

## **Casein Kinase 1.2 Over Expression Restores Stress Resistance to *Leishmania donovani* HSP23 Null Mutants**

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## Supplementary Information

Table S1

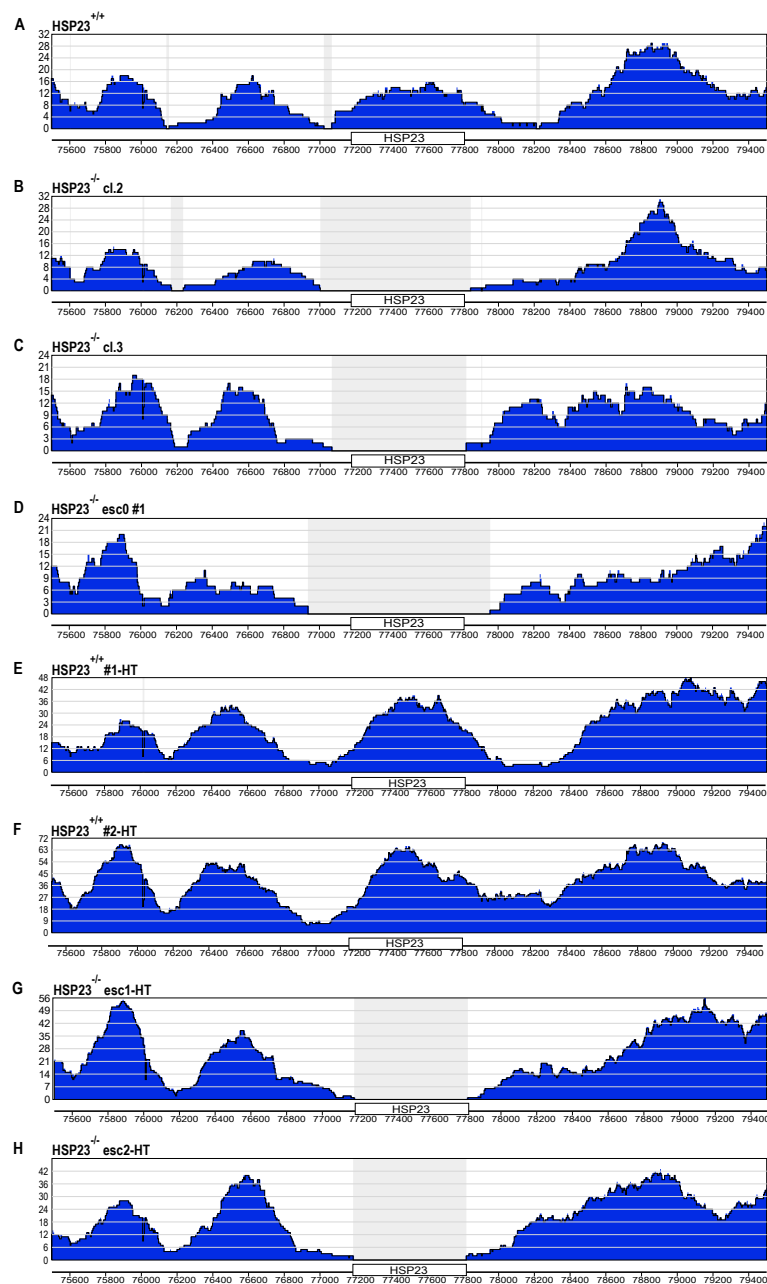
Primers used for CK1.2 study	Primer sequence 5'-3'
<b>qPCR</b>	
LdBPK_340230.1_fwd	CCATTCCTGGATTTGGCTCGG
LdBPK_340230.1_rev	GCATATCGCCGTCGTCATCTCC
LdBPK_351030.1_fwd	CACGTGTACATCATCGACTTTGG
LdBPK_351030.1_rev	CTCTTGCCCTTCCTTGATGGGAT
LdBPK_351040.1_fwd	AACTCTCCCTTCGCATTATCAG
LdBPK_351040.1_rev	CTCGATGAAGTAGATCGGTTGCT
LdBPK_351050.1_fwd	TTTTGGATTAAGCGCCTGCAAC
LdBPK_351050.1_rev	GAAAGTAAATGGTCGTGTAGGCG
LdBPK_351060.1_fwd	CAGTACTACACACACAACCCGTA
LdBPK_351060.1_rev	CGACAACCTTCTGGTAGACTGGA
LdBPK_351070.1_fwd	TAGTCTTCTACGTGCTTCTCTGC
LdBPK_351070.1_rev	GTGATTTGGGTTGAAGAGGACAC
LdBPK_351080.1_fwd	CGAGTACGGCAACTTCAACGATA
LdBPK_351080.1_rev	CCGGTCGTCTATGTCATCTTCTT
<b>over expression plasmids construction</b>	
351030_KpnI_fwd	GGAGGGTACCATGAACGTGGAAGTGC
351030_NdeI_rev	GGAGCATATGCTACTGCTGTTCTTGCGCAC
351040_KpnI_fwd	GGAGGGTACCATGCACACGCCACCCCTCTC
351040_NdeI_rev	GGAGCATATGTCACATGAAGCGAGCCGCCA
351050_KpnI_fwd	GGAGGGTACCATGACCTCCCCGGCAGCCGC
351050_NdeI_rev	GGAGCATATGCTAGATTCTGTTGTCTGTAAG
351060_NdeI_fwd	GGAGCATATGATGACGAGCCAGTCGCACGTG
351060_BglII_rev	GGAGAGATCTTTAGGGCTCCATCACCATCG
351070_KpnI_fwd	GGAGGGTACCATGACCGTTCTGCCACCCAAG
351070_NdeI_rev	GGAGCATATGTCACCTTGCCAAAGCGTCGG
351080_KpnI_fwd	GGAGGGTACCATGGGTAAGCGAAACGAAAG
351080_NdeI_rev	GGAGCATATGCTACATGGATGCGACCACGC
<b>pJC65 multiple cloning site</b>	
Link-pJC65+	CATGGGCCATCATCATCATCATCATCATCACGGTGGTACCA
Link-pJC65-	AGCTTGGTACCACCGTGATGATGATGATGATGATGATGATGATGGCC

## Supplementary Information

Table S2: Ploidy values for chromosomes based on NGS sequence read coverages.

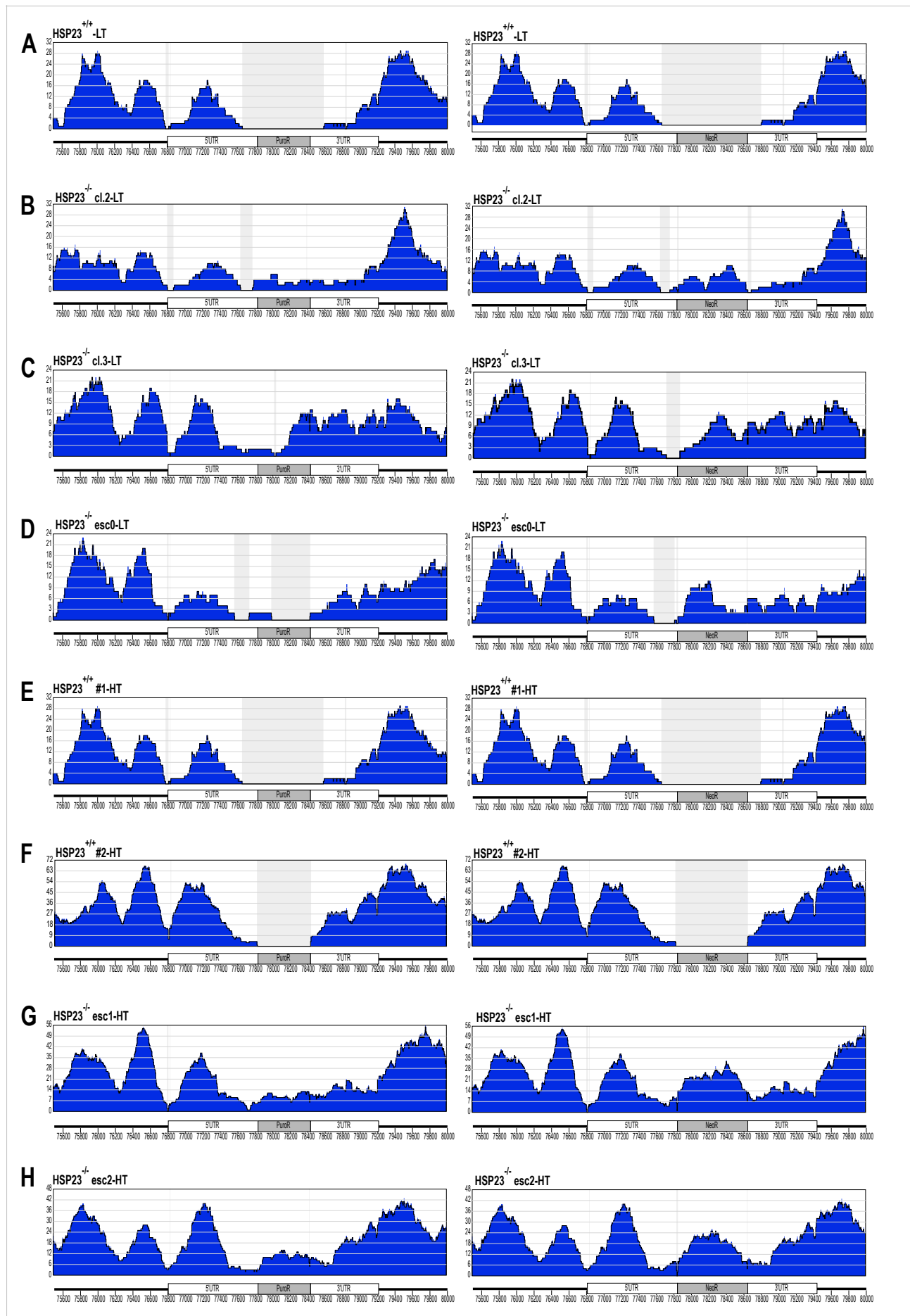
CHR	HSP23+/+ #1-LT	HSP23+/+ #2-LT	HSP23+/+ #1-HT	HSP23+/+ #2-HT	HSP23-/ cl.2-LT	HSP23-/ cl.3-LT	HSP23-/ esc #1-LT	HSP23-/ esc #2-LT	HSP23-/ esc #1-HT	HSP23-/ esc #2-HT
1	1.91	1.61	1.56	1.63	1.85	1.89	1.66	1.70	1.74	1.69
2	2.12	1.95	1.96	2.06	2.35	2.20	2.03	2.09	2.13	2.00
3	1.80	1.78	1.87	1.81	1.97	2.02	1.69	1.84	1.86	2.37
4	1.70	1.79	1.80	1.80	1.98	1.93	1.87	1.85	1.87	1.83
5	1.82	1.74	1.73	2.22	1.86	2.02	1.77	1.87	1.85	1.78
6	1.95	1.95	1.94	1.96	2.06	2.10	1.93	1.98	2.06	1.95
7	2.07	1.92	1.95	1.93	2.08	2.00	1.98	1.98	2.05	1.96
8	2.66	2.78	2.00	2.48	3.06	3.07	2.92	2.84	2.79	2.55
9	1.77	1.86	1.80	1.76	1.97	1.94	1.88	1.92	1.88	1.87
10	1.94	1.99	2.01	1.97	2.07	2.08	1.96	1.99	1.97	1.99
11	2.09	2.14	2.26	2.12	2.05	2.09	2.15	2.05	2.08	2.12
12	3.04	3.11	2.92	2.51	3.03	3.01	2.05	1.99	2.01	2.05
13	2.02	1.90	2.10	1.90	1.97	1.91	1.96	1.96	1.92	1.88
14	1.82	1.86	1.95	2.22	1.97	1.91	2.71	2.71	2.66	2.02
15	1.94	1.84	1.97	2.17	1.96	1.91	1.98	1.97	1.92	1.86
16	1.99	2.01	2.04	1.97	2.19	2.03	2.07	2.10	2.07	2.08
17	2.10	2.05	1.99	1.96	2.09	2.02	1.98	2.05	2.05	1.99
18	2.02	2.07	2.11	2.29	1.98	1.93	1.99	2.00	1.96	1.98
19	1.93	1.98	1.96	1.96	1.97	2.00	2.06	1.97	1.96	1.93
20	1.95	2.06	1.84	1.50	2.08	2.02	2.00	1.96	2.01	2.04
21	1.98	1.99	2.04	2.01	2.07	2.01	1.99	2.02	2.02	2.00
22	2.01	1.98	1.99	2.01	2.06	2.01	2.00	2.00	1.99	1.95
23	3.11	3.22	3.25	2.67	3.15	3.16	2.18	2.08	2.11	2.14
24	2.01	1.96	2.00	2.01	1.98	1.93	1.98	2.00	1.98	1.98
25	1.92	1.99	1.98	2.00	1.99	1.94	1.99	1.97	2.01	1.97
26	2.95	3.05	2.98	2.82	3.15	2.87	3.11	3.03	3.02	3.01
27	2.14	2.16	2.17	2.00	2.07	2.01	2.17	2.06	2.05	2.07
28	1.96	2.11	2.17	2.00	2.00	1.94	2.10	2.05	2.04	2.03
29	2.17	2.18	2.12	2.00	1.99	2.01	2.09	2.05	2.01	2.04
30	2.10	2.20	2.19	2.01	1.98	2.00	2.17	2.05	2.05	2.12
31	4.11	4.30	4.37	4.07	4.16	3.88	4.32	4.16	4.17	4.18
32	2.12	2.17	2.18	2.02	2.00	1.94	2.18	2.02	2.09	2.08
33	2.09	2.16	2.17	2.03	2.06	1.94	2.17	2.00	1.97	2.01
34	2.09	2.12	2.11	1.96	1.98	1.92	2.16	2.00	1.94	1.97
35	2.11	2.13	2.13	1.91	2.93	2.87	2.36	2.26	1.98	2.02
36	1.95	2.16	2.19	1.91	1.97	1.91	2.16	2.00	1.98	2.05

