

Supplementary Figures

Figure S1. Bacterial LPS induces early expression of SAG mRNA and protein in J774 cells.

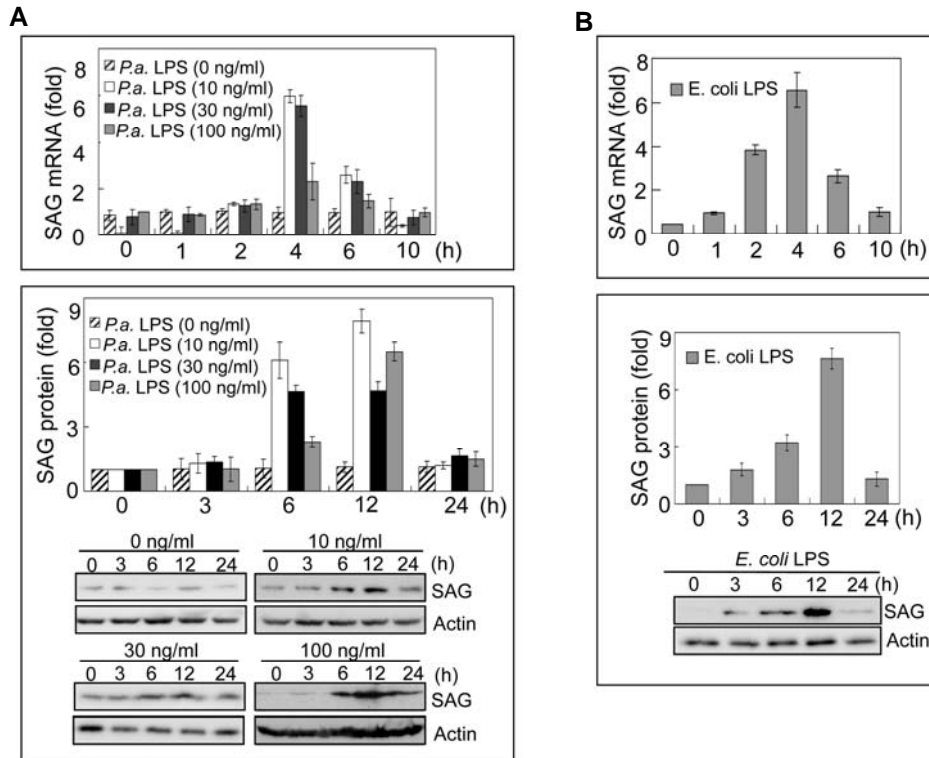
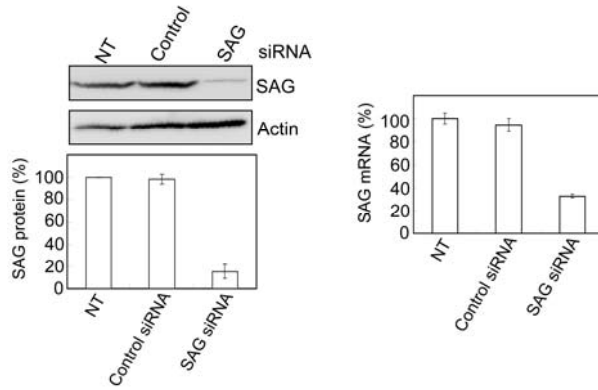
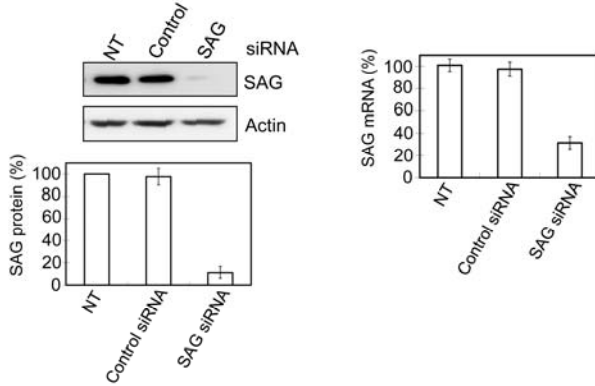


Figure S2. SAG-knockdown in J774, RAW and BMDM cells.

