Supplemental data

Table. ST1

Contribution of tyrosine residues (color coded with amino acid sequence in supplemental Fig S2) from different domains of Shc towards average absorbance (A_{450}) during tyrosine phosphorylation of recombinant Shc by recombinant Jak3.

ShcA-constructs	Domain/s lost	Tyrosine residues (Y) lost	Average loss of A_{450}	Number of Y phosphorylated ($=A_{450}/0.175$)
ShcA	none	0	0.7	4
ShcA-W378*	SH2	**2**	**0.35**	2
ShcA-E230*	SH2+CH1	**2**+**3**	0.55 (**0.35**+**0.20**)	1
ShcA-M46*	SH2+CH1+PID	**2**+**3**+**5**	0.73 (**0.35**+**0.20**+**0.18**)	1

Supplemental data

Fig. S1

p52ShcA-PID-domain

```
       020        030        040        050        060        070
RTRVEGGQLG GEEWTRHGSF VNKPTRGWLH PNDKVMGPGV SYLVRYMGCV EVLQSMRALD

       080        090        100        110        120        130
FNTRTQVTRE AISLVCEAVP GAKGATRRRK PCSRPLSSIL GRSNLKFAGM PITLTVSTSS

       140        150        160        170        180        190
LNLMAADCKQ IIANHHMQSI SFASGGDPDT AEYVAYVAKD PVNQRACHIL ECPEGLAQDV

       200        210        220        230        240        250
ISTIGQAFEL RFKQYLRNPP KLVTPHDRMA GFDGSAWDEE EEEPPDHQYY NDFPGKEPPL
```

p52ShcA-CH1-domain

```
       200        210        220        230        240        250
ISTIGQAFEL RFKQYLRNPP KLVTPHDRMA GFDGSAWDEE EEEPPDHQYY NDFPGKEPPL

       260        270        280        290        300        310
GGVVDMRLRE GAAPGAARPT APNAQTPSHL GATLPVGQPV GGDPEVRKQM PPPPPCPGRE

       320        330        340        350        360        370
LFDDPSYVNV QNLDKARQAV GGAGPPNPAI NGSAPRDLFD MKPFEDALRV PPPPQSVSMA
```

p52ShcA-SH2-domain

```
       380        390        400        410        420        430
EQLRGEPWFH GKLSRREAEA LLQLNGDFLV RESTTTPGQY VLTGLQSGQP KHLLLVDPEG

       440        450        460        470
VVRTKDHRFE SVSHLISYHM DNHLPIISAG SELCLQQPVE RKL
```

