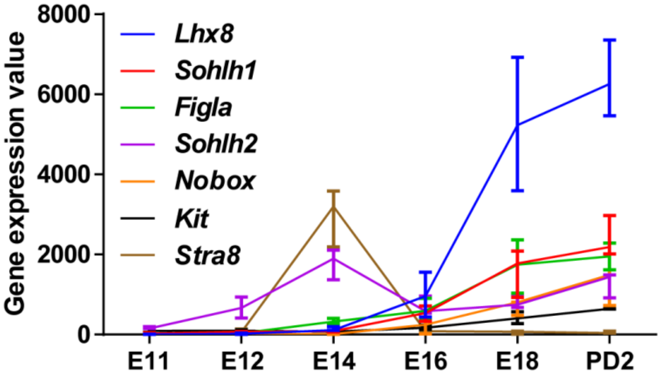
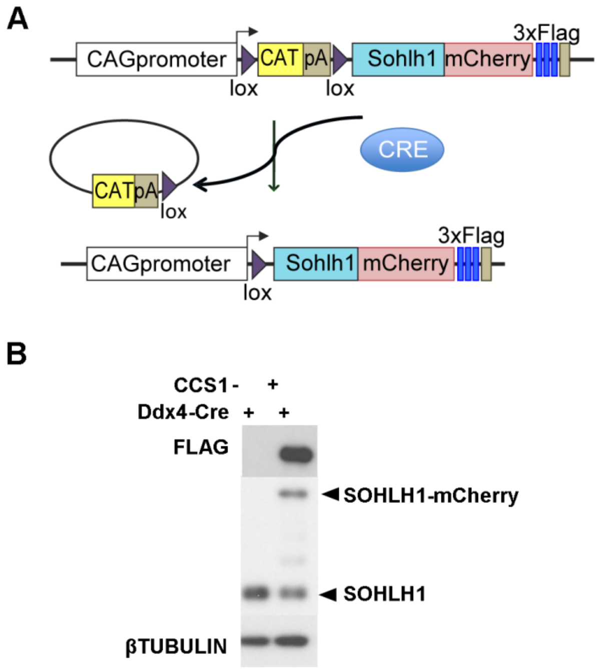
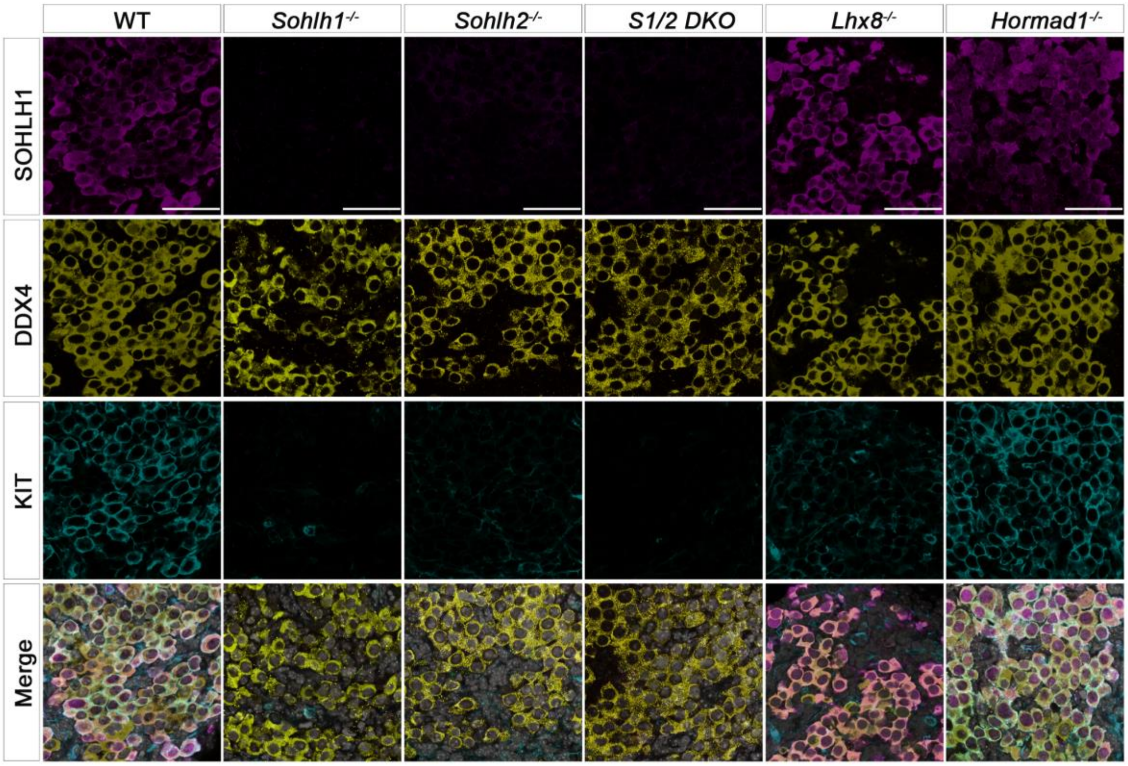
Supplemental Figures and Figure Legends



