Profiling the Human Phosphoproteome to Estimate the True Extent of Protein Phosphorylation and Conservation

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Figure S1. Mean % conservation across found orthologues of likely (A) Ser, (B) Thr, (C) Tyr phosphosites and corresponding likely non-



Figure S2. Normalised counts of proximal amino acids positioned at (A) + 1 around Ser; (B) - 1 around Ser; (C) + 1 around Thr; (D) - 1 around Thr; (E) + 1 around Tyr; (F) - 1 around Tyr sites of various phosphorylation likelihood based on evidence in PSP and PA, which are significantly (Bonferroni corrected p-value <0.001) enriched in the "*High in PSP and PA*" compared to the "*Not phosphorylated*" set and to the expected amino acid distribution in the human proteome (represented by dotted baseline).



Evidence source in UniProt	Ser count	Ser % FDR (95% Cl)	Ser TP count	Thr count	Thr % FDR (95% Cl)	Thr TP count	Tyr count	Tyr % FDR (95% Cl)	Tyr TP count
Large scale study data	21588	7 (± 8)	20077	3479	22 (± 14)	3910	494	6 (± 7)	464



Figure S3. The analysis of UniProt (UP) sites. Counts of proximal amino acids positioned at (**A**) +1 around Ser; (**B**) +1 around Thr; (**C**) +1 around Tyr; (**D**) -1 around Ser; (**E**) -1 around Thr; (**F**) -1 around Tyr sites with different phosphorylation evidence types across PhosphoSitePlus (PSP), PeptideAtlas (PA) and UP, normalised to observed distribution of those amino acids in human proteome (represented by dotted baseline fixed at 1). Significant (Bonferroni corrected p-value <0.001) enrichment of proximal amino acids in the "*5*+ *pieces of evidence in PSP and PA*" set is highlighted by the caret symbol (^) when compared against the "*Not phosphorylated*" set, and an asterisk symbol (*) when compared to the expected amino acid distribution. (**G**) False discovery rate (FDR) and counts of estimated true positive (TP) Ser, Thr and Tyr phosphosites within a set of UniProt sites identified by large scale proteomics studies and which are found in the reference human proteome. Box plots show conservation percentages (%) across 100 eukaryotic species of human (**H**) Ser, (**I**) Thr sites categorised based on evidence type in PSP/PA and UniProt (UP). Within each box, a horizontal line represents median % conservation, an (x) symbol represents mean % conservation per group. Each box extends from the 25th to the 75th percentile of each set's distribution of conservation in "5+ *pieces of evidence in PSP and PA*" set for visual comparison.



Figure S4. Count of significant (Benjamini–Hochberg adj. p-value <0.05) functional groups identified in DAVID for protein sets containing different highest ranked (**A**) Ser, (**B**) Thr, (**C**) Tyr sites based on phosphorylation likelihood sets in PSP and PA. The number of proteins in each set is presented by *n*.



Figure S5. Top 10 functional categories for which protein sets containing various highest ranked (A) Ser, (B) Thr, (C) Tyr sites based on the amount of available phosphorylation evidence were significantly enriched in DAVID (Benjamini–Hochberg corrected p-value <0.05). For each protein set, the percentage of proteins (%) enriched for a particular functional category is given as well as the log2(fold enrichment) for that category. The number of proteins in each set is presented by *n*.



pST conservation (%) (n=19725)

Α



SI Figure S6. Conservation patterns of (A) Ser/Thr and (B) Tyr phosphosites from human proteins across the groups of eukaryotic species. Each row in the heatmap represents an individual phosphosite and its conservation across specific species groups which are separated into columns. Conservation is scored as a percentage out of all species per group and reflected by a colour gradient divided at quarterly intervals. Phosphosites were clustered based on their similarity in conservation patterns using the Euclidean distance method. For each cluster, a label is assigned which describes the most observed conservation pattern (i.e., at least 50% of sites in the cluster follow the described phosphosite conservation pattern), where high and medium conservation refers to conservation scores of \geq 75% and \geq 50%, respectively. The total number of analysed phosphosites is given by *n*

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SI Figure S7. Top 10 functional categories for which protein sets with different (A) Ser/Thr and (B) Tyr phosphosite conservation patterns were significantly enriched in DAVID (Benjamini– Hochberg corrected p-value <0.05). For each protein set, the % of proteins enriched for a particular functional category is given as well as the log2(fold enrichment) for that set. The number of proteins in each set is presented by n.

Table S3. Proteomes of eukaryotic species analysed in the conservation analysis.

