# Supplementary Methods

This document provides supplementary information for the manuscript "The Ortholog Conjecture Revisited: the Value of Orthologs and Paralogs in Function Prediction" by Moses Stamboulian, Rafael F. Guerrero, Matthew W. Hahn, and Predrag Radivojac.

**Maryland bridge similarity.** Let $A$ and $B$ be two sets of GO terms denoting the propagated functional annotations for the proteins $a$ and $b$, respectively. The Maryland bridge similarity [1] is defined as

$$s_{\mathrm{M}}(A, B) = \frac{1}{2} \cdot \left( \frac{|A \cap B|}{|A|} + \frac{|A \cap B|}{|B|} \right), \tag{1}$$

and can be interpreted as an average of precision and recall when the terms associated with protein $a$ are used as a prediction of the terms associated with protein $b$, and *vice versa*. The root term was excluded from these calculations, in order to exploit the full $[0, 1]$ range for functional similarity.

**Schlicker semantic similarity.** The Schlicker similarity used here is a slight modification of the original similarity measure [2], as proposed in [3]. To calculate the similarity between two leaf GO terms, $t_i$ and $t_j$, this measure uses Lin's similarity measure defined as

$$s_{\mathrm{L}}(t_i, t_j) = \frac{2 \cdot s_{\mathrm{R}}(t_i, t_j)}{\log {^1}/_{P(t_i)} + \log {^1}/_{P(t_j)}}, \tag{2}$$

where $P(t)$ is the probability of observing a term $t$ in a randomly selected protein, and $s_{\mathrm{R}}(t_i, t_j)$ is Resnik's similarity [4] between the terms, calculated as

$$s_{\mathrm{R}}(t_i, t_j) = \max_{t \in \mathcal{A}(t_i, t_j)} \frac{1}{\log P(t)}. \tag{3}$$

In Eq. (3), $\mathcal{A}(t_i, t_j)$ indicates the set of common ancestor terms between terms $t_i$ and $t_j$ in the ontology. Term probabilities were estimated using their relative frequencies in the annotations of all species in the UniProt-GOA database.

As gene products are often annotated with more than one leaf term, functional similarity for a protein pair was calculated using the best match average method, proposed originally in Schlicker et al. [2], giving the following Schlicker similarity

$$s_{\mathrm{S}} = \frac{1}{|p_1| + |p_2|} \cdot \left( \sum_{i \in p_1} \max_{j \in p_2}(s_{\mathrm{L}}(i, j)) + \sum_{j \in p_2} \max_{i \in p_1}(s_{\mathrm{L}}(i, j)) \right) \tag{4}$$

Combining the terms in Eq. (4) differs from the original formula suggested in [2] in the way that the two maximum terms are averaged. This particular implementation was chosen to closely match the semantic similarity from [3].

**Topological metrics.** The performance of protein function prediction was evaluated using a leave-one-out strategy based on one topological and one information-theoretic accuracy measure. We used the $F_{\max}$ topological metric, a criterion regularly seen in CAFA [5, 6]. To summarize this performance evaluation process, we consider a prediction algorithm on a

set of $n$ proteins, where each protein $i$ is assigned a score (say, between 0 and 1) for each functional term $v$ in the ontology. We now define an average precision $(pr)$ and recall $(rc)$ at some score threshold $\tau$ as

$$pr(\tau) = \frac{1}{m(\tau)} \sum_{i=1}^{m(\tau)} \frac{\sum_v \mathbb{1}(v \in P_i(\tau) \wedge v \in T_i)}{\sum_v \mathbb{1}(v \in P_i(\tau))} \tag{5}$$

$$rc(\tau) = \frac{1}{n} \sum_{i=1}^{n} \frac{\sum_v \mathbb{1}(v \in P_i(\tau) \wedge v \in T_i)}{\sum_v \mathbb{1}(v \in T_i)}, \tag{6}$$

where $P_i(\tau)$ contains predicted terms with a score greater than or equal to $\tau$ for the $i$-th protein, $T_i$ is the experimental annotation for the $i$-th protein, $m(\tau)$ is the number of proteins having at least one annotation transferred with a score greater than or equal to $\tau$, and $\mathbb{1}$ is an indicator function. The $F_{\max}$ score is defined as

$$F_{\max} = \max_{\tau} \left\{ \frac{2 \cdot pr(\tau) \cdot rc(\tau)}{pr(\tau) + rc(\tau)} \right\} \tag{7}$$

and represents the maximum harmonic mean between average precision and average recall over all thresholds.
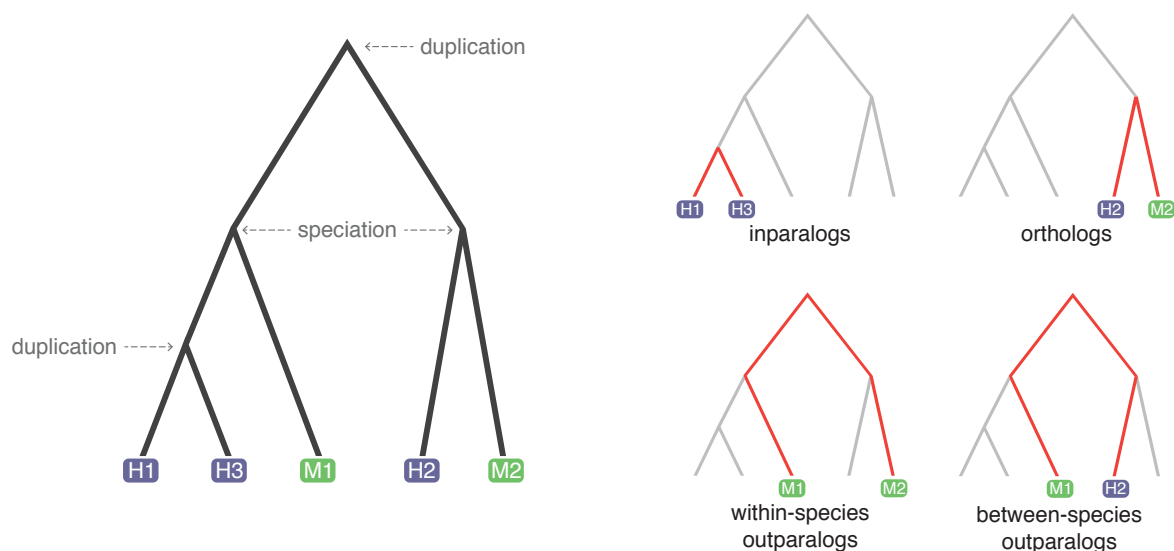
# Supplementary Figures



