Supplementary Materials and Methods

Phosphorylation studies

Phosphorylation of Syk (15min treatment), SHP1, SHP2, SHIP1 or SHIP2 (1, 5 or 30min treatment) in the treated macrophages were measured using the MSD platform (Meso Scale Diagnostics), following the Manufacturer`s instructions.

Flow cytometry

The immuno-phenotyping of macrophages was monitored by flow cytometer using fluorochrome-conjugated mouse anti-human antibodies (BD Pharmingen): CD16-BV421, CD32-FITC, CD64-BUV737, CD40-APC, CD80-AF700, HO-1-AF647, CD206-BV510 and NRP1-BV421. Fluorescence intensities were measured and analyzed by LSRII flow cytometer (BD Biosciences Immunocytometry Systems). Data analysis was performed using FlowJo software v10 (Tree Star).

Enzyme-linked immunosorbent assay

The production of pro-inflammatory cytokines was measured from supernatants of treated macrophages using the MSD 10-cytokine Pro-Inflammatory Panel (Meso Scale Diagnostics), following the Manufacturer`s instruction.

Antioxidant capacity

Human blood plasma samples from healthy individuals (in-house blood donor program) or inhibitor-negative and inhibitor-positive HemA patients (HRF, Inc.; George King Bio-Medical, Inc.) were tested using the Total Antioxidant Capacity Assay Kit (Abcam), following the Manufacturer`s instruction.

Supplementary Figure legends

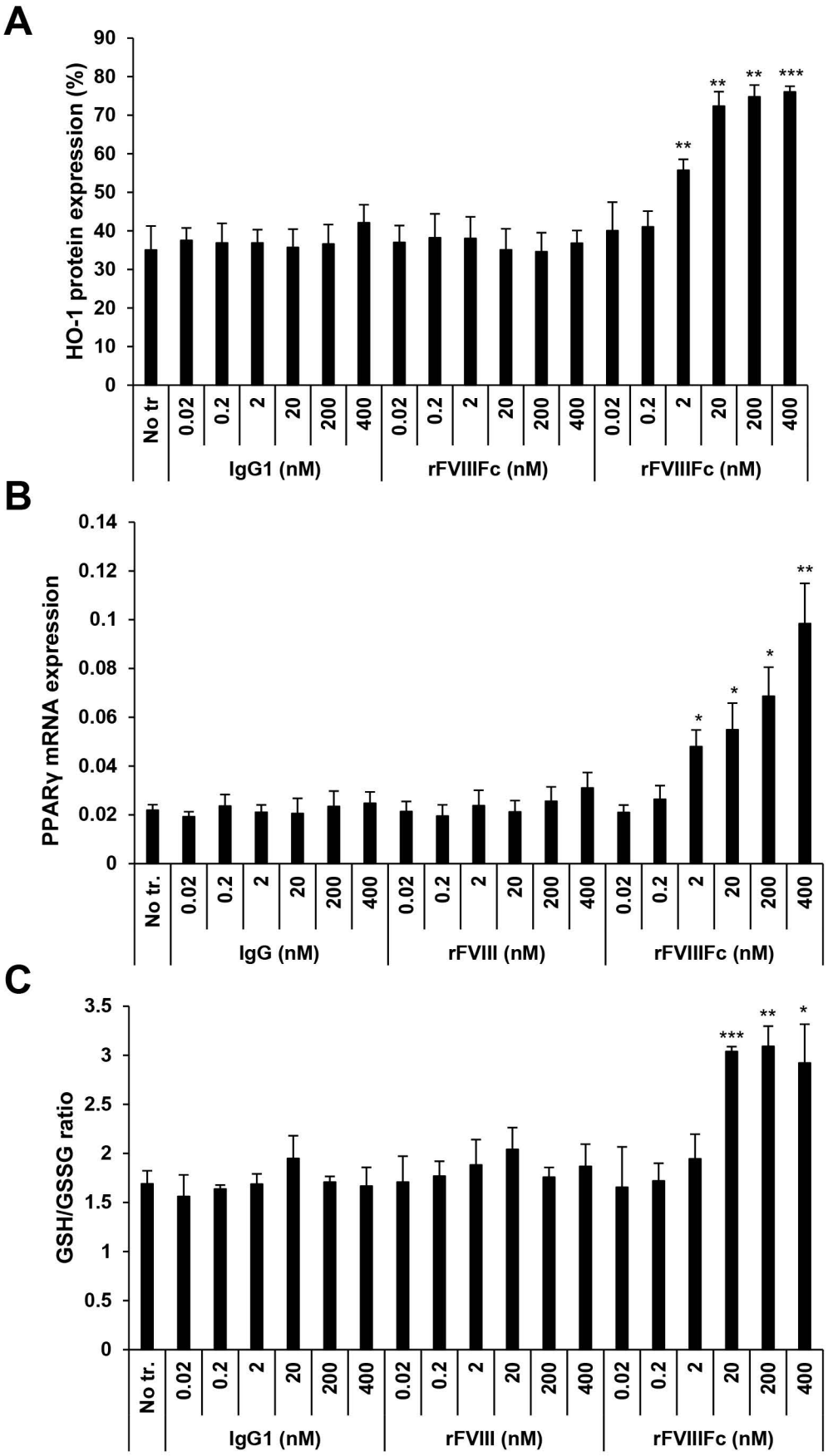
Supplementary Figure 1. Dose correlation of HO-1 protein expression, *PPARγ* gene expression and reduced glutathione production in macrophages. Macrophages (n=3) were treated with IgG1, rFVIII or rFVIIIFc at the indicated concentrations. (**A**) HO-1 protein expression was measured by flow cytometry after 24h treatment. (**B**) *PPARγ* mRNA expression was measured after 6h of treatment by Q-PCR. (**C**) Glutathione production was measured from lysates of cells treated for 24h. Mean±SE; *p≤0.05, **p≤ 0.01, ***p≤ 0.005, calculated as compared to untreated cells.

