

# Supplementary Information

## Characterization of MYC-Induced Tumorigenesis by *in situ* Lipid Profiling

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## Materials and Methods

*Transgenic Mouse Liver Tissue.* The Tet-system previously was used to generate transgenic mice that conditionally express human c-MYC cDNA in murine hepatocytes. Briefly, transgenic LAP-tTA/TetO-MYC mice were bred and maintained with doxycycline (DOX, 100  $\mu\text{g}/\text{ml}$ ), and liver-specific MYC expression was induced subsequently by removing the DOX from the drinking water of adult, 6-week old mice. Liver tissue was collected prior to oncogene activation, at 2 or 4 months of MYC expression, or upon 4 months of MYC activation followed by 3 months of MYC inactivation. Extracted frozen tissue was sectioned at 15  $\mu\text{m}$ , and histology was examined by hematoxylin-eosin (H&E) staining. All animals were maintained and treated in accordance with the policies of Stanford University.

*Desorption Electrospray Ionization Mass Spectrometry Imaging.* Frozen mouse liver tissue sections that were deposited on a glass slide were defrosted and placed in a desiccator. After drying, the glass slide was placed on an XY translation stage. The DESI source spray was a solution of *N,N*-dimethylformamide:acetonitrile (1:1 by volume). The solution was infused at a rate of 0.7  $\mu\text{L}/\text{min}$  and the  $\text{N}_2$  flow rate was 0.6  $\text{L}/\text{min}$ . The step size of the XY translation stage was 150  $\mu\text{m}$  in the *x* and *y* directions. Mass spectra were acquired on an Orbitrap Exactive in negative ion mode. Tandem mass spectrometry data (accumulation time = 500 ms; activation time = 100 ms; normalized collision energy = 30%) was acquired on an LTQ linear ion trap mass spectrometer after DESI-MSI using the same tissue section.

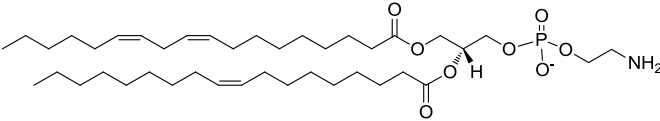
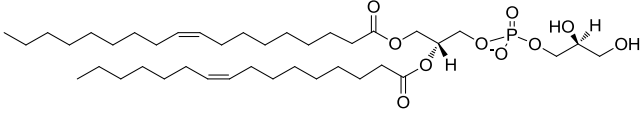
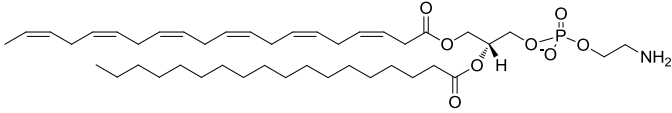
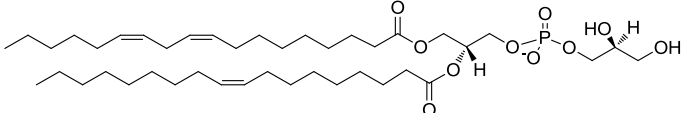
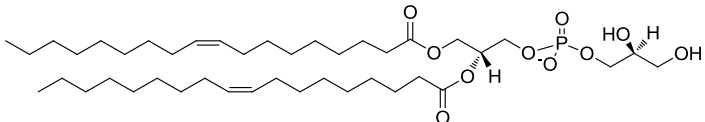
*Cancer Cell Lines.* A cell line (EC4) was generated from a MYC-induced HCC isolated from a LAP-tTA/TetO-MYC transgenic mouse. Cells ( $1 \times 10^6$ ) were treated with 20  $\text{ng}/\text{mL}$  DOX for 24 or 48 hrs to suppress human c-MYC expression, as confirmed by Western blot and

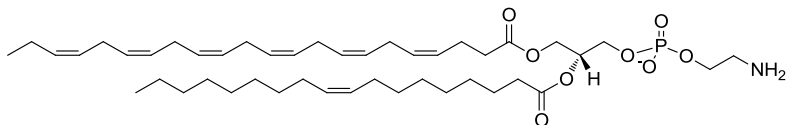
quantitative real-time PCR (qPCR). Cells were resuspended in 100  $\mu$ L PBS, and homogenized in an ultrasonic bath. Cellular lipids were extracted using 2:1 chloroform/methanol and submitted for high performance liquid chromatography (HPLC).