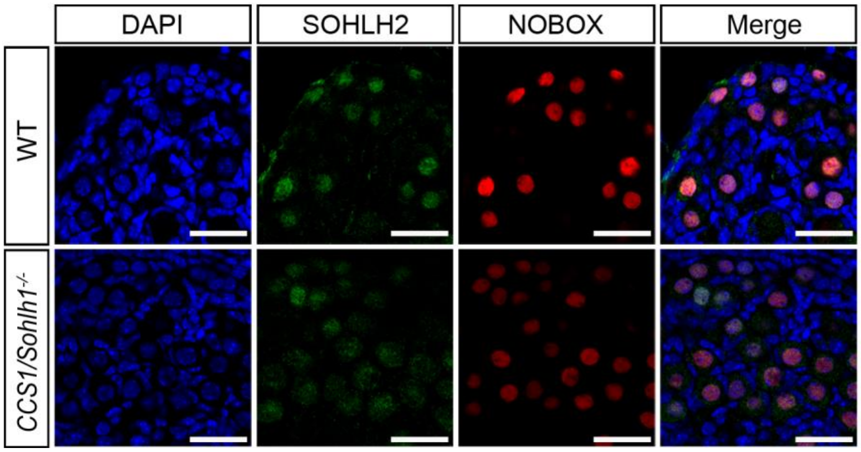
Supplemental Figure 1. Germ cell–specific gene expression profile during embryonic ovarian development. Gene expression values were derived from the GDS2203 GEO data set. The GDS2203 data set consists of RNA expression data derived from three independent experiments at each time point on the Affymetrix Mouse Genome 430 platform at various stages of development, commencing at Embryonic Day 11 (E11) and ending at Postnatal Day 2 (PD2). *Lhx8* (blue), *Sohlh1* (red), *Figla* (green), *Sohlh2* (purple), *Nobox* (orange), *Kit* (gray), and *Stra8* (brown) expressions are plotted against E11-PD2 time points. Note that expression of *Lhx8*, *Kit*, *Sohlh1*, *Figla*, and *Nobox* rise in parallel and commence circa embryonic day 16 (E16).



Supplemental Figure 2. CAG-loxp-CAT-loxp Sohlh1-mCherry *(CCS1)* transgene. (A) Diagram of the *CCS1* transgene shows chicken beta actin hybrid promoter (CAG), a floxed chloramphenicol acetyltransferase gene that prevents *Sohlh1* expression in the absence of *Cre*, mCherry, and FLAG tag. (B) CSC1 in the presence of *Ddx4-Cre* expresses SOHLH1-mCherry protein as shown by Western immunoblotting with anti-FLAG and anti-SOHLH1 antibodies (*n* = 3 per group). Data are representative of at least 3 independent experiments.



Supplemental Figure 3. KIT expression is disrupted by *Sohlh1*, *Sohlh2*, and *Lhx8* deficiencies. Immunofluorescence staining with SOHLH1 (magenta), DDX4 (yellow), and KIT (cyan) in ovaries derived from wild type, *Sohlh1*$^{-/-}$, *Sohlh2*$^{-/-}$, double knockout (*S1/2DKO*), *Lhx8*$^{-/-}$, and *Hormad1*$^{-/-}$ mice. *Hormad1*$^{-/-}$ did not affect KIT or DDX4 expression (*n* = 3 per group). DNA was stained with DAPI (gray). Data are representative of at least 3 independent experiments. Scale bar = 50 µm.



Supplemental Figure 4. The expression of SOHLH2 and NOBOX in wild-type and *CCS1/Sohlh1*$^{-/-}$*/Ddx4Cre* ovaries at PD3. NOBOX only expresses in the oocyte nucleus. In wild-type ovaries, SOHLH2 preferentially expresses in the oocyte nucleus, whereas both in the oocyte nucleus and cytoplasm in *CCS1/Sohlh1*$^{-/-}$*/Ddx4Cre* ovaries. Scale bars: 30 μm.

