Supporting Information

Sun and Lin 10.1073/pnas.0802701105

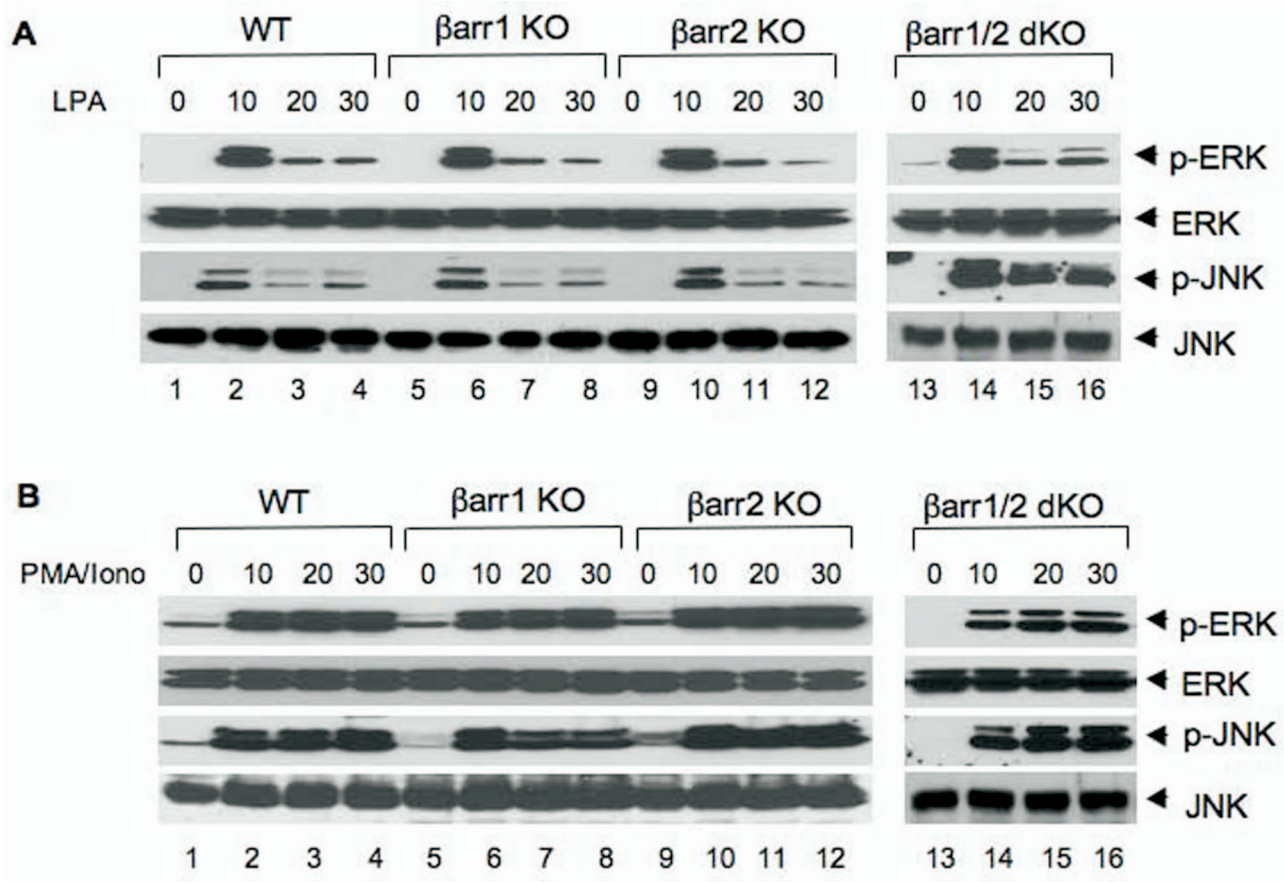

Fig. S1. MAP kinase activation induced by lysophosphatidic acid (LPA) and phorbol 12-myristate 13-acetate plus Ionomycin (PMA/Iono) is independent of β-arrestins. Wild-type, β-arrestin 1 knockout (βarr1 KO), β-arrestin 1 knockout (βarr2 KO), and β-arrestin 1/β-arrestin 2 double knockout (βarr1/2 dKO) murine embryonic fibroblast (MEF) cells were stimulated with LPA (10 μM) (*A*) and PMA (40 ng/ml) plus Ionomycin (100 ng/ml) (*B*) for the indicated time course. Phosphorylation of ERK and JNK, as well as their loading controls, was examined by Western blotting using the indicated antibodies.

