Supplemental Figure Legends

Supplemental Figure 1. Expression and purification of GST fusion protein of PLCγ1 SH2 C and its control (GST). 20µl of whole cell lysate from cells transformed with a GST expression vector or GST-PLCγ1-SH2-C vector before (UI) or after (IND) induction with IPTG were subjected to SDS-PAGE. The gel was stained with coomasie brilliant blue to visualize the protein expression (lane 3-6). 20µg of purified GST or GST-PLCγ1-SH2-C were subjected to SDS-PAGE and stained with coomasie brilliant blue (Lanes 1-2).

Supplemental Figure 2. PLCβ1 and PLCδ1 are not expressed in fibroblasts. Sprouty 1,2,4 null or control fibroblasts were serum starved and treated with PDGF:BB (20ng/ml) for the indicated time. 50µg of Cell lysate was immunoblotted for PLCγ1, PLCβ1, and PLCδ1.

Supplemental Figure 3. A) Sprouty1 expression upon Doxycycline induction in NIH 3T3 cells. NIH 3T3 cells engineered to express Spry1 under doxycycline control treated overnight with doxycycline in starvation media, treated with PDGF:BB (20ng/ml) for the indicated time and 50µg of cell lysate was immunoblotted with antibodies directed against anti-Spry1 and anti-beta actin. **B) Genomic PCR analysis for Spry gene integrity**. Fibroblasts created from Spry1,2,4$^{flox/flox}$ mouse embryos were immortalized by passage under a 3T3 protocol and infected with recombinant adenoviruses AdCMV5-eGFP or AdCMVCre-eGFP. Genomic PCR analysis confirms the deletion of Spry1, 2 and 4 from the fibroblasts. **C) Genomic PCR analysis for Spry2 genes expression in Spry2$^{+/+}$ and Spry2$^{-/-}$ Mast cells**. Mast cells derived from Spry2$^{+/+}$ and Spry2$^{-/-}$ bone marrow cultured for four weeks in Iscove modified Dulbecco media + glutamine containing 10ng/mL IL3 and 20ng/ml murine stem cell factor (SCF). Genomic PCR analysis performed to test their presence and absence of Spry2 in mast cells derived from Spry2$^{+/+}$ and Spry2$^{-/-}$ mice. **D)** Sprouty 1,2,4 null or control fibroblasts were serum starved and treated with PDGF BB (20ng/ml) for the indicated times. 50µg of Cell lysate were immunoblotted for Spry2 antibody.

Supplemental Figure 4. A) siRNA depletion of PLCγ1 and PLCγ2 in Spry1 overexpressing cells. NIH 3T3 cells engineered to express Spry1 under doxycycline control were depleted for endogenous PLCγ1 and PLCγ2 using siGENOME SMARTpool duplex RNA oligonucleotides for 24h, followed by transient transfection with NFAT luciferase reporter (5µg) and Renilla (50ng) plasmids using Fugene (Amersham) in duplicate. Serum starved (0.2% FBS containing serum media along with doxycycline) cells were either left unstimulated or stimulated with PDGF BB (20ng/ml) for 4h. 50µg of whole cell lyate was immunoblotted for PLCγ1, PLCγ2, Spry1 and GAPDH. **B) siRNA depletion of PLCγ1 and PLCγ2 in Spry2 overexpressing cells.** NIH 3T3 cells were treated with siGENOME SMARTpool directed against PLCγ1 and PLCγ2 for 24 hours followed by transient transfection with a NFAT luciferase reporter (5µg), Renilla (50ng) and Spry2 (1µg) expression plasmids using Fugene (Amersham) in duplicates. Serum starved (0.2% FBS containing serum media) cells were either left unstimulated or stimulated with PDGF BB (20ng/ml) for 4h. 50µg of whole cell lysate was immunoblotted for PLCγ1, PLCγ2, Spry2 and GAPDH. **C) The effect of PLCγ depletion of calcium-dependent transcription. Top-**NIH 3T3

