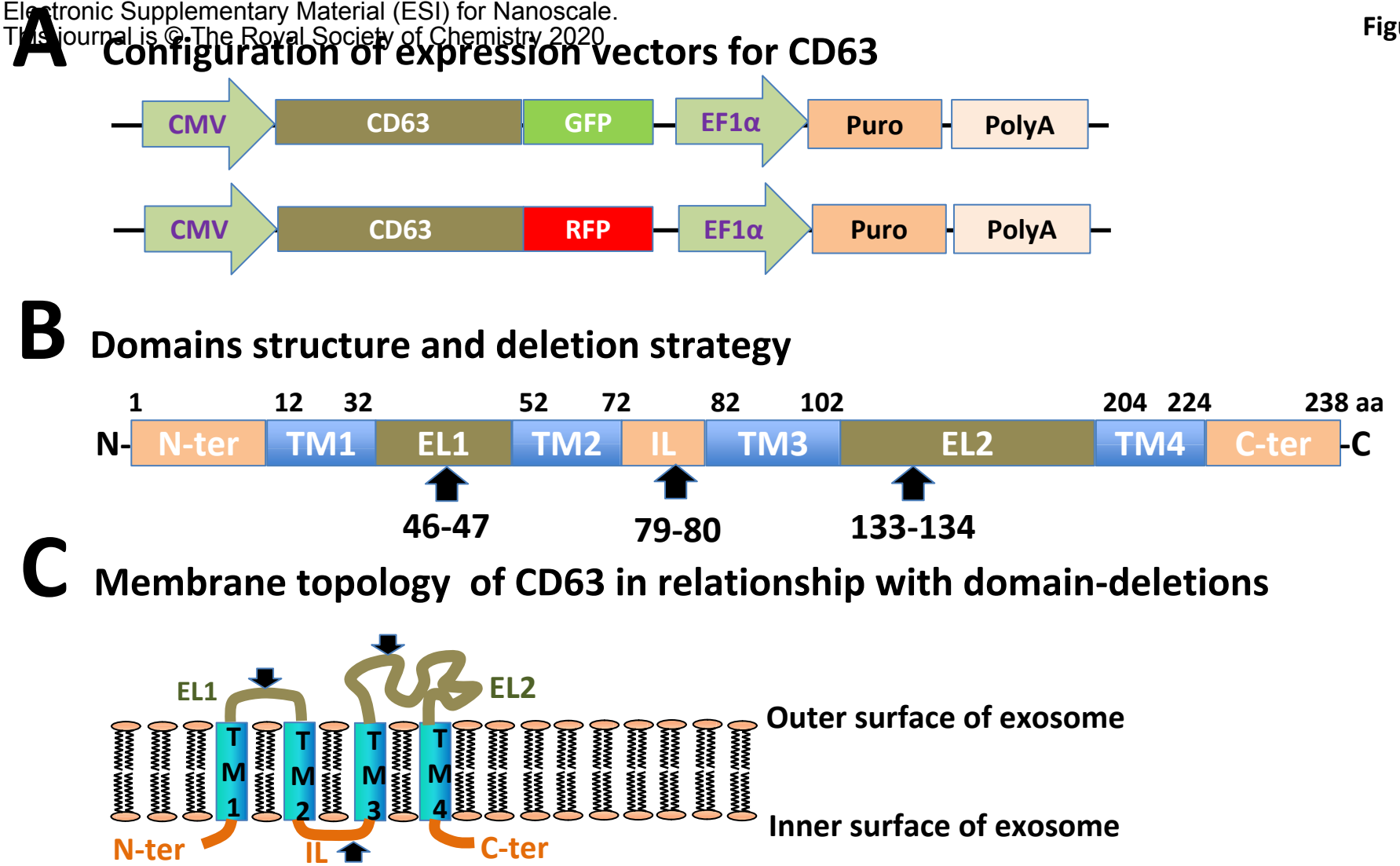
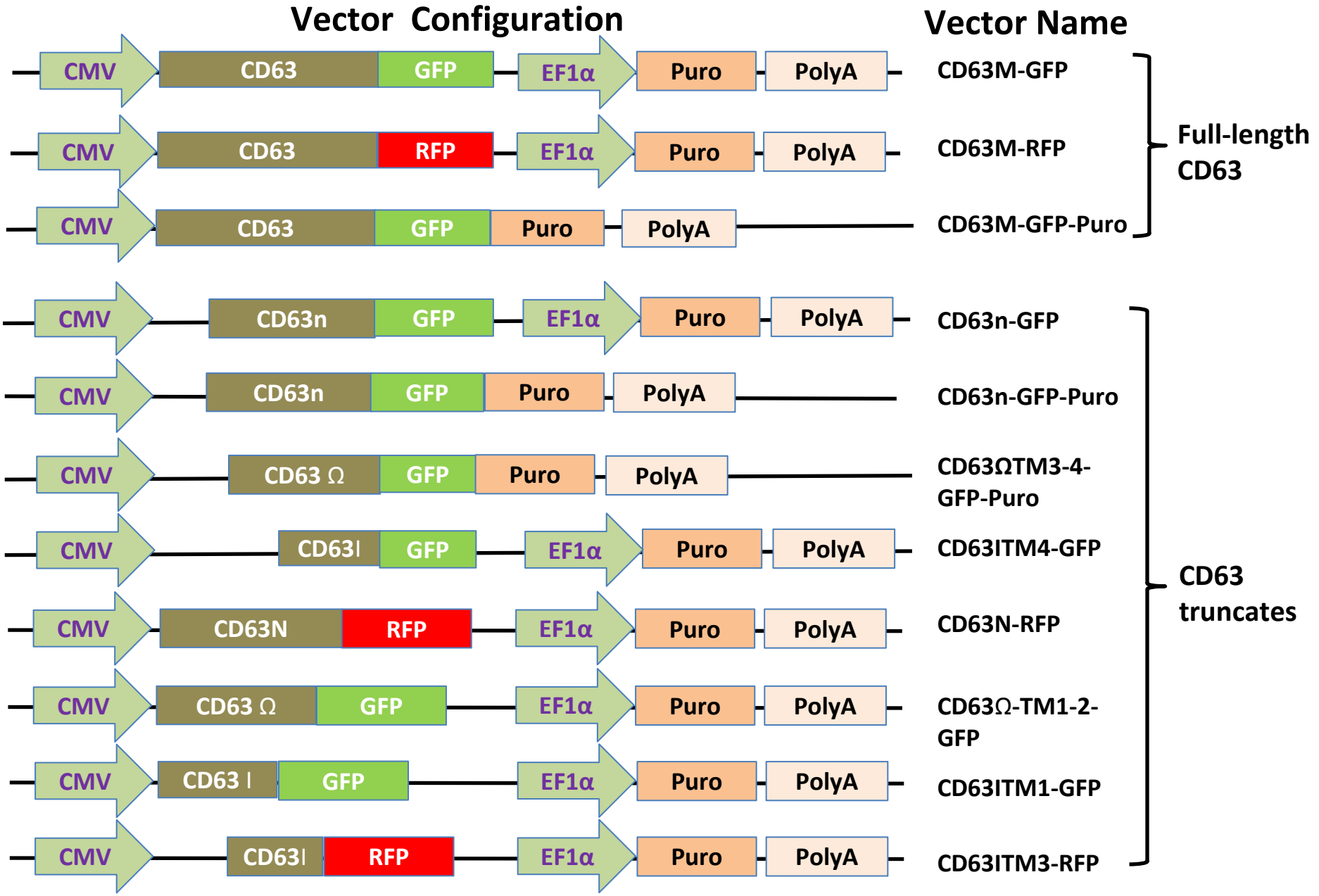
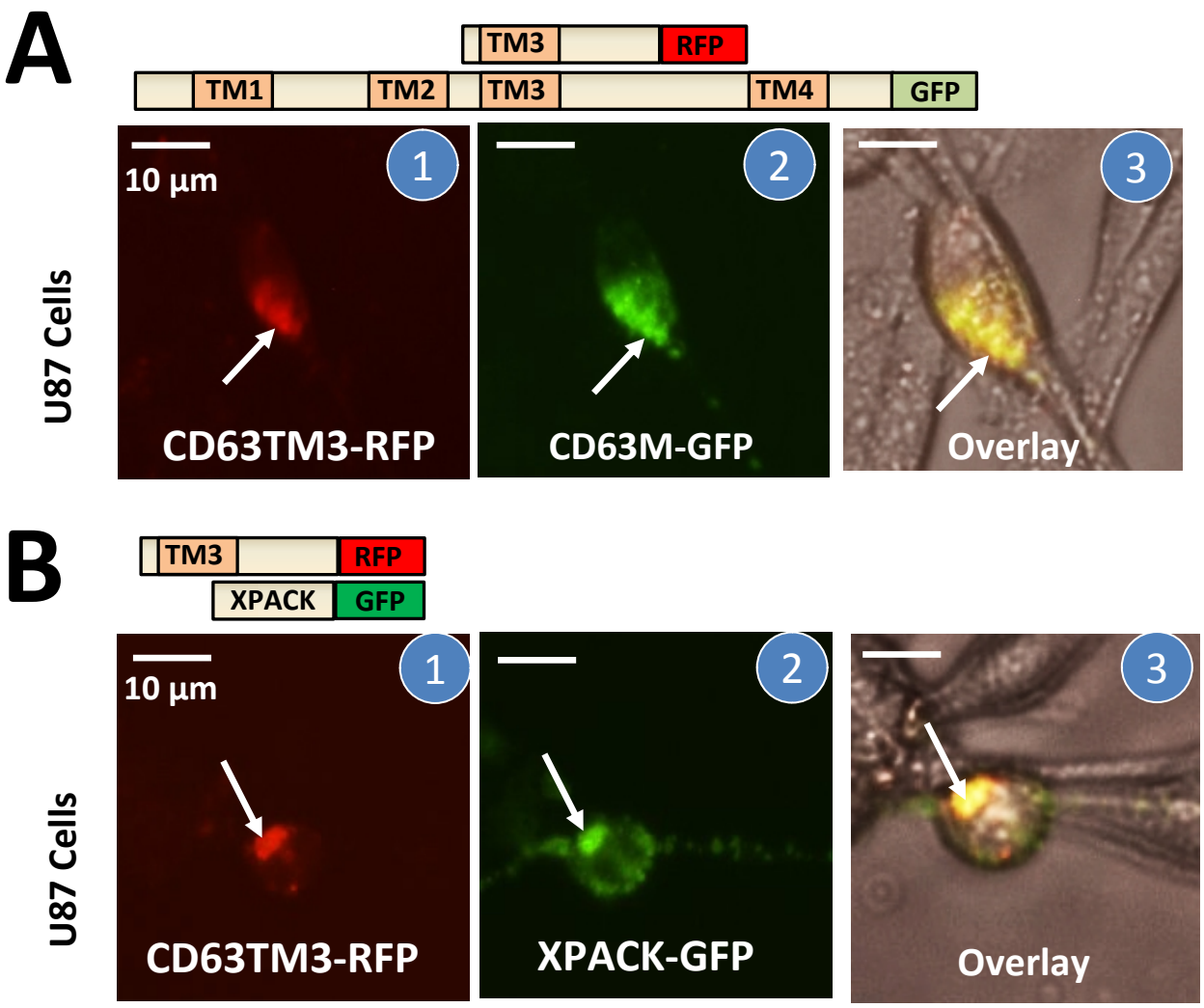


Supplementary Figure S1. Construction of domain-deleted CD63. (**A**) Configuration of expression vectors for the human full-length CD63-GFP/RFP fusion proteins. (**B**) Domain structure and deletion strategy . (C) Membrane topology of the full-length CD63 and its relationship with domain deleted CD63-truncates. CMV: cytomegalovirus promoter; EF1α: elongation factor 1-alpha promoter; EL1, external loop1; EL2: external loop2; IL: internal loop; N-ter: N-terminal sequences; C-ter: C-terminal sequences; TM1-4: transmembrane helixes. Black arrows point to the selected sites for making deletions. Black arrows indicate the cutting sites for making domain deletions. The sites are between are numbered according to amino-acid sequences and indicated by black arrows.