Organism name from Uniprot	Proteome ID	Organism	BUSCO score	Gene count
Pan troglodytes (Chimpanzee)	UP000002277	9598	C·98 3%[S·48 4% D·49 9%] F·0 5% M·1 1% n·6192	23003
Pan paniscus (Pygmy chimpanzee)	UP000240080	9597	C·97 4%[S·54 4% D·43%] F·1 4% M·1 1% n·6192	21211
Gorilla gorilla gorilla (Western	UP000001519	9595	C:97.3% [S:52%, D:45.3%],F:1.8%, M:1%, n:6192	21787
Pongo abelii (Sumatran orangutan)	LIP000001595	9601	C·94 5% [S·88 5% D·6%] F·4 2% M·1 3% p·6192	21992
Nomascus leucogenys (Northern	UP00001073	61853	C.96.3%[S.57.7% D.38.6%] F.2.6% M.1.1% n.6192	20753
white-cheeked gibbon)	0100001075	01055	C.90.576[5.57.170;D.50.070];F.2.070;MI.1.170;H.0192	20755
Macaca mulatta (Rhesus macaque)	UP000006718	9544	C:89.6% [S:47.8%, D:41.8%], F:1.5%, M:8.9%, n:6192	21868
Cercocebus atys (Sooty mangabey)	UP000233060	9531	C:98%[S:48.3%,D:49.7%],F:1.1%,M:0.9%,n:6192	20874
Rhinopithecus bieti (Black snub-nosed monkey)	UP000233180	61621	C:95.1% [S:49.6%, D:45.5%], F:2.9%, M:2%, n:6192	20845
<i>Chlorocebus sabaeus</i> (Green monkey)	UP000029965	60711	C:95.4%[S:94.4%.D:1%].F:3.4%.M:1.2%.n:6192	19136
Macaca fascicularis (Crab-eating	UP000233100	9541	C:98.2%[S:48.8%,D:49.3%],F:1.1%,M:0.8%,n:6192	22278
macaque)				
Papio anubis (Olive baboon)	UP000028761	9555	C:98.4%[S:51.7%,D:46.7%],F:0.8%,M:0.8%,n:6192	21559
Mandrillus leucophaeus (Drill)	UP000233140	9568	C:95.5% [S:55.1%, D:40.3%], F:3%, M:1.6%, n:6192	20767
Saimiri boliviensis boliviensis	UP000233220	39432	C:96.3%[S:50.1%,D:46.2%],F:2.2%,M:1.5%,n:6192	19356
(Bolivian squirrel monkey)				
<i>Callithrix jacchus</i> (White-tufted-ear marmoset)	UP000008225	9483	C:97.9%[S:52.7%,D:45.2%],F:1%,M:1.1%,n:6192	22587
Aotus nancymaae (Ma's night	UP000233020	37293	C:97.4%[S:50.9%,D:46.5%],F:1.3%,M:1.3%,n:6192	20363
monkey)				
Tarsius syrichta (Philippine tarsier)	UP000189704	1868482	C:75.6% [S:60%, D:15.6%], F:5.7%, M:18.7%, n:6192	19956
Otolemur garnettii (Small-eared	UP000005225	30611	C:96.8%[S:94%,D:2.8%],F:2.1%,M:1.1%,n:6192	19443
galago)				
Propithecus coquereli (Coquerel's sifaka)	UP000233160	379532	C:92%[S:63.6%,D:28.4%],F:4.1%,M:3.9%,n:6192	17876
Ictidomys tridecemlineatus (Thirteen-	UP000005215	43179	C:94.2%[S:71.1%,D:23%],F:2.6%,M:3.2%,n:6192	18440
Cavia poreellus (Guipes pig)	11000005447	10141	C:04.20% [S:70.20% D:22.00%] E:2.00% M:2.00% p:6102	19247
Mus musculus (Mouse)	UP000005447	10141	C.94.2% [S:70.3%, D.23.3%], $F.2.3%$, $M.2.3%$, $M.2.5%$, $M.0.1%$	21082
