

Fig. S1. *tgo*^{RNAi} is efficient to deplete Tgo expression throughout oogenesis.

(A-F) Tgo expression (red in A-F and white in A'-H') in stage-7 (A), stage-8 (B), stage-11 (C), stage 10A (D), stage 12 (E), stage 13 (F-G), and stage-14 (H) follicles with flip-out Gal4 clones (marked by GFP) overexpressing *tgo*^{RNAi1} (*act > tgo*^{RNAi1}; A,C,E,G) or *tgo*^{RNAi2} (*act > tgo*^{RNAi2}; B,D,F,H). Clone boundary is outlined by a blue dashed line. Nuclei are marked by DAPI in blue.

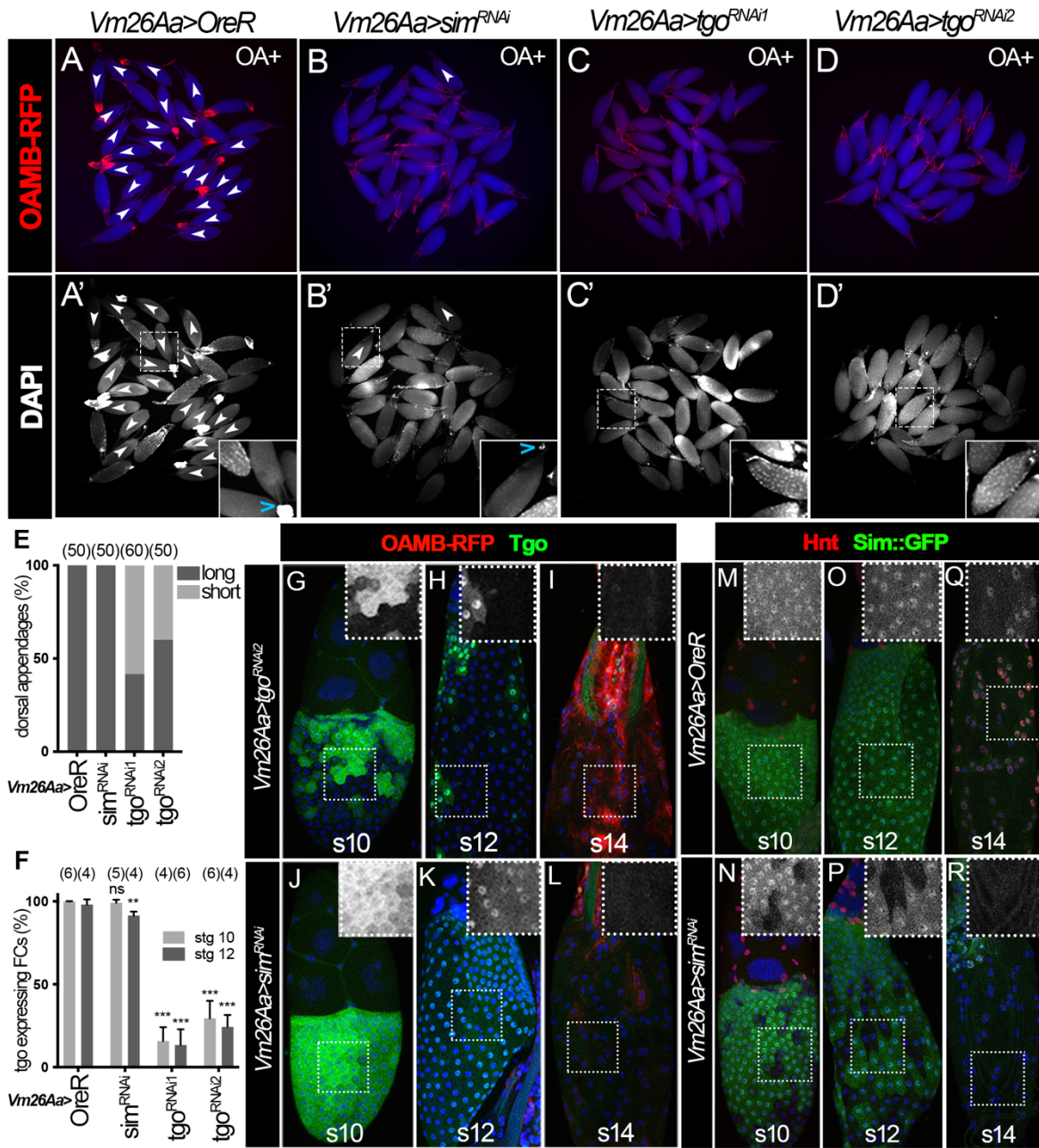


Fig. S2. Representative images of follicle rupture and the efficiency of *sim* and *tgo* knockdown with *Vm26Aa-Gal4* (A-D) Representative images show mature follicles from control (A), *sim*^{RNAi} (B), *tgo*^{RNAi1} (C) and *tgo*^{RNAi2} (D) females with *Vm26Aa-Gal4* and *Oamb-RFP* (red) after 3h culture with 20 μM OA. Bright field images are shown in blue and isolated DAPI signal is shown in white in A'-D'. Ruptured follicles are labeled with white arrowheads that face the dorsal appendage. Insets show higher magnification images of selected areas. Blue arrows point to corpus luteum. Ruptured follicles lack DAPI signal indicating follicle cell nuclei. (E) Quantification of dorsal appendage defect in control, *sim*^{RNA}, *tgo*^{RNAi1}, and *tgo*^{RNAi2} ovaries with *Vm26Aa-Gal4*. Representative examples of short and long dorsal appendages are shown in Figure 3 DIC images. The number of follicles quantified is noted above each bar. (F) Quantification of Tgo expression in mainbody follicle cells in stage 10 (light gray) and stage 12 (dark gray) follicles from control, *sim*^{RNA}, *tgo*^{RNAi1}, and *tgo*^{RNAi2} females with *Vm26Aa-Gal4*. The number of follicles quantified is noted above each bar. (G-L) Tgo expression (green) in stage-10, stage-12, and stage-14 follicles from *tgo*^{RNAi2} (G-I) and *sim*^{RNAi} (J-L) females with *Vm26Aa-Gal4* and *Oamb-RFP*. OAMB-RFP is shown in red. The insets are higher magnification of Tgo expression (white) in squared areas. (M-R) Sim::GFP expression (green, detected by GFP antibody) and Hnt expression (red) in stage-10 (M-N), stage-12 (O-P), and stage-14 (Q-R) follicles from control (M, O, Q) and *sim*^{RNAi} (N, P, R) females with *Vm26Aa-Gal4*. The insets are higher magnification of Sim::GFP expression (white) in squared areas. Nuclei are marked by DAPI in blue. ***p<0.001, **p<0.01 (Student's t-test).

