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Supplemental Material to:

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PRKAA1/AMPKα1 is required for autophagy-dependent mitochondrial clearance during erythrocyte maturation

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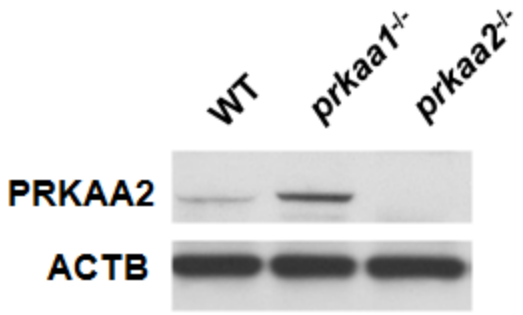

Figure S1. Western blot analysis of the expression of PRKAA2/AMPKα2 in MEFs. Anti-PRKAA2 antibody was used to determine the expression of PRKAA2 in WT, *prkaa1*$^{-/-}$, and *prkaa2*$^{-/-}$ MEFs.

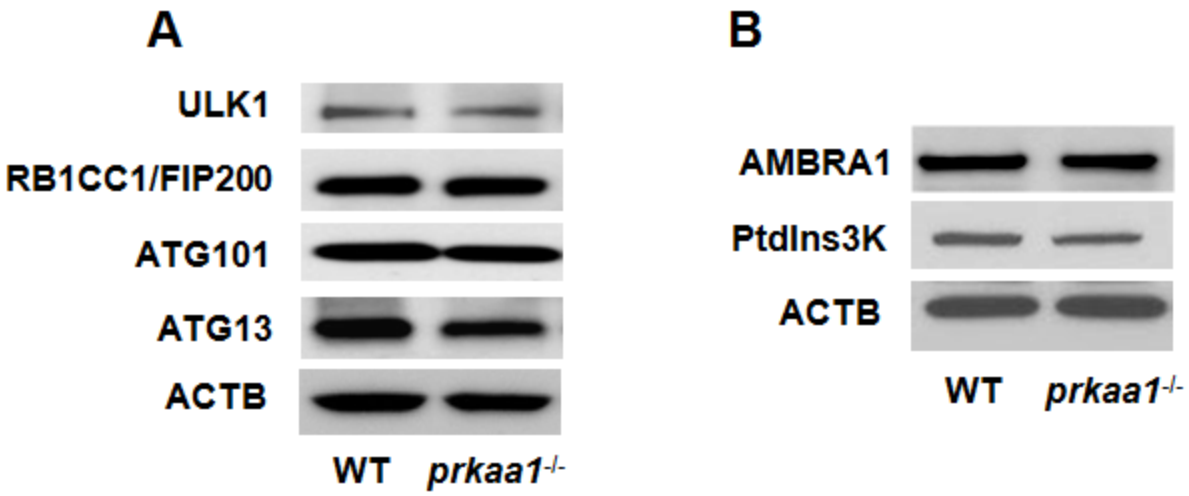

Figure S2. Reduced ULK1 phosphorylation has no effect on the expression of the components in ULK1 and BECN1-PtdIns3K complexes in MEFs. (**A**) The expression of ULK1, RB1CC1/FIP200, ATG101, and ATG13 in WT and *prkaa1*$^{-/-}$ MEFs. (**B**) The expression of AMBRA1 and PtdIns3K in WT and *prkaa1*$^{-/-}$ MEFs was analyzed by western blotting.