Orvetolagus cuniculus (Rabbit)	UP00000389	9986	C.99.7%[5.32.1%,D.47.0%],F.0.2%,M.0.1%,H.0192	21982
Cricetulus griseus (Chinese hamster)	UP000001075	10029	C:63 7%[S:63% D:0 7%] F:19 2% M:17 1% n:6192	23874
Fukomvs damarensis (Damaraland	UP000028990	885580	C:82.4%[S:81.9%,D:0.5%],F:7.7%,M:9.9%,n:6192	20401
mole rat)				
Mesocricetus auratus (Golden	UP000189706	10036	C:77.6% [S:54.7%, D:22.9%], F:1.9%, M:20.5%, n:6192	20418
hamster)				
Dipodomys ordii (Ord's kangaroo rat)	UP000081671	10020	C:93.7% [S:66.1%,D:27.6%],F:5.4%,M:0.9%,n:6192	19730
Heterocephalus glaber (Naked mole	UP000006813	10181	C:87%[S:85.5%,D:1.5%],F:6.7%,M:6.2%,n:6192	21445
rat) Vombatus unsinus (Common wombat)	110000214097	20120	$C_{0} = C_{0} = C_{0$	10972
Myotis lucifugus (Little brown bat)	UP000314987	59463	C.90.0% [S.50.2%, D.40.4%], $F.1.8%$, $M.1.0%$, $H.4104C.95.1%$ [S.90% D.5.1%] F.3.5% M.1.4% p.6253	19672
Canis lucy familiaris (Dog)	UP000001074	9615	C·97%[S·45 5% D·51 5%] F·1 7% M·1 3% n·6253	20624
Capra hircus (Goat)	UP000291000	9925	C:98%[S:57.9% D:40.1%].F:1.2% M:0.8% n:6253	21149
Ovis aries (Sheep)	UP00002356	9940	C:98%[S:86.8%,D:11.2%],F:1.4%,M:0.5%,n:6253	21212
Sus scrofa (Pig)	UP000008227	9823	C:95.3% [S:49%,D:46.3%],F:2.4%,M:2.3%,n:6253	22130
Felis catus (Cat)	UP000011712	9685	C:97% [S:67%,D:30%],F:1.4%,M:1.6%,n:6253	19645
Ailuropoda melanoleuca (Giant	UP000008912	9646	C:97.4%[S:91.7%,D:5.8%],F:2%,M:0.6%,n:6253	19332
panda)				
Pteropus alecto (Black flying fox)	UP000010552	9402	C:84.2%[S:83.7%,D:0.5%],F:9%,M:6.8%,n:6253	19520
Erinaceus europaeus (Western	UP000079721	9365	C:95.2%[S:66.7%,D:28.4%],F:3.6%,M:1.2%,n:6253	19242
European hedgehog)				
<i>Equus caballus</i> (Horse)	UP000002281	9796	C:97.8%[S:57.5%,D:40.3%],F:1.3%,M:0.9%,n:6253	20845
Bos taurus (Bovine)	UP000009136	9913	C:98.2%[S:34.4%,D:63.8%],F:1.2%,M:0.6%,n:6253	23844
Mustela putorius furo (European	UP000000715	9669	C:96.2%[S:94.9%,D:1.3%],F:2.4%,M:1.4%,n:6253	19902
domestic ferret)	110000265200	110707	C.09 50/ [S.74 20/ D.24 20/] E.1 10/ M.O.20/	10012
dolphin)	01000205300	118/9/	C.90.3%[5:/4.2%,D:24.3%],F:1.1%,MI:U.3%,n:0253	18840
Lentonychotes weddellii (Weddell	UP000245341	9713	C·85%[S·50 5% D·34 5%] F·14% M·1% n·6253	13162
seal)	010002-00-1	7115	0.00/0.000/0.00/00/	15102
Ursus maritimus (Polar bear)	UP000261680	<u>2</u> 9073	C:97.1%[S:71.8%,D:25.3%],F:2.6%,M:0.2%,n:6253	19368
Delphinapterus leucas (Beluga whale)	UP000248483	9749	C:98.6% [S:44%, D:54.6%], F:1.1%, M:0.4%, n:6253	17043

