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Supplementary information

Mouse genome rewriting and tailoring of three important disease loci

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Mouse genome rewriting and tailoring of three important disease loci

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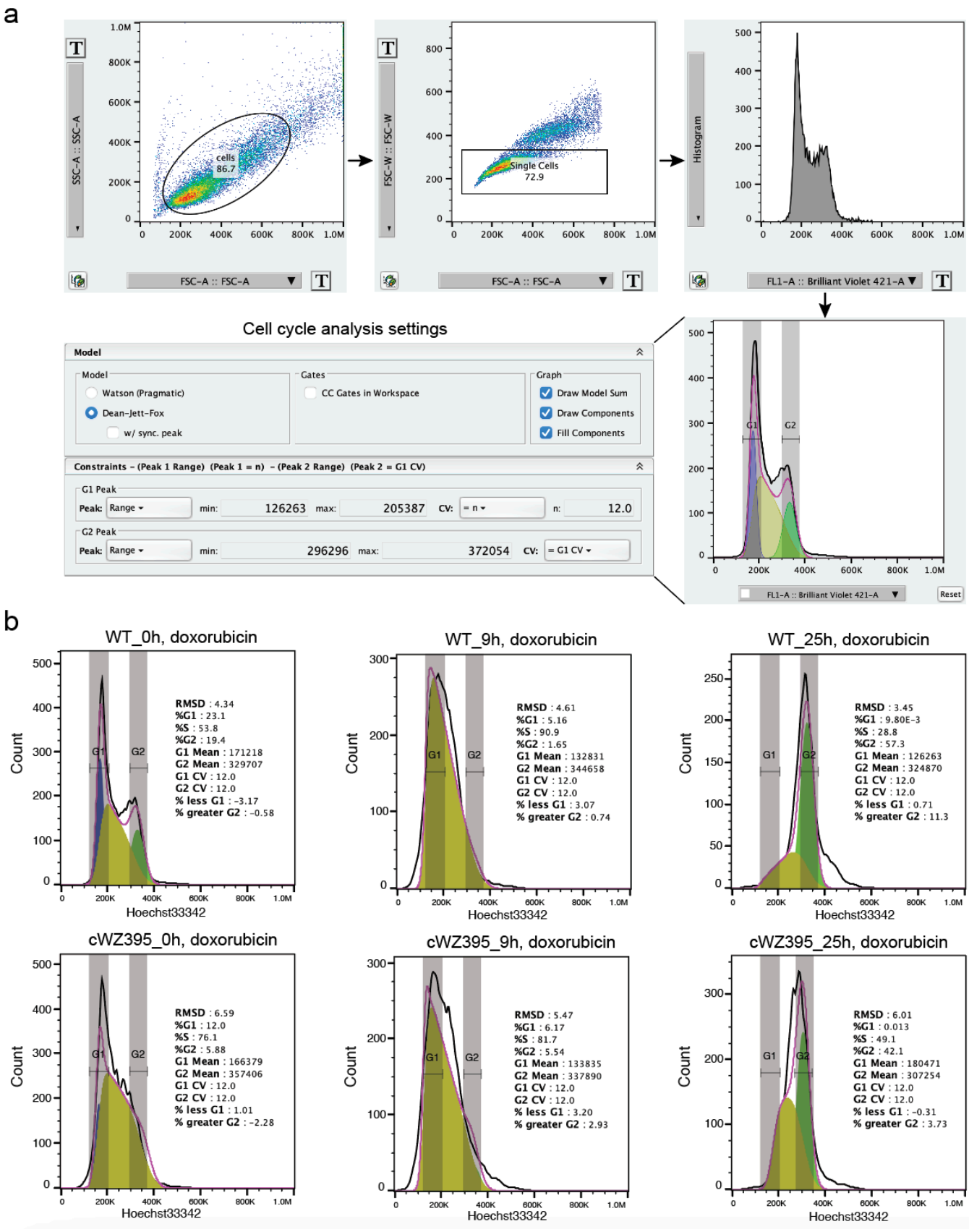
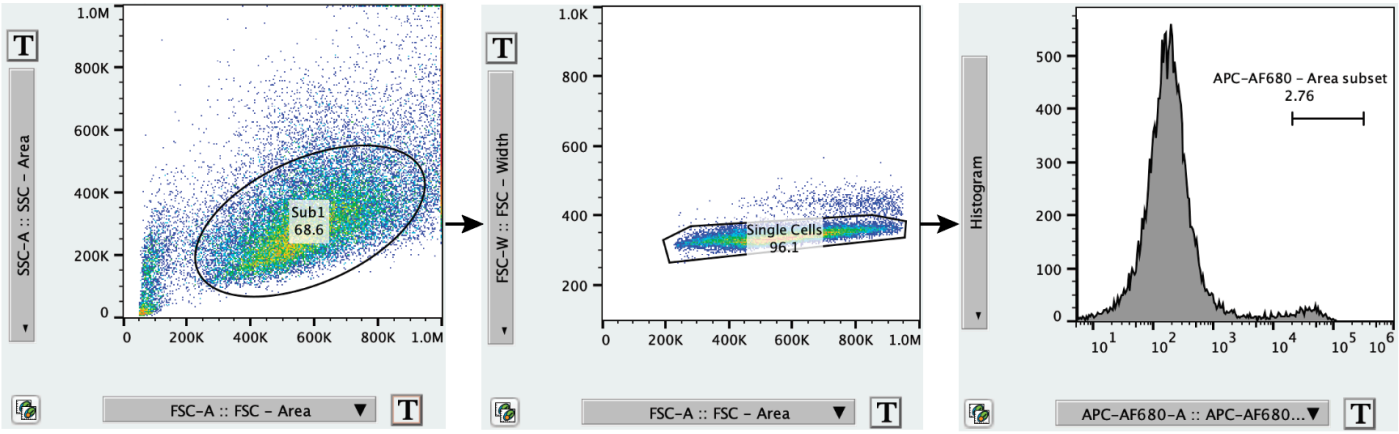


