Supplementary Methods

Force-distance curve analysis

Force-distance curves were acquired using contact mode. The obtained force-distance curves were analyzed using the SPM image processing v.3 software (JPK instruments). For the cantilever with a bead, the "Sphere" (1) and "Paraboloid (the original Hertz model)" (Kuznetsova et al., 2007) (2) models were applied according to the manufacturer's instruction and compared for the calculation of Young's moduli.

$$F = \frac{E}{1-\nu^2}\left[\frac{a^2+R^2}{2}\ln\frac{R+a}{R-a} - aR\right] \quad (1)$$

$$F = \frac{E}{1-\nu^2}\frac{4\sqrt{R}}{3}\delta^{3/2} \quad (2)$$

$$F = \frac{E}{1-\nu^2}\frac{2\tan\alpha}{\pi}\delta^2 \quad (3)$$

The values obtained using Equations (1) and (2) were essentially the same at the indentation depth that we examined (Figs. S3 for tissue slices and S5 for singular cells). Only the values obtained with Equation (1) are indicated, unless described in the Results and Table 1. For the sharpened pyramidal tip, Sneddon model (3) (Sneddon, 1965; Kuznetsova et al., 2007) was applied. The variables are as follows: Radius of the bead, *R*; Applied force, *F*; Young's modulus, *E*; Poisson's ratio, *v*; radius of contact

circle, a; the half-opening angle of the tip of the cantilever, α and depth of indentation, δ. The Poisson's ratio was chosen to be 0.5, as used in cellular (Kataoka et al., 2002) and tissue studies (Elkin et al., 2007).

Cell height measurement

Dissociated living cortical cells were stained with PKH26 (SIGMA) and Hoechst33258 (SIGMA) before plating according to the company's instructions. Z stack images were captured using a confocal laser microscope under incubation. The cell height was calculated from the obtained Z stack images using Volocity Visualization (Perkin-Elmer).

References

Elkin, B. S., Azeloglu, E. U., Costa, K. D. and Morrison, B., 3rd (2007) 'Mechanical heterogeneity of the rat hippocampus measured by atomic force microscope indentation', *J. Neurotraum.* 24(5): 812-22.

Kataoka, N., Iwaki, K., Hashimoto, K., Mochizuki, S., Ogasawara, Y., Sato, M., Tsujioka, K. and Kajiya, F. (2002) 'Measurements of endothelial cell-to-cell and cell-to-substrate gaps and micromechanical properties of endothelial cells during monocyte adhesion', *Proc. Nat. Acad. Sci. U. S. A.* 99(24): 15638-43.

Kuznetsova, T. G., Starodubtseva, M. N., Yegorenkov, N. I., Chizhik, S. A. and Zhdanov, R. I. (2007) 'Atomic force microscopy probing of cell elasticity', *Micron* 38(8): 824-33.

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Supplementary figure legends

Figure S1

(**A)** Schematics of cantilevers tested in our experiments. Types of cantilevers are indicated. Grey circle, a bead attached with cantilever; grey triangle, a pyramidal shaped tip of cantilever; blue semicircle, a cortical cell on a dish.

(B) Comparison of cantilevers for measurement of the stiffness of cortical cells. The Young's modulus of the singular neural cells derived from cortices at E14.5 were measured using cantilevers with **(a)** a 20-μm (in diameter) borosilicate bead, **(b)** a 5-μm borosilicate bead, **(c)** a 4.5-μm polystyrene bead, and **(d)** a sharpened pyramidal tip (OMCL-TR400-PB, Olympus). For the fitting model for each cantilever, see the Supplemental Methods. Note that the absolute value and the linearity of the stiffness along the indentation depth differed depending on the tip shape. Among four cantilever types, (a) the 20-μm borosilicate bead exhibited the highest linearity of the stiffness along the indentation depth.

Figure S2

(A) Agar frame maintains the structure of sliced embryonic brain. A living cortical slice of E18.5 mouse brain with 2% agar frame (**b** and **d**) maintained its intact structure for 3 hours during the culture for AFM measurement. In contrast, collapsed structures were identified in the brain slice without agar frame (**a** and **c**). (c) and (d), white rectangles in (a) and (b), respectively. Arrowhead in (b), the edge of the agar frame. Arrow in (c), the collapsed structure of the cortical plate. Bar = 500 μm.

