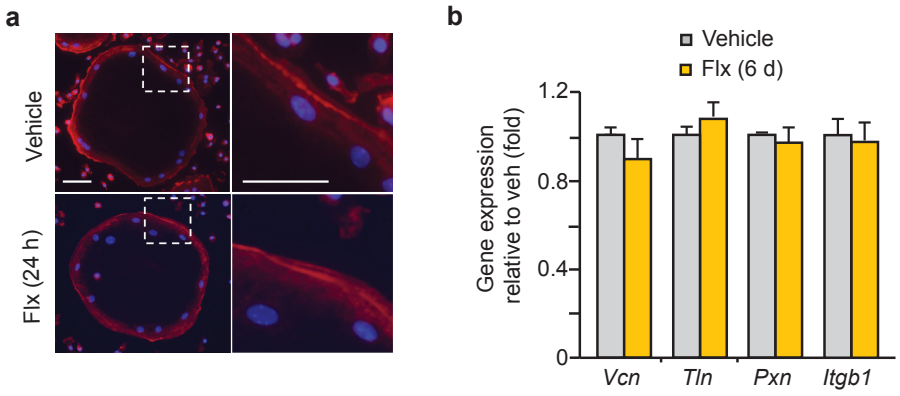
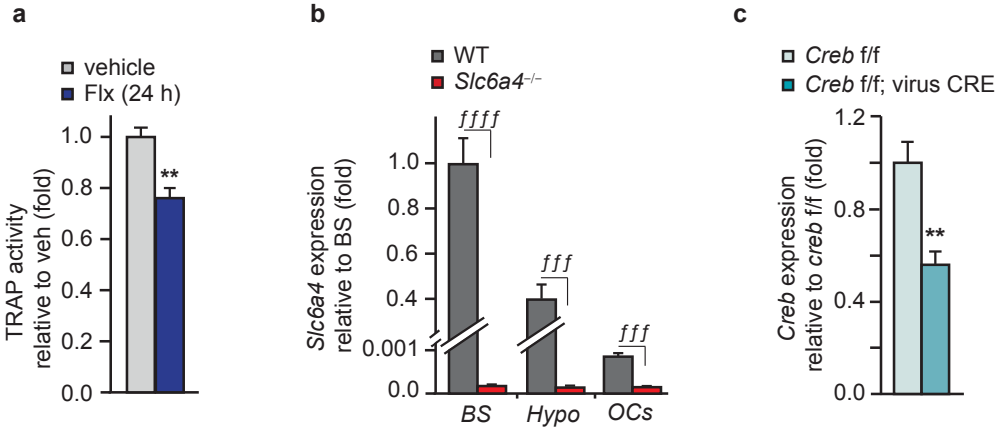
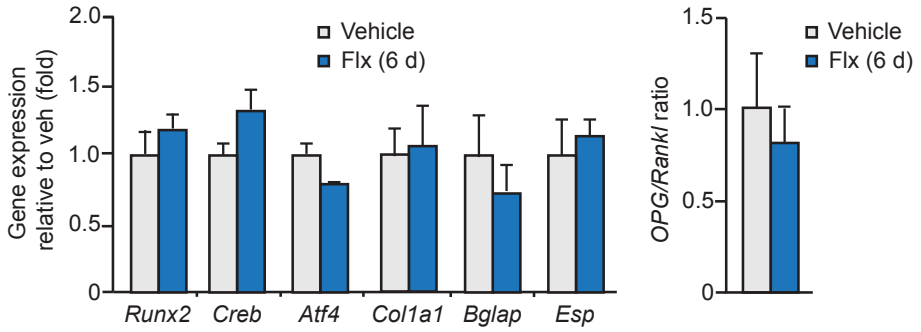


