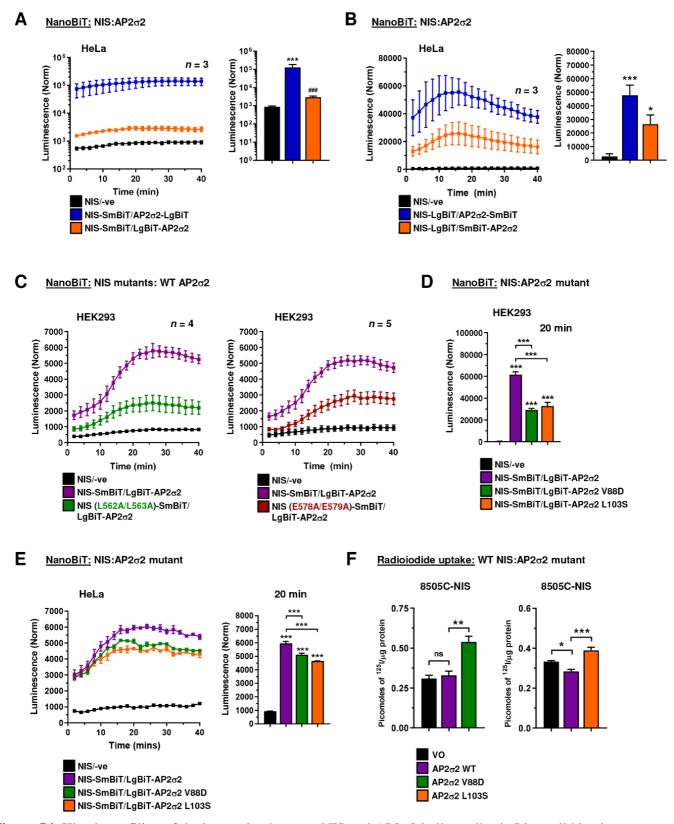
## **SUPPLEMENTARY FIGURE S4**



**Figure S4.** Kinetic profiling of the interaction between NIS and AP2 $\sigma$ 2 in live cells. **A**, Live cell kinetic measurement using the NanoBiT assay to evaluate protein-protein interactions between NIS and AP2 $\sigma$ 2 tagged with LgBiT in HeLa cells. (*right*) NanoBiT assay results at 20 minutes post-addition of Nano-Glo substrate. **B**, Same as **A** but AP2 $\sigma$ 2 tagged with SmBiT. **C**, Same as **A** but using NIS mutants L562A/L563A (*left*) and E578A/E579A (*right*) in HEK293 cells. **D**, Same as **A** but using AP2 $\sigma$ 2 mutants V88D and L103S in HEK293 cells. NanoBiT assay results are shown at 20 minutes post-addition of Nano-Glo substrate. **E**, Same as **D** but in HeLa cells. **F**, RAI uptake in 8505C-NIS cells transfected with WT AP2 $\sigma$ 2, AP2 $\sigma$ 2 mutant V88D and AP2 $\sigma$ 2 mutant L103S. Data presented as mean ± S.E.M., *n* = 4 - 5, one-way ANOVA followed by Tukey's post hoc test (ns, not significant; \**P* < 0.05; \*\**P* < 0.01; \*\*\**P* < 0.001) or unpaired two-tailed t-test (###*P* < 0.001).