Lysosomal Phospholipase A2 As Companion Diagnostic Biomarker for Phospholipidosis, a Drug-Induced Toxicity Event

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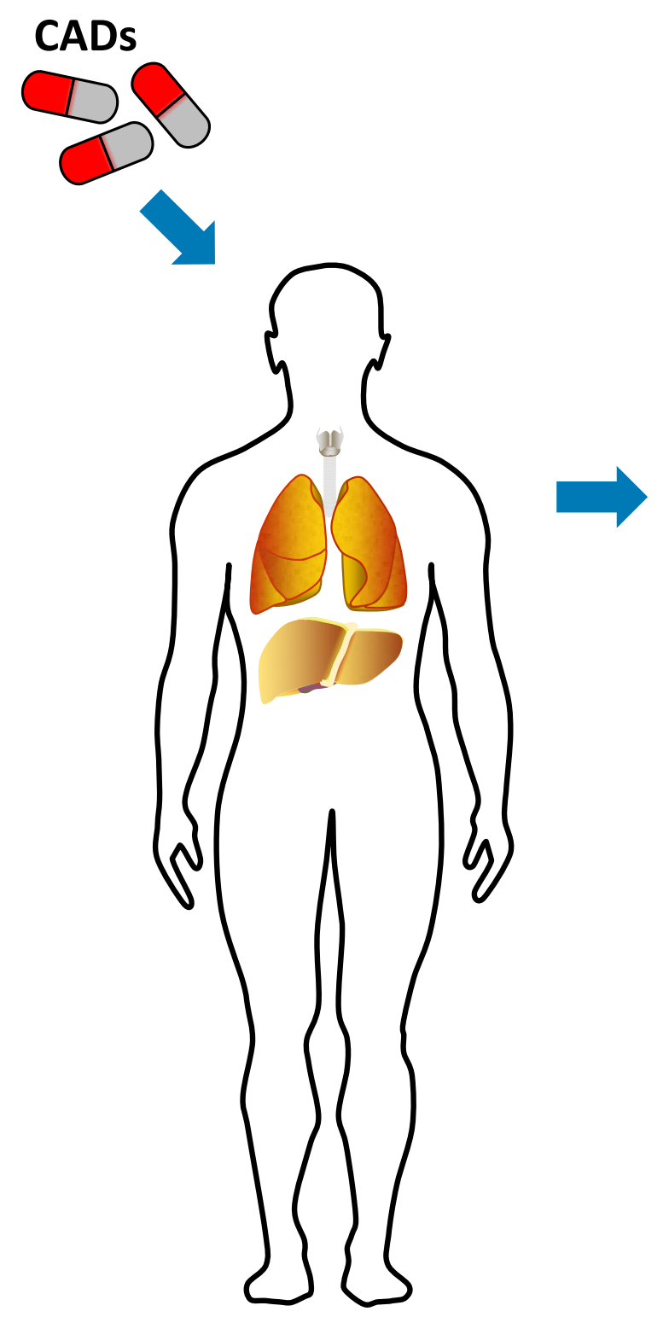
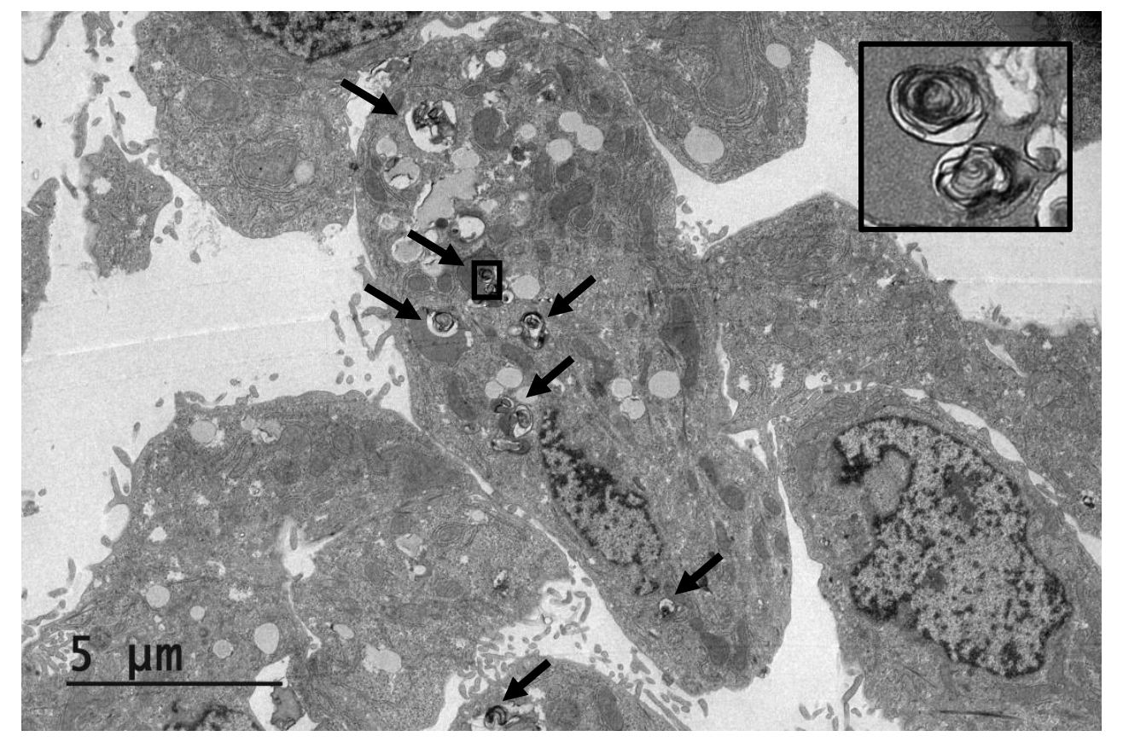
1. Overview

- ➢ **Lysosomal phospholipase A2 (LPLA2) is involved in drug-induced phospholipidosis (DIPL)**
- ➢ **Evaluated if a compound's ability to inhibit LPLA2 activity can be used as a drug toxicity screen to predict DIPL**
- ➢ **Studied plasma LPLA2 concentration and activity using an LPLA2 antibody & a quenched fluorogenic probe specifically designed for LPLA2 in an acidic environment**

