Table S1: The Sequences of Proteins Used in this Study (Listed from N- to C-terminus).

Protein Name	Protein sequence	Source
HPC1	MLDVDKLGALEERRKVLQVKIRNLLKEKESRENSRRQAKAR GEDIEPLRLEVRKLSEELDAAHAELDALQAEIRDIALTIPW	**Recursive PCR**
HPC2	MLDVDKLGALEERRKVLQVKTEKLQKLRNGASNLIGATKA LGEDIEPVRLLVNKLGEELDAAKAELDALQAEIRDIALTIPW	**Recursive PCR**
HPC2-L57K	MLDVDKLGALEERRKVLQVKTEKLQKLRNGASNKIGATKA LGEDIEPVRLLVNKLGEELDAAKAELDALQAEIRDIALTIPW	**SDM on HPC2**
SRS-ACC	MLDVDKLGALEERRKVLQVKTENLQAERNSRSKSIGQAKAR GEDIEPLRLEVNKLGEELDAAKAELDALQAEIRDIALTIPW	**Oakley&Kim**
PKN-ACC1	QSEPRSWSLLEQLGLAGADLAAPGVQQQLELERERLRREIRK ELKLKEGAENLRRATTDLGRSLGPVELLLRGSSRRLDLLHQQ LQELHAHVVLPDPAATHDGPQSPGAGGPTCSATNLSRVAGL EKQLAIELKVKQGAENMIQTYSNG	**Asymmetric PCR**
PKN-ACC1-L59K	QSEPRSWSLLEQLGLAGADLAAPGVQQQLELERERLRREIRK ELKLKEGAENKRRATTDLGRSLGPVELLLRGSSRRLDLLHQQ LQELHAHVVLPDPAATHDGPQSPGAGGPTCSATNLSRVAGL EKQLAIELKVKQGAENMIQTYSNG	**SDM on PKN-ACC1**
WT RhoA	MAAIRKKLVIVGDGACGKTCLLIVFSKDQFPEVYVPTVFENY VADIEVDGKQVELALWDTAGQEDYDRLRPLSYPDTDVILMC FSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKDLRNDEH TRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDG VREVFEMATRAALQARRGKKKSGC LVL	**Dr. Feig**
RhoA[b]	MAAIRKKLVIVGDVACGKTCLLIVFSKDQFPEVYVPTVFENY VADIEVDGKQVELALWDTAGQEDYDRLRPLSYPDTDVILMC FSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKDLRNDEH TRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDG VREVFEMATRAALQARRGKKKSGC LVL	**SDM on WT RhoA**
RhoA-F39A[b]	MAAIRKKLVIVGDVACGKTCLLIVFSKDQFPEVYVPTVAENY VADIEVDGKQVELALWDTAGQEDYDRLRPLSYPDTDVILMC FSIDSPDSLENIPEKWTPEVKHFCPNVPIILVGNKKDLRNDEH TRRELAKMKQEPVKPEEGRDMANRIGAFGYMECSAKTKDG VREVFEMATRAALQARRGKKKSGC LVL	**SDM on RhoA-G14V**

[b] Contains G14V mutation.

Table S2: Oligonucleotides Used for Total Gene Construction (Listed From 5' to 3').

