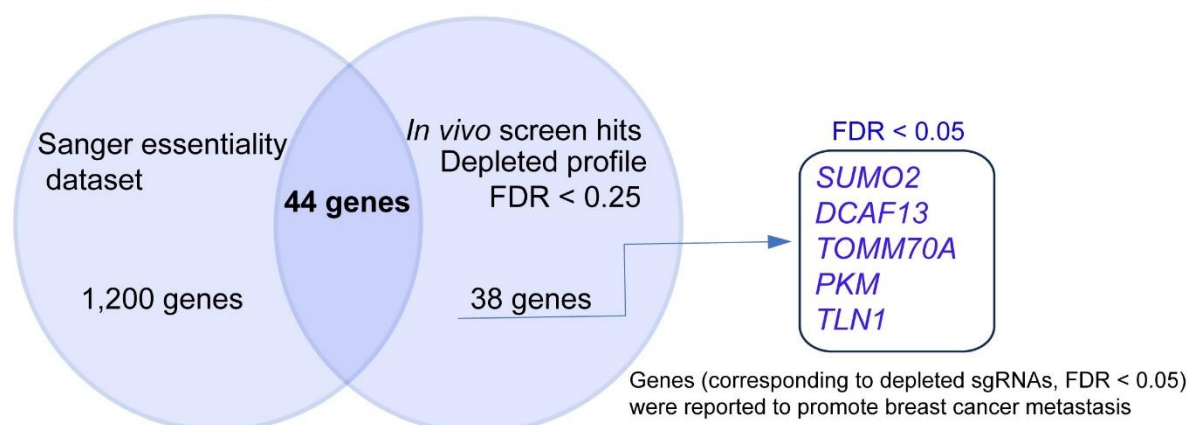


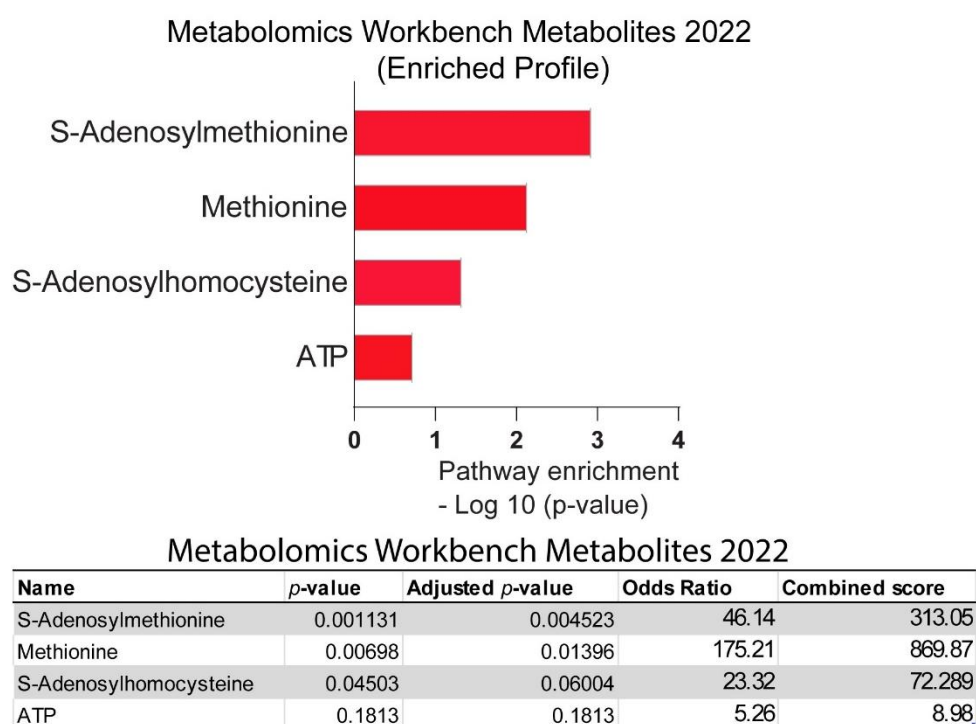
***In Vivo* CRISPR Screening Identifies Metastasis Suppressors in Triple-Negative Breast Cancer**

Supplementary data

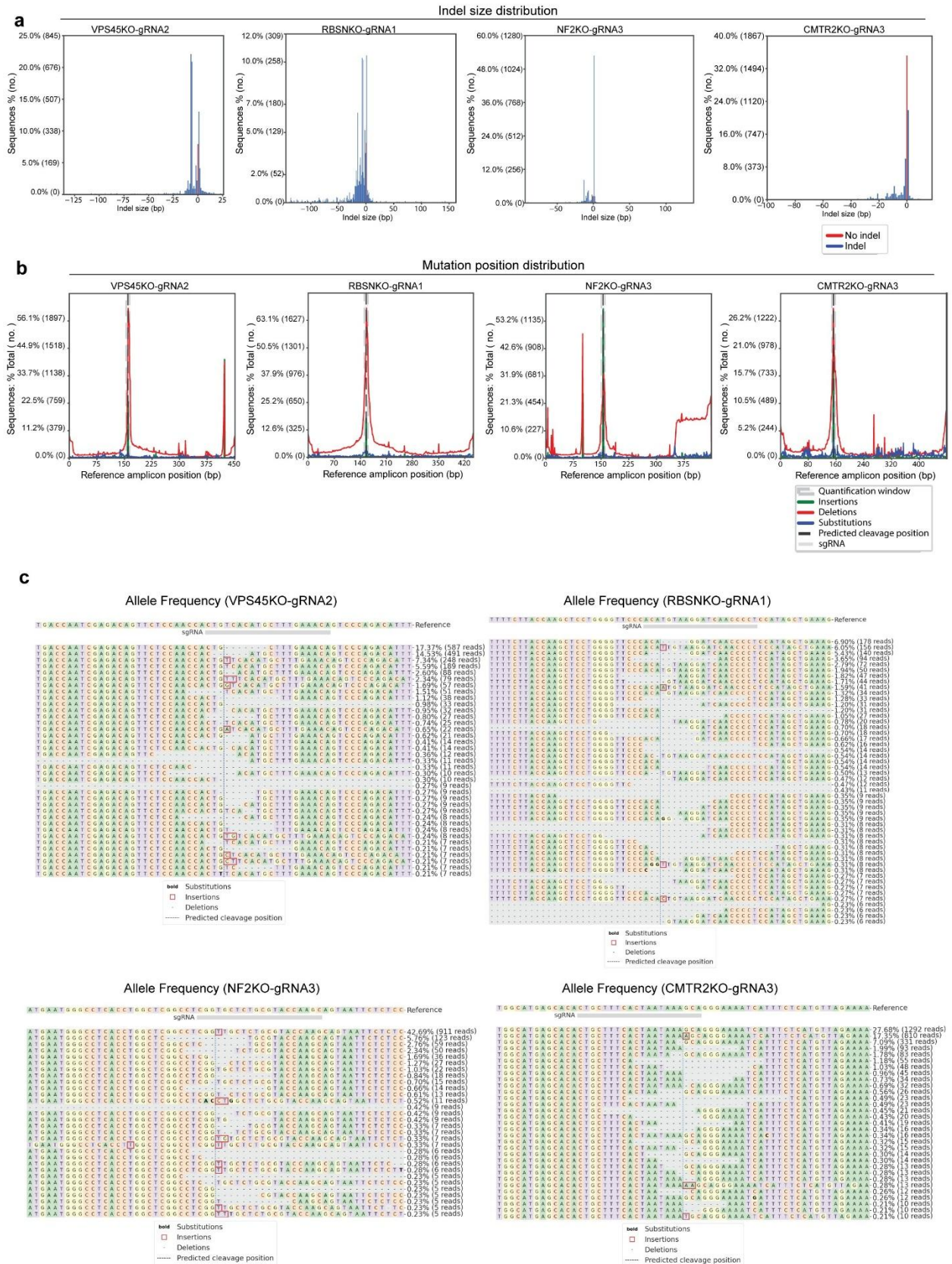
- a** Sanger CRISPR essentiality screen = 1,244 genes
In vivo lung metastasis screen hits (negative selection)
 FDR < 0.25 = 82 genes



