Supplemental figure legends

Supplemental Figure 1: BRAF/ERK signaling pathway controls metabolism, NAD+ level, and proliferation in BRAF^{V600E} melanoma cells. (A-F) Deregulated dipeptides, long chain fatty acids and mono acyl glycerols in PLX4032-treated versus non-treated cells. Note that these metabolites are poorly annotated in the KEGG or HMDB databases. (G) Schematic summary of the effects of PLX4032 on melanoma metabolism. Metabolites highlighted in yellow are downregulated, while those highlighted in red are upregulated. *Note that glucose 6-P and pyruvate are downregulated by PLX4032 with a Log Fc of -1.9 and -2.4 respectively, and a FDR=0.058.

Supplemental Figure 2: PLX4032 does not affect NAMPT expression in BRAF^{WT} melanoma cells. (A) Dose response curves of melanoma cells exposed to increasing concentration of PLX4032 for 72 h. Cell number is shown. Values represent the means +/-SD of three independent experiments. (B) Proliferation assays of BRAF^{WT} and BRAF^{V600E} human melanoma cells exposed to PLX4032 (5 µM, 72 h). (C) Intracellular NAD+ levels in A375 human melanoma cells, exposed to MEK (GSK1120212, U0126, and PD98059) or ERK (SCH772984) inhibitors. Values represent the means +SD of three independent experiments. (D) Intracellular NAD+ levels in glucose-deprived human melanoma cells. Values represent the means +SD of three independent experiments.

Supplemental Figure 3: The BRAF/ERK signaling pathway controls NAMPT expression. (A) NAMPT mRNA and NAD+ levels in BRAF^{V600E} melanoma cells

(A375, WM9, and UACC62) exposed or not to PLX4032 (5 μM, 24 and 48 h) were measured using QPCR. Values represent the means +SD of three independent experiments. (B) Western blot analysis of NAMPT, pERK and ERK2 in human HMVII melanoma cells exposed to PLX4032. (C) Western blot analysis of NAMPT expression in A375 melanoma cells exposed to BRAF (PLX4032), MEK (GSK1120212, U0126, and PD98059) or ERK (SCH772984) inhibitors. Phospho-ERK analysis shows the efficiency of kinase inhibitors, and ERK2 was used as loading control. (D) Analysis of publicly available data sets GSE51115 (n=26) of melanoma cells exposed to MEK inhibitor (MEKi). Scatter plots showing the means +/-SD of the NAMPT mRNA expression. (E) Western blot analysis of NAMPT expression in normal human melanocytes (MHN) after transduction with empty (EV) or $BRAF^{V600E}$ adenovirus. Where indicated, the cells were exposed to MEK (GSK1120212 and U0126) or ERK (SCH772984) inhibitors. Western blot analysis shows the transduction efficiency based on BRAF expression. Phospho-ERK analysis shows the efficiency of kinase inhibitors, and HSP90 was used as loading control. (F) Intracellular NAD+ levels in MHN after transduction with empty (EV) or $BRAF^{V600E}$ adenovirus Values represent the means +SD of three independent experiments.

Supplemental Figure 4: The BRAF/ERK signaling pathway, through STAT5 activation, controls NAMPT at the transcriptional level. (A) Activity of NAMPT promoter in A375 cells exposed to increasing doses of PLX4032 for 48h. Data are shown as the means +/-SD of 3 experiments. (B) Activity of NAMPT promoter reporter vector in A375 cells exposed to MEK (GSK1120212 and U0126) or ERK (SCH772984) inhibitors. Values represent the means +SD of three independent

experiments. (C) Activity of NAMPT promoter fragments with different lengths in A375 cells exposed to PLX4032. Values represent the means +SD of three independent experiments. (D) Activity of NAMPT promoter reporter in 501mel cells transfected with a control vector or a vector encoding $BRAF^{V600E}$ and left untreated or exposed to STAT5 inhibitor (STAT5i, 100 μM, 48 h). Values represent the means +SD of three independent experiments. (E) Intracellular NAD+ levels of control or STAT5i treated A375 and WM9 melanoma cells. Values represent the means +SD of three independent experiments. (F) Western blot analysis of NAMPT, STAT5 and ERK in HMVII cells control or exposed to PLX4032 (5 μM) or STAT5i (100 μM) for 48 h. (G) Intracellular NAD+ levels of control or STAT5i treated HMVII melanoma cells. Values represent the means +SD of three independent experiments.

Supplemental Figure 5: NAMPT controls melanoma cell proliferation. (A) Western blot analysis of NAMPT expression in A375 and UACC62 melanoma cells transfected with control or 2 different NAMPT siRNA or exposed to NAMPT inhibitor (FK866). HSP90 was used as loading control. (B) Intracellular NAD+ levels in A375 and UACC62 melanoma cells treated as in (A). Values represent the means +SD of three independent experiments. (C) Dose response curves of melanoma cells exposed to increasing concentration of FK866 for 72 h. Intracellular NAD+ is shown. Values represent the means +/-SD of three independent experiments. (D) Dose response curves of melanoma cells exposed to increasing concentration of FK866 for 48 h. Cell number is shown. Values represent the means +/-SD of three independent experiments. (E) Proliferation of A375 and UACC62 melanoma cells treated as in (A). Cells were trypsinized and counted each day. (F) Intracellular NAD+ levels in A375

and UACC62 melanoma cells exposed to FK866 (5 μM) or FK866 plus NAD+ (500 μM) for 48 h. Values represent the means +SD of three independent experiments.

Supplemental Figure 6: NAMPT mediates melanoma cell survival. (A) A375 or WM9 melanoma cells exposed to 2 different NAMPT siRNAs or to FK866 (10 μM, 48 h), were then exposed to DAPI and % of dead cells up-taking DAPI measured by FACS. Histograms represent the means +SD of three independent experiments. (B) NAMPT mRNA level in tumors established by subcutaneous injection of WM9 cells. (C) NAD+ level in the corresponding tumors.

Supplemental Figure 7: NAMPT inhibition resensitizes PLX4032-resistant melanoma cells to PLX4032. (A) PLX4032-resistant A375 melanoma cells were transfected with control or NAMPT siRNA and subsequently exposed to PLX4032 (5 μM). After 48 h, the cells were counted. The histogram represents the means +SD of three independent experiments. (B) Cell number of PLX4032-resistant A375 melanoma cells exposed to FK866 (5 μM) or FK866 plus NAD+ (500 μM) for 72 h. Values represent the means +SD of three independent experiments. (C) Growth curve of tumor xenografts of PLX4032-resistant A375 melanoma cells after subcutaneous injection. Mice (6 per group) were treated with vehicle, PLX4032 (25 mg/kg) or FK866 (1.5 mg/kg and 15 mg/kg) alone or with the low FK866 dose in combination with PLX4032. Black arrow indicates beginning of the treatment. Data are presented as the means +/-SD. (D) NAD+ level in the corresponding tumors.

Supplemental Figure 8: Regulation of NfkB and TGFb signaling pathways by NAMPT.(A) Activity of NFkB and (B) TGFb promoter reporter in A375 or WM9 melanoma cells transduced with an empty (EV) adenovirus or an adenovirus encoding NAMPT (Ad NAMPT). Data are shown as the means +SD of three experiments**.**

Supplemental Figure 9: NAMPT induces changes in H3K27me3, H3K27ac and H3K4me3 histone marks. Profiles of H3K27me3, H3K27ac and H3K4me3 histone marks showing the regulation of each mark within the specified cluster.

Supplemental Figure 10: Venn diagrams displaying the intersection between genes and histone marks regulated by NAMPT. (A) Intersection of genes up-regulated by NAMPT with active histone marks down-regulated and repressive histone marks up-regulated by NAMPT. (B) Intersection of genes down-regulated by NAMPT with active histone marks up-regulated and repressive histone marks down-regulated by NAMPT.

Supplemental Figure 11: NAMPT promotes histone modifications. UCSC browser view of H3K27ac and H3K4me3 ChIP-Seq binding density at (A) CDK2/PMEL, (B) BIRC7, (C) ITGB4 and (D) WNT5A loci.

Supplemental Figure 12: NAMPT enhances the motile features of melanoma cells. (A-B) Boyden chamber experiments with 501mel or SKmel28 melanoma cells transduced with an empty (EV) adenovirus or an adenovirus encoding NAMPT. Values represent mean + SD of three independent experiments. (C-D) Western blot analysis of NAMPT, fibronectin (FN) and MITF in 501mel or in SKmel28. (E)

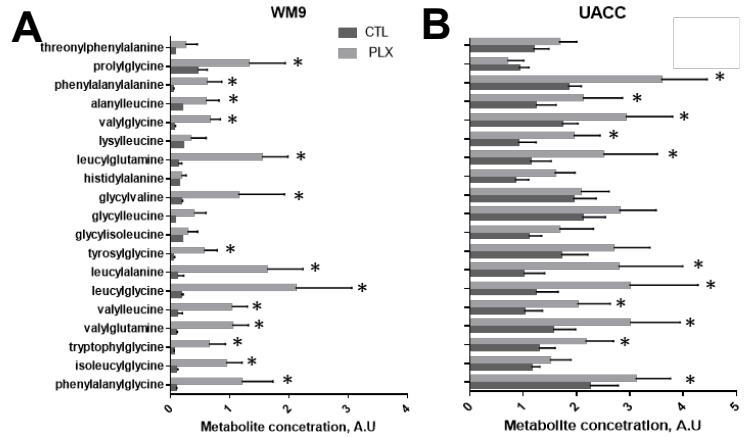
melanoma cells transduced with an empty (EV) adenovirus or an adenovirus encoding NAMPT. (E) UCSC browser view of H3K27ac and H3K4me3 ChIP-Seq binding density at the ZEB1 locus.

