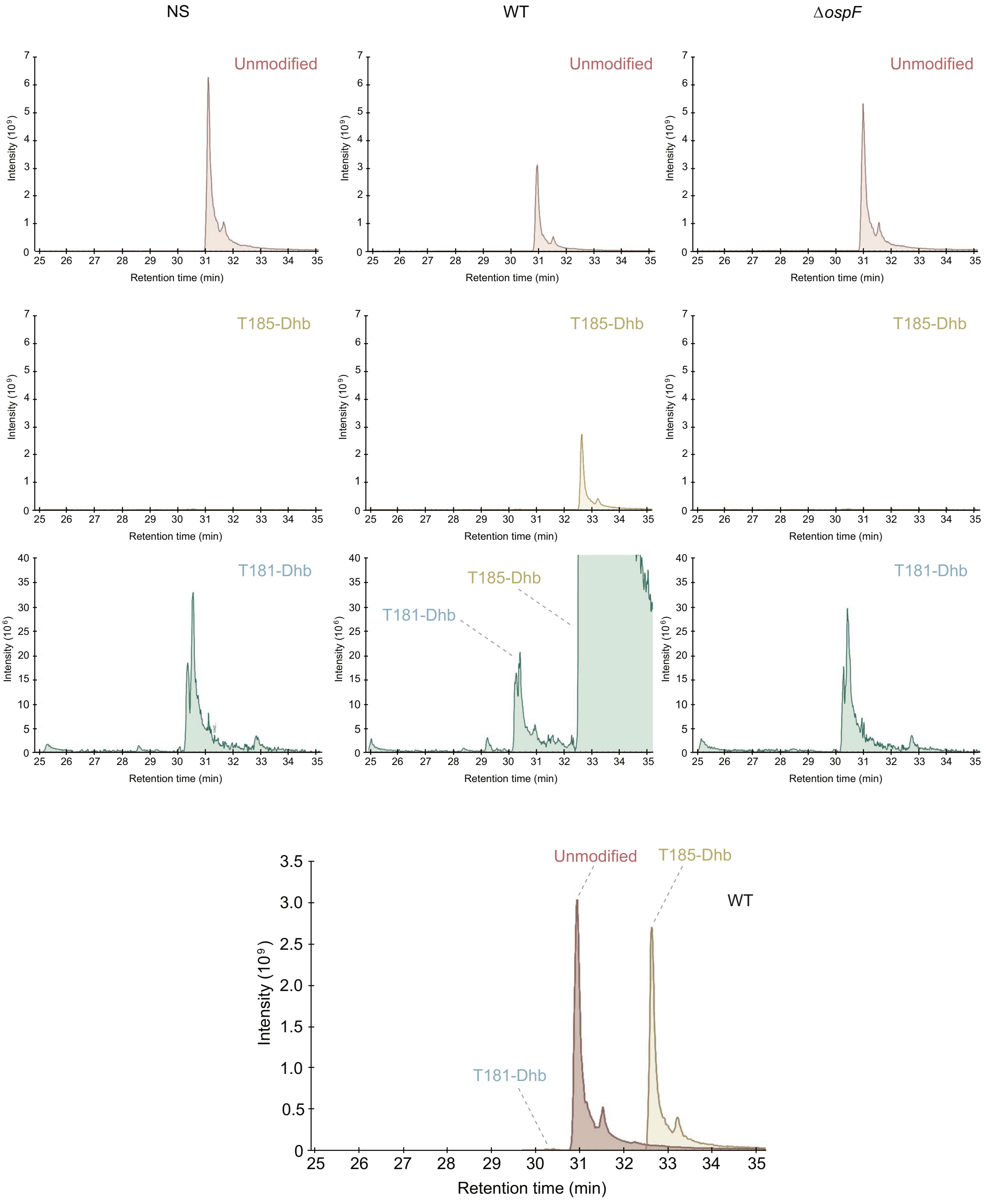
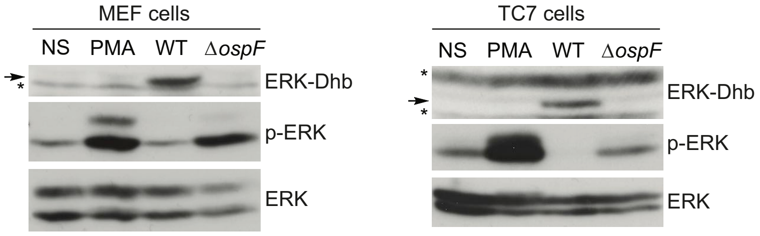
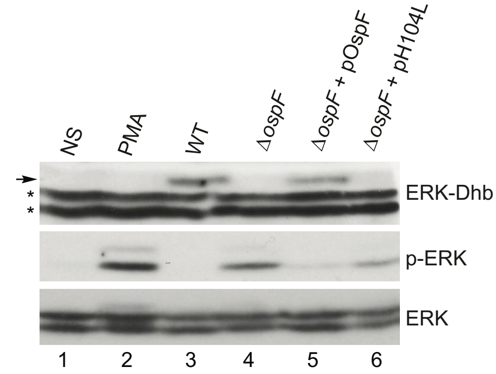


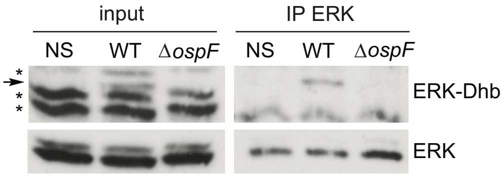
**Supplementary Figure 1. ERK is constitutively modified on T181 Dhb.** Extracted ion chromatogram (XIC) of the precursor ion (first three isotopes, z=3) for the ERK peptide 173-191 in HeLa cells non stimulated (NS) or infected by the wild-type *Shigella* (WT) or the mutant for the *ospF* gene (Δ*ospF*) for 2 hours. We separately report the signal for the non modified (top), the peptide carrying a Dhb modification on T185 (middle) and the peptide carrying a Dhb modification on T181. Of note, the peptide Dhb 185 and Dhb 181 have the same precursor masses but different retention time. Overlap of the XIC signal of the non modified, Dhb 185 and Dhb 181 of the peptide 173-191 in the sample WT, showing the difference in intensity and retention times of these three forms.



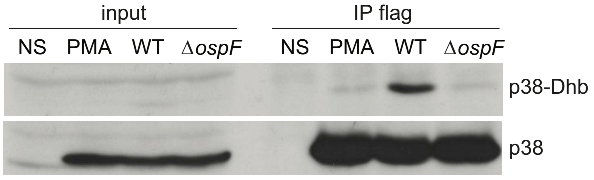
