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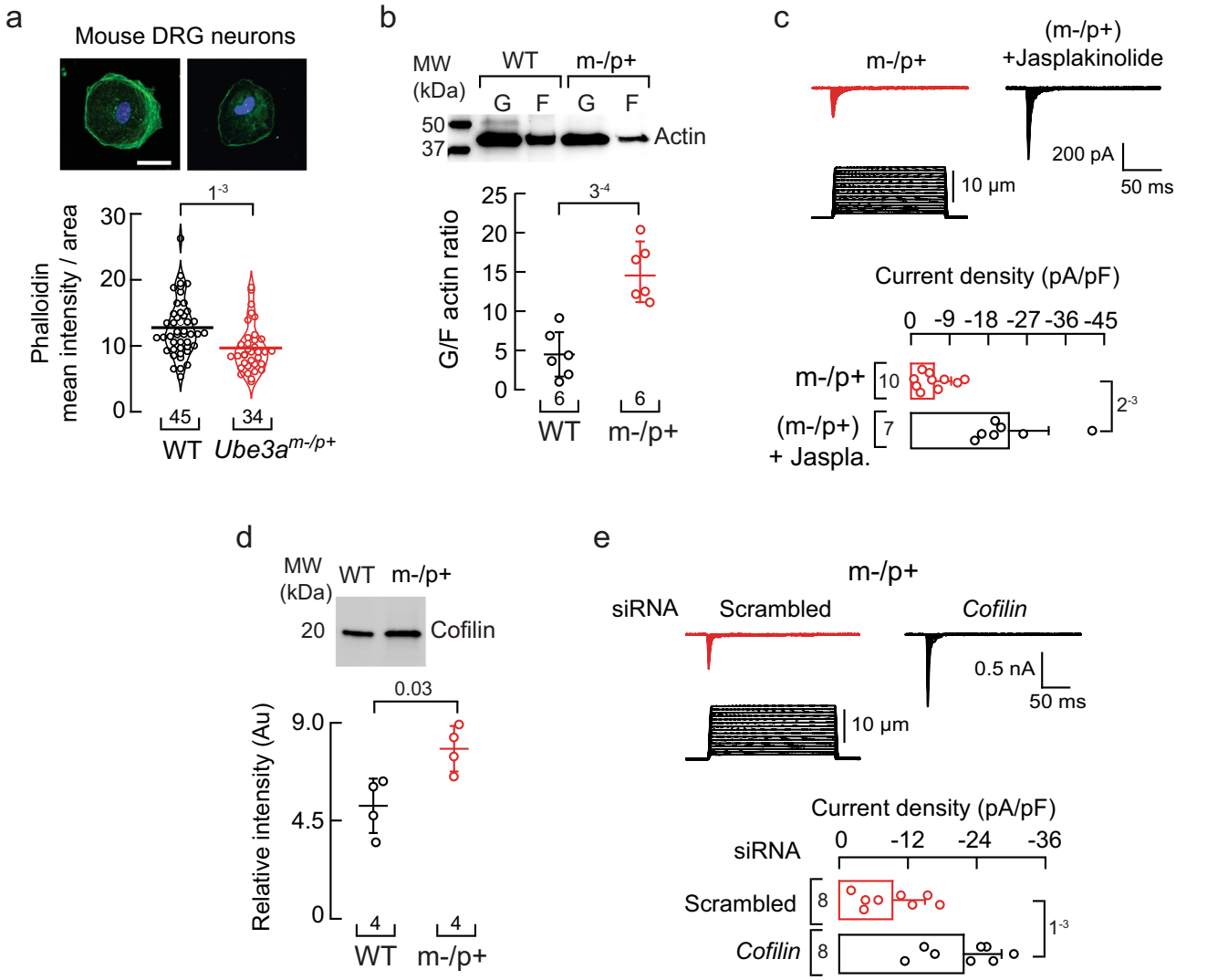

