Supplemental Fig. 1. ATP-dependent transport of [^{33}P]-labeled S1P into rat IOVs. Experiments were performed as described in Experimental Procedures and Fig. 2, except that [^{33}P]S1P transported into the IOVs was extracted and analyzed by using TLC. (A) [^{33}P]S1P bands image separated by TLC were visualized with a FLA3000G Bioimaging Analyzer. (B) Time-dependent [^{33}P]S1P transport into rat IOVs was quantified with FLA3000G. Closed circles and triangles indicate S1P uptake in the presence and absence of 2 mM ATP, respectively.

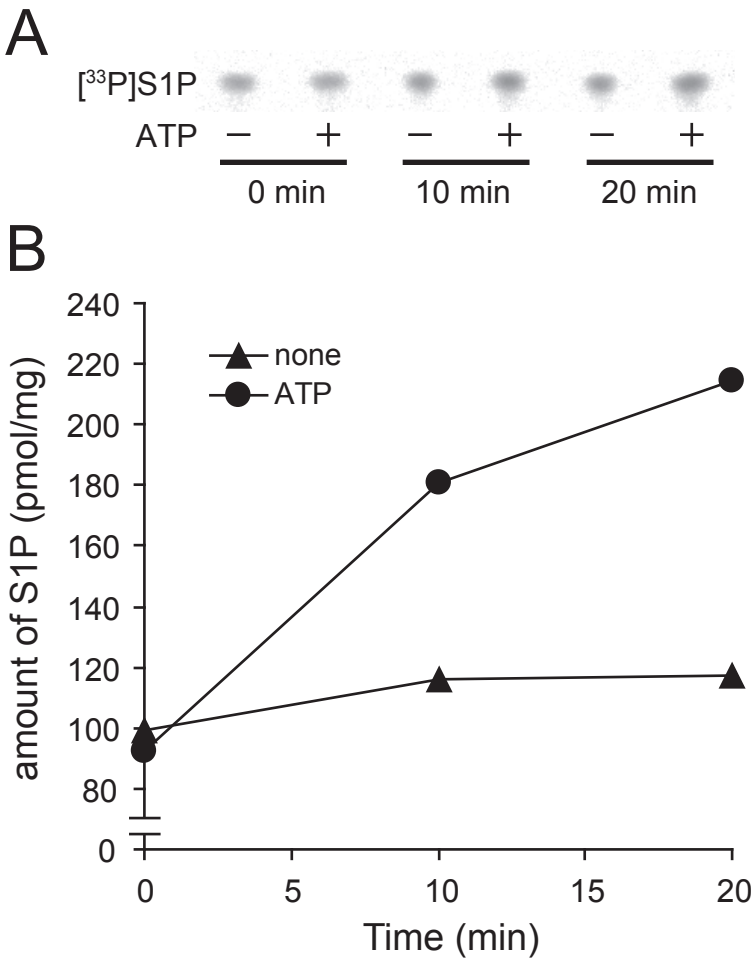
Supplemental Fig. 2. Reverse flow of the S1P from the IOVs. Experiments were performed as described in Experimental Procedures. Amount of the S1P inside the vesicles were expressed as the difference between the amounts of S1P in the presence and absence of ATP in the first incubation. Buffers were changed to S1P-free incubation buffer (none), incubation buffer with 2mM ATP (ATP) or incubation buffer with 2mM ATP and 1mM vanadate (ATP+Vana). Then, second incubation was performed as indicated. Experiments were performed more than three times, and the error bars indicate the standard deviation.

Supplemental Fig. 3. BSA enhances the ATP-dependent S1P transport into IOVs. A: The transport assay was performed as described in Experimental Procedures. The amounts of the transported S1P in the 0.1% BSA-preloaded IOVs in the presence and absence of ATP are indicated as open circles and open triangles, respectively. Closed circles and closed triangles indicate the transported S1P in IOVs without preloaded BSA in the presence and absence of ATP, respectively. B: BSA-dependent cGMP uptake was also tested. Experiments were performed as described above except that 1 μM [^{3}H]cGMP was used instead of [^{33}P]S1P.

