

Supplementary Methods

Zebrafish genomic DNA extraction: Genomic DNA was extracted from fin-clips using QIAGEN DNeasy® Blood and Tissue Kit (Qiagen, Manchester, UK) according to manufacturer's instructions. Samples were eluted into distilled water and stored at -20°C until later use.

Site Specific Polymerase Chain Reaction: Allele-specific pcr single nucleotide polymorphism (SNP) assays were used for genotyping F3 individuals for mutations known to be present in the ENU-mutagenized F1 generation. Four primer pairs were designed to carry out pcr genotyping as previously described (61). The list of loss-of-function mutations in the AJBQM1 and AJBQM2 lines is detailed in Supplementary Table 1. For each line, a primer was designed with 3' complementary to the ENU-SNP with a second primer ~100bp downstream. The second pair had one primer with 3' complementary to the wild-type base with a second primer ~200bp upstream. The resulting pcr results in a 300bp fragment that spans the region and acts as an internal control for the pcr plus one 100bp fragment if homozygous for the mutation, 2 bands of 100bp and 200bp if heterozygous, and one 200bp fragment if homozygous wild-type. The 4-primer groups were designed with melting temperatures as close as possible using the NCBI primer design tool and were ordered from Eurofins, MWG operon (Ebersberg, DE).

Supplementary Table 1: List of loss-of-function mutations in the AJBQM1 (A) and AJBQM2 (B) lines. List was derived from exome sequencing and provided by the Wellcome Sanger Trust, Hinxton, Cambridge.

A)

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SNP Name	Allele Number	Location	Description
cacna1ba (Cacna1b)	sa1562	Zv9:5:31016641	voltage-dependent N-type calcium channel subunit alpha-1B
vcana (VCAN)	sa1563	Zv9:5:48057817	novel protein similar to vertebrate chondroitin sulfate proteoglycan 2
si:ch211-157f15.1 (EVPL)	sa1564	Zv9:6:21645941	envoplakin
mobkl2a (MOBKL2A)	sa1565	Zv9:8:20954361	mps one binder kinase activator-like 2A
ENSDARG00000068026 (PRKG1)	sa1566	Zv9:8:53199402	protein kinase, cGMP-dependent, type I
glis3 (GLIS3)	sa1567	Zv9:10:663606	zinc finger protein GLIS3
si:dkey-220f10.4 (TULP2)	sa1568	Zv9:12:21973687	novel tub family member protein
slit3 (SLIT3)	sa1569	Zv9:14:25591202	slit homolog 3 protein
dchs1 (DCHS1)	sa1570	Zv9:15:31441900	dachsous 1
flad1 (FLAD1)	sa1571	Zv9:16:25049338	Molybdenum cofactor biosynthesis protein-like region FAD synthase region
si:ch211-199m3.2 (AKD1)	sa1572	Zv9:20:33741430	adenylate kinase domain containing 1
si:dkey-4c23.3 (???)	sa1573	Zv9:22:25367694	novel protein similar to vitellogenin 1 (Vg1)
magi2 (MAGI2)	sa1574	Zv9:25:21478784	membrane associated guanylate kinase, WW and PDZ domain containing 2
zgc:101050 (TRIMM55)	sa158	Zv9:23:17631394	hypothetical protein LOC445187 (tripartite motif-containing 55)

27 **B)**

SNP Name	Allele Number	Location	Description
capn3 (CAPN3)	sa150	Zv9:17:45493087	calpain-3
chrna9 (CHRNA9)	sa975	Zv9:1:22190803	cholinergic receptor, nicotinic, alpha 9
snrnp70 (SNRNP70)	sa976	Zv9:3:32068963	U1 small nuclear ribonucleoprotein 70 kDa
zgc:158677 (SV2B)	sa977	Zv9:7:16060160	synaptic vesicle protein 2B homolog
wu:fa96e12 (AC103686.1)	sa978	Zv9:7:44124381	DNA-dependent protein kinase catalytic subunit
kctd4 (KCTD4)	sa980	Zv9:9:19495015	potassium channel tetramerisation domain containing 4
LOC557854 (SLC19A3)	sa981	Zv9:15:34443534	solute carrier family 19, member 3
tspan3a (TSPAN3)	sa984	Zv9:18:26858396	tetraspanin 3
rapsn (RAPSN)	sa985	Zv9:18:20289900	43 kDa receptor-associated protein of the synapse
si:ch211-132b12.1 (SLC6A11)	sa986	Zv9:18:38859333	hypothetical protein LOC100034467
pkhd1l1 (PKHD1L1)	sa987	Zv9:19:23349482	polycystic kidney and hepatic disease 1 (autosomal recessive)-like 1
klf11a (KLF-11)	sa988	Zv9:20:29529553	kruppel-like factor 11a

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30 **Supplementary Table 2.** Gene ID and primer sequences used in gene expression analysis.

