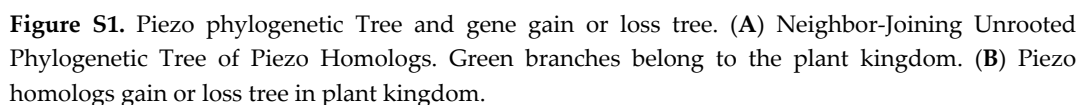
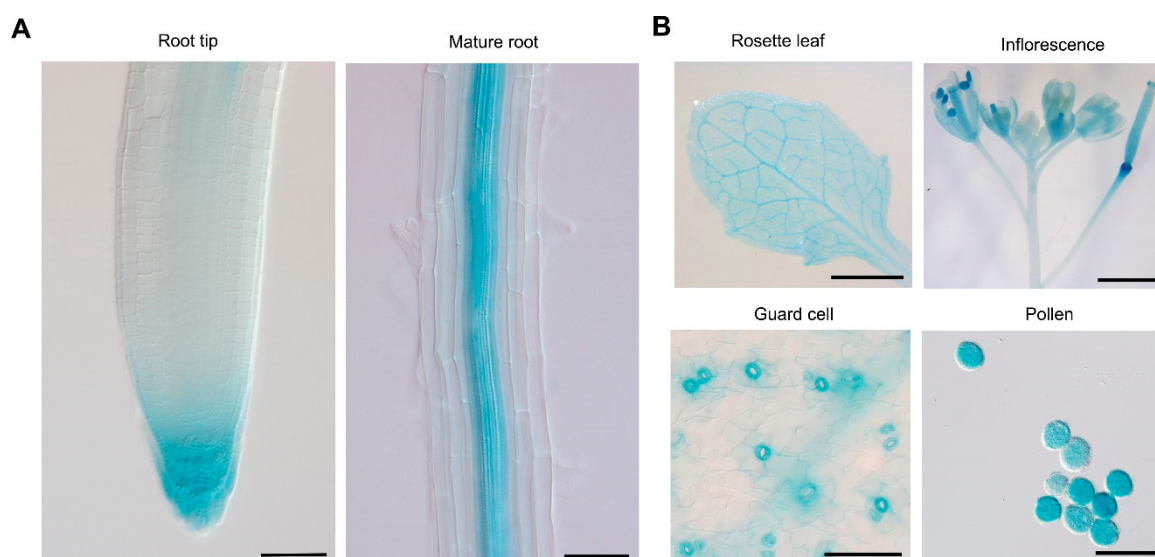


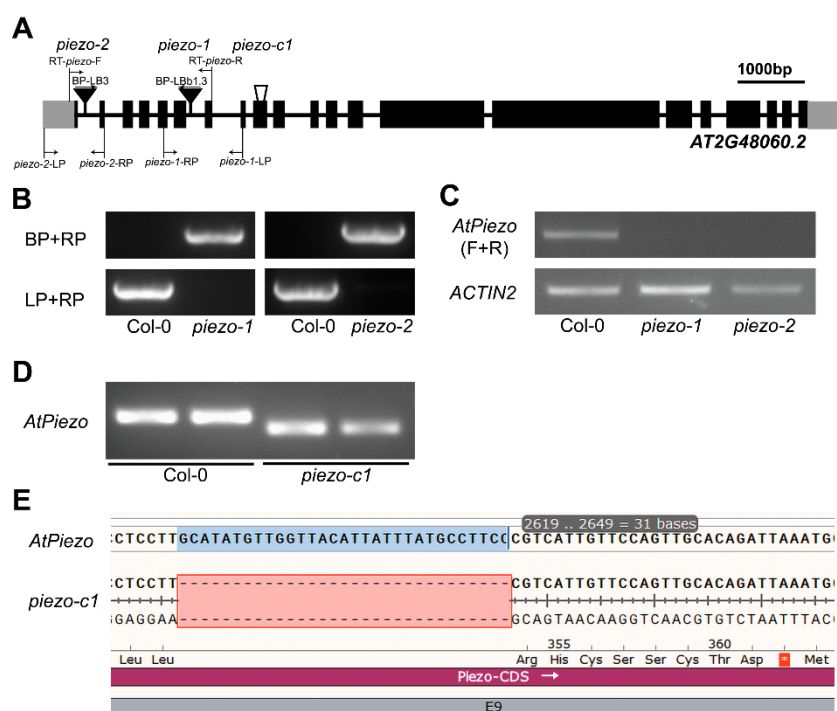
## AtPiezo plays an important role in root cap mechanotransduction

