Online Data Supplement

Cardiac physiologic and genetic predictors of hyperoxia-induced acute lung injury in mice.

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Supplemental methods

Surgical implantation of radio-telemetry transmitter. Inhaled isoflurane was used to anaesthetise mice during surgery, and buprenorphin (0.1 mg/Kg) was administered for post-surgical pain relief. In the skin, a 3 cm midline dorsal incision was made and a blunt instrument was used to make a subcutaneous tissue pocket. An ETA-F20 ECG transmitter (DSI; Arden Hills, MN) was placed inside the tissue pocket and sutured to the left latissimus dorsi muscle. The positive and negative ECG leads were sutured over the left superficial gluteus and right trapezius muscles respectively and routed subcutaneously. All incisions were closed using wound clips and animals were allowed five days to recover.

Hyperoxia exposure and Measurements. Conscious, unrestrained animals were placed individually in whole body plethysmographs (Buxco Electronics, Wilmington, NC) and allowed at least 30 minutes to acclimate before baseline ECG and pulmonary function were recorded. Circadian variation in baseline HR, HRV and pulmonary function was accounted for by recording at the same time of day. Following twenty minutes of baseline recording, mice were exposed to hyperoxia (100% oxygen) in the whole body plethysmographs. 100% oxygen gas was delivered from a liquid oxygen tank, warmed to room temperature and humidified by passing the gas through a sealed flask containing water. Mice were exposed to hyperoxia 23.5 hr/day for a maximum of 96 hours, until moribund or when HR had declined to ~200 bpm. Mouse chow and water were provided *ad libitum*.

The term HRV refers to the variance in the time intervals (typically R-R interval) between successive, normal cardiac cycles. Spectral analysis of the R-R interval series provides a unique insight into the relative variability of both the sympathetic and parasympathetic limbs of the autonomic nervous system on the sinoatrial node (1). This measurement has been used extensively in the clinical research setting to assess a number of human health concerns including aging and cardiovascular disease, and has been shown to be reproducible (2). Low HRV has become a well-established indicator of increased cardiovascular disease risk (3, 4).

The methods used for calculating HR and HRV have been described in detail previously (5). Briefly, using specialist software (Dataquest ART analysis v3.1), R-R intervals were extracted and HR was calculated after arrhythmias were removed. HRV was assessed in the frequency domain using the Lomb periodogram method. The low frequency (LF) HRV range was set at 0.2-1.5 Hz and high frequency (HF) HRV was 1.5-5.0 Hz. Total power (TP) was determined by summation of the LF and HF range values. Minute ventilation ($\dot{V}_E$) was also calculated using specialist software (Buxco Electronics, Wilmington, NC).

Additional mice used for assessment of lung injury were acclimated in unrestrained in stainless steel cages within Hazelton 1000 chambers (Lab Products, Maywood, NJ) for 23.5 hrs/day for 72 hrs. Mice were exposed continuously to >95% O_2 for 48, 60, or 72 hr. Parallel exposures to filtered air were done in a separate chamber. Each animal was provided mouse chow and water *ad libitum* during the exposure. Constant chamber air temperature (72 ± 3° F) and relative humidity (50 ± 15%) were maintained. Immediately after the end of each exposure, mice were euthanized by sodium pentobarbital overdose (104 mg/Kg). On necropsy, right lungs from each mouse were subjected to bronchoalveolar lavage (BAL) analysis. Left lung from each mouse was fixed with Zn formalin for histopathology. All animal use was approved by the NIEHS Animal Care and Use Committee.

Bronchoalveolar lavage (BAL) analysis. Bronchoalveolar lavage (BAL) was performed *in situ* four times with Hanks' balanced salt solution (HBSS, 0.5 ml/20g b.w.; pH 7.2–7.4) and the recovered BAL fluid (BALF) was immediately cooled in ice. BALF returns were centrifuged (1000*g*, 10 min at 4^{o}C), and the supernatant from the first BALF return was separated for determination of total protein (a marker of lung protein permeability). Protein concentration was measured using a Bradford reagent as indicated in the manufacturer's procedure (Bio Rad laboratories, Hercules, CA). Pooled cell pellets from all BALF were combined and resuspended in 1 ml of HBSS, and the total cell number per ml BAL was counted with a hemacytometer.

Lung tissue preparation for histopathology. The left lung lobe isolated from each mouse was inflated gently and fixed with zinc formalin, trimmed at the level of G5 (proximal) and G11 (distal), embedded in paraffin, and sectioned (5 nm). Tissue sections were histochemically stained with hematoxylin and eosin (H&E) for morphological analysis of pulmonary injury.