## Supplementary Information



**Fig S1 Verification of the *HSP23* gene replacement.** Sequence reads from each analysed strain (A-H) were aligned to the reference DNA sequence consisting of chromosome 34 (TriTrypDB-46\_LdonovaniBPK282A1\_Genome.fasta). The Y-axis represents the number of reads and the X-axis shows the nucleotide position (bp) on chromosome 34. Grey shaded areas denote complete lack of aligned reads.

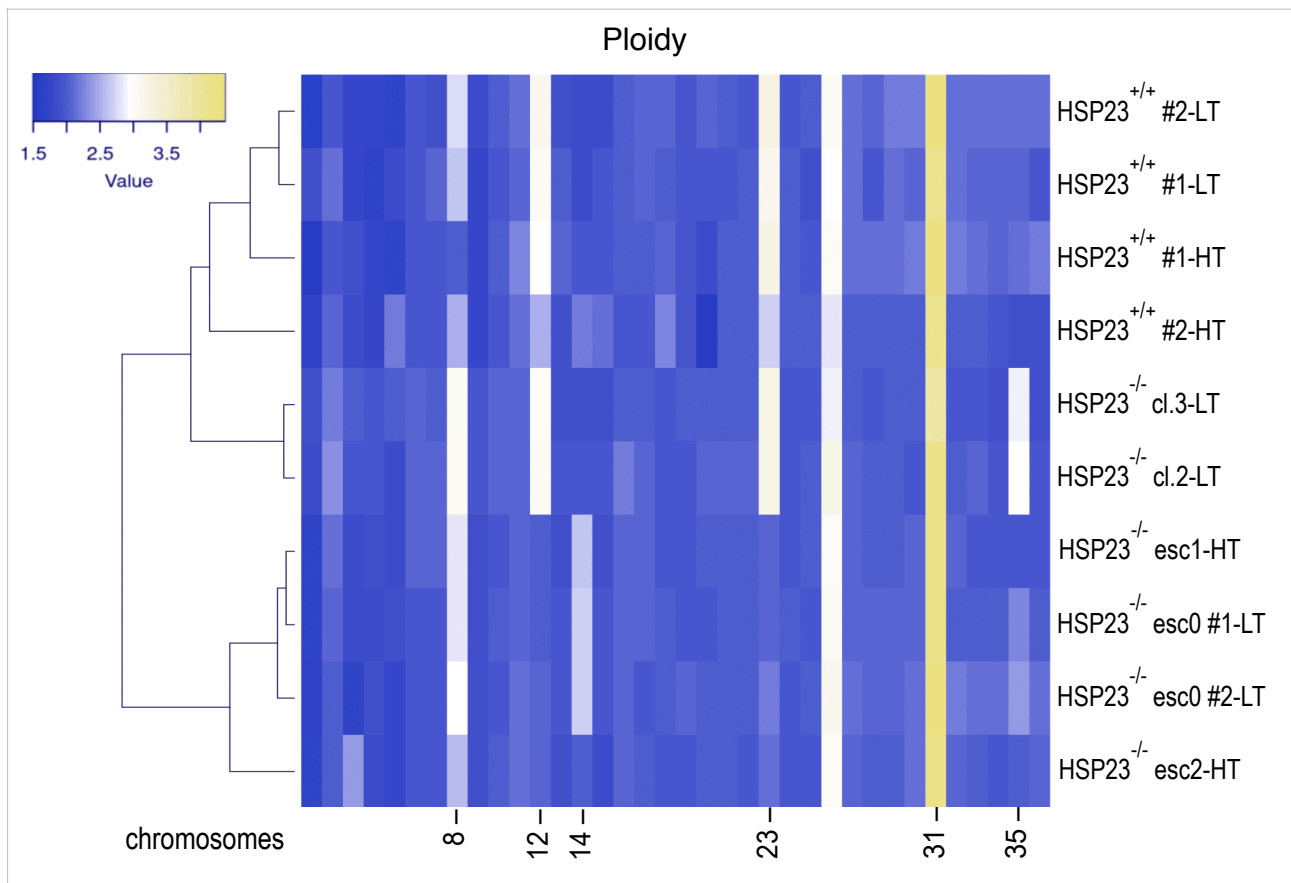
# Supplementary Information



## Supplementary Information

### Fig S2 Verification of the *HSP23* gene replacement by the respective resistance cassettes.

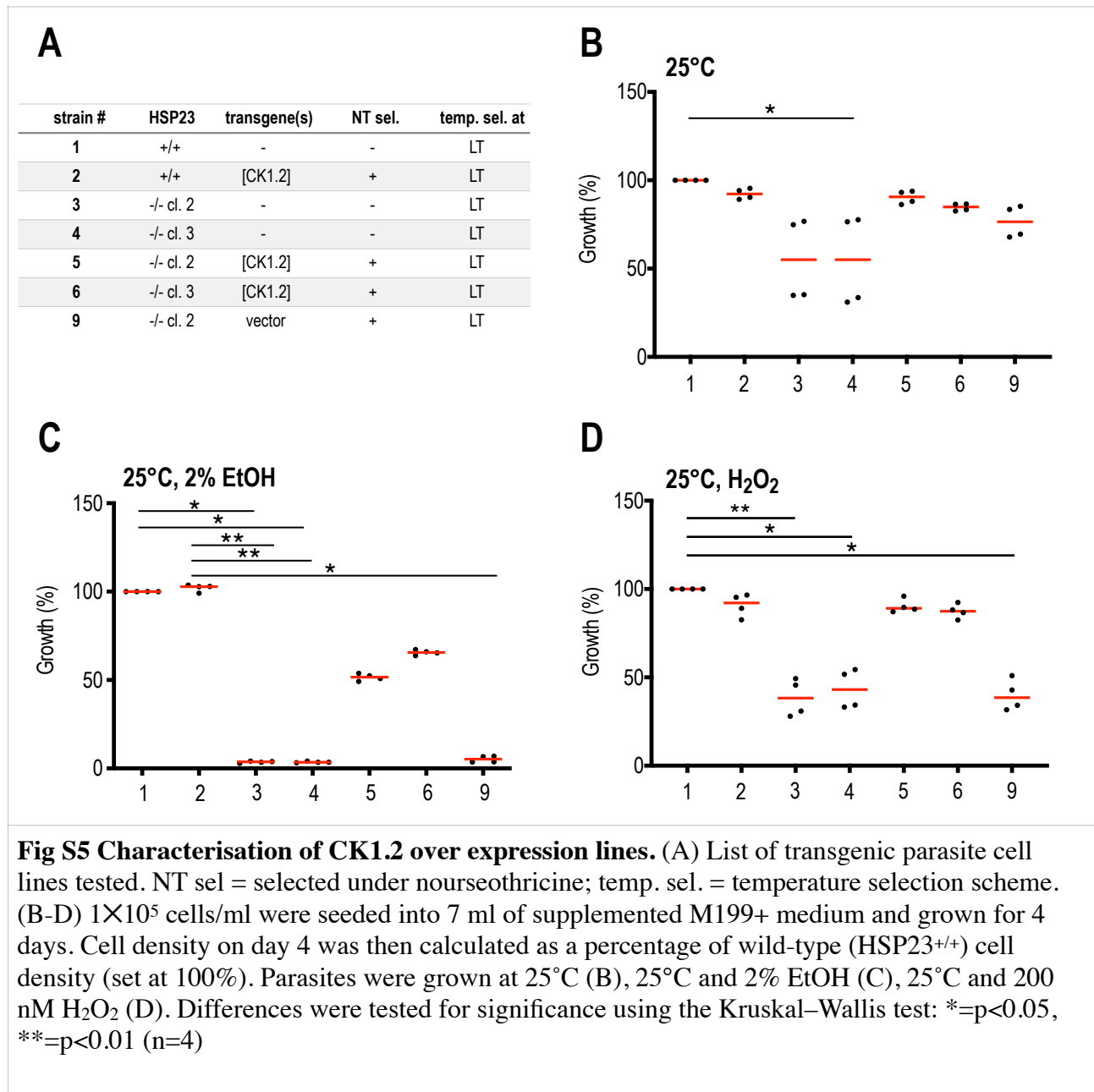
Sequence reads from each analysed strain (A-H) were aligned to the *in silico*-designed reference DNA sequences consisting of the *L. donovani* genome (DNA) sequence of chromosome 34 (TriTrypDB-46\_LdonovaniBPK282A1\_Genome.fasta) with the expected insertion of antibiotic resistance cassettes (NeoR = Neomycin; PuroR = Puromycin). The Y-axis represents the number of reads and the X-axis shows the nucleotide position (bp) on chromosome 34. Grey shaded areas denote complete lack of aligned reads.



