Supplementary Figures and Table

Gregory, Han, *et al.*: Multi-omic blood analysis reveals differences in innate inflammatory sensitivity between species.

This supplement contains:

Figs. S1-S8
Table S1
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The following data tables are provided as separate files:

Data S1. Plasma proteins with differing abundance in between sensitive and resilient animals.

Data S2. HDL proteins with differing abundance in between sensitive and resilient animals.

Data S3. Genes with significantly different expression between sensitive and resilient animals in unstimulated leukocytes.

Data S4. Genes with no overlap in expression between sensitive and resilient animals in unstimulated leukocytes.

Data S5. Genes with significantly different expression between sensitive and resilient animals in leukocytes stimulated with 10 ng/mL LPS for 2 hours.

Data S6. Genes with significantly different expression between sensitive and resilient animals in leukocytes stimulated with 10 ng/mL LPS for 6 hours.

Data S7. Genes with significantly different expression between sensitive and resilient animals in leukocytes stimulated with 10 ng/mL LPS for 24 hours.

Data S8. Genes with no overlap in expression between sensitive and resilient animals in leukocytes stimulated with 10 ng/mL LPS for 2 hours.

Data S9. Genes with no overlap in expression between sensitive and resilient animals in leukocytes stimulated with 10 ng/mL LPS for 6 hours.

Data S10. Genes with no overlap in expression between sensitive and resilient animals in leukocytes stimulated with 10 ng/mL LPS for 24 hours.

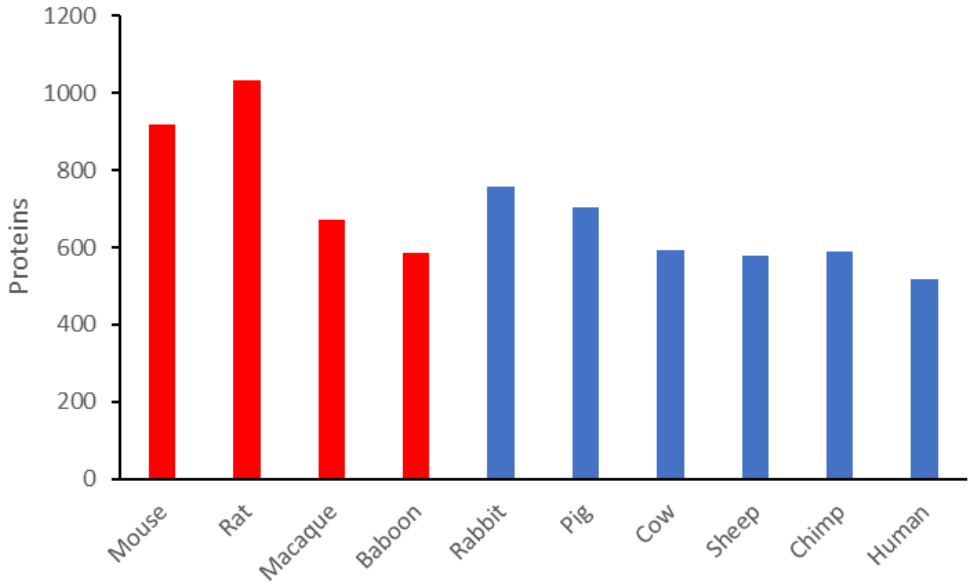

Fig. S1

Total number of proteins identified and quantified in unstimulated whole plasma for each species after LC-MS/MS analysis. Resilient species are indicated in red, sensitive animals in blue.

