

# Proteomic Analysis of Domestic Cat Blastocysts and Their Secretome Produced in an In Vitro Culture System without the Presence of the Zona Pellucida

Daniel Veraguas-Dávila <sup>1,2,\*</sup>, Camila Zapata-Rojas <sup>2</sup>, Constanza Aguilera <sup>3</sup>, Darling Saéz-Ruiz <sup>2</sup>, Fernando Saravia <sup>2</sup>, Fidel Ovidio Castro <sup>2</sup> and Lleretny Rodriguez-Alvarez <sup>2</sup>

<sup>1</sup> Escuela de Medicina Veterinaria, Departamento de Ciencias Agrarias, Facultad de Ciencias Agrarias y Forestales, Universidad Católica del Maule, Km 6 Los Niches, Curicó 3340000, Chile

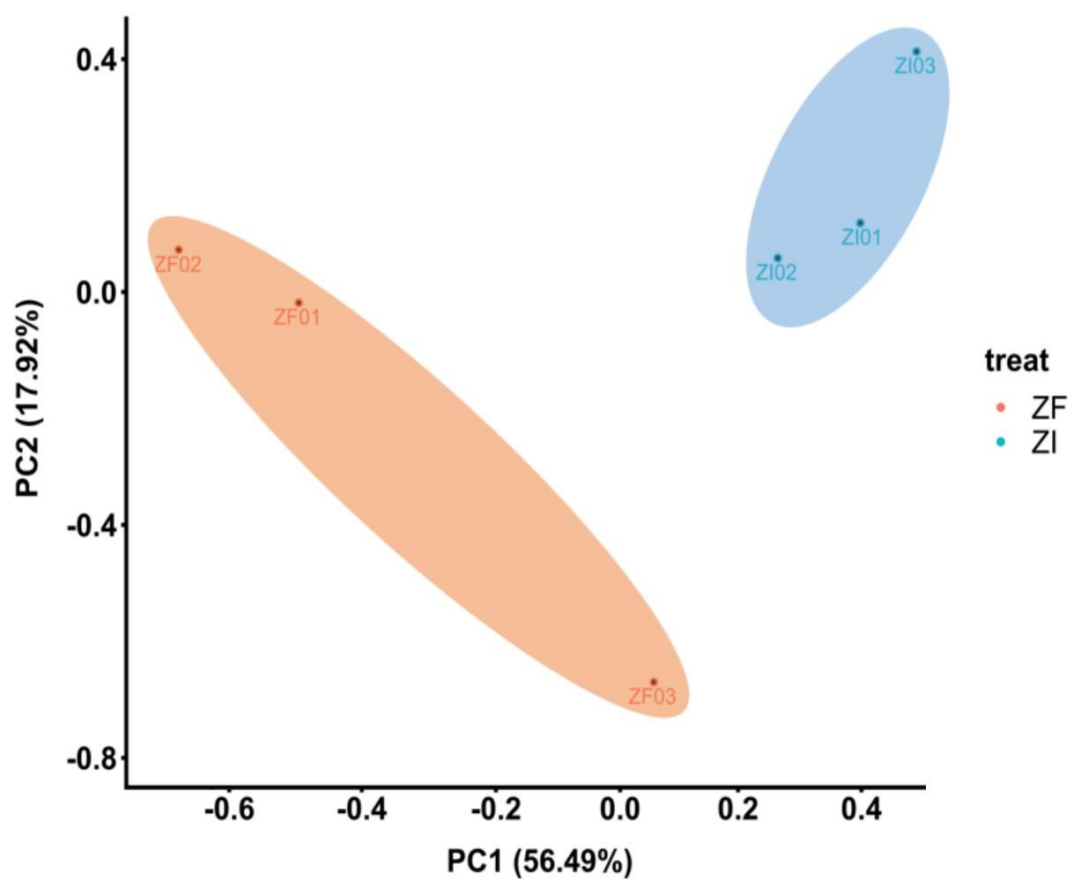
<sup>2</sup> Department of Animal Science, Faculty of Veterinary Sciences, Universidad de Concepción, Av. Vicente Méndez 595, Chillan 3780000, Chile; mv.camilazapatarojas@gmail.com (C.Z.-R.); darlin.saez.r@gmail.com (D.S.-R.); fsaravia@udec.cl (F.S.); fidcastro@udec.cl (F.O.C.); llrodriguez@udec.cl (L.R.-A.)

<sup>3</sup> School of Veterinary Medicine, Faculty of Natural Sciences, San Sebastián University, Concepción 4081339, Chile; constanza.aguilera@uss.cl

\* Correspondence: dveraguas@ucm.cl or daniveraguasdv@gmail.com

## Supplementary files

<b>S1. Principal component analysis (PCA)</b> .....	2
<b>S2. Quantifiable proteins of ZF and ZI blastocysts</b> .....	3-8
<b>S3. Detailed functional annotation of DEPs of blastocysts</b> .....	9-11
<b>S3.1-6. GO of proteins of ZF blastocysts (S3.1, S3.2, S3.3) and ZI blastocysts (S3.4, S3.5, S3.6)</b> .....	12-17
<b>S4. Proteins identified in the culture medium of blastocysts from the ZF and ZI groups</b> ...	18-19
<b>S5. GO annotation of the proteins identified in the culture</b> .....	20-23
<b>S6. Detailed functional annotation of proteins identified in conditioned medium</b> .....	24-26



**Figure S1. Principal component analysis (PCA).** PCA of the total proteins detected in each replicate from the ZF (ZF01, ZF02 and ZF03) and ZI (ZI01, ZI02 and ZI03). The PCA shows clusters of expressed proteins that differ across the samples.

**Table S2. Quantifiable proteins of ZF and ZI blastocysts.** Quantifiable proteins identified in domestic cat blastocysts from the ZF and ZI groups. Differentially expressed proteins (DEPs)  $P < 0.05$ .

	Protein (ID)	Log2FC	P. Value
1	M3VZQ3 M3VZQ3_FELCA	3.17734479	8.8332E-05
2	A0A337SVF6 A0A337SVF6_FELCA	-2.81974135	0.00076552
3	A0A2I2UXE2 A0A2I2UXE2_FELCA	-2.65692623	0.00244662
4	A0A2I2U1C5 A0A2I2U1C5_FELCA	3.98060042	0.00311181
5	M3WCZ9 M3WCZ9_FELCA	-2.25459988	0.00357124
6	A0A5F5XCJ6 A0A5F5XCJ6_FELCA	-2.11846902	0.00388714
7	M3WHX6 M3WHX6_FELCA	-1.74681032	0.00401573
8	A0A337SCY3 A0A337SCY3_FELCA	-1.9626275	0.00586399
9	A0A2I2UZM2 A0A2I2UZM2_FELCA	1.95964111	0.00625748
10	A0A2I2U221 A0A2I2U221_FELCA	2.23313259	0.00612021
11	A0A337SW00 A0A337SW00_FELCA	-2.0509994	0.00760704
12	A0A2I2UXE4 A0A2I2UXE4_FELCA	2.31927503	0.00962896
13	M3VYI7 M3VYI7_FELCA	2.71239201	0.00899639
14	M3VX46 M3VX46_FELCA	1.61502009	0.01107067
15	A0A337S6R5 A0A337S6R5_FELCA	2.42297848	0.01078582
16	M3W9Y8 M3W9Y8_FELCA	2.01100493	0.01179752
17	M3WAW0 M3WAW0_FELCA	-1.48787899	0.01293448
18	A0A5F5XWX3 A0A5F5XWX3_FELCA	-1.82092779	0.01365135
19	A0A5F5XQQ6 A0A5F5XQQ6_FELCA	-1.9562502	0.01636457
20	M3WCX7 M3WCX7_FELCA	1.87232765	0.01809163
21	A0A5F5Y0Y9 A0A5F5Y0Y9_FELCA	1.89221244	0.01811358
22	A0A2I2UL05 A0A2I2UL05_FELCA	-1.5655253	0.01909538
23	M3VW27 M3VW27_FELCA	-1.46532	0.01962583
24	M3WD17 M3WD17_FELCA	-1.36580332	0.0206993
25	A0A337SJ9 A0A337SJ9_FELCA	1.42487437	0.02292737
26	M3W1X7 M3W1X7_FELCA	-1.60814943	0.02304837
27	A0A5F5XWD4 A0A5F5XWD4_FELCA	2.23313382	0.02424332
28	M3W9X1 M3W9X1_FELCA	3.21413703	0.02400918
29	A0A2I2UMD6 A0A2I2UMD6_FELCA	1.48842654	0.02362781
30	M3X4Y3 M3X4Y3_FELCA	-1.32858383	0.02750559
31	M3WFT4 M3WFT4_FELCA	1.34473579	0.02860092
32	M3WDF5 M3WDF5_FELCA	-1.44449596	0.03319027
33	Q66RN5 EF1A1_FELCA	1.51020066	0.03705747
34	M3WVC7 M3WVC7_FELCA	1.35282905	0.04453224
35	A0A5F5XYM1 A0A5F5XYM1_FELCA	-2.29930597	0.03963366
36	A0A2I2U7Z5 A0A2I2U7Z5_FELCA	1.60935135	0.0440415
37	M3WIA4 M3WIA4_FELCA	-1.28571479	0.04705984
38	A0A2I2V068 A0A2I2V068_FELCA	-1.35619626	0.04892405
39	M3WJL8 M3WJL8_FELCA	1.29586264	0.04988445
40	M3WRT2 M3WRT2_FELCA	2.0962291	0.04569637

