Gene number expansion and contraction in vertebrate genomes with respect to invertebrate genomes

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Functional bias in vertebrate and invertebrate gene groups (gene type number *vs.* gene copy number)

In general, the patterns of functional bias for the number of gene types and for the number of gene copies are similar, though more significantly differential categories are observed for gene copy number than for gene type number because there are more gene copies than gene types (Fig. 6 and Supplemental Fig. 4S). However, for some functional categories we observe different functional bias patterns between gene type number and gene copy number. For the biological process categories, the magnitude of gene copy number overrepresentation in *Vonly* for each of the signal transduction, multicellular organismal development and "biological_process" categories is larger than the magnitude of gene type number overrepresentation. Moreover, the gene copy numbers for the signal transduction and "biological_process" categories are significantly overrepresented in *Vonly*, *VFProk*, and *V.A50* and underrepresented in *V.MCL* (Supplemental Fig. 4S), whereas the gene type numbers in these two categories are significantly overrepresented only in *Vonly* and *V.A50* (Fig 6). The gene copy number in the regulation of biological process category is overrepresented in both *Vonly* and *V.MCL* but underrepresented in *V.A50* and *VFProk* (Supplemental Fig 4S). However, the number of gene types in this category is overrepresented in *Vonly* but underrepresented in *V.MCL* (Fig. 6). The inconsistent patterns of functional bias between the number of gene types and the number of gene copies are observed only for the genes involved in transcription. In

this category, gene types are significantly overrepresented in *Vonly* but significantly underrepresented in *V.MCL*; however, gene copies are significantly underrepresented in *Vonly* but overrepresented in *V.MCL* (Fig 6 vs. Supplemental Fig. 4S).

For the cellular component categories, the functional bias patterns for gene type number and for gene copy number are very similar. However, different patterns are observed for the proteins localized to chromosome or to cytoskeleton: the number of gene copies is significantly *under*represented in *Vonly* and *over*represented in *V.MCL*, but the number of gene types is *over*represented in *Vonly* and *under*represented in *V.MCL* (Supplemental Figs 5S and 6S).

In addition, a similar comment applies to functional bias patterns for the numbers of the invertebrate gene copies and gene types (Supplemental Figs 7S vs. 8S and Figs. 9S vs. 10S). In particular, the opposite functional bias patterns between the number of gene copies and the number of gene types are observed for the invertebrate genes involved in the transcription, multicellular organismal development, and anatomical structure morphogenesis categories, where the number of gene copies is significantly overrepresented in *Ionly* (and underrepresented in *I.MCL*) but the number of gene types is underrepresented in *Ionly* (and overrepresented in *I.MCL*) (Figs 7S and 8S).

In summary, except for some functional categories, the functional bias patterns for the gene copy number and for the gene type number are similar.

Supplemental Tables:

Table 1S. The numbers of genes (and gene types [a]) in invertebrate gene groups.

Invertebrate gene group	ciona	sea urchin	yellow fever mosquito	African mosquito	fruitfly	worm
I.MCL	9151 (4497)	16958 (5592)	9192 (4334)	7997 (4290)	8125 (4362)	7428 (3575)
I.A50	137 (88)	415 (120)	335 (94)	302 (97)	194 (70)	185 (35)
Ionly	4895 (3937)	4104 (2777)	5873 (4113)	4885 (3943)	5701 (4812)	12420 (7818)
Ionly genes shared by ≥ 2 invertebrate genomes	141 (66)	201 (93)	2868 (1617)	2273 (1562)	1406 (938)	98 (53)
All genes	14183 (8522)	21477 (8489)	15400 (8541)	13184 (8330)	14020 (9244)	20033 (11428)

[a] number of gene types is the sum of the number of singletons and the number of gene families.

Supplemental Figures:

Figure 1S. Proportions of invertebrate genes grouped by homology in the 12 vertebrate genomes (4 fish plus 8 land vertebrate genomes). For each invertebrate genome on the x-axis, the invertebrate gene groups are represented, from the top to bottom, by segments of a bar for the genes that can be found in ≥1 genome of the 12 invertebrate genomes with standard homology search criteria (denoted as *I.MCL*), for those that are not in *I.MCL* but have homolog in the vertebrate genomes when the homology search criteria are relaxed (denoted as *I.A50*), and for those that are in none of the above two groups (denoted as *Ionly*).

Figure 2S. Proportions of the invertebrate *I.MCL*, *I.A50*, and *Ionly* genes that are singletons, in the two-gene families, or in the multigene families.

Figure 3S. The slopes from a simple linear model (*A*) and a robust linear model (*B*) between the average vertebrate family size (n_v) against the average invertebrate family size (n_i) for each GOSlim cellular component category in the *V.MCL* gene families. The error bar represents a 95% confidence interval of a slope from 1,000 bootstrap replicates. The slope from the original data and the mean of the bootstrapped slopes for each category are indicated by the circular and triangular points, respectively. The x-axis shows the cellular component categories with the *p*-value < 0.01 for a null hypothesis of the estimated slopes = 0 in either the simple linear model or the robust linear model. These categories are ordered by the mean of their bootstrapped slopes in panel *A*. Most of the proteins with GOSlim in the "cellular_component" category are those in extracellular matrix, synapse, viral capsid, and viral envelope. Furthermore, most of these gene families are also assigned to other GOSlim categories (mainly to cell, cytoplasmic membrane-bound vesicle, plasma membrane, cytoplasm, extracellular space or extracellular region).

Figure 4S. Functional bias in each GOSlim biological process category is shown for the *V.MCL* and *Vonly* groups (represented, respectively, by the circular and triangular points) in the top panel and for the *V.A50* and *VFProk* gene groups (represented, respectively, by the diamond and square points) in the bottom panel. The significant

functional bias categories at the 5% false discovery rate are marked by red S. The magnitude for the under- or over-represented gene copies (below or above 0, respectively) from the overall average is indicated on the y-axis. Most of the proteins with GOSlim in the "biological_process" category are those involved in cell adhesion, response to stimulus, sensory perception of smell, immune response, homophilic cell adhesion, and defense response. Furthermore, most of these gene families are also assigned to other GOSlim categories (mainly to signal transduction, regulation of biological process, response to stress or multicellular organismal development).