Supplemental table 1. Differential metabolite level in PLX4032-treated melanoma cells when compared to control cells.

Supplemental table 2. Analysis of enriched metabolic function in PLX4032-treated melanoma cells when compared to control cells using MetaboAnalyst.

Supplemental table 3. Top 10 differentially regulated cellular functions in melanoma cells with forced expression of NAMPT when compared to control melanoma cells analysed using Ingenuity Pathway analysis.

Ohanna_Supplemental Fig1



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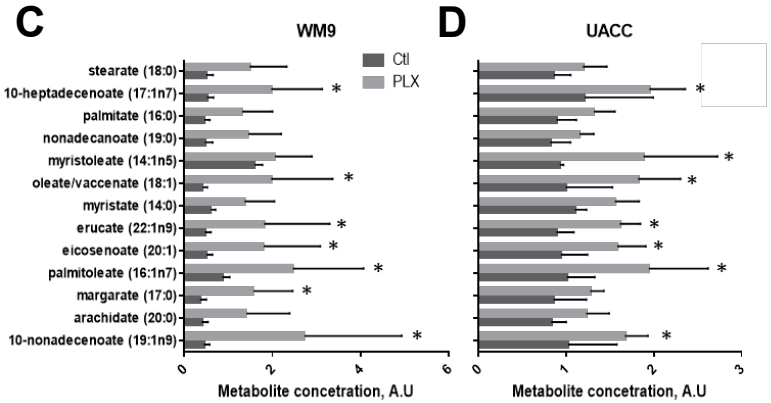

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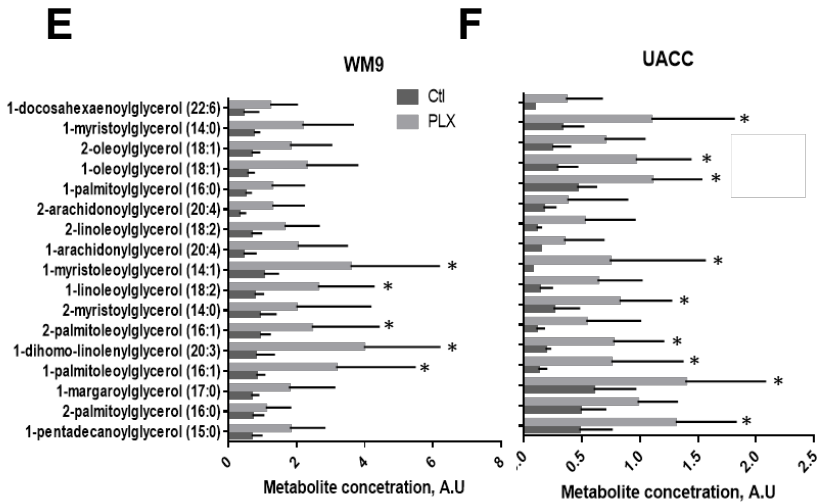

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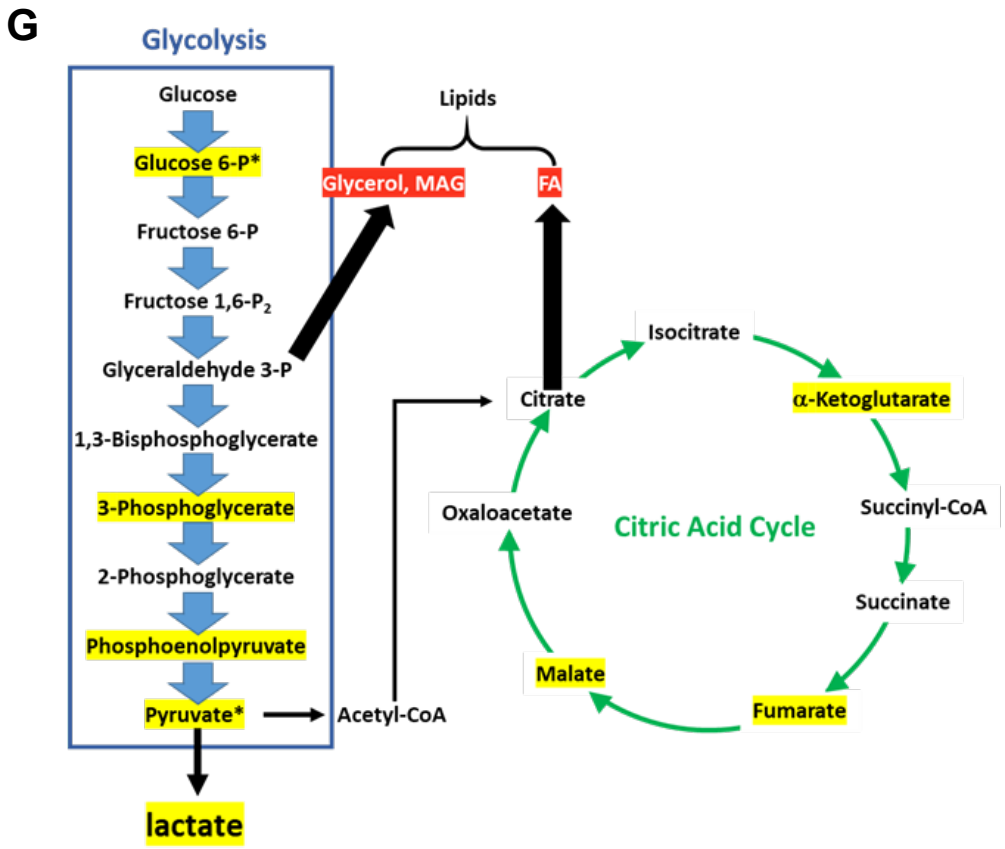

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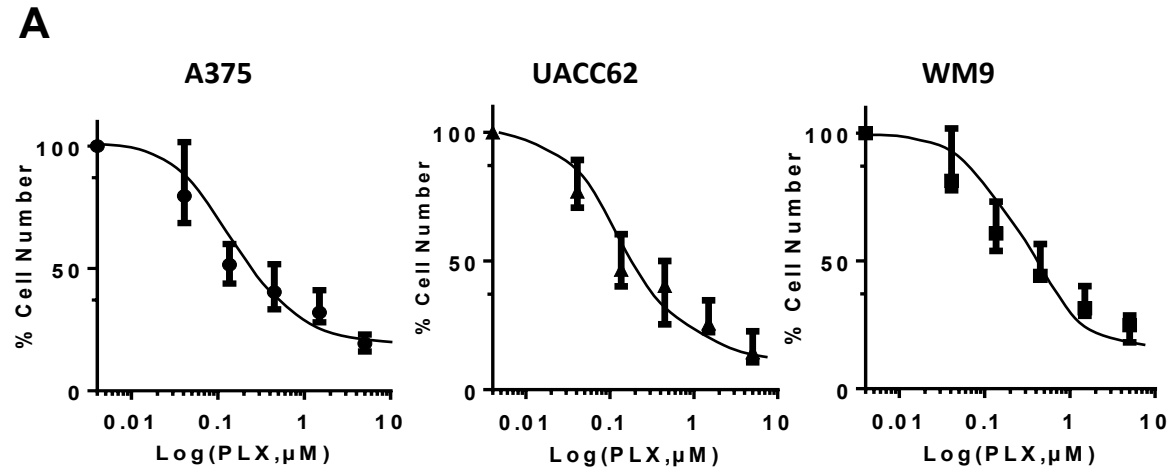

