

## Supplementary Information

### **Morpho/Proteomic Comparative between High Grade Pleomorphic Sarcoma and Metastasis Diagnosed in an Old Captive Common Hippo**

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**Table S1.** Proteins identified in healthy skeletal muscle tissue sample

Identified homologous proteins							
Accession	Gene	Protein	-10logP	Organism	Peptides identified	-10logP	ppm
sp_Q3ZC07	ACTC1	actin, alpha cardiac muscle 1	143	<i>Bos taurus</i>	HQGVMVGMGQKDSYVGDEAQSKR	112	0.64
					AGFAGDDAPRAVFPISIVGRPR	44	1.3
tr_W5NYJ1	ACTA1	actin	271	<i>Ovis aries</i>	HQGVMVGMGQKDSYVGDEAQSKR	200	0.7
					AGFAGDDAPRAVFPISIVGRPR	67.59	1.0
					GILTLKYPIEHGIITNWDDM(+15.99)EKIWHHTFYNELR	45.74	4.3
					HQ(+0.98)GVMVGMGQKDSYVGDEAQSKR	34.82	5.9
tr_A1E281	ACTB	beta actin	81.74	<i>Mus musculus</i>	HQ(+0.98)GVMVGMGQKDSYVGDEAQSKR	46.77	5.9
					AGFAGDDAPR	38.11	4.6
tr_F1SS62	MYH1	myosin-1	72.17	<i>Sus scrofa</i>	DLEEATLQHEATAATLRKK	58.61	5.0
					A(sub T)LEDQLSELKTKEEEQQR	29.82	0.9
sp_Q076A5	MYH4	myosin-4	71.91	<i>Canis familiaris</i>	DLEEATLQHEATAATLRKK	51.65	5.0
					SMSKANSEVAQWR	40.52	-2.6
tr_I6L963	Myh2	Myh2 protein	51.65	<i>Mus musculus</i>	DLEEATLQHEATAATLRKK	51.65	5.0
tr_A0A091DQA6	H920_05415	microtubule-associated protein 4	25.73	<i>Fukomys damarensis</i>	LGAPAM(+15.99)K	25.73	-1.0
tr_L8I8N9	M91_01539	tropomyosin beta chain	45.43	<i>Bos mutus</i>	IQLVEEELDRAQER	45.43	-9.7
tr_F1N757	TTN	titin	128.82	<i>Bos Taurus</i>	IIIPEC(+58.01)K	41.37	-2.6
					K(+42.01)ELSATPSAQKVT(+79.97)K	38.31	8.1
					I(+43.01)PAAVPK	37.13	-1.1

**Table S1.** Proteins identified in healthy skeletal muscle tissue sample (cont.)

Accession	Gene	Protein	-10logP	Organism	Peptides identified	-10logP	ppm
gi_307717700	Aspm	abnormal spindle-like microcephaly-associated protein	95.04	<i>Hippopotamus</i>	VAFRRHRTR	31.21	8.2
				<i>amphibious</i>	YKGMK(+71.04)	29.60	3.7
					FLS(+79.97)LRK(+14.02)	26.18	6.5
Identified protein for <i>de novo</i> sequencing							
Accession	Gene	Protein	E-value	Organism	Peptides identified	ALC / %	ppm
P84856.1	ACTB	beta actin <sup>a</sup>	3e-06	<i>Chlorocebus pygerythrus</i>	D(+42.01)GNAKDSYVGDEAQSQR	81	-2.0
Q7LBE3.1	SLC26A9	Solute carrier family 26 member 9	1.3	<i>Homo sapiens</i>	MEGVHQLVPGSVMSK	78	-2.6
Q9WUP7.2	Uchl5	Ubiquitin carboxyl-terminal hydrolase isozyme L5	0.003	<i>Mus musculus</i>	NQLVEEPMRAEQR	71	-4.5
Q076A5.1	MYH4	myosin-4 <sup>a</sup>	7e-07	<i>Canis lupus familiaris</i>	C(+57.02)NVVKFSMSKANSEVAQWR	76	1.9
Q62838.1	Musk	muscle, skeletal receptor tyrosine protein kinase	0.73	<i>Rattus norvegicus</i>	WAAAMEGKTDHQLHK	68	7.7
P27824.2	CANX	calnexin	0.001	<i>Homo sapiens</i>	NQLVEEMKDAELPGR	68	0.9
Q6ZPY7.2	Kdm3b	lysine-specific demethylase 3B	1.6	<i>Mus musculus</i>	M(+15.99)M(+15.99)M(+15.99)NKNNWKK	65	5.1
Q7TSC1.1	Prrc2a	protein PRRC2A	0.25	<i>Mus musculus</i>	VPPVSPGVAPVLLR	73	7.5