A. J774 cells



B. RAW cells



C. BMDM cells

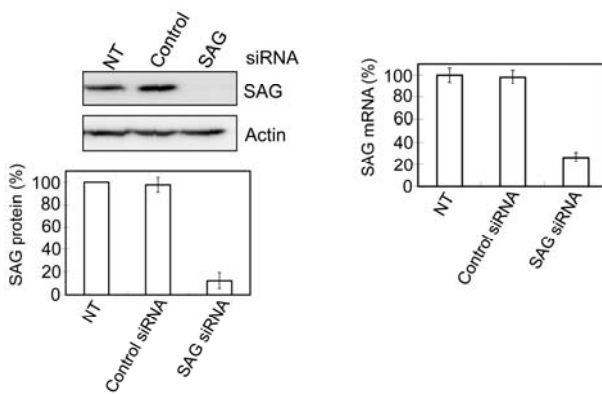
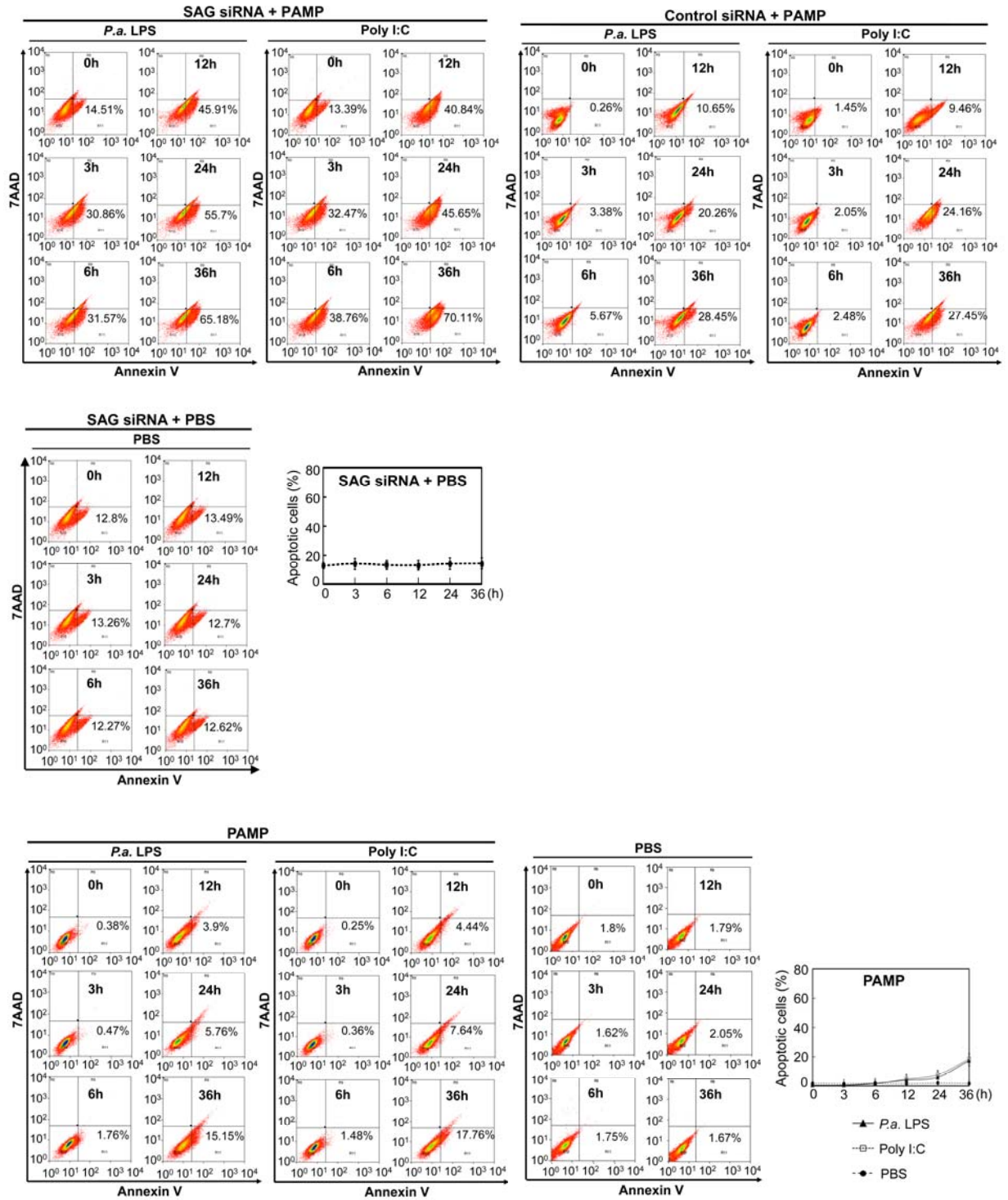
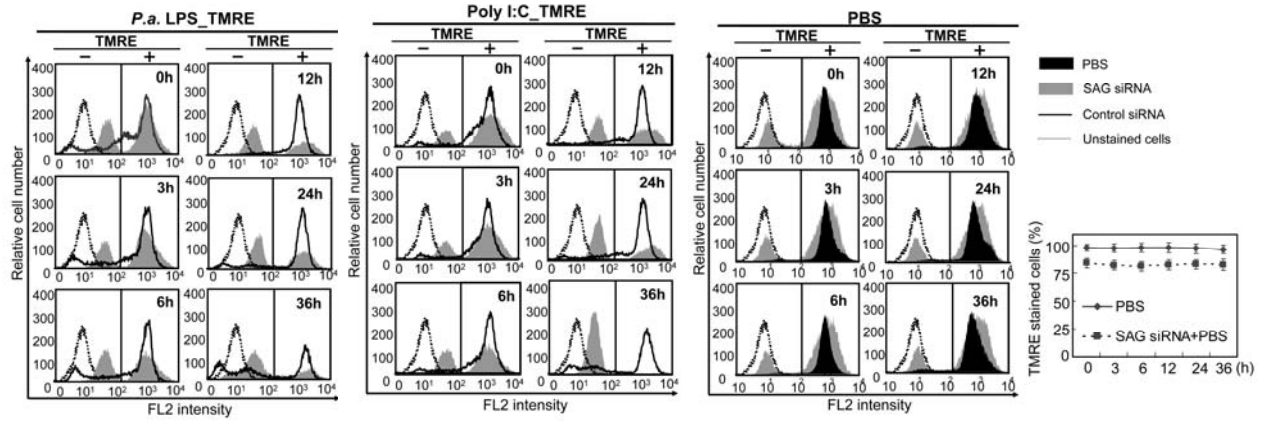


Figure S3. FACS analysis of apoptosis after PAMP-treatments of J774 cells with and without SAG-knockdown.

