Figure S1. RAS activation increases concentration of secreted exosomes in human breast cancer cells. (A) MCF-7 cells were transfected with the control vector (Con) or K-RASV12 vector. The expression levels of Ras and p-ERK1/2 was determined using western blot analysis. (B) T47D cells were transfected with the control vector (Con), K-RASV12, H-RASV12, or N-RASV12 vector. The expression levels of Ras and p-ERK1/2 was determined using western blot analysis. (C) T47D cells were transfected with the control vector (Con), HER2 vector. The expression level of HER2 was determined using western blot analysis. (D) Nanoparticle tracking analysis of exosomes derived from MCF-7 cells transfected with the control vector (Con), K-RASV12, H-RASV12, or N-RASV12 vector. (E) Nanoparticle tracking analysis of exosomes derived from T47D cells transfected with the control, K-RASV12, H-RASV12, or N-RASV12 vector. (F) Nanoparticle tracking analysis of exosomes derived from T47D cells transfected with the control or HER2 vector. (G) Nanoparticle tracking analysis of exosomes derived from MDA-MB-231 cells treated with the control or salirasib (10 μM). HER2, human epidermal growth factor receptor 2.

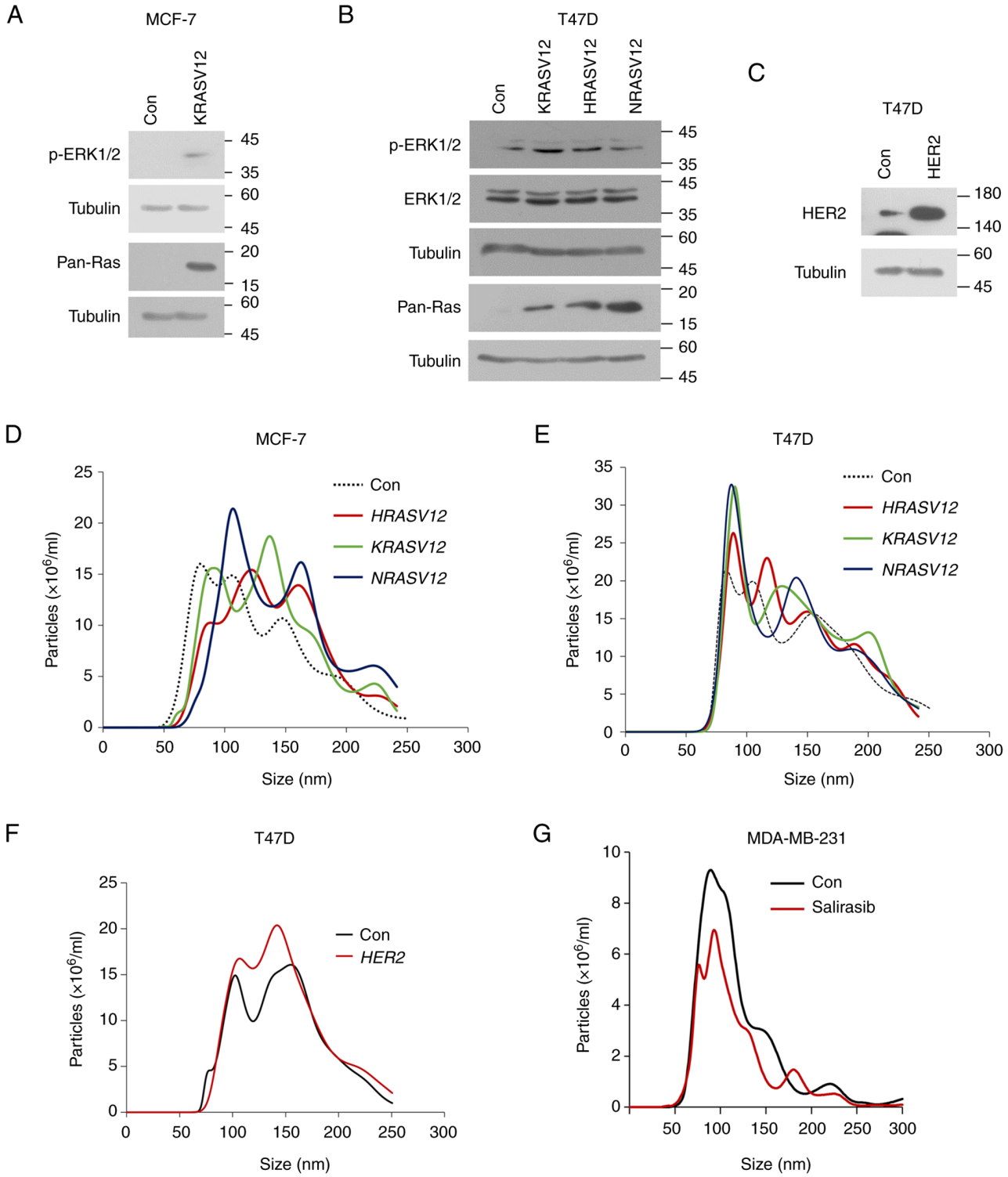