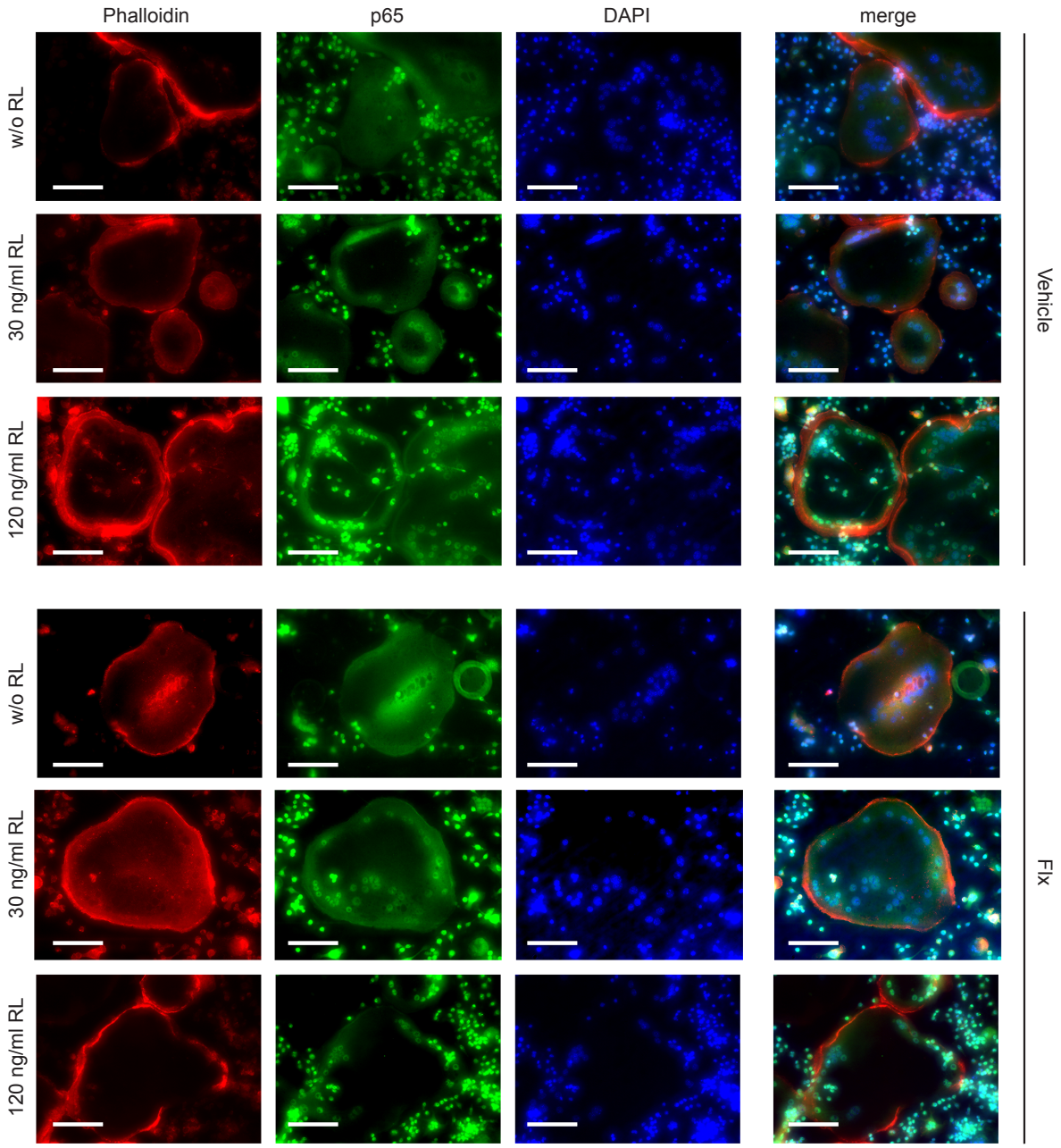
Supplementary Figure 1. Flx does not affect actin ring formation in primary osteoclast cultures (OCs). OCs treated for 6 days with vehicle or Flx. **(a)** Staining of actin-ring with phalloidin-rodamine (red) and nuclei with DAPI (blue). Left panels show magnification of the boxed areas (*n* = 10 images per condition). Scale bar: 100 μm. **(b)** Gene expression of markers of osteoclast actin ring formation (*n* = 6). *Vcn, Vinculin. Tln, Talin. Pxn, Paxillin. Itgb1, Integrin β1.*



