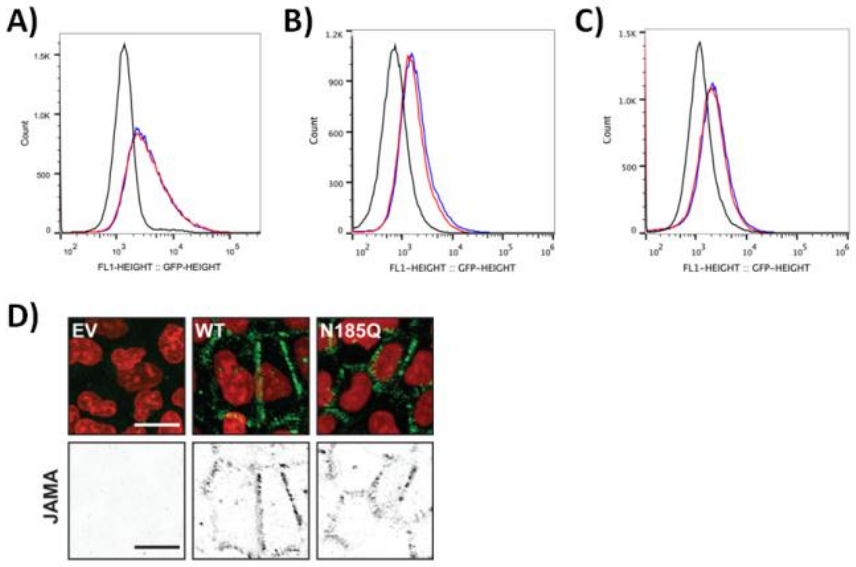
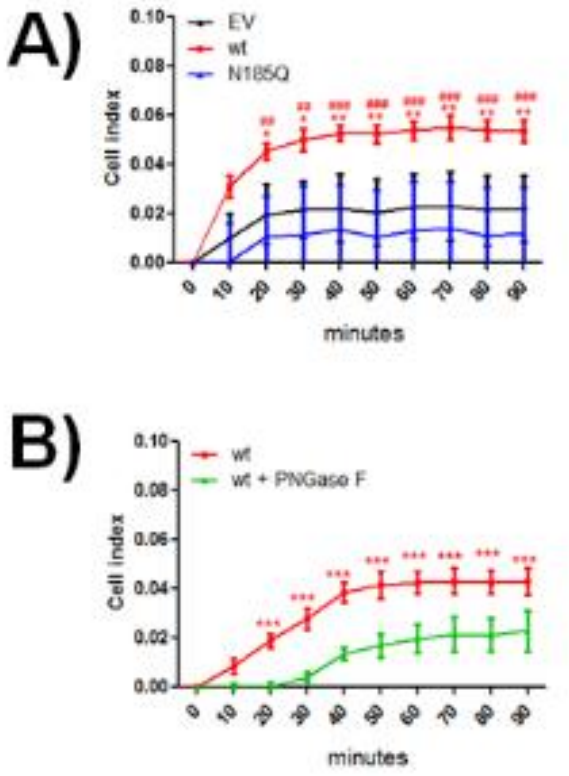
Supplemental Materials

Molecular Biology of the Cell

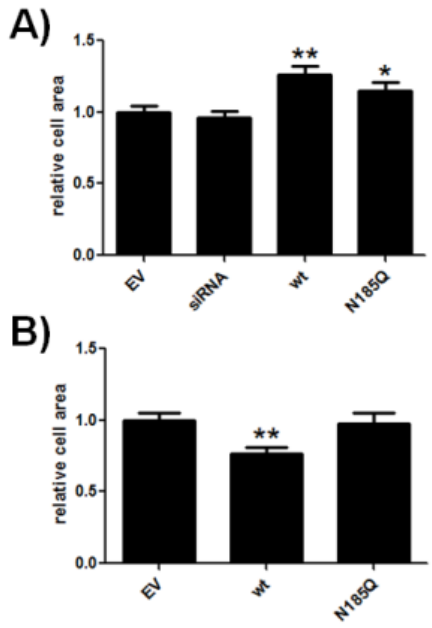
Scott et al.



