

Figure S1

Figure S1. Phylogenetic tree of the p38K genes in *Drosophila*. The evolutionary relationships between all three p38K genes across 22 species of *Drosophila*. Black denotes the p38Ka genes, red the p38Kb genes, and grey the p38Kc genes. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

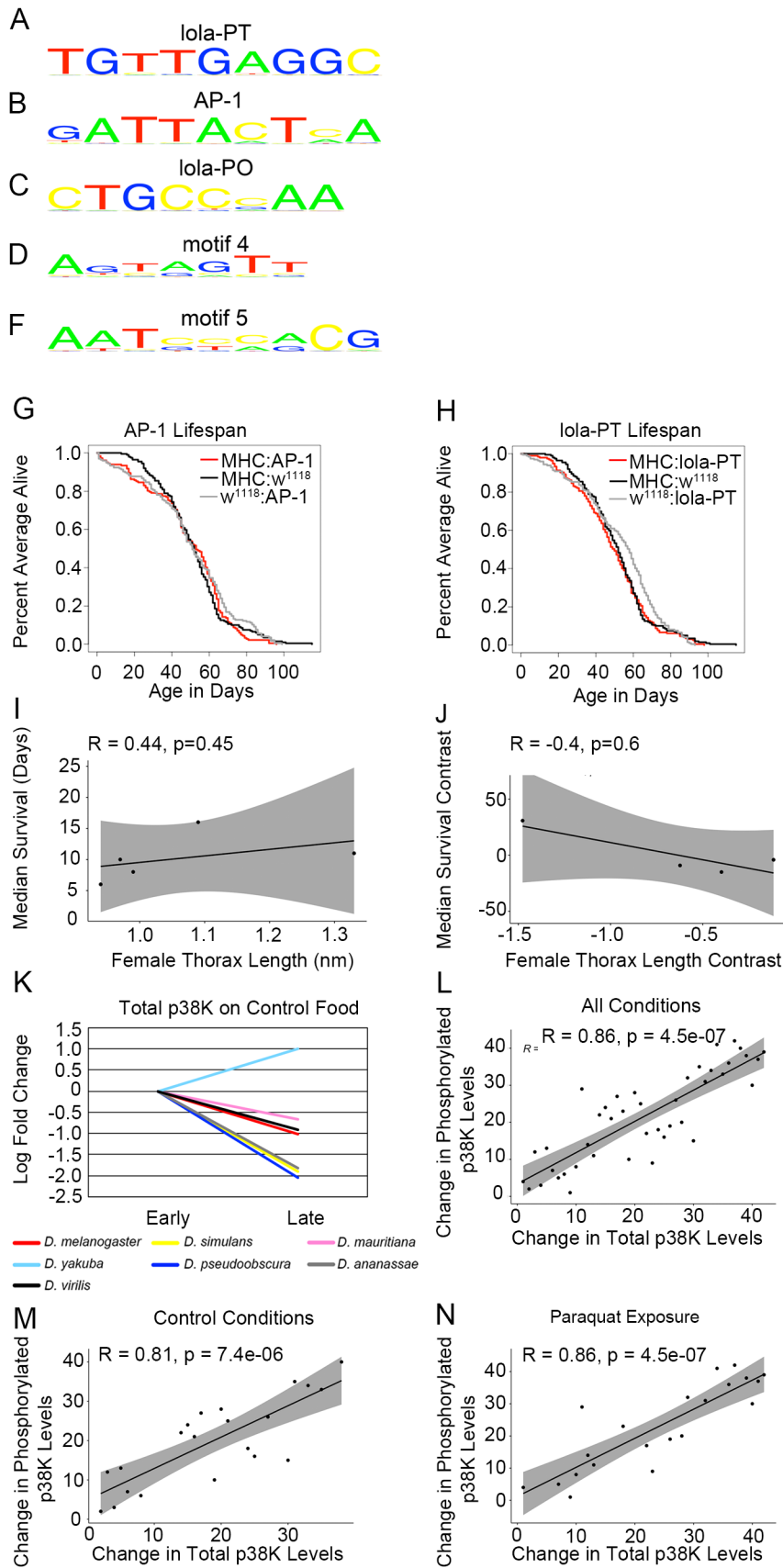


Figure S2

Figure S2. Relationship between predicted transcription factor binding sites and p38K