Name	Chromosome	WT RPKM	S1KO RPKM	S2KO RPKM	S1/2 DKO RPKM	S1KO/WT	S2KO/WT	S1/2DKO/WT
AU015836	X	16.40	2.69	3.83	2.46	0.16	0.23	0.15
Kif14	1	12.65	2.80	2.84	2.36	0.22	0.22	0.19
Mvp	7	9.79	2.17	2.20	1.60	0.22	0.22	0.16
Rab3d	9	8.04	1.87	1.78	1.48	0.23	0.22	0.18
Lsm14b	2	39.37	7.56	8.65	7.80	0.19	0.22	0.20
Ctse	1	9.79	0.67	2.02	0.14	0.07	0.21	0.01
Bfsp1	2	8.55	1.70	1.75	1.66	0.20	0.20	0.19
Tnfrsf14	4	6.77	0.96	1.38	0.06	0.14	0.20	0.01
Cdc25b	2	7.36	1.32	1.47	0.85	0.18	0.20	0.12
Ppp1r15a	7	14.07	2.47	2.77	2.48	0.18	0.20	0.18
Tle6	10	13.23	2.24	2.55	2.63	0.17	0.19	0.20
Ctsg	14	8.21	1.45	1.57	0.53	0.18	0.19	0.06
Padi6	4	26.97	3.15	5.11	4.43	0.12	0.19	0.16
Spta1	1	10.31	0.92	1.91	0.04	0.09	0.19	0.00
Trim71	9	9.66	1.31	1.78	1.47	0.14	0.18	0.15
Rhd	4	7.06	0.88	1.29	0.30	0.12	0.18	0.04
Sycp2l	13	6.40	0.91	1.16	1.00	0.14	0.18	0.16
Ermap	4	12.81	1.06	2.31	0.05	0.08	0.18	0.00
Elane	10	7.96	1.70	1.43	0.76	0.21	0.18	0.10
Cpox	16	19.27	2.15	3.46	1.61	0.11	0.18	0.08
Hmga1	17	21.11	3.20	3.66	2.72	0.15	0.17	0.13
Nlrp5	7	52.62	9.40	9.09	8.20	0.18	0.17	0.16
Klf1	8	7.20	0.68	1.19	0.04	0.09	0.17	0.01
Fam46c	3	11.58	2.25	1.82	1.47	0.19	0.16	0.13
Esrp1	4	11.39	1.86	1.67	2.12	0.16	0.15	0.19
Hemgn	4	13.36	0.76	1.85	0.02	0.06	0.14	0.00
Slc25a37	14	14.82	1.56	1.98	1.13	0.11	0.13	0.08
Mpo	11	19.06	2.66	2.47	0.94	0.14	0.13	0.05
Hbb-bs	7	450.84	103.45	53.64	32.64	0.23	0.12	0.07
Gypa	8	25.02	1.60	2.97	0.12	0.06	0.12	0.00
Lonrf3	X	15.76	2.39	1.74	2.18	0.15	0.11	0.14
Tcl1	12	8.16	0.53	0.87	1.00	0.06	0.11	0.12
Olfm4	14	15.40	2.82	1.48	0.42	0.18	0.10	0.03
Sptb	12	9.94	0.59	0.87	0.28	0.06	0.09	0.03
Alas2	X	48.36	9.40	4.06	1.90	0.19	0.08	0.04
Uchl1	5	43.54	3.24	3.61	3.33	0.07	0.08	0.08
Kit	5	40.69	2.83	2.97	2.01	0.07	0.07	0.05
Sohlh1	2	10.71	0.28	0.66	0.24	0.03	0.06	0.02
Lhx8	3	13.63	0.46	0.63	0.70	0.03	0.05	0.05
Slc4a1	11	36.82	1.29	1.54	0.05	0.03	0.04	0.00
Zp3	5	8.92	0.24	0.37	0.37	0.03	0.04	0.04

Ccno	13	14.51	0.13	0.29	0.20	0.01	0.02	0.01

Supplemental Table 1. Genes commonly downregulated in mutants (≧2-fold, q<0.05 against wild type.