2. Introduction

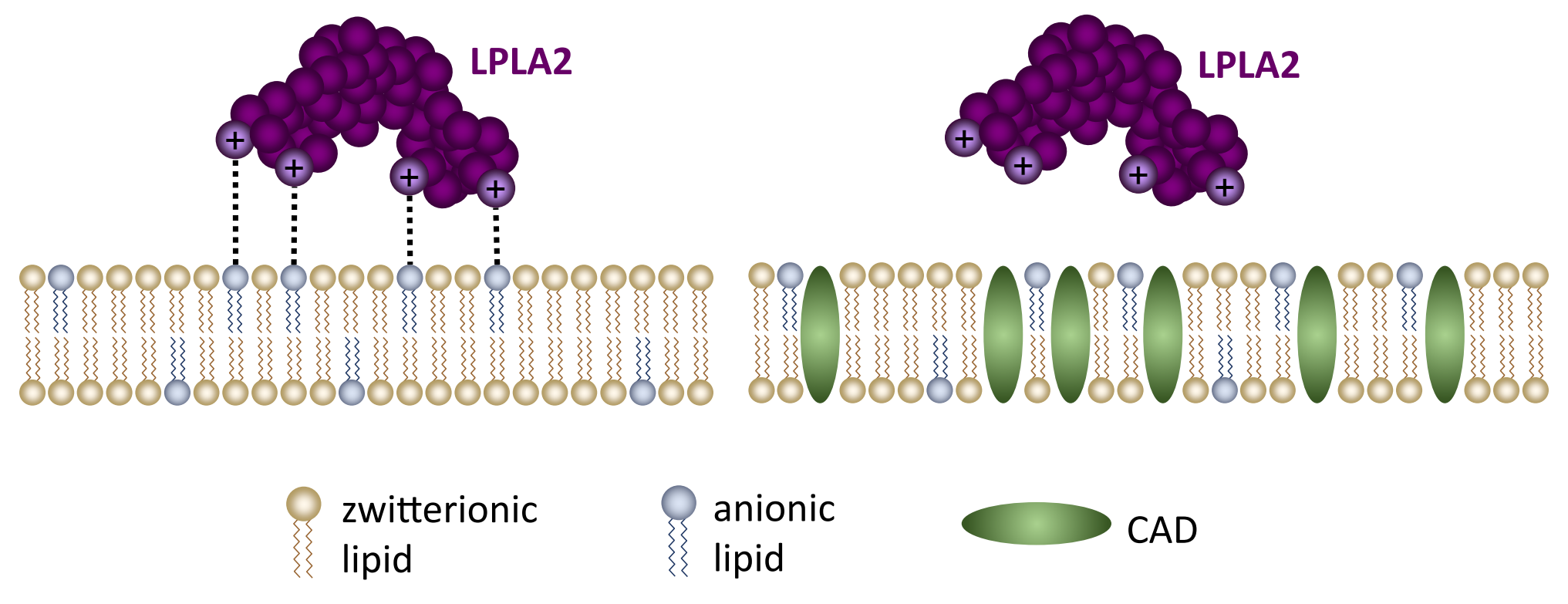




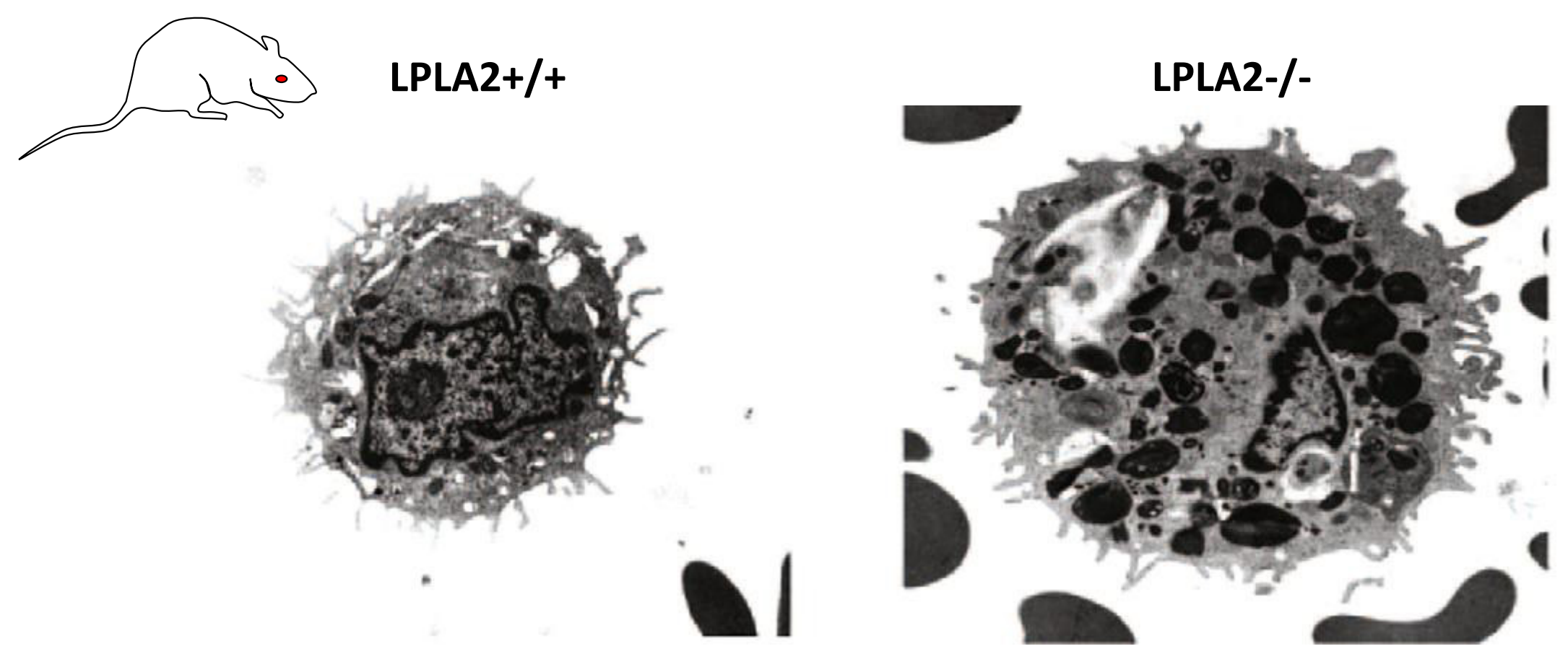
- ❑ Phospholipidosis (PL) is a condition of excessive accumulation of intracellular phospholipids
- ❑ Drug-induced phospholipidosis (DIPL) is often caused by common cationic amphiphilic drugs (CADs) on the market
- ❑ DIPL can be deadly if not caught in time
- ❑ Lamellar bodies (LBs,🡅) are a hallmark of PL

