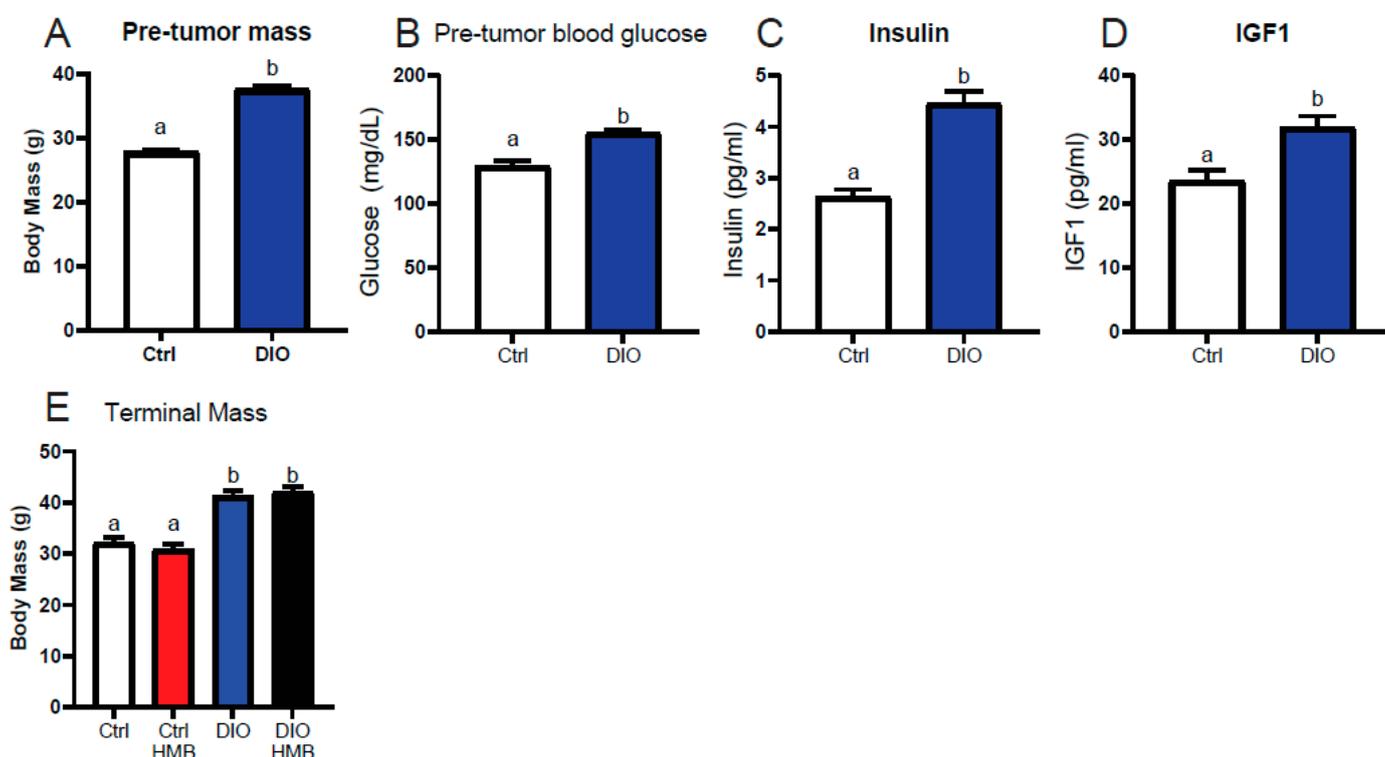
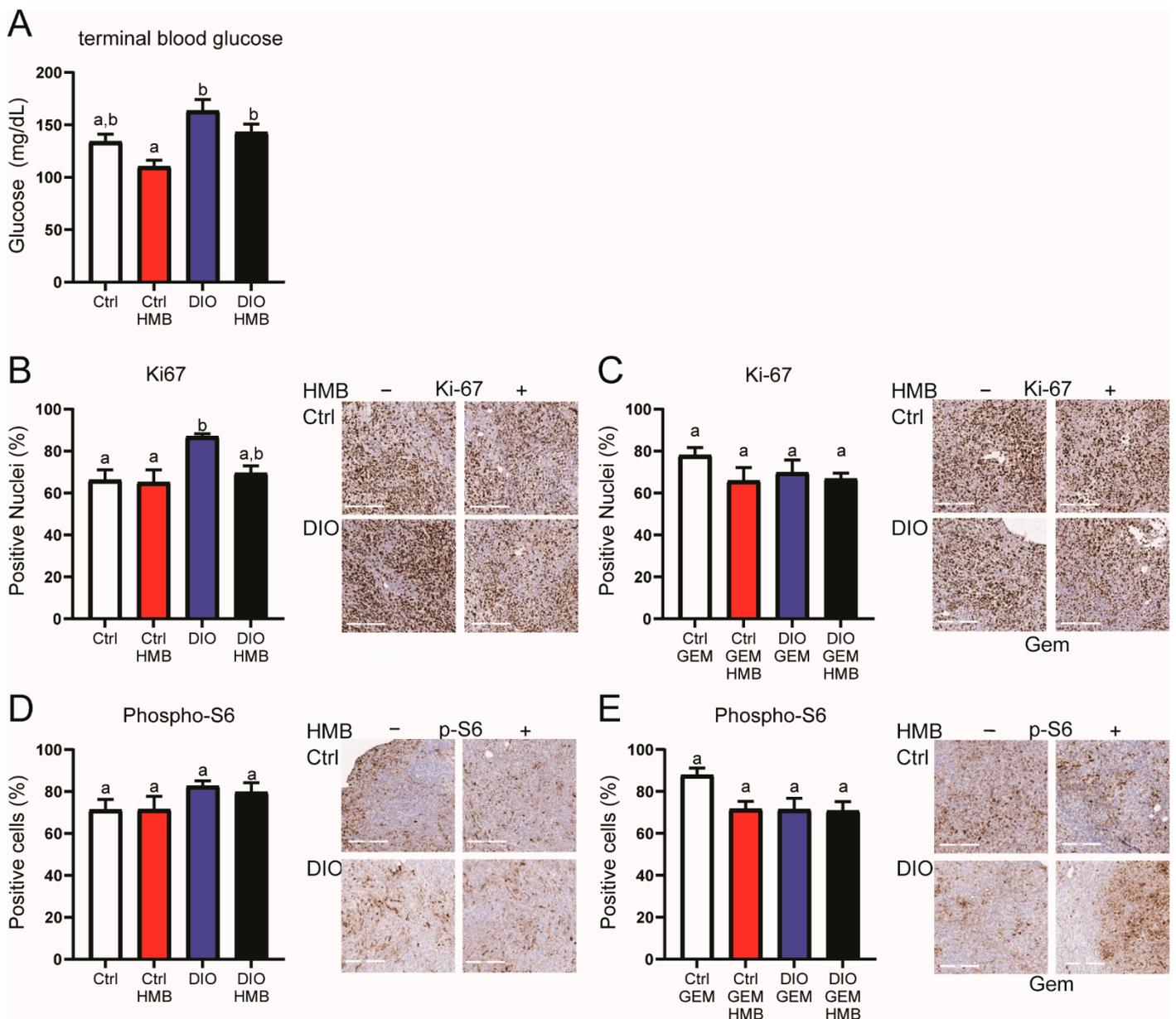


