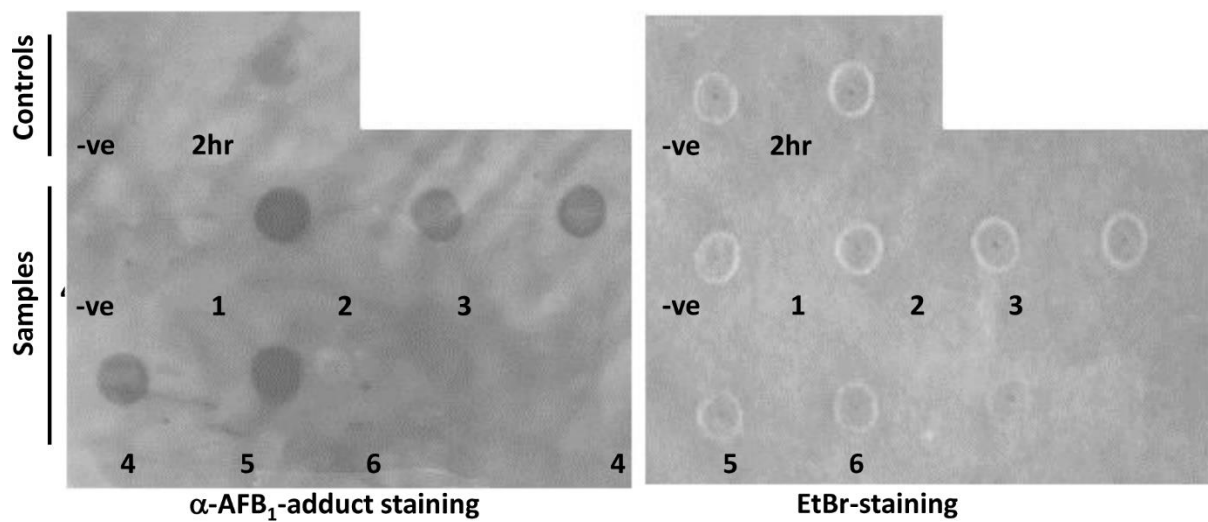
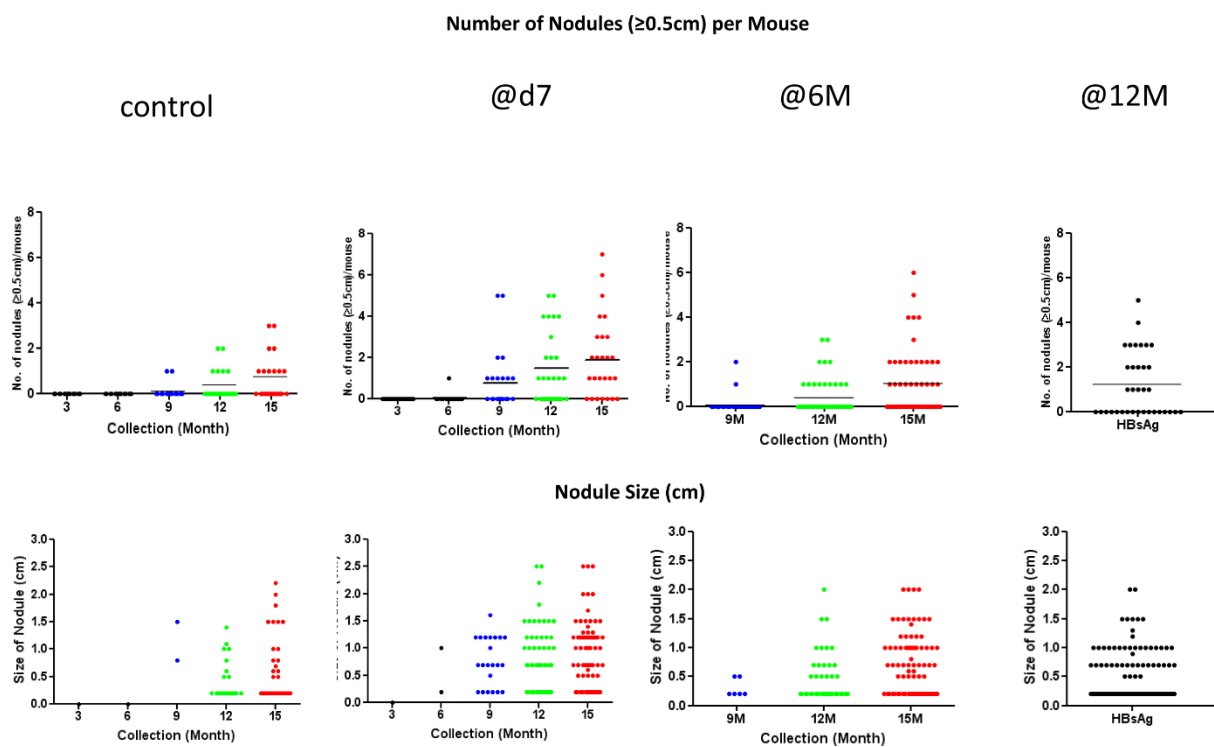


