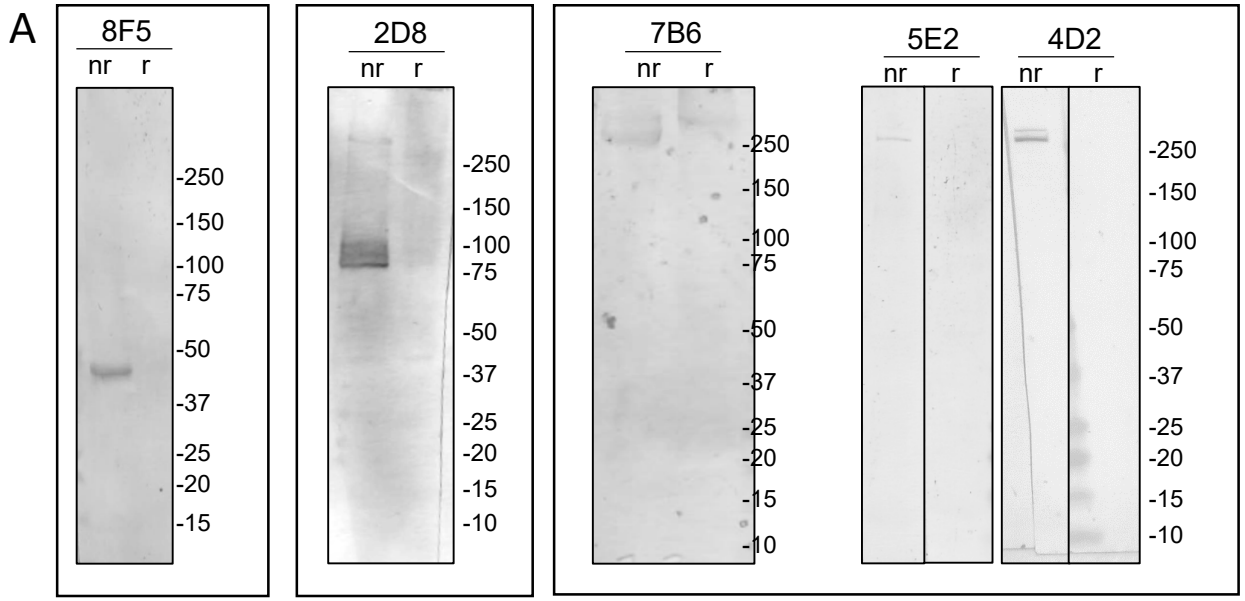
Fig S1



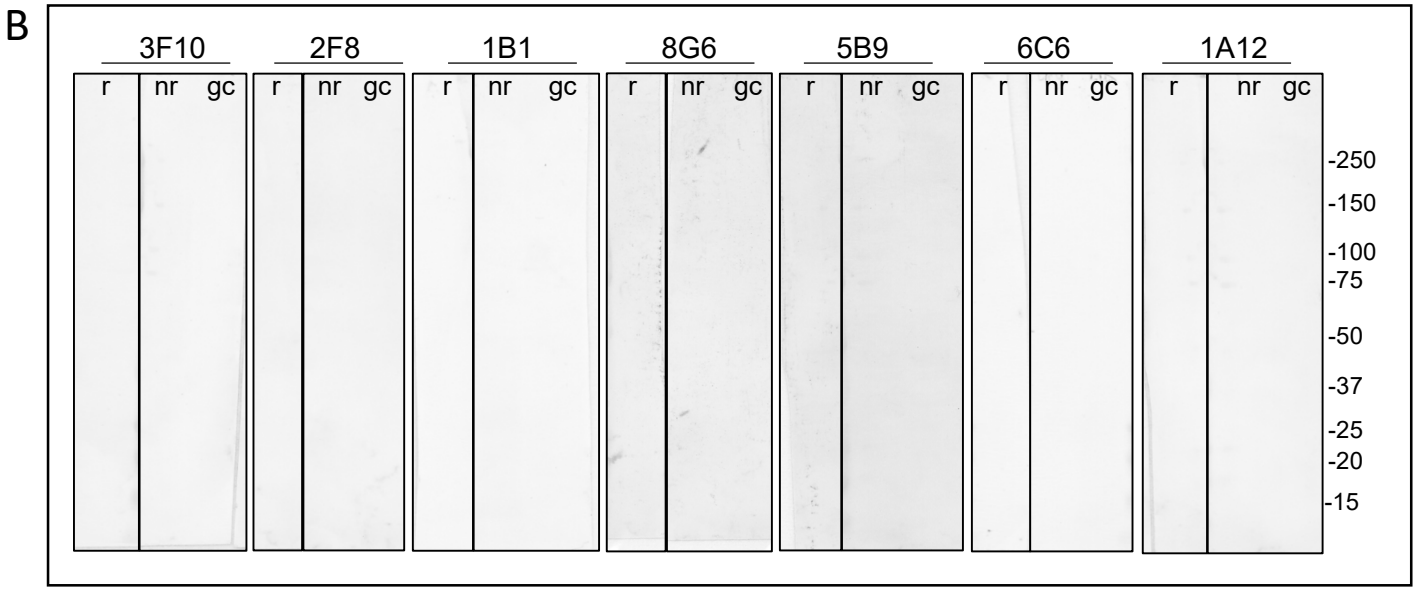
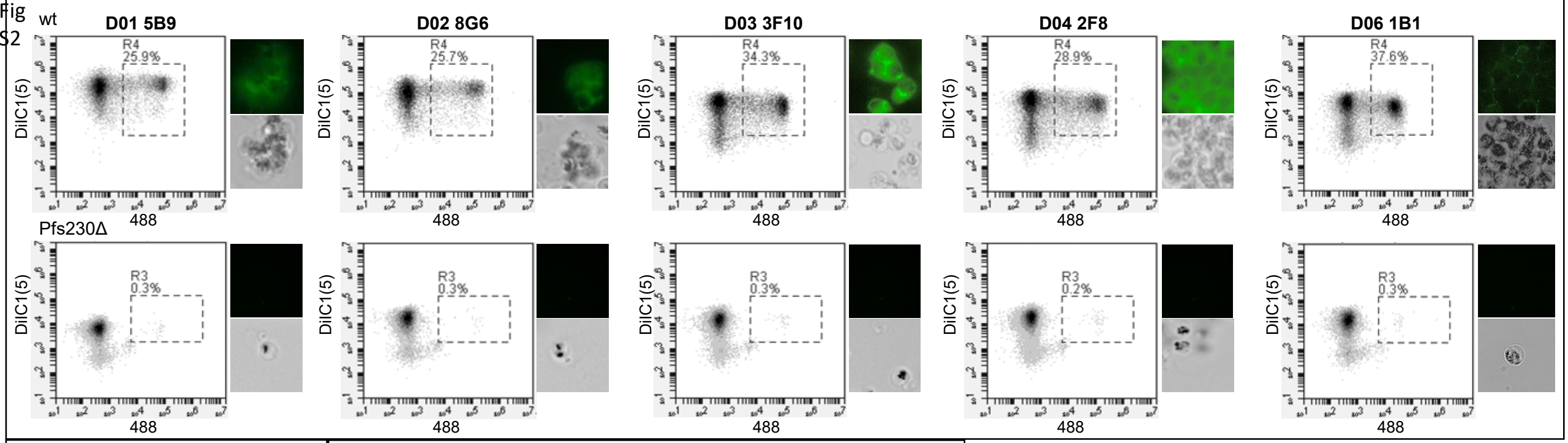


Figure S1: Monoclonal (mAb) sexual stage parasite reactivity. Immunoblots of purified gamete (gm) Triton X-100 extract with (r) and without (nr) β-mercaptoethanol (A&B) and purified gametocyte (gc) Triton X-100 extract without β-mercaptoethanol (B) were probed with the indicated mAb and the bands visualized with 5-Bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT).

