Supplementary Tables

Table S1. Strains used in this study.

Strain	Genotype	Source	Figure
YL515	[BY4741/2] *MATα; his3Δ1, leu2Δ0, ura3Δ0*	[1]	1A-F
MB27	[YL515] *gtr1Δ::HIS3*	[1]	1A-F, 2C
MB28	[YL515] *gtr2Δ::HIS3*	[1]	1A-F, 2C
NP52-2A	[YL515] *ego2Δ::kanMX*	This study	1A-F
NP44	[YL515] *ego4Δ::kanMX*	This study	1A-F
NP51-3C	[YL515] *ego2Δ::kanMX, gtr1Δ::kanMX*	This study	1A, C- D, F, 2C
NP54-4D	[YL515] *ego2Δ::kanMX, gtr2Δ::kanMX*	This study	1A, C- D, F, 2C
NP48-5C	[YL515] *ego4Δ::kanMX, gtr1Δ::kanMX*	This study	1A, C- D, F, 2C
NP60-10D	[YL515] *ego4Δ::kanMX, gtr2Δ::kanMX*	This study	1A, C- D, F, 2C
KT1961	*MAT***a***; trp1, leu2, his3, ura3-52*	[2]	5C-D
CDV213	[KT1961] *EGO1-GFP::TRP1*	[3]	2A
CDV221-1A	[KT1961] *ego3Δ::natMX, EGO1-GFP::TRP1*	This study	2A
KP12-1C	[KT1961] *ego2Δ::kanMX, EGO1-GFP::TRP1*	This study	2A, 3B
KP37-4B	[KT1961] *ego2Δ::kanMX, ego3Δ::natMX EGO1-GFP::TRP1*	This study	2A
KP22-2C	[KT1961] *ego4Δ::kanMX, EGO1-GFP::TRP1*	This study	2A, 3B
CDV214	[KT1961] *EGO3-GFP::TRP1*	[3]	2A
CDV219-4A	[KT1961] *ego1Δ::natMX, EGO3-GFP::TRP1*	This study	2A, 5B
KP13-2D	[KT1961] *ego2Δ::kanMX, EGO3-GFP::TRP1*	This study	2A, 3B
KP39-6D	[KT1961] *ego2Δ::kanMX, ego1Δ::natMX, EGO3-GFP::TRP1*	This study	2A
KP35-2D	[KT1961] *ego4Δ::kanMX, EGO3-GFP::TRP1*	This study	2A, 3B
CDV210	[KT1960] *ego1Δ::natMX*	[3]	2B, 5C-D
KP27-5A	[KT1961] *ego1Δ::natMX, ego2Δ::kanMX*	This study	2B, 3F, 5B
KP33-3C	[KT1961] *ego1Δ::natMX, ego4Δ::kanMX*	This study	2B
MP268-2B	[KT1961] *gtr1Δ::natMX, gtr2Δ::natMX*	This study	2D-E, 5C-D, 6A-C
KP29-3B	[KT1961] *gtr1Δ::natMX, gtr1Δ::natMX, ego2Δ::kanMX*	This study	2D-E, 3F
KP34-1A	[KT1961] *gtr1Δ::natMX, gtr1Δ::natMX, ego4Δ::kanMX*	This study	2D-E
NMY51	*MAT***a***; his3Δ200, trp1-901, leu2-3,112, ade2, LYS2::(lexAop)4-HIS3, ura3::(lexAop)8- lacZ, ade2::(lexAop)8-ADE2, GAL4*	Dualsystems	3A, 5A
FLJ1	[NMY51] *ego3Δ::kanMX*	[4]	3G
KP12-2D	[KT1961] *ego2Δ::kanMX*	This study	3E-F
KP02	[KT1961] *ego4Δ::kanMX*	This study	3E-F
KP28-3D	[KT1961] *ego2Δ::kanMX, ego3Δ::natMX*	This study	3F
MP279-18B	[KT1960] *gtr1Δ::natMX, ego1Δ::kanMX*	This study	5B
MP279-18A	[KT1961] *gtr2Δ::natMX, ego1Δ::kanMX*	This study	5B
KP41-9C	[KT1961] *gtr1Δ::natMX, gtr2Δ::natMX, ego1Δ::kanMX, ego2Δ::kanMX, ego3Δ::kanMX* (*egocΔ*)	This study	6A-C
KP41-10C	[KT1961] *gtr1Δ::natMX, ego1Δ::kanMX, ego2Δ::kanMX, ego3Δ::kanMX*	This study	6D-E

Table S2. Plasmids used in this study.

