Supporting Figures

Structural analysis of the P132L disease mutation in caveolin-1 reveals its role in assembly of oligomeric complexes

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Running title: Structural basis for the P132L mutation in caveolin-1

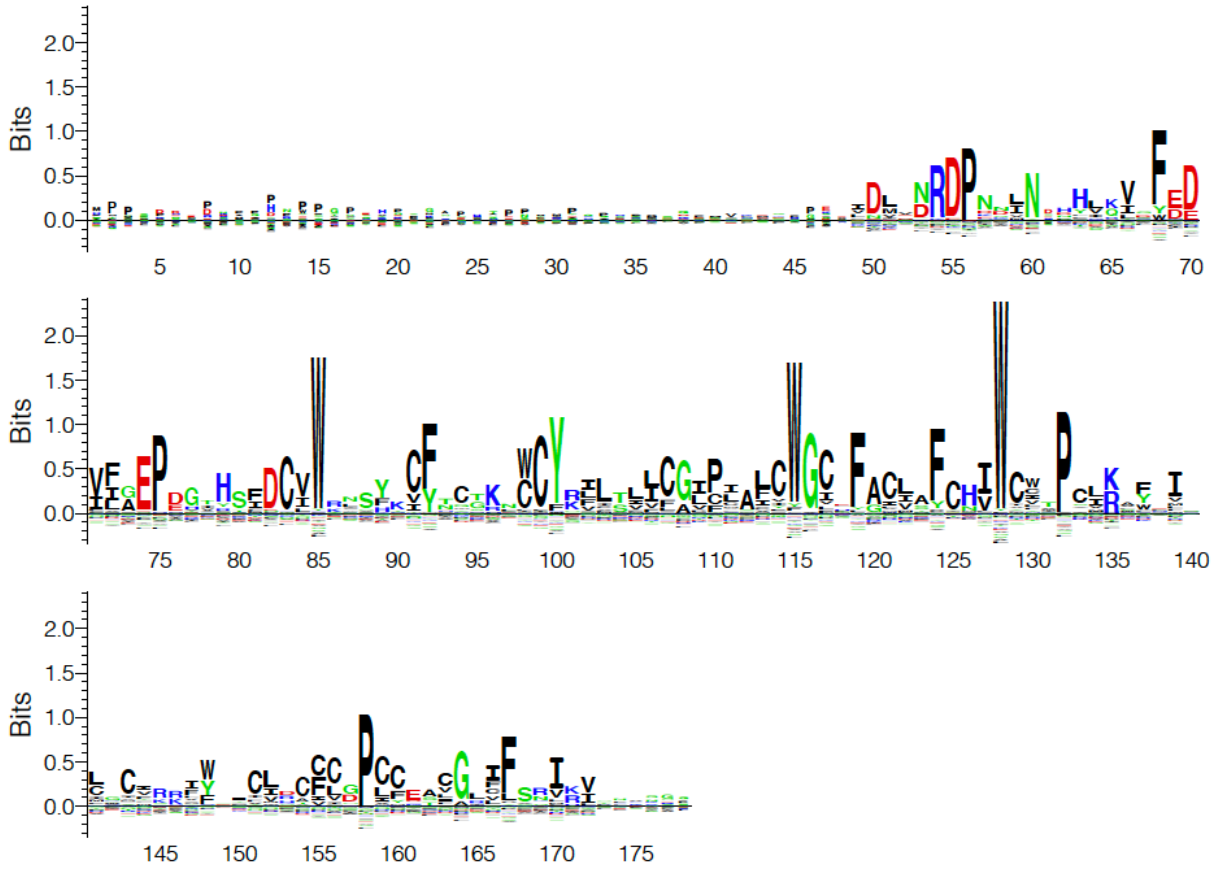


Figure S1. Sequence logo of CAV1 among metazoan caveolins. Logo was created with Seq2Logo.

