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Data article

# Evidence for Dsg3 in regulating Src signaling by competing with it for binding to caveolin-1



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## ABSTRACT

This data article contains extended, complementary analysis related to the research articles entitled "Desmoglein 3, via an interaction with E-cadherin, is associated with activation of Src" (Tsang et al., 2010) [1] and figures related to the review article entitled "Desmoglein 3: a help or a hindrance in cancer progression?" (Brown et al., 2014) [2]. We show here that both Src and caveolin-1 (Cav-1) associate with Dsg3 in a non-ionic detergent soluble pool and that modulation of Dsg3 levels inversely alters the expression of Src in the Cav-1 complex. Furthermore, immunofluorescence analysis revealed a reduced colocalization of Cav-1/total Src in cells with overexpression of Dsg3 compared to control cells. In support, the sequence analysis has identified a region within the carboxylterminus of human Dsg3 for a likelihood of binding to the scaffolding domain of Cav-1, the known Src binding site in Cav-1, and this region is highly conserved across most of 18 species as well as within desmoglein family members. Based on these findings, we propose a working model that Dsg3 activates Src through competing with its inactive form for binding to Cav-1, thus leading to release of Src followed by its auto-activation.

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Subject area More specific subject	Biology Cell signaling
area	
Type of data	Western blotting, image, sequence alignment, graph
How data was acquired	Confocal microscope, co-immunoprecipitation, proximity ligation assay, bioinformatics
Data format	filtered, analyzed
Experimental factors	Cell culture, RNAi mediated knockdown of Dsg3, calcium depletion and repletion
Experimental features	in vitro analyses of protein-protein interaction and protein sequence alignment
Data source location	Original source of protein sequences used for alignment is from the NCBI Proteins
Data accessibility	Data is with this article

## **Specifications Table**

## Value of the data

- Dsg3 forms a complex with Src, caveolin-1 and E-cadherin.
- Dsg3 competes with Src for binding to caveolin-1 and thus elicits Src auto-activation.
- A highly conserved putative region within the carboxyl-terminus of Dsg3 is identified for binding to the scaffolding domain of caveolin-1.

## 1. Data

The desmosomal cadherin, Desmoglein 3 (Dsg3) is a calcium-dependent adhesion protein in epithelial cells and has recently been identified to associate with E-cadherin/Src and act as an upstream regulator in E-cadherin/Src signaling [1–5]. However, the molecular mechanism by which Dsg3 regulates Src remains poor understood. *In vitro* study showed that Dsg3 is internalized through a lipid raft-mediated pathway upon PV-IgG binding [6] and lipid raft contains caveolin protein. Interestingly, the Dsg3 associated family member Dsg2 is recently found to interact directly with the scaffold domain of caveolin-1 [7]. Hence, we speculated that Dsg3 also forms a complex with caveolin-1 along with Src. To investigate this possibility, we performed co-IP experiments with mouse antibody against Dsg3 in Triton-soluble and insoluble fractions of HaCaT cells, respectively, using the same procedures as previously described [1,4]. Western blotting of immunoprecipitates revealed that both caveolin-1 and Src co-purified with Dsg3, alongside E-cadherin and actin, in particular from Triton-soluble fraction (Fig. 1A). The proximity ligation assay (PLA) showed that, compared with the negative control, there was a substantial increase of PLA signals in cells probed with either Dsg3/ caveolin-1 or Dsg3/Src antibody combinations (Fig. 1B left bar chart) and Dsg3 silencing resulted in a reduced PLA signals as expected (data not shown).

Several lipid-regulated signaling molecules, including Src, G $\alpha$  subunits and H-Ras, bind caveolin [8,9]. Src of inactivated form is identified to bind to a membrane-anchored scaffolding domain of caveolin; the 20aa stretch within a membrane-proximal region of the cytosolic N-terminal domain of caveolin; the 20aa stretch within a membrane-proximal region of the cytosolic N-terminal domain of caveolin [8] (see cartoon in Fig. 5B). This 20aa residues functionally inhibit the auto-activation of c-Src and Fyn tyrosine kinases [8]. Hence, we hypothesized that Dsg3 may compete with inactive Src for the same binding site on caveolin. To test this hypothesis, we analyzed the immune complexes purified with caveolin-1 antibody in A431-Vect control and A431-hDsg3.myc cells with overexpression of Dsg3. Western blotting of caveolin-1 immunoprecipitates showed that overexpression of Dsg3 increased its association with caveolin-1 while reducing the amounts of Src in such a complex, compared to vector control cells (Fig. 1C left panels). In parallel, co-IP was performed in HaCaTs with or without Dsg3 depletion. Western blotting analysis of immunoprecipitates showed that Dsg3 silencing resulted in an increase in the amount of Src in



**Fig. 1. Dsg3 competes with Src for binding to caveolin-1.** (A) Western blotting analysis of the Dsg3 immunoprecipitates from Triton-soluble and insoluble fractions of HaCaT keratinocytes and probed with the indicated antibodies. (B) Proximity ligation assay (PLA), left, for Dsg3 and Src or caveolin-1 (Cav-1) that showed the enhanced protein interaction signals for both Dsg3/Src and Dsg3/Cav-1 and the representative images of PLA are displayed on the right. (C) Western blotting of immune complexes purified with Cav-1 antibody from A431 and HaCaT cell lysates that showed the overexpression of Dsg3 resulted in a reduction of Src in the caveolin complex, and an inverse effect was observed in cells with Dsg3 silencing (RNAi), compared to the respective control cells (arrows). The densitometry of the Src was indicated above the Src blots. Scram: scrambled control siRNA, HC: antibody heavy chain. (D) Confocal microscopy showed enhanced colocalisation of Dsg3/Cav-1 in cells with overexpression of Dsg3 (A431-D3) compared to vector control cells (A431-V). Top panels are the projected images of the stacks (with maximum intensity) and bottom ones are the orthogonal views if stacks. Scale bar, 10 µm.

the complex purified by caveolin-1 antibody (Fig. 1C right panels). Furthermore, confocal analysis indicated enhanced co-localization of Dsg3 and caveolin-1 at the plasma membrane in cells with overexpression of Dsg3 relative to vector control cells (Fig. 1D).

To test our hypothesis further, we performed double immunostaining with antibody combination for Cav-1/phospho-Src and Cav-1/total Src, respectively, followed by colocalization analysis with ImageJ. As shown in Fig. 2, there was little colocalization for Cav-1/pSrc at the cell borders in A431 cells and pSrc was predominantly expressed in the membrane protrusions. However, a marked increase in the colocalization of Cav-1 and total Src was detected at the cell borders in A431-V cells but this was found to be reduced in Dsg3 overexpressing cells (A431-D3) (see the colocalization images and profiles in Fig. 2B). Interestingly, a reduced expression level of Cav-1 was also observed in A431-D3 cells compared to A431-V control in which an enhanced Cav-1 staining at cell borders was noticeable.

