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Supporting Information

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for

Isolation of Phospholipase D Mutants Having Phosphatidylinositol-Synthesizing Activity with Positional Specificity on *myo*-Inositol

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Construction of XYR mutants: Out of 20 XYR variants, DYR, AYR and MYR were ones isolated by the screening. WYR was constructed by ligating a 5' half portion of WT-PLD gene (containing W187 and Y191) with a 3' half potion of DYR gene (containing 385R mutation) at a *Bam* HI site. The rest 16 mutants were constructed by overlapping PCR using the primers listed in Table S1 and the DYR gene as the template.

Table S1. Primers used for construction of XYR variants.[a]

| Primer	Sequence (5' to 3')
W187V	ATCACGGGCGGGATCAACGGC<u>GTC</u>AAGGACGACTACC
W187I	ATCACGGGCGGGATCAACGGC<u>ATC</u>AAGGACGACTACC
W187Y	ATCACGGGCGGGATCAACGGC<u>TAC</u>AAGGACGACTACC
W187F	ATCACGGGCGGGATCAACGGC<u>TTC</u>AAGGACGACTACC
W187P	ATCACGGGCGGGATCAACGGC<u>CCG</u>AAGGACGACTACC
W187T	ATCACGGGCGGGATCAACGGC<u>ACG</u>AAGGACGACTACC
W187S	ATCACGGGCGGGATCAACGGC<u>AGC</u>AAGGACGACTACC
W187C	ATCACGGGCGGGATCAACGGC<u>TGC</u>AAGGACGACTACC
W187G	ATCACGGGCGGGATCAACGGC<u>GGG</u>AAGGACGACTACC
W187N	ATCACGGGCGGGATCAACGGC<u>AAC</u>AAGGACGACTACC
W187H	ATCACGGGCGGGATCAACGGC<u>CAC</u>AAGGACGACTACC
W187Q	ATCACGGGCGGGATCAACGGC<u>CAG</u>AAGGACGACTACC
W187E	ATCACGGGCGGGATCAACGGC<u>GAG</u>AAGGACGACTACC
W187K	ATCACGGGCGGGATCAACGGC<u>AAG</u>AAGGACGACTACC
W187R	ATCACGGGCGGGATCAACGGC<u>CGC</u>AAGGACGACTACC
W187L	ATCACGGGCGGGATCAACGGC<u>CTG</u>AAGGACGACTACC

The codons for mutations are underlined.
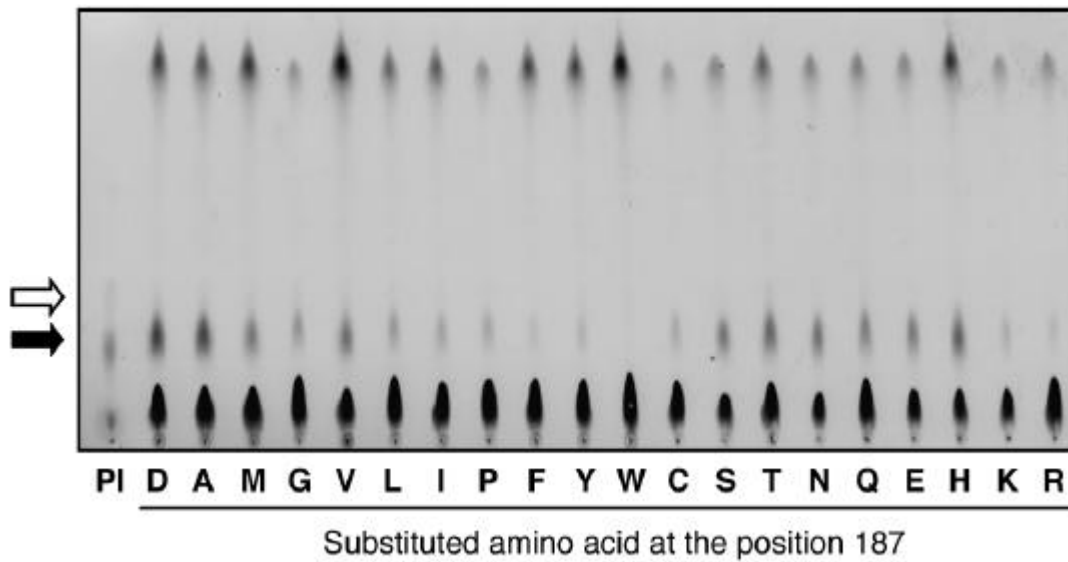
Figure S1. TLC analysis of the phospholipids generated by the XYR variants. The mutant enzymes were expressed by single symbols denoting the substituted amino acid at the position 187. The lane "PI" denotes the soybean PI as the standard. The black and white arrows indicate the position of 1(3)-PI and 4(6)-PI (if present), respectively.