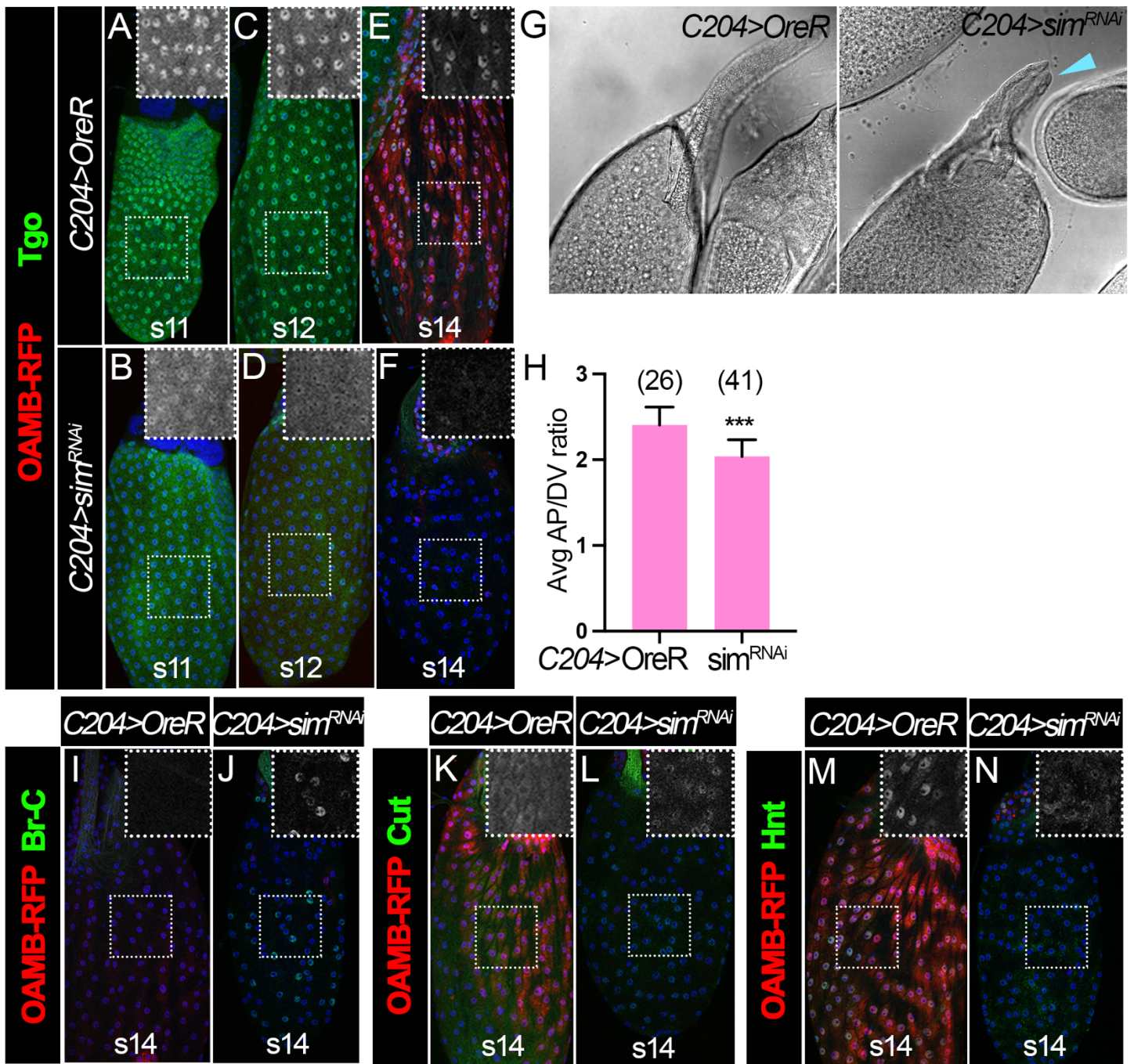


Fig. S3. Depletion of Sim using *C204-Gal4* results in defective follicle differentiation and maturation

(A-F) Tgo expression (green) in stage-11 (A-B), stage-12 (C-D), and stage-14 (E-F) follicles from control (A, C, E) and *sim^{RNAi}* (B, D, F) females with *C204-Gal4* and *Oamb-RFP*. OAMB-RFP is shown in red, and the insets are higher magnification of Tgo expression (white) in squared areas. (G) Representative DIC images show dorsal appendage morphology in control and *sim^{RNAi}* stage-14 follicles with *C204-Gal4*. Blue arrowheads indicate stunted dorsal appendages. (H) Quantification of the AP (anterior-posterior) to DV (dorsal-ventral) ratio in control and *sim^{RNAi}* stage-14 follicles with *C204-Gal4*. Total number of follicles is noted above each bar. ****p*<0.001 (Student's t-test). (I-N) Expression of transcription factors Br-C (green in I-J), Cut (green in K-J) and Hnt (green in M-N) was detected in control (I, K, M) and *sim^{RNAi}* (J, L, N) stage-14 follicles with *C204-Gal4* and *Oamb-RFP*. OAMB-RFP is shown in red, and the insets are higher magnification of the respective antibody expression (white) in squared areas.

