

Fig. S1. Treatment of MDA-MB-231 (A) and 4T1 (B) cells with doxycycline (Dox), tetracycline (Tet), and minocycline (Min) at 1 $\mu\text{g/ml}$ for 24 h increased their ecto-LPP activities. The molar concentrations for 1 $\mu\text{g/ml}$ doxycycline, tetracycline and minocycline are 2.0, 2.1, 2.0 μM respectively. Results are means \pm SD from three independent experiments. Significant differences between drug-treated and control cells are shown as *, $p < 0.05$.

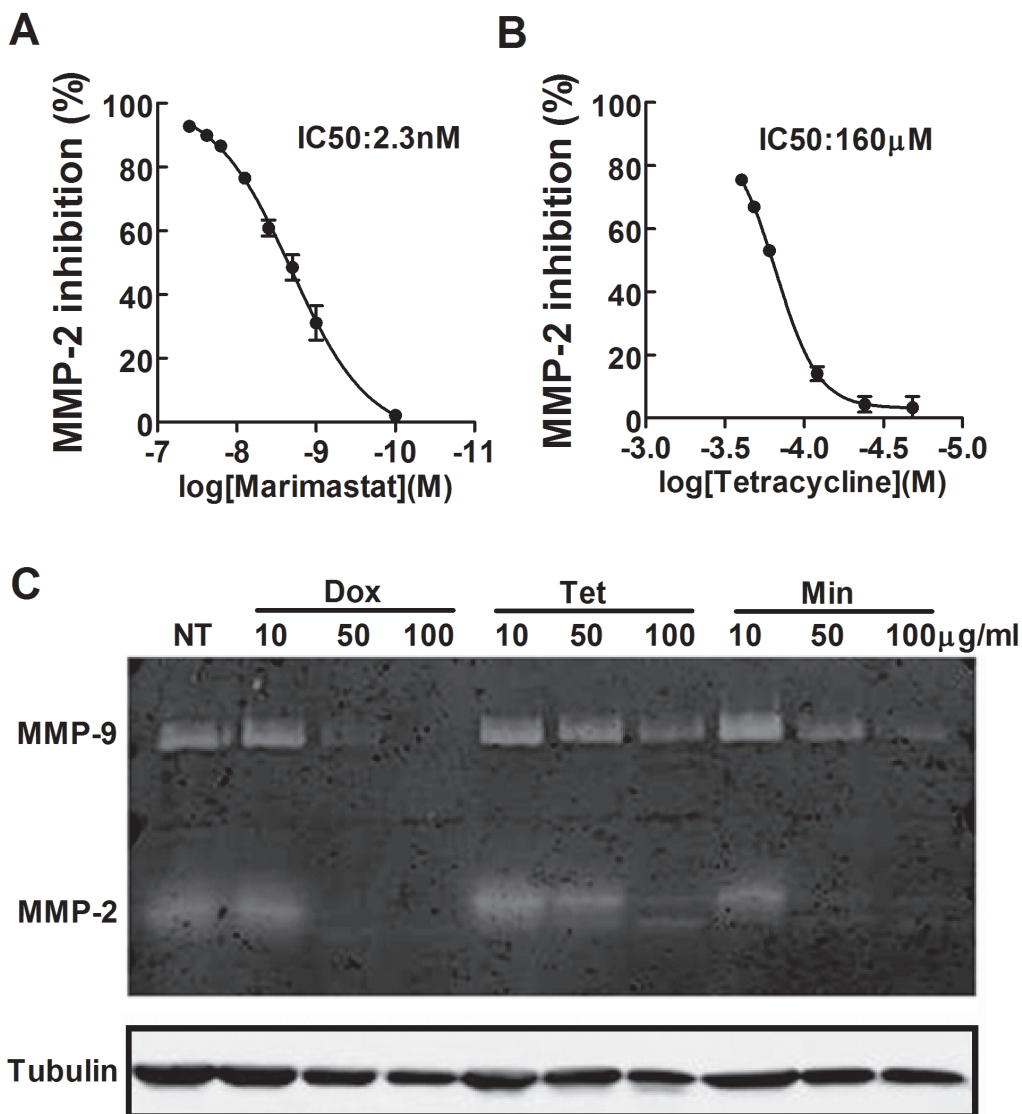


Fig. S2. The inhibition of recombinant human MMP2 (PF023, EMD Millipore) by tetracycline and marimastat were measured with DQ-gelatin (D12054, Life Technologies) in 96-well plate, the reaction system was in 200 μ l volume containing 100 μ g/ml DQ-gelatin, 50 ng/ml recombinant human MMP2 and different concentrations of tetracycline or marimastat diluted with reaction buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM CaCl₂, pH 7.6). Samples were excited at 490 nm and emissions at 510 nm were measured. IC₅₀ of marimastat (A) and tetracycline (B) were calculated using probit analysis. The effect of tetracyclines on secretion of MMP2 and MMP9 was determined by