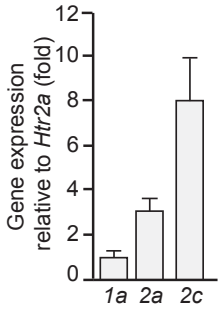
Supplementary Figure 2. Flx affects osteoclastogenesis in a 5HTT- independent manner**.** **(a)** TRAP activity in primary osteoclast cultures derived from *Tph1*$^{-/-}$ mice (*n* = 4). **(b)** *Slc6a4* expression in brain and primary osteoclast cultures (OCs) of *Slc6a4*$^{-/-}$ samples compared to wt (BS wt *n* = 7, *Slc6a4*$^{-/-}$ *n* = 11; Hypo *n* = 9; OCs: *n* = 9). BS, brainstem. Hypo, hypothalamus. OCs, primary osteoclasts. *Slc6a4*, gene encoding 5HTT. **(c)** CRE recombination of primary osteoclast cultures derived from *Creb* $^{fl/fl}$ mice measured as *Creb* expression. (empty *n* = 8, CRE *n* = 6). Values are mean ± SEM. To veh (*) or wt (^{f}) *$P \leq 0.05$, **$P \leq 0.01$, ***/$^{fff}P \leq 0.001$ using Student's test.



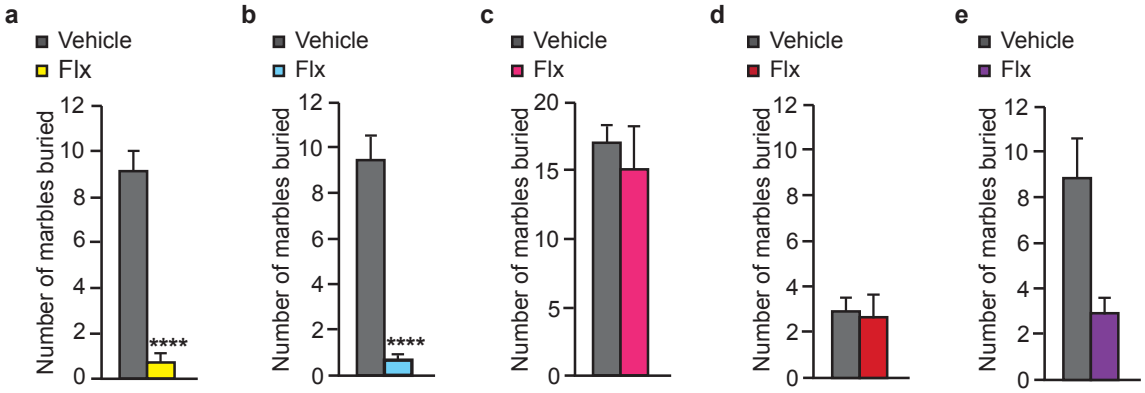
Supplementary Figure 3. Flx does not affect differentiation or function of primary osteoblast cultures. Gene expression (*n* = 9). *Col1a1, Type 1 Collagen alpha 1. Ocn, Osteocalcin.* The *OPG/Rankl* ratio, an indicator of osteoblast-driven osteoclast differentiation, is shown on the right. Values are mean ± SEM.



Supplementary Figure 4. Brain serotonin-dependent increase in RANKL can rescue the impairment in osteoclastogenesis caused by Flx. NFκB p65 subcellular localization analysis by immunocytochemistry in osteoclast-like RAW264.7 cultures. Staining with AF-548 phalloidine (red), NFκB p65 (green) and nuclei with DAPI (blue) (*n* = 10 images per condition). RL, RANKL. Scale bar: 100 μm.



Supplementary Figure 5. Gene expression of *Htr21a*, *2a* and *2c* hypothalamic serotonin receptors in neuron-like Neuro2A cell line (*n* = 5). Values are mean ± SEM.



Supplementary Figure 6. Marble burying behavioral test. **(a, b)** WT females treated for 3 weeks **(a)** or 6 weeks **(b)** (*n* = 13). **(c)** *Tph2*$^{-/-}$ females treated for 6 weeks (*n* = 5). **(d)** *Slc6a4*$^{-/-}$ females treated for 3 weeks (*n* = 7). **(e)** WT males treated for 6 weeks (*n* = 10). Values are mean ± SEM. To veh ****$P \leq 0.0001$ using Student's test.