Table S1. Antibody list. List of all antibodies used in this study.

| | Immunogen | Antibody Source | Clone | Dilution | Manufacturer | Application | Reference |
|--|--|--------------------------|-------|----------|---|-------------|-----------|
| PTPIP51 (P51ab) The specificity was controlled by preabsorption experiments. | Human recombinant PTPIP51 protein encoding amino acids (aa) 131-470 | Rabbit polyclonal | | 1:500 | Prof. HW Hofer, Biochemical Department, University Konstanz, Germany | WB, DPLA | [1-4] |
| PTPIP51- anti- pTyr176 PTPIP51 The specifity was controlled by RP-HPLC MassSpectrum | KLH-coupled peptide with the sequence: CDAESEGG[pT]TAN AE | Guinea pig polyclonal | | 1:1000 | Biolux, Stuttgart, Germany | IHC | [4] |
| PTPIP51 anti-pSer46 PTPIP51 The specifity was controlled by RP-HPLC MassSpectrum | KLH-coupled peptide with the sequence: CQRHGRSQ[pS]LP NS | Guinea pig polyclonal | | 1:500 | Genosphere Biotechnologies, Paris, France | IHC | [5] |
| PTPIP51 anti- pSer212 PTPIP51 | KLH-coupled peptide with the sequence: | Rabbit polyclonal | | 1:500 | Genosphere Biotechnologies, Paris, France | IHC | |

| | CETVKMGRKD[pS] | | | | | | |
|---|--|---------------------|--------------|-------|---|------|------|
| The specifity was controlled by RP-HPLC MassSpectrum | LDLE | | | | | | |
| PTP1B | Recombinant protein corresponding to aa 1-321 of human PTP1B | Mouse monoclonal | 107AT5 31 | 1:200 | Abnova Cat.# MAB1152 Lot: SDG070208D | DPLA | [6] |
| 14-3-3ß | Epitope mapping between aa 220-244 at the C-terminus of human 14-3-3ß | Mouse monoclonal | A-6 | 1:100 | Santa Cruz Biotechnology Cat.# sc-25276 Lot: F1011 | DPLA | [7] |
| Raf-1 | Epitope mapping the C-terminus of Raf-1 | Mouse monoclonal | E-10 | 1:100 | Santa Cruz Biotechnology Cat.# sc-7267 Lot: E0411 | DPLA | [8] |
| EGFR | raised against plasma membranes of A431 cells | Mouse monoclonal | 2E9 | 1:100 | Santa Cruz Biotechnology Cat.# sc-57091 Lot: B1307 | DPLA | [9] |
| ERK1/2 | raised against synthetic peptide corresponding to | Mouse Monoclonal | MK12 | 1:100 | | DPLA | [10] |

| (p42/44) | aa336-356 of human ERK1 | | | | Merck Millipore Cat.# 05-1152 Lot: 1956740 | | |
|--|--|---------------------|-------|-------|---|------|------|
| pERK | Epitope corresponding to a sequence containing Tyr204 phophorylated ERK of human origin | Mouse monoclonal | E-4 | 1:100 | Santa Cruz Biotechnology Cat. # sc-7383 Lot: F2613 | DPLA | [11] |
| CG199 | epitope mapping at the N-terminus of CGI-99 of human origin | Goat polyclonal | N-14 | 1:100 | Santa Cruz Biotechnology Cat. # sc-104834 Lot: C0310 | DPLA | [4] |
| Nuf-2 (cdcA1) | raised against aa 1–300 mapping at the N-terminus of CdcA1 human origin | Mouse monoclonal | E-6 | 1:100 | Santa Cruz Biotechnology Cat.# sc-271251 Lot: E2510 | DPLA | [12] |
| ReIA validated in EMSA, FC, ICC, IF, IHC, IHC(P), WB to detect NFkB | Specfic for an epitope overlapping the nuclear location signal of the p65 subunit of the NFKB heterodimer | Mouse monoclonal | 12H11 | 1:100 | Millipore Cat.# MAB3026 Lot: 2580674 | DPLA | [13] |

| also known as Rel A | | | | | | | |
|---|---|---------------------|--------|-------|---|------|------|
| Rac1 routinely evaluated by immunoblot on rat brain microsomal protein validated for use in Immunohistoch emistry (IHC), Immunoprecipit ation (IP) and Western Blotting (WB) for the detection of Rac1 protein | Recombinant protein containing the full length human Rac1 | Mouse monoclonal | 23A8 | 1:100 | Merck-Millipore Cat.# 05-389 Lot: E0411 | DPLA | [14] |
| VAPB | E. coli-derived recombinant human VAP-B. Ala2-Pro132 | Mouse monoclonal | 736904 | 1:100 | R&D systems Cat.# MAB58551 | DPLA | [15] |
| IR (ß-subunit) Routinely evaluated by Western Blot on NIH/3T3 lysates. | Recombinant- fragment including C-terminal 100 aa of human insulin receptor ß-subunit | Mouse monoclonal | CT-3 | 1:100 | Merck-Millipore Cat.#05-1104 | DPLA | [16] |

