



**Supplementary Figure 1.** Initial screening of 26 Nbs (periplasmic extracts) targeting moLAG-3. **(a)** Table summarizing the different selection steps i.e., ELISA, flow cytometry, and SPR. **(b)** Binding of 50  $\mu$ L periplasmic extract on 293T-WT (grey line) versus 293T-LAG-3 cells (red line). **(c)** Evaluation of kinetics of periplasmic extract of the Nbs on immobilized Fc-tagged recombinant LAG-3 protein. Each sensogram represents a different concentration of the same Nb i.e., undiluted extract (red line), 1/4 diluted extract (orange line), 1/8 diluted extract (blue line), and a blank run (grey line). Nanobodies depicted in orange were further evaluated.