Figure S2. Identification of K-RASV12-dependent miRNAs in exosomes. (A) Heatmap of the differentially expressed miRNAs (>2-fold) in exosomes derived from MCF-7 cells transfected with the control (MCF-7/Con) or KRASV12 vector (MCF-7/KRASV12). (B) Scatter plot of miRNA expression by miRNA microarray between MCF-7/Con and MCF-7/KRASV12. (C) The number of miRNAs significantly upregulated and downregulated between MCF-7/Con and MCF-7/KRASV12 (>2-fold). (D) BMMs were transfected with the indicated miRNA mimics (20 nM each). The expression level of each miRNA was determined using reverse transcription-quantitative PCR (n=3). NC, miRNA mimic negative control. The results were normalized to U6 snRNA. *P<0.05, **P<0.01 and ***P<0.001. (E) C2C12 cells transfected with the indicated miRNA mimics (20 nM each) were incubated with BMP2 (30 ng/ml) for 3 days. The ALP activity of C2C12 cells was determined (n=3). NC, miRNA mimic negative control. **P<0.01. BMMs, bone marrow-derived macrophages; BMP2, bone morphogenetic protein 2; ALP, alkaline phosphatase.

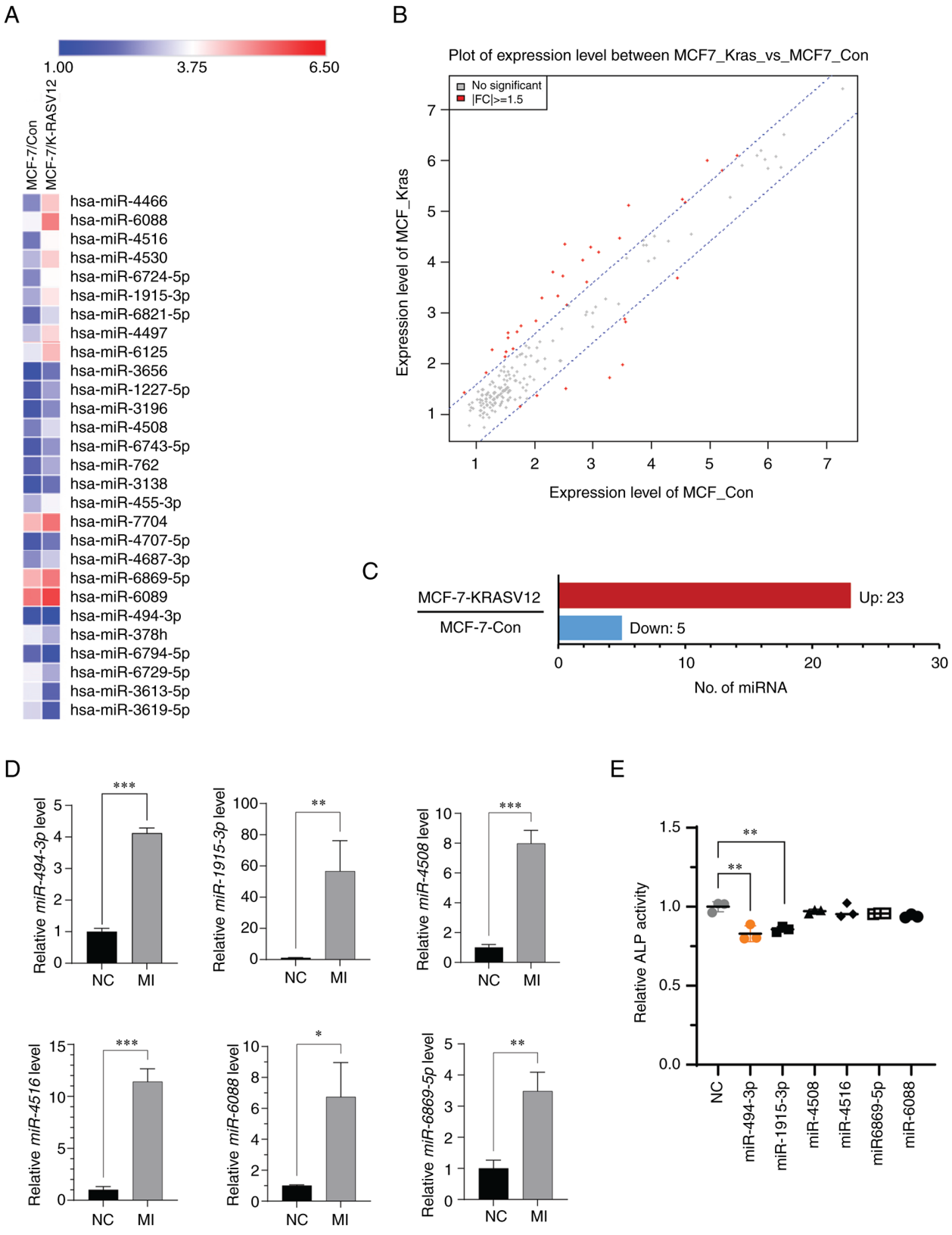

Figure S3. Effects of miR-494-3p inhibitor on RANKL-induced NF-κB activation. (A) RAW264.7 cells were transfected with miRNA mimic negative control (NC1), miRNA inhibitor negative control (NC2), miR-494-3p