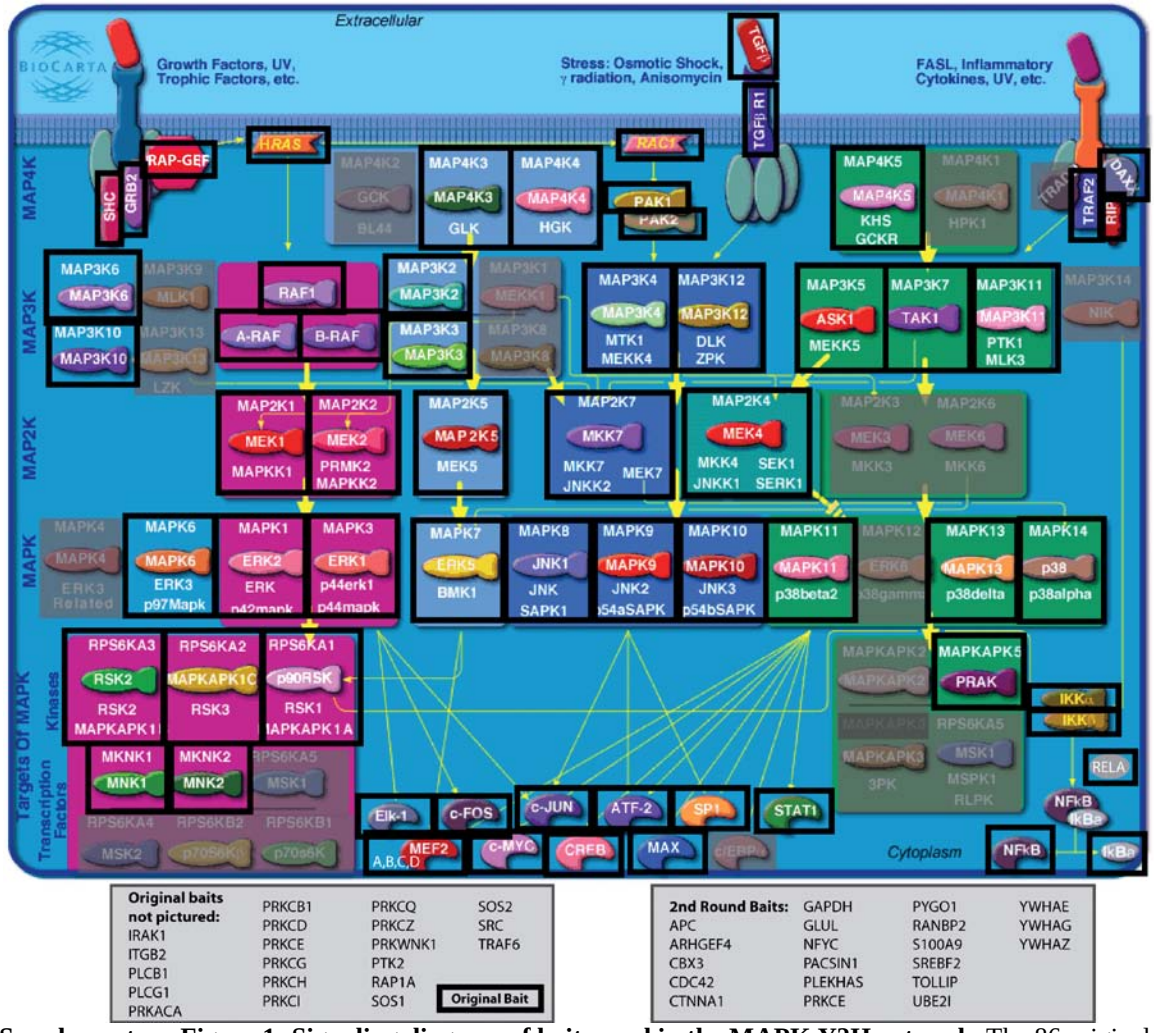
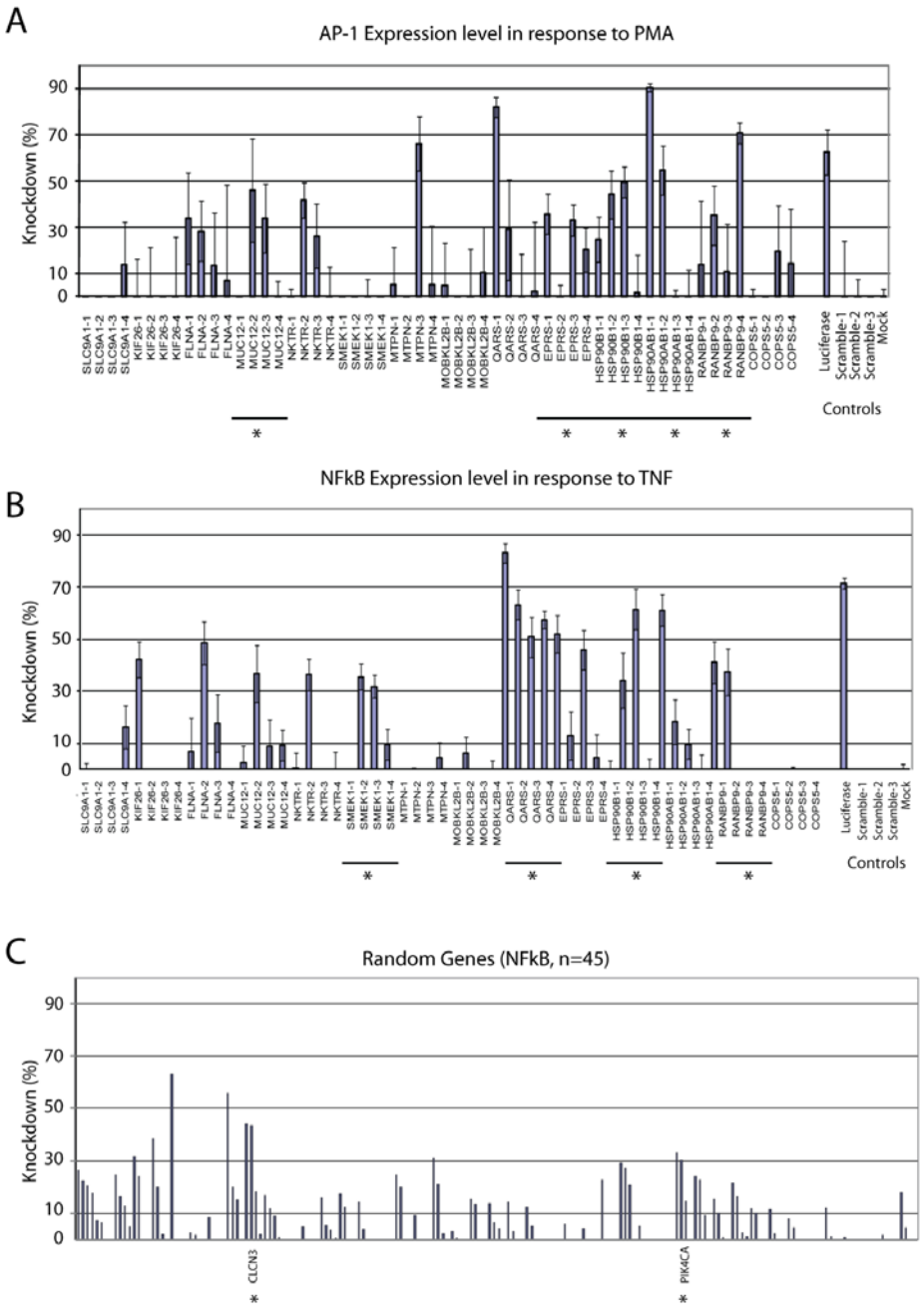
