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Supplemental Information

Direct inhibition of phosphate transport by immune signaling in *Arabidopsis*

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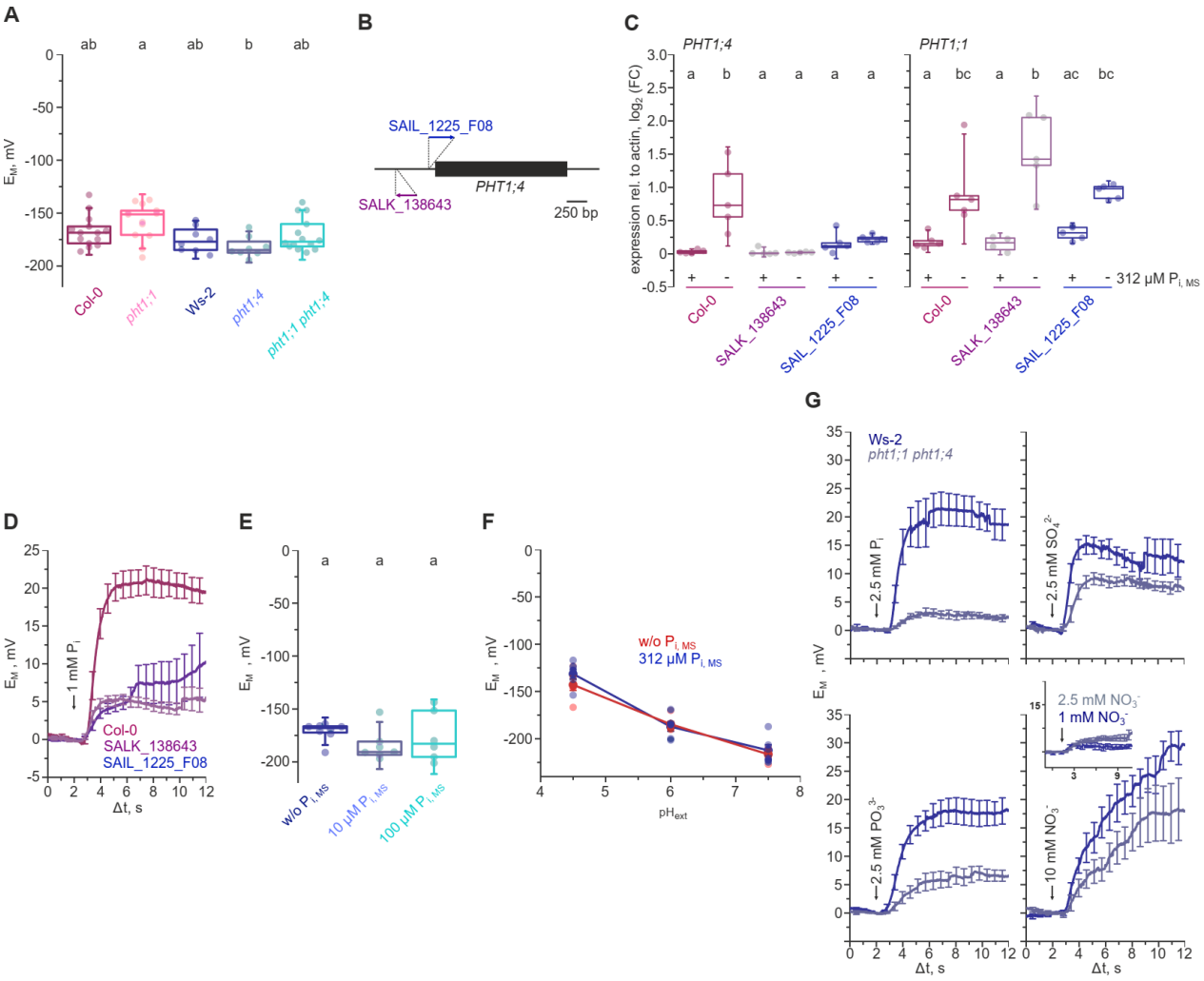

