

SUPPLEMENTARY MATERIAL**Table S1. Paired tests comparing ω and d_N values of upstream genes with their downstream targets controlling for a number of variables**

Parameter	Controlling variable	n^a	Upstream > Downstream ^b	Upstream < Downstream ^c	P
ω	Expression level	763	418	345	0.009**
	Expression breadth	763	418	344	0.008**
	ENC	800	435	365	0.015*
	Connectivity	709	360	349	0.707
	Number of paralogs	800	442	358	0.003**
d_N	Expression level	763	419	344	0.007**
	Expression breadth	763	429	331	4.34×10^{-4} ***
	ENC	800	447	353	0.001**
	Connectivity	709	351	358	0.822
	Number of paralogs	800	452	348	2.71×10^{-4} ***

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

^aNumber of genes with at least one direct downstream target (out-degree > 0). Only genes with available information for the controlled factor were considered.

^bNumber of genes with a higher value than the central value of its downstream targets.

^cNumber of genes with a lower value than the central value of its downstream targets.

Table S2. Comparison of genes occupying extreme upstream and downstream positions controlling for a number of variables

Parameter	Controlling variable	Upstream			Downstream			<i>P</i>
		<i>n</i>	Median	Average	<i>n</i>	Median	Average	
ω	Expression level	322	-0.0243	0.0105	243	-0.0350	0.0000	0.098
	Expression breadth	322	-0.0225	0.0095	243	-0.0348	-0.0008	0.048*
	ENC	333	-0.0200	0.0120	249	-0.0349	-0.0018	0.041*
	Connectivity	293	-0.0302	0.0087	225	-0.0378	-0.0001	0.099
	Number of paralogs	333	-0.0227	0.0119	249	-0.0349	-0.0017	0.043*
d_N	Expression level	322	-0.0182	0.0073	243	-0.0254	-0.0018	0.110
	Expression breadth	322	-0.0168	0.0066	243	-0.0225	-0.0025	0.035*
	ENC	333	-0.0165	0.0080	249	-0.0251	-0.0026	0.049*
	Connectivity	293	-0.0225	0.0049	225	-0.0266	-0.0018	0.172
	Number of paralogs	333	-0.0168	0.0079	249	-0.0249	-0.0025	0.048*

P-values were obtained from the Mann-Whitney *U* test. *, *P* < 0.05.

Table S3. Partial correlations between evolutionary rates and extreme upstream/downstream location

Variable 1	Controlling variable	<i>n</i>	ρ	<i>P</i>
ω	Expression level	565	0.071	0.092
	Expression breadth	565	0.075	0.075
	ENC	582	0.091	0.028*
	Connectivity	518	0.057	0.191
	Number of paralogs	582	0.086	0.037*
d_N	Expression level	565	0.071	0.093
	Expression breadth	565	0.075	0.075
	ENC	582	0.082	0.048*
	Connectivity	518	0.041	0.348
	Number of paralogs	582	0.081	0.049*

Upstream/downstream location was encoded as a binary variable (1 for upstream, 0 for downstream). *, $P < 0.05$.

Table S4. Partial correlations between parameters of interest and measures of hierarchical positions of genes in the network controlling for a number of variables

Variable 1	Variable 2	Controlling variable	<i>n</i>	ρ	<i>P</i>
ω	in-degree	Expression level	1016	-0.112	$3.36 \times 10^{-4}***$
		Expression breadth	1016	-0.105	0.001***
		ENC	1049	-0.119	$1.04 \times 10^{-4}***$
		Connectivity	951	-0.058	0.073
		Number of paralogs	1049	-0.114	$2.10 \times 10^{-4}***$
	out-degree	Expression level	1016	-0.082	0.009**
		Expression breadth	1016	-0.069	0.028*
		ENC	1049	-0.068	0.028*
		Connectivity	951	-0.036	0.261
		Number of paralogs	1049	-0.065	0.034*
	<i>H</i>	Expression level	1016	0.054	0.085
		Expression breadth	1016	0.051	0.103
		ENC	1049	0.071	0.021*
		Connectivity	951	0.052	0.106
		Number of paralogs	1049	0.070	0.024*
d_N	in-degree	Expression level	1016	-0.114	$2.64 \times 10^{-4}***$
		Expression breadth	1016	-0.106	0.001***
		ENC	1049	-0.117	$1.37 \times 10^{-4}***$
		Connectivity	951	-0.045	0.170
		Number of paralogs	1049	-0.116	$1.68 \times 10^{-4}***$
	out-degree	Expression level	1016	-0.080	0.011*
		Expression breadth	1016	-0.066	0.036*
		ENC	1049	-0.072	0.019*
		Connectivity	951	-0.031	0.332
		Number of paralogs	1049	-0.068	0.027*
	<i>H</i>	Expression level	1016	0.058	0.064
		Expression breadth	1016	0.055	0.080
		ENC	1049	0.067	0.030*
		Connectivity	951	0.050	0.127
		Number of paralogs	1049	0.069	0.025*

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table S5. Evolutionary rates in the mammalian Ras pathway genes

<i>Drosophila</i> gene	Human orthologs ^a	Human protein	Pathway position ^b	ω ^c
<i>drk</i>	<i>GRB2</i>	Grb2	0	0.0047
<i>Sos</i>	<i>SOS1</i>	SOS1	1	0.0234
	<i>SOS2</i>	SOS2	1	0.0461
<i>Ras85D</i>	<i>HRAS</i>	H-Ras	2	0.0001
	<i>KRAS</i>	K-Ras	2	0.0222
	<i>NRAS</i>	N-Ras	2	0.0058
<i>p11</i>	<i>ARAF</i>	A-Raf	3	0.0472
	<i>BRAF</i>	B-Raf	3	0.0396
	<i>RAF1</i>	C-Raf	3	0.0262
<i>Dsor1</i>	<i>MAP2K1</i>	MEK1	4	0.0088
	<i>MAP2K2</i>	MEK2	4	0.0196
<i>rl</i>	<i>MAPK1</i>	ERK2	5	0.0043
	<i>MAPK3</i>	ERK1	5	0.0181
<i>S6kII</i>	<i>RPS6KA1</i>	RSK1	6	0.0108
	<i>RPS6KA2</i>	RSK3	6	0.0225
	<i>RPS6KA3</i>	RSK2	6	0.0022
<i>ksr</i>	<i>KSR1</i>	KSR1	–	0.0645
	<i>KSR2</i>	KSR2	–	0.0295
<i>csw</i>	<i>PTPN6</i>	SHP1	–	0.0308
	<i>PTPN11</i>	SHP2	–	0.0029

^aHuman genes known to be involved in the Ras pathway.

^bPathway position is defined as the number of steps required to transduce the signal from Grb2 to each of the pathway components. Genes encoding the SHP and KSR proteins were not assigned a precise pathway position, as they do not directly participate in the core signaling cascade (see Fig. 2A and refs. 60 and 82).

^cNonsynonymous to synonymous divergence ratio ($\omega = d_N/d_S$) estimated from the comparison of human-mouse pairs of orthologs.