

Electronic Supplementary Information

The tungsten metallome of *Pyrococcus furiosus*

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Figure S1 Calibration curve of the phosphor screen for autoradiography. Volumes of 10 μl of different dilutions of the ^{187}W stock solution were applied to filter paper to which the phosphor screen was subsequently exposed for 2-h after 4 half-lives. Parallel samples of the dilution range were measured with a gamma counter. A linear relationship between the phosphor-screen intensity and radiation was seen over the whole concentration range.

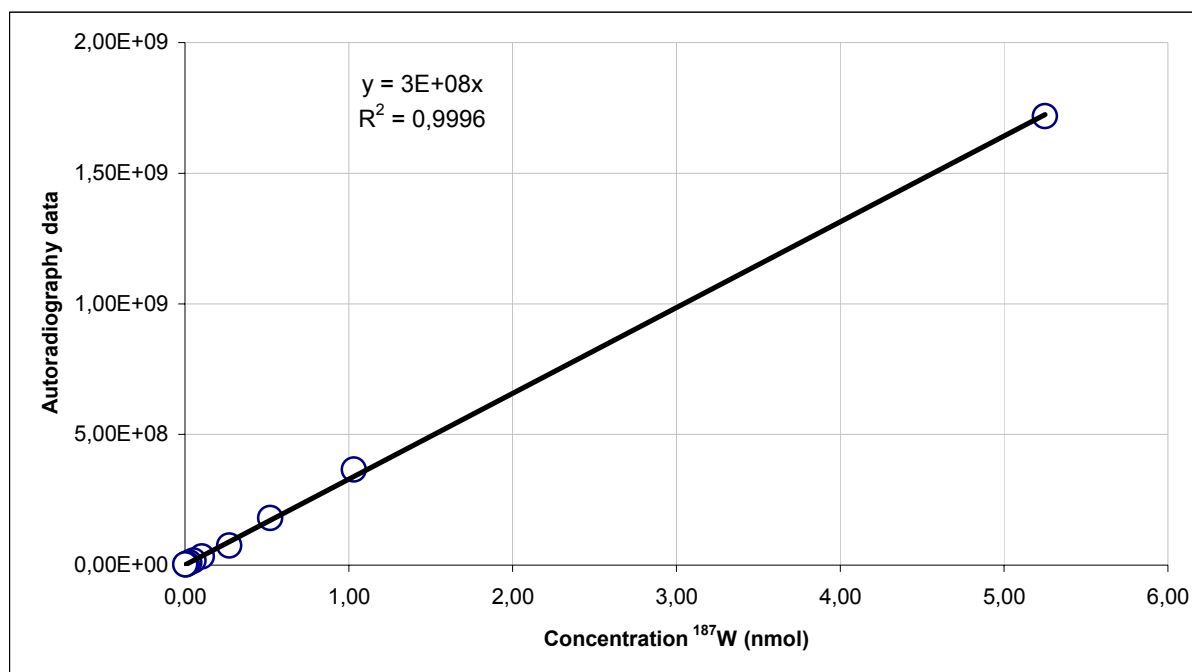


Figure S2 Native–native 2D–PAGE of *P. furiosus* solubilised membrane proteins. **a**, Autoradiogram of native–native 2D–PAGE. **b**, Coomassie brilliant blue (CBB) stained gel of native–native 2D–PAGE. The extract was obtained from cells grown under normal growth conditions for 14 h at 95° C (135 µg protein) of *P. furiosus*.