Fig. 3 | *Ube3a*$^{m-/p+}$ DRG neurons display reduced F-actin and jasplakinolide treatment increases PIEZO2 currents. a Top, representative micrographs of cultured WT and *Ube3a*$^{m-/p+}$ DRG neurons fixed and stained with phalloidin (green) and DAPI (blue). Scale bar 20 µm. Bottom, phalloidin mean intensity normalized by the neuron's area is depicted as a violin plot with the means shown as horizontal bars. Two-tailed unpaired *t*-test ($t = 3.41$). **b** Top, western blot of soluble and insoluble actin (G and F, respectively) of WT and *Ube3a*$^{m-/p+}$ DRGs. Bottom, mean/scatter-dot plot showing G/F actin ratios. Lines are mean ± SD. Two-tailed unpaired *t*-test ($t = 5.43$). **c** Top, representative whole-cell patch-clamp recordings of PIEZO2 currents elicited by mechanical stimulation (−60 mV) of control and jasplakinolide (0.5 µM; 18 h)-treated *Ube3a*$^{m-/p+}$ DRG neurons. Bottom, current densities elicited by maximum displacement. Bars are mean ± SD. Two-tailed unpaired *t*-test with Welch's correction ($t = 4.68$). **d** Top, representative western blot (anti-cofilin) of the cytosolic fractions of WT and *Ube3a*$^{m-/p+}$ DRGs. Bottom, mean/scatter-dot plot showing relative intensities of cofilin content. Lines are mean ± SD. Two-tailed Mann-Whitney test ($U = 0$). **e** Top, representative whole-cell patch-clamp recordings of currents elicited by mechanical stimulation (−60 mV) of *Ube3a*$^{m-/p+}$ DRG neurons transfected with scrambled or *cofilin* siRNAs. Bottom, current densities elicited by maximum displacement of siRNA-transfected *Ube3a*$^{m-/p+}$ DRGs. Bars are mean ± SD. Two-tailed unpaired *t*-test ($t = 4.02$). n is denoted in each panel. Post-hoc *p*-values are denoted in the corresponding panels. Source data are provided as a Source Data file.

demonstrate that the essential dietary fatty acid LA (C18:2) increases PIEZO2 activity.

LA increases membrane structural disorder

Western blot analyses revealed that LA supplementation did not increase PIEZO2 membrane expression in MCC13 cells (Fig. 5a-b). Furthermore, perfusion of free LA (150 µM) onto *Piezo1*$^{-/-}$ N2A cells transfected with PIEZO2 did not change channel function (Fig. 5c-d). Hence, it is possible that LA increases PIEZO2 function by modifying the membrane's mechanical properties. To determine the effect of LA on membranes, we used differential scanning calorimetry (DSC) and measured changes in the heat-capacity profiles (Cp) of synthetic membranes (1,2-dipalmitoyl-sn-glycero-3-phosphocholine, DPPC) containing various fatty acids. From the DSC thermograms, we obtained melting transition temperature (Tm) and cooperativity (κ) as a readout of both lipid-lipid interaction and membrane organization (Fig. 5e)[54,55]. LA displayed the lowest melting temperature (i.e., less temperature required to transition from the gel to the liquid phase) compared to DPPC alone or liposomes containing SA, OA, γLA, DγLA, AA, or DTA (Fig. 5e-f). This result indicates that LA elicits the largest increase in membrane structural disorder. We also determined that liposomes containing LA display the largest cooperative unit, indicating that there are more lipids undergoing the phase transition simultaneously (Fig. 5g). The effect of LA versus the other fatty acids is made further apparent when plotting the current densities (from Fig. 4b) as a function of cooperativity (Pearson's r: 0.93; Fig. 5h).

Next, we tested the effect of LA supplementation on the bacterial mechanosensitive ion channel of large conductance (MscL), whose gating relies solely on the mechanical properties of the plasma membrane[56]. To this end, we measured pressure-activated currents