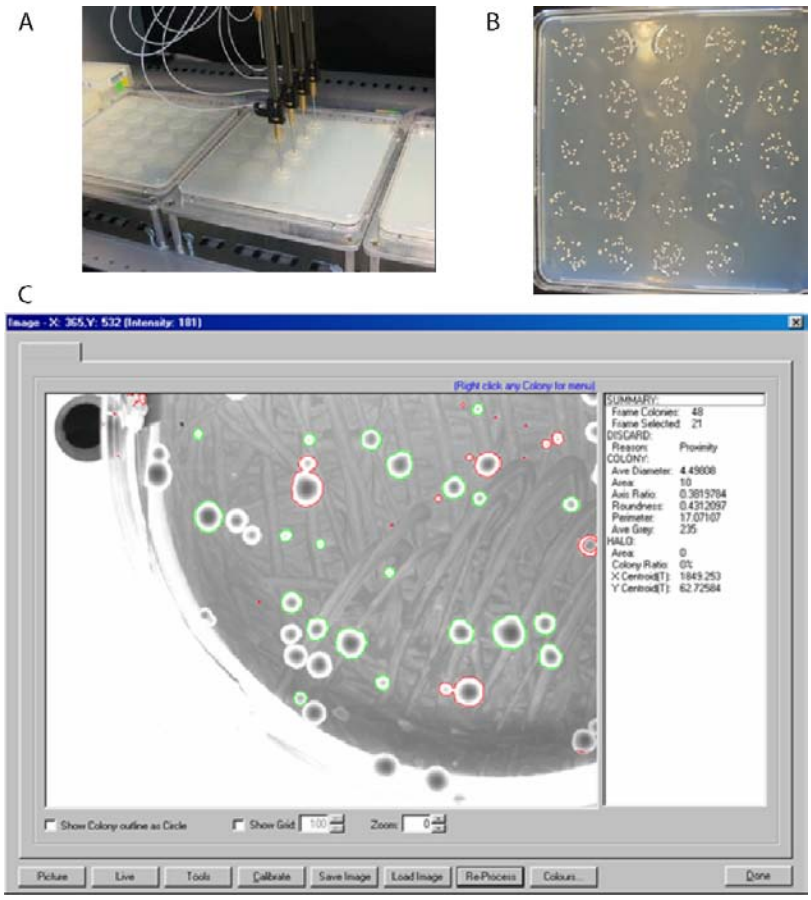
Supplementary Figures



