Table S1a Clinico-pathological information (PR mapping).

Sample number	Age	Age group	Sex	PMD (h)	CTD	CSF pH	BW (g)	Cause of death
CM1	40gw	Perinatal period	M	14:05	19:55	N.A.	320	Respiratory failure
CM2	4y	Childhood	M	23:55	9:35	N.A.	1565	Sepsis
CM3	17y	Adolescence	M	41:00	0:00	N.A.	1499	Myocarditis
CM4	27y	Youth	M	N.A.	N.A.	N.A.	N.A.	Drowning
CM5	55y	Middle age	M	7:15	11:05	N.A.	1393	Intestinal ischemia
CM6	79y	Elderly age	M	6:30	10:00	6.71	1121	Legal euthanasia
CF1	40gw	Perinatal period	F	30:45	3:30	N.A.	380	Asphyxia
CF2	7y	Childhood	F	9:45	7:15	N.A.	1320	Respiratory failure
CF3	15y	Adolescence	F	20:00	13:00	N.A.	1480	Lung edema
CF4	26y	Youth	F	6:35	2:25	N.A.	1156	Respiratory arrest
CF5	54y	Middle age	F	5:35	15:15	6.40	1118	Liver cirrhosis
CF6	83y	Elderly age	F	3:20	16:30	6.80	1173	Legal euthanasia

Abbreviations: BW, brain weight; CF, control females; CM, control males; CSF, cerebral spinal fluid; CTD, clock time of death; F, female; g, gram; gw, gestational week; h, hour; M, male; N.A., not available; PMD, postmortem delay; y, year.

Table S1b Clinico-pathological information (fetal hypothalami).

Sample	Age (gw)	Sex	PMD (h)	CTD	BW (g)	Cause of death
1	22	M	15:16	17:44	N.A.	N.A.
2	30	M	17:00	0:00	150	Pneumonia
3	37	M	17:00	0:00	400	Congenital heart syndrome

Abbreviations: BW, brain weight; CTD, clock time of death; g, gram; gw, gestational week; h, hour**;** M, male; N.A., not available; PMD, postmortem delay.

Table S1c Clinico-pathological information (two-group comparison).

Sample number	Psychiatric diagnosis	Age (years)	Sex	PMD (h)	CTD	CSF pH	BW (g)	Suicide attempt	Antipsychotics in last three months	Cause of death
MD1	MDD	50	M	N.A.	N.A.	N.A.	1380	Yes	N.A.	Suicide
MD2	MDD	39	M	41:00	00:00	N.A.	1670	Yes	TCA, SSRI	Suicide
MD3	BD	39	M	48:00	11:30	N.A.	1220	Yes	BZD, RIMA, SSRI	Suicide: intoxication
MD4	MDD	79	M	21:10	17:50	N.A.	1530	Yes	BZD, SSRI	Suicide: high fall
MD5	MDD	74	M	62:55	17:05	N.A.	1444	Yes	BZD, Cisordinol, SSRI	Suicide: hanging
MD6	MDD	62	F	20:35	00:00	6.53	1199	Yes	BZD	Suicide: suffocation
MD7	MDD	56	F	10:30	14:00	6.42	1120	Yes	BZD	Suicide: intoxication
MD8	MDD	62	F	11:40	16:15	6.18	1155	Yes	BZD	Suicide: strangulation
MD9	MDD	58	M	4:30	9:40	6.80	1470	Yes	SGA, SSRI, TCA	Suicide: intoxication
MD10	MDD	90	F	3:55	11:20	6.62	1220	No	BZD, INN	Suicide: intoxication
MD11	BD	81	M	6:40	20:00	6.70	1283	No	BZD, Li, TCA, VPA	Legal euthanasia
MD12	MDD	77	F	8:40	11:20	6.77	1120	Yes	BZD, INN, SSRI	Legal euthanasia
MD13	MDD	50	M	9:40	13:00	6.80	1420	No	BZD	Legal euthanasia
MD14	MDD	38	M	6:15	11:45	6.79	1525	No	BZD	Legal euthanasia