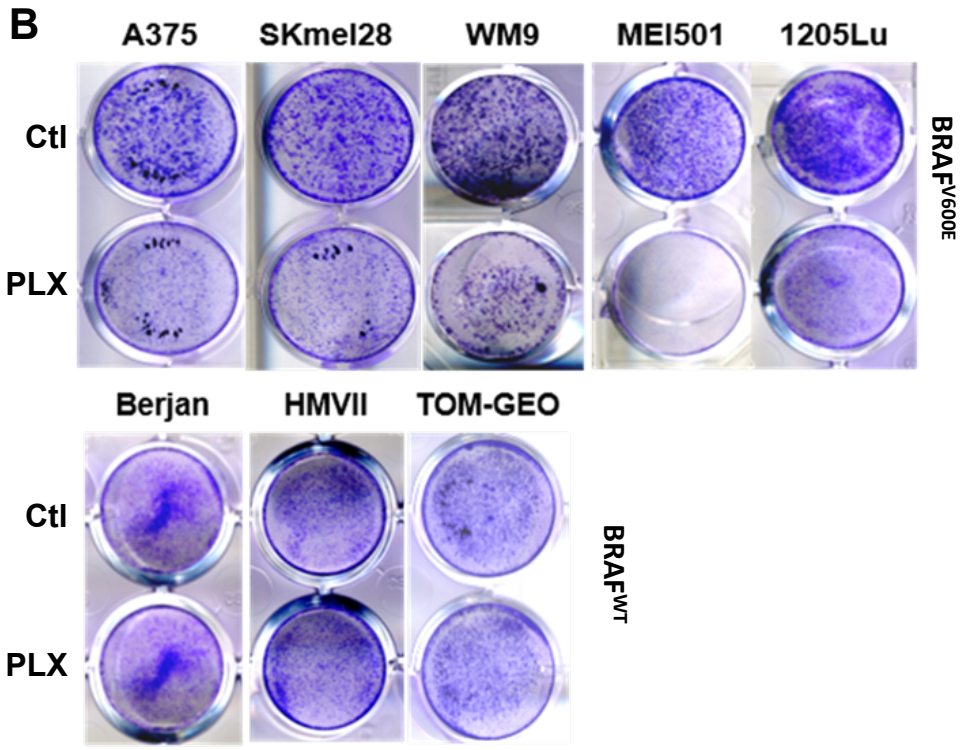

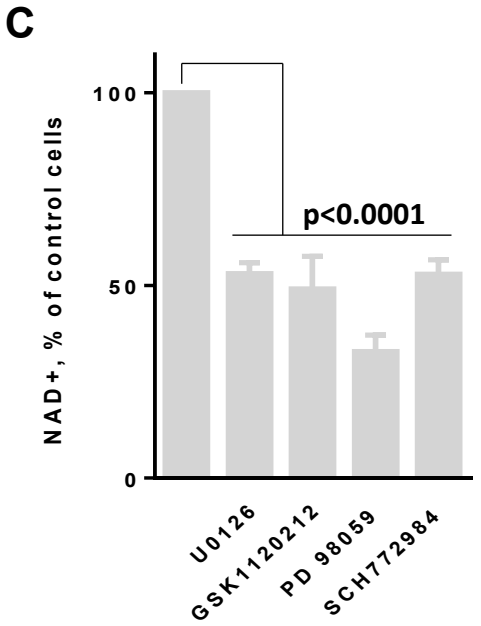

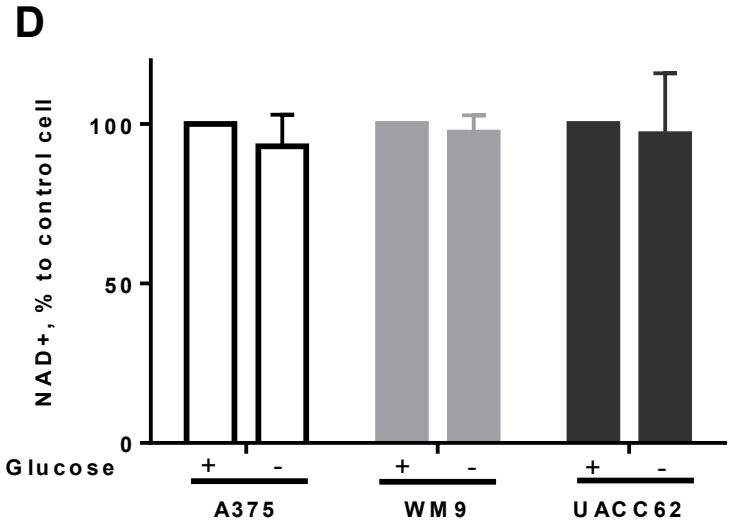

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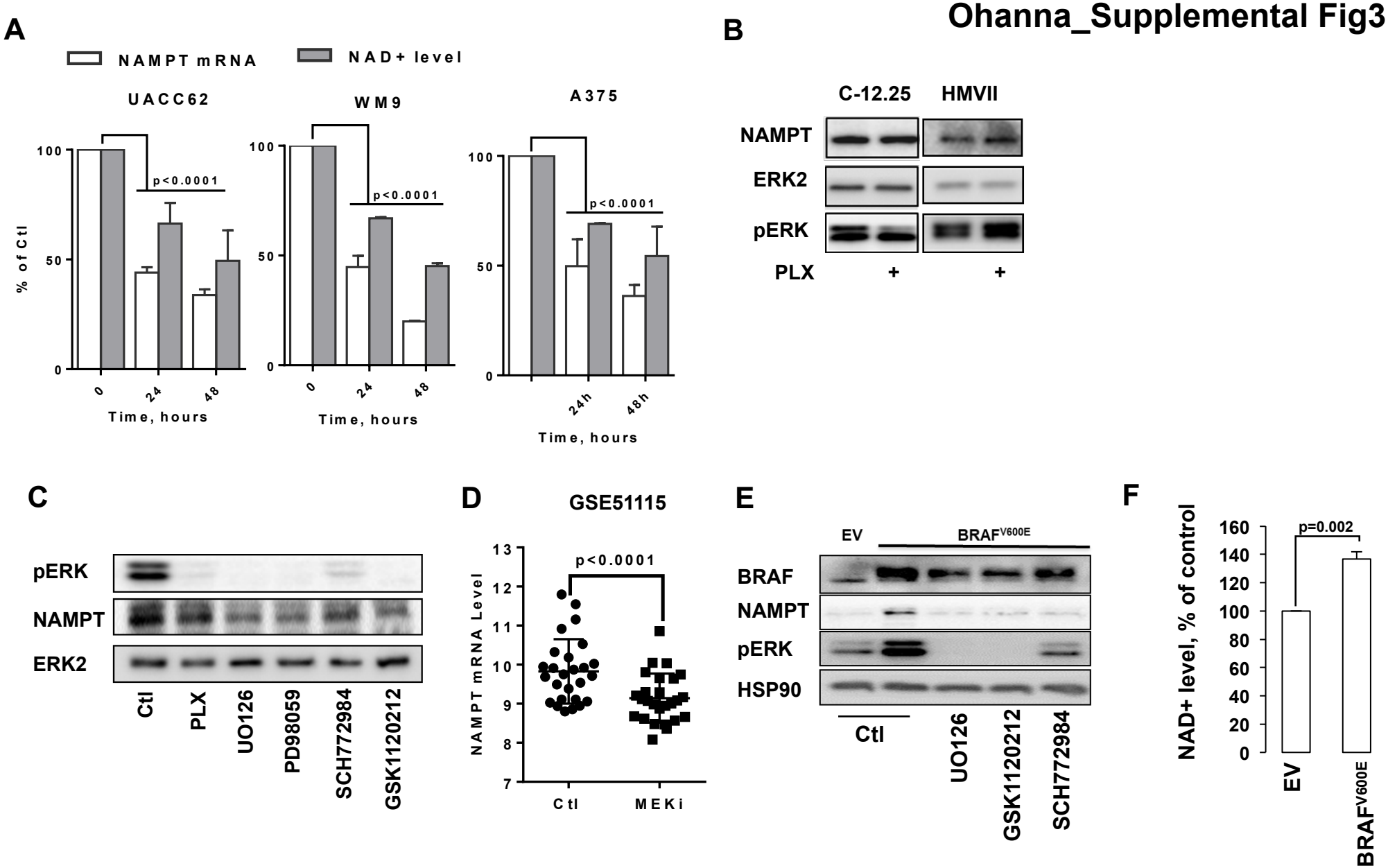

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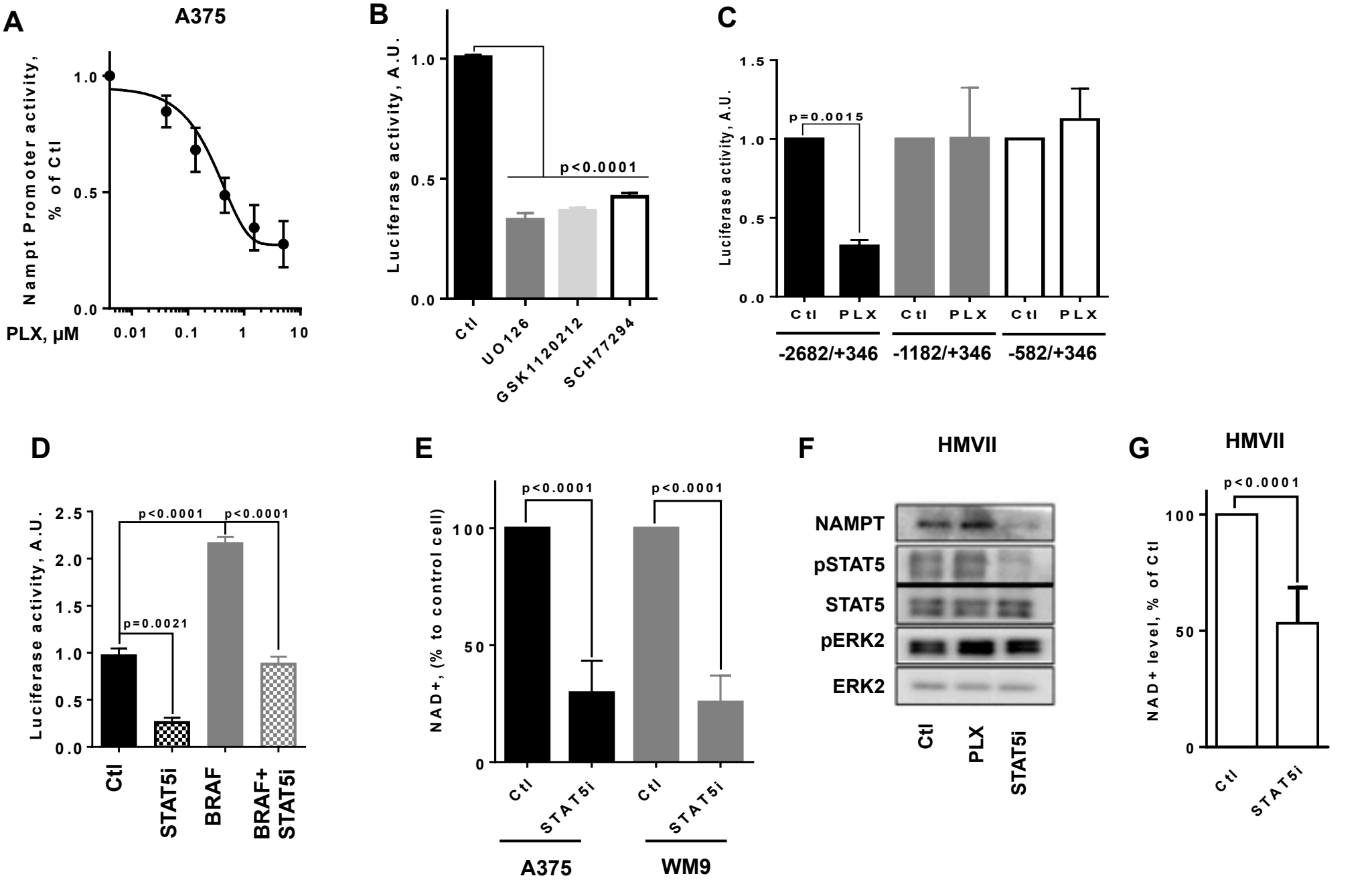