Figure S1: Four different types of homology relations. A family of five genes sampled from human (in blue) and mouse (in green) evolves through speciation and duplication events (left-hand tree). The relationships among genes are highlighted in the four trees on the right. Human genes H1 and H3 are inparalogs, arising from a duplication event after the most recent speciation event in the tree. Genes H2 and M2 are orthologs, as they are related through a speciation event. Gene pairs M1-M2 and M1-H2 are outparalogs because they arose from a duplication that predates the reference speciation event. Pair M1-M2 are within-species outparalogs, while M1-H2 are between-species outparalogs.
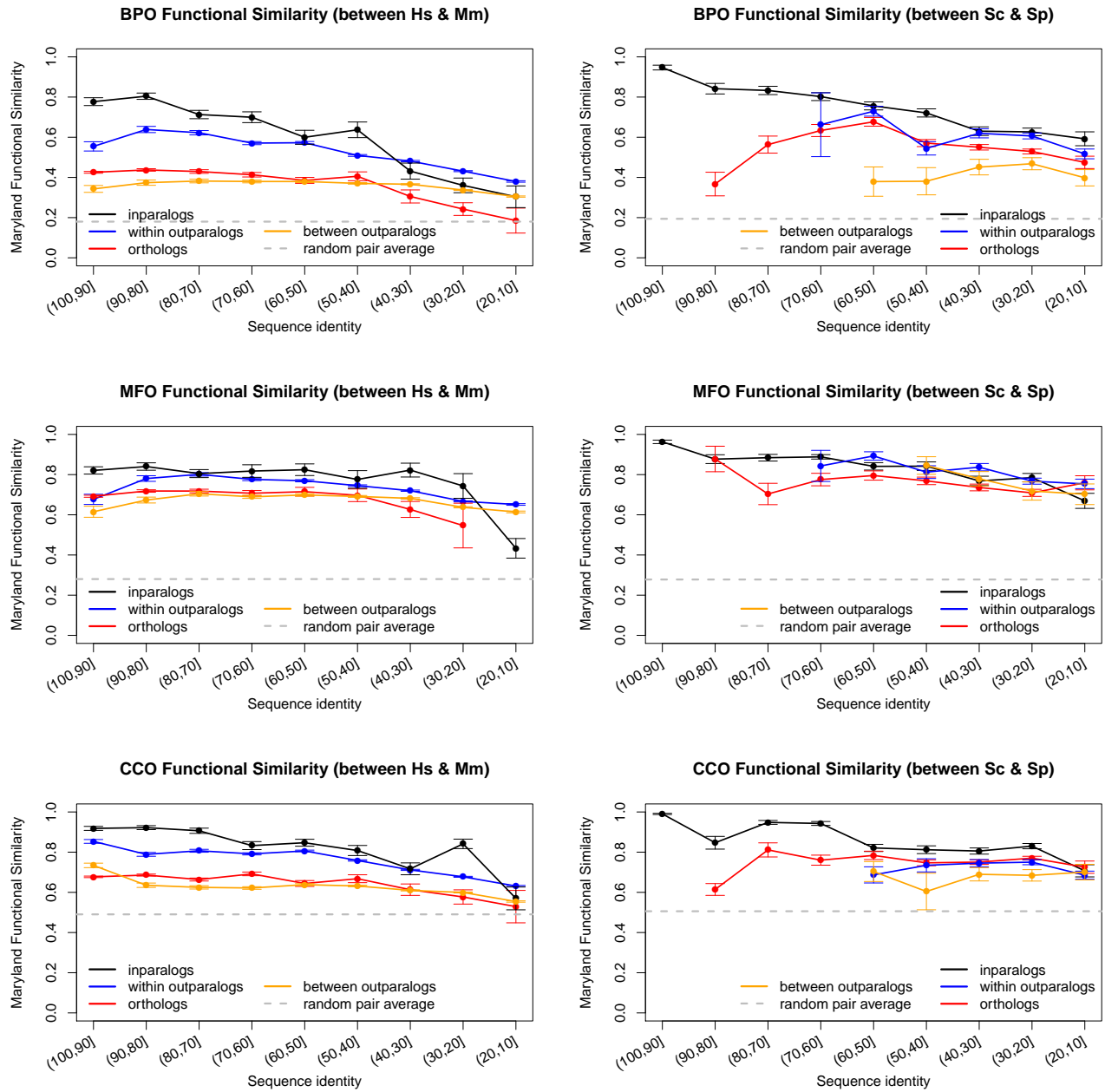
Figure S2: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe homologs for all three ontologies, using Maryland bridge similarity.
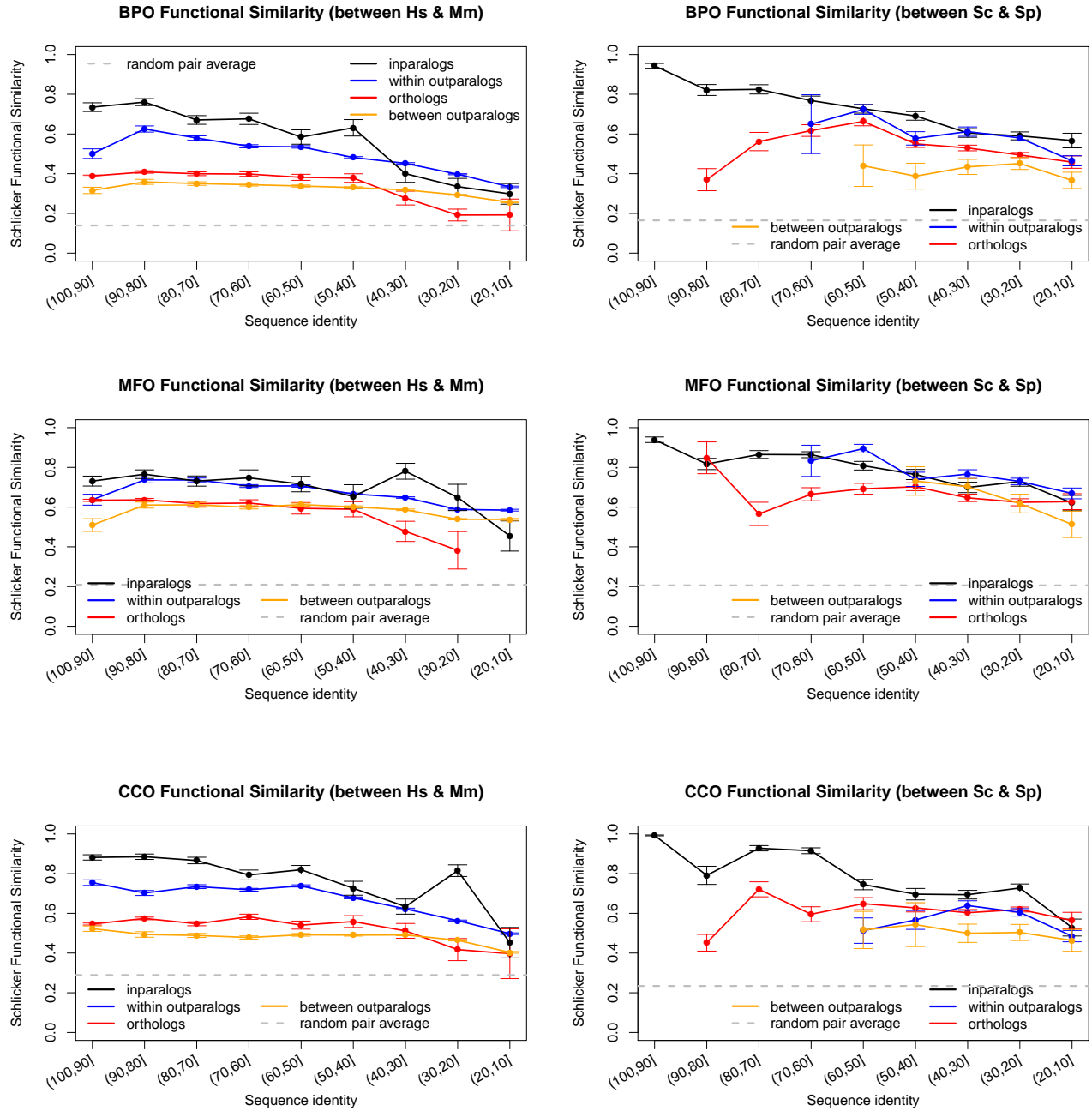
Figure S3: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe homologs for all three ontologies, using Schlicker's similarity.
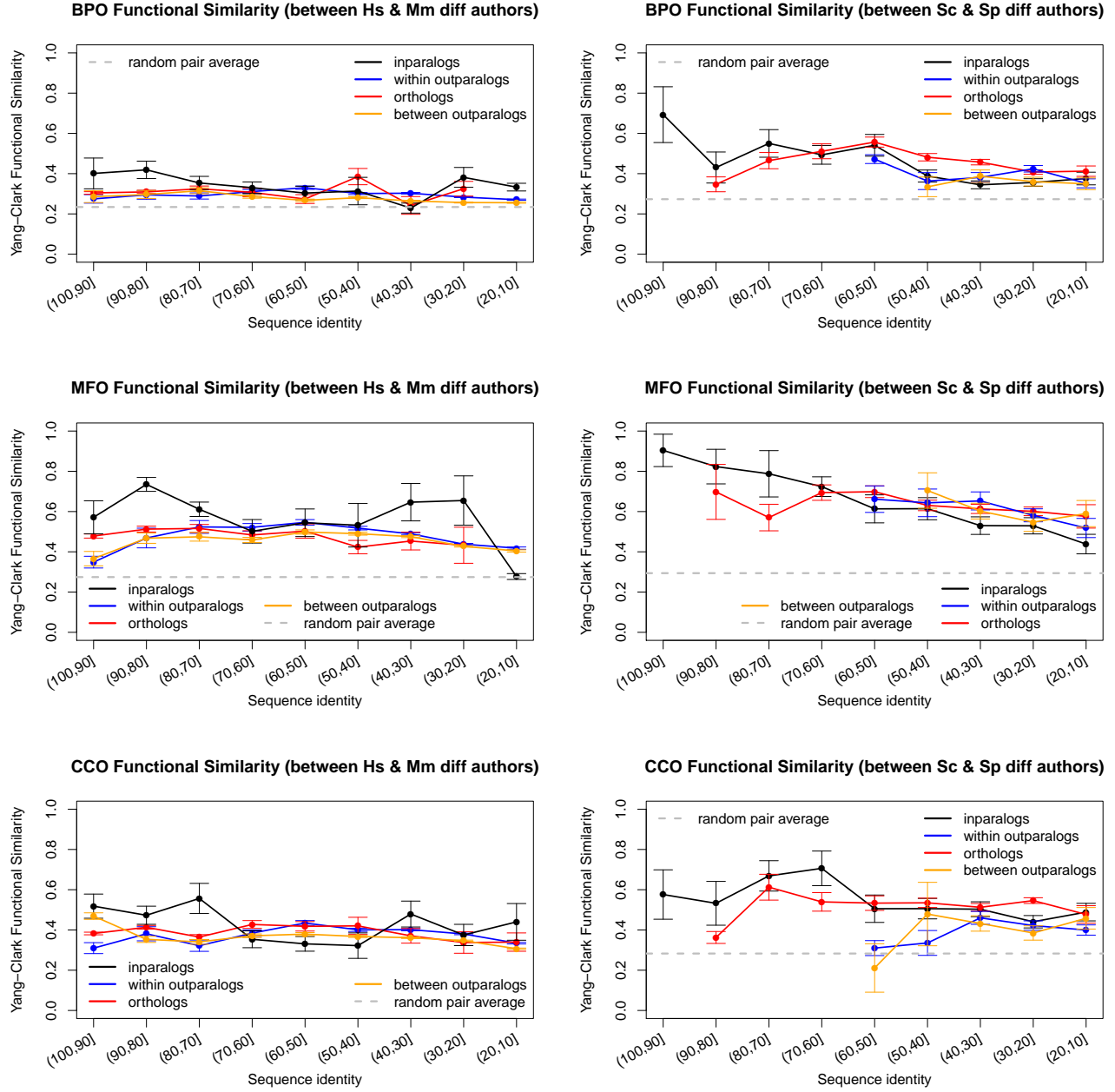
Figure S4: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe pairs annotated by different authors, for all three ontologies, using Yang-Clark similarity.
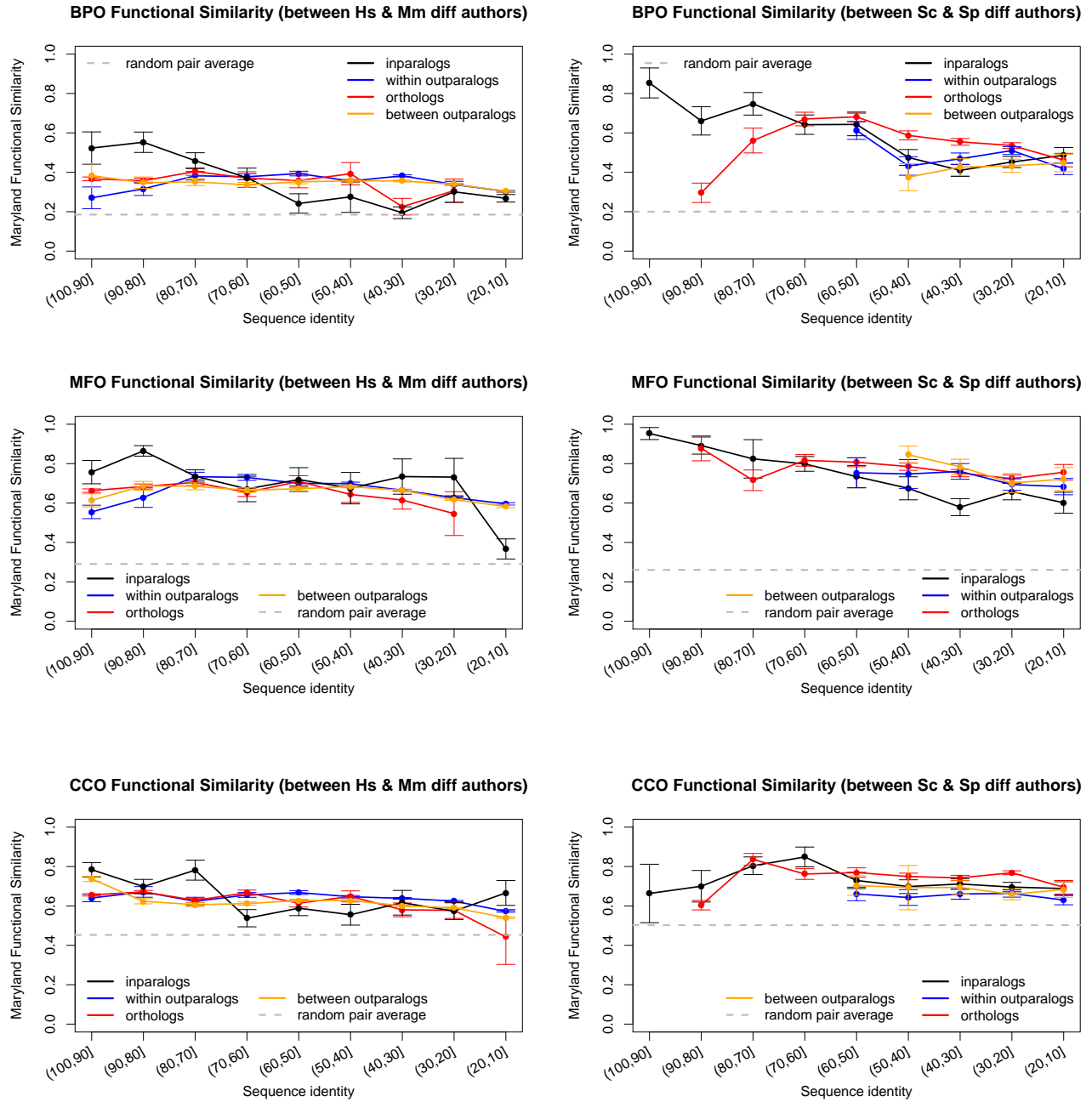
Figure S5: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe pairs annotated by different authors, for all three ontologies, using Maryland bridge similarity.
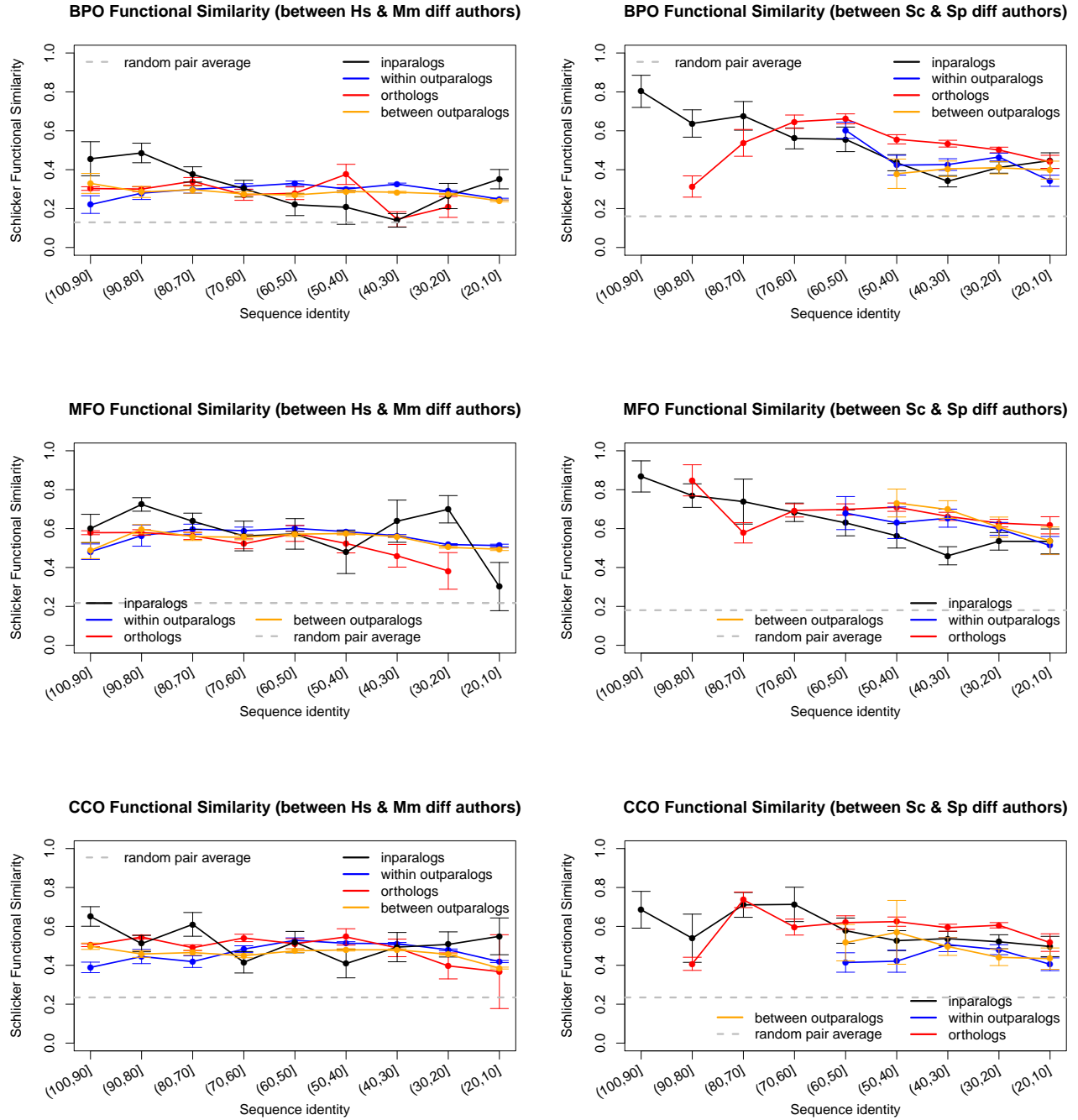
Figure S6: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe pairs annotated by different authors, for all three ontologies, using Schlicker's similarity.