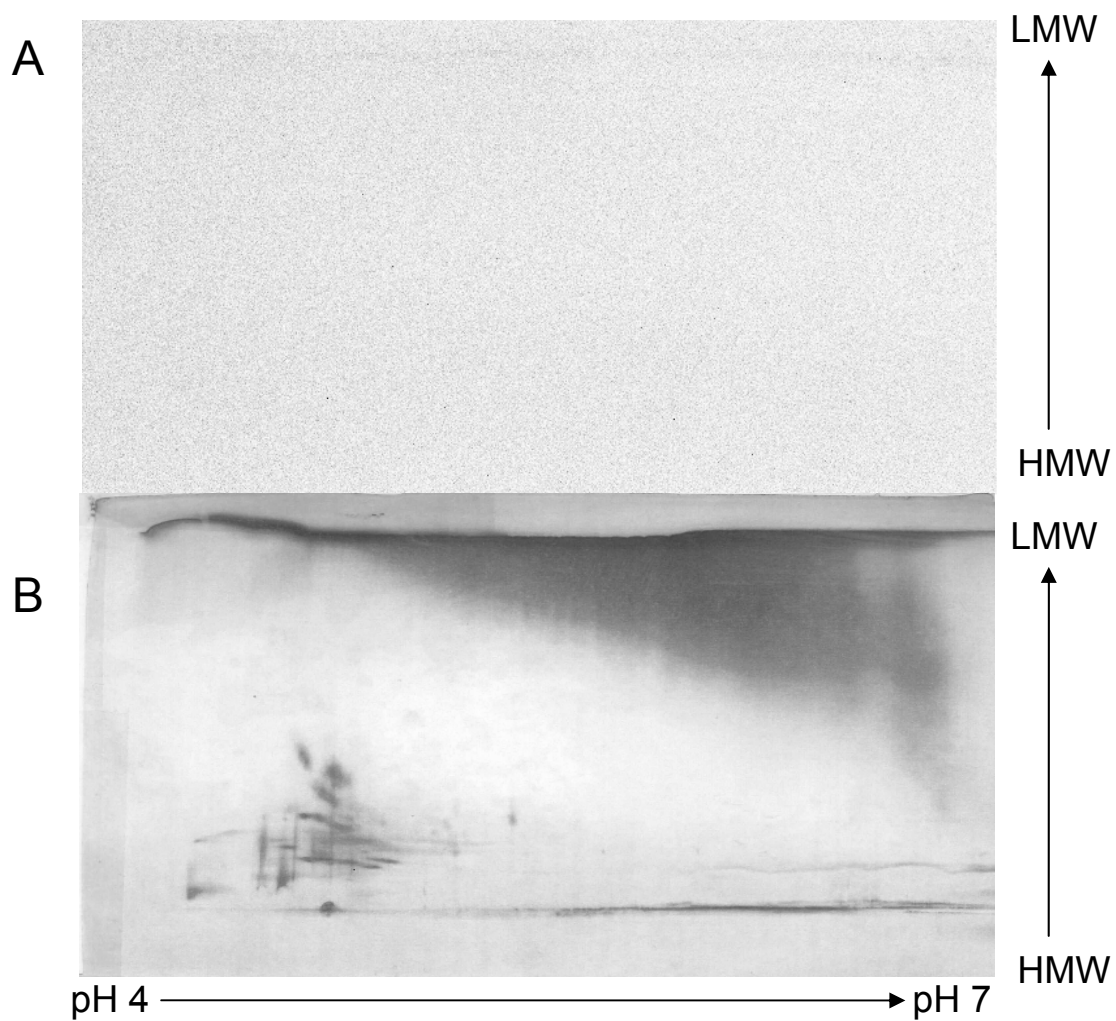


Figure S3 Autoradiogram of native–native 2D–PAGE of ^{187}W solution.



Table S1 Protein identification using mass spectrometry from native–native 2D–PAGE separation of *P. furiosus* soluble protein extract, growth 14 h 95° C.