Supplementary Figure 1. (a) Wild type and *synTrp53* mESCs cell cycle arrest flow cytometry gating strategy. **(b)**. Cell cycle analysis using FlowJo's build-in tool, wild-type *Trp53* (MK6) without doxorubicin treatment was used for defining the G1 and G2 gates.



Supplementary Figure 2. Wild type and *synTrp53* mESCs cell apoptosis analysis flow cytometry gating strategy.

Fig. 4c. *hACE2* testis, panel 1

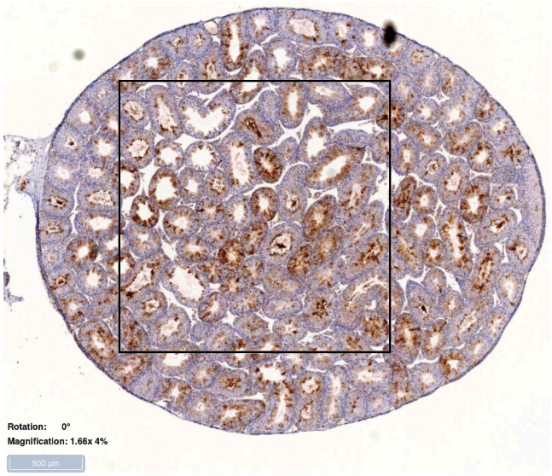


Fig. 4c. WT testis, panel 2

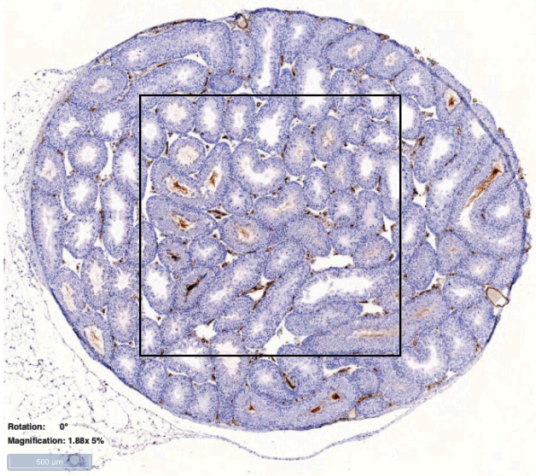


Fig. 4c. *hACE2* lung, panel 3