Genetic linkage analysis. Genome wide scans for quantitative trait loci (QTLs) were performed using the phenotypes from 27 RI lines and the Gene Network (www.genenetwork.org) WebQTL resource. AXB19a and AXB19b were excluded from the linkage analyses as they are considered sister strains with AXB19 (http://jaxmice.jax.org/jaxnotes/504/504e.html) and thus do not provide additional informative genetic insight. Marker regression analysis was used across the genome at the location of available marker loci using the RI strain phenotype (basal HR, LF, HF, TP, pulmonary function and HRTI, LFTI, HFTI, TPTI and $\dot{V}_E$ TI) data. Hypothetical QTLs were evaluated at each marker locus and the statistical significance of the QTLs was estimated by the base-10 likelihood of the odds (LOD) score. Data that were not distributed normally were power transformed before using the data for linkage analyses. 1000 permutation tests were used to establish the suggestive and significance thresholds for the genome wide linkage map curves. LOD curves on the linkage map are provided for each chromosome, and graphically represent the approximate position of identified QTL(s).

References.

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Supplemental Table E1. Strain, age (wks) and body mass (g ± SEM) of inbred and recombinant inbred strains used in the study.

Strain	**n**	**Age (wks)**	**Mass ± SEM (g)**
A/J	4	10.5	21.5 ± 1.0
B57BL/6J	4	10	24.4 ± 0.5
C3H/HeJ	4	12.4	25.6 ± 0.8
DBA/2J	4	14.2	27.3 ± 1.4
AXB1/PgnJ	4	12.6	29.0 ± 2.8
AXB2/PgnJ	4	11.9	25.3 ± 0.8
AXB4/PgnJ	4	10.9	19.5 ± 0.2
AXB5/PgnJ	4	8.9	23.2 ± 0.7
AXB6/PgnJ	4	9.4	25.5 ± 0.6
AXB8/PgnJ	2	7.9	19.6 ± 0.5
AXB10/PgnJ	4	10.3	23.6 ± 0.9
AXB12/PgnJ	4	11.9	24.4 ± 1.0
AXB13/PgnJ	4	7.5	20.3 ± 0.5
AXB15/PgnJ	4	12.7	27.1 ± 0.7
AXB17/PgnJ	4	12.7	24.8 ± 0.9
AXB19a/PgnJ	4	8.7	21.2 ± 0.7
AXB19/PgnJ	4	10.6	21.1 ± 0.5
AXB19b/PgnJ	3	11.6	21.9 ± 1.3
AXB23/PgnJ	4	9.4	24.5 ± 0.1
AXB24/PgnJ	4	11.3	22.4 ± 0.4
BXA1/PgnJ	4	11.1	29.4 ± 0.4
BXA2/PgnJ	4	20.9	38.4 ± 1.4
BXA4/PgnJ	3	21.4	27.5 ± 0.5
BXA7/PgnJ	4	13.6	29.7 ± 0.4
BXA8/PgnJ	4	12.9	25.0 ± 0.8
BXA11/PgnJ	4	16.4	34.6 ± 2.6
BXA12/PgnJ	2	13.1	22.7 ± 1.9
BXA13/PgnJ	4	13.0	24.8 ± 1.1
BXA14/PgnJ	4	17.4	28.5 ± 0.7
BXA16/PgnJ	4	15.4	25.2 ± 0.5
BXA24/PgnJ	4	12.7	29.7 ± 0.6
BXA25/PgnJ	4	12.6	25.8 ± 0.2
BXA26/PgnJ	4	12.7	22.3 ± 0.4

Supplemental Table E2. Genes located with the significant [chromosomes 3 (A) and 5 (B)] and suggestive [chromosome 9 (C)] QTLs. Bolded genes contain non-synonymous coding SNPs that are informative in A/J and B6 mice, and associated with differential responses to hyperoxia in the RI strains. The green shaded genes (chromosome 9) delineate those that were within the statistically significant interval of the entire QTL.

A. Chromosome 3 QTL genes.

Gene Symbol	Mbp Start (mm9)	Gene Length (Kb)	SNP Count	SNP Density (SNP/Kb)	Human Chr	Gene Description
4932443G14	49.129601	41.68	94	2.255278		hypothetical protein 49324...
Pcdh18	**49.547217**	**14.021**	**8**	**0.570573**	**4**	**protocadherin 18**
Slc7a11	**50.168857**	**78.678**	**423**	**5.376344**	**4**	**solute carrier family 7 (c...**
5830415G21Rik	50.379107	1.384	4	2.890173		RIKEN cDNA 5830415G21 gene
Ccrn4l	51.028368	27.208	90	3.307851	4	CCR4 carbon catabolite rep...
Elf2	51.059586	70.323	61	0.867426	4	E74-like factor 2
4930577N17Rik	51.080682	1.529	2	1.308044		RIKEN cDNA 4930577N17 gene
4930583H14Rik	51.192337	8.094	34	4.200642		RIKEN cDNA 4930583H14 gene
Ndufc1	51.2094	3.477	14	4.02646	4	NADH dehydrogenase (ubiqui...
Narg1	51.219937	59.969	69	1.150594	4	NMDA receptor-regulated ge...
Rab33b	51.287887	12.247	0	0	4	RAB33B, member of RAS onco...
Setd7	51.319239	45.506	1	0.021975		SET domain containing (lys...
5430433H01Rik	51.386227	0.542	0	0		RIKEN cDNA 5430433H01 gene
Mgst2	51.465114	21.483	1	0.046548	4	microsomal glutathione S-t...
Maml3	51.491536	417.392	10	0.023958	4	mastermind like 3 (Drosoph...
3110080O07Rik	51.633308	0.995	0	0		RIKEN cDNA 3110080O07 gene
5031434O11Rik	51.654787	0.05	0	0		RIKEN cDNA 5031434O11 gene