Supplementary Figure 1: Signaling diagram of baits used in the MAPK Y2H network. The 86 original baits and the 20 additional baits are shown. Image adapted from BioCarta "MAPKinase signaling pathway" (http://www.biocarta.com/pathfiles/h_mapkPathway.asp)



Supplementary Figure 2. Functional validation of MAPK interactors using siRNA screening. Four siRNAs against fourteen genes contained in the MAPK interactome were selected and tested for knockdown of luciferase-fused reporter constructs for AP-1 (**A**) and NFkB (**B**). Mock represents no siRNA control, Scramble indicates "scrambled" non-specific siRNA sequences, and Luciferase indicates siRNAs against the luciferase gene. Starred genes indicate those with two or more siRNAs which induce >30% knockdown. siRNAs that showed no knockdown phenotype are omitted for clarity. (**C**) Forty-five random genes are tested for functional interactions with NFkB. The two genes that show multiple siRNAs with greater than 30% knockdown are indicated.



Supplementary Figure 3. **High-throughput Y2H screening process.** (**A**) Plating of mated Y2H clones. (**B**) View of plated colonies. (**C**) Image analysis of Y2H positive colonies for automated picking.

Supplementary Tables

Supplementary Table 3: List of candidate scaffolds identified in this study.

GENE_ID	NAME	Percent of interactions with kinases	Total number of interactions	Interactions with number of different MAPK levels
10048	RANBP9	42.9	14	2
408	ARRB1	42.9	7	2
11064	CEP1	50	4	2
2316	FLNA	50	14	2
3181	HNRPA2B1	50	6	2
6154	RPL26	50	6	2
57459	GATAD2B	60	5	2
1387	CREBBP	66.6	6	2
6548	SLC9A1	88.9	9	4
26153	KIF26A	100	4	3

Supplementary Table 6: List of cDNA sources used in this study

	Human Tissue
1	Adipose
2	Brain
3	Brain, caudate nucleus
4	Brain, cerebellum
5	Brain, hippocampus
6	Colon
7	Colorectal adenocarcinoma
8	Fetal Brain
9	Fetal lung
10	Kidney
11	Leukocyte
12	Liver
13	Lung
14	Lung carcinoma
15	Mammary Gland
16	Melanoma
17	Pancreas
18	Placenta
19	Prostate Gland
20	Spinal Cord
21	Spleen
22	Testis

Supplementary Table 7: List of siRNA sequences used in this study.

Entrez Gene Id	NCBI gene symbol	mRNA Accessions	siRNA Target Sequence	Quiagen Product Id	Product Name
6548	SLC9A1	NM_003047	CAAGCTCAACCGGTTTAATAA	SI00047446	Hs_SLC9A1_2
6548	SLC9A1	NM_003047	CAGACCAATCTTAGTTTCTAA	SI00047460	Hs_SLC9A1_4
6548	SLC9A1	NM_003047	ACGAAGCGCTCCATCAACGAA	SI03040877	Hs_SLC9A1_5
6548	SLC9A1	NM_003047	CAGCATCATCTCGGCCGTGGA	SI03065888	Hs_SLC9A1_6
26153	KIF26A	XM_050278 XM_941210	CTGAGGATCGTGCATAGCATA	SI00462462	Hs_KIF26A_3
26153	KIF26A	XM_050278 XM_941210	GCCGTTCGAGATCAAGGTGTA	SI00462469	Hs_KIF26A_4
