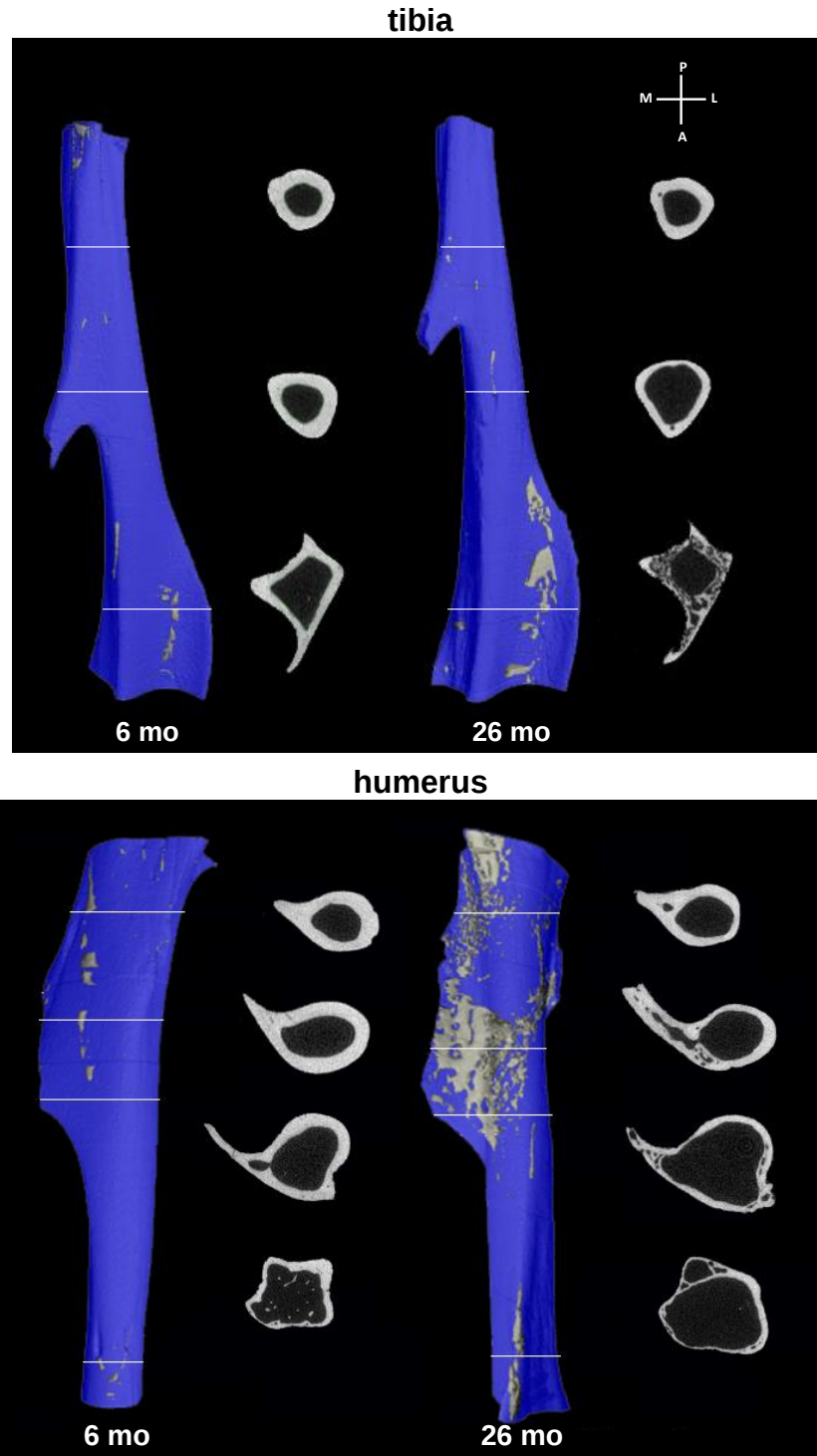
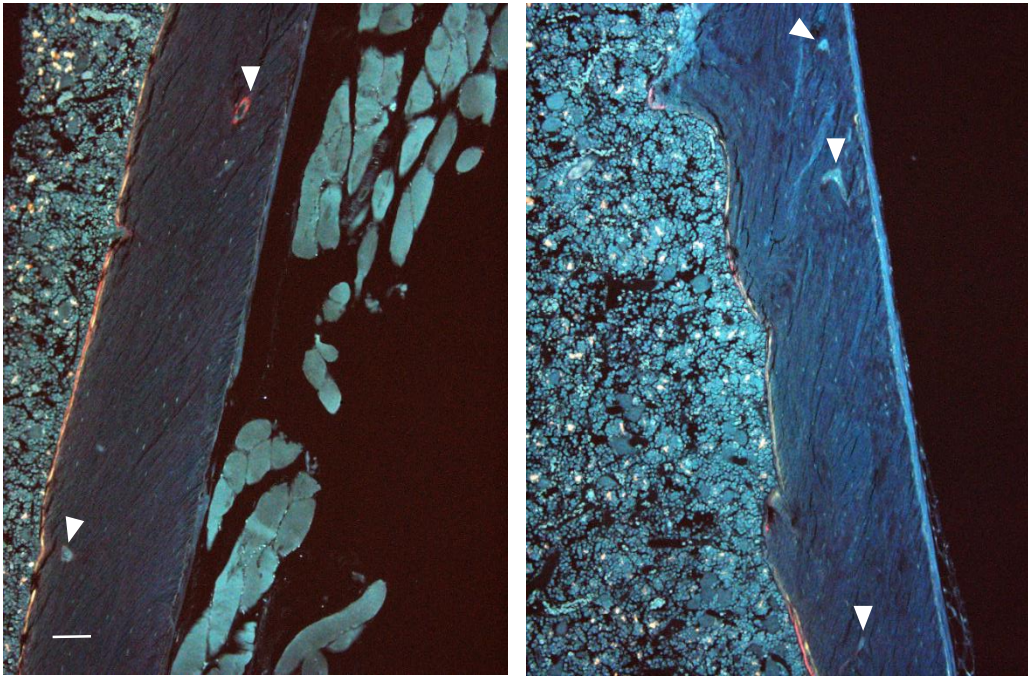