Four proposed hypotheses for the mechanism of DIPL:

- ❖ **CADs bind to phospholipids**
- ❖ CADs bind to lysosomal phospholipases
- ❖ CADs stimulate phospholipid synthesis in the cells
- ❖ CADs induce the dissociation of a lysosomal hydrolase from the lysosomal membrane

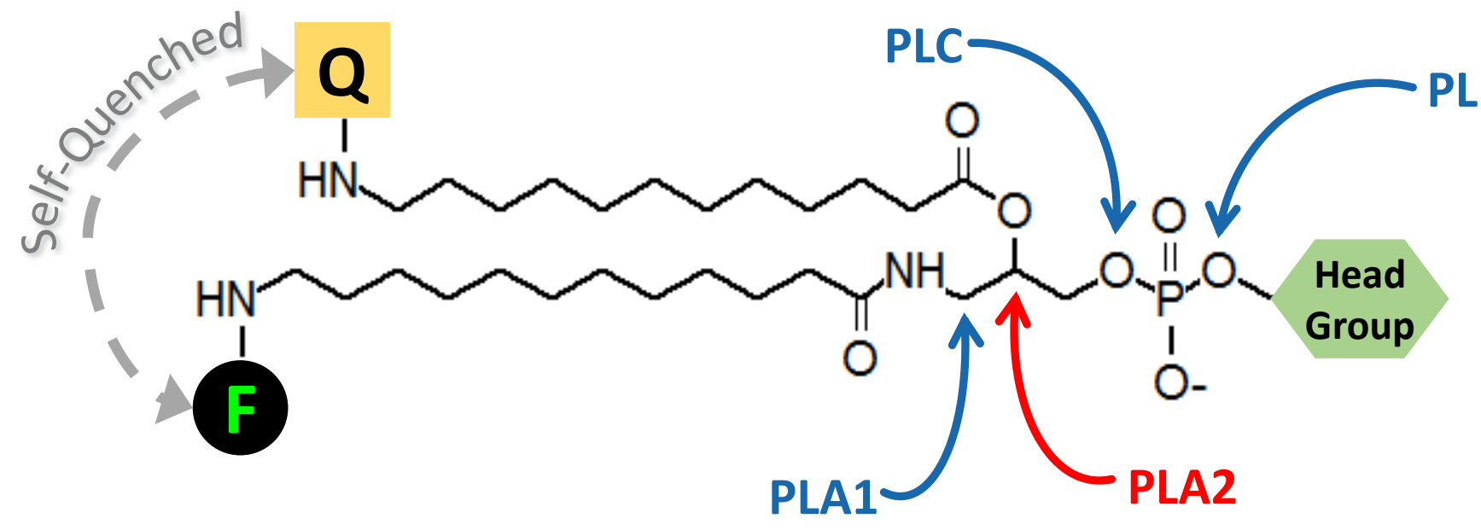


3. LPLA2 & DIPL

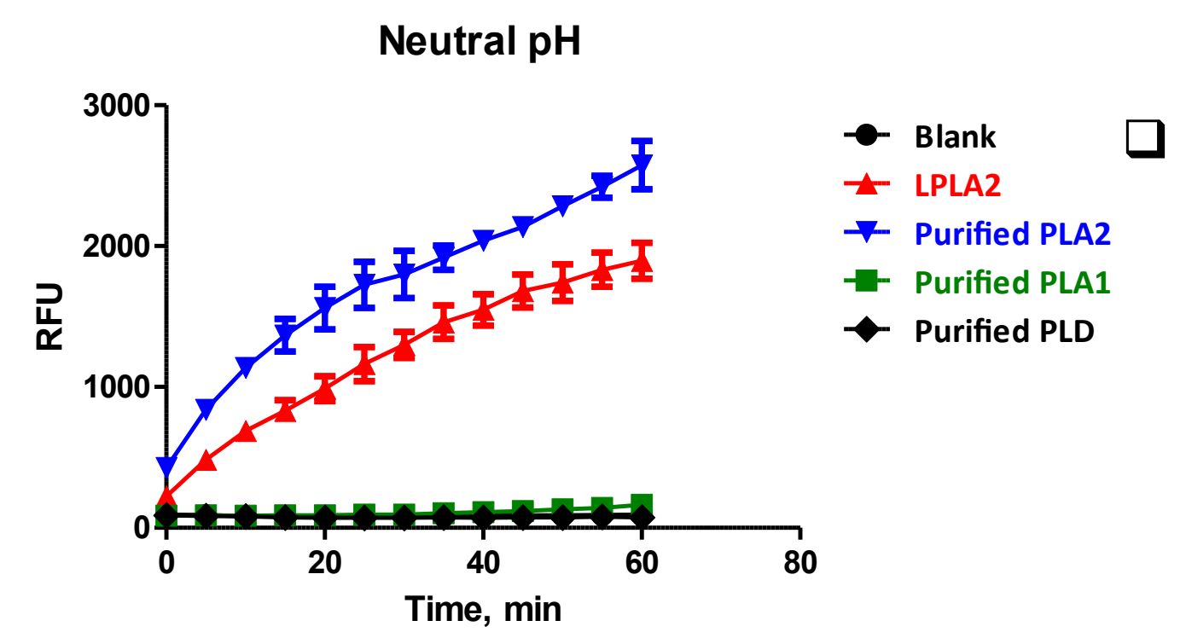


- ❑ LPLA2 activity is optimal at pH 4.5 as found in the lysosome
- ❑ Phospholipid-containing LBs, a hallmark of PL, originate from lysosomes or lipid droplets
- ❑ Transmission electron micrographs (TEM) show increased number and size of LBs within alveolar macrophages in the LPLA2 knockout (KO) mice
- ❑ LPLA2 KO mice also develop phenotypes similar to systemic lupus erythematosus (SLE)
- ❑ All of the above suggests LPLA2 association with DIPL & SLE