gelatin zymography (C). MDA-MB-231 cells were incubated in serum- and BSA-free DMEM medium for 24 h with or without different doses of doxycycline (Dox), tetracycline (Tet) and minocycline (Min). The conditioned medium was collected and concentrated by 5 times using 10 kDa cut off centrifugal filters (UFC501024, Millipore). Cells from each sample were collected and lysed, protein concentrations of cell lysates were measured for calculating the loading amount for each sample. Concentrated conditioned media were separated with 10% polyacrylamide gels co-polymerized with 0.1% gelatin. Gels were renatured with 2.5% Triton X-100 and developed in 50 mM Tris-HCl, 200 mM NaCl, 5 mM CaCl₂, 0.2% Brij35. Gels were stained with 0.5% Coomassie Blue R-250, 5% methanol, 10% acetic acid, and destained with 5% methanol, 10% acetic acid. Digestion of gelatin by MMP2 and MMP9 were shown as white bands against blue background.

Fig. S2

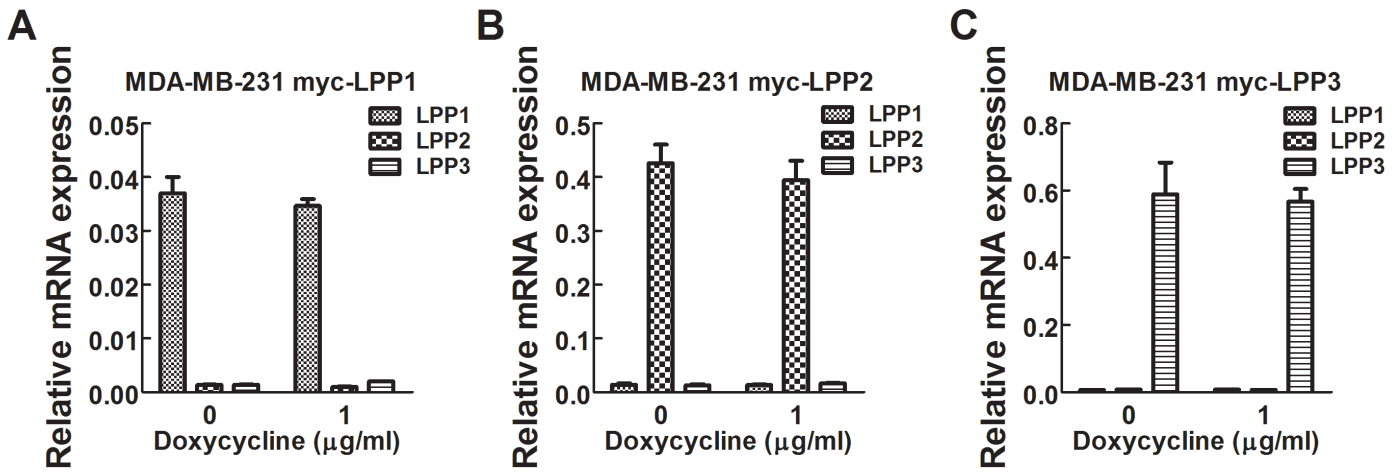


Fig. S3. mRNA levels of LPP1, LPP2 and LPP3 relative to GAPDH mRNA in MDA-MB-231 cells stably expressing myc-LPP1 (A), myc-LPP2 (B) and myc-LPP3 (C) with or without 1 µg/ml (2 µM) doxycycline treatment for 24 h.