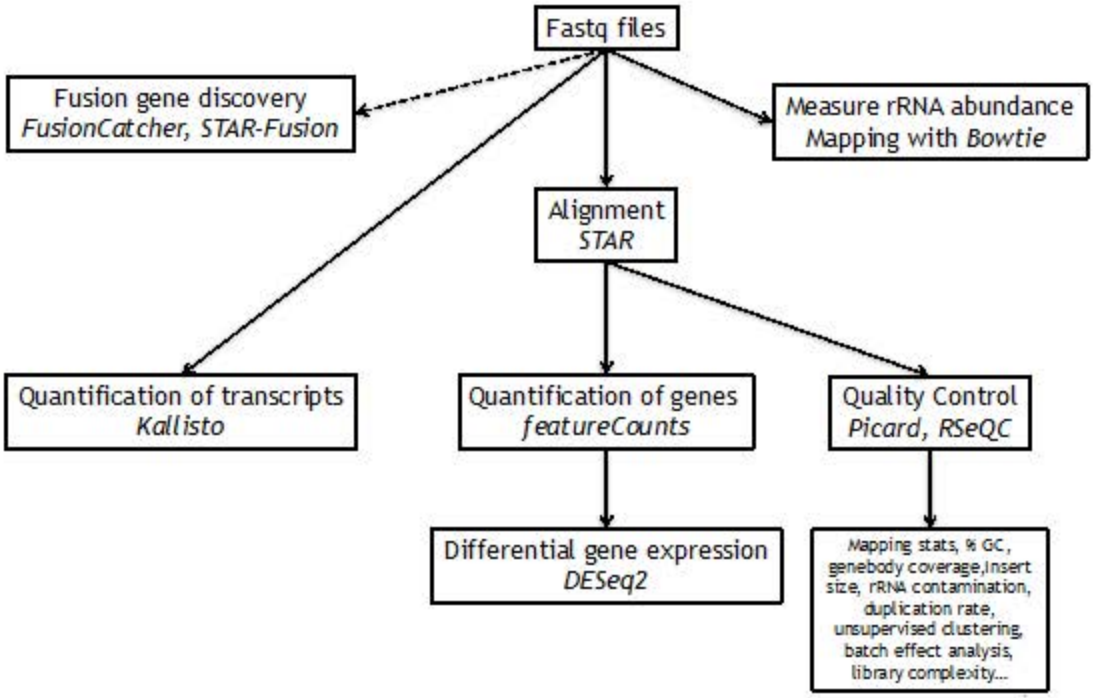
Supplementary Figure 2. RNA sequencing pipeline. Bioinformatics analyses: the reads were aligned with STAR (version 2.4.0c) [58], and genes annotated in Gencode v18 were quantified with featureCounts (v1.4.3-p1) [59]. Normalization and differential expression was done with the Bioconductor package DESeq2 [60]. p≤0.05 was taken as significant difference.

Supplementary Figure 3. Metabolite screening summary of lysates of IgG1-, rFVIII- or rFVIIIFc-treated macrophages. Green: indicates significant difference – downregulation (p≤0.05); red: indicates significant difference – upregulation (p≤0.05); light green: narrowly missed cutoff for significance - downregulation; 0.05<p<0.10; light red: narrowly missed cutoff for significance - upregulation; 0.05<p<0.10; Non-colored text and cell: mean values are not significantly different for that comparison.

