SUPPLEMENTARY MATERIAL

Age-related alterations in fertilization-induced Ca^{2+} oscillations depend on the genetic background of mouse oocytes

by

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Supplemental Figure legends

Supplemental Figure S1

Characteristics of the Oregon Green 488 BAPTA 1-AM staining

(A) Initial fluorescence intensity of the Oregon Green 488 BAPTA staining recorded for oocytes from young (black squares) and old (white squares) F1, SWISS, and C57BL/6 females. Each small square reflects a mean fluorescence intensity calculated for a single experiment (either in vitro fertilization or TG/A23187 treatment). Big squares reflect average intensities for all experiments conducted for a given type of oocytes. Error bars indicate standard deviations. (B) Representative image sequences showing Oregon Green 488 BAPTA fluorescence in oocytes from young and old F1, SWISS, and C57BL/6 females at the beginning of the recording and just before and during the 1st and the 3rd Ca^{2+} transients. Time is measured from the beginning of the recording and is shown in minutes and seconds. There is no visible compartmentalization of the dye. Scale bar 10 μm.

Supplemental Figure S2

Representative patterns of Ca^{2+} oscillations in fertilized oocytes from young and old F1, SWISS and C57BL/6 mice

(A-B) The representative patterns of Ca^{2+} oscillations in fertilized oocytes obtained from (A) young and (B) old F1 females. (C-D) The representative patterns of Ca^{2+} oscillations in fertilized oocytes obtained from (C) young and (D) old SWISS females. (E-F) The representative patterns of Ca^{2+} oscillations in fertilized oocytes obtained from (E) young and (F) old C57BL/6 females.

Supplemental Figure S3

Characteristics of the 3rd Ca^{2+} transient in fertilized oocytes from young and old F1, SWISS and C57BL/6 mice

(A) Duration and (B) amplitude of the 3rd Ca^{2+} transient in young (n = 56 F1, 92 SWISS, 36 C57BL/6) and old (n = 29 F1, 73 SWISS, 16 C57BL/6) oocytes. (C) Rate of Ca^{2+} increase during the 3rd Ca^{2+} transient in young (n = 56 F1, 92 SWISS, 36 C57BL/6) and old (n = 29 F1, 74 SWISS, 16 C57BL/6) oocytes. (D) Rate of Ca^{2+} decrease during the 3rd Ca^{2+} transient in young (n = 56 F1, 92 SWISS, 36 C57BL/6) and old (n = 29 F1, 74 SWISS, 16 C57BL/6) oocytes. (A-D) Graphs present medians and the 1st and the 3rd quartile values. The ends of the whiskers are set at 1.5*IQR above the third quartile and 1.5*IQR below the first quartile. Dots show the minimum and maximum values if they are outside the range (outliers).

Supplemental Figure S4

ITPR and ATP2A2 in oocytes from young and old F1 females

(A) A representative immunoblot showing the amount of ITPR protein in oocytes from young and old F1 females. The dashed line shows the area presented in Fig. 3 F (upper panel). The contrast and brightness have not been adjusted. (B) Quantification of the ITPR immunostaining in young (n=49) and old (n=13) F1 oocytes. (C) Quantification of the ATP2A2 immunostaining in young (n=29) and old (n=28) F1 oocytes. All intensity values in (B-C) were normalized with the mean fluorescence intensity calculated for the control, young oocytes imaged with the same settings. Graphs present medians and the 1st and the 3rd quartile values. The ends of the whiskers are set at 1.5*IQR above the third quartile and 1.5*IQR below the first quartile. Dots show the minimum and maximum values if they are outside the range (outliers).

Supplemental Table S1. The pattern of Ca^{2+} oscillations and time of pronuclei formation in fertilized oocytes obtained from young and old females of different genetic background

