SUPPLEMENTAL FIGURES

Supplemental Figure 1. CD151 clustering at areas of cell-cell contact *in vivo*. Chick embryos bearing a tumor composed of HEp3 cells were treated with Alexa 488-conjugated mAB 1A5 (5 µg/animal). Accumulation of fluorescently-labeled antibody in the tumor was document with time-lapse imaging of Alexa 488 fluorescence. The bottom panels represent the full micrograph at 0 and 340 minutes. The central panel shows a full time-lapse of the cropped region (dashed white lines). The top panels are magnified to reveal accumulation at intermediate time points. A white arrow highlights accumulation of mAB 1A5 at the area of cell-cell contact between two tumor cells invading stroma adjacent to the tumor.

Supplemental Figure 2. Cell-cell adhesion in response to CD151 clustering. (A) Jurkat and U937cells cultured in the absence or presence of either control IgG or mAB 1A5. (B) MAB 1A5 (A and B) or control IgG (C and D) were added to Jurkat cells after (A and C) or 1 hr before fixing the cell with paraformaldehyde. The antibody location was detected using Alexa 546-conjugated anti-mouse IgG.

Supplemental Figure 3. MAB 1A5 binds to CD151 not engaged with α3 integrin in HEp3 cells. CD151 was immunoprecipitated from HEp3 cell lysates using three distinct anti-CD151 antibodies (1A5, 8C3 and 11G5A). The precipitated CD151 and the co-immunoprecipation α3 was detected by immunoblotting.

Supplemental Figure 4. MAb 1A5 recognizes the integrin-binding domain of CD151. (A) Schematic of CD151 highlighting the extracellular domains of the protein including the small extracellular loop (SEL) and the large extracellular loop (LEL). The LEL as depicted is divided into 2 regions a constant region and a variable region. The variable region can be further divided into 3 subdomains denoted site 1, site 2 and site 3. (B) Representative flow cytometric dot plot of NIH3T3 cells gated for GFP and Alexa 647. The three panels show NIH 3T3 cells the were mock transfected, transfected with a wildtype CD151-GFP, or transfected with mutant CD151-GFP not recognized by the antibody. (C) Table adapted from Yamada et. al (23) grouping the various anti-CD151 monoclonal antibodies including mAB 1A5 according to their abilities to bind to the CD151-LEL mutants as demonstrated by two color flow cytometry.

Supplemental Figure 5. Antibodies that recognize the integrin-binding domain of CD151 mediate clustering at areas of cell-cell contact. A549 cells transiently transfected with CD151-GFP were treated with the control IgG or the anti-CD151 antibodies 1A5, 8C3 or 11G5A at 5 μg/ml. Localization of the tetraspanin was documented by fluorescent microscopy. The * defines cells with excessive expression which saturates the image.

Supplemental Figure 6. CD151 expression in normal and prostate cancer tissue. (A) Evaluation of CD151 expression in prostate tissue using the publicly available GDS3113 (n=3/tissue). Tissues are arranged according to expression levels following the universal human reference. The dashed line corresponds to the level of expression found in normal prostate. (B-C) A comparison of CD151 expression in prostate cancer vs. normal or benign tissue was accomplished using GSE6099 (D, n=102), GSE2545 (C, n=171), and GDS1439 (n=19) respectively.

Supplemental Figure 7. Expression of integrin α3 is not altered in cancers of the bladder or skin. Expression of CD151 (top) and integrin α3 (bottom) in cancers of the bladder and skin with a comparison of normal and tumor tissue (representative example of 12 patients/cancer). Histological staining is publicly available through ProteinAtlas.org.

Supplemental Figure 8. $CD151^{free}$ detectable in benign and normal prostate tissue does not correspond with patient outcome. Kaplan-Meier curves of recurrence-free survival generated using the $CD151^{free}$ immunoreactivity in tissue sections of prostate tissue obtained from patients that received a RRP after diagnosis whom were monitored for biochemical recurrence (cohort #1, N=99). A analysis of $CD151^{free}$ staining on adjacent benign (A) and distant normal tissue (B).

Supplemental Movie 1. CD151 clustering at areas of cell-cell contact in HEp3 tumor cells. HEp3 cells cultured to 80% confluency were monitored by time-lapse microscopy before, during and after treatment with 1μg/ml Alexa 488-conjugated mAB 1A5. Cell behaviour was monitored by differential interference contrast (DIC) imaging while antibody localization was monitored by fluorescence microscopy.