HPC1
GCGTTAGGCATATGCTGG
GGAATTCTTACCAAGGGATG
GCGTTAGGCATATGCTGGATGTAGATAAGCTGGGCGCTCTTGAAGAGCGTCGTAAAGTAT TGCAGGTCAAAATTCGT
ACCGCGCGCTTTCGCCTGACGACGGGAGTTTTCGCGGGATTCTTTCTCTTTCAGCAGGTT ACGAATTTTGACCTGCAATAC
CGTCAGGCGAAAGCGCGCGGTGAAGATATCGAGCCTTTACGTCTGGAAGTGCGTAAACTG AGCGAAGAGCTGGATGCAGCA
GGAATTCTTACCAAGGGATGGTCAGCGCGATATCGCGAATTTCAGCCTGTAAAGCATCCA GCTCGGCATGTGCTGCATCCAGCTCTTCGCT
HPC2
GCGTTAGGCATATGCTGGATGTAGATAAGCTGGGCGCTCTTGAAGAGCGTCGTAAAGTAT TGCAGGTCAAAACGGAA
ACCCAGCGCTTTGGTCGCGCCAATCAGGTTCGACGCGCCGTTACGCAGTTTTTGTTCTTT TTCCGTTTTGACCTGCAATAC
GGCGCGACCAAAGCGCTGGGTGAAGATATCGAGCCTGTGCGTCTGCTGGTGAACAAACTG GGCGAAGAGCTGGATGCAGCA
GGAATTCTTACCAAGGGATGGTCAGCGCGATATCGCGAATTTCAGCCTGTAAAGCATCCA GCTCGGCTTTTGCTGCATCCAGCTCTTCGCC
PKN-ACC1
ATATATCCGCGGCAG
TTAATGGATCCTTAGCCATTG
ATATATCCGCGGCAGAGTGAGCCGCGCAGCTGGTCCCTGCTGGAGCAGCTGGGCCTGGCCGGG GCAGACCTGGCGGCCCCGGG
CGTGAAATCCGCAAAGAGCTGAAACTGAAAGAGGGTGCGGAGAACCTGCGTCGTGCCACCA CCGACCTGGGCCGCAGCCT
CTGGACCTGCTGCACCAGCAGCTGCAGGAGCTGCACGCCCACGTGGTGCTGCCGGACCCGGC GGCCACCCACGATGGCCC
AACCTGAGCCGCGTGGCGGGCCTGGAGAAACAGCTGGCCATTGAGCTGAAAGTGAAACAGG GGGCGGAGAACATGATCCA
TTAATGGATCCTTAGCCATTGCTGTAGGTCTGGATCATGTTCTCCGCCCC
CCCGCCACGCGGCTCAGGTTGGTGGCCGAGCAGGTCGGGCCACCCGCACCCGGGGACTGCG GGCCATCGTGGGTGGCCGC
TGCTGGTGCAGCAGGTCCAGGCGGCGCGAGGAGCCACGCAGCAGCAGCTCCACCGGGCCCA GGCTGCGGCCCAGGTCGGT
AGCTCTTTGCGGATTTCACGACGCAGACGCTCACGCTCCAGCTCCAGCTGCTGCTGCACCCC CGGGGCCGCCAGGTCTGC

Table S3. Oligonucleotides for Site-Directed-Mutagenesis (Listed from 5' to 3').

RhoA-G14V
GTGATTGTTGGTGATGTTGCCTGTGGAAAGACATG
CATGTCTTTCCACAGGCAACATCACCAACAATCAC
RhoA-F39A
GTATGTGCCCACAGTGGCTGAGAACTATGTGGC
GCCACATAGTTCTCAGCCACTGTGGGCACATAC
PKN-L59K
GAAAGAGGGTGCGGAGAACAAACGTCGTGCCACCACCGAC
GTCGGTGGTGGCACGACGTTTGTTCTCCGCACCCTCTTTC
HPC2-L57K
GTAACGGCGCGTCGAACAAAATTGGCGCGACCAAAG
CTTTGGTCGCGCCAATTTTGTTCGACGCGCCGTTAC