B. Chromosome 5 QTL genes.

Gene Symbol	Mbp Start (mm9)	Gene Length (Kb)	SNP Count	SNP Density (SNP/Kb)	Human Chr	Gene Description
Ppp1cc	122.608287	16.991	1	0.058855	12	protein phosphatase 1, cat...
Hvcn1	**122.659745**	**32.561**	**5**	**0.153558**	**12**	**hydrogen voltage-gated cha...**
Tect1	122.691523	22.946	1	0.043581	12	tectonic 1
Pptc7	122.734406	39.884	12	0.300873	12	PTC7 protein phosphatase h...
Rad9b	122.775516	28.688	40	1.394311	12	RAD9 homolog B (S. cerevis...
Vps29	122.804421	8.875	27	3.042254	12	vacuolar protein sorting 2...
Atpbd1c	122.822516	10.26	53	5.165692	12	ATP binding domain 1 famil...
Arpc3	122.841936	14.251	82	5.753982	12	actin related protein 2/3 ...
Anapc7	122.872489	22.099	88	3.982081	12	anaphase promoting complex...
Atp2a2	122.903521	48.713	123	2.524993	12	ATPase, Ca++ transporting,...
Ift81	123.000212	64.315	45	0.699681	12	intraflagellar transport 8...
P2rx7	123.093919	11.35	23	2.026432	12	purinergic receptor P2X, l...
P2rx4	123.157565	21.486	56	2.606348	12	purinergic receptor P2X, l...
Camkk2	123.183206	31.097	132	4.244782	12	calcium/calmodulin-depende...
Anapc5	123.237469	33.879	172	5.076891	12	anaphase-promoting complex...
Rnf34	**123.300196**	**18.758**	**52**	**2.772151**	**12**	**ring finger protein 34**
Fbxl10	123.320686	118.415	56	0.472913	12	F-box and leucine-rich rep...
A930024E05Rik	123.439362	8.986	2	0.222568		RIKEN cDNA A930024E05 gene
Orai1	123.465082	15.379	1	0.065024	12	ORAI calcium release-activ...
Morn3	123.487135	9.694	25	2.578915	12	MORN repeat containing 3
Tmem120b	123.526283	41.171	62	1.505914	12	transmembrane protein 120B
Rhof	123.568188	14.45	0	0	12	ras homolog gene family, m...

Hpd	123.621815	10.88	2	0.183824	12	4-hydroxyphenylpyruvic aci...
Psmd9	123.678198	21.936	0	0	12	proteasome (prosome, macro...
Wdr66	123.728136	1.458	4	2.743484	12	WD repeat domain 66
Bcl7a	123.794542	29.546	28	0.947675	12	B-cell CLL/lymphoma 7A
Mlxip	123.844827	63.113	143	2.265777	12	MLX interacting protein
Il31	123.930166	2.428	9	3.706755	12	interleukin 31
Lrrc43	123.939333	18.88	23	1.21822	12	leucine rich repeat contai...
B3gnt4	123.960468	1.423	6	4.216444	12	UDP-GlcNAc:betaGal beta-1,...
Diablo	123.961338	12.835	17	1.324503	12	diablo homolog (Drosophila...
Vps33a	123.97911	43.905	65	1.480469	12	vacuolar protein sorting 3...
Clip1	124.029078	105.222	195	1.853225	12	CAP-GLY domain containing ...
Zcchc8	124.149806	21.143	58	2.743225	12	zinc finger, CCHC domain c...
Rsrc2	124.178438	20.983	42	2.00162	12	arginine/serine-rich coile...
Kntc1	**124.199734**	**71.868**	**103**	**1.433183**	**12**	**kinetochore associated 1**
Gpr109a	124.313585	1.92	6	3.125	12	G protein-coupled receptor...
Gpr81	124.326986	3.026	0	0	12	G protein-coupled receptor...
Denr	124.357283	21.558	2	0.092773	12	density-regulated protein
Hip1r	124.423647	29.562	4	0.135309	12	huntingtin interacting pro...
Vps37b	124.454649	27.62	3	0.108617		vacuolar protein sorting 3...
Abcb9	124.511865	33.942	46	1.355253	12	ATP-binding cassette, sub-...
Ogfod2	124.562346	3.139	11	3.504301	12	2-oxoglutarate and iron-de...
Arl6ip4	124.566116	2.088	7	3.35249	12	ADP-ribosylation factor-li...
Pitpnm2	124.568698	97.729	85	0.869752	12	phosphatidylinositol trans...
Mphosph9	124.700967	77.493	70	0.903307	12	M-phase phosphoprotein 9
2810006K23Rik	124.778097	12.033	19	1.578991		RIKEN cDNA 2810006K23 gene
Cdk2ap1	124.795447	9.19	5	0.54407	12	CDK2 (cyclin-dependent kin...