Name	Chromosome	WT RPKM	S1KO RPKM	S2KO RPKM	S1/2 DKO RPKM	S1KO/WT	S2KO/WT	S1/2DKO/WT
Stfa2l1	16	30.83	13.29	13.71	1.86	0.43	0.44	0.06
Hmbs	9	13.11	4.21	5.28	2.95	0.32	0.40	0.23
BC100530	16	25.11	9.00	9.77	1.95	0.36	0.39	0.08
H2afx	9	23.87	9.37	9.04	5.19	0.39	0.38	0.22
Camp	9	38.78	16.93	14.34	1.87	0.44	0.37	0.05
Gm5483	16	26.73	11.81	9.66	2.44	0.44	0.36	0.09
Tuba1c	15	16.64	5.76	5.91	4.09	0.35	0.36	0.25
Cd24a	10	133.33	36.09	47.35	27.91	0.27	0.36	0.21
Map3k11	19	4.65	1.63	1.65	1.16	0.35	0.35	0.25
S100a9	3	273.48	104.53	95.20	23.48	0.38	0.35	0.09
Cd47	16	7.94	2.23	2.69	1.87	0.28	0.34	0.23
Glrx5	12	12.43	3.26	4.16	2.39	0.26	0.33	0.19
E2f4	8	10.63	3.65	3.52	2.51	0.34	0.33	0.24
Hist2h4	3	5.05	2.31	1.65	0.96	0.46	0.33	0.19
Lyz2	10	21.91	9.07	6.91	4.18	0.41	0.32	0.19
S100a8	3	99.27	32.17	30.01	7.96	0.32	0.30	0.08
Stfa2	16	17.44	6.00	5.25	1.09	0.34	0.30	0.06
Myb	10	6.74	2.12	1.98	0.33	0.32	0.29	0.05
Cdr2	7	7.07	2.03	2.03	1.45	0.29	0.29	0.21
AI662270	11	6.96	1.84	1.92	0.31	0.26	0.28	0.04
Ngp	9	57.11	19.40	15.72	2.12	0.34	0.28	0.04
Chil3	3	6.50	1.69	1.68	0.09	0.26	0.26	0.01
Lcn2	2	6.40	1.93	1.64	0.23	0.30	0.26	0.04
Stfa3	16	36.21	12.97	9.08	1.17	0.36	0.25	0.03
Hist2h3c2	3	12.86	7.01	9.03	3.17	0.55	0.70	0.25
F630028O10Rik	X	5.64	3.31	2.91	0.61	0.59	0.52	0.11
Hist1h4m	13	17.20	10.31	6.42	1.09	0.60	0.37	0.06
Hist2h2ac	3	122.18	78.72	31.56	25.56	0.64	0.26	0.21
Hist1h2ag	13	57.30	37.67	24.63	11.41	0.66	0.43	0.20
Stfa1	16	19.55	20.26	16.91	1.73	1.04	0.87	0.09

Supplemental Table 2. Genes significantly downregulated in Sohlh1/2 DKO (≧2-fold, q<0.05 against wild type).