Supplemental data

Fig. S2

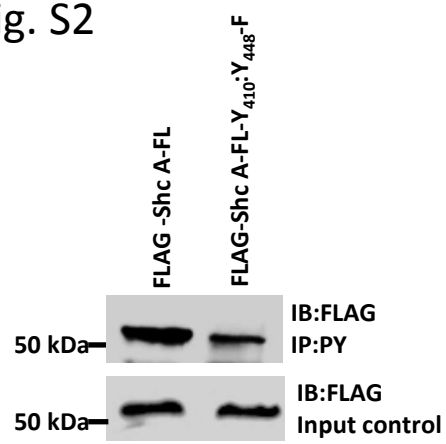


Fig. S3

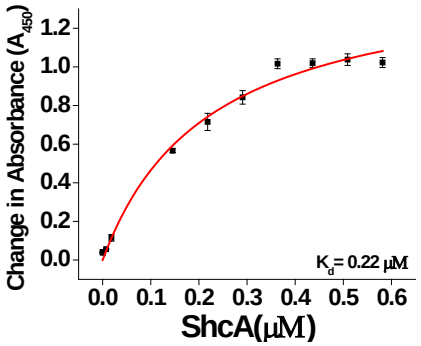


Fig. S4

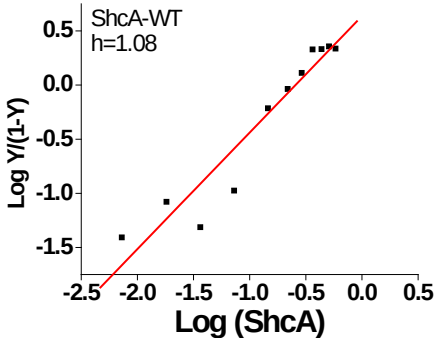


Fig. S5

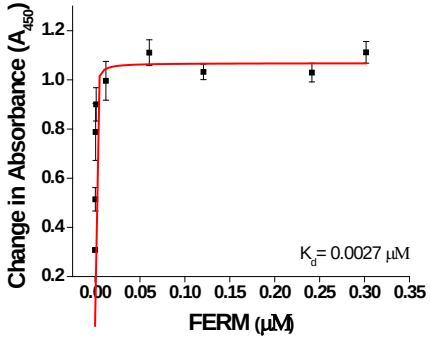


Fig. S6

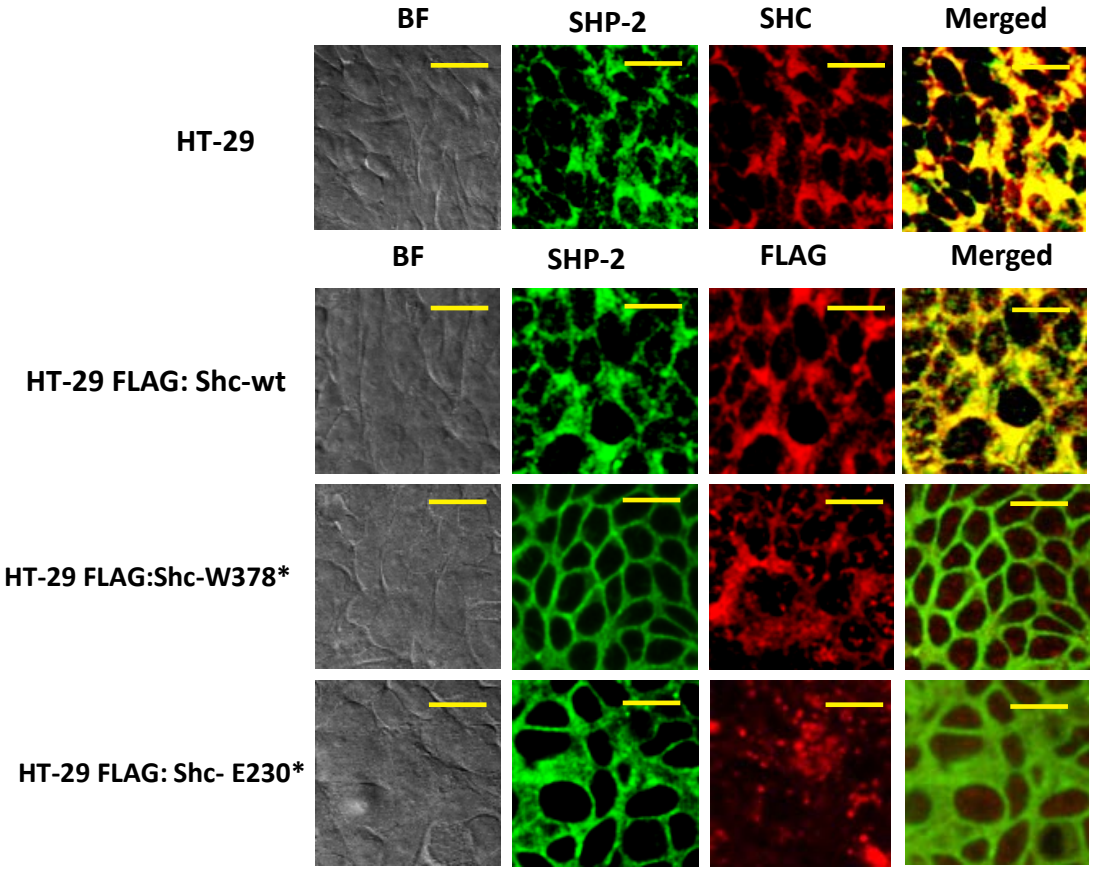

BF
SHP-2
SHC
Merged
HT-29
BF
SHP-2
FLAG
Merged
HT-29 FLAG: Shc-wt
HT-29 FLAG:Shc-W378*
HT-29 FLAG: Shc- E230*