| Western Blot Analysis: 1:500 dilution of this lot detected Insulin Receptor, beta subunit on 10 µg of NIH/3T3 Iysates. | | | | | | | |
|--|--|----------------------|------|--------|--|------|------|
| Grb2 | raised against amino acids 54-164 mapping to a central domain of GRB2 of mouse origin | Mouse monoclonal | C-7 | 1:100 | Santa Cruz Biotechnology Cat.# sc-8034 Lot: 2614 | DPLA | [17] |
| c-Src | raised against full- length recombinant c-Src of human origin | Mouse monoclonal | H-12 | 1:100 | Santa Cruz Biotechnology Cat.# sc-5266 Lot: K2509 | DPLA | [18] |
| GSK3β | raised against amino acods 345-420 mapping at the C- terminus of GSK3β of human origin | Mouse monoclonal | E11 | 1:100 | Sanat Cruz Biotechnology Cat.# sc-377213 Lot: A3114 | DPLA | [19] |
| Phospho- Akt (Ser473) | a synthetic phosphopeptide corresponding to | Rabbit monoclonal | D9E | 1:2500 | Cell signaling technology | WB | [20] |

| Phospho- p42/p44 MAPK | residues surrounding Ser473 of mouse Akt a synthetic phosphopeptide corresponding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase | Rabbit monoclonal | D13.14. 4E | 1:2500 | Cat.# 4060 Lot: 19 Cell signaling technology Cat.# 4370 Lot: 12 | WB | [21] |
|---------------------------------------|---|----------------------|---------------|--------|--|----|------|
| Phospho- cdc2 (Tyr15) (CDK1) | synthetic phosphopeptide corresponding to residues surrounding Tyr15 of human cdc2 | Rabbit monoclonal | 10A11 | 1:2500 | Cell signaling technology Cat.# 4539 Lot:2 | WB | [22] |
| Phospho- PKCα (Thr638) | A synthetic phospho- peptide corresponding to residues surrounding Thr638 of human PKC alpha. | Rabbit monoclonal | E195 | 1:2500 | Abcam Cat.# ab32502 Lot: GR13679-1 | WB | [23] |

| Phospho- GSK3β (S9) | synthetic phosphopeptide corresponding to the sequence of human GSK-3β. | Rabbit monoclonal | 5B3 | 1:2500 | Cell signalling technology Cat.# 9323 Lot: 13 | WB | [24] |
|--|---|----------------------|-----|--------|--|-----|------|
| Alexa 555 Coupled to anti-rabbit antibody | Rabbit gamma immunoglobins heavy and light chains | Goat polyclonal | | 1:1600 | Life Technology Cat.# A21428 Lot: | IHC | |
| Alexa 488 Coupled to anti-mouse antibody | lgG heavy chains from mouse | Goat polyclonal | | 1:800 | Life Technology Cat# A11029 Lot: | IHC | |
| Cy3 donkey anti-guinea pig | lgG (H+L) from guinea pig | Donkey polyclonal | | 1:400 | Dianova Cat.# 706166148 Lot: | IHC | |
| HRP goat anti-rabbit | IgG isolated from rabbit serum | Goat polyclonal | | 1:5000 | Dako Cat.# P0448 Lot: 20027913 | WB | |

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Supplementary information, Figure S1. Immunoblot analyses of the phosphotyrosine 176 and phosphoserine 212 status of PTPIP51.

- A. Phosphorylation of the tyrosine 176 residue. Quantification data of the blot shown in C. Each detected isoform was quantified separately. Total protein was calculated as the sum of the three detected isoforms.
- B. Phosphorylation of the serine 212 residue. Quantification data of the blot shown in D. Each detected isoform was quantified separately. Total protein was calculated as the sum of the three detected isoforms.
- C. Immunoblot using the specific antibody raised against phosphorylated tyrosine
 176 residue.

- D. Immunoblot using the specific antibody raised against phosphorylated tyrosine 212 residue.
- E. Stain-free blot for quantification of the phosphorylated tyrosine 176 residue.
- F. Stain-free blot for quantification of the phosphorylated tyrosine 212 residue.



Supplementary information, Figure S2. MTT assay of HaCaT cells treated with LDC-4 and LDC-9 and the LDC-4 and LDC-9 effects on the time dependent interaction profile of PTPIP51 and PTP1B.

A. Cell viability of LDC-4 treated HaCaT cells assayed by MTT.

B. Cell viability of LDC-9 treated HaCaT cells assayed by MTT.

To exclude the toxic effect of DMSO, a second curve was established applying gradient amounts of DMSO comparable to the amount added with the rising concentrations of the effector added to the test system. The values for LDC-4 and LDC-9 treated cells were calculated as the percental quotient of the LDC-4 or LDC-9 value and the DMSO value.