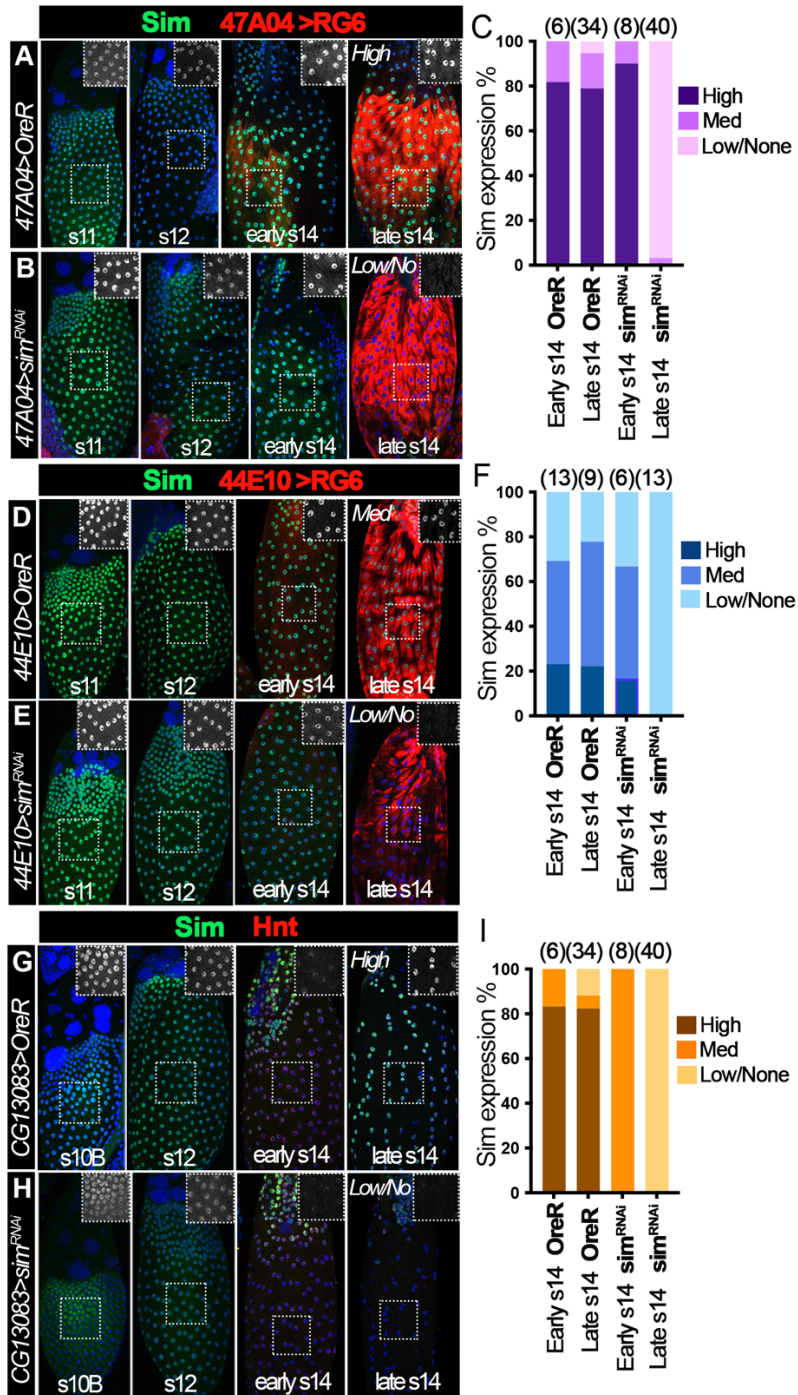


Fig. S4. *sim* knockdown efficiency using 47A04-Gal4, 44E10-Gal4 and CG13083-Gal4.

(A-B) Sim expression (green, detected by Sim antibody) in stage-11, stage-12, early stage-14 (stage 14A and 14B; low/no RFP in main body expression) and late stage-14 follicles (stage 14C, high RFP in main body expression) from control (A) and *sim*^{RNAi} (B) females with 47A04-Gal4 and UAS-RG6. **(C)** Quantification of Sim expression levels in early and late stage-14 follicles from control and *sim*^{RNAi} females with 47A04-Gal4 and UAS-RG6. The number of follicles is above each bar. **(D-E)** Sim expression (green) in stage-11, stage-12, early stage-14 and late stage-14 follicles from control (D) and *sim*^{RNAi} (E) females with 44E10-Gal4 and UAS-RG6. **(F)** Quantification of Sim expression levels in early and late stage-14 follicles from control and *sim*^{RNAi} females with 44E10-Gal4 and UAS-RG6. The number of follicles is noted above each bar. **(G-H)** Sim expression (green) and Hnt expression (red) in stage-10B, stage-12, early stage-14 (characterized by high Hnt expression either at anterior and posterior or all follicle cells) and late stage-14 follicles (characterized by med/low Hnt expression) from control (G) and *sim*^{RNAi} (H) females with CG13083-Gal4. **(I)** Quantification of Sim expression in early and late stage-14 follicles from control and *sim*^{RNAi} females with CG13083-Gal4. Italicized labels for expression intensity examples at late stage 14 are provided next to the expression inset. The number of follicles quantified is noted above each bar. For all images, the insets are higher magnification of Sim expression (white) in squared areas, and DAPI is shown in blue.

