In vivo metabolic imaging identifies lipid vulnerability in a preclinical model of Her2+/Neu breast cancer residual disease and recurrence

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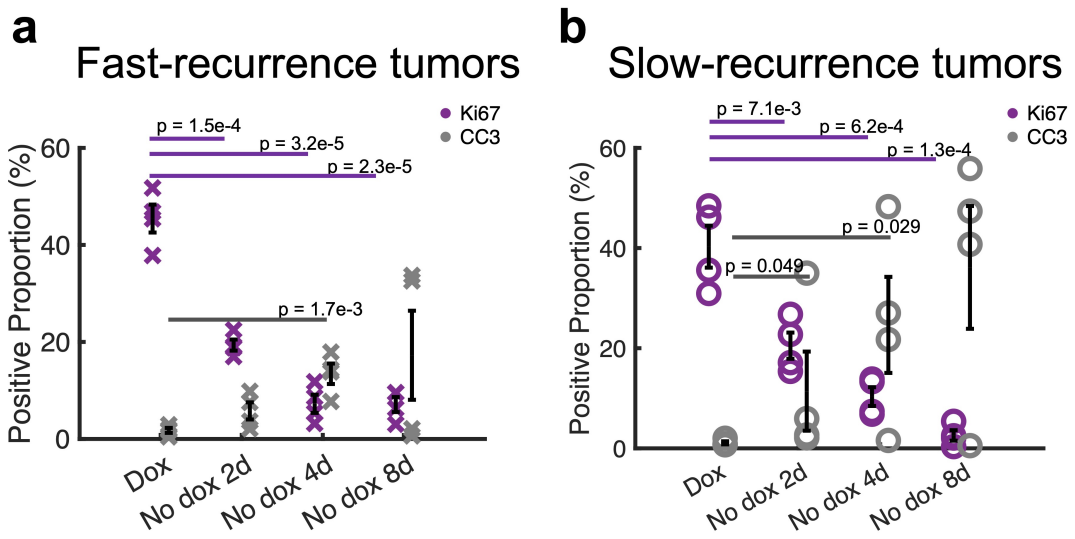
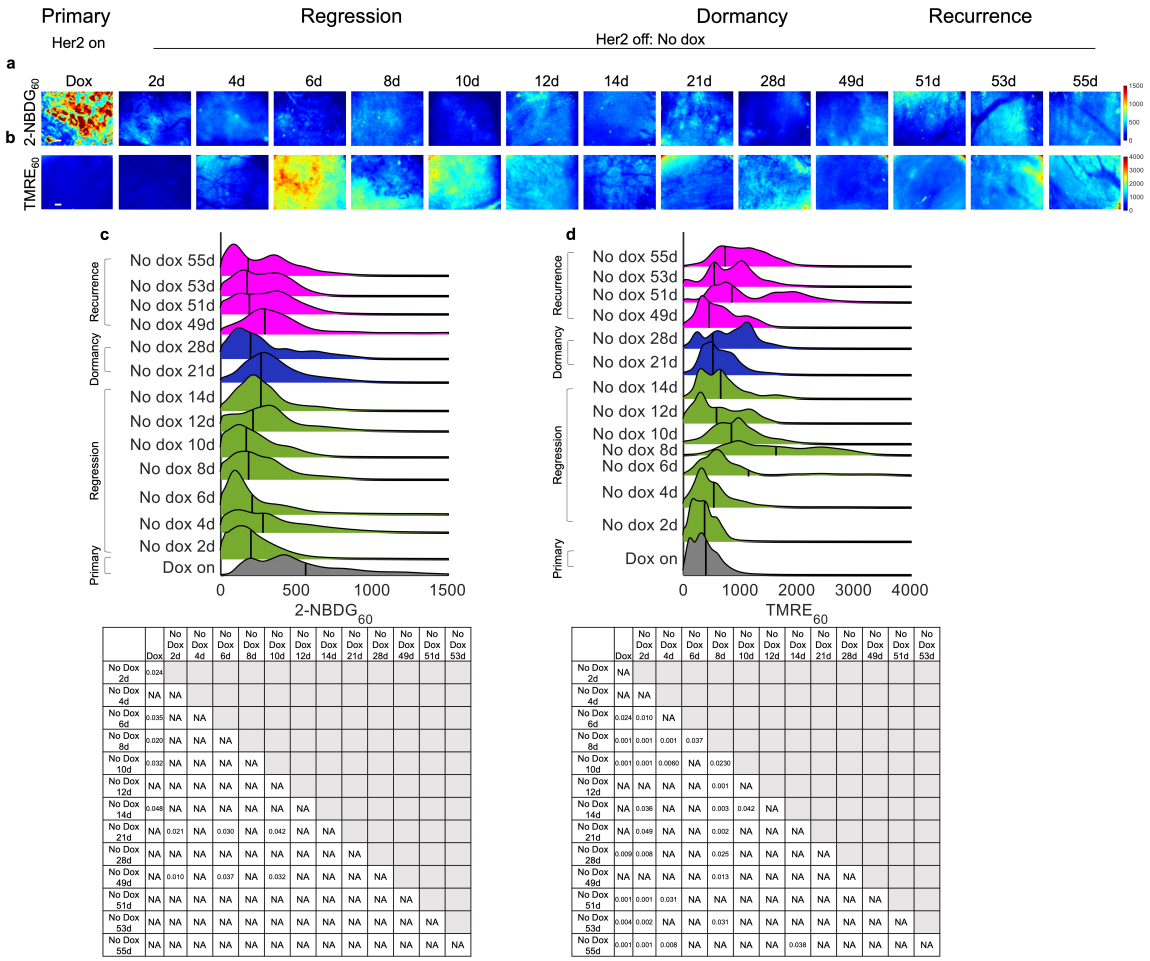
Supplementary Figure 1