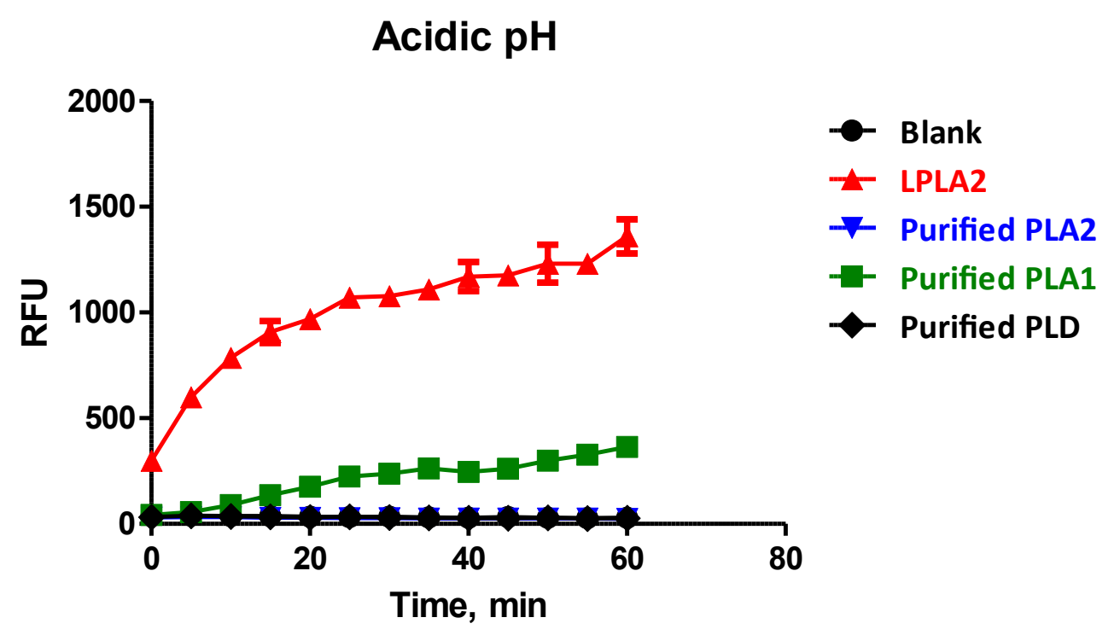
4. Smart Probe Technology



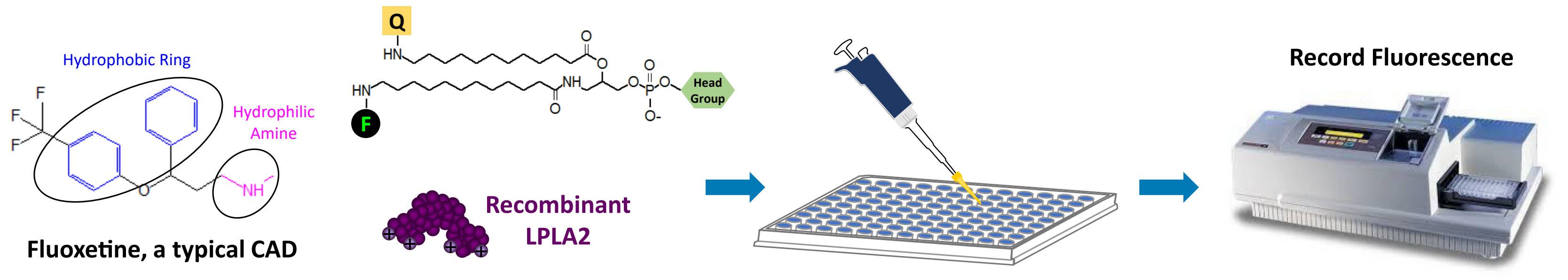
- ❑ The self-quenched fluorophore quencher pair allows direct fluorescence detection when phospholipase A2 (PLA2) cleaves the smart probe at the *sn-2* position