Supplementary Figure 1. MicroCT imaging of porosity in the tibia and humerus of female B6 mice. Bones were scanned at 8 μm isotropic voxel size. Void areas are depicted in grey within a transparent bone matrix. Images of single microCT slices, before binarization, from the indicated areas are shown. Orientation of the slices is shown at the top (M, medial; P, posterior; L, lateral; A, anterior).

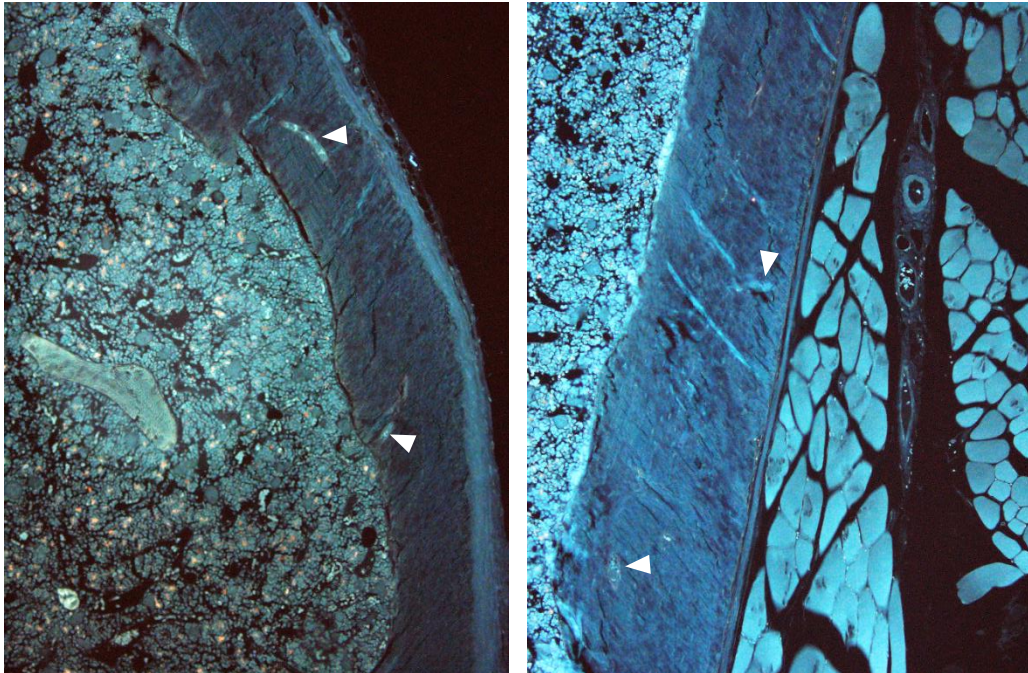
Vehicle