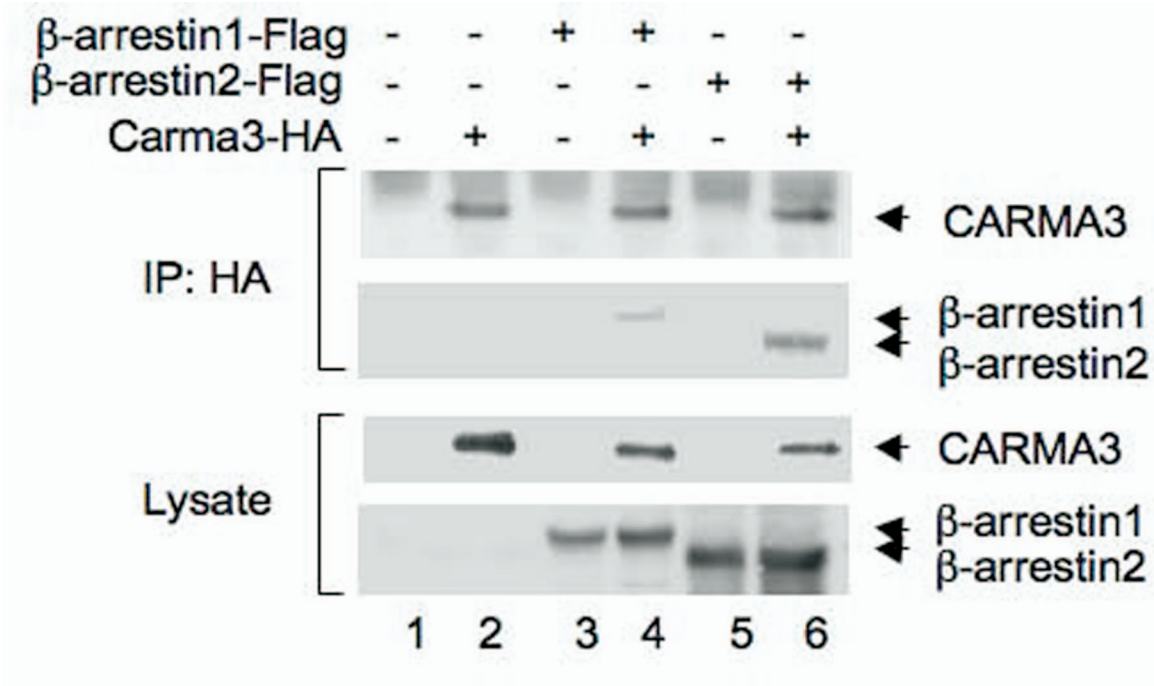

Fig. S2. CARMA3 can form a complex with both β-arrestin 1 and β-arrestin 2. HEK293T cells were transfected with expression vectors encoding HA-tagged CARMA3, FLAG-tagged β-arrestin 1, or FLAG-tagged β-arrestin 2 in different combinations. Twenty hours after transfection, cell lysates were subjected to immunoprecipitation (IP) with anti-HA antibody-conjugated beads, and immunoprecipitated proteins and cell lysates were subjected to SDS/PAGE and analyzed by immunoblotting using the indicated antibodies.