C. Using LDC4 for 4 h in increasing concentrations progressively depressed the rate of PTPIP51/PTP1B interaction. Yet, highly significant differences to normal controls were only seen with concentrations starting from 10μ M up to 100μ M (p<0.0001). Extending the time of exposure to 12 h changed the pattern only at concentrations of 50μ M reaching near normal values and at 100μ M where the concentrations were higher than those of the controls.

Prolongation to 24h of incubation time displayed a pattern comparable to that seen after 12h of LDC4 application.

D. Application of LDC9 in increasing concentrations led to significant changes of the PTPIP51/PTP1B. After 4 h incubation the interactions slightly increased under the influence of 0.5 μ M LDC9 and were normalized under 5 μ M. Using concentrations of 10 μ M and higher negatively influenced the number of interactions to values being significantly different from the controls (10 μ M p<0.001, 25 μ M p<0.01, 50 μ M p<0.05, 60 μ M p<0.0001, 100 μ M p<0.05). Prolongation of the incubation time to 12h significantly reduced the number of interactions for all applied concentrations (0.5 μ M, 10 μ M, 50 μ M, 60 μ M and 100 μ M p<0.0001, 5 μ M and 25 μ M p<0.001). After 24 h of incubation with LDC9 all concentrations decreased the number of interactions, but only high concentrations were able to exert a significant reduction (25 μ M p<0.001, 50 μ M p<0.001, 100 μ M p<0.05).



Supplementary information, Figure S3. Effects of LDC-3 on the receptor tyrosine kinase signaling and associated adapter molecules and on the MAPK signaling pathway.

Quantitative analysis of the Duolink proximity ligation assay of PTPIP51/EGFR (A), PTPIP51/IR (B), PTPIP51/c-Src (C) and PTPIP51/Grb2 (D). The interactions were evaluated

by Duolink Image Tool software in untreated controls and in cells treated with 5μ M, 50μ M and 100μ M LDC-3 for 12h.

Quantitative analysis of the Duolink proximity ligation assay of PTPIP51/14-3-3 (E), PTPIP51/Raf-1 (F), PTPIP51/pErk1/2 (G) and 14-3-3/Raf-1 (H). The interactions were evaluated by Duolink Image Tool software in untreated controls and in cells treated with 5µM, 50µM and 100µM LDC-3 for 12h.

The resulting data were analyzed by GraphPad Prism 6 software, the significance of the results was tested by Dunnett's multiple comparisons test. * (p < 0.05), ** (p < 0.01), *** (p < 0.001)



Supplementary information, Figure S4. Effects of LDC-3 on the mitotic interactome of PTPIP51, the NFkB pathway, the Ca²⁺ homestasis and the cell motility.

Quantitative analysis of the Duolink proximity ligation assay of PTPIP51/CGI-99 (A) and PTPIP51/Nuf2 (B). The interactions were evaluated by Duolink Image Tool software in untreated controls and in cells treated with 5µM, 50µM and 100µM LDC-3 for 12h.

Quantitative analysis of the Duolink proximity ligation assay of PTPIP51/VAPB (C), PTPIP51/ GSK3β (D), PTPIP51/Rac-1 (E), PTPIP51/ReIA (F). The interactions were evaluated by Duolink Image Tool software in untreated controls and in cells treated with 5µM, 50µM and 100µM LDC-3 for 12h. The resulting data were analyzed by GraphPad Prism 6 software, the significance of the results was tested by Dunnett's multiple comparisons test. * (p < 0.05), ** (p < 0.01), *** (p < 0.001)



Supplementary information, Figure S5. 3D reconstruction of HaCaT cells treated with LDC-3 and immunostained with the antibody specific to phospho-Tyr176-PTPIP51.

The cells do not show any shift in the protein localization, rather than an increase in total amount of tyrosine 176 phosphorylated PTPIP51. The reconstruction was performed for the control group and each concentration of LDC-3: control (A), 5μ M (B), 50μ M (C), 100μ M (D). The grid indicates the length in μ m.



Supplementary information, Figure S6. Stain-free blot for quantification. Using this method no loading control is needed to quantify the data. (A) Stainfree-blot for the western blot analyses of phospho-MAPK and phospho-Akt (left blot) as well as of phospho-GSK3b and phospho-PKCa (right blot). (B) Stain-free blot for the siRNA knockdown experiments. Left blot: no LDC-3 treatment. Right blot: LDC-3 treatment.



Supplementary information, Figure S7. Semiquantitative analyses of the tyrosine 176 phosphorylation status of PTPIP51. The cells were marked in ImageJ and the results of ten images (mean values of the encircled cells) were used for statistical analyses. (A) control, (B) 5μ M LDC-3, (C) 50μ M LDC-3, (D) 100μ M LDC-3.