The protein sequence analysis identifies a putative region for binding the caviolin-1 scaffolding domain enriched in aromatic amino acids [10] in Dsg3 which is highly conserved across most of 18 species (Fig. 3) as well as within the Dsg family members (Fig. 4). This potential binding site is located within the ICS domain of C-terminus of Dsg3 at 788-798aa, an 11aa stretch which contains 4 aromatic aa residues and which likely competes with the inactive Src for binding to the scaffolding domain in caveolin-1 [10]. Interestingly, this region is overlapped with previously identified segment by Andi and Stanley [11], the 87aa sequence within the ICS domain downstream from the 779aa, that is



**Fig. 2. The Dsg3 overexpressing cells showed reduced colocalization of caveolin-1 and total Src at cell periphery.** Immunofluorescence and colocalization analysis of caveolin-1 (Cav-1, red)/phospho-Src (pSrc, green) (A) and Cav-1 (red)/total Src (green) (B) in A431 cell lines. Cells were seeded at sub-confluent density for one day and fixed with ice cold Methanol for 10 min before proceeding immunofluorescence (IMF) staining. (A) Little colocalization of Cav-1/pSrc was detected at the cell periphery (highlighted in white pixels in the bottom two images). Phospho-Src was found predominantly expressed in the membrane protrusions and was not co-localized with Cav-1. Reduced expression of Cav-1 was observed in A431-D3 cells compared to A431-V control in which an enhanced Cav-1 staining at cell borders was noticeable. (B) A significantly enhanced colocalization of Cav-1/Src was seen in A431-V cells as compared with that for Cav-1/pSrc staining. The Dsg3 overexpressing cells (A431-D3) showed relatively less colocalization than that of A431-V cells for Cav-1/total Src staining (highlighted white pixels in the bottom two images). The profiles of IMF staining at cell borders for the marked cells (a and b) in A and B are displayed on the right, respectively. The percentage of colocalization in each image was indicated at the bottom right corner. Scale bar, 10 µm.

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**Fig. 3. A conserved putative binding site is identified within the carboxyl-terminus of Dsg3 by sequence alignment.** Alignment of Dsg3 across 18 species using the program MUSCLE [14]. The putative binding site (box with red line), at amino acid sequence between 788-798 with four aromatic amino acid residues (asterisks), for the scaffolding domain of caveolin-1 [10] is depicted to be conserved across 10 of 18 species. The amino acids are coloured according to the Clustal X Colouring Scheme [15].

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DSG3/1 999 e)[114672673/1 981 g)[297275144/1 999 g)[296222463/1 1003 g)[149271044/1 983 g)[5050209/1 993 g)[2950209/1 993 g)[29139242/1 1015 g)[2913924/1 993 g)[12429741/1 993 g)[12429744/1 493 g)[1242744/1 493 g)[12627140/1 505 g)[1262634/1 900 g)[1262745961 856 g)[14224591/1 853 g)[1537030821 842 g)[156119862/1 856 g)[2257196671 856	575 ROEMERSLILE VOODNRGIDGTSY PTTSPOTRYGREHSGRLOPAAIGLLLL GLLLLLAPLLLLTODCGASTGG VTGG 654 574 ROEMEGLTLE VOODNRGIDGTSY PTTSPOTRYGREHSGRLOPAAIGLLLL GLLLLLAPLLLTODCGASTGG VTGG 654 575 ROEMEGLTLE VOODNRGVGTSY PTTYPOTRYGRENG GRESSGRLOPAAIGLLLL GLLLLLAPLLLTODYGGSTGG VTGG 654 575 LOETTE SLTLE VOODNRGVGTSY PTTYPOTRYGRSF GRLOPAAIGLLL GLLLLLAPLLLTODYGGSTGG VTGG 654 574 LOETTE SLTLE VOODNRGVGTSY PTTDPGNYGVGTSPGRLOPAAIGLLL GLLLLLAPLLLTODYGGSTGG VTGG 654 574 LOETTE SLTLE VOODNRGVGTSY PTTDPGNYGVGTSPGRLOPAAIGLLL GLLLLLAPLLLTODYGGSTGG VTGG 655 574 LOETTE SLTLE VOODNRGIDGTSY PTTDPGNYGVGTSPGRLOPAAIGLLL GLLLLLAPLLLTODYGGSTGG VTGG 653 575 LOETTE SLTLE VOODNRGIDGTSY PTTDPGNYGVGTSS SVRLOPAAIGLLL GLLLLLAPLLLTODYGGSTGG VTGG 653 576 LOETTE SLTLE VOODNRGIDGTSGS FLORGSS SVRLOPAAIGLLL GLLLLLLAPLLLTODYGGS GFLGG 576 LOETTE SLTLE VOODONRGIDGTSGS FLORGSS SVRLOPAAIGLLL GLLLLLLAPLLLTODGGSGFIGGATGG 576 LOETTE SLTLE VOODONRSTGRS FLORGSS SVRLOPAAIGLLL GLLLLLLAPLLLTODGGSGFIGGATGG 576 LOETTE SLTLE VOODONSS SVRLOPAS 576 LOETTE SLTLE VOODONSS SVRLOPAS 576 LOETTE SLTLE VOODONSS SVRLOPAS 576 LOETTE SLTLE VOODONSS SVRLOPAS 577 LOETTE SLTLE VOODONSS SVRLOPAS 578 LOETTE SLTLE SLTLE VOODONSS SVRLOPAS 578 LOETTE SLTLE VOODONS SVRLOPAS 578 LOETTE SLTLE SL
DSG3/1 999 q1[14672675/1 981 q1[29727514/1 998 g1[29727514/1 998 g1[29822463/1 1003 g1[149721049/1 983 g1[291394242/1 1015 g1[20079310/1 988 q1[24297841/1 993 g1[20079741/1 975 g1[202821440/1 1005 g1[292624564/1 865 g1[48224991/1 863 g1[1621780886/1 865 g1[262719967/1 858	655 F I PVPD  GSE GT I HOWG I EGA HPEDKE I TN I CV PVT A NGADF MES SE VCTNTYARGT AVEGT SGMENTTIK L GAATE EGGA 734    654 F I PVPD  GSE GT I HOWG I EGA HPEDKE I TN I CV PVT A NGADF MES SE VCTNTYARGT AVEGT SGMENTTIK L GAATE EGGA 734    655 F I PVPD  GSE GT I HOWG I EGA HPEDKE I TN I CV PIT I A NGADF MES SE VCTNTYARGT AVEGT SGMENTTIK L GAATE EGGA 734    655 F I PVPD  GSE GT I HOWG I EGA HPEDKE I TN I CV PI I TN NGADF MES SE VCTNTYARGT AVEGT SGMENTTIK L GAATE EGGA 734    654 F I PVPD  GSE GT I HOWG I EGA HPEDKE I TN I CV PI I TS NADF MES SE VCTNTYARGT VEGA SGMEL TIK L GAAT GSGA 733    654 F I PVPD  GSE GT I HOWG I EGA HPEDKE I TN I CV PI I TS NADF MES SE VCTNTYARGT VEGA SGMEL TIK L GAAT GSGA 733    654 F I PVPD  GSE GT I HOWG I EGA OPEDKE I TN I CV PI I TS NADF MES SE VCTNTYA GGT VEGA SGMEL TIK L GAAT GSGA 634    654 F I PVPD  GSE GT I HOWG I EGA OPEDKE I TN I CV PI I TA NGDF MES SE VCTNTYA GGT VEGA SGMEL TIK L GAAT GSGA 634    654 F I PVPD  GSE GT I HOWG I EGA OPEDKE I TN I CV PI I TA NGDF MES SE VCTNTYA GGT VEGA SGMEL TIK L GAAT GSGA 734    655 F I PVPD  GSE GT I HOWG I EGA OPEDKE I TN I CV PI I TA NGDF MES SE VCTNTYA GGT VEGA SGMEL TIK L GAAT GSGA 734    655 F I PVPD  GSE GT I HOWG I EGA OPEDKE I TN I CV PI VI'S NADAP MES SE VCTNTYA GGT VEGA SGMEL TIK L GAAT GSGA 734    655 F I PVPD  GSE GT I HOWG I EGA OPEDKE I TN I CV PI VI'S NADAP MES SE VCTNTYA WGT VEGA SGMET K L GAAT 34    656 F I PVPD  GSE GT I HOWG I EGA OPEDKE I TN I CV PI VI'S NA