protein levels. A-E) Consensus sequences the p38Kb transcription factor binding sites. The consensus sequence of the A) lola-PT, B) AP-1, C) lola-PO, D) Motif 4, and E) Motif 5 transcription factor binding sites. For each consensus binding site, the size of the letter indicates the proportion of confirmed sites that use that base. G-H) Over-expression of AP-1 or lola-PT has no effect on lifespan by Log-Rank test. G) Over-expression of AP-1 (red) or H) lola-PT (red) reared on control food has no significant effect on lifespan as compared to outcrossed GAL4 (black) and transgene (grey) controls. In G-H the following number of animals and replicates per genotype were as follows: MHC:AP-1 n=196, 10 replicates, W₁₁₁₈:AP-1 n=182, 12 replicates, MHC:lola-PT n=197, 12 replicates, W₁₁₁₈:lola-PT n=195, 10 replicates, and MHC:W₁₁₁₈ n=199, 11 replicates. I-J) Body size is weakly correlated with paraquat resistance. I) Scatterplot of correlation between female thorax length and median survival. Correlation between resistance and size was calculated for 5 species using Pearson's product-moment correlation. J) Scatterplot of correlation between phylogenetically independent contrasts of female thorax length and median survival. Correlation between resistance and size was calculated for phylogenetically independent contrasts calculated from 5 species using Spearman rho correlation. K-N) Levels of total and phosphorylated p38K have a linear relationship. K) Log₂ fold change of levels of p38K protein on control food for 7 species. Scatter plots showing the relationship calculated by Pearson's product-moment correlation between changes in total and phosphorylated p38K under L) all conditions, M) control conditions and N) exposure to 20mM paraquat.

Accession numbers for analyzed DNA sequences		
Species	Gene Name	NCBI Accession #
<i>D. ananassae</i>	p38Ka	XM_001955664.2
<i>D. biarmipes</i>	p38Ka	XM_017097085.1
<i>D. bipectinata</i>	p38Ka	XM_017249637.1
<i>D. elegans</i>	p38Ka	XM_017258256.1
<i>D. erecta</i>	p38Ka	XM_001982000.3
<i>D. eugracilis</i>	p38Ka	XM_017228726.1
<i>D. ficusphila</i>	p38Ka	XM_017191561.1
<i>D. grimshawi</i>	p38Ka	XM_001989942.1
<i>D. kikkawai</i>	p38Ka	XM_017180763.1
<i>D. mauritiana</i>	p38Ka	NIGA01000004.1 (Genome accession ID)
<i>D. melanogaster</i>	p38Ka	NM_057815.5
<i>D. miranda</i>	p38Ka	XM_017289227.1
<i>D. mojavensis</i>	p38Ka	XM_001998772.2
<i>D. persimilis</i>	p38Ka	XM_002013400.2
<i>D. pseudoobscura</i>	p38Ka	XM_001358779.4
<i>D. rhopaloa</i>	p38Ka	XM_017125963.1
<i>D. sechellia</i>	p38Ka	XM_002032419.1
<i>D. simulans</i>	p38Ka	XM_016174818.1
<i>D. takahashii</i>	p38Ka	XM_017160399.1
<i>D. virilis</i>	p38Ka	XM_002058599.2
<i>D. willistoni</i>	p38Ka	XM_002070167.3
<i>D. yakuba</i>	p38Ka	XM_002099248.2
<i>D. ananassae</i>	p38Kb	XM_001964952.2
<i>D. biarmipes</i>	p38Kb	XM_017106505.1
<i>D. bipectinata</i>	p38Kb	XM_017250102.1
<i>D. elegans</i>	p38Kb	XM_017276942.1
<i>D. erecta</i>	p38Kb	XM_001969550.3
<i>D. eugracilis</i>	p38Kb	XM_017212644.1
<i>D. ficusphila</i>	p38Kb	XM_017192371.1
<i>D. grimshawi</i>	p38Kb	XM_001988300.1
<i>D. kikkawai</i>	p38Kb	XM_017181846.1
<i>D. mauritiana</i>	p38Kb	NIGA01000001.1 (Genome accession ID)
<i>D. melanogaster</i>	p38Kb	NM_058013.5
<i>D. miranda</i>	p38Kb	XM_017296766.1
<i>D. mojavensis</i>	p38Kb	XM_002004004.2