Name	Chromosome	WT RPKM	S1KO RPKM	S2KO RPKM	S1/2 DKO RPKM	S1KO/WT	S2KO/WT	S1/2DKO/WT
Gpm6a	8	2.63	5.61	6.50	5.29	2.13	2.47	2.01
Ptn	6	5.69	16.51	16.74	11.65	2.90	2.94	2.05
Gm10471	5	4.85	12.47	10.13	10.01	2.57	2.09	2.06
Tktl1	X	9.34	20.55	20.54	20.54	2.20	2.20	2.20
1700018B24Rik	3	1.52	4.09	3.92	3.41	2.69	2.58	2.24
Hist1h2aa	13	9.11	21.77	24.74	20.45	2.39	2.72	2.24
D1Pas1	1	1.17	2.97	3.21	2.69	2.55	2.76	2.31
Hddc3	7	1.40	2.92	3.30	3.32	2.09	2.36	2.38
Cst12	2	3.46	6.98	7.76	8.28	2.02	2.24	2.39
5031410I06Rik	5	15.38	47.80	44.34	36.93	3.11	2.88	2.40
Syce3	15	1.04	2.99	2.89	2.54	2.88	2.79	2.45
Xlr	X	1.31	2.98	3.53	3.31	2.27	2.69	2.52
Rpl26	11	118.52	305.87	284.58	299.06	2.58	2.40	2.52
Acyp2	11	1.65	3.38	3.98	4.28	2.06	2.41	2.60
Ugt8a	3	1.17	2.88	2.92	3.09	2.46	2.51	2.65
Taf9b	X	2.89	6.45	6.90	7.77	2.23	2.39	2.69
Sap25	5	0.14	0.88	0.48	0.38	6.39	3.51	2.76
Usp26	X	1.05	2.85	2.82	2.96	2.71	2.68	2.81
Taf7l	X	19.12	39.83	46.57	54.75	2.08	2.44	2.86
Topaz1	9	1.70	4.84	4.26	4.88	2.85	2.51	2.87
Sycp2	2	8.04	18.79	18.65	23.27	2.34	2.32	2.90
Fmr1nb	X	3.09	8.38	8.87	8.98	2.71	2.87	2.90
Gab3	X	1.12	3.07	3.43	3.27	2.75	3.07	2.93
Gm364	X	5.73	14.08	16.40	16.95	2.46	2.86	2.96
Hfm1	5	2.42	5.90	5.18	7.15	2.44	2.14	2.96
Ccdc152	15	6.13	16.23	15.84	18.47	2.65	2.58	3.01
Ccnb3	X	3.42	10.01	7.41	10.45	2.92	2.17	3.05
Ccdc173	2	1.36	3.71	3.12	4.21	2.73	2.30	3.10
9230104L09Rik	2	0.18	0.95	0.60	0.59	5.25	3.30	3.22
Rhox2a	X	1.13	4.10	5.23	3.67	3.62	4.61	3.24
Nup62cl	X	1.54	4.06	4.34	5.17	2.64	2.82	3.36
Pcna-ps2	19	3.26	11.67	12.73	10.96	3.58	3.90	3.36
Xlr5a	X	3.20	8.39	10.21	10.82	2.62	3.19	3.38
Hist1h2ba	13	10.96	43.46	38.97	37.99	3.97	3.56	3.47
Barx1	13	0.18	1.08	0.76	0.61	6.17	4.30	3.48
Vmn2r13	5	0.18	1.10	0.51	0.62	6.27	2.89	3.52
Gm1140	X	2.83	5.94	6.90	10.00	2.10	2.44	3.53
Rad51ap2	12	0.61	3.29	1.98	2.18	5.38	3.24	3.57
Gm11837	4	1.35	3.83	3.49	4.93	2.83	2.59	3.65
Efhc2	X	0.91	2.90	2.99	3.34	3.19	3.29	3.67
Apoc1	7	1.73	5.65	5.67	6.38	3.27	3.28	3.69
Xlr5b	X	1.02	3.47	3.34	3.91	3.40	3.28	3.83
AV320801	X	1.16	4.41	3.44	4.59	3.81	2.96	3.95
Pramel3	X	1.37	4.98	5.48	5.46	3.62	3.98	3.97

