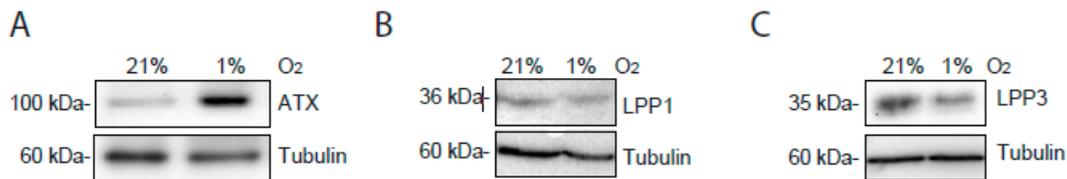
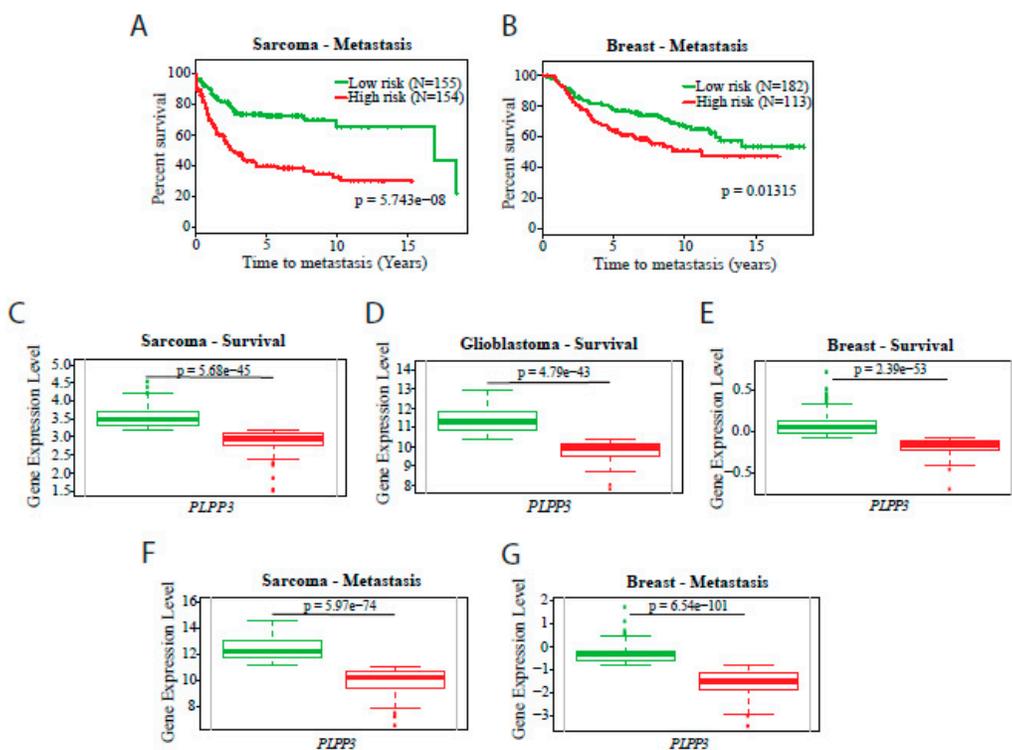


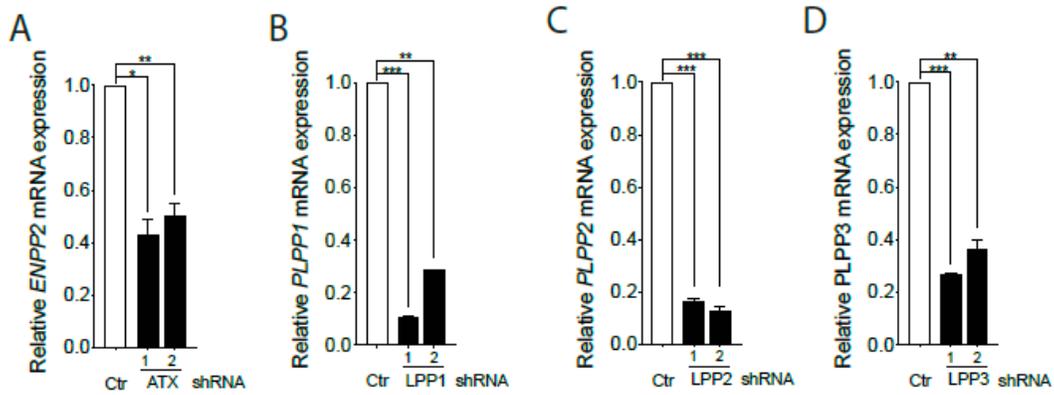
Supplementary Materials: Hypoxia Downregulates LPP3 and Promotes the Spatial Segregation of ATX and LPP1 During Cancer Cell Invasion