Figure S1: Electrophysiological characterization of PHT1-mediated P_i transport. Related to Figure 1. (**A**) Boxplot of resting E_M values averaged over the 5 s before P_i application. Values correspond to measurements shown in Figure 2A and B. (**B**) Illustration highlighting the genomic locus of *PHT1;4* and the position of analysed T-DNA insertion mutants. (**C**) Transcript analysis of *PHT1;1* (right panel) and *PHT1;4* (left panel) in Col-0 and the homozygous *PHT1;4* T-DNA insertion mutants SALK_138643 and SAIL_1225_F08 grown on media supplemented without or with 312 µM Pi. (**D**) Averaged responses of the PM potential to application of 1 mM inorganic P_i at pH 6. The response was tested in Col-0 and the homozygous *PHT1;4* T-DNA insertion mutants SALK_138643 and SAIL_1225_F08 grown on media supplemented without Pi. Curves are normalized and aligned to the point of P_i application (Δt = 2). Error bars show ±SEM (n = 6). (**E**) Boxplot of resting E_M values for seedlings grown at the indicated P_i conditions. Values were averaged over the 5 s before P_i application. Values correspond to measurements shown in Figure 2D. (**F**) Straight lines connect average resting E_M values averaged over the 5 s before P_i application for external pH values of 4.5, 6 and 7.5 and seedlings grown on media supplemented without or with 312 µM Pi. Values correspond to measurements shown in Figure 2E. Light colored points highlight individual measurements. Error bars show ±SEM. (**G**). Averaged responses of the PM potential to application of 2.5 mM Pi, phosphite, sulfate and 10 mM nitrate at pH 6. The measurements correspond to Figure 2G and H. The responses were tested in Ws-2 and in the double mutant *pht1;1 pht1;4*. Curves are normalized and aligned to the point of P_i application (Δt = 2). The inset in the left panel shows the tested nitrate concentrations <10 mM on Ws-2 seedlings. Error bars show ±SEM (n = 6 to 8). Boxes indicate the 25 and 75 percentile, the horizontal line shows the median, error bars represent ±SD. Closed circles show individual measurements. When indicated significance was tested with a one-way ANOVA and a post-hoc Tukey test. Equal letters at the top of the panel indicate p >0.05.