Sbno1	124.81871	57.213	218	3.810323	12	sno, strawberry notch homo...
Setd8	124.889938	22.378	0	0	12	SET domain containing (lys...
Rilpl2	124.913273	14.971	0	0	12	Rab interacting lysosomal ...
Rilpl1	124.943088	38.312	0	0	12	Rab interacting lysosomal ...
Tmed2	124.990799	9.713	4	0.411819	12	transmembrane emp24 domain...
Ddx55	125.002872	16.075	11	0.684292	12	DEAD (Asp-Glu-Ala-Asp) box...
Eif2b1	125.020222	8.844	1	0.113071	12	eukaryotic translation ini...
Gtf2h3	125.029179	17.671	11	0.622489	12	general transcription fact...
Tctn2	125.048757	28.901	55	1.903048	12	tectonic family member 2
Atp6v0a2	125.079647	122.87	241	1.961423	12	ATPase, H+ transporting, l...
Dnahc10	125.281834	32.838	116	3.532493	12	dynein, axonemal, heavy ch...
Ccdc92	125.314801	27.79	64	2.302987	12	coiled-coil domain contain...
Zfp664	125.343074	25.926	40	1.542853	12	zinc finger protein 664
3110032G18Rik	125.483844	9.073	32	3.526948		RIKEN cDNA 3110032G18 gene
Ncor2	125.497524	162.06	208	1.283475	12	nuclear receptor co-repres...
Ubg	125.675638	0.048	1	20.833333		ubiquitin, beta-galactosid...
EG216818	125.675715	0.227	0	0		predicted gene, EG216818
Scarb1	125.757459	63.985	115	1.797296	12	scavenger receptor class B...
Ubc	125.866334	4.053	1	0.246731	12	ubiquitin C
Dhx37	125.894773	19.645	37	1.883431	12	DEAH (Asp-Glu-Ala-His) box...
Bri3bp	125.921937	19.318	28	1.449425	12	Bri3 binding protein
Aacs	125.956242	41.531	180	4.334112	12	acetoacetyl-CoA synthetase
Tmem132b	126.102857	76.286	128	1.677896	12	transmembrane protein 132B
1700048F04Rik	126.409148	23.852	63	2.641288		RIKEN cDNA 1700048F04 gene
4933438B17Rik	127.511158	57.741	111	1.922378		RIKEN cDNA 4933438B17 gene
4930572K03Rik	127.650934	13.792	4	0.290023		RIKEN cDNA 4930572K03 gene

4930548G05Rik	127.681064	13.637	2	0.14666		RIKEN cDNA 4930548G05 gene
2810482M11Rik	127.722195	45.511	127	2.790534		RIKEN cDNA 2810482M11 gene
Tmem132c	127.722195	323.965	535	1.651413	12	transmembrane protein 132C
1700085B03Rik	127.737231	24.724	62	2.507685		RIKEN cDNA 1700085B03 gene
Slc15a4	128.076035	21.727	24	1.104616	12	solute carrier family 15, ...
Glt1d1	128.112631	75.259	124	1.647643	12	glycosyltransferase 1 doma...
Tmem132d	128.26386	675.077	1221	1.808683	12	transmembrane protein 132D
Fzd10	129.10698	2.988	12	4.016064	12	frizzled homolog 10 (Droso...
Piwil1	129.24212	19.229	9	0.468043	12	piwi-like homolog 1 (Droso...
Rimbp2	129.266275	192.962	379	1.964117	12	RIMS binding protein 2
Stx2	129.490435	24.004	74	3.08282	12	syntaxin 2
Ran	129.52603	4.166	18	4.320691	12	RAN, member RAS oncogene f...
Gpr133	129.602624	107.85	435	4.03338	12	G protein-coupled receptor...

