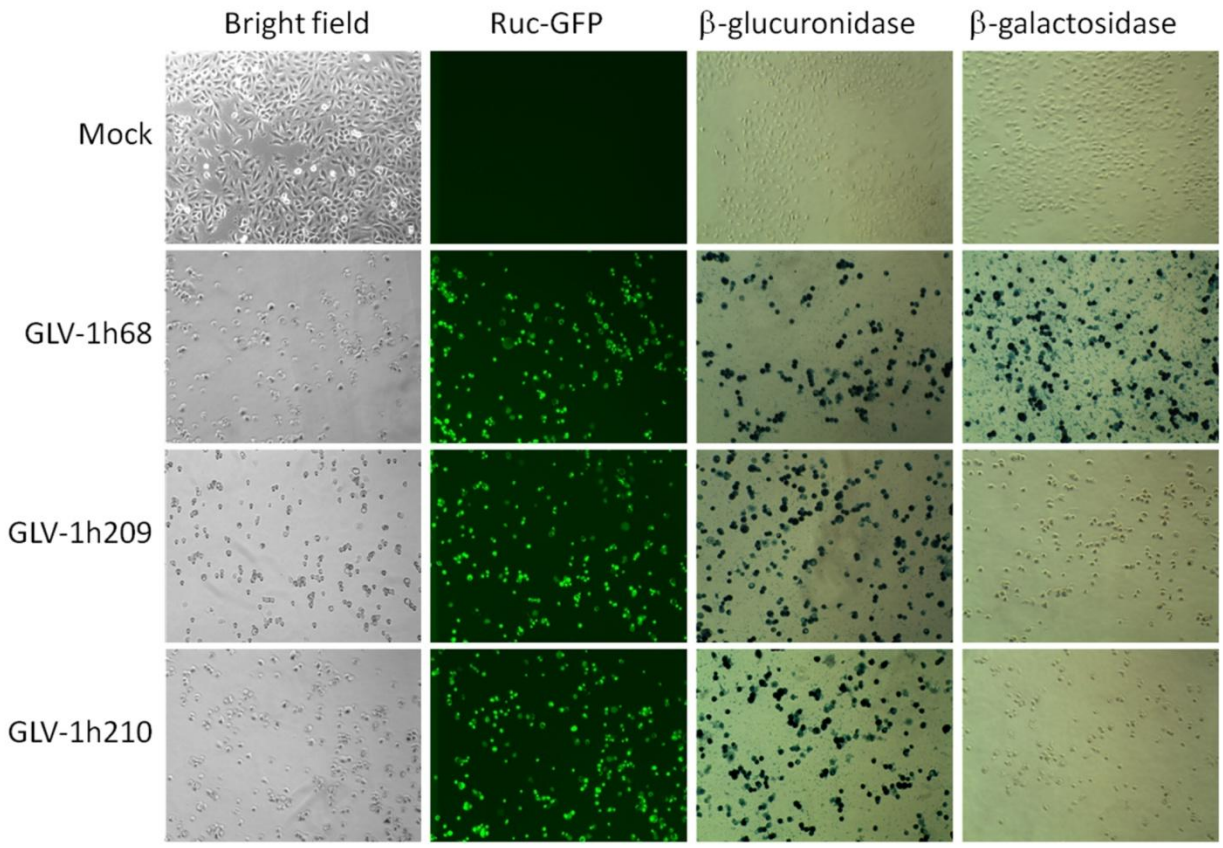
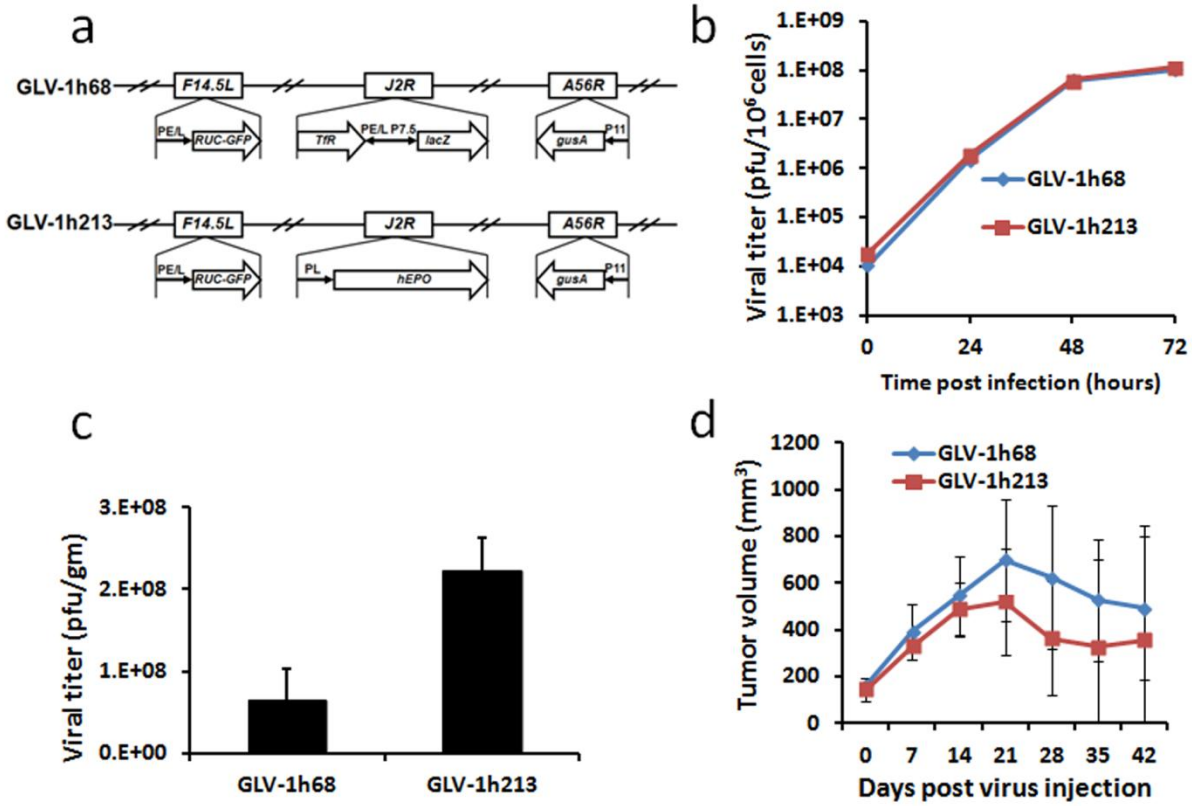
