

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Plasmid constructions

The full-length coding region of human HIF2 α or HIF1 α genes (gifts from Dr. Richard Bruick and Dr. Amato Giaccia, respectively) was PCR amplified in frame with a forward linker encoding HA tag downstream a NheI restriction site and a reverse linker with a NotI site downstream cDNA sequence (see supplemental table 3 for oligonucleotide sequences). The amplified PCR fragments and pCMV hygro 2xFLAG expression vector (gift from Dr. Pierre Lutz, IPBS, Toulouse) were digested by NheI and NotI restriction enzymes, purified and sub-cloned. Fidelity of PCR and identity of the constructs were confirmed by sequencing the inserts.

Reporter Gene Assays

501mel cells were co-transfected with 100 ng of pHRE-luc, a firefly luciferase reporter gene under control of three HRE binding sites (gift from Dr. Jacques Pouyssegur), 50 ng of a renilla luciferase construct (pCMV-RL, Promega France) and 100 ng of pCMV-FLAG/HA HIF α or 100 ng of pCMV-hygro 2xFLAG (pCMV-Flag) empty vector. After 48h of culture, firefly and renilla luciferase activities were assayed with the dual luciferase assay system (Promega France). Firefly luciferase activity was normalized to renilla luciferase activity to take into account transfection efficiency. Each assay was performed in duplicate in three independent experiments.

Immunofluorescence

Cells were grown on coverslips at 37°C for at least 16h and subsequently washed, fixed for 15 min in 3.7% paraformaldehyde, permeabilized (0.1% triton X100, 5 min), blocked and incubated for 2h with an HA antibody (supplemental Table 3) in PBS containing 1% BSA (PBS-BSA). Subsequently, coverslips were washed with PBS-BSA, incubated with FITC-conjugated secondary antibody in PBS-BSA for 1h at room temperature, washed again with PBS, incubated for 5 min with DAPI, air-dried

and embedded in Dako Cytomation Fluorescent Mounting Medium. Fluorescence was detected using a Leica microscope (Leitz DMRB; 100x objective). Images were acquired as sets of color-images and prepared using Photoshop software.

RNA extraction and RT-PCR

Total RNA was extracted using the RNeasy Extraction Kit (Qiagen). Each RNA sample was reverse transcribed with the Superscript III First Strand Synthesis Kit (Invitrogen) starting from 1 µg of total RNA. Quantitative real-time PCR was performed on an ABIPrism 7000 (Applied Biosystems) using β -actin, HPRT and YWHAZ as internal standards to control variability in cDNA quantity. Primer sequences are available on request. Each 20 µl reaction consisted of 2 µl of cDNA, 1x iQ SYBR Green Supermix (Bio-Rad) and 500 nM of primers. The amount of the target transcript was related to that of the reference genes by the $\Delta\Delta C_t$ method. All cDNA samples were assayed in triplicate and at least three independent experiments were carried out.

Supplemental table 1: Lists of the already identified partners of HIF subunits 1 α , 2 α and 1 β .

Protein lists have been obtained from two different internet facilities: biogrid (<http://thebiogrid.org/>) and Human Protein Reference Database (<http://www.hprd.org/>). Proteins validated in the current study according to our screening criteria are in bold in the last two columns of the table. HIF1 α , HIF2 α and HIF1 β : respectively first, second and third worksheet.

Supplemental table 2: Lists of peptide sequences identified and quantified in all gel bands analyzed. Worksheets A1 to A11 correspond to peptide data identification in the eleven fractions cut along the migration lines for experiment 1. For each fraction level, identification and quantification data (peptide sequence, theoretical mass, charge, measured m/z, retention time, Mascot score and measured XIC area) are given. Identical data are provided for the other analyses (worksheets B1 to B11 for experiment 2, C1 to C11 for experiment 3 and D1 to D11 for experiment 4).

Supplemental table 3: List of antibodies and oligonucleotides used in this study.

Supplemental Fig. 1: Flag/HA tagged HIF2 α exhibits normal sub-cellular localization and transactivation properties.

Full-length HIF2 α cDNA was cloned downstream to Flag and HA tags in a pCMV vector.

A- Plasmid map of pCMV-Flag/HA HIF2 α vector.

B- Transactivation of HRE-luciferase reporter gene by Flag/HA-HIF2 α . Correct functionality of the fusion protein was tested by transient co-transfection of 501mel cells with pCMV Flag/HA HIF2 α and a luciferase reporter gene under the control of HRE (Hypoxia Responsive Element). 501mel cells were transiently co-transfected with both pHRE-luc (firefly luciferase gene under control of 3 HRE) and pCMV-RL (renilla luciferase gene) vectors and with the pCMV-Flag/HA-HIF2 α vector or with the

empty plasmid (Mock). Cells were cultured for 48h under normoxia before luciferase assay. Firefly luciferase activities were normalized to renilla luciferase activities. Data are representative of three independent experiments undertaken in duplicate (means \pm SEM). Transient Flag/HA HIF2 α expression in 501mel cells strongly increased luciferase activity in comparison to empty vector-transfected cells.

C- Immunofluorescence analysis of HIF2 α localization. Nuclear localization of exogenous HIF2 α was checked by immunofluorescence using an anti-HA antibody. Cells were seeded on glass coverslips and transfected with pCMV-Flag/HA-HIF2 α vector (HIF2 α) or with the empty plasmid (Mock). Immunofluorescence experiments were performed 24h after transfection. Exogenous HIF2 α was labeled using an anti-HA antibody and a FITC-conjugated secondary antibody. Flag/HA HIF2 α was predominantly located in the cell nucleus as confirmed by DAPI staining.

D- Western-blot analysis of HIF proteins expression. Protein extracts prepared from untransfected 501mel or from 501mel cells stably transfected with pCMV-Flag/HA HIF2 α (H2) or with the empty vector (Mock) were probed by western blotting with the indicated antibodies. Actin was used as a loading control. Hypoxic conditions (H) were obtained by incubating cells with the hypoxia-mimetic CoCl₂. N: normoxic conditions. As expected, HIF2 α expression was enhanced in the presence of CoCl₂ but could also be observed in normoxia for clone H2.

Supplemental Fig. 2: Four way Venn diagrams comparing number of protein identified in each biological replicate.

A- Venn diagram showing the number of proteins identified in the four experiments.

B- Venn diagram showing the number of validated proteins in the four experiments according to screening criteria used in the current study.

Supplemental Fig. 3: Scatter plots comparing protein abundance in Mock and H2 assays.

Protein abundance index (PAI) was determined from label free quantification of anti-Flag immunoprecipitated proteins from Mock compared to H2 nuclear extracts. Four independent experiments were performed. Data from the log(protein amount in H2 sample) (y axis) are plotted *versus* log(protein amount in Mock sample) (x axis). Each diamond represents single protein identification. The red line defines a ratio of 1 (unchanged protein abundance in the two assays, H2 vs Mock). The green line, included in the insert of each experiment, indicates the applied threshold value *i.e.* a fold change of 4. Proteins located above the green line are considered to be HIF2 α specific partners. Green diamonds point out proteins only present in H2 assays. HIF2 α (EPAS1), MITF, SOX10 and AP2 α (TFAP2A) are indicated in red.

Supplemental Fig. 4: Schematic representation of two transcriptional complexes found as HIF2 α interactors.

A- The Mediator complex. Three modules can be defined in the structure of Mediator: head, middle and tail. The head presents variability (for MED27, MED28 and MED29). MED21, which is indicated by a red arrow, is the only subunit not identified in HIF2 α interactome. MED18 and MED20 (indicated by blue arrows) are labile subunits.

B- BRM/BRG1 complex. These two complexes are members of the SWI/SNF family. SMARCA2 (BRM) is encountered in the BRM complex whereas SMARCA4 (BRG1) is found in the BRG1 complex. SMARCD1 or SMARCD2 are present indifferently in the two complexes. All the proteins of these two complexes have been identified as HIF2 partners. SMARCA2: BRM; SMARCA4: BRG1.

Supplemental Fig. 5: Alignment of CBP and P300 protein sequences.

CBP (CREBBP) and P300 (EP300) protein sequences have been aligned. Stars indicate sequence identities. Bold blue sequences are MS/MS identified peptides exclusively found in CBP or P300

amino acid sequence. Bold red sequences are MS/MS identified peptides common to both CBP and P300.