C. Chromosome 9 QTL genes.

Gene Symbol	**Mb Start (mm9)**	**Gene Length (Kb)**	**SNP Count**	**SNP Density (SNP/Kb)**	**Human Chr**	**Gene Description**
Ddx10	52.987979	0.05	0	0	11	DEAD (Asp-Glu-Ala-Asp) box...
4930510E17Rik	53.072719	16.371	1	0.061084		RIKEN cDNA 4930510E17 gene
Exph5	53.109774	75.845	5	0.065924	11	exophilin 5
Kdelc2	53.192127	17.845	1	0.056038	11	KDEL (Lys-Asp-Glu-Leu) con...
4930550C14Rik	53.21339	27.518	2	0.07268		RIKEN cDNA 4930550C14 gene
Atm	53.245226	99.55	6	0.060271	11	ataxia telangiectasia muta...
Npat	53.345151	38.581	5	0.129597	11	nuclear protein in the AT ...
1110050P16Rik	53.375857	1.369	0	0		RIKEN cDNA 1110050P16 gene
Acat1	53.388626	29.829	4	0.134098	11	acetyl-Coenzyme A acetyltr...
Cul5	53.422686	52.926	14	0.26452	11	cullin 5
Rab39	53.492214	22.123	0	0	11	RAB39, member RAS oncogene...
Slc35f2	53.619341	46.627	7	0.150128	11	solute carrier family 35, ...
Sln	53.698057	3.599	4	1.11142	11	sarcolipin
Elmod1	53.759266	63.842	65	1.018139	11	ELMO domain containing 1
Tnfaip8l3	53.873412	42.806	61	1.425034	15	tumor necrosis factor, alp...
Cyp19a1	54.013743	27.506	48	1.745074	15	cytochrome P450, family 19...
1700104A03Rik	54.113914	3.192	0	0		RIKEN cDNA 1700104A03 gene
Gldn	54.134292	55.292	12	0.21703	15	gliomedin
4933412E14Rik	54.38679	6.037	4	0.662581		RIKEN cDNA 4933412E14 gene
Cib2	54.393158	14.728	2	0.135796	15	calcium and integrin bindi...
Idh3a	54.434317	18.152	9	0.495813	15	isocitrate dehydrogenase 3...
Acsbg1	54.452803	56.889	7	0.123047	15	acyl-CoA synthetase bubble...
Dnaja4	54.547365	16.754	0	0	15	DnaJ (Hsp40) homolog, subf...

Wdr61	54.564961	17.34	0	0	15	WD repeat domain 61
Crabp1	54.612614	8.302	0	0	15	cellular retinoic acid bin...
Ireb2	54.711595	46.244	10	0.216244	15	iron responsive element bi...
C630028N24Rik	54.765096	32.635	0	0		RIKEN cDNA C630028N24 gene
Psma4	54.798665	7.172	0	0	15	proteasome (prosome, macro...
Chrna5	54.828903	25.478	0	0	15	cholinergic receptor, nico...
Chrna3	54.859149	15.217	0	0	15	cholinergic receptor, nico...
Chrnb4	54.875962	20.389	4	0.196184	15	cholinergic receptor, nico...
1700071A11Rik	54.996288	0.904	0	0		RIKEN cDNA 1700071A11 gene
Ube2q2	54.997184	58.151	0	0	15	ubiquitin-conjugating enzy...
D9Wsu74e	55.00276	0.05	0	0		DNA segment, Chr 9, Wayne ...
Fbxo22	55.056741	15.499	1	0.06452	15	F-box protein 22
Nrg4	55.068028	63.404	15	0.236578	15	neuregulin 4
AI118078	55.174755	111.396	59	0.529642		expressed sequence AI11807...
Etfa	55.302242	57.808	4	0.069195	15	electron transferring flav...
9430072B17Rik	55.30294	1.283	0	0		RIKEN cDNA 9430072B17 gene
Isl2	55.388955	5.03	0	0	15	insulin related protein 2 ...
Scaper	55.397689	388.233	419	1.079249	15	S phase cyclin A-associate...
8430427G23Rik	55.596521	0.05	0	0		RIKEN cDNA 8430427G23 gene
1110019B24Rik	55.746763	1.04	3	2.884615		RIKEN cDNA 1110019B24 gene
4930563M21Rik	55.810395	18.344	70	3.815962		RIKEN cDNA 4930563M21 gene
Rcn2	55.889651	17.239	43	2.494344	15	reticulocalbin 2
Pstpip1	55.937782	38.915	46	1.182063	15	proline-serine-threonine p...
Tspan3	55.98369	25.165	6	0.238426	15	tetraspanin 3
C230081A13Rik	56.048935	216.922	52	0.239718		RIKEN cDNA C230081A13 gene
Hmg20a	56.266652	78.091	57	0.729918	15	high mobility group 20A