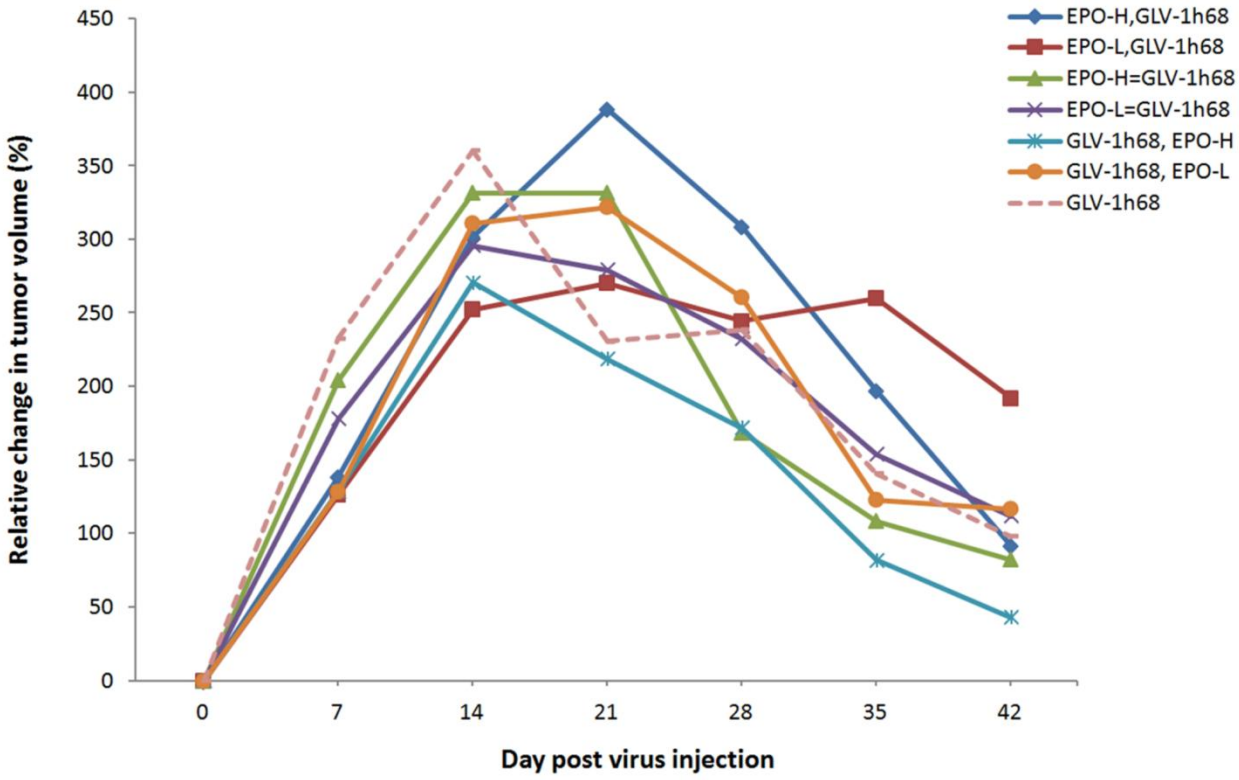