A

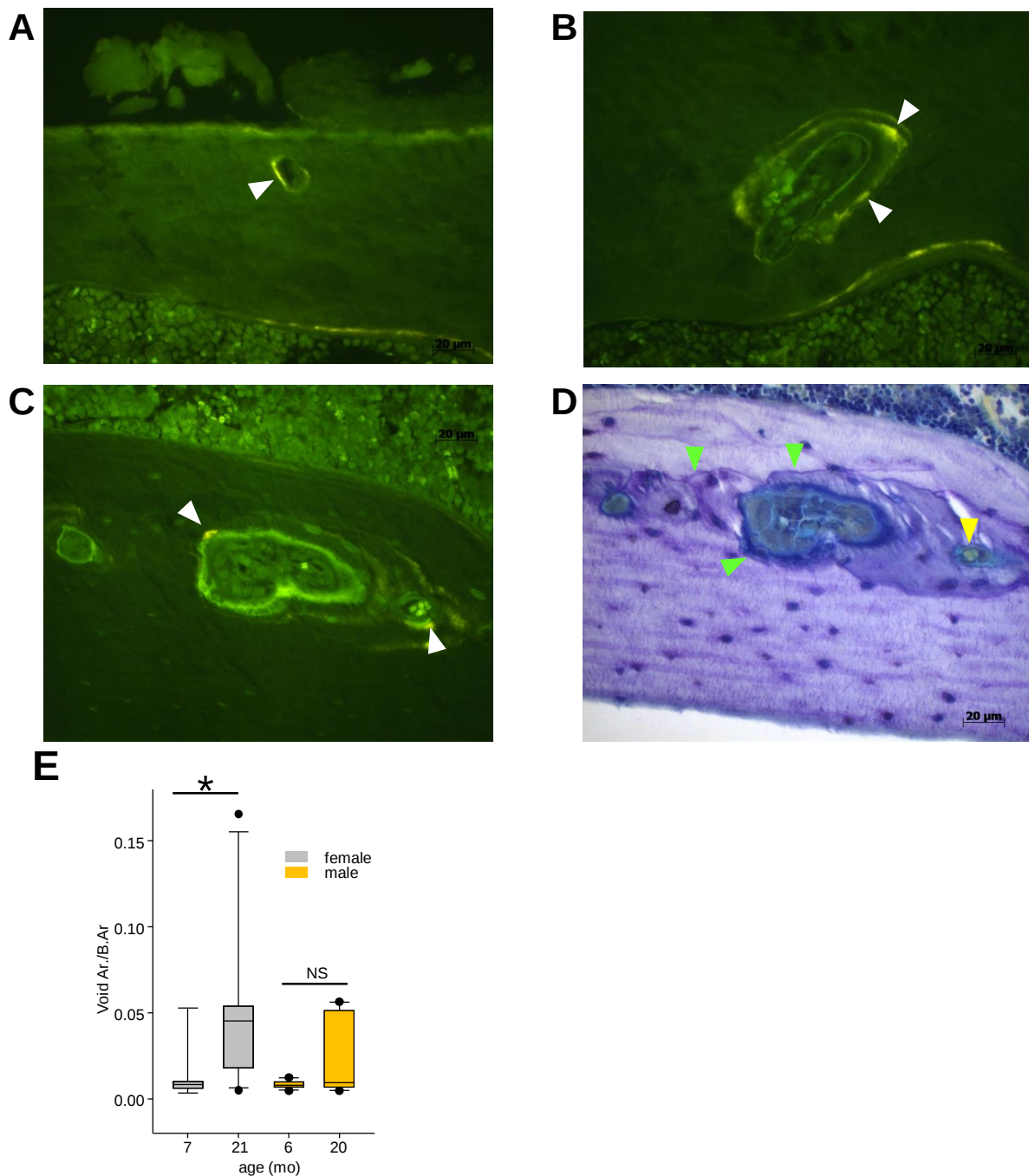


OPG

B



Supplemental Figure 2. OPG eliminates intracortical bone formation in adult mice. Swiss-Webster female mice (7 mo) were injected with (A) vehicle or (B) OPG. Unstained longitudinal sections of the femur were made to visualize alizarin (red) and calcein (yellow) fluorochromes. Images from two mice of each treatment group are shown. White arrowheads indicate location of pores. Bar = 20 μ m.