Supplementary Figure 1. Influence of hypoxia on ATX, LPP1, and LPP3 protein expression.

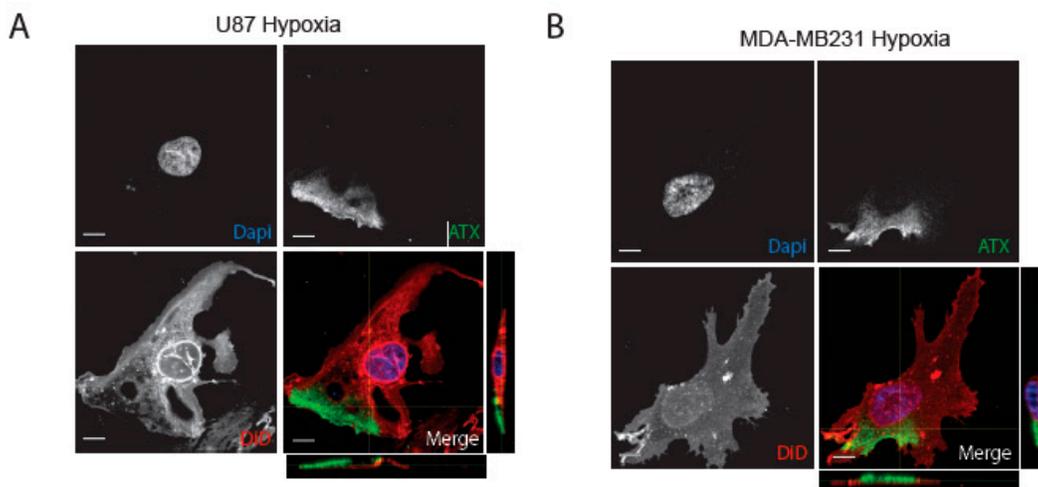
HT-1080 cells were incubated overnight under normoxic (21% O₂) or hypoxic conditions (1% O₂) for 16 h and protein levels of (A) ATX, (B) LPP1 or (C) LPP3 were analyzed by western blotting. Tubulin was used as a loading control.





