Supporting information for PONE-D-22-03926

1) Project tracking for all animals and preparations. This includes names and genotypes of all animals used. Genotyping, as specified in the Methods, was done independently by Jax Labs in a contract arrangement.

A. Original mouse names and breeding information.

| Backcrossed | Mice ID | Gender | Orai1-fl | runx2-cre | Orai1 | Birth Date | Parents M/F | Sacrifice |
|-------------|---------|--------|----------|-----------|-------|------------|-------------|-----------|
| R9          | Rx72A   | F      | mut/mut  | wt/mut    | -/-   | 7/20/16    | Rx61A       | Rx62A     | Rx68A     | Oral1KO   | 1/25/17   |
|             | Rx72D   | F      | mut/mut  | wt/mut    | -/-   | 7/20/16    | Rx61A       | Rx62A     | Rx68A     | Oral1KO   | 1/25/16   |
| R9          | Rx90A   | M      | mut/mut  | wt/mut    | -/-   | 10/25/16   | Rx61A       | Rx87A     | Rx72D     | Oral1KO   | 2/15/16   |
| R9          | Rx90C   | M      | mut/mut  | wt/mut    | -/-   | 10/25/16   | Rx61A       | Rx87A     | Rx72D     | Oral1KO   | 2/15/16   |
| R10         | Rx86B   | F      | mut/mut  | wt/wt     | +/-   | 9/12/16    | Rx69B       | Rx70D     |           |           |           |
| R9          | Rx90A   | M      | mut/mut  | wt/wt     | +/-   | 11/18/16   | Rx82B       | Rx83E     | Rx87B     | calcein injections for sacrifice March 13 |
| R9          | Rx83E   | F      | mut/mut  | wt/wt     | +/-   | 9/12/16    | OC83B       | Rx66B     | Rx66C     | Calcein injections for sacrifice March 22 |
| R9          | Rx87B   | F      | mut/mut  | wt/wt     | +/-   | 9/14/16    | Rx67B       | Rx66A     |           | calcein injections for sacrifice March 22 |
| R9          | Rx73A   | M      | mut/mut  | wt/mut    | -/-   | 8/3/16     | Rx67B       | Rx66A     | Rx68D     | Oral1KO   |           |
| R9          | Rx87A   | F      | mut/mut  | wt/mut    | -/-   | 9/14/16    | Rx67B       | Rx66A     |           | Oral1KO   | calcein injections for sacrifice March 29 |
| R9          | Rx87C   | F      | mut/mut  | wt/mut    | -/-   | 9/14/16    | Rx67B       | Rx66A     |           | Oral1KO   | calcein injections for sacrifice March 29 |
| R9          | Rx87D   | F      | mut/mut  | wt/mut    | -/-   | 9/14/16    | Rx67B       | Rx66A     |           | Oral1KO   | calcein injections for sacrifice March 29 |
| R9          | Rx90B   | M      | mut/mut  | wt/wt     | +/-   | 11/18/16   | Rx82B       | Rx83E     | Rx87B     | Calcein injections for sacrifice March 22 |
| R9          | Rx91D   | F      | mut/mut  | wt/wt     | +/-   | 11/18/16   | Rx82B       | Rx83E     | Rx87B     | Calcein injections for sacrifice March 13 |
| R10         | Rx92A   | F      | mut/mut  | wt/wt     | +/-   | 11/21/16   | Rx85E       | Rx86B     |           | Calcein injections for sacrifice March 15 |
| R10         | Rx92B   | F      | mut/mut  | wt/wt     | +/-   | 11/21/16   | Rx85E       | Rx86B     |           | Calcein injections for sacrifice March 15 |
| R9          | Rx93A   | M      | mut/mut  | wt/mut    | -/-   | 12/10/16   | Rx73A       | Rx72A     | Rx87D     | Oral1KO   | Calcein injections for sacrifice April 10 |
| R9          | Rx93B   | M      | mut/mut  | mut/mut   | -/-   | 12/10/16   | Rx73A       | Rx72A     | Rx87D     | Oral1KO   | Calcein injections for sacrifice April 10 |

