Supplementary Figure Legends

Supplementary Figure S1. PC3-MM2/Trop-2-GFP disseminated in liver parenchyma. A, FACS profiles of parental PC3-MM2 cells stained for Trop-2 (left panel, continuous black line), and of PC3-MM2/GFP (middle panel, continuous blue line) and PC3-MM2/Trop-2-GFP (right panel, continuous green line) cells are shown. Dotted black line, unstained parental PC3-MM2. B, Fluorescence microscopy pictures of liver sections (left) have been subjected to spectral deconvolution using the Nuance Software as described in Supplementary Materials and Methods in order to obtain color-coded pictures (right). A representative picture before and after spectral deconvolution is shown. Arrows, fluorescent cells.

Supplementary Figure S2. Trop-2 promotes prostate cancer cell migration on FN. A, Migration assays using PC3-2 transfectants seeded on FN-coated transwell chambers: representative pictures of cells attached and cells migrated are shown on top and bottom panels, respectively. B, LNCaP/Trop-2 (top) and LNCaP/Mock (bottom) transfectants seeded on FN-coated plates were observed for 17 hours by time-lapse video microscopy. The movements of individual cells were followed using cell-tracking software, and the panels are presented as overlays of trajectories described by cells during their migration (left); randomly selected migration tracks were copied and combined into individual panels to avoid empty spaces (middle). Bar graphs showing directionality, velocity, total distance migrated and linear displacement of LNCaP/Trop-2 (n=93 cells) and LNCaP/Mock (n=114 cells) transfectants (right). Values are reported as means ± SEM. **, $P<0.001$ as determined by a Student's t test.

Supplementary Figure S3. Surface levels of β_1, α_v and α_5 integrins are not affected by Trop-2. A, Surface levels of β_1 (left) and β_3 (right) integrins in PC3-2/Trop-2 (continuous black lines) and PC3-2/Mock (dotted black lines) transfectants. PC3-2/Trop-2 cells stained with a non-immune mouse IgG (continuous gray lines) were used as negative controls. B, Surface levels of β_1 (left) and α_v (right) integrins in PC3-1/Trop-2 shRNA (continuous black lines), PC3-1/ctr. shRNA (dotted black lines) and parental PC3-1 (discontinuous black lines) cells. PC3-1/Trop-2 shRNA cells stained with a non-immune mouse IgG (continuous gray lines) were used as negative controls. C, Surface levels of α_5 integrin in PC3-2/Trop-2 (continuous black lines) and PC3-2/Mock (dotted black lines) transfectants. PC3-2/Trop-2 cells stained with a non-immune mouse IgG (continuous gray lines) were used as negative controls.

Supplementary Figure S4. The association between Trop-2 and β_1 integrins occurs independently on β_1 activation or adhesion to ECM ligands. A, PC3-1 cells were incubated with PBS alone (-) and PBS supplemented with Mn^{2+} ions (+); IP was then performed as described in Supplementary Materials and Methods, using an Ab against β_1. IB analysis was performed using a goat pAb against Trop-2. A mouse IgG (Neg.Ctr.) was used as a negative control Ab for IP. B, PC3-1 cells were seeded on FN, VN, and collagen-I before lysis, and IP was then performed as described in Supplementary Materials and Methods, using a mAb against β_1. Cells seeded on these three ECM ligands were compared with cells in regular culture conditions (FBS). IB analysis was performed using a goat pAb against Trop-2. A mouse IgG (Neg.Ctr.) was used as a negative control Ab for IP.

Supplementary Figure S5. Trop-2 co-localizes with α_5 and β_1 integrin subunits in trafficking vesicles. A, Internalization and recycling of membrane receptors were induced in Trop-2-endogenously expressing DU145 cells as described in Supplementary Materials and Methods. Cells

were fixed at the various stages of internalization / recycling, and analyzed by confocal microscopy. Co-localization between Rab4 (green) and Trop-2 (red) upon internalization (PCC = 0.596±0.027) is shown in the top panels. Co-localization between Rab11 (green) and Trop-2 (red) upon recycling (PCC = 0.625±0.034) is shown in the bottom panels. B, Trop-2 (red) co-localization with integrin subunits (green) in trafficking vesicles was assessed by confocal microscopy upon induction of internalization (top) and recycling (bottom). Insets on the bottom of each figure represent a projection on the Z-axis. Arrows, internal vesicles in which Trop-2 and integrin subunits are co-localized. Left, co-localization between Trop-2 and $\alpha 5$. Right, co-localization between Trop-2 and $\beta 1$.

Supplementary Figure S6. Surface expression levels of wild type and Δcyto Trop-2 in PC3-2 transfectants. PC3-2 cells were transiently transfected with wild type and Δcyto Trop-2, and surface expression of Trop-2 variants were analyzed by FACS. Continuous black lines, expression profiles of PC3-2/wild type Trop-2 (left) and PC3-2/Δcyto Trop-2 (right) transfectants stained using an Ab against the extracellular domain of the molecule. Dotted black lines, staining with a non-immune mouse IgG.