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Supplementary information

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CRISPR-StAR enables high-resolution genetic screening in complex in vivo models

In the format provided by the authors and unedited

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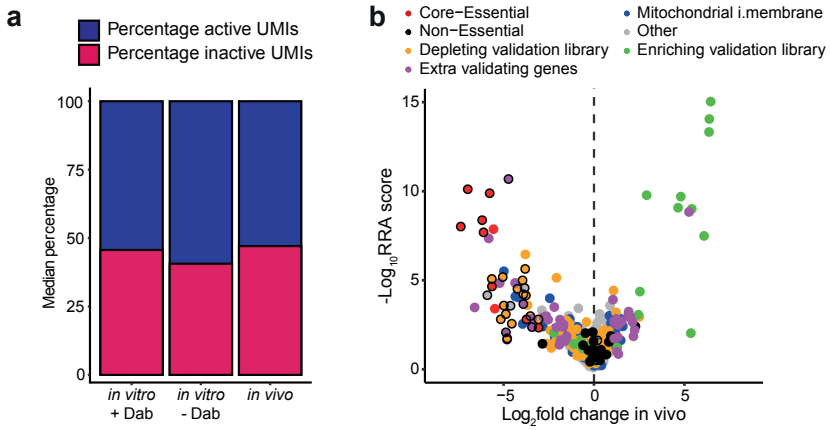
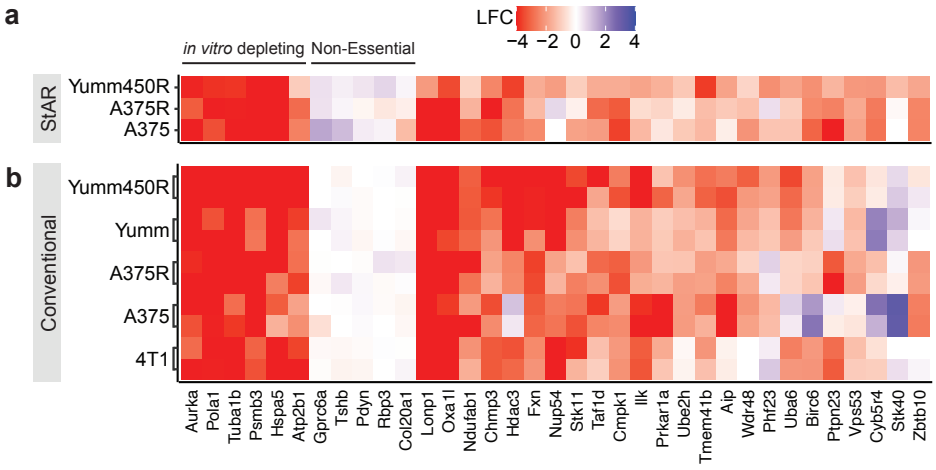


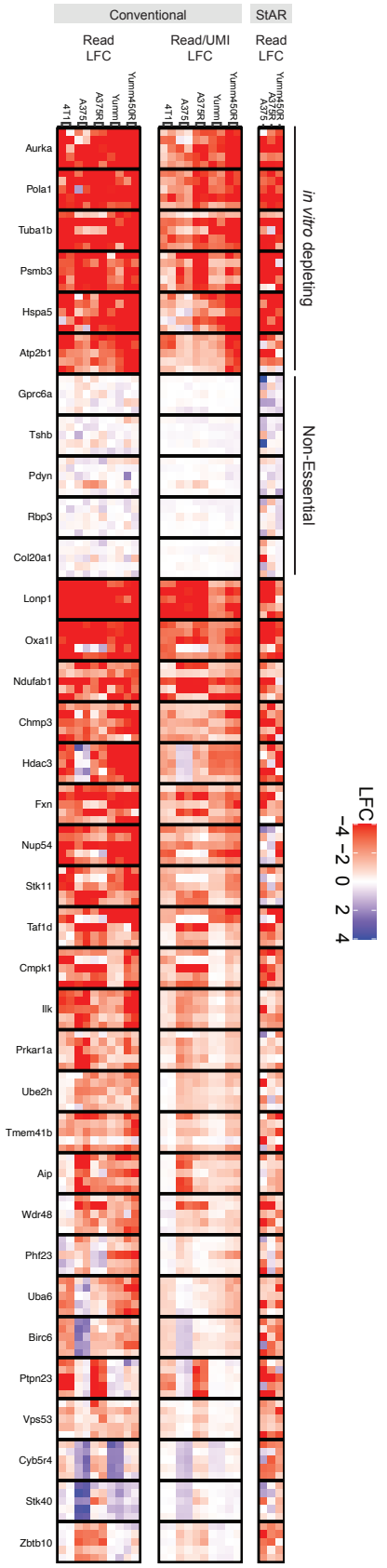
Supplementary Figure 1 | Median ratio active/inactive UMIs in validation screen and lower stringency validation set *in vivo*