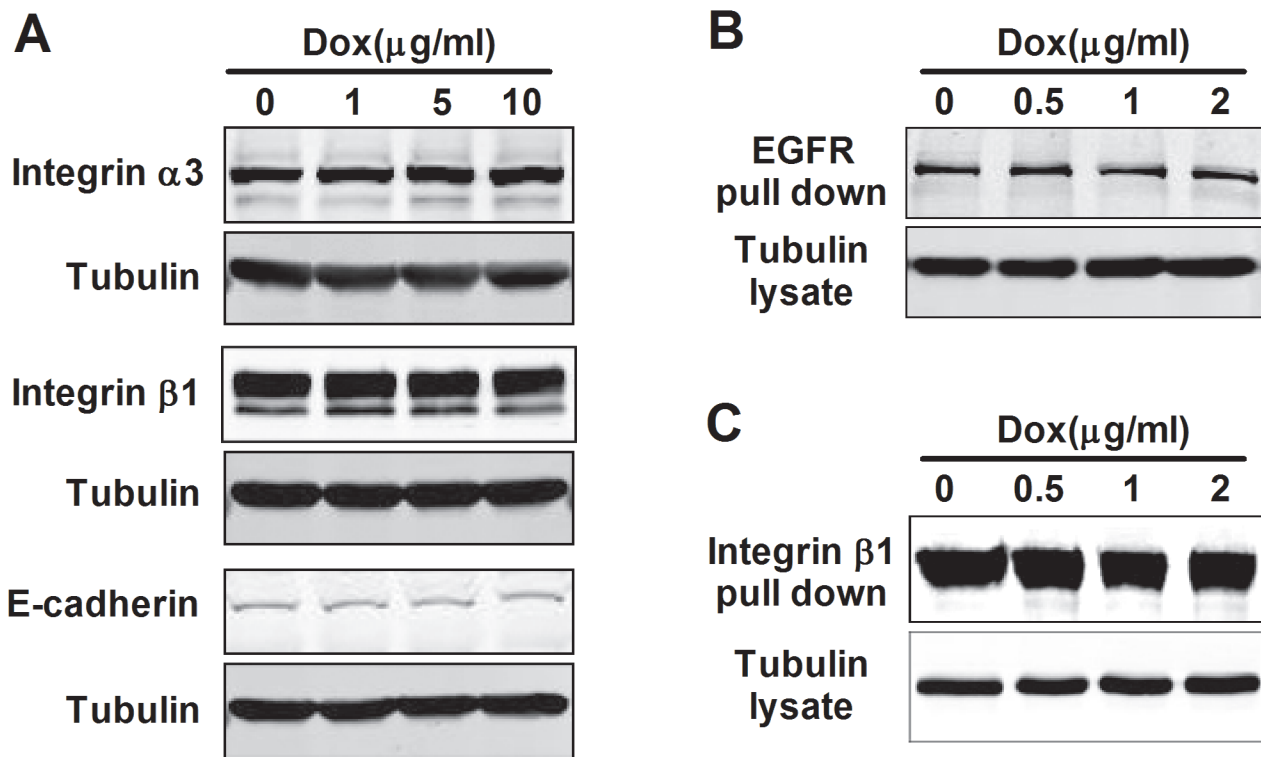


Fig. S4. (A): Protein expression of integrin $\alpha 3$, $\beta 1$ and E-cadherin in MDA-MB-231 cell lysates after 24 h treatment with doxycycline (Dox) at 1, 5, 10 $\mu\text{g/ml}$ (2, 10, 20 μM). (B) and (C): Plasma membrane EGFR and Integrin $\beta 1$ in MDA-MB-231 cells after 24h treatment with doxycycline (Dox) at 1, 5, 10 $\mu\text{g/ml}$ (2, 10, 20 μM)

