

## Supplemental Information

### 2-D Standardisation

Every attempt was made to standardize all procedures as much as possible, which can be divided into several sub-sections; (i) Sample acquisition, (ii) Membrane preservation and storage, (iii) Membrane preparation as for transplantation, (iv) protein extraction and purification, (v) 1-DE, (vi) 2-DE, (vii) gel staining, and, (viii) image acquisition and analysis. Standardisation of (i)-(iii) is discussed below in comment 10. For all subsequent procedures all chemicals, reagents and IPG strips were obtained from reputable sources in appropriate sized batches to complete the study. These were then tested before use. Where possible, all buffers and solution used were made as batches in enough ready-made aliquots and stored at -80°C until needed. If not, they were made fresh. For protein extraction and purification (iv), where possible samples were prepared in batches, following a well established and optimised protocols by the same experienced person; quantitated in batches and then aliquoted into single-use volumes and stored at -80°C. Once thawed and used any remaining buffers/samples were discarded and not re-used. 1-D gels were prepared and run as batches. Purification of samples was performed identically, and replicate samples were pooled before 1-D loading. (v) Replicate samples were run within a single batch and all batches were focused under identical conditions and parameters. Likewise, in the 2-D. (vi). Samples were equilibrated and run as batches of replicate gels following identical procedures and parameters. Where possible, several replicate 1-D sets were run within the same batch of 2-D gel. Gels were stained identically following an optimized procedure for each protein load. Replicate sets of gels were stained using a single batch of buffers. Gels were imaged immediately after staining, and using identical parameters.

**Supplemental Table I. Human Amniotic Membrane proteins identified from 2-D gels by peptide mass fingerprinting.** Acc. No., accession number in the Swiss-Prot/TrEMBL databases. Spot/Gel ID corresponds to spot given in the annotated gel images (Fig. 2) and the gel used for mass spectrometric identification. Theoretical molecular mass (Mr, kDa) of the full-length protein against the observed experimental running molecular mass. Protein score, no. of matching peptides, E-value, sequence coverage (%) of the identified proteins, and the randomised (r) score are shown.

**Supplemental Table II. Proteins identified from 2-D gels by nano-ESI-Q-TOF MS/MS.** Acc. No., accession number in the Swiss-Prot/TrEMBL databases. Spot/Gel ID corresponds to spot given in the annotated gel images (Fig. 2) and the gel used for mass spectrometric identification. Mr(expt),

experimental molecular mass of the peptide (Da). Mr(calc), the calculated molecular mass of the peptide (Da),  $\Delta$ Mr, Mr(expect)-Mr(calc) (Da).

**Supplemental Table III. Complete list of proteins identified in human amniotic membrane.**

Functional group, proteins are categorised according to their primary function as reported in the current literature. Alternative reported functions are also given. Blood/plasma (B), classical blood proteins; Calcium binding (CB), calcium binding proteins; Cell metabolism (CM), proteins involved in cell metabolism and general turnover; Cell protection (CP), protection of cell against free radicals and apoptosis; Cell Structure (CS), associated with maintaining and modulating cell structure; Extracellular (EC), proteins associated with the ECM; Extracellular function (EF), intra-cellular proteins or proteins located on the outside of the cell surface that have a reported extracellular function; Immune (I), proteins involved in the immune response; Protein turnover (PT), protein synthesis, folding, and degradation; Miscellaneous (Misc), Unknown function in AM. Acc. No., accession number in the Swiss-Prot/TrEMBL databases. Primary location and alternative location, Sub-cellular/extracellular location categorised as reported in the current literature. Cytoplasmic & Other (CO), found in cytoplasm and in other sub-cellular/extracellular locations; Cytoplasm cytoskeleton (CK), forms or associated with cytoskeleton; Cytoplasmic (CP), found in cytoplasm only; Extracellular (EC), extracellular proteins and extracellular matrix proteins; Endoplasmic Reticulum (ER), located in the endoplasmic reticulum; Golgi (G), located in the Golgi body; Mitochondria (M), located in the Mitochondria; Membrane proteins (Mb), proteins associated with organelle and the plasma membrane; Nucleus (N), located in the nucleus; Unknown (U) Unknown location. Spot no. Spot number corresponds to spot given in the annotated gel images (Fig. 2). Identified in AM, proteins are categorised according to previous identifications in mammalian amniotic membrane by PMF or MS/MS; Y, protein previously identified in mammalian AM by PMF or MS/MS; N, protein never identified or only identified previously identified by an indirect (e.g. antibody-based) method in mammalian amnion.

