

# PROTEOMICS

**Supporting Information**

**for Proteomics**

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**Proteomic analysis of the chicken egg vitelline  
membrane**

## **SUPPLEMENTARY MATERIAL**



00572470.1 00680749.1 00684971.1	Q92108	+	Annexin I 38kDa protein 39kDa protein	<b>y,s</b>	166	2	4	6%	8	0.2
00591852.1 00577739.1	O52ZLO6 Q5ZMD1	+	14-3-3 protein $\alpha/\beta$ chain 14-3-3 protein $\theta$ ; all 14-3-3 entries share peptides		119 92	1 1	2 1	5% 6%	9	0.2 0.2
00601983.1			Similar to CRTAC1 protein; aa269-592 similar to cartilage acidic protein		81	1	1	2%	6	0.2
00596673.1	P09102		Protein disulfide-isomerase	<b>s</b>	214	2	2	3%	6	0.2
00597105.1 00682304.1			Hypothetical protein XP_415598/31kDa/similar to meteorin	<b>w,s</b>	107	1	1	3%	9	0.2
00571469.4 00591422.1	P14315-2 P14315-1	+	Isoform 2 of F-actin-capping protein subunit $\beta$ , isoforms 1 and 2		101	1	1	3%	9	0.2
00580166.1	P20136	+	Glutathione S-transferase 2	<b>w,s</b>	65	1	2	5%	9,10	0.2
00578026.1 00683830.1 00580166.1		+	Similar to fibulin-5/51kDa protein		91	1	2	2%	6	0.2
00571478.3 00593804.2 00786262.1 00573643.1	Q1KME6		GTP-binding protein/RAB6A		109	1	3	5%	9,10	0.2
00570652.1 00589837.2 00602725.2			Similar to ectonucleotide pyrophosphatase/phosphodiesterase 2/102kDa protein		83	1	1	2%	4	0.1
00576977.1			Similar to histone protein 2h3c1	<b>y,w,s</b>	134	2	2	2%	9,16	0.1
00597520.1	Q8QGG9	(+)	Similar to Slit homolog 1		112	1	5	>1%	3,4	>0.1
<b>KERATINS</b>										
00570671.2 00584075.2		(+)	Protein/otokeratin/type II cytoskeletal cochlear	<b>y,w,s</b>	408	4	17	7%	2,4-6,9,14, 16	0.7
00570671.2			Protein/keratin	<b>y,w,s</b>	408	4	17	10%	2	0.3

00577384.2 00581466.2	(+)	57kDa/similar to $\alpha$ -keratin IIC	<b>y,w,s</b>	189	2	8	4%	1,2,9,14,16	0.2
00574393.2 00581314.1		61kDa/type II $\alpha$ -keratin IIA	<b>y,w</b>	170	2	4	3%	9,16	0.2
00588495.1	(+)	Similar to type II keratin K6h	<b>y,w</b>	113	1	21	<1%	1,3,6,9,10, 15,16	<0.1

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**Table S2****The proteome of salt-washed vitelline membranes.**

Proteins are ordered according to decreasing emPAI. The protein score is the sum of MS/MS and MS<sup>3</sup> scores calculated using MSQuant. The sequence coverage was calculated for mature proteins. If a protein was identified in more than three gel sections, only sections yielding more than 5% of the total peptide number are indicated. #, proteins previously identified as VM components by sequence analysis, immunological methods, enzymatic activities or binding activities. +, proteins also identified in water-washed VM (in brackets if tentative identification). The average mass accuracy was 0.6ppm (p<0.05). Y, egg yolk; w, egg white; s, eggshell.

