

## SUPPLEMENTARY MATERIALS AND METHODS

### *Generation of concentrated conditioned media and LOX Amplex Red Activity Assays.*

Conditioned medium was concentrated essentially as previously described [1] with the following changes: Cells were seeded at ~80% confluency in 10 cm tissue culture plates. After ~12 hours cells were washed with PBS before addition of 10ml serum-free and phenol red-free DMEM supplemented with L-glutamine, gentamycin sulfate, amphotericin B, CuSO<sub>4</sub> (10 μM for HEK293 cells and 5 μM for MDA-MB-231 cells) and 0.1% BSA [1]. After 48 hours the conditioned medium was collected and concentrated 20 fold using 10k centrifugal filters. The resulted concentrated conditioned medium was used for LOX activity assays or immunoblotting. The LOX activity assay was also performed as previously described [1], with the following changes: Two separate solutions were made; the S solution containing Amplex® Red Reagent (A12222, Invitrogen) and DAP (1,5-diaminopentane, Cadaverin 95%, D22606-5G, Sigma. Acting as the substrate for LOX) in Assay Buffer (50 mM sodium borate, pH 8.0), and the E solution containing horseradish peroxidase in Assay Buffer. Solution E (50 μL) was added to 10μL of the LOX containing concentrated conditioned medium in All-black 96-well plates (Costar, Corning Inc). The irreversible LOX inhibitor  $\alpha$ -aminopropionitrile (BAPN) [2] was then added to the control wells. Following addition of 40 μL of the S solution the plate was loaded into a FLUOstar Galaxy Multi-functional Microplate Reader (BMG Labtechnologies). Final concentrations of all reagents are as described in [1]. A kinetic top read of fluorescence was performed every 1 minute for a total of 179 minutes (excitation of 540 nm and emission 580 nm). Assays were done in duplicates. Activity was determined as the difference between the excitation and emission after 179 min of wells that contained or did not contain BAPN.

**A**

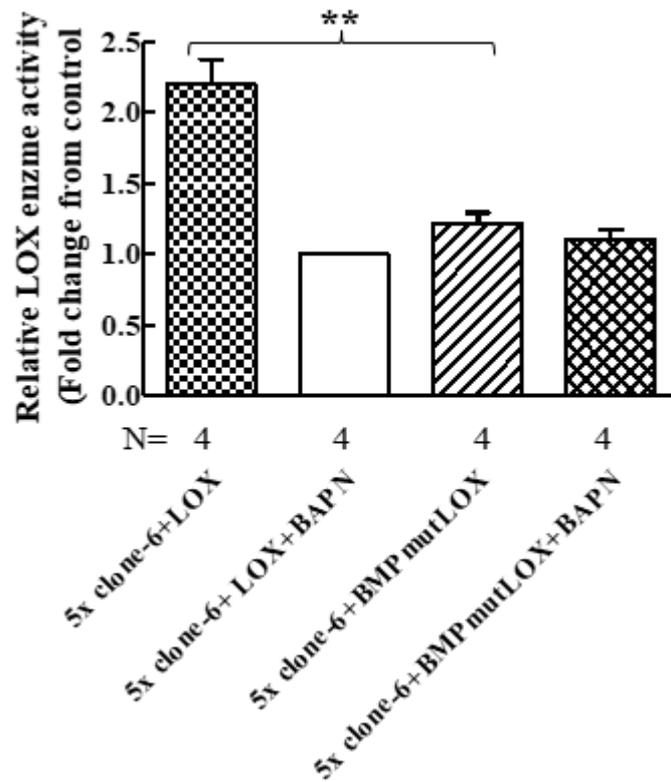
WT hLOXL2	<b>Ala</b>	<b>Pro</b>	<b>Ala</b>	<b>Asn</b>	<b>Val</b>	<b>Ala</b>	<b>Lys</b>	<b>Ile</b>	
	49	50	51	52	53	54	55	56	
#11 allele	<b>Ala</b>	<b>Pro</b>	<b>Ala</b>	<b>Gln</b>	<b>Arg</b>	<b>Gly</b>	<b>Gln</b>	<b>Asp</b>	Insertion +1
Only one chromosome / two identical									
WT hLOXL3	<b>Arg</b>	<b>Val</b>	<b>Glu</b>	<b>Ile</b>	<b>Gln</b>	<b>Arg</b>	<b>Ala</b>	<b>Gly</b>	
	57	58	59	60	61	62	63	64	
#1 allele 1	<b>Arg</b>	<b>Val</b>	<b>Glu</b>	<b>Ilu</b>	<b>Gln</b>	<b>Arg</b>	<b>Ter*</b>		Deletion -5
#1 allele 2	<b>Arg</b>	<b>Val</b>	<b>His</b>	<b>Leu</b>	<b>Arg</b>	<b>Ter*</b>			Deletion -29
WT hLOXL4	<b>Lys</b>	<b>Tyr</b>	<b>Gly</b>	<b>Gln</b>	<b>Gly</b>	<b>Glu</b>	<b>Gly</b>	<b>Pro</b>	
	87	88	89	90	91	92	93	94	
#1 allele 1	<b>Lys</b>	<b>Tyr</b>	<b>Gly</b>	<b>Ser</b>	<b>Arg</b>	<b>Gly</b>	<b>Gly</b>	<b>Ter*</b>	Insertion +1
#1 allele 2	<b>Lys</b>	<b>Tyr</b>	<b>Ala</b>	<b>Arg</b>	<b>Gly</b>	<b>Gly</b>	<b>Ter*</b>		Deletion -2
WT hLOX	<b>Ala</b>	<b>Ser</b>	<b>Arg</b>	<b>Ala</b>	<b>Glu</b>	<b>Asn</b>	<b>Gln</b>	<b>Thr</b>	
	139	140	141	142	143	144	145	146	
#28 allele 1	<b>Ala</b>	<b>Ser</b>	<b>Arg</b>	<b>Arg</b>	<b>Gly</b>	<b>Glu</b>	<b>Pro</b>	<b>Asp</b>	Insertion +1
#28 allele 2	<b>Ala</b>	<b>Ser</b>	<b>Arg</b>	<b>Arg</b>	<b>Arg</b>	<b>Thr</b>	<b>Arg</b>	<b>Gln</b>	Deletion -1
WT hLOXL1	<b>Ser</b>	<b>Glu</b>	<b>Tyr</b>	<b>Val</b>	<b>Pro</b>	<b>Ala</b>	<b>Gly</b>	<b>Pro</b>	
	59	60	61	62	63	64	65	66	
#6 allele 1	<b>Ser</b>	<b>Glu</b>	<b>Tyr</b>	<b>Ala</b>	<b>Gly</b>	<b>Arg</b>	<b>Thr</b>	<b>Ser</b>	Deletion -2
#6 allele 2	<b>Ser</b>	<b>Glu</b>	<b>Tyr</b>	<b>Cys</b>	<b>Arg</b>	<b>Pro</b>	<b>Asp</b>	<b>Leu</b>	Deletion -1