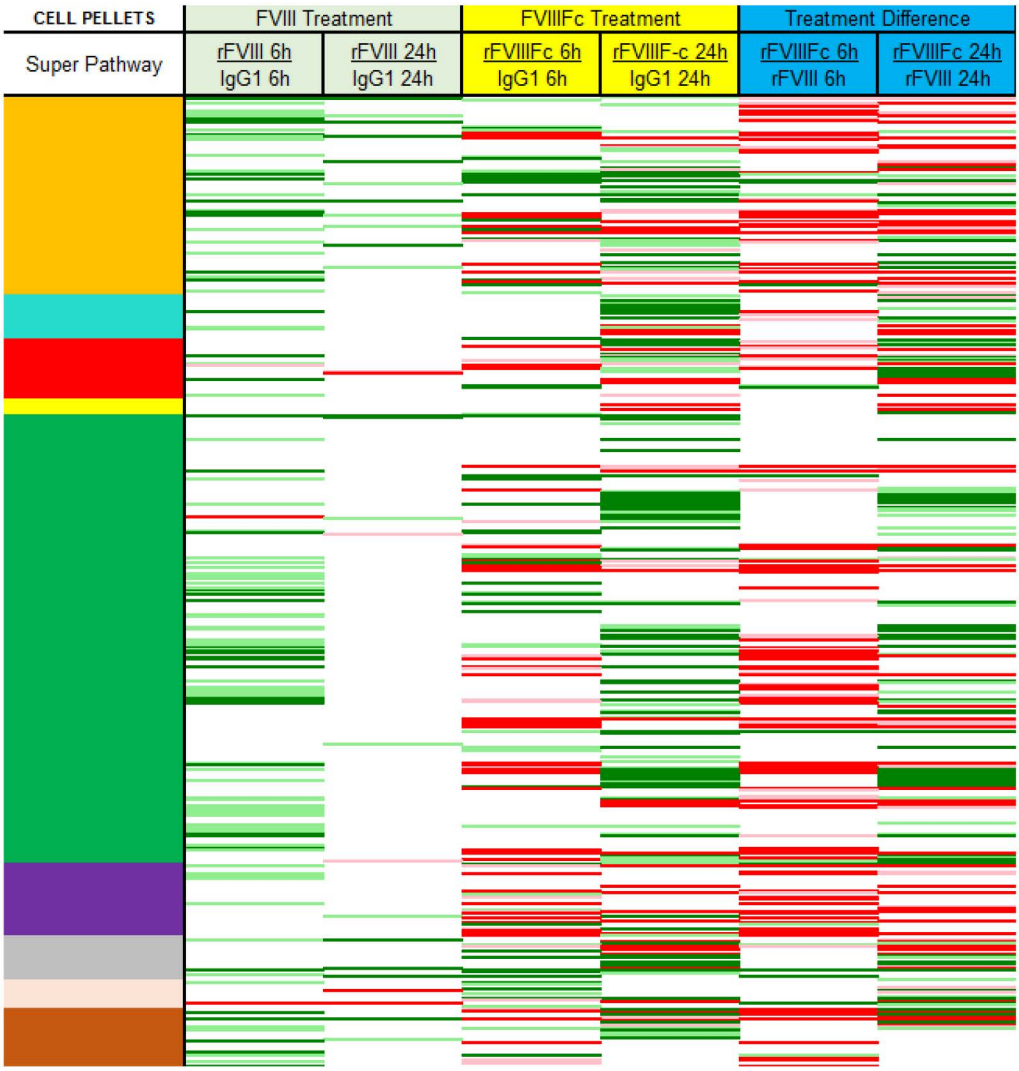
Supplementary Figure 4. Fatty acid screening of IgG1-, rFVIII- or rFVIIIFc-treated macrophage lysates. Green: indicates significant difference – downregulation ($p \leq 0.05$); red: indicates significant difference – upregulation ($p \leq 0.05$); light green: narrowly missed cutoff for significance - downregulation; $0.05 < p < 0.10$; light red: narrowly missed cutoff for significance - upregulation; $0.05 < p < 0.10$; Non-colored text and cell: mean values are not significantly different for that comparison.