MD15	MDD	61	F	8:40	15:35	7.07	1205	No	BZD	Legal euthanasia
MD16	MDD	61	M	5:45	14:05	N.A.	1210	No	SSRI	Legal euthanasia
MD17	MDD	67	M	3:30	11:10	6.72	1445	No	BZD, Hal	Legal euthanasia
MD18	MDD	70	M	20:00	19:00	N.A.	1500	No	BZD, DA stabilizer, SSRI	Heart attack
MD19	MDD	73	F	22:00	19:00	N.A.	1287	No	BZD, TCA	Bronchopneumonia
MD20	MDD	71	M	13:30	22:30	N.A.	1109	No	BZD, Li, MAOI, PTZ	Respiratory insufficiency
MD21	MDD	61	M	60:20	4:40	N.A.	1424	No	PTZ	Pneumonia
MD22	MDD	81	M	6:00	15:30	6.50	1280	No	Hal	Renal insufficiency
MD23	MDD	68	M	8:55	5:45	6.82	1510	Yes	BZD, SSRI	Sudden death
MD24	BD	70	M	6:50	21:00	6.19	1275	No	BZD, LTG, VPA	Pulmonary infection and renal insufficiency
MD25	BD	73	M	13:45	19:45	6.24	1480	No	BZD	Infection
MD26	BD	94	F	5:10	4:00	7.55	985	No	BZD, SSRI	Respiratory and cadiac failure
MD27	MDD	58	F	7:20	23:00	5.61	1295	No	BZD, DA stabilizer, SSRI	Renal insufficiency
MD28	BD	65	M	4:50	18:30	N.A.	1305	No	BZD, Li, SGA	Colon carcinoma
Median	-	66	-	08:55	14:05	6.70	1291	-	-	-
CTR1	-	68	F	5:45	12:15	6.97	1135	No	None	Legal euthanasia

CTR2	-	83	F	3:20	16:30	6.80	1173	No	None	Legal euthanasia
CTR3	-	49	M	6:15	17:30	6.15	1364	No	BZD	Legal euthanasia
CTR4	-	79	M	6:30	10:00	6.71	1121	No	BZD	Legal euthanasia
CTR5	-	83	M	5:45	19:35	7.28	1195	No	None	Legal euthanasia
CTR6	-	41	M	17:00	00:00	N.A.	1150	No	None	Renal insufficiency
CTR7	-	72	F	17:30	16:30	N.A.	N.A.	No	None	Bronchopneumonia
CTR8	-	49	M	21:40	19:20	N.A.	1629	No	None	Cardiac infarction
CTR9	-	74	F	7:25	9:50	6.95	1167	No	BZD	Intestinal necrosis
CTR10	-	66	M	41:00	00:00	N.A.	1461	No	INN	Septic shock
CTR11	-	88	F	5:55	7:00	6.05	1115	No	BZD	Cardiac arrest
CTR12	-	39	M	16:30	00:30	N.A.	1400	No	None	Cardiac infarction
CTR13	-	55	M	7:15	11:05	N.A.	1393	No	BZD, INN	Intestinal ischemia
CTR14	-	78	F	4:35	22:40	6.41	1226	No	BZD, INN	Bronchopneumonia
CTR15	-	73	M	8:00	16:15	5.37	1553	No	BZD, INN	Pneumonia
CTR16	-	83	M	5:15	11:15	6.60	1372	No	BZD	Cardiac infarction
CTR17	-	67	M	9:00	15:05	6.48	1292	No	BZD, INN	Aortic aneurysm
Median	-	72	-	07:15	12:15	6.60	1259	-	-	-

***P* value**	-	0.45	0.83	0.34	n.sig.	0.75	0.47	-	-	-

Abbreviations: BD, bipolar disorder; BW, brain weight; BZD, benzodiazepine; CTD, clock time of death; CTR, control; DA, dopamine; F, female; Hal, haloperidol; INN, nemifitide; Li, lithium; LTG, lamotrigine; M, male; MAOI, monoamine oxidase inhibitor; MD, mood disorders; MDD, major depressive disorder; N.A., not available; PMD, postmortem delay; PTZ, pentylenetetrazole; RIMA, reversible inhibitor of monoamine oxidase-A; SGA, second-generation antipsychotics; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; VPA, valproate.