A. FACS histograms of apoptosis assay



B. FACS histograms of TMRE staining



C. FACS histograms of caspase assay

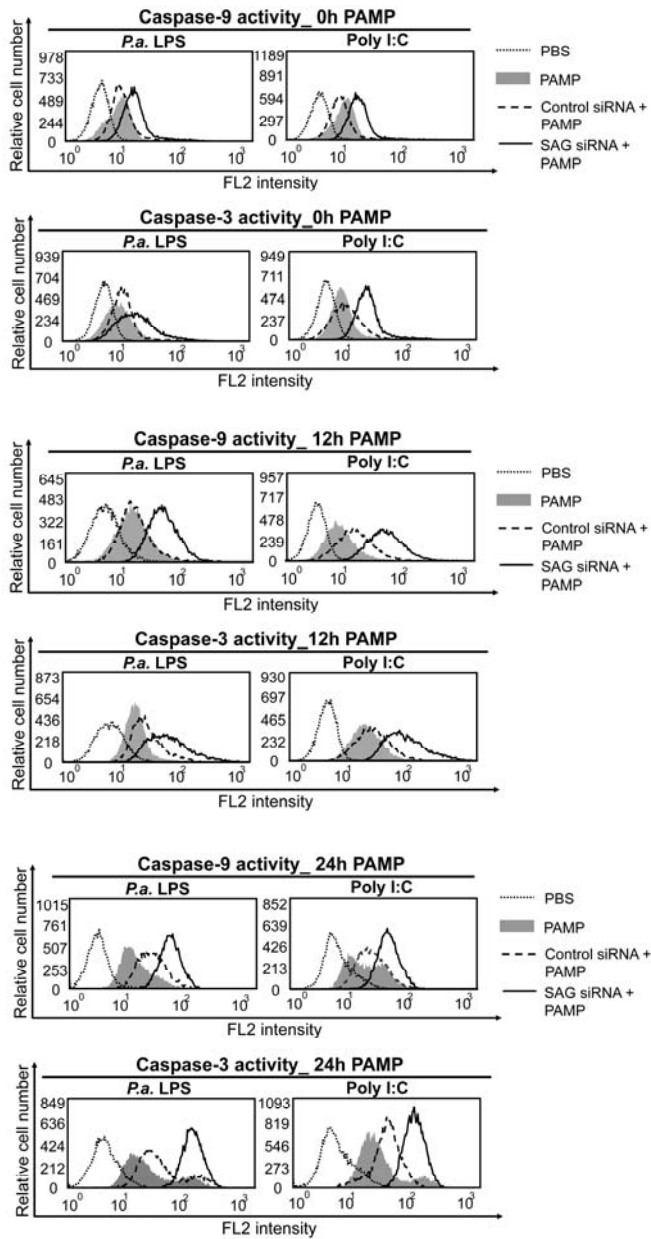
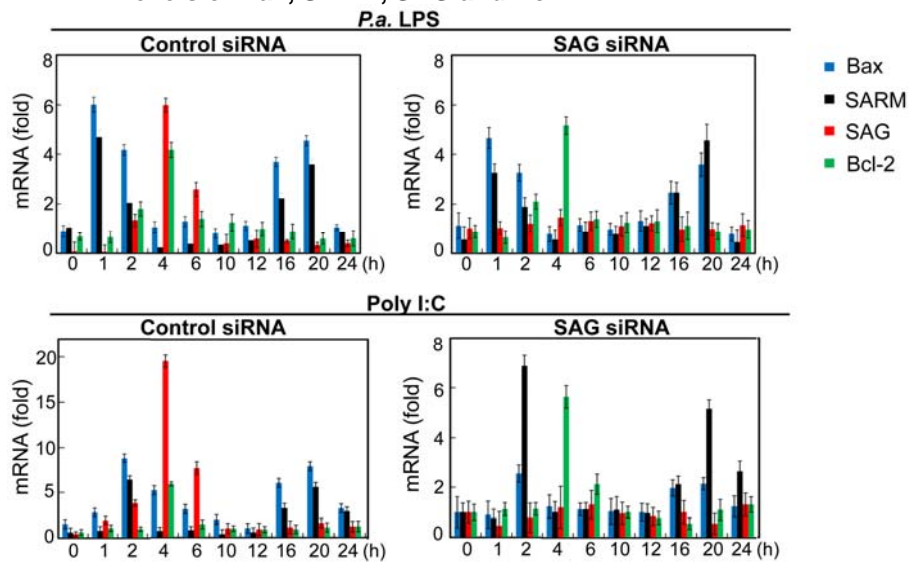
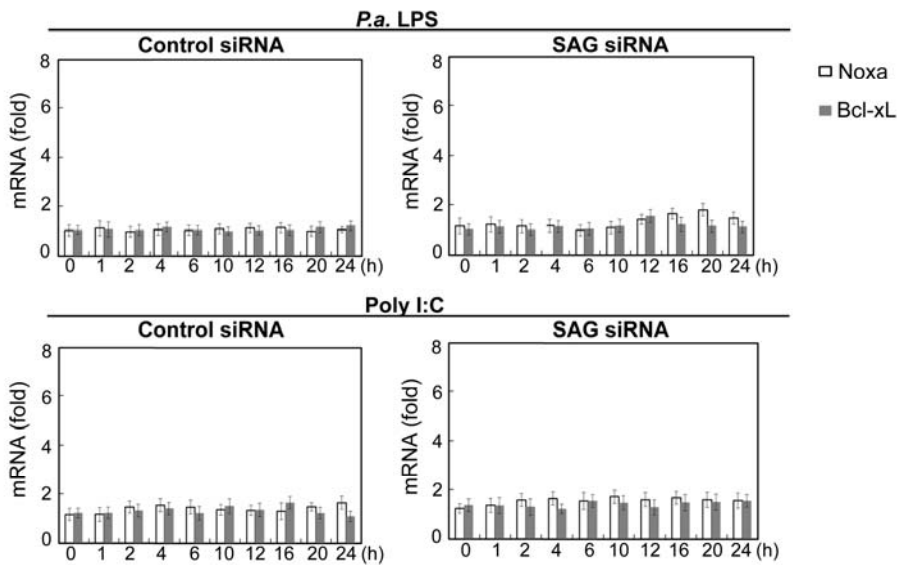


