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(54) Title: POLYPEPTIDES COMPRISING VINCULIN BINDING SITES FOR THE TREATMENT OF PROLIFERATION AND/OR ADHESION RELATED DISEASES

(57) Abstract: The present invention relates to polypeptides comprising at least one vinculin binding sites, to nucleic acid sequences encoding thereof and to their use for treating a proliferation and/or adhesion related disease.

POLYPEPTIDES COMPRISING VINCULIN BINDING SITES FOR THE TREATMENT OF PROLIFERATION AND/OR ADHESION RELATED DISEASES

FIELD OF INVENTION

The present invention relates to the treatment of proliferation and/or adhesion related diseases, such as cancer and metastasis. In particular, the present invention relates to polypeptides that comprise vinculin binding sites (VBSs), and to the use thereof for treating proliferation and/or adhesion related diseases.

BACKGROUND OF INVENTION

Cancer and tumor cells are characterized by dysregulation of the cellular cycle leading to abnormal proliferation. These cells also exhibit dysregulation in adherence properties which are characteristics associated with increased invasive and migratory properties determining the metastatic potential of cells.

Generally, anticancer drugs include protein kinases inhibitors, microtubules inhibitors, or anti-metabolite agents targeting cells that divide rapidly. Therefore, besides cancer cells, blood cells are also targeted. When blood cells are affected, patients are more likely to get infections, or bleed easily, and may feel unusually weak and very tired. Rapidly dividing cells in hair roots and cells that line the digestive tract may also be affected. Therefore, there is a need to develop novel strategies to fight cancer and to avoid this cytotoxic effect.

Another characteristic of cancer cells is a decrease in adhesion properties. The Applicant's strategy thus consists in targeting the anchoring to the cellular matrix. The purpose of this alternative treatment is thus inhibiting the proliferation, modulating the adhesion properties and the invasiveness of the cancer cells. In particular, the Applicant aimed at developing novel agents for inducing strong adherence of cells independently of the mechanosensing phenomenon, thereby inducing cellular adherence *de novo* (for

example of non-adherent cells, especially of metastasis) or thereby reinforcing already existent cellular adherence.

Shigella is a bacterium injecting proteins into host cells that reorganize the cytoskeleton, to trigger its internalization by epithelial cells. Among these proteins, IpaA is involved in bacterial internalization by promoting anchorage of the bacteria on host cells. Analysis demonstrated that the C-terminus of IpaA was involved in these processes due the presence of vinculin binding sites (VBSs) (Orchard R.C. & Alto N.M., **2012.** *Cell Microbiol.* **14**:10-8; Izard T. et *al,* **2006.** *J. Cell Biol.* **175**:465-75; Tran VanNhieu G. & Izard T., **2007.** *EMBOJ.* **26**:4588-96; Park H. *et al,* **2011.** *J. Biol. Chem.* **286**:23214-21). The binding of IpaA to vinculin induces changes in vinculin's conformation from an inactive to an active state.

The Applicant surprisingly demonstrates that a peptide derived from IpaA, and in particular a peptide comprising the 3 VBSs of IpaA, induces a novel conformational state of vinculin, defined as a "supra-activation state", thereby inducing cell anchoring to a support. This supra-activation of vinculin is only observed in presence of VBS3, but not when only VBS1 and VBS2 are comprised in the peptide. This result was particularly unexpected, because VBS3 was previously described in the art as functionally redundant with the other two IpaA-VBSs (Park H. *et al.* **2011.** *J. Biol. Chem.* **286**:23214-21).

Moreover, the results of the Applicant presented in the present application demonstrate an unexpected synergistic effect between the three VBSs of IpaA. Strikingly, cell anchorage induced by the peptide of the invention is independent of the mechanosensing phenomenon.

Therefore, a peptide comprising the 3 VBSs of IpaA may be used for treating cancer, and in particular for treating or preventing metastasis.

SUMMARY

One object of the present invention is a polypeptide comprising the three following vinculin binding sites (VBS):

VBS1: IYKAAKDVTTSLSKVLKNI (SEQ ID NO: 2) or a fragment or variant thereof;

VBS2: IYEKAKEVSSALSKVLSKI (SEQ ID NO: 3) or a fragment or variant thereof;

VBS3: IFEASKKVTNSLSNLISLI (SEQ ID NO: 4) or a fragment or variant thereof;

or any sequence having at least 60% identity with SEQ ID NO: 2; 3 and 4;

wherein said polypeptide is not SEQ ID NO: 1.

In one embodiment, said polypeptide is SEQ ID NO: 5 or a variant thereof.

Another object of the present invention is a nucleic acid comprising the three following domains:

VBS 1 (SEQ ID NO: 8);

VBS2 (SEQ ID NO: 9);

VBS3 (SEQ ID NO: 10);

or any nucleic acid sequence having at least 60% identity with SEQ ID NO: 8; 9 and 10;

wherein said nucleic acid is not SEQ ID NO: 7.

In one embodiment, said nucleic acid is SEQ ID NO: 6.

Another object of the present invention is a vector encoding the polypeptide as described above, or comprising the nucleic acid sequence as described above.

Another object of the present invention is a composition comprising the polypeptide as described above or the nucleic acid sequence as described above or the vector as described above.

Another object of the present invention is a pharmaceutical composition comprising the polypeptide as described above or the nucleic acid sequence as described above or the vector as described above and at least one pharmaceutically acceptable excipient.

Another object of the present invention is a medicament comprising the polypeptide as described above or the nucleic acid sequence as described above or the vector as described above.

In one embodiment, the composition as described above or the pharmaceutical composition as described above or the medicament as described above are for use in the treatment of a proliferation and/or adhesion related disease.

In another embodiment, the proliferation and/or adhesion related disease is a cancer.

In another embodiment, the proliferation and/or adhesion related disease is a tumor.

In another embodiment, the proliferation and/or adhesion related disease is metastasis.

In another embodiment, the composition, the pharmaceutical composition or the medicament as described above, is to be administered in combination with another anti-cancer agent.

DEFINITIONS

In the present invention, the following terms have the following meanings:

- "**Binding Site**" refers to a domain responsible for selectively binding to a polypeptide. Binding domains or binding regions comprise at least one binding site. Exemplary binding sites comprise VBS1 (SEQ ID NO: 2), VBS2 (SEQ ID NO: 3) and VBS3 (SEQ ID NO: 4).
- "**Identity**" when used in a relationship between the sequences of two or more nucleic acid sequences or amino acid sequences, refers to the degree of sequence relatedness between the sequences, as determined by the number of matches between strings of two or more base pairs of nucleic acid or amino acid residues. "Identity" measures

the percent of identical matches between the smaller of two or more sequences with gap alignments (if any) addressed by a particular mathematical model or computer program (i.e., "algorithms"). Identity of related nucleic acid sequences or amino acid sequences can be readily calculated by known methods. Such methods include, but are not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M. Stockton Press, New York, 1991; and Carillo et al, SIAM J. Applied Math. 48, 1073 (1988). Preferred methods for determining identity are designed to give the largest match between the sequences tested. Methods of determining identity are described in publicly available computer programs. Preferred computer program methods for determining identity between two sequences include the GCG program package, including GAP (Devereux et al, Nucl. Acid. Res. \2, 387 (1984); Genetics Computer Group, University of Wisconsin, Madison, Wis.), BLASTP, BLASTN, and FASTA (Altschul et al, J. Mol. Biol. 215, 403-410 (1990)). The BLASTX program is publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul et al. NCB/NLM/NIH Bethesda, Md. 20894; Altschul et al., supra). The well-known Smith Waterman algorithm may also be used to determine identity.

- **"Nucleic acid sequence"** encompasses nucleic acids having the sequences set forth below as well as variants thereof including for example fragments, deletions, insertions and substitutions that comprise the VBS domains as described in the present invention.

- **"Polypeptide"** is used in its conventional meaning, i.e., as a sequence of amino acids. Unless otherwise specified, a polypeptide is not limited to a specific length of the product. Peptides, oligopeptides, and proteins are included within the definition of polypeptide, and such terms may be used interchangeably herein unless specifically indicated otherwise. This term may also include post-expression modifications of the

polypeptide, for example, glycosylations, acetylations, phosphorylations and the like, as well as other modifications known in the art, both naturally occurring and non-naturally occurring. A polypeptide may be an entire protein, or a subsequence thereof. Particular polypeptides of interest in the context of this invention are amino acid subsequences comprising VBSs. An "isolated polypeptide" is one that has been identified and separated and/or recovered from a component of its natural environment. In preferred embodiments, the isolated polypeptide will be purified (1) to greater than 95% by weight of polypeptide as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver staining. Isolated polypeptide includes the polypeptide in situ within recombinant cells since at least one component of the polypeptide's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

- **"Pharmaceutically acceptable excipient"** refers to an excipient that does not produce an adverse, allergic or other untoward reaction when administered to an animal, preferably a human. It includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. For human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by regulatory offices, such as, for example, FDA Office or EMA.

- **"Therapeutically effective amount"** means level or amount of agent that is aimed at, without causing significant negative or adverse side effects to the target, (1) delaying or preventing the onset of a disease, disorder, or condition related to proliferation and/or adhesion; (2) slowing down or stopping the progression, aggravation, or deterioration of one or more symptoms of the disease, disorder, or condition related to proliferation and/or adhesion; (3) bringing about ameliorations of the symptoms of the disease, disorder, or condition related to proliferation and/or adhesion; (4) reducing the severity or incidence of the disease, disorder, or condition related to proliferation and/or adhesion; or (5) curing the disease, disorder, or

condition related to proliferation and/or adhesion. A therapeutically effective amount may be administered prior to the onset of the disease, disorder, or condition related to proliferation and/or adhesion, for a prophylactic or preventive action. Alternatively or additionally, the therapeutically effective amount may be administered after initiation of the disease, disorder, or condition related to proliferation and/or adhesion, for a therapeutic action.

- "**Treating**" or "**treatment**" refer to both therapeutic treatment and prophylactic or preventative measures; wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented. A subject or mammal is successfully "treated" for the targeted pathologic condition or disorder if, after receiving a therapeutic amount of the polypeptide according to the present invention, the subject shows observable and/or measurable reduction in or absence of one or more of the following: reduction in the number of pathogenic cells; reduction in the percent of total cells that are pathogenic; and/or relief to some extent, of one or more of the symptoms associated with the specific disease or condition; reduced morbidity and mortality, and improvement in quality of life issues. The above parameters for assessing successful treatment and improvement in the disease are readily measurable by routine procedures familiar to a physician.

- "**Subject**" refers to an animal, including a human. In the sense of the present invention, a subject may be a patient, i.e. a person receiving medical attention, undergoing or having underwent a medical treatment, or monitored for the development of a disease. In one embodiment, the subject is a male. In another embodiment, the subject is a female.

- "**About**" preceding a figure means plus or less 10% of the value of said figure.

DETAILED DESCRIPTION

One object of the invention is a polypeptide comprising at least one of the following VBSs:

VBS1: IYKAAKDVTTSLSKVLKNI (SEQ ID NO: 2) or IYKAAKDVTTSLSKVLKNINKD (SEQ ID NO: 66) or a fragment or variant thereof;

VBS2: IYEKAKEVSSALSKVLSKI (SEQ ID NO: 3) or IYEKAKEVSSALSKVLSKIDD (SEQ ID NO: 67) or a fragment or variant thereof;

VBS3: IFEASKKVTNSLSNLISLI (SEQ ID NO: 4) or TRETIFEASKKVTNSLSNLISLIGT (SEQ ID NO: 68) or a fragment or variant thereof;

or any fragment or variant thereof having at least 60; 65; 70; 75; 80; 85; 90; 95; 96; 97; 98; 99% identity with SEQ ID NO: 2; 3; 4; 66; 67 and 68.

In one embodiment, the polypeptide comprises at least one of the following VBSs:

VBS1: IYKAAKDVTTSLSKVLKNI (SEQ ID NO: 2) or a fragment or variant thereof;

VBS2: IYEKAKEVSSALSKVLSKI (SEQ ID NO: 3) or a fragment or variant thereof;

VBS3: IFEASKKVTNSLSNLISLI (SEQ ID NO: 4) or a fragment or variant thereof;

or any fragment or variant thereof having at least 60; 65; 70; 75; 80; 85; 90; 95; 96; 97; 98; 99% identity with SEQ ID NO: 2; 3 or 4.

In one embodiment, the polypeptide comprises at least one of the following VBSs:

VBS1: IYKAAKDVTTSLSKVLKNINKD (SEQ ID NO: 66) or a fragment or variant thereof;

VBS2: IYEKAKEVSSALSKVLSKIDD (SEQ ID NO: 67) or a fragment or variant thereof;

VBS3: TRETIFEASKKVTNSLSNLISLIGT (SEQ ID NO: 68) or a fragment or variant thereof;

or any fragment or variant thereof having at least 60; 65; 70; 75; 80; 85; 90; 95; 96; 97; 98; 99% identity with SEQ ID NO: 66; 67 or 68.

In one embodiment, the polypeptide of the invention comprises two VBS (or a fragment or variant thereof) selected from SEQ ID NO: 2-4. In one embodiment, the polypeptide of the invention comprises two VBS (or a fragment or variant thereof) selected from SEQ ID NO: 66-68. In another embodiment, the polypeptide of the invention comprises the three VBSs (or a fragment or variant thereof) as described hereinabove.

In one embodiment, the polypeptide of the invention comprises, from Nterm to Cterm:

- optionally an amino acid sequence comprising from 1 to 20 amino acids, preferably from 1 to 10 amino acids;
- a sequence consisting of SEQ ID NO: 4 or SEQ ID NO: 68 or a variant thereof;
- optionally an amino acid sequence comprising from 1 to 100 amino acids, preferably from 1 to 60 amino acids;
- a sequence consisting of SEQ ID NO: 3 or SEQ ID NO: 67 or a variant thereof;
- optionally an amino acid sequence comprising from 1 to 50 amino acids, preferably from 1 to 30 amino acids;
- a sequence consisting of SEQ ID NO: 2 or SEQ ID NO: 66 or a variant thereof, and
- optionally an amino acid sequence comprising from 1 to 20 amino acids, preferably from 1 to 10 amino acids, more preferably from 1 to 5 amino acids.

In one embodiment, the polypeptide of the invention comprises, from Nterm to Cterm:

- 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acids;
- a sequence consisting of SEQ ID NO: 4 or SEQ ID NO: 68 or a variant thereof;
- 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55 amino acids;
- a sequence consisting of SEQ ID NO: 3 or SEQ ID NO: 67 or a variant thereof;
- 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or 27 amino acids;
- a sequence consisting of SEQ ID NO: 2 or SEQ ID NO: 66 or a variant thereof, and
- 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acids.

In one embodiment, the polypeptide of the invention comprises, from Nterm to Cterm:

- optionally, an amino acid sequence comprising or consisting of GDTYLTRET (SEQ ID NO: 40) or a variant thereof;
- a sequence consisting of SEQ ID NO: 4 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of GTKSGTQERELQEKSKDITKSTTEHRINNKLKVTDANIRNYVTETNADTIDKNHA (SEQ ID NO: 41) or a variant thereof;
- a sequence consisting of SEQ ID NO: 3 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of DDTSAELLTDDISDLKNNNDITAENNN (SEQ ID NO: 42) or a variant thereof;
- a sequence consisting of SEQ ID NO: 2 or a variant thereof; and
- optionally, an amino acid sequence comprising or consisting of NKD or a variant thereof.

In one embodiment, the polypeptide of the invention comprises, from Nterm to Cterm:

- optionally, an amino acid sequence comprising or consisting of GDTYL (SEQ ID NO: 72) or a variant thereof;
- a sequence consisting of SEQ ID NO: 68 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of KSGTQERELQEKSKDITKSTTEHRINNKLKVTDANIRNYVTETNADTIDKNHA (SEQ ID NO: 73) or a variant thereof;
- a sequence consisting of SEQ ID NO: 67 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of TSAELLTDDISDLKNNNDITAENNN (SEQ ID NO: 74) or a variant thereof, and
- a sequence consisting of SEQ ID NO: 66 or a variant thereof.

In one embodiment, the polypeptide of the invention comprises, from Nterm to Cterm:

- an amino acid sequence comprising or consisting of SEQ ID NO: 40 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 4 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 41 or a fragment or a variant thereof;

- a sequence consisting of SEQ ID NO: 3 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 42 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 2 or a variant thereof; and
- an amino acid sequence comprising or consisting of NKD or a variant thereof.

In one embodiment, the polypeptide of the invention comprises, from Nterm to Cterm:

- an amino acid sequence comprising or consisting of SEQ ID NO: 72 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 68 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 73 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 67 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 74 or a fragment or a variant thereof; and
- a sequence consisting of SEQ ID NO: 66 or a variant thereof.

In one embodiment, the polypeptide of the invention comprises, from Cterm to Nterm:

- optionally an amino acid sequence comprising from 1 to 20 amino acids, preferably from 1 to 10 amino acids;
- a sequence consisting of SEQ ID NO: 4 or SEQ ID NO: 68 or a variant thereof;
- optionally an amino acid sequence comprising from 1 to 100 amino acids, preferably from 1 to 60 amino acids;
- a sequence consisting of SEQ ID NO: 3 or SEQ ID NO: 67 or a variant thereof;
- optionally an amino acid sequence comprising from 1 to 50 amino acids, preferably from 1 to 30 amino acids;
- a sequence consisting of SEQ ID NO: 2 or SEQ ID NO: 66 or a variant thereof; and
- optionally an amino acid sequence comprising from 1 to 20 amino acids, preferably from 1 to 10 amino acids, more preferably from 1 to 5 amino acids.

In one embodiment, the polypeptide of the invention comprises, from Cterm to Nterm:

- 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acids;
- a sequence consisting of SEQ ID NO: 4 or SEQ ID NO: 68 or a variant thereof;

- 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, or 55 amino acids;
- a sequence consisting of SEQ ID NO: 3 or SEQ ID NO: 67 or a variant thereof;
- 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 or 27 amino acids;
- a sequence consisting of SEQ ID NO: 2 or SEQ ID NO: 66 or a variant thereof, and
- 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 amino acids.

In one embodiment, the polypeptide of the invention comprises, from Cterm to Nterm:

- optionally, an amino acid sequence comprising or consisting of SEQ ID NO: 40 or a variant thereof;
- a sequence consisting of SEQ ID NO: 4 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of SEQ ID NO: 41 or a variant thereof;
- a sequence consisting of SEQ ID NO: 3 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of SEQ ID NO: 42 or a variant thereof;
- a sequence consisting of SEQ ID NO: 2 or a variant thereof; and
- optionally, an amino acid sequence comprising or consisting of NKD or a variant thereof.

In one embodiment, the polypeptide of the invention comprises, from Cterm to Nterm:

- optionally, an amino acid sequence comprising or consisting of SEQ ID NO: 72 or a variant thereof;
- a sequence consisting of SEQ ID NO: 68 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of SEQ ID NO: 73 or a variant thereof;
- a sequence consisting of SEQ ID NO: 67 or a variant thereof;
- optionally, an amino acid sequence comprising or consisting of SEQ ID NO: 74 or a variant thereof; and
- a sequence consisting of SEQ ID NO: 66 or a variant thereof.

In one embodiment, the polypeptide of the invention comprises, from Cterm to Nterm:

- an amino acid sequence comprising or consisting of SEQ ID NO: 40 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 4 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 41 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 3 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 42 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 2 or a variant thereof; and
- an amino acid sequence comprising or consisting of NKD or a variant thereof.

In one embodiment, the polypeptide of the invention comprises, from Cterm to Nterm:

- an amino acid sequence comprising or consisting of SEQ ID NO: 72 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 68 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 73 or a fragment or a variant thereof;
- a sequence consisting of SEQ ID NO: 67 or a variant thereof;
- an amino acid sequence comprising or consisting of SEQ ID NO: 74 or a fragment or a variant thereof; and
- a sequence consisting of SEQ ID NO: 66 or a variant thereof.

In one embodiment, a variant of SEQ ID NO: 2, 3, 4, 40, 41, 42, 66, 67 or 68 comprises conservative amino acid substitutions as compared to the sequence of SEQ ID NO: 2, 3, 4, 40, 41, 42, 66, 67 or 68, respectively.

In another embodiment, a variant of SEQ ID NO: 2, 3, 4, 40, 41, 42, 66, 67 or 68 is a polypeptide having a sequence identity of at least 70%, preferably of at least 75, 80, 85, 90, 95, 96, 97, 98, 99% or more with SEQ ID NO: 2, 3, 4, 40, 41, 42, 66, 67 or 68, respectively.

In another embodiment, a variant of SEQ ID NO: 2, 3, 4, 40, 41, 42, 66, 67 or 68 is a polypeptide wherein 1, 2, 3, 4, or 5 amino acids from the sequence of SEQ ID NO: 2, 3, 4, 40, 41, 42, 66, 67 or 68 (respectively) is/are absent, or substituted by any amino acid, or wherein 1, 2, 3, 4 or 5 amino acids (either contiguous or not) is/are added.

In one embodiment, a variant of SEQ ID NO: 2 or SEQ ID NO: 66 comprises amino acid residues K6 or K14 of SEQ ID NO: 2 or 66. In another embodiment, a variant of SEQ ID NO: 2 or SEQ ID NO: 66 comprises amino acid residues K6 and K14 of SEQ ID NO: 2 or 66.

In one embodiment, a variant of SEQ ID NO: 2 or SEQ ID NO: 66 is SEQ ID NO: 47 (LFQAATQTTQALSSLIDTVG). SEQ ID NO: 47 corresponds to VBS1 of the translocated actin recruiting phosphoprotein from *Chlamydia trachomatis serovar L2.*
In one embodiment, a variant of SEQ ID NO: 2 or SEQ ID NO: 66 is SEQ ID NO: 49 (LLEAARNTTTMLSKTLSKV). SEQ ID NO: 49 corresponds to VBS1 of the translocated actin recruiting phosphoprotein from *Chlamydophila caviae, strain GPIC.*

In one embodiment, a variant of SEQ ID NO: 2 or SEQ ID NO: 66 is SEQ ID NO: 52 (LADAARNVTTQLSKTLSKA). SEQ ID NO: 52 corresponds to VBS1 of the translocated actin recruiting phosphoprotein from *Chlamydophila abortus.*

In one embodiment, a variant of SEQ ID NO: 2 or SEQ ID NO: 66 is SEQ ID NO: 55 (LFDAAKQTTAQLSKMIYRA). SEQ ID NO: 55 corresponds to VBS1 of the translocated actin recruiting phosphoprotein from *Chlamydophila felis.*

In one embodiment, a variant of SEQ ID NO: 2 or SEQ ID NO: 66 is SEQ ID NO: 58 (LFAAARATTQSLSSLIGTV). SEQ ID NO: 58 corresponds to VBSI of the translocated actin recruiting phosphoprotein from *Chlamydia muridarum.*

In one embodiment, a variant of SEQ ID NO: 3 or SEQ ID NO: 67 comprises amino acid residues K6 or K14 of SEQ ID NO: 3 or 67. In another embodiment, a variant of SEQ ID NO: 3 or SEQ ID NO: 67 comprises amino acid residues K6 and K14 of SEQ ID NO: 3 or 67.

In one embodiment, a variant of SEQ ID NO: 3 or SEQ ID NO: 67 is SEQ ID NO: 48 (LFQAAAVTQALGNVAGKVNLAIQG). SEQ ID NO: 48 corresponds to VBS2 of the translocated actin recruiting phosphoprotein from *Chlamydia trachomatis serovar L2.*

In one embodiment, a variant of SEQ ID NO: 3 or SEQ ID NO: 67 is SEQ ID NO: 50 (IPGAAANVTATLSSVANKI). SEQ ID NO: 50 corresponds to VBS2 of the translocated actin recruiting phosphoprotein from *Chlamydophila caviae, strain GPIC.*

In one embodiment, a variant of SEQ ID NO: 3 or SEQ ID NO: 67 is SEQ ID NO: 53 (IPEAAGNVIQALSNVAKKI). SEQ ID NO: 53 corresponds to VBS2 of the translocated actin recruiting phosphoprotein from *Chlamydophila abortus.*

In one embodiment, a variant of SEQ ID NO: 3 or SEQ ID NO: 67 is SEQ ID NO: 56 (IPQAAANVTQTLSNVTQKL). SEQ ID NO: 56 corresponds to VBS2 of the translocated actin recruiting phosphoprotein from *Chlamydophila felis.*

In one embodiment, a variant of SEQ ID NO: 3 or SEQ ID NO: 67 is SEQ ID NO: 59 (LYDAAKNVTQALTSVTNKV). SEQ ID NO: 59 corresponds to VBS2 of the translocated actin recruiting phosphoprotein from *Chlamydia muridarum.*

In one embodiment, a variant of SEQ ID NO: 4 comprises amino acid residue K7 of SEQ ID NO: 4. In one embodiment, a variant of SEQ ID NO: 68 comprises amino acid residue K11 of SEQ ID NO: 68.

In one embodiment, a variant of SEQ ID NO: 4 or SEQ ID NO: 68 is SEQ ID NO: 51 (LHGAAKGVADSLSNLLQAA). SEQ ID NO: 51 corresponds to VBS3 of the translocated actin recruiting phosphoprotein from *Chlamydophila caviae, strain GPIC.*

In one embodiment, a variant of SEQ ID NO: 4 or SEQ ID NO: 68 is SEQ ID NO: 54 (LHGAARDVASSLSNLLEAA). SEQ ID NO: 54 corresponds to VBS3 of the translocated actin recruiting phosphoprotein from *Chlamydophila abortus.*

In one embodiment, a variant of SEQ ID NO: 4 or SEQ ID NO: 68 is SEQ ID NO: 57 (LYAAAGNVADSLSNLLQAA). SEQ ID NO: 57 corresponds to VBS3 of the translocated actin recruiting phosphoprotein from *Chlamydophila felis.*

In one embodiment, a variant of SEQ ID NO: 4 or SEQ ID NO: 68 is SEQ ID NO: 60 (YTKKELIECARRVSEKVSHVLAALQA), corresponding to talin H46.

In one embodiment, a variant of SEQ ID NO: 40 or SEQ ID NO: 72 comprises amino acid residue D2 of SEQ ID NO: 40 or 72.

In one embodiment, a variant of SEQ ID NO: 40 is SEQ ID NO: 61 (GDPYLTRET). In one embodiment, a variant of SEQ ID NO: 72 is SEQ ID NO: 75 (GDPYL).

In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residues K3, K16, K20, K30, K32, E44, D48, D51 or K61 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residues Kl, K14, K18, K28, K30, E42, D46, D49 or K59 ofSEQ ID NO: 73.

In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residues K3, K16, K20, K30, K32, E44, D48, D51 and K61 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residues Kl, K14, K18, K28, K30, E42, D46, D49 and K59 of SEQ ID NO: 73.

In one embodiment, a variant of SEQ ID NO: 41 comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9) amino acid residues selected from the group consisting of K3, K16, K20, K30, K32, E44, D48, D51 and K61 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 73 comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9) amino acid residues selected from the group consisting of Kl, K14, K18, K28, K30, E42, D46, D49 and K59 of SEQ ID NO: 73.

In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue K3 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue K16 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue K20 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue K30 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue K32 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue E44 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises

amino acid residue D48 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue D51 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 41 comprises amino acid residue K61 of SEQ ID NO: 41. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue K1 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue K14 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue K18 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue K28 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue K30 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue E42 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue D46 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue D49 of SEQ ID NO: 73. In one embodiment, a variant of SEQ ID NO: 73 comprises amino acid residue K59 of SEQ ID NO: 73.

In one embodiment, a variant of SEQ ID NO: 41 is SEQ ID NO: 62 (GTKSGTQERELQEKSKDITKSTTEHRINNKLKITDANTINYVTETNADTIDKNHA). In one embodiment, a variant of SEQ ID NO: 73 is SEQ ID NO: 76 (KSGTQERELQEKSKDITKSTTEHRINNKLKITDANTINYVTETNADTIDKNHA). In one embodiment, a variant of SEQ ID NO: 41 is SEQ ID NO: 63 (GTKSGTQERELQEKSKDITKSTTEHRINNKLKVTDANTINYVTETNADTIDKNHA). In one embodiment, a variant of SEQ ID NO: 73 is SEQ ID NO: 77 (KSGTQERELQEKSKDITKSTTEHRINNKLKVTDANTINYVTETNADTIDKNHA). In one embodiment, a variant of SEQ ID NO: 41 is SEQ ID NO: 64 (GTKSGTQERELQEKSKDITKSTTEHRINNKLKITDANTRNYVTETNADTIDKNHA). In one embodiment, a variant of SEQ ID NO: 73 is SEQ ID NO: 78 (KSGTQERELQEKSKDITKSTTEHRINNKLKITDANTRNYVTETNADTIDKNHA).

In one embodiment, a variant of SEQ ID NO: 42 comprises amino acid residues E6, D10, D11, or K16 of SEQ ID NO: 42. In one embodiment, a variant of SEQ ID NO: 74 comprises amino acid residues E4, D8, D9, or K14 of SEQ ID NO: 74.

In one embodiment, a variant of SEQ ID NO: 42 comprises amino acid residues E6, D10, D11, and K16 of SEQ ID NO: 42. In one embodiment, a variant of SEQ ID NO: 74 comprises amino acid residues E4, D8, D9, or K14 of SEQ ID NO: 74.

In one embodiment, a variant of SEQ ID NO: 42 comprises at least one (e.g., 1, 2, 3, 4) amino acid residues selected from the group consisting of E6, D10, D11, and K16 of SEQ ID NO: 42. In one embodiment, a variant of SEQ ID NO: 74 comprises at least one (e.g., 1, 2, 3, 4) amino acid residues selected from the group consisting of E4, D8, D9, and K14 of SEQ ID NO: 74.

In one embodiment, a variant of SEQ ID NO: 42 comprises amino acid residue E6 of SEQ ID NO: 42. In one embodiment, a variant of SEQ ID NO: 42 comprises amino acid residue D10 of SEQ ID NO: 42. In one embodiment, a variant of SEQ ID NO: 42 comprises amino acid residue D11 of SEQ ID NO: 42. In one embodiment, a variant of SEQ ID NO: 42 comprises amino acid residue K16 of SEQ ID NO: 42. In one embodiment, a variant of SEQ ID NO: 74 comprises amino acid residue E4 of SEQ ID NO: 74. In one embodiment, a variant of SEQ ID NO: 74 comprises amino acid residue D8 of SEQ ID NO: 74. In one embodiment, a variant of SEQ ID NO: 74 comprises amino acid residue D9 of SEQ ID NO: 74. In one embodiment, a variant of SEQ ID NO: 74 comprises amino acid residue K14 of SEQ ID NO: 74.

In one embodiment, a variant of SEQ ID NO: 42 is SEQ ID NO: 65 (DDTSAELLTEDISNLKNNNDITAENNN). In one embodiment, a variant of SEQ ID NO: 74 is SEQ ID NO: 79 (TSAELLTEDISNLKNNNDITAENNN).

In one embodiment, the polypeptide of the invention does not comprise or consist of the IpaA protein full-length (SEQ ID NO: 1, EMBL accession AL391753.1; NCBI Reference Sequence: WP_005063225.1), which is known to be cytotoxic upon cell transfection.

SEQ ID NO: 1

MHNVNNTQAPTFLYKATSPSSTEYSELKSKISDIHSSQTSLKTPASVSEKENFAT
SFNQKCLDFLFSSSGKEDVLRSIYSNSMNAYAKSEILEFSNVLYSLVHQNGLNFE
NEKGLQKIVAQYSELIIKDKLSQDSAFGPWSAKNKKLHQLRQNIEHRLALLAQQ

HTSGEALSLGQKLLNTEVSSFIKNNILAELKLSNETVSSLKLDDLVDAQAKLAF
DSLRNQRKNTIDSKGFGIGKLSRDLNTVAVFPELLRKVLNDILEDIKDSHPIQDG
LPTPPEDMPDGGPTPGANEKTSQPVIHYHINNDNRTYDNRVFDNRVYDNSYHE
NPENDAQSPTSQTNDLLSRNGNSLLNPQRALVQKVTSVLPHSISDTVQTFANNS
ALEKVFNHTPDNSDGIGSDLLTTSSQERSANNSLSRGHRPLNIQNSSTTPPLHPE
GVTSSNDNSSDTTKSSASLSHRVASQINKFNSNTDSKVLQTDFLSRNGDTYLTR
ETIFEASKKVTNSLSNLISLIGTKSGTQERELQEKSKDITKSTTEHRINNKLKVTD
ANIRNYVTETNADTIDKNHAIYEKAKEVSSALSKVLSKIDDTSAELLTDDISDLK
NNNDITAENNNIYKAAKDVTTSLSKVLKNINKD.

In another embodiment, the polypeptide of the invention does not comprise or consist of SEQ ID NO: 43 *{Shigella sonnei SipA* protein, NCBI reference Sequence WP_052992066.1).

SEQ ID NO: 43

NIEHRLALLAQQHTSGEALSLGQKLLNTEVSSFIKNNILAELKLSNETVSSLKLD
DLVDAQAKLAFDSLRNQRKNTIDSKGFGIGKLSRDLNTVAVFPELLRKVLNDIL
EDIKDSHPIQDGLPTPPEDMPDGGPTPGANEKTSQPVIHYHINNDNRTYDNRVF
DNRVYDNSYHENPENDAQSPTSQTNDLLSRNGNSLLNPQRALVQKVTSVLPHSI
SDTVQTFANNSALEKVFNHTPDNSDGIGSDLLTTSSQERSANNSLSRGHRPLNIQ
NSSTTPPLHPEGVTSSNDNSSDTTKSSASLSHRVASQINKFNSNTDSKVLQTDFF
SRNGDTYLTRETIFEASKKVTNSLSNLISLIGTKSGTQERELQEKSKDITKSTTEH
RINNKLKVTDANTINYVTETNADTIDKNHAIYEKAKEVSSALSKVLSKIDDTSA
ELLTDDISDLKNNNDITAENNNIYKAAKDVTTSLSKVLKNINKD.