(B) Concentration of the agar does not affect to the tissue stiffness. The stiffness of E18.5 mouse brain cortical slices was measured with agar (0.5%, 2%, and 5%) or without an agar frame (20 points in each condition). Note that the brain slice without agar exhibits lower stiffness, likely reflecting the collapsed structure shown in (A). Error bars in the graphs: S.E.M. *P<0.05, **P<0.01, and ***P<0.001, no mark indicates a non-significant result, t-test.

Figure S3

Profiles between the indentation depth (0.25, 0.5, 1, 3, and 5 μm) and the obtained

Young's modulus of the cortical tissue at various developing stages (E12.5 (**a**), 14.5 (**b**), 16.5 (**c**), and 18.5 (**d**)). Upon increasing the indentation, the measured Young's modulus showed a non-linear response that decreased initially in the depth range from 0.25 to 0.5 μm and increased at depths greater than 0.5 μm. Comparison of the Young's modulus of the cortical tissue calculated by different models is displayed in each panel. The obtained force-distance curves were analyzed by the SPM image processing software using the "Sphere" and "Paraboloid" models (see the Supplemental Methods). The calculated values are almost identical (4.4% difference at maximum) at an indentation depth of 3 μm in all developing stages. The Young's moduli acquired from all layers are contained (n = 20 for each stage). Error bars in the graphs: S.E.M.

Figure S4

(A) Effect of coating conditions on the stiffness of cortical cells. Several different ECMs were used to coat plastic culture dishes to test their effect on the stiffness of E14.5 cortical cells: PLO, poly-L ornithine; FN, fibronectin; L, laminin; Col, collagen type-1. Stiffness of cortical cells on 0.3% collagen gel (1 mm in height) is indicated for

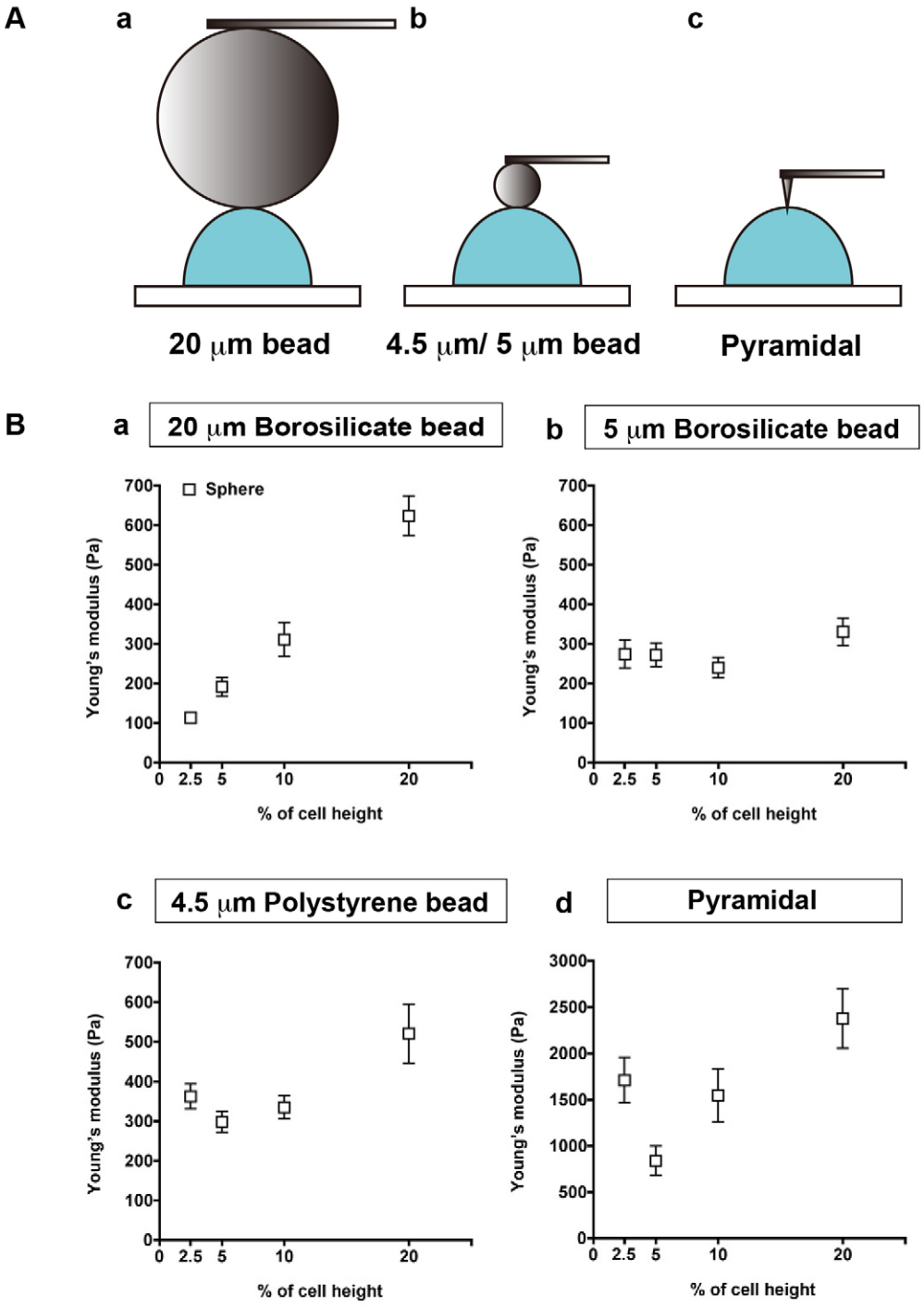
the comparison to the cellular stiffness on the plastic dish coated with poly-L ornithine + collagen. *P<0.05, **P<0.01, and ***P<0.001, no mark indicates a non-significant result, t-test.