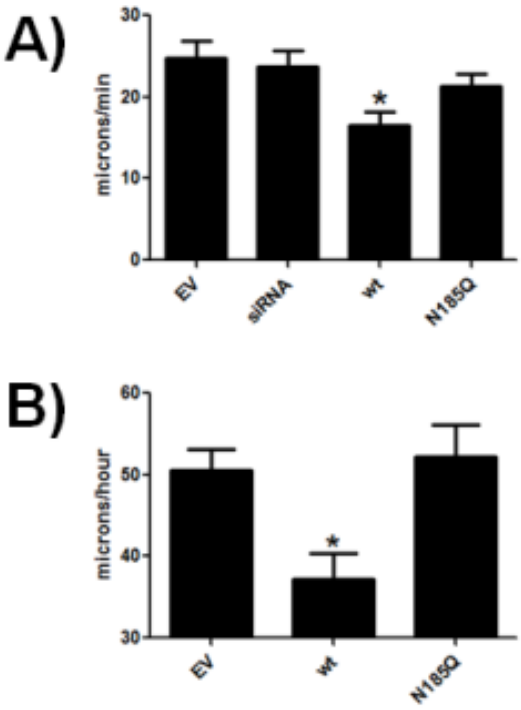
Supplemental Figure 1. Cell surface analysis of wt and N185Q JAM-A. CHO (A), HUVEC (B), or MDA-MB-231 (C) were transfected with empty vector (black line), wt (red line), or N185Q JAM-A (blue line) and assessed for cell surface expression of JAM-A by flow cytometric analysis. (D) CHO cells transfected with empty vector (EV), wild type (wt), or N185Q mutant JAM-A were assessed for expression and junctional localization of JAM-A by confocal microscopy. Top row represents pseudocolored staining of JAM-A (green) and nuclei (red). Bottom row represents inverted grayscale of JAM-A staining to highlight junctional localization.



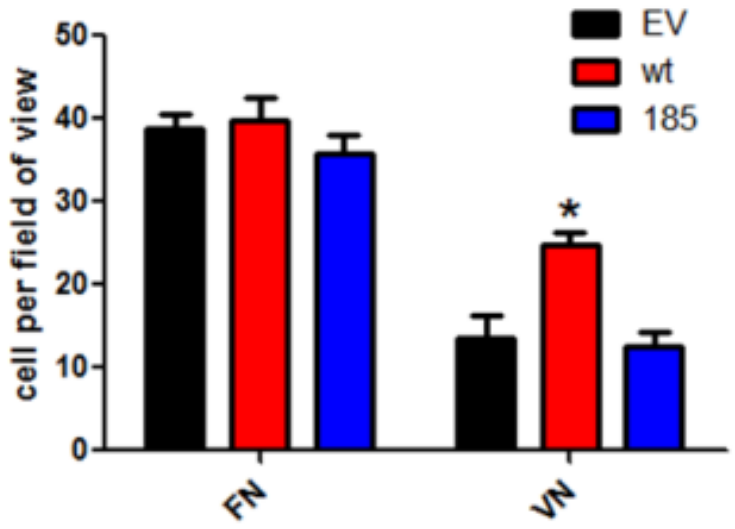
Supplemental Figure 2. N-glycans are required for JAM-A binding to JAM-A/fc. CHO cells expressing empty vector (EV), wild type (wt) or N185Q JAM-A were allowed to adhere and spread to JAM-A/fc (A). Cho cells expressing wt JAM-A were allowed to adhere and spread onto JAM-A/fc or to the same protein that had been incubated with PNGaseF to remove N-glycans (B). Data are representative of two separate experiments run in quadruplicate. In (A), * represent $p<0.05$ verus EV, ** represent $p<0.01$ versus EV, ## represent $p<0.01$ veruss N185Q, and ### represents $p<0.001$ versus N185Q. In (B), *** represent $p<0.01$ versus PNGaseF treatment. All analysis were conducted using two-way ANOVA with Bonferroni post-test.