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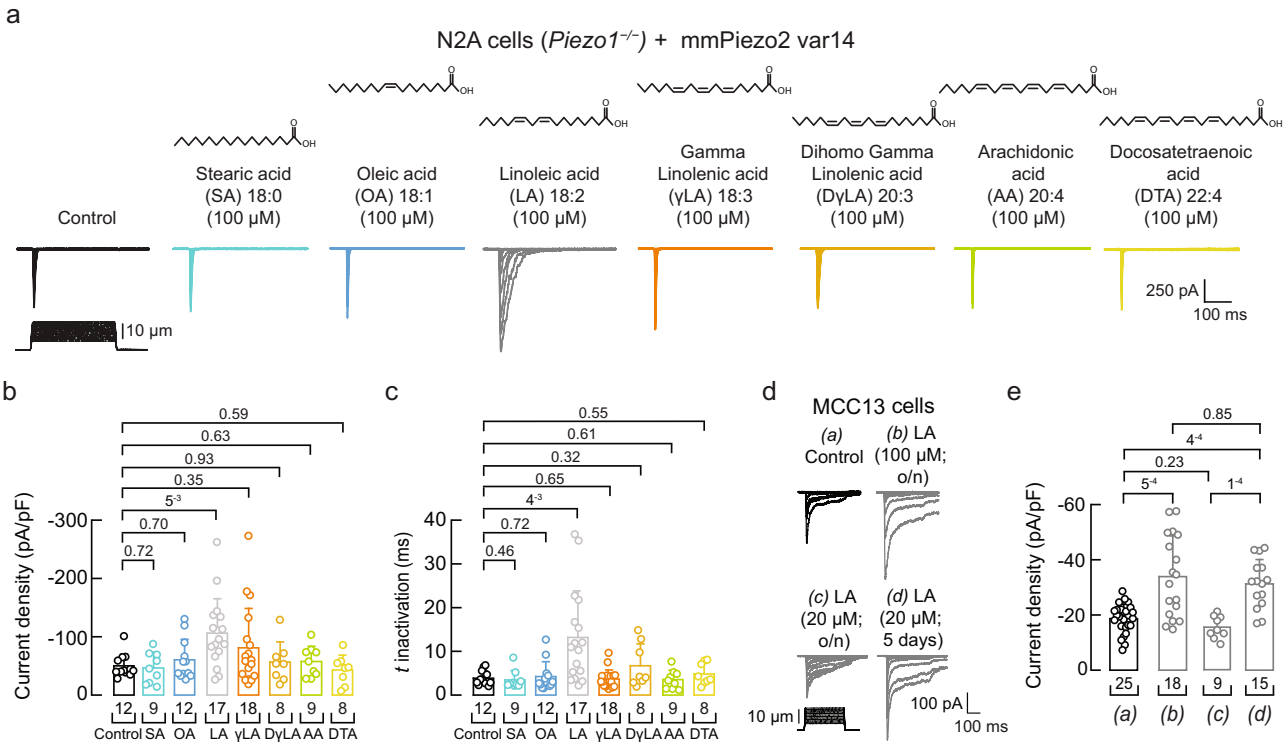

Fig. 4 | LA increases PIEZO2 activity in *Piezo1*$^{-/-}$ N2A and MCC13 cells. **a** Representative whole-cell patch-clamp recordings of currents elicited by mechanical stimulation (−60 mV) in control, SA, OA, LA, γLA, DγLA, AA, and DTA (100 μM; 24 h)-treated *Piezo1*$^{-/-}$ N2A cells transfected with *Piezo2* variant 14 (var14). **b** Current densities elicited by maximum displacement of control or fatty acid-treated *Piezo1*$^{-/-}$ N2A cells transfected with *Piezo2* var14. Bars are mean ± SD. Kruskal-Wallis (H = 15.7; $p = 0.028$) and Dunn's multiple comparisons test. **c** Time constants of inactivation elicited by maximum displacement of control or fatty acid-treated *Piezo1*$^{-/-}$ N2A cells transfected with *Piezo2* var14. Bars are mean ± SD. Kruskal-Wallis (H = 22.41; $p = 0.0022$) and Dunn's multiple comparisons test. **d** Representative whole-cell patch-clamp recordings elicited by mechanical stimulation (−60 mV) of (*a*) control, (*b*) LA (100 μM; o/n), (*c*) LA (20 μM; o/n), and (*d*) LA (20 μM each day for five days)-treated MCC13 cells. **e** Current densities elicited by maximum displacement of control and LA-treated MCC13 cells. Bars are mean ± SD. Kruskal-Wallis (H = 27.03; $p = 5.8^{-6}$) and Dunn's multiple comparisons test. n is denoted in each panel. Post hoc *p*-values are denoted above the bars. Source data are provided as a Source Data file.

of MscL transfected in *Piezo1*$^{-/-}$ N2A cells, with or without LA. Similar to what has been reported for MscL reconstituted in liposomes containing LA[57], supplementing N2A cells with this fatty acid increased the function of MscL, when compared to untreated cells, as determined by the leftward shift in the pressure required to open the channel (Fig. 5i-j). Taken together, these results support the notion that the effect of LA on increasing PIEZO2 function is likely through membrane remodeling (i.e., higher membrane fluidity and cooperativity) rather than changes in protein expression or the interaction of free LA with the channel.

