Supplementary Figure 1. The MSL1 precursor is not imported into isolated chloroplasts *in vitro*. Radiolabelled MSL1 precursor was incubated with purified chloroplasts under conditions that support protein uptake (first panel). Following import, thermolysin treatment was conducted as indicated. The uptake of small subunit of RUBISCO (SSU) was used as a positive control for chloroplast import (second panel). p, precursor protein; m, processed mature protein.

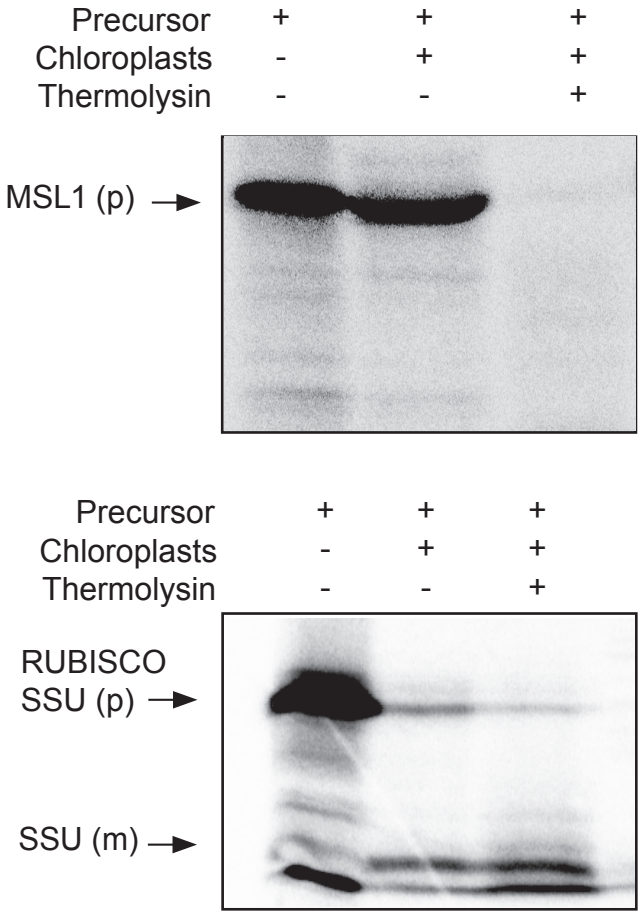
Supplementary Figure 2. Characterization of *msl1-1*-knockout and complemented lines of Arabidopsis. (A) Schematic showing location of the T-DNA insertion in the *MSL1* gene. (B) Semi-quantitative RT-PCR showing loss of transcript in *msl1-1* and its restoration in the complemented line. (C) The loss and restoration of MSL1 protein in the mutant (*msl1-1*) and complemented line (*msl1-1*/gMSL1) is demonstrated by western blot of peripheral and membrane fractions of mitochondria using antibody against MSL1 (also refer to Figure 1C for a more detailed figure legend). (D) Phenotype of *msl1-1*-knockout and complemented lines grown on agar plates and soil.

Supplementary Figure 3. Verification of MSL1 expression in *E. coli*. (A) SDS-PAGE of Triton X-100-soluble (lane A, total cytoplasmic and membrane fraction) and –insoluble (lane B, fraction of inclusion bodies) proteins following overexpression of MSL1 in *E. coli*. Proteins were either visualized directly by Coomassie blue staining (left) or transferred onto a PVDF membrane for immunoblotting with an antibody against penta-histidine tag (right). The location of MSL1 band on the gel is indicated by a black triangle. (B) 1D Blue Native/SDS-PAGE of Triton X-100-solubilized fraction from *E. coli* expressing either RAC-kinase (lane 1, control) or mature MSL1 (lane 2). Proteins were either visualized directly by Coomassie blue staining (left) or transferred onto a PVDF membrane for immunoblotting with a peptide antibody raised against MSL1 (right). The location of MSL1 band on the Coomassie-stained gel is indicated by a black triangle.

