Label-free Quantification for Proteins in Single Embryonic Cells with Neural Fate in the Cleavage-stage Frog (*Xenopus laevis*) Embryo using CE-ESI-HRMS

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Table S2. Representative peptides identified from vitellogenin b1 (Vtgb1). Separation of these peptides is shown **in Figure 2B.** A comprehensive set of peptides identified from Vtgb1 is listed in **Table S1A**. Key: *z*, charge; MT, migration time; N, theoretical number of plates for separation.

m/z Measured	*z*	MT (min)	Peptide Sequence	"-mer" ID	Start Seq.	End Seq.	Missed Cleavage	N
460.2548	2+	16.11	(R)SGIKLNCK(A)	8-mer	50	57	1	131,322
527.2951	2+	21.93	(K)FIFSLSSPR(T)	9-mer	1482	1490	0	368,764
563.2950	2+	22.36	(K)FLPGFSSGASR(L)	11-mer	499	509	0	363,435
580.7909	2+	17.92	(R)NVGQQDSEKR(M)	10-mer	921	930	1	239,870
621.3696	2+	23.82	(K)SPTVVITAQAVR(N)	12[a]-mer	1345	1356	0	363,435
460.9132	3+	16.09	(K)QAKFIFSLSSPR(T)	12[b]-mer	1479	1490	1	143,602
641.8655	2+	18.89	(K)AIGNAGQPASIKR(I)	13-mer	483	495	1	227,959
932.5036	2+	26.17	(R)VALNIMNPVF DQLSFR(Y)	16-mer	613	628	0	246,759
782.7384	3+	28.72	(R)NGQVGNIFAA EDVSETALNI QR(G)	22-mer	113	134	0	229,784
780.4001	4+	19.4	(K)NAESQVVETL QHLVQNNQEL VHGESTSK(F)	28-mer	305	332	0	279,770

Table S3. Enhancing peptide sequencing to boost protein identifications in limited amounts, ~20 ng, of protein digests from the 16-cell *Xenopus* embryo. Each sample was measured in technical triplicate. Asterisk (*) marks optimal experimental conditions (see identified proteins in **Table S1B**).

| Experimental Condition	Peptides		Protein groups	
	Average	Cumulative	Average	Cumulative
Sheath Flow				
50% MeOH, 0.1% FA	292 ± 28	369	54 ± 7	74*
10% DMSO in 50% MeOH, 0.1% FA	246 ± 12	277	27 ± 5	32
100 mM sulfolane in 50% MeOH, 0.1% FA	156 ± 6	177	12 ± 2	20
MS^1 Acquisition Rate				
2 Hz	283 ± 36	368	45 ± 11	73
4 Hz	292 ± 28	369	54 ± 12	74*
8 Hz	311 ± 54	347	42 ± 6	56
12 Hz	254 ± 41	348	37 ± 14	56
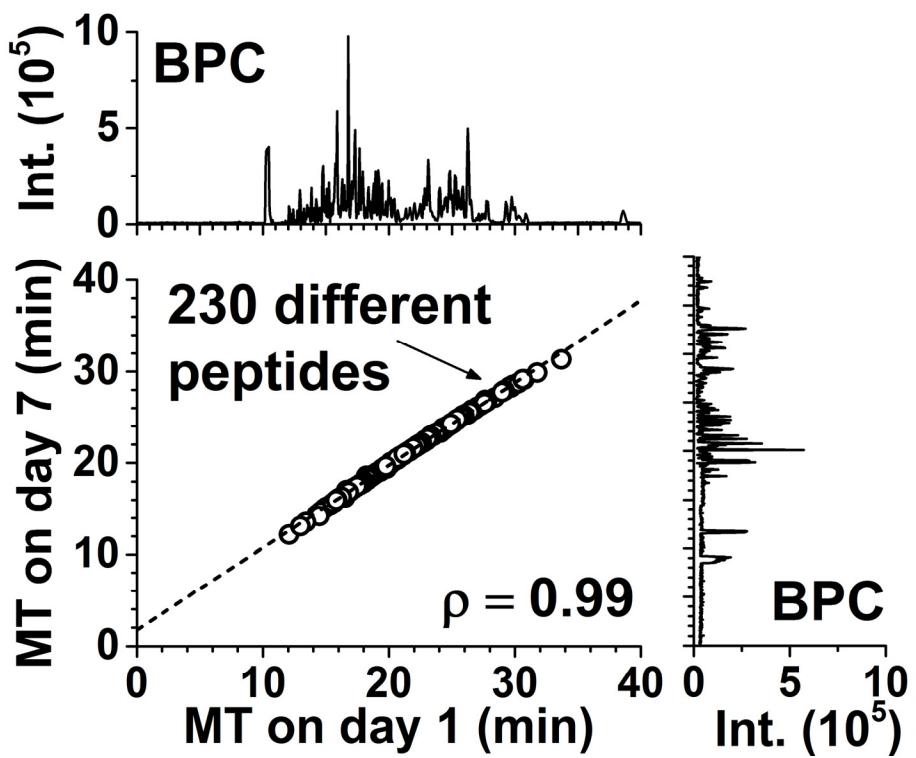
Figure S1. Robust separation of peptides in 20 ng protein digest from the 16-cell *Xenopus laevis* embryo. A high Pearson cross-correlation coefficient (ρ) calculated for 230 randomly selected different peptides revealed reproducible migration times across 7 days of experiments.
