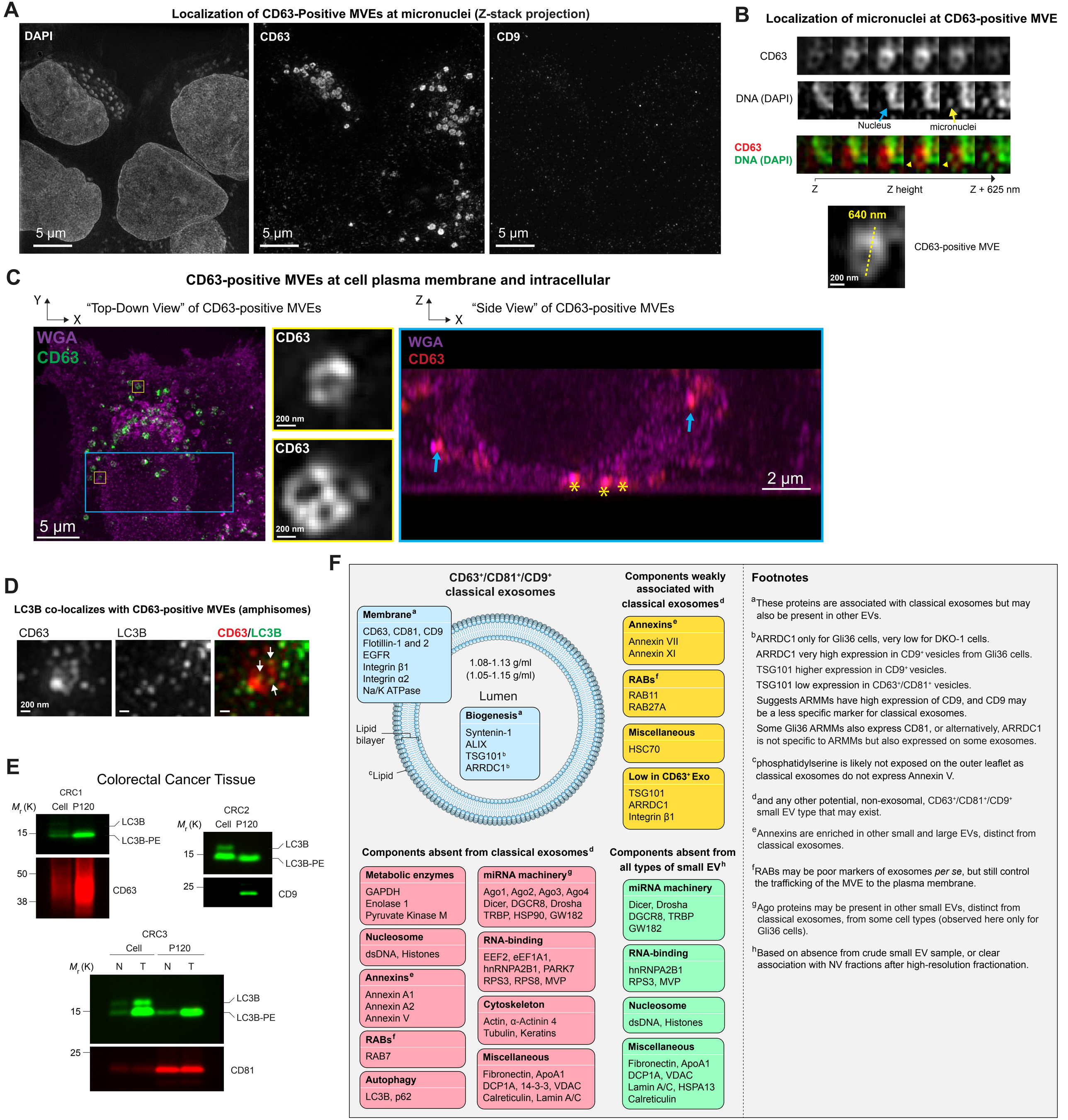
A
Localization of CD63-Positive MVEs at micronuclei (Z-stack projection)
DAPI
CD63
CD9
5 µm
5 µm
5 µm
B
Localization of micronuclei at CD63-positive MVE
CD63
DNA (DAPI)
Nucleus
micronuclei
CD63
DNA (DAPI)
Z
Z height
Z + 625 nm
640 nm
200 nm
CD63-positive MVE
C
CD63-positive MVEs at cell plasma membrane and intracellular
Y
X
"Top-Down View" of CD63-positive MVEs
Z
X
"Side View" of CD63-positive MVEs
WGA
CD63
5 µm
CD63
200 nm
CD63
200 nm
WGA
CD63
2 µm
D
LC3B co-localizes with CD63-positive MVEs (amphisomes)
CD63
LC3B
CD63/LC3B
200 nm
E
Colorectal Cancer Tissue
CRC1
Cell P120
M_r (K)
15
50
38
LC3B
LC3B-PE
CD63
CRC2
Cell P120
M_r (K)
15
25
LC3B
LC3B-PE
CD9
CRC3
Cell
P120
M_r (K)
N T N T
15
25
LC3B
LC3B-PE
CD81
F
CD63+/CD81+/CD9+ classical exosomes
Membrane[a]
CD63, CD81, CD9
Flotillin-1 and 2
EGFR
Integrin β1
Integrin α2
Na/K ATPase
1.08-1.13 g/ml
(1.05-1.15 g/ml)
Lumen
Biogenesis[a]
Syntenin-1
ALIX
TSG101[b]
ARRDC1[b]
Lipid bilayer
[c]Lipid
Components weakly associated with classical exosomes[d]
Annexins[e]
Annexin VII
Annexin XI
RABs[f]
RAB11
RAB27A
Miscellaneous
HSC70
Low in CD63+ Exo
TSG101
ARRDC1
Integrin β1
Components absent from classical exosomes[d]
Metabolic enzymes
GAPDH
Enolase 1
Pyruvate Kinase M
Nucleosome
dsDNA, Histones
Annexins[e]
Annexin A1
Annexin A2
Annexin V
RABs[f]
RAB7
Autophagy
LC3B, p62
miRNA machinery[g]
Ago1, Ago2, Ago3, Ago4
Dicer, DGCR8, Drosha
TRBP, HSP90, GW182
RNA-binding
EEF2, eEF1A1,
hnRNPA2B1, PARK7
RPS3, RPS8, MVP
Cytoskeleton
Actin, α-Actinin 4