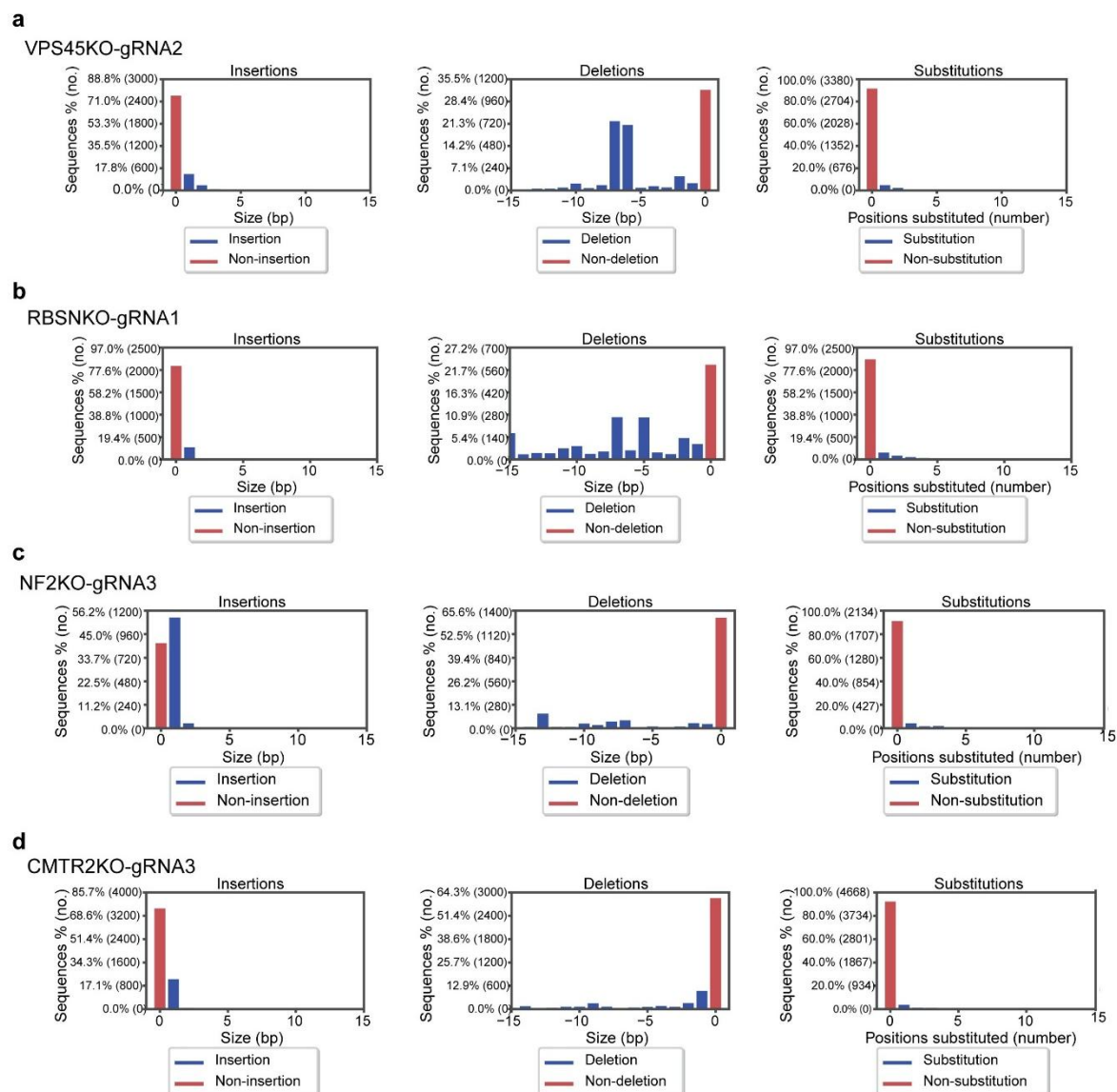
b



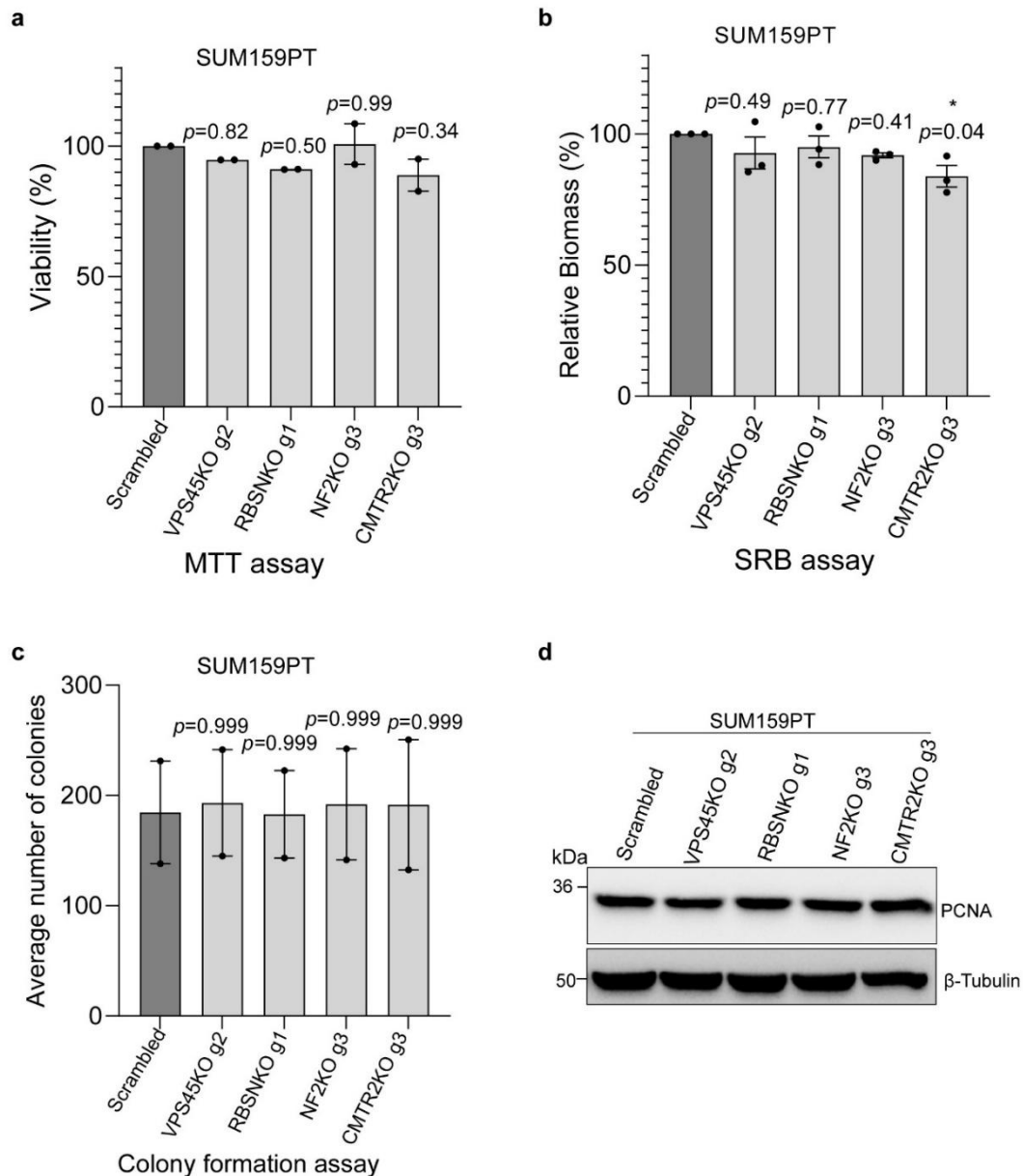
Supplementary Fig. 1. a Venn diagram comparing the top 82 genes with a false discovery rate (FDR) < 0.25 from the negative selection profile of our CRISPR screen with Sanger CRISPR essentiality dataset (1,244 genes). As shown, a total of 44 essential genes overlap between the two screens. **b** Pathway enrichment analysis of the top 20 enriched genes shows S-Adenosylmethionine as the most significantly dysregulated metabolite ****** $p < 0.01$. Source data are provided as a Source Data file.



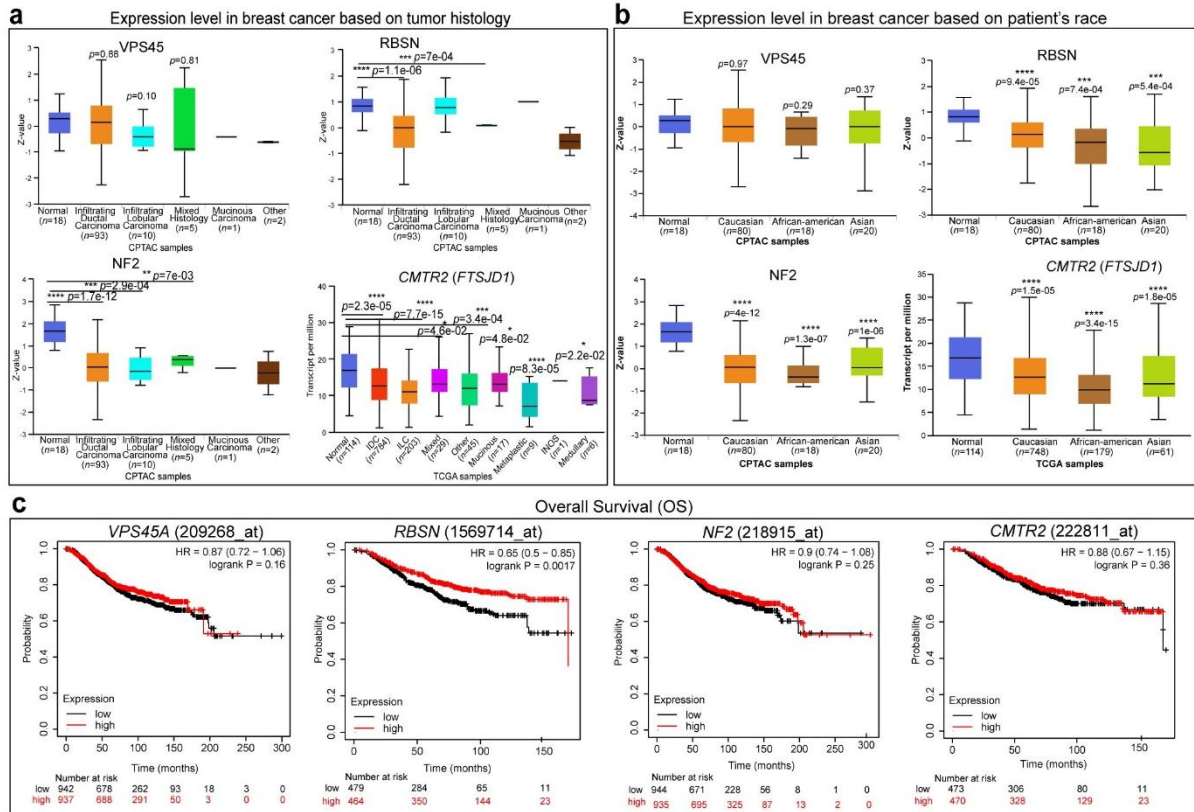
Supplementary Fig. 2 Validation of efficient CRISPR/Cas9-mediated gene disruption in *VPS45*, *RBSN*, *NF2*, and *CMTR2*. Genomic DNA from cells transduced with sgRNAs targeting *VPS45*, *RBSN*, *NF2*, and *CMTR2* (with a non-targeting sgRNA control for each gene) was PCR-amplified and subjected to targeted amplicon sequencing, followed by analysis using CRISPResso2. **a** Indel size distribution showing the frequencies of insertions and deletions across different sizes at each target locus. **b** Insertion, deletion, and substitution maps indicating the positions of sequence modifications relative to the sgRNA target site and the predicted Cas9 cleavage site. **c** Allele frequency tables around the sgRNA target region showing the most frequent edited alleles and their corresponding abundances.