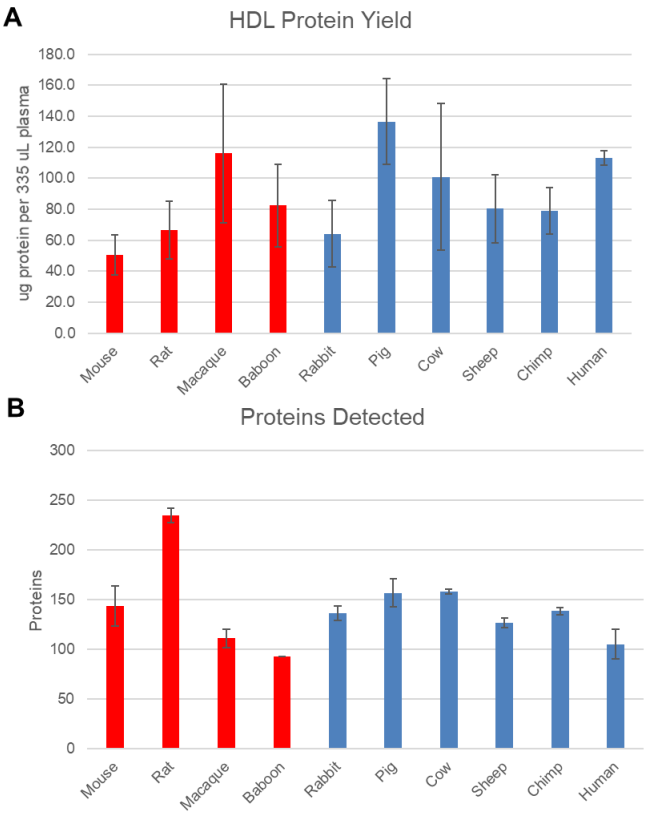

Fig. S2.

Measured protein yields from HDL protein isolation and detection. A) yield of total protein amount per species after isolation of HDL from replicate plasma samples without LPS stimulation. B) unique HDL proteins detected and identified per species after LC-MS/MS analysis. Error bars represent biological triplicate analysis.

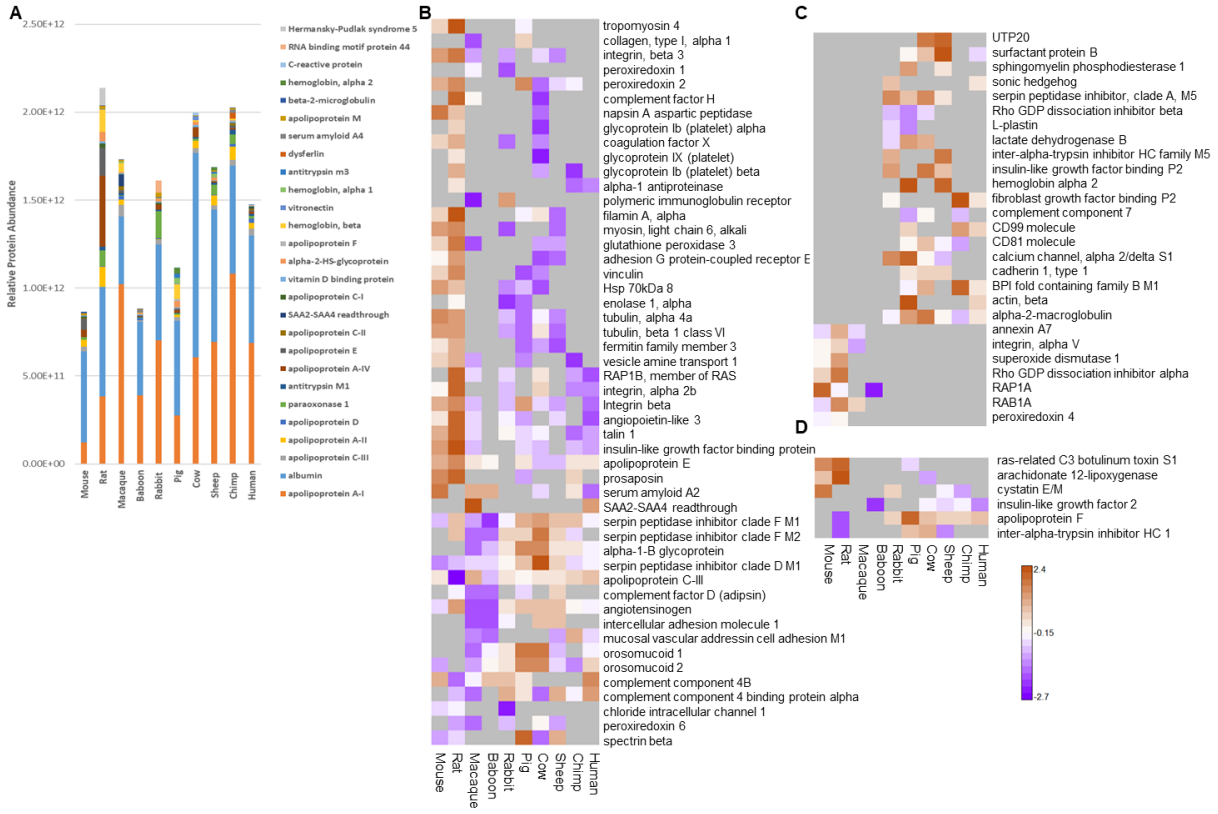

Fig. S3.

