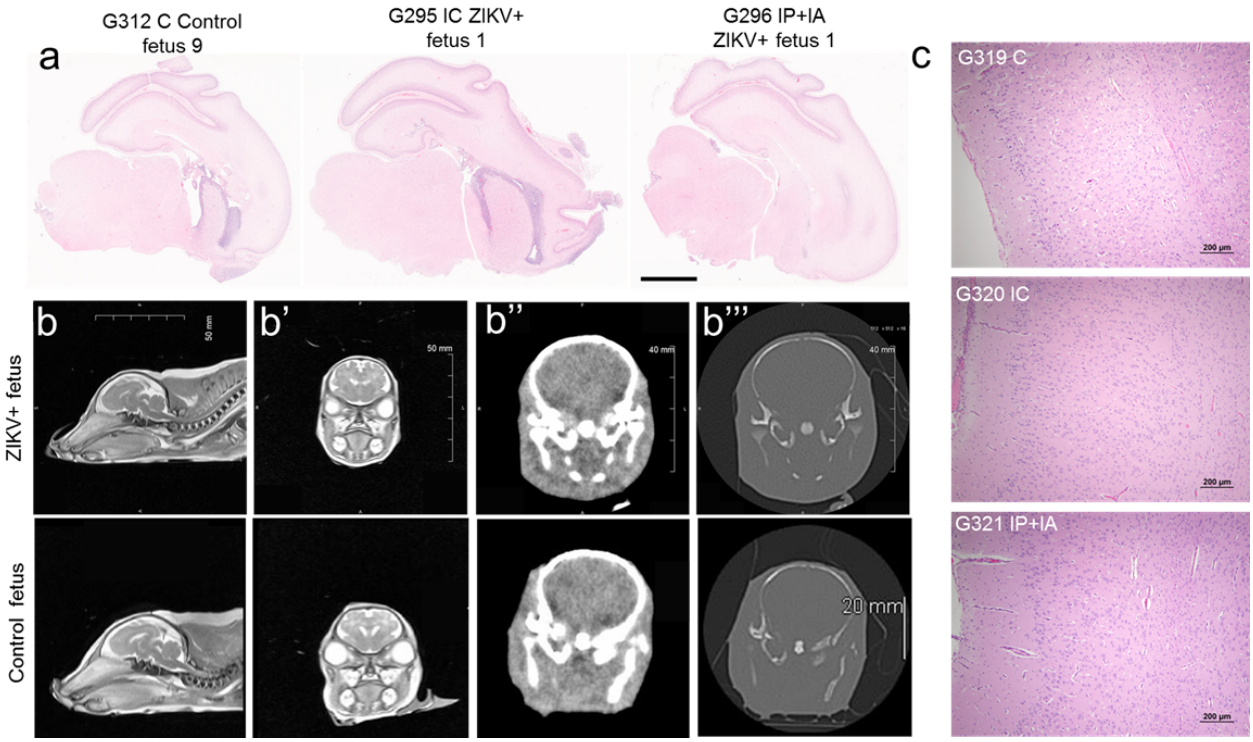
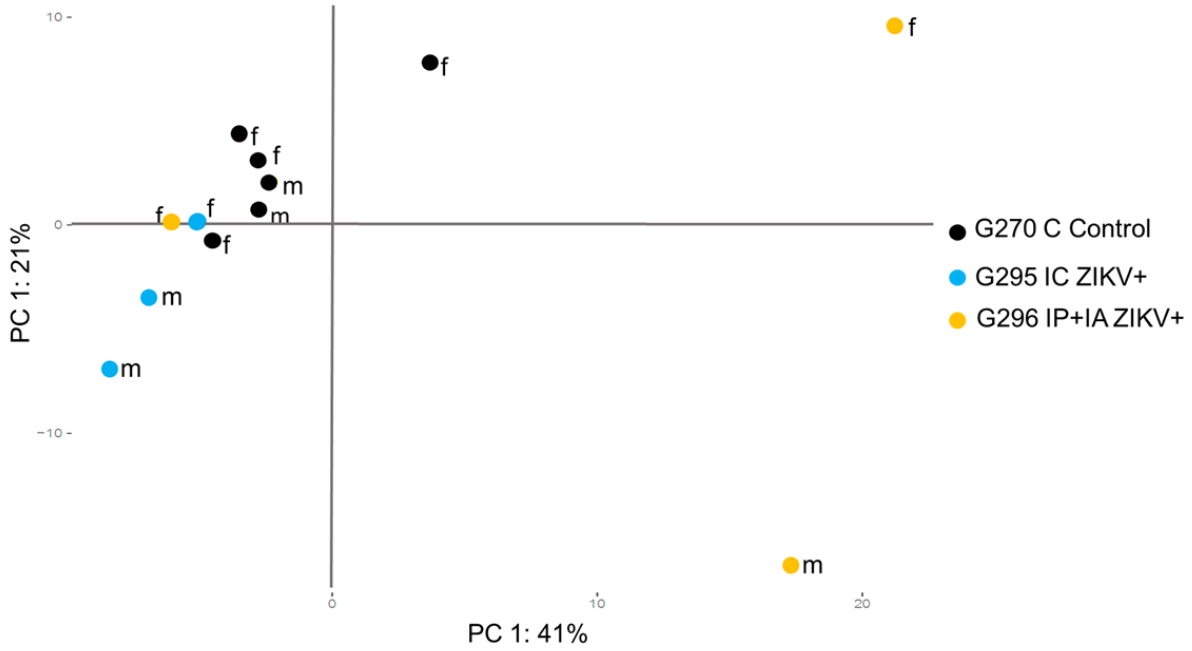