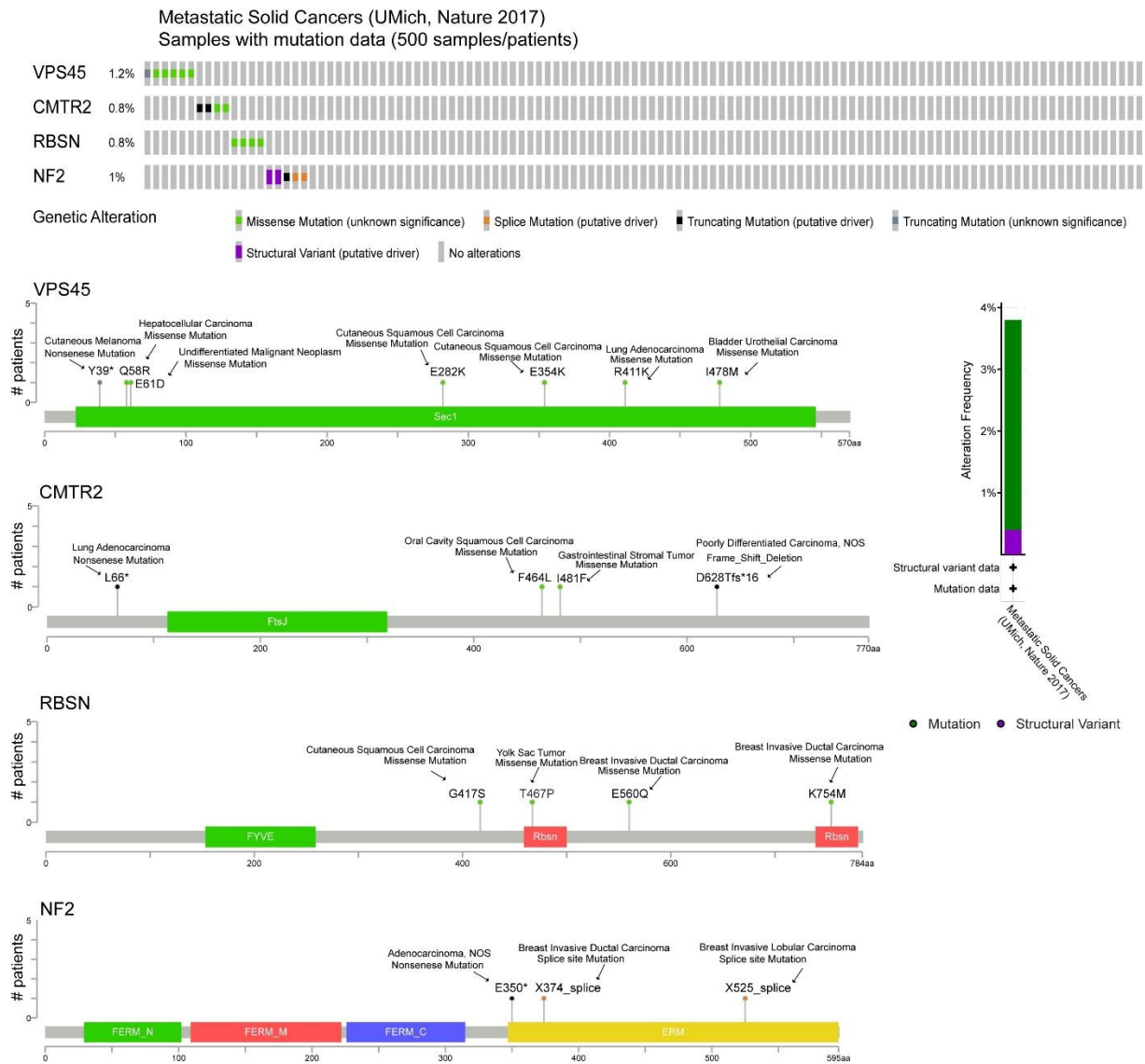
Supplementary Fig. 3 a–d Histograms showing the size distributions of insertions, deletions, and substitutions at the CRISPR target loci of *VPS45*, *RBSN*, *NF2*, and *CMTR2*, as determined by CRISPResso2 analysis of targeted amplicon sequencing data.



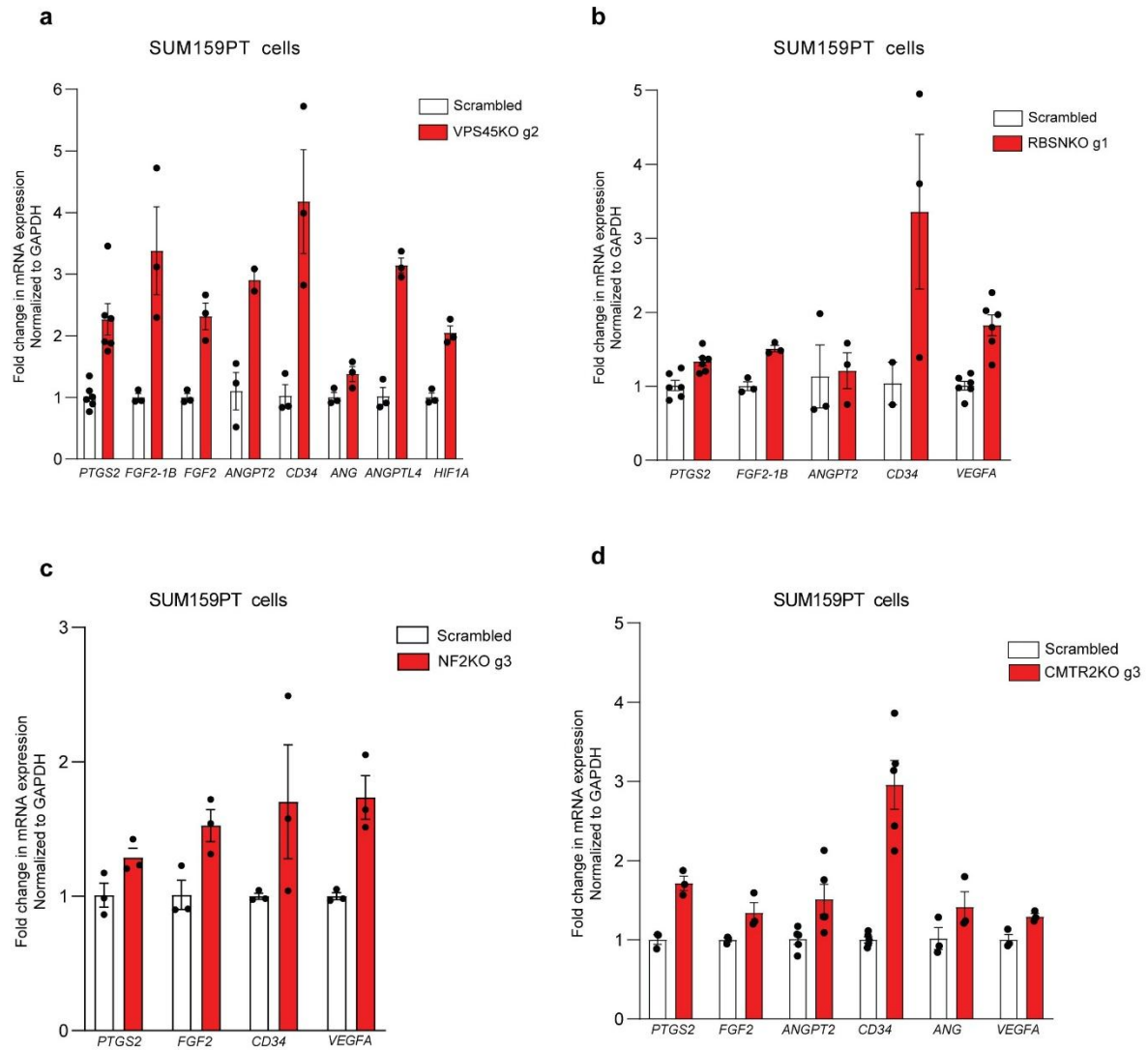
Supplementary Fig. 4 Loss of MSGs does not enhance intrinsic proliferative capacity in SUM159PT cells. **a, b** MTT and SRB assays show no increase in viability and cell growth in VPS45KO, RBSNKO, NF2KO, and CMTR2KO SUM159 cells relative to scrambled controls, with modest reductions observed in some knockouts. $n = 2$ independent biological replicates for MTT assay and $n = 3$ independent biological replicates for SRB assay. For statistical significance, one-way ANOVA followed by Dunnett's multiple comparisons test was performed. Data are presented as mean $\pm$ SEM **c** Colony formation assays reveal no significant differences in clonogenic potential among all groups. $n = 2$ independent biological replicates. Data are presented as mean $\pm$ SEM; statistical significance was determined by one-way ANOVA. **d** Representative immunoblot analysis of proliferating cell nuclear antigen (PCNA) expression in knockout and control cells confirms unchanged proliferation marker expression ($n = 2$ independent biological replicates). Scrambled control and the indicated single knockout samples were resolved on the same gel and probed with anti-PCNA and anti- β -tubulin antibodies. Source data are provided as a Source Data file.