Spot	Accession	Protein	Mass	# of unique peptides	Mascot Protein Score
1	PF1203	FOR	69,072	7	269
	PF0547	Hypothetical protein	50,895	6	257
2	PF1540	ADP forming acetyl coenzyme A synthetase	50,047	21	1,160
	PF0861	Phospho–sugar mutase	49,715	17	882
	PF1838	Hypothetical protein	51,903	18	850
			65,025	19	740
	PF1480	WOR5			
	PF1787	Acetyl–CoA synthetase	25,862	14	727
	PF0966	Pyruvate ferredoxin oxidoreductase alpha–2	44,216	11	654
	PF0588	Cofactor–independent phosphoglycerate mutase	45,342	16	615
	PF1077	Hypothetical protein	49,458	9	411
	PF0421	Hypothetical protein	44,146	8	376
	PF1266	Cystathionine gamma–synthase	41,349	7	358
	PF1477	Methylmalonyl–CoA mutase	64,322	8	337
	PF0139	Indolepyruvate ferredoxin oxidoreductase	20,009	6	292
	PF0965	Ferredoxin oxidoreductase beta subunit	36,580	3	289
	PF1837	Hypothetical protein	26,427	7	285
	PF0265	8–amino–7–oxononanoate synthase	43,840	4	255
	PF0207	Argininosuccinate synthase	46,058	4	231
	PF0966	Pyruvate ferredoxin oxidoreductase subunit delta	12,460	4	202
	PF1946	Methylmalonyl–CoA mutase, subunit alpha	15,641	3	192
	PF0890	Leucyl–tRNA synthetase	113,819	5	186
	PF0236	Ribose–phosphate pyrophosphokinase	30,928	4	137
	PF1076	Hypothetical TLDD protein	53,701	4	133
	PF1479	Putative oxidoreductase, Fe–S subunit	19,796	3	128
	PF1328	Hydrogenase gamma subunit	30,968	2	119
	PF0935	Acetolactate synthase	62,064	3	104
	PF1232	4–aminobutyrate aminotransferase	50,782	2	100
	PF1975	Hypothetical protein	54,281	2	74
	PF0372	Molybdenum cofactor biosynthesis protein	18,562	2	72
	PF1283	Rubrerythrin	19,716	2	66
	PF1327	Glutamate synthase [NADPH] small chain	53,152	2	56
	PF0533	Indolepyruvate ferredoxin oxidoreductase subunit a	71,938	2	56
	3	PF0346	AOR	66,931	9
4	PF0043	Similar to <i>E. coli</i> pyruvate, water dikinase	90,713	15	797

	PF1974	Thermosome, single subunit	60,001	14	623
	PF0346	AOR	66,931	12	605
	PF0402	UDP- or dTTP-glucose 4- epimerase or 4-6-dehydratase	35,396	11	570
	PF1394	Phosphoglycerate dehydrogenase	33,858	7	305
	PF1516	GMP synthase subunit B	35,260	7	248
	PF1104	Homoserine dehydrogenase	34,976	7	246
	PF1187	Hypothetical protein	38,983	7	245
	PF0966	Pyruvate ferredoxin oxidoreductase alpha-2	44,216	5	197
	PF1787	Acetyl-CoA synthetase	25,862	2	116
	PF1102	Bifunctional short chain isoprenyl diphosphate synthase	38,337	2	104
	PF0077	Biotin-protein ligase	25,691	3	98
	PF1602	Chain A, Structure Of Glutamate Dehydrogenase	46,953	3	95
	PF0308	Adenylosuccinate synthetase	37,226	2	80
	PF0421	Hypothetical protein	44,146	2	71
	PF1729	Phospho-sugar mutase	26,537	2	67
	PF1472	Aspartate/serine transaminase	42,956	2	67
	PF0751	Flavoprotein	47,429	2	49
5	PF1974	Thermosome, single subunit	60,001	13	611
	PF0346	AOR	66,931	9	294
	PF0966	Pyruvate ferredoxin oxidoreductase alpha-2	44,216	3	135
	PF1516	GMP synthase subunit B	35,260	3	96
6	PF1961	WOR4	69,610	9	251
	PF1454	Hypothetical protein	69,304	3	95
	PF1283	Rubryerythrin	19,716	3	93
	PF0464	GAPOR	74,089	2	65
7	PF1961	WOR4	69,610	2	76
8	PF0464	GAPOR	74,089	2	60
	PF1961	WOR4	69,610	2	43
9	PF0464	GAPOR	74,089	14	563
	PF0426	Phosphoribosylaminoimidazole carboxylase ATPase subunit	41,130	5	224
	PF1203	FOR	69,072	5	199
	PF0794	UDP-n-acetylglucosamine 2- epimerase	40,772	6	154
	PF1532	NADH oxidase	48,155	2	106
	PF0422	Phosphoribosylamine-glycine ligase	49,009	3	105
	PF1974	Thermosome, single subunit	60,001	3	84
	PF1920	Triosephosphate isomerase	24,165	2	74
	PF0265	2-amino-3-ketobutyrate coenzyme A ligase	43,840	2	73
	PF0935	Acetolactate synthase	62,064	2	56
	PF1232	4-aminobutyrate aminotransferase	50,782	2	52
10	PF1535	Alpha-glucan phosphorylase	97,815	17	680
	PF1602	Glutamate dehydrogenase	47,141	13	663
	PF0043	Similar to <i>E. coli</i> pyruvate, water dikinase	90,713	12	563
	PF0966	Pyruvate ferredoxin oxidoreductase	44,216	11	522