Fig. S3. **Histology and MRI/CT scanning. (a)** The representative images of whole scanned fetal brain tissue sections. Examination of H&E stained fetal brain tissue sections did not reveal histopathology. The bar is 5 mm. Representative images of computed tomography (CT) and magnetic resonance imaging (MRI). Sagittal **(b)** and axial **(b')** MRI images of ZIKV-infected and control fetal brains. Axial tissue (**b''**) and bone (**b'''**) window CT images of virus-infected and control fetal brains. MRI and CT did not show pathology in fetal brains. (**c**) Examination of H&E stained neonatal brain tissue sections did not reveal histopathology.



338

339 **Fig. S4.** Principal Component Analyses of RNA-seq data.

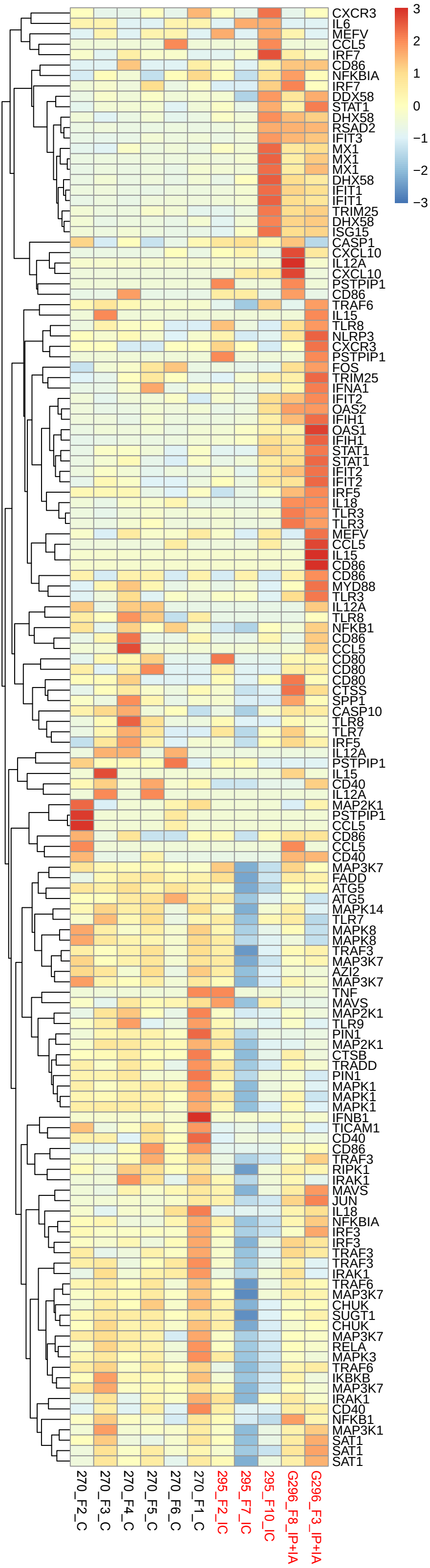


Fig. S5. Hitmap of the enriched gene set related to antiviral responses. We tested a set of genes related to antiviral responses assembled from previous ZIKV publications (Bayer et al., 2016; Hamel et al., 2015; Li et al., 2016; Quicke et al., 2016; references are in the manuscript text), and found that infected fetuses from the IP+IA and IC groups were enriched in this term (False discovery rate (FDR) corrected p-value = 0.102 for the IP+IA group and FDR p-value = 0.175 for the IC group, where FDR p-value < 0.25 is considered significant).

