(a) Bait: empty, Madm, Madm full-length, N-terminus, N-terminus + KLD, KLD + C-terminus, C-terminus. Prey: BunA.

(b) Bait: empty, Madm, Madm full-length, aa 398-566, aa 458-566, aa 458-530, Madm R525H. Prey: BunA.

(c) IP: anti-HA

HA: GFP, Madm, Madm-N, Madm-N-KLD, Madm-KLD-C

WB: anti-BunA

WB: anti-HA

Input: < GFP-BunA (250kDa), < HA-Madm (95kDa), < HA-Madm-KLD-C (80kDa), < HA-Madm-N-KLD (75kDa), < HA-Madm-C (45kDa), < HA-Madm-N (42kDa), < HA-GFP (40kDa).

(d) IP: anti-HA

HA: GFP, Madm aa 398-566, Madm aa 458-566, Madm aa 458-530, Madm R525H

WB: anti-BunA

WB: anti-HA

Input: < GFP-BunA (250kDa), < HA-Madm (95kDa), < HA-aa 398-566 (32kDa), < HA-aa 458-566 (28kDa), < HA-aa 458-530 (20kDa).
Supplemental Figure 2

The C-terminal Madm peptide aa 458-566 is sufficient for the interaction with BunA. (a,b) Y2H assays (Materials and methods) were performed with the Madm constructs shown in Figure 2e. (a) The Madm full-length protein as well as the Madm kinase-like domain (KLD) plus C-terminus and the C-terminus alone interact with full-length BunA, whereas neither KLD plus N-terminus nor N-terminus alone interacts. Thus, the BunA interacting sequence resides within the C-terminus of Madm. (b) Madm peptides encompassing the C-terminal amino acids 398-566 and 458-566 strongly interact with BunA. Since a peptide containing amino acids 458-530 no longer binds to BunA, amino acids 530-566 are not dispensable for the interaction. Moreover, a mutated full-length version of Madm containing the amino acid exchange R525H does not interact with BunA. (c,d) Co-IP experiments (Materials and methods). HA-tagged proteins were immunoprecipitated using anti-HA beads. (c) Consistent with the Y2H results, only HA-tagged Madm peptides including the C-terminus – Madm full-length, Madm-KLD-C and Madm-C – pull down GFP-BunA. Thus, the Madm C-terminus is sufficient for the interaction with BunA. (d) The C-terminal Madm peptides aa 398-566 and 458-566 are able to pull down BunA whereas the peptide 458-530 and the mutated full-length Madm protein (R525H) do not bind to BunA. Note that the IP Western blot was exposed longer than the input Western blot to detect the anti-BunA signal in (c) (exposure times for the detection of the anti-HA signal were the same). See Materials and methods and Additional data file 4 for the anti-BunA antibody.