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Small Interfering RNA Screen for Phosphatases Involved in IgE-Mediated Mast Cell Degranulation

Juan Zhang, Mary Mendoza, Michel F. Guiraldelli, Emilia Alina Barbu, and Reuben P. Siraganian

Mast cells play pivotal roles in the initiation of the allergic response. To gain an understanding of the functions played by phosphatases in IgE-mediated mast cell activation, a small interfering RNA (siRNA) library that targets all mouse phosphatase genes was screened in a mouse mast cell line, MMC-1. Of 198 targets, 10 enhanced and 7 inhibited FcεRI-induced degranulation. For seven of the strongest hits, four different siRNAs per target were tested, and at least two out of the four single siRNA per target had similar effects as the pool suggesting that these were true hits. Bone marrow-derived mast cells from normal mice further validated these results for six definite positive targets. The mechanism of the reduced mast cell degranulation due to calcineurin B deficiency was investigated. Calcineurin B deficiency reduced the phosphorylation of MAPKs and the phosphorylation of protein kinase D/protein kinase C μ and protein kinase C δ , which are involved in FcεRI signaling. The screen, therefore, has identified several new molecules that are critical for FcεRI-induced degranulation. Regulating the function of these proteins may be potential targets for the treatment of allergic inflammation. The result also indicates that the system used is efficient for searching molecules implicated in complex receptor-induced signaling. *The Journal of Immunology*, 2010, 184: 000–000.

Mast cells play an important role in allergic reactions and IgE-associated immune responses. Aggregation of FcεRI on these cells initiates a biochemical cascade, which eventually results in mast cell functional responses, such as degranulation and release of inflammatory mediators (1–5). The activation pathway leading from FcεRI stimulation to degranulation is a complex one that involves many molecules, some of which are directly involved in the release of granules, whereas others regulate this process. The role of several molecules has been established by using cell lines that are deficient in one of these molecules. Such cells are usually derived from either in vitro selection of established cell lines or from genetically modified mice that lack those molecules (6–16). However, the development of small interfering RNA (siRNA) technology has made it possible to perform genetic screens in mammalian cell lines, although such screening has not been commonly applied to such complex pathways.

Protein phosphorylation is one of the earliest detectable events after FcεRI aggregation and plays an essential role in this signal transduction pathway. The extent of FcεRI-induced phosphorylation is regulated by the balance between protein kinases and phosphatases. Significant advances have been made recently in understanding the functions of kinases. It is clear now that many

kinases, for example, Lyn, Fyn, Syk, Btk, PI3K, MAPK, sphingosine kinase, and different isoforms of protein kinase C are involved in FcεRI signal transduction (17–27). However, there is only limited information about the role of protein phosphatases in mast cells. So far, studies of phosphatases have been restricted to several well-characterized molecules, such as Src homology region 2 domain-containing phosphatase (SHP) 1, SHP2, SHIP1, SHIP2, and phosphatase and tensin homologue deleted on chromosome 10 (PTEN) (6, 28–33). We therefore used an siRNA screening approach using a 198-member siRNA library to identify phosphatase genes that are involved in FcεRI-stimulated mast cell degranulation. The screen identified several molecules that regulate FcεRI-induced degranulation; the reduced expression of these molecules either enhanced or inhibited degranulation. Most of these molecules had not been recognized as having a role in this pathway. The results also indicate that this is an efficient system for screening for molecules that are important in complex immune-receptor signaling pathways leading to granular secretion.

Materials and Methods

Abs

The HRP-conjugated anti-phosphotyrosine Ab (PY20), anti-protein kinase C (PKC) δ and anti-protein kinase D (PKD)/PKC μ Abs were purchased from BD Biosciences (San Jose, CA). The anti-Syk (N-19), anti-SHIP1, anti-SHIP2, anti-SHP1, anti-SHP2, and anti-phospho-JNK Abs were from Santa Cruz Biotechnology (Santa Cruz, CA); the anti-phospho-p44/42 MAPK, anti-p44/42 MAPK, anti-phospho-p38, anti-p38, anti-phospho-PKC δ (Ser643), anti-phospho-PKC δ (Thr505), and anti-phospho-PKD/PKC μ (Ser916) Abs were from Cell Signaling Technology (Beverly, MA); anti-JNK and anti-PTEN Abs were from Upstate Biotechnology (Lake Placid, NY); anti-calcineurin B Ab was from Abcam (Cambridge, MA). All other Abs used were previously described (34).

Cell culture

The mouse mast cell line MMC-1 (CXBI-I-CA5) was maintained in DMEM supplemented with 20% heat-inactivated FBS, 2 mM L-glutamine, 5×10^{-5} M 2-ME, 10% NCTC 109 media, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and antibiotics as described previously (35). Cells were subcultured every 2 to 3 d to maintain their high viability and good degranulation response.

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Abbreviations used in this paper: BMDC, bone marrow-derived mast cell; CsA, cyclosporin A; PI, phosphatidylinositol; PI(3,4,5)P $_3$, phosphatidylinositol 3,4,5-trisphosphate; PI(4,5)P $_2$, phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PKD, protein kinase D; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SHP, Src homology region 2 domain-containing phosphatase; siRNA, small interfering RNA.

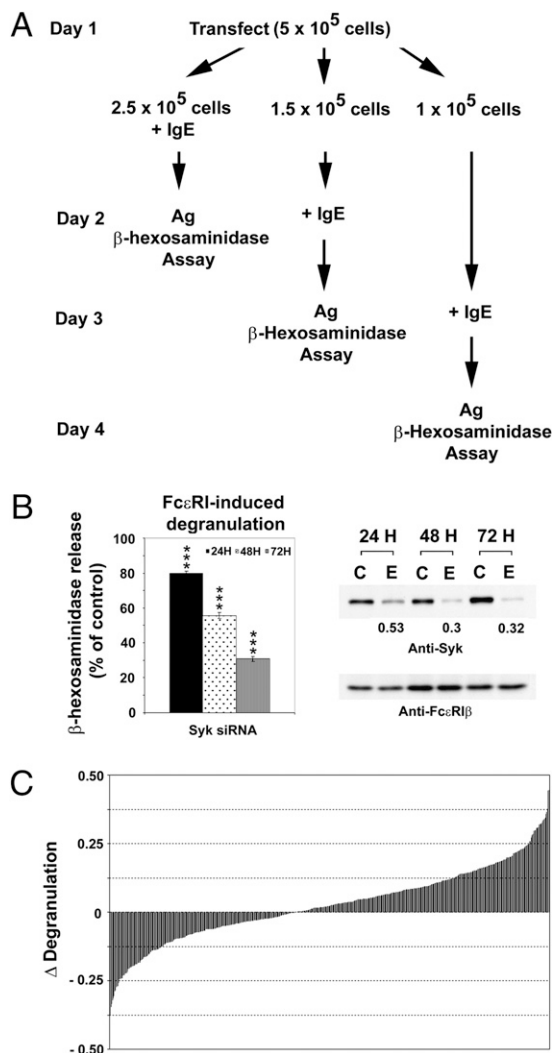


FIGURE 1. siRNA screening of FcεRI-stimulated degranulation in mast cells. **A**, Experimental scheme for functional analysis of genes involved in FcεRI signaling. Posttransfection, cells were divided into three aliquots as indicated, cultured with IgE, and at 24, 48 and 72 h tested for Ag-induced β-hexosaminidase release. **B**, Syk was used as positive control. Cells transfected with control scrambled (C) or Syk (E) siRNA were sensitized with IgE and then stimulated by Ag as outlined in **A**. The supernatants were used for β-hexosaminidase assay and the cell pellets for immunoblotting. Values are mean $\pm$ SEM. $n > 20$. Immunoblots are representative of >10 separate experiments. The fraction of the protein left in the transfected cells compared with the controls was determined by densitometry postnormalization for gel loading. The Ag-induced β-hexosaminidase release in the Syk-transfected cells is expressed as a percentage of that in the control cells. **C**, Distribution of degranulation indices of library screening. The degranulation indices in transfected cells were calculated as described in *Materials and Methods*, with that of control cells as 0 and inhibition (–) or enhancement (+) expressed as a fraction of the control release. The result was sorted from negative to positive and includes all data from all 3 d of assays (24 h, 48 h, and 72 h) for each siRNA pool. The Ag-induced β-hexosaminidase release for control cells transfected with scrambled siRNA were $37\% \pm 5.5\%$ (SD) at 24 h, $31\% \pm 4.4\%$ (SD) at 48 h, and $31\% \pm 3.5\%$ (SD) at 72 h.

Bone marrow cells from C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were cultured in the same medium supplemented with 30 ng/ml IL-3 and 25 ng/ml stem cell factor. Bone marrow-derived mast cells (BMMCs) were used for these experiments after 5–7 wk of culture. The usage of mice has been reviewed and approved by National Institute of Dental and Craniofacial Research institutional review committee (Bethesda, MD).

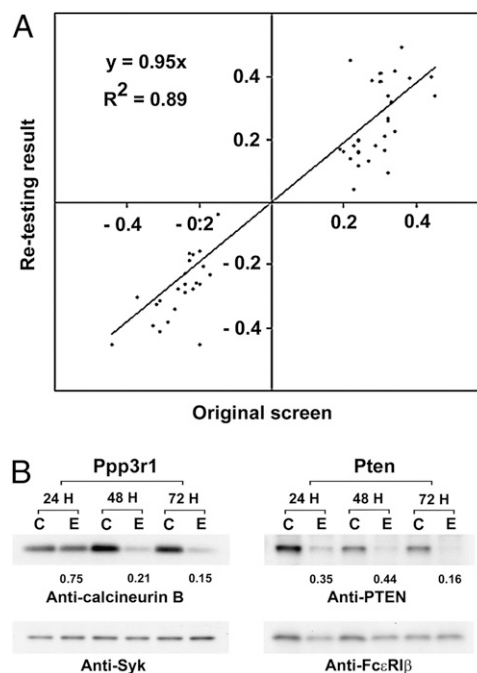


FIGURE 2. Confirmation of positive pools. **A**, Based on the library screening, all siRNA pools that enhanced or inhibited degranulation ≥ 1.5 times the SD were selected for retesting. The change in Ag-induced degranulation is expressed as a fraction of control release as described in *Materials and Methods*; decrease (minus) or increase (positive) as a fraction of total release. The maximum degranulation indices of the repeat experiments were plotted against that of original library screening. The equation and R^2 value of the trend line are shown in the plot. **B**, After the degranulation assay, cells transfected with the indicated siRNA were analyzed by immunoblotting with the corresponding Abs to show the siRNA-induced protein knockdown. Anti-Syk or anti-FcεRIβ was used as loading controls. Data are representative of at least two separate experiments.

siRNA transfection

The phosphatase siRNA library was purchased from Dharmacon (Chicago, IL). The library contains pools of four siRNA duplexes per gene that target 198 mouse enzymes with known or predicted phosphatase activity. The list of genes, Entrez gene ID, locus number, catalog number, and sequences can be found in Supplemental Table I. Negative control siRNA was the siCONTROL nontargeting pool that contains four nontargeting siRNAs, which has minimal targeting of known genes in human, mouse, and rat cells (Dharmacon). Mouse *Syk* (NM_011518) siGENOME SMARTpool was also from Dharmacon. For transfection, cells were seeded in 96-well dishes and transfected with $\sim 0.53 \mu\text{g}$ siRNA/ 10^6 cells using the Amaxa Nucleofector 96-well Shuttle System according to the manufacturer's protocols (Amaxa Biosystems, Cologne, Germany) using the FF-138 program for MMC-1 cells and DC-100 for the BMMCs. In each experiment, there were negative control cells transfected with scrambled siRNA and positive control cells with siRNA for *Syk*.

Cell stimulation

Cells were sensitized with $0.3 \mu\text{g/ml}$ Ag-specific IgE (anti-trinitrophenyl mAb) in culture medium for 24 h. For cell activation, MMC-1 cells were incubated for 45 min at 37°C with DMEM-TB (DMEM containing 0.1% BSA and 10 mM Tris [pH 7.5]) with or without 30 ng/ml of Ag, whereas BMMCs were stimulated with 3 ng/ml of Ag for 45 min. Duplicate wells were used for each treatment with a reaction volume of $200 \mu\text{l/well}$. The overview of the procedures used for sensitization and stimulation is summarized in Fig. 1A.

The measurement of degranulation and immunoblotting

Poststimulation, cells were centrifuged, and the supernatants were used for measuring the enzymatic activities of β-hexosaminidase. The cell pellets from wells incubated only with DMEM-TB were solubilized with 1% Triton X-100 in DMEM-TB (blanks) and used to determine the total cellular content of β-hexosaminidase. The cell pellets from extra wells that

Table I. Positives identified by the siRNA screen of phosphatase genes

Gene Symbol	Name (Alternate Name)	Maximum Effect ^a (%)
<i>Pten</i>	Phosphatase and tensin homolog (TEP1)	−45
<i>Mtmr4</i>	Myotubularin related protein 4 (FYVE zinc finger phosphatase)	−41
<i>Ptpn14</i>	Protein tyrosine phosphatase, nonreceptor type 14	−39
<i>Ptpn4</i>	Protein tyrosine phosphatase, nonreceptor type 4 (hPTP-MEG)	−38
<i>Ppp3r1</i>	Protein phosphatase 3, regulatory subunit B, α isoform (calcineurin B, type I)	−37
<i>Epm2a</i>	Epilepsy, progressive myoclonic epilepsy, type 2 gene α (laforin)	−32
<i>Ppp3cc</i>	Protein phosphatase 3, catalytic subunit, γ isoform (PP2BA γ)	−31
<i>Ppm1f</i>	Protein phosphatase 1F (PP2C domain containing)	33
<i>Ptpn9</i>	Protein tyrosine phosphatase, nonreceptor type 9 (Meg2)	33
<i>Prtg</i>	Protogenin homolog (<i>Gallus gallus</i>)	34
1810034K20RIK	RIKEN cDNA 1810034K20 gene	39
<i>Dusp5</i>	Dual specificity phosphatase 5	40
<i>Dusp7</i>	Dual specificity phosphatase 7	41
<i>Ptp4a2</i>	Protein tyrosine phosphatase 4a2 (Prl-2)	42
<i>Ptpn20</i>	Protein tyrosine phosphatase, nonreceptor type 20 (typ)	44
<i>Psph</i>	Phosphoserine phosphatase (PSPase)	45
<i>Inpp5b</i>	Inositol polyphosphate-5-phosphatase B (75 kDa)	49

This is a list of the targets that induced ≥ 2.0 SD change in the original screen and that on the original or by retesting had $>30\%$ change in IgE-induced degranulation.
^aThe maximum percent inhibition (−) or enhancement (+) observed during the original or in repeat screening.

had been transfected but not further manipulated were combined with those from Ag-stimulated cells, lysed with SDS-PAGE sample buffer, and used for Western blotting to determine changes in the level of expression of proteins (34). The β -hexosaminidase enzymatic activity was measured with *p*-nitrophenyl *N*-acetyl- β -D-glucosaminide in 0.1 M sodium citrate (pH 3.5) for 60 min at 37°C. After stopping the reaction by adding 0.4 M glycine (pH 10.7), the released product 4-*p*-nitrophenol was detected by absorbance at 405 nm. The extent of Ag-induced degranulation was calculated as follows: 4-*p*-nitrophenol absorbance in the supernatant of Ag-stimulated cells minus 4-*p*-nitrophenol absorbance in the supernatant of blank cells (spontaneous release), which was then divided by the sum of 4-*p*-nitrophenol absorbance in the supernatants and cell pellets of blank cells. The release was expressed as a ratio of that induced by scrambled siRNA-transfected cells, and in some graphs this value was subtracted from 1.00.

RNA isolation and quantitative PCR analysis

Total RNA from the cells transfected with scrambled or MTMR4-specific siRNA was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA) and reverse transcribed using SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA). The mRNA expression of MTMR4 and Syk (as an internal control) was detected with ABI Prism 7000 Sequence Detection System by using SYBR Green PCR Master Mix (Applied

Biosystems, Foster City, CA). The primers used for MTMR4 are: forward primer 5'-AAAGACTCTGTCATCAACGTGC-3' and reverse primer 5'-CCGGCTTAGCCTTGAGAGC-3'. The primers used for Syk are: forward primer 5'-CTACCTGCTACGCCAGAGC-3' and reverse primer 5'-GCC-ATTAAGTTCCCTCTCGATG-3'. The results were analyzed using ABI Prism Software (Applied Biosystems), and the expression of MTMR4 mRNA was normalized to that of Syk.

