SUPPLEMENTAL INFORMATION FOR

FUNCTIONAL INTERACTIONS BETWEEN ERYTHROID KRUPPEL-LIKE FACTOR (EKLF/KLF1) AND PROTEIN PHOSPHATASE PPM1B/PP2Cß

Yvette Y. Yien and James J. Bieker

FIGURE S1. **Induction of EKLF and M2-agarose pulldown of EKLF-interacting proteins.** MEL stable cell lines expressing Zn-inducible FL-EKLF were treated with 100 µM Zn for 18 hours to express FL-EKLF. Cell lysates from untreated (-Zn) or treated (+Zn) samples were immunoprecipitated with anti-FLAG M2 agarose. Bound proteins were eluted with FLAG peptide and concentrated using a centricon unit with a 10kDa cutoff. Top: After SDS-PAGE, the gel was fixed with 10% methanol and stained with colloidal blue. Six bands, labelled 1 to 6 as shown, were sliced out of the gel and subjected to mass spectrometric analysis. Molecular weight markers are on the left, and major proteins in each band on the right (see Table S1). Bottom: 5% of cell lysate were subjected to SDS-PAGE electrophoresis and immunolotting with M2-HRP (Sigma) to verify that FL-EKLF was induced.

FIGURE S2. **EKLF likely interacts with multiple isoforms of Ppm1b.** Amino acid alignment of the four Ppm1b isoforms are shown. Of the 10 Ppm1b peptides identified from the gel in Fig. S1 (and tabulated in Table S2), 9 peptide sequences are present in all 4 isoforms; one is unique to the largest isoform (isoform 1).

TABLE S1. **Proteins identified by mass spectrometry in FL-EKLF pulldown experiment shown in Fig. S1.** Accession numbers are given for protein identity in the International Protein Index, http://www.ebi.ac.uk/IPI.

TABLE S2. **Peptides corresponding to non-keratin proteins that were identified by mass spectrometry from the gel in Fig. S1.**

TABLE S3. **Nucleotide sequences for siPpm1b that were used for nucleofection in Fig. 2.**

FIGURE S1

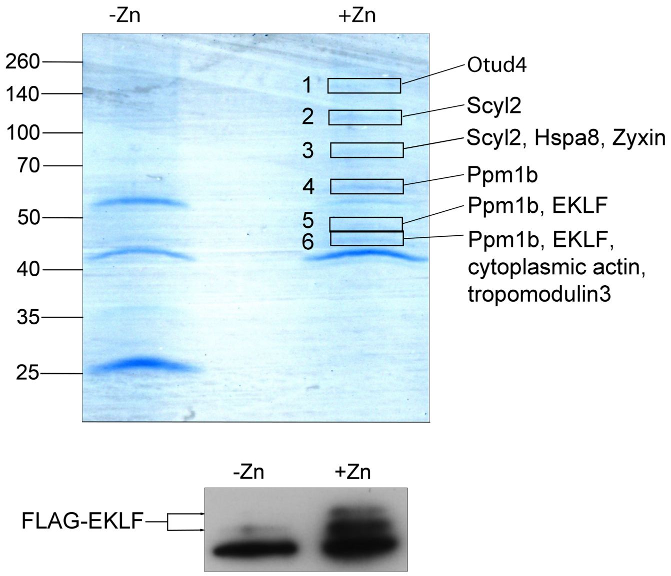
-Zn
+Zn
260
140
100
70
50
40
35
25
1
2
3
4
5
6
Otud4
Scyl2
Scyl2, Hspa8, Zyxin
Ppm1b
Ppm1b, EKLF
Ppm1b, EKLF, cytoplasmic actin, tropomodulin3
-Zn
+Zn
FLAG-EKLF

FIGURE S2

Alignment of mouse Ppm1b isoforms

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isoform_2       MGAFLDKPKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAVVGIPHGLDNWSFFAVYD 60
isoform_1       MGAFLDKPKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAVVGIPHGLDNWSFFAVYD 60
isoform_3       MGAFLDKPKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAVVGIPHGLDNWSFFAVYD 60
Isoform_4       MGAFLDKPKTEKHNAHGAGNGLRYGLSSMQGWRVEMEDAHTAVVGIPHGLDNWSFFAVYD 60
                ************************************************************

