Supplemental Information appendix

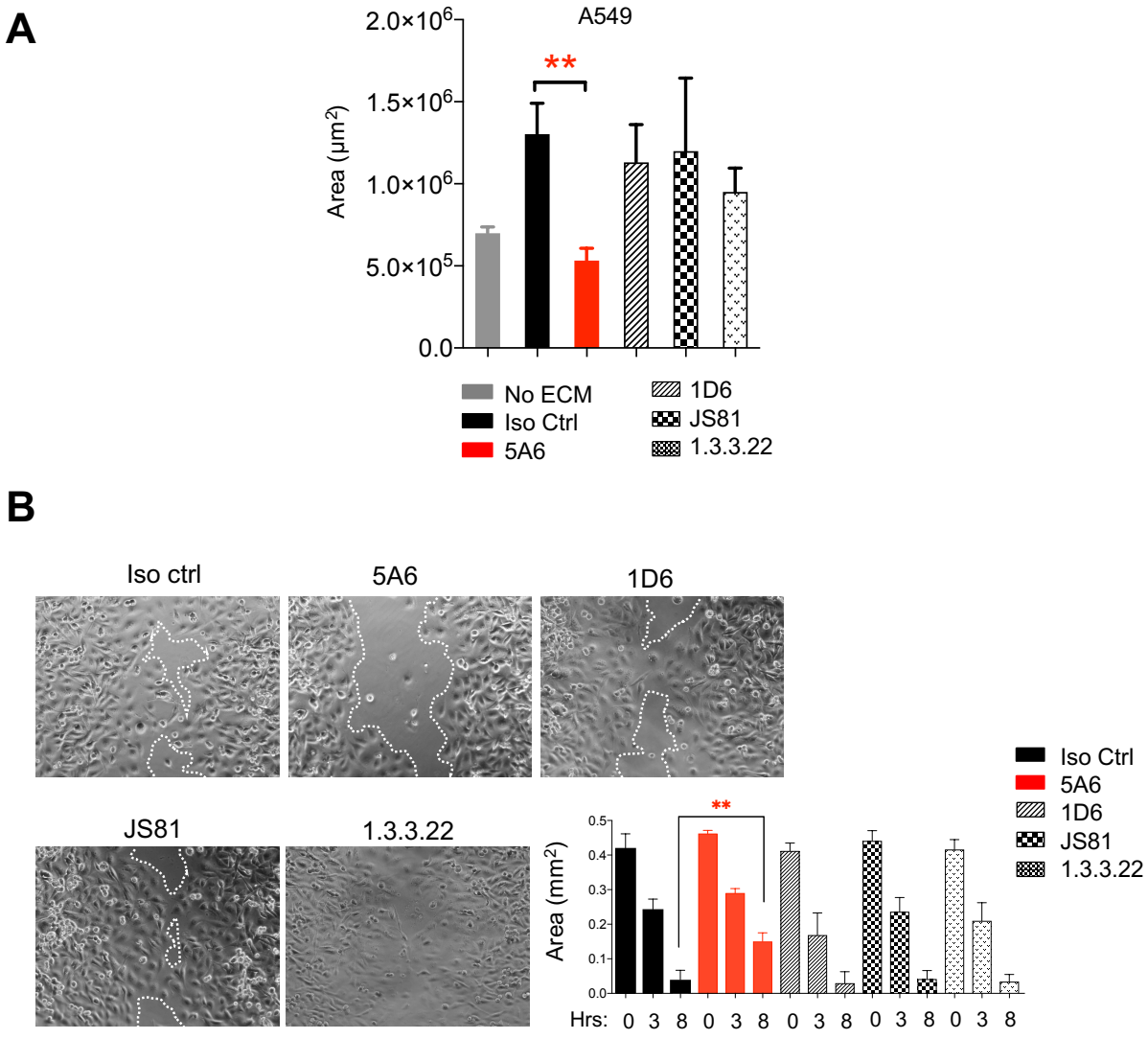

Figure S1. 5A6 inhibits migration of tumor cell lines. (A) 3D invasion assay of A549 cells. 3x10^3 cells were seeded in spheroid formation media and then incubated with extracellular matrix containing various anti-CD81 mAbs. Invaded areas were quantified after 72 hrs using imageJ software. Shown is mean ± SEM, Student's t test, *p=0.0065***. **(B)** *In vitro* scratch assay. A monolayer of MDA-MB-231 cells were scratched and then treated with 5A6, 1D6, JS81, 1.3.3.22 or isotype control mAb, quantify migrated areas were analyzed after 3 and 8 hrs by light microscopy. Shown is mean ± SEM, Student's *t test*, *p=0.0028***.