Data analysis

Paired two-sided Student *t* tests were used to test significance with a comparison with the control without correction for multiple comparisons. The significance for *p* values between 0.05 and 0.01 is marked with one asterisk (*), values of *p* between 0.01 and 0.001 are marked with two asterisks (**), and values of *p* < 0.001 are marked with three asterisks (***). All experiments were repeated between three and six times. In immunoblots, densitometry of the bands was used to calculate the decrease in the protein expression as a percentage of control cells transfected with nontargeting siRNA corrected for gel loading. The annotations in the tables are derived from a search of the Mouse Genome Database at the Mouse Genome Informatics Web site, The Jackson Laboratory; World Wide Web Mouse Genome Database (www.informatics.jax.org) last checked in September 2008.

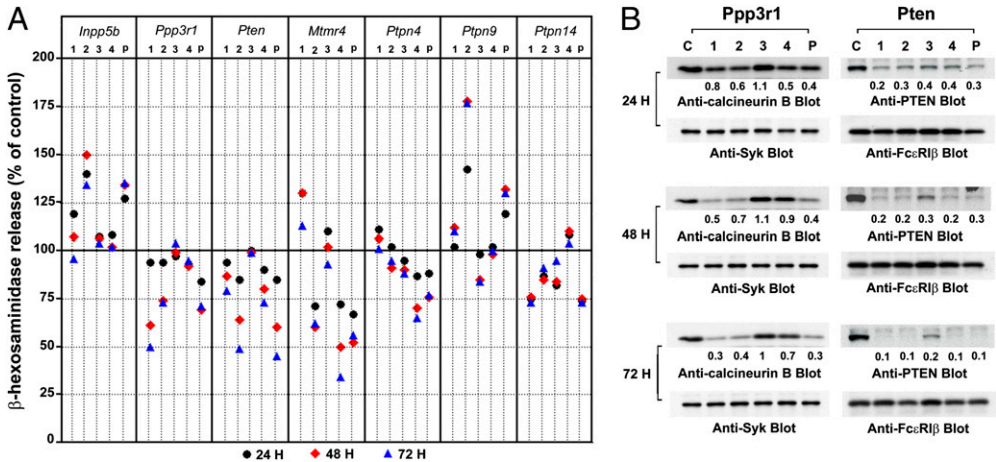


FIGURE 3. Confirmation of on target effect of positive hits by testing of the four individual siRNAs from each pool. *A*, FcεRI-induced degranulation in the cells transfected by individual siRNA. Four individual siRNAs included in the original siRNA pools for *Inpp5b*, *Ppp3r1*, *Pten*, *Mtmr4*, *Ptpn4*, *Ptpn9*, and *Ptpn14* was separately tested for their effects on IgE-Ag-induced degranulation at 24, 48, and 72 h (individual siRNA indicated by 1, 2, 3, and 4, and the pool of the four by P). The results are the average of at least three different experiments. The Ag-induced release for control scrambled siRNA-transfected cells was 29.2% $\pm$ 1.9% (SD) at 24 h, 27% $\pm$ 2.9% (SD) at 48 h, and 25.5% $\pm$ 3.2% (SD) at 72 h. *B*, Cell lysates were prepared from the same cells used in *A* and blotted with the corresponding Abs to show the targeted protein knockdown. Anti-Syk or anti-FcεRI β was used as loading controls. Data are representative of at least three separate experiments.

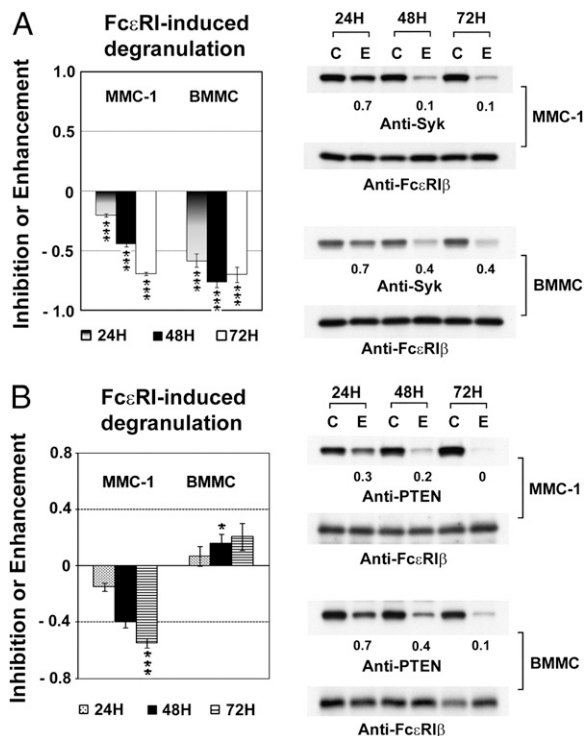


FIGURE 4. The comparisons of targeting Syk and PTEN in MMC-1 and BMMC. **A**, Syk was used as a positive control for BMMC transfection. MMC-1 or BMMC were transfected with scrambled (C) or Syk (E) siRNA, sensitized with IgE, and then stimulated by Ag as outlined in Fig. 1A. The supernatants were used for β -hexosaminidase assay and the cell pellets for immunoblotting. The Ag-induced β -hexosaminidase release in the Syk siRNA-transfected cells is expressed as the percentage of inhibition or enhancement of the release in controls as described in the *Materials and Methods*. The values are the mean of >7 separate experiments. The immunoblots are representative of >6 separate experiments. **B**, The effects of PTEN deficiency in MMC-1 and BMMCs. Cells were transfected with the indicated siRNA (C for scramble and E for *Pten*), stimulated, and tested as described in **A**. Values are mean of >3 different experiments, and the immunoblots are representative of at least three separate experiments.

Results

Screen of mouse phosphatase siRNA library

To identify phosphatases involved in FcεRI signaling, we screened a siRNA library targeting 198 known or predicted mouse phosphatases (Supplemental Table I). Pools of four different siRNA duplexes per targeting gene were used to increase the chance of siRNA-targeted protein knockdown, and IgE-Ag-induced mast cell degranulation was used as a functional readout for each targeted phosphatase. The mouse mast cell line MMC-1 was used as model system, as these cells were found to maintain their high viability and stable degranulation response posttransfection. Efficient transient transfection of these cells was achieved by using the Amaxa transfection system (Amaxa Biosystems); under the optimized conditions, enhanced GFP-expression vector resulted in $>80\%$ of the MMC-1 cells becoming fluorescent after 24 h of transfection.

Mouse Syk siRNA pool of four duplexes was used as a positive control to test the screening system, whereas the negative control siRNA was a pool of four nontargeting siRNAs that has been microarray-confirmed to minimally target known genes in human, mouse, and rat cells (Dharmacon). To test this screening system, MMC-1 cells were transfected with Syk or nontargeting siRNA pool in a 96-well plate format (Fig. 1A). Posttransfection, cells were divided into triplicate plates and sensitized with Ag-specific IgE immediately or after 24 or 48 h, respectively. After the 24 h of

culture with IgE, the sensitized cells were stimulated by Ag, and the release of β -hexosaminidase was used as a measure of the extent of degranulation. In parallel, as positive controls, the efficiency of the knockdown in Syk protein expression was determined by immunoblotting. Transfection with the Syk siRNA pool effectively reduced Syk protein expression, with the largest reduction observed at 72 h posttransfection (Fig. 1B). As expected from previous reports, Syk protein knockdown decreased FcεRI-initiated mast cell degranulation, and this reduced release was proportionally related to the decrease in Syk protein expression (19).

In preliminary experiments, using siRNA targeting Syk and several other signaling molecules, there were different lag times between siRNA transfection and the maximum decrease in protein expression, which would be expected because of the variability in $t_{1/2}$ of different proteins. Therefore, in the phosphatase siRNA library screening, the functional readout of FcεRI-induced degranulation was tested repeatedly at 24, 48, and 72 h posttransfection with each siRNA pool (Fig. 1A).

The results of the library screen are summarized in Supplemental Table II, which shows the effects of each siRNA transfection at 24, 48, and 72 h. There was variation in the effects of the different siRNA with $>25\%$ inhibition or enhancement by some samples (Fig. 1C), the results having a normal distribution curve (Supplemental Fig. 1). In analyzing the data from all 3 d of testing, 27 out of the 198 siRNA enhanced or inhibited the FcεRI-induced degranulation at >2 SD. These results suggested that siRNA-based target knockdown can be used as an efficient screening tool to study Ag-receptor signal transduction. Furthermore, it suggested that multiple phosphatases were involved in FcεRI-induced mast cell degranulation.

Confirmation of positive siRNA pools

The same siRNA gave similar results on the three different test days, suggesting the reproducibility of the results and that the knockdown had effects for several days (Supplemental Fig. 2). As outlined in Fig. 1A, the screening method used in the current experiments involved multiple handling procedures. To eliminate the possibility that false positives are due to experimental errors, we retested the siRNA pools that enhanced or inhibited degranulation at >1.5 SD. The results of the two separate experiments were closely related, as shown by the correlation coefficient of 0.94 (Fig. 2A). Supplemental Table III shows the results of the original and repeat determinations for all the siRNA that induced >2 SD effects in the original screen; for these, the correlation coefficient was 0.90. These results demonstrate that the majority of the hits from the original screen still remained positive in the repeat experiments, which strongly suggests that the observed effects were the true results of the siRNA transfections.

The targets that were positive on original screening that were confirmed on retesting to show $>30\%$ change in degranulation are in Table I. Among the 17 positives, 10 enhanced and 7 inhibited degranulation. The majority of these were poorly characterized molecules that on the basis of their sequence had been classified as protein tyrosine, serine, or dual-specific phosphatases. However, there were also some well-characterized proteins that inhibited degranulation [e.g., the siRNA that targeted both the regulatory (Ppp3r1) and the catalytic (Ppp3cc) subunit of calcineurin and PTEN].

Western blotting was then used to investigate if the observed functional changes were indeed due to siRNA-targeted decrease in protein expression. Different Abs for the eight positive hits for which commercial Abs were available were tested. Unfortunately, only anti-PTEN and anti-calcineurin B (another name for Ppp3r1) out of the >10 tested Abs detected the specific proteins. Fig. 2B

shows the results of immunoblotting for the cells transfected with *Pten* or *Ppp3r1* siRNA, which indicated that in these cells, the decreased mast cell degranulation was directly related with the reduced expression of targeted proteins.

Some siRNAs have off-target effects due to partial homology to other transcripts that could result in the inhibition or enhancement of degranulation. A recommended strategy to preclude this problem is to test four independent siRNAs against the same transcript; if at least two of these are positive, then it is unlikely that two independent siRNAs against the same gene target a common off-target transcript for an effect. Therefore, each of the four different siRNAs included in the original siRNA pools were separately tested for five genes that showed the strongest inhibition and two genes that enhanced. These were *Inpp5b*, *Ppp3r1* (calcineurin B), *Pten*, *Mtmr4*, *Ptpn4*, *Ptpn9*, and *Ptpn14*. Their effects on IgE-Ag-induced degranulation of each single siRNA from the chosen pools was examined several times; the summary of these results is shown graphically in Fig. 3A, and the values are in Supplemental Table IV. Out of the seven tested as single siRNAs, at least two out of the four single siRNA showed similar effects as their pool, suggesting that these were true hits.

Additionally, as shown in Fig. 3B, the on-target effects of the single siRNA for *Pten* and *Ppp3r1* (calcineurin B) were further confirmed by the specifically decreased protein expression. For *Mtmr4*, the on-target effects of single siRNA were confirmed by real-time PCR (Supplemental Fig. 3).

Positive target validation by using BMMCs

To test if the knockdown of these positive targets had the same effect in primary cells, BMMCs grown from normal C57BL/6J mice were used for further validation. Syk siRNA was used as a positive control to test the BMMC transfection system. As shown in Fig. 4A, the transfection of BMMC with Syk siRNA specifically reduced the Syk protein expression, which correlated with the decreased FcεRI-induced degranulation.

It has been reported that PTEN deficiency enhances the IgE-Ag-induced mast cell degranulation (33). However, in MMC-1 cells, reduced expression of PTEN resulted in decreased release (Figs. 2, 3). To clarify these discordant results, BMMCs were transfected

and tested for FcεRI-induced degranulation and PTEN protein expression with the same scrambled and *Pten* siRNA that had been used in MMC-1 cells. Compared to control cells, the transfection of *Pten* siRNA reduced PTEN protein expression in BMMC and slightly enhanced IgE-Ag-induced degranulation (Fig. 4B). The discrepancy between MMC-1 and BMMC in the degranulation response to the siRNA-induced decrease in PTEN expression is probably due to a difference in the status of PI3K activation in the two cell types. Indeed, it had been observed that MMC-1 cells have a high basal PI3K activity as indicated by the increased Akt phosphorylation even in nonstimulated cells (E.A. Barbu, J. Zhang, and R.P. Siraganian, unpublished observations).

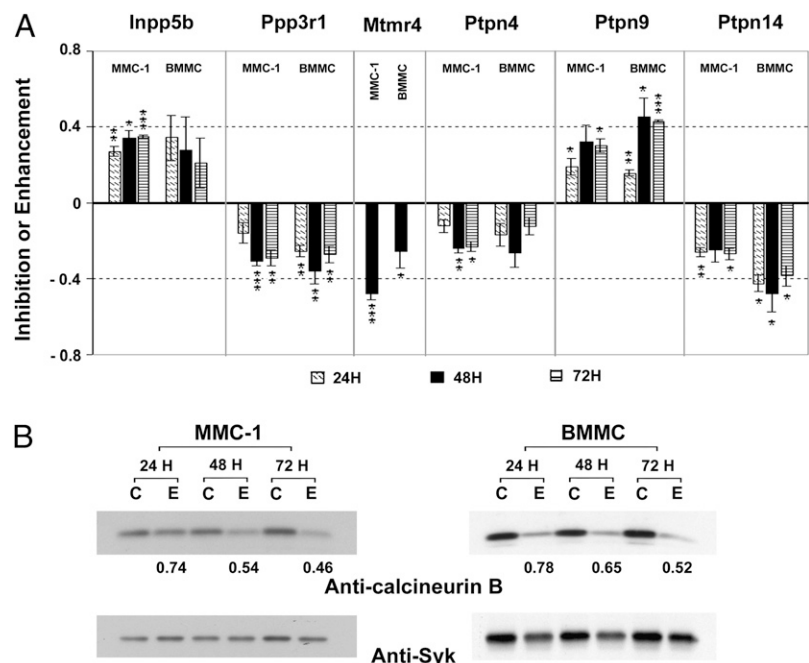
All of the positive hits shown in Fig. 3A were retested with BMMC. Except for PTEN, transfection of these positive pool siRNA in both BMMC and MMC-1 cells had similar effects on FcεRI-induced mast cell degranulation (Fig. 5A). Thus, *Inpp5b* or *Ptpn9* siRNA transfection enhanced IgE-Ag-initiated degranulation, whereas *Ppp3r1*, *Mtmr4*, *Ptpn4*, and *Ptpn14* inhibited this response. Furthermore, immunoblotting with specific anti-PPP3R1 Ab (anti-calcineurin B) indicated that *Ppp3r1* siRNA specifically reduced its target protein expression in both MMC-1 and BMMC cells (Fig. 5B).

Knockdown of calcineurin B impairs FcεRI signaling in mast cells

To understand the mechanism by which calcineurin B (*Ppp3r1*) deficiency impaired IgE-Ag-initiated degranulation, cellular protein phosphorylation was examined. Fig. 6 compared the FcεRI-induced total cellular protein tyrosine phosphorylation in MMC-1 cells transfected with control scrambled siRNA to those with calcineurin B siRNA. The reduced expression of calcineurin B slightly decreased total cellular protein tyrosine phosphorylation.