cells engineered to express Spry1 under doxycycline control were depleted for PLCγ1 and PLCγ2 using siGENOME SMARTpool RNA oligonucleotides for 24h followed by transient transfection in duplicate with a NFAT luciferase reporter (5μg) and Renilla (50ng) plasmids using Fugene (Amersham) in triplicates. Serum starved (0.2% FBS containing serum media along with doxycycline) cells were either left unstimulated or stimulated with PDGF BB (20ng/ml) for 4h. The cells were then assayed using the Dual Luciferase Assay kit (Promega), normalizing firelfly luciferase activity to Renilla luciferase activity. **C) Bottom-** NIH 3T3 cells were depleted for PLCγ1 and PLCγ2 as above followed by transient transfection in duplicate with a NFAT luciferase reporter (5μg), Renilla (50ng) and Spry2 (1μg) expression plasmids using Fugene (Amersham). Serum starved (0.2% FBS containing serum media) cells were either left unstimulated or stimulated with PDGF BB (20ng/ml) for 4h. The cells were then assayed using the Dual Luciferase Assay kit (Promega), normalizing luciferase activity to Renilla luciferase activity.

Supplemental Methods

Genomic PCR analysis

The following Primers used to identify the genotype of Spry 1,2, 4 wt and Spry 1,2, and 4 null MEFs as well as Spry2$^{+/+}$ and Spry2$^{-/-}$ Mast cells.

Spy1

71: 5'-CTCAATAGGAGTGGACTGTGAAACTGC-3'

72: 5'-GGGAAAACCGTGTTCTAAGGAGTAGC-3'

73: 5'-GTTCTTTGTGGCAGACACTCTTCATTC-3'

Spy2

12: 5'-GGATGGCTCTGATCTGATCC-3'

138: 5'-GCATGGGCTATTCACAAAC-3'

148: 5'-TTGAGAACATGCCTCGACC-3'

Spy4

F1: 5'-CAGGACTTGGGAGTGCTTCCTTAG-3'

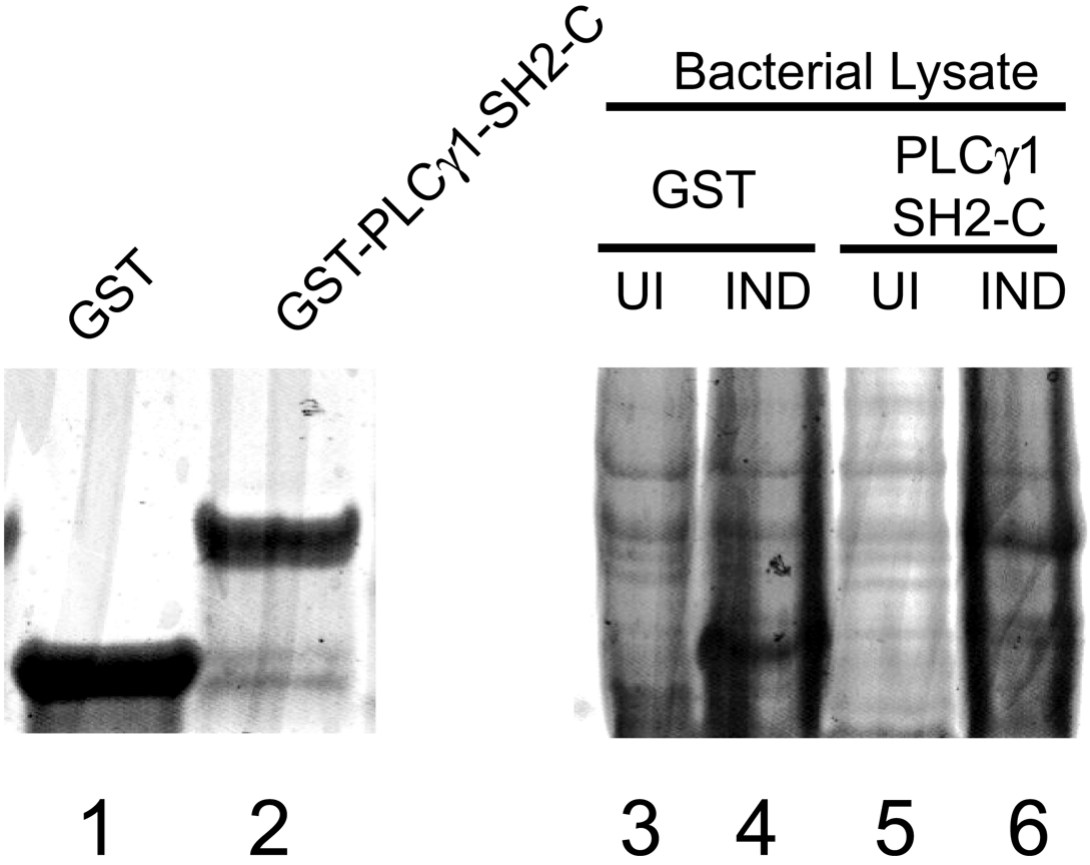
B3: 5'-CCTCCTAGTACCTTTTTGGGGAGAG-3'