a) Median ratio percentage of active and inactive UMIs in the *in vitro* with and without Dabrafenib (Dab) validation screening condition and the *in vivo* validation screen. **b)** Volcano plot of *in vivo* validation screen with in black non-essential genes, in blue genes that are part of the cellular component GOterm “mitochondrial inner membrane” (GO:0005743), in orange *in vivo* depleting library, in green *in vivo* enriching library, in purple the genes with lower stringency cut offs that are validating (45/267, 16.3% LFC>1 or LFC *in vivo* <-1.5, *in vitro* >1.5), and in grey the genes that are not defined by these aforementioned groups, called other. LFC on x-axis and -Log_{10}RRA score on y-axis.



Supplementary Figure 2 | Cross-species validation of genetic dependencies using NGS read comparison

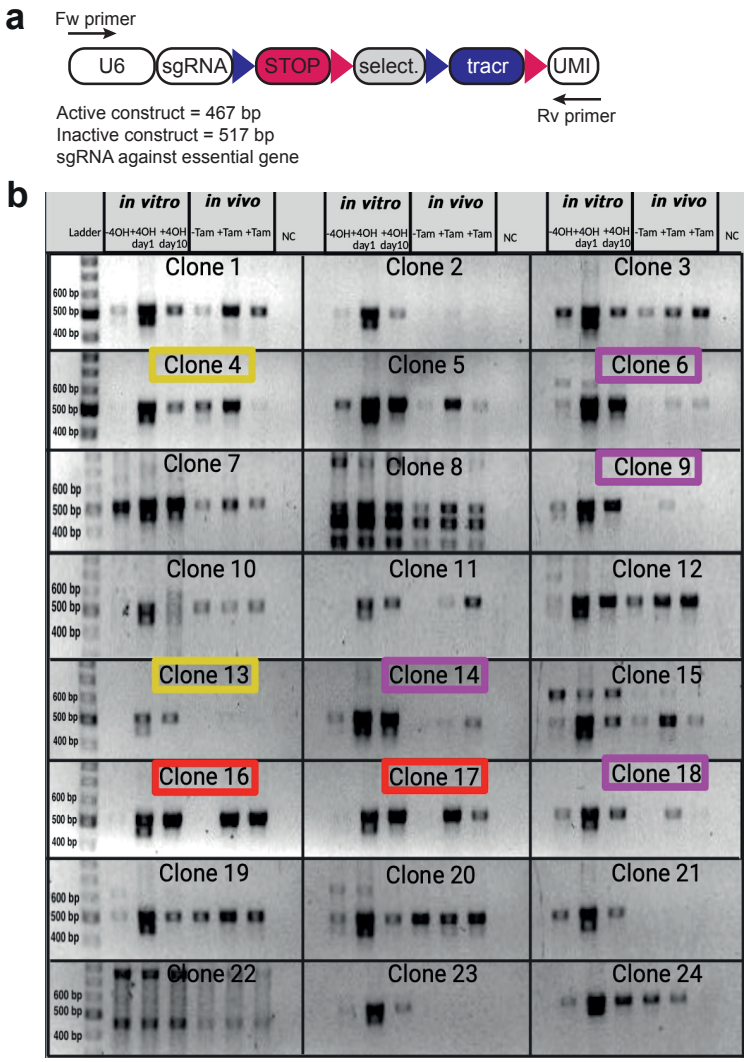
a) Same heatmap as in fig. 5, showing gene effects measured by CRISPR-StAR screening *in vivo* with three different melanoma cell lines. Yumm450R; Braf inhibitor resistant murine melanoma, A375R; Braf inhibitor resistant human melanoma, A375; Braf inhibitor sensitive human melanoma. Color range depicts the LFC gene effect, red for depleting, white for no effect and blue for enriching. **b)** Heatmap of conventional CRISPR screening *in vivo* with Yumm450R, A375R, A375 and additionally Yumm; Braf inhibitor sensitive murine melanoma and 4T1; murine mammary carcinoma. LFC was calculated by comparison of the NGS reads in the harvested tumor at the end of the screening time versus the NGS reads in the cells at the day of engraftment. This dropout of reads relative to engraftment is a proxy for cell fitness, whereas the LFC calculated by the comparison of the number of UMIs in the tumor to the NGS reads in the cells at the day of engraftment in fig 5, can be seen as a proxy for cell death.



