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Supplemental Information

An All-Recombinant Protein-Based Culture System Specifically Identifies Hematopoietic Stem Cell Maintenance Factors

Aki Ieyasu, Reiko Ishida, Takaharu Kimura, Maiko Morita, Adam C. Wilkinson, Kazuhiro Sudo, Toshinobu Nishimura, Jun Ohehara, Yoko Tajima, Chen-Yi Lai, Makoto Otsu, Yukio Nakamura, Hideo Ema, Hiromitsu Nakauchi, and Satoshi Yamazaki

Supplementary Experimental Procedures

Mice

C57BL/6 (B6-Ly5.2) mice were purchased from SLC (Shizuoka, Japan) and B6-Ly5.1 from Sankyo Lab Service (Tsukuba, Japan). All animal care was in accordance with the guidelines of the University of Tokyo.

Purification of murine hematopoietic stem cells

Mouse CD34⁻KSL HSCs were purified from BM cells of 8 to 10 week-old mice. Whole bone marrow cells were stained with an antibody cocktail consisting of biotinylated anti-Gr-1 (#13593185), -Mac-1 (#13011285), -CD4 (#13004285), -IL-7R (13127185), and -Ter-119 (#13592185) (eBioscience, San Diego, CA), and -B220 (#13045285) and -CD8 (#13008185) monoclonal antibodies (BioLegend, San Diego, CA) (lineage cocktail). Lineage-positive cells were depleted with anti-Biotin MicroBeads (MiltenyiBiotec, Auburn, CA) using LS columns (MiltenyiBiotec). The lineage-depleted fraction was then further stained with fluorescein isothiocyanate (FITC)-conjugated anti-CD34 (#11034185, BD Bioscience, California, CA), phycoerythrin (PE)-conjugated anti-Sca-1 (#108108, eBioscience), and allophycocyanin (APC)-conjugated anti-c-Kit antibodies (#17117183, BioLegend). Biotinylated antibodies were detected with streptavidin-APC-Cy7 (#47431782, BioLegend). Analysis and cell sorting were performed on a FACS Aria (BD Bioscience) and results were analyzed with FlowJo software (Tree Star, Ashland, OR).

Hematopoietic stem cell culture system

CD34⁻KSL HSCs were deposited into 96-well micro-titer plates containing 200 μ l of serum free medium S-Clone SF-03 (Sanko JunyakuInc, Tokyo, Japan) supplemented with 1% BSA (Sigma, and Wako, Japan), MSA or HSA (Albumin Bioscience, Sigma, Bioverde) and cytokines (50 ng/ml mouse SCF, 50 ng/ml human TPO).

Competitive repopulation assays

40 CD34⁺KSL cells from B6-Ly5.1 and the BM competitor cells (1×10^6) from B6-F1 mice were transplanted into B6-Ly5.2 mice irradiated at a dose of 9.8 Gy. Following transplantation, peripheral blood (PB) cells of the recipients were stained with PE-conjugated anti-Ly5.1 (BioLegend) and FITC-conjugated anti-Ly5.2 (BD Bioscience). The cells were further stained with PE-Cy7-conjugated anti-Mac-1 and -Gr-1, Pacific Blue (PB)-conjugated anti-B220 and APC-Cy7-conjugated anti-CD3 antibodies (BioLegend) and analyzed using a FACS Aria. Secondary BM transplantation assays were performed by transferring 1×10^6 BM cells from the primary recipient mice into lethally irradiated B6-Ly5.2 mice. PB cells were collected from secondary recipient mice at 4, 8, 12 and 16 weeks post-transplantation and analyzed as above. For limiting dilution assays, 1000, 100, and 10 (seven-day) cultured cells were aliquoted by FACS and transplantation into lethally-irradiated mice together with 2×10^5 BM competitor cells. PB cells were collected at 4 weeks post-transplantation and analyzed as above.

Purification samples for Mass Spectrometry analysis

Depletion of albumin from BSA-FV was performed using Melon Gel IgG Spin Purification Kit (Thermo Scientific) according to the manufacturer's instructions. Briefly, 500 μ l of 10% BSA-FV in 10 mM HEPES pH7.3 was diluted 1:10 in Melon Gel Purification Buffer and applied to Melon Gel spin columns. After incubation for 5 min at room temperature with rotation, spin columns were centrifuged and flow through was used for mass spectrometry analysis (Medical & Biological Laboratories CO., LTD.).

Western blotting

500 μ g of BSA-FV was subjected to albumin depletion. Total flow-through of Melon Gel was applied to SDS-PAGE, transferred to PVDF membranes and blots were incubated with rabbit polyclonal anti-hemopexin antibody (#ab133415, 1:1000, Abcam) for 30 min at room temperature. Following incubation with an appropriate secondary antibody, immunoreactivity was detected by chemiluminescence using Image Quant LAS4000 (GE Healthcare Life Science).

ROS detection assay

CD34⁺KSL cells were collected after 2 days of culture and stained with HySOx (excitation: 555 nm, emission: 575 nm; Goryo Chemical, Hokkaido, Japan), for 30 minutes at 37°C. After washing, cells were analyzed for ROS accumulation by FACS Aria. Cellular ROS were quantified by mean fluorescence intensity (MFI).

