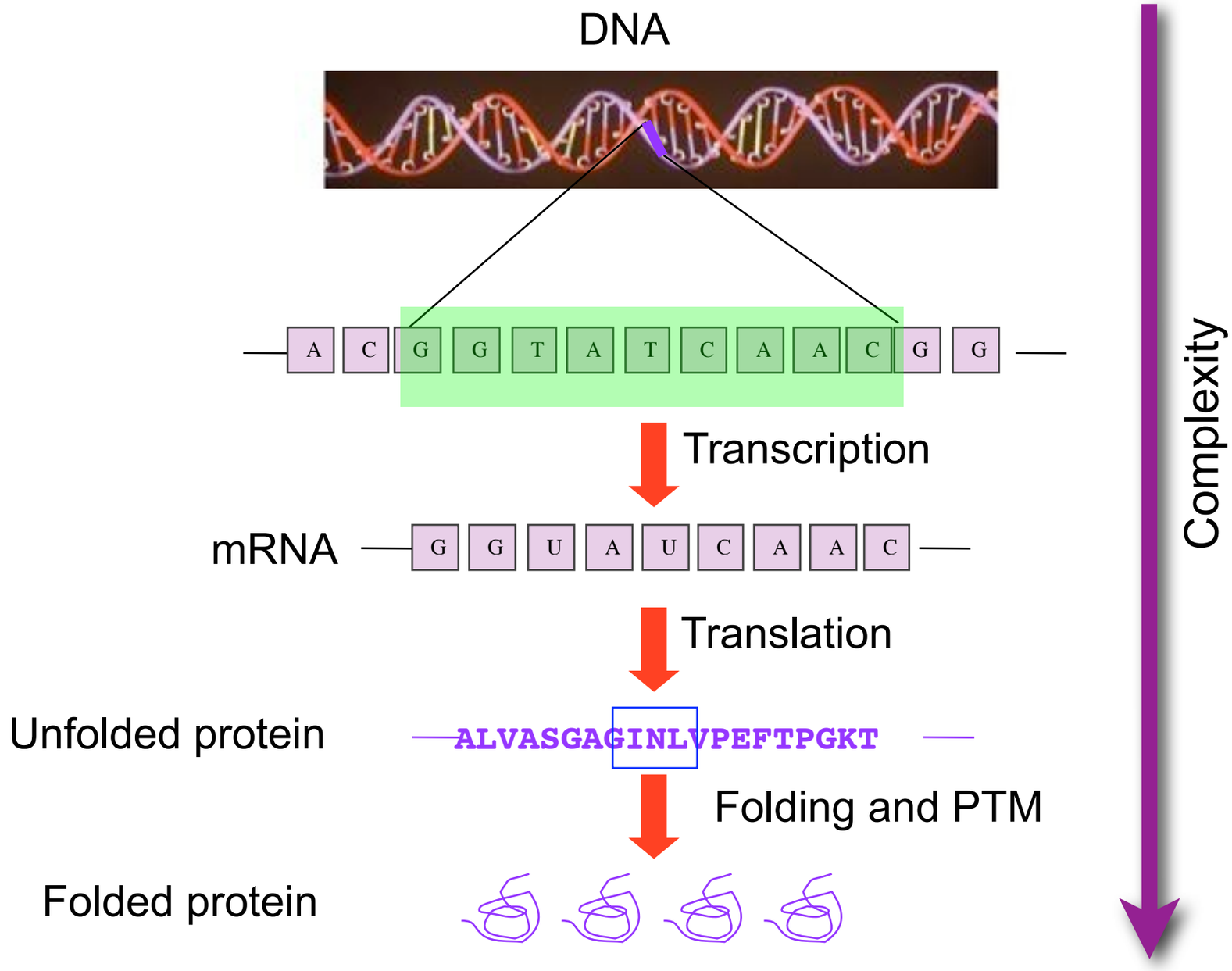
A proteomics strategy for protein expression profiling and biomarker discovery in wildlife: effects of endocrine disrupting chemicals in frog (*Xenopus laevis*)

Anders Goksøyr [1,2], Christina C Tolfsen [1], Anneli Bohne Kjersem [1], Tina Søfteland, Torbjørn Midtun [1], Ralph Urbatzka, Werner Kloas [3], & Bjørn Einar Grøsvik [1,4]

[1]Department of Molecular Biology, University of Bergen, Bergen, Norway
[2]Biosense Laboratories AS, Bergen, Norway
[3]Department of Inland Fisheries, Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany
[4]Institute of Marine Research, Bergen, Norway

Proteomics and the proteome

Proteomics = the study of all proteins expressed by the genome of a given cell or tissue of an organism



EASYRING biomarker discovery strategy

(**EASYRING - E**nvironmental **A**gent **S**usceptibilit**y** Assessment Utilizing Existing and Novel Biomarkers as **R**apid Non **In**vasive Testing Methods) - EU FP5 project associated with the CREDO cluster (2003-2005)

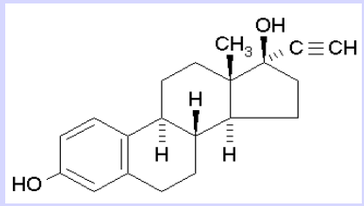
Test species

Common carp
(*Cyprinus carpio*)

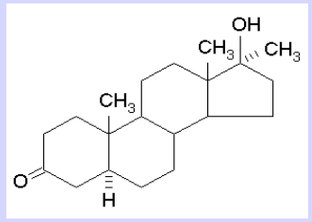
African clawed frog
(*Xenopus laevis*)

Aquaria/ cell culture exposures

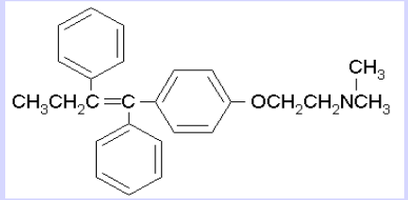
EE2



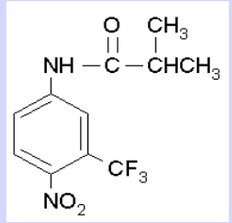
MDHT



TAM



FLU

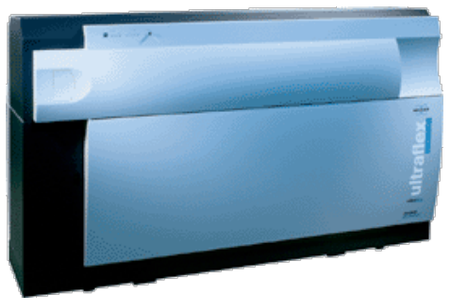


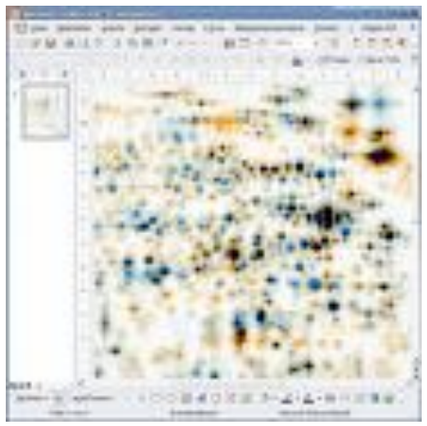
+ Lambro river water (Italy)

Sampling

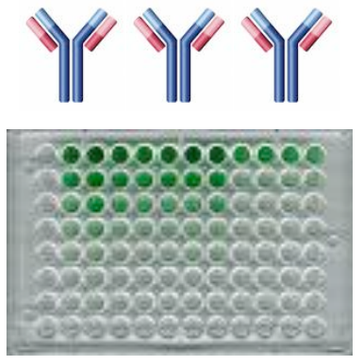
- Mucus
- Liver
- Plasma
- Hepatocytes
- MVLN cells
- Culture medium

2-DE/MS





Biomarker candidates



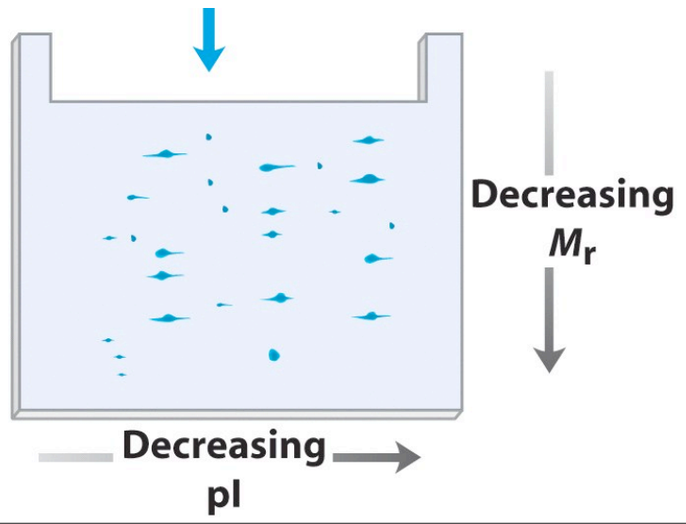
Ab production and assay development

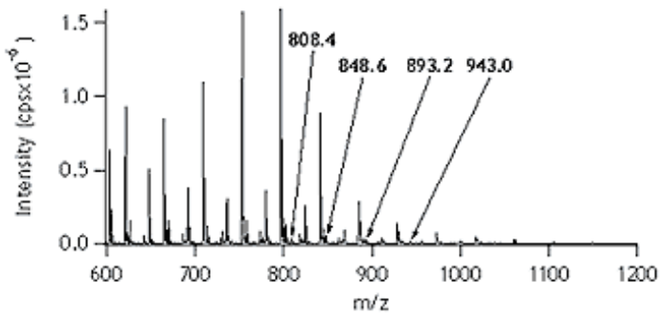
Carp and Xenopus exposures

Control	EE2	Tamoxifen	MDHT	Flutamide	Lambro R.

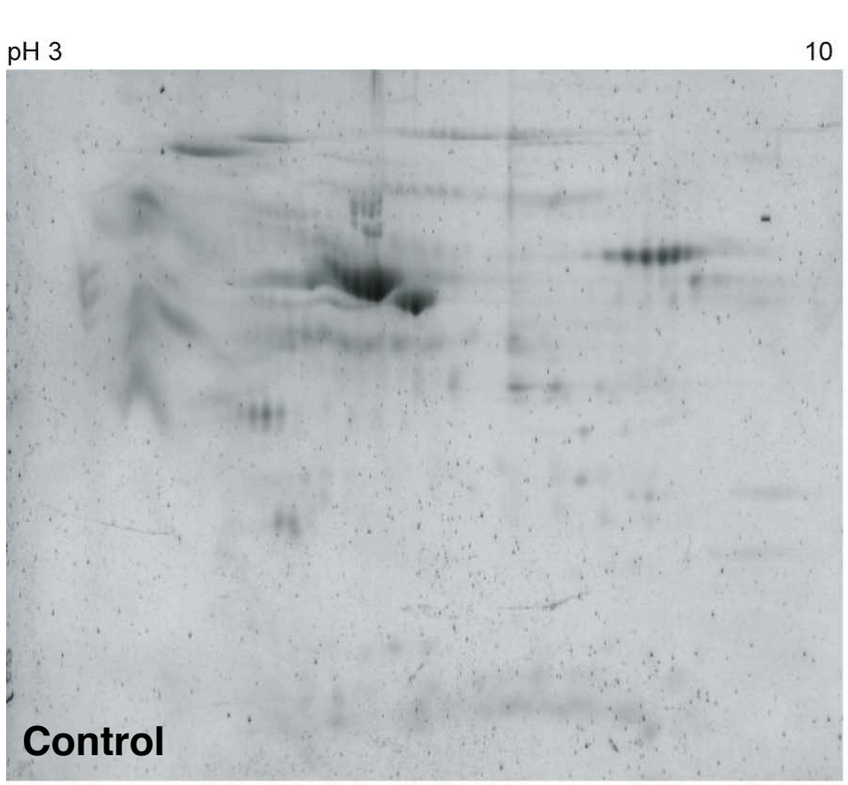
Carp: 4 doses, 2 weeks, continuous

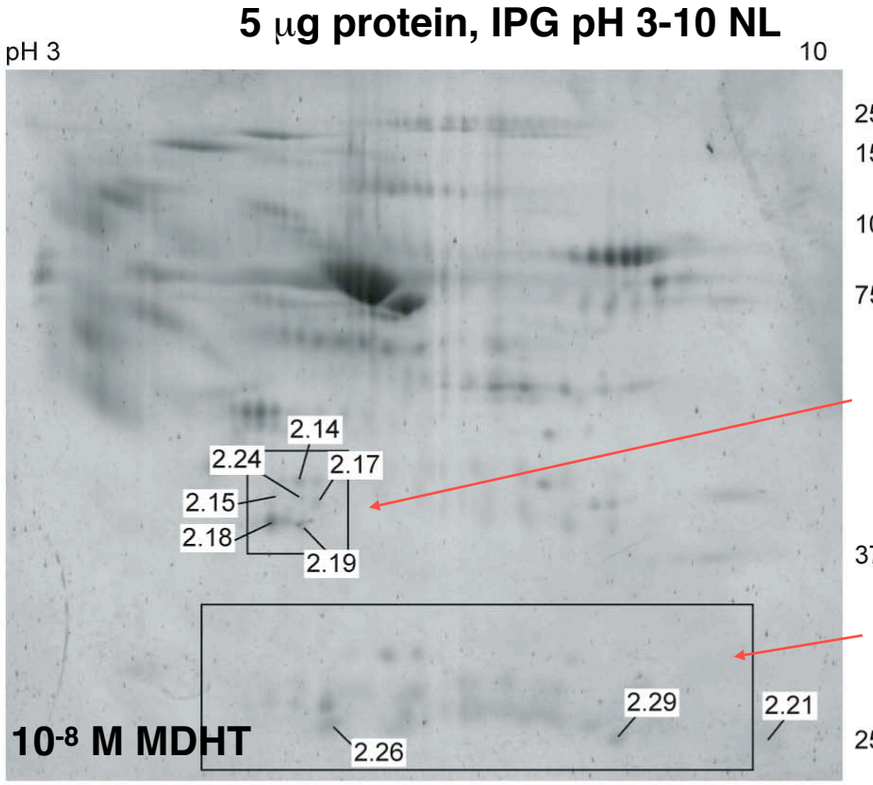
Xenopus: 1 dose (10^{-8}M), 4 weeks, semi-static





Mini 2-DE of X. laevis plasma





250 kDa
150 kDa
100 kDa
75 kDa

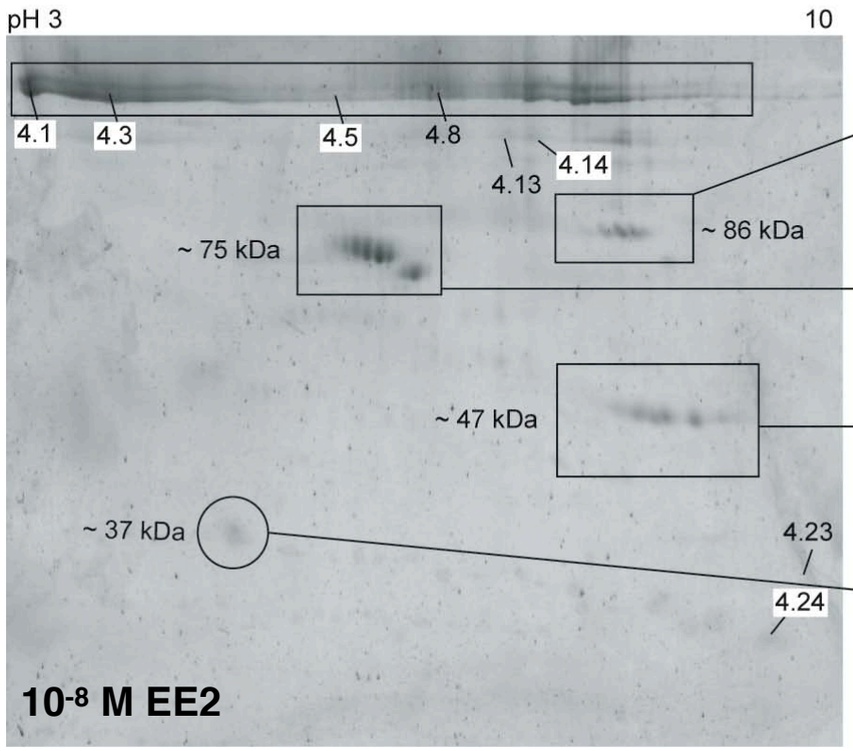
Complement C3
Actin

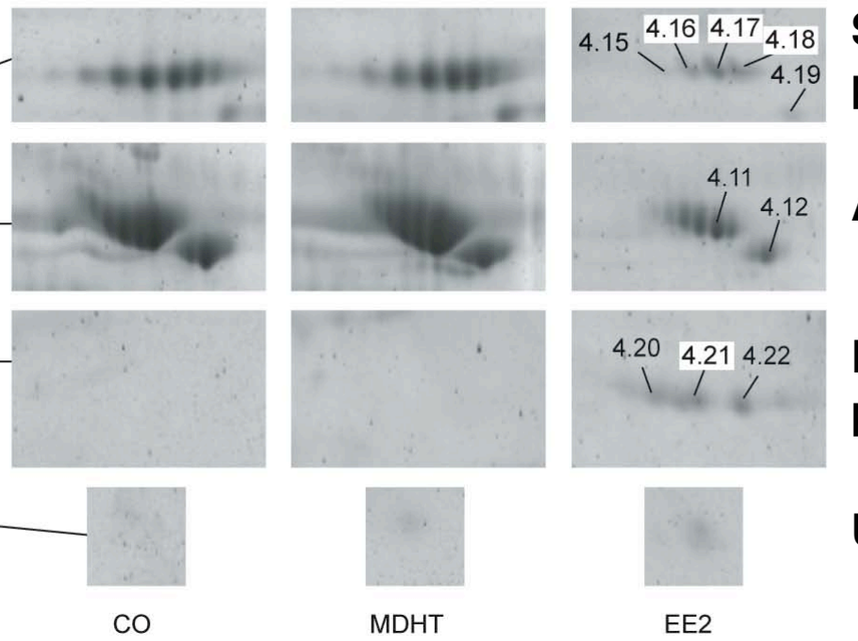
37 kDa

Immunoglobulin light chain

25 kDa

Vitellogenin





Serotransferrin precursor

Albumin

Estrogen regulated protein Ep45

Unidentified

Table 1. Proteins identified from *Xenopus laevis* liver by preparative 2-DE and MALDI-TOF MS followe

Mascot searches in the NCBInr database.

Spot #[a.]	Protein name (Family)	Accession #[a.]	Theor. Mr/pI[a.]	Scored	Expect	Seq. Cov. (%)[a.]	Queries (matched)
1	Carbamoyl-phosphate synthetase I and	ABA01549	165355/5.98	198	2.2e-16	28	100(42)
	Retinoid X receptor beta	AAH99003	50623/8.55	63	0.0067	23	100(14)
2	Carbamoyl-phosphate synthetase I	ABA01549	165355/5.98	176	3.5e-14	28	100(41)
3	Carbamoyl-phosphate synthetase I and	ABA01549	165355/5.98	289	1.8e-25	33	100(54)
	Hypothetical protein MGC78867 (Leukotriene A4 hydrolase, Peptidase family M1)*	AAH72036	69318/6.34	56	0.031	21	100(14)
6	Heat shock protein gp96 (Hsp90 family)	AAO21339	92828/4.77	144	5.6e-11	38	85(29)
8	p97 subunit of 15S Mg(2+) – ATPase	CAA38146	89760/5.16	163	7e-13	41	100(30)
11	78 kDa glucose-regulated protein precursor (Hsp70 family) and	Q91883	72705/4.96	163	7e-13	46	100(27)
	Hypothetical LOC495268 (Lactamase, beta 2)*	AAH84364	32627/6.11	57	0.029	34	100(10)
12	Unknown (protein for MGC:52648) (Hsp70 family)	AAH41200	72489/5.03	171	1.1e-13	45	100(28)
15	74 kDa serum albumin and	AAH81223	72476/5.57	157	2.8e-12	54	100(26)
	Serum albumin B precursor (74 kDa serum albumin)	P14872	72503/5.57	133	7e-10	28	51(17)
21	Serum albumin A precursor	P08759	72563/5.53	56	0.037	12	19(7)
22	Calreticulin	CAA47866	48542/4.39	74	0.0005	26	80(14)
23	Phosphoglucomutase 1	AAH43876	61904/5.81	226	3.5e-19	49	95(32)
27	Catalase	AAH54964	60620/7.66	164	5.6e-13	38	55(19)
28	Catalase	AAH54964	60620/7.66	229	1.8e-19	59	100(30)
29	Catalase	AAH54964	60620/7.66	141	1.1e-10	39	88(22)
30	Catalase	AAH54964	60620/7.66	154	5.6e-12	37	49(18)
31	Hypotetical protein MGC64309 protein (Protein disulfide isomerase)[b]	AAH54954	57060/4.72	121	1.1e-08	37	100(22)
32	P4hb protein (Protein disulfide isomerase)	AAH46736	58380/4.81	115	4.4e-08	32	86(20)
33	P4hb protein (Protein disulfide isomerase)	AAH46736	58380/4.81	190	1.4e-15	47	100(28)
34	Beta-Tubulin at 56D	AAH54297	50240/4.79	95	4e-06	36	90(18)
34	Tubulin, beta, 5	AAH74549	50152/4.78	170	1.4e-13	45	68(26)
35	Tubulin, alpha 7	AAH61260	50532/4.96	110	1.4e-07	48	73(17)
37	Keratin 8	AAH44116	56029/5.25	90	1.5e-05	35	88(18)
38	Unknown (protein for MGC:64458) (Formiminotransferase cyclodeaminase)*	AAH54248	52149/6.06	80	0.00012	39	100(16)
39	ATP synthase, H^+ transporting mitochondrial F1 complex, beta subunit	AAH46741	563957/5.25	136	3.5e-10	69	70(28)
40	67kD laminin receptor precursor	AAW62261	34208/4.81	93	6.9e-06	45	69(12)
41	Vimentin 4	CAA34742	53521/5.08	93	7.5e-09	30	67(17)
42	Magho-nashi homolog and	AAH53764	17124/5.95	62	0.0087	46	99(9)
	Similar to aldehyde dehydrogenase 9 family, member A1	AAH44080	56811/6.58	57	0.027	27	100(15)
43	Similar to aldehyde dehydrogenase 9 family, member A1	AAH44080	56811/6.58	109	1.8e-07	31	60(17)
44	Similar to aldehyde dehydrogenase 9 family, member A1	AAH44080	56811/6.58	133	7e-10	33	71(20)
45	Similar to aldehyde dehydrogenase 9 family, member A1	AAH44080	56811/6.58	58	0.02	26	93(14)
46	Aldehyde dehydrogenase class 1	BAA76412	55750/7.51	138	2.2e-10	39	100(26)
49	ARP3 actin-related protein 3 homolog (yeast)	AAH47983	47716/5.67	66	0.0037	29	71(12)
5	Enolase 1, alpha	AAH54169	47817/6.17	178	2.2e-14	51	71(22)
52	Alpha-enolase	CAA68706	47930/5.92	104	5.6e-07	37	66(16)
54	S-adenosyl-L-homocysteine hydrolase	CAA07706	48170/5.98	125	4.4e-09	34	45(14)
55	Adenosylhomocysteinase	AAH73400	48172/6.04	181	1.1e-14	42	78(22)
56	Similar to aldehyde dehydrogenase 9 family, member A1	AAH44080	56811/6.58	67	0.0026	27	89(15)
57	MGC80785 protein (Aldehyde dehydrogenase 2 family)*	AAH77908	57727/7.55	68	0.0022	25	71(12)

