Robust, durable gene activation *in vivo* via mRNA-encoded activators

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Supplementary Tables and Figures

Supplementary Tables

Table S1: Sequences of sgRNA used in the study

B4galnt2 sgRNA	**Sequence (5' - 3')**
1	mC*mA*mC*rArUrCrCrUrGrGrArCrGrCrGrArGrGrCrA
2	mC*mU*mA*rUrUrUrArArGrUrUrGrGrUrCrCrArCrUrG
3	mU*mG*mG*rArArCrArCrArUrCrCrUrGrGrArCrGrCrG
4	mG*mC*mG*rUrCrCrArGrGrArUrGrUrGrUrUrCrCrArA
5	mG*mU*mU*rGrGrUrCrCrArCrUrGrUrGrGrCrUrGrUrG
Non Target	mG*mC*mA*rCrUrArCrCrArGrArGrCrUrArArCrUrCrA
Epo sgRNA	**Sequence (5' - 3')**
1	mC*mC*mC*rUrGrGrCrCrArArGrGrGrUrCrCrGrUrUrC
2	mU*mG*mA*rGrArCrArCrArGrCrCrArCrCrGrGrCrCrA
3	mU*mG*mU*rCrUrCrArCrUrGrUrGrUrUrCrCrCrGrArA
4	mC*mC*mC*rGrArArCrGrGrArCrCrCrUrUrGrGrCrCrA
5	mC*mC*mC*rUrGrGrCrCrArArGrGrGrUrCrCrGrUrUrC
Modified sgRNA scaffold	rGrUrUrUrUrArGrAmGmCmUmAmGmAmAmAmUrAmGmCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrAmAmCmUmUmGmAmAmAmAmAmGmUmGmGmCmAmCmCmGmAmGmUmCmGmGmUmGmCmU*mU*mU*mU

Abbreviations: *r= ribonucleotide base, m=phosphorothioated 2' O-methyl ribonucleotide base, *= Phosphorothioate bond*

Table S2: Gene activator protein coding sequences and mCherry DNA sequence

VPH-dCas9-SS18 (Flag-tag, VP64, p65, HSF1, SV40 NLS, dCas9, SS18, T2A, mCherry)
MDYKDHDGDYKDHDIDYKDDDDKHVDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLD MLGSDALDDFDLDMLGSLPSASVEFEGSGGPSGQISNQALALAPSSAPVLAQTMVPSSAMV PLAQPPAPAPVLTPGPPQSLSAPVPKSTQAGEGTLSEALLHLQFDADEDLGALLGNSTDPGV FTDLASVDNSEFQQLLNQGVSMSHSTAEPMLMEYPEAITRLVTGSQRPPDPAPTPLGTSGL PNGLSGDEDFSSIADMDFSALLSQISSSGGQGGGGSGFSVDTSALLDLFSPSVTVPDMSLPDL DSSLASIQELLSPQEPPRPPEAENSSPDSGKQLVHYTAQPLFLLDPGSVDTGSNDLPVLFEL GEGSYFSEGDGFAEDPTISLLTGSEPPKAKDPTVSNPKKKRKVGRGMDKKYSIGLAIGTNSV GWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRI CYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLV DSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVD AKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTY DDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLK ALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLL RKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRIPYYVGPLARGNSRFA WMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLPKHSLLYEYFTVYNELT KVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKIECFDSVEISGVEDRFN ASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLKTYAHLFDDKVMKQLKR RRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLTFKEDIQKAQVSG QGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQKGQKNS RERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVD AIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLT KAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSD FRKDFQFYKVREINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQ EIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQ VNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGK SKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAG ELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVIL ADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTIDRKRYTSTKEVLDAT LIHQSITGLYETRIDLSQLGGDSRADPKKKRKVASMSVAFAAPRQRGKGEITPAAIQKMLDDN NHLIQCIMDSQNKGKTSECSQYQQMLHTNLVYLATIADSNQNMQSLLPAPPTQNMPMGPGG MNQSGPPPPPRSHNMPSDGMVGGGPPAPHMQNQMNGQMPGPNHMPMQGPGPNQLNM TNSSMNMPSSSHGSMGGYNHSVPSSQSMPVQNQMTMSQGQPMGNYGPRPNMSMQPNQ GPMMHQQPPSQQYNMPQGGGQHYQGQQPPMGMMGQVNQGNHMMGQRQIPPYRPPQQ GPPQQYSGQEDYYGDQYSHGGQGPPEGMNQQYYPDGNSQYGQQQDAYQGPPPQQGYP PQQQQYPGQQGYPGQQQGYGPSQGGPGPQYPNYPQGQGQQYGGYRPTQPGPPQPPQ QRPYGYDQGQYGNYQQASGSGEGRGSLLTCGDVEENPGPMVSKGEEDNMAIIKEFMSFKV HMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPA DIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQK KTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKL DITSHNEDYTIVEQYERAEGRHSTGGMDELYK*
dCas9-VP64 (dCas9, SV40 NLS, VP64, T2A, mCherry)

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRL
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YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
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PYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLP
KHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI
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DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM
ARENQTTQKGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYV
DQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQL
LNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
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KVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV
AYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPIDFLEAKGYKEVKKDLIIKLPKYSLF
ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHY
LDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTID
RKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDPIAGSKASPKKKRKVGRADALDDFDLD
MLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLASGSGEGRGSLLTCGDVEE
NPGPASGSGEGRGSLLTCGDVEENPGPMVSKGEEDNMAIIKEFMSFKVHMEGSVNGHEFEI
EGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGF
KWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERM
YPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQ
YERAEGRHSTGGMDELYK*

dCas9-VPR (dCas9, SV40 NLS, VPR (VP64, RelA(p65), Rta), P2A, mCherry)

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRL
KRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNF
DLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM
DGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRI
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ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHY
LDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTLTNLGAPAAFKYFDTTID
RKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDSRADPKKKRKVSPGIRRLDALISTSLYK
KAGYKEASGSGRADALDDFDLDMLGSDALDDFDLDMLGSDALDDFDLDMLGSDALDDFDL
DMLINSRSSGSPKKKRKVGSQYLPDTDDRHRIEEKRKRTYETFKSIMKKSPFSGPTDPRPPP
RRIAVPSRSSASVPKPAPQPYPFTSSLSTINYDEFPTMVFPSGQISQASALAPAPPQVLPQAP
APAPAPAMVSALAQAPAPVPVLAPGPPQAVAPPAPKPTQAGEGTLSEALLQLQFDDEDLGA
LLGNSTDPAVFTDLASVDNSEFQQLLNQGIPVAPHTTEPMLMEYPEAITRLVTGAQRPPDPA
PAPLGAPGLPNGLLSGDEDFSSIADMDFSALLGSGSGSRDSREGMFLPKPEAGSAISDVFEG
REVCQPKRIRPFHPPGSPWANRPLPASLAPTPTGPVHEPVGSLTPAPVPQPLDPAPAVTPE
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DLNLDSPLTPELNEILDTFLNDECLLHAMHISTGLSIFDTSLFGSGATNFSLLKQAGDVEENPG
PGSGATNFSLLKQAGDVEENPGPMVSKGEEDNMAIIKEFMSFKVHMEGSVNGHEFEIEGEG
EGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWE
RVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMYPED
GALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIVEQYERA
EGRHSTGGMDELYK*

