

Supplementary Materials and Methods

Plasmids

RE(AB)pEGFP (Schmitz et al., 2000)

RE(AB)pGBKT7, was amplified via PCR with forward primer (277): TTTTTCATATGCCGGTTCACAGCAGG and reverse primer (264): TTTCTCGAGCTATTGCTCGTTGGGGTGCTC using rRIBEYE as template (Schmitz et al., 2000). PCR product was cloned after restriction digest with NdeI/XhoI into the NdeI/SalI sites of pGBKT7.

RIBEYE(A) plasmids

RE(A)pEGFP (Schmitz et al., 2000)

If not denoted otherwise, the following plasmids were amplified via PCR with the indicated primers and rRE(A)pEGFP (845) as template and subsequently cloned into the EcoRI and XhoI sites of pACT2.

RE(A)1-41pACT2: forward primer (388):

TTTGAATTCTTATGCCGGTTCACAGCAG; reverse primer (393): TTTCTCGAGGCCATAAGTCAGAGAACTT.

RE(A)1-105pACT2: forward primer (388): see above; reverse primer (399):

TTTCTCGAGGTCGCCATAGTAGTCTTGT.

RE(A)1-105pGEX, was generated by excising RE(A)1-105 from RE(A)1-105pACT2 with EcoRI and XhoI and subsequently cloned into the EcoRI and XhoI sites of pGEX-KG.

RE(A)1-105pMal-C2, was generated by excising RE(A)1-105 from RE(A)1-105pGEX-KG with BamHI and XhoI and subsequently cloned into the BamHI and SalI sites of pMal-C2.

RE(A)1-162pACT2 forward primer (388): see above; reverse primer (398):

TTTCTCGAGACTCCACAGATTCCCGGTA

RE(A)1-216pACT2 forward primer (388): see above; reverse primer (397):

TTTCTCGAGTAGAGTCAACGGGTCTTTC.

RE(A)1-291pACT2, forward primer (388): see above; reverse primer (396):

TTTCTCGAGCTCAGCAGGGCCAAAAC.

RE(A)1-368pACT2, forward primer (388): see above; reverse primer (391):

TTTCTCGAGGTGGCTGAAGCTACT.

RE(A)1-424 pACT2, forward primer (388): see above; reverse primer (395):

TTTCTCGAGGCAGAGTAAGTAGCTGCT.

RE(A)1-496 pACT2, forward primer (388): see above; reverse primer (394):

TTTCTCGAGGTTCCTCTTAAGCAGTTC.

RE(A)1-563 pACT2, forward primer (388): see above; reverse primer (356):

TTTCTCGAGACTTGTTCTGGTGTTAGCA.

RE(A)438-563pACT2, forward primer (401):

TTTGAATTCTTTCTGGATATAGCTCTCCTA; reverse primer (390):

TTTCTCGAGACTTGTTCTGGTGTTAGC.

RE(A)438-563 pGEX, was generated by excising RE(A)438-536 from RE(A)438-536 pACT2 with EcoRI and XhoI and subsequently cloned into the EcoRI and XhoI sites of pGEX-KG.

RE(A)106-363pACT2, forward primer (879):

TTTGAATTCTTCCATCCAGGGGCACCA; reverse primer (881):

TTTCTCGAGTGGACCGATGGCGGGGT.

RE(A)106-363pGEX, was generated by excising RE(A)106-363 from RE(A)106-363pACT2 with EcoRI and XhoI and subsequently cloned into the EcoRI and XhoI sites of pGEX-KG.

RE(A)106-363 pMal-C2, was generated by excising RE(A)106-363 from RE(A)106-363pGEX-KG with BamHI and XhoI and subsequently cloned into the BamHI and SalI sites of pMal-C2.

RE(A)1-563pGBKT7, forward primer (98):

TTAGAATTCATGCCGGTTCCCAGCAGG; reverse primer (119):

TTCCTCGAGACTTGGTTCTGGTGTTAGCAT. The PCR product was cloned into the EcoRI/SalI sites of pGBKT7.

RE(A)106-363pGBKT7, was generated by excising the respective insert from RE(A)106-363pACT2 with BamHI and XhoI and by the subsequent cloning of the insert into the BamHI/SalI sites of pGBKT7.

RE(A)364-563pACT2/pGBKT7, forward primer (418):

TTTGGATCCTTAGTAGCTTCAGCCACCGA; reverse primer (390):

TTTCTCGAGACTTGGTTCTGGTGTTAGC. PCR product was cloned into the BamHI/XhoI sites of pACT2 and BamHI/SalI sites of pGBKT7.

RE(A)1-105pGBKT7, was generated by excising RE(A)1-105 from RE(A)1-105pACT2 with BamHI and XhoI and subsequently cloned into the BamHI and SalI sites of pGBKT7.

RE(A)pmRFP, forward primer (839):

TTTTCTCGAGTGCCACCATGCCGGTTCCCAGCAG; reverse primer (836):

TTTTGGATCCCTACTTGGTTCTGGTGTTAGC; using rRIBEYE(A)EGFP as template (Schmitz et al., 2000). The PCR product was cloned into the XhoI and BamHI sites of pmRFP.

rRE(A)pPIC3.5K, forward primer (734): TTTGAATTCATGCCGGTTCCCAGCAG; reverse primer (735): TTTTGCGGCCGCTTAACCTGGTTCTGGTGTTAG using rRE(A)pEGFP as template. The PCR product was cloned into the EcoRI/NotI site of pPIC3.5K (Invitrogen).

RE(A)1-563pMal-C2, forward (840): TTTTGAATTCATGCCGGTTCCCAGCAG; reverse (841): TTTTTGTGCGACACTTGGTTCTGGTGTTAGC using rRE(A)pEGFP as template. The PCR product was cloned into the EcoRI/SalI of pMal-C2 (NEB).

RE(A)1-563pGEX, was generated by excising the respective insert from RE(A)1-563pACT2 with EcoRI/XhoI and subsequent cloning of the insert into the EcoRI/XhoI sites of pGEX-KG.

RE(A)343-536pMal-C2, forward (384): TTTGAATTCTGCAGGACAGAGATGCAGTT; reverse (385): TTTTCTAGATGTTGGGAGACTTGCCTG using rRE(A)-EGFP as template. The PCR product was cloned into the EcoRI and XbaI sites of pMal-C2. The respective fusion protein was used for raising antibodies against the A-domain of rat RIBEYE.

RIBEYE(B) plasmids

RE(B)pGEX (Schmitz et al., 2000).

RE(B)pEGFP (Schmitz et al., 2000).

RE(B)pMal-C2, insert was generated via PCR with forward primer (96):

TTCACAATTGATCCGCCCCCAGATCATG; reverse primer (97):

TTCACTAGTCTATTGCTCGTTGGGGTGC using rRE(B)pGEX as template, digested with *MunI*/*SpeI* and subsequently cloned into the *EcoRI*/*XbaI* sites of pMal-C2.

If not denoted otherwise, the following plasmids were amplified via PCR with the indicated primers and RE(B)pGEX as template and subsequently cloned into the *NcoI* and *XhoI* sites of pACT2 or the *NcoI* and *Sall* sites of pGBKT7, respectively.

RE(B)pGBKT7, forward primer (406):

TTTTCCATGGTTATCCGCCCCCAGATCATGA; reverse primer (405):

TTTTCTCGAGCTATTGCTCGTTGGGGTGCT

RE(B)pACT2, forward primer (406), see above; reverse primer (405), see above.

RE(B)NBDpGEX, forward primer (508):

TTTTGAATTCTTATCCCATCTGCTGCACT; reverse primer (509):

TTTTCTCGAGGCTGTACCAGGCTGTGT. The PCR product was cloned into the *EcoRI*/*XhoI* sites of pGEX-KG.

RE(B)NBDpACT2, was excised from RE(B)NBDpGEX with *EcoRI* and *XhoI* and cloned into the respective sites of pACT2.

RE(B)SBDpACT2, was generated by overlapping PCR using the following primers:

outward forward primer (353): GTTCCATGGAGATCCGCCCCCAGATCAT; forward primer (523): AACATCCCATCTGCTGCAGGAGGATCTTACAGCGAACAAGCATCA; reverse primer (522):

TGATGCTTGTTTCGCTGTTAGATCCTCCTGCAGCAGATGGGATGTT; outward reverse primer (354): GTTCTCGAGCTATTGCTCGTTGGGGT.

RE(B)564-938pGBKT7, forward primer (406):

TTTTCCATGGTTATCCGCCCCCAGATCATGA; reverse primer (404):

TTTTCTCGAGCCACACCTACAATGCCT.

RE(B)E844QpGBKT7, outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT; mutated forward primer (868):

CATGAGTCTCAGCCCTTCAGCTTT; mutated reverse primer (869):

AAAGCTGAAGGGCTGAGACTCATG; outward reverse primer (354):

GTTCTCGAGCTATTGCTCGTTGGGGT using rRE(B)pSK as template.

RE(B)E844QpGEX, was cloned by inserting the amplified point-mutated PCR product (see above) into the *NcoI*/*XhoI* sites of pGEX-KG.

RE(B)F848WpGEX, outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT; mutated forward primer (870):

CCCTTCAGCTGGGCTCAGGGCCCA; mutated reverse primer (871):

TGGGCCCTGAGCCCAGCTGAAGGG; outward reverse primer (354):

GTTCTCGAGCTATTGCTCGTTGGGGT using rRE(B)pSK as template and cloned into the *NcoI*/*XhoI* sites of pGEX-KG.

RE(B)F848WpGBKT7, was cloned by inserting the amplified mutated PCR product (see above) into the *NcoI*/*Sall* site of pGBKT7.