Immunofluorescence staining of BM sections

Frozen BM sections were prepared and immunostained according to the Kawamoto method (Kawamoto, 2003). BM sections were fixed using dry ice/ethanol or 4% paraformaldehyde (PFA). Immunofluorescence data were obtained and analyzed with a TCS SP2 AOBS confocal microscope (Leica, Microsystems, Tokyo). The immunofluorescence-microscopy images of BM sections were automatically obtained, using integrated reader software, from multiple replicates. Markers and antibodies used were: Alexa 488-conjugated goat anti-Rabbit IgG, and Alexa 647-conjugated goat anti-rabbit IgG (Molecular Probes, Carlsbad, CA, USA); rabbit anti-GFAP (#Z0334, Dako, Glostrup, Denmark); sheep anti-hemopexin (#MAB7007, R&D systems); 4, 6-diamidino-2-phenylindole (DAPI), a DNA marker.

3D imaging

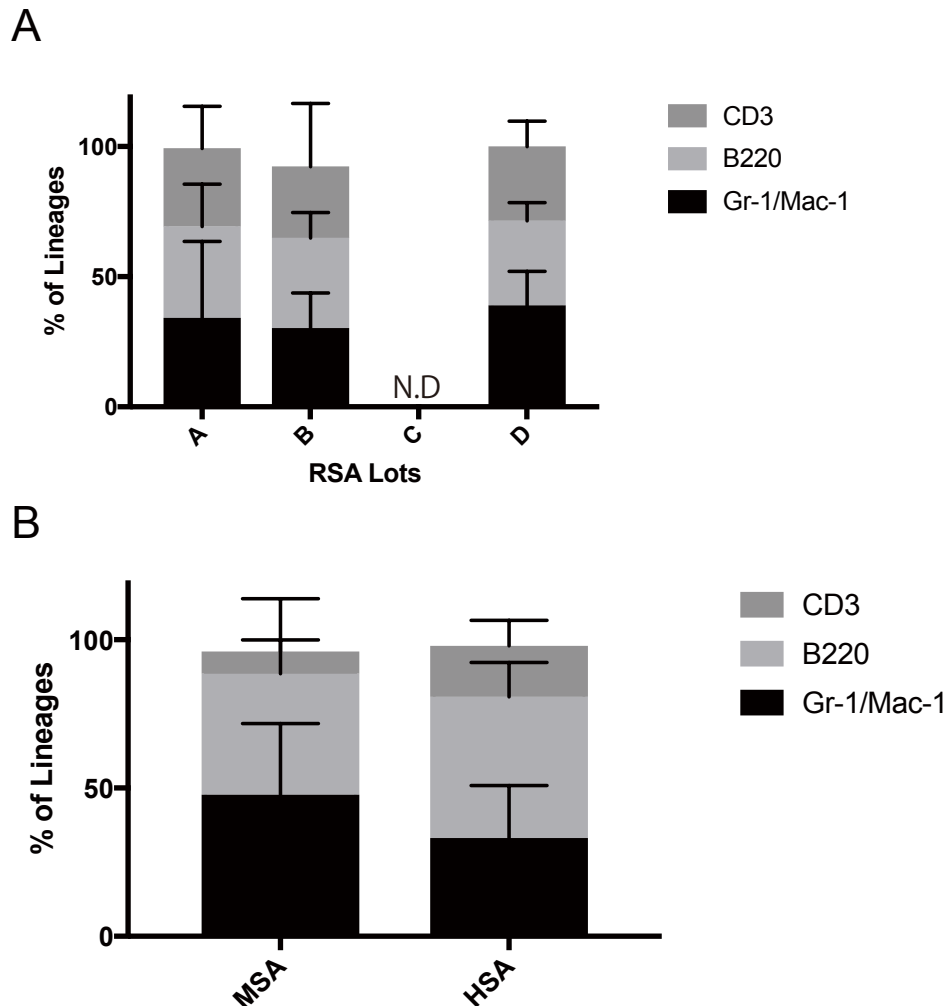
3D imaging was performed as previously described (Susaki et al., 2015). In brief, after tibias were collected and fixed in 4% PFA solution, bone marrow plugs were extracted from bones by flushing. Upon DAPI staining, marrow plugs were treated with ScaleCUBIC-1 (Reagent-1) for one week followed by ScaleCUBIC-2 (Reagent-2) for another week. To visualize GFAP⁺ cells and hemopexin⁺, cells were stained overnight with antibodies. Images were acquired using a ZEISS Z1 Lightsheet microscope (ZEISS) and 3D reconstitutions were performed with Imaris software (Bitplane).

Statistical analysis

Mean values of two groups were compared using two-tail unpaired t testing. All statistical analyses were performed on Prism 7 software (Graphpad, San Diego, CA).

