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Supplemental Material to:

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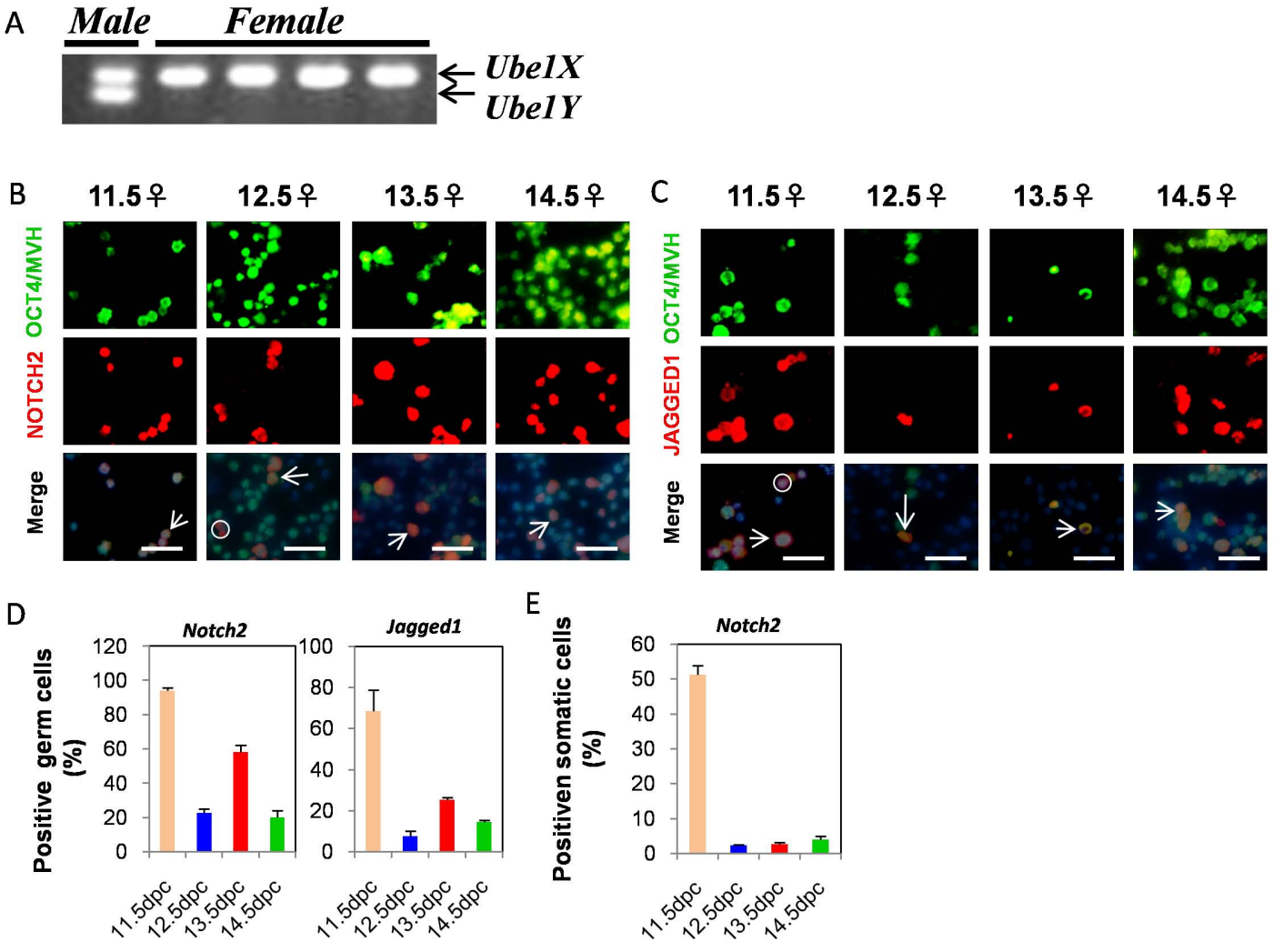
Notch pathway regulates female germ cell meiosis progression and early oogenesis events in fetal mouse