Fig. 3. (continued)

	gi 225719967/1-858	710 LL I DPEDDVRDN I LK - Y DEEGGGEEDQDYDL SQL QQPDT VEPDA	AIK PVGIRRLDERPIHAEPQYPVRSAAPHPGD783
III 222301 4961 495    TYD BC VT DB YV AC Y BOTTLA FEB AT TAXL.    BC VD DB WARD DB WARD VD	DSG3/1-999 gi[11472675/1-981 gi[29275144/1-999 gi[29275144/1-999 gi[296222463/1-1003 gi[16950209/1-983 gi[2019300/1-988 gi[20173900/1-988 gi[20073900/1-988 gi[20073900/1-988 gi[2007411/1-975 gi[120621440/1-1005 gi[12066934/1-900 gi[797500521-905	735  ACF AT GT VS GAAS GF GAAT GVG I C S SOC GTMRTE HIST GCT NC    734  AGF AT GT VS GAAS GF GAAT GVG I C S SOC GTMRTE HIST GCT NC    735  AGF AT GT VS AAS GF GAAT GVG I C S SOC GTMRTE HIST GCT NC    736  GF AT GT VS AAAS GF GAAT GLG I C S SOC GTMRTE HIST GCT NC    737  GF AT GT VS AAAS GF GAAT GLG I C S SOC GTMRTE HIST GCT NC    736  GF AT GT VS AAAS GF GAAT GLG I C S SOC GTMRTE HIST GCT NC    737  GF AT GT VS AAAG F GAAT GLG I C S SOC GTMRTE HIST GCT NC    734  AGF	
Ip1197020091-042  Te5 ID C0  In Edu KaaN BT AP VOLLUVE 910    Ip1297198417635  Te6 ID C0  In Edu KaaN BT AP VOLLUVE 910    Ip1297198417635  Te6 ID C0  In Edu KaaN BT AP VOLLUVE 910    Ip11971086177637  Stop ID AP AP VOLUCE 910  In Edu KaaN BT AP VOLUCE 910    Ip11971086177637  Stop ID AP AP VOLUCE 910  ID P AP AP AP VOLUCE 910  ID P AP AP AP VOLUCE 910    Ip11971086177647  Stop ID AP AP AP VOLUCE 910  ID P AP AP AP VOLUCE 910  ID P AP AP AP AP VOLUCE 910    Ip1197210471749  BTO ID AP AP AP AP VOLUCE 910  ID P AP AP AP AP VOLUCE 910  ID P AP A	gi 292624596/1-856 gi 148224991/1-863	747 DQY YTSGRYDNKIYGNGTLQ - KFSNTGTLD 757 AED NRGGLTLTSMGGGGQQQFFDVNRVNTI	WRTN GCYLDRKLAYFGEOEDGRYADDLLKNY 807
(1)    (1	gi 163780986/1-842	768   GD	FINEGLKAADNDPTAPPYDSLLVF 794
95031499  91 To PE - BADAT GERVOR YOL - GER KADL DOF LOD COP FUNAL ELIC VADES (VADES KOLD Y LESCHIELEA	gi 196119862/1-858 gi 225719967/1-858	784 IGD 784 IGD	FINEGLKAADNDPIAPPYDSLLVF 810 FINEGLKAADNDPTAPPYDSLLVF 810
a):252:224:31-1003  a):7  N.E. 40.A):T.BY VEX.VG: - C.F. I ADEL DOB'T. LOUIL OPFERLA. AL ILLUI LOUDEAU OSY TETSC.BISKEV	DSG3/1-999 gi 114672675/1-981 gi 297275144/1-999	817 DNE - GADAT GSPVGSVGC CSFIADDLDDSFLDSLGPKFKK/ 816 DNE - GADAT GSPVGSVGC CSFIADDLDDSFLDSLGPKFKK/ 817 DNE - GADAT GSPVGSVGC CSFIADDLDDSFLDSLGPKFKK/	NEISLGVDGEGKEVOPPSKDSGYGIESCGHPIEV 890 NEISLGVDGEGKOVOPPSKDSGYGIESCGHPIEA 889 NEISLGVDGESKOVOPPSKDSGYGIESCGRSTEV 890
91053602011-033  90 0 PH = DBAG C S VISTL GE - C F LADE LODEF LODEF LODEF LODE A LEVEL AS CME S KASS SMESCH ST S LEVEL	gi 296222463/1-1003	817 DNE - GADAT GSPVGSVGC CSFIADDLDDSFLDSLGPKFKKLA	AEISLGIDDEGKQVQPPSKDRCYGTESCGHSKEV 890
a)2234221-1015  832  R)2 ENCOMAVES BY VOLE G: C. C.F. I ADD DOBE-LEDIL OPERTIL AL ELS LONG ADRAEL SADE MAREL SADE MAR	gi 50950209/1-993	810 DNE - GMGAPS SPVGSLSC CSFTADELDDSFLDSLGPKFKKL/	AEISLGUDDEAKQSQPLSKASLSGMESCGYSLEV 883
9)99999999999999999999999999999999999	gi 291394242/1-1015	832 DNEEDMAVPSSPVGSLGC CSFIADDLDDSFLDSLGPKFKRL	AE ISL GMDEEA GRARLSSQDSGARSESRGPAPDT 906
19/107274111-975  755 DD EGECAA PR EP A LS - C E FLADD DDD FL DBL GPK-FKL AE LC SVDDEA VK KERNOSS SE DA CARFT FLA E B B B B B B B	gi 124297841/1-993	796 DDEGEDAAPHSP TLSS CSIFADDLDDNFLDSLGPKFKKL/	AEICLGIDDEAKQAKPGPKDSGSGADTCARSMEVPQSGS 873
0 12632140(1-1005  510 005 - 91 - 68 - 61 - 68 - 67 - 051 - 600 004 - 004 - 074 - 074 004 - 004 - 074 - 074 004 - 004 - 074 004 - 004 - 074 004 - 004 - 074 004 -	gi 300797411/1-975	795 DDEGEDAAPRSPA LSS CSFMADDLDDSFLDSLGPKFKKL/	AEICLGVDDEAKQVKPGPKDSSSGADACARPTEA 867
1797500221-095  27.1 Bit	gi 126321440/1-1005 gi 118086934/1-900	810 DDE - GIEAPSSPVGSVGC CSFIADDFDDSFLDSLGPKFKKLA 707 SOG	ADISMGIDDEPKPSQAPEKTNIAVPGTSGSQSAS 883 AFICIGRRIDMKDASSKNESSEGVNEOKAESSKOTSASG 782
9 22224596/1-856 9 16370996/1-857 9 16370986/1-852 9 16370986/1	gi 79750062/1-905	721 DIE GEGSPAGSVGC CSFIGEDLDDSFLDTLGPKFKKLA	ADISLGKETEPYPDPDPSWPPQSTDPICPPQGTEP 792
9119324293111003  0.00000  0.00000  0.0000  0	gi 292624596/1-856		ADICYTTNKTGK 856
III196119862/1-858  811 DY E	gi 148224991/1-863 gi 163780986/1-842	795 DYE GSGSTAGSVGC CSDFRDEDRMDFLNNLEPNFRTLA	ADMY GGGDD
g1225719967/1-858  611 DY E	gi 196119862/1-858	811 DYE GSGSTAGSLSSLNSSSSGGEQDYDYLNDWGPRFKKL/	AEMY GGGDD 858
DSG311999  891  OT G'VK GOT S G  SCGAPA SAGEV - OPAXS  PDL DHGYL VTETYSA - SGL VOPSTA 449    gil14727514411999  890  OT G'VK GOT S G  SCGAPA SAGEV - OPAXS  PDL DHGYL VTETYSA - SGL VOPSTA 449    gil29222463111033  891  OS SG KK OT S G  SCGAPA SAGEV - OPAXS  PDL DHGYL VTETYSA - SGL VOPSTA 449    gil29622246311103  891  OS SG KK OT S G  SCGAPA SAGEV - OPAXS  PDL DHGYL VTETYSA - SGL VOPSTA 449    gil20502011-933  864  OS SEARN OT SG  SCGAPA SAGEV - OPAXS  PDL DHGYL VTETYSA - SGL VOPSTA 449    gil201394242741105  907  CCGAET MRGOT SG  SCGAPA SAGEV - OPAXS  PDL DHGYL VTETYSA - SGL VOPSTA 449    gil201394247411193  874  NEYTEYSA  SCGAPA SAGEV - OPAXS  PDL DHGYL VTETYSA - SGL VOPSTA 449    gil201394247411195  874  NEYTEYSA  SCGAPA SAGEV - PAXA  PASS INVETTYSA - SGL VOPTA 449    gil201297411195  874  NEYTEYSA  SCGAPA SAGEV - PAXA  PASS INVETTYSA - SGL VOPTA 449    gil2122571990711-96  874  NEYTEYSA - SGL VOPTA 449  SGVOPTA 440  SGVOPTA 440    gil22571990711-97  733  ISSCHPTI SGVOPTA 440  SGVOPTA 440  SGVOPTA 440    gil22571990711-858  SG	gi 225719967/1-858	811 UYE GSGSTAGSLSSLNSSSSGGEQUYDYLNDWGPRFKKL/	<b>ADM</b> Y <b>GGGDD</b> 858
0  147/26/5/1-961  990  00  00  00  100  00  100  00  100  00  100  00  100	DSG3/1-999	891 QQTGFVKCQTLSG SQGASALSAS	SGSV - QPAVS I PDPL QHGNYL VTETYSA - SGSL VQPSTA 949
