Supplemental Data

Positive Regulation of Apoptosis Signal-regulating Kinase 1 Signaling by ZPR9, a Zinc Finger Protein

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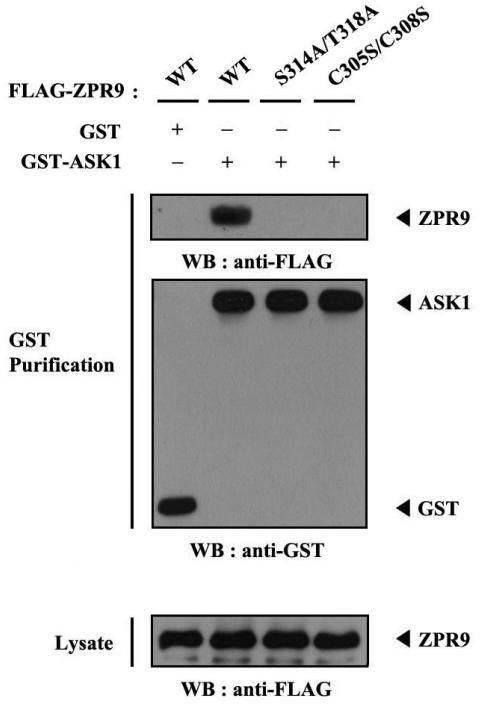

FIG. S1. **Effect of ASK1-mediated phosphorylation of ZPR9 at Ser^{314} and Thr^{318} on the association between ASK1 and ZPR9.** HEK293 cells were transfected with the indicated combinations of expression vectors. GST fusion proteins were purified on glutathione-Sepharose beads (*GST Purification*) and the degree of complex formation was determined by anti-FLAG antibody immunoblot (*top*).

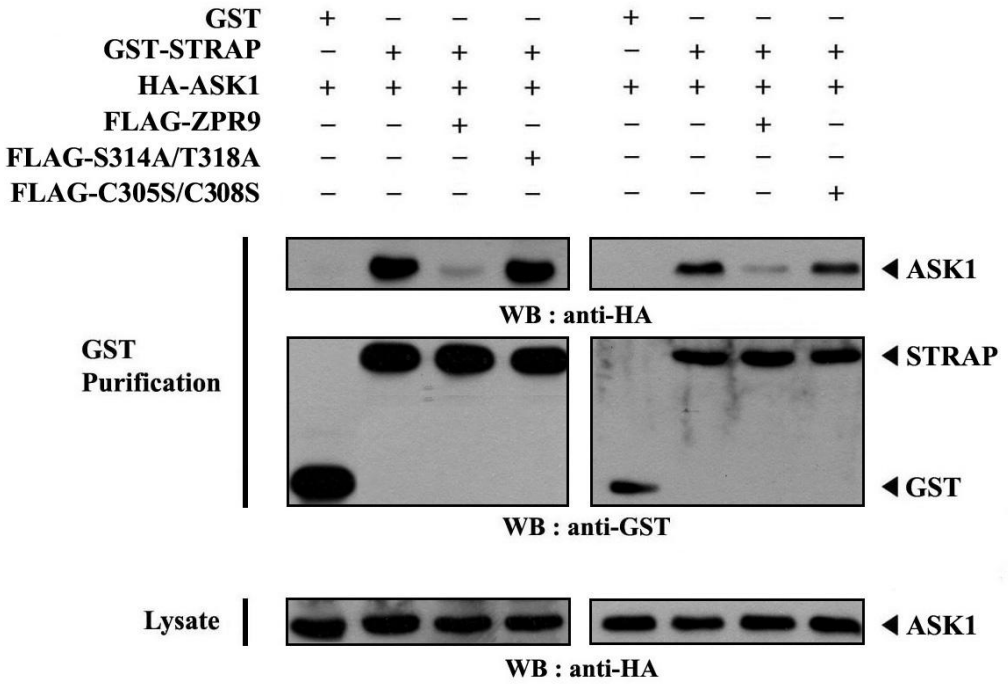

FIG. S2. **Effect of ZPR9 on the physical association between ASK1 and its negative regulator STRAP.** HEK293 cells were transiently transfected with the indicated combinations of expression vectors. GST fusion proteins were purified on glutathione-Sepharose beads (*GST Purification*) and the complex formation between ASK1 and STRAP was determined by anti-HA antibody immunoblot (*top*). The same bolt was stripped and reprobed with an anti-GST antibody to confirm the expression levels of the GST fusion proteins (*middle*). The expression levels of ASK1 in the total cell lysate (*Lysate*) were determined by anti-HA antibody immunoblot (*bottom*).

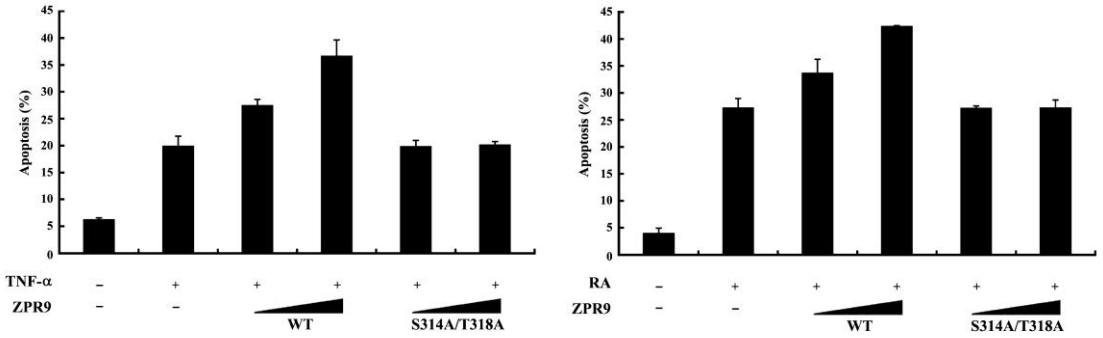

FIG. S3. **Mutation to S314A/T318A abrogates ZPR9-dependent apoptosis.** 293T cells were transfected with increasing amounts of wild-type and mutant (S314A/T318A) ZPR9 (3 and 6 μg), along with an expression vector encoding GFP (2 μg), and incubated for 14 h with or without TNF-α (20 ng/ml) and cycloheximide (10 μg/ml). SK-N-BE(2)C cells transfected with the indicated expression vectors, as described above, were treated with or without retinoic acid (RA: 5 μM, 2–3 days). GFP-positive cells were analyzed for the presence of apoptotic nuclei using a fluorescence microscope.