Gene name	Transcript ID	Sequence (5' -> 3')	Fragment size (bp)
<i>Serotonergic pathway</i>			
<i>Htr1aa</i>	NM_001123321.1	Forward: TTCTACATCCCGCTCATCCTCA Reverse: CCTCCAAGTTTTACCCACCTCTC	180
<i>Htr1ab</i>	NM_001145766.1	Forward: AAACACCGAGGCGAAGAGGAA Reverse: GGCAGCCAACACAGAATGAAAGT	99
<i>Htr2a</i>	XM_684208.9	Forward: TACGGTGGCTGGGAACATTTTAG Reverse: GGGACACAGTGATGCAGGGAAA	187
<i>Htr5a</i>	NM_001126410.2	Forward: TGGATCAAAGAGGACCAACACC Reverse: CTGAAACGTCACCGTGGCAT	118
<i>Htr2cl1</i>	NM_001129893.1	Forward: AACTTCTTCCTCCGCTCACTCG Reverse: ATGGCACACAGGTGCATGATGG	179
<i>Htr2cl2</i>	XM_001339004.7	Forward: CACAACCCACCAACTTCTTCC Reverse: ACGTCCAGAAAGATCCACAGCG	152
<i>Htr4</i>	XM_021481160.1 XM_009291062.3	Forward: GTTTCTTTCCAAGCGCCTC Reverse: ACTTCTTCCATCTCAGGCATC	168
<i>Htr6</i>	XM_009297078.3	Forward: ACTACAGTCATCAGGAGCCACC Reverse: GCCAGGCACTGAAGAATAGTCC	147
<i>Htr7</i>	XM_003199584.5	Forward: TGGATGTGATGTGCTGTACCGC Reverse: GCCATGCACTTTCCACTCTGTCT	118
<i>slc6a4/ SERT</i>	NM_001039972.1	Forward: ACCAGGGGCGAAGCCAAGCA Reverse: GCCACAGGCCCCCGCTGTTA	117
<i>Htr1b</i>	NM_001128709.1	Forward: CCTTGTCGTCAGTTCTGGGT Reverse: ATCAGAAAGTTCCGCCGGTGT	112
<i>Nicotinic pathway</i>			
<i>Chrna2</i>	NC_007128.7	Forward: TGGCTGCAGATCAGTCAAAGAC Reverse: CCCTCTAACTGTCCCTTCACAA	271
<i>Chrna3</i>	Not found in BLAST	Forward: TGTACATCCGCCGATTACCGCT Reverse: TCCGCAGTCGGAGGGCAGTA	?
<i>Chrna4b</i>	ENSDART00000018614.7	Forward: TTACAAGAGGTTTGGGCGCT Reverse: ACAGACCAGTAGATCATCACTCC	90
<i>Chrna5</i>	NM_001017885.1	Forward: GGCTCCCAGGTGACATTCTC Reverse: AACCCCGGTTACCAGTGGCCT	103
<i>Chrna6</i>	NM_001042684	Forward: AGGCTCTTTCGTCGTTTATTC Reverse: TCTCAGCCAAAGGTTTGTTC	156
<i>Chrna7</i>	ENSDART00000171463.3 and ENSDART00000166391.2	Forward: ACCGTGTCACATTGTTCACTCTC Reverse: ACAGGTCTCTCCAGTGGGTTA	105