House with Rx83E, Rx87B on February 21

calcein injections for sacrifice March 13

Calcein injections for sacrifice March 22

calcein injections for sacrifice March 22

Calcein injections for sacrifice March 29

calcein injections for sacrifice March 29

calcein injections for sacrifice March 29

Calcein injections for sacrifice March 22

calcein injections for sacrifice March 13

calcein injections for sacrifice March 15

calcein injections for sacrifice March 15

Calcein injections for sacrifice April 10

Calcein injections for sacrifice April 10
|   |   |   |   |   |   |
|---|---|---|---|---|---|
| R9 | Rx94A | F | mut/mut | wt/mut | +/- |
|   | Rx94B | F | mut/mut | wt/mut | +/- |
|   | Rx96D | F | mut/mut | wt/mut | +/- |
|   | Rx95A | M | mut/mut | mut/mut | +/- |
|   | Rx95B | M | mut/mut | wt/mut | +/- |
|   | Rx95C | M | mut/mut | wt/mut | +/- |
|   | Rx95D | M | mut/mut | wt/mut | +/- |
|   | Rx95E | M | mut/mut | wt/mut | +/- |
| R9 | Rx97A | M | mut/mut | wt/wt | +/- |
| R9 | Rx97B | M | mut/mut | wt/wt | +/- |
| R9 | Rx97C | M | mut/mut | wt/wt | +/- |
| R9 | Rx97D | M | mut/mut | wt/wt | +/- |
| R9 | Rx97E | M | mut/mut | wt/wt | +/- |
| R9 | Rx98A | M | mut/mut | wt/wt | +/- |
| R9 | Rx98B | M | mut/mut | mut/mut | +/- |
| R9 | Rx98C | M | mut/mut | wt/wt | +/- |
| R9 | Rx98D | M | mut/mut | mut/mut | +/- |
| R9 | Rx98E | M | mut/mut | wt/wt | +/- |
| R9 | Rx99A | F | mut/mut | wt/mut | +/- |
| R9 | Rx99B | F | mut/mut | wt/wt | +/- |
| R9 | Rx100A | M | mut/mut | wt/wt | +/- |
| R9 | Rx100B | M | mut/mut | wt/wt | +/- |
| R9 | Rx100C | M | mut/mut | wt/wt | +/- |
| R9 | Rx100D | M | mut/mut | wt/wt | +/- |
| R9 | Rx101A | F | mut/mut | wt/wt | +/- |
| R9 | Rx101B | F | mut/mut | wt/wt | +/- |
| R9 | Rx101C | F | mut/mut | wt/wt | +/- |
| R9 | Rx102A | M | mut/mut | wt/wt | +/- |

**Orai1KO** calcein injections for sacrifice April 11

**Rx73A** calcein injections for sacrifice April 11

**Rx72A** calcein injections for sacrifice April 12

**Rx87D** calcein injections for sacrifice April 12

**Rx72D** calcein injections for sacrifice April 12

**Rx90C** calcein injections for sacrifice April 13

**Rx90C** calcein injections for sacrifice April 13

**Rx90C** calcein injections for sacrifice April 17

**Rx90C** calcein injections for sacrifice April 17

**Rx90C** calcein injections for sacrifice April 19

**Rx90C** calcein injections for sacrifice April 19

**Rx90C** calcein injections for sacrifice April 19

**Rx90C** calcein injections for sacrifice April 19

**Rx90C** calcein injections for sacrifice May 1

**Rx90C** calcein injections for sacrifice May 1

**Rx90C** calcein injections for sacrifice May 3

**Rx90C** calcein injections for sacrifice May 3

**Rx90C** calcein injections for sacrifice May 4

**Rx90C** calcein injections for sacrifice May 4

**Rx90C** calcein injections for sacrifice May 15

**Rx90C** calcein injections for sacrifice May 15

**Rx90C** calcein injections for sacrifice May 16

**Rx90C** calcein injections for sacrifice May 16

**Rx90C** calcein injections for sacrifice May 17

**Rx90C** calcein injections for sacrifice May 17

**Rx90C** calcein injections for sacrifice May 17

**Rx90C** calcein injections for sacrifice May 31
### B. Mice with genotypes and major assays including micro CT.

| Notes* | Mouse ID | Orai1-fl | runx2-cre | Orai1 | Sex | Birthdate | Date of sac | Age @ sac | # vials BM | # vials non-adh | other | msc |
|---------|----------|----------|-----------|-------|-----|-----------|-------------|-----------|------------|--------------|--------|-----|
| pooled @ Pitt | 14A | wt/wt | wt/mut | +/- | M | 5/29/14 | 7/30/14 | 9 weeks | | | | |
| pooled @ Pitt | 17A | wt/wt | wt/mut | +/- | F | 6/2/14 | 7/30/14 | 9 weeks | | | | |
| pooled @ Pitt | 17C | wt/wt | wt/mut | +/- | F | 6/2/14 | 7/30/14 | 9 weeks | | | | |
| pooled @ Pitt | 14D | mut/mut | wt/mut | +/- | M | 5/29/14 | 7/30/14 | 9 weeks | | | | 
| pooled @ Pitt | 15B | mut/mut | wt/mut | +/- | M | 6/4/14 | 7/30/14 | 9 weeks | | | | 
| pooled @ Pitt | 15C | mut/mut | wt/mut | +/- | M | 6/4/14 | 7/30/14 | 9 weeks | | | |
| pooled @ Pitt | 15C | mut/mut | wt/mut | +/- | M | 6/4/14 | 7/30/14 | 9 weeks | | | |
| pooled @ Pitt | 15C | mut/mut | wt/mut | +/- | M | 6/4/14 | 7/30/14 | 9 weeks | | | |

**6mo WT**

| Mouse ID | Orai1-fl | runx2-cre | Orai1 | Sex | Birthdate | Date of sac | Age @ sac | # vials BM | # vials non-adh | other | msc |
|----------|----------|-----------|-------|-----|-----------|-------------|-----------|------------|--------------|--------|-----|
| Rx 72A | mut/mut | wt/mut | +/- | F | 7/20/16 | 1/25/17 | 27w | 1 | 2 | | |
| Rx 72D | mut/mut | wt/mut | +/- | F | 7/20/16 | 1/25/17 | 27w | 2 | 2 | | |

