

# Review of: "ANXA2 enhances virus replication through negatively regulating cGAS-STING and RLRs2 mediated signal pathway"

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The authors describe a new role of membrane scaffold protein ANXA2 in regulating the production of type I IFN against RNA and DNA virus infection. The finding is interesting that ANXA2 targets on multiple signal molecules, such as MAVS, STING and IRF3. However, the mechanism of ANXA2 must be more strongly supported with additional evidence. There are a number of concerns with this study which include the following:

1. HEK293 and HeLa cells are known that respond only to RNA virus and not to DNA virus, because they both contain viral oncoproteins that antagonize the cGAS-STING pathway (refer to *Science* 250:568). However, in the present manuscript, HEK293 and HeLa cells are frequently used in HSV infection or cGAMP stimulation, and the relative results showed the normal activation of cGAS-STING pathway in these two cells. The authors should recheck the cells and results, and give solid evidence in other cells.
2. Because the subcellular localization of MAVS, STING and IRF3 is important for their function, the dynamic interaction and colocalization of ANXA2 with these molecules are certainly concerned. The manuscript showed some effort but was not sufficient to make subcellular localization of ANXA2 clear, as all the confocal observation was based on ectopic expression (Fig. 4I, 5G, 6F, S6D). It is hard to conclude the colocalization of two proteins because the immunofluorescent dots filled the cytoplasm. The authors should also determine whether the endogenous interaction of ANXA2 with MAVS, STING or IRF3 is dependent on virus infection or not.
3. Does ANAX2 interact with TRAF3? As co-IP was frequently used in the manuscript, *in vitro* pull-down assay should be performed to determine whether the interaction of two proteins is direct or indirect.
4. The study performed viral infection assays in mice. Does ANAX2 deficiency affect the mortality of mice upon viral infection?
5. As the authors mentioned that "ANXA2 exists as a heterotetrameric complex composed of S100A10

dimer and two ANXA2", does S100A10 play similar role in RIG-I or cGAS pathway?