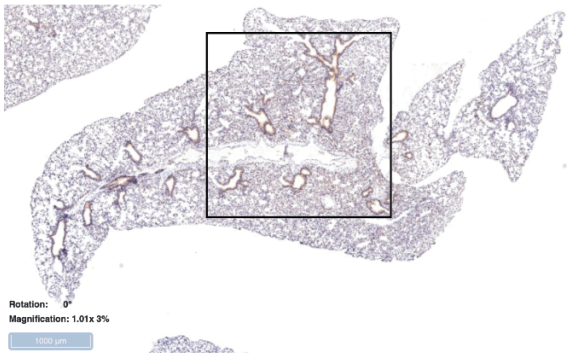


Fig. 4c. WT lung, panel 4

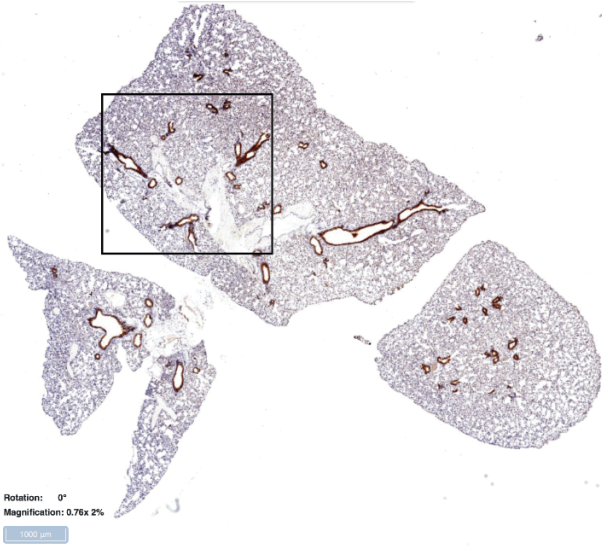


Fig. 5f. WT lung, panel 1

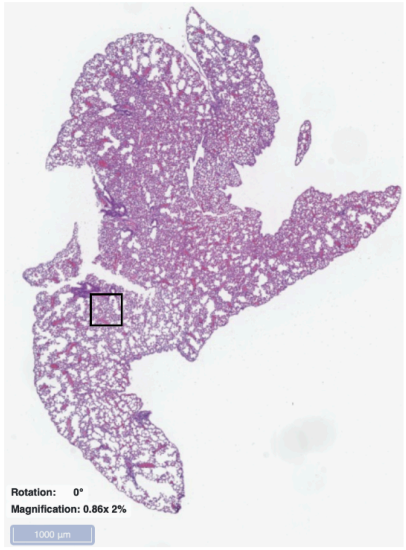


Fig. 5f. *K18-hACE2* lung, panel 2

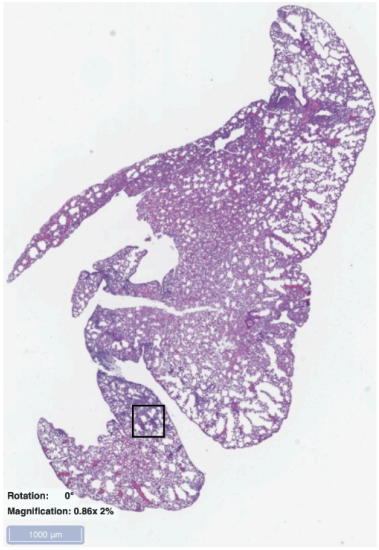
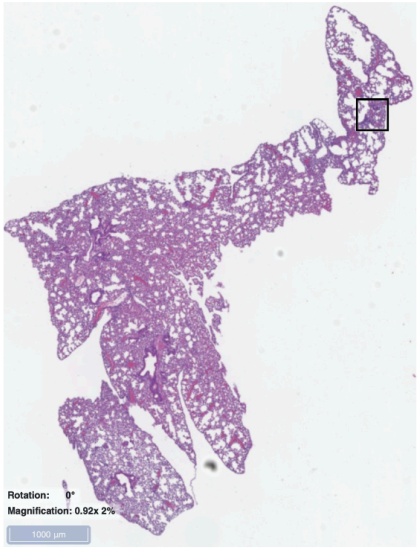


Fig. 5f. *hACE2* lung, panel 3



Supplementary Figure 3. Uncropped lungs, testes immunohistochemistry staining with ACE2 antibody, and H&E staining of infected lungs.

Supplementary Table 1. PCRTag and insertion coordinates for the subsequent mSwAP-In of *synTrp53*.