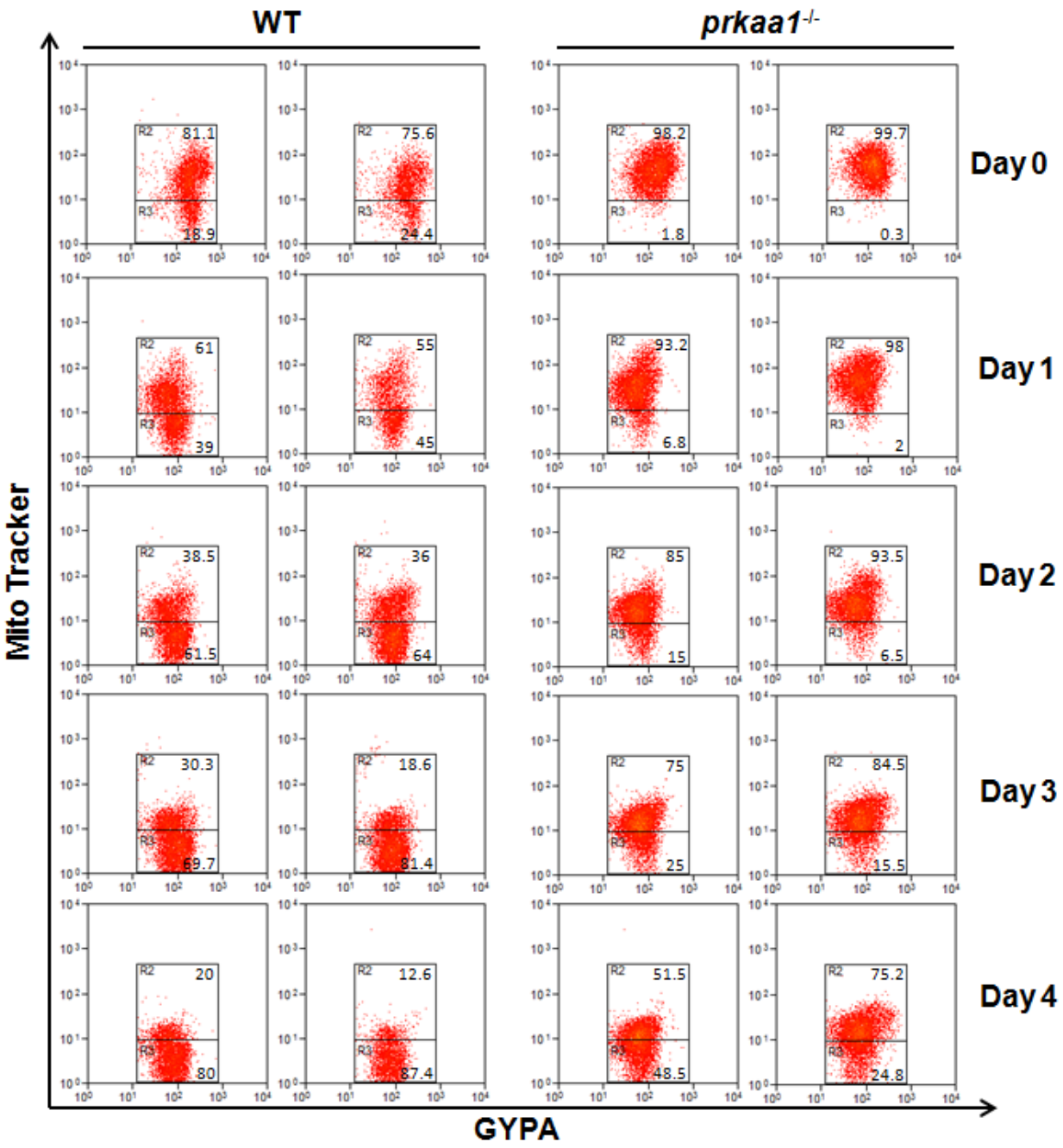

Figure S3. Contour plots of flow cytometry analysis of reticulocytes undergoing erythroid maturation. Reticulocytes were cultured for in vitro maturation for 0, 1, 2, 3, and 4 days. The cells were stained for MitoTracker Deep Red and GYPA, and analyzed by flow cytometry.

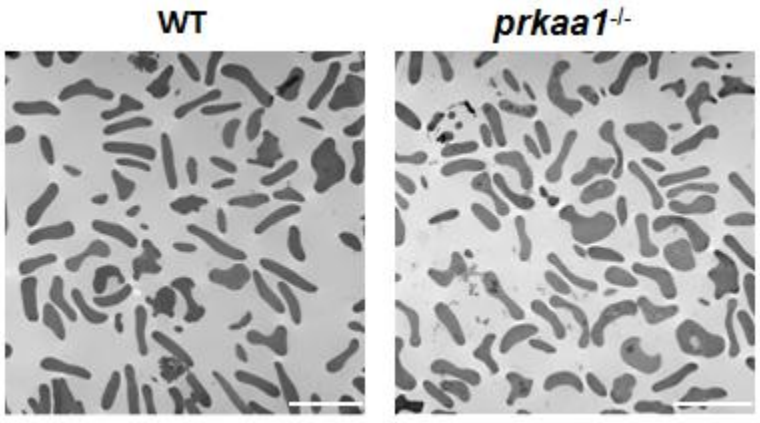

Figure S4. Representative electron micrographs of WT and *prkaa1*$^{-/-}$ peripheral blood erythrocytes. Electron micrographs of erythrocytes from WT and *prkaa1*$^{-/-}$ mice. Scale bar: 10 µm.