Supplementary information, Figure S8. Semiquantitative analyses of the serine 46 phosphorylation status of PTPIP51. The cells were marked in ImageJ and the results of ten images (mean values of the encircled cells) were used for statistical analyses. (A) control, (B) 5μ M LDC-3, (C) 50μ M LDC-3, (D) 100μ M LDC-3.



Supplementary information, Figure S9. Semiquantitative analyses of the serine 212 phosphorylation status of PTPIP51. The cells were marked in ImageJ and the results of ten images (mean values of the encircled cells) were used for statistical analyses. (A) control, (B) 5μ M LDC-3, (C) 50μ M LDC-3, (D) 100μ M LDC-3.

Semiguantitative analyses of the serine and tyrosine residues of PTPIP51

phosphorylated tyrosine 176 residue of PTPIP51

| control (untreated) | |
|---------------------|--|
|---------------------|--|

| | | , | | | |
|---|------|--------|--------|-----|-----|
| | Area | | Mean | Min | Max |
| | 1 | 20.253 | 32.614 | 18 | 208 |
| | 2 | 11.817 | 40.703 | 18 | 193 |
| | 3 | 13.786 | 32.327 | 17 | 191 |
| | 4 | 10.397 | 29.756 | 17 | 135 |
| | 5 | 10.397 | 29.756 | 17 | 135 |
| | 6 | 10.376 | 35.077 | 19 | 189 |
| | 7 | 21.254 | 40.540 | 20 | 242 |
| | 8 | 11.746 | 48.147 | 23 | 227 |
| | 9 | 10.617 | 39.913 | 19 | 181 |
| 1 | LO | 10.137 | 50.353 | 28 | 197 |

| | Area | Mean | Min | Max |
|----|--------|---------|-----|-----|
| 1 | 21.316 | 106.514 | 47 | 198 |
| 2 | 21.959 | 108.042 | 48 | 233 |
| 3 | 14.200 | 103.601 | 51 | 204 |
| 4 | 9.609 | 117.317 | 50 | 199 |
| 5 | 20.017 | 99.299 | 50 | 181 |
| 6 | 15.017 | 111.852 | 48 | 200 |
| 7 | 11.216 | 112.814 | 50 | 184 |
| 8 | 7.928 | 119.871 | 47 | 172 |
| 9 | 10.572 | 119.908 | 53 | 177 |
| 10 | 16.041 | 121.942 | 45 | 190 |

50µM LDC-3

100µM LDC-3

5µM LDC-3

| | Area I | Mean | Min | Max |
|----|--------|---------|-----|-----|
| 1 | 18.518 | 100.783 | 46 | 193 |
| 2 | 14.028 | 96.900 | 49 | 184 |
| 3 | 20.584 | 89.783 | 48 | 160 |
| 4 | 15.790 | 93.578 | 47 | 188 |
| 5 | 15.975 | 92.446 | 48 | 191 |
| 6 | 8.609 | 102.564 | 52 | 171 |
| 7 | 18.472 | 97.261 | 50 | 215 |
| 8 | 19.544 | 100.263 | 51 | 190 |
| 9 | 14.052 | 133.898 | 48 | 240 |
| 10 | 9.872 | 131.884 | 49 | 219 |

| | Area | Mean | Min | Max |
|----|--------|--------|-----|-----|
| 1 | 18.936 | 90.302 | 49 | 171 |
| 2 | 13.985 | 72.923 | 31 | 160 |
| 3 | 8.988 | 78.876 | 43 | 172 |
| 4 | 7.788 | 80.493 | 43 | 196 |
| 5 | 11.937 | 78.526 | 38 | 196 |
| 6 | 4.702 | 92.515 | 47 | 202 |
| 7 | 11.897 | 84.214 | 44 | 171 |
| 8 | 3.306 | 98.974 | 45 | 194 |
| 9 | 12.950 | 96.581 | 44 | 181 |
| 10 | 9.946 | 85.396 | 43 | 181 |

| | Area | Mean | | Min | Max | |
|---|------|-----------------|--------|-----|-----|-----|
| 1 | 13.2 | 38 5 | 57.451 | 2 | 27 | 255 |
| 2 | 6.3 | 54 6 | 59.872 | 2 | 28 | 255 |
| 3 | 9.6 | 84 5 | 56.552 | 2 | 20 | 255 |
| 4 | 5.0 | 60 6 | 56.079 | 2 | 24 | 255 |
| 5 | 20.7 | 13 4 | 42.016 | 1 | L8 | 255 |
| 6 | 15.3 | 61 6 | 53.183 | 2 | 26 | 214 |
| 7 | 15.9 | 22 5 | 59.555 | 2 | 22 | 255 |
| 8 | 6.3 | 42 6 | 52.505 | 2 | 25 | 255 |
| 9 | 3.5 | 05 6 | 53.457 | 2 | 23 | 255 |
| | | | | | | |