Supplementary Figure 1: Quantification of immunohistochemistry results confirm changes in proliferation and apoptosis. Ki67 and cleaved caspase 3 (CC3) were imaged from sectioned primary (+dox) and regression (No dox 2, 4, 8 days) for (**a**) fast-recurrence tumors and (**b**) slow-recurrence tumors. Ki67 and CC3 proportions were quantified by binary thresholding each cell as either positive or negative for staining. Additionally, statistical analysis using a one-way ANOVA followed by a Tukey test for multiple comparisons shows the highest proportion of Ki67 positive cells in primary tumors (Dox) and an increase in CC3 positive cells in regressing cells (No dox). n = 4 tumors. Error bar = standard error of the mean.

Supplementary Figure 2

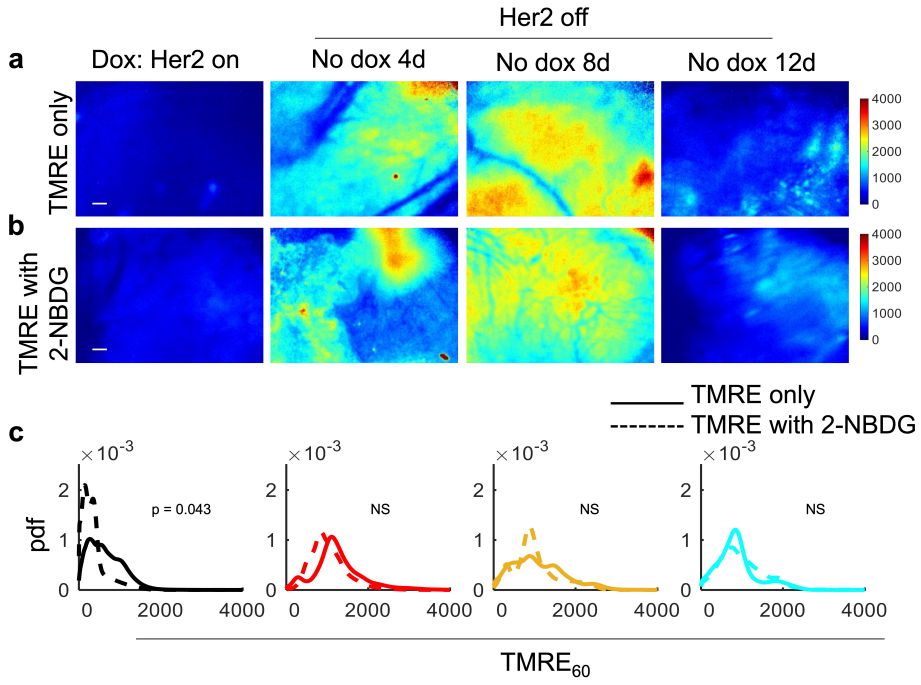


	Dox	No Dox 2d	No Dox 4d	No Dox 6d	No Dox 8d	No Dox 10d	No Dox 12d	No Dox 14d	No Dox 21d	No Dox 28d	No Dox 49d	No Dox 51d	No Dox 53d
No Dox 2d	0.024												
No Dox 4d	NA	NA											
No Dox 6d	0.035	NA	NA										
No Dox 8d	0.020	NA	NA	NA									
No Dox 10d	0.032	NA	NA	NA	NA								
No Dox 12d	NA	NA	NA	NA	NA	NA							
No Dox 14d	0.048	NA	NA	NA	NA	NA	NA						
No Dox 21d	NA	0.021	NA	0.030	NA	0.042	NA	NA					
No Dox 28d	NA	NA	NA	NA	NA	NA	NA	NA	NA				
No Dox 49d	NA	0.010	NA	0.037	NA	0.032	NA	NA	NA	NA			
No Dox 51d	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
No Dox 53d	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
No Dox 55d	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

	Dox	No Dox 2d	No Dox 4d	No Dox 6d	No Dox 8d	No Dox 10d	No Dox 12d	No Dox 14d	No Dox 21d	No Dox 28d	No Dox 49d	No Dox 51d	No Dox 53d
No Dox 2d	NA												
No Dox 4d	NA	NA											
No Dox 6d	0.024	0.010	NA										
No Dox 8d	0.001	0.001	0.001	0.037									
No Dox 10d	0.001	0.001	0.0060	NA	0.0230								
No Dox 12d	NA	NA	NA	NA	0.001	NA							
No Dox 14d	NA	0.036	NA	NA	0.003	0.042	NA						
No Dox 21d	NA	0.049	NA	NA	0.002	NA	NA	NA					
No Dox 28d	0.009	0.008	NA	NA	0.025	NA	NA	NA	NA				
No Dox 49d	NA	NA	NA	NA	0.013	NA	NA	NA	NA	NA			
No Dox 51d	0.001	0.001	0.031	NA	NA	NA	NA	NA	NA	NA	NA		
No Dox 53d	0.004	0.002	NA	NA	0.031	NA	NA	NA	NA	NA	NA	NA	
No Dox 55d	0.001	0.001	0.008	NA	NA	NA	NA	0.038	NA	NA	NA	NA	NA