**Supplementary Figure 2. ERK-Dhb detection in various cell types.** MEF and TC7 cells were stimulated with PMA for 60 minutes, or were infected by the wild type *Shigella* (WT) or the mutant for the *ospF* gene (Δ*ospF*) for 60 minutes. "*" indicates non-specific bands (not OspF-dependent). The arrow designs the specific band.



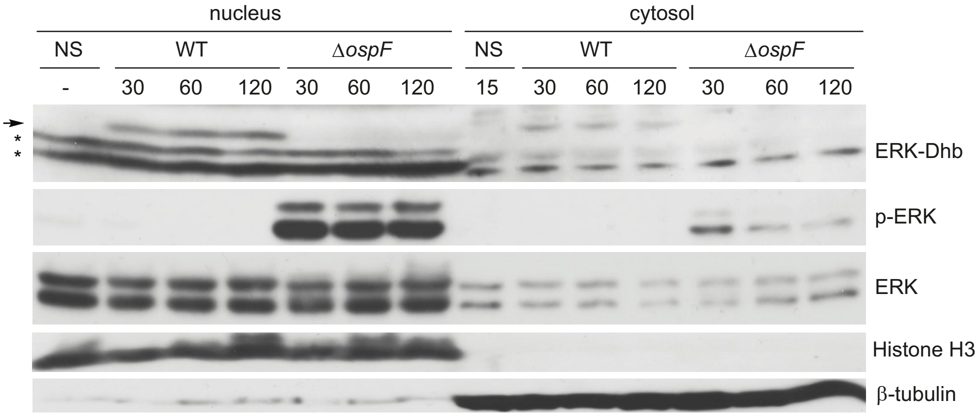
**Supplementary Figure 3. The detection of ERK-Dhb is strictly dependant on OspF catalytic activity.** HeLa cells lysates left untreated (NS), stimulated with 1 µg/mL of PMA or infected with the wild type *Shigella* (WT), the *ospF* mutant (Δ*ospF)*, or the *ospF* mutant transformed with a pUC18 plasmid allowing the expression of OspF (Δ*ospF*-pOspF) or the catalytically inactive form of OspF (Δ*ospF*-pH104L) for 60 minutes. "*" indicates non-specific bands (not OspF-dependent). The arrow designs the specific band. The arrow designs the specific band.