41	M3W9D5 M3W9D5_FELCA	-1.20664542	0.04717624
42	A0A337S416 A0A337S416_FELCA	1.3425531	0.04971628
43	M3WJZ7 M3WJZ7_FELCA	-1.28876733	0.05618766
44	M3W6H0 M3W6H0_FELCA	-1.51076432	0.05877985
45	A0A2I2U4B2 A0A2I2U4B2_FELCA	-1.21562436	0.06292421
46	A0A5F5Y5E1 A0A5F5Y5E1_FELCA	-1.94225307	0.06737603
47	M3X1T7 M3X1T7_FELCA	2.8291405	0.07447896
48	A0A5F5XQT3 A0A5F5XQT3_FELCA	-1.03582209	0.06176732
49	M3W6X3 M3W6X3_FELCA	1.11369034	0.06106935
50	M3WXV4 M3WXV4_FELCA	1.14585085	0.07820449
51	M3WRZ7 M3WRZ7_FELCA	1.19497296	0.07464491
52	A0A5F5XFM1 A0A5F5XFM1_FELCA	-1.32299395	0.08053958
53	A0A2I2UNG3 A0A2I2UNG3_FELCA	1.76317592	0.07434401
54	M3XDX6 M3XDX6_FELCA	-1.11660959	0.07863656
55	A0A337SQQ5 A0A337SQQ5_FELCA	-1.30598621	0.0871821
56	A0A2I2UD00 A0A2I2UD00_FELCA	-1.18176787	0.08979881
57	A0A2I2UUV9 A0A2I2UUV9_FELCA	1.09493502	0.09261486
58	A0A5F5XM88 A0A5F5XM88_FELCA	-1.33422208	0.08927905
59	A0A5F5XNM3 A0A5F5XNM3_FELCA	-0.94863444	0.07606561
60	M3WUL6 M3WUL6_FELCA	1.24481136	0.09206694
61	A0A337RZN8 A0A337RZN8_FELCA	1.01758194	0.09952202
62	A0A2I2U5M9 A0A2I2U5M9_FELCA	1.01501299	0.10796034
63	A0A2I2UQ30 A0A2I2UQ30_FELCA	-1.10497045	0.1026632
64	M3W6N1 M3W6N1_FELCA	-0.87423562	0.09685437
65	M3WRM0 M3WRM0_FELCA	-0.95478767	0.12936561
66	M3W103 M3W103_FELCA	-1.1497569	0.12663303
67	A0A337SKJ9 A0A337SKJ9_FELCA	-0.88254023	0.1291582
68	M3XAK5 M3XAK5_FELCA	-0.93351261	0.12121576
69	M3VVI7 M3VVI7_FELCA	0.88137742	0.12709696
70	A0A337SU81 A0A337SU81_FELCA	-2.42330235	0.12053453
71	M3WQJ0 M3WQJ0_FELCA	-0.955751	0.11180913
72	A0A2I2UIR6 A0A2I2UIR6_FELCA	-1.43879033	0.13210278
73	A0A337SGK3 A0A337SGK3_FELCA	-0.97457468	0.13432965
74	A0A337S2X4 A0A337S2X4_FELCA	1.45180618	0.12727104
75	M3VWC7 M3VWC7_FELCA	-2.8404019	0.11667173
76	A0A2I2V1C1 A0A2I2V1C1_FELCA	-2.50453648	0.13117932
77	M3XE20 M3XE20_FELCA	1.18236665	0.13884542
78	M3VW05 M3VW05_FELCA	0.87555343	0.15495318
79	A0A337SDT4 A0A337SDT4_FELCA	-0.94151244	0.14172712
80	M3W340 M3W340_FELCA	1.00393666	0.15457413
81	M3WWP2 M3WWP2_FELCA	1.0519005	0.1552471
82	M3W1E8 M3W1E8_FELCA	-0.71424771	0.13951312
83	M3WMR1 M3WMR1_FELCA	-0.98450477	0.14937222
84	A0A337SJM7 A0A337SJM7_FELCA	1.430515	0.16597559
85	D3U660 D3U660_FELCA	-0.89811593	0.17749818

86	A0A337RXE8 A0A337RXE8_FELCA	1.39230122	0.17120384
87	M3WFR2 M3WFR2_FELCA	0.74858779	0.16297069
88	A0A5F5Y0E9 A0A5F5Y0E9_FELCA	0.87421188	0.16993118
89	M3W8D3 M3W8D3_FELCA	-1.11652385	0.19614802
90	M3W6N3 M3W6N3_FELCA	-0.84229759	0.20542554
91	A0A337STW1 A0A337STW1_FELCA	-1.18300428	0.19807428
92	A0A337SK34 A0A337SK34_FELCA	-0.71507341	0.1977503
93	A0A337SEI8 A0A337SEI8_FELCA	0.83705413	0.187847
94	A0A5F5XJ28 A0A5F5XJ28_FELCA	1.0281029	0.20696765
95	M3XCN6 M3XCN6_FELCA	0.74630243	0.18057895
96	M3VVN9 M3VVN9_FELCA	-1.91235804	0.18946062
97	M3W5B5 M3W5B5_FELCA	1.15469796	0.19471384
98	M3WIJ6 M3WIJ6_FELCA	0.85896043	0.19457348
99	M3WZB0 M3WZB0_FELCA	0.79449334	0.20071081
100	M3WIY9 M3WIY9_FELCA	0.68731582	0.21730774
101	A0A2I2U4U9 A0A2I2U4U9_FELCA	-0.6948812	0.23504809
102	M3XAB2 M3XAB2_FELCA	-0.63181561	0.21039822
103	M3WEG0 M3WEG0_FELCA	0.73614418	0.21328651
104	A0A337S1C3 A0A337S1C3_FELCA	-0.61691418	0.19212901
105	A0A337S574 A0A337S574_FELCA	-0.87744834	0.22741398
106	A0A2I2U5B1 A0A2I2U5B1_FELCA	0.6893842	0.23889908
107	M3WES0 M3WES0_FELCA	1.33559327	0.24972994
108	A0A5F5XZJ4 A0A5F5XZJ4_FELCA	0.68525792	0.27332331
109	A0A2I2V4V0 A0A2I2V4V0_FELCA	-1.22412716	0.25283299
110	M3W0Z9 M3W0Z9_FELCA	1.25556378	0.25675866
111	M3WXZ4 M3WXZ4_FELCA	0.74101269	0.23774364
112	M3W0S1 M3W0S1_FELCA	-0.74563497	0.18870746
113	M3W314 M3W314_FELCA	0.63825065	0.25602845
114	M3W616 M3W616_FELCA	-1.61546534	0.24485034
115	M3WFE1 M3WFE1_FELCA	0.84877822	0.19648222
116	M3W6I2 M3W6I2_FELCA	-0.7070366	0.24716385
117	M3XFH0 M3XFH0_FELCA	0.89905949	0.25148967
118	M3WG78 M3WG78_FELCA	0.69794975	0.26145728
119	A0A5F5XXV6 A0A5F5XXV6_FELCA	-0.72392569	0.30454032
120	A0A337S6S4 A0A337S6S4_FELCA	0.60932632	0.26272722
121	A0A2I2V257 A0A2I2V257_FELCA	0.65866376	0.26273789
122	A0A5F5XLI3 A0A5F5XLI3_FELCA	-1.20921034	0.30052652
123	M3X673 M3X673_FELCA	1.29524112	0.29625437
124	A0A0A0MPX9 A0A0A0MPX9_FELCA	0.56673339	0.2935787
125	A0A2I2UFN5 A0A2I2UFN5_FELCA	0.5739124	0.345949
126	A0A5F5XTM3 A0A5F5XTM3_FELCA	-0.77298411	0.30682978
127	M3VWM9 M3VWM9_FELCA	-0.73283126	0.37583264
128	A0A5F5XI95 A0A5F5XI95_FELCA	-0.69785452	0.33005281
129	M3VX06 M3VX06_FELCA	-0.63290582	0.40177814
130	A0A337S0E1 A0A337S0E1_FELCA	-1.59264676	0.35190383

131	M3W8Q7 M3W8Q7_FELCA	0.54243869	0.40418621
132	M3X7Z9 M3X7Z9_FELCA	0.63226259	0.40663471
133	A0A337SBS4 A0A337SBS4_FELCA	0.61730868	0.36518077
134	A0A5F5XDM3 A0A5F5XDM3_FELCA	-1.04796814	0.38457297
135	A0A2I2V2E9 A0A2I2V2E9_FELCA	-0.54045608	0.37838812
136	M3WN96 M3WN96_FELCA	-0.97199818	0.39857618
137	A0A337S5W7 A0A337S5W7_FELCA	-0.6470899	0.44761128
138	A0A337SKI4 A0A337SKI4_FELCA	0.79674309	0.42717936
139	A0A2I2V256 A0A2I2V256_FELCA	-1.21278287	0.41387961
140	A0A5F5XE26 A0A5F5XE26_FELCA	0.72716569	0.40729381
141	M3WM64 M3WM64_FELCA	0.78744828	0.44932791
142	A0A5F5XVC1 A0A5F5XVC1_FELCA	-0.74508586	0.45003252
143	A0A2I2UP60 A0A2I2UP60_FELCA	0.48528117	0.39241787
144	M3VY74 M3VY74_FELCA	-0.51296096	0.48588117
145	M3W9U6 M3W9U6_FELCA	-1.00312623	0.46131722
146	A0A2I2UG69 A0A2I2UG69_FELCA	0.66929167	0.49381499
147	M3VY56 M3VY56_FELCA	-0.53077197	0.51477886
148	A0A2I2UNB8 A0A2I2UNB8_FELCA	-0.39333699	0.44012135
149	A0A337SAA8 A0A337SAA8_FELCA	-1.15669399	0.4616709
150	M3VWY7 M3VWY7_FELCA	0.54230663	0.41443626
151	M3WNV1 M3WNV1_FELCA	0.6966889	0.49031456
152	A0A2I2UJE2 A0A2I2UJE2_FELCA	0.66064069	0.4133928
153	A0A5F5XQH4 A0A5F5XQH4_FELCA	0.5550161	0.43508366
154	M3WGE6 M3WGE6_FELCA	0.74753285	0.41061425
155	M3X2Q9 M3X2Q9_FELCA	-0.4897043	0.48225859
156	A0A2I2U902 A0A2I2U902_FELCA	0.73084154	0.53015201
157	A0A2I2UDQ5 A0A2I2UDQ5_FELCA	0.55171635	0.49419813
158	M3W7N2 M3W7N2_FELCA	0.47614104	0.415903
159	A0A5F5XE84 A0A5F5XE84_FELCA	0.5693967	0.43053054
160	M3VV33 M3VV33_FELCA	-0.43455161	0.42186376
161	A0A2I2UHR0 A0A2I2UHR0_FELCA	0.55252544	0.50489897
162	A0A2I2V2I3 A0A2I2V2I3_FELCA	-0.84502585	0.55533422
163	M3WSM7 M3WSM7_FELCA	0.55817512	0.47007981
164	M3VX55 M3VX55_FELCA	0.4155886	0.47936794
165	M3XCQ1 M3XCQ1_FELCA	-0.46820336	0.47874947
166	A0A337SFA0 A0A337SFA0_FELCA	0.5220583	0.50596542
167	M3WU44 M3WU44_FELCA	-0.5840467	0.40386813
168	A0A337S2D3 A0A337S2D3_FELCA	0.27982289	0.65995173
169	A0A337S266 A0A337S266_FELCA	-0.43491997	0.59816099
170	P63052 RL40_FELCA	0.40760661	0.45350201
171	M3VZE2 M3VZE2_FELCA	0.45272905	0.53028123
172	M3VYQ2 M3VYQ2_FELCA	0.34418969	0.48789018
173	A0A5F5XEX8 A0A5F5XEX8_FELCA	-0.42198273	0.56543779
174	M3W484 M3W484_FELCA	-0.31737862	0.71123519
175	M3WAQ4 M3WAQ4_FELCA	0.41428386	0.59670788