*High Performance Liquid Chromatography.* High performance liquid chromatography mass spectrometric analyses of the lipids in lysed EC4 samples were carried out using the approach described in *Methods in Molecular Biology*, **2011**, 708, 247. An Orbitrap Exactive mass spectrometer was used and the resolution was set to 100,000 at  $m/z$  400 for the analyses.

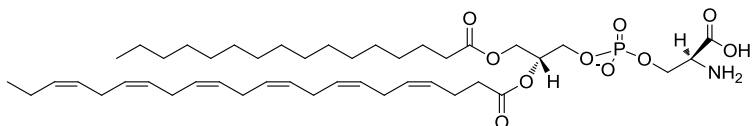
*Data Analysis.* The intensities of lipid species in specific sections of tissues were obtained from BioMap.<sup>1</sup> A region of interest was selected on the tissue, then the average intensity was multiplied by the number of points to give the total intensity for a given lipid species. These abundances were used to calculate the relative ratios shown in Table S1. For experiments involving EC4 cell lines, the abundance of lipid species were determined from areas in extracted ion chromatograms.

**Table S1.** Identification of lipids that exhibit changes associated with MYC-induced HCC tumor onset. Identified isomeric lipids and their compositions from MS<sup>n</sup> data are shown. The types of tissues are: MYC activated for 2 months (MYC ON/2), MYC activated for 4 months (MYC ON/4), and MYC activated for 4 months and then deactivated for 3 months (MYC ON/4-OFF/3). Cancer cell lines (EC4) had MYC deactivated for 24 hours (MYC OFF/24h) and 48 hours (MYC OFF/48h). MYC ON/4-MYC OFF/3 intensities were also compared relative to the average of MYC ON/2 and MYC ON/4. These values are shown in brackets in the MYC ON/4-MYC OFF/3 column (negative values indicate a reduction).

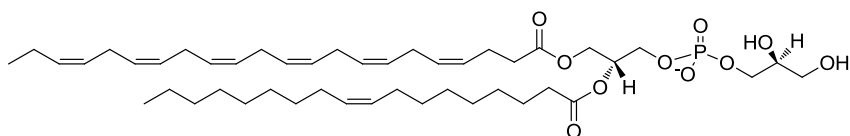
[M-H] <sup>-</sup>	Class	Species	%	Abundance Relative to WT (Normalized)				Abundance Relative to EC4 MYC ON	
				MYC ON/2	MYC ON/2 (adjacent)	MYC ON/4	MYC ON/4-MYC OFF/3	EC4: MYC OFF/24h	EC4: MYC OFF/48h
740.5265	PE	18:1/18:2 16:0/20:3 18:0/18:3	90 9 1	4	6	11	6 (1)	1	3
									