**Rx 90AKO**

| Mouse ID | Orai1-fl | runx2-cre | Orai1 | Sex | Birthdate | Date of sac | Age @ sac | # vials BM | # vials non-adh | other | msc |
|----------|----------|-----------|-------|-----|-----------|-------------|-----------|------------|--------------|--------|-----|
| Rx 90C | mut/mut | wt/mut | +/- | M | 10/25/16 | 2/15/17 | 16w 1d | 1 | 1 | NA |

**Rx 90AZ**

| Mouse ID | Orai1-fl | runx2-cre | Orai1 | Sex | Birthdate | Date of sac | Age @ sac | # vials BM | # vials non-adh | other | msc |
|----------|----------|-----------|-------|-----|-----------|-------------|-----------|------------|--------------|--------|-----|
| *snow arr. 3/17 | Rx 90AZ | mut/mut | wt/wt | +/- | M | 11/18/16 | 3/13/17 | 16w 3d | 2 | 0 | NA |
| *snow arr. 3/17 | Rx 91D | mut/mut | wt/wt | +/- | F | 11/18/16 | 3/13/17 | 16w 3d | 2 | 0 | NA |

| Mouse ID | Orai1-fl | runx2-cre | Orai1 | Sex | Birthdate | Date of sac | Age @ sac | # vials BM | # vials non-adh | other | msc |
|----------|----------|-----------|-------|-----|-----------|-------------|-----------|------------|--------------|--------|-----|
| Rx 92A | mut/mut | wt/wt | +/- | F | 11/21/16 | 3/15/17 | 16w 2d | 3 | 0 | m-flox-wt | NA |
| Rx 92B | mut/mut | wt/wt | +/- | F | 11/21/16 | 3/15/17 | 16w 2d | 2 or 3 | 0 | m-het flox-cre | |

*snow arr. 3/17
| Rx   | Genotype 1 | Genotype 2 | Sex | Start Date | End Date | Age | Notes  |
|------|------------|------------|-----|------------|----------|-----|--------|
| Rx 90B | mut/mut    | wt/wt      | +/- | 11/18/16   | 3/21/17  | 17w 4d | 2 NA   |
| Rx 83E | mut/mut    | wt/wt      | +/- | 9/12/16    | 3/21/17  | 27w 1d | 5 flox-wt |
| Rx 87B | mut/mut    | wt/wt      | +/- | 9/14/16    | 3/21/17  | 26w 6d | 2 NA   |
| Rx 86B | mut/mut    | wt/wt      | +/- | 9/12/16    | 3/21/17  | 28w 2d | 2 NA   |
| Rx 87A | mut/mut    | wt/mut     | +/- | 9/14/16    | 3/29/17  | 27w 1d | 3 NA   |
| Rx 87C | mut/mut    | wt/mut     | +/- | 9/14/16    | 3/29/17  | 28w   | 3 NA   |
| Rx 87D | mut/mut    | wt/mut     | +/- | 9/14/16    | 3/29/17  | 28w   | 2 NA   |
| Rx 93A | mut/mut    | wt/mut     | +/- | 12/10/16   | 4/10/17  | 17w 2d | 3 m-flox-cre |
| Rx 93B | mut/mut    | mut/mut    | +/- | 12/10/16   | 4/10/17  | 17w 2d | 3 NA   |
| Rx 94A | mut/mut    | wt/mut     | +/- | 12/10/16   | 4/10/17  | 17w 2d | 2 NA   |
| Rx 94B | mut/mut    | wt/mut     | +/- | 12/10/16   | 4/10/17  | 17w 2d | 2 NA   |
| Rx 95A | mut/mut    | mut/mut    | +/- | 12/11/16   | 4/11/17  | 17w 2d | 2 m-flox-cre |
| Rx 95B | mut/mut    | mut/mut    | +/- | 12/11/16   | 4/11/17  | 17w 2d | 3 m-flox-?wt |
| Rx 95C | mut/mut    | mut/mut    | +/- | 12/11/16   | 4/11/17  | 17w 2d | 2 s-?fox-cre |
| Rx 96A | mut/mut    | mut/mut    | +/- | 12/11/16   | 4/11/17  | 17w 2d | 2 NA   |
| Rx 96D | mut/mut    | mut/mut    | +/- | 12/11/16   | 4/12/17  | 17w 1d | 2 s-?fox-cre |
| Rx 97A | mut/mut    | wt/wt      | +/- | 12/28/16   | 4/17/17  | 15w 5d | 2 NA   |
| Rx 97B | mut/mut    | wt/wt      | +/- | 12/28/16   | 4/17/17  | 15w 5d | 2 NA   |
| Rx 97C | mut/mut    | wt/wt      | +/- | 12/28/16   | 4/19/17  | 16w   | 2 NA   |
| Rx 97D | mut/mut    | wt/wt      | +/- | 12/28/16   | 4/19/17  | 16w   | 2 NA   |
| Rx 97E | mut/mut    | wt/wt      | +/- | 12/28/16   | 4/19/17  | 16w   | 2 NA   |
| Rx 98A | mut/mut    | wt/wt      | +/- | 1/10/17    | 5/1/17   | 15w 6d | 2 NA   |

