

Appendix

Proteasomal degradation within endocytic organelles mediates antigen cross-presentation

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Appendix Figure Legends

Appendix Figure S1. Expression of Rab mutants does not alter the cell surface H2-K^b levels or BMDC differentiation.

A, B Plasmids encoding GFP, GFP-Rab5ACA and GFP-Rab22ACA were expressed in BMDCs under the control of a TetON inducible promoter. BMDCs were cultured in Doxycycline (1 µg/ml) for 16hr after which cell lysates were analyzed by anti-GFP immunoblotting (**A**). Myc-Rab7ADN was expressed in BMDCs under the control of constitutive promoter and the expression was assessed by anti-Myc immunoblotting (**B**).

C IL-2 production by B3Z cells in response to paraformaldehyde-fixed BMDCs pulsed with varying doses of SIINFEKL peptide for 1hr prior to fixation.

D Cell surface H2-K^b levels of BMDCs expressing Rab5ACA, Rab22ACA and Rab7ADN were assessed by flow cytometry.

E Cell surface expression of CD11c was determined to analyze the effect of the Rab mutants on BMDC differentiation.

Data Information: In C, representative experiments are shown for each Rab mutants expressed in BMDC. The mean±SD of assay triplicates are plotted.

Appendix Figure S2. Cell surface expression of hβ2m in BMDCs.

A-C Cell surface expression of transduced human β2-microglobulin (hβ2m) on wild-type and TAP1^{-/-} BMDCs expressing GFP, GFP-Rab5ACA (**A**), GFP-Rab22ACA (**B**) and Rab7ADN (**C**) was evaluated by flow cytometry using the monoclonal antibody, BBM.1.

Appendix Figure S3. Representative Immunoelectron microscopy images.

A Immunoblot analysis of WT and LMP2 knockout MEF cells using anti-LMP2 antibody.

B Five representative images captured by TEM of double immunogold labeling of BMDC, WT MEF and LMP2 KO MEF with anti-LMP2(15nm) and anti-LAMP1(5nm) (Bar 500nm).

C Five representative images of double immunogold labeling of BMDC with antibodies(15nm) raised against $\alpha 5$, $\beta 5$ and 19S S2 subunits of proteasomes, respectively, and anti-LAMP1(5nm) (Bar 500nm).

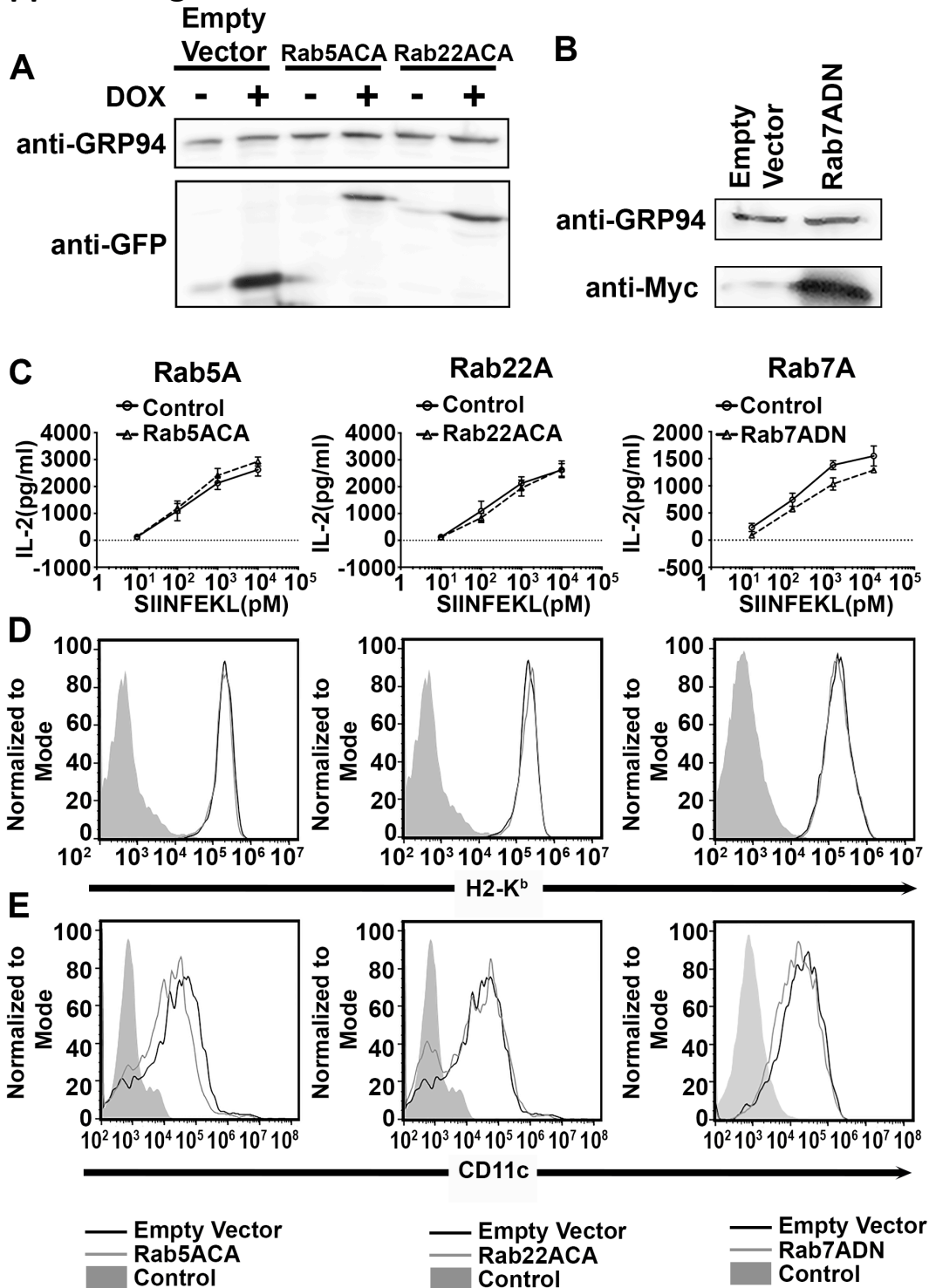
Appendix Figure S4. Proteasomes remain associated with purified latex beads post membrane solubilization by Triton X-100.