## Supplementary figures



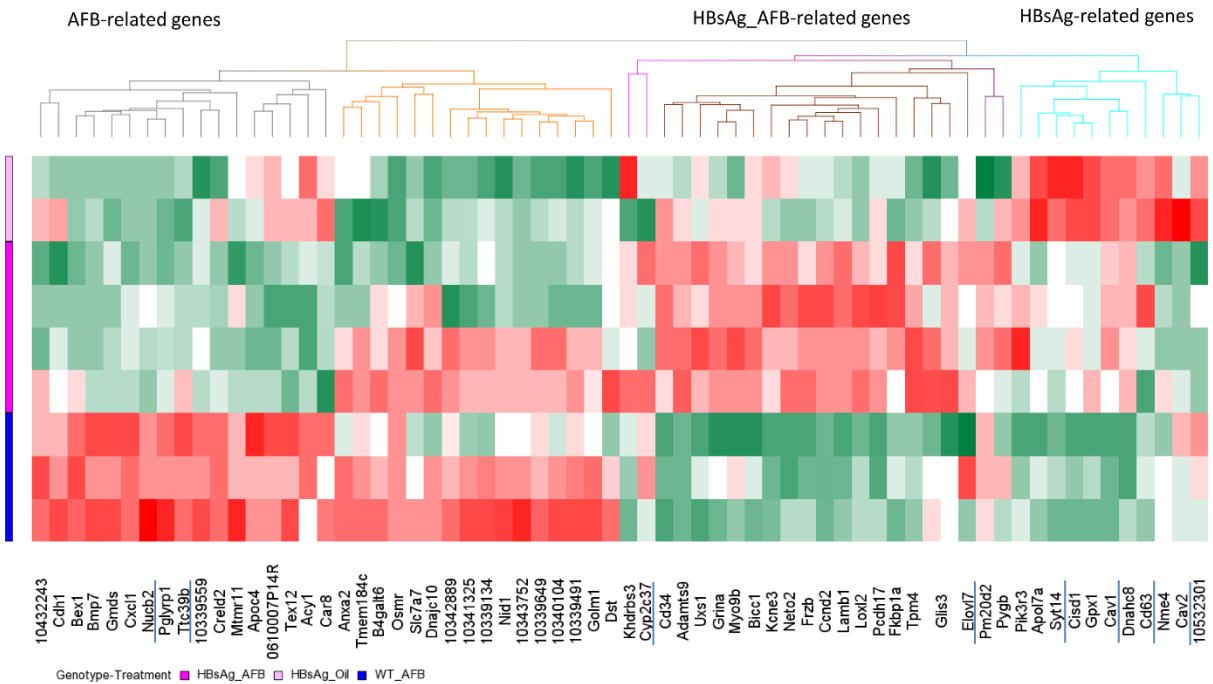
**Figure S1. Dot-blot analysis of AFB<sub>1</sub>-induced adducts**

South-western dot-blot analysis was performed using 4 µg genomic DNA (gDNA) from livers of the control or AFB<sub>1</sub>-injected (samples) mice (@D7) and the anti-AFB<sub>1</sub>-adduct antibody. Loading for DNA was visualized by ethidium bromide (EtBr) staining. –ve represents without the anti-AFB<sub>1</sub> antibody. gDNA samples 1-6 are from six individual mice, harvested 2 h post AFB<sub>1</sub>-injection.