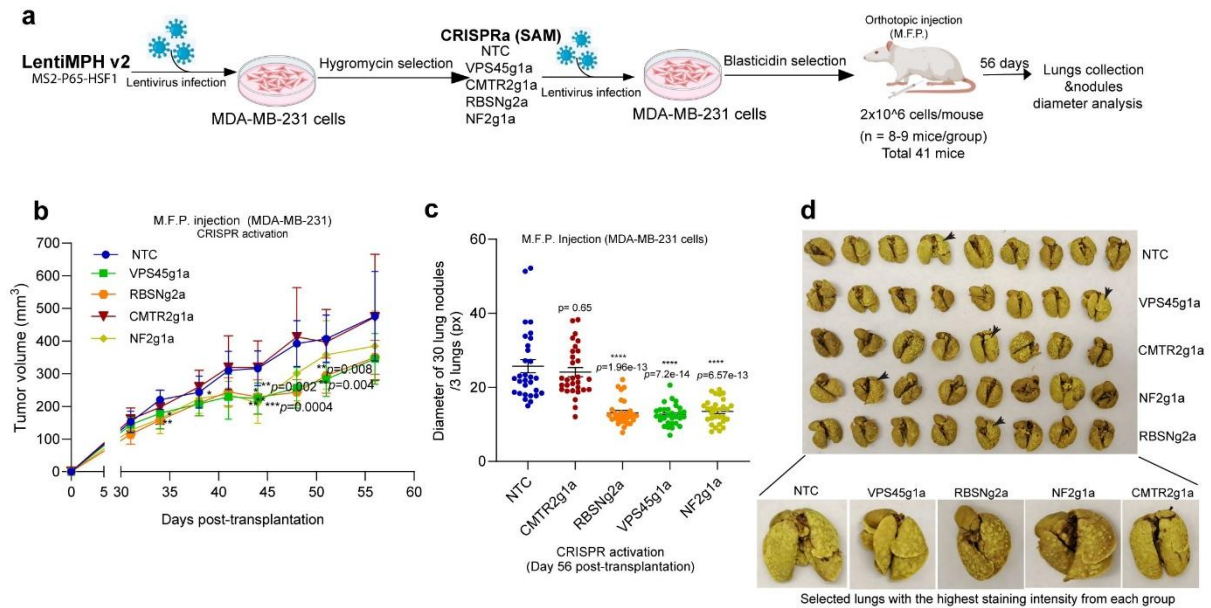
Supplementary Fig. 5 Prognostic value of the 4 target genes *VPS45*, *RBSN*, *NF2*, and *CMTR2* in breast cancer. **a** and **b** Box-and-Whisker plots show the protein expression levels of *VPS45*, *RBSN*, and *NF2*, and mRNA expression level of *CMTR2*, across histological subtypes of breast cancer and normal breast tissues using the Clinical Proteomic Tumor Analysis Consortium (CPTAC) and The Cancer Genome Atlas Program (TCGA) datasets. For all box-and-whisker plots, the central line denotes the median, the box spans the 25th to 75th percentiles (interquartile range), and the whiskers indicate the minimum and maximum values. Significance was calculated using a two-sided Welch's *t*-test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$). IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; INOS, infiltrating carcinoma not otherwise specified. **c** Kaplan-Meier overall survival (OS) analysis of the indicated genes in breast cancer patients using KM Plotter (mRNA gene chip analysis of all breast cancer subtypes). Patients were stratified into high expression (red line) and low expression (black line) cohorts for *VPS45*, *RBSN*, *NF2*, and *CMTR2*. The OS was evaluated across the respective gene expression cohorts ($n = 1,879$ for *VPS45*, $n = 943$ for *RBSN*, $n = 1,879$ for *NF2*, and $n = 943$ for *CMTR2*). Each plot shows the corresponding Affymetrix probe ID, the log-rank p-value for statistical significance, and the hazard ratio (HR) indicating the relative risk of relapse. The cumulative number of patients at risk for each cohort at baseline (Time 0) and at specific time intervals is listed below the horizontal axis. * p -value < 0.05 was statistically significant and generated by the Mantel-Cox log-rank test.



