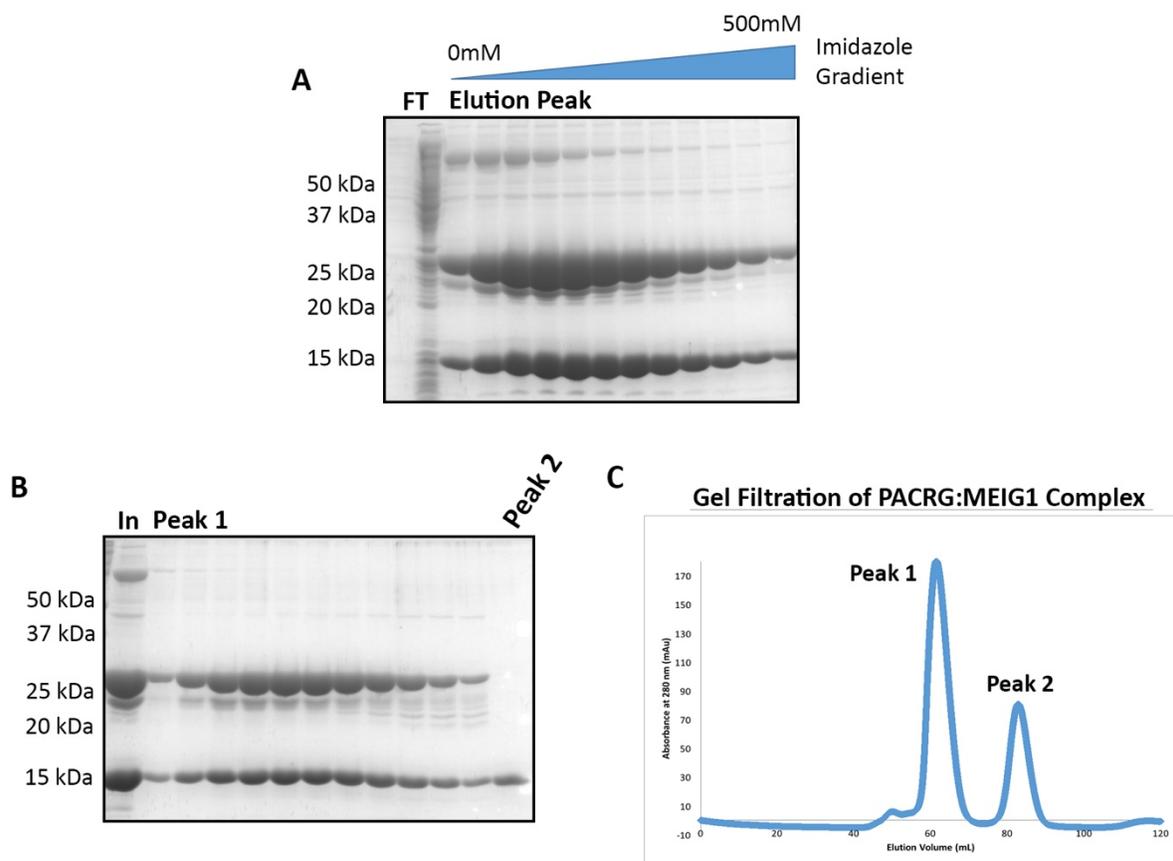


Supplemental Figure S1. PACRG_{FL}:MEIG1 co-expression in *E. coli*, related to Figure 1. (A) Cells expressing MEIG1 or PACRG alone showed little to no signal at 29 kDa, PACRG_{FL} expected size, when blotted against PACRG. When PACRG was co-expressed with MEIG1, proteins levels significantly increased. Lysates were normalized by total protein concentration. **(B)** Cell lysates were blotted against PACRG and the his-tag on MEIG1 to indicate that both proteins were indeed expressed. Immunoblotting was performed with PBS with 0.1% Tween 20 with either PACRG mouse monoclonal antibody (1:2000, Santa Cruz Biotechnology, Inc.) or His-tag polyclonal rabbit antibody (1:2000, Cell Signaling Technology) in 15 mL of PBST with 2% bovine serum albumin (BSA), shaking over night at 4°C.



Supplemental Figure S2. Purification of co-expressed PACRG_{FL}:MEIG1, related to Figure 1. (A). A richer media, TB, was used for *E. coli* growth and protein production followed by Ni-NTA gradient purification. The flow-through removed many impurities. An imidazole gradient (0-500 mM, blue bar) was used to separate the complex from *E. coli* proteins that bound to the column non-specifically. The complex was expressed in large amounts, PACRG_{FL} at 29 kDa and MEIG1 at 16 kDa. The *E. coli* Lac Operon Repressor protein, large band above 50 kDa, was still present. (B). Gel filtration was performed on the elutions from the Ni-NTA purification to remove large impurities like the Lac Operon Repressor. SDS-PAGE of gel filtration peaks. Peak1 corresponded to the complex, it was relatively pure enough to stop at this purification step. Peak 2 corresponded to free MEIG1 that did not bind to PACRG_{FL}. (C) Chromatogram of gel filtration, input was the elution fractions from Ni-NTA purification. The gel filtration separated the proteins successfully, the shoulder to the left of peak 1 corresponds to the Lac Operon Repressor. FT: flow through, In: input.