Supplemental Fig. 6: Flag/HA tagged HIF1 α exhibits normal subcellular localization and transactivation properties.

The strategy to assess the presence of proteins in HIF1 α -containing protein complexes was similar to that used for HIF2 α .

A- Transactivation of HRE-luciferase reporter gene by the pCMV-Flag/HA-HIF1 α . 501mel cells were transiently co-transfected with both pHRE-luc (firefly luciferase) and pCMV-RL vectors with the pCMV Flag/HA HIF1 α vector or with the empty pCMV plasmid (Mock). Firefly luciferase activities were measured in normoxia conditions and normalized to renilla luciferase activities. Data are representative of three independent experiments undertaken in duplicate (means \pm SEM).

B- Immunofluorescence analysis of HIF1 α localization. Cells were seeded on glass coverslips and transfected with pCMV-Flag/HA-HIF1 α vector (HIF1 α) or with the empty plasmid (Mock). Immunofluorescence experiment was performed, 24h after transfection, using an anti-HA antibody.

C- Western blot analysis of HIF1 α protein expression. Protein extracts, prepared from untransfected 501mel or from 501mel cells stably transfected with pCMV Flag/HA HIF1 α (H1) or with the empty pCMV vector (Mock), were probed by western blotting with the indicated antibodies. Actin was used as a loading control. Hypoxic conditions (H) were obtained by incubating cells with the hypoxia mimetic CoCl₂. N: normoxic conditions.

Supplemental Fig. 7: HIF α and MITF gene expression following up or down regulation of HIF α subunits.

A- Reverse transcriptase (RT)-qPCR analysis of HIF1 α , HIF2 α and MITF expression following the indicated overexpressions or siRNA-mediated knockdowns. Mock is for the 501mel cell line stably

transfected with an empty vector, H1 and H2 are for cell lines overexpressing respectively HIF1 α and HIF2 α . Cells were treated (H) or not (N) with CoCl₂ 36h after siRNA transfection and cultured for 12 additional hours. siC, siH1, siH2 are for cell lines transiently transfected by respectively a non-targeting siRNA, siRNA targeting HIF1 α and HIF2 α mRNA. Results from cells cultured in normoxia or under hypoxia-mimicking conditions are respectively in dark grey or in light grey. The changes in expression of the indicated genes were expressed relatively to the control (Mock for overexpression and siC for knock-down).

B- Immunoblot of total extracts from cells following transfection of siRNAs against HIF α subunits or non-targeting siRNA as control, β -actin is used as loading control. Whatever the expression level of HIF α genes was (augmented in H1 and H2 clones and almost extinct in 501mel cells treated with siH1 or siH2), MITF expression was not affected, neither at mRNA (A) nor protein (B) level.

*: p value < 0.01; **: p value < 0.001.

Supplemental Fig. 8: Migration and invasion capacities of six human melanoma cell lines measured under normoxia.

A- Equal number of melanoma cells was subjected to migration assays. Total and migratory cell numbers were evaluated. The results were expressed as percentage of migrating cells.

B- Invasion assays were performed using Matrigel invasion chambers. Three sets of independent experiments were carried out, each in duplicate. Data are presented as mean \pm SEM (n = 3). SK28 is for SK-MEL-28 and Lu for Lu1205.

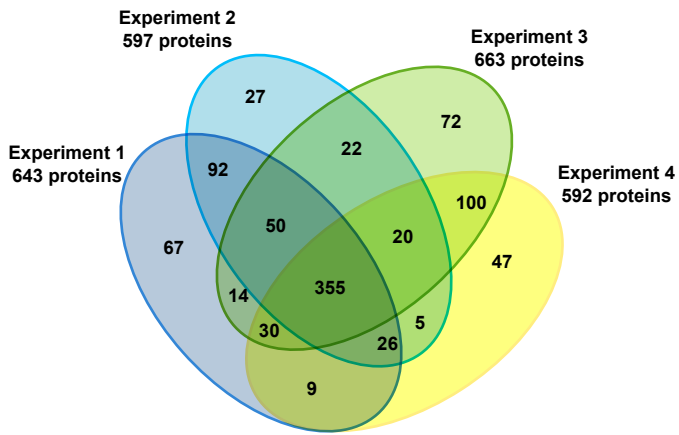
Supplemental Fig. 9: Annotated MS/MS spectra of single peptide-based protein identifications.

Supplemental table 3

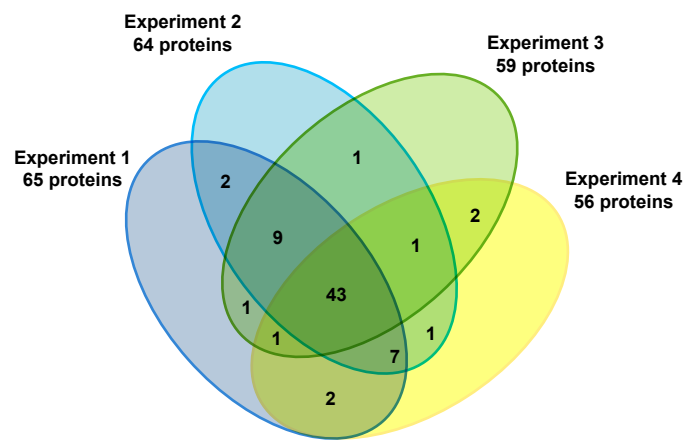
Antibodies				
Protein target	Furnishers	Product reference	IP concentration	WB or IP dilution
HIF1 α	Novus	NB100-134	2 μ g/ml	1/1000
HIF1 β	Santa Cruz Biotechnology	sc-5580		1/200
HIF2 α	Santa Cruz Biotechnology	sc-46691		1/200
HIF2 α	Novus	NB100-122	2 μ g/ml	
SOX10	Santa Cruz Biotechnology	sc-17342	2 μ g/ml	1/200
MITF	Santa Cruz Biotechnology	sc-56725		1/100
MITF	Santa Cruz Biotechnology	sc-10999X	2 μ g/ml	
Actin	Santa Cruz Biotechnology	sc-1616		1/200
HA	Covance	MMS-101P		1/1000
AP2 α	Santa Cruz Biotechnology	sc-12726X	2 μ g/ml	1/1000
Primers				
qRT-PCR oligonucleotides				
HIF2 α – Forward	5'	TGGCCGCTCAGCCTATGAAT	3'	
HIF2 α – Reverse	5'	TGGGTCTCCAGCCACACGTA	3'	
β actin - Forward	5'	TTCGCAAAGGCTATGCGC	3'	
β actin - Reverse	5'	GTTACTACCCAGGTCAGGCCAG	3'	
YWHAZ - Forward	5'	ACTTTTGGTACATTGTGGCTTCAA	3'	
YWHAZ - Reverse	5'	CCGCCAGGACAAACCAGTAT	3'	
Cloning oligonucleotides				
HIF2 α – Forward	5'	GATCGCTAGCT TACCCATACGATGTTCCAGATTACGCTT CTAGAATGACAGCTGAC	3'	
HIF2 α – Reverse	5'	CGCGCGGCCGCTCAGGTGGCCTGGTCCAGGGCTCT	3'	
HIF1 α – Forward	5'	GATCGCTAGCT TACCCATACGATGTTCCAGATTACGCTT CTAGCATGGAGGGCGCC	3'	
HIF1 α – Reverse	5'	CGCGCGGCCGCTCAGTTAACTTGATCCAAAGCTCT	3'	
<i>HA tag sequence is in bold</i>				