Supplementary Figure S1. **Expression of marker genes from EPO-VACVs detected by fluorescence microscopy and X-gal, X-GLcA staining**. A549 cells in 24-well plates were mock infected or infected with GLV-1h68, GLV-1h209 or GLV-1h210 at an MOI of 1. At 24 hpi, cells were pictured for the GFP signal under the fluorescence microscope, and stained with 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside (X-gal) and 5-bromo-4-chloro-3-indolyl-b-D-glucuronic acid (X-GlcA) for detection of β-galactosidase and β-glucuronidase, respectively.

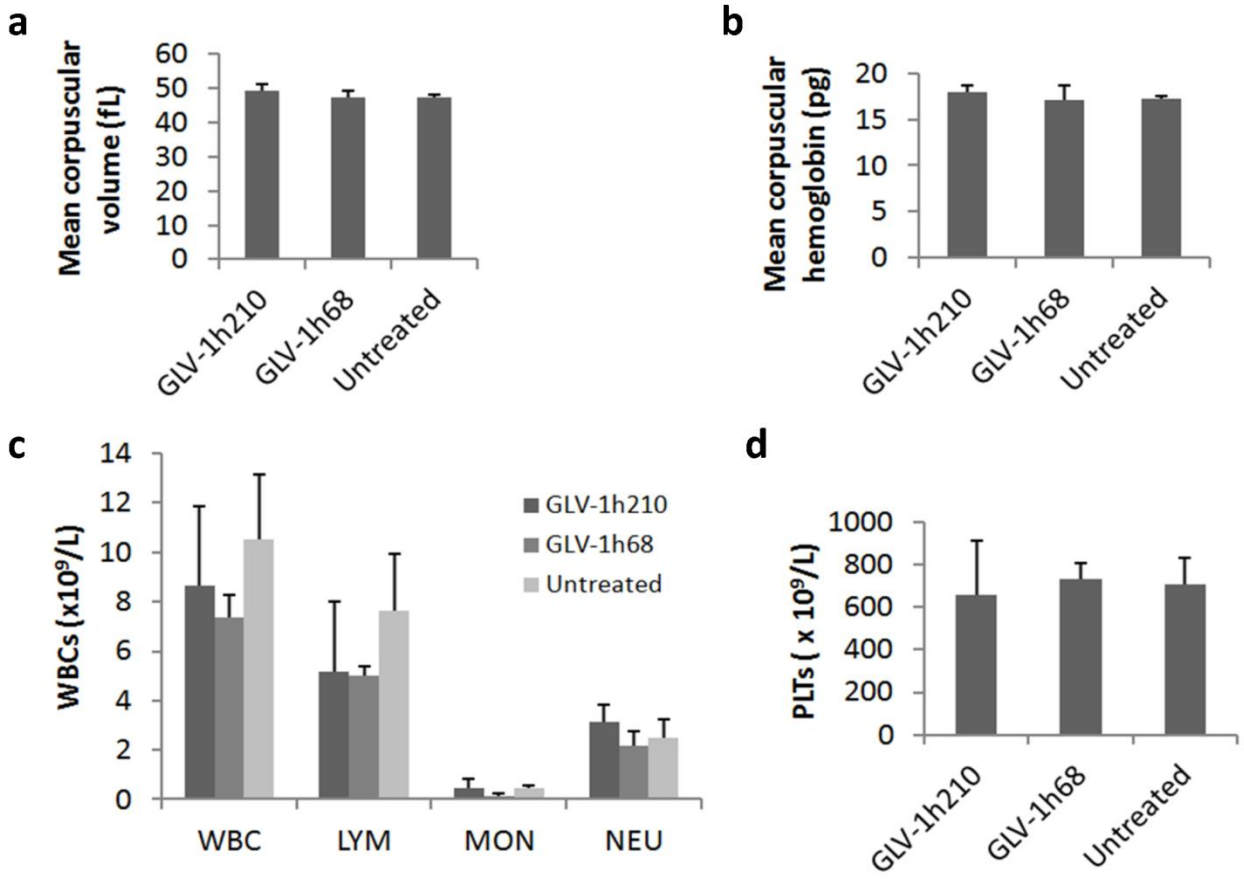


Supplementary Figure S2. Virally expressed hEPO enhances virus replication in tumors, resulting accelerated tumor regression. (**a)** Schematic representation of GLV-1h68 and GLV-1h213 constructs. GLV-1h213 was derived from the parental virus GLV-1h68. The β-galactosidase expression cassette in GLV-1h68 was replaced with the hEPO expression cassette under the control of the vaccinia synthetic late promoter PL to yield GLV-1h213. Ruc-GFP is the *Renilla* luciferase-Aequorea GFP fusion protein. PE/L, P11, and P7.5 are VACV synthetic early/late, 11-K late, and 7.5-K early/late promoters, respectively. TfR is the