Figure S7: Background similarity for different types of homology relationships for human-mouse (left panels) and cerevisiae-pombe (right panels), using Yang-Clark similarity.
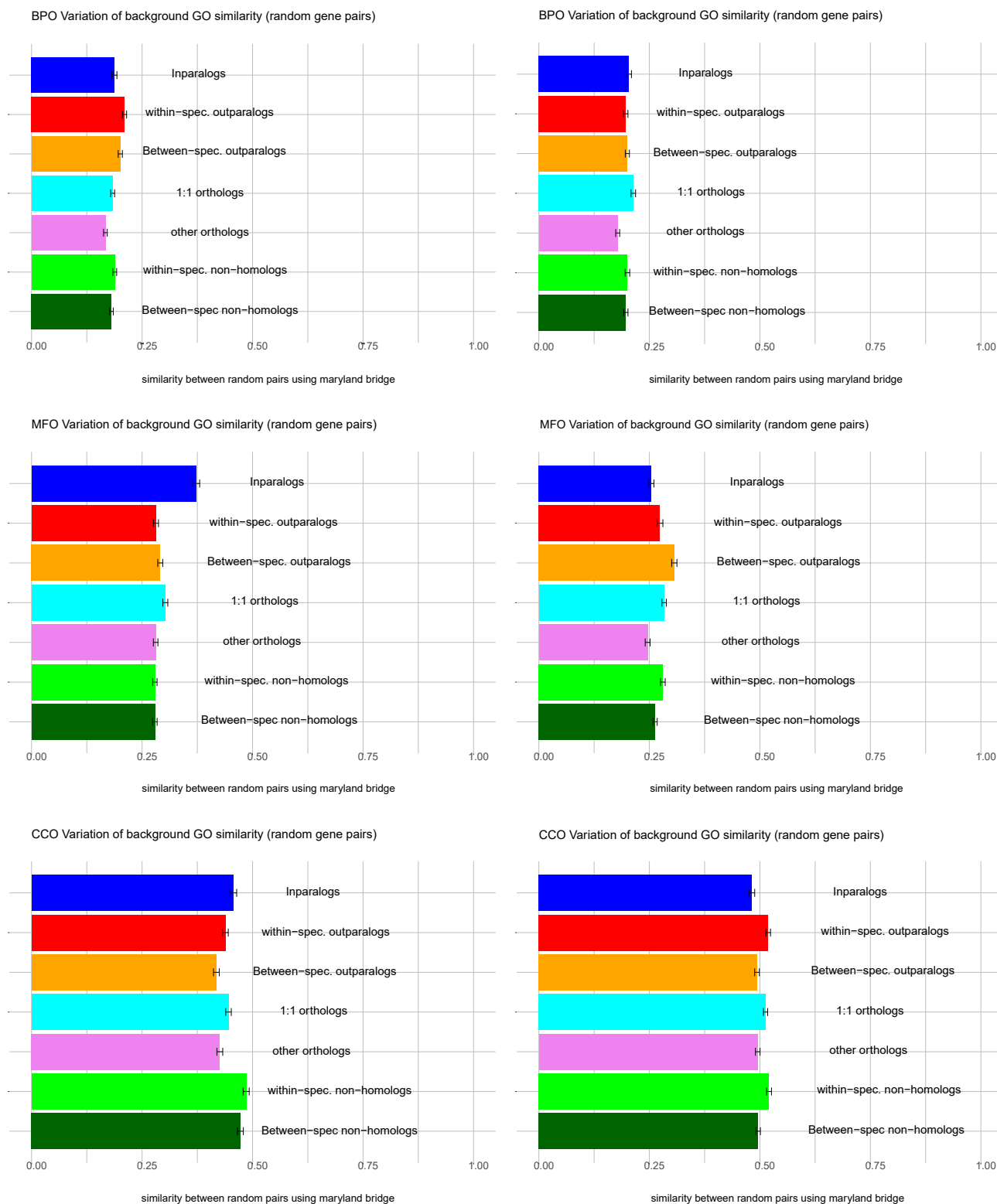
Figure S8: Background similarity for different types of homology relationships for human-mouse (left panels) and cerevisiae-pombe (right panels), using Maryland bridge similarity.