176	M3X6Y9 M3X6Y9_FELCA	-0.5272906	0.70475752
177	A0A2I2U0U0 A0A2I2U0U0_FELCA	0.49135937	0.66422395
178	A0A2I2UD94 A0A2I2UD94_FELCA	-0.27499601	0.74736059
179	M3X8C2 M3X8C2_FELCA	0.27777717	0.68644143
180	M3WFX6 M3WFX6_FELCA	0.34794819	0.70357863
181	A0A2I2U4W2 A0A2I2U4W2_FELCA	0.46827761	0.50778625
182	M3WGK3 M3WGK3_FELCA	-0.35853682	0.49388268
183	M3XB84 M3XB84_FELCA	-0.54178942	0.5353923
184	M3WLH5 M3WLH5_FELCA	-0.15826925	0.81602143
185	A0A337S2E2 A0A337S2E2_FELCA	-0.23048676	0.71707321
186	A0A337SAT1 A0A337SAT1_FELCA	-0.40752042	0.54197182
187	M3WAB5 M3WAB5_FELCA	0.14051258	0.84858983
188	M3VXZ1 M3VXZ1_FELCA	0.30650203	0.66882377
189	A0A5F5XKD4 A0A5F5XKD4_FELCA	0.16216635	0.78687204
190	P61246 RS3A_FELCA	-0.42039794	0.54178873
191	M3X3M7 M3X3M7_FELCA	-0.4027443	0.82418817
192	M3VXE9 M3VXE9_FELCA	0.48421677	0.5614932
193	M3VVZ2 M3VVZ2_FELCA	0.07473626	0.94205655
194	A0A2I2UFE0 A0A2I2UFE0_FELCA	0.19756537	0.78368149
195	M3XCH0 M3XCH0_FELCA	0.33154207	0.69930009
196	M3WAZ1 M3WAZ1_FELCA	0.0688818	0.95978421
197	M3WY95 M3WY95_FELCA	0.34809153	0.61956759
198	M3X2J1 M3X2J1_FELCA	0.38207968	0.55067371
199	A0A2I2UWM9 A0A2I2UWM9_FELCA	0.05780446	0.9254989
200	A0A2I2U1M4 A0A2I2U1M4_FELCA	0.47114034	0.61481224
201	M3VUH3 M3VUH3_FELCA	-0.08414469	0.94342427
202	A0A5F5XKM9 A0A5F5XKM9_FELCA	-0.241069	0.65125065
203	A0A2I2URI6 A0A2I2URI6_FELCA	-0.60474723	0.69245125
204	M3W668 M3W668_FELCA	0.24647312	0.66201051
205	A0A2I2U5K7 A0A2I2U5K7_FELCA	-0.20079229	0.71490421
206	A0A2I2V460 A0A2I2V460_FELCA	-0.23749236	0.70739198
207	A0A2I2U8W9 A0A2I2U8W9_FELCA	-0.4689799	0.64817985
208	A0A2I2UXL1 A0A2I2UXL1_FELCA	-0.02707674	0.97024054
209	A0A2I2U9Q8 A0A2I2U9Q8_FELCA	-0.35372991	0.62093985
210	A0A2I2U9C6 A0A2I2U9C6_FELCA	0.43543891	0.68076518
211	M3W4G0 M3W4G0_FELCA	0.29203187	0.62429355
212	A0A2I2UY31 A0A2I2UY31_FELCA	0.24485541	0.6303822
213	M3VZT0 M3VZT0_FELCA	0.28066303	0.63175032
214	A0A337SGS6 A0A337SGS6_FELCA	-0.21434273	0.713302
215	A0A2I2V0F1 A0A2I2V0F1_FELCA	0.0269977	0.97111462
216	A0A5F5XE47 A0A5F5XE47_FELCA	-0.33766502	0.70028922
217	A0A2I2UJH0 A0A2I2UJH0_FELCA	0.32258437	0.67086999
218	A0A5F5XV94 A0A5F5XV94_FELCA	-0.29761638	0.60725388
219	M3W584 M3W584_FELCA	0.16394197	0.80233478
220	A0A337S3P4 A0A337S3P4_FELCA	0.19294274	0.69024359

221	M3WCU9 M3WCU9_FELCA	-0.22366223	0.75470213
222	M3WGN8 M3WGN8_FELCA	0.29436657	0.68817701
223	M3W9K1 M3W9K1_FELCA	-0.41375661	0.62547059
224	Q9N2D5 G3P_FELCA	0.28868361	0.71679951
225	A0A337SJV5 A0A337SJV5_FELCA	0.20340093	0.69208425
226	M3WFL8 M3WFL8_FELCA	0.26081364	0.6323484
227	A0A337S498 A0A337S498_FELCA	0.10694867	0.88774223
228	M3WWI9 M3WWI9_FELCA	0.00561339	0.9923296
229	A0A2I2UFB9 A0A2I2UFB9_FELCA	0.2004487	0.81835163
230	A0A2I2UTE6 A0A2I2UTE6_FELCA	-0.06685416	0.9407761
231	M3WZA4 M3WZA4_FELCA	0.36516127	0.78176555
232	M3W059 M3W059_FELCA	-0.08800975	0.90622452
233	M3W6M1 M3W6M1_FELCA	0.19046829	0.79644861
234	M3WEF9 M3WEF9_FELCA	0.04460123	0.93370804
235	A0A2I2V3D5 A0A2I2V3D5_FELCA	-0.04726234	0.95249919
236	A8WA76 A8WA76_FELCA	-0.00154657	0.99752947
237	K0J107 MDHM_FELCA	-0.09861651	0.84915846
238	A0A2I2U8U0 A0A2I2U8U0_FELCA	0.2415807	0.78211448
239	M3W5T1 M3W5T1_FELCA	-0.10418937	0.87296297
240	M3XFZ1 M3XFZ1_FELCA	-0.04177231	0.93146945
241	Q66WF5 RL24_FELCA	-0.08975336	0.90495434
242	A0A5F5Y6Z4 A0A5F5Y6Z4_FELCA	-0.06822556	0.89754353
243	A0A2I2ULA7 A0A2I2ULA7_FELCA	-0.13665265	0.80711816
244	A0A337SMT8 A0A337SMT8_FELCA	0.09408336	0.91752904
245	M3VUG5 M3VUG5_FELCA	-0.14139884	0.87214441
246	M3WVY3 M3WVY3_FELCA	-0.02400945	0.95681854
247	M3X4F1 M3X4F1_FELCA	0.01954638	0.96919767
248	M3W064 M3W064_FELCA	0.07851178	0.89797205
249	M3WMZ2 M3WMZ2_FELCA	-0.18206107	0.70626683
250	M3XA07 M3XA07_FELCA	-0.00398996	0.99387616

---

**Table S3. Detailed functional annotation of DEPs (Fig.6 Pie charts).** Upregulated proteins: A) Molecular function, B) Biological Process, and C) Cellular component. Downregulated proteins: D) Molecular function, E) Biological Process, F) Cellular component.

**A) Upregulated proteins. Molecular function GO:0003674**

1. Cytoskeletal motor activity GO:0003774 (MYH9).	1
2. Binding GO:0005488 (MYH9, TUFM, IGF2BP1, HNRNPH1, HSP90B1, CCT8, RPL8, RPL18, HSPA8, EZR, COX5B, EEF1A1, EEF1B2, HDLBP, RNPS1, YWHAZ, HSPA5).	17
3. Catalytic activity GO:0003824 (PDIA4, TUFM, ATP5PF, HSP90B1, CCT8, HSPA8, EEF1A1, EEF1B2, HSPA5, ALDOA).	10
4. Translation regulator activity GO:0045182 (EEF1A1).	1
5. Molecular adaptor activity GO:0060090 (IGF2BP1, TLE6).	2
6. Structural molecule activity GO:0005198 (RPL8, RPL18).	2
7. Molecular function regulator activity GO:0098772 (EEF1B2).	1
8. ATP-dependent activity GO:0140657 (HSP90B1, CCT8, HSPA8, HSPA5).	4
9. Molecular sequestering activity GO:0140313 (YWHAZ).	1
10. Transporter activity GO:0005215 (ATP5PF).	1
11. Transcription regulator activity GO:0140110 (TUFM, TLE6).	2
12. Protein folding chaperone GO:0044183 (HSP90B1, CCT8, HSPA8, HSPA5).	4
13. Unassigned (BCAP31).	1

**B) Upregulated proteins. Biological process GO:0008150**

1. Cellular process GO:0009987 (PDIA4, TUFM, IGF2BP1, ATP5PF, HSP90B1, CCT8, RPL8, RPL18, HSPA8, EEF1B2, COX5B, BCAP31, EEF1A1, RNPS1, YWHAZ, HSPA5, ALDOA).	17
2. Response to stimulus GO:0050896 (PDIA4, YWHAZ, HSPA5, IGF2BP1, HSP90B1, EEF1A1).	6
3. Metabolic process GO:0008152 (PDIA4, TUFM, IGF2BP1, ATP5PF, RPL8, RPL18, HSPA8, EEF1B2, COX5B, EEF1A1, RNPS1, HSPA5, ALDOA, HSP90B1, CCT8, YWHAZ).	16
4. Reproduction GO:0000003 (CCT8, TLE6).	2
5. Biological regulation GO:0065007 (IGF2BP1, YWHAZ, HSPA5, TLE6, HSP90B1, CCT8, EEF1A1).	7
6. Developmental process GO:0032502 (IGF2BP1, YWHAZ, HSPA5, TLE6).	4
7. Multicellular organismal process GO:0032501 (IGF2BP1, HSPA5).	2
8. Localization GO:0051179 (IGF2BP1, HSPA8, BCAP31, HSP90B1, CCT8, YWHAZ, HSPA5).	7
9. Unassigned (MYH9, HNRNPH1, EZR, HDLBP).	4