Overview of HDL isolated proteins from plasma and identified as differentially abundant in comparison of resilient versus sensitive species. A) top 10 most abundant HDL proteins per species. Three different comparisons were performed (B-D) resulting in 83 total HDL proteins identified as differentially abundant. Quantitative protein level values are based upon scaled LFQ intensities combined from peptide level intensities. Color scale represented as scaled quantitative abundance differences as shown with red representing higher abundance and purple lower abundance for each individual protein. B) mapping of 50 proteins at p <0.05, Pearson correlated. C) mapping of 27 proteins with yes/no abundance based upon a minimum of 3 species observations, bimodal correlation. D) mapping of 6 proteins based upon higher fold-change abundance (+/-3.0 in $\log_2$ phase, minimum of 4 occurrences), bimodal correlation.

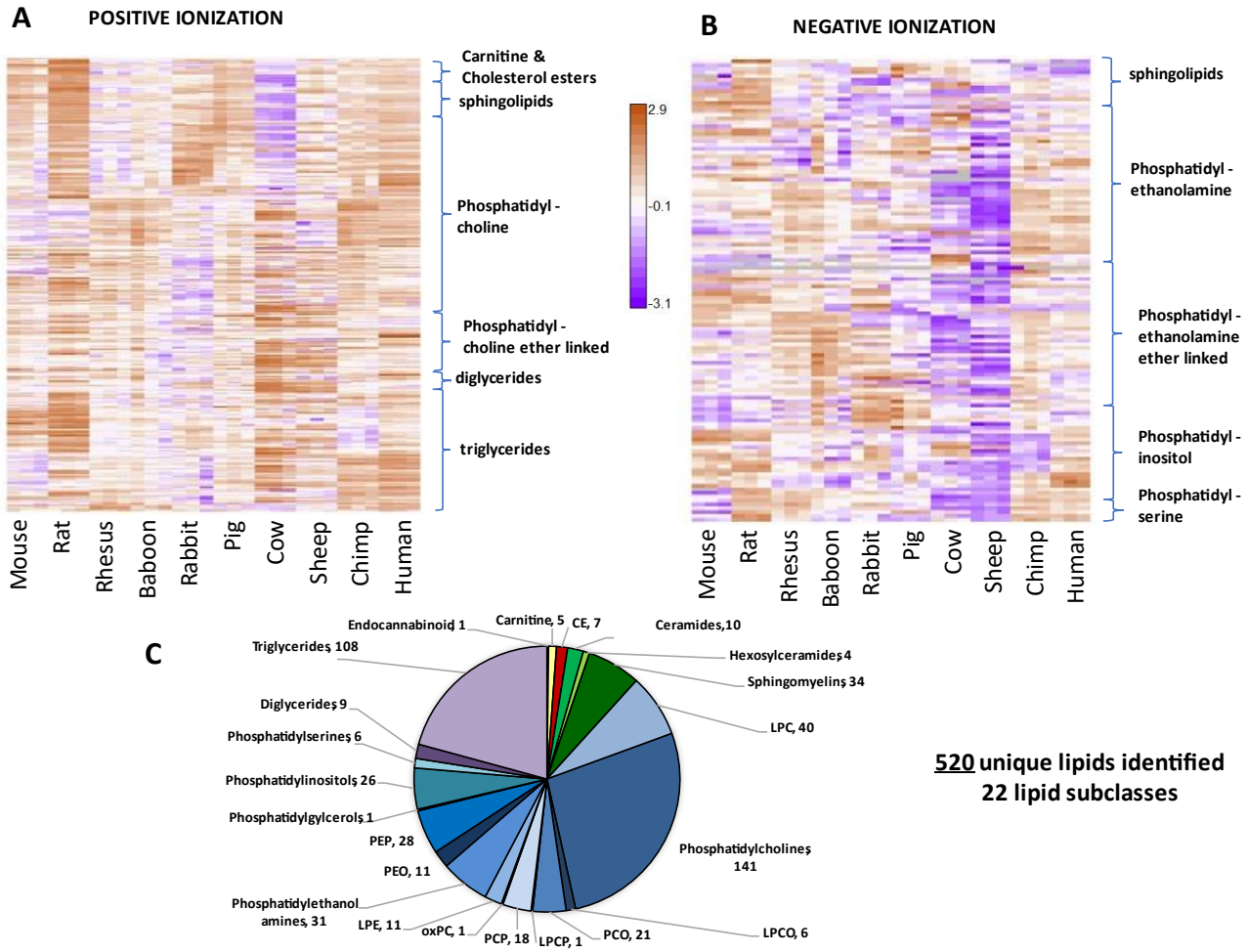

Fig. S4.

Plasma lipidomes. Abundance of individual lipids that form positive (A) or negative (B) ions detected and quantified via LC-ESI-MS/MS is shown for each of 3 individuals per species, relative to the global mean signal intensity for each lipid. (C) Total number of distinct lipids identified, by lipid subclass. CE: cholesterol esters; LPC: lyso-phosphatidylcholines; LPCO: monoalkylglycerophosphocholines; PCO 1-alkyl,2-acylglycerophosphocholines; LPCP: 1Z-alkenylglycerophosphocholines; PCP: 1-(1Z-alkenyl),2-acylglycerophosphocholines; oxPC: oxidized phosphatidylcholines; LPE: lyso-phosphatidylethanolamines.