5830432F11Rik	56.296511	1.052	0	0		RIKEN cDNA 5830432F11 gene
Lingo1	56.466281	66.779	23	0.34442	15	leucine rich repeat and Ig...
Odf3l1	56.696465	3.305	0	0	15	outer dense fiber of sperm...
Cspg4	56.71291	34.767	5	0.143815	15	chondroitin sulfate proteo...
Sh3px3	56.765006	11.172	3	0.268528	15	SH3 and PX domain containi...
4930442G15Rik	56.779437	1.915	0	0		RIKEN cDNA 4930442G15 gene
Imp3	56.785306	0.899	0	0	15	IMP3, U3 small nucleolar r...
Snupn	56.79873	32.276	4	0.123931	15	snurportin 1
B930032C10Rik	56.82168	0.865	0	0		RIKEN cDNA B930032C10 gene
Ptpn9	56.842774	67.84	24	0.353774	15	protein tyrosine phosphata...
Sin3a	56.919846	56.329	67	1.189441	15	transcriptional regulator,...
2700012I20Rik	56.923022	0.798	0	0		RIKEN cDNA 2700012I20 gene
2410133F24Rik	56.928715	0.05	0	0		RIKEN cDNA 2410133F24 gene
Man2c1	56.978583	11.434	36	3.148504	15	mannosidase, alpha, class ...
Neil1	56.991062	3.779	12	3.175443	15	nei endonuclease VIII-like...
Commd4	57.002847	3.259	0	0	15	COMM domain containing 4
1700041C23Rik	57.023081	10.829	25	2.308616		RIKEN cDNA 1700041C23 gene
14-Sep	57.050459	10.131	43	4.244398		septin 14
Trcg1	57.084362	13.309	22	1.653017		taste receptor protein 1
4930430J02Rik	57.238876	9.295	2	0.215169		RIKEN cDNA 4930430J02 gene
Ppcdc	57.26047	27.451	35	1.274999	15	phosphopantothenoylcystein...
Scamp5	57.289133	26.698	22	0.824032	15	secretory carrier membrane...
Rpp25	57.351908	1.346	0	0	15	ribonuclease P 25 subunit ...
Cox5a	57.369038	11.195	3	0.267977	15	cytochrome c oxidase, subu...
Mpi	57.392074	8.485	0	0	15	mannose phosphate isomeras...
Scamp2	57.40875	27.855	4	0.143601	15	secretory carrier membrane...

Ulk3	57.437274	6.759	2	0.295902	15	unc-51-like kinase 3 (C. e...
Cplx3	57.447798	6.241	1	0.160231	15	complexin 3
Lman1l	57.454839	13.742	3	0.218309	15	lectin, mannose-binding 1 ...
Csk	57.474452	18.535	2	0.107904	15	c-src tyrosine kinase
Cyp1a2	57.524743	6.719	0	0	15	cytochrome P450, family 1,...
Cyp1a1	57.535734	15.894	2	0.125834	15	cytochrome P450, family 1,...
Edc3	57.556375	41.594	11	0.264461	15	enhancer of mRNA decapping...
Clk3	57.598517	15.15	7	0.462046	15	CDC-like kinase 3
Arid3b	57.638315	43.726	25	0.571742	15	AT rich interactive domain...
Ubl7	57.758792	18.983	10	0.526787	15	ubiquitin-like 7 (bone mar...
Sema7a	57.787941	22.731	46	2.023668	15	sema domain, immunoglobuli...
Cyp11a1	57.862823	12.007	37	3.081536	15	cytochrome P450, family 11...
Ccdc33	57.876488	73.544	204	2.77385	15	coiled-coil domain contain...
Stra6	57.977155	24.605	11	0.447064	15	stimulated by retinoic aci...
Islr	58.004075	2.874	16	5.567154	15	immunoglobulin superfamily...
Islr2	58.044104	8.022	0	0	15	immunoglobulin superfamily...
Pml	58.064986	32.607	6	0.18401	15	promyelocytic leukemia
Stoml1	58.100998	9.327	1	0.107216	15	stomatin-like 1
Loxl1	58.136407	24.586	3	0.122021	15	lysyl oxidase-like 1
Tbc1d21	58.20761	10.566	3	0.28393	15	TBC1 domain family, member...
1700072B07Rik	58.21831	3.68	1	0.271739		RIKEN cDNA 1700072B07 gene
4930461G14Rik	58.302979	14.451	49	3.390769		RIKEN cDNA 4930461G14 gene
6030419C18Rik	58.336409	11.14	15	1.346499		RIKEN cDNA 6030419C18 gene
Cd276	58.372106	16.641	20	1.201851	15	CD276 antigen
Nptn	58.430046	70.64	122	1.727067		neuroplastin
2410076I21Rik	58.500689	88.674	270	3.044861		RIKEN cDNA 2410076I21 gene