dCas9-p300 (dCas9**,** Nucleoplasmin NLS, p300, T2A, mCherry**)**

MDKKYSIGLAIGTNSVGWAVITDEYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRL
KRTARRRYTRRKNRICYLQEIFSNEMAKVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVA
YHEKYPTIYHLRKKLVDSTDKADLRLIYLALAHMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTY
NQLFEENPINASGVDAKAILSARLSKSRRLENLIAQLPGEKKNGLFGNLIALSLGLTPNFKSNF
DLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKNLSDAILLSDILRVNTEITKAPLSAS
MIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYIDGGASQEEFYKFIKPILEKM
DGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYPFLKDNREKIEKILTFRI
PYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFDKNLPNEKVLP
KHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKEDYFKKI
ECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEERLK
TYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHD
DSLTFKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEM
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DQELDINRLSDYDVDAIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQL
LNAKLITQRKFDNLTKAERGGLSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLI
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KVYDVRKMIAKSEQEIGKATAKYFFYSNIMNFFKTEITLANGEIRKRPLIETNGETGEIVWDKG
RDFATVRKVLSMPQVNIVKKTEVQTGGFSKESILPKRNSDKLIARKKDWDPKKYGGFDSPTV
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ELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKGSPEDNEQKQLFVEQHKHY
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RKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDKRPAATKKAGQAKKKKGSIFKPEELRQ
ALMPTLEALYRQDPESLPFRQPVDPQLLGIPDYFDIVKSPMDLSTIKRKLDTGQYQEPWQYV
DDIWLMFNNAWLYNRKTSRVYKYCSKLSEVFEQEIDPVMQSLGYCCGRKLEFSPQTLCCYG
KQLCTIPRDATYYSYQNRYHFCEKCFNEIQGESVSLGDDPSQPQTTINKEQFSKRKNDTLDP
ELFVECTECGRKMHQICVLHHEIIWPAGFVCDGCLKKSARTRKENKFSAKRLPSTRLGTFLE
NRVNDFLRRQNHPESGEVTVRVVHASDKTVEVKPGMKARFVDSGEMAESFPYRTKALFAF
EEIDGVDLCFFGMHVQEYGSDCPPPNQRRVYISYLDSVHFFRPKCLRTAVYHEILIGYLEYVK
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KNNKKTSKNKSSLSRGNKKKPGMPNVSNDLSQKLYATMEKHKEVFFVIRLIAGPAANSLPPIV
DPDPLIPCDLMDGRDAFLTLARDKHLEFSSLRRAQWSTMCMLVELHTQSQDGSEGRGSLLT
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EFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSF
PEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEAS
SERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDY
TIVEQYERAEGRHSTGGMDELYK*

AcrIIA4 (AcrIIA4, P2A, BFP)

MNLKELVREIKNKDYTAKLSGTDSNSITQLIIHVNNDGNEYGISESNFESIVEKFVSTFENGWD
GAYEDEEEFYNDMQDIVNRHFKGSGATNFSLLKQAGDVEENPGPGSGATNFSLLKQAGDV
EENPGPSELIKENMHMKLYMEGTVDNHHFKCTSEGEGKPYEGTQTMRIKVVEGGPLPFAFD
ILATSFLYGSKTFINHTQGIPDFFKQSFPEGFTWERVTTYEDGGVLTATQDTSLQDGCLIYNV
KIRGVNFTSNGPVMQKKTLGWEAFTETLYPADGGLEGRNDMALKLVGGSHLIANIKTTYRSK
KPAKNLKMPGVYYVDYRLERIKEANNETYVEQHEVAVARYCDLPSKLGHKLN*

mCherry (DNA sequence)

ATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGAGCTTCAA
GGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGA
GGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCC
CCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACG
TGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAG
TGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCT
CCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTC
CGACGGCCCCGTAATGCAGAAGAAAACCATGGGCTGGGAGGCCTCCTCCGAGCGGATG
TACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGAC
GGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGCAGC
TGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGACTAC
ACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGAC
GAGCTGTACAAGTAGTAA

Table S3: Ordinary Two-way ANOVA & Post Test Results

%mCherry+

Ordinary Two-way ANOVA		
Source of Variation	**% of total variation**	**P value**
Interaction	23.3	0.0051
gRNA	12.32	0.0136
Activator	37.99	<0.0001

Tukey's multiple comparisons test			
Comparison	**Mean Diff.**	**95.00% CI of diff.**	**Adjusted P Value**
VPR vs. VP64	-6.906	-12.34 to -1.467	0.0101
VPR vs. P300	-15.97	-21.41 to -10.53	<0.0001
VP64 vs. P300	-9.067	-14.51 to -3.628	0.0007

mCherry MFI

Ordinary Two-way ANOVA		
Source of Variation	**% of total variation**	**P value**
Interaction	15.17	<0.0001
gRNA	2.948	0.0596
Activator	72.89	<0.0001

Tukey's multiple comparisons test			
Comparison	**Mean Diff.**	**95.00% CI of diff.**	**Adjusted P Value**
VPR vs. VP64	-359.1	-449.9 to -268.2	<0.0001
VPR vs. P300	-632.8	-723.7 to -542.0	<0.0001
VP64 vs. P300	-273.8	-364.6 to -182.9	<0.0001

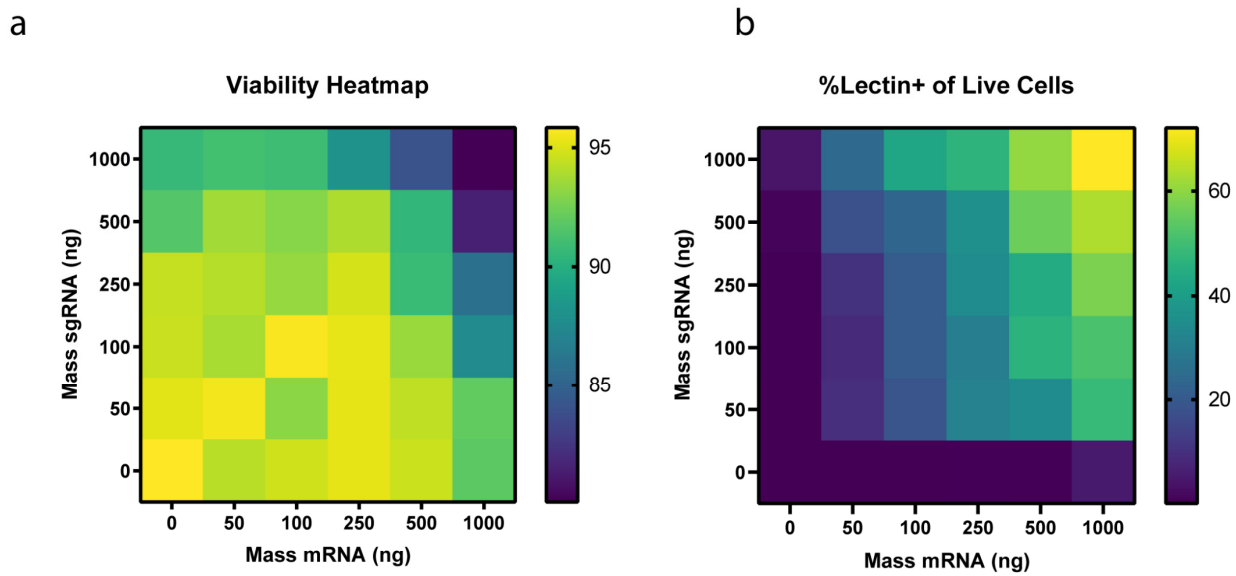
Table S4: sgRNA guide combinations used for the study in Figure 1b

		Number of Guides																																		
		5	4					3										2										1								0
	sgRNA	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	NT	Control
B4galnt2	1	x		x	x	x	x	x	x	x		x	x		x						x			x		x	x	x							
	2	x	x		x	x	x	x	x		x	x		x		x				x			x		x		x		x						
	3	x	x	x		x	x	x		x	x		x	x			x		x			x			x	x				x					
	4	x	x	x	x		x		x	x	x				x	x	x	x				x	x	x								x				
	5	x	x	x	x	x						x	x	x	x	x	x	x	x	x	x												x			
	NT																																	x	

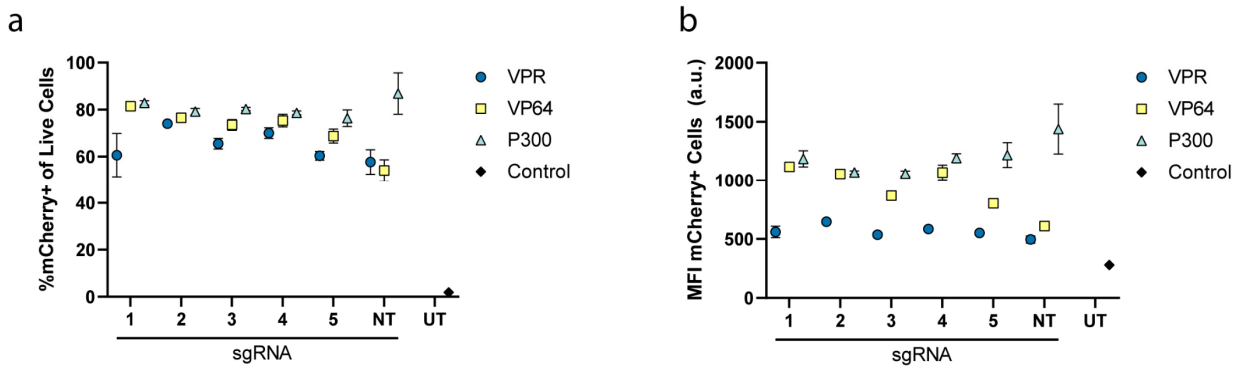
Table S5: Primer-Probe set used in the study