Homo 1 -MVAEKETLSLNKCPDKMPKRTKLLAQQL - - - - 29
Sus 1 -MVAEKETLSLSKCPDKMPKRTKLLAQQL - - - - 29
Rattus 1 -MVAEKETLSLNKCPDKMPKRTKLLPQQT I - - - - 29
Mus 1 - - - - -MPKRTKLLPQQT F - - - - 13
Gallus 1 -MVVEEAGCGPAKPNRRPQKQEP - - - LG - - - 26
Anolis 1 -MVAEKEGLG - - -PHRSRPPQLQEP - - - LR - - - 23
Xenopus 1 -MVYE - - - - -TSKGT EAG - - - - 12
Danio 1 -MRT F - - - - - 4
Branchiostoma 1 -MSSE - - - - - 4
Aplysia 1 - - - - -M - - - - 1
Camponatus 1 -MVNEKE - - - - -FWTE I RK - - - -DIP - - - 16
Drosophila 1 - - - - -MAMAQTARTATARRP THDYHRP TRS - - - -KSANPAQLRPL -SGI HGA AVSRP 48
Chlamydomonas 1 -MNGDVAGS LFTS TYRNVKLAGKAPPAANLSGTGSC FDTTSLPARAGAHKALDVQKDEL - - - -PVWSKSTLSYKYPAG - - - - 74
Tetrahymena 1 MLPKI PQQGNTVGS I SKRENVASQL I VEDHLKNI L - - - - -AKS TNSNYKPKWI PQAPKNHSP FGD FPK 64
Trypanosoma 1 -MSYE I QP I LKGT KRDPATRYKNRAAGKGF FGA FPP 35

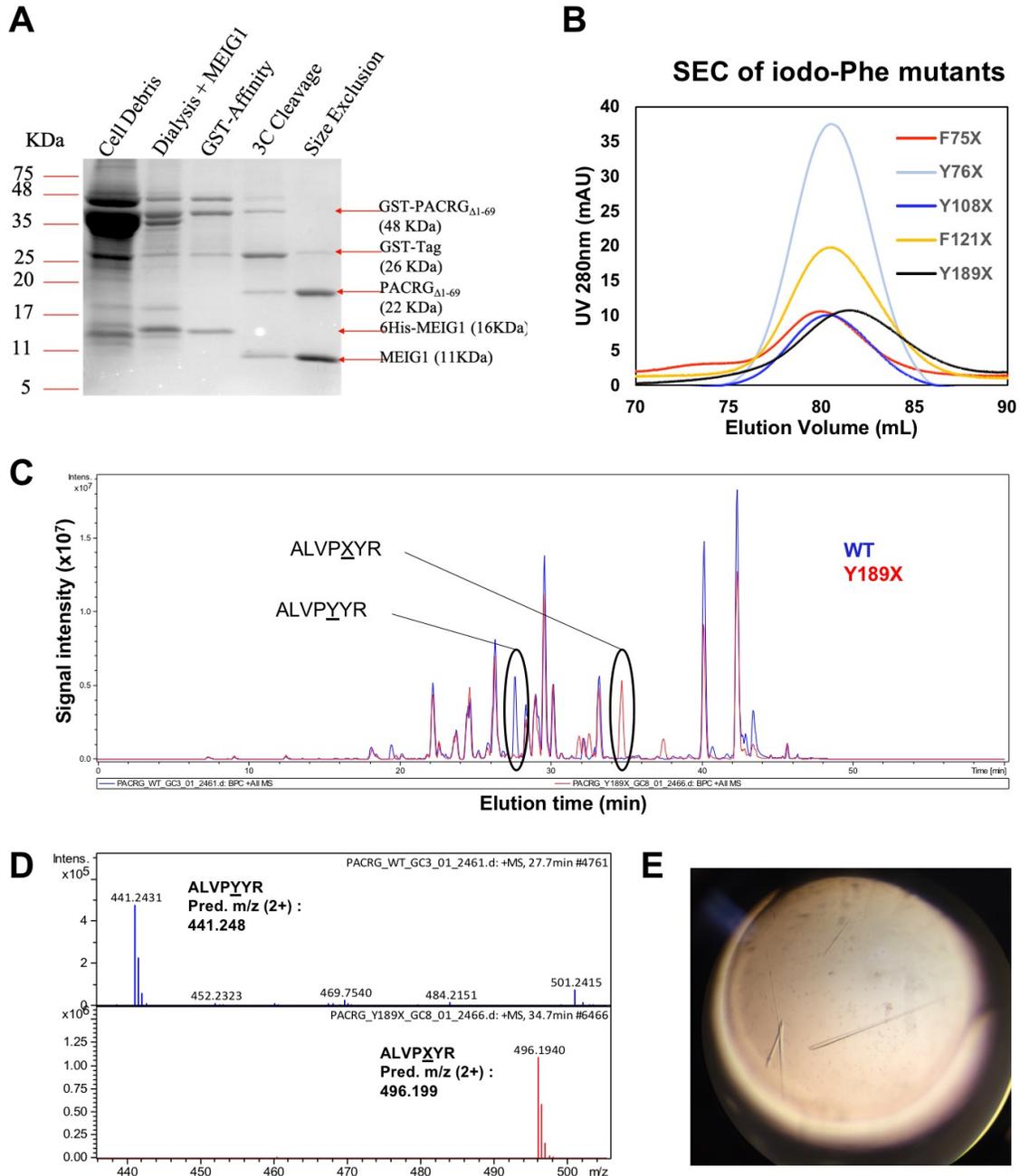
T70

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Sus 30 -PVHQPHSLVSEGFTV - - - - -K - - - - -AMMKNS - - -VVRGPPAAGAFKERPT -KP TA FRK FYER 78
Rattus 30 -QVHQPHSLVSEGFTV - - - - -K - - - - -AMMKNS - - -VVRGPPAAGAFKERPT -KP TA FRK FYER 78
Mus 14 -QVHQPHSLVSEGFTV - - - - -K - - - - -AMMKNS - - -VVRGPPAAGAFKERPT -KP TA FRK FYER 62
Gallus 27 -HVKKTKQVSDGFTV - - - - -K - - - - -AMMKNT - - -VVRGPPAAGAFKERPT -KP TA FRK FYER 75
Anolis 24 -QSKRTRKQVSDGFTV - - - - -K - - - - -AMMKNT - - -VVRGPPAAGAFKERPT -KP TA FRK FYER 72
Xenopus 13 -SNSKGGKSDSEGFTV - - - - -K - - - - -AMMKNS - - -VVRGPPAAGAFKERPT -KP TA FRK FYER 61
Danio 5 -EP LAKGELKTQGFTV - - - - -M - - - - -STMKNS - - -VVRGPPAAGAFKERPT -KP TA FRK FYER 53
Branchiostoma 5 -VLI KGSRME TEGFTV - - - - -K - - - - -SRLRNA - - -KV LAPPNAGAFKERPT -KP TA FRK FYER 53
Aplysia 2 -PGRSVDLRQTV P FTHL - - - - -V - - - - -DI FEKNNLAKPEPP LSGAFKVRDT -PMTS FRK FYER 53
Camponatus 17 -RYKKRKRPRVPAFTI - - - - -Q - - - - -ALQENT - - -VVAKPPRCGLYPRPP -KPST FRK FYER 65
Drosophila 49 RYVPPFS I QSQKNTV I DGP I HETAPKTAS - - - - -ARSRVNP K I LRRQK - - - -SMS TFLMGLNGCSTGGANPDGRGTL FRM YDR 129
Chlamydomonas 75 - - - - -RPNPTGFLKKG DGEMI - - - - -K - - - - -TKTGGFEERKPSPPQAGAYKRRNPPNTA FRR YER 127
Tetrahymena 65 EYLPKSKLKLSEQHAPV FEDSQAA TVVNFQGLR - - - - -QGTGGKTS - - - - -TQLPVKQPFQAPNP I CGAFKRT I -PVSE FRR YDR 140
Trypanosoma 36 GYAPKQEKPS I P - - - - -IEGVP AVQGRVRFAYKGT VQRTGGTTTS LYKGRQGHESA V AFT TNGAGDSKPPKAGAFKRR I I -PPTE FRR YDR 120

