SUPPLEMENTARY INFORMATION

An improved method for growing neurons: comparison with standard methods

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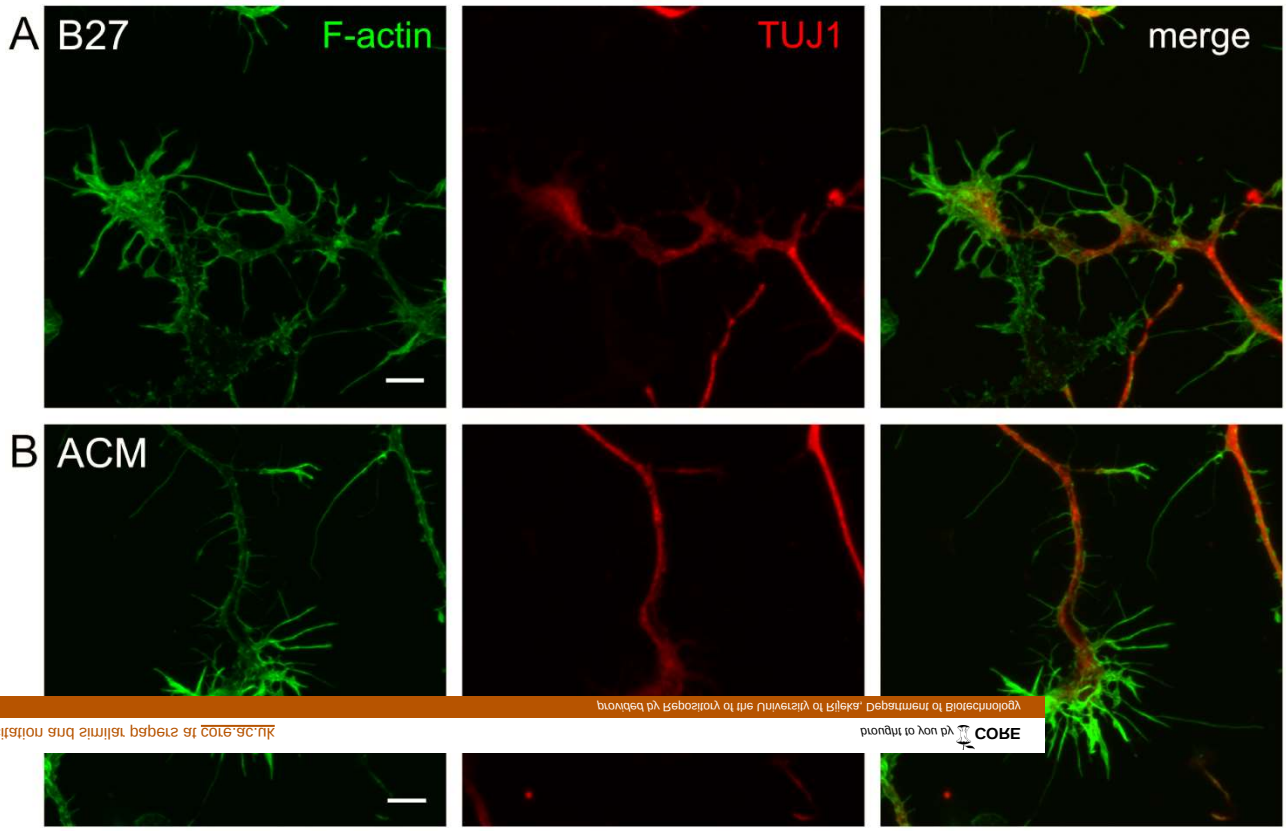
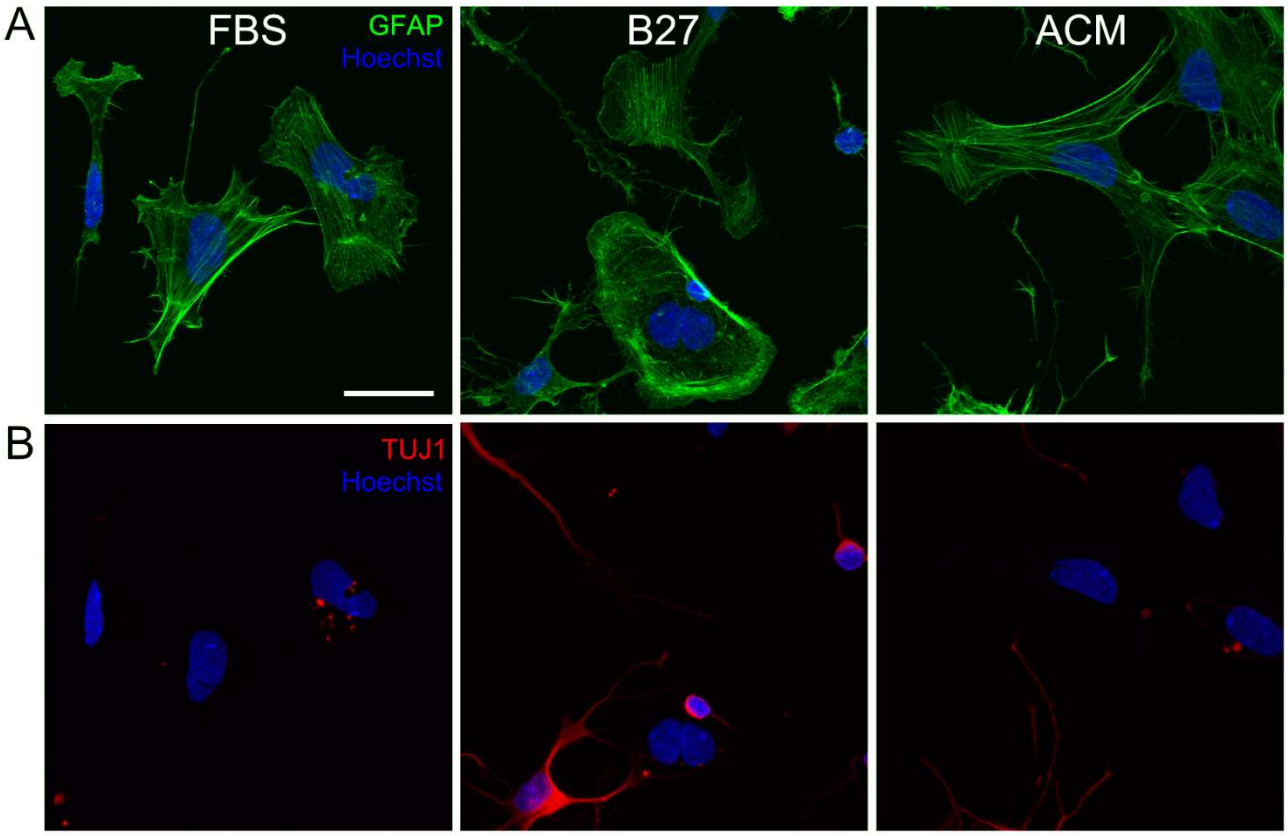
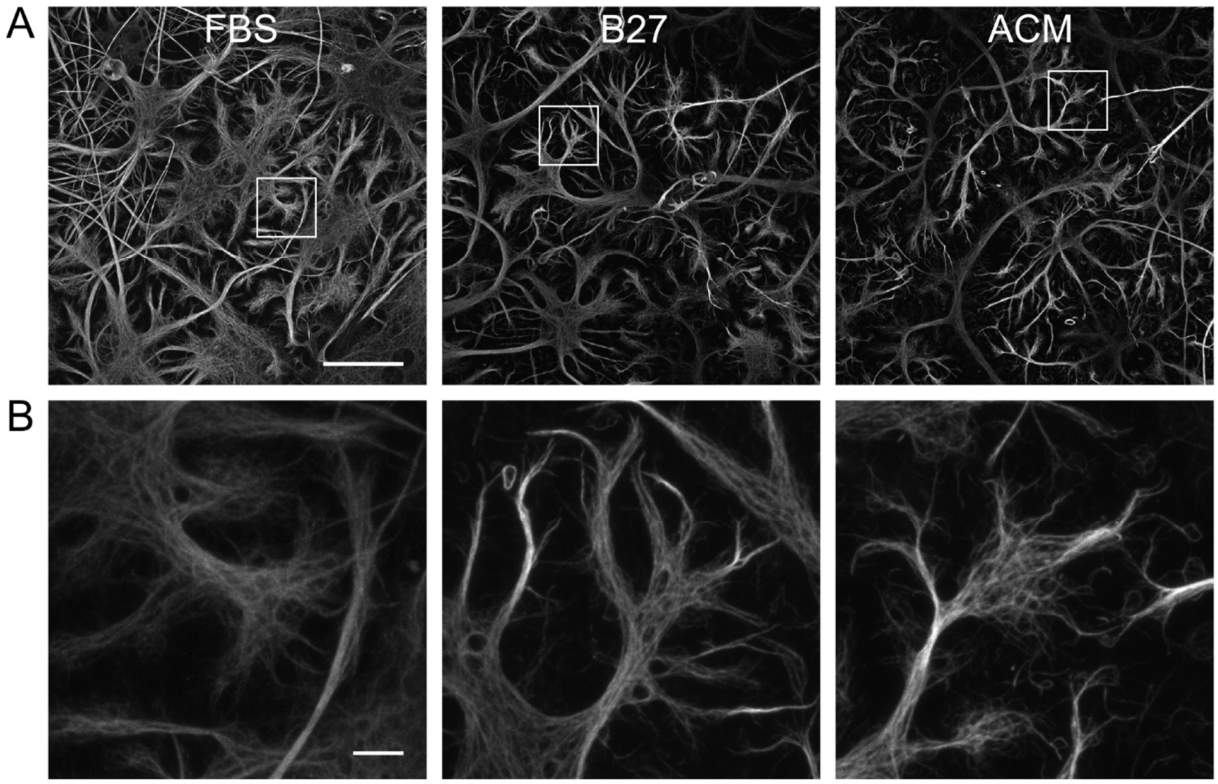