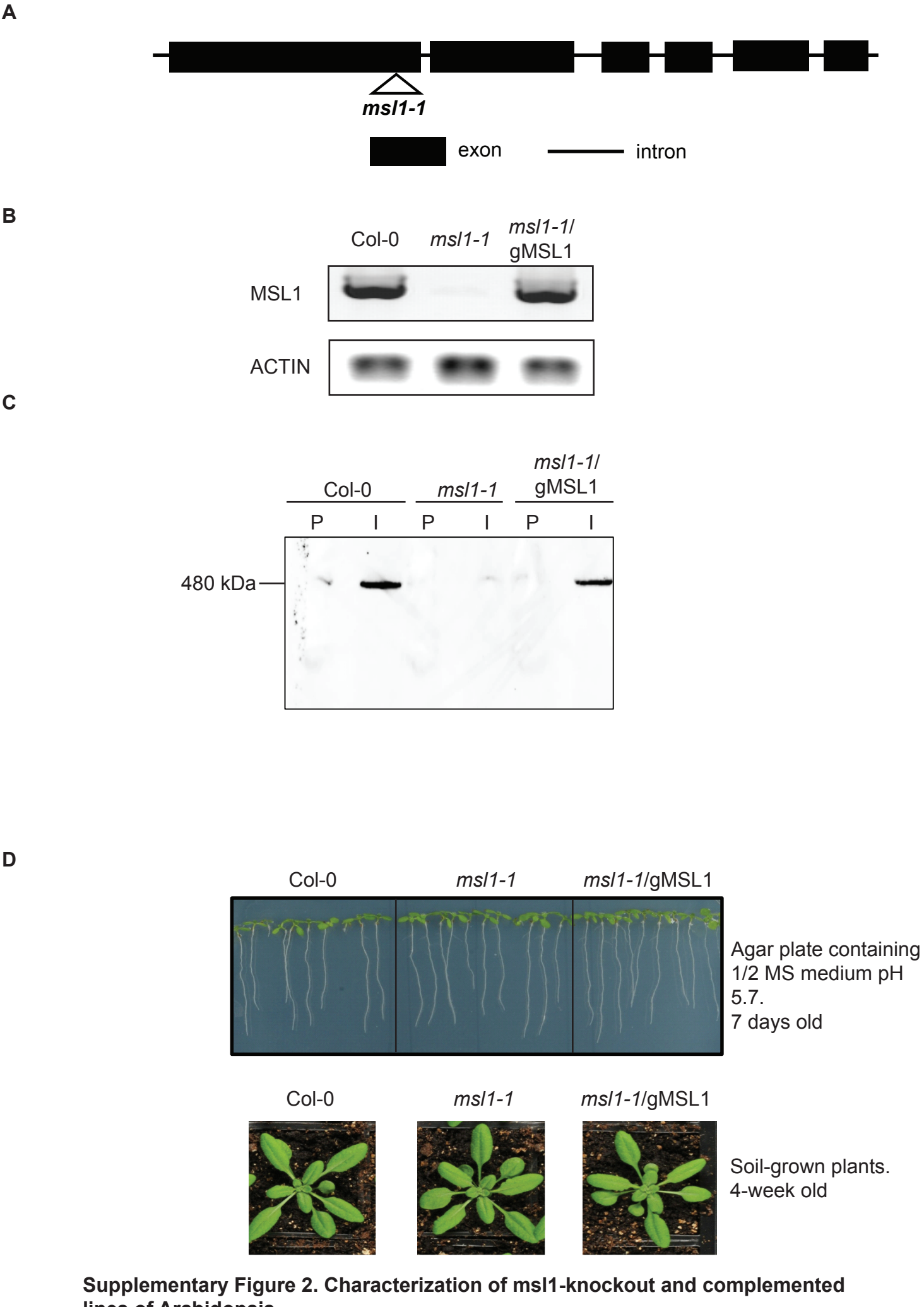
Supplementary Figure 4. Comparison of tension-gated MSL1 channel activity under positive and negative membrane potentials.

Supplementary Data 1. Identification of MSL1 by Mass spectrometry. Excel file includes a list of peptides detected in this study and in published literature. Three MGF files contain the identity of all peptides/proteins in the MSL1-containing protein spot extracted from three independent fractionation of isolated mitochondria on Blue-Native/SDS-PAGE.