Gene	Primer Probe set ID	Forward primer	Reverse primer	TaqMan probe
B4galnt2	Mm00484661_m1	-	-	-
Epo	Mm01202755_m1			
Gapdh	Mm99999915_g1	-	-	-
mCherry	-	TGAGGTCAAGACCACCTACA	CTGTTCCACGATGGTGTAGTC	TTGGACATCACCTCCCACAACGAG

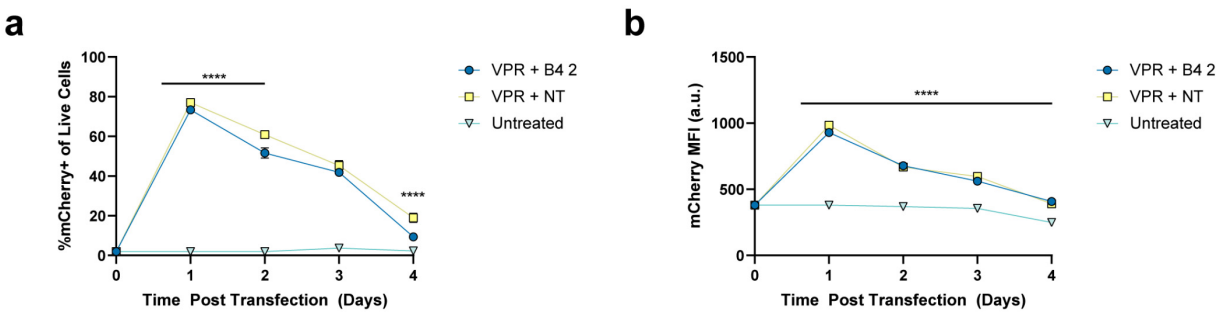
Supplementary Figures



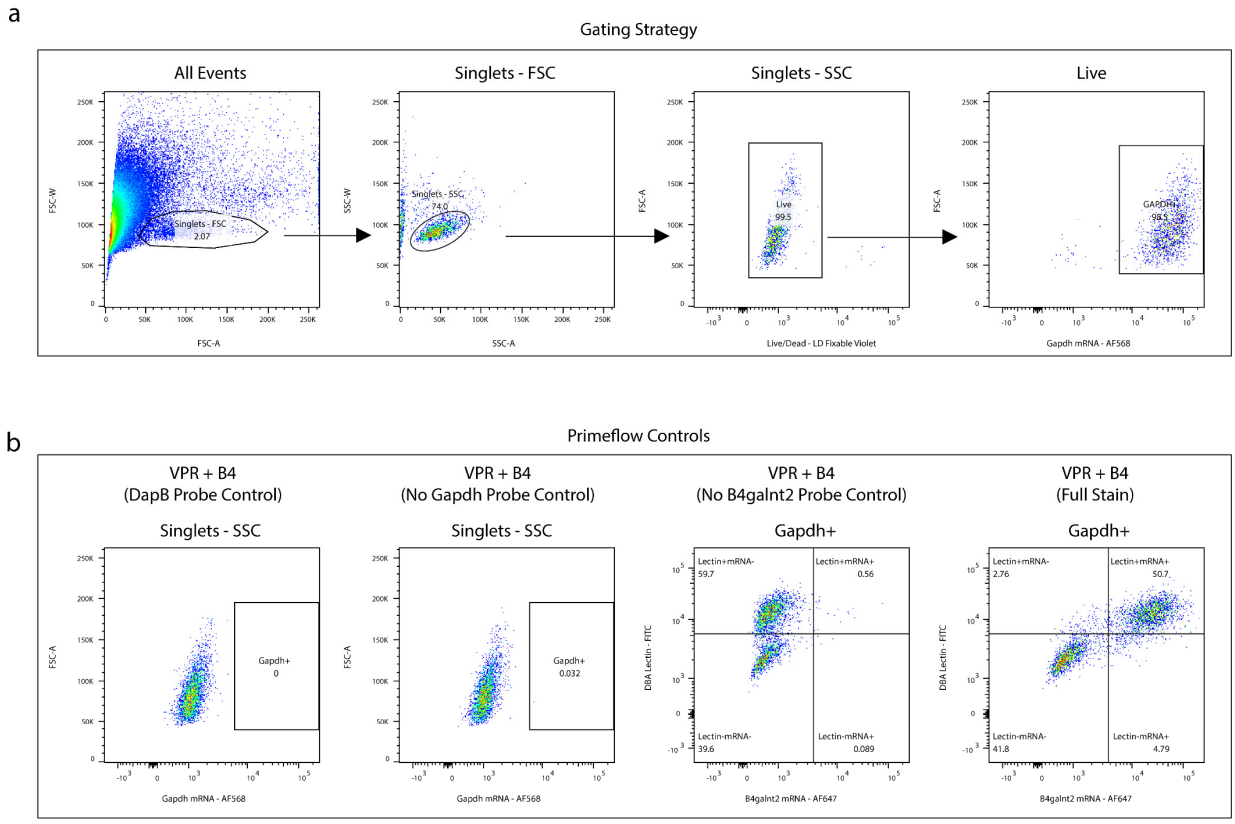
Sup. Fig. 1 | *In vitro* dose optimization in AML12 cells by flow cytometry. a, Heatmap showing the percentage of live cells (**a**) and lectin+ cells (**b**) with varying doses of VPR mRNA and sgRNA



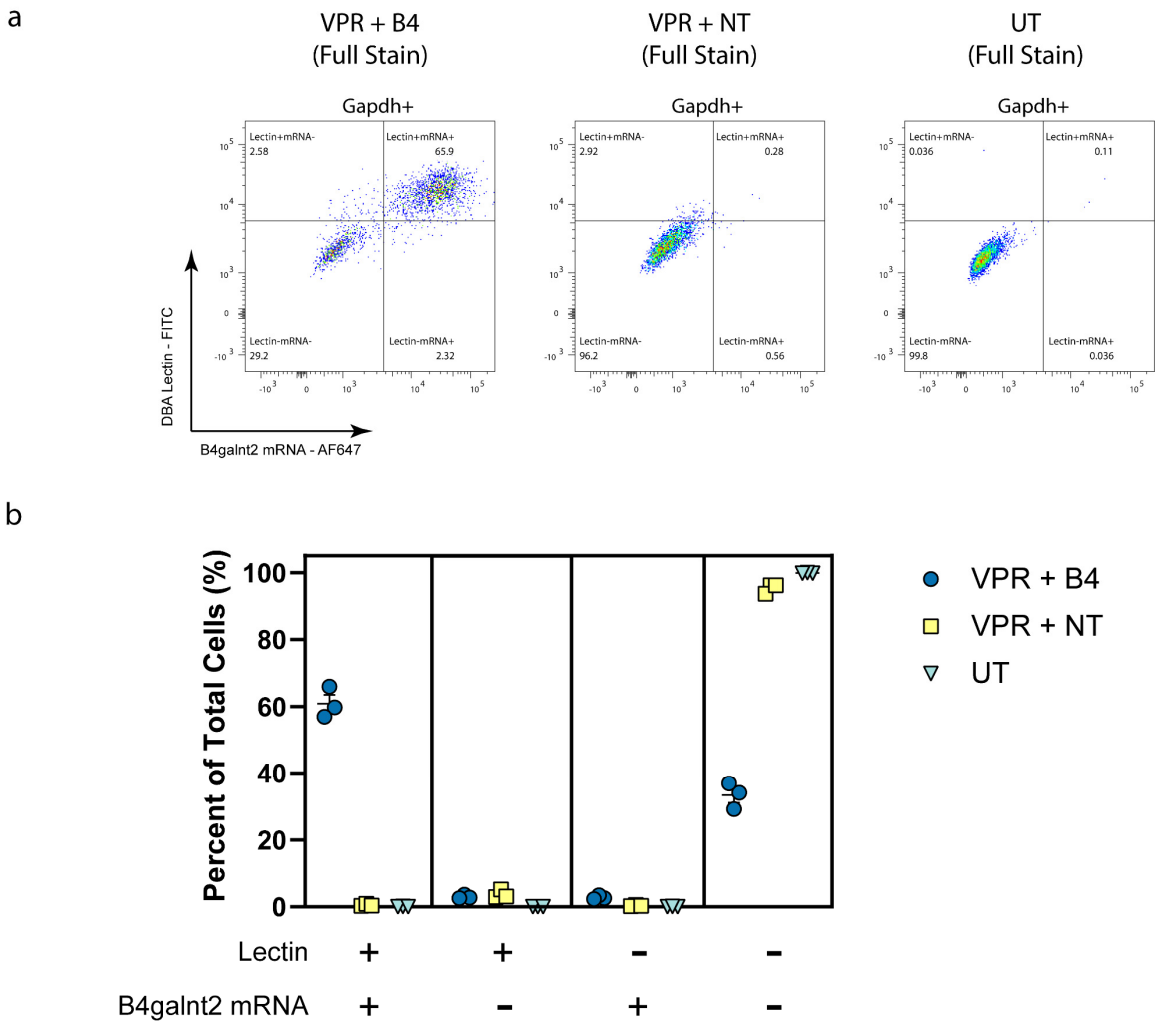
Sup. Fig. 2 | mCherry expression from in vitro activator screen. Each activator mRNA construct contained a cleavable mCherry sequence. **a,** Percentage of mCherry+ cells. **b,** MFI of mCherry+ cells. To assess differences in protein expression between the activator mRNA constructs, an ordinary two-way ANOVA was performed with Tukey's multiple comparisons test (**Table S2**) Data represent mean ± SEM (n = 3 biological replicates).