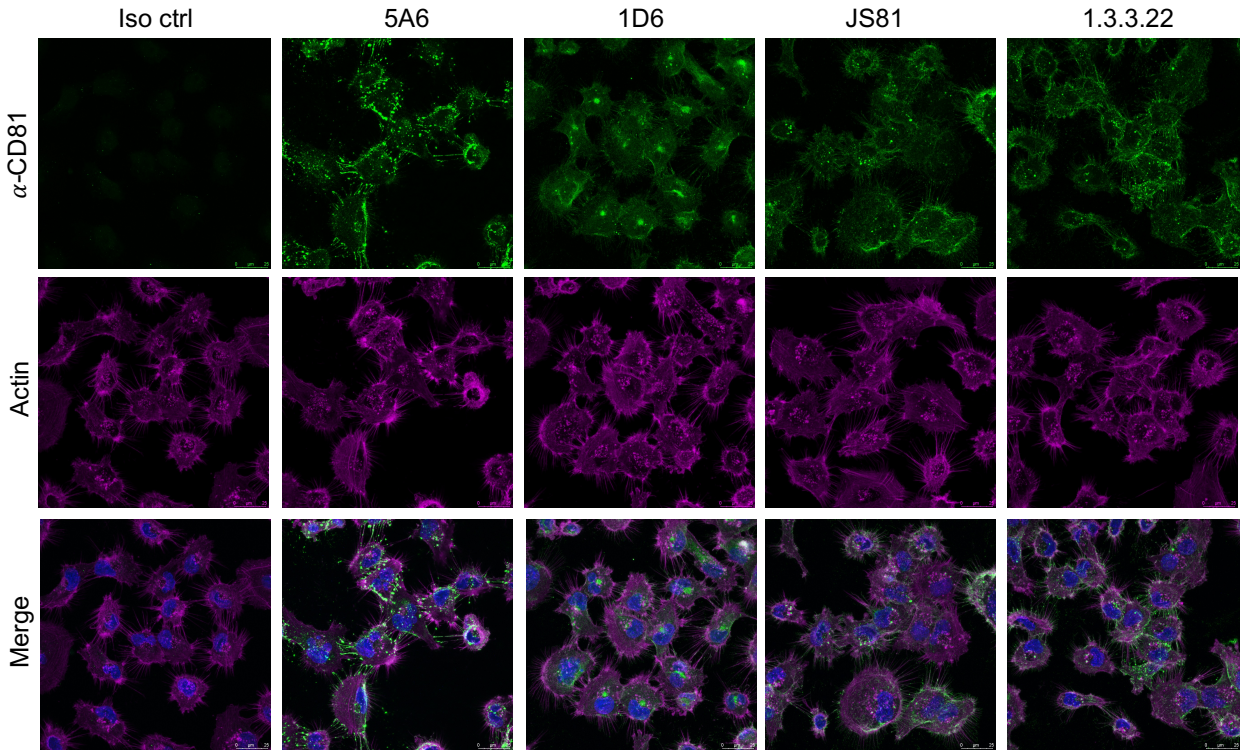

Figure S2. 5A6 but no other anti-CD81 mAbs clusters CD81 at the cell to cell contact areas. (A) MDA-MB-231 cells were plated on fibronectin-coated coverslips and incubated at 37°C for 1 hr with the indicated unconjugated mouse mAbs, then fixed and permeabilized; followed by anti-mouse FITC secondary antibody and with Phalloidin-Alexa 647 to stain the actin cytoskeleton. Coverslips were mounted in ProGold media with DAPI and analyzed by confocal microscopy. (photos were taken at 63X magnification).