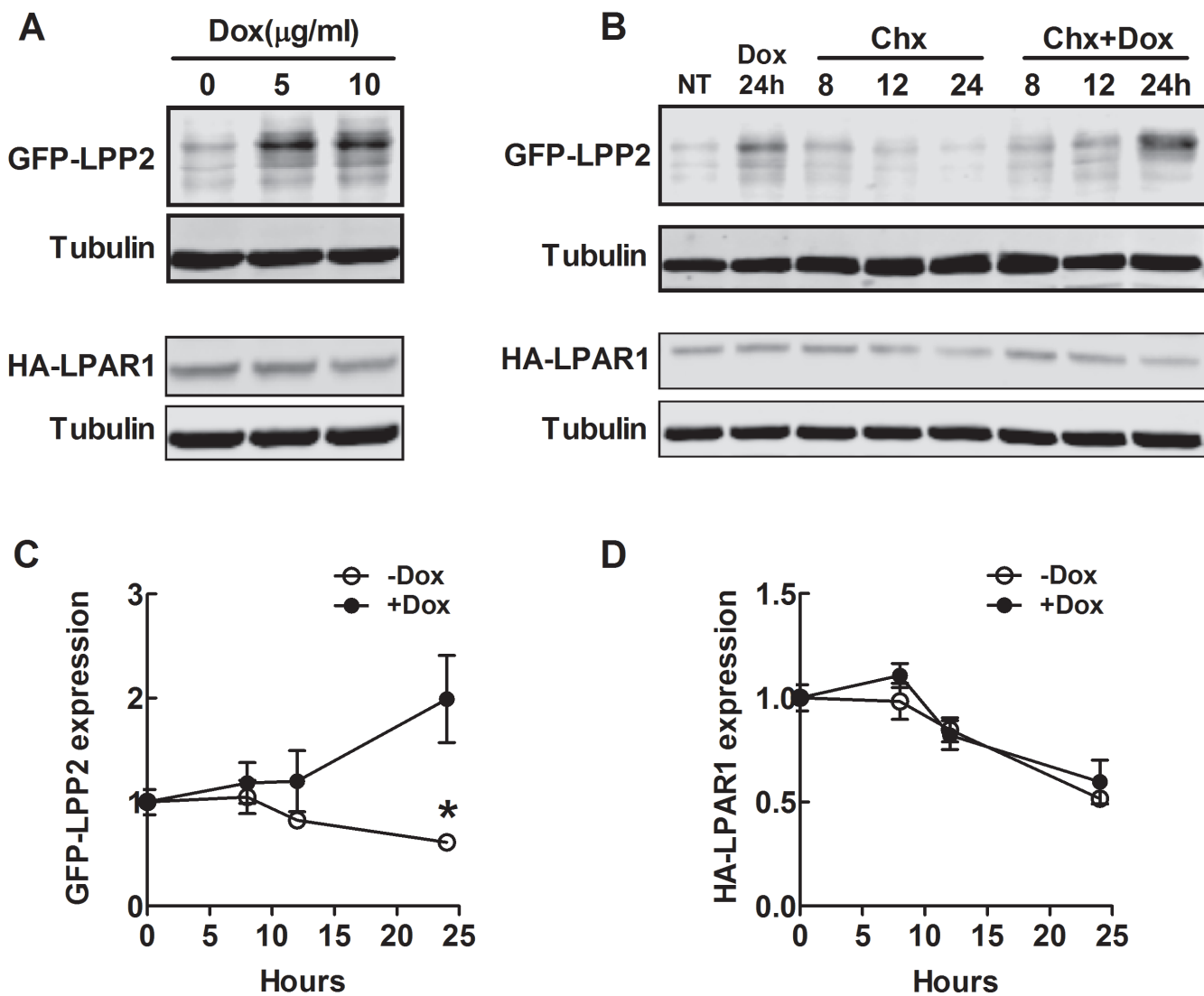


Fig. S5. Doxycycline increases protein level and inhibits degradation of GFP-LPP2 but not HA-EDG2. (A): Protein levels of GFP-LPP2 and HA-EDG2 in HEK293 cells treated with 5 and 10 $\mu\text{g/ml}$ doxycycline for 24 h. (B) Protein levels of GFP-LPP2 and HA-EDG2 in HEK293 cells treated with 50 mg/ml cycloheximide (Chx) for 8, 12, 24 h in the presence or absence of 5 $\mu\text{g/ml}$ doxycycline (Dox). The first two lanes on the left side are cells without treatment (NT) and cells treated with 5 $\mu\text{g/ml}$ doxycycline for 24 h. (C) and (D) are quantification of protein levels of GFP-LPP2 and HA-EDG2 in panel B. Results are means \pm SD from three independent experiments. Significant differences between cells with or without doxycycline treatment are shown as *, $p < 0.05$.