Linoleic acid supplementation increases mechanocurrents in *Ube3a*$^{m-/p+}$ DRG neurons

We tested whether LA could increase mechano-responses in DRG neurons dissected and cultured from *Ube3a*$^{m-/p+}$ mice. The culture media was supplemented with LA (50 μM for the first 24 h, followed by 100 μM for the second 24 h period) for two days before measuring currents. A two-day supplementation protocol improved neuronal viability and the ability to perform patch-clamp recordings. Like DRG neurons measured after 24 h (Fig. 1b), *Ube3a*$^{m-/p+}$ DRG neurons displayed reduced mechanocurrents compared with WT or *Ube3a*$^{m+/p-}$ DRG neurons, after 48 h in culture (Supplementary Fig. 5a). Notably, LA supplementation increased all the mechanocurrents in cultured DRG neurons from WT, *Ube3a*$^{m-/p+}$, and *Ube3a*$^{m+/p-}$ mice (Fig. 6a-d and Supplementary Fig. 5b). *Ube3a*-deficient neurons supplemented with LA displayed a decrease in the displacement threshold required to elicit mechanocurrents when compared to control, whereas no effect was measured for WT and *Ube3a*$^{m+/p-}$ neurons (Fig. 6e). Parenthetically, we found that DRG neurons supplemented with LA have similar membrane capacitance, action potential amplitudes, resting potentials, input resistances, and minimal current threshold required to elicit an action potential, compared to control neurons (Supplementary Fig. 5c-h), suggesting that LA does not affect neuronal electrical excitability. Additionally, LA supplementation does not alter the function of the sensory receptors TRPV1, TRPA1, and TRPM8 (Supplementary Fig. 5i-n). Together, these results demonstrate that LA increases mechanocurrents, including those from PIEZO2, while decreasing the mechanical threshold for *Ube3a*$^{m-/p+}$ DRG neurons.

A linoleic acid-enriched diet recovers *Ube3a*$^{m-/p+}$ neuronal mechano-currents and -excitability

LA is an essential *ω*-6 fatty acid and a structural component of plasma membranes[58]. It is commonly found in safflower, soybean, sunflower, corn, and canola oils[59–61]. Since LA supplementation in the culture media increases mechanocurrents, we postulated that including LA in the animal's diet may rescue the mechano-deficits of *Ube3a*$^{m-/p+}$ DRG neurons. To this end, we used a non-western-style diet enriched in safflower oil as a delivery method to increase LA in DRG neurons. Oil from the seeds of the safflower plant has been shown to contain over 70% LA[62]. LA can accumulate when its consumption in the diet is increased due to its limited conversion by the delta-6 desaturase enzyme[59]. We pair-fed *Ube3a*$^{m-/p+}$ mice for 12 weeks with a LA-enriched diet and found that their DRG neurons had higher LA membrane content (-twofold) when compared to WT and *Ube3a*$^{m-/p+}$ mice fed with a standard diet (Fig. 7a), as determined by LC-MS. On the other hand, the content of downstream