59	MGC80785 protein (Aldehyde dehydrogenase 2 family)*	AAH77908	57727/7.55	210	1.4e-17	45	47(21)
60	MGC80785 protein (Aldehyde dehydrogenase 2 family)*	AAH77908	57727/7.55	192	8.8e-16	42	68(23)
62	Actin, cytoplasmic type 5	P53505	42165/5.30	164	5.8e-13	51	100(27)
64	Aminoacylase 1	AAH77639	46403/5.43	74	0.0006	35	95(13)
68	GDP dissociation inhibitor 2	AAH78017	50967/5.44	91	1.2e-05	50	100(17)
66	Hypotetical LOC494713 (Isocitrate dehydrogenase 1)*	AAH82651	47219/5.77	150	1.4e-11	53	81(21)
67	Hypothetical LOC494713 (Isocitrate dehydrogenase)	AAH82651	47219/5.77	161	1.1e-12	54	74(23)
69	Mixture of Arginosuccinate synthase and	AAH46941	47119/7.57	142	8.9e-11	-	36(18)
	Elongation factor 1 gamma	CAA44367	50101/7.55	-	-	-	-
70	Arginosuccinate synthetase 1	AAH46941	47119/7.57	145	4.4e-11	49	94(23)
78	Senescence marker protein-30	BAA93719	33451/5.18	166	3.5e-13	68	100(24)
79	Mixture of Fructose-1,6-bisphosphatase and	AAH53784	37185/5.78	233	7e-20	-	100(36)
	Phosphotriesterase related	AAH56120	39126/5.68	-	-	-	-
81	MGC82058 protein (Ezrin/radixin/moesin family)[b]	AAH79712	69131/5.79	62	0.0081	25	61(16)
82	Arginase	AAH43635	35455/6.21	117	2.8e-08	45	90(17)
83	Fructose-1,6-bisphosphatase	AAH53784	37185/5.78	67	0.0028	30	100(15)
84	Hypothetical protein MGC68644 (3-hydroxyanthranilate 3,4-dioxygenase)*[b]	AAH61663	32714/5.54	79	0.00017	48	91(15)
85	Hypotetical protein MGC68644 (3-hydroxyanthranilate 3,4-dioxygenase)*	AAH61663	32714/5.54	99	1.7e-06	53	96(18)
340	Glycerol kinase	AAH56091	62594/5.93	105	4.4e-07	32	60(15)
86	Hypotetical protein MGC53995 (RNA pol II accessory factor, Cdc73 family) and	AAH43965	60701/9.68	59	0.018	24	94(16)
	Hypotetical protein MGC83218 (Ribose 5-phosphate isomerase)*[b]	AAH68951	25890/6.11	56	0.034	39	94(10)
87	L-lactate dehydrogenase B chain	P42119	36677/6.25	126	3.5e-09	47	68(16)
88	L-lactate dehydrogenase B chain	P42119	36677/6.25	140	1.4e-10	49	77(17)
89	L-lactate dehydrogenase B chain	P42119	36677/6.25	110	3.8e-05	35	47(12)
90	Lactate dehydrogenase A and	AAH45015	36734/8.17	191	1.1e-15	46	46(18)
	Unknown (protein for MGC:115135) (Myelin basic protein)	AAH92318	19767/10.96	58	0.024	42	100(11)
91	Lactate dehydrogenase A	AAH45015	36734/8.17	75	0.00044	36	100(14)
104	Unknown (protein for IMAGE:4681515) (Tetratricopeptide repeat domain)	AAH94440	42747/5.41	58	0.025	34	96(13)
109	Phosphoglucomutase 1	AAH43876	61904/5.81	113	7e-08	30	47(16)
111	Heat shock protein gp96 (Hsp90 family)	AAO21339	92828/4.77	90	1.5e-05	30	81(21)
114	Glucose regulated protein, 58 kDa (Protein disulfide isomerase)*	AAH46707	56486/5.72	140	1.4e-10	41	100(24)
127	Aminoacylase 1 [b] and	AAH77639	46403/5.43	74	0.00057	36	1000(14)
	Translation initiation factor IF4A II	AAH45237	46057/5.32	85	4.4e-05	37	87(15)
137	Unknown (protein for IMAGE:5513341) (Arginyl-tRNA synthetase)[b]	AAH97633	74691/6.31	186	3.5e-15	40	63(28)
149	Fumarylacetoacetate hydrolase	AAH54283	46978/6.22	63	0.0075	36	87(14)
150	Glutamate dehydrogenase 1[b]	AAH77910	59928/8.03	64	0.0061	31	100(18)
155	MGC83638 protein (Phosphoethanolamine N-methyltransferase)* or	AAH78119	57278/5.25	59	0.019	22	66(10)
	Mixture of MGC79068 protein (Protein disulfide isomerase, A, P5 subfamily) and	AAH77228	48153/5.11	186	3.5e-15	-	97(34)
	Keratin 18[b]	AAH54993	48031/5.20	-	-	-	-
156	Keratin 8[b] and	AAH44116	56029/5.25	71	0.0012	32	55(12)
	Protein disulfide isomerase-related protein (Thioredoxin domain)	AAH46867	48228/5.05	125	4.4e-09	30	37(13)
157	MGC79068 protein (Protein disulfide isomerase)	AAH77288	48153/5.11	149	1.8e-11	38	33(14)
	Fibrinogen gamma chain precursor	P17634	50659/5.43	55	0.043	24	87(11)
158	LOC495086 protein (ATP citrate lyase)*[b]	AAH84253	120830/6.59	64	0.0058	11	61(16)
159	Similar to ubiquinol-cytochrome c reductase core protein 1	AAH49288	52826/5.83	65	0.0049	37	53(18)
161	An2 (F0F1-type ATP synthase, alpha subunit)[b]	AAH80064	59965/9.13	135	4.4e-10	35	45(19)
162	Unknown (protein for MGC:52648) (Hsp70 family)	AAH41200	72489/5.03	120	1.4e-08	36	96(22)
163	Unknown (protein for MGC:52648) (Hsp70 family)	AAH41200	72489/5.03	135	4.4e-10	40	86(22)
166	Glycyl-tRNA synthetase	AAH77232	84957/6.98	92	9.9e-06	31	95(21)
167	Transketolase	AAH56101	68378/6.27	80	0.00016	30	75(14)

168	Hypothetical LOC495275 (Betaine-homocysteine methyl S-transferanse)*	AAH84414	44723/6.40	86	3.6e-05	35	61(12)
169	Expoxide hydrolase 2, cytoplasmic	AAH78066	62949/5.98	203	7e-17	53	87(30)
170	MGC81570 protein (Argininosuccinate lyase)*	AAH81012	49780/8.09	69	0.0018	32	64(13)
172	Hypothetical protein MGC64592 (Phosphorylase family 2) *	AAH54317	27824/8.09	57	0.03	38	83(10)
173	Hypothetical protein MGC64592 (Phosphorylase family 2)	AAH54317	27824/8.09	65	0.0044	44	98(13)
176	Unknown (protein for IMAGE:4173753) (Carboxylesterase)*	AAI08856	65027/4.98	25	0.00063	25	66(14)
180	Zinc finger, CW- type with coiled-coil domain 3 (Histidine kinase-like ATPases) and	AAH77542	103075/5.71	62	0.0081	20	79(16)
	Actin, cytoplasmic type 8	P53506	42163/5.31	58	0.24	27	79(10
183	Arginosuccinate synthetase 1	AAH46941	47119/7.57	92	8.6e-06	36	91(16)
184	Aldehyde dehydrogenase class 1	AAH76716	55556/7.03	144	5.6e-11	43	86(24
185	Aldehyde dehydrogenase class 1	AAH76716	55556/7.03	80	0.00016	33	100(19)
187	Carbamoyl-phosphate synthetase I	ABA01549	165355/5.98	93	7.2e-06	19	93(26)
189	Carbamoyl-phosphate synthetase I	ABA01549	165355/5.98	89	1.6e-05	21	100(26)
190	Hypothetical LOC495275 (Betaine-homocysteine S-methyltransferase)*[b]	AAH84414	44723/6.40	56	0.039	33	69(10)
191	Phosphoglycerate kinase 2	AAH77781	44953/6.62	120	1.4e-08	53	87(19)
194	Hypotetical protein LOC594868 (COMM domain containing 4)*[b]	AAH77441	22417/7.60	57	0.029	35	87(10)
195	Carbamoyl-phosphate synthetase I	ABA01549	165355/5.98	107	2.8e-07	21	100(32)
206	LOC443721 protein (Cathepsin D)*[b]	AAH75134	43750/5.50	57	0.026	27	69(10)
224	Catalase	AAH54964	60620/7.66	105	4.4e-07	35	78(19)
225	Glucose regulated protein, 58 kDa (Protein disulfide isomerase)*	AAH46707	56486/5.72	59	0.016	19	52(11)
230	Mixture of UDP-glucose pyrophosphorylase 2 and	AAH77213	55392/6.88	94	5.6e-06	33	95(17)
	Aldehyde dehydrogenase class 1	AAH76716	55556/7.03	88	2.4e-05	33	95(19)
231	Unknown (protein for IMAGE:7393486) (Glutaminyl-tRNA synthetase)*[b]	AAI10785	88198/6.95	68	0.0021	25	100(20)
252	Unknown (protein for MGC:52648) (Hsp70 family)	AAH41200	72489/5.03	190	1.4e-15	44	100(29)
253	Unknown (protein for MGC:52648) (Hsp70 family)	AAH41200	72489/5.03	115	4.4e-08	32	75(18)
254	Hypothetical protein MGC68448 (Molecular chaperone, Hsp90 family)	AAH60352	93242/4.73	65	0.0043	26	99(21)
255	Hsp90beta (Hsp90 family)	AAV41061	83304/4.97	163	7e-13	36	63(26)
260	Catalase	AAH54964	60620/7.66	149	1.8e-11	48	89(22)
261	Catalase	AAH54964	60620/7.66	177	2.8e-14	52	100(27)
263	Unknown (protein for MGC:52655) (Hsp70 family)	AAH41201	71372/5.43	169	1.8e-13	50	97(27)
268	Hypotetical LOC495278 (Chaperonin GroEL (Hsp60 family))	AAH84429	52377/6.20	118	2.2e-08	40	68(17)
269	Seryl-aminoacyl-tRNA synthetase 1	AAH43975	58823/5.71	63	0.0072	26	77(13)
270	Aconitase	AAH68910	86241/6.65	146	3.5e-11	37	73(22)
271	Enolase 1, alpha	AAH54169	47817/6.17	201	1.1e-16	57	97(27)
275	Similar to enolase 1, alpha non-neuron	AAH41279	47888/5.61	145	4.4e-11	50	100(21)
277	Thioredoxin domain containing 5 (Protein disulfide isomerase)	AAH45245	46658/5.82	68	0.0024	26	40(9)
278	Hypothetical LOC495702 (Vitellogenin)*	AAI06663	38289/5.78	123	7.e-09	46	70(17)
279	Acetyl-CoA acetyltransferase 2	AAH56089	41802/6.62	165	4.4e-13	60	100(26)
281	Mixture of Hypothetical protein LOC398623 (Fructose-1,6-bisphosphate aldolase) and	AAH84132	39940/8.67	135	4.4e-10	-	96(25)
	Glycine C-acetyltransferase	AAH47258	45837/7.59	-	-	-	-
286	MGC83669 protein (Inorganic pyrophosphatase)	AAH73722	33282/5.34	153	7e-12	66	100(20)
287	Acidic ribosomal protein P0	AAH42268	34247/5.41	97	2.9e-06	46	88(15)
288	Fructose-1,6-bisphosphatase	AAH53784	37185/5.78	97	2.9e-06	44	99(17)
290	Hypothetical LOC495702 (Vitellogenin)*[b]	AAH85204	34072/5.26	73	0.00066	47	93(13)
292	Hypothetical LOC495702 (Vitellogenin)*	AAH85204	34072/5.26	96	3.7e-06	52	87(17)
296	Mixture of MGC81885 protein (Hydroxysteroid (17-beta) dehydrogenase 4)* and	AAH74145	80749/7.62	183	7e-15	-	72(31)
	Annexin 4 or	AAH60389	36234/6.40	-	-	-	-
	Hypothetical LOC495275 (Betaine-homocysteine methyl S-transferase)* and	AAH84414	44723/6.40	87	3.1e-05	41	84(14)
	MGC84363 protein (Esterase)	AAH81180	32035/5.98	57	0.03	28	84(9)
299	Hypothetical protein LOC414512 (NmrA-like family)	AAH68857	32730/8.48	64	0.005	41	83(11)

300	Guanine nucleotide binding protein, beta 2, related sequence 1 (G-protein)	AAH41541	35503/7.60	201	1.1e-16	62	42(18)
333	MGC82032 protein (Adenosine kinase)	AAH75155	40854/5.63	57	0.027	29	89(11)
338	Adenosylhomocysteinase and	AAH73400	48712/6.04	88	2.4e-05	33	84(15)
	MGC81895 protein (Methylcrotonoyl-Coenzyme A carboxylase 1 (alpha))*,b	AAH74151	79961/6.16	60	0.015	22	84(16)

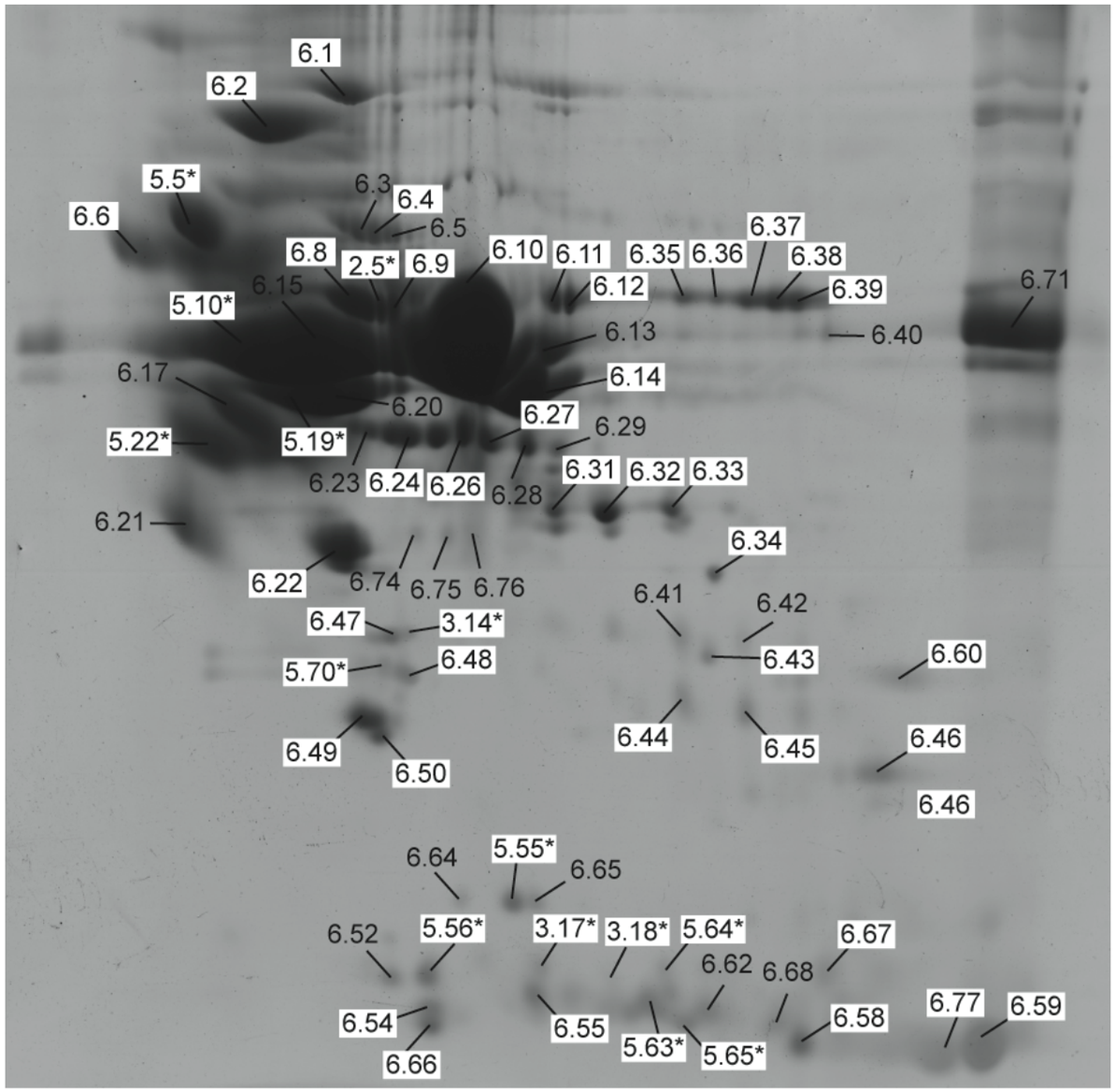
a) Abbreviations used: #, number; Theor., theoretical; Seq. Cov (%), percent sequence coverage.

b) Protein identified with a peptide tolerance of 150 ppm. Unless otherwise stated all other proteins were identified using a peptide tolerance of 100 ppm.

c) *, Information on protein name/family was obtained by homology searches using Blastp.

d) Protein scores greater than 54 were significant ($p<0.05$).

Proteins identified in X. laevis plasma



Control, pooled male plasma, 500 ug, CBB-G250

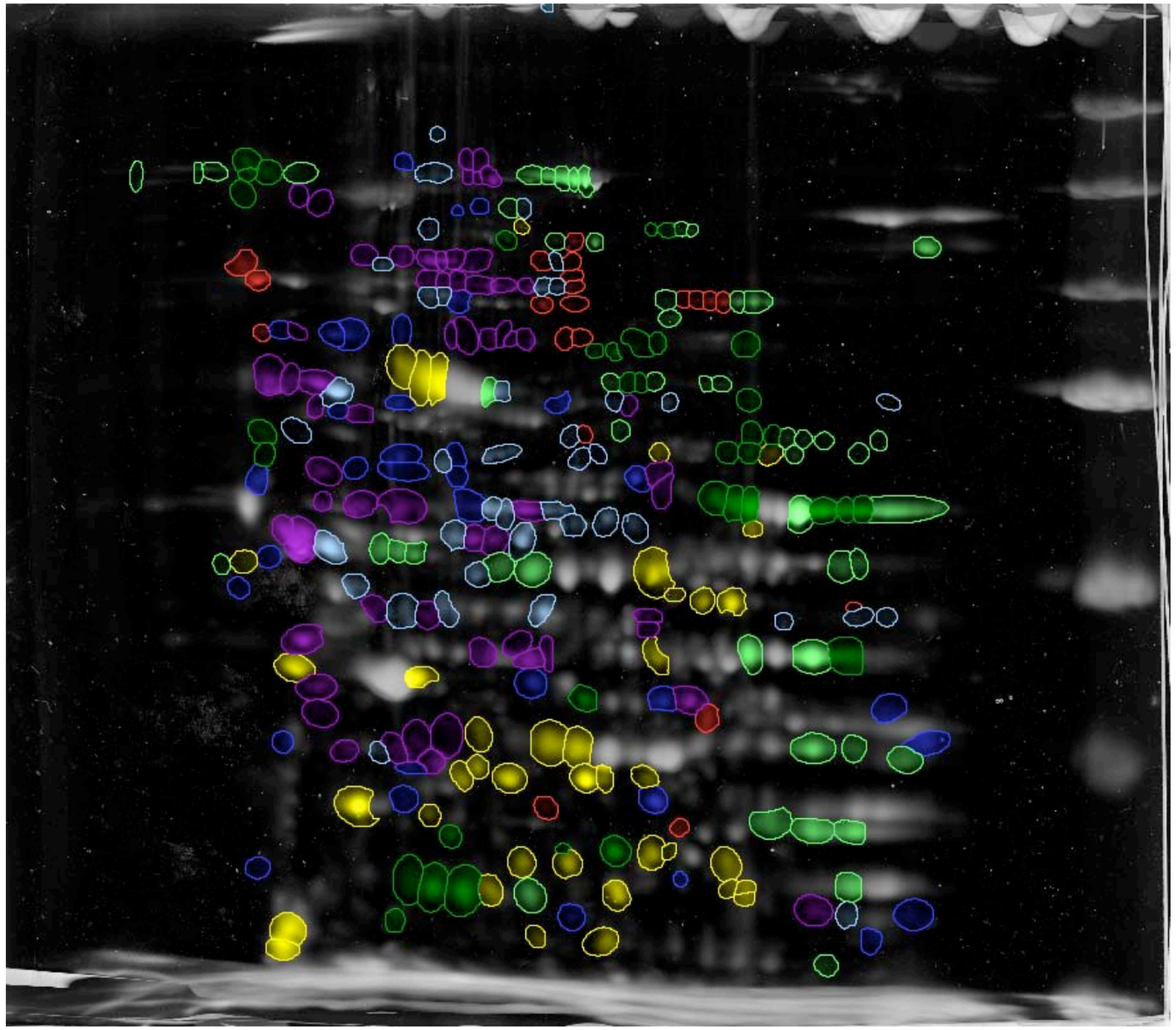
Differentially regulated proteins

- **123 identified proteins**
- **Constitutive + differentially regulated**
- **19 protein families**

- **Albumin**
- **Endodermin**
- **Alpha-2-macroglobulin**
- **Apolipoprotein A1**
- **Alpha-1-antitrypsin**
- **Complement C3/ C4**
- **Enolase 3**
- **Creatine kinase**
- **Estrogen regulated protein Ep45 precursor**
- **Immunoglobulin heavy and light chain**
- **Fibrinogen (alpha, beta, gamma)**
- **Glyceraldehyde-3-phosphate dehydrogenase**
- **Fructose-1,6-bisphosphate aldolase**
- **Actin**
- **Serotransferrin precursor**
- **Triosephosphate isomerase**
- **Vimentin**
- **Vitellogenin B1**
- **Ficolin-1**
- **Fetuin**
- **Hypotetical proteins**

Differential expression in X. laevis liver

Male, 10^{-8} M EE2, pooled/individual, ≥ 2-fold regulation



↑, EE2 pooled and individual

↑, EE2 individual only

↑, EE2 pooled only

↑↓, Contradictory results EE2 pooled/ individual

↓, EE2 pooled and individual

↓, EE2 individual only

↓, EE2 pooled only

Complex pattern of expression

430 proteins differentially regulated by EDC treatment

106 differentially regulated spots represented identified proteins

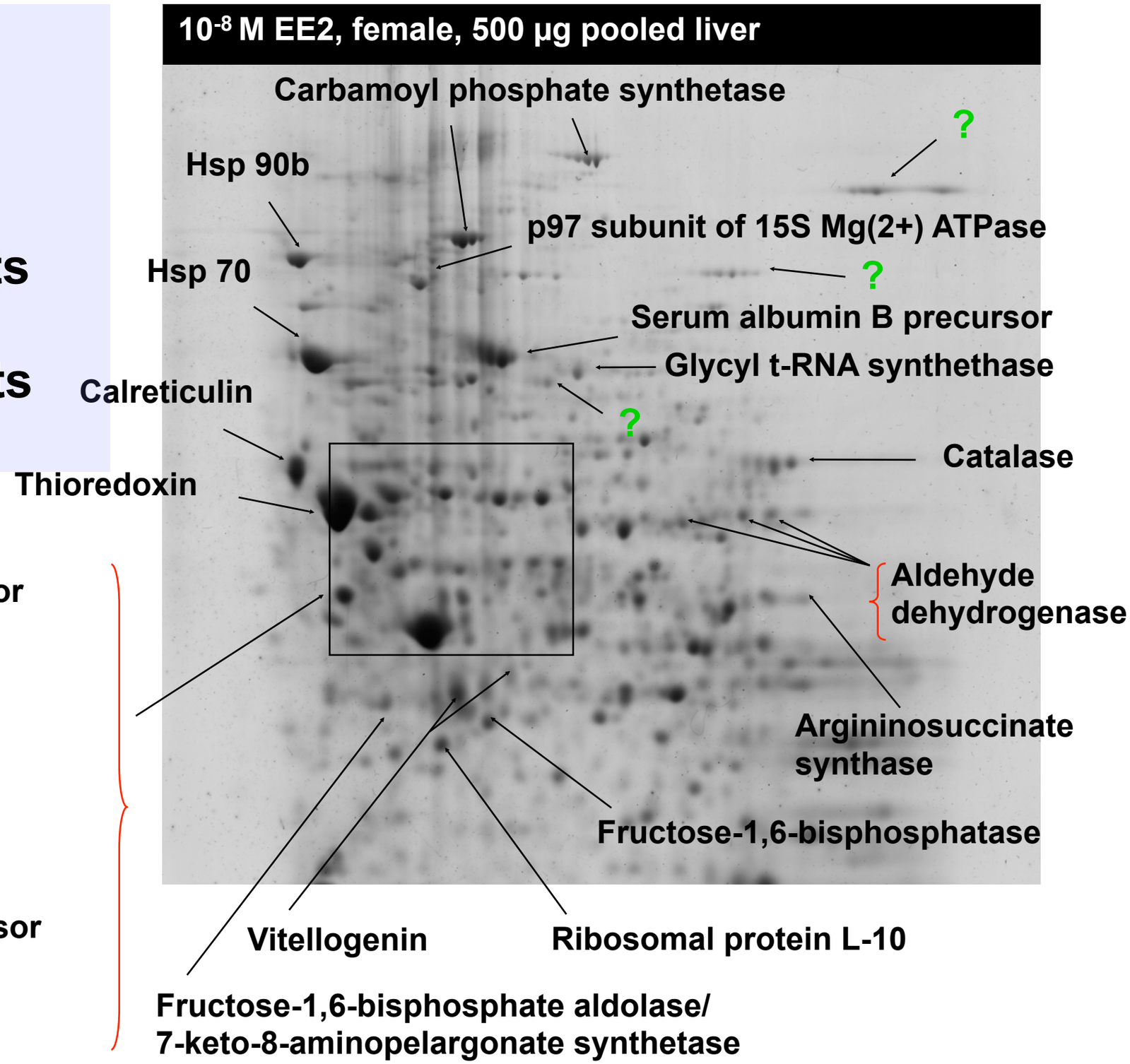
Proteins identified in X. laevis liver

241 analyzed proteins

196 identities

131 unique protein spots

17 % of the protein spots contained > 1 protein



67 kDa laminin receptor precursor
ATP synthase alpha/ beta
Tubulin, beta 5 and 7 alpha
Keratin
Thioredoxin
Catalase
DEAD box helicase
GDP dissociation factor
Glutaminyl t-RNA synthetase
Fibrinogen gamma chain precursor
Insulinase