In another embodiment, the polypeptide of the invention does not comprise or consist of SEQ ID NO: 44 *{Shigella sonnei SipA* protein, NCBI reference Sequence WP_05298 1248.1).

SEQ ID NO: 44

VAQYSELIIKDKLSQDSAFGPWSAKNKKLHQLRQNIEHRLALLAQQHTSGEALS
LGQKLLNTEVS SFIKNNILAELKLSNETVSSLKLDDLVDAQAKLAFD SLRNQRK

NTIDSKGFGIGKLSRDLNTVAVFPELLRKVLNDILEDIKDSHPIQDGLPTPPEDMP
DGGPTPGANEKTSQPVIHYHINNDNRTYDNRVFDNRVYDNSYHENPENDAQSP
TSQTNDLLSRNGNSLLNPQRALVQKVTSVLPHSISDTVQTFANNSALEKVFNHT
PDNSDGIGSDLLTTS SQERSANNSLSRGHRPLNIQNS STTPPLHPEGVTS SNDNS S
DTTKSSASLSHRVASQINKFNSNTDSKVLQTDFFSRNGDTYLTRETIFEASKKVT
NSLSNLISLIGTKSGTQERELQEKSKDITKSTTEHRINNKLKVTDANTINYVTETN
ADTIDKNHAIYEKAKEVSSALSKVLSKIDDTSAELLTDDISDLKNNNDITAENNN
IYKAAKD VTTSLSKVLK NINKD.

In another embodiment, the polypeptide of the invention does not comprise or consist of SEQ ID NO: 45 *{Shigella sonnei* 5JG's YopE protein, referenced under SEED reference fig|2 **1**6599. 1.peg. **1**346).

SEQ ID NO: 45

MSEKESFATSFNQKCLDFLFSSSGKEDVLRSIYSNSMNAYAKSEILEFSNVLYSL
VHQNGLNFENEKGLQKIVAQYSELIIKDKLSQDSAFGPWSAKNKKLHQLRQNIE
HRLALLAQQHTSGEALSLGQKLLNTEVSSFIKNNILAELKLSNETVSSLKLDDLV
DAQAKLAFDSLRNQRKNTIDSKGFGIGKLSRDLNTVAVFPELLRKVLNDILEDI
KDSHPIQDGLPTPPEDMPDGGPTPGANEKTSQPVIHYHINNDNRTYDNRVFDNR
VYDNSYHENPENDAQSPTSQTNDLLSRNGNSLLNPQRALVQKVTSVLPHSISDT
VQTFANNSALEKVFNHTPDNSDGIGSDLLTTSSQERSANNSLSRGHRPLNIQNSS
TTPPLHPEGVTSSNDNSSDTTKSSASLSHRVASQINKFNSNTDSKVLQTDFFSRN
GDTYLTRETIFEASKKVTNSLSNLISLIGTKSGTQERELQEKSKDITKSTTEHRIN
NKLKVTDANTINYVTETNADTIDKNHAIYEKAKEVSSALSKVLSKIDDTSAELL
TDDI SDLKNNNDIT AENNNI YKAAKD VTT SL SKVLKNINKD .

In another embodiment, the polypeptide of the invention is a fragment of SEQ ID NO: 1 and comprises or consists of a sequence starting from amino acids 2; 3; 4; 5; 6; 7; 8; 9; 10; 11; 12; 13; 14; 15; 17; 18; 19; 20; 21; 22; 23; 24; 25; 26; 27; 28; 29; 30; 31; 32; 33; 34; 35; 36; 37; 38; 39; 40; 41; 42; 43; 44; 45; 46; 47; 48; 49; 50; 51; 52; 53; 54; 55; 56; 57; 58; 59; 60; 61; 62; 63; 64; 65; 66; 67; 68; 69; 70; 71; 72; 73; 74; 75; 76; 77; 78; 79; 80; 81; 82; 83; 84; 85; 86; 87; 88; 89; 90; 91; 92; 93; 94; 95; 96; 97; 98; 99; 100; 101;

102; 103; 104; 105; 106; 107; 108; 109; 110; 111; 112; 113; 114; 115; 116; 117; 118; 119; 120; 121; 122; 123; 124; 125; 126; 127; 128; 129; 130; 131; 132; 133; 134; 135; 136; 137; 138; 139; 140; 141; 142; 143; 144; 145; 146; 147; 148; 149; 150; 151; 152; 153; 154; 155; 156; 157; 158; 159; 160; 161; 162; 163; 164; 165; 166; 167; 168; 169; 170; 171, 172; 173; 174; 175; 176; 177; 178; 179; 180; 181; 182; 183; 184; 185; 186; 187; 188; 189; 190; 191; 192; 193; 194; 195; 196; 197; 198; 199; 200; 201; 202; 203; 204; 205; 206; 207; 208; 209; 210; 211; 212; 213; 214; 215; 216; 217; 218; 219; 220; 221; 222; 223; 224; 225; 226; 227; 228; 229; 230; 231; 232; 233; 234; 235; 236; 237; 238; 239; 240; 241; 242; 243; 244; 245; 246; 247; 248; 249; 250; 251; 252; 253; 254; 255; 256; 257; 258; 259; 260; 261; 262; 263; 264; 265; 266; 267; 268; 269; 270; 271; 272; 273; 274; 275; 276; 277; 278; 279; 280; 281; 282; 283; 284; 285; 286; 287; 288; 289; 290; 291; 292; 293; 294; 295; 296; 297; 298; 299; 300; 301; 302; 303; 304; 305; 306; 307; 308; 309; 310; 311; 312; 313; 314; 315; 316; 317; 318; 319; 320, 321; 322; 323; 324; 325; 326; 327; 328; 329; 330; 331; 332; 333; 334; 335; 336; 337; 338; 339; 340; 341; 342; 343; 344; 345; 346; 347; 348; 349; 350; 351; 352; 353; 354; 355; 356; 357; 358; 359; 360; 361; 362; 363; 364; 365; 366; 367; 368; 369; 370; 371; 372; 373; 374; 375; 376; 377; 378; 379; 380; 381; 382; 383; 384; 385; 386; 387; 388; 389; 390; 391; 392; 393; 394; 395; 396; 397; 398; 399; 400; 401; 402; 403; 404; 405; 406; 407; 408; 409; 410; 411; 412; 413; 414; 415; 416; 417; 418; 419; 420; 421; 422; 423; 424; 425; 426; 427; 428; 429; 430; 431; 432; 433; 434; 435; 436; 437; 438; 439; 440; 441; 442; 443; 444; 445; 446; 447; 448; 449; 450; 451; 452; 453; 454; 455; 456; 457; 458; 459; 460; 461; 462; 463; 464; 465; 466; 467; 468; 469; 470; 471; 472; 473; 474; 475; 476; 477; 478; 479; 480; 481; 482; 483; 484; 485; 486; 487; 488; 489; 490; 491 or 492 and ending at amino acid 631, 632 or 633 of SEQ ID NO: 1.

In another embodiment, the polypeptide of the invention is a fragment of SEQ ID NO: 1 and comprises or consists of a sequence starting from amino acids 477, 478, 479, 480, 481, 482; 483; 484; 485; 486; 487; 488; 489; 490; 491 or 492 and ending at amino acid 631, 632 or 633 of SEQ ID NO: 1.

In another embodiment, the polypeptide of the invention is a fragment of SEQ ID NO: 1 and comprises or consists of a sequence starting from amino acids 492 and ending at amino acid 631 of SEQ ID NO: 1.

In one embodiment, the polypeptide of the invention comprises or consists of 19 to 500 amino acids, preferably from 70 to about 400 amino acids, more preferably from about 90 to about 300 amino acids, even more preferably from about 110 to about 200 amino acids and still even more preferably from about 140 to about 160 amino acids.

As used herein, the term "polypeptide" means molecules formed from the linking, in a defined order, of amino acids, and of at least 19, 38, 57, 60, 70, 80, 90, 100, 125 or 150 amino acids.

In one embodiment, the polypeptide according to the invention has a length of at least 19, 38, 57, 60, 70, 80, 90, 100, 125 or 150 amino acids. In one embodiment, the polypeptide of the invention has a length of at least 151 amino acids.

In one embodiment, the polypeptide of the invention is SEQ ID NO: 5. SEQ ID NO: 5 consists in amino acids 483 to 633 of SEQ ID NO: 1.

SEQ ID NO: 5

GDTYLTRETIFEASKKVTNSLSNLISLIGTKSGTQERELQEKSKDITKSTTEHRIN
NKLKVTDANIRNYVTETNADTIDKNHAIYEKAKEVSSALSKVLSKIDDTSAELL
TDDISDLKNNNDITAENNNIYKAAKDVTTSLSKVLKNINKD.

In one embodiment of the invention, said peptide has about 151, 155, 160, 175, 200, 250, 300 or 350 amino acids length and comprises the peptide sequence SEQ ID NO: 5.

In one embodiment, the polypeptide of the invention comprises SEQ ID NO: 5 and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids in C-term, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acids in N-term.

As used herein, "amino acids" are represented by their full name, their three letter code or their one letter code as well known in the art. Amino acid residues in peptides are

abbreviated as follows: Phenylalanine is Phe or F; Leucine is Leu or L; Isoleucine is Ile or I; Methionine is Met or M; Valine is Val or V; Serine is Ser or S; Proline is Pro or P; Threonine is Thr or T; Alanine is Ala or A; Tyrosine is Tyr or Y; Histidine is His or H; Glutamine is Gin or Q; Asparagine is Asn or N; Lysine is Lys or K; Aspartic Acid is Asp or D; Glutamic Acid is Glu or E; Cysteine is Cys or C; Tryptophan is Trp or W; Arginine is Arg or R; and Glycine is Gly or G.

As used herein, the term "amino acids" includes both natural and synthetic amino acids, and both D and L amino acids. "Standard amino acid" or "naturally occurring amino acid" means any of the twenty standard L-amino acids commonly found in naturally occurring peptides. "Non-standard amino acid residue" means any amino acid, other than the standard amino acids, regardless of whether it is prepared synthetically or derived from a natural source. For example, naphtlylalanine can be substituted for tryptophan to facilitate synthesis. Other synthetic amino acids that can be substituted include, but are not limited to, L-hydroxypropyl, L-3,4-dihydroxyphenylalanyl, alpha-amino acids such as L-alpha-hydroxylysyl and D-alpha-methylalanyl, L-alpha-methylalanyl, beta-amino acids, and isoquinolyl.

As used herein, "amino acid" also encompasses chemically modified amino acids, including but not limited to salts, amino acid derivatives (such as amides), and substitutions. Amino acids contained within the polypeptides of the present invention, and particularly at the carboxy- or amino-terminus, can be modified by methylation, amidation, acetylation or substitution with other chemical groups which can change the polypeptide's circulating half-life without adversely affecting their activity. Additionally, a disulfide linkage may be present or absent in the polypeptides of the invention.

The polypeptides of the invention may comprise naturally standard amino acids or non-standard amino acids. Polypeptide mimetics include polypeptides having the following modifications: i) polypeptides wherein one or more of the peptidyl -C(0)NR- linkages (bonds) have been replaced by a non-peptidyl linkage such as a -CFb-carbamate linkage (-CH_2OC(0)NR-), a phosphonate linkage, a -CH_2-sulfonamide (-CH_2-S(0)$_2$NR-) linkage, a urea (-NHC(O)NH-) linkage, a -CH_2-secondary amine linkage, or with an alkylated peptidyl linkage (-C(O)NR-) wherein R is $_{C1-C4}$ alkyl; ii) polypeptides wherein

the N-terminus is derivatized to a -NRR^1 group, to a -NRC(0)R group, to a -NRC(0)OR group, to a -NRS(0)$_2$R group, to a -NHC(0)NHR group where R and R^1 are hydrogen or C1-C4 alkyl with the proviso that R and R^1 are not both hydrogen; iii) polypeptides wherein the C terminus is derivatized to -C(0)R^2 where R^2 is selected from the group consisting of C1-C4 alkoxy, and -NR^{3}R^4 where R^3 and R^4 are independently selected from the group consisting of hydrogen and C1-C4 alkyl.

In one embodiment, the polypeptide of the invention comprises or consists in SEQ ID NO: 5 or a variant thereof. In one embodiment, a variant of SEQ ID NO: 5 binds to vinculin and/or talin with an equivalent affinity to the one of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 conserves the activity of SEQ ID NO: 5 on cell proliferation and/or anchoring.

In one embodiment, a variant of SEQ ID NO: 5 comprises conservative amino acid substitutions as compared to the sequence of SEQ ID NO: 5.

As used herein, the term "conservative amino acid substitution" is defined herein as an amino acid exchange within one of the following five groups:

I. Small aliphatic, nonpolar or slightly polar residues: Ala, Ser, Thr, Pro, Gly;

II. Polar, negatively charged residues and their amides: Asp, Asn, Glu, Gin;

III. Polar, positively charged residues: His, Arg, Lys;

IV. Large, aliphatic, nonpolar residues: Met, Leu, Ile, Val, Cys;

V. Large, aromatic residues: Phe, Tyr, Trp.

In another embodiment, a variant of SEQ ID NO: 5 is a polypeptide having a sequence identity of at least 70%, preferably of at least 75, 80, 85, 90, 95, 96, 97, 98, 99% or more with SEQ ID NO: 5.

In another embodiment, a variant of SEQ ID NO: 5 is a polypeptide wherein 1, 2, 3, 4, or 5 amino acids from the sequence of SEQ ID NO: 5 is/are absent, or substituted by any amino acid, or wherein 1, 2, 3, 4 or 5 amino acids (either contiguous or not) is/are added.

In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residues D2, K16, K31, K44, K48, K58, K60, E72, D76, D79, K80, K89, K97, E108, D112, D113, K118, K135, K143 or K150 of SEQ ID NO: 5.

In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residues D2, K16, K31, K44, K48, K58, K60, E72, D76, D79, K80, K89, K97, E108, D112, D113, K118, K135, K143 and K150 of SEQ ID NO: 5.

In one embodiment, a variant of SEQ ID NO: 5 comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20) amino acid residues selected from the group consisting of D2, K16, K31, K44, K48, K58, K60, E72, D76, D79, K80, K89, K97, E108, D112, D113, K118, K135, K143 and K150 of SEQ ID NO: 5.

In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue D2 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K16 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K31 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K44 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K48 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K58 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K60 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue E72 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue D76 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue D79 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K80 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K89 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K97 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue E108 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue D112 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue D113 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K118 of SEQ ID NO: 5. In one embodiment, a variant of

SEQ ID NO: 5 comprises amino acid residue K135 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K143 of SEQ ID NO: 5. In one embodiment, a variant of SEQ ID NO: 5 comprises amino acid residue K150 of SEQ ID NO: 5.

The polypeptides described herein can be produced synthetically by chemical synthesis or enzymatic synthesis as it is well known in the art. Alternatively, nucleotide sequences encoding the polypeptides of the invention can be introduced into a protein expression vector and produced in a suitable host organism (e.g., bacteria, insect cells, etc.), then purified. An additional polypeptide ("tag") can be added on for the purpose of purifying or identifying or purifying the polypeptides. Protein tags make it possible, for example, for the polypeptides to be adsorbed, with high affinity, to a matrix, and for the matrix then to be washed stringently with suitable buffers without the complex being eluted to any significant extent, and for the adsorbed complex subsequently to be eluted selectively. Examples of protein tags which are known to the skilled person are a $(His)_6$ tag, a Myc tag, a FLAG tag, a hemagglutinin tag, a glutathione transferase (GST) tag, intein having an affinity chitin- binding tag or maltose-binding protein (MBP) tag. These protein tags can be located N- terminally, C -terminally and/or internally.

In one embodiment of the invention, the polypeptides as described here above are modified by means well-known in the art, for instance by the addition of one or more functional group such as a phosphate, acetate, lipid or carbohydrate group, and/or by the addition of one or more protecting group.

For example, the polypeptides can be modified by the addition of one or more functional groups such as phosphate, acetate, or various lipids and carbohydrates. The polypeptides of the invention can also exist as polypeptide derivatives. The term "polypeptide derivative" refers to compound having an amino group (—NH—), and more particularly, a peptide bond. Polypeptides may be regarded as substituted amides. Like the amide group, the peptide bond shows a high degree of resonance stabilization. The C--N single bond in the peptide linkage has typically about 40 percent double-bond character and the C=0 double bond about 40 percent single-bond character. "Protecting groups" are those groups that prevent undesirable reactions (such as proteolysis) involving unprotected

functional groups. Specific examples of amino protecting groups include formyl; trifluoroacetyl; benzyloxycarbonyl; substituted benzyloxycarbonyl such as (ortho- or para-) chlorobenzyloxycarbonyl and (ortho- or para-) bromobenzyloxycarbonyl; and aliphatic oxycarbonyl such as t-butoxycarbonyl and t-amiloxycarbonyl. The carboxyl groups of amino acids can be protected through conversion into ester groups. The ester groups include benzyl esters, substituted benzyl esters such as methoxybenzyl ester; alkyl esters such as cyclohexyl ester, cycloheptyl ester or t-butyl ester. The guanidino moiety may be protected by nitro; or arylsulfonyl such as tosyl, methoxybenzensulfonyl or mesitylenesulfonyl, even though it does not need a protecting group. The protecting groups of imidazole include tosy, benzyl and dinitrophenyl. The indole group of tryptophan may be protected by formyl or may not be protected.

The modification of the polypeptides aims in particular to improve their life time *in vivo.* One type of modification is the addition to the N or C termini of the polypeptides of polyethylene glycol (PEG). PEG is known by the person skilled in the art to have many properties that make it an ideal carrier for polypeptides such as high water solubility, high mobility in solution and low immunogenicity. This modification also protects the polypeptides from exopeptidases and therefore increases their overall stability *in vivo.*

The other modifications used to prevent degradation of the polypeptides by endopeptidases or exopeptidases include N-terminal modifications such as acetylation or glycosylation, C-terminal modifications such as amidation and use of unnatural amino acids (β-amino and a-trifluoromethyl amino acids) at particular sites within the polypeptides.

Another alternative to increase polypeptide molecular size is the genetic fusion of the polypeptides to the Fc domain of human gamma immunoglobulin or the fusion of the polypeptides to albumin.

In one embodiment, the polypeptide of the invention binds to vinculin.

Vinculin (SEQ ID NO: 31) is composed by three repetitions (D1-D3 (SEQ ID NO: 11-13)) of a conserved domain consisting of two bundles of four helices, and a fourth D4 domain (SEQ ID NO: 14) containing only one helical bundle connected to a proline-rich

unstructured region and the carboxyterminal F-actin binding domain. Under its inactive folded state, intramolecular interactions between the D1 (SEQ ID NO: 11) and D4 (SEQ ID NO: 14) domains prevent the access of VBSs to vinculin.

In one embodiment, the polypeptide of the invention binds to vinculin with an estimated K_D in the femtoM range. The affinity between a polypeptide of the invention and vinculin may be characterized by any conventional technique known by the skilled artisan. Binding properties of a polypeptide to another polypeptide or to cells or tissues may generally be determined and assessed using immunodetection methods including, for example, ELISA, immunofluorescence-based assays, such as immuno-histochemistry (IHC) and/or fluorescence-activated cell sorting (FACS) or by surface plasmon resonance (SPR, BIAcore) or by isothermal titration calorimetry.

In one embodiment, the interaction of the polypeptide of the invention with vinculin induces the activation of vinculin, i.e., induces a shift from a resting or inactive state to an active state.

The term "resting state" or "inactive state" refers to the folded conformation of vinculin wherein the D1 amino-terminal a-helical bundle domain interacts with the carboxy-terminal tail domain, hindering ligand binding sites.

The term "activation" of vinculin refers to the disruption of the interaction between the head and tail of vinculin. As a consequence, vinculin conformation evolves in an open "active" state able to bind to F-actin in order to reinforce the anchorage of the actin cytoskeleton to membrane receptors and cell adhesion.

In one embodiment, the interaction of the polypeptide of the invention with vinculin induces the supra-activation of vinculin.

The term "supra-activation" of vinculin as used herein refers to the unraveling of additional sites of binding to D2 domain and possibly other domains of vinculin. As a consequence, vinculin promotes the scaffolding of large talin-vinculin complexes significantly reinforcing the association of the cytoskeleton to membrane receptors in the absence of mechanosensing.

For IpaA VBSl, as for all VBSs described to date, vinculin activation occurs through binding to the first helical bundle of the D1 domain, promoting major conformational changes that disrupt the D1-D4 intramolecular interactions and frees the vinculin F-actin binding region. The IpaA VBS2, on the other hand, interacts with the second helical bundle of D1 and its association with IpaA VBS 1 results in a very high affinity and stable binding to D1 with an estimated K_D in the femtoM range. Functional evidence seems to indicate that IpaA VBS3 cooperates with IpaA VBS 1-2 to stimulate bacterial internalization by host cells, but that intriguingly, it may act as IpaA VBSl in promoting vinculin activation through interaction with the vinculin D1 first helical bundle.

In another embodiment, the polypeptide of the invention binds to talin.

In one embodiment, the polypeptide of the invention binds to vinculin and talin.

In one embodiment, the polypeptide of the invention binds to talin with an estimated K_D in the nM range. The affinity between a polypeptide of the invention and talin may be characterized by any conventional technique known by the skilled artisan. Binding properties of a polypeptide to another polypeptide or to cells or tissues may generally be determined and assessed using immunodetection methods including, for example, ELISA, immunofluorescence-based assays, such as immuno-histochemistry (IHC) and/or fluorescence-activated cell sorting (FACS) or by surface plasmon resonance (SPR, BIAcore) or by isothermal titration calorimetry.

Another object of the invention is a nucleic acid encoding a polypeptide as described herein above.

In one embodiment, the nucleic acid of the invention comprises at least one, preferably at least 2, and more preferably the three VBS encoding sequences SEQ ID NO: 8-10:

VBSl:

ATATATAAAGCAGCAAAAGATGTAACCACTTCCCTATCAAAAGTATT AAAGAATATC (SEQ ID NO: 8);

VBS2:

ATCTATGAAAAGGCAAAAGAAGTATCTAGCGCCCTCAGCAAGGTATT GTCAAAAATT (SEQ ID NO: 9);

VBS3:
ATATTTGAAGCTTCAAAAAAAGTAACAAACTCCCTAAGTAATCTTAT
ATCTCTCATT (SEQ ID NO: 10);
or any nucleic acid sequence having at least 60; 65; 70; 75; 80; 85; 90; 95; 96; 97; 98; 99% identity with SEQ ID NO: 8; 9 and 10.

In one embodiment, the nucleic acid of the invention comprises at least one, preferably at least 2, and more preferably the three VBS encoding sequences SEQ ID NO: 69-71 :

VBS1:
ATATATAAAGCAGCAAAAGATGTAACCACTTCCCTATCAAAAGTATT
AAAGAATATCAATAAGGAT (SEQ ID NO: 69);
VBS2:
ATCTATGAAAAGGCAAAAGAAGTATCTAGCGCCCTCAGCAAGGTATT
GTCAAAAATTGACGAT (SEQ ID NO: 70);
VBS3:
ACACGGGAAACGATATTTGAAGCTTCAAAAAAAGTAAC AAACTCCCT
AAGTAATCTTATATCTCTCATTGGAACT (SEQ ID NO: 71);
or any nucleic acid sequence having at least 60; 65; 70; 75; 80; 85; 90; 95; 96; 97; 98; 99% identity with SEQ ID NO: 69; 70 and 71.

In one embodiment, the nucleic acid of the invention is SEQ ID NO: 6.

In another embodiment, the nucleic acid of the invention does not comprise or consist of full-length IpaA nucleic acid sequence (SEQ ID NO: 7).

In another embodiment, the nucleic acid of the invention does not comprise or consist of nucleic acid residues 3109 to 5010 of pINV_F6_M1382, referenced under NCBI accession number AY206439.1 (SEQ ID NO: 46).

In one embodiment, the nucleic acid of the invention comprises or consists in 171 to 1500 nucleotides, preferably from 210 to about 1200 nucleotides, more preferably from about 270 to about 900 nucleotides, even more preferably from about 330 to about 600 nucleotides and still even more preferably from about 420 to about 480 nucleotides.

In one embodiment, the nucleic acid sequence according to the invention has a length of at least 171, 180, 190, 200, 300, 400 or 450 nucleotides. In one embodiment, the nucleic acid sequence of the invention has a length of at least 453 nucleotides.

Another object of the invention is an expression vector comprising a nucleic acid sequence encoding the polypeptide as described here above. In one embodiment, said nucleic acid sequence is a nucleic acid as described here above. Examples of vector include, but are not limited to, a plasmid, a bacteriophage, a virus, a cationic vesicle or any other type of vector.

Another object of the invention is a composition comprising or consisting of at least one polypeptide, nucleic acid and/or vector of the invention.

Another object of the invention is a pharmaceutical composition comprising or consisting of or consisting essentially of at least one polypeptide, nucleic acid and/or vector of the invention and at least one pharmaceutically acceptable excipient.

Another object of the invention is a medicament comprising or consisting of or consisting essentially of at least one polypeptide, nucleic acid and/or vector of the invention.

As used herein, the term "consisting essentially of, with reference to a pharmaceutical composition or medicament, means that the at least one polypeptide, nucleic acid and/or vector of the invention is the only one therapeutic agent or agent with a biologic activity within said pharmaceutical composition or medicament.

Pharmaceutically acceptable excipients include water, saline, Ringer's solution, dextrose solution, and solutions of ethanol, glucose, sucrose, dextran, mannose, mannitol, sorbitol, polyethylene glycol (PEG), phosphate, acetate, gelatin, collagen, Carbopol®, vegetable oils, and the like. One may additionally include suitable preservatives, stabilizers, antioxidants, antimicrobials, and buffering agents, such as, for example, BHA, BHT, citric acid, ascorbic acid, tetracycline, and the like.

Other examples of pharmaceutically acceptable excipients that may be used in the composition of the invention include, but are not limited to, ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer

substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene- polyoxypropylene- block polymers, polyethylene glycol and wool fat.

In addition, pharmaceutically acceptable excipients may comprise some excipients, such as, for example, surfactants (e.g. hydroxypropylcellulose); suitable carriers, such as, for example, solvents and dispersion media containing, for example, water, ethanol, polyol (e.g. glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils, such as, for example, peanut oil and sesame oil; isotonic agents, such as, for example, sugars or sodium chloride; coating agents, such as, for example, lecithin; agents delaying absorption, such as, for example, aluminum monostearate and gelatin; preservatives, such as, for example, benzalkonium chloride, benzethonium chloride, chlorobutanol, thimerosal and the like; buffers, such as, for example, boric acid, sodium and potassium bicarbonate, sodium and potassium borates, sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like; tonicity agents, such as, for example, dextrose, potassium chloride, propylene glycol, sodium chloride; antioxidants and stabilizers, such as, for example, sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like; nonionic wetting or clarifying agents, such as, for example, polysorbate 80, polysorbate 20, poloxamer 282 and tyloxapol; viscosity modifying agents, such as, for example dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxmethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose; and the like.

Another object of the invention is a polypeptide, a nucleic acid or a vector as described here above for treating or for use in the treatment of a proliferation and/or adhesion related disease.

Another object of the invention is a composition, a pharmaceutical composition or a medicament as described here above for treating or for use in the treatment of a proliferation and/or adhesion related disease.

Another object of the invention is a method for treating a proliferation and/or adhesion related disease, wherein the method comprises administering to the subject the composition, the pharmaceutical composition or the medicament of the invention.

Indeed, the Applicant herein demonstrated that the expression of a peptide of the invention by a cell induces the rapid anchoring of said cell to the support, independently of the stiffness of the substrate. Moreover, said expression slows down the division time and the velocity of migration of said cell. These results thus strongly support the therapeutic use of a peptide of the invention for treating a proliferation and/or adhesion related disease.

Without willing to be bound to a theory, the Applicant suggests that the expression of a peptide of the invention may lead to the anchoring of diseased cells to the substrate, and thereby (i) avoid dissemination of these cells (such as, for example, metastatic cells), and (ii) facilitate access to these cells by therapeutic agents or methods.

As used herein, the term "proliferation and/or adhesion related disease" refers to pathologies wherein abnormal cell proliferation and/or dysfunction in cell adhesion are observed. Abnormal cell proliferation and dysfunction in cell adhesion are mechanisms well known in the state of the art.

Proliferation and/or adhesion related diseases include but are not limited to, cancer, tumor, metastasis, inflammatory diseases and/or auto-immune disease.

Examples of cancers include but are not limited to, tumors, metastasis, carcinoma, melanoma, lymphoma, glioma, myeloma, neoplasm, leukemia, soft tissue cancer (such as, for example, soft tissue sarcoma), anal cancer, bladder cancer, bone cancer, breast cancer, cervical cancer, endometrial cancer, esophageal cancer, extrahepatic bile duct cancer, eye cancer, gall bladder cancer, gastric cancer, head and neck cancer, hypopharyngeal cancer, kidney cancer, laryngeal cancer, lip and oral cavity cancer, liver

cancer, lung cancer, mesothelioma, metastatic squamous head and neck cancer, nasopharyngeal cancer, neuroblastoma, oral cancer, oropharyngeal cancer, ovarian cancer, pancreatic cancer, sinus and nasal cancer, parathyroid cancer, penile cancer, pheochromocytoma cancer, pituitary cancer, plasma cell neoplasm, prostate cancer, rectal cancer, salivary gland cancer, skin cancer, stomach cancer, testicular cancer, thymoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer.

Examples of tumors include but are not limited to, malignant tumors, epithelial tumor, connective tissue neoplasm, sarcomas, fibroma, fibrosarcoma, dermatofibrosarcoma protuberans, desmoplastic fibroma, aggressive infantile fibromatosis, aponeurotic fibroma, collagenous fibroma, diffuse infantile fibromatosis, familial myxovascular fibromas, fibroma of tendon sheath, fibromatosis colli, infantile digital fibromatosis, juvenile hyaline fibromatosis, plantar fibromatosis, pleomorphic fibroma, oral submucous fibrosis, malignant fibrous histiocytoma, atypical fibroxanthoma, solitary fibrous tumor, myxoma/myxosarcoma, Brenner tumor, fibroadenoma, phyllodes tumor, synovial sarcoma, clear-cell sarcoma, lipoma/liposarcoma, chondroid lipoma, intradermal spindle cell lipoma, pleomorphic lipoma, lipoblastomatosis, spindle cell lipoma, hibernoma, myoma/myosarcoma, brain tumor, endocrine tumor, myeloma, extracranial germ cell tumor, Ewing's tumor, germ cell tumor, gestational trophoblastic tumor, carcinoid tumor, Wilm's tumor, Krukenberg tumor, bone tumor, cartilage tumor, osteoid osteoma, osteoblastoma, osteoma/osteosarcoma, chondroblastoma, giant cell tumor bone, Mullerian tumor, rhabdomyoma/rhabdomyosarcoma: embryonal rhabdomyosarcoma, sarcoma botryoides, alveolar rhabdomyosarcoma, leiomyoma/leiomyosarcoma, Askin's tumor, malignant hemangioendothelioma, soft tissue sarcomas.

Examples of carcinomas include, but are not limited to, basal cell carcinoma, adenocarcinoma, adrenocortical carcinoma, breast carcinoma, colon carcinoma, Merkel cell carcinoma, rhabdomyosarcoma, renal cell carcinoma, islet cell carcinoma, basal cell carcinoma, squamous cell carcinoma, ductal carcinoma *in situ* (DCIS), invasive ductal carcinoma.

Examples of sarcomas include, but are not limited to, soft tissue sarcoma, Kaposi's sarcoma, osteosarcoma, liposarcoma, sarcoma botryoides, Askin's tumor, chondrosarcoma, Ewing's tumor, malignant hemangioendothelioma, osteosarcoma, soft tissue sarcomas, alveolar soft part sarcoma, angiosarcoma, cystosarcoma phyllodes, dermatofibrosarcoma, desmoid Tumor, desmoplastic small round cell tumor, epithelioid sarcoma, extraskeletal chondrosarcoma, extraskeletal osteosarcoma, fibrosarcoma, gastrointestinal stromal tumor, hemangiopericytoma, hemangiosarcoma, leiomyosarcoma, liposarcoma, lymphangiosarcoma, lymphosarcoma, malignant fibrous histiocytoma, malignant peripheral nerve sheath tumor, neurofibrosarcoma, rhabdomyosarcoma, synovial sarcoma.

Examples of lymphomas include, but are not limited to, T-cell lymphoma, Hodgkin lymphomas, non-Hodgkin lymphomas, Burkitt lymphoma.

In one embodiment, the polypeptide, nucleic acid or vector of the invention is used for preventing or treating metastasis.

Examples of inflammatory diseases include, but are not limited to, acne vulgaris, asthma, inflammatory autoimmune diseases, autoinflammatory diseases, celiac disease, chronic prostatitis, glomerulonephritis, hypersensitivities, inflammatory bowel diseases, pelvic inflammatory disease, reperfusion injury, rheumatoid arthritis, sarcoidosis, transplant rejection, vasculitis, interstitial cystitis, atherosclerosis, allergies, myopathies, leukocyte defects and cancers.