Figure S4. Expression profile of SAG, Bcl-2, Bax, SARM, Noxa and Bcl-xL in J774 cells after PAMP treatments (with and without SAG-knockdown).

A. mRNA levels of Bax, SARM, SAG and Bcl-2



B. mRNA levels of Noxa and Bcl-xL



C. Western blotting analysis of Bax, SARM, SAG, Bcl-2, Noxa and Bcl-xL

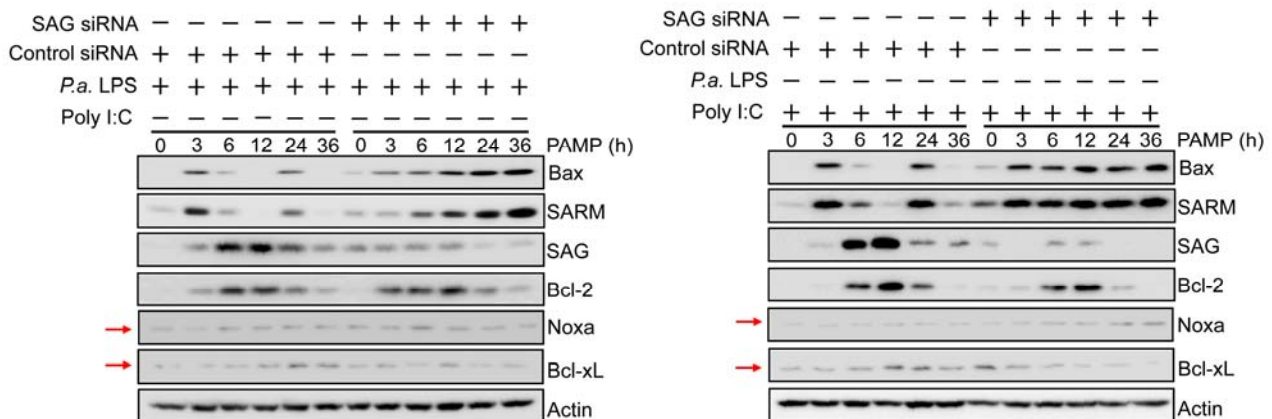
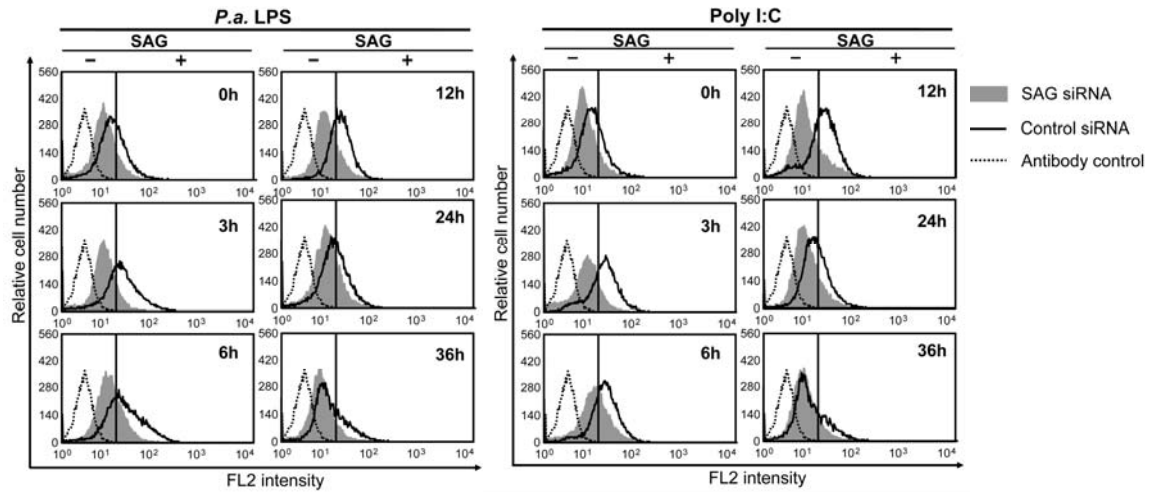
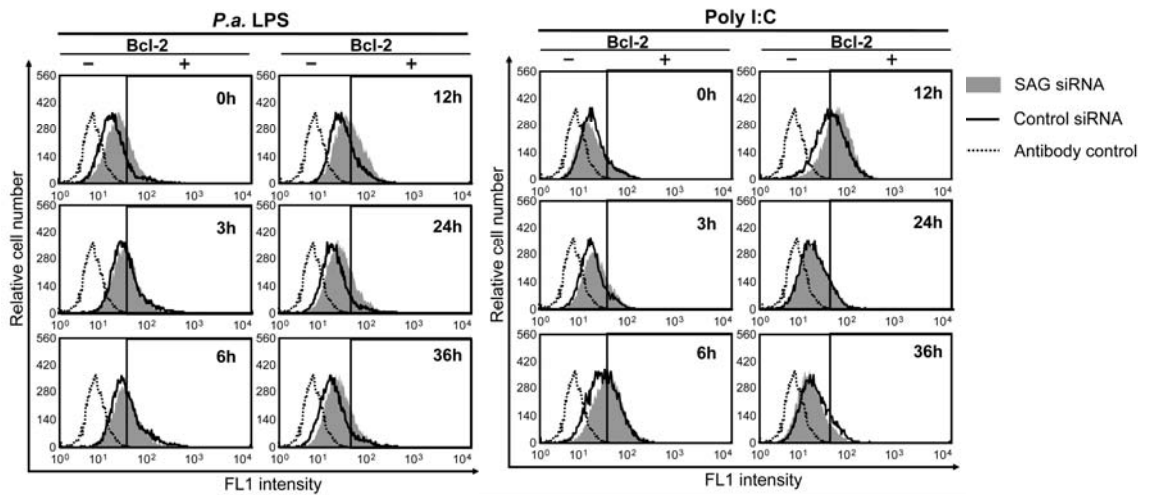