IPI_Chick	Swiss Prot/Trembl accession		Protein	Also present in	Protein score	Unique peptides	Accepted peptides	Sequence coverage	Gel section	emPAI
00575264.1	P79762 Q9PWF8	#,+	Zona pellucida protein 3 (ZP3)/ZPC;Unigene Gga.7210 (ZP3A of [10])		1961	21	2182	68%	6-11	4922.9
00600859.1	P00698	#,+	Lysozyme C	y,w,s	1460	15	472	84%	11,12	2781.6
00572084.1 00655503.2	P53478 P60706	+	Actin, cytoplasmic 1/5	y,w,s	1782	20	247	84%	6,7,9-12	386.5
00578517.1	Q6WV24 Q9DER4	#,+	Zona pellucida protein 1 (ZP1); Unigene Gga.224	y	1809	17	4283	24%	3-9	340.5
00583974.1	P01012	#,+	Ovalbumin	y,w	1668	16	335	66%	6-11	340.5
00574804.1	Q9PS49	#,+	VMO-II/β-defensin 11	w,s	495	6	44	73%	10-12	157.5
00584841.1	Q8AV77	+	Hep21	y,w,s	495	7	155	71%	10-12	157.5
00598229.1	O42273	+	Tenp	y,w,s	1176	11	492	41%	6-11	118.4
00578622.1	Q90Y11	+	Ovocalyxin-32	s	642	9	120	37%	3,6-11	99.0

00604279.1	Q6PTX2	+	Clusterin	<b>y,w,s</b>	1801	20	173	51%	6-10	52.4
00577021.1	P20763	+	Ig $\lambda$ chain C region	<b>y,w,s</b>	526	6	127	45%	9-11	50.8
00582126.1	P04210									
00591488.1	P01875	+	Immunoglobulin $\mu$ chain C-region	<b>y,w,s</b>	1291	13	209	41%	5-7	49.8
00595847.1		+	Similar to $\alpha$ 2-macroglobulin-1	<b>w,s</b>	6685	68	1411	50%	1-3,5	48.4
00596818.1	Q766V2	#+	Zona pellucida protein D; Unigene Gga.14454		809	10	260	24%	2,3,6-8	45.4
00580509.1	P02659	+	Apovitellenin-1	<b>y,s</b>	445	4	28	45%	4,11,12	30.6
00596201.1	Q25C36	+	Olfactomedin-Noelin-Tiarin factor 1	<b>w</b>	1263	13	128	40%	6-8	23.5
00591483.1	P09244	+	Tubulin $\beta$ 7	<b>s</b>	1474	13	40	64%	6,7	23.0
00570724.1			Similar to tubulin $\beta$ 2							
00580626.1	P09206		Tubulin $\beta$ 3; all 3 entries show partially identical peptide sets							
00584997.2	Q98UI9	#+	Ovomucin $\alpha$ -subunit	<b>w,s</b>	7263	77	1471	42%	1-3	22.1
00681274.1		+	7kDa/gallin	<b>w,s</b>	314	3	44	78%	4,11,12	20.5
00578012.2	Q4ADG4	+	Ovotransferrin BC/ovotransferrin	<b>y,w,s</b>	3309	20	183	58%	5,6	12.9
00683271.1	P02789									
00588908.1		+	Similar to ovulatory protein 2 (aa256-398); domains: 3 WAP	<b>w</b>	696	8	59	34%	8-12	11.3
00578559.1		+	Similar to polymeric immunoglobulin receptor (aa371-1005, 100% identity); extracellular: aa377-871	<b>w,s</b>	1435	17	253	27%	5-7,11,12	10.0
00573506.2	Q53HW8	+	Ovocalyxin-36	<b>s</b>	727	7	58	26%	6-10	9.0
00577587.1		+	Similar to ZPAX; Unigene Gga.11855; (ZPAX2 of [10])		1291	14	151	22%	1,3-6	8.2
00585021.1	P01013	+	Similar to ovalbumin-related protein Y (Ovalbumin X), aa109-496	<b>y,w,s</b>	1084	12	44	39%	6-8	7.9
00577274.1	O42390	+	RGD-CAP	<b>s</b>	1475	20	61	40%	5,6,10	7.4
00596061.1	P41366	#+	Similar to VMO-I (VMO-I is aa 142-324)	<b>y,w,s</b>	764	9	32	40%	10,11	7.3
00600265.1	Q5G8Y9	+	Apolipoprotein D	<b>y,w,s</b>	270	5	6	36%	11	6.2