Supplemental Figure and Figure Legends

Supplemental Figure 1

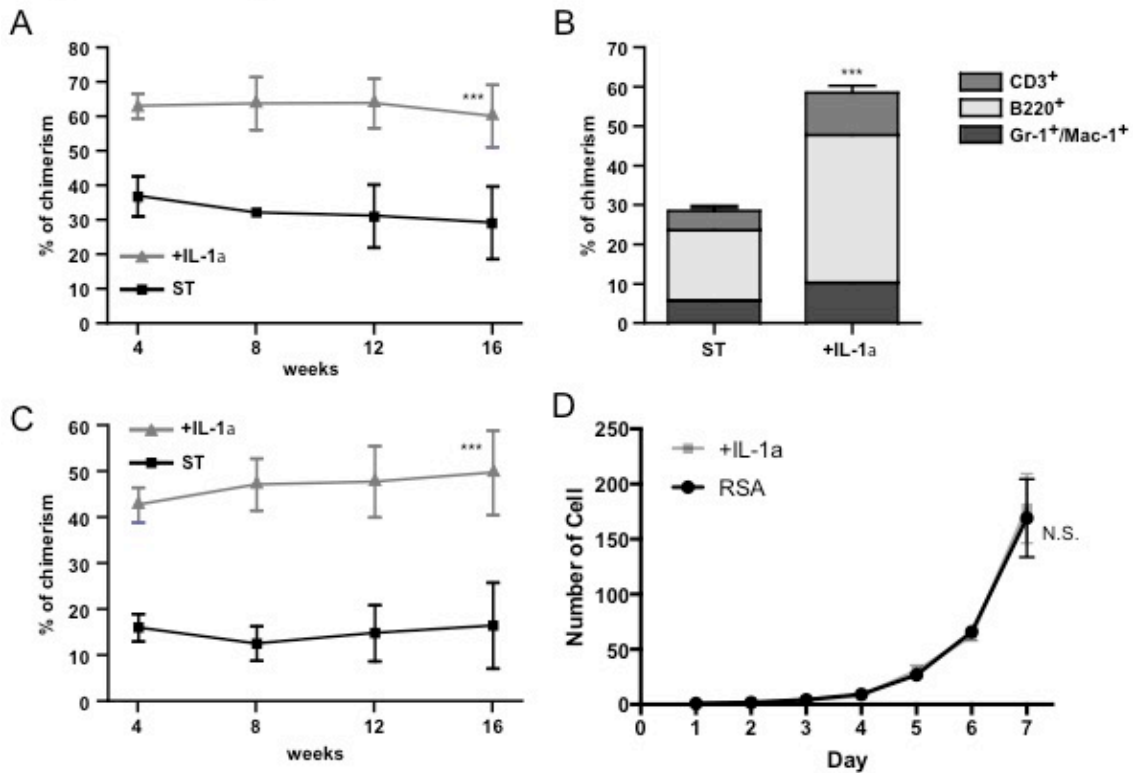


Supplementary Figure 1: Multi-lineage HSC output following RSA-based culture

(A) Ratio of myeloid (Gr-1/Mac-1⁺), B cell (B220⁺) and T cell (CD3⁺) PB chimerism from 40 CD34-HSCs cultured for seven days in various lots of RSA (A-D) supplemented with SCF and TPO. Mean ratios \pm SDs at 12 weeks post-transplantation, as described in Figure 3A (n = 5 per BSA-FV culture condition).

(B) Ratio of myeloid (Gr-1/Mac-1⁺), B cell (B220⁺) and T cell (CD3⁺) PB chimerism from 40 CD34-HSCs cultured for seven days in mouse RSA (MSA) or human RSA (HSA) supplemented with SCF and TPO. Mean ratios \pm SDs at 12 weeks post-transplantation, as described in Figure 3B (n = 5 per RSA culture condition).

Supplemental Figure 2



Supplementary Figure 2: IL-1 α enhances *in vitro* HSC maintenance

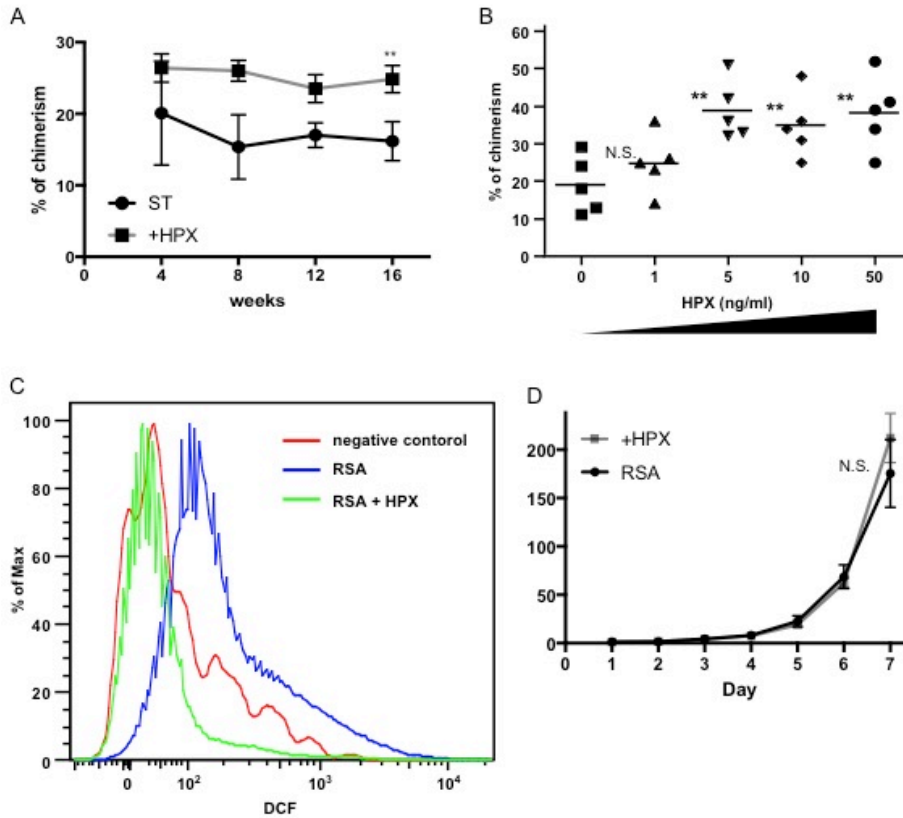
(A,B) 40 CD34⁺KSL HSCs were cultured for one week with 1% HSA, SCF, TPO and 20 ng/ml IL-1 α before transplantation into lethally irradiated mice together with 10⁶ BM competitor cells. (A) Time course analysis of PB chimerism. (B) The donor-derived PB cell ratio 12 weeks after transplantation. The data presented are the mean ratios \pm SDs of two independent experiments (n = 5 per culture condition).