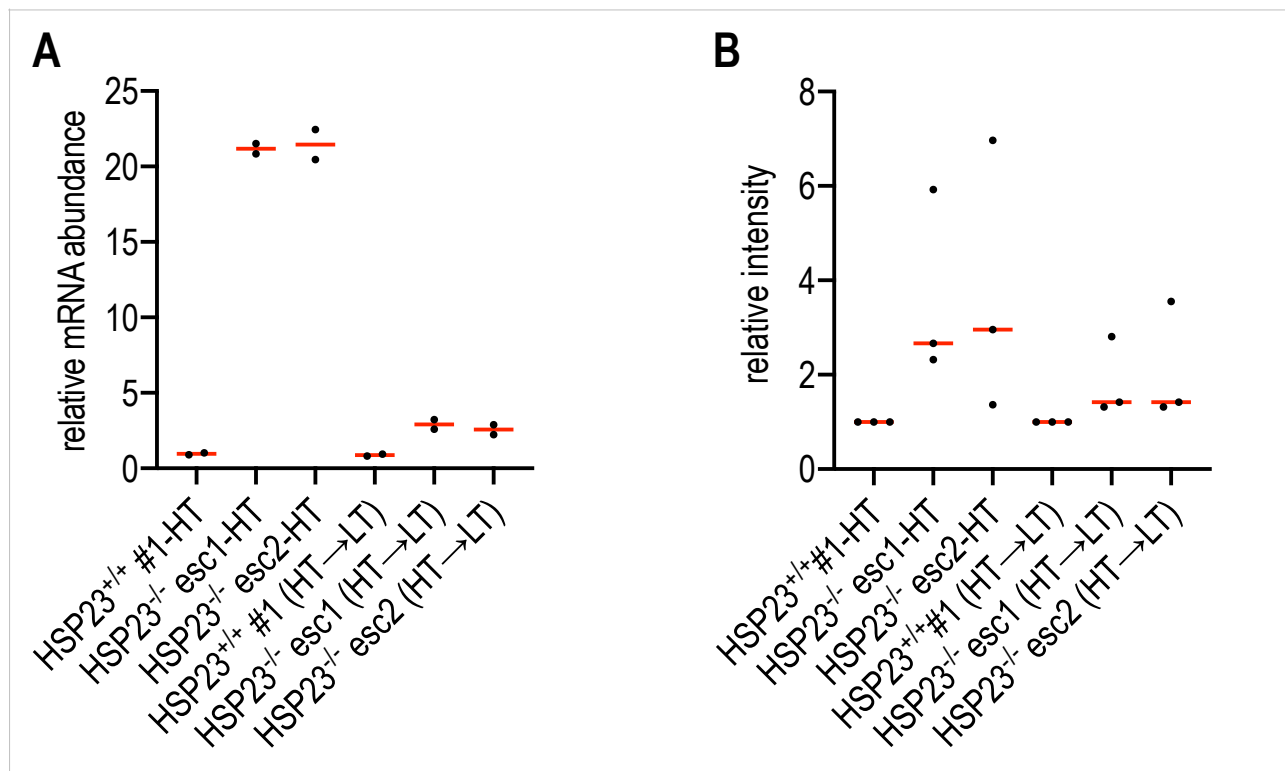
**Fig S3: Dendrogram of chromosomal ploidy profiles.** Heat map showing ploidy levels across all samples. The x-axis indicates individual chromosomes of *L. donovani*. Color scale indicates low (blue) to high (yellow) ploidy. The dendrogram shows clustering of the strains based on similarity of ploidy profiles and was generated by Pearson distance measurements.

## Supplementary Information

**Fig S4 Abundance of mRNA levels in transgenic over expression lines.** mRNA was isolated from transgenic over expression lines HSP23<sup>-/-</sup> [Mix]-LT and HSP23<sup>-/-</sup> [Mix]-HT, selected at 25°C or 37°C, respectively. mRNA levels were determined for all genes of the six-gene cluster on chromosome 35 (LdBPK\_351030-351080) and normalised to WT levels (set to 1).





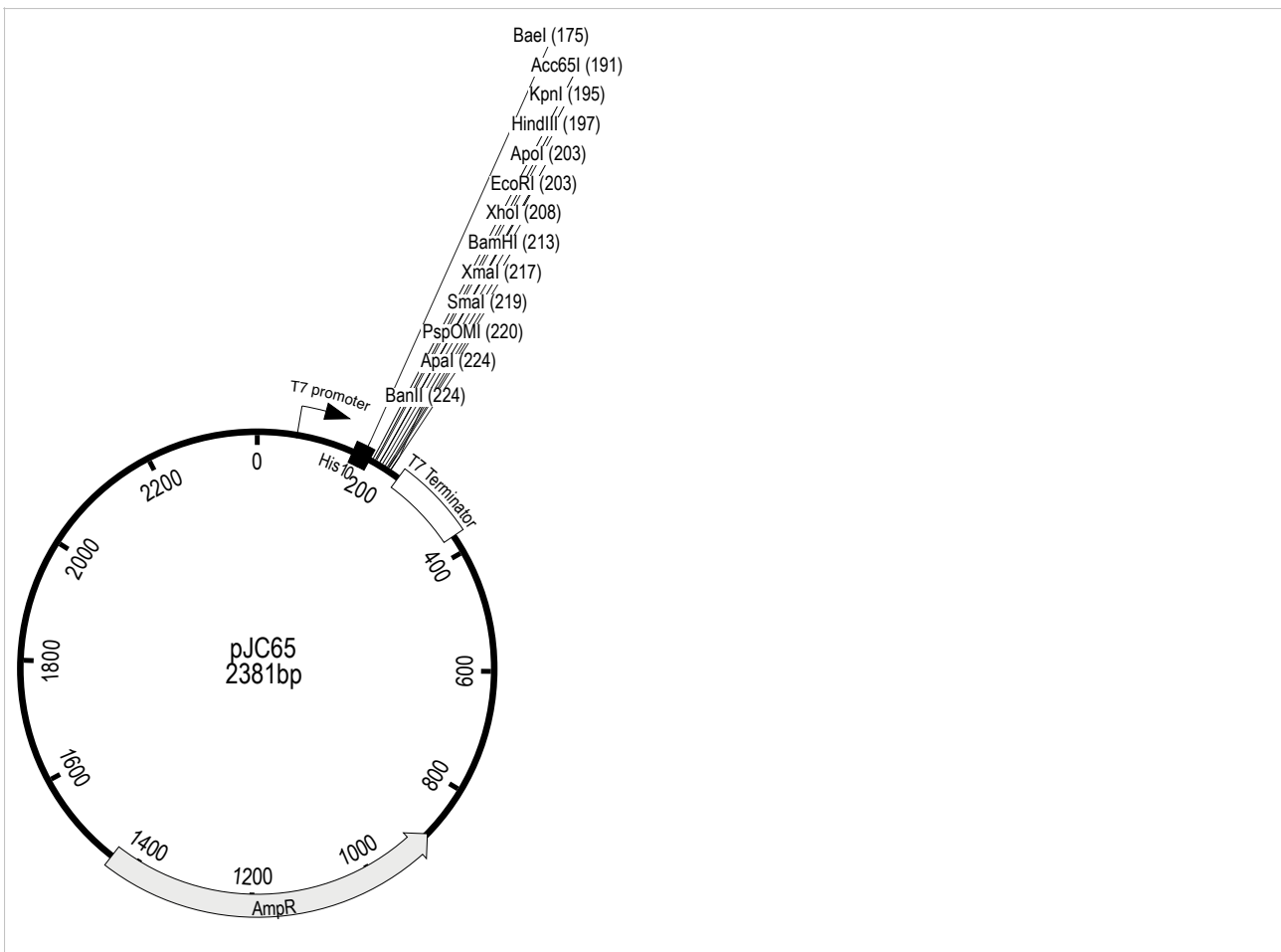


**Fig S6 Abundance of CK1.2 in HSP23<sup>-/-</sup> esc1/2 mutants under different temperatures.** CK1.2 mRNA (n=2) (A) and protein (n=3) (B) abundance were determined for HSP23<sup>-/-</sup> esc1/2 mutants that were selected first at HT (37°C) and were then transferred back to LT (25°C)(HT→LT). mRNA and protein samples were taken 30 days after transfer to LT conditions. For Western blot analysis, signal intensities were quantified using the Image J Software and were normalised to the WT (set to 1).

	CK1 = S/TXXS/T	CK1 = S/TXXS/T
LdHsp23	1 MSTSGPLVPPISWAQRPEYVLITIPLDQT <u><b>TGV</b></u> SVEIKDEGRELC---FACSSPEHKQYACTIHFYGVIS <u><b>SEESQ</b></u> HVVVRPRQIELKLRKFKSKSLEDANDDEVEWPRLTKE	107
LmjHsp23	1 MSSSGPLAPPISWAQRPEYVLITIPLDQT <u><b>TGV</b></u> SVEIKDEGRELH---FACSSPEHKQYACTIHFYGVIS <u><b>SEESQ</b></u> HVVVRPRQIELKLRKFKSKSLEDANDDEVEWPRLTKE	107
LbrHsp23	1 MSASGSLVPPISWAQRPEYVLITIPLDQT <u><b>TGV</b></u> TVEIKDEGRELL---FACCAPEGKQYACTIHFYGAIS <u><b>SEESQ</b></u> HVVVRPRQIELKLRKFKSKSLEDANDDEVEWPRLTKE	106
LmxHsp23	1 MSTSSPLAPPISWAQRPEYVLITIPLDQTTVCVVEIKDEGKELR---FACSSPEHKQYACTIHFYGAIS <u><b>SEESQ</b></u> HVVVRPRQIELKLRKFKSKSLEDANDDEVEWPRLTKE	107
LdP23	1 MS----HLPKWAERKDRLFITVEA <u><b>STPT</b></u> DVQVNFQEKTVSISNGITAKGSQPHALKDELHLLKEIVPEESTFKVLGMAIQICAIK-----EKGYNRLVDE	95
LdHsp23	108 KAKYPN--ISIDWSKWKDENDDECAADDLGNFGLSGGDAMDQYSEMLSQLMQAQQQKDAEESAGLPPGTIPFGSARGQEATHGACAVAGDDDGMPPLEEDT---	209
LmjHsp23	108 KSKYPN--ITIDWSKWKDENDDECAADDLGNFGLSGGDAMDQYSEMLSQLMQAQQQKDAEESADLPPGTIPFGSARGQEATHGAS <u><b>SAET</b></u> GDDDGMPPLEEDT---	209
LbrHsp23	107 KVKYPN--ITIDWSKWKDE--DEGATDDLGNFGLSGGDAMDQYSEMLSQLMQTQKDAEELAGLPPGTIPFGSARGQEATHGASAAAGDDSEMPPLEDDM---	207
LmxHsp23	108 KVKYPN--ITIDWSKWKDENDDECAADDLGNFGLSGGDAMDQYSEMLSQLMQAQQQDAEESAGLPTGTIPFGSARGQEATHGTSAGAGDDDGMPPLEEDT---	209
LdP23	96 <u><b>STKATKSWLS</b></u> ADWNLWKDEDEAEVAAASNFGGYG--DMGGMDMASMMGGMGMDMESMMAS--MGKDAGGDSDEBELDDGAEADGCTEEEPAAIDSLNA	199

**Fig S7 Protein sequence alignments of HSP23 orthologs in Leishmania spp. and L. donovani P23 protein.** Putative CK1 phosphorylation sites (S/TXXS/T) are underlined and in bold font.

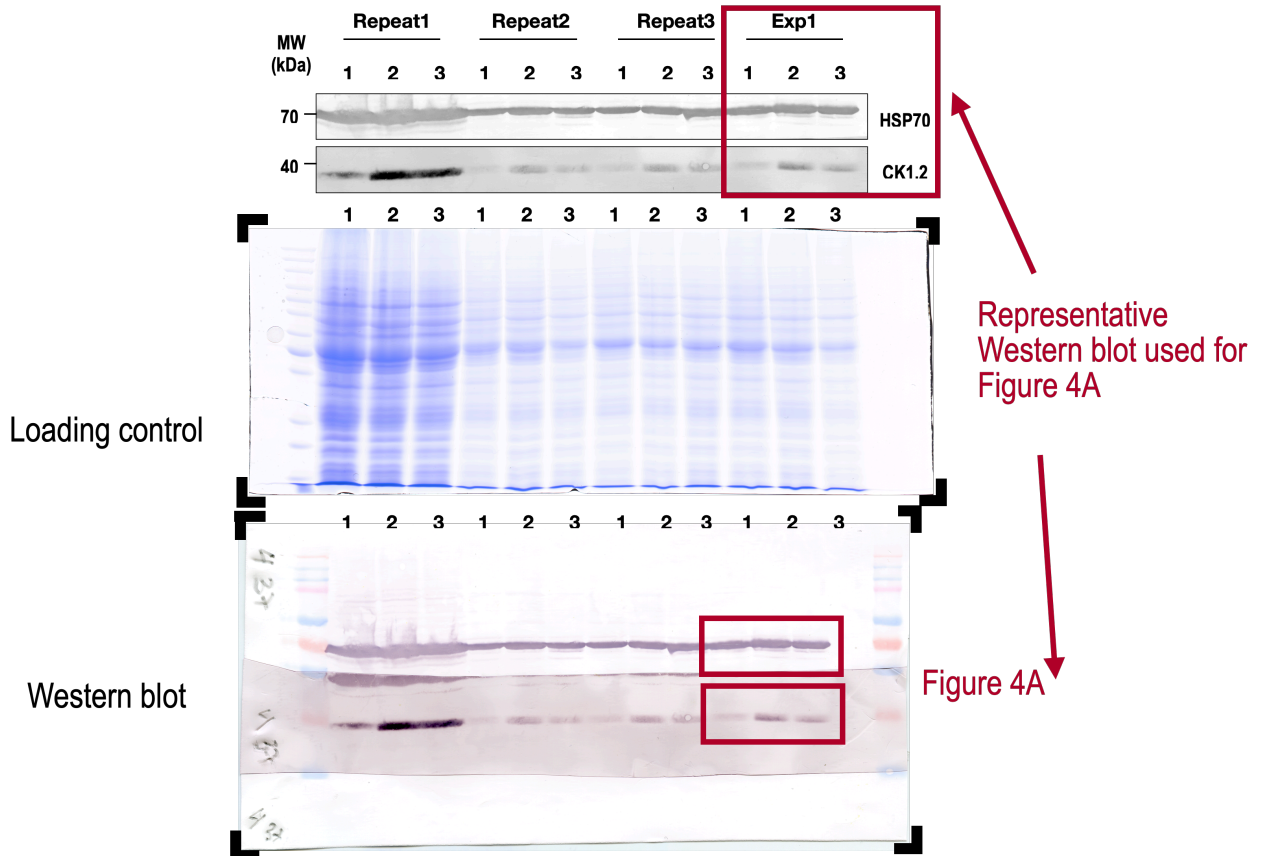
## Supplementary Information



**Fig S8 Schematic depiction plasmid used for protein expression in *E.coli*.** pJC65 is derived from pJC45 (Schlüter et al., 2000), with a newly designed multiple cloning site.