inhibitor (IN), or miR-494-3p mimic (MI). The expression level of miR-494-3p in BMMs was determined using reverse transcription-quantitative PCR (n=3). The results were normalized to U6 snRNA. *P<0.05. (B) RAW264.7 cells were incubated with RANKL (100 ng/ml) for the the indicated period and the expression level of IκBα was determined using western blot analysis (upper panel). RAW264.7 cells were transfected with miRNA inhibitor negative control (NC, 100 nM) and miR-494-3p inhibitor (IN,100 nM). After 48 h, the cells were incubated with RANKL (100 ng/ml) for 10 min and the expression level of IκBα was determined using western blot analysis (lower panel). (C) RAW264.7 cells transfected with miRNA inhibitor negative control (NC) or miR-494-3p inhibitor (IN, 100 nM) were stimulated with RANKL and M-CSF for 60 min. The cells were stained with an anti-RelA/p65 antibody. DAPI was used to stain the nuclei. RANKL, receptor activator of nuclear factor-κB ligand; M-CSF, macrophage colony-stimulating factor; BMMs, bone marrow-derived macrophages; LGR4, leucine-rich repeat-containing G-protein coupled receptor 4. (D) RAW264.7 cells were transfected with control siRNA or *LGR4* siRNA. The expression levels of LGR4 was determined using western blot analysis.