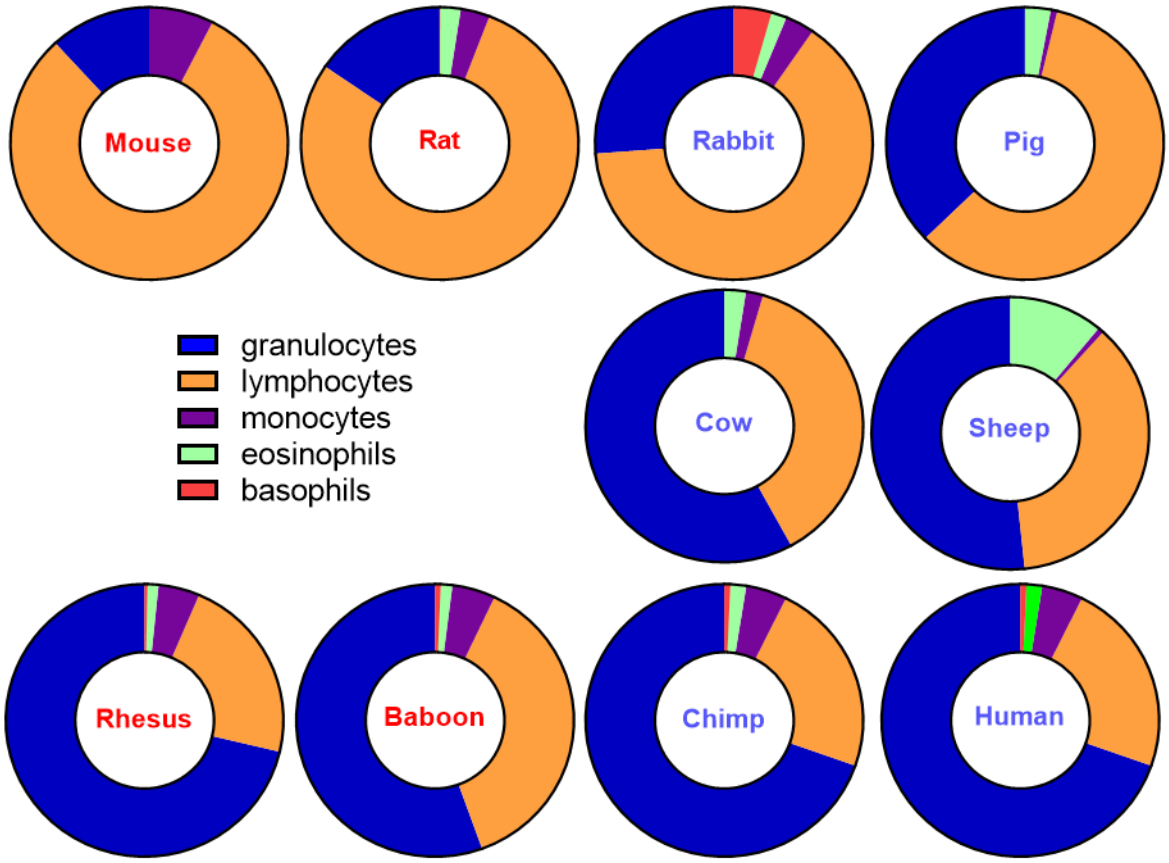

Fig. S5.

Blood differential counts obtained from the blood used in this study. Due to safety limitations, data