Supplementary Materials

EGCG disrupts the LIN28B/Let-7 interaction and reduces neuroblastoma aggressiveness

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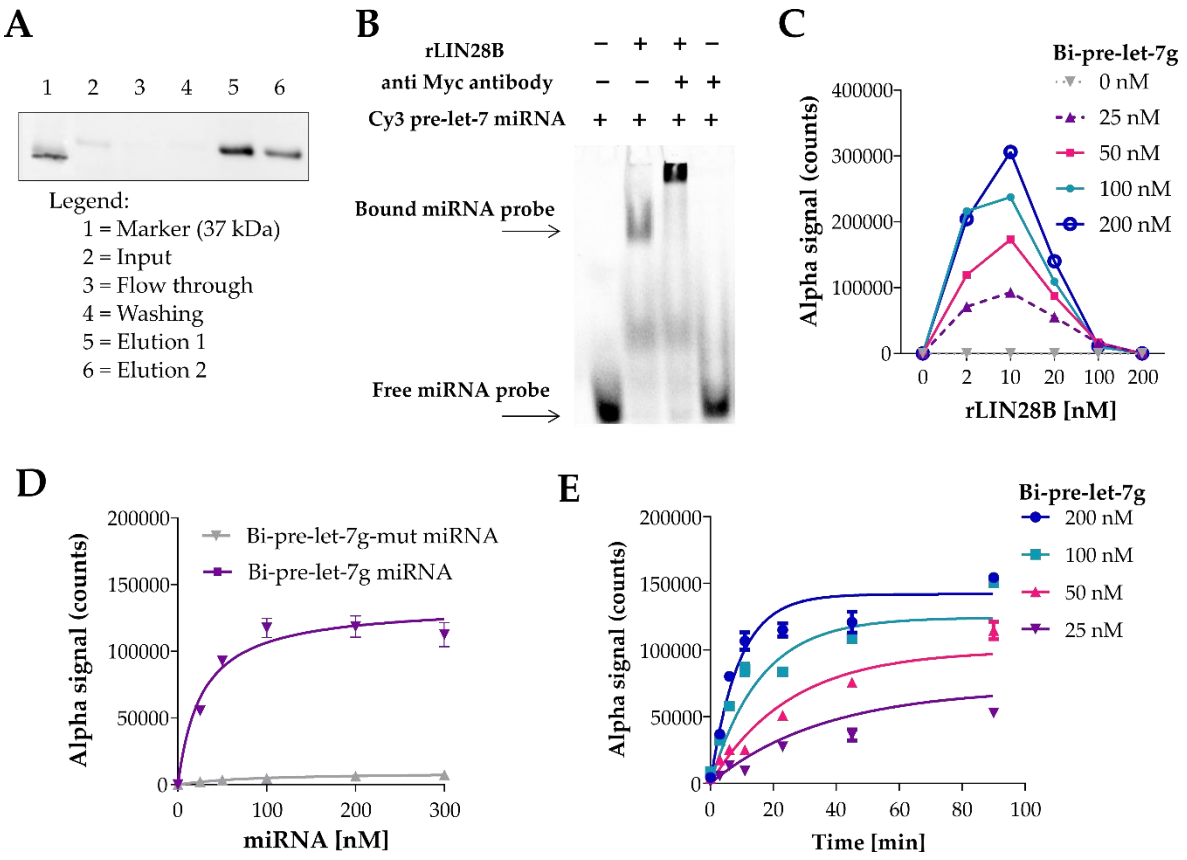

Figure S1. Purification and functional binding of rLIN28B to pre-let-7g miRNA by AlphaScreen and REMSA. (**A**) Immunoblot detection of His6-cMyc-tagged human recombinant LIN28B protein (rLIN28B) produced in HEK293T cells using a monoclonal anti-Myc antibody. (**B**) Representative REMSA results showing the formation of a rLIN28B/Cy3-labelled-pre-let-7g miRNA complex. The free Cy3-labelled-pre-let-7g miRNA probe alone is detected at lower molecular weights. Anti-Myc antibody addition in the binding reaction produces a complex supershift. (**C**) rLIN28B titration curves obtained with different concentrations of the biotinylated pre-let-7g miRNA probe performed to identify the 'hook' point of the AlphaScreen assay. (**D**) Saturation binding experiment to determine the apparent K*d* value for rLIN28Bprotein/biotinylated pre-let-7g miRNA interaction. K*d* = 34.7 ± 3.6 nM. n = 3 replicates. Mean ± SD. (**E**) The time course of the binding reaction showing that the binding of rLIN28B to the biotinylated pre-let-7g miRNA probe is both time and dose-dependent. n = 2 replicates. Mean ± SD.