Fig S2



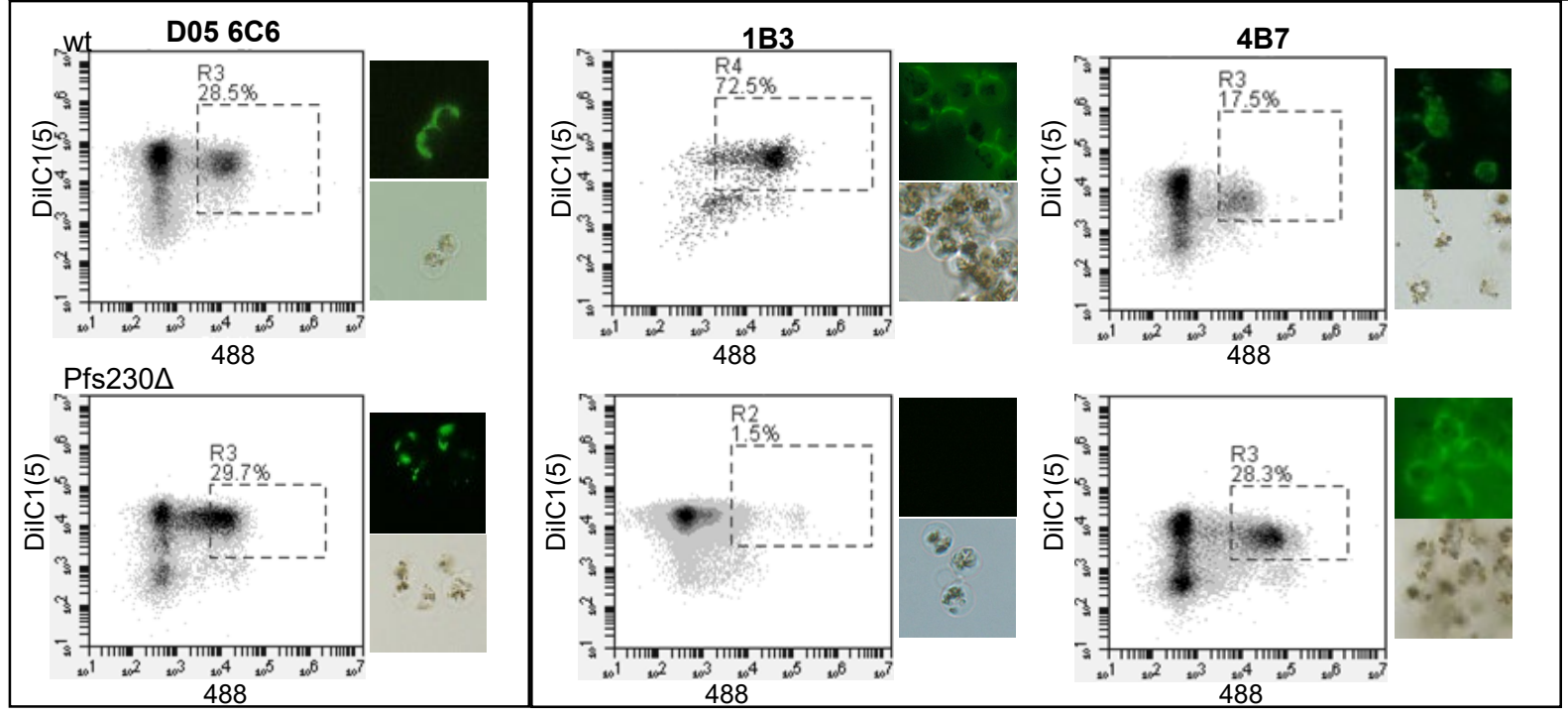


Figure S2: Gamete surface reactivity of immunoblot negative monoclonals (mAbs). Purified wild type strain NF54 (wt) or Pfs230Δ$_{452\text{-}3135}$ (Pfs230Δ) gametes were incubated with the indicated mAb, washed, and then stained with Alexa Fluor 488-labeled anti-mouse IgG (488) and membrane potential dye DiIC1(5). Fluorescence was monitored by flow cytometry and fluorescence microscopy. For each antibody and gamete population the DiIC1(5) and Alex Fluor 488 fluorescence of each cell is plotted and representative fluorescent and bright field images are shown. Pfs230-specific mAb 1B3 and Pfs25-specific mAb 4B7 are included as controls.