A Phagosomes isolated from 293T-FcR-K^b expressing empty vector or Rab mutants were incubated with 0.5% Triton X-100 in PBS on ice for 15min.

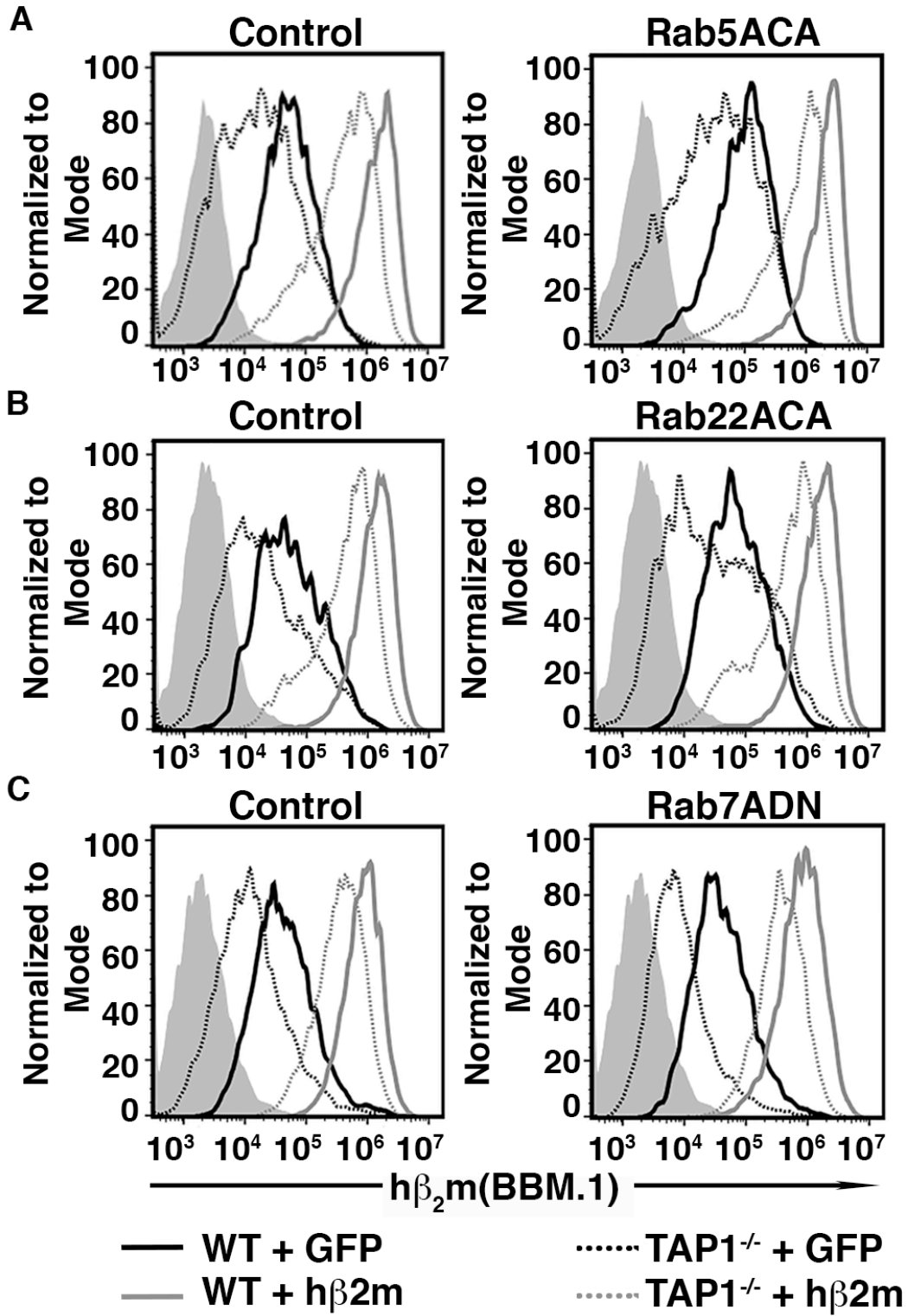
Residual proteasomes associated with the latex beads or proteasomes released into the supernatant were detected by immunoblotting for the $\beta 5$ subunit. The efficiency of membrane solubilization was assessed by immunoblotting for the lysosomal membrane protein LAMP1.