RE(B)K854QpGEX, outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT, mutated forward primer (872):

GGCCCATTCAGGATGCTCCAAAT; mutated reverse primer (873):

ATTTGGAGCATCCTGCAATGGGCC; outward reverse primer (354):

GTTCTCGAGCTATTGCTCGTTGGGGT using rRE(B)pSK as template and cloned into the NcoI/XhoI sites of pGEX-KG.

RE(B)K854QpGBKT7 was cloned by inserting the amplified mutated PCR product (see above) into the NcoI/SalI site of pGBKT7.

RE(B)D758NpGEX, outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT, mutated forward primer (866):

TACTTACAGAACGGGATAGAGCGG; mutated reverse primer (867):

CCGCTCTATCCCGTTCTGTAAAGTA; outward reverse primer (354):

GTTCTCGAGCTATTGCTCGTTGGGGT using rRE(B)pSK as template and cloned into the NcoI/XhoI sites of pGEX-KG.

RE(B)D758NpGBKT7 was cloned by inserting the amplified mutated PCR product (see above) into the NcoI/SalI site of pGBKT7.

RE(B)I796ApGEX, outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT; mutated forward primer (876):

CACCACCTCGCCAATGACTTCACC; mutated reverse primer (877):

GGTGAAGTCATTGGCGAGGTGGTG; outward reverse primer (354):

GTTCTCGAGCTATTGCTCGTTGGGGT using RE(B)pSK as template and cloned into the NcoI/XhoI sites of pGEX-KG.

RE(B)I796ApGBKT7 was cloned by inserting the amplified mutated PCR product (see above) into the NcoI/SalI site of pGBKT7.

RE(B)C683SpGEX: outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT; mutated forward primer (816):

CGACAGTCTCCCATATCCTC;

mutated reverse primer (817): GAGGATATGGGAGACTGTCTG; outward reverse primer (354): GTTCTCGAGCTATTGCTCGTTGGGGT using rRE(B)pSK as template and cloned into the NcoI/XhoI sites of pGEX-KG.

RE(B)C683SpGBKT7 was cloned by inserting the amplified mutated PCR product (see above) into the NcoI/SalI site of pGBKT7.

RE(B)G730ApGEX, outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT; mutant forward primer (158):

CTCATTGCCTTTGGTTCGCACTGGG; mutant reverse primer (159):

ACCAAAGGCAATGAGGCCAGTGTT; outward reverse primer (354):

GTTCTCGAGCTATTGCTCGTTGGGGT using RE(B)pGEX as template and cloned into the NcoI/XhoI sites of pGEX-KG.

RE(B)G730ApGBKT7 was cloned by excision of the insert from RE(B)G730ApGEX with NcoI/XhoI and cloning into the NcoI/SalI site of pGBKT7.

RE(B)F904WpGEX, outward forward primer (353):

GTTCCATGGAGATCCGCCCCCAGATCAT; mutated forward primer (483):

CAACAAAGAATGGTTTGTACTTCAAC; mutated reverse primer (484):

GTTGAAGTAACAAACCATTTCTTTGTTG; outward reverse primer (354):

GTTCTCGAGCTATTGCTCGTTGGGGT using RE(B)pGEX as template and cloned into the NcoI/XhoI sites of pGEX-KG.

RE(B)F904WpGBKT7, was cloned by excision of the insert from RE(B)F904WpGEX with NcoI/XhoI and cloning into the NcoI/SalI site of pGBKT7.

RE(B) Δ HDLpGEX the insert was generated by overlapping PCR using the following primers and RE(B)pGEX as template: outward forward primer (353): GTTCCATGGAGATCCGCCCCCAGATCAT; forward primer (505): TGCCATATCCTCAATCTGGGAGGAGGAGCTGCTCGGATCC; reverse primer (504): GGATCCGAGCAGCTCCTCCTCCCAGATTGAGGATATGGCA; outward reverse primer (354): GTTCTCGAGCTATTGCTCGTTGGGGT and cloned into the NcoI/XhoI site of pGEX-KG.

RE(B) Δ HDLpGADT7 was cloned by excision of the respective insert from RE(B) Δ HDLpGEX with NcoI/XhoI and cloned into the NcoI/XhoI sites of pGADT7.

RE(B)mRFP, was amplified via PCR with the indicated primers and RIBEYE(B)pGEX (Schmitz et al. 2000) as template and cloned into the XhoI and BamHI sites of pmRFP-N1 (Clontech): forward primer (837): TTTTCTCGAGTGCCACCATGATCCGCCCCCAGATCAT; reverse primer (838): TTTTAGATCTCTTTGCTCGTTGGGGTGCT.

Bacterial strains:

E. coli DH 10B, genotype: [F- *mcrA* Δ (*mrr-hsdRMS-mcrBC*) Φ 80*lacZ* Δ M15 Δ *lacX74* *recA1 endA1 araD139* Δ (*ara, leu*)7697 *galU galK* _- *rpsL nupG*].

E. coli JC201, genotype: *plsC thr-1 ara- Δ (gal-att λ)-99 hisG4 rpsL136 xyl-5 mtl-1 lacY1 tsx-78 eda-50 rfbD1 thi-1* (Coleman, 1990).

E. coli BL21 (DE 3), genotype: [F⁻ *ompT hsdS_B(r_B⁻m_B⁻) gal dcm* (DE3)].

Antibodies

The following antibodies were used in the present study: mouse monoclonal anti-GST (Sigma), mouse monoclonal anti-MBP (NEB), anti-RIBEYE(B)domain (U2656, Schmitz et al., 2000), anti-rRIBEYE(A)-domain (“Tau”, generated against ratRIBEYE(A)343-536)-MBP, polyclonal antibody against rat RIBEYE(A)-domain (tom Dieck et al., 2005), polyclonal antibody against bassoon (Synaptic Systems), monoclonal antibody to β -tubulin (Sigma).

Supplementary Figures

Supplementary Figure 1.

(A) Interaction of RIBEYE(A) with RIBEYE(A) in the YTH system: Quantification of β -galactosidase activities in liquid assays of the indicated yeasts (see also Fig.1). The indicated yeast clones growing on selective medium either for the presence of bait- and prey plasmids (-LW dropout medium) or selective for protein - protein interaction (-ALWH dropout medium). For convenience, experimental bait-prey pairs are underlayered in color (green in case of interacting bait-prey pairs); control matings are non-colored. Error bars represent s.e.m..

(B) Western blot analysis of RIBEYE(A)-RIBEYE(A) pulldowns. Immobilized RIBEYE(A)-GST binds RIBEYE(A) from a crude extract of RIBEYE(A)-transgenic *Pichia pastoris* yeasts. RIBEYE(A)-GST (lane 6) but not GST alone (lane 8) pulls-down untagged RIBEYE(A). The western blot was developed with an antibody against RIBEYE(A)-domain that simultaneously detects both untagged RIBEYE(A) which was used as prey protein as well as the GST-tagged RIBEYE(A) which served as bait protein. GST served as the control bait protein and did not pull down RIBEYE(A) from the respective *Pichia pastoris* extract. The pull-downed untagged RIBEYE(A) is labelled with an arrow in lane 6, the GST-tagged RIBEYE(A) is labelled with an arrowhead (Suppl.Fig.1Ba). The asterisk denotes an unspecific band detected by the antiserum against RIBEYE(A). Suppl. Fig. 1Bb shows the same blot as in Suppl. Fig. 1Ba after stripping and reprobing of the nitrocellulose with anti-GST antibodies to show equal loading of bait proteins. Abbreviations: RE, RIBEYE; RE(A), RIBEYE(A)-domain.

Supplementary Figure 2. Mapping of RIBEYE(A)-RIBEYE(A)-interaction in the YTH system. For convenience, experimental bait-prey pairs are underlayered in color (green in case of interacting bait-prey pairs; yellow in case of non-interacting bait-prey pairs); control matings are non-colored. The indicated yeast clones growing on selective medium either for the presence of bait- and prey plasmids (-LW dropout medium) or selective for protein-protein interaction (-ALWH dropout medium). In the right panels, β -galactosidase activities were monitored by filter lift assays. Blue color indicates β -galactosidase activity. Mating #1 represents the positive control (RIBEYE(A)-RIBEYE(A)-interaction; mating #17 demonstrates interaction of RIBEYE(A2) with full-length RIBEYE(A), mating #38 demonstrates interaction of RIBEYE(A1) with full-length RIBEYE(A) and mating #41 demonstrates interaction of RIBEYE(A3) with full-length RIBEYE(A). All constructs that contained either „A1“, „A2“ or „A3“ interacted with full-length RIBEYE(A)-domain. All the tested RIBEYE constructs did not show auto-activation (matings #2-5,7-8,10-13,15,16,18-21,23,24,26-29,31,32,34-37,39,40,42-45). Abbreviations: RE, RIBEYE; RE(A), RIBEYE(A)-domain.

Supplementary Figure 3.

(A) RIBEYE(B) homo-dimerizes with RIBEYE(B) in the YTH system. For convenience, experimental bait-prey pairs are underlayered in color (green in case of interacting bait-prey pairs; yellow in case of non-interacting bait-prey pairs); control matings are non-colored. The indicated yeast clones growing on selective medium either for the presence of bait- and prey plasmids (-LW dropout medium) or selective for protein-protein

interaction (-ALWH dropout medium). RIBEYE(B) interacts with RIBEYE(B) (mating #6) as well as with RIBEYE(AB) (mating #1). Matings #2-5,7-8 show control matings demonstrating that the respective bait and prey constructs are not auto-activating.

