Science Advances

▲AAAS

Supplementary Materials for

Structure and assembly of CAV1 8*S* complexes revealed by single particle electron microscopy

Bing Han, Jason C. Porta, Jessica L. Hanks, Yelena Peskova, Elad Binshtein, Kelly Dryden, Derek P. Claxton, Hassane S. Mchaourab, Erkan Karakas, Melanie D. Ohi*, Anne K. Kenworthy*

*Corresponding author. Email: mohi@umich.edu (M.D.O.); akk7hp@virginia.edu (A.K.K.)

This PDF file includes:

Figs. S1 to S6
Tables S1 to S3

Extracellular side

N-terminus

61   82   101   134

Scaffolding domain

Oligomerization domain   Intramembrane domain
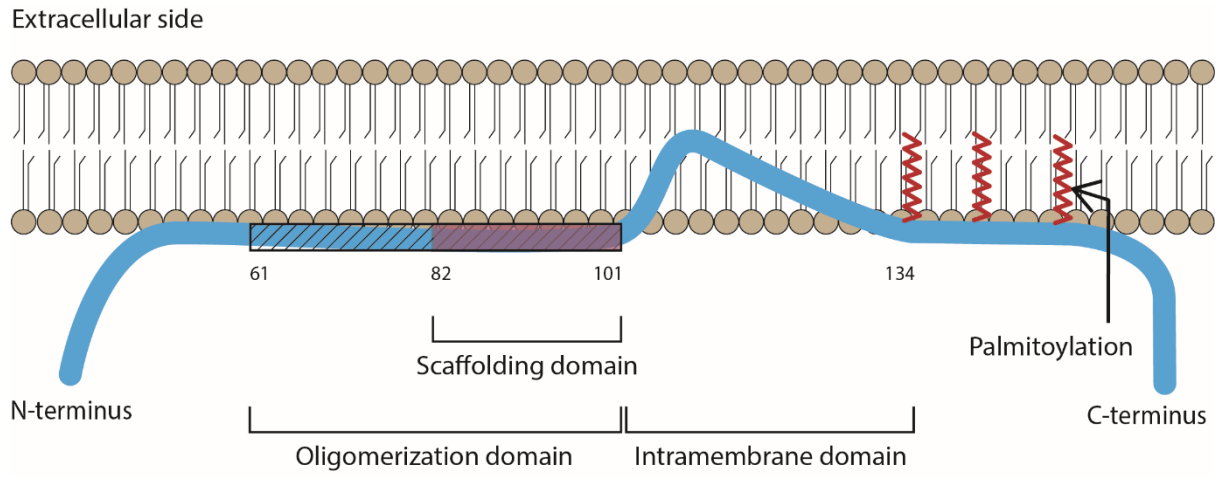
Palmitoylation