(B) Indentation depth-dependent stiffness of the cortical cells on the fibronectin-coated dish. Stiffness of E14.5 cortical cells showed fine linearity along the indentation depth (2.5%, 5%, 10%, and 20% of cell height) on the dish coated with poly-L ornithine + fibronectin, but not with poly-L ornithine + collagen. The “Sphere” model was used for the fitting (see the Supplemental Methods). Error bars in the graphs: S.E.M.

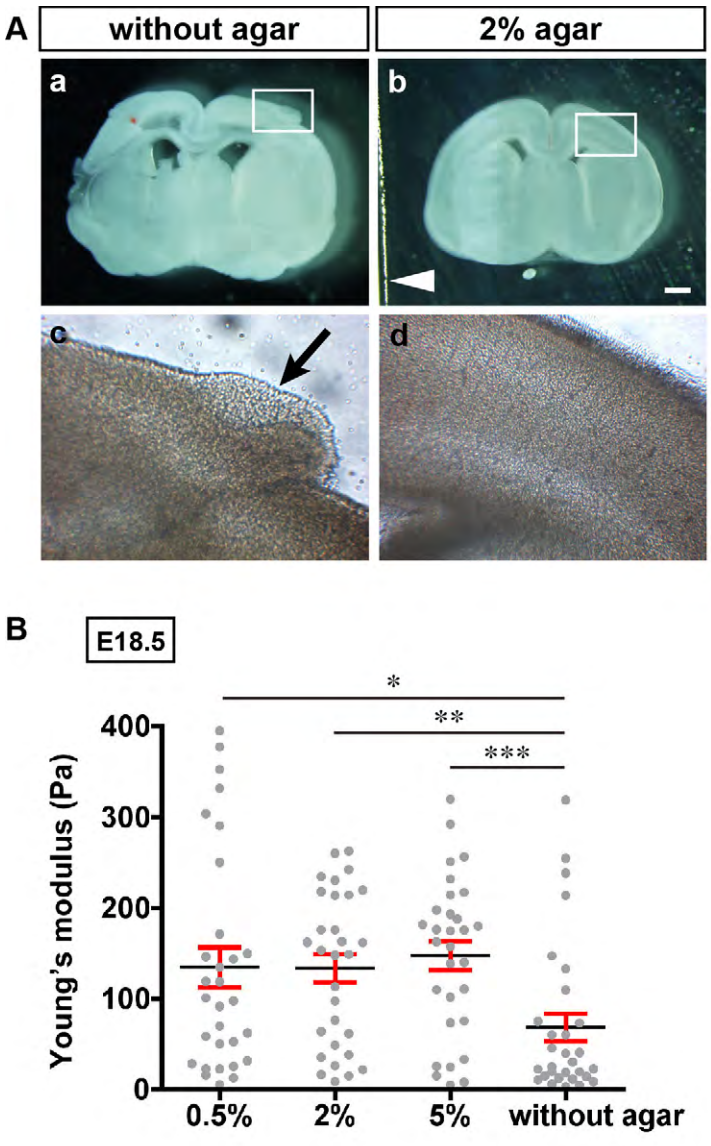
Figure S5

Profiles between the indentation depth (2.5%, 5%, 10%, and 20% of cell height) and the obtained Young’s modulus of the single neural cells dissociated from cortices of various developing stages (E12.5 (**a**), 14.5 (**b**), 16.5 (**c**), and 18.5 (**d**). Comparison