Table S1. Statistical test using set of independent paralogons and 1,000 simulations

Threshold for removal of a paralogon where any ohnolog has this many ohnolog pairs	Paralogons		Paralogons with PPIs		Percentile[a]
	Number of sets of non-overlapping paralogons	Total number of paralogons in the set	Number of sets of non-overlapping paralogons	Total number of paralogons in the set	
Not removed	139	1,110	56	602	100
> 7	185	970	94	505	99.6
> 6	227	901	132	458	100
> 5	273	773	169	380	100
> 4	264	601	157	282	100
> 3	229	418	127	189	100

[a]Percentage of replicates in which paralogons have *cis*-PPIs significantly much more than *trans*-PPIs (Benjamini-Hochberg False Discovery Rate corrected $P < 0.05$, Wilcoxon signed rank test with continuity correction).

Table S2. Short distance *cis*- and *trans*-interactions between singletons within yeast paralogons inferred by ancestral gene order

PPI data	Size	#*cis*-PPIs	#*trans*-PPIs	#paralogon pairs (PGs)	#PGs (#*cis*-PPIs > #*trans*-PPIs)	#PGs (#*cis*-PPIs = #*trans*-PPIs)	#PGs (#*cis*-PPIs < #*trans*-PPIs)	*p*-value
BioGRID	≤ 5	2,153	1,787	302	160	36	106	2.9×10^{-5}
	6 - 10	1,224	1,196	179	88	19	72	not significant
	11 - 20	1,257	1,216	117	53	9	55	not significant
	> 20	1,307	1,134	66	28	10	28	not significant
DIP	≤ 5	179	105	148	90	13	45	1.5×10^{-5}
	6 - 10	85	105	89	30	15	44	not significant
	11 - 20	97	94	73	32	10	31	not significant
	> 20	100	82	32	13	4	15	not significant

Table S3. *Cis*- and *trans*-interactions between singletons within paralogons inferred by ancestral gene order

PPI data	#*cis*-PPIs	#*trans*-PPIs	#paralogon pairs (PGs)	#PGs (#*cis*-PPIs > #*trans*-PPIs)	#PGs (#*cis*-PPIs = #*trans*-PPIs)	#PGs (#*cis*-PPIs < #*trans*-PPIs)	*p*-value
BioGRID	3,029	2,833	149	62	14	73	not significant
DIP	238	231	100	38	17	45	not significant

Table S4. Number of *cis*-relationships for interacting singletons compared to the expected number of *cis*-relationships for non-interacting singletons in a paralogon

Species	PPI data	Window size for identifying ohnologs	#paralogon pairs (PGs; #*cis*-PPIs > #*trans*-PPIs)	#PGs (number of observed *cis* > number of expected *cis*)	#PGs (number of observed *cis* = number of expected *cis*)	#PGs (number of observed *cis* < number of expected *cis*)	*p*-value
Human	HPRD	100	483	356	15	112	*
		30	323	271	20	32	**
Plant	AtPIN	-	85	84	0	1	*
Yeast	BioGRID	-	153	70	57	26	3.4×10^{-5}
	DIP	-	95	74	20	1	5.8×10^{-14}

* Not amenable to statistical analysis, see main text

** Data subsampled to make independent paralogon pairs consistently showed statistical significance (Table S5)

Table S5. Statistical test using set of Independent paralogons by 1,000 simulations

Size of hub ohnologs for removing paralogons including them	Paralogons		#paralogons (#*cis*-PPIs > #*trans*-PPIs)		Percentile[a]
	Number of set of non-overlapping paralogons	Total number of paralogons in the set	Number of set of non-overlapping paralogons	Total number of paralogons in the set	
Not removed	139	1,110	34	323	100
> 7	185	970	62	273	100
> 6	227	901	90	252	100
> 5	273	773	117	210	100
> 4	264	601	106	157	100
> 3	229	418	84	106	100

[a]Percentage of replicates in which paralogons have observed *cis*-PPIs significantly much more than expected *cis*-PPIs (Benjamini-Hochberg False Discovery Rate corrected $P < 0.05$, Wilcoxon signed rank test with continuity correction).

Table S6. *Cis*- and *trans*-interacting gene pairs between singletons within paralogons in yeast genomes

PPI data	species	Conserved gene pairs		*S. cerevisiae cis*-interacting gene pairs			*S. cerevisiae* trans-interacting gene pairs		
		#cis	*#trans*	#orthologous *cis*	#paralogous *cis*	*#trans*	*#cis*	#orthologous *trans*	#paralogous *trans*
BioGRID	*Vanderwaltozyma polyspora*	4749	4713	4704	1	88	44	4625	0
	Tetrapisispora phaffii	4917	4743	4867	0	89	50	4654	0
	Tetrapisispora blattae	4733	4671	4689	0	84	44	4587	0
	Naumovozyma dairenensis	5257	5041	5206	0	97	51	4944	0
	Naumovozyma castellii	5245	5039	5208	0	72	37	4967	0
	Saccharomyces castellii	5138	4911	5097	1	93	40	4818	0
	Kazachstania naganishii	5329	5002	5272	0	104	57	4898	0
	Kazachstania africana	5172	4889	5108	1	115	63	4774	0
	Candida glabrata	5576	5159	5518	0	107	58	5052	0
	Saccharomyces bayanus	5236	4745	5175	2	123	59	4622	0
DIP	*Vanderwaltozyma polyspora*	373	341	372	0	10	1	331	0
	Tetrapisispora phaffii	384	341	383	0	5	1	336	0
	Tetrapisispora blattae	388	333	384	0	4	4	329	0
	Naumovozyma dairenensis	408	355	403	0	6	5	349	0
	Naumovozyma castellii	413	353	410	0	4	3	349	0
	Saccharomyces castellii	399	355	398	0	5	1	350	0
	Kazachstania naganishii	427	368	424	0	6	3	362	0
	Kazachstania africana	396	357	395	0	6	1	351	0
	Candida glabrata	431	372	428	0	6	3	366	0
	Saccharomyces bayanus	417	335	413	2	10	2	325	0