Plasmid	Genotype	Source	Figure
pRS413	*CEN, HIS3*	[5]	1A, D-F, 2A-E, 5B-D
pRS414	*CEN, TRP1*	[5]	3E-F, 5B-D
pRS415	*CEN, LEU2*	[5]	1A-C, 2A-B, D-E, 3B, E-F, 5B-D
pRS416	*CEN, URA3*	[5]	1A-F, 2A, C, 3B, E-F, 5B-D
pJU1064	[pRS413] $SCH9^{T570A}$-HA_5	[6]	1B-C, 5D
pJU1058	[pRS415] $SCH9^{T570A}$-HA_5	[6]	1D-E
YCplac111	*CEN, LEU2*	[7]	
pNP2529	[YCplac111] *ADH1p-EGO2*	This study	1C
pNP2530	[YCplac111] *ADH1p-EGO4*	This study	1C
YCplac33	*CEN, URA3*	[7]	
pMB1394	[YCplac33] Tet_{ON}-$GTR1^{Q65L}$	[1]	1D-E
pMB1395	[YCplac33] Tet_{ON}-$GTR1^{S20L}$	[1]	1D-E
pPM1619	[YCplac33] Tet_{ON}-$GTR2^{Q66L}$	This study	1D-E
pPM1620	[YCplac33] Tet_{ON}-$GTR2^{S23L}$	This study	1D-E
pJU650	[pRS416] *GTR1*	[4]	1F, 2D-E
pJU653	[pRS416] $GTR1^{Q65L}$	[1]	1F
pJU652	[pRS416] $GTR1^{S20L}$	[1]	1F
pJU651	[pRS416] *GTR2*	[1]	1F
pJU655	[pRS416] $GTR2^{Q66L}$	[1]	1F
pJU654	[pRS416] $GTR2^{S23L}$	[1]	1F
pFLJ1973	[YCplac33] *EGO1-GST*	This study	2B
pNP2572	[pRS416] *ADH1p-EGO1-TAP*	This study	2B
pMP1639	[pRS415] *GTR1-GFP*	[1]	2C, 5B
pMP1642	[pRS415] *GTR2-GFP*	[1]	2C, 5B
pMP1640	[pRS415] $GTR1^{Q65L}$-*GFP*	This study	2C
pMP1644	[pRS415] $GTR2^{S23L}$-*GFP*	This study	2C
pNP2441	[pRS413] *EGO2-GFP*	This study	3E-F, 5B
pNP2442	[pRS413] *EGO4-GFP*	This study	3E-F, 5B
pMB1344	[YCplac33] *GTR1-TAP*	[1]	2D-E
pMB1371	[YCplac33] $GTR1^{S20L}$-*TAP*	[1]	2E
pMB1372	[YCplac33] $GTR1^{Q65L}$-*TAP*	[1]	2E
pMP2177	[pRS414] *GTR2-V5-6HIS*	[4]	2D-E
pAI-Alg5	*2μ, ADH1-HA-NUB1, TRP1*	Dualsystems	3A, G, 5A
pDL2-Alg5	*2μ, ADH1-HA-NUBG, TRP1*	Dualsystems	3A, G, 5A
pCabWT	*CEN, CYC1-CUB-LexA, LEU2*	Dualsystems	3A, G, 5A
pFLJ2393	[pCabWT] *CYC1-EGO2-CUB-LexA*	This study	3A, G, 5A
pFLJ2394	[pCabWT] *CYC1-EGO4-CUB-LexA*	This study	3A, G
pPR3-N	*2μ, CYC1-NUBG-HA, TRP1*	Dualsystems	3A, G
pNP1689	[pPR3-N] *CYC1-NUBG-HA-GTR1*	[1]	3A, G
pNP1690	[pPR3-N] *CYC1-NUBG-HA-*$GTR1^{S20L}$	[1]	3A, G
pNP1691	[pPR3-N] *CYC1-NUBG-HA-*$GTR1^{Q65L}$	[1]	3A, G
pNP1692	[pPR3-N] *CYC1-NUBG-HA-GTR2*	This study	3A, G
pNP1693	[pPR3-N] *CYC1-NUBG-HA-*$GTR2^{S23L}$	This study	3A, G
pNP1694	[pPR3-N] *CYC1-NUBG-HA-*$GTR2^{Q66L}$	[4]	3A, G
pNP1696	[pPR3-N] *CYC1-NUBG-HA-EGO1*	[1]	3A, G, 5A