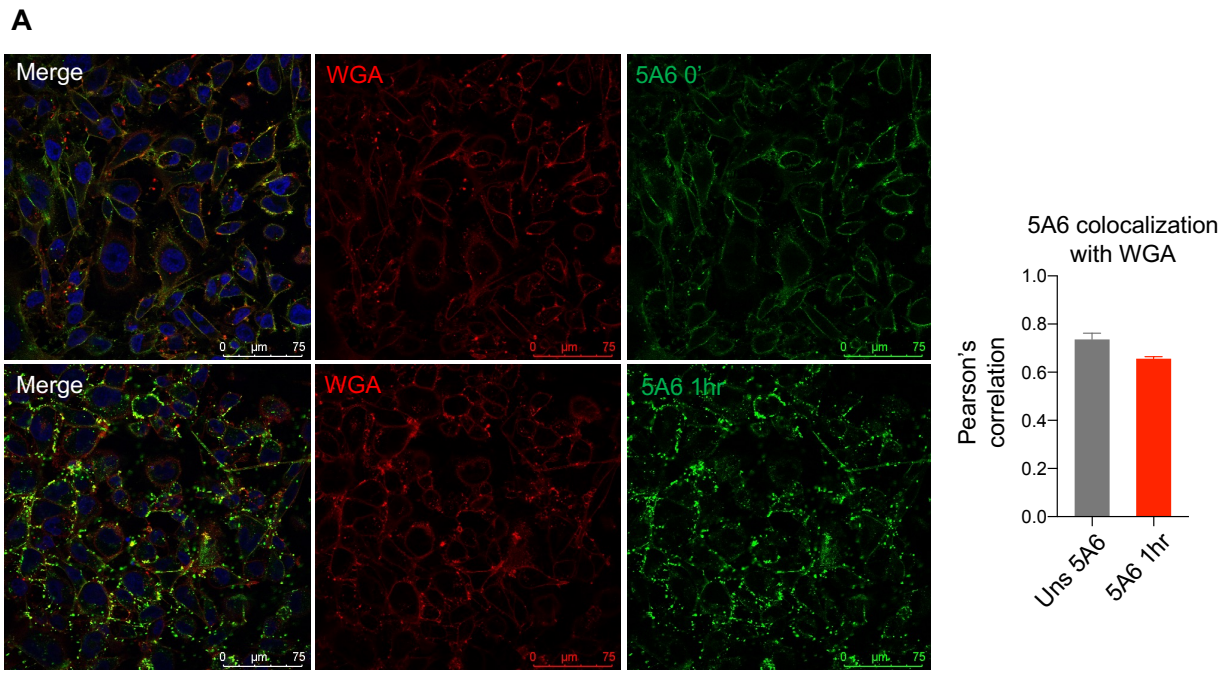

Figure S3. 5A6 induces CD81 clustering at the cell surface membrane. MDA-MB-231 cells were plated on fibronectin-coated coverslips and left unstimulated followed by fixation (5A6 0') or incubated with 5A6-alexa 488 fo1 hr at 37°C and then fixed. Both stimulated and unstimulated cells were then stained with the cell surface membrane marker wheat germ agglutinin-Alexa 647 labeled (WGA). Coverslips were mounted in ProGold media with DAPI and analyzed by confocal microscopy. (photos were taken at 40X magnification). Person's correlation coefficient between WGA and 5A6 was analyze using imageJ software.