(C) Secondary competitive repopulation analysis of BM from mice in (A). Data are the mean \pm SD of donor-derived PB cells (n = 10 per culture condition).

(D) Proliferation of HSCs with RSA with IL-1 α *in vitro*. Single CD34⁺KSL HSCs were cultured for one week in 96-well micro-titer plates in S-Clone SF-03 supplemented with SCF, TPO and 1% RSA (black line) with or without 20 ng/ml IL-1 α . Cell numbers were counted every 24 hours under a microscope. Data are the mean \pm SEM (n = 40 per culture condition).

Statistical significance denoted by ** ($P < 0.05$), *** ($P < 0.005$) or N.S. (not significant) as determined by unpaired t testing.

Supplemental Figure 3



Supplementary Figure 3: Hemopexin enhances engraftment of *in vitro* cultured HSCs

(A) Secondary competitive repopulation analysis of BM from mice in Figure 4A. Data are the mean ± SD of donor-derived PB cells (n = 10 per culture condition).

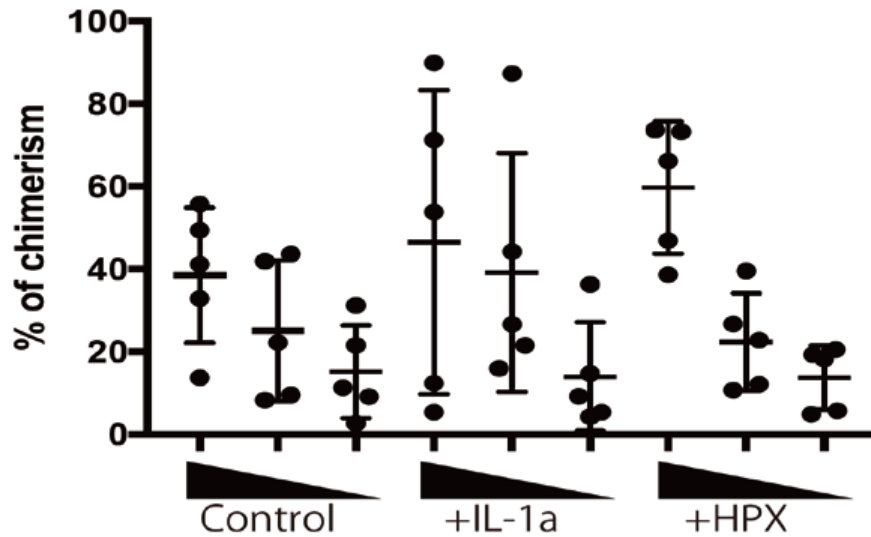
(B) 40 CD34⁺KSL cells were cultured for 1 week with 1% MSA, SCF, TPO and various concentrations of HPX before transplantation into lethally irradiated recipient mice together with 10⁶ BM competitor cells. The donor-derived chimerism was measured 12 weeks after transplantation. Data are the mean ± SD of donor-derived cells in the PB (n = 5 per culture condition) of two independent experiments.

(C) Representative flow cytometric plots displaying HySOx staining (a measure of ROS levels) of HSCs cultured *in vitro* with or without HPX.

(D) Proliferation of HSCs with RSA with HPX *in vitro*. Single CD34⁺KSL HSCs were cultured for one week in 96-well micro-titer plates in S-Clone SF-03 supplemented with SCF, TPO and 1% RSA (black line) with or without 10 ng/ml HPX. Cell numbers were counted every 24 hours under a microscope. Data are the mean ± SEM (n = 40 per culture condition).

Statistical significance denoted by ** ($P < 0.05$), *** ($P < 0.005$) or N.S. (not significant) as determined by unpaired t testing.

Supplemental Figure 4



Supplementary Figure 4: Limiting dilution analysis of RSA-cultured HSCs

40 CD34⁺KSL HSCs were cultured for one week with 1% HSA, SCF, TPO with or without 20 ng/ml IL-1 α or 10 ng/ml HPX. 1000 (left bar), 100 (middle bar) and 10 (right bar) cultured cells were then aliquoted by FACS and transplantation into lethally irradiated mice together with 2×10^5 BM competitor cells. PB chimerism at 4 weeks post-transplantation are displayed.