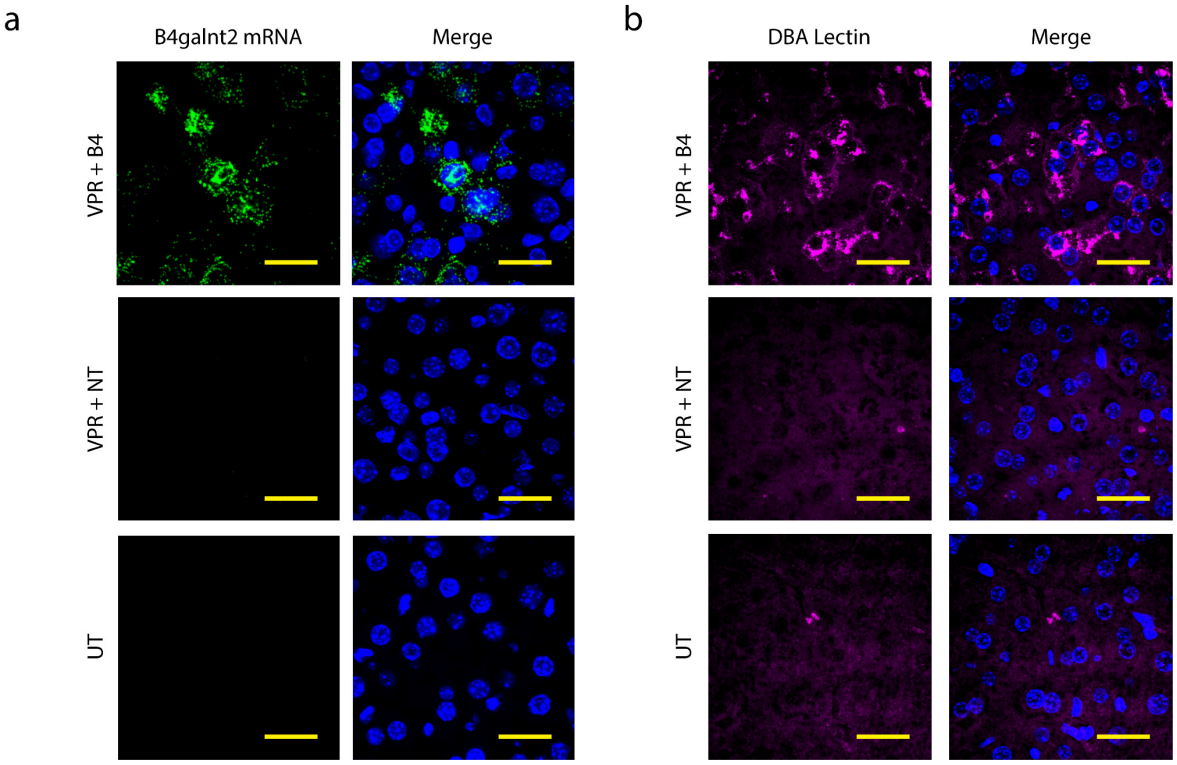
Sup. Fig. 3 | mCherry expression from in vitro time course. Each activator mRNA construct contained a cleavable mCherry sequence. **a,** Percentage of mCherry+ cells over time. **b,** MFI of mCherry+ cells. To assess differences in protein expression between the activator mRNA constructs over time. An ordinary two-way ANOVA was performed with Tukey's multiple comparisons test. Data represent mean ± SEM (n = 3 biological replicates).



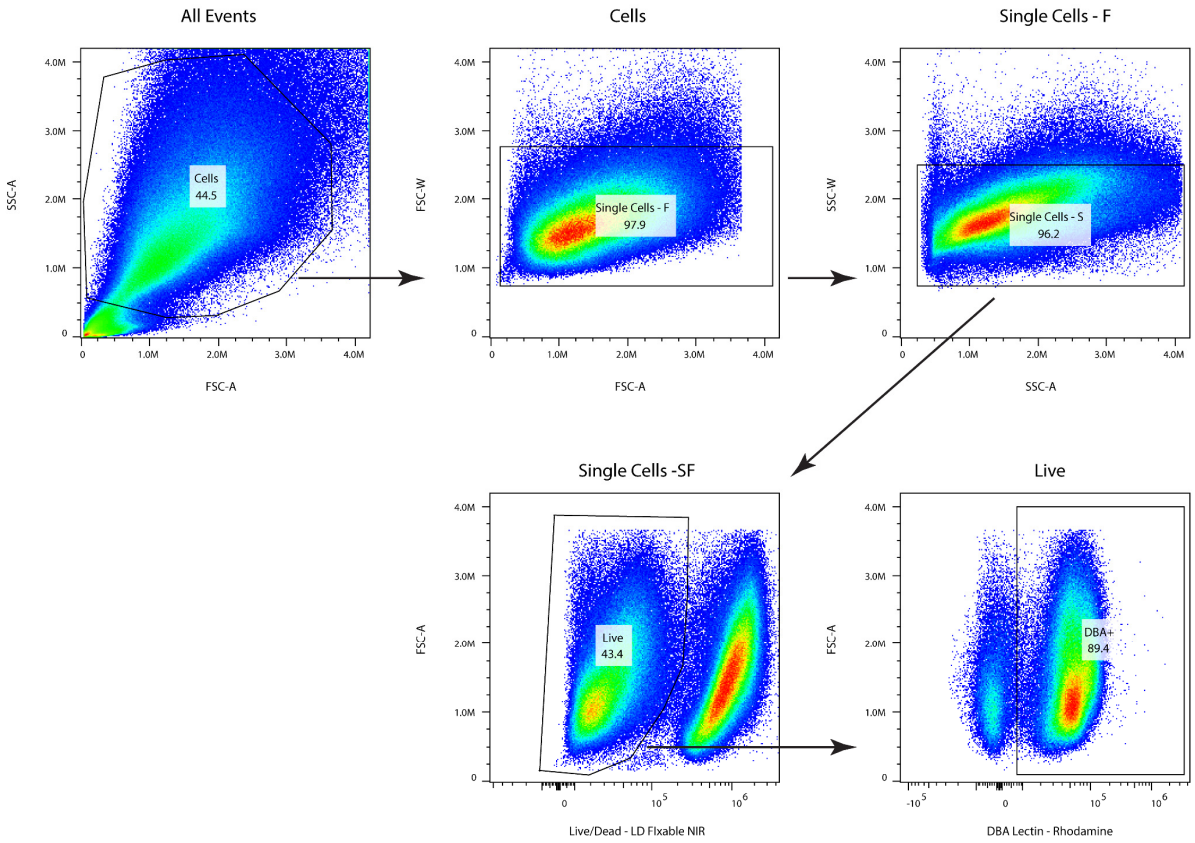
Sup. Fig. 4 | Flow cytometry gating scheme and controls for PrimeFlow assay in AML12 cells. a, Gating strategy **b**, PrimeFlow controls demonstrating probe specificity (DapB control and no Gapdh control) and successful DBA lectin staining with simultaneous PrimeFlow mRNA signal.