<i>Chrb2b</i>	ENSDART00000041625.7 and ENSDART00000185728.1	Forward: CACAAAGTCACGCTCCGATAC Reverse: CCGTCGCTCTGAGCAGATAA	160
<i>Chrb3b</i>	ENSDARG00000038508.5	Forward: CAGGAGTCAACCTCCGCTTT Reverse: TGAATCTGAACGCACTGGCT	106
<i>Chrb4</i>	NC_007129.7	Forward: ATGTGAATGAATGGCGGTGTGTG Reverse: ATGCGCGTGTTCAGATTTACCC	203
<i>Dopaminergic pathway</i>			
<i>Drd1b</i>	NM_001135976.2	Forward: TGGTTCCTTTCTGCAACCCA Reverse: AGTGATGAGTTCGCCCAACC	100
<i>Drd2a</i>	XM_009291617.3 and XM_005157501.4	Forward: TCCACAAAATCAGGAAAAGCGT Reverse: CAGCCAATGTAAACCGGCAA	106
<i>Drd3</i>	XM_021470111.1 and XM_005162673.4	Forward: ATCGAGTTTCGCAGAGCCTT Reverse: TCCACAGTGTCTGAAAGCCG	95
<i>Slc6a3</i>	NM_131755.1	Forward: GCCTGGTTTTACGGAGTGGA Reverse: GGAGGATTGAAGGTGGCGAA	66
<i>Adrenergic pathway</i>			
<i>Adra1aa</i>	NM_001324454.1	Forward: AAGAAGGCCGCAAGACTTT Reverse: GTCCGAGGGTCTGTACGTTG	114
<i>Adra1d</i>	XM_691951.6	Forward: AAGCTGCTAAAACCCTCGCC Reverse: GGCTTCAGAGCTGGGAAGAAT	103
<i>Adra2b</i>	NM_207638.1	Forward: AAAAGCCAGGCCTCCTCAACTT Reverse: GGGCTTGCAGAAGGTTGTTG	92
<i>Adra2c</i>	NM_207639.1	Forward: CGCCGTTTTAACGAGCAGAG Reverse: AGTGTGGCCACCAGAATGTC	87
<i>Adra2da</i>	NM_194364.2	Forward: CATCATCCTCGTGGTGTCCC Reverse: ATCCCATGATCTCGTTGGCG	188
<i>Adra2db</i>	NM_194365.1	Forward: TGCCACTTTGGTCATTCCGT Reverse: AGCCAGGTAGAAAGCACACC	88

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33 **Supplementary Table 3: Sample characteristics of the human cohorts.** Detailed inclusion
 34 and exclusion criteria for the London cohorts can be found in <https://clinicaltrials.gov>.
 35 Further details about recruitment and definition of medical phenotypes can be found
 36 elsewhere (69–71). **Differences in sample size for Finnish cohorts was due to hard-call
 37 genotype probability threshold. DSM-IV nicotine dependence symptoms, Fagerström scores
 38 and cigarettes smoked each day (N = 1715). Sensation felt after smoking first cigarette (N =
 39 1915). Time to first cigarette in the morning (N= 1726).

<i>Cohort name</i>	<i>N</i>	<i>Country</i>	<i>Cohort description</i>	<i>ClinicalTrials.gov ID</i>	<i>Mean age (years)</i>	<i>% female</i>	<i>Smoking phenotypes investigated</i>	<i>N heavy smokers</i>
ViDiCO	272	UK	Subjects with mild, moderate or severe chronic obstructive pulmonary disease (COPD) treated with the same bi-monthly 3mg vitamin D3 intervention.	NCT00977873	64.6	40	Tobacco consumption; smoking cessation (current vs ever smokers)	249
ViDiAs	293	UK	Adult patients with asthma treated with inhaled corticosteroids treated with a bi-monthly 3mg vitamin D3 intervention	NCT00978315	47	56	Tobacco consumption; smoking cessation (current vs ever smokers)	17
ViDiFLU	298	UK	Adults in sheltered accommodation given 10 mcg vitamin D3 daily as well as bi-monthly 3mg vitamin D3 interventions	NCT01069874	66.8	66	Tobacco consumption; smoking cessation (current vs ever smokers)	66
Finnish Twins	1915, 1715, 1726*	Finland	Study sample ascertained from the Finnish Twin Cohort study (N=35834 adult twins) concordant for moderate to heavy smoking	NA	55	48	DSM-IV nicotine dependence symptoms; Fagerström scores; cigarettes smoked each day; sensation felt after smoking first	NA

40 **Phenotype definitions for the London cohorts:** *Amount smoked* was defined as the average
41 number of cigarettes smoked per day (CPD) for each participant. Participants met criteria for
42 *smoking cessation* if they reported being ‘ever smokers’ and reported **not** smoking currently.
43 The percentage of current smokers in the cohort was 42%, 7% and 18% for ViDiCO, ViDiAs
44 and ViDiFLU, respectively.