**Table S1.** Proteins identified in healthy skeletal muscle tissue sample (cont.)

Identified protein for <i>de novo</i> sequencing							
Accession	Gene	Protein	<i>E</i> -value	Organism	Peptides identified	ALC / %	ppm
O75369.2	FLNB	filamin-B	0.047	<i>Homo sapiens</i>	HYVNAREAMQK	78	-8.3
Q8NHS4.3	CLHC1	clathrin heavy chain linker domain-containing protein 1	0.37	<i>Homo sapiens</i>	L(+42.01)FATSHAFSC(+57.02)K	74	-7.8
P19973.2	Lsp1	lymphocyte-specific protein 1	0.91	<i>Mus musculus</i>	HPYHRDWSQKPQQR	73	-5.1
Q8NFN8.2	GPR156	probable G-protein coupled receptor 156	0.009	<i>Homo sapiens</i>	LYLPKMFPYMVRR	72	1.1
Q96JB1.2	DNAH8	dynein heavy chain 8, axonema	0.27	<i>Homo sapiens</i>	HM(+15.99)M(+15.99)LYTVANDTVSER	71	-9.4
Q8CJ27.2	Aspm	Abnormal spindle-like microcephaly-associated protein homolog <sup>a</sup>	0.25	<i>Mus musculus</i>	ATRRWRDHKK	68	-7.3
Q7YRK1.1	COX6C	citocromo c oxidase subunidade 6C	1.7	<i>Trachyp cristatus</i>	SNTPKPQMR	68	-2.3
Q62829.1	Pak3	Serine/threonine-protein kinase PAK 3	0.13	<i>Rattus norvegicus</i>	Y(+42.01)LDSYVGDEALMVR	67	-7.9
P11215.2	ITGAM	integrin alpha-M	0.31	<i>Homo sapiens</i>	FFFPNAFLELYR	66	8.6

**Table S1.** Proteins identified in healthy skeletal muscle tissue sample (cont.)

Identified protein for <i>de novo</i> sequencing							
Accession	Gene	Protein	<i>E</i> -value	Organism	Peptides identified	ALC / %	ppm
A2RT91.1	Ankar	ankyrin and armadillo repeat- containing protein	1.7	<i>Mus musculus</i>	M(+42.01)M(+15.99)SQMHNSGQK	65	9.5
P84856.1	ACTB	beta actin <sup>a</sup>	0.003	<i>Chlorocebus pygerythrus</i>	AGFAGDDAPR	95	7.8
Q076A5.1	MYH4	myosin-4 <sup>a</sup>	4e-07	<i>Canis familiaris</i>	SMSKANSEVAQWR	92	2.4
P84856.1	ACTB	beta actin <sup>a</sup>	0.022	<i>Chlorocebus pygerythrus</i>	HQGVVMVGMNAK	80	0.2
Q9TV61.1	MYH1	myosin-1 <sup>a</sup>	0.001	<i>Sus scrofa</i>	LQTESGEYSR	75	2.5

Annotated MS/MS spectra and corresponding proteins and peptides in database or *de novo* sequencing using BLASTp (NCBI). <sup>a</sup>Redundance. Possible post-translational modifications (PTM): oxidation (+15.99 Da); deamidation (+0.98 Da); carboxymethyl (+58.01 Da); acetylation (+42.01 Da); phosphorylation (+79.97 Da); carbamylation (+43.01 Da); propionamide (+71.04 Da); methyl ester (+14.02 Da); carbamidomethylation (+57.02 Da); substitution A → T.