human transferrin receptor inserted in the reverse orientation with respect to the promoter PE/L. **(b)** Virus replication curves. A549 cells in 6-well plates were infected with GLV-1h68 or GLV-1h210 at an MOI of 0.01 in triplicate. Cells and supernatants were collected at 24, 48, and 72 hpi for virus titration using plaque assays in CV-1 cells. GLV-1h213 and GLV-1h68 showed almost identical replication efficiency in A549 cells. **(c)** Viral titers in tumors at 14 dpi. 5-6 week-old-male athymic nude mice were implanted s.c. with 5 x 10^6 A549 cells on the right hind flank. About 3 weeks later, tumor-bearing mice were r.o. injected with a single dose of 2

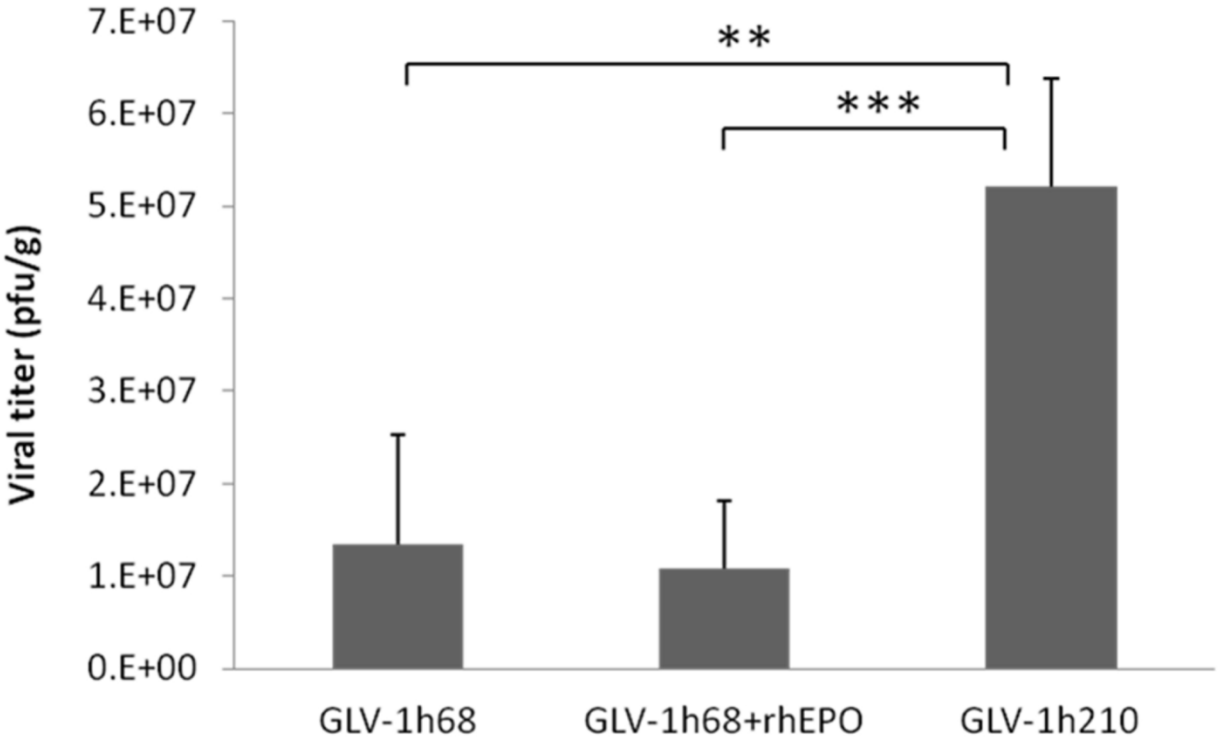
x 10^6 pfu/mouse of each virus. Tumors were excised at 14 days post virus injection. Viral titers were determined by plaque assays on CV-1 cells. Data is presented as average values of 4 individual samples per gram with tissue standard deviations. The GLV-1h213 titer in tumors was 3.5 times as high as the GLV-1h68 titer. The difference was statistically significant (p=0.0014). Consistently, GLV-1h213-treated tumors showed accelerated regression compared with tumors treated with GLV-1h68 (**d)**.