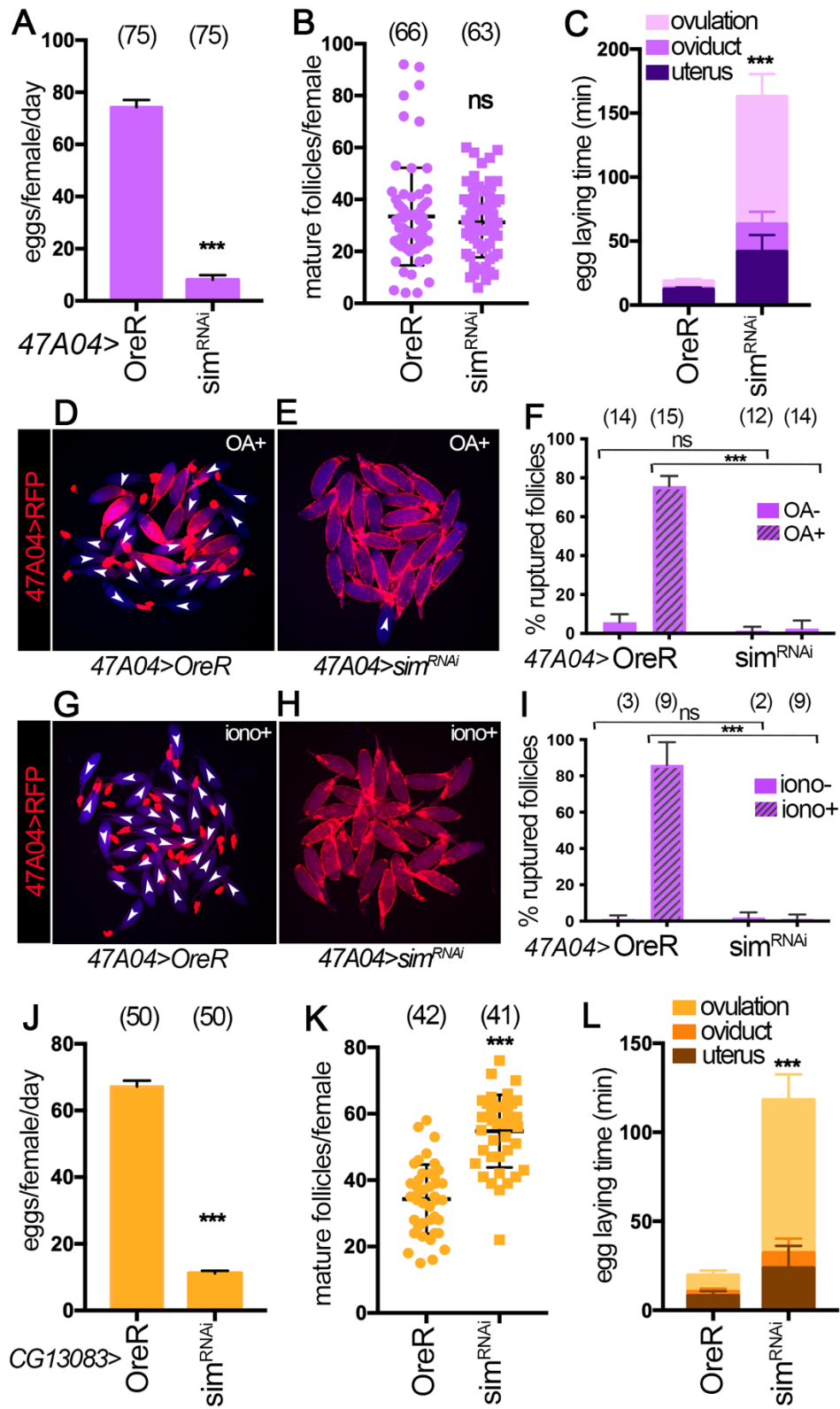


Fig. S5. Sim depletion with 47A04-Gal4 or CG13083-Gal4 results in ovulation defects.

(A-C) Quantification of egg laying (A), mature follicle numbers after egg laying (B) and egg laying time (C) in control and *sim^{RNAi}* females with *47A04-Gal4* and *UAS-RFP*. The number of females is noted above each bar. **(D-I)** Representative images show mature follicles from control (D, G) and *sim^{RNAi}* (E, H) females with *47A04-Gal4* and *UAS-RFP* after 3h culture with 20 μ M OA (OA+; D-E) or 2 μ M ionomycin (iono+; G-H). Oamb-RFP is shown in red and bright-field signal is shown in blue. Ruptured follicles are labeled with white arrowheads. Quantification of OA- and ionomycin-induced follicle rupture is shown in F and I, respectively. The number of wells analyzed is noted above each bar. **(J-L)** Quantification of egg laying (J), mature follicle numbers after egg laying (K) and egg laying time (L) in control and *sim^{RNAi}* females with *CG13083-Gal4* and *OAMB-RFP*. The number of females is noted above each bar. *** $p < 0.001$ (Student's t-test).