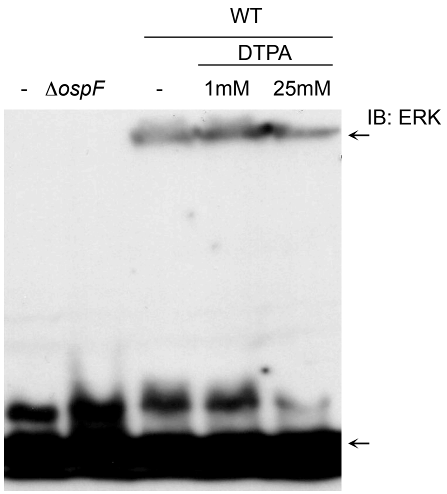
**Supplementary Figure 4 Immunoprecipitation of endogenous ERK showed Dhb formation in a OspF-dependant manner.** Immunoprecipitation performed with an ERK antibody in HeLa cells non treated (NS) or infected by the wild type *Shigella* (WT) or the mutant for the *ospF* gene (Δ*ospF*) for 60 minutes. "*" indicates non-specific bands (not OspF-dependent). The arrow designs the specific band.



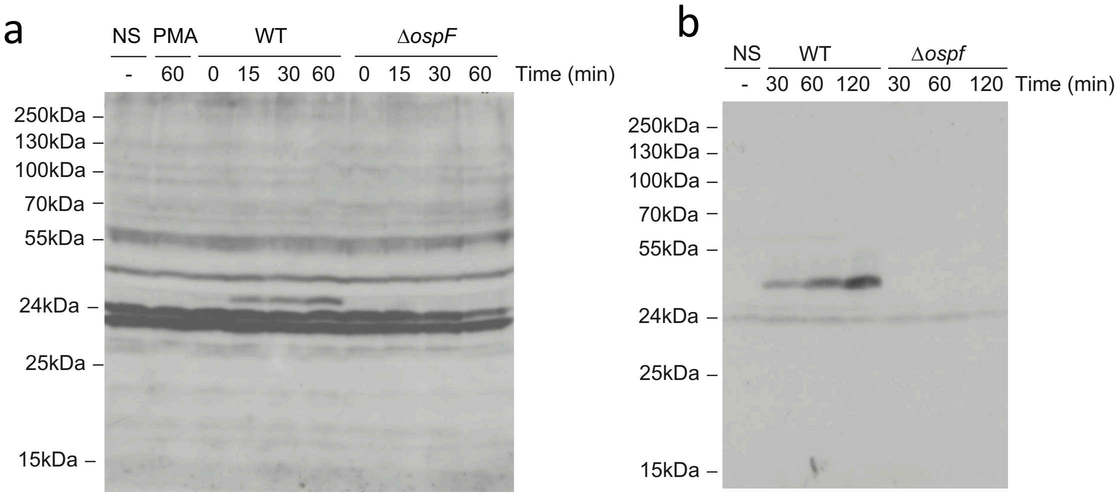
**Supplementary Figure 5 Detection of p38-Dhb.** Immunoprecipitation was performed with a flag antibody in HeLa cells transfected with a pcDNA-flag-p38 construct, then stimulated with PMA for 60 minutes, or infected by the wild type *Shigella* (WT) or the mutant for the *ospF* gene (Δ*ospF*) for 60 minutes.