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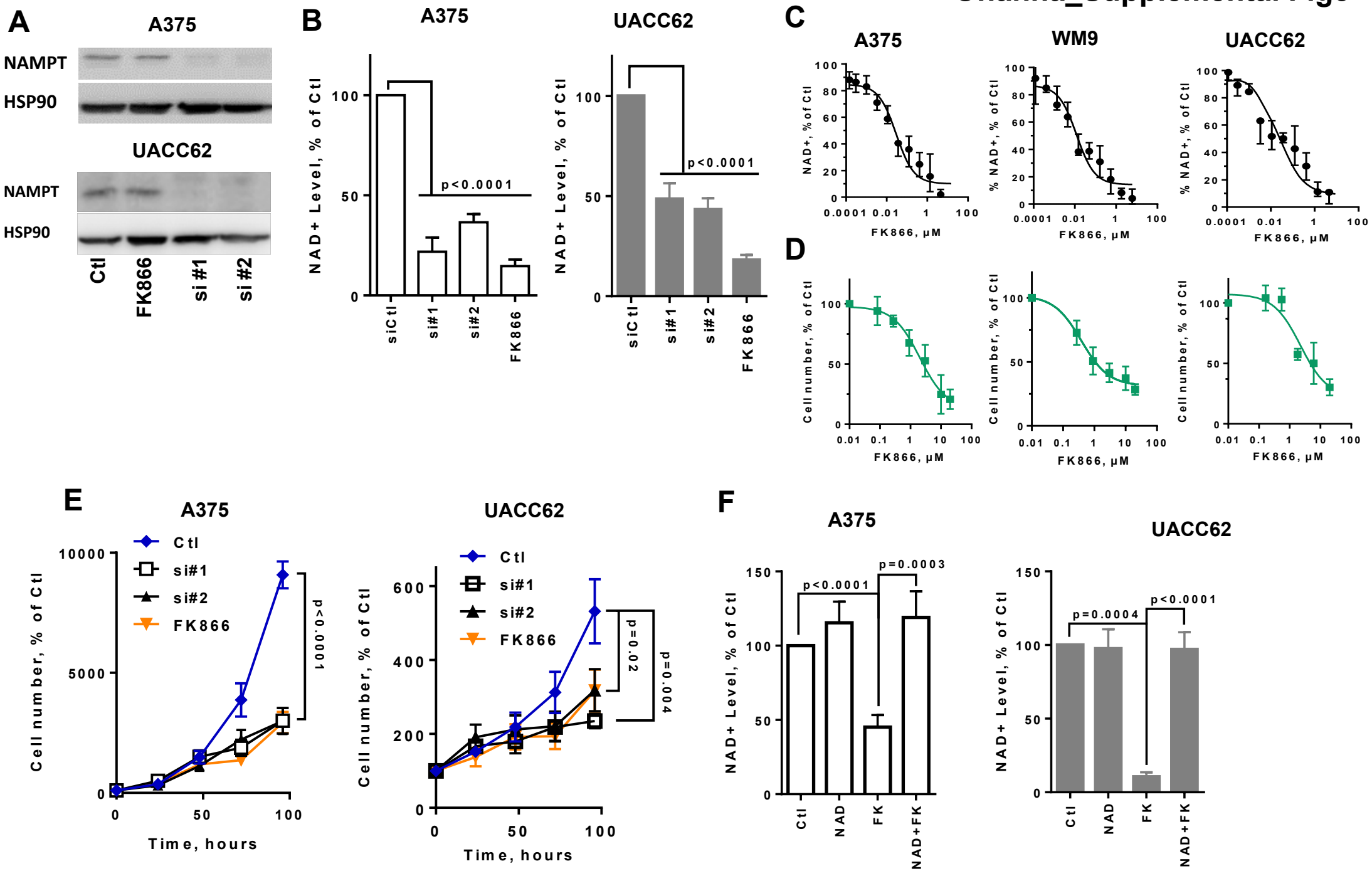

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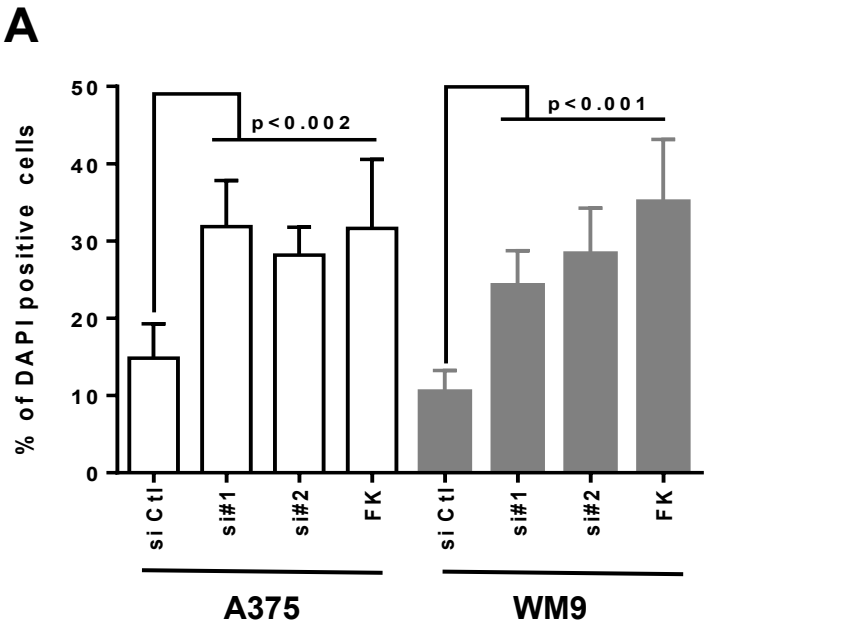

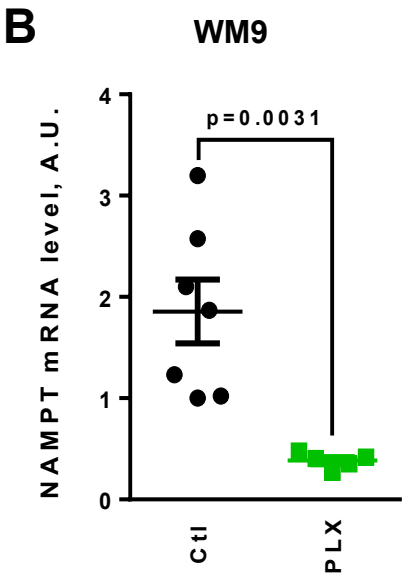

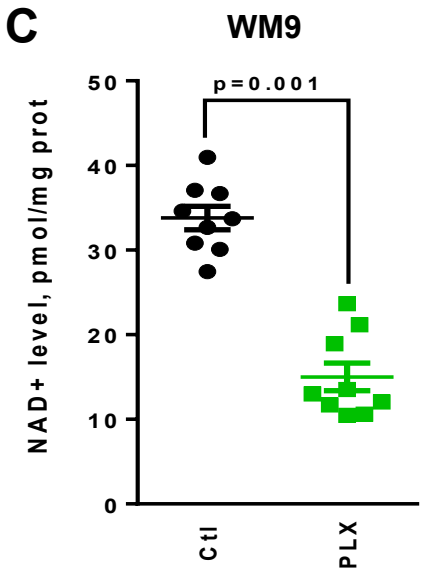

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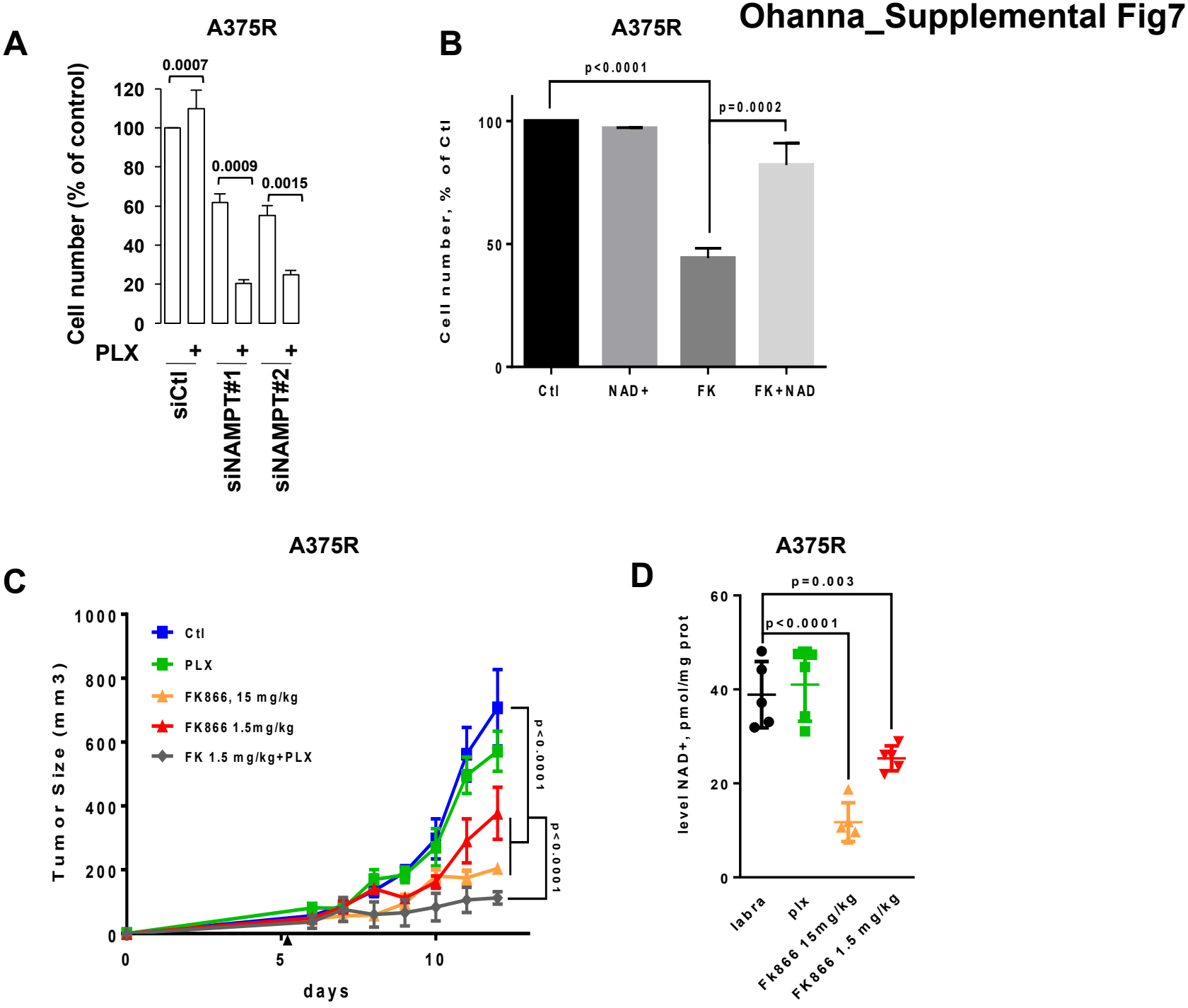