Supplemental figure 2

A

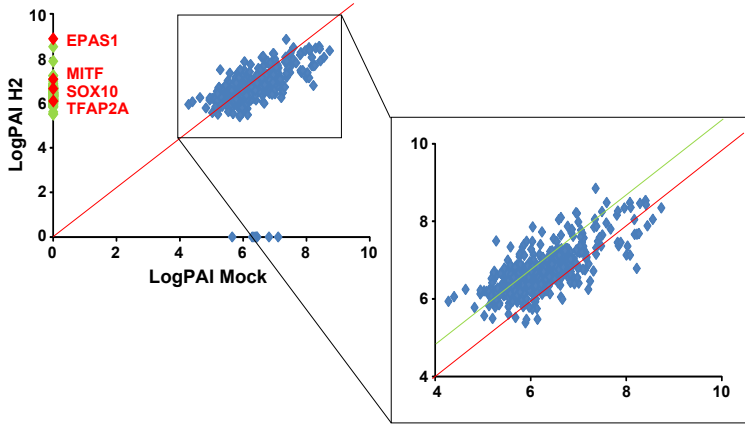


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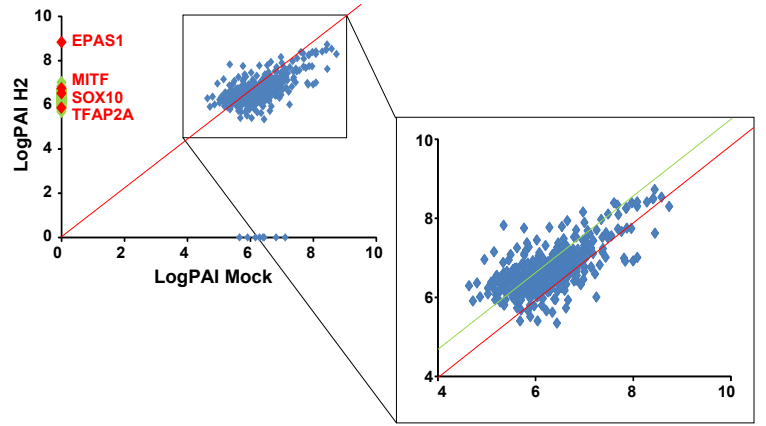


Supplemental figure 3

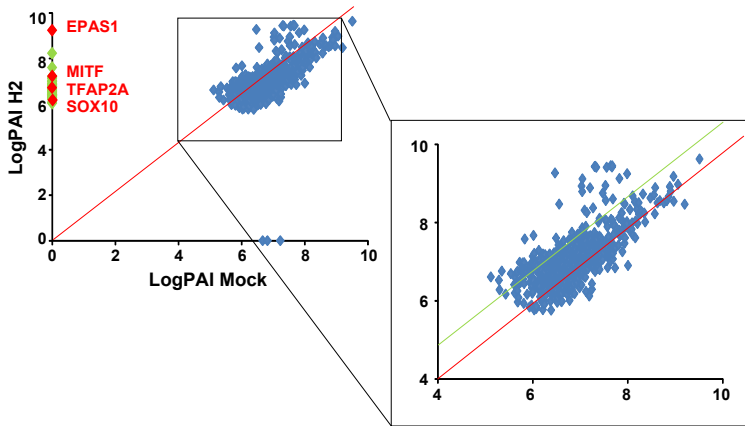
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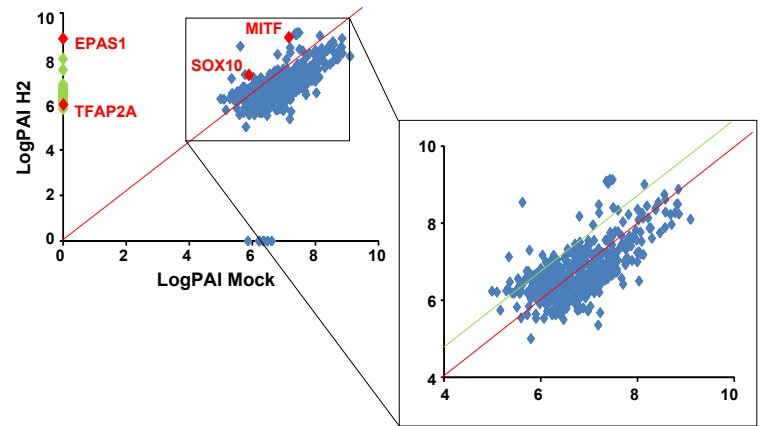
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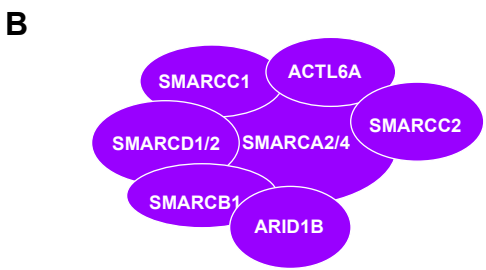
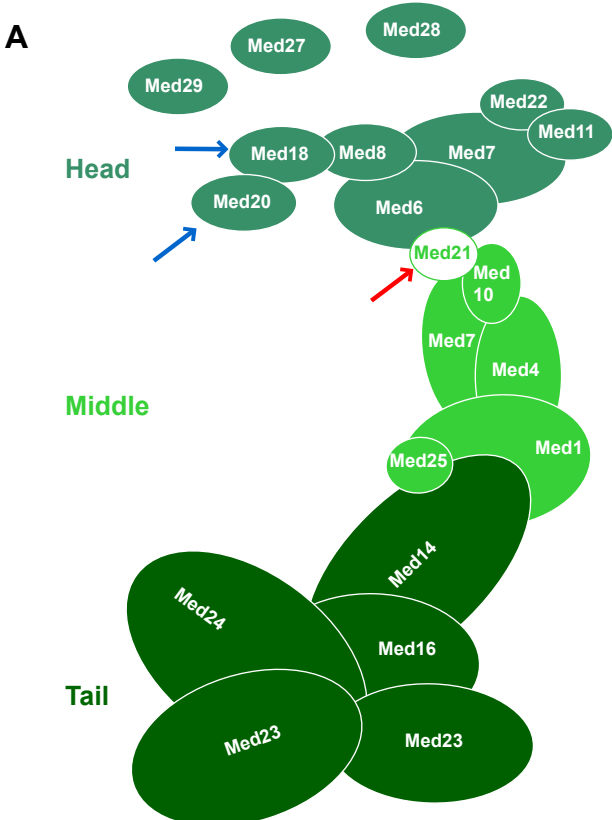
Experiment 3



Experiment 4



Supplemental figure 4



1205 EFSFQTLCCYGKQLCTIPRDAAYSYQNRHYHFCEKCFTEIQGENVTGLDDPSQPQTISK 1264 CREBBP

1169 EFSFQTLCCYGKQLCTIPR**DATYYSYQNR**HYHFCEKCFNEIQGESVSLGDDPSQPQTINK 1228 EP300
1265 DQFEKKNNDTLDPEFPVDCCEKGRKMHQICVLHYDIIWPSGFVCDNCLKKTGRPRKENKF 1324 CREBBP
* * * * *
1229 EQFSKRKNDTLDPELFEVCTEGRKMHQICVLHHEIIWPAGFVCDGCLKKSARTRKENKF 1288 EP300
1325 SAKRLQ**TRLGNHLEDR**VNKFLRRQNHPEAGEVFRVAVASSDK**TVEVKPGMK**SRFVDSGE 1384 CREBBP

1289 SAK**RLPSTR**LGTFLERNVDFLR**RQNHPESEGEVTVR**VVHASDK**TVEVKPGMK**ARFVDSGE 1348 EP300
1385 MSESFPYRTKALFAFEEIDGVDCFFGMHVQEYSGDCPPNTRRVYISYLDSEIHFRRPC 1444 CREBBP
* * * * *
1349 MAESFPYRTKALFAFEEIDGVDCFFGMHVQEYSGDCPPNQRVYISYLDSEVHFRPKC 1408 EP300
1445 LRTAVYHEILIGYLEYVKKLGYVTGHIWACPPSEGDDYIFHCHPPDQKIPKPKRLQEWYK 1504 CREBBP

1409 LRTAVYHEILIGYLEYVKKLGYVTGHIWACPPSEGDDYIFHCHPPDQKIPKPKRLQEWYK 1468 EP300
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1469 KMLDKAVSER**IVHDYKDIKQ**ATEDRLTSAKELPYFEGDFWPNVLEESIK**ELEQEEERK** 1528 EP300
1565 KEESTAASETTEGSQDGSNAKKNKTKNKNKSSISRANKKPSMPNVSNDLSQKLYAT 1624 CREBBP
* * * * *
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1588 MEKHKEVFFVIRLIAGPAANSLPPIVDPDPLPCDLMGRDAFLTLARDKHLEFSSLRRA 1647
1685 KWSTLCMLVELHTQGQDRFVYTCNECKHHVETRWHCTVCEDYDLCINCYNTKSHAHKMVK 1744 CREBBP
* * * * *
1648 QWSTMCMLVELHTQSQDRFVYTCNECKHHVETRWHCTVCEDYDLCITCYNTK**NHDHMEK** 1707 EP300
1745 WGLGLDDESSQGEPPQSKSPQESRRLSIQRCIQSLVHACQCR**NANCSLPCQK**MKRVVQH 1804 CREBBP