**HCB genotype**

aka 96A

| Rx   | Genotype 1 | Genotype 2 | Sex | Start Date | End Date | Age | Notes  |
|------|------------|------------|-----|------------|----------|-----|--------|
| Rx 95D | mut/mut    | wt/mut     | +/- | 12/11/16   | 4/12/17  | 17w 1d | 2 s-?fox-cre |
| Rx 95E | mut/mut    | wt/mut     | +/- | 12/11/16   | 4/12/17  | 17w 1d | 2 s-?fox-cre |

NA: Not applicable
### Table: Mouse IDs and Genotypes

| MSC | Mouse ID | fox     | cre     | orai | sex | birth date | sac date | age at sac | genotype       | WVU genotype |
|-----|----------|---------|---------|------|-----|------------|----------|------------|----------------|---------------|
| fl-wt | Rx 98B   | mut/mut | mut/mut | +/-  | M   | 1/10/17    | 5/1/17   | 15w 6d     | orai pres      | NA            |
| fl/fl | Rx 98C   | mut/mut | wt/wt   | +/-  | M   | 1/10/17    | 5/1/17   | 15w 6d     | orai pres      | NA            |
| ~fl/wt | Rx 98D  | mut/mut | mut/mut | +/-  | M   | 1/10/17    | 5/3/17   | 16w 1d     | orai pres      | NA            |
| wt    | Rx 98E   | mut/mut | wt/wt   | +/-  | M   | 1/10/17    | 5/3/17   | 16w 1d     | orai pres      | NA            |
| fl/fl | Rx 99A   | mut/mut | wt/wt   | +/-  | M   | 1/10/17    | 5/4/17   | 16w 2d     | orai pres      | NA            |
| fl-wt | Rx 99B   | mut/mut | wt/wt   | +/-  | M   | 1/10/17    | 5/4/17   | 16w 2d     | no orai        | NA            |

C. Derived cell lines, MSC first and then differentiated OB (not used in work shown)

| MSC | Mouse ID | fox     | cre     | orai | sex  | birth date | sac date | age at sac | genotype | WVU genotype |
|-----|----------|---------|---------|------|------|------------|----------|------------|----------|---------------|
| Rx 94A   | mut/mut | wt/mut   | +/-    | F    | 12/10/16 | 4/10/17 | 17w 2d(s-cre?) |          |               |
| Rx 95A   | mut/mut | mut/mut  | +/-    | M    | 12/11/16 | 4/11/17 | 17w 2d m-flox-cre |          |               |
| Rx 95B   | mut/mut | wt/mut   | +/-    | M    | 12/11/16 | 4/11/17 | 17w 2d m-flox-wt |          |               |
| Rx 95C   | mut/mut | wt/mut   | +/-    | M    | 12/11/16 | 4/11/17 | 17w 2d s-flox-? |          |               |
| Rx 95D   | mut/mut | wt/mut   | +/-    | M    | 12/11/16 | 4/12/17 | 17w 1d s-flox-? |          |               |
| Rx 97C   | mut/mut | wt/wt    | +/-    | M    | 12/28/16 | 4/19/17 | 16w |          |               |
| Rx 98C   | mut/mut | wt/wt    | +/-    | M    | 1/10/17  | 5/1/17  | 15w 6d |          |               |
| Rx 99A   | mut/mut | wt/mut   | +/-    | M    | 1/10/17  | 5/4/17  | 16w 2d orai pres |          |               |
| Rx 72A   | mut/mut | wt/mut   | +/-    | F    | 7/20/16  | 1/25/17 | 27w |          | flox-cre        |
| Rx 72D   | mut/mut | wt/mut   | +/-    | F    | 7/20/16  | 1/25/17 | 27w |          | flox-cre        |
| Rx 83E   | mut/mut | wt/wt    | +/-    | F    | 9/12/16  | 3/21/17 | 27w 1d |          | flox-cre        |
| Rx 87B   | mut/mut | wt/wt    | +/-    | F    | 9/14/16  | 3/21/17 | 26w 6d |          |               |

Ob Mouse IDs:

| Mouse ID | fox     | cre     | orai | sex | birth date | sac date | age at sac | genotype       | WVU genotype |
|----------|---------|---------|------|-----|------------|----------|------------|----------------|---------------|
| Rx 90AKO | mut/mut | wt/mut  | +/-  | M   | 10/25/16   | 2/15/17  | 16w 1d     |               | flox-cre       |
| Rx 90AZ  | mut/mut | wt/wt   | +/-  | M   | 11/18/16   | 3/13/17  | 16w 3d     |               | flox-cre       |
| Rx 90B   | mut/mut | wt/wt   | +/-  | M   | 11/18/16   | 3/21/17  | 17w 4d     |               | flox-cre       |
| Rx 95A   | mut/mut | mut/mut | +/-  | M   | 12/11/16   | 4/11/17  | 17w 2d m-flox-cre |               |               |
| Rx 95C   | mut/mut | wt/mut  | +/-  | M   | 12/11/16   | 4/11/17  | 17w 2d s-flox-cre |               |               |
| RX   | Genotype 1 | Genotype 2 | Sex | Start Date | End Date | Age | Notes        |
|------|------------|------------|-----|------------|----------|-----|--------------|
| 98B  | mut/mut    | mut/mut    | M   | 1/10/17    | 5/1/17   | 15w 6d orai pres |
| 98E  | mut/mut    | wt/wt      | M   | 1/10/17    | 5/3/17   | 16w 1d         |
| 99A  | mut/mut    | wt/mut     | F   | 1/10/17    | 5/4/17   | 16w 2d orai pres |
| 72D  | mut/mut    | wt/mut     | F   | 7/20/16    | 1/25/17  | 27w     | fox-cre      |
| 87B  | mut/mut    | wt/wt      | F   | 9/14/16    | 3/21/17  | 26w 6d         |
| 87C  | mut/mut    | wt/mut     | F   | 9/14/16    | 3/29/17  | 28w     | fox-cre      |
2) Original data plotted in Fig 2 quantification of micro CT images.

| Group | ID    | Measurements | AVE | SD  | Group | ID    | Measurements | Ribs | AVE | SD  |
|--------|-------|--------------|-----|-----|-------|-------|--------------|------|-----|-----|
| Gr1    | 90B (M) (17w) | 154          |     |     | Gr1   | 90B (M) (17w) | 94.3 | A   |     |     |
|        | 154   | 154          |     |     |       | 90.38 | A   |     |     |     |
|        | 154   | 148          |     |     |       | 95.9  | A   |     |     |     |
|        | 166   | 166          |     |     |       | 93.93 | B   |     |     |     |
|        | 166   | 160          |     |     |       | 94.5  | B   |     |     |     |
|        |       |              |     |     |       | 96.01 | B   |     |     |     |
|        |       |              |     |     |       | 94.81 |     |     |     |     |
| Gr2    | 95D (M) (17w) | 178          |     |     | Gr2   | 95D (M) (17w) | 73.5 | A   |     |     |
|        | 190   | 178          |     |     |       | 71.94 | A   |     |     |     |
|        | 148   | 172          |     |     |       | 73.95 | A   |     |     |     |
|        | 148   | 172          |     |     |       | 74    | B   |     |     |     |
|        | 172   | 166          |     |     |       | 72.53 | B   |     |     |     |
|        | 178   | 166          |     |     |       | 73.65 | B   |     |     |     |
| 98D (M) (16w) | 160 | 98D (M) (16w) | 84.13 | C |
|---------------|-----|---------------|-------|---|
| 166           |     | 80.4          | C     | 81.04 |
| 160           |     | 78.59         | C     |     |
| 178           |     | 87.34         | D     |     |
| 178           | 162 | 88.15         | D     |     |
| 172           | 176 | 88.42         | D     | 87.97 |
| 99A (F) (16w) | 184 | 99A (F) (16w) | 87.26 | E |
| 172           |     | 89.15         | E     |     |
| 172           | 176 | 89.4          | E     | 88.60 |
| 148           |     | 90.22         | F     |     |
| 148           |     | 90.89         | F     |     |
| 142           | 146 | 90.68         | F     | 90.60 |
3) Original uncropped and unprocessed western blots from Fig 3. As per Plos instructions

Figure 4D top panel Western Blot

Method used: Proteins were separated on a 4-12% gradient bis-tris gel and transferred to polyvinylidene difluoride (PVDF) membranes. Target protein was detected by enhanced chemiluminescence on autoradiography film. Primary antibody rabbit anti-Orai1 antibody Alomone Labs (ACC-062, Jerusalem, Israel) (1:200). Secondary antibody horseradish peroxidase conjugated anti-rabbit (1:40,000, Jackson ImmunoResearch)

The position of the standards (colored, not labeled by antibody) is traced from the original pdf membrane. Selected standards shown were identified by color.

Loading order and sample identity are labeled on the blot image
Actin Blot per Plos instructions

Figure 4D bottom panel Western blot

Method used: Proteins were separated on a 4-12% gradient bis-tris gel and transferred to polyvinylidene difluoride (PVDF) membranes. Target protein was detected by enhanced chemiluminescence on autoradiography film.

Mouse monoclonal beta-actin antibody A2228 (1:1,000, Sigma), with secondary horse radish peroxidase conjugated anti-mouse (1:40,000) was used. The actin re-blot was done three days later after removing the antibody with Restore Western Blot stripping solution (Fischer).