Calcineurin B is regulated by calcium influx. Therefore, it was expected that its deficiency may have some effect on the downstream part of the FcεRI signaling pathway. This was observed when the IgE-Ag-induced phosphorylation of several MAPKs was examined. As shown in Fig. 6, the reduced expression of calcineurin B clearly decreased FcεRI-initiated phosphorylation of p42/44 Erk and p38 MAPKs, especially at late time points. In the cells transfected by

FIGURE 5. The validation of positive pools with BMMC. **A**, Comparison of FcεRI-induced degranulation between MMC-1 and BMMC transfected by different positive siRNA pools. Cells transfected with scrambled or the indicated siRNA were stimulated with IgE plus Ag, and their degranulation was detected by β-hexosaminidase assay. The changes of Ag-induced release in the different siRNA transfected cells are expressed as the fraction of inhibition or enhancement of the release in controls. Values are mean of at least three separate experiments. **B**, Lysates were prepared from the cells transfected by scrambled and *Ppp3r1* siRNA and immunoblotted with anti-calcineurin B and anti-Syk Abs, respectively. Data are representative of at least three different experiments.



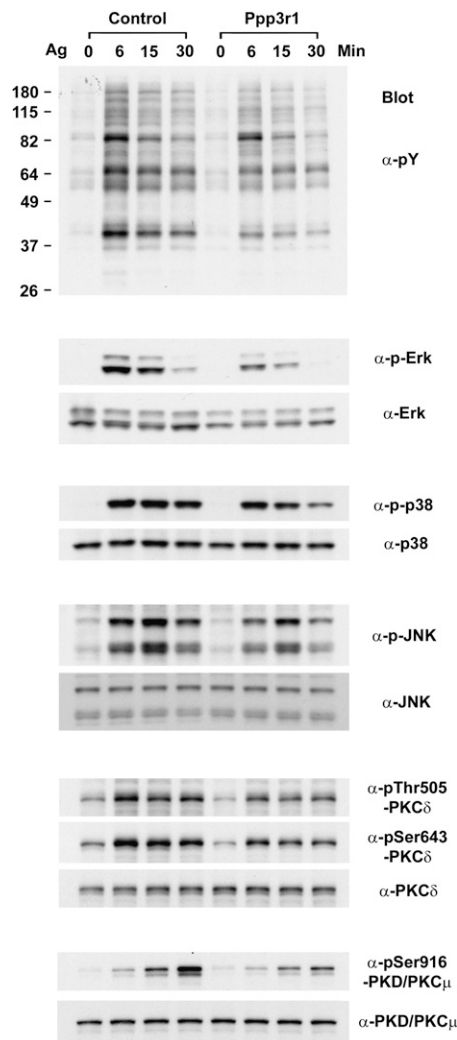


FIGURE 6. The calcineurin B (Ppp3r1) deficiency reduced FcεRI-initiated phosphorylation of MAPKs and PKC. Cells transfected with the specified siRNA were stimulated by IgE plus Ag for the indicated times. The cell lysates were blotted with anti-phosphotyrosine Ab, anti-phospho-p44/42 MAPK and anti-p44/42 MAPK Abs, anti-phospho-p38 and anti-p38 Abs, anti-phospho-JNK and anti-JNK Abs, anti-phospho-PKCδ (Thr505), anti-phospho-PKCδ (Ser643) and anti-PKCδ Abs, and anti-phospho-PKD/PKCμ (Ser916) and anti-PKD/PKCμ Abs. Data are representative of at least three separate experiments.

calcineurin B siRNA, the FcεRI-induced phosphorylation of JNK was also reduced, but its extent was much less.

PKC plays an important role in mast cell degranulation. We therefore also measured the effect of calcineurin B deficiency on the phosphorylation of PKCs. The reduced calcineurin B expression decreased FcεRI-induced PKCδ phosphorylation at Thr505 and Ser643 sites, as shown in Fig. 6. PKD/PKCμ is a distant relative of the PKC isozymes, which function downstream of PKCs (36). Phosphorylation of Ser916 of PKD/PKCμ is directly related with its enzymatic activity (37). The calcineurin B deficiency inhibited IgE-Ag-initiated phosphorylation of Ser916 of PKD/PKCμ (Fig. 6). Altogether, these results suggest that calcineurin B is involved in the regulation of FcεRI pathways at multiple steps.

Discussion

In this study, we demonstrated the utility of an siRNA functional screen to identify phosphatases involved in the regulation of FcεRI signaling. Following siRNA transfection, among 198 known or

predicted mouse phosphatases, there were 17 that on retesting were confirmed to enhance or inhibit the IgE-Ag-induced mast cell degranulation (Table I). This number of positives is probably due to the fact that a phosphatase library was used in this screen of a signaling pathway in which a critical event is phosphorylation. A subset of the positive hits was then validated by testing separately each of the four different siRNAs included in the original siRNA pool, which confirmed the on-target effect of these positive hits. In addition, BMMCs grown from normal C57BL/6J mice were used for the further validation of the positive pools. Except for *Pten*, all of the other six positive hits including *Inpp5b*, *Ppp3r1*, *Mtmr4*, *Ptpn4*, *Ptpn9*, and *Ptpn14* had similar effects on FcεRI-induced mast cell degranulation in both MMC-1 and BMMC cells.

Although degranulation is a complex event that depends on many cellular molecules, it can be determined by a robust and specific assay. This assay is quantitative and allows for identifying of both weak and strong hits; however, weak phenotypes can be due to partial knockdown of a gene with a strong effect or a strong knockdown of a gene with a weak effect. Among the positive hits, there was approximately an equal number that inhibited or enhanced degranulation (Table I). There was a >40% decrease or increase in secretion by these siRNAs, which indicates that these are important regulators of this signaling pathway. The putative phosphatases included molecules that act on phosphoinositides or on proteins phosphorylated on tyrosine, serine, or threonine residues.

siRNA library screening has been used as an efficient and useful approach to identify targets involved in different cell functions, although most of these have been with simpler assays (38–40). However, the utility of such siRNA screens for studies of Ag receptor signaling has been hampered by the difficulty of transfecting differentiated immune cells and the complexity of Ag-receptor signaling. In preliminary experiments, we tested different mast cell lines and siRNA delivery systems in attempts to establish a suitable transfection system that would work for large-scale screening. The virus-based transfection that has obvious advantages was tested by using lentiviral transduction particles. However, the results with both GFP and Syk short hairpin RNA transduction suggested that the transfection efficiency of the lentiviral system was too low and not efficient in decreasing the target protein expression in transient transfection systems (J. Zhang and R.P. Siraganian, unpublished observations). To increase transfection efficiency, small chemical synthesized siRNA was tested using different delivery methods followed by immunoblotting to monitor the targeted protein knockdown and FcεRI-induced degranulation to evaluate the effect of transfection on mast cell function. The protocol that was eventually developed for the current study yielded >80% transfection efficiency, and, as demonstrated with control and Syk siRNA, it dramatically decreased targeted protein expression without having nonspecific effects on mast cell function. As compared with stable transfected cell lines, this transient transfection system is suitable for large-scale screening, and it also eliminated the possibility of clonal variation.

Because the MMC-1 line was derived from transformed tumor cells that grow independent of growth factors, BMMC obtained from normal mice were used to further validate the seven positive hits; six out of the seven positive pools had very similar effects in both cell types, suggesting that the observed changes were due to the siRNA treatment. The positive hit that showed discrepant results in the two cell types was PTEN; transfection of *Pten* siRNA strongly inhibited FcεRI-induced degranulation in MMC-1 cells, whereas it slightly enhanced it in BMMC. Immunoblotting confirmed the specific PTEN protein knockdown by siRNA in both cell types. The knockdown of PTEN mediated by lentivirus transfection of short hairpin RNA was reported to increase FcεRI-induced degranulation

in CD34⁺ peripheral blood-derived human mast cells (33). PTEN regulates phosphatidylinositol (PI) phosphate levels in cells by dephosphorylating several of the PIs at the 3' position. It dephosphorylates PI 3,4,5-trisphosphate [PI(3,4,5)P₃], which would increase PI 4,5-bisphosphate [PI(4,5)P₂] levels and decrease the level of PI(3,4,5)P₃. The intracellular levels of both PI(4,5)P₂ and PI(3,4,5)P₃ are important in mast cell signaling that results in degranulation. Receptor aggregation results in phospholipase C stimulation, which cleaves PI(4,5)P₂ to form inositol (1,4,5)P₃ and 1,2-diaclyglycerol that release intracellular calcium and activate PKC, respectively, which are critical for FcεRI-induced degranulation. The difference in response to PTEN protein knockdown between MMC-1 and BMMC cells could be explained by the different activation status of PI3K in these cells. In MMC-1 cells, the increased basal PI3K activity would shift the balance between PI(4,5)P₂ and PI(3,4,5)P₃ with an increase in PI(3,4,5)P₃ levels. The decreased expression by siRNA of PTEN in these cells would further enhance this shift and result in less PI(4,5)P₂ substrate for phospholipase C, which could explain the reduced degranulation, whereas in BMMC, the balance between PI(4,5)P₂ and PI(3,4,5)P₃ is different because of the lower basal activity of PI3K in these cells. The decrease by siRNA in PTEN in BMMC would then shift the balance toward the accumulation of PI(3,4,5)P₃ and result in enhanced secretion.

Calcineurin is a calcium-dependent serine/threonine protein phosphatase consisting of catalytic and regulatory subunits. The siRNA that targeted both the regulatory and the enzymatic subunits of calcineurin inhibited degranulation (37% for the regulatory Ppp3r1 and 31% for the enzymatic Ppp3cc). Calcineurin B (Ppp3r1) is the 19-kDa regulatory subunit of calcineurin and plays an important role in the activation of calcineurin phosphatase activity. Increased intracellular calcium activates calcineurin, which binds and dephosphorylates proteins, including the transcription factor NFAT, resulting in its nuclear translocation and subsequent gene expression. However, the function of calcineurin B in cells has not been easily defined because germ line mutation of the calcineurin B gene results in lethal defects in vascular patterning (41). Calcineurin enzymatic activity is inhibited by the cyclosporin A (CsA), which has been used to study the function of calcineurin in mast cells. CsA inhibits the FcεRI-dependent degranulation of bone marrow-derived mouse mast cells, human lung mast cells, or RBL-2H3 cells and blocks anti-IgE-induced histamine and cytokine releases from basophils (42–44). CsA binds to the cytosolic protein cyclophilin, which inhibits calcineurin. However, as cyclophilin is also present in mitochondrial membrane, CsA may also have an effect on mitochondria, such as preventing the formation of mitochondrial permeability transition pore. In the present experiments, siRNA knockdown of calcineurin B expression indicates a specific function of this protein in regulating FcεRI-induced degranulation.

The mechanism by which calcineurin controls NFAT activation has been extensively studied, but how this protein regulates mast cell degranulation is unclear. A possible mechanism could be by the regulation of PKC. AKAP79, a mammalian scaffold protein, coordinates the formation of a complex of PKC and calcineurin B (45). In the current study, we observed that decreased expression of calcineurin B reduced the phosphorylation of PKCδ and PKD/PKCμ, a substrate of PKC. Because PKC activation is required for mast cell degranulation, the reduced PKC activity could be responsible for the decreased degranulation in the calcineurin B-deficient cells. In thymocytes, calcineurin B deficiency reduced CD3/CD28 initiated Erk phosphorylation during thymocyte development (46). Similarly, we observed that the knockdown of calcineurin B reduced IgE-Ag-induced Erk and p38 phosphor-

ylation in mast cells. Therefore, calcineurin B is implicated in the regulation of multiple molecules involved in FcεRI signaling.

In summary, these experiments demonstrated the utility of siRNA-based functional screening to identify molecules that positively or negatively regulate degranulation. Many of the phosphatase genes that were found to regulate this complex event have not been previously associated with degranulation. Further analysis of how they regulate these signaling pathways should provide new insights into this important basic cellular function. Furthermore, these studies have identified several new molecules that are critical for FcεRI-mediated mast cell degranulation, which may serve as potential therapeutic targets for the treatment of asthma and allergic diseases.

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Disclosures

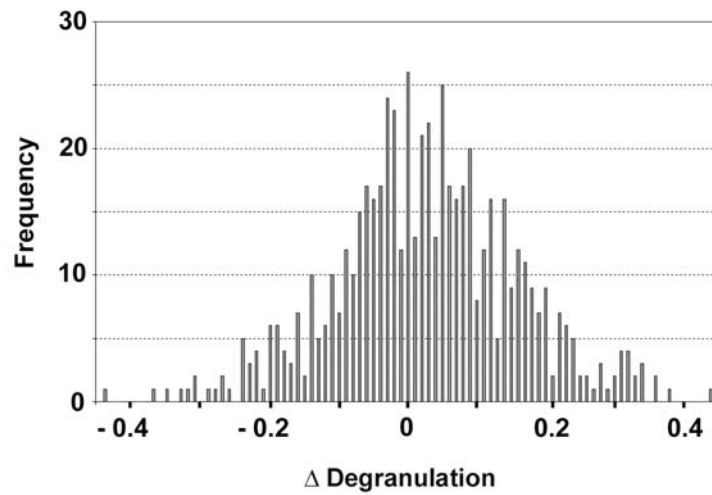
The authors have no financial conflicts of interest.

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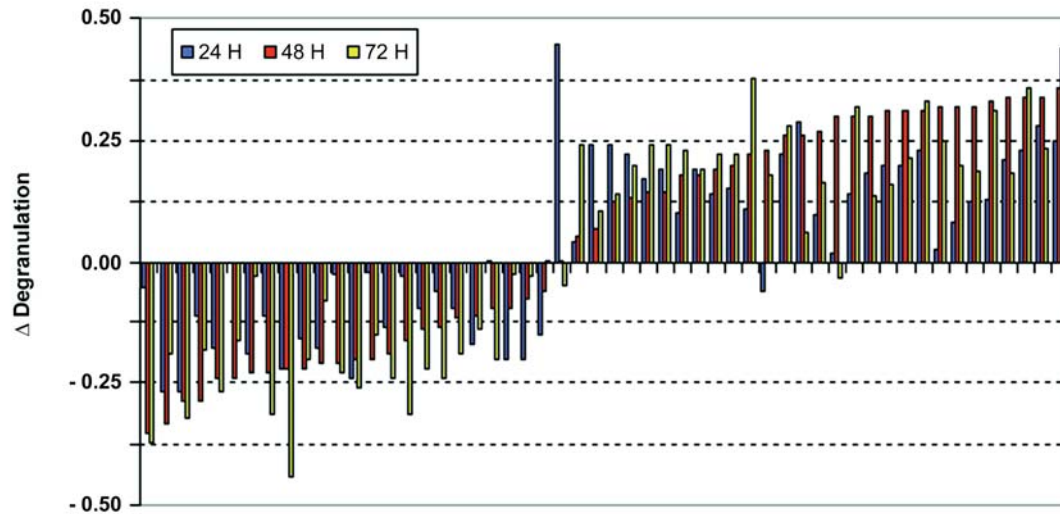
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Supplemental Figure S1



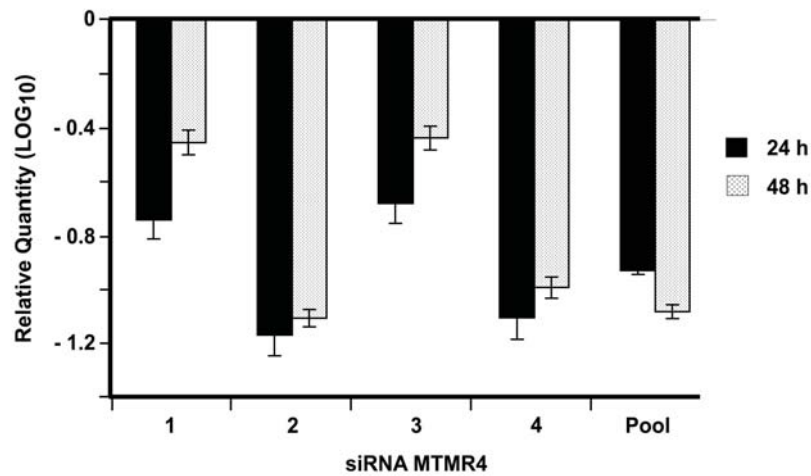
The distribution frequency of degranulation indices. Following library screening, different siRNA pools were grouped according to their effect on degranulation, and graphed to show the distribution frequency. The graph includes all the results obtained from the 3 days of assays (24h, 48h, and 72h) for each siRNA pool.