Name	Coordinates	Inserted sequences
PCRTag-1	Chr11: 69,597,884	GCTTTGAGCTGACAATTTGCCACCACCG
PCRTag-2	Chr11: 69,611,965	TCTGACGCCGTGGCTACTAACGGCAATA
PCRTag-3	Chr11: 69,625,010	TCTCTCCTGCCAGATGACACCTGAAGTG
PCRTag-4	Chr11: 69,637,459	CAAGGTTGACTTAAAAGCAGCTCTAGCG
PCRTag-5	Chr11: 69,650,231	CCCAAGCGTCATCATTAGCGGCAGTAAA
PCRTag-6	Chr11: 69,663,762	GTAAGCGCTTCTACGGGTGATACTTCTA
PCRTag-7	Chr11: 69,676,478	GGTGCCACTTGATAAGCTAGTTTGATCG
PCRTag-8	Chr11: 69,689,789	CGAAACCTTGAGTGGCTTGAGAATCGTC

Supplementary Table 2. Summary of tetraploid complementation injection success rate.

Tetraploid injected mESC clones	No. of Injected Embryos	No. of Pups	Birth Rate
116 kb *hACE2*-cWZ271	25	5	20%
116 kb *hACE2*-cWZ272	25	2	8%
180 kb *hACE2*-cWZ329	25	5	20%
180 kb *hACE2*-cWZ328	20	6	30%
180 kb *hACE2*-cWZ323	25	5	20%
TMPRSS2+116 kb *hACE2*-cWZ529	23	3	13%

Supplementary Table 3. Guide RNA sequences.

Guide RNA purposes	Sequences
gRNA targets UGT1	GCUUCAUGUGGUCGGGGUAG
gRNA targets UGT2	CACGAGGGUGGGCCAGGGCA
gRNA mediates MC1 insertion for *ACE2* humanization	AGGGUCUUCUCUACUCAAGG
Custom gRNA for *ACE2* humanization	UUAUUACUAGAGUAGCAGGG
gRNA mediates MC1 insertion for “CG-less” *Trp53* engineering	UACUGCCGUGUAUCGUAUUG
Custom gRNA for *synTrp53* engineering	UUGUAUAGGACCCUCGGGCA
Custom gRNA for engineering the 40 kb payload downstream of *synTrp53*	CAUCUCACCAGCCUAGCAGG
Custom gRNA for engineering the 75 kb payload downstream of *synTrp53*	UCAUUAACCCAGGAGCCACG
Custom gRNA for engineering the 115 kb payload downstream of *synTrp53*	ACCUGCUUCACAGAUAACUG

Supplementary Table 4. Amplicon sequencing primers.

Name	Sequence	Notes
oWZ1995	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCACCATCGGAGCAGCGCTCATGG	R155R172 P7 primer with UMI
oWZ1996	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNNGCGGAAATTTTCTTCTTCTGTACG	R270R279 P7 primer with UMI
oWZ1997	TTTCCCTACACGACGCTCTTCCGATCTNCACCTCCAGCTGGGAGCCGTGTC	R155R172 P5 primer for *Trp53*$^{wt/wt}$
oWZ1998	TTTCCCTACACGACGCTCTTCCGATCTNcTCCTGCATGGGGGGCATGAAC	R245R246 P5 primer for *Trp53*$^{wt/wt}$
oWZ2002	TTTCCCTACACGACGCTCTTCCGATCTNGCTTTCTTACTGCCTTGTGCTGGT	R270R279 P5 primer for *Trp53*$^{wt/wt}$
oWZ2004	CTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNNNNNNNCGCGCCTTCCTACCTGGAGT	R245R246 P7 primer with UMI
oWZ2006	TTTCCCTACACGACGCTCTTCCGATCTNNCACCTCCAGCTGGGAGCCGTGTC	R155R172 P5 primer for *Trp53*$^{syn/syn}$
oWZ2007	TTTCCCTACACGACGCTCTTCCGATCTNNcTCCTGCATGGGGGGCATGAAC	R245R246 P5 primer *Trp53*$^{syn/syn}$
oWZ2008	TTTCCCTACACGACGCTCTTCCGATCTNNGCTTTCTTACTGCCTTGTGCTGGT	R270R279 P5 primer *Trp53*$^{syn/syn}$

Supplementary Table 5. RT-qPCR primers and probes.