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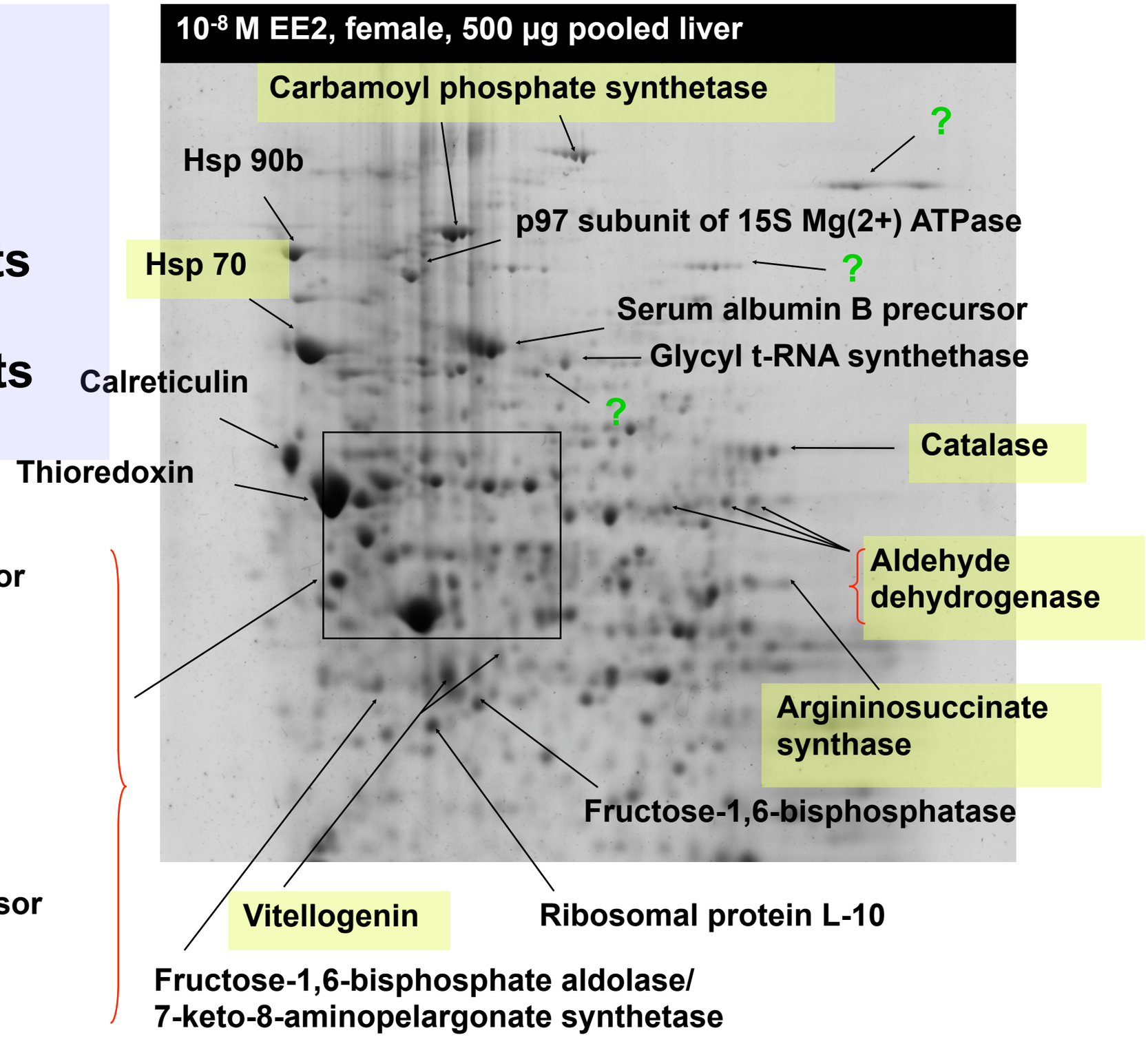

67 kDa laminin receptor precursor
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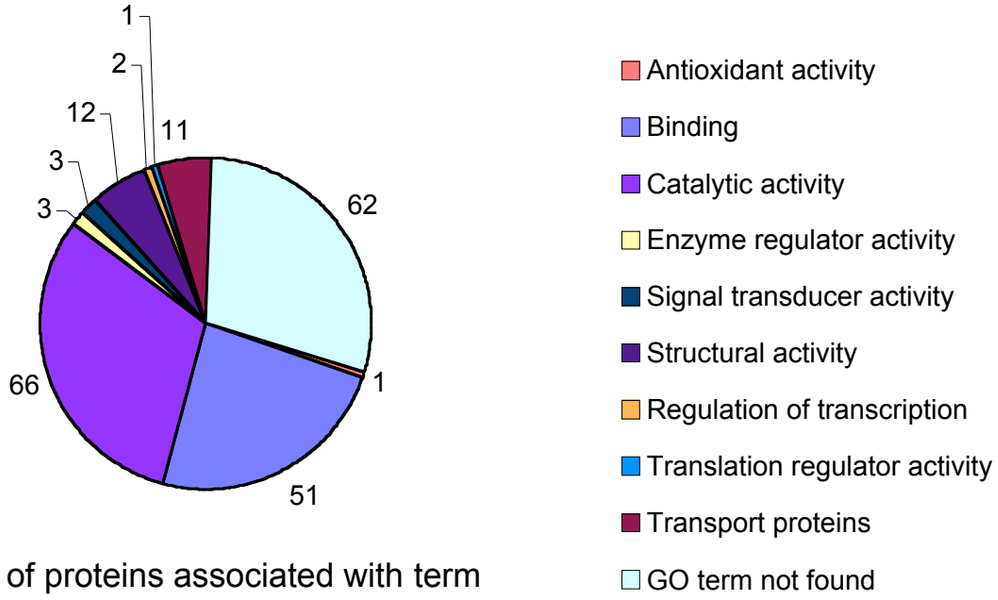
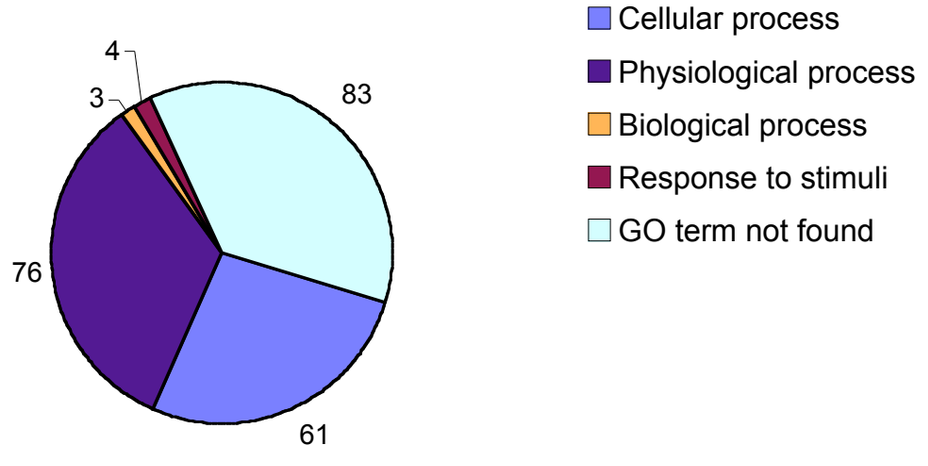
Keratin
Thioredoxin
Catalase
DEAD box helicase
GDP dissociation factor
Glutaminyl t-RNA synthetase
Fibrinogen gamma chain precursor
Insulinase

Gene Ontology terms for X. laevis liver proteins

Molecular function; 97 identified, 62 unknown

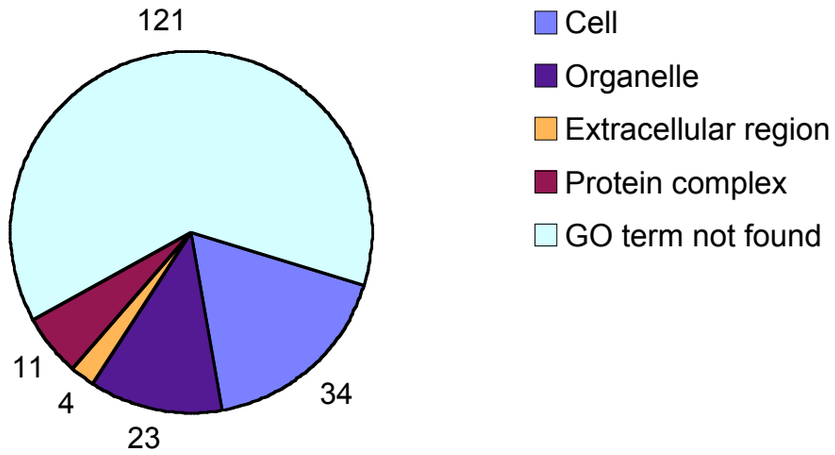


Biological process; 76 identified, 83 unknown



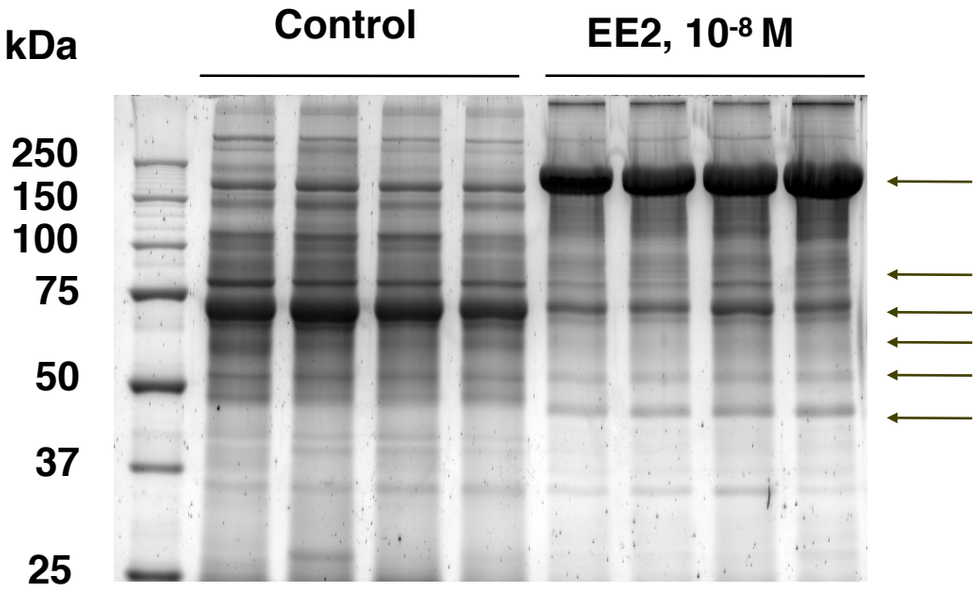
Gene ontology (GO), is a controlled vocabulary used to describe molecular functions, biological processes and the location of gene products

Cellular component; 38 identified, 121 unknown

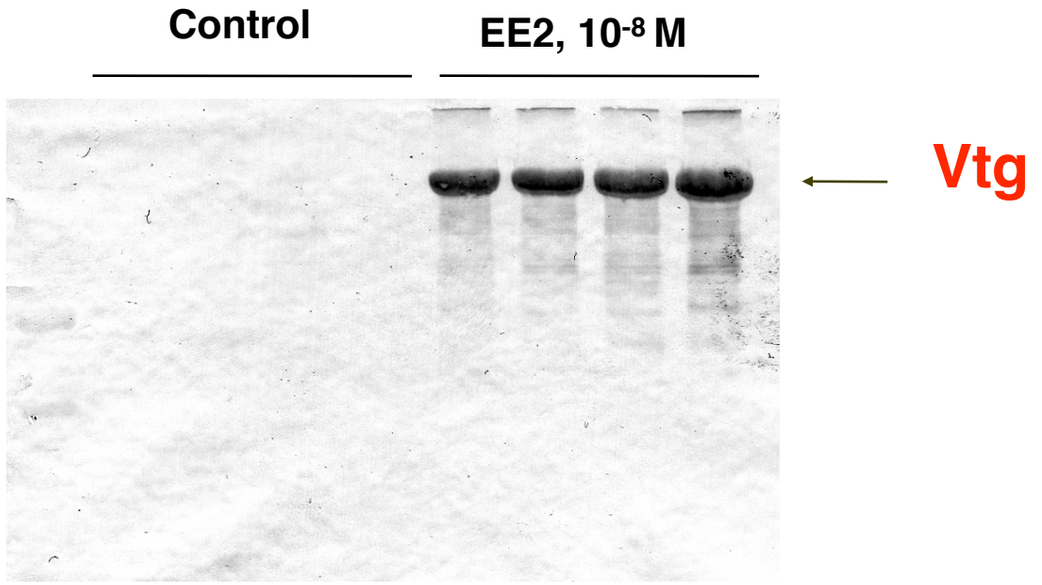


1-D SDS-PAGE and Vtg-western blot of plasma

Female

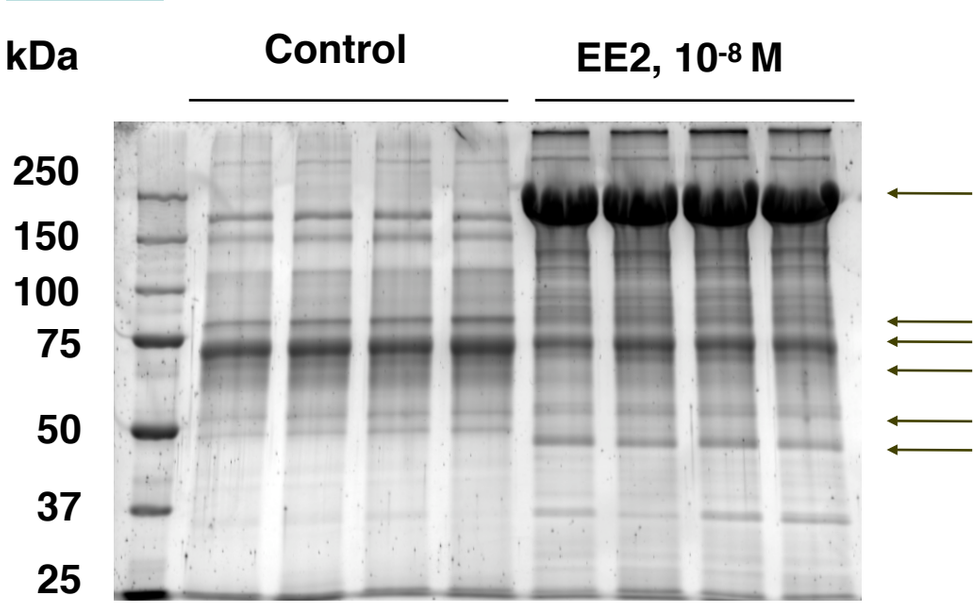


7.5 % PA

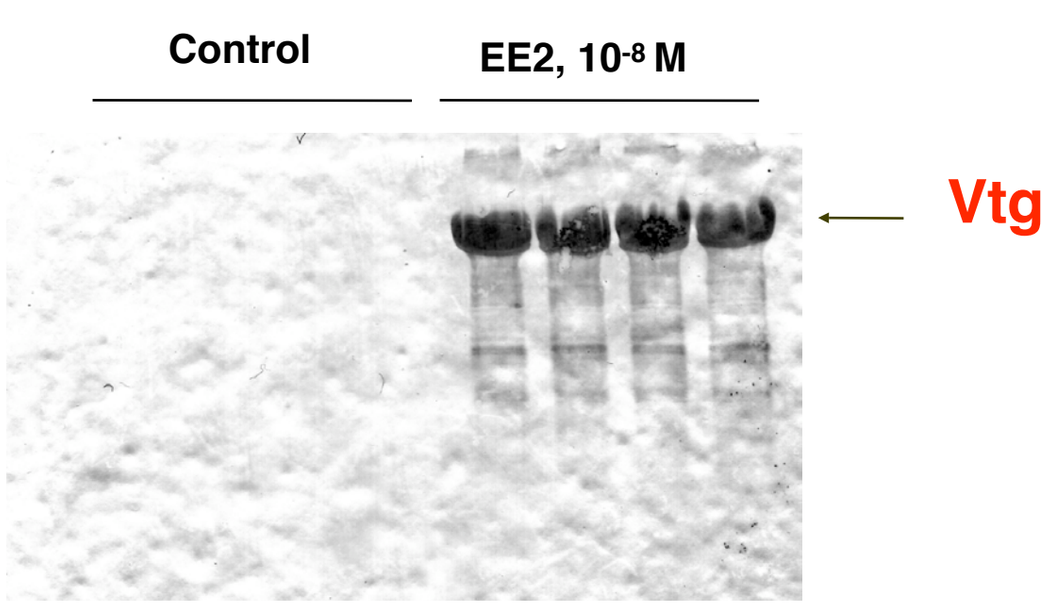


Anti-xenopus Vtg 1:2000

Male



7.5 % PA

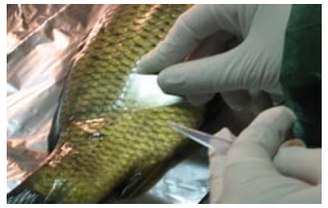


Anti-xenopus Vtg 1:2000

Development of dipstick for endocrine disruption monitoring - non-disruptive sampling of fish mucus

easyring

biosense LABORATORIES

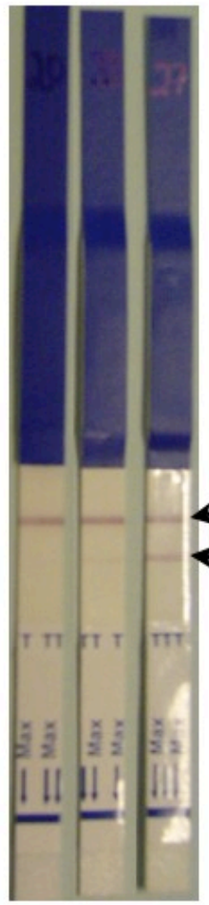


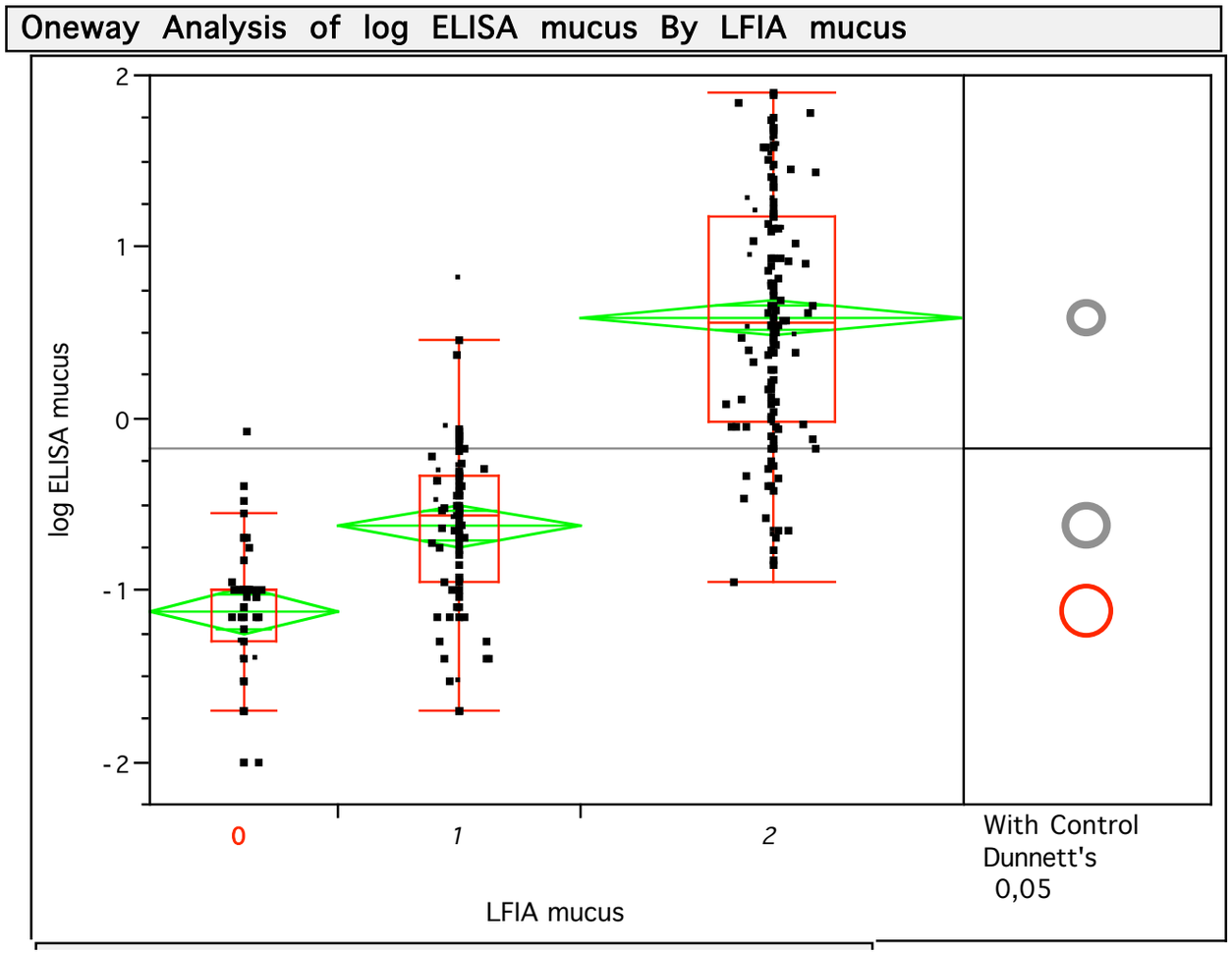
Detection of Vtg in carp mucus

Development and testing of Vtg LFIA

5 min

Dip-stick in mucus/
PBS extract



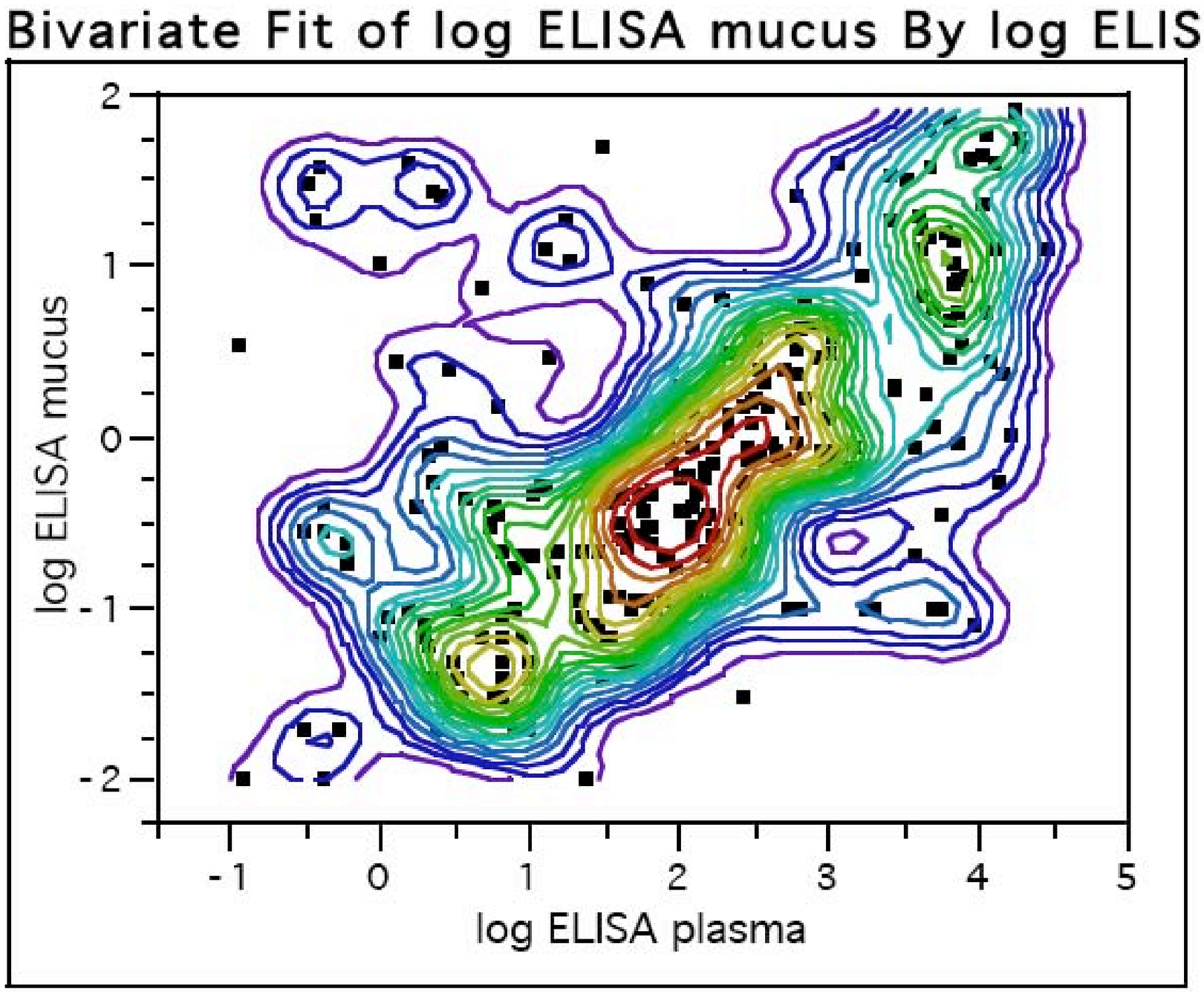


LFIA = Lateral Flow Immunoassay

Rapid non-invasive testing method for detection of Vtg in carp mucus.