C-terminus

Figure S1

Figure S1.   Cartoon of caveolin domain structure.   See text for details.
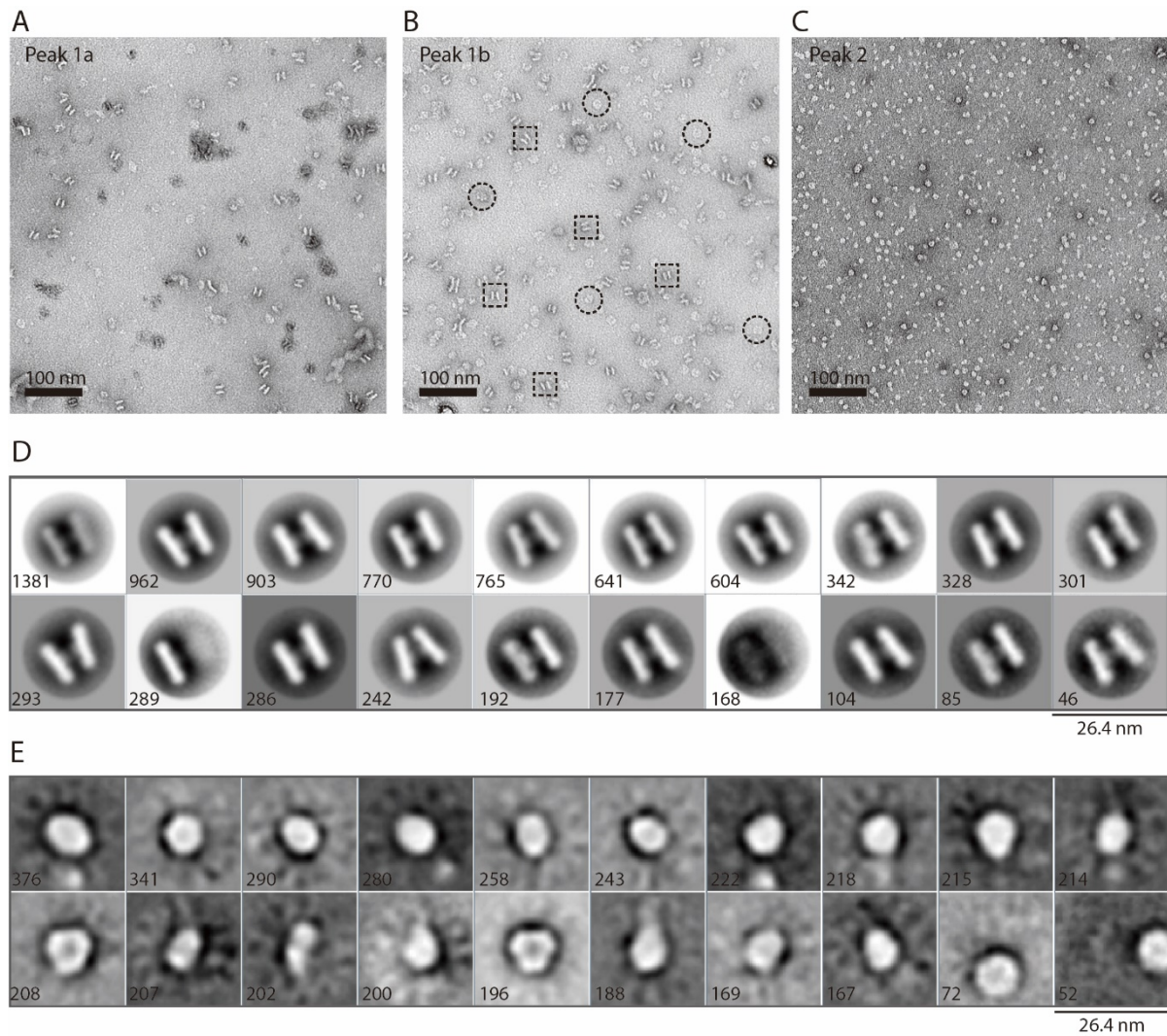
Figure S2