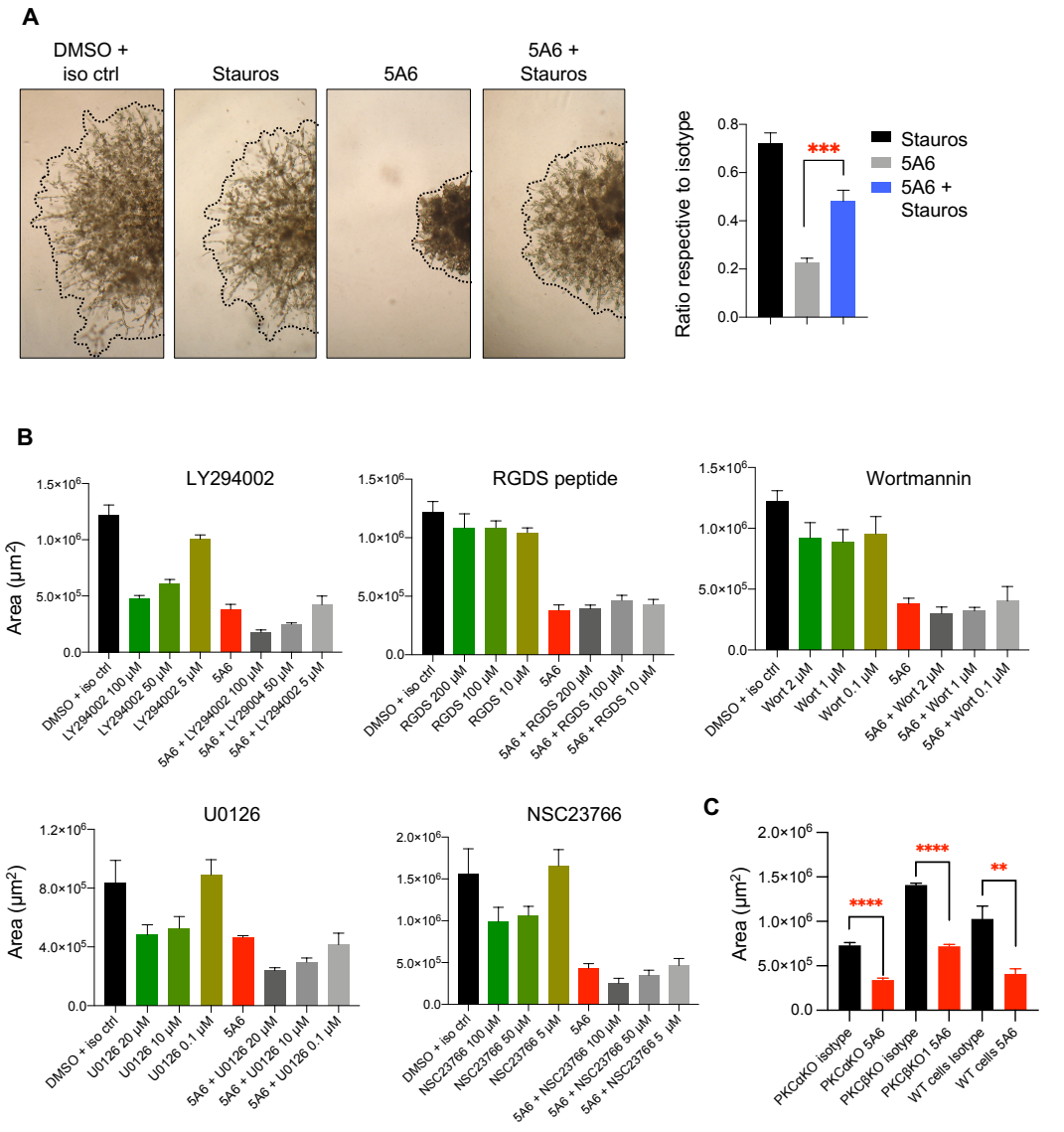

Figure S4. Inhibition of invasion by 5A6 is partially inhibited by Staurosporine, but does not depend on PI-3K, MEK1/2, Rac1, PKCα/β nor RGDS-dependent Integrins. **(A-B)** MDA-MB-23 cells or **(C)** MDA-MB-231 PKCαKO or MDA-MB-231 PKCβKO cells were seeded in a 96 well round bottom plate in 1X spheroid formation ECM and incubated for 48-72 hrs to promote sphere formation. Invasion matrix was added containing 10µg/ml of 5A6 mAb or isotype control mAb in the presence of **(A-B)** DMSO and the presence of absence of either, Staurosporine, Wortmannin, LY94002, RGDS peptide, U0126 or NSC23766 inhibitors at the indicated concentrations. Photographs were then taken at 72-96 hrs. (A) shown photograph taken at 96hrs and the invaded areas were analyzed at (A) 96hrs or (B) 72hrs using imageJ software Shown is mean ± SEM, Student's *t test* p=0.0001***. Experiments were done in triplicates. **(C)** Shown invaded areas at 72 hrs analyzed by imageJ software. Shown is mean ± SEM. Student's *t test,* p=0.0001****, p=0.0028**.