Fig. S7

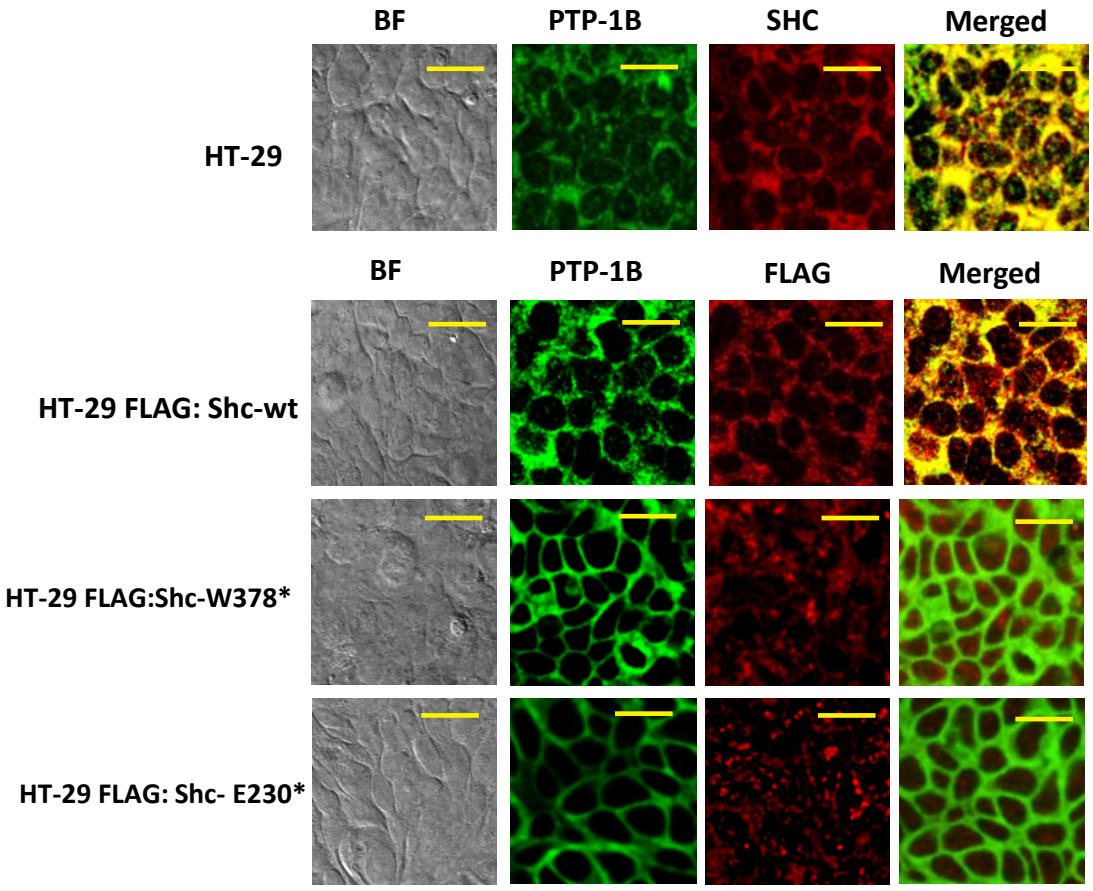

BF
PTP-1B
SHC
Merged
HT-29
BF
PTP-1B
FLAG
Merged
HT-29 FLAG: Shc-wt
HT-29 FLAG:Shc-W378*
HT-29 FLAG: Shc- E230*

Fig. S8

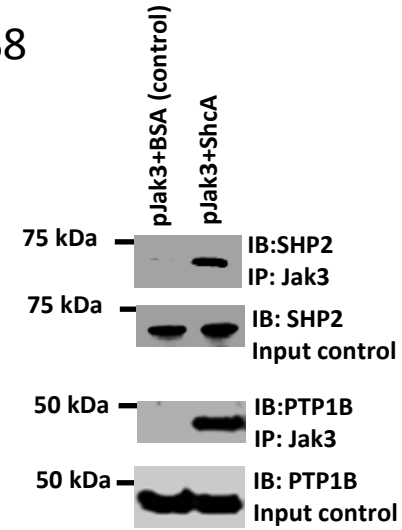
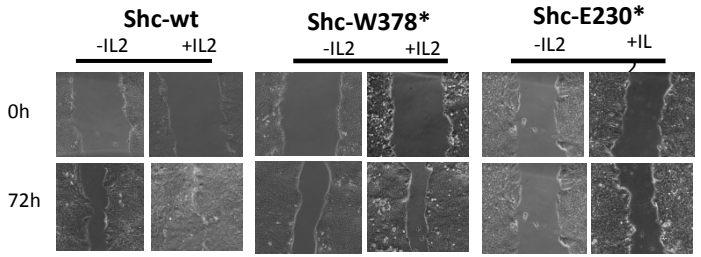