**B**

WT hLOX	<b>Ala</b>	<b>Ser</b>	<b>Arg</b>	<b>Ala</b>	<b>Glu</b>	<b>Asn</b>	<b>Gln</b>	<b>Thr</b>	
	139	140	141	142	143	144	145	146	
#17 allele 1	<b>Ala</b>	<b>Ser</b>	<b>Arg</b>	<b>Arg</b>	<b>Gly</b>	<b>Glu</b>	<b>Pro</b>	<b>Asp</b>	Insertion +1
#17 allele 2	<b>Ala</b>	<b>Ser</b>	<b>Arg</b>	<b>Arg</b>	<b>Gln</b>	<b>Arg</b>	<b>Arg</b>	<b>Glu</b>	Deletion -10

**FIGURE S1**

*The effects of the frame shift mutations introduced into the alleles of the five lysyl-oxidases genes of the clone-6 5x knock-out cells and of 1x clone 17 knock-out cells on the amino-acid sequences immediately adjacent to the frame shift mutation: A. The amino-acid sequence of the various wild type (WT) lysyl-oxidases near the location of the frame shift mutations is shown on top for each lysyl-oxidase. Underneath are indicated the predicted changes in the amino-acid sequences in each of the alleles of the lysyl-oxidase genes that were caused by the frame shift mutations. Changes in amino-acid composition are shown in red. B. The amino acid changes introduced into the proteins encoded by each of the alleles of the LOX gene in clone 17 knock-out cells. Changed amino-acids are in red.*



**FIGURE S2**

*Changes in the amino-acid sequences of the lysyl-oxidases in clone-6 5x knock-out cells: The enzyme activity of the BMPmut LOX is inhibited: LOX enzyme activity was determined in concentrated conditioned medium derived from the indicated cell lines in the presence or absence of BAPN. Shown is the mean excitation/emission ration at 179min of each sample. All sample ratios are normalized in reference to clone 6+LOX+BAPN. Statistical significance was evaluated using unpaired student's T-test with Welch's correction.\*\*  $p < 0.01$ .*