**C) Upregulated proteins. Cellular component GO:0005575**

1. Cytoskeleton GO:0005856 (MYH9, CCT8, EZR).	3
2. Extracellular region GO:0005576 (PDIA4).	1
3.	
4. Endoplasmic reticulum GO:0005783 (PDIA4, HSP90B1, RPL18, EEF1B2, BCAP31, HSPA5).	6
5. Organelle lumen GO:0043233 (PDIA4, IGF2BP1, HNRNPH1, HSP90B1, CCT8, EEF1A1, RNPS1, HSPA5).	8
6. Mitochondrion GO:0005739 (TUFM, ATP5PF, COX5B, HSPA5).	4
7. Nucleus GO:0005634 (IGF2BP1, HNRNPH1, CCT8, HSPA8, EEF1A1, HDLBP, RNPS1, HSPA5, TLE6).	9
8. Cytosol GO:0005829 (IGF2BP1, HSP90B1, CCT8, RPL8, RPL18, HSPA8, EEF1B2, YWHAZ, HSPA5).	9
9. Protein-containing complex GO:0032991 (IGF2BP1, ATP5PF, HSP90B1, CCT8, RPL8, RPL18, COX5B, RNPS1, HSPA5, TLE6).	10
10. Cell projection GO:0042995 (IGF2BP1, CCT8, HSPA8, EZR).	4
11. non-membrane-bounded organelle GO:0043228 (IGF2BP1, RPL8, RPL18, EEF1A1).	4
12. Organelle envelope GO:0031967 (ATP5PF, COX5B).	2
13. Cytoplasmic vesicle GO:0031410 (HSP90B1, HSPA5).	2
14. Vacuole GO:0005773 (HSPA8).	1
15. Plasma membrane GO:0005886 (HSPA8, EZR, EEF1A1, HSPA5).	4
16. Unassigned (ALDOA).	1

**D) Downregulated proteins. Molecular function GO:0003674**

1. Catalytic activity GO:0003824 (UQCRFS1, EMG1, PLK1).	3
2. Binding GO:0005488 (LGALS1, UQCRFS1, RPL12, TRA2B, RRP9, EMG1, PLK1, KHDC3L, H2BC3, H2BC18, GNG12, HMGA1).	12
3. Structural molecule activity GO:0005198 (RPL12, RPS25, RPL36A, COL1A2, H2BC3, H2BC18).	6
4. Antioxidant activity GO:0016209 (PRXL2A).	1
5. Molecular carrier activity GO:0140104 (KPNA2).	1
6. Transcription regulator activity GO:0140110 (HMGA1).	1
7. Transporter activity GO:0005215 (UQCRFS1).	1
8. Molecular adaptor activity GO:0060090 (HMGA1).	1
9. Unassigned (COA3, PSME3IP1, YIPF3).	4

**E) Downregulated proteins. Biological process GO:0008150**

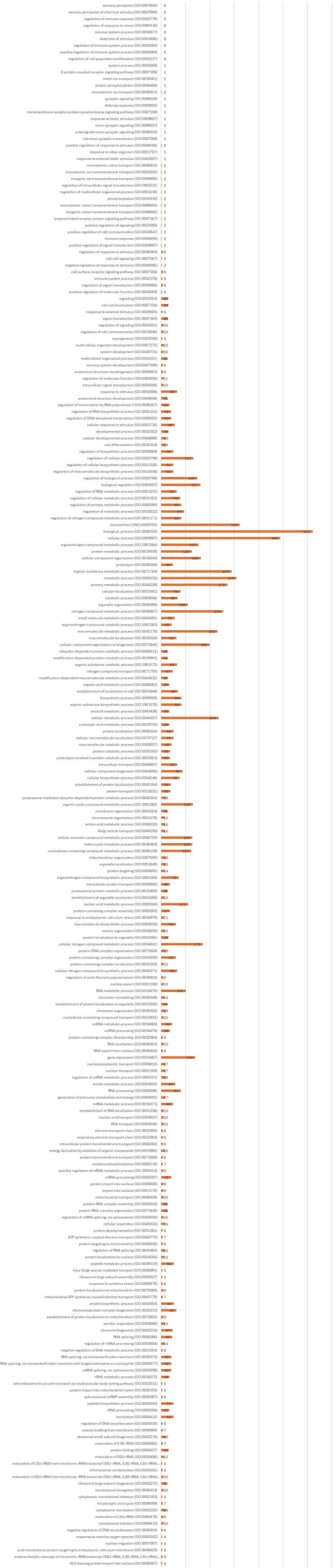
1. Cellular process GO:0009987 (COA3, UQCRFS1, RPL12, TRA2B, RPS25, RRP9, PRXL2A, EMG1, PLK1, KPNA2, RPL36A, GNG12, HMGA1).	13
2. Biological regulation GO:0065007 (COA3, TRA2B, PLK1, GNG12, HMGA1).	5
3. Metabolic process GO:0008152 (COA3, UQCRFS1, RPL12, TRA2B, RPS25, RRP9, EMG1, PLK1, RPL36A, HMGA1).	10
4. Response to stimulus GO:0050896 (TRA2B, PRXL2A, PLK1, GNG12, HMGA1).	5
5. Homeostatic process GO:0042592 (TRA2B).	1
6. Developmental process GO:0032502 (TRA2B, EMG1).	2
7. Multicellular organismal process GO:0032501 (TRA2B, PLK1).	2
8. Localization GO:0051179 (PLK1, KPNA2).	2

9. Reproduction GO:0000003 (PLK1).	1
10. Unassigned (LGALS1, PSME3IP1, KHDC3L, H2BC3, YIPF3, H2BC18, COL1A2).	7

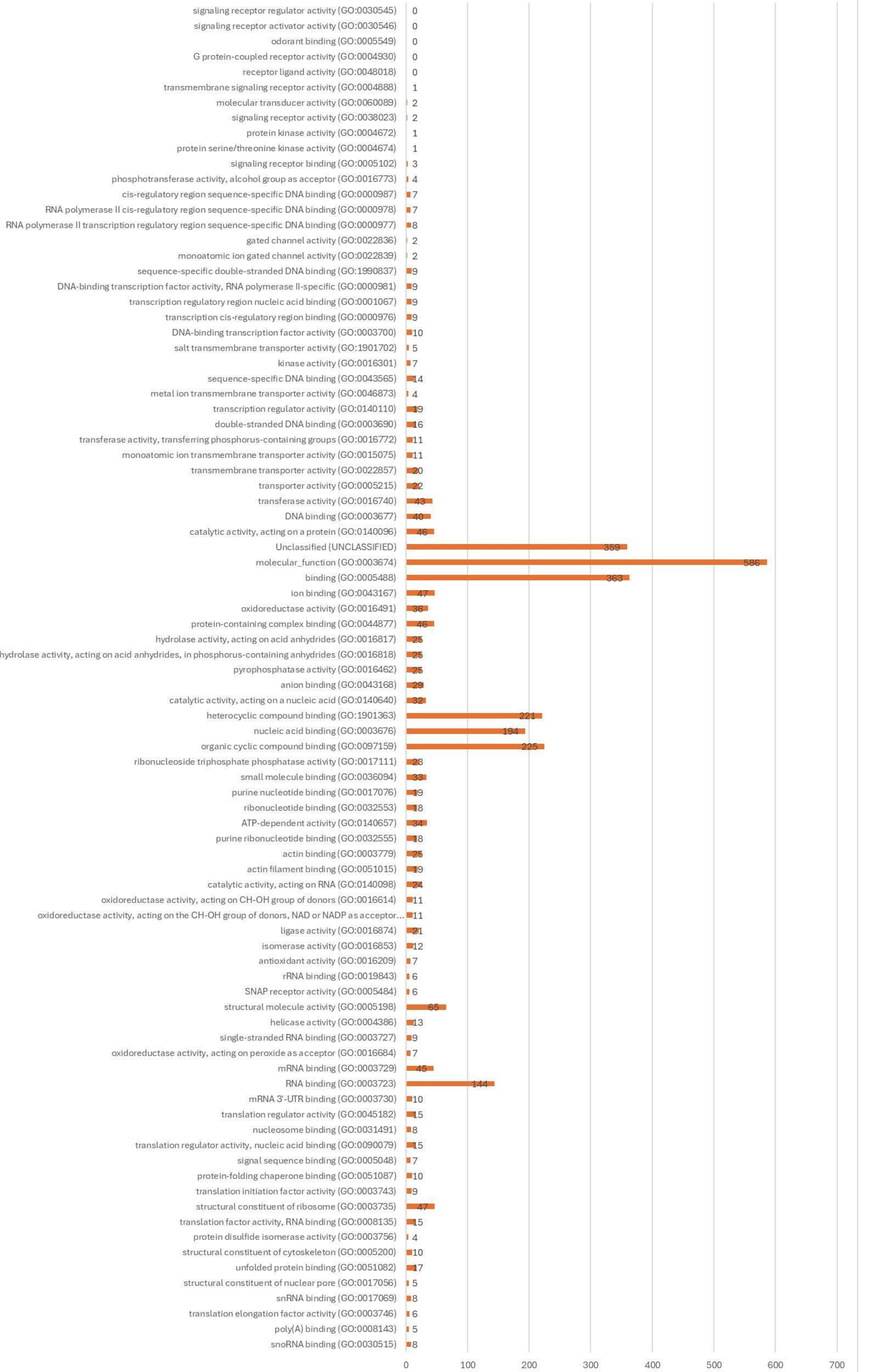
**F) Downregulated proteins. Cellular component GO:0005575**

1. Mitochondrion GO:0005739 (COA3, UQCRFS1).	2
2. Organelle envelope GO:0031967 (UQCRFS1, TRA2B).	2
3. Protein-containing complex GO:0032991 (UQCRFS1, TRA2B, RPS25, RRP9, EMG1, KHDC3L, RPL36A, H2BC3, H2BC18, GNG12).	10
4. Membrane GO:0016020 (PSME3IP1, YIPF3).	2
5. Cytosol GO:0005829 (RPL12, RPS25, H2BC3, H2BC18).	4
6. Ribosome GO:0005840 (RPL12).	1
7. Nucleus GO:0005634 (TRA2B, RRP9, EMG1, PLK1, H2BC3, H2BC18, HMGA1).	7
8. Organelle lumen GO:0043233 (TRA2B, RRP9, EMG1, H2BC3, H2BC18).	5
9. non-membrane-bounded organelle GO:0043228 (RPS25, RRP9, EMG1, RPL36A, H2BC3, H2BC18).	6
10. Cytoplasm GO:0005737 (PRXL2A).	1
11. Nuclear chromosome GO:0000228 (PLK1).	1
12. Chromosome GO:0005694 (PLK1, HMGA1).	2
13. Microtubule organizing center GO:0005815 (PLK1).	1
14. Cytoskeleton GO:0005856 (PLK1).	1
15. Golgi apparatus GO:0005794 (YIPF3).	1
16. Cytoplasmic vesicle GO:0031410 (YIPF3).	1
17. Plasma membrane GO:0005886 (GNG12).	1
18. Unassigned (LGALS1, KPNA2, COL1A2).	3

