**Supplementary Material for Varberg et al., 2020**

**Figure S1: Criteria used to determine positive interactions**.

A) (*left*) Representative images of MYTH colony growth after 4 days of growth on SD-leu-trp-ade-his + 25mM 3-AT test plates. At least 100 individual colonies were manually scored and binned into four categories based on colony size/strength of interaction: strong, medium, weak and negative Summary statistics for each category are listed in Table S2. (*center*) A density cutoff was set at the 25th percentile value for "weak" colonies, depicted by dashed line. (*right*) Colony densities for each bait showing distribution of densities, with those falling above the threshold cutoff highlighted in blue. B) Comparison of normalized density values extracted using our custom ImageJ plugin with those available in the SGATools software package (http://sgatools.ccbr.utoronto.ca). C) Representative images depicting methods used to generate normalization values to correct for differences in diploid colony sizes. Thresholding was performed to generate a binary mask of diploid colonies and extract the corresponding colony area values in ImageJ. These values were used to correct colony densities from test plates as described in Materials and Methods.

**Figure S2: Quality control of MYTH baits.** A)Growth assays for MYTH baits that failed quality control screening. N-terminal Sad1 bait fusion protein showed growth on selection media when co-expressed with the empty MYTH prey plasmid (pPR3-N), indicating autoactivation. No growth was observed for C-terminal baits of Sad1 or Man, or for the N-terminal Man1 bait, when co-expressed with the positive control prey construct (Ost1-NubI), which contains a Nub fragment that retains its affinity for Cub. B) Growth assays confirming the expression and functionality of the Cut11 mutant MYTH bait proteins.

**Figure S3: Validation of high-throughput screen by haploid dilution assays.** Growth assays of haploid strains expressing both bait and prey plasmids were performed by spotting a ten-fold serial dilution series onto SD-leu-trp (SD-LT) or SD-leu-trp-ade-his (SD-LTAH) plates supplemented with 25 mM 3-AT and monitoring growth at 30°C. Bait-prey combinations from each category of hits identified in the high-throughput screen were conducted to confirm strength and bait specificity. Density values from the high-throughput screen are shown in the heatmap on the left, with the corresponding growth assay shown on the right.

**Table S1: *S. pombe* MYTH prey library.** Each prey in libray is listed, with columns for Pombase StrainID, Gene, Product, presence of transmembrane (TM) domain, *S. cerevisiae* and *H. sapiens* orthologs, and the location of each protein as determined using a nucleoplasmic split-GFP reporter screen in *S. cerevisiae*. “INM” indicates observed localization to the inner nuclear membrane, “Nucleoplasmic” indicates soluble nuclear localization, “Negative” indicates no signal detected with nucleoplasmic reporter, and “Unknown” indicates that the protein was not included in the screen.

**Table S2: Interactors identified by MYTH screen.** All 377 prey identified as a positive interactor for either Cut11, Ima1 or Lem2 bait proteins. Values represent averaged density values for all technical replicates for each prey. Summary statistics for each manually defined growth category are provided on Sheet 2.

**Table S3: Interactomes of wild-type and mutant Cut11.** Prey identified as interactors for Cut11 bait proteins. Values represent averaged densities for all technical replicates for each prey. An additional column is provided showing fold-change relative to wild-type (WT) for each mutant bait (L521F, C525R and T498I).