26153	KIF26A	XM_050278 XM_941210	not disclosed - validated	SI03019863	Hs_KIF26A_5
26153	KIF26A	XM_050278 XM_941210	not disclosed - validated	SI03019870	Hs_KIF26A_6
2316	FLNA	NM_001456	CTCGGTCGAGTACATCCCTTA	SI00419748	Hs_FLNA_1
2316	FLNA	NM_001456	CCGCTTCACCATCGACACCAA	SI00419755	Hs_FLNA_2
2316	FLNA	NM_001456	CACCCATGGAGTAGTGAACAA	SI02655912	Hs_FLNA_7
2316	FLNA	NM_001456	not disclosed - validated	SI02654722	Hs_FLNA_5
10071	MUC12	XM_379904 XM_499351 XM_936315	AAGGAACGAAGTCGCAAATGA	SI00651511	Hs_MUC12_1
10071	MUC12	XM_379904 XM_499351 XM_936315	CGGCCTTGAGAACGCCTACAA	SI00651518	Hs_MUC12_2
10071	MUC12	XM_379904 XM_499351 XM_936315	TAGCATCGTGGTCAAGAACGA	SI00651525	Hs_MUC12_3
10071	MUC12	XM_379904 XM_499351 XM_936315	TTCGGAGATTGCTCAACGGTA	SI00651532	Hs_MUC12_4
4820	NKTR	NM_001012651 NM_005385	CACAGTCTTATTCTAGAGGAA	SI00076342	Hs_NKTR_1
4820	NKTR	NM_001012651 NM_005385	CACGCTTAAACCGTAGACCAA	SI00076349	Hs_NKTR_2
4820	NKTR	NM_001012651 NM_005385	CAGGATTGGCAGTAAGAGATA	SI00076356	Hs_NKTR_3
4820	NKTR	NM_001012651 NM_005385	CCAGATCAACCTCATTGATAA	SI00076363	Hs_NKTR_4
55671	SMEK1	NM_017936 NM_032560	AAGAAAGATTATTGGATTAAA	SI00462140	Hs_KIAA2010_1
55671	SMEK1	NM_017936 NM_032560	CAGATTTGTTTGCACAACTAA	SI00462147	Hs_KIAA2010_2
55671	SMEK1	NM_017936 NM_032560	CAGCCAGTCATCTACAACAAA	SI00462161	Hs_KIAA2010_4
55671	SMEK1	NM_017936 NM_032560	CTCATACTTGGTTGAATATAA	SI03199560	Hs_KIAA2010_5
136319	MTPN	NM_145808	CTCCACTGAAGTAATACTTAA	SI00651315	Hs_MTPN_1
136319	MTPN	NM_145808	CAGATTGTGGATAATAATTGA	SI00651322	Hs_MTPN_2
136319	MTPN	NM_145808	CTGGGATTCCTGATCAAGTAA	SI00651336	Hs_MTPN_4
136319	MTPN	NM_145808	CTCCAGATAAACATCATATTA	SI03200043	Hs_MTPN_5
79817	MOBKL2B	NM_024761	CTGGTTATTGATGGAAGCAAA	SI00647031	Hs_MOBKL2B_1
79817	MOBKL2B	NM_024761	AAGTATTTATGTGGTATTAAA	SI00647045	Hs_MOBKL2B_3
79817	MOBKL2B	NM_024761	AAGCATTTGACTATAAGGAAA	SI00647052	Hs_MOBKL2B_4
79817	MOBKL2B	NM_024761	CGGGAGCGTTGGGCATGATTA	SI03196914	Hs_MOBKL2B_5
5859	QARS	NM_005051	CACGTGGTGGATGCAGCATTA	SI00696703	Hs_QARS_1
5859	QARS	NM_005051	CACCATGAATCTACTAAAGCA	SI00696710	Hs_QARS_2
5859	QARS	NM_005051	CCGGTTCCCGCCAGAACCCAA	SI00696724	Hs_QARS_4
5859	QARS	NM_005051	TAGCCTATCGAGTCAAGTATA	SI03111129	Hs_QARS_5
2058	EPRS	NM_004446	CAGGAGGAGACTATACAACTA	SI00063833	Hs_EPRS_1
2058	EPRS	NM_004446	AAGCATGAAGAGCTAATGCTA	SI00063840	Hs_EPRS_2
2058	EPRS	NM_004446	AAGGCGATTACTCAGTGTTAA	SI00063847	Hs_EPRS_3
2058	EPRS	NM_004446	AAGGAGGTGGGCTCTCATCAA	SI00063854	Hs_EPRS_4
7184	HSP90B1	NM_003299	AGGACGGATGATGAAGTAGTA	SI03044566	Hs_HSP90B1_1
7184	HSP90B1	NM_003299	AAGTTGATGTGGATGGTACAG	SI00302008	Hs_TRA1_5
7184	HSP90B1	NM_003299	AAGTTGATGTGGATGGTACAT	SI02655177	Hs_TRA1_8
7184	HSP90B1	NM_003299	TCGCCTCAGTTTGAACATTGA	SI02663738	Hs_TRA1_9