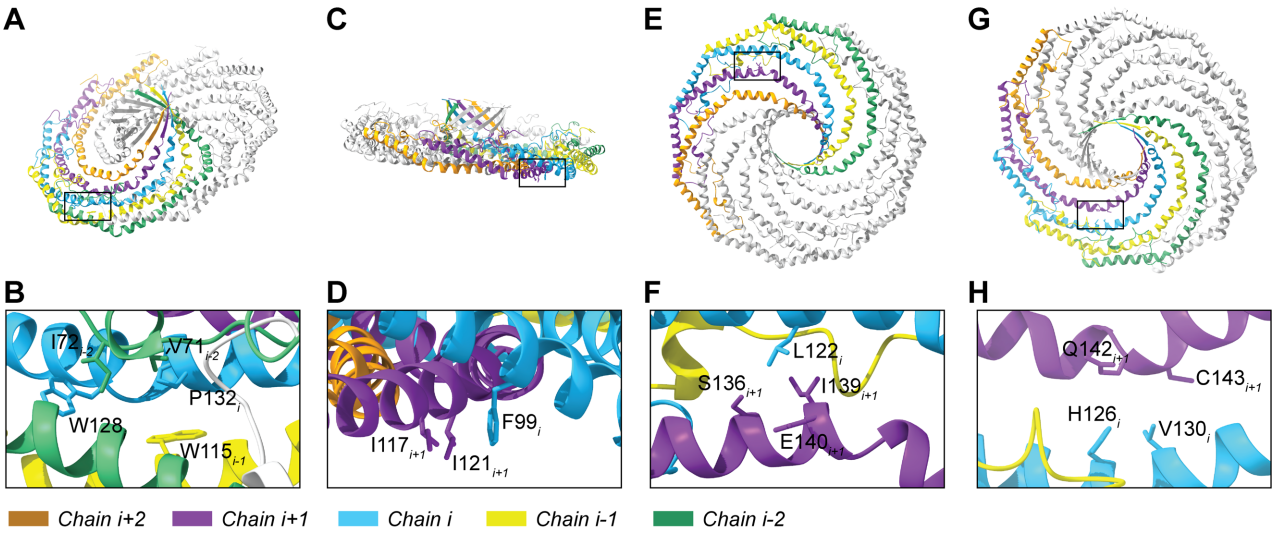


Figure S2. Positions of P132, F99, L122, and V130 in the CAV1 complex. Chains are color coded as follows: *i+2*, brown; *i+1*, purple; *i*, cyan; *i-1*, yellow, and *i-2*, green. (**A, B**) P132. (**C, D**) F99. (**E, F**) L122. (**G, H**) V130.

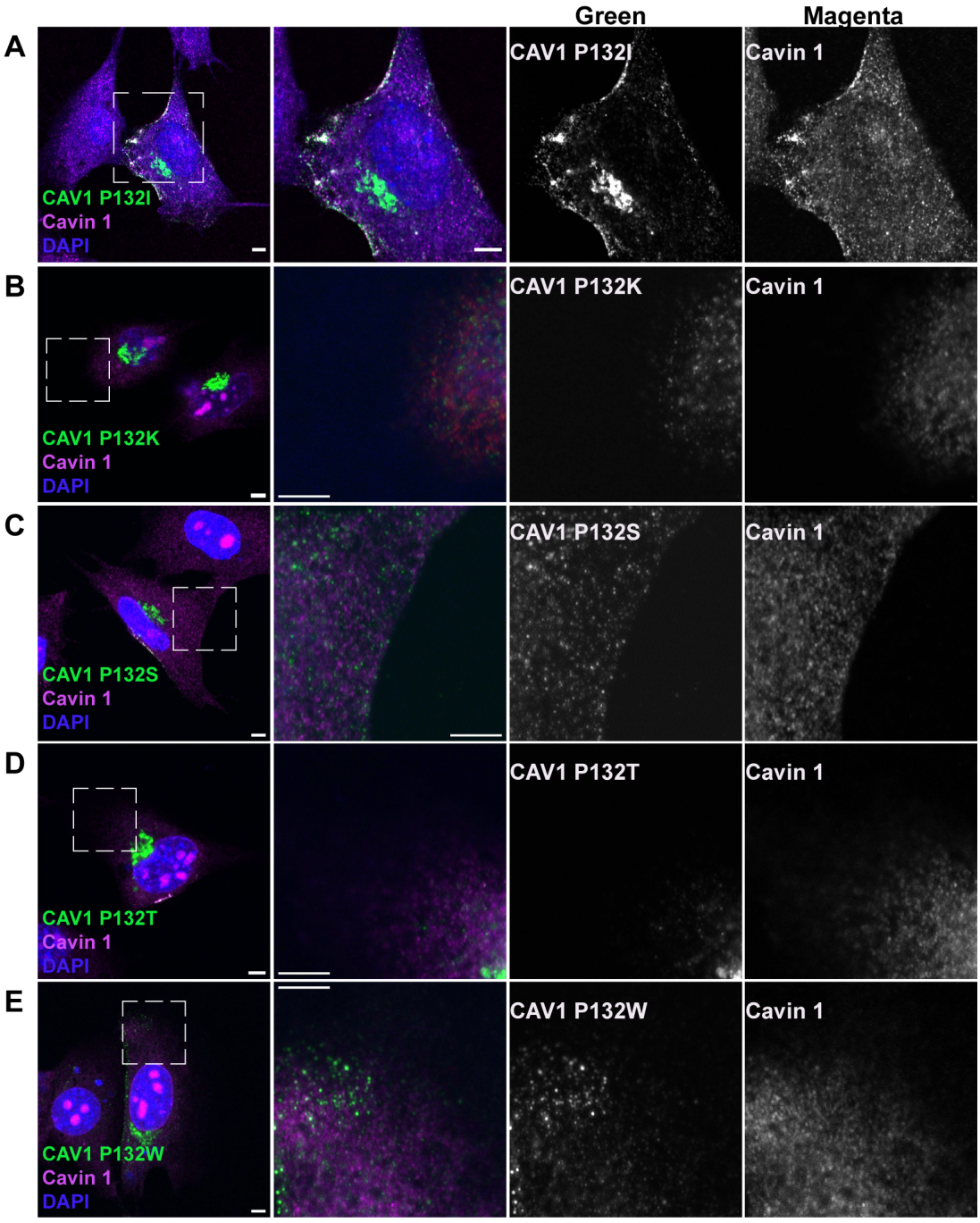


Figure S3. Single amino acid substitutions to P132 cause CAV1 to accumulate in the Golgi complex. Representative confocal images are shown for CAV1 $^{-/-}$ MEF cells expressing myc-tagged (**A**) P132I, (**B**) P132K, (**C**) P132S, (**D**) P132T, and (**E**) P132W. Cells were allowed to express the indicated constructs for 24h, fixed, and immunostained for endogenous cavin-1 (magenta) and myc (green) prior to imaging. Dashed boxes indicate position of zoomed images highlighting the low levels of staining outside of the Golgi complex. Note that low levels of plasma membrane staining are observed for P132I in addition to a substantial Golgi pool. Bars, 5 μm.