00592851.1	Q5ZMA0	+	Hypothetical protein/ADP-ribosylation factor 1;	<b>w,s</b>	625	9	19	43%	10-12	7.1
00575684.1	P49702		ADP-RF 5							
00592098.1	Q5ZKR9		ADP-RF 4; partially identical peptide sets							
00598054.1		+	Immunoglobulin J linker	<b>w,s</b>	448	5	10	32%	10,11	5.8
00577039.1	P17785	+	Annexin A2; N-acetylated	<b>s</b>	1225	15	44	55%	8,9	5.7
00585604.1		+	Similar to mucin 5	<b>s</b>	4577	47	576	27%	1-6	5.4
00597482.1	Q6L608	#+	Similar to ovomucin $\beta$ -subunit/ovomucin $\beta$ -subunit (fragment)	<b>w</b>	3778	35	300	34%	1-3	5.0
00588683.2	P09572	#+	Na <sup>+</sup> /K <sup>+</sup> -transporting ATPase, $\alpha$ 1 chain; all peptides from 3 potential cytoplasmic domains (6-90, 151-286, 337-773)		2020	24	78	51%	1-6	5.0
00599562.1			7kDa protein/guanine nucleotide-binding protein $\gamma$ 10; N-term. N-acetyl Ser2		193	3	5	57%	12	4.6
00599279.1	P24479		S100-A11	<b>s</b>	190	2	10	22%	10	4.6
00581002.1	P62973	+	Ubiquitin/polyubiquitin	<b>y,w,s</b>	319	3	16	44%	3,4,10-12	4.6
00594278.1	P79781									
00578632.1	Q5ZKC9	+	14-3-3 protein $\zeta$ chain, N-acetyl Met1		695	8	16	39%	9,10	4.2
00579092.1	Q5ZMT0		14-3-3 protein $\epsilon$ chain		384	5	8	18%		1.5
00591852.1	O52ZLO6		14-3-3 protein $\alpha/\beta$ chain		242	3	4	18%		0.8
00577739.1	Q5ZMD1		14-3-3 protein $\theta$ ; all entries share peptides		559	6	7	28%		2.5
00589360.2		+	Similar to plasma GSH peroxidase	<b>y,w,s</b>	361	3	20	23%	10-12	4.6
00600353.1	8QFM7	+	Chondrogenesis-associated lipocalin	<b>w,s</b>	303	4	11	30%	10,11	3.6
00594564.1		+	Similar to nexin-1/plasminogen activator inhibitor type 1 member 1	<b>s</b>	1283	14	44	37%	6-8	3.6
00571529.1	Q6LC82		GTPase cRac1A	<b>s</b>	226	4	4	16%	11	3.6
00574703.1		+	Similar to Ig $\lambda$ chain	<b>y,w,s</b>	256	2	10	34%	9-12	3.6
00577820.1			Similar to ZPC; Unigene Gga.23380; all peptides in ZP domain (aa78-336); (ZP3B of [10])		430	5	12	27%	7,8	3.3
00594653.1	P00355	#+	Glyceraldehyde-3-phosphate dehydrogenase	<b>y,s</b>	498	6	26	31%	8,9	3.2