was not obtained from the human subjects; established clinical reference values are given instead.

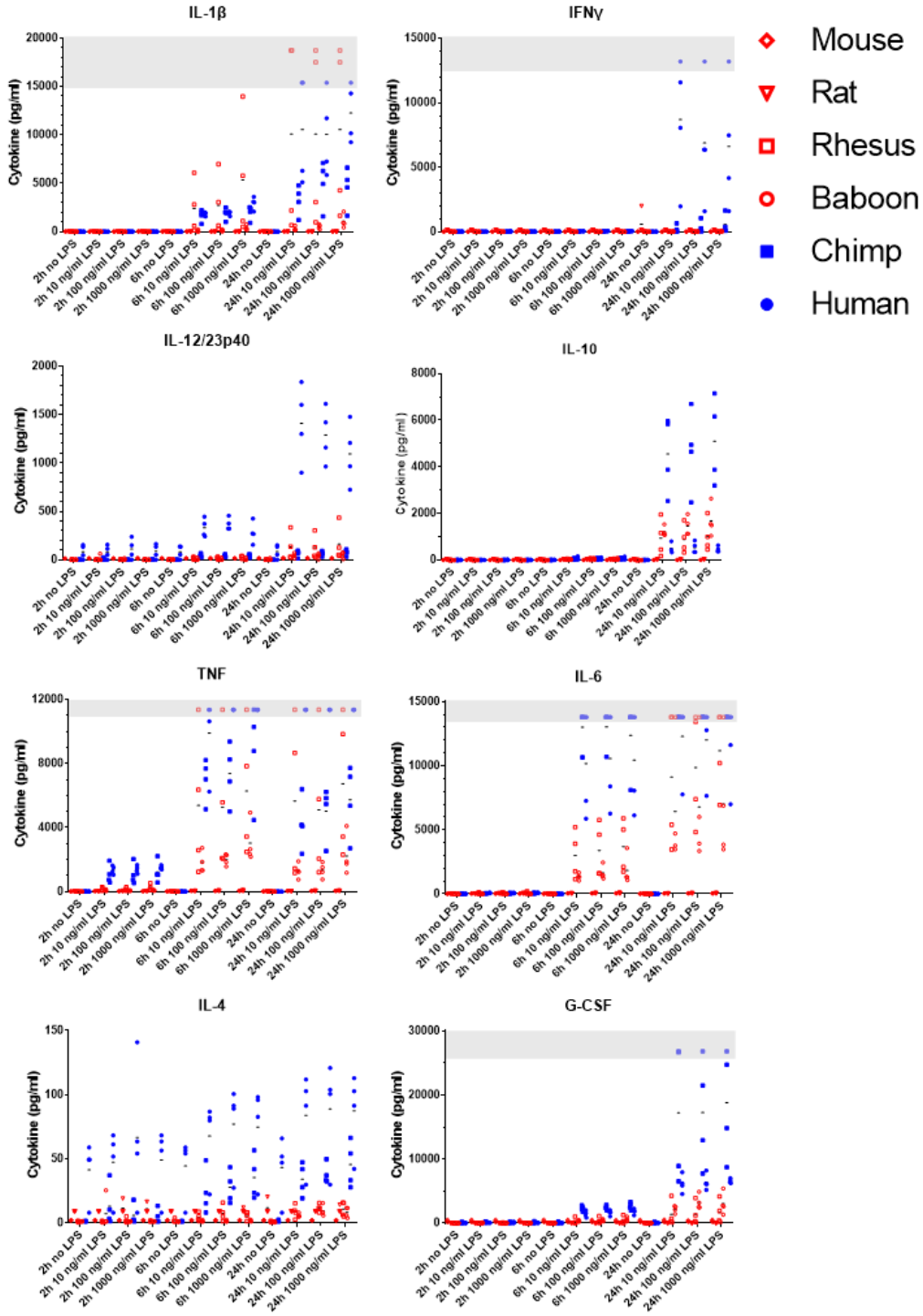

Fig. S6.