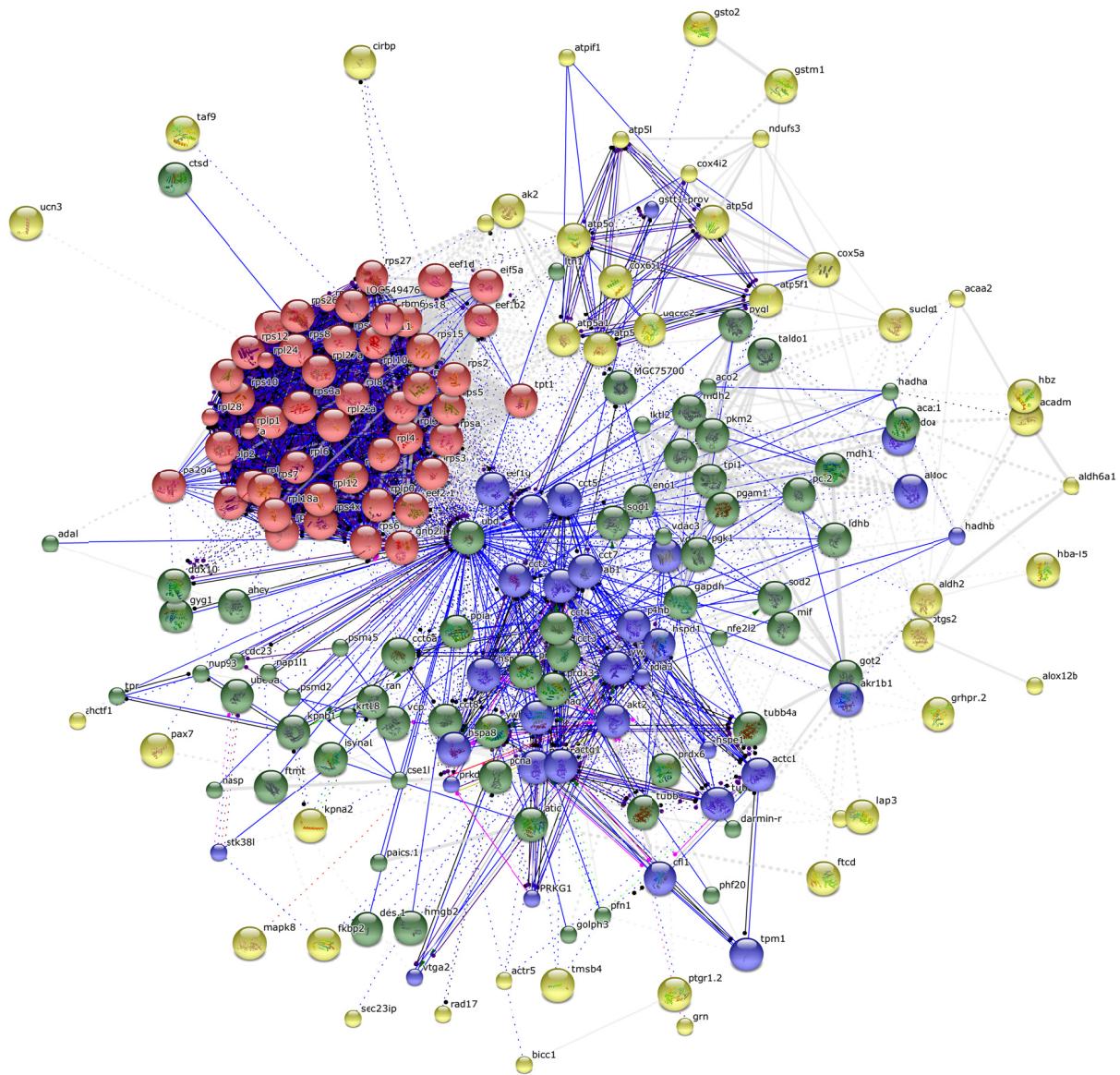
Figure S2. STRING interaction networks for proteins that were quantified between the single D11 blastomeres. STRING parameters: actions view shown; disconnected proteins removed; k-means clustering threshold = 3. Labels indicate gene names.