Name	Sequence	Notes
oWZ1588	GGACCCAGGAAATGTTCAGA	Human *ACE2* qPCR primer F1
oWZ1589	GGCTGCAGAAAGTGACATGA	Human *ACE2* qPCR primer R1
oWZ1590	GGGATCAGAGATCGGAAGAAGAAA	Human *ACE2* qPCR primer F2
oWZ1591	AGGAGGTCTGAACATCATCAGTG	Human *ACE2* qPCR primer R2
oWZ1592	AAACATACTGTGACCCCGCAT	Human *ACE2* qPCR primer F3
oWZ1593	CCAAGCCTCAGCATATTGAACA	Human *ACE2* qPCR primer R3
oWZ1596	TCCATTGGTCTTCTGCCATCC	Mouse *ACE2* qPCR primer F1
oWZ1597	AACGATCTCCCGCTTCATCTC	Mouse *ACE2* qPCR primer R1
oWZ1598	TGATGAATCAGGGCTGGGATG	Mouse *ACE2* qPCR primer F2
oWZ1599	ATTCTGAAGTCTCCGTGTCCC	Mouse *ACE2* qPCR primer R2
oWZ1600	GATCAAGATCATTGCTCCTCCTGA	Mouse *Actb* reference primer for RT-qPCR
oWZ1601	AAGGGTGTAAAACGCAGCTCA	Mouse *Actb* reference primer for RT-qPCR

oWZ1655	AGTCCGCCTAGAAGCACTTGCGGTG	*Actb* probe labeled with Cy5
N1-F	GACCCCAAAATCAGCGAAAT	SARS-CoV-2 nucleocapsid forward primer
N1-R	TCTGGTTACTGCCAGTTGAATCTG	SARS-CoV-2 nucleocapsid reverse primer
N1-probe	ACCCCGCATTACGTTTGGTGGACC	SARS-CoV-2 nucleocapsid probe labeled with FAM
oWZ1683	CCTCATTAGCTTTGTCACACGAGCC	Mouse *Actb* reference primer for copy number qPCR
oWZ1684	CCATAGGACTCCCTTCTATGAGCTG	Mouse *Actb* reference primer for copy number qPCR
oWZ1771	ATCGGGTGACAGAAGACCAATGGA	*ACE2* short isoform detection primer
oWZ1772	GCTTGTGAGAGCCTTAGGTTGGATT	*ACE2* short isoform detection primer
oWZ1781	GTCAGCCAACTTTTTCCCAAGAGTG	*ACE2* long transcript variant detection primer
oWZ1782	CCGTATCAATGATGCTTTCCGTCTG	*ACE2* long transcript variant detection primer
oWZ1826	CATCCTGGTGAGGAACGAAAGG	*Isg15* mouse RT-qPCR primer-F
oWZ1827	CTCAGCCAGAACTGGTCTTCGT	*Isg15* mouse RT-qPCR primer-R
oWZ1828	CCGAGTAACGGCTGCGACAAAG	*Cxcl11* mouse RT-qPCR primer-F
oWZ1829	CCTGCATTATGAGGCGAGCTTG	*Cxcl11* mouse RT-qPCR primer-R
oWZ1830	TGGACATTGCTACCACAGAGGC	*Mx1* mouse RT-qPCR primer-F
oWZ1831	TTGCCTTCAGCACCTCTGTCCA	*Mx1* mouse RT-qPCR primer-R
oWZ1855	CCTCTAACTGGTGTGATGGCGT	Human *TMPRSS2* RT-qPCR primer-F
oWZ1856	TGCCAGGACTTCCTCTGAGATG	Human *TMPRSS2* RT-qPCR primer-R
oWZ1857	AAGTCCTCAGGAGCACTGTGCA	Mouse *Tmprss2* RT-qPCR primer-F
oWZ1858	CAGAACCTCCAAAGCAAGACAGC	Mouse *Tmprss2* RT-qPCR primer-R
oWZ2100	GTGAAAGCGGGTGTGAGGAgc	Human *TMPRSS2* isoform 1 RT-qPCR primer forward
oWZ2101	CTGTGCGGGATAGGGGTTTTcc	Human *TMPRSS2* isoform 1 RT-qPCR primer reverse
oWZ2102	GCGAGGGGCGGGGAGCGCCg	Human *TMPRSS2* isoform 2 RT-qPCR primer forward
oWZ2103	cgGGTAGTACTGAGCCGGATGC	Human *TMPRSS2* isoform 2 RT-qPCR primer reverse