Figure S2. Characterization of CAV1 complexes purified from *E. coli*. Representative negative stain images of particles found in Peaks 1a (**A**), 1b (**B**), and 2 (**C**) of gel filtration profile of CAV1-His6 purification. Peak 1b was used for further structural characterization. Examples of *en face* views are circled, and boxes mark examples of side views of dimerized 8S complexes. Scale bar, 100 nm. (**D**) Class averages of dimers of CAV1-His6 in peak 1a. (**E**) Class averages of CAV1 particles in peak 2. Bottom left numbers in D and E indicate the number of particles in each class. Scale bars in D and E, 26.4 nm.
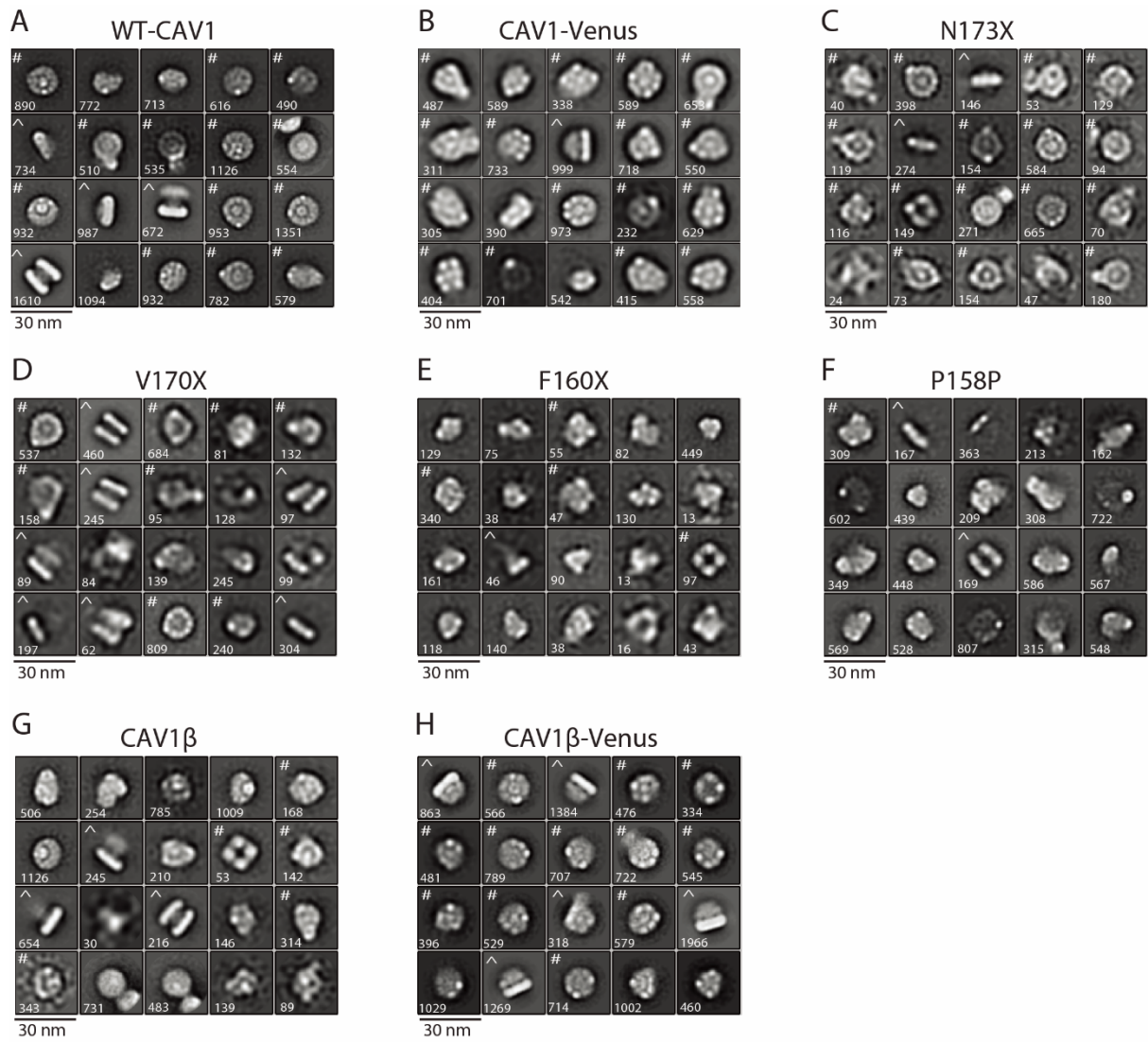
Figure S3. Negative stain averages of CAV1 constructs. Negative class averages of (**A**) WT-CAV1, (**B**) CAV1-Venus, (**C**) N173X, (**D**) V170X, (**E**) F160X, (**F**) P158P, (**G**) CAV1β, and (**H**) CAV1β-Venus. #, *en* face; ^, side view; bottom left number, particles per average. Scale bars, 30 nm.
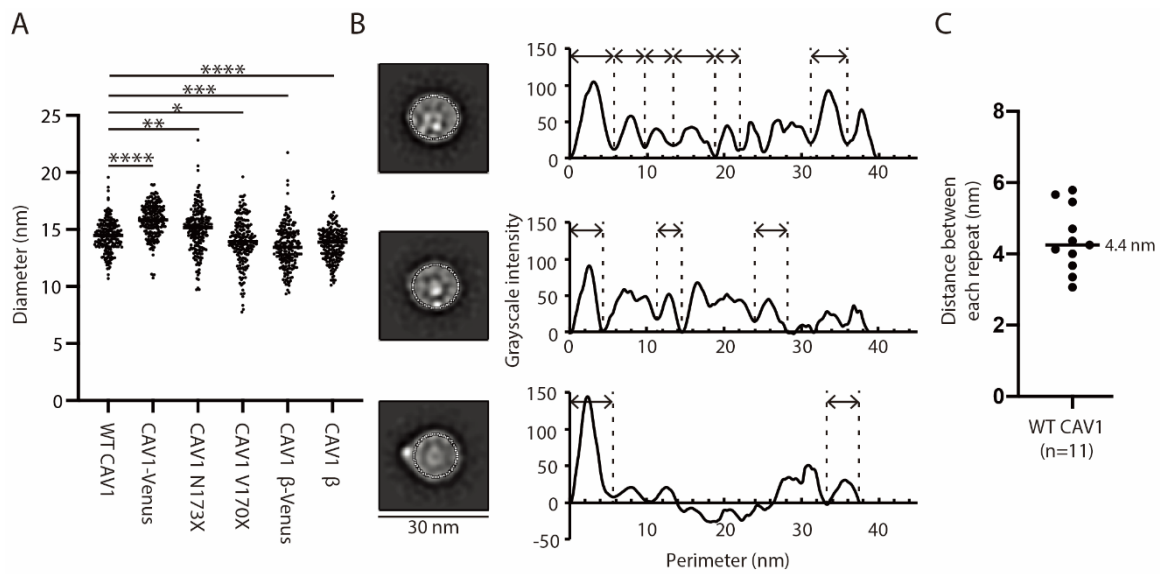
Figure S4