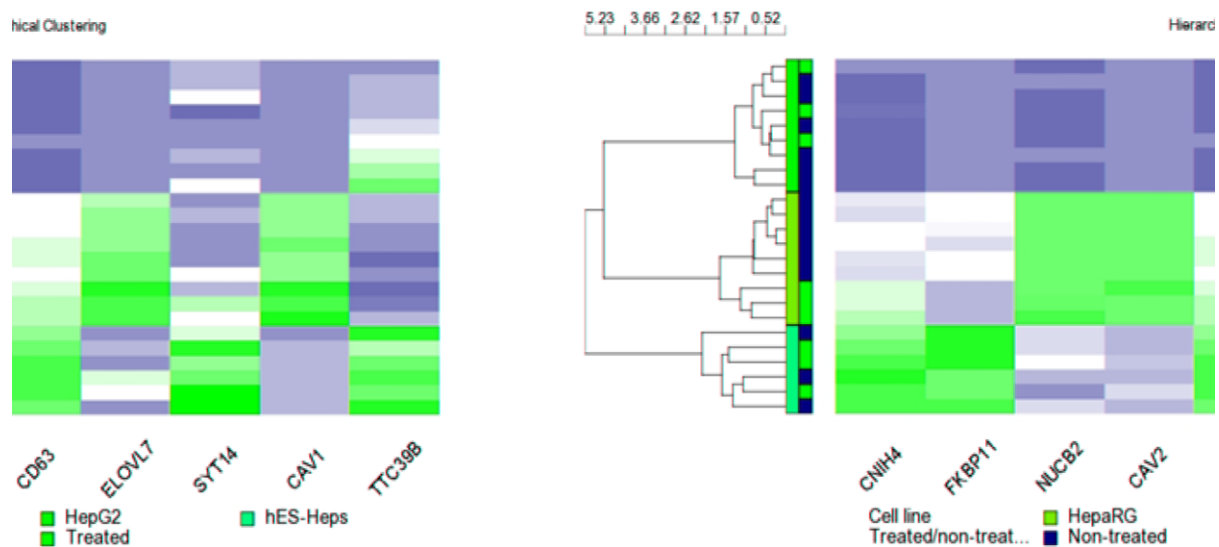
**Figure S2. Characterization of liver nodules**

Liver samples were scored and plotted as described in the main legends (Figure 2). Direct comparison of HBsAg mice that were AFB<sub>1</sub>-injected at D7, 6M or 12M are shown, together with D7 oil controls.