**Table S2.** Proteins identified in primary tumor tissue sample

Identified homologous proteins							
Accession	Gene	Protein	-10logP	Organism	Peptides identified	-10logP	ppm
tr_G5C990	GW7_06468	histone H2B	103.96	<i>Heterocephalus glaber</i>	HAVSEGTKAVTKYTSSK	85.78	-2.0
tr_G3HHM2	I79_010126	histone H3	96.81	<i>Cricetulus griseus</i>	HAVSEGTKAVTKYTSSK	85.78	-2.0
tr_G3HPV7	I79_012828	histone H4	85.78	<i>Cricetulus griseus</i>	HAVSEGTKAVTKYTSSK	85.78	-2.0
tr_F6VG02	TTN	titin	157.66	<i>Equus caballus</i>	V(+42.01)TAVNAAGNSEPSEAS(+79.97)NFISC(+58.01)R	51.28	0.2
					C(+58.01)N(+0.98)LPQKLQ(+0.98)K	46.44	1.2
					SNLKMQIR(+14.02)	68.70	8.3
					EPNRK(+15.99)	58.53	4.4
sp_P02543	VIM	vimentin	115.37	<i>Sus scrofa</i>	S(+27.99)TRTVSSSSYR	76.79	-7.6
					MFGGPGTASRPSSRS	33.92	1.1
tr_L8IV51	M91_15600	collagen alpha-1(I) chain	76.68	<i>Bos mutus</i>	SGDRGETGPAGPAGPIGPVGAR	76.68	-9.6
tr_F1MUZ9	HSPD1	60 kDa heat shock protein (hsp60)	76.50	<i>Bos Taurus</i>	ALMLQGVDLLADAVAVTMGPKGR	76.50	10.0
tr_F2VRF3	CXCR4	chemokine (C-X-C motif) receptor 4	47.79	<i>Hippopotamus amphibious</i>	SC(+58.01)YC(+58.01)IIISK	25.28	9.7

**Table S2.** Proteins identified in primary tumor tissue sample (cont.)

Accession	Gene	Protein	-10logP	Organism	Peptides identified	-10logP	ppm
sp_P19015	HBA	hemoglobin subunit alpha	40.18	<i>Hippopotamus amphibious</i>	VLSANDKSNVK	30.85	8.6
tr_L8I7H6	M91_11211	methionyl-tRNA synthetase	44.55	<i>Bos mutus</i>	GKPLETA(sub S)K	34.06	7.8
Identified homologous proteins							
Accession	Gene	Protein	-10logP	Organism	Peptides identified	-10logP	ppm
tr_E2JAI3	Aspm	abnormal spindle-like microcephaly- associated protein	107.75	<i>Hippopotamus amphibious</i>	RE(+14.02)HYVR	34.69	4.3
					YKGMK(+71.04)	28.56	3.7
					TGIPR(+14.02)	28.84	8.0
					SIFTK	28.75	8.4
					LT(-18.01)AHK	28.85	0.1
					F(+43.01)RTLVMRR	26.79	-3.0
					T(+79.97)GIPR(+14.02)	25.70	4.9
					DRHG(sub L)WK	25.70	0.6
sp_P68285	CRYAA	alpha-crystallin A chain	27.15	<i>Hippopotamus amphibious</i>	ALGPFYPSR	27.15	9.1
gi_511970298	GPR116	probable G-protein coupled receptor 116 isoform X5	77.72	<i>Mustela putorius furo</i>	DLSVSH(sub T)GK	66.05	-7.9
gi_554561260	APOB	apolipoprotein B-100 isoform X2	65.20	<i>Myotis brandtii</i>	VLNALY(sub K)SNK	65.20	5.0