Supplemental Figure S2



Responses at the three time points (24, 48 and 72 h) for siRNA in the library screen that were used for retesting. The results are degranulation indices from library screening where there was ≥ 1.5 times the standard deviation change in degranulation. The graph shows the results for each target plotted with the 24, 48 and 72 h data next to each other. The indices were sorted based on the release at 48 h after siRNA transfection.

Supplemental Figure S3



Confirmation by quantitative PCR of “on target” effect by four single siRNA specific for MTMR-4. MMC-1 cells were transfected with four individual siRNA (labeled 1-4) and the siRNA pool specific for *Mtmr4*. After 24h and 48h RNA was prepared and used for quantitative PCR as described under Materials and Methods. The results are the average of 4 different experiments. Bars indicate SE.

Supplemental table S1.

siRNA oligo-sequences of mouse phosphatase library						
Pool Number	Cat Number	Gene Name	Gene ID	Accession	GI Number	OLI Sequence
M-041634-00	D-041634-01	ACP1	11431	NM_021330	31542069	GAGCUAUGAUCCACAGAAA
M-041634-00	D-041634-02	ACP1	11431	NM_021330	31542069	UCAGAUAAUUGGAGGAUAG
M-041634-00	D-041634-03	ACP1	11431	NM_021330	31542069	GCACAUUGCACGGCAGAUU
M-041634-00	D-041634-04	ACP1	11431	NM_021330	31542069	CGGUAACAUUUGCCGGUCA
M-042366-00	D-042366-01	ACP2	11432	NM_007387	29150252	GAACACCUCUUACCACAGG
M-042366-00	D-042366-02	ACP2	11432	NM_007387	29150252	UGAAGAACCUGACCCUAAU
M-042366-00	D-042366-03	ACP2	11432	NM_007387	29150252	GCAACGCUACCAUGGCUUU
M-042366-00	D-042366-04	ACP2	11432	NM_007387	29150252	CGGAGUCUACGCUUUGUUA
M-043522-00	D-043522-01	ACP5	11433	NM_007388	6680623	GAACUUGCGACCAUUGUUA
M-043522-00	D-043522-02	ACP5	11433	NM_007388	6680623	GGUAUGUGCUGGCUGGAAA
M-043522-00	D-043522-03	ACP5	11433	NM_007388	6680623	GCACAGAUUGCAUACUCUA
M-043522-00	D-043522-04	ACP5	11433	NM_007388	6680623	GGAAGCCUCUGGAAAUCA
M-049533-00	D-049533-01	ACP6	66659	NM_019800	9790058	GAAAGGGUCUGCCGUCAUC
M-049533-00	D-049533-02	ACP6	66659	NM_019800	9790058	CAACAACGGUGAUGACGUG
M-049533-00	D-049533-03	ACP6	66659	NM_019800	9790058	GGAAUGACUUGUUUACACA
M-049533-00	D-049533-04	ACP6	66659	NM_019800	9790058	CGAAACCUCAUUCUCAUUA
M-065438-00	D-065438-01	ACPP	56318	NM_019807	46575783	GCGAGAGUGUUCACAAUUU
M-065438-00	D-065438-02	ACPP	56318	NM_019807	46575783	AGAUGGCGCUAGAUGUUUA
M-065438-00	D-065438-03	ACPP	56318	NM_019807	46575783	CUAUAAAGCAUGAUCAGAUU
M-065438-00	D-065438-04	ACPP	56318	NM_019807	46575783	CCGAGGACGCCAUGAUUAA
M-040047-00	D-040047-01	Acyp1	66204	NM_025421	13384809	GAGCAAACUUCAACAAUGA
M-040047-00	D-040047-02	Acyp1	66204	NM_025421	13384809	GAAAAGUUACCACGUAUUA
M-040047-00	D-040047-03	Acyp1	66204	NM_025421	13384809	UCGCAAACUUGGAUUUAUUC
M-040047-00	D-040047-04	Acyp1	66204	NM_025421	13384809	GAACUAUUCUGUGCUCAGU
M-040048-00	D-040048-01	ACYP2	75572	NM_029344	27229218	GGAAUACUCUGAUUUUAGU
M-040048-00	D-040048-02	ACYP2	75572	NM_029344	27229218	GGUGUUUGCUUCAGAAUGU
M-040048-00	D-040048-03	ACYP2	75572	NM_029344	27229218	CAAGGUGUUUGCUUCAGAA
M-040048-00	D-040048-04	ACYP2	75572	NM_029344	27229218	AGAAGGAGAAGCUAAGAAA
M-048015-00	D-048015-01	AKAP11	219181	XM_127854	38076403	CAAAUAAUCUUUCGUGUGA
M-048015-00	D-048015-02	AKAP11	219181	XM_127854	38076403	GCAAGUCGCUGUUUGAUUG
M-048015-00	D-048015-03	AKAP11	219181	XM_127854	38076403	GAGAGUGUCACCUACAUUA
M-048015-00	D-048015-04	AKAP11	219181	XM_127854	38076403	GACCCGCAAUUAAGGAUUA
M-043592-00	D-043592-01	2010001C14RIK	76768	XM_129951	38050325	GAGUGCAGCUGCUCGAUUA
M-043592-00	D-043592-02	2010001C14RIK	76768	XM_129951	38050325	GCAAGGACAUCGCCACUCA
M-043592-00	D-043592-03	2010001C14RIK	76768	XM_129951	38050325	GAAAUAUGAUGCCAACCGA
M-043592-00	D-043592-04	2010001C14RIK	76768	XM_129951	38050325	GAAGGACACGCUAAUACUC
M-043406-00	D-043406-01	AKP2	11647	NM_007431	6671532	GGAAAUACAUGUACCCGAA
M-043406-00	D-043406-02	AKP2	11647	NM_007431	6671532	GGACAUCGCAUUCAGCUA
M-043406-00	D-043406-03	AKP2	11647	NM_007431	6671532	UAACGGGCCUGGCUACAAG
M-043406-00	D-043406-04	AKP2	11647	NM_007431	6671532	AGUAUGAAUUGAAUCGGAA
M-058286-00	D-058286-01	AKP5	11650	NM_007433	34328098	GUACUCGGAUGCACAAAUG
M-058286-00	D-058286-02	AKP5	11650	NM_007433	34328098	GCUACAGGAUGGCUGCAAA
M-058286-00	D-058286-03	AKP5	11650	NM_007433	34328098	CAACGGUCCUGGUUACAAG
M-058286-00	D-058286-04	AKP5	11650	NM_007433	34328098	AAUAUGAAAUCCACCGAGA

M-043397-00	D-043397-01	AKP3	11648	NM_007432	6671534	GGAAUCUGGUGCAGGAAUG
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M-059466-00	D-059466-02	ANP32E	66471	NM_023210	31542130	UAUAAUUUCUGGAGGCUUG
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M-041210-00	D-041210-03	BPNT1	23827	NM_011794	39652625	UACAGUUCUCAUUGGGAU
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M-040027-00	D-040027-01	D19ERTD703E	52036	NM_029456	22726196	GAACAGCAUUGGCGUCAUA
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M-050528-00	D-050528-02	1700047I17RIK	73385	NM_028527	37537561	GUACGCCAUUGAUGAGUAU
M-050528-00	D-050528-03	1700047I17RIK	73385	NM_028527	37537561	CGAGGAGGGUCAUCCACUU
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M-049062-00	D-049062-02	ORF5	53858	NM_016924	8393888	GGAAGGACAGCGGAAUUU
M-049062-00	D-049062-03	ORF5	53858	NM_016924	8393888	GCUGAACACAGAUUCUGAUA
M-049062-00	D-049062-04	ORF5	53858	NM_016924	8393888	UGGGAAACCUGGUGUUGUU
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M-061575-00	D-061575-02	GSBS	19051	NM_011153	31982770	UGACAAGCUAGCUAUUUAA
M-061575-00	D-061575-03	GSBS	19051	NM_011153	31982770	UGAGAGUGCAGGAGUGAUU
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M-048809-00	D-048809-01	CDC14A	229776	XM_149387	38076999	GGACAUUGAUAGCCUGUUA
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M-048809-00	D-048809-03	CDC14A	229776	XM_149387	38076999	GGCCCAGACUUUCAGAUUA
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M-056071-00	D-056071-01	CDC14B	218294	NM_172587	31982553	CAUUUAACCUUGACGAAUA
M-056071-00	D-056071-02	CDC14B	218294	NM_172587	31982553	GGCCAUGGUUUACAGAUAU
M-056071-00	D-056071-03	CDC14B	218294	NM_172587	31982553	GAAGACGCGUAUAGAACAU
M-056071-00	D-056071-04	CDC14B	218294	NM_172587	31982553	GCAUAGAUAAACGAGCUAGA
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M-044293-00	D-044293-02	CDC25A	12530	NM_007658	6680895	GCGAGAAUUUAAAGAAUGA
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M-050832-00	D-050832-02	CDC25B	12531	NM_023117	12963486	GGAGAGUGAUUUAAAGGAU
M-050832-00	D-050832-03	CDC25B	12531	NM_023117	12963486	CCACGAAUGUGCCGCUUCA
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M-049191-00	D-049191-02	CDC25C	12532	NM_009860	6753361	UGUGUGAGCUGGAAAUGAA
M-049191-00	D-049191-03	CDC25C	12532	NM_009860	6753361	GAUUGUCGCUAUCCGUAUG
M-049191-00	D-049191-04	CDC25C	12532	NM_009860	6753361	GUAGUACUCCGAAUGCUAU
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M-047568-00	D-047568-02	CDKN3	72391	XM_354809	38076060	GGUUGUAAGUUUAAAGAUG
M-047568-00	D-047568-03	CDKN3	72391	XM_354809	38076060	GAACUAAAGAGCUACGGGA
M-047568-00	D-047568-04	CDKN3	72391	XM_354809	38076060	ACGAAGAACCUGUUGAUGA
M-055605-00	D-055605-01	CILP	214425	NM_173385	27734195	GAGCUAGUGUUAACUCUUAU
M-055605-00	D-055605-02	CILP	214425	NM_173385	27734195	GCAAUGGGCCUAUCUAUGC
M-055605-00	D-055605-03	CILP	214425	NM_173385	27734195	GGAAUAACCGCGUGAGCAU
M-055605-00	D-055605-04	CILP	214425	NM_173385	27734195	CCGUGUAGACCGCACAUUA
M-056979-00	D-056979-01	CTDP1	67655	NM_026295	34328279	GACGAUACCCGCACACAUA
M-056979-00	D-056979-02	CTDP1	67655	NM_026295	34328279	CGAAUAUUGUCUAGAGAUG
M-056979-00	D-056979-03	CTDP1	67655	NM_026295	34328279	CGACGAUGACCACUUGAUA
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M-051464-00	D-051464-02	CTDSPL	69274	NM_133710	19526887	CGUUUAAGCCCAUUAGUAA
M-051464-00	D-051464-03	CTDSPL	69274	NM_133710	19526887	UGACGGUGCUCGACUAUGG
M-051464-00	D-051464-04	CTDSPL	69274	NM_133710	19526887	GAAAU CGACGGAACCAUAC
M-046656-00	D-046656-01	1700001E16RIK	69305	NM_027030	21312255	GAAGCUGACCGGAUUGUUU
M-046656-00	D-046656-02	1700001E16RIK	69305	NM_027030	21312255	UACACUACCUGCCCUCUUA
M-046656-00	D-046656-03	1700001E16RIK	69305	NM_027030	21312255	UAUCAACAGCGCACUCUUA
M-046656-00	D-046656-04	1700001E16RIK	69305	NM_027030	21312255	CCAAGGCAUCUGAGUGAUA
M-048450-00	D-048450-01	PTPN20	19256	NM_008978	6679550	GCUCAGAUUCGACCAUUAA
M-048450-00	D-048450-02	PTPN20	19256	NM_008978	6679550	GUUCGAGUAUUUCAGAUUG
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M-048450-00	D-048450-04	PTPN20	19256	NM_008978	6679550	GGUCUGCUAUGCGGGAUUG
M-040234-00	D-040234-01	A930026L03RIK	243382	NM_175523	31342096	GAAAUUCGGUGGGUUUGUA
M-040234-00	D-040234-02	A930026L03RIK	243382	NM_175523	31342096	GGAGAAAUGUGUUAUGGAU
M-040234-00	D-040234-03	A930026L03RIK	243382	NM_175523	31342096	CAGAAGAGGUGCUAUACUU
M-040234-00	D-040234-04	A930026L03RIK	243382	NM_175523	31342096	GAAGAAGAGAGUGUUGUUA
M-059657-00	D-059657-01	DNAJC6	72685	NM_198412	38259193	GCACAGACUUUGAACGAAU
M-059657-00	D-059657-02	DNAJC6	72685	NM_198412	38259193	CGACAUUGCUGGAGGUUGG
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M-040753-00	D-040753-01	Dusp1	19252	NM_013642	7305422	CAACGAGGCUAUUGACUUC
M-040753-00	D-040753-02	Dusp1	19252	NM_013642	7305422	CCAUGGGCCUGGAGCAUAU
M-040753-00	D-040753-03	Dusp1	19252	NM_013642	7305422	AAGCAGAGGCGGAGUAUCA
M-040753-00	D-040753-04	Dusp1	19252	NM_013642	7305422	GGACUAACCGGGUAAAGCU
M-060807-00	D-060807-01	DUSP10	63953	NM_022019	31560302	CUGCGAAUCUGACGUUAU
M-060807-00	D-060807-02	DUSP10	63953	NM_022019	31560302	GGCCUUUCAUGGAGUACAA
M-060807-00	D-060807-03	DUSP10	63953	NM_022019	31560302	AGGCAAACGACCAAUUAU
M-060807-00	D-060807-04	DUSP10	63953	NM_022019	31560302	ACGCAGAGCUAACGCCCAU
M-065353-00	D-065353-01	DUSP11	72102	NM_028099	46519172	AGAAAGAACCGGAAUGUUA
M-065353-00	D-065353-02	DUSP11	72102	NM_028099	46519172	CAA AUGUGCAGUUAAGAA
M-065353-00	D-065353-03	DUSP11	72102	NM_028099	46519172	UCAGCGCUAUUAUAAAGUA
M-065353-00	D-065353-04	DUSP11	72102	NM_028099	46519172	GGACUAUUCACAAAGAAGA
M-040049-00	D-040049-01	DUSP12	80915	NM_023173	12963552	GAUGGAAUCUACUCUGUUG
M-040049-00	D-040049-02	DUSP12	80915	NM_023173	12963552	CAGAGUGGAUGAAAUGAAA
M-040049-00	D-040049-03	DUSP12	80915	NM_023173	12963552	GAAACUGUAUGAGGCAAUG
M-040049-00	D-040049-04	DUSP12	80915	NM_023173	12963552	CAGGAACUCUUCGCUGUUG
M-042257-00	D-042257-01	DUSP13	27389	NM_013849	34328222	GAACACCUCUGGAGUACUA
M-042257-00	D-042257-02	DUSP13	27389	NM_013849	34328222	GAGAUGCCCUCAAUAUUCC