The position of the standards (colored, not labeled by antibody) is traced from the original pdf membrane

Selected standards shown were identified by color

Loading order and sample identity are labeled on the blot image
4) Original data for all micro CT measurements shown in Fig 3.

| Bone Volume/Total Volume (BV/TV) | Trabecular Thickness (µm; Tb.Th) | Trabecular Number (mm-1; Tb.N) | Trabecular spacing (Tb.Sp) |
|---------------------------------|---------------------------------|--------------------------------|-----------------------------|
| Wild Type                       | Orai1 cKO                       | Wild Type                       | Orai1 cKO                   | Wild Type                       | Orai1 cKO                   | Wild Type                       | Orai1 cKO                   |
| 25.99                           | 23.86                           | 50.26                           | 49.79                       | 0.0052                         | 0.0048                         | 143.17                         | 158.97                       |
| 25.61                           | 13.98                           | 49.80                           | 43.19                       | 0.0051                         | 0.0032                         | 144.75                         | 265.45                       |
| 25.53                           | 22.96                           | 50.16                           | 50.00                       | 0.0051                         | 0.0046                         | 146.31                         | 167.87                       |
| 23.80                           | 13.49                           | 49.00                           | 42.44                       | 0.0049                         | 0.0032                         | 156.76                         | 272.02                       |
| 25.37                           | 19.98                           | 51.16                           | 46.62                       | 0.0050                         | 0.0043                         | 150.46                         | 186.48                       |
| 23.69                           | 19.53                           | 49.23                           | 46.05                       | 0.0048                         | 0.0042                         | 158.67                         | 189.80                       |
| 21.44                           | 21.71                           | 50.29                           | 46.49                       | 0.0043                         | 0.0047                         | 184.45                         | 167.65                       |

ave | ave | ave | n=7 | sd | sd | sd | n=7 |
24.50 | 19.36 | 49.98 | 46.37 | 0.72 | 2.90 | 0.0077 | graphpad |
1.62 | 4.13 | 24.98 | 19.36 | 0.72 | 2.90 | 0.0077 | graphpad |

p= 0.0099
graphpad

| Fraction Surface Labeled | Bone Formation (mm3/d; BFR) |
|--------------------------|-----------------------------|
| Wild Type                | Orai1 cKO                   | Wild Type                | Orai1 cKO                   |
| 0.39                     | 0.30                        | 10.29                    | 5.93                        |
| 0.34                     | 0.30                        | 8.96                     | 5.89                        |
| 0.30                     | 0.32                        | 7.82                     | 6.29                        |
| 0.35                     | 0.41                        | 9.25                     | 8.01                        |

ave | ave | n=4 | n=4 | sd | sd |
0.35 | 0.33 | 9.08 | 6.53 | 0.02 | 1.00 |
p= NS | p= 0.0118 | graphpad | graphpad |
5) Original data for PCR measurements in Fig 5, beginning with list of all primers used in [12] and [16] and this manuscript