Odobenus rosmarus divergens	UP000245340	9708	C:99% [S:64%,D:35%],F:0.9%,M:0.1%,n:6253	19331
(Pacific walrus)	LIP000248484	0755	C·86.3% [S·53.7% D·32.6%] F·0.8% M·12.0% p·6253	20100
whale)	01000248484	9155	C.80.5%[5.55.7%,D.52.0%],F.0.8%,WI.12.9%,II.0255	20100
Tursiops truncatus (Atlantic bottle- nosed dolphin)	UP000245320	9739	C:84.1%[S:42.6%,D:41.4%],F:8.4%,M:7.6%,n:6253	17075
Loxodonta africana (African elephant)	UP000007646	9785	C:97.4%[S:73.3%,D:24.1%],F:1.9%,M:0.6%,n:4104	20015
Trichechus manatus latirostris (Elorida manatee)	UP000248480	127582	C:98.1%[S:55.4%,D:42.7%],F:1.6%,M:0.3%,n:4104	19079
Ornithorhynchus anatinus (Duckbill	UP000002279	9258	C:75.7% [S:68%,D:7.7%],F:18.8%,M:5.5%,n:4104	21677
Meleagris gallopavo (Wild turkey)	UP000001645	9103	C:91.1%[S:81.4%,D:9.7%],F:5.4%,M:3.5%,n:4915	14164
Taeniopygia guttata (Zebra finch)	UP000007754	59729	C:95.4% [S:91.2%, D:4.3%], F:3.6%, M:0.9%, n:4915	17428
Anas platyrhynchos (Mallard)	UP000296049	8839	C:79.4%[S:78.5%,D:0.9%],F:7.8%,M:12.8%,n:4915	16574
Dryobates pubescens (Downy woodpecker)	UP000053875	118200	C:94.6%[S:93.7%,D:1%],F:2.2%,M:3.2%,n:4915	13097
<i>Tinamus guttatus</i> (White-throated tinamou)	UP000053641	94827	C:89.8% [S:88.5%, D:1.3%], F:5.5%, M:4.7%, n:4915	13377
Amazona aestiva (Blue-fronted Amazon parrot)	UP000051836	12930	C:86.6% [S:85.4%,D:1.2%],F:7.5%,M:5.9%,n:4915	16092
Calypte anna (Anna's hummingbird)	UP000054308	9244	C:95.4%[S:94.5%,D:0.9%],F:1.7%,M:2.9%,n:4915	13267
Columba livia (Rock dove)	UP000053872	8932	C:93%[S:78.5%,D:14.4%],F:5%,M:2.1%,n:4915	14619
Callipepla squamata (Scaled quail)	UP000198323	9009	C:77.6%[S:75.8%,D:1.8%],F:14.5%,M:7.9%,n:4915	16973
Aptenodytes forsteri (Emperor penguin)	UP000053286	9233	C:97.9%[S:97%,D:0.9%],F:0.8%,M:1.3%,n:4915	13704
Opisthocomus hoazin (Hoatzin)	UP000053605	30419	C:95.3%[S:94.7%,D:0.6%],F:2.1%,M:2.6%,n:4915	12773
Egretta garzetta (Little egret)	UP000053119	188379	C:96.7%[S:96%,D:0.8%],F:1%,M:2.3%,n:4915	13489
Alligator mississippiensis (American alligator)	UP000050525	8496	C:88.6%[S:67.4%,D:21.2%],F:6.8%,M:4.6%,n:3950	24656
Alligator sinensis (Chinese alligator)	UP000189705	38654	C:74.4% [S:55.1%, D:19.3%], F:2.4%, M:23.2%, n:3950	19111
Anolis carolinensis (Green anole)	UP000001646	28377	C:92.1%[S:88.4%,D:3.7%],F:5.3%,M:2.6%,n:3950	18525
Pelodiscus sinensis (Chinese softshell turtle)	UP000007267	13735	C:93.5%[S:79.1%,D:14.4%],F:4.9%,M:1.6%,n:3950	18109
Salmo salar (Atlantic salmon)	UP000087266	8030	C:97.9%[S:23.9%,D:74%],F:1.5%,M:0.6%,n:4584	47717
Oncorhynchus mykiss (Rainbow trout)	UP000193380	8022	C:77.1%[S:46.4%,D:30.7%],F:10.9%,M:12%,n:4584	46447
Gasterosteus aculeatus (Three-spined stickleback)	UP000007635	69293	C:97.5% [S:74.5%, D:23%], F:1.9%, M:0.6%, n:4584	20665
Seriola dumerili (Greater amberjack)	UP000261420	41447	C:97.6% [S:69.5%, D:28.1%], F:1.5%, M:0.8%, n:4584	23238
<i>Takifugu rubripes</i> (Japanese pufferfish)	UP000005226	31033	C:95.1%[S:65.4%,D:29.7%],F:2.7%,M:2.2%,n:4584	20591
Xenopus laevis (African clawed frog)	UP000186698	8355	C:95.6%[S:41.5%,D:54.1%],F:1.6%,M:2.8%,n:3950	43235
Xenopus tropicalis (Western clawed frog)	UP000008143	8364	C:62.9%[S:37.4%,D:25.4%],F:2.4%,M:34.8%,n:3950	35973
Daphnia pulex (Water flea)	UP00000305	6669	C:96.1%[S:93.8%,D:2.3%],F:1.4%,M:2.5%,n:1066	30118
Tribolium castaneum (Red flour beetle)	UP000007266	7070	C:98.6%[S:91.5%,D:7.1%],F:1.2%,M:0.2%,n:1658	16568
Bombyx mori (Silk moth)	UP000005204	7091	C:89.9%[S:89.4%,D:0.5%],F:6.8%,M:3.3%,n:1658	14773
Anopheles darlingi (Mosquito)	UP00000673	43151	C:89.4%[S:89%,D:0.4%],F:2.6%,M:7.9%,n:2799	10447
Drosophila melanogaster (Fruit fly)	UP00000803	7227	C:99.3%[S:38.2%,D:61.1%],F:0.4%,M:0.3%,n:2799	13790
Harpegnathos saltator (Jerdon's jumping ant)	UP000008237	610380	C:90.3%[S:90%,D:0.3%],F:6.4%,M:3.3%,n:4415	15029
Ooceraea biroi (Clonal raider ant)	UP000053097	2015173	C:95.9%[S:95.4%,D:0.4%],F:3.4%,M:0.8%,n:4415	16497
Papilio xuthus (Asian swallowtail butterfly)	UP000053268	66420	C:96.3%[S:94.9%,D:1.4%],F:2.4%,M:1.3%,n:1658	15265
Zootermopsis nevadensis (Dampwood termite)	UP000027135	136037	C:93.9% [S:92.8%,D:1.1%],F:1.6%,M:4.5%,n:1658	14539
Operophtera brumata (winter moth)	UP000037510	104452	C:75.9%[S:73.4%,D:2.5%],F:15%,M:9.2%,n:1658	16814
Lucilia cuprina (Green bottle fly)	UP000037069	7375	C:92.5%[S:91.5%,D:1%],F:1.8%,M:5.6%,n:2799	14353
Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's	UP000002311	559292	C:98.9%[S:98.3%,D:0.6%],F:1.1%,M:0%,n:1711	6049
<i>Emericella nidulans</i> (strain FGSC A4 / ATCC 38163 / CBS 112.46 / NRRL 194 / M139) (Aspergillus nidulans)	UP00000560	227321	C:93%[S:92.9%,D:0.1%],F:4.5%,M:2.5%,n:4046	10557
Neurospora crassa (strain ATCC 24698 / 74-OR23-1A / CBS 708.71 / DSM 1257 / FGSC 987)	UP000001805	367110	C:99%[S:88.6%,D:10.4%],F:0.8%,M:0.2%,n:3725	9759