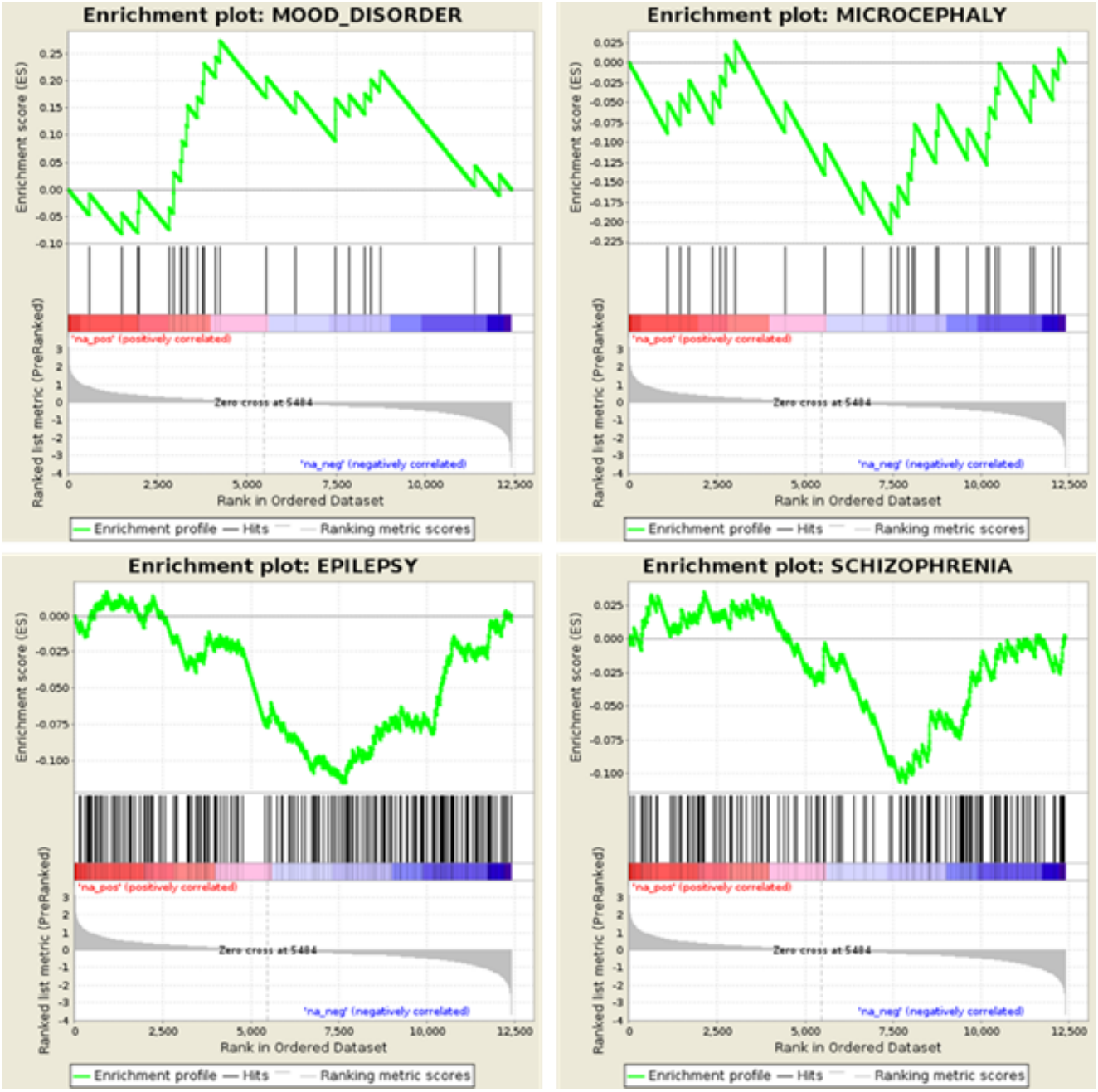


Fig. S6. Enrichment plots of gene sets with the known or potential association to congenital Zika syndrome clinical representations in human fetuses and neonates. We tested custom gene sets (Table S4) using GSEA and found that infected fetuses from the IC group were enriched in Mood Disorder term, and depleted in terms Microcephaly, Epilepsy, and Schizophrenia. For individual genes, see Dataset S2-Sheet 9.