A130026P03Rik	58.514471	2.178	5	2.295684		RIKEN cDNA A130026P03 gene
Hcn4	58.671318	37.444	59	1.575686	15	hyperpolarization-activate...
Neo1	58.722486	161.762	381	2.355312	15	neogenin
Adpgk	59.139378	24.629	47	1.908319	15	ADP-dependent glucokinase
Bbs4	59.169772	31.495	36	1.143039	15	Bardet-Biedl syndrome 4 ho...
Arih1	59.23636	97.821	108	1.104057	15	ariadne ubiquitin-conjugat...
Tmem202	59.366491	6.817	1	0.146692	15	transmembrane protein 202
Hexa	59.387473	25.439	0	0	15	hexosaminidase A
Brunol6	59.426143	28.956	0	0	15	bruno-like 6, RNA binding ...
Parp6	59.46509	32.986	0	0	15	poly (ADP-ribose) polymera...
Pkm2	59.504414	22.768	0	0	15	pyruvate kinase, muscle
1700043A12Rik	59.50819	0.777	0	0		RIKEN cDNA 1700043A12 gene
4933407I18Rik	59.527184	1.436	0	0		RIKEN cDNA 4933407I18 gene
Gramd2	59.55557	8.661	0	0	15	GRAM domain containing 2
Senp8	59.583692	14.725	1	0.067912	15	SUMO/sentrin specific pept...
Myo9a	59.726791	0.05	0	0	15	myosin IXa
Nr2e3	59.790577	7.309	8	1.094541	15	nuclear receptor subfamily...
Thsd4	**59.817525**	**171.417**	**317**	**1.849291**	**15**	**thrombospondin, type I, do...**
B930001P03Rik	60.001122	1.93	3	1.554404		RIKEN cDNA B930001P03 gene
5930427L02Rik	60.090883	2.197	0	0		RIKEN cDNA 5930427L02 gene
2010001M07Rik	60.181948	0.827	2	2.41838		RIKEN cDNA 2010001M07 gene
9230112J17Rik	60.371463	0.737	2	2.713704		RIKEN cDNA 9230112J17 gene
Lrrc49	60.435045	100.896	13	0.128846	15	leucine rich repeat contai...
B930082K07Rik	60.535525	2.679	0	0		RIKEN cDNA B930082K07 gene
Larp6	60.560927	25.681	2	0.077879	15	La ribonucleoprotein domai...
1700036A12Rik	60.590926	27.156	4	0.147297		RIKEN cDNA 1700036A12 gene

Uaca	60.642354	85.823	47	0.547639	15	uveal autoantigen with coi...
A430102L10	60.864875	2.729	0	0		hypothetical protein A4301...
4933433G08Rik	60.950818	2.374	9	3.79107		RIKEN cDNA 4933433G08 gene
I730028E13Rik	60.986321	6.745	10	1.48258		RIKEN cDNA I730028E13 gene
Tle3	61.220172	46.132	9	0.195092	15	transducin-like enhancer o...
Rplp1	61.761089	1.228	7	5.700326	15	ribosomal protein, large, ...
Kif23	61.7653	29.264	95	3.246309	15	kinesin family member 23
Paqr5	61.801544	73.053	8	0.10951	15	progestin and adipoQ recep...
9530006O14Rik	61.854485	1.216	0	0		RIKEN cDNA 9530006O14 gene
Glce	61.905055	13.358	1	0.074862	15	glucuronyl C5-epimerase
1700013K18Rik	62.115182	0.468	1	2.136752		RIKEN cDNA 1700013K18 gene
Spesp1	62.118535	11.451	31	2.707187	15	sperm equatorial segment p...
Anp32a	62.189149	37.46	135	3.603844	15	acidic (leucine-rich) nucl...
Coro2b	62.267298	117.553	256	2.177741	15	coronin, actin binding pro...
Itga11	62.525661	106.125	188	1.771496	15	integrin, alpha 11
Fem1b	62.639635	19.82	1	0.050454	15	feminization 1 homolog b (...
Cln6	62.686593	13.216	3	0.226998	15	ceroid-lipofuscinosis, neu...
Calml4	62.70591	17.814	1	0.056136	15	calmodulin-like 4
Pias1	62.727883	100.803	8	0.079363	15	protein inhibitor of activ...
Lbxcor1	62.985982	8.805	8	0.908575	15	ladybird homeobox 1 homolo...
Map2k5	63.011576	214.083	404	1.887119	15	mitogen-activated protein ...
2300009A05Rik	63.192492	0.05	0	0		RIKEN cDNA 2300009A05 gene
Iqch	63.269263	181.024	414	2.28699	15	IQ motif containing H
2310007F21Rik	63.450461	39.235	110	2.803619		RIKEN cDNA 2310007F21 gene
Smad3	63.494573	111.228	130	1.16877	15	MAD homolog 3 (Drosophila)
Smad6	63.800949	68.179	140	2.053418	15	MAD homolog 6 (Drosophila)