Examples of autoimmune diseases include, but are not limited to, myocarditis, postmyocardial infarction syndrome, postpericardiotomy syndrome, subacute bacterial endocarditis, anti-glomerular basement membrane nephritis, interstitial cystitis, lupus nephritis comorbidity, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, antisynthetase syndrome, alopecia areata, autoimmune angioedema, autoimmune progesterone dermatitis, autoimmune urticarial, bullous pemphigoid, cicatricial pemphigoid, dermatitis herpetiformis, discoid lupus erythematosus, epidermolysis bullosa acquisita, erythema nodosum, gestational pemphigoid, hidradenitis suppurativa, lichen sclerosus, pemphigus vulgaris, pityriasis

lichenoides and varioliformis acuta, Mucha-Habermann disease, psoriasis, systemic scleroderma, vitiligo, Addison's disease, autoimmune polyendocrine syndrome, autoimmune polyendocrine syndrome type 2, autoimmune polyendocrine syndrome type 3, autoimmune pancreatitis, diabetes mellitus type 1, autoimmune thyroiditis, Ord's thyroiditis, Graves' disease, autoimmune oophoritis, endometriosis, autoimmune orchitis, Sjogren's syndrome, autoimmune enteropathy, celiac disease, Crohn's disease, microscopic colitis, ulcerative colitis, antiphospholipid syndrome, aplastic anemia, autoimmune hemolytic anemia, autoimmune lymphoproliferative syndrome, autoimmune neutropenia, autoimmune thrombocytopenic purpura, cold agglutinin disease, essential mixed cryoglobulinemia, evans syndrome, IgG4-related systemic disease, paroxysmal nocturnal hemoglobinuria, pernicious anemia, pure red cell aplasia, thrombocytopenia, adiposis dolorosa, adult-onset Still's disease, ankylosing spondylitis, CREST syndrome, drug-induced lupus, enthesitis-related arthritis, eosinophilic fasciitis, Felty syndrome, juvenile arthritis, Lyme disease (chronic), mixed connective tissue disease, palindromic rheumatism, Parry Romberg syndrome, Parsonage-Turner syndrome, psoriatic arthritis, reactive arthritis, relapsing polychondritis, retroperitoneal fibrosis, rheumatic fever, rheumatoid arthritis, sarcoidosis, Schnitzler syndrome, systemic lupus erythematosus, undifferentiated connective tissue disease, dermatomyositis, fibromyalgia, inclusion body myositis, myositis, myasthenia gravis, neuromyotonia, paraneoplastic cerebellar degeneration, polymyositis, acute disseminated encephalomyelitis, acute motor axonal neuropathy, anti-N-Methyl-D-Aspartate receptor encephalitis, Balo concentric sclerosis, Bickerstaff s encephalitis, chronic inflammatory demyelinating polyneuropathy, Guillain-Barre syndrome, idiopathic inflammatory demyelinating diseases, Lambert-Eaton myasthenic syndrome, multiple sclerosis, narcolepsy, pediatric autoimmune neuropsychiatric disorder associated with Streptococcus, progressive inflammatory neuropathy, restless leg syndrome, stiff person syndrome, Sydenham chorea, transverse myelitis, autoimmune retinopathy, autoimmune uveitis, Cogan syndrome, Graves ophthalmopathy, intermediate uveitis, ligneous conjunctivitis, Mooren's ulcer, neuromyelitis optica, opsoclonus myoclonus syndrome, optic neuritis, scleritis, Susac's syndrome, sympathetic ophthalmia, Tolosa-Hunt syndrome, autoimmune inner ear disease, Meniere's disease, anti-neutrophil cytoplasmic antibody-associated vasculitis, Beliefs disease, Churg-Strauss syndrome, giant cell

arteritis, Henoch-Schonlein purpura, Kawasaki's disease, leukocytoclastic vasculitis, lupus vasculitis, rheumatoid vasculitis, microscopic polyangiitis, polyarteritis nodosa, polymyalgia rheumatic, urticarial vasculitisa and vasculitis.

Examples of inflammatory autoimmune diseases include, but are not limited to, intestinal inflammatory condition such as Crohn's disease and ulcerative colitis; arthritis condition such as rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis and juvenile idiopathic arthritis; multiple sclerosis; uveitis; Wegener's disease; primary biliary cirrhosis; primary sclerosing cholangitis; asthma, transplant rejection (host versus graft disease); diabetes or graft versus host disease.

In one embodiment, the composition, the pharmaceutical composition or the medicament of the invention is to be administered orally, by injection, topically, nasally, buccally, rectally, vaginaly, intratracheally, by endoscopy, transmucosally, or by percutaneous administration.

The disclosed polypeptides or nucleic acids or vectors can be delivered to the target cells in a variety of ways. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example *in vivo* or *in vitro.* The skilled artisan will be able to adapt the delivery of the polypeptides or nucleic acid sequences of the invention.

In one embodiment, the composition, the pharmaceutical composition or the medicament of the invention is to be orally administered. Examples of formulations adapted to oral administration include, but are not limited to: solid forms, liquid forms and gels. Examples of solid forms adapted to oral administration include, but are not limited to, pill, tablet, capsule, soft gelatine capsule, hard gelatine capsule, caplet, compressed tablet, cachet, wafer, sugar-coated pill, sugar coated tablet, or dispersing/or disintegrating tablet, powder, solid forms suitable for solution in, or suspension in, liquid prior to oral administration and effervescent tablet. Examples of liquid form adapted to oral administration include, but are not limited to, solutions, suspensions, drinkable solutions, elixirs, sealed phial, potion, drench, syrup and liquor.

In one embodiment, the composition, the pharmaceutical composition or the medicament of the invention is to be administered by injection, preferably systemically injected. Examples of formulations adapted to systemic injections include, but are not limited to: liquid solutions or suspensions, solid forms suitable for solution in, or suspension in, liquid prior to injection. Examples of systemic injections include, but are not limited to, intravenous, subcutaneous, intramuscular, intradermal, intravitreal, and intraperitoneal injection, or perfusion. In another embodiment, when injected, the composition, the pharmaceutical composition or the medicament of the invention is sterile. Methods for obtaining a sterile pharmaceutical composition include, but are not limited to, GMP synthesis (GMP stands for "Good manufacturing practice").

In another embodiment, the composition, the pharmaceutical composition or the medicament of the invention is to be topically administered. Examples of formulations adapted to topical administration include, but are not limited to, sticks, waxes, creams, lotions, ointments, balms, gels, masks, leave-on washes and/or the like.

In one embodiment of the invention, the ointment is an oleaginous ointment; an emulsified ointment such as, for example, oil-in-water or a water-in-oil ointment; or a water-soluble ointment, preferably is an oleaginous ointment.

In one embodiment of the invention, the oleaginous ointment uses bases such as, for example, plant and animal oils; plant and animal fats; waxes; vaseline, such as, for example, white vaseline or vaseline oil; and paraffin such as, for example, liquid paraffin or paraffin oil.

In another embodiment, the composition of the invention can also be applied topically using a transdermal system, such as one of an acrylic-based polymer adhesive with a resinous crosslinking agent impregnated with the composition and laminated to an impermeable backing. Examples of formulations adapted to transdermal administration include, but are not limited to, ointment, paste, cream, film, balm, patch, such as, for example, transdermal patch, gel, liposomal forms and the like.

In one embodiment, the composition of the present invention can be administered topically as a transdermal patch, more particularly as a sustained-release transdermal

patch. The transdermal patches can include any conventional form such as, for example, adhesive matrix, polymeric matrix, reservoir patch, matrix or monolithic-type laminated structure, and are generally comprised of one or more backing layers, adhesives, penetration enhancers, an optional rate controlling membrane and a release liner which is removed to expose the adhesives prior to application. Polymeric matrix patches also comprise a polymeric-matrix forming material. Suitable transdermal patches are described in more detail in, for example, U.S. Patent Nos. 5,262,165; 5,948,433; 6,010,715 and 6,071,531, the disclosure of which are incorporated herein in their entirety.

In one embodiment, the composition, pharmaceutical composition or medicament of the invention may be used in conjunction with delivery systems that facilitate delivery of the agents to targeted organs or cells affected by a pathologic condition. For example, the composition, pharmaceutical composition or medicament of the invention may be PEGylated.

In one embodiment, the composition, the pharmaceutical composition or the medicament is administered in a sustained-release form. In another embodiment, the composition, the pharmaceutical composition or the medicament comprises a delivery system that controls the release of the agent.

Depending on the cell targeted, the skilled artisan can determine the technology needed for the introduction of the delivered nucleic acid sequences of the present application in the targeted cells.

The "targeted cells" or "targeted organ" as used herein refer to cells or organs affected by the diseases described below. In particular, targeted cells may include cells with abnormal proliferation or dysregulated adhesive properties.

For example, the polypeptides or the nucleic acids of the invention can be delivered through a number of direct delivery systems such as: electroporation, sonoporation, lipofection, calcium phosphate, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection include viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion

of nucleic acid sequences. Technics for delivering nucleic acids to cells as used in the present application are well known by the person skilled in the art. These technics are described in Guide to Molecular Cloning Technics (Berger S.L. and Kimmel A.R., **1987.** *Methods in Enzymology.* **152**:359-371). In particular cases, the methods will be modified and adapted to large nucleic acid molecules. Further, these methods can be used to target cells and in particular cell populations by using the targeting characteristics of the carrier.

There are a number of compositions and methods that can be used to deliver nucleic acids to cells, either *in vitro* or *in vivo.* These methods and compositions can largely be classified into two classes: viral based delivery systems and non-viral based delivery systems.

Viral based delivery systems for introducing nucleic acid molecules into cells involve the use of viral vectors. Since viral growth depends on the ability to get the viral genome into cells, viruses have devised clever and efficient methods for doing it. One such virus widely used for protein production is an insect virus, baculovirus. Baculovirus attracted the attention of researchers because during infection, it produces one of its structural proteins (the coat protein) to spectacular levels. If a foreign gene were to be substituted for this viral gene, it too ought to be produced at high level. Baculovirus, like vaccinia, is very large, and therefore foreign genes must be placed in the viral genome by recombination. To express a foreign gene in baculovirus, the gene of interest is cloned in place of the viral coat protein gene in a plasmid carrying a small portion of the viral genome. The recombinant plasmid is cotransfected into insect cells with wild-type baculovirus DNA. At a low frequency, the plasmid and viral DNAs recombine through homologous sequences, resulting in the insertion of the foreign gene into the viral genome. Virus plaques develop, and the plaques containing recombinant virus look different because they lack the coat protein. The plaques with recombinant virus are picked and expanded. This virus stock is then used to infect a fresh culture of insect cells, resulting in high expression of the foreign protein. Various viral vectors have also been used to transfect cells, such as bacteriophage, vaccinia virus, adenovirus, and retrovirus.

Non-viral based delivery systems are also known in the art for introducing nucleic acid molecules into targeted cells. One of these methods is microinjection, in which nucleic

acid sequences is injected directly into the nucleus of cells through fine glass needles. Alternatively, nucleic acid sequences can be incubated with an inert carbohydrate polymer (dextran) to which a positively charged chemical group (DEAE, for diethylaminoethyl) has been coupled. The nucleic acid sequence sticks to the DEAE-dextran via its negatively charged phosphate groups. These large nucleic acid-containing particles stick in turn to the surfaces of cells, which are thought to take them in by a process known as endocytosis. Some of the nucleic acid evades destruction in the cytoplasm of the cell and escapes to the nucleus, where it can be transcribed into RNA like any other gene in the cell. In another method, cells efficiently take in nucleic acid sequence in the form of a precipitate with calcium phosphate. In electroporation, cells are placed in a solution containing nucleic acid and subjected to a brief electrical pulse that causes holes to open transiently in their membranes. Nucleic acid sequence enters through the holes directly into the cytoplasm, bypassing the endocytotic vesicles through which they pass in the DEAE-dextran and calcium phosphate procedures (passage through these vesicles may sometimes destroy or damage the nucleic acid sequence). The nucleic acid sequence can also be incorporated into artificial lipid vesicles, liposomes, which fuse with the cell membrane, delivering their contents directly into the cytoplasm. In an even more direct approach, used primarily with plant cells and tissues, the nucleic acid sequence is absorbed to the surface of tungsten micro projectiles and fired into cells with a device resembling a shotgun.

Another object of the invention is a capsule or a liposome that targets specifically a cell, an organ, a tissue or a site affected by a proliferation and/or adhesion related disease.

In one embodiment, the capsule or the liposome of the invention comprises at least one polypeptide, nucleic acid or vector as described here above.

Thus, the polypeptides or nucleic acid sequences can be encapsulated or vectorized for example, lipids such as liposomes, such as cationic liposomes (e. g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. The polypeptide or the nucleic acid sequences of the invention and a cationic liposome can be administered to the blood afferent to a target organ or target cells affected by the diseases described above.

In one embodiment, the capsule or the liposome of the invention comprises a polypeptide, a nucleic acid, a vector, a composition, a pharmaceutical composition or a medicament of the invention.

In one embodiment, the composition, the pharmaceutical composition or the medicament of the invention is to be administered at a dose determined by the skilled artisan and personally adapted to each subject.

In one embodiment, a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention is administered to a subject in need thereof.

It will be understood that the total daily usage of the composition, pharmaceutical composition or medicament of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective amount for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the specific composition employed, the age, body weight, general health, sex and diet of the subject; the time of administration, route of administration, the duration of the treatment; drugs used in combination or coincidental with the polypeptide or nucleic acid sequence employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of a therapeutic compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

In one embodiment, a therapeutically effective amount of the composition, pharmaceutical composition or medicament of the invention is administered at least once a day, twice a day, or at least three times a day.

In another embodiment, a therapeutically effective amount of the composition, pharmaceutical composition or medicament of the invention is administered every two, three, four, five, or six days.

In another embodiment, a therapeutically effective amount of the composition, pharmaceutical composition or medicament of the invention is administered every week, twice a week, every two weeks, or once a month.

In another embodiment, a therapeutically effective amount of the composition, pharmaceutical composition or medicament of the invention is administered every month for a period at least 2; 3; 4; 5; or 6 months.

In another embodiment, a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention ranges from about 1 µg to 5 g.

In another embodiment, a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention to be administered ranges from about 0.1 µg/kg to 1 g/kg.

In one embodiment, the method of the invention is for a chronic treatment, i.e., the composition, pharmaceutical composition or medicament of the invention, is administered for a prolonged period of time, such as, for example, for at least about 1 week, 1 month, 1 year or more.

In another embodiment, the method of the invention is for an acute treatment, such as, for example, a treatment with only 1, 2 or 3 administrations of the composition, pharmaceutical composition or medicament of the invention.

In another embodiment, the composition, pharmaceutical composition or medicament as described here above is to be administered in combination with another treatment for proliferation and/or dysfunction in cell adhesion, preferably another anti-cancer or an anti-tumoral agent.

Examples of anti-tumoral agent comprise but are not limited to: chemotherapy, radiation, surgery, protein kinases inhibitors, microtubules inhibitors, anti-metabolite agents a tumor vaccine or an immunostimulatory antibody.

In one embodiment of the invention, the method for treating cancer in a subject in need thereof, comprises administering to the subject the composition, the pharmaceutical

composition or the medicament prior to, concurrent to and/or posterior to another anti-tumoral agent or cancer treatment.

In another embodiment, the subject is affected, preferably is diagnosed with a proliferation and/or adhesion related disease. In another embodiment, the subject of the invention is at risk of developing a proliferation and/or adhesion related disease. Examples of risk factor include, but are not limited to, genetic predisposition, familial history of proliferation and/or adhesion related disease or environmental factors.

In another embodiment, the subject of the invention is affected, preferably is diagnosed with a cancer. In another embodiment, the subject of the invention is at risk of developing a cancer. In another embodiment, the subject of the invention is in a remission stage following a cancer.

In another embodiment, the subject of the invention is affected, preferably is diagnosed with a tumor. In another embodiment, the subject of the invention is at risk of developing a tumor. In another embodiment, the subject of the invention is in a remission stage following a tumor.

In another embodiment, the subject of the invention is affected, preferably is diagnosed with metastasis. In another embodiment, the subject of the invention is at risk of developing metastasis. In another embodiment, the subject of the invention is in a remission stage following metastasis.

In another embodiment, the subject of the invention is affected, preferably is diagnosed with an inflammatory disease.

In another embodiment, the subject of the invention is affected, preferably is diagnosed with an auto-immune disease.

Another object of the invention is a method for inducing supra-activation of vinculin in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention.

Another object of the invention is a method for inhibiting proliferation and/or migration of cells, preferably of cancer cells and/or tumor cells in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention. In one embodiment, the method of the invention is for inhibiting migration of cells. In another embodiment, the method of the invention is for inhibiting the migration rate of cells. In another embodiment, the method of the invention is for inhibiting proliferation of cells.

Another object of the invention is a method for modulating (i.e., for inhibiting or activating) cell adhesion in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention. In one embodiment, the method of the invention is a method for modulating cell adhesion independently of mechanosensing. Consequently, in one embodiment, the method of the invention is a method for modulating cell adhesion independently of the stiffness of the substrate, i.e., soft or hard substrate. In one embodiment, the method of the invention is a method for modulating cell adhesion whilst by-passing mechanosensing normally associated with the initial steps of cell adhesion.

Another object of the invention is a method for modulating, preferably for slowing down, the division time of cells, comprising administering a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention.

Another object of the invention is a method for modulating, preferably for slowing down, the velocity of migration of cells, comprising administering a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention.

Another object of the invention is a method for reinforcing cell adhesion, preferably for accelerating the dynamics of cell adhesion, comprising administering a therapeutically effective amount of the polypeptide, nucleic acid or vector of the invention. In one embodiment, the method of the invention is a method for reinforcing cell adhesion independently of the stiffness of the substrate, i.e., soft or hard substrate. In one embodiment, the method of the invention is a method for reinforcing cell adhesion whilst by-passing mechanosensing normally associated with the initial steps of cell adhesion.

The Applicant has demonstrated (see Examples) that the polypeptide of the invention allows rapid anchoring of a cell expressing said polypeptide to a support comprising a receptor for beta-1 integrin, such as, for example, fibronectin. Indeed, anchoring is detected about 1 minute or less after insertion of the polypeptide within a cell.

Therefore, the present invention further relates to non-therapeutic applications of the polypeptide of the invention, resulting from this rapid effect on cell adhesion.

For example, the polypeptide of the invention may be used for forming a coating of cells on a support coated with a receptor for beta-1 integrin, such as, for example, fibronectin. It may also be used in the field of bioprinting, wherein cells are anchored on a support in order to generate spatially-controlled cell patterns. Such applications may apply to, without limitation, the development of tissue-on-a-chip and organ-on-a-chip in tissue engineering, coating of prosthesis with cells, etc.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a set of schemes and graphs showing the interaction of A483 (SEQ ID NO: 5) and A524 (SEQ ID NO: 15) with full-length vinculin (HV). **A,** Scheme of folded vinculin. The binding sites and corresponding ligands in HVD1 (SEQ ID NO: 11) first and second helical bundles are indicated. **B,** Scheme of constructions with corresponding domains full-length vinculin or IpaA (IpaA-FL). Empty box: HVD 1 domain; dark grey box: HVD2 (SEQ ID NO: 12) domain. IpaA VBS1 (grey); IpaA VBS2 (dark grey); IpaA VBS3 (solid). The numbers indicate the start residue of each domain. **C-F,** Solid phase binding assays between vinculin and IpaA derivatives. **C,** coating: HV; ligands: A483 (solid squares); A524 (empty circles). **D,** coating: A483 (solid squares) or A524 (empty circles); ligand: HV as ligand. **E,** coating: HVD1 (solid squares, SEQ ID NO: 11) or HVD1D2 (SEQ ID NO: 35) (empty circles); ligand: A483. **F,** coating: HVD1 (empty circles), HVD1D2 (solid squares), HVD1D2 K276R (SEQ ID NO: 36) (empty squares) or HVD1D2 E305Q (SEQ ID NO: 37) (empty triangles); ligand: A483.

Figure 2 is a set of photographs showing the formation of vinculin and IpaA derivatives complexes analyzed by BN-PAGE and Coomassie staining analysis of. Lanes: 1-10,

protein mixture at the molar ratio indicated above each lane. **A,** HVD1 and A524; **B,** HVD1 and A483; **C,** HVD1-D2 and A524; **D,** HVD1-D2 and A483. **A,** and **B,** 6-18% polyacrylamide gradient gels; **C,** and **D,** 4.5-16% polyacrylamide gradient gels. Arrowheads indicate protein alone, or complex migration at the indicated molar ratio.

Figure 3 is a set of schemes representing IpaA that binds to the first helical bundle (D2a) of the vinculin D2 domain (HVD2). Proteolytic map from mass spectrometry analysis of HVD1D2 and A483 alone, or following cross-linking of the HVD1D2-A483 1:2 complex (arrows). Note that first bundle of D2 domain (D2a) is protected from trypsin proteolysis in the HVD1D2-A483 complex compared to proteins alone. HVD1D2 (vinculin D1-D2 alone), A483 (A483 alone), HVD1D2 *Comp (complex 1:2 HVD1D2 and A483) and A483 *Comp (complex 1:2 HVD1D2 and A483). Empty boxes: domains protected from proteolysis; grey boxes: domains sensitive to proteolysis; brackets: A483-HVD1D2 interactive domains that are protected upon complex formation but not in proteins alone.

Figure 4 is a set of images and graphs showing vinculin-mediated cell adhesion enhanced by IpaA. **A** and **G,** Immunofluorescence analysis of C2.7 cells (CTRL) and GFP-A483 transfectants (A483). GFP fluorescence (IpaA); vinculin; actin. **A,** cells plated for 16 hours. Arrowheads: large peripheral focal adhesions (FAs). Arrows: ventral FAs. **B,** The percentage of cells showing FAs with a small (S), medium (M) and large (L) FAs was scored (Exp. Procedures). The results are representative of n(control) = 37 and n(GFP-A483) = 27 cells in 3 independent experiments. Distributions were compared using a Pearson's Chi-squared test ($p = 5.41 \times 10^{-9}$). **C,** Box-plot of the median time between two divisions for GFP (93 cells, N = 4) and GFP-A483 transfectants (40 cells, N = 4). **: p = 0.002. **D,** Box-plot of the mean velocity of GFP (268 cells, N = 4) and GFP-A483 transfectants (199 cells, N = 4). ***p = 0.0007. **E,** Root of Median Square of displacement over time for GFP-(SlopeCTRL = 355998 μm.hr, $R^2 = 0.97$) and GFP-A483 transfectants (SlopeA483=300097 μm.hr, $R^2=0.98$). The slopes were analyzed using a covariance test (ANCOVA, $p < 2x10^{-16}$). **F,** Box-plot of the median cell surface for GFP control (444 cells, N = 4) and GFP-A483 transfectants (300 cells, N = 4), ****: $p < 2 \times 10^{-16}$. **G,** cells plated for 15 min on Fn-coated. Arrowheads: large peripheral vinculin-rich structures. **H,** Cells were plated for 7 minutes prior to washing and processing for DAPI

staining. Left, representative micrographs of indicated cells. Right, box plot of the median number of adherent cells per sample for control (4586 cells, n=6) and GFP-A483 transfectants (6383 cells, n=6). *: p = 0.015. **I,** cells were perfused in a microfluidic chamber and allowed to adhere for 1 minute prior to shear stress application. Boxplot of the median resistance to shear stress for control (34 cells, N = 2) and GFP-A483 (15 cells, N = 1). ***: p = 0.00039.

Figure 5 is a graph showing the result of binding assays of A483 and A525 to vinculin. Solid phase binding assays between vinculin and IpaA derivatives showing that A483 have similar immunoreactivity to the alpaA antibody. Coating: A483 (solid square) and A525 (empty circle); ligand: HV.

Figure 6 is a set of photographs and graphs showing stoichiometry analysis of the A483 - HVD1D2 complexes. **A,** SDS-PAGE (15%) second dimension electrophoresis showing the amount of HVD1D2 (upper band) and A483 (lower band) recovered from BN-PAGE sliced complexes. Left: complex 1:1 of HVD1D2 and A524; center: complex 1:1 of HVD1D2 and A483; right: complex 2:1 of A483 and HVD1D2. **B,** Amount of IpaA and HVD1D2 in 1:1 and 1:2 complexes measured by densitometry. IpaA524-633:HVDlD2 ratio for the 1:1 complex (Dark, left), IpaA483-633:HVDlD2 for 1:1 complex (Light grey, center) and for 2:1 complex (Dark grey, right). Kruskal-Wallis Chi-squared test (p-value = 0.01458).

Figure 7 is a set of photographs and graphs showing the effect of A483 on the cell adhesion properties **A,** and **B,** Comparison between the total cell surface of GFP control and GFP-A483 cells. **A,** Measure of the total cell surface at three time points (25%/50%/75%) per recorded cell track for GFP control (Left panel) and GFP-A483 (Right panel) expressing cells. **B,** Kernel density (Gaussian) estimation function (Frequency) of the mean cell surface area ($\mu\eta^2$) between GFP control (Black lane -GFP) and GFP-A483 (pointed lane -IpaA_483) expressing cells. C, and **D,** Comparison between the time of division of GFP control and GFP-A483 cells showing that GFP-A483 cells are divided in two groups with different median times of division. C, Kernel density (Gaussian) estimation function (Frequency) of the time of division between GFP control (Black lane -GFP) and GFP-A483 (dotted lane -IpaA_483) cells. **D,** Box-plot comparing

the time between two divisions for both GFP control (GFP), Group I GFP-A483 (GFP-A483 I) and Group II GFP-A483 (GFP-A483 II) expressing cells. Kruskal-Wallis Chi-squared test (p-value = $1.8\,lxl\,0^{-06}$). **E,** Immunofluorescence analysis of C2.7 cells (CTRL) and GFP-A483 transfectants (A483). GFP fluorescence (IpaA); vinculin (vinculin); vinculin with enhanced contrast (vinculin X) and actin (actin) plated for 7 and 30 minutes on Fn-coated coverslips. **F,** Cells were plated for 15 min prior to washing and processing for DAPI staining. Box plot of the median number of adherent cells per sample for GFP control (4822 cells, n = 6) and GFP-A483 transfectants (6745 cells, n = 6). n.s.: p =0.132.

Figure 8 is a set of photographs showing the interaction between IpaA VBS3 and talin (H1-H4). **A,** IpaA constructs used in this study. The VBSs are depicted as boxes in IpaA full length (IpaA-FL), with the residue number corresponding to the first residue of the corresponding VBS. The first and last IpaA residues are annotated for the IpaA truncated derivatives. V5: V5 epitope. **B,** HeLa cells were transfected with IpaA derivatives, fixed and processed for immunofluorescence staining of the V5 epitope and talin. Representative fluorescence images are shown, with a larger magnification of the inset boxed in the left panels. Scale bar = 5 μιη. **C,** Native PAGE analysis of IpaA VBSs and talin H1-H4 interaction. 1. Purified talin H1-H4 was incubated with the indicated IpaA VBS peptides and analyzed using 6-18% gradient native PAGE. The talin H1-H4: IpaA VBS molar ratio is indicated, with 1 corresponding to a final concentration of 25 μM. The migration of talin H1-H4 and of the talin Hl-H4:IpaA VBS3 complex are indicated.

Figure 9 is a set of photographs and graphs showing the binding of IpaA VBS3 to talin with high affinity. **A,** Isothermal titration calorimetry (ITC) analysis of the interaction between talin H1-H4 and the indicated IpaA VBSs. The estimated KD are 15.17 μM and 174 nM for IpaA VBS1 and IpaA VBS3, respectively. **B,** ITC analysis of the interaction between the A483:HVD1 complex and talin H1-H4, KD = 2.94 μM. **C,** Native PAGE analysis of A483 and A524 interaction with talin H1-H4. Purified talin H1-H4 was incubated with A483 or A524 at the indicated talin Hl-H4:IpaA derivative molar ratio, with 1 corresponding to a final concentration of 25 μM.

Figure 10 is a set of photographs and graphs showing A483 ternary complex with HVD1 and talin H1-H4. Size exclusion chromatography (SEC) analysis of interaction between IpaA derivatives, talin H1-H4 and HVD1. Proteins indicated on the left were incubated for 60 minutes in column buffer prior to SEC analysis using a Superdex 75 column. Eluted fractions annotated above each lane were analyzed by SDS-PAGE using a 12.5% polyacrylamide gel and Coomassie blue staining. SEC analysis of A, Talin H1-H4 (top panel), talin H1-H4 and A524 (middle panel), talin H1-H4 and A483 (bottom panel); **B,** HVD1 and talin H1-H4 (top panel), HVD1 and A524 (middle panel), HVD1 and A483 (bottom panel); **C,** HVD1 and talin H1-H4 and A524 (top panel), HVD1 and talin H1-H4 and A483 (bottom panel). The traces represent the densitometry analysis of the indicated protein / protein complex species.

Figure 11 is a set of photographs and graphs showing Talin's requirement for IpaA-dependent *Shigella* anchoring to actin foci and invasion. HeLa cells were challenged with the indicated bacterial strain. Samples were fixed and processed for immunofluorescent staining of bacterial LPS (bacteria), talin and actin. The left panel corresponds to a lower magnification with the inset boxed. A, Representative micrographs of cells challenged with WT *Shigella* for the time indicated in minutes on the left, with a larger magnification of the inset boxed in the left panel. The arrows point to talin "cups" at bacterial cell contacts. Scale bar = 5 μιη. **B,** The average intensity of talin staining at actin foci was quantified and the recruitment expressed as a ratio relative to a corresponding cell control area. **C, D,** and **E,** Cells were treated with anti-talin siRNA prior to bacterial challenge. **C,** Relative intensity of actin foci. **D,** Representative micrographs of cells infected for 15 minutes with WT *Shigella.* Scale bar = 5 μιη. **E,** Percentage of internalized bacteria. **F, G,** and **H,** Cells were challenged for 15 minutes with the indicated bacterial strain. IpaA/FL-A: *Shigella ipaA* mutant complemented with full length IpaA; IpaA/DTBS: *Shigella ipaA* mutant complemented with IpaA AVBS3 (deleted for residues IpaA 489-511); IpaA/DCT: *Shigella ipaA* complemented with IpaA AVBSl-2 (deleted for residues IpaA 550-633); IpaA/DTBS-DCT: *Shigella ipaA* complemented with IpaA AVBSl-2-3 (deleted for residues IpaA 489-511 and 550-633). **F,** Representative micrographs for WT and *ipaA* infected cells. The arrows point to talin "cups" at bacterial cell contacts. Scale bar = 5 μιη. **G,** Average intensity of talin recruitment ± SD. **H,** Average percentage of

actin foci forming talin "cups" ± SD. Wilcoxon rank sum test (p-value = 1.319×10^{-5}). Pearson's Chi-squared test (p-value = 0.0006048).

Figure 12 is a set of photographs and graphs showing that IpaA A483 enhances vinculin-mediated cell adhesion compared A524 without inducing membrane ruffling. **A, B,** and **C,** Immunofluorescence analysis of C2.7 cells (Control), GFP-A483 transfectants (IpaA 483-633) and GFP-A524 transfectants (IpaA 524-633). GFP fluorescence (IpaA); vinculin (red); actin (green). **B,** and **C,** cells plated for 16 hours. **B,** The percentage of cells showing FAs with a small (Small), medium (Medium) and large (Large) FAs was scored. The results are representative of ncontrol = 37, nGFP-A483 = 27 cells and nGFP-A524 = 29 in 3 independent experiments. Distributions were compared using a Pearson's Chi-squared test. **C,** The percentage of cells showing few (None/Few), some (Some) and abundant (Abundant) membrane ruffling was scored. The results are representative of n(control) = 37, n(GFP-A483) = 27 cells and n(GFP-A524) = 30 in 3 independent experiments. Distributions were compared using a Pearson's Chi-squared test.

Figure 13 is a set of graphs showing that IpaA VBS3 promotes binding of the IpaA carboxyterminal domain to talin H1-H4. Size exclusion chromatography-multi angle light scattering (SEC-MALS) analysis of interaction between IpaA derivatives and talin Hl-H4. Indicated proteins were incubated for 60 minutes in column buffer prior to SEC analysis using a Superdex 200 10/300 GL Increase column. Traces: absorbance at 280 nm of the indicated proteins or complex species. Dotted lines: molecular mass of the indicated proteins or complexes determined by MALS.

Figure 14 is an image representing talin H1-H4 forms a covalent dimer in solution. SDS-PAGE (12.5%) electrophoresis analysis of a solution containing 20 μιη of purified talin H1-H4 protein denatured in the presence (left) or in the absence (right) of a reducing agent (1 mM β-Mercaptoethanol). The percentages of talin H1-H4 dimer and monomer were quantified by densitometry.

Figure 15 is a set of scheme, image and table showing that talin H46 (VBS3) a-helix (SEQ ID NO: 60) is an hybrid VBS/TBS *in vitro*. **A,** Sequence alignment of IpaA VBS3/TBS and endogenous talin H46 (VBS3) a-helix by Clustal W (1.83) multiple

sequence alignment. **B,** Talin-VBSs binding to the 4-helix bundle of talin. Native gel analysis showing binding of talin VBS3 to the talin 4-helix bundle H1-H4. Control: talin H1-H4 alone; tin H4: talin Hl-H4:talin H4; tin H46: talin Hl-H4:talin H46. The asterisks indicate complex formation. C, Isothermal titration calorimetry (ITC) analysis of the interaction between talin H1-H4 and talin H46 (VBS3) or IpaA VBS3 a-helixes. The estimated KD are 60 and 174.2 nM for talin H46 (VBS3) and IpaA VBS3/TBS, respectively.

