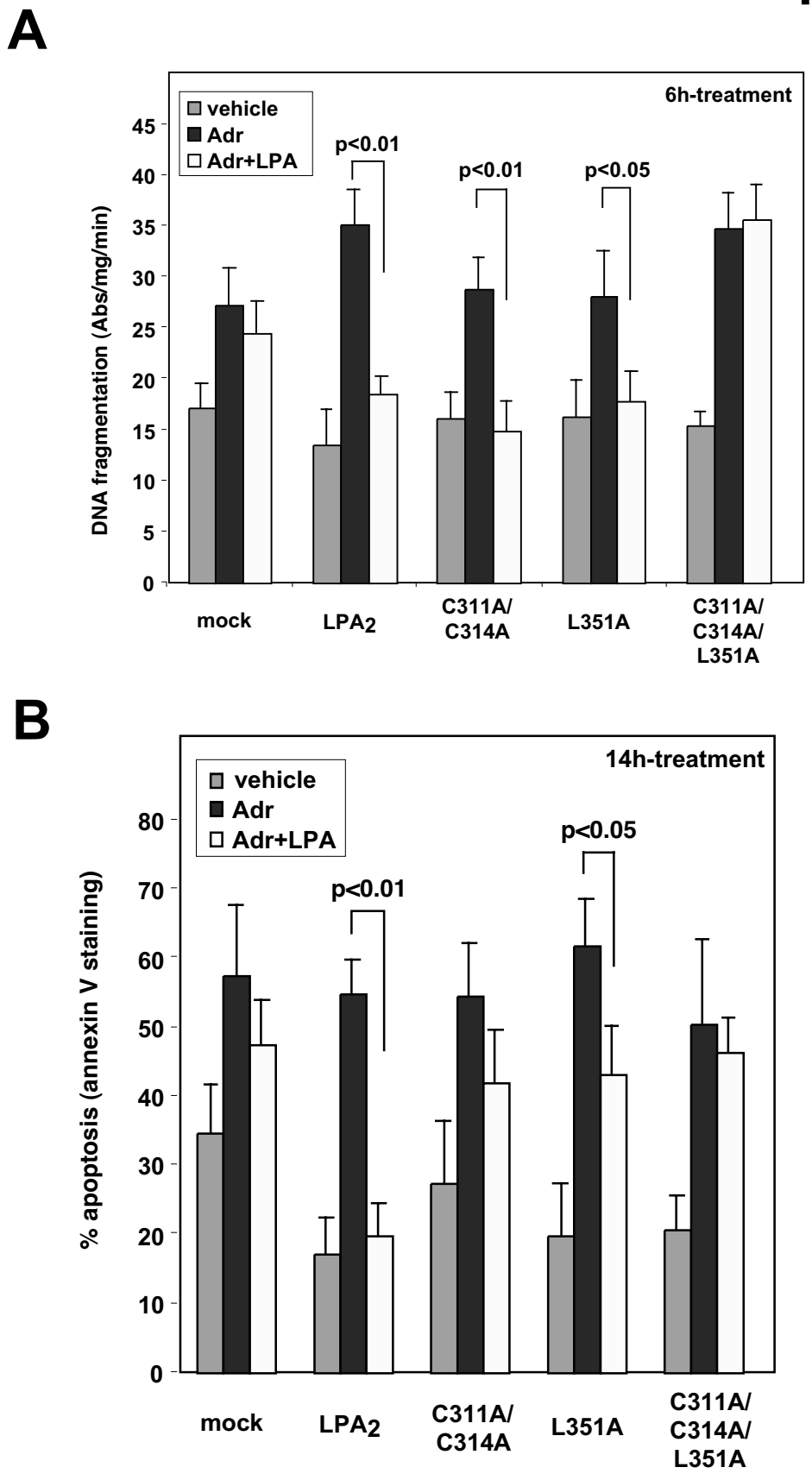
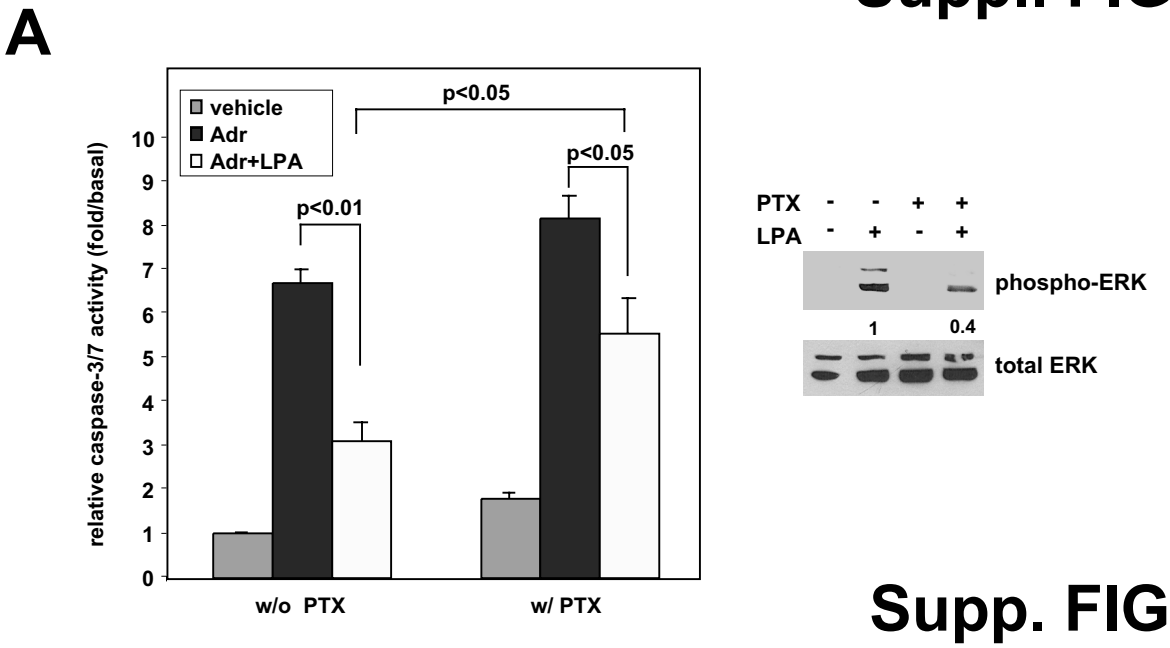
SUPPLEMENTAL MATERIAL