**Supplementary Figure 6. Subcellular localization of ERK-Dhb.** Nuclear or cytoplasmic lysates of HeLa cells non stimulated (NS), stimulated with PMA for 60 minutes or infected with the wild-type Shigella (WT) or the *ospF* mutant (ΔospF) for different durations. "*" indicates non-specific bands (not OspF-dependent). The arrow designs the specific band.



**Supplementary Figure 7 – The 170kDa ERK2-containing complex is stable** Whole cell extracts of HeLa cells transfected with a pcDNA-flag-ERK2 construct, then infected by the wild type *Shigella* (WT) or the mutant for the *ospF* gene (Δ*ospF*) for 60 minutes, lysed in 8M Urea, 2,5% β-mercaptoethanol, and treated when indicated with DTPA, a strong chelating agent, at concentrations of 1mM or 25mM.



**Supplementary figure S8:** Western blots using the anti Erk-Dhb antibody. (a) is related to Figure 2D and (b) to Figure 2C.

| PROTEIN | | | PEPTIDE | | | | | | | | | PEPTIDE INTENSITY | | | | | | | | | | | | MODIFIED SITES | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Pre-immune Serum (S) | | | NS | | | WT | | | ΔospF | | | | |
| Proteins | Protein names | Gene names | Sequence | MC | Mass | Start | End | Unique (Proteins) | Charges | PEP | Score | n1 | n2 | n3 | n1 | n2 | n3 | n1 | n2 | n3 | n1 | n2 | n3 | Dehydrated (ST) site IDs | Phospho (STY) site IDs |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | AAAAAAGAGPEMVR | 0 | 1283,6292 | 2 | 15 | yes | 2 | 0,01 | 120,84 | | | | | | | 7,13 | 6,98 | 8,07 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | AAAAAQGGGGGGEPR | 0 | 1210,5691 | 2 | 15 | yes | 2 | 0,01 | 83,351 | | | | | | | 7,12 | | 8,12 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | ALDLLDR | 0 | 814,45487 | 303 | 309 | yes | 2 | 0,01 | 91,9 | | | | | | | | | 7,90 | | | | | |
| P28482;P27361 | Mitogen-activated protein kinase 1;Mitogen- | MAPK1;MAPK3 | APEIMLNSK | 0 | 1001,5216 | 195 | 203 | no | 2 | 0,01 | 111,61 | | | 7,25 | | | | | | 7,97 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | ASTLEAMR | 0 | 877,43275 | 109 | 116 | yes | 2 | 0,01 | 77,732 | | | | | | | | | 7,93 | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | DLKPSNLLLNTTCDLK | 1 | 1843,9713 | 149 | 164 | yes | 2;3 | 0,01 | 98,421 | | | | | | | 7,56 | 6,70 | 8,20 | | | | | |
| P28482;P27361 | Mitogen-activated protein kinase 1;Mitogen- | MAPK1;MAPK3 | DVYIVQDLMETDLYK | 0 | 1843,8914 | 100 | 114 | no | 3 | 0,002532 | 31,089 | | | | | | | 6,74 | 6,46 | | | | | | |
| P28482;P27361 | Mitogen-activated protein kinase 1;Mitogen- | MAPK1;MAPK3 | DVYIVQDLMETDLYK | 0 | 1859,8863 | 100 | 114 | no | 2 | 0,01 | 53,008 | | | | | | | 6,67 | | | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | ELIFEETAR | 0 | 1106,5608 | 345 | 353 | yes | 2 | 0,01 | 101,9 | | | | | | | | | 7,64 | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | FDMELDDLPK | 0 | 1221,5587 | 331 | 340 | yes | 2 | 0,01 | 60,255 | | | | | | | | | 7,28 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | FQPGVLEAP | 0 | 956,49673 | 371 | 379 | yes | 2 | 0,01 | 35,269 | | | | | | | | | 7,51 | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | FRHENIIGINDIIR | 1 | 1708,9373 | 78 | 91 | yes | 4 | 0,01 | 47,301 | | | | | | | | | 7,35 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | FRHENVIGIR | 1 | 1239,6836 | 95 | 104 | yes | 2;3 | 0,01 | 61,765 | | | | | | | | | 7,75 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | GQPFDVGPR | 0 | 971,48248 | 33 | 41 | yes | 2 | 0,01 | 36,424 | | | | | | | | | 