9 28224341-1003  891  OL GF VK OT LS G  S GG AAB AS AS GSV -L DAVS L PPL OHS FV TETY SA - SG LX OP TA 949    9 149721049/1-983  874  OC SE SARVOT LS G  S GG AAB AS AS GSV -L DAVS L OPALS I PPL OHS FV TETY SA - SG LX OP TA 943    9 12912409/1-983  874  OC SE SARVOT LS G  S GG AAB AS AS SV LO AN IS I PPL OHS FV TETY SA - SG LX OP TA 943    9 1291242421-1015  907  OC AAT MRGOT LS G  S GG AAB AS AS SV LO AN IS I PPL OHS FW VTETY SA - SG LV OP TA 943    9 124227841/1-993  874 NRYOT L PG SS VR OT LS G  S GG AAB AS AS SV LO AN IS I PPL OHS FW VTETY SA - SG LV OP TA 943    9 124227841/1-993  874 NRYOT L PG SS VR OT LS G  S GG AAB AS AS GS V LO AN IS I PPL OHS FW VTETY SA - SG LV OP TA 949    9 124227841/1-905  864  A ST H T C PPT INVT CPE AT FYSNED T PA SG V OP AN IP PPL OL ON VL TETY TS - SG F A OP TI 943    9 126321440/1-1005  884  A ST H T C PPT INVT CPE AT FYSNED T PA SG V OP AN IP PPL OL ON VL TETY TT - SG F A OP TI 952    9 174500671-905  733  ST L S SV P OT INV TETY VL SS SG SO - HOAL PL OL ON VL TETY TT - SG F A OP TI 952    9 174502671-905  733  ST S PA SG SV SG V N SE SV SG CO - HOAL PL OL ON VL TETY TT - SG F A OP TI 952    9 17462249911-863  940 GF DPL L TON VI VTERVICP IS SV P - GN AG - PTO L  PTO L RES HTML CHED CS RL I    9 1747200571-981  940 GF DPL L TON VI VTERVICP	gi 114672675/1-981 gi 297275144/1-999	890 QUIGFVKCQILSG SUGASALSA 891 QOSGFIKCQTLSG GOGASALSA	SGSV - OPAVSIPDPL OHGNYLVTETYSA - SGSLVOPSTA 948
i]1427210491-983  B74  COSES ARMOT LSG  SIGUALS ARGS/LOPALS IPPOLATSEV/CETYAL SCSUL ADDS 18 93    i]0595020971-993  B84  COSES ARMOT LG  SIGUALS ARGS/LOPALS IPPOLATSEV/CETYAL SCSUL OPALS IPPOLATELYAL SCSUL OPALS IPPOLATION IPPOLAT	gi 296222463/1-1003	891 QQLGFVKCQTLSG SQGASALSAS	SGSV - L PAVS I PDPL QHGNYL VTETYSA - SGSL V QPSTA 949
gij291394242/1-1015  907  QCAATTMR GOTLS G  SB GVTLLSA SGM - QPAIS I PDPL QHAMYLVTETYSA - S SLV QPASY 965    gij3007939001-988  878  COSES VRY OTLSG  SB GAALSA SSVLOPAVS I TDPLLGAGSYLVTETYSA - S SLV QPAA 938    gij124297811-933  874 NRYOTL GCSLE UT DTS KI CHTLSG  NB ET VX SSGW - QPAV SI TDPLLGAGYLVL TETYST - SCSF QPT 1944    gij12429781401-1005  884  L CACSKV CHVLPG  NB ET VX SSGW - HA 1A I PDPLLGAFVLT ETYST - SCSF QPT 1952    gij1282264361-866  L CACSKV CHVLPG  NB ET VX SSGW - HA 1A I PDPLLGAFVLT ETYST - SCSF QPT 1952    gij1282264361-866  SG HPPLS HI GTTT	gi 149721049/1-983 gi 50950209/1-993	874 QQSESARYQTLSG SQGASALSAS 884 SQGASALSAS	SGSVL QPATSTPEPLQHSSFVVTETYSA - SGSLAQPSTA 933
g 307939001-988  87.8  COSES VPM OT LSG  S GA AL SASS V CPAVS I TOP LDHGYLV LETYTS S COV 0 PAA 938    g 124278411-1933  87.4  NRVOT LEGS LE VT TTS S I CAT TTS S I CAT TTS S I CAT TTS S I CAT TTY 944    g 3007974111-975  86.8  L ALGS V CHV LGC  ND ET VMS S COV PAA 14 I POP LD CAVL I ETYTS - S COS POP TTA 924    g 124278411-1005  88.4  ALS THE CPT I INVT CPA TTY S LOS COV - HAVAL I POP LD CAVL I TETYT - S COS F OP TTA 926    g 1780589341-900  78.3  ST I S VOAGGAV USEAVSOEV I METT IS LOS COV - HAVAL I POP LD CAVL I TETYT - S COS F OP TTA 926    g 1780589341-900  78.3  ST I S S HE T CPT I INVT CPA TTY S COS OV HAT TTY S LOS COV - HAVAL I THY FT I S COS CHAA 850    g 178058961-842  I S S HP I S PH I S FH I G TTT - V I S ETYP S CPGV HH - MI POP LS YGW I METEYT - S C I L KPS V 853    g 1252719671-858	gi 291394242/1-1015	907 QCAGTMRGQTLSG SQGVFTLSA	SGSM - QPAISIPDPLQHANYLVTETYSA - SSSLVQPSSV 965
911242304111935  01 MINIOLE EXISTING IN LOG  NILD TAYLE SGN - HPATA POPULAGINELL GIVEL TENT TO SGN - GON A CONTROL OF A DECISION OF A DECIS	gi 300793900/1-988		SSVLOPAVSITOPLOHOSYLVTETYSASSOSLVOPPAA 938
iii 12821440/1-1005  84  AB ST HGT CPPT INVT CEPA TFYSNEPTL BASCSV DEALEPT DELOGHELVTEYTT - SCSF COPETU 952    iii 180869341/-900  783 GYL  SHT SV0AGCAV SEAVOGEV INTT SLOSGO-HOART TT PLOES COLL OF INTT PLOES GGAVTEAS 653    iii 12925245961/-856	gi 124297841/1-995 gi 300797411/1-975	868 LQAGSKVCHVLPG NQDTSVLSS	SSV - HPAVATPDPLQL GNTLLTETTST - SGSFAQPTTV 944 SGSV - HPATATPDPLQL GNYLLTETYTT - SGSFVQPTTA 926
iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii	gi 126321440/1-1005	884 APSTHGTCPPTINVTCPPATFYSNEPSTLPAS	SGSVFQPAIPIPDPLLQGNFLVTESYTT-SGSFTQPST1952
gi[292624596/1-856    gi[1482249911-863    gi[15770968/1-842    gi[15770968/1-842    gi[25719967/1-858	gi 118086934/1-900 gi 79750062/1-905	783 GYL SHTPSVQAGGAVGSEAVSQEVIMETTSSLQS 793 IGSGHPPISPHIGTTT VISESTYPS	GQ HDARTL TTPFTETNVTMTETYS GGGPTCSAAS 850 BPGVHHP-MPIPDPISYGNVTMTESYTT-SG-UKPSVH853
iiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiiii	gi 292624596/1-856		
91103030104201042    911031030201042    912571967/1-858    912571967/1-858    91297275144/1-999    950 GF DPLL TONV IV TERVICP ISSVP    91297275144/1-999    91297275144/1-999    91297275144/1-999    950 GF DPLL TONV IV TERVICP ISSVP    91297275144/1-999    91297275144/1-999    91297275144/1-999    91297275144/1-999    91297275144/1-999    91297275144/1-999    912922463/1-1030    950 GF DPLL TONV IV TERVICP ISSVP    91293942421/1015    966 V DPLL TONV TV FERVICP ISSVP    91293942421/1015    966 V DPLL TONV IV TERVICP ISSVF    9129134242421/1015    912929721/1015    912929721/1015    912929721/1015    912929721/1015    912929721/1015    912929721/1015    912929721/1015    91297 F D HV ONVT V ERVICP ISSC GLOSUN ISTEDPC	gi 148224991/1-863 gi 163780986/1-842		
gil225719967/1-858	gi 196119862/1-858		