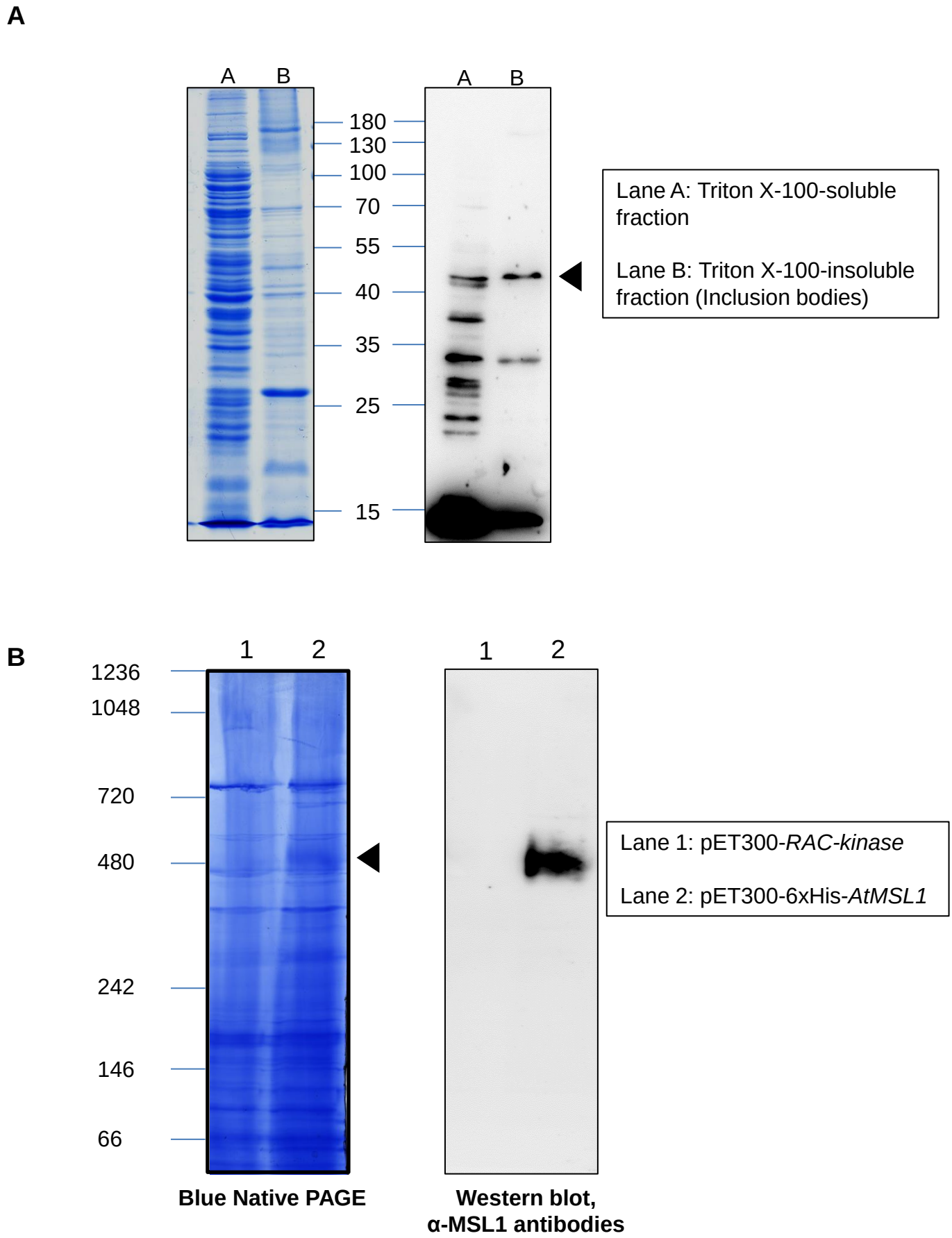
Supplementary Table 1. A list of primers used in this study.



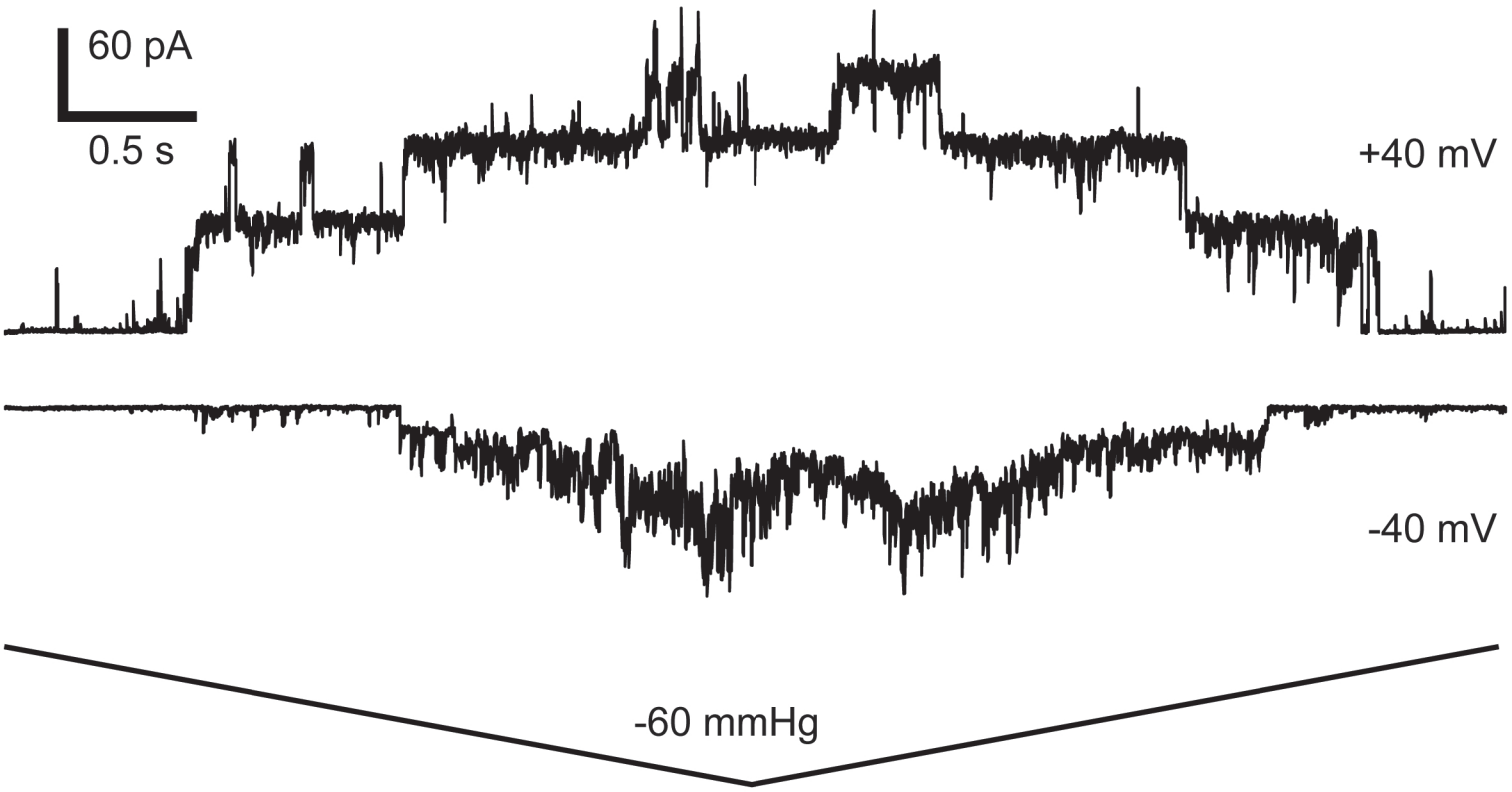
Supplementary Figure 1. The MSL1 precursor protein is not imported into isolated chloroplasts *in vitro*