<sup>1</sup> Ministry of Education Key Laboratory of Cell Activities and Stress Adaptations, School of Life Sciences, Lanzhou University, Lanzhou 730000, China; fangxm18@lzu.edu.cn (X.F.); liubb19@lzu.edu.cn (B.L.); shaoqsh19@lzu.edu.cn (Q.S.); huangxm17@lzu.edu.cn (X.H.); lijia@lzu.edu.cn (J.L.)  
<sup>2</sup> Department of Plant and Microbial Biology, University of California, Berkeley, California 94720, USA  
\* Correspondence: hekai@lzu.edu.cn (K.H.); sluan@berkeley.edu (S.L.)

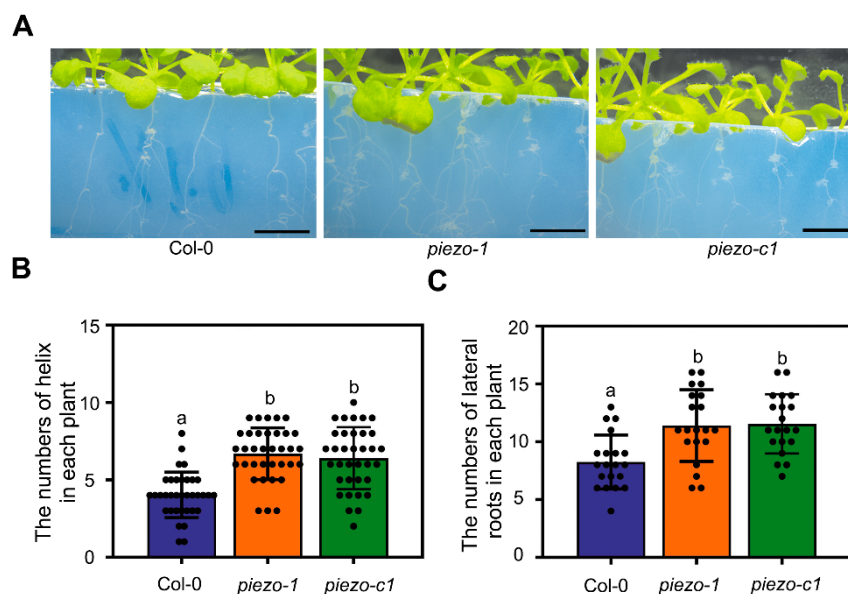




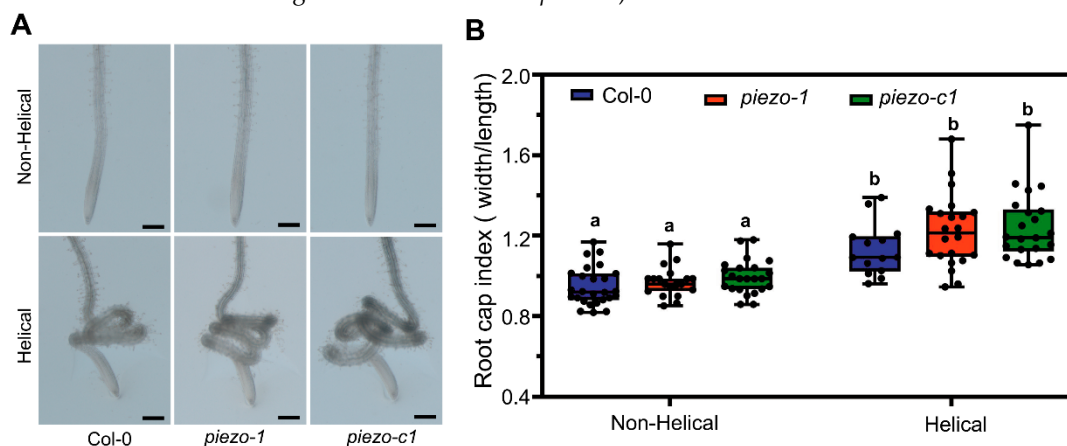
**Figure S2.** Representative images of the expression pattern of *AtPiezo* in *Arabidopsis*. (A) Histochemical GUS staining is shown in root tip and mature zone of primary root of 4-day-old seedling. Bars= 50µm. (B) Expression patterns of *pAtPiezo::GUS* in different organs. Histochemical GUS staining is shown in rosette leaf, guard cell, inflorescence and pollen. Bars=1mm (rosette leaf), 100µm (guard cell), 2mm (inflorescence), and 50µm (pollen).



