supplementary Fig 1

a

screening step	experiments	the number of positive clones	notes
0	suppression subtractive hybridization (using mouse primary cultured neuron)	480 (96 x 5)	[cDNA from tunicamycin-treated primary cultured neuron] minus [cDNA from normal primary cultured neuron]
1	dot blot analysis	147	
2	sequencing and database research	100	removing genes whose functions are already known (see Supplemental Fig 1B)
3	northern hybridization (using mouse primary cultured neuron)	23	see Supplemental Fig 1C
4	northern hybridization (using BALB/c 3T3)	7	including TRAPγ

b

dot blot series (each 96 clones)	the number of clones		
	total positive clones	known genes (BiP)	functionally unknown or est clones
1	29	12 (2)	17
2	28	8 (2)	20
3	39	10 (1)	29
4	20	6 (0)	14
5	31	11 (2)	20
total	147	47 (7)	100

c

clone No.	Tm induction		GENBANK accession Number	brief character
	neuronal cell	Balb/c 3T3		
ER1-2	+	NS	AI182245	est
ER1-16	+	+/-	AI115301	Mus musculus Fbxl5
ER1-19 (=ER1-61)	++	++	W62980	est
ER1-59	++	++	AA272065	Mus musculus Sdf2l1
ER1-83	++	-	X91656	Mus musculus Srp20
ER1-90	++	-	C78819	est
ER2-B7	+	-	AU067312	Mus musculus trans-golgi network protein (Tgoln1)
ER2-C4	+	-	AA934170	Mus musculus tropomodulin 3 (Tmod3)
ER2-D3	+/-	-	AA144989	est
ER2-F5	+/-	-	AA467609	Mus musculus FtsJ homolog 3 (Ftsj3)
ER2-G2	+	NS	AA990432	est
ER2-G3	+	-	AA139085	Mus musculus down-regulator of transcription 1 (Dr1)
ER2-I4	++	-	AA987183	Mus musculus matrin 3 (Matr3)
ER3-A8	+	+	R74635	Mus musculus TSPY-like 4 (Tspyl4)
ER3-B3	++	+	AA574497	Mus musculus leucine rich repeat containing 59 (Lrrc59)
ER3-C4	+	+	AW228009	Mus musculus translocon associated protein, γ (TRAPγ)
ER3-E1	++	++	AA437769	Mus musculus minor histocompatibility antigen precursor (H47)
ER3-E2	+	-	AI182245	est
ER4-D8	+/-	-	AI006586	Mus musculus ubiquitin specific peptidase 3
ER4-E5	+	-	AA500741	Mus musculus exportin 5
ER4-G6	+/-	+	AI049465	est
ER4-K1	+	++	AA409017	est
ER5-G2	+	+/-	AA798537	Mus musculus importin 11 (Ipo11)

supplementary Fig 1. Strategy and results for screening novel ER stress inducible genes. (A) Scheme of screening strategy based on suppression subtractive hybridization (SSH) and northern hybridization. cDNA library for screening was synthesized from mouse primary cultured neuron with or without tunicamycin treatment (10 μg mL^{-1}, 12 h). cDNAs encoding BiP and Grp94 were removed from cDNA library by pre-hybridization to avoid high background in the screening step. ER stress-responsible cDNA library was made by SSH method: [cDNAs from tunicamycin-treated primary cultured neuron] – [cDNAs from normal primary cultured neuron]. Following screening steps were composed of four experimental procedures; 1) dot blot analysis using 96 x 5 SSH positive clones (brief genetical profiles of the positive clones of this step were shown in B), 2) sequencing and database search to remove the genes whose functions are already known, 3) northern blot hybridization using total RNA obtained from primary cultured neuron, and 4) northern blot hybridization using total RNA obtained from BALB/c 3T3 cells. Finally, we obtained 7 positive clones, including TRAPγ, that were induced by ER stress both in primary cultured neuron and in BALB/c 3T3 cells, and their functions under ER stress response are unknown. (B) Table showing the number of positive clones in SSH and dot blot analysis, and brief genetical profiles of them. We isolated 147 clones including 7 clones encoding BiP cDNA, triplicate of ERdj4 cDNA and 3 pairs of duplicate cDNA. (C) Table showing the list of GENBANK accession number and a brief character for positive clones of third screening we obtained, and results of fourth screening. In the table, ++, +, +/- and - indicated over 3-fold, around 2-fold, weak and no induction by tunicamycin treatment, respectively. NS indicated no signal.