| | Area | Mean | Min | Max |
|----|--------|--------|-----|-----|
| 1 | 24.548 | 39.384 | 19 | 255 |
| 2 | 15.197 | 43.823 | 20 | 255 |
| 3 | 23.834 | 44.049 | 24 | 255 |
| 4 | 17.840 | 46.307 | 19 | 255 |
| 5 | 13.930 | 59.123 | 25 | 255 |
| 6 | 8.932 | 49.415 | 26 | 255 |
| 7 | 6.759 | 38.388 | 20 | 249 |
| 8 | 9.103 | 55.484 | 28 | 255 |
| 9 | 18.109 | 54.784 | 27 | 255 |
| 10 | 14.831 | 58.836 | 26 | 255 |

50µM LDC-3

| Area | | Mean | Min | Max |
|------|--------|--------|-----|-----|
| 1 | 3.957 | 85.812 | 40 | 255 |
| 2 | 7.716 | 69.270 | 27 | 255 |
| 3 | 15.006 | 59.534 | 27 | 255 |
| 4 | 6.185 | 64.390 | 27 | 255 |
| 5 | 15.225 | 55.365 | 29 | 255 |
| 6 | 14.194 | 57.797 | 24 | 255 |
| 7 | 6.181 | 59.167 | 24 | 255 |
| 8 | 7.856 | 45.199 | 20 | 231 |
| 9 | 16.518 | 65.125 | 29 | 255 |
| 10 | 5.090 | 80.327 | 30 | 255 |

| | Area | Mean | Min | Max |
|----|--------|--------|-----|-----|
| 1 | 23.520 | 39.196 | 18 | 255 |
| 2 | 7.222 | 35.480 | 16 | 223 |
| 3 | 15.414 | 25.477 | 15 | 254 |
| 4 | 14.827 | 39.521 | 22 | 246 |
| 5 | 10.635 | 45.857 | 19 | 194 |
| 6 | 24.114 | 41.503 | 24 | 243 |
| 7 | 12.688 | 43.222 | 25 | 255 |
| 8 | 7.002 | 56.014 | 30 | 255 |
| 9 | 6.076 | 77.574 | 40 | 235 |
| 10 | 12.596 | 40.210 | 17 | 230 |

phosphorylated serine 212 residue of PTPIP51 control (untreated)

| Α | rea Mo | ean Min | Max | |
|---|--------|---------|-----|-----|
| 1 | 28.444 | 75.846 | 47 | 253 |
| 2 | 16.316 | 52.955 | 36 | 224 |
| 3 | 30.535 | 47.358 | 31 | 228 |
| 4 | 29.523 | 48.576 | 32 | 253 |

5µM LDC-3

100µM LDC-3

| | Area I | Mean | Min | Max |
|---|--------|--------|-----|-----|
| 1 | 27.216 | 46.313 | 29 | 253 |
| 2 | 25.870 | 44.365 | 29 | 253 |
| 3 | 31.511 | 39.094 | 25 | 253 |
| 4 | 25.711 | 44.683 | 27 | 246 |

| 5 | 18.545 | 52.662 | 34 | 198 |
|----|--------|--------|----|-----|
| 6 | 24.271 | 49.509 | 34 | 223 |
| 7 | 20.051 | 55.668 | 36 | 191 |
| 8 | 18.882 | 55.347 | 37 | 253 |
| 9 | 32.889 | 51.089 | 31 | 253 |
| 10 | 20.443 | 59.528 | 35 | 227 |

| 5 | 5 | 17.937 | 40.963 | 28 | 214 |
|----|---|--------|--------|----|-----|
| 6 | 5 | 24.833 | 40.838 | 29 | 233 |
| 7 | 7 | 28.079 | 39.163 | 26 | 253 |
| 8 | 3 | 12.062 | 48.815 | 31 | 247 |
| 9 |) | 18.222 | 47.780 | 33 | 124 |
| 10 |) | 26.745 | 40.898 | 27 | 206 |
| | | | | | |

100µM LDC-3

50µM LDC-3

| | Area | a | Mean | Min | Max |
|---|------|--------|--------|-----|-----|
| | 1 | 25.623 | 50.726 | 32 | 253 |
| | 2 | 29.479 | 44.642 | 29 | 253 |
| | 3 | 30.718 | 39.432 | 26 | 253 |
| | 4 | 21.104 | 45.354 | 29 | 193 |
| | 5 | 31.686 | 43.433 | 28 | 253 |
| (| 6 | 14.508 | 39.423 | 27 | 253 |
| | 7 | 15.001 | 38.341 | 26 | 233 |
| ł | 8 | 31.859 | 38.626 | 26 | 241 |
| 9 | 9 | 16.590 | 40.600 | 27 | 197 |
| 1 | 0 | 21.100 | 37.513 | 27 | 212 |