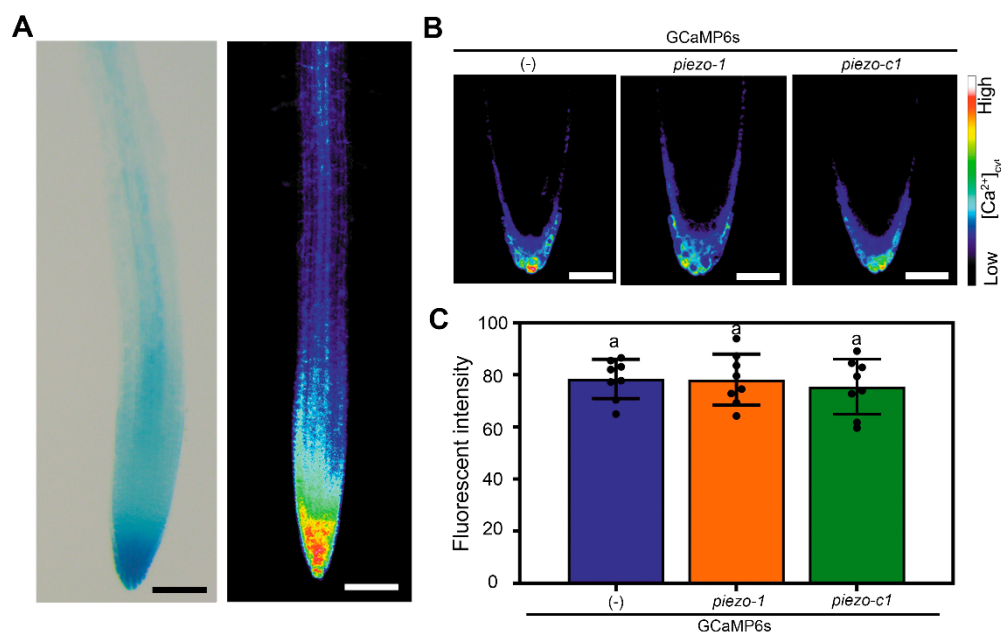
**Figure S3.** Isolation of *atpiezo* mutants. (A) Schematic diagram of *AtPiezo* gene structure with the localization of T-DNA insertions and primers used for mutant identification (B) T-DNA insertion in *piezo-1* and *piezo-2* was determined by PCR. (C) RT-PCR confirmed *piezo-1* and *piezo-2* are null alleles. *ACTIN2* was used as a positive control. (D) Deletion of genomic DNA fragment in *piezo-c1* mutant was determined by PCR. (E). *piezo-c1* has a 31-bp deletion in the genomic DNA of *AtPiezo*.



**Figure S4.** AtPiezo affects the root architecture in the medium. (A) Representative images of root growth status of different plants in the medium. 14-day-old seedlings of WT and *atpiezo* mutants were grown in the medium with 0.8% agar. Bar=1cm. (B) Statistic analysis of the helical roots in 14-day-old seedlings of WT and *atpiezo* mutants. Data are presented as mean  $\pm$  SD ( $n \geq 30$ ). The results were analyzed with one-way ANOVA and Tukey's multiple comparison test (Different lowercase letters indicate significant differences at  $p < 0.05$ ). (C) Statistics analysis of the lateral roots in 14-day-old seedlings of WT and *atpiezo* mutants. Data are presented as mean  $\pm$  SD ( $n \geq 30$ ). The results were analyzed with two-way ANOVA and Tukey's multiple comparison test (Different lowercase letters indicate significant differences at  $p < 0.05$ ).