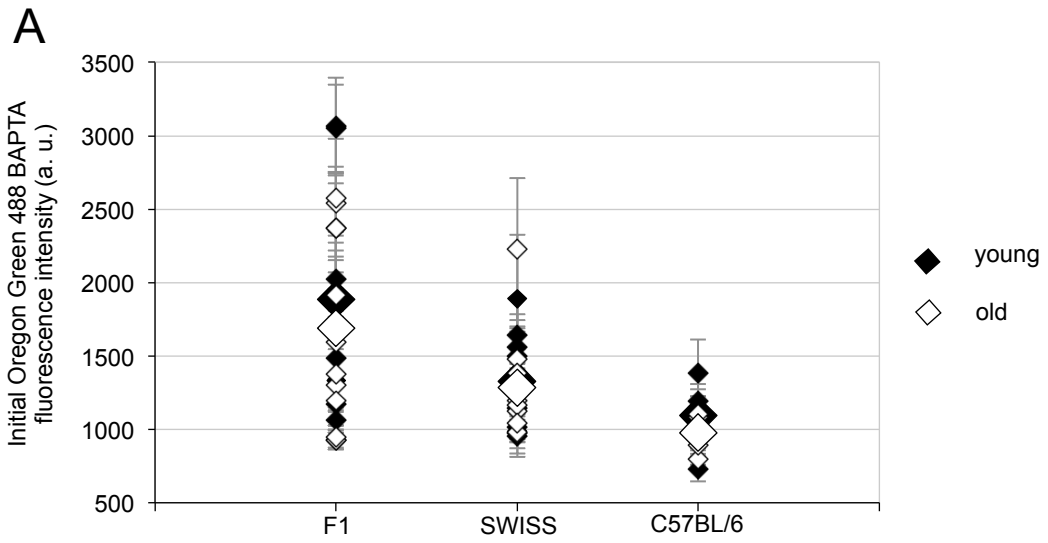
	Median (Q1; Q3) *Number of analyzed embryos*					
	F1		SWISS		C57BL/6	
Parameter	young *(3 exp)#*	old *(8 exp)*	young *(4 exp)*	old *(4 exp)*	young *(2 exp)*	old *(2 exp)*
No. of Ca^{2+} transients	**10.0** (8.0; 11.0) *n=57*	**7.0**[a] (7.0; 8.0) *n=30*	**9.0** (7.0; 12.0) *n=93*	**9.0** (7.0; 11.0) *n=74*	**8.5** (8.0; 9.3) *n=36*	**7.0**[c] (5.0; 8.0) *n=17*
Total duration of Ca^{2+} oscillations (min)	**153.2** (130.8; 172.5) *n=57*	**162.7** (122.7; 176.3) *n=29*	**162.1** (142.7; 194.3) *n=92*	**152.4**[d] (132.9; 177.6) *n=74*	**153.0** (133.3; 171.6) *n=36*	**111.8**[b] (66.0; 143.0) *n=17*
Mean interval between Ca^{2+} transients* (min)	**15.5** (13.3; 17.8) *n=57*	**21.9**[a] (18.9; 23.4) *n=29*	**16.9** (13.7; 20.8) *n=92*	**17.8** (15.1; 20.6) *n=74*	**17.6** (15.2; 20.6) *n=36*	**15.3** (12.3; 19.7) *n=17*
Duration of the 1st Ca^{2+} transient (min)	**2.3** (2.0; 2.4) *n=55*	**2.8**[a] (2.5; 3.1) *n=27*	**3.2** (2.7; 3.7) *n=92*	**2.5**[a] (2.0; 2.8) *n=73*	**2.2** (2.0;2.5) *n=36*	**2.6**[c] (2.2; 2.7) *n=18*
Amplitude of the 1st Ca^{2+} transient ($\Delta F/F_0$)	**0.8** (0.6; 0.9) *n=55*	**0.9** (0.8; 1.0) *n=27*	**0.8** (0.8; 0.9) *n=92*	**0.8** (0.8; 0.9) *n=73*	**1.0** (1.0; 1.1) *n=36*	**1.0**[d] (0.9; 1.0) *n=18*
Duration of the 3rd Ca^{2+} transient (min)	**0.7** (0.7; 0.8) *n=56*	**0.8**[a] (0.8; 1.0) *n=29*	**1.0** (0.8; 1.2) *n=92*	**0.8**[c] (0.8; 1.0) *n=73*	**0.8** (0.7; 0.8) *n=36*	**0.7** (0.5; 0.7) *n=16*
Amplitude of the 3rd Ca^{2+} transient ($\Delta F/F_0$)	**0.7** (0.6; 0.9) *n=56*	**0.8**[c] (0.8; 0.8) *n=29*	**0.8** (0.7; 0.8) *n=92*	**0.8** (0.7; 0.8) *n=73*	**0.8** (0.7; 0.9) *n=36*	**0.9** (0.8; 0.9) *n=16*
Rate of the 1st Ca^{2+} increase (min^{-1})	**3.0** (2.4; 3.9) *n=54*	**3.0** (2.5; 3.8) *n=27*	**3.2** (2.8; 3.9) *n=92*	**3.1** (2.6; 3.8) *n=74*	**5.0** (3.7; 5.9) *n=36*	**3.7**[c] (2.9; 4.8) *n=18*
Rate of the 1st Ca^{2+} decrease (min^{-1})	**-3.9** (-4.5; -2.9) *n=56*	**-3.9** (-4.3; -3.5) *n=30*	**-3.5** (-3.8; -3.2) *n=92*	**-3.5** (-3.9; -3.0) *n=74*	**-4.5** (-4.9; -3.9) *n=36*	**-4.0**[c] (-4.3; -3.8) *n=18*
Rate of the 3rd Ca^{2+} increase (min^{-1})	**4.0** (3.1; 5.0) *n=56*	**4.5**[c] (4.2; 4.8) *n=29*	**3.7** (3.3; 4.1) *n=92*	**3.8** (3.4; 4.2) *n=74*	**4.8** (4.2; 5.4) *n=36*	**5.1** (4.6; 5.3) *n=16*
Rate of the 3rd Ca^{2+} decrease (min^{-1})	**-4.1** (-4.8; -3.4) *n=56*	**-4.5**[d] (-4.7; -4.1) *n=29*	**-3.7** (-4.2; -3.5) *n=92*	**-3.7** (-4.0; -3.3) *n=74*	**-4.4** (-5.4; -4.1) *n=36*	**-4.9** (-5.3; -4.5) *n=16*
Time of pronuclei formation (min)	**189.4** (170.4; 229.6) *n=26*	**242.7**[b] (214.0; 261.7) *n=13*	**206.5** (179.5; 253.2) *n=42*	**247.4**[b] (229.4; 269.3) *n=22*	**225.3** (216.5; 251.6) *n=28*	**201.5**[b] (188.3; 211.1) *n=12*