Figure S5. FACS histograms of SAG, Bcl-2, Bax and SARM in J774 cells after PAMP treatments (with and without SAG-knockdown).

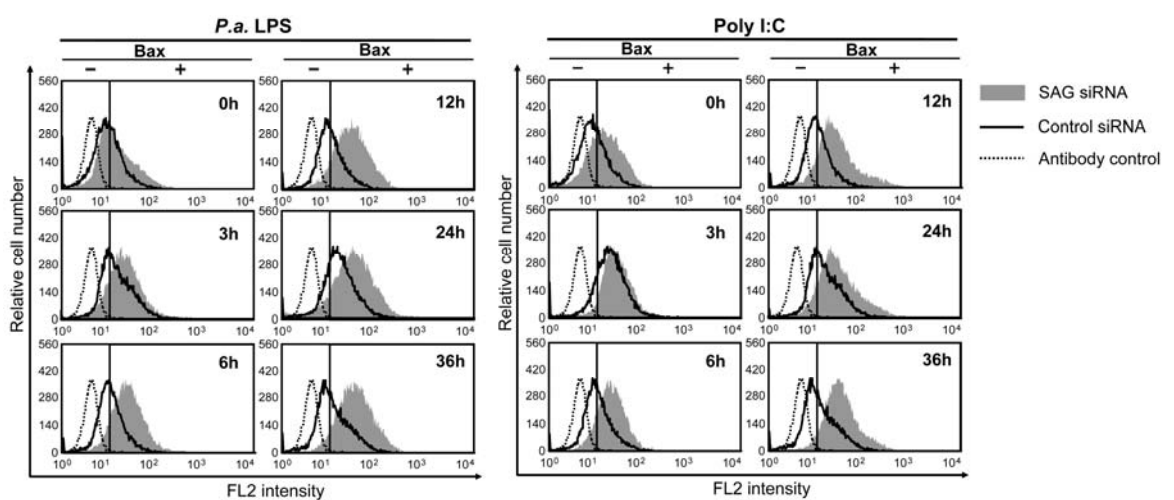
A. Representative histograms of SAG



B. Representative histograms of Bcl-2



C. Representative histograms of Bax