alpha-2				
PF1529	Pyridoxine biosynthesis protein	36,933	13	460
PF0766	Putative dehydrogenase	35,467	7	355
PF0965	Ferredoxin oxidoreductase beta subunit	36,580	6	343
PF1592	Tryptophan synthase subunit beta	49,642	7	328
PF1974	Thermosome, single subunit	60,001	6	263
PF0613	Hypothetical protein	41,801	5	239
PF1627	Glycyl-tRNA synthetase	66,698	5	213
PF1375	Elongation factor Tu	47,605	5	199
PF0102	Hypothetical protein	28,088	4	171
PF1975	Hypothetical protein	54,281	4	128
PF1030	Methionyl-tRNA synthetase	85,393	3	115
PF1351	Threonyl-tRNA synthetase	73,333	3	111
PF0969	2-ketovalerate ferredoxin oxidoreductase subunit alpha	43,989	2	95
PF0248	Hypothetical protein	17,705	2	94
PF0139	Indolepyruvate ferredoxin oxidoreductase	20,009	3	82
PF0321	Related to inosine monophosphate dehydrogenase	44,230	2	76
PF1778	Serine hydroxymethyltransferase	48,013	2	72
PF0430	5'-phosphoribosylglycinamide transformylase	48,315	2	64

The position of the spot number is indicated in Fig. 3A.

This table contains just proteins with a number of unique peptides ≥ 2 .

Table S2 Protein identification with mass spectrometry after native–native 2D–PAGE separation of *P. furiosus* soluble protein extract obtained from cold–shock growth conditions 5 hours at 72° C in addition to 14 h 95° C.

Spot	Accession	Protein	Mass	# of unique peptides	Mascot Protein Score	
1	PF1203	FOR	69,072	17	791	
	PF1422	Thioredoxin reductase	38,354	4	215	
	PF0547	Hypothetical protein	50,895	3	157	
2	PF1540	ADP forming acetyl coenzyme A synthetase	50,047	23	1,577	
	PF1480	WOR5	65,025	25	1,330	
	PF1787	Acetyl–CoA synthetase	25,862	16	1,033	
	PF0421	Hypothetical protein	44,146	14	633	
	PF0861	Phospho–sugar mutase	49,715	11	145	
	PF1838	Hypothetical protein	51,903	9	416	
	PF1479	Putative oxidoreductase, Fe–S subunit	19,796	5	261	
	PF0965	Ferredoxin oxidoreductase beta subunit	36,580	5	222	
	PF1771	2–oxoglutarate ferredoxin oxidoreductase	44,206	5	221	
	PF0139	Indolepyruvate ferredoxin oxidoreductase	20,009	3	221	
	PF1477	Methylmalonyl–CoA mutase	64,322	6	202	
	PF1208	Beta–mannosidase	59,089	6	201	
	PF0547	Hypothetical protein	50,895	3	177	
	PF0588	Cofactor–independent phosphoglycerate mutase	45,342	5	167	
	PF0016	5′–methylthioadenosine phosphorylase	29,486	3	124	
	PF1853	3–ketoacyl–(acyl–carrier–protein) reductase	28,520	3	123	
	PF1837	Hypothetical protein	26,427	3	95	
	PF1203	FOR	69,072	3	90	
	PF1076	Hypothetical TLDD protein	53,701	2	83	
	3	PF0346	AOR	66,931	33	2,184
		PF1241	Uracil phosphoribosyltransferase	26,147	17	795
		PF0318	Hypothetical acylaminoacyl–peptidase	72,991	18	682
		PF1540	ADP forming acetyl coenzyme A synthetase	50,047	8	475
PF1535		Alpha–glucan phosphorylase	97,815	11	451	
PF1920		Triosephosphate isomerase	24,165	8	443	
PF0751		Flavoprotein	47,429	10	381	
PF0175		Trk system potassium uptake – like protein	25,496	7	347	
PF1787		Acetyl–CoA synthetase	25,862	3	268	
PF0272		Alpha–amylase	76,375	7	246	
PF1480		WOR5	65,025	3	207	
PF1778		Serine hydroxymethyltransferase	48,013	5	165	
PF0183		V–type ATP synthase subunit B	51,940	3	161	
PF0182		V–type ATP synthase subunit A	113,609	2	134	
PF1532		NADH oxidase	48,155	2	91	