SI Tables

TABLE S1 Bio-plex assay reagents

Cytokine	**Capture Antibody; Supplier**	**Detection Antibody; Supplier; Dilution**	**Standard; Supplier; Initial Concentration**	**Bead; Supplier**
IFN*α*	MAb anti porcine IFNα; GeneTex GTX11408	MAb anti pig IFNα; PBL 27105-1; biotinylated in house; 1/5000 dilution	Recombinant porcine IFNα; Genentech (gift); 200 pg/mL	Region 45; BioRad MC10045-01
IFNγ	MAb anti porcine IFNγ; Fisher ENMP700	MAb anti porcine IFNγ; Fisher ENPP700; biotinylated in-house; 1/400 dilution	Recombinant porcine IFNγ; Ceiba Geigy (gift); 2000 pg/ml	Region 43; BioRad MC10043-01
IL-1β	MAb anti porcine IL1β/ IF2; R&D MAB6811	Goat anti porcine IL1β/IF2 biotin; R&D BAF681; 0.5 ug/ml	recombinant porc IL1β/IF2; R&D 681-PI-10; 5000 pg/ml	Region 26; BioRad MC10026-01
Il-6	Goat anti porcine IL-6; R&D AF686	Goat anti porcine IL-6 biotin; R&D BAF686; 0.5 ug/mL	Recombinant porcine IL-6; R&D 686-PI-025; 5000 pg/mL	Region 65; BioRad MC10065-01
IL-8	MAb anti sheep IL8 (86.9% homology); AbD Serotec MCA1660	MAb anti porcine CXCL8/IL8 biotin; R&D MAB5351; biotinylated in house; 1/400 dilution	Recombinant porcine IL-8; Kingfisher RP0109S-005; 200 pg/ml	Region 27; BioRad MC10027-01
IL-10	MAb anti swine IL-10; Invitrogen ASC0104	MAb anti swine IL-10 biotin; Invitrogen ASC9109; 0.5 ug/mL	Recombinant swine IL-10; Invitrogen PSC0104; 5000 pg/mL	Region 28; BioRad MC10028-01
IL-12	MAb anti porcine IL-12; Kingfisher MA0413S-100	MAb anti porcine IL12/IL23 p40 biotin; R&D BAM9122; 0.5 ug/mL	Recombinant porcine IL-12; R&D 912-PL-025; 5000 pg/mL	Region 36; BioRad MC10036-01
IL-13	Goat anti swine IL-13; Kingfisher PB0094S-100	Goat anti swine IL-13 biotin; Kingfisher PBB0096S-050; 0.5 ug/ml	Recombinant swine IL-13; Kingfisher RP0007S-005; 5000 pg/ml	Region 52 ; BioRad MC10052-01
IL-17A	Rabbit anti porcine IL-17A; Kingfisher KP0498S-100	Rabbit anti porcine IL-17A biotin; Kingfisher KPB0499S-050; 0.1 ug/mL	Recombinant porcine IL-17A; Kingfisher RP0128S-005; 2000 pg/mL	Region 62; BioRad MC10062-01

TABLE S2 Body measurements in fetuses

Litter	Fetus		Body length (mm)		Cranium diameter (mm)
G312 C*					
	1M		220		36
	2F		220		38
	3M		220		38
	4M		210		35
	5M		215		36
	6F		240		37
	7M		220		36
	8F		220		36
	9F		215		37
	10M		205		37
	11F		200		36
	12M		210		39
	13F		195		35
	14M		225		37
G314 IC*					
	1F		235		38
	2F		255		37
	3F		225		36
	4M		230		38
	5M		240		37
	6F		240		37
	7M		220		21
	8M		235		38
	9F		230		36
	10F		240		38
	11F		200		37
	12F		190		33
	13F		202		33
	14F		220		40
G313 IP+IA*					
	1F		225		39
	2F		220		38
	3F		205		39
	4na		m		m
	5na		m		m
	6na		m		m
	7F		225		38
	8M		225		31
	9M		195		36
	10M		225		38
	11F		210		36
	12na		m		m
	13F		230		40
	14na		m		m
	15na		m		m

*IC – three conceptuses (in red) were inoculated with Zika virus (ZIKV) intracerebrally or mock-inoculated (in green) with virus-free media; IP+IA – three conceptuses (in red) were inoculated with ZIKV intraperitoneally + intra-amniotic or mock-inoculated (in green) with virus-free media; C - a control gilt; four conceptuses (in green) in the uterus were inoculated with media IC and IP+IA. All inoculations were made at 50 days of gestation and litters were sampled 28 days later.

M and F – Male and Female. m – Mummified fetuses. na – Not available.