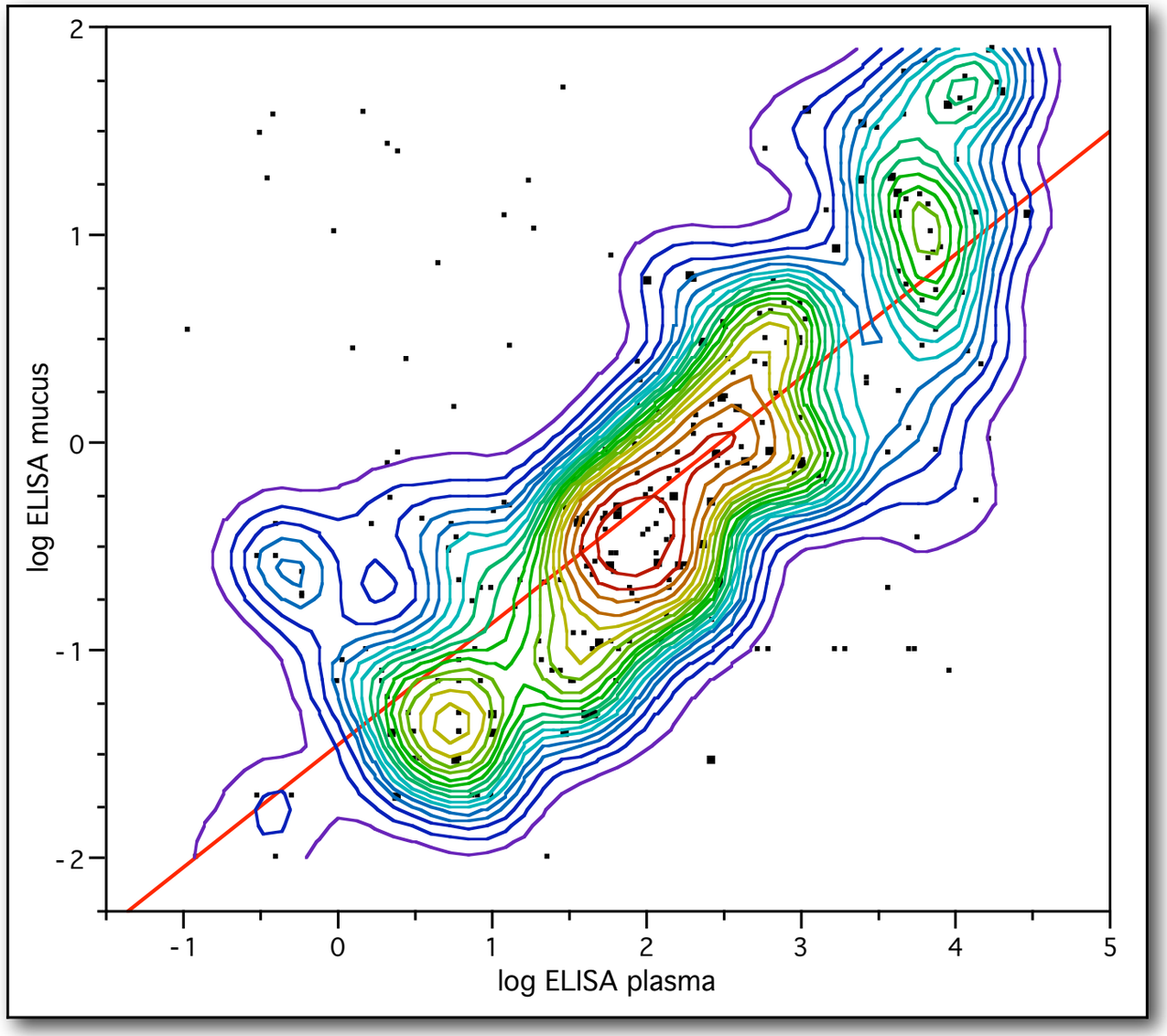
biosense LABORATORIES

Vtg in plasma vs. mucus

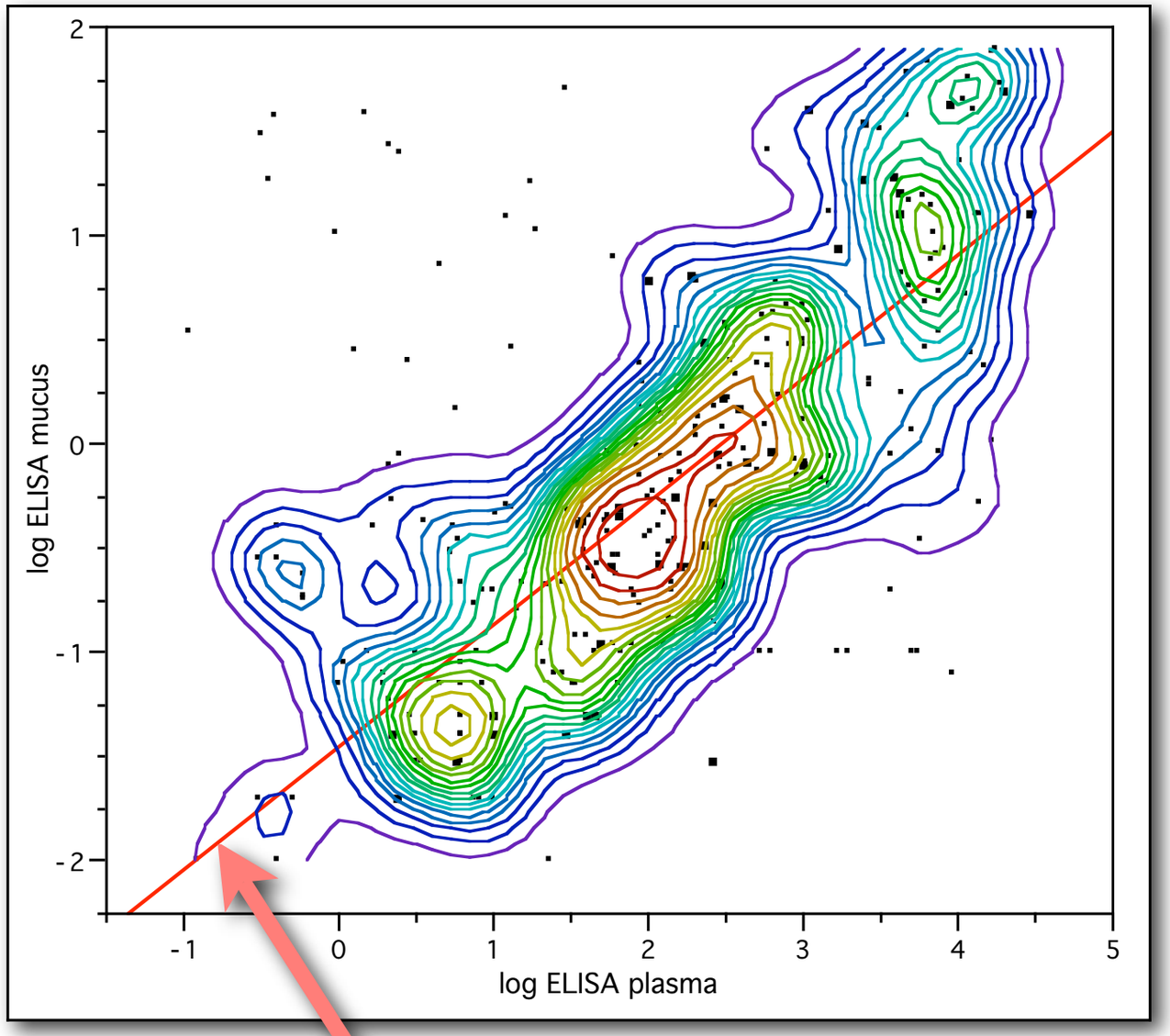


Vtg in plasma vs. mucus

Vtg in plasma vs. mucus

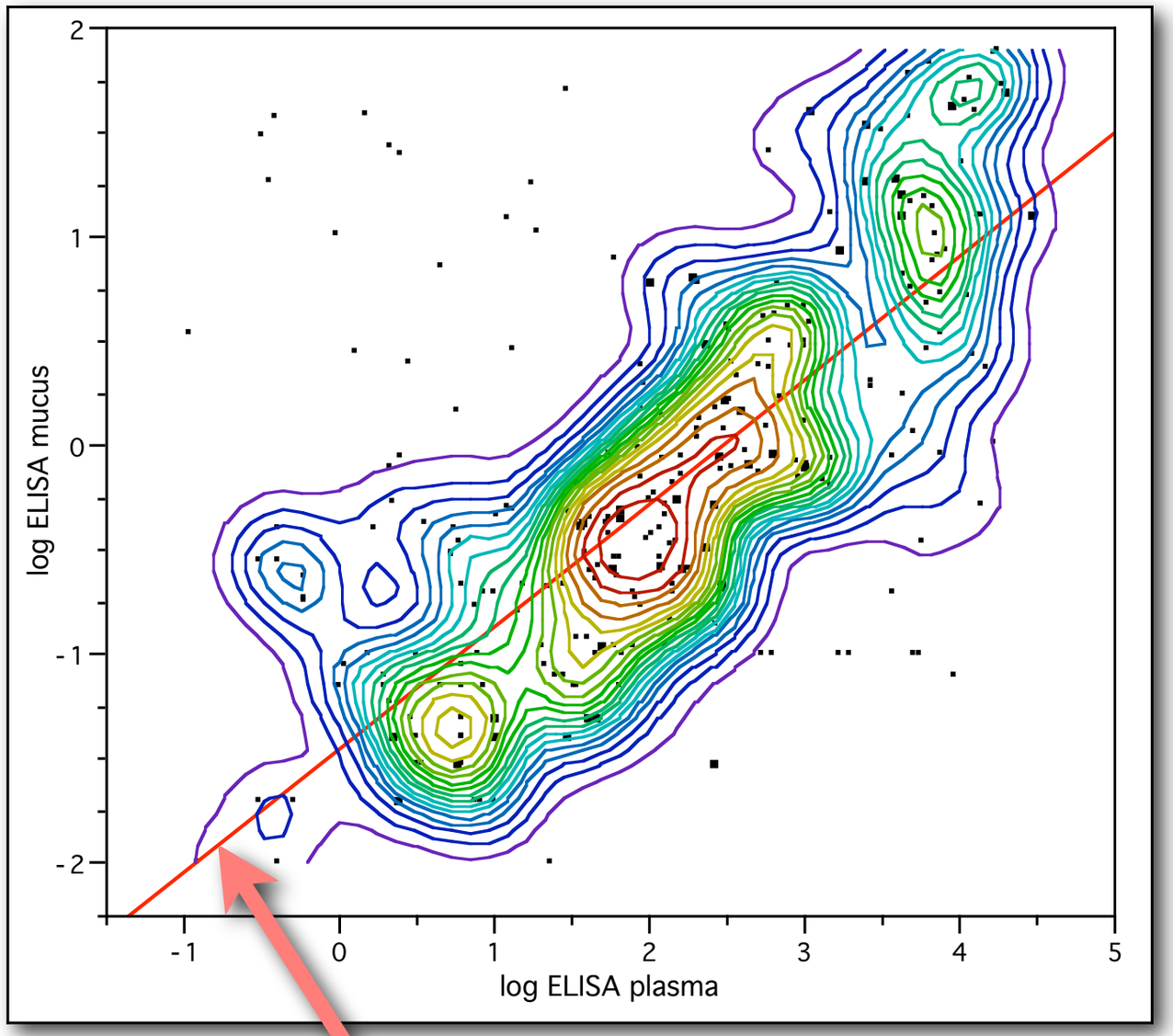


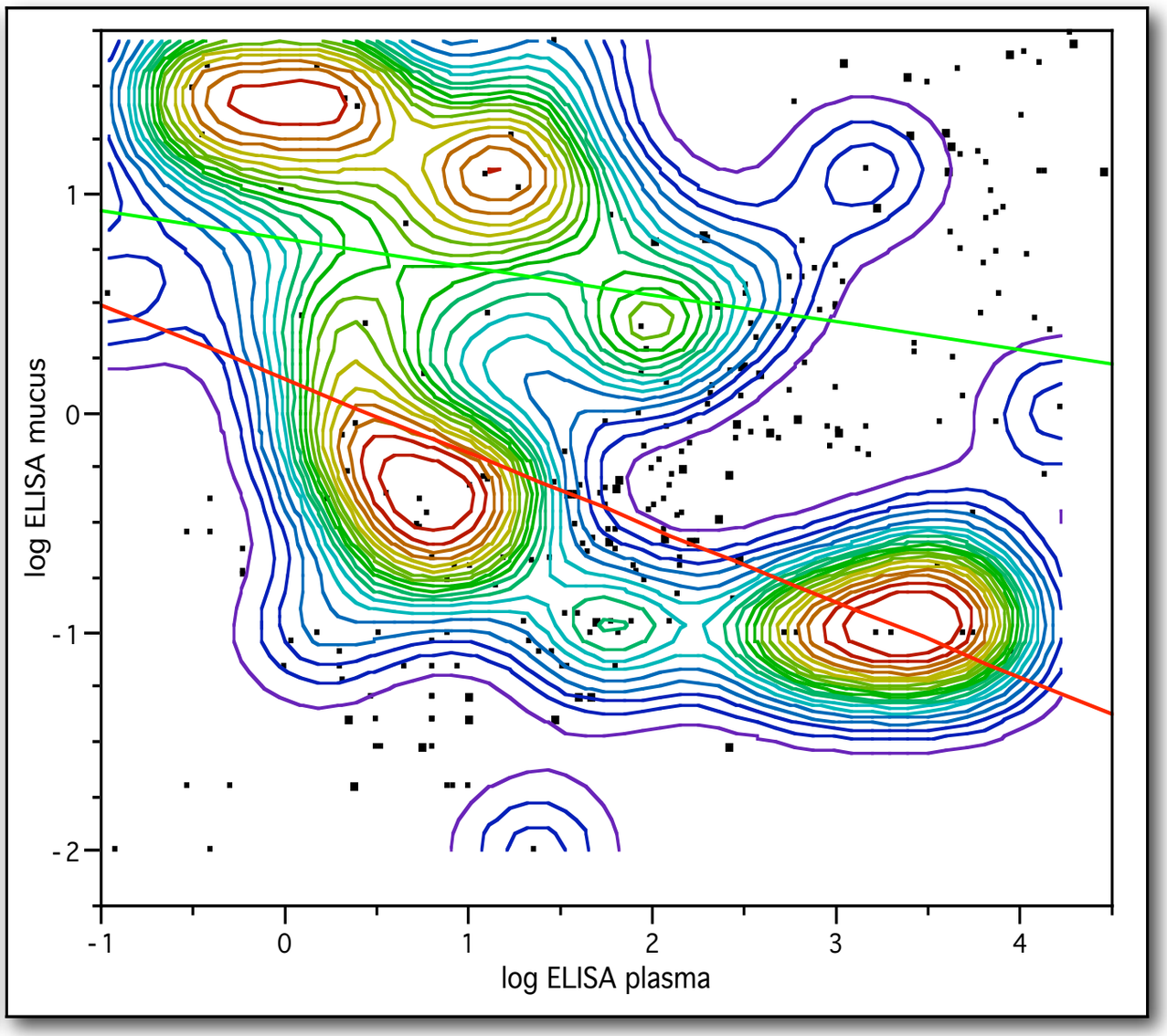
Vtg in plasma vs. mucus



EE2, MDHT, FLU

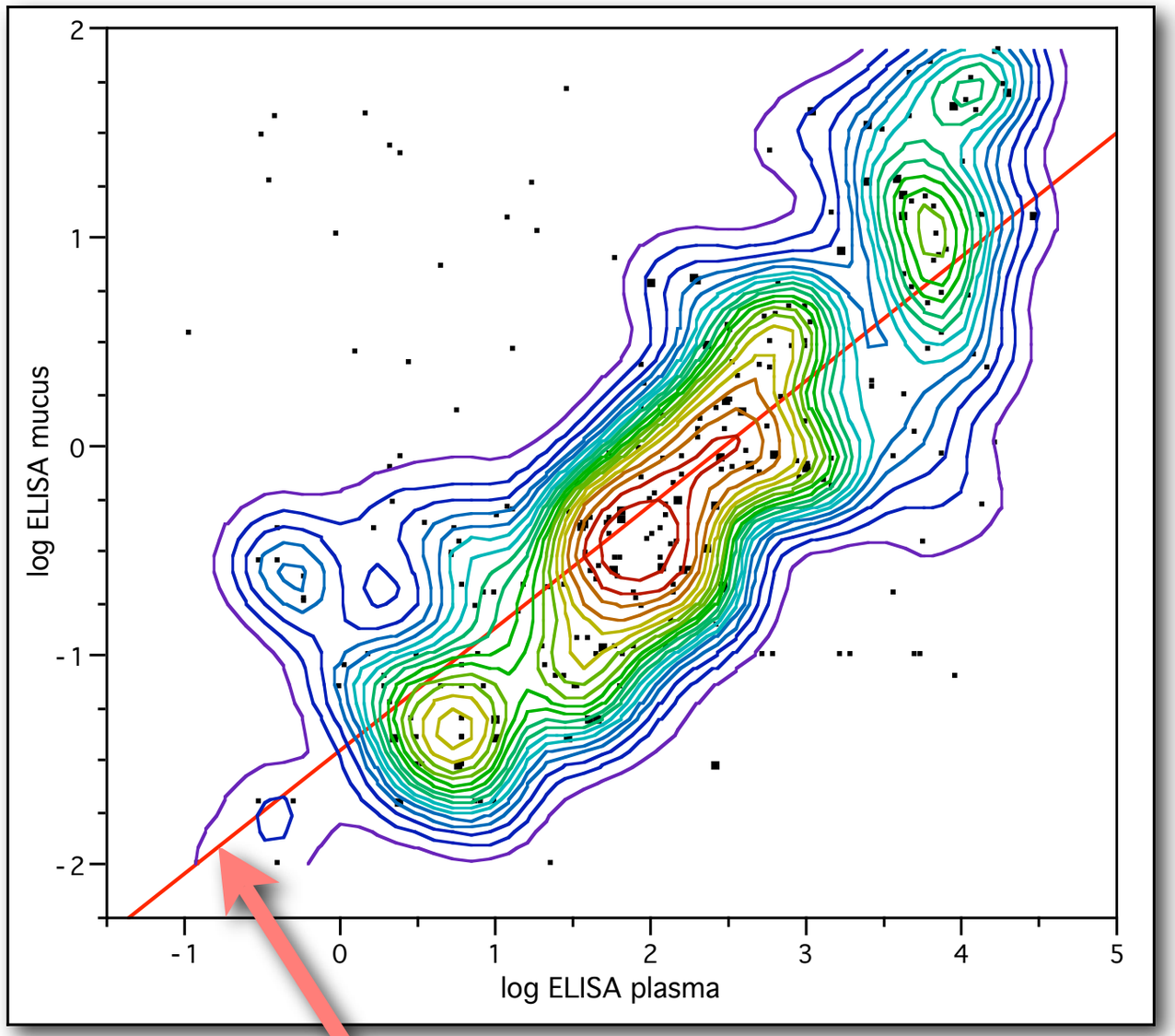
Vtg in plasma vs. mucus



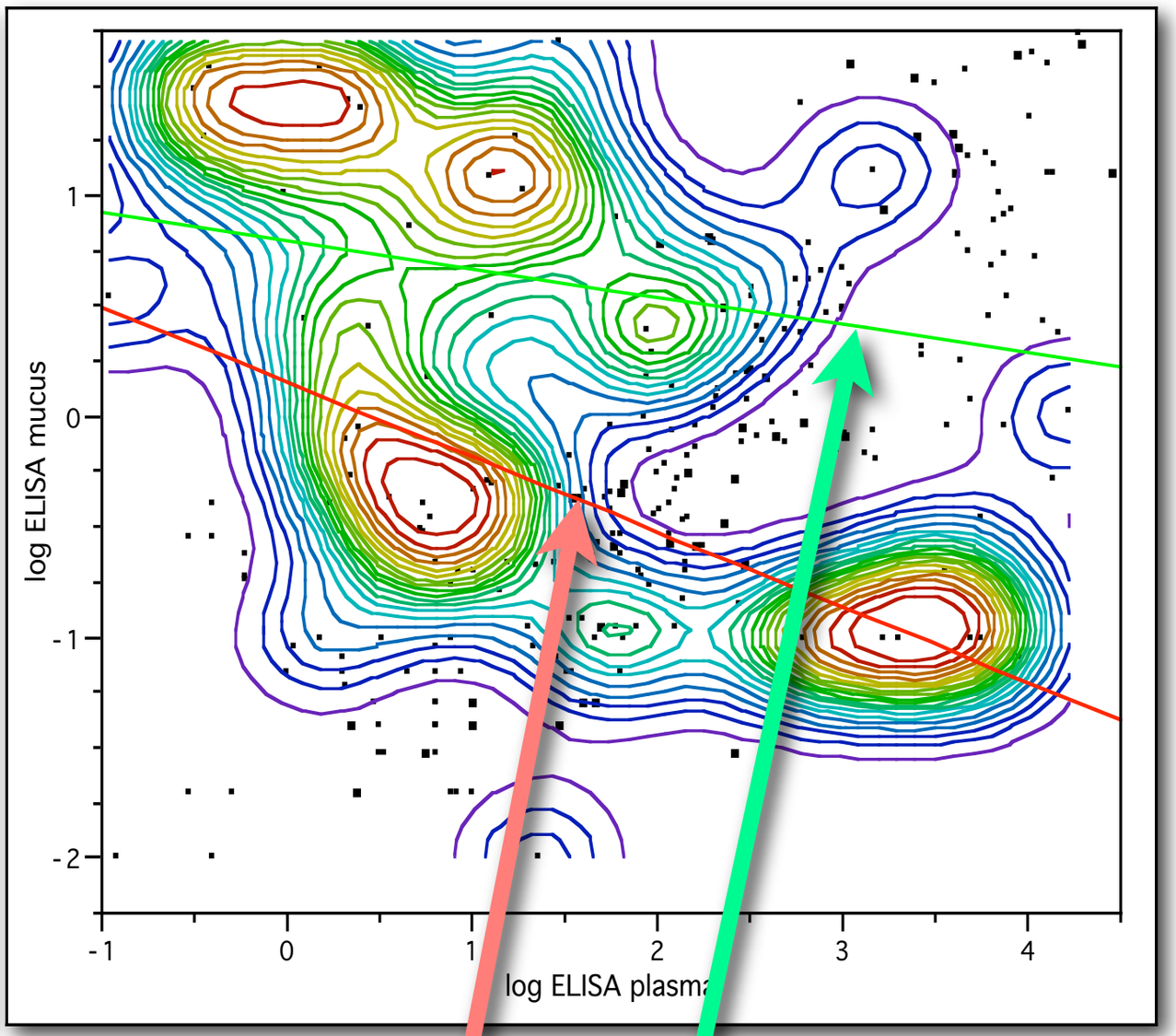


EE2, MDHT, FLU

Vtg in plasma vs. mucus

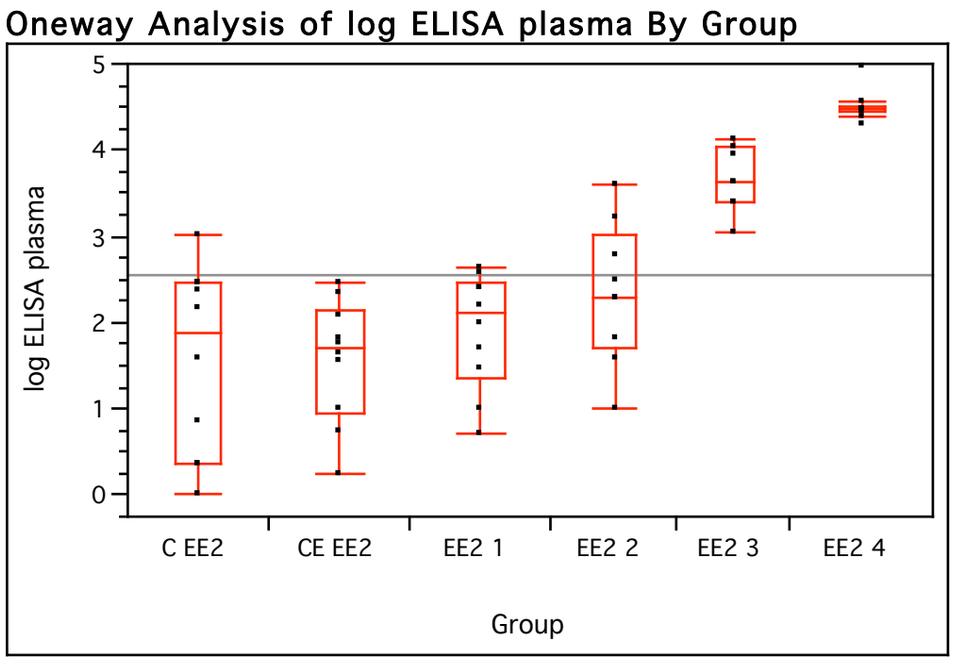


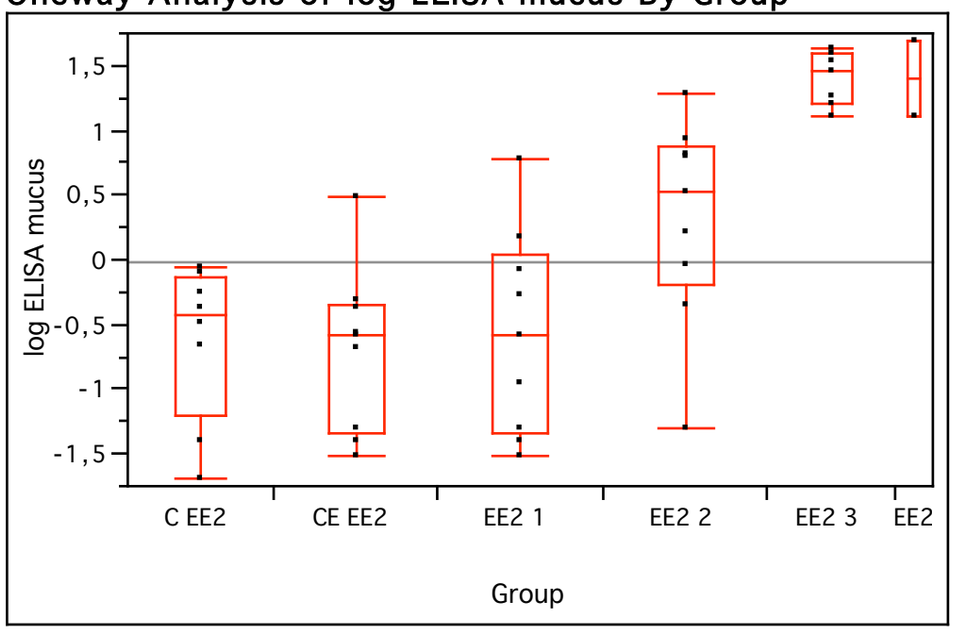
EE2, MDHT, FLU



TAM Field

Vtg in carp plasma vs. mucus





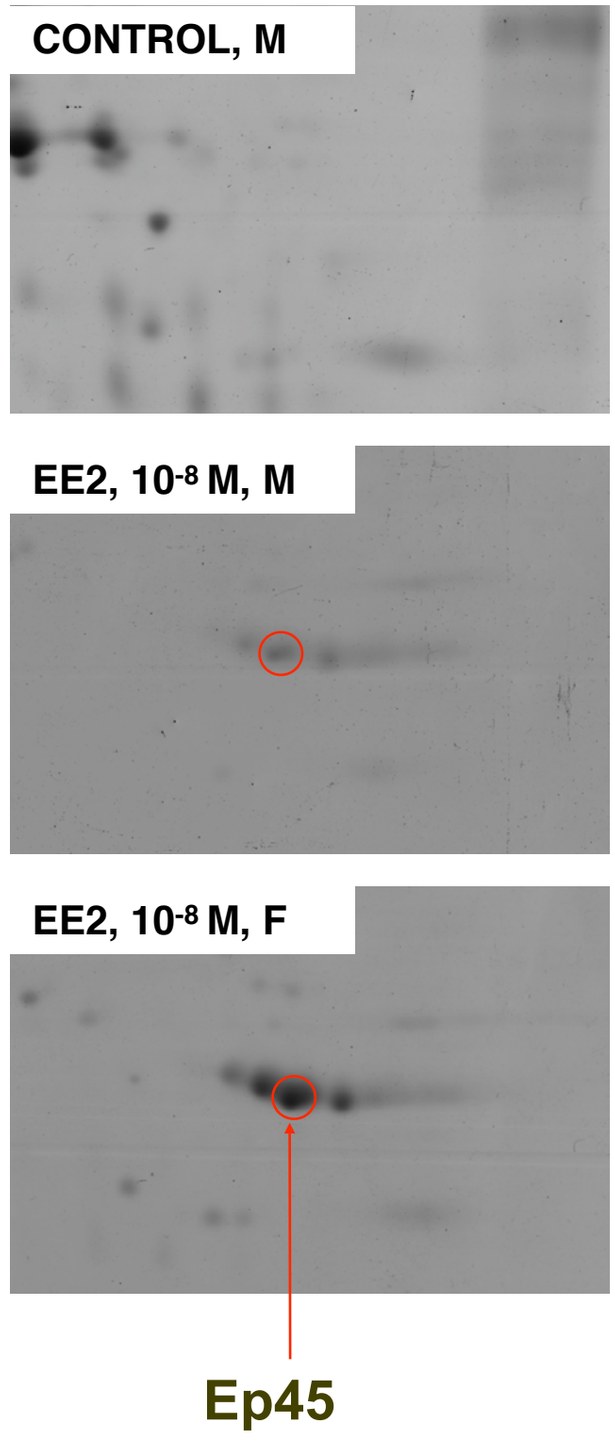
	Plasma Vtg µg/ml ±SD (N)	Mucus Vtg µg/ml ±SD (N)
Untreated control	202±310 (10)	0.41±0.32 (8)
Solvent control	86±99 (10)	0.54±0.97 (9)
EE2 – 1 ng/l	169±157 (10)	1.04±1.93 (9)
EE2 – 4 ng/l	775±1278 (9)	5.25±6.11 (9)*
EE2 – 16 ng/l	5935±4453 (8)*	27.6±11.6 (7)*
EE2 – 64 ng/l	35355±20978 (10)*	30.8±25.5 (2)*

* Significantly different from untreated control, Dunnetts test of log-transformed data ($p<0.05$)

Carp Vtg ELISA kit (Biosense)

Estrogen regulated protein Ep45 precursor (Ep45)

- Belongs to the serpin superfamily of proteinase inhibitors
- Similarity to Hu alpha-1-antitrypsin, the major plasma serpin[2]
- Absent in control, 6-fold increase in expression within 8 days by E2 exposure[1]
- Induction parallels that of Vtg[1]
- Possible role in female reproduction by protecting Vtg from proteolytic cleavage during transport[2]