Supplementary Figure S3. Relative changes in tumor volume after treatment with GLV-1h68 in combination with rhEPO (Epoetin alfa). Tumor-bearing mice were injected r.o. with a single dose of 2x10^6 pfu of GLV-1h68 or GLV-1h68 combined with different rhEPO doses given at different times. 1000 U/kg (EPO-H) and 100 U/kg (EPO-L) were injected s.c. 1 week before, at the same time, or 1 week after GLV-1h68 administration. Data shows relative changes in median tumor volume (n=6). No statistically significant differences among groups were found.



Supplementary Figure S4. The effects of virally expressed hEPO on blood cell populations. Tumor-bearing mice were injected r.o. with a single dose of 2 x 10^6 pfu of GLV-1h210 or GLV-1h68. At 14 dpi, Mice were euthanized by CO_2 inhalation and 0.1 mL of whole blood was taken r.o. from each mouse for blood testing. (**a)** Mean corpuscular volume, (**b)** Mean corpuscular hemoglobin, (**c)** White blood cell populations (WBC); lymphocytes (LYM), monocytes (MON), neutrophils (NEU), and (**d)** Platelets (PLT) are shown as average values with SD.



Supplementary Figure S5. Virus titer after treatment with GLV-1h68, GLV-1h210 and GLV-1h68 in combination with rhEPO. Mice were injected with 2 x 10^6 pfu of GLV-1h68 or GLV-1h210. Concomitantly, 500 U/kg rhEPO was injected s.c. for 9 consecutive days post virus injection (Day 1-9) into the GLV-1h68+rhEPO group. Four mice were killed at 14 dpi for virus titration in tumors.

Supplementary Table S1. Virus distribution in organs after treatment with GLV-1h68, GLV-1h209, and GLV-1h210. Organs from tumor-bearing mice treated with the indicated viruses, were excised at 14 days post virus injection. Tissues were homogenized, frozen/thawed, sonicated. Virus particles were determined by plaque assays on CV-1 cells. Data is presented as average values of 4-5 individual samples per gram tissue or per milliliter (serum) with standard deviation (SD). n.d stands for non-detectable, * indicates p = 0.016 when compared to GLV-1h68.

Virus	**Viral titer (pfu/g)** (numbers of animal detected virus/total tested animal)															
	Tumor		**Lung**		**Spleen**		**Brain**		**Liver**		**Kidney**		**Heart**		**Serum**	
	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD	Average	SD
GLV-1h68	**2.73E+06 (4/4)**	3.16E+06	110 (2/4)	165	n.d		n.d		n.d		26 (1/4)	52	48 (2/4)	56	n.d	
GLV-1h209	**1.25E+07 (4/4)**	1.01E+07	21 (1/4)	46	n.d		n.d		n.d		n.d		n.d		n.d	
GLV-1h210	**2.34E+07 * (5/5)**	1.48E+07	126 (2/5)	237	32 (1/5)	72	n.d		n.d		36 (1/5)	80	n.d		n.d	

Supplementary Table S2. Hemoglobin levels return to normalcy in mice treated with GLV-1h210 after tumor eradication. Tumor-bearing mice were injected r.o. with a single dose of 2 x 10^6 pfu of GLV-1h210. Tumor volumes, GFP signal and Hb levels measured at day 40 and 90 post virus injection are displayed for individual mice. Mice with tumor volumes <50 mm^3 are considered tumor eradicated (grey shading).