Table S7. *Cis*- and *trans*-interacting gene pairs between singletons conserved in sister FSGD paralogons

Species	#*cis*	#*trans*	#identified FSGD paralogon pairs (PGs) containing FSGD singletons with PPIs	#PGs (#*cis*-PPIs > #*trans*-PPIs)	#PGs (#*cis*-PPIs = #*trans*-PPIs)	#PGs (#*cis*-PPIs < #*trans*-PPIs)	*p*-value
stickleback	1,884	1,221	99	85	5	9	5.1×10^{-13}
tetraodon	1,628	843	52	41	5	6	2.1×10^{-8}
medaka	1,655	1,068	106	87	7	12	6.1×10^{-13}
zebrafish	1,347	567	154	123	12	19	$< 2.2 \times 10^{-16}$

Table S8. Function of extant-paired ohnologs in human

Significant difference	GO IDs	Term	Obs.	Mean	S.D.	Z score	*p*-value[a]
Over representation	GO:0007275	multicellular organismal development	1424	1073.2	23.0	15.2	1.87E-52
	GO:0007154	cell communication	2069	1746.6	26.4	12.2	3.84E-32
	GO:0006810	transport	1387	1132.0	23.4	10.9	4.88E-27
	GO:0050789	regulation of biological process	3277	2959.4	30.3	10.5	9.52E-26
	GO:0030154	cell differentiation	735	569.6	17.9	9.2	4.48E-20
	GO:0009987	cellular process	3364	3113.6	28.0	8.9	5.15E-16
	GO:0006928	cell motion	289	202.8	10.6	8.1	1.85E-14
	GO:0043062	extracellular structure organization	85	51.6	5.4	6.2	2.36E-08
	GO:0007610	behavior	198	163.9	9.5	3.6	6.85E-03
	GO:0032501	multicellular organismal process	812	750.0	19.2	3.2	2.01E-02
	GO:0046903	secretion	163	136.0	8.6	3.1	3.52E-02
Under representation	GO:0050896	response to stimulus	846	1094.7	22.6	-11.0	5.12E-27
	GO:0006519	cellular amino acid and derivative metabolic process	89	138.2	8.6	-5.7	1.77E-07
	GO:0009056	catabolic process	443	507.5	16.5	-3.9	9.09E-04

[a] The estimated *P* values were adjusted by Bonferroni correction.

Table S9. Function of extant-paired ohnologs in plant and yeast

Species	Significant difference	GO IDs	Term	Obs.	Mean	S.D.	Z score	*p*-value[a]
Plant	Over representation	GO:0006464	protein modification process	263	210.4	11.0	4.8	1.28E-04
		GO:0006412	translation	153	118.0	8.6	4.1	2.00E-03
		GO:0019725	cellular homeostasis	30	17.2	3.5	3.6	1.34E-02
		GO:0009628	response to abiotic stimulus	29	17.5	3.5	3.3	4.66E-02
	Under representation	GO:0008152	metabolic process	189	234.4	12.2	-3.7	1.99E-03
		GO:0006629	lipid metabolic process	48	74.0	7.1	-3.7	3.53E-03
		GO:0006950	response to stress	100	129.1	9.3	-3.1	2.31E-02
Yeast	Over representation	GO:0006412	translation	143	83.1	7.6	7.8	5.15E-12
		GO:0006091	generation of precursor metabolites and energy	52	24.7	4.3	6.3	4.05E-07
		GO:0044262	cellular carbohydrate metabolic process	95	60.4	6.8	5.1	2.73E-05
		GO:0031505	fungal-type cell wall organization	48	26.5	4.5	4.8	3.18E-04
		GO:0007165	signal transduction	90	58.8	6.7	4.6	2.31E-04
		GO:0042221	response to chemical stimulus	98	67.2	7.0	4.4	6.27E-04
		GO:0019725	cellular homeostasis	53	33.0	5.0	4.0	5.24E-03
	Under representation	GO:0007005	mitochondrion organization	24	58.4	6.7	-5.1	3.99E-07
		GO:0051276	chromosome organization	43	81.0	7.7	-4.9	4.12E-06
		GO:0006259	DNA metabolic process	50	86.2	7.9	-4.6	3.19E-05
		GO:0016070	RNA metabolic process	194	236.8	11.7	-3.6	6.50E-03
		GO:0007059	chromosome segregation	15	31.5	4.9	-3.3	8.92E-03
		GO:0070271	regulates	23	41.2	5.6	-3.3	1.64E-02
		GO:0044257	cellular protein catabolic process	23	41.4	5.7	-3.2	1.15E-02

[a] The estimated *P* values were adjusted by Bonferroni correction.