**Table 1. PCR primers (Mouse)**

| Gene   | Primers                                                                 | Product size | Reference |
|--------|-------------------------------------------------------------------------|--------------|-----------|
| ALP    | F - 5'-ATCGGAACAACCTGACTGACCTTTT-3' R - 5'-ACCTCATGATGCTGGTTCAAAT-3'     | 131 bp       | [12]      |
| Col1a1 | F - 5'-TCTCTCTGGCAAGAGACGACGTTA-3' R - 5'-AGGAAGCTGAGTCATAACCGCCA-3'     | 159 bp       | [16]      |
| Osx    | F - 5'-GATTGGCGGTCTCTGCTGTT-3' R - 5'-ACCCTCATGATGTCCGTGGTCAAT-3'         | 146 bp       |           |
| RANKL  | F - 5'-GCTCCGAGCTGTTGACATA-3' R - 5'-GATGGCTTGGCTCAGTCT-3'               | 83 bp        |           |
| LysM   | F - 5'-ATGATGCAACTGCACTGCTAC-3' R - 5'-ACTGAGCTGGTGTGGTGAAGA-3'           | 105 bp       |           |
| Atp1a2 | (old name ATPa3) F - 5'-TGACCAGAAGGCTGTGAGCT-3' R - 5'-AGAAGCTGAGACACCT-3'| 163 bp       |           |
| ATP6v0d2| F - 5'-CCAAGGCTTCACATATGGAGA-3' R - 5'-TCACCGTGATCCTTGCAGAAAT-3'         | 192 bp       |           |
| Cathepsin-K | F - 5'-CAACAGAGGATGGTGTACTATG-3' R - 5'-CGTCCTGCTTATTCGAGC-3'             | 174 bp       |           |
| TRAP   | F - 5'-CACGGAGAAGGCTGCTGCTGTC-3' R - 5'-AGTTGGTGTGGCAGTACCTTCCA-3'       | 174 bp       |           |
| PPARBEI| F - 5'-CGCTGATGCACTGCTATGAGA-3' R - 5'-AGAAGCTGAGACACCT-3'               | 100 bp       |           |
| Osteocalcin | F - 5'-ACCCTTTTTGCTCAGTCTGT-3' R - 5'-TTTCCAGTCCTTGGAGCATG-3'             | 118 bp       | [16]      |
| Actin  | F - 5'-GAGATATCGACTGCCTGCTGCTG-3' R - 5'-ACCAGGACGCTTATGAGAAGG-3'        | 275 bp       |           |
| GAPDH  | F - 5'-GTGGTCTTCTGACTGGTTC-3' R - 5'-GAGGTTGCTGACATGAGG-3'                | 184 bp       |           |
| Orai1  | F - 5'-TACTTAAGCCCGCCAAGCTGATAGA-3' R - 5'-GCAGGTGCTAGTCTGTGTTG-3'       | 192 bp       |           |
| OPG    | F - 5'-TACCTTAAAGCCGCGCAACTGCTGTTGAGG-3' R - 5'-GCAGGTGCTGACATGAGG-3'    | 211 bp       |           |
| ATF4   | (CREB2) F - 5'-CCTGAACAGGAGTGGTCTGGTTGACTGAGG-3' R - 5'-ACTGAGCTGAG-3'   | 134 bp       |           |
| RunX2  | F - 5'-ATGATGACACTGCACTGCGCTTCTGAG-3' R - 5'-ACTGAGCTGAG-3'              | 105 bp       |           |
|       | GAPDH | Orai1 | OCN | ALP | RUNX2 | Col1 A1 |
|-------|-------|-------|-----|-----|-------|---------|
| cKO   |       |       |     |     |       |         |
| 5A-mut/mut,M | 17.54 | 0.687770909 | 29.72 | 0.000215504 |       |         |
| 5A-mut/mut,M | 17.58 | 0.668963777 | 29.82 | 0.000206725 |       |         |
| 95D-wt/mut,M | 18.33 | 0.397768242 | 28.03 | 0.001202289 |       |         |
| 95D-wt/mut,M | 18.3 | 0.406126198 | 27.77 | 0.001410087 | 26.72 | 0.001153313 |
| 72A-wt/mut,F | 19.42 | 0.186856156 | 29.41 | 0.000983355 | 26.79 | 0.001145347 |
| 72A-wt/mut,F | 19.4 | 0.189464571 | 29.6 | 0.000850147 | 27.83 | 0.00085606 |
| 72D-wt/mut,F | 19.62 | 0.162667332 | 28.69 | 0.001860621 | 27.86 | 0.000804288 |
| 72D-wt/mut,F | 19.61 | 0.163799175 | 28.72 | 0.001809742 | 22.61 | 0.01897195 |
| 95A-CRE | 16.96 | 1.028113827 | 26.72 | 0.001153313 | 22.58 | 0.01991501 |
| 95A-CRE | 17.02 | 0.986232704 | 26.79 | 0.001145347 | 24.13 | 0.003670011 |
| 95C-CRE | 17.64 | 0.641712949 | 27.83 | 0.00085606 | 28.84 | 0.000372622 |
| 95C-CRE | 17.58 | 0.668963777 | 27.86 | 0.000804288 | 28.74 | 0.000410594 |
| 95D-CRE | 16.89 | 1.079228237 | 22.61 | 0.01897195 | 22.4 | 0.02005353 |
| 95D-CRE | 16.93 | 1.049716684 | 22.58 | 0.01991501 | 22.5 | 0.01991501 |
| WT    |       |       |     |     |       |         |
| 97C   | 16.32 | 1.602139755 | 22.8 | 0.011202775 |       |         |
| 83E   | 16.71 | 1.172834949 | 22.46 | 0.019370433 | 21.24 | 0.03794359 |
| 83E-WT| 16.72 | 1.214194884 | 22.44 | 0.01897195 | 21.18 | 0.040666933 |
| 83E-WT| 16.52 | 1.394743666 | 21.24 | 0.03794359 | 19.44 | 0.211686328 |
| 83E-WT| 16.56 | 1.356604327 | 21.18 | 0.040666933 | 19.41 | 0.207329886 |
| 98B-WT| 17.2 | 0.870550563 | 19.44 | 0.211686328 | 18.1 | 0.757858283 |
| 98B-WT| 17.14 | 0.907519155 | 19.41 | 0.207329886 | 18.06 | 0.768437591 |
| 97C-WT| 17.7 | 0.615572207 | 18.1 | 0.757858283 | 20.57 | 0.038207509 |
| 97C-WT| 17.68 | 0.624165274 | 18.06 | 0.768437591 | 20.6 | 0.038207509 |

n=14

| Av  | sd    | p     | p     |
|-----|-------|-------|-------|
| 0.88517087 | 0.19899326 | 0.003670306 | 0.006701963 | 0.009372225 | 0.20713557 | 0.194808938 |
| 0.003670306 | 0.006701963 | 0.009372225 | 0.20713557 | 0.000410594 | 0.020713557 | 0.209139422 |

n=10

| Av  | sd    | p     | p     |
|-----|-------|-------|-------|
| 0.874882305 | 0.343060924 | 0.208428878 | 0.038207509 | 0.389769823 | 0.045562933 | 0.369380436 |