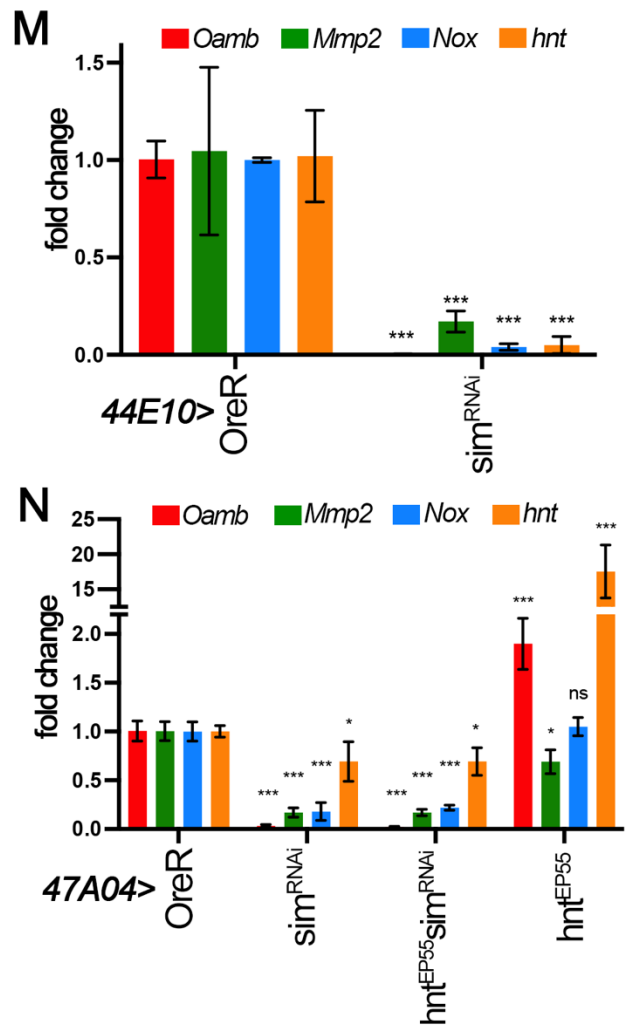
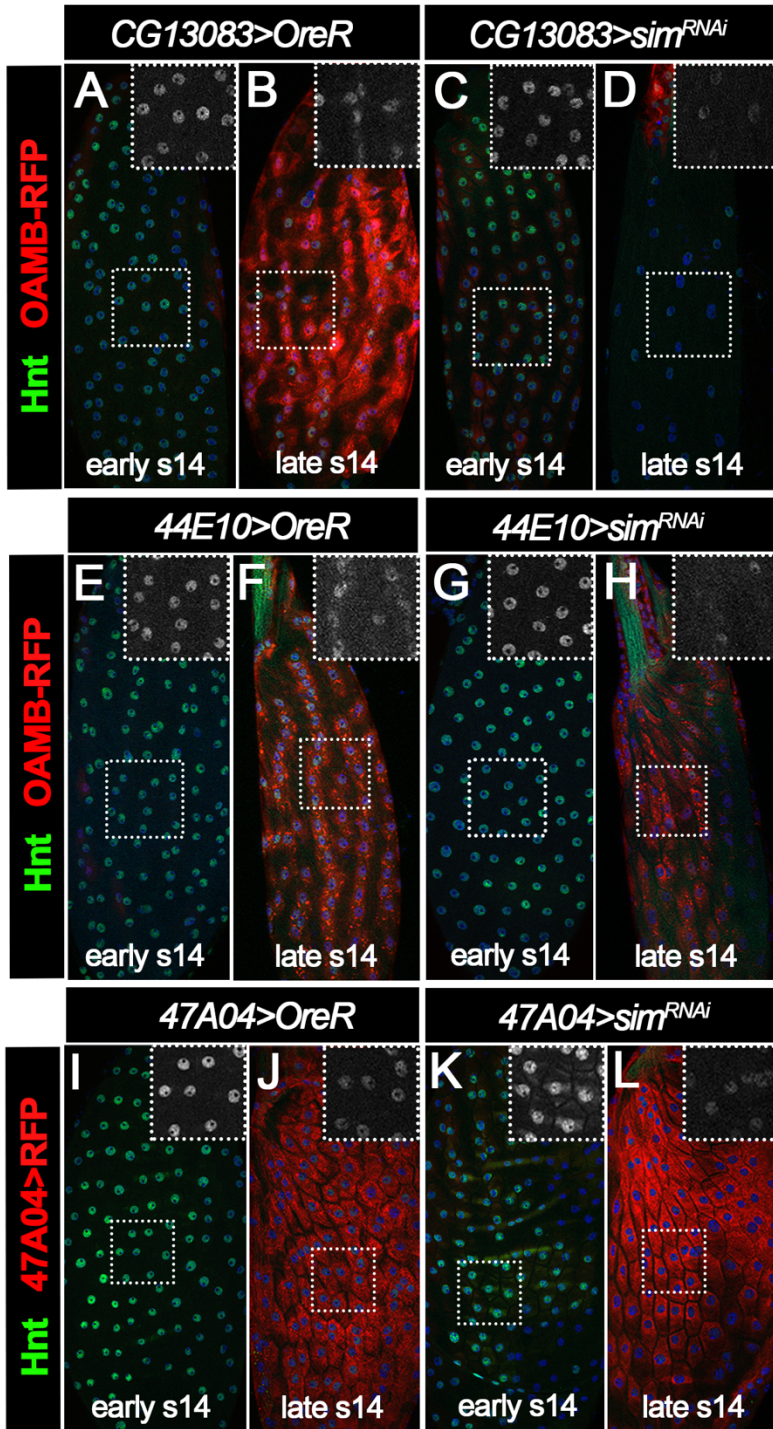


Fig. S6. Effect of *sim*-knockdown on *hnt*, *Oamb*, *Mmp2* and *Nox* expression.

(A-D) Hnt (green) and OAMB-RFP (red) expression in control (A-B) and *sim*^{RNAi} (C-D) early and late stage-14 follicles with *CG13083-Gal4* and *Oamb-RFP*. All images from A-D were acquired using the same microscopic settings. **(E-H)** Hnt (green) and OAMB-RFP (red) expression in control (E-F) and *sim*^{RNAi} (G-H) early and late stage-14 follicles with *44E10-Gal4* and *Oamb-RFP*. All images from E-H were acquired using the same microscopic settings. **(I-L)** Hnt (green) and RFP (red; driven by *47A04-Gal4*, *47A04>RFP*) expression in control (I-J) and *sim*^{RNAi} (K-L) early and late stage-14 follicles with *47A04-Gal4* and *UAS-RFP*. All images from I-L were acquired using the same microscopic settings. The insets are higher magnification of Hnt expression (white) in squared areas. Nuclei are marked by DAPI in blue. **(M)** Quantification of *Oamb*, *Mmp2*, *Nox*, and *hnt* mRNA levels in control and *sim*^{RNAi} late stage-14 follicles with *44E10-Gal4* and *Oamb-RFP* using quantitative RT-PCR. **(N)** Quantification of *Oamb*, *Mmp2*, *Nox*, and *hnt* mRNA levels in control, *sim*^{RNAi}, *hnt*^{EP55}; *sim*^{RNAi} and *hnt*^{EP55} late stage-14 follicles with *47A04-Gal4* and *UAS>RFP* using quantitative RT-PCR.