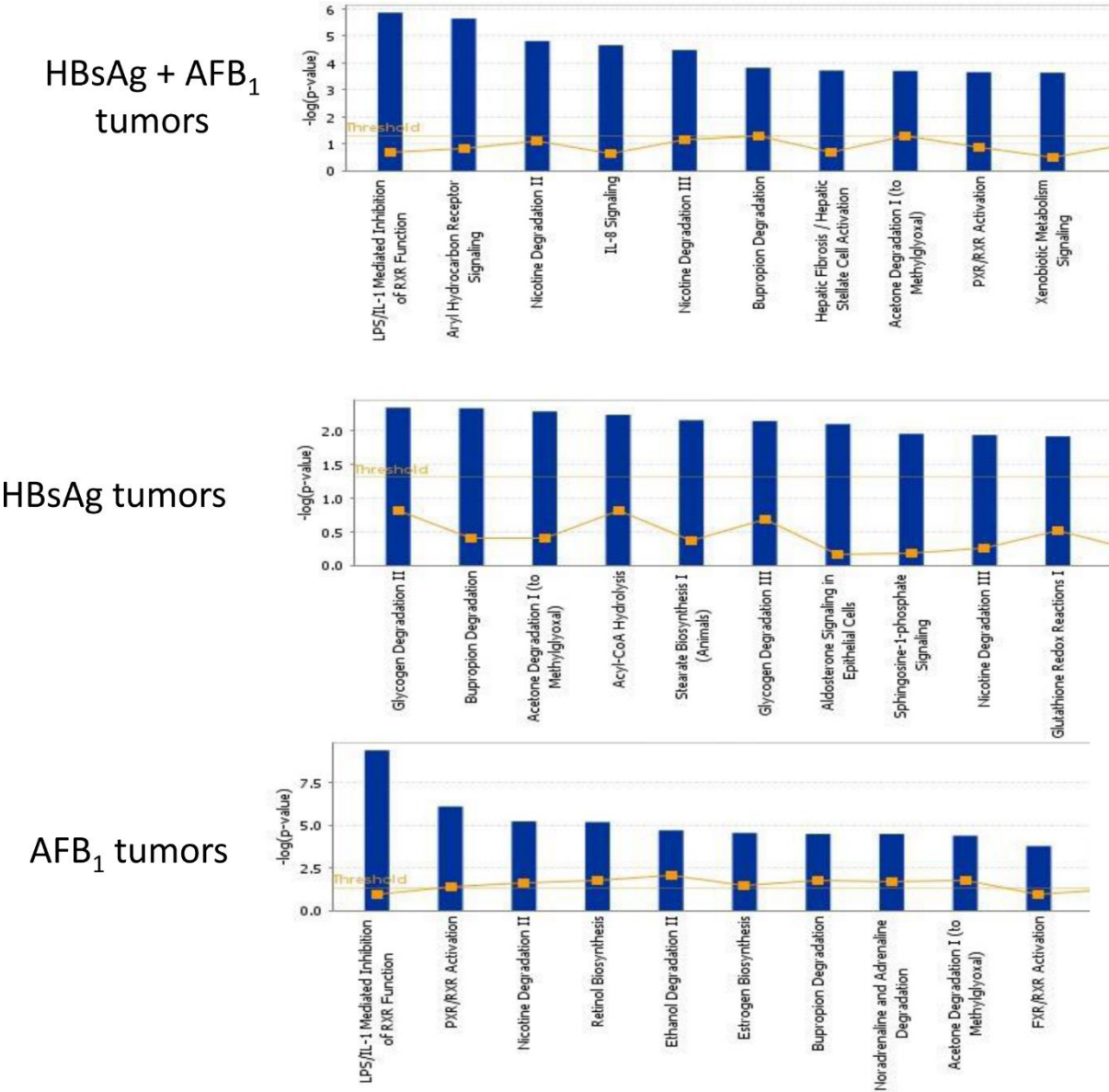


**Figure S3. Divergent pathways are deregulated by HBsAg and AFB<sub>1</sub>**

Genes obtained from HBsAg\_AFB\_T vs WT\_Oil\_N, HBsAg\_Oil\_T vs WT\_Oil\_N, and WT\_AFB\_T vs WT\_Oil\_N ( $n=66$ , Bonferroni corrected) showed a stepwise model based on genotype and treatment. Note: 8 of the 9 HCC genes are in the list (underlined).

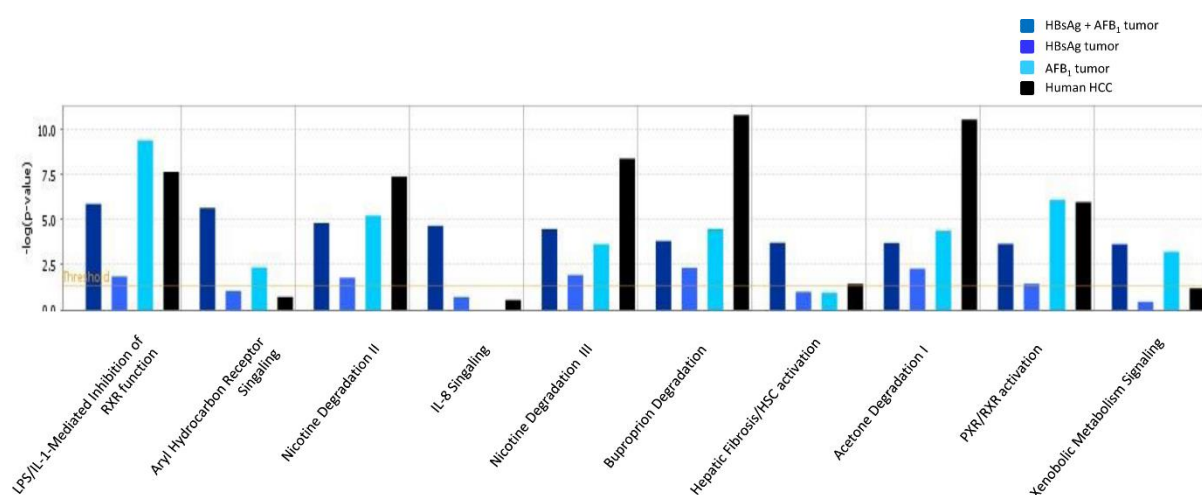


(A,B) Differential clustering between tumor and normal HCCs was observed when segregated by the HCC gene signature pattern, similar to the mouse HCC model (A). However, the HCC gene signature was able to distinguish between primary (HepaRG or embryonic stem cell [hES]-derived) and transformed (HepG2) hepatocytes (B). ‘Treated’ refers to AFB<sub>1</sub> treatment.