**Table S2.** Proteins identified in primary tumor tissue sample (cont.)

Identified protein for <i>de novo</i> sequencing							
Accession	Gene	Protein	<i>E</i> -value	Organism	Peptides identified	ALC / %	ppm
Q99J94.1	Slco1a6	solute carrier organic anion transporter family member 1A6	0.49	<i>Mus musculus</i>	RSDLSTMSTVLTQLHR	84	-9.9
Q9HBR0.2	SLC38A10	putative sodium-coupled neutral amino acid transporter 10	0.006	<i>Homo sapiens</i>	GVPRGQAAADGK	76	-5.0
Q5RC67.1	DDX23	probable ATP-dependent RNA helicase DDX23	0.37	<i>Pongo abelii</i>	ARPLEHMPVSK	73	8.9
P84856.1	ACTB	beta actin	1.0	<i>Cercopithecus pygerythrus</i>	FDTYSNYTGDEAQSQR	71	0.8
Q00715.2	H2B	histone H2B type 1 <sup>a</sup>	0.006	<i>Rattus norvegicus</i>	KVVPQMKAVTKYTSSK	69	8.9
A2APF3.1	Ctcf1	transcriptional repressor CTCFL	0.52	<i>Mus musculus</i>	C(+57.02)C(+57.02)GQLMAAGEVAVTMGYFK	67	-8.5
Q969W3.2	FAM104A	protein FAM104A	1.3	<i>Homo sapiens</i>	LHSSSSSVSPDR	66	-7.6
Q14246.3	EMR1	EGF-like module-containing mucin-like hormone receptor-like 1	1.3	<i>Homo sapiens</i>	V(+42.01)SVESMSTVLTGYWK	78	2.3



**Table S2.** Proteins identified in primary tumor tissue sample (cont.)

Accession	Gene	Protein	<i>E</i> -value	Organism	Peptides identified	ALC / %	ppm
Q52KR3.2	Prune2	protein prune homolog 2	0.73	<i>Mus musculus</i>	C(+57.02)C(+57.02)GQLMAAGEVAVTMGFYK	69	-8.5
Q922D4.1	Ppp6r3	serine/threonine- protein phosphatase 6 regulatory subunit 3	1.6	<i>Mus musculus</i>	C(+57.02)LLM(+15.99)KLYAYK	67	-2.4
O00635.1	TRIM38	E3 ubiquitin-protein ligase TRIM38	0.67	<i>Homo sapiens</i>	NYSVSPLFLTPPGHK	66	-3.1
Q8IU80.3	TMPRSS6	transmembrane protease serine 6	0.92	<i>Homo sapiens</i>	EESMSSTVLTSDGM(+15.99)K	66	-7.6
P54278.2	PMS2	mismatch repair endonuclease PMS2	0.85	<i>Homo sapiens</i>	GNELVGTAANTNDMK	68	4.9
P84856.1	ACTB	beta actin <sup>a</sup>	0.12	<i>Chlorocebus pygerythrus</i>	KFAGDDAPR	97	-6.8
Q00715.2	H2B	histone H2B type 1 <sup>a</sup>	0.006	<i>Rattus norvegicus</i>	HAVKVM(+15.99)KAVTKYTSSK	80	3.8
Q60775.1	Elf1	ETS-related transcription factor Elf-1	0.73	<i>Mus musculus</i>	VCLASSNSQAAVK	65	-3.5

Annotated MS/MS spectra and corresponding proteins and peptides in database. <sup>a</sup>Redundance. Possible post-translational modifications (PTM): acetylation (+42.01 Da); phosphorylation (+79.97 Da); carboxymethyl (+58.01 Da); deamidation (+0.98 Da); formylation (+27.99 Da); methyl ester (+14.02 Da); propionamide (+71.04 Da); pyro-glu (-18.01 Da); carbamylation (+43.01 Da); carbamidomethylation (+57.02 Da); levuglandinyl – arginine hydroxylatam adduct (+15.99 Da); substitution: A → S; G → L; H → T; Y → K.