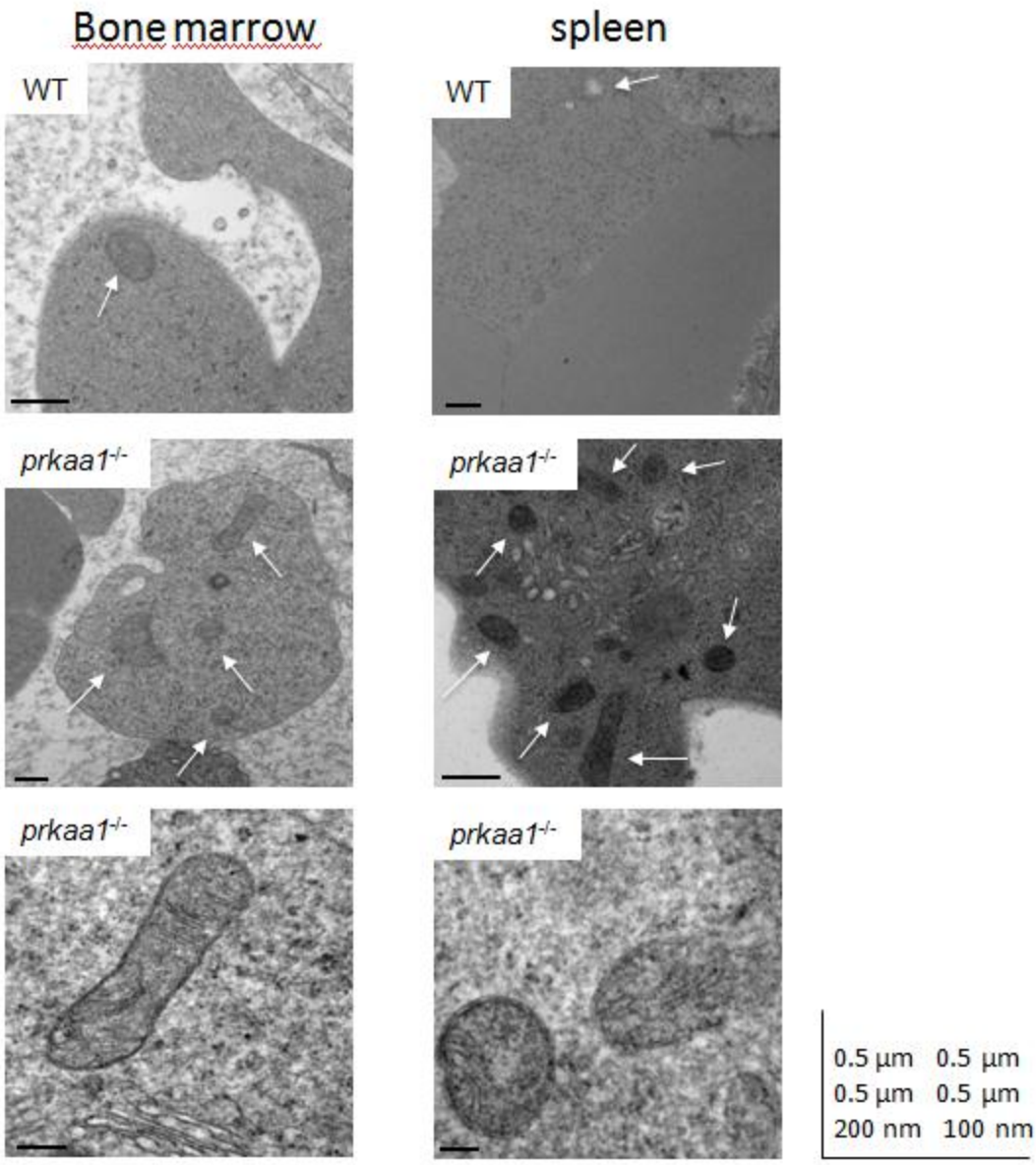

Figure S5. Representative electron micrographs of WT and *prkaa*1$^{-/-}$ erythroid cells isolated from bone marrow and spleen. Electron micrographs of bone marrow and spleen erythroid cells isolated from WT and *prkaa*1$^{-/-}$ mice. Arrow indicates mitochondrial structures.