D. Representative histograms of SARM

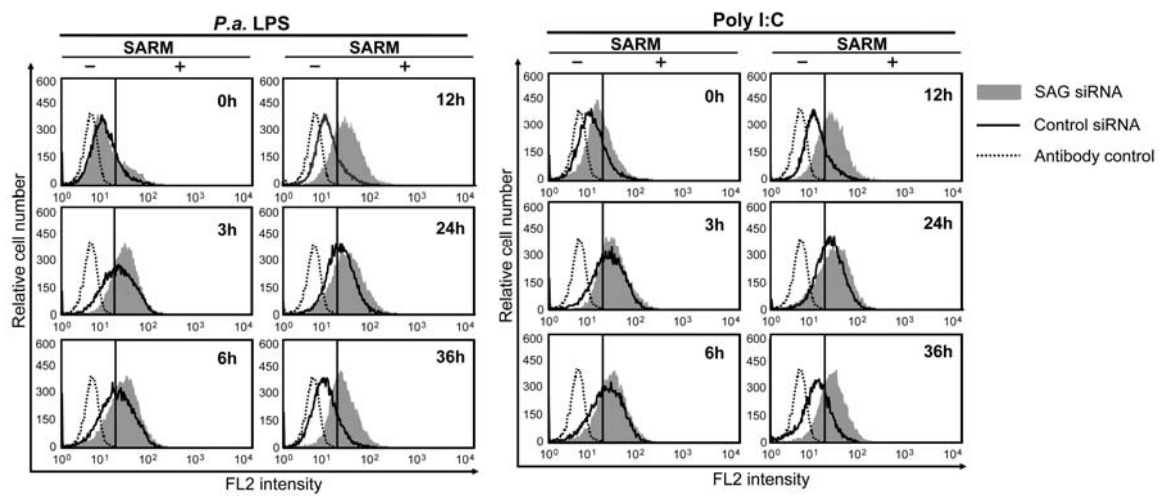
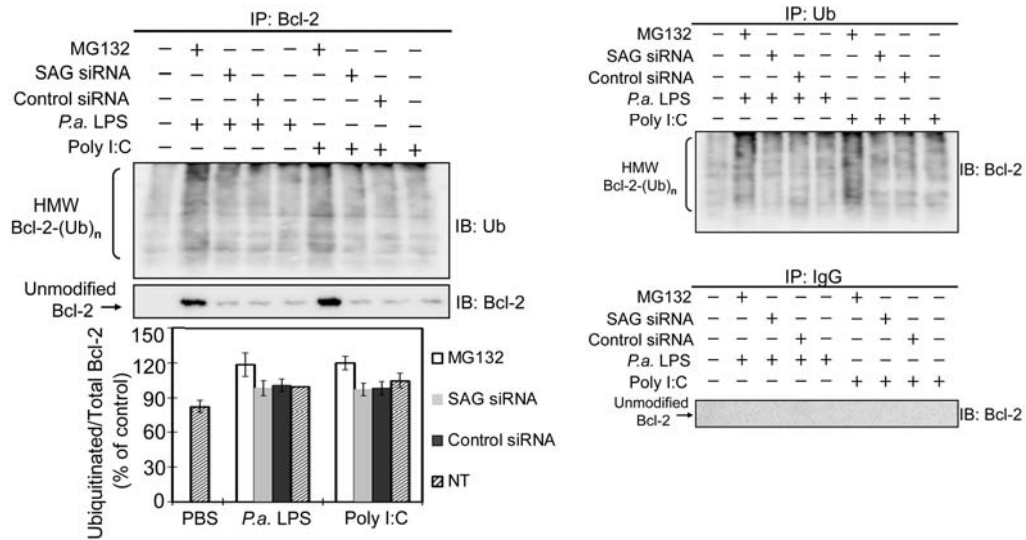
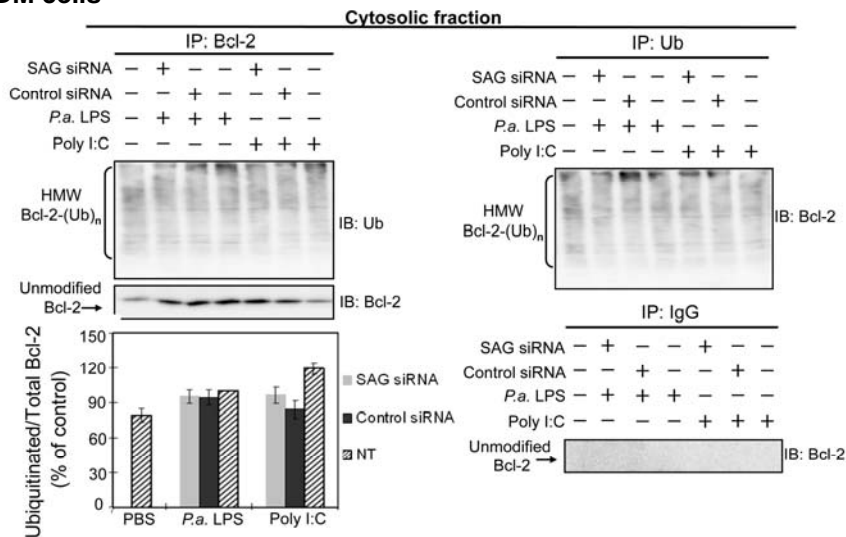


Figure S6. SAG-knockdown did not affect the level of ubiquitinated Bcl-2 in J774, RAW and BMDM cells.