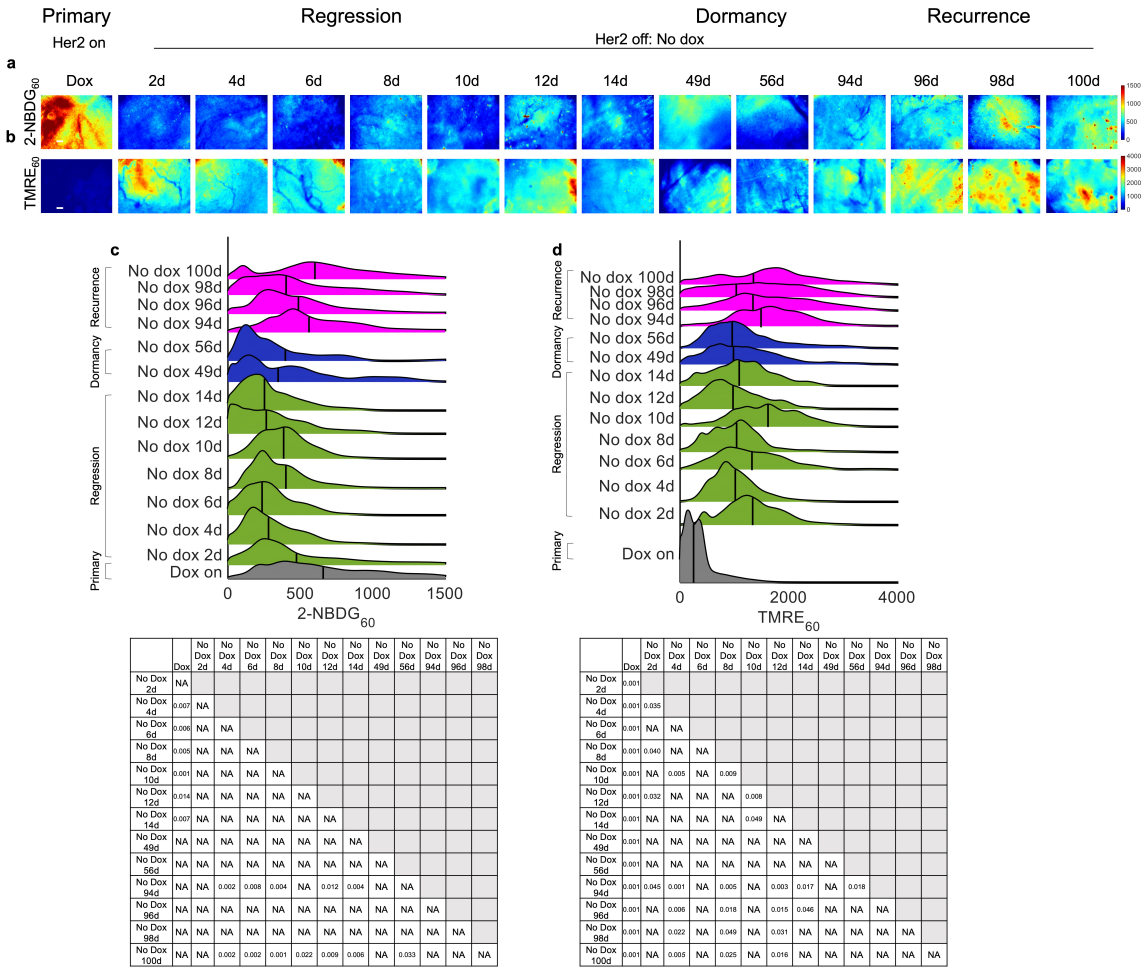
Supplementary Figure 2: Metabolic imaging following Her2 downregulation in tumors with a short period of dormancy. Representative images of (**a**) 2-$NBDG_{60}$ and (**b**) $TMRE_{60}$ in a mammary tumor for Her2 on (+ dox) (n = 10) and Her2-off imaged every other day for 14 days (n = 10), during sustained growth arrest (No dox 21 and 28 days dox) (n = 8), and during regrowth (No dox 49-55 days) (n = 7). Scale bar = 200 μm. (**c**) 2-$NBDG_{60}$ and (**d**) $TMRE_{60}$ PDF ridgeline plots for Her2 on (+ dox) or Her2 off (- dox) across all pixels and all mice at each time point. Vertical lines superimposed on PDF curves report the average fluorescence. Statistical comparisons were performed using a Kolmogorov-Smirnov test; NS = not significant.

Supplementary Figure 3



Supplementary Figure 3: Simultaneous imaging does not mask metabolic changes following Her2 downregulation. Primary (Her2 on) and Her2 downregulated tumors in mammary window chambers were imaged following TMRE only or TMRE and 2-NBDG injections. (**a**) Representative $TMRE_{60}$ images following TMRE only injection from mammary tumors for Her2 on (+ dox) (n = 10 mice) or Her2-off (4, 8, and 12 days after dox was removed, n = 12, 10, 7, respectively). (**b**) Representative $TMRE_{60}$ images following TMRE + 2-NBDG injection from mammary tumors for Her2 on (+ dox) or Her2-off (4, 8, and 12 days after dox was removed) (all groups; n = 10 mice). Scale bar = 200 μm. (**c**) $TMRE_{60}$ PDFs for Her2 on (+ dox) or Her2 off (- dox) across all pixels and all mice in each group. Statistical comparisons were performed using a Kolmogorov-Smirnov test; NS = not significant.