Table S1. Compounds selected following the primary and the confirmatory Alpha Screenings.

ID	Compound Name	CAS number	Formula	Mol. Wt.	CANONICAL SMILES
EGCG	epigallocatechin-3-monogallate	989-51-5	$C_{22}H_{18}O_{11}$	458.38	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C(=C3)O)O)O)OC(=O)C4=CC(=C(C(=C4)O)O)O
TFMG	theaflavin monogallates	30462-34-1 [3-isomer], 28543-07-9 [3′-isomer]	$C_{36}H_{28}O_{16}$	716.61	C1C(C(OC2=CC(=CC(=C21)O)O)C3=CC(=C(C4=C(C(=O)C=C(C=C34)C5C(CC6=C(C=C(C=C6O5)O)O)OC(=O)C7=CC(=C(C(=C7)O)O)O)O)O)O)O
GA	gallic acid	149-91-7	$C_7H_6O_5$	170.12	C1=C(C=C(C(=C1O)O)O)C(=O)O
ATA	aurin tricarboxylic acid	4431-00-9	$C_{22}H_{14}O_9$	422.35	C1=CC(=C(C=C1C(=C2C=CC(=O)C(=C2)C(=O)O)C3=CC(=C(C=C3)O)C(=O)O)C(=O)O)O
1	terbutaline hemisulfate	23031-32-5	$C_{12}H_{21}NO_7S$	323.37	CC(C)(C)NCC(C1=CC(=CC(=C1)O)O)O.CC(C)(C)NCC(C1=CC(=CC(=C1)O)O)O.OS(=O)(=O)O
2	thioguanine	154-42-7	$C_5H_5N_5S$	167.19	C1=NC2=C(N1)C(=S)N=C(N2)N
3	thioridazine hydrochloride	130-61-0	$C_{21}H_{27}ClN_2S_2$	407.04	CN1CCCCC1CCN2C3=CC=CC=C3SC4=C2C=C(C=C4)SC.Cl
4	suramin hexasodium salt	129-46-4	$C_{51}H_{34}N_6Na_6O_{23}S_6$	1429.19	CC1=C(C=C(C=C1)C(=O)NC2=C3C(=CC(=CC3=C(C=C2)S(=O)(=O)[O-])S(=O)(=O)[O-])S(=O)(=O)[O-])NC(=O)C4=CC(=CC=C4)NC(=O)NC5=CC=CC(=C5)C(=O)NC6=C(C=CC(=C6)C(=O)NC7=C8C(=CC(=CC8=C(C=C7)S(=O)(=O)[O-])S(=O)(=O)[O-])S(=O)(=O)[O-])C.[Na+].[Na+].[Na+].[Na+].[Na+].[Na+]
5	diflubenzuron	35367-38-5	$C_{14}H_9ClF_2N_2O_2$	310.69	C1=CC(=C(C(=C1)F)C(=O)NC(=O)NC2=CC=C(C=C2)Cl)F
6	*N*-hydroxymethylnicotinamide	3569-99-1	$C_7H_8N_2O_2$	152.15	C1=CC(=CN=C1)C(=O)NCO
7	salicylanilide	87-17-2	$C_{13}H_{11}NO_2$	213.24	C1=CC=C(C=C1)NC(=O)C2=CC=CC=C2O
8	dibutyl phthalate	84-74-2	$C_{16}H_{22}O_4$	278.35	CCCCOC(=O)C1=CC=CC=C1C(=O)OCCCC
9	aminosalicylate sodium	6018-19-5	$C_7H_6NNaO_3$	175.12	C1=CC(=C(C=C1N)O)C(=O)[O-].O.O.[Na+]