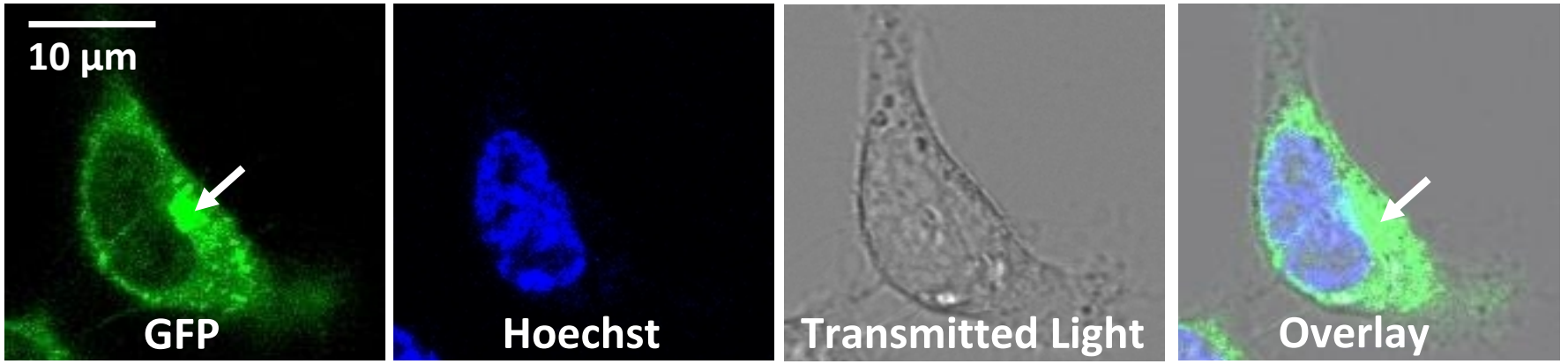


Supplementary Figure S2. Configuration of CD63-fusion protein expression vectors. CMV and EF1α are constitutive promoters. PolyA, polyadenylation signaling sequences.

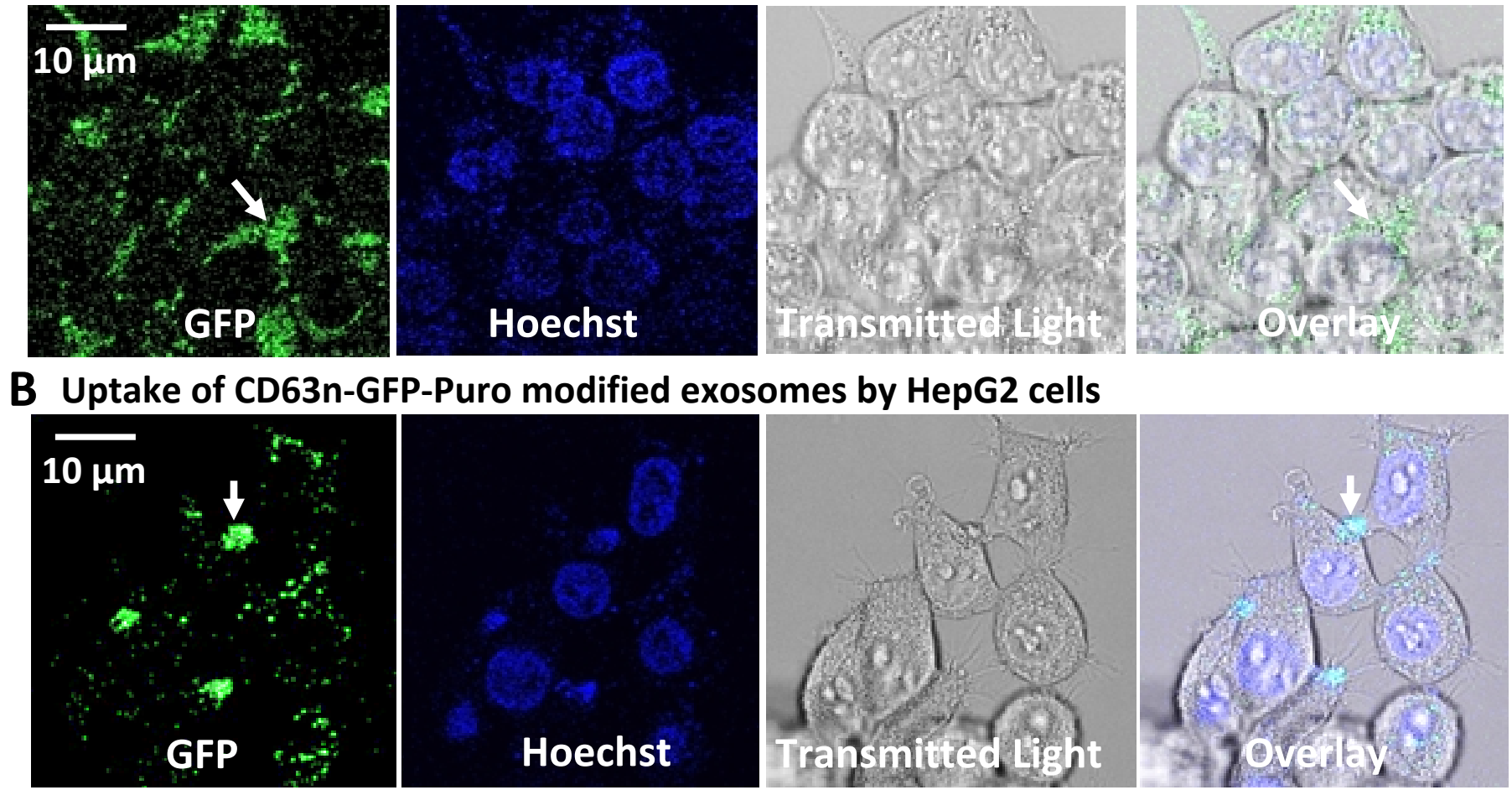


Supplementary Figure S3. U87 cells were co-transfected with fusion protein CD63TM3-RFP (TM3-only) along with either the full-length CD63M-GFP (**A**) or exosomal marker XPACK-GFP (**B**). On Day 3, TM3-only truncate exhibits punctuated GFP fluorescence in cells, where it co-colocalizes with either the full-length CD63M-GFP or exosome marker XPACK-GFP in human U87 cells. Arrows indicate endosome/exosome/MVB structures.

Confocal image of CD63n-GFP-Puro in HepG2 cells



Supplementary Figure S4. Subcellular localization of CD63n-GFP-Puro in living human hepatic cells of HepG2. Cells transfected with CD63n-GFP-Puro for 72 hours were stained with Hoechest that show cell nuclei (blue) and subjected to confocal imaging. CD63n-GFP-Puro is mainly localized at cytosol as green fluorescent punctates, indicated by arrows.



Supplementary Figure S5. Cellular uptake of CD63 variant modified exosomes by various recipient human cells. CD63n-GFP-Puro labelled exosomes were isolated from the conditioned medium as describe in Materials and Methods after the transient transfection. CD63n-GFP-Puro labelled exosomes were added into the culture medium (300 µg/mL protein) and incubated for 72 hours before cells were stained with Hoechst to show their nuclei. Confocal images of cellular uptake of CD63 variant modified exosomes (indicated by arrows) in human kidney HEK293 (**A**) or human hepatic HepG2 (**B**) cells.