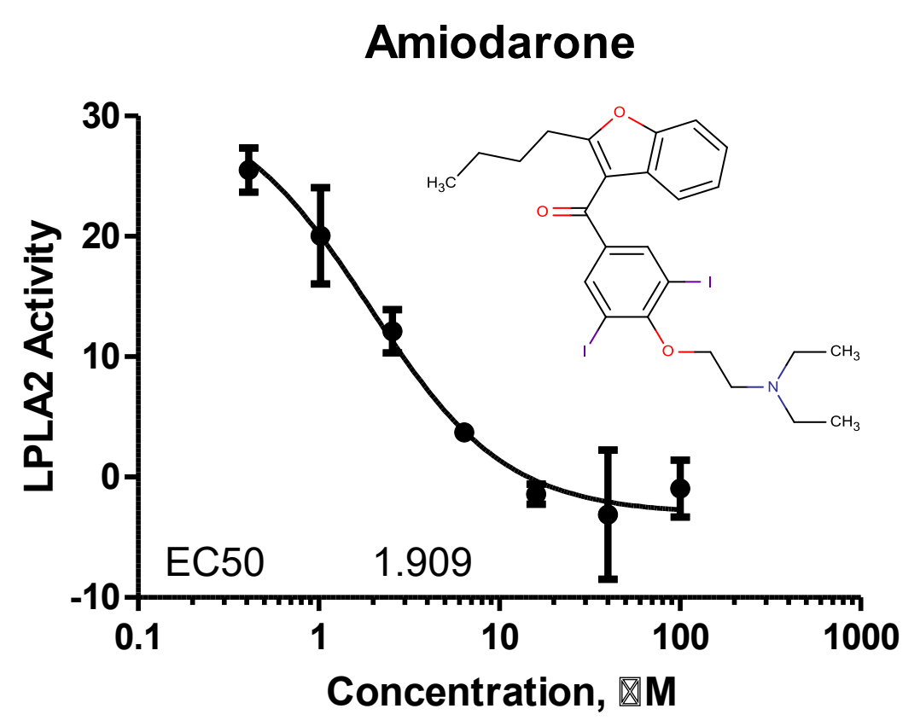


- ❑ An amide is incorporated at the *sn-1* position of the smart probe to avoid non-specific hydrolysis by phospholipase 1 (PLA1)
- ❑ The smart probe is designed to work at acidic pH to prevent non-specific hydrolysis by the other PLA2s