3326	HSP90AB1	NM_007355	CACAACGATGATGAACAGTAT	SI03055304	Hs_HSP90AB1_1
3326	HSP90AB1	NM_007355	CAGAAGACAAGGAGAATTACA	SI03062990	Hs_HSP90AB1_2
3326	HSP90AB1	NM_007355	TACGTTGCTCACTATTACGTA	SI03110100	Hs_HSP90AB1_3
3326	HSP90AB1	NM_007355	not disclosed - validated	SI02780561	Hs_HSPCB_5
10048	RANBP9	NM_005493	ACGGTGTGGTTATGACCTTTA	SI00078057	Hs_RANBP9_2
10048	RANBP9	NM_005493	CAGTGCAATATTAGAAACCCA	SI00078071	Hs_RANBP9_4
10048	RANBP9	NM_005493	not disclosed - validated	SI02662289	Hs_RANBP9_6
10048	RANBP9	NM_005493	CAGGTTGGGATAAGCATTCAT	SI02662842	Hs_RANBP9_7
10987	COPS5	NM_006837	TAGGACATACCCAAAGGGCTA	SI00092274	Hs_COPS5_3
10987	COPS5	NM_006837	AAGAACAATATCCGCAGGGAA	SI03030832	Hs_COPS5_5
10987	COPS5	NM_006837	ATGCAATCGGGTGGTATCATA	SI03049774	Hs_COPS5_6
10987	COPS5	NM_006837	CTGGACTAAGGATCACCATTA	SI03096905	Hs_COPS5_7
1432	MAPK14	NM_001315 NM_139012 NM_139013 NM_139014	AACTGCGGTTACTTAAACATA	SI00300769	Hs_MAPK14_5
1432	MAPK14	NM_001315 NM_139012 NM_139013 NM_139014	CAGAGAACTGCGGTTACTTAA	SI00605157	Hs_MAPK14_6
4214	MAP3K1	XM_001128827 XM_042066	not disclosed - validated	SI02659958	Hs_MAP3K1_10
4214	MAP3K1	XM_001128827 XM_042066	not disclosed - validated	SI02659965	Hs_MAP3K1_11
5608	MAP2K6	NM_002758 NM_031988	AAGGCTTGCATTTCTATTGGA	SI02222997	Hs_MAP2K6_5
5608	MAP2K6	NM_031988	TAGACCTATGATAAATAACCA	SI02223004	Hs_MAP2K6_6