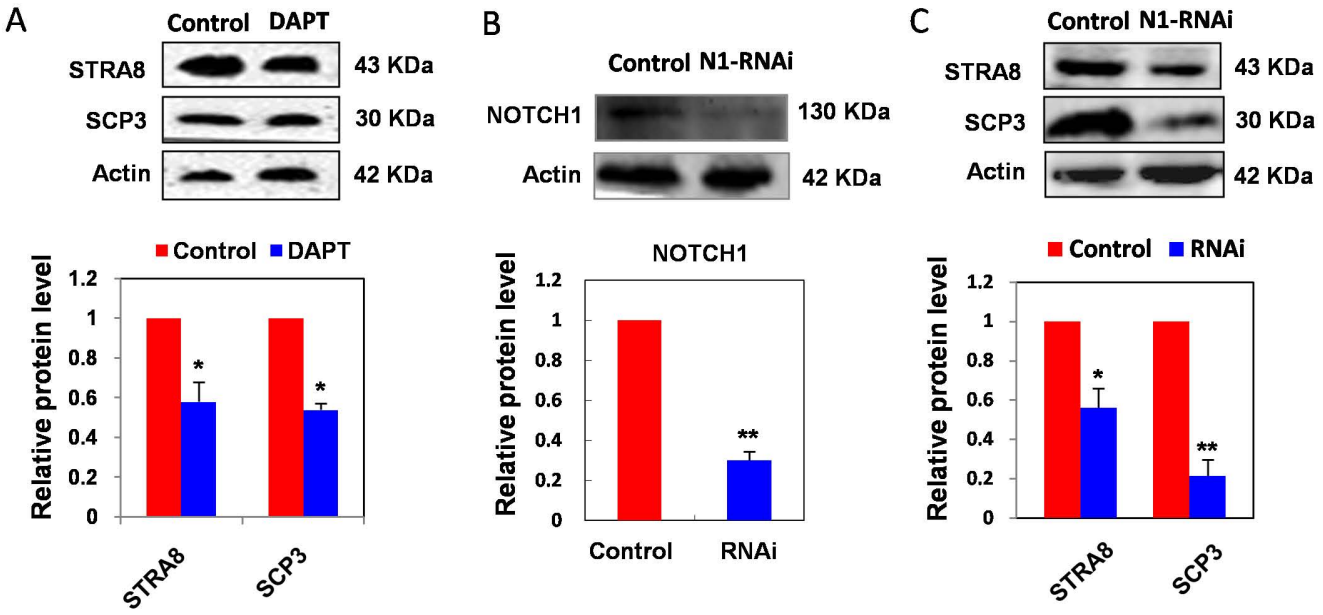
Cell Cycle 2014; 13(5)
http://dx.doi.org/10.4161/cc.27708

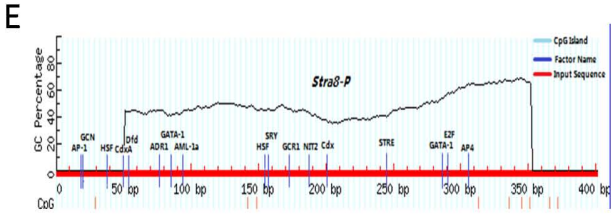
http://www.landesbioscience.com/journals/cc/article/27708

Supplemental Figure 1



Supplemental Figure 2





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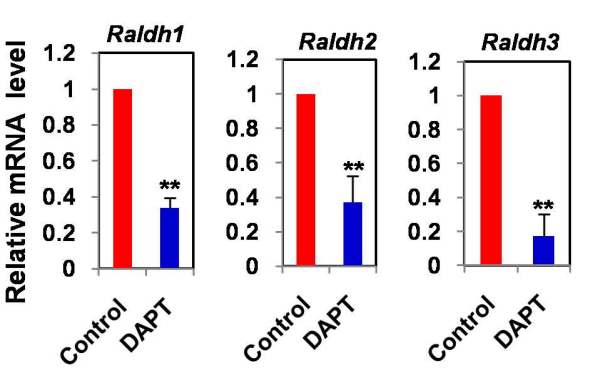