Figure 16 is a set of graphs showing that IpaA VBS3/TBS targets vinculin- and talin-adhesion structures. HeLa cells were transfected with GFP-IpaA VBS3 and vinculin talin. Cells were fixed and processed for fluorescence staining of actin. Cells were treated with anti-vinculin or anti-talin siRNA prior to transfection with GFP-IpaA VBS3. Representative micrographs corresponding to a basal confocal plane are shown. Scale bar = 5 μιη. The area of focal adhesions **(A,** n > 1000, N = 2) and the number of focal adhesions per cell **(B,** n as indicated, N = 2) were quantified using a semi-automated protocol in ICY. Geometric mean ± 95% confidence intervals of measurements are shown. One-way Anova test (p-value < 0.001, ***).

Figure 17 is a set of graphs showing that talin is required for IpaA-dependent *Shigella* anchoring to actin foci and invasion. **A,** Talin-GFP and vinculin-mCherry transfected cells challenged with *Shigella* strains. Representative scatter plots are showing the intensity correlation of pixels with vinculin and talin co-localization at bacterial coats for WT and *IpaA* mutant strains. **B,** The median of the regression coefficient of the intensity of talin and vinculin at bacterial entry foci (n(WT)=33 and n(IpaA)=18) were measured as 0.80 ± 0.16 and 0.14 ± 0.04 respectively, and compared using a Wilcoxon rank sum test (p-value = 5 xlO^{-9}, ****).

Figure 18 is a graph showing the role of IpaA VBSs in talin and vinculin recruitment during *Shigella* invasion. HeLa cells were transfected with talin-GFP and challenged with *Shigella* strains for 30 minutes at 37°C. Cells challenged with the *IpaA* mutant strain complemented with full length IpaA (FL), vector alone (2.1), IpaAAVBS3 and IpaAAVBSl-2. The arrows point at talin coat structures surrounding invading bacteria. Scale bar = 5 μιη. Average percentage of actin foci forming talin-coat structures induced

by the indicated bacterial strain ± SD (Exp. Procedures). For each samples, n > 35 foci in at least three independent experiments. Chi-squared test with *post-hoc* comparison (FDR correction for p-value).

Figure 19 is a set of graphs and schemes showing that A483 increases the rate of force generation and cell adhesion strength. **A,** The parallel plates setup: the traction force (F) generated by a single cell is monitored by the deflection (d) of the flexible microplate of calibrated stiffness (k). The flexible microplate can also be used to pull on the cell to test its adherence to the plates. **B,** Representative traces showing single-cell traction forces as a function of time. CTRL: control cells; IpaA-VBD: GFP-A483 transfectants. The rates (dF/dt) were measured as 0.12 and 0.40 for control cells and GFP-A483 transfectants, respectively, indicating faster force generation in GFP-A483 transfectants. **C,** The force F (in nN) and contact area A (arrow, in $\mu\iota\eta^2$) at detachment are recorded to determine the "adhesion stress" a=F/A. σ= 0.3 and 6.5 $\eta N/\mu\eta\iota^2$ for control cells and GFP-A483 transfectants, respectively, indicating an increased cell adhesion strength in GFP-A483 transfectants.

Figure 20 is a set of graphs and images showing that A483 enhances vinculin-mediated cell adhesion in the absence of mechanosensing. **A,** Suspended cells were incubated for the indicated time with fibronectin-coated wells prior to washing. The number of adherent cells is indicated. **B,** Cells were perfused in a microfluidic chamber and allowed to adhere for 1 minute prior to shear stress application. Boxplot of the median resistance to shear stress for control (34 cells, N = 2) and GFP-A483 (15 cells, N = 1). ***: p = 0.00039. **C,** Cells were plated on fibronectin-coated PDMS membranes with the indicated stiffness. Note the formation of myotubes in A483 transfectants at low substrate stiffness. CTRL: control cells; IpaA-VBD: GFP-A483 transfectants.

Figure 21 is a set of images showing that IpaA prevents the disassembly of focal adhesions induced by the relaxation of the actomyosin contraction. MEF cells were transfected with focal adhesion proteins Paxillin-Cherry (PAX), Vinculin-Cherry (VIN) or VASP-Cherry (VASP). CTRL: single transfection; A483: cells were co-transfected with GFP-A483. The intensity of focal adhesions proteins is shown at the indicated time points in seconds after the addition of the ROCK inhibitor Y-27632 by TIRF microscopy.

A483 prevents the destabilization of FA markers induced by the ROCK inhibitor observed in control cells.

Figure 22 is a set of schemes depicting a model for the roles of talin and vinculin in mechanosensing and mechanotransduction during integrin-mediated cell adhesion. In a first step of mechanosensing (left panel), myosin II-mediated cytoskeletal tension promotes the FAK-mediated phosphorylation of paxillin, and subsequent association of phosphorylated paxillin with vinculin. Signaling linked to this complex in response to mechanosensing leads to the recruitment of talin, under its folded form. In a second step of mechanotransduction (right panel), each vinculin molecule contributes to tethering a talin VBS with F-actin, strengthening cytoskeletal linkage therefore enabling higher traction forces.

Figure 23 is a set of EDC crosslink maps from mass spectrometry analysis (LC-ESI/TOF) of vinculin domains D1 **(A** and **B)** and D1D2 **(C** and D) with A524 **(A** and **C)** and A483 **(B** and **D)** following the extraction of HVDlD2:IpaA 1:1 complexes separated by SDS-PAGE electrophoresis. Black dashed box: interaction of the IpaA VBS3 with the first bundle of the vinculin D2 domain (HVD2).

Figure 24 is a set of micrographs and graph, showing the inhibition of melanocytes migration and matrigel invasion upon transfection with A483 or A524. **A,** Representative micrographs of melanocytes after transfection with A483, A524 or Mock-transfection (CTRL), showing the spread of A483 and A524-transfected cells. The transfection efficacy is estimated to about 20-30%, as scored by GFP fluorescence. **B,** Number of cells which migrated across a matrigel, for A483-, A524- and Mock (CTRL)-transfected melanocytes. The graph clearly indicates a strong inhibitory effect of both A483 and A524 on the migration of these melanocytes. Results are expressed as the average percentage ± SD relative to control cells of three independent experiments. CTRL: 1232 cells, A524: 96 cells; A483: 120 cells. Dunn rank test (*: $p < 0.025$).

EXAMPLES

The present invention is further illustrated by the following examples.

Materials and Methods

Generation of expression constructs ***(Fig. IB)***

Human vinculin constructs were generated by polymerase chain reaction using an upstream 5' GCGCATATGCCAGTGTTTCATACG-3 ' (SEQ ID NO: 17) and two downstream 5'-CGTCGACTCACCAGGCATCTTCATC-3' (SEQ ID NO: 18) / 5'-CGTCGACTCAGTGTACAGCTGCTTTG-3 ' (SEQ ID NO: 19) primers for HVD1 (residues 1-258) and HVD1D2 (residues 1-492) respectively, using a plasmid containing full-length human vinculin as template. The amplified sequences were digested with Ndel/Sall (New England Biolabs) restriction enzymes and ligated (T4 ligase, New England Biolabs) into a pET15b (Novagen) plasmid following manufacturer's recommendations. The HVD 1D2 K276R and E305Q point mutations were obtained using the QuickChange II (Stratagene) method with 5'-GCATTGGCCTCCATAGACTCCCGTCTGAACCAGGCCAAAGG-3' (SEQ ID NO: 20) / 5'-CCTTTGGCCTGGTTCAGACGGGAGTCTATGGAGGCCAATGC-3 ' (SEQ ID NO: 21) and 5'-GGCCATCAGACAGATCTTAGATCAAGCTGGAAAAGTTGGTG-3' (SEQ ID NO: 22) / 5'-CACCAACTTTTCCAGCTTGATCTAAGATCTGTCTGATGGCC-3 ' (SEQ ID NO: 23) primers for HVD1D2 K276R and HVD1D2 E305Q respectively.

IpaA 483-633 (A483) constructs were generated by polymerase chain reaction using 5'-GCGATATCATGGCCAGCAAAGG-3 ' (SEQ ID NO: 24) / 5'-GCGCGGCCGCTTAATCCTTATTGATATTC-3' (SEQ ID NO: 25) and 5' GGCGAATTCCCGGAGACACATATTTAACACG-3' (SEQ ID NO: 26) / 5'-GCCGTCGACTTAATCCTTATTGATATTCT-3' (SEQ ID NO: 27) primers for GFP-A483 (SEQ ID NO: 28) and GST-A483 (SEQ ID NO: 29) respectively. GFP-A483 amplicon was cloned into a pcDNA3.1 NT-GFP Topo (Invitrogen) following manufacturer's recommendations. GST-A483 amplicon was digested with EcoRI/Sall restriction enzymes (Invitrogen and New England Biolabs) and ligated (T4 ligase, New

England Biolabs) into a pGEX-4T-2 (GE Lifesciences) T7 expression plasmid. GST-A524 expression plasmids used in this work were previously described by Romarao *et al.* *(FEBSLett.* **2007** Mar 6;**581(5**):853-7).

Yeast double hybrid analysis

The yeast two-hybrid analysis was performed using IpaA or IpaA 1-482 as baits to screen a human placental RP1 library, according to standard procedures and the Y2H protocole (Hybrigenics services).

Protein purification

BL21 (DE3) chemically competent *E. coli* (Life Technologies) were transformed with pGEX 4T2-A483 (A483 is encoded by SEQ ID NO: 6), pGEX 4T2-A524 (A524 is encoded by SEQ ID NO: 32), pET15b-HVDl (HVDl is encoded by SEQ ID NO: 33) or pET15b-HVDlD2 (HVD1D2 is encoded by SEQ ID NO: 34) plasmids. The HVDl and HVD1D2 were purified essentially as described (Izard *et al.*, **2006;** Papagrigoriou *et al.*, **2004.** *EMBO J.* **23(15**):2942-51). For the IpaA derivatives, bacteria were grown until $\mathbf{OD_{600nm}}$ = 1.0 and further grown in the presence of 0.5 mM IPTG for another 3 hours. Bacteria were pelleted and washed in binding buffer 25 mM Tris pH 7.4, 100 mM NaCl and 1 mM β-Mercaptoethanol, containing Complete™ protease inhibitor. Bacterial pellets were resuspended in 1/50th of the original culture volume and lyzed using a cell disruptor (One shot model, Constant System Inc.). Proteins were purified by affinity chromatography using a GSTrap HP affinity column (GE Healthcare) followed by size exclusion chromatography (HiLoad S200, GE Healthcare). Protein concentration was determined using the BCA assay (Thermoscientific). Samples were dialyzed in binding buffer and stored aliquoted at -80°C concentrations ranging from 1 to 10 mg/ml.

Native-PAGE analysis

Talin H1-H4 and IpaA peptides/proteins were incubated in binding buffer for 1 hour at 4°C. After incubation, the protein/peptides were mixed in a 2x Native loading buffer (62.5 mM Tris pH 6.8, Glycerol 25%) and separated by Tris-Glycine Native-PAGE electrophoresis. Gels were stained using standard colloidal Coomassie stain.

SEC-MALS

The purified proteins IpaA 483-633 (A483), IpaA 524-633 (A524) and Talin H1-H4 (TlnHl-H4) were used at 20 μM equimolar concentrations and incubated at 4°C for one hour in binding buffer (25 mM Tris-HCL pH 7.0, 100 mM NaCl and 1 mM β-Mercaptoethanol). The protein mixtures (200 μL) were analyzed by size-exclusion chromatography (SEC) on a Superdex 200 10/300 GL (GE Healthcare) using a Shimadzu Prominence HPLC. Multi-angle laser light scattering (MALS) was measured with a MiniDAWN TREOS (Wyatt Technology). Refractometry was monitored using an Optilab T-rEX (Wyatt Technology).

Crystallization, structure determination, and crystallographic refinement

The IpaA/talin complex was screened against commercial crystallization solutions using the TTP LabTech Mosquito crystallization robot. Multiple crystallization conditions were identified. Reproducible plate shape crystals were grown in 0.1 mM Li sulfate, 0.1 mM Tris/HCl (pH 8.5), 30% PEG4000 at room temperature. The crystals were harvested and frozen after briefly soaking them into the reservoir solution supplemented with 20% ethylene glycol. A complete X-ray diffraction data set to 2.3 A Bragg spacings was collected at 100 K using a wavelength of 1 A on the SER-CAT beamline 22ID at the Advanced Photon Source at Argonne National Laboratory (Argonne, IL). The dataset was merged and scaled using AutoProc and phased using Phaser. The refinement of the model structure was conducted with BUSTER. Manual rebuilding and model adjustment during the refinement was conducted with COOT. The final structure was validated with MolProbity.

ELISA interaction assay

96-well Maxisorp (Nunc) ELISA plates were coated with 30 nM full-length vinculin, vinculin constructs or IpaA proteins in binding buffer (25 mM Tris pH 7.4, 100 mM NaCl and 1 mM β-Mercaptoethanol). The wells were blocked with PBS-BSA 2%, washed and incubated with half series dilutions of IpaA or vinculin proteins in binding buffer + 0.2% BSA at room temperature for one hour. After incubation the plates were washed and incubated with an anti-IpaA (dilution: 1/2000) polyclonal primary antibody (Tran Van

Nhieu *et al,* **1997.** EMBO J. **16(10)**:2717-29) or anti-vinculin (dilution: 1/2000) ml 1.5 monoclonal primary antibody (ref. V4505, Sigma-Aldrich) in binding buffer + 0.2% BSA for one hour at room temperature. Plates were washed and incubated with an HRP-coupled secondary anti-rabbit or anti mouse Ig antibodies (dilution: 1/32000, Jackson ImmunoResearch) for one hour. The reaction was revealed by adding 100 µL of Tetramethylbenzidine (TMB) Liquid Substrate (ref. T0440, Sigma-Aldrich) for 15 minutes, stopped by adding 50 µL of 0.66 N H_2SO_4 (VWR), and the plates were read at 450 nm wavelength (Dynatech MR400).

Blue native (BN)-polvacrylamide gel electrophoresis (PAGE)protein native gel analysis and complex crosslinking

25 µM of vinculin constructs were incubated with different molar ratios of IpaA proteins in a 1x BN-PAGE buffer (250 mM aminocaprionic acid, 25 mM Bis-Tris pH 7.0) at 4°C for one hour. The protein mixtures were separated in a one-dimension native BN-PAGE electrophoresis. For vinculin-IpaA protein ratio assay, vinculin-IpaA bands containing the complexes separated by BN-PAGE were cut, sliced and boiled in a denaturant 2x Laemmli SDS buffer, then separated by standard SDS-PAGE electrophoresis. The second dimension SDS-PAGE gels were stained (colloidal Coomassie staining) and the density of the bands corresponding to vinculin and IpaA proteins (Image J) compared. The normalized vinculinTpaA ratio of the complexes was compared using a non-parametric Kruskal-Wallis rank sum test (R statistical software).

For vinculin-IpaA complex crosslinking, the bands containing the complexes were cut, sliced and electroeluted in native conditions (15 mM Bis-Tris pH 7.0, 50 mM Tricine) inside a closed dialysis membrane (SpectraPor). The soluble complexes were recovered and their buffer exchanged twice into an amine-free crosslink buffer (25 mM Hepes, 100 mM NaCl) using 10MWCO ZEBA desalting columns (Thermo Scientific). The fractions containing the complexes were incubated for 1 hour at 4°C with 10 mM sulfo-NHS and 5 mM EDC (Sigma-Aldrich) following manufacturer's recommendations. The crosslink reaction was stopped by adding 50 mM Tris pH 7.4, incubated for 20 minutes and denaturated in 2x SDS Laemmli buffer for 5 minutes at 95°C.

Talin H1-H4 and IpaA peptides/proteins at different molar ratios were incubated in binding buffer (25 mM Tris pH 7.4, 100 mM NaCl and 1 mM β-Mercaptoethanol) for 1 hour at 4°C. After incubation, the protein/peptides were mixed in a 2x Native loading buffer (62.5 mM Tris pH 6.8, Glycerol 25%) and separated by Tris-Glycine Native-PAGE electrophoresis. The Native PAGE electrophoresis gels were stained using standard colloidal Coomassie stain.

Immunostaining and cell adhesion kinetics

C2.7 mice myoblasts cells were seeded at $2.5x10^4$ cells in 5 mm rounded cell culture coverslips. Cells were transfected with 3 µg of pcDNA3.1 NT GFP-A483 plasmid, with 6 µL JetPEI transfection reagent (Polyplus) overnight following manufacturer's recommendations. Control and GFP-A483 transfected cells were fixed (3.7% PFA-PBS), permeabilized (0.1% Triton X-100, Sigma-Aldrich) and incubated with a 1/200 monoclonal ml 1.5 anti-vinculin antibody (ref. V4505, Sigma-Aldrich). Coverslips were washed and incubated with a 1/200 anti-mouse secondary antibody coupled to Alexa 546 (Jackson Research) and 1/200 Phalloidin A633 (Invitrogen), washed and mounted on slides using Dako mounting medium (Invitrogen). Samples were observed using a confocal microscopy with rotary disc (Roper Scientific®). Peripheral and ventral focal adhesion sizes (vinculin) were separated in three different categories (Small/None, Medium and Large) and the size of their focal adherences scored from three independent experiments. The focal adhesion size's distributions between the control and GFP-A483 transfected cells were compared using a Pearson's Chi-squared test.

C2.7 mice myoblasts cells were seeded at $2.5x10^5$ cells in a 10 cm cell culture petri dish (TPP). Cells were transfected with 15 µg of pcDNA3.1 NT GFP-A483 plasmid, with 45 µL JetPEI transfection reagent (Polyplus) overnight following manufacturer's recommendations. Control and transfected cells were detached and washed in a large volume of DMEM/Hepes (ref. 21063-045, Life Technologies) with 0.2% BSA and 50 ng/mL LPA (ref. L7260, Sigma-Aldrich) without FCS. Some 15,000 control/IpaA cells per well were seeded (DMEM/BSA/LPA, no FCS) in 24-well plates added with 5 mm rounded coverslips coated with 25 µg/mL fibronectin and blocked with 2% BSA-PBS lx. Plates containing cells were centrifuged at 1000 g for 2 minutes, incubated at

37°C and fixed (3.7% PFA-PBS) at different time points. Coverslips were stained with an anti-vinculin antibody + Phalloidin as described before (Immunostaining) or with a solution containing 1/200 DAPI (1 mg/mL) for number-of-cells adhesion tests. All coverslips were washed and mounted on slides using Dako mounting medium (Invitrogen) and observed using a confocal microscopy with rotary discs (Roper Scientific®).

FACS sorting

C2.7 mice myoblasts cells were seeded at 2.5-5xl0 ^{5} cells in a 10 cm cell culture petri dish (TPP). Cells were transfected with 15 µg of pcDNA3.1-GFP or pcDNA3.1-NTGFP A483 plasmids, with 45 µL JetPEI transfection reagent (Polyplus) overnight following manufacturer's recommendations. Control and transfected (GFP or GFP-A483) cells were detached and washed in a large volume of DMEM + Hepes (ref. 21063-045, Life Technologies) with 0.2% BSA without FCS, centrifuged and -lxlO ^{6} cells recovered in 2.5 mL DMEM 0.2% BSA medium for sorting. Flow cytometer FACSARIA II (BD Biosciences) was washed and the lasers and sorting system calibrated following manufacturer's recommendations. GFP and GFP-A483 transfected populations were sorted (Mode: Purity) and recovered in DMEM + 10% FCS with lx Penicillin/Streptomycin (Invitrogen). Around 15000 control GFP and GFP-A483 sorted cells were seeded per well in 24-well cell culture plates and left overnight for recovery.

Live cell videomicroscopy and tracking

GFP and GFP-A483 sorted-cells were washed and added with 600 µL of sterile mineral oil (Ibidi). The plates were installed in a 37°C 5%-C0 $_{2}$ chamber of a Leica DRMB microscope and cell-containing fields were selected at random using a 20x objective in phase contrast. One acquisition was made each 3 minutes over 24 hours for control GFP and GFP-A483. Division, migration and cell surface parameters were obtained from four independent experiments. All statistical analysis was done in R statistical software. The mean velocity of migration was measured as the whole distance traveled by the cell divided by the time of migration for both GFP control (n(GFP) = 268) and GFP-A483 (n(A483) = 199) for all tracks followed for at least 5 hours. Medians were compared using

a Wilcoxon rank sum test and dispersion by Median absolute dispersion (MAD) parameter. The measure of the two-dimensional (x and y) distance traveled by these cells was obtained from a function that quantifies the Median Square of displacement:

$$MdSD = \mathbf{f(t)} = ([(x\text{-}xo)tf + [(y\text{-}yo)t]^{2})t,i$$

MdSD: Median Square of displacement (μη/hΠ).

Md: Median of the cell square of displacement population i at t time point.

x: X coordinate of the nucleus of the cell at t time point.

xo: X coordinate of the nucleus of the cell at zero time point.

y: Y coordinate of the nucleus of the cell at t time point.

yo: Y coordinate of the nucleus of the cell at zero time point.

The root square of MdSD over time for GFP control and GFP-A483 was plotted over time and both fitted by linear regression. The slopes of the linear fit were compared using an ANCOVA test (linear model). The median cell surface was quantified as the mean of the surface for three time points (25%, 50% and 75%) of the whole cell track for control GFP and GFP-A483 cells, and dispersion measured by the Median absolute dispersion (MAD).

Microfluidics cell adhesion assay

A microfluidic system similar previously was installed in a 37°C temperature controlled Leica DRMB microscope and the microfluidic channels observed with a 20x phase contrast objective. The microfluidics chips were washed extensively with a sterile PBS 1x solution, coated with 25 μg/mL fibronectin (Calbiochem) and blocked with PBS lx, 2%> BSA (Sigma-Aldrich). Cell containing solution was applied to the chip system driven by hydrostatic pressure, and the flow verified. Hydrostatic pressure equal zero point was determined (zero flow) and the difference of pressure applied on the circuit was then equivalent to the difference of height between the zero point (h=0) and the final height using a calibrated stand.

Size exclusion chromatography

The purified proteins HVD1, A483, A524 and Talin H1-H4 were used at an equimolar concentration of 20 μM and incubated at 4°C for one hour in binding buffer. The protein

mixtures (200 µL) were injected in a FPLC chromatography system (FPLC Pharmacia) at 16°C in a Superdex 75 HR 10/30 column (GE Healthcare) previously equilibrated with 10 volumes of binding buffer. The SEC system was calibrated with a Low Molecular Weight Gel Filtration Kit (ref. 17-0442-01 , GE Healthcare) and the hydrodynamic radius (Angstroms) of the reference proteins (BSA, OVA, ChyA and RibA) correlated with the elution volume **(Ve)** using an Euler exponential equation: Hydrodynamic radius (Ang) = 210.46 $e^{-0.189}$ **Ve** (mL), with a correlation squared coefficient of R^2 = 0.9973. Fractions corresponding to elution volume 8-10 mLs were recovered, and analyzed by SDS-PAGE using 15% polyacrylamide gels.

Isothermal titration calorimetry

Vinculin, IpaA proteins and peptides were extensively dialyzed in binding buffer (25 mM Tris pH 7.4, 100 mM NaCl and 1 mM β-Mercaptoethanol). The final concentration of all proteins/peptides was measured using a BCA test (Thermo Scientific). Talin-IpaA binding was measured by microcalorimetry using an ITC200 calorimeter (MicroCal) at 25°C. 200 µL of 20-100 µM of talin H1-H4 protein was added to the cell and binding in the presence of different concentrations of IpaA VBS1, VBS2 or VBS3 peptides was measured. In other experiments, 200 µL of a 16 µM equimolar mix of A483:HVD1 was incubated for one hour at room temperature prior to addition to the cell. Binding to talin H1-H4 (100 µM) was measured. Typically, 20-40 injections of 2 µL of ligand were made with intervals of 320 seconds between each addition, with a reference power of 12 µcal/sec. Data were analyzed using the MicroCal software provided by manufacturer.

Cell culture and Shigella bacterial strains

All *Shigella flexneri* strains used in this work were previously described. *Shigella* WT (Tran Van Nhieu *et al.,* **1997),** the *ipaA/lpaA* mutants complemented with a recombinant plasmid encoding full length IpaA, *ipaA/pcr2A* containing an empty pCR2.1 plasmid, *ipaA/*IpaAACter with IpaA deleted for the carboxy terminal region Δ550-633 were described in Izard *et al.,* **2006.** *ipaA*/IpaAAVBS3 with IpaA deleted for residues Δ489-511 and ipaA/IpaAAA with IpaA deleted for residues Δ489-511 and Δ550-633 were described in Park *et al.,* **2011.** *J. Biol. Chem.* **286(26)**:23214-21. Bacterial strains were

cultured in trypticase soy broth (TCS) medium at 37°C, when specified; antibiotics were added at the following concentrations: carbenicilin 100 µg/mL, kanamycin 20 µg/mL. HeLa cells were from ATCC. HeLa cells were incubated in RPMI (Roswell Park Memorial Institute) medium containing 5% FCS (fetal calf serum, Gibco®) in an incubator with 5% CO_2.

siRNA transfection

HeLa cells were seeded at a density of 10^5 cells in wells containing a 22 x 22 mm coverslip, in a 6-well plates. The following day, cells were transfected with of anti-talin 1 siRNA (Stealth Select RNAi, catalog no. 1299003, Invitrogen, oligo 804; sequence: 5-CCAAGAACGGAAACCUGCCAGAGUU-3 ' (SEQ IDNO: 30)) duplex at the indicated concentrations and time periods. The efficiency of talin expression inhibition was tested using cell crude lysates and anti-talin Western-blot analysis.

Cell challenge with Shigella strains

HeLa cells were seeded at lxl 0^5 cells in 33 mm-diameter wells in diameter containing a sterile coverslip. One day after and immediately before infection, cells were washed three times with EM medium at 22°C. Different *Shigella* bacterial strains were grown in TCS medium to exponential phase, washed in EM medium and diluted to OD_{600nm} = 0.1. The IpaA *Shigella* strains were incubated for 10 minutes in the presence of 10 µg/mL polylysine, washed and used to infect HeLa cell line prepared as described before. Infected cell cultures were incubated for 15 minutes at 22°C to allow adhesion of the bacteria, and then transferred at 37°C for 5, 10, 12, 15 and 30 minutes. After incubation, the different infected cell lines were fixed (3.7% PFA-PBS), permeabilized (0.1% Triton X-100, Sigma-Aldrich) and incubated with 1/400 monoclonal 8d4 anti-talin antibody (ref. T3287 Sigma-Aldrich). Coverslips were washed and incubated with a 1/200 anti-mouse secondary antibody coupled to Alexa 546 (Jackson ImmunoResearch) and 1/200 Phalloidin A633 (Invitrogen), washed and mounted on slides using Dako mounting medium (Dako, Agilent Technologies). Samples were observed using a confocal microscopy with rotary disc (Roper Scientific®) using a 63x objective.

Samples were also observed in an epifluorescence microscope DMRIBe (Leica) equipped with a 63x objective and an EM-CCD Cascade 512B camera (Roper Scientific). For each sample, more than thirty stack fields were acquired for each condition in two independent experiments. The results are expressed as the fluorescence intensity ratio of talin between the foci and the cytoplasm (R) using the following formula:

$$R = (I_F - I_o) / (I_c - I_o)$$

IF: Medium intensity of talin at the entry foci.

Ic: Average intensity of talin in the cytoplasm of the infected cell.

Io: Medium intensity of background in a region containing neither cells nor bacteria.

Bacterial-induced foci formation and invasion

Control and siRNA talin transfected cells were infected with WT *Shigella* strain for 30 minutes, fixed, permeabilized and incubated with 1/500 polyclonal anti-LPS antibody (Flex V). Coverslips were washed and incubated with a 1/200 anti-rabbit secondary antibody coupled to Alexa 546 (Jackson ImmunoResearch) and 1/200 Phalloidin A647 (Invitrogen), washed and mounted on slides in Dako mounting medium (Dako, Agilent Technologies). Samples were analyzed using an Eclipse Ti microscope (Nikon) equipped with a 63x objective, a CSU-X1 spinning disk confocal head (Yokogawa), and a Coolsnap HQ2 camera (Roper Scientific Instruments), controlled by the Metamorph 7.7 software. The percent of internalized bacteria was scored as the ratio between the number of internalized bacteria per foci and the number of total bacteria in found in the foci (n(control) = 27, n(siRNA^{Tln}) = 23) in two independent experiments. The percent of bacteria showing an actin intimate recruitment was scored as the ratio between the number of bacteria with actin intimate recruitment per foci and the number of total bacteria in the foci in two independent experiments. For control and anti-talin siRNA transfected cells, the percent of internalized bacteria and the percent of bacteria showing an actin intimate recruitment was compared using a non-parametric Wilcoxon rank sum test.

To quantify the size distribution of *Shigella* actin foci, we divided the foci in three classes: Small, Medium and Large. The size of control (n(control) = 27) and siRNA talin (n(siRNA^{Tln}) = 23) *Shigella-induced* foci were classified into this three categories,

arranged in a contingency table and its distributions compared using a Pearson's Chi-squared test.

Example 1: Vinculin supra-activation by the Shigella type III invasion effector IpaA

The Shigella type III effector IpaA induces a novel conformation of vinculin unveiling additional binding sites.

We first measured the interaction of A483 (SEQ ID NO: 5) or A524 (SEQ ID NO: 15), containing IpaA VBS1/3 or IpaA VBS1/2, respectively, with full-length vinculin (HV) in solid phase assays. Classical hyperbolic saturation binding curves were obtained for A483 and A524. Strikingly, a large difference was observed in the plateau between the two proteins, indicating that human vinculin construct (HV) (SEQ ID NO: 16) harbored more binding sites for A483 (SEQ ID NO: 5) compared to A524 (SEQ ID NO: 15). These results were unexpected, since vinculin activating ligands have been described to bind to a single site on the D1 domain of vinculin. When reverse binding assays were performed, whereby IpaA constructs were immobilized on the solid phase and HV used as a soluble ligand, similar saturation curves were observed for A483 and A524, plateauing at similar ligand concentrations (25 nM) **(Fig. 1 C** and **D).** These results suggested that, despite the presence of the IpaA VBS3 in A483, the stoichiometry of A483:HV and A524:HV was similar at saturating concentrations of HV, probably due to steric hindrance. The difference in the A483:HV stoichiometry observed for A483 depending on its immobilized or soluble state was consistent with more binding of soluble A483 molecules per immobilized HV molecule. This suggested that while A524 bound to the HVD1 domain (SEQ ID NO: 11), the presence of IpaA VBS3 revealed novel sites on other HV domains. The immunoreactivity of the anti-IpaA antibodies was found to be similar for A482 and A524 **(Fig. 5).** To test this, we assayed the binding of A483 to vinculin derivatives containing only amino acid residues 1 to 257 of HV (HVD1) (SEQ ID NO: 11) or amino acid residues 1 to 484 of HV (HVD1D2) (SEQ ID NO: 35) As shown in **Fig. IE,** A483 bound to HVD1 and HVD1D2, but as for HV, a higher binding plateau was observed for HVD1D2 relative to HVD1, consistent with novel binding sites on the vinculin D2 domain (HVD2).

Similar to HVDl (SEQ ID NO: 11), HVD2 (SEQ ID NO: 12) contains two four-helical-bundles, with the amino-terminal bundle (D2a) sharing most similarities with the first bundle to HVDl (Dla) **(Fig. 3).** The IpaA VBS3 peptide alone was proposed to interact with the HVDl first bundle **(Fig. 1A).** However, in the context of A483, this interaction is unlikely to occur because of the very high affinity of IpaA VBS 1 associated with IpaA VBS2 for the first and second helical bundles of HVDl, respectively **(Fig. 1A).** We reasoned that when constrained during the A483:HVD1D2 interaction, IpaA VBS3 could interact with the HVD2 first helical bundle. We thus designed mutations predicted from the structural alignment of the HVDl and HVD2 first bundles, based on the previous identification of contact residues between IpaA VBS 1 and the first bundle of HVD 1. An HVD1D2 derivative containing the conservative mutations K276R and E305Q in the D2 domain was generated and tested for binding to A483. As shown in **Fig. IF,** A483 bound to HVD1D2-K276R and HVD1D2-E305Q with a similar affinity as HVD1D2, but saturation occurred at a lower plateau indicating a decreased number of binding sites.

Together, the results are consistent with the IpaA VBS3 unveiling new sites on the vinculin D2 domain.

We next studied the formation of different IpaA-vinculin complexes in solution using BN-PAGE native gels. One complex was observed when HVDl was incubated with A483, or when HVDl or HVD1D2 was incubated with A524 at the lowest molar ratio of 1:0.025 **(Fig. 2A-C,** arrowhead 1:1). When HVD1D2 was incubated with A483, however, additional complexes could be observed at molar ratio of 1:1 and above **(Fig. 2D,** arrowheads). The analysis of these complexes following protein band excision from the BN gel, by further analysis by SDS-PAGE and Coomassie staining revealed a stoichiometry consistent with 1:1 for complexes observed in all samples at a 1:0.25 molar ratio **(Fig. 6A-B).** In contrast, the additional HVD1D2:A483 complex showed a 1:2 stoichiometry **(Fig. 2D,** arrowhead; **Fig. 6B).** These results were consistent with the solid-phase assays indicating that A483 binds to additional sites on HVD2. The sequential formation of 1:1 and 1:2 HVD1D2:A483 complexes with increasing molar ratio suggested allosteric changes, whereby binding of A483 to HVDl occurred first, revealing an additional binding site on HVD2.