00598959.1	Q90694		Cell division control protein 42 homolog		360	4	7	25%	10,11	3.2
00585627.1		+	74kDa/similar to bactericidal/permeability-increasing protein-like 2	<b>w</b>	1031	12	41	17%	5,6,10	3.0
00587313.2 00681776.1	P10184	+	Ovoinhibitor/52kDa protein	<b>y,w,s</b>	706	9	14	26%	6,7,9	2.9
00580765.1	P08250	+	Apolipoprotein A-I	<b>y,w,s</b>	860	10	15	42%	10	2.9
00574658.1	P26652	+	Metalloproteinase inhibitor 3 (TIMP 3)	<b>w,s</b>	419	6	37	38%	9-11	2.8
00596111.1	Q5ZL15	+	Hypothetical protein/guanine nucleotide binding protein (G protein), beta polypeptide; N-term: acetylated Ser2	<b>s</b>	912	10	25	26%	8,9	2.8
00572470.1 00680749.1 00684971.1	Q92108	(+)	Annexin I 38kDa protein 39kDa protein	<b>y,s</b>	1051	9	33	30%	8,9	2.7
00578305.1			Similar to hypothetical A430083B19; domain: S-100	<b>s</b>	255	3	6	22%	11	2.7
00578026.1 00683830.1		(+)	Similar to fibulin-5/51kDa protein		541	7	12	19%	6,7	2.4
00573738.1	P01014	+	Ovalbumin-related protein Y	<b>y,w,s</b>	594	8	28	25%	6,7,11	2.4
00576782.1	P01038	+	Cystatin	<b>y,w,s</b>	188	2	6	24%	11	2.2
00680005.1 00594471.1		+	31kDa protein/Similar to erythrocyte band 7.2b stomatin (~aa379-657)		543	7	7	39%	9,10	2.2
00595925.1		(+)	Similar to Ig $\mu$ , heavy chain disease protein (BOT), partial	<b>y,s</b>	79	1	2	14%	5,6	2.2
00575913.1		+	Similar to complement C8 $\gamma$ -chain,partial	<b>y,w,s</b>	190	2	3	14%	11	2.2
00575989.1 00585941.1	P02552	+	Similar to tubulin $\alpha$ 2 (~aa1-500) Similar to tubulin $\alpha$ 3/ $\alpha$ 7	<b>s</b>	1243	13	32	35%	6-8	2.1
00594561.1	Q90W22	+	Similar to Slit homolog 2	<b>s</b>	1826	19	69	22%	2,3	2.0
00601120.2 00680878.1 00586548.2		+	41kDa/43kDa/54kDa protein; domain: fibrinogen-related (FR $\alpha$ D); partially identical peptide sets	<b>w,s</b>	816	9	28	23%	5,6	2.0

00601033.1	P00337	#,+	L-lactate dehydrogenase B chain	<b>y,s</b>	533	6	12	21%	8,9	1.9
00593541.2	Q1XIH7	+	Renin/prorenin receptor; extracellular ~aa23-300		586	6	6	18%	10	1.9
00598720.1	Q5ZHX1		Hypothetical protein; domain: Rap1		388	4	8	26%	11	1.8
00585276.2		+	13kDa; domains: 1 IG	<b>y,w</b>	322	3	72	17%	3,5,6,8,11, 12	1.8
00600667.2										
00683916.1										
00571825.2		+	62kDa/similar to oviductin protease; domains: 1 Tryp_SPc (trypsin-like serine protease), 2 CUB		595	8	34	20%	6,7,9,10	1.8
00601768.1	Q10751	+	Similar to angiotensinogen converting enzyme (extracellular domain (1-1156)	<b>w</b>	1745	20	50	25%	2,3	1.7
00598709.1			Similar to Ig heavy chain V-region	<b>y,s</b>	226	2	7	17%	5,6	1.7
00590375.1	Q90593	+	78kDa glucose-regulated protein/HSP70-5/BiP	<b>y,s</b>	1280	13	33	28%	6,7,9,10	1.7
00603309.1	O73885		Heat shock cognate protein 70; partially identical peptide sets							
00582091.4	P08106									
00589747.1	P20740	+	Ovostatin	<b>y,w,s</b>	1981	24	52	23%	2,3	1.7
00591229.1		+	Similar to Ig $\lambda$ chain	<b>y,w,s</b>	645	7	17	15%	10,11	1.6
00590719.3	O12945		Vitronectin	<b>y,s</b>	518	5	15	20%	6,7	1.6
00583337.1	P24367	+	Peptidyl-prolyl cis-trans isomerase B	<b>w,s</b>	379	4	5	21%	10	1.5
00586484.1	Q5ZH4W	(+)	Hypothetical protein/Ras-related protein Rab	<b>s</b>	421	4	7	22%	10-12	1.5
00596261.1	Q5ZIP7		5B/Rab-5C-like							
00600808.1	Q98932									
00580059.1		+	Similar to peroxiredoxin 1	<b>s</b>	263	3	7	14%	10,11	1.5
00600069.1	P21760	+	Extracellular fatty acid-binding protein	<b>y,w,s</b>	285	3	4	18%	11	1.4
00591421.2			Similar to ZPC; domain: partial ZP (C-terminal part), (ZP3B, LOC415305 of [10]); Unigene Gga.32611		327	3	8	35%	7,8	1.4
00681412.1	O93467		22kDa protein/similar to RhoA GTPase; domain: RhoA-like		266	3	8	26%	10-12	1.4
00578325.2										
00589985.3	Q90835	+	Elongation factor 1- $\alpha$ 1	<b>y,s</b>	397	5	16	14%	7,9-10	1.4