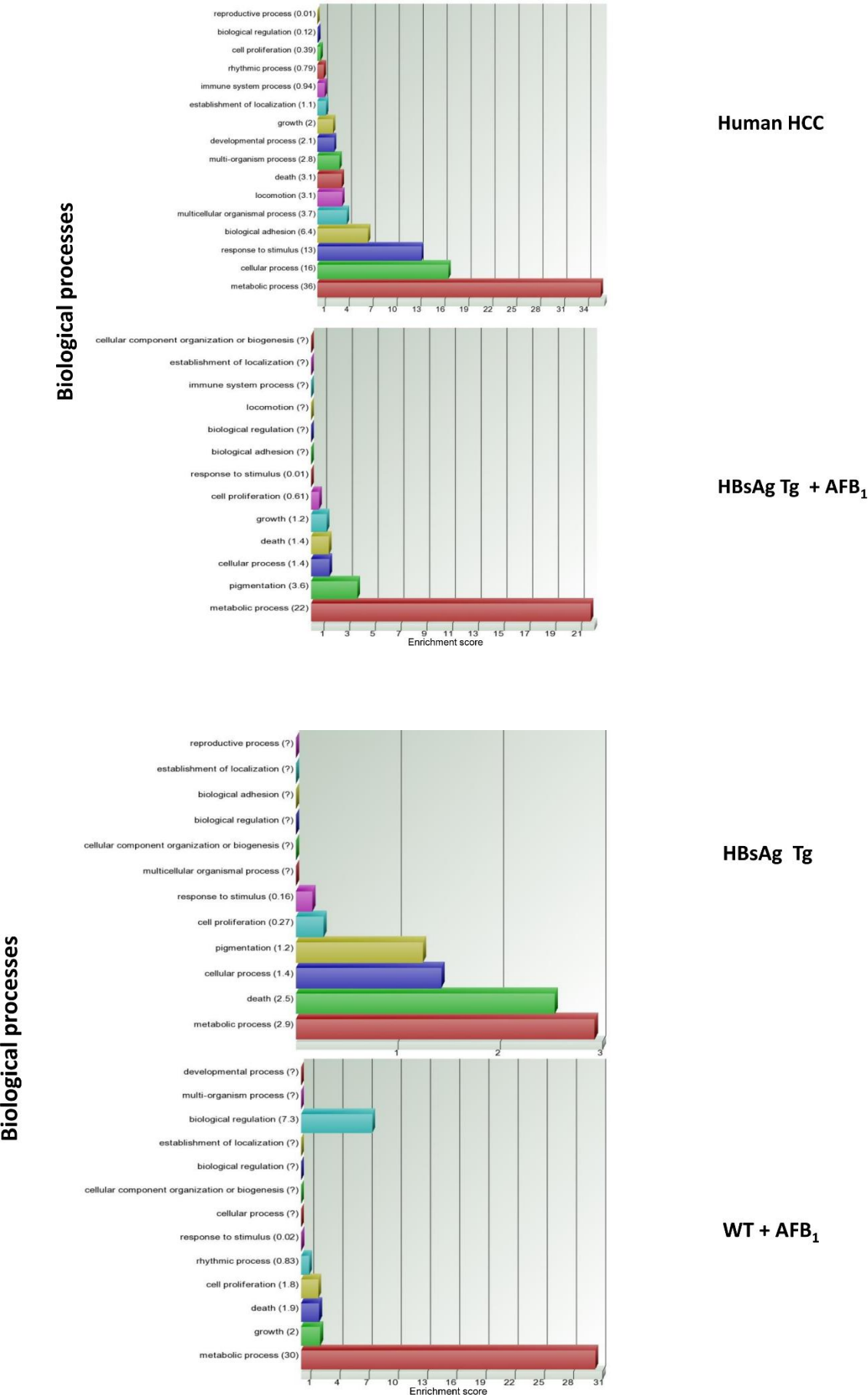
**Figure S5. Molecular characterization of liver nodules from mouse models**

Top canonical pathways of upstream regulators in the tumors from the various categories are indicated.