A. J774 cells



B. BMDM cells



C. RAW cells

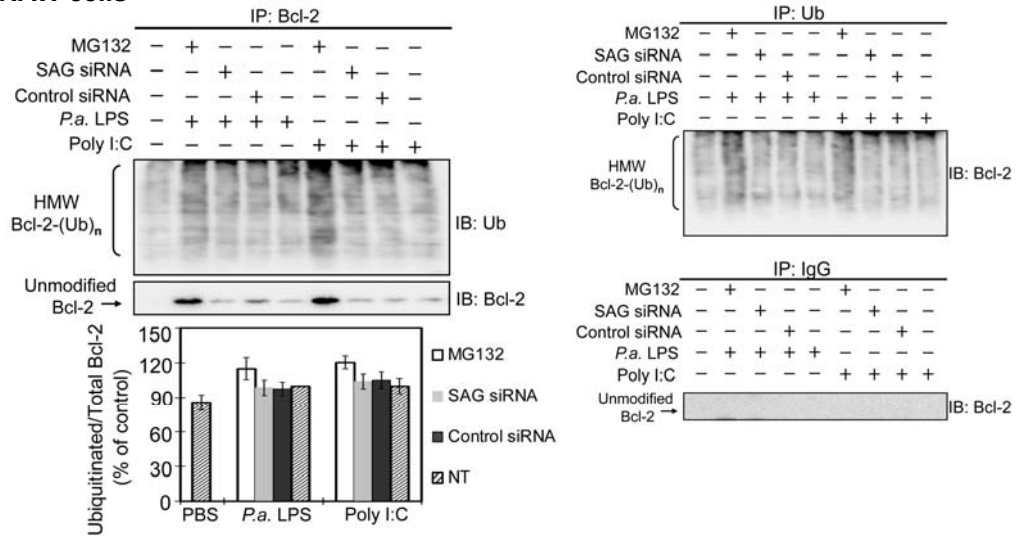
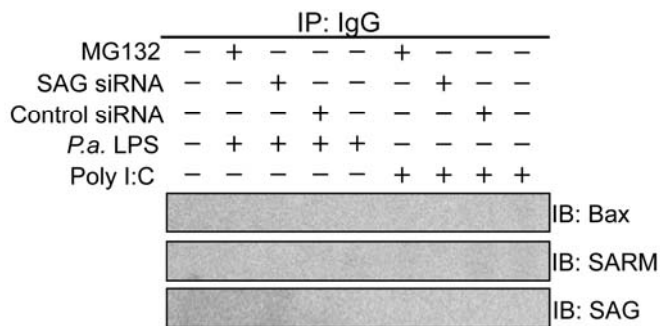


Figure S7. A control IgG tested in J774 and BMDM cells demonstrates the specificity of immunoprecipitation.

A. J774 cells



B. BMDM cells

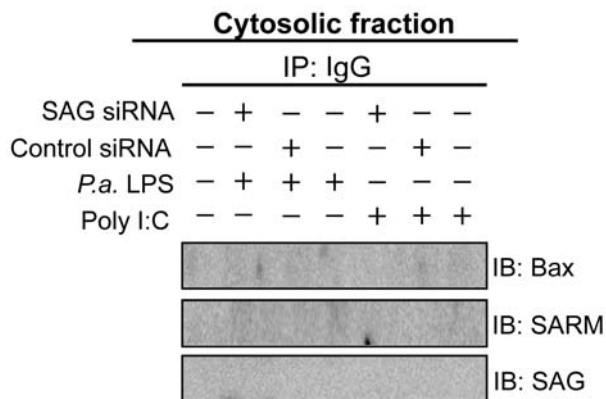
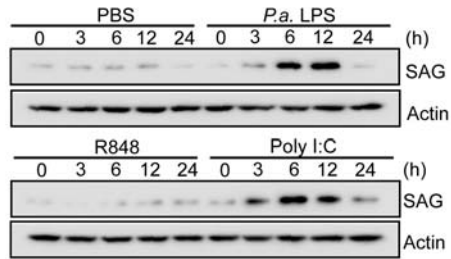
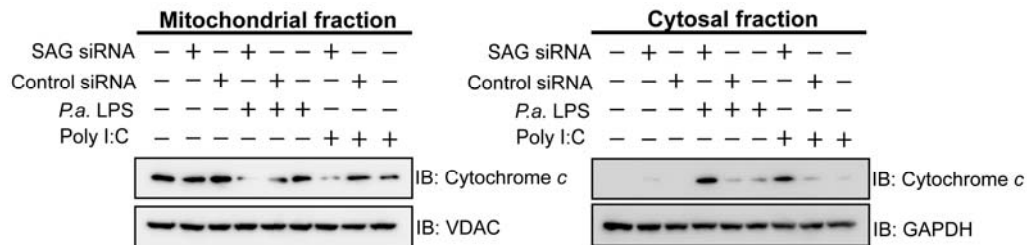


Figure S8. RAW264.7 cells challenged with PAMPs show induction of SAG which ubiquitinates Bax and SARM.

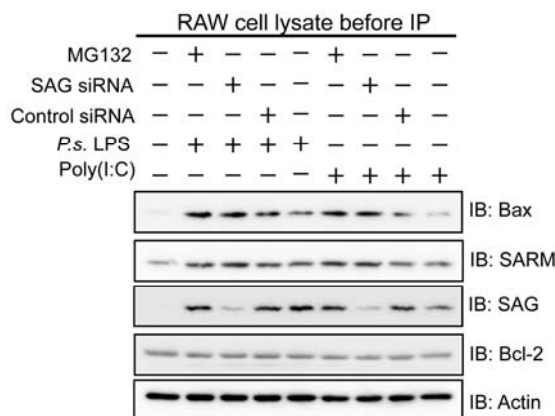
A. SAG induction upon PAMP stimulation in RAW264.7 cells