Cytokine release following LPS stimulation in the subset of species where antibodies were available, measured by Luminex. Resilient animals are indicated with a red symbol; blue symbols indicate sensitive animals. Black bars indicate mean values for each species. Grey bars indicate readings above quantifiable range.

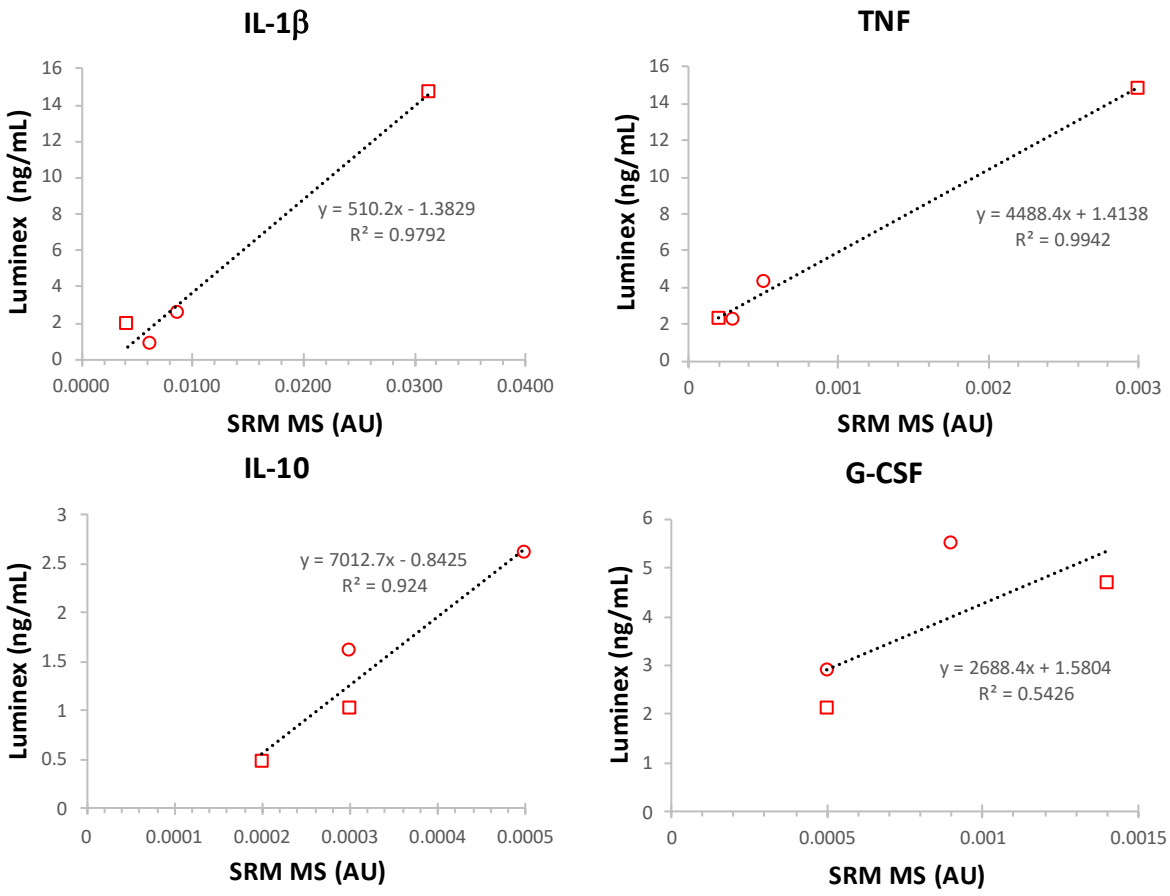

Fig. S7.