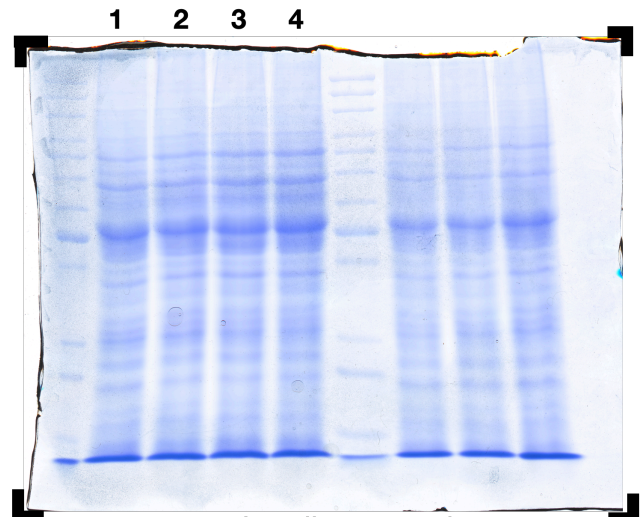
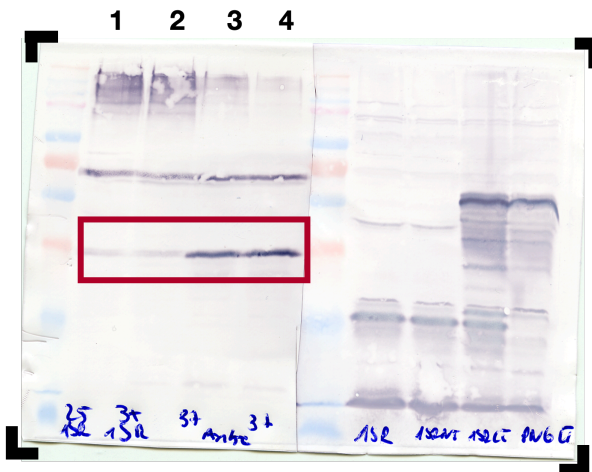
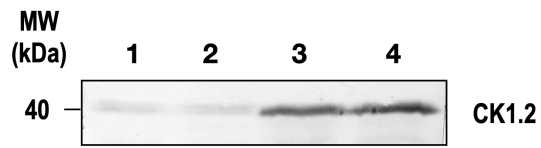
## Raw Images used for Figures

Quantification of CK1.2 protein levels shown in Fig 4A+Fig4B Repeat 1-4



Supplementary Information

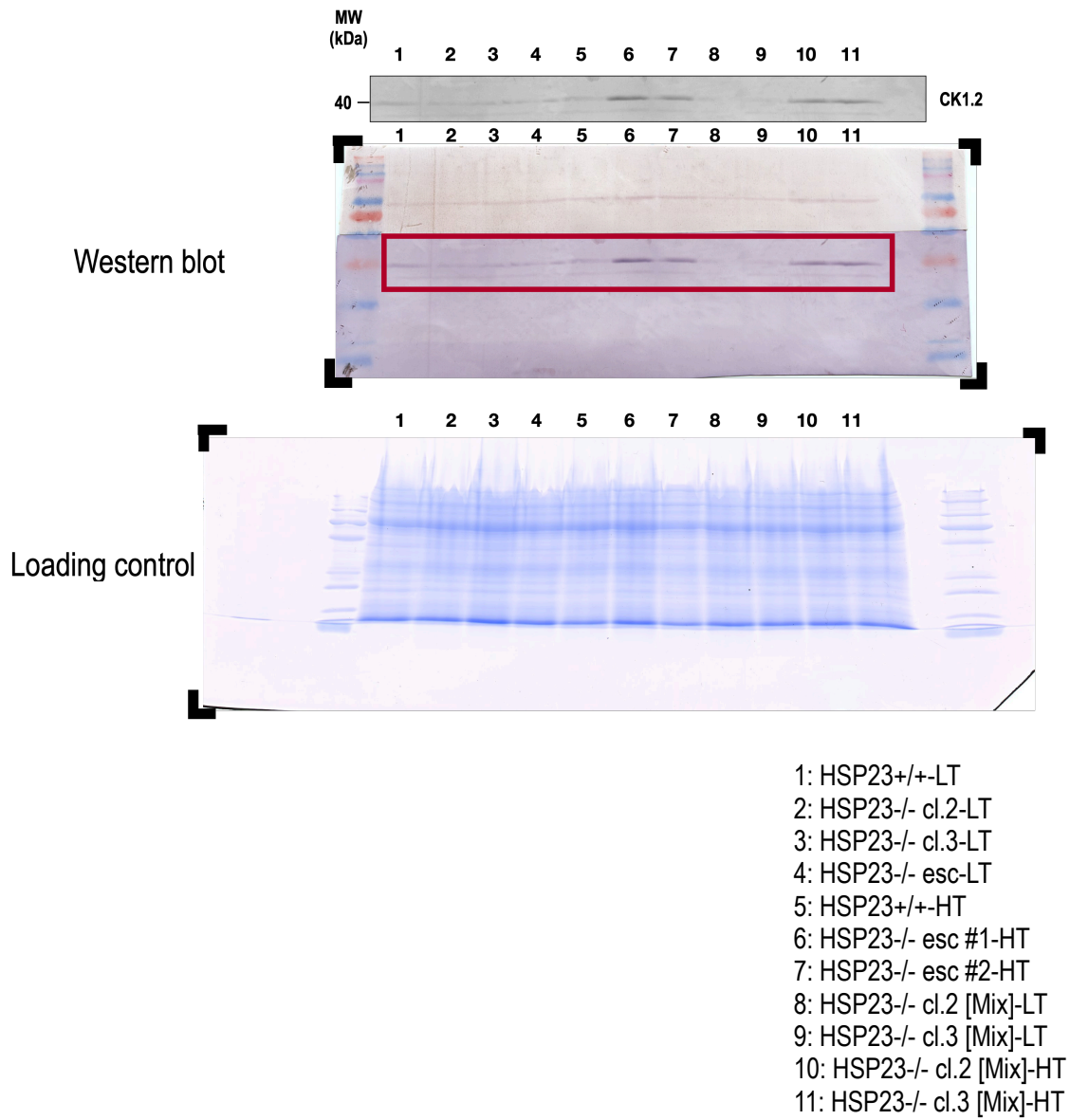
Quantification of CK1.2 protein levels shown in Fig.4B Repeat4



- 1: HSP23<sup>+/+</sup> -LT
- 2: HSP23<sup>+/+</sup>-HT
- 3: HSP23<sup>-/-</sup> esc #1-HT
- 4: HSP23<sup>-/-</sup> esc #2-HT

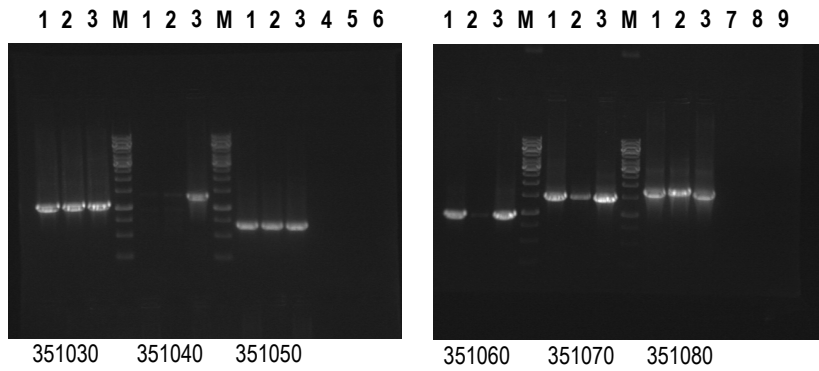
## Supplementary Information

Quantification of CK1.2 protein levels shown in Fig 4B Repeat 5

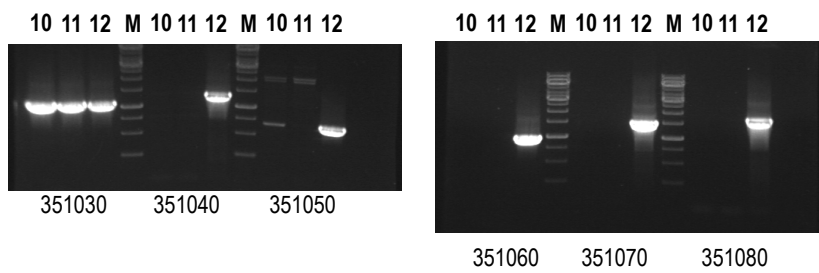


# Supplementary Information

Raw images Fig 5B (left panels)



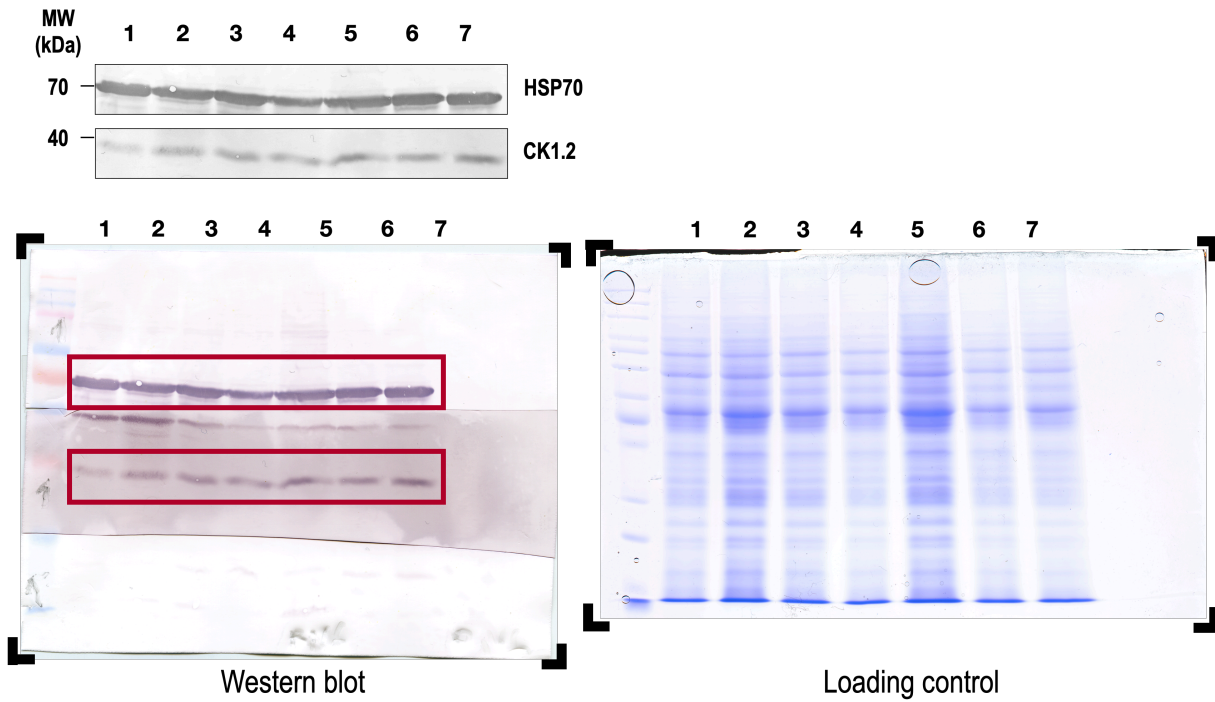
Raw image Fig 5B (right panels)