10	amoxicillin	26787-78-0	$C_{16}H_{19}N_3O_5S$	365.41	CC1(C(N2C(S1)C(C2=O)NC(=O)C(C3=CC=C(C=C3)O)N)C(=O)O)C
11	amphotericin B	1397-89-3	$C_{47}H_{73}NO_{17}$	924.10	CC1C=CC=CC=CC=CC=CC=CC(CC2C(C(CC(O2)(CC(CC(C(CCC(CC(CC(=O)OC(C(C1O)C)C)O)O)O)O)O)O)O)C(=O)O)OC3C(C(C(C(O3)C)O)N)O
12	anthralin	480-22-8, 1143-38-0	$C_{14}H_{10}O_3$	226.23	C1C2=C(C(=CC=C2)O)C(=O)C3=C1C=CC=C3O
13	chloramphenicol	530-43-8	$C_{27}H_{42}Cl_2N_2O_6$	561.55	CCCCCCCCCCCCCCCC(=O)OCC(C(C1=CC=C(C=C1)[N+](=O)[O-])O)NC(=O)C(Cl)Cl
14	chlorcyclizine hydrochloride	1620-21-9	$C_{18}H_{22}Cl_2N_2$	337.30	CN1CCN(CC1)C(C2=CC=CC=C2)C3=CC=C(C=C3)Cl.Cl
15	dapsone	80-08-0	$C_{12}H_{12}N_2O_2S$	248.31	C1=CC(=CC=C1N)S(=O)(=O)C2=CC=C(C=C2)N
16	ethionamide	536-33-4	$C_8H_{10}N_2S$	166.25	CCC1=NC=CC(=C1)C(=S)N
17	telenzepine hydrochloride	80880-90-6	$C_{19}H_{23}ClN_4O_2S$	406.94	CC1=C2C(=CS1)C(=O)NC3=CC=CC=C3N2C(=O)CN4CCN(CC4)C
18	medroxyprogesterone acetate	71-58-9	$C_{24}H_{34}O_4$	386.54	CC1CC2C(CCC3(C2CCC3(C(=O)C)OC(=O)C)C)C4(C1=CC(=O)CC4)C
19	piperazine	110-85-0	$C_4H_{10}N_2$	86.14	C1CNCCN1
20	procaine hydrochloride	51-05-8	$C_{13}H_{21}ClN_2O_2$	272.78	CCN(CC)CCOC(=O)C1=CC=C(C=C1)N.Cl
21	acedapsone	77-46-3	$C_{16}H_{16}N_2O_4S$	332.38	CC(=O)NC1=CC=C(C=C1)S(=O)(=O)C2=CC=C(C=C2)NC(=O)C
22	doxorubicin	23214-92-8	$C_{27}H_{29}NO_{11}$	543.53	CC1C(C(CC(O1)OC2CC(CC3=C2C(=C4C(=C3O)C(=O)C5=C(C4=O)C(=CC=C5)OC)O)(C(=O)CO)O)N)O
23	dehydro (11,12)ursolic acid lactone		$C_{30}H_{46}O_3$	454.70	CC1CCC23CCC4(C5(CCC6C(C)(C(CCC6(C5C=CC4(C3C1C)OC2=O)C)O)C)C)C
24	coralyne chloride	38989-38-7	$C_{22}H_{22}ClNO_4$	399.88	CC1=C2C=C(C(=CC2=CC3=[N+]1C=CC4=CC(=C(C=C43)OC)OC)OC)OC.[Cl-]
25	2',5'-dihydroxy-4-methoxychalcone	6342-92-3	$C_{18}H_{18}O_6$	330.34	COC1=CC=C(C=C1)C=CC(=O)C2=C(C=CC(=C2)O)O