**Supplemental Table I.** Spots identified by peptide mass fingerprinting.

Protein Name	Spot/Gel ID	Acc. No.	Mr (kDa) Observed/ theoretical	pI Observed/ theoretical	Score	No. of matching peptides	E-Value	Sequence Coverage (%)	R Score
4-PH alpha-1	41/M	P13674	62/59.1	5.9/5.7	161	12	4.40E-12	30	46
60 kDa heat shock protein	18/M	P10809	64/57.9	5.2/5.2	99	8	6.60E-06	20	51
Actin; Cytoplasmic Chain1	36/B	P60709	47/43	5.2/5.3	94	6	2.30E-05	22	44
Alpha crystallin B chain	12/M	P02511	21/20.1	7/6.7	79	5	7.30E-04	23	42
Alpha enolase	26/B	P06733	50/47	6.7/7.0	126	9	1.40E-08	27.9	39
Alpha enolase	27/M	P06733	50/47	6.9/6.9	125	9	1.70E-08	27.3	40
Annexin A1	22/B	P04083	35.1/38.6	6.4/6.6	67	5	1.10E-02	20	37
Annexin A1	65/W	P04083	38/38.5	6.4/6.6	158	9	8.70E-12	37	48
Annexin A4	17/M	P09525	33/35.8	5.8/5.8	132	11	3.50E-09	31	42
Annexin II	20/M	P07355	35/38.6	6/6.6	52	7	0.32	18	32
Annexin II	23/M	P07355	36/38.5	7.0/7.6	254	18	2.20E-21	58	37
Annexin II	24/B	P07355	36/38.5	7.3/7.6	130	9	5.50E-09	30	36
BIG-H3	40/M	Q15582	63/73.1	7.0/7.4	127	10	1.10E-08	18.6	37
Chain 2; Gelsolin	66/M	P06396	83/80.5	5.6/5.5	85	7	1.70E-04	14	59
Chain 2; Gelsolin	67/M	P06396	83/80.5	5.7/5.5	113	10	2.80E-07	17	56
Citrate Hydrol-lyse	51/M	Q99798	79/82.4	7.0/6.9	157	13	1.10E-11	21	36
Collagen type $\pm$ 1(VI)	54/M	P12109	116/108.5	5.0/5.3	161	14	4.40E-12	18	40
Collagen type VI $\pm$ 2(VI)	53/M	P12110	111/97.4	6.1-6.2/5.4	72	10	0.0032	22.5	39
Elongation Factor 2	25/M	P49411	46/45	6.7/6.3	162	11	3.50E-12	33	41
Elongation Factor 2	69/M	P13639	93/95.2	6.9/6.4	78	8	9.80E-04	10	39
Endoplasmic precursor	63/W	P14625	98/90.17	4.7/4.7	104	11	2.20E-06	14	41
Ezrin (villin 2)	49/M	P15311	77/69.2	6.3/5.9	136	12	1.40E-09	20.3	40
Flavin reductase	58/W	P30043	23/21.9	7.3/7.3	93	6	3.00E-05	36	46
Galectin I	11/M	P09382	13/14.6	5.7/5.3	59	3	0.069	32	45
Glutamate dehydrogenase 1	28/M	P00367	53/61	6.9/6.7	153	13	8.70E-12	26	41
Glutathione S-transferase P	60/W	P09211	23.2/25	5.6/5.4	143	8	2.80E-10	51	39
GRP58	37/M	P30101	58/54.2	5.8/5.6	125	11	1.70E-08	23	38
Heat shock 70kDa protein 1	43/M	P08107	70/70.0	5.4/5.5	81	8	4.20E-04	9.7	41