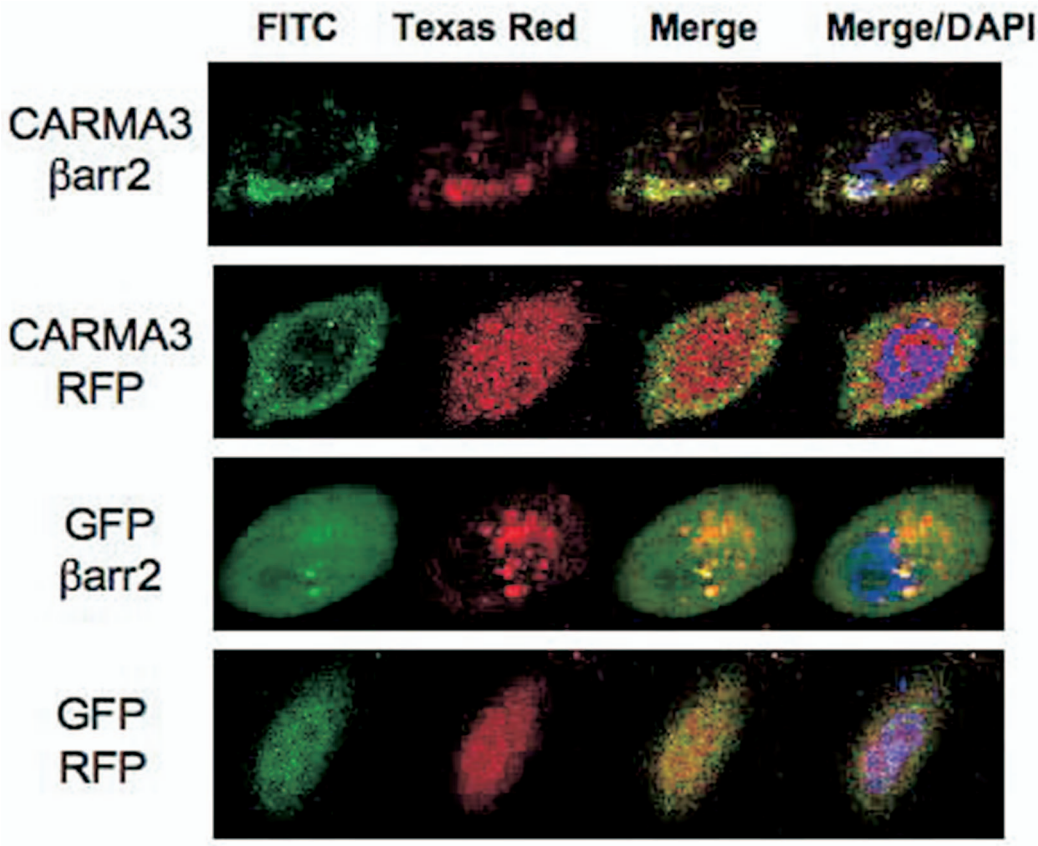

Fig. S3. Colocalization of CARMA3 and β-arrestin 2. HeLa cells were transfected with GFP-CARMA3, ref fluorescent protein (RFP)-β-arrestin 2, GFP, or RFP in different combinations together with HA-tagged LPA receptor (LPA_1). Twenty-four hours later, the cells were fixed and analyzed on the laser confocal fluorescence microscope.

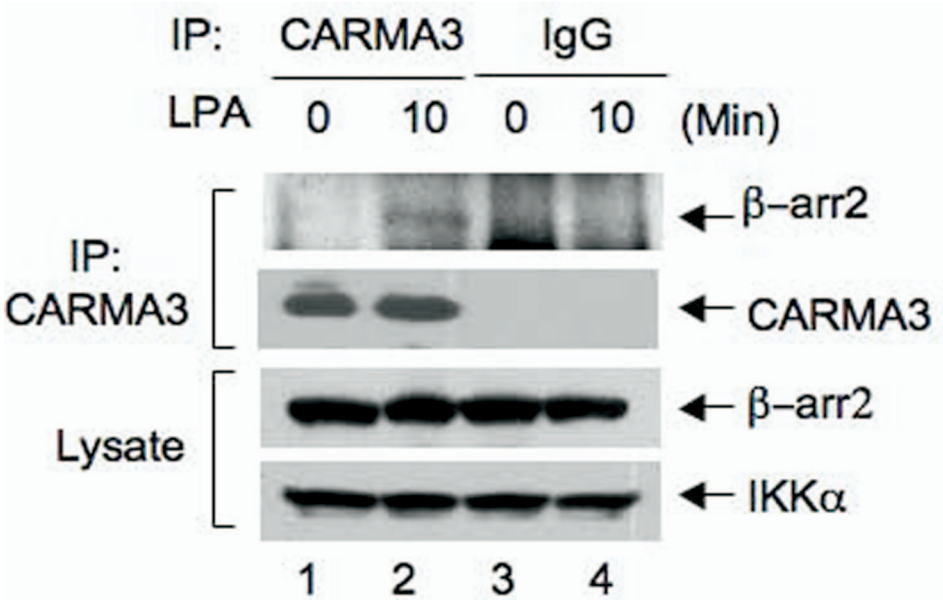

Fig. S4. CARMA3 associates with β-arrestin 2. Wild-type MEF cells (15-cm plate, 80% confluent) were stimulated with or without LPA for 10 min and then were scraped off and lysed with lysis buffer (containing 1% Nonidet P-40 and 250 mM NaCl). The resulting lysates were subjected to immunoprecipitation (IP) with CARMA3 antibody-conjugated beads or IgG control beads. The obtained immunocomplexes were washed three times and subjected to SDS/PAGE and Western blotting using anti-β-arrestin 2 or CARMA3.