isoform_2       GHAGSRVANYCSTHLLEHITTNEDFRAADKSGSALEPSVESVKTGIRTGFLKIDEYMRNF 120
isoform_1       GHAGSRVANYCSTHLLEHITTNEDFRAADKSGSALEPSVESVKTGIRTGFLKIDEYMRNF 120
isoform_3       GHAGSRVANYCSTHLLEHITTNEDFRAADKSGSALEPSVESVKTGIRTGFLKIDEYMRNF 120
Isoform_4       GHAGSRVANYCSTHLLEHITTNEDFRAADKSGSALEPSVESVKTGIRTGFLKIDEYMRNF 120
                ************************************************************

isoform_2       SDLRNGMDRSGSTAVGVMVSPTHMYFINCGDSRAVLCRNGQVCFSTQDHKPCNPVEKERI 180
isoform_1       SDLRNGMDRSGSTAVGVMVSPTHMYFINCGDSRAVLCRNGQVCFSTQDHKPCNPVEKERI 180
isoform_3       SDLRNGMDRSGSTAVGVMVSPTHMYFINCGDSRAVLCRNGQVCFSTQDHKPCNPVEKERI 180
Isoform_4       SDLRNGMDRSGSTAVGVMVSPTHMYFINCGDSRAVLCRNGQVCFSTQDHKPCNPVEKERI 180
                ************************************************************

isoform_2       QNAGGSVMIQRVNGSLAVSRALGDYDYKCVDGKGPTEQLVSPEPEVYEIVRAEEDEFVVL 240
isoform_1       QNAGGSVMIQRVNGSLAVSRALGDYDYKCVDGKGPTEQLVSPEPEVYEIVRAEEDEFVVL 240
isoform_3       QNAGGSVMIQRVNGSLAVSRALGDYDYKCVDGKGPTEQLVSPEPEVYEIVRAEEDEFVVL 240
Isoform_4       QNAGGSVMIQRVNGSLAVSRALGDYDYKCVDGKGPTEQLVSPEPEVYEIVRAEEDEFVVL 240
                ************************************************************

isoform_2       ACDGIWDVMSNEELCEFVKSRLEVSDDLENVCNWVVDTCLHKGSRDNMSVVLVCFSNAPK 300
isoform_1       ACDGIWDVMSNEELCEFVKSRLEVSDDLENVCNWVVDTCLHKGSRDNMSVVLVCFSNAPK 300
isoform_3       ACDGIWDVMSNEELCEFVKSRLEVSDDLENVCNWVVDTCLHKGSRDNMSVVLVCFSNAPK 300
Isoform_4       ACDGIWDVMSNEELCEFVKSRLEVSDDLENVCNWVVDTCLHKGSRDNMSVVLVCFSNAPK 300
                ************************************************************

isoform_2       VSEEAVKRDSELDKHLESRVEEIMQKSGEEGMPDLAHVMRILSAENIPNLPPGGGLAGKR 360
isoform_1       VSEEAVKRDSELDKHLESRVEEIMQKSGEEGMPDLAHVMRILSAENIPNLPPGGGLAGKR 360
isoform_3       VSEEAVKRDSELDKHLESRVEEIMQKSGEEGMPDLAHVMRILSAENIPNLPPGGGLAGKR 360
Isoform_4       VSEEAVKRDSELDKHLESRVEEIMQKSGEEGMPDLAHVMRILSAENIPNLPPGGGLAGKR 360
                ************************************************************

isoform_2       HVIEAVYSRLNPHKDNDGGAGDLEDS------LVAL------------------------ 390
isoform_1       HVIEAVYSRLNPHKDNDGASDEAEEGGSQGKLVEALRQMRVNHRGNYRQLLEEMLTSYRL 420
isoform_3       HVIEAVYSRLNPHKDNDGMADLSTS-------ICKPS----------------------- 390
isoform_4       HVIEAVYSRLNPHKDNDGFYQPSIAYSDN---VFLL------------------------ 393
                ******************              :

isoform_2       ---------------------------------------------------------
isoform_1       AKVEGEESPADPAAAAASSNSDGGNPVAMQERDTEGGPAGLDSRNEDAGTKRSAENI 477
isoform_3       ---------------------------------------------------------
Isoform_4       ---------------------------------------------------------
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SUPPLEMENTARY TABLE 1