DSG3/1-999    950 GF DP LL TONV IV TERVICP ISSVP    GNL A G    PTOLRGENT MLCTEDPCRLI    999      g 114727575/1-981    949 GF DP LL TONV IV TERVICP ISSVP    GNL A G    PTOLRGENT MLCTEDPCRLI    991      g 1297275144/1-999    950 GF DP LL TONV IV TERVICP ISSVP    GNL A G    PTOLRGENT MLCTEDPCRLI    991      g 129275144/1-999    950 GF DP LL TONV IV TERVICP ISSVP    GNL A G    PTOLRESIT MLCTEDPC RLI    993      g 129275144/1-993    950 GF DP LL TONV IV TERVICP ISSVP    GNL A G    PTOLRESIT MLCTEDPC RLI    1003      g 169214941-983    954 V DP LL TONV IV TERVICP ISSVP    GNL A G    PTOLRESIT MLCTEDPC RLI    103      g 10505020/1-993    944 V L PL LTONV IV TERVICP ISSVP    GNL A G    PME IRGSRMICTEDPC RLI    993      g 12913942421-1015    966 V SD LL TOSVIT VERVICP ISS IT    GNL A G    PME IRGSRMICTEDPC RLI    993      g 124237841/1-993    945 T F D HVTONVT VTERVICP ISS IT    GNL A G    PTELRESVENT TEER SER    993      g 124237841/1-993    945 T F D HVTONVT VTERVICP LSS SES TO GNL A G    PTELRESVENT TEER SER    993      g 12521440/1-1005    953 V D RL TENVT VERVICP LSS SES GS GS GNL A G    PTELRESVENT YTEE CH L <td< td=""><td>gi 225719967/1-858</td><td></td><td></td></td<>	gi 225719967/1-858		
i 114722751/1-981  949 GF DPLL TONV IVTERVI CP IS SVP  GNL AG  PTCL-RESIT MLCTEDPCCRL  981    i 29275144/1-993  950 GF DPLL TONV IVTERVI CP IS SVP  GNL AG  PTCL-RESIT MLCTEDPCCRL  1003    gi 149721049/1-983  954 VF DPLL TONV IVTERVI CP IS SVP  GNL AG  PTCL-RESIT MLCTEDPCCRL  1003    gi 149721049/1-983  934 VF DPLL TONV IVTERVI CP IS SVP  GNL AG  PMELROS RT MLCTEDPC RL  1003    gi 150950209/1-993  934 VF DPLL TONV IVTERVI CP IS SVP  GNL AG  PMELROS RT MLCTEDPC RL  983    gi 20191394242/1-1015  966 VS DPLL TONV IVTERVI CP IS SVP  GNL AG  PMELROS RNIL CTEDPC RL  1015    gi 3007930001-988  939 VF DPLL TONV IVTERVI CP IS ST  GSL IV  PTELROS RNIL CTEDPC RL  1015    gi 3007937001-988  935 VF DPHVTONVTV TERVI CP IS ST  GSL IV  PTELROS RNIL CTEDPC RL  988    gi 124278141/1-993  945 TF DPHVTONVTV TERVI CP IS SSI TO SSI TEDPC SRL  993  988    gi 124278141/1-905  953 VF DPHL TONVI VTERVI CP IS SSI SSI VA  PTELROS TML TONVI VTERVI CP IS SSI SSI SSI VA  PTELROS TML TONVI VTERVI CP IS SSI SSI SSI VA  993    gi 126179500621-905  953 VF DPHL ENVT VTERVI CP IS SSI SSI SSI VA  PTELROS TML TONVI VTERVI CP IS SSI SSI SSI SSI VA	DSG3/1-999	950 GFDPLLTONVIVTERVICPISSVP GNLAG PTOLRGS	HTMLCTEDPCSRLI 999
gi[2]2952246311-1003  950 G D LL TONVIV TERVIC PUSNE  GNLA GET CLEDITIC LICENTIAL COLLECTION CLEDITIC CL	gi 114672675/1-981 gi 297275144/1-999	949 GF DPLLTONVIVIERVICPISSVP GNLAG PTQL	
g 142721040/1-983  934 V E DPLL TONVTV ERVICE VS VE GNL HG PME IRCS RT MICTEDPCS RL I  983    g 50950209/1-993  944 V E DPLL TONVTV ERVICE ISNOS GNL OT PME IRCS RT MICTEDPCS RL I  993    g 291394242/1-1015  966 V SDPLL TOSVTV TERVICE ISNOS GNL OT PME IRCS RT MICTEDPCS RL I  993    g 307393001-988  939 V E DPLL TONVIV TERVICE TS IT GNL HG PTELRES RS IL CTEDPCS RL I  983    g 12427841/1-933  945 T E DPHVTONVIV TERVICE ISNOS GSL VA PTELRES TSML TTECPLS RL I  983    g 12427841/1-935  927 T E DPHVTONVIV TERVICE LSS SS E I GONE NG PTELRES TSML THE CS HL 993  993    g 126324249/1-1005  953 V F DPLT TONVIV TERVICE LSS SS E I GONE NG PTELRES TSML THE CS HL 975  9112 E DPLY TONVIV TERVICE LSS SS E I GONE NG PTELRES TSML THE CS HL 975    g 128251440/1-1005  953 V F DPLT TENVITERVICE LSS SG I GONE NG PTELRES TSML THE CS HL 975  9118086934/1-900  900    g 172975062/1-905  854 V HONROAS NVVTERVICE LSS SG I GONE NG ANL HGML EMPDL ROS NVITERVICE LSS SG 1 GONE NG ANL HGML EMPDL ROS NVITERVITER VIAP NSS  905    g 148224991/1-863	gi 296222463/1-1003	950 GF DPLLT QNVIVTERVICPISSVP GNLAGPT QLPTQLRGS	RTML CTEDPCSRLI 1003
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gij200793000/1-988  93 Y E DP L L D N I V ERVICETES IT ON H G PTELRGSDSKI FTEDPSRLI  988    gij124297841/1-993  945 T F DP HVT ON VT V ERVICP LP SAS SSI V A PTELRGSTM LY TKETCSHL.  993    gij20073411/1-975  927 T F DP HVT ON VT V ERVICP LP SAS SSI V A PTELRGSTM LY TKETCSHL.  975    gij20573411/1-975  927 T F DP HVT ON VT V ERVICP LSSS SSI V A PTELRGSTM LY TKETCSHL.  975    gij205731001705  953 V D RL TEN VT V ERVICP LSSS GLOON NG PTELRGSCNUT CTODPCSRLI  1005    gij205731001705  954 V HD NROAKNIN VTERVICP LSSS GLOON NG PTELRGSCNVTCHDPCSRLI  900    gij22624596/1-856	gij30950209/1-993 gij291394242/1-1015	966 VSDPLLTOSVTVTERVVCPRAAVS GSLIV PMELROS	RSILCTEDPCSRLI 993
9 12429/641/1-993 945 TF DPHVT ON VTV TERV I CPL PSAS SSIVA PTELROS YN MLY TKE TC CPL 993 9 100797411/1-975 927 TF DPHVT ON VTV TERV I CPL PSAS SSIVA PTELROS YN MLY TKE TC CPL 975 9 126321440/1-1005 953 VF DPRL TENVT VT ERV I CPL SSAS GSIVA PTELROS YN MLY TKE CSHL 975 9 12808934/1-900 851 F DPRL TENVT VT ERV I CPL SSAS 982 PS SK NNV YT ERV TSK 992 PS SK NNV YT ERV I CPL SSAS 993 9 129750052/1-905 854 V F DPRL TENVT VT ERV I CPL SSAS SSIVA PSL PS SK NNV YT ERV I SSAS 9 12975730986/1-842	gi 300793900/1-988	939 VFDPLLTONVIVTERVICPTPSIT GNLHG PTELRGS	DSKIFTEDPSSRLI 988
gi 126321440/1-1005    553 V F DP RL TENVT VT ENV I CP IS GVS & I OGNUNS PTOLGOS CNV I CT OD PC SRL 1    1005      gi 13086934/1-900    851 F L DP CF KEN I VV TENV LAP AS CLR EMV E I PSL PS GKNNVV TENUT TE EDP    900      gi 79750062/1-905    854 V HD NROA SMVV TERV V CP IS G AN L HGML EMP DL RD GS NV I VT ERV I APNSS    905      gi 148224991/1-863	gi 124297841/1-993 gi 300797411/1-975	945 IF DPHVTONVTVTERVICPLPSAS SSIVA PTELRGS	(NMLYTKETCSHL- 993 (SMLYTKEACSHL- 975
gi[11086934/1-900    851 F_DBC/FKENT[VV[FERVLAPASCLR EMVE] PSLPSCKNNVVTERNLT[FEBP]    900      gi[79750062/1-905    854 VHD/NRQASMVVTERVVCPISC	gi 126321440/1-1005	953 VF DPRLTENVTVTERVICPISGVSGIQGNLNG PTOLGGS	CNVTCTODPCSRLI 1005
gi]2526245961/1-863 gi]163780986/1-842 gi]252719967/1-858	gi 118086934/1-900 gi 79750062/1-905		MVVTERMLTSEGP 900
gi[14224991/1-863	gi 292624596/1-856		905
y 153/04/500/1-642	gi 148224991/1-863		
gi 225719967/1-858	gi 196119862/1-858		
	gi 225719967/1-858		