	40 dpi			90 dpi		
Mouse ID	**Hb (g/dL)**	**GFP**	**Tumor volume (mm^3)**	**Hb (g/dL)**	**GFP**	**Tumor volume (mm^3)**
14854	21.89	+	224.16	12.59	-	30.70
14855	22.61	++	204.52	24.44	-	106.50
14856	22.41	+++	737.18	23.53	+	259.36
14827	21.92	++	176.65	15.78	-	0.00
14828	24.10	++	321.30	25.27	+++	321.77
14829	23.96	+++	206.43	25.13	+	175.59

Supplementary Table S3. Comparison of mouse immune-related antigens in tumors after treatment with GLV-1h210, GLV-1h209 or GLV-1h68. Displayed are the fold differences in mouse immune antigens expressed in tumors at 14 days post treatment with 2 x 10^6 pfu of GLV-1h210, GLV-1h209 or GLV-1h68. Equal amounts of protein from 4 tumors in each group were pooled into 1 representative sample and examined for 58 immune-related antigens (RodentMAP, Mariad RBM, Austin, TX, USA).

	Antigen	Units	LDD	GLV-1h210/ GLV-1h209 ratio	GLV-1h210/ GLV-1h68 ratio	GLV-1h209/ GLV-1h68 ratio
1	Apolipoprotein A-I (Apo A-I)	ug/mL	0.24	**0.8**	**1.3**	**1.7**
2	C-Reactive Protein Mouse (CRP Mouse)	ug/mL	0.023	**1.2**	**1.0**	**0.9**
3	CD40 (CD40)	pg/mL	4.5	**0.6**	**1.8**	**3.3**
4	CD40 Ligand (CD40-L)	pg/mL	414	**0.8**	**0.8**	**1.0**
5	Endothelin-1 (ET-1)	pg/mL	36	**2.0**	**1.1**	**0.6**
6	Eotaxin	pg/mL	5.0	**0.8**	**1.3**	**1.6**
7	Epidermal Growth Factor Mouse (EGF Mouse)	pg/mL	9.3	**1.0**	**0.9**	**0.9**
8	Factor VII	ng/mL	28	**1.3**	**1.0**	**0.8**
9	Fibrinogen	ug/mL	7.7	**0.6**	**0.7**	**1.2**
10	Fibroblast Growth Factor 9 (FGF-9)	ng/mL	2.1	**1.0**	**0.9**	**0.9**
11	Fibroblast Growth Factor basic (FGF-basic)	ng/mL	23	**1.0**	**0.8**	**0.8**
12	Granulocyte Chemotactic Protein-2 Mouse (GCP-2 Mouse)	ng/mL	0.019	**1.0**	**1.8**	**1.8**
13	Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	pg/mL	4.2	**1.2**	**<LOW>**	**<LOW>**
14	Growth-Regulated Alpha Protein (KC/GRO)	ng/mL	0.024	**1.0**	**1.1**	**1.2**
15	Haptoglobin	ug/mL	0.60	**0.8**	**0.7**	**0.9**
16	Immunoglobulin A (IgA)	ug/mL	0.52	**1.0**	**0.7**	**0.7**
17	Interferon gamma (IFN-gamma)	pg/mL	23	**0.8**	**1.2**	**1.5**
18	Interferon gamma Induced Protein 10 (IP-10)	pg/mL	14	**0.9**	**1.3**	**1.5**

