Supplementary Information

Chebulinic Acid Suppresses Adipogenesis in 3T3-L1 Preadipocytes by Inhibiting PPP1CB Activity

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MADGELNVDSLITRLLEVRGCRPGKIVQMTEAEVRGLCIKSREIFLSQPILLELEAPLKICGDIHGQYTDLLRLFEYGGFPPEANYLFLGDYVDR
GKQSLETICLLLAYKIKYPENFFLLRGNHECASINRIYGFYDECKRRFNIKLWKTFTDCFNCLPIAAIVDEKIFCCHGGLSPDLQSMEQIRRIMRPT
DVPDTGLLCDLLWSDPDKDVQGWGENDRGVSFTFGADVVSKFLNRHDLDLICRAHQVVEDGYEFFAKRQLVTLFSAPNYCGEFDNAGGMM
SVDETLMCSFQILKPSEKKAKYQYGGLNSGRPVTPPRTANPPKKR

Figure S1. Sequence of purified PPP1CB (1-266) was highlighted in Red color.

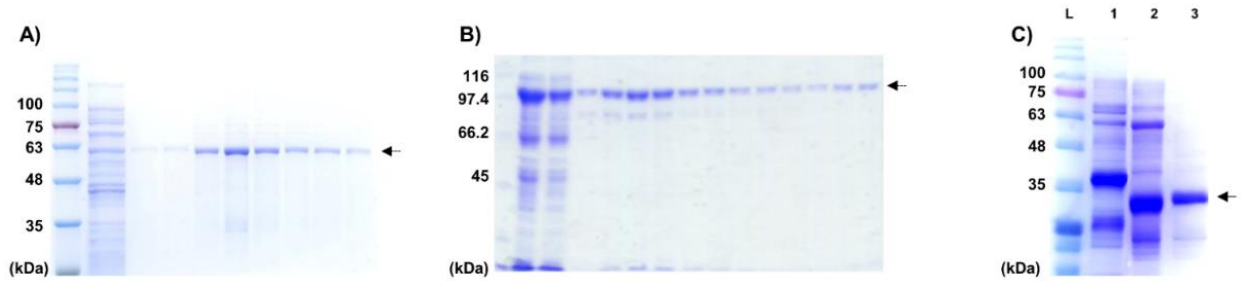

Figure S2. Purified proteins according to the affinity tags. (A) Glutathione-S-transferase (GST)-tagged protein phosphatase-1 catalytic subunit beta (PPP1CB) was purified using the columns packed with glutathione resins. (B) Maltose-binding protein (MBP)-tagged PPP1CB was purified using amylose resins. (C) 1 - 6xHis-tagged PPP1CB was purified using talon resins. 2 - 6xHis tag was cleaved using caspase-3. 6xHis tag and caspase-3 were eliminated using talon resins. 3 - PPP1CB without any affinity tag was subjected to further purification using Superose-6.

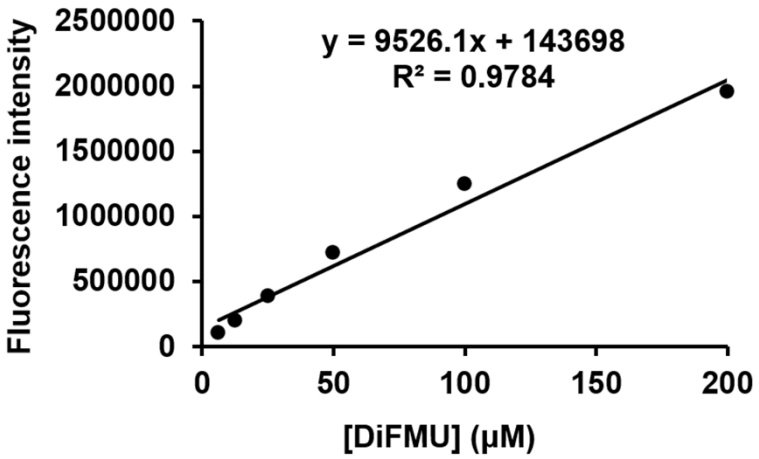

Figure S3. Calibration curve of the fluorescence intensity versus DiFMU from 6.25 to 200 μM. DiFMU with various concentrations (6.25, 12.5, 25, 50, 100, 200 μM) in the reaction buffer (20 mM Tris pH 7.0, 150 mM NaCl, 0.01% Triton X-100, and 1 mM MnCl2) was detected by a Victor X4 Multilabel Plate Reader at excitation and emission wavelengths of 355 and 460 nm.

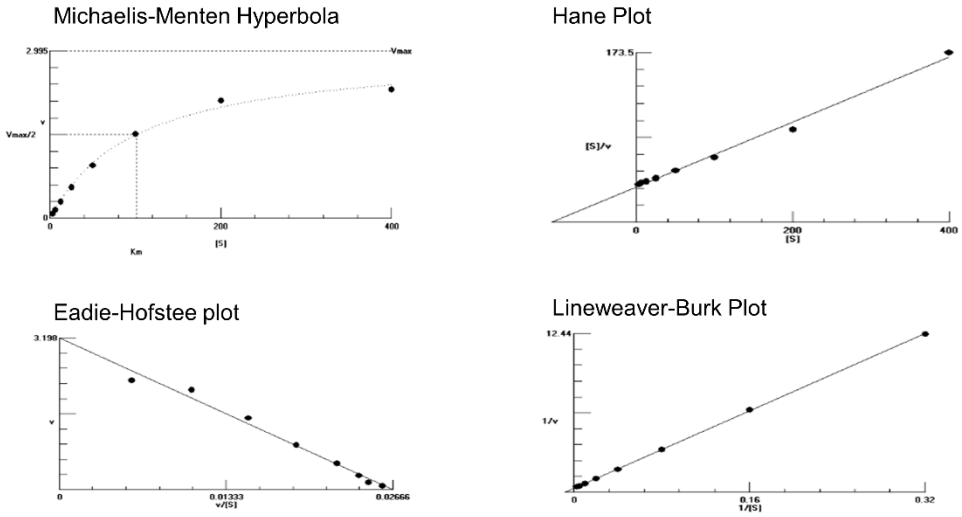

Figure S4. Kinetic graph of GST-Tagged-PPP1CB. Protein was added to reaction buffer containing each concentration of DifMUP (400, 200, 100, 50, 25, 12.5, 6.25, and 3.13 μM).