- 1 : HSP23<sup>-/-</sup> cl.2 [Mix]-LT
- 2: HSP23<sup>-/-</sup> cl.3 [Mix]-LT
- 3: (+) ctrl
- 4: (-) ctrl for LdBPK\_351030
- 5: (-) ctrl for LdBPK\_351040
- 6: (-) ctrl for LdBPK\_351050
- 7: (-) ctrl for LdBPK\_351060
- 8: (-) ctrl for LdBPK\_351070
- 9: (-) ctrl for LdBPK\_351080
- 10: HSP23<sup>-/-</sup> cl.2 [Mix]-HT
- 11: HSP23<sup>-/-</sup> cl.3 [Mix]-HT
- 12: (+) ctrl
- M : 1kb DNA ladder

## Supplementary Information

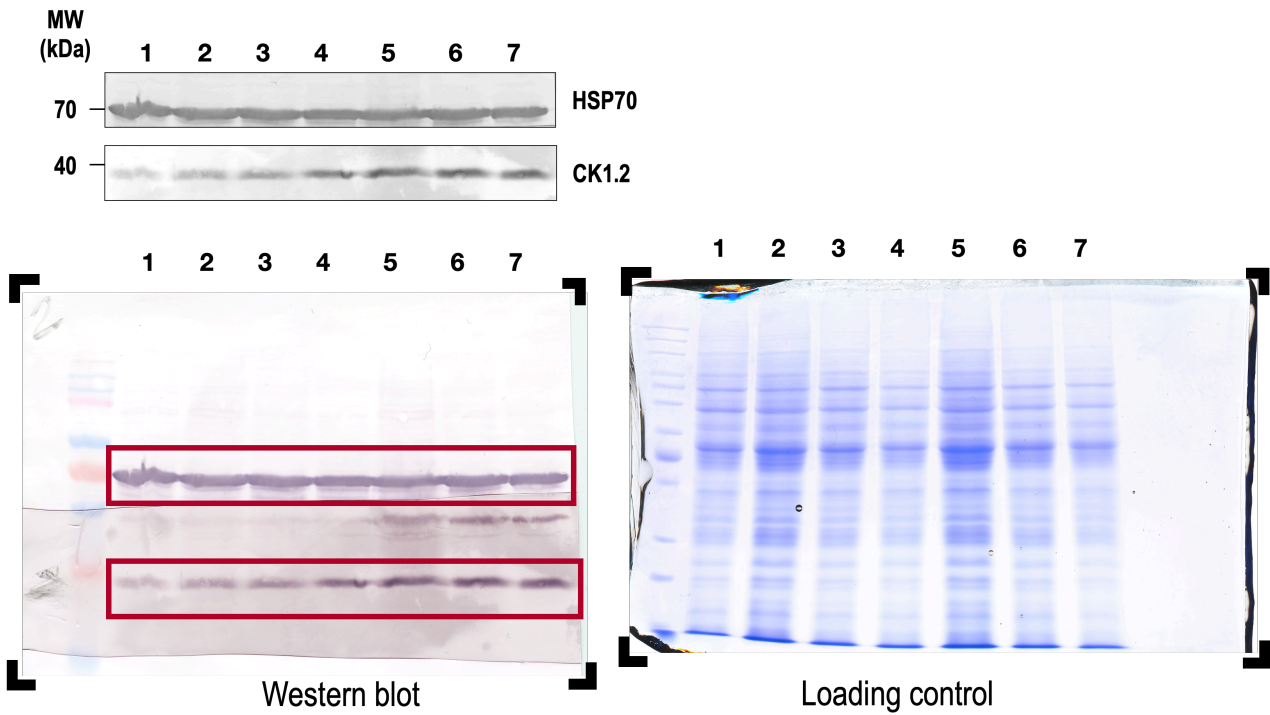
Quantification of CK1.2 protein levels shown in Fig 6F cl.2 Repeat 1



- 1: HSP23<sup>+/+</sup> -LT
- 2: HSP23<sup>-/-</sup> cl.2 [Mix ]-LT
- 3: HSP23<sup>-/-</sup> cl.2 [Mix ]-HT
- 4: HSP23<sup>-/-</sup> cl.2 [Mix ]-LT→HT
- 5: HSP23<sup>-/-</sup> cl.2 [CK1.2 ]-LT
- 6: HSP23<sup>-/-</sup> cl.2 [CK1.2 ]-HT
- 7: HSP23<sup>-/-</sup> cl.2 [CK1.2 ]-LT→ HT

Supplementary Information

Quantification of CK1.2 protein levels shown in Fig 6F cl.3 Repeat 1

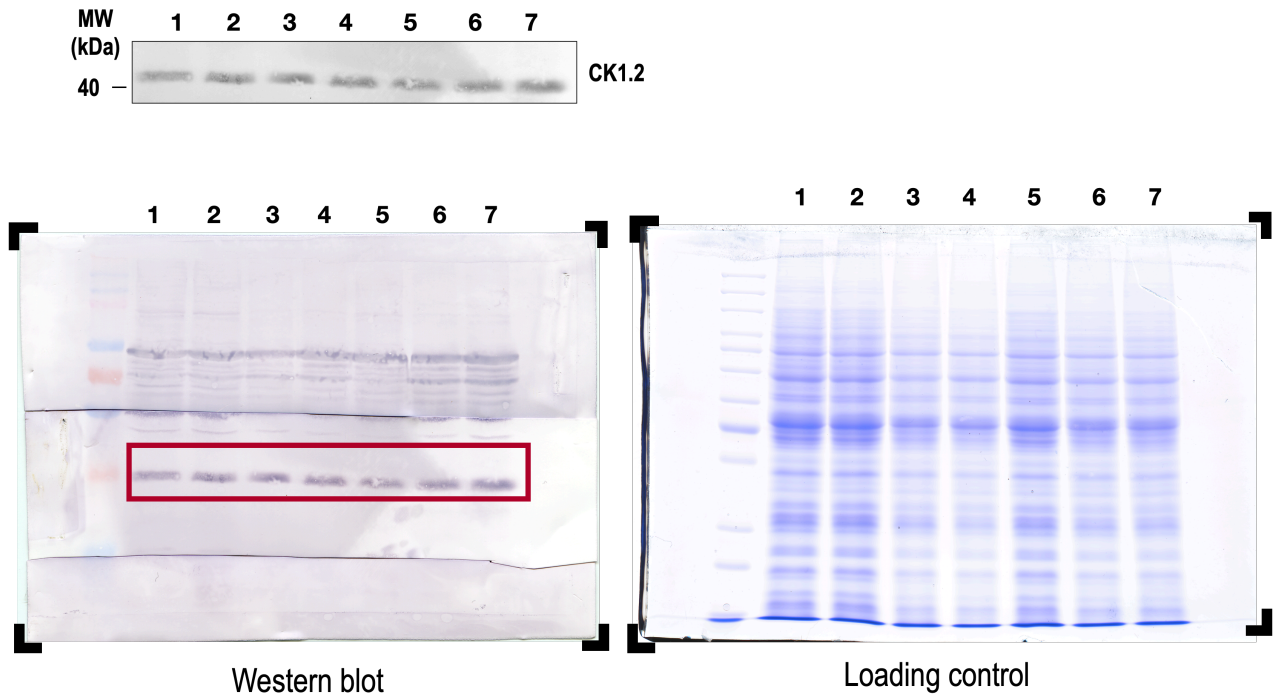


- 1: HSP23<sup>+/+</sup> -LT
- 2: HSP23<sup>-/-</sup> cl.3 [Mix ]-LT
- 3: HSP23<sup>-/-</sup> cl.3 [Mix ]-HT
- 4: HSP23<sup>-/-</sup> cl.3 [Mix ]-LT→HT
- 5: HSP23<sup>-/-</sup> cl.3 [CK1.2 ]-LT
- 6: HSP23<sup>-/-</sup> cl.3 [CK1.2 ]-HT
- 7: HSP23<sup>-/-</sup> cl.3 [CK1.2 ]-LT→ HT



Supplementary Information

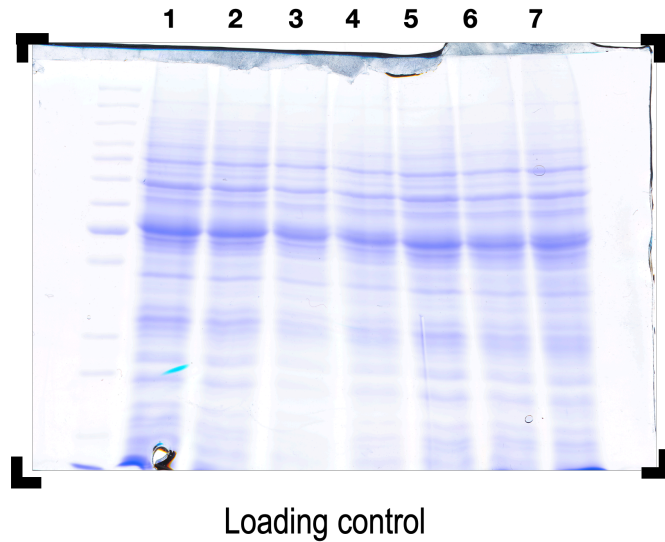
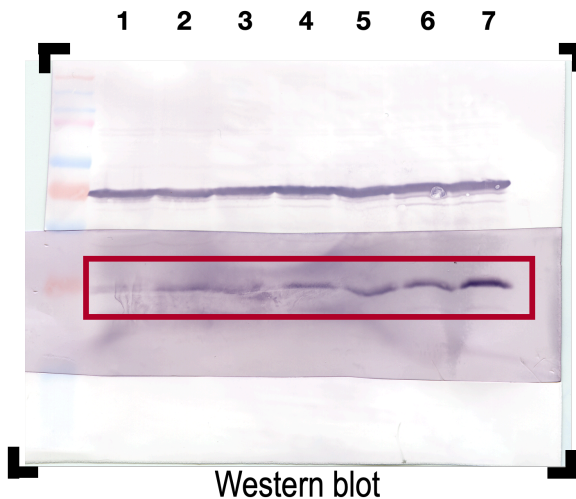
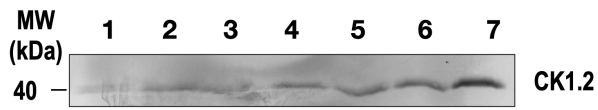
Quantification of CK1.2 protein levels shown in Fig 6F cl.2 Repeat 2



- 1: HSP23<sup>+/+</sup> -LT
- 2: HSP23<sup>-/-</sup> cl.2 [Mix ]-LT
- 3: HSP23<sup>-/-</sup> cl.2 [Mix ]-HT
- 4: HSP23<sup>-/-</sup> cl.2 [Mix ]-LT→HT
- 5: HSP23<sup>-/-</sup> cl.2 [CK1.2 ]-LT
- 6: HSP23<sup>-/-</sup> cl.2 [CK1.2 ]-HT
- 7: HSP23<sup>-/-</sup> cl.2 [CK1.2 ]-LT→ HT