1708 LGLGLDDESNNQAAATQSPGDSRRLSIQRCIQSLVHACQCR**NANCSLPCQK**MKRVVQH 1767 EP300
1805 TKGCKRKTNGGCPVCKQLIALCCYHAKHCQENKCPVPFCLNIKHLRQQQIQHR**LQQAQL** 1864 CREBBP

1768 TKGCKRKTNGGCPICKQLIALCCYHAKHCQENKCPVPFCLNIKQLRQQQLQHR**LQQAQM** 1827 EP300
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1828 **LR**RRMASMQRTGVVQQQLPSPTPATPTPTPTGQQPTTPQTPPTSQPQTPPNSMP 1884 EP300
1922 PSVARTQPPTTVSTGKPTSQV PAPPPPAQPAAVEAARQIER**EAQQQOHL**YR 1974 CREBBP
* * * * *
1885 PYLPR**TQAAGPVSQK**AAAGVTPPTPPQTAQPPLPGPPAAVEMAMQIQRAAETQRQMAH 1944 EP300
1975 VNINNSMPPGRTGMGTGPGSQMAPVSLNVPRPNQV SGPVMPSPMPGQW QQ APL 2026 CREBBP
* * * * *
1945 VQIFQR PIQHQMPPM TPMAPGMNPPMTRGSPGHLEP GMGPTGMQQPPWSQGG 2000 EP300
2027 PQ QQPMPGLPRPVISMQAQ AAVAGPRMPSVQP PRSISPSALQDLLRRTLKS 2077 CREBBP
* * * * *
2001 PQPQQQLQSGMPRPAMMSVAHQGPLNMAPQPGLGQVGISPLKPGTVSQQALQNLRLRLS 2060 EP300
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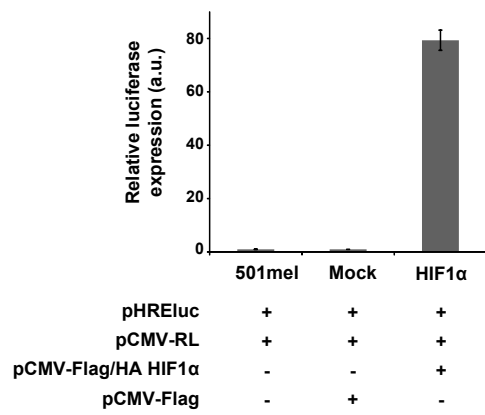
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* * * * *
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2183 ASMNPOYREMLRRQLLQQQQQQQQQQQQQSSAGMAGGMAGHGQFQQPQPGGYPP 2242 CREBBP
* * * * *
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* * * * *
2271 GSPVQPNPMSPPQHMLPNQAQSPHLQGGQIPNSLSNQVRSQPVPSPRPQSQPPHS 2325 EP300
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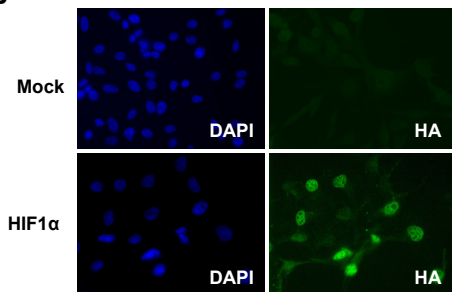
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2421 **SSELSLVGDTTGD**TLEKFEVGL 2442 CREBBP
* * * * *
2386 HGASATDLGLSTDNSDLNSNLSQSTLDIH 2414 EP300

Supplemental figure 6

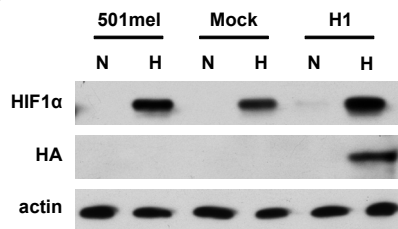
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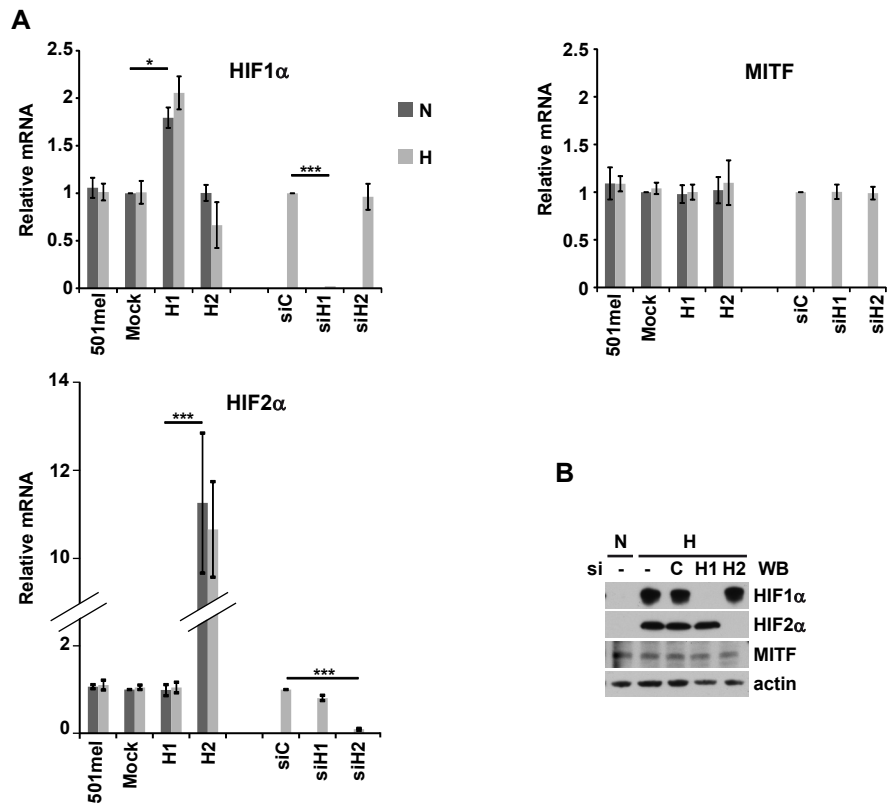
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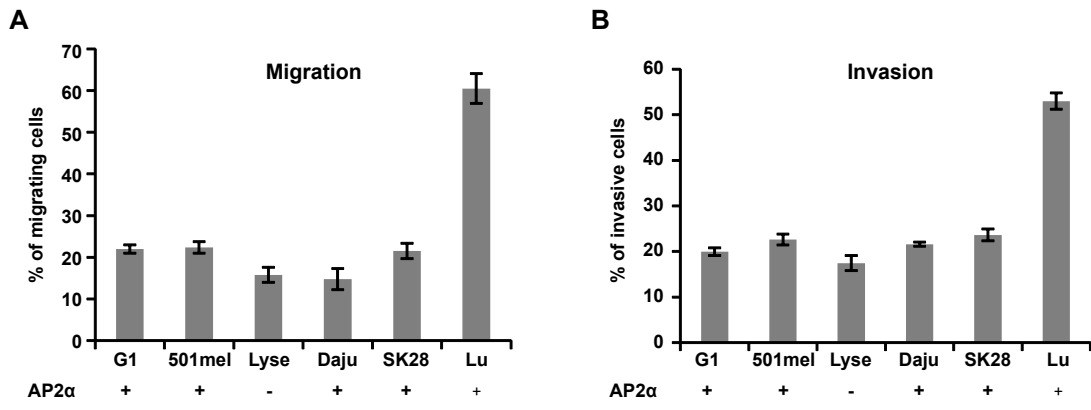
C



Supplemental figure 7



Supplemental figure 8



Peptide View

MS/MS Fragmentation of **AYLDQTVVPILLOGLAVLAK**

Found in **IPI00028109**, Tax_Id=9606 Gene_Symbol=DPY30 Protein dpy-30 homolog

Experiment: FDR1% HIF2 2eme 091227_A3 **Fraction:**

Match to Query 3219: 2124.259448 from(1063.137000,2+)