Table S2. Plasmids used in this study - continued

Plasmid	Genotype	Source	Figure
pMPG2221	[pPR3-N] *CYC1-NUBG-HA-EGO3*	[4]	3A, G, 5A
pKP2623	[pRS413] *EGO2-HA$_3$*	This study	3B
pKP2622	[pRS413] *EGO4-HA$_3$*	This study	3B
pET-15b	*PT7lac, HIS6, lacI, ApR*	Novagen	
pNP2564	[pET-15b] *HIS6-EGO1, EGO2, EGO3, EGO4*	This study	3C-D
pFLJ2220	[pCabWT] *CYC1-EGO3-CUB-LexA*	[4]	5A
pFLJ2734	[pPR3-N] *CYC1-NUBG-HA-EGO1$^{\Delta 152-184}$*	This study	5A
pFLJ2735	[pPR3-N] *CYC1-NUBG-HA-EGO1$^{\Delta 169-184}$*	This study	5A
pKP2736	[YCplac33] *EGO1-HA$_3$*	This study	5B-C
pKP2737	[YCplac33] *EGO1$^{\Delta 152-184}$-HA$_3$*	This study	5B-C
pKP2738	[YCplac33] *EGO1$^{\Delta 169-184}$-HA$_3$*	This study	5B-C
pKP2739	[YCplac33] *EGO1*	This study	5C-D
pKP2740	[YCplac33] *EGO1$^{\Delta 152-184}$*	This study	5C-D
pKP2741	[YCplac33] *EGO1$^{\Delta 169-184}$*	This study	5C-D
pKP2801	[YCplac33] *EGO1^{NT}-vhhGFP4*	This study	6A-D
pNSlm-vhhGFP4	*pcDNA3-NSlmb-vhhGFP4*	[8]	
pRH2776	[pPRS413] *VAC8-vhhGFP4*	This study	6E

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Supplementary Figures

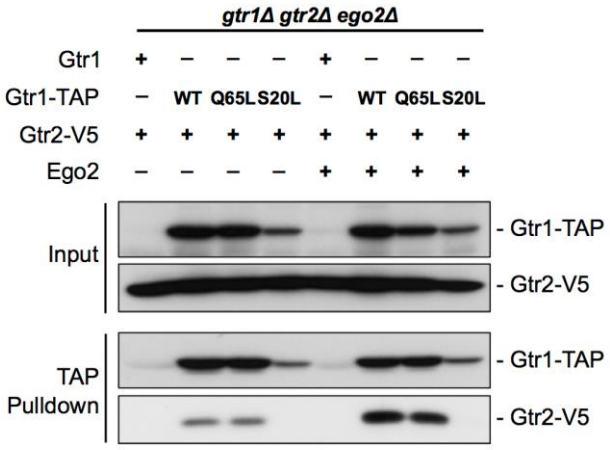

Figure S1. TAP pull-down experiments showing that the reduced interaction between Gtr1-TAP and Gtr2-V5 in an *ego2*Δ strain can be rescued by re-introduction of *EGO2* expressed from a plasmid. Lysates from cells expressing the indicated fusion proteins (input) and TAP pull-down fractions were analyzed by immunoblotting with anti-TAP and anti-V5 antibodies. Cells expressing untagged Gtr1 were used as a control.

