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Supplemental Material to:

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Substrate-attached materials are enriched with tetraspanins and are analogous to the structures associated with rear-end retraction in migrating cells

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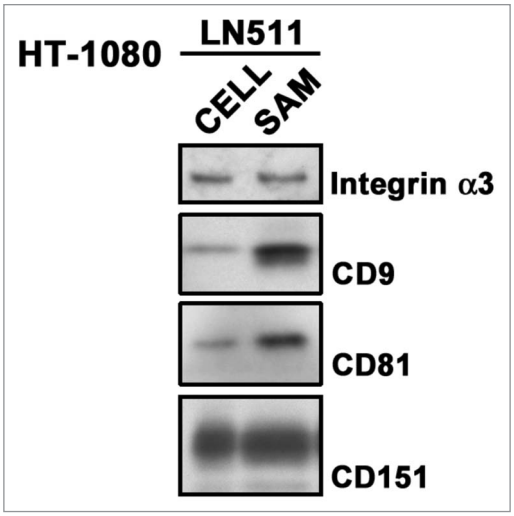

Figure S1. SAMs prepared from HT-1080 cells adhering to laminin-511. HT-1080 cells were cultured on laminin-511-coated dishes for 2h30min. SAMs were prepared after detaching the cells by treatment with EGTA as described in "Materials and Methods". Lysates were also prepared from detached cells (CELL). SAMs and lysates were separated by SDS-PAGE and immunoblotted with the antibodies indicated on the right of the blots.

Movie S1. Time-lapse imaging of A549 cells migrating on laminin-511 in the absence of inhibitor. A549 cells were plated in serum-free medium on plastic dishes coated with laminin-511. One hour later, the medium was replaced with medium containing 1% FBS without inhibitor. Two hours after the medium change, cell migration was monitored at 5 min intervals for 6 h by time-lapse phase-contrast microscopy with a 10× objective.

Movie S2. Time-lapse imaging of A549 cells migrating on laminin-511 in the presence of Y-27632. A549 cells were plated in serum-free medium on plastic dishes coated with laminin-511. One hour later, the medium was replaced with medium containing 1% FBS plus 10 μM Y-27632. Two hours after the medium change, cell migration was monitored at 5 min intervals for 6 h by time-lapse phase-contrast microscopy with a 10× objective.

Movie S3. Time-lapse imaging of A549 cells migrating on laminin-511 in the presence of blebbistatin. A549 cells were plated in serum-free medium on plastic dishes coated with laminin-511. One hour later, the medium was replaced with medium containing 1% FBS plus 20 μM (±)-blebbistatin. Two hours after the medium change, cell migration was monitored at 5 min intervals for 6 h by time-lapse phase-contrast microscopy with a 10× objective.

Movie S4. Time-lapse imaging of A549 cells migrating on laminin-511 in the presence of dynasore. A549 cells were plated in serum-free medium on plastic dishes coated with laminin-511. One hour later, the medium was replaced with medium containing 1% FBS plus 100 μM dynasore. Two hours after the medium change, cell migration was monitored at 5 min intervals for 6 h by time-lapse phase-contrast microscopy with a 10× objective.

Movie S5. Time-lapse imaging of A549 cells migrating on laminin-511. A549 cells were plated in serum-free medium on glass-bottom dishes coated with laminin-511. One hour later, the medium was replaced with medium containing 1% FBS. Two hours after the medium change, cell migration was monitored at 30 sec intervals for 30 min by time-lapse phase-contrast microscopy with a 63× objective.

Movie S6. Time-lapse imaging of A549 cells migrating on type I collagen. A549 cells were plated in serum-free medium on glass-bottom dishes coated with type I collagen. One hour later, the medium was replaced with medium containing 1% FBS. Two hours after the medium change, cell migration was monitored at 30 sec intervals for 30 min by time-lapse phase-contrast microscopy with a 63× objective.

Movies S1–S6 can be found at www.landesbioscience.com/journals/celladhesion/article/25041

Table S1. Analysis 1; Proteins detected by LC-MS/MS analysis of SAMs.

Table S1.xls can be found at www.landesbioscience.com/journals/celladhesion/article/25041

SAMs were prepared from A549 cells cultured on laminin-511 and analyzed by LC-MS/MS as described in "Materials and Methods".

Table S2. Analysis 2; Proteins detected by LC-MS/MS analysis of SAMs.

Table S2.xls can be found at www.landesbioscience.com/journals/celladhesion/article/25041

SAMs were prepared from A549 cells cultured on laminin-511 and analyzed by LC-MS/MS as described in "Materials and Methods".

Table S3. Analysis 3; Proteins detected by LC-MS/MS analysis of SAMs.

Table S3.xls can be found at www.landesbioscience.com/journals/celladhesion/article/25041

SAMs were prepared from A549 cells cultured on laminin-511 and analyzed by LC-MS/MS as described in "Materials and Methods".

Table S4. Proteins reproducibly detected by LC-MS/MS analysis.

Table S4.xls can be found at www.landesbioscience.com/journals/celladhesion/article/25041

Proteins reproducibly detected in three independent analyses are listed. The proteins were categorized by cellular localization and functions based on the UniProt Knowledgebase.