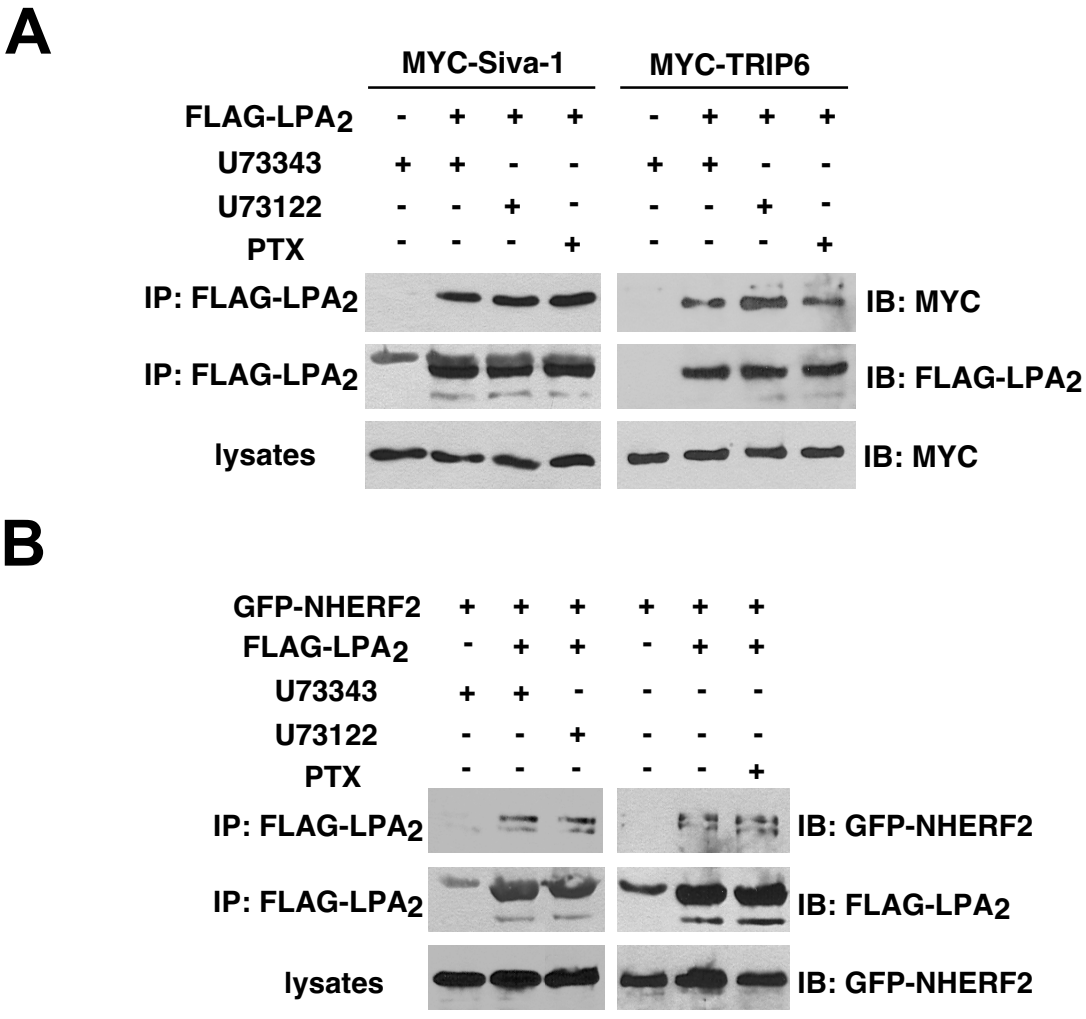
Suppl. FIG. S1



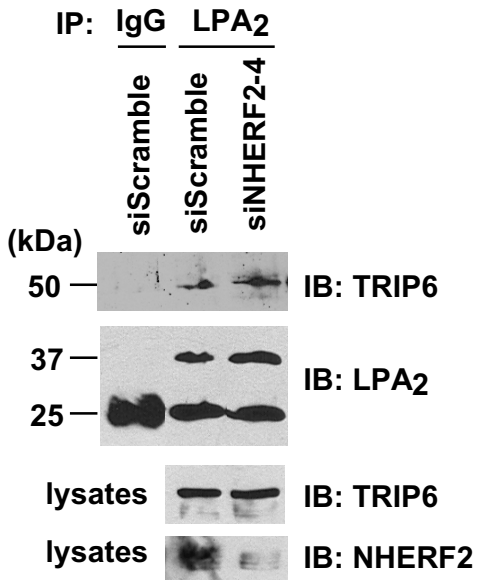
Suppl. FIG. S2



Supp. FIG. S3



Suppl. FIG. S4


IP:
IgG
LPA2
siScramble
siScramble
siNHERF2-4
(kDa)
50
37
25
IB: TRIP6
IB: LPA2
lysates
IB: TRIP6
lysates
IB: NHERF2

SUPPLEMENTAL FIGURE LEGENDS

Suppl. FIG. S1. Both CXXC- and PDZ-binding motifs are required for LPA_2-mediated protection from adriamycin-induced apoptosis.

A. Mutation of both CXXC- and PDZ-binding motifs eliminates the mitigating effect of LPA on adriamycin-induced apoptosis. Stable $LPA_{1/2}$ DKO MEFs were starved in 0.5% FBS-containing DMEM for 1 h followed by the addition of 3 μM adriamycin. Two μM LPA were added 1 h later. After 6 h treatment, DNA fragmentation assay was performed. Data shown are the mean ± SEM of four to five independent experiments.

B. LPA-mediated protection from adriamycin-induced apoptosis is inhibited by mutation of both CXXC and PDZ-binding motifs. Different $LPA_{1/2}$ DKO MEFs were pretreated with LPA in 0.1% fatty acid-free BSA-containing medium for 1 h, followed by addition of 1.5 μM adriamycin for 14 h. Apoptosis was determined by annexin V-FITC staining followed by flow cytometry analysis. Data show the mean ± SEM of three independent experiments.

Suppl. FIG. S2. Pertussis toxin (PTX) attenuates LPA_2-mediated ERK activation and protection from adriamycin-induced apoptosis. DKO-LPA_2 MEFs were pretreated with 100 ng/ml PTX in 10% serum-containing DMEM overnight. After incubation with 10 μM LPA with or without 100 ng/ml PTX in 0.1% fatty acid-free DMEM for 1 h, 2 μM adriamycin was added for another 7 h. Caspase-3/7 activity was determined. Data show the mean ± SEM of three independent experiments. One set of the MEFs were starved in the presence or absence or PTX for 7 h and then treated with 2 μM LPA for 10 min. Immunoblotting was performed to detect phospho-ERK and total ERK