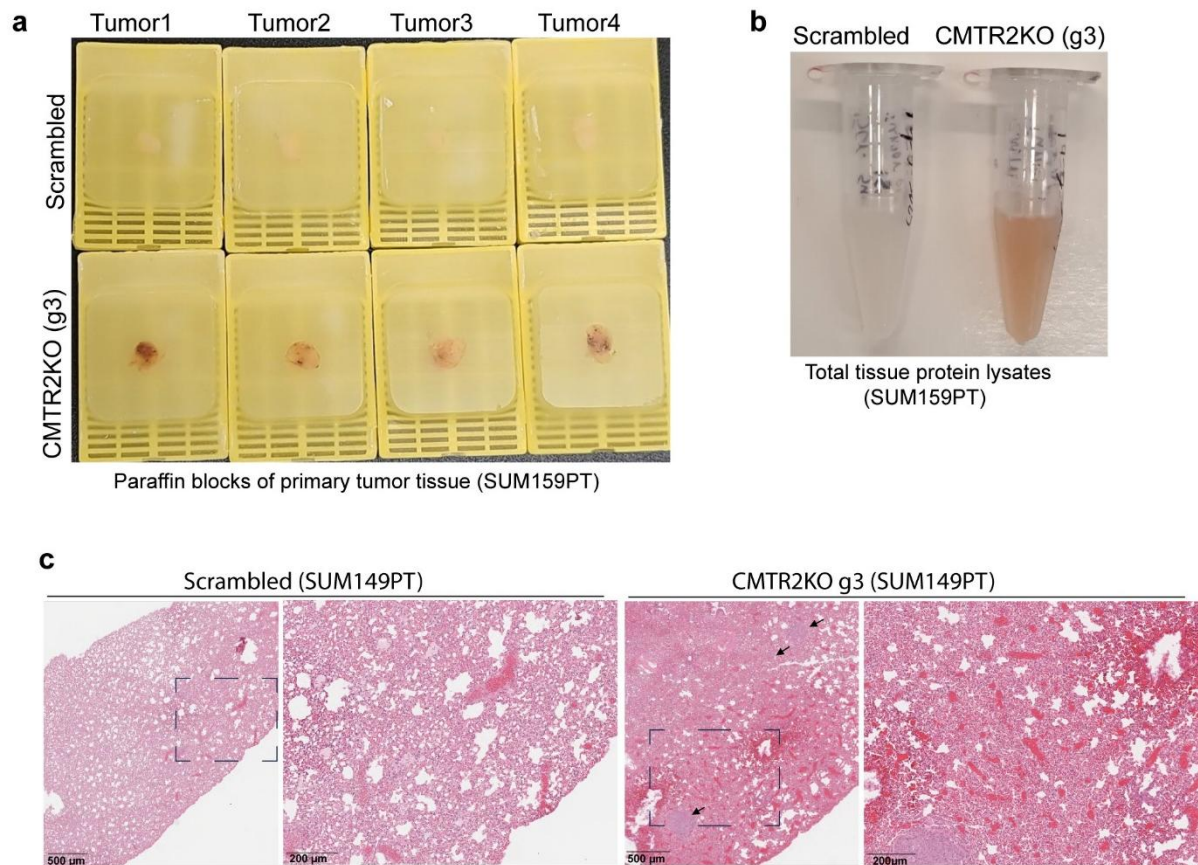
Supplementary Fig. 6 Genetic alterations of the top enriched target candidates from the *metastatic solid cancer dataset (UMich, Nature 2017)*. The figures show the frequency of genomic alterations, mutation types, protein alteration sites, and primary tumor sites for each gene.



Supplementary Fig. 7 Loss of VPS45, RBSN, CMTR2, and NF2 induces expression of angiogenesis-associated genes. a-d Real-time qPCR analysis of the mRNA expression of angiogenesis-related genes using total RNA isolated from VPS45KO, RBSNKO, NF2KO, and CMTR2KO SUM159 cells and normalized to *GAPDH* mRNA and expressed relative to non-targeting (scrambled) control. Depletion of the four target genes resulted in increased expression of multiple angiogenesis-associated genes. Data are presented as mean $\pm$ SEM. The experiment has been repeated 3 times with similar results. Source data are provided as a Source Data file.



Supplementary Fig. 8 Spontaneous lung metastasis model using the MDA-MB-231 TNBC cell line with endogenous activation of *VPS45*, *RBSN*, *CMTR2*, and *NF2*. **a** Schematic representation of the mammary fat pad (M.F.P.) injection of NSG female mice using a CRISPR endogenous activation system targeting non-targeting control (NTC), *VPS45*, *RBSN*, *CMTR2*, and *NF2* in MDA-MB-231 cells. "Schematic representation created in BioRender. Chaltel, L. (2026) <https://BioRender.com/v9d6qtf>". **b** Mammary tumor volume was assessed at the indicated days post-transplantation using two-way repeated-measures ANOVA, followed by Dunnett's multiple comparisons test (n = 8-9 mice per group and one independent *in vivo* experiment was performed). P-values indicate comparisons of each group versus the NTC group at each time point. ****p < 0.0001. **c** Diameter measurements (pixels) of 30 lung nodules from three lungs per group. Statistical analysis was performed using one-way ANOVA with multiple comparisons. ****p < 0.0001. **d** Representative images of lungs were collected at day 56 following M.F.P. injection of transduced MDA-MB-231 cells. All error bars represent SEM. n = 8-9 mice per group. Source data are provided as a Source Data file.



Supplementary Fig. 9 CMTR2 knockout promotes angiogenesis in TNBC xenograft models. **a** Paraffin-embedded primary tumor tissues from scrambled control and CMTR2KO SUM159PT xenografts display a more reddish appearance in CMTR2KO tumors compared with the scrambled controls, consistent with increased vascularization ($n = 4$ mice/group). **b** Representative protein lysates were extracted from scrambled and CMTR2KO SUM159PT tumors. CMTR2KO tumor lysates samples exhibit reddish coloration compared with the scrambled control, consistent with increased blood content and enhanced vascularization observed in tumor sections. **c** Hematoxylin and eosin (H&E) stained lung sections from scrambled control and CMTR2KO SUM149PT tail vein xenograft models. CMTR2KO lungs show increased metastatic colonization, with a notable increase in vascularization surrounding tumor foci. Scale bars: 200 μ m and 500 μ m, as indicated. $n = 3$ serial lung sections from 1 mouse (proximal, middle, distal).

Supplementary Table 1. sgRNA sequences used in GeCKOv2 library.

sgRNAs used in the GeCKOv2 library			
Gene	Rank	gRNA ID	gRNA sequence (KO)
<i>VPS45</i>	1	HGLibA_53793	TGACCATTCTAGTGCTCTCC
		HGLibA_53794	TGTCTCGATTGGTCAGTGAA
		HGLibA_53795	CTTTATTTAGTGGACATATC
<i>CMTR2</i>	2	HGLibA_10235	AGGAAACTCCGAAGTCTATG
		HGLibA_10236	GCTCCCGCAATGAATGTAGA
		HGLibA_10237	GAACATGGTGTATATCATCC
<i>ZFYVE20</i> (<i>RBSN</i>)	3	HGLibA_55278	ACATCCTGGTCGTTGACCCA
		HGLibA_55279	GACAGGTTGTTGAAACGAGA
		HGLibA_55280	TCCTTACATGTGGGAACCCC
<i>NF2</i>	4	HGLibA_31760	CCTGGCTTCTTACGCCGTCC
		HGLibA_31761	AAACATCTCGTACAGTGACA
		HGLibA_31762	CTTATTAACACGAAGCTTTG

Supplementary Table 2. sgRNA sequences used for cloning into the LentiCRISPRv2 backbone.