Supplemental Fig. 3. De novo osteonal remodeling in 21-mo-old male B6 mice. (A-C) Nondecalcified unstained longitudinal femoral sections show intracortical pores with tetracycline labeling (white arrowheads). (D) Toluidine blue-stained section adjacent to the section shown in panel C to visualize cement lines (green arrowheads) and capillaries (yellow arrowhead). (E) Histomorphometric determination of porosity in B6 females (reproduced from Figure 8I of the main text), and in 7 mo-old (n=9) and 21 mo-old (n=10) B6 males. * $P < 0.05$ using a permutation approach to Student's two tailed t-test. NS, not significant ($P=0.70$).

Supplemental videos. Three-dimensional depiction of cortical porosity in the distal femur.

Images proceed proximally from the distal metaphysis of the femur to the mid-diaphysis of female B6 mice, from Fig. 6B of the manuscript. The porosity is 0.06% for the 6 mo example (Suppl Video_6 mo), 2.31% for the 18 mo example (Suppl Video_16 mo), and 4.68% for the 26 mo example (Suppl Video_26 mo).

Supplementary Table 1. Age-related changes in body weight in B6 mice

B6					
	Female			Male	
	6 mo	18 mo	26 mo	7 mo	21 mo
	(20)	(20)	(19)	(10)	(10)
Body weight, g	23 ± 2	29 ± 4	28 ± 3*	33 ± 2	39 ± 4*

Supplementary Table 2. Age-related changes in body weight in CB6F1 mice

CB6F1						
	Female			Male		
	7 mo	19 mo	30 mo	7 mo	19 mo	30 mo
	(10)	(10)	(10)	(10)	(10)	(8)
Body weight, g	29 ± 2	38 ± 4*	36 ± 4*	40 ± 4	51 ± 5*	39 ± 4

Data were analyzed by ANOVA or Students t-test. *, $p < 0.05$ vs 6 or 7 mo-old of the same sex and strain.

Supplementary Table 3. Age-related changes in cortical bone of B6 mice

	Female			Male	
	6 mo (20)	18 mo (20)	26 mo (18)	7 mo (10)	21 mo (10)
VERTEBRA (L4)					
Ct.Th , mm	0.084 ± 0.006	0.073 ± 0.008*	0.070 ± 0.006*	0.074 ± 0.004	0.063 ± 0.004*
FEMUR	6 mo (7)	18 mo (8)	26 mo (7)	7 mo (10)	21 mo (10)
Ct.Th. mm (dia)	0.226 ± 0.007	0.201 ± 0.013*	0.189 ± 0.009**	0.231 ± 0.010	0.197 ± 0.016*
T.Ar, mm ² (dia)	1.808 ± 0.057	2.269 ± 0.195*	2.415 ± 0.130*	2.314 ± 0.201	2.516 ± 0.148*
Med.Ar, mm ² (dia)	0.993 ± 0.177	1.322 ± 0.130*	1.497 ± 0.106**	1.203 ± 0.154	1.483 ± 0.113*
Ct.Th, mm (meta)	0.212 ± 0.009	0.175 ± 0.009*	0.141 ± 0.015**	0.183 ± 0.010	0.154 ± 0.023*
T.Ar, mm ² (meta)	2.142 ± 0.093	2.148 ± 0.227	2.402 ± 0.274*	2.574 ± 0.207	2.394 ± 0.252
Med.Ar, mm ² (meta)	1.098 ± 0.078	1.268 ± 0.149*	1.601 ± 0.274**	1.509 ± 0.187	1.497 ± 0.232

