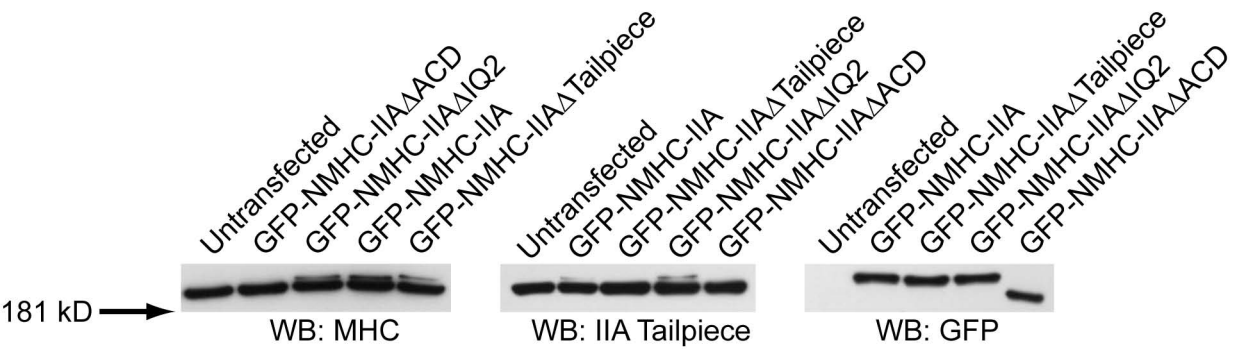
E08-04-0372 Egelhoff

Supplemental Figure S1. Transfected proteins are expressed at tracer amounts relative to endogenous MHC IIA. Western blots of total cell lysates from cells of typical transfected GFP brightness. Flow cytometry was used to collect cell populations of each set of transfections, with gates set to collect cells of similar brightness between each group, which in each case included the average range of brightness within the population. The left panel shows a western blot probed with a total MHC antibody. The top band of the doublet in transfected samples represents the GFP conjugated constructs, indicating that GFP constructs are expressed at tracer amounts relative to endogenous. Due to its reduced size (see right panel), the GFP-NMHCΔACD protein comigrates with the endogenous NMHC-IIA, so the doublet is not observed. The middle panel shows a western blot performed with an antibody specific to the nonhelical tailpiece of the NM-IIA. Note the absence of bands corresponding to the Δtailpiece and ΔACD constructs, as these two mutants lack the carboxy-terminal segment of MHC that contains the epitope for this antibody. The right panel shows a western blot performed with an anti-GFP antibody, revealing uniform expression levels for all constructs. Note the significant shift of the ΔACD deletion, indicating its reduced size compared to the other constructs.


Untransfected
GFP-NMHC-IIAΔACD
GFP-NMHC-IIAΔIQ2
GFP-NMHC-IIA
GFP-NMHC-IIAΔTailpiece
181 kD
WB: MHC
Untransfected
GFP-NMHC-IIA
GFP-NMHC-IIAΔTailpiece
GFP-NMHC-IIAΔIQ2
GFP-NMHC-IIAΔACD
WB: IIA Tailpiece
Untransfected
GFP-NMHC-IIA
GFP-NMHC-IIAΔTailpiece
GFP-NMHC-IIAΔIQ2
GFP-NMHC-IIAΔACD
WB: GFP