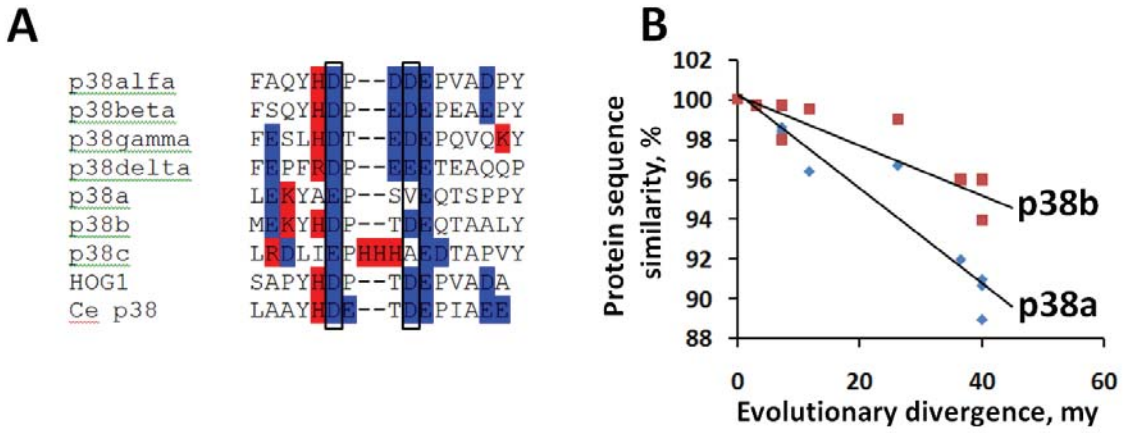

FIGURE S1. **Protein sequence conservation in the *Drosophila* p38 MAP kinases.** (A) CD domain sequences of p38 kinases from various species: human p38α, β, γ, and δ, *Drosophila* p38a, b, and c, *S. cerevisiae* HOG1, and *C.elegans* (Ce) p38. Positively charged residues are shown in red, and negatively charged residues – in blue. Asp316 and Asp319 (numbers correspond to *Drosophila* p38b) shown in boxes, are conserved throughout metazoan evolution, but are mutated in *Drosophila* p38a and c. (B) Rates of divergence of the *Drosophila* p38 MAPKs. Protein sequence similarity between the *D. melanogaster* p38 kinases and homologues from other *Drosophila* species is plotted as a function of time since the last ancestor between the respective species. Divergence rates are estimated from the slopes of linear trend lines. Plot points correspond to the following species: *D. simulans* (3.1 my), *D. sechellia* (3.1 my), *D. erecta* (7.2 my), *D. yakuba* (7.2 my), *D. ananassae* (11.7 my), *D. pseudoobscura* (26.2 my), *D. willistoni* (36.5 my), *D. mojavensis* (40 my), *D. virilis* (40 my), and *D. grimshawi* (40 my). p38 protein sequences were obtained from FlyBase, and aligned with the respective *D. melanogaster* sequences.

TABLE S1. **Proteomic quantification of the components of the p38b complex using spectral counting.** Total spectral counts for each component under indicated conditions are shown.

	Unstimulated				**37°C heat shock**			**Oxidative stress**	
Replicate	1	2	3	4	1	2	3	1	2
p38b	334	246	270	241	291	361	322	399	168
MK2	86	72	94	70	85	85	112	93	31
GS	24	26	57	54	25	75	48	67	40
Clbn	10	9	17	18	12	22	7	17	9
GYG	4	5	9	4	2	6	2	2	0
lic	5	3	0	2	0	6	4	4	0
Mekk1	0	0	0	0	5	6	1	0	0
puc	0	0	1	1	5	4	3	4	0
PTP-ER	1	2	0	2	0	0	0	0	0
Mts	0	0	1	1	0	0	0	0	0
S6KII	0	0	0	0	0	2	0	2	0