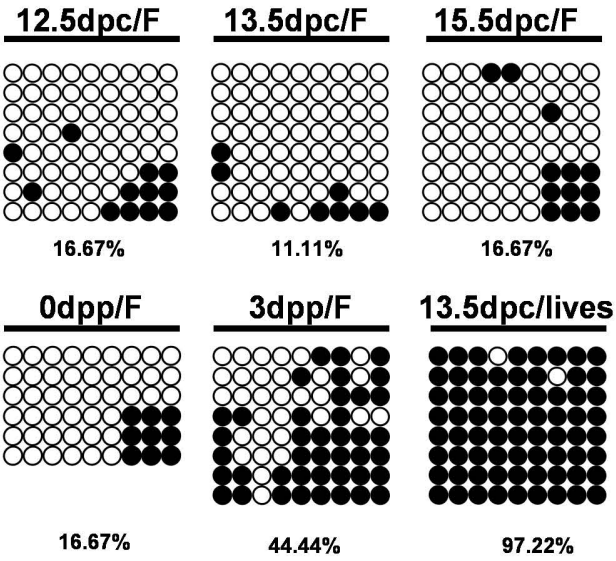

Supplemental Figure 3

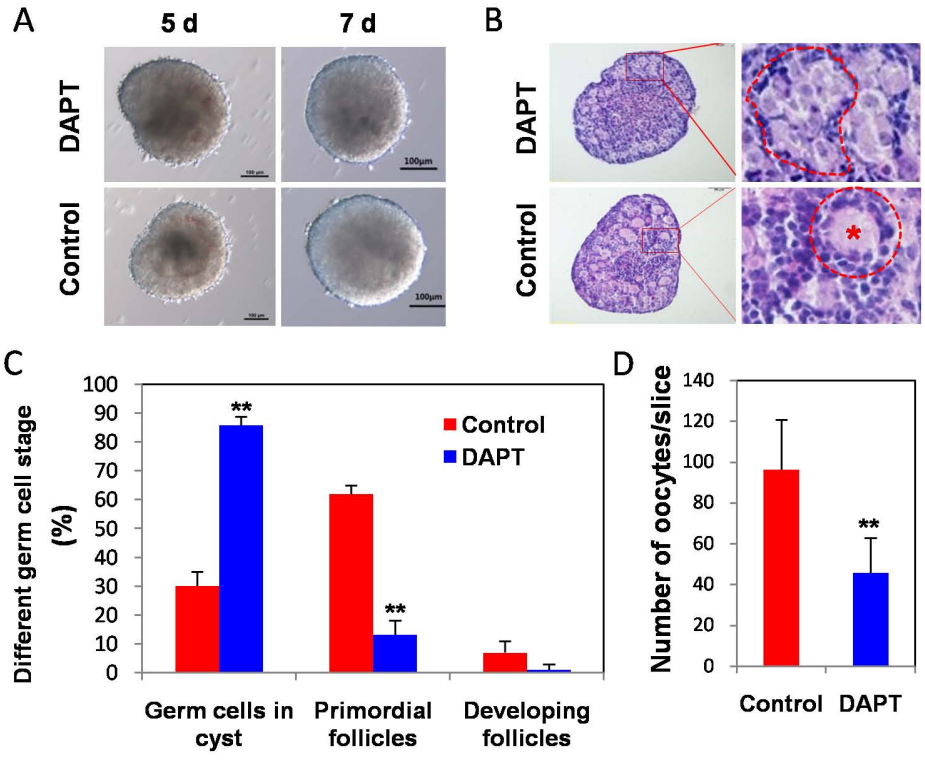

Table S1 the primers used for RT-PCR amplification of meiotic gene cDNA fragments

Gene	Primer sequence	Amplified fragment length(bp)	Gene Locus
Stra8	F : 5′-CTCCTCCTCCACTCTGTTGC-3	134	XM_004046273
	R : 5′-GCGGCAGAGACAATAGGAAG-3		
Dazl	F : 5′-TGACGTGGATGTGCAGAAGAT-3′	244	NM_010021
	R : 5′-AGGAGGATATGCCTGAACATACT-3′		
Scp3	F : 5′- GGGGCCGGACTGTATTTACT -3′	169	NM_011517
	R : 5′- AGGCTGATCAACCAAAGGTG -3′		
Rec8	F : 5′-CTACCTAGCTTGCTTCTTCCCA-3′	114	NM_000080
	R : 5′-GCCTCTAAAAGGTGTCGAATCTG-3′		
Dmc1	F: 5′-CCCTCTGTGTGACAGCTCAAC-3′	113	NM_010059
	R: 5′-GGTCAGCAATGTCCCGAAG-3′		

Table S2 the primers used for RT-PCR amplification of Notch signal molecular cDNA fragment