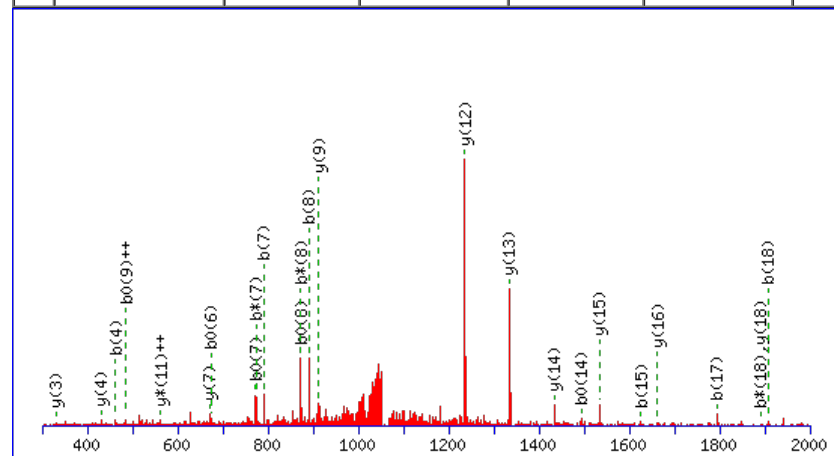
Title: OTMMD091227_24.6750.6750.2.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip Masse2 Hif 2 091227\OTMMD091227_24.RAW

Monoisotopic mass of neutral peptide Mr(calc): 2124.255814 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 46

Expect: 8.1e-005 **Matches :** 25/186 fragment ions using 51 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ⁺⁺⁺	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ⁺⁺⁺	y ⁰	y ⁰⁺⁺	#
1	72.044390	36.525833					A							20
2	235.107719	118.057498					Y	2054.225993	1027.616634	2037.199444	1019.103360	2036.215428	1018.611352	19
3	348.191783	174.599529					L	1891.162664	946.084970	1874.136115	937.571696	1873.152099	937.079687	18
4	463.218726	232.113001			445.208161	223.107719	D	1778.078600	889.542938	1761.052051	881.029664	1760.068035	880.537656	17
5	591.277304	296.142290	574.250755	287.629016	573.266739	287.137008	Q	1663.051657	832.029467	1646.025108	823.516192	1645.041092	823.024184	16
6	692.324983	346.666130	675.298434	338.152855	674.314418	337.660847	T	1534.993079	768.000178	1517.966530	759.486903	1516.982514	758.994895	15
7	791.393397	396.200337	774.366848	387.687062	773.382832	387.195054	V	1433.945400	717.476338	1416.918851	708.963063			14
8	890.461811	445.734544	873.435262	437.221269	872.451246	436.729261	V	1334.876986	667.942131	1317.850437	659.428857			13
9	987.514575	494.260926	970.488026	485.747651	969.504010	485.255643	P	1235.808572	618.407924	1218.782023	609.894650			12
10	1100.598639	550.802958	1083.572090	542.289683	1082.588074	541.797675	I	1138.755808	569.881542	1121.729259	561.368268			11
11	1213.682703	607.344990	1196.656154	598.831715	1195.672138	598.339707	L	1025.671744	513.339510	1008.645195	504.826236			10
12	1326.766767	663.887021	1309.740218	655.373747	1308.756202	654.881739	L	912.587680	456.797478	895.561131	448.284204			9
13	1454.825345	727.916311	1437.798796	719.403036	1436.814780	718.911028	Q	799.503616	400.255446	782.477067	391.742172			8
14	1511.846809	756.427042	1494.820260	747.913768	1493.836244	747.421760	G	671.445038	336.226157	654.418489	327.712883			7
15	1624.930873	812.969074	1607.904324	804.455800	1606.920308	803.963792	L	614.423574	307.715425	597.397025	299.202151			6
16	1695.967987	848.487631	1678.941438	839.974357	1677.957422	839.482349	A	501.339510	251.173393	484.312961	242.660119			5
17	1795.036401	898.021838	1778.009852	889.508564	1777.025836	889.016556	V	430.302396	215.654836	413.275847	207.141562			4
18	1908.120465	954.563870	1891.093916	946.050596	1890.109900	945.558588	L	331.233982	166.120629	314.207433	157.607355			3
19	1979.157579	990.082427	1962.131030	981.569153	1961.147014	981.077145	A	218.149918	109.578597	201.123369	101.065323			2
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Peptide View

MS/MS Fragmentation of **LNFIVTGLQIDK**

Found in **IPI00030481**, Tax_Id=9606 Gene_Symbol=MED10 Mediator of RNA polymerase II transcription subunit 10

Experiment: FDR1% HIF2 3ème 100401_A3 **Fraction:**

Match to Query 3428: 1474.804448 from(738.409500,2+)

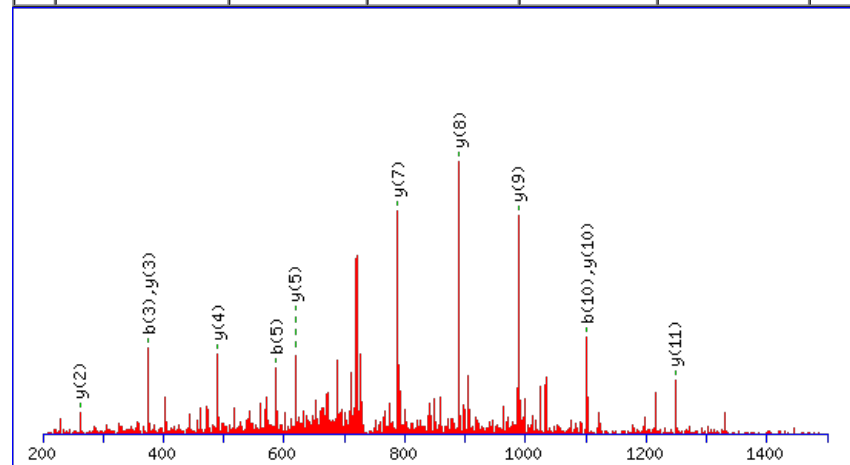
Title: OTMMD100401_26.5554.5554.2.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip Masse3 Hif2 100401\OTMMD100401_26.RAW

Monoisotopic mass of neutral peptide Mr(calc): 1474.803146 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 78

Expect: 8.3e-007 **Matches :** 12/130 fragment ions using 13 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{*++}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{*++}	y ⁰	y ⁰⁺⁺	#
1	114.091340	57.549308					L							13
2	228.134267	114.570771	211.107718	106.057497			N	1362.726358	681.866817	1345.699809	673.353543	1344.715793	672.861535	12
3	375.202681	188.104978	358.176132	179.591704			F	1248.683431	624.845354	1231.656882	616.332079	1230.672866	615.840071	11
4	488.286745	244.647010	471.260196	236.133736			I	1101.615017	551.311147	1084.588468	542.797872	1083.604452	542.305864	10
5	587.355159	294.181218	570.328610	285.667943			V	988.530953	494.769115	971.504404	486.255840	970.520388	485.763832	9
6	688.402838	344.705057	671.376289	336.191783	670.392273	335.699775	T	889.462539	445.234908	872.435990	436.721633	871.451974	436.229625	8
7	745.424302	373.215789	728.397753	364.702515	727.413737	364.210507	G	788.414860	394.711068	771.388311	386.197794	770.404295	385.705786	7
8	858.508366	429.757821	841.481817	421.244547	840.497801	420.752539	L	731.393396	366.200336	714.366847	357.687062	713.382831	357.195054	6
9	986.566944	493.787110	969.540395	485.273836	968.556379	484.781828	Q	618.309332	309.658304	601.282783	301.145030	600.298767	300.653022	5
10	1101.593887	551.300582	1084.567338	542.787307	1083.583322	542.295299	D	490.250754	245.629015	473.224205	237.115741	472.240189	236.623733	4
11	1214.677951	607.842614	1197.651402	599.329339	1196.667386	598.837331	I	375.223811	188.115543	358.197262	179.602269	357.213246	179.110261	3
12	1329.704894	665.356085	1312.678345	656.842811	1311.694329	656.350803	D	262.139747	131.573512	245.113198	123.060237	244.129182	122.568229	2
13							K	147.112804	74.060040	130.086255	65.546765			1



