Supplemental Figure Legends

Figure S1. AT suppresses growth of MYC-induced HCC cell line EC4. a) AT inhibits the proliferation of MYC-induced murine HCC tumor-derived cell line, EC4. MTT assay was performed every 24 hours for 4 days on EC4 cells treated with 10 μM AT, AT plus 100μM MV, or DMSO control. Cells were also treated with 20ng/mL Dox to inactivate transgenic MYC as a positive control. All experiments were repeated 3 times ($p<0.0001$). **b)** MTT shows a dose-dependent suppression of EC4 cell growth. Cells were treated with AT, AT plus MV, DMSO, or Dox for 96 hours. **c)** Immunofluorescence for Ki67 on EC4 cells treated with 10μM AT for 24 hr demonstrates that statin treatment inhibits HCC proliferation ($p<0.0001$). **d)** FACS analysis of AnnexinV staining of AT-treated murine HCC cell line EC4 shows a significant increase in apoptosis due to AT treatment ($p<0.0001$). Cell were treated with 10μM AT for 48 hours. **e)** AT induces cell cycle arrest in murine HCC as assessed by FACS analysis of PI stained cells ($p=0.01$). Cells were treated with 10μM AT for 48 hours. Together, these data indicate that MYC-induced HCC cell lines are similarly responsive to growth suppression via AT treatment.

Figure S2. AT suppresses growth of murine and human HCC cell lines in a dose-dependent manner. a) MTT shows a dose-dependent suppression of growth of MYC-induced murine HCC cell line 3-4. Cells were treated with AT, AT plus MV, AT plus 10μM GGPP, AT plus 10μM FPP, DMSO, or Dox for 96hrs. DMSO *versus* Dox, DMSO *versus* 10 μM AT; *p<0.0001*. **b)** MTT reveals dose-dependent suppression of growth of human HCC cell line Huh7. Cells were treated with AT, AT plus MV, AT

plus 10μM GGPP, AT plus 10μM FPP, or DMSO for 96hrs. DMSO *versus* 10 μM AT; *p<0.0001*.

Figure S3. AT demonstrates reduced efficacy in murine cell line TP3. a) The TP3 cell line, though derived from a MYC-dependent tumor, demonstrates minimal expression of the human c-MYC transgene and therefore is resistant to growth inhibition via Dox treatment. Accordingly, AT shows minimal inhibition of the proliferation of murine cell line, TP3. MTT assay was performed every 24 hours for 4 days on TP3 cells treated with AT, AT plus MV, DMSO, or Dox. All experiments were repeated 3 times. DMSO *versus* Dox, *p=0.19*; DMSO *versus* 10 μM AT, *p=0.10*, DMSO *versus* AT+MV, *p=0.42*. **b)** MTT shows suppression of TP3 cell growth only at high doses. Cells were treated for with AT, AT plus MV, DMSO, or Dox 96hrs. TP3 cells are not as sensitive to AT treatment as other murine and human HCC cell lines, suggesting that AT may be more effective on HCC cells that are maintained by MYC expression.

Figure S4. Optimization of AT dose for *in vivo* treatment. a) FVB/N mice were treated 3 times per week with PBS, 100mg/kg AT, or 300mg/kg AT for 2 weeks. H&E staining shows that the liver maintained normal hepatic structure upon 100mg/kg AT treatment but was disrupted upon 300mg/kg AT treatment. b) Serum bilirubin level was measured as an indicator of AT-induced liver damage. Levels remained largely unchanged at 100mg/kg, showing a dramatic increase at 300mg/kg. The absence of hepatotoxicity thereby indicates that 100mg/kg AT can be utilized *in vivo*.

Figure S5. Bioluminescence c-MYC phospho-sensor can detect AT-dependent inhibition of MYC in additional human HCC cell lines. a) HepG2 human HCC cells were transiently transfected with the c-Myc phosphorylation sensor for 24hrs and treated with AT at the indicated concentrations for 18hrs followed by bioluminescent imaging (BLI) as described in Material and Methods. FL activity was normalized to the RL activity and the total protein and plotted against the drug concentration, $p<0.0001$.

Figure S6. Time course of control Renilla Luciferase expression/activity following hydrodynamic injection. a) The time course of transient expression of the control renilla luciferase (RL) plasmid was determined by BLI of an FVB/N mouse after hydrodynamic injection. Mice demonstrated efficient RL activity as early as 18 hours, persisting to 66 hours. **b)** Photon output of RL activity was plotted against hours after injection.

Figure S7. Time course of c-MYC bioluminescence phospho-sensor expression following hydrodynamic injection. a) The time course of transient expression of the c-Myc phosphorylation sensor was determined by BLI of a control FVB/N mouse (Test mouse) and two LAP-tTA/TRE-MYC transgenic mice (Experimental mouse) after hydrodynamic injection of the sensor plasmid. Test mouse demonstrated highest and most focal FL activity 22 hours post injection. Experimental mice showed high, focal activity of FL 24 hours post injection. Together with RL expression (Fig. S6), we determined 22 hours post injection to be appropriate for assessing c-Myc phosphorylation

in vivo using our phospho-sensor. **b)** Photon output of FL activity was plotted against the hours after injection.

Figure S8. AT suppresses MYC transcriptional activity. a) Liver tissue from LAP-tTA/TRE-MYC transgenic mice treated with PBS, 100mg/kg AT, or AT plus 20mg/kg MV was assessed for phospho-MYC and protein expression of MYC target genes Cdk4 and E2F1. Immunoblot reveals AT inhibition of MYC phosphorylation and transcriptional activity *in vivo*. Graph represents the relative intensity of bands on the immunoblots, normalized to Tubulin and relative to PBS control. **b)** Human Huh7 cells were treated with PBS, 10μM AT, or AT plus 100μM MV for 24hrs. Immunoblot shows suppression of MYC phosphorylation and transcriptional activity via reduced Cdk4 and E2F1 target gene expression. Band intensity was normalized to Tubulin and relative to PBS control.

Figure S9. Verification of adenovirus infection in HCC cells. a) Successful infection of Ad-MYC^{WT}, Ad-MYC^{S62A} or Ad-MYC^{T58A} in tumor masses as assessed by co-expression of GFP. Tumor sections were analyzed by immunofluorescence of GFP encoded by the adenovirus. **b)** Inactivation of endogenous MYC upon Dox treatment. HCC cells were treated with or without Dox (20ng/ml). Cell lysates were immunoblotted with antibodies to total c-MYC and tubulin.

Figure S10. GGPP abrogates AT suppression of MYC transcriptional activity. a) qRT-PCR of MYC target genes was performed in HCC cells treated with PBS, 10μM AT

AT, and 10μM AT with either 100μM MV, 10μM FPP, or 10μM GGPP. Co-treatment with MV recovered Cdk4 and E2F1 expression to untreated levels, while GGPP rescued above untreated controls and FPP failed to rescue the effect of AT on MYC transcriptional activation of Cdk4 and E2F1. For CDK4, PBS *versus* AT, $p<0.0001$; PBS *versus* AT+MV, $p=0.06$, PBS *verus* AT+FPP, $p<0.0001$, PBS *versus* AT+GGPP, $p<0.0001$ with the rescued expression surpassing the control-treated expression. For E2F1, PBS *versus* AT, $p<0.0001$; PBS *versus* AT+MV, $p<0.01$, PBS *verus* AT+FPP, $p<0.0001$, PBS *versus* AT+GGPP, $p<0.0001$ with the rescued expression surpassing the control-treated expression.