TABLE S3 Virological data in fetuses sampled for RNA-seq analysis

Gilt	Group	Fetal #	Distance[a]	ZIKV PCR titers[b]					Infectious ZIKV[c]	Serology[d]	
				Brain (cerebrum)	Plasma	PL	AM	AF	AF	IgM	IgG
G270	Control										
		1-6	na	neg	neg	neg	neg	neg	neg	neg	neg
G295	IC										
		2	12	2	neg	1.1	1.1	neg	neg	neg	neg
		7	7	1.7	neg	1.1	4	2	neg	neg	neg
		10	4	1.7	1.1	na	na	4.3	neg	neg	neg
G296	IP+IA										
		3	10	0.8	neg	3.8	3.8	4.5	1.2	neg	neg
		8	5	0.9	0.5	3.2	6.4	5.1	1.9	neg	neg
		11	2	0.7	neg	4.4	5.8	5.1	2.2	3	3

[a]Samples from non-manipulated fetuses which acquired Zika virus (ZIKV) via trans-fetal infection were selected for RNA-seq analyses. The distance is the number of fetuses to a closest sibling directly challenged with ZIKV.

[b]ZIKV RNA U per ml or g. [c]$\log_{10}$ $TCID_{50}$/ml of ZIKV. [d]$\log_2$

"neg" negative.

IC – three fetuses in the uterus were inoculated with ZIKV intracerebrally.

IP+IA – three fetuses in the uterus were inoculated with ZIKV intraperitoneally+intra-amniotic.

na – not available. PL – placenta. AM – amniotic membrane. AF – amniotic fluid.

TABLE S4 Custom gene sets

Microcephaly
ASPM
CASC5
CENPF
MCPH1
RBBP8
STIL
TBR2
CEP152
WDR62
BUB1B
ORC1
PLK4
KIF11
PLK1
EOMES
INCENP
NDE1
SLC25A19
MFSD2A
NBN
CENPJ
ACTB
WHSC1
BCCIP
HCEC1
CEP135
ACTG1
DIAPH1
IER3IP1
ZNF335
CTCF
BRAT1

CDK5RAP2 POMT2 EFTUD2 PNKP TRMT10A EPC2 MBD5 VRK2 NAALADL2 NLGN1 XRCC4 CASK
Epilepsy
SCN1A SCN8A STXBP1 TRIM8 FHF1 ARX SLC25A22 KCNQ2 CDKL5 PCDH19 STX1B CACNA1A ATP1A2 ALDH7A1 BRD2 CACNA1H CACNB4 CASR CHRNA2 CHRNA4 CHRNB2 CLCN2CSTB

EFHC1
EPM2A
GABRA1
GABRB3
GABRD
GABRG2
GPR98
GRIN2A
GRIN2B
KCNMA1
KCNQ3
KCTD7
MBD5
ME2
NHLRC1
PRICKLE1
PRICKLE2
SCARB2
SCN1B
SCN2A
SCN9A
SLC2A1
TBC1D24
ARHGEF9
CNTNAP2
FOXG1
MAPK10
MECP2
NRXN1
PNKP
RNASEH2A
RNASEH2B
RNASEH2C
SAMHD1
SLC9A6
SPTAN1

TCF4
TREX1
UBE3A
ZEB2
ARFGEF2
COL18A1
COL4A1
CPT2
DCX
EMX2
EOMES
FGFR3
FKRP
FKTN
FLNA
GPR56
LAMA2
LARGE
PAFAH1B1
PAX6
PEX7
POMGNT1
POMT1
POMT2
PQBP1
RAB3GAP
RELN
SNAP29
SRPX2
TUBA1A
TUBA8
TUBB2B
VDAC1
WDR62
ASPM
ATR

BUB1B
CASK
CDK5RAP2
CENPJ
CEP152
LIG4
MCPH1
MED17
NHEJ1
PCNT
RARS2
SLC25A19
STIL
TSEN2
TSEN34
TSEN54
VRK1
AHI1
ARL13B
CC2D2A
CEP290
INPP5E
NPHP1
RPGRIP1L
TMEM216
TMEM67
FGF8
GLI2
GLI3
PTCH1
SHH
SIX3
TGIF
ZIC2
ATP6AP2
ATRX

CUL4B
CXORF5
FGD1
GPC3
GRIA3
HSD17B10
JARID1C
OPHN1
PAK3
PHF6
PLP1
RAB39B
SMC1A
SMS
SYP
ABCC8
ACY1
ADSL
ALDH4A1
ALDH5A1
ARG1
ATIC
BTD
DPYD
ETFA
ETFB
ETFDH
FH
FOLR1
GAMT
GCDH
GCSH
GCST
GLDC
HPD
L2HGDH