Fig S3

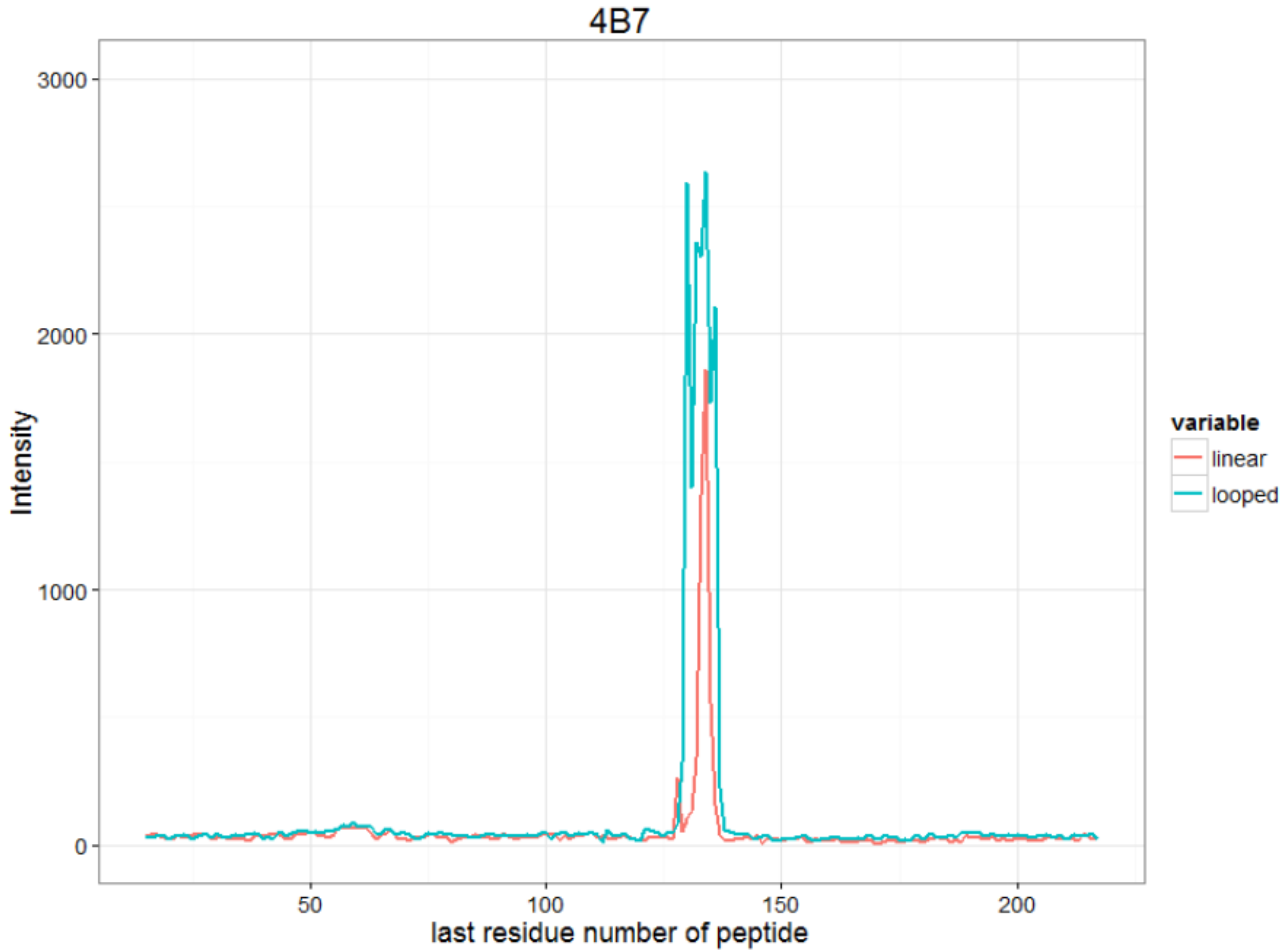


Figure S3: Peptides bound by monoclonal (mAb) 4B7. The fluorescence intensity of mAb 4B7 binding to two sets of peptides corresponding to Pfs25. One set was linear fifteen aa peptides with an offset of one residue (orange) and the other set was looped by adding cysteine residues at both ends of the one aa offset 15 aa Pfs25 peptides to allow disulfide binding (cyan).

Table S1: Standard membrane feeding assay using monoclonals purified from standard hybridoma media

Immuno-blot	Sample name	IgG conc [ug/ml]	% Un-infect	Oocyst		% Inhibition			
				Range	Ave	Estimate	95%CI Lo	95%CI Hi	p-value
	Human serum	0	10	0-39	13.1				
Pfs25	4B7	93.8	90	0-1	0.1	99.2	97.3	100	0.001
Neg Ctr	F5 15F7	375.0	30	0-87	16.7				
Pfs230	F3 09F4 5E1	375.0	50	0-21	4.5	73.4	23.5	90.9	0.015
	F5 6D11 7E5	375.0	85	0-6	0.4	97.6	91.9	99.5	0.001
	F5 05A6 2F5	375.0	75	0-4	0.4	97.6	92.5	99.3	0.001
Pfs48/45	F2 14E5 1G9	375.0	25	0-16	3	79.5	42.7	93.3	0.002
Pfs25	F5 04F5 2C7	375.0	20	0-25	5.1	69.5	8.2	89.6	0.036
Neg	F3 14A1 2H11	375.0	30	0-18	5.0	70.1	11.7	89.8	0.031

Table S2: Mass Spectrometry results for the material immunoprecipitated from Triton X-100 gamete extracts using the indicated monoclonal (mAb)

Accession	Description	Sum PEP Score[a]	Coverage	# Pep-tides	# PSMs	# Unique Peptides	# Protein Groups	# AAs	MW [kDa]	calc. pI	Confidence	Area: F1: Sample	emPAI	Score Sequest HT	# Peptides Sequest HT
mAb 2H11															
PF3D7_0209000	6-cysteine protein (P230)	35.13	6.86	17	30	17	1	3135	363	5.45	High	7.32E+06	0.279	77.72	17
PF3D7_1008700	tubulin beta chain	19.25	19.33	7	21	7	1	445	49.7	4.83	High	6.22E+06	1.462	58.99	7
PF3D7_1246200	actin I (ACT1)	16.92	23.67	7	30	7	1	376	41.8	5.34	High	5.04E+06	1.754	68.97	7