B01		
Identified Proteins (4)	**Accession Number**	**Molecular Weight**
Keratin, type II cytoskeletal 1	IPI00625729	66 kDa
Keratin complex 1, acidic, gene 10	IPI00755181 (+2)	57 kDa
Otud4 protein (Otud 4)	IPI00137459	123 kDa
Keratin type I cytoskeletal 19	IPI00112947 (+3)	45 kDa
B02		
Identified Proteins (5)	**Accession Number**	**Molecular Weight**
Isoform 1 of SCY1-like protein 2 (Scyl 2)	IPI00229287	103 kDa
Keratin complex 1, acidic, gene 10	IPI00755181 (+2)	57 kDa
Keratin, type II cytoskeletal 1	IPI00625729	66 kDa
Keratin, type II cytoskeletal 79	IPI00124499	58 kDa
Krt19 Keratin, type I cytoskeletal 19	IPI00112947 (+3)	45 kDa
B03		
Identified Proteins (6)	**Accession Number**	**Molecular Weight**
Zyxin	IPI00387422 (+1)	61 kDa
Keratin, type II cytoskeletal 1	IPI00625729	66 kDa
Heat shock cognate 71 kDa protein (Hspa 8)	IPI00323357 (+2)	71 kDa
Isoform 1 of SCY1-like protein 2 (Scyl 2)	IPI00229287	103 kDa
Keratin 15, isoform CRA_a	IPI00225378 (+1)	49 kDa
Keratin, type II cytoskeletal 2 epidermal	IPI00622240 (+1)	71 kDa
B04		
Identified Proteins (4)	**Accession Number**	**Molecular Weight**
Ppm1b, Protein phosphatase 1B	IPI00118736	52 kDa
Keratin, type II cytoskeletal 5	IPI00139301	62 kDa
Keratin, type II cytoskeletal 1	IPI00625729	66 kDa
Keratin complex 1, acidic, gene 10	IPI00755181 (+2)	57 kDa
B05		
Identified Proteins (4)	**Accession Number**	**Molecular Weight**
Keratin complex 1, acidic, gene 10	IPI00755181 (+2)	57 kDa
Ppm1b, Protein phosphatase 1B	IPI00118736 (+5)	52 kDa
Keratin, type II cytoskeletal 1	IPI00625729	66 kDa
Keratin, type I cytoskeletal 42	IPI00468696	50 kDa
Kruppel-like factor 1 (EKLF)	IPI00230364	40 kDa
B06		
Identified Proteins (6)	**Accession Number**	**Molecular Weight**
Ppm1b, Isoform Beta-1 of Protein phosphatase 1B	IPI00876176	43 kDa
Actin, cytoplasmic 1	IPI00110850	42 kDa
Keratin, type II cytoskeletal 1	IPI00625729	66 kDa
Tropomodulin-3	IPI00119478	40 kDa
Keratin, type II cytoskeletal 5	IPI00139301	62 kDa
Keratin 15, isoform CRA_a	IPI00225378	49 kDa
Klf1 Kruppel-like factor 1 (EKLF)	IPI00230364	40 kDa