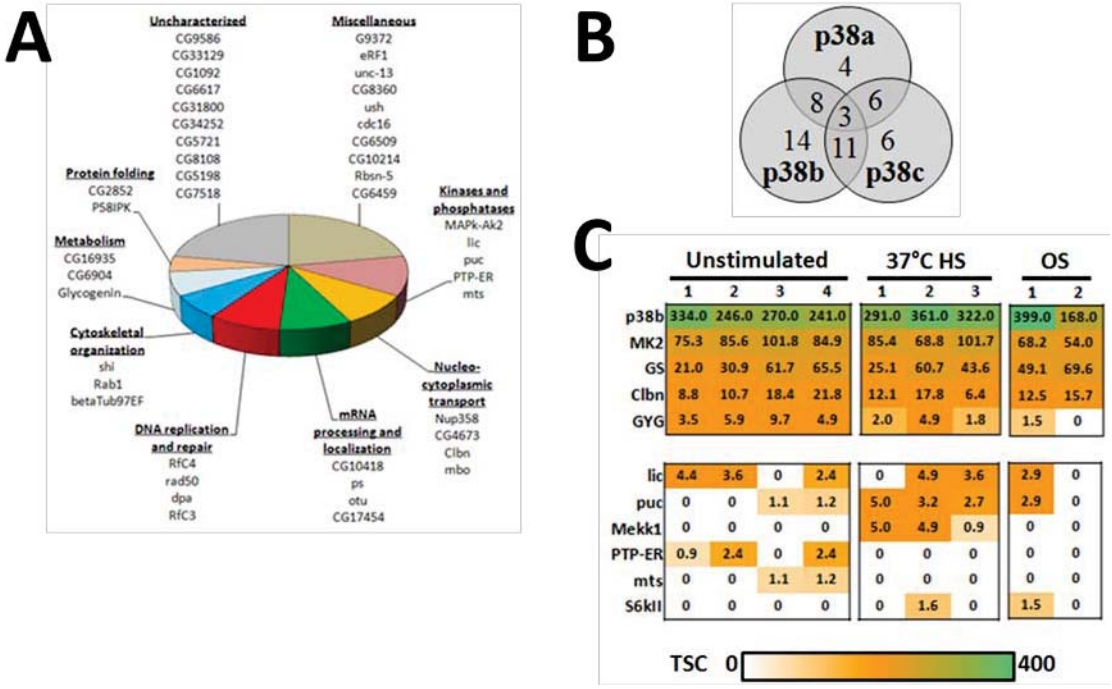

FIGURE S2. ***Drosophila* p38 interactome.** (A) Pie chart representing the distribution of functional classes of p38 interacting proteins. Sectors are color-coded to match the colors in the table in Figure 4. (B) Venn diagram illustrating the numbers of unique and shared p38 interacting proteins. (C) Total spectral counts (TSCs) of peptides derived from p38b-binding proteins in samples from unstimulated, heat shocked (HS), and oxidative stress-treated (OS) S2 cells. Numbers above the table represent individual replicates. TSC values are normalized to the respective bait TSC in each sample, and presented as a heat map in the range from 0 to 400.

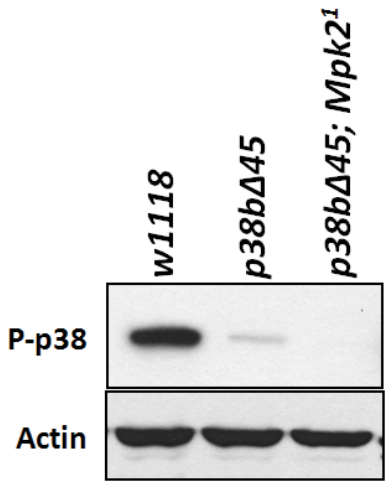

FIGURE S3. **p38 MAPK is active in early *Drosophila* embryos.** The expression of doubly phosphorylated (active) p38 was detected by Western blotting of total lysates from the wild-type *w1118* embryos. The P-p38 band represents both p38a and p38b isoforms. P-p38b is the predominant species as the signal is greatly reduced in *p38bΔ45* (p38b null) embryos, and completely absent in double p38a (*Mpk2*^{1}) and p38b mutants.

TABLE S2. **Experimentally observed phosphorylation sites in p38b-interacting proteins, and computationally predicted p38 phosphorylation sites.** Experimental phosphosites were extracted from two datasets, described in J Proteome Res. (2008), 7(4):1675-82 and Nat Biotechnol. (2008), 26(12):1339-40. Computational predictions were made using Scansite program (Nucleic Acids Res. (2003), 31(13):3635-41). Phosphosites found in both experimental datasets are shown in bold. Observed phosphosites matching the predicted p38 sites are underlined.