Supplementary Table 4. Age-related changes in cortical bone of CB6F1 mice

	Female			Male		
	7 mo (8)	19 mo (10)	30 mo (10)	7 mo (8)	21 mo (10)	30 mo (10)
VERTEBRA (L4)						
Ct.Th , mm	0.080 ± 0.005	0.075 ± 0.006*	0.054 ± 0.009*	0.068 ± 0.005	0.075 ± 0.004*	0.061 ± 0.006**
FEMUR	7 mo (8)	19 mo (10)	30 mo (10)	7 mo (8)	21 mo (10)	30 mo (10)
Ct.Th. mm (dia)	0.263 ± 0.011	0.250 ± 0.006	0.259 ± 0.020	0.239 ± 0.010	0.247 ± 0.011	0.240 ± 0.018
T.Ar, mm ² (dia)	1.832 ± 0.055	2.277 ± 0.097*	2.457 ± 0.144**	2.194 ± 0.356	2.756 ± 0.196	2.872 ± 0.152**
Med.Ar, mm ² (dia)	0.774 ± 0.133	1.135 ± 0.074*	1.209 ± 0.086**	1.114 ± 0.247	1.491 ± 0.150*	1.549 ± 0.166**
Ct.Th, mm (meta)	0.226 ± 0.007	0.209 ± 0.012*	0.173 ± 0.020**	0.189 ± 0.013	0.190 ± 0.013	0.163 ± 0.014*
T.Ar, mm ² (meta)	2.138 ± 0.110	2.115 ± 0.074	2.214 ± 0.112	2.525 ± 0.319	2.714 ± 0.196	2.886 ± 0.303*
Med.Ar, mm ² (meta)	1.045 ± 0.056	1.114 ± 0.095	1.263 ± 0.166**	1.438 ± 0.231	1.598 ± 0.162	1.854 ± 0.235**

Cortical architecture was measured by microCT of the lumbar vertebra L4, the femoral mid-diaphysis (“dia”), and femoral distal metaphysis (“meta”). Cortical thickness (Ct.Th), cross-sectional area (T.Ar), and medullary area (Med.Ar) were determined. The number of animals is indicated in the parentheses. Data were analyzed by ANOVA or Students t-test. *, p<0.05 vs 6-7 mo old mice of the same sex; ** p<0.001 vs 6-7 mo and 18-21 mo old mice of the same sex.

Supplementary Table 5. Age-related changes in trabecular bone of B6 mice

	female			male	
VERTEBRA (L4)	6 mo (20)	18 mo (20)	26 mo (18)	7 mo (10)	21 mo (10)
BV/TV (%)	25.8 ± 3.5	20.7 ± 5.1*	16.2 ± 6.1*	32.4± 2.6	23.3 ± 3.8 [#]
Tb.N, mm ³	3.8 ±0.4	2.8 ±0.4*	2.5 ± 0.5*	5.1 ± 0.2	3.8 ± 0.3 [#]
Tb.Th, mm (x10 ³)	62.2 ± 2.3	64.3 ± 3.8	60.2 ± 5.4*	62.0± 4.0	61.0 ± 4.0
Tb.Sp, mm	0.25 ±0.03	0.38 ± 0.06*	0.43 ± 0.08*	0.18 ±0.01	0.24 ±0.02 [#]
FEMUR	6 mo (8)	18 mo (8)	26 mo (7)	7 mo (10)	21 mo (10)
BV/TV (%)	6.2 ± 2.0	1.1 ± 0.6*	0.6 ± 0.6*	21.3 ± 4.2	6.5 ± 1.6*
Tb.N, mm ³	2.9 ± 0.4	1.5 ± 0.2	1.3 ± 0.2	4.2 ±0.2	2.7 ± 0.2*
Tb.Th, mm (x10 ³)	51.8 ± 2.1	54.9 ± 8.2	51.7 ± 3.80	66.0 ± 5.0	61.0 ±6.0
Tb.Sp, mm	0.34 ± 0.04	0.69 ± 0.12*	0.78 ± 0.10*	0.22 ± 0.01	0.36 ±0.03 [#]