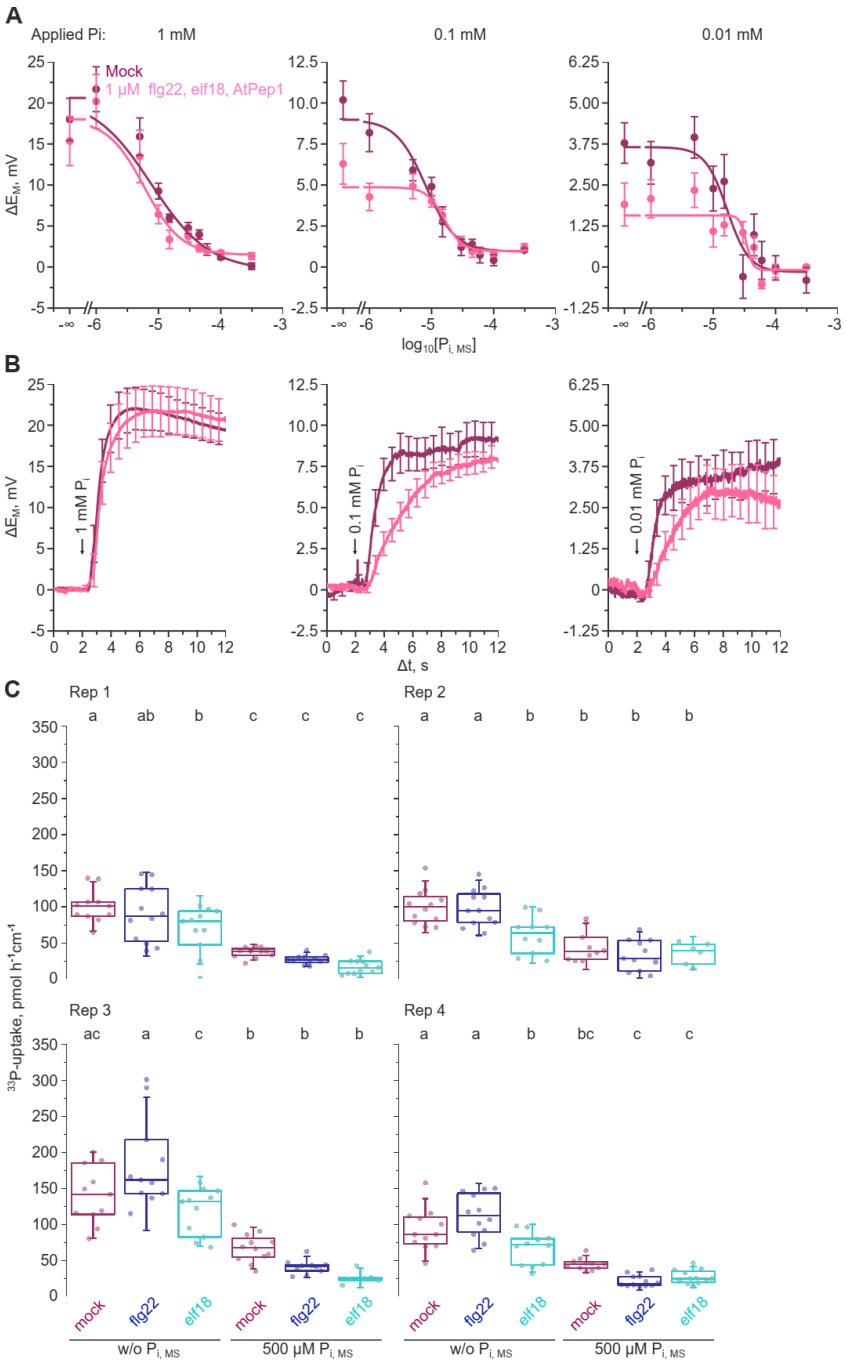

Figure S2: Effect of elicitor treatment on P_i transport. Related to Figure 2. (**A**) Dose response curves for P_i -induced depolarization of Col-0 root hairs in dependence of the P_i concentration in the growth media. Applied P_i concentration was 1 mM, 0.1 mM or 0.01 mM, as indicated. Responses were measured without or after a 15 to 30 min pretreatment with an elicitor mix of 1 µM flg22, elf18 and AtPep1. Values correspond to the average ΔE_M values at Δt = 4.5 to 5.5 Error bars show ±SEM. Solid lines show fits of sigmoidal dose-response curves. Error bars show ±SEM (n = 5 to 11). (**B**) Averaged responses of the PM potential to application of 1 mM, 0.1 mM, or 0.01 mM P_i in Col-0 root hairs grown at 1 µM P_i. Curves are normalized and aligned to the point of phosphate application (Δt = 2). Measurements correspond to those in (A). Error bars show ±SEM (n= 5 to 11). (**C**) Biologically-independent replicates of radioactive P_i-uptake experiments corresponding to Figure 2B. Boxes indicate the 25 and 75 percentile, the horizontal line shows the median, error bars represent ±SD. Closed circles show individual measurements. Significance was tested with a one-way ANOVA and a post-hoc Tukey test. Equal letters at the top of the panel indicate p >0.05.