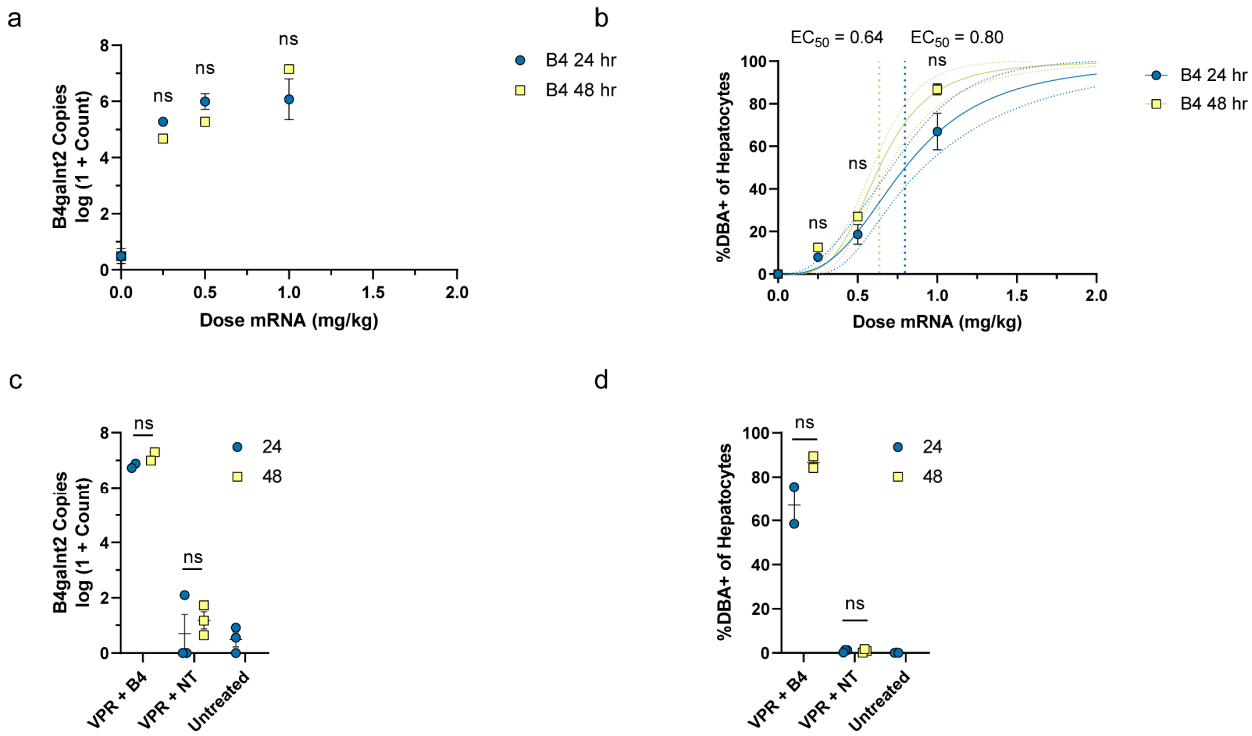
Sup. Fig. 5 | Demonstration of B4galnt2 mRNA upregulation by PrimeFlow in AML12 cells. **a**, Representative flow plots depicting the cell populations observed between treatment conditions as defined by B4galnt2 mRNA signal and lectin staining. **b**, Quantification of the percentage of cells according to B4galnt2 mRNA upregulation and lectin staining status. Data represent mean ± SEM (n = 3 biological replicates).



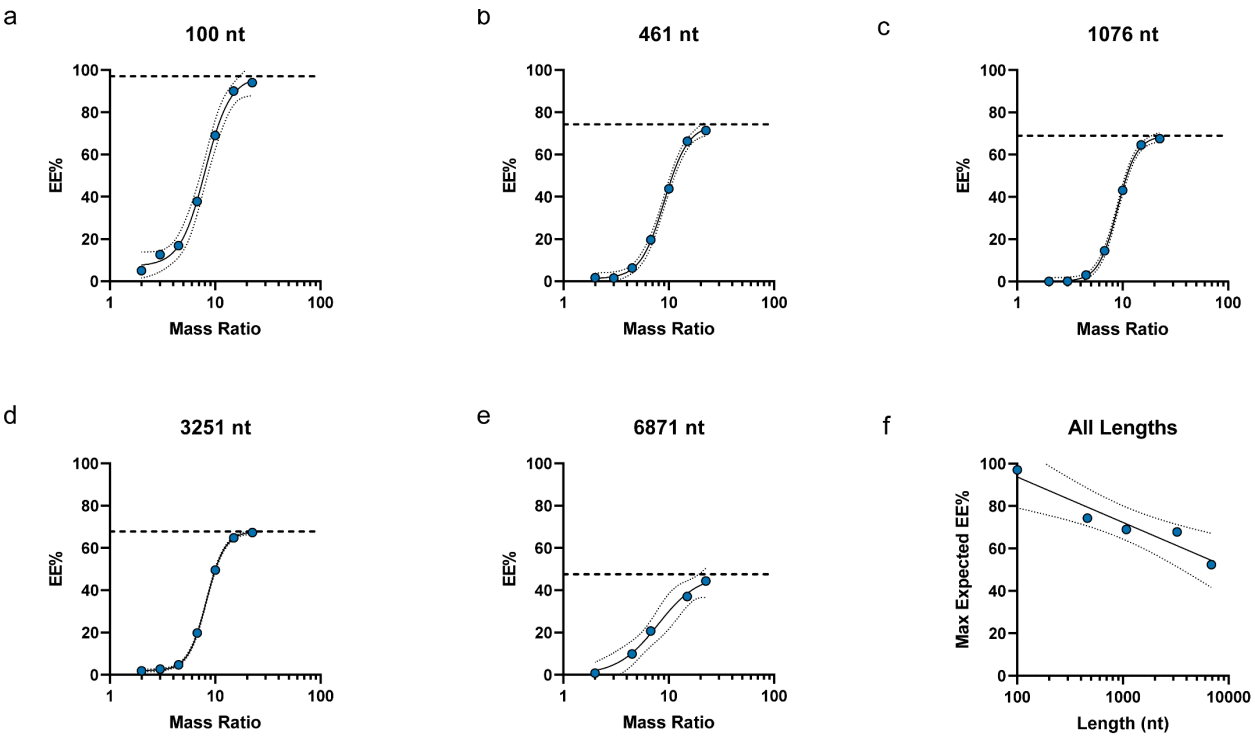
Sup. Fig. 6 | Initial demonstration of successful B4galnt2 gene activation in liver samples. Representative images showing RNAscope staining for B4galnt2 mRNA (green) (**a**), DBA lectin (magenta) (**b**), and DAPI (blue) at 1 day post injection. Scale bars are all 25 μm



Sup. Fig. 7 | Flow cytometry gating scheme for hepatocytes from liver cell suspensions. Representative flow plots depict the gating strategy based on side-scatter and live/dead staining to gate on live hepatocytes.

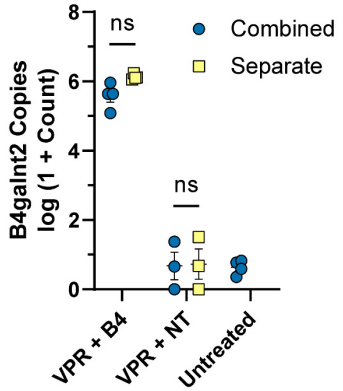


Sup. Fig. 8 | Initial dose response results using combined LNP formulation approach. B4galnt2 mRNA copy numbers (**a**) and percentage of activated hepatocytes (**b**) from liver cell suspensions at varying doses of VPR mRNA and B4-targeted sgRNA. (**c,d**) Comparison of B4galnt2 mRNA copy numbers and percentage of activated hepatocytes between treatment groups receiving either VPR mRNA + B4 sgRNA (1 mg/kg mRNA), VPR mRNA + NT sgRNA (1 mg/kg mRNA), or no treatment. Statistical significance was assessed using a two-way ANOVA followed by Dunnett's multiple comparison between 24 and 48 hours. (ns, P>0.05). 4PL curves were fit to data in b (solid lines = best fit curve, dotted lines = 95% CI). An extra sum-of-squares F-test was performed to assess statistical significance between the EC_{50} values. When P>0.05, a combined EC_{50} values was reported for the curves.