Fig. 3. (continued)

required for binding to plakoglobin and recruitment of Dsg3 to desmosomes. Study based on plakoglobin null keratinocytes by Green and colleagues has suggested that plakoglobin plays a role to suppress Src activity [12]. Thus it is possible that when plakoglobin is ablated the excess free Dsg3 molecules compete with Src for binding the scaffolding domain of caveolin-1 thus elicits Src autoactivation. In accord, when Dsg3 is overexpressed the same result would occur with a consequence of Src activation.

Dsgl	1 MDW SFFRVVAM LF IFLVVVEVN SEFR IOVR - DYN TKNGT IKWH SIRROKREW IK FAAAC REGEDNSKRNP IAK IH SD	76
Deg2	1 - MAR SOCRA VALUET LE RAVE SCHLAVISTRAFAK LEVENDAU TA VALUE REPORTS VALUET RECORTS SEEND TA VALUET RECORTS SEEND TA VALUET RECORTS SEEND TA VALUET RECORDS SEEND TA	76
DSG2		70
D8g3	I MMG LEPKIIGALA IPVVV ILVHGELR IEHKGO DEEEMIMO -QAKRKOKKEWVKFAKPCKEGEDNSKKNPIAKIISD	76
Dsg4	1 MDW LFFRN IC LL I ILMVVM EVN SEF IV EVK – EFD IEN G <b>ITK</b> WQ TV <b>RROKR</b> EW IK FAAAC <b>REGEDN SKRN P</b> IAK IR SD	76
Dagl	77 CAANO - OVITYN ISGVG ID OPPYG IFV IN OKIGE IN ITS IVDREV TPFFI IYCRALN SMGOD LERPLELRVRVLD IN	151
Decal	77 TA PERTURNAL TRANSPORTED FOR THE PARTY PARTY PARTY PARTY PARTY AND A DAMAGE PARTY	152
Dagz	THE ERGIN THE TOKE THEFTE TO TO THE DAY TO THE FETTED TO THE DARGENEVER FUELD, IN THE	155
Dsg3	77 XQATQ KITYR ISGVG IDQPPFG IFVVDKNTGD IN ITA IVDREETPSFL ITCRALNAQG LDVEKPL ILTVK ILD IN	151
Dsq4	77 CESNO KITYR ISGVG IDR PPYGVFT IN PRTGE IN ITSVVDRE ITPLFL IYCRALN SRGED LERPLELRVKVMD IN	151
De ut		000
Dagi	152 DN PPV PSMA TPAGO TEEN SNAN TLVM TLNA TDAD EPNN LN SK TAPK TTRDEP SD SPM PTTNKN TGE TRIMNN PLDKB	228
Dsg2	154 DNEPVFTQDVFVGSVEELSAAHTLVMK INATDADEPNTLNSK ISYR IVSLEPAYPPVFYLNKDTGE IYTTSVTLDRE	230
Dsq3	152 DN PPV F SOO IFMGE IEEN SA SN S LVM ILNA TD A D EPNH LN SK, IA FK, IV SO E PAG T PM F LL SK N TG EVR T L TN S LD R E	228
Dsg4	152 DNA PV FSO SV YTA S TEEN SDANT LVVK LCATDADEENH LNSK TAYK IV SO EPSGAPMET LNR YTG EVC TM SSELDRE	228
Dsgl	229 Q YG Q YA LAVRG SD BDGG - A DGM SA EC ECN IK ILD VNDN IPYM EQ S SYT IE IQ EN T LN SN LLE IR V ID LD EEF SA NWM	304
Dsg2	231 EH S SYTLIVEARDGNGEVIDK PVKQAQVQIR ILDVNDN IPVVENKVLEGMVEENQVNVEVIR IKVFDADEIGSDNWL	307
Dsq3	229 OASSYR LVV SGADKDG EGLSTOCECN INVKDVNDN FPM FRD SOYSAR IEEN ILSSELLR FOVTDLDEEYTDNWL	302
Daga	229 OH SMAN LVVR G SDRD GA - ADG LS SECOCR TKV LDVNDN PPT LEK TSVSA S TEENCLS SEL TR LOA TO LD FEG TONW L	304
bogi		001
Dsgl	305 AV IFF I <mark>s</mark> gn Egnwfe Iemn er tnvg Ilkvvk pldyeamoslo LS Igvenka Effh S Im <mark>s</mark> o yk Lka sa Isv tv LNV Ib	381
Dsg2	308 AN FTFA SGN EGGYFH IETDAO TN EG IV TL IK EVDYEEMKN LD FSV IV AN KAA FHK S IR SK YK P TP IP IK V K V K N V K E	384
Dag3	303 A V Y F F T S C N E G NW F E TO TO PR TN E G T L K V V K A LO Y F O LO SVK L S TA V K N K A F F H O SV T S P V O S T P V T TO V TN V B F	379
Dand	205 NO WE TE STORE TO BE	201
DSG4		201
Dsgl	382 G PV F M PGISK TVV TGIN MGI SN D K – – – – VGD F V A TD LD TG R PS T IV N YV MGIN N PA D L LA V D SN TGK L T L K <u>N K V</u> TN P <u>O</u> YN	454
Dsg2	385 G IH FK S SY IS IY SESMOR SSK - GQ IIGN FQAFD ED TG LPA - HAR YVK LEDRDN W ISVD SV TSB IK LAR LPD F ESR Y	459
Dsg3	380 G TA FR PA SK TET VOK G TS SKK LUD Y TLG T VOA TO FD TN KAASN VK VVMGRND G YLM TO FTA FTK EV EN MIN DO FTA	456
5395		450
DSg4	382 G PA FH P STMA F SVR EG IKG S S L N YV LG THTA ID LDTGN PATOVKTI IGHDAG SW LK ID SKIG E IQ F SKIEFD KIK KKKK	458
Dsgl	455 M LGGK YQGT ILS I-DDN LQRTC TGT IN IN IQSFGNDDRTN TEPNTK ITTN TGR -QESTSSTN YD TSTT	520
Dsg2	460 YONG TYTYK TVA TSED YPR KT TTGTVL TNVED TNDNC PTL TEPVOTICHDA EYVNVTA ED LDGH PN SC PESESY TDK	536