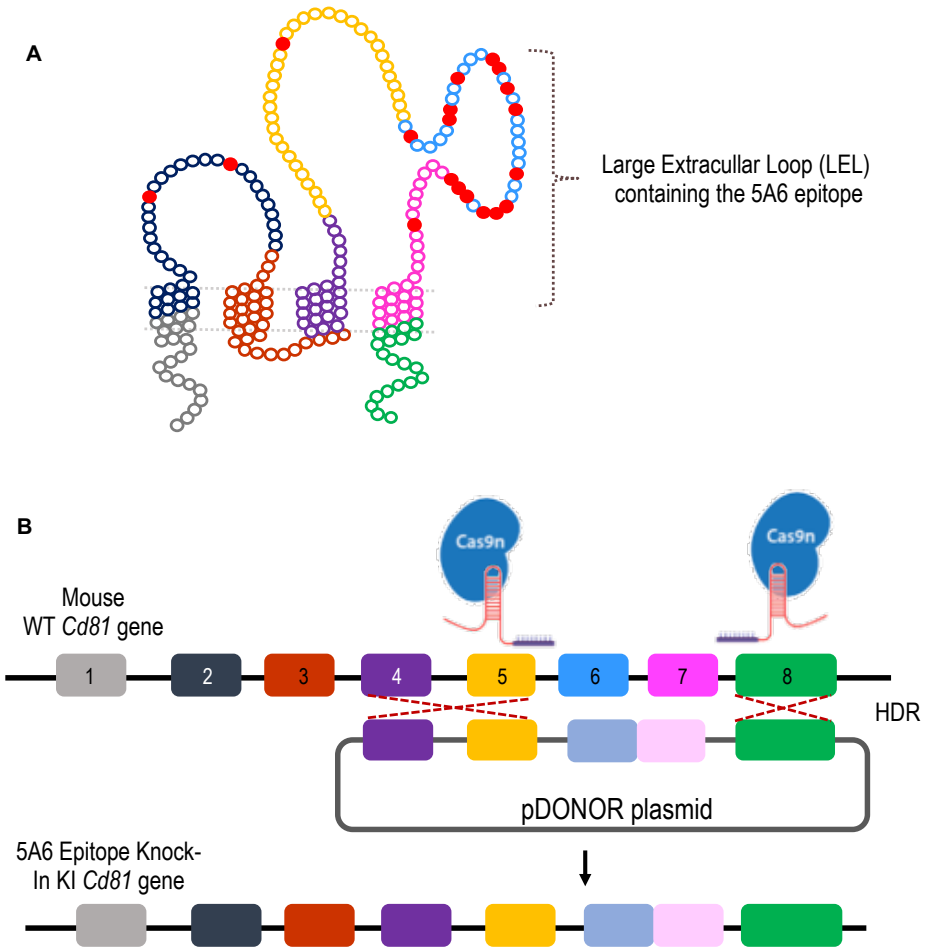

Figure S5. Schematic diagram of CD81 protein/genomic DNA structure and CRISPR/Cas9 epitope knock-In strategy. (A) The CD81 protein is encoded by 8 exons (shown by different color-coded amino acids) corresponding to the genomic DNA map shown below. Red circles represent amino acids that differ between mouse and human, mostly located in exons 6 (blue) and 7(pink). **(B)** Schema used to generate 5A6-epitope knock-in 4T1 cells that express CD81 driven by its endogenous promoter. The location of the guide RNAs/Cas9 complex (gRNA) flanking exons 6 and 7 is indicated, as well as donor template design (pDONOR plasmid) that includes homology arms to exons 4/5 and 8 and human exon 6 and 7 in tandem without intron.