Gene	Human-specific sgRNAs oligos for cloning into LentiCRISPR v2 backbone (Knockout)	
	(5' to 3') Top Oligo	(5' to 3') Bottom Oligo
VPS45 sgRNA#1	CACCGTTTGAATTCCGTCGGACAG	AAACCTGTCCGACGGAATTCAAACC
VPS45 sgRNA#2	CACCGTGTTCAAAGCATGTGACAG	AAACCTGTCACATGCTTTGAAACAC
VPS45 sgRNA#3	CACCGAGCTATCTAGAACAACCTCAA	AAACTTGAGTTGTTCTAGATAGCTC
CMTR2 sgRNA#1	CACCGCCAATGACAAGGAAACCGAT	AAACATCGGTTTCCTTGTCATTGGC
CMTR2 sgRNA#2	CACCGAGGAAACTCCGAAGTCTATG	AAACCATAGACTTCGGAGTTTCCTC
CMTR2 sgRNA#3	CACCGCTGCTTTCACATAATAAGCG	AAACCGCTTTATTAGTGAAAGCAGC
RBSN sgRNA#1	CACCGAGGGGTTGATCCTTACATGT	AAACACATGTAAGGATCAACCCCTC
RBSN sgRNA#2	CACCGTGACATAATAGACCCGCAG	AAACCTGCGGGTCTATTATGTGCAC
RBSN sgRNA#3	CACCGGAGTGCGCACTTCATCCATG	AAACCATGGATGAAGTGCGCACTCC
NF2 sgRNA#1	CACCGGGAGGAGGCTGAACGCACGA	AAACTCGTGCGTTCAGCCTCCTCCC
NF2 sgRNA#2	CACCGGACCCAGTGTTCAACAAGCG	AAACCGCTTGTTGAACACTGGGGTCC
NF2 sgRNA#3	CACCGGCTTGGTACGCAGAGCACCG	AAACCGGTGCTCTGCGTACCAAGCC

Gene	Mouse-specific sgRNAs oligos for cloning into LentiCRISPRv2 backbone (Knockout)	
	(5' to 3') Top Oligo	(5' to 3') Bottom Oligo
VPS45 sgRNA#1	CACCGAAAGCTGATAACGAATCATG	AAACCATGATTCGTTATCAGCTTTC
VPS45 sgRNA#2	CACCGTCTGACCTGGTTGAGCAGCG	AAACCGCTGCTCAACCAGGTCAGAC
VPS45 sgRNA#3	CACCGTGTCTCGGTTGGTCAGTGAG	AAACCTCACTGACCAACCGAGACAC
CMTR2 sgRNA#1	CACCGGCCGAAGTACCAGCAGTGCA	AAACTGCACTGCTGGTACTTCGGCC
CMTR2 sgRNA#2	CACCGAGGCGCCCGGAGCTTCACAC	AAACGTGTGAAGCTCCGGGCGCCTC
CMTR2 sgRNA#3	CACCGGTAAGCCACTCGATAATGAG	AAACCTCATTATCGAGTGGCTTACC
RBSN sgRNA#1	CACCGACATGATAGACCCACAGAGG	AAACCTCTGTGGGTCTATCATGTC
RBSN sgRNA#2	CACCGGCACACTTCAGGAGAACCTG	AAACAGGTTCTCCTGAAGTGTGCC
RBSN sgRNA#3	CACCGAGGCTGCTCTGGAGTCACGG	AAACCCGTGACTCCAGAGCAGCCTC
NF2 sgRNA#1	CACCGGTATACAATCAAGGACACGG	AAACCCGTGTCCTTGATTGTATACC
NF2 sgRNA#2	CACCGGCTTGGTATGCGGAGCACCG	AAACCGGTGCTCCGCATACCAAGCC
NF2 sgRNA#3	CACCGCGAGATGGAGTTCAACTGCG	AAACCGCAGTTGAACTCCATCTCGC

Supplementary Table 3. sgRNA sequences used for cloning into the LentiSAMv2 backbone.

Gene & sgRNA#	sgRNAs oligos for cloning into LentiSAMv2 backbone (Activation)	
	(5' to 3') Top Oligo	(5' to 3') Bottom Oligo
VPS45 sgRNA#1	CACCG TGGAGAATAACGCCCGCGCG	AAAC CGCGCGGGCGTTATTCTCCAC
VPS45 sgRNA#2	CACCG ATAACGCCCGCGCGTGGCAG	AAAC CTGCCACGCGCGGGCGTTATC
VPS45 sgRNA#3	CACCG CCTGGGCGGCTGCCACGCGC	AAAC GCGCGTGGCAGCGGCCAGGC
CMTR2 sgRNA#1	CACCG ACTCCGCGAAGCAAAACCTA	AAAC TAGGTTTTGCTTCGCGGAGTC
CMTR2 sgRNA#2	CACCG TACTCCGCGAAGCAAAACCT	AAAC AGGTTTTGCTTCGCGGAGTAC
CMTR2 sgRNA#3	CACCG AAGCAAAACCTAGGGAGCTG	AAAC CAGCTCCCTAGGTTTTGCTTC
RBSN sgRNA#1	CACCG GATACGGATTCTGAATAGTGG	AAAC CCACTATTCTGAATCCGTATCC
RBSN sgRNA#2	CACCG ATACGGATTCTGAATAGTGA	AAAC TCCACTATTCTGAATCCGTATC
RBSN sgRNA#3	CACCG GCGGATACGGATTCTGAATAG	AAAC CTATTCTGAATCCGTATCCGCC
NF2 sgRNA#1	CACCG TAGTGGTCTGGGCGACCTGA	AAAC TCAGGTCGCCCAGACCACTAC
NF2 sgRNA#2	CACCG GTAGTGGTCTGGGCGACCTG	AAAC CAGGTCGCCCAGACCACTACC
NF2 sgRNA#3	CACCG TGGTCTGGGCGACCTGAGGG	AAAC CCCTCAGGTCGCCCAGACCAC

Supplementary Table 4. Oligo sequences used for the Surveyor nuclease assay.

