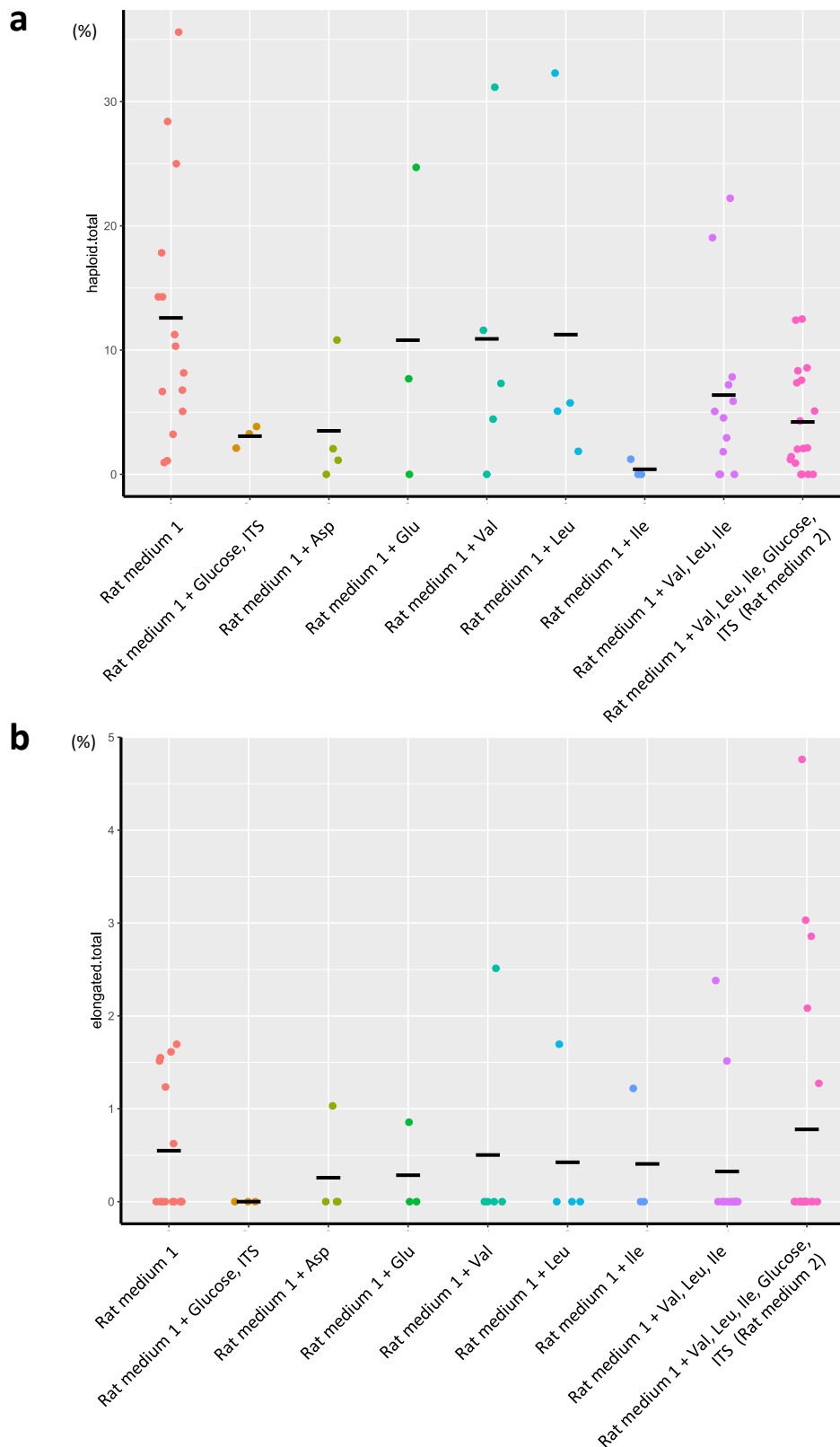
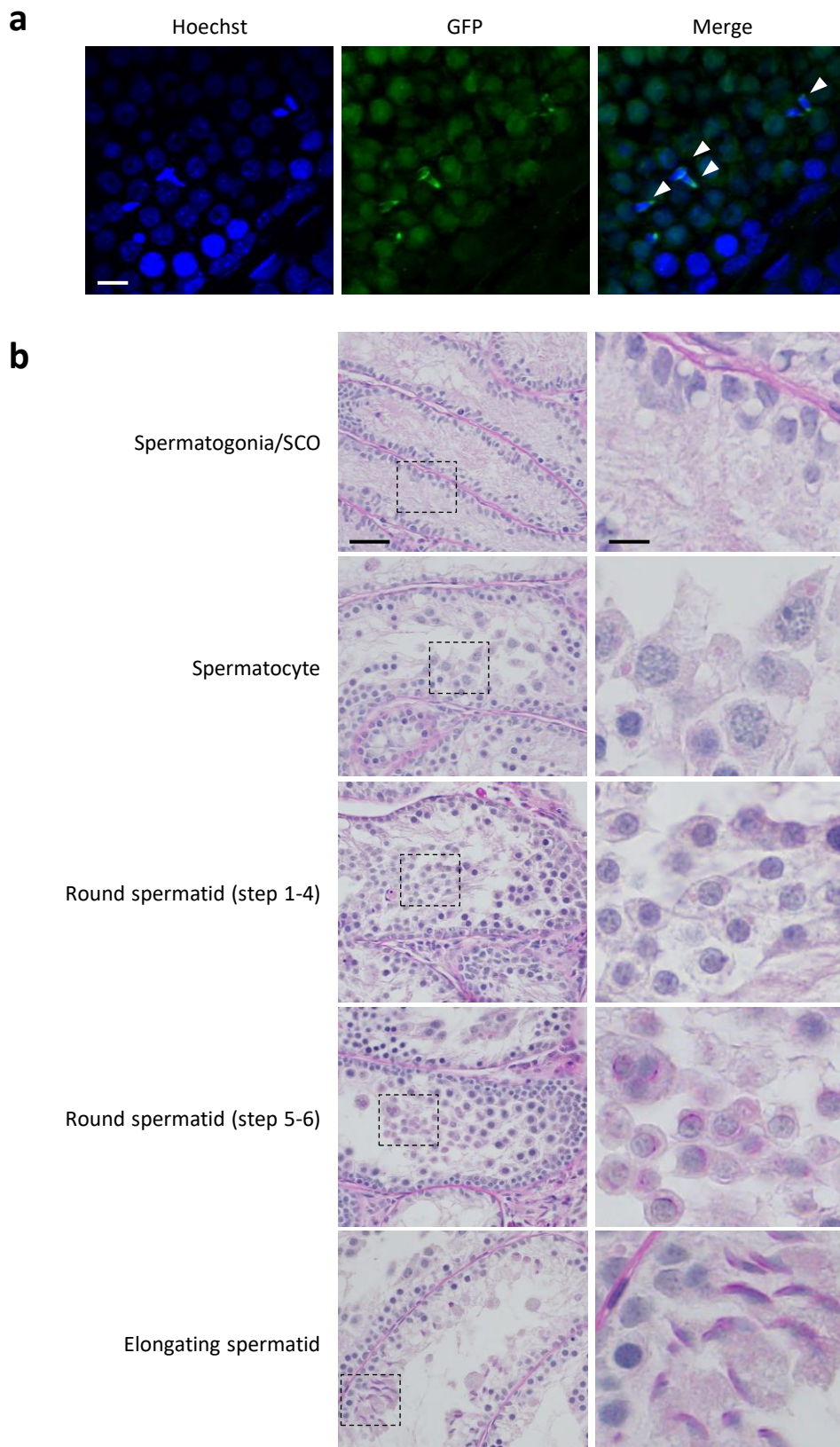




