Supplemental Materials

Molecular Biology of the Cell

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Supplemental Figures

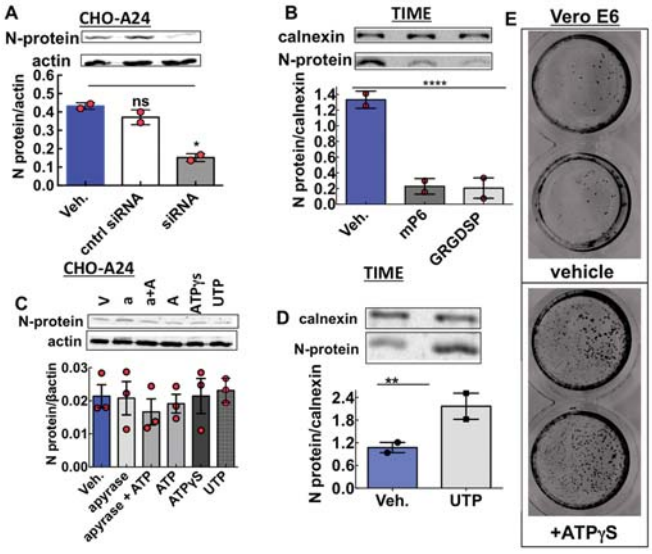

Fig S1. Occupancy of the PSI domain induces physiological integrin activation and infection. **A.** siRNA transfected CHO-A24 cells were infected within 24 hours after transfection and analyzed for viral N-protein. Infection decreased by 60% in cells with siRNA knockdown of $P2Y_2R$, *P < 0.05.**B.** $^{RGD}P2Y_2R$ cis-interaction with β_3 integrin and $G\alpha_{13}$binding to the β_3 integrin cytoplasmic domain is required for SNV entry in endothelial cells.Cells were treated with mP6 (250 μM), or GRGDSP 10 μM) for 30 min before infection. Analysis was conducted by Western blot. Values are means ± SD of duplicate measurements. Statistical significance was determined by Dunnett ****P < 0.0001.**C.** Cells are known to release ATP under mechanical stress or biological activation (Corriden et al., 2010). To test whether extracellular ATP plays a role in $P2Y_2R$ activation during infection,apyrase (150 ng/ml), a nucleotide- hydrolyzing enzyme was incubated with CHO-A24 cells for 1hr before infection, nucleotides (100 μM ATPγs and 1mM ATP, 100μM UTP), were co-administered with SNV. **D.** 100μM UTP was co-administered with 0.1 moiSNV in TIME cells. In all cases cells were washed 3 times in low pH media, after initial infection and incubated in normal culture media for 24 hours and analyzed for SNV N-protein by Western blot. Error bars reflect standard error for 2 measurements.**E**. GPCR stimulated inside-out integrin activation potentiates production of progeny SNV in permissive Vero E6 cells. Images of focus-forming units (FFU) in 48 well plates, showing mock and ATPγS treated samples collected on day 7 post infection (pi). Focus Reduction Neutralization Test (FRNT) was used to quantify focus-forming units. Images were taken with a Canon camera model EOS Rebel T3i.Cells were inoculated with 0.01 moi SNV. Supernatants from infected cells were collected for 9 days, diluted 1:1000 and then subjected to FRNT.Day 7 pisamples,yielded the highest quantity of progeny SNV compared to all other days post infection, with $1.6 \pm 0.12 \times 10^5$ FFU/ml in normal mediaand $2.86 \pm 0.44 \times 10^6$ FFU/ml produced in ATPγs replete samples.Errors represent SD from duplicate wells, shown in the images.