**Figure S5.** Root cap shape of the root foraging in the medium. (A) The root cap shape of WT and *atpiezo* mutants in the agar medium. Four-day-old seedlings were grown in medium with 0.8% agar. Bar=0.2mm. (B) Statistic analysis of root cap index for helical and non-helical roots of WT and *atpiezo* mutants in medium. Boxplots span the first to the third quartiles of the data. A line in the box represents the median ( $n \geq 14$ ). The results were analyzed with two-way ANOVA and Tukey's multiple comparison test (Different lowercase letters indicate significant differences at  $p < 0.05$ ).



**Figure S6.**  $\text{Ca}^{2+}$  gradient in the root cap. (A) The distribution of  $\text{Ca}^{2+}$  is similar to the expression pattern of *AtPiezo* gene in the root cap. Histochemical GUS staining is shown in 4-days-old seedling of *pAtPiezo::GUS* lines (left).  $\text{Ca}^{2+}$  signal was detected in the root cap of WT plants harboring GCaMP6s (right). Bar=100 $\mu\text{m}$ . (B)  $\text{Ca}^{2+}$  signal in root caps of WT and the *atpiezo* mutants. Bar=50 $\mu\text{m}$ . (C) Fluorescent intensity of  $\text{Ca}^{2+}$  in WT and *atpiezo* mutants. Data are presented as mean  $\pm$  SD ( $n = 8$ ). The results were analyzed with one-way ANOVA and Tukey's multiple comparison test (Different lowercase letters indicate significant differences at  $p < 0.05$ ).