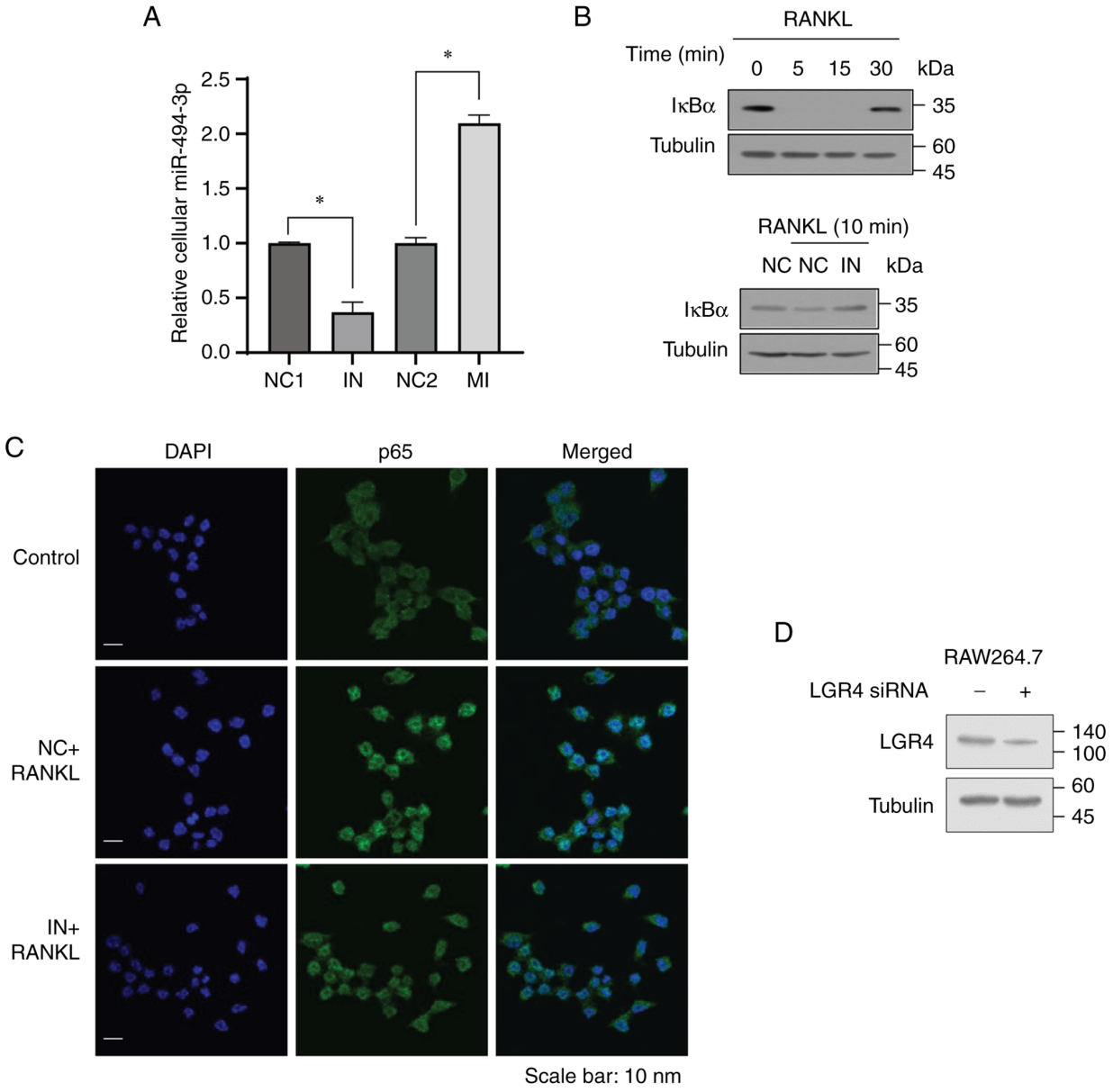

Figure S4. Effect of *miR-494-3p* mimic or *miR-494-3p* