Supplemental Materials

Molecular Biology of the Cell

Kasioulis et al.

Supplemental Figure 1: Nuclear and cytoplasmic distribution of Tagged Kdm3a isoforms
(A) Summary diagram illustrating the position of GFP and RFP tags for each construct. FL: full length, i2: newly identified isoform 2. (B) Full length Kdm3a can be exclusively nuclear (Nu) or both nuclear-cytoplasmic (Nu/Cyt). GFP tagged isoform 2 is mostly nuclear and cytoplasmic. Few cells present strong nuclear staining (arrow). The subcellular distribution of Kdm3a isoforms is not affected by the position of the Tag (not shown). (C) The relative abundance of overexpressed Kdm3a isoforms in each subcellular compartment was quantified by a script that would automatically segment the nuclear area of a given cell based on its DAPI staining followed by quantification of H3K9me2 (FITC) and TxRd (RFP) staining intensities in the segmented nucleus and manually defined cytoplasmic area seen in the merged image. The results of RFP distribution before and after Hsp90 inhibition are shown in Figure 6F.

Supplemental Figure 2: Protein loadings and analysis.
(A) TrueBlue™ stain of 9 aliquots (1-17) from testes sucrose gradients to evaluate fractionation pattern of samples and loadings. Input is the cytoplasmic extract used for each gradient. (B) Membranes were simultaneously blotted with Hsp90ab1 and γ-tubulin. A long exposure illustrate how only γ-tubulin is found in soluble fractions in *Kdm3a*$^{\Delta JC/\Delta JC}$. (C) X-ray shown in Figure 8 were superimposed and scanned and the indicated areas (yellow rectangles) were measured for γ tubulin profile plots by ImageJ (Figure 8C) Hsp90ab1 and Cct3 (Supplemental Figure 2D). Exposure times between genotypes are equal. The sum of intensities across the indicated fractions is considered 100%. The numbers over the indicated fractions illustrate the disproportionately low presence of Hsp90ab1 in the fourth fraction of homozygous gradients. (D) Illustrate the relative distribution of the indicated Hsp90ab1 and Cct3 across 5 fractions of WT and *Kdm3a*$^{\Delta JC/\Delta JC}$ extracts. A and B, point to the difference between WT and *Kdm3a*$^{\Delta JC/\Delta JC}$ gradients for Cct3 and Hsp90ab1 respectively.

Supplemental Figure 3: A second antibody to the C-terminal end of Kdm3a validates its cytoplasmic fractionation.
(A) Discontinuous sucrose gradients of cytoplasmic wild type testes blotted with the indicated antibodies confirms Kdm3a complexed state beyond fraction 7 and its partial overlap with Cct and Hsp90 chaperones. Input is the cytoplasmic extract used for this gradient were microtubules were destabilised to reduce indirect effects. (B) Trueblue™ stain illustrates protein distribution along the early fractions of the gradient.

Supplemental Figure 4: Chaperone complexes in somatic cells with destabilized microtubules
(A) Membranes were simultaneously blotted with Hsp90ab1 and Cct3. As cells were treated with Nocodazole and cytochalasin D, γ-tubulin is present in early fractions only. (B) Quantitation of each of these bands along the gradient shows their relative partition in wild type and *Kdm3a*$^{\Delta JC/\Delta JC}$ cells. Note altered fractionation of Hsp90ab1 and Tubulin between wild type and homozygous gradients (black line). (C) TrueBlue™ stain of 10 aliquots (1-19) from this gradient illustrates the identical distribution of bulk proteins, indicating that the difference shown in B is specific to these two proteins.

Supplemental Figure 5: Mass-spectrometric evidence for the PTM state of proteins from *Kdm3a*$^{\Delta JC/\Delta JC}$ testes.
(A) The single band shown in Figure 8 D was cut and digested in gel. Fragment ions of Actbl2 identified from *Kdm3a*$^{\Delta JC/\Delta JC}$ testes with a pool of antibodies to mono, di and tri-methylated lysines. (B) Total Hsp90 pull-downs from each genotype were digested on beads. Actbl2 peptides found this way from *Kdm3a*$^{\Delta JC/\Delta JC}$ extracts (yellow). Modified peptide found in A was not found in this analysis (red box). (C) Fragment ions for Cct2 found in Hsp90 purified complexes from *Kdm3a*$^{\Delta JC/\Delta JC}$ testes using Scaffold 4(v4.2.1) support lysine methylation.

Supplemental Figure 6: Hsp90 interaction with another JmjC containing protein.
(A) Positive clones identified from a large two-hybrid screen using Hsp90 as baits were purified, re-

transformed into yeast and mated to re-assess interactions. The catalytic domain of Kdm6a interacts with Hsp90aa1. Diagram illustrates JmjC regions contained in two-hybrid and GST constructs. (B) Bacterially expressed catalytic domain of JmjC proteins identified in the two-hybrid screens were expressed in GST vectors and incubated with mammalian cell extracts. Proteins bound to glutathione beads were resolved and blotted with the indicated antibodies to determine interactions of GST constructs with Hsp90.

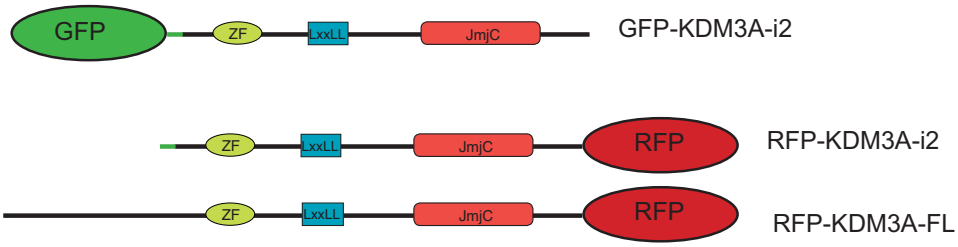
Supplemental Table 2: Hsp90 pull downs of total testes extracts. The table displays exclusive unique spectrum counts of proteins absents in WT, *Kdm3a*$^{\Delta JC/-}$ and *Kdm3a*$^{\Delta JC/\Delta JC}$ controls.

Supplemental Table 3: Cct chaperonins in Hsp90 pull downs of total testes extracts.
The table displays exclusive unique spectrum counts of Cct/Tcp proteins in all samples including controls. Subtractions of the spectral number found in each respective control suggest an enrichment of Cct spectral counts in *Kdm3a*$^{\Delta JC/\Delta JC}$ samples (boxed counts).

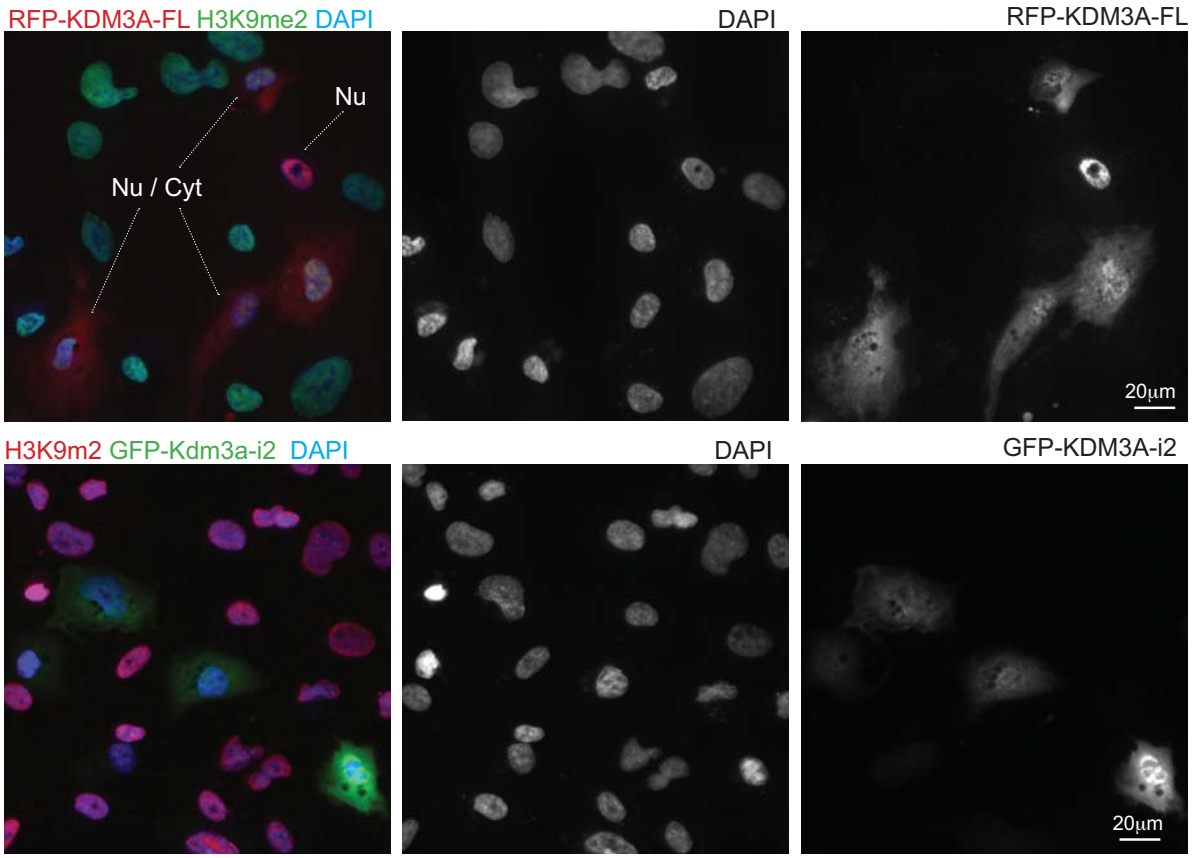
Supplemental Table 4: Potentially methylated peptides found in *Kdm3a*$^{\Delta JC/\Delta JC}$ only.
The table displays exclusive unique modified spectrum counts.

Supplemental Figure 1:

A

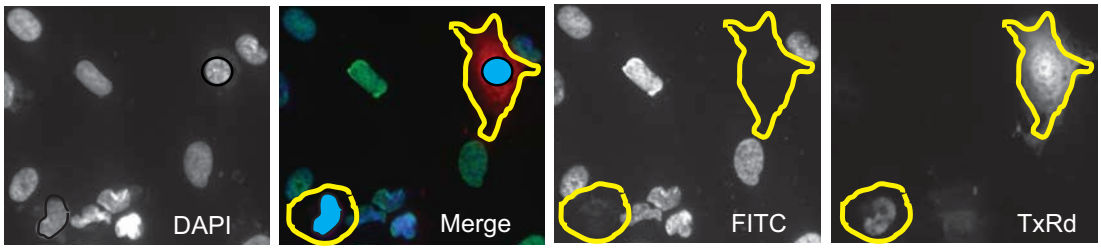


B

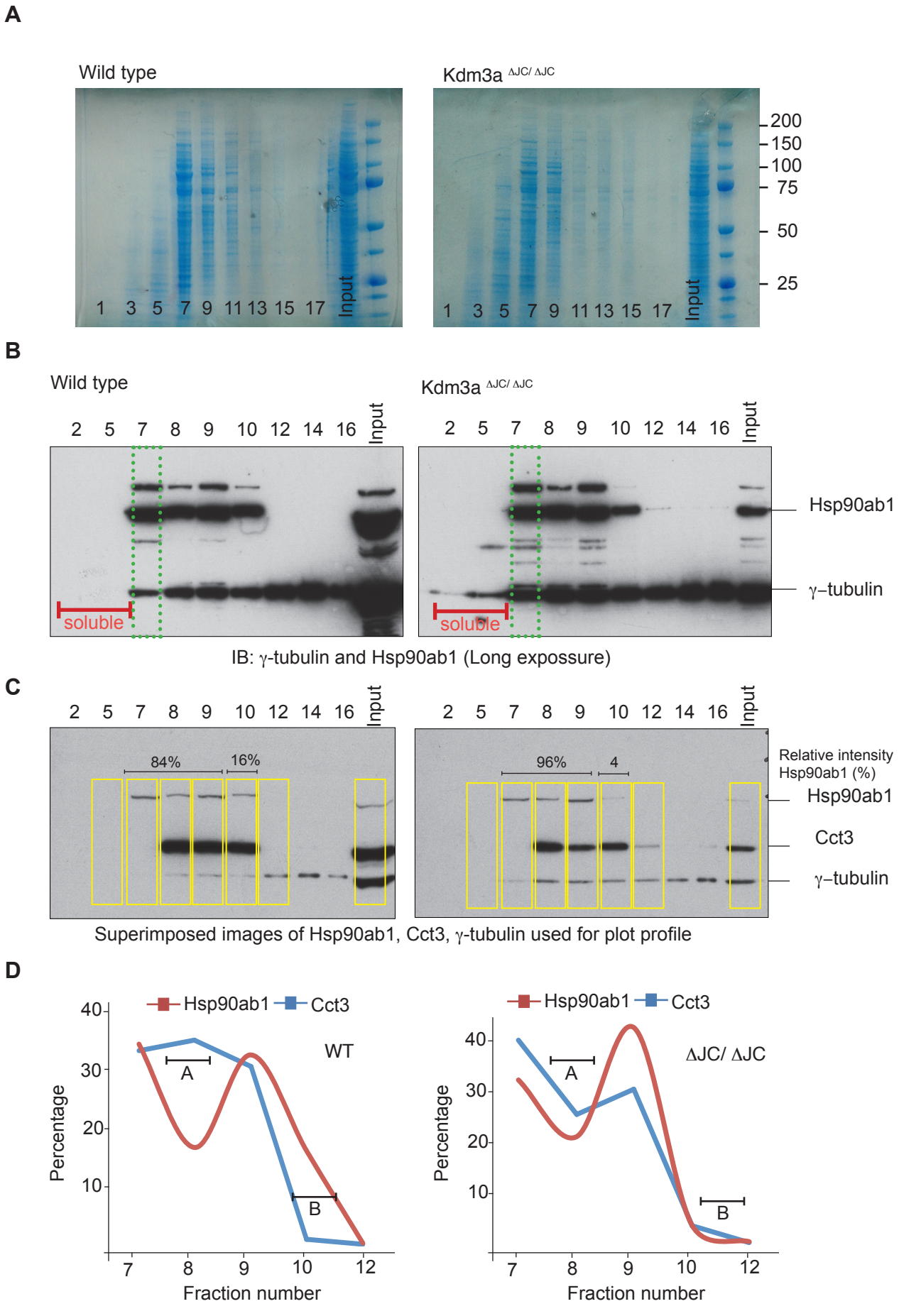


C

Autosegmentation of nuclei, manual demarcation of cytoplasm and measurement of FITC (H3K9me2) and TxRd (RFP) in defined areas

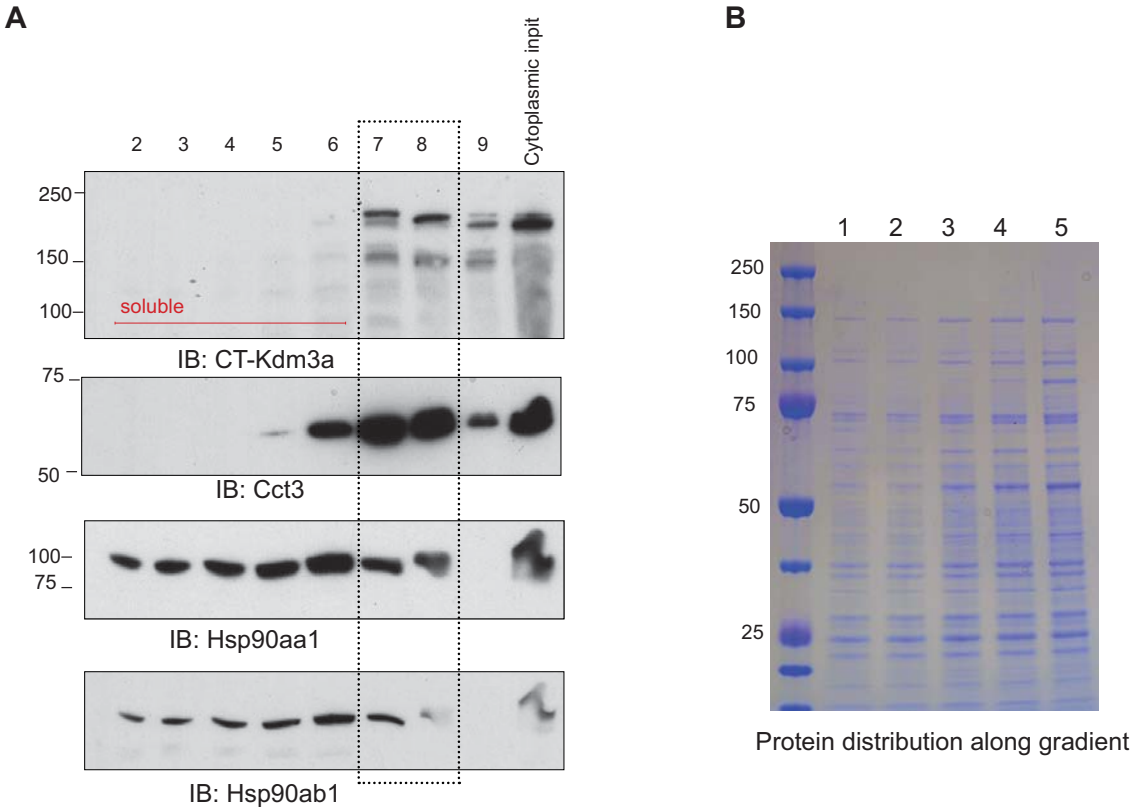


Supplemental Figure 2:



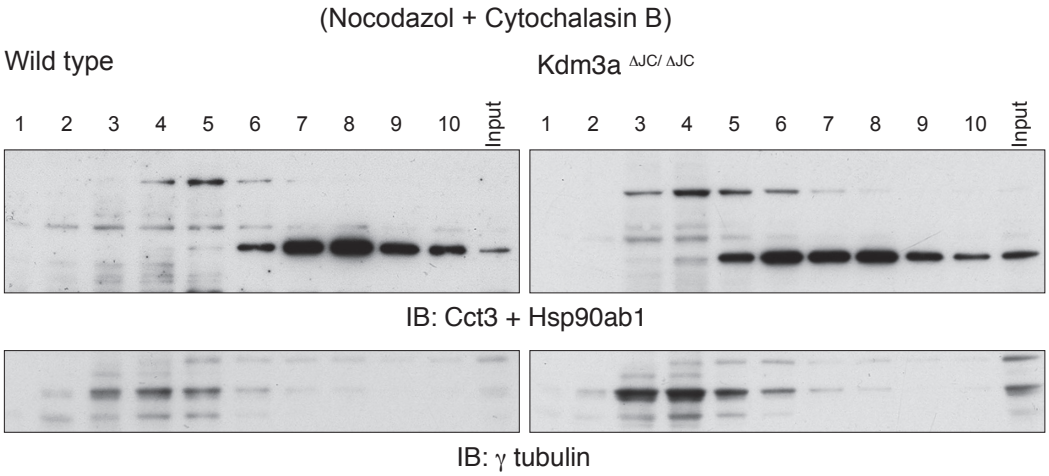
Supplemental Figure 3:

Cytoplasmic gradients of Wild type adult testes (Cytochalasin B + Nocodazole)



Supplemental Figure 4:

A



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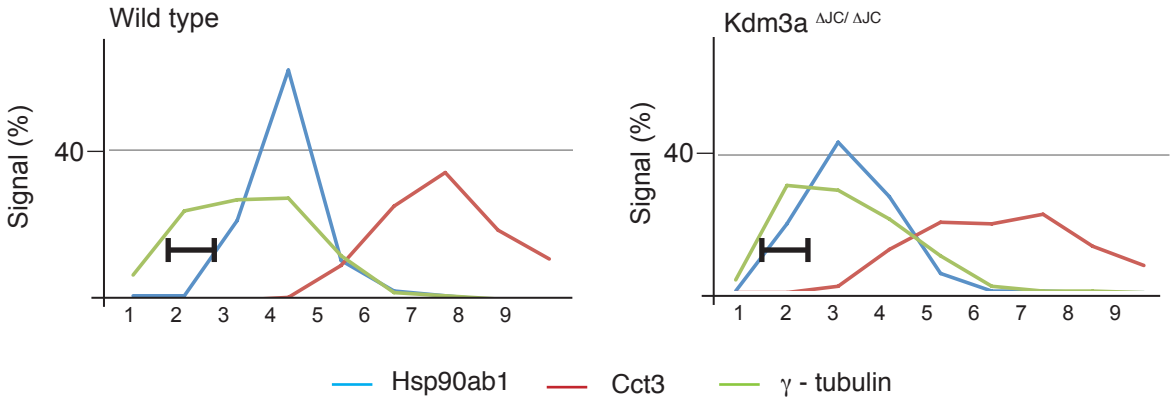
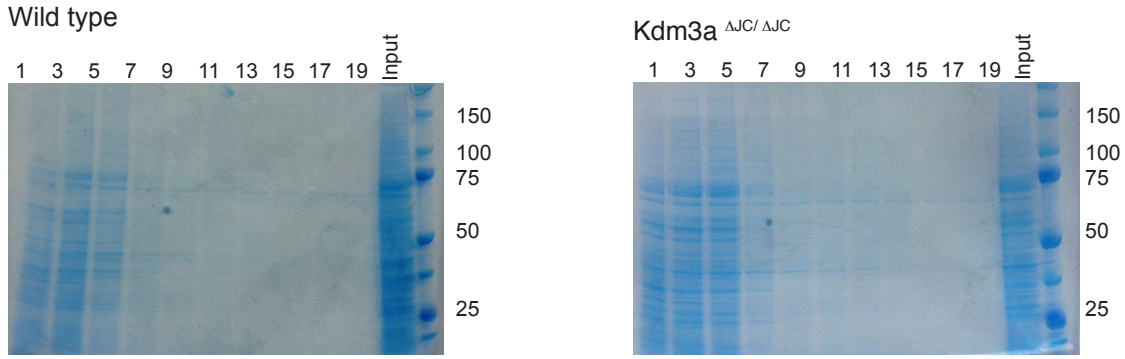


C



Supplemental Figure 5:

A

Fragment ions for Actbl2 di-methylated peptide (Mascot display)

Monoisotopic mass of neutral peptide Mr(calc): 1591.8313
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
K3 : Dimethyl (K)
M13 : Oxidation (M), with neutral losses 63.9983(shown in table), 0.0000
Ions Score: 27 **Expect:** 0.069
Matches : 32/220 fragment ions using 69 most intense peaks (help)

#	b	b^{++}	b*	b^{*++}	b^{0}	b^{0++}	Seq.	y	y^{++}	y*	y^{*++}	y^{0}	y^{0++}	#
1	132.0478	66.5275					M							14
2	*260.1063*	130.5568	243.0798	122.0435			Q	1397.7999	699.4036	1380.7733	690.8903	1379.7893	690.3983	13
3	*416.2326*	208.6199	399.2061	200.1067			K	1269.7413	635.3743	1252.7147	626.8610	1251.7307	626.3690	12
4	*545.2752*	273.1412	528.2486	264.6280	527.2646	264.1360	E	1113.6150	557.3111	1096.5885	548.7979	1095.6045	548.3059	11
5	*658.3593*	329.6833	641.3327	321.1700	640.3487	320.6780	I	984.5724	492.7899	967.5459	484.2766	966.5619	483.7846	10
6	*759.4069*	380.2071	742.3804	371.6938	741.3964	371.2018	T	871.4884	436.2478	854.4618	427.7345	853.4778	427.2425	9
7	*830.4441*	415.7257	813.4175	407.2124	812.4335	406.7204	A	770.4407	385.7240	753.4141	377.2107	752.4301	376.7187	8
8	943.5281	472.2677	926.5016	463.7544	925.5176	463.2624	L	*699.4036*	350.2054	682.3770	341.6921	681.3930	341.2001	7
9	1014.5652	507.7863	997.5387	499.2730	996.5547	498.7810	A	*586.3195*	293.6634	569.2930	285.1501	568.3089	284.6581	6
10	1111.6180	556.3126	1094.5914	547.7994	1093.6074	547.3074	P	*515.2824*	258.1448	498.2558	249.6316	497.2718	249.1396	5
11	1198.6500	599.8286	1181.6235	591.3154	1180.6395	590.8234	S	*418.2296*	209.6185	401.2031	201.1052	400.2191	200.6132	4
12	1299.6977	650.3525	1282.6712	641.8392	1281.6871	641.3472	T	*331.1976*	166.1024	314.1710	157.5892	313.1870	157.0972	3
13	1382.7348	691.8710	1365.7083	683.3578	1364.7243	682.8658	M	*230.1499*	115.5786	213.1234	107.0653			2
14							K	147.1128	74.0600	130.0863	65.5468			1

B

ACTBL_MOUSE (100%), 42,005.1 Da
Beta-actin-like protein 2 OS=Mus musculus GN=Actbl2 PE=1 SV=1
2 exclusive unique peptides, 3 exclusive unique spectra, 4 total spectra, 108/376 amino acids (29% coverage)

M V D D E L T A L V V D N G S G M C K A G F G G D D A P R A V F P S M V G R P R H Q G V M V G M G Q K D C Y V G D E A Q S K R G I L T L K Y
P I E H G V V T N W D D M E K I W Y H T F Y N E L R V A P D E H P I L L T E A P L N P K I N R E K M T Q I M F E A F N T P A M Y V A I Q A V
L S L Y A S G R T T G I V M D S G D G V T H T V P I Y E G Y A L P H A I L R L D L A G R D L T D Y L M K I L T E R G Y N F T T T A E R E I V
R D V K E K L C Y V A L D F E Q E M V T A A A S S S L E R S Y E L P D G Q V I T I G N E R F R C P E A I F Q P S F L G I E S R G I H E T T F
N S I M K C D V D I R K D L F A N T V L S G G S T M Y P G I A D R M Q K E I V T L A P S T M K I K I I A P P E R K Y S V W I G G S I L A S L
S T F Q Q M W I S K Q E Y D E A G P P I V H R K C F

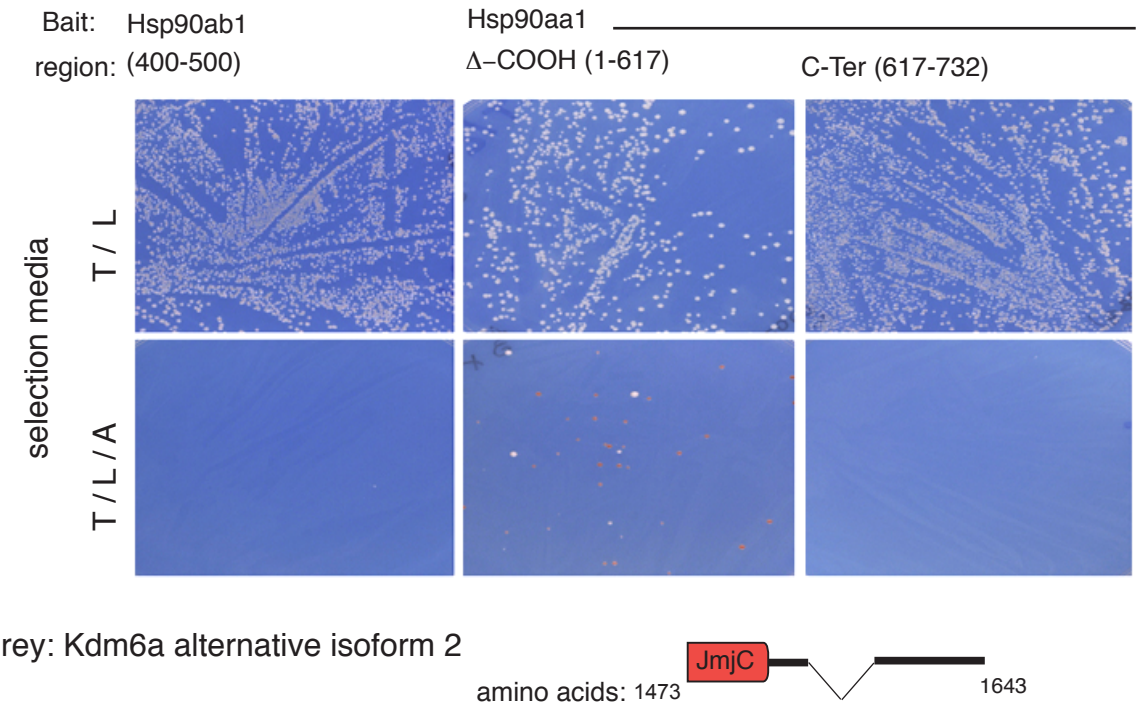
C

Fragment ions for Cct2 mono-methylated peptide (Scaffold™ display)

B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	114.1	57.5			L	1,492.9	746.9	1,475.9	1,474.9	14
2	242.2	121.6	225.2		K	1,379.8	690.4	1,362.8	1,361.8	13
3	299.2	150.1	282.2		G	1,251.7	626.4	1,234.7	1,233.7	12
4	386.2	193.6	369.2	368.2	S	1,194.7	597.8	1,177.7	1,176.7	11
5	443.3	222.1	426.2	425.3	G	1,107.7	554.3	1,090.6	1,089.6	10
6	557.3	279.2	540.3	539.3	N	1,050.6	525.8	1,033.6	1,032.6	9
7	670.4	335.7	653.4	652.4	L	936.6	468.8	919.6	918.6	8
8	799.4	400.2	782.4	781.4	E	823.5	412.3	806.5	805.5	7
9	870.5	435.7	853.4	852.5	A	694.5	347.7	677.4		6
10	983.6	492.3	966.5	965.5	I	623.4	312.2	606.4		5
11	1,120.6	560.8	1,103.6	1,102.6	H	510.3	255.7	493.3		4
12	1,219.7	610.3	1,202.7	1,201.7	V	373.3	187.1	356.3		3
13	1,332.8	666.9	1,315.7	1,314.8	I	274.2	137.6	257.2		2
14	1,492.9	746.9	1,475.9	1,474.9	K+14	161.1	81.1	144.1		1

Supplemental Figure 6:

A



B