745.5051	PG	16:1/18:1 16:0/18:2 16:2/18:0	54 45 1	1	1	4	2 (2)	1	3
									
762.5105	PE	18:0/20:6 16:0/22:6 18:1/20:5 18:2/20:4 16:1/22:5	50 46 2 1 1	-3	2	1	2 (6)	1	2
									
771.5208	PG	18:1/18:2 18:0/18:3 16:0/20:3 16:1/20:2	95 3 1 1	93	15	296	59 (-2)	2	6
									
773.5365	PG	18:1/18:1 18:0/18:2 16:0/20:2 16:1/20:1	100 1 0.5 0.5	16	3	45	4 (-4)	1	6
									
788.5262	PE	18:1/22:6	100	4	3	3	2 (-2)	1	2



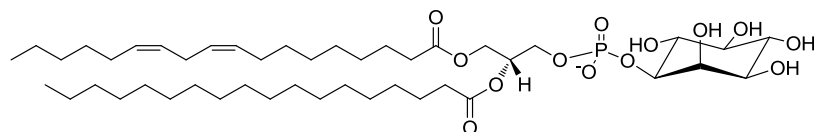
806.5005	PS	16:0/22:6	100	3	1	9	3 (1)	0	0
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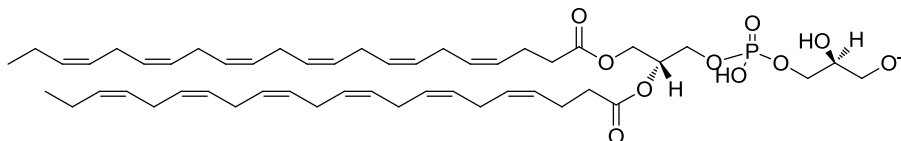
819.5207	PG	18:1/22:6 18:2/22:5 20:3/20:4	63 13 24	13	3	39	5 (-3)	2	5
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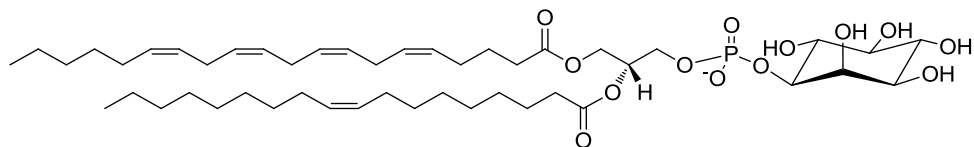
861.5528	PI	18:0/18:2 16:0/20:2 18:1/18:1	88 4 8	2	3	2	6 (3)	1	3
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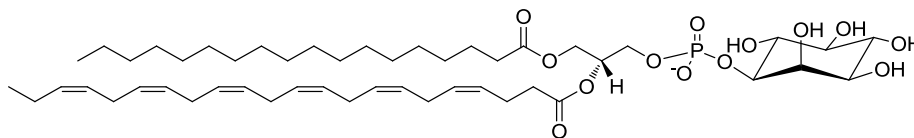
865.5057	PG	22:6/22:6	100	-6	2	3	3 (18)	0	0
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883.5373	PI	18:1/20:4 18:2/20:3 18:0/20:5	93 2 5	3	2	6	4 (2)	2	5
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909.5530	PI	18:0/22:6 20:2/20:4	95 5	2	2	3	5 (3)	1	1
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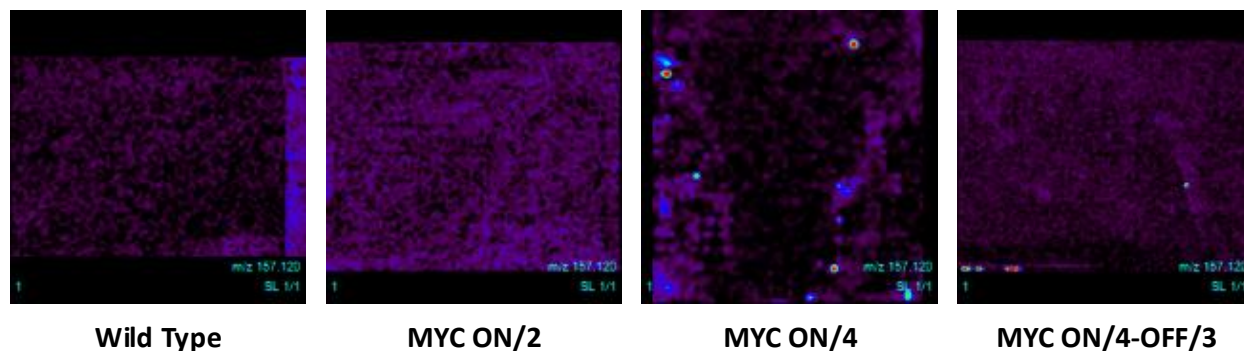
### Possible Phospholipid Correlations with MYC Expression