Figure S4. Size measurements of negatively stained CAV1 complexes. (**A**) Plots of diameters of 200 individual 8S complexes of WT CAV1, CAV1-Venus, N173X, V170X, CAV1β-Venus, and CAV1β seen in negative stain images. An ordinary one-way Analysis of Variance (ANOVA) test was performed to determine whether the difference in the means were statistically significant, whereas Tukey's multiple comparisons test was performed for pairwise comparisons. ****$P \leq 0.0001$; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$. (**B**) Oval plot profiles for three WT CAV1 *en face* class averages. Positions of the oval used for analysis are shown in white dotted lines on the particle images. On the graphs, dotted lines mark the boundaries between adjacent globular domains, which we interpret to represent CAV1 monomers. Note that only globular domains with distinct boundaries between them were included in the analysis. (**C**) Plots of average distance each globular domain occupies for WT CAV1 as measured in panel B.
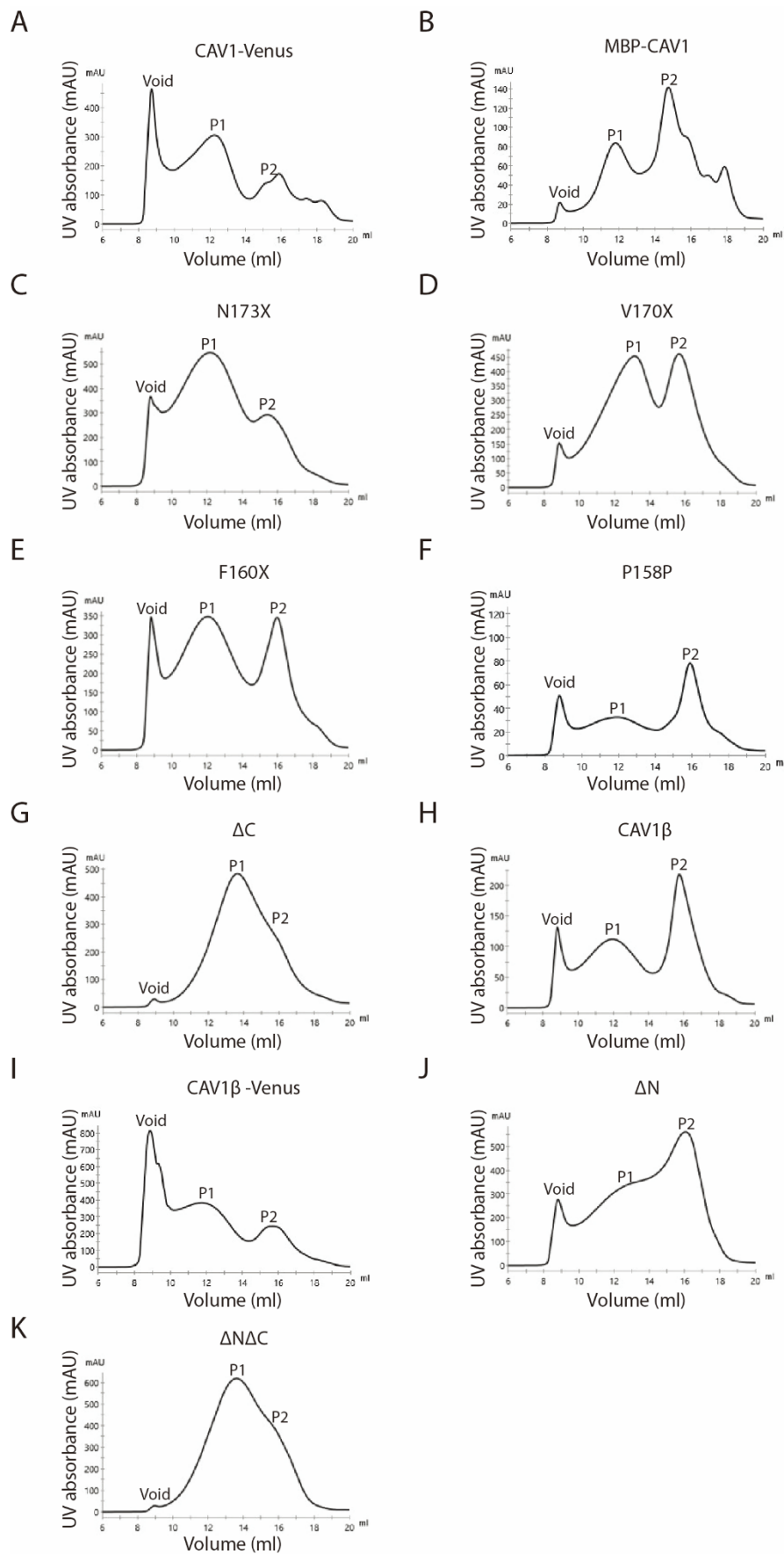
Figure S5. FPLC traces of CAV1 constructs. The indicated proteins were purified from E. coli membranes and applied to a Superose®6 Increase 10/300 GL column. Shown are the elution profiles for CAV1-Venus (**A**), MBP-CAV1 (**B**), N173X (**C**), V170X (**D**), F160X (**E**), P158P (**F**), ΔC (**G**), CAV1β (**H**), CAV1β-Venus (**I**), ΔN (**J**), and ΔNΔC (**K**). For all of the traces listed above, Peak 1 contains the fully organized oligomers and was used for structural characterization.