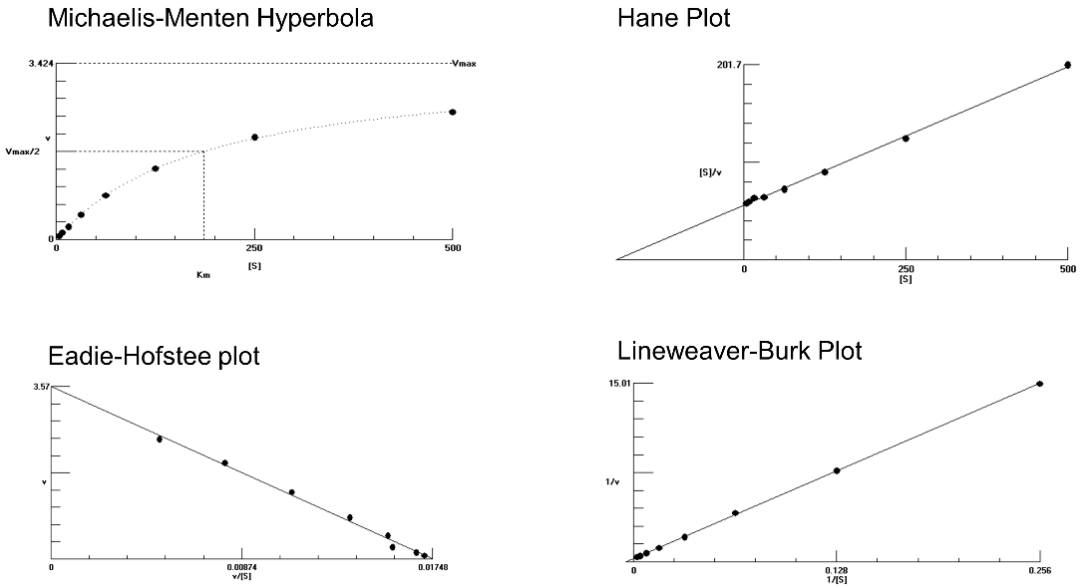

Figure S5. Kinetics graph of PPP1CB without tag. Protein was added to reaction buffer containing each concentration of DifMUP (500, 250, 125, 62.5, 31.25, 15.63, 7.81, and 3.91 μM).

Table S1. Information of compounds inhibiting PPP1CB.

Compound number	Compound name	CAS number
1	1,2,3,4,6-o-Pentagalloylglucose	30964-13-7
53	Acetylshikonin	24502-78-1
65	Alpha-Boswellic acid	471-66-9
69	Amentoflavone	1617-53-4
100	Baicalein	491-67-8
132	Cantharidin	56-25-7
142	Celastrol	34157-83-0
146	Chebulagic acid	23094-71-5
147	Chebulinic acid	18942-26-2
164	Coptisine Chloride	6020-18-4
176	Crocetin	27876-94-4
177	Crocin I	42553-65-1
178	Crocin II	55750-84-0
189	Cyanidin chloride	528-58-5
190	Cyanidin-3-glucoside chloride	7084-24-4
235	Elllagic Acid	476-66-4
242	Epigallocatechin gallate	989-51-5
261	Fisetin	528-48-3
271	Gallic Acid Ethyl Ester	831-61-8
272	Gallocatechin gallate	4233-96-9
284	Geraniin	60976-49-0
331	Hypericin	548-04-9
335	Icariside I	56725-99-6
340	Irinotecan Hydrochloride	100286-90-6
362	Juglone	481-39-0
410	Medicagenic acid	599-07-5
428	Myricetin	529-44-2
429	Myricetrin	17912-87-7
471	Palmatine Chloride	10605-02-4
526	Punicalagin	65995-63-3
527	Punicalin	65995-64-4
528	Purpurin	81-54-9
531	Quercetin 7-rhamnoside	22007-72-3
558	Salvianolic acid C	115841-09-3
574	Scopoletin	92-61-5
576	Scutellarein	529-53-3
582	Sennoside A	81-27-6
583	Sennoside B	128-57-4
670	Ligustilide	81944-09-4
721	Sulforaphene	592-95-0
750	Dimethoxylapigenin	5128-44-9
991	Epiberberine	6873-09-2
1044	Luteolin	491-70-3
1053	Norcantharidin	29745-04-8
1088	Soyasaponin Ba	114590-20-4

Table S2. Sequences of primers.

Gene	Forward (5'-3')	Reverse (5'-3')
Mouse *PPARγ*	AGGGCGATCTTGACAGGAAA	GATCGAAACTGGCACCCTTG
Mouse *C/EBPα*	GTGGACAAGAACAGCAACGAG	TTGACCAAGGAGCTCTCAGG
Mouse *FAS*	GCTGCGGAAACTTCAGGAAA	GAGTTGAGCTGGGTTAGGGT
Mouse *SCD*	CGAGAGAAGGTGAAGACGGT	TAGAGCTTGCAGGAGGGAAC

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Figure S6. Certificate of editing.