0		600
DSg3	457 IVNKI IIAEVLA I-DETIGNISIGIVEVRVPDENDICPIAVLEKDAVCSSSPSVVSARILN-NRIIGPIIFALEDQ	221
Dsg4	459 I ING IYTA E ILA I-DDG SGK TA TGT IC IEV PD INDYC PN IFPERRTIC ID SPSV LISVN E - H SYG SPFTEC VVD E	531
Dsgl	521 STD SSOVYSSEPGNGAKDLLS	545
Deg2	537 PROMA FRWK TAROFST SVITO -OSEKKIGRSFTOFT ISANOG FSC PEKOVITT TVC FC LHGSGC FA	602
DSG2	337 FEMALERAR TARGEST OF DEC 23 ERREDARSE TO FET TO A CELER OF DEC VET TO CELER OF DEC VET	002
Dsg3	532 PVK LPAVWS ITTLNATSALLRAQEQ IPPGVYH ISLVLTD SQNNRCEMPRSLTLEVCQCDNRG ICGTSYPTTS	603
Dsg4	532 PPG IAD MWDVR STNA TSA IL TAKQV LSPG FYE IP ILVKD SYN RAC E LAQMVQ LYACDCDDNHMC LD SGAAG IYTED I	608
Dsg1	546	601
D		663
Dsgz	603 QHD STAKGF IP IP GT IEM LA PLELELE VPLELE MCHCGKGAKGF IP IP GT IEM LA PWNN EG	001
Dsg3	604 PG TR YGR PH SGR LG PAA IG LLLLG LLLLLA PLLLL TCDCGAG STGGV TGGF I PV PDG SEGT IH QWG I EG	673
Dsg4	609 TGD TYG PV TEDQAGV SNVG LG PAG IGMMV LG ILLL ILA PLLLLLCCCKQRQ PEG LG TR FA PV PEGGEGVMQ SWR IEG	685
Dag1		658
Dogi		200
Dagz	662 A PPEDRVVPSFLPVDQGGSLVGRNGVGGMAREA IMRGSSSASIVRGQHEMSEMDGRWEEHRSLLS	120
Dsg3	674 A H P ED K E ITN IC V P P V TANGAD FM ESSEVCTN TYARGTAVE-G TSGMEM TTK LGAA TESGGAAGFA TGTV S	743
Dsg4	686 A H P ED R D V SN IC A PM TA SN TQ D R M D SS E IY TN TYAA G G TV EGG V SG V E LN T	736
Deci		719
Dagi		719
DSGZ	12/ GRA 10 FTGA 16 - A IM I TE TAK TAKA I GA SKOMAGAQAAAVA LMEEF LKNEF TONAAST EED ENH TAKOC LL	191
Dsg3	744 GAASG FGAATGV-GICSSGQ SGIM R TRHSTGGINKD YADGAISMNFLDSYFSQKA FACAEEDDGQ EANDCLL	814
Dsg4	737 GMG TAVG LMAAGAAGA GAAGA KKRSSTMGT LED YADA D INMAFLD SYFSEEA YA YAD ED EGRPANDC LL	804
Decil		780
Dago		0.00
Dagz	130 Y LOUEL I ED INA D LOUEDF LEO BLODIKF LOU LOUEFN TLA EVC LOUK ID INKE IEURUK PA TE TSMN TA SHS LC	8/1
Dsg3	815 IYDN EGADA TG SPV G SV G CC SF IADD LDD SFLD SLG PK FK KLAE ISLG VD G EGK EV O P - PSKD SG YG IE S C	884
Dsg4	805 IYDHEGVGSPVGSIGCCSWIVDDLDESCMETLDPKFRTLAEICLNTEIEPFPS	857
Deci		047
Dodi	TOT DEVE DEVE TE	04/
Dsg2	872 EQ TMIVN SEN TYSSESSFEVEK SLQEAN A EKVTQE IVTERSIO A QKVA TELPOPMA SRNV IA TETSYVTESIM	946
Dsg3	885 G HP IEVQ Q - TGEVKCQ TLSGSQ GASA - LSTSGSVQ PAVS IPDPLQHGNYLVTET - YSASGSL	943
Dsq4	858 HQAC IP ISTD	917
Deri		017
nagi	040 NE SVRVRURKEA SRVVVVTEKVVGPISGAD LHGMLEMED LKUGSSRVIVTEKVIA PSSSLPTSLTIHHPRES	917
Dsg2	947 🦻 🕈 TV ILG 🖻 SQ 🖻 Q SL IV TER V YA 🖻 A STLVD Q 🖻 YA N EG TVVV TER V I 10 🦻 H GG G 💁 N 🖻 L EG TQ H LQD V	101
Dsg3	944	956
Dsg4		930
-		
Dsgl	918 SHAVVVIEH VIQPISGMIGSLSMHPELANAHNVIVICENVSGAGVIGISGITGISGGIGSSGLVGISMGAGSGALS	992
Dsg2	1012 <b>B</b> Y M V R <b>EN</b> E S <b>F LA P</b> S <b>S</b> G V O <b>P</b> T LA M P - <u>N</u> IA V G O N V T V T E R V LA PA S T L O S S Y O I P T E N SM TA R N T T V S	107
Dsq3	957 ONVIVITERVICPISSVPGNLAGPTOLRGSHTMLCTED	993
Dsg4	931 PNVVVTEAVMA PVVD TOON TOVPA BLAD YNNV TVA PRVTA SPOVED MEN SSTTECOMOD	992
2091		176
DSGI	993 GA G ISGGGIGING ISS LGG TA SIGHMR SSSDHHFNQ TIG SA SP STAR SRITKYS TVQ YS K -	104
Dsg2	1078 <b>ga gv pg p</b> 1 <b>p</b> 0 <b>pg</b> L E E S <b>G H S</b> N S <b>T 1</b> T <b>T</b> S S T <b>T v</b> 0 <b>H S</b> Y S	111:
Dsg3	994	999
Dsc4	993 GN TIVG PETOVMOM	104
	2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	T 0 4

**Fig. 4. The putative binding site is conserved within human Dsg subfamily.** The four family members of human Dsgs were aligned using the program MUSCLE [14]. The putative binding site for the scaffolding domain of caveolin [10] is highlighted in the box with a dotted line, in which four aromatic amino acid residues are depicted to be highly conserved across the family members (asterisks).