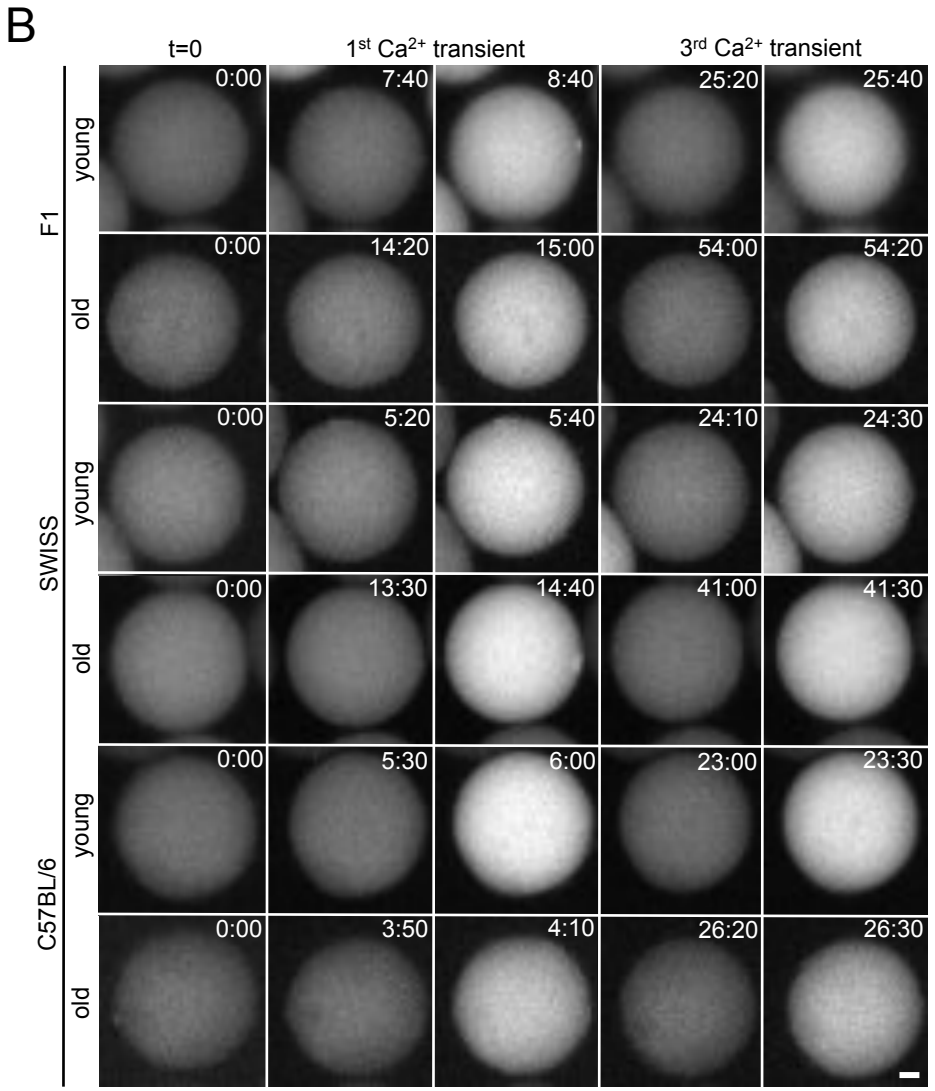
*calculated for the first 2 hrs of Ca^{2+} oscillations