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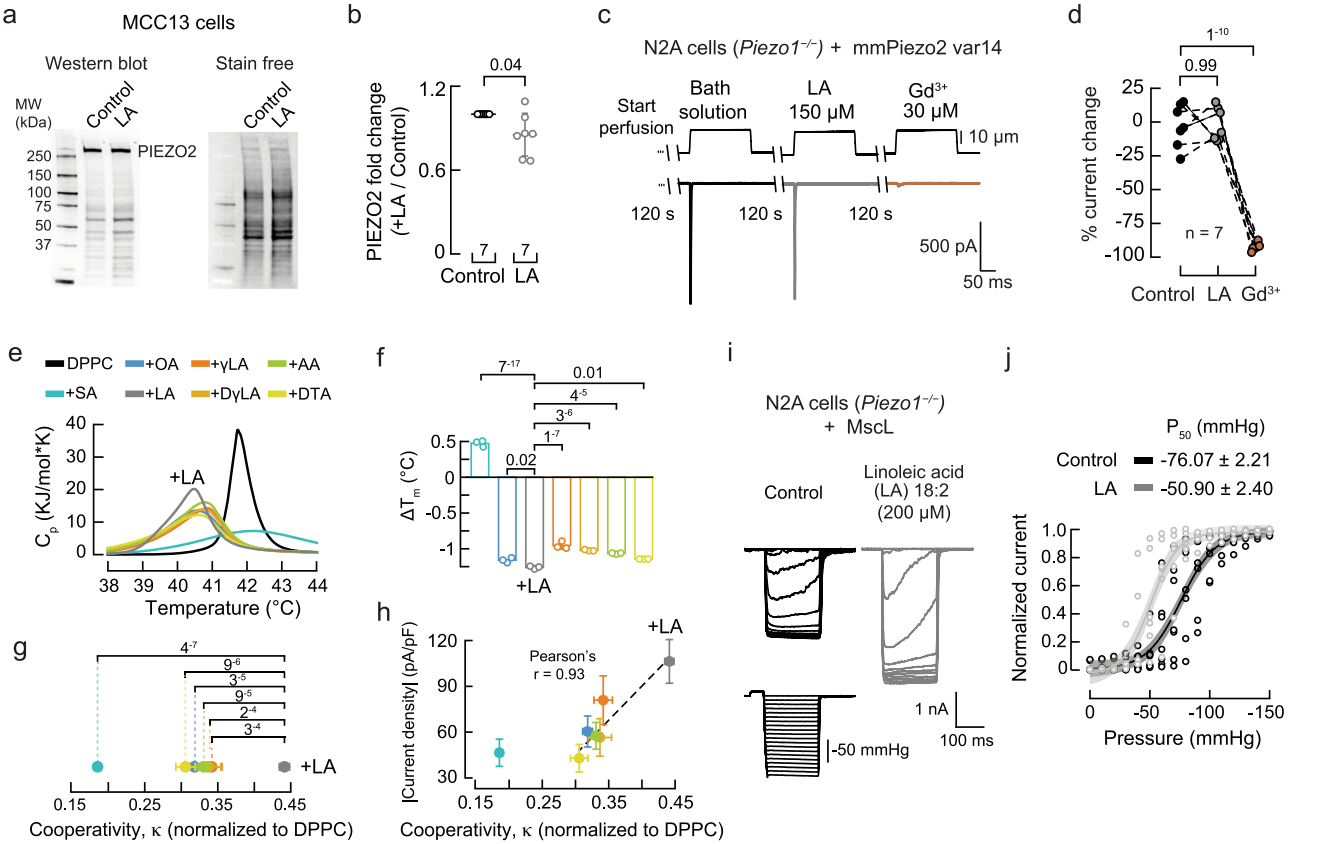