M-042257-00	D-042257-03	DUSP13	27389	NM_013849	34328222	GAGUCGCUCUGCCACAAUU
M-042257-00	D-042257-04	DUSP13	27389	NM_013849	34328222	ACACAGGUGCCAAGUUCUA
M-060717-00	D-060717-01	DUSP14	56405	NM_019819	31560387	CCAUUAGACUGUACUUUGA
M-060717-00	D-060717-02	DUSP14	56405	NM_019819	31560387	UUGCGUACCUGAUGAAAUU
M-060717-00	D-060717-03	DUSP14	56405	NM_019819	31560387	GACACUUGAUGCCUUAUUG
M-060717-00	D-060717-04	DUSP14	56405	NM_019819	31560387	GAGGCAUACAACUGGGUGA
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M-053554-00	D-053554-02	DUSP15	252864	NM_145744	21919413	GAUAUCACCUACCUUCGAA
M-053554-00	D-053554-03	DUSP15	252864	NM_145744	21919413	AAGAAUGCGUCCACUUUUAU
M-053554-00	D-053554-04	DUSP15	252864	NM_145744	21919413	GUCUGAUACUCCUGAGGUA
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M-064263-00	D-064263-02	DUSP18	75219	NM_173745	31982064	CUACGAGGAUAUCCAGUAU
M-064263-00	D-064263-03	DUSP18	75219	NM_173745	31982064	AGAAUACAAUGCAGAUAGAU
M-064263-00	D-064263-04	DUSP18	75219	NM_173745	31982064	UCUCAGUAGAGGUAGCAAA
M-063567-00	D-063567-01	DUSP19	68082	NM_024438	31980900	CACGCUAACUGGAAAGAAA
M-063567-00	D-063567-02	DUSP19	68082	NM_024438	31980900	UAAGGUGACUCAUAUUCUC
M-063567-00	D-063567-03	DUSP19	68082	NM_024438	31980900	AAAGCAUUCUCCCGGGAUA
M-063567-00	D-063567-04	DUSP19	68082	NM_024438	31980900	GAGGCGAGACCAUCCAUAU
M-040331-00	D-040331-01	DUSP2	13537	NM_010090	46560572	GGACGAGGCCUUUGACUUU
M-040331-00	D-040331-02	DUSP2	13537	NM_010090	46560572	UCACAGCAGUUCUCAUGU
M-040331-00	D-040331-03	DUSP2	13537	NM_010090	46560572	GCAUUCCAGUAGAAGAUAA
M-040331-00	D-040331-04	DUSP2	13537	NM_010090	46560572	UAGACUCGGUGAAGAAUAG
M-058060-00	D-058060-01	L700094E07RIK	73547	XM_135794	38086087	GGGAACAACUGAUUCAUUA
M-058060-00	D-058060-02	L700094E07RIK	73547	XM_135794	38086087	GCGAUGCUCCUAAUUCUUA
M-058060-00	D-058060-03	L700094E07RIK	73547	XM_135794	38086087	GGAAUACCGUUCGCAUGAU
M-058060-00	D-058060-04	L700094E07RIK	73547	XM_135794	38086087	GUAACUCUGCGGUAGCUAA
M-047024-00	D-047024-01	DUSP3	72349	NM_028207	21312313	CCUCAGUGCUUACUUUGAA
M-047024-00	D-047024-02	DUSP3	72349	NM_028207	21312313	UCAACGACCUGCUCUCGGA
M-047024-00	D-047024-03	DUSP3	72349	NM_028207	21312313	CCAAUGAUACGCAGGAGUU
M-047024-00	D-047024-04	DUSP3	72349	NM_028207	21312313	CAACGCUAGUUAUCGCCUA
M-061306-00	D-061306-01	DUSP4	319520	NM_176933	31581570	GUACAUCGACGCAGUAAAG
M-061306-00	D-061306-02	DUSP4	319520	NM_176933	31581570	GGACAUCUGCCUGCUUAAA
M-061306-00	D-061306-03	DUSP4	319520	NM_176933	31581570	GGACUGCAGCGUGCUCUAAA
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M-057231-00	D-057231-01	LOC240672	240672	XM_140740	38085196	GCAGCCAGCUCCACGUUUA
M-057231-00	D-057231-02	LOC240672	240672	XM_140740	38085196	CAUCUCGGCUCUAUCUCAGA
M-057231-00	D-057231-03	LOC240672	240672	XM_140740	38085196	CCAAGCGUCUAACACAAGA
M-057231-00	D-057231-04	LOC240672	240672	XM_140740	38085196	GGAAGUGCCUACCACGCAU
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M-040050-00	D-040050-02	DUSP6	67603	NM_026268	13399313	CAUCGAAUCUGCCA UUAU
M-040050-00	D-040050-03	DUSP6	67603	NM_026268	13399313	CCUCGGACAUUGAGUCUGA
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M-040051-00	D-040051-01	DUSP7	235584	NM_153459	23618874	GAAGCCCGCUCCAAGAAGU
M-040051-00	D-040051-02	DUSP7	235584	NM_153459	23618874	GGCAUCAGCCGUUCAGUGA
M-040051-00	D-040051-03	DUSP7	235584	NM_153459	23618874	CAUCAGCCGUUCAGUGACA
M-040051-00	D-040051-04	DUSP7	235584	NM_153459	23618874	CAUAACCUGU UCCCGAUCA
M-048028-00	D-048028-01	DUSP8	18218	NM_008748	6679155	GGAUAAGCUAUGUCCUCA
M-048028-00	D-048028-02	DUSP8	18218	NM_008748	6679155	GGAAGGUGAUGGACGCAAA
M-048028-00	D-048028-03	DUSP8	18218	NM_008748	6679155	GGUCCUUCGUGGAGUAUAA

M-048028-00	D-048028-04	DUSP8	18218	NM_008748	6679155	UGACGACGCAUACAGGUUU
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M-064007-00	D-064007-02	DUTP	110074	NM_023595	31981691	CAAUAUACCCCAUGGAGAA
M-064007-00	D-064007-03	DUTP	110074	NM_023595	31981691	GAGCUGGUGUCAUAGACGA
M-064007-00	D-064007-04	DUTP	110074	NM_023595	31981691	GCUCCACCGGGAAGAAUUA
M-042719-00	D-042719-01	ENPP1	18605	NM_008813	12597622	CGAUUUGGGUGACCGCUAA
M-042719-00	D-042719-02	ENPP1	18605	NM_008813	12597622	AAAGUGCUGUCGCUGGUUU
M-042719-00	D-042719-03	ENPP1	18605	NM_008813	12597622	CUAAUAUAGUGCCAAUGUA
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M-048995-00	D-048995-01	ENPP2	18606	NM_015744	7657450	CGAGAGAAGUUUAACCAUA
M-048995-00	D-048995-02	ENPP2	18606	NM_015744	7657450	CUAAAUAACGCCUUCAUA
M-048995-00	D-048995-03	ENPP2	18606	NM_015744	7657450	CAUAUGAGAGCGAGAUUUA
M-048995-00	D-048995-04	ENPP2	18606	NM_015744	7657450	UGAGAGGCCUUCAGUUUAU
M-062709-00	D-062709-01	ENPP3	209558	XM_125594	38090399	AAUCAGGACUCGUAUAUA
M-062709-00	D-062709-02	ENPP3	209558	XM_125594	38090399	GAAUACGCCUGGUCUAGAA
M-062709-00	D-062709-03	ENPP3	209558	XM_125594	38090399	GAGUCGAUAUGAUGCCUUA
M-062709-00	D-062709-04	ENPP3	209558	XM_125594	38090399	GAAGUUGCGAUCGAUAUU
M-050655-00	D-050655-01	ENPP4	224794	NM_199016	39841030	CAACACAGCAGUCGAAUUC
M-050655-00	D-050655-02	ENPP4	224794	NM_199016	39841030	GAUGACCCUUUAAUUGAUU
M-050655-00	D-050655-03	ENPP4	224794	NM_199016	39841030	GAAUGGAGCGGAGCCUAUU
M-050655-00	D-050655-04	ENPP4	224794	NM_199016	39841030	GUGCAUUAUUCUCCCAGAA
M-046066-00	D-046066-01	ENPP5	83965	NM_032003	14030778	GAUGAAGGGUGGUUAUAUU
M-046066-00	D-046066-02	ENPP5	83965	NM_032003	14030778	CCGCUGGGAUUACUUAUAU
M-046066-00	D-046066-03	ENPP5	83965	NM_032003	14030778	GAGGAAUCGUACCCUUAUU
M-046066-00	D-046066-04	ENPP5	83965	NM_032003	14030778	CGACCUAUUAUCUACCUUA
M-058090-00	D-058090-01	ENPP6	320981	NM_177304	31343292	GCACGGAUAUGACAACGAA
M-058090-00	D-058090-02	ENPP6	320981	NM_177304	31343292	GCUCUCGACUCAUUAAGA
M-058090-00	D-058090-03	ENPP6	320981	NM_177304	31343292	UAGCAGCCAUAUACCAUGA
M-058090-00	D-058090-04	ENPP6	320981	NM_177304	31343292	CCAACUUACUGCCUAGAAU
M-058252-00	D-058252-01	EPB4.1L5	226352	NM_145506	30841013	GAGCAUACCUUUGUCUUUA
M-058252-00	D-058252-02	EPB4.1L5	226352	NM_145506	30841013	CGACUAGGAUCCCGAUUUA
M-058252-00	D-058252-03	EPB4.1L5	226352	NM_145506	30841013	GACCUACCAUUGACAUUAA
M-058252-00	D-058252-04	EPB4.1L5	226352	NM_145506	30841013	GUGAGGAGCUAACCCGGUA
M-041117-00	D-041117-01	EPM2A	13853	NM_010146	6753763	GAAGAAGGCUUGUCCUACA
M-041117-00	D-041117-02	EPM2A	13853	NM_010146	6753763	GCACAACAGACUUCUAUUU
M-041117-00	D-041117-03	EPM2A	13853	NM_010146	6753763	GACCGUUGCUGCACAUUA
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M-055549-00	D-055549-02	L600019O04RIK	72007	NM_173182	27502352	CCUUAGAACUCGUUUAUCA
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M-055549-00	D-055549-04	L600019O04RIK	72007	NM_173182	27502352	GCGCAGGGCCAUAUAGUGA
M-042888-00	D-042888-01	FBP1	14121	NM_019395	9506588	GCAAUAUAGUUGUCUGUUU
M-042888-00	D-042888-02	FBP1	14121	NM_019395	9506588	GGACAAAGAUUAUUAUAGAC
M-042888-00	D-042888-03	FBP1	14121	NM_019395	9506588	CAAUUGCCAUCAUAAUCGA
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M-045301-00	D-045301-01	FBP2	14120	NM_007994	6679760	GGAAUAUAGUGGUUUGCUU
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M-045301-00	D-045301-03	FBP2	14120	NM_007994	6679760	AAAGAUGUCCGGAUUAAGA
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M-041872-00	D-041872-01	FHIT	14198	NM_010210	6753861	UGAAGUGGCCGAUUUGUUU
M-041872-00	D-041872-02	FHIT	14198	NM_010210	6753861	GGACAGUGGUGGAGAAGCA
M-041872-00	D-041872-03	FHIT	14198	NM_010210	6753861	GAAUAGGAAACCCGUUGUA
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M-041904-00	D-041904-02	FKBP1B	14226	NM_016863	40254557	GAUGUGGCCUAUGGAGCUA
M-041904-00	D-041904-03	FKBP1B	14226	NM_016863	40254557	AGAU AUGUGUGGUGCACUA
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M-042293-00	D-042293-01	5430404L10RIK	66793	NM_025769	21313313	CGGACUAGAUCGUAAUGUA
M-042293-00	D-042293-02	5430404L10RIK	66793	NM_025769	21313313	CAGAGUAUUUCGAGGCUUU
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M-042293-00	D-042293-04	5430404L10RIK	66793	NM_025769	21313313	GAUUAUCACUGUUUCUUCG
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M-044918-00	D-044918-02	DUSP23	68440	NM_026725	55926214	CCAUUGAGACGUAUGAACA
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M-054149-00	D-054149-02	C130099A20RIK	235534	NM_153420	23510308	CCGUAGACCCGGUUUAUGA
M-054149-00	D-054149-03	C130099A20RIK	235534	NM_153420	23510308	UCAAGACGCACCAGAUAGA
M-054149-00	D-054149-04	C130099A20RIK	235534	NM_153420	23510308	ACCCUAAAACUAGAAGCUUU
M-052556-00	D-052556-01	4921523A10RIK	110332	NM_173449	27734091	GAUCAUAUCUGGAGUACUA
M-052556-00	D-052556-02	4921523A10RIK	110332	NM_173449	27734091	GCGAAGCAGUGAUCAGUUC
M-052556-00	D-052556-03	4921523A10RIK	110332	NM_173449	27734091	CUUCAUAGAGAGUGUAUAA
M-052556-00	D-052556-04	4921523A10RIK	110332	NM_173449	27734091	GAUCAAAAGUGUGGGAUGA
M-056497-00	D-056497-01	A230098A12RIK	235472	NM_175485	31342005	CCUAAGACCUCCCGAAAUU
M-056497-00	D-056497-02	A230098A12RIK	235472	NM_175485	31342005	CGACACGACUCAUUA CAUC
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M-042150-00	D-042150-01	2810423O19RIK	67905	NM_198931	39573723	CGAAGAAGAUCAAGAGUGG
M-042150-00	D-042150-02	2810423O19RIK	67905	NM_198931	39573723	GUUCAGGGAUCAUCACAUG
M-042150-00	D-042150-03	2810423O19RIK	67905	NM_198931	39573723	GCAGGUAUCCUACGACGAU
M-042150-00	D-042150-04	2810423O19RIK	67905	NM_198931	39573723	AAACAGAGCGGCAGCGGAU
M-051740-00	D-051740-01	MGC60968	384619	NM_201374	41235780	GCAAGCAGCUGCGAAGUAC
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M-047978-00	D-047978-02	FNDC3	319448	NM_207636	46519046	GU AUCAGUCUUCACAAGUA
M-047978-00	D-047978-03	FNDC3	319448	NM_207636	46519046	GCUUAUAACUCAGAAGGUA
M-047978-00	D-047978-04	FNDC3	319448	NM_207636	46519046	UCGCACGGCUUUAAAAUUA
M-047468-00	D-047468-01	LOC268729	268729	XM_193753	38075911	GCACAUCCAUGGAGAACAA
M-047468-00	D-047468-02	LOC268729	268729	XM_193753	38075911	CAAAGCAUACU UCCGAUU
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M-047468-00	D-047468-04	LOC268729	268729	XM_193753	38075911	GAUGCACGUCUAU UCCUUA
M-058931-00	D-058931-01	6030440G05RIK	232288	NM_145148	21553102	GAACAUGAUUUACCUAAGA
M-058931-00	D-058931-02	6030440G05RIK	232288	NM_145148	21553102	GGAGAGCAAUUUCCUGAUA
M-058931-00	D-058931-03	6030440G05RIK	232288	NM_145148	21553102	GCACUUCGCUGUCAGGUUC
M-058931-00	D-058931-04	6030440G05RIK	232288	NM_145148	21553102	GCACGGAGA UCCUUGACGA
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M-065373-00	D-065373-02	AW742319	57874	NM_021345	28916662	GAGCGCAAUUGAAAUCUUC
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M-065373-00	D-065373-04	AW742319	57874	NM_021345	28916662	GGAAAGGCUCACCAAGCAA
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M-045385-00	D-045385-02	4930403L05RIK	67395	NM_026128	13385637	GAAGUUACACUACAACGAA
M-045385-00	D-045385-03	4930403L05RIK	67395	NM_026128	13385637	GGAGCUACAAUCUGAAGUA
M-045385-00	D-045385-04	4930403L05RIK	67395	NM_026128	13385637	GGAAGAAAUCGCAGAAGUG
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M-059587-00	D-059587-04	IGBP1	18518	XM_196586	38087214	UCAAGACAGGCUAAAAUA
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M-048470-00	D-048470-02	0710007A14RIK	67444	NM_023343	39930414	CAUCAUCUCUCAUJACUCG
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M-063094-00	D-063094-02	IMPA1	55980	NM_018864	31980941	GAGAUGGAUGUCAUGAUUA
M-063094-00	D-063094-03	IMPA1	55980	NM_018864	31980941	GUGCAUCGGUUUCCCUUUG
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M-046445-00	D-046445-01	IMPA2	114663	NM_053261	16716594	GCUCAUAGCUCAGGCCCUA
M-046445-00	D-046445-02	IMPA2	114663	NM_053261	16716594	CGACGGCACCUGCAACUUU
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M-061296-00	D-061296-02	INPP4B	234515	XM_134427	38089360	GGACAUGGCAGUCGGCAUU
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M-061387-00	D-061387-01	C130044A18RIK	244650	XM_146511	38089433	GACCUUAGCUCCUUAUGUA