11 **Table S1d Clinico-pathological information (five-group comparison).**

Sample number	Psychiatric diagnosis	Age (years)	Sex	PMD (h)	CTD	CSF pH	BW (g)	Suicide attempt	Antipsychotics in last three months	Cause of death
MDS1	MDD	50	M	N.A.	N.A.	N.A.	1380	Yes	N.A.	Suicide
MDS2	MDD	39	M	41:00	00:00	N.A.	1670	Yes	TCA, SSRI	Suicide
MDS3	BD	39	M	48:00	11:30	N.A.	1220	Yes	BZD, RIMA, SSRI	Suicide: intoxication
MDS4	MDD	79	M	21:10	17:50	N.A.	1530	Yes	BZD, SSRI	Suicide: high fall
MDS5	MDD	74	M	62:55	17:05	N.A.	1444	Yes	BZD, Cisordinol, SSRI	Suicide: hanging
MDS6	MDD	62	F	20:35	00:00	6.53	1199	Yes	BZD	Suicide: suffocation
MDS7	MDD	56	F	10:30	14:00	6.42	1120	Yes	BZD	Suicide: intoxication
MDS8	MDD	62	F	11:40	16:15	6.18	1155	Yes	BZD	Suicide: strangulation
MDS9	MDD	58	M	4:30	9:40	6.80	1470	Yes	SGA, SSRI, TCA	Suicide: intoxication
MDS10	MDD	90	F	3:55	11:20	6.62	1220	No	BZD, INN	Suicide: intoxication
Median	-	60	-	20:35	11:30	6.53	1300	-	-	-
MDE1	BD	81	M	6:40	20:00	6.70	1283	No	BZD, Li, TCA, VPA	Legal euthanasia
MDE2	MDD	77	F	8:40	11:20	6.77	1120	Yes	BZD, INN, SSRI	Legal euthanasia
MDE3	MDD	50	M	9:40	13:00	6.80	1420	No	BZD	Legal euthanasia

MDE4	MDD	38	M	6:15	11:45	6.79	1525	No	BZD	Legal euthanasia
MDE5	MDD	61	F	8:40	15:35	7.07	1205	No	BZD	Legal euthanasia
MDE6	MDD	61	M	5:45	14:05	N.A.	1210	No	SSRI	Legal euthanasia
MDE7	MDD	67	M	3:30	11:10	6.72	1445	No	BZD, Hal	Legal euthanasia
Median	-	61	-	6:40	13:00	6.78	1283	-	-	-
MDN1	MDD	70	M	20:00	19:00	N.A.	1500	No	BZD, DA stablizer, SSRI	Heart attack
MDN2	MDD	73	F	22:00	19:00	N.A.	1287	No	BZD, TCA	Bronchopneumonia
MDN3	MDD	71	M	13:30	22:30	N.A.	1109	No	BZD, Li, MAOI, PTZ	Respiratory insufficiency
MDN4	MDD	61	M	60:20	4:40	N.A.	1424	No	PTZ	Pneumonia
MDN5	MDD	81	M	6:00	15:30	6.50	1280	No	Hal	Renal insufficiency
MDN6	MDD	68	M	8:55	5:45	6.82	1510	Yes	BZD, SSRI	Sudden death
MDN7	BD	70	M	6:50	21:00	6.19	1275	No	BZD, LTG, VPA	Pulmonary infection and renal insufficiency
MDN8	BD	73	M	13:45	19:45	6.24	1480	No	BZD	Infection
MDN9	BD	94	F	5:10	4:00	7.55	985	No	BZD, SSRI	Respiratory and cadiac failure
MDN10	MDD	58	F	7:20	23:00	5.61	1295	No	BZD, DA stabilizer, SSRI	Renal insufficiency
MDN11	BD	65	M	4:50	18:30	N.A.	1305	No	BZD, Li, SGA	Colon carcinoma