B The gating strategy used to identify intact phagosomes to measure the proteasome dependent degradation of Alexa647-OVA. Based on FSC-SSC the bead population was identified. OVA negative gating defines the intact phagosomes.

Appendix Figure S1

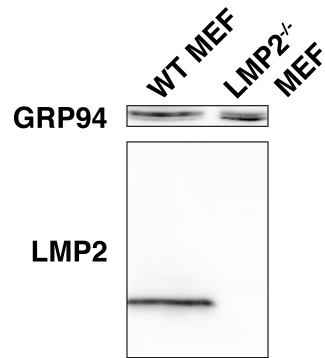


Appendix Figure S2

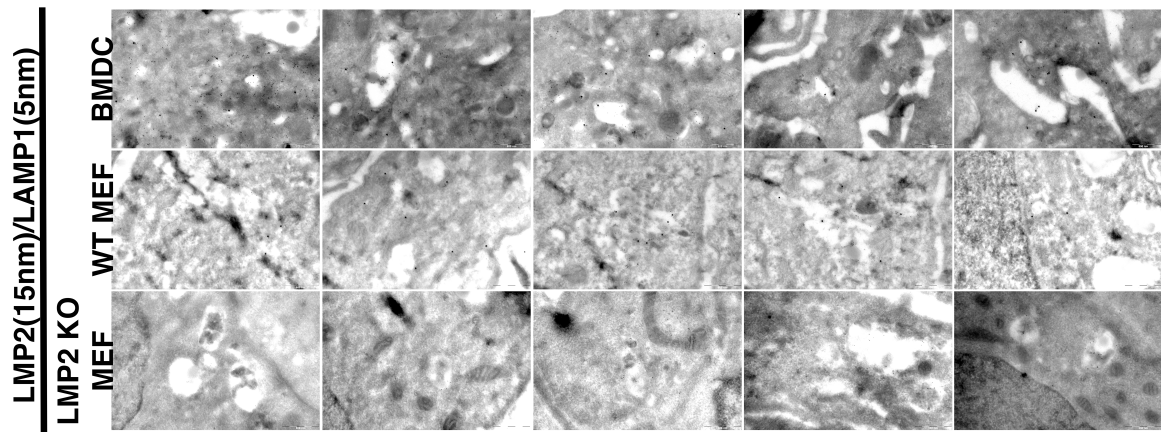


Appendix Figure S3

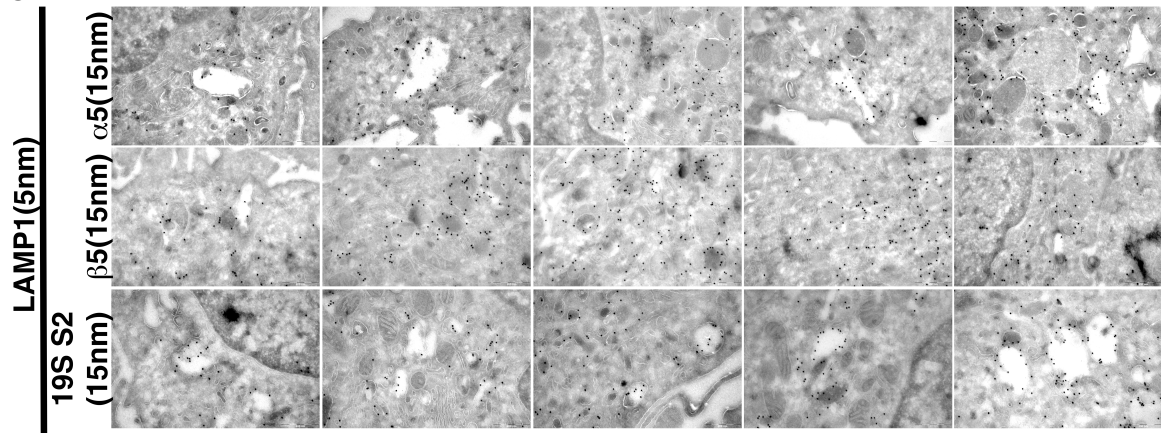
A



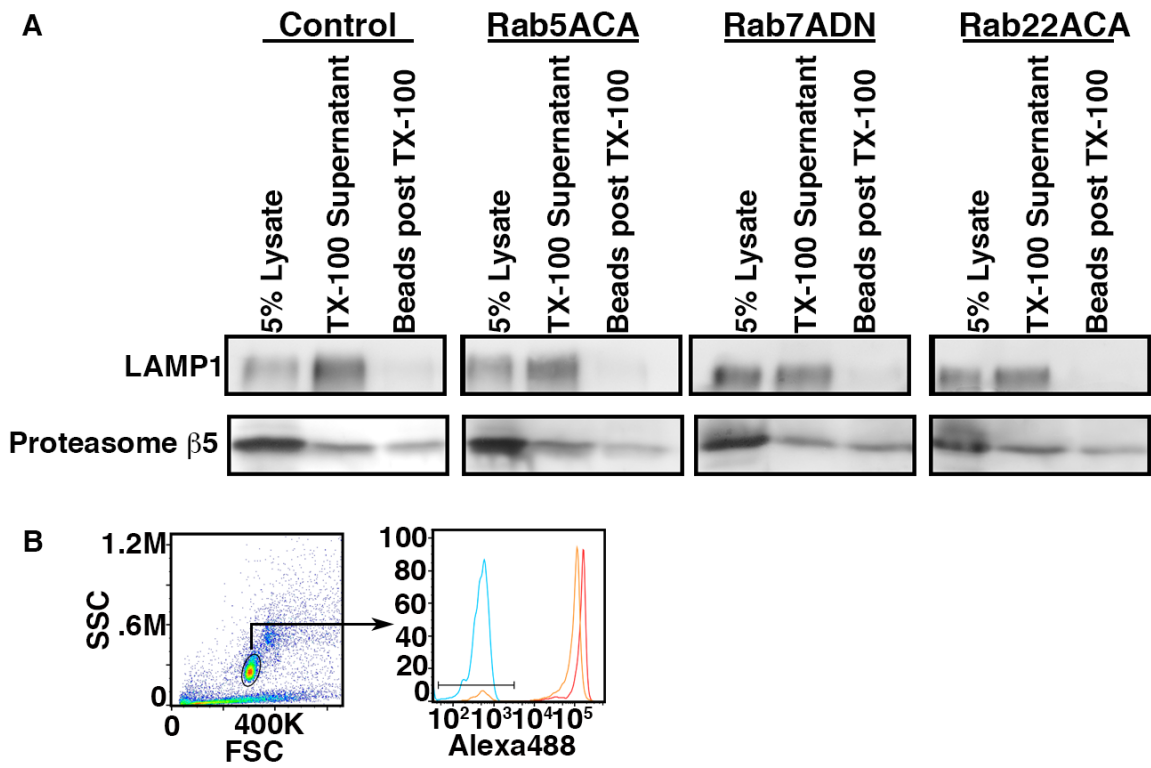
B



C



Appendix Figure S4



Appendix Table S1.**Raw data analyzed to generate proteasome activity curve in Fig 6A.**

Empty Vector

ATP	-	-	+	+
Epoxomicin	-	+	-	+
0hr	4547	4514.000001	4287.000001	4278
1hr	4523	4384.000002	8977.999997	8716
2hr	7351	7438.000003	10600	12898
3hr	7238	6433.000002	18324	12172

Rab5ACA

ATP	-	-	+	+
Epoxomicin	-	+	-	+
0hr	5038.745237	5182	5013.999998	4818
1hr	6891.584213	6955	16092	9460
2hr	9942.682771	10062	24030	12920
3hr	10383.55741	10140	25000	13728

Rab22ACA

ATP	-	-	+	+
Epoxomicin	-	+	-	+
0hr	5550.999998	5643	5336	5224
1hr	7172.000001	6229	20340	9699
2hr	8723.000001	12122	35959.99999	18189
3hr	12713.99999	13230	46065.99999	21620

Rab7ADN

ATP	-	-	+	+
Epoxomicin	-	+	-	+
0hr	5210	5433	4936	4790
1hr	6857.000002	6034	16732	6782
2hr	8430.999996	9100	30510	11323
3hr	9141.000004	9381	37180	13224