19	Interleukin-1 alpha (IL-1 alpha)	pg/mL	182	**1.3**	**1.2**	**0.9**
20	Interleukin-1 beta (IL-1 beta)	ng/mL	6.0	**<LOW>**	**<LOW>**	**<LOW>**
21	Interleukin-2 (IL-2)	pg/mL	60	0.5	**1.1**	**2.0**
22	Interleukin-3 (IL-3)	pg/mL	6.8	**0.7**	**1.4**	**2.0**
23	Interleukin-4 (IL-4)	pg/mL	96	**1.0**	**0.8**	**0.8**
24	Interleukin-5 (IL-5)	ng/mL	0.41	**<LOW>**	**<LOW>**	**<LOW>**
25	Interleukin-6 (IL-6)	pg/mL	6.5	**0.9**	**1.5**	**1.6**
26	Interleukin-7 (IL-7)	ng/mL	0.073	**1.4**	**1.2**	**0.9**
27	Interleukin-10 (IL-10)	pg/mL	224	**0.8**	**1.3**	**1.6**
28	Interleukin-11 (IL-11)	pg/mL	137	**0.8**	**0.7**	**0.9**
29	Interleukin-12 Subunit p70 (IL-12p70)	ng/mL	0.18	**0.8**	**1.4**	**1.8**
30	Interleukin-17A (IL-17A)	ng/mL	0.012	**0.9**	**1.6**	**1.9**
31	Interleukin-18 (IL-18)	ng/mL	4.3	**0.7**	**2.0**	**2.7**
32	Leukemia Inhibitory Factor (LIF)	pg/mL	707	**1.4**	**0.9**	**0.6**
33	Lymphotactin	pg/mL	26	**0.6**	**1.5**	**2.4**
34	Macrophage Colony-Stimulating Factor-1 (M-CSF-1)	ng/mL	0.0088	**0.7**	**1.1**	**1.5**
35	Macrophage-Derived Chemokine (MDC)	pg/mL	44	**1.5**	**3.1**	**2.0**
36	Macrophage Inflammatory Protein-1alpha (MIP-1 alpha)	ng/mL	2.9	**1.8**	**0.9**	**0.5**
37	Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	pg/mL	50	**0.8**	**1.1**	**1.4**
38	Macrophage Inflammatory Protein-1 gamma (MIP-1 gamma)	ng/mL	0.046	**1.1**	**1.0**	**0.9**
39	Macrophage Inflammatory Protein-2 (MIP-2)	pg/mL	5.9	**0.7**	**1.2**	**1.7**
40	Macrophage Inflammatory Protein-3 beta (MIP-3 beta)	ng/mL	0.22	**1.1**	**1.3**	**1.2**
41	Matrix Metalloproteinase-9 (MMP-9)	ng/mL	0.39	**0.5**	**0.9**	**1.9**
42	Monocyte Chemotactic Protein 1 (MCP-1)	pg/mL	5.0	**0.8**	**2.0**	**2.4**
43	Monocyte Chemotactic Protein 3 (MCP-3)	pg/mL	8.1	**1.0**	**1.8**	**1.9**
44	Monocyte Chemotactic Protein-5 (MCP-5)	pg/mL	3.9	**0.9**	**2.3**	**2.5**
45	Myeloperoxidase (MPO)	ng/mL	0.60	**1.1**	**0.9**	**0.9**
46	Myoglobin	ng/mL	0.34	**1.2**	**1.1**	**0.9**
47	Oncostatin-M (OSM)	ng/mL	0.15	**0.7**	**0.9**	**1.2**

48	Serum Amyloid P-Component (SAP)	ug/mL	0.091	**1.3**	**1.4**	**1.1**
49	Serum Glutamic Oxaloacetic Transaminase (SGOT)	ug/mL	53	**0.9**	**1.3**	**1.6**
50	Stem Cell Factor (SCF)	pg/mL	130	**0.8**	**1.0**	**1.1**
51	T-Cell-Specific Protein RANTES (RANTES)	pg/mL	0.076	**1.3**	**3.0**	**2.3**
52	Thrombopoietin	ng/mL	22	**1.2**	**0.1**	**0.1**
53	Tissue Factor (TF)	ng/mL	3.6	**1.1**	**0.8**	**0.7**
54	Tissue Inhibitor of Metalloproteinases 1 Mouse (TIMP-1 Mouse)	ng/mL	0.057	**1.6**	**1.3**	**0.8**
55	Tumor Necrosis Factor alpha (TNF-alpha)	ng/mL	0.052	**1.0**	**1.0**	**0.9**
56	Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/mL	0.14	**1.0**	**1.2**	**1.2**
57	Vascular Endothelial Growth Factor A (VEGF-A)	pg/mL	45	**1.0**	**0.6**	**0.6**
58	von Willebrand factor (vWF)	ng/mL	0.76	**0.8**	**1.5**	**1.8**

(LDD) the least detectable dose

<LOW> reflect samples not measurable on the standard curve.