Validation of cytokine measurements. Concentrations of the indicated cytokines were established by sequence specific targeted SRM MS of unique peptides for two rhesus (squares) and two baboon (circles) plasma samples. Concentrations are expressed relative to peptide standard in arbitrary units (AU). Values are correlated with those obtained by Luminex for the same samples.

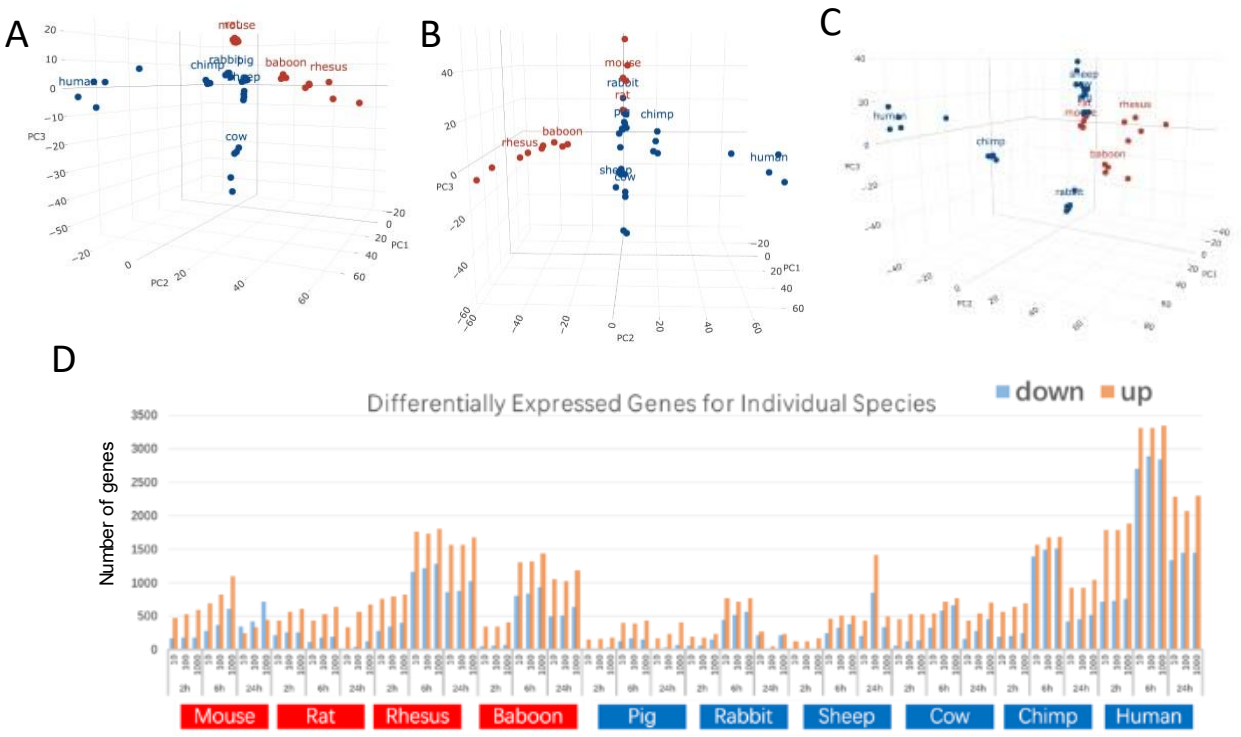

Fig. S8.

Gene expression responses of leukocytes to LPS stimulation. Principal component analysis showing overall distribution of fold change in individual mRNA abundance at A) 2h, B) 6h, or C) 24h following 10 ng/mL LPS stimulation. D) Number of genes significantly (2-fold change, FDR<0.05) changed relative to baseline at each time point after stimulation with 10, 100, or 1000 ng/mL LPS.

Table S1.