7,79 | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | GQVFDVGPR | 0 | 973,49813 | 16 | 24 | yes | 2 | 0,01 | 44,863 | | | | | | | | | 7,77 | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | HENIIGINDIIR | 0 | 1405,7678 | 80 | 91 | yes | 3 | 0,01 | 38,3 | | | | | | | | | 7,13 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | IADPEHDHTGFLTEYVATR | 0 | 2153,0178 | 190 | 208 | yes | 2;3 | 0,01 | 125,23 | | | | | | | 7,34 | 7,33 | 8,14 | | | | T202 | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | IEVEQALAHPYLEQYYDPSDEPIAEAPFK | 0 | 3361,6031 | 302 | 330 | yes | 3 | 0,01 | 68,96 | | | | | | | 7,01 | 6,38 | | | | | | |
| P28482;P27361 | Mitogen-activated protein kinase 1;Mitogen- | MAPK1;MAPK3 | ISPFEHQTYCQR | 0 | 1564,7093 | 56 | 67 | no | 2;3 | 0,01 | 140,14 | | | | | | | 6,26 | | 8,22 | | | | | |
| P28482;P27361 | Mitogen-activated protein kinase 1;Mitogen- | MAPK1;MAPK3 | ISPFEHQTYCQRTLR | 1 | 1934,9421 | 56 | 70 | no | 2 | 0,01 | 11,918 | | | | | | | 7,21 | | | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | LKELIFEETAR | 1 | 1347,7398 | 343 | 353 | yes | 3 | 0,01 | 45,28 | | | | | | | | | 7,22 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | LKELIFQETAR | 1 | 1346,7558 | 360 | 370 | yes | 3 | 0,01 | 71,076 | | | | | | | | | 7,79 | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | MLTFNPHK | 0 | 986,50077 | 293 | 300 | yes | 2 | 0,01 | 10,71 | 7,09 | | | | | | 7,01 | 7,02 | | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | MLTFNPHKR | 1 | 1142,6019 | 293 | 301 | yes | 2 | 0,01 | 89,542 | | | | | | | | | 7,07 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | MLTFNPNKR | 1 | 1119,5859 | 310 | 318 | yes | 2 | 0,01 | 89,698 | | | | | | | | | 7,64 | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | NYLLSLPHK | 0 | 1083,6077 | 262 | 270 | yes | 2 | 0,01 | 44,392 | | | | | | | | | 7,51 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | RTEGVGPGVPGEVEMVK | 1 | 1739,8876 | 16 | 32 | yes | 2 | 0,01 | 114,99 | | | | | | | | | 7,56 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | TEGVGPGVPGEVEMVK | 0 | 1583,7865 | 17 | 32 | yes | 2 | 0,01 | 32,465 | | | | | | | | 6,70 | | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | TEGVGPGVPGEVEMVK | 0 | 1599,7814 | 17 | 32 | yes | 2 | 0,01 | 41,088 | | | | | | | 6,65 | | | | | | | |
| P28482 | Mitogen-activated protein kinase 1 | MAPK1 | VADPDHDHTGFLTEYVATR | 0 | 2124,9865 | 173 | 191 | yes | 3 | 0,01 | 89,917 | | | | | | | 7,42 | | | | | | T185 | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | YTQLQYIGEGAYGMVSSAYDHVR | 0 | 2607,2064 | 139 | 148 | yes | 3 | 0,01 | 130,98 | | | | | | | 6,71 | 7,00 | 7,98 | | | | | |
| P27361 | Mitogen-activated protein kinase 3 | MAPK3 | YTQLQYIGEGAYGMVSSAYDHVR | 0 | 2623,2013 | 42 | 64 | yes | 3 | 0,01 | 63,979 | | | | | | | 7,63 | | | | | | | |

**Table S1 (referred to as Table 3): Detailed list of MAPK1 and MAPK3 peptides identified in the anti-Dhb-ERK immunoprecipitates.** We report the peptides sequence, the number of trypsin missing cleavages (MC) the peptide identification score (score), the log(2) ofthe intensity of the signal obtained for each peptide attributed to MAPK1 and MAPK3 (confident protein identification requires at least two unique peptides fragmented per proteinper sample). Common peptides to MAPK1 and MAPK3 are reported as non unique to the protein (Unique (Proteins)=no). Modified sites reports the DHB sites identified. n1, n2 and n3 are three biological replicates.