M-061387-00	D-061387-02	C130044A18RIK	244650	XM 146511	38089433	GGAGAAACUUACCAUGUUA
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M-061387-00	D-061387-04	C130044A18RIK	244650	XM 146511	38089433	GGCCUGAACUUGUCCCAUA
M-057271-00	D-057271-01	4930579P15RIK	75276	NM 172420	29150267	ACACAAGAAUCGCAGAAUG
M-057271-00	D-057271-02	4930579P15RIK	75276	NM 172420	29150267	CAGCAUCCCUUGUGAUUUCU
M-057271-00	D-057271-03	4930579P15RIK	75276	NM 172420	29150267	GCGUUAAGCAUCUGAAAGA
M-057271-00	D-057271-04	4930579P15RIK	75276	NM 172420	29150267	AUGAGAAGAGAGUGACUAA
M-064489-00	D-064489-01	LOC238011	238011	XM 137914	38092576	CACAACAUGUUCUACAAUA
M-064489-00	D-064489-02	LOC238011	238011	XM 137914	38092576	GAACGUCACCUACCAAGGA
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M-064489-00	D-064489-04	LOC238011	238011	XM 137914	38092576	GUACAGUGACCUCGGCUAU
M-059386-00	D-059386-01	D3BWG0562E	229791	NM 177664	33186920	UGACAGACCUCAAUCAAGA
M-059386-00	D-059386-02	D3BWG0562E	229791	NM 177664	33186920	GAAGAUUUUUGUUCAGGAU
M-059386-00	D-059386-03	D3BWG0562E	229791	NM 177664	33186920	UAAGAACCAUCCAGUCGAU
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M-042328-00	D-042328-01	MAP3K7IP1	66513	NM 025609	34328267	GACGAGCUCUUUCGGCUUU
M-042328-00	D-042328-02	MAP3K7IP1	66513	NM 025609	34328267	GCAACUGGGUUUAGACGCA
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M-047047-00	D-047047-02	2310043K02RIK	66959	NM 025869	27229023	GGAGUGAGCAGAUCCGCUA
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M-043285-00	D-043285-04	MINPP1	17330	NM 010799	6754697	AAAGAAAGUUGCAGCUACU
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M-048244-00	D-048244-02	1700011C14RIK	76571	NM 029659	15805021	GCUCUUACAUAUCAAGCUU
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M-045040-00	D-045040-01	MTMR1	53332	NM 016985	8393793	GAAAGUGCUUACCCUAAUG
M-045040-00	D-045040-02	MTMR1	53332	NM 016985	8393793	GUGAAAGACGUCAUUUAUA
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M-040162-00	D-040162-01	MTMR4	170749	NM_133215	18875367	GAAGCCACCUGAACAGUUU
M-040162-00	D-040162-02	MTMR4	170749	NM_133215	18875367	AAAGAGUACUUGAGCAAUA
M-040162-00	D-040162-03	MTMR4	170749	NM_133215	18875367	CCAAGGAACUGGUGAAGGA
M-040162-00	D-040162-04	MTMR4	170749	NM_133215	18875367	GCAAGAGGCCGAGCAAUAA
M-064801-00	D-064801-01	9530014D17RIK	320634	NM_177215	46195806	GAAGAUAUUCGUCCGCAUUA
M-064801-00	D-064801-02	9530014D17RIK	320634	NM_177215	46195806	GUUGAAAUUUGACCAGUUA
M-064801-00	D-064801-03	9530014D17RIK	320634	NM_177215	46195806	GAACUUAGCAGGAGAGAGU
M-064801-00	D-064801-04	9530014D17RIK	320634	NM_177215	46195806	CUUCAGAUGUGUUCAAGAA
M-061583-00	D-061583-01	LOC382051	382051	XM_358697	38089574	GAUCAGAAAUCCAAUCAGG
M-061583-00	D-061583-02	LOC382051	382051	XM_358697	38089574	GGAGACGCCUUAUCUCAAG
M-061583-00	D-061583-03	LOC382051	382051	XM_358697	38089574	CCUACUGGAUCUUCAAUUC
M-061583-00	D-061583-04	LOC382051	382051	XM_358697	38089574	GCGGGAGGGAUUUUCUUU
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M-041829-00	D-041829-02	PFKFB2	18640	NM_008825	34147044	CUAAGAAACUAACACGCUA
M-041829-00	D-041829-03	PFKFB2	18640	NM_008825	34147044	CCAAUAUUCUGGAGGUAAA
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M-050027-00	D-050027-01	PFKFB3	170768	NM_133232	18875393	CAACGAAAGUGUUCAUGU
M-050027-00	D-050027-02	PFKFB3	170768	NM_133232	18875393	CCAAGAAGCUGACUCGCUA
M-050027-00	D-050027-03	PFKFB3	170768	NM_133232	18875393	GUUCUACGCUGCCUACUAG
M-050027-00	D-050027-04	PFKFB3	170768	NM_133232	18875393	CGAAUUGUAUACUACCUGA
M-054640-00	D-054640-01	C230090D14	270198	NM_173019	40254264	CUAAGAAGCUGACGCGGUA
M-054640-00	D-054640-02	C230090D14	270198	NM_173019	40254264	AGAGUCGCAUCGUUUUAUUA
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M-054640-00	D-054640-04	C230090D14	270198	NM_173019	40254264	AUUCAACGUUGGUCAGUAU
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M-045595-00	D-045595-02	PHPT1	75454	XM_203853	28484798	GUACCACGCGGACAUCUAU
M-045595-00	D-045595-03	PHPT1	75454	XM_203853	28484798	AAUCGUGCGUGGCUACAAA
M-045595-00	D-045595-04	PHPT1	75454	XM_203853	28484798	GUGUAUGGCUACUCUAUGG
M-041181-00	D-041181-01	PIB5PA	170835	NM_172439	27369945	GACCUGAACUCCGCAUUG
M-041181-00	D-041181-02	PIB5PA	170835	NM_172439	27369945	GAUCUUGUAUUCUGGUUUG
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M-048382-00	D-048382-01	2700017M01RIK	72590	NM_028292	30794137	GGACGGAAGCGGGACUUUA
M-048382-00	D-048382-02	2700017M01RIK	72590	NM_028292	30794137	GGGCUUAUCCAAUCUCUUU
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M-048382-00	D-048382-04	2700017M01RIK	72590	NM_028292	30794137	CGGCAGCAAUCAUCAGUAG
M-062377-00	D-062377-01	PON1	18979	NM_011134	7242182	GACGGGUGCUGAAGACUUA
M-062377-00	D-062377-02	PON1	18979	NM_011134	7242182	UGGCAAGUAUGUCUAUAUA
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M-062377-00	D-062377-04	PON1	18979	NM_011134	7242182	UUACUACAGUCCAGAUAAA
M-063336-00	D-063336-01	PON2	330260	NM_183308	34303978	GACCAUAUAUGAUAGGAAA
M-063336-00	D-063336-02	PON2	330260	NM_183308	34303978	UGACAACGGUGUAUAUCAA
M-063336-00	D-063336-03	PON2	330260	NM_183308	34303978	GGAUCAAUUUUCACCUGA
M-063336-00	D-063336-04	PON2	330260	NM_183308	34303978	AGACAUACUUGAACCUACA
M-048597-00	D-048597-01	PPAP2A	19012	NM_008247	45592927	UGACUGACAUCGCUAAGUA
M-048597-00	D-048597-02	PPAP2A	19012	NM_008247	45592927	CAACUGCAGUGAUGGCUAU
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M-048597-00	D-048597-04	PPAP2A	19012	NM_008247	45592927	UGUCGGCAAUCCCUACAUA
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M-040454-00	D-040454-02	PPAP2B	67916	NM_080555	18017589	ACAAGGCGAUCGUCCCUGA
M-040454-00	D-040454-03	PPAP2B	67916	NM_080555	18017589	GGAAUUCUACCGGAUCUAU
M-040454-00	D-040454-04	PPAP2B	67916	NM_080555	18017589	AAACGAUGCUGUGCUCUGU
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M-047777-00	D-047777-02	PPAP2C	50784	NM_015817	7657470	GUACAUGAUUGGCCGUCUU
M-047777-00	D-047777-03	PPAP2C	50784	NM_015817	7657470	UGUCAUCCUUGUCUCAUUG
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M-059311-00	D-059311-01	LOC237178	237178	XM_142281	38086988	GGUCAUACAUGGUGGGUAU
M-059311-00	D-059311-02	LOC237178	237178	XM_142281	38086988	GCAUUAGUAACUCGAAUUU
M-059311-00	D-059311-03	LOC237178	237178	XM_142281	38086988	CGCCAAAGAGAUACAUA
M-059311-00	D-059311-04	LOC237178	237178	XM_142281	38086988	GCGGAAGUGUUGACUACAU
M-060194-00	D-060194-01	PPEF2	19023	NM_011148	31560803	CCAAGGAAGUGAUGCAUAA
M-060194-00	D-060194-02	PPEF2	19023	NM_011148	31560803	CGGAUAACGUGUUGGAAUA
M-060194-00	D-060194-03	PPEF2	19023	NM_011148	31560803	CGAUACGUCUUGAAUCUUC
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M-040052-00	D-040052-01	PPM1A	19042	NM_008910	31560649	GAACAGGGUUUCUGGAGAU
M-040052-00	D-040052-02	PPM1A	19042	NM_008910	31560649	GAUGAACACAUGAGAGUUA
M-040052-00	D-040052-03	PPM1A	19042	NM_008910	31560649	GAAACAUGGUGCAGAUAGA
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M-040053-00	D-040053-01	PPM1B	19043	NM_011151	33859599	GAUCCCGAGUGGCAAAUUA
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M-040053-00	D-040053-04	PPM1B	19043	NM_011151	33859599	GAACGGACAGGUCUGCUUU
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M-060674-00	D-060674-03	PPM1D	53892	NM_016910	31560441	GUGAUGGACUUUGGAAUAU
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M-057052-00	D-057052-01	PPM1F	68606	NM_176833	31341186	GGCAGUGGCUGGUCUCUAU
M-057052-00	D-057052-02	PPM1F	68606	NM_176833	31341186	GCACAAGGGCAAUGGGAUG
M-057052-00	D-057052-03	PPM1F	68606	NM_176833	31341186	GGCAGUGUCUAGAGCCA UU
M-057052-00	D-057052-04	PPM1F	68606	NM_176833	31341186	GCGUGGAUGCAGCAAGGUA
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M-040054-00	D-040054-02	PPM1G	14208	NM_008014	31982360	UCAGAAGGCUUUACAAGAU
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M-040054-00	D-040054-04	PPM1G	14208	NM_008014	31982360	UGAGGAGGCUGCAUUGUUG
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M-057952-00	D-057952-02	PPM1L	242083	NM_178726	30520152	GAAGAAGCGGUUCGAUUCA
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M-057952-00	D-057952-04	PPM1L	242083	NM_178726	30520152	GAUAGAGGAUACAAUGACU
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M-050145-00	D-050145-02	LOC381511	381511	XM_355470	38078071	GUUCAAUUCUCAUGUUGUA
M-050145-00	D-050145-03	LOC381511	381511	XM_355470	38078071	GCAGAAA UUUUACCUCACA
M-050145-00	D-050145-04	LOC381511	381511	XM_355470	38078071	GCCAUGCUGUAGGCAAUAA
M-040960-00	D-040960-01	PPP1CA	19045	NM_031868	13994194	UCAGAUACCCGGAGAAUUU
M-040960-00	D-040960-02	PPP1CA	19045	NM_031868	13994194	CCAAGUUCUGCACAAAGCA
M-040960-00	D-040960-03	PPP1CA	19045	NM_031868	13994194	UGACCGUGGUGUCUCCUUU
M-040960-00	D-040960-04	PPP1CA	19045	NM_031868	13994194	GAAGAUACAACAUCAAACU

M-040211-00	D-040211-01	PPP1CB	19046	NM_172707	28173567	GAAGAUUUUGUGGAGACAUU
M-040211-00	D-040211-02	PPP1CB	19046	NM_172707	28173567	GGAAGACAUUCACUGAUUG
M-040211-00	D-040211-03	PPP1CB	19046	NM_172707	28173567	GAAACCAUCUGAAAAGAAA
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M-040212-00	D-040212-01	PPP1CC	19047	NM_013636	31980771	GAACUUCUUUCUUCUCAGA
M-040212-00	D-040212-02	PPP1CC	19047	NM_013636	31980771	GGAGUGUCCUUCACAUUUG
M-040212-00	D-040212-03	PPP1CC	19047	NM_013636	31980771	UCAAUAGGAUCUACGGAU
M-040212-00	D-040212-04	PPP1CC	19047	NM_013636	31980771	GCAGUUAGUCACUCUGUUU
M-048224-00	D-048224-01	PPP1R11	76497	NM_029632	18390326	GAGCCUAACUAUGAAACU
M-048224-00	D-048224-02	PPP1R11	76497	NM_029632	18390326	GACAACGGUUACUGAGACA
M-048224-00	D-048224-03	PPP1R11	76497	NM_029632	18390326	AACGCAAGCCAGAGAAGAA
M-048224-00	D-048224-04	PPP1R11	76497	NM_029632	18390326	CUGUGGACAAUGAGCAU
M-055949-00	D-055949-01	PPP1R16B	228852	NM_153089	27819637	GAACCAACCUAUACCGGAA
M-055949-00	D-055949-02	PPP1R16B	228852	NM_153089	27819637	GAAGAUCCUCGUUCAGU
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M-040427-00	D-040427-02	PPP1R1B	19049	NM_144828	21536255	CCAUUAGCAACUUGAGU
M-040427-00	D-040427-03	PPP1R1B	19049	NM_144828	21536255	AUUAGCAACUUGAGUGA
M-040427-00	D-040427-04	PPP1R1B	19049	NM_144828	21536255	AUGGAAACUCUGAGGAC
M-042431-00	D-042431-01	PPP1R2	66849	NM_025800	18859586	UAAGUUAGCCAGACAGU
M-042431-00	D-042431-02	PPP1R2	66849	NM_025800	18859586	GAAGACGCCUAUAGUGA
M-042431-00	D-042431-03	PPP1R2	66849	NM_025800	18859586	GAGACAGCAUGAAUGU
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M-044456-00	D-044456-01	PPP1R3C	53412	NM_016854	33468956	ACAAUGAGGGUCAGAAU
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M-046960-00	D-046960-02	PPP1R7	66385	NM_023200	12963568	GAAUUGGAGGUUCUAGA
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M-053022-00	D-053022-02	6330548N22RIK	100336	NM_146154	22122684	CAUCAGCAGUGGCCAUAAA
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M-041905-00	D-041905-01	PPP2CB	19053	NM_017374	40254559	GAUACGAACUACCUAUUCA
M-041905-00	D-041905-02	PPP2CB	19053	NM_017374	40254559	UGGAAAUACUUUACAGAU
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M-060647-00	D-060647-02	PPP2R1A	51792	NM_016891	31560470	CAGCAACCUUAAGAAAUUA
M-060647-00	D-060647-03	PPP2R1A	51792	NM_016891	31560470	GAUUCGCUCUAUCCUAUUG
M-060647-00	D-060647-04	PPP2R1A	51792	NM_016891	31560470	CCGAAUGACUACACUCUUC
M-049607-00	D-049607-01	2900026H06RIK	72930	NM_028392	21312160	CAACCACACGGGAGAGUUA