Supplementary Figure 1: Augmentation of consumed substances in rat medium 1. (a) In the histological examination, seminiferous tubules containing round or elongating spermatids were counted as positive, and the positivity rates were calculated by dividing the number of positive tubules by the number of total tubules examined. (b) The rate of elongating spermatid-containing seminiferous tubules was calculated in the same manner as in (a). ITS: insulin-transferrin-selenate; Asp: aspartate; Glu: glutamic acid; Val: valine; Leu: leucine; Ile: isoleucine.



Supplementary Figure 2: Histological analysis of cultured tissues.

(a) Immunostaining with anti-GFP antibody (green) and Hoechst 33342 nuclear staining (blue). (b) PAS-stained view of germ cells in P7 rat tissues cultured for 41 days using rat medium 1. Scale bars: 20 μ m (A), 50 μ m (B left), 10 μ m (B right).

Rat medium 1		
AlbuMAX I	20	mg/mL
Testosterone	1	μM
3,3',5-Triiodo-L-thyronine sodium	2	ng/mL
LH	1	ng/mL
FSH	1	ng/mL
L-Ascorbic acid 2 glucoside	0.5	mM
DL-α-Tocopherol acetate	0.5	mM
L-Glutathione reduced	0.5	mM
L-α-Lysophosphatidylcholine	100	μg/mL
Lysophosphatidic acid	10	μM

Ingredients were added to the α-MEM.

Supplementary Table 1: Composition of rat medium 1

Substances supplemented		Concentration added (µg/mL)
D(+)-Glucose		1000
ITS	Insulin	1
	Transferrin	0.55
	Sodium Selenite	0.00067
L-Aspartic acid (Asp)		30
L-Glutamic Acid (Glu)		75
L-Valine (Val)		46
L-Isoleucine (Ile)		52
L-Leucine (Leu)		52

Supplementary Table 2: Substances augmented in Rat medium 1.