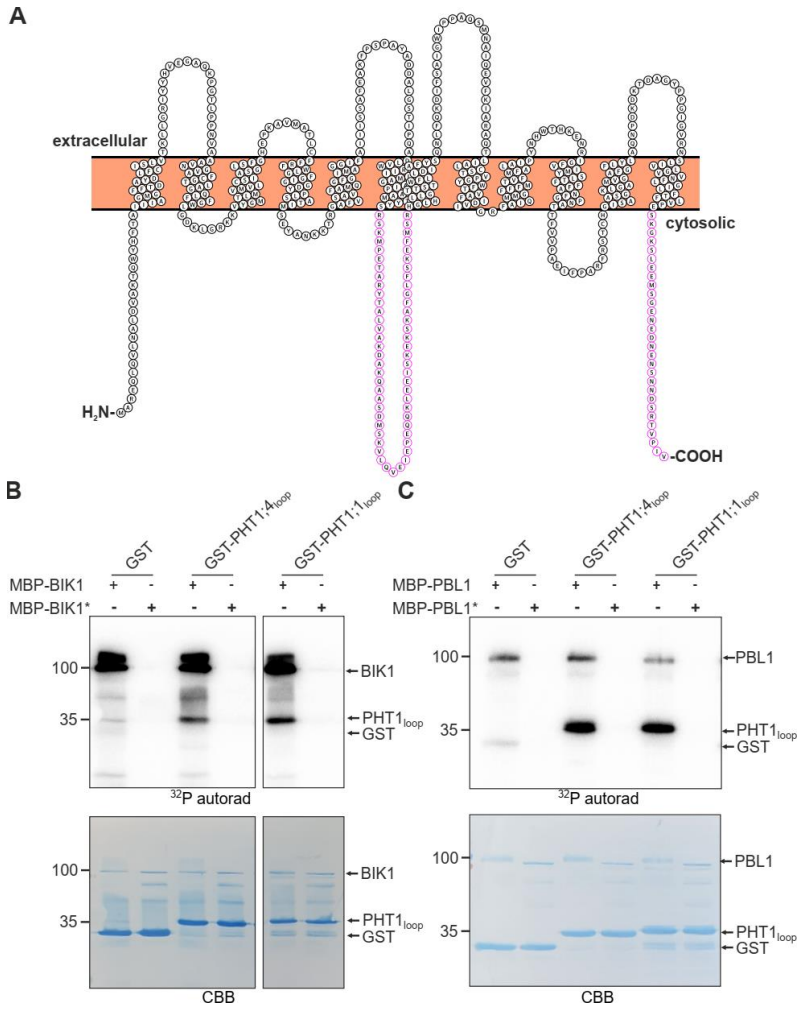

Figure S3: PHT1;4 and PHT1;4 are substrates for BIK1 and PBL1. Related to Figure 3. (**A**) Predicted topology of PHT1;4 with the large cytosolic loop and C-terminus highlighted in magenta. Prediction with: https://wlab.ethz.ch/protter. (**B** and **C**) ^{32}P kinase assay showing *in vitro* transphosphorylation of the PHT1;4 and PHT1;1 cytosolic loops by BIK1 (B) and PBL1 (C). Asterisk indicates kinase-dead variant. Kinase assays were repeated at least twice with similar results.