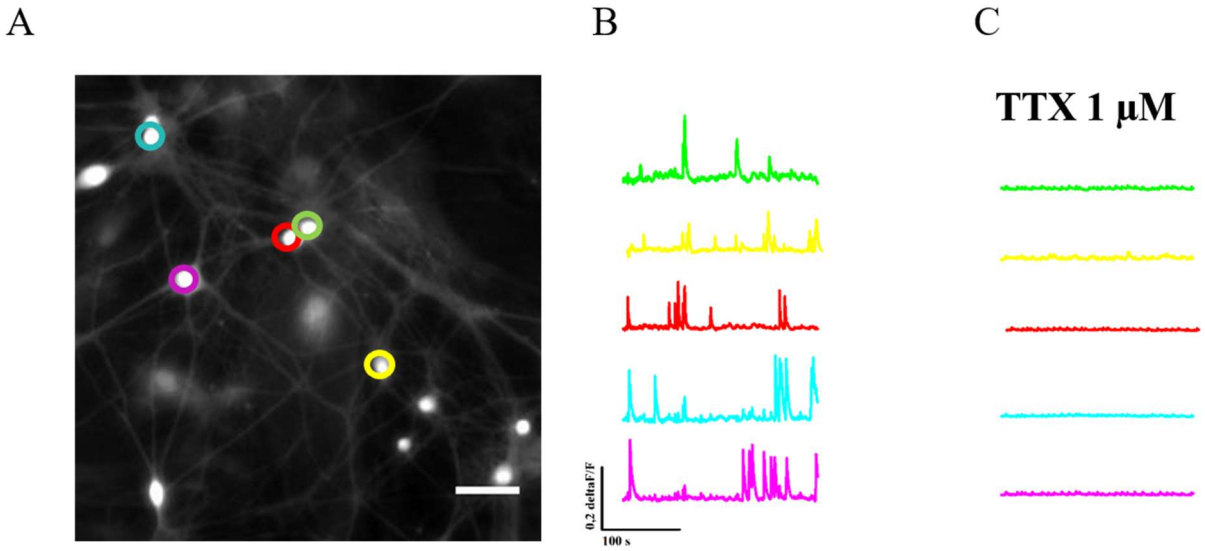
Supplementary Figure 1. Elaborated growth cone morphology in Neurobasal/B27-based media. Hippocampal culture at DIV1 in B27 (A) and ACM (B) stained for F-actin (green) and β-tubulin III (TUJ1, red). Such complex morphology was never observed in FBS-based medium at DIV1. Scale bar, 5µm.



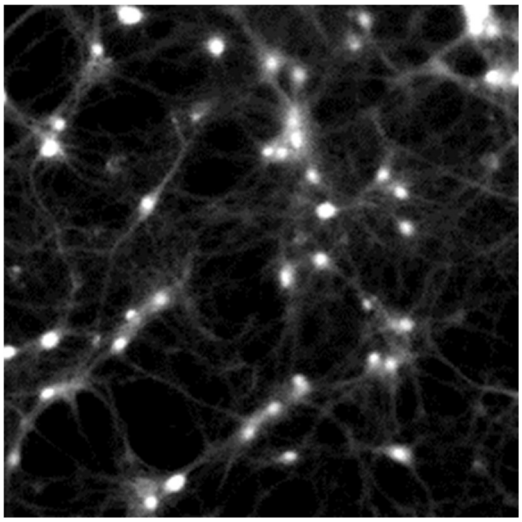
Supplementary Figure 2. Glial cell morphology at DIV1. (A) Hippocampal culture in FBS (left), B27 (middle) and ACM (right) stained for F-actin (green) and Hoechst nuclear stain (blue). (B) Fluorescent confocal images of the same fields in A but for neuronal marker β-tubulin III (TUJ1, red) and Hoechst nuclear stain (blue). Glial cells are identified as F-actin-positive and β-tubulin III-negative cells. Scale bar, 25 μm.