(B) RIBEYE(B) homo-dimerizes with RIBEYE(B) in the YTH system as demonstrated by quantitative analysis of β -galactosidase activity of the indicated yeasts. Error bars represent s.e.m..

(C) RIBEYE(B) homo-dimerization is dependent on the HDL loop as shown in the YTH system. The interaction between RIBEYE(B) and RIBEYE(B) is abolished when the HDL loop is deleted (matings #1,2). Mating #5 shows the positive control (RIBEYE(B)-RIBEYE(B) interaction), the other matings represent the indicated respective auto-activation controls.

(D) Structure model of RIBEYE(B) domain (wildtype protein, Figure Da) and RIBEYE(B) Δ HDL with a deleted homo-dimerization loop (Figure Db). The arrow points to the RIBEYE(B) homo-dimerization loop (HDL). The NADH bound to RIBEYE is depicted in yellow.

Supplementary Figure 4.

(A) RIBEYE(B) hetero-dimerizes with RIBEYE(A) in the YTH system: Quantification of β -galactosidase activities in liquid assays. Abbreviations: ***, highly significant ($\alpha=0.001$). Error bars represent s.e.m..

(B) Mapping of the RIBEYE(B)-binding site on RIBEYE(A) using the YTH system. For convenience, experimental bait-prey pairs are underlayered in color (green in case of interacting bait-prey pairs; yellow in case of non-interacting bait-prey pairs); control matings are non-colored. The indicated yeast clones growing on selective medium either for the presence of bait- and prey plasmids (-LW dropout medium) or selective for protein-protein interaction (-ALWH dropout medium). In the right panels, β -galactosidase activities of the indicated yeasts (filter tests) are depicted. RIBEYE(B) interacts with the carboxyterminal region of the A-domain (mating #6, #33) which is denoted as RIBEYE(A2) in the text. Mating #1 represents the positive control (RIBEYE(B)-RIBEYE(A) interaction), the other matings (#2-5, #7-32, #34-35) are the respective indicated auto-activation controls and other indicated RIBEYE constructs-containing mated yeasts which were not interacting.

(C) Mapping of the RIBEYE(A)-binding site on RIBEYE(B) with the YTH system: RIBEYE(B)-RIBEYE(A) interaction is independent on the RIBEYE(B) HDL loop. If the HDL loop is deleted RIBEYE(B) can still interact with RIBEYE(A) (mating #1). In contrast, homo-dimerization of RIBEYE(B) is entirely dependent on the HDL loop (Supplementary Figure 3C). The NBD-subdomain of RIBEYE(B) is responsible for interaction with RIBEYE(A): RIBEYE(A) interacted with the NBD-subdomain of RIBEYE(B) (mating #4) but not with the SBD-subdomain of RIBEYE(B) (mating #7).

Supplementary Figure 5.

Mapping of the binding site of RIBEYE(A) on RIBEYE(B) using point mutants of RIBEYE(B). The indicated yeast clones growing on selective medium either for the presence of bait- and prey plasmids (-LW dropout medium) or selective for protein-protein interaction (-ALWH dropout medium). For convenience, experimental bait-prey pairs are underlayered in color (green in case of interacting bait-prey pairs; yellow in case

of non-interacting bait-prey pairs); control matings are non-colored. **(A)** The RIBEYE point mutant RIBEYE(B)C683S can no longer interact with RIBEYE(A) (mating #6) but can still interact with RIBEYE(B) (mating #9). Mating #1 shows the positive control (RIBEYE(A)-RIBEYE(B) interaction; the other matings (#2-5, #7-8, #10-11) are auto-activation controls. **(B)** The RIBEYE point mutant RIBEYE(B)K854Q interacts both with RIBEYE(A) (mating #9) as well as with RIBEYE(B) (Suppl. Fig. 6, mating #15). RIBEYE(B)F904W also interacts both with RIBEYE(A) (mating #6) as well as with RIBEYE(B) (Suppl. Fig. 6, mating #21). In contrast, RIBEYE(B)D758N, RIBEYE(B)E844Q, RIBEYE(B)F848W and RIBEYE(B)I796A did no longer interact with RIBEYE(A) (matings #12, #15, #18, #21) although all of these point mutants still interacted with RIBEYE(B) (Suppl. Fig. 6; matings #6,9,12,18). Mating #1 represents the positive control (RIBEYE(A)-RIBEYE(B) interaction). Matings #2-5, 7,8,10,11,13,14,16,17,19,20,22,23 represent the respective autoactivation controls. None of the used constructs is auto-activating. **(C)** The NADH binding-deficient RIBEYE(B)G730A can no longer interact with RIBEYE(A) (mating #6) although it still can interact with RIBEYE(B) wildtype protein (mating #9). Matings #2-5,7,8,10,11 are the indicated auto-activation controls. None of the RIBEYE constructs was auto-activating in YTH. **(D)** Binding of radioactive NAD⁺ to RIBEYE(B)-GST, RIBEYE(B)G730A-GST and GST alone was determined as previously described (Schmitz et al., 2000). Binding of NAD⁺ to RIBEYE(B)-GST was set to 100%. RIBEYE(B)G730A (column 3) does not bind significant amounts of radioactive NAD⁺ and is defective in NAD⁺ binding (n=7). Error bars represent s.e.m..

Supplementary Figure 6.

Interaction analyses of RIBEYE(B) point mutants for their capability to homo-dimerize with RIBEYE(B) wildtype protein in the YTH system. These RIBEYE(B) point mutants were tested in Suppl. Fig. 5 for their interaction with RIBEYE(A). The present figure summarizes the capability of these mutants to interact with RIBEYE(B) in the YTH system. For convenience, experimental bait-prey pairs are underlayered in color (green in case of interacting bait-prey pairs); control matings are non-colored. RIBEYE(B)D758N, RIBEYE(B)E844Q, RIBEYE(B)F848W, RIBEYE(B)K854Q, RIBEYE(B)I796A and RIBEYE(B)F904W interacted with RIBEYE(B) wildtype protein in YTH (matings #6,9,12,15,18,21). Mating #1 is a positive control (RIBEYE(B)-RIBEYE(B) interaction); the other matings (matings #2-5,7,8,10,11,13,14,16,17,19,20,22,23) are the indicated auto-activation controls. None of the constructs were auto-activating.

Supplementary Figure 7.

Interaction analyses of RIBEYE(AB) (full-length RIBEYE) with RIBEYE(B) Δ HDL in the YTH system. For convenience, experimental bait-prey pairs are underlayered in color (green in case of interacting bait-prey pairs); control matings are non-colored. RIBEYE(B) Δ HDL is deficient in interaction with RIBEYE(B) (Supplementary Figure 3C) but can still interact with RIBEYE(AB) (mating #1) indicating that RIBEYE(A)-RIBEYE(B) interaction can compensate for the loss of RIBEYE(B)-RIBEYE(B) interaction. Matings #2-5 are control matings with the indicated plasmids to demonstrate lack of autoactivation of the respective constructs.

Supplementary Figure 8.

In Fig. 6 we demonstrated that NADH and NAD⁺ inhibit RIBEYE(A)-RIBEYE(B) interactions at various physiological concentrations of NADH and NAD⁺. The present figure summarizes that at all NAD⁺/NADH concentrations used in these experiments, there is no unspecific binding between the immobilized control bait protein (GST alone) and the prey protein RIBEYE(B)-MBP. 0.3μM of immobilized GST fusion protein (control protein) or RE(A)-GST fusion protein were incubated with 0.3μM of RE(B)-MBP in the presence of the indicated concentrations of NAD⁺ (A,C) or NADH (B,D) for 3hrs at 4°C in binding buffer (100mM Tris-HCl, pH 8.0, 150mM NaCl, 1mM EDTA, 1% Triton X-100). After several washes with binding buffer, the pellets were boiled with SDS-sample buffer and analyzed for binding of RIBEYE(B)-MBP by western blot analyses and probing of the western blots with anti-MBP antibodies. As already shown in Fig. 6, both NAD⁺ and NADH strongly inhibited binding of RIBEYE(B) to RIBEYE(A). At 25 nM of NAD⁺ (A) or 25nM NADH (B) we observed a strong inhibition of binding of RIBEYE(B)-MBP to RIBEYE(A)-GST (lane 9) in comparison to no NADH/NAD⁺ added (lane 8 in A and B). Also at the lowest concentration of NADH/NAD⁺ used (25nM, lane 6 in A and B)) we did not observe any unspecific binding of RE(B)MBP to GST alone. This was also the case for all other concentrations of NADH/NAD⁺ used (Suppl. Fig. 8 C,D) as well as in the absence of any NADH/NAD⁺ added (Fig. 4). Suppl. Figs 8Ab- Db show the respective loading controls in which the same blots as shown in Suppl. Figs. Aa-BDa were incubated with GST antibodies after stripping of the respective blots. Abbreviations: RE, RIBEYE; RE(B), RIBEYE(B)-domain; RE(A), RIBEYE(A)-domain.

Supplementary Figure 9.