M-049607-00	D-049607-02	2900026H06RIK	72930	NM_028392	21312160	GGACAAUCCGGCUGUGUGA
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M-049607-00	D-049607-04	2900026H06RIK	72930	NM_028392	21312160	CGUAAUAUUUCAGCGAGAA
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M-062227-00	D-062227-02	3222402P14RIK	235542	XM_135153	38090054	GACCGUAGAUUUUGACAGAA
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M-040055-00	D-040055-01	PPP2R4	110854	NM_138748	31981676	GGAAGUUGCAGAAGACAUA
M-040055-00	D-040055-02	PPP2R4	110854	NM_138748	31981676	GGACCAGCCUUCCCGGUUU
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M-040213-00	D-040213-02	PPP2R5A	226849	NM_144880	21450120	AAAGAACACUGGAAUCAGA
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M-042528-00	D-042528-03	PPP2R5B	225849	NM_198168	37718992	CGCAUGAUAUUCAGUAAUA
M-042528-00	D-042528-04	PPP2R5B	225849	NM_198168	37718992	CCUCUGAGUUGCAUGAGUU
M-058585-00	D-058585-01	PPP2R5C	26931	NM_012023	23956065	GACAAUACACGGCUUGAUA
M-058585-00	D-058585-02	PPP2R5C	26931	NM_012023	23956065	ACGAGUACAUCAUGAGUUU
M-058585-00	D-058585-03	PPP2R5C	26931	NM_012023	23956065	UAAUUGAACCAUCAGAGUU
M-058585-00	D-058585-04	PPP2R5C	26931	NM_012023	23956065	CCUUUAUACCGCAACUCAA
M-041653-00	D-041653-01	PPP2R5D	21770	NM_009358	33942058	UCAAUAGAGAUUGGUGGAGUA
M-041653-00	D-041653-02	PPP2R5D	21770	NM_009358	33942058	CCUAUAAACUGAAGAAGGA
M-041653-00	D-041653-03	PPP2R5D	21770	NM_009358	33942058	UCGCAUCUAUGGCAAGUUU
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M-053789-00	D-053789-01	PPP2R5E	26932	NM_012024	33859659	GACACGCUAUCUGAUCUUA
M-053789-00	D-053789-02	PPP2R5E	26932	NM_012024	33859659	GCGCUCCACUCUUAUGAA
M-053789-00	D-053789-03	PPP2R5E	26932	NM_012024	33859659	CGAGAACGGGACUAUUUAA
M-053789-00	D-053789-04	PPP2R5E	26932	NM_012024	33859659	GGAUGUGAUUGAACCGUCA
M-055714-00	D-055714-01	PPP3CA	19055	NM_008913	42415472	GCAAAGCGCUACUGUUGAG
M-055714-00	D-055714-02	PPP3CA	19055	NM_008913	42415472	ACAGAGUAUUUCACGUUUA
M-055714-00	D-055714-03	PPP3CA	19055	NM_008913	42415472	UAACAGAGGGUGCUUCGAU
M-055714-00	D-055714-04	PPP3CA	19055	NM_008913	42415472	CCUGGUCGCUGCCAUUUGU
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M-040744-00	D-040744-01	Ppp3r1	19058	NM_024459	13277369	GAACAGAAGUUGAGGUUUG
M-040744-00	D-040744-02	Ppp3r1	19058	NM_024459	13277369	GGAUGGAGAUGGAAGAAUA
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M-041185-00	D-041185-02	PTP4A1	19243	NM_011200	6755225	GAACAUGCGAUUUCUUAUU
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M-040086-00	D-040086-02	PTPN14	19250	NM_008976	6679548	AGAAACAUCUGGACAAGUU
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M-040086-00	D-040086-04	PTPN14	19250	NM_008976	6679548	GUACAUUUUCUCGGUUGUUU
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M-043905-00	D-043905-02	PTPN18	19253	NM_011206	6755235	GAACUGCGCUCCAAUCUGC
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M-055896-00	D-055896-02	PTPN8	19260	NM_008979	6679554	GAGUAAGAGUUUGAAGAUU
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M-055896-00	D-055896-04	PTPN8	19260	NM_008979	6679554	UCUGGGGAUAUGCGUUGUUA
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M-041172-00	D-041172-02	HCPH	15170	NM_013545	7305132	GAACAAAUGUGUCCCAUAC
M-041172-00	D-041172-03	HCPH	15170	NM_013545	7305132	GCAAGAACCGCUACAAGAA
M-041172-00	D-041172-04	HCPH	15170	NM_013545	7305132	UGACACAGCAGAAUACAAA
M-056850-00	D-056850-01	C920001D21RIK	320139	NM_177081	31342761	GAAGAUUCCCUCAAACUUU
M-056850-00	D-056850-02	C920001D21RIK	320139	NM_177081	31342761	UGAAAGAGCACCCAGAAUA
M-056850-00	D-056850-03	C920001D21RIK	320139	NM_177081	31342761	GGAGAAAUGUGUACACUAC
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M-049539-00	D-049539-02	PTPN9	56294	NM_019651	9790180	CGAGUGCCCUAUUCAAUCA
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M-040761-00	D-040761-02	Ptpra	19262	NM_008980	6679556	CAACAGAAUUGGCUAAGGA
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M-040062-00	D-040062-01	PTPRR	19279	NM_011217	6755247	CAAGAGGCAUACAAACAU
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M-048432-00	D-048432-04	PTPRT	19281	NM_021464	10946855	CGACAUGGCUGAGAAUGAA
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M-056825-00	D-056825-04	PTPRU	19273	NM_011214	6755245	GGAAGGGUUCUCCGUAUCA
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M-055571-00	D-055571-02	PTPRZ1	19283	XM_358362	38083676	GAUCAGGGGUCCUUAUUA
M-055571-00	D-055571-03	PTPRZ1	19283	XM_358362	38083676	CGUAUGCUCUAAUGGUUUA
M-055571-00	D-055571-04	PTPRZ1	19283	XM_358362	38083676	UAACUAGGGUGGAUAAGUC
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M-046008-00	D-046008-03	RWDD2	69519	NM_027100	21312303	CAACAUCGAUUUUGUCAUC
M-046008-00	D-046008-04	RWDD2	69519	NM_027100	21312303	GAUCUAUAGCCACCACAUA
M-045630-00	D-045630-01	SAG	20215	NM_009118	6677844	AAGAAAGUGUUCAGGAUGA
M-045630-00	D-045630-02	SAG	20215	NM_009118	6677844	UCAAGAAGGUCUCUCGGGA
M-045630-00	D-045630-03	SAG	20215	NM_009118	6677844	UACCUACCCUGUUCAGUGA
M-045630-00	D-045630-04	SAG	20215	NM_009118	6677844	CCAAGUAGAGCCUGUAGAU
M-048933-00	D-048933-01	SBF1	77980	XM_358316	38077092	GCAAGUCAGUGUGGGAGUA
M-048933-00	D-048933-02	SBF1	77980	XM_358316	38077092	GCGCUUGGAGACAGAGUUA
M-048933-00	D-048933-03	SBF1	77980	XM_358316	38077092	CAACACGUCGCGUUUACAA
M-048933-00	D-048933-04	SBF1	77980	XM_358316	38077092	GAUACUAUGACCACCGAAU
M-051936-00	D-051936-01	AW538196	101869	NM_133952	19527171	GCAACGAGCUGUUCAAAUG
M-051936-00	D-051936-02	AW538196	101869	NM_133952	19527171	GAUCCACGCUGCCGGCAAA
M-051936-00	D-051936-03	AW538196	101869	NM_133952	19527171	GAAUAUAGACUCAUCCAAC
M-051936-00	D-051936-04	AW538196	101869	NM_133952	19527171	GCACGCUGGUCGGCAUUUG
M-042756-00	D-042756-01	SSH3	245857	NM_198113	37674268	GGUCAAAAGCCGAAUCUUA
M-042756-00	D-042756-02	SSH3	245857	NM_198113	37674268	CCUGAGCGCUUACCUAUU
M-042756-00	D-042756-03	SSH3	245857	NM_198113	37674268	ACACAUCGAUUAUUGAGG
M-042756-00	D-042756-04	SSH3	245857	NM_198113	37674268	GCUACUUACUACCAGGAGA
M-040044-00	D-040044-01	STYX	56291	NM_019637	9789980	GCAGGUAUUCUCUAGAAGUG
M-040044-00	D-040044-02	STYX	56291	NM_019637	9789980	GAUGGAAGCUUACAAAUG
M-040044-00	D-040044-03	STYX	56291	NM_019637	9789980	GGAGUGGACUUAACCUAUG
M-040044-00	D-040044-04	STYX	56291	NM_019637	9789980	GAGAUGCAUUUGCUUAUGU
M-053808-00	D-053808-01	LOC385666	104015	XM_358889	38081524	CCAAAGUACUGGAUGCAUA

M-053808-00	D-053808-02	LOC385666	104015	XM 358889	38081524	GGACUUCACGGAAAGAUAA
M-053808-00	D-053808-03	LOC385666	104015	XM 358889	38081524	GAUAAUAUAGGGCGUAAUC
M-053808-00	D-053808-04	LOC385666	104015	XM 358889	38081524	UGAAUCUGCUUGGGUCUAA
M-058579-00	D-058579-01	SYNJ2	20975	NM 011523	21362256	GGAAUUAUGGGACGAUUAU
M-058579-00	D-058579-02	SYNJ2	20975	NM 011523	21362256	GCUCAGGGGUGUUCUAUUU
M-058579-00	D-058579-03	SYNJ2	20975	NM 011523	21362256	GCGAAGGGCGAGAAUGUAA
M-058579-00	D-058579-04	SYNJ2	20975	NM 011523	21362256	UGUCUCGACUGUCUGGAUA
M-050678-00	D-050678-01	TPTE	234129	NM 199257	40549439	GAAACGAAUUGGAUAAUAC
M-050678-00	D-050678-02	TPTE	234129	NM 199257	40549439	GUUGUGCGGUUCCUGGAUA
M-050678-00	D-050678-03	TPTE	234129	NM 199257	40549439	UCGGUUGCCUUCAGAAUAU
M-050678-00	D-050678-04	TPTE	234129	NM 199257	40549439	UAACAGGCUUUAUCUACCA
M-059671-00	D-059671-01	ZFHX1B	24136	NM 015753	40254563	GUAAAUGGCCGAAUGAGAA
M-059671-00	D-059671-02	ZFHX1B	24136	NM 015753	40254563	GCGACACGGCCAUUAUUUA
M-059671-00	D-059671-03	ZFHX1B	24136	NM 015753	40254563	GCAGGUAACCGCAAGUUCA
M-059671-00	D-059671-04	ZFHX1B	24136	NM 015753	40254563	UAGAUUUGGUCACUGAUGA

Supplemental Table S2.
Mouse phosphatase library screening results

Gene Symbol	β -hex* (24H)	SD* (24H)	β -hex (48H)	SD (48H)	β -hex (72H)	SD (72H)
0710007A14RIK	0.07	0.58	-0.02	-0.13	-0.12	-0.86
1600019O04RIK	0.12	1.02	0.16	1.08	0.10	0.69
1700001E16RIK	0.07	0.61	0.05	0.35	0.22	1.51
1700011C14RIK	0.05	0.48	0.01	0.09	0.01	0.04
1700047I17RIK	-0.16	-1.41	-0.03	-0.24	0.06	0.40
1700094E07RIK	0.05	0.43	0.05	0.32	-0.04	-0.28
1810034K20RIK	0.18	1.61	0.30	2.07	0.13	0.94
2010001C14RIK	0.03	0.30	0.12	0.82	0.15	1.06
2310043K02RIK	0.00	0.02	0.02	0.17	0.05	0.34
2700017M01RIK	0.06	0.57	0.14	1.00	0.09	0.61
2810423O19RIK	0.13	1.15	0.08	0.58	0.05	0.32
2900026H06RIK	-0.03	-0.23	0.05	0.32	-0.08	-0.57
3222402P14RIK	-0.06	-0.49	-0.02	-0.16	0.04	0.26
4921523A10RIK	0.22	1.93	0.26	1.79	0.28	1.96
4930403L05RIK	-0.01	-0.08	-0.05	-0.38	0.01	0.08
4930579P15RIK	-0.10	-0.87	-0.06	-0.42	-0.01	-0.08
5430404L10RIK	-0.03	-0.29	-0.14	-0.94	-0.05	-0.35
6030440G05RIK	0.09	0.81	-0.02	-0.13	-0.02	-0.16
6330548N22RIK	-0.07	-0.59	-0.07	-0.50	-0.07	-0.50
9530014D17RIK	-0.03	-0.29	-0.18	-1.26	-0.05	-0.35
A930026L03RIK	0.12	1.05	0.04	0.29	0.08	0.56
ACP1	-0.07	-0.66	0.05	0.35	-0.05	-0.33
ACP2	-0.02	-0.18	-0.06	-0.42	0.00	-0.03
ACP5	0.04	0.32	0.00	0.03	-0.01	-0.07
ACP6	0.03	0.25	-0.03	-0.24	0.00	0.02
ACPP	-0.01	-0.07	0.01	0.05	0.01	0.10
Acyp1	-0.07	-0.61	-0.04	-0.28	0.09	0.60
ACYP2	-0.05	-0.42	-0.08	-0.54	0.06	0.42
AKAP11	0.09	0.83	0.11	0.75	-0.03	-0.24
AKP2	0.15	1.34	0.15	1.02	0.18	1.25
AKP3	0.02	0.15	-0.03	-0.21	-0.09	-0.64
AKP5	0.05	0.41	-0.03	-0.20	-0.04	-0.25
ANP32E	0.14	1.22	-0.02	-0.11	0.01	0.10
AW456874	-0.14	-1.21	-0.19	-1.34	-0.24	-1.69
AW538196	-0.10	-0.84	-0.12	-0.85	-0.11	-0.75
AW742319	-0.03	-0.28	-0.19	-1.28	0.07	0.46
BPNT1	-0.08	-0.72	-0.05	-0.32	-0.05	-0.35
C130044A18RIK	0.09	0.78	0.11	0.73	0.06	0.45
C130099A20RIK	-0.12	-1.10	-0.13	-0.92	-0.05	-0.34
C230090D14	0.15	1.29	0.20	1.39	0.22	1.51
C920001D21RIK	0.02	0.14	0.02	0.11	0.10	0.73
CDC14A	0.06	0.57	0.06	0.40	-0.09	-0.60
CDC14B	0.22	1.97	0.13	0.92	0.20	1.40
CDC25A	-0.08	-0.67	-0.11	-0.74	-0.12	-0.83
CDC25B	-0.09	-0.84	-0.14	-0.96	-0.22	-1.52
CDC25C	0.11	1.01	-0.02	-0.12	0.00	0.02

CDKN3	-0.02	-0.17	-0.03	-0.22	0.11	0.76
CILP	0.07	0.64	0.03	0.20	0.06	0.45
CTDP1	0.04	0.40	0.14	0.99	0.12	0.81
CTDSPL	0.18	1.61	0.15	1.01	0.15	1.04
D19ERTD703E	-0.10	-0.86	-0.11	-0.75	-0.16	-1.13
D3BWG0562E	-0.08	-0.71	0.20	1.37	0.12	0.84
DNAJC6	0.00	-0.01	-0.06	-0.42	0.01	0.10
DUSP1	-0.06	-0.52	-0.14	-0.94	-0.24	-1.69
DUSP2	0.05	0.43	0.03	0.23	-0.03	-0.18
DUSP3	0.05	0.46	0.05	0.35	0.06	0.43
DUSP4	-0.06	-0.54	0.07	0.49	-0.01	-0.04
DUSP5	0.11	0.94	0.22	1.53	0.38	2.63
DUSP6	0.12	1.10	-0.02	-0.15	0.03	0.24
DUSP7	0.02	0.14	0.30	2.06	-0.03	-0.23
DUSP8	-0.02	-0.20	0.17	1.17	0.23	1.60
DUSP10	0.09	0.76	0.05	0.33	-0.04	-0.28
DUSP11	-0.02	-0.22	0.06	0.41	0.04	0.29
DUSP12	0.00	-0.02	-0.04	-0.25	0.19	1.34
DUSP13	-0.09	-0.81	-0.06	-0.43	-0.09	-0.65
DUSP14	0.12	1.03	0.02	0.11	-0.09	-0.64
DUSP15	0.21	1.89	0.34	2.35	0.18	1.29
DUSP18	0.07	0.65	0.11	0.74	-0.06	-0.39
DUSP19	-0.02	-0.20	-0.20	-1.37	-0.15	-1.06
DUSP23	-0.19	-1.72	-0.23	-1.58	-0.03	-0.22
DUT	-0.18	-1.55	-0.24	-1.66	-0.27	-1.88
ENPP1	0.13	1.13	0.04	0.27	0.00	-0.01
ENPP2	-0.02	-0.19	-0.02	-0.11	-0.14	-0.97
ENPP3	0.11	1.02	0.03	0.21	0.08	0.57
ENPP4	0.06	0.55	-0.02	-0.17	-0.11	-0.75
ENPP5	0.02	0.17	0.08	0.58	0.07	0.46
ENPP6	0.04	0.36	0.05	0.38	0.24	1.68
EPB4.1L5	0.00	-0.01	-0.24	-1.69	-0.16	-1.15
EPM2A	-0.27	-2.41	-0.28	-1.97	-0.32	-2.26
FBP1	-0.03	-0.25	0.02	0.13	-0.02	-0.13
FBP2	0.02	0.15	0.05	0.32	-0.07	-0.51
FHIT	-0.04	-0.39	-0.09	-0.65	-0.06	-0.39
FKBP1B	0.09	0.84	0.02	0.11	0.07	0.51
FNDC3	0.04	0.32	0.02	0.13	-0.10	-0.67
GPD11	0.08	0.72	0.32	2.21	0.20	1.39
GSBS	-0.17	-1.54	-0.11	-0.76	-0.14	-0.96
HCPH	0.05	0.40	0.04	0.26	0.12	0.82
IGBP1	0.02	0.18	0.00	0.03	0.08	0.59
ILVBL	-0.07	-0.59	-0.08	-0.55	-0.13	-0.91
IMPA1	-0.04	-0.39	0.03	0.19	-0.06	-0.43
IMPA2	0.09	0.77	-0.09	-0.59	0.03	0.24
INPP1	0.14	1.27	0.08	0.57	0.03	0.21
INPP4B	0.02	0.15	0.10	0.66	0.11	0.74
INPP5B	0.23	2.00	0.34	2.32	0.36	2.50
INPP5D	-0.14	-1.23	0.00	0.00	0.12	0.82
LOC237178	0.19	1.69	0.14	0.99	0.24	1.71
LOC238011	-0.04	-0.35	0.07	0.49	-0.01	-0.08
LOC268729	0.05	0.47	0.03	0.23	0.02	0.14

