**Supplemental Material**

**Elkin et al: A systematic analysis of cancer cells reveals heterogeneous changes in their endocytic activities**

**Supplemental Figure Legends:**

**Supplemental Figure 1: TfnR and CD8 internalization as measured by In-Cell ELISA.** **A**) Representative images of D65 and anti-CD8 treated ARPE-19 cells, incubated for different time points (0-20 min), after adding the respective secondary-HRP conjugated antibodies and developing and stopping reagents. Total surface bound D65 (total) and background (acid washed cells) was used to calculate the relative percentage of TfnR and CD8 internalization. **B**) Continuous internalization of TfnR and CD8 in ARPE-19 cells. The rate of uptake of TfnR and CD8 was measured using the anti-transferrin receptor antibody HTR.D65 and a specific anti-CD8 antibody, respectively. Percentage of TfnR and CD8 uptake was calculated relative to the initial total surface bound ligand at 4oC (*n=3*). Data represents mean +/- SD.

**Supplemental Figure 2: Relationship between endocytic activity and total surface expression of receptors.** Linear regression analysis comparing endocytic activity and total surface expression of markers used for CME, CavME, CIE-CD44, and CIE-CD59. Correlation was assessed by r2 values, as indicated.

**Supplemental Figure 3: Relationship between endocytic activity and genetic alteration associated with cancer cell oncogenic transformation. A)** Correlation between p53 mutation status and endocytic activity in all four endocytic pathways. **B**) Correlation between overexpression (OE) status of MYC and endocytic activity in all four pathways. Wilcoxon rank sum tests were used to assess statistical significance, as indicated.

**Supplemental Figure 4: Relationship between endocytic activity and proliferation.** Spearman correlation of proliferation compared to endocytic activity of CME (not significant), CavME (not significant), CIE-CD44 (p<.05), CIE-CD59 (p=0.07).

**Supplemental Figure 5: Relationship between endocytic activity and cancer cell properties.** Linear regression analysis was used to compare endocytic activities with **A)** 3D migration and **B)** growth in soft agar. Correlation was assessed by r2 values, as indicated.

**Supplemental Figure 6: Relationship between endocytic activity and adhesion. A)** Cells were ordered by increasing adhesive properties on different substrates (See Methods). While there were significant differences in measure activities, these differences did not directly correlate with changes in endocytic activities. **B)** Adhesion on one substrate correlated with adhesion on the other substrates tested using a Pearson Correlation. Each histogram displays averages of three independent experiments each performed in triplicate.

**Supplemental Figure 7: Comparison of internalization and total surface binding of endocytic markers between cluster 1 and cluster 2.** Comparison of the extent of internalization after 5 min at 37°C and total surface binding, measured at 4°C, of **A)** CIE-CD595 **B)** CavME, **C)** CME. Significance, as indicated, was tested using the Wilcoxon rank sum test.