1700055C04Rik	63.886271	3.001	6	1.999334		RIKEN cDNA 1700055C04 gene
1110036E04Rik	63.897635	4.272	9	2.106742		RIKEN cDNA 1110036E04 gene
2600006L11Rik	63.928567	7.285	0	0		RIKEN cDNA 2600006L11 gene
Lctl	63.964953	20.972	0	0	15	lactase-like
Zwilch	63.984952	35.786	3	0.083832	15	Zwilch, kinetochore associ...
Rpl4	64.021193	5.176	0	0	15	ribosomal protein L4
Snapc5	64.027103	3.392	0	0	15	small nuclear RNA activati...
Map2k1	64.033599	67.813	98	1.445151	15	mitogen-activated protein ...
Uchl4	64.083007	1.162	0	0	15	ubiquitin carboxyl-termina...
Tipin	**64.129413**	**23.186**	**89**	**3.838523**	**15**	**timeless interacting prote...**
Dis3l	64.154815	34.182	82	2.398923	15	DIS3 mitotic control homol...
Megf11	64.233432	315.349	201	0.637389	15	multiple EGF-like-domains ...
Rab11a	64.563106	22.457	48	2.137418	15	RAB11a, member RAS oncogen...
F730015K02Rik	64.659266	29.65	49	1.652614		RIKEN cDNA F730015K02 gene
Slc24a1	64.770667	28.747	0	0	15	solute carrier family 24 (...
2010321M09Rik	64.808638	26.143	3	0.114753		RIKEN cDNA 2010321M09 gene
Ptplad1	64.834789	34.735	4	0.115158	15	protein tyrosine phosphata...
Dpp8	64.880264	50.194	6	0.119536	15	dipeptidylpeptidase 8
Nope	64.949301	36.449	2	0.054871	15	neighbor of Punc E11
Punc	64.988995	44.682	105	2.34994	15	putative neuronal cell adh...
Parp16	65.062496	24.53	57	2.323685	15	poly (ADP-ribose) polymera...
Cilp	65.112986	15.426	40	2.593025	15	cartilage intermediate lay...
Clpx	65.142101	36.364	71	1.95248	15	caseinolytic peptidase X (...
Pdcd7	65.193874	13.576	17	1.25221	15	programmed cell death prot...
5430433E21Rik	65.23649	2.969	6	2.020882		RIKEN cDNA 5430433E21 gene
Rasl12	65.246343	14.162	26	1.835899	15	RAS-like, family 12

Ostb	65.260559	10.021	39	3.891827	15	organic solute transporter...
Ankdd1a	65.282674	0.05	0	0	15	ankyrin repeat and death d...
Mtfmt	65.283588	17.273	63	3.647311	15	mitochondrial methionyl-tR...
Spg21	65.308743	27.534	28	1.016925	15	spastic paraplegia 21 homo...
Plekho2	65.40219	25.651	69	2.689954	15	pleckstrin homology domain...
Pif1	65.435011	8.758	22	2.511989	15	PIF1 5'-to-3' DNA helicase...
Rbpms2	65.478388	29.937	56	1.870595	15	RNA binding protein with m...
Oaz2	65.524354	13.753	33	2.399476	15	ornithine decarboxylase an...
Zfp609	65.539389	135.982	205	1.507552	15	zinc finger protein 609
Trip4	65.680031	76.562	113	1.475928	15	thyroid hormone receptor i...
2810417H13Rik	65.738129	13.229	18	1.360647		RIKEN cDNA 2810417H13 gene
D030028M11Rik	65.743728	2.342	5	2.134927		RIKEN cDNA D030028M11 gene
Csnk1g1	65.756816	136.005	343	2.521966	15	casein kinase 1, gamma 1
Ppib	65.907975	6.461	10	1.547748	15	peptidylprolyl isomerase B
Snx22	65.912982	4.556	2	0.438982	15	sorting nexin 22
Snx1	65.935933	36.76	60	1.632209	15	sorting nexin 1
5730536A07Rik	65.974417	12.358	36	2.913093		RIKEN cDNA 5730536A07 gene
Dapk2	66.006032	114.017	211	1.850601	15	death-associated kinase 2
Herc1	66.305392	0.05	0	0	15	hect (homologous to the E6...
Fbxl22	66.356265	6.118	1	0.163452	15	F-box and leucine-rich rep...
Usp3	66.365687	75.1	11	0.146471	15	ubiquitin specific peptida...
Car12	66.561492	53.159	35	0.658402	15	carbonic anyhydrase 12
Aph1b	66.623293	19.937	1	0.050158	15	anterior pharynx defective...
Aph1c	66.6628	19.713	2	0.101456		anterior pharynx defective...
Rab8b	66.69147	76.024	20	0.263075	15	RAB8B, member RAS oncogene...
Rps27l	66.793924	3.392	0	0	15	ribosomal protein S27-like

Lactb	66.803199	20.092	17	0.846108	15	lactamase, beta
Tpm1	66.870399	26.621	48	1.803088	15	tropomyosin 1, alpha
Tln2	67.064891	342.619	640	1.867964	15	talin 2
3300001A09Rik	67.606382	2.358	1	0.424088		RIKEN cDNA 3300001A09 gene
Vps13c	67.688202	155.239	58	0.373617	15	vacuolar protein sorting 1...
4930502A04Rik	68.318708	49.299	98	1.98787		RIKEN cDNA 4930502A04 gene