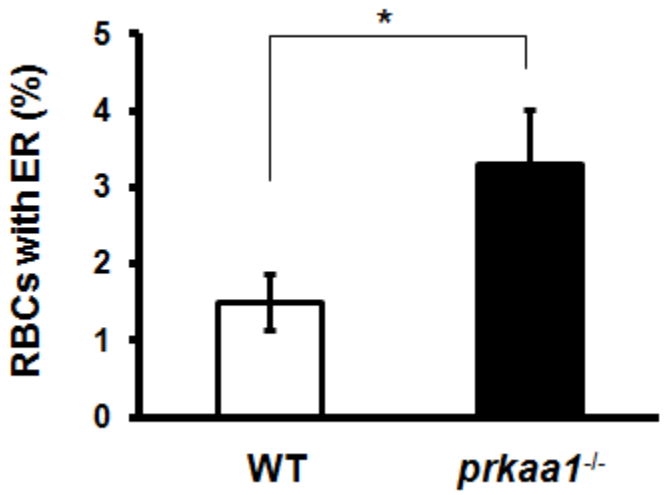

Figure S6. Percentage of erythrocytes containing ERs. Peripheral blood erythrocytes were staining by ER-Tracker Green and analyzed by flow cytometry. n = 8, * $P < 0.05$ vs. WT.

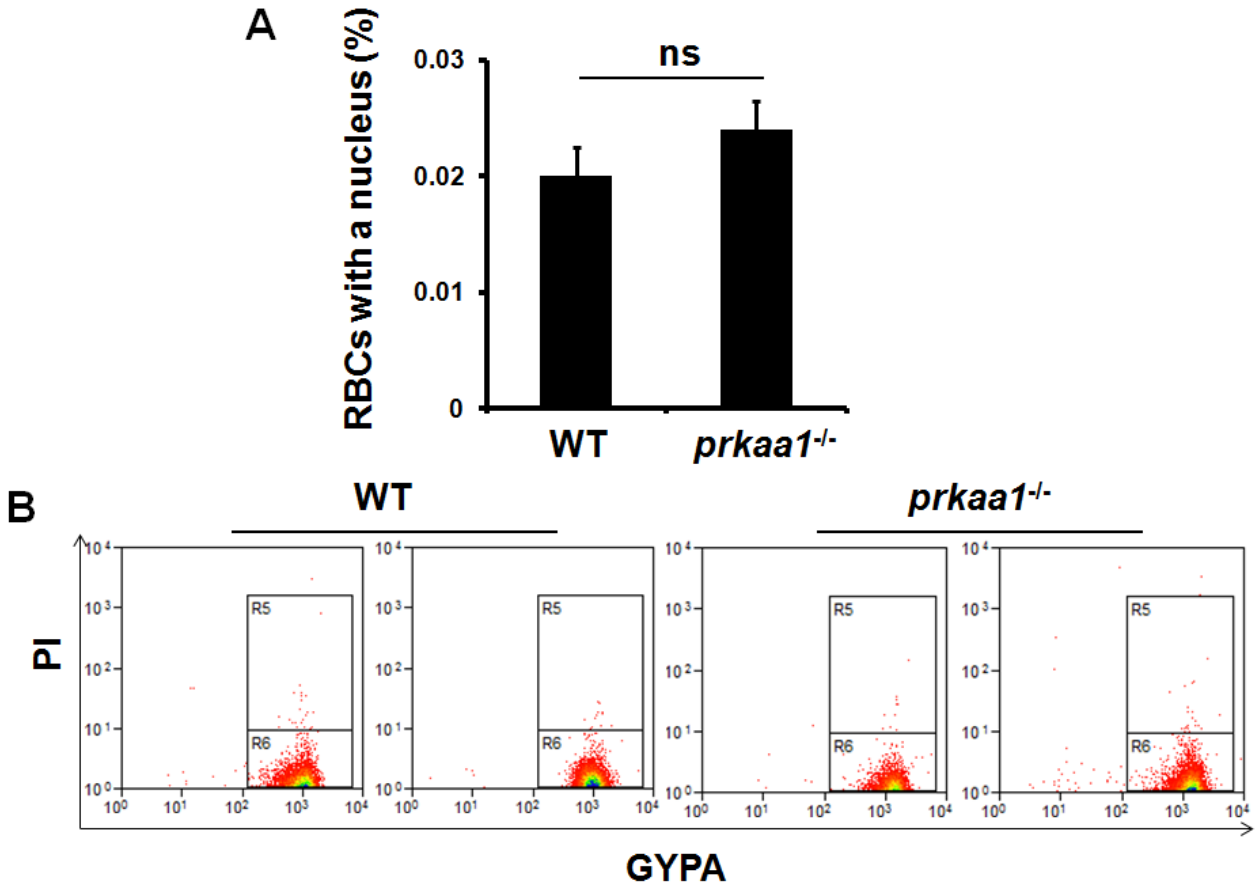

Figure S7. Percentage of erythrocytes containing nuclei. RBCs were labeled with APC -GYPA, nuclei were stained with PI (Invitrogen) and cells were analyzed by flow cytometry. n = 8; ns, not significant.

