Cellular and biophysical studies of the Ebola virus matrix protein eVP40 protein-lipid interactions interfaces.

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Ebola virus (EBOV) is a negative-sense filamentous RNA virus which causes severe hemorrhagic fever. It is one of the most dangerous known pathogens with a high fatality rate. Ebola outbreaks are recurrent in humans because the virus is present in animal reservoirs. There are limited vaccines or therapeutics for prevention and treatment of EBOV, so it is important to get a detailed understanding of the virus lifecycle to illuminate new drug targets. EBOV encode for the matrix protein, VP40, which regulates assembly and budding of new virions from the inner leaflet of the host cell plasma membrane. The trafficking and assembly of the VP40 dimer to the plasma membrane requires a network of protein-protein and protein-lipid interactions (PPIs and PLIs). In this work we use molecular dynamic simulation, confocal microscopy, and surface plasmon resonance (SPR) to study the effects of VP40 mutations that occur at these PLI interfaces on VP40 plasma membrane dynamics and function. Our key finding is that these mutations affect viral assembly and budding by altering VP40 membrane binding capabilities. Mutations that increase VP40 net positive charge (G198R, G201R and D193A/K) increase eVP40 affinity for phosphatidylserine (PS) in the host cell plasma membrane. This increased affinity enhances plasma membrane association and budding efficiency leading to more infectious particles released to infect new cells. In contrast, mutations that decrease this charge (G198D) lead to a decrease in assembly and budding because of decreased interactions with PS in the membrane. Mutations at this interface however do not influence VP40-GP VLP entry into cells. Taken together our results highlight the importance of electrostatic interactions on VP40 assembly and budding. Understanding the effects of single amino-acid substitutions on viral budding and assembly will be useful for explaining changes in the infectivity and virulence of different EBOV strains and for long-term drug discovery aimed at EBOV assembly and budding.

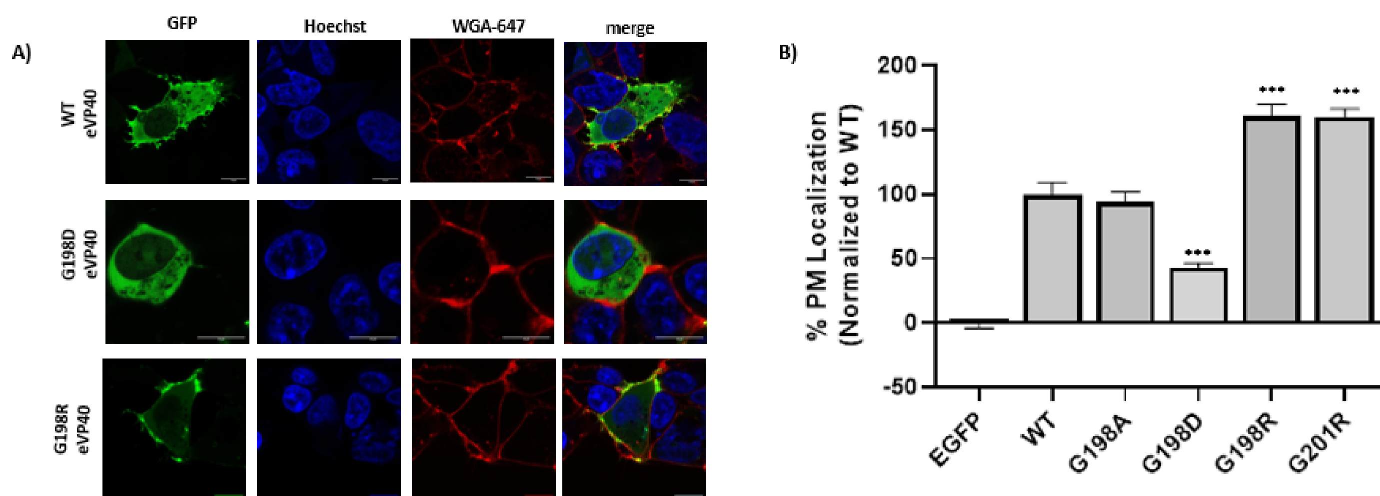

Figure 1: Exploring plasma membrane association (A) Representative images of select mutants (WT, G198D and G198R) showing eVP40 localization to the plasma membrane, transfected cells were stained with Hoechst 3342 nuclear stain and the WGA plasma membrane stain. Imaging was performed on a confocal microscope and B) Quantitative analysis of plasma membrane localization in HEK293 cells transfected with 2.5 µg of DNA