B. Cytochrome c release in RAW264.7 cells



C. Presence of apoptotic proteins in RAW264.7 cells (before IP)



D. Ubiquitin assay in RAW264.7 cells

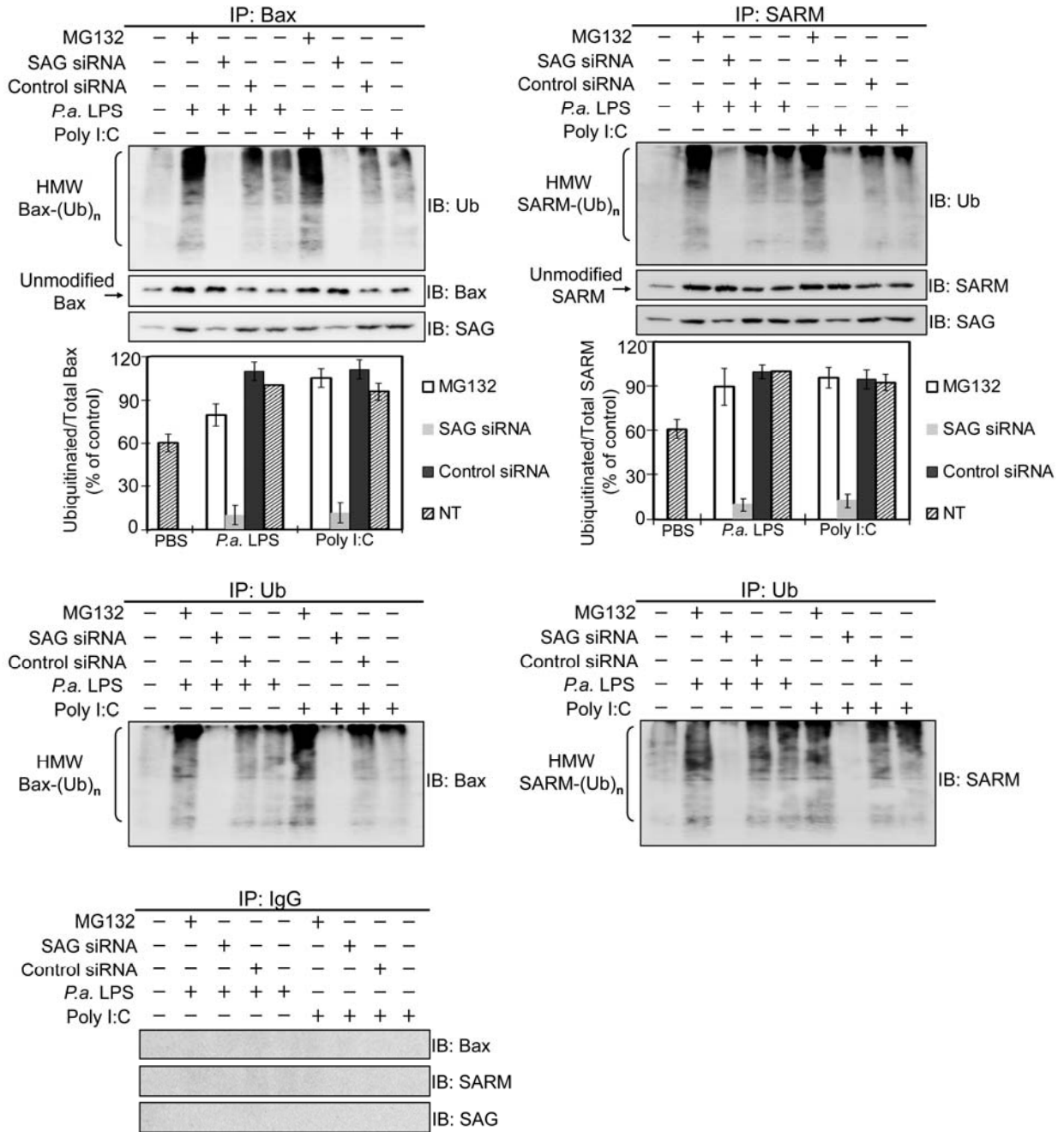


Figure S9. SAG is an evolutionarily conserved protein.

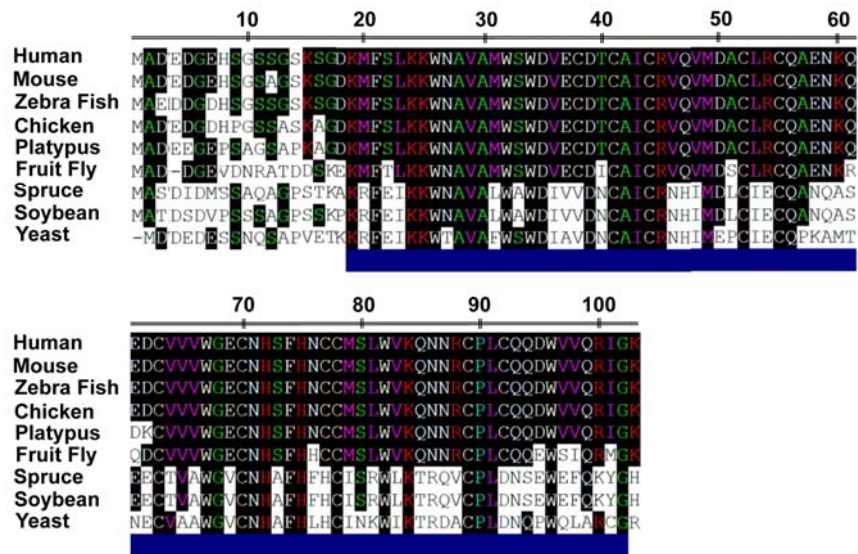


Figure S10. SAG overexpression in primary BMDM cells.