**Table S1. Information of protein sequences in phylogenetic analysis.**

| No. | Species                            | Protein name          | Predicted Size (aa) | Sequence identifies |
|-----|------------------------------------|-----------------------|---------------------|---------------------|
| 1   | <i>Homo sapiens</i>                | <b>HsPiezo1</b>       | 2521                | NP_001136336.2      |
| 2   | <i>Homo sapiens</i>                | <b>HsPiezo2</b>       | 2752                | NP_071351.2         |
| 3   | <i>Mus musculus</i>                | <b>MmPiezo1</b>       | 2546                | NP_001032375.1      |
| 4   | <i>Mus musculus</i>                | <b>MmPiezo2</b>       | 2824                | NP_001034574.4      |
| 5   | <i>Xenopus laevis</i>              | <b>XlPiezo1</b>       | 2475                | XP_018096992.1      |
| 6   | <i>Xenopus laevis</i>              | <b>XlPiezo2</b>       | 2830                | XP_018123363.1      |
| 7   | <i>Xenopus laevis</i>              | <b>XlPiezo3</b>       | 2776                | XP_018092291.1      |
| 8   | <i>Danio rerio</i>                 | <b>DrPiezo1</b>       | 2538                | XP_696355.4         |
| 9   | <i>Danio rerio</i>                 | <b>DrPiezo2a</b>      | 2752                | XP_021325930.1      |
| 10  | <i>Danio rerio</i>                 | <b>DrPiezo2b</b>      | 3005                | XP_021323945.1      |
| 11  | <i>Danio rerio</i>                 | <b>DrPiezo3</b>       | 2634                | XP_021334475.1      |
| 12  | <i>Drosophila melanogaster</i>     | <b>DmPiezo1</b>       | 2533                | NP_001188719.1      |
| 13  | <i>Drosophila melanogaster</i>     | <b>DmPiezo2 (Pzl)</b> | 2326                | NP_001303493.1      |
| 14  | <i>Caenorhabditis elegans</i>      | <b>CePiezo</b>        | 2402                | NP_001293979.1      |
| 15  | <i>Dictyostelium discoideum</i>    | <b>DdPiezo</b>        | 3080                | XP_640187.1         |
| 16  | <i>Galdieria sulphuraria</i>       | <b>GsPiezo</b>        | 2823                | XP_005703632.1      |
| 17  | <i>Chondrus crispus</i>            | <b>CcPiezo</b>        | 2929                | XP_005712062.1      |
| 18  | <i>Chlorella variabilis</i>        | <b>CvPiezo</b>        | 2946                | XP_005847868.1      |
| 19  | <i>Ostreococcus lucimarinus</i>    | <b>OlPiezo</b>        | 2372                | XP_001418409.1      |
| 20  | <i>Marchantia polymorpha</i>       | <b>MpPiezo</b>        | 2626                | A0A2R6XC�6          |
| 21  | <i>Physcomitrella patens</i>       | <b>PpPiezo1a</b>      | 2575                | XP_024385645.1      |
| 22  | <i>Physcomitrella patens</i>       | <b>PpPiezo1b</b>      | 2572                | XP_024369685.1      |
| 23  | <i>Selaginella moellendorfi</i>    | <b>SmPiezo</b>        | 2399                | XP_024535480.1      |
| 24  | <i>Zea mays</i>                    | <b>ZmPiezo</b>        | 2505                | XP_020405822.1      |
| 25  | <i>Sorghum bicolor</i>             | <b>SbPiezo</b>        | 2504                | XP_021311180.1      |
| 26  | <i>Oryza sativa Japonica Group</i> | <b>OsPiezo</b>        | 2557                | XP_025881504.1      |
| 27  | <i>Brachypodium distachyon</i>     | <b>BdPiezo</b>        | 2503                | XP_010230893.1      |
| 28  | <i>Amborella trichopoda</i>        | <b>AtrPiezo</b>       | 2485                | XP_020526919.1      |
| 29  | <i>Medicago truncatula</i>         | <b>MtPiezo1</b>       | 2478                | XP_024639854.1      |
| 30  | <i>Medicago truncatula</i>         | <b>MtPiezo2</b>       | 2462                | XP_003626328.2      |
| 31  | <i>Glycine max</i>                 | <b>GmPiezo1a</b>      | 2483                | XP_014618394.1      |
| 32  | <i>Glycine max</i>                 | <b>GmPiezo1b</b>      | 2482                | XP_006575092.1      |
| 33  | <i>Glycine max</i>                 | <b>GmPiezo2</b>       | 2310                | XP_025982938.1      |
| 34  | <i>Vigna radiata</i>               | <b>VrPiezo1</b>       | 2465                | XP_022642325.1      |
| 35  | <i>Vigna radiata</i>               | <b>VrPiezo2</b>       | 2483                | XP_022640429.1      |
| 36  | <i>Vitis vinifera</i>              | <b>VvPiezo1</b>       | 2487                | XP_019076639.1      |
| 37  | <i>Vitis vinifera</i>              | <b>VvPiezo2</b>       | 2481                | XP_010660075.1      |
| 38  | <i>Gossypium raimondii</i>         | <b>GrPiezo1a</b>      | 2478                | XP_012476188.1      |
| 39  | <i>Gossypium raimondii</i>         | <b>GrPiezo1b</b>      | 2476                | XP_012490496.1      |

|    |                             |                   |      |                |
|----|-----------------------------|-------------------|------|----------------|
| 40 | <i>Gossypium raimondii</i>  | <b>GrPiezo2</b>   | 2469 | XP_012462417.1 |
| 41 | <i>Coffea arabica</i>       | <b>CaPiezo1</b>   | 2475 | XP_027116044.1 |
| 42 | <i>Coffea arabica</i>       | <b>CaPiezo1</b>   | 2468 | XP_027114534.1 |
| 43 | <i>Populus trichocarpa</i>  | <b>PtPiezo1</b>   | 2482 | XP_024440850.1 |
| 44 | <i>Populus trichocarpa</i>  | <b>PtPiezo2</b>   | 2461 | XP_024456948.1 |
| 45 | <i>Nicotiana attenuata</i>  | <b>NaPiezo</b>    | 2473 | XP_019228329.1 |
| 46 | <i>Helianthus annuus</i>    | <b>HaPiezo1a</b>  | 2441 | XP_022035444.1 |
| 47 | <i>Helianthus annuus</i>    | <b>HaPiezo1b</b>  | 2480 | XP_022010653.1 |
| 48 | <i>Cucumis sativus</i>      | <b>CsPiezo1</b>   | 2438 | XP_011659324.1 |
| 49 | <i>Solanum lycopersicum</i> | <b>SlPiezo1</b>   | 2473 | XP_010326620.1 |
| 50 | <i>Solanum tuberosum</i>    | <b>StPiezo1</b>   | 2473 | XP_006358438.1 |
| 51 | <i>Camellia sinensis</i>    | <b>CsiPiezo1a</b> | 2468 | XP_028067352.1 |
| 52 | <i>Camellia sinensis</i>    | <b>CsiPiezo1b</b> | 2489 | XP_028090451.1 |
| 53 | <i>Brassica oleracea</i>    | <b>BoPiezo1a</b>  | 2481 | XP_013635988.1 |
| 54 | <i>Brassica oleracea</i>    | <b>BoPiezo1b</b>  | 2485 | XP_013635938.1 |
| 55 | <i>Brassica rapa</i>        | <b>BrPiezo1b</b>  | 2485 | XP_009142485.1 |
| 56 | <i>Brassica rapa</i>        | <b>BrPiezo1a</b>  | 2482 | XP_009118144.2 |
| 57 | <i>Capsella rubella</i>     | <b>CrPiezo</b>    | 2485 | XP_006293550.1 |
| 58 | <i>Arabidopsis thaliana</i> | <b>AtPiezo</b>    | 2485 | NP_001323909.1 |
| 59 | <i>Papaver somniferum</i>   | <b>PsPiezo1</b>   | 2485 | XP_026456484.1 |
| 60 | <i>Papaver somniferum</i>   | <b>PsPiezo2</b>   | 2480 | XP_026450010.1 |