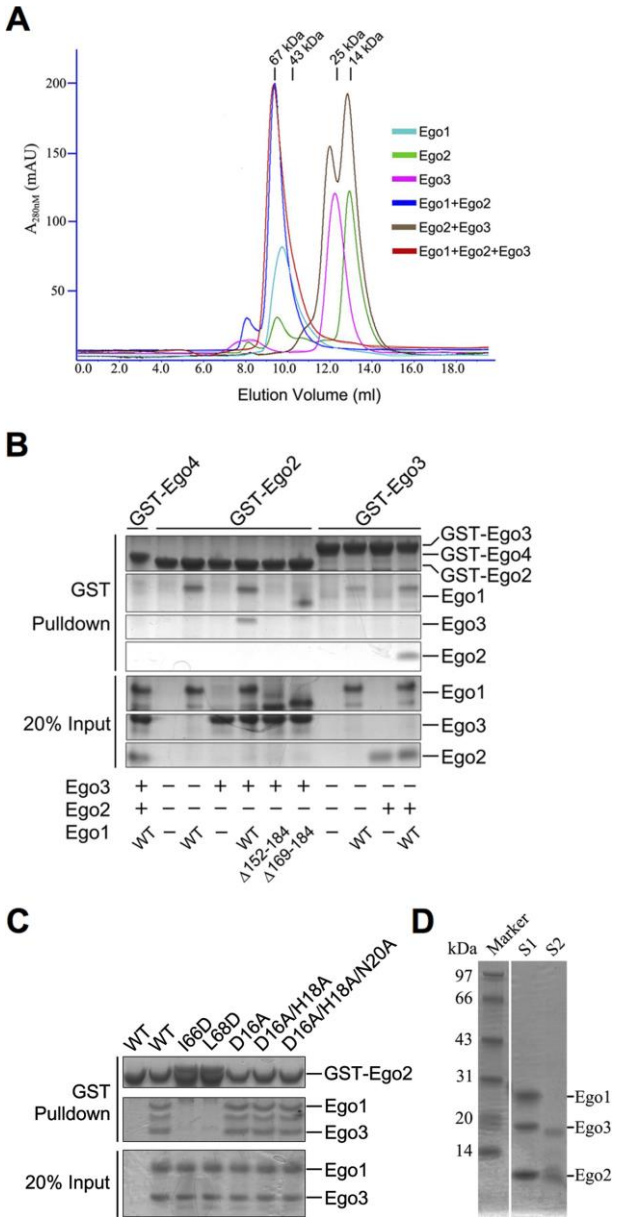

Figure S2. *In vitro* functional analyses of the Ego1-Ego2-Ego3 ternary complex (EGO-TC). **(A)** Analysis of the oligomeric state of the Ego proteins in solution by size-exclusion chromatography. The Ego1-Ego2 complex was purified in a similar way as the Ego1-Ego2-Ego3 complex as described in the experimental procedures. The Ego1, Ego2, and Ego3 proteins were fused with a C-terminal His_6-tag. Absorbance at 280 nm is plotted against the elution volume. Positions of the molecular weight standards are indicated. **(B)** *In vitro* GST-pull down binding assays of GST-fused Ego2, Ego3, or Ego4 with full-length or truncated Ego1 and/or Ego2 and Ego3. The results were visualized by Coomassie blue staining on SDS-PAGE. **(C)** *In vitro* GST-pull down binding assays of GST-fused wild-type or mutant Ego2 with Ego1 and Ego3. The results were visualized by Coomassie blue staining on SDS-PAGE. **(D)** SDS-PAGE of the purified EGO-TC used in the crystallization (S1) and the protein sample obtained from the crystallization solution (S2). The N-terminal region of Ego1 was degraded in the crystallization solution. The loaded proteins were visualized by Coomassie blue staining. Molecular weight markers are indicated on the left.