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46 **Phenotype definition for the Finnish twin cohort study:** Definitions of the phenotypes
47 were adapted from Broms et al (74).

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49 *Amount smoked*

50 Cigarettes per day (CPD) constitutes of eight categories: 1-2, 3-5, 6-10, 11-15, 16-19, 20-25,
51 26-39, ≥ 40 CPD. In the statistical analyses of the CPD variables, original categorical
52 observations were replaced with class means of CPD (1.5, 3.5, 8, 13, 17.5, 22.5, 32.5, and 45
53 cigarettes per day, respectively). Regression coefficients can therefore be interpreted as the
54 average change in number of cigarettes smoked per day when the number of minor allele is
55 increased by one.

- 56
- **CPD:** Number of cigarettes smoked per day during month of heaviest smoking.
57 Values ranged from 1 to >40 with mean=19.8 cigarettes per day.
 - **Maximum CPD:** Maximum number of cigarettes ever smoked during one day (24h
58 period). Values ranged from 2 to 98 with mean=30 cigarettes per day.
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61 *Smoking initiation*

- 62 • **Age of onset of weekly smoking:** Age (years) when started to smoke weekly (“How
63 old were you when you first smoked a cigarette at least once a week for at least two
64 months in a row?”). Values ranged from 6 to 54, mean=17.3 years.
- 65 • **First time sensation.** Sensation felt after smoking the first cigarette or first puffs.
66 Sensation measured as: ”While smoking your very first cigarettes, did you (1) like the
67 taste or smell of the cigarette, (2) cough, (3) feel dizzy or light-headed, (4) feel more
68 relaxed, (5) get a headache, (6) feel a pleasurable rush or buzz, (7) feel your heart
69 racing, (8) feel nauseated, like vomiting, (9) feel your muscles tremble or become
70 jittery, (10) feel burning in your throat”). Sum score of 10 questions (items #1, #4,
71 and #6 were reverse-scored before summation): 0 points if answered “No”, 1 = ”A
72 little bit”, 2=”Some”, 3= ”Quite a bit”, 4=”A great deal”. Cronbach’s alpha = 0.70.
73 Values ranged from 3.6 to 15.8. Mean =10.2.

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75 Nicotine dependence

- 76 • **DSM-IV ND diagnosis:** Nicotine dependence by DSM-IV diagnosis (≥ 3 symptoms
77 out of 7 occurring within a year). Prevalence = 53.5%.
- 78 • **DSM-IV ND symptoms:** Number of DSM-IV ND symptoms from 0 to 7. Mean=3
- 79 • **FTND (≥ 4):** Nicotine dependent if ≥ 4 out of 10 points in Fagerström Test for
80 Nicotine Dependence. Prevalence = 50.4%
- 81 • **FTND score:** Fagerström Test for Nicotine Dependence (FTND) score: 0 to 10
82 points. Mean=3.7.
- 83 • **FTND time to first cigarette (TTF):** Time to first cigarette in the morning (one item
84 of the FTND scale). Five categories: 0-5 min, 6-15 min, 16-30 min, 31-60 min,
85 >60 min. Categorization differs from original four categories (3), i.e., 6-30 minutes is
86 split into 6-15 min and 16-30 min. In our data set 46% of smokers belong to the group

87 of 6-30 min, and from the smoking behaviour point of view there is a significant
88 difference whether one smokes the first cigarette within 6 minutes or 30 minutes from
89 waking up. In this data set 22% of smokers belong to the 6-15 min and 24% to the 16-
90 30 min group. Values ranged from 1 to 5 with a mean=3.1.

1 **Supplementary Table 4.** Primer and reporter sequences used for human genotyping.