Figure 5S. Functional bias in each GOSlim cellular compartment category is shown for the *V.MCL* and *Vonly* groups (represented, respectively, by the circular and triangular points) in the top panel and for the *V.A50* and *VFProk* gene groups (represented, respectively, by the diamond and square points) in the bottom panel. The significant functional bias categories at the 5% false discovery rate are marked by red S. The magnitude for the under- or over-represented families (below or above 0, respectively) from the overall average is indicated on the y-axis. Most of the proteins with GOSlim in the "cellular_component" category are those in extracellular matrix, synapse, viral capsid, and viral envelope. Furthermore, most of these gene families are also assigned to other GOSlim categories (mainly to cell, cytoplasmic membrane-bound vesicle, plasma membrane, cytoplasm, extracellular space or extracellular region).

Figure 6S. Functional bias in each GOSlim cellular component category is shown for the *V.MCL* and *Vonly* groups (represented, respectively, by the circular and triangular points) in the top panel and for the *V.A50* and *VFProk* gene groups (represented, respectively, by the diamond and square points) in the bottom panel. The significant functional bias categories at the 5% false discovery rate are marked by red S. The magnitude for the under- or over-represented gene copies (below or above 0, respectively) from the overall average is indicated on the y-axis. Most of the proteins with GOSlim in the "cellular_component" category are those in extracellular matrix, synapse, viral capsid, and viral envelope. Furthermore, most of these gene families are also assigned to other GOSlim categories (mainly to cell, cytoplasmic membrane-bound vesicle, plasma membrane, cytoplasm, extracellular space or extracellular region).

Figure 7S. Functional bias in each GOSlim biological process category is shown for the *I.MCL*, *I.A50* and *Ionly* groups (represented, respectively, by the circular, diamond and triangular points). The significant functional bias categories at the 5% false discovery rate are marked by red S. The magnitude for the under- or over-represented families (below or above 0, respectively) from the overall average is indicated on the y-axis. Most of the proteins with GOSlim in the "biological_process" category are those involved in cell adhesion, homoiothermy, sensory perception of smell, homophilic cell adhesion, immune response, response to stimulus, ciliary or flagellar motility, defense response, and visual perception. Furthermore, most of these gene families are also assigned to other GOSlim categories (mainly to response to stress, response to abiotic stimulus, signal transduction, regulation of biological process, multicellular organismal development, cell differentiation, response to external stimulus, or anatomical structure morphogenesis).

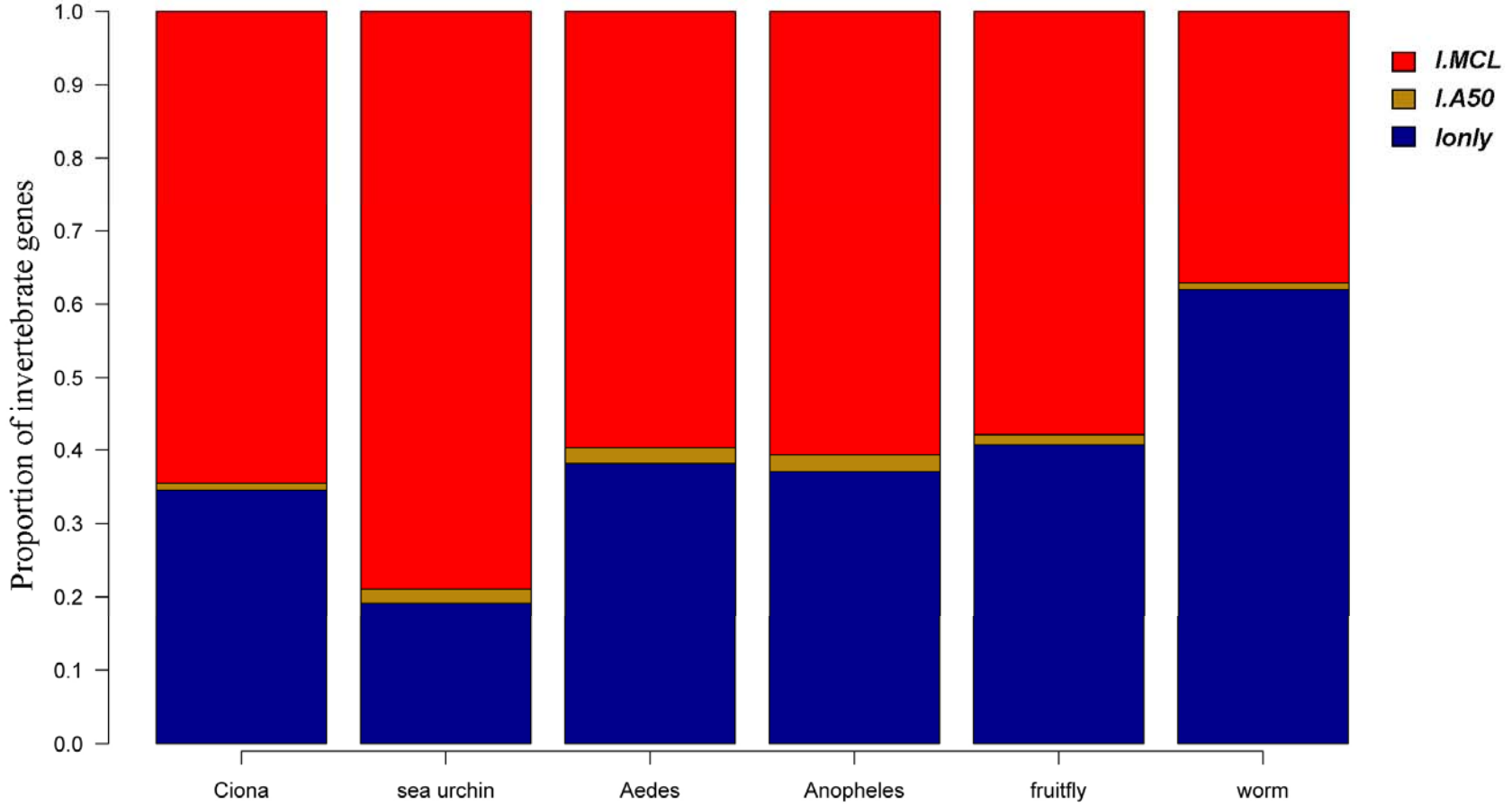
Figure 8S. Functional bias in each GOSlim biological process category is shown for the *I.MCL*, *I.A50* and *Ionly* groups (represented, respectively, by the circular, diamond and triangular points). The significant functional bias categories at the 5% false discovery rate are marked by red S. The magnitude for the under- or over-represented gene copies (below or above 0, respectively) from the overall average is indicated on the y-axis. Most of the proteins with GOSlim in the "biological_process" category are those involved in cell adhesion, homoiothermy, sensory perception of smell, homophilic cell adhesion, immune response, response to stimulus, ciliary or flagellar motility, defense response, and visual perception. Furthermore, most of these gene families are also assigned to other GOSlim categories (mainly to response to stress, response to abiotic stimulus, signal transduction, regulation of biological process, multicellular organismal development, cell differentiation, response to external stimulus, or anatomical structure morphogenesis).