**Table S3.** Proteins identified in heart metastasis tissue sample

Identified protein for <i>de novo</i> sequencing							
Accession	Gene	Protein	-10logP	Organism	Peptides identified	-10logP	ppm
sp_p19016	HBB	hemoglobin	256.37	<i>Hippopotamus</i>	VHLTAEKDAVLGLWGKVVNVQEVGGEALGR	83.56	2.4
		subunit beta		<i>amphibius</i>	TP(+13.98)ELQAAYQKVVAGVANALAHRYH	110.31	2.7
					T(+226.08)FGK(+42.01)EFTPELQAAYQK	83.53	4.3
tr_L5K231	PAL_GLEAN	60 kDa heat shock protein (hsp60)	36.94	<i>Pteropus alecto</i>	ALMLQGVDLLADAVAVTMGPKGR	36.94	-2.9
tr_G5B0U1	GW7_13516	titin	32.86	<i>Heterocephalus glaber</i>	IIIPEC(+58.01)K	32.86	-2.6
tr_M3YI93	COL4A1	collagen alpha-1(IV) chain isoform	29.90	<i>Mustela putorius furo</i>	GLPGSDGIPGSKGEAGLPGKPGPTGPAGQKGEPGSDGIPGSAGEK	29.90	3.8
tr_F7DJL4	EPSTI1	epithelial-stromal interaction protein	26.69	<i>Callithrix jacchus</i>	AVHLAPR	26.69	-9.5
gi_675785278	EIF2AK4	eukaryotic translation initiation factor 2-alpha kinase 4	20.64	<i>Pan paniscus</i>	LEELAKKHC(+58.01)GEVM(+15.99)IFELAYHVQSFLSEHNKPPPK	20.64	1.7

**Table S3.** Proteins identified in heart metastasis tissue sample (cont.)

Accession	Gene	Protein	-10logP	organism	peptides identified	-10logP	ppm
tr_Q3U5G4	Ciita	class II transactivator, isoform CRA	20.55	<i>Mus musculus</i>	GLLAGIFQR	20.55	1.9
gi_223096	0506206A	histone H2B	69	<i>Rattus norvegicus</i>	AMGIMNSFVNDIFQRIAGEASR	55	6.30
Identified protein for <i>de novo</i> sequencing							
Accession	Gene	Protein	<i>E</i> -value	Organism	Peptides identified	ALC / %	ppm
P13631.1	RARG	retinoic acid receptor gamma	0.084	<i>Homo sapiens</i>	EEM(+15.99)PSSPSPGPR	80	1.6
P84856.1	ACTB	beta actin	6e-05	<i>Chlorocebus pygerythrus</i>	M(+15.99)HDKDSYVGDEAQSQR	79	-6.8
Q5NC32.1	Slc16a11	monocarboxylate transporter 11	0.26	<i>Mus musculus</i>	KPAGVVAASAFSVLK	67	0.3
Q1KL86.1	EPHA2	ephrin type-A receptor 2	0.048	<i>Macaca fascicularis</i>	LGLRTDNVMNDR	71	-1.7
P84856.1	ACTB	beta actin <sup>a</sup>	0.003	<i>Chlorocebus pygerythrus</i>	AGFAGDDAPR	96	-0.8
Q2KIP2.1	GSG2	serine/threonine- protein kinase haspin	0.79	<i>Bos taurus</i>	SAVFPSLASPGPR	88	4.6

**Table S3.** Proteins identified in heart metastasis tissue sample (cont.)

Accession	Gene	Protein	<i>E</i> -value	Organism	Peptides identified	ALC / %	ppm
Q13972.2	RASGRF1	ras-specific guanine nucleotide- releasing factor 1	1.5	<i>Homo sapiens</i>	AALSASSAFAPPK	71	-2.5
O14646.2	CHD1	chromodomain- helicase-DNA- binding protein 1	0.93	<i>Homo sapiens</i>	FPYLLVVPLLR	68	7.4

Annotated MS/MS spectra and corresponding proteins and peptides in database. <sup>a</sup>Redundance. Possible post-translational modifications (PTM): proline oxydation to pyroglutamic acid (+13.98 Da); biotinylation (+226.08 Da); acetylation (+42.01 Da); oxidation (+15.99 Da); carboxymethyl (+58.01 Da).