Figure S9: Background similarity for different types of homology relationships for human-mouse (left panels) and cerevisiae-pombe (right panels), using Schlicker's similarity.
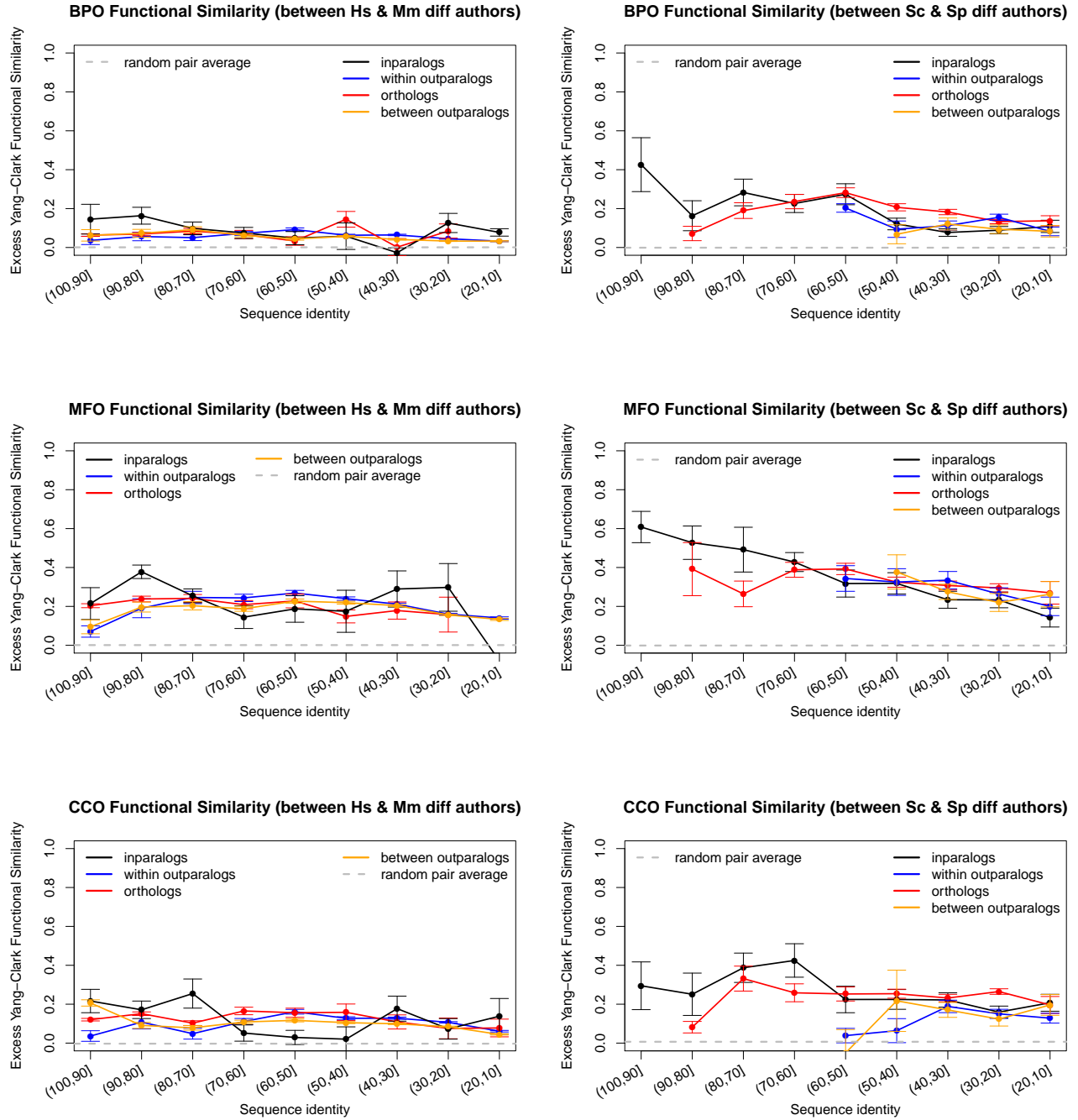
Figure S10: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe pairs annotated by different authors after removing background similarities, for all three ontologies, using Yang-Clark similarity.
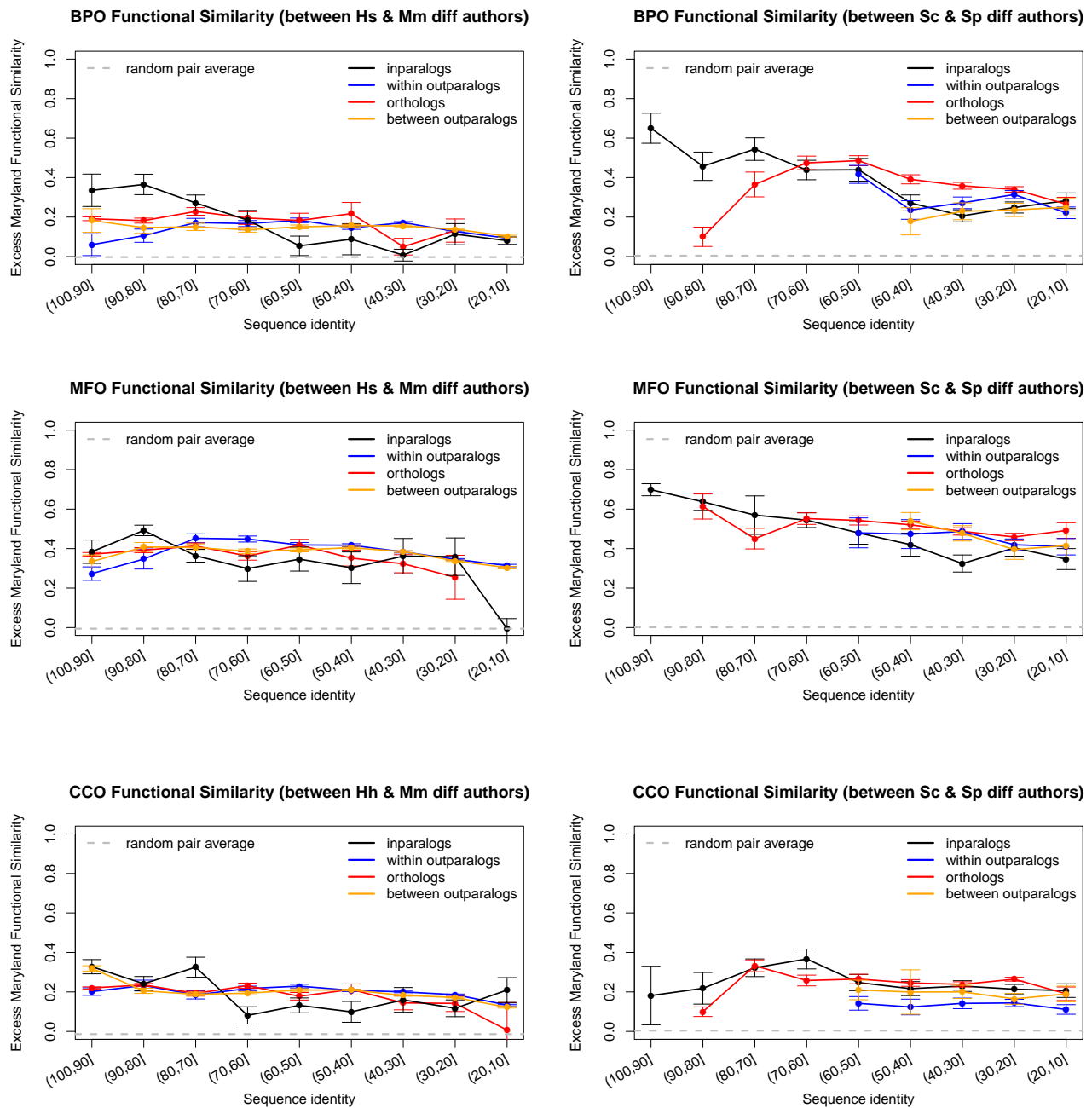
Figure S11: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe pairs annotated by different authors after removing background similarities, for all three ontologies, using Maryland bridge similarity.
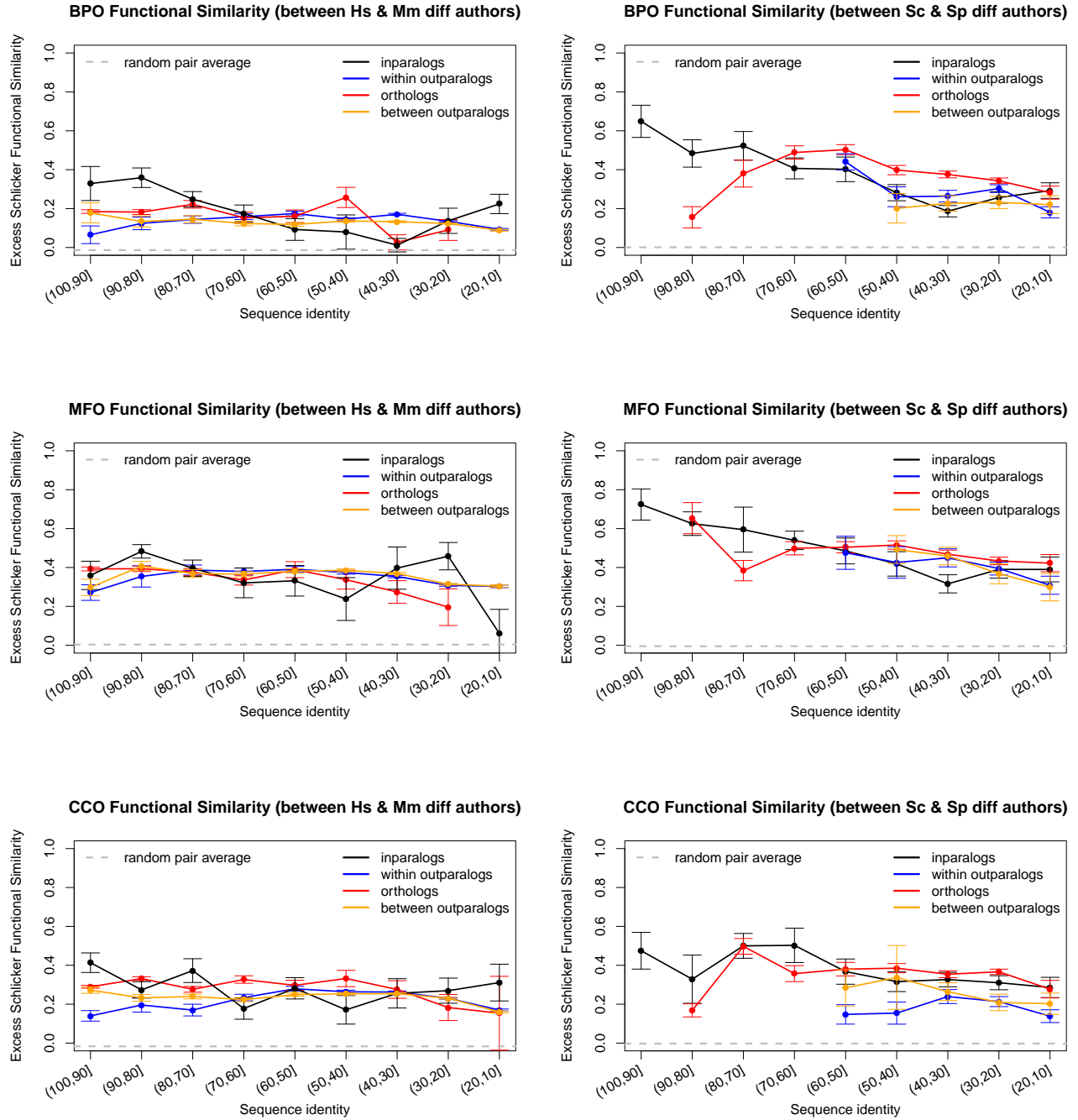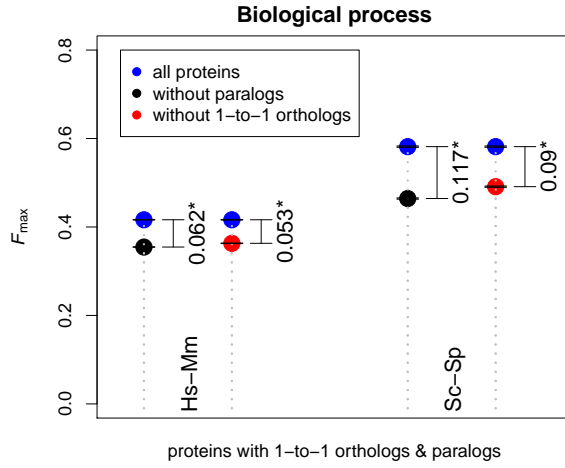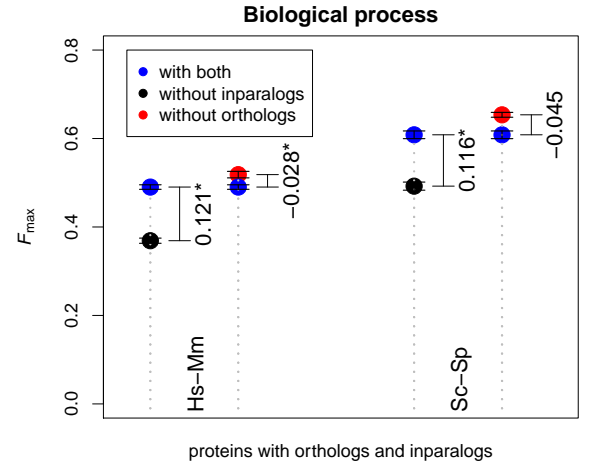
Figure S12: Relationship between functional similarity and sequence identity for human-mouse and cerevisiae-pombe pairs annotated by different authors after removing background similarity, for all three ontologies, using Schlicker's similarity.
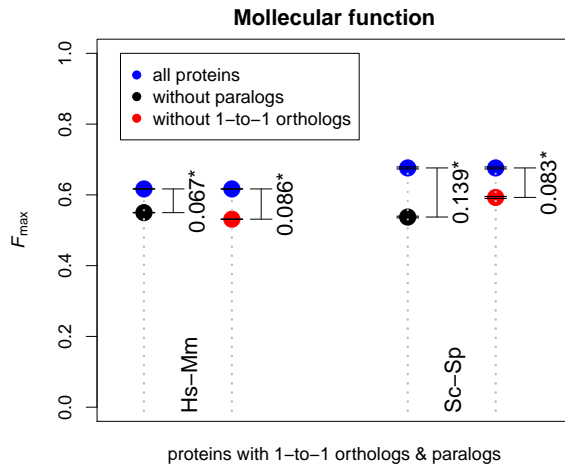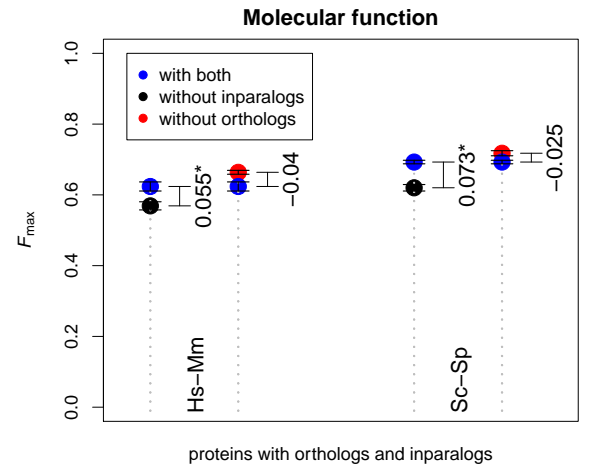
(a)