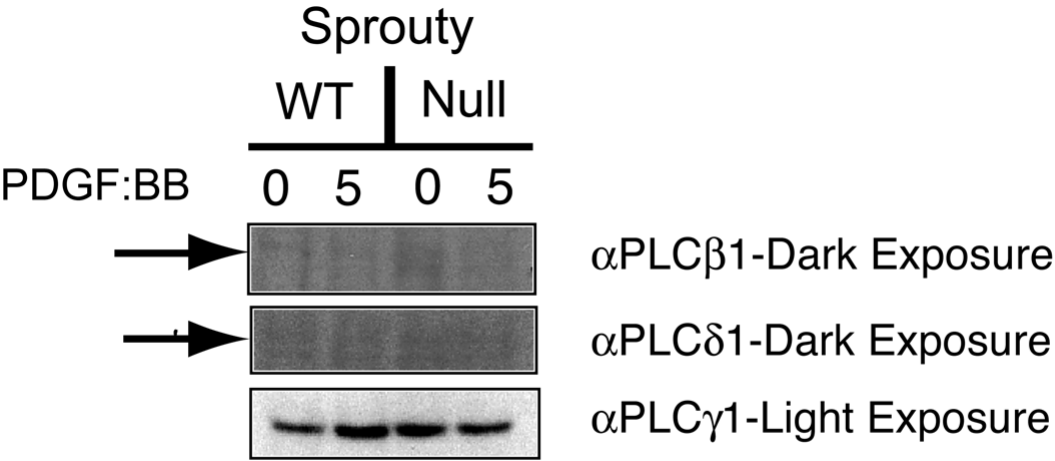
B4: 5'-TACAGCAGGAATGGCTACGGTG-3'