Median	-	70	-	8:55	19:00	6.37	1295	-	-	-
CE1	-	68	F	5:45	12:15	6.97	1135	No	None	Legal euthanasia
CE2	-	83	F	3:20	16:30	6.80	1173	No	None	Legal euthanasia
CE3	-	49	M	6:15	17:30	6.15	1364	No	BZD	Legal euthanasia
CE4	-	79	M	6:30	10:00	6.71	1121	No	BZD	Legal euthanasia
CE5	-	83	M	5:45	19:35	7.28	1195	No	None	Legal euthanasia
Median	-	79	-	5:45	16:30	6.80	1173	-	-	-
CN1	-	41	M	17:00	00:00	N.A.	1150	No	None	Renal insufficiency
CN2	-	72	F	17:30	16:30	N.A.	N.A.	No	None	Bronchopneumonia
CN3	-	49	M	21:40	19:20	N.A.	1629	No	None	Cardiac infarction
CN4	-	74	F	7:25	9:50	6.95	1167	No	BZD	Intestinal necrosis
CN5	-	66	M	41:00	00:00	N.A.	1461	No	INN	Septic shock
CN6	-	88	F	5:55	7:00	6.05	1115	No	BZD	Cardiac arrest
CN7	-	39	M	16:30	00:30	N.A.	1400	No	None	Cardiac infarction
CN8	-	55	M	7:15	11:05	N.A.	1393	No	BZD, INN	Intestinal ischemia
CN9	-	78	F	4:35	22:40	6.41	1226	No	BZD, INN	Bronchopneumonia
CN10	-	73	M	8:00	16:15	5.37	1553	No	BZD, INN	Pneumonia

CN11	-	83	M	5:15	11:15	6.60	1372	No	BZD	Cardiac infarction
CN12	-	67	M	9:00	15:05	6.48	1292	No	BZD, INN	Aortic aneurysm
Median	-	70	-	8:30	11:10	6.45	1372	-	-	-
***P* value**	-	0.43	0.97	0.07	Sig.	0.21	0.47	-	-	-

Abbreviations: BD, bipolar disorder; BW, brain weight; BZD, benzodiazepine; CTD, clock time of death; CTR, control; DA, dopamine; F, female; Hal, haloperidol; INN, nemifitide; Li, lithium; LTG, lamotrigine; M, male; MAOI, monoamine oxidase inhibitor; MD, mood disorders; MDD, major depressive disorder; N.A., not available; PMD, postmortem delay; PTZ, pentylenetetrazole; RIMA, reversible inhibitor of monoamine oxidase-A; SGA, second-generation antipsychotics; Sig., significance; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; VPA, valproate.

16 **Table S2 Specification of the antibodies used.**

Antibody	Species	Manufacturer	Catalog number and specificity
PR	rabbit monoclonal	Abcam	ab32085
α-MSH	rabbit polyclonal	NIN	α-MSH 4372 #23.04.75 [1]
NPY	rabbit polyclonal	NIN	Niepke #26.11.88 [2]
CRH	rat monoclonal	Gift NIN	PFU 83 [3]
TRH	rabbit polyclonal	Novus Biologicals	NBP2-34014
GFAP	rabbit polyclonal	Agilent DAKO	GA524
Iba1	rabbit polyclonal	FUJIFILM Wako	019-19741
GFAP-δ	rabbit polyclonal	Gift NIN	100501 [4]
Nestin	mouse monoclonal	Chemicon	MAB5326
OXT	mouse monoclonal	Gift	OT-A-I-28 [5]
AVP	mouse monoclonal	Gift	VPIII-D-7 [6]
SOM	rabbit polyclonal	NIN	Somaar [7]
GAL	rabbit polyclonal	NIN	Gaaltje [8]
KISS1	sheep polyclonal	Gift	GQ2 [9]
TH	mouse monoclonal	Sigma-Aldrich	MAB318

DYN	rabbit polyclonal	Gift	Dynorphin (1-8) #73 [10]

Note: 1 Specificities of commercial antibodies were provided by the manufacturers. The specificity of ab32085 refers to *Wagner, J., Rapsomaniki, M. A., Chevrier, S., Anzeneder, T., Langwieder, C., Dykgers, A., ... & Bodenmiller, B. (2019). A single-cell atlas of the tumor and immune ecosystem of human breast cancer. Cell, 177(5), 1330-1345*.

2 POMC-ir neurons were stained by α-MSH antibody [11].

Anti-CRH antibody is a kind gift from Prof. Dr. F.J.H. Tilders;

Anti-GFAP- δ antibody is a kind gift from Prof. Dr. E.M. Hol;

Anti-OXT antibody is a kind gift from Prof. Dr. F.W. van Leeuwen who obtained it from Dr. A. Hou Yu;

Anti-AVP antibody is a kind gift from Prof. Dr. F.W. Van Leeuwen who obtained it from Dr. A. Silverman;

Anti-KISS1 antibody is a kind gift from Prof. Dr. W.S. Dhillo;

Anti-DYN antibody is a kind gift from Prof. Dr. S.J. Watson.