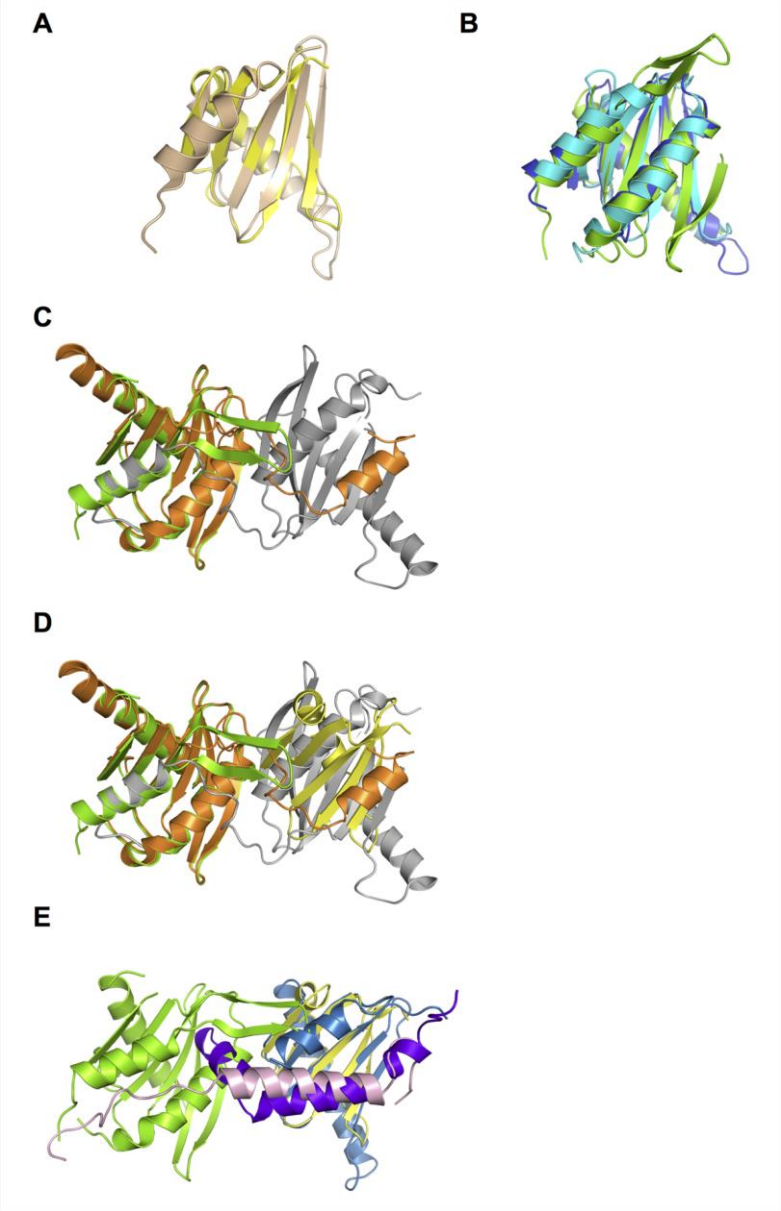

Figure S3. Structural comparisons of the Ego proteins and several representative Roadblock domain-containing proteins. **(A)** Superposition of Ego2 (colored in yellow) and LAMTOR5 (colored in wheat). **(B)** Superposition of the Ego3 monomer in the EGO-TC (colored in green), LAMTOR2 (PDB code 3CPT, colored in blue) and LAMTOR3 (PDB code 3CPT, colored in cyan). **(C)** Superposition of the Ego3 monomer in the EGO-TC (colored in green) and the Ego3 homodimer (PDB code 4FTX, one monomer colored in orange and the other in gray). **(D)** Superposition of Ego2 (colored in yellow) and Ego3 (colored in green) in the EGO-TC with the Ego3 homodimer showing that the position for Ego2 binding was occupied by the other monomer in the Ego3 homodimer. **(E)** Superposition of the EGO-TC with *Drosophila melanogaster* dynein intermediate chain and light chain complex (PDB code 3L9K). Ego1, Ego2 and Ego3 of the EGO-TC are colored in pink, yellow and green, respectively, and the dynein intermediate chain and light chain are colored in purple and skyblue, respectively.