of the Young’s modulus of the single neural cells calculated by different models is displayed in each panel. The obtained force-distance curves were analyzed using the “Sphere” and “Paraboloid” models. The calculated values are almost identical (1.6% difference at

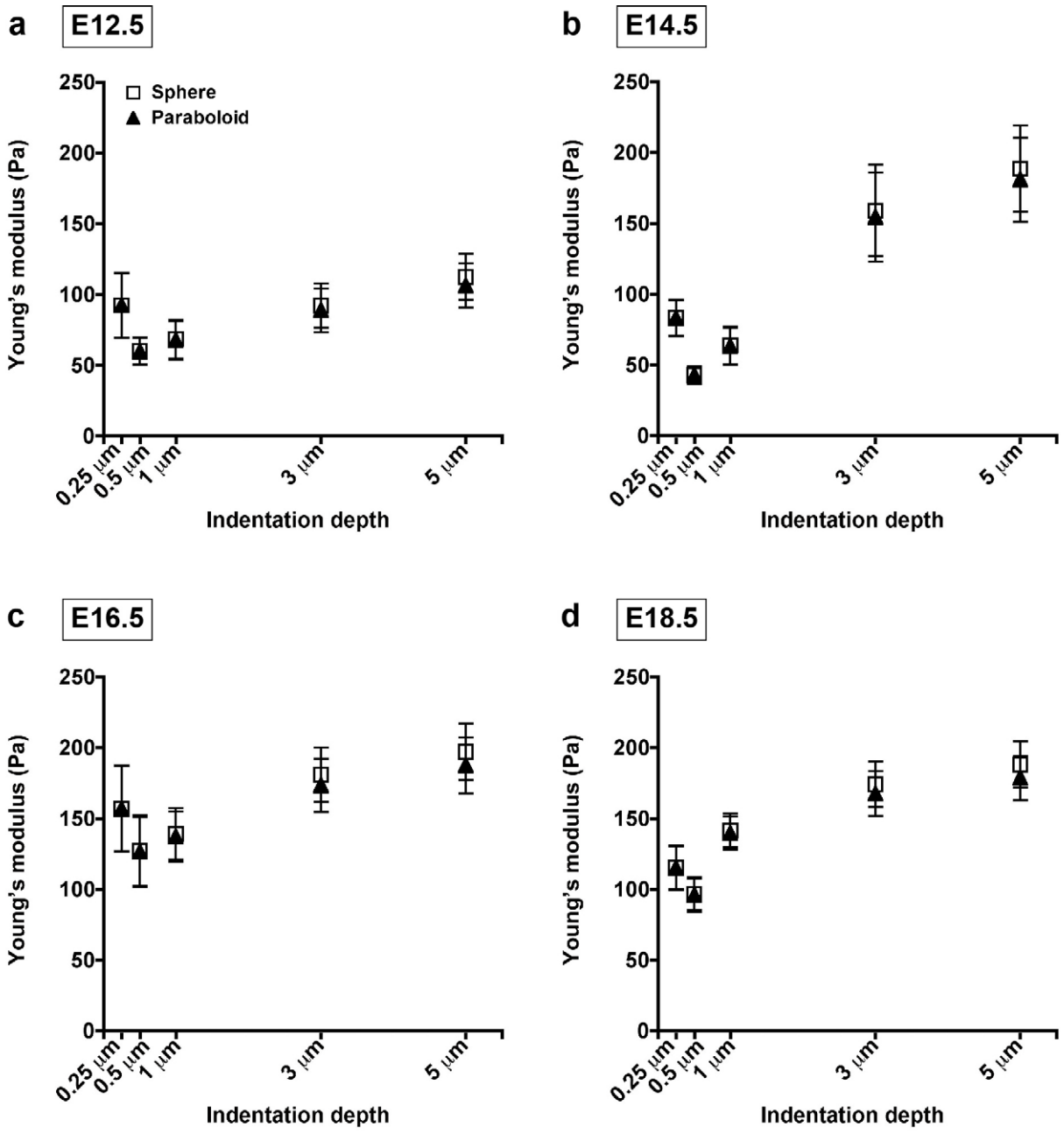
maximum) at 5% of cell height as an indentation depth in all developing stages. Note the fine linearity in the range of 2.5-10% of the cell height as an indentation depth at all developmental stages (R^2 = 0.999 (a), 0.995 (b), 0.999 (c), and 0.994 (d), the “Sphere” model). The Young’s moduli acquired from all cell types are shown (n = 30 for each stage). Error bars in the graphs: S.E.M. (b) is the same data as shown in Fig S1B (a).