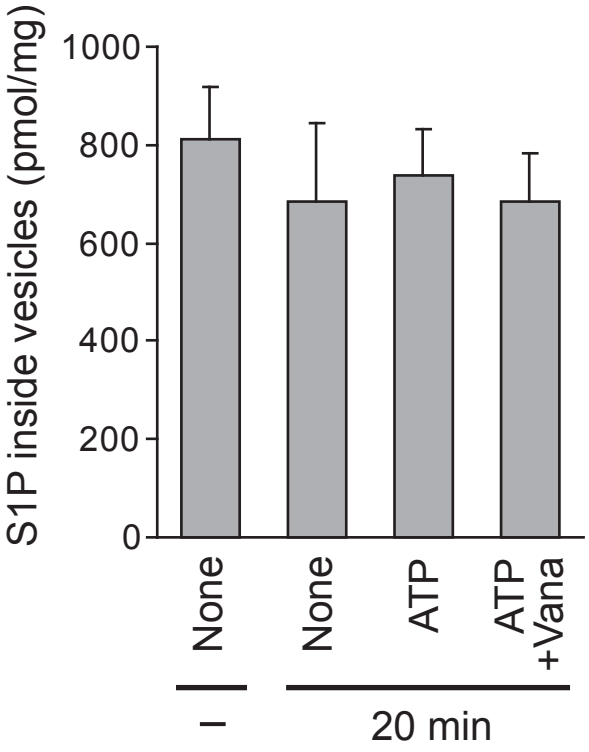
Supplemental Fig. 4. Dihydrosphingosine uptake and dihydrosphingosine-1-phosphate release in rat erythrocytes. Experiments were performed as described in Fig. 1 except that [^{3}H]Sphingosine was replaced with [^{3}H]Dihydrosphingosine (DHSph). Closed and open circles indicate [^{3}H]DHSph and [^{3}H]Dihydrosphingosine 1-phosphatae (DHS1P), respectively. The total amounts of DHSph and DHS1P at 0.2 min were set at 100%.

Supplemental Fig. 5. Effects of glyburide and vanadate on the ATP-dependent transport of S1P into IOVs. Experiments were performed as described for Fig. 7 except that the concentration of glyburide was 200 μM. Experiments were performed more than three times, and the error bars indicate the standard deviation.