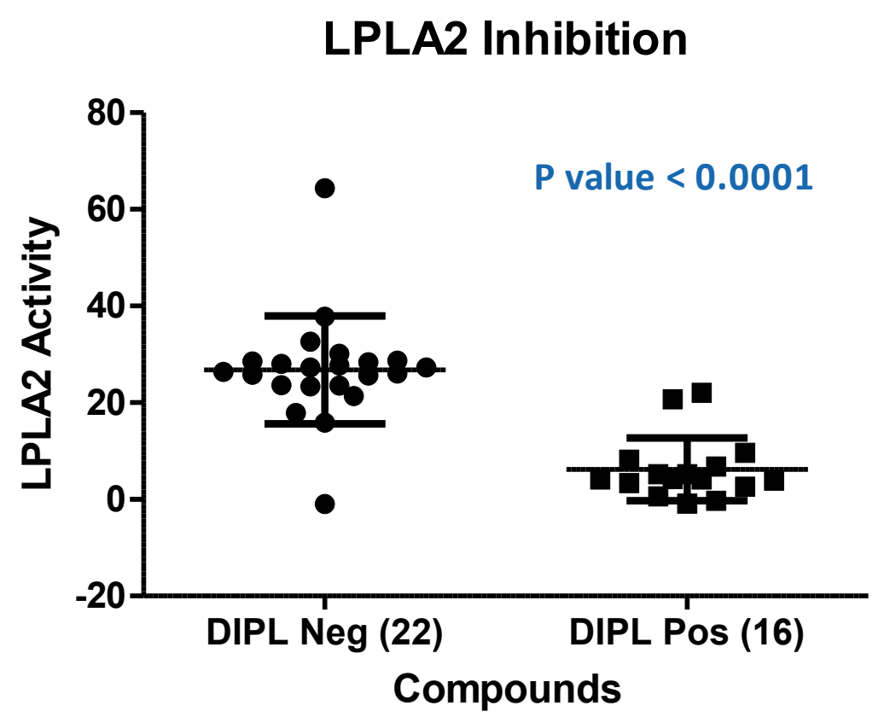


5. LPLA2 Inhibition by CAD

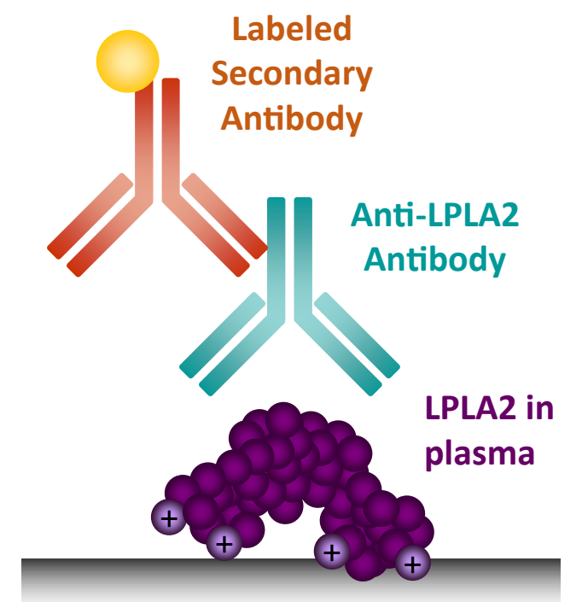


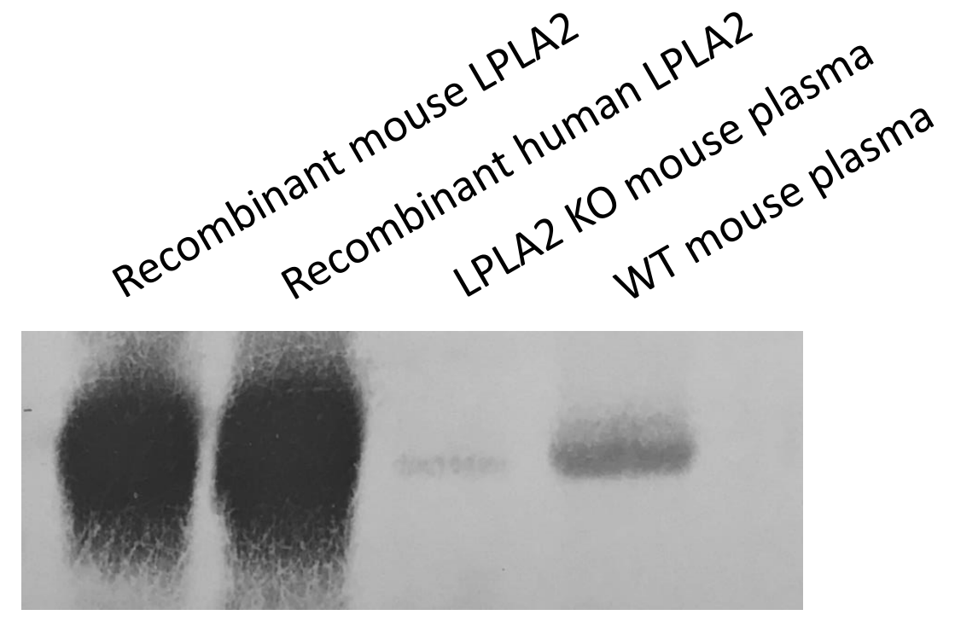


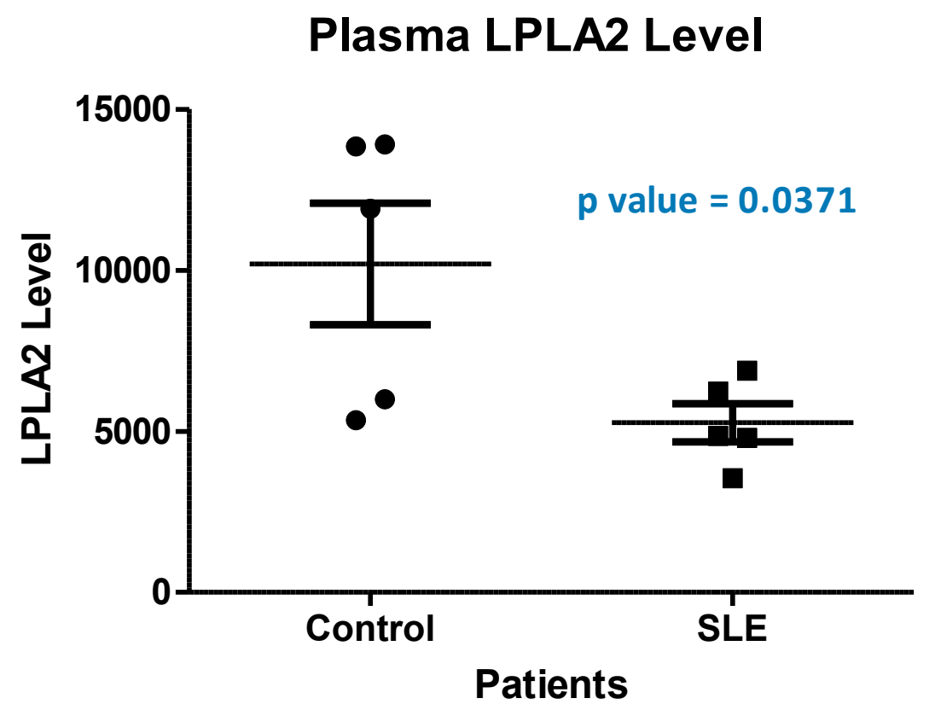
- ❑ Amiodarone, an antiarrhythmic agent & CAD known to cause DIPL, showed significant inhibition of LPLA2
- ❑ DIPL positive compounds showed significant LPLA2 inhibition while DIPL negative compounds had no effect on LPLA2
- ❑ LPLA2 activity inhibition can be used as drug toxicity screen for DIPL