Tubulin, Keratins
Miscellaneous
Fibronectin, ApoA1
DCP1A, 14-3-3, VDAC
Calreticulin, Lamin A/C
Components absent from all types of small EV[h]
miRNA machinery
Dicer, Drosha
DGCR8, TRBP
GW182
RNA-binding
hnRNPA2B1
RPS3, MVP
Nucleosome
dsDNA, Histones
Miscellaneous
Fibronectin, ApoA1
DCP1A, VDAC
Lamin A/C, HSPA13
Calreticulin
Footnotes
[a]These proteins are associated with classical exosomes but may also be present in other EVs.
[b]ARRDC1 only for Gli36 cells, very low for DKO-1 cells.
ARRDC1 very high expression in CD9+ vesicles from Gli36 cells.
TSG101 higher expression in CD9+ vesicles.
TSG101 low expression in CD63+/CD81+ vesicles.
Suggests ARMMs have high expression of CD9, and CD9 may be a less specific marker for classical exosomes.
Some Gli36 ARMMs also express CD81, or alternatively, ARRDC1 is not specific to ARMMs but also expressed on some exosomes.
[c]phosphatidylserine is likely not exposed on the outer leaflet as classical exosomes do not express Annexin V.
[d]and any other potential, non-exosomal, CD63+/CD81+/CD9+ small EV type that may exist.
[e]Annexins are enriched in other small and large EVs, distinct from classical exosomes.
[f]RABs may be poor markers of exosomes *per se*, but still control the trafficking of the MVE to the plasma membrane.
[g]Ago proteins may be present in other small EVs, distinct from classical exosomes, from some cell types (observed here only for Gli36 cells).
[h]Based on absence from crude small EV sample, or clear association with NV fractions after high-resolution fractionation.