Supplementary Information

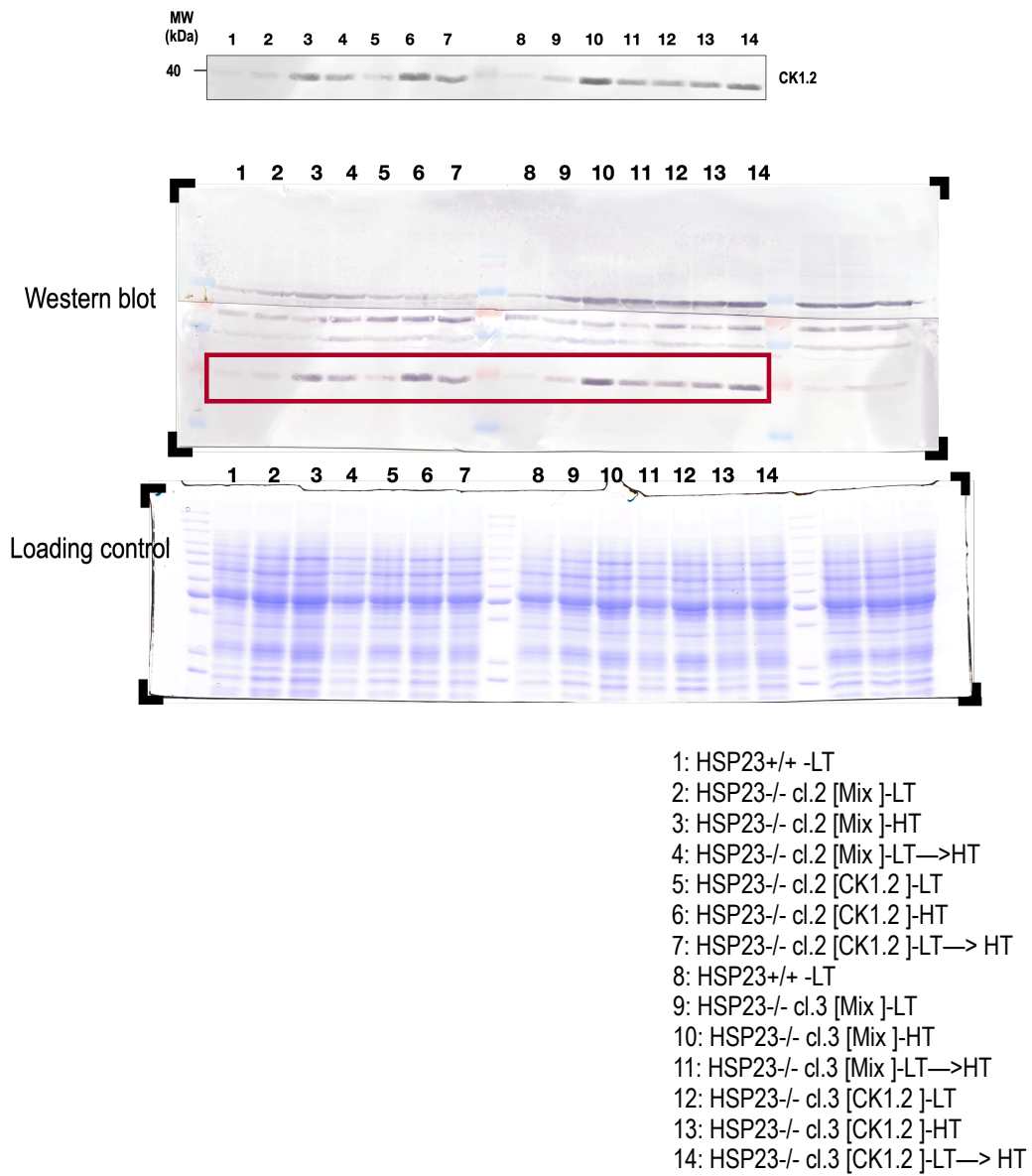
Quantification of CK1.2 protein levels shown in Fig 6F cl.3 Repeat 2



- 1: HSP23<sup>+/+</sup> -LT
- 2: HSP23<sup>-/-</sup> cl.3 [Mix ]-LT
- 3: HSP23<sup>-/-</sup> cl.3 [Mix ]-HT
- 4: HSP23<sup>-/-</sup> cl.3 [Mix ]-LT→HT
- 5: HSP23<sup>-/-</sup> cl.3 [CK1.2 ]-LT
- 6: HSP23<sup>-/-</sup> cl.3 [CK1.2 ]-HT
- 7: HSP23<sup>-/-</sup> cl.3 [CK1.2 ]-LT→ HT