To map interactions between A483 and HVD1D2, protein complexes eluted from native gels were cross-linked, subjected to proteolysis and analyzed using LC-mass spectrometry. The proteolytic digestion profiles of individual proteins compared to the HVD1D2:A483 1:2 complex shown in **Fig. 3,** were clearly indicative of protection consistent with interaction between A483 and HVD1D2. Protection was observed for the all IpaA VBS (1, 2 and 3) when in complex with HVD1D2, which was not observed for A483 alone **(Fig. 3).** Protection was observed in the first four-helix-bundle of HVDl (Dla), as expected from previous structural characterization of the IpaA VBS1:HVD1 complex. In contrast to what could be expected from the structural characterization of IpaA VBS2 in complex with HVDl, however, little difference in protection could be detected in the second HVDl bundle between the proteins alone and the cross-linked complex, which may be explained by the low density of reactive groups exposed to the cross-linking agent in this particular bundle. Interestingly, protection was observed for IpaA VBS3 and the first four-helix-bundle of HVD2 (D2a) in the HVD1D2:A483 1:2 complex **(Fig. 3).** These results suggest an interaction between IpaA VBS3 and the first bundle of HVD2, consistent with the solid phase and native gel assays' results.

Analysis of the cross-linked peptides enhanced intermolecular links between A524 or A483 and HVDl or HVD2 **(Table 1** and **Fig. 23).** For the A524:HVD1 complex, peptide links were detected consistent with the "canonical" conformer expected from previous structural studies indicating interactions between IpaA VBSl and VBS2 with the HVDl first and second four-helix-bundles, respectively. Links confirming this canonical interaction were observed for all complexes. Isolated links could be detected, suggestive of a conformer with an opposite interaction between A524 and HVDl, with IpaA VBSl interaction and IpaA VBS2 with the second bundle and first bundle of Dl, respectively. For the A524:HVD1D2 complex, two additional links were also between IpaA VBS 1 and the D2 second bundle. For A483, an important number of links indicated the existence of the opposite-interaction conformer with IpaA VBS3 interacting with the D1 first bundle and IpaA VBSl interacting with the second bundle of D2. This conformer is consistent with the similar mode of interaction between IpaA VBSl or VBS3 and HVDl (Park *et al.,* **2011),** and further suggests that A483 can engage HVD1D2 in two opposite orientations. Importantly, in the canonical interaction where IpaA VBSl and VBS2

interact with the HVD1 first and second bundle, respectively, IpaA VBS3 was found to interact with the first bundle of D2. These results are consistent with the solid phase and native gel assays' results, indicating the unveiling of binding sites on the HVD2 domain.

HVD1D2 with IpaA A483/A524				HVD1 with IpaA A483/A524			
HVD1D2:A483		HVD1D2:A524		HVD1:A483		HVD1:A524	
HVD1D2	**A483**	**HVD1D2**	**A524**	**HVD1**	**A483**	**HVD1**	**A524**
E31	K150	E200	K109	E60	G-5	E200	K17
E200	K143	E31	K109	D67	G-5	E60	K18
E128	K135	E200	K102	E128	G-5	E200	K56
K464	D112	E128	K94	E200	G-5	K170	K71
K170	D112	K170	D81	E240	K16	K170	D72
K352	E108	E66	K77	E66	K31	D67	K77
K170	E108	K366	D75	D67	K31	K170	D81
E100	K97	K173	D75	E235	K31	K173	D81
E66	K97	K464	E67	E240	K31	E200	K94
E200	K89	E66	K60	E66	K58	E240	K102
E66	K80	E200	K56	E31	K60	E200	K105
E60	K80	E66	K56	E200	K60	D39	K109
K59	D79	E60	K39	E200	K80	E31	K109
K170	D76	K173	D35	E200	K89	E28	K109
K219	E72	K170	E31	E200	K97		
K170	E72	E128	K19	G1	E108		
E66	K60	E128	K17	K170	D112		
E66	K58	E66	K7	K170	D113		
E66	K48			D67	K118		
E128	K44			E128	K135		
E66	K44			E200	K143		
E66	K31						
E66	K16						
K366	D2						
D389	G-5						
D361	G-5						
E181	G-5						
D176	G-5						
D121	G-5						
D67	G-5						
E60	G-5						

Table 1. Cross-linked residues of the interactions between vinculin and IpaA domains.

Vinculin is involved in cell proliferation, migration, as well as in adhesive processes with the extracellular matrix and at cell-cell junctions. The A483-mediated unveiling of sites

on the vinculin D2 domain is expected to promote strong effects on these vinculin-dependent processes. Consistently, when cells were transfected with GFP-A483, large vinculin-rich peripheral and ventral focal adhesions (FA) could be detected, that were not present in control cells **(Fig. 4A-B).** An increase in the cell surface was also observed in GFP-A483 transfected cells, which showed a median surface of 1846.9 ± 655 μm^2 as opposed to 1156.2 ± 767 μm^2 for GFP control cells, indicative of the higher adhesive properties of GFP-A483 transfectants **(Fig. 4F, Fig. 7A-B).**

To test the effects of GFP-A483 on dynamic processes, cells were FACS-sorted following transfection and analyzed by time-lapse videomicroscopy and the time between two division events was determined. The density profile of division times for GFP-transfected cells showed a gamma distribution, with a median time of 10.2 ± 1.45 hours **(Fig. 4C** and **Fig. 7D;** 93 cells, N = 4). In contrast, the density profile of the GFP-A483 population showed two distinct groups, one with a median time of not statistically different than that of control cells (30 cells, N = 4), and another considerably longer of 17.1 ± 1.6 hours (10 cells, N = 4) **(Fig. 7C-D).**

The median velocity of migration was also decreased in GFP-A483 transfected cells compared to GFP-transfected controls, with median values corresponding to 30.7 ± 6.48 $\mu m/hour$ (199 cells, N = 4) compared to 35.1 ± 7.51 $\mu m/hour$ (268 cells, N = 4), respectively **(Fig. 4D).** The decreased median velocity was also associated with a decrease in the total explored area, with a rate of the root square of the Median Square of Displacement of 300097 ± 2668 $\mu m.hr$ (R^2 = 0.98) for GFP-A483 transfected cells, and 355998 ± 3447 $\mu m.hr$ (R^2 = 0.97) for GFP transfected controls **(Fig. 4E).**

Cell adhesion is subjected to mechanosensing, acto-myosin dependent stretching of talin exposing its VBSs being a key step in vinculin scaffolding (del Rio *et al.,* **2009.** *Science.* **323**:638-41; Yao *et al.,* **2014.** *Sci. Rep.* **4**:4610) **(Fig. 22).** We wanted to test whether A483-mediated vinculin supra-activation favored the kinetics of cell adhesion, because of the "wider opening" of vinculin. For this, transfected cells were resuspended by trypsinization and plated for defined periods on fibronectin-coated surfaces. Strikingly, the formation of large adhesion structures could be detected in GFP-A483 transfected cells as early as 7 minutes following plating, at a time where no adhesion structures was

observed for GFP-transfected cells **(Fig. 7E).** When cell adhesion was quantified as a function of time following plating, a considerable difference was evidenced in GFP-A483 transfected cells, which show significant levels of adhesion as early as **7** minutes following plating **(Fig. 4G-H** and **Fig. 7F).** These results suggested that GFP-A483 transfected could perform strong adhesion in the absence of mechanosensing. To further confirm this, the cell resistance to shear stress was measured as a function of plating time, using a microfluidic chamber device. Consistent with dynamic adhesion assays and as opposed to control cells, most of GFP-A483 transected cells resisted a shear stress up to 2.5 dynes/cm^2, when allowed to spread onto the fibronectin coated surfaces for as little as 1 minute compared to control GFP transfected cells **(Fig. 41).**

Together, these results indicate that the supra-activation of vinculin by A483 requires the presence of the three VBS (VBS1, VBS2 and VBS3), since it is not observed with A524 (containing only VBS1 and VBS2, **Figure 12).** A483-mediated supra-activation results in the formation of large and highly stable focal adhesions that are not observed with A524, and not only reinforces, but also accelerates the dynamics cell adhesion.

Example 2: The Shigella type III effector IpaA promotes vinculin-talin scaffolds through a unique hybrid binding site

The Shigella type III effector IpaA interacts with the focal adhesion protein Talin via its VBS3.

We used a yeast two-hybrid approach to identify molecular partners of the *Shigella* effector IpaA. The screen used an established human placental cDNA library totaling 82.02 million prey clones and a construct containing amino acid residues 1 to 565 of IpaA (SEQ ID NO: 1), devoid of the VBS1 and 2 sites (559-633) as bait. This screen identified 150 clones representing 16 different genes. Among these genes, 2 were with very high confidence in the interaction, which include "talin" identified in 95 prey clones corresponding to different in-frame regions of talin VBS1 (482-636) (SEQ ID NO: 38) and VBS3 (1944-1969) (SEQ ID NO: 39) previously identified as *bona fide* VBSs. As expected, because of the presence of IpaA VBS3 in the IpaA(l-565) bait, vinculin was also identified albeit with a lesser confidence than talin.

To confirm and extend these results, IpaA derivatives tagged with the V5 epitope were constructed and tested for their ability to associate with talin following their transfection and cellular expression **(Fig. 8A).** As shown in **Fig. 8B,** IpaA 7-522 localized to focal adhesions and other talin-containing structures. In contrast, IpaA 7-422, in which the IpaA VBS3 was deleted, showed a diffuse staining and did not co-localize with talin, indicating that the IpaA VBS3 was required for talin association **(Fig. 8B).**

To directly show that IpaA VBS3 acted as a Talin Binding Site (TBS), we performed native protein gel shift assays using purified synthetic IpaA VBSs. IpaA VBS3 promoted the dose-dependent formation of talin Hl-H4:IpaA VBS3/TBS complexes that could be detected at an IpaA VBS3 concentration of 25 μM. In contrast, even at high concentrations, IpaA VBS1 and IpaA VBS2 did not induce the formation of such talin H1-H4 complexes **(Fig. 8C).**

Together, these results indicate that IpaA VBS3 also act as a TBS, a property that is not shared by the IpaA VBS1 and IpaA VBS2.

IpaA VBS3/TBS binds to talin H1-H4 in the presence of IpaA VBS1-2.

The three IpaA VBSs are closely spaced within the carboxy-terminal 145 residues of IpaA, A483 **(Fig. 8A).** To test whether IpaA VBS3/TBS binding to talin H1-H4 could still occur in the presence of IpaA VBS1/2, we performed native gel shift assays with A483. As shown in **Fig. 9C,** a clear migration shift could be detected with A483 at concentrations as low as 5 μM, when mixed with talin H1-H4 **(Fig. 9C,** arrow), that was accompanied by a depletion of the species corresponding to talin H1-H4 alone consistent with the formation of an A483:talin H1-H4 complex **(Fig. 9C,** left). When A524, containing only IpaA VBS1/2 but not IpaA VBS3/TBS was incubated with talin H1-H4, no migration shift could be detected **(Fig. 9C,** right).

Then, we further characterized the interaction of IpaA VBSs with talin H1-H4 by isothermal titration calorimetry (ITC).

Consistent with native gel shift assays, ITC measurements indicated that IpaA VBS3/TBS bound to talin H1-H4 with a high affinity (KD = 174 ± 19 nM), whereas IpaA VBS2 did

not show any interaction with talin H1-H4 **(Fig. 9A).** IpaA VBS3/TBS interaction with talin H1-H4 was exothermic, with a predominant enthalpic contribution (AHVBS3= -6393 cal/mol) and a significant entropic contribution (-TASVBS3= -2826 cal/mol) **(Table 2).** However, in contrast to native gel assays, IpaA VBS1 was found to interact with talin H1-H4, although with much lower affinity than IpaA VBS3/TBS (KD = 15.2 ± 1.1 μM). When binding of A483 to talin H1-H4 was studied, A483 :talin H1-H4 complex formation could also be detected, with an estimated affinity (KD = 24.9 ± 6.8 nM), that was even higher than that of IpaA VBS3/TBS alone **(Table 2,** A483 (VBS3)).

Cell	**Talin H1-H4**	**Talin H1-H4**	**HVD1:A483**	**A483 (VBS3)**	**A524**
Titrant	**IpaA VBS1**	**IpaA VBS3**	**Talin H1-H4**	**Talin H1-H4**	**Talin H1-H4**
N	1.22	0.893	0.459	1	1.5
K_D	15.17 μM (±1.14)	174.52 nM (±18.9)	2.94 μM (±0.89)	24.93 nM (±6.84)	2.03 μM (±0.50)
ΔH (cal/mol)	-4104	-6393	-9047 (±1386)	-6229 (±103)	-3979
-TΔS (cal/mol)	-2471	-2826	-1368.4815	-3796	-3469
ΔG (cal/mol)	-6575	-9219	-7678.5185	-10025	-7448

Table 2. Non-linear fit values for ITC measurements. N (stoichiometry), KD (affinity constant), ΔH (cal/mol) enthalpy, -TAS (cal/mol) entropic contribution and AG (cal/mol) free enthalpy. IpaA binding to talin H1-H4 is exothermic (AG < 0) and mainly driven by enthalpy (AH < 0 and AH < -TAS). Talin H1-H4 binding to IpaA peptides VBS1 and VBS2 shows an important enthalpic and minor entropic contribution. Binding of talin H1-H4 to the A483:HVD1 1:1 complex is exclusively enthalpically-driven, with an entropic penalty (-TAS > 0).

These results confirm that IpaA VBS3 can act as a *bona fide* TBS, and that IpaA VBS1 may cooperate with IpaA VBS3/TBS to promote binding to talin H1-H4.

IpaA VBS3/TBSforms a new a-helix bundle with talin H1-H4.

The crystal structure of talin H1-H4 domain in complex with IpaA VBS3/TBS peptide was determined to a 2.3 A resolution **(Table 3).** The structure of the complex revealed a drastic conformational restructuration of talin H1-H4 when bound to IpaA VBS3/TBS, where the four a-helices of talin H1-H4 fold around IpaA VBS3/TBS. IpaA VBS3/TBS thus represent an entirely novel mode of interaction with talin H1-H4. Indeed, previous structural works reported that the binding of HVDl (SEQ ID NO: 11) occurred on a loosely folded conformation of talin H1-H4, in which only the fourth a-helix (H4) of talin VBSl interacts with the amino-terminal four a-helix bundle of HVDl (SEQ ID NO: 11) (Papagrigoriou *et al.,* **2004).**

X-ray data reduction	
Space group	*P* 2i 2i 2
Unit cell dimensions	
a, *b*, *c* (A)	90.24, 53.12, 69.58
α, β, γ (°)	90, 90, 90
Resolution (A)	90.26 - 2.31 (2.47 - 2.31) [a]
Total measured reflections	55,338
Unique reflections	7,995
Rmeige [%]	4.5 (53.5) [a]
I/c(I)	35.7 (1.4) [a]
Completeness (%)	99.3 (99.8) [a]
Redundancy	7.0 (3.1) [a]
Crystallographic refinement	
Resolution (A)	20.7 - 2.31 (2.47 - 2.31) [a]
Number of reflections	15,199 (2,697)
R_{work} / R_{free} [b]	26.0/31.0 (27.7/30.1) [a]
Number of atoms	
Protein	2,338
Solvent	71
R *.m.s.* deviations from ideal geometry	
Bond lengths (A)	0.01
Bond angles (°)	1.24

[a] Values in parentheses are for the highest resolution shell.

[b] R_{free} was calculated as R_{work} using 4.9% of reflections that were selected randomly and omitted from refinement.

Table 3. X-ray data collection and crystallographic refinement statistics

The compact folding of the complex formed by talin H1-H4 and IpaA VBS3/TBS was reminiscent of the inactive talin VBSl t(Hl-H5), whereby the hydrophobic H4 a-helix is buried in the presence of the H5 a-helix. When structurally aligned, an important homology between the talin Hl-H4:IpaA VBS3/TBS complex and t(Hl-H5) could be observed.

This structural similarity occurred in spite of the absence of obvious conservation between the residues of IpaA VBS3/TBS and the talin VBSl H5 helix, suggesting that IpaA VBS3/TBS and talin H5 share punctual key stabilizing contacts with the talin Hl-H4 helixes.

These findings indicate that IpaA VBS3/TBS acts as a TBS which folds talin HI-H4 into a compact structure, homologous to inactive tVBSl.

The asymmetric unit comprised two talin Hl-H4:IpaA VBS3/TBS complexes and the electron density was well resolved overall, except for the loop regions (amino acid residues from 518 to 526) connecting the a-helices HI and H2 in both molecules, indicating that these regions were dynamic. The two talin Hl-H4:IpaA VBS3/TBS complexes in the asymmetric unit form a dimer via a disulfide bridge provided by talin Cys575 from each talin subunit. IpaA VBS3/TBS is nested in a groove between a-helices H2 and H4 of talin H1-H4 via hydrophobic and hydrogen bonding interactions. Specifically, the side chains of residues Val499, Leu506 and Leu509 of IpaA VBS3/TBS pack against Thr554, Val558, Leu622, Val547, Ile550, Leu615, Met587, Val591, Leu608, and Ala612 of talin H1-H4. Furthermore, the side chains of Ser502 and Asn505 engage in hydrogen bonding interactions with the side chain of Thr554 and the backbone carbonyl of Gly614, respectively.

Superposition of IpaA VBS3/TBS in its talin bound state onto its vinculin bound state reveals that the interaction of IpaA with talin a-helix H4 is unique to IpaA binding to talin and not engaged in interactions in its vinculin bound state, while the interaction of IpaA with vinculin a-helix a1 is unique to IpaA binding to vinculin and not interacting in its talin bound state. However, superposition of talin H1-H4 in its IpaA bound state onto

talin H1-H5 revealed a remarkable molecular mimicry although there is no obvious conservation between IpaA VB3/TBS and the talin H5 helix.

IpaA483 mediates the folding of talin H1-H4 through IpaA VBS3.

The compact folding of IpaA VBS3/TBS:talin H1-H4 represents a novel mode of TBS-talin interaction that limits steric hindrance, and would be potentially compatible with the establishment of other interactions with IpaA VBS1/2. To test if talin H1-H4 folding could be triggered by A483, we performed size-exclusion chromatography (SEC, **Fig. 10).** The formation of the protein complexes was observed by comparing the elution profiles of protein mixtures versus the proteins alone.

The hydrodynamic radius (RH) of A483 and talin H1-H4 were measured and normalized to RG, the estimated hydrodynamic radius of globular proteins with the same MW **(Table 4).** The RH/RG ratio showed that A483 and A524 alone are both loosely folded, with values of 1.83, 1.88, respectively.

Stoichiometry	Protein	MW (Da)	$Radius_m$ (Ang)	$Radius_g$ (Ang)	$Radius_m$ / $Radius_g$
	A483	17304	32.86	17.99	1.83
	A524	12762	28.93	15.36	1.88
	HVD1	31069	28.25	24.36	1.16
1:1	TlnH1-H4	18344	33.33	18.54	1.80
1:1	TlnH4:A483	35648	35.78	26.15	1.37
1:1	TlnH4:A524	31106	33.33	24.37	1.37
1:1	TlnH4:HVD1	49413	35.78	30.97	1.16
1:1	HVD1:A483	48373	40.27	30.63	1.31
1:1:1	HVD1:A524	43831	38.41	29.11	1.32
1:1:1	HVD1:A483:TlnH4	66717	40.27	36.18	1.11
1:1:1	HVD1:A524:TlnH4	62175	36.64	34.88	NC

Table 4. The formation of a tripartite A483:talin H1-H4:HVD1 complex is associated with an important conformational refolding. The estimated hydrodynamic radius Radiusm and the ratio (Radiusm/Radiusg) between this measured value and the hydrodynamic radius of a globular protein with the same MW (RadiuSg) are indicated.

Consistent with previous reports, talin H1-H4 was also loosely folded with a RH/RG ratio equal to 1.80 **(Table 4;** Papagrigoriou *et al.* **2004).** In contrast, and as expected from

previous reports, the RH/RG ratio of the D1 domain of vinculin (HVD1) (SEQ ID NO: 11) corresponds to a tightly folded globular protein (Borgon *et al.*, **2004).** When incubated with talin H1-H4, however, the formation of 1:1 A483:talin H1-H4 as well as A524:talin H1-H4 could be detected. The formation of these complexes led to a substantial refolding of both proteins, since the RH/RG ratio switched from 1.80 and 1.83 for A483 and talin H1-H4 alone, respectively, to 1.37 for the A483:talin H1-H4 complex **(Fig. 10** and **Table 4)·**

Binding of talin to IpaA VBS3/TBS.

Further investigation regarding the interaction between talin and IpaA were performed.

To study the interaction of talin H1-H4 with IpaA A483 and A524 in solution, samples were analyzed by size-exclusion chromatography coupled with multi-angle static light scattering system (SEC-MALS, **Fig. 13).** Both IpaA derivatives A483 and A524 were monomers in solution, as indicated by the molecular mass determination by MALS **(Fig. 13** and **Table 5).** Consistent with the structure resolution, SEC-MALS analysis indicated that talin H1-H4 formed a globular dimer in solution **(Fig. 13** and **Table 5),** resulting from the covalent linking of two talin H1-H4 molecules by disulfide bridging **(Fig. 14).** When mixed, single species corresponding to the talin H1-H4 dimer and A524 monomers were still recovered in the SEC-MALS analysis, consistent with the absence of complex formation **(Fig. 13).** In contrast, a compact globular complex corresponding to one talin H1-H4 dimer bound to one A483 molecule was observed **(Fig. 13** [H1-H4 + A483] and **Table 5).** These results are in agreement with IpaA VBS3/TBS binding to talin H1-H4 **(Fig. 9** and **Table 4).**

	Stoichiometry	Predicted MM (kDa)	Measured MM (kDa)	R_g (nm)
A483	1	17.3	17.9	n.d.
A524	1	12.8	12.5 ± 0.7	n.d.
Talin H1-H4	2	36.4	35.2 ± 0.2	3.3 ± 0.3
Talin H1-H4 + A483	2:1	53.7	46.8 ± 0.2	3.7 ± 0.3

Table 5. SEC-MALS analysis of the interaction between talin H1-H4 and IpaA proteins. The molecular mass predicted by the primary sequence (Predicted MM) of talin

H1-H4 and IpaA proteins and complex was compared and the measured molecular mass (Measured MM) of these proteins and complexes by SEC-MALS in the indicated stoichiometries. The hydrodynamic radius (R_g) of the complex was determined by refractometry.

We then tested whether the property of IpaA VBS3/TBS to bind vinculin and talin was unique, or if it was shared by "physiological" VBSs. By comparing the sequence of IpaA VBS3/TBS with the talin VBSs, we found a remarkable homology between IpaA VBS3/TBS and the 46th a-helix of the talin rod domain corresponding to talin VBS3 (H46, **Fig. 15A).** To test the capacity of talin H46 to interact with talin H1-H4, we performed native gel assays. As shown in **Fig. 15B,** talin H46, but not talin H4, formed a complex with talin H1-H4 **(Fig. 15B,** tin H46). These results were confirmed by ITC measurements, showing a high affinity interaction between talin H46 and talin H1-H4 with an estimated $K_D = 60 \pm 0.8$ nM. Binding of talin H46 to talin H1-H4 was exothermic with a predominant enthalpic contribution (ΔH = -16970 cal/mol) and an unfavorable (-TAS = + 7200 cal/mol) entropic contribution **(Fig. 15C).** Interestingly, the affinity of talin H46 and IpaA VBS3/TBS for talin H1-H4 were comparable (KD = 60 ± 0.8 nM and 174.52 ±18.9 nM, respectively; **Fig. 9),** further suggesting that these talin H46 could also act as a TBS.

These results indicate that a subclass of VBSs including IpaA VBS3/TBS and talin H46, but not talin VBS 1 or IpaA VBS2, act as hybrid VBS/TBSs.

To test the capacity of IpaA VBS3/TBS to act as a TBS and a VBS *in vivo,* we analyzed the localization of a GFP-lpaA VB3/TBS fusion respective to that of talin and vinculin. GFP-lpaA VBS3/TBS predominantly labeled peripheral focal adhesion (FA) structures as indicated by its strict co-localization with talin and vinculin. Consistent with its binding to active configurations of talin, GFP-VBS3/TBS preferentially labeled FA structures, even in cells showing a large fraction of cytosolic talin.

We then tested the respective roles of vinculin and talin in the localization of GFP-lpaA VBS3/TBS. Cells were depleted for against talin and vinculin using siRNAs, and the recruitment of GFP-lpaA VBS3/TBS at adhesion structures was analyzed. Western blot

analysis showed that in these experiments, the expression of vinculin and talin was depressed by at least 80%. Vinculin depletion led to a reduction in the median size of talin-labeled FAs by 28% relative to control cells. In these vinculin-depleted cells, IpaA VBS3/TBS co-localized with these talin-labeled structures confirming that it could act as a TBS. As expected, adhesion structures were more altered in talin-depleted cells, with a median size and numbers of adhesion structures reduced by 32% and 51%, respectively, relative to control cells **(Fig. 16B).** IpaA VBS3/TBS still co-localized in the vinculin-labeled structures in the talin-depleted cells, confirming its VBS function. A similar decrease was observed for cells depleted for vinculin and talin suggesting that IpaA VB3/TBS preferentially revealed talin-containing focal adhesions in these assays **(Fig. 16A).** Together, these results are consistent with IpaA VBS3/TBS acting as a hybrid site that binds to talin and vinculin.

A483 form a ternary complex with talin and vinculin.

We next verified whether the compact folding of talin H1-H4 promoted by IpaA VBS3/TBS was compatible with binding of IpaA VBS 1/2 to the vinculin D1 domain (HVD1) (SEQ ID NO: 11). As shown in **Fig. IOC,** following incubation of the three proteins prior to SEC analysis, the formation of a A483:HVDl:talin H1-H4 ternary complex could readily be detected from the co-elution of the proteins in early fractions compared to proteins alone or binary A483:HVD1 or A483:talin H1-H4 complexes **(Fig. 10B** and **11A).** Interestingly, this ternary complex eluted in the same volume as the A483:HVD1 binary complex alone **(Fig. IOC,** grey and black peaks) suggesting an additional folding upon A483 simultaneous binding to HVD1 (SEQ ID NO: 11) and talin H1-H4. The estimated RH/RG ratio further decreased from 1.3 for the A483:HVD1 1:1 complex to 1.1 for the A483 :HVD 1:talin H 1-H4 1:1:1 ternary complex.

The A483:HVD1 :talin H1-H4 ternary complex formation was also analyzed by ITC. As shown in **Fig. 9B** and **Table 2,** the affinity of A483:HVD1 for talin H1-H4 was lower than that of the IpaA VBS3/TBS peptide alone, with an estimated KD of 2.94 ± 0.89 μM compared to 150 nM, respectively. This decreased affinity could be explained by decreased in exothermic free enthalpy for talin H1-H4 in the ternary complex from $\Delta G_{VBS3/TBS}$ = -9070 ± 103 cal/mol to ΔG_{temary} = -7678 ± 1386 cal/mol, mostly due to a

substantial decrease in the entropic contribution (-TAS) from -1529 cal/mol for IpaA VBS3/TBS alone to +1368 cal/mol for A483:HVD1 **(Table 2,** HVD1:A483). This entropic penalty suggested a restrained motion of the ternary complex partially compensated by an increase in the AH enthalpy of talin H1-H4 binding (AH = -9047 ± 1386 cal/mol). Such restrained motion could account for the two-fold decrease in the number of binding sites (N) observed for the ternary complex to 0.459 ± 0.05. Alternatively, this decreased in N may result from competitive inhibition of HVD1 and talin H1-H4 binding to IpaA VBS3/TBS in A483.

These results confirm the unique property of IpaA VBS3 to act as a TBS, and show that its binding to talin H1-H4 is constraint but not impeded by the binding of the adjacent IpaA VBSl/2 to HVD1. Importantly, the formation of an A483:HVDl:talin H1-H4 tertiary complex, and the absence of stoichiometry other than 1:1 for the A483:HVD1 binary complex argue that in the context of IpaA, when IpaA VBSl/2 associate with vinculin, IpaA VBS3 acts as a TBS.

Talin is neededfor efficient Shigella invasion of epithelial cells.

In previous works, we established the role of vinculin and IpaA in *Shigella* invasion (Izard *et al.,* **2006;** Tran Van Nhieu *et al.,* **2007;** Park *et al.,* **2011).** Our results suggest that talin is also a major target of IpaA, in association with vinculin.

To test the role of talin during *Shigella* invasion, we first analyzed its recruitment at sites of bacterial entry. As shown in **Fig. 11A** and **1IB,** talin could be detected at bacterial sites in ruffles surrounding the bacteria, as early as 5 minutes following challenge. As bacterial internalization proceeded, talin was enriched at actin-rich membrane extensions extending at the bacterial vicinity. Rapidly, however, talin recruitment was detected at the intimate bacterial-cell contact site to form coat-like structures around internalized bacteria **(Fig. 11A,** arrowhead). The formation of these coat-like structures, referred to in previous works as focal-adhesion like structures (Tran Van Nhieu *et al.,* **2007),** occurred concomitantly with actin depolymerization in membrane ruffles upon completion of the bacterial internalization process and was virtually detected in all foci at 30 minutes post-challenge.

To evaluate the importance of talin in *Shigella* invasion, cells were transfected with anti-talin siRNA prior to bacterial challenge. Upon talin inhibition, the frequency of actin foci formation per cell was not significantly different from control cells, but the dynamics of bacterial induced actin polymerization at invasion sites were drastically altered. While in control cells, completion of bacterial invasion was associated with actin depolymerization and formation of coat structures, such structures did not form in anti-talin siRNA treated cells and actin foci still expanded after 20 minutes of bacterial challenge **(Fig. 11C** and **11F).** Strikingly, upon talin inhibition, actin polymerization did not appear proficient for bacterial invasion, with bacteria remaining associated at the periphery of actin foci **(Fig. llC** and **11D).** As shown in **Fig. 11D** and **HE,** the percentage of internalized bacteria was reduced by ca. 10-fold in anti-talin siRNA treated cells compared to control cells. When cells were challenged with a *Shigella IpaA* mutant, as previously reported, a ca. 10-fold decrease in bacterial internalization was observed in control cells (Izard T. *et al,* **2006).**

To investigate the role of IpaA domains in talin recruitment, cells were challenged with *ipaA* mutant strains, complemented with IpaA construct derivatives. Consistent with the role of IpaA and IpaA VBSl/3 in talin recruitment, coat structures were not detected for the *ipaA* mutant strain, or the *ipaA* mutant strain complemented with IpaA Nterminal domain deleted for its C-terminal VBSl/3, as opposed to WT *Shigella* **(Figures 11G** and **11H).** Complementation of the *ipaA* mutant strain with IpaAA550-633, containing only VBS3, or IpaAA489-51 1 containing only VBSl/2, partially trigger talin recruitment at the site of intimate bacterial-cell contact and coat-structure formation but less frequently compared to full length IpaA **(Fig. 11G** and **11H).** These results are consistent with a redundant role for IpaA VBSl/2 and IpaA VBS3/TBS and underline the importance of vinculin-talin scaffolds during *Shigella* invasion.

A strong correlation in recruitment of talin and vinculin was observed at actin structures coating WT Shigella. The correlation in the intensity of talin-vinculin co-localization was observed throughout the coat structures for WT Shigella, but not the *ipaA* mutant **(Fig. 17A** and **17B).**

To investigate the role of IpaA domains in the formation of talin coat structures forming at the intimate bacterial-cell contact, cells were challenged with *ipaA* mutant strains complemented with different IpaA derivatives. Talin "coat" structures were detected for WT Shigella or the *ipaA* mutant strain complemented with gene encoding full length IpaA, but not for *ipaA* mutant strains complemented with vector alone (2.1) or with gene encoding IpaA deleted for its VBS 1/3 (AVBS1-3) **(Fig. 18).** Complementation of the *ipaA* mutant strain with genes encoding full length IpaA, IpaAAVBSl/2 or IpaAAVBS3, restored the formation of foci with talin coat structures. However, a significant decrease in the percentage of foci forming talin coat structures was observed for *ipaA* mutant strains expressing IpaAAVBSl/2 or IpaAAVBS3 compared to full length IpaA, with 19 ± 0.7% and 25 ± 5% relative to 52 ± 6.7%, respectively **(Fig. 18).**

These results are consistent with a joint role for IpaA VBS 1/2 and IpaA VBS3/TBS in talin/vinculin recruitment and underline the importance of multiple interactions with cytoskeletal linkers during *Shigella* invasion.

Example 3: A483 induces vinculin supra-activation and cell adhesion in the absence of mechanosension

A483 increases the rate of force generation and cell adhesion strength.

To quantify the effects of vinculin supra-activation on the strength of traction forces and cell adhesion, we used a parallel-plates technique **(Fig. 19A)** allowing one to measure the traction forces generated by single cells, while simultaneously monitoring focal adhesions (FAs)' growth, as previously described (Fouchard *et al.*, **2014.** *PNAS).* It can also be used to apply traction forces to the cell in order to measure the adhesion strength.