Supplementary Figure 4

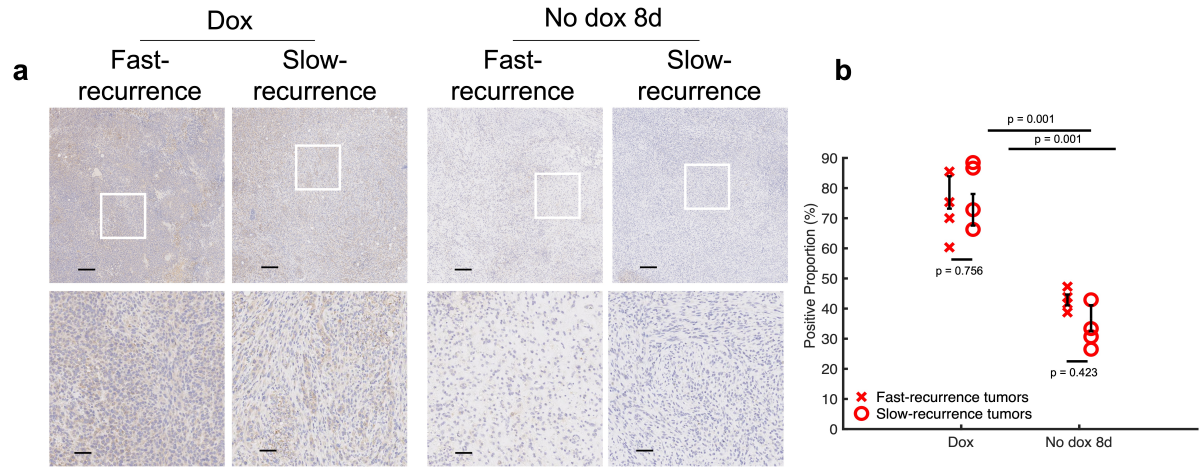


	Dox	No Dox 2d	No Dox 4d	No Dox 6d	No Dox 8d	No Dox 10d	No Dox 12d	No Dox 14d	No Dox 49d	No Dox 56d	No Dox 94d	No Dox 96d	No Dox 98d
No Dox 2d	NA												
No Dox 4d	0.007	NA											
No Dox 6d	0.006	NA	NA										
No Dox 8d	0.005	NA	NA	NA									
No Dox 10d	0.001	NA	NA	NA	NA								
No Dox 12d	0.014	NA	NA	NA	NA	NA							
No Dox 14d	0.007	NA	NA	NA	NA	NA	NA						
No Dox 49d	NA	NA	NA	NA	NA	NA	NA	NA					
No Dox 56d	NA	NA	NA	NA	NA	NA	NA	NA	NA				
No Dox 94d	NA	NA	0.002	0.008	0.004	NA	0.012	0.004	NA	NA			
No Dox 96d	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
No Dox 98d	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
No Dox 100d	NA	NA	0.002	0.002	0.001	0.022	0.009	0.006	NA	0.033	NA	NA	NA

	Dox	No Dox 2d	No Dox 4d	No Dox 6d	No Dox 8d	No Dox 10d	No Dox 12d	No Dox 14d	No Dox 49d	No Dox 56d	No Dox 94d	No Dox 96d	No Dox 98d
No Dox 2d	0.001												
No Dox 4d	0.001	0.035											
No Dox 6d	0.001	NA	NA										
No Dox 8d	0.001	0.040	NA	NA									
No Dox 10d	0.001	NA	0.005	NA	0.009								
No Dox 12d	0.001	0.032	NA	NA	NA	0.008							
No Dox 14d	0.001	NA	NA	NA	NA	0.049	NA						
No Dox 49d	0.001	NA	NA	NA	NA	NA	NA	NA					
No Dox 56d	0.001	NA	NA	NA	NA	NA	NA	NA	NA				
No Dox 94d	0.001	0.045	0.001	NA	0.005	NA	0.003	0.017	NA	0.018			
No Dox 96d	0.001	NA	0.006	NA	0.018	NA	0.015	0.046	NA	NA	NA		
No Dox 98d	0.001	NA	0.022	NA	0.049	NA	0.031	NA	NA	NA	NA	NA	
No Dox 100d	0.001	NA	0.005	NA	0.025	NA	0.016	NA	NA	NA	NA	NA	NA