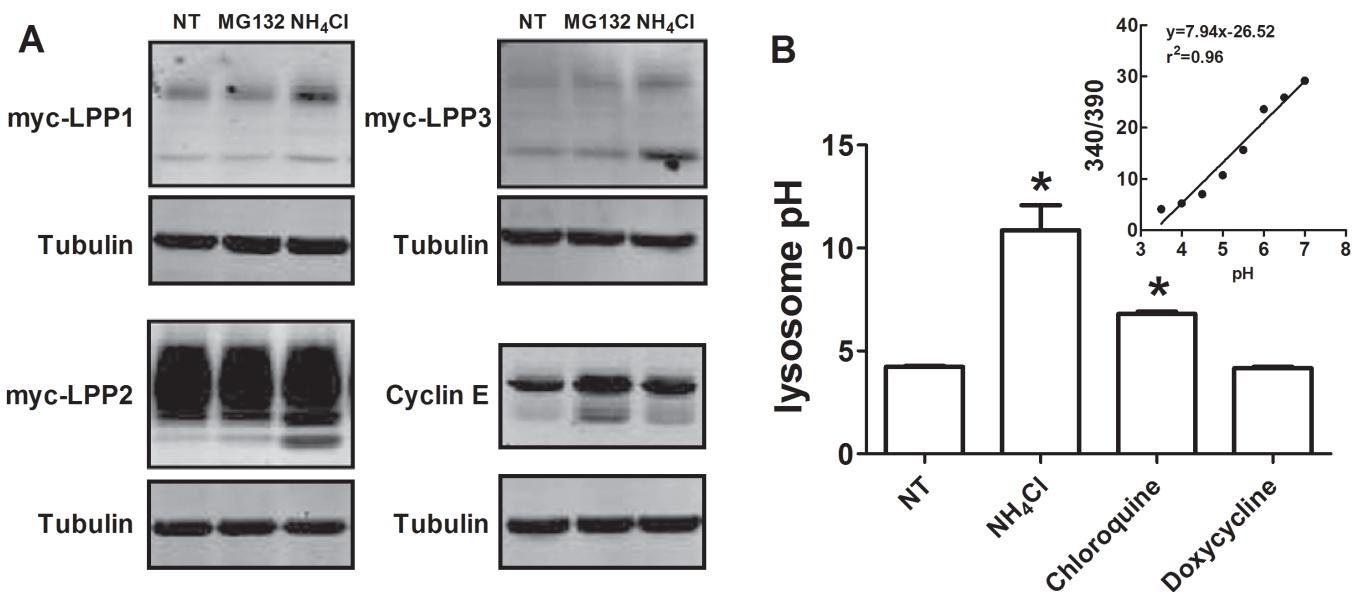


Fig. S6. (A): Protein expression of myc-LPP1, myc-LPP2 and myc-LPP3 in stable MDA-MB-231 cells treated with proteasome inhibitor MG132 (10 μ M) or lysosome inhibitor NH₄Cl (50 mM) for 16 h. Cyclin E is known to be degraded by the proteasome, which was used as a control. (B): Lysosomal pH in MDA-MB-231 cells pre-treated with 50 mM NH₄Cl, 20 μ M chloroquine and 1 μ g/ml (2 μ M) doxycycline for 24 h. Cells were labeled with 1 μ M LysoSensor Yellow/Blue DND-160. Emission at 490 nm caused by excitation at 340 nm (E1) and 390 nm (E2) were measured, and the ratio of E1/E2 was calculated. The small insert in panel B is a standard curve of the ratio of E1/E2 at different pH. The lysosomal