Supplemental Table 1

BSA-FV #1			BSA-FV #8			BSA-FV #15		
Accession number	Name	Peptides (95%)	Accession number	Name	Peptides (95%)	Accession number	Name	Peptides (95%)
gi 30794280	serum albumin precursor [Bos taurus]	1129	gi 30794280	serum albumin precursor [Bos taurus]	823	gi 30794280	serum albumin precursor [Bos taurus]	686
gi 154425704	ALB protein [Bos taurus]	971	gi 154425704	ALB protein [Bos taurus]	700	gi 74267962	ALB protein [Bos taurus]	575
gi 296490958	TPA: serotransferrin precursor [Bos taurus]	654	gi 27806789	transferrin precursor [Bos taurus]	216	gi 99028969	complement C3 preproprotein [Bos taurus]	130
gi 2501351	RecName: Full=Serotransferrin; Short=Transferrin; AltName: Full=Beta-1 metal-binding globulin; AltName: Full=Siderophilin; Flags: Precursor	636	gi 1699167	IgG2a heavy chain constant region [Bos taurus]	63	gi 27806789	transferrin precursor [Bos taurus]	92
gi 77736171	hemopexin precursor [Bos taurus]	186	gi 99028969	complement C3 preproprotein [Bos taurus]	55	gi 109939993	Apolipoprotein H (beta-2-glycoprotein I) [Bos taurus]	88
gi 114051225	protein HP-20 homolog precursor [Bos taurus]	96	gi 94966811	alpha-1-acid glycoprotein precursor [Bos taurus]	42	gi 74353860	IGL@ protein [Bos taurus]	53
gi 114050753	protein HP-25 homolog 1 precursor [Bos taurus]	55	gi 296490958	TPA: serotransferrin precursor [Bos taurus]	41	gi 151556360	Unknown (protein for MGC:159378) [Bos taurus]	51
gi 27806789	transferrin precursor [Bos taurus]	53	gi 74353860	IGL@ protein [Bos taurus]	40	gi 15088675	immunoglobulin lambda light chain [Bos taurus]	51
gi 114052108	protein HP-25 homolog 2 precursor [Bos taurus]	39	gi 296486756	TPA: complement factor I [Bos taurus]	37	gi 77735883	serum amyloid P-component precursor [Bos taurus]	49
gi 6	beta-2-glycoprotein I [Bos taurus]	37	gi 77735367	ribonuclease UK114 [Bos taurus]	36	gi 297461373	PREDICTED: complement factor H-related protein 2 [Bos taurus]	42
gi 92096965	Immunoglobulin light chain, lambda gene cluster [Bos taurus]	34	gi 108750	Ig heavy chain precursor (B/MT.4A.17.H5.A5) - bovine	28	gi 296490958	TPA: serotransferrin precursor [Bos taurus]	38
gi 116812902	hemoglobin subunit alpha [Bos taurus]	32	gi 296478893	TPA: proteoglycan 4 [Bos taurus]	24	gi 75812954	fibrinogen alpha chain precursor [Bos taurus]	16
gi 27819608	hemoglobin subunit beta [Bos taurus]	31	gi 296487872	TPA: keratin 6A [Bos taurus]	19	gi 343197018	immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3b [Bos taurus]	16
gi 77735935	complement C2 precursor [Bos taurus]	30	gi 343197018	immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3b [Bos taurus]	18	gi 77404252	collagen alpha-1(I) chain precursor [Bos taurus]	15
gi 91982959	immunoglobulin gamma 1 heavy chain constant region [Bos taurus]	27	gi 27807007	insulin-like growth factor-binding protein 3 precursor [Bos taurus]	16	gi 164414427	collagen alpha-1(II) chain isoform 2 precursor [Bos taurus]	14
gi 59858077	aspartate aminotransferase 1 [Bos taurus]	26	gi 297461373	PREDICTED: complement factor H-related protein 2 [Bos taurus]	15	gi 28189426	similar to ubiquitin-S27a fusion protein [Bos taurus]	12
gi 108750	Ig heavy chain precursor (B/MT.4A.17.H5.A5) - bovine	23	gi 296484341	TPA: protein AMBP precursor [Bos taurus]	14	gi 330688394	folate receptor alpha precursor [Bos taurus]	11
gi 270483766	beta-hexosaminidase subunit beta preproprotein [Bos taurus]	22	gi 153791660	extracellular matrix protein 1 precursor [Bos taurus]	12	gi 2323380	immunoglobulin light chain variable region [Bos taurus]	11
gi 95147674	complement factor B precursor [Bos taurus]	21	gi 95147674	complement factor B precursor [Bos taurus]	12	gi 27807007	insulin-like growth factor-binding protein 3 precursor [Bos taurus]	10
gi 296475479	TPA: fumarylacetoacetase [Bos taurus]	17	gi 310893435	immunoglobulin light chain [Bos taurus]	12	gi 2323402	immunoglobulin light chain variable region [Bos taurus]	10