<i>Yarrowia lipolytica</i> (strain CLIB 122 / E 150) (Yeast) (Candida lipolytica)	UP000001300	284591	C:87.9%[S:87.3%,D:0.6%],F:9.8%,M:2.3%,n:1711	6449
Arachis hypogaea (Peanut)	UP000289738	3818	C:93.7% [S:17.2%, D:76.5%], F:1.7%, M:4.7%, n:1440	71122
Musa acuminata subsp. Malaccensis	UP000012960	214687	C:86.7% [S:76.5%, D:10.3%], F:4.7%, M:8.5%, n:1440	36474
(Wild banana)				
Arabidopsis thaliana (Mouse-ear	UP000006548	3702	C:99.6% [S:59.1%, D:40.5%], F:0.2%, M:0.2%, n:1440	27466
cress)				
Oryza sativa subsp. indica (Rice)	UP000007015	39946	C:94.8%[S:93.6%,D:1.2%],F:2.4%,M:2.8%,n:1440	37344
Zea mays (Maize)	UP000007305	4577	C:96.4%[S:49.4%,D:46.9%],F:2.1%,M:1.5%,n:1440	39400
Triticum aestivum (Wheat)	UP000019116	4565	C:99.4%[S:1.4%,D:98%],F:0.1%,M:0.5%,n:1440	105061
Physcomitrella patens subsp. patens	UP000006727	3218	C:67.8% [S:52%, D:15.8%], F:3.3%, M:28.9%, n:1440	30857
(Moss)				
Emiliania huxleyi (Pontosphaera	UP000013827	2903	C:74.9%[S:0.7%,D:74.3%],F:14.2%,M:10.9%,n:303	35676
huxleyi)				
Dictyostelium discoideum (Slime	UP000002195	44689	C:96% [S:92.4%,D:3.6%],F:0.7%,M:3.3%,n:303	12739
mold)				
Chlamydomonas reinhardtii	UP000006906	3055	C:96% [S:90.1%,D:5.9%],F:2.3%,M:1.7%,n:303	17614
(Chlamydomonas smithii)				
Thalassiosira pseudonana (Marine	UP000001449	35128	C:34.2%[S:33.3%,D:0.9%],F:1.3%,M:64.5%,n:234	11717
diatom)				
Plasmodium falciparum (isolate 3D7)	UP000001450	36329	C:25.6% [S:25.2%, D:0.4%], F:0.4%, M:73.9%, n:234	5376