

Supplemental figures

Supplemental figure 1. FTY720 treatment does not affect the association between

S1P₁ and WWP2. A, effect of FTY720P treatment. HEK293 cells expressing S1P₁-GFP or S1P₁-S5A-GFP or S1P₁-K4R-GFP were treated with FTY720P or not for 30 min. Cell extracts were immunoprecipitated (IP) with anti-S1P₁ antibody followed by an immunoblot (IB) analysis with anti-WWP2 antibody.

Supplemental figure 2. Effect of S1P and FTY720P treatment on S1P₁ degradation in

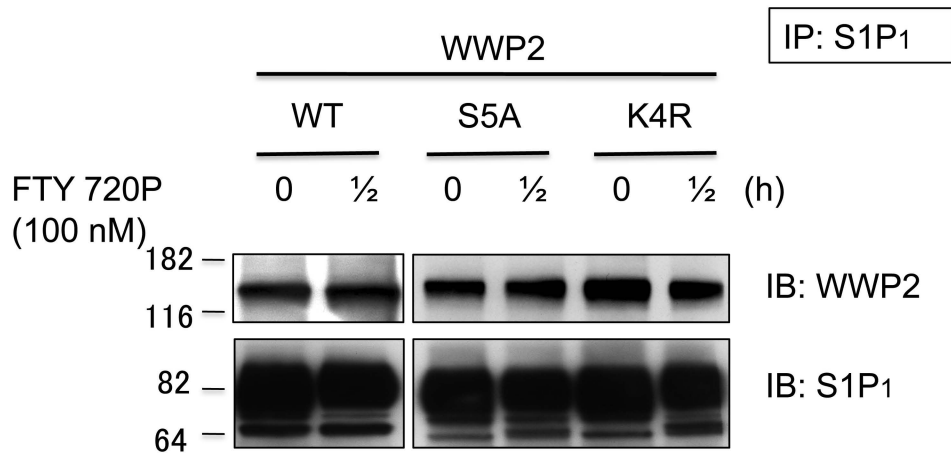
WWP2-expressing HEK293 cells. HEK293 cells expressing S1P₁-GFP were transfected with a WWP2 expression vector and treated with S1P (200 nM) or FTY720P (100 nM) for indicated time periods. Expression of S1P₁ and WWP2 were analyzed by immunoblot analysis of total cell extracts.

Supplemental figure 3. Effect of E3 ubiquitin ligases NEDD4-1, NEDD4-2, Cbl, or AIP4

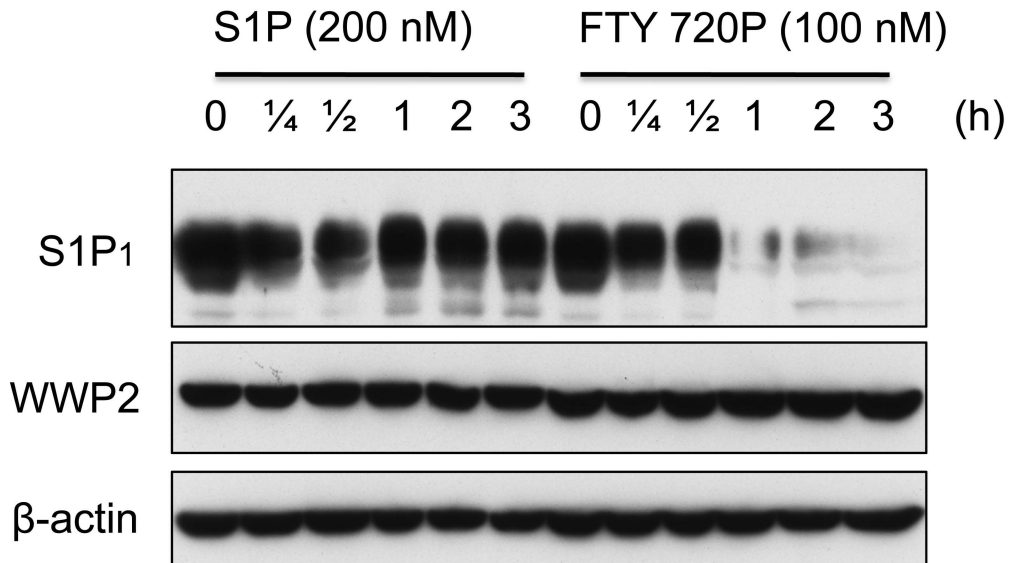
over-expression on FTY720P-induced S1P₁ degradation. HEK293 cells expressing S1P₁-GFP were transfected with indicated E3 ubiquitin ligase expression vectors, and treated with FTY720P (100 nM) for indicated time periods. Expression of S1P₁ was determined by immunoblot analysis of total cell extracts.