Further examples of R28 cells transfected with the indicated EGFP- or mRFP-tagged RIBEYE constructs. If RIBEYE(B) was co-transfected with full-length RIBEYE (RIBEYE(AB)) (A-B), RIBEYE(B) completely re-distributed from a diffuse to a spot-like distribution typical for RIBEYE(AB) and co-localized with RIBEYE(AB) (arrows in Suppl. Fig. 9AB). Interestingly, if RIBEYE(AB)EGFP / RIBEYE(B)-transfected cells were analyzed already a few hours after transfection (A), the RIBEYE-containing aggregates were smaller and more numerous (arrowheads in A) than at later time points (B, see also Figs. 7,9) suggesting that the smaller protein clusters could mature/coalesce to the bigger protein aggregate. The arrows in (A,B) point to intracellular RIBEYE aggregates that contain both types of the indicated differentially tagged RIBEYE proteins. (C) shows a further example of a R28 cell double-transfected with RIBEYE(B) and RIBEYE(A). RIBEYE(B) re-distributed from a diffuse distribution to a spot-like distribution typical for RIBEYE(A) and co-localized with RIBEYE(A) (arrows in C); a portion of RIBEYE(B) remained diffusely distributed (arrowhead in C); see also Fig. 7D. Abbreviations: n, nucleus. Scale bars: 10μm. D) Western blot analysis with a polyclonal antibody against bassoon demonstrates the presence of bassoon in R28 cells at the protein level. Expression of bassoon in R28 cells was also demonstrated at the mRNA level by RT-PCR experiments (data not shown).

Supplementary Figure 10.

COS cells were transfected with the indicated mRFP- or EGFP-tagged RIBEYE constructs. Transfected cells were analyzed for the intracellular distribution of the respective proteins via direct epifluorescence microscopy. (Suppl. Fig. 10A,B): RIBEYE(AB)-EGFP co-aggregated and co-localized with RIBEYE(A)-mRFP (Suppl. Fig. 10A) and RIBEYE(B)-mRFP (Suppl. Fig. 10B). RIBEYE(B) is diffusely expressed in single-transfected cells (Schmitz et al., 2000; Suppl. Fig. 10D, see also Suppl. Fig. 11). The arrowhead in Suppl. Fig. 10B shows a single transfected cell that is transfected only with RIBEYE(AB)-EGFP but not with RIBEYE(B)-mRFP. If RIBEYE(B) is co-transfected with RIBEYE(AB) (Suppl. Fig. 10B) RIBEYE(B) virtually completely redistributed from a diffuse distribution into a spot-like, RIBEYE(AB)-typical distribution and colocalized with RIBEYE(AB) (arrows in Suppl. Fig. 10B). Arrowheads in Suppl. Fig. 10A,B,D point to single-transfected cells. RIBEYE(B) also redistributed from a diffuse to spot-like distribution if co-transfected with RIBEYE(A) (Suppl. Fig. 10D,E); part of RIBEYE(B) remained diffusely distributed (small arrow in E). The higher degree of co-distribution of RIBEYE(B) with RIBEYE(AB) in comparison to RIBEYE(A) probably represents the fact that more type of interactions can be formed between RIBEYE(B) and RIBEYE(AB) than between RIBEYE(B) and RIBEYE(A) alone (for summary, see Fig. 11). Arrowheads point to single-transfected cells. RIBEYE(A) also co-aggregated and co-localized with RIBEYE(A) (Suppl. Fig. 10C). For further examples of transfected COS cells, see also Suppl. Fig. 11. R28 cells transfected with the respective plasmids produced qualitatively identical results (Fig. 7 and Suppl. Fig. 9). Abbreviation: n, nucleus. Scale bars: 10µm.

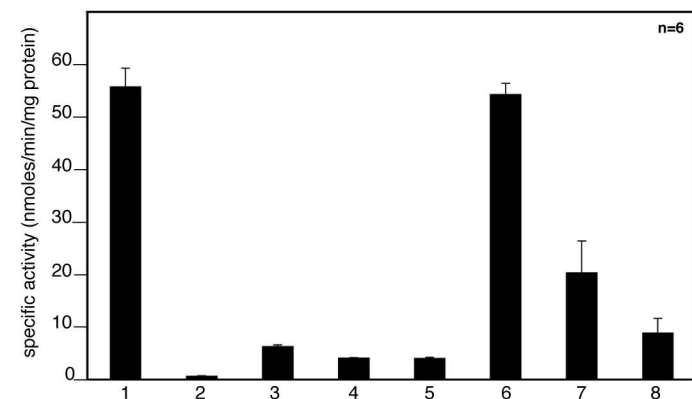
Supplementary Figure 11.

Co-clustering of RIBEYE in transfected COS cells. Further examples of transfected COS cells transfected with the indicated EGFP- or mRFP-tagged RIBEYE constructs. Single transfected RIBEYE(B) is diffusely distributed, as previously described (Schmitz et al., 2000) (arrowheads **Suppl. Fig. 11 C,D**). The distribution is independent of the used fluorescent tag. The arrow in (C) indicates a cell double-transfected with RIBEYE(B)-EGFP and RIBEYE(B)-mRFP. In contrast, RIBEYE(A) and RIBEYE(AB) show a discrete, punctate distribution (**Suppl. Fig. 11 A,B**) as previously described (Schmitz et al., 2000). If RIBEYE(A)EGFP was co-transfected with RIBEYE(A)mRFP both proteins co-aggregated into the same protein clusters (arrows, **Suppl. Fig. 11A**). If RIBEYE(B) was co-transfected with RIBEYE(AB) (**Suppl. Fig. 11B**), RIBEYE(B) nearly completely re-distributed to a spot-like distribution typical for RIBEYE(AB) (arrows, **Suppl. Fig. 11B**). Arrowheads indicate single transfected cells. In **Suppl. Fig. 11D**, cells are shown that are transfected either only with RIBEYE(AB)-EGFP or only with RIBEYE(B)-mRFP to demonstrate the distribution of the respective proteins in single transfected cells. Arrowheads point to these single-transfected cells. Abbreviations: n, nucleus. Scale bars: 10µm.

Supplementary Figure 1

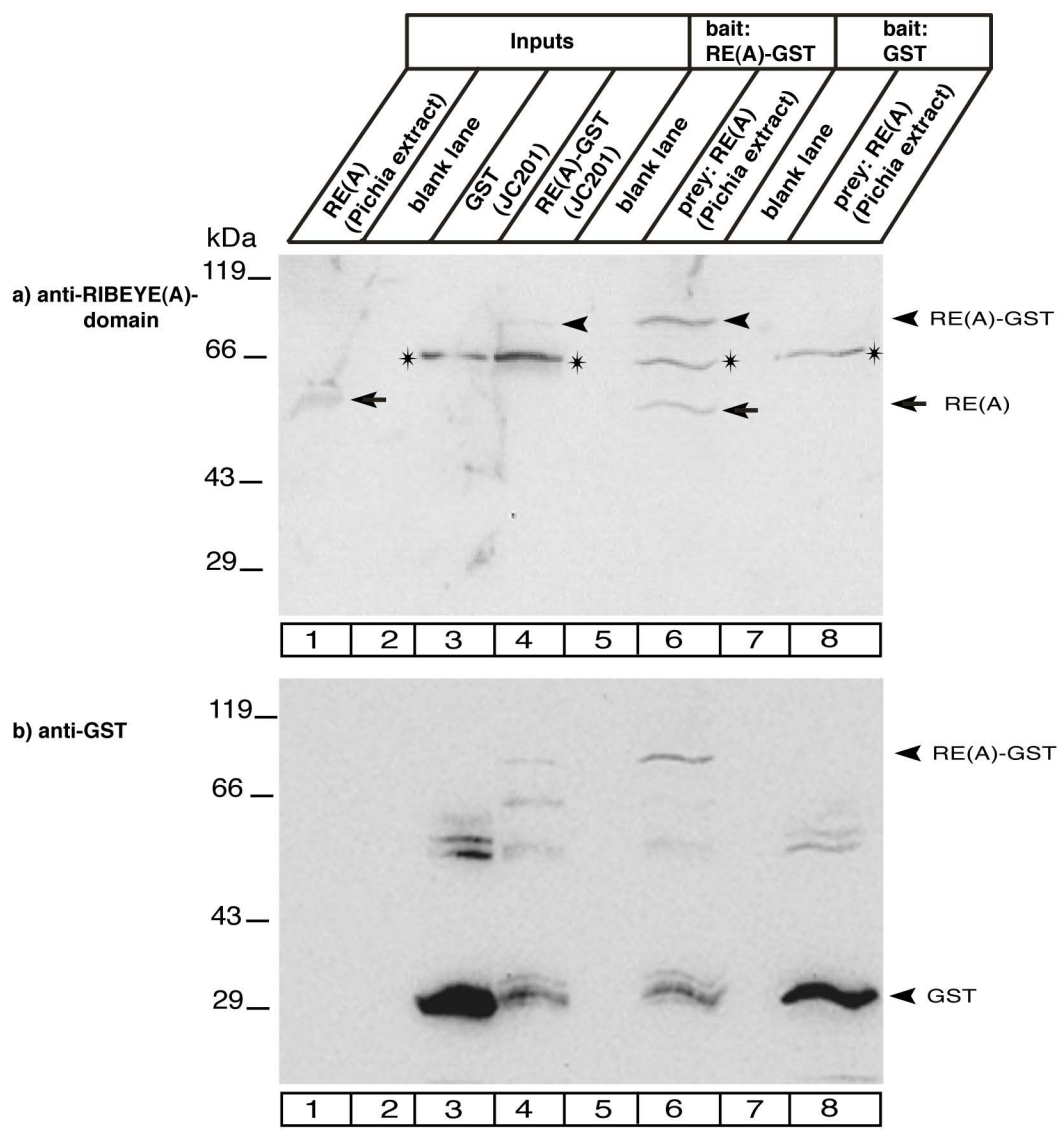
A) RIBEYE(A) interacts with RIBEYE(A) in YTH

β -galactosidase assays (liquid assays)