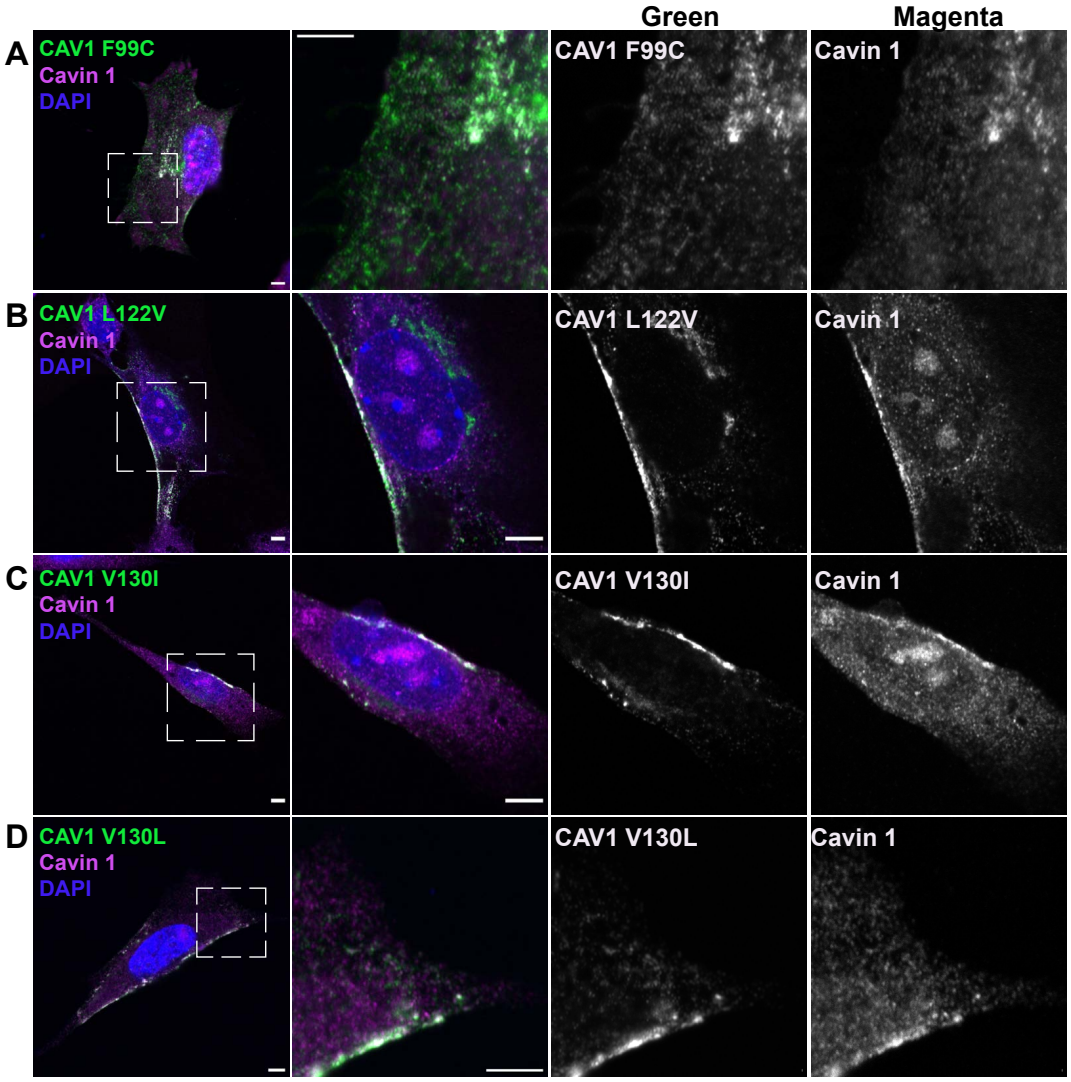


Figure S4. Single amino acid substitutions to F99, L122, or V130 do not interfere with the delivery of CAV1 to the plasma membrane. Representative confocal images are shown for CAV1 $^{-/-}$ MEF cells expressing myc-tagged (**A**) F99C, (**B**) L122V, (**C**) V130I, and (**D**) V130L. Cells were allowed to express the indicated constructs for 24h, fixed, and immunostained for endogenous cavin-1 (magenta) and myc (green) prior to imaging. Dashed boxes indicate position of zoomed images highlighting cell surface pools of CAV1 that colocalize with cavin-1. Bars, 5 μm