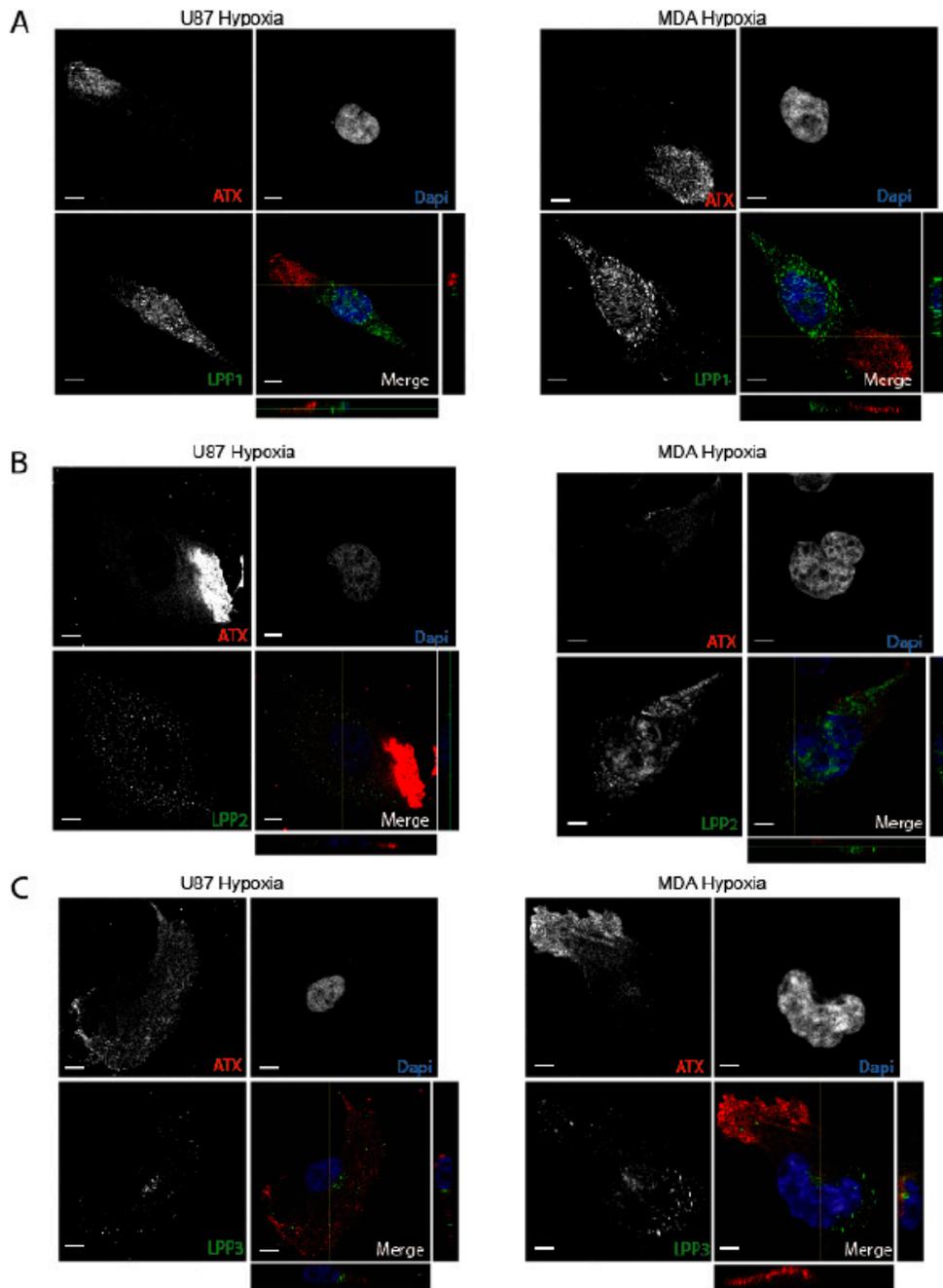
Supplementary Figure 3. Validation of ATX- and LPPs-targeted shRNAs by qPCR. (A-D)

HT1080 cells were transfected with scramble, ATX-, LPP1-, LPP2-, or LPP3-targeted shRNAs. mRNA levels of (A) *ENPP2*, (B) *PLPP1*, (C) *PLPP2*, or (D) *PLPP3* were measured and quantified by qPCR. *RPLP0* was used to normalize the data (n=3). Bars represent the mean \pm SEM (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).



Supplementary Figure 4. ATX localization in hypoxic U87 and MDA-MB231 cell lines. (A-B)

Representative immunofluorescence images of (A) U87 or (B) MD-MB231 cells cultured on non-fluorescent gelatin for 4h in hypoxia (1%O₂) are shown. Images show ATX (green) and DiD (red) staining in non-permeabilized cells. Nuclei were stained with DAPI (blue). Magnification 60X, scale bars, 5 μ m.



Supplementary Figure 5. Localization of both ATX and LPPs in hypoxic U87 and MDA-MB231 cell lines. (A-C) Representative immunofluorescence images of U87 or MDA-MB231 cells cultured on non-fluorescent gelatin for 4h in hypoxia (1%O₂) are shown. Cells were stained for (A) LPP1, (B) LPP2, or (C) LPP3 (green) and ATX (red). Nuclei were stained with DAPI (blue). Magnification 60X, scale bars, 5 μm.