# Supplementary Materials: $\beta$ -Hydroxy- $\beta$ -Methylbutyrate Supplementation Promotes Antitumor Immunity in An Obesity Responsive Mouse Model of Pancreatic Ductal Adenocarcinoma

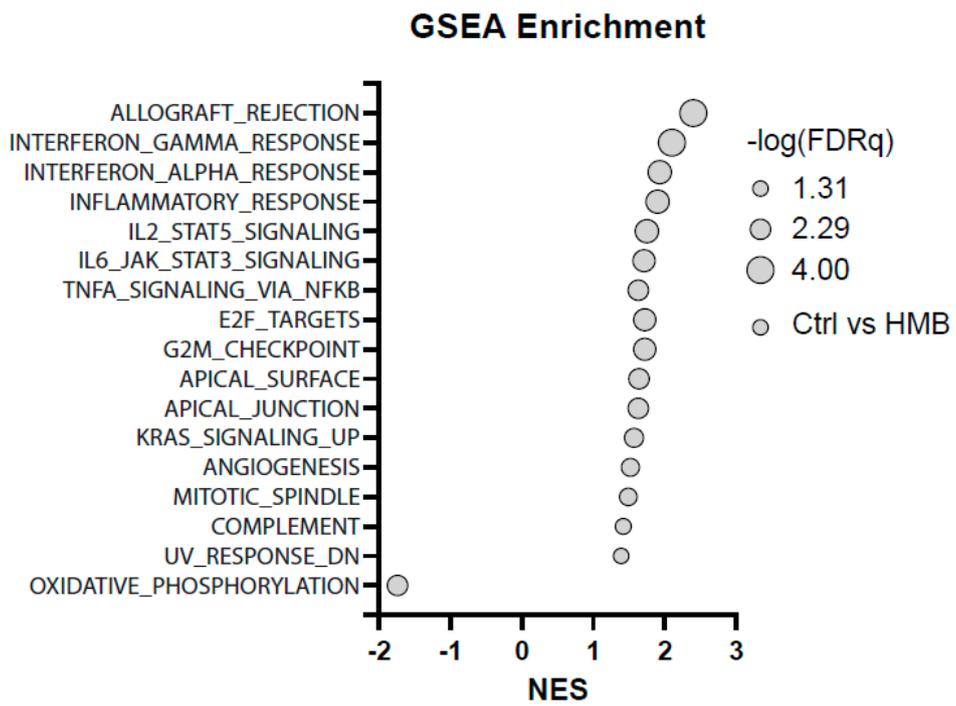
Michael F Coleman, Kristyn A Liu, Alexander J Pfeil, Suhas K Etigunta, Xiaohu Tang, Salvador Fabela, Laura M Lashinger, Zhengrong Cui and Stephen D Hursting