PF3D7_1130200	60S ribosomal protein P0 (PfP0)	4.32	6.96	2	11	2	1	316	34.9	6.71	High	6.49E+05	0.245	26.94	2
PF3D7_0903700	alpha tubulin 1	3.97	6.84	2	3	2	1	453	50.3	5.06	High	1.04E+06	0.212	5.41	2
PF3D7_0917900	heat shock protein 70 (HSP70-2)	3.94	3.53	2	2	2	1	652	72.3	5.31	High	2.74E+06	0.125	5.93	2
mAb 11A6															
PF3D7_0209000	6-cysteine protein (P230)	298.92	36.91	93	170	93	1	3135	363	5.45	High	3.73E+07	3.276	452.62	93
PF3D7_1346800	6-cysteine protein (P47)	135.79	54.90	25	189	25	1	439	50.8	8.31	High	3.24E+08	108.648	427.90	25
PF3D7_0917900	heat shock protein 70 (HSP70-2)	45.83	39.57	18	23	18	1	652	72.3	5.31	High	9.02E+06	2.07	58.69	18
PF3D7_1008700	tubulin beta chain	41.58	38.20	12	15	12	1	445	49.7	4.83	High	1.00E+07	3.489	45.36	12
PF3D7_0818900	heat shock protein 70 (HSP70)	30.09	19.65	12	18	12	1	677	73.9	5.67	High	3.08E+06	1.239	49.11	12
mAb 2D8															
PF3D7_0818900	heat shock protein 70 (HSP70)	141.35	43.87	29	99	29	1	677	73.9	5.67	High	7.08E+07	12.335	277.96	29
PF3D7_0917900	heat shock protein 70 (HSP70-2)	117.62	47.70	28	101	28	1	652	72.3	5.31	High	5.18E+07	9	280.92	28
PF3D7_0708400	heat shock protein 90 (HSP90)	114.90	31.95	25	103	25	1	745	86.1	5.01	High	1.17E+08	5.434	278.28	25
PF3D7_1008700	tubulin beta chain	106.67	38.88	16	142	16	1	445	49.7	4.83	High	2.03E+08	19.153	431.32	16
PF3D7_0903700	alpha tubulin 1	93.02	40.84	15	84	4	1	453	50.3	5.06	High	3.09E+07	12.335	238.81	15
PF3D7_0422300	alpha tubulin 2	91.96	43.56	15	101	4	1	450	49.7	5.01	High	1.62E+07	15.876	281.57	15
PF3D7_1222300	endoplasmin, putative (GRP94)	90.90	33.62	25	65	25	1	821	95	5.41	High	3.94E+07	2.682	173.23	25
PF3D7_1302100	gamete antigen 27/25 (G27/25)	90.29	57.60	16	180	16	1	217	26	6.89	High	9.25E+08	118.378	417.36	16
PF3D7_1246200	actin I (ACT1)	86.20	42.29	18	90	16	1	376	41.8	5.34	High	8.69E+07	11.023	283.43	18