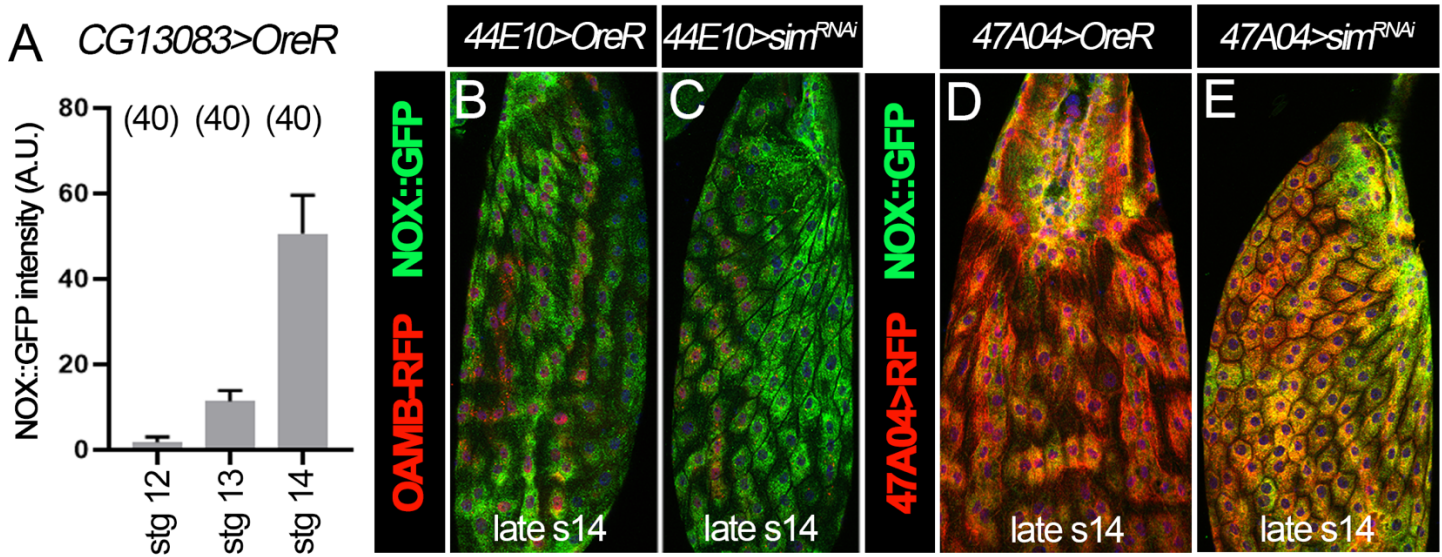


Fig. S7. Sim depletion in late stage-14 does not affect NOX::GFP expression

(A) NOX::GFP signal intensity quantification at stage 12, stage 13 and late stage 14 follicle cells from control females with *CG13083-Gal4*. The number of follicle cells analyzed is noted above each bar. Two follicles of each stage were used for the quantification. **(B–C)** NOX::GFP (green, detected by GFP antibody) in late stage-14 follicles from control (B) and *sim^{RNAi}* (C) females with *44E10-Gal4* and *Oamb-RFP*. **(D–E)** NOX::GFP (green) in late stage-14 follicles from control (D) and *sim^{RNAi}* females (E) with *47A04-Gal4* and *UAS-RFP*. Nuclei are marked by DAPI in blue for all panels. Images B-C and D-E, respectively, were acquired using the same microscopic settings.