Supplementary Methods

Y2H network generation

Both bait and prey were cloned as double fusions in plasmids pOBD.111 and pOAD.102. The Y2H procedure is as described in LaCount et al. (*1*). The cDNA was cloned between the 2-hybrid domain on the 5' end of the insert and an in-frame selection marker on the 3' end of the insert. Bait cDNA was cloned between the GAL4 binding domain and the TRP1 coding region. Preys were cloned between the GAL4 transcriptional activation domain and URA3. Both bait and prey cDNA libraries were prepared by random primed cDNA synthesis from polyA-selected RNA isolated from the human tissues (outlined Supplementary Table 6) followed by the PCR addition of yeast recombination tails. These cDNAs were then cloned into linearized expression vectors by recombination in yeast. Transformed bait yeast were plated on medium lacking tryptophan to select for in-frame TRP1 fusions, and prey were selected without uracil for in-frame URA3 fusions. Y2H screens were performed in 96-well plates by mating in each well $5x10^6$ cells of a yeast clone expressing a single bait with $5x10^6$ clonally diverse cells from a prey library. After mating overnight, the well contents were plated on medium that selected simultaneously for successful mating, expression of the ORF-selection markers, and activity of the metabolic reporter genes ADE2 and HIS3 (Supplementaty Figure 3A,B). Two-hybrid-positive diploids were counted and up to 48 colonies per mating were picked and transferred to liquid medium (Supplementary Figure 3C). Searches that yielded more than 200 positives were considered to be self activators (i.e. resulting from bait plasmids that activated transcription in the absence of specific protein-protein interactions) and were not analyzed further. The liquid cultures were then used as template for separate PCR reactions to amplify insert sequence from bait and prey plasmids for subsequent sequence determination. This sequence information was processed to perform vector and adaptor clipping, read assembly, repeat masking and contamination filtering. Subsequently, sequences were BLASTed against RefSeq and the top hit used for identification and Entrez Gene mapping.

Incorporation of other networks and identification of network modules

Literature-curated human protein interactions were obtained from BIND (*4*) and HPRD (*5*) and interactions in the BioCarta Database (*6*) (Supplementary Table 2). To identify conserved human interactions, yeast literature curated protein-protein interactions were taken from Reguly et al. (*8*) and combined with Ptacek et al. (*9*), Gavin et al. (*10*), Ito et al. (*11*), Ho et al. (*12*) and Uetz et al. (*13*).

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