Supplementary Material

Figure S1. Primary sequence alignment of biotin-binding proteins in fungi. Protein names or identifiers are displayed for each sequences found in *Pleurotus cornucopiae* (Pc), *Galerina marginata* (Gm), *Hypholoma sublateritium* (Hs). Conserved residues are highlighted. Residues that interact with biotin in tamavidin 2 are indicated (*).

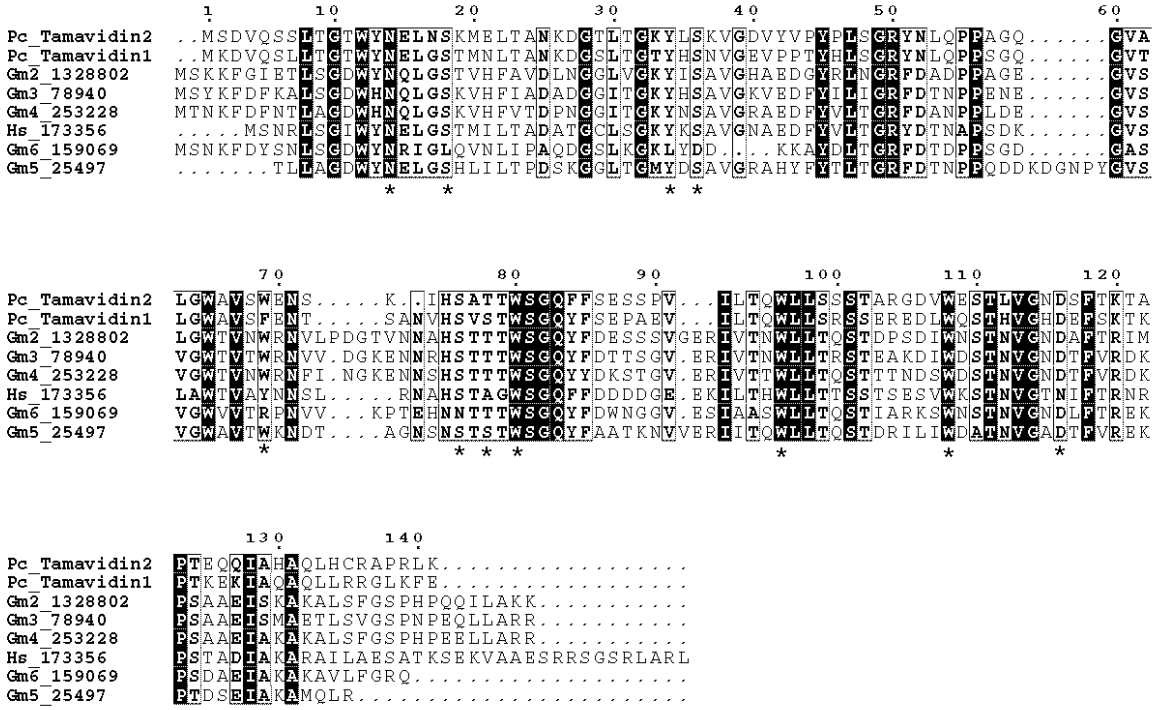
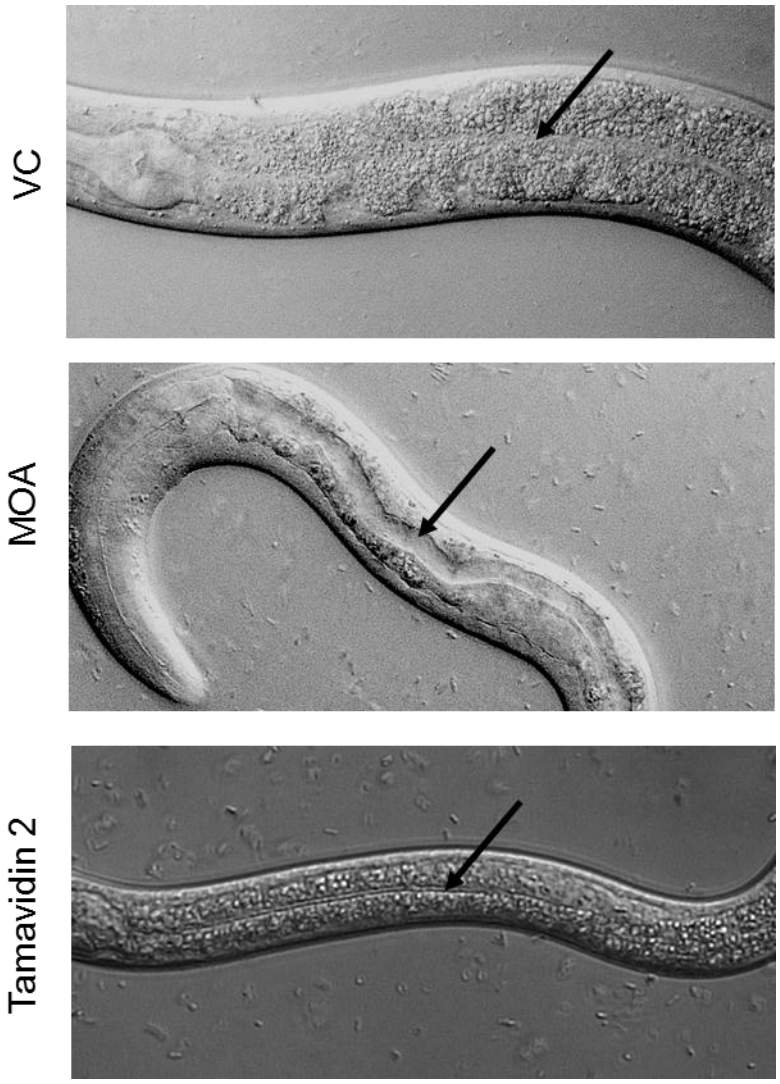


