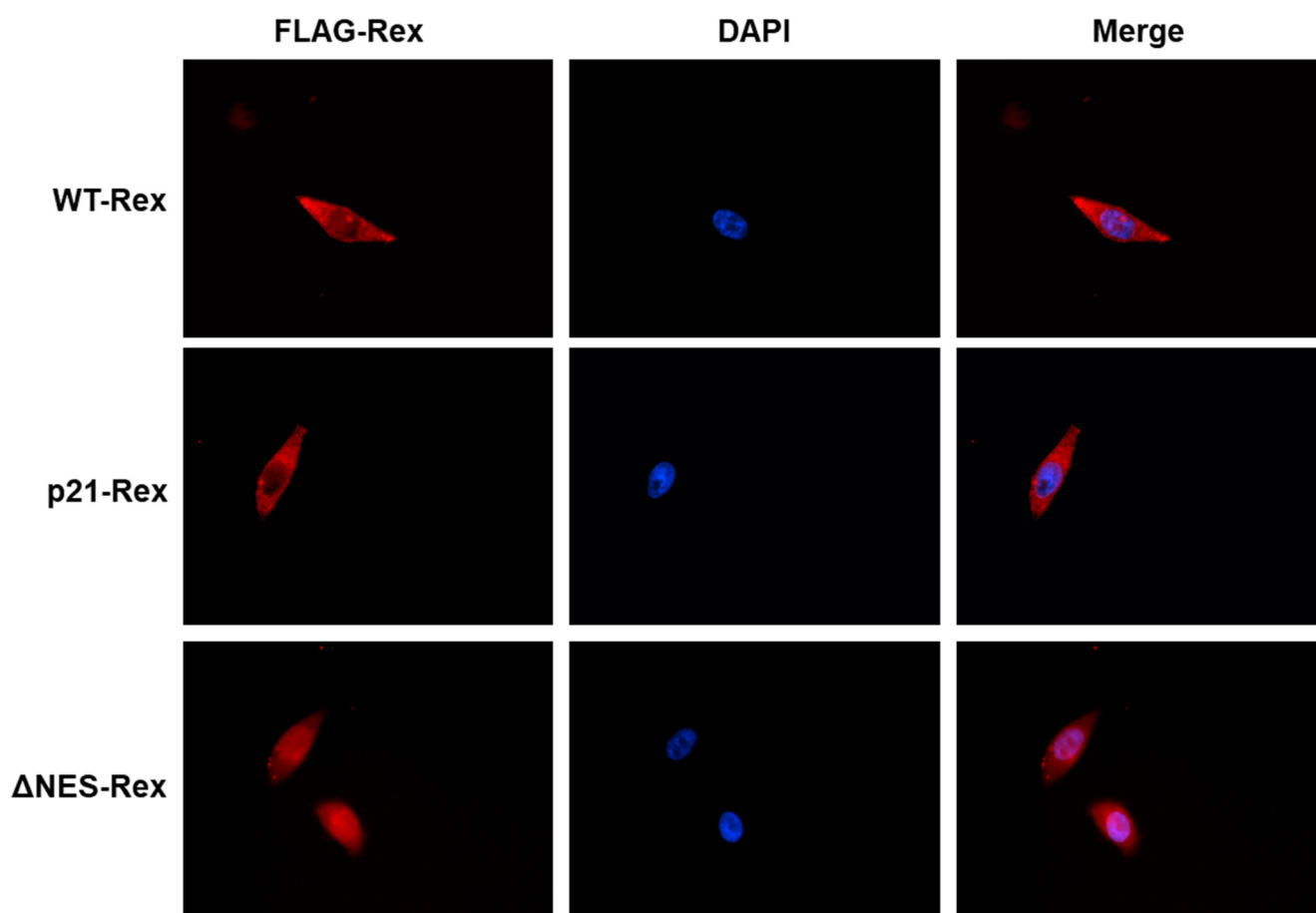


**Figure S1. Y-region contains a motif important for stabilization of Rex.**

**A.** Ten Rex deletion mutants in Y-region, of which function has not been clarified by using the 10 deletion mutants of Rex as shown. **B.** Expression levels of those 10 deletion mutants were compared with WT-Rex by Western blotting. Deletion of aa150-159 in the Y-region resulted in almost no protein expression (mutant-#3 and #4 compared with #2). Deletion of only the stability domain (aa170-189) (mutant-#5) had no significant effect on protein expression, whereas deletion of aa160-169 with the stability domain markedly reduced protein expression (mutant-#6 and #7 compared with #5, #9, #10). **C.** The NMD activity was compared between HeLa cells expressing WT-Rex or mutant-Rex-#10 ( $\Delta$ aa150-169). The graph shows that the mutant-#10 suppresses the NMD activity of the cells to the same extent as WT-Rex comparing with Mock cells. (n=6, Mean $\pm$ SD, \*\*\*p<0.001)



**Figure S2. Subcellular localization of FLAG-Rex in HeLa cells.**

FLAG-tagged WT-Rex, P21Rex, and  $\Delta$ NES-Rex were overexpressed in HeLa cells and the subcellular localization was examined by immunocytochemistry with anti-FLAG antibody. WT-Rex distributes both in nucleus and cytoplasm, while p21-Rex without NLS region is only in cytoplasm, as already reported.  $\Delta$ NES-Rex is mainly in nucleus but also in cytoplasm in a lower level.