| | Area | Mean | Min | Max |
|----|--------|--------|-----|-----|
| 1 | 30.264 | 49.451 | 32 | 253 |
| 2 | 17.462 | 56.793 | 35 | 184 |
| 3 | 17.891 | 53.113 | 32 | 239 |
| 4 | 18.292 | 60.607 | 37 | 244 |
| 5 | 29.754 | 54.007 | 35 | 236 |
| 6 | 28.200 | 60.935 | 42 | 253 |
| 7 | 26.180 | 57.396 | 36 | 243 |
| 8 | 20.222 | 51.877 | 35 | 253 |
| 9 | 27.550 | 48.015 | 32 | 253 |
| 10 | 16.252 | 48.988 | 34 | 253 |

Western Blot analysis

<u>phospho-MAPK</u>

| | Band No. | | Relative Fron | Volume (Int) Bar | id % | I | Lane % | Norm. Factor | Norm. Vol. (Int) | | |
|--------|----------|---|---------------|------------------|------|---|-----------|--------------|------------------|---|-------------|
| Lane 1 | | 1 | 0,723776 | 45360 | 10 | 0 | 0,976162 | 1 | 45360 | C | ontrol |
| Lane 2 | | 1 | 0,724476 | 113352848 | 10 | 0 | 89,165007 | 0,880588 | 99817102 | C |),5μM LDC-3 |
| Lane 3 | | 1 | 0,725175 | 221391744 | 10 | 0 | 92,457641 | 1,620132 | 358683797 | 5 | μM LDC-3 |
| Lane 4 | | 1 | 0,723776 | 101714480 | 10 | 0 | 85,252976 | 2,445313 | 248723788 | 2 | 25μM LDC-3 |
| Lane 5 | | 1 | 0,718881 | 119398720 | 10 | 0 | 87,195497 | 2,399076 | 286446556 | 5 | ομ LDC-3 |
| Lane 6 | | 1 | 0,711189 | 135538032 | 10 | 0 | 89,265911 | 2,934817 | 397779261 | 1 | .00µM LDC-3 |

<u>phospho-Akt (S473)</u>

| | Band No. | ŀ | Relative Fron | Volume (Int) Band | % | Lane % | Norm. Factor | Norm. Vol. (Int) | |
|--------|----------|---|---------------|-------------------|-----|-----------|--------------|------------------|-------------|
| Lane 1 | | 1 | 0,429371 | 47269521 | 100 | 20,802828 | 1 | 47269521 | control |
| Lane 2 | | 1 | 0,421678 | 62230880 | 100 | 22,564182 | 0,880588 | 54799736 | 0,5μM LDC-3 |
| Lane 3 | | 1 | 0,420979 | 28790002 | 100 | 4,354193 | 1,620132 | 4664359 | 5μM LDC-3 |
| Lane 4 | | 1 | 0,424476 | 18657208 | 100 | 1,358712 | 2,445313 | 4562272 | 25µM LDC-3 |
| Lane 5 | | 1 | 0,421678 | 1145046 | 100 | 1,422396 | 2,399076 | 2747051 | 50μ LDC-3 |
| Lane 6 | | 1 | 0,418182 | 771584 | 100 | 0,779425 | 2,934817 | 2264457 | 100µM LDC-3 |

phospho-GSK3 beta

| | Band No. | | Relative Fron V | /olume (Int) Band % | , D | Lane % | Norm. Factor | Norm. Vol. (Int) | |
|--------|----------|---|-----------------|---------------------|--------|--------------------|--------------|------------------|-------------|
| Lane 1 | | 1 | 0,554882 | 602822 | 100 | 98,119393 | 1 | 602822 | control |
| Lane 2 | | 1 | 0,557307 | 6493348 | 100 | 99,635666 | 0,933933 | 6064352 | 0,5μM LDC-3 |
| Lane 3 | | 1 | 0,556095 | 6590974 | 100 | 99,605927 | 0,902391 | 5947633 | 5μM LDC-3 |
| Lane 4 | | 1 | 0,556095 | 4306780 | 100 | 98,365815 | 0,935498 | 4028982 | 25µM LDC-3 |
| Lane 5 | | 1 | 0,554275 | 31370912 | 100 | 99 <i>,</i> 865025 | 0,551956 | 17315378 | 50µ LDC-3 |
| Lane 6 | | 1 | 0,553062 | 24451868 | 100 | 99,954936 | 0,719338 | 17589162 | 100µM LDC-3 |