Figure 9S. Functional bias in each GOSlim cellular compartment category is shown for the *I.MCL*, *I.A50* and *Ionly*groups (represented, respectively, by the circular, diamond and triangular points). The significant functional bias categories at the 5% false discovery rate are marked by red S. The magnitude for the under- or over-represented families (below or above 0, respectively) from the overall average is

indicated on the y-axis. Most of the proteins with GOSlim in the "cellular_component" category are those in extracellular matrix, viral nucleocapsid, viral capsid, synapse and viral envelope. Less than half of these gene families are also assigned to other GOSlim categories (e.g., cytoplasm, cell, extracellular region, plasma membrane, lysosome, intracellular, or proteinaceous extracellular matrix).

Figure 10S. Functional bias in each GOSlim cellular compartment category is shown for the *I.MCL*, *I.A50* and *Ionly*groups (represented, respectively, by the circular, diamond and triangular points). The significant functional bias categories at the 5% false discovery rate are marked by red S. The magnitude for the under- or over-represented gene copies (below or above 0, respectively) from the overall average is indicated on the y-axis. Most of the proteins with GOSlim in the "cellular_component" category are those in extracellular matrix, viral nucleocapsid, viral capsid, synapse and viral envelope. Less than half of these gene families are also assigned to other GOSlim categories (e.g., cytoplasm, cell, extracellular region, plasma membrane, lysosome, intracellular, or proteinaceous extracellular matrix).

Figure 1S.


Proportion of invertebrate genes
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0.9
0.8
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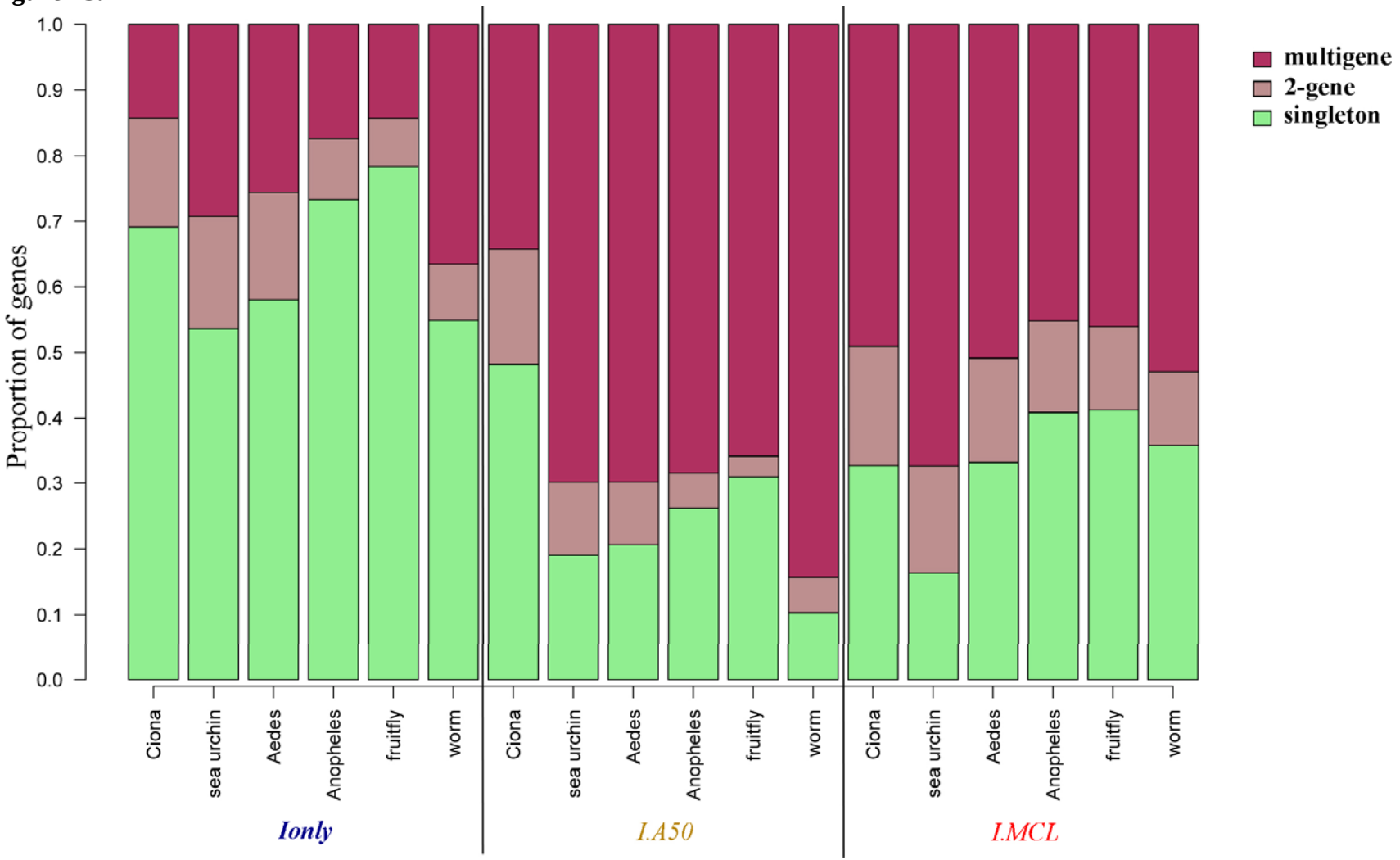
sea urchin
Aedes
Anopheles
fruitfly
worm
I.MCL
I.A50
Ionly