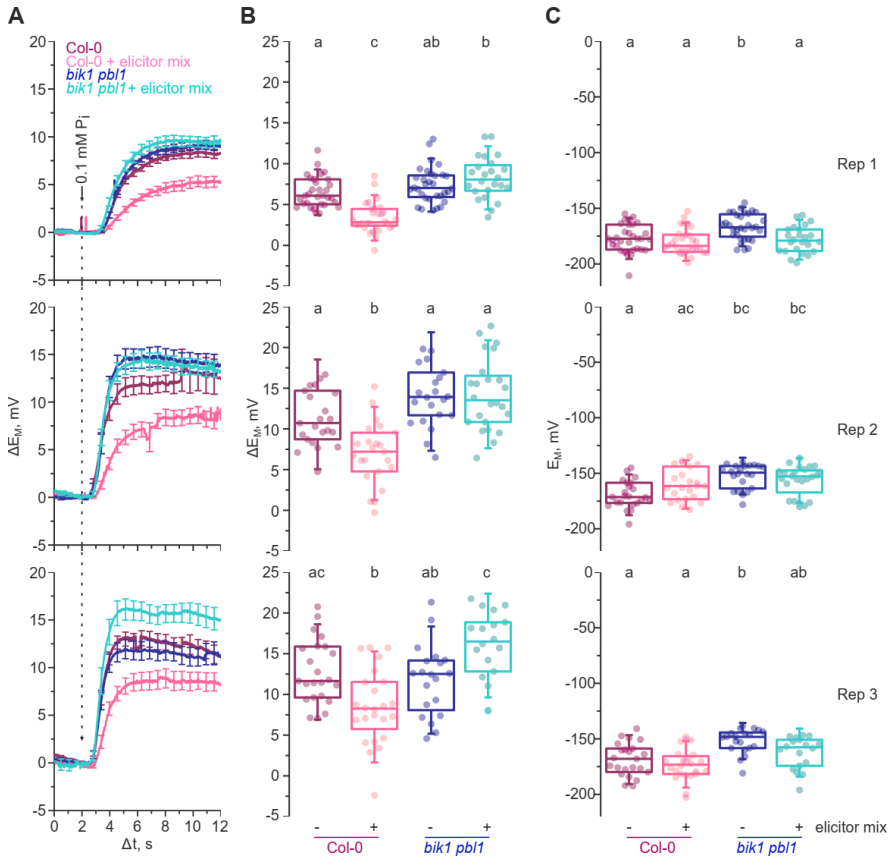

Figure S4: **Biologically-independent replicates corresponding to Figure 3D and E.** (**A**) Averaged responses of the PM potential to application of 0.1 mM P_i at pH 6 without or after a 15 to 30 min pretreatment with an elicitor mix of 1 µM flg22, elf18 and AtPep1. The response was tested in Col-0 and the *bik1 pbl1* double mutant. Curves are normalized and aligned to the point of P_i application (Δt = 2). Error bars show ±SEM (n = 18 to 32). (**B**) Boxplot of ΔE_M values at Δt = 5.5 to 6.5 from curves in (A). (**C**) Boxplot of resting E_M values averaged over the 5 s before P_i application. Values correspond to measurements shown in Figure 3A and B. Boxes indicate the 25 and 75 percentile, the horizontal line shows the median, error bars represent ±SD. Closed circles show individual measurements. When indicated significance was tested with a one-way ANOVA and a post-hoc Tukey test. Equal letters at the top of the panel indicate p >0.05.

Name	Sequence 5`to 3`	purpose
PHT1;4_fwd	GCGCAAAGCATGAACGCAATTC	qPCR
PHT1;4_rev	CGTGCTACACAAGGCGATTAGC	qPCR
PHT1;1_fwd	GGAGCCATTGTTGGAGCCTTTG	qPCR
PHT1;1_rev	CGTCTACCTTGGCCTTGTCTTGTG	qPCR
ACTIN2_fwd	CTTGTTCCAGCCCTCGTTTGTG	qPCR
ACTIN2_rev	CCTTGGAGATCCACATCTGCTG	qPCR
Ubox_fwd	TGCGCTGCCAGATAATACACTATT	qPCR
Ubox_rev	TGCTGCCCAACATCAGGTT	qPCR
ZAT12_fwd	TTGGTTACACGCGCTTTGTTGC	qPCR
ZAT12_rev	ACAAGCCACTCTCTTCCCACTG	qPCR
PLP2_fwd	AACCCGGCTTTGTTGGCCATTG	qPCR
PLP2_rev	TTCCGGTTCCAAGCGAAAGCAC	qPCR
PHT1;4_234_SalI-f	TAGTCGACtctcaaggtcgaagatgcc	pGEX cloning
PHT1;4_294_NotI-r	TAGCGGCCGCTTAATGGCGACTCATGAATTC	pGEX cloning
PHT1;4_503_SalI-f	TAGTCGACtcgtacctgaatctaaagg	pGEX cloning
PHT1;4_NotI-r	TAGCGGCCGCTTAAACTATTGGGACCGTTC	pGEX cloning
PHT1.1_233_SalI-f	TAGTCGACtccgtatgaagatgcctg	pGEX cloning
PHT1.1_293_NotI-r	TAGCGGCCGCTTAatggcgtctaaggaattc	pGEX cloning
PHT1.4_B1-f	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTatggcaagggaacaattac	pDONR cloning
PHT1.4-B2-r	GGGGACCACTTTGTACAAGAAAGCTGGGTCAACTATTGGGACCGTTC	pDONR cloning
338F	ACTCCTACGGGAGGCAGCA	16s rRNA seq
806R	GGACTACHVGGGTWTCTAAT	16s rRNA seq
Illumina-PCR-F	AATGATACGGCGACCACCGAGATCTACACGCCTCCCTCGCGCCATCAGAGATGTG	16s rRNA seq

Table S1. Primers used in this study. Related to STAR Methods.