Supplementary Figure 5. Mouse bone marrow-derived macrophages respond to rFVIIIFc treatment with polarization to Mox/M2-like cells. Bone marrow from 12-week-old C57BL/6 mice was collected and cultured in complete RPMI medium supplemented with 20ng/ml rmM-CSF for 6 days. Mouse macrophages (n=5) then were treated with 200nM each of IgG1, rFVIII or rFVIIIFc. (**A**) HO-1 protein expression was measured by flow cytometry from 24h-treated samples. (**B**) Arginase 1 (*Arg1*) mRNA expression was measured after 6h of treatment by Q-PCR. (**C**) Glutathione production was measured from lysates of cells treated for 24h. Mean±SE; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.005$.


A
HO-1 protein expression (%)
90
80
70
60
50
40
30
20
10
0
**
**
**
***
No tr
0.02
0.2
2
20
200
400
IgG1 (nM)
rFVIIIFc (nM)
B
PPARγ mRNA expression
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
*
*
*
**
No tr.
IgG (nM)
rFVIII (nM)
rFVIIIFc (nM)
C
GSH/GSSG ratio
3.5
3
2.5
2
1.5
1
0.5
0
***
**
*
No tr.
IgG1 (nM)
rFVIII (nM)
rFVIIIFc (nM)

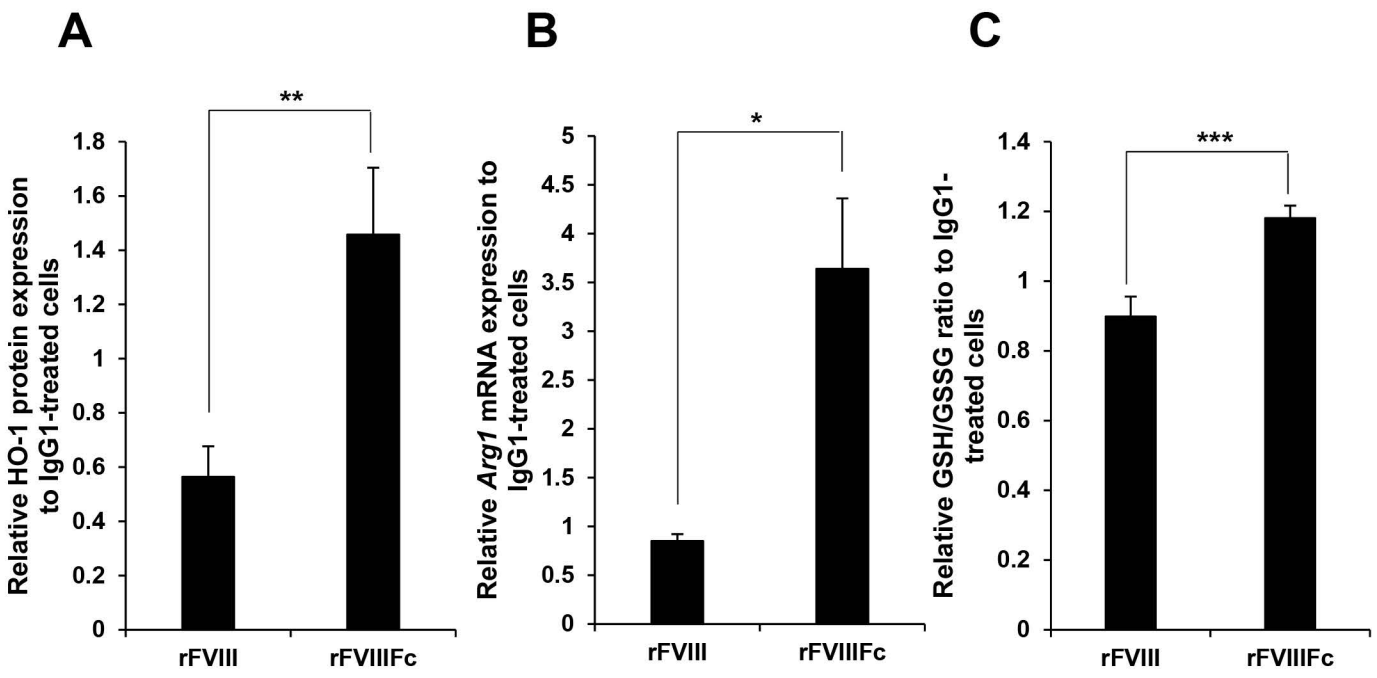

Fastq files
Fusion gene discovery
FusionCatcher, STAR-Fusion
Measure rRNA abundance
Mapping with Bowtie
Alignment
STAR
Quantification of transcripts
Kallisto
Quantification of genes
featureCounts
Quality Control
Picard, RSeQC
Differential gene expression
DESeq2
Mapping stats, % GC, genebody coverage,Insert size, rRNA contamination, duplication rate, unsupervised clustering, batch effect analysis, library complexity...