<i>D. persimilis</i>	p38Kb	XM_026987565.1
<i>D. pseudoobscura</i>	p38Kb	XM_001356214.3
<i>D. rhopaloa</i>	p38Kb	XM_017134148.1
<i>D. sechellia</i>	p38Kb	XM_002035711.1
<i>D. simulans</i>	p38Kb	XM_016180114.1
<i>D. takahashii</i>	p38Kb	XM_017159206.1
<i>D. virilis</i>	p38Kb	XM_002057421.2
<i>D. willistoni</i>	p38Kb	XM_002066223.3
<i>D. yakuba</i>	p38Kb	XM_002088597.2
<i>D. pseudoobscura</i>	p38Kb2	XM_002132725.1
<i>D. ananassae</i>	p38Kc	XM_001955665.2
<i>D. biarmipes</i>	p38Kc	XM_017096660.1
<i>D. bipectinata</i>	p38Kc	XM_017249656.1
<i>D. elegans</i>	p38Kc	XM_017258257.1
<i>D. erecta</i>	p38Kc	XM_026983955.1
<i>D. eugracilis</i>	p38Kc	XM_017228501.1
<i>D. ficusphila</i>	p38Kc	XM_017191241.1
<i>D. kikkawai</i>	p38Kc	XM_017180769.1
<i>D. mauritiana</i>	p38Kc	NIGA01000004.1 (Genome accession ID)
<i>D. melanogaster</i>	p38Kc	NM_206554.2
<i>D. miranda</i>	p38Kc	XM_017289226.1
<i>D. persimilis</i>	p38Kc	XM_002013401.2
<i>D. pseudoobscura</i>	p38Kc	XM_002137454.2
<i>D. rhopaloa</i>	p38Kc	XM_017125964.1
<i>D. sechellia</i>	p38Kc	XM_002032418.1
<i>D. simulans</i>	p38Kc	XM_016174820.1
<i>D. takahashii</i>	p38Kc	XM_017160398.1
<i>D. yakuba</i>	p38Kc	XM_002099249.2

Accession numbers for analyzed protein sequences		
Species	Gene Name	NCBI Accession #
<i>H. sapiens</i>	ERK1 (MAPK 3)	NP_002737.2
<i>H. sapiens</i>	ERK2 (MAPK 1)	NP_002736.3
<i>M. musculus</i>	ERK1 (MAPK 3)	NP_036082.1
<i>M. musculus</i>	ERK2 MAPK 1	NP_001033752.1
<i>D. rerio</i>	ERK1 (MAPK 3)	NP_958915.1
<i>D. rerio</i>	ERK2 (MAPK 1)	NP_878308.2
<i>D. melanogaster</i>	rolled	NP_001015122.1