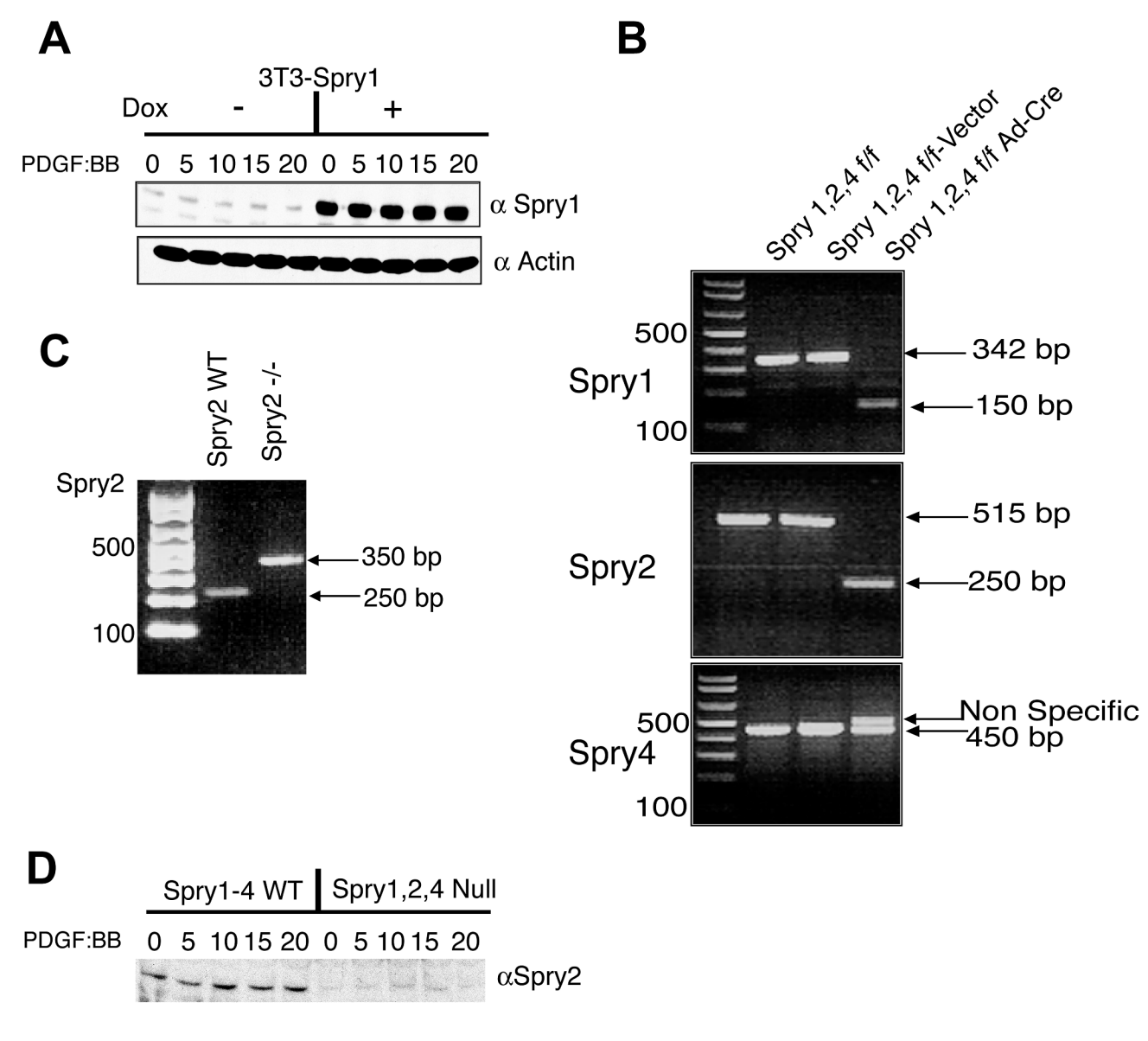
PCR cycle parameters were as follows: initial step 95 C for 5 min and then 33 cycles of 94 C for 30 s, annealing temperature 57 C for 30 s, and the final step of 72 C for 45 s using the above mentioned primers. Following table used to identify the specific gene amplification.

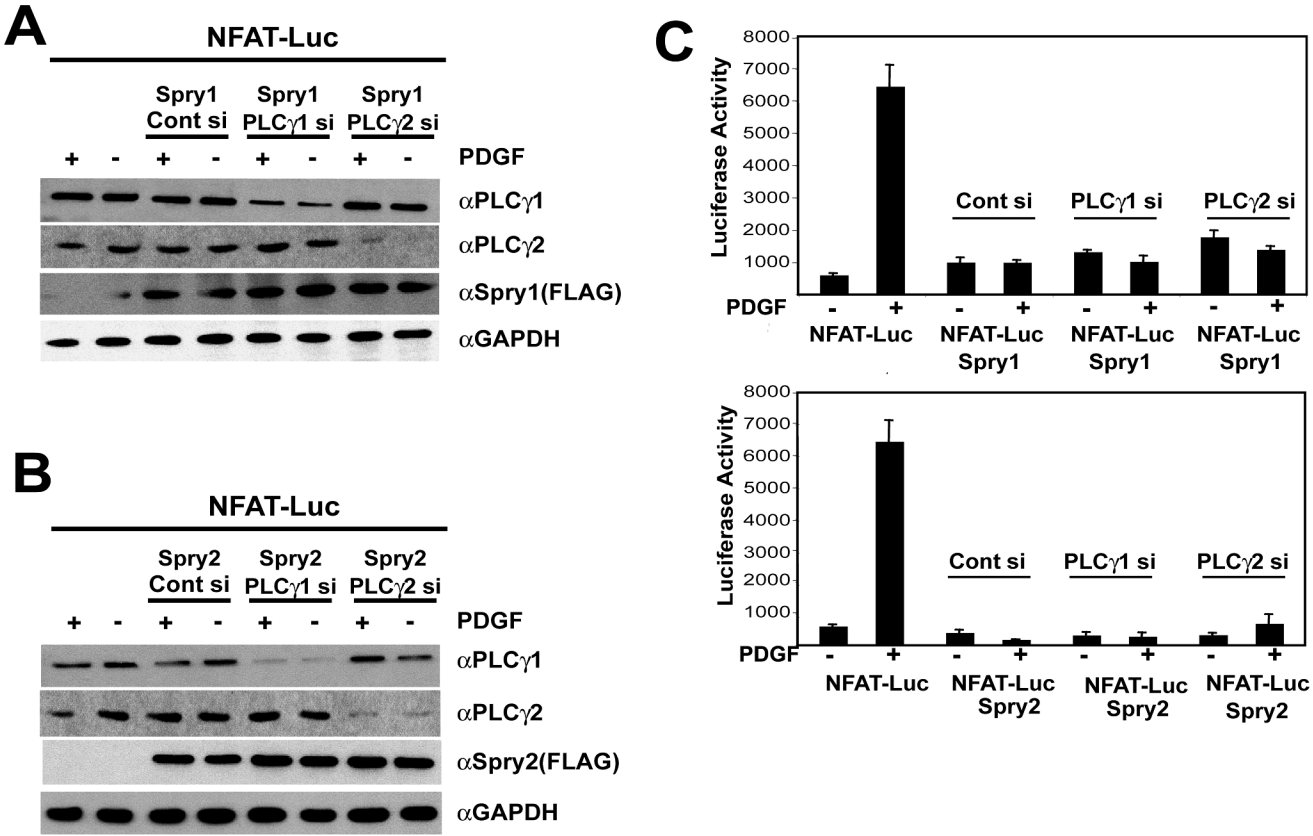
Gentotype	Spry1	Spry2	Spry4
Wt	**314 bp**	**350 bp**	**300 bp**
Mut	**150 bp**	**250 bp**	**450 bp**
Flox	**342 bp**	**515 bp**	**450 bp**