[#]no. of experiments conducted for the respective group of oocytes

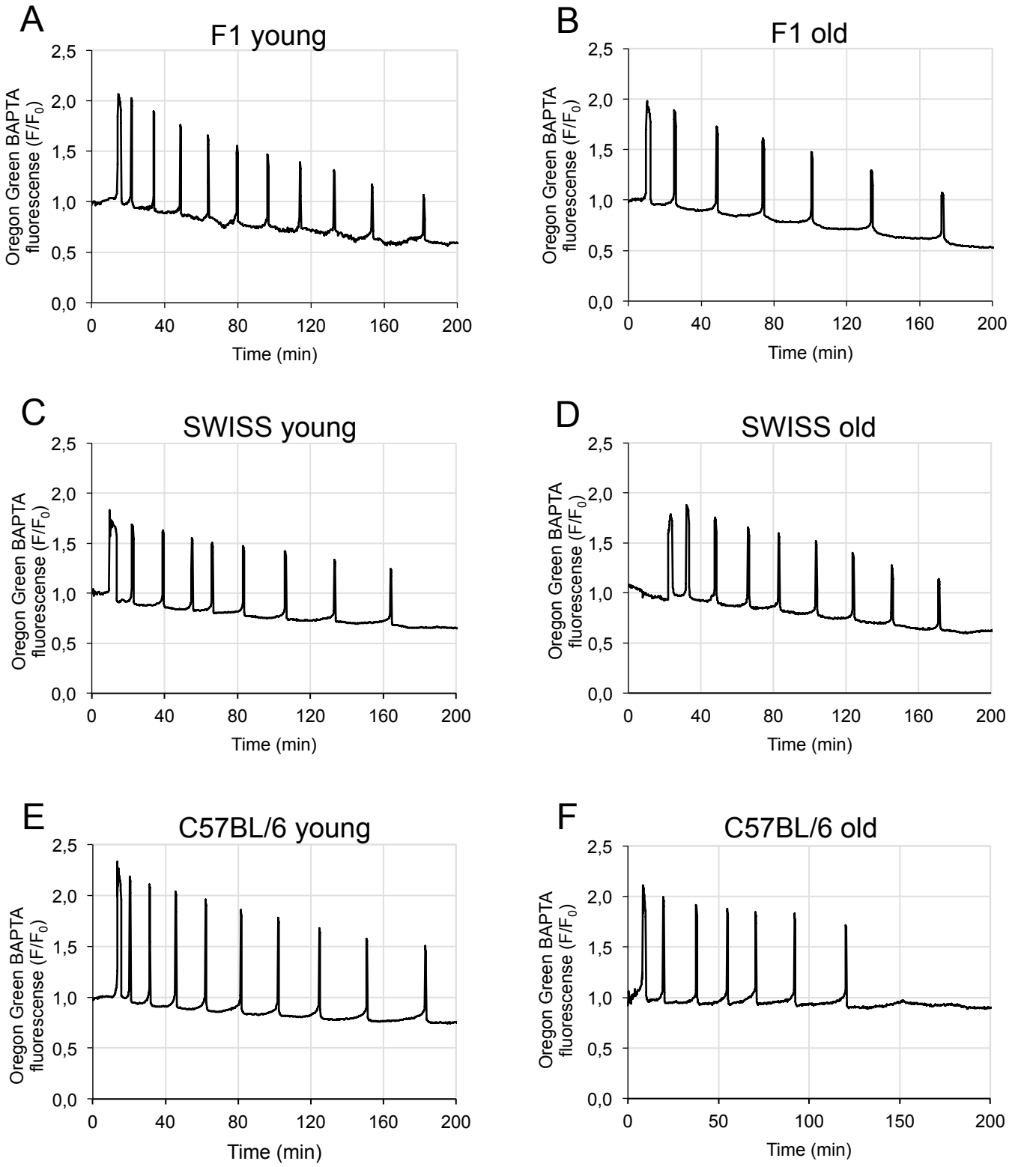
[a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$, [d]$p=0.05$ *vs*. oocytes from young females of the respective background