Fig. 5 | LA does not increase PIEZO2 expression but alters the physical properties of the membranes. a Western (anti-PIEZO2) and stain-free blots of the membrane fractions of control and LA-treated MCC13 cells. **b** Mean/scatter-dot plot showing relative intensities of PIEZO2 protein normalized to the level of PIEZO2 in the control group. Lines are mean ± SD. Two-tailed one-sample *t*-test ($t = -2.5$). **c** Representative currents elicited by 10 µm displacement of *Piezo2* var14 transfected cells after perfusing bath solution with LA and Gd^{3+}. **d** Percent current change from independent cells recorded with the protocol shown in (**c**). Paired data points represent individual cells. One-sided repeated measures ANOVA ($F = 186.94$; $p = 9.51^{-6}$) and Tukey test. **e** Thermotropic characterization of the DPPC/fatty acid systems using DSC: control (Tm = 41.75 ±0.05 °C; mean ± sd), SA (42.23±0.04 °C), OA (40.59±0.03 °C), LA (40.53±0.06 °C), γLA (40.80±0.05 °C), DγLA (40.73±0.01 °C), AA (40.79±0.01 °C), and DTA (40.61±0 °C). **f** Effects of DPPC/fatty acids on melting temperatures (ΔTm) with respect to DPPC membranes. $n = 3$. Bars are mean ± SD. One-way ANOVA (F = 1,177; $p = 0$) and Bonferroni test. **g** Cooperative unit (κ) of the main transition of DPPC/fatty acid systems extracted from the thermotropic curves shown in (**e**), normalized to DPPC. Circles are mean ± SD. $n = 3$. Two-way ANOVA ($F = 45.76896$; $p = 2.09^{-8}$) and Tukey multiple-comparisons test. **h** Mean current densities of control or fatty acid-treated *Piezo1*$^{-/-}$ N2A cells transfected with *Piezo2* var14 vs. the cooperative unit (κ) of the main transition of DPPC/fatty acid systems. Circles are mean ± SEM. n = 3. A Pearson correlation was fitted to the unsaturated fatty acids data. **i** Inside-out recordings of currents elicited by negative pressure (at −10 mV) in LA (200 µM; 24 h)-treated cells transfected with MscL. **j** Normalized current responses to pressure changes of control ($n = 7$) and LA (200 µM; 24 h; $n = 6$)-treated cells transfected with MscL. A Boltzmann function was fitted to the data (continuous lines). The shadows indicate the 95% confidence bands. n is denoted in each panel. Post-hoc *p*-values are denoted in the corresponding panels. Source data are provided as a Source Data file.

PUFAs (γLA, DγLA, AA, and DTA) remained constant (Supplementary Fig. 6a). Remarkably, cultured DRG neurons from *Ube3a*$^{m-/p+}$ mice fed with a LA-enriched diet displayed robust mechanocurrents and a lower displacement threshold when compared to neurons from *Ube3a*$^{m-/p+}$ mice fed with standard or high-fat diets (Fig. 7b-d and Supplementary Fig. 6b). Although the LA-enriched diet enhanced PIEZO2- and intermediate inactivating mechanocurrents, it had no effect on those that were slowly inactivating (Fig. 7c, right panel). However, further supplementing DRG neurons from *Ube3a*$^{m-/p+}$ mice fed with a LA-enriched diet with additional LA (during culture) increased the slowly inactivating currents (Supplementary Fig. 6c-d). Next, we tested DRG neurons of the *Ube3a*$^{m-/p+}$ mice fed with a LA-enriched diet for their ability to elicit mechanically activated action potentials. These neurons required smaller indentation steps (≤10µm, like WT and *Ube3a*$^{m+/p-}$ mice) to elicit action potentials compared to neurons from *Ube3a*$^{m-/p+}$ mice on standard or high-fat diets (≥12 µm; Fig. 7e-f).

We also tested the effect of a LA-enriched diet on WT animals and found that LA increased PIEZO2 currents in neurons compared to those from animals fed with a standard diet, but did not change the displacement threshold or threshold for mechanically activated action potentials (Supplementary Fig. 6e-i). Of note, WT, *Ube3a*$^{m-/p+}$ mice fed with a standard diet, and *Ube3a*$^{m-/p+}$ mice fed with a LA-enriched diet had similar body weights after pair-feeding (Supplementary Fig. 7a). Furthermore, *Ube3a*$^{m-/p+}$ mice fed with a LA-enriched diet displayed similar behavioral responses to noxious mechanical (pinprick and tail clip) and thermal stimuli (hot plate and Hargreaves), suggesting that a LA-enriched diet does not enhance nociception (Supplementary Fig. 7b-e). We also determined that feeding the LA-enriched diet to *Ube3a*$^{m-/p+}$ mice did not alter their cytokine profile compared to those fed with the control diet, indicating that the non-western safflower oil-enriched diet does not induce inflammation (Supplementary Fig. 7f). These results demonstrate that a LA-enriched diet increases mechanocurrents, including those from PIEZO2, and is sufficient to recover the mechanical excitability of *Ube3a*$^{m-/p+}$ DRG neurons.

A linoleic acid-enriched diet ameliorates gait ataxia in a mouse model of AS

We sought to determine the effect of the LA-enriched diet on the gait of *Ube3a*$^{m-/p+}$ mice. Mouse gait measurements are consistent, commonly used to assess locomotion in several human disease models,