Homo 79 GDFP I ALEHDSKGNK IAWKVE I EKLDYHHYLP LFFDGLCE MTFPYE FFARQGI HDMLEHGGNK I LPV I PQL I IPI KNALNLRNRQV I CVTL 169
Sus 79 GDFP I ALEHDSKGNK IAWKVE I EKLDYHHYLP LFFDGLCE MTFPYE FFARQGI HDMLEHGGNK I LPV I PQL I IPI KNALNLRNRQV I CVTL 169
Rattus 79 GDFP I ALEHDSKGNK IAWKVE I EKLDYHHYLP LFFDGLCE MTFPYE FFARQGI HDMLEHGGNK I LPV I PQL I IPI KNALNLRNRQV I CVTL 169
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Gallus 76 GDFP I AIEHDTKGNR IAWKVE I EKLDYHHYLP LFFDGLCE MTFPYE FFARQGI HDMLEHGGNK I LPV I PQL I IPI KNALSLNRNRQV I C I TL 166
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Xenopus 62 GDFP I ALEHDTKGNK IAWKVE I EKLDYHHYLP LFFDGLCE TTHPYE FFARQGVHDMLEHGGPK I LPV I PQL I IPI KNALNLRNRQV I C I TL 152
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Drosophila 130 GDLP I KMEYLCGGDK I GWTVD I EKLDYSLYLP LFFDGLAE TKHPYKTYARQGV TDLL LAGGEK IHPV I PQL I IPI KNALSLNRNRQV I C I TL 220
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Tetrahymena 141 GDLP I KVDHQGSVNK I I W I QPDQLDYHHYLP I FFDGLREKLDPYRFLA I LGTYDLLEKGSNK I LPV I PQL I IPI K T ALNTRDNE I I G I ML 231
Trypanosoma 121 GDLP I LSAVHGN -RPT I DWKVDVERLDYHHYLP I FFDG I RETEYPMFLARQGC L D L KRGPK I LP I T I PQL I IPI K T ALNTRHPE I I C A TL 210

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Sus 170 KVLQHLVLSAEMVGGKTLVPPYRQILPVLN I FKNMN - -VNSGDG I DYSQQKRENI GDLIQETLEAFERYGGEDAFINIKYMPVPTYESCLLN - 257
Rattus 170 KVLQHLVLSAEMVGGKTLVPPYRQILPVLN I FKNMN - -VNSGDG I DYSQQKRENI GDLIQETLEAFERYGGEDAFINIKYMPVPTYESCLLN - 257
Mus 154 KVLQHLVLSAEMVGGKTLVPPYRQILPVLN I FKNMN - -VNSGDG I DYSQQKRENI GDLIQETLEAFERYGGEDAFINIKYMPVPTYESCLLN - 241
Gallus 167 KVLQHLVLSAEMVGGKTLVPPYRQILPVLN I FKNMN - -VNSGDG I DYSQQKRENI GDLIQETLEAFERYGGEDAFINIKYMPVPTYESCLLN - 254
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Chlamydomonas 219 QL LQKLVLSADLVGALVPPYRQILPVLN I FKNMN - -VNSGDG I DYSQQKRENI GDLIQETLEAFERYGGEDAFINIKYMPVPTYESCLLN - 307
Tetrahymena 232 K I LQR L V S G D M I G E A L V P Y R Q I L P V L N I F K N M N - - V N S G D G I D Y G R K K L T L G D I Q E T L E A F E R Y G G E D A F I N I K Y M P V P T Y E S C V H N - 319
Trypanosoma 211 R I L Q Q L I V S G D L I G E A L V P Y R Q I L P M F N L F K S R H K N R A R G D A I D F G R K R D D V G D L V I E T L Q L L E V H G G D A Y I N I K Y M P V P T Y E S C I F S - 300

Supplemental Figure S3. Sequence alignment of PACRG protein orthologs from different species, related to Figure 1. PACRG N-terminal is highly variable across species, whereas the C-terminus is more conserved. Deletion constructs were made by deleting amino acids 1-69 (a.a. Thr70 labeled in red) in human PACRG. Positions labeled with black arrows indicate site where p-iodo-L-phenylalanine was incorporated for phasing. Asterisks indicate residues important for binding MEIG1. Black circles highlight residues that interact with tubulin in the axonemal doublet tubule structure.



Supplemental Figure S4. Purification of *p*-iodo-*L*-phenylalanine mutants of PACRG Δ^{1-69} bound to MEIG1, related to Figure 1. (A) Fractions from the Y189X PACRG Δ^{1-69} mutant inclusion body purification migrated on SDS-PAGE gel and stained with Coomassie Brilliant Blue. The fractions consisted of protein from the cell debris collected after removing the cleared lysate (Cell Debris), after dialysis and addition of MEIG1 (Dialysis+MEIG1), after GST-affinity chromatography (GST-Affinity), following overnight 3C protease cleavage (3C cleavage), and after size-exclusion chromatography (Superdex 75). (B). UV 280 nm intensity for all eluted mutants on size-exclusion chromatography. (C) Extracted ion chromatograms of tryptic peptides from the wild type (WT) and Y189X mutant of PACRG Δ^{1-69} bound to MEIG1. All peptides are identical except the peptide spanning amino acids 185-191, which contains iodo-phenylalanine at position 189. (D) The precursor spectra indicate the mass shift induced by the incorporation of iodo-phenylalanine. (E) Crystals of the MEIG1:PACRG Δ^{1-69} Y189X complex.