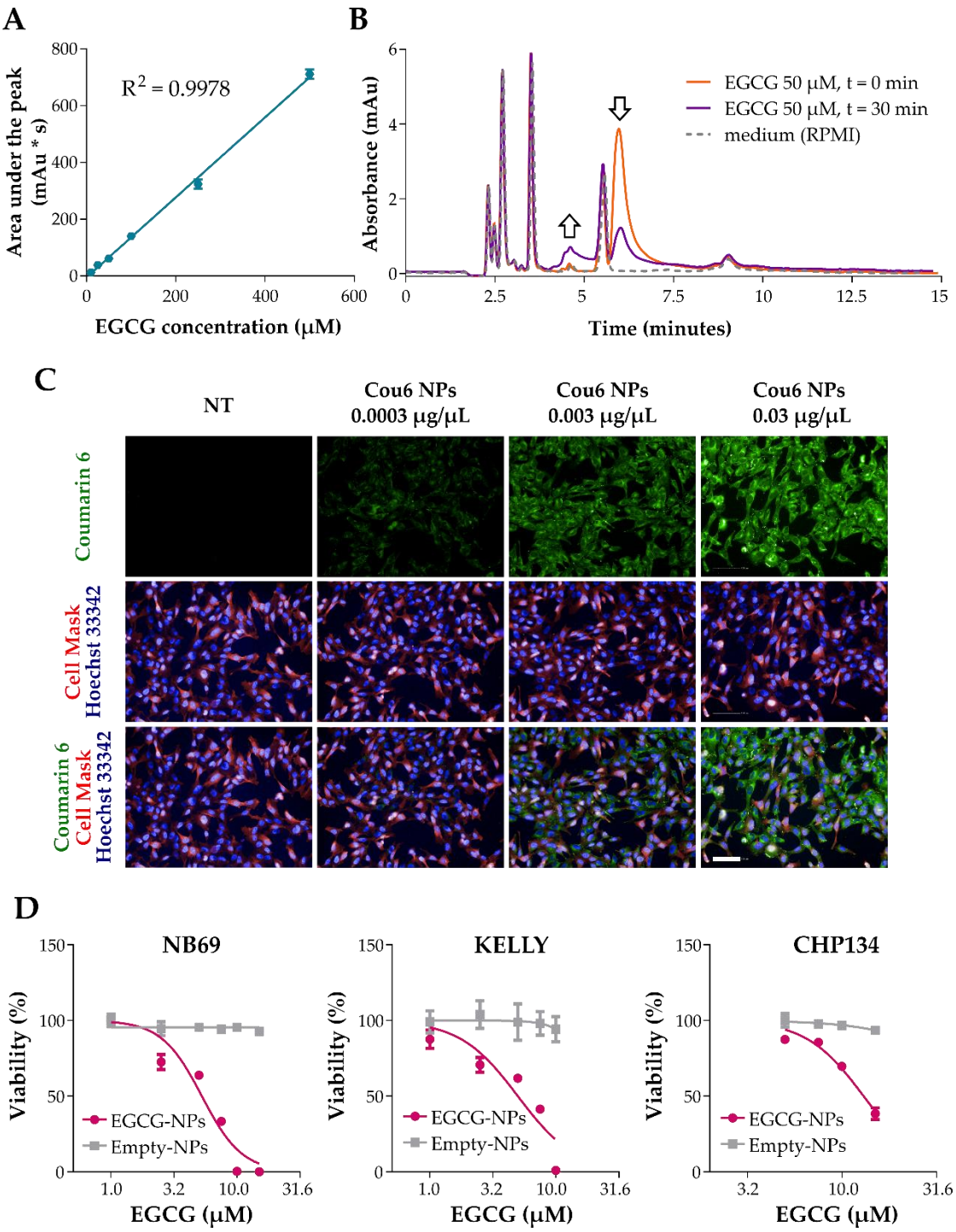

Figure S2. EGCG stability evaluation by HPLC and assessment of EGCG-NP penetration and effect in NB cells. (**A**) EGCG calibration curve. A series of EGCG solutions with different known concentrations (10 μM, 25 μM, 50 μM, 100 μM, 250 μM, 500 μM) was injected in an Agilent 1200 High HPLC system. A Phenomenex Gemini 5 μm C18 110 Å column was used in isocratic conditions with 80:20 water:acetonitrile with 0.01% TFA (pH 4-4.5). The flow rate and the detection were set at 1 mL·min^{-1}, and at 280 nm, respectively. n = 3 replicates, mean ± SD. R^2 = 0.9978. (**B**) Representative HPLC chromatogram of EGCG (50 μM) in RPMI-1640 at t = 0 and 30 minutes. EGCG retention time is 5.9-6.0 min. The chromatogram of the EGCG solution injected at t = 0 min is depicted in orange, whereas that of the EGCG solution at t = 30 min is depicted in purple. Cell culture medium (RPMI-1640) signals remain constant over time (grey). Arrows indicate EGCG instability in aqueous media

and the formation of degradation products. n = 3 replicates. (**C**) Representative immunofluorescence images of NB69 cells treated with different amounts of Cou6-NPs (green). Nuclei were stained with Hoechst 33342 (blue) and the cytoplasm with the CellMask™ Deep Red Stain (red). Images were acquired using the Operetta-High Content Imaging System. Scale bar = 100 µm. (**D**) Representative dose-response curves for EGCG-NPs and empty-NPs on three NB cell lines (NB69, KELLY, CHP134). After 48 h of treatment, cell viability was measured using the CellTiter-Glo® Luminescent Cell Viability Assay. Data are expressed as the percentage of viability normalized on non-treated cells. The graph shows one representative result of n = 3 biological replicates, n = 3 technical replicates each.