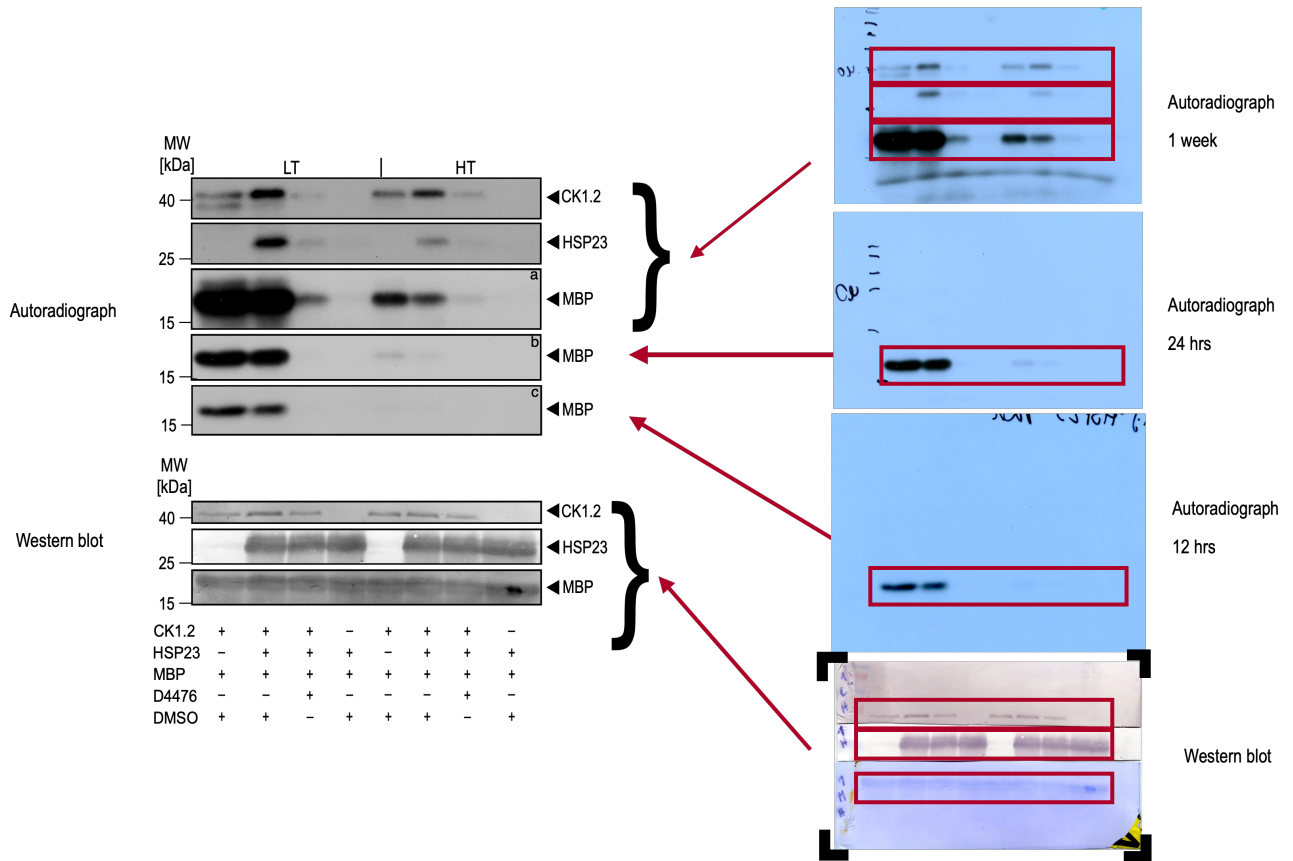
# Supplementary Information

Quantification of CK1.2 protein levels shown in Fig 6F cl.2+3 Repeat 3



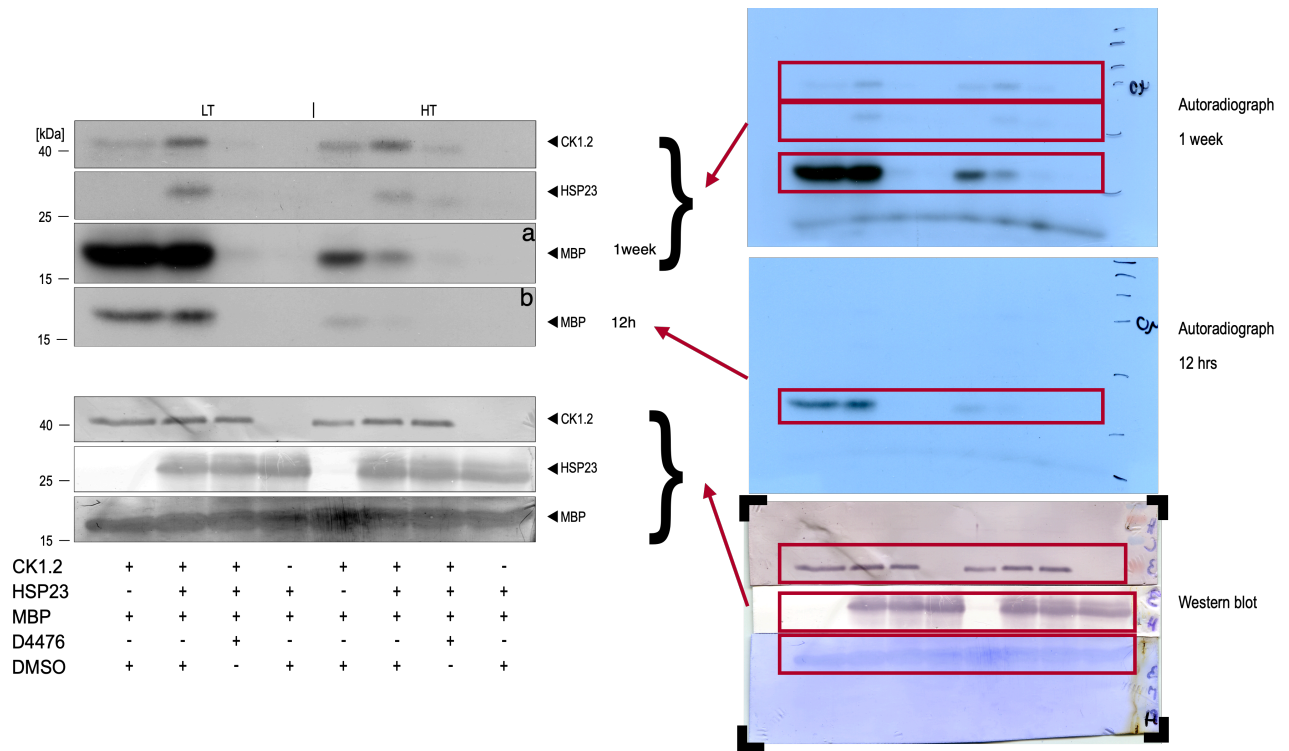
Supplementary Information

Representative Autoradiograph and Westernblot shown in Figure 7B: Repeat 1 HSP23



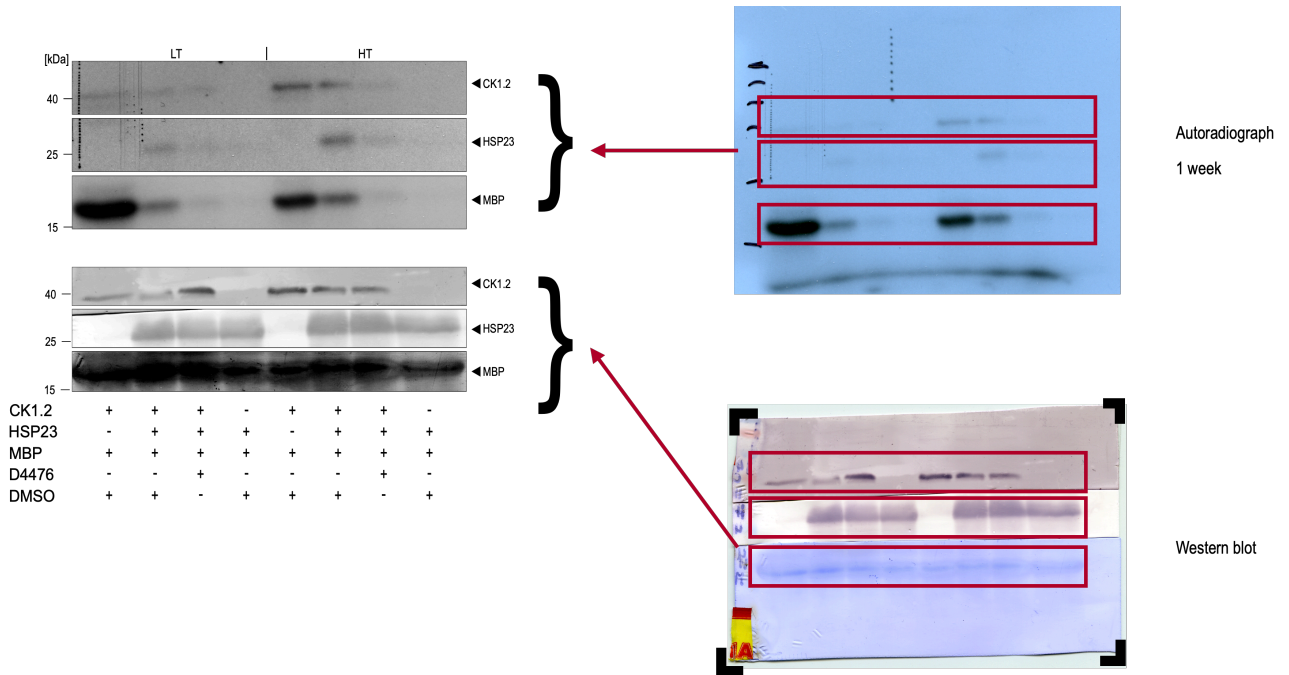
Supplementary Information

Additional data set for Fig.7: Repeat 2 HSP23



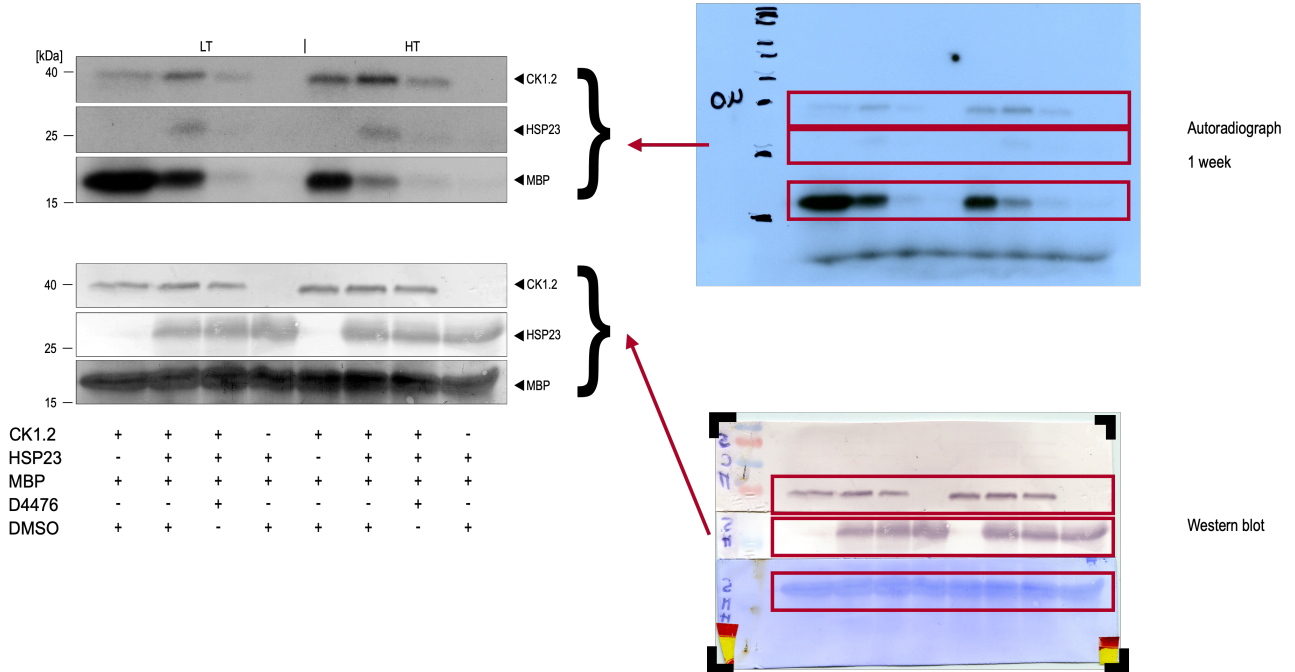
# Supplementary Information

## Additional data set for Fig.7: Repeat 3 HSP23



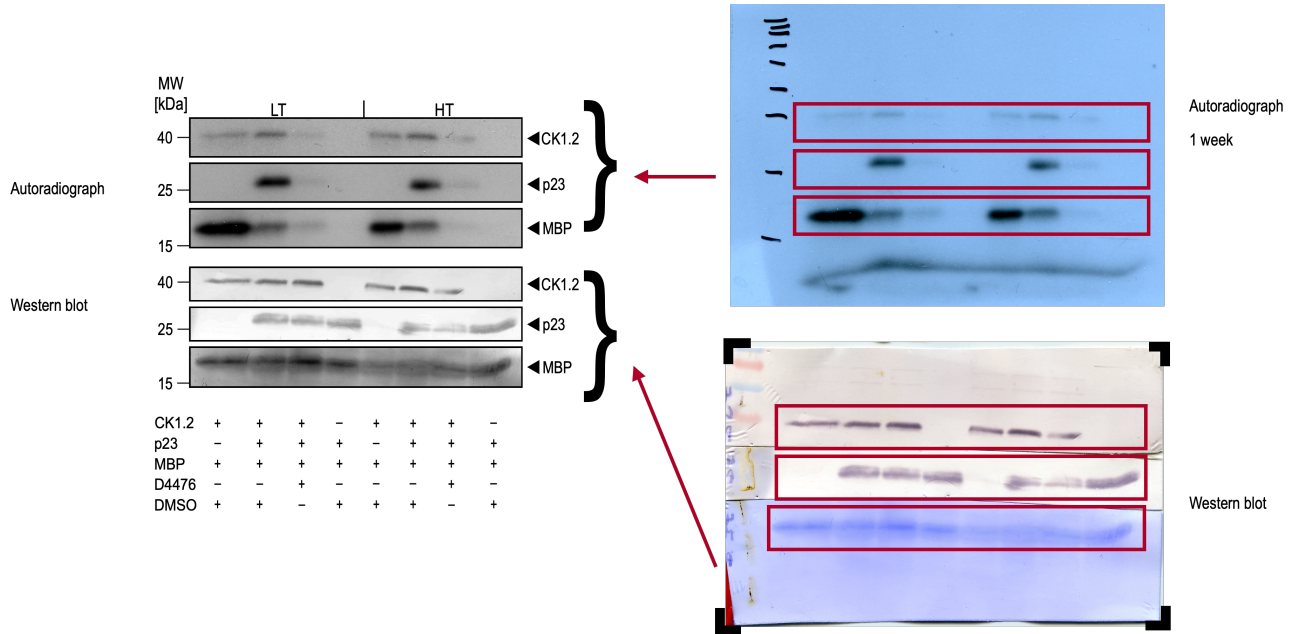
Supplementary Information

Additional data set for Fig.7: Repeat 4 HSP23



Supplementary Information

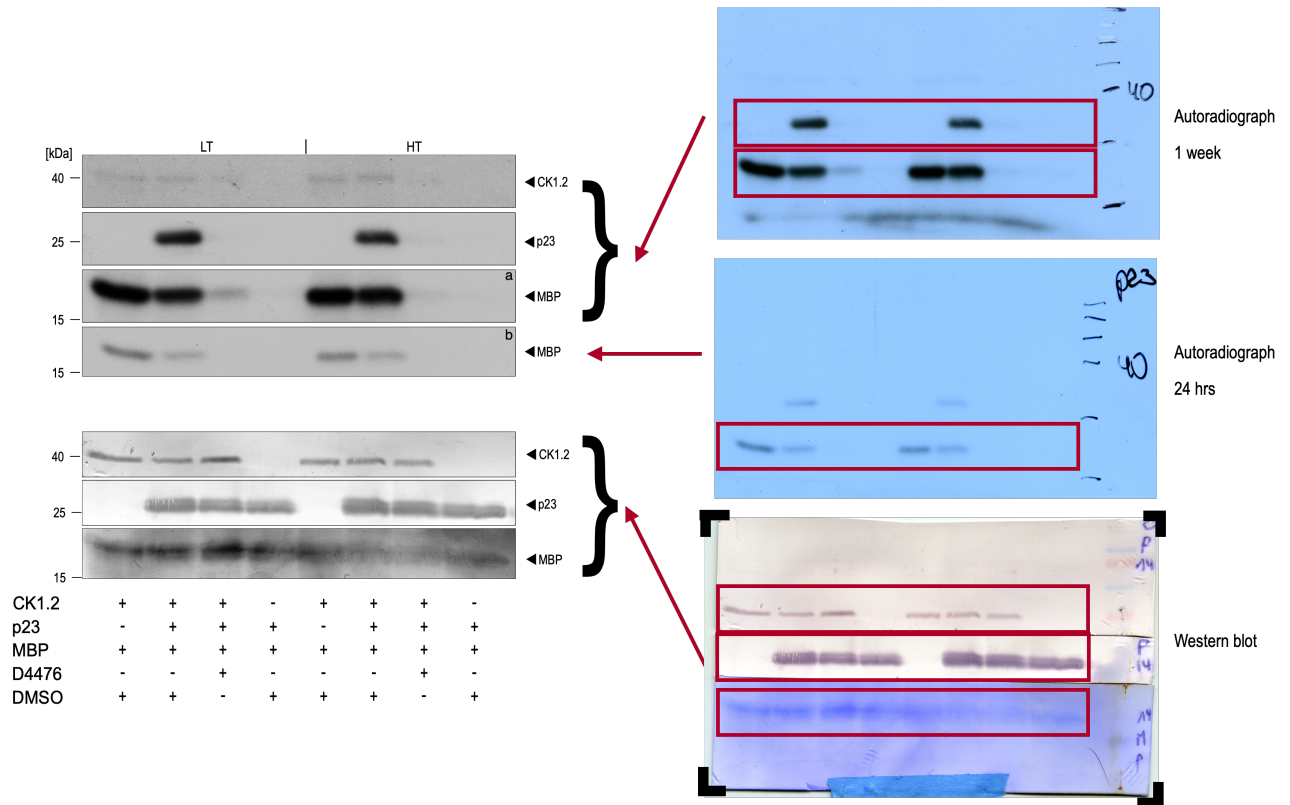
Representative Autoradiograph and Westernblot shown in Figure 7C: Repeat 1 p23