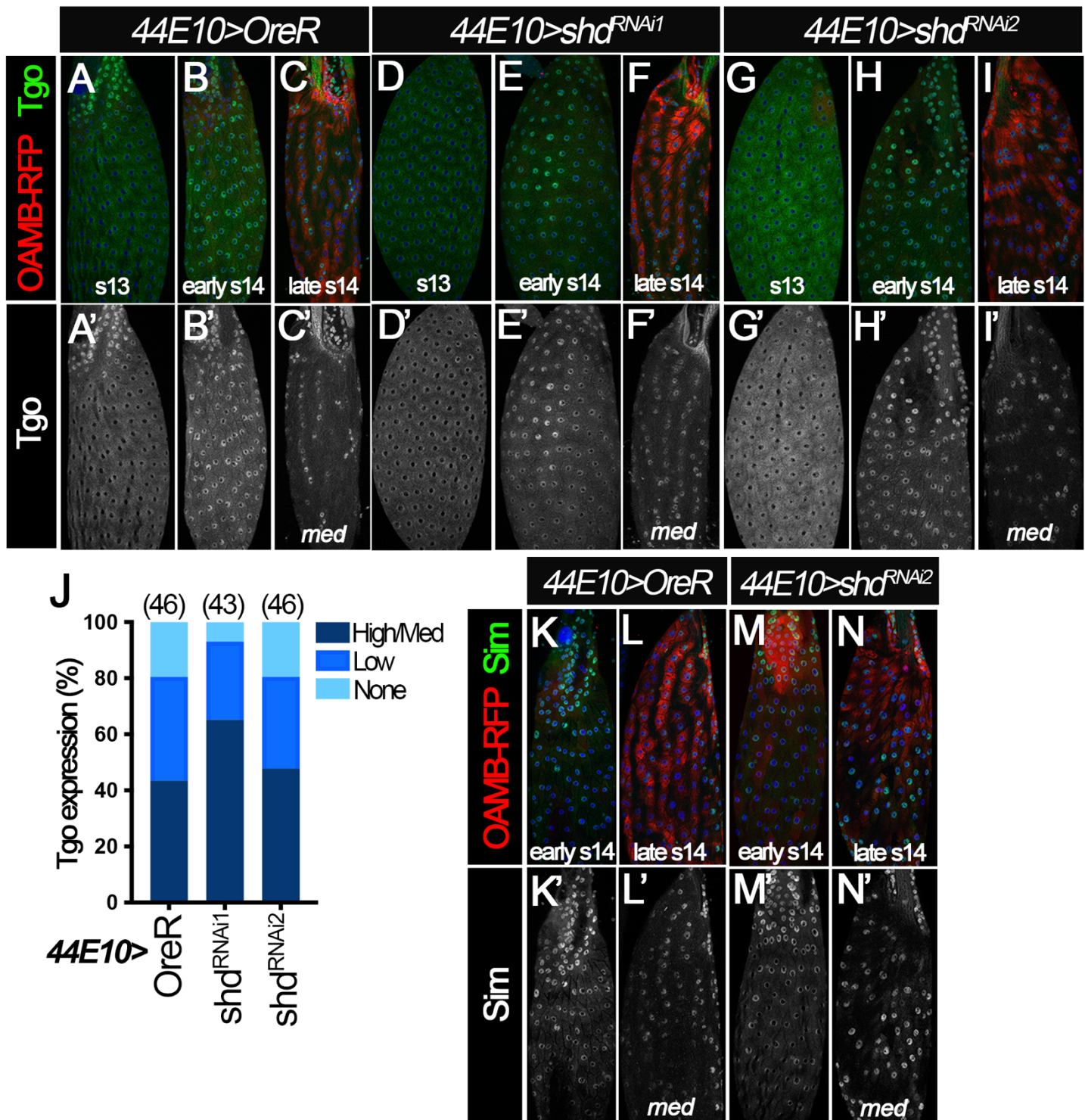


Fig. S8. Effect of *shd*-knockdown on Tgo expression in stage-14 follicle cells.

(A-I) Tgo (green in A-I and white in A'-I') and OAMB-RFP (red) expression in control (A-C), *shd*^{RNAi1} (D-F), and *shd*^{RNAi2} (G-I) follicles with *44E10-Gal4* and *Oamb-RFP*. All images from A-I were acquired using the same microscopic settings. Nuclei are marked by DAPI in blue. (J) Quantification of Tgo expression levels in control, *shd*^{RNAi1}, and *shd*^{RNAi2} late stage-14 follicles from flies expressing *44E10-Gal4* and *Oamb-RFP*. The number of follicles quantified is noted above each bar. (K-N) Sim (green in K-N and white in K'-N') and OAMB-RFP (red) expression in control (K-L) and *shd*^{RNAi2} (M-N) follicles with *44E10-Gal4* and *Oamb-RFP*. All images from K-N were acquired using the same microscopic settings. Nuclei are marked by DAPI in blue.

Table S1. Egg laying, egg distribution in the reproductive tract, and egg laying time across multiple genetic conditions.

Genotype	Egg laying in 2 days		Egg distribution in 6h			Chi-square p-value	Egg laying time (min)			
	N	Eggs/female/day	N	Ovary with egg %	Oviduct with egg %		Uterus with egg %	Ovulation time	Oviduct time	Uterus time
<i>Vm26Aa-Gal4,UAS-dcr2/OreR;OAMB-RFP[F8]/+</i>	50	70.33 ± 2.75	108	28.70 ± 8.53	3.70 ± 3.56	67.59 ± 8.83		5.39 ± 1.61	0.70 ± 0.67	12.69 ± 1.69
<i>Vm26Aa-Gal4,UAS-dcr2/+;OAMB-RFP[F8]/sim[V26888]</i>	50	2.91 ± 0.48***	71	76.06 ± 9.93	11.27 ± 7.35	12.68 ± 7.74	p<0.00001	345.0 ± 57.34	51.11 ± 33.77	57.50 ± 35.60
<i>Vm26Aa-Gal4,UAS-dcr2/+;OAMB-RFP[F8]/tgo[BL53351]</i>	45	7.00 ± 1.61***	61	75.41 ± 10.81	3.28 ± 4.47	21.31 ± 10.28	p<0.00001	142.2 ± 29.52	6.18 ± 8.48	40.19 ± 20.30
<i>Vm26Aa-Gal4,UAS-dcr2/+;OAMB-RFP[F8]/+;tgo[BL26740]/+</i>	50	2.44 ± 0.32***	79	65.82 ± 10.46	5.06 ± 4.83	29.11 ± 10.02	p<0.00001	356.09 ± 63.3	27.39 ± 26.25	157.5 ± 55.65
<i>44E10-Gal4,UAS-dcr2,OAMB-RFP[F6]/OreR</i>	50	76.27 ± 3.41	111	41.44 ± 9.16	1.8 ± 2.47	56.76 ± 9.22		7.17 ± 1.60	0.31 ± 0.43	9.82 ± 1.62
<i>w1118;44E10-Gal4,UAS-dcr2,OAMB-RFP[F6]/+</i>	25	83.98 ± 1.51	93	38.71 ± 9.90	2.15 ± 2.95	59.14 ± 9.99	p<0.00001	6.08 ± 1.56	0.34 ± 0.46	9.3 ± 1.58
<i>sim[V26888]/+;44E10-Gal4,UAS-dcr2,OAMB-RFP[F6]/+</i>	50	16.20 ± 1.09***	84	78.57 ± 8.77	3.57 ± 3.97	17.86 ± 8.19	p<0.00001	64.02 ± 7.6	2.91 ± 3.24	14.6 ± 6.7
<i>OAMB-RFP[F9]/OreR;CG13083-Gal4,UAS-dcr2/+</i>	75	70.47 ± 2.78	104	29.81 ± 8.79	5.77 ± 4.48	64.42 ± 9.20		5.58 ± 1.65	1.08 ± 0.84	12.07 ± 1.74
<i>OAMB-RFP[F9]/+;CG13083-Gal4,UAS-dcr2/hnt[V3788]</i>	75	8.10 ± 1.76***	129	61.24 ± 8.41	13.18 ± 5.84	25.58 ± 7.53	p<0.00001	99.80 ± 17.54	21.48 ± 9.80	41.69 ± 13.10
<i>OAMB-RFP[F9]/hnt[EP55];CG13083-Gal4,UAS-dcr2/+</i>	50	67.16 ± 1.82	51	47.06 ± 13.70	11.76 ± 8.84	41.18 ± 13.51		9.25 ± 2.70	2.31 ± 1.74	8.09 ± 2.66
<i>OAMB-RFP[F9]/+;CG13083-Gal4,UAS-dcr2/hnt[EP55]</i>	50	11.17 ± 0.74***	55	72.73 ± 11.77	7.27 ± 6.86	20.0 ± 10.57	p<0.00001	85.94 ± 14.35	8.59 ± 8.12	23.63 ± 12.53
<i>OAMB-RFP[F9]/OreR;CG13083-Gal4,UAS-dcr2/+</i>	58	59.46 ± 1.88	58	44.83 ± 12.8	8.62 ± 7.22	46.55 ± 12.84		9.95 ± 2.85	1.91 ± 1.60	10.33 ± 2.86
<i>OAMB-RFP[F9]/+;CG13083-Gal4,UAS-dcr2/sim[V26888]</i>	65	12.67 ± 1.16***	70	74.29 ± 10.24	8.57 ± 6.56	17.14 ± 8.83	p<0.001	77.40 ± 11.35	8.93 ± 6.85	17.86 ± 9.24
<i>OAMB-RFP[F9]/+;CG13083-Gal4,UAS-dcr2/hnt[V3788]</i>	69	29.84 ± 1.30***	80	73.75 ± 9.64	1.25 ± 2.43	25.00 ± 9.49	p<0.001	32.63 ± 4.33	0.55 ± 1.08	11.06 ± 4.21
<i>OAMB-RFP[F9]/hnt[EP55];CG13083-Gal4,UAS-dcr2/sim[V26888]</i>	74	26.16 ± 2.83***	81	54.32 ± 10.85	6.17 ± 5.24	39.51 ± 10.65	p<0.001	27.41 ± 5.68	3.11 ± 2.65	19.94 ± 5.48
<i>OAMB-RFP[F9]/hnt[EP55];CG13083-Gal4,UAS-dcr2/+</i>	69	48.51 ± 1.70***	92	54.35 ± 10.18	8.70 ± 5.76	36.96 ± 9.86	p<0.001	14.79 ± 2.78	2.37 ± 1.57	10.06 ± 2.69
<i>44E10-Gal4,UAS-dcr2,OAMB-RFP[F6]/OreR</i>	65	65.29 ± 1.76	49	42.86 ± 13.86	4.08 ± 5.54	53.06 ± 13.97		8.66 ± 2.80	0.83 ± 1.12	10.73 ± 2.83
<i>sim[V26888]/+;44E10-Gal4,UAS-dcr2,OAMB-RFP[F6]/+</i>	70	21.11 ± 1.88***	49	69.29 ± 12.90	0	30.61 ± 12.90	p<0.001	43.39 ± 8.32	0	19.14 ± 8.12
<i>hnt[EP55]/+;sim[V26888]/+;44E10-Gal4,UAS-dcr2,OAMB-RFP[F6]/+</i>	75	28.97 ± 3.06***	61	80.33 ± 9.98	3.28 ± 4.47	16.39 ± 9.29	p<0.001	36.6 ± 4.95	1.49 ± 2.04	7.27 ± 4.25
<i>hnt[EP55]/+;44E10-Gal4,UAS-dcr2,OAMB-RFP[F6]/+</i>	30	53.52 ± 2.46***	19	31.58 ± 20.90	0	68.42 ± 20.90	p<0.001	7.79 ± 5.16	0	16.88 ± 5.19