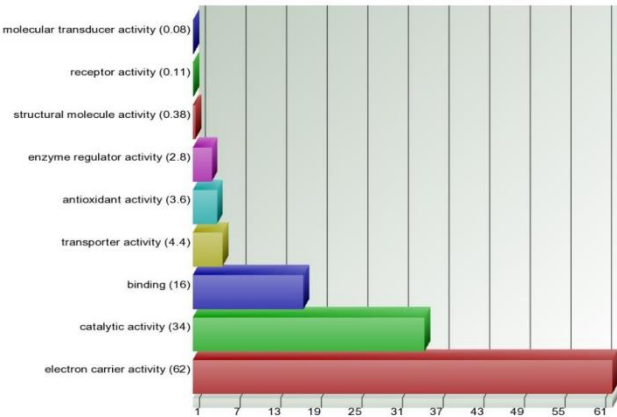


**Figure S6. Molecular characterization of liver nodules from mouse models and human HCC**

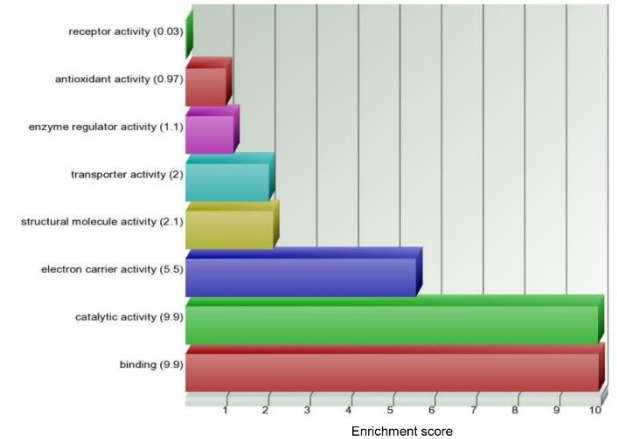
Direct comparison of canonical pathways affected in the human HCCs and tumors from the various mice categories are listed.