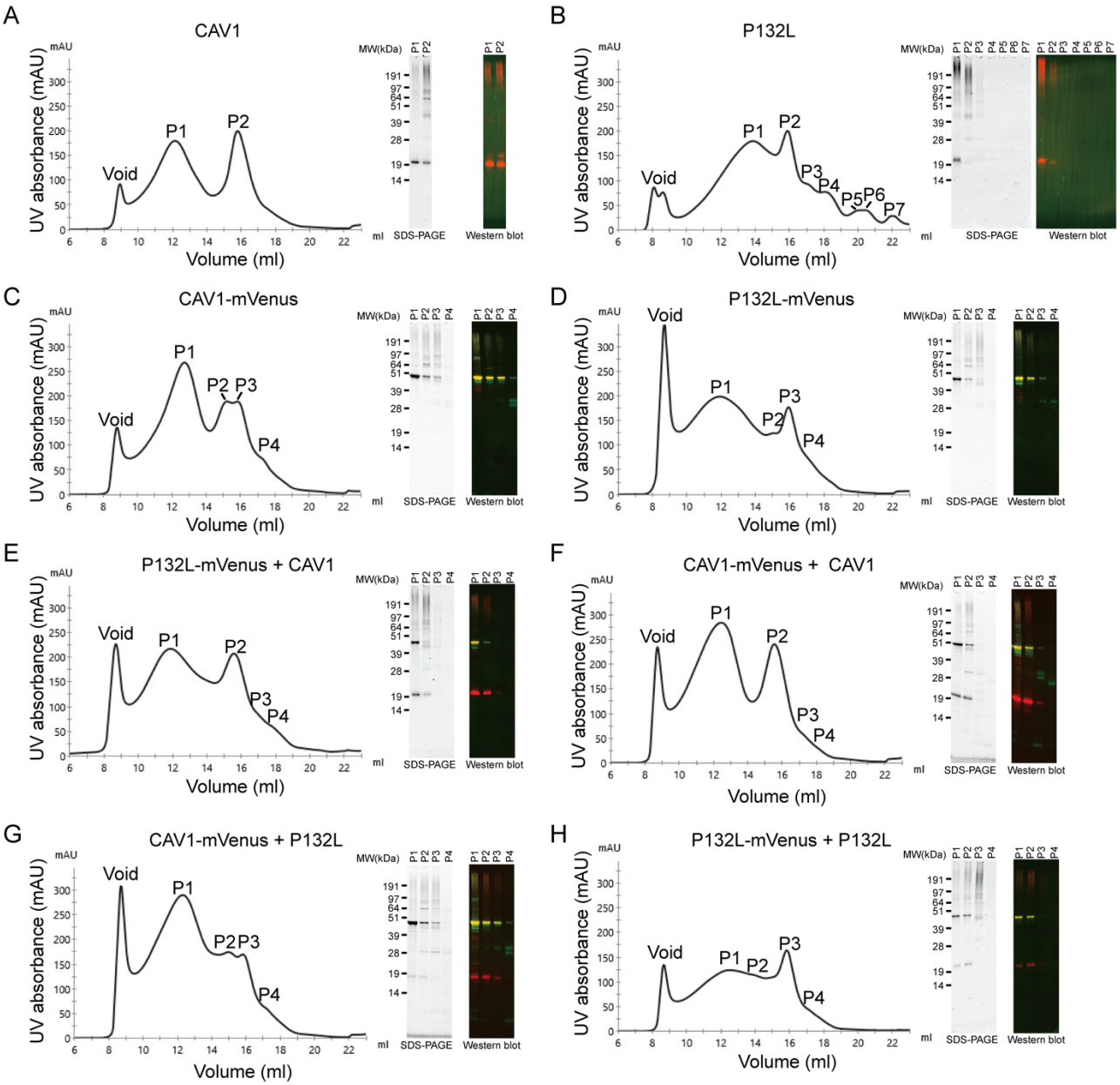


Figure S5: FPLC traces, SDS-PAGE electrophoresis and Western blotting of CAV1 constructs used in this study. The indicated Cav1 proteins were purified from *E. coli* membranes and applied to a Superose®6 10/300 GL column. Elution profiles, SDS-PAGE and Western blotting results are shown for (**A**) CAV1, (**B**) P132L, (**C**) CAV1-mVenus, (**D**) P132L-mVenus, (**E**) co-expressed P132L-mVenus and CAV1, (**F**) co-expressed CAV1-mVenus and CAV1, (**G**) co-expressed CAV1-mVenus and P132L, and (**H**) co-expressed P132L and P132L-mVenus. In the Western blotting results, mVenus signal is shown in green and CAV1 signal is in red.