Supplementary Table 6. Age-related changes in trabecular bone of CB6F1 mice

		female			male	
VERTEBRA (L4)	7 mo (8)	19 mo (10)	30 mo (9)	7 mo (10)	19 mo (10)	30 mo (8)
BV/TV (%)	25.8 ± 2.6	15.7 ± 3.4*	17.6 ± 7.9*	32.1 ± 2.2	28.1 ± 2.0*	22.9 ± 3.7**
Tb.N, mm ³	3.7 ± 0.4	2.3 ± 0.3*	2.6 ± 1.2*	5.3 ± 0.3	4.5 ± 0.3*	4.1 ± 0.5*
Tb.Th, mm (x10 ³)	62.0 ± 1.0	61.0 ± 3.0	56.0 ± 2.0*	57.4 ± 3.1	59.7 ± 2.4	57.0 ± 4.5
Tb.Sp, mm	0.27 ± 0.03.	0.45 ± 0.05*	0.045 ± 0.14*	0.17 ± 0.1	0.20 ± 0.2*	0.22 ± 0.03*
FEMUR	7 mo (8)	19 mo (10)	24 mo (9)	7 mo (10)	19 mo (10)	24 mo (8)
BV/TV (%)	6.6 ± 1.5	1.1 ± 1.0*	0.6 ± 0.4*	16.4 ± 3.1	8.3 ± 2.4*	3.4 ± 0.01**
Tb.N, mm ³	2.8 ± 0.2	1.5 ± 0.2*	1.2 ± 0.2*	4.3 ± 0.3	3.1 ± 0.4*	2.1 ± 0.5 [#]
Tb.Th, mm (x10 ³)	55.0 ± 2.0	53.0 ± 7.0	53.0 ± 13.0	55.7 ± 4.1	62.4 ± 3.2*	60.5 ± 5.5
Tb.Sp, mm	0.35 ± 0.03	0.69 ± 0.1*	0.85 ± 0.2*	0.22 ± 0.02	0.31 ± 0.04*	0.48 ± 0.11**

Trabecular architecture architecture was measured by microCT of the lumbar vertebra L4, and of the femoral distal metaphysis. Trabecular bone volume (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th), and trabecular separation (Tb.Sp) were determined. The number of animals is indicated in the parentheses. Data were analyzed by ANOVA or Students t-test. *, p<0.05 vs 6-7 mo old mice of the same sex; ** p<0.001 vs 6-7 mo and 18-21 mo old mice of the same sex.

Supplementary Table 7. Primer-probe pairs for quantification of gene expression by Taqman PCR.

NCBI gene name	Taqman #
RANKL	Mm00441908_m1
OPG	Mm00435452_m1
Wnt16	Mm00446420_m1
Wnt5a	Mm00437347_m1
Sost	Mm00470479_m1
Dkk1	Mm00438422_m1
Wnt10b	Mm00442104_m1
Wnt6	Mm00437353_m1
Wnt4	Mm00437341_m1
p16	Mm00494449_m1
Sdf1	Mm00445553_m1
MMP-13	Mm00439491_m1
ChoB	Mm00475529_m1
Actin-beta	Mm00607939_s1
beta2M	Mm00437762_m1
18S	4319413E
Hprt1	Mm00446968_m1