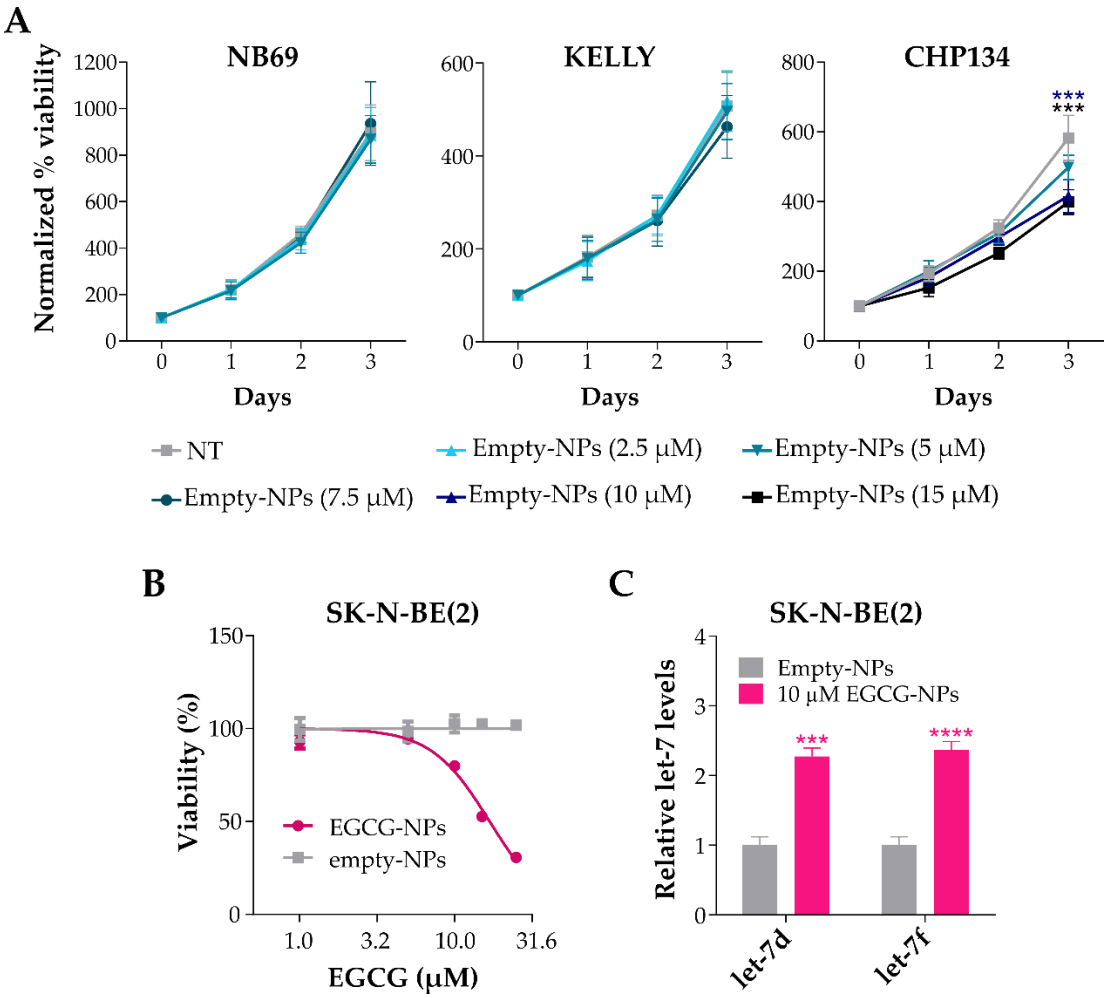

Figure S3. Empty-NP treatment only slightly affects NB cell proliferation, and EGCG-NP treatment increases let-7 miRNA levels in SK-N-BE(2) cells. (**A**) Proliferation curves of NB69, KELLY and CHP134 cells treated with several doses of empty-NPs corresponding to the doses of EGCG-NPs used in Figure 5B. Cell viability was measured using the CellTiter-Glo® Luminescent Cell Viability Assay and normalized on the day of the treatment (day 0). NT = non-treated cells. n = 3 biological replicates, n = 3 technical replicates each. Mean ± SEM. Two-way ANOVA followed by Fisher's LSD test (*** $p < 0.001$). (**B**) Representative dose-response curves for EGCG-NPs and empty-NPs in SK-N-BE(2) cell line. After 48 h of treatment, cell viability was measured using the CellTiter-Glo® Luminescent Cell Viability Assay. Data are expressed as the percentage of viability normalized on non-treated cells. A representative result is shown, n = 2 biological replicates, n = 3 technical replicates each. Mean ± SD. (**C**) qPCR analysis of let7-f and let7-d miRNAs levels in SK-N-BE(2) cells treated with empty-NPs or EGCG-NPs. Expression level is shown as fold change relative to empty-NPs, data were normalised on U6 internal reference gene. n = 3 technical replicates. Mean ± SD. Unpaired two-tailed t-test analysis (*** $p < 0.001$, **** $p < 0.0001$).

Table S2. Primer sequences

Gene	Forward	Reverse
LIN28B	5′ GAGTCAATACGGGTAACAGGAC 3′	5′CACCACAGTTGTAGCATCTATCT 3′
SOX2	5′ GTATCAGGAGTTGTCAAGGCAGAG 3′	5′ CTAGTCTTAAAGAGGCAGCAAAC 3′