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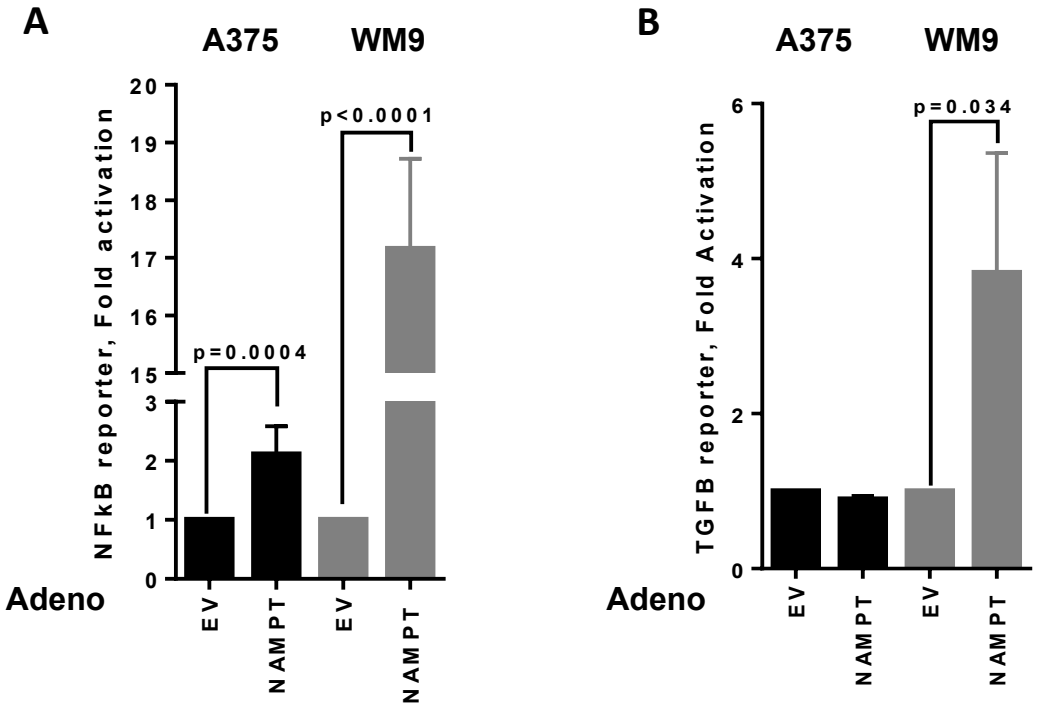

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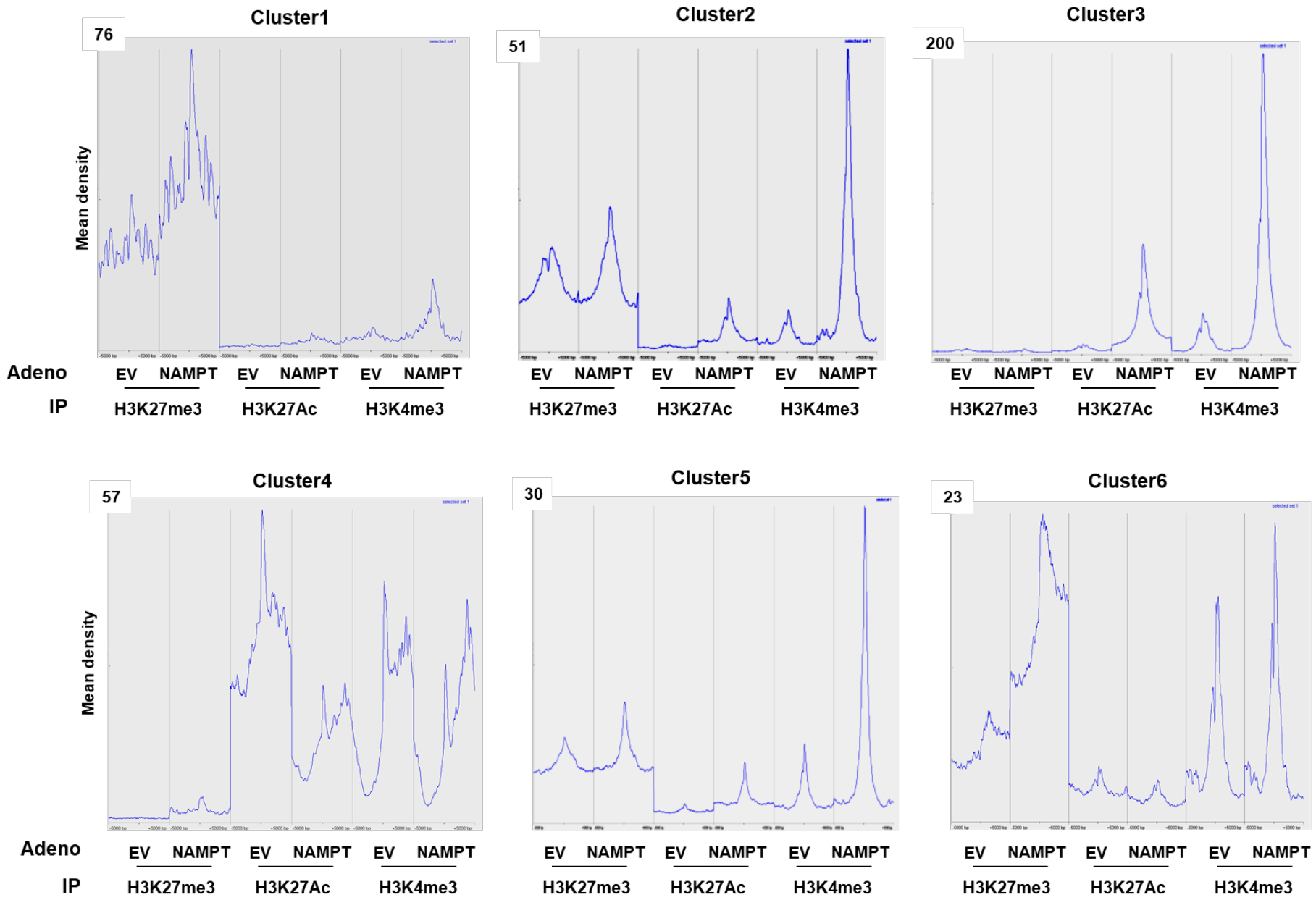

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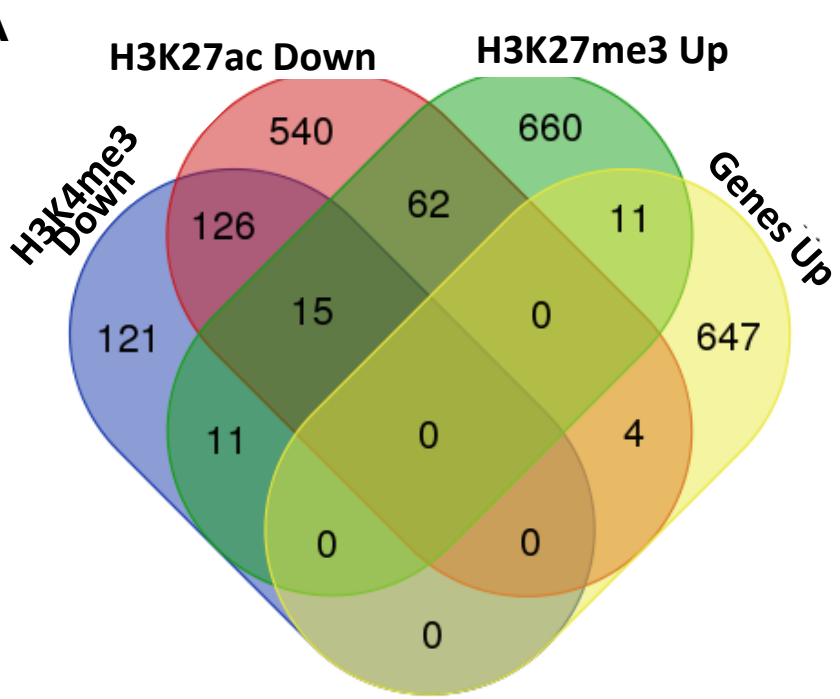