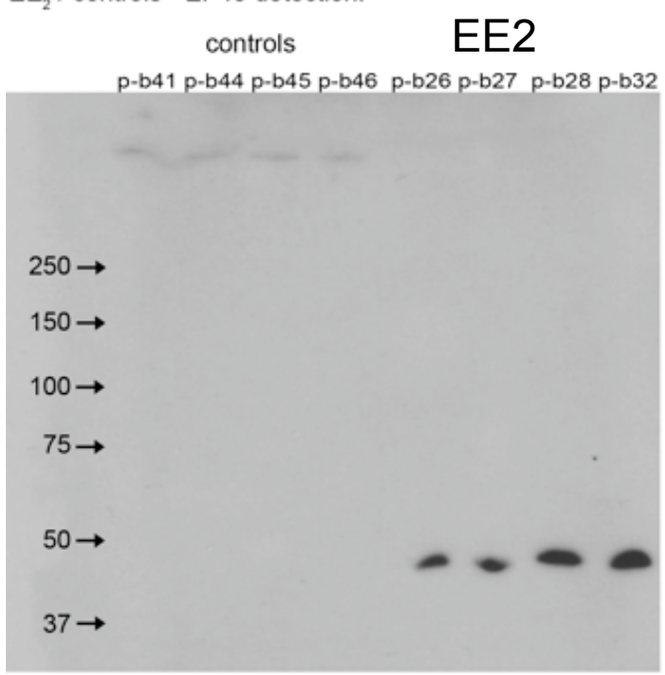


[1]Holland LJ, Wangh LJ. 1987. Mol. Cell. Endocrinol. 49:63-7
[2]Holland et al. 1992. Journ. of Biol.Chem. 267:7053-7059

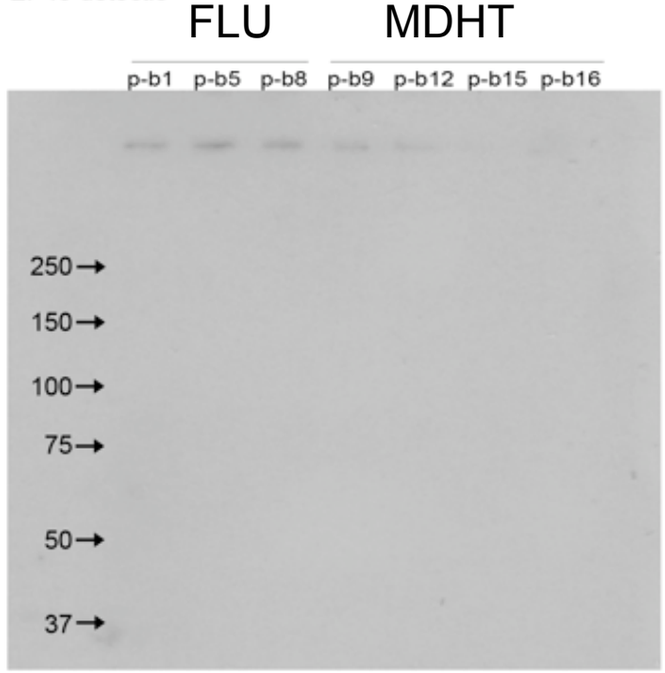
Xenopus plasma: Anti-Ep45-peptide western blots

♀

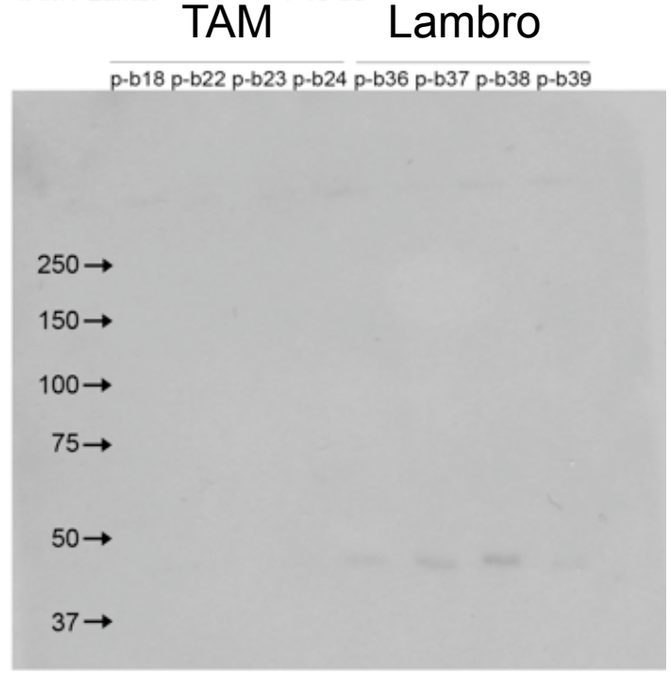
Xenopus laevis plasma; females exposed to EE_2 / controls - EP45 detection.



Xenopus laevis plasma; females exposed to Flu / MDHT EP45 detectio

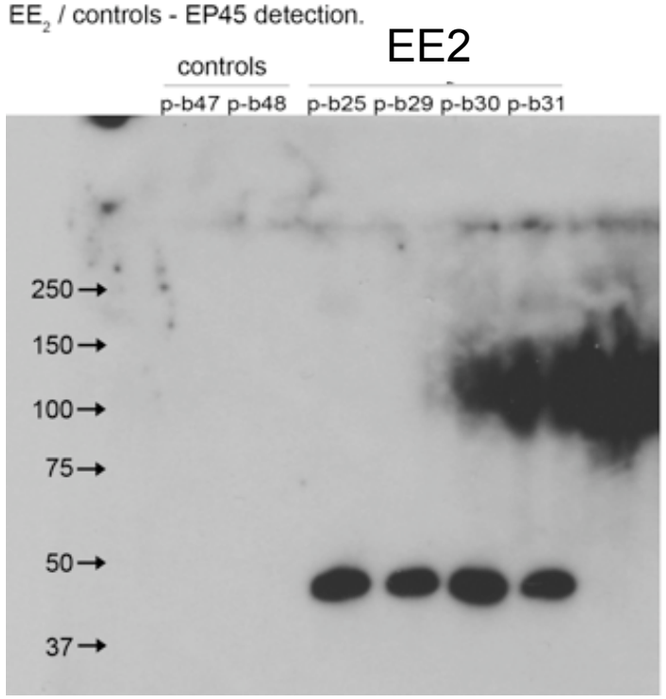


Xenopus laevis plasma; females exposed to TAM / Lambro-water - EP45 detection



♂

Xenopus laevis plasma; males exposed to EE_2 / controls - EP45 detection.



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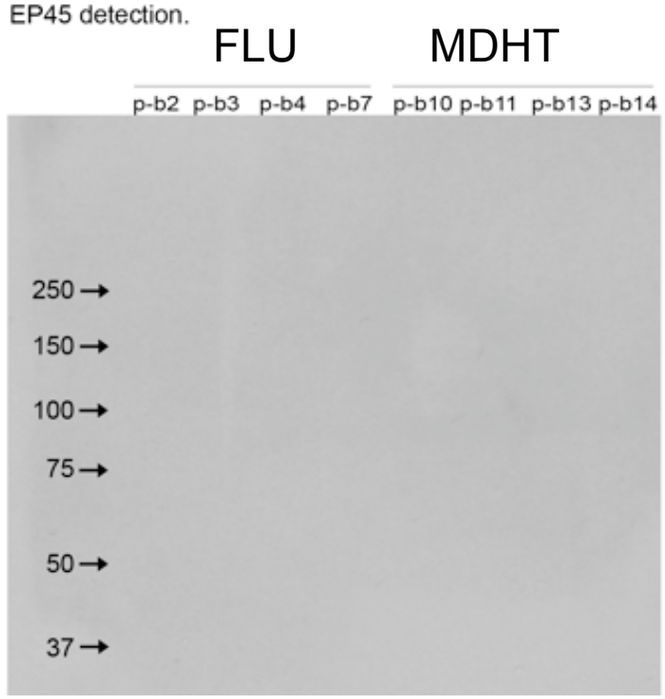

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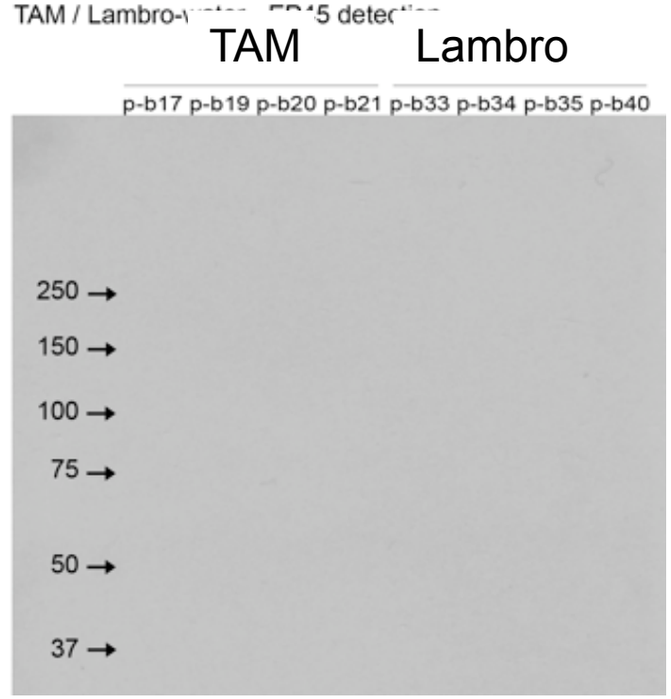
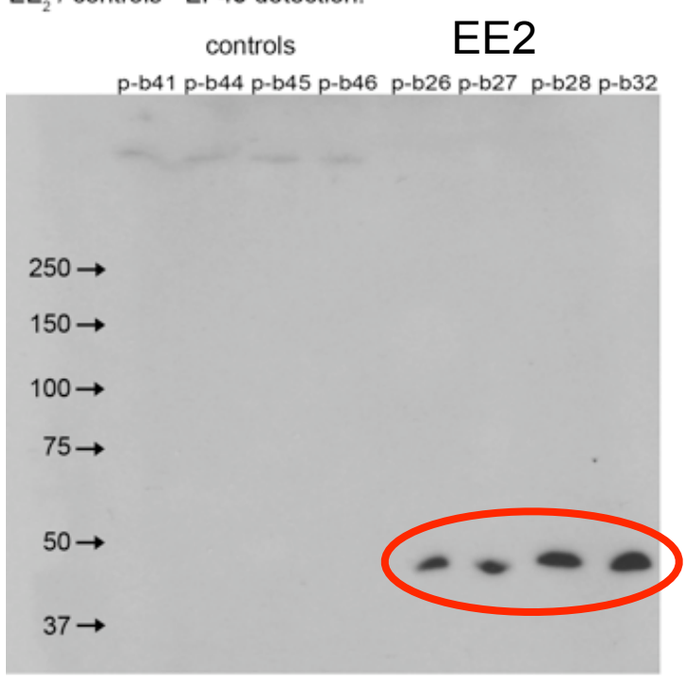


Xenopus plasma: Anti-Ep45-peptide western blots

♀

Xenopus laevis plasma; females exposed to EE_2 / controls - EP45 detection.



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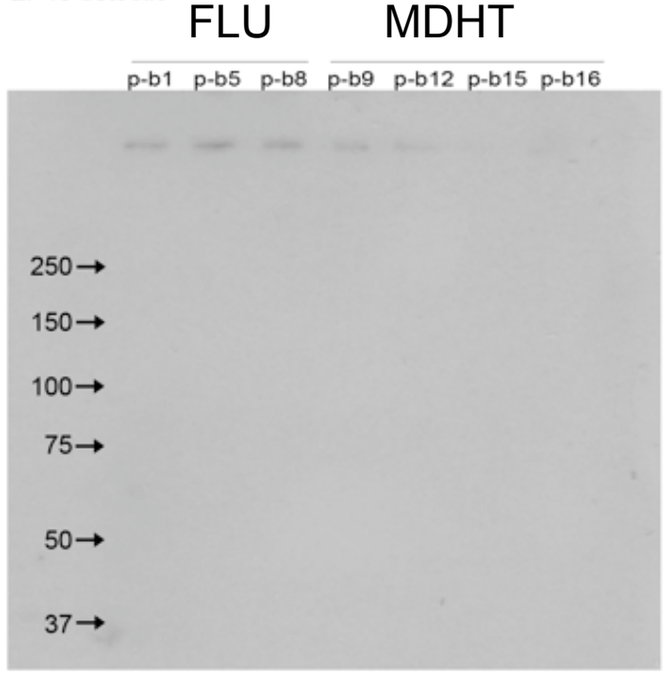
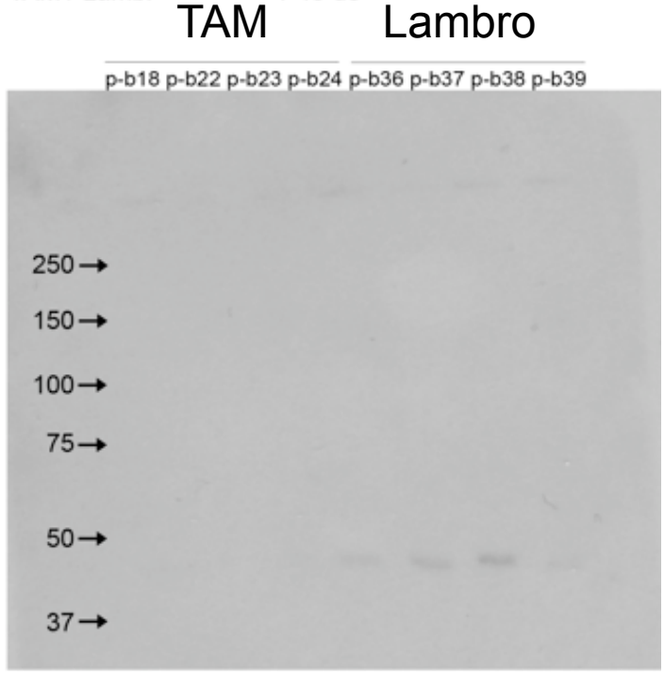
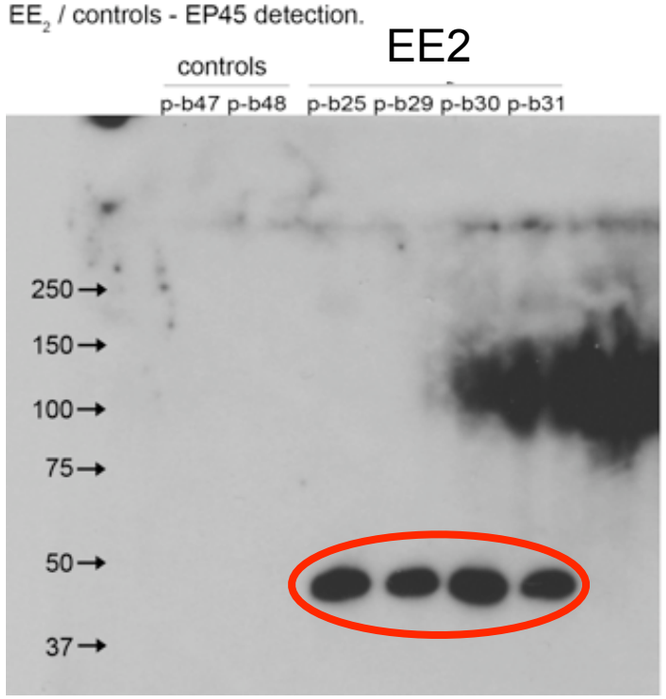


Xenopus laevis plasma; females exposed to TAM / Lambro-water - EP45 detection

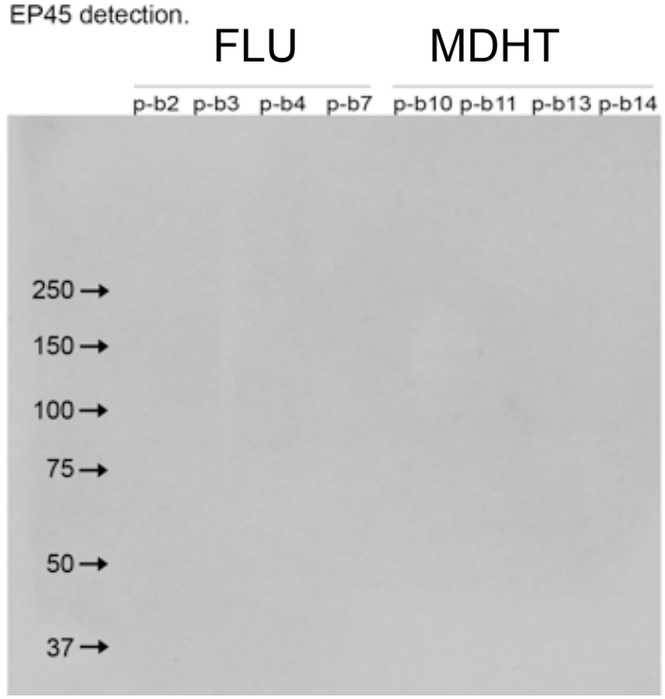


♂

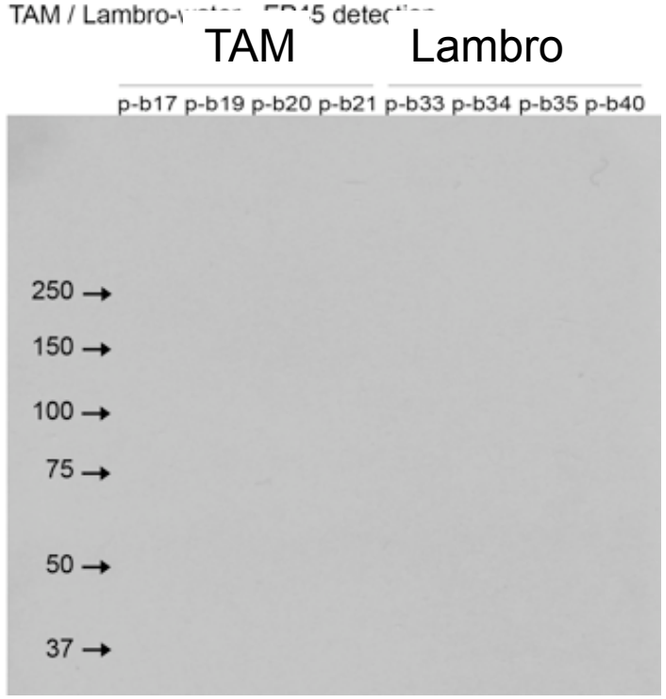
Xenopus laevis plasma; males exposed to EE_2 / controls - EP45 detection.