Supplementary Figure 3 | Cross-species validation of genetic effect per sgRNA
Heatmaps show LFC for each of the 5 sgRNAs of a targeted gene. Analysis performed with CRISPR-StAR (top), conventional analysis comparing reads with UMI dropout (middle) and read LFC (bottom).

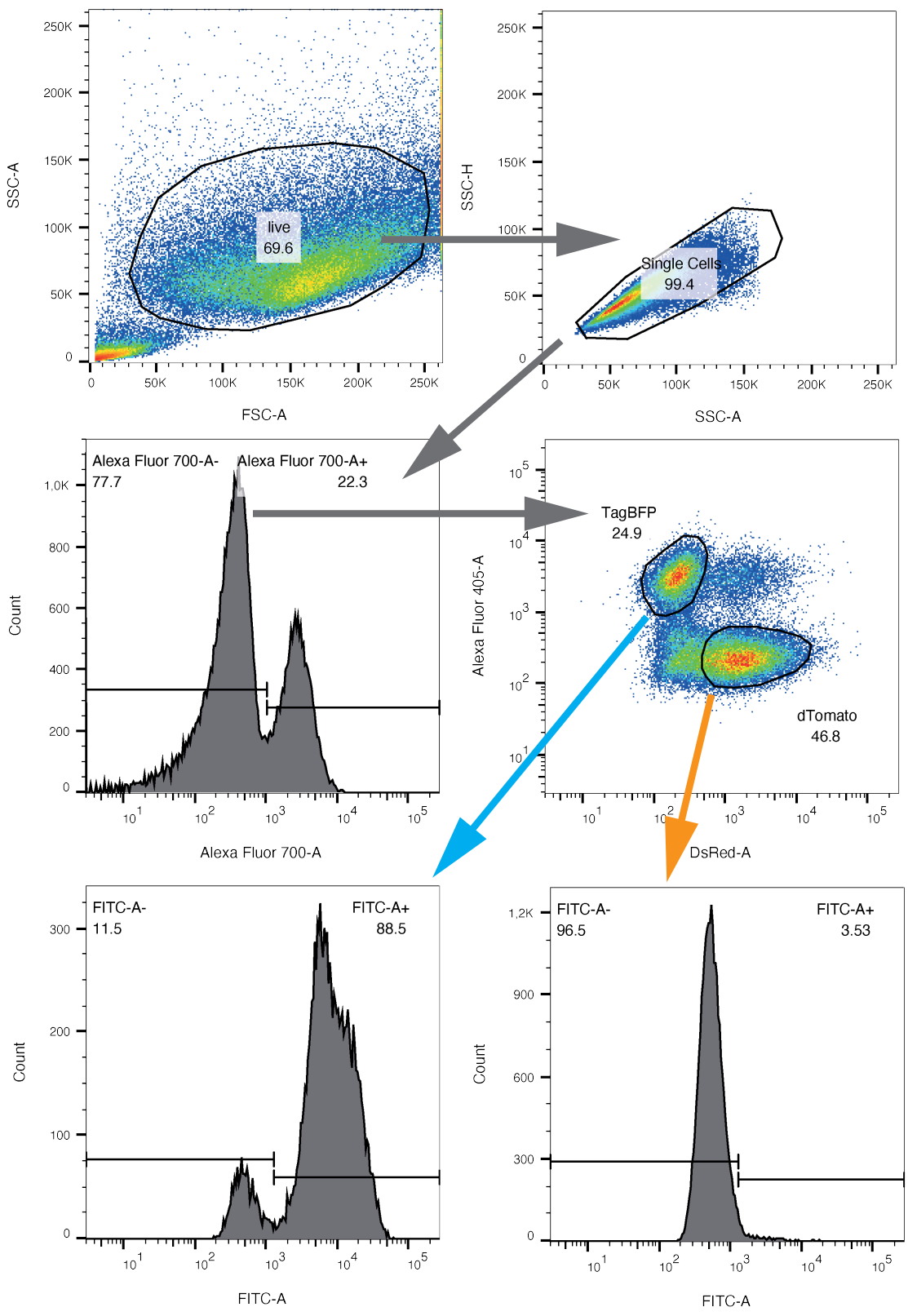
Supplementary Figure 4 | cBioPortal for Cancer Genomics data visualization of Melanoma studies