Name	Sequence 5' to 3'
PCR_R_bc1	CAAGCAGAAGACGGCATACGAGAT TTACCGACG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc2	CAAGCAGAAGACGGCATACGAGAT ATTGGACAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc3	CAAGCAGAAGACGGCATACGAGAT TCGCATGGA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc4	CAAGCAGAAGACGGCATACGAGAT AGCGAACCT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc5	CAAGCAGAAGACGGCATACGAGAT AGCTTCGAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc6	CAAGCAGAAGACGGCATACGAGAT GTCAGCCGT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc7	CAAGCAGAAGACGGCATACGAGAT TCCAGATAG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc8	CAAGCAGAAGACGGCATACGAGAT GAGAGTCCA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc9	CAAGCAGAAGACGGCATACGAGAT GCTCACAAT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc10	CAAGCAGAAGACGGCATACGAGAT TTGACGACA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc11	CAAGCAGAAGACGGCATACGAGAT CTTAGAACG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc12	CAAGCAGAAGACGGCATACGAGAT CGGTTCACA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc13	CAAGCAGAAGACGGCATACGAGAT CGATAGGCC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc14	CAAGCAGAAGACGGCATACGAGAT GCTATATCC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc15	CAAGCAGAAGACGGCATACGAGAT GTCTTCAGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc16	CAAGCAGAAGACGGCATACGAGAT TAGACACCG GTGACTGGAGTTCAGACGTGTGCTC

PCR_R_bc17	CAAGCAGAAGACGGCATACGAGAT TCAGCTGAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc18	CAAGCAGAAGACGGCATACGAGAT TAAGTCGGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc19	CAAGCAGAAGACGGCATACGAGAT GCTCCTTAG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc20	CAAGCAGAAGACGGCATACGAGAT ATGGCCTGA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc21	CAAGCAGAAGACGGCATACGAGAT TTGCAAGTA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc22	CAAGCAGAAGACGGCATACGAGAT CCTAGTAAG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc23	CAAGCAGAAGACGGCATACGAGAT CTAGGATCA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc24	CAAGCAGAAGACGGCATACGAGAT TATGAACGT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc25	CAAGCAGAAGACGGCATACGAGAT CTTGTGCGA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc26	CAAGCAGAAGACGGCATACGAGAT CACGATGGT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc27	CAAGCAGAAGACGGCATACGAGAT ACGTGCCTT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc28	CAAGCAGAAGACGGCATACGAGAT TGAACTAGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc29	CAAGCAGAAGACGGCATACGAGAT TATTCAGCG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc30	CAAGCAGAAGACGGCATACGAGAT TAATCGGTG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc31	CAAGCAGAAGACGGCATACGAGAT GCGTCCATG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc32	CAAGCAGAAGACGGCATACGAGAT CGTAAGATG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc33	CAAGCAGAAGACGGCATACGAGAT CTGTTACAG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc34	CAAGCAGAAGACGGCATACGAGAT ACGATCATC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc35	CAAGCAGAAGACGGCATACGAGAT GTAACGGCT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc36	CAAGCAGAAGACGGCATACGAGAT CCATGCTTA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc37	CAAGCAGAAGACGGCATACGAGAT GTACGCACA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc38	CAAGCAGAAGACGGCATACGAGAT TTAGAGCCA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc39	CAAGCAGAAGACGGCATACGAGAT ATAAGGTCG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc40	CAAGCAGAAGACGGCATACGAGAT AGTGGCACT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc41	CAAGCAGAAGACGGCATACGAGAT CCAGAAGTG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc42	CAAGCAGAAGACGGCATACGAGAT CTACTAGCG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc43	CAAGCAGAAGACGGCATACGAGAT TAGCGTTCC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc44	CAAGCAGAAGACGGCATACGAGAT GTGAGTCAT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc45	CAAGCAGAAGACGGCATACGAGAT TGGTCCTAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc46	CAAGCAGAAGACGGCATACGAGAT TACGCGTAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc47	CAAGCAGAAGACGGCATACGAGAT GAGCCATCT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc48	CAAGCAGAAGACGGCATACGAGAT CGTCCGTAT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc49	CAAGCAGAAGACGGCATACGAGAT GATACGTTC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc50	CAAGCAGAAGACGGCATACGAGAT CAGCTGGTT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc51	CAAGCAGAAGACGGCATACGAGAT TTAAGCGCC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc52	CAAGCAGAAGACGGCATACGAGAT CCTGCGAAG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc53	CAAGCAGAAGACGGCATACGAGAT TTGTAGCCG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc54	CAAGCAGAAGACGGCATACGAGAT TCTGTAGAG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc55	CAAGCAGAAGACGGCATACGAGAT CTATTAAGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc56	CAAGCAGAAGACGGCATACGAGAT CTCTGAGGT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc57	CAAGCAGAAGACGGCATACGAGAT CAGGATTCG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc58	CAAGCAGAAGACGGCATACGAGAT TCACTGCTA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc59	CAAGCAGAAGACGGCATACGAGAT ACATGTCAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc60	CAAGCAGAAGACGGCATACGAGAT ATTCTGCCG GTGACTGGAGTTCAGACGTGTGCTC