Peptide View

MS/MS Fragmentation of **EIGAILQNAGTVILELSK**

Found in **IP100000305**, Tax_Id=9606 Gene_Symbol=MED11 Mediator of RNA polymerase II transcription subunit 11

Experiment: FDR1% HIF2 3ème 100401_A3 **Fraction:**

Match to Query 4201: 1868.066048 from(935.040300,2+)

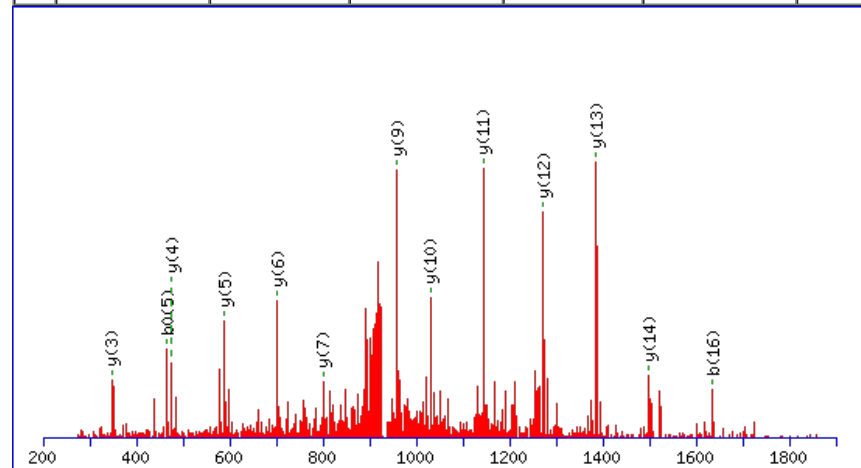
Title: OTMMD100401_26.6977.6977.2.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip Masse3 Hif2 100401\OTMMD100401_26.RAW

Monoisotopic mass of neutral peptide Mr(calc): 1868.061844 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 89

Expect: 2.2e-008 **Matches :** 13/190 fragment ions using 16 most intense peaks ([help](#))

#	b	b ⁺⁺	b*	b ⁺⁺⁺	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y*	y ⁺⁺⁺	y ⁰	y ⁰⁺⁺	#
1	130.049869	65.528572			112.039304	56.523290	E							18
2	243.133933	122.070605			225.123368	113.065322	I	1740.026563	870.516920	1723.000014	862.003645	1722.015998	861.511637	17
3	300.155397	150.581336			282.144832	141.576054	G	1626.942499	813.974888	1609.915950	805.461613	1608.931934	804.969605	16
4	371.192511	186.099894			353.181946	177.094611	A	1569.921035	785.464156	1552.894486	776.950881	1551.910470	776.458873	15
5	484.276575	242.641926			466.266010	233.636643	I	1498.883921	749.945598	1481.857372	741.432324	1480.873356	740.940316	14
6	597.360639	299.183958			579.350074	290.178675	L	1385.799857	693.403567	1368.773308	684.890292	1367.789292	684.398284	13
7	725.419217	363.213247	708.392668	354.699972	707.408652	354.207964	Q	1272.715793	636.861535	1255.689244	628.348260	1254.705228	627.856252	12
8	839.462144	420.234710	822.435595	411.721436	821.451579	411.229428	N	1144.657215	572.832246	1127.630666	564.318971	1126.646650	563.826963	11
9	910.499258	455.753267	893.472709	447.239993	892.488693	446.747985	A	1030.614288	515.810782	1013.587739	507.297507	1012.603723	506.805500	10
10	967.520722	484.263999	950.494173	475.750725	949.510157	475.258717	G	959.577174	480.292225	942.550625	471.778951	941.566609	471.286943	9
11	1068.568401	534.787839	1051.541852	526.274564	1050.557836	525.782556	T	902.555710	451.781493	885.529161	443.268218	884.545145	442.776210	8
12	1167.636815	584.322046	1150.610266	575.808771	1149.626250	575.316763	V	801.508031	401.257654	784.481482	392.744379	783.497466	392.252371	7
13	1280.720879	640.864078	1263.694330	632.350803	1262.710314	631.858795	I	702.439617	351.723447	685.413068	343.210172	684.429052	342.718164	6
14	1393.804943	697.406109	1376.778394	688.892835	1375.794378	688.400827	L	589.355553	295.181415	572.329004	286.668140	571.344988	286.176132	5
15	1522.847536	761.927406	1505.820987	753.414132	1504.836971	752.922124	E	476.271489	238.639383	459.244940	230.126108	458.260924	229.634100	4
16	1635.931600	818.469438	1618.905051	809.956164	1617.921035	809.464155	L	347.228896	174.118086	330.202347	165.604811	329.218331	165.112803	3
17	1722.963628	861.985452	1705.937079	853.472178	1704.953063	852.980169	S	234.144832	117.576054	217.118283	109.062780	216.134267	108.570772	2
18							K	147.112804	74.060040	130.086255	65.546765			1



Peptide View

MS/MS Fragmentation of **LHHQNQQQIQQQQQQLOR**
 Found in **IPI00879996**, Tax_Id=9606 Gene_Symbol=MED15 Protein
Experiment: FDR1% HIF2 3èmeBis 100419_A3 **Fraction:**

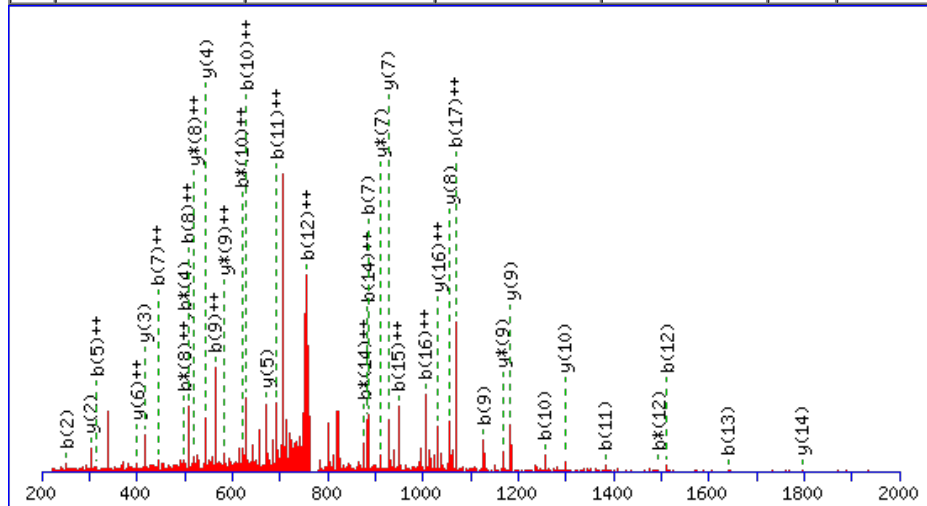
Match to Query 3635: 2310.174072 from(771.065300,3+)

Title: OTMMD100419_18.1409.1409.3.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip masse3 injection2 HIF2 100419\OTMMD100419_18.RAW

Monoisotopic mass of neutral peptide Mr(calc): 2310.168961 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 43 **Expect:** 0.0029 **Matches :** 38/130 fragment ions using 79 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{***}	Seq.	y	y ⁺⁺	y [*]	y ^{***}	#
1	114.091340	57.549308			L					18
2	251.150252	126.078764			H	2198.092189	1099.549732	2181.065640	1091.036458	17
3	388.209164	194.608220			H	2061.033277	1031.020276	2044.006728	1022.507002	16
4	516.267742	258.637509	499.241193	250.124235	Q	1923.974365	962.490821	1906.947816	953.977546	15
5	630.310669	315.658973	613.284120	307.145698	N	1795.915787	898.461532	1778.889238	889.948257	14
6	758.369247	379.688262	741.342698	371.174987	Q	1681.872860	841.440068	1664.846311	832.926794	13
7	886.427825	443.717551	869.401276	435.204276	Q	1553.814282	777.410779	1536.787733	768.897505	12
8	1014.486403	507.746840	997.459854	499.233565	Q	1425.755704	713.381490	1408.729155	704.868216	11
9	1127.570467	564.288872	1110.543918	555.775597	I	1297.697126	649.352201	1280.670577	640.838927	10
10	1255.629045	628.318160	1238.602496	619.804886	Q	1184.613062	592.810169	1167.586513	584.296895	9
11	1383.687623	692.347450	1366.661074	683.834175	Q	1056.554484	528.780880	1039.527935	520.267606	8
12	1511.746201	756.376739	1494.719652	747.863464	Q	928.495906	464.751591	911.469357	456.238317	7
13	1639.804779	820.406028	1622.778230	811.892753	Q	800.437328	400.722302	783.410779	392.209028	6
14	1767.863357	884.435317	1750.836808	875.922042	Q	672.378750	336.693013	655.352201	328.179739	5
15	1895.921935	948.464606	1878.895386	939.951331	Q	544.320172	272.663724	527.293623	264.150449	4
16	2009.005999	1005.006638	1991.979450	996.493363	L	416.261594	208.634435	399.235045	200.121160	3
17	2137.064577	1069.035927	2120.038028	1060.522652	Q	303.177530	152.092403	286.150981	143.579128	2
18					R	175.118952	88.063114	158.092403	79.549839	1