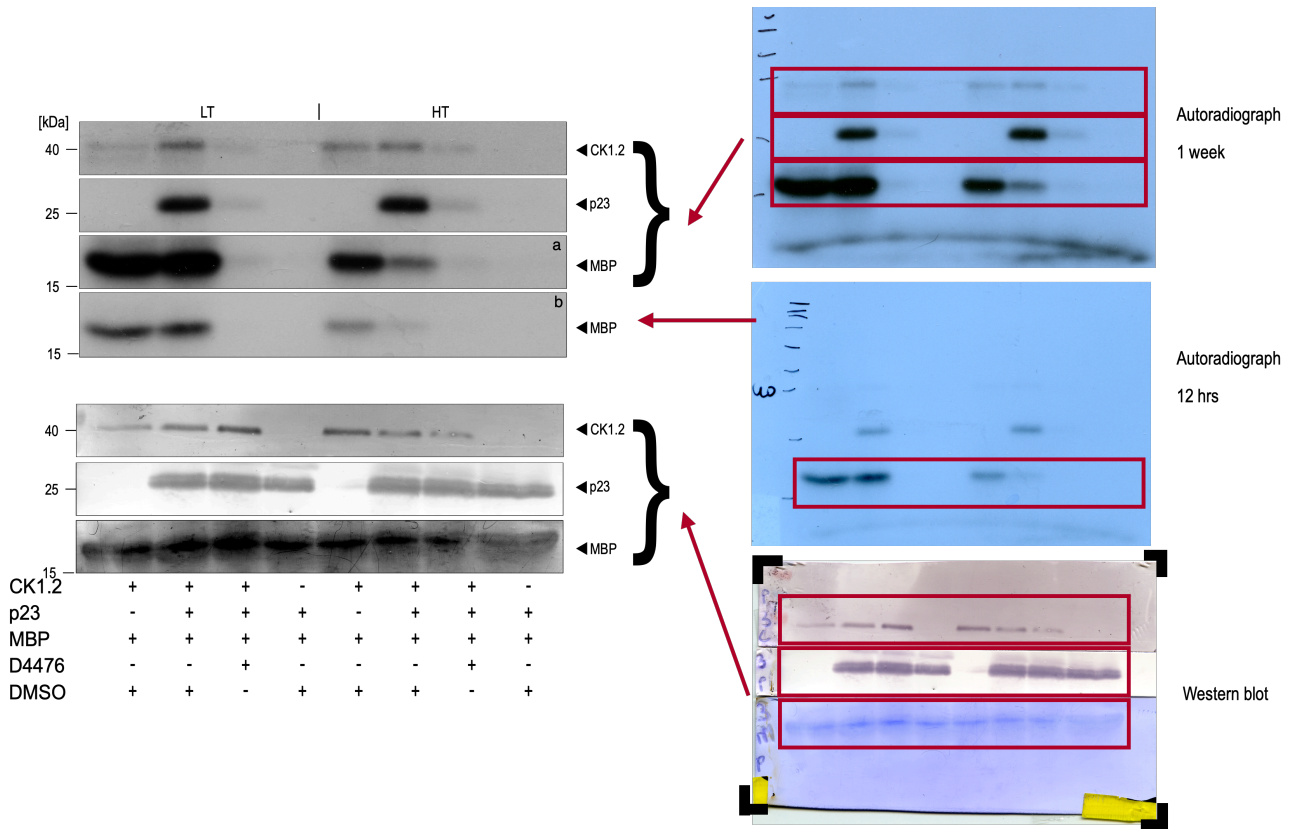
Supplementary Information

Additional data set for Fig.7:Repeat 2 p23



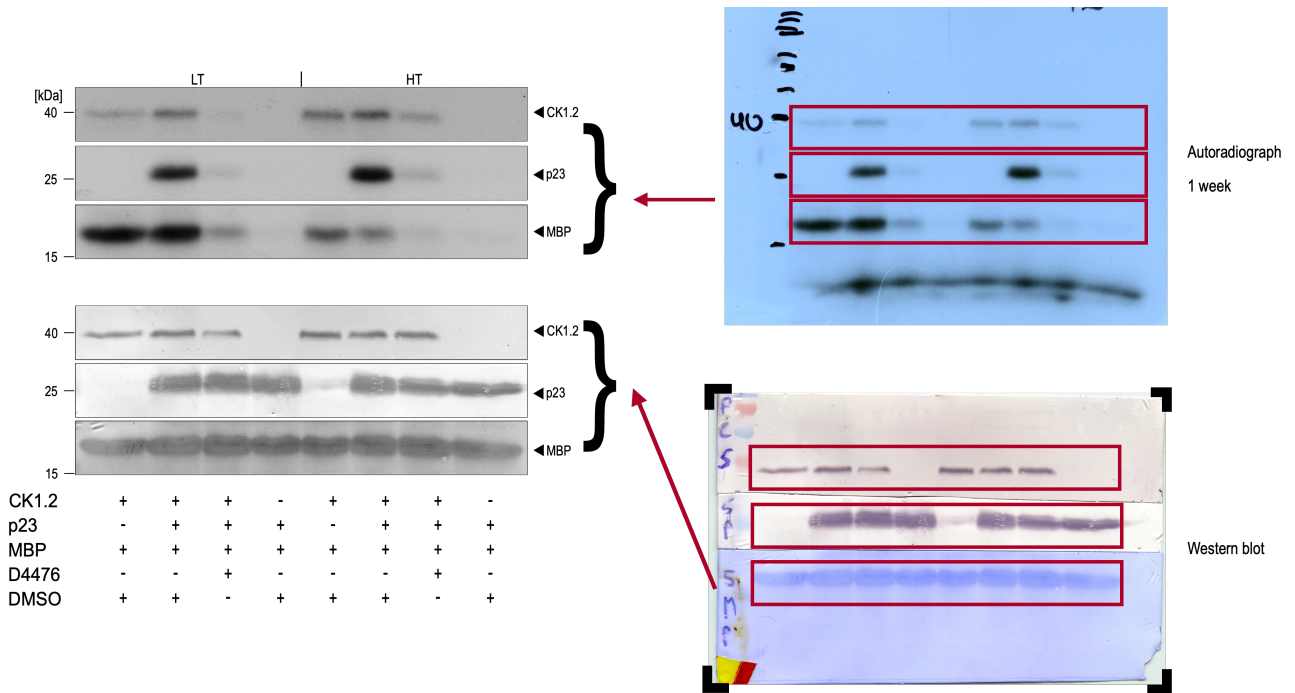
Supplementary Information

Additional data set for Fig.7: Repeat 3 p23



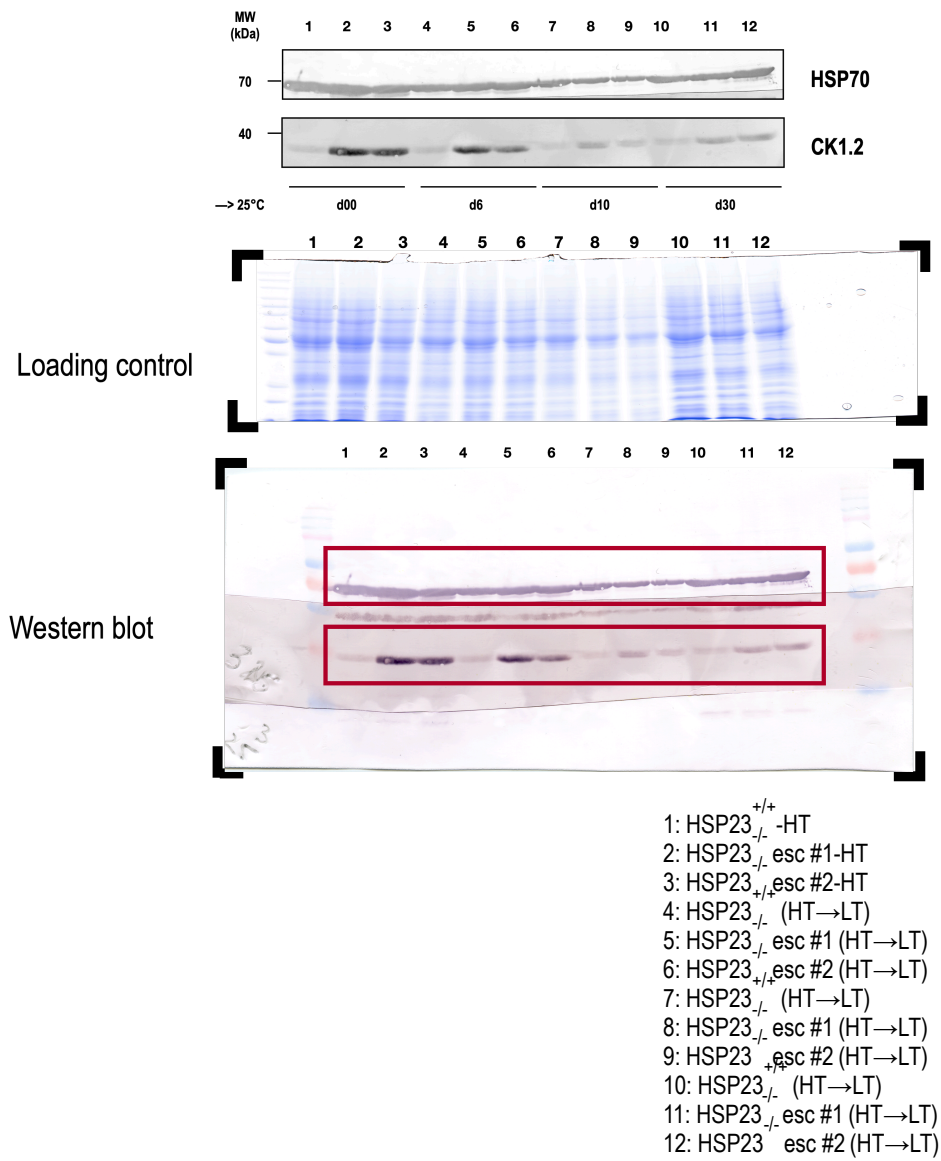
Supplementary Information

Additional data set for Fig.7: Repeat 4 p23



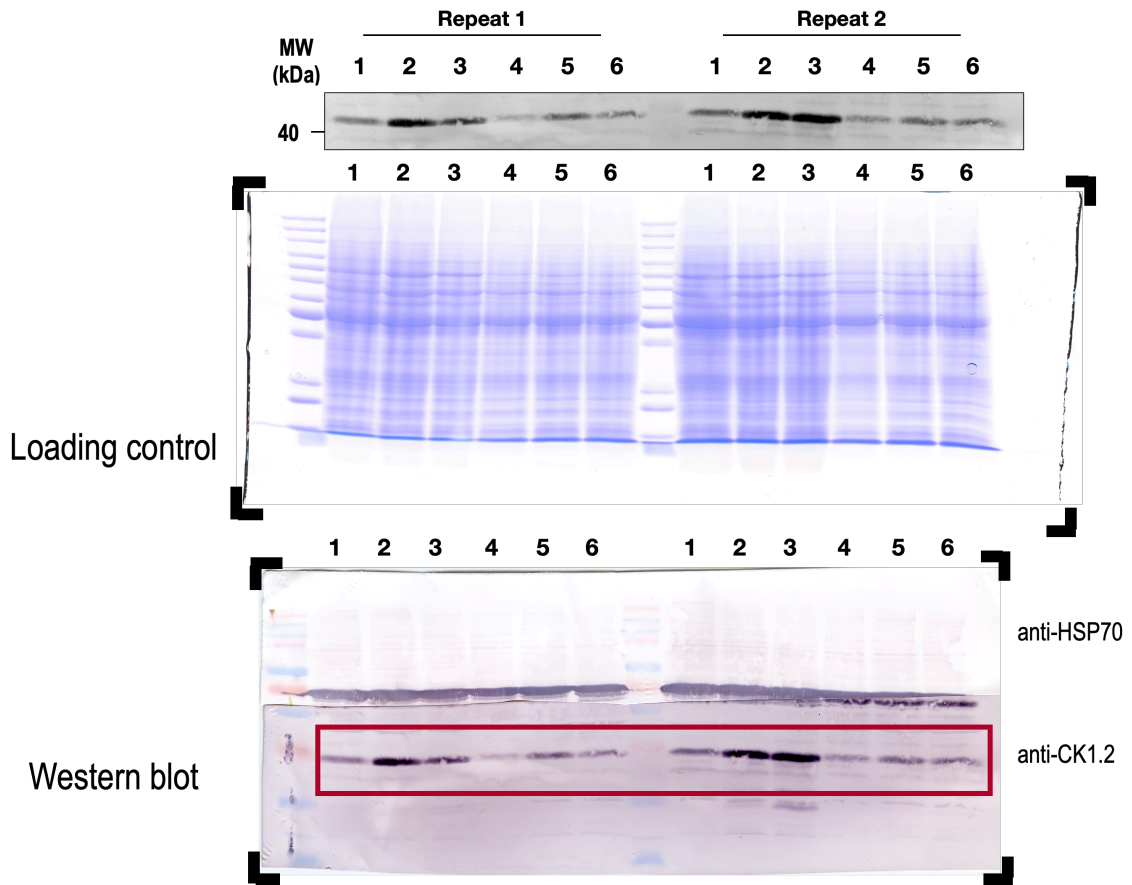
# Supplementary Information

Quantification of CK1.2 protein level shown in Fig S6 Repeat 1



Supplementary Information

Quantification of CK1.2 protein level shown in Fig S6 Repeat 2+3



- 1: HSP23<sup>+/+</sup>-HT
- 2: HSP23<sup>-/-</sup> esc #1-HT
- 3: HSP23<sup>-/-</sup> esc #2-HT
- 4: HSP23<sup>+/+</sup> (HT→LT)
- 5: HSP23<sup>-/-</sup> esc #1 (HT→LT)
- 6: HSP23<sup>-/-</sup> esc #2 (HT→LT)