Supplementary Figure 3. Glial cells morphology after two weeks of culture. (A) Hippocampal culture at DIV14 in FBS (left), B27 (middle) and ACM (right) stained for glial fibrillary acidic protein (GFAP). Scale bar, 50 µm. (B) Astrocytes processes at higher magnification. Corresponding insets are indicated in A. Scale bar, 5 µm.



Supplementary Figure 4. The blocking action of TTX: the neuronal origin of calcium transients in a representative calcium imaging experiment on cells grown in FBS medium for 2 months and loaded with 4 µM Oregon Green 488 BAPTA - 1® calcium indicator. Cells analyzed in (A) were identified as neurons because their spontaneous calcium transients (B) were blocked by the addition of 1 µM TTX, a well-known specific antagonist of voltage gated sodium channels (C).



Supplementary Video 1. Fluorescent images of neuronal cultures grown for 1.5 months in ACM, loaded with 4 μM Oregon Green 488 BAPTA - 1® and imaged with an EM –CCD camera at a speed of 7 Hz for 10 min.

	Neuronal medium (MEM w/Glutamax® – based)	**Neurobasal/B27 (Neurobasal® - based)**
Amino Acids	**Concentration (mg/l)**	**Concentration (mg/l)**
L – Arginine hydrochloride	126.0	84.0
L - Cysteine	24.0	31.5
L-Histidine hydrochloride-H_2O	42.0	42.0
L - Isoleucine	52.0	105.0
L - Leucine	52.0	105.0
L – Lysine hydrochloride	73.0	146.0
L - Methionine	15.0	30.0
L - Phenylalanine	32.0	66.0
L - Threonine	48.0	95.0
L - Tryptophan	10.0	16.0
L - Tyrosine	36.0	72.0
L - Valine	46.0	94.0
L – Alanyl - Glutamine	406.0	108.5
Glycine		30.0
L - Alanine		2.0
L – Asparagine – H_2O		0.83
L - Proline		7.76
L - Serine		42.0
Vitamins		
Choline chloride	1.0	4.0
D – Calcium pantothenate	1.0	4.0
Folic Acid	1.0	4.0
Niacinamide	1.0	4.0
Pyridoxal hydrochloride	1.0	4.0
Thiamine hydrochloride	1.0	4.0

Riboflavin	0.1	0.4
i- Inositol	2.0	7.2
Vitamin B12	1.5	0.0068
Inorganic salts		
Calcium chloride	264.0	200.0
Sodium Bicarbonate	2200.0	2200.0
Sodium chloride	6800.0	4000.0
Sodium phosphate monobasic	158.0	125.0
Magnesium sulfate	200.0	
Ferric nitrate		0.1
Magnesium chloride		77.3
Potassium Chloride	400.0	400.0
Zinc sulfate		0.194
Other components		
D - glucose	7000.0	4500.0
Phenol red	10.0	8.1
HEPES	3600.0	2600.0
Sodium Pyruvate		25.0
Apo - transferrin	100	***B27***
Biotin	0.1	***B27***
Insulin	30.0	***B27***

Supplementary Table 1. Chemical composition of Minimum Essential Medium (MEM, cat.no.41090028)-based Neuronal medium and Neurobasal medium (cat.no.21103049, both from ThermoFisher). The two media differ in terms of concentration and number of components. Most of the amino acids and vitamins are more concentrated in Neurobasal comparing to MEM, while inorganic salts in MEM are less numerous than in Neurobasal but more concentrated. Note that components such as apo – transferrin, biotin and insulin were added at specific concentration to the neuronal medium formulation supplemented with FBS, while in Neurobasal/B27 they are included in the commercial B27 supplement at unknown concentration (for complete composition list of the B27 supplement please see cat.no.17504044, Thermo Fisher Scientific). In both cases, the antibiotic gentamycin was added at a concentration of 2.5 μg/l.