| Pre-immune Serum | | | NS* | | | WT* | | | ΔospF* | | | Identification | | | Modified sites | | ID | | | Statistical test | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | | | | | | WT vs NS | | | WT vs *ΔospF* | | |
| S_n1 | S_n2 | S_n3 | NS_n1 | NS_n2 | NS_n3 | WT_n1 | WT_n2 | WT_n3 | ΔospF_n1 | ΔospF_n2 | ΔospF_n3 | Score | Intensity | MS/MS Count | Phospho (STY) site | Dehydrated (ST) site | Protein IDs | Protein names | Gene names | t-test Significant | t-test p value | t-test Difference | t-test Significant | t-test p value | t-test Difference |
| 0 | 0 | 0 | 31,3107 | 30,9622 | 32,0819 | 31,835 | 30,2926 | 33,1764 | 31,3954 | 29,0968 | 32,5951 | 99,03 | 41913000000 | 175 | | | P02792 | Ferritin light chain | FTL | | 0,7419 | -0,3164 | | 0,6060 | 0,7389 |
| 0 | 0 | 0 | 28,3733 | 26,8078 | 29,0879 | 28,5607 | 28,5458 | 29,8681 | 29,1904 | 26,5777 | 29,7436 | 141,85 | 3469600000 | 120 | | | P23526 | Adenosylhomocysteinase | AHCY | | 0,3244 | -0,9019 | | 0,6723 | 0,4876 |
| 0 | 0 | 0 | 17,0686 | 14,7363 | 16,9376 | 26,7407 | 27,4819 | 28,9781 | 19,0379 | 19,5685 | 18,3855 | 72 | 763740000 | 22 | | 202 | P27361 | Mitogen-activated protein kinase 3 | MAPK3 | + | 0,0003 | -11,4861 | + | 0,0003 | 8,7363 |
| 0 | 0 | 0 | 17,0877 | 14,7592 | 16,9578 | 27,5647 | 26,8714 | 29,4702 | 19,0546 | 19,5827 | 18,4041 | 108,6 | 938150000 | 36 | | 185 | P28482 | Mitogen-activated protein kinase 1 | MAPK1 | + | 0,0004 | -11,7005 | + | 0,0005 | 8,9550 |
| 0 | 0 | 0 | 26,7095 | 25,8364 | 26,6694 | 26,6223 | 26,8446 | 27,2741 | 26,8057 | 20,0977 | 27,2317 | 78 | 737970000 | 39 | | | P46736 | Lys-63-specific deubiquitinase BRCC36 | BRCC3 | | 0,2122 | -0,5086 | | 0,3960 | 2,2020 |
| 0 | 0 | 0 | 17,7791 | 15,5914 | 28,8413 | 19,118 | 18,6054 | 16,7006 | 28,7414 | 28,6264 | 29,4364 | 42,271 | 2499200000 | 33 | | | P54762 | Ephrin type-B receptor 1 | EPHB1 | | 0,5670 | 2,5959 | + | 0,0002 | -10,7934 |
| 0 | 0 | 0 | 27,2618 | 26,5128 | 26,7448 | 27,4675 | 27,2866 | 27,7028 | 27,2559 | 26,5225 | 27,4481 | 104,95 | 1007200000 | 45 | | | Q08257 | Quinone oxidoreductase | CRYZ | | 0,0625 | -0,6458 | | 0,2521 | 0,4101 |
| 0 | 0 | 0 | 27,7815 | 27,3513 | 26,2581 | 29,0431 | 18,6221 | 16,7221 | 19,6586 | 19,6531 | 19,0783 | 138 | 1080700000 | 38 | | | Q14789 | Golgin subfamily B member 1 | GOLGB1 | | 0,2156 | 5,6679 | | 0,6297 | 1,9991 |
| 0 | 0 | 0 | 27,5808 | 26,7419 | 27,6921 | 27,2728 | 27,8361 | 27,5278 | 27,3131 | 26,043 | 27,5728 | 102 | 1119100000 | 74 | | | Q15018 | BRISC complex subunit Abro1 | FAM175B | | 0,5764 | -0,2073 | | 0,3184 | 0,5693 |