Per study the mutations in the genes of interest (enriching in the validation screen) per patient are shown. Colors in the first line represent the different studies of origin. The different color pattern in the lines per gene represent the kind of mutation that is found.



Supplementary Figure 5 | PCR based strategy for functional clone selection

a) Schematic representation of PCR strategy for clonal selection. Clones carry different UMIs and for each UMI there is a matching reverse (Rv) primer. A PCR with a forward (Fw) primer binding the U6 promoter with the Rv primer on a recombined construct will result in a band of 457 base pairs for the active construct versus 517 base pairs for the inactive construct. Using an sgRNA against an essential gene Cas9 functionality can be assessed.
b) Gel electrophoresis of PCRs to assess the engraftment, the recombination and the Cas9 functionality. Yellow framed clones show no CreERT2 leakiness *in vitro* only; violet framed clones show no CreERT2 leakiness *in vivo* only and in red there is tight CreERT2 activity *in vitro* as well as *in vivo* visible.



Supplementary Figure 6 | FACS gating strategy
Example of an in vitro D7 sample with sgEGFP. First gated for life cells, followed by single cell gating. Subsequently Alexa Fluor 700 negative cells were gated to assess the recombination event. Recombined cells with TagBFP or dTomato were separately examined for FITC-A.

Supplementary Table 1 | FACS data with individual SD

sgEmpty	in vivo D14	in vitro D7	in vitro D14
	1.73023256	2.30516432	2.46728972
	2.00460829	2.30046948	2.31111111
	2.23837209	2.26851852	2.40540541
SD	0.254340106	0.019940829	0.078647781
p value	0.1111		
	0.0585		

sgEGFP	in vivo D14	in vitro D7	in vitro D14
	1.69565217	1.87951807	2
	1.6437247	1.904	1.99604743
	0.96753247	1.94262295	2.10460251
SD	0.406220525	0.031815413	0.061565024
p value	0.1147		
	0.0653		

sgTuba1b	in vivo D14	in vitro D7	in vitro D14
	0.00665434	0.02907303	0.01518325
	0.01788079	0.02486188	0.01508453
	0.003	0.03319088	0.01515152
SD	0.00775484	0.004164587	5.03986E-05
p value	0.0174		
	0.2539		

sgAip	in vivo D14	in vitro D7	in vitro D14
	0.01435257	1.23384615	0.52545825
	0.00892116	1.18844985	0.51844262
	0.37939698	1.15727003	0.54124748
SD	0.212343768	0.038507375	0.011680329
p value	0.0011		
	0.0326		

sgBirc6	in vivo D14	in vitro D7	in vitro D14
	0.09072581	1.17682927	0.64823009
	0.14	1.13069909	0.65
	0.11083481	1.13690476	0.62389381
SD	0.024775411	0.025034877	0.014588353
p value	<0.0001		
	<0.0001		

sgUba6	in vivo D14	in vitro D7	in vitro D14
	0.02281553	0.90884718	0.38433515
	0.03502825	0.88533333	0.39703154
	0.09032882	0.83028721	0.36996337
SD	0.035975334	0.040320891	0.013542724
p value	<0.0001		
	0.0001		

sgStk40	in vivo D14	in vitro D7	in vitro D14
	0.17118644	1.1143695	0.59538784
	0.20583942	1.12244898	0.51383399
	0.12676056	1.11304348	0.58436214
SD	0.03963995	0.005090837	0.044247065
p value	<0.0001		
	0.0003		