Figure S5

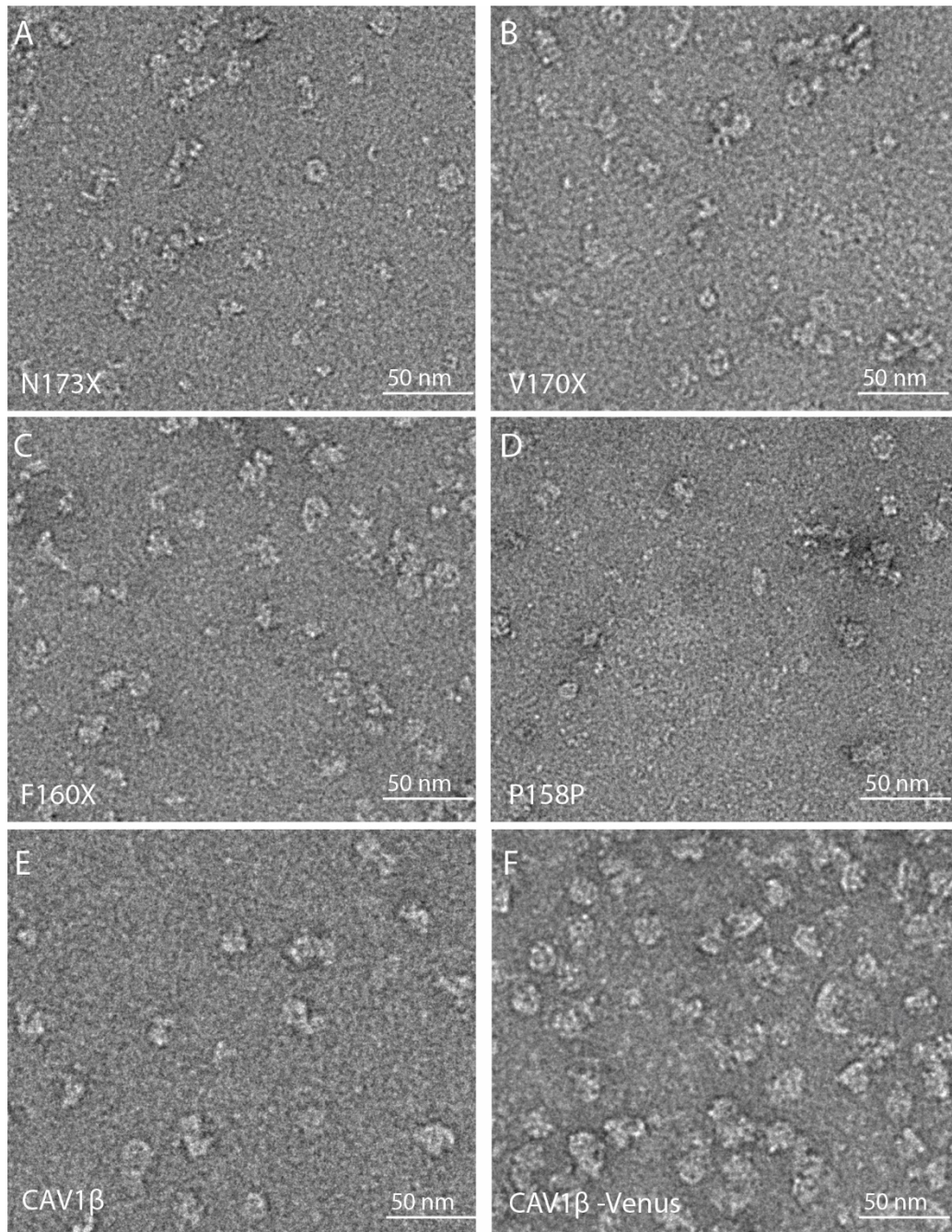Figure S6

Figure S6. Negative stain analysis of CAV1 C-terminus truncations and CAV1β. Representative negative stain images of (**A**) N173X, (**B**) V170X, (**C**) F160X, (**D**) P158P, (**E**) CAV1β, and (**F**) CAV1β-Venus. Scale bars, 50 nm.

Table S1. Summary of 8S complex diameter measurements.

	WT CAV1	CAV1-Venus	N173X	V170X	CAV1β-Venus	CAV1β
N	200	200	200	200	200	200
Mean ± SD (nm)	14.4 ± 1.4	15.8 ± 1.4	15.0 ± 2.0	14.0 ± 2.0	13.7 ± 1.4	13.5 ± 1.9

Table S2.   Constructs used in this study.