<i>C. elegans</i>	mpk-1	NP_001022584.1
<i>S. cerevisiae</i>	FUS3	NP_009537.1
<i>S. cerevisiae</i>	KSS1	NP_011554.3
<i>H. sapiens</i>	JNK1 (MAPK 8)	NP_001310231.1
<i>H. sapiens</i>	JNK2 (MAPK 9)	NP_001351536.1
<i>H. sapiens</i>	JNK3 (MAPK 10)	NP_001304998.1
<i>M. musculus</i>	JNK1 (MAPK 8)	NP_057909.1
<i>M. musculus</i>	JNK2 (MAPK 9)	NP_001157144.1
<i>M. musculus</i>	JNK3 (MAPK 10)	NP_001075036.1
<i>D. rerio</i>	JNK1 (MAPK 8)	NP_571796.1
<i>D. rerio</i>	JNK2 (MAPK 9)	XP_001919688.1
<i>D. rerio</i>	JNK3 (MAPK 10)	NP_001305247.1
<i>D. melanogaster</i>	basket	NP_001162930.1
<i>C. elegans</i>	jnk-1	NP_001021270.1
<i>S. cerevisiae</i>	HOG1	NP_013214.1
<i>H. sapiens</i>	p38Ka (MAPK14)	NP_001306.1
<i>H. sapiens</i>	p38Kb (MAPK11)	NP_002742.3
<i>H. sapiens</i>	p38Kg (MAPK12)	NP_002960.2
<i>H. sapiens</i>	p38Kd (MAPK13)	NP_002745.1
<i>M. musculus</i>	p38Ka (MAPK14)	NP_036081.1
<i>M. musculus</i>	p38Kb (MAPK11)	NP_035291.4
<i>M. musculus</i>	p38Kg (MAPK12)	NP_038899.1
<i>M. musculus</i>	p38Kd (MAPK13)	NP_036080.2
<i>D. rerio</i>	p38Ka (MAPK14)	NP_571797.1
<i>D. rerio</i>	p38Kb (MAPK11)	NP_001002095.1
<i>D. rerio</i>	p38Kg (MAPK12)	NP_571482.1
<i>D. rerio</i>	p38Kd (MAPK13)	XP_001337833.2
<i>D. melanogaster</i>	p38Ka	NP_001163711.1
<i>D. melanogaster</i>	p38Kb	NP_477361.1
<i>D. melanogaster</i>	p38Kc	NP_996277.1
<i>C. elegans</i>	pmk-1	NP_501365.1
<i>C. elegans</i>	pmk-2	NP_741457.2
<i>C. elegans</i>	pmk-3	NP_501363.1
<i>D. ananassae</i>	basket	XP_001963064.2
<i>D. ananassae</i>	rolled	XP_001965786.2
<i>D. virilis</i>	basket	XP_002052403.1
<i>D. virilis</i>	rolled	XP_002052382.1
<i>D. yakuba</i>	basket	XP_002089054.1
<i>D. yakuba</i>	rolled	XP_002086048.2

<i>D. ananassae</i>	p38Ka	XP_001955700.1
<i>D. ananassae</i>	p38Kb	XP_001964988.1
<i>D. ananassae</i>	p38Kc	XP_001955701.1
<i>D. virilis</i>	p38Ka	XP_002058635.2
<i>D. virilis</i>	p38Kb	XP_002057457.1
<i>D. yakuba</i>	p38Ka	XP_002099284.1
<i>D. yakuba</i>	p38Kb	XP_002088633.1
<i>D. yakuba</i>	p38Kc	XP_002099285.1

Table S1. Accession numbers for DNA and protein sequences analyzed

Comparisons of different batches of 20mM paraquat				
Genotype	Average Age batch 1	Average Age batch 2	Median Age batch 1	Median Age batch 2
MHC-GAL4::w1118	8.2 days	3.8 days	9 days	3 days
MHC-GAL4::AP-1	10.3 days	7.5 days	10 days	8.5 days
MHC-GAL4::lola PT	9.3 days	5.7 days	10 days	6 days

Lifespan of AP-1 and lola-PT over-expression					
Genotype	Average Age	Median Age	n	p value vs outcrossed GAL4	p value vs outcrossed transgene
MHC-GAL4::w1118	51.5 days	52 days	199	n/a	n/a
w1118::AP-1	50.5 days	51 days	196	0.54	n/a
MHC-GAL4::AP-1	49 days	53 days	182	0.98	0.39
w1118::lola PT	53.0 days	58 days	197	0.154	n/a
MHC-GAL4::lola PT	49.1 days	50 days	195	0.472	0.099
$\chi^2=1.8$, $p=0.4$ for AP-1 and $\chi^2=5.5$, $p<0.06$ for lola-PT					