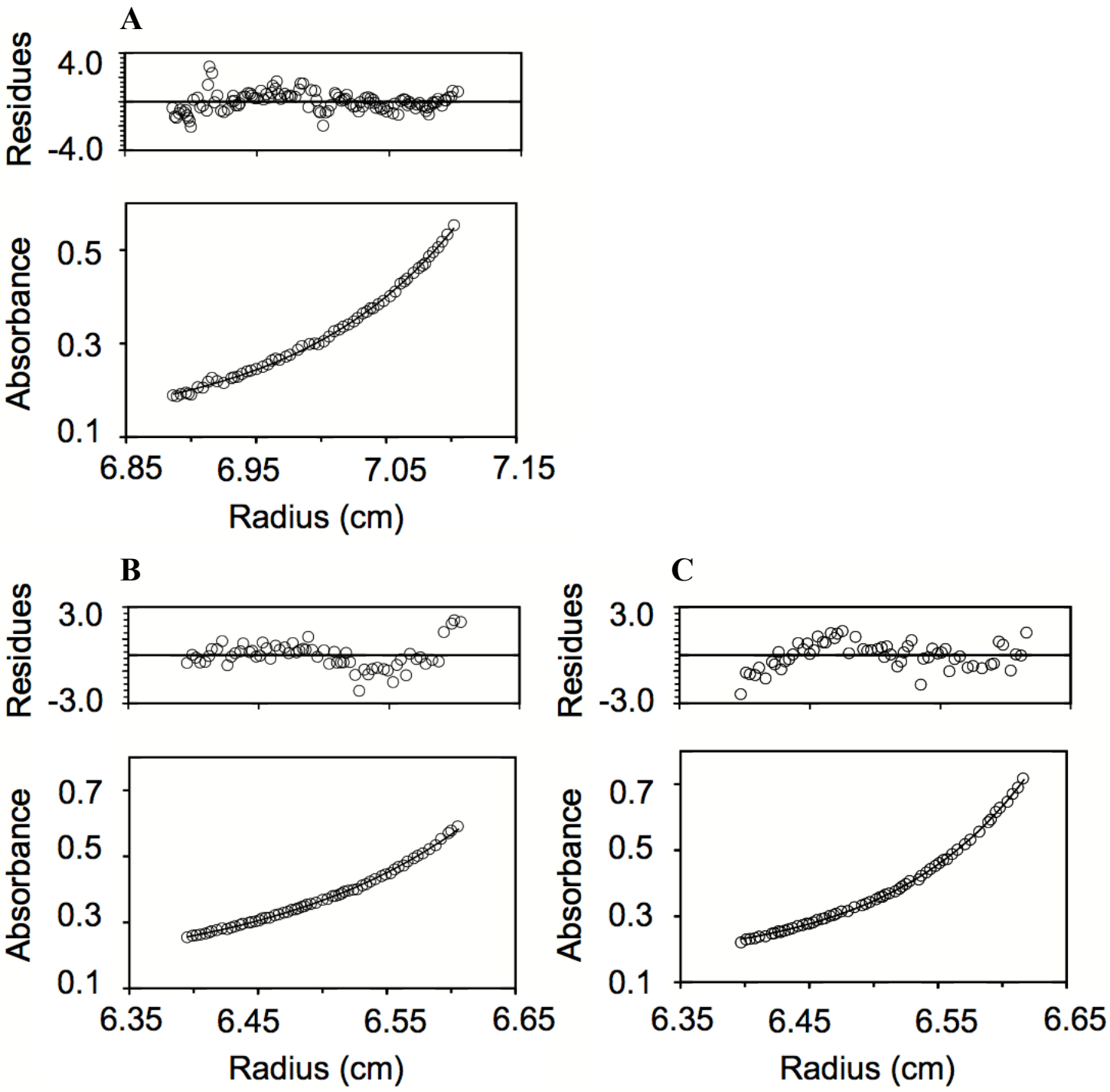


Fig S1. Analytical ultracentrifuge sedimentation data for HPC2 (4 ºC, PBS buffer, pH 7.4). At each protein concentration, data from two rotor speeds are globally fitted with an ideal single species model. (**A**) 7 μM, 28 krpm; (**B**) 15 μM, 23 krpm; (**C**) 15 μM, 28 krpm.