Our preliminary results indicate that, under the parameters used, A483 accelerates the kinetics of force generation **(Fig. 19B),** and clearly increases the maximum stress before detachment, e.g. the adhesion strength **(Fig. 19C).**

IpaA-VBD enhances vinculin-mediated cell adhesion in the absence of mechanosensing.

To confirm this latter result at the scale of a whole cell population, we performed assays where cells, resuspended by trypsinization, were allowed to adhere for define amounts of

time on Fn-coated substrates before washing. Then the number of cells that remained attached to the substrate was measured.

As previously observed, A483 induced the formation of large adhesion structures in C2.7 cells **(Fig. 20A).** While A483 transfectants induced a spectacular 5.6-fold increase in the number of adherent cells after 60 seconds of incubation, this increase was reduced to 1.3-fold when adhesion was tested after 30 min. These findings were quantified by measuring the resistance to shear stress of cells under flow as a function of the incubation time with the substrate in microfluidic chambers **(Fig. 20B).** Thus, through its direct binding via IpaA VBS3/TBS or indirect unveiling of binding sites on vinculin "supra-activated", A483 may "by-pass" mechanosensing normally associated with the initial steps of cell adhesions' formation. While we cannot rule out the possibility that A483 induces an original *ex-nihilo* adhesion structure, our current results rather argue that it allows the bypassing of actomyosin-dependent mechanosensing steps that would lead to vinculin supra-activation at high actomyosin contraction forces. To further test this, we plated the C2.7 myoblastic cells on various fibronectin-coated PDMS membranes showing different stiffness. As expected, at low substrate stiffness (1.5 kPa), control cells remained spread and isolated, whereas at high substrate stiffness (15 kPa), cells differentiated into myotubes **(Fig. 20C).** Strikingly, however, A483 expressing cells formed myotubes not only at high, but also at low substrate stiffness, consistent with bypassing of mechanosensing. Indeed, while A483 expressing cells show increased FAs' extension and adhesion, preliminary results indicate no difference in force generation between control and A483 cells.

These results indicate thus the by-passing of actomyosin-dependent mechanosensing steps, even on low-stiffness substrates, suggesting an efficient role of A483 in the treatment of metastasis and other soft-tissue cancers.

Effects of A483-mediated vinculin supra-activation on adhesion structure composition and dynamics.

Focal adhesion (FA) markers such as FAK, paxillin and vinculin are associated with nascent adhesions while VASP, a-actinin or zyxin are characteristic of mature FAs

(Fig. 22). The kinetics and hierarchy of disappearance of these markers following actomyosin relaxing drugs are in good agreement with the recruitment of these markers during FA maturation as a result of increased traction forces. Following MAPK-dependent phosphorylation, however, zyxin has been shown to redistribute to stress fibers, along with VASP and a-actinin, and promote their stabilization. The identification of markers recruited by supra-active vinculin at FA will provide with information on whether A483 bypasses mechanosensing steps or generates a specific adhesion structure. Beyond cell adhesion, FA components have been involved in various processes including signaling linked to cell proliferation, cell death as well as inflammation.

Vinculin activation depending on actomyosin contraction was shown to be required for the scaffolding of mature FA markers. However, FA markers appear to differ in their requirements for vinculin-dependent recruitment, since upon drug-based actin relaxation experiments, some like vinexin and ponsins are stabilized by active mutant forms of vinculin deficient for HVDl-tail interaction, while others like a-actinin are not. The reason for these differences are not known, but in lights of evidence for vinculin supra-activation, it is possible that the recruitment of some of these FA markers is directly mediated by vinculin domains other than HVD1 specifically exposed on the supra-active form of vinculin.

In preliminary experiments, we have analyzed the role of actomyosin contraction on the recruitment of FA markers linked to the supra-activation of vinculin, by treating cells with the Rho-kinase inhibitor Y-27632. The disappearance of markers from FAs was analyzed following in real-time using total internal reflection (TIRF) microscopy. As shown in **Fig. 21,** A483 transfection led to a stabilization of all FA markers studied compared to control cells. Strikingly, VASP, a marker of mature FAs, was also stabilized by A483, while previous studies had shown that this FA marker was not stabilized by active vinculin, deficient for head-tail intra-molecular interaction.

Together, these experiments support the notion that A483 promotes the establishment of stable FAs in the absence of acto-myosin contraction required for mechanosensing and reinforce the efficient use of A483 in the treatment of cancers, including soft-tissue cancers.

Example 4: A483 inhibits melanocytes migration and matrigel invasion

Cells were transfected by GFP-A524, GFP-A483 or Mock-transfected (without DNA, as a control).

After transfection, control cells show a typical fusiform shape. A524- and A483-cells however were more spread, with both transfected constructs labeling peripheral adhesion structures **(Fig. 24A).**

Cells were further trypsinized, collected, and deposited in a matrigel insert. Following a 24-hour incubation, the matrigel was remove, the membrane was fixed and processed for fluorescence straining of cell nuclei with DAPI. Cells that transmigrated across the matrigel were scored **(Fig. 24B).** In despite of the 20-30% levels of transfection estimated from the scoring of GFP fluorescence for both A483 and A524 in **Fig. 24A,** the inhibition of cell migration in matrigel invasion assays associated with the constructs reached up to 90%.

Without willing to be bound by a theory, the Applicant suggests that this discrepancy could be explained by the underestimation of the actual levels of transfectants, combined with an inhibitory effect of these constructs on cell replication.

Preliminary experiments from the analysis of 6 movies for each sample in time lapse experiments indicate that the transfection of A483 shows a strong effect on cell migration. Remarkably, transfection with GFP-A524 did not drastically alter the motility of melanocytes. In these experiments, however, long term incubation (6 hours) were associated with a significant fraction of cells rounding up in the A524 and A483 transfected cells, suggesting a general stress on the cell fitness linked to the combine effects of the constructs and imaging parameters.

CLAIMS

1. A polypeptide comprising the three following vinculin binding sites (VBS):

VBS1: IYKAAKDVTTSLSKVLKNI (SEQ ID NO: 2) or a fragment or variant thereof;

VBS2: IYEKAKEVSSALSKVLSKI (SEQ ID NO: 3) or a fragment or variant thereof;

VBS3: IFEASKKVTNSLSNLISLI (SEQ ID NO: 4) or a fragment or variant thereof;

or any sequence having at least 60% identity with SEQ ID NO: 2; 3 and 4;

wherein said polypeptide is not SEQ ID NO: 1.

2. The polypeptide according to claim **1,** wherein said polypeptide is SEQ ID NO: 5 or a variant thereof.

3. A nucleic acid comprising the three following domains:

VBS 1 (SEQ ID NO: 8);

VBS2 (SEQ ID NO: 9);

VBS3 (SEQ ID NO: 10);

or any nucleic acid sequence having at least 60% identity with SEQ ID NO: 8; 9 and 10;

wherein said nucleic acid is not SEQ ID NO: 7.

4. The nucleic acid according to claim **3,** wherein said sequence is SEQ ID NO: 6.

5. A vector encoding the polypeptide according to any one of claims **1** to **2,** or comprising the nucleic acid sequence according to any one of claim **3** or **4.**

6. A composition comprising the polypeptide according to any one of claims **1** to **2** or the nucleic acid sequence according to any one of claim **3** or **4** or the vector of claim **5.**

7. A pharmaceutical composition comprising the polypeptide according to any one of claims **1** to **2** or the nucleic acid sequence according to any one of claim **3** or **4** or the vector of claim **5** and at least one pharmaceutically acceptable excipient.

8. A medicament comprising the polypeptide according to any one of claims **1** to **2** or the nucleic acid sequence according to any one of claim **3** or **4** or the vector of claim **5.**

9. The composition according to claim **6** or the pharmaceutical composition according to claim **7** or the medicament according to claim **8** for use in the treatment of a proliferation and/or adhesion related disease.

10. The composition, pharmaceutical composition or medicament according to claim **9,** wherein said proliferation and/or adhesion related disease is a cancer.

11. The composition, pharmaceutical composition or medicament according to claim **9,** wherein said proliferation and/or adhesion related disease is a tumor.

12. The composition, pharmaceutical composition or medicament according to claim **9,** wherein said proliferation and/or adhesion related disease is metastasis.

13. The composition, pharmaceutical composition or medicament according to any one of claims **10** to **12,** wherein said composition or pharmaceutical composition or medicament is to be administered in combination with another anti-cancer agent.

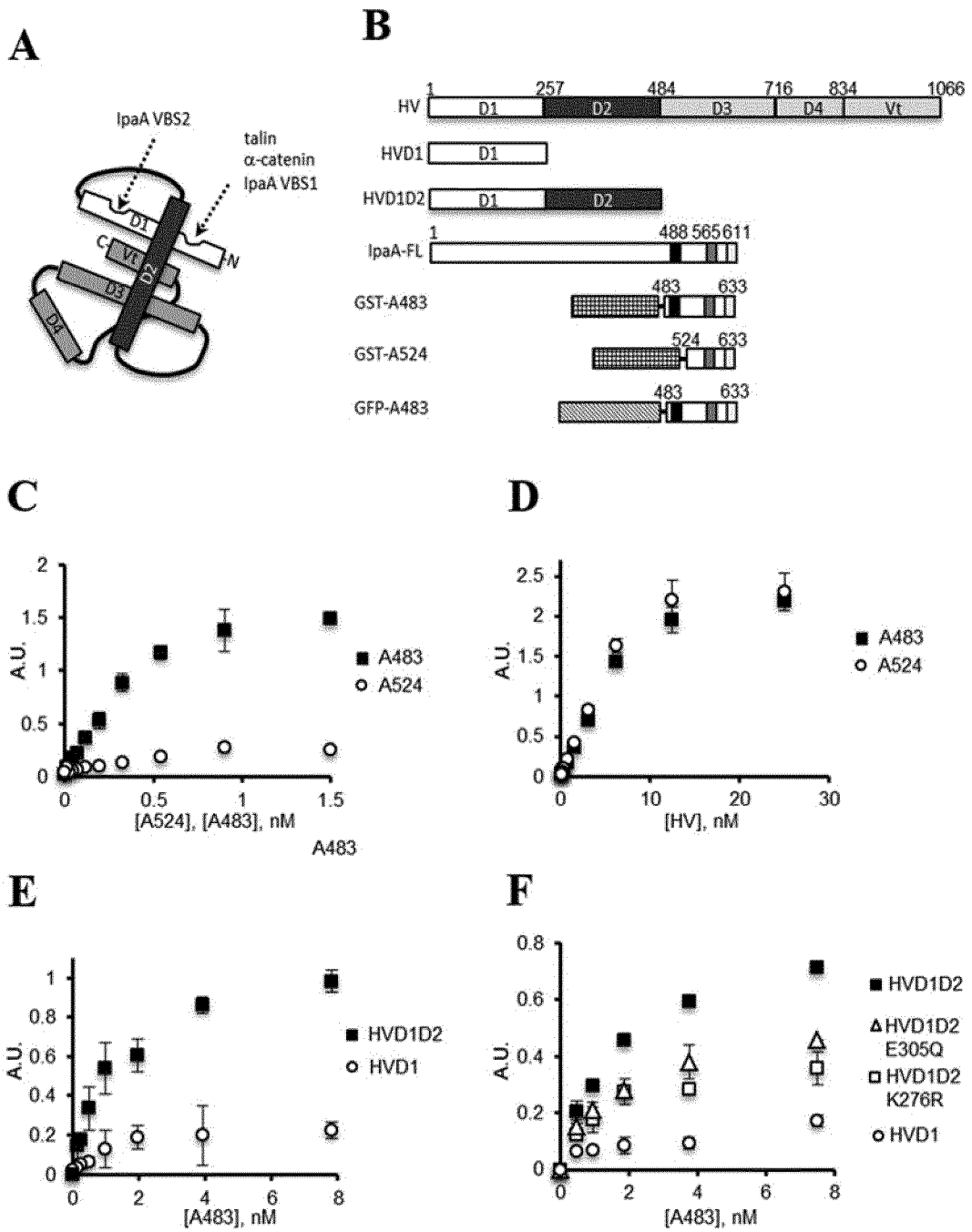

A
IpaA VBS2
talin
α-catenin
IpaA VBS1
D1
C
Vt
D2
N
D3
D4
B
HV
HVD1
HVD1D2
IpaA-FL
GST-A483
GST-A524
GFP-A483
1
257
484
716
834
1066
D1
D2
D3
D4
Vt
488 565 611
483 633
524 633
C
A.U.
[A524], [A483], nM
A483
A524
D
[HV], nM
E
HVD1D2
HVD1
[A483], nM
F
HVD1D2
HVD1D2 E305Q
HVD1D2 K276R
HVD1

FIG. 1

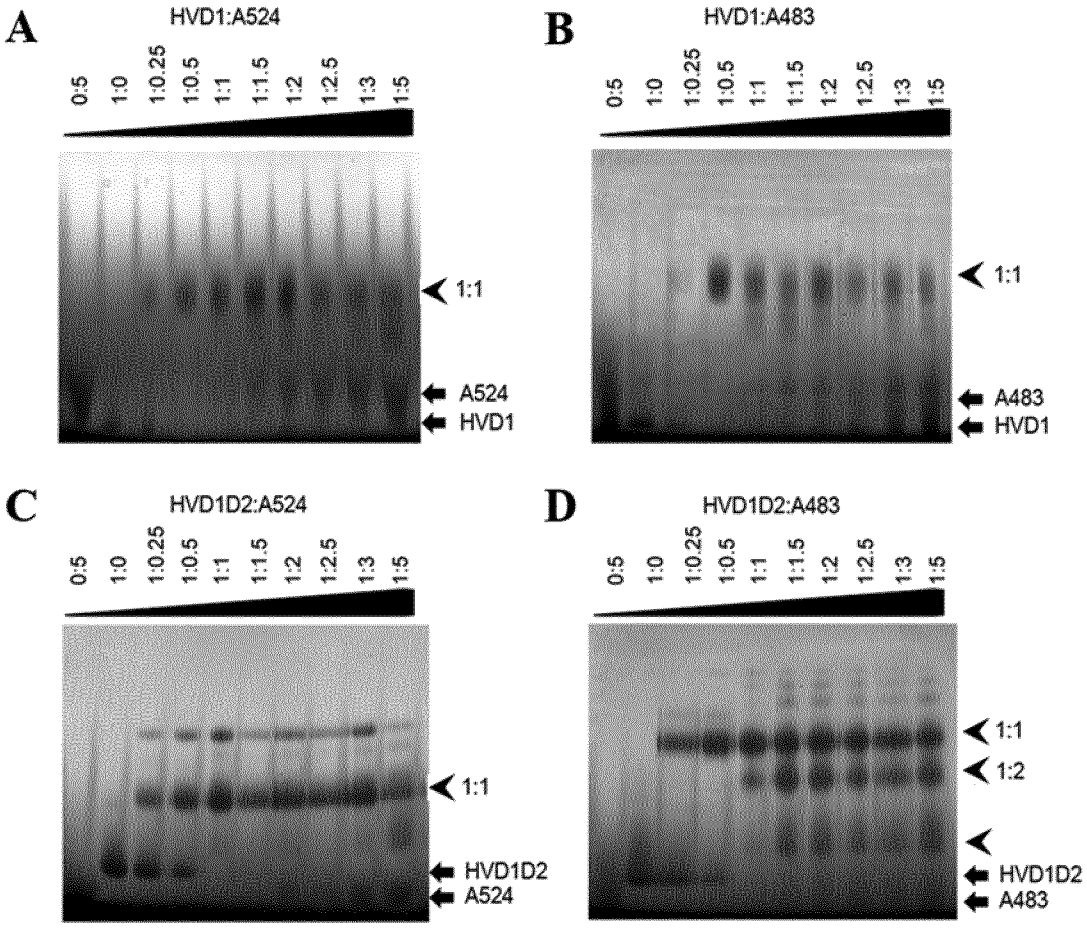

A
HVD1:A524
0:5
1:0
1:0.25
1:0.5
1:1
1:1.5
1:2
1:2.5
1:3
1:5
1:1
A524
HVD1
B
HVD1:A483
0:5
1:0
1:0.25
1:0.5
1:1
1:1.5
1:2
1:2.5
1:3
1:5
1:1
A483
HVD1
C
HVD1D2:A524
0:5
1:0
1:0.25
1:0.5
1:1
1:1.5
1:2
1:2.5
1:3
1:5
1:1
HVD1D2
A524
D
HVD1D2:A483
0:5
1:0
1:0.25
1:0.5
1:1
1:1.5
1:2
1:2.5
1:3
1:5
1:1
1:2
HVD1D2
A483

FIG. 2

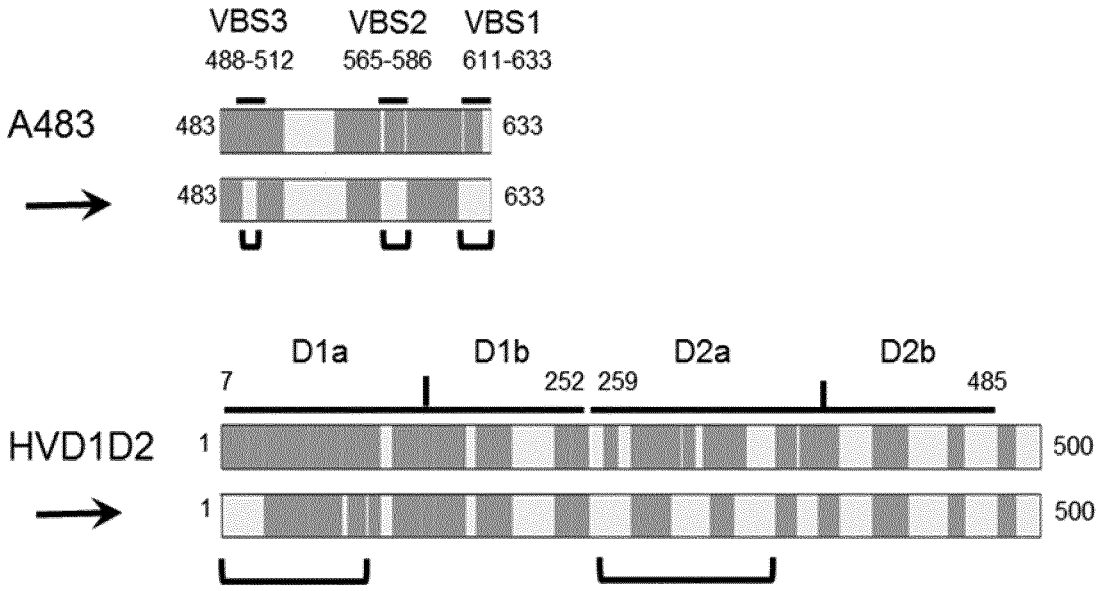

VBS3
488-512
VBS2
565-586
VBS1
611-633
A483
483
633
483
633
D1a
D1b
D2a
D2b
7
252
259
485
HVD1D2
1
500
1
500

FIG. 3

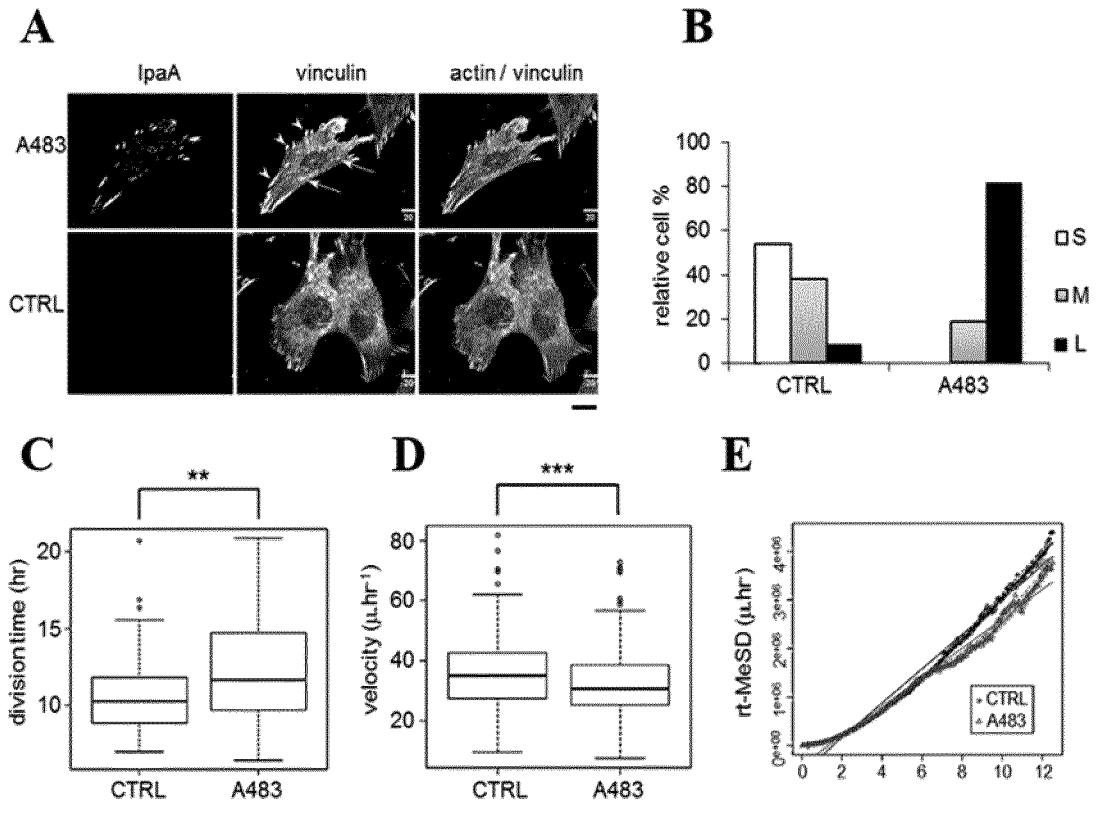

A
IpaA
vinculin
actin / vinculin
A483
CTRL
B
relative cell %
100
80
60
40
20
0
CTRL
A483
S
M
L
C
**
division time (hr)
20
15
10
CTRL
A483
D
***
velocity (µ.hr⁻¹)
80
60
40
20
CTRL
A483
E
rt-MeSD (µ.hr⁻¹)
CTRL
A483
0
2
4
6
8
10
12

FIG. 4A-E

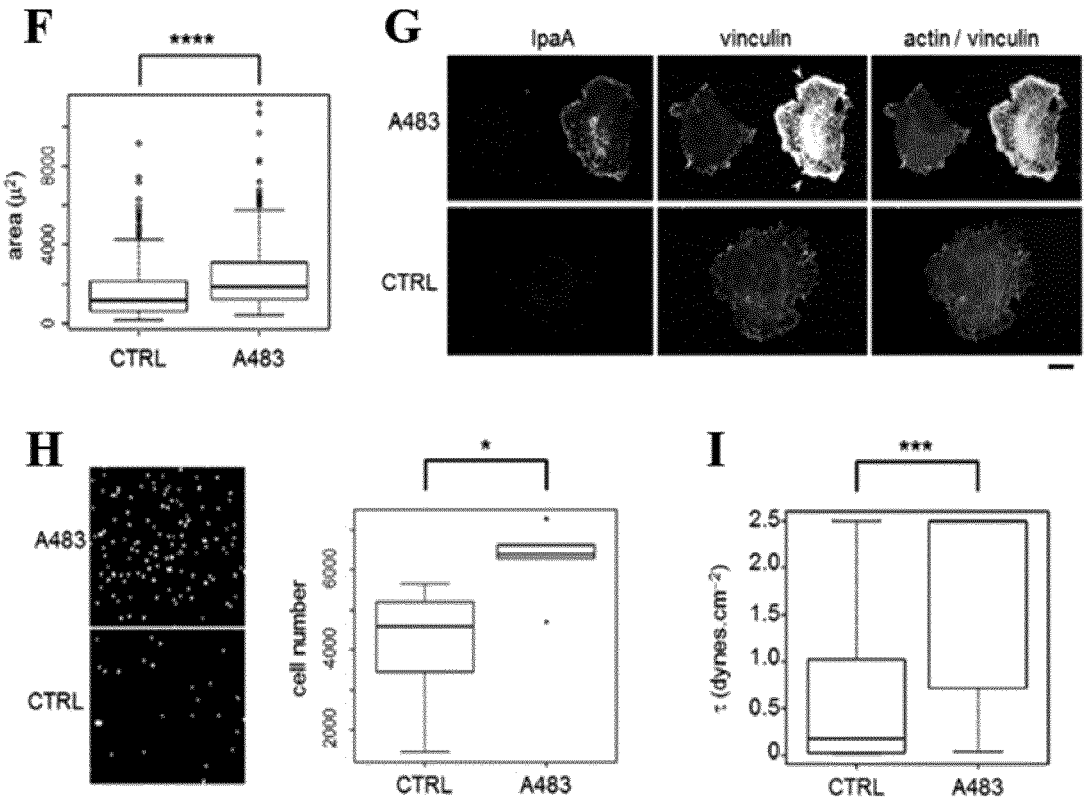

F
****
area (µ²)
8000
4000
0
CTRL
A483
G
IpaA
vinculin
actin / vinculin
A483
CTRL
H
A483
CTRL
*
cell number
6000
4000
2000
CTRL
A483
I
***
τ (dynes.cm⁻²)
2.5
2.0
1.5
1.0
0.5
0
CTRL
A483

FIG. 4F-I

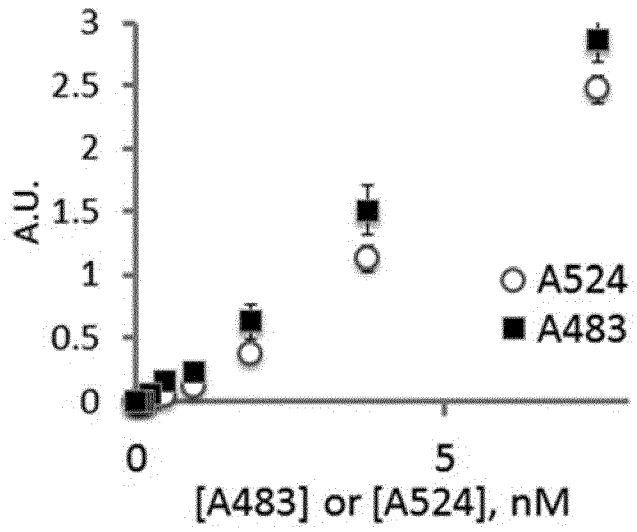

A.U.
3
2.5
2
1.5
1
0.5
0
0
5
[A483] or [A524], nM
A524
A483

FIG. 5

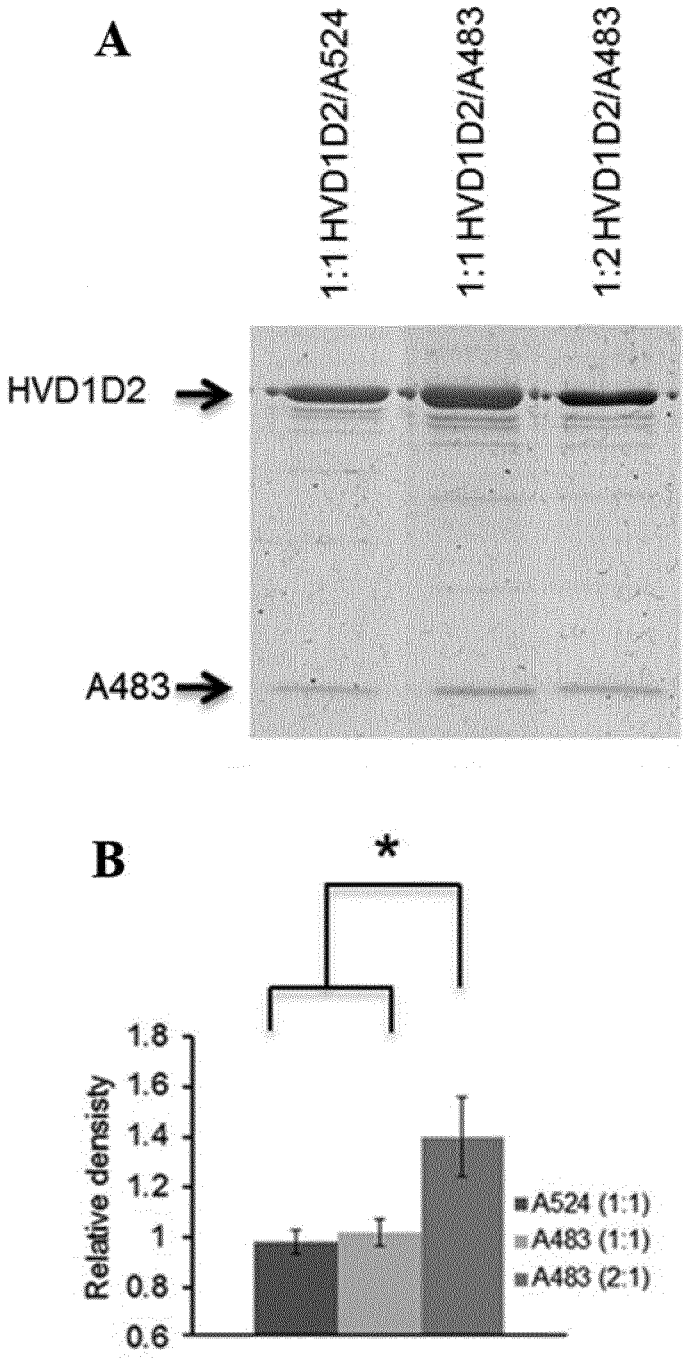

A
1:1 HVD1D2/A524
1:1 HVD1D2/A483
1:2 HVD1D2/A483
HVD1D2
A483
B
*
Relative densisty
1.8
1.6
1.4
1.2
1
0.8
0.6
A524 (1:1)
A483 (1:1)
A483 (2:1)

FIG. 6

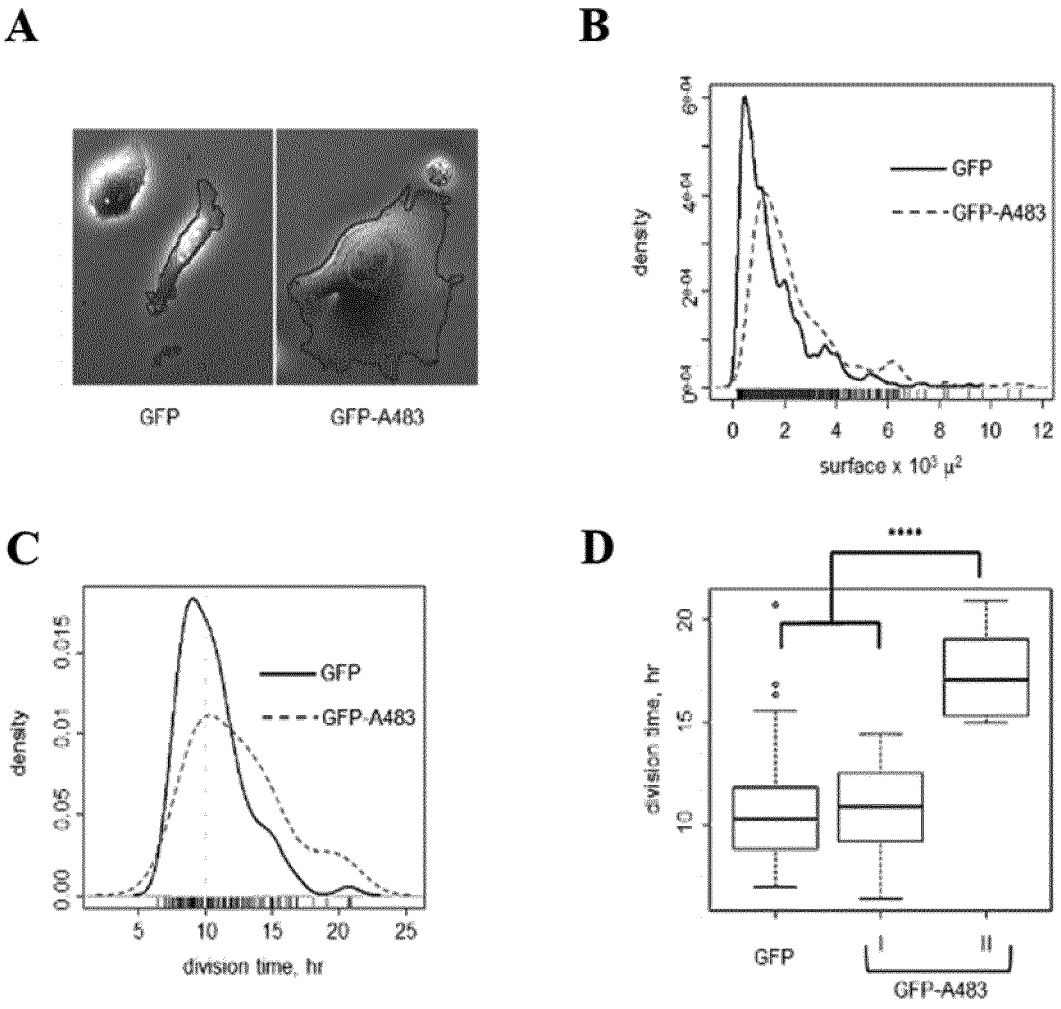
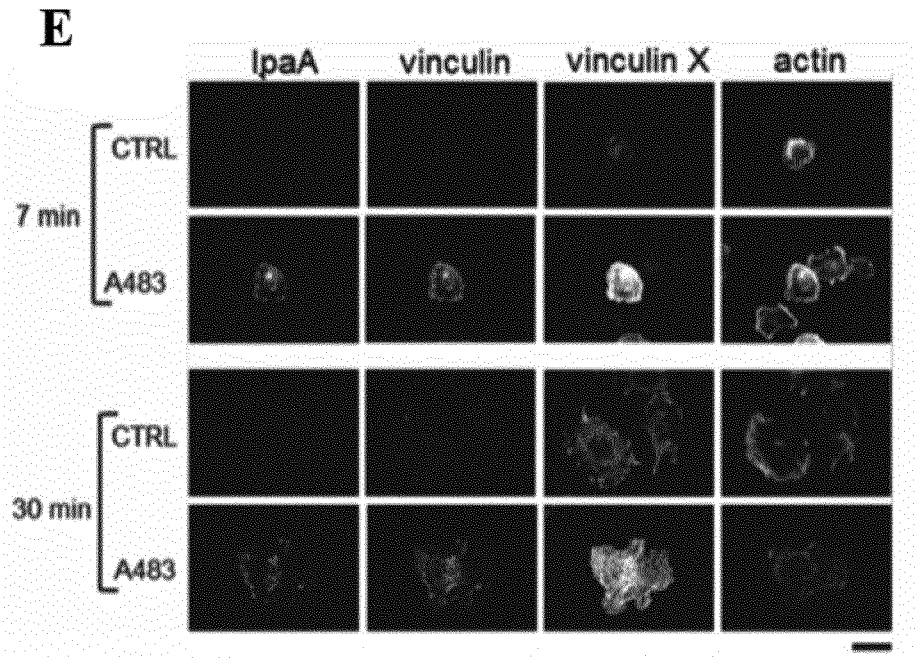