Abbreviations: α-MSH, alpha-melanocyte-stimulating hormone; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; DYN, dynorphin; GAL, galanin; GFAP, glial fibrillary acidic protein; GFAP-δ, glial fibrillary acidic protein-delta; NIN, Netherlands Institute for Neuroscience; HLA-DP, DQ, DR, human leukocyte antigen-DP, DQ, DR isotypes; KISS1, kisspeptin; NPY, neuropeptide Y; OXT, oxytocin; PR, progesterone receptor; SOM, somatostatin; TH, tyrosine hydroxylase; TRH, thyrotropin-releasing hormone.

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Table S3a Immunohistochemistry for single staining.

Antibody	Washing buffer	Antigen retrieval	Blocking buffer	Incubating buffer	Dilution	Incubating time
PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI [1]	1:100	72 h
α-MSH	3×TBS	0.03M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI-HS [2]	1:8000	24 h
NPY	1×TBS	-	1×TBS-5% milk	SUMI-milk [3]	1:1000	24 h

Note: 1 SUMI: supermix, 0.25 g gelatin in 100 ml 1×TBS, pH 7.6 heat the gelatin, mix until it dissolves, add Triton-X 0.5 ml. Store in fridge.

2 SUMI-HS: supermix-high salt, 0.25 g gelatin in 100 ml 3×TBS, pH 7.6 heat the gelatin, mix until it dissolves, add Triton-X 0.5 ml. Store in fridge.

3 SUMI-milk: add 5 g milk powder in 100 ml SUMI, mix until it dissolves. Prepare before use.

Abbreviations: α-MSH, alpha-melanocyte-stimulating hormone; NPY, neuropeptide Y; PR, progesterone receptor; TBS, tris-buffered saline.

71 **Table S3b Immunohistochemistry for double staining.**

Combo	Antibodies	Washing buffer	Antigen retrieval	Blocking buffer	Incubating buffer	Dilution	Incubating time	Color development
PR+α-MSH	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	α-MSH	3×TBS	-	-	SUMI-HS	1:4000	24 h	DAB
PR+NPY	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	NPY	1×TBS	-	1×TBS-5% milk	SUMI-milk	1:500	24 h	DAB
PR+CRH	PR	1×TBS	0.01M citrate buffer (pH 6.0)	-	SUMI	1:100	72 h	DAB-Ni

			microwave 800 w 20 min					
	CRH	1×TBS	-	-	SUMI	1:50000	24 h	DAB
PR+TRH	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	TRH	3×TBS	-	-	SUMI-HS	1:2000	24 h	DAB
PR+GFAP	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	GFAP	1×TBS	-	-	SUMI	1:10000	24 h	DAB
PR+ iba1	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni

	Iba1	1×TBS	-	-	SUMI	1:500	24 h	DAB
PR+GFAP-δ	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	GFAP-δ	1×TBS	-	-	SUMI	1:250	24 h	DAB
PR+nestin	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	nestin	1×TBS	-	-	SUMI	1:200	24 h	DAB
PR+OXT	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	OXT	1×TBS	-	-	SUMI	1:500	24 h	DAB

PR+AVP	PR	1×TBS	0.05M Tris-HCl buffer (pH 9.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	AVP	1×TBS	-	-	SUMI	1:100	24 h	DAB
PR+SOM	PR	1×TBS	0.05M Tris-HCl buffer (pH 9.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	SOM	3×TBS	-	-	SUMI-HS	1:800	24 h	DAB
PR+GAL	PR	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	GAL	1×TBS	-	-	SUMI	1:800	24 h	DAB
PR+KISS1	PR	1×TBS	0.01M citrate buffer (pH 6.0)	-	SUMI	1:100	72 h	DAB-Ni

			microwave 800 w 20 min					
	KISS	-	-	-	SUMI	1:50000	24 h	DAB
PR+TH	PR	1×TBS	0.05M Tris-HCl buffer (pH 9.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	TH	1×TBS	-	-	SUMI	1:750	24 h	DAB
PR+DYN	PR	1×TBS	0.05M Tris-HCl buffer (pH 9.0) microwave 800 w 20 min	-	SUMI	1:100	72 h	DAB-Ni
	DYN	1×TBS	-	-	SUMI	1:200	24 h	DAB
α-MSH+NPY	α-MSH	3×TBS	0.03M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI-HS	1:8000	24 h	DAB-Ni