(b)

Figure S13: $F_{max}$ values for proteins having orthologs, proteins having orthologs and other homologs (a) and proteins having 1-to-1 orthologs and paralogs (b) in human-mouse and cerevisiae-pombe using biological process ontology (* indicates values are statistically significant).



(a)

(b)

Figure S14: $F_{max}$ values for proteins having orthologs, proteins having orthologs and other homologs (a) and proteins having 1-to-1 orthologs and paralogs (b) in human-mouse and cerevisiae-pombe using molecular function ontology (* indicates values are statistically significant).
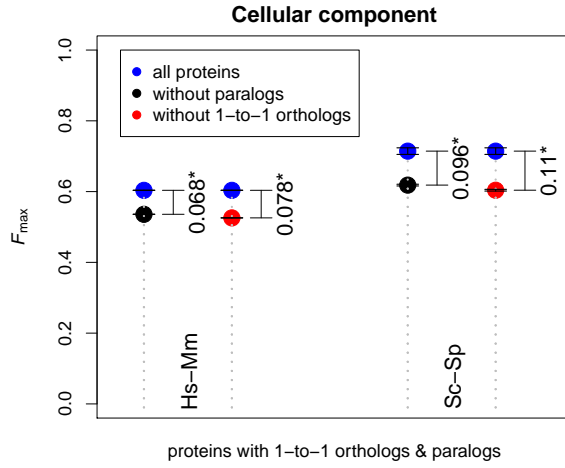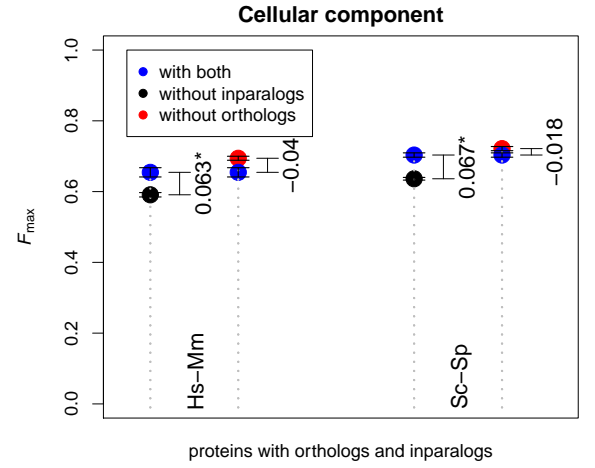
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(a)

(b)

Figure S15: $F_{max}$ values for proteins having orthologs, proteins having orthologs and other homologs (a) and proteins having 1-to-1 orthologs and paralogs (b) in human-mouse and cerevisiae-pombe using cellular context ontology (* indicates values are statistically significant).



(a)

(b)

Figure S16: $S_{max}$ values for proteins having inparalogs, proteins having inparalogs and other homologs (a), and proteins having both inparalogs and orthologs (b) in human-mouse and cerevisiae-pombe using biological process ontology (* indicates values are statistically significant).