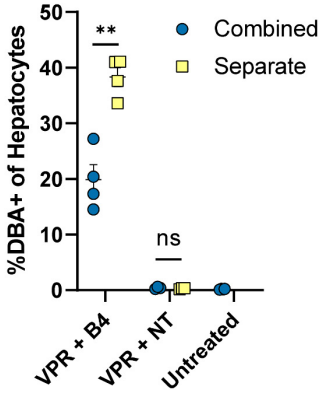


Sup. Fig. 9 | Mass ratio and mRNA length influence encapsulation efficiency of LNPs. Encapsulation efficiencies for cKK-E12 LNPs formulated with sgRNA (**a**) or different mRNAs with increasing lengths (**b-e**) at lipid:mRNA mass ratios ranging from 2 to 20. 4PL curves were fit to data in a-e (solid lines = best fit curve, dotted lines = 95% CI, horizontal dashed line = asymptotic top value). The asymptotic top values from each graph was plotted against RNA length (**f**), demonstrating a decrease in the theoretical maximum encapsulation efficiency with increasing length. Linear regression was performed for the combined data set (R^2 = 0.913, solid line = best fit, dotted lines = 95% CI). Pearson correlation analysis was also performed for the combined data set (r = -0.955, P = 0.011). All data points represent single replicates.