Survival of AP-1 and lola-PT over-expression with 20mM paraquat exposure					
Genotype	Average Age	Median Age	n	p value vs outcrossed GAL4	p value vs outcrossed transgene
MHC-GAL4::w1118	3.8 days	3 days	75	n/a	n/a
w1118::AP-1	5.7 days	5 days	145	4.80E-05	n/a
MHC-GAL4::AP-1	7.5 days	8.5 days	120	8.10E-13	2.50E-09
w1118::lola PT	5.2 days	5 days	106	0.01258	n/a
MHC-GAL4::lola PT	5.7 days	6 days	123	0.00085	0.06162
$\chi^2=70$, $p=6E-16$ for AP-1 and $\chi^2=15.8$, $p=4E-04$ for lola-PT					

Survival of AP-1 and lola-PT over-expression with 10mM paraquat exposure					
Genotype	Average Age	Median Age	n	p value vs outcrossed GAL4	p value vs outcrossed transgene
MHC-GAL4::w1118	11.5 days	12 days	66	n/a	n/a
w1118::AP-1	14.3 days	14 days	58	0.03915	n/a
MHC-GAL4::AP-1	16.9 days	17.5 days	90	1.80E-09	0.00043
w1118::lola PT	10.7 days	11 days	68	0.00067	n/a
MHC-GAL4::lola PT	14.6 days	13 days	98	0.00116	5.30E-07
$\chi^2=35.1$, $p=2E-08$ for AP-1 and $\chi^2=33.5$, $p=5E-08$ for lola-PT					

Survival of Drosophila species with 10mM paraquat exposure				
Species	Average Age	Median Age	n	Confidence Group
<i>D. melanogaster</i>	7.5 days	8 days	210	a
<i>D. mauritiana</i>	9.4 days	8 days	207	e
<i>D. ananassae</i>	5.7 days	6 days	212	b
<i>D. simulans</i>	9.8 days	10 days	211	e,f
<i>D. pseudoobscura</i>	15.3 days	16 days	200	c
<i>D. virilis</i>	12.8 days	11 days	245	d
<i>D. yakuba</i>	9.2 days	9 days	209	e, g
$\chi^2=547$, $p<2e-16$				

Table S2. Lifespan and Survival under control and paraquat conditions

ERK MAPK genes across taxa	
Species	Gene Name
<i>H. sapiens</i>	ERK1 (MAPK 3)
<i>H. sapiens</i>	ERK2 (MAPK 1)
<i>M. musculus</i>	ERK1 (MAPK 3)
<i>M. musculus</i>	ERK2 MAPK 1
<i>D. rio</i>	ERK1 (MAPK 3)
<i>D. rio</i>	ERK2 (MAPK 1)
<i>D. melanogaster</i>	rolled
<i>C. elegans</i>	mpk-1
<i>S. cerevisiae</i>	FUS3
<i>S. cerevisiae</i>	KSS1

JNK MAPK genes across taxa	
Species	Gene Name
<i>H. sapiens</i>	JNK1 (MAPK 8)
<i>H. sapiens</i>	JNK2 (MAPK 9)
<i>H. sapiens</i>	JNK3 (MAPK 10)
<i>M. musculus</i>	JNK1 (MAPK 8)
<i>M. musculus</i>	JNK2 (MAPK 9)
<i>M. musculus</i>	JNK3 (MAPK 10)
<i>D. rio</i>	JNK1 (MAPK 8)
<i>D. rio</i>	JNK2 (MAPK 9)
<i>D. rio</i>	JNK3 (MAPK 10)
<i>D. melanogaster</i>	basket
<i>C. elegans</i>	jnk-1
<i>S. cerevisiae</i>	HOG1

p38K MAPK genes across taxa	
Species	Gene Name
<i>H. sapiens</i>	p38K alpha (MAPK14)
<i>H. sapiens</i>	p38K beta (MAPK11)
<i>H. sapiens</i>	p38K gamma (MAPK12)
<i>H. sapiens</i>	p38K delta (MAPK13)
<i>M. musculus</i>	p38K alpha (MAPK14)
<i>M. musculus</i>	p38K beta (MAPK11)
<i>M. musculus</i>	p38K gamma (MAPK12)
<i>M. musculus</i>	p38K delta (MAPK13)