Figure S17: $S_{\mathrm{max}}$ values for proteins having inparalogs, proteins having inparalogs and other homologs (a), and proteins having both inparalogs and orthologs (b) in human-mouse and cerevisiae-pombe using molecular function ontology (* indicates values are statistically significant).
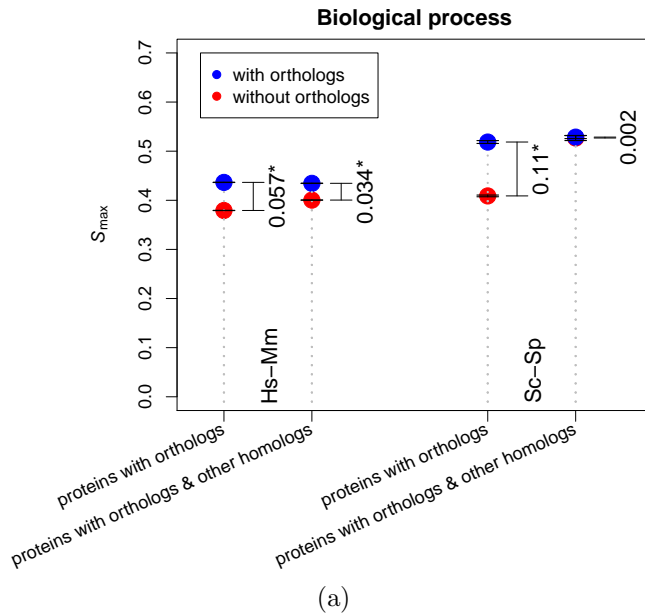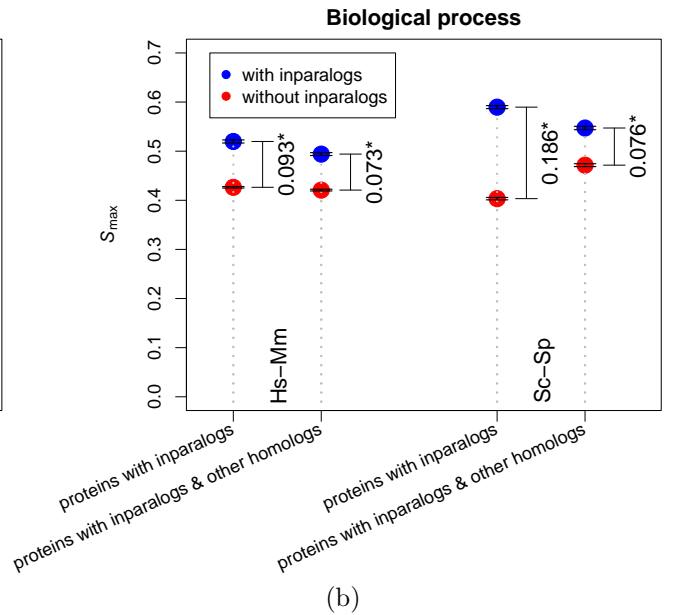


Figure S18: $S_{\mathrm{max}}$ values for proteins having inparalogs, proteins having inparalogs and other homologs (a), and proteins having both inparalogs and orthologs (b) in human-mouse and cerevisiae-pombe using cellular context ontology (* indicates values are statistically significant).

Figure S19: $F_{\max}$ values for proteins having inparalogs, proteins having inparalogs and other homologs (a), and proteins having both inparalogs and orthologs (b) in human-mouse and cerevisiae-pombe using biological process ontology (* indicates values are statistically significant).
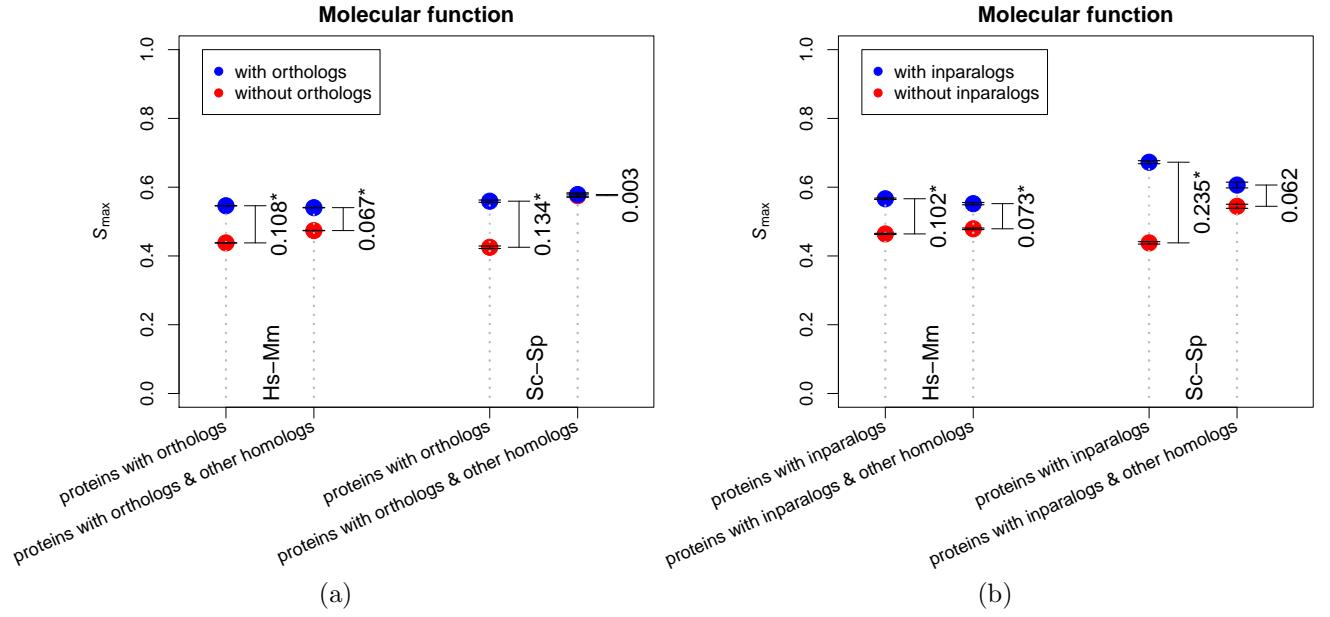


Figure S20: $F_{\max}$ values for proteins having inparalogs, proteins having inparalogs and other homologs (a), and proteins having both inparalogs and orthologs (b) in human-mouse and cerevisiae-pombe using molecular function ontology (* indicates values are statistically significant).
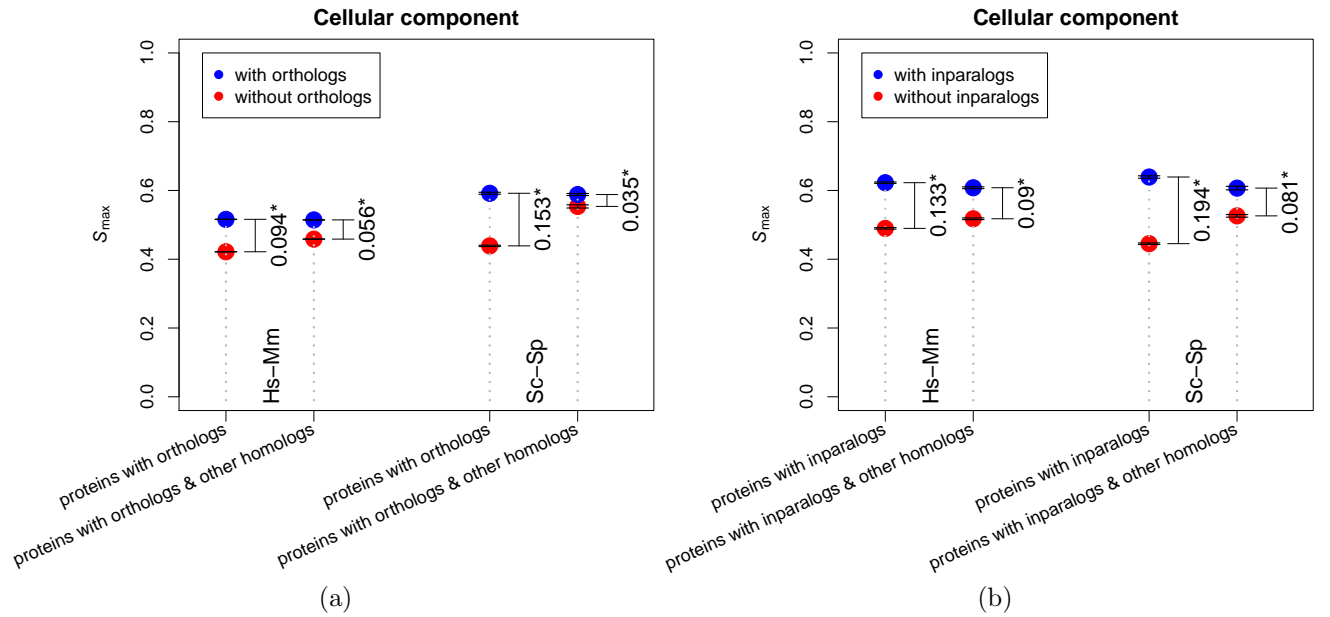
Figure S21: $F_{\max}$ values for proteins having inparalogs, proteins having inparalogs and other homologs (a), and proteins having both inparalogs and orthologs (b) in human-mouse and cerevisiae-pombe using cellular context ontology (* indicates values are statistically significant).
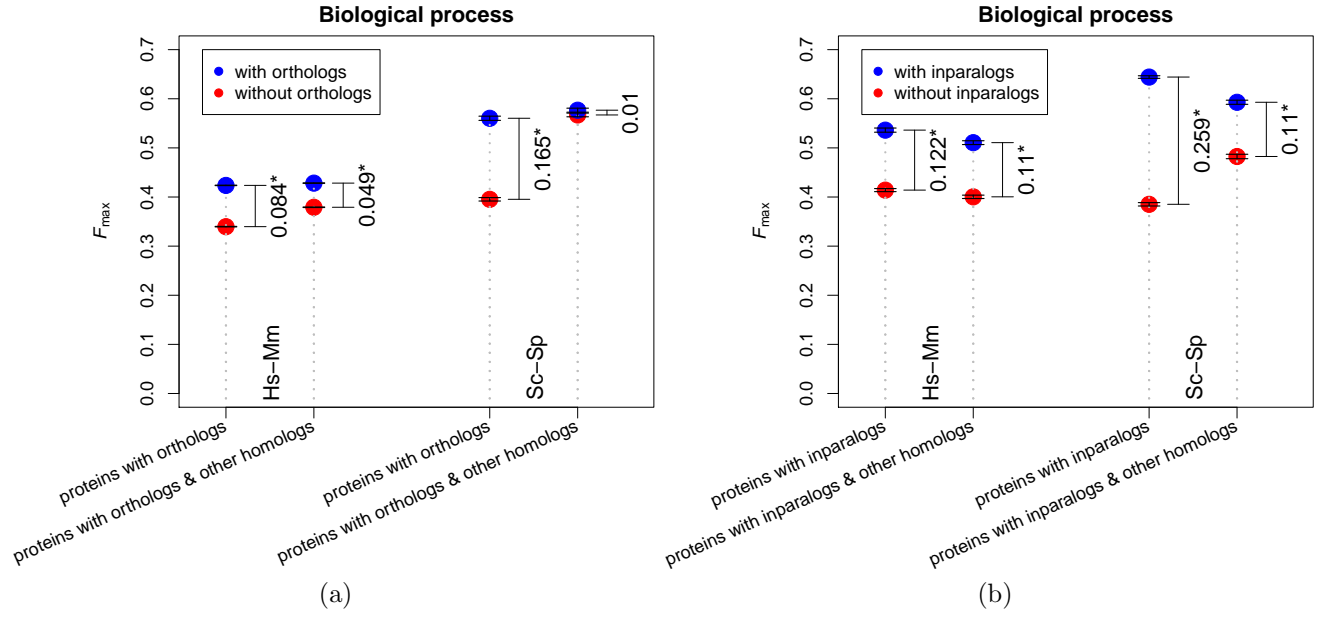