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PACRG_short      1 MVAEKETLS LNKCPDKMPKRTKLLAQQLPVHQPHSLVSEGFTVKAMMKNSVVRGPPAAGAFKER 65
PACRG_long       1 MVAEKETLS LNKCPDKMPKRTKLLAQQLPVHQPHSLVSEGFTVKAMMKNSVVRGPPAAGAFKER 65

PACRG_short     66 PTKP TAFRKFYERGDFF I ALEHDSKGNK I AWKVE I EKLDYHHY L P L FFDGLCEMTFPYE F FARQG 130
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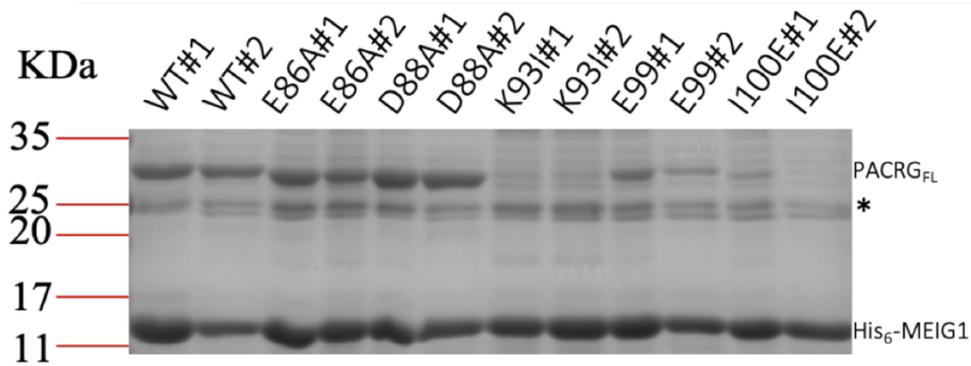
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PACRG_long      131 I HDMLEHGGNK I LPVLPQL I I P I KNALNLRNRQV I CVTLKVLQHLLVSAEMVGKALVPYYRQ I LP 195

PACRG_short     196 VLN I FKNMN - - - - - VNSGDG I DYSQQKRE N I 221
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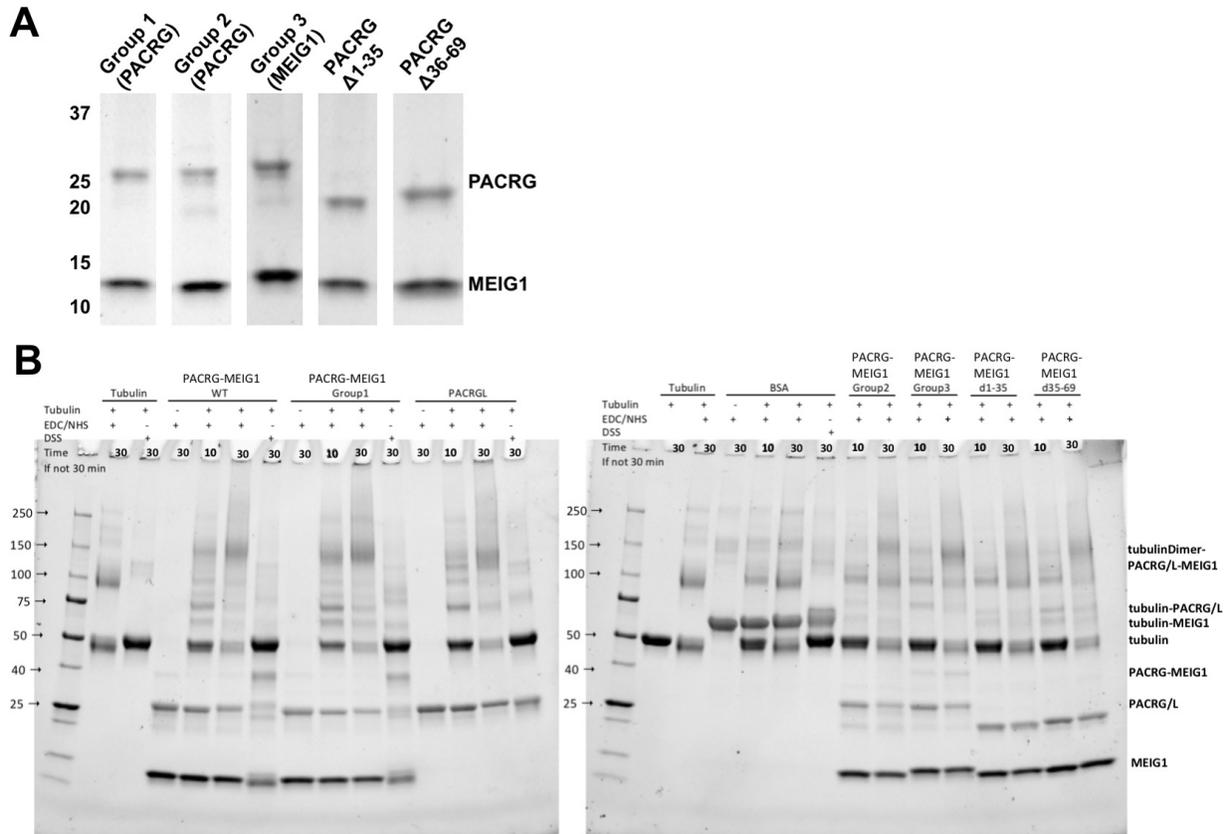
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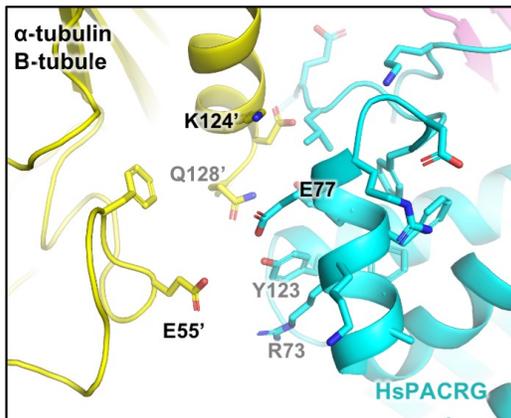
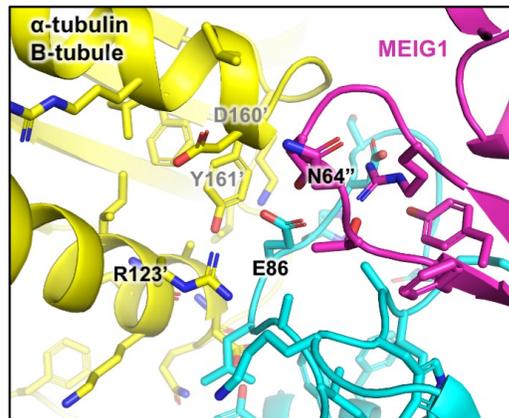
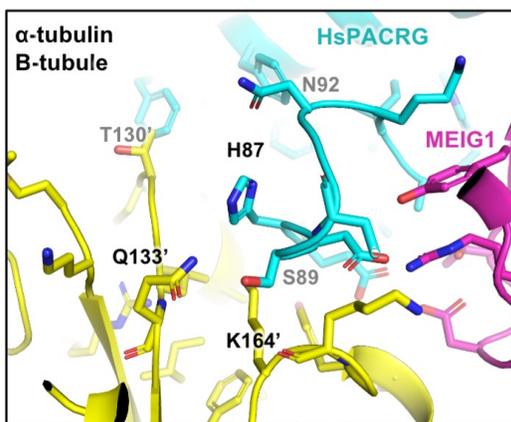
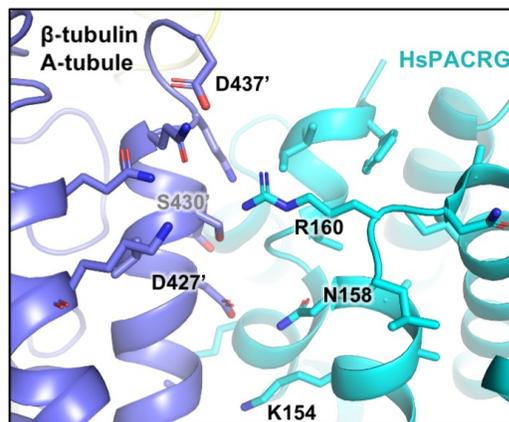
Supplemental Figure S5. Sequence alignment of PACRG isoforms, related to Figure 1. The disordered loop in between $\alpha 6$ and $\alpha 7$ in the crystal structure of the short isoform of PACRG is highlighted with a blue box.