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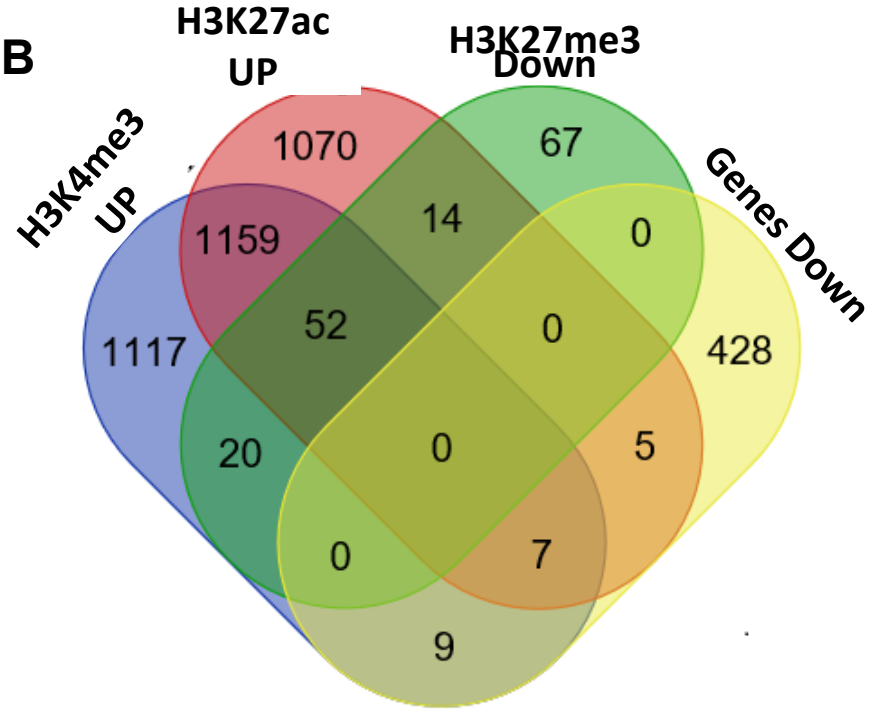

Ohanna_Supplemental Fig11

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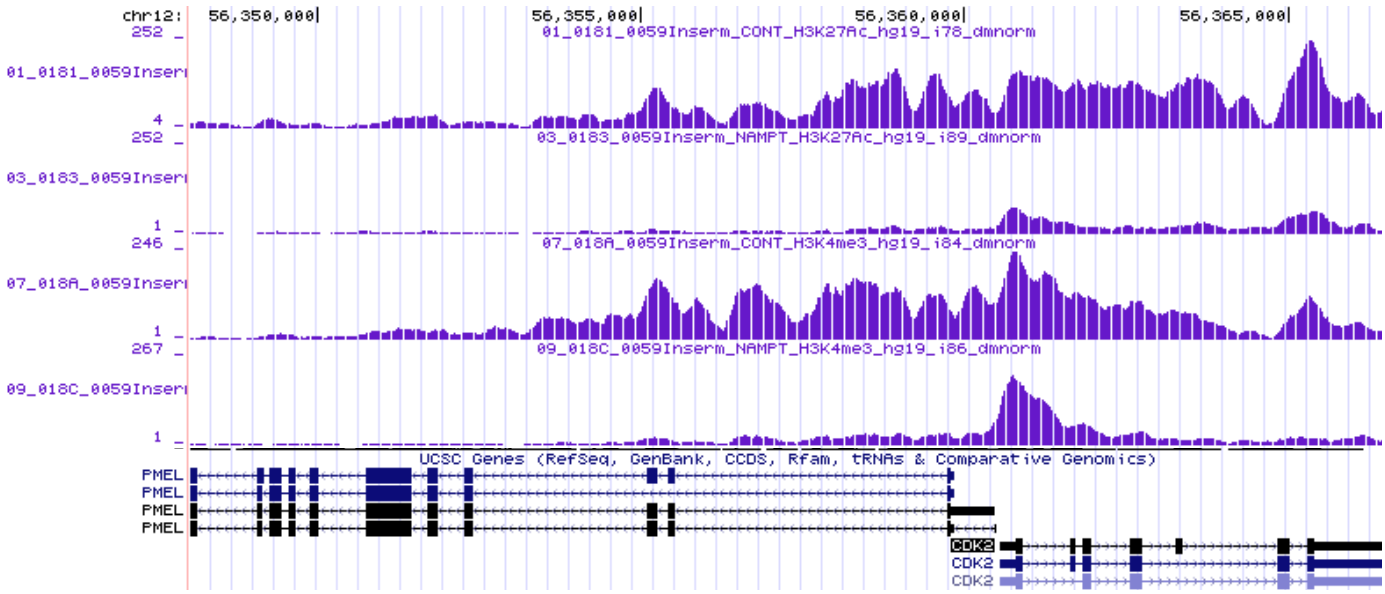

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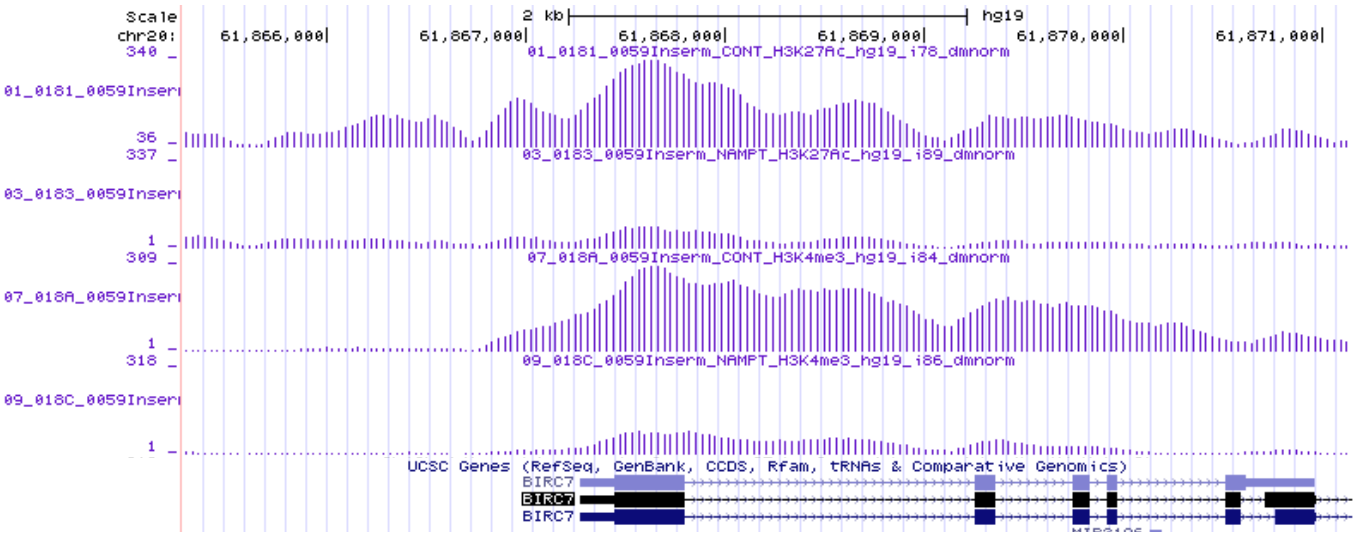

Ohanna_Supplemental Fig11

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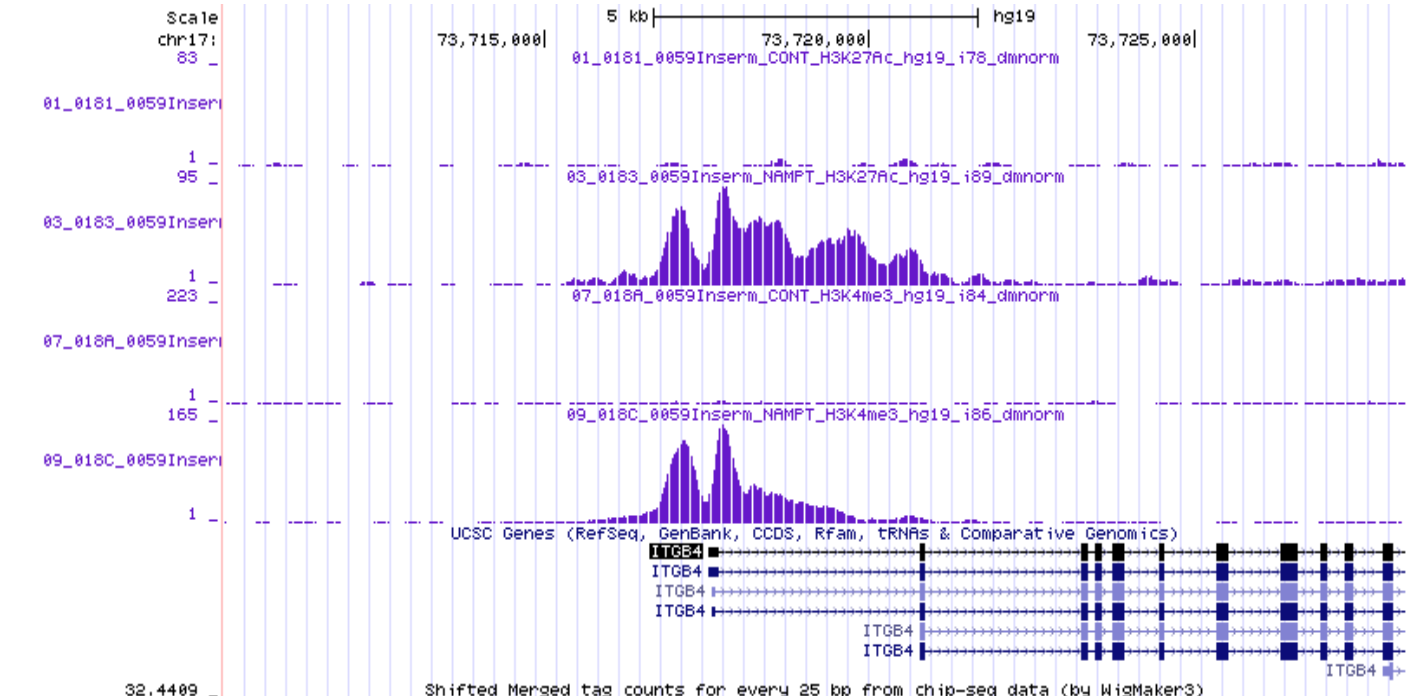