| 0 | 0 | 0 | 26,1458 | 14,8729 | 26,5803 | 26,8141 | 26,4167 | 25,9196 | 26,8491 | 19,3301 | 26,9252 | 216,45 | 4658600000 | 56 | | | Q8IWJ2 | GRIP and coiled-coil domain-containing protein 2 | GCC2 | | 0,3728 | -3,8504 | | 0,4707 | 2,0153 |
| 0 | 0 | 0 | 24,237 | 24,2103 | 27,5071 | 19,1341 | 25,1885 | 27,5663 | 19,1371 | 19,5055 | 28,1566 | 36,003 | 740850000 | 30 | | | Q8WXD5 | Gem-associated protein 6 | GEMIN6 | | 0,6466 | 1,3552 | | 0,6838 | 1,6965 |
| 0 | 0 | 0 | 26,2472 | 14,3511 | 25,9704 | 26,8275 | 26,3291 | 26,5619 | 26,7433 | 19,6182 | 26,673 | 54 | 746550000 | 62 | | | Q92523 | Carnitine O-palmitoyltransferase 1, muscle isoform | CPT1B | | 0,3264 | -4,3833 | | 0,4000 | 2,2280 |
| 0 | 0 | 0 | 28,6026 | 26,6525 | 27,6935 | 28,3317 | 28,2627 | 28,2971 | 28,8453 | 26,8603 | 28,4433 | 66,573 | 1654900000 | 58 | | | Q9BV19 | Uncharacterized protein C1orf50 | C1orf50 | | 0,3147 | -0,6476 | | 0,7039 | 0,2476 |
| 0 | 0 | 0 | 27,413 | 29,8151 | 30,5763 | 27,065 | 30,3017 | 17,4991 | 18,7583 | 19,9518 | 29,4692 | 36 | 7929700000 | 31 | 78 | | Q9H0W7 | THAP domain-containing protein 2 | THAP2 | | 0,3373 | 4,3129 | | 0,6860 | 2,2288 |
| 0 | 0 | 0 | 27,5172 | 14,6345 | 27,4293 | 27,6484 | 27,9391 | 27,8262 | 27,7801 | 19,801 | 28,1995 | 42 | 1198800000 | 37 | | | Q9NPJ3 | Acyl-coenzyme A thioesterase 13;Acyl-coenzyme A thioesterase 13, N | ACOT13 | | 0,3420 | -4,6109 | | 0,4047 | 2,5444 |

**Table S2 (referred to as Table 4)- Detailed list of proteins immunoprecipited by the anti- ERK-Dhb antibody.** We report the log(2)LFQ (label-free intensity) of the proteins identified by high resolution mass spectrometry in the immunoprecipitation. Gray cells represent non-detected intensities (missing values). Only proteins non-detected in the in the pre-immune serum were selected. For statistical analysis, missing values were imputed using a gaussian distribution of values (width 0.3 and downshift of 6 SD of the distribution of the intensity of all the proteins of each sample). Identification parameters include the identification score, the total protein intensity, and the total number of peptides used for the identification (MS/MS count). Modified sites reports the aa position for the Dhb and phosphorylation sites identified for each protein. Statistical test reports the p-value and the difference of the averages for WT vs NS and for WT vs ΔospF.* The proteins listed in these columns were never identified in pre-immune serum. n1, n2 and n3 are three biological replicates.