Supplemental Figure S1

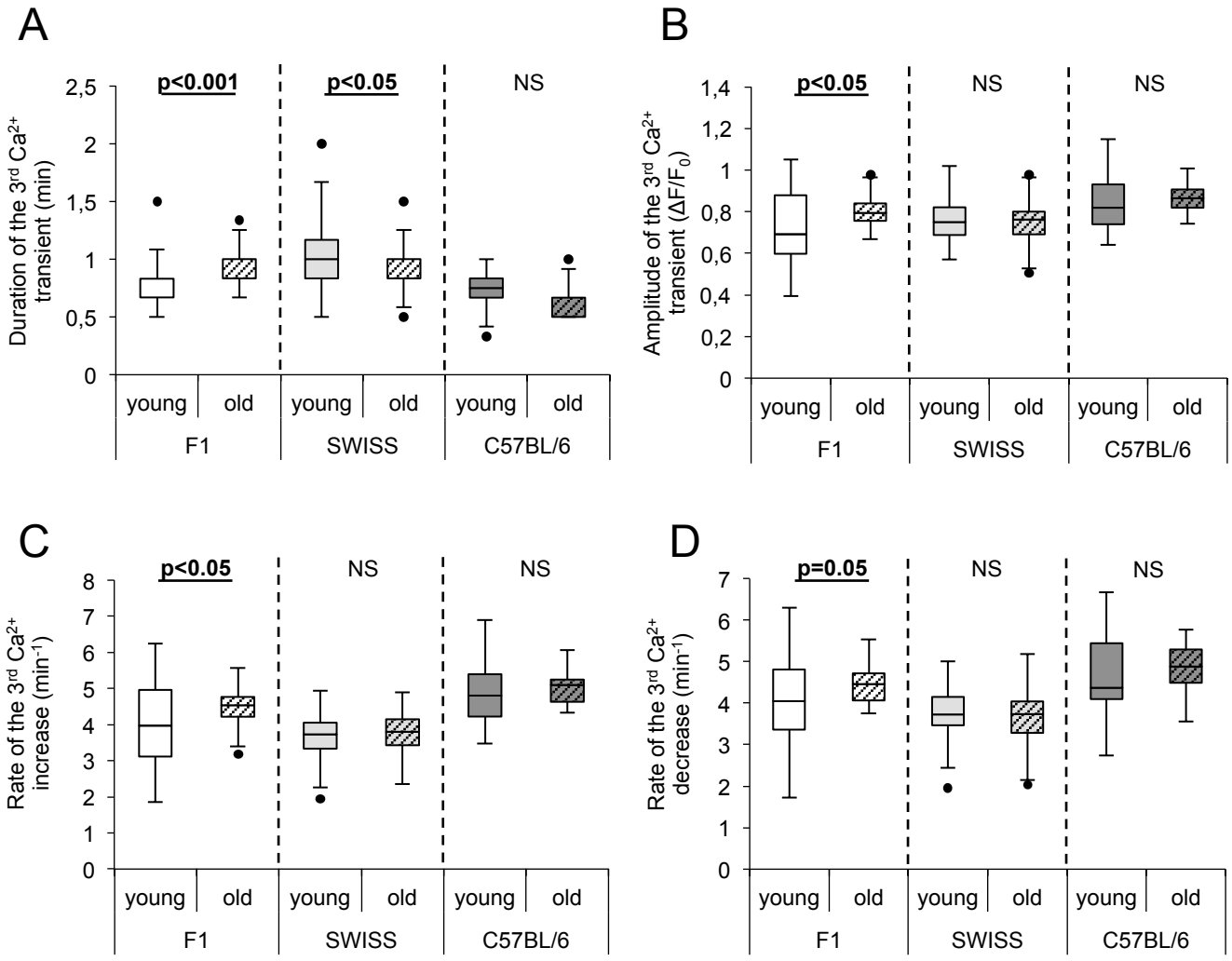




Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4