A
GFP
GFP-A483
B
density
GFP
GFP-A483
surface x 10³ µ²
C
density
GFP
GFP-A483
division time, hr
D
division time, hr
GFP
I
II
GFP-A483

FIG. 7A-D


E
IpaA
vinculin
vinculin X
actin
CTRL
7 min
A483
CTRL
30 min
A483

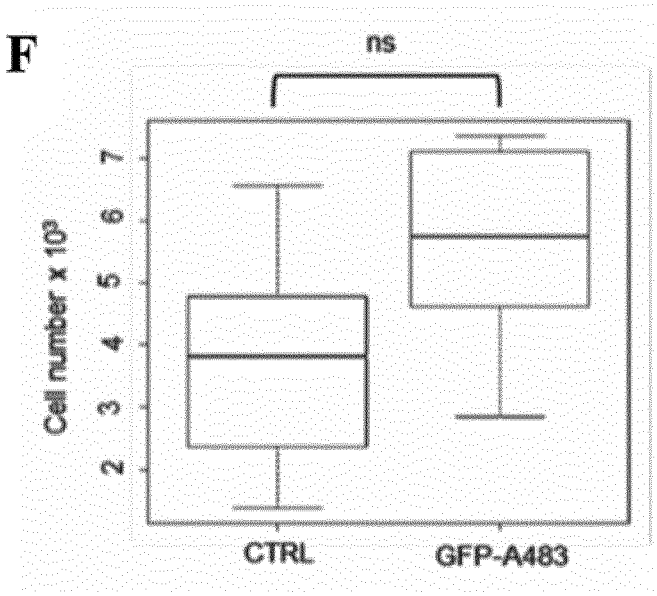

F
ns
Cell number x 10³
2
3
4
5
6
7
CTRL
GFP-A483

FIG. 7E-F

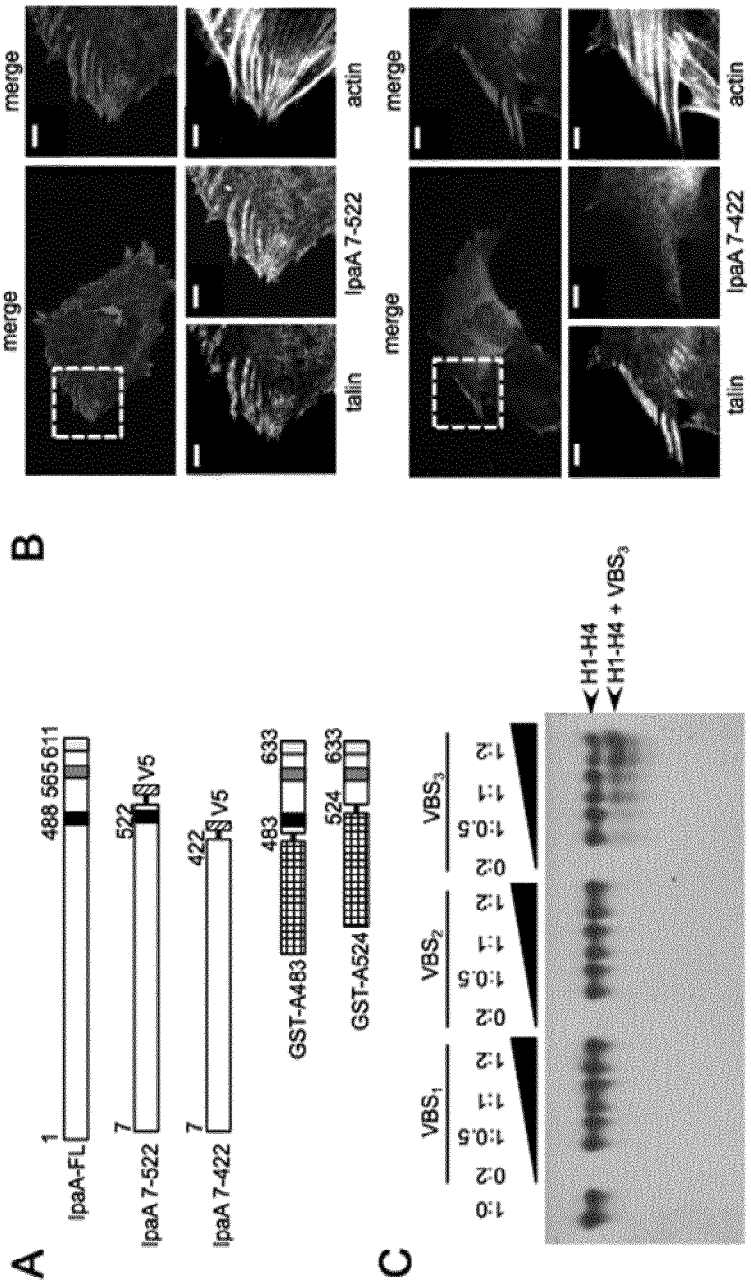

A
IpaA-FL
IpaA 7-522
IpaA 7-422
GST-A483
GST-A524
V5
B
merge
talin
actin
IpaA 7-522
IpaA 7-422
C
VBS1
VBS2
VBS3
H1-H4
H1-H4 + VBS3

FIG. 8

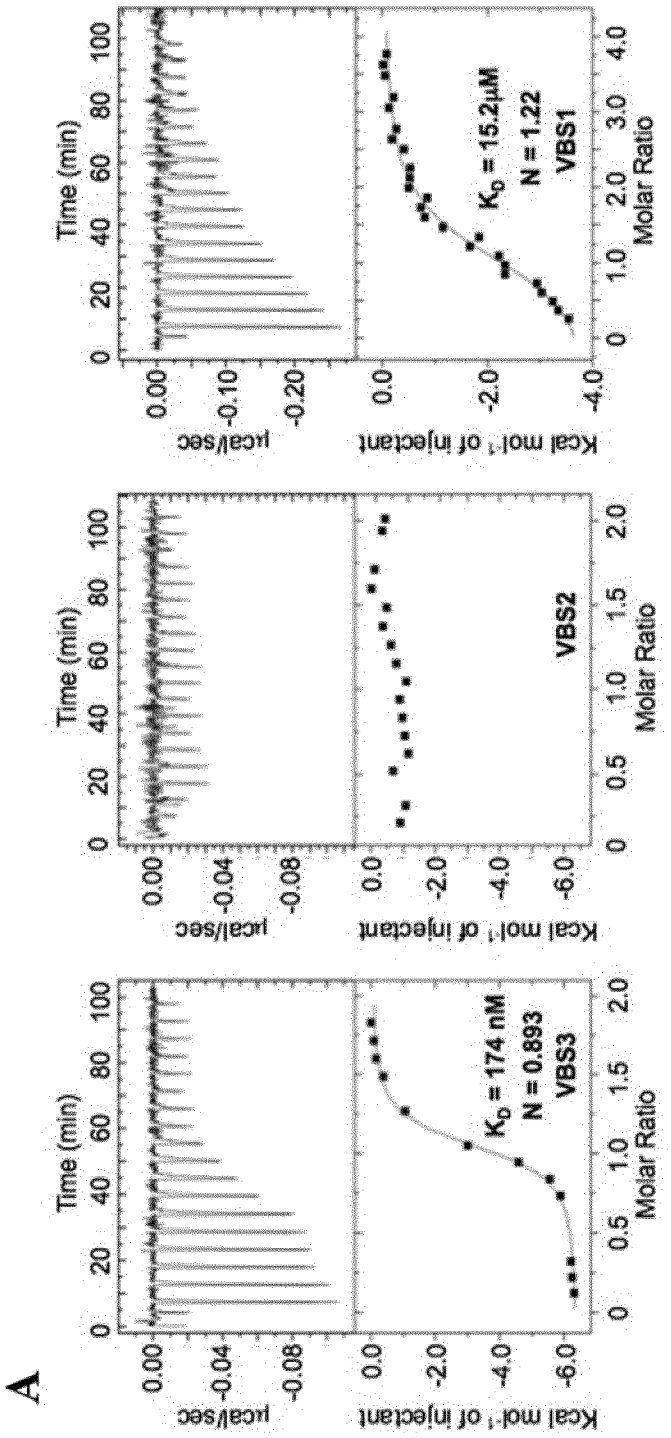

A
Time (min)
µcal/sec
Kcal mol⁻¹ of injectant
Molar Ratio
K_D = 174 nM
N = 0.893
VBS3
K_D = 15.2µM
N = 1.22
VBS1
VBS2

FIG. 9A

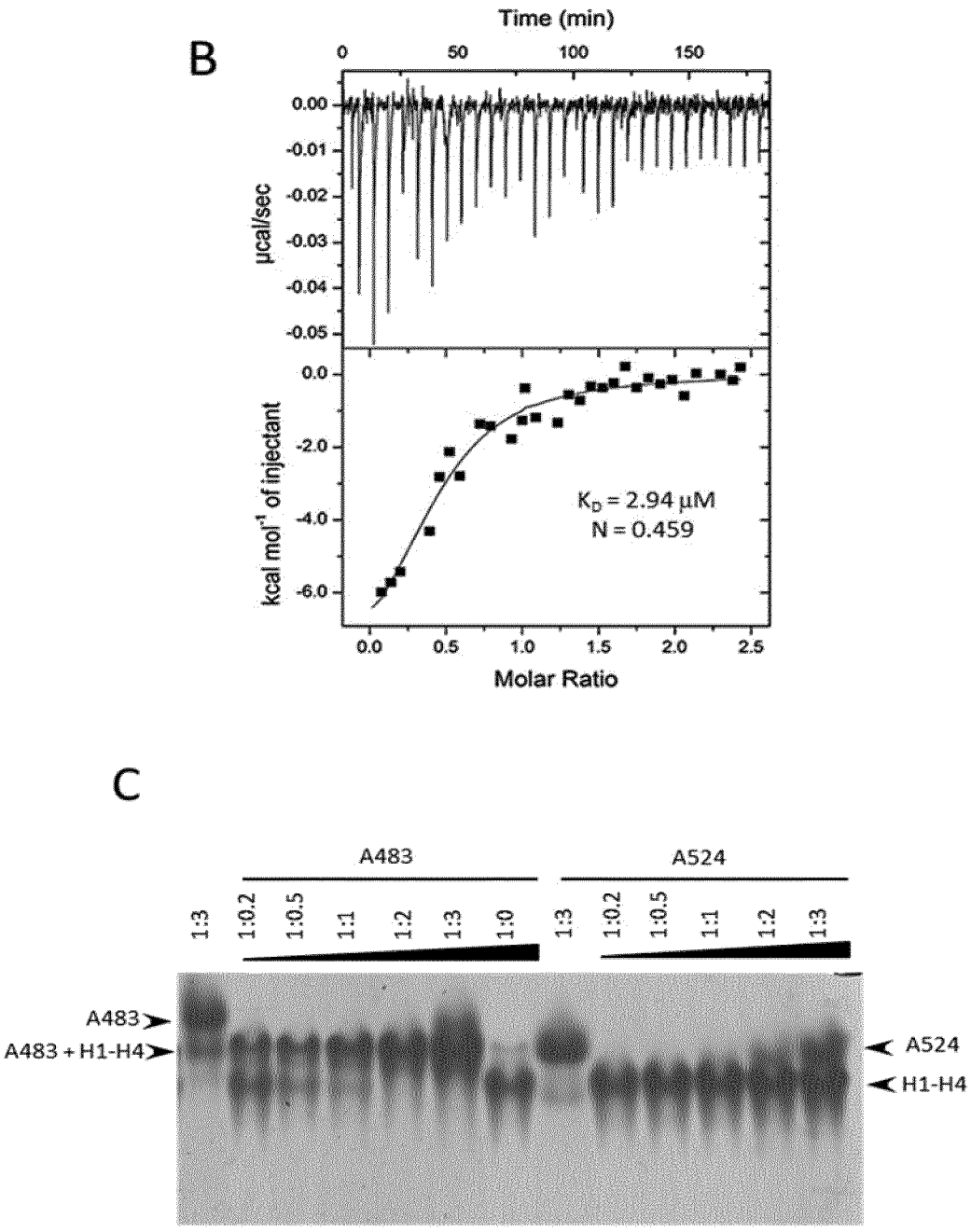

B
Time (min)
0
50
100
150
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
µcal/sec
0.0
-2.0
-4.0
-6.0
kcal mol-1 of injectant
KD = 2.94 µM
N = 0.459
0.0
0.5
1.0
1.5
2.0
2.5
Molar Ratio
C
A483
A524
1:3
1:0.2
1:0.5
1:1
1:2
1:3
1:0
1:3
1:0.2
1:0.5
1:1
1:2
1:3
A483
A483 + H1-H4
A524
H1-H4

FIG. 9B-C

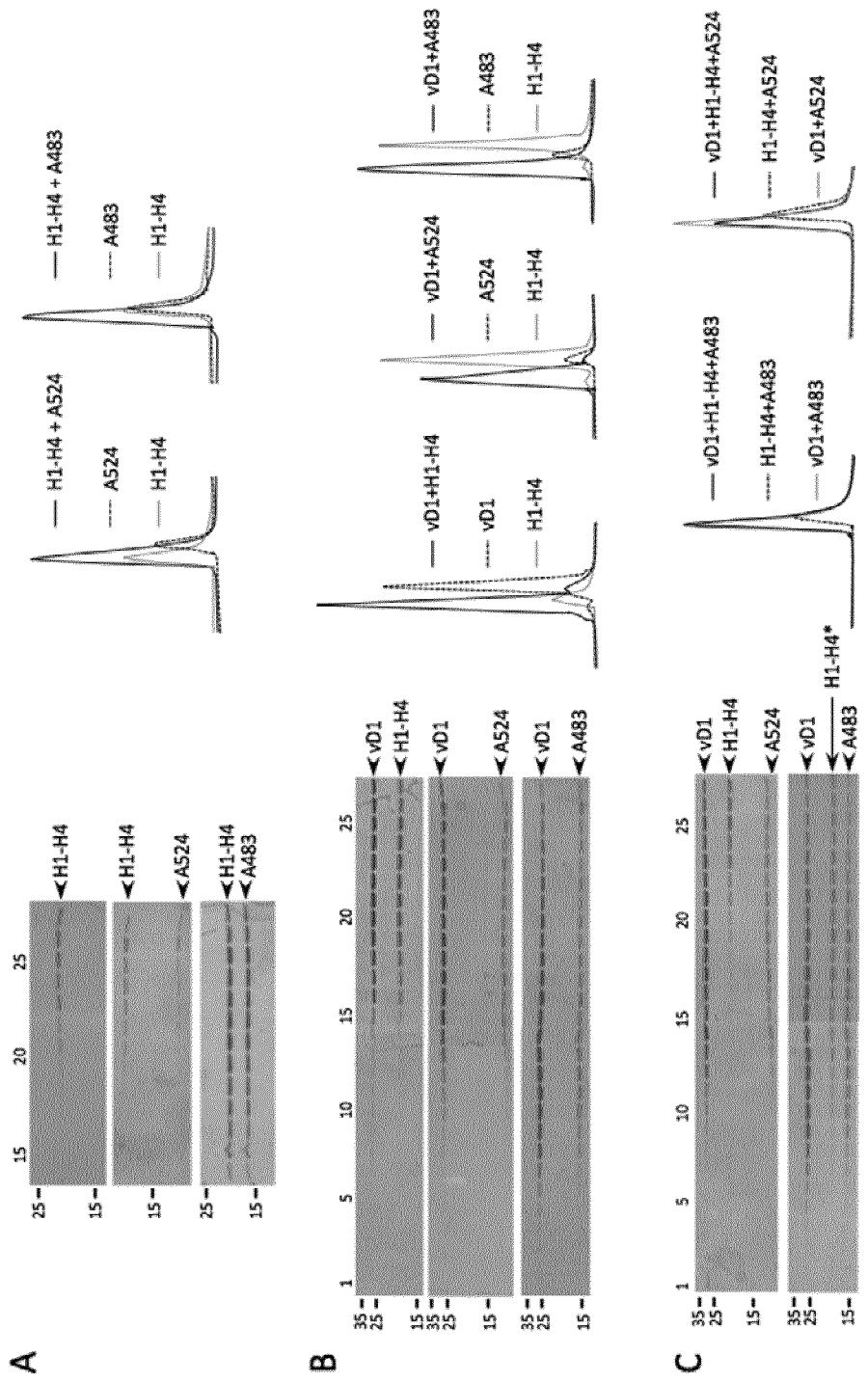

A
H1-H4
H1-H4
A524
H1-H4
A483
H1-H4 + A524
A524
H1-H4
H1-H4 + A483
A483
H1-H4
B
vD1
H1-H4
vD1
A524
vD1
A483
vD1+H1-H4
vD1
H1-H4
vD1+A524
A524
H1-H4
vD1+A483
A483
H1-H4
C
vD1
H1-H4
A524
vD1
H1-H4*
A483
vD1+H1-H4+A483
H1-H4+A483
vD1+A483
vD1+H1-H4+A524
H1-H4+A524
vD1+A524

FIG. 10

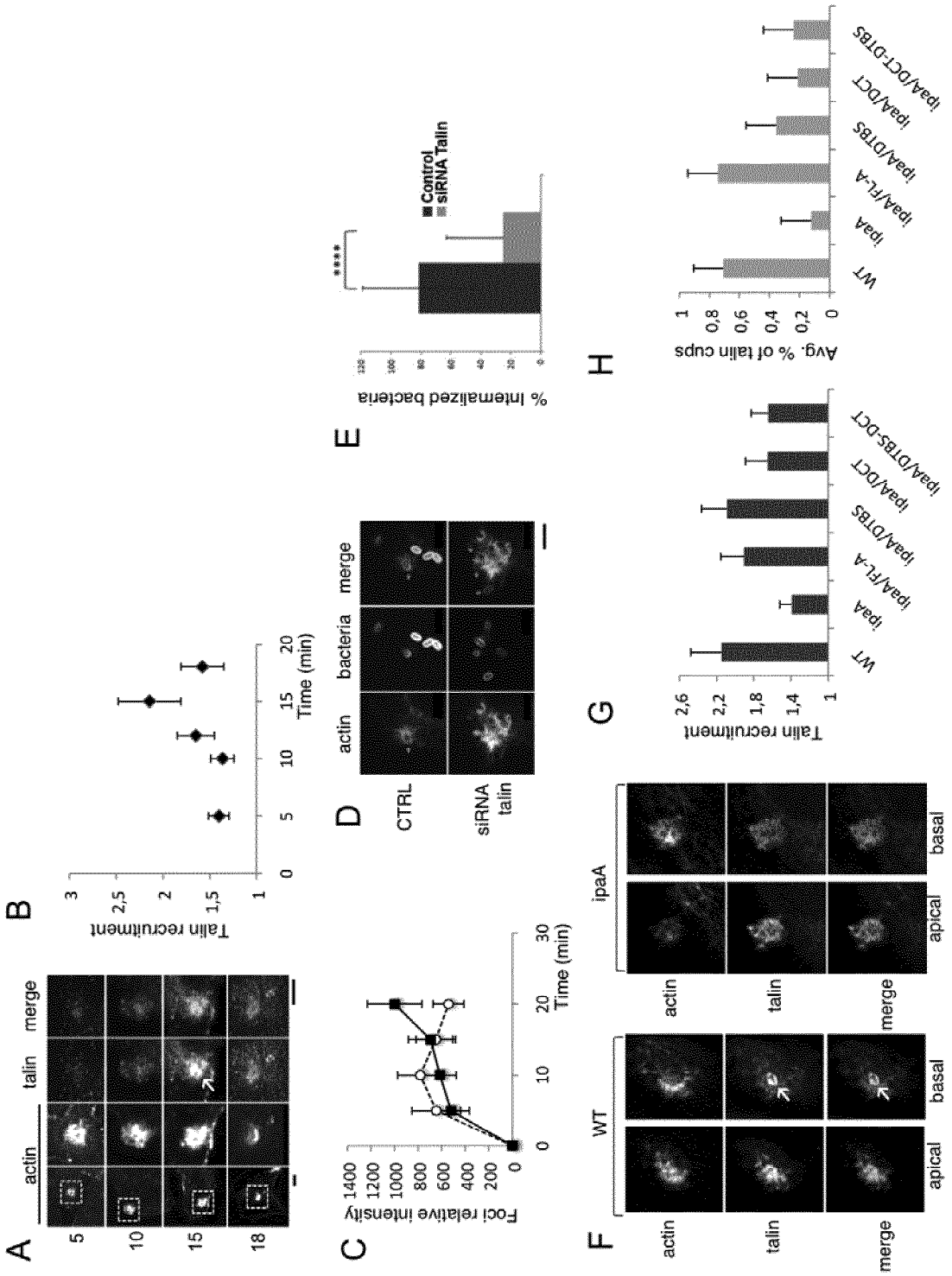

A
actin
talin
merge
5
10
15
18
B
Talin recruitment
Time (min)
C
Foci relative intensity
Time (min)
D
actin
bacteria
merge
CTRL
siRNA talin
E
% Internalized bacteria
Control
siRNA Talin
****
F
WT
ipaA
actin
talin
merge
apical
basal
G
Talin recruitment
WT
ipaA
ipaA/FL-A
ipaA/DTBS
ipaA/DCT
ipaA/DTBS-DCT
H
Avg. % of talin cups
WT
ipaA
ipaA/FL-A
ipaA/DTBS
ipaA/DCT
ipaA/DCT-DTBS

FIG. 11

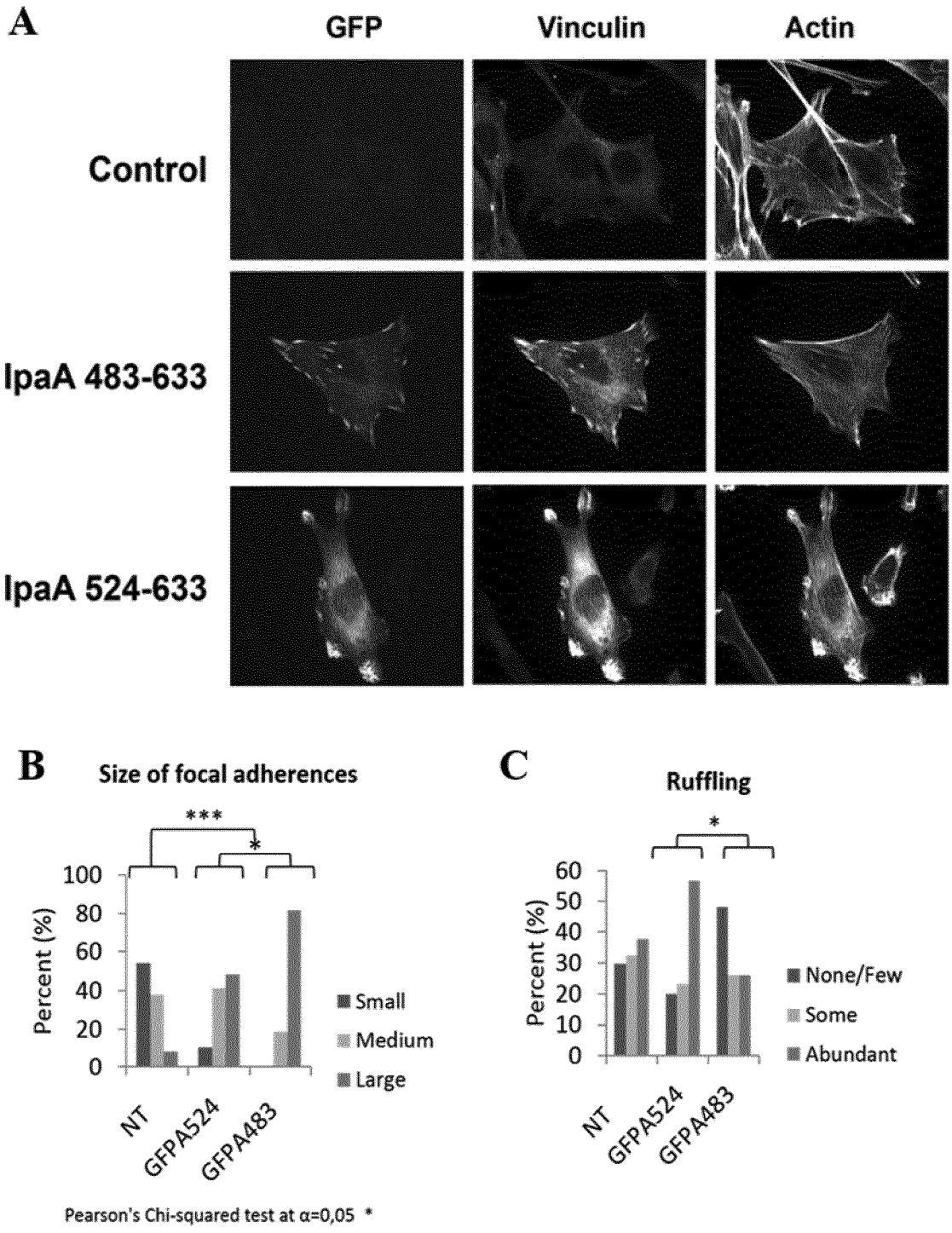

A
GFP
Vinculin
Actin
Control
IpaA 483-633
IpaA 524-633
B
Size of focal adherences
***
*
100
80
60
40
20
0
Percent (%)
Small
Medium
Large
NT
GFPA524
GFPA483
Pearson's Chi-squared test at α=0,05 *
C
Ruffling
*
60
50
40
30
20
10
0
Percent (%)
None/Few
Some
Abundant
NT
GFPA524
GFPA483

FIG. 12

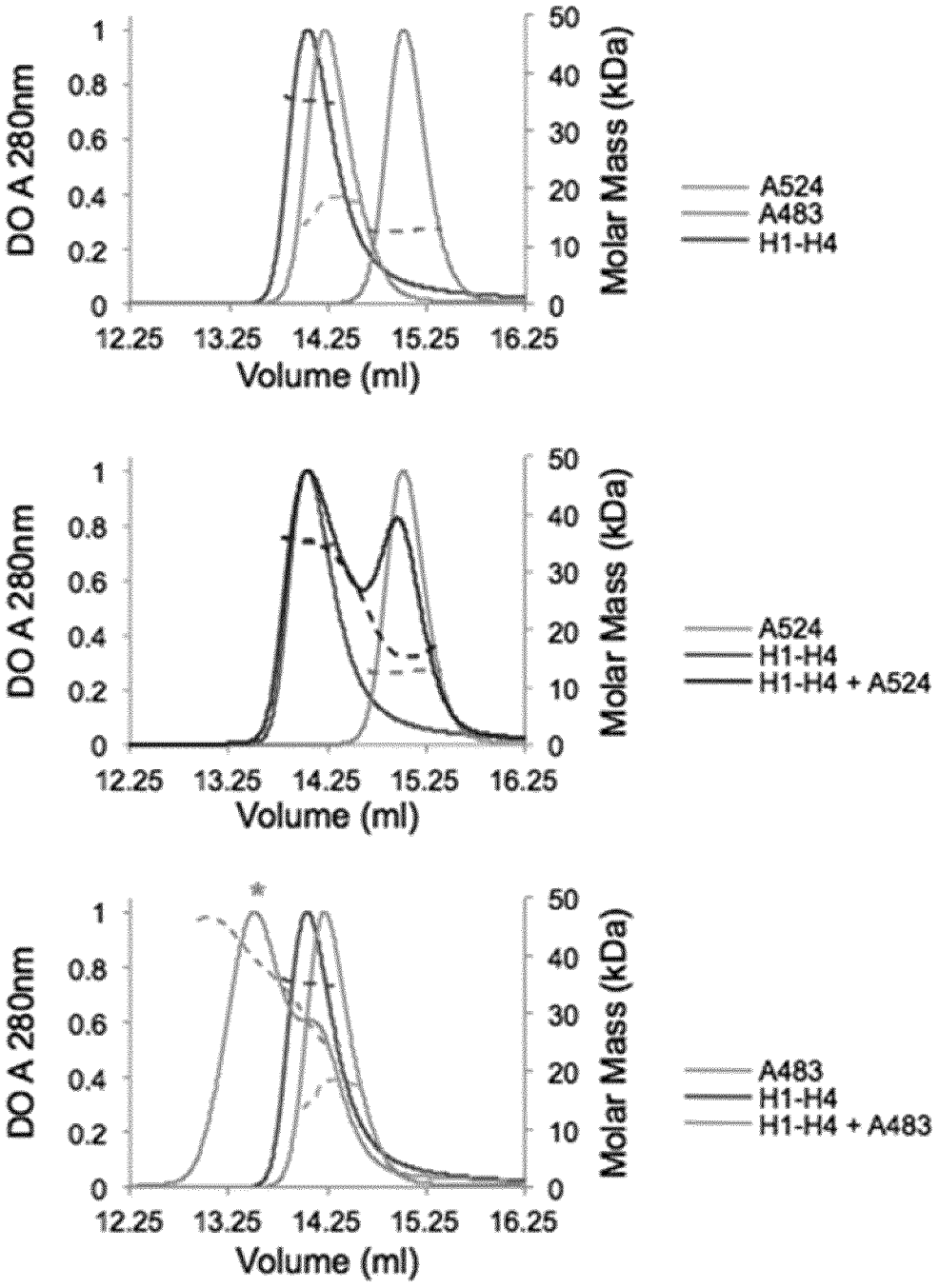

DO A 280nm
Molar Mass (kDa)
Volume (ml)
12.25
13.25
14.25
15.25
16.25
A524
A483
H1-H4
H1-H4 + A524
H1-H4 + A483

FIG. 13

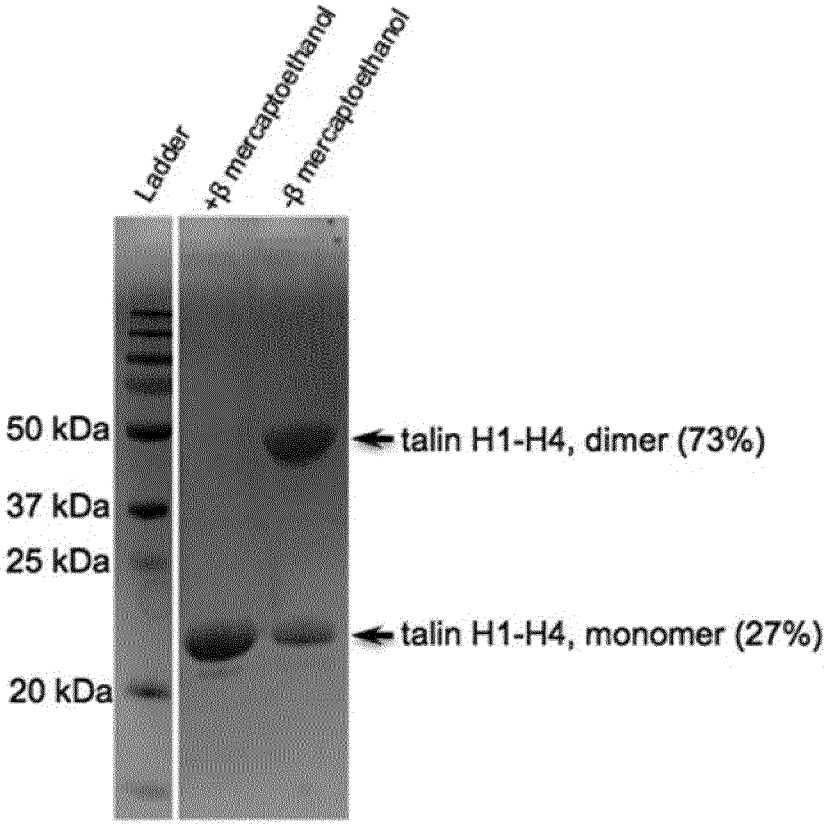

Ladder
+β mercaptoethanol
-β mercaptoethanol
50 kDa
37 kDa
25 kDa
20 kDa
talin H1-H4, dimer (73%)
talin H1-H4, monomer (27%)