in the whole cell lysates. The relative levels of phospho-ERK were quantified and normalized by the total levels of ERK in each sample.

Suppl. FIG. S3. The association of LPA_2 with Siva-1, TRIP6 or NHERF2 is not altered by treatment with pertussis toxin or U73122 phospholipase C inhibitor.

FLAG-LPA_2 was co-expressed with MYC-Siva-1 (A), MYC-TRIP6 (A) or GFP-NHERF2 (B) in HEK 293T cells. Cells were pretreated or not with 100 ng/ml pertussis toxin (PTX) overnight, and then starved in 0.1% fatty acid-free BSA-containing DMEM with or without PTX for another 4 h. After treatment with or without 5 μM U73122 phospholipase C inhibitor or the inactive U73343 analogue for 10 min, cells were stimulated with 2 μM LPA for another 10 min. Co-immunoprecipitation of FLAG-LPA_2 with MYC-Siva-1, MYC-TRIP6 or GFP-NHERF2 was performed. Data shown are representative of three separate experiments.

Suppl. FIG. S4. The association of LPA_2 with TRIP6 is not altered by knockdown of NHERF2 expression.

SKOV-3 cells transiently expressing a scrambled siRNA or a NHERF2 siRNA (siNHERF2-4) were starved overnight followed by the addition of 2 μM LPA for 10 min. The endogenous LPA_2 receptor was immunoprecipitated with an anti-LPA_2 rat antibody or a control rat IgG. The co-immunoprecipitated TRIP6 was detected with an anti-TRIP6 rabbit polyclonal antibody. The bottom two panels show the expression of TRIP6 and NHERF2 in the whole cell lysates. The result shown is representative of two independent experiments.