Figure 2S.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Ionly
I.A50
I.MCL

Figure 3S.

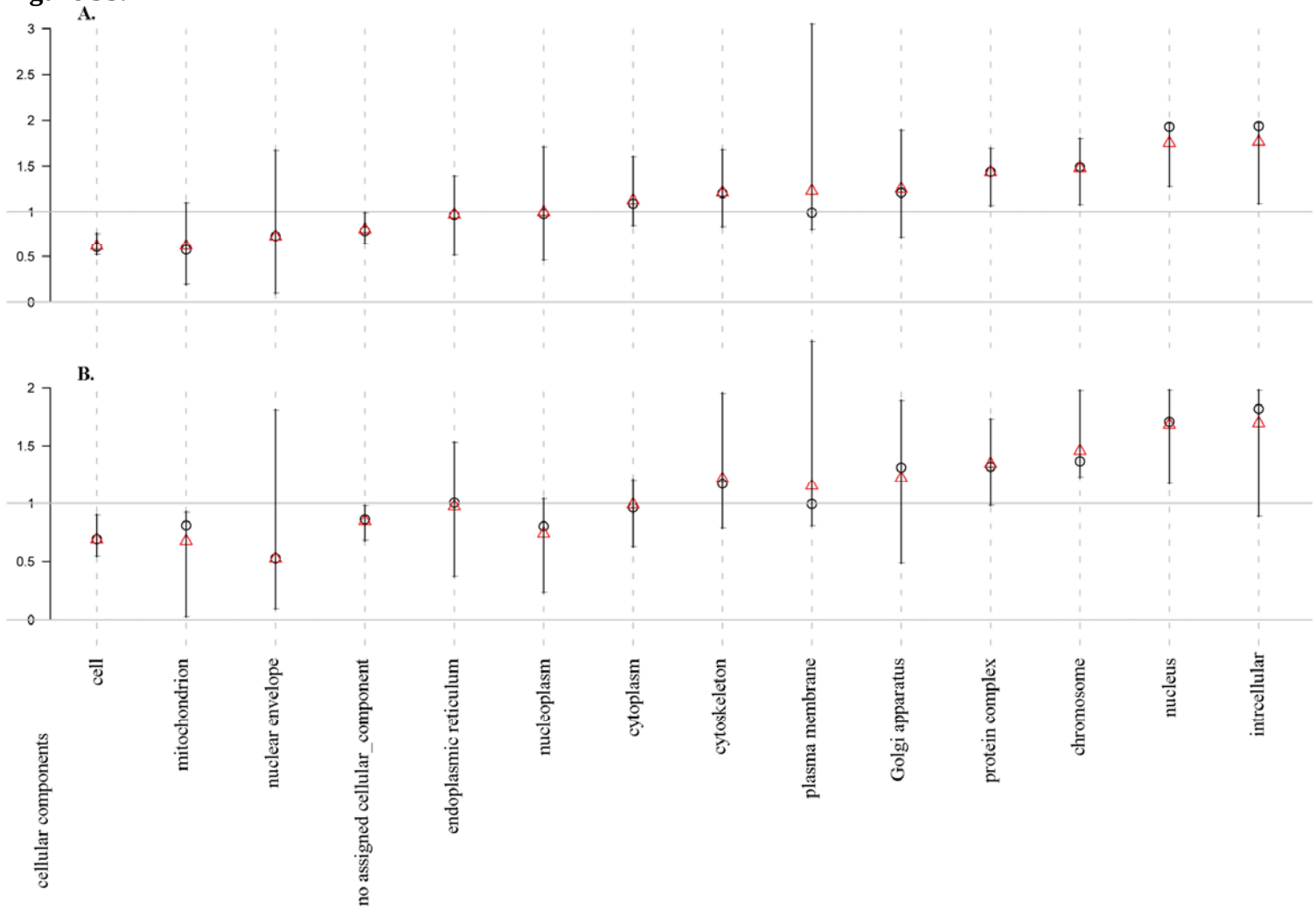
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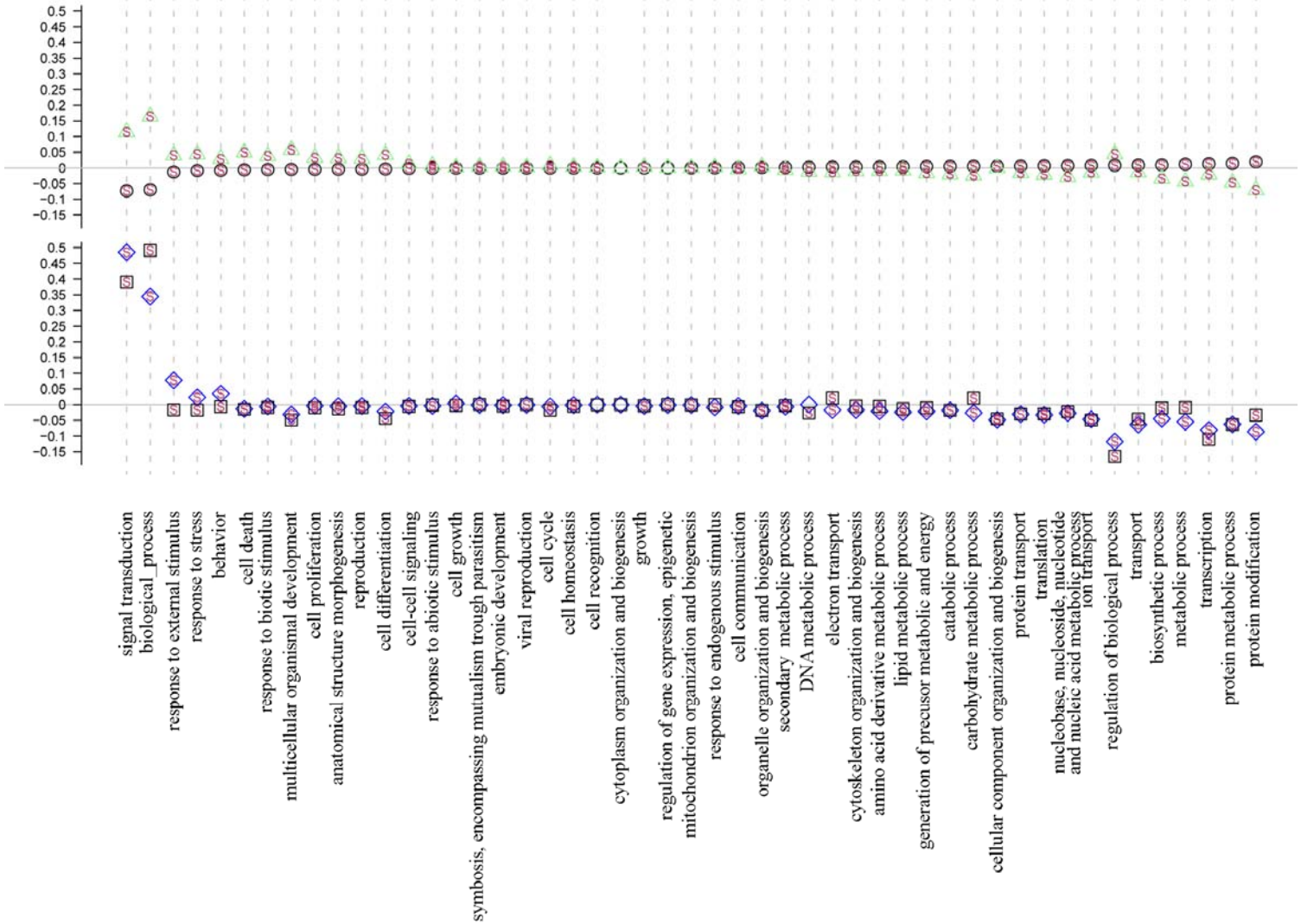
Figure 4S.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response to external stimulus
response to stress
behavior
cell death
response to biotic stimulus
multicellular organismal development
cell proliferation
anatomical structure morphogenesis
reproduction
cell differentiation
cell-cell signaling
response to abiotic stimulus
cell growth
symbosis, encompassing mutualism trough parasitism
embryonic development
viral reproduction
cell cycle
cell homeostasis
cell recognition
cytoplasm organization and biogenesis