Super Pathway	
Amino Acid	
Peptide	
Carbohydrate	
Energy	
Lipid	
Nucleotide	
Cofactors and Vitamins	
Xenobiotics	
Unnamed	



Summary Counts	FVIII Treatment		FVIIIFc Treatment		Treatment Differences	
	rFVIII 6H IgG1 6H	rFVIII 24H IgG1 24H	rFVIIIFc 6H IgG1 6H	rFVIIIFc 24H IgG1 24H	rFVIIIFc 6H rFVIII 6H	rFVIIIFc 24H rFVIII 24H
Total number of biochemicals with p≤0.05	43	15	88	131	107	139
% of biochemicals with p≤0.05	7.0	2.4	14.3	21.3	17.4	22.6
Biochemicals (↑|↓)	2 | 41	3 | 12	49 | 39	37 | 94	97 | 10	65 | 74
Total number biochemicals 0.05<p<0.10	79	12	42	62	44	57
% of biochemicals with 0.05<p<0.10	12.8	2.0	6.8	10.1	7.2	9.3
Biochemicals (↑|↓)	1 | 78	3 | 9	18 | 24	16 | 46	36 | 8	24 | 33

Cell Lysate	FVIII Treatment		FVIIIFc Treatment	
Biochemical Name	rFVIII 6H IgG1 6H	rFVIII 24H IgG1 24H	rFVIIIFc 6H IgG1 6H	rFVIIIFc 24H IgG1 24H
butyrylcarnitine (C4)	0.84	0.92	1.03	1.21
propionylcarnitine (C3)	0.72	0.72	0.46	0.53
acetylcarnitine (C2)	0.83	0.86	0.72	0.84
3-hydroxybutyrylcarnitine (1)	0.88	1.08	1.21	1.17
3-hydroxybutyrylcarnitine (2)	0.91	0.84	0.99	1.35
hexanoylcarnitine (C6)	0.93	1.15	1.81	1.04
octanoylcarnitine (C8)	0.84	1.12	1.67	0.86
decanoylcarnitine (C10)	0.73	1.49	1.14	1
5-dodecenoylcarnitine (C12:1)	1.19	1.14	1.56	0.6
laurylcarnitine (C12)	1.13	1.11	2.58	0.76
myristoylcarnitine (C14)	1.01	1.08	1.73	0.66
palmitoylcarnitine (C16)	1.01	0.87	1.52	0.51
palmitoleoylcarnitine (C16:1)*	1.28	1	1.3	0.38
stearoylcarnitine (C18)	0.91	0.88	1.32	0.55
linoleoylcarnitine (C18:2)*	1.18	0.77	0.8	0.21
3-hydroxyoleoylcarnitine	1.03	0.98	1.16	0.57
oleoylcarnitine (C18:1)	1.41	1.01	1.25	0.34
myristoleoylcarnitine (C14:1)*	1.23	1.16	1.3	0.4
adipoylcarnitine (C6-DC)	0.72	0.83	0.64	0.99
arachidoylcarnitine (C20)*	0.85	0.79	0.95	0.59
arachidonoylcarnitine (C20:4)	1.28	0.84	0.81	0.23
adrenoylcarnitine (C22:4)*	1.08	0.72	0.59	0.31
behenoylcarnitine (C22)*	0.82	0.97	0.9	0.71
dihomo-linoleoylcarnitine (C20:2)*	1.11	0.84	1.1	0.52
eicosenoylcarnitine (C20:1)*	0.85	0.86	0.87	0.64
erucoylcarnitine (C22:1)*	0.8	0.82	0.82	0.48
docosatrienoylcarnitine (C22:3)*	1.55	0.78	1.16	1.26
tetracosadienoylcarnitine (C24:2)*	0.84	0.71	0.77	0.57
lignoceroylcarnitine (C24)*	0.78	0.87	0.91	0.86
margaroylcarnitine (C17)*	1.13	0.9	1.71	0.64
pentadecanoylcarnitine (C15)*	1.18	0.92	1.81	0.54
3-hydroxypalmitoylcarnitine	0.93	1	1.37	0.71
N6,N6,N6-trimethyllysine	0.81	0.77	1.19	0.72
deoxycarnitine	0.8	0.87	0.77	1.06
carnitine	0.78	0.94	0.77	1.12
3-hydroxybutyrate (BHBA)	0.96	1.43	1.18	0.99


A
B
C
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*
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Relative HO-1 protein expression to IgG1-treated cells
Relative Arg1 mRNA expression to IgG1-treated cells
Relative GSH/GSSG ratio to IgG1-treated cells
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
0.5
2
2.5
3
3.5
4
4.5
5
rFVIII
rFVIIIFc