Figure S22: $S_{\max}$ values for proteins both orthologs and within species homologs (a), Fmax values for proteins having both orthologs and within species homologs in human-mouse and cerevisiae-pombe using biological process ontology (* indicates values are statistically significant).

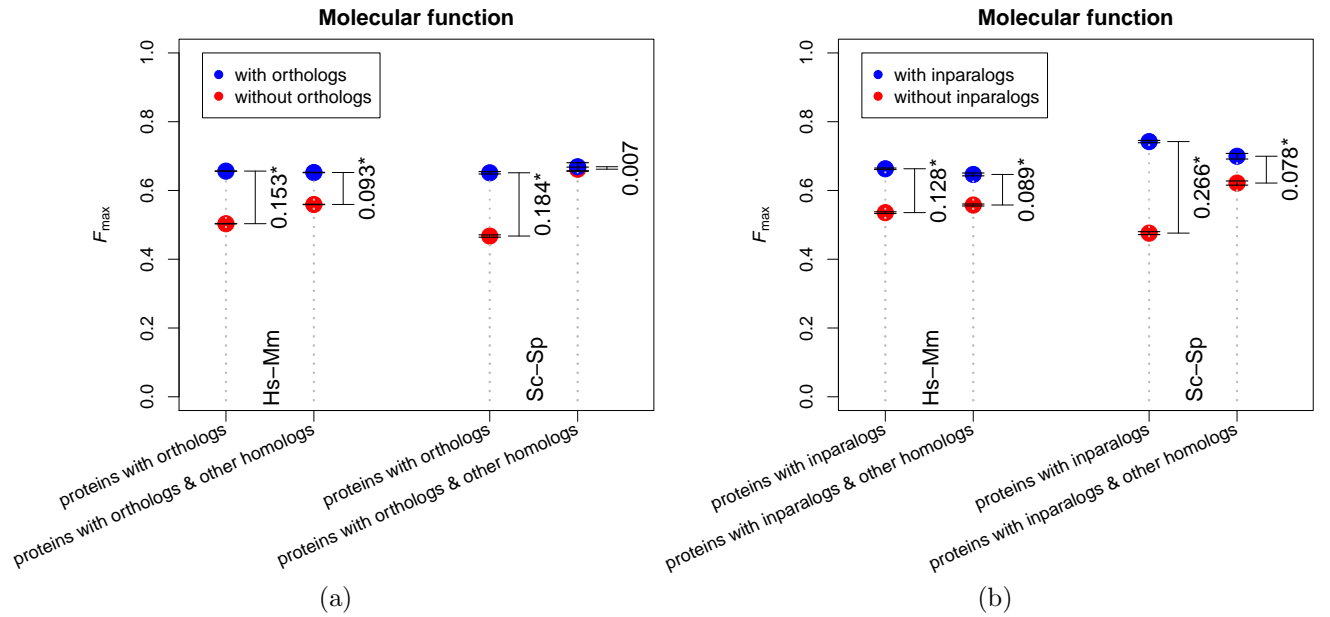(a)                                           (b)

Figure S23: $S_{\max}$ values for proteins both orthologs and within species homologs (a), Fmax values for proteins having both orthologs and within species homologs in human-mouse and cerevisiae-pombe using molecular function ontology (* indicates values are statistically significant).
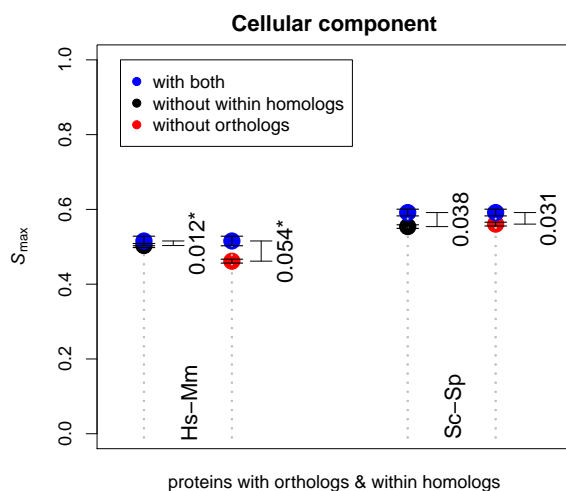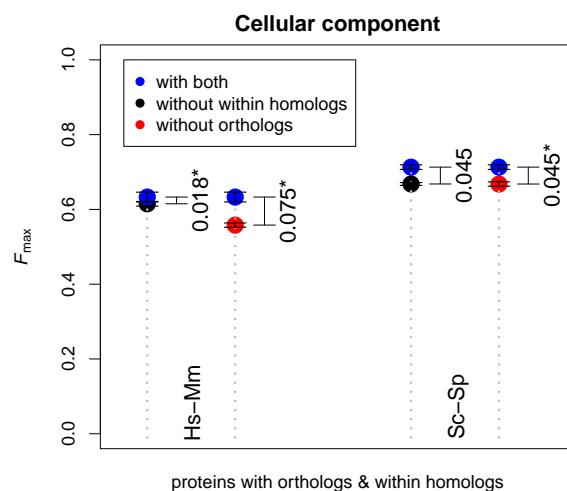