Supplementary Figure 2. Characterization of msl1-knockout and complemented lines of Arabidopsis



Supplementary Figure 3. Verification of MSL1 expression in *E. coli*.



Supplementary Figure 4. Comparison of tension-gated MSL1 channel activity under positive and negative membrane potentials.

Pimer name	Primer sequence	Purpose
MSL1_F	ATGGCCGGAGTTAGGTTATCGC	Genotyping of MSL1 transgenic lines with genomic DNA
MSL1_R	CTTCTCCCTATCAAGCCCACC	Genotyping of MSL1 transgenic lines with genomic DNA
T-DNA_F	ATATTGACCATCATACTCATTGC	Genotyping of MSL1 transgenic lines with genomic DNA
T-DNA_R	ATAGCGAAAAGACCAACAGATGAT	Genotyping of MSL1 transgenic lines with genomic DNA
MSL1_sqPCR_F	ATGGCCGGAGTTAGGTTATCGC	Semi-quantitative PCR of MSL1
MSL1_sqPCR_R	TCACAATGTAGAATTGTCCCAGGTG	Semi-quantitative PCR of MSL1
MSL1_qPCR_F	TGGAGGGTAGCCACCGCATTTG	Quantitative PCR of MSL1
MSL1_qPCR_R	CCTTCTACGGATCCAGCTTTGATAG	Quantitative PCR of MSL1
LHO710	CACC GCT CTG ATT GAC ATA GAA GAA G	For generating genomic complementation transgene MSL1g
LHO817	CCT GTA CAT AGG AAT TGT TAG AAC CCT ATA AAT ACC TAC	For generating genomic complementation transgene MSL1g
MSL1-GFP_F	CACCATGGCCGGAGTTAGGTTATCG	For generating MSL1-GFP construct
MSL1-GFP_R	GGAGACCAGAACACGATTTTGC	For generating MSL1-GFP construct
His-MSL1-m_F	CACCAGCTCCAAATCTGATGATTTC	Generation of MSL1-carrying vector for bacterial expression and protein extraction
His-MSL1-m_R	TCACAATGTAGAATTGTCCCAGGTGG	Generation of MSL1-carrying vector for bacterial expression and protein extraction
LHO2458	AGC TTC TCG AGA ATT ATG CAT CAT CAT CAT CAT CAC ATC ACA AG	Generation of MSL1-carrying vector for bacterial expression, osmotic shock assay and electrophysiology
LHO2459	CGT CCT TGT AGT CGA TCA CAA TGT AGA ATT GTC CCA GGT GG	Generation of MSL1-carrying vector for bacterial expression, osmotic shock assay and electrophysiology
LHO2460	AGC TTC TCG AGA ATT ATG AGC TCC AAA TCT GAT GAT TTC GGA AG	Generation of MSL1-carrying vector for bacterial expression, osmotic shock assay and electrophysiology
LHO2462	CGT CCT TGT AGT CGA ATG TAG AAT TGT CCC AGG TGG TAC C	Generation of MSL1-carrying vector for bacterial expression, osmotic shock assay and electrophysiology