pJak3+BSA (control)
pJak3+ShcA
75 kDa
IB:SHP2
IP: Jak3
75 kDa
IB: SHP2
Input control
50 kDa
IB:PTP1B
IP: Jak3
50 kDa
IB: PTP1B
Input control

Fig. S9


Shc-wt
-IL2
+IL2
Shc-W378*
-IL2
+IL2
Shc-E230*
-IL2
+IL
0h
72h

Supplemental Figure Legends

Supplemental Fig S1: *Amino acid sequence of Shc indicating individual domains (colored yellow) and the tyrosine residues present in each domain color coded same as in Table ST1.*

Supplemental Fig S2: *Y^{410} and Y^{448} are the Jak3-mediated phosphorylation sites in full-length Shc.* Western analysis of co-immunoprecipitates from cell lysates of stably transfected cells of FLAG-tagged full-length (FL) Shc or point mutants for Y^{410} and Y^{448} to phenylalanine (F) in FLAG-tagged full-length (FL) Shc, treated with IL-2 (50 U/ml) were done using indicated antibodies for phosphotyrosine (pY), or FLAG using previously reported methods (1).

Supplemental Fig S3-S5: P-Jak3 interacts with P-p52ShcA: **(S3)** P-Jak3 interactions with P-p52ShcA were determined by pair-wise binding assay using a 96-well multi-plate pre-coated with P-p52ShcA-wt. These plates were incubated with increasing concentrations of P-GST-Jak3-wt proteins and the bound P-GST-Jak3-wt was detected using anti-GST-antibody with GST alone as control. **(S4)** The Hill equation is rearranged as reported before (2) and plotted to show the relation between the log $Y/(1-Y)$ and log (Jak3), where Y is the fractional saturation of Absorbance. The Hill coefficient (h) was derived from the slope. (**S5)** Binding kinetics of GST-Jak3-G257* with P-ShcA-wt were determined as in "**S3**". (**S3** and **S5**) Curve-fitting were done as reported before (2) using Hyperbol-fit program in MicroCal Origin to calculate K_d.

Supplemental Fig S6-S7: *Shc interacts with SHP2 and PTP1B.* Immunofluorescence microscopy on control, FLAG-tagged-Shc-wt, FLAG-tagged-Shc-378*, and FLAG-tagged-Shc-230* were done to determine the interactions between endogenous Shc or its domains and SHP2 (S3) or PTP1B (S4) using indicated primary antibodies and FITC (green) or Cy3 (red) conjugated secondary antibodies and Nikon C1-pluc laser confocal microscope. Yellow color in the merged panels indicates interactions between proteins. Scale bar:14μM.

Supplemental Fig S8: *Jak3 interacts with SHP2 and PTP1B in the presence of Shc.* Pure proteins of Jak3 were allowed to interact with SHP2 or PTP1B in the presence of absence of purified Shc proteins followed by IP and IB by indicated antibodies using previously reported methods (1).

Supplemental Fig S9: *Disruption of Jak3 interactions with p52ShcA decreases wound repair by IEC***:** Stably transfected confluent cells of Shc-wt or indicated mutants were scraped using a micropipette tip and the migration of the remaining cells into the wounded area was measured as reported before (1) in the presence or absence of IL-2 to determine the role of Jak3-Shc interactions on autocrine or IL-2-induced wound closure.

Reference;

1. Kumar, N., Mishra, J., Narang, V. S., and Waters, C. M. (2007) Janus kinase 3 regulates interleukin 2-induced mucosal wound repair through tyrosine phosphorylation of villin. *J Biol Chem* **282**, 30341-30345
2. Kumar, N., Tomar, A., Parrill, A. L., and Khurana, S. (2004) Functional dissection and molecular characterization of calcium-sensitive actin-capping and actin-depolymerizing sites in villin. *J Biol Chem* **279**, 45036-45046