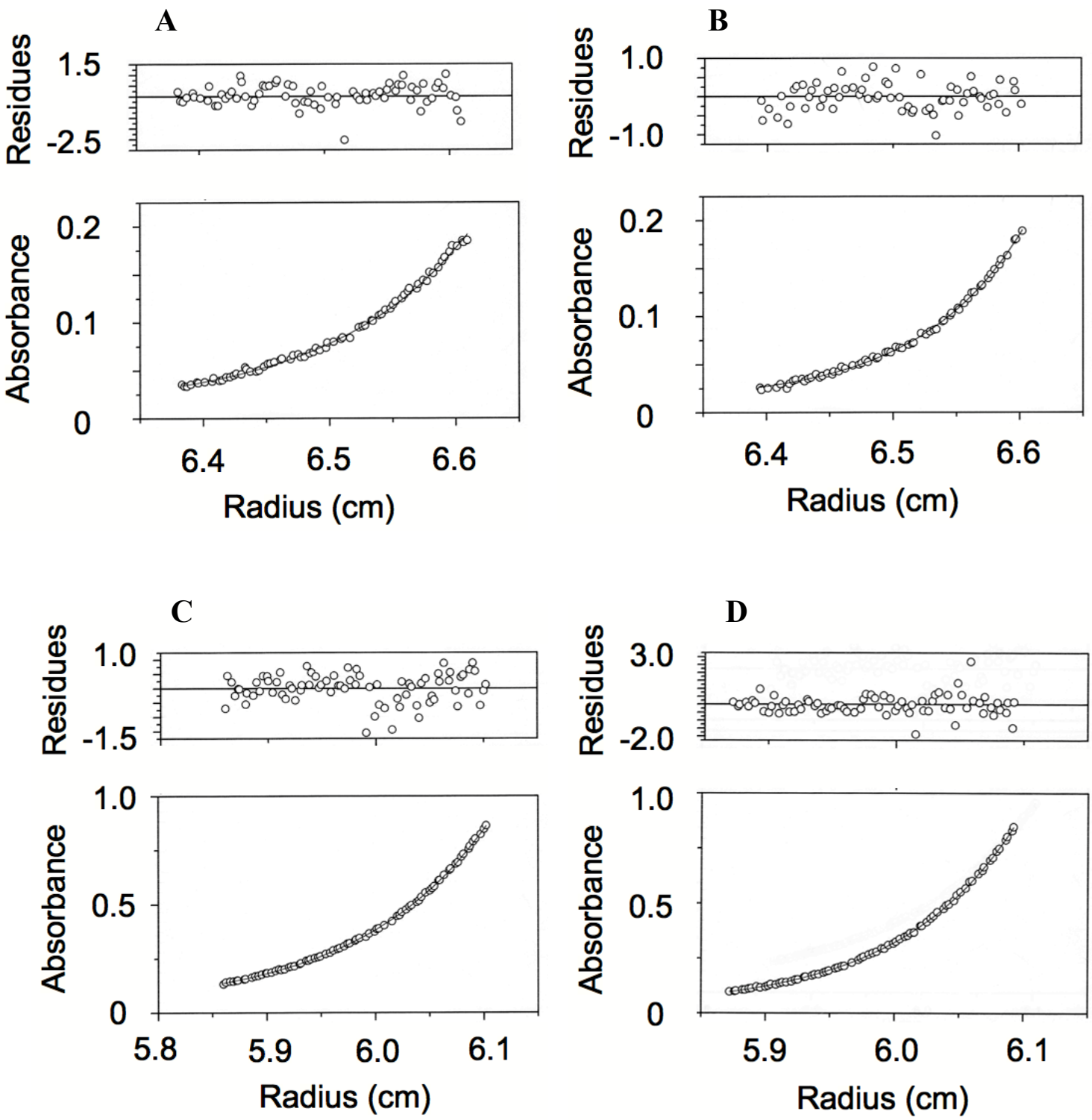


Fig S2. Analytical ultracentrifuge sedimentation data for HPC1 (4 ºC, PBS buffer, pH 7.4). At each protein centration, data from two rotor speeds are globally fitted with an ideal single species model. (**A**) 20 μM, 33 krpm; (**B**) 20 μM, 38 krpm; (**C**) 85 μM, 33 krpm; (**D**) 85 μM, 38 krpm;