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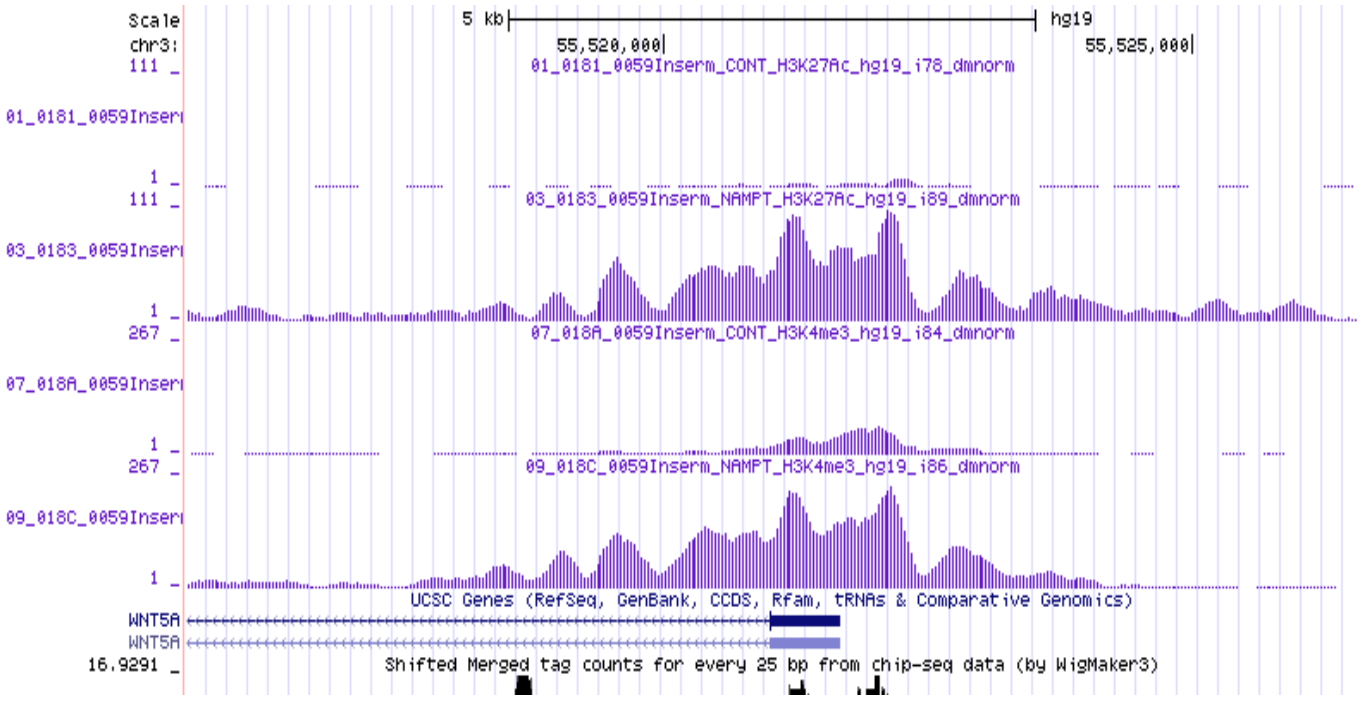
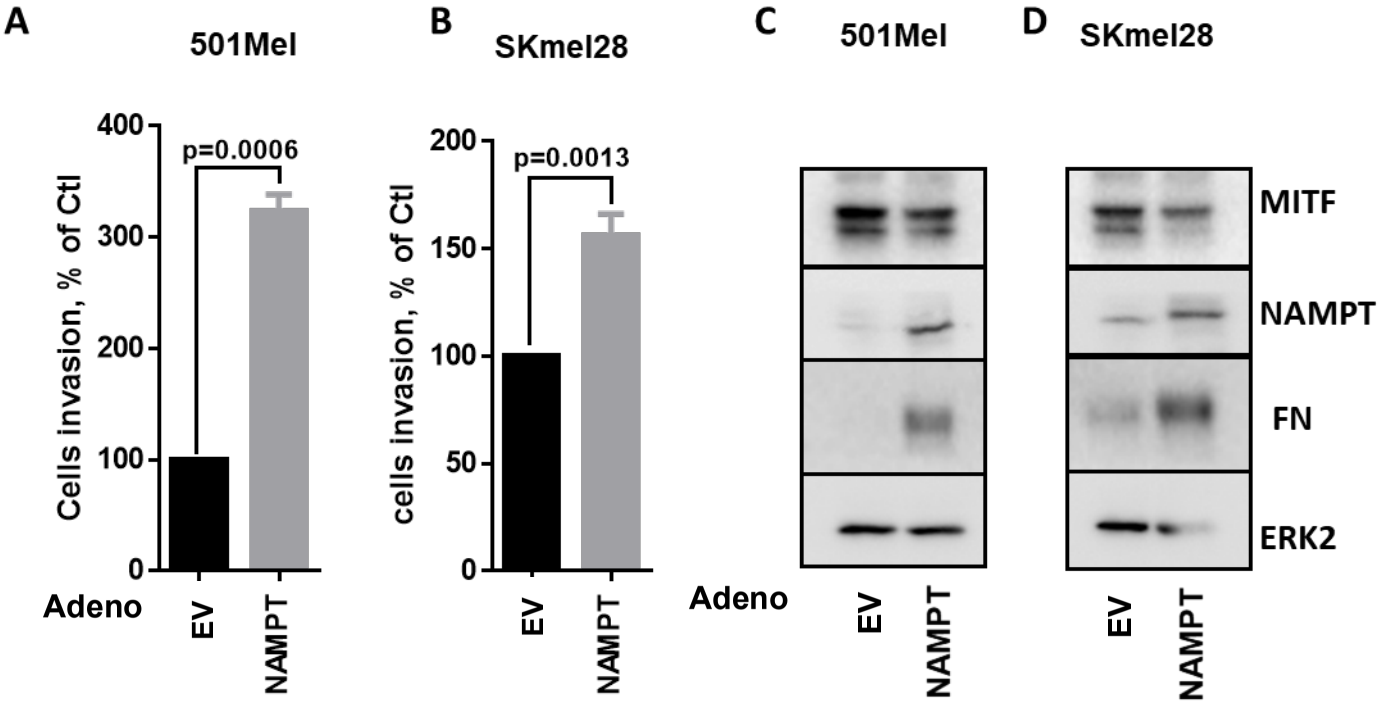


Ohanna_Supplemental Fig12



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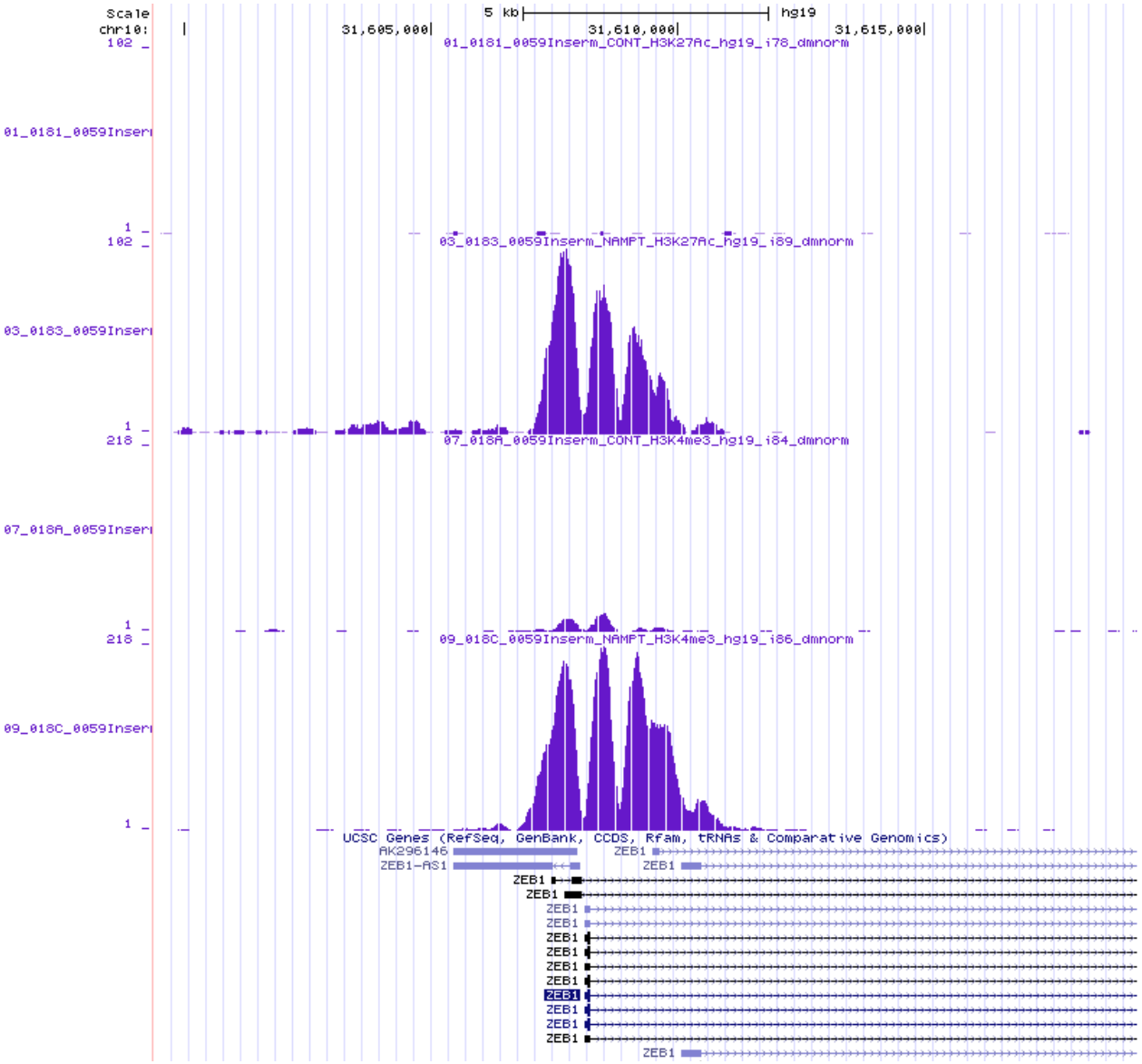

