

### Primers used to generate point mutation constructs of Rho1

Rho1-1	CGACCTGGAGGTATTTGAGACC
Rho1-2	CAGCTGGCATTGACGGACAGC
Rho1-A (Q15V)	GCTCACGACCATCAGGCGAC <b>GTT</b> GCTTGTGGTAAAACATGTCTG
Rho1-B (Q15V)	CAGACATGTTTTACCACAAGC <b>AAC</b> GTGCGCTGATGGTCGTGAGC
Rho1-C (Q64L)	CATTGTGGGATACTGCTGG <b>ACTG</b> GTGCGTGGTAATTGTCGCCC
Rho1-D (Q64L)	GGGCGACAATTACCACGCAC <b>CAG</b> TCCAGCAGTATCCCACAATG
Rho1-3	GTTCCGAGTATTTCTTTGACCcaggaaacagctatgaccatg
Rho1-4	catggtcatagctgttctgGGTCGAAAGAAATACTCGGAAC
Rho1-5	cactggccgtcgttttacaacTTACTTGCATCTTGCCCCAAC
Rho1-6	GTTGGGGCAAGATGCAAGTGTA <b>Ag</b> ttgtaaaacgacggccagt
Rho1-7	ACGTATGCTATGCGTGTTAGCC
Rho1-8	GAAATTGCACCTGAGGATTGGCC

### Primers used to generate $P_{CTR4}$ -RHO1

Pcu1	cactggccgtcgttttacaacCGATGGTCCATATGGACCAATTGG
Pcu2	CCAATTGGTCCATATGGACCATCGgttgtaaaacgacggccagt
1-Rho1 Pcu	GCGTTGCTATTCTTCTTTGAGAGCGCC
2-Rho1 Pcu	CACATTTAGTTCACCGTCTTCCTGACC
3-Rho1 Pcu	GGAGGTATTTGAGACCAGAGAcaggaaacagctatgaccatg
4-Rho1 Pcu	catggtcatagctgttctgTCTCTGGTCTCAAATACCTCC
5-Rho1 Pcu	CAACGACTTCACCAATCCATATG <b>ATGTCCGGAGAAATCAGGAGAAAGC</b>
6-Rho1 Pcu	<b>GCTTTCTCCTGATTTCTCCGGACAT</b> CATATGGATTGGTGAAGTGGTTG
7-Rho1 Pcu	CAGCGACGAACAAGATAAAGTACAACCTC
8-Rho1 Pcu	GGGCCAAGGCGTGAGAATGGC
9-Rho1 Pcu	CGAGCATTACAGGGCATAACTAC

### Primers used to generate deletion constructs of RHO10, RHO11, CDC42 and CDC420

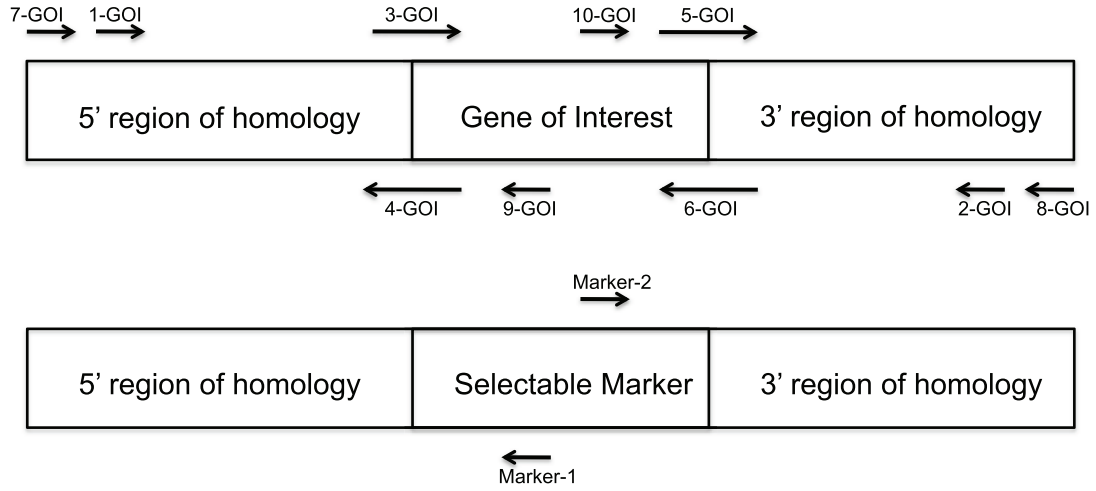
1-Rho10	TGTCAGCATCGGACGATACATGGACACT
2-Rho10	AACTGGGTTGGAAAGATGAGCTGGAGGA
3-Rho10	GCTGGGACCCGAAACACCTCCAcaggaaacagctatgaccatg
4-Rho10	catggtcatagctgttctgTGGAGGTGTTTCGGGTCCCAGC
5-Rho10	cactggccgtcgttttacaacGGATGTTTGGGAGAAATTACGA
6-Rho10	TCGTAATTCTCCCCAAACATCCgttgtaaaacgacggccagt
7-Rho10	AGATGAAAGGTTTGGTACCGGGAGGCAT
8-Rho10	GAGACATGTTCTGCGAAGGTGATGCTGA
9-Rho10	CGTCTCATGGGTGCGCTGTCGT
10-Rho10	TGAAGGGCAGGAAGTGCCTGG
1-Rho11	TCCTAATCCCACCCGACCTACTTCATCG
2-Rho11	ATGACCGATTGACTTGGCATCTCTCCCT
3-Rho11	CAAGTCCCAACAATCGTGCAGTGcaggaaacagctatgaccatg
4-Rho11	catggtcatagctgttctgCACTGCACGATTGTTGGGACTTG