gi 1699167	IgG2a heavy chain constant region [Bos taurus]	16	gi 75812954	fibrinogen alpha chain precursor [Bos taurus]	11	gi 95147674	complement factor B precursor [Bos taurus]	10
gi 343197026	immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3c [Bos taurus]	15	gi 296476317	TPA: keratin, type I cytoskeletal 14 [Bos taurus]	10	gi 153791660	extracellular matrix protein 1 precursor [Bos taurus]	9
gi 358422418	PREDICTED: WASH complex subunit 7-like [Bos taurus]	13	gi 77736171	hemopexin precursor [Bos taurus]	10	gi 358420619	PREDICTED: complement C4-A [Bos taurus]	9
gi 156120479	fructose-bisphosphate aldolase A [Bos taurus]	12	gi 358421409	PREDICTED: keratin, type II cytoskeletal 1 [Bos taurus]	9	gi 84000165	complement factor I precursor [Bos taurus]	8
gi 27807261	acidic mammalian chitinase precursor [Bos taurus]	11	gi 114051856	keratin, type II cytoskeletal 7 [Bos taurus]	9	gi 75832056	apolipoprotein A-I preproprotein [Bos taurus]	7
gi 114051379	leucine-rich alpha-2-glycoprotein precursor [Bos taurus]	10	gi 358422499	PREDICTED: keratin, type I cytoskeletal 10-like [Bos taurus]	9	gi 296485168	TPA: serine peptidase inhibitor, Kazal type 5 [Bos taurus]	7
gi 2323386	immunoglobulin light chain variable region [Bos taurus]	10	gi 160395544	RecName: Full=Keratin, type I cytoskeletal 17; AltName: Full=Cytokeratin-17; Short=CK-17; Short=K17	9	gi 164450479	kininogen-2 isoform 1 precursor [Bos taurus]	7
gi 30038325	cathepsin C [Bos taurus]	9	gi 156120885	CD5 antigen-like precursor [Bos taurus]	8	gi 115497340	serum amyloid A protein precursor [Bos taurus]	6
gi 77735921	fructose-bisphosphate aldolase B [Bos taurus]	6	gi 358417209	PREDICTED: extracellular peptidase inhibitor-like [Bos taurus]	8	gi 255003702	fibronectin precursor [Bos taurus]	5
gi 296475228	TPA: serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 [Bos taurus]	6	gi 2323386	immunoglobulin light chain variable region [Bos taurus]	8	gi 8100793	insulin-like growth factor I [Bos taurus]	4
gi 154425814	IGK protein [Bos taurus]	5	gi 77735883	serum amyloid P-component precursor [Bos taurus]	7	gi 296484341	TPA: protein AMBP precursor [Bos taurus]	4
gi 397740864	vitamin D binding protein [Bos taurus]	5	gi 358420619	PREDICTED: complement C4-A [Bos taurus]	6	gi 59858077	aspartate aminotransferase 1 [Bos taurus]	4
gi 114052298	apolipoprotein A-II precursor [Bos taurus]	5	gi 296475228	TPA: serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 [Bos taurus]	5	gi 94966811	alpha-1-acid glycoprotein precursor [Bos taurus]	4
gi 358421409	PREDICTED: keratin, type II cytoskeletal 1 [Bos taurus]	4	gi 27806741	beta-2-glycoprotein 1 precursor [Bos taurus]	5	gi 296487872	TPA: keratin 6A [Bos taurus]	4
gi 194685481	PREDICTED: keratin, type I cytoskeletal 10-like isoform 2 [Bos taurus]	4	gi 59858077	aspartate aminotransferase 1 [Bos taurus]	4	gi 29648975	TPA: CD5 molecule-like [Bos taurus]	4
gi 30466252	carbonic anhydrase 2 [Bos taurus]	4	gi 27807341	cathelicidin-1 precursor [Bos taurus]	2	gi 395268	conglutinin [Bos taurus]	3
gi 78369426	prostaglandin reductase 1 [Bos taurus]	4	gi 296480310	TPA: apolipoprotein A-I-like [Bos taurus]	2	gi 397740864	vitamin D binding protein [Bos taurus]	3
gi 41386683	beta-2-microglobulin precursor [Bos taurus]	4	gi 375065868	ceruloplasmin precursor [Bos taurus]	2	gi 114051856	keratin, type II cytoskeletal 7 [Bos taurus]	3
gi 83405800	Legumain [Bos taurus]	4	gi 78369352	complement component C9 precursor [Bos taurus]	2	gi 78369352	complement component C9 precursor [Bos taurus]	3
gi 302179501	epidermal growth factor receptor [Bos taurus]	4	gi 300797776	DNA-directed RNA polymerase I subunit RPA1 [Bos taurus]	1	gi 27807009	insulin-like growth factor-binding protein 4 precursor [Bos taurus]	2