GENE	SNP	Sequence name	Sequence
<i>CYP3A4</i>	rs2740574	Forward	CCAGGCATAGGTAAAGATCTGTAGGT
		Reverse	CTCAAGTGGAGCCATTGGCATA
		Reporters	ACAAGGGCAAGAGAG and ACAAGGGCAGGAGAG
<i>CUBN</i>	rs3740165	Forward	GCAATGAGATTAATCTTCAGGAAACACA
		Reverse	CTGGAGGTATAGGAAGCAGTGAAG
		Reporters	CCGCCATATGGCCTG and CGCCATACGGCCTG
<i>RXRA</i>	rs7861779	Forward	TGGCCCATGCACGAGTAG
		Reverse	ACCGAGACAGGCCAAACTC
		Reporters	CAGCAGAGGTGGCCGA and CAGCAGAGATGGCCGA

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Supplementary results

6 **Supplementary Table 5: Site specific pcr genotyping of AJBQM1 (A) and AJBQM2 (B)**
 7 **outlier siblings.** The siblings were genotyped at each of the candidate loci using site specific
 8 pcr and results compared with each individual place preference change scores. P-values result
 9 from independent two-sample t-tests comparing preference change scores between wildtype
 10 and subjects with a copy of mutant allele at each locus.

11 **(A)**

Gene name	CPP Change Score										P-value
	0	0.01	0.07	0.15	0.32	0.43	0.44	0.47	0.51	0.6	
<i>Slit3</i>	WT	WT	WT	WT	HET	HET	HET	HET	HET	HET	7.6592x10 ⁻⁵
<i>Cacne</i>	WT	HET	WT	WT	HOM	HET	WT	WT	WT	WT	0.691
<i>Vcan</i>	HET	HET	WT	HET	HOM	WT	HET	HET	WT	WT	0.259
<i>Evpl</i>	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	-
<i>Mob3a</i>	HET	HET	HET	HOM	HOM	HET	WT	HET	WT	HET	0.236
<i>Prkg1</i>	HET	HET	HET	HET	HET	HET	HET	HET	HOM	HOM	-
<i>Glis3</i>	WT	HET	HET	HET	WT	WT	WT	HET	WT	HET	0.602
<i>Tulp2</i>	HOM	HOM	WT	HET	WT	HET	HET	HET	WT	WT	0.481
<i>Dchs1</i>	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	-
<i>Flad1</i>	HOM	HOM	WT	HET	WT	WT	WT	HET	HET	HET	0.981
<i>Akd1</i>	HOM	HOM	WT	HET	WT	HET	HET	HET	WT	WT	0.418
<i>MagI2</i>	HET	WT	HET	WT	HET	HOM	WT	WT	HOM	HET	0.73
<i>Trimm55</i>	WT	HET	WT	HET	WT	WT	WT	WT	HET	WT	0.51

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13 (B)

Gene name	CPP Change Score														P-value
	-0.38	-0.28	-0.27	-0.23	-0.21	-0.18	-0.17	-0.17	-0.12	-0.09	-0.09	-0.07	0.04	0.07	
<i>Tspan3a</i>	HET	HET	HET	WT	WT	WT	HET	HET	WT	WT	WT	HET	HET	HET	0.583
<i>Raspn</i>	WT	HOM	WT	WT	WT	HOM	HOM	HOM	WT	WT	WT	HOM	WT	HOM	0.792
<i>A9</i>	WT	WT	HET	WT	WT	WT	HET	WT	HET	HET	HET	HET	HET	HET	0.339
<i>Capn3</i>	HET	HET	HET	WT	HET	WT	WT	HET	WT	HET	WT	WT	WT	WT	0.911
<i>Klf11a</i>	WT	WT	WT	HET	WT	WT	WT	HET	WT	WT	HET	WT	HET	WT	0.318
<i>Kctd4</i>	HET	WT	WT	HET	WT	WT	HET	WT	HET	HET	HET	HET	WT	WT	0.252
<i>Slc6a11</i>	HET	HET	HET	WT	HET	WT	HET	HET	WT	WT	WT	WT	HET	WT	0.697
<i>Pkhd11l</i>	WT	WT	WT	HET	HOM	HOM	HET	WT	WT	WT	WT	WT	WT	HOM	0.499
<i>Slc19a3</i>	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	-
<i>Sv2b</i>	WT	WT	HET	WT	WT	WT	WT	HET	HOM	HET	HOM	HOM	HET	HOM	0.269
<i>Snrnp70</i>	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	-
<i>Ac10103686</i>	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	-

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16 **Supplementary Table 6:** Results of association analysis of *SLIT3* SNPs on **smoking**
 17 **initiation.** Logistic regression of initiation vs non-initiation on additive genotype, controlling
 18 for age, sex and cohort. OR: Odds ratio. >1 value indicates that the minor allele increases
 19 odds of persistent smoking relative to the major allele, SE: standard error, L95: lower limit of
 20 95% confidence interval, U95: upper limit of 95% confidence interval. Benjamini Hochberg
 21 cut off at $0.1 = 0.00526$.