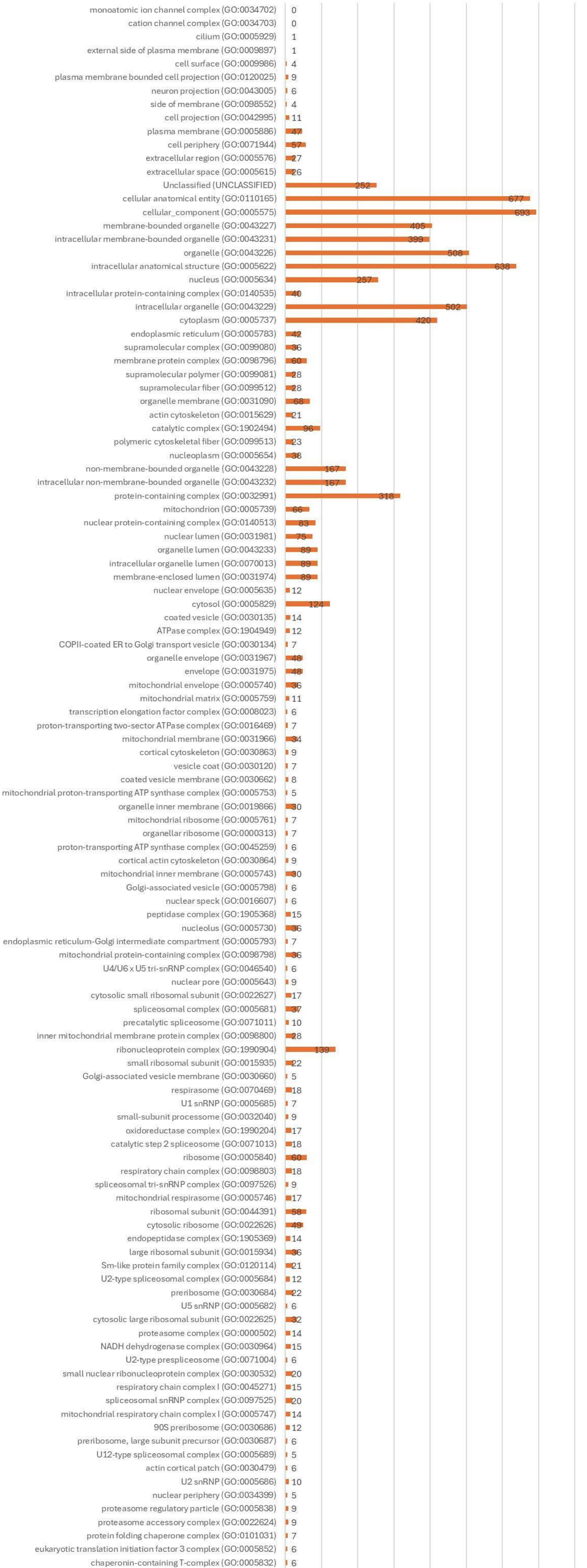
PANTHER GO-Slim Biological Process - ZF Blastocysts



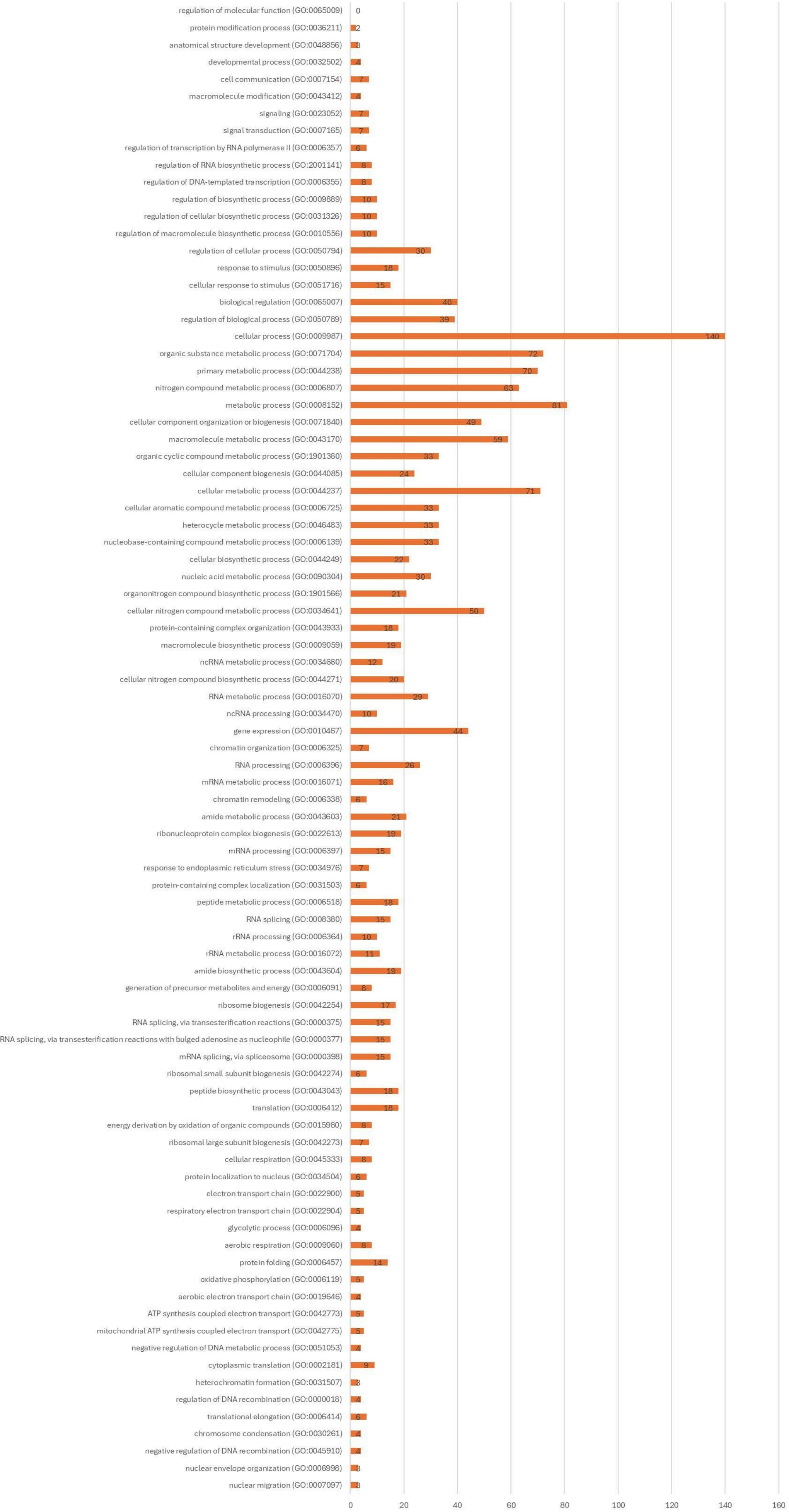
PANTHER GO-Slim Molecular Function - ZF Blastocysts



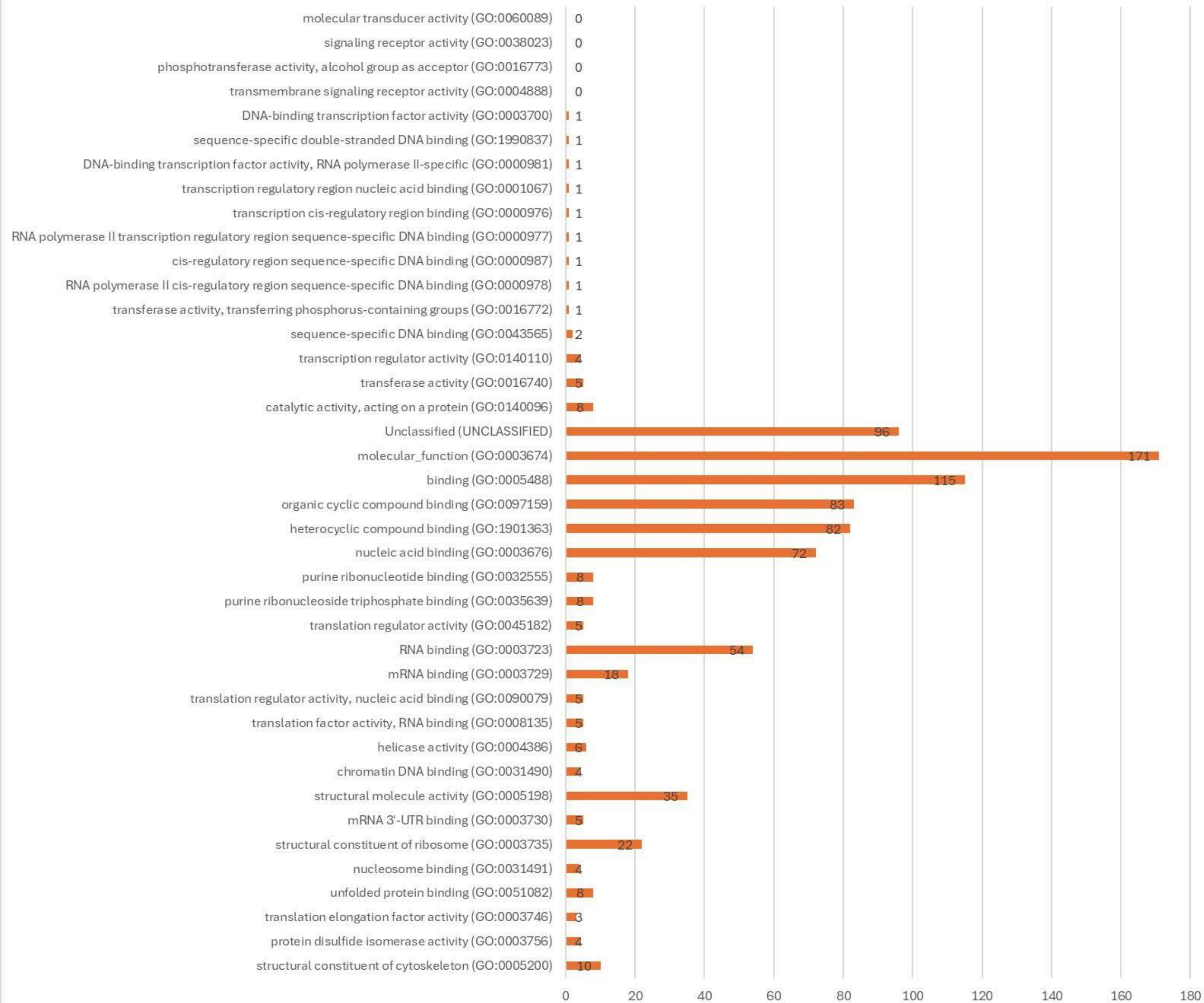
PANTHER GO-Slim Cellular Component ZF blastocysts