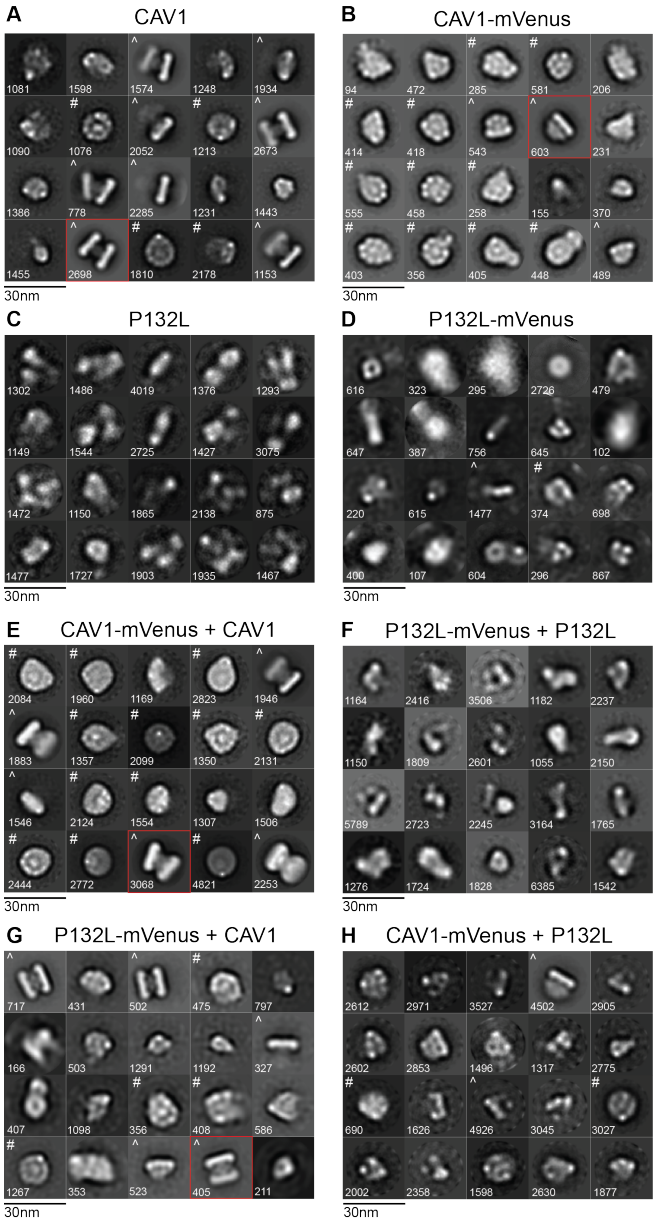


Figure S6: Negative stain averages of Cav1 constructs. Negative stain class averages of (**A**) CAV1, (**B**) CAV1-mVenus, (**C**) P132L, (**D**) P132L-mVenus, (**E**) co-expressed CAV1-Venus and CAV1 (**F**), co-expressed P132L-mVenus and P132L, (**G**) co-expressed P132L-mVenus and CAV1, and (**H**) co-expressed CAV1-Venus and P132L. # denotes en face averages, ^ denotes side view averages, and the number of particles per average are noted in the bottom left. Scale bars, 30 nm. Classes in red boxes are displayed in **Figure 7**.