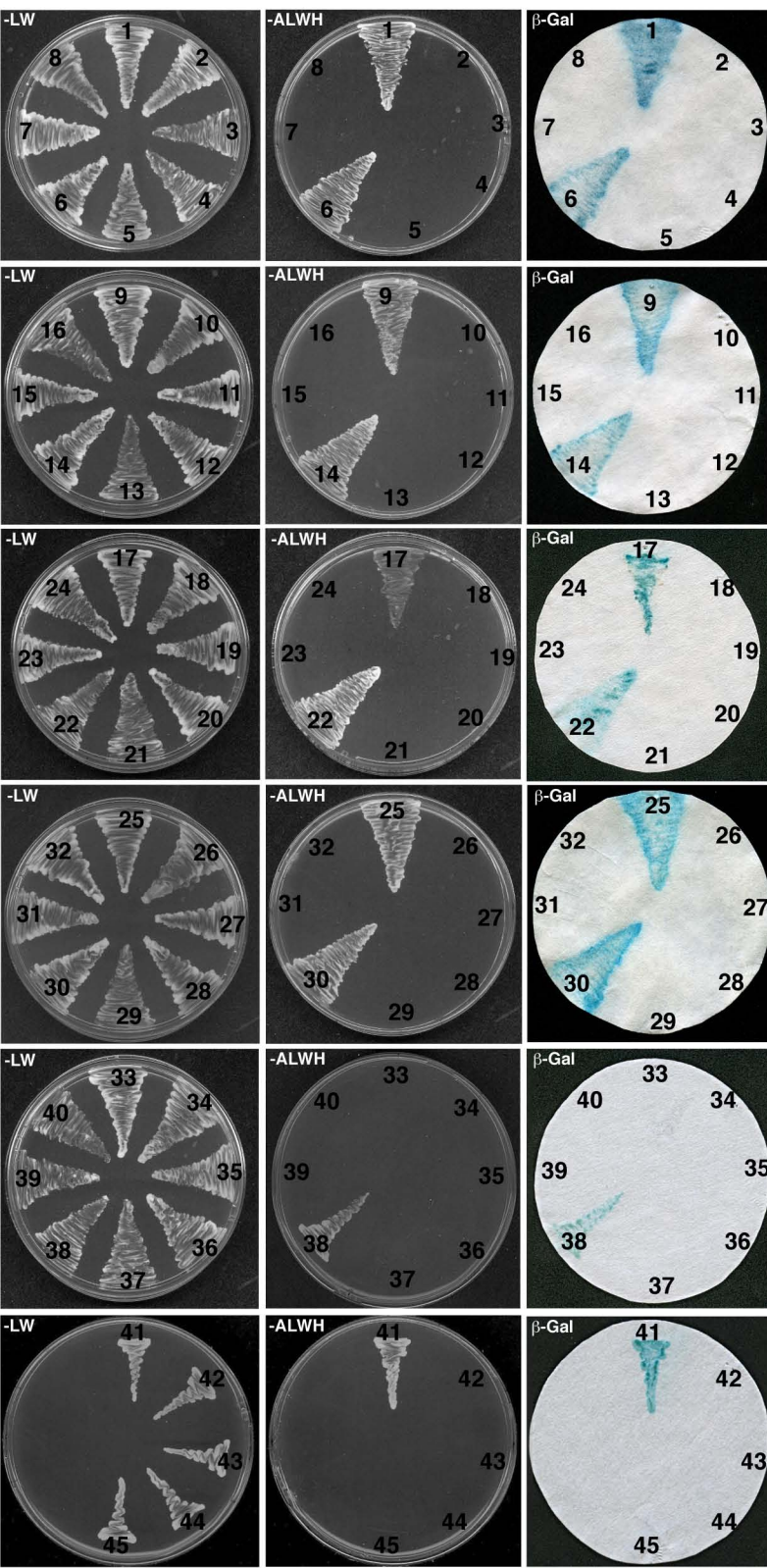
mating	bait	prey	-ALWH	β Gal
1	RE(A)	RE(A)	+	+
2	pGBKT7	RE(A)	-	-
3	pSE1112	RE(A)	-	-
4	RE(A)	pACT2	-	-
5	RE(A)	pSE1111	-	-
6	RE(AB)	RE(A)	+	+
7	RE(AB)	pACT2	-	-
8	RE(AB)	pSE1111	-	-

B) RIBEYE(A)-GST binds RIBEYE(A) from *Pichia pastoris* extract in GST pull-down assays



Supplementary Figure 2

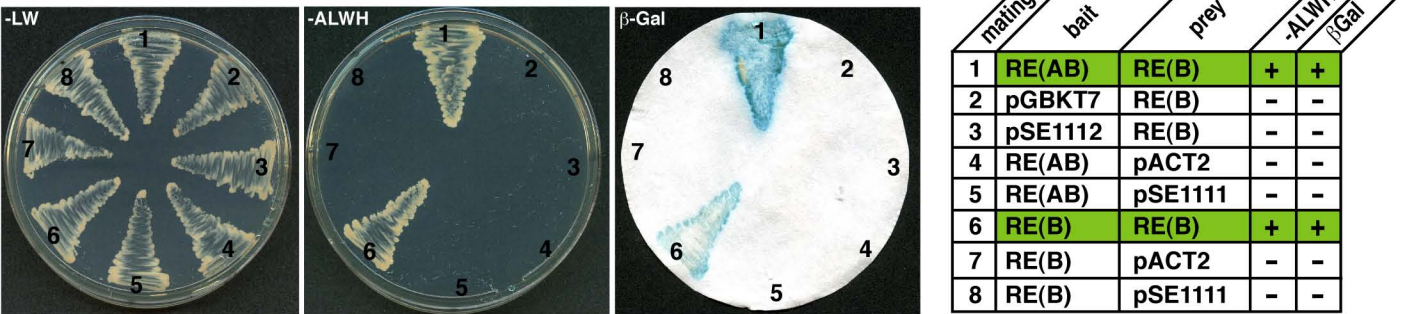
Mapping of RIBEYE(A)-RIBEYE(A) interactions in YTH



	mating	bait	prey	-ALWH	βGal
1		RE(A)1-563	RE(A)1-563	+	+
2		pGBKT7	RE(A)1-563	-	-
3		pSE1112	RE(A)1-563	-	-
4		RE(A)1-563	pACT2	-	-
5		RE(A)1-563	pSE1111	-	-
6		RE(A)1-563	RE(A)1-291	+	+
7		pGBKT7	RE(A)1-291	-	-
8		pSE1112	RE(A)1-291	-	-
9		RE(A)1-563	RE(A)364-563	+	+
10		pGBKT7	RE(A)364-563	-	-
11		pSE1112	RE(A)364-563	-	-
12		RE(A)1-563	pACT2	-	-
13		RE(A)1-563	pSE1111	-	-
14		RE(A)1-563	RE(A)1-216	+	+
15		pGBKT7	RE(A)1-216	-	-
16		pSE1112	RE(A)1-216	-	-
17		RE(A)1-563	RE(A)438-563	+	+
18		pGBKT7	RE(A)438-563	-	-
19		pSE1112	RE(A)438-563	-	-
20		RE(A)1-563	pACT2	-	-
21		RE(A)1-563	pSE1111	-	-
22		RE(A)1-563	RE(A)1-424	+	+
23		pGBKT7	RE(A)1-424	-	-
24		pSE1112	RE(A)1-424	-	-
25		RE(A)1-563	RE(A)1-496	+	+
26		pGBKT7	RE(A)1-496	-	-
27		pSE1112	RE(A)1-496	-	-
28		RE(A)1-563	pACT2	-	-
29		RE(A)1-563	pSE1111	-	-
30		RE(A)1-563	RE(A)1-162	+	+
31		pGBKT7	RE(A)1-162	-	-
32		pSE1112	RE(A)1-162	-	-
33		RE(A)1-563	RE(A)1-41	-	-
34		pGBKT7	RE(A)1-41	-	-
35		pSE1112	RE(A)1-41	-	-
36		RE(A)1-563	pACT2	-	-
37		RE(A)1-563	pSE1111	-	-
38		RE(A)1-563	RE(A)1-105	+	+
39		pGBKT7	RE(A)1-105	-	-
40		pSE1112	RE(A)1-105	-	-
41		RE(A)1-563	RE(A)106-363	+	+
42		pGBKT7	RE(A)106-363	-	-
43		pSE1112	RE(A)106-363	-	-
44		RE(A)1-563	pACT2	-	-
45		RE(A)1-563	pSE1111	-	-