Heat shock 70kDa protein 9B	44/M	P38646	73/68.6	5.4/5.4	113	8	2.80E-07	16	40
Hemoglobin beta chain	5/B	P68871	15/16	7.1/7.2	118	7	8.70E-08	54	43
HSRBC protein	33/M	Q969G5	41/27.6	5.6/5.9	91	7	1.20E-04	28	44
HSRBC protein	32/M	Q969G5	41/27.6	5.8/5.9	91	7	1.20E-04	28	44
Integrin alpha-6	55/M	P23229	105/101.7	4.3-5.6/5.5	85	8	1.90E-04	11.5	36
Keratin 24	47/M	Q9NXG7	59/55.6	4.9/4.9	153	13	7.00E-11	21	40
Keratin, type I cytokeratin 18	30/M	P05783	44/47.9	6.3/5.3	115	9	1.70E-07	24.2	43
Keratin, type I cytoskeletal 17	42/M	Q04695	54/49	5.0/5.0	233	18	2.80E-19	42	44
Keratin, type II cytoskeletal 6A	38/M	P02538	59/60	6.6/8.1	94	8	2.20E-05	13.5	40
Keratin, type II cytoskeletal 6A	39/M	P02538	56/59.9	7.3/8.1	87	7	0.00011	16.7	40
Keratin, type II cytoskeletal 8	68/M	P05787	99/53	5.2/5.55	153	12	1.10E-11	28	40
Lamin A/C	50/M	P02545	72-79/74.1	6.6-6.9/6.6	211	18	4.40E-17	31	42
Macrophage capping protein	64/W	P40121	44/38.51	6.0/5.8	55	5	1.70E-01	16	40
Mimcan [Precursor]	19/M	P20774	34/34	5.3/5.4	90	6	5.00E-05	23	44
Neutral alpha-glucosidase AB	57/M	Q14697	94/107	5.8/5.7	233	20	2.80E-19	27	45
Ornithine aminotransferase, mitochondrial precursor	29/M	P04181	47/48	6.3/6.57	100	6	6.20E-06	19	40
Peroxiredoxin 6	16/M	P30041	26/25	6.2/6.0	157	9	1.10E-11	51	45
Phosphoglycerate Kinase 1	70/W	P00558	43/44.4	8.2/8.3	163	11	2.80E-12	35	39
Phosphoglycerate mutase 1	15/B	P18669	28/28.7	6.8/6.7	67	5	0.01	24	40
pp-GalNAc-T5	52/M	Q7Z7M9	107/106.2	7.0-7.1/9.5	32	7	32	10	37
Protein disulphide-Isomerase	45/M	P07237	64/57	4.6/4.7	196	15	1.40E-15	35	56
Septin 2	31/M	Q15019	45/41.5	6.3/6.2	57	4	1.10E-01	13	44
Serum albumin precursor	48/M	P02768	70/66.4	5.9/5.7	137	11	1.10E-09	18	40
Superoxide dismutase	13/M	P04179	21/22.2	7/6.7	116	6	1.40E-07	36	49
Thrombospondin 1	21/M	P07996	31/127.5	5.7/4.7	120	10	5.50E-08	10.3	40
Thrombospondin-1 Precursor	62/W	P07996	31/127.5	5.7/4.7	103	8	2.80E-06	9	38
Triosephosphate isomerase 1	14/B	Q8WWD0	26/26.6	6.6/6.5	207	12	1.10E-16	62	44
Triosephosphate isomerase 1	59/W	Q8WWD0	26/26.6	6.7/6.5	153	9	2.80E-09	49.4	37
Vimentin	46/M	P08670	65/53.5	4.9/5.1	152	14	3.50E-11	32	42