Xenopus laevis plasma; males exposed to Flu / MDHT EP45 detection.



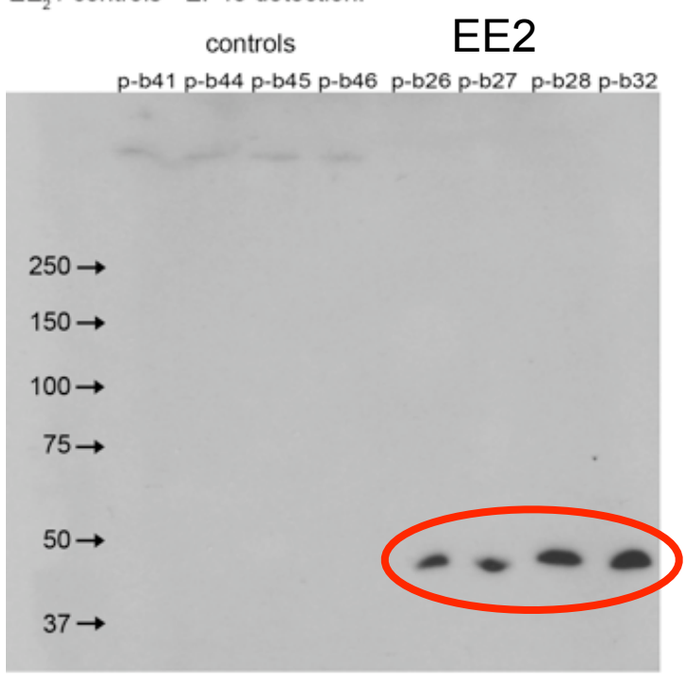
Xenopus laevis plasma; males exposed to TAM / Lambro-water - EP45 detection



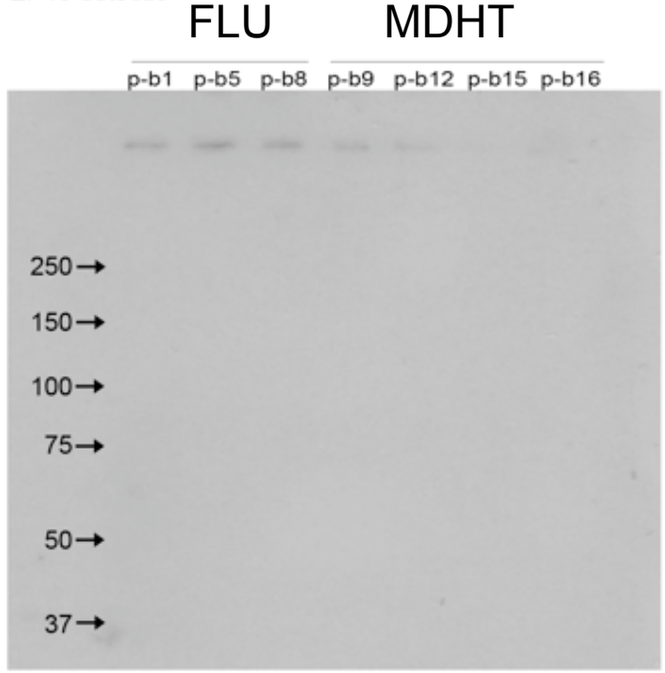
Xenopus plasma: Anti-Ep45-peptide western blots

♀

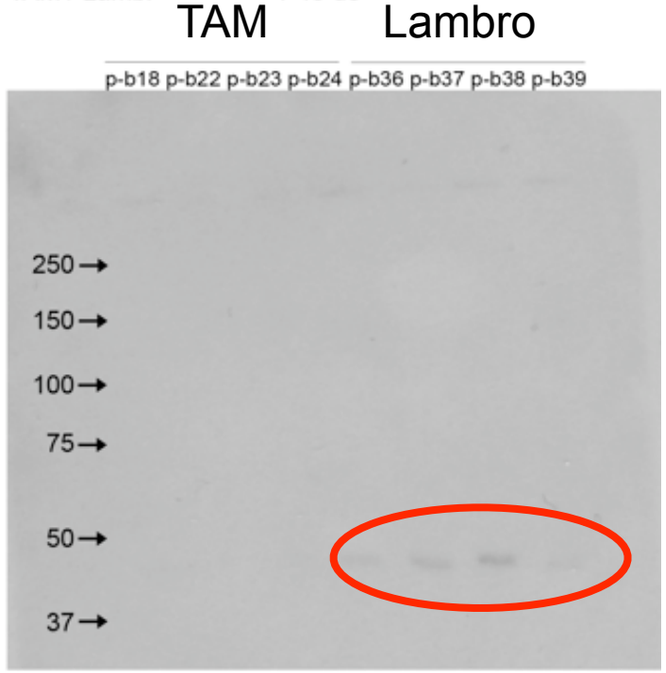
Xenopus laevis plasma; females exposed to EE_2 / controls - EP45 detection.



Xenopus laevis plasma; females exposed to Flu / MDHT EP45 detectio

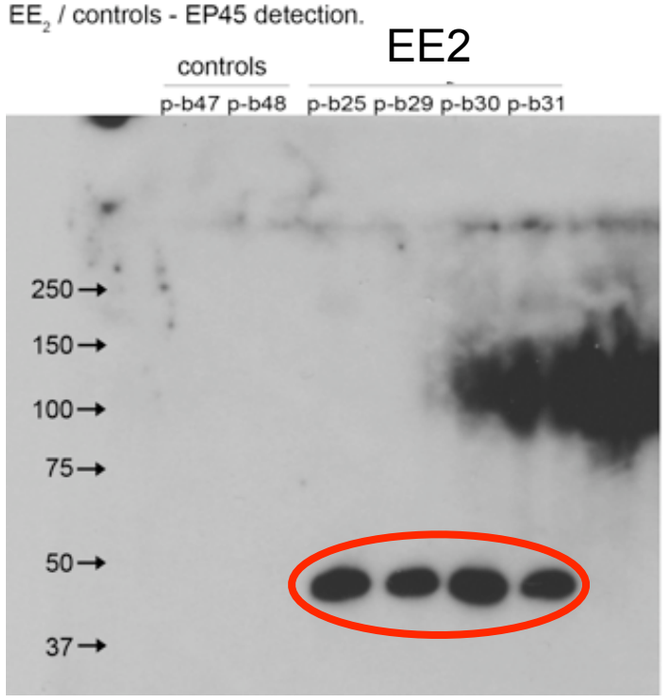


Xenopus laevis plasma; females exposed to TAM / Lambro-water EP45 detection

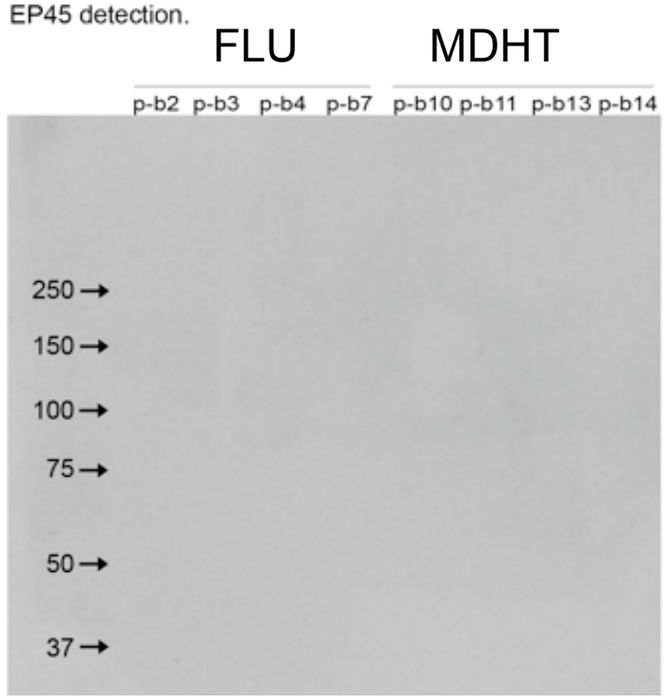


♂

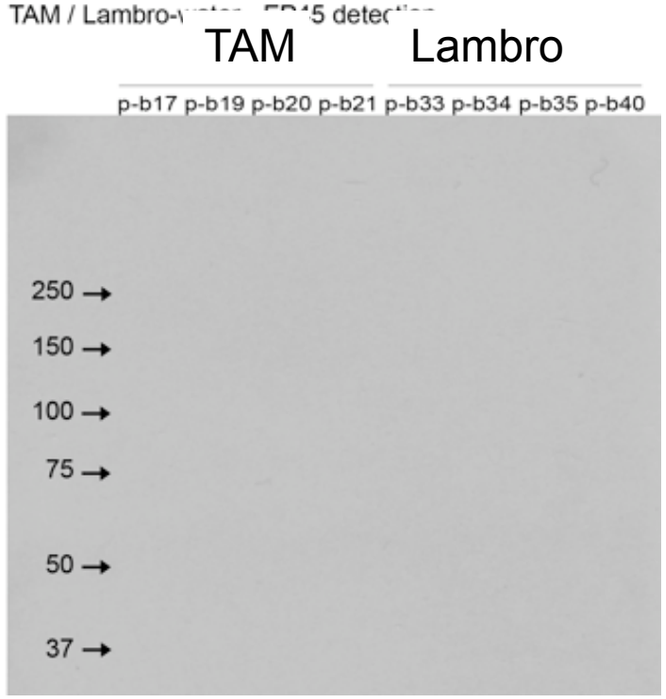
Xenopus laevis plasma; males exposed to EE_2 / controls - EP45 detection.



Xenopus laevis plasma; males exposed to Flu / MDHT EP45 detection.



Xenopus laevis plasma; males exposed to TAM / Lambro-water EP45 detection



Xenopus plasma: Anti-Ep45-peptide western blots

♀

Xenopus laevis plasma; females exposed to EE_2 / controls - EP45 detection.

controls EE2

p-b41 p-b44 p-b45 p-b46 p-b26 p-b27 p-b28 p-b32

250 → 150 → 100 → 75 → 50 → 37 →

Xenopus laevis plasma; females exposed to Flu / MDHT EP45 detectio

FLU MDHT

p-b1 p-b5 p-b8 p-b9 p-b12 p-b15 p-b16

Xenopus laevis plasma; females exposed to TAM / Lambr… …P45 de…

TAM Lambro

p-b18 p-b22 p-b23 p-b24 p-b36 p-b37 p-b38 p-b39

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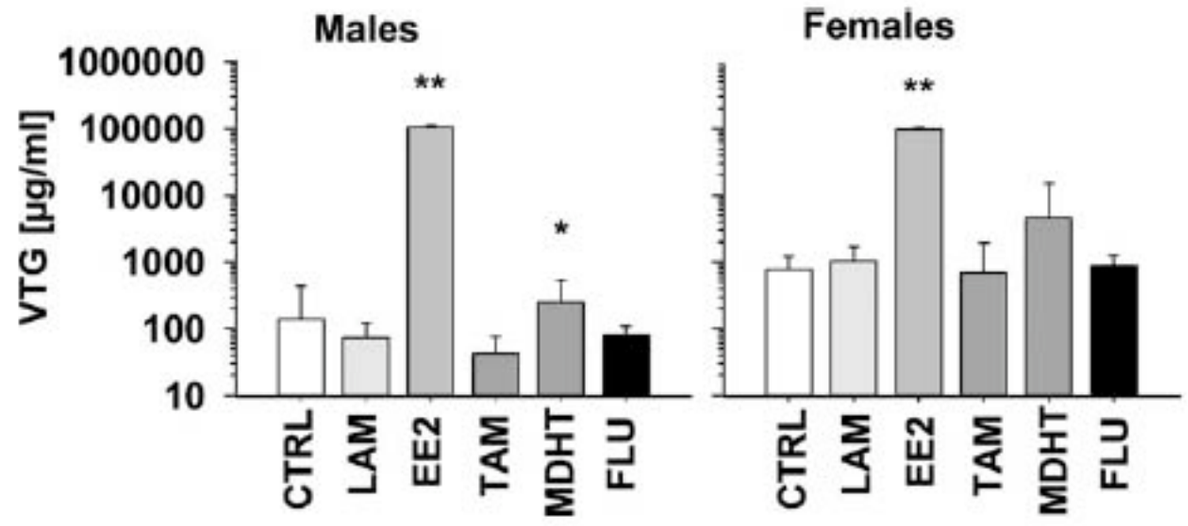

Fig. 3. Vitellogenin (VTG) plasma protein levels of male and female *X. laevis* according to Figs. 1 and 2. The concentration is given as µg/mL. Significant deviations from the control were tested by one-way ANOVA, Dunnett's-test and are indicated by asterisks (** = $p<0.01$, * = $p<0.05$).

♂

Xenopus laevis plasma; males expos… EE_2 / controls - EP45 detection.

controls EE…

p-b47 p-b48 p-b25 p-b29

250 → 150 → 100 → 75 → 50 → 37 →

…ma; males exposed to …P45 detec…

…AM Lambro

…p-b20 p-b21 p-b33 p-b34 p-b35 p-b40

100 → 75 → 50 → 37 →

100 → 75 → 50 → 37 →

Xenopus plasma: Anti-serotransferrin-peptide western blots

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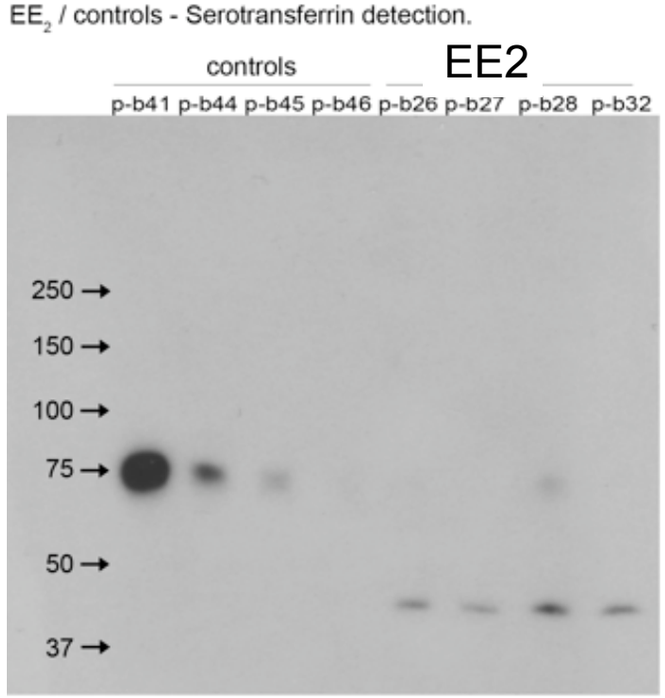

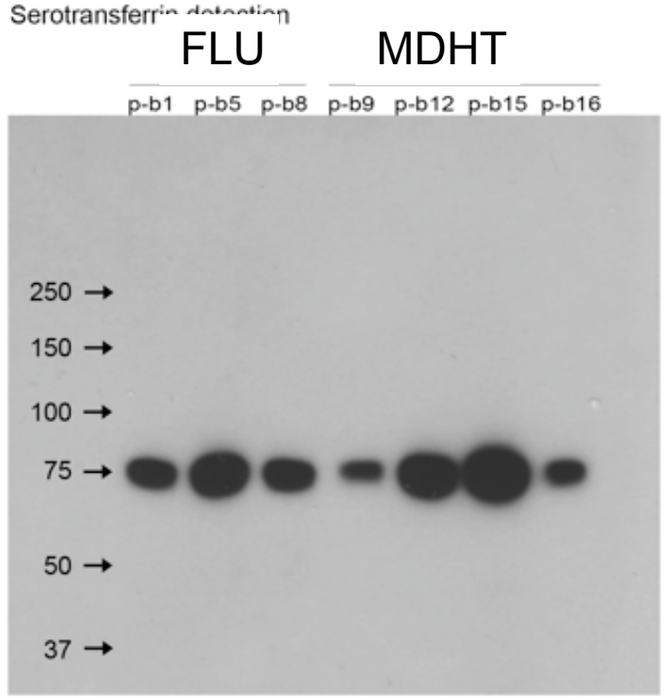

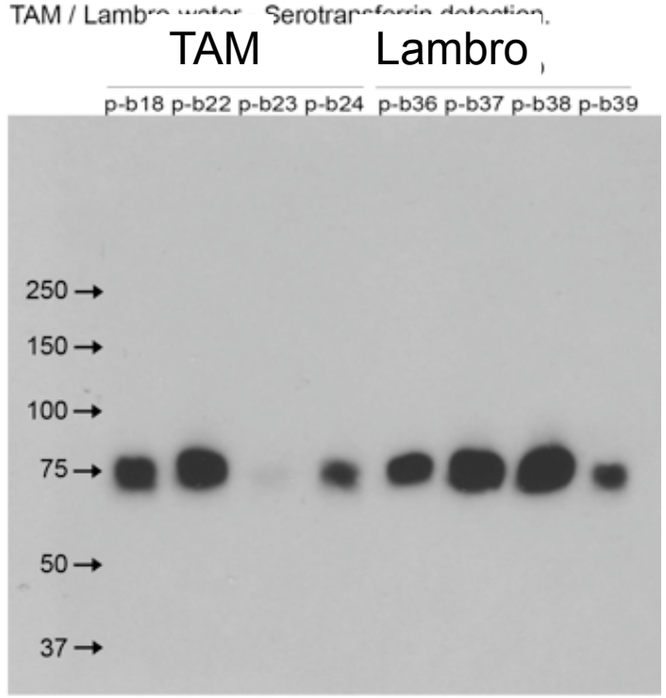

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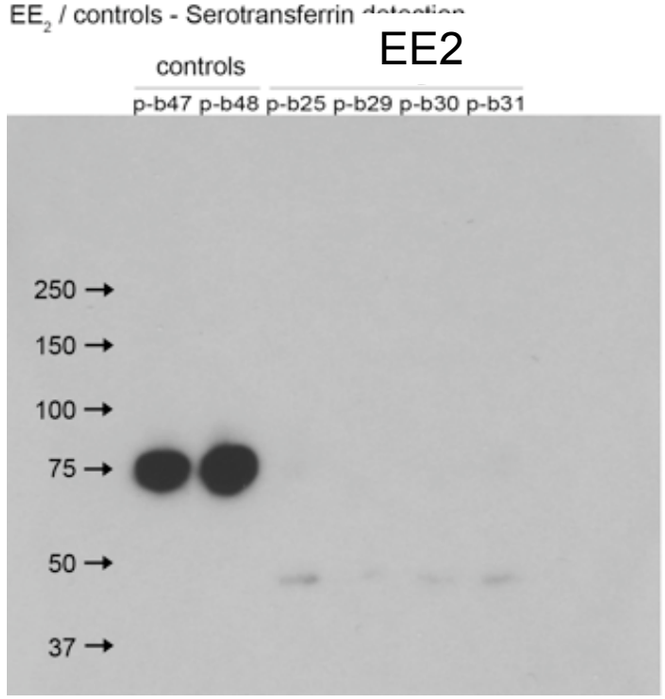

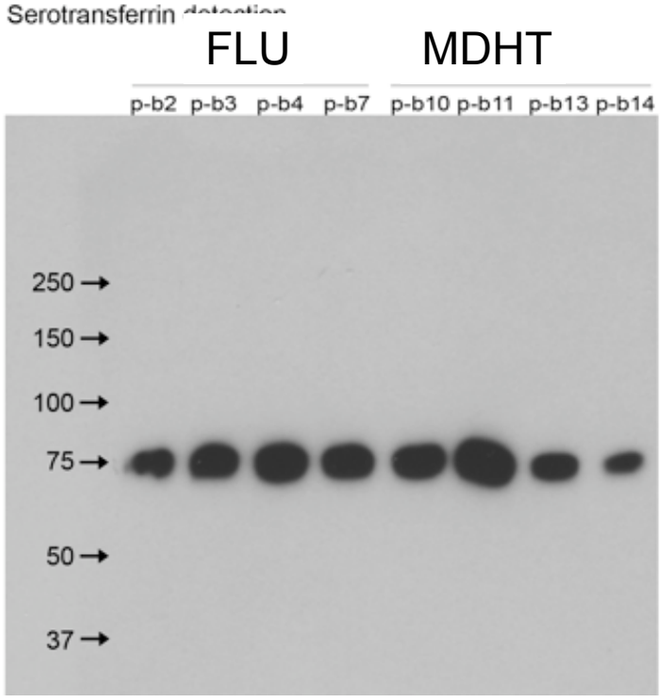

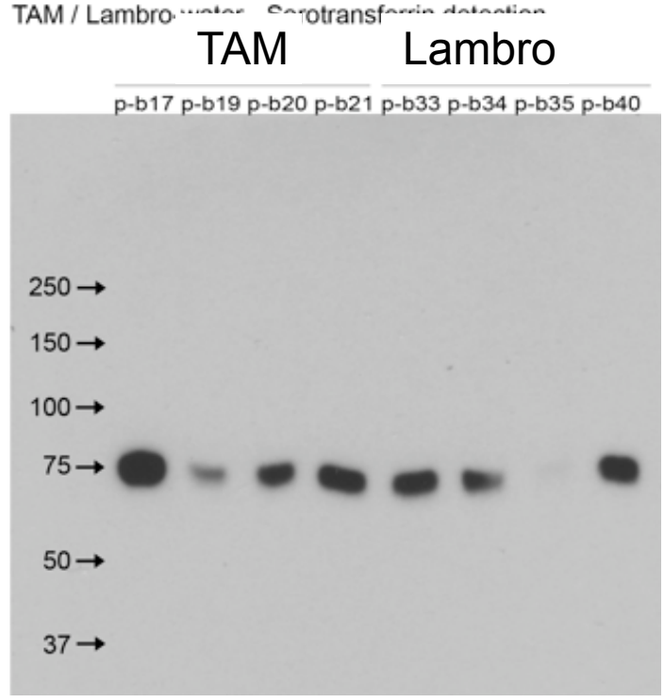

Xenopus plasma: Anti-serotransferrin-peptide western blots

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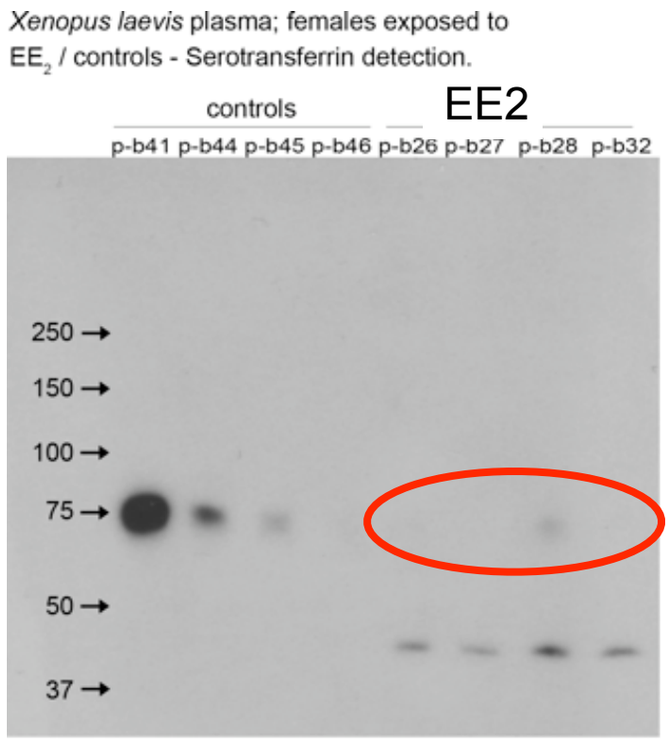