Construct	Species	Backbone	CAV1 Sequence	Tags	Source
WT CAV1	*Homo sapiens*	pET20	Full-length	-LE-6His	This study
N173X	*Homo sapiens*	pET20	1-172	-LE-6His	This study
V170X	*Homo sapiens*	pET20	1-169	-LE-6His	This study
F160X	*Homo sapiens*	pET20	1-159	-LE-6His	This study
P158P	*Homo sapiens*	pET20	1-158+novel C-terminus	-LE-6His	This study
ΔC	*Homo sapiens*	pET20	1-147	-LE-6His	This study
CAV1β	*Homo sapiens*	pET20	32-178	-LE-6His	This study
ΔN	*Homo sapiens*	pET20	1, 49-178	-LE-6His	This study
ΔNΔC	*Homo sapiens*	pET20	1, 49-147	-LE-6His	This study
MBP-CAV1	*Homo sapiens*	pET28	Full-length	6His-MBP-TEV-6His-Factor Xa-	This study
CAV1-Venus	*Homo sapiens*	pET28	Full-length	-TEV-Venus-10His	This study
CAV1β -Venus	*Homo sapiens*	pET28	32-178	-TEV-Venus-10His	This study

Table S3. Primers used for plasmid construction in this study.

	WT CAV1
Forward	5'GCGGCCCATATGTCTGGTGGTAAATACGTTGACTCTGAAGG3'
Reverse	5'GCGGCCCTCGAGGATTTCTTTCTGCAGGTTGATACG3'
	N173X
Forward	5'GCGGCCCATATGTCTGGTGGTAAATACGTTGACTCTGAAGG3'
Reverse	5'GCGGCCCTCGAGGATACGAACGTTAGAGAAGATTTTACC3'
	V170X
Forward	5'GCGGCCCATATGTCTGGTGGTAAATACGTTGACTCTGAAGG3'
Reverse	5'GCGGCCCTCGAGGTTAGAGAAGATTTTACCAACCGCTTCG3'
	F160X
Forward	5'GCGGCCCATATGTCTGGTGGTAAATACGTTGACTCTGAAGG3'
Reverse	5'GCGGCCCTCGAGCAGCGGGTCGCAAACGGTGTGAACG3'
	P158P
Forward	5'GCGGCCCATATGTCTGGTGGTAAATACGTTGACTCTGAAGG3'
Reverse	5'GCGGCCCTCGAGCTTATATTTCTTTCTGCAAGTTGATGCGG3'
	ΔC
Forward	5'GCGGCCCATATGTCTGGTGGTAAATACGTTGACTCTGAAGG3'
Reverse	5'GCGGCCCTCGAGAACACGAGAGATGCACTGG3'
	CAV1β
Forward	5'GCGGCCCATATGGCGGACGAACTGTCTG3'
Reverse	5'GCGGCCCTCGAGGATTTCTTTCTGCAGGTTGATACG3'
	ΔN
Forward	5'GCGGCCCATATGATCGACCTGGTTAACCG3'
Reverse	5'GCGGCCCTCGAGGATTTCTTTCTGCAGGTTGATACG3'
	ΔNΔC
Forward	5'GCGGCCCATATGATCGACCTGGTTAACCG3'
Reverse	5'GCGGCCCTCGAGAACACGAGAGATGCACTGG3'
	MBP-CAV1
Forward	5'CGGGATCCCATCATCATCATCATCATATCGAAGGTCGTATGTCTGGGGGGCAAATACGTAGAC3'
Reverse	5'CGGAATTCTTATATTTCTTTCTGCAAGTTGATGCG3'
	CAV1β -Venus
Forward	5'GATATACCATGGCGGACGAACTGTCTG3'
Reverse	5'CACGCCTCGAGATCTAAGCTTTCCGGAGCCCTG3'