Rat medium 2		
AlbuMAX I	20	mg/mL
Testosterone	1	μM
3,3',5-Triiodo-L-thyronine sodium	2	ng/mL
LH	1	ng/mL
FSH	1	ng/mL
L-Ascorbic acid 2 glucoside	0.5	mM
DL-α-Tocopherol acetate	0.5	mM
L-Glutathione reduced	0.5	mM
L-α-Lysophosphatidylcholine	100	μg/mL
Lysophosphatidic acid	10	μM
D(+)-Glucose	1	mg/mL
Insulin *	1	μg/mL
Transferrin *	0.55	μg/mL
Sodium Selenite *	0.00067	μg/mL
L-Valine	46	μg/mL
L-Isoleucine	52	μg/mL
L-Leucine	52	μg/mL

Ingredients were added to the α-MEM.

* These 3 were added as ITS (Thermo Fisher Scientific).

Supplementary Table 3: Composition of rat medium 2

Components	Day 0	Day 3	Day 7	Day 17	Day 21	Day 24	Day 28
Threonine	342	378.7	359.7	388.6	427.6	419.4	415.1
Cysteine	760.3	579.9	460	244.7	511.3	528.5	543.2
Alanine	203.7	307.3	308.4	282.6	311.7	332.9	337
Histidine	140	167	169.7	160.9	199.7	196.9	192.2
Methionine	71.2	79.1	75.5	73	83.5	83.2	80.4
Tyrosine	139.8	149	146.1	161.2	169.9	176	172.3
Cyanocobalamin	0.9	0.9	0.9	0.9	1.1	1	1
Folic_acid	1	1	1	1	1.1	1.1	1.1
Tryptophan	33.8	34.8	32.3	34.5	36.8	38.2	36.8
Threonic_acid	7.9	26.8	30	33.7	43.7	38.8	43
Cystyl-Cysteine	48.8	78.2	103.9	134.4	173.4	161.3	180.9
Hydroxy Proline		1.6	3.5	3.7	5.8	4.3	4.9
Glycine	427.6	564.3	625.6	701.9	908.9	846.6	999.7
Glutamine	1282.1	1334.5	1438.2	1625.4	1926.1	1857	1922.2
Methionine_sulfoxide	1.4	2.5	3.4	3.5	4.3	4.7	4.6
Citruline		2.5	4	3.4	4.3	3.4	3.5
Ornithine	0.2	7.9	15.1	9.8	10.1	8.9	9.8
Proline	238	253.8	260.9	289.2	328.1	338.7	317.6
2AE		12.8	14	15	25.3	22.7	23
Lysine	262.1	302	318.7	328.2	397.5	390.8	385.1
Lactate		435.5	967.9	1050.8	1254.5	1155.4	1091
Uracil		1.4	6.8	10.7	15.5	9.2	10.1
Citrate	16.7	11.9	32.9	36.1	54.7	44.4	54.1
Uric_acid		1.5	13	18.5	32.9	19.4	28
Oxoproline	93	230.3	402.7	448.8	733.7	609.5	773.6
Uridine		2.5	2.8	4.4	6	4.8	5.7
Choline	5.9	17.9	22.2	17.4	24.9	20.6	24
Putrescine	0	6.2	19.3	14.1	19.3	11.7	12.3
Thymidine		2.5	3.9	4.9	6.6	4.8	6.6
Cytidine		2.9	4.3	4.1	5.7	4.3	5.2
Deoxycytidine		2.7	4.8	4.6	6.1	4.4	5.2
Biotin	0.5	0.4	0.5	0.4	0.4	0.4	0.6
Phenylalanine	133.1	143	140.1	154.2	174.3	174.3	172.9
Glucose	4253.9	4450.3	3620.6	2196.7	1598.8	2386.2	1361.5
Asparagine	571.8	574.6	515.9	491.2	512.2	521.9	448
Aspartic acid	166.6	159.6	113.4	77.4	32.2	69.2	19.7
Serine	158.2	178	150.4	128.9	141.7	153.9	139.3
Glyceric_acid		16.7	15.1	13.7	18.5	18.7	12.5
Glutamic acid	397.2	472.9	321.6	236.2	116.2	199.2	59.4
Ascorbate	81.1	3.6	0	0	0	0	0
Pyruvate	571.4	552.6	414.7	307	168.4	231.6	165.9
Arginine	425.2	428.9	389.7	344.9	335.2	378.2	324.1
Glutathione	40.9	32.3	22.7	19	20.3	21	14.5
Valine	307.8	308.2	232.5	166.9	114.6	181.9	102.4
Niacinamide	6.3	6.6	5.6	5.2	5.1	5.5	4.2
Oxidized_glutathione	486	461.1	418.4	401.3	375.9	391	316.7
Riboflavin	0.5	0.4	0.4	0.4	0.5	0.3	0.3
Pyridoxal	8.1	6.4	4.3	3.3	2	2.6	1.4
Isoleucine	373.7	327.2	211.5	146.2	65.6	144.6	57.4
Leucine	379.4	332	211.2	150.8	74	154.2	75.2

Supplementary Table 4: Concentrations of medium components (μM)