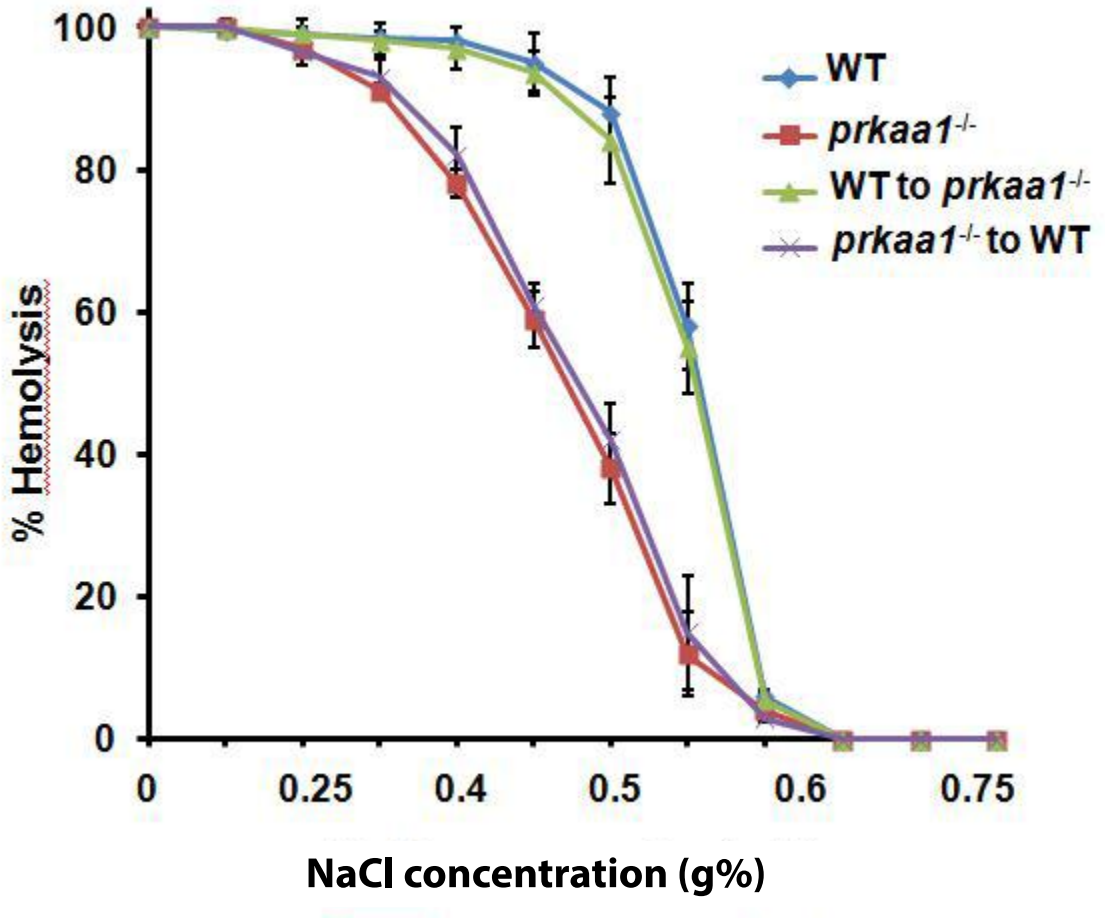

Figure S8. Assay of osmotic fragility of erythrocytes. The osmotic fragility of erythrocytes was assessed in WT and *prkaa1*$^{-/-}$ mice, as well as WT recipients transplanted with *prkaa1*$^{-/-}$ bone marrow cells and *prkaa1*$^{-/-}$ recipients transplanted with WT bone marrow cells. Peripheral blood erythrocytes were exposed to various concentrations of NaCl (%), the supernatant fraction was collected for assessing hemolysis by spectrometry. A fragility curve was generated by plotting various NaCl concentrations versus hemolysis (n = 5).