gi 129277510	extracellular superoxide dismutase [Cu-Zn] precursor [Bos taurus]	3	gi 91982959	immunoglobulin gamma 1 heavy chain constant region [Bos taurus]	25	gi 126607	RecName: Full=Lysozyme C; AltName: Full=1,4-beta-N-acetylmuramidase C; Flags: Precursor	2
gi 114052314	plasma kallikrein precursor [Bos taurus]	3	gi 148356276	keratin, type II cytoskeletal 4 [Bos taurus]	6	gi 47564119	apolipoprotein C-III precursor [Bos taurus]	1
gi 333360891	hepatocyte growth factor activator preproprotein [Bos taurus]	3	gi 22773998	immunoglobulin delta heavy chain membrane bound form [Bos taurus]	4	gi 343197026	immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3c [Bos taurus]	15
gi 84000195	phosphoglycerate mutase 2 [Bos taurus]	3	gi 75832116	inter-alpha-trypsin inhibitor heavy chain H4 precursor [Bos taurus]	2	gi 310893435	immunoglobulin light chain [Bos taurus]	8
gi 94966763	haptoglobin precursor [Bos taurus]	3	gi 397740864	vitamin D binding protein [Bos taurus]	2	gi 2323384	immunoglobulin light chain variable region [Bos taurus]	7
gi 75812940	phosphatidylethanolamine-binding protein 1 [Bos taurus]	2	gi 291490675	CD44 antigen precursor [Bos taurus]	2	gi 358421409	PREDICTED: keratin, type II cytoskeletal 1 [Bos taurus]	3
gi 61888856	triosephosphate isomerase [Bos taurus]	2	gi 300795742	synaptic vesicle membrane protein VAT-1 homolog [Bos taurus]	2	gi 77735935	complement C2 precursor [Bos taurus]	3
gi 375065868	ceruloplasmin precursor [Bos taurus]	2	gi 432134242	DNA excision repair protein ERCC-6-like 2 [Bos taurus]	1	gi 45429979	spleen trypsin inhibitor 1 precursor [Bos taurus]	3
gi 358421417	PREDICTED: keratin, type II cytoskeletal 3 [Bos taurus]	2	gi 329664128	protocadherin-17 precursor [Bos taurus]	1	gi 41386685	thrombospondin-1 precursor [Bos taurus]	2
gi 156120583	arylsulfatase G [Bos taurus]	2	gi 164448719	sterile alpha motif domain-containing protein 3 [Bos taurus]	1	gi 296487901	TPA: insulin-like growth factor-binding protein 6 precursor [Bos taurus]	2
gi 27806591	glutathione peroxidase 1 [Bos taurus]	2	gi 156120521	kinesin-like protein KIFC1 [Bos taurus]	1	gi 27806907	clusterin preproprotein [Bos taurus]	2
gi 34538498	immunoglobulin heavy chain constant region [Bos taurus]	2	gi 27806751	alpha-2-HS-glycoprotein precursor [Bos taurus]	1	gi 194670528	PREDICTED: ribonuclease 4 [Bos taurus]	2
gi 15088675	immunoglobulin lambda light chain [Bos taurus]	29	gi 114051379	leucine-rich alpha-2-glycoprotein precursor [Bos taurus]	1	gi 27806751	alpha-2-HS-glycoprotein precursor [Bos taurus]	2
gi 148744128	Unknown (protein for MGC:159455) [Bos taurus]	29	gi 359070805	PREDICTED: collagen alpha-1(V) chain-like [Bos taurus]	1	gi 157954059	Fe fragment of IgG, low affinity IIc, receptor for (CD32) [Bos taurus]	2
gi 343197018	immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3b [Bos taurus]	9	gi 1351907	RecName: Full=Serum albumin; AltName: Full=BSA; AltName: Allergen=Bos d 6; Flags: Precursor	821	gi 6006423	hemoglobin alpha chain [Bos taurus]	2
gi 310893435	immunoglobulin light chain [Bos taurus]	7	gi 74267962	ALB protein [Bos taurus]	702	gi 297488636	PREDICTED: protein phosphatase 1M [Bos taurus]	1
gi 296487283	TPA: hyaluronan synthase 2-like [Bos taurus]	3	gi 83764016	prepro complement component C3 [Bos taurus]	55	gi 27807335	cathelicidin-7 precursor [Bos taurus]	1
gi 187171271	dual specificity mitogen-activated protein kinase kinase 2 [Bos taurus]	2	gi 4093220	complement component 3 [Bos taurus]	55	gi 114050753	protein HP-25 homolog 1 precursor [Bos taurus]	1
gi 83764016	prepro complement component C3 [Bos taurus]	1	gi 122697593	alpha-1-acid glycoprotein precursor [Bos taurus]	42	gi 85681876	RecName: Full=Protein-lysine 6-oxidase; AltName: Full=Lysyl oxidase; Flags: Precursor	1
gi 148745450	Fibrinogen alpha chain [Bos taurus]	1	gi 114326282	serotransferrin precursor [Bos taurus]	40	gi 296487283	TPA: hyaluronan synthase 2-like [Bos taurus]	1
gi 300797661	transcription elongation factor SPT6 [Bos taurus]	1	gi 2501351	RecName: Full=Serotransferrin; Short=Transferrin; AltName: Full=Beta-1 metal-binding globulin; AltName: Full=Siderophilin; Flags: Precursor	40	gi 114051379	leucine-rich alpha-2-glycoprotein precursor [Bos taurus]	1
gi 164452943	gelsolin isoform a precursor [Bos taurus]	1	gi 84000165	complement factor 1 precursor [Bos taurus]	37	gi 1351907	RecName: Full=Serum albumin; AltName: Full=BSA; AltName: Allergen=Bos d 6; Flags: Precursor	685
gi 300795058	gamma-glutamyltransferase 5 precursor [Bos taurus]	1	gi 151556360	Unknown (protein for MGC:159378) [Bos taurus]	37	gi 154425704	ALB protein [Bos taurus]	573
gi 151554455	MSH3 protein [Bos taurus]	1	gi 343197030	immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3d [Bos taurus]	26	gi 83764016	prepro complement component C3 [Bos taurus]	130
gi 157954059	Fe fragment of IgG, low affinity IIc, receptor for (CD32) [Bos taurus]	1	gi 331284120	proteoglycan 4 precursor [Bos taurus]	20	gi 2506196	RecName: Full=Beta-2-glycoprotein 1; AltName: Full=Apolipoprotein H; Short=Apo-H; AltName: Full=Beta-2-glycoprotein I; Short=B2GPI; Short=Beta(2)GPI; Flags: Precursor	88
gi 77735466	complement factor D precursor [Bos taurus]	1	gi 343197008	immunoglobulin lambda light chain constant region 2 allotypic variant IGLC2c [Bos taurus]	20	gi 6	beta-2-glycoprotein I [Bos taurus]	88
gi 1351907	RecName: Full=Serum albumin; AltName: Full=BSA; AltName: Allergen=Bos d 6; Flags: Precursor	1125	gi 134085706	keratin, type II cytoskeletal 6A [Bos taurus]	19	gi 92096965	Immunoglobulin light chain, lambda gene cluster [Bos taurus]	48
gi 74267962	ALB protein [Bos taurus]	978	gi 343197026	immunoglobulin lambda light chain constant region 3 allotypic variant IGLC3c [Bos taurus]	16	gi 139948632	immunoglobulin lambda-like polypeptide 1 precursor [Bos taurus]	45
gi 114326282	serotransferrin precursor [Bos taurus]	643	gi 163190	insulin-like growth factor binding protein-3 [Bos taurus]	14	gi 2501351	RecName: Full=Serotransferrin; Short=Transferrin; AltName: Full=Beta-1 metal-binding globulin; AltName: Full=Siderophilin; Flags: Precursor	37
gi 2506196	RecName: Full=Beta-2-glycoprotein 1; AltName: Full=Apolipoprotein H; Short=Apo-H; AltName: Full=Beta-2-glycoprotein I; Short=B2GPI; Short=Beta(2)GPI; Flags: Precursor	37	gi 27806743	protein AMBP precursor [Bos taurus]	14	gi 114326282	serotransferrin precursor [Bos taurus]	37
gi 109939993	Apolipoprotein H (beta-2-glycoprotein 1) [Bos taurus]	37	gi 2323404	immunoglobulin light chain variable region [Bos taurus]	12	gi 148745450	Fibrinogen alpha chain [Bos taurus]	16
gi 74353860	IGL@ protein [Bos taurus]	33	gi 1276627	immunoglobulin lambda light chain variable region, partial [Bos taurus]	12	gi 164414425	collagen alpha-1(II) chain isoform 1 precursor [Bos taurus]	14
gi 139948632	immunoglobulin lambda-like polypeptide 1 precursor [Bos taurus]	32	gi 148745450	Fibrinogen alpha chain [Bos taurus]	11	gi 3789962	fibrinogen A-alpha chain [Bos taurus]	14
gi 296474257	TPA: complement component 2 precursor [Bos taurus]	30	gi 3789962	fibrinogen A-alpha chain [Bos taurus]	11	gi 329665078	polyubiquitin-C [Bos taurus]	14
gi 11120280	complement component 2 precursor [Bos taurus]	30	gi 262118301	keratin, type I cytoskeletal 14 [Bos taurus]	10	gi 350537449	putative ubiquitin C variant 5 [Taeniopygia guttata]	13
gi 6006425	hemoglobin alpha chain [Bos taurus]	30	gi 116004057	keratin, type II cytoskeletal 75 [Bos taurus]	10	gi 296478548	TPA: ubiquitin C [Bos taurus]	13