0.0119209207

Note two outliers significant without these.
| Replic | Dye   | Threshold | Primers | cDNA   | Ct (dR) | Proportion |
|--------|-------|-----------|---------|--------|---------|------------|
| 1      | SYBR  | 51.519    | GAPDH   | 97C-WT | 16.57   |             |
| 2      | SYBR  | 51.519    | GAPDH   | 97C-WT | 16.65   |             |
| 3      | SYBR  | 51.519    | GAPDH   | 83E-WT | 15.36   |             |
| 4      | SYBR  | 51.519    | GAPDH   | 83E-WT | 15.52   |             |
| 5      | SYBR  | 51.519    | GAPDH   | 95D-KO | 16.91   |             |
| 6      | SYBR  | 51.519    | GAPDH   | 95D-KO | 17.12   |             |
| 7      | SYBR  | 51.519    | GAPDH   | 95C-KO | 18.25   |             |
| 8      | SYBR  | 51.519    | GAPDH   | 95C-KO | 18.52   |             |
| 9      | SYBR  | 51.519    | Osterix | 97C-WT | 20.8    | 0.0533     |
| 10     | SYBR  | 51.519    | Osterix | 97C-WT | 20.76   | 0.0579     |
| 11     | SYBR  | 51.519    | Osterix | 83E-WT | 18.76   | 0.0947     |
| 12     | SYBR  | 51.519    | Osterix | 83E-WT | 18.63   | 0.1158     |
| 13     | SYBR  | 51.519    | Osterix | 95D-KO | 21.99   | 0.0296     |
| 14     | SYBR  | 51.519    | Osterix | 95D-KO | 22.07   | 0.0324     |
| 15     | SYBR  | 51.519    | Osterix | 95C-KO | 25.04   | 0.0090     |
| 16     | SYBR  | 51.519    | Osterix | 95C-KO | 25.01   | 0.0111     |
| 17     | SYBR  | 51.519    | ATF-4   | 97C-WT | 18.73   | 0.2238     |
| 18     | SYBR  | 51.519    | ATF-4   | 97C-WT | 18.83   | 0.2207     |
| 19     | SYBR  | 51.519    | ATF-4   | 83E-WT | 18.01   | 0.1593     |
| 20     | SYBR  | 51.519    | ATF-4   | 83E-WT | 17.95   | 0.1856     |
| 21     | SYBR  | 51.519    | ATF-4   | 95D-KO | 19.81   | 0.1340     |
| 22     | SYBR  | 51.519    | ATF-4   | 95D-KO | 20.03   | 0.1330     |
| 23     | SYBR  | 51.519    | ATF-4   | 95C-KO | 20.93   | 0.1560     |
| 24     | SYBR  | 51.519    | ATF-4   | 95C-KO | 20.98   | 0.1817     |
| 25     | SYBR  | 51.519    | ATF-4   | 97C-WT | 18.28   | 0.3057     |
| 26     | SYBR  | 51.519    | ATF-4   | 97C-WT | 18.45   | 0.2872     |
| 27     | SYBR  | 51.519    | ATF-4   | 83E-WT | 17.4    | 0.2432     |
| 28     | SYBR  | 51.519    | ATF-4   | 83E-WT | 17.35   | 0.2813     |
| 29     | SYBR  | 51.519    | ATF-4   | 95D-KO | 19.73   | 0.1416     |
| 30     | SYBR  | 51.519    | ATF-4   | 95D-KO | 19.32   | 0.2176     |
| 31     | SYBR  | 51.519    | ATF-4   | 95C-KO | 20.23   | 0.2535     |
| 32     | SYBR  | 51.519    | ATF-4   | 95C-KO | 20.36   | 0.2793     |
| 33     | SYBR  | 51.519    | H2O     | H2O    | No Ct   |             |
| 34     | SYBR  | 51.519    | H2O     | H2O    | No Ct   |             |
### ATF PCR data

| WT     | KO  |
|--------|-----|
| 0.0533 | 0.0296 |
| 0.0579 | 0.0324 |
| 0.0947 | 0.009 |
| 0.1158 | 0.0111 |

| WT     | KO  |
|--------|-----|
| 0.2238 | 0.134 |
| 0.2207 | 0.133 |
| 0.1593 | 0.156 |
| 0.1856 | 0.1817 |
## Quantitative data for Fig 6

| Stain | wt | ko | wt | ko |
|-------|----|----|----|----|
| note -- all are inverses |
| **VK** | direct | | | |
| 1 | 146 | 207 | 110.00 | 49.00 |
| 2 | 145 | 206 | 111.00 | 50.00 |
| 3 | 109 | 209 | 147.00 | 47.00 |
| 4 | 120 | 211 | 136.00 | 45.00 |
| mean | | | 126.00 | 47.75 |
| sd | | | 18.46 | 2.22 |

| **Alk phos** | | | |
| 1 | 109 | 183 | 147.00 | 73.00 |
| 2 | 114 | 186 | 142.00 | 70.00 |
| 3 | 133 | 181 | 123.00 | 75.00 |
| 4 | 132 | 181 | 124.00 | 75.00 |
| mean | | | 134.00 | 73.25 |
| sd | | | 12.30 | 2.36 |

| **ORO** | | | |
| 1 | 185 | 183 | 71.00 | 73.00 |
| 2 | 185 | 183 | 71.00 | 73.00 |
| 3 | 199 | 206 | 57.00 | 50.00 |
| 4 | 208 | 201 | 48.00 | 55.00 |
| mean | | | 61.75 | 62.75 |
| sd | | | 11.30 | 12.01 |