Supplemental Figure S6. Interaction assay for the binding of PACRG mutants to His₆-MEIG1, related to Figure 2. WT or mutants PACRG^{FL} were expressed with His₆-MEIG1 from a single pRSF-DUET plasmid. Following expression in E.coli, the cell lysate was incubated with Co-NTA affinity resin to pull-down on His₆-MEIG1. The eluate was loaded directly on SDS-PAGE and stained with Coomassie (example shown here). Densitometry was performed on both the PACRG^{FL} and His₆-MEIG1 bands to calculate the ratios showed in Figure 2B. Each mutant was tested 4 times independently. The asterisk indicates a contaminant protein co-eluting non-specifically on Co-NTA.



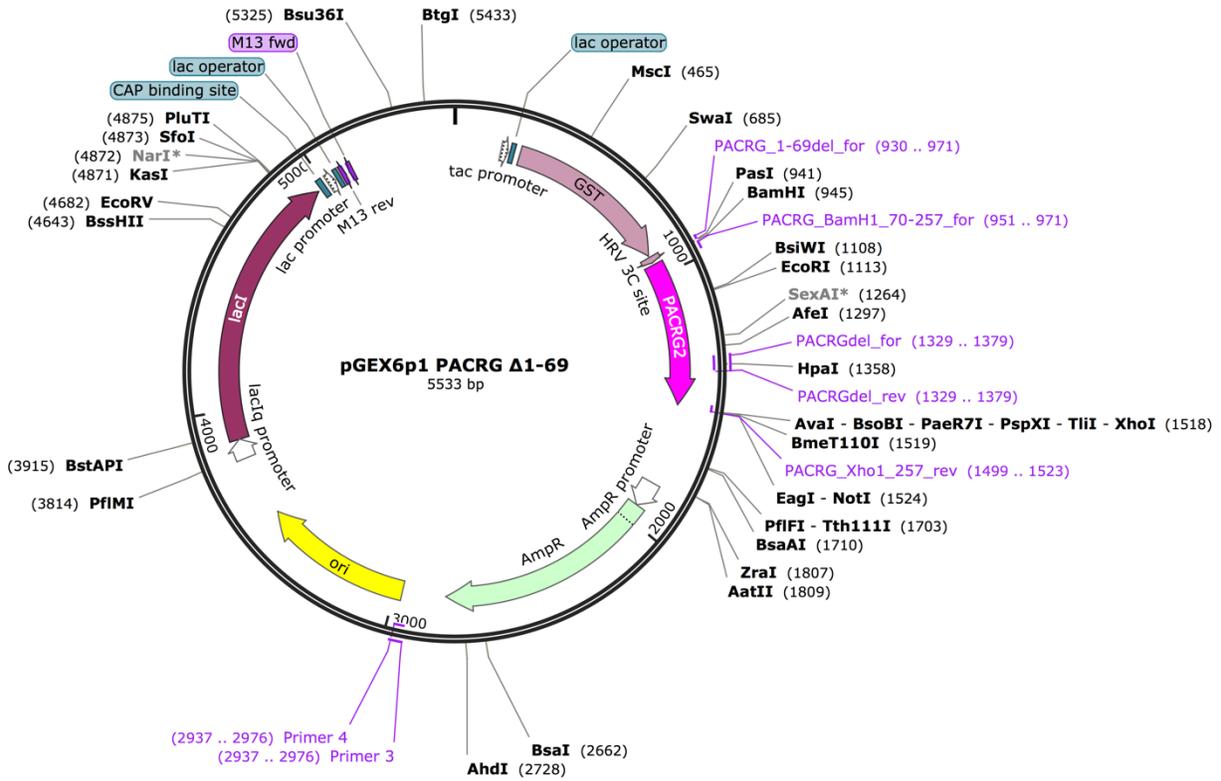
Supplemental Figure S7. Crosslinking studies show that PACRG:MEIG1 and PACRGL bind to tubulin, related to Figure 4 and 5. (A) Purification of PACRG:MEIG1 group mutants used in the tubulin recruitment and crosslinking assays. Proteins were purified by affinity chromatography, 3C cleavage and size-exclusion chromatography. Products were resolved on SDS-PAGE and visualized using Bio-rad stain-free UV 4-20% pre-cast gels. Group 1: PACRG Y76F-E77K-E86K-H87A; group 2: PACRG K154E-R160Q-R191E; group 3: MEIG1 H13A-K78A-H81A-K82A-Y86A. **(B)** SDS-PAGE analysis of crosslinking reactions between tubulin (5 μ M) and different candidate microtubule-associated proteins (12.5 μ M), using either EDC/NHS or DSS as crosslinker (10 and 30 min). Gels were imaged by UV-induced fluorescence (Biorad TGX Stain-Free technology).