PCR_R_bc61	CAAGCAGAAGACGGCATACGAGAT TACACGCTG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc62	CAAGCAGAAGACGGCATACGAGAT TGCATACAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc63	CAAGCAGAAGACGGCATACGAGAT ACGCAATGT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc64	CAAGCAGAAGACGGCATACGAGAT GCTCGAAGA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc65	CAAGCAGAAGACGGCATACGAGAT AGACGTTGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc66	CAAGCAGAAGACGGCATACGAGAT TAGAGCTGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc67	CAAGCAGAAGACGGCATACGAGAT GGTAACCTC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc68	CAAGCAGAAGACGGCATACGAGAT GACTTCATG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc69	CAAGCAGAAGACGGCATACGAGAT CTGCATACT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc70	CAAGCAGAAGACGGCATACGAGAT TAAGGCATC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc71	CAAGCAGAAGACGGCATACGAGAT AGTATTCGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc72	CAAGCAGAAGACGGCATACGAGAT TTCGCAGAT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc73	CAAGCAGAAGACGGCATACGAGAT GCACCTGTT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc74	CAAGCAGAAGACGGCATACGAGAT CTCATGGTA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc75	CAAGCAGAAGACGGCATACGAGAT ACTAGTTGG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc76	CAAGCAGAAGACGGCATACGAGAT GCGGACTAT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc77	CAAGCAGAAGACGGCATACGAGAT ATCGCTTAA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc78	CAAGCAGAAGACGGCATACGAGAT TCAGGACGT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc79	CAAGCAGAAGACGGCATACGAGAT GCATTACTG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc80	CAAGCAGAAGACGGCATACGAGAT GCTATGGAA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc81	CAAGCAGAAGACGGCATACGAGAT GATTGTGCA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc82	CAAGCAGAAGACGGCATACGAGAT AGCCTCATG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc83	CAAGCAGAAGACGGCATACGAGAT AACTCCTGT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc84	CAAGCAGAAGACGGCATACGAGAT TAGAAGGCT GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc85	CAAGCAGAAGACGGCATACGAGAT GACTAGTCA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc86	CAAGCAGAAGACGGCATACGAGAT GGATACTCG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc87	CAAGCAGAAGACGGCATACGAGAT CCGACATTG GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc88	CAAGCAGAAGACGGCATACGAGAT TCGTGACGC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc89	CAAGCAGAAGACGGCATACGAGAT GGCCTATAA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc90	CAAGCAGAAGACGGCATACGAGAT GTAGCACTC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc91	CAAGCAGAAGACGGCATACGAGAT CTAAGACGT GTGACTGGAGTTCAGACGTGTGCTC
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PCR_R_bc93	CAAGCAGAAGACGGCATACGAGAT TGTAACGCC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc94	CAAGCAGAAGACGGCATACGAGAT ATGCGAGAC GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc95	CAAGCAGAAGACGGCATACGAGAT CCGTCAAGA GTGACTGGAGTTCAGACGTGTGCTC
PCR_R_bc96	CAAGCAGAAGACGGCATACGAGAT TAGTAGCAC GTGACTGGAGTTCAGACGTGTGCTC

Table S2. Reverse primers for bacterial 16s rRNA sequencing. Related to STAR Methods.