pH in cells was calculated based on the standard curve. Significant difference between cells with or without drug treatment is shown as *, $p < 0.05$.

Fig. S6

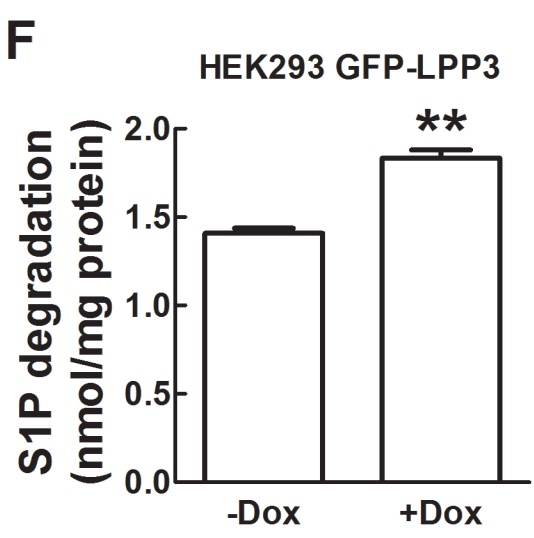
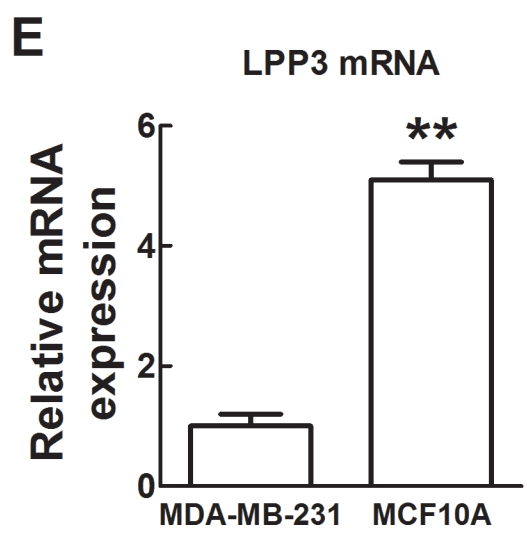
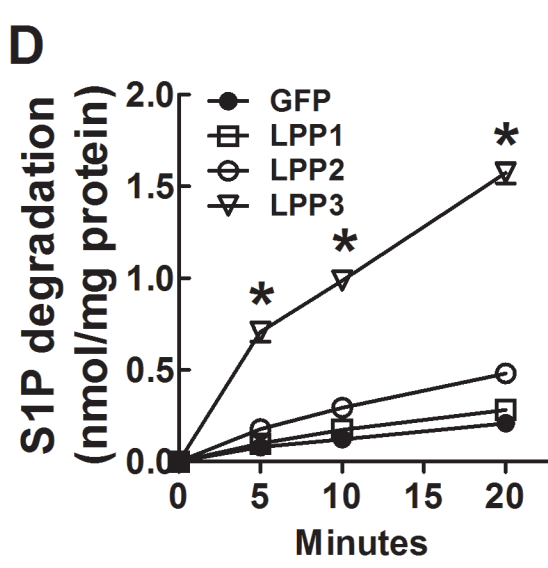
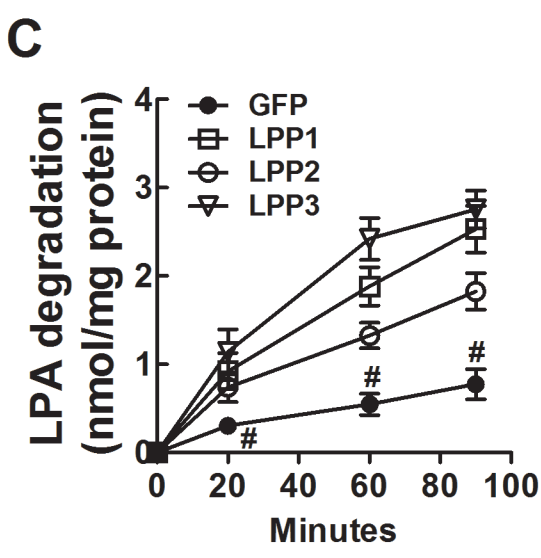
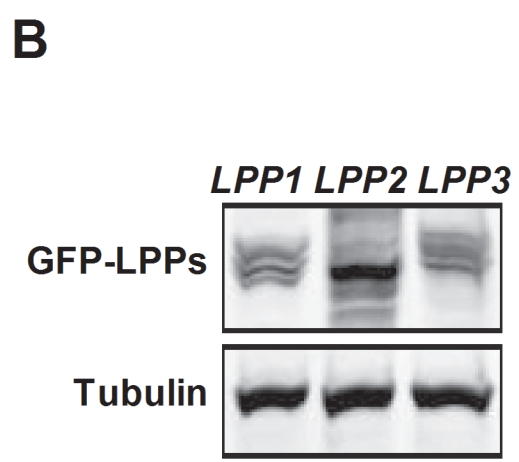
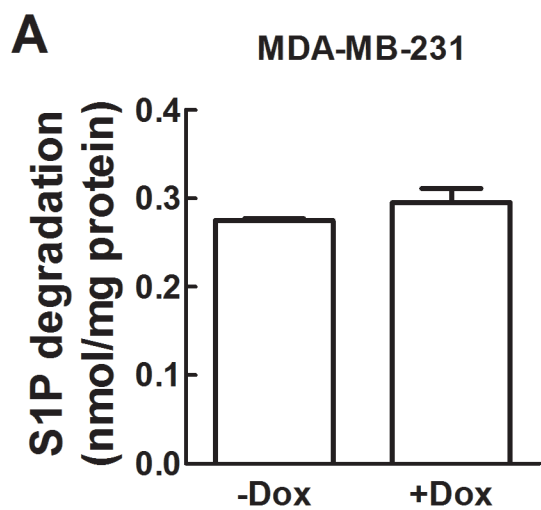


Fig S7

Fig. S7. Effects of different LPPs on the dephosphorylation of extracellular LPA and S1P. (A): Dephosphorylation of exogenous S1P by MDA-MB-231 cell with or without 1 $\mu\text{g/ml}$ (2 μM) doxycycline (Dox) treatment for 24 h. (B): Expression of GFP-tagged human LPP1, 2 and 3 in HEK293 cells detected by GFP antibody. (C): Degradation of exogenous LPA by HEK293 cells overexpressing GFP, or GFP-tagged LPP1, 2 and 3. Significant difference between cells expressing GFP and LPPs is shown as #, $p < 0.05$. (D): Degradation of exogenous S1P by HEK293 cells overexpressing GFP, or GFP-tagged LPP1, 2 and 3. Significant difference between LPP3-expressing cells and other cells is shown as *, $p < 0.05$. (E): LPP3 mRNA levels relative to GAPDH mRNA in MDA-MB-231 and MCF10A cells. Significant difference is shown as **, $p < 0.01$. (F): Degradation of exogenous S1P by HEK293 cell overexpressing GFP-LPP3 with or without 1 $\mu\text{g/ml}$ (2 μM) doxycycline (Dox) treatment for 24 h. Significant difference is shown as **, $p < 0.01$.