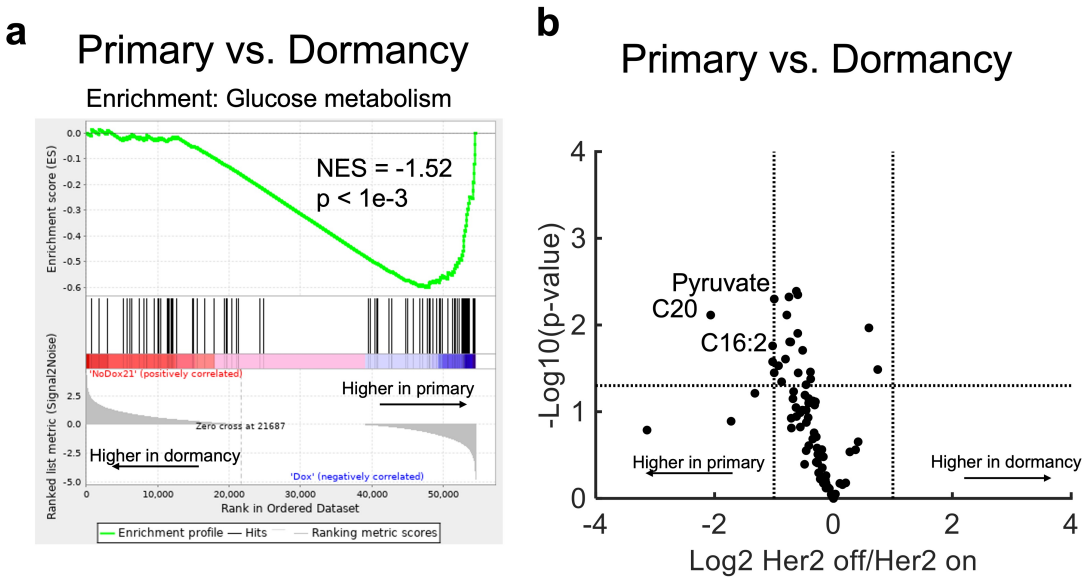
Supplementary Figure 4: Metabolic imaging following Her2 downregulation in tumors with a long period of dormancy. Representative images of (**a**) 2-NBDG$_{60}$ and (**b**) TMRE$_{60}$ in a mammary tumor for Her2 on (+ dox) (n = 10) and Her2-off imaged every other day for 14 days (n = 10), during sustained growth arrest (No dox 49 and 56 days – dox) (n = 8), and during regrowth (No dox 94-100 days – dox) (n = 9). Scale bar = 200 μm. (**c**) 2-NBDG$_{60}$ and (**d**) TMRE$_{60}$ PDF ridgeline plots for Her2 on (+ dox) or Her2 off (- dox) across all pixels and all mice at each time point. Vertical lines superimposed on PDF curves report the average fluorescence. Statistical comparisons were performed using a Kolmogorov-Smirnov test; NS = not significant.

Supplementary Figure 5



Supplementary Figure 5: Immunohistochemistry and quantification of ATP5A1 as a surrogate for mitochondrial content comparison between fast and slow recurring tumors. ATP5A1 (brown), a key subunit of the ATP synthase complex localized within the mitochondria, was (**a**) imaged and (**b**) quantified from sectioned primary (Dox) and regressing tumors. White boxes indicate magnified section shown in bottom panels. Scale bars represent 200μm (top panels) and 50μm (bottom panels). ATP5A1 proportions were quantified by binary thresholding each cell as either positive or negative for staining. Additionally, statistical analysis using a one-way ANOVA followed by a Tukey test for multiple comparisons shows no significant difference in ATP5A1 positive cells in slow- compared to fast-recurrence primary (Dox) tumors and slow- compared to fast-recurrence regression. n = 4 tumors. Error bar = standard error of the mean.

Supplementary Figure 6



Supplementary Figure 6: Changes in transcription and metabolite levels in dormant tumors compared to primary tumors. (**a**) Gene set enrichment analysis (GSEA) of primary (dox) and dormant (no dox 21 days) tumors showing enrichment of the glucose metabolism gene set in primary tumors compared to dormant tumors, similar to results seen in primary tumors compared to regressing tumors. Enrichment scores were calculated using the KS statistic and p-values were calculated using permutation testing with 1000 permutations. (**b**) Volcano plot of fold-change in metabolite levels between dormant and primary tumors. Dashed lines represent $p<0.05$ via two-sided t-test and $|Log2FoldChange|>1$. Significantly different metabolites are labeled within graph. n = 5 per group.