Molecular Functions

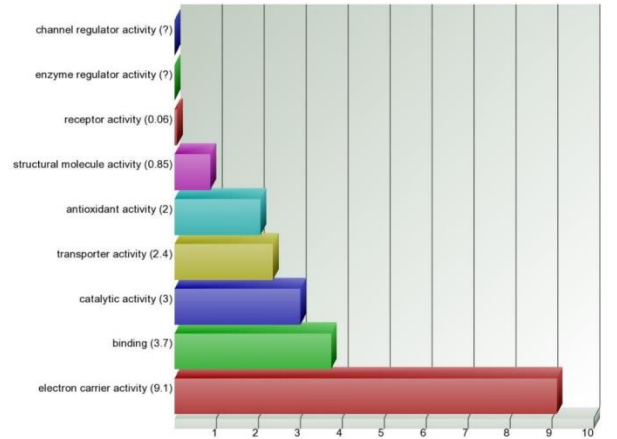


Human HCC

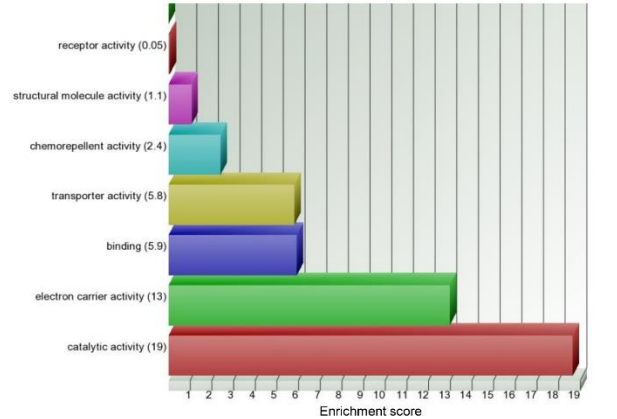


HBsAg Tg + AFB<sub>1</sub>

Molecular Functions



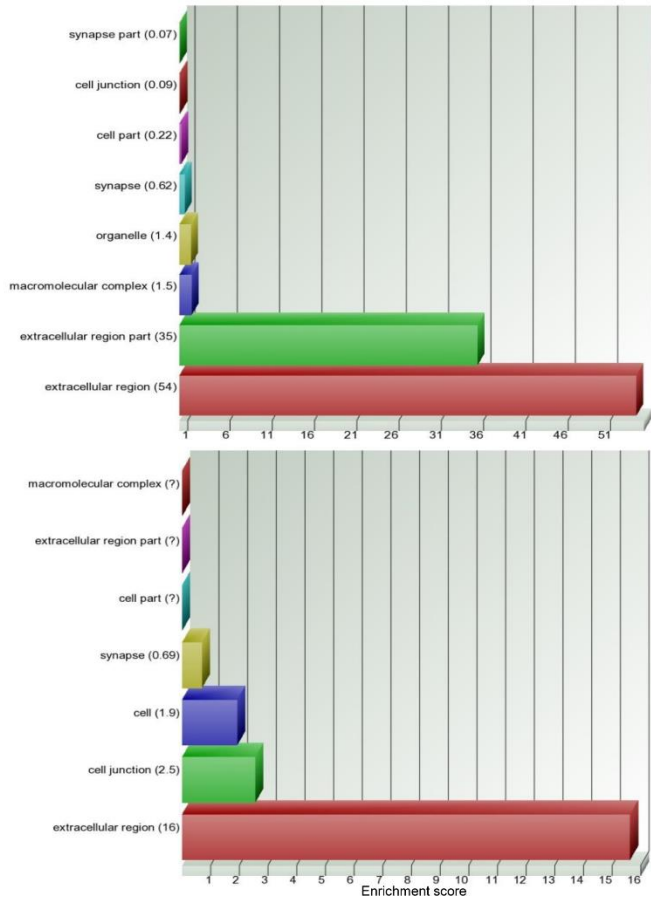
HBsAg Tg



WT + AFB<sub>1</sub>



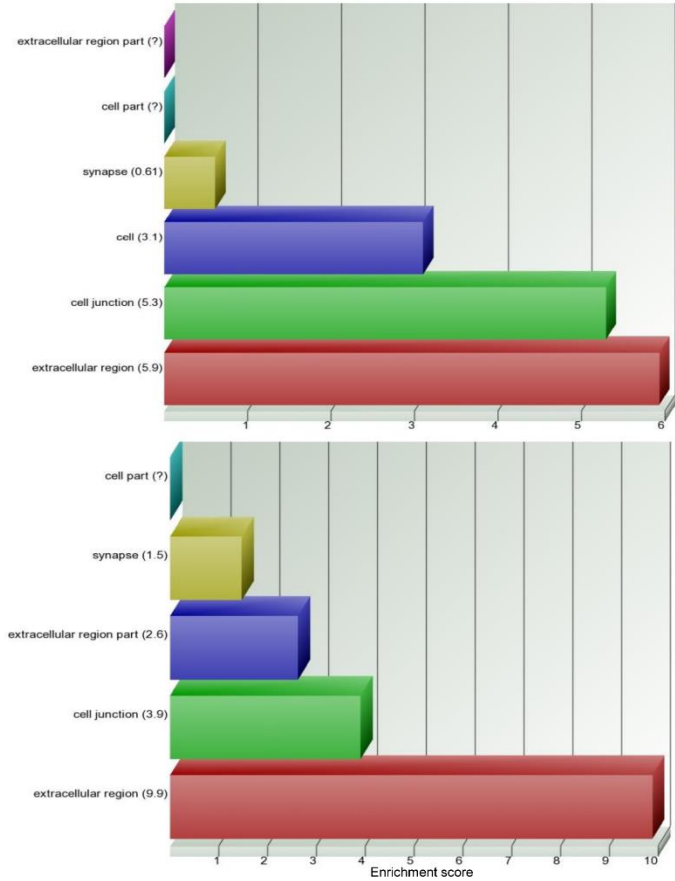
Cellular Components



Human HCC

HBsAg Tg + AFB<sub>1</sub>

Cellular Components



HBsAg Tg

WT + AFB<sub>1</sub>

## Figure S7. Gene Ontology analysis of molecular and cellular functions affected in liver tumors

Top biological process, molecular functions and cellular components identified through gene-ontology analysis, are shown. The major perturbations are highly similar between human HCCs and the liver nodules from the three categories of mice.