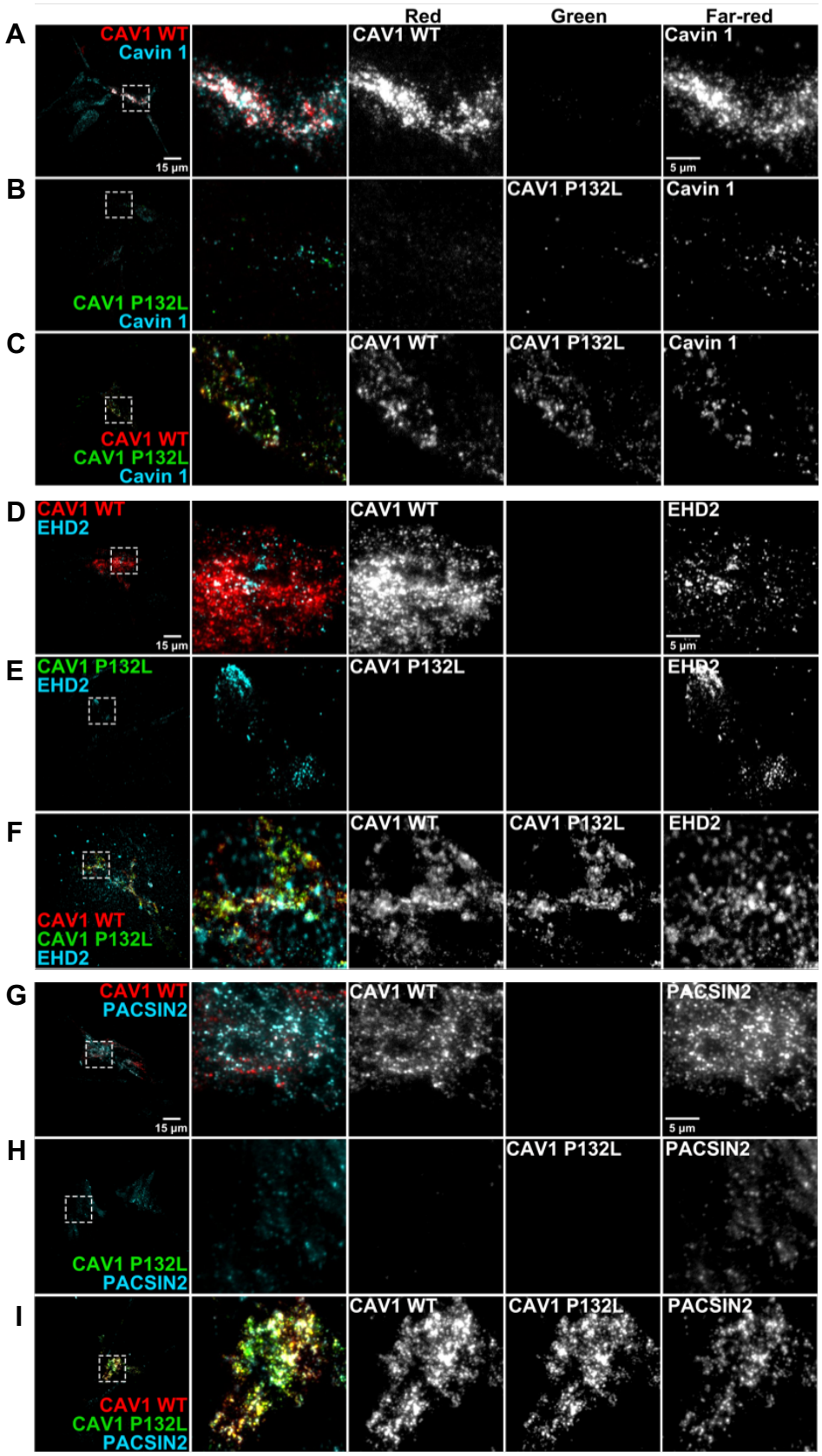

Red
Green
Far-red
A
CAV1 WT
Cavin 1
15 µm
CAV1 WT
Cavin 1
5 µm
B
CAV1 P132L
Cavin 1
CAV1 P132L
Cavin 1
C
CAV1 WT
CAV1 P132L
Cavin 1
CAV1 WT
CAV1 P132L
Cavin 1
D
CAV1 WT
EHD2
15 µm
CAV1 WT
EHD2
5 µm
E
CAV1 P132L
EHD2
CAV1 P132L
EHD2
F
CAV1 WT
CAV1 P132L
EHD2
CAV1 WT
CAV1 P132L
EHD2
G
CAV1 WT
PACSIN2
15 µm
CAV1 WT
PACSIN2
5 µm
H
CAV1 P132L
PACSIN2
CAV1 P132L
PACSIN2
I
CAV1 WT
CAV1 P132L
PACSIN2
CAV1 WT
CAV1 P132L
PACSIN2

Figure S7. When co-expressed with WT CAV1, P132L is recruited to cell surface puncta that partially co-localize with endogenous cavin-1, EHD2, and PACSIN-2 in CAV1$^{-/-}$ MEFs as visualized using TIRF microscopy.