6. Plasma LPLA2 Detection

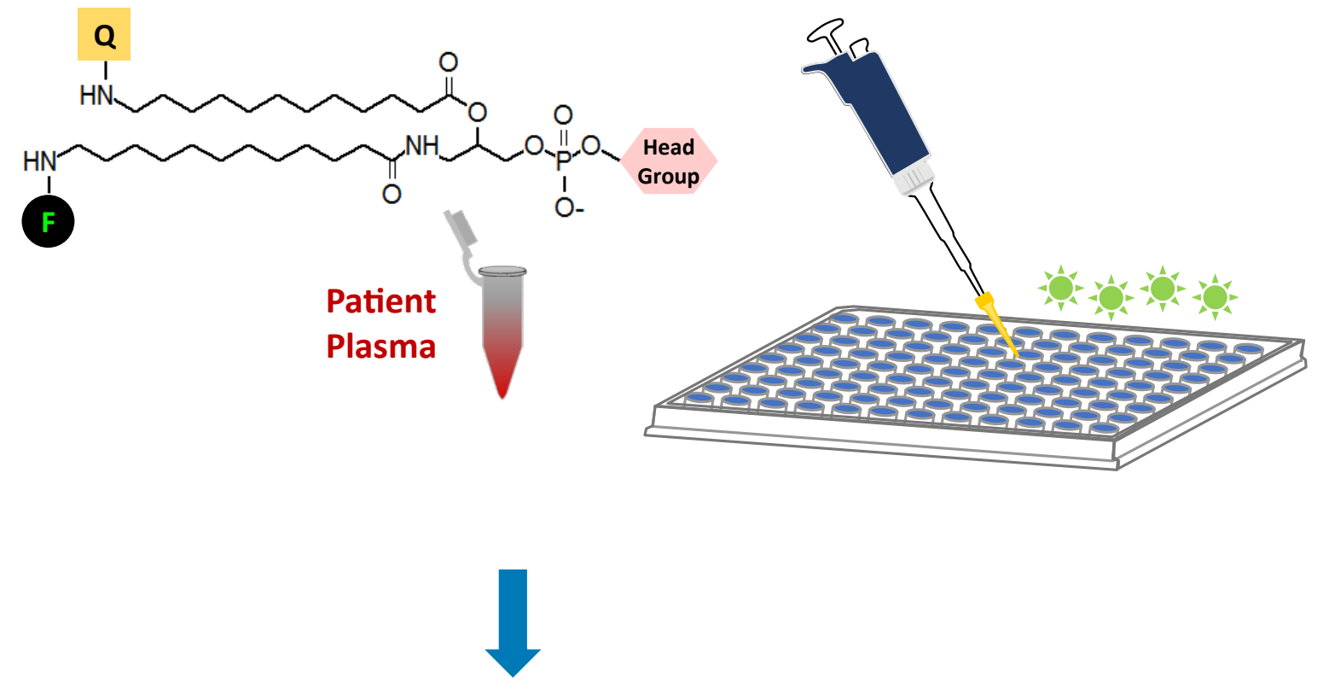




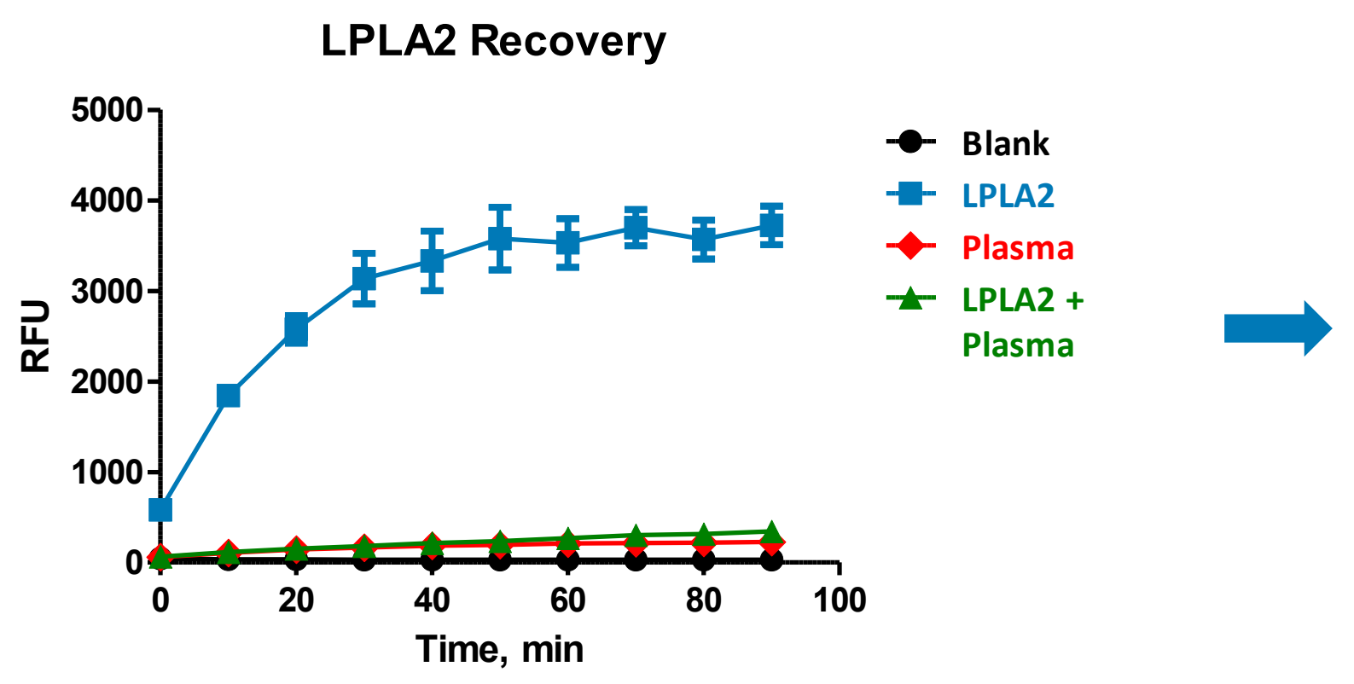


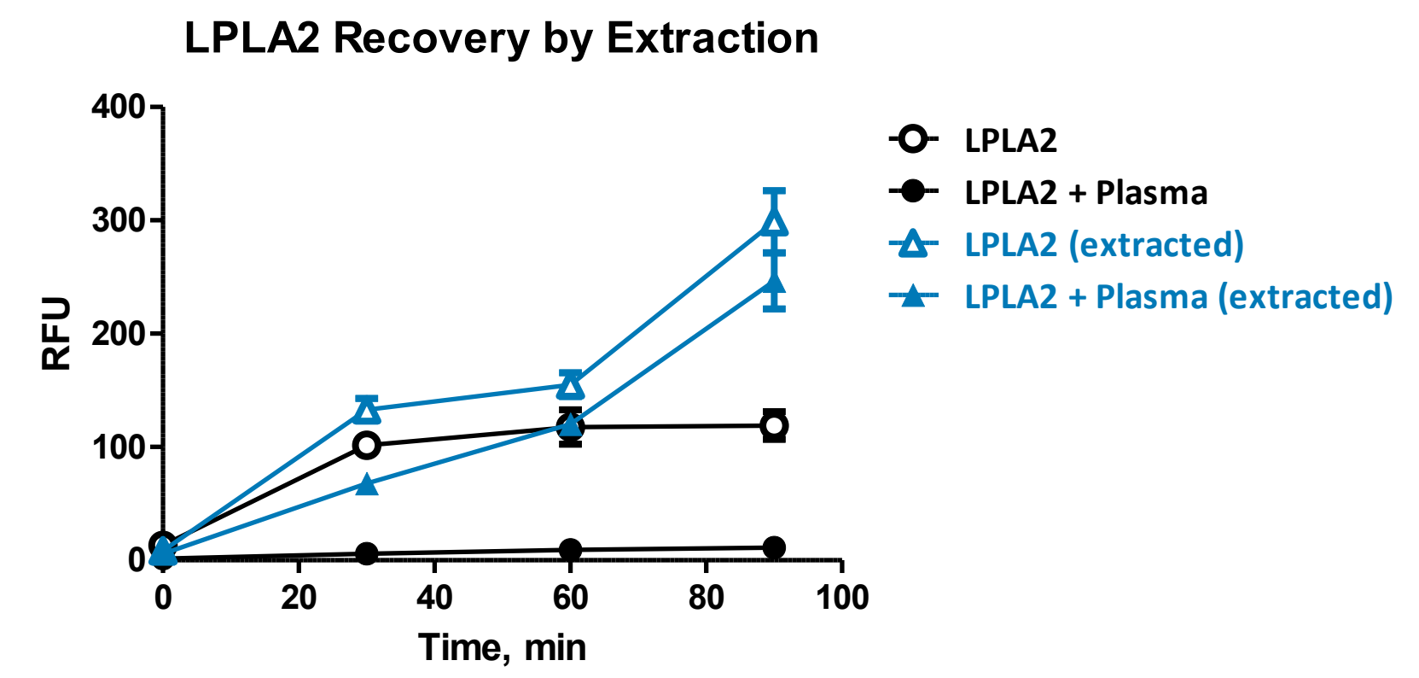
- ❑ No LPLA2 detected from LPLA2 KO plasma, validating the specificity of the anti-LPLA2 antibody
- ❑ A significant difference in the plasma LPLA2 levels between controls and SLE patients further suggests LPLA2 involvement in SLE and DIPL