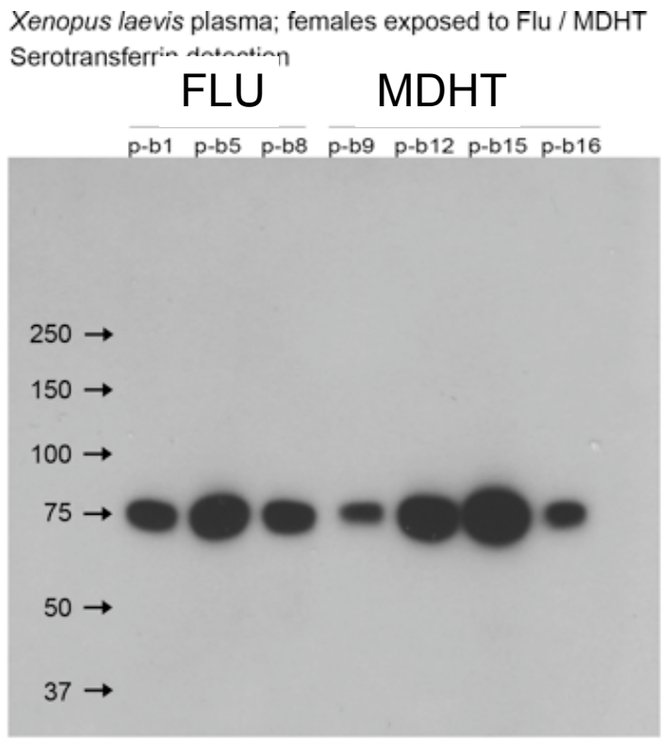

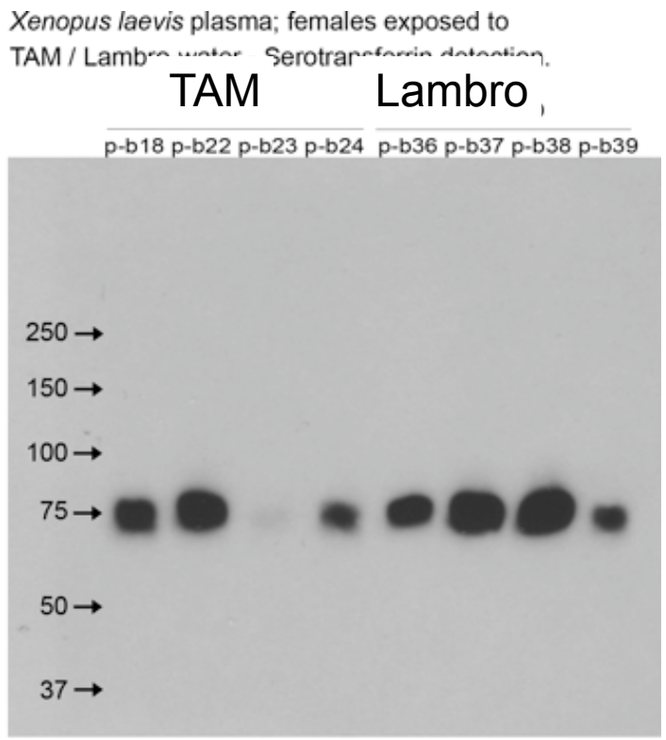

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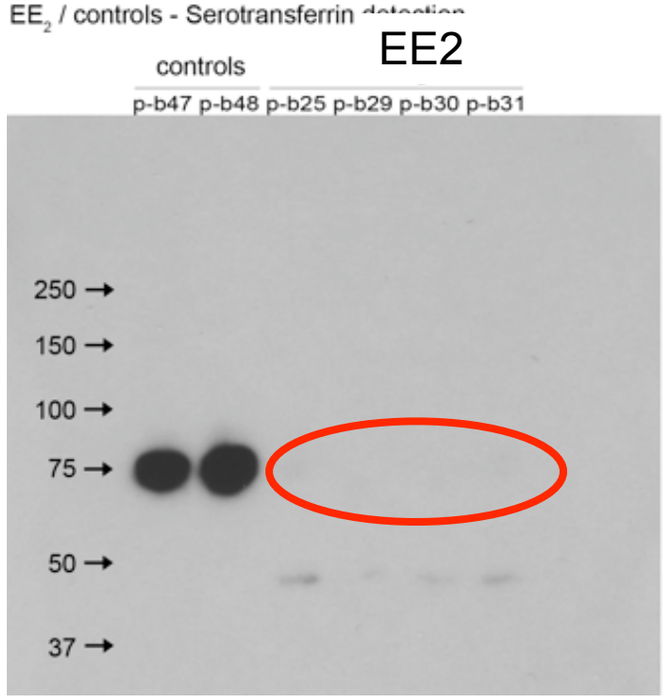

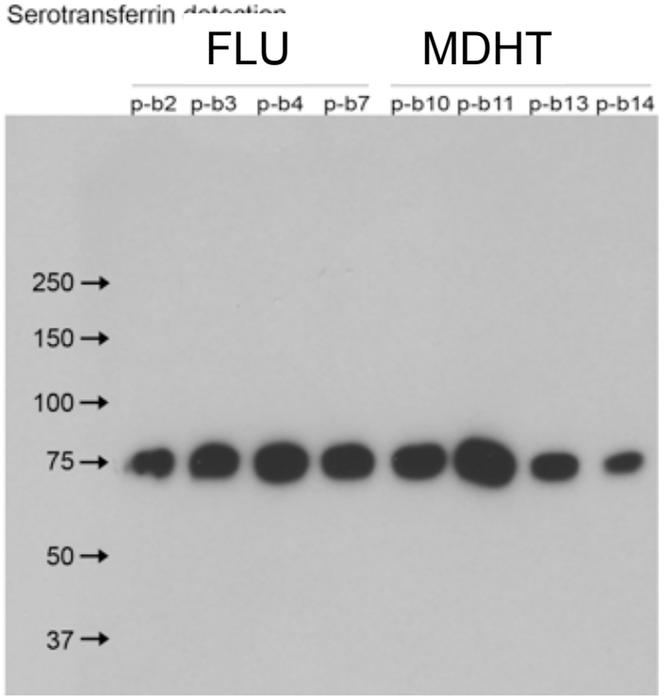

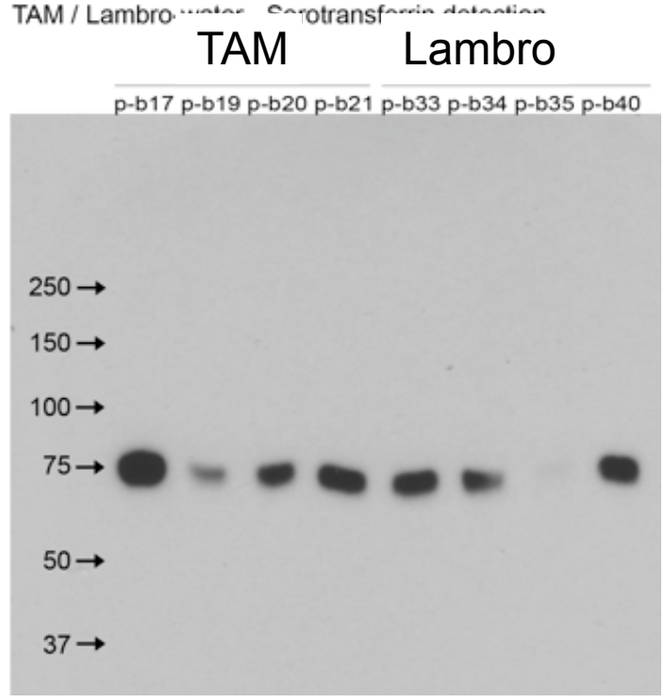

Xenopus plasma: Anti-fibrinogen β -peptide western blots

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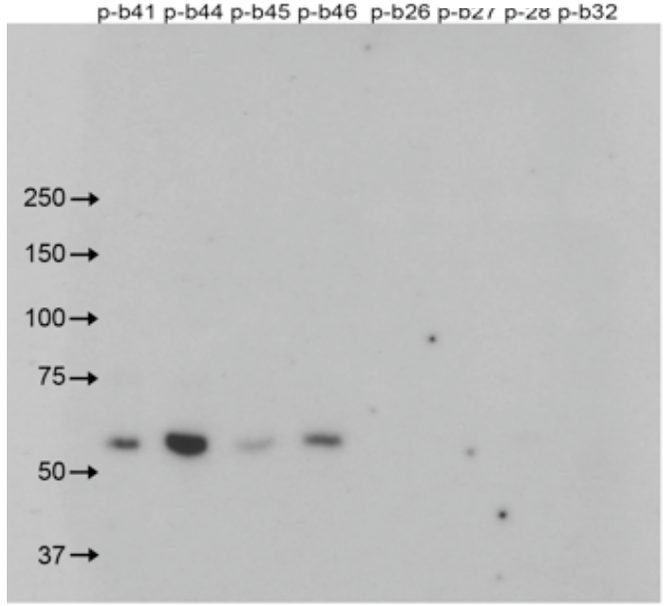
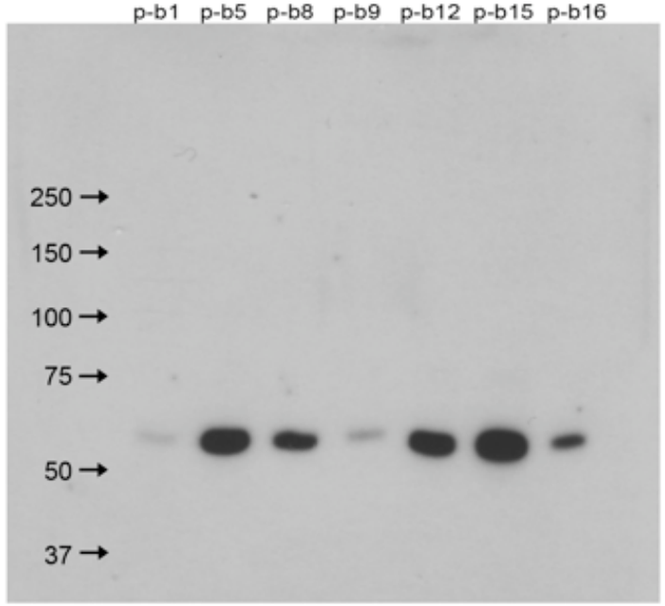
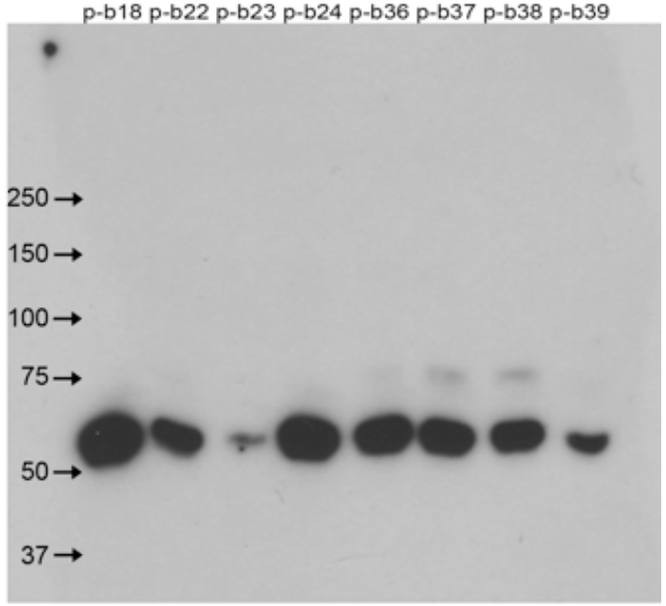
Xenopus laevis plasma; females exposed to EE_2 / controls - fibrinogen beta detection.



Xenopus laevis plasma; females exposed to Flu / MDHT fibrinogen beta detection



Xenopus laevis plasma; females exposed to TAM / Lambro-water - fibrinogen beta detection



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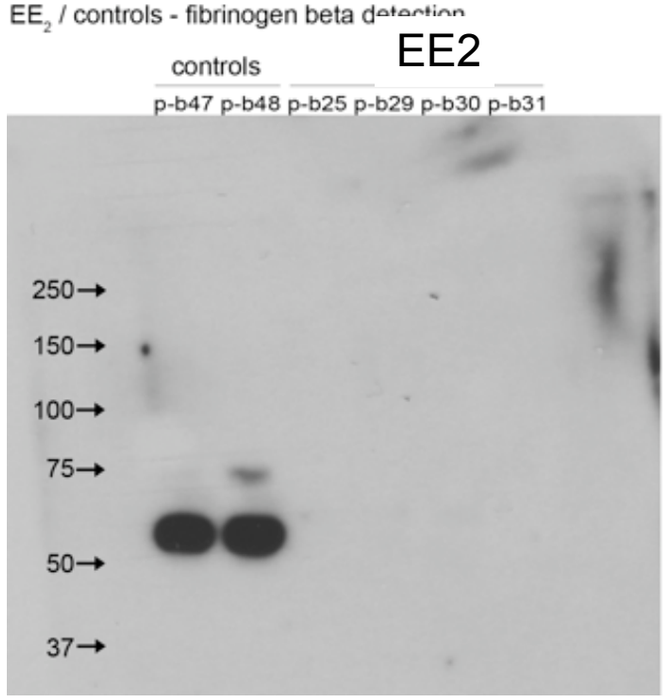
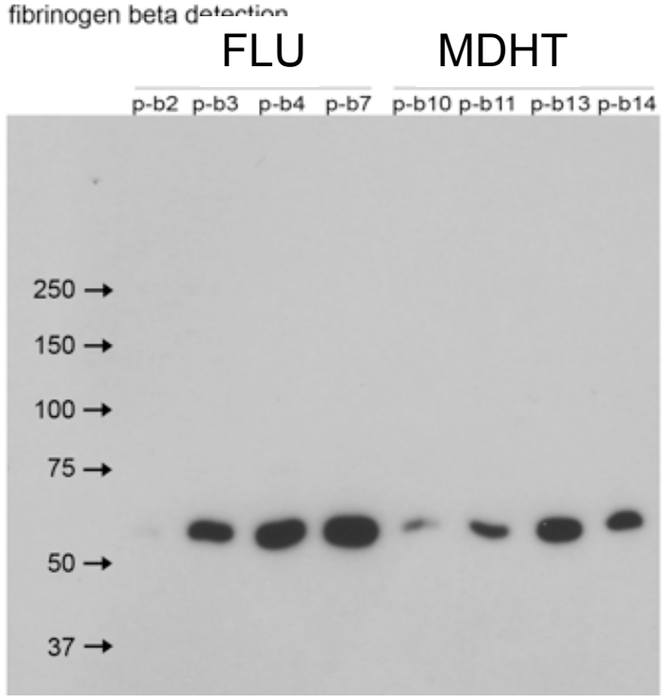
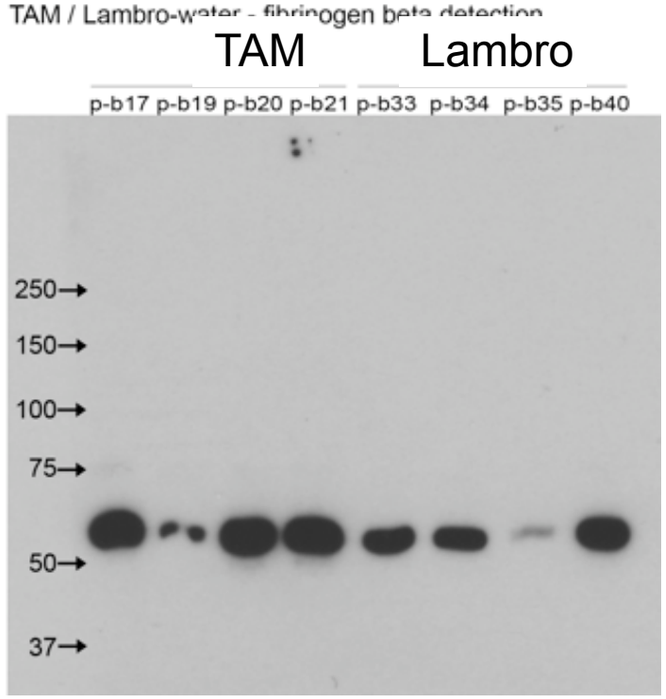
Xenopus laevis plasma; males exposed to EE_2 / controls - fibrinogen beta detection



Xenopus laevis plasma; males exposed to Flu / MDHT fibrinogen beta detection



Xenopus laevis plasma; males exposed to TAM / Lambro-water - fibrinogen beta detection



Xenopus plasma: Anti-fibrinogen β -peptide western blots

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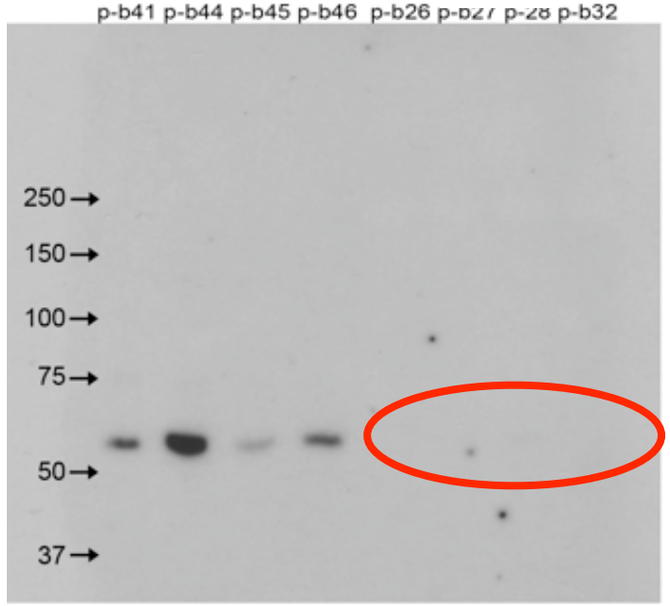
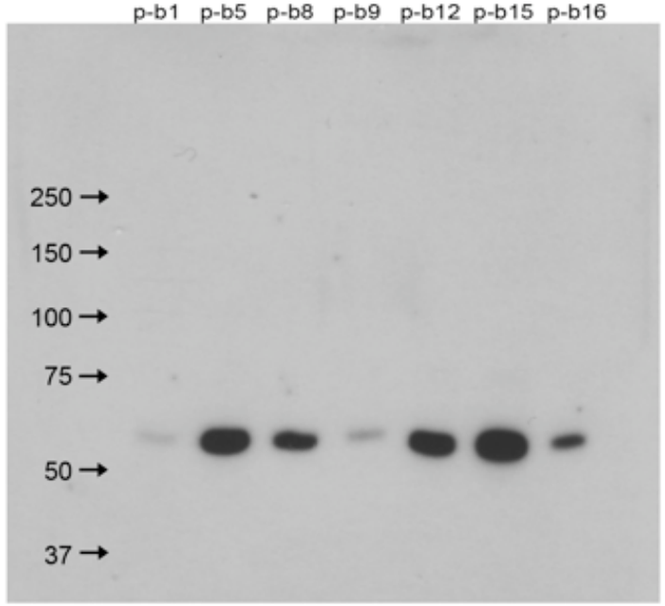
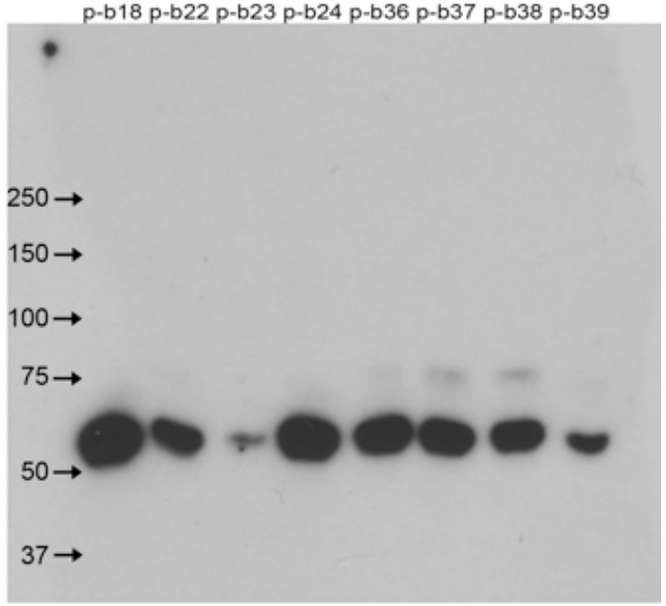
Xenopus laevis plasma; females exposed to EE_2 / controls - fibrinogen beta detection.



Xenopus laevis plasma; females exposed to Flu / MDHT fibrinogen beta detection



Xenopus laevis plasma; females exposed to TAM / Lambro-water - fibrinogen beta detection



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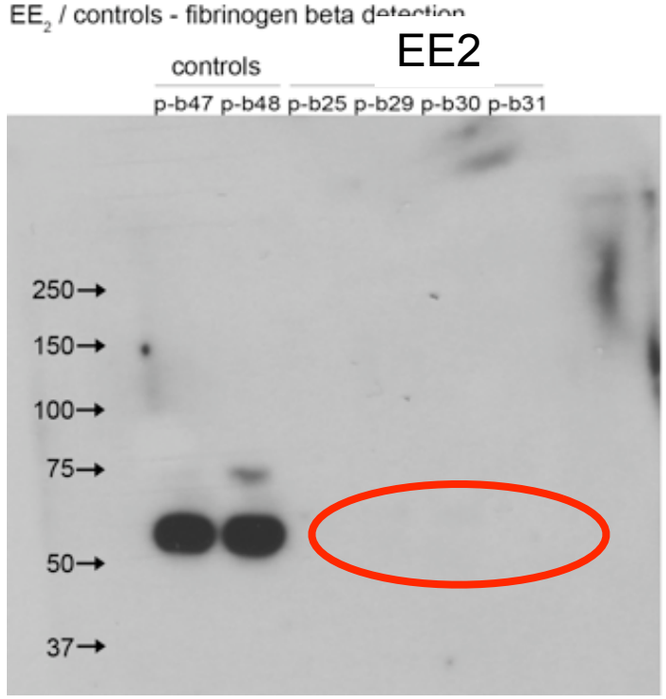
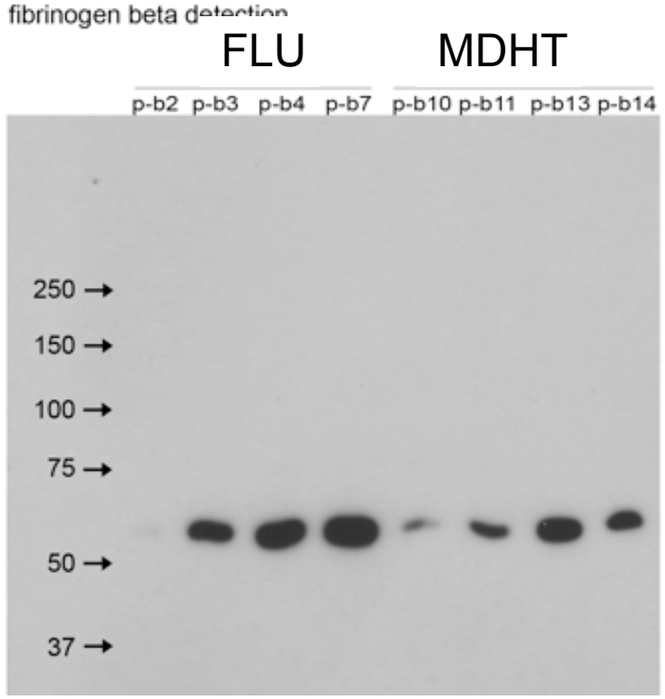
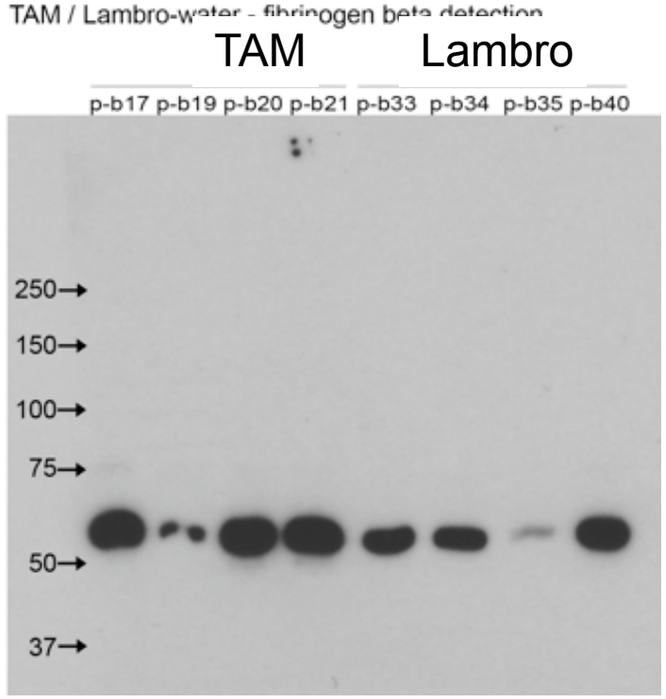
Xenopus laevis plasma; males exposed to EE_2 / controls - fibrinogen beta detection