growth
regulation of gene expression, epigenetic
mitochondrion organization and biogenesis
response to endogenous stimulus
cell communication
organelle organization and biogenesis
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DNA metabolic process
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cytoskeleton organization and biogenesis
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generation of precusor metabolic and energy
catabolic process
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cellular component organization and biogenesis
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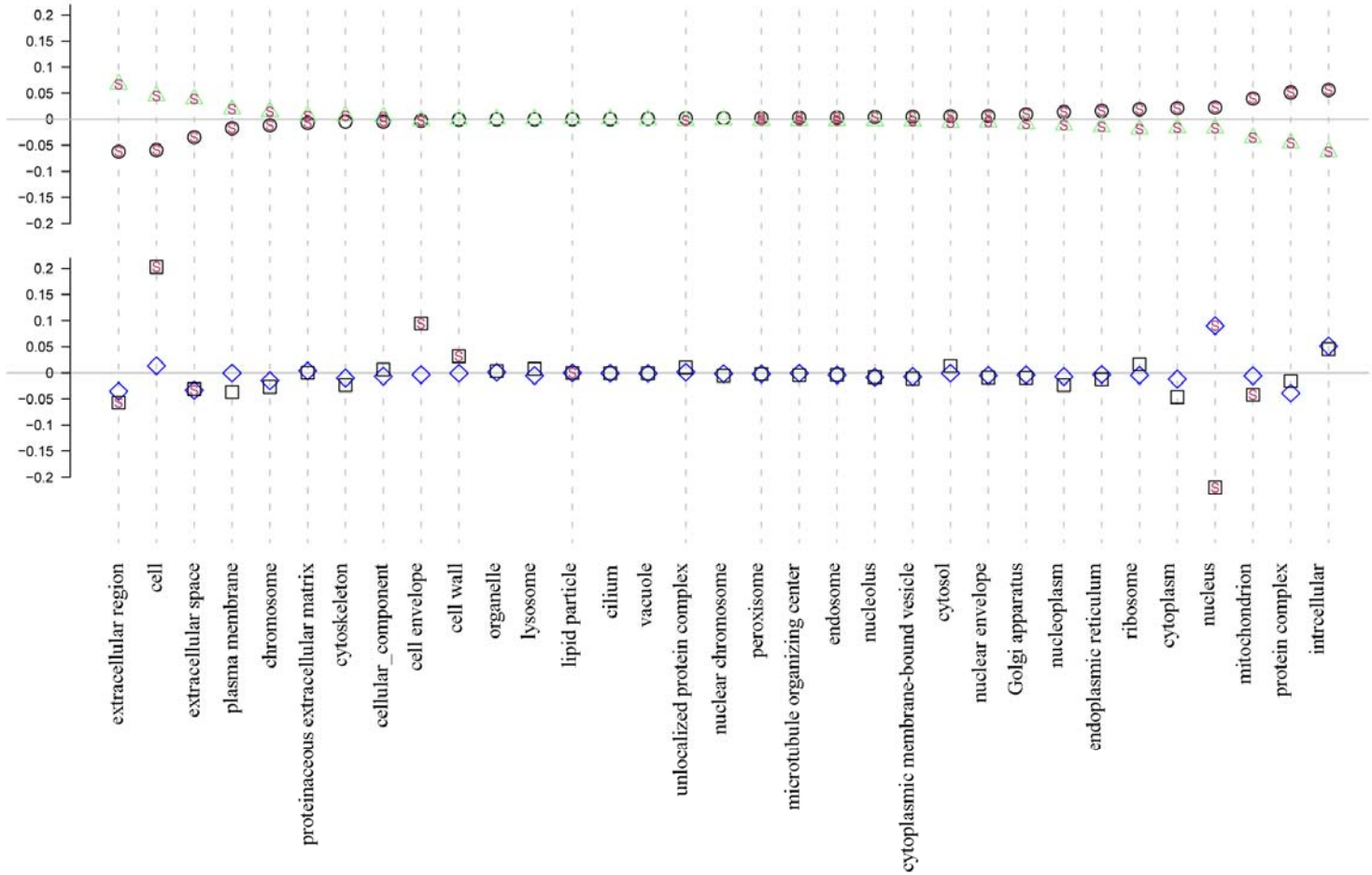
metabolic process
transcription
protein metabolic process
protein modification

