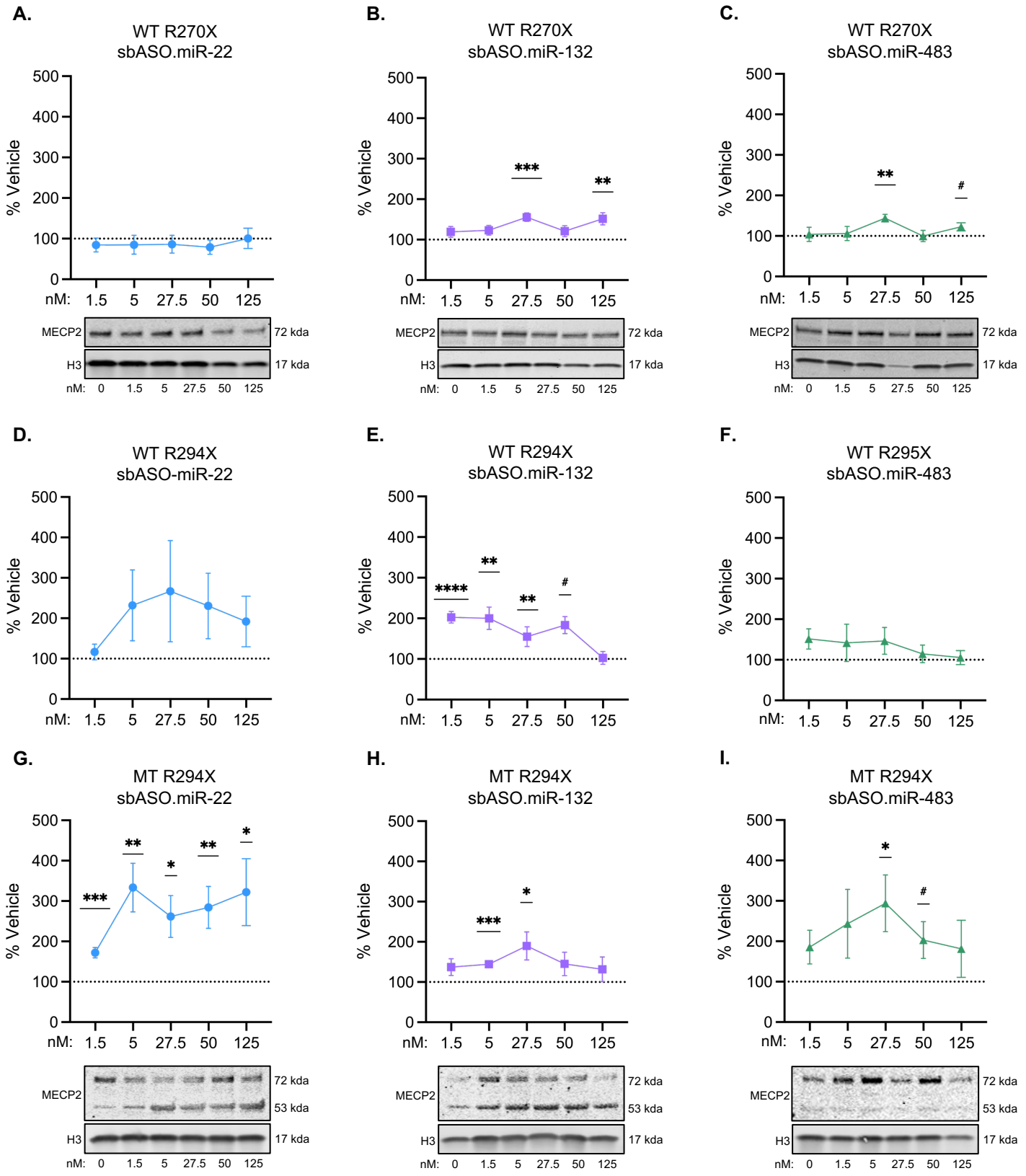
A.
WT R270X
sbASO.miR-22
B.
WT R270X
sbASO.miR-132
C.
WT R270X
sbASO.miR-483
D.
WT R294X
sbASO-miR-22
E.
WT R294X
sbASO.miR-132
F.
WT R295X
sbASO.miR-483
G.
MT R294X
sbASO.miR-22
H.
MT R294X
sbASO.miR-132
I.
MT R294X
sbASO.miR-483
% Vehicle
nM: 1.5 5 27.5 50 125
MECP2
H3
72 kda
53 kda
17 kda
nM: 0 1.5 5 27.5 50 125