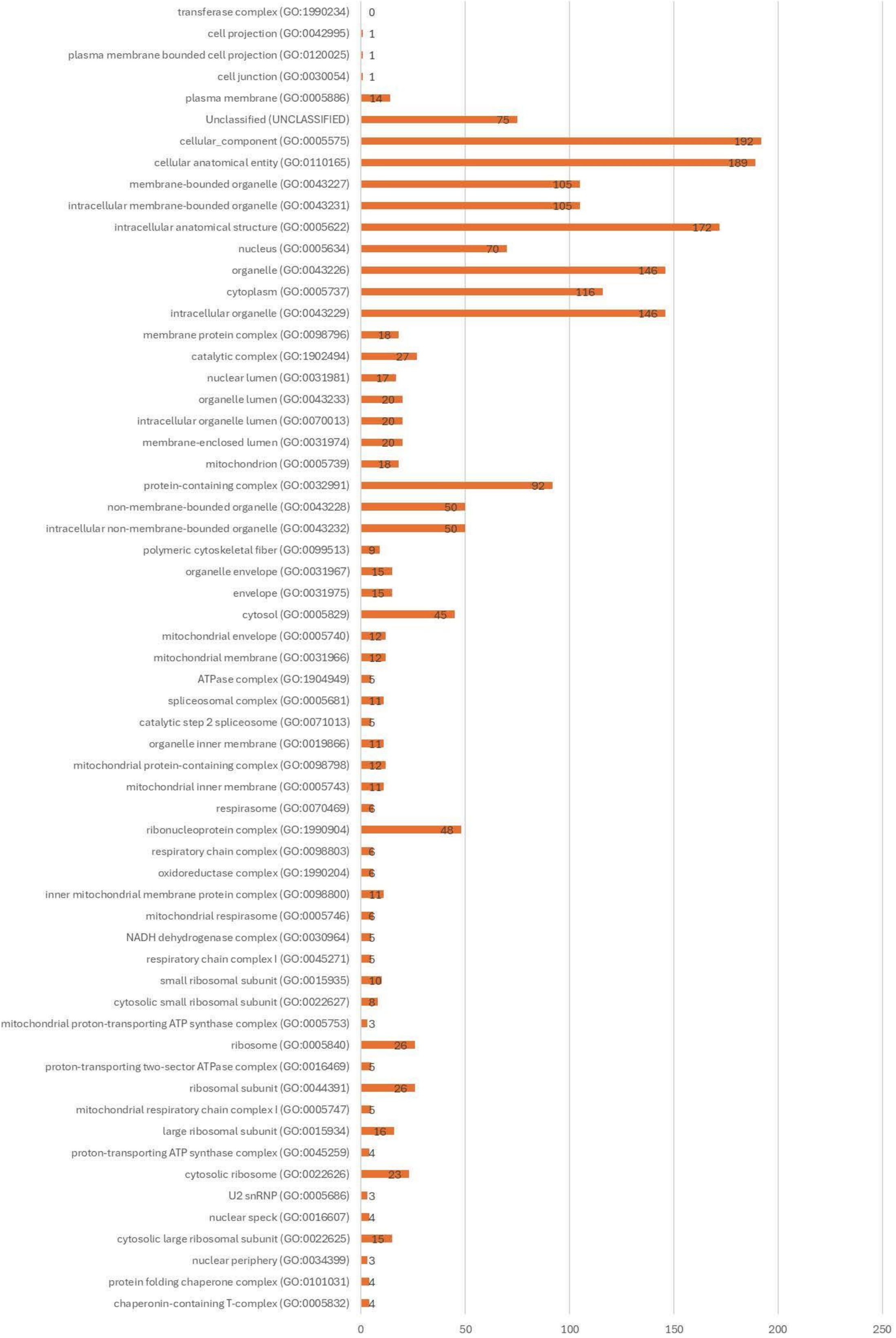
PANTHER GO-Slim Biological Process - Zl Blastocysts



# PANTHER GO-Slim Molecular Function - ZI Blastocysts



PANTHER GO-Slim Cellular Component - ZI Blastocysts



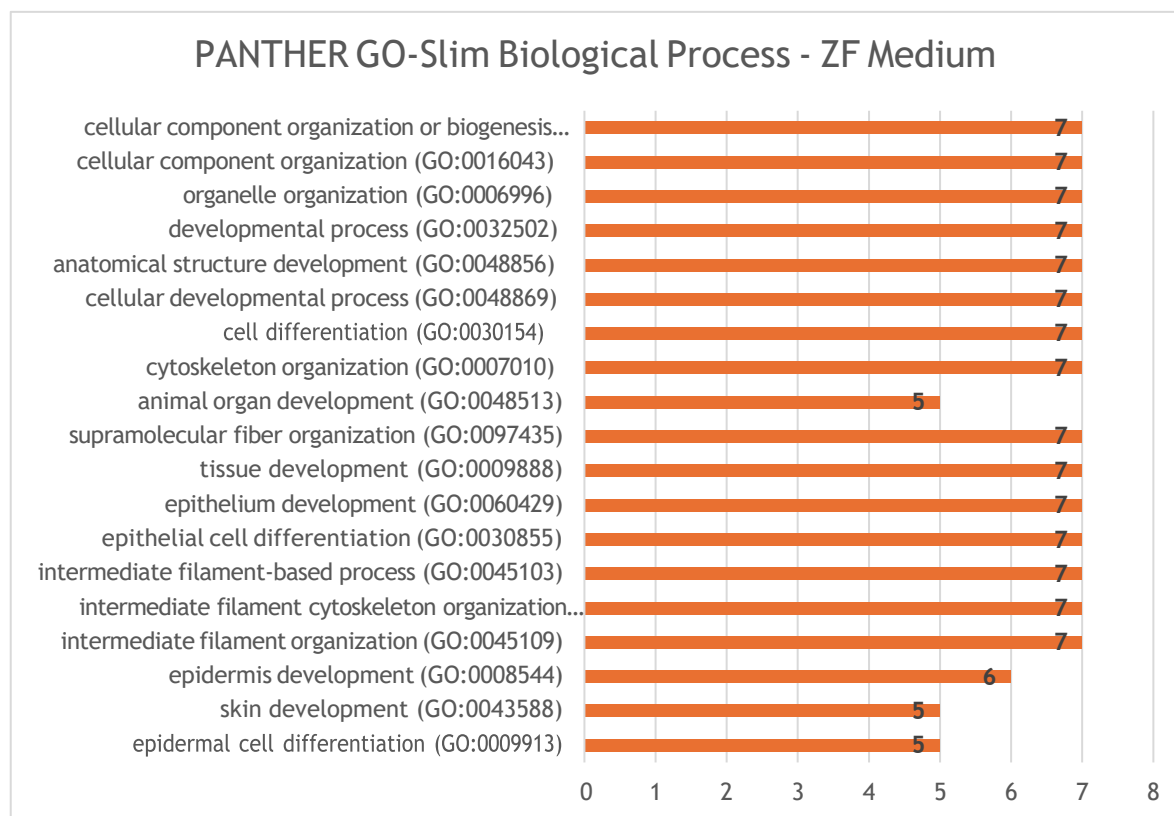
**Table S4. Proteins identified in the culture medium of blastocysts from the ZF and ZI groups.**

	Protein ID	Description	Name	Group
1	A0A5F5XYD4_FELCA	Keratin, type I cytoskeletal	KRT15	ZF and ZI
2	M3VXR1_FELCA	Keratin 10	KRT10	ZF and ZI
3	M3X1M2_FELCA	Hemicentin 1	HMCN1	ZF and ZI
4	P49064_FELCA	Albumin	ALB	ZF and ZI
5	A0A337S8S6_FELCA	Protease, Serine, 2 (Trypsin 2)	TRY2	ZF and ZI
6	A0A337SCP9_FELCA	Keratin, type I cytoskeletal 13	KRT13	ZF and ZI
7	M3VXK4_FELCA	Keratin, type I cytoskeletal 17	KRT17	ZF and ZI
8	M3VUG7_FELCA	Keratin 76	KRT76	ZF and ZI
9	A0A5F5XLA0_FELCA	Keratin, type I cytoskeletal 17	KRT17	ZF and ZI
10	M3VUF9_FELCA	Keratin 74	KRT74	ZF and ZI
11	M3VUG8_FELCA	Keratin 3	KRT3	ZF and ZI
12	M3VUG4_FELCA	Intermediate filament protein	unassigned	ZF and ZI
13	A0A2I2U8D2_FELCA	Centrosomal protein of 162 kDa	CEP162	ZF and ZI
14	A0A337SFR9_FELCA	GDNF family receptor alpha 3	GFRA3	ZF and ZI
15	A0A5F5XDM4_FELCA	Actin beta	ACTB	ZF and ZI
16	A0A5F5XNI1_FELCA	Transferrin	TF	ZF and ZI
17	M3WLU0_FELCA	Cathepsin B	CTSB	ZF and ZI
18	A0A5F5XLU4_FELCA	Pantetheinase	VNN1	ZF and ZI
19	A0A2I2UZM2_FELCA	Heat shock protein family A (Hsp70) member 8	HSPA8	ZF and ZI
20	M3X9A1_FELCA	Triosephosphate isomerase	TPI1	ZF and ZI
21	M3VUU7_FELCA	Calreticulin	CALR	ZF and ZI
22	M3WZD7_FELCA	Belongs to the intermediate filament family	KRT89	ZF and ZI
23	A0A5F5XZ97_FELCA	Fatty acid-binding protein 5	FABP5	ZF and ZI
24	A0A337SJA7_FELCA	Calmodulin 2	CALM2	ZF and ZI
25	A0A337S1R3_FELCA	Prothymosin alpha	PTMA	ZF and ZI
1	A0A337SNF5_FELCA	Alpha-ketoglutarate-dependent dioxygenase	FT5	ZF
2	A0A337S839_FELCA	Protein Mdm4	MDM4	ZF
3	A0A337S8L1_FELCA	L-lactate dehydrogenase	LDHA	ZF
4	A0A337S103_FELCA	Metallothionein	MT1X	ZF
5	M3W8Q7_FELCA	Glutamate dehydrogenase [NAD(P)(+)]	GLUD1	ZF
1	A0A2I2USF3_FELCA	Histone H2B	H2BC17	ZI
2	A0A5F5XCD9_FELCA	Belongs to the intermediate filament family	KRT86	ZI
3	A0A337SCD2_FELCA	Annexin	ANXA2	ZI
4	A0A2I2UBN8_FELCA	Junction plakoglobin	JUP	ZI
5	M3W114_FELCA	Proteolipid protein 2	PLP2	ZI
6	A0A2I2UGZ3_FELCA	Keratin 32	KRT32	ZI
7	A0A5F5XSP5_FELCA	Leucine aminopeptidase 3	LAP3	ZI
8	M3W2I5_FELCA	Plakophilin 1	PKP1	ZI
9	M3WCK3_FELCA	Desmoplakin	DSP	ZI
10	M3VUF5_FELCA	Keratin 82	KRT82	ZI
11	M3W584_FELCA	H1.5 linker histone, cluster member	H1-5	ZI
12	A0A5K1VP01_FELCA	Lamin A/C	LMNA	ZI