gi 12206517	RecName: Full=Aspartate aminotransferase, cytoplasmic; AltName: Full=Glutamate oxaloacetate transaminase 1; AltName: Full=Transaminase A	26	gi 297474460	PREDICTED: keratin, type II cytoskeletal 1 [Bos taurus]	9	gi 28189917	similar to polyubiquitin [Bos taurus]	13
gi 15470790	fumarylacetoacetase [Bos taurus]	17	gi 296476308	TPA: keratin, type I cytoskeletal 10 [Bos taurus]	9	gi 28189839	similar to polyubiquitin [Bos taurus]	13
gi 154425761	FAH protein [Bos taurus]	17	gi 27805977	keratin, type I cytoskeletal 10 [Bos taurus]	9	gi 27806505	polyubiquitin-B [Bos taurus]	13
gi 7547266	IgG1 heavy chain constant region [Bos taurus]	16	gi 194685481	PREDICTED: keratin, type I cytoskeletal 10-like isoform 2 [Bos taurus]	9	gi 163575	polyubiquitin, partial [Bos taurus]	13
gi 119892690	PREDICTED: WASH complex subunit 7 [Bos taurus]	13	gi 358416465	PREDICTED: immunoglobulin lambda-like polypeptide 5-like [Bos taurus]	9	gi 163573	polyubiquitin, partial [Bos taurus]	13
gi 343197004	immunoglobulin lambda light chain constant region 2 allotypic variant IGLC2b [Bos taurus]	13	gi 343196996	immunoglobulin lambda light chain constant region 2 allotypic variant IGLC2a [Bos taurus]	9	gi 110282963	RecName: Full=Folate receptor alpha; Short=FR-alpha; AltName: Full=Folate receptor 1; AltName: Full=Folate-binding protein 1; Short=FBP; AltName: Full=Milk folate-binding protein; Flags: Precursor	11
gi 343197008	immunoglobulin lambda light chain constant region 2 allotypic variant IGLC2c [Bos taurus]	11	gi 157427776	keratin, type I cytoskeletal 17 [Bos taurus]	9	gi 2323374	immunoglobulin light chain variable region [Bos taurus]	11
gi 2323380	immunoglobulin light chain variable region [Bos taurus]	10	gi 296489775	TPA: CDS molecule-like [Bos taurus]	8	gi 2323376	immunoglobulin light chain variable region [Bos taurus]	11
gi 2323376	immunoglobulin light chain variable region [Bos taurus]	10	gi 2323380	immunoglobulin light chain variable region [Bos taurus]	8	gi 2323386	immunoglobulin light chain variable region [Bos taurus]	11
gi 2323374	immunoglobulin light chain variable region [Bos taurus]	10	gi 2323376	immunoglobulin light chain variable region [Bos taurus]	8	gi 296478557	TPA: ubiquitin B-like [Bos taurus]	10
gi 75812938	dipeptidyl peptidase 1 precursor [Bos taurus]	9	gi 358421417	PREDICTED: keratin, type II cytoskeletal 3 [Bos taurus]	8	gi 163190	insulin-like growth factor binding protein-3 [Bos taurus]	10
gi 359064628	PREDICTED: protein piccolo-like [Bos taurus]	9				gi 296479849	TPA: folate receptor 1-like [Bos taurus]	10
						gi 2323400	immunoglobulin light chain variable region [Bos taurus]	10
						gi 296486756	TPA: complement factor I [Bos taurus]	8
						gi 975844	immunoglobulin lambda light chain variable region, partial [Bos taurus]	8