Supplemental Table 1A-C. Inositol phosphate (IP) levels of three different experiments from NIH 3T3 cells overexpressing Spry1 (A), Spry1-4 wt and Spry 1-4 null cells (B) and Spry2 wt and Spry2 null Mast cells (C).


GST
GST-PLCγ1-SH2-C
Bacterial Lysate
GST
PLCγ1
SH2-C
UI
IND
UI
IND
1
2
3
4
5
6


Sprouty
WT
Null
PDGF:BB
0 5 0 5
αPLCβ1-Dark Exposure
αPLCδ1-Dark Exposure
αPLCγ1-Light Exposure


A
3T3-Spry1
Dox
-
+
PDGF:BB
0 5 10 15 20 0 5 10 15 20
α Spry1
α Actin
B
Spry 1,2,4 f/f
Spry 1,2,4 f/f-Vector
Spry 1,2,4 f/f Ad-Cre
500
Spry1
100
342 bp
150 bp
Spry2
515 bp
250 bp
500
Spry4
100
Non Specific
450 bp
C
Spry2 WT
Spry2 -/-
Spry2
500
100
350 bp
250 bp
D
Spry1-4 WT
Spry1,2,4 Null
PDGF:BB
0 5 10 15 20 0 5 10 15 20
αSpry2


A
NFAT-Luc
Spry1 Cont si
Spry1 PLCγ1 si
Spry1 PLCγ2 si
+ - + - + - + -
PDGF
αPLCγ1
αPLCγ2
αSpry1(FLAG)
αGAPDH
B
NFAT-Luc
Spry2 Cont si
Spry2 PLCγ1 si
Spry2 PLCγ2 si
+ - + - + - + -
PDGF
αPLCγ1
αPLCγ2
αSpry2(FLAG)
αGAPDH
C
Luciferase Activity
8000
7000
6000
5000
4000
3000
2000
1000
Cont si
PLCγ1 si
PLCγ2 si
PDGF
- + - + - + - +
NFAT-Luc
NFAT-Luc Spry1
NFAT-Luc Spry1
NFAT-Luc Spry1
Luciferase Activity
8000
7000
6000
5000
4000
3000
2000
1000
Cont si
PLCγ1 si
PLCγ2 si
PDGF
- + - + - + - +
NFAT-Luc
NFAT-Luc Spry2
NFAT-Luc Spry2
NFAT-Luc Spry2