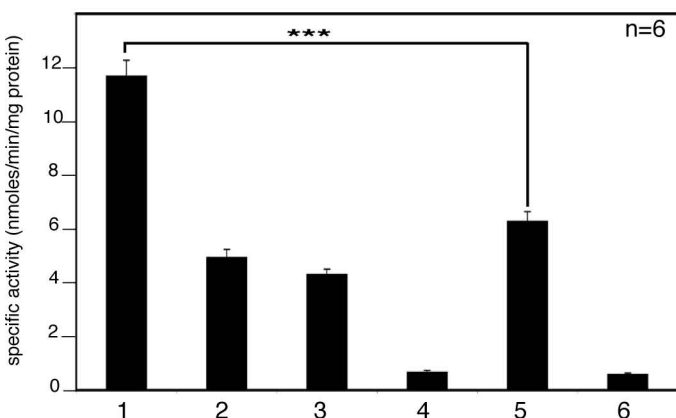
Supplementary Figure 3

A) RIBEYE(B) homo-dimerizes with RIBEYE (B) in YTH



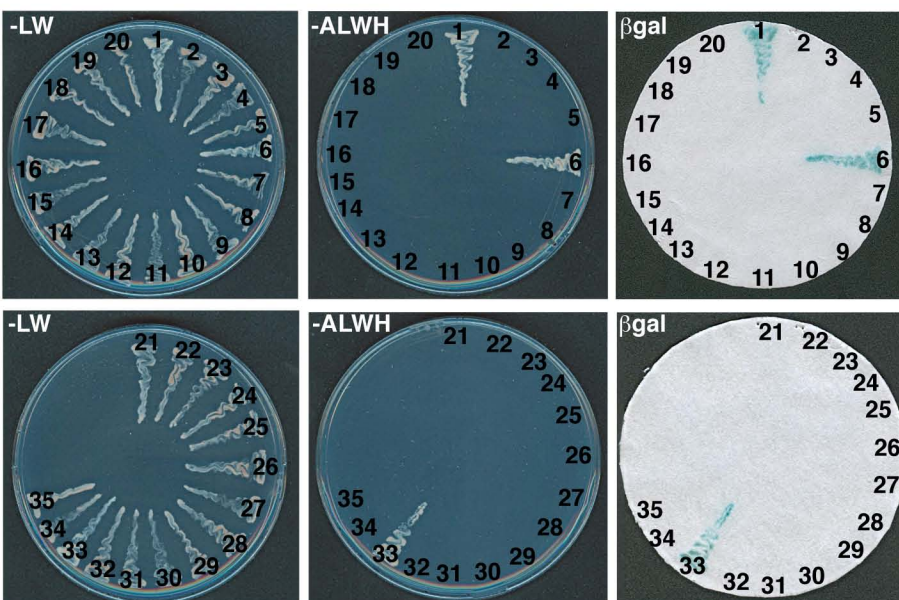
Supplementary Figure 4

A) Quantification of RIBEYE(A) - RIBEYE(B) interaction in YTH: β -galactosidase assays (liquid assays)



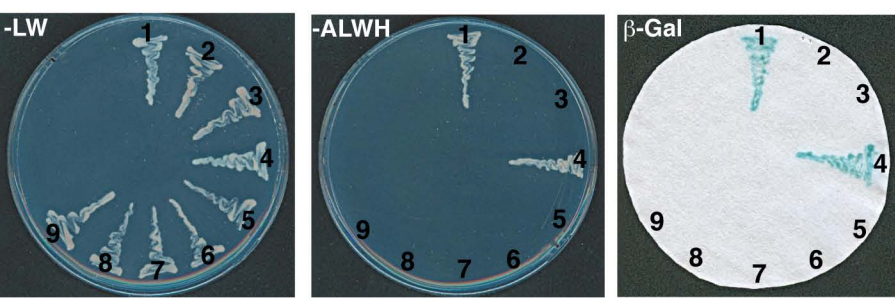
	mating	bait	prey	-ALWH	β Gal
1		RE(B)	RE(A)	+	+
2		RE(B)	pACT2	-	-
3		RE(B)	pSE1111	-	-
4		pGBKT7	RE(A)	-	-
5		pSE1112	RE(A)	-	-
6		pGBKT7	pACT2	-	-

B) Mapping of the RIBEYE(B)-binding site on RIBEYE(A) in YTH



	mating	bait	prey	-ALWH	β Gal
1		RE(B)564-938	RE(A)1-563	+	+
2		RE(B)564-938	pACT2	-	-
3		RE(B)564-938	pSE1111	-	-
4		pGBKT7	RE(A)1-563	-	-
5		pSE1112	RE(A)1-563	-	-
6		RE(B)564-938	RE(A)364-563	+	+
7		pGBKT7	RE(A)364-563	-	-
8		pSE1112	RE(A)364-563	-	-
9		RE(B)564-938	RE(A)1-496	-	-
10		pGBKT7	RE(A)1-496	-	-
11		pSE1112	RE(A)1-496	-	-
12		RE(B)564-938	RE(A)1-424	-	-
13		pGBKT7	RE(A)1-424	-	-
14		pSE1112	RE(A)1-424	-	-
15		RE(B)564-938	RE(A)1-368	-	-
16		pGBKT7	RE(A)1-368	-	-
17		pSE1112	RE(A)1-368	-	-
18		RE(B)564-938	RE(A)1-291	-	-
19		pGBKT7	RE(A)1-291	-	-
20		pSE1112	RE(A)1-291	-	-
21		RE(B)564-938	RE(A)1-216	-	-
22		pGBKT7	RE(A)1-216	-	-
23		pSE1112	RE(A)1-216	-	-
24		RE(B)564-938	RE(A)1-162	-	-
25		pGBKT7	RE(A)1-162	-	-
26		pSE1112	RE(A)1-162	-	-
27		RE(B)564-938	RE(A)1-105	-	-
28		pGBKT7	RE(A)1-105	-	-
29		pSE1112	RE(A)1-105	-	-
30		RE(B)564-938	RE(A)1-41	-	-
31		pGBKT7	RE(A)1-41	-	-
32		pSE1112	RE(A)1-41	-	-
33		RE(B)564-938	RE(A)438-563	+	+
34		pGBKT7	RE(A)438-563	-	-
35		pSE1112	RE(A)438-563	-	-

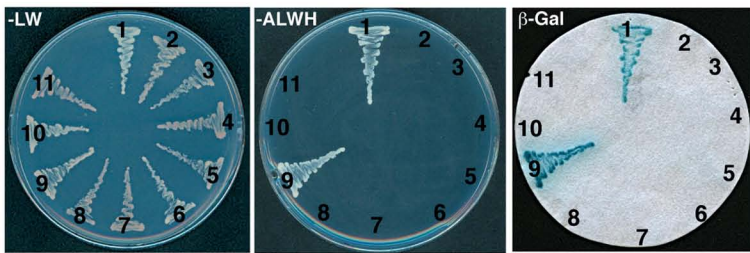
C) Mapping of the RIBEYE(A)-binding site on RIBEYE(B) in YTH



	mating	bait	prey	-ALWH	β Gal
1		RE(A)	RE(B) Δ HDL	+	+
2		pGBKT7	RE(B) Δ HDL	-	-
3		pSE1112	RE(B) Δ HDL	-	-
4		RE(A)	RE(B)NBD	+	+
5		pGBKT7	RE(B)NBD	-	-
6		pSE1112	RE(B)NBD	-	-
7		RE(A)	RE(B)SBD	-	-
8		pGBKT7	RE(B)SBD	-	-
9		pSE1112	RE(B)SBD	-	-

Supplementary Figure 5

Mapping of the binding site of RIBEYE(A) on RIBEYE(B) using point mutants of RIBEYE(B) in YTH



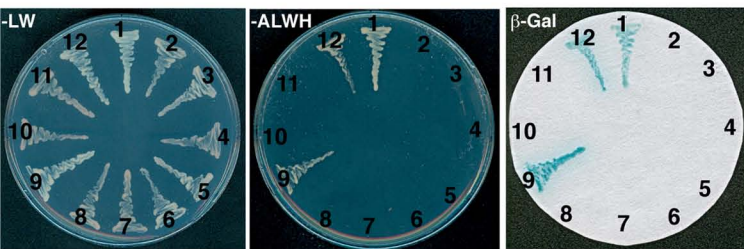
	mating	bait	prey	-ALWH	βGal
1	RE(B)		RE(A)	+	+
2	RE(B)		pACT2	-	-
3	RE(B)		pSE1111	-	-
4	pGBKT7		RE(A)	-	-
5	pSE1112		RE(A)	-	-
6	RE(B)C683S		RE(A)	-	-
7	RE(B)C683S		pACT2	-	-
8	RE(B)C683S		pSE1111	-	-
9	RE(B)C683S		RE(B)	+	+
10	pGBKT7		RE(B)	-	-
11	pSE1112		RE(B)	-	-

B)



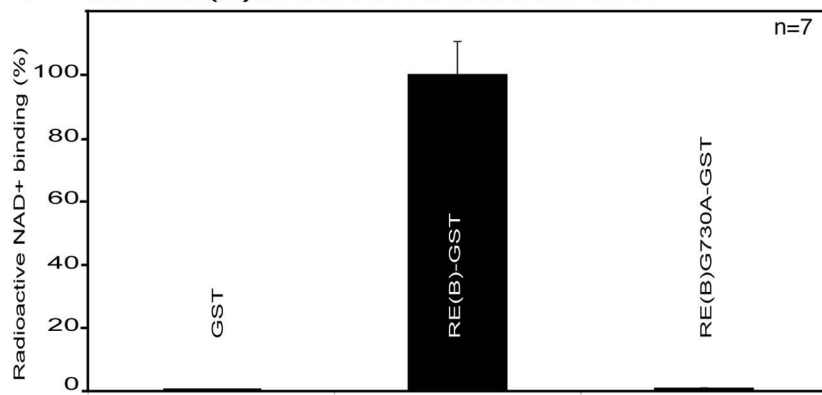
	mating	bait	prey	-ALWH	βGal
1	RE(B)		RE(A)	+	+
2	pGBKT7		RE(A)	-	-
3	pSE1112		RE(A)	-	-
4	RE(B)		pACT2	-	-
5	RE(B)		pSE1111	-	-
6	RE(B)F904W		RE(A)	+	+
7	RE(B)F904W		pACT2	-	-
8	RE(B)F904W		pSE1111	-	-
9	RE(B)K854Q		RE(A)	+	+
10	RE(B)K854Q		pACT2	-	-
11	RE(B)K854Q		pSE1111	-	-
12	RE(B)D758N		RE(A)	-	-
13	RE(B)D758N		pACT2	-	-
14	RE(B)D758N		pSE1111	-	-
15	RE(B)E844Q		RE(A)	-	-
16	RE(B)E844Q		pACT2	-	-
17	RE(B)E844Q		pSE1111	-	-
18	RE(B)F848W		RE(A)	-	-
19	RE(B)F848W		pACT2	-	-
20	RE(B)F848W		pSE1111	-	-
21	RE(B)I796A		RE(A)	-	-
22	RE(B)I796A		pACT2	-	-
23	RE(B)I796A		pSE1111	-	-