Summary of animals bled in this study

Species	Age	Sex	Samples	Sedation	Source	Housing
Mouse	10 week	Male	4× pools of 25	Ketamine-xylazine	Cardiac	Specific pathogen-free barrier facility
Rat	14 week	Male	4× pools of 4	Isoflurane	Cardiac	Specific pathogen-free barrier facility
Rhesus	4 years	Male	5× individuals	Ketamine	Venous	Open air, specific pathogen-free
Baboon	13-18 years	Male	4× individuals	Ketamine	Venous	Open air, specific pathogen-free
Pig	Adult	Male (castrated)	4× individuals	None	Venous	Farm
Rabbit	22 week	Male	4× individuals	Isoflurane	Cardiac	Specific pathogen-free barrier facility
Sheep	Adult	Female	4× individuals	None	Venous	Farm
Cow	Adult	Female	5× individuals	None	Venous	Farm
Chimp	19-26 years	Male	4× individuals	Telazol/ xylazine	Venous	Open air, specific pathogen-free
Human	Adult	Male	5× individuals	None	Venous	Unrestricted

Table S2.

Details of studies used to define species as resilient or sensitive to 1 mg/kg LPS.

Species	Strain	Route	Study	Reference
Mouse	NMRI	i.p.	McCuskey 1984	(1)
Mouse	Balb/c	i.v.	Remick 1995	(2)
Mouse	CD-1	i.v.	Craig 1974	(3)
Mouse	C57/B6	i.p.	Hill 1992	(4)
Mouse	Swiss Webster	i.p.	Su 1997	(5)
Rat		i.p.	Berczi 1966	(6)
Rat	PVG	i.p,	Clark 1982	(7)
Rat	Wistar	i.v	McCuskey 1984	(1)
Rat	Sprague-Dawley	i.v.	Whalley 1992	(8)
Rat	Wistar	i.v.	Kustanova 2006	(9)
Rhesus		i.v.	Sheagren 1967	(10)
Rhesus		i.v.	Coalson 1970	(11)
Macaque		i.v.	Dinbar 1971	(12)
Rhesus		i.v.	Fiser 1974	(13)
Rhesus		i.v.	Premaratne 1995	(14)
Baboon		i.v.	Fletcher 1980	(15)
Baboon		i.v.	Casey 1985	(16)

Baboon		i.v.	Fischer 1992	(17)
Baboon		i.v.	Kneidinger 1996	(18)
Rabbit		i.p.	Berczi 1966	(6)
Rabbit	NZ White	i.v.	Mathison 1988	(19)
Rabbit	NZ White	i.v.	Barrett 1988	(20)
Rabbit	NZ White	i.v.	Whalley, 1992	(8)
Rabbit	NZ White	i.v.	Semeraro 1993	(21)
Rabbit	NZ White	i.v.	Carvalho 1997	(22)
Rabbit	NZ White	i.v.	Kishnamurti	(23)
Pig	Yucatan minipig	i.v.	Hand 1983	(24)
Pig	Minipig	i.v., cumulative	Beller 1985	(25)
Pig	Yorkshire	i.v.	Goldfarb, 1986	(26)
Pig	Mixed breed	i.v.	Schrauwen, 1988	(27)
Pig		Intra-arterial, cumulative	Mozes, 1991	(28)
Pig	Cross-bred	i.v.	Majetschak, 2004	(29)
Cow		i.p.	Berczi, 1966	(6)
Cow	Holstein	i.v.	Ohtsuka, 1997	(30)
Cow	Holstein	i.v.	Gerros, 1995	(31)
Cow	Holstein	i.v.	Yilmaz, 2016	(32)
Sheep		i.v.	Esbenshade, 1982	(33)
Sheep	Mixed breed	Intra-arterial	Golenbock,1987	(34)
Sheep	Suffolk/Merino	i.v, cumulative	Doty, 1988	(35)
Sheep	Suffolk	i.v.	Whyte, 1989	(36)
Sheep	Mixed breed	i.v., cumulatuve	Perkowski, 1996	(37)
Sheep	Mixed breed	i.v., cumulative	Schiffer, 2002	(38)
Chimp		i.v.	Tully, 1965	(39)
Chimp		i.v.	Van der Poll, 2008	(40)
Chimp		i.v.	Van fer Poll, 2008	(41)
Human		i.v.	Sauter, 1980	(42)
Human		i.v.	Elin, 1981	(43)
Human		i.v.	Van Deventer, 1990	(44)
Human		i.v.	Martich, 1991	(45)
Human		i.v.	Taveira da Silva, 1993	(46)

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