A Glu77 (E77K)**B** Glu86 (E86K)**C** His87 (H87A)**D** Arg160 (R160Q)

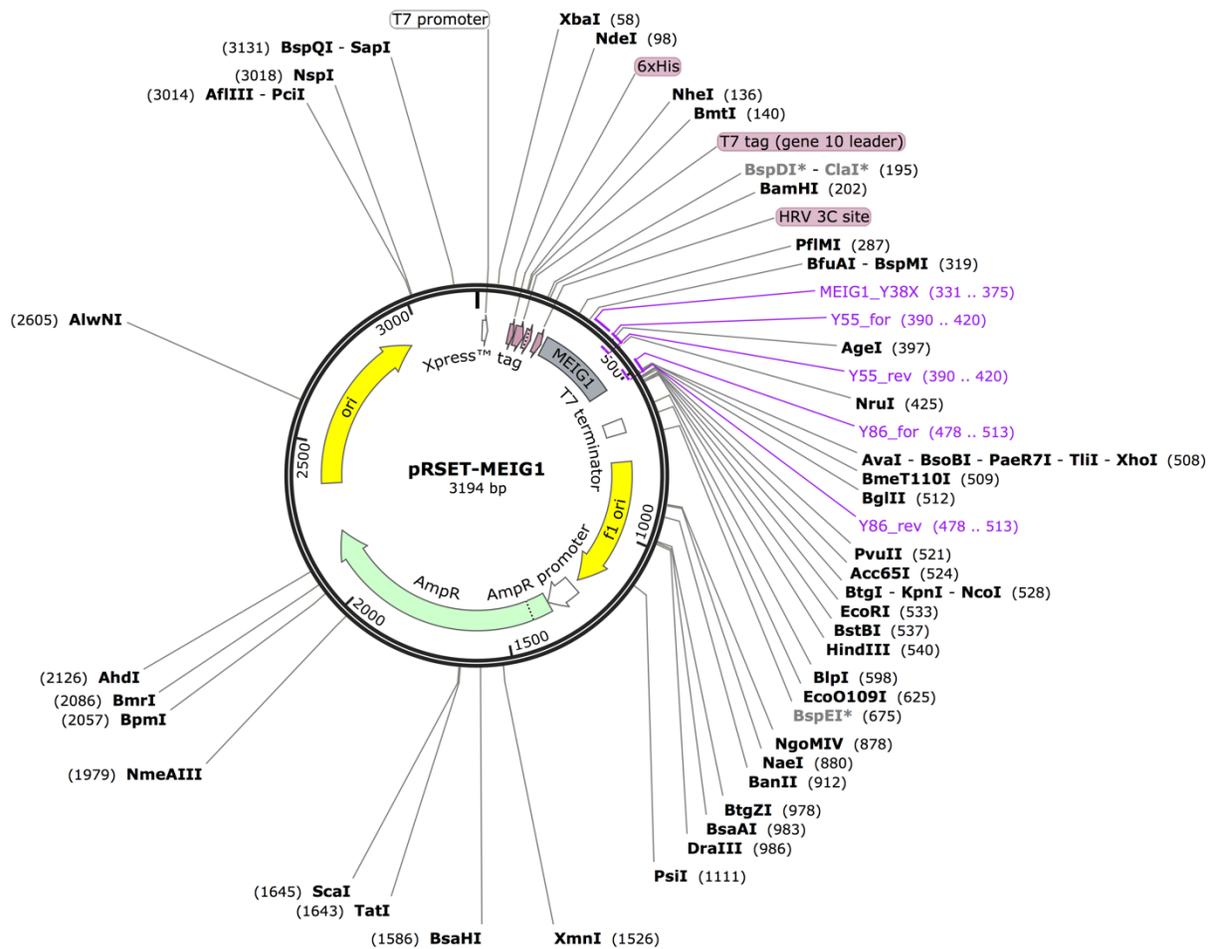
Supplemental Figure S8. Critical sites for the interaction of human PACRG:MEIG1 with human tubulin in homology model of the axonemal doublet microtubule, related to Figure 3, 4, and 5. PACRG residues are labeled with amino acid and number, tubulin residues are labeled additionally with a single prime ('), MEIG1 residues with a double prime (''). **(A)** The mutation E77K enhances tubulin binding in our assays. In the model, Glu77 is adjacent to Glu55' in α -tubulin (B-tubule). Thus the mutation E77K could lead to formation of a salt bridge. **(B)** The rare human variant E86K reduces tubulin binding. In the model, Glu86 is adjacent to Arg123' in α -tubulin (B-tubule). The mutation E86K would break a salt bridge. **(C)** The mutation H87A reduces tubulin binding. The H87A mutation could break hydrogen bonds with Gln133' or Lys164'. **(D)** The rare human variant R160Q dramatically reduces tubulin binding. Arg160 may interact with Asp427' or Asp437' in β -tubulin, and thus the R160Q mutation may break favorable interactions.