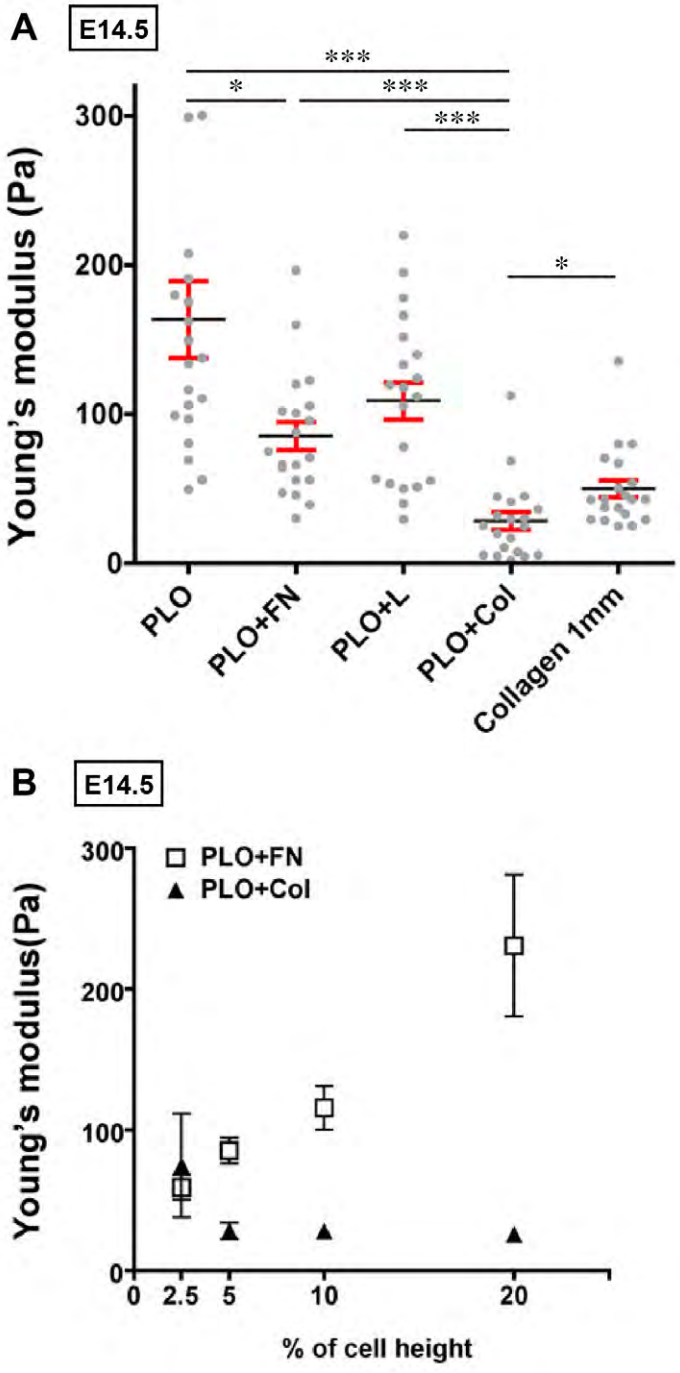
Supplemental Figure 1 Iwashita et al



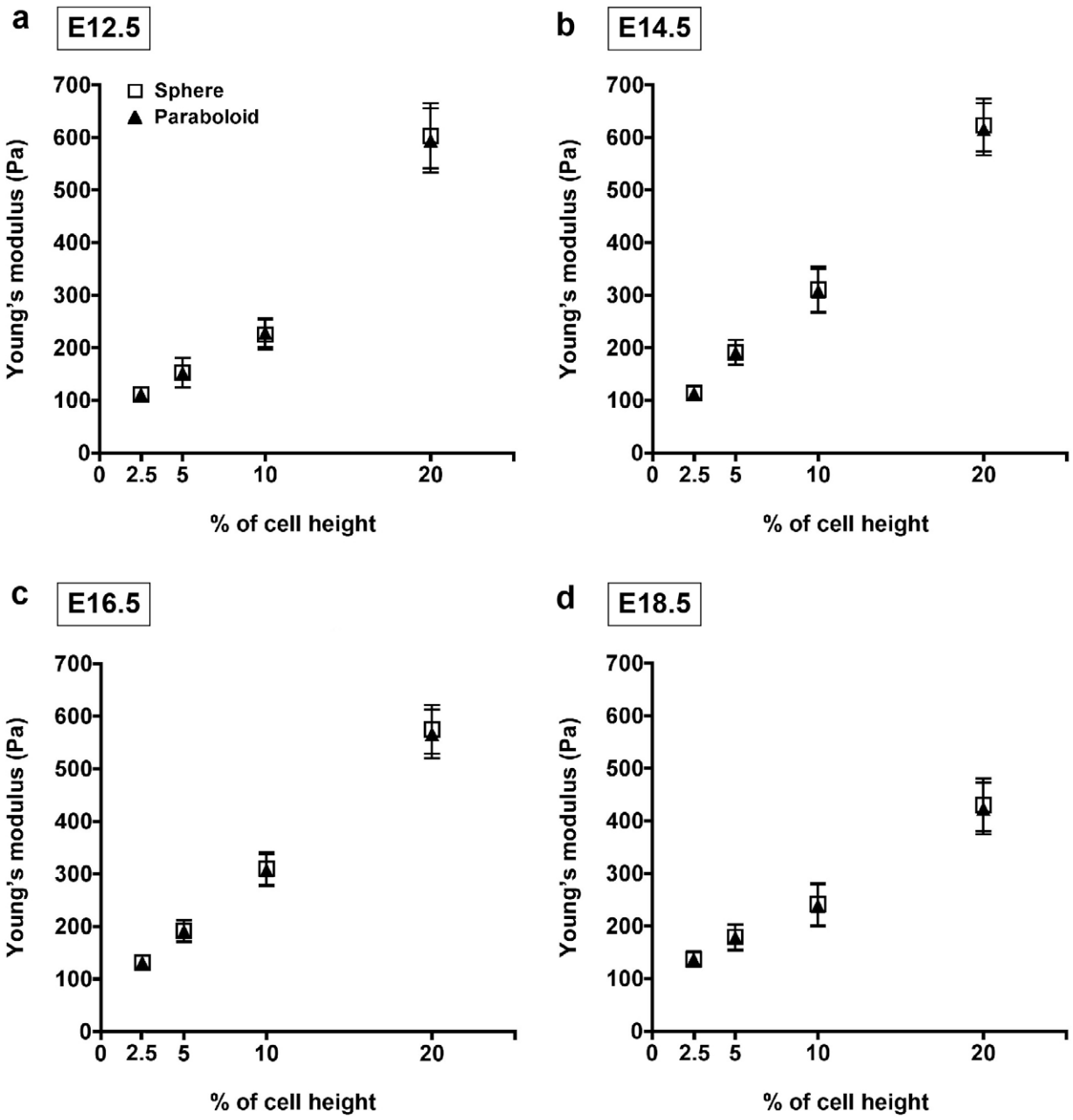
Supplemental Figure 2 Iwashita et al



Supplemental Figure 3 Iwashita et al.



Supplemental Figure 4 Iwashita et al.



Supplemental Figure 5 Iwashita et al.