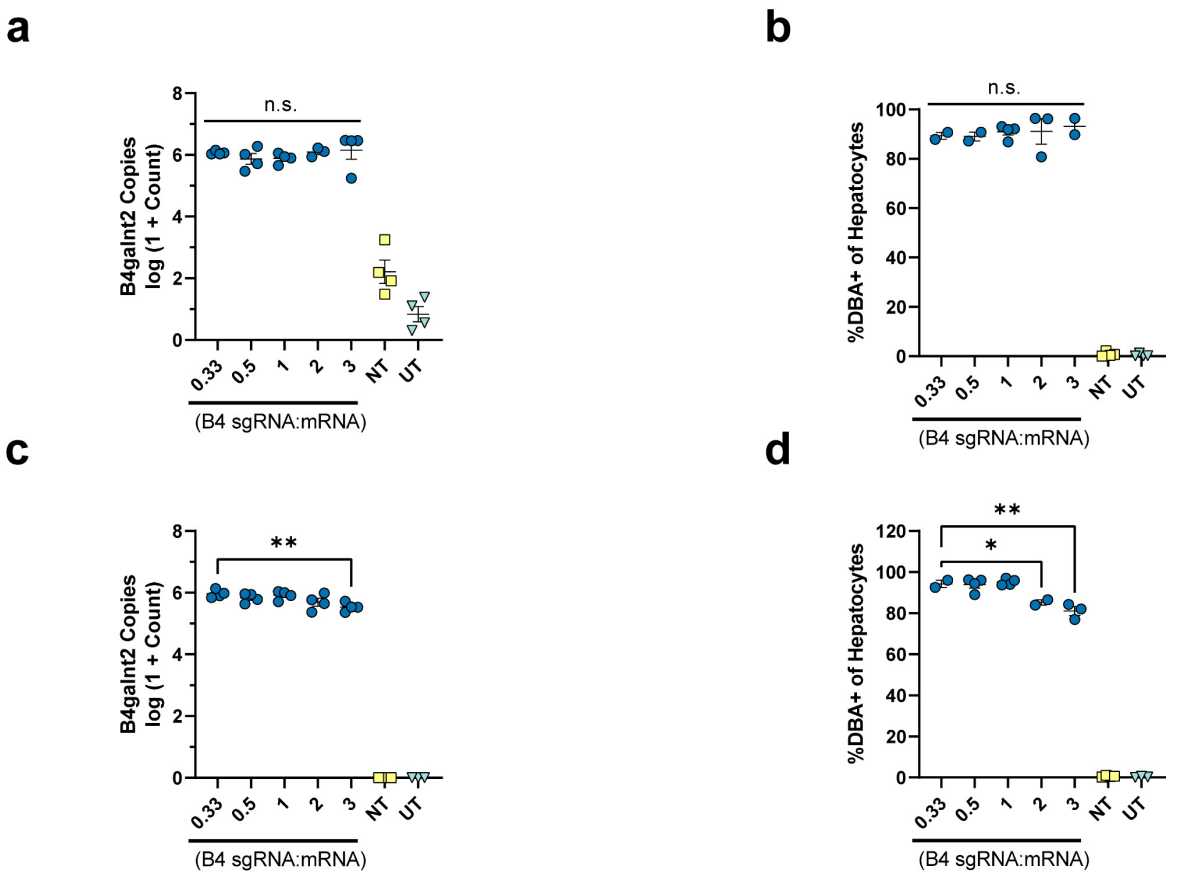
a



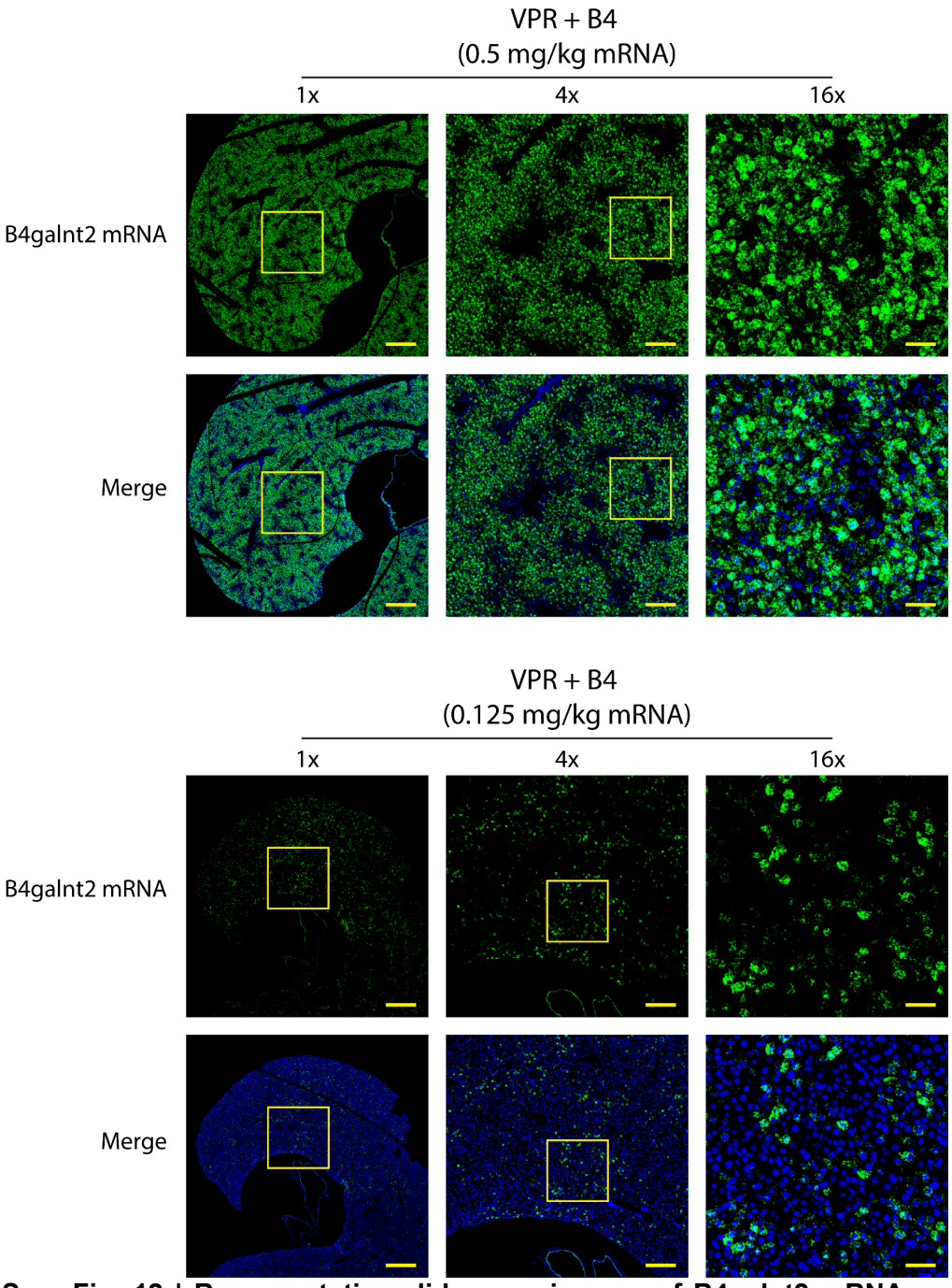
b



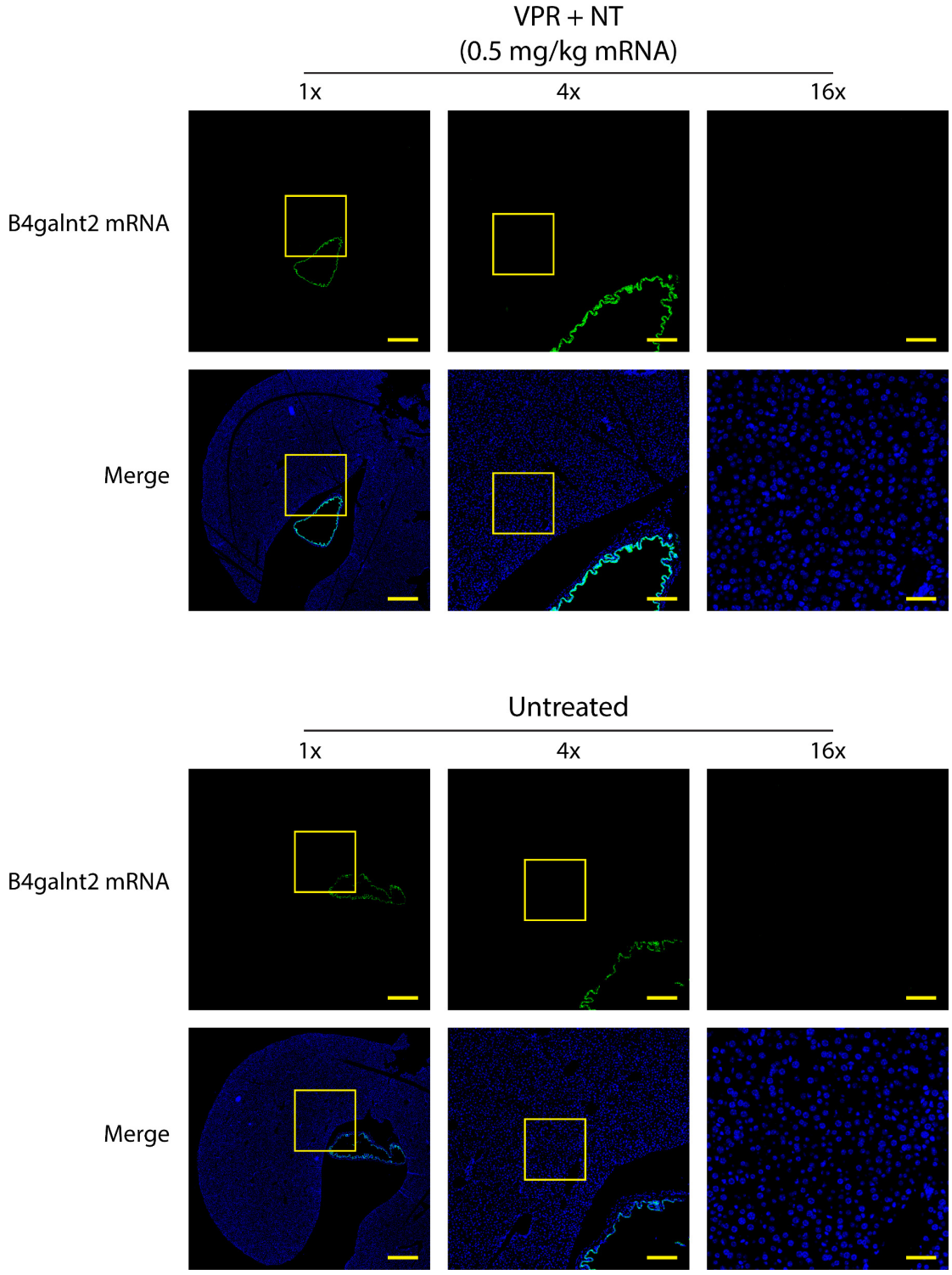
Sup. Fig. 10 | Comparison of LNP formulation approaches. a, B4galnt2 mRNA copy numbers in bulk hepatocytes (**a**) and percentage of activated hepatocytes (**b**) between combined and separate formulation approaches at a dose of 1 mg/kg VPR mRNA. Data represent mean ± SEM (n = 3-4 mice). Statistical significance was assessed using a two-way ANOVA followed by Dunnett's multiple comparison between formulation approaches.



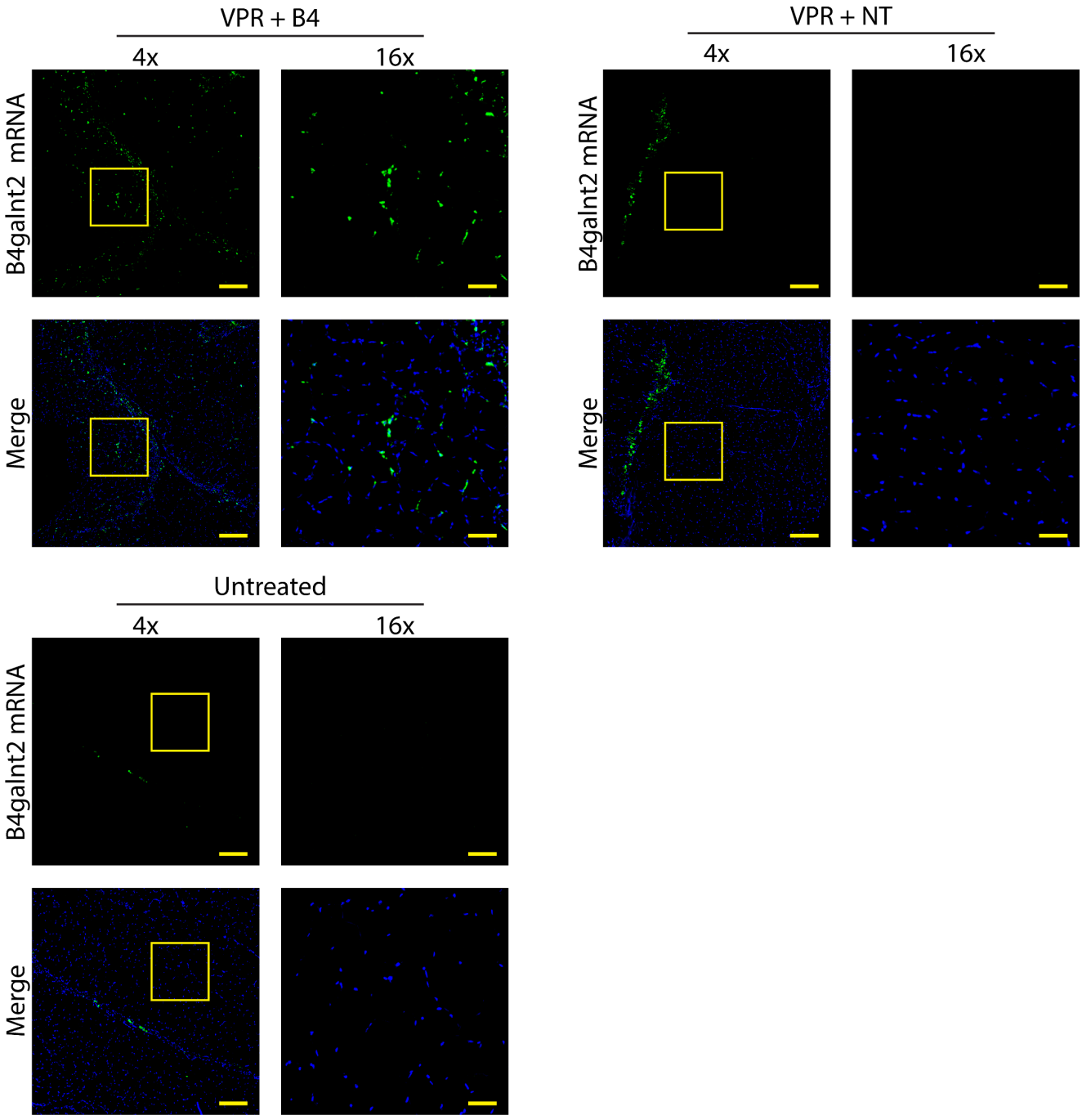
Sup. Fig. 11 | mRNA and sgRNA titration for B4galnt2 gene activation. (**a**, **b**) Titration results from fixed mRNA at 0.5 mg/kg and varying sgRNA:mRNA ratio between 1:3 and 3:1. (**c**, **d**) Titration results from fixed sgRNA at 0.5 mg/kg and varying sgRNA:mRNA ratio between 1:3 and 3:1. Data represent mean ± SEM (n = 3-4 mice). Statistical significance was assessed using a one-way ANOVA followed by Dunnett's multiple comparison between each B4 treatment dose and a sgRNA:mRNA mass ratio of 3 (**a**, **b**) or a mass ratio of 1:3 (**c**, **d**). (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).