We examined how various phospholipids might be correlated with MYC expression by comparing the DESI-MSI to HPLC-MS analyses using a MYC-HCC derived cancer cell line, EC4. We performed HPLC-MS analyses of EC4 cancer cell lines under three conditions: (a) MYC ON, (b) MYC OFF/24h, and (c) MYC OFF/48h (Table S1). For example we found that the PG 18:1/18:2 is up-regulated in a HCC-derived cell line upon MYC inactivation. Importantly, in the tissue sections, the opposite behavior was observed. Thus, the average intensity of PG 18:1/18:2 for MYC ON/2 was compared to that of MYC ON/4-OFF/3, and we found a decrease by a factor of 2. A comparison to MYC ON/4 supported the same conclusion. These results suggest that PG 18:1/18:2 is regulated both in the host tumor microenvironment and in the tumor by MYC expression, but changes in the host appear to dominate. Notably, our results are reminiscent of recent studies suggesting that PGs inhibit DNA polymerase, DNA topoisomerase, and human cancer cell growth.<sup>2</sup> One intriguing possibility is that that PG 18:1/18:2 is released as part of a host response to tumor formation. However, additional studies will be required to address this.

We identified several additional species including m/z 740.5265 (18:1/18:2 PE), m/z 773.5365 (PG 18:1/18:1), m/z 788.5262 (phosphatidylethanolamine (PE) 18:1/22:6) and m/z 819.5207 (PG 18:1/22:6) (Table S1). Note that these behave similarly to PG 18:1/18:2 upon MYC activation and tumor progression. We note that PEs are of considerable interest because of their potential role in cell growth control via the enzyme phospholipase C, which hydrolyzes PEs to phosphoethanolamine and diacylglycerol, both of which are well known to be elevated in

human tumors, the latter of which is involved in cell cycle signaling by initiating apoptosis.<sup>3,4</sup> Interestingly, the species at  $m/z$  806.5005 (phosphatidylserine (PS) 16:0/22:6 are translocated to the outer leaflet of solid tumors) and  $m/z$  865.5057 (PG 22:6/22:6) are completely absent in the HCC-derived cell line indicating that they are likely regulated in the host in response to the tumor. Other lipids identified include phosphatidylinositol (PI 18:1/20:4), which was found to increase in both the HCC cell line and tumor tissue by an average of MYC ON/2 and MYC ON/4 compared to MYC ON/4-OFF/3. This implies these species are directly MYC-regulated in the cancer. Our results demonstrate that DESI-MSI can distinguish lipids that are specific both to the host tumor microenvironment as well as the tumor. We recognize that additional work will be required to determine the causal relationship between these changes and induction of MYC expression.

### Normalization



**Figure S1.** DESI-MS images of the background ion at  $m/z$  157.1229.

Normalization was carried out using the background ion at  $m/z$  157.1229 (Figure S1), which had global average absolute intensities of 49,76, 54, and 87 (average = 57; relative standard deviation = 27%) for wild type, MYC ON/2, MYC ON/4, and MYC ON/4-OFF/3, respectively.

## References

1. [http://www.maldi-msi.org/index.php?Itemid=32&id=14&option=com\\_content&view=article](http://www.maldi-msi.org/index.php?Itemid=32&id=14&option=com_content&view=article)
2. Ishimaru C., Takeuchi T., Sugawara F., Yoshida H., Mizushima Y. *Med. Chem.* **2010**, *6*, 114-122.
3. Agarwal M. L., Larkin H. E., Zaidi S. I. A., Mukhtar H., Oleinick N.L. *Cancer Res.* **1993**, *53*, 5897-5902.
4. Arias-Mendoza F., Smith M. R., Brown T.R. *Acad. Radiol.* **2004**, *11*, 368-376.