Supplemental figure 4. Effect of FTY720P on S1P₁ mutants in WWP2 expressing

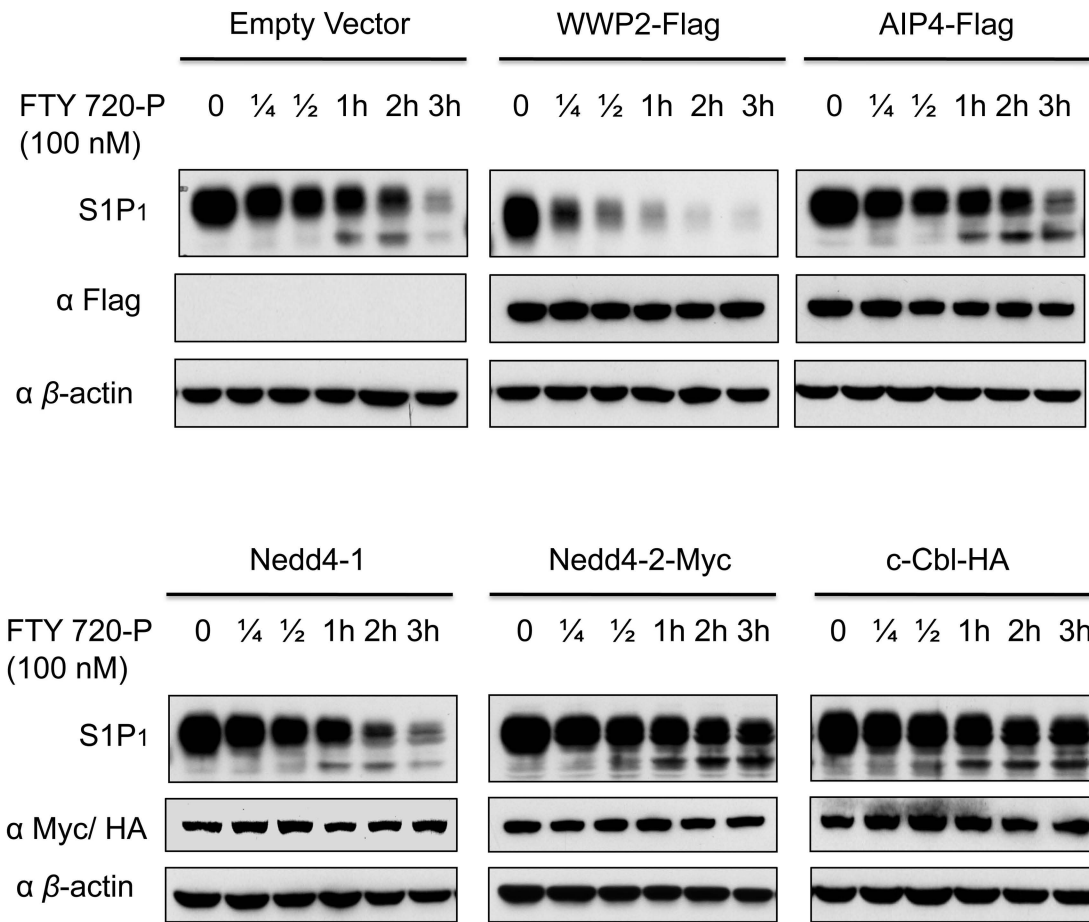
HEK293 cells. HEK293 cells expressing S1P₁-S5A-GFP or S1P₁-K4R-GFP were transfected with WWP2 expression vector and treated with FTY720P (100 nM) for indicated time periods. Expression of S1P₁ mutants was determined by immunoblot analysis as described.



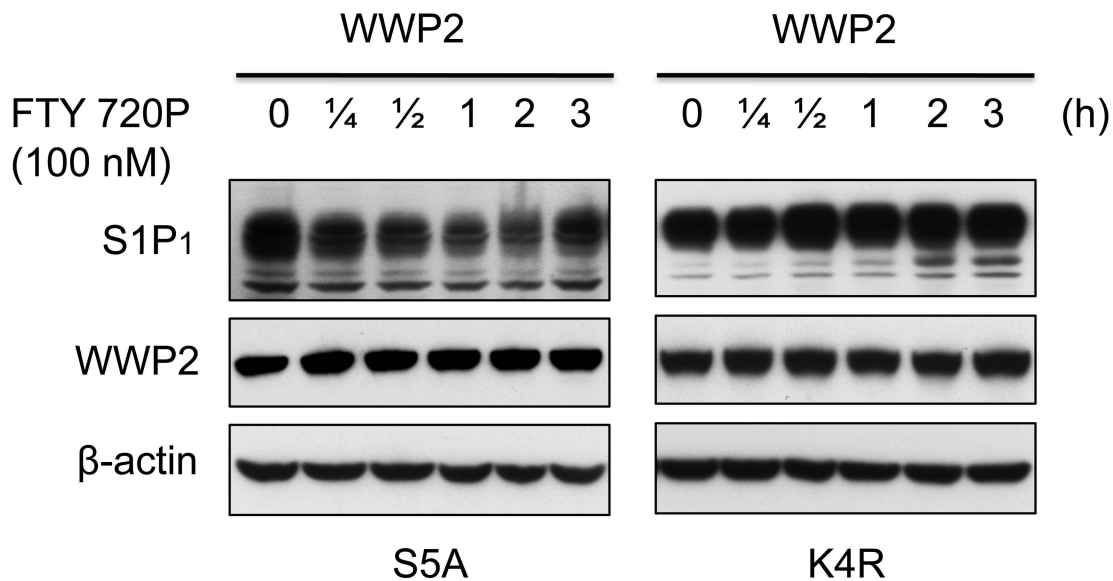
supplemental figure 1



Supplemental figure 2



supplemental figure 3



Supplemental figure 4