SUPPLEMENTAL INFORMATION

EXPERIMENTAL PROCEDURES

Cell culture-CHO/$S1P_2$ and $S1P_4$ cells were cultured in α-minimal essential medium (MEM) containing 10% (v/v) FCS (1). Stable CHO/$S1P_5$ cell line was generated after transfection with flag-tagged human $S1P_5$ plasmid, which is kindly gifted from Dr. Kihara (Hokkaido University), and selection with 3 μg/ml puromycin. The expression of human $S1P_5$ was detected by Western blot analysis (data not shown).

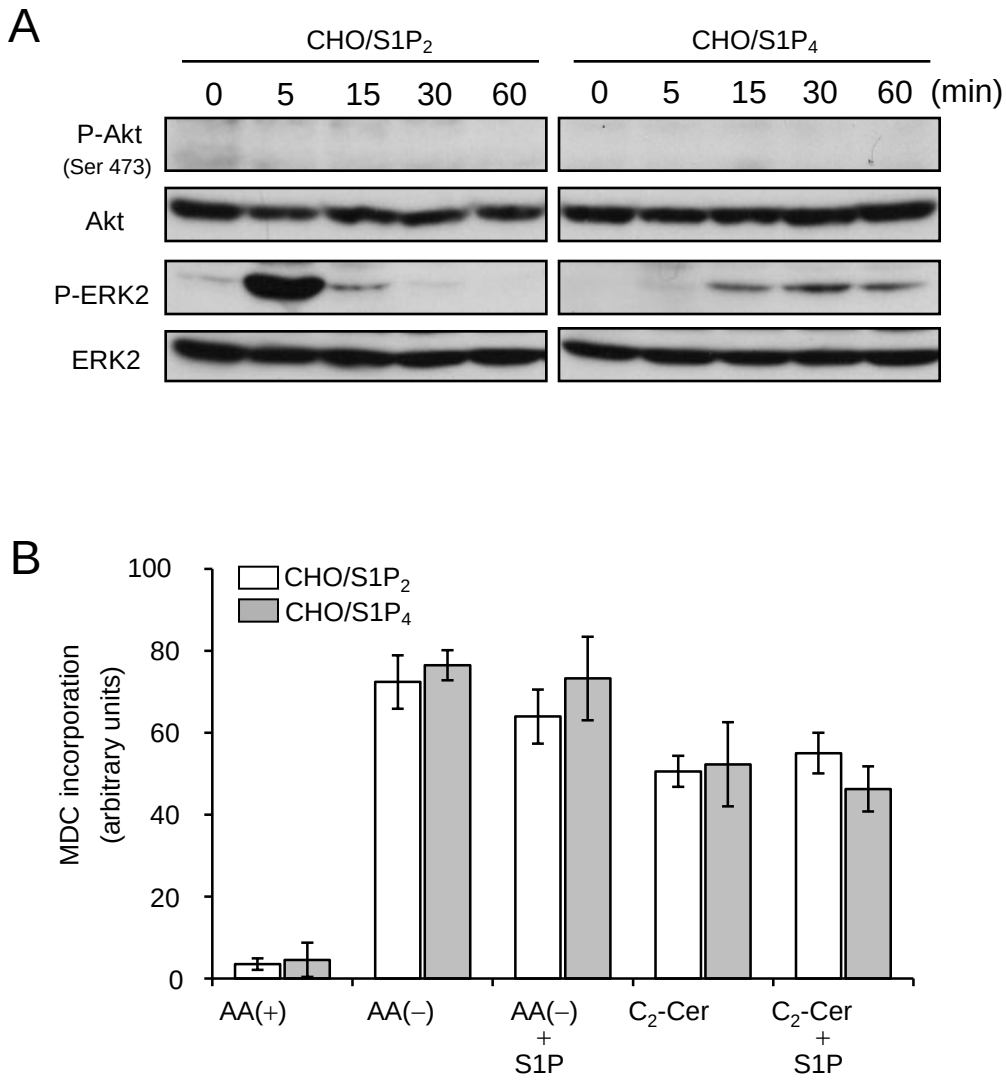
REFFERENSES

1. Kohno, T., and Igarashi, Y. (2003) *J Biochem* **134**, 667-673
2. Bligh, E. G., and Dyer, W. J. (1959) *Can J Biochem Physiol* **37**, 911-917

FIGURE LEGENDS

Supplemental Fig. S1. Effect of $S1P_2$ and $S1P_4$ on counteracting autophagy. (A) CHO/$S1P_2$ and CHO/$S1P_4$ cell lines were cultured in AA(-) with 20 μM S1P for indicated time. Activation of ERK2 was detected by Western blot analysis. (B) Cells were cultured in AA(-) or serum free medium (AA(+)) containing 50 μM C_2-ceramide (C_2-Cer) with or without 10 μM S1P for 30 min as described in Fig. 6E. MDC incorporation was examined by fluorescence microscopy, and the density of MDC was calculated. Images were obtained by fluorescent microscopy, and cells having at least five dots were counted as autophagic cells. Values are the mean ± SD from three independent experiments.

Supplemental Fig. S2. Effect of $S1P_5$ on inhibition of autophagy. (A) CHO and CHO/$S1P_5$ cells were cultured in AA(-) with 20 μM S1P for indicated time. Akt and ERK2 activations were detected by immunoblotting. (B) Cells were cultured in AA(-) with or without S1P (2 or 20 μM) for 30 min. Indicated proteins were detected by Western blot analysis. The data was representative of three independent experiments.


A
CHO/S1P2
CHO/S1P4
0 5 15 30 60 0 5 15 30 60 (min)
P-Akt
(Ser 473)
Akt
P-ERK2
ERK2
B
CHO/S1P2
CHO/S1P4
MDC incorporation
(arbitrary units)
100
80
60
40
20
0
AA(+)
AA(–)
AA(–) + S1P
C2-Cer
C2-Cer + S1P

Supplemental Figure S2