phospho-PKC alpha

| | Band No. | | Relative Fron V | /olume (Int) Band % | | Lane % | Norm. Factor | Norm. Vol. (Int) | |
|--------|----------|---|-----------------|---------------------|-----|-----------|--------------|------------------|-------------|
| Lane 1 | | 1 | 0,478421 | 1057954 | 100 | 1,328877 | 1 | 1057954 | control |
| Lane 2 | | 1 | 0,476584 | 1470737 | 100 | 0,811643 | 0,933933 | 1373570 | 0,5μM LDC-3 |
| Lane 3 | | 1 | 0,471993 | 11184163 | 100 | 4,863087 | 0,902391 | 10092484 | 5μM LDC-3 |
| Lane 4 | | 1 | 0,471074 | 1221127 | 100 | 0,717778 | 0,935498 | 1142361 | 25µM LDC-3 |
| Lane 5 | | 1 | 0,471074 | 14976818 | 100 | 4,673779 | 0,551956 | 8266551 | 50μ LDC-3 |
| Lane 6 | | 1 | 0,46281 | 24379376 | 100 | 10,419498 | 0,719338 | 17537016 | 100μM LDC-3 |

siRNA-knockdown experiments

no LDC-3 treatement

<u>PTPIP51</u>

| | Band No. | | Relative Fron | Volume (Int) Band | % | Lane % | Norm. Factor | Norm. Vol. (Int) | .) | | |
|--------|----------|---|---------------|-------------------|-----|-----------|--------------|------------------|---------|--|--|
| Lane 1 | | 1 | 0,583567 | 132448000 | 100 | 73,151765 | 1 | 132448000 | control | | |
| Lane 2 | | 1 | 0,586368 | 69771500 | 100 | 50,487424 | 1,162271 | 81093387 | siRNA A | | |
| Lane 3 | | 1 | 0,587302 | 209030000 | 100 | 77,991554 | 0,984957 | 205885659 | siRNA B | | |
| Lane 4 | | 1 | 0,579832 | 114891400 | 100 | 60,225561 | 0,744547 | 85542023 | siRNA C | | |

<u>phospho-MAPK</u>

| | Band No. | | Relative Fron | Volume (Int) | Band % | I | Lane % | Norm. Factor | | |
|--------|----------|---|---------------|--------------|--------|-----|-----------|--------------|-----------|---------|
| Lane 1 | | 1 | 0,473389 | 115415980 | | 100 | 27,06594 | 1 | 115415980 | control |
| Lane 2 | | 1 | 0,471522 | 79615200 | | 100 | 24,249739 | 1,162271 | 92534434 | siRNA A |
| Lane 3 | | 1 | 0,467787 | 55238193 | | 100 | 9,850461 | 0,984957 | 54407270 | siRNA B |
| Lane 4 | | 1 | 0,455649 | 101645160 | | 100 | 19,807519 | 0,744547 | 75679577 | siRNA C |

LDC-3 treatement

<u>PTPIP51</u>

| | Band No. | | Relative Fron | Volume (Int) Band % | | Lane % | Norm. F | actorl | Norm. Vol. (Int) | | |
|--------|----------|---|---------------|---------------------|-----|-----------|---------|--------|------------------|---|--------|
| Lane 1 | | 1 | 0,544563 | 221723635 | 100 | 74,333622 | | 1 | 221723635 | С | ontrol |

| Lane 2 | 1 | 0,544563 | 66884769 | 100 | 45,830706 | 1,307511 | 87452553 | siRNA A |
|--------|---|----------|-----------|-----|-----------|----------|-----------|---------|
| Lane 3 | 1 | 0,545455 | 268376444 | 100 | 78,214121 | 1,083438 | 290769256 | siRNA B |
| Lane 4 | 1 | 0,533868 | 90577389 | 100 | 59,933153 | 1,506996 | 136499730 | siRNA C |

phospho-MAPK

| | Band No. | | Relative Fron | Volume (Int) Band % | | Lane % | Norm. Factor | Norm. Vol. (Int) | |
|--------|----------|---|---------------|---------------------|-----|-----------|--------------|------------------|---------|
| Lane 1 | | 1 | 0,414373 | 325092040 | 100 | 37,457005 | 1 | 325092040 | control |
| Lane 2 | | 1 | 0,417431 | 166906040 | 100 | 34,044608 | 1,307511 | 218231439 | siRNA A |
| Lane 3 | | 1 | 0,420489 | 202981100 | 100 | 21,887193 | 1,083438 | 219917450 | siRNA B |
| Lane 4 | | 1 | 0,415138 | 110646760 | 100 | 29,059485 | 1,506996 | 166744184 | siRNA C |