Ohanna_Supplemental Table S1

Metabolite	Log FC	FDR
N6-succinyladenosine	-3.34	0.027
N2-methylguanosine	-2.92	0.018
leucylalanine	-1.97	0.017
N6-carbamoylthreonyladenosine	-1.96	0.017
1-1-enyl-palmitoyl-2-palmitoleoyl-GPC P-160/161	-1.94	0.017
leucylglycine	-1.91	0.017
2'-deoxycytidine	-1.78	0.046
retinol	-1.74	0.028
leucylglutamine	-1.71	0.017
1-linoleoylglycerol 182	-1.68	0.044
1-1-enyl-palmitoyl-2-oleoyl-GPC P-160/181	-1.67	0.017
5-methylcytidine	-1.67	0.017
methionine sulfoxide	-1.66	0.017
2-linoleoylglycerol 182	-1.63	0.041
1-oleoylglycerol 181	-1.62	0.034
1-docosahexaenoylglycerol 226	-1.55	0.048
1-myristoylglycerol 140	-1.49	0.037
valylleucine	-1.41	0.017
valylglutamine	-1.40	0.023
mannose	-1.36	0.017
palmitoleate 161n7	-1.35	0.026
2-oleoylglycerol 181	-1.31	0.038
docosahexaenoate	-1.31	0.030
alpha-lipoate	-1.28	0.017
tryptophylglycine	-1.28	0.032
sulfate	-1.26	0.017
1-oleoyl-GPS 181	-1.23	0.034
eicosapentaenoate	-1.23	0.028
N-acetylmethionine sulfoxide	-1.19	0.030
C-glycosyltryptophan	-1.19	0.032
N-palmitoyl-sphingadienine d182/160	-1.14	0.017
isoleucylglycine	-1.14	0.032
myristoleate 141n5	-1.11	0.017
1-pentadecanoylglycerol 150	-1.11	0.026
docosapentaenoate	-1.08	0.045
erucate 221n9	-1.08	0.038
tyrosylglycine	-1.07	0.044
N-stearoyl-sphingadienine d182/180	-1.06	0.034
linolenate alpha or gamma	-1.05	0.017
heme	-1.05	0.037
eicosenoate 201	-1.03	0.037
N-behenoyl-sphingadienine d182/220	-1.00	0.017
oleoyl ethanolamide	-1.00	0.038
N-stearoyl-sphingosine d181/180	-0.98	0.030
linoleate 182n6	-0.97	0.042
1-palmitoylglycerol 160	-0.96	0.037
N-myristoyl-sphingosine d181/140	-0.95	0.017
2-palmitoleoyl-GPC 161	-0.94	0.017

1-stearoyl-GPI 180	-0.94	0.025
1-1-enyl-oleoyl-GPE P-181	-0.91	0.044
cytidine	-0.89	0.030
phenylalanylglycine	-0.86	0.032
lactosyl-N-nervonoyl-sphingosine d181/241	-0.86	0.032
myristate 140	-0.86	0.023
1-1-enyl-oleoyl-2-linoleoyl	-0.85	0.048
palmitoyl ethanolamide	-0.84	0.017
lactosyl-N-palmitoyl-sphingosine d181/160	-0.84	0.017
oleoyl-oleoyl-glycerol 181/181 1	-0.82	0.017
stearoyl-linoleoyl-glycerol 180/182 1	-0.82	0.028
nicotinamide riboside	-0.80	0.043
carnitine	0.80	0.017
1.2-dipalmitoyl-GPC 160/160	0.82	0.017
1.2-dipalmitoyl-GPG 160/160	0.83	0.028
coenzyme A	0.85	0.017
glutamate	0.91	0.027
deoxycarnitine	0.92	0.017
adenosine 3'.5'-cyclic monophosphate	0.95	0.025
1-palmitoyl-2-stearoyl-GPC 160/180	0.95	0.017
uridine 5'-monophosphate UMP	0.98	0.038
ribitol	0.99	0.017
nicotinamide adenine dinucleotide	1.02	0.026
3-4-hydroxyphenyllactate	1.03	0.023
arabitol/xylitol	1.04	0.032
N-formylmethionine	1.07	0.017
pantothenate	1.09	0.035
guanosine 5'-diphospho-fucose	1.11	0.017
N-acetylserine	1.12	0.028
adenosine 5'-monophosphate	1.14	0.046
N-acetylglutamate	1.15	0.017
phenyllactate	1.21	0.032
uridine 5'-diphosphate	1.24	0.044
3-phosphoglycerate	1.25	0.017
UDP-glucuronate	1.25	0.048
allantoin	1.27	0.037
beta-alanine	1.30	0.032
glutathione. reduced GSH	1.31	0.028
N-acetylcysteine	1.32	0.023
phosphoenolpyruvate	1.32	0.017
glucose 1-phosphate	1.32	0.023
gluconate	1.35	0.029
N-acetylthreonine	1.37	0.017
succinylcarnitine	1.38	0.036
taurine	1.40	0.017
indolelactate	1.41	0.036
N-acetyltaurine	1.44	0.017
N-acetylglutamine	1.44	0.046
cytidine triphosphate	1.46	0.046
N-acetylglucosaminyl asparagine	1.46	0.025
alpha-ketoglutarate	1.47	0.034
cytidine diphosphate	1.48	0.046

5-methyltetrahydrofolate 5MeTHF	1.52	0.036
N-acetylaspartate	1.53	0.030
beta-guanidinopropanoate	1.57	0.044
3-hydroxybutyrylcarnitine 2	1.63	0.017
uridine 5'-triphosphate UTP	1.65	0.019
imidazole lactate	1.66	0.026
ribonate	1.69	0.035
adenosine 5'-diphosphate	1.71	0.032
lactate	1.74	0.017
carnosine	1.76	0.046
glycerate	1.79	0.038
N-acetylglucosamine 6-phosphate	2.04	0.027
hypotaurine	2.07	0.017
cysteinylglycine	2.31	0.026
gamma-glutamylcysteine	2.33	0.047
inosine 5'-monophosphate	2.35	0.035
UDP-glucose	2.57	0.024
acetylphosphate	2.68	0.037
UDP-galactose	2.93	0.023

Ohanna_Supplemental Table S2

Metabolite function	Total Compounds	Hits	Enrichment	Raw p	FDR
VITAMIN B6 METABOLISM	10	5	2.851	0.022	0.046
ARGININE AND PROLINE ME	26	8	2.210	0.018	0.040
INSULIN SIGNALLING	19	5	3.279	0.016	0.037
GLYCEROLIPID METABOLISM	13	4	3.263	0.014	0.034
BETA-ALANINE METABOLISM	13	5	3.265	0.011	0.029
BETAINE METABOLISM	10	5	3.438	0.011	0.029
PENTOSE PHOSPHATE PATH	18	5	4.201	0.009	0.026
NICOTINATE AND NICOTINA	13	4	4.810	0.008	0.025
MITOCHONDRIAL ELECTRON	15	5	3.945	0.008	0.025
ALANINE METABOLISM	6	3	5.395	0.005	0.019
PURINE METABOLISM	45	19	3.041	0.004	0.015
ALPHA LINOLENIC ACID AND	9	6	4.192	0.002	0.010
PYRUVATE METABOLISM	20	4	4.886	0.002	0.009
GLUCOSE-ALANINE CYCLE	12	5	4.925	0.002	0.009
CITRIC ACID CYCLE	23	10	4.780	0.000	0.009
NUCLEOTIDE SUGARS META	9	4	4.879	0.000	0.009
UREA CYCLE	20	11	4.272	0.001	0.009
GLYCINE SERINE AND THREO	26	10	4.145	0.001	0.009
GLUCONEOGENESIS	27	11	4.969	0.001	0.009
PYRIMIDINE METABOLISM	36	18	3.459	0.001	0.009
RNA TRANSCRIPTION	9	6	4.697	0.001	0.009
GLYCOLYSIS	21	9	4.993	0.001	0.009
ASPARTATE METABOLISM	12	6	3.751	0.001	0.009
AMMONIA RECYCLING	18	10	4.181	0.001	0.009
MALATE-ASPARTATE SHUTTI	8	4	4.807	0.000	0.008
BILE ACID BIOSYNTHESIS	49	3	6.610	0.000	0.001
TAURINE AND HYPOTAURINI	7	3	8.453	0.000	0.001

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	p-Value	z-score
Cell movement	1.66E-23	4.065
Migration of cells	5.99E-23	3.435
Development of vasculature	1.40E-17	2.527
Angiogenesis	4.96E-17	2.527
Morbidity or mortality	1.04E-16	-5.451
Organismal death	1.44E-16	-5.395
Cell movement of tumor cell lines	1.60E-16	4.135
Invasion of tumor cell lines	2.81E-16	3.323
Invasion of cells	2.91E-16	3.556
Migration of tumor cell lines	6.73E-15	3.768