[a]Sum PEP Score: The protein score, which is the sum of the scores of the individual peptides using SEQUEST search algorithm, for which the score is the sum of all peptide Xcorr[b] values above the specified score threshold. The score threshold is calculated as follows: 0.8 + peptide_charge × peptide_relevance_factor where peptide_relevance_factor is a parameter with a default value of 0.4. For each spectrum and sequence, the Proteome Discoverer application uses only the highest scored peptide. When it performs a search using dynamic modifications, one spectrum might have multiple matches because of permutations of the modification site.

Coverage: The percent coverage calculated by dividing the number of amino acids in all found peptides by the total number of amino acids in the entire protein sequence.

Peptides: The number of distinct peptide sequences in the protein group.

PSMs: The total number of identified peptide sequences (peptide spectrum matches) for the protein, including those redundantly identified.

Unique peptides: The number of peptide sequences unique to a protein group.

Protein Groups: The number of identified proteins in the protein group of a master protein. Proteins are grouped based on sequence homology and/or isoforms as explained below.

AAs, MW [kDa], calc. pI: The calculated parameters of the protein based on the amino acid sequence in the FASTA database used to generate the report. The Proteome Discoverer application calculates the molecular weight without considering post-translational modifications.

Confidence: Confidence that PSMs are from theidentified protein

Area: The area of the peaks from the spectra for each peptide

emPAI: Exponentially modified protein abundance index (emPAI) for estimation of absolute protein amount in proteomics by the number of sequenced peptides per protein

Score Sequest HT: The sum of the scores of the individual peptides.

Peptides Sequest HT: # of peptides found by Sequest HT, which is the search engine used in Proteome Discoverer

[b]Xcorr: A search-dependent score. It scores the number of fragment ions that are common to two different peptides with the same precursor mass and calculates the cross-correlation score for all candidate peptides queried from the database by SEQUEST searches. (Jimmy K. Eng, Ashley L. McCormack, and John R. Yates, III; An Approach to Correlate Tandem Mass Spectral Data of Peptides with Amino Acid Sequences in a Protein Database. J. Am. Soc. Mass Spectrom. 1994, 5, 976-989)