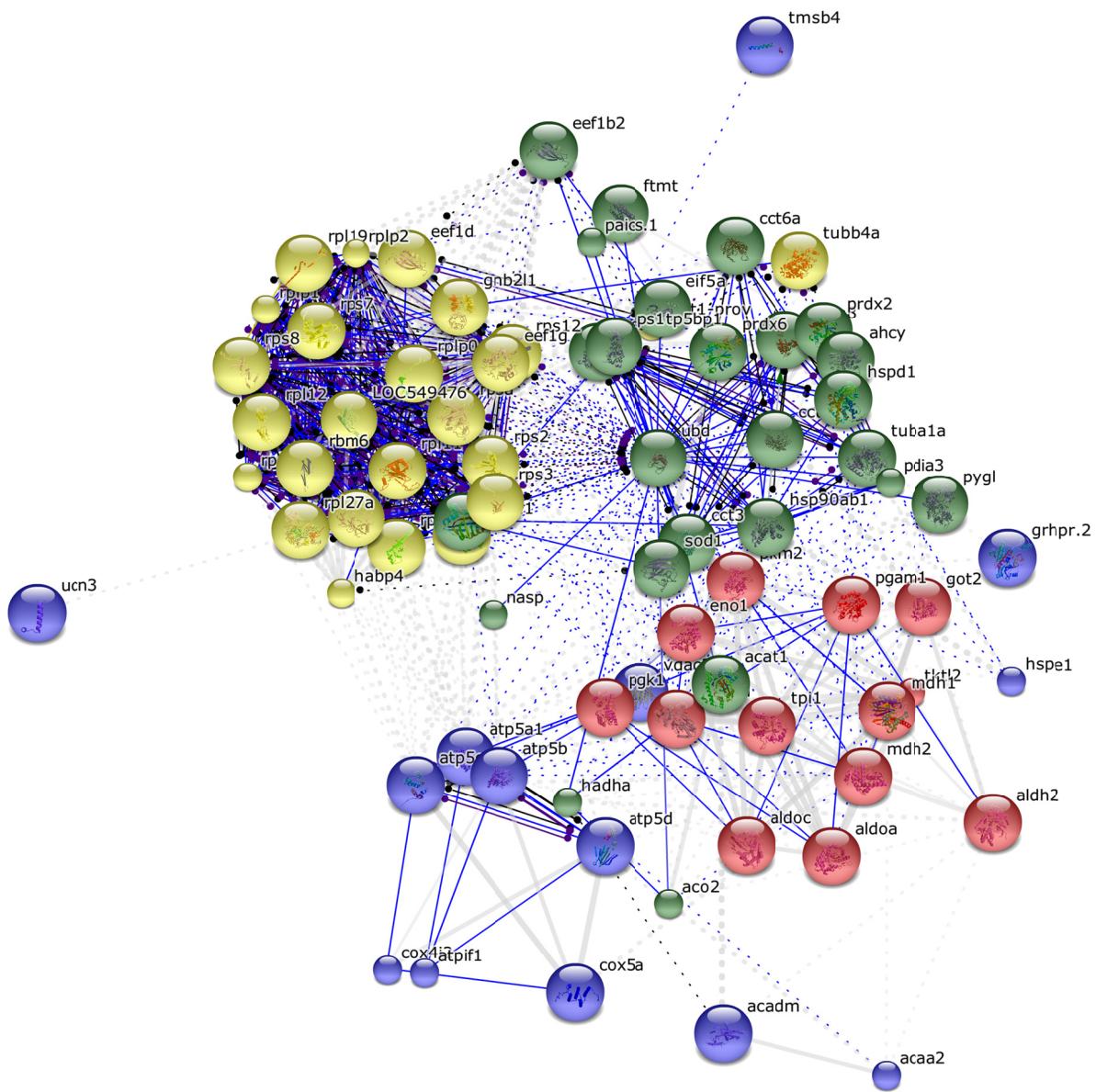
Figure S3. STRING interaction networks for proteins with stable expression between single D11 blastomeres (zoom-in of **Figure 6)**. STRING parameters: actions view shown; disconnected proteins removed; k-means clustering threshold = 3. Labels indicate gene names.