Figure 5S.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vacuole
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Golgi apparatus
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ribosome
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Figure 6S.


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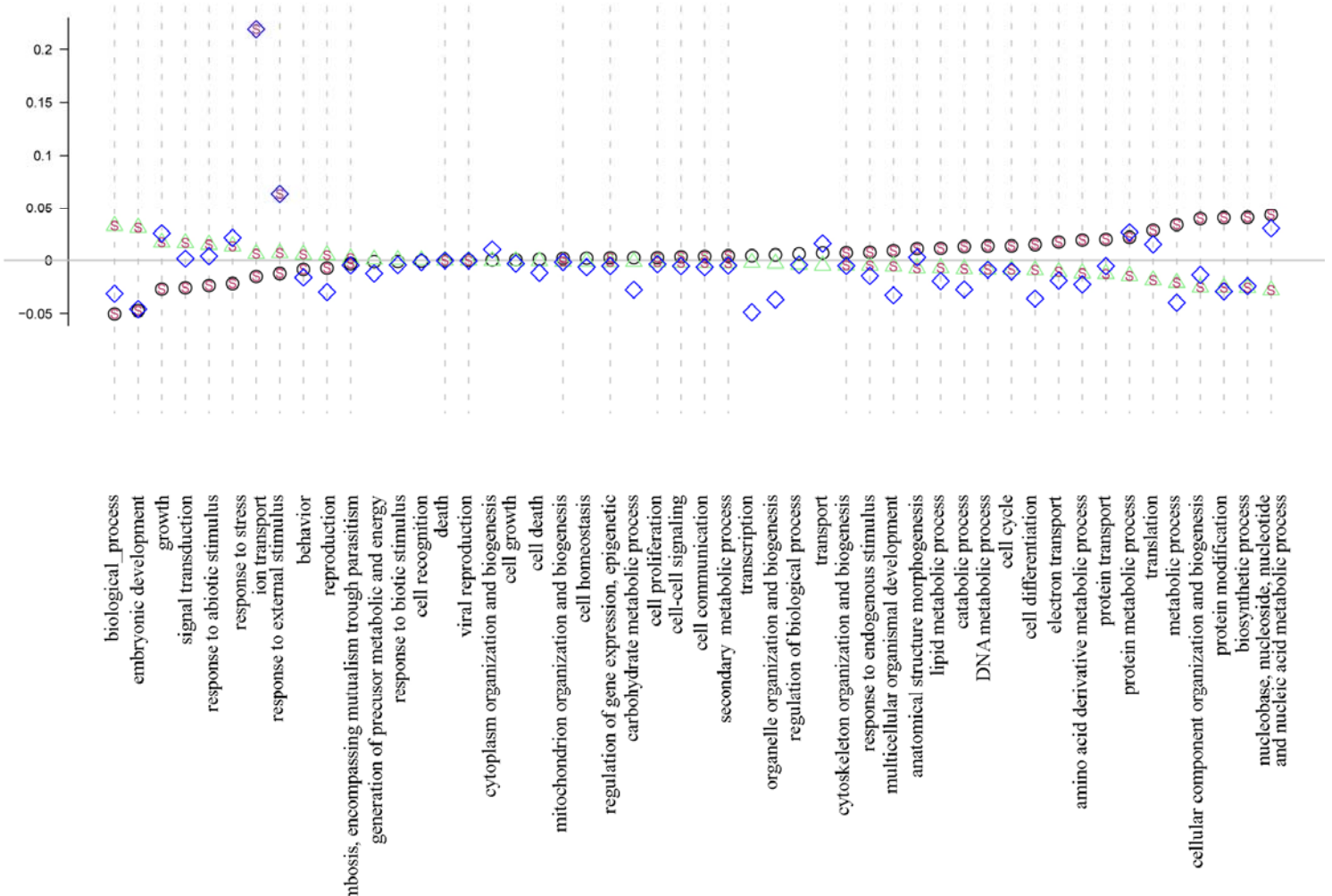
Figure 7S.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generation of precusor metabolic and energy
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nucleobase, nucleoside, nucleotide
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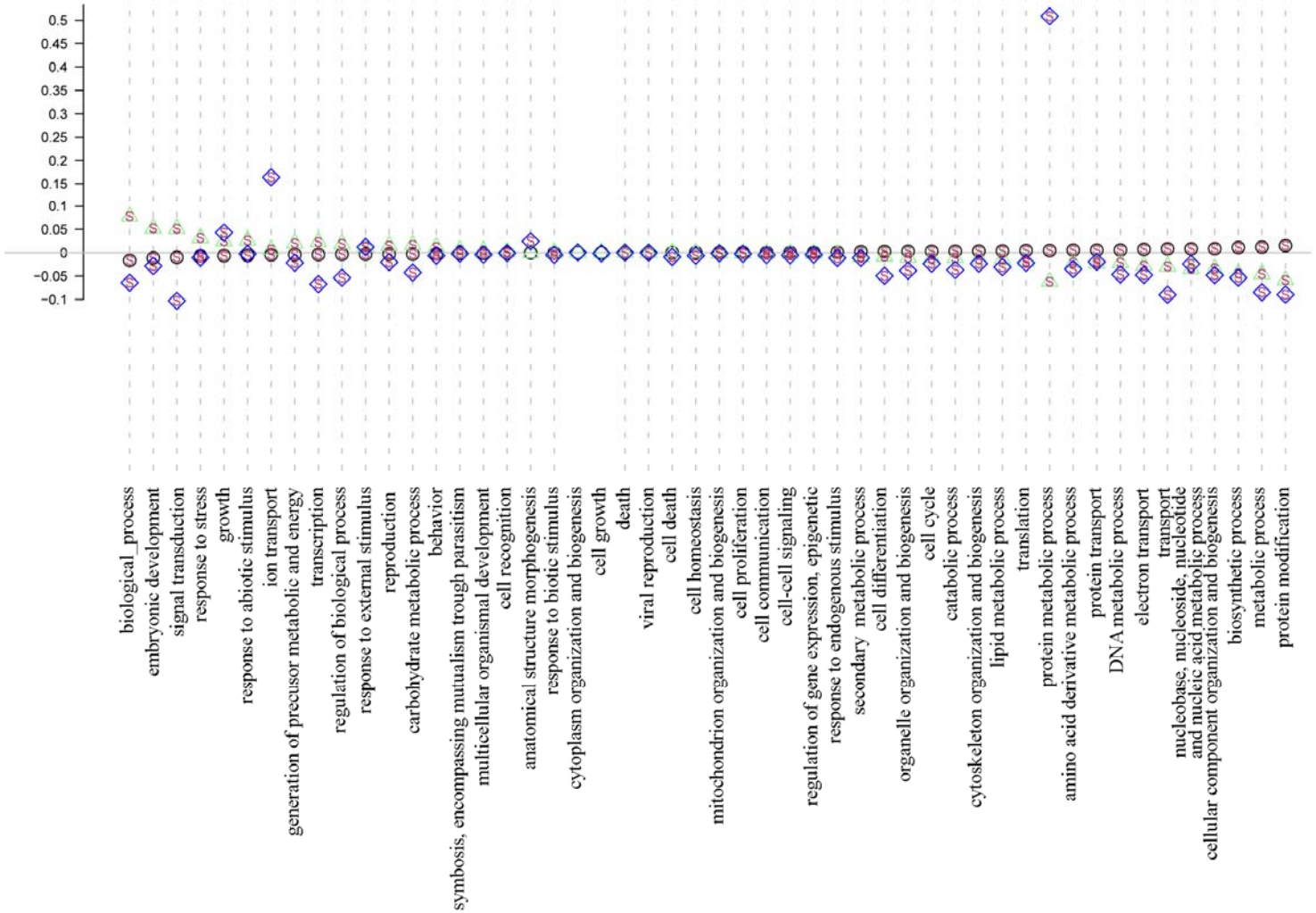