Immunoblotting for the phosphorylation status of PTPIP51

Immunoblot pSer212-PTPIP51

| | - | | | | | | | Norm. Vol. | |
|------|----------|---|-----------------------|--------------|-----------|-----------|--------------|------------|--------------------------|
| Lane | Band No. | | Relative Front | Volume (Int) | Band % | Lane % | Norm. Factor | (Int) | |
| | 1 | 1 | 0,543014 | 22889187 | 37,531729 | 4,000402 | 1 | 2623156 | control 52kDA |
| | 1 | 2 | 0,682725 | 13621163 | 62,468271 | 6,658319 | 1 | 4366013 | control 38 kDa |
| | | | | | | | | 6989169 | control Total protein |
| | 2 | 1 | 0,551273 | 12610083 | 71,22605 | 3,042012 | 1,432632 | 1864613 | 10μM LDC-3 52kDa |
| | 2 | 2 | 0,68479 | 5851861 | 28,77395 | 1,228914 | 1,432632 | 753267 | 10μM LDC-3 38kDa |
| | | | | | | | | 2617880 | 10μM LDC-3 Total protein |
| | 3 | 1 | 0,559532 | 7778408 | 61,398213 | 2,175317 | 2,520859 | 2012515 | 25μM LDC-3 52kDa |
| | 3 | 2 | 0,707502 | 9184029 | 38,601787 | 1,367648 | 2,520859 | 1265292 | 25μM LDC-3 38kDa |
| | | | | | | | | 3277807 | 25μM LDC-3 Total protein |
| | 4 | 1 | 0,573297 | 16287023 | 70,27511 | 7,483294 | 1,581675 | 4288042 | 50μM LDC-3 52kDa |
| | 4 | 2 | 0,712319 | 15215341 | 29,72489 | 3,165276 | 1,581675 | 1813751 | 50μM LDC-3 38kDa |
| | | | | | | | | 6101793 | 50μM LDC-3 Total protein |
| | 5 | 1 | 0,580179 | 24847134 | 31,220198 | 6,10717 | 0,901083 | 3519773 | 100μM LDC-3 52kDa |
| | 5 | 2 | 0,721266 | 30967936 | 68,779802 | 13,454429 | 0,901083 | 7754253 | 100μM LDC-3 38kDa |

11274026

| Immunoblot pTvr176-PTP | PIP51 |
|------------------------|-------|
|------------------------|-------|

| | | | | | | | | Norm. Vol. | |
|------|----------|----|--------------|--------------|-----------|-----------|--------------|------------|---------------------------|
| Lane | Band No. | Re | lative Front | Volume (Int) | Band % | Lane % | Norm. Factor | (Int) | |
| | 1 | 1 | 0,496134 | 50333500 | 15,947454 | 8,446312 | 1 | 18953760 | control 52kDA |
| | 1 | 2 | 0,556701 | 35998620 | 5,523725 | 2,925552 | 1 | 6565020 | control 45 kDa |
| | 1 | 3 | 0,719072 | 138245520 | 78,528821 | 41,591526 | 1 | 93332540 | control 38 kDa |
| | | | | | | | | 118851320 | control Total protein |
| | 2 | 1 | 0,483247 | 61056800 | 69,678565 | 23,462668 | 0,885364 | 22468106 | 10μM LDC-3 52kDa |
| | 2 | 2 | 0,552835 | 5186020 | 2,687722 | 0,905029 | 0,885364 | 866665 | 10μM LDC-3 45kDa |
| | 2 | 3 | 0,706186 | 30177280 | 27,633713 | 9,305023 | 0,885364 | 8910591 | 10μM LDC-3 38kDa |
| | | | | | | | | 32245362 | 10μM LDC-3 Total protein |
| | 3 | 1 | 0,487113 | 61432280 | 78,572481 | 24,460857 | 2,690292 | 64302758 | 25µM LDC-3 52kDa |
| | 3 | 2 | 0,559278 | 5828060 | 3,864032 | 1,202934 | 2,690292 | 3162276 | 25µM LDC-3 45kDa |
| | 3 | 3 | 0,713918 | 27669600 | 17,563488 | 5,467792 | 2,690292 | 14373743 | 25µM LDC-3 38kDa |
| | | | | | | | | 81838777 | 25μM LDC-3 Total protein |
| | 4 | 1 | 0,488402 | 62225380 | 22,222728 | 13,601749 | 1,967189 | 52417834 | 50µM LDC-3 52kDa |
| | 4 | 2 | 0,541237 | 74020380 | 15,946085 | 9,760037 | 1,967189 | 37612810 | 50µM LDC-3 45kDa |
| | 4 | 3 | 0,706186 | 131115180 | 61,831187 | 37,844691 | 1,967189 | 145844241 | 50µM LDC-3 38kDa |
| | | | | | | | | 235874885 | 50μM LDC-3 Total protein |
| | 5 | 1 | 0,484536 | 109812500 | 18,475327 | 10,332215 | 1,384209 | 48541699 | 100µM LDC-3 52kDa |
| | 5 | 2 | 0,552835 | 115350900 | 15,457976 | 8,64478 | 1,384209 | 40613973 | 100µM LDC-3 45kDa |
| | 5 | 3 | 0,708763 | 212909200 | 66,066698 | 36,947404 | 1,384209 | 173582304 | 100µM LDC-3 38kDa |
| | | | | | | | | 262737976 | 100μM LDC-3 Total protein |