SOX9	5′ GTACCCGCACTTGCACAAC 3′	5′ TCTCGCTCTCGTTCAGAAGTC 3′
GAP43	5′ GGCCGCAACCAAAATTCAGG 3′	5′CGGCAGTAGTGGTGCCTTC 3′
TUBB3	5′ TCAGCGTCTACTACAACGAGGC 3′	5′ GCCTGAAGAGATGTCCAAAGGC 3′
NESTIN	5′ CCTCAAGATGTCCCTCAGCC 3′	5′ TCCAGCTTGGGGTCCTGAAA 3′
MYCN	5′ CCGGGCATGATCTGCAA 3′	5′ CCGCCGAAGTAGAAGTCATCTT 3′
TH	5′ TCATCACCTGGTCACCAAGTT 3′	5′ GGTCGCCGTGCCTGTACT 3′
HPRT1	5′ TGACACTGGCAAAACAATGCA 3′	5′ GGTCCTTTTCACCAGCAAGCT 3′
SDHA	5′ TGGGAACAAGAGGGCATCTG 3′	5′ CCACCACTGCATCAAATTCATG 3′

Table S3. Protein purification: buffers composition.

Buffer Name	Composition
Equilibration buffer (EQ)	25 mM Tris-Cl pH 8; 100 mM NaCl; 0.05% NP-40; 1 mM DTT; 3 mM $MgCl_2$; 10 mM imidazole; Protease inhibitors (diluted 1:100)
Wash1 (W1)	25 mM Tris-Cl pH 8; 200 mM NaCl; 0.05% NP-40; 1 mM DTT; 3 mM $MgCl_2$; 50 mM imidazole.
Wash2 (W2)	25 mM Tris-Cl pH 8; 300 mM NaCl; 0.05% NP-40; 1 mM DTT; 3 mM $MgCl_2$; 50 mM imidazole
Wash3 (W3)	25 mM Tris-Cl pH 8; 300 mM NaCl; 0.05% NP-40; 1 mM DTT; 3 mM; $MgCl_2$; 70 mM imidazole
Wash4 (W4)	25 mM Tris-Cl pH 7; 500 mM NaCl; 0.05% NP-40; 1 mM DTT; 3 mM $MgCl_2$; 70 mM Imidazole
Elution buffer (EL)	25 mM Tris-Cl pH 8; 250 mM NaCl; 3 mM $MgCl_2$; 400 mM imidazole
Dialysis buffer	25 mM HEPES pH 7.4; 110 mM KCl; 10Mm NaCl; 1 mM $MgCl_2$; 15 μM $ZnCl_2$; 0.02% Tween; 0.1 % p/v ultrapure BSA
Storage buffer (S)	25 mM HEPES pH 7.4; 110 mM KCl; 10Mm NaCl; 1 mM $MgCl_2$; 15 μM $ZnCl_2$; 0.02% Tween; 0.1 % p/v ultrapure BSA, 15% glycerol, pH 7.5

The purification buffers were prepared in DEPC water.