Supplemental Table S1. Evolution of PACRG-Parkin head-to-head (H2H) gene structure and conservation of PACRG and MEIG1, related to Figure 7.

Species name (latin)	Common name	PACRG-Parkin H2H?	Space (kb)	%ID PACRG	%ID MEIG1	Comments
<i>Homo sapiens</i>	Human	Yes	0	100.0	100.0	
<i>Pan troglodytes</i>	Chimp	Yes	0	100.0	97.7	
<i>Gorilla gorilla</i>	Gorilla	Yes	0	100.0	96.6	
<i>Sus scrofa</i>	Pig	Yes	240	96.9	94.3	
<i>Rattus norvegicus</i>	Rat	Yes	0	96.1	92.1	
<i>Mus musculus</i>	Mouse	Yes	0	92.1	87.5	
<i>Ursus maritimus</i>	Polar bear	Yes	200	97.3	95.5	
<i>Myotis lucifugus</i>	Little brown bat	Yes	0	90.0	90.9	
<i>Gallus gallus</i>	Chicken	Yes	0	70.4	77.0	
<i>Calypte anna</i>	Hummingbird	Yes	150	85.0	80.7	
<i>Anolis carolinensis</i>	Green anole	Yes	0	92.2	77.0	
<i>Alligator mississippiensis</i>	American alligator	Yes	0	81.4	81.8	
<i>Terrapene mexicana triunguis</i>	Three-toed box turtle	Yes	0	82.9	87.5	
<i>Xenopus laevis</i>	African clawed frog	n.a.	n.a.	90.0	75.0	No parkin/PINK1
<i>Xenopus tropicalis</i>	Tropical clawed frog	n.a.	n.a.	91.3	75.0	No parkin/PINK1
<i>Danio rerio</i>	Zebrafish	No	n.a.	89.6	71.6	
<i>Callorhynchus milii</i>	Elephant shark	Yes	0	76.8	79.5	
<i>Salmo salar</i>	Atlantic salmon	No	n.a.	88.7	75.3	
<i>Aplysia californica</i>	Sea slug	No	n.a.	82.3	61.0	
<i>Drosophila melanogaster</i>	Fruit fly	No	n.a.	59.9	none	No MEIG1
<i>Caenorhabditis elegans</i>	Nematode worm	No	n.a.	33.5	none	No MEIG1
<i>Chlamydomonas reinhardtii</i>	Green algae	n.a.	n.a.	63.7	none	No Parkin/PINK1, MEIG1
<i>Tetrahymena thermophila</i>	Ciliate	n.a.	n.a.	60.5	none	No Parkin/PINK1, MEIG1
<i>Paramecium tetraurelia</i>	Ciliate	n.a.	n.a.	58.7	none	No Parkin/PINK1, MEIG1
<i>Trypanosoma brucei</i>	Kinetoplastids	n.a.	n.a.	57.4	none	No Parkin/PINK1, MEIG1
<i>Oxyrrhis marina</i>	Dinoflagellate	n.a.	n.a.	None	38.9	No Parkin/PINK1, PACRG