Supplemental Figure 5. Dose-Dependent Activity of sbASOs in Human-Derived Fibroblasts with Rett Syndrome Truncation Mutations. Western blot analysis of the dose-dependent activity of sbASOs on MECP2 protein levels in the nuclear fraction of human fibroblast cells derived from Rett syndrome patients with truncation mutations R270X and R294X. Protein quantification was normalized to Histone H3 (H3) levels, with data represented as the mean percentage relative to vehicle-treated controls. The sbASOs demonstrated a dose-dependent increase in MECP2 levels in the cells with truncation mutations. Representative Western blots for each mutation and treatment condition are displayed below the corresponding bar graphs, indicating the levels of MECP2 (upper band at 71 kDa; R294X truncated band at 53 kDA) and H3 (lower band at 17 kDa). Outliers were identified and excluded using Grubb's test with a significance level of $\alpha = 0.1$. Data are presented as mean ± SEM.

sbASO.miR-22: **(A)** R270X: 1.5nM [t (df,7) = 1.054, p = 0.3268], 5nM [t (df,7) = 0.7487, p = 0.4784], 27.5nM [t (df,7) = 0.7236, p = 0.4928], 50nM [t (df,7) = 1.388, p = 0.2076], and 125nM [t (df84) = 0.0161, p = 0.9875].**(D)** WT R294X: 1.5nM [t (df,7) = 0.9687, p = 0.3650], 5nM [t (df,8) = 1.506, p = 0.1707], 27.5nM [t (df,8) = 6.125, p = 0.2184], 50nM [t (df,8) = 1.613, p = 0.1455], and 125nM [t (df,8) = 1.470, p = 0.1796].

(G) MT R294X: 1.5nM [t (df,7) = 6.366, ***p = 0.0004], 5nM [t (df,8) = 3.882, **p = 0.0047], 27.5nM [t (df,8) = 3.115, *p = 0.0143], 50nM [t (df,8) = 3.544, **p = 0.0076], and 125nM [t (df,8) = 2.682, **p = 0.0278].

sbASO.miR-132: **(B)** R270X: 1.5nM [t (df,7) = 1.616, p = 0.1501], 5nM [t (df,8) = 1.901, p = 0.0938], 27.5nM [t (df,8) = 5.314, ***p = 0.0007], 50nM [t (df,8) = 1.501, p = 0.1716], and 125nM [t (df,7) = 3.936, **p = 0.0056].

(E) WT R294X: 1.5nM [t (df,7) = 8.130, ****p < 0.0001], 5nM [t (df,8) = 3.668, **p = 0.0063], 27.5nM [t (df,8) = 2.257, p = 0.0539], 50nM [t (df,8) = 3.976, **p = 0.0041], and 125nM [t (df,8) = 0.1776, p = 0.8634].

(H) MT R294X: 1.5nM [t (df,7) = 1.941, p = 0.0934], 5nM [t (df,8) = 6.827, ***p = 0.0002], 27.5nM [t (df,8) = 1,517, *p = 0.0360], 50nM [t (df,8) = 1.495, p = 0.1734], and 125nM [t (df,8) = 0.9768, p = 0.3573].

sbASO.miR-483: **(C)** R270X: 1.5nM [t (df,8) = 0.2307, p = 0.8234], 5nM [t (df,8) = 0.346, p = 0.7381], 27.5nM [t (df,8) = 4.688, **p = 0.0016], 50nM [t (df,7) = 0.0205, p = 0.9842], and 125nM [t (df,8) = 2.267, p = 0.0531].

(F) WT R294X: 1.5nM [t (df,7) = 2.076, p = 0.0716], 5nM [t (df,8) = 1.032, p = 0.3363], 27.5nM [t (df,8) = 1.419, p = 0.1937], 50nM [t (df,8) = 0.6789, p = 0.5163], and 125nM [t (df,8) = 0.3647, p = 0.7261].

(I) MT R294X: 1.5nM [t (df,7) = 2.063, p = 0.0730], 5nM [t (df,8) = 1.690, p = 0.1269], 27.5nM [t (df,8) = 2.769, *p = 0.0243], 50nM [t (df,8) = 2.261, p = 0.0537], and 125nM [t (df,8) = 1.311, p = 0.2313].