**Table S1. sgRNA sequences used for CRISPR/Cas9 mediated knock-out of LOX family genes.**

Gene	Top	Bottom
LOX (1)	<b>CACCg</b> TGTCTGGTTCTCCGCGCGCG	<b>AAAC</b> CGCGCGCGGAGAACCAGACA <b>c</b>
LOX (2)	<b>CACCg</b> ACGCGGACGGCCGGCTCATC	<b>AAAC</b> GATGAGCCGGCCGTCCGCGT <b>c</b>
Loxl2	<b>CACCg</b> AATCTTGGCCACGTTGGCGG	<b>AAAC</b> CCGCCAACGTGGCCAAGATT <b>c</b>
Loxl3	<b>CACCg</b> CGCGTGGAGATACAGCGAGC	<b>AAAC</b> GCTCGCTGTATCTCCACGCG <b>c</b>
Loxl4	<b>CACCg</b> ACAGTGCCAAGTACGGCCAA	<b>AAAC</b> TTGGCCGTACTTGGCACTGT <b>c</b>
Loxl1	<b>CACCg</b> CTCGGGCTCAGAGTACGTGC	<b>AAAC</b> GCACGTACTCTGAGCCCGAG <b>c</b>

**Table S2. Primers used for construction of expression vectors directing expression of recombinant LOX and loxl genes using the NEBuilder HiFi DNA Assembly kits.**

Primer description	Primer sequence
5' for inserting hlox12 into NSPI	tgtggtggaattctgcagatatggagaggcctctgtgctc
3' for inserting hlox12 into NSPI	cggccgcccactgtgctggatttactcggggacagctggt
5' for inserting hlox13 into NSPI	tccagcacagtggcggccgcatgcgacctgtcagtgtctg
3' for inserting hlox13 into NSPI	gttcgaagggccctctagacttagataatctggttctggtctgg
5' for inserting hlox14 into pENTR1A	cgactggatctcgagctcaatggcgtggtccccaccagc
3' for inserting hlox14 into pENTR1A	ggtctagatatctcgagtgtcagatgaggttgttctga
5' for inserting hLOX into NSPI (was also used for inserting hLOX, and	tgtggtggaattctgcagatatgcgcttcgcctggaccgt

the 1/3 part of LOXmut, BMPmutLOX and ADAmutLOX into NSPI with a MycHis tag)	
3' for inserting hLOX into NSPI	cggccgccactgtgctggatctaatacgggtgaaattgtgc
3' for inserting the 1/3 part of LOXmut, BMPmutLOX and ADAmutLOX into NSPI with a MycHis tag	gctgtctggttctccgcgcgcgaggc
5' for inserting the 2/3 part of LOXmut, BMPmutLOX and ADAmutLOX into NSPI with a MycHis tag	gcctcgcgcgcggagaaccagacagc
3' for inserting the 2/3 part of LOXmut, BMPmutLOX and ADAmutLOX into NSPI with a MycHis tag	ataatctctgacatctgcctgtat
5' for inserting the 3/3 part of LOXmut, BMPmutLOX and ADAmutLOX into NSPI with a MycHis tag	atacagggcagatgtcagagattat
3' for inserting hLOX, and the 3/3 part of LOXmut, BMPmutLOX and ADAmutLOX into NSPI with a MycHis tag	cggccgccactgtgctggattatacgggtgaaattgtgcag

*Table S3. Sequences of the Taq-Man primers used for Quantitative real-time PCR analyses.*

LOX	Hs00942480
-----	------------

lox12	Hs00158757
lox13	Hs01046945
lox14	Hs00260059
STOX2	Hs01391114
AGR2	Hs00356521
DNAB11	Hs00212527
DNAJC3	Hs00939346
HSP90B1	Hs00427665
DERL3	HS00992980
HSPA5	HS00607129
RPLPO	Hs99999902

## REFERENCES

1. Hutchinson, J. H.; Rowbottom, M. W.; Lonergan, D.; Darlington, J.; Prodanovich, P.; King, C. D.; Evans, J. F.; Bain, G. Small Molecule Lysyl Oxidase-like 2 (LOXL2) Inhibitors: The Identification of an Inhibitor Selective for LOXL2 over LOX. *ACS Med. Chem. Lett.* 2017 **8**, 423-427
2. Tang, S. S.; Trackman, P. C.; Kagan, H. M. Reaction of aortic lysyl oxidase with beta-aminopropionitrile. *J. Biol. Chem.* 1983 **258**, 4331-4338