	PF0966	Pyruvate ferredoxin oxidoreductase alpha-2	44,216	2	78
4	PF0402	UDP- or dTTP-glucose 4-epimerase or 4-6-dehydratase	35,396	13	613
	PF0346	AOR	66,931	10	476
	PF0077	Biotin-protein ligase	25,691	6	375
	PF0980	Putative aspartate racemase	25,268	8	362
	PF1187	Hypothetical protein	38,983	5	352
	PF1516	GMP synthase subunit B	35,260	6	233
	PF1893	Transcriptional regulatory protein, asnC family	8,582	3	149
	PF1289	Putative thiamine biosynthesis protein	21,937	4	137
	PF0722	Alkyl hydroperoxide reductase subunit c	21,603	3	116
	PF1343	Xaa-pro dipeptidase (proline dipeptidase)	39,420	2	111
	PF0598	Aspartate carbamoyltransferase regulatory subunit	17,107	2	91
	PF1394	Phosphoglycerate dehydrogenase	33,858	2	78
5	PF1974	Thermosome, single subunit	60,001	18	1,043
	PF1787	Acetyl-CoA synthetase	25,862	15	838
	PF0966	Pyruvate ferredoxin oxidoreductase alpha-2	44,216	11	667
	PF0346	AOR	66,931	9	405
	PF0432	Putative sugar-catabolism phosphotransferase	30,598	5	133
	PF0940	3-isopropylmalate dehydrogenase 2	39,387	3	86
	PF1281	Superoxide reductase	14,372	3	86
	PF1102	Bifunctional short chain isoprenyl diphosphate synthase	38,337	2	83
	PF0009	Hypothetical protein	25,590	2	78
	PF1105	Pyrazinamidase/nicotinamidase pxnc	20,038	2	65
6	PF1961	WOR4	69,610	27	2,332
	PF0464	GAPOR	74,089	14	792
	PF0422	Phosphoribosylamine-glycine ligase	49,009	12	624
	PF1203	FOR	69,072	15	498
	PF1532	NADH oxidase	48,155	6	338
	PF0980	Putative aspartate racemase	25,268	6	298
	PF1454	Hypothetical protein	69,304	8	266
	PF1283	Rubrerythrin	19,716	7	234
	PF0097	43 kDa subunit bacteriochlorophyll synthase-like protein	44,110	5	204
	PF0440	Ribonucleotide-diphosphate reductase alpha subunit	201,603	5	185
	PF0931	Nucleoside diphosphate kinase	18,494	4	159
	PF1355	NDP-sugar dehydrogenase	48,186	4	157
	PF0098	NAD(+) synthetase	28,874	4	144
	PF0265	8-amino-7-oxononanoate synthase	43,840	3	137

	PF0016	5'-methylthioadenosine phosphorylase	29,531	3	116
	PF1560	Transcription elongation factor NusA	18,377	3	111
	PF1299	Pyrrolidone Carboxyl Peptidase	22,775	2	93
7	PF1961	WOR4	69,610	28	1,803
	PF0422	Phosphoribosylamine—glycine ligase	49,009	14	692
	PF0464	GAPOR	74,089	10	424
	PF1532	NADH oxidase	48,155	8	333
	PF1488	Hypothetical protein	26,520	4	185
		Putative aspartate aminotransferase	47,198	3	141
	PF1283	Rubrerythrin	19,716	4	125
	PF1454	Hypothetical protein	69,304	4	120
	PF0016	5'-methylthioadenosine phosphorylase	29531	3	107
	PF1137	Translation initiation factor IF-2	110,933	3	102
	PF0097	43 kDa subunit bacteriochlorophyll synthase-like protein	44,110	2	94
	PF1355	NDP-sugar dehydrogenase	48,186	2	85
	PF0265	8-amino-7-oxononanoate synthase	43,840	2	77
	PF1327	Glutamate synthase [NADPH] small chain	53,152	2	75
	PF0595	Diphthine synthase	30,255	2	66
8	PF1961	WOR4	69,610	32	1,616
	PF1355	NDP-sugar dehydrogenase	48,186	10	404
	PF1137	Translation initiation factor IF-2	110,933	9	361
	PF1532	NADH oxidase	48,155	7	298
	PF1987	Hypothetical protein	69,072	5	176
	PF1327	Glutamate synthase [NADPH] small chain	53,152	3	100
	PF1283	Rubrerythrin	19,716	3	97
	PF0016	5'-methylthioadenosine phosphorylase	29,531	2	91
	PF0422	Phosphoribosylamine—glycine ligase	49,009	2	65
9	PF0464	GAPOR	74,089	12	530
	PF1961	WOR4	69,610	3	207
	PF1246	Sarcosine oxidase, subunit beta	42,533	4	153
	PF1016	2-hydroxyhepta-2,4-diene-1,7-dioate isomerase	25,248	2	112
	PF0747	Putative proline dipeptidase	40,871	3	78
	PF0422	Phosphoribosylamine—glycine ligase	49,009	2	64
	PF2038	Cob(I)yrinic acid a,c-diamide adenosyltransferase	19,728	2	60
10	PF0139	Indolepyruvate ferredoxin oxidoreductase	20,009	3	243
	PF0042	Metal-dependent hydrolase	24,554	2	135
	PF0861	Phospho-sugar mutase	49,715	2	91
	PF0464	GAPOR	74,089	2	83