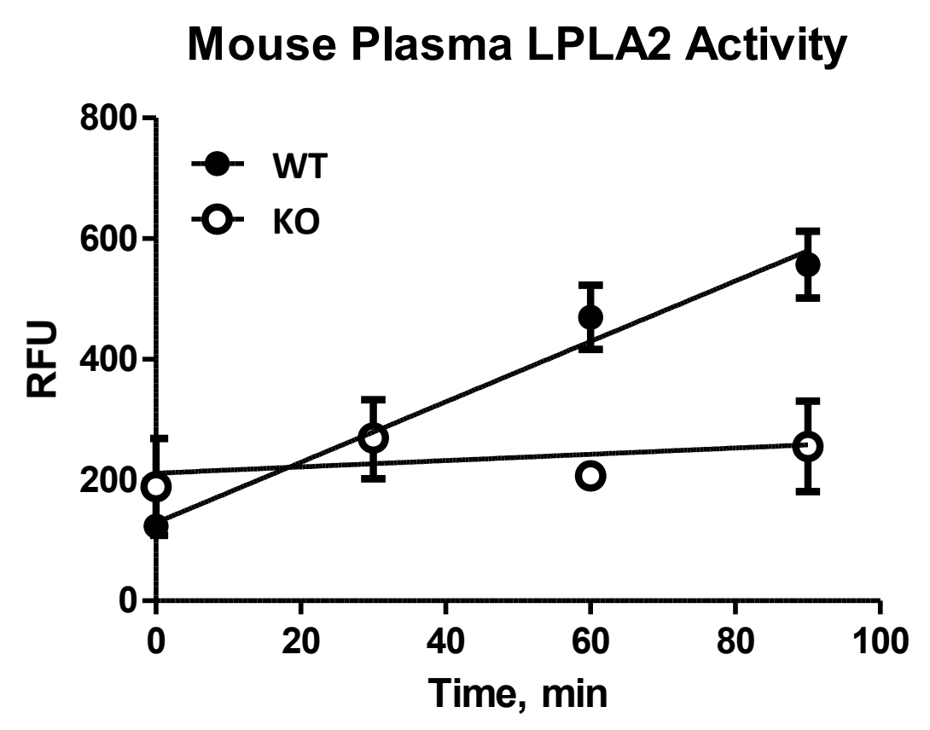
7. Plasma LPLA2 Activity Detection

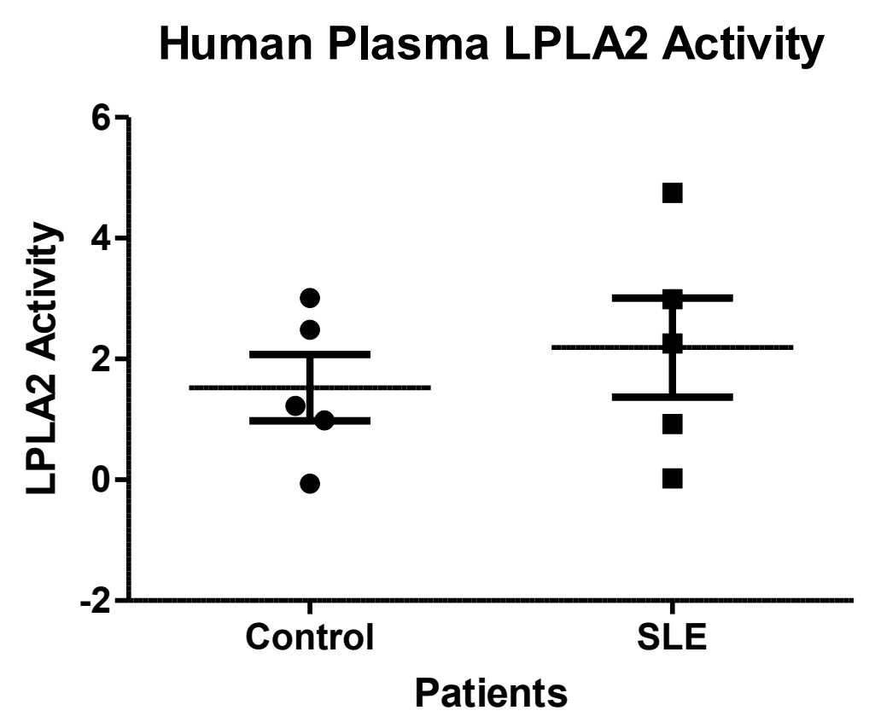


- ❑ Unknown interfering substances appear to inhibit the activity of the spiked LPLA2 in plasma
- ❑ The activity of the spiked LPLA2 in plasma can be recovered by extracting the enzyme reaction product, the cleaved probe, using organic solvents









- ❑ LPLA2 activity was detected from the WT mice but not from the LPLA2 KO mice
- ❑ No significant difference in the plasma LPLA2 activities between controls and SLE patients
- ❑ These findings suggest organic extraction may not completely resolve plasma interference. Further assay development is necessary to accurately measure LPLA2 activity in biological samples.

8. Conclusions & Acknowledgements

- ➢ **CAD inhibition of LPLA2 activity is a promising mechanism for DIPL**
- ➢ **LPLA2 activity can be used as drug toxicity screen for DIPL predict**
- ➢ **LPLA2 levels and activities vary between individuals and may serve as a potential companion diagnostic biomarker for DIPL**

- ➢ **We appreciate Dr. James M Willard from FDA in sharing the Phospholipidosis Working Group database**
- ➢ **We thank our collaborators Dr. Piotr W. Rzepecki for advice**
- ➢ **This study is supported by FDA, SBIR Contract, 5R44FD004052**

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