one day = 22h at 29°C
 *p<0.05, **p<0.01, ***p<0.001
 All data are mean ± 95% confidence interval. Ttest was used for egg laying and Chi-square test was used for egg distribution

Table S2. Sim binding motifs DDRCGTG and ACGTG found in the Nox gene region

Nox extends sequence 2kb 5' and 3'.									
Nox-RC is the major isoform expressed in follicle cells. From 1 to 3880 is the promoter region and 3881 is the transcription start site.									
motif_id	motif_alt_id	sequence_name	start	stop	strand	score	p-value	q-value	matched_sequence
1	DDRCGTG	Nox	1332	1338	+	9.47273	0.000683	1	TAGCGTG
1	DDRCGTG	Nox	1448	1454	-	9.47273	0.000683	1	TTGCGTG
1	DDRCGTG	Nox	1667	1673	+	9.73939	0.000256	0.606	TGGCGTG
1	DDRCGTG	Nox	3181	3187	-	9.73939	0.000256	0.606	GTGCGTG
1	DDRCGTG	Nox	3208	3214	+	9.73939	0.000256	0.606	TGGCGTG
1	DDRCGTG	Nox	3355	3361	-	9.47273	0.000683	1	GAACGTG
1	DDRCGTG	Nox	4128	4134	+	9.47273	0.000683	1	GTACGTG
1	DDRCGTG	Nox	4236	4242	+	9.73939	0.000256	0.606	GTGCGTG
1	DDRCGTG	Nox	4370	4376	-	10.0061	3.60E-05	0.47	GGCGTG
1	DDRCGTG	Nox	4378	4384	-	9.47273	0.000683	1	GAACGTG
1	DDRCGTG	Nox	5612	5618	+	9.20606	0.000944	1	TTACGTG
1	DDRCGTG	Nox	6056	6062	-	9.73939	0.000256	0.606	GTGCGTG
1	DDRCGTG	Nox	6068	6074	+	9.73939	0.000256	0.606	TGGCGTG
1	DDRCGTG	Nox	7864	7870	+	9.20606	0.000944	1	TAACGTG
1	DDRCGTG	Nox	8324	8330	-	9.47273	0.000683	1	AGACGTG
1	DDRCGTG	Nox	9044	9050	-	9.73939	0.000256	0.606	TGGCGTG
1	DDRCGTG	Nox	9055	9061	-	10.0061	3.60E-05	0.47	GGCGTG
1	DDRCGTG	Nox	11753	11759	+	9.73939	0.000256	0.606	TGGCGTG
1	DDRCGTG	Nox	12890	12896	+	9.20606	0.000944	1	AAACGTG
1	DDRCGTG	Nox	13032	13038	-	9.73939	0.000256	0.606	GTGCGTG

FIMO (Find Individual Motif Occurrences): Version 5.5.0 compiled on Dec 19 2022 at 09:36:54
 # The format of this file is described at <https://meme-suite.org/meme/doc/fimo-output-format.html>.
 # fimo --oc . --verbosity 1 --thresh 0.001 motifs.meme sequences.fa

ACGTG is highlighted in yellow