11	PF1394	Phosphoglycerate dehydrogenase	33,858	11	521	
	PF0464	GAPOR	74,089	15	479	
	PF1126	Hypothetical protein	32,609	8	330	
	PF2038	Cob(I)yrinic acid a,c-diamide adenosyltransferase	19,728	5	195	
	PF0042	Metal-dependent hydrolase	24,554	4	172	
	PF0139	Indolepyruvate ferredoxin oxidoreductase	20,009	3	129	
12	PF0861	Phospho-sugar mutase	49,715	23	1,164	
	PF1540	ADP forming acetyl coenzyme A synthetase	50,047	18	1,097	
	PF1480	WOR5	65,025	15	637	
	PF1477	Methylmalonyl-CoA mutase	64,322	17	630	
	PF0588	Cofactor-independent phosphoglycerate mutase	45342	10	558	
	PF1787	Acetyl-CoA synthetase	25,862	8	482	
	PF1771	2-oxoglutarate ferredoxin oxidoreductase	44,206	9	440	
	PF0533	Indolepyruvate ferredoxin oxidoreductase subunit a	71,938	8	355	
	PF0965	Ferredoxin oxidoreductase beta subunit	36,580	6	334	
	PF1946	Methylmalonyl-CoA mutase, subunit alpha	15,641	5	287	
	PF1837	Hypothetical protein	26,427	6	242	
	PF0935	Acetolactate synthase	62,064	5	237	
	PF0139	Indolepyruvate ferredoxin oxidoreductase	20,009	5	236	
	PF1838	Hypothetical protein	51,903	5	221	
	PF1076	Hypothetical TLDD protein	53,701	3	194	
	PF1077	Hypothetical protein	49,458	3	169	
	PF0547	Hypothetical protein	50,895	5	167	
	PF0534	Indolepyruvate ferredoxin oxidoreductase	23,673	4	139	
	PF0075	Alcohol dehydrogenase	42,938	3	124	
	PF0308	Adenylosuccinate lyase	51,724	2	113	
	PF1479	Putative oxidoreductase, Fe-S subunit	19,796	2	60	
	13	PF1375	Elongation factor Tu	47,605	7	225
		PF1535	Alpha-glucan phosphorylase	97,815	5	210
PF1547		Endoglucanase	38,616	3	94	
PF1861		Endo-1,4-beta-glucanase-like protein	38,247	3	81	
PF2012		Elongation factor EF-2	82,374	2	76	
PF0594		Ornithine carbamoyltransferase	35,273	2	63	
14		PF0043	Similar to <i>E. coli</i> pyruvate, water dikinase	90,713	4	150
	PF1375	Elongation factor Tu	47,605	3	111	
	PF0966	Pyruvate ferredoxin oxidoreductase alpha-2	44,216	2	106	
	PF1266	Cystathionine gamma-synthase	41,349	2	85	

The position of the spot number is indicated in Fig. 3B.

This table contains just proteins with a number of unique peptides ≥ 2 .