Fig 9A : g-H2AX

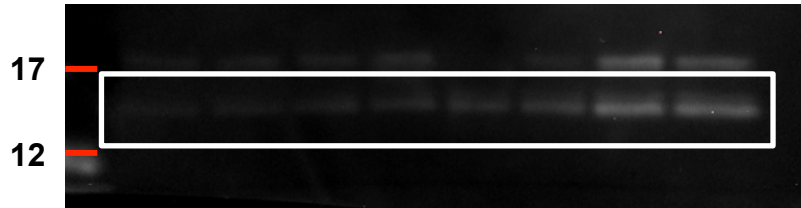


Fig 9A : GATA4

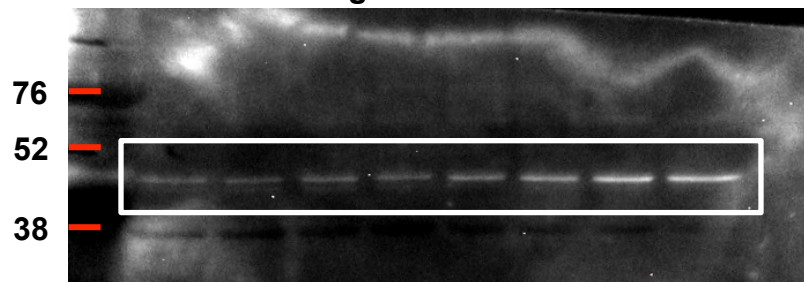


Fig 9A : p62

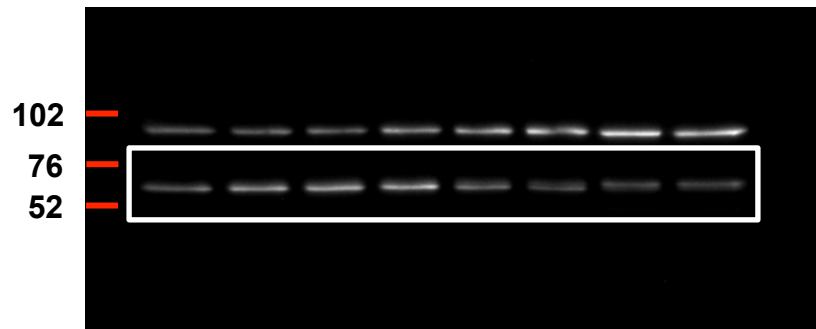


Fig 9A : b-Actin

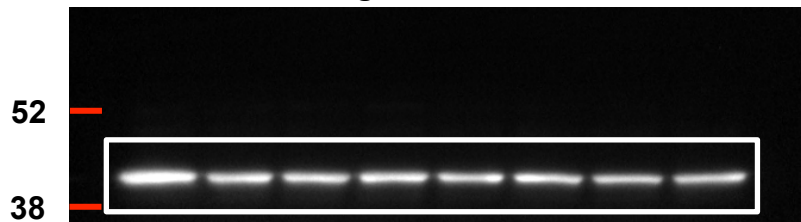


Fig 9D : g-H2AX

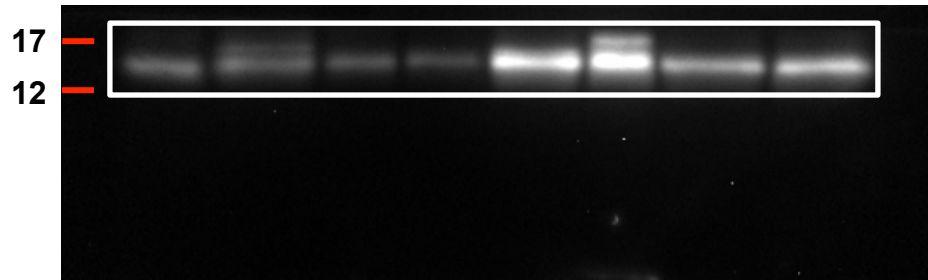


Fig 9D : GATA4

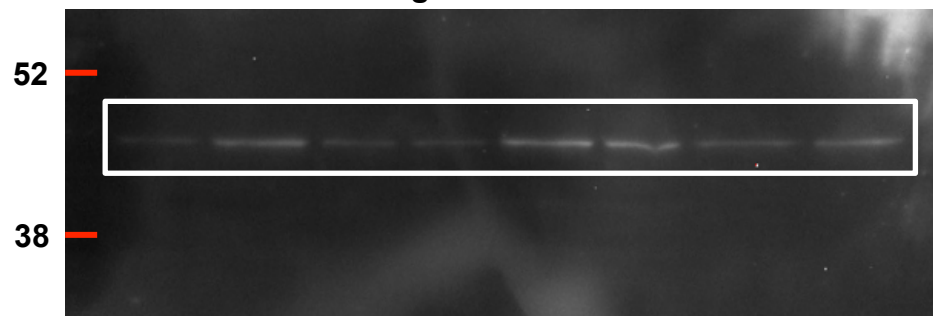


Fig 9D : p62

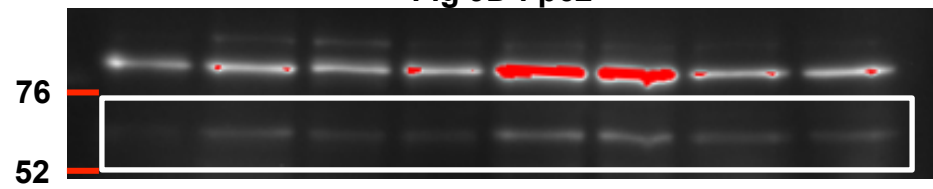


Fig 9D : b-Actin