FIG. 14

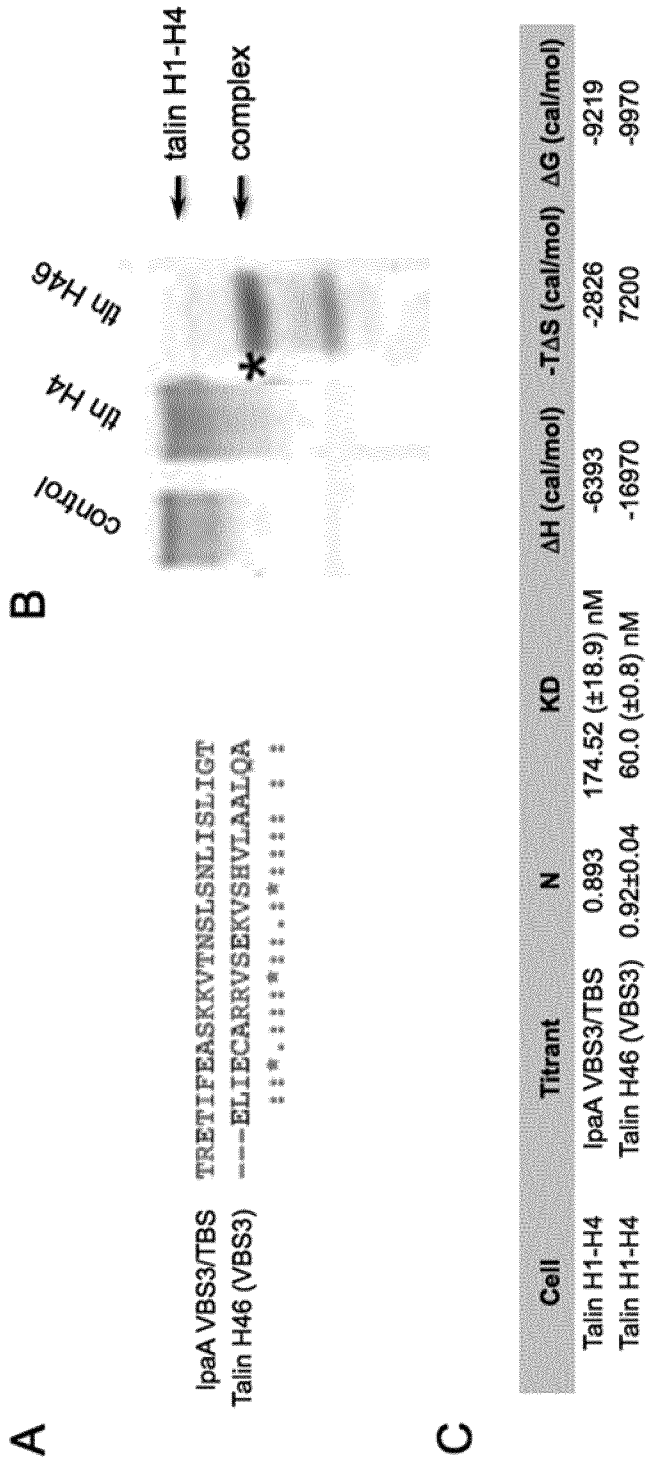

A
IpaA VBS3/TBS TRETIFEASKKVTNSLSNLISLIGT
Talin H46 (VBS3) ---ELIECARRVSEKVSHVLAALQA
B
control
talin H4
talin H46
talin H1-H4
complex
*
C
Cell Titrant N KD ΔH (cal/mol) -TΔS (cal/mol) ΔG (cal/mol)
Talin H1-H4 IpaA VBS3/TBS 0.893 174.52 (±18.9) nM -6393 -2826 -9219
Talin H1-H4 Talin H46 (VBS3) 0.92±0.04 60.0 (±0.8) nM -16970 7200 -9970

FIG. 15

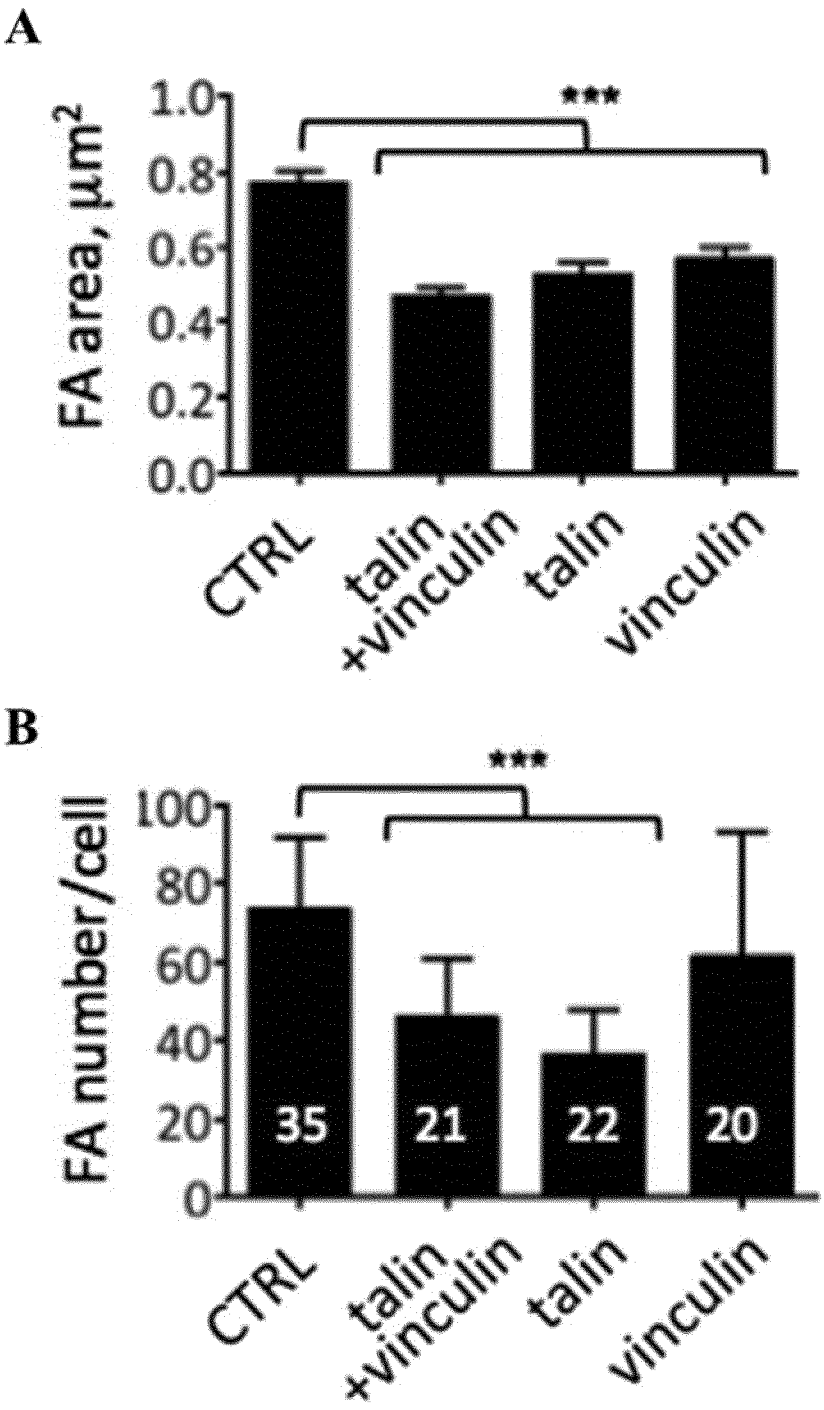

A
FA area, µm²
1.0
0.8
0.6
0.4
0.2
0.0
***
CTRL
talin +vinculin
talin
vinculin
B
FA number/cell
100
80
60
40
20
0
***
35
21
22
20
CTRL
talin +vinculin
talin
vinculin

FIG. 16

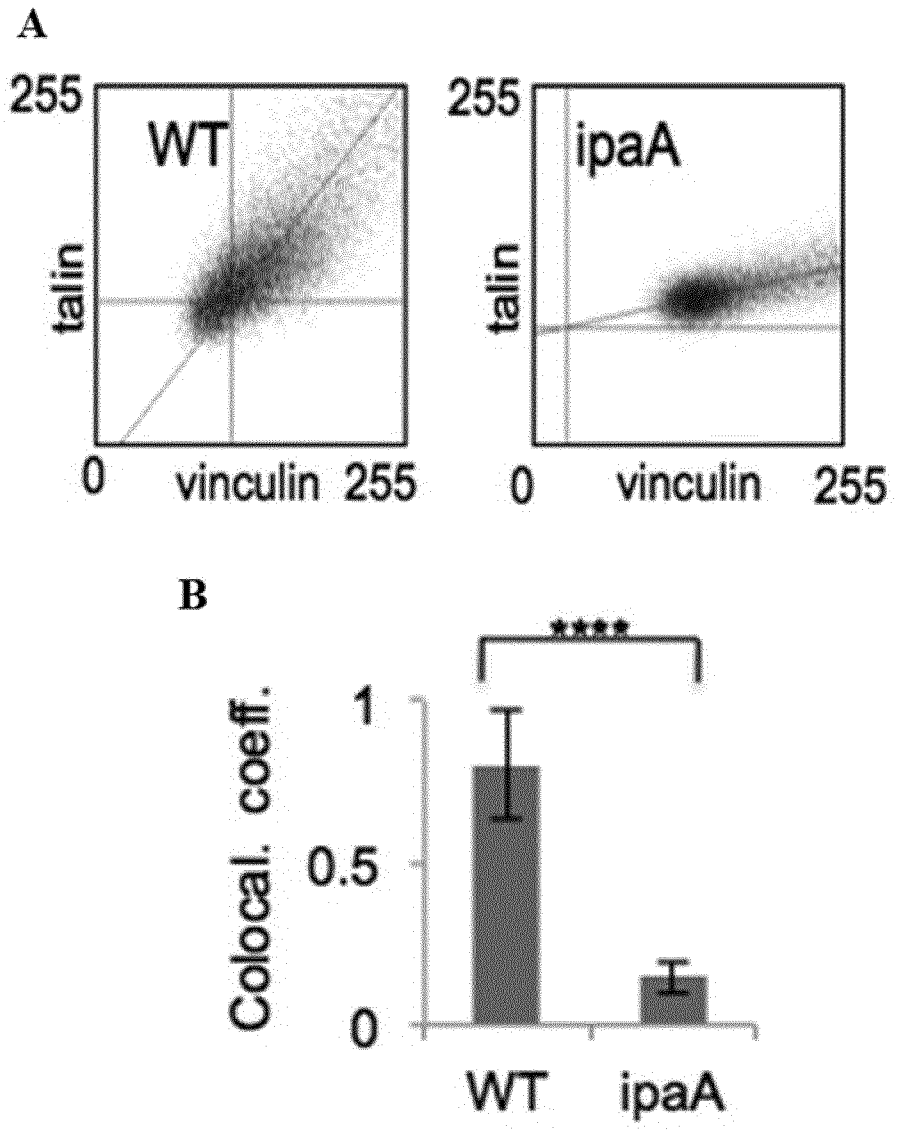

A
255
WT
talin
0
vinculin
255
255
ipaA
talin
0
vinculin
255
B
****
Colocal. coeff.
1
0.5
0
WT
ipaA

FIG. 17

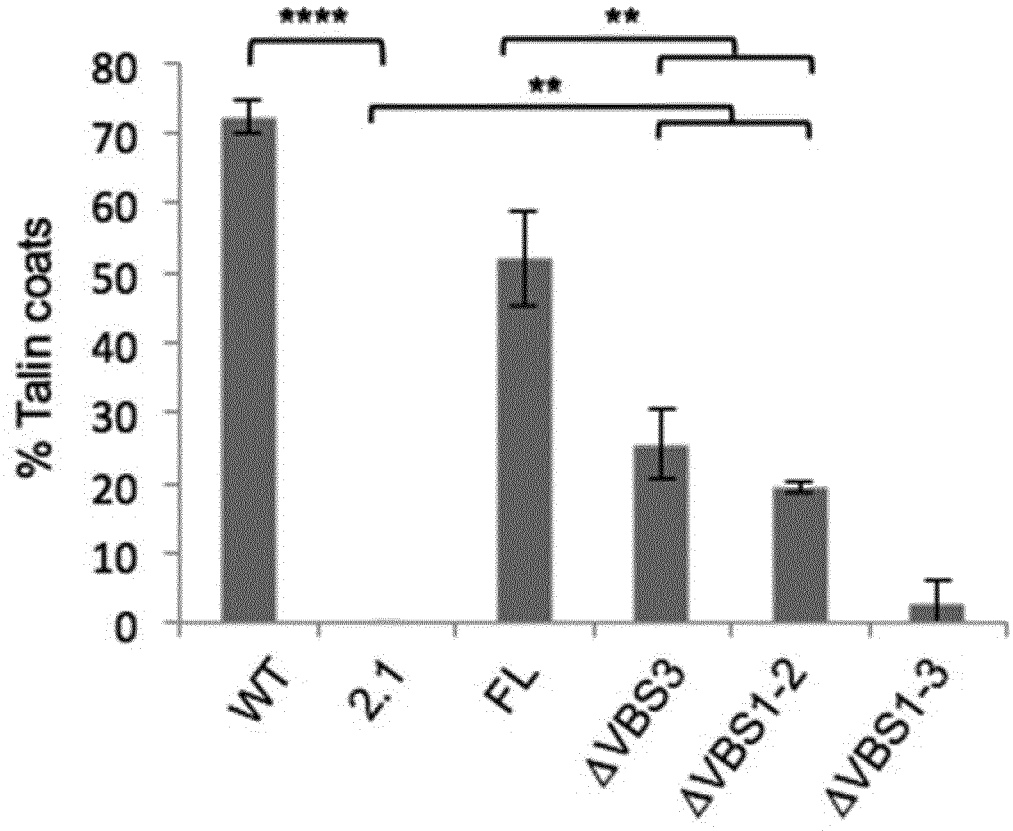

****
**
**
% Talin coats
80
70
60
50
40
30
20
10
0
WT
2.1
FL
ΔVBS3
ΔVBS1-2
ΔVBS1-3

FIG. 18

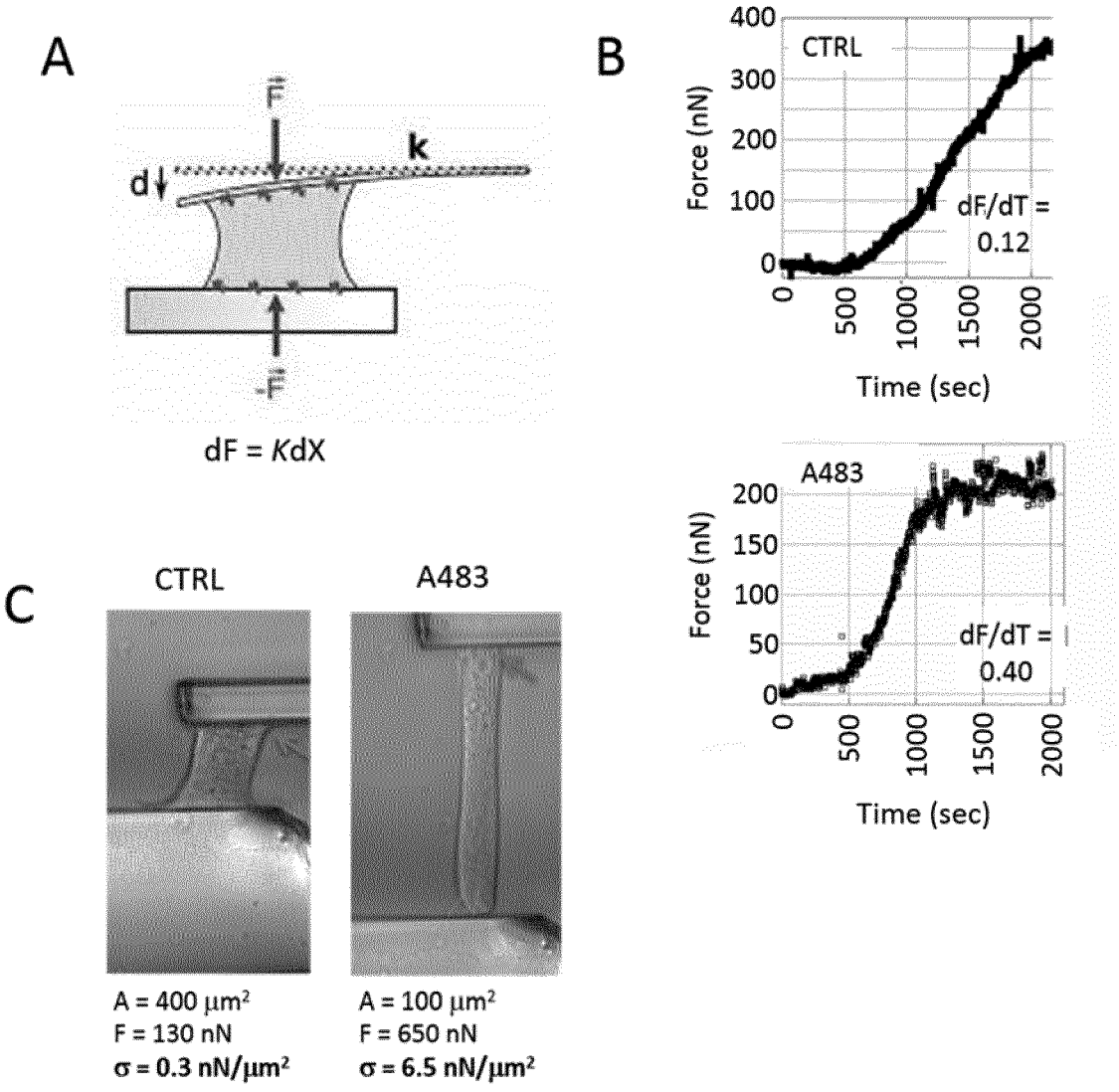

A
F
k
d
-F
dF = KdX
B
CTRL
Force (nN)
400
300
200
100
0
dF/dT = 0.12
0
500
1000
1500
2000
Time (sec)
A483
Force (nN)
200
150
100
50
0
dF/dT = 0.40
0
500
1000
1500
2000
Time (sec)
C
CTRL
A483
A = 400 µm²
F = 130 nN
σ = 0.3 nN/µm²
A = 100 µm²
F = 650 nN
σ = 6.5 nN/µm²

FIG. 19

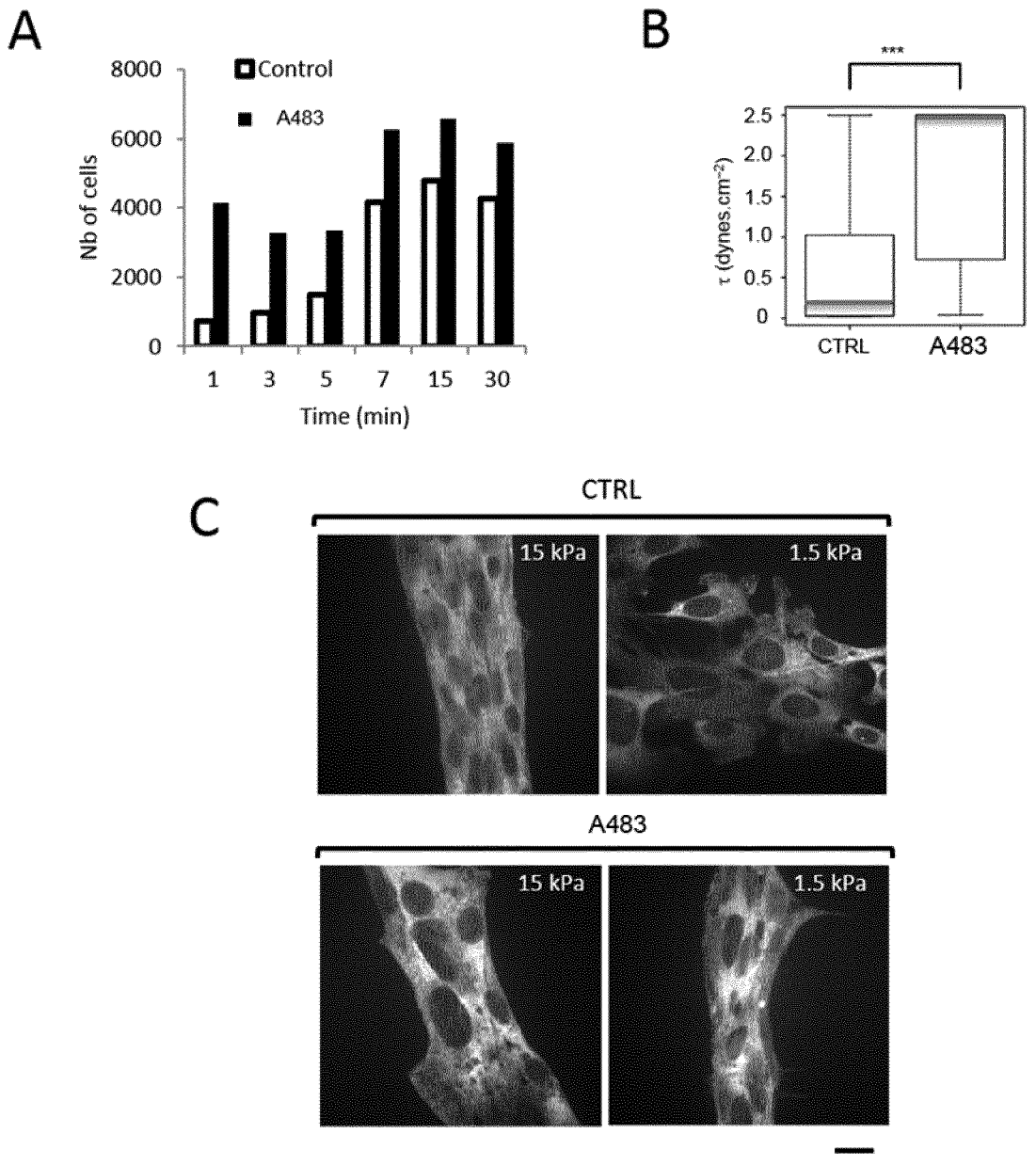

A
8000
6000
4000
2000
0
Nb of cells
Control
A483
1
3
5
7
15
30
Time (min)
B
***
2.5
2.0
1.5
1.0
0.5
0
τ (dynes.cm⁻²)
CTRL
A483
C
CTRL
15 kPa
1.5 kPa
A483
15 kPa
1.5 kPa

FIG. 20

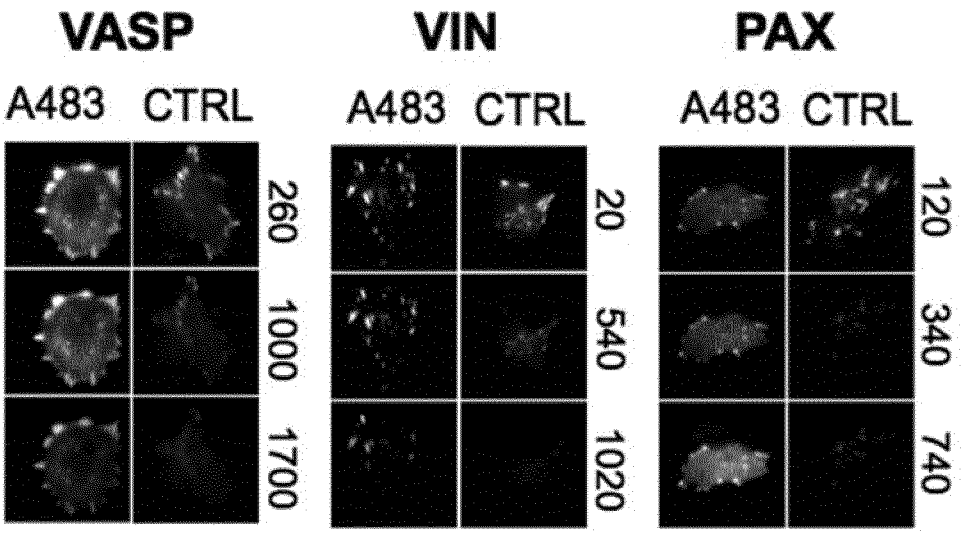

VASP
A483 CTRL
260
1000
1700
VIN
A483 CTRL
20
540
1020
PAX
A483 CTRL
120
340
740

FIG. 21

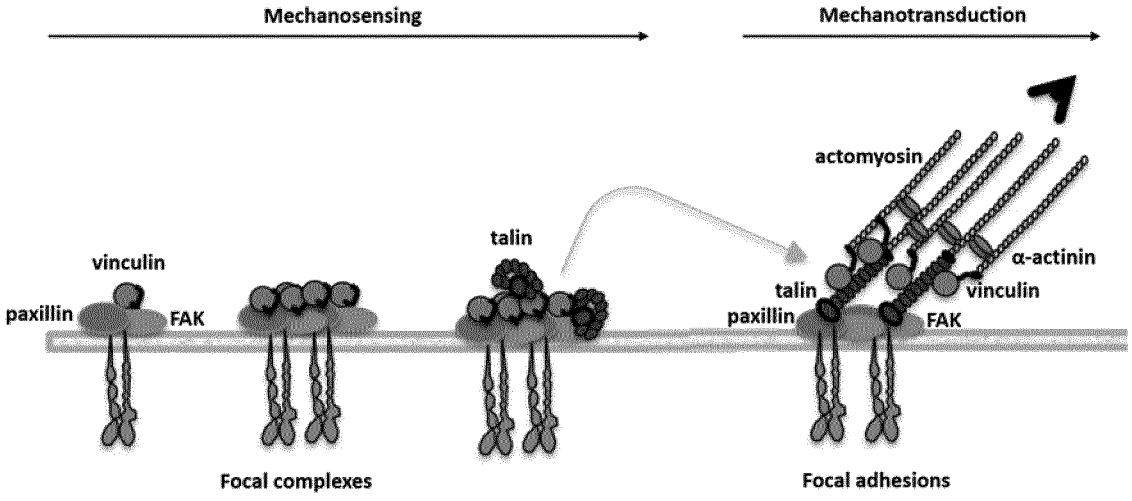

Mechanosensing
Mechanotransduction
actomyosin
talin
vinculin
paxillin
FAK
α-actinin
talin
vinculin
paxillin
FAK
Focal complexes
Focal adhesions

FIG. 22

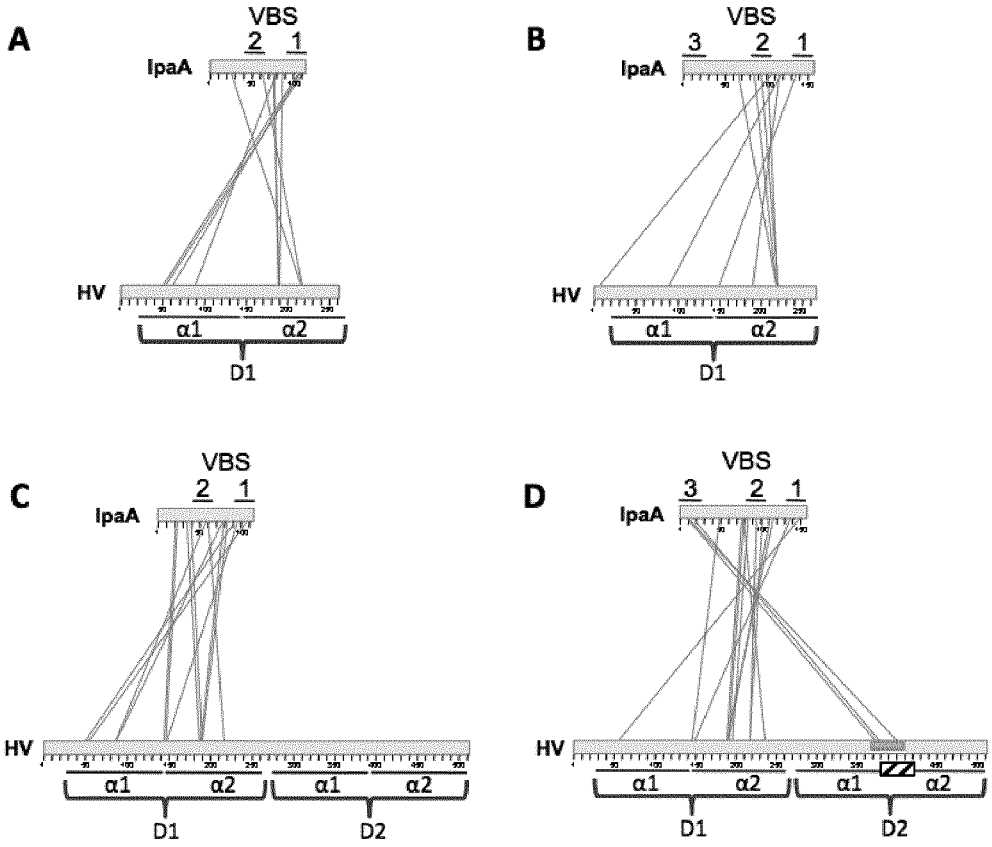

A
VBS
2 1
IpaA
HV
α1 α2
D1
B
VBS
3 2 1
IpaA
HV
α1 α2
D1
C
VBS
2 1
IpaA
HV
α1 α2 α1 α2
D1 D2
D
VBS
3 2 1
IpaA
HV
α1 α2 α1 α2
D1 D2

FIG. 23

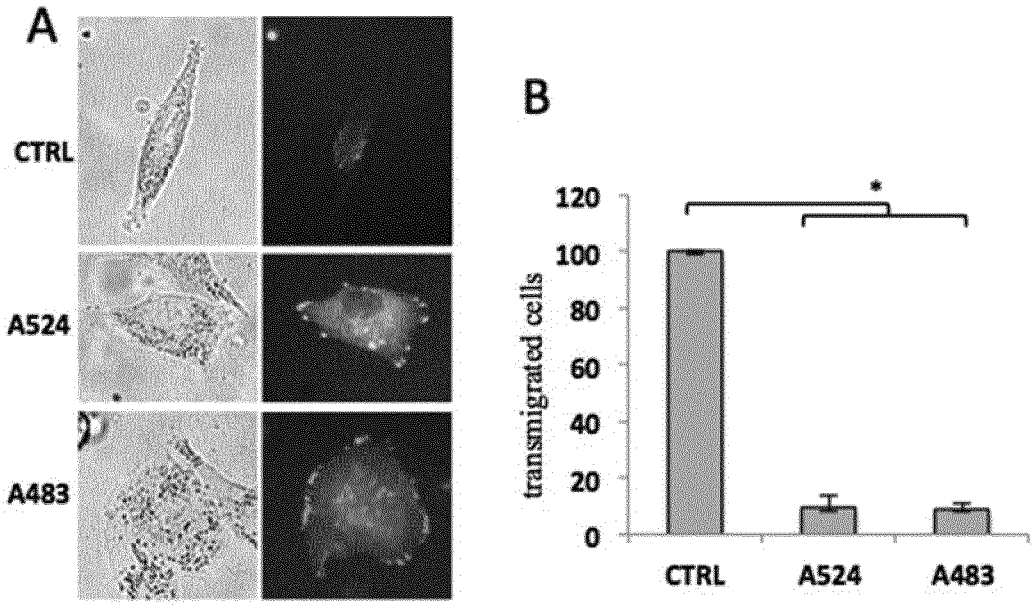

A
CTRL
A524
A483
B
transmigrated cells
120
100
80
60
40
20
0
*
CTRL
A524
A483

FIG. 24

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/073287

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K4/04 C07K14/25
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous : "UPI00069AA064" , , 22 August 2015 (2015-08-22) , XP055237548, Retri eved from the Internet: URL: http://www. uni prot.org/uni parc/UPI 0006 9AA064 [retri eved on 2015-12-18] sequence -----	1,2,6-8
X	Anonymous : "UPI00069B6C94" , , 22 August 2015 (2015-08-22) , XP055237551 , Retri eved from the Internet: URL: http://www. uni prot.org/uni parc/UPI 0006 9B6C94 [retri eved on 2015-12-18] sequence ----- -/- ·	1,2,6-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
15 November 2016	30/11/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Hoff, Cel i ne

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous : "UPI 000326416B ", , 19 March 2013 (2013-03-19) , XP055237552 , Retri eved from the Internet: URL: http://www. uni prot.org/uni parc/UPI 0003 26416B [retri eved on 2015-12-18] sequence	1, 2 ,6-8
X	Anonymous : "EM_STD:AY206439" , , 23 October 2003 (2003-10-23) , XP055237584, Retri eved from the Internet: URL: http://i bi s. i nternal .epo.org/exam/dbfe tch .j sp?i d=EM_STD:AY206439 [retri eved on 2015-12-18] sequence	3-8
A	A. R. GINGRAS ET AL: "Mappi ng and Consensus Sequence Identi f i cati on for Mul t i pl e Vi ncul i n Bi ndi ng Si tes wi thi n the Tal i n Rod" , JOURNAL OF BIOLOGICAL CHEMISTRY, vol . 280, no. 44, 30 August 2005 (2005-08-30) , pages 37217-37224, XP055237717 , US ISSN : 0021-9258, D0I : 10. 1074/jbc.M508060200 the whol e document	1-13
A	H. PARK ET AL: "Novel Vi ncul i n Bi ndi ng Si t e of the IpaA Invasi n of Shi gel l a" , JOURNAL OF BIOLOGICAL CHEMISTRY, vol . 286, no. 26, 27 Apri l 2011 (2011-04-27) , pages 23214-23221 , XP055237752 , US ISSN : 0021-9258, D0I : 10. 1074/ jbe. M 10. 184283 the whol e document	1-13

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