00602594.1	P18359	+	Destrin (actin-depolymerizing factor); N-term. acetylated	<b>s</b>	212	3	11	20%	11	1.3
00577014.2	Q4JK63		20-hydroxysteroid dehydrogenase		400	5	5	25%	10	1.3
00604297.1			Similar to chloride intracellular channel 4	<b>s</b>	319	4	4	23%	10	1.2
00679854.1										
00680447.1										
00579242.1	O93601		Apolipoprotein A-IV		660	7	9	24%	8,9	1.0
00760358.1	Q31414		MHC class II $\beta$		318	3	5	17%	10	1.0
00782845.2	O19497									
00782850.1	O19496									
00575013.2			52kDa protein/similar to MGC75581/similar to milk fat globule-EGF factor 8; domains: 2 EGF, 2 FA5/8C (coagulation factor 5/8 C-term (discoidin)	<b>y,s</b>	502	6	11	16%	6	1.0
00588573.1										
00593485.3	Q5ZM28		Hypothetical protein/sorcin; domains: EF-hand		226	3	3	16%	11	1.0
00684474.1										
00584318.2			Similar to ras-related v-ral simian leukemia viral oncogene		205	3	3	15%	10	0.9
00684262.1			14kDa; domain Ig	<b>y</b>	195	2	5	23%	1,5,6	0.9
00580166.1	P20136	(+)	Glutathione S-transferase 2	<b>w,s</b>	382	4	4	23%	10	0.9
00579518.1	P21566		Cofilin-2; N-term: N-acetyl Ala2		201	3	3	25%	11	0.9
00601265.3	P02701	+	Avidin	<b>y,w,s</b>	169	2	2	14%	11	0.9
00599180.1		+	Similar to leucine-rich repeat containing 19	<b>s</b>	238	3	7	9%	8,9	0.9
00591299.2	P02845	+	Vitellogenin-2	<b>y,s</b>	1599	19	34	13%	3-5,10	0.8
00596765.1	Q9W6J2		Glutathione S-transferase class $\alpha$	<b>s</b>	139	3	3	15%	11	0.8
00598984.1	Q5ZLC9		Golgin subfamily A member 7		176	2	3	18%	12	0.8
00585601.1	Q5F3B5	+	Hypothetical protein/guanine nucleotide binding protein (G protein), q polypeptide		426	4	16	16%	7,8,10	0.8





00572461.1	Q90734	+	$\alpha$ -actinin	<b>s</b>	288	3	9	3%	4	0.1
00588764.1	P05094-2									
00597432.1	P05094-1									
00593582.2			Similar to Leucyl-cystinyl aminopeptidase/oxytocinase		197	2	2	2%	3	0.1
00576318.1	Q90574		Filamin		357	5	6	2%	3,10	0.1
00576308.1			Similar to carboxypeptidase D; domains: 3 peptidase_M14 (Zn-carboxypeptidase)	<b>w</b>	239	3	4		3	0.1
00575584.1	P51913		$\alpha$ -enolase	<b>y,s</b>	134	1	3	4%	7	0.1

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**Table S3****Tentatively identified proteins from salt-treated vitelline membrane samples.**

Proteins with high quality spectra and high Mascot scores identified tentatively because of low or missing MS<sup>3</sup> scores, insufficient quality of MS<sup>3</sup> spectra or MS<sup>3</sup> data from very short ions. Keratins were included in this table because they usually showed overlapping sets of peptides and had also peptides in common with closely related human keratins. Y, egg yolk; w, egg white; s, eggshell. +, proteins also identified in water-washed VM (in brackets if tentative identification).