Gene	Primer sequence	Amplified Fragment length(bp)	Gene Locus
Notch1	F : 5′- CGTGGATTCATCTGTAGGTGC-3′	134	NM_008714
	R : 5′- CATAGGCAGGTGGGACTACG-3′		
Notch2	F : 5′-GCTGTCAATAATGTGGAGGCG-3′	125	NM_010928
	R : 5′-TTGGCCGCTTCATAACTTCC-3′		
Notch3	F : 5′-CTTCCACTGTGAGATTGACTTGC-3′	133	NM_008716
	R : 5′-CTCGTATTGGCAGTGTGTGC-3′		
Jagged1	F : 5′-TGGATTCAAGTGTGTGTGCC-3′	138	NM_013822
	R : 5′-GGAAGGCAATCACAGTAGTAGC-3′		
Jagged2	F : 5′-GCTTTGAATGCCACTGTCCG-3′	129	NM_010588
	R : 5′-AGATGCACTCGAAGCCGTCC-3′		
Delta-like 1	F : 5′-GGTTGCTCTGTGTTCTGCCG-3′	142	NM_007865

	R : 5′-GTTGGTCATCACACCCTGGC-3′		
Delta-like 3	F : 5′-CTGGACCTTGTGATGGGAACC-3′	112	NM_007866
	R : 5′-CTCACCTCACATCGAAGCCC-3′		
Delta-like 4	F : 5′-AAGAATAGCGGCAGTGGTCG-3′	141	NM_019454
	R : 5′-GATGAGAGAGTTTCCTGGCG-3′		
Hes1	F :5′-GAGCACAGAAAGTCATCAAAGCC-3′	133	NM_008235
	R : 5′-TCTCTAGCTTGGAATGCCGG-3′		
Hes2	F : 5′-CGGATCAACGAGAGCCTAAGC-3′	115	NM_008236
	R : 5′-AGCGCACAGTCATTTCCAGG-3′		
Hes3	F : 5′-GCATCAACGTGTCACTGGAGC-3′	116	NM_008237
	R : 5′-CATGTACTTAACACTCAGCTCCAGG-3′		
Hes5	F : 5′-GTGGTGGAGAAGATGCGTCG-3′	150	NM_010419
	R : 5′-GCTGTGTTTCAGGTAGCTGACG-3		

Fig. S1 Sexing mouse embryos by *Ube-1* genotyping, and immunofluorescence (IF) localization of Notch2 and Jagged1 in female germ cells.

(A) Two distinct bands of 217 and 198 bp identify male embryos, while a single band of 217 bp was present in female embryos. (B-C) Monodispersed cells were obtained from female gonadal ridges (11.5 dpc) and ovaries (12.5-14.5 dpc). Representative fields of IF for Notch2 and Jagged1. Anti-Oct4 antibody and anti-MVH antibodies were used as markers of 11.5-12.5 dpc and 13.5-14.5 dpc germ cells, respectively. Scale bar: 10 μm; circles indicate somatic cells, arrows germ cells. (D-E) Histograms showing the percentage of the germ cells and somatic cells positive for Notch2 and Jagged1.

Fig. S2. Relationship between Notch signaling and *Stra8*.

(A-C) Tissues from 12.5 dpc ovaries were cultured for 3 days with/without DAPT, or with/without Notch1-siRNA. Representative Western blot for NOTCH1, STRA8 and SCP3. This analysis was repeated three times with similar results. Graphs represent the average ± SD of samples in triplicate from three to five experiments. $p<0.05$;** $p<0.01$. (D) Quantitative RT-PCR analyses of *Raldh1-3* transcripts in 12.5 dpc ovaries were cultured for 3 days with/without DAPT. (E) Analysis of *Stra8* gene methylation state. Upper panelpotential transcription factor binding sites and CpG rich regions of the 385 bp fragment of the Stra8 gene analyzed in the present work. Redhorizontal line input sequence; blue vertical bars, potential transcriptionfactor binding sites; red vertical lines positions of the 9 CpG sites. Lower panel detailed Stra8 sequence from the -238 to 147bp locus. Top and bottom sequences correspond to bisulfate sequencing and original sequence, respectively; red horizontal lines location of primers. (F) Bisulfite sequencing analysis of the methylation state of the *Stra8* between the promoter region and the first exon were performed in oocytes obtained from 12.5 dpc, 13.5dpc, 15.5 dpc, 0 dpp and 3 dpp ovaries; black boxes methylated sites, white boxes unmethylated sites.

Fig.S3. Inhibition of Notch signaling reduces oocyte cyst breakdown and primordial follicle assembly.

(A) Morphologies of 16.5 dpc ovarian tisuuse cultured for 7 days in the presence or absence of DAPT. (B) Representative tissue sections of the ovarian tissues shown in A. (C) Percentage of oocytes in cyst and differentclasses of follicles in control and DAPT treated groups. (D) Number of oocytes for slices in control and DAPT treated groups

.The results were presented as mean ± SD of at least three experiments. * $p<0.05$;** $p<0.01$.