MOCS1
MOCS2
PGK1
PRODH
QDPR
SLC25A15
SLC46A1
SUOX
APTX
ATPAF2
BCS1L
C12ORF65
C8ORF38
CABC1
COQ2
COQ9
COX10
COX15
DLD
LRPPRC
NDUFA2
NDUFS1
NDUFS3
NDUFS4
NDUFS7
NDUFS8
NDUFV1
PC
PDHA1
PDSS1
PDSS2
POLG
SCO2
SDHA
SURF1
TACO1

TMEM70
CLN3
CLN5
CLN6
CLN8
CTSD
MFSD8
PPT1
TPP1
AGA
ARSA
ARSB
CTSA
FUCA1
GALC
GALNS
GLB1
GNE
GNPTAB
GNPTG
GNS
GUSB
HEXA
HEXB
HGSNAT
HYAL1
IDS
IDUA
MCOLN1
NAGLU
NEU1
NPC1
NPC2
PSAP
SGSH
SLC17A5

SMPD1
SUMF1
ALG1
ALG12
ALG2
ALG3
ALG6
ALG8
ALG9
B4GALT1
COG1
COG7
COG8
DPAGT1
DPM1
DPM3
GCS1
MGAT2
MPDU1
MPI
PMM2
RFT1
SLC35A1
SLC35C1
TMEM15
PEX1
PEX12
PEX14
PEX2
PEX26
PEX3
PEX5
PEX6
ASPA
EIF2B1
EIF2B2

EIF2B3
EIF2B4
EIF2B5
GFAP
MLC1
NOTCH3
BRAF
CBL
HRAS
KRAS
MAP2K1
MAP2K2
MYST4
NF1
NRAS
PTPN11
RAF1
SHOC2
SOS1
SPRED1
GLRA1
GLRB
GPHN
SLC6A5
A2BP1
ATP2A2
ATP6V0A2
CCDC88C
CLCNKA
CLCNKB
COH1
KCNA1
KCNJ1
KCNJ10
KIAA1279
LBR

LGI1 MLL2 NIPBL PANK2 PI12 PIGV PLA2G6 RAI1 SETBP1 SLC4A10 SMC3 SYNGAP1 TBX1 TSC1 TSC2 VPS13A
Dysphagia
DMPK MEGF10 COX10 NDUFS7 NDUFS8 SURF1 SLC52A3 NDUFAF5
Clubfoot
CHST14 PITX1 TPM2 TBX4 MYH3 TNNT3 HOXD10 CHST3

HOXD13 ZNF664 CHST3 MYBPC1 WNT7A HOXA9 TPM1 FOXN3 SORCS1 MMP7 TMEM123 CHST14 CAND2 NAT1 NAT2 CASP10
Arthrogryposis
TNNI2 TNNT3 TPM2 MYH3 MYH8 UBE1 DMPK LMNA MYBPC1 PIEZO2 FBN2 MYBPC2 ADGRG6 MYH6 IGHD4-11 SLC35A3
Guillain-Barre Syndrome

PMP22
TNF
CD79A
MBP
ALB
GALE
HLA-DQB1
HP
TNFRSF1A
MPZ
TNFRSF1B
HLA-DQA1
CD40LG
Schizophrenia
DISC1
HTR2A
COMT
RGS4
DRD3
MTHFR
SYN2
RTN4R
CHI3L1
CD40LG
ETM1
TAAR6
DTNBP1
DAOA
NRG1
DAO
DISC2
PRODH
DRD2
AKT1
SCZD6

SCZD1
SCZD2
SCZD7
SCZD8
SCZD3
SCZD11
SCZD5
SCZD12
APOL2
RELN
NPAS3
APOL4
DRD4
CHRNA7
ECSCR
SHANK3
NOTCH4
PRL
CLINT1
GABRB1
KCNN3
BDNF
TPH1
ZDHHC8
SLC6A4
PDE4B
QKI
ZNF804A
NRXN1
GRIK3
GRM3
HTR1A
NRG3
FZD3
HTR2C
CYP2D6

SLC1A1
GAD1
MC4R
SULT4A1
SLC6A3
PPP3CC
GRIN1
DBH
CPLX2
DGCR6
LRRTM1
DRD1
YWHAH
GRIN2B
HTR3A
NTF3
TSNAX-DISC1
NOS1
CACNG2
FEZ1
NTS
GRM1
DRD5
GRIA4
CHRM1
GRIK4
GRIA1
ARVCF
GNAL
PDLIM5
SYN3
GRM2
FOLH1
MAOA
MED12
PVALB