Xenopus laevis plasma; males exposed to Flu / MDHT fibrinogen beta detection



Xenopus laevis plasma; males exposed to TAM / Lambro-water - fibrinogen beta detection



Xenopus plasma: Anti-fibrinogen β -peptide western blots

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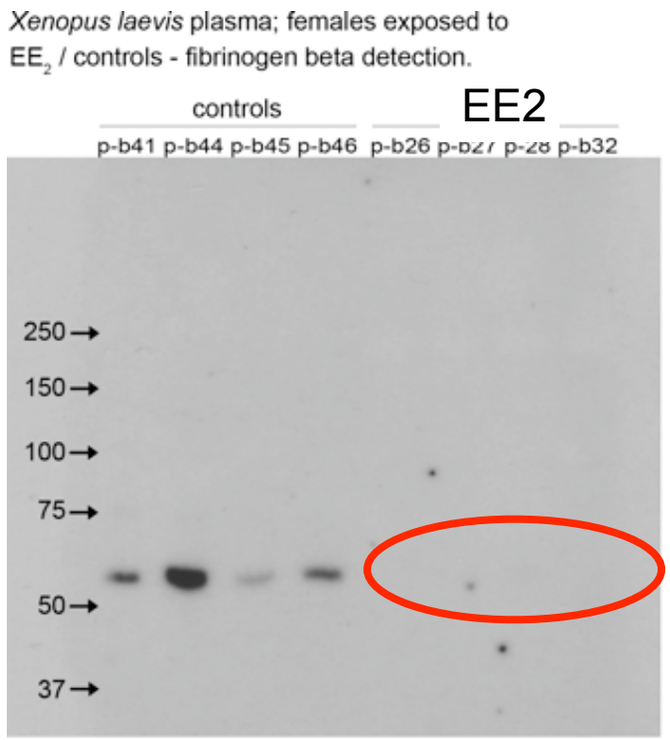

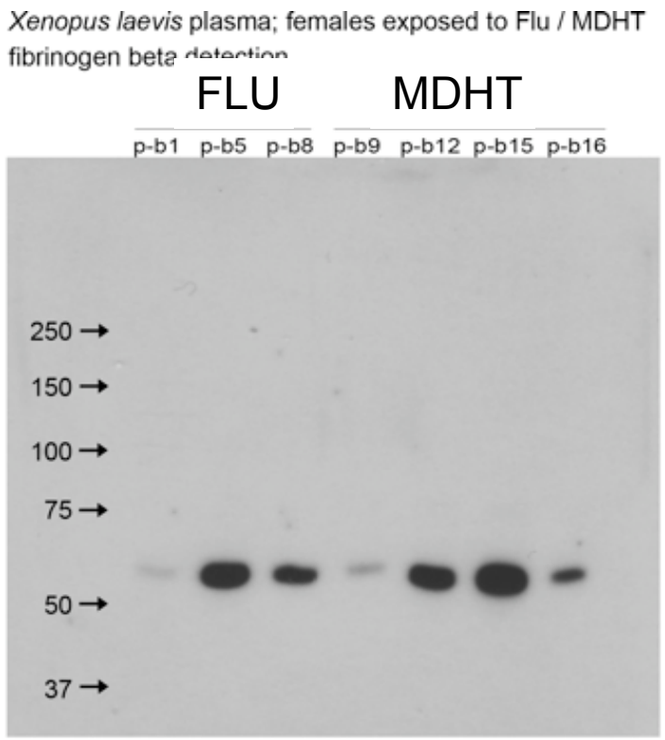

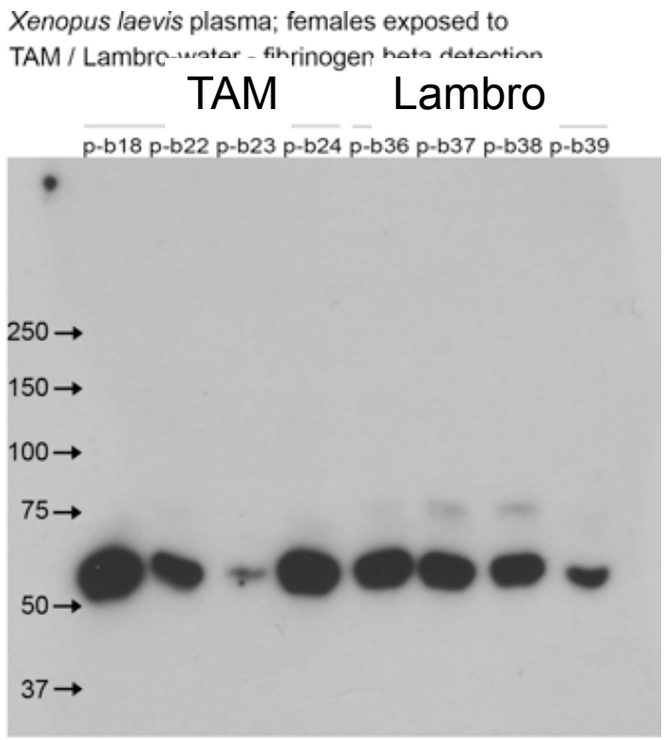

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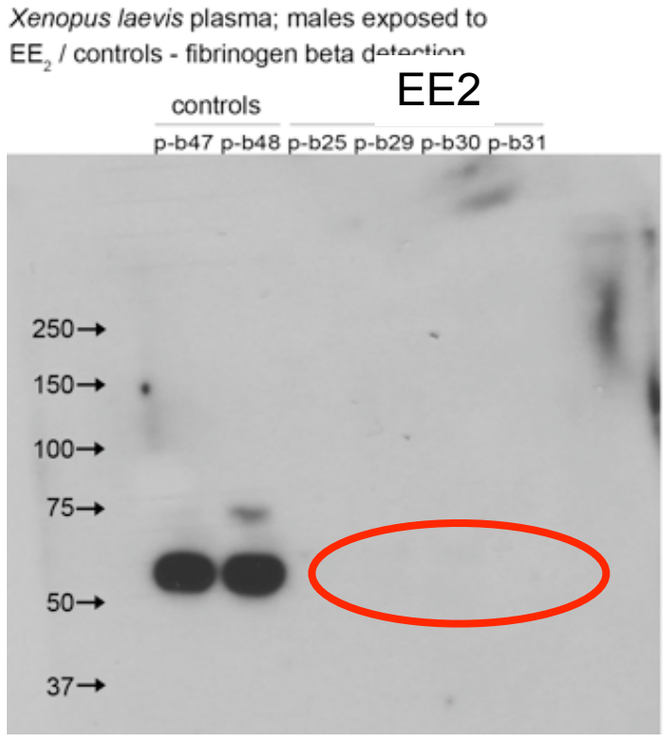
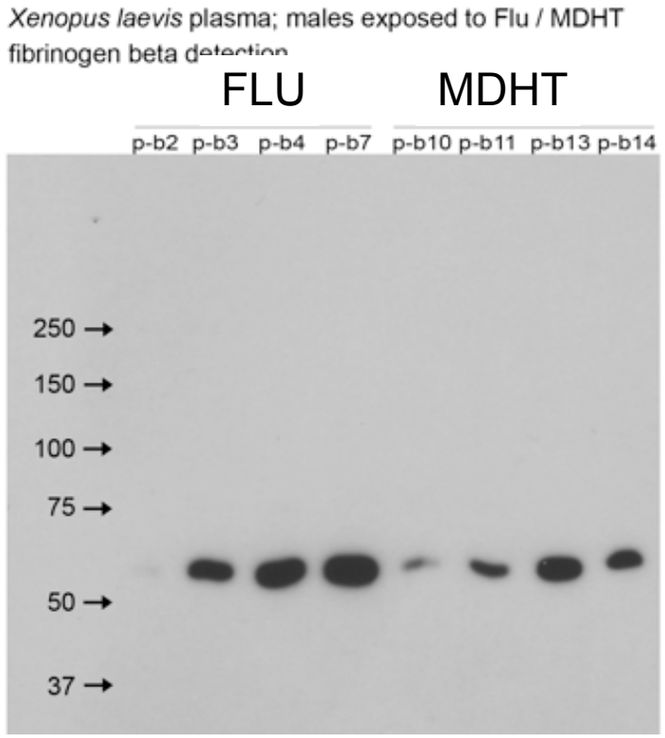
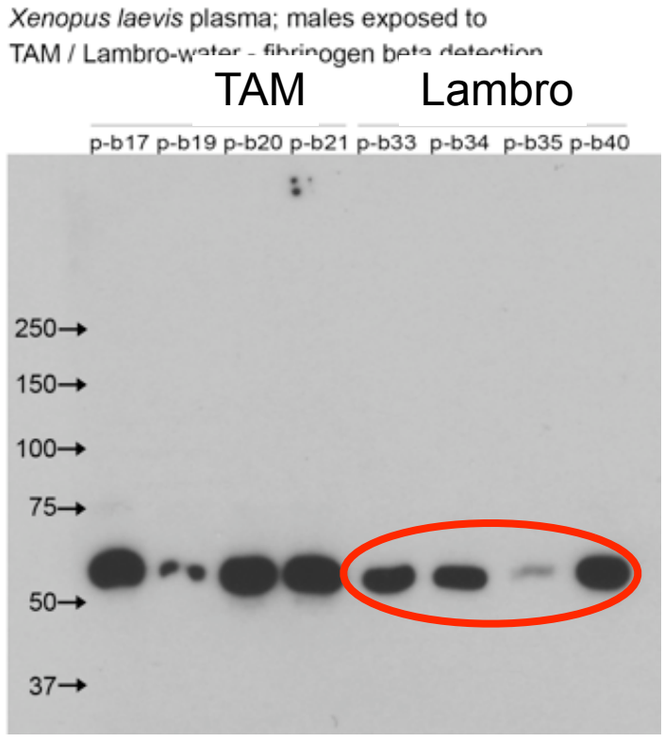




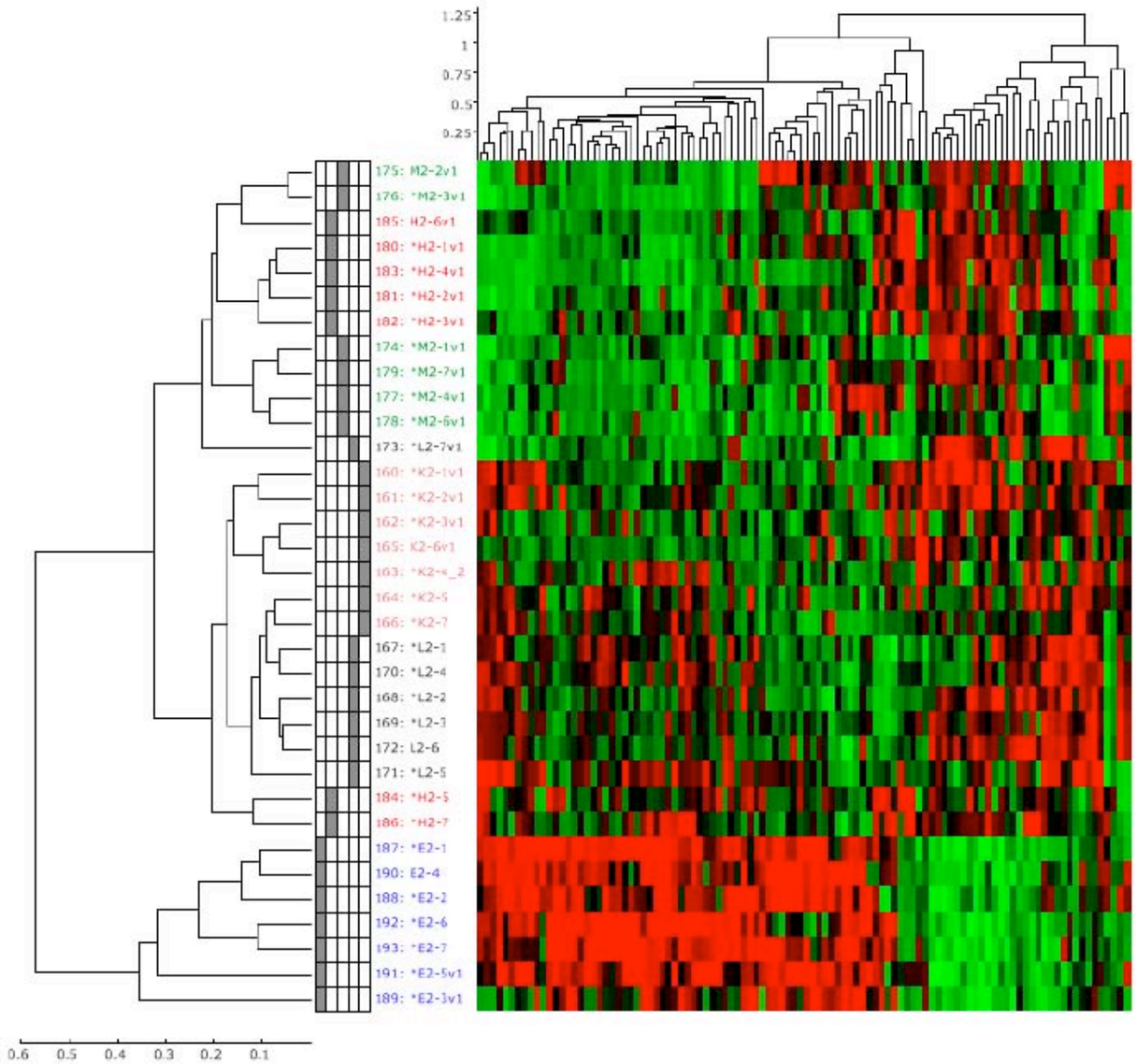


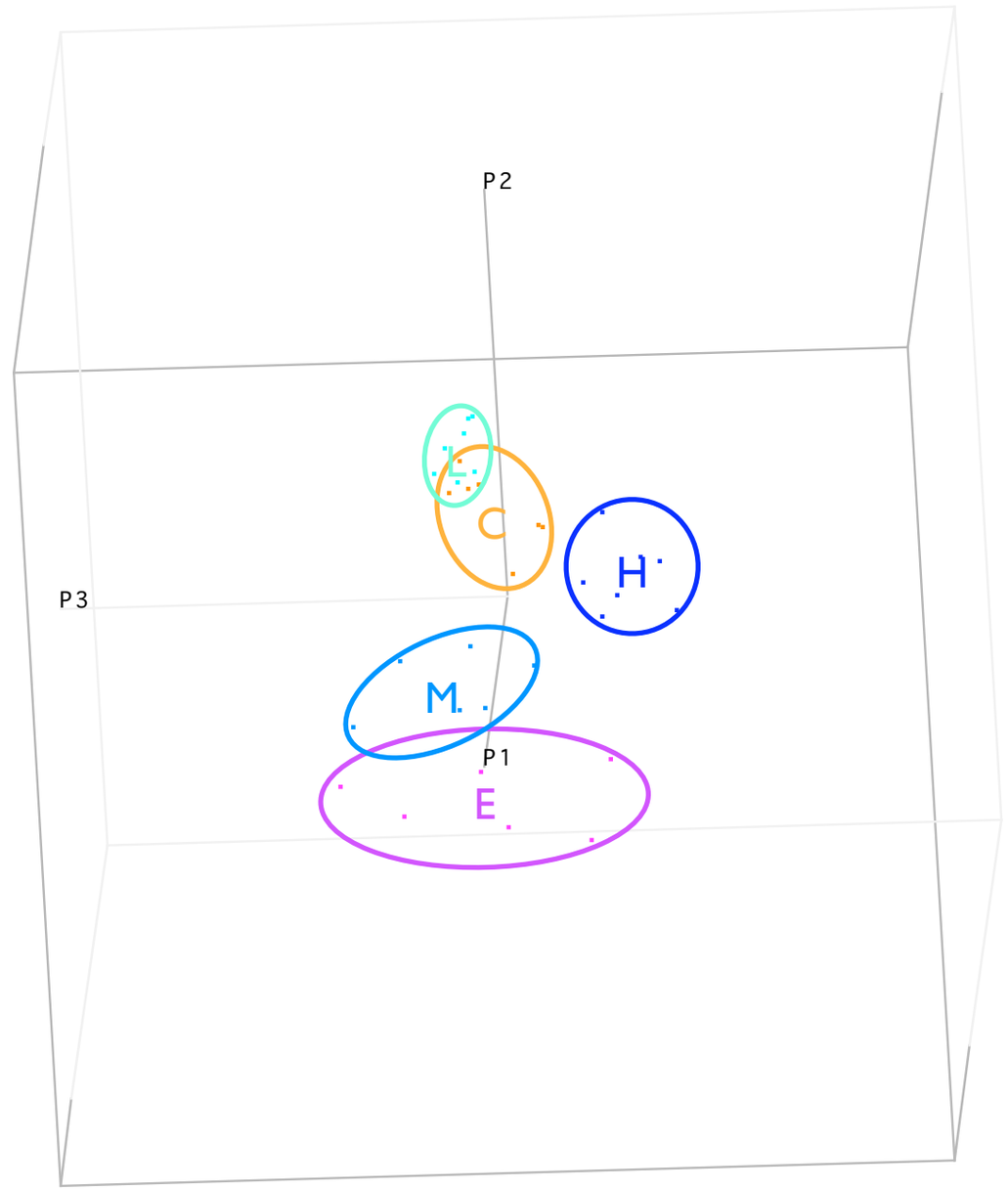
Proteome changes in Atlantic cod larvae exposed to produced water

Photo: J. Skadal and I. Rønnestad

Biomarker discovery in Atlantic cod fry liver after continuous exposure to produced water

Figure 2





Kjersem (2007), PhD thesis

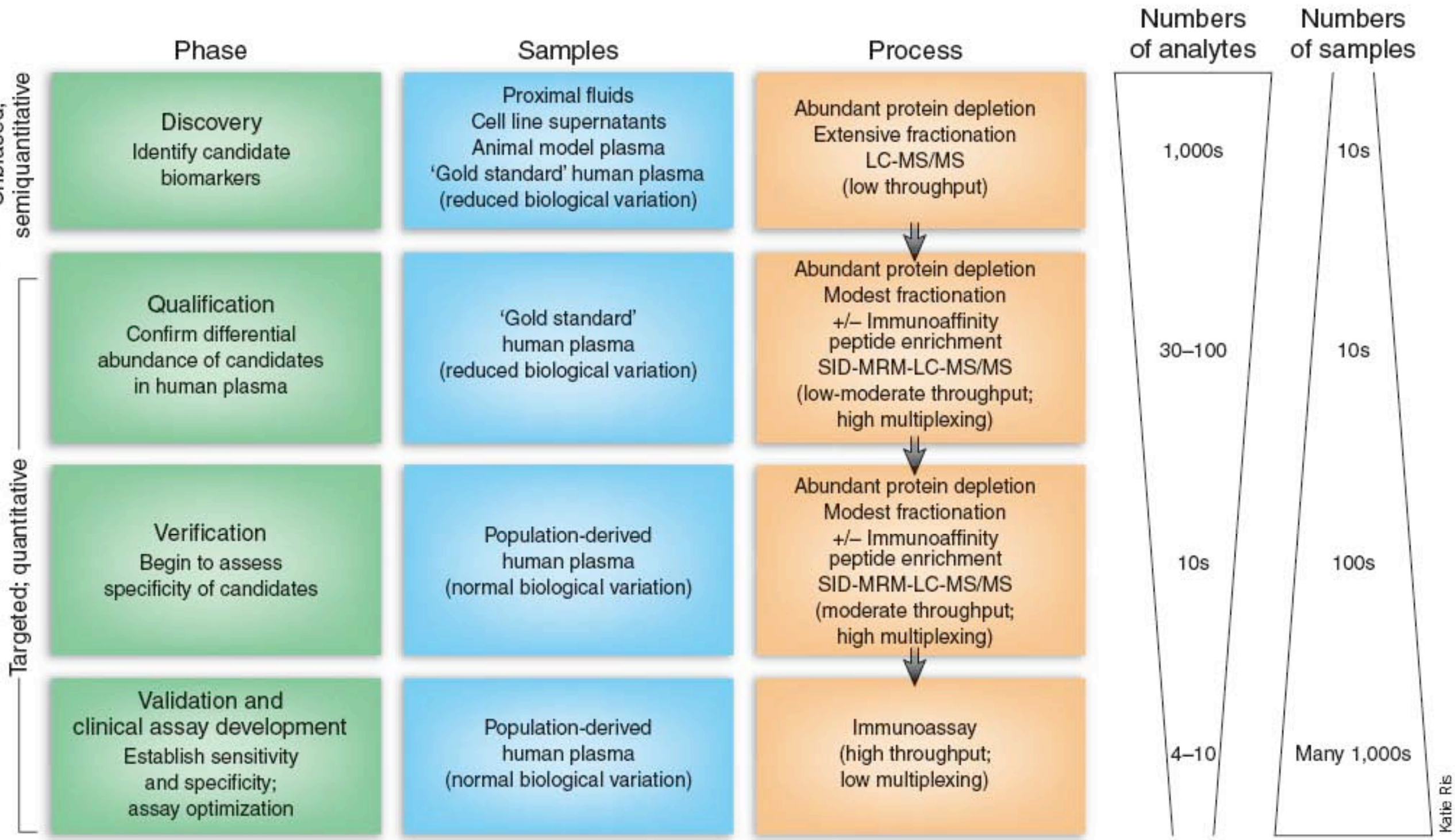
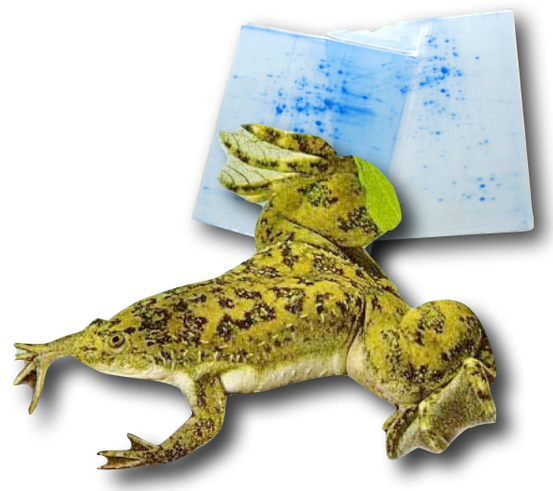
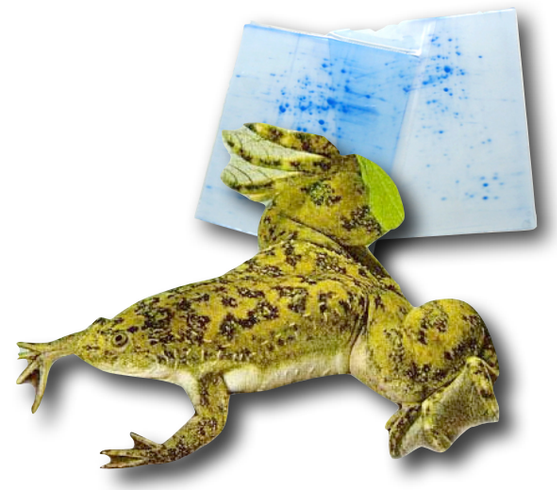


Figure 1 Process flow for the development of novel protein biomarker candidates. 'Numbers of analytes' refers to the number of proteins expected to be evaluated as candidate biomarkers in each phase of development. 'Numbers of samples' refers to the sample requirements for each phase. LC-MS/MS, liquid chromatography tandem mass spectrometry; SID, stable isotope dilution; MRM, multiple reaction monitoring.

Conclusions





Conclusions

- A toxicoproteomic strategy has been established to identify biomarker candidates under various exposure regimes in different species
- Higher identification success rates are obtained in species with better genomic coverage (e.g. X. laevis > carp > cod)
- Proteome changes linked to annotated databases and Gene Ontology terms may help elucidates toxicological mechanisms and modes of action
- In general, responses specific to a single chemical are interesting as biomarker candidates, however suites of biomarkers may prove more informative in field studies targeting emerging pollutants - e.g. applied in protein/antibody arrays

Acknowledgements

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Alberta Mandich, University of Genova, Italy
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