SNP	OR	SE	L95	U95	P value
rs2938774	0.7253	0.1418	0.5493	0.9578	0.02357
rs11742567	1.328	0.1538	0.9825	1.796	0.06496
rs4282339	0.7277	0.1815	0.5099	1.039	0.07991
rs297886	1.328	0.176	0.9405	1.875	0.1071
rs9688032	1.269	0.1495	0.9467	1.701	0.111
rs7728604	1.198	0.1446	0.9024	1.591	0.2112
rs1345588	0.7788	0.2046	0.5215	1.163	0.2218
rs17734503	0.7362	0.2632	0.4395	1.233	0.2445
rs12515725	0.8612	0.1458	0.6471	1.146	0.3052
rs11749001	0.8698	0.1951	0.5933	1.275	0.4746
rs3733975	1.116	0.1641	0.8092	1.54	0.5029
rs12521041	1.116	0.1641	0.8092	1.54	0.5029
rs11134527	0.9212	0.155	0.6798	1.248	0.5964
rs12654448	0.8718	0.2654	0.5182	1.467	0.6052
rs1559051	0.9257	0.1575	0.6799	1.26	0.6241
rs17665158	0.9304	0.171	0.6654	1.301	0.673
rs1421763	0.9423	0.1704	0.6748	1.316	0.7271
rs295994	0.9732	0.1404	0.7391	1.281	0.8464
22 rs10036727	1.01	0.1522	0.7491	1.361	0.9503