sgWdr48	in vivo D14	in vitro D7	in vitro D14
	0.27372263	1.13473054	0.83415842
	0.29478458	1.20307692	0.83816425
	0.24271845	1.19937695	0.82758621
SD	0.026190796	0.038436258	0.005340655
p value	<0.0001		
	<0.0001		

sgZbtb10	in vivo D14	in vitro D7	in vitro D14
	0.58383234	1.812	2.06451613
	0.47191011	1.78571429	2.06374502
	0.2039604	1.74609375	2.06
SD	0.195203451	0.0331772	0.002415754
p value	0.0003		
	0.0001		

Supplementary table 2 | Oligos for CRISPR-StAR vector generation.

#	Oligo name	Sequence 5'-3'
1	Lox5171 in XBaI F	CTAGCATAACTTCGTATAATGTGTACTATACGAAGTTAT
2	Lox5171 in XBaI R	CTAGATAACTTCGTATAGTACACATTATACGAAGTTATG
3	Lox5171-PacI in EcoRI F	AATTATAACTTCGTATAATGTGTACTATACGAAGTTATTAATT
4	Lox5171-PacI in EcoRI R	AATTAATTAATAACTTCGTATAGTACACATTATACGAAGTTAT
5	SbfIBlastiF	ACTGTCCTGCAGGAATTCTACCGGGTAGGGGAGG
6	SbfIBlastiR	ACTGTCCTGCAGGTCAGCCCTCCCACACATAACC
7	NheI-StAR_Fw	CTACAGCTAGCCTCTAGGCGCCGGAATTG
8	NheI-StAR_Rv	CAATGTGCTAGCCGGTAGAATTACGCGTCAATTG

Supplementary table 3 | Oligos for sgRNA pool amplification

Oligo name	Sequence 5'-3'
A1.drugged_Fw	CCCCGGTTGCTAACAAAAGCa
A1.drugged_Rv	GAGGTTGTGATCGCTGAGGTA
A2.cell.surface_Fw	CAGCGGAAACCCCAACAGTTA
A2.cell.surface_Rv	GAGGGTCAGTAGAACATGCGT
A3.metabolism_Fw	CaCAATGTGTCGGTGTTCGAG
A3.metabolism_Rv	CtCGGGTAAGGGTTCATCTGG
A4.other.druggable_Fw	gATCATTGTGGACCCCTTGAG
A4.other.druggable_Rv	TTGCCCATGTACTCCGAAAGC
B1.TF.epigenetics_Fw	GGGCTTCGTGGAGCATcTTCT
B1.TF.epigenetics_Rv	GACGGCGATGATAGACAGGTC
B2.Signaling_Fw	GGGATGAACACATCTACCCCT
B2.Signaling_Rv	GTCTTCCCGGCAGTAGTTGTA
B3.RNA.biology_Fw	AgcAATCCCGATGGCACcATG
B3.RNA.biology_Rv	CTTCCCACGGAGTCCCTTgTC
B4.other.nuclear_Fw	TTTGTGGGCCTGAAGAAAACT
B4.other.nuclear_Rv	AGGGCTGTCCTGAATAAGCAG
E1.rest_Fw	AAGGTCCCGGTCAAGttACAG
E1.rest_Rv	CGTTCTGCGTCACACCATTGC

Supplementary table 4 | Oligos for Next Generation Sequencing

#	Oligo name	Sequence 5'-3'
1	sgRNA amplification_ barcoded-sample index_Fw	AATGATACGGCGACCACCGAGATCTACACXXXXXXXXGAGG GCCTATTTCCCATGATTCCTTC
2	sgRNA amplification_ barcoded- sample index _Rv	CAAGCAGAAGACGGCATACGAGATXXXXXXXXACCGTTGATG AGTAGCTCTCAGATGAGG
3	NGS_Read1_sgRNA	CGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACC G
4	NGS_Index1_sample index2	CCTCATCTGAGAGCTACTCATCAACGGT
5	NGS_Read2_UMI _barcode	CCGTTGATGAGTAGCTCTCAGATGAGGAC
6	NGS_Index2_sample index1	CAAATATGAAGGAATCATGGGAAATAGGCCCTC