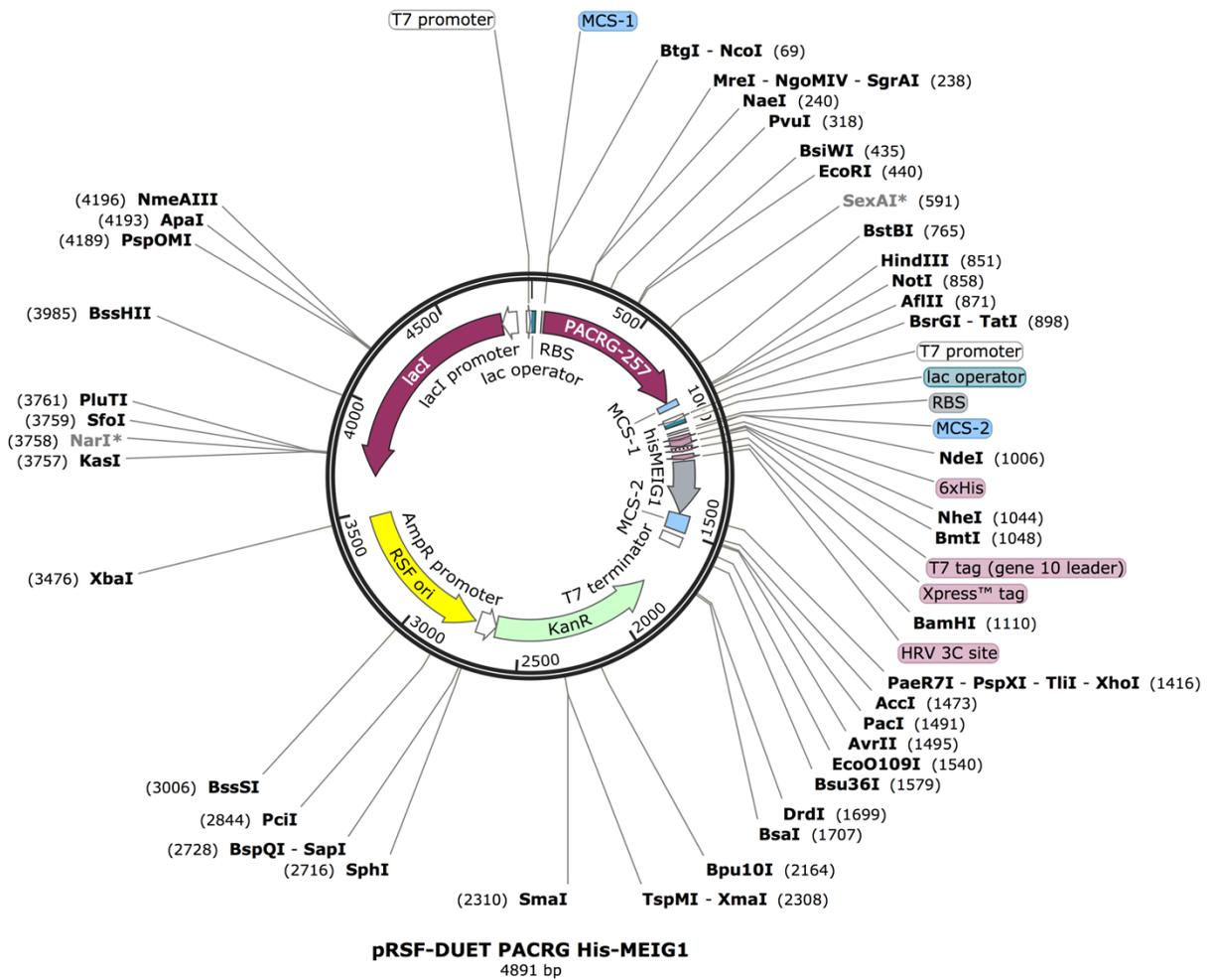


Supplemental Data S2. Vector map of pGEX6p1-PACRG ^{Δ 1-69}, related to the STAR Methods.

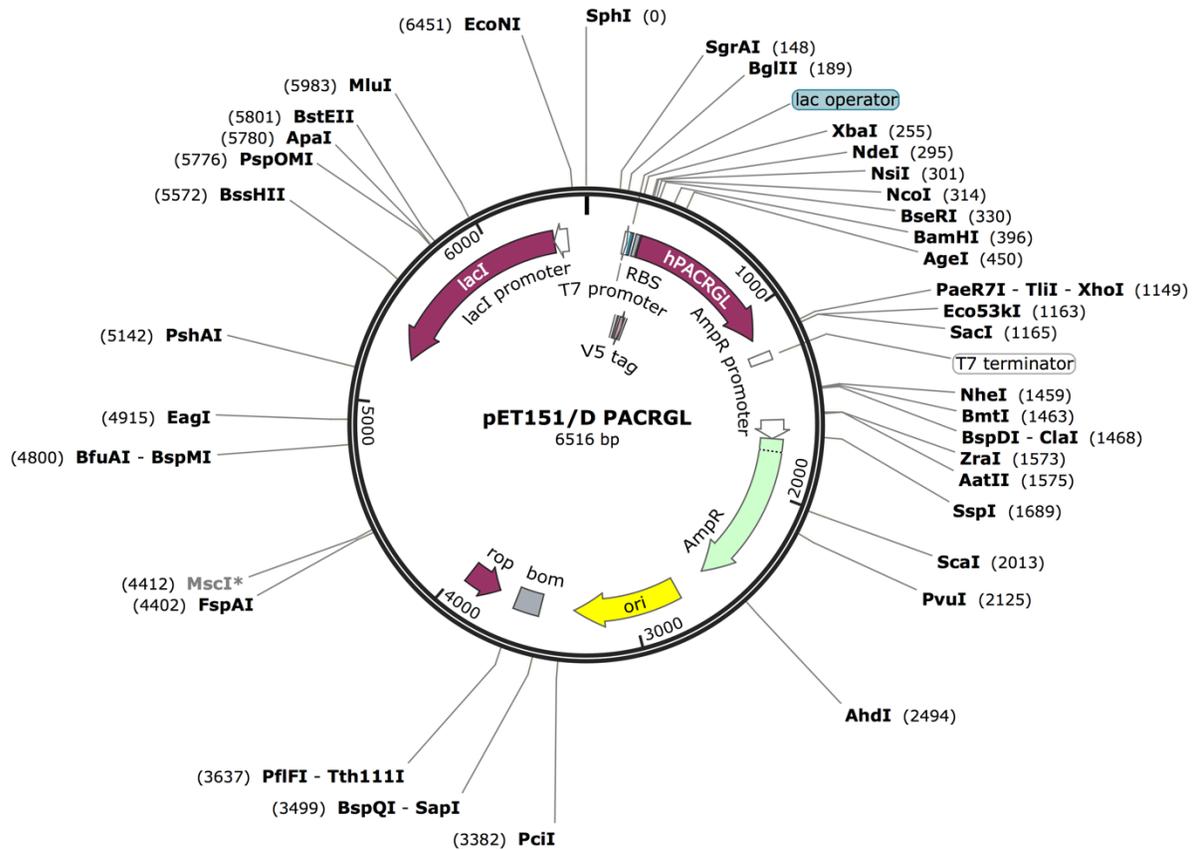


CATATGCGGGGTTCT**CATCATCATCATCATCAT**GGTATGGCTAGCATGACTGGTGGACAGC
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 TGTTTCAGGGTCCGCTGGGTAGCAT**GTGCAAGCAGTGATGTTAAACCGAAAAGCGTTAGCC**
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GTGCGACGATAAAGAAGTGCATAAAGTAAAATCTATGCCTATTA**ACTCGAG**

Supplemental Data S3. Vector map of pRSET-His₆-MEIG1, related to the STAR Methods. The sequence of the open-reading frame comprising the His₆-tag (red), Nde1 and Xho1 sites (underlined) and codon-optimized sequence of MEIG1 (blue) is shown below. The initiator (Met) and stop codons are in bold.



Supplemental Data S4. Vector map of pRSF-DUET PACRG^{FL}-His₆MEIG1, related to the STAR Methods.



CATATGCATCATCACCATCACCATGGTAAGCCTATCCCTAACCCCTCTCCTCGGTCTCGATT
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GGTGGTAGCGGTAGCCTGTCAATTATCAAAGCAAATTCCGACCTATTGCAGCATCTGCT
GTAACTCGAG

Supplemental Data S5. Vector map of pET-151/D His₆-PACRGL, related to the STAR Methods. The sequence of the open-reading frame comprising the His₆-tag (red), Nde1 and Xho1 sites (underlined) and codon-optimized sequence of human PACRGL (blue) is shown below. The initiator (Met) and stop codons are in bold.