C)



	mating	bait	prey	-ALWH	βGal
1	RE(B)		RE(A)	+	+
2	RE(B)		pACT2	-	-
3	RE(B)		pSE1111	-	-
4	pGBKT7		RE(A)	-	-
5	pSE1112		RE(A)	-	-
6	RE(B)G730A		RE(A)	-	-
7	RE(B)G730A		pACT2	-	-
8	RE(B)G730A		pSE1111	-	-
9	RE(B)G730A		RE(B)	+	+
10	pGBKT7		RE(B)	-	-
11	pSE1112		RE(B)	-	-
12	RE(B)		RE(B)	+	+

D) RIBEYE(B)G730A does not bind NAD⁺



Supplementary Figure 6

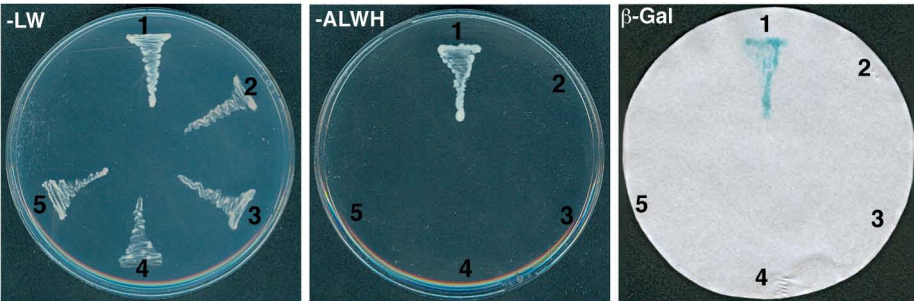
Point mutants of RIBEYE(B) that abolish interaction with RIBEYE(A) do not abolish RIBEYE(B) homo-dimerization



mating	bait	prey	-ALWH	βGal
1	RE(B)	RE(B)	+	+
2	pGBKT7	RE(B)	-	-
3	pSE1112	RE(B)	-	-
4	RE(B)	pACT2	-	-
5	RE(B)	pSE1111	-	-
6	RE(B)D758N	RE(B)	+	+
7	RE(B)D758N	pACT2	-	-
8	RE(B)D758N	pSE1111	-	-
9	RE(B)E844Q	RE(B)	+	+
10	RE(B)E844Q	pACT2	-	-
11	RE(B)E844Q	pSE1111	-	-
12	RE(B)F848W	RE(B)	+	+
13	RE(B)F848W	pACT2	-	-
14	RE(B)F848W	pSE1111	-	-
15	RE(B)K854Q	RE(B)	+	+
16	RE(B)K854Q	pACT2	-	-
17	RE(B)K854Q	pSE1111	-	-
18	RE(B)I796A	RE(B)	+	+
19	RE(B)I796A	pACT2	-	-
20	RE(B)I796A	pSE1111	-	-
21	RE(B)F904W	RE(B)	+	+
22	RE(B)F904W	pACT2	-	-
23	RE(B)F904W	pSE1111	-	-

Supplementary Figure 7

Interaction between RIBEYE(AB) and RIBEYE(B) persists even when RIBEYE(B) homo-dimerization is blocked

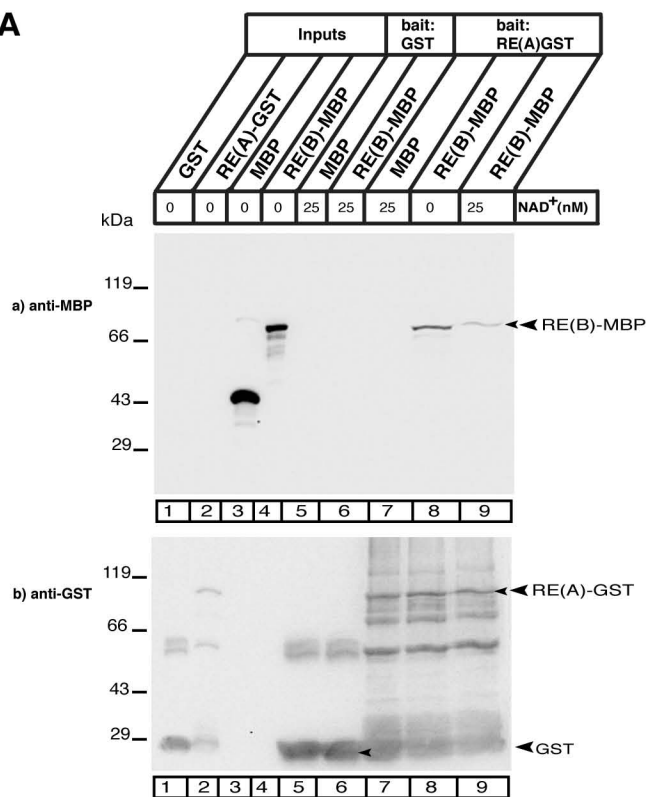


mating	bait	prey	-ALWH	βGal
1	RE(AB)	RE(B)ΔHDL	+	+
2	RE(AB)	pGADT7	-	-
3	RE(AB)	pSE1111	-	-
4	pGBKT7	RE(B)ΔHDL	-	-
5	pSE1112	RE(B)ΔHDL	-	-

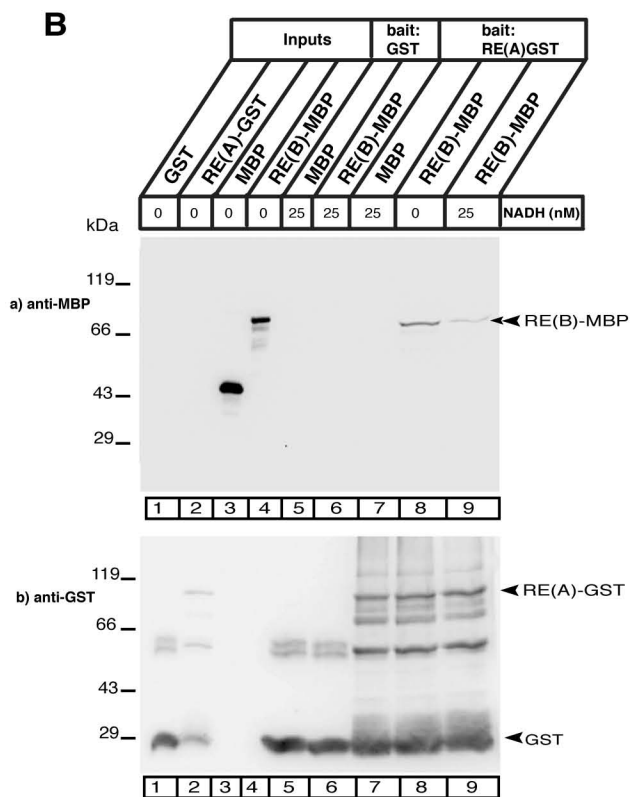
Supplementary Figure 8

NADH and NAD⁺ inhibit RIBEYE(B)-MBP - RIBEYE(A)-GST interaction without affecting control pulldowns