GRIN2C
RTN4
HTR7
CYP1A2
PIP4K2A
CHRNA4
FXYD6
HTR6
NDUFV2
DPYSL2
GABBR1
NTNG1
CNTNAP2
GRIN2A
HRH2
MAP6
ITIH3
VRK2
GRM5
PPP1R1B
GRIN3B
ERBB4
SP4
ERVW-1
CHRFAM7A
SRR
TH
MIAT
DLG4
NOS1AP
CACNG5
MEGF10
UHMK1
SLC6A9
CNR1

RSRC1
ASTN2
SMARCA2
MAOB
ATP2A2
TSNAX
SELENBP1
CDC42SE2
PLA2G4A
KYAT1
MIR137
TBX1
SNAP25
SLC6A1
GRM4
GRIK5
CACNA1C
SEMA3A
SLC17A7
GPR78
ADRA2A
NR4A2
CNP
MOBP
CALB1
HRH3
CHN2
GRIK1
DLG3
CNIH1
GRID1
CYP3A4
CALB2
GRIK2
CNIH2
INS

ANK3
HCRT
APOD
PAFAH1B1
GRIA2
PICK1
SLC18A1
IMPA2
HTR1B
CCK
NRGN
SLC18A2
TPH2
PLXNA2
NCS1
CHL1
EGR3
CHRNB2
ADRA1A
GABRA4
CCKAR
GRIA3
SLC1A2
HINT1
MLC1
GABRB3
DGKH
SST
WWC1
SCZD10
CRP
GNB3
GAP43
ACSL6
APOL1
NPY

PCNT
GSK3B
GABRA5
GAD2
FAN1
NTRK2
SLC6A2
VIPR2
MICB
ADORA2A
MAP2
GSTT2
PIK3C3
SLC6A5
ST8SIA2
TACR3
TIMELESS
PLA2G6
SCZD4
SCZD13
CALY
SLC1A3
PAX6
CIT
IL2RB
TDO2
HTR1D
DNMT1
MAG
APP
MBP
MARCKS
PSAT1
ADGRA3
NMBR
ULK4

SLC27A5
NPAS2
ARNTL
C9orf72
SLC17A6
GABRG2
Attention Deficit-Hyperactivity Disorder
DRD4
DRD5
MECP2
ADHD4
ADHD1
ADHD2
ADHD3
SLC6A3
TPH2
COMT
SLC9A9
SLC6A2
ADRA2A
DOCK3
SLC6A4
DBH
HTR1B
MAOA
CHRNA4
DRD2
DRD1
SNAP25
ADRA2C
BDNF
HTR2A
THRB
HTR2C
DRD3

SLC18A2
ADRA1A
MAOB
GRIN2A
CHRNA7
FMR1
TPH1
CYP2D6
HRH3
ADGRL3
HK1
TTF2
CDH13
GRM7
CHRFAM7A
HTR1A
TH
HTR1E
CALY
GFOD1
CLOCK
GIT1
PRTG
TACR1
HTR1D
NTF3
SYN3
FOS
PPP1R1B
STS
IMMP2L
CES1
DCDC2
CNTNAP2
ARRB2
SLC6A1

IPO11 GRM5 NTRK2
Anxiety Disorder
SLC6A4 HTR1A HTR2A BDNF MAOA COMT TPH2 CRHR1 DRD2 CCK SLC6A3 NPY SLC6A2 TPH1 RGS2 ADCYAP1R1 TSPO HTR3A DRD4 OXT OXTR PRL DRD3
Mood Disorder
BDNF SLC6A4 HTR1A HTR2A MAOA COMT

DRD4 CRH CACNA1C DRD2 CREB1 NR3C1 PER3 IL6 POMC HTR1B MAOB TH SLC18A2 XK NPY TACR1 PLA2G4A ACE DNMT1 ADCY8 CNR1 GLUL MAPK1 GFAP IL2 NGFR
Learning Disability
CDH8 MECP2 COLEC11 PSAP NPAS3 MEIS2 PURA MASP1

MAPT ZIC1 CNTF OMG KIAA0319 DYX1C1 DCDC2 EIF4A3 NT5C2 CLPP MYCN
Psychotic Disorder
DISC1 COMT BDNF RGS4 SLC6A4 PRODH SLC1A1 PRL S100B SLC18A2 AKT1 MAOB GABRB3 HLA-A SMARCA2 SHANK3 DTNBP1 NRG1 HTR2A FXYD6 DRD2 GRM2 PVALB

