



Supplementary Figure 2. Jxtm1 fragment show essentially the same results with F-3 fragment. (A) HeLa cells were transfected with indicated constructs and pEGFP as a control. The representative confocal images show the subcellular distribution of the indicated fusion proteins. Either singly or doubly substituted mutant constructs significantly abolished the nuclear translocation compared with wild type Jxtm1. $n=150\text{--}250$. Error bars ; standard deviations of three independent experiments. * $P < 0.05$, ** , $P < 0.01$.

(B) HeLa cells transiently transfected with Jxtm1 fusion protein were treated with or without $10\mu\text{M}$ nigericin during indicated durations. The number of cells was counted following immunocytochemistry using anti-GFP antibody. Bar graph illustrates the percentage of cells showing indicated subcellular distribution ($n=145\text{--}250$). Nuclear accumulation of Jxtm1 fusion protein follows time dependent manner of nigericin treatment. Error bars ; standard deviations in three independent experiments. * $P < 0.05$, ** , $P < 0.01$.

(C) HeLa cells transiently transfected with Jxtm1 fusion construct were treated with or without $10\mu\text{M}$ nigericin during indicated durations. Western blot analysis was performed using anti-GFP antibody after cellular fractionation. Accumulation of Jxtm1 fusion protein in nuclear fraction was increased in time dependent manner. Tubulin and laminB were used as nuclear and cytoplasmic markers respectively. Protein densitometry graphs below each panel show their respective subcellular distribution of the protein in fold change. **(D)** Densitometric Bar graph depicts the amount of the cargo-binding importin β in fold change. For this, immunoprecipitation was carried out in the same way as mentioned in Fig 3B from the lysate of Jxtm1 transfected HeLa cells.