Figure 8S.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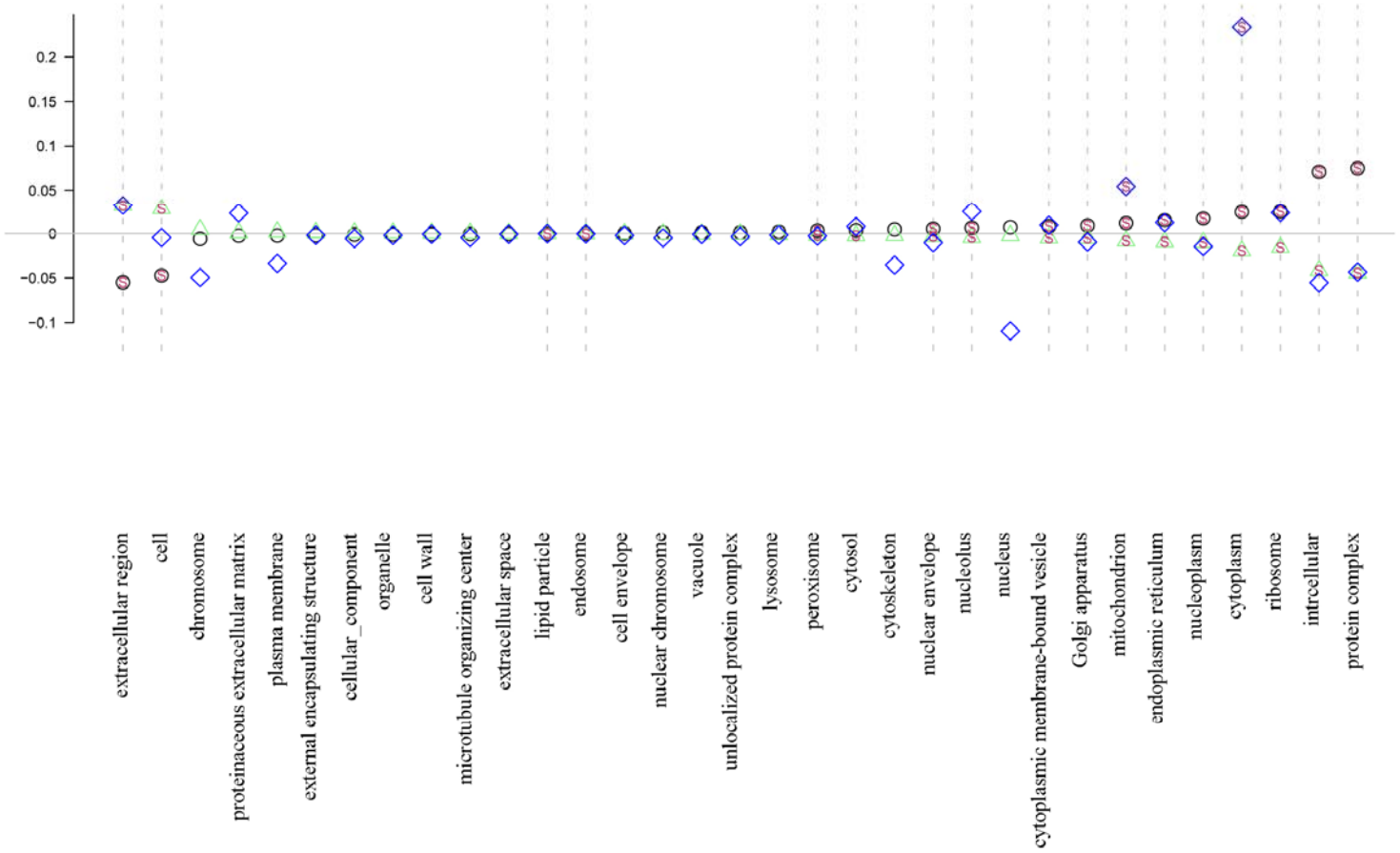
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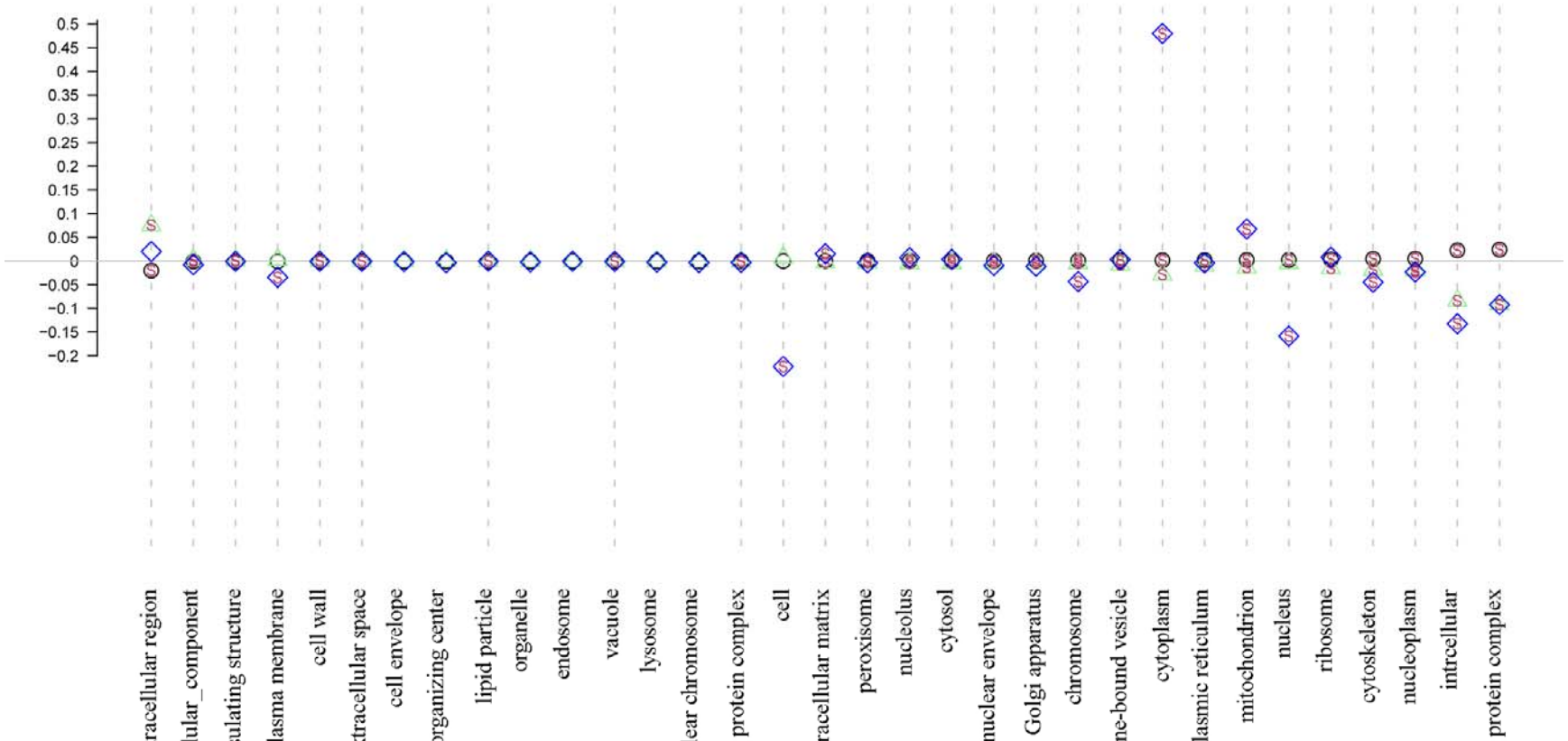
Figure 10S.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