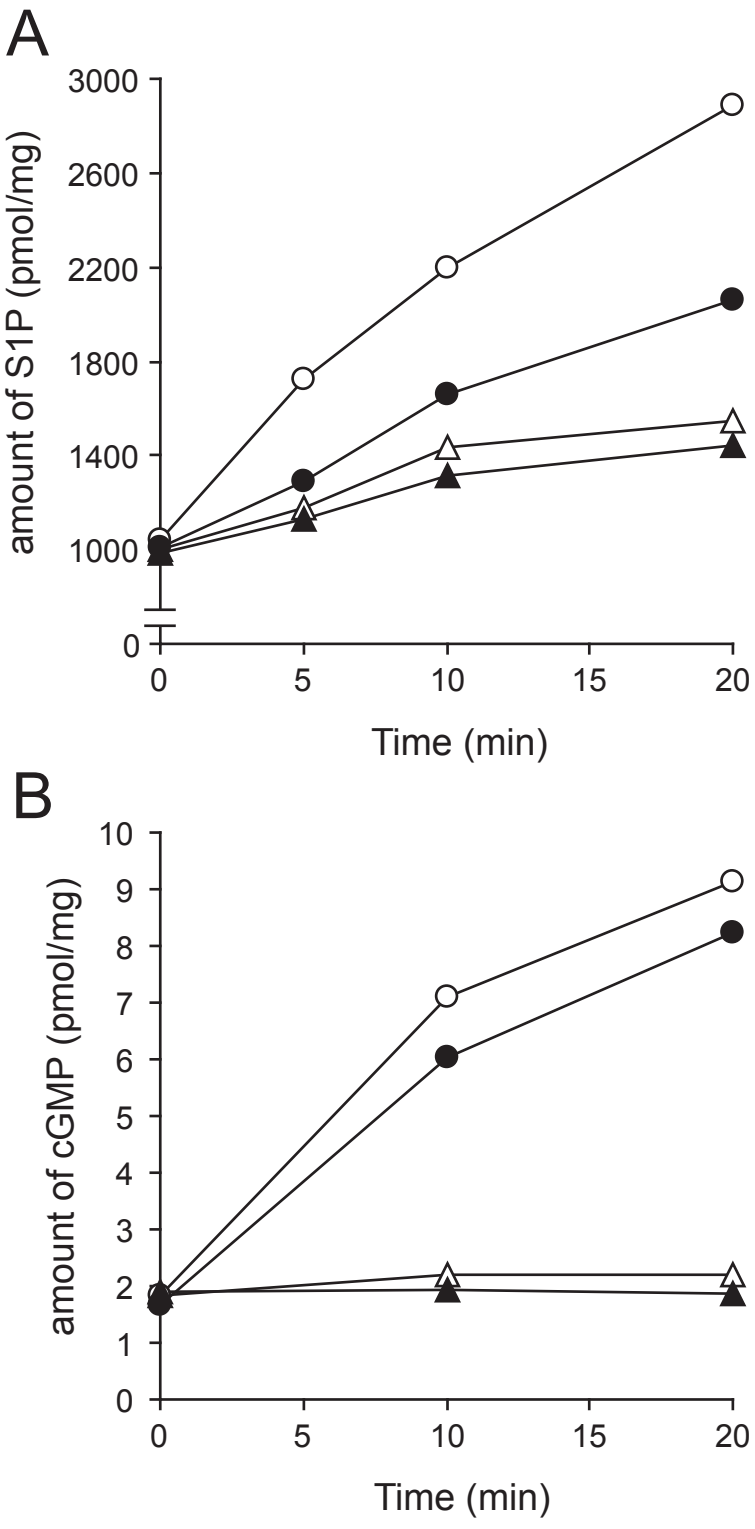
Supplemental Figure 1



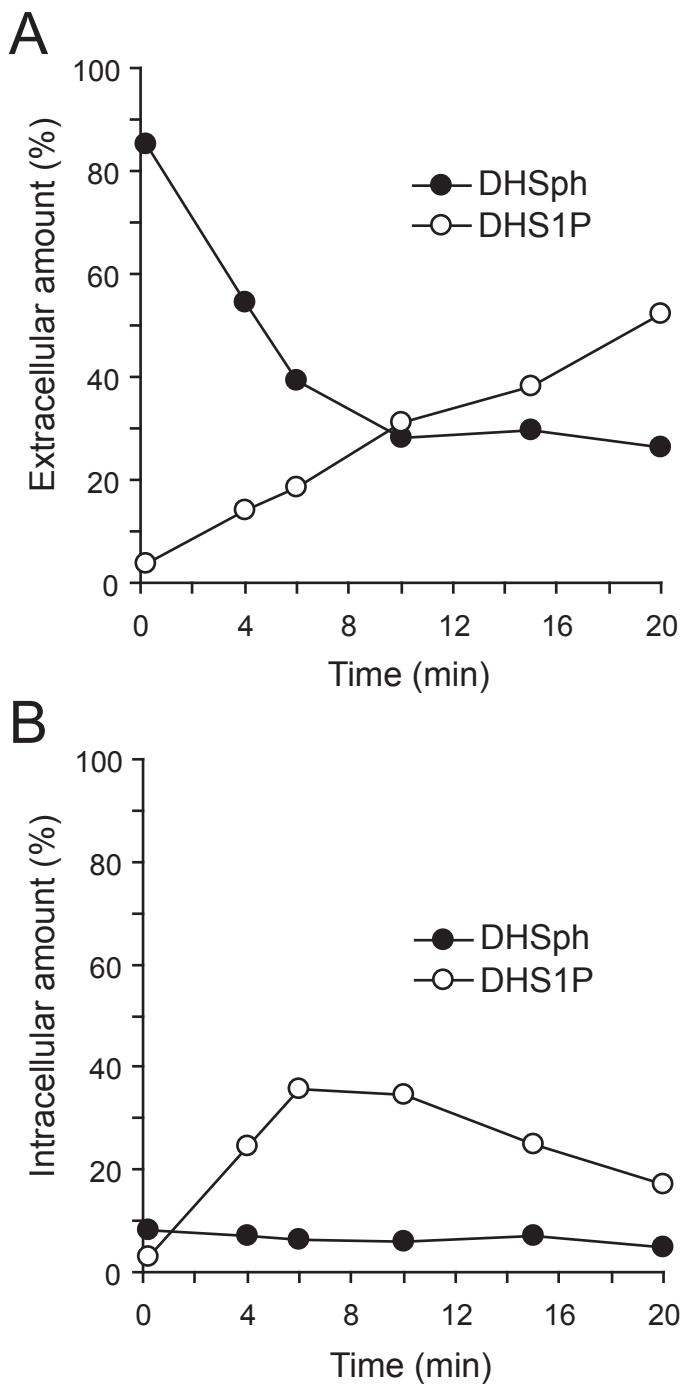
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5