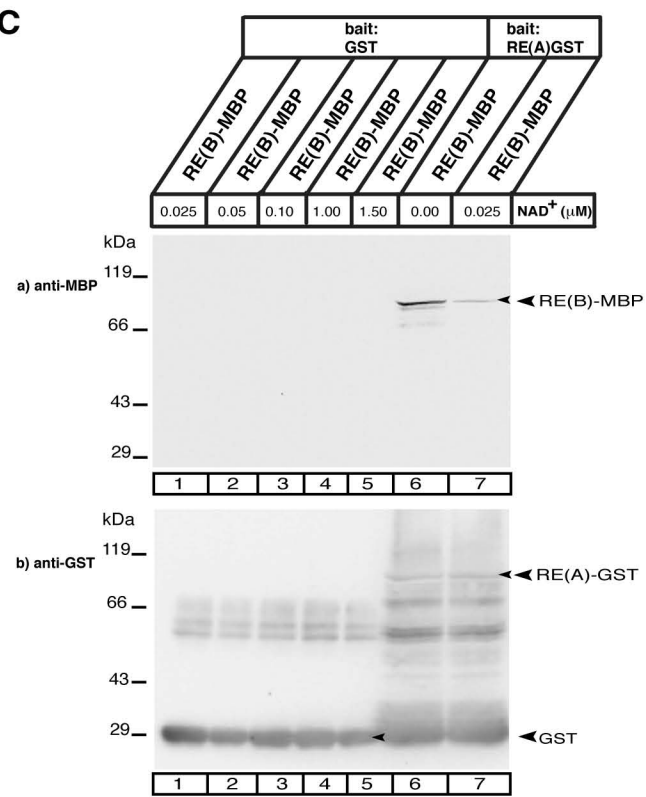
A



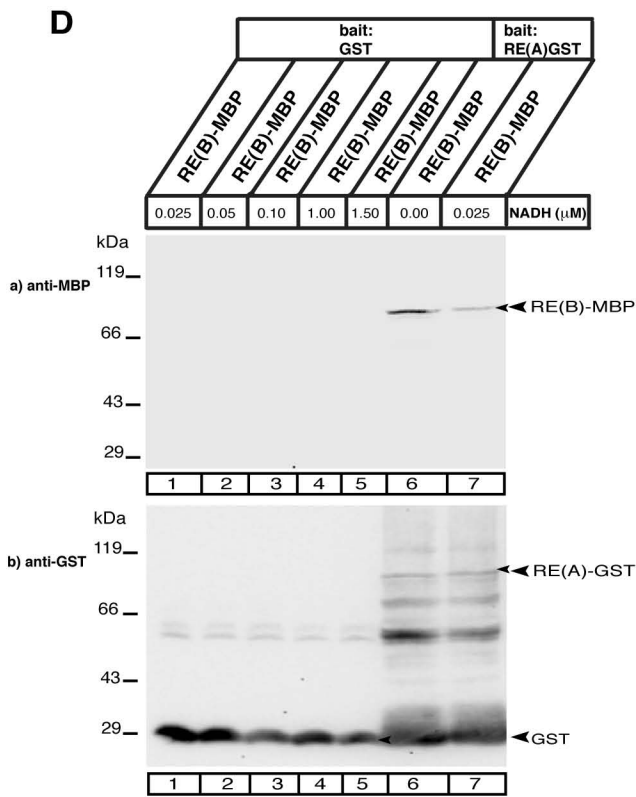
B



C

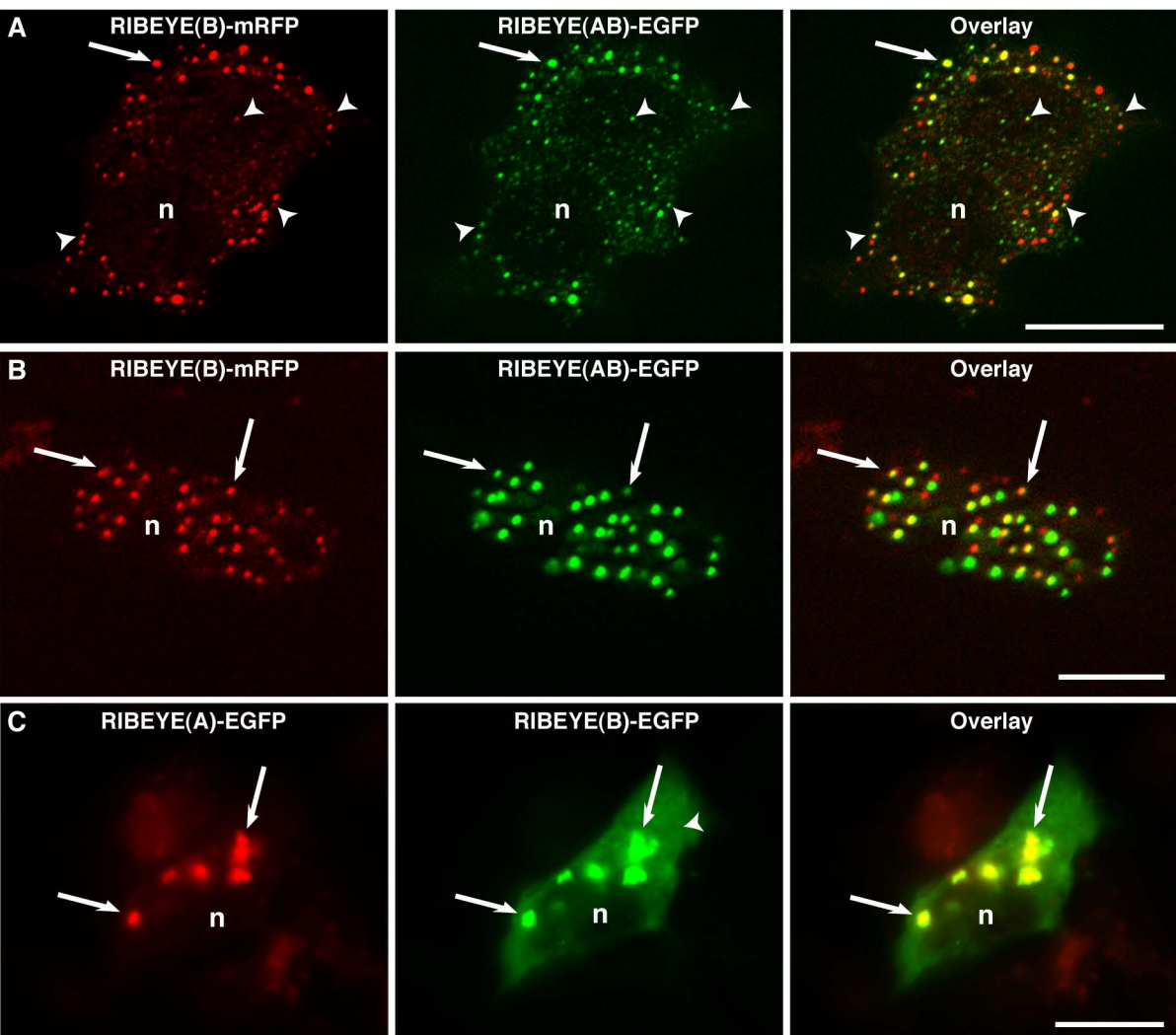


D

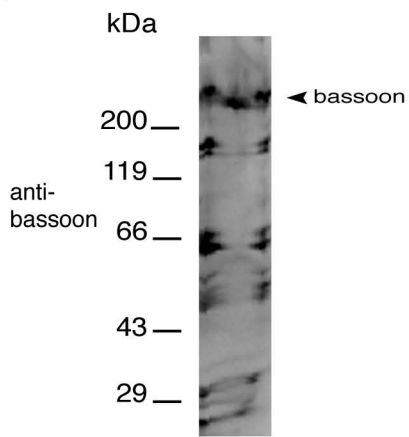


Supplementary Figure 9

Co-clustering of RIBEYE in transfected R28 cells

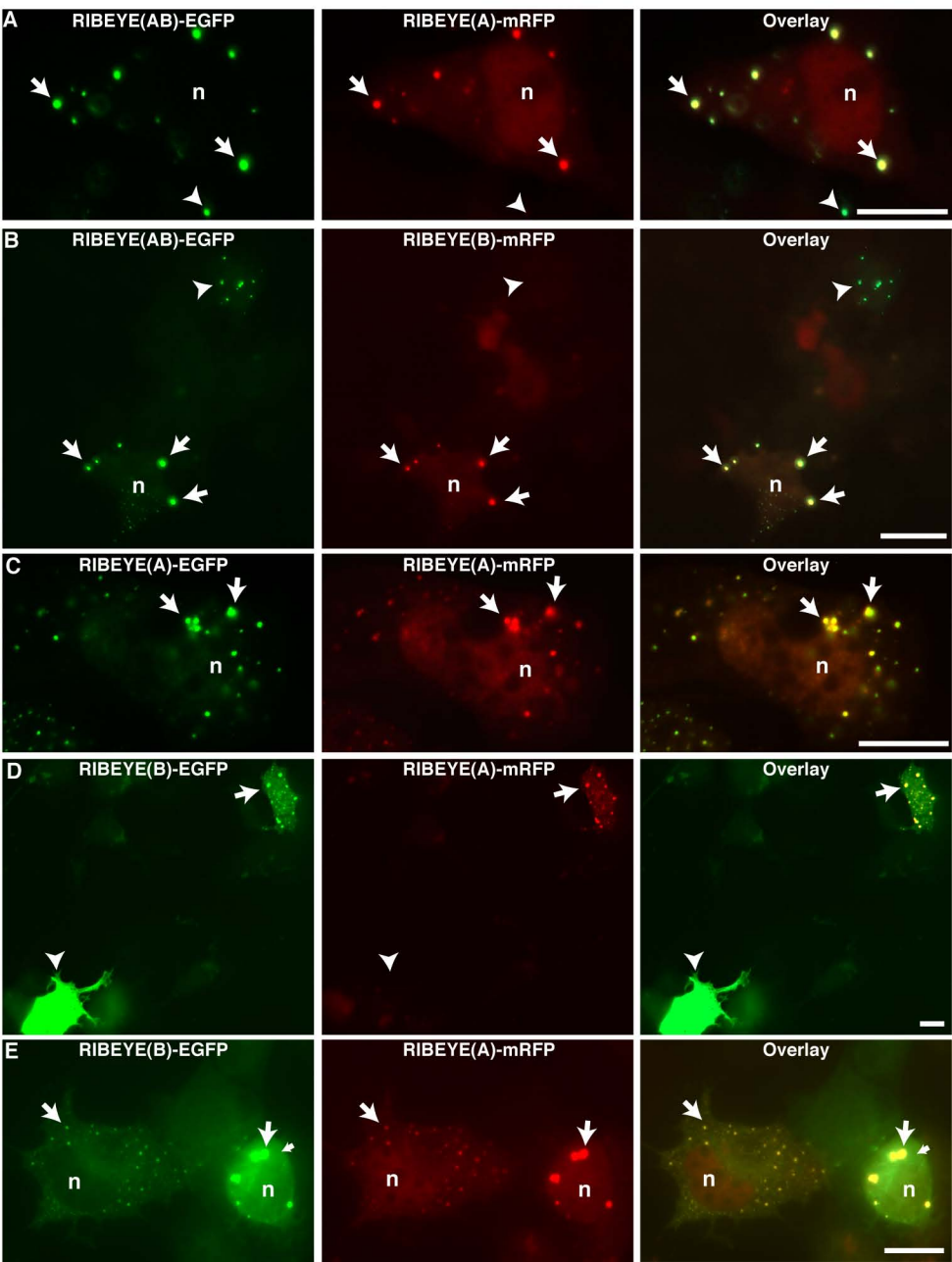


D



Supplementary Figure 10

Co-clustering of RIBEYE in transfected COS cells



Supplementary Figure 11

Co-clustering of RIBEYE in transfected COS cells