<i>D. rio</i>	p38K alpha (MAPK14)
<i>D. rio</i>	p38K beta (MAPK11)
<i>D. rio</i>	p38K gamma (MAPK12)
<i>D. rio</i>	p38K delta (MAPK13)
<i>D. melanogaster</i>	p38Ka
<i>D. melanogaster</i>	p38Kb
<i>D. melanogaster</i>	p38Kc
<i>C. elegans</i>	pmk-1
<i>C. elegans</i>	pmk-2
<i>C. elegans</i>	pmk-3
<i>S. cerevisiae</i>	HOG1

Table S3. MAPK family genes across taxa

Genome coordinates for the predicted transcription factor binding sites upstream of p38Ka				
Species	Motif	Genome motif start	Genome motif end	Genome Accession ID
<i>D. melanogaster</i>	lola-PO	24152039	24152047	NT_033777.3
<i>D. melanogaster</i>	homeobox	24151977	24151985	NT_033777.3
<i>D. melanogaster</i>	lola-PK	24152025	24152033	NT_033777.3
<i>D. simulans</i>	lola-PO	19514993	19515001	NT_479536.1
<i>D. simulans</i>	homeobox	19514930	19514938	NT_479536.1
<i>D. simulans</i>	lola-PK	19514979	19514987	NT_479536.1
<i>D. sechellia</i>	lola-PO	22693221	22693229	NIFZ01000004.1
<i>D. sechellia</i>	homeobox	22693158	22693166	NIFZ01000004.1
<i>D. sechellia</i>	lola-PK	22693207	22693215	NIFZ01000004.1
<i>D. erecta</i>	lola-PO	8296314	8296322	NW_020825194.1
<i>D. erecta</i>	homeobox	8296252	8296260	NW_020825194.1
<i>D. erecta</i>	lola-PK	8296300	8296308	NW_020825194.1
<i>D. erecta</i>	motif3	8296287	8296295	NW_020825194.1
<i>D. yakuba</i>	lola-PO	26525390	26525398	NT_167065.1
<i>D. yakuba</i>	homeobox	26525328	26525336	NT_167065.1
<i>D. yakuba</i>	lola-PK	26525376	26525384	NT_167065.1
<i>D. ananassae</i>	lola-PO	22766070	22766078	QMES02000001.1
<i>D. ananassae</i>	lola-PK	22766056	22766064	QMES02000001.1
<i>D. biarmipes</i>	lola-PO	328703	328711	NW_016033678.1
<i>D. biarmipes</i>	homeobox	328641	328649	NW_016033678.1
<i>D. biarmipes</i>	lola-PK	328689	328697	NW_016033678.1
<i>D. bipectinata</i>	lola-PO	637272	637280	NW_016089276.1
<i>D. bipectinata</i>	lola-PK	637258	637266	NW_016089276.1
<i>D. elegans</i>	lola-PO	282346	282354	NW_016083516.1
<i>D. elegans</i>	homeobox	282283	282291	NW_016083516.1
<i>D. elegans</i>	lola-PK	282332	282340	NW_016083516.1
<i>D. eugracilis</i>	lola-PK	412191	412199	NW_016078341.1
<i>D. ficusphila</i>	lola-PO	1145970	1145978	NW_016073234.1
<i>D. ficusphila</i>	homeobox	1145908	1145916	NW_016073234.1
<i>D. ficusphila</i>	lola-PK	1145956	1145964	NW_016073234.1
<i>D. rhopaloa</i>	lola-PO	56330	56338	NW_016061501.1
<i>D. takahashii</i>	lola-PO	63876	63884	NW_016038156.1
<i>D. takahashii</i>	homeobox	63814	63822	NW_016038156.1
<i>D. takahashii</i>	lola-PK	63862	63870	NW_016038156.1
<i>D. mauritiana</i>	lola-PO	21108464	21108472	NIGA01000004.1
<i>D. mauritiana</i>	homeobox	21108401	21108409	NIGA01000004.1
<i>D. mauritiana</i>	lola-PK	21108450	21108458	NIGA01000004.1