IPI_Chick	Swiss Prot/Trembl accession	Protein	Also present in y	Prot-ein score	unique peptides	accepted peptides	Sequence coverage	Gel section	emPAI
00681344.1		8kDa protein/similar to Kunitz-like protease inhibitor		95	1	22	18%	8	9.0
00583446.2 00581978.1 00681289.1 00735151.1	Q7LZ64 (+)	Vesicle-associated membrane protein (VAMP) 2 Similar to VAMP 2 14kDa protein VAMP 3		140	1	4		11	1.5
00590428.1		Similar to Ig $\alpha$ chain,partial	y,s	271	3	6	14%	5	1.3
00584017.1	P26990	ADP-ribosylation factor 6	s	163	2	3	12%	11,12	0.9
00586861.1		Similar to actin-related protein 2/3 complex subunit 4	s	157	2	2	14%	11	0.9
00582708.1	Q5ZJ03	Hypothetical protein/guanine nucleotide –binding protein $\gamma$ 2		95	1	1	30%	11	0.8

00589456.1	P02607-2		Myosin light chain polypeptide 6		207	2	5	14%	1,2	0.7
00598184.1	P02607-1									
00603045.1			Similar to phosphoethanolamine-binding protein-1	s	217	2	2	16%	11	0.7
00591935.1		(+)	Similar to solute carrier family 39 (zinc transporter) member 8; peptide is not in solute carrier-resembling sequence but in aa1-160	s	92	1	7	9%	3,6-8	0.6
00586294.3	Q02960		Macrophage migration-inhibiting factor		121	1	4	14%	11,12	0.5
00580686.1	Q9DEQ4		Secreted frizzled-related protein 1		138	2	2	8%	8	0.5
00572756.1	Q9PRS8	+	Ovocleidin-17	y,s	93	1	2	7%	11,12	0.4
00602255.2	P09648		Similar to cathepsin L (catalytic domain, aa101-331)	s	111	1	2	6%	9	0.4
00596504.1			Hypothetical protein/Ras viral oncogene homolog 2-related		132	2	2	11%	10	0.4
00601567.2			Similar to Na <sup>+</sup> /Cl <sup>-</sup> -dependent neutral and basic amino acid transporter B		197	2	4	4%	6	0.3
00600831.2			Similar to solute carrier family 12 member 2		201	2	2	3%	10	0.2
00574866.1	Q08392		Glutathione S-transferase	s	96	1	1	4%	11	0.2
00588923.1	P26697									
00603445.1	Q9W6J3									
00680510.1										
00592470.1	P17153		Annexin A5	s	141	2	2	5%	9	0.2
00578917.1	O42163	+	Cochlin	s	192	2	4	4%	7	0.2
00574613.1		#	Similar to 5-nucleotidase		110	1	1	3%	6	0.2
00595344.1			Similar to testican-1		140	2	2	3%	6	0.1
00583749.1			Similar to PAPS synthase 2		99	1	1	2%	6	0.1
00603766.1			Similar to insulin receptor tyrosine kinase 53kDa substrate		173	2	2	2%	10	0.1



00603573.2	P31696-1	+	Agrin, peptide set matches isoforms 1-6	s	187	2	3	1%	1	0.1
00592771.2	P31696-2									
00579891.2	P31696-3									
00597536.2	P31696-4									
00590466.2	P31696-5									
00786263.1	P31696-6									
00597520.1	Q8QGG9	(+)	Similar to Slit homolog 1		104	1	6	>1%	1-3	>0.1
00602986.2		+	Similar to desmoplakin	y,s,w	255	3	3	>1%	7	>0.1
00597868.1			Similar to laminin $\alpha$ 3		216	2	2	1%	3	>0.1
00597846.1	Q6KDZ1		Perlecan/basement membrane-specific heparin sulfate proteoglycan	s	163	2	2	>1%	2	>0.1
00575808.2	Q5F3V2 Q9PU50		Hypothetical protein/ATP citrate lyase		93	1	1	>1%	10	>0.1
<b>KERATINS</b>										
00570671.2		(+)	Protein/otokeratin/type II cytoskeletal cochlear	y,w,s	420	4	13	5%	6,7,12	0.3
00584075.2										
00577384.2		(+)	57kDa/similar to $\alpha$ -keratin IIC	y,w,s	124	1	4	2%	7	0.1
00581466.2										
00588495.1		(+)	Similar to type II keratin K6h	y,w	130	1	7	>1%	2,4,5,7,11, 12	>0.1

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