Gene & sgRNA#	Surveyor assay primer sequence		Cut site	Amplicon Size	Base Before Digest	Base After Digest
	PCR primer left	PCR primer right				
VPS45 sgRNA#1	GGTACAGGAGAGATGAAGCAGAAAG	GCAGAGTGCCTTAAGGTATGGC	150077711	768	273	494
VPS45 sgRNA#2	TCTGCCTGGCACAGTGCCTT	CTGGGCAACAAGAGCAAACTCTG	150082776	450	160	289
VPS45 sgRNA#3	CCTACCTCTTTTCGCGGAAACC	CCCACGTTGACTGTTGTTACCC	150077132	615	233	381
CMTR2 sgRNA#1	CAAGTGAACAGTAGCCATGCTGC	CCAGTGAGATTTTCACCTGTGACC	71285436	548	208	339
CMTR2 sgRNA#2	CGCCTTTCCCATGCACTCAAATAG	ACCTTGCACTGGTGGTACTTTGG	71284981	579	224	354
CMTR2 sgRNA#3	TGGCAGTTACCAGATCCCAGTG	CCAAAGTACCACAGTGCAAGG	71285639	479	152	326
RBSN sgRNA#1	CAAGGGCATAAGGTCCATGAGAAG	TAGTGTGTTGTGTGCTTGCTCCTGC	15090420	443	156	286
RBSN sgRNA#2	GCATAGTACCTAGTCTGTAGTGGG	GGGATGACAGATGAGACTTGAGG	15084787	602	221	380
RBSN sgRNA#3	CCCGTTCACGCAACATCTGCA	AGAGGGTGGGTGACAGTGTG	15074700	452	148	303
NF2 sgRNA#1	AAGGTCCCCAAAGGGGAGACTG	AAGGCAACCTCGTGGTGAAAGTG	29671880	450	159	290
NF2 sgRNA#2	GAGATCCCACACTGTTTACTGGAG	GGAAGTGACTGCTTTCTTTGAGGG	29654685	516	189	326
NF2 sgRNA#3	CTCCCCAGCTCTCCCCTTTT	GAGGGTTGCACCTAGGTCTG	29655661	450	157	292

Supplementary Table 5. Primer sequences used for RT-qPCR analysis.

Gene	RT-qPCR primers sequences	
	(5' to 3') Forward Oligo	(5' to 3') Reverse Oligo
<i>VPS45</i>	GTCCTGGGAGGCACCACAGT	CTGTGCCCTTCCCCCAACCA
<i>RBSN</i>	AGAGTCTGTGCCCTCCCAGAT	TGCCACTTCAGCCACTGGGG
<i>CMTR2</i>	GCGTGGGCGAAGTTGCTGG	GGGCTGAATGACGCGGGACT
<i>NF2</i>	GCTGAAGAGGAGCTGGTTCAGG	TGGCCTGGACGGCGTAAGAA
<i>GAPDH</i>	GAAGGTGAAGGTCGGAGTCAA	CGTTCTCAGCCTTGACGGT
<i>EPCAM</i>	TGCAGGGTCTAAAAGCTGGTG	TCTTCGTCCCACGCACACAC
<i>CDH1</i>	AGCTACACGTTACGGTGCCC	CCAGAAACGGAGGCCTGATGGG
<i>MUC1</i>	ACAGCTACCACAGCCCCTA	TTGGAGAGGCCCAGAAAACC
<i>TJP1</i>	TAACAGCAATGGGGGCGTGC	ACAGTGTGGTAAGCGCAGCTC
<i>CAPZA1</i>	AGCCCAGGTGGTTGGCGT	TTGGTGCGGGTAACTGGAAGC
<i>KRT7</i>	CAGGAACTCATGAGCGTGAA	GAATAAGCCTTCAGGAGCCC
<i>KRT17</i>	AGCAGCAGAACCAGGAATACA	CACAATGGTACGCACCTGAC
<i>KRT80</i>	CCCACTTCCCAAGAATCTCA	CTTGAAGCTCCCAGACTTG
<i>SNAIL</i>	TCGGAAGCCTAACTACAGCGA	AGATGAGCATTGGCAGCGAG
<i>FNI</i>	CCATCGCAAACCGCTGCCAT	AACACTTCTCAGCTATGGGCTT
<i>SNAIL2</i>	AAGCATTTCAACGCCTCCAAA	GGATCTCTGGTTGTGGTATGACA
<i>LAMC2</i>	AGCCAAGAACGCTGGGGTTACA	TCTGGGTCTTGCTCGGGAA
<i>MMP1</i>	CAGGCCCAGGTATTGGAGGG	ATGGGCTGGACAGGATTTTGGGA
<i>MMP3</i>	GCGTGAATCTGTATCTTGCCGGTCA	TGCACACGAGTGCTTCCCCTTC
<i>SPARC</i>	TTGGCAGCCCCCTCAGCAAGA	GGCAGGGATTTTCCGCCACC
<i>CTGF</i>	GGCCTTGCGAAGCTGACCTG	CGGCAGGGTGGTGGTTCTGT
<i>KAT2B</i>	AAGCGCAACTACGCTCCGCTC	GGGGAGTGGGTGAGGGGTTAGG
<i>PLAU</i>	CCAAAGGCAGCAATGAACTT	GTTGCACCAGTGAATGTTGG
<i>GPR56</i>	GCAATGTGTGTTCTGGGTTG	GAGACCATCAGCACTGCAAA
<i>ACTA2</i>	TCGCATCAAGGCCCAAGAAA	TTGTACACACCAAGGCAGT
<i>EPHB2</i>	GTGTGTAACAGACGGGGGTT	CCCGACTTGAGCGTCTTGAT
<i>CD34</i>	TCCTAACGCCCTCCGCCTTT	GAGGGGTGGGGGATTCTTGCT
<i>ANGPT2</i>	GCTCTTCTCCAGCCCCTACG	GCCGTCTGGTTCTGTACTGCATTC
<i>Vegfa</i>	CCGGGCCTCGGTTCCA	TGCTCTCCTTCTGTCTGTTGGT
<i>Ptgs2</i>	CTTTGCCCAGCACTTCACCCA	GGGGGATACACCTCTCCACCA
<i>HIF1A</i>	CCGCCCCTTCTCTCTAGT	CGCCGAGATCTGGCTGCAT
<i>FGF2</i>	GTCCGGGAGAAGAGCGACCC	CTGCCAGTTCGTTTCAGTGCC
<i>FGF2-1b</i>	CGGCCACTTCAAGGACCCCA	TCTTCTCCCGGACCCCGTCA
<i>ANG</i>	CCACACCTCACCACGCCC	CAAAACGCCCAGGCCCATCAC
<i>ANGPTL4</i>	CGACCTCCCGTTAGCCCCTG	GCTGGGCCACCTTGTGGAAGA
<i>PTGS2</i>	AGCTTTCACCAACGGGCTGGG	AAGACCTCCTGCCCCACAGCAA
<i>VEGFA</i>	GACGAGGGCCTGGAGTGTGTG	AGCAGCGGGCACCAACGTA