Table 1: Details of numbers of mice used in the study

A		Treatment	Genotype	Collection (months)									
				3		6		9		12		15	
				♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
10	Corn Oil	WT		7	11	11	9	9	10	8	6	9	11
			HBsAg Tg			10	8	7	13	8	12	11	10
		AFB <sub>1</sub> at day 7	WT			9	8	9	9	8	10	8	9
13			HBsAg Tg			8	7	15	10	11	15	13	14

B		Treatment	Genotype	Collection (months)							
				9		12		15			
				♂	♀	♂	♀	♂	♀		
16	19	AFB <sub>1</sub> at 6 mths	WT	11	15	14	20	14	15	AFB <sub>1</sub> at 12 mths	
19	26		HBsAg Tg	18	24	24	31	30	29		

Table S1A-B. Teoh et al.

Table S1A-B - Teoh et al.

## D7 injection and collection at 9 months

Table Analyzed	WT ctrl vs HbsAg ctrl			WT ctrl vs wt AFB <sub>1</sub>			WT ctrl vs HbsAg AFB <sub>1</sub>		
P value	1			1			0.0027		
P value summary	ns			ns			**		
One- or two-sided	Two-sided			Two-sided			Two-sided		
Statistically significant? (alpha<0.05)	No			No			<u>Yes</u>		
Data analyzed	WT Ctrl	HbsAg Ctrl	Total	WT Ctrl	WT AFB <sub>1</sub>	Total	WT Ctrl	HbsAg AFB <sub>1</sub>	Total
without nodules	18	17	35	18	18	36	18	14	32
with nodules	1	2	3	1	0	1	1	13	14
Total	19	19	38	19	18	37	19	27	46

Table Analyzed	HbsAg Ctrl vs WT AFB <sub>1</sub>			HbsAg Ctrl vs HbsAg AFB <sub>1</sub>			WT AFB <sub>1</sub> vs HbsAg AFB <sub>1</sub>		
P value	0.4865			0.0104			0.0004		
P value summary	ns			*			***		
One- or two-sided	Two-sided			Two-sided			Two-sided		
Statistically significant? (alpha<0.05)	No			<u>Yes</u>			<u>Yes</u>		
Data analyzed	HbsAg Ctrl	WT AFB <sub>1</sub>	Total	HbsAg Ctrl	HbsAg AFB <sub>1</sub>	Total	WT AFB	HbsAg AFB <sub>1</sub>	Total
without nodules	17	18	35	17	14	31	18	14	32
with nodules	2	0	2	2	13	15	0	13	13
Total	19	18	37	19	27	46	18	27	45

Table S2A - Teoh et al.

## D7 injection and collection at 15 months

Table Analyzed	WT ctrl vs HBsAg ctrl			WT ctrl vs wt AFB <sub>1</sub>			WTt ctrl vs HBsAg AFB <sub>1</sub>		
P value	< 0.0001			0.0480			< 0.0001		
P value summary	***			*			***		
One- or two-sided	Two-sided			Two-sided			Two-sided		
Statistically significant? (alpha<0.05)	<u>Yes</u>			<u>Yes</u>			<u>Yes</u>		
Data analyzed	WT Ctrl	HBsAg Ctrl	Total	WT Ctrl	WT AFB <sub>1</sub>	Total	WT Ctrl	HBsAg AFB	Total
without nodules	18	4	22	18	15	33	18	5	23
with nodules	0	17	17	0	5	5	0	23	23
Total	18	21	39	18	20	38	18	28	46

Table Analyzed	HBsAg Ctrl vs WT AFB <sub>1</sub>			HBsAg Ctrl vs HbsAg AFB <sub>1</sub>			WT AFB vs HBsAg AFB <sub>1</sub>		
P value	0.0005			1			0.0001		
P value summary	***			ns			***		
One- or two-sided	Two-sided			Two-sided			Two-sided		
Statistically significant? (alpha<0.05)	<u>Yes</u>			No			<u>Yes</u>		
Data analyzed	HBsAg Ctrl	WT AFB <sub>1</sub>	Total	HBsAg Ctrl	HBsAg AFB <sub>1</sub>	Total	WT AFB <sub>1</sub>	HBsAg AFB <sub>1</sub>	Total
without nodules	4	15	19	4	5	9	15	5	20
with nodules	17	5	22	17	23	40	5	23	28
Total	21	20	41	21	28	49	20	28	48

Table S2B - Teoh et al.