**Table S2. Primer sequences used in this study.**

| Primer Name                         | Sequence (5'→3')                            |
|-------------------------------------|---------------------------------------------|
| RT-ACTIN2-F                         | TGCTGTGATTTCTTTGCTCATACG                    |
| RT-ACTIN2-R                         | CAGTGGTCGTACAACCGGTATTG                     |
| RT- <i>AtPiezo</i> -F               | AGCAGAATCCCCTCCGTACT                        |
| RT- <i>AtPiezo</i> -R               | ATGGCCTGGAGAGCTAGTGA                        |
| BP-LBb1.3                           | ATTTTGCCGATTTCGGAAC                         |
| BP-LB3                              | TAGCATCTGAATTTTCATAACCAATCTCGATACAC         |
| <i>piezo-1</i> -LP                  | CCCCTCAGTAAAACATTGGTG                       |
| <i>piezo-1</i> -RP                  | TCTTGTGGACTGGTCCTTGAC                       |
| <i>piezo-2</i> -LP                  | GTCCTTGGGTTCTAATCCGTC                       |
| <i>piezo-2</i> -RP                  | CAGGCTTACCAATTTCAGGTG                       |
| RT- <i>piezo</i> -F                 | CTTTTGGAAGAAATTGAGGTCG                      |
| RT- <i>piezo</i> -R                 | CTTGATTCTCGAATAAAAAATG                      |
| Cas9- <i>piezo-c1</i> -T1-BsF       | ATATATGGTCTCGATTGAACTGGAACAATGACGGGAGTT     |
| Cas9- <i>piezo-c1</i> -T1-F0        | TGAACTGGAACAATGACGGGAGTTTTAGAGCTAGAAATAGC   |
| Cas9- <i>piezo-c1</i> -T2-R0        | AACCCTTGCATATGTTGGTTACCAATCTCTTAGTCGACTCTAC |
| Cas9- <i>piezo-c1</i> -T2-BsR       | ATTATTGGTCTCGAAACCCTTGCATATGTTGGTTACCAA     |
| Identify- <i>piezo-c1</i> -F        | TATTTGCGTTCATGATTGCCAG                      |
| Identify- <i>piezo-c1</i> -R        | AGGCCACATTGAATATATACGT                      |
| Promoter- <i>AtPiezo</i> -F         | AAAAAGCAGGCTTCTACAAGAAATATTCCTTAAGG         |
| Promoter- <i>AtPiezo</i> -R         | AGAAAGCTGGGTCTGGAACTTTTGTCTTAACGA           |
| Infusion-Kpn I - <i>Piezo</i> CDS-F | TTAAACTTAAGCTTGGTACCATGGCGAGTTTTTGGTGGGCT   |
| Infusion-Xba I - <i>Piezo</i> CDS-R | AAACGGGCCCTCTAGAAGCATCATAGTCTAGCTTTGTAT     |

**Publisher's Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).