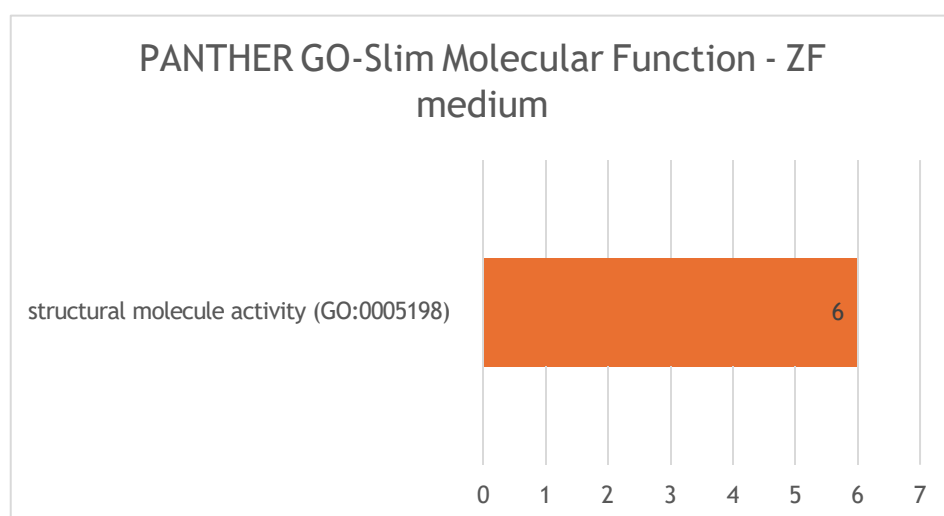
13	A0A337SS59_FELCA	Sad1 and UNC84 domain containing 2	SUN2	ZI
14	A0A2I2U7V8_FELCA	Four and a half LIM domains 1	FHL1	ZI

---

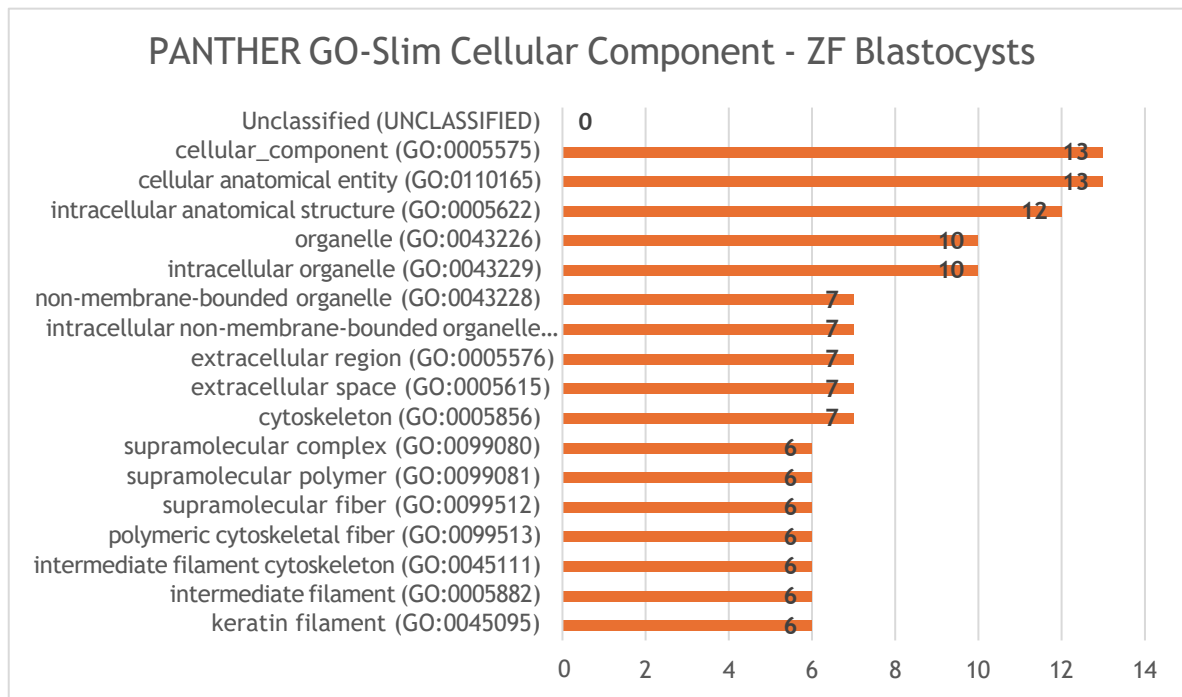
**Figure S5. GO annotation of the proteins identified in the culture medium of ZF and ZI blastocysts.**



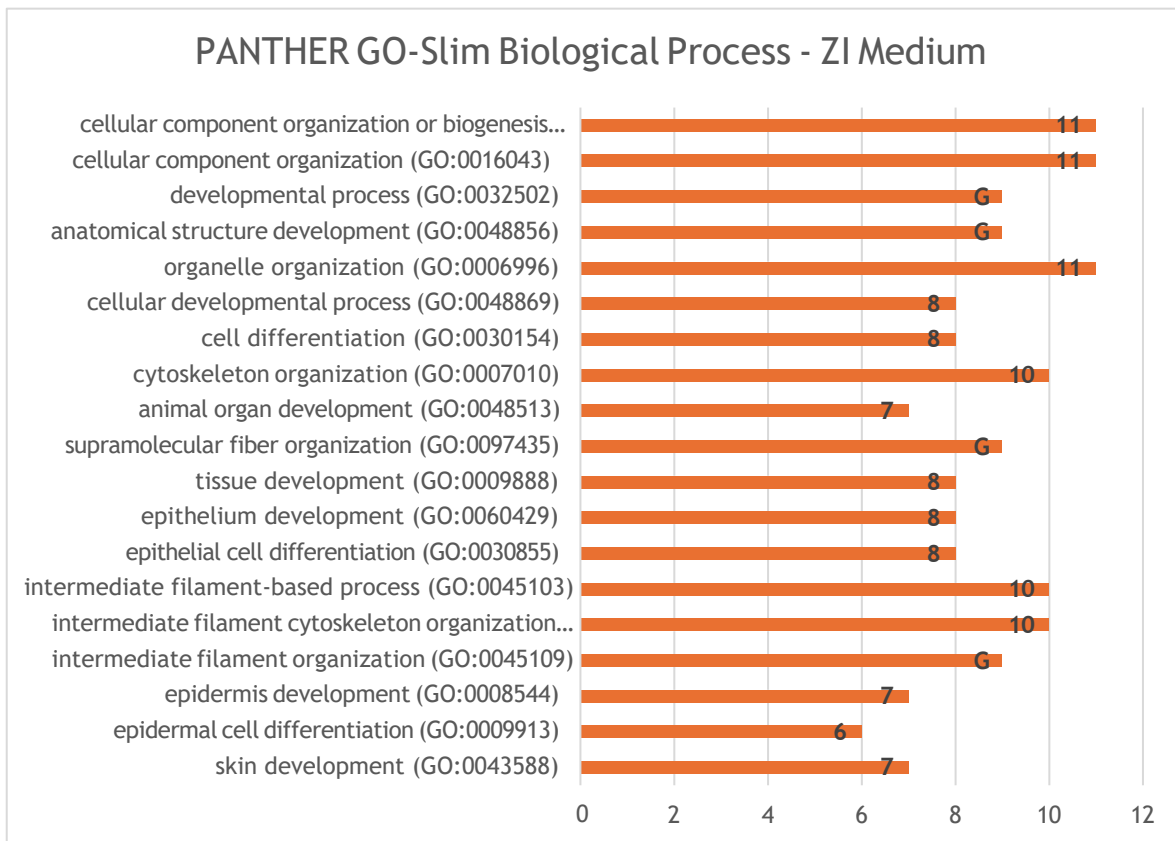
**S5.1. GO Biological Process annotation of all the proteins identified in the medium samples of ZF Blastocysts. The PANTHER18.0 Classification System was used for analysis.**



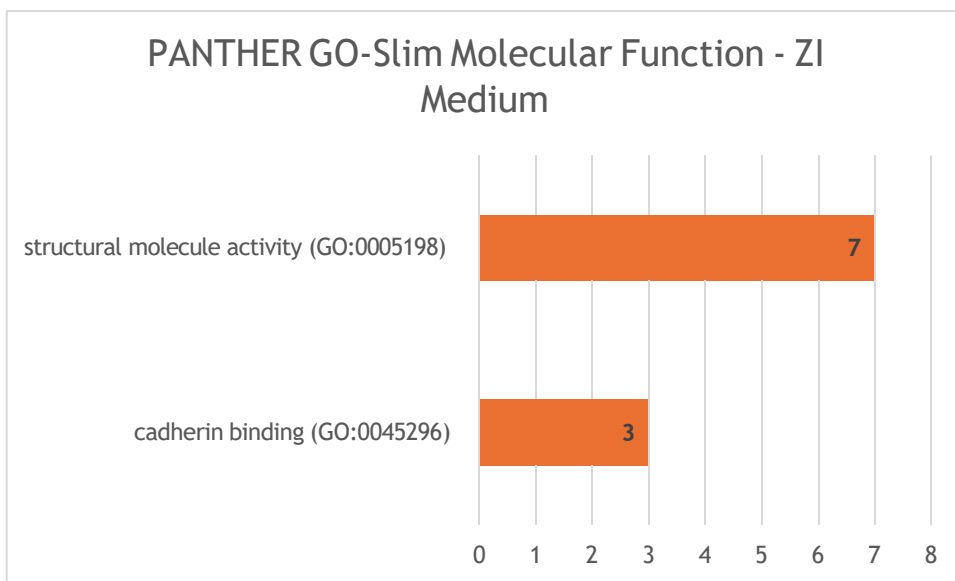
**S5.2. GO Molecular Function annotation of all the proteins identified in the medium samples of ZF Blastocysts. The PANTHER18.0 Classification System was used for analysis.**