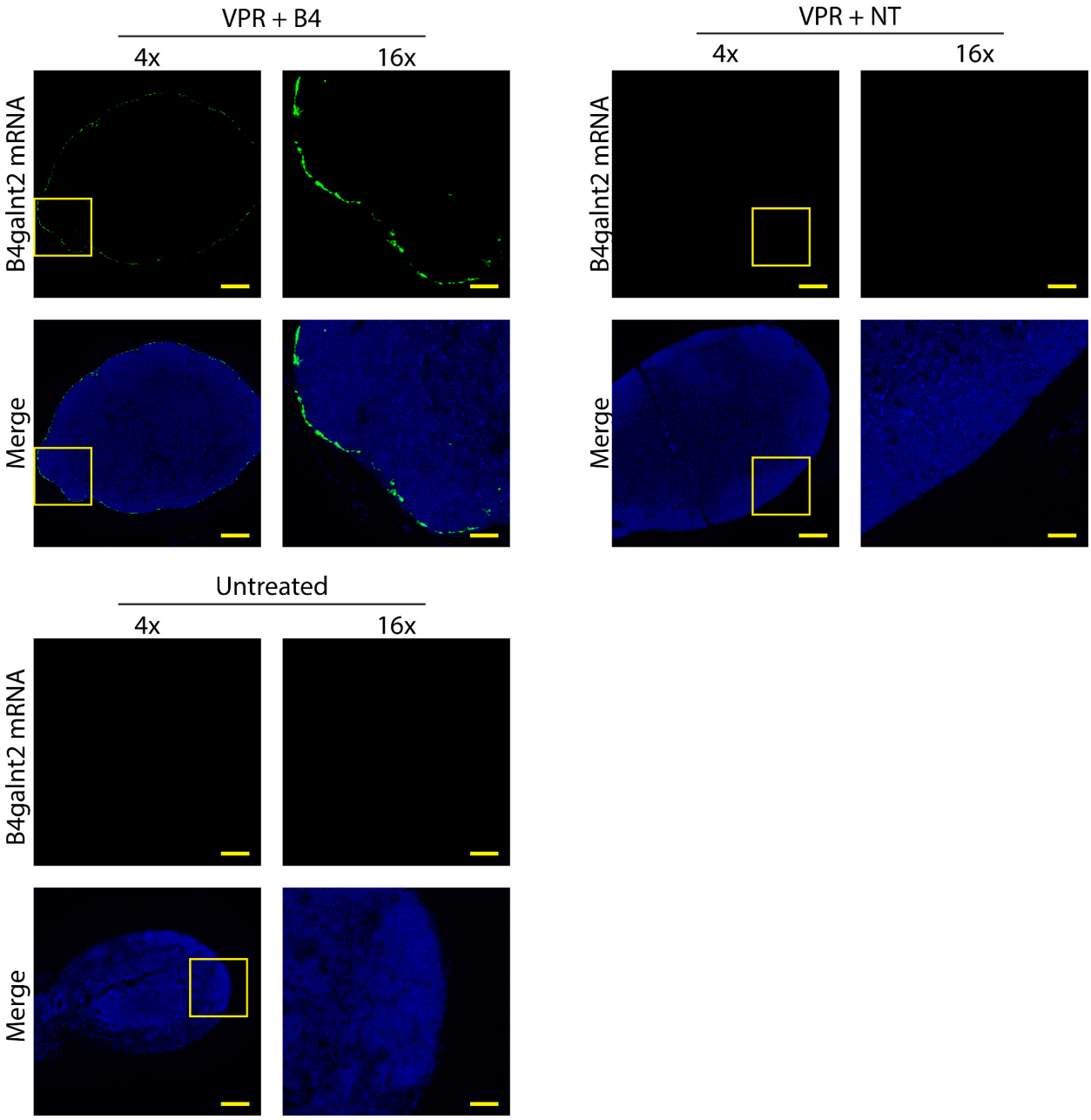
Sup. Fig. 12 | Representative slide scan images of B4galnt2 mRNA upregulation at 0.5 mg/kg and 0.125 mg/kg mRNA dose. Representative slide scan images of liver sections showing RNAscope staining for B4galnt2 mRNA (green) and DAPI (blue). The 0.125 mg/kg dose included in this figure is not contained in Figure 3a. Insets depict the relative locations of 4x and 16x views. Scale bars are 800 μm for 1x, 200 μm for 4x, and 50 μm for 16x images.



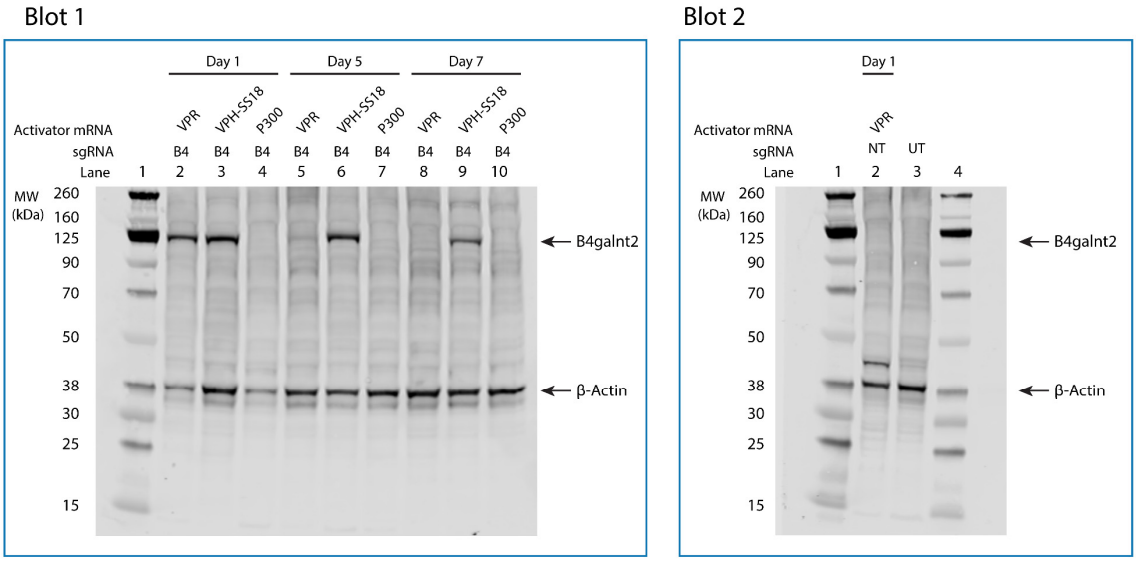
Sup. Fig. 13 | Representative slide scan images of non-targeted control and untreated livers. Representative slide scan images of liver sections showing RNAscope staining for B4galnt2 mRNA (green) and DAPI (blue). The untreated condition included in this figure is not contained in Figure 3a. Insets depict the relative locations of 4x and 16x views. Scale bars are 800 μm for 1x, 200 μm for 4x, and 50 μm for 16x images. Basal B4galnt2 mRNA expression occurs in untreated mice in the gallbladder.



Sup. Fig. 14 | Representative slide scan images of B4galnt2 mRNA upregulation in the quadriceps muscle. Representative slide scan images of quadriceps muscle sections showing RNAscope staining for B4galnt2 mRNA (green) and DAPI (blue). The VPR mRNA + B4 sgRNA treatment group displays multiple activated cells within the muscle fibers. . Insets depict the relative locations of 16x views. Scale bars are 200 μm for 4x and 50 μm for 16x images



Sup. Fig. 15 | Representative slide scan images of B4galnt2 mRNA upregulation in the paraaortic lymph node. Representative slide scan images of paraaortic lymph node showing RNAscope staining for B4galnt2 mRNA (green) and DAPI (blue). The VPR mRNA + B4 sgRNA treatment group displays a strong ring of activated cells surrounding the lymph node. Insets depict the relative locations of 16x views. Scale bars are 200 μm for 4x and 50 μm for 16x images



Sup. Fig. 16 | Western blot images of B4galnt2 protein upregulation in liver samples. Liver lysates were assessed for B4galnt2 protein, the dimeric protein (~125 kDa) is visible at ~125 kDa (63.25 kDa monomer) and actin (~38 kDa). Lanes are labeled with the activator mRNA and sgRNA injected into each animal. Blot 1, lane 1 and Blot 2 lanes 1 and 4 contain molecular weight ladders. Blot 2, lane 3 represents an untreated mouse.

Sup. Fig. 17 | Spleen images of from all mice 7 days post injection. Ambient light images of spleens are organized by rows. Scale markers represent 1 cm.