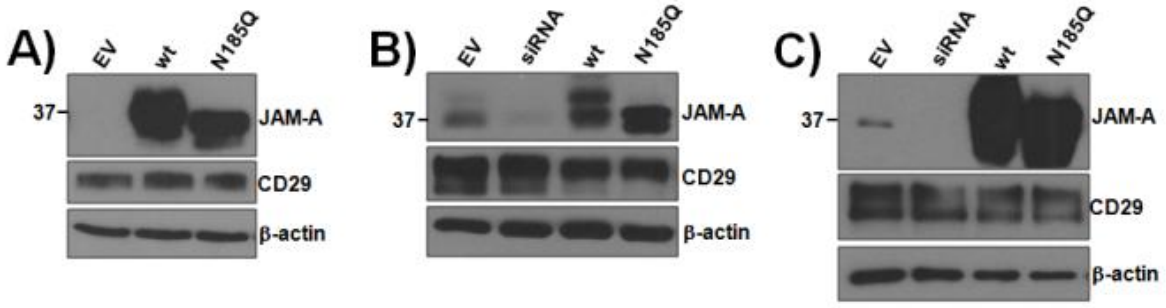
Supplemental Figure 3. N-glycosylation regulates JAM-A mediated effects on cell spreading. HUVEC (A) or MDA-MB-231 (B) cells were transfected with empty vector (EV), siRNA against JAM-A (siRNA), wild type (wt) or N185Q JAM-A and cell size were determined for Alexa488 phalloidinstained cells plated on fibronectin for two hours. *indicates $p<0.05$ versus EV and ** indicated $p<0.01$ versus EV by one-way ANOVA with Tukey's post-test. The number of cells analyzed in (A) were: EV 68, siRNA 59, wt 71, N185Q 63. The number of cell analyzed in (B) were, EV 34, wt 34, N185Q 32.



Supplemental Figure 4. N-glycosylation regulates JAM-A-mediated effects on cell motility. HUVEC (A) or MDA-MB-231 (B) cells were transfected with empty vector (EV), siRNA against JAM-A (siRNA), wild type (wt) or N185Q JAM-A and random cell motility (velocity) was determined by time-lapse microscopy. The number of cells analyzed in (A) was; EV 65, siRNA 72, wt 65, N185Q 68. The number of cells analyzed in (B) was EV 38, wt 39, N185Q 28. *indicates $p<0.05$ versus EV by one-way ANOVA with Tukey's post-test

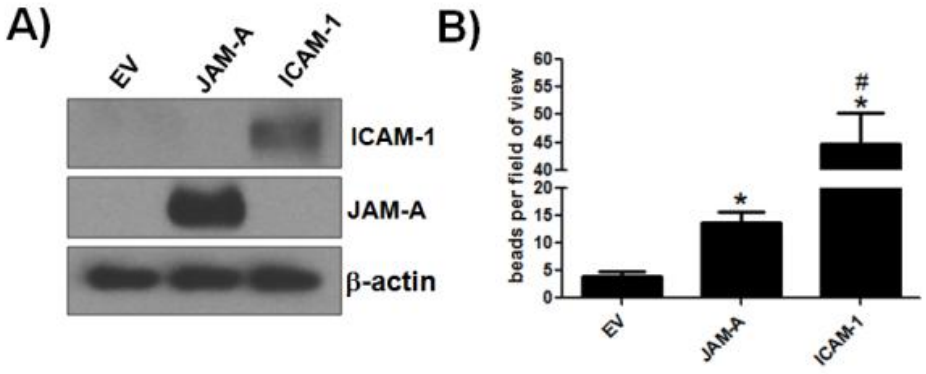


Supplemental Figure 5. JAM-A-mediated N-glycan dependent migration is matrix specific**.** HUVECs were transfected with empty vector (EV), wild type (wt) or N185Q JAM-A, and allowed to migrate towards fibronectin (FN) or vitronectin (VN) as described in methods. * indicates $p<0.01$ versus EV by one-way ANOVA with Tukey's post test. Data represent mean ± SEM from two separate experiments run in triplicate.



Supplemental Figure 6. Altering JAM-A expression does not change β1 integrin expression. CHO (A), HUVEC (B), or MDA-MB-231 (C) cells were transfected with empty vector (EV),

siRNA against JAM-A (siRNA), wild type (wt) or N185Q JAM-A. Cells were lysed and analyzed for expression of β1 integrin (CD29), JAM-A, and β-actin by western blot analysis. Data are representative of at least three separate experiments.



Supplemental Figure 7. JAM-A increases binding of LFA-1/fc coated beads. CHO cells were transfected with empty vector (EV), JAM-A, or ICAM-1. Cells were incubated with LFA-1/fc coated beads as described in methods. * indicates $p<0.01$ versus EV and # indicated $p<0.01$ versus JAM-A by one-way ANOVA with Tukey's post-test. Data represent mean ± from two separate experiments run in duplicate.