5- Rho11	cactggccgctgttttacaacGGCAACATCCGAGAATCGGGC
6- Rho11	GCCCGATTCTCGGATGTTGCCgttgtaaacgacggccagtg
7- Rho11	CTTGCAAATCACCTGTGTCAGTCACCTC
8- Rho11	CTACCTTTCCCGGATCCCATCATTGAGA
9- Rho11	CACAATTGCGTTCGCGTCAGC
10-Rho11	GGCGCCACGAATGGGAGCTTT
1-Cdc42	CGATTCATACGACAGTGACAGCAGCACA
2-Cdc42	CTCAGTCATCAAAGCTTCCACCCCTTCC
3-Cdc42	TCGGAGACGGTGCCGTTGGAAcaggaaacagctatgacctg
4-Cdc42	catggtcatagctgtttcctgTTCCAACGGCACCGTCTCCGA
5-Cdc42	cactggccgctgttttacaacTCCTTTTGGCTTCCCTTTGCGCA
6-Cdc42	TGGCGAAAGGGAAGCCAAAAGGAgttgtaaacgacggccagtg
7-Cdc42	AGAGACGGAGAGCGACGATTTCAAGTCC
8-Cdc42	GCCCCTTCTTCTTCTCTCGTATCCACC
9-Cdc42	GGCGAGAGGCTCATGCGGAAT
10-Cdc42	CGTGGCGGCATTAGAACCCCT
1-Cdc420	CAGCGACGATGTTGATGAGGGTAAGCAT
2-Cdc420	CAACTCATCCCCATCCCCTCACCAC
3-Cdc420	GGGTGACGGCGCTGTTGGAAAcaggaaacagctatgacctg
4-Cdc420	catggtcatagctgtttcctgTTTCCAACAGCGCCGTCACCC
5-Cdc420	cactggccgctgttttacaacCCGCCAGTCGTGAAAAAGACCA
6-Cdc420	TGGTCTTTTTTACGACTGGCGGgttgtaaacgacggccagtg
7-Cdc420	AGTACCAAGGCCAAGAGATGGATTCCCC
8-Cdc420	GCAAGTCTTTTCTCTCCCATCAAGCCGA
9-Cdc420	ACAGTGGGGCGAACGGGACGTA
10-Cdc420	TGATGAGGTGAGTGGGCGTGGA

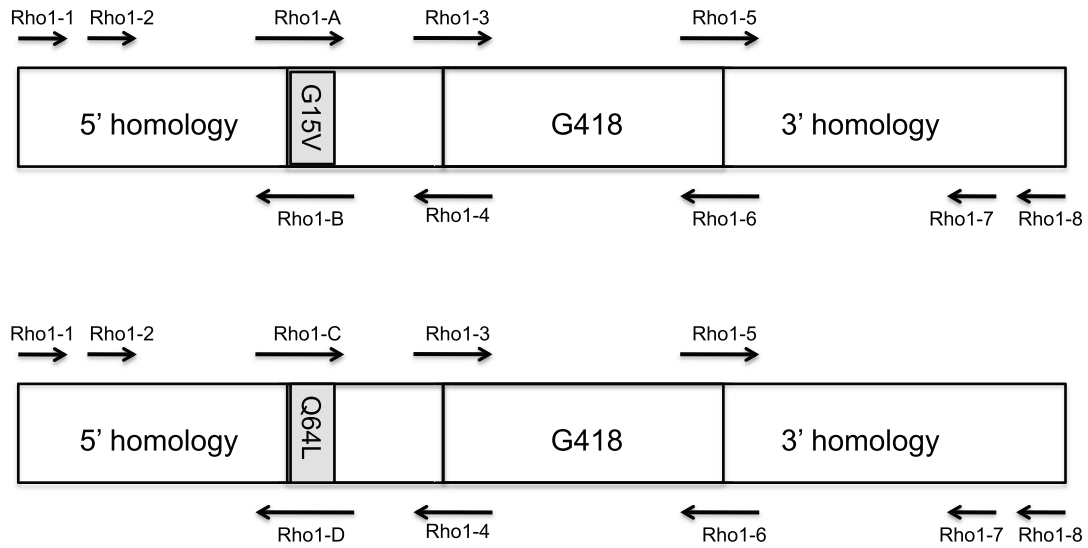
Table S1. **Primers used in this study.** Upper case correspond to the gene indicated. Italics upper case refer to *CTR4* promoter. Lower case refer to invariant regions. Underlined in bold refer to nucleotide changes.

Figure S1

A.



B.



C.

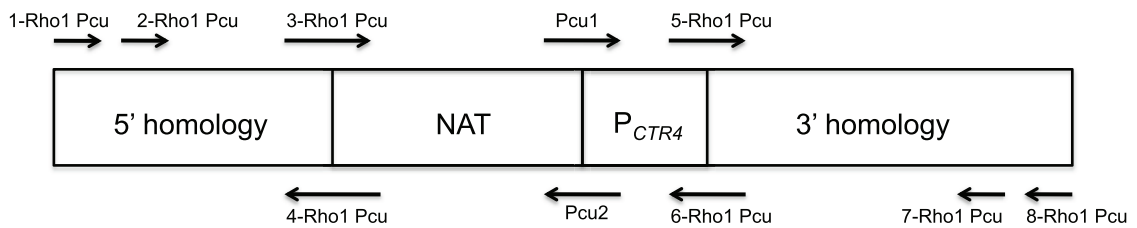


Figure S1. **Schematic diagram of gene deletion, point mutants and promoter replacement cassettes used for biolistic transformation.** Construct design and corresponding primers for generation of strains used in this study, A. gene deletions of *RHO10* and *RHO11*, and B. point mutations in *RHO1* and C. replacement of the endogenous *RHO1* promoter with  $P_{CTR4}$ .