Rdm1	11	1.04	5.00	5.41	4.14	4.81	5.19	3.98
Tecrl	5	0.84	3.63	2.78	3.39	4.34	3.32	4.05
Trap1a	X	2.11	9.49	8.89	8.58	4.50	4.21	4.06
Pet2	X	3.54	13.29	11.75	14.90	3.75	3.32	4.21
Tex19.2	11	0.20	1.15	0.69	0.85	5.74	3.46	4.23
Oacyl	18	0.21	1.15	0.84	0.89	5.59	4.09	4.32
Gramd1c	16	0.95	3.66	3.59	4.12	3.86	3.78	4.35
Gm5128	X	2.30	9.04	9.50	10.01	3.94	4.14	4.36
Rhox7a	X	0.27	1.40	1.24	1.20	5.25	4.64	4.49
Tex101	7	3.73	17.49	15.10	17.65	4.68	4.05	4.73
Hand2	8	0.23	3.58	0.84	1.11	15.39	3.63	4.78
Tex16	X	1.88	8.53	7.82	9.03	4.53	4.15	4.80
Cpa3	3	6.56	20.46	32.74	32.38	3.12	4.99	4.94
3830403N18Rik	X	0.53	2.86	2.27	2.73	5.34	4.25	5.10
1700013H16Rik	X	1.47	9.56	7.61	7.57	6.49	5.16	5.14
Myom3	4	0.13	0.69	0.59	0.68	5.23	4.53	5.18
Mcpt4	14	1.41	4.03	9.98	7.63	2.86	7.09	5.42
Rhox7-ps	X	0.41	3.12	2.40	2.21	7.66	5.90	5.43
Folr4	9	0.27	1.37	1.88	1.48	5.04	6.94	5.45
Gm595	X	1.02	7.01	6.23	5.59	6.89	6.12	5.50
Cma1	14	4.17	16.68	24.41	23.20	4.00	5.85	5.56
Timd4	11	0.24	1.89	1.58	1.37	7.89	6.61	5.75
Sis	3	0.74	1.62	3.64	4.41	2.19	4.90	5.94
Tpsb2	17	1.82	11.75	13.29	10.81	6.46	7.30	5.94
Prss50	9	0.19	2.07	2.02	1.12	11.02	10.76	5.95
Nlrp4e	7	0.12	0.70	0.58	0.75	5.63	4.66	6.06
Cers3	7	0.20	1.25	1.39	1.24	6.21	6.88	6.16
Rhox2g	X	0.10	0.68	0.81	0.63	6.63	7.87	6.16
4933425L06Rik	13	0.33	2.14	1.70	2.06	6.50	5.16	6.25
Hist1h1t	13	1.87	9.35	12.74	11.81	4.99	6.80	6.30
4933403O08Rik	X	0.14	0.81	0.95	0.93	5.70	6.73	6.53
Gm1564	11	1.40	10.52	6.15	9.33	7.50	4.38	6.65
Xlr5c	X	0.89	5.43	6.03	6.03	6.12	6.80	6.80
Scml1	X	0.38	2.24	2.06	2.63	5.90	5.43	6.93
1700080O16Rik	X	0.43	2.93	3.05	3.03	6.73	7.02	6.98
Svopl	6	0.49	3.68	4.27	3.49	7.58	8.81	7.19
Olfr461	6	0.23	1.20	1.07	1.68	5.18	4.60	7.24
Rhox5	X	0.62	3.21	5.03	4.69	5.22	8.16	7.61
Rhox2c	X	0.10	0.49	0.83	0.73	5.18	8.73	7.64
Cox7b2	5	0.50	3.05	2.90	3.85	6.05	5.76	7.64
Dpep3	8	1.02	9.02	8.52	7.89	8.81	8.32	7.71
Rhox2d	X	0.12	1.29	0.45	0.93	10.77	3.78	7.77
SCHIP1	3	0.13	0.92	0.82	1.03	7.23	6.44	8.11
Rhox6	X	0.35	1.93	2.25	3.05	5.48	6.39	8.66
Rhox7b	X	0.13	1.47	0.97	1.09	11.77	7.74	8.68
Tas2r137	6	0.22	1.15	1.42	1.91	5.28	6.49	8.74
Stra8	6	0.06	0.74	0.54	0.54	12.22	8.97	8.84

Rhox2b	X	0.16	1.27	1.20	1.43	8.01	7.55	9.02
Steap1	5	0.10	0.59	0.52	0.91	5.97	5.29	9.16
Gm4926	11	0.05	0.69	0.61	0.50	13.53	11.96	9.92
Gml	15	0.21	1.24	1.46	2.36	5.80	6.82	11.08
Dynlrb2	8	0.17	1.96	1.40	1.86	11.88	8.50	11.26
Reg2	6	0.08	0.52	0.82	0.89	6.63	10.39	11.26
Gm773	X	1.70	19.55	17.04	19.94	11.53	10.05	11.76
4930524B15Rik	11	0.08	0.71	0.88	0.98	8.70	10.70	11.93
Fam3b	16	0.04	0.61	0.70	0.50	15.33	17.47	12.46
Ly6k	15	0.20	3.22	2.55	2.76	16.30	12.90	13.93
H2bfm	X	0.03	0.60	0.64	0.38	23.20	24.55	14.47
RP24-424K5.1	1	0.06	0.53	0.73	0.98	8.29	11.33	15.27
1700019B21Rik	X	0.77	13.18	11.18	13.61	17.06	14.46	17.61
Svs5	2	0.04	0.32	0.52	0.75	7.46	12.27	17.69
Trpa1	1	0.28	4.62	4.93	5.01	16.38	17.49	17.78
Tpsab1	17	0.22	3.98	2.85	4.15	17.94	12.83	18.73
Magea8	X	0.05	0.50	0.67	1.00	9.94	13.53	20.10
Ctcfl	2	0.07	2.44	2.05	2.01	35.94	30.21	29.54

Supplemental Table 3. Genes commonly upregulated in mutants (≧2-fold, $q<0.05$ against wild type).