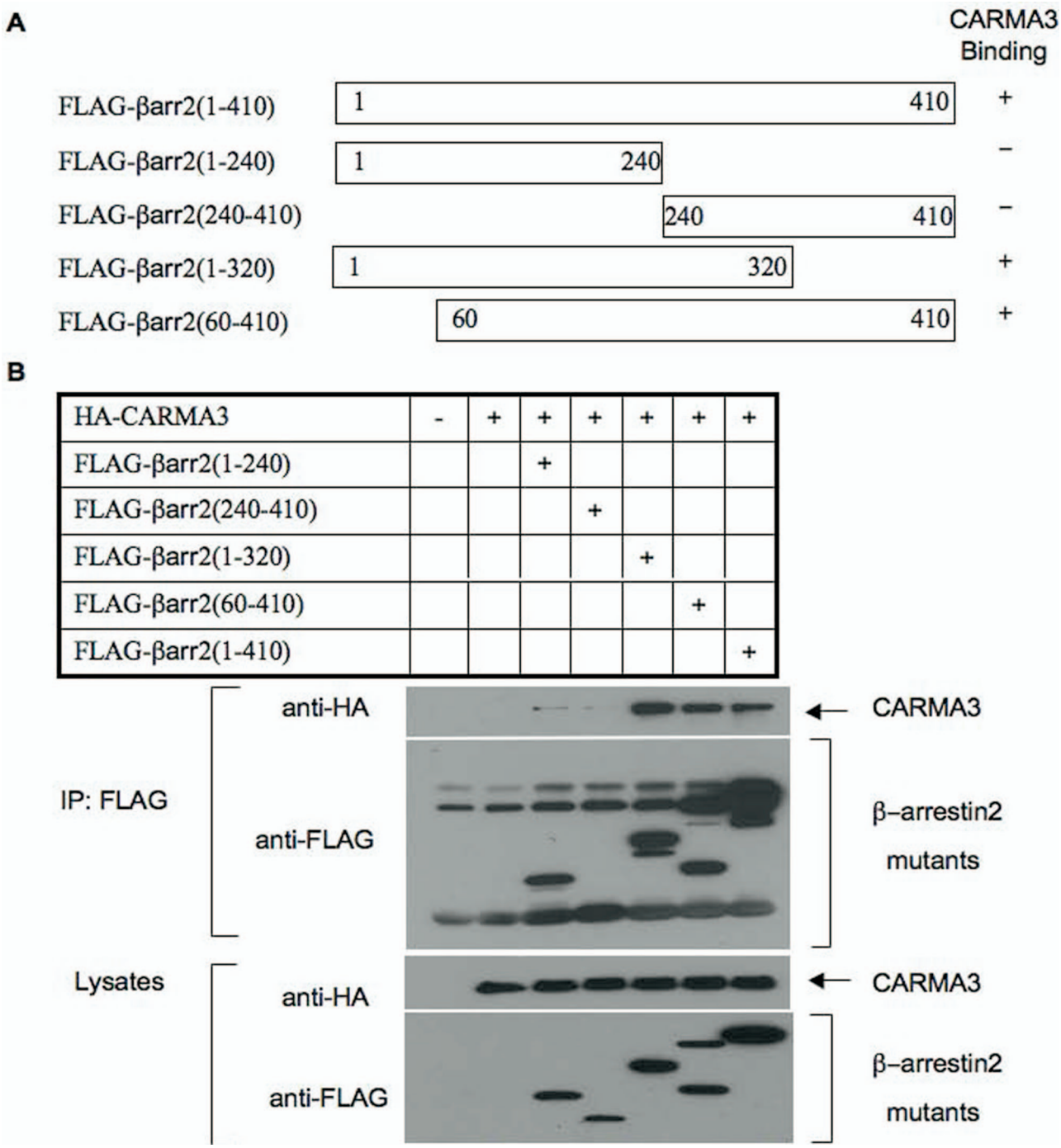

Fig. S5. The domain of β-arrestin 2 interacted with CARMA3. (*A*) Diagram of full-length and deletion mutants of β-arrestin 2. (*B*) FLAG-tagged full-length or deletion mutants of β-arrestin 2 together with HA-tagged CARMA3 was transfected into HEK293T cells. Twenty hours after transfection, cell lysates were subjected to immunoprecipitation (IP) with anti-FLAG antibody-conjugated beads, and immunoprecipitated proteins and cell lysates were subjected to SDS/PAGE and analyzed by immunoblotting using indicated antibodies.