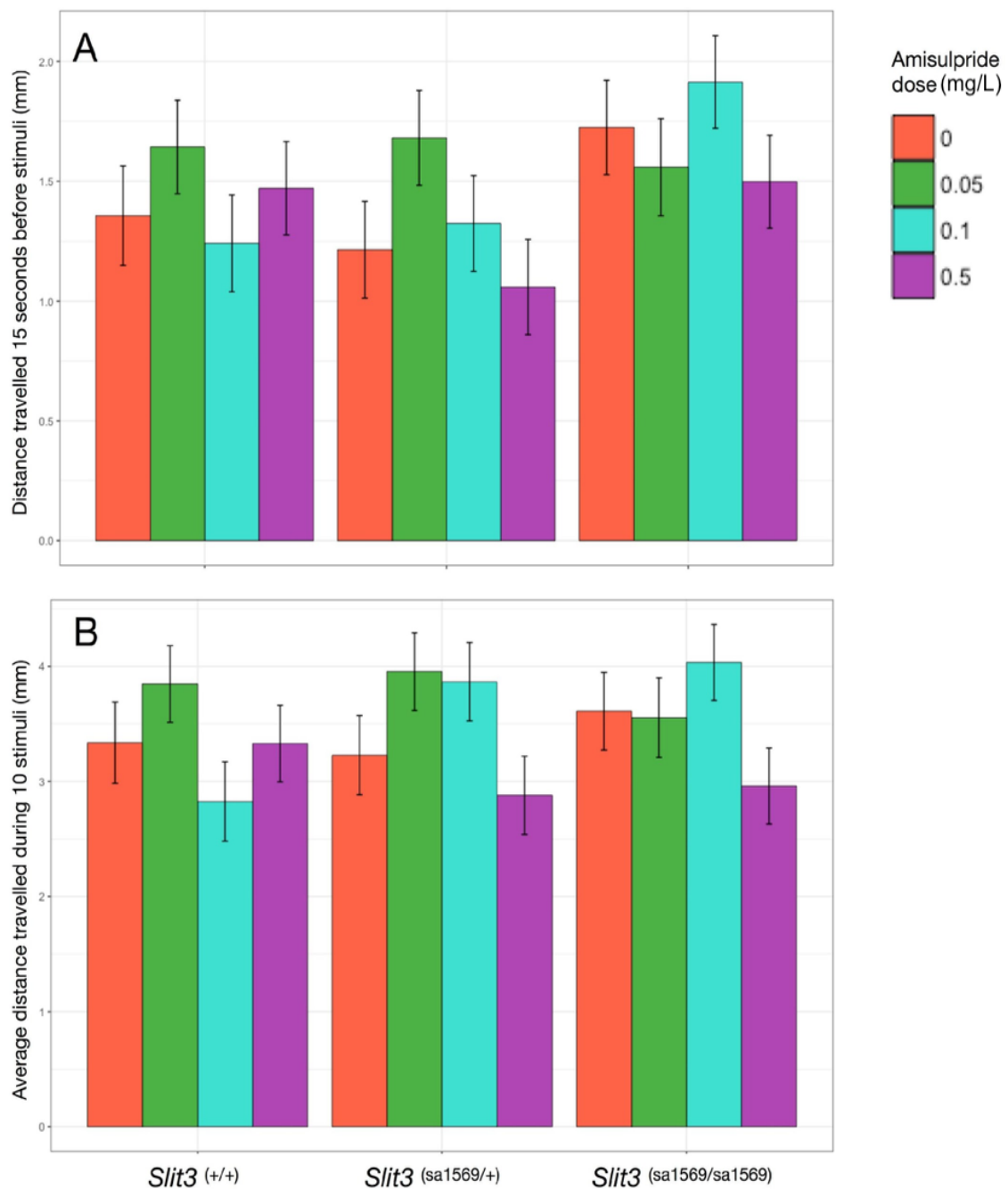
23

24 **Supplementary Table 7:** Results of association analysis of *SLIT3* SNPs on **persistent**
 25 **smoking.** Logistic regression of initiation vs non-initiation on additive genotype, controlling
 26 for age, sex and cohort. OR: Odds ratio. >1 value indicates that the minor allele increases
 27 odds of persistent smoking relative to the major allele, SE: standard error, L95: lower limit of
 28 95% confidence interval, U95: upper limit of 95% confidence interval. Benjamini Hochberg
 29 cut off at $0.1 = 0.00526$.

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SNP	OR	SE	L95	U95	P value
rs11134527	1.428	0.1573	1.049	1.943	0.02359
rs12521041	0.6871	0.1736	0.489	0.9655	0.03061
rs11742567	0.7288	0.1547	0.5382	0.987	0.04089
rs3733975	0.7165	0.1712	0.5123	1.002	0.05146
rs17734503	0.6142	0.2631	0.3667	1.029	0.06398
rs1345588	0.6786	0.2145	0.4457	1.033	0.07068
rs17665158	1.338	0.163	0.9722	1.842	0.07393
rs12654448	0.6225	0.2671	0.3688	1.051	0.07597
rs2938774	1.232	0.1394	0.9373	1.619	0.1348
rs295994	1.214	0.1443	0.9147	1.61	0.1796
rs7728604	1.152	0.1434	0.8699	1.526	0.3232
rs1559051	1.115	0.1549	0.8231	1.511	0.4821
rs12515725	0.9108	0.1456	0.6846	1.212	0.521
rs297886	0.9342	0.1726	0.6661	1.31	0.6932
rs4282339	0.9391	0.19	0.6471	1.363	0.7411
rs10036727	0.9596	0.1516	0.713	1.292	0.7857
rs1421763	1.029	0.1671	0.7417	1.428	0.8633
rs9688032	1.013	0.1511	0.7531	1.362	0.9328
31 rs11749001	0.9943	0.1969	0.676	1.463	0.977

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34 **Supplementary Figure 1.** Average distance moved before (Figure 1A) and during startle

35 stimuli (Figure 1B) in wildtype and *Slit3*^{sa1569} mutant five day old zebrafish larvae. **A)**

36 Distance moved as function of amisulpride dose, fish *Slit3* genotype and their interaction.

37 The effect of dose and genotype was tested in a linear mixed model. Timepoint, well where

38 the fish were placed and plate were also included as fixed factors and the fish ID as random

39 factor. **B)** Distance moved during taps as function of amisulpride dose and fish *Slit3*

40 genotype. Drug and genotype effects were examined in a linear mixed model including
41 stimulus number, well, plate used and distance moved before stimuli as fixed factors and Fish
42 ID as random factor. Zebrafish larvae did not differ in the distance travelled before or during
43 startle stimuli as a function of amisulpride dose nor genotype ($p > 0.05$). Bars represent
44 estimated marginal means \pm SEM (n=42-48 fish per group).

45

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