Table S2. Overview of characterized avidin-like, biotin-binding proteins.

Name	Organism	pI	MW (kDa) /Oligomeric	Localization	Reference
Avidin	*Gallus gallus*	10.0	15.6 / tetramer	Secreted	(5)
Streptavidin	*Streptomyces svidinii*	6.1	16.5 / tetramer	Secreted	(2)
Bradavidin I	*Bradyrhizobium japonicum*	6.3	14.3 / tetramer	Secreted	(10)
Bradavidin II	*Bradyrhizobium japonicum*	9.6	12.56 / tetramer	Secreted	(6)
Rhizavidin	*Rhizobium etli*	4.0	14.0 / dimer	Secreted	(7)
Burkavidin	*Burkholderia pseudomallei*	7.4	14.2 / tetramer	Secreted	(11)
Tamavidin 2	*Pleurotus cornucopiae*	7.4	15.5 / tetramer	Cytoplasmic	(13)
Xenavidin	*Xenopus tropicalis*	9.2	18.6 / tetramer	Secreted	(8)

Method S3. Purification of recombinant tamavidin 2. A plasmid encoding a N-terminally His-tagged version of the protein was constructed and expressed in *E.coli* (BL21). The protein was purified via metal affinity chromatography using the Ni-NTA resin (Qiagen) according to manufacturer's protocol.

Figure S4. Comparison of the gut phenotype of *C. elegans* when fed either on recombinant *E.coli* expressing the nematotoxic lectin MOA and Tamavidin 2. *E.coli* expressing a vector control (VC) was used as control. Arrows point to the intestine.



Discussion S5

Considering the ability of these proteins to sequester biotin, the question arises of how organisms producing BBPs avoid reduction or depletion of biotin required for normal metabolism. In the case of plants, where no BBPs have been identified, most biotin exists as a free pool in the cytoplasm, and the rest in protein-bound form in the soluble fractions of chloroplasts and mitochondria (1). For this reason, heterologous expression of BBPs for biological control has to be targeted to the apoplast (12) or the vacuoles (9) in order not to interfere with plant viability. In *E. coli*, most of intracellular biotin is bound to proteins making the intracellular pool of free biotin very low (3, 4). This may explain why recombinant expression of tamavidins and other BBPs in the cytoplasm of *E. coli* is not toxic for the cell. The same may be true for fungi. The tamavidins are most likely synthesized in the cytoplasm of *P. cornucopiae* as they do not have a signal sequence for secretion. Accordingly, recombinant expression of tamavidins in the cytoplasm of the ascomycete *Ashbya gossypii* is possible without deleterious effects for this fungus (unpublished data). This result suggests that also in fungi, free-biotin pools are absent or minimal in the cytoplasm.

References

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