ZNF804A
EP300
GRM3
HTR1A
DRD3
RELN
HTR2C
GAD1
CHRNA4
SLC27A5
DAOA
PNP
PIK3CD
CHRFAM7A
CHRNA7

TABLE S5 Body measurements in neonates exposed to ZIKV *in utero*

Litter	Piglet		Body length (cm)			Body weight (g)			Brain weight (g)		Brain/Body weight ratio
G319 C*			**1 day**	**21 days**		**1 day**	**21 days**		**21 days**		**21 days**
	1F		37.7	53.5		1500	5900		4.9		0.08
	2F		40.1	54		1500	6700		4.84		0.07
	3M (**e-1**)		32.2	na		700	na		na		na
	4M		37.3	51		1100	5200		4.8		0.09
	5F		38.9	57.5		1600	7200		4.75		0.07
	6M		35.3	41		900	2200		4.01		0.18
	7F		38.8	56.5		1700	7200		5.10		0.07
	8M		39.1	55.5		1700	7100		5.17		0.07
	100M (d-0)		34.5	na		na	na		na		na
	m		na	na		na	na		na		na
	m		na	na		na	na		na		na
	m		na	na		na	na		na		na
G320 IC*											
	9M		36.7	48		1300	4000		4.75		0.12
	10M		33.9	44		1000	3000		4.42		0.15
	11M		33.6	47		1000	3800		4.28		0.11
	12F		36.7	48		1200	3700		4.31		0.12
	13M		39.6	52		1700	5700		4.65		0.08
	14M		40.4	53.5		1800	5700		4.55		0.08
	15F		39.2	51		1500	4700		4.99		0.11
	16M		37.7	52.5		1500	5000		5.6		0.11
	17F		44	56.5		1900	7100		5.15		0.07
	18F		37.5	50		1800	4500		4.77		0.11
	19F		31.2	40.5		1300	1700		3.94		0.23
	20M		36.5	49		1400	4100		4.43		0.11
	21M (**d-3**)		30	na		600	na		Na		na
	22F		37.2	53		1600	5200		4.91		0.09
	140F (d-0)		34	na		na	na		na		na
	170F (d-0)		39.5	na		na	na		na		na
	160M (d-0)		33.5	na		na	na		na		na
	150 decomposed		27	na		na	na		na		na
G321 IP+IA*											
	23M (**e-1**)		29.5	na		700	na		na		na
	24F		30.2	41		800	2600		3.56		0.14
	25F		32.9	45		800	3400		3.79		0.11
	26F		34.4	49		1200	5200		4.18		0.08
	100M (e-0)		28.1	na		563	na		na		na

*G319 C – Control; four fetuses in the uterus were inoculated with media; IC – three fetuses in the uterus were inoculated with Zika virus (ZIKV) intracerebrally.
IP+IA – Three fetuses in the uterus were inoculated with ZIKV intraperitoneally + intra-amniotic. All inoculations were done at 50 days of gestation.
M and F – Male and Female. (d/e-0/1/3) – Died/euthanized at birth (0) or at 1-3 days post birth. m – Mummified fetuses. na – Not available.

SI Datasets

Dataset S1 CT and MRI data.

Dataset S2 Differentially expressed genes.

SI Videos

Video S1. Severe seizures in a neonate #100 at birth. G321 IP+IA litter.

Video S2. Neonate #100 had severe seizures, did not recover and was euthanized shortly after birth. The entire G321 IP+IA litter was weak. For comparison, see **Video S11**.

Video S3. Splayed back legs in neonate #23 from the G321 IP+IA litter at 1 day after birth. Other siblings remained weak for the extended time. For comparison, see **Video S7** and **Video S8**.

Video S4. During the study neonates from the G319 C litter remained healthy and playful. The video was recorded at 14 days after birth.

Video S5. Neonates from the G321 IP+IA litter were less active and depressed. The video is recorded at 14 days after birth.

Video S6. Neonate #24 in the G321 IP+IA litter deteriorated during the study and remained predominantly recumbent with continued seizure-like activities.

Video S7. The 319 C (control) litter at 1 day after birth.

Video S8. Neonates in the G320 IC litter had the potential increase in activity in comparison to control neonates since first days of life. The video is recorded at 1 day after birth.

Video S9. During the study, neonates in the G320 IC litter were continuously and aggressively fighting. The video is recorded at 14 days after birth.

Video S10. During the study, neonates in the G320 IC litter were continuously and aggressively fighting. The video is recorded at 21 days after birth.

Video S11. The G320 IC litter recorded shortly after birth.