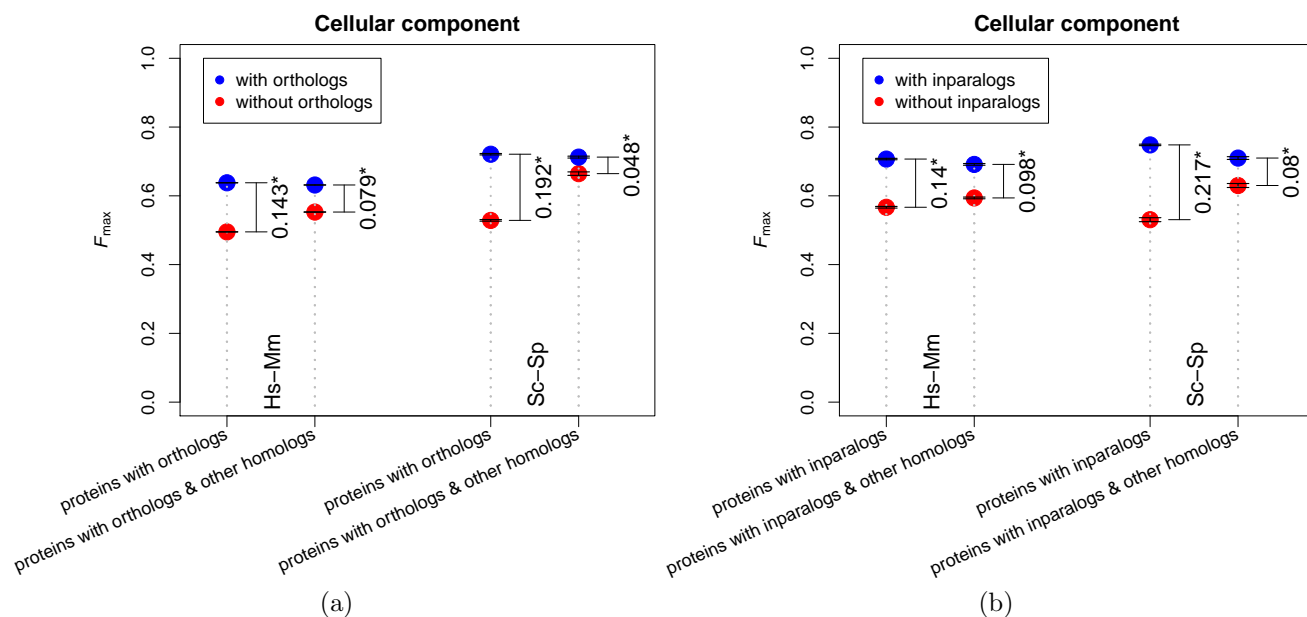(a)                                           (b)
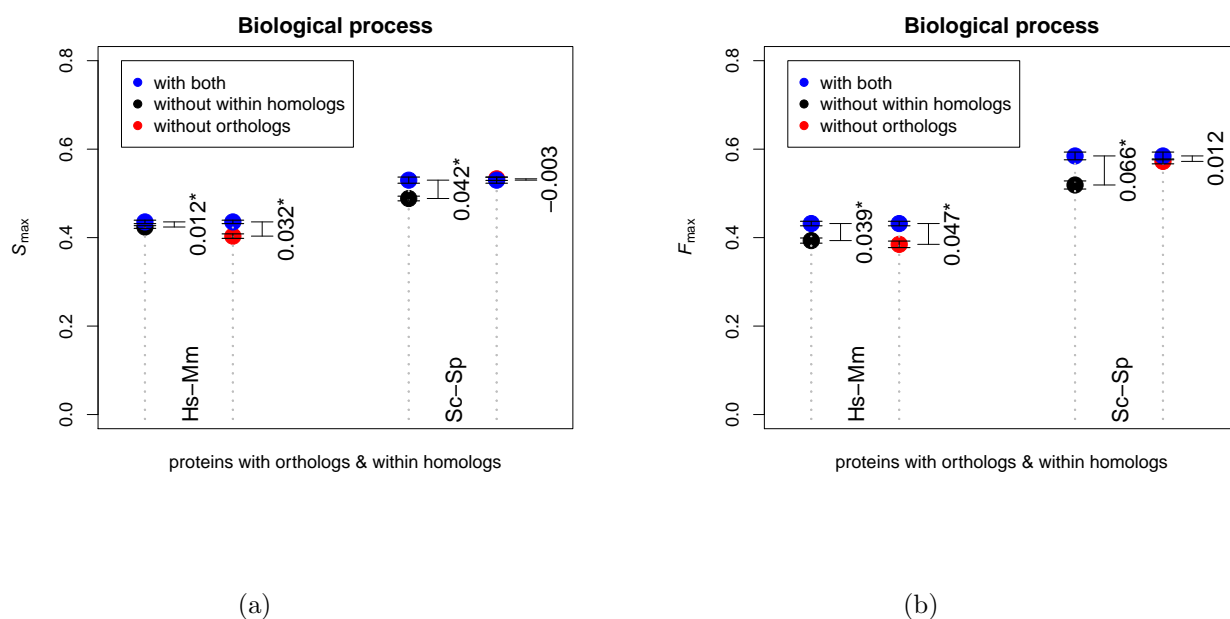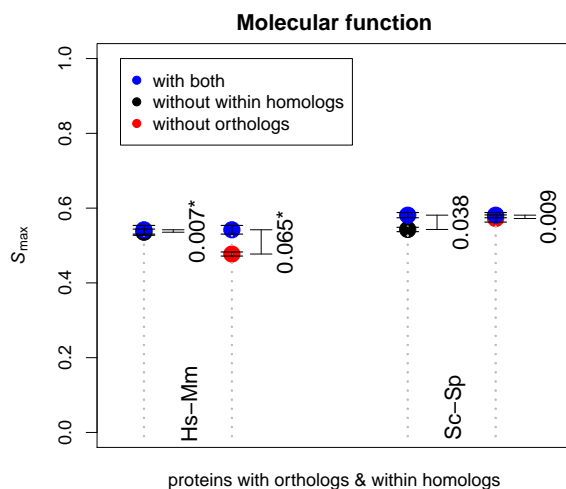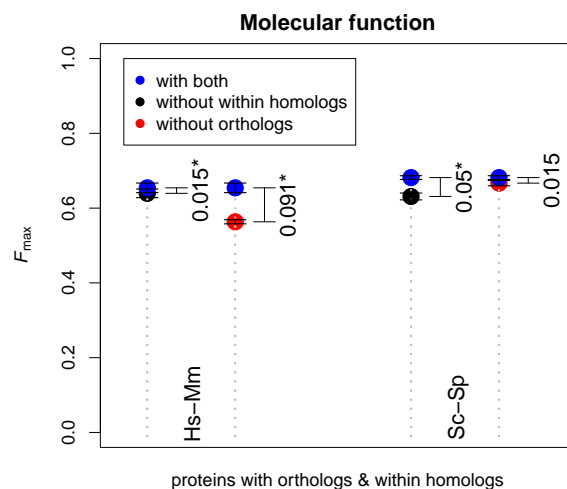
Figure S24: $S_{\max}$ values for proteins both orthologs and within species homologs (a), Fmax values for proteins having both orthologs and within species homologs in human-mouse and cerevisiae-pombe using cellular context ontology (* indicates values are statistically significant).

**a.** *H. sapiens* and *M. musculus* genomes

8,606 gene families (40,595 genes)

within-species paralogs

orthologs

573 (2,423)

84 (317)

4,744 (9,488)

3,109 (27,515)

84 (828)

7 (14)

5 (10)

between-species paralogs
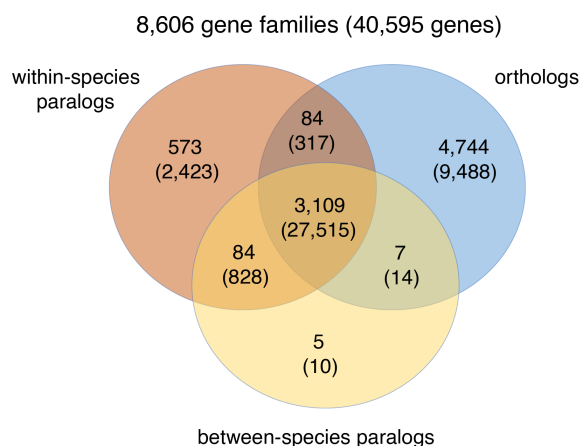
**b.** *S. cerevisiae* and *S. pombe* genomes

3,059 gene families (7,030 genes)

within-species paralogs

orthologs

948 (2,613)

48 (152)

1,969 (3,938)

49 (167)

34 (140)

9 (18)

2 (4)

between-species paralogs
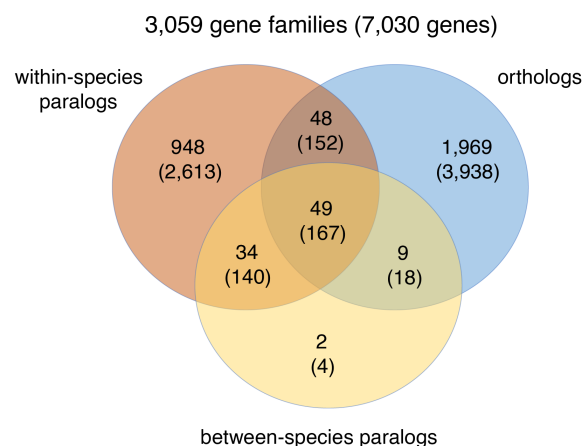
Figure S25: The numbers of gene families with different types of homologous relationships in them, where orthologs represent only one-to-one orthologs (Figure 3 in the main text considers all types of orthologs). The numbers in parentheses represent the counts of genes in the respective gene families. (a) gene families containing *H. sapiens* and *M. musculus* proteins, and (b) gene families containing *S. cerevisiae* and *S. pombe* proteins.

Table S1: Number of genes from human-mouse forming gene families, homologous pairs and number of families with different types of homologies.

| | | |
|---|---|---|
| Gene families (from gene trees) | 8686 | |
| genes making gene families | 41501 | (19,731 human + 21,770 mouse) |
| genes making homologous pairs | 40912 | (19,514 human + 21,398 mouse) |
| intersection (gene fams & hom pairs) | 40,884 | |
| protein coding genes | 44,603 | (22,376 human + 22,227 mouse) |
| intersection (p_coding genes & hom_genes) | 40,722 | |
| intersection (p_coding genes & gene families) | 40,741 | |
| total # of homologous pairs found in gene trees | 21,8254 | |
| ortholog pairs | 24,106 | |
| within species inparalog pairs | 33,741 | |
| within species outparalog pairs | 88,625 | |
| between specie outparalog pairs | 71,782 | |
| clusters containing homolgous pairs (at least 1 pair) | | |
| ortholog pairs | 8,606 | 99.07% |
| within inparalog pairs | 1,299 | 14.95% |
| between outparalog pairs | 3,205 | 36.89% |
| within outparalog pairs | 3,249 | 37.40% |
| both within inparalog and ortholog pairs | 1299 | 14.95% |
| both between homolog and within homolog | 3,850 | 44.32% |
| ortholog only families | 4,744 | 55.12% |

Table S2: Number of genes from cerevisiae-pombe forming gene families, homologous pairs and number of families with different types of homologies.

| | | |
|---|---|---|
| Gene families (from gene trees) | 3,061 | |
| genes making gene families | 7,335 | (4,042 yeast + 3,293 pombe) |
| genes making homologous pairs | 7,691 | (4,208 yeast + 3,483 pombe) |
| intersection (gene fams & hom pairs) | 7,334 | |
| protein coding genes | 11,839 | (6,693 yeast + 5,146 pombe) |
| intersection (p_coding genes & hom_genes) | 7,690 | |
| intersection (p_coding genes & gene families) | 3,061 | |
| total # of homologous pairs found in gene trees | 8,621 | |
| ortholog pairs | 3,249 | |
| within species inparalog pairs | 5,019 | |
| within species outparalog pairs | 202 | |
| between specie outparalog pairs | 151 | |
| clusters containing homolgous pairs (at least 1 pair) | | |
| ortholog pairs | 2,488 | 81.33% |
| within inparalog pairs | 999 | 32.60% |
| between outparalog pairs | 94 | 3.00% |
| within outparalog pairs | 130 | 4.20% |
| both within inparalog and ortholog pairs | 463 | 15.12% |
| both between homolog and within homolog | 1,079 | 35.27% |
| ortholog only families | 1,969 | 64.03% |

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