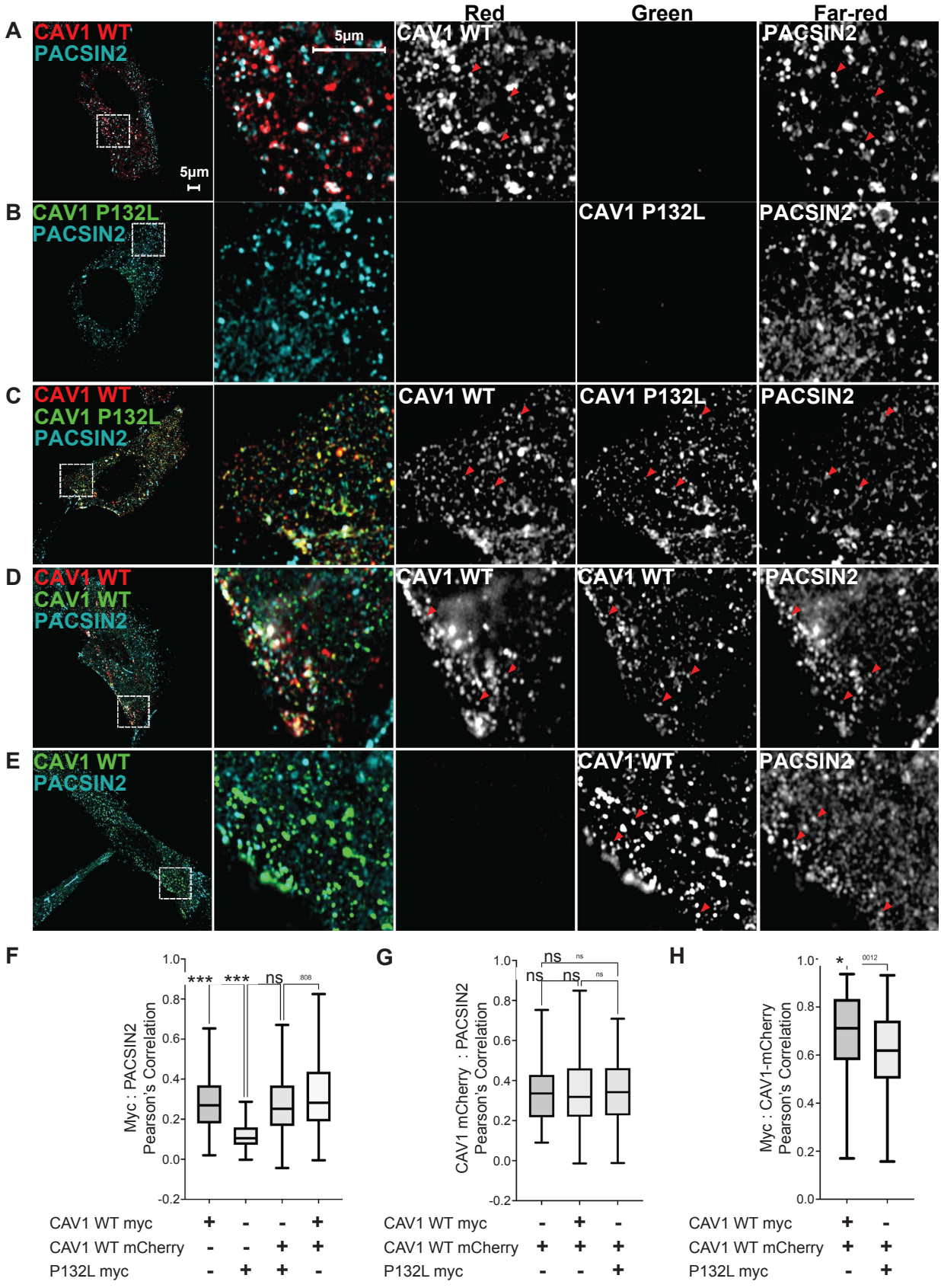
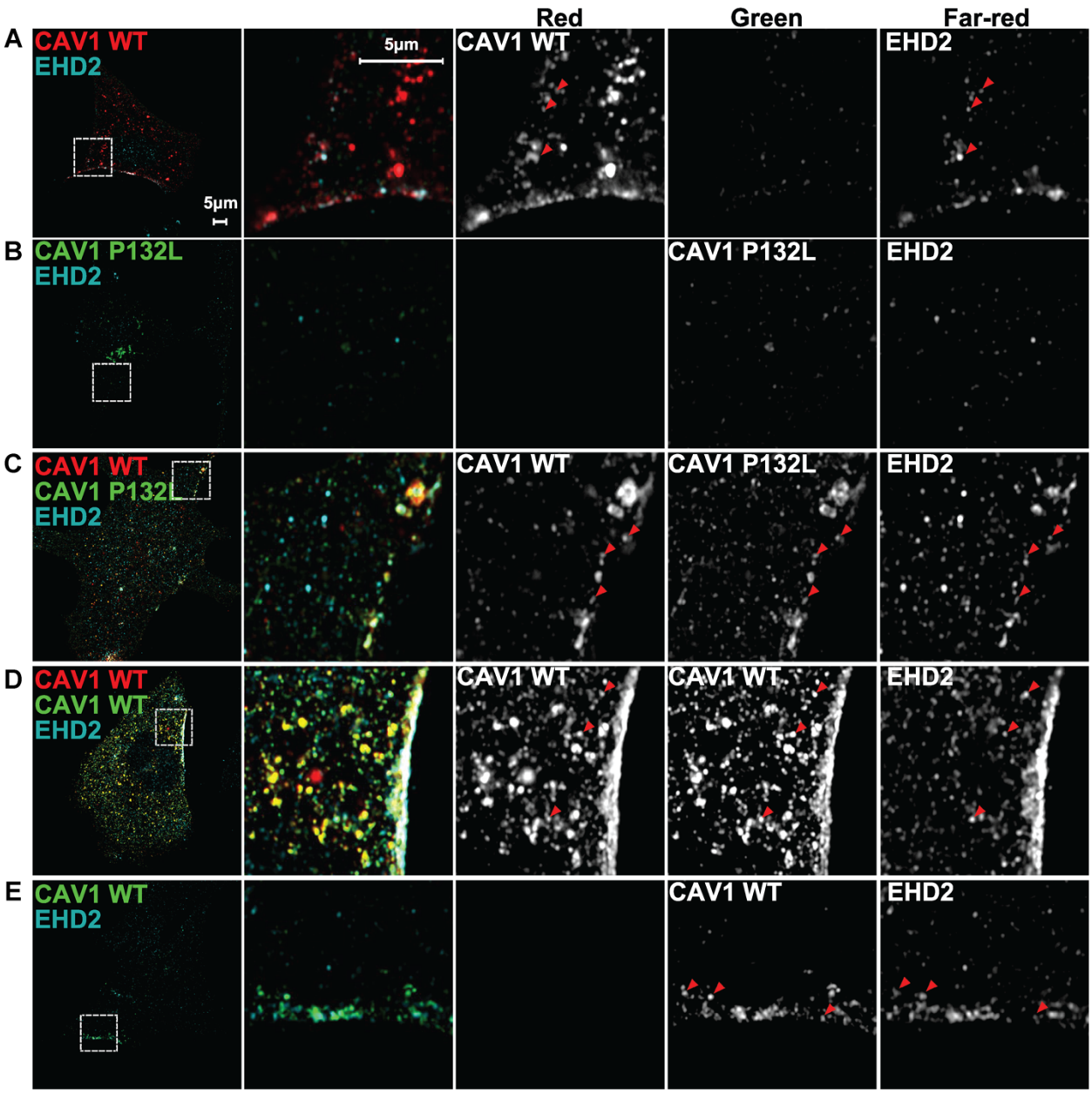