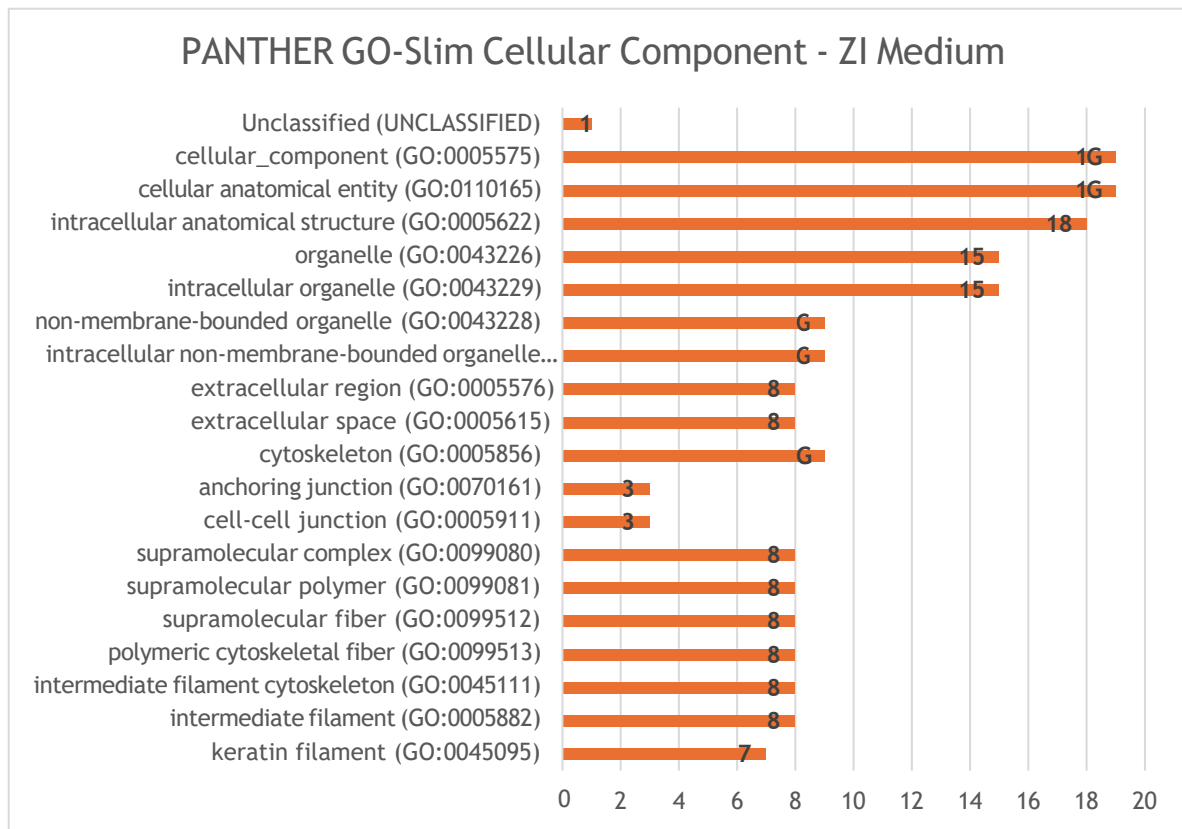
**S5.3.** GO Cellular Component annotation of all the proteins identified in the medium samples of ZF Blastocysts. The PANTHER18.0 Classification System was used for analysis.



**S5.4.** GO Biological Process annotation of all the proteins identified in the medium samples of ZI Blastocysts. The PANTHER18.0 Classification System was used for analysis.



**S5.5.** GO Molecular Function annotation of all the proteins identified in the medium samples of ZI Blastocysts. The PANTHER18.0 Classification System was used for analysis.



**S5.6.** GO Cellular Component annotation of all the proteins identified in the medium samples of ZI Blastocysts. The PANTHER18.0 Classification System was used for analysis.

**Table S6. Detailed functional annotation of DEPs (Fig.8 Pie charts).** Proteins identified in the culture medium of ZF blastocysts (A, B, and C), ZI blastocysts (D, E, and F), and proteins shared between the conditioned medium of ZF and ZI blastocysts (G, H, and I).

<b>A) ZF-medium. Molecular function GO:0003674</b>		
1. Binding GO:0005488 (FT5, MDM4, MT1X).		3
2. Catalytic activity GO:0003824 (FT5, LDHA, GLUD1).		3
<b>B) ZF-medium. Biological process GO:0008150</b>		
1. Metabolic process GO:0008152 (FT5, LDHA, GLUD1).		3
2. Cellular process GO:0009987 (FT5, MDM4, LDHA, GLUD1).		4
3. Response to stimulus GO:0050896 (FT5).		1
4. Biological regulation GO:0065007 (FT5, MDM4).		2
5. Developmental process GO:0032502 (FT5).		1
6. Growth GO:004007 (FT5).		1
7. Localization GO:0051179 (GLUD1).		1
8. Unassigned (MT1X).		1
<b>C) ZF-medium. Cellular component GO:0005575</b>		
1. Cytoplasm GO:0005737 (FT5).		1
2. Nucleoplasm GO:0005654 (FT5).		1
3. Nucleus GO:0005634 (MDM4).		1
4. Mitochondrion GO:0005739 (LDHA, GLUD1).		2
5. Organelle lumen GO:0043233 (GLUD1).		1
6. Unassigned (MT1X).		1
<b>D) ZI-medium. Molecular function GO:0003674</b>		
1. Binding GO:0005488 (H2BC17, ANXA2, JUP, PKP1, H1-5, FHL1).		6
2. Structural molecule activity GO:0005198 (KRT86, KRT32, KRT82).		3
3. Molecular function regulator activity GO:0098772 (ANXA2).		1
4. Transporter activity GO:0005198 (ANXA2).		1
5. Transcription regulator activity GO:0140110 (JUP).		1
6. Catalytic activity GO:0003824, (LAP3).		1
7. Unassigned (PLP2, DSP, LMNA, SUN2).		4

**E) ZI-medium. Biological process GO:0008150**

1. Biological regulation GO:0065007 (JUP, H1-5).	2
2. Cellular process GO:0009987 (JUP, PKP1, DSP, KRT82, H1-5).	5
3. Response to stimulus GO:0050896 (JUP).	1
4. Metabolic process GO:0008152 (LAP3).	1
5. Developmental process GO:0032502 (DSP, KRT82, FHL1).	3
6. Multicellular organismal process GO:0032501 (KRT82).	1
7. Unassigned (H2BC17, KRT86, ANXA2, PLP2, KRT32, LMNA, SUN2).	7

**F) ZI-medium. Cellular component GO:0005575**

1. Nucleus GO:0005634 (H2BC17, ANXA2, JUP, PKP1, H1-5, LMNA).	6
2. Protein-containing complex GO:0032991 (H2BC17, ANXA2, H1-5).	3
3. non-membrane-bounded organelle GO:0043228 (H2BC17, ANXA2, H1-5).	3
4. Cytoskeleton GO:0005856 (KRT86, JUP, KRT32, KRT82, LMNA, DSP).	6
5. Extracellular region GO:0005576 (ANXA2).	1
6. Endosome GO:0005768 (ANXA2).	1
7. Vacuole GO:0005773 (ANXA2).	1
8. Cytosol GO:0005829 (ANXA2, JUP, LAP3).	3
9. Plasma membrane GO:0005886 (ANXA2, JUP, PKP1, DSP).	4
10. Organelle GO:0043226 (JUP).	1
11. Membrane GO:0016020 (PLP2, SUN2).	2
12. Nucleoplasm GO:0005635 (PKP1).	1
13. Organelle lumen GO:0043233 (H1-5).	1
14. Nuclear envelope GO:0005635 (LMNA).	1
15. Unassigned (FLH1).	1

**G) ZF and ZI medium. Molecular function GO:0003674**

1. Structural molecule activity GO:0005198 (KRT15, KRT10, KRT13, KRT17, KRT76, KRT74, KRT3, LOC101097497, KRT89).	10
2. Binding GO:0005488 (KRT10, HMCN1, ALB, ACTB, CTSB, HSPA8, TPI1, CALR, FABP5, CALM2, PTMA).	11
3. Catalytic activity GO:0003824 (TRY2, CTSB, VNN1, HSPA8, TPI1).	5
4. Molecular transducer activity GO:0060089 (GFRA3).	1
5. ATP-dependent activity GO:0140657 (HSPA8).	1
6. Protein folding chaperone GO:0044183 (HSPA8).	1
7. Molecular carrier activity GO:0140104 (CALR).	1
8. Unassigned (CEP162, TF).	2

**H) ZF and ZI medium. Biological process GO:0008150**

1. Cellular process GO:0009987 (KRT10, HMCN1, ALB, KRT13, KRT76, KRT74, KRT3, LOC101097497, CEP162, CTSB, HSPA8, TPI1, CALR, KRT89, PTMA).	15
2. Metabolic process GO:0008152 (TRY2, CTSB, VNN1, HSPA8, TPI1, CALR, PTMA).	7
3. Developmental process GO:0032502 (KRT10, KRT13, KRT76, KRT74, KRT3, LOC101097497, CTSB, CALR, KRT89).	9
4. Response to stimulus GO:0050896 (HMCN1, ALB, KRT13, CTSB, CALR).	5
5. Biological regulation GO:0065007 (ALB, KRT13, KRT74, CALR, PTMA).	5
6. Multicellular organismal process GO:0032501 (KRT74, LOC101097497, CTSB, CALR).	4
7. Reproduction GO:0000003 (CTSB, CALR).	2
8. Localization GO:0051179 (HSPA8, CALR).	2
9. Immune system process GO:0002376 (CALR).	1
10. Unassigned (KRT15, KRT17, GFRA3, ACTB, TF, FABP5, CALM2).	7

**I) ZF and ZI medium. Cellular component GO:0005575**

1. Cytoskeleton GO:0005856 (KRT15, KRT10, KRT13, KRT17, KRT76, KRT74, KRT3, LOC101097497, CEP162, KRT89).	10
2. Cytoplasm GO:0005737 (KRT10, ALB, KRT74, CEP162, FABP5).	5
3. Plasma membrane GO:0005886 (KRT10, KRT74, GFRA3, CTSB, HSPA8, CALR).	6
4. Extracellular region GO:0005576 (HMCN1, CTSB, FABP5).	3
5. Extracellular matrix GO:0031012 (HMCN1).	1
6. Extracellular space GO:0005615 (ALB, TF, CALR).	3
7. Protein-containing complex GO:0032991 (ALB, CTSB).	2
8. Microtubule organizing center GO:0005815 (CEP162).	1
9. Vacuole GO:0005773 (CTSB, HSPA8).	2
10. Cellular anatomical entity GO:0110165 (VNN1).	1
11. Nucleus GO:0005634 (HSPA8, PTMA).	2
12. Cytosol GO:0005829 (HSPA8, TPI1, CALR, PTMA).	4
13. Cell projection GO:0042995 (HSPA8).	1
14. Nuclear envelope GO:0005635 (CALR).	1
15. Endoplasmic reticulum GO:0005783 (CALR).	1
16. Unassigned (TRY2, ACTB, CALM2).	3