Peptide View

MS/MS Fragmentation of **LAHECLSQSCDSA**K

Found in **IPI00032780**, Tax_Id=9606 Gene_Symbol=MED29 cDNA FLJ54860, highly similar to Homo sapiens intersex-lik (IXL), mRNA

Experiment: FDR1% HIF2 3ème 100401_A3 **Fraction:**

Match to Query 4133: 1604.695448 from(803.355000,2+)

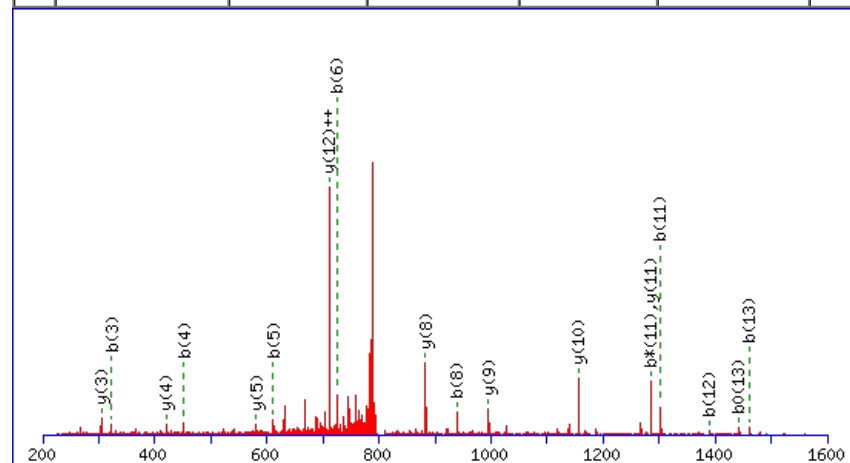
Title: OTMMD100401_25.1290.1290.2.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip Masse3 Hif2 100401\OTMMD100401_25.RAW

Monoisotopic mass of neutral peptide Mr(calc): 1604.692276 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 54

Expect: 8.1e-005 **Matches :** 18/132 fragment ions using 27 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{*++}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{*++}	y ⁰	y ⁰⁺⁺	#
1	114.091340	57.549308					L							14
2	185.128454	93.067865					A	1492.615504	746.811390	1475.588955	738.298116	1474.604939	737.806108	13
3	322.187366	161.597321					H	1421.578390	711.292833	1404.551841	702.779559	1403.567825	702.287551	12
4	451.229959	226.118617			433.219394	217.113335	E	1284.519478	642.763377	1267.492929	634.250103	1266.508913	633.758095	11
5	611.260608	306.133942			593.250043	297.128660	C	1155.476885	578.242081	1138.450336	569.728806	1137.466320	569.236798	10
6	724.344672	362.675974			706.334107	353.670692	L	995.446236	498.226756	978.419687	489.713482	977.435671	489.221474	9
7	811.376700	406.191988			793.366135	397.186706	S	882.362172	441.684724	865.335623	433.171450	864.351607	432.679442	8
8	939.435278	470.221277	922.408729	461.708003	921.424713	461.215995	Q	795.330144	398.168710	778.303595	389.655436	777.319579	389.163428	7
9	1026.467306	513.737291	1009.440757	505.224017	1008.456741	504.732009	S	667.271566	334.139421	650.245017	325.626147	649.261001	325.134139	6
10	1186.497955	593.752616	1169.471406	585.239341	1168.487390	584.747333	C	580.239538	290.623407	563.212989	282.110133	562.228973	281.618125	5
11	1301.524898	651.266087	1284.498349	642.752813	1283.514333	642.260805	D	420.208889	210.608083	403.182340	202.094808	402.198324	201.602800	4
12	1388.556926	694.782101	1371.530377	686.268827	1370.546361	685.776819	S	305.181946	153.094611	288.155397	144.581337	287.171381	144.089329	3
13	1459.594040	730.300658	1442.567491	721.787384	1441.583475	721.295376	A	218.149918	109.578597	201.123369	101.065322			2
14							K	147.112804	74.060040	130.086255	65.546765			1



Peptide View

MS/MS Fragmentation of **YQEQLVQEQELAK**

Found in **IPI00984935**, Tax_Id=9606 Gene_Symbol=NCAPD3 Uncharacterized protein

Experiment: FDR1% HIF2 2ème 091227_A3bis **Fraction:**

Match to Query 2547: 1604.805448 from(803.410000,2+)

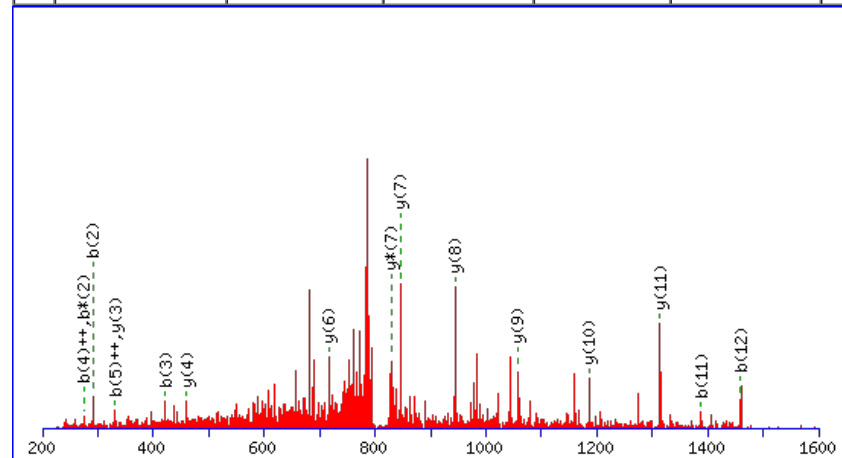
Title: OTMMD091227_29.2432.2432.2.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip Masse2 Hif 2 091227\OTMMD091227_29.RAW

Monoisotopic mass of neutral peptide Mr(calc): 1604.804565 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 46

Expect: 0.0022 **Matches :** 16/132 fragment ions using 28 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{***}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{***}	y ⁰	y ⁰⁺⁺	#
1	164.070605	82.538940					Y							13
2	292.129183	146.568230	275.102634	138.054955			Q	1442.748551	721.877913	1425.722002	713.364639	1424.737986	712.872631	12
3	421.171776	211.089526	404.145227	202.576252	403.161211	202.084244	E	1314.689973	657.848625	1297.663424	649.335350	1296.679408	648.843342	11
4	549.230354	275.118815	532.203805	266.605541	531.219789	266.113533	Q	1185.647380	593.327328	1168.620831	584.814054	1167.636815	584.322045	10
5	662.314418	331.660847	645.287869	323.147573	644.303853	322.655565	L	1057.588802	529.298039	1040.562253	520.784765	1039.578237	520.292757	9
6	761.382832	381.195054	744.356283	372.681780	743.372267	372.189772	V	944.504738	472.756007	927.478189	464.242732	926.494173	463.750724	8
7	889.441410	445.224343	872.414861	436.711069	871.430845	436.219061	Q	845.436324	423.221800	828.409775	414.708526	827.425759	414.216518	7
8	1018.484003	509.745640	1001.457454	501.232365	1000.473438	500.740357	E	717.377746	359.192511	700.351197	350.679237	699.367181	350.187229	6
9	1146.542581	573.774928	1129.516032	565.261654	1128.532016	564.769646	Q	588.335153	294.671215	571.308604	286.157940	570.324588	285.665932	5
10	1275.585174	638.296225	1258.558625	629.782951	1257.574609	629.290943	E	460.276575	230.641925	443.250026	222.128651	442.266010	221.636643	4
11	1388.669238	694.838257	1371.642689	686.324983	1370.658673	685.832974	L	331.233982	166.120629	314.207433	157.607354			3
12	1459.706352	730.356814	1442.679803	721.843540	1441.695787	721.351531	A	218.149918	109.578597	201.123369	101.065322			2
13							K	147.112804	74.060040	130.086255	65.546765			1