LOC381511	0.03	0.27	-0.09	-0.65	-0.05	-0.32
LOC382051	0.03	0.23	0.00	0.01	0.08	0.56
LOC385666	0.00	0.01	0.02	0.16	0.05	0.36
MAP3K7IP1	0.08	0.68	0.03	0.21	0.08	0.58
MGC60968	0.07	0.64	0.03	0.22	0.12	0.85
MINPP1	0.28	2.52	0.26	1.78	0.06	0.44
MTM1	0.00	-0.04	0.11	0.75	-0.01	-0.09
MTMR1	-0.02	-0.15	0.03	0.22	-0.02	-0.17
MTMR2	-0.11	-0.99	0.04	0.25	0.17	1.22
MTMR3	-0.03	-0.27	0.09	0.64	0.14	0.98
MTMR4	-0.11	-0.99	-0.23	-1.58	-0.31	-2.19
ORF5	0.08	0.74	0.05	0.32	0.07	0.52
PFKFB2	0.16	1.40	-0.04	-0.25	-0.06	-0.42
PFKFB3	0.09	0.79	0.20	1.38	0.16	1.15
PHPT1	0.08	0.73	0.05	0.37	-0.11	-0.77
PIB5PA	0.12	1.10	0.02	0.11	0.08	0.54
PON1	-0.06	-0.51	0.23	1.60	0.18	1.23
PON2	-0.01	-0.07	0.17	1.19	0.20	1.39
PPAP2A	0.17	1.47	0.16	1.09	0.16	1.11
PPAP2B	0.14	1.24	0.19	1.33	0.22	1.53
PPAP2C	0.14	1.23	0.17	1.15	0.06	0.40
PPEF2	-0.05	-0.45	-0.07	-0.48	-0.01	-0.05
PPM1A	-0.24	-2.14	-0.20	-1.37	-0.26	-1.81
PPM1B	-0.13	-1.15	-0.15	-1.04	-0.13	-0.90
PPM1D	-0.03	-0.26	0.00	0.00	0.07	0.52
PPM1F	0.23	2.00	0.31	2.13	0.33	2.35
PPM1G	0.09	0.77	0.17	1.21	0.03	0.23
PPM1L	-0.08	-0.74	-0.19	-1.31	-0.13	-0.94
PPP1CA	0.12	1.08	0.32	2.21	0.19	1.32
PPP1CB	0.14	1.24	0.29	2.04	0.32	2.26
PPP1CC	0.03	0.25	-0.04	-0.27	-0.04	-0.29
PPP1R11	-0.16	-1.44	-0.22	-1.53	-0.20	-1.41
PPP1R16B	0.03	0.23	-0.02	-0.17	0.05	0.34
PPP1R1B	-0.03	-0.31	0.00	-0.02	0.07	0.49
PPP1R2	0.03	0.29	0.09	0.65	0.17	1.22
PPP1R3C	0.00	-0.01	-0.01	-0.07	-0.03	-0.23
PPP1R7	0.09	0.75	0.06	0.42	0.08	0.53
Ppp2ca	0.09	0.84	0.27	1.88	0.16	1.13
PPP2CB	0.11	0.96	0.22	1.54	0.14	0.96
PPP2R1A	-0.04	-0.36	0.04	0.25	-0.08	-0.56
PPP2R4	-0.10	-0.91	-0.03	-0.20	0.02	0.15
PPP2R5A	0.10	0.85	0.18	1.28	0.23	1.60
PPP2R5B	-0.06	-0.54	-0.14	-0.99	-0.04	-0.31
PPP2R5C	-0.04	-0.36	-0.05	-0.34	0.00	-0.02
PPP2R5D	0.05	0.42	-0.05	-0.35	0.01	0.06
PPP2R5E	0.17	1.50	0.14	0.98	0.24	1.69
PPP3CA	0.09	0.79	0.05	0.35	0.04	0.26
PPP3CC	-0.03	-0.25	-0.16	-1.13	-0.31	-2.15
PPP3R1	-0.05	-0.47	-0.35	-2.39	-0.37	-2.63
PPP4C	-0.07	-0.64	-0.14	-0.95	-0.07	-0.49
PPP5C	-0.20	-1.73	-0.08	-0.54	-0.03	-0.20
PPP6C	0.00	0.01	-0.10	-0.67	-0.20	-1.43

PRTG	0.02	0.22	0.32	2.21	0.25	1.73
PSPH	0.45	3.94	0.00	0.01	-0.05	-0.35
PTEN	-0.22	-1.98	-0.22	-1.54	-0.44	-3.06
PTP4A1	-0.03	-0.23	-0.21	-1.43	-0.23	-1.62
PTP4A2	0.28	2.51	0.34	2.38	0.23	1.63
PTP4A3	0.02	0.18	0.09	0.61	0.07	0.50
Ptpla	0.00	0.03	0.15	1.04	0.12	0.81
Ptpn1	-0.09	-0.84	-0.12	-0.80	-0.19	-1.33
PTPN2	0.08	0.69	-0.07	-0.46	-0.07	-0.51
Ptpn3	0.16	1.44	0.06	0.41	0.08	0.59
PTPN4	-0.11	-0.98	-0.29	-2.00	-0.18	-1.28
PTPN5	0.00	0.00	-0.07	-0.51	-0.16	-1.09
PTPN8	-0.04	-0.40	-0.16	-1.13	-0.06	-0.43
PTPN9	0.13	1.14	0.33	2.26	0.31	2.17
PTPN11	0.05	0.43	0.18	1.26	0.19	1.34
PTPN12	0.12	1.06	0.02	0.12	0.11	0.78
PTPN13	-0.05	-0.47	-0.06	-0.42	0.01	0.04
PTPN14	-0.27	-2.39	-0.33	-2.31	-0.19	-1.33
PTPN18	0.15	1.34	0.07	0.47	0.10	0.71
PTPN20	0.25	2.22	0.36	2.52	0.44	3.11
PTPN21	-0.06	-0.55	-0.17	-1.18	-0.17	-1.21
PTPN23	0.14	1.25	0.09	0.62	0.06	0.41
Ptpa	-0.03	-0.23	-0.01	-0.04	-0.03	-0.21
PTPRB	-0.07	-0.64	-0.12	-0.80	-0.05	-0.33
PTPRC	0.20	1.80	0.31	2.11	0.16	1.13
PTPRE	0.09	0.77	0.09	0.64	0.08	0.56
PTPRF	-0.14	-1.28	-0.06	-0.42	0.00	0.02
PTPRG	0.05	0.45	0.08	0.57	0.16	1.15
PTPRH	0.14	1.23	0.10	0.68	0.00	0.03
PTPRJ	0.20	1.75	0.31	2.12	0.21	1.50
PTPRK	-0.02	-0.21	-0.10	-0.68	-0.04	-0.29
PTPRM	0.01	0.09	0.17	1.19	0.04	0.29
PTPRN	0.01	0.13	-0.11	-0.76	0.00	-0.02
PTPRN2	0.16	1.42	0.17	1.18	0.20	1.43
PTPRO	0.14	1.26	-0.02	-0.12	0.10	0.71
PTPRR	0.24	2.09	0.07	0.49	0.11	0.74
PTPRS	-0.02	-0.21	0.01	0.08	-0.02	-0.14
PTPRT	-0.20	-1.78	-0.09	-0.65	-0.02	-0.17
PTPRU	0.16	1.41	0.17	1.19	0.12	0.85
PTPRZ1	0.06	0.56	0.14	0.97	0.18	1.25
RWDD2	-0.07	-0.62	-0.09	-0.63	0.02	0.13
SAG	0.24	2.15	0.12	0.86	0.14	0.98
SBF1	-0.18	-1.62	-0.21	-1.42	-0.08	-0.56
SSH3	0.03	0.27	-0.04	-0.31	-0.06	-0.42
STYX	0.06	0.55	0.15	1.06	0.10	0.71
SYNJ2	0.16	1.43	0.00	0.03	0.01	0.08
TPTE	0.19	1.65	0.18	1.25	0.19	1.30
ZFHX1B	0.03	0.23	-0.01	-0.10	0.09	0.66

β -hex*: β -hexosaminidase release

SD*: standard deviation

The numbers are indices of Fc ϵ RI-induced degranulation with that of control cells as 0. The antigen-

induced β -hexosaminidase for control cells transfected with scrambled siRNA were $37\% \pm 5.5\%$ (SD) at 24 hr, $31\% \pm 4.4\%$ (SD) at 48 hr, and $31\% \pm 3.5\%$ (SD) at 72 hr.

Supplemental Table S3. Targets that induced >2.0 SD changes in degranulation.

Gene Symbol	Accession	b-hex release, 24H		b-hex release, 48H		b-hex release, 72H	
		Screening	Repeat	Screening	Repeat	Screening	Repeat
1810034K20RIK	NM_023397	0.18*	0.13	0.30**	0.22	0.13	0.39
DUSP5	XM_140740	0.11	0.28	0.22*	0.29	0.38**	0.40
DUSP7	NM_153459	0.02	0.22	0.30**	0.41	-0.03	0.10
DUSP15	NM_145744	0.21*	0.14	0.34**	0.22	0.18	0.01
EPM2A	NM_010146	-0.27**	-0.32	-0.28*	-0.29	-0.32**	-0.25
GPD1I	NM_175380	0.08	0.24	0.32**	0.26	0.20	0.20
INPP5B	NM_008385	0.23**	0.13	0.34**	0.34	0.36**	0.49
MINPP1	NM_010799	0.28**	0.18	0.26*	0.17	0.06	0.07
MTMR4	NM_133215	-0.11	-0.20	-0.23*	-0.41	-0.31**	-0.31
PPM1A	NM_008910	-0.24**	-0.20	-0.20	-0.22	-0.26*	-0.27
PPM1F	NM_176833	0.23**	0.16	0.31**	0.32	0.33**	0.28
PPP1CA	NM_031868	0.12	0.25	0.32**	0.27	0.19	0.16
PPP1CB	NM_172707	0.14	0.04	0.29**	0.09	0.32**	0.05
PPP3CC	NM_008915	-0.03	0.06	-0.16	-0.17	-0.31**	-0.31
PPP3R1	NM_024459	-0.05	-0.11	-0.35**	-0.26	-0.37**	-0.30
PRTG	NM_175485	0.02	0.17	0.32**	0.34	0.25*	0.29
PSPH	NM_133900	0.45**	0.34	0.00	0.13	-0.05	0.02
PTEN	NM_008960	-0.22*	-0.07	-0.22*	-0.18	-0.44**	-0.45
PTP4A2	NM_008974	0.28**	0.10	0.34**	0.26	0.23*	0.42
PTPN4	NM_019933	-0.11	-0.09	-0.29**	-0.31	-0.18	-0.38
PTPN9	NM_019651	0.13	0.20	0.33**	0.32	0.31**	0.24
PTPN14	NM_008976	-0.27**	-0.35	-0.33**	-0.33	-0.19	-0.39
PTPN20	NM_008978	0.25**	0.40	0.36**	0.31	0.44**	0.40
PTPRC	NM_011210	0.20*	0.18	0.31**	0.21	0.16	0.02
PTPRJ	NM_008982	0.20*	0.16	0.31**	0.14	0.21*	0.16
PTPRR	NM_011217	0.24**	0.19	0.07	0.13	0.11	0.00
SAG	NM_009118	0.24**	0.12	0.12	0.10	0.14	-0.05

This is a list of the targets and the results of the original and repeat testing. The enhancement or inhibition (-) is expressed as a fraction of the control release. For the screening results, * indicates SD was ≥ 1.5 and ** that SD was ≥ 2 . The antigen-induced β -hexosaminidase release for scrambled control cells were $40\% \pm 3.8\%$ (SD) for 24 hr, $36\% \pm 3.1\%$ (SD) for 48 hr, and $32\% \pm 2.4\%$ (SD) for 72 hr.

Supplemental Table 4.**FcεRI-Induced Degranulation with Single and Pooled siRNA**

	INPP5B 1	INPP5B 2	INPP5B 3	INPP5B 4	INPP5B Pool
24hr	0.19*	0.40*	0.07	0.08	0.27**
48hr	0.07	0.50**	0.06	0.02	0.34*
72hr	-0.04	0.34**	0.04*	0.02	0.35***
	PPP3R1 1	PPP3R1 2	PPP3R1 3	PPP3R1 4	PPP3R1 Pool
24hr	-0.06**	-0.06	-0.03	-0.07	-0.16
48hr	-0.39***	-0.26***	-0.01	-0.08	-0.31***
72hr	-0.50***	-0.27***	0.04	-0.05	-0.29**
	PTEN 1	PTEN 2	PTEN 3	PTEN 4	PTEN Pool
24hr	-0.06	-0.15***	0.00	-0.10**	-0.15**
48hr	-0.13	-0.36***	0.01	-0.20***	-0.40***
72hr	-0.21**	-0.51***	-0.01	-0.27***	-0.55***
	MTMR4 1	MTMR4 2	MTMR4 3	MTMR4 4	MTMR4 Pool
24hr	0.30**	-0.29***	0.10	-0.28***	-0.33***
48hr	0.30***	-0.40***	0.02	-0.50***	-0.48***
72hr	0.13*	-0.38***	-0.07	-0.66***	-0.44***
	PTPN4 1	PTPN4 2	PTPN4 3	PTPN4 4	PTPN4 Pool
24hr	0.11	0.02	-0.05	-0.13	-0.12
48hr	0.06	-0.09	-0.10	-0.30**	-0.24**
72hr	0.01	-0.05	-0.12	-0.35**	-0.23*
	PTPN9 -1	PTPN9 2	PTPN9 3	PTPN9 4	PTPN9 Pool
24hr	0.02	0.42**	-0.02	0.02	0.19*
48hr	0.12	0.78**	-0.15	-0.02	0.32
72hr	0.10	0.77**	-0.16*	0.00	0.30*
	PTPN14 1	PTPN14 2	PTPN14 3	PTPN14 4	PTPN14 Pool
24hr	-0.25**	-0.13**	-0.18*	0.08	-0.26**
48hr	-0.24***	-0.15	-0.16*	0.10	-0.25
72hr	-0.27*	-0.09	-0.05	0.04	-0.27*

Comparison to control scrambled siRNA transfected cells, the enhancement or inhibition (-) is expressed as a fraction of the control release. Results are the average of at least 3 experiments; * P is < 0.05; ** P is < 0.01; and *** P is < 0.001. See legend of Fig 3A for more details.