Genome coordinates for the predicted transcription factor binding sites upstream of p38Kb				
Species	Motif	Genome motif start	Genome motif end	Genome Accession ID
<i>D. melanogaster</i>	lola-PT	13780871	13780879	NT_033779.5
<i>D. melanogaster</i>	AP-1	13780852	13780860	NT_033779.5
<i>D. melanogaster</i>	lola-PO	13780928	13780935	NT_033779.5
<i>D. simulans</i>	lola-PT	13327283	13327291	NT_479533.1
<i>D. simulans</i>	AP-1	13327265	13327273	NT_479533.1
<i>D. simulans</i>	lola-PO	13327340	13327347	NT_479533.1
<i>D. simulans</i>	motif 5	13327317	13327325	NT_479533.1
<i>D. sechellia</i>	lola-PT	13470991	13470999	NIFZ01000001.1
<i>D. sechellia</i>	AP-1	13470973	13470981	NIFZ01000001.1
<i>D. sechellia</i>	lola-PO	13471048	13471055	NIFZ01000001.1
<i>D. sechellia</i>	motif 5	13471025	13471033	NIFZ01000001.1
<i>D. erecta</i>	AP-1	11957923	11957931	NW_020825200.1
<i>D. yakuba</i>	AP-1	10192656	10192664	NT_167062.1
<i>D. pseudoobscura2</i>	lola-PT	5966412	5966420	NW_001589963.2
<i>D. pseudoobscura2</i>	AP-1	5966393	5966401	NW_001589963.2
<i>D. pseudoobscura2</i>	motif 4	5966386	5966392	NW_001589963.2
<i>D. pseudoobscura2</i>	motif 4	5966464	5966470	NW_001589963.2
<i>D. pseudoobscura</i>	lola-PT	6621127	6621135	NW_001589964.2
<i>D. pseudoobscura</i>	AP-1	6621108	6621116	NW_001589964.2
<i>D. pseudoobscura</i>	motif 4	6621101	6621107	NW_001589964.2
<i>D. pseudoobscura</i>	motif 4	6621179	6621185	NW_001589964.2
<i>D. persimilis</i>	motif 4	12327403	12327409	NW_020825294.1
<i>D. biarmipes</i>	lola-PT	2546854	2546862	NW_016033946.1
<i>D. biarmipes</i>	AP-1	2546834	2546842	NW_016033946.1
<i>D. biarmipes</i>	motif 5	2546888	2546896	NW_016033946.1
<i>D. elegans</i>	lola-PT	1393125	1393133	NW_016083846.1
<i>D. elegans</i>	AP-1	1393106	1393114	NW_016083846.1
<i>D. elegans</i>	motif 5	1393150	1393158	NW_016083846.1
<i>D. eugracilis</i>	lola-PT	352754	352762	NW_016077995.1
<i>D. eugracilis</i>	AP-1	352735	352743	NW_016077995.1
<i>D. eugracilis</i>	motif 5	352788	352796	NW_016077995.1
<i>D. ficusphila</i>	lola-PT	212001	212009	NW_016073247.1
<i>D. ficusphila</i>	AP-1	211982	211990	NW_016073247.1
<i>D. miranda</i>	lola-PT	675897	675905	NC_030306.1
<i>D. miranda</i>	AP-1	675878	675886	NC_030306.1
<i>D. miranda</i>	motif 4	675949	675955	NC_030306.1

<i>D. rhopaloa</i>	lola-PT	298248	298256	NW_016062131.1
<i>D. rhopaloa</i>	AP-1	298229	298237	NW_016062131.1
<i>D. takahashii</i>	lola-PT	52415	52423	NW_016039789.1
<i>D. takahashii</i>	AP-1	52396	52404	NW_016039789.1
<i>D. takahashii</i>	motif 4	52309	52315	NW_016039789.1
<i>D. takahashii</i>	motif 5	52449	52457	NW_016039789.1
<i>D. mauritiana</i>	lola-PT	13455431	13455439	NIGA01000001.1
<i>D. mauritiana</i>	AP-1	13455413	13455421	NIGA01000001.1
<i>D. mauritiana</i>	lola-PO	13455488	13455495	NIGA01000001.1
<i>D. mauritiana</i>	motif 5	13455465	13455473	NIGA01000001.1

Table S4. Genomic coordinates for the predicted transcription factor binding sites upstream of p38Ka and p38Kb