	NPY	1×TBS	-	1×TBS-5% milk	SUMI-milk	1:500	24 h	DAB
α-MSH+CRH	α-MSH	3×TBS	0.03M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI-HS	1:8000	24 h	DAB-Ni
	CRH	1×TBS	-	-	SUMI	1:50000	24 h	DAB
α-MSH+TRH	α-MSH	3×TBS	0.03M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI-HS	1:8000	24 h	DAB-Ni
	TRH	3×TBS	-	-	SUMI-HS	1:2000	24 h	DAB
NPY+α-MSH	NPY	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	1×TBS-5% milk	SUMI-milk	1:1000	24 h	DAB-Ni
	α-MSH	3×TBS	-	-	SUMI-HS	1:4000	24 h	DAB

NPY+CRH	NPY	1×TBS	-	1×TBS-5% milk	SUMI-milk	1:1000	24 h	DAB-Ni
	CRH	1×TBS	-	-	SUMI	1:50000	24 h	DAB
NPY+TRH	NPY	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	1×TBS-5% milk	SUMI-milk	1:1000	24 h	DAB-Ni
	TRH	3×TBS	-	-	SUMI-HS	1:2000	24 h	DAB
CRH+α-MSH	CRH	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100000	24 h	DAB-Ni
	α-MSH	3×TBS	-	-	SUMI-HS	1:4000	24 h	DAB
CRH+NPY	CRH	1×TBS	-	-	SUMI	1:100000	24 h	DAB-Ni
	NPY	1×TBS	-	1×TBS-5% milk	SUMI-milk	1:500	24 h	DAB

CRH+TRH	CRH	1×TBS	0.01M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI	1:100000	24 h	DAB-Ni
	TRH	3×TBS	-	-	SUMI-HS	1:2000	24 h	DAB
TRH+α-MSH	TRH	3×TBS	0.03M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI-HS	1:4000	24 h	DAB-Ni
	α-MSH	3×TBS	-	-	SUMI-HS	1:4000	24 h	DAB
TRH+NPY	TRH	3×TBS	0.03M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI-HS	1:4000	24 h	DAB-Ni
	NPY	1×TBS	-	1×TBS-5% milk	SUMI-milk	1:500	24 h	DAB

TRH+CRH	TRH	3×TBS	0.03M citrate buffer (pH 6.0) microwave 800 w 20 min	-	SUMI-HS	1:4000	24 h	DAB-Ni
	CRH	1×TBS	-	-	SUMI	1:50000	24 h	DAB

Note: 1 SUMI: supermix, 0.25 g gelatin in 100 ml 1×TBS, pH 7.6 heat the gelatin, mix until it dissolves, add Triton-X 0.5 ml. Store in fridge.

2 SUMI-HS: supermix-high salt, 0.25 g gelatin in 100 ml 3×TBS, pH 7.6 heat the gelatin, mix until it dissolves, add Triton-X 0.5 ml. Store in fridge.

3 SUMI-milk: add 5 g milk powder in 100 ml SUMI, mix until it dissolves. Prepare before use.

Abbreviations: α-MSH, alpha-melanocyte-stimulating hormone; AVP, arginine vasopressin; CRH, corticotropin-releasing hormone; DAB, 3,3'-diaminobenzidine; DAB-Ni, 3,3'-diaminobenzidine-nickel; DYN, dynorphin; GAL, galanin; GFAP, glial fibrillary acidic protein; GFAP-δ, glial fibrillary acidic protein-delta; HLA-DP, DQ, DR, human leukocyte antigen-DP, DQ, DR isotypes; KISS1, kisspeptin; NPY, neuropeptide Y; OXT, oxytocin; PR, progesterone receptor; SOM, somatostatin; TBS, tris-buffered saline; TRH, thyrotropin-releasing hormone.