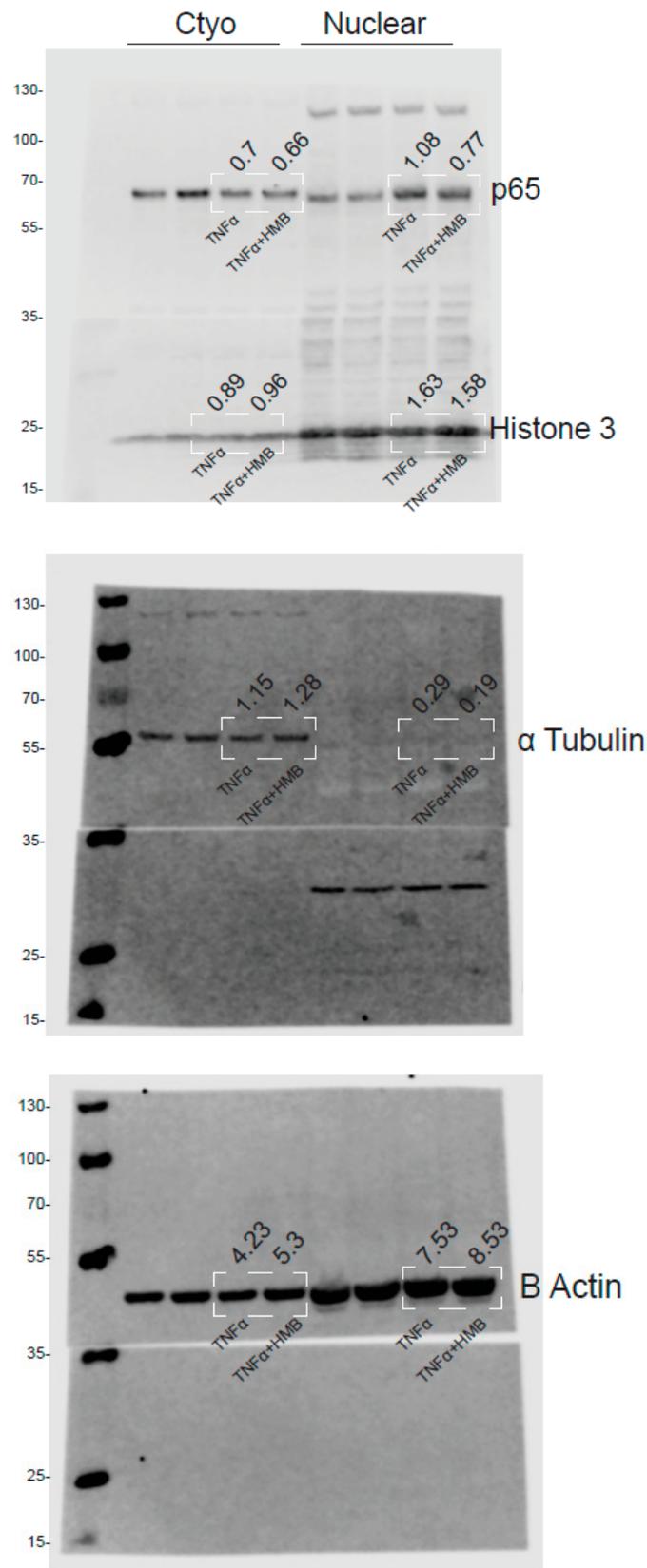
**Figure S1.** Metabolic effects of DIO and HMB supplementation. Following 10 weeks of DIO diet regimen metabolic alterations in control and DIO mice was assessed. (A) mouse body mass (n=28-30/group). (B-D) Blood glucose (n=28-30/group), serum insulin(n=26-30/group), and serum IGF-1(n=24-26/group) following 6 hour fast. (E) Terminal mouse body mass following HMB supplementation (n=14-15/group). All data are presented as mean  $\pm$  SEM. Differences are considered significant if  $p < 0.05$  as indicated by different letters within the same graph.



**Figure S2.** Blood glucose, proliferation, and apoptosis are not altered by HMB. (A) Fasted blood glucose following 6 hour fast (n=14/group). (B-E) immunohistochemistry staining of tumor sections for Ki67 (B-C) and phosphor-S6 (D-E), in treatment naïve (B&D) or gemcitabine treated tumors (C&E) (n=5-6/group). All data are presented as mean ± SEM. Differences are considered significant if  $p < 0.05$  as indicated by different letters within the same graph.



**Figure S3.** HMB supplementation alters GSEA Hallmark enrichments in muscles. Significant GSEA enrichments from Hallmark gene sets resulting from comparing Control vs HMB (grey), FDRq is denoted by bubble size (n=7/group).



**Figure S4.** Uncropped Western blots for Figure 7E. C2C12 myotubes treated with HMB for 18 h in serum-free media containing 10 ng/ml TNF $\alpha$ . Western blot of nuclear p65 protein levels.