p38b interacting protein	Phosphosites from w^{1118} embryos	Phosphosites from Kc cells	Predicted p38 phosphorylation sites
GS	**S6**, **S10**, S636, **S640**, S647, **S651**, **S655**, T657, S659, T662, T663, S667, **S671**, **S676**, T681	**S6**, **S10**, T403, **S640**, **S651**, **S655**, **S671**, **S676**	S655
MK2	-	T115, T158, T178, T290, T294, T340	T178, T290
Clbn	-	-	-
Glycogenin	**S206**, S374, T379	**S206**	-
lic	**T8**, **S200**	**T8**, **S200**	-
CG6509	S664, S985, S988, **S991**, S994, S1206, S1208, **S1278**, **S1390**, S1397, T1399	T89, S301, T719, T733, S881, S886, S958, **S991**, T1054, S1172, S1188, S1256, **S1278**, **S1390**, S1395, S1411, T1671	T668, S1077, S1390
CG5198	T22, **S25**, **S30**, **S41**	S17, **S25**, **S30**, **S41**, S116, S117, T164	-
puc	-	T186, S413	S135, T280, T360, S441
RfC3	-	-	-
CG5721	T9	T457	-
cdc16	**S336**, T343	S248, T287, **S336**	T287, S607
eRF1	S341	S70, T340	-
CG4673	T114, **S115**, T117, S134, **S138**	S111, **S115**, **S138**	-
PTP-ER	S128, S130, S166, S170, **S172**, **S176**, T178, T188, **S258**, T516, T521, T522, **S728**	**S172**, **S176**, **S258**, S381, **S728**, S788	T21, S111, T307, S788
ush	S21, S24, S109, S111, T112, S116, S118, S119, S243, T254, **S548**, **S1013**, S1015, **S1017**, S1154, S1156	T131, S134, **S548**, S556, S633, **S1013**, **S1017**, S1069	T112, S116, S119, T131, S607
unc-13	-	S2136, S2734	S219, S544, T697

mts	-	-	-
CG10418	-	-	-
CG8108	T121, S122, S123, S124, S132, S133, S140, S141, S341, S370, S374, **S381**, **S603**, **S605**, **S608**, T769, S805, **S851**, T854, **T855**, T857, **T872**	S61, S181, S305, **S381**, S400, **S603**, **S605**, **S608**, T829, S843, **S851**, **T855**, **T872**, S899	T855
otu	-	-	S546, T765

TABLE S3. **List of the p38 interacting proteins from the DPiM database that were also isolated in this study.** Color codes are as follows: Green, common specific interactors; Red, interactors present only in control purifications in this study; Blue, interactors present both in control and bait purifications in this study; Grey, proteins that can not be reliably distinguished from the respective baits due to high sequence identity.

p38a	p38b	p38c
CG8602	Atg18	Atx2
Ipk1	CG8602	bocksbeutel
128up	CkIIα	CG4278
Arc-p34	HLHm3	isoQC
CG10306	h	Jafrac2
CG3817	Ipk1	mRpS24
Imp	Atg12	Ote
isoQC	CG6094	Pdi
LRP1	eIF-5A	Saf-B
p16-ARC	MAPk-Ak2	SmD3
Rpb8	Mpk2	Tm1
p38b	mRpL10	U4-U6-60K
	mRpL19	
	mRpL43	
	mRpL44	
	mRpL4	
	mRpL51	
	mts	
	Psi	
	Rbp1-like	
	RpS21	
	Saf-B	
	SC35	
	SmG	
	Vha55	
	wdb	
	Zn72D	
	PCID2	
	porin	
	puc	
	Rab10	
	skpA	
	Snr1	

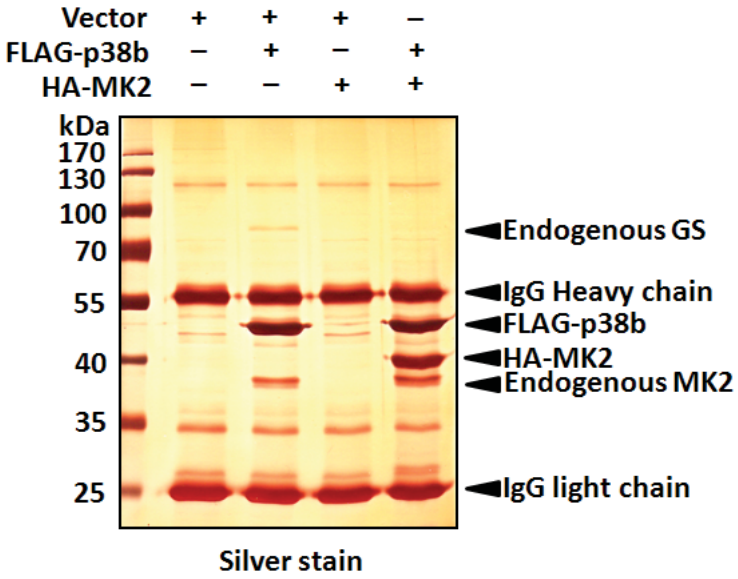

FIGURE S4. **MK2 competes with GS for binding to p38b, suggesting two mutually exclusive complexes.** S2 cells were transfected with indicated constructs and used for co-immunoprecipitation with FLAG-p38 as bait. Immunoprecipitated samples were run on an SDS-PAGE gel and visualized by silver staining. Note the disappearance of the endogenous GS band in the presence of over-expressed MK2.