Based on these findings we have proposed a working model that Dsg3 competes with inactive Src for binding to the scaffolding domain of caveolin-1, and thus causes Src to release from binding to caveolin-1, that leading to its auto-activation (Fig. 5B). This working model opens up a new avenue for future research.



**Fig. 5. A proposed working model of how Dsg3 activates Src.** (A) Amino acid sequence alignment of Dsg family members showing highly conserved putative region (dotted line) for binding to the scaffolding domain of caveolin-1 [7,10]. Asterisks indicate conserved aromatic amino acid residues across the family members. (B) Cartoon illustrating a possible mechanism by which Dsg3 regulates Src activity, *i.e.* expression and membrane distribution of Dsg3 induced by calcium renders its binding to Cav-1 within the caveolae and thus causes release of Src from its interaction with Cav-1, leading to auto-activation of Src that likely associates with E-cadherin (E-cad). Cav: caveolin-1, N: N-terminus of caveolin, C: C-terminus of caveolin, pSrc: phospho-Src.

#### 2. Experimental design, materials and methods

#### 2.1. Antibodies

The mouse monoclonal and rabbit polyclonal Abs used were: 5H10, mouse Ab against Dsg3 (Santa Cruz Biotechnology, Inc); HECD-1, mouse anti-E-cadherin (Abcam); Src (32G6) rabbit mAb and phospho-Src family (Tyr416) (Cell Signaling); rabbit caveolin-1 Ab (Cell Signaling); mouse mAb against caveolin-1 (Santa Cruz Biotechnology); rabbit anti beta actin-loading control ab8227 (Abcam); Secondary Abs were Alexa Fluor 488 conjugated goat anti-mouse IgG and Alexa Fluor 546 conjugated goat anti-rabbit IgG (Invitrogen).

## 2.2. Transient Dsg3 siRNA transfection

The siRNA sequence corresponding to nucleotides 620–640 of the respective coding region in human Dsg3 mRNA (Accession: NM\_001944.1) (AAATGCCACAGATGCAGATGCA) was used to knockdown Dsg3. The scrambled control siRNA was a randomized sequence of RNAi (AACGATGATA-CATGACACGAG). Both sequences were synthesized by Dharmacon Research (USA). The transfection procedures were described previously [13].

## 2.3. Co-immunoprecipitation (co-IP) and Western blotting

For analysis of protein in Triton X-100 -soluble and -insoluble fractions, cells were grown to freshly confluence in 100 mm Petri dishes prior to protein extraction in 500  $\mu$ l of Trition X-100 lysis buffer (10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 2 mM ethyleneglycol-bis-( $\beta$ -aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 5 mM ethylenediamine tetraacetic acid (EDTA), 1%Triton X-100, 1 mM phenylmethylsufonyl fluoride and protease inhibitor cocktail (Boehringer Mannheim)) for 10 min at 4 °C. After centrifugation the supernatant was denoted as the Triton X-100 soluble fraction and the undissolved pellet subsequently was extracted in 200  $\mu$ l RiPA buffer. After centrifugation the supernatant was collected and lysate was denoted as the Triton X-100 insoluble fraction. Protein con-

natant was collected and lysate was denoted as the Triton X-100 insoluble fraction. Protein concentration for each fraction was determined. 500  $\mu$ g of protein in each fraction was used for co-IP experiment. For total cell lysate preparation, confluent cell cultures were washed with ice-cold PBS and lysed in ice cold RIPA buffer (Upstate) containing a protease-inhibitor cocktail (Calbiochem), for 10 min at 4 °C. Then lysates were clarified by microcentrifugation. The amount of 500  $\mu$ g of total protein in each sample, as determined by *DC* protein assay (Bio-Rad), was used for IP. The rabbit caveolin antibody were coupled with Dynabeads (Invitrogen) for 3 h before addition into each lysate and incubated overnight at 4 °C with rotation. Immunoprecipitates were washed thoroughly prior to resuspension in 2 × Laemmli sample buffer and boiled for 3 min. Aliquots of the denatured proteins were separated by SDS-PAGE and processed for Western blotting.

## 2.4. Duolink in situ proximity ligation assay for protein:protein interactions

Duolink proximity ligation assay (PLA) kit, composed of anti-mouse PLA probe plus, anti-rabbit PLA probe minus and detection kit 563, was purchased from Olink Bioscience (Cambridge Bioscience, UK). PLA assay was conducted following the protocol described with the kit. Briefly, cells grown on coverslips were fixed and then blocked with 10% goat serum (Sigma) for 15 min followed by incubation with primary antibody for 1 h at 37 °C. After this, coverslips were subjected to sequential incubation with PLA (plus + minus) probes for 2 h, hybridization for 15 min, ligation with ligase for 15 min, amplification with polymerases for 90 min and finally detection for 1 h. All these incubations were carried out at 37 °C and the coverslips were washed with 1 × TBS-T under gentle agitation for 2–5 min between each incubation step. Finally, the samples were mounted and analyzed using a Leica DM5000 epi-fluorescence microscope. The negative control in this assay was A431-V cells labeled with mouse Dsg3 (5H10) and rabbit anti-Myc tag Ab. The experimental samples were HaCaT cells labeled with mouse Dsg3 (5H10) and rabbit Cav-1 Ab or rabbit anti-Src, respectively. Statistical analysis was performed with Student's *t*-test and p < 0.05 was considered statistically significant.

## 2.5. Immunofluorescence, confocal microscopy and colocalization analysis

For confocal analysis, cells were seeded at confluent or sub-confluent density on coverslips for one day. Then, cells were fixed with ice cold methanol and immune-fluorescently labeled using the mouse Dsg3 Ab (5H10)/ rabbit anti-caveolin-1 (in Fig. 1D) or the indicated antibody combinations (in Fig. 2). Coverslips were blocked with 10% goat serum for 20 min before the primary antibody incubation for 1 h at room temperature, followed by three washes for 5 min in PBS with 0.2% Tween 20. The secondary antibodies were Alexa 488 mouse IgG/ Alexa 546 rabbit IgG (Fig. 1D) or Alexa 488 rabbit IgG/ Alexa 546 mouse IgG (Fig. 2) and all were incubated for 1 h at room temperature. After two washes, coverslips were counterstained with DAPI for 10 min before a final wash and then mounted on slide. All antibodies were diluted at 1:100 in blocking buffer. Image stacks were acquired with Zeiss510 confocal microscope and analyzed with ImageJ for image processing and colocalization of double stained proteins. The presented images in Fig. 1D were Z projection with maximum intensity and in Fig. 2 were single confocal slice acquired at the highest protein expressing level in each region. The Colocalization Finder tool was used for colocalization analysis and the profiles of IMF staining at the cell borders were analyzed with a Segmented Lines tool with a line width of 20 pixels. All graphs were plotted in Excel.

## 2.6. Protein sequence alignment

The protein sequence alignment analysis was performed using the MUSCLE program [14]. The amino acids are colored according to the Clustal X Coloring Scheme [15].

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