SUPPLEMENTARY TABLE 2

Protein name	Protein accession numbers	No. unique peptides	Peptide sequence	Best Peptide identification probability	Peptide start index	Peptide stop index
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	ALGDYDYK	95.00%	201	208
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	DSELDKHLESR	95.00%	309	319
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	GPTEQLVSPEPEVYEIVR	95.00%	214	231
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	HVIEAVYSR	95.00%	361	369
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	ILSAENIPNLPPGGGLAGK	95.00%	341	359
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	IQNAGGSVMIQR	95.00%	180	191
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	QLLEEMLTSYR	95.00%	409	419
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	SGEEGMPDLAHVMR	95.00%	327	340
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	VNGSLAVSR	95.00%	192	200
IPI:IPI00118736.3 Tax_Id=10090 Gene_Symbol=Ppm1b Putative uncharacterized protein	IPI00118736	10	YGLSSMQGWR	95.00%	24	33
IPI:IPI00387422.3 Tax_Id=10090 Gene_Symbol=Zyx zyxin	IPI00387422,IPI00408218	6	FAPVAPK	95.00%	257	263
IPI:IPI00387422.3 Tax_Id=10090 Gene_Symbol=Zyx zyxin	IPI00387422,IPI00408218	6	FAPVVAPKPK	95.00%	26	35
IPI:IPI00387422.3 Tax_Id=10090 Gene_Symbol=Zyx zyxin	IPI00387422,IPI00408218	6	FSPGAPSGPGPQPNQK	95.00%	271	286
IPI:IPI00387422.3 Tax_Id=10090 Gene_Symbol=Zyx zyxin	IPI00387422,IPI00408218	6	GPLSQAPTPAPK	95.00%	245	256
IPI:IPI00387422.3 Tax_Id=10090 Gene_Symbol=Zyx zyxin	IPI00387422,IPI00408218	6	SPGGPGPLTLK	95.00%	336	346
IPI:IPI00387422.3 Tax_Id=10090 Gene_Symbol=Zyx zyxin	IPI00387422,IPI00408218	6	VNPFRPGDSEPPVAAGAQR	95.00%	36	54
IPI:IPI00229287.3 Tax_Id=10090 Gene_Symbol=Scyl2 Isoform 1 of SCY1-like protein 2	IPI00229287	5	LGSSSLTSIPEEVR	95.00%	288	301
IPI:IPI00229287.3 Tax_Id=10090 Gene_Symbol=Scyl2 Isoform 1 of SCY1-like protein 2	IPI00229287	5	LILPELGPVFK	95.00%	408	418
IPI:IPI00229287.3 Tax_Id=10090 Gene_Symbol=Scyl2 Isoform 1 of SCY1-like protein 2	IPI00229287	5	QEVAVFVFDKK	95.00%	59	69
IPI:IPI00229287.3 Tax_Id=10090 Gene_Symbol=Scyl2 Isoform 1 of SCY1-like protein 2	IPI00229287	5	VTADVTSAVMGNPVTR	95.00%	15	30
IPI:IPI00229287.3 Tax_Id=10090 Gene_Symbol=Scyl2 Isoform 1 of SCY1-like protein 2	IPI00229287	5	YGLLQVSEGLSFLHSSVK	95.00%	156	173
IPI:IPI00110850.1 Tax_Id=10090 Gene_Symbol=Actb Actin, cytoplasmic 1	IPI00110850	4	AGFAGDDAPR	95.00%	19	28
IPI:IPI00110850.1 Tax_Id=10090 Gene_Symbol=Actb Actin, cytoplasmic 1	IPI00110850	4	EITALAPSTMK	95.00%	316	326
IPI:IPI00110850.1 Tax_Id=10090 Gene_Symbol=Actb Actin, cytoplasmic 1	IPI00110850	4	GYSFTTTAER	95.00%	197	206
IPI:IPI00110850.1 Tax_Id=10090 Gene_Symbol=Actb Actin, cytoplasmic 1	IPI00110850	4	VAPEEHPVLLTEAPLNPK	95.00%	96	113
IPI:IPI00119478.1 Tax_Id=10090 Gene_Symbol=Tmod3 Tropomodulin-3	IPI00119478	3	MLEENTNILK	95.00%	308	317
IPI:IPI00119478.1 Tax_Id=10090 Gene_Symbol=Tmod3 Tropomodulin-3	IPI00119478	3	QQLGTSVELEMAK	95.00%	295	307
IPI:IPI00119478.1 Tax_Id=10090 Gene_Symbol=Tmod3 Tropomodulin-3	IPI00119478	3	SNDPVAVAFADMLK	95.00%	239	252
IPI:IPI00137459.6 Tax_Id=10090 Gene_Symbol=Otud4 Otud4 protein	IPI00137459	2	AVAEQVLHSQSR	95.00%	49	60
IPI:IPI00137459.6 Tax_Id=10090 Gene_Symbol=Otud4 Otud4 protein	IPI00137459	2	KADIDLASGSSAVEGK	95.00%	939	954
IPI:IPI00230364.7 Tax_Id=10090 Gene_Symbol=Klf1 Kruppel-like factor 1	IPI00230364	2	AGSVGGFFPR	95.00%	187	196
IPI:IPI00230364.7 Tax_Id=10090 Gene_Symbol=Klf1 Kruppel-like factor 1	IPI00230364	2	SEETQDLGPGPPNPTGPSL	95.00%	51	74
IPI:IPI00323357.3 Tax_Id=10090 Gene_Symbol=Hspa8 Heat shock cognate 71 kDa protein	IPI00323357,IPI00457741,IPI	2	DAGTIAGLNVLR	95.00%	160	171
IPI:IPI00323357.3 Tax_Id=10090 Gene_Symbol=Hspa8 Heat shock cognate 71 kDa protein	IPI00323357,IPI00457741,IPI	2	TTPSYVAFTDTER	95.00%	37	49

SUPPLEMENTARY TABLE 3

	Catalog no.	Target sequence
si1	SI02759197	TAGCCTAACTACACACATCAA
si2	SI02658677	CGACATAACATGAGTATTGTA
si3	SI04436362	ATCACAAACCTTGCAATCCAA