Red
Green
Far-red
A
CAV1 WT
PACSIN2
5μm
5μm
CAV1 WT
PACSIN2
B
CAV1 P132L
PACSIN2
CAV1 P132L
PACSIN2
C
CAV1 WT
CAV1 P132L
PACSIN2
CAV1 WT
CAV1 P132L
PACSIN2
D
CAV1 WT
CAV1 WT
PACSIN2
CAV1 WT
CAV1 WT
PACSIN2
E
CAV1 WT
PACSIN2
CAV1 WT
PACSIN2
F
Myc : PACSIN2
Pearson's Correlation
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
***
***
ns
808
CAV1 WT myc + - - +
CAV1 WT mCherry - - + +
P132L myc - + + -
G
CAV1 mCherry : PACSIN2
Pearson's Correlation
ns
ns
ns
ns
ns
CAV1 WT myc - + -
CAV1 WT mCherry + + +
P132L myc - - +
H
Myc : CAV1-mCherry
Pearson's Correlation
*
0012
CAV1 WT myc + -
CAV1 WT mCherry + +
P132L myc - +

Figure S8. When co-expressed with WT CAV1, P132L partially co-localizes with endogenous PACSIN2. CAV1 $^{-/-}$ MEFs were transiently transfected with **(A)** CAV1-mCherry, **(B)** P132L, **(C)** CAV1-mCherry plus P132L, **(D)** CAV1-mCherry plus CAV1, or **(E)** CAV1 for 24h, fixed, and immunostained prior to imaging. Representative AiryScan images are shown. Bar, 5 μm. **(F-H)** Pearson's correlation analysis was carried out using ImageJ (n = 90 ROIs from 3 independent experiments). A one-way ANOVA with Tukey's test (≥3 groups) or an unpaired Student's t test (2 groups) was used to calculate P-values. n.s., not significant.


Red
Green
Far-red
A
CAV1 WT
EHD2
5μm
5μm
CAV1 WT
EHD2
B
CAV1 P132L
EHD2
CAV1 P132L
EHD2
C
CAV1 WT
CAV1 P132L
EHD2
CAV1 WT
CAV1 P132L
EHD2
D
CAV1 WT
CAV1 WT
EHD2
CAV1 WT
CAV1 WT
EHD2
E
CAV1 WT
EHD2
CAV1 WT
EHD2

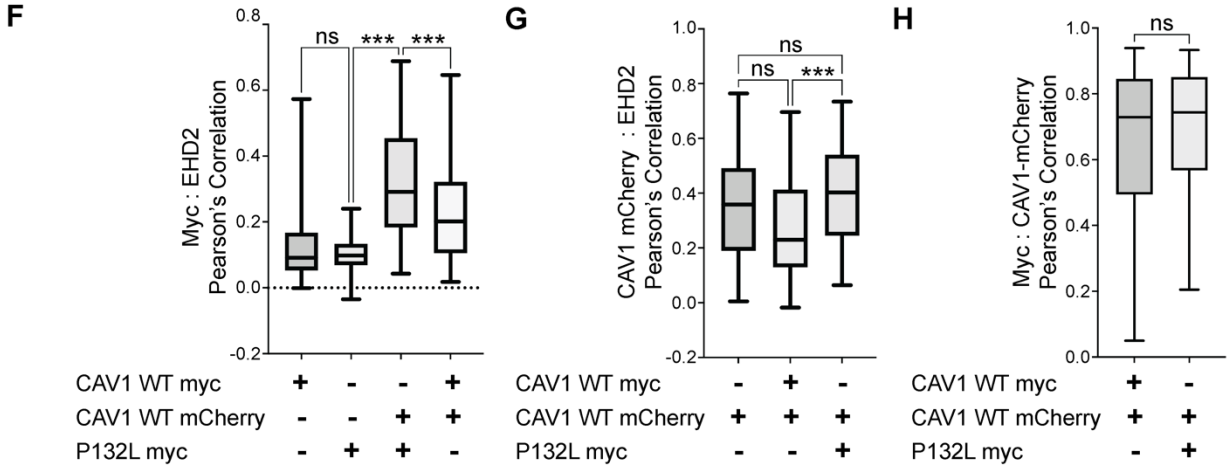

F
Myc : EHD2
Pearson's Correlation
0.8
0.6
0.4
0.2
0.0
-0.2
ns
***
***
CAV1 WT myc + - - +
CAV1 WT mCherry - - + +
P132L myc - + + -
G
CAV1 mCherry : EHD2
Pearson's Correlation
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
ns
ns
***
CAV1 WT myc - + -
CAV1 WT mCherry + + +
P132L myc - - +
H
Myc : CAV1-mCherry
Pearson's Correlation
1.0
0.8
0.6
0.4
0.2
0.0
ns
CAV1 WT myc + -
CAV1 WT mCherry + +
P132L myc - +

Figure S9. P132L partially colocalizes with endogenous EHD2 when co-expressed with WT CAV1. CAV1 $^{-/-}$ MEFs were transiently transfected with **(A)** CAV1-mCherry, **(B)** P132L, **(C)** CAV1-mCherry plus P132L, **(D)** CAV1-mCherry plus CAV1, or **(E)** CAV1 for 24h, fixed, and immunostained prior to imaging. Representative AiryScan images are shown. Bar, 5 μm. **(F-H)** Pearson's correlation analysis was carried out using ImageJ (n = 90 ROIs from 3 independent experiments). A one-way ANOVA with Tukey's test (≥3 groups) or an unpaired Student's t test (2 groups) was used to calculate P-values. n.s., not significant.