Peptide View

MS/MS Fragmentation of **NSPNNISGISNPPGTPR**

Found in **IPI00157304**, Tax_Id=9606 Gene_Symbol=SSBP3 Isoform 1 of Single-stranded DNA-binding protein 3

Experiment: FDR1% HIF2 2ème 091227_A3bis **Fraction:**

Match to Query 2755: 1720.849448 from(861.432000,2+)

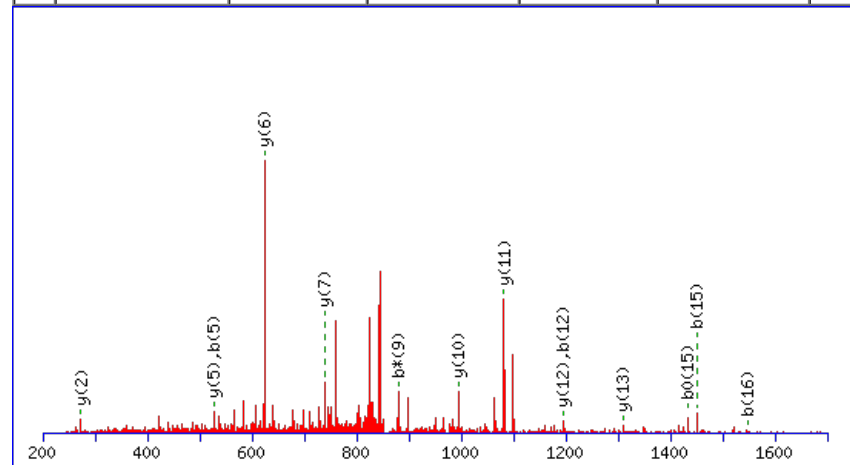
Title: OTMMD091227_35.1838.1838.2.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip Masse2 Hif 2 091227\OTMMD091227_35.RAW

Monoisotopic mass of neutral peptide Mr(calc): 1720.849243 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 66

Expect: 1.8e-005 **Matches :** 14/186 fragment ions using 15 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{*++}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{*++}	y ⁰	y ⁰⁺⁺	#
1	115.050203	58.028740	98.023654	49.515465			N							17
2	202.082231	101.544753	185.055682	93.031479	184.071666	92.539471	S	1607.813608	804.410442	1590.787059	795.897168	1589.803043	795.405160	16
3	299.134995	150.071135	282.108446	141.557861	281.124430	141.065853	P	1520.781580	760.894428	1503.755031	752.381154	1502.771015	751.889146	15
4	413.177922	207.092599	396.151373	198.579324	395.167357	198.087316	N	1423.728816	712.368046	1406.702267	703.854772	1405.718251	703.362764	14
5	527.220849	264.114063	510.194300	255.600788	509.210284	255.108780	N	1309.685889	655.346583	1292.659340	646.833308	1291.675324	646.341300	13
6	640.304913	320.656095	623.278364	312.142820	622.294348	311.650812	I	1195.642962	598.325119	1178.616413	589.811845	1177.632397	589.319837	12
7	727.336941	364.172109	710.310392	355.658834	709.326376	355.166826	S	1082.558898	541.783087	1065.532349	533.269813	1064.548333	532.777805	11
8	784.358405	392.682841	767.331856	384.169566	766.347840	383.677558	G	995.526870	498.267073	978.500321	489.753799	977.516305	489.261791	10
9	897.442469	449.224873	880.415920	440.711598	879.431904	440.219590	I	938.505406	469.756341	921.478857	461.243067	920.494841	460.751059	9
10	984.474497	492.740887	967.447948	484.227612	966.463932	483.735604	S	825.421342	413.214309	808.394793	404.701035	807.410777	404.209027	8
11	1098.517424	549.762350	1081.490875	541.249076	1080.506859	540.757068	N	738.389314	369.698295	721.362765	361.185021	720.378749	360.693013	7
12	1195.570188	598.288732	1178.543639	589.775458	1177.559623	589.283450	P	624.346387	312.676832	607.319838	304.163557	606.335822	303.671549	6
13	1292.622952	646.815114	1275.596403	638.301840	1274.612387	637.809832	P	527.293623	264.150450	510.267074	255.637175	509.283058	255.145167	5
14	1349.644416	675.325846	1332.617867	666.812572	1331.633851	666.320564	G	430.240859	215.624067	413.214310	207.110793	412.230294	206.618785	4
15	1450.692095	725.849686	1433.665546	717.336411	1432.681530	716.844403	T	373.219395	187.113335	356.192846	178.600061	355.208830	178.108053	3
16	1547.744859	774.376068	1530.718310	765.862793	1529.734294	765.370785	P	272.171716	136.589496	255.145167	128.076221			2
17							R	175.118952	88.063114	158.092403	79.549839			1



Peptide View

MS/MS Fragmentation of **RPAAAAAAGSASPR**

Found in **IPI00102997**, Tax_Id=9606 Gene_Symbol=WRNIP1 Isoform 2 of ATPase WRNIP1

Experiment: FDR1% HIF2 2ème 091227_A3 **Fraction:**

Match to Query 1755: 1252.662648 from(627.338600,2+)

Title: OTMMD091227_18.640.640.2.dta

Data file \\tol-brandir\Masse\Data LTQ-Orbitrap\Manuelle Ducoux\Manip Masse2 Hif 2 091227\OTMMD091227_18.RAW

Monoisotopic mass of neutral peptide Mr(calc): 1252.663589 **Fixed modifications:** Carbamidomethyl (C) (apply to specified residues or termini only) **Ions Score:** 42

Expect: 0.0037 **Matches :** 26/134 fragment ions using 55 most intense peaks ([help](#))

#	b	b ⁺⁺	b [*]	b ^{***}	b ⁰	b ⁰⁺⁺	Seq.	y	y ⁺⁺	y [*]	y ^{***}	y ⁰	y ⁰⁺⁺	#
1	157.108387	79.057831	140.081838	70.544557			R							14
2	254.161151	127.584213	237.134602	119.070939			P	1097.569798	549.288537	1080.543249	540.775263	1079.559233	540.283255	13
3	325.198265	163.102770	308.171716	154.589496			A	1000.517034	500.762155	983.490485	492.248880	982.506469	491.756872	12
4	396.235379	198.621328	379.208830	190.108053			A	929.479920	465.243598	912.453371	456.730324	911.469355	456.238316	11
5	467.272493	234.139884	450.245944	225.626610			A	858.442806	429.725041	841.416257	421.211766	840.432241	420.719758	10
6	538.309607	269.658442	521.283058	261.145167			A	787.405692	394.206484	770.379143	385.693209	769.395127	385.201201	9
7	609.346721	305.176999	592.320172	296.663724			A	716.368578	358.687927	699.342029	350.174652	698.358013	349.682644	8
8	680.383835	340.695556	663.357286	332.182281			A	645.331464	323.169370	628.304915	314.656095	627.320899	314.164087	7
9	737.405299	369.206288	720.378750	360.693013			G	574.294350	287.650813	557.267801	279.137539	556.283785	278.645531	6
10	824.437327	412.722302	807.410778	404.209027	806.426762	403.717019	S	517.272886	259.140081	500.246337	250.626806	499.262321	250.134798	5
11	895.474441	448.240859	878.447892	439.727584	877.463876	439.235576	A	430.240858	215.624067	413.214309	207.110792	412.230293	206.618784	4
12	982.506469	491.756872	965.479920	483.243598	964.495904	482.751590	S	359.203744	180.105510	342.177195	171.592235	341.193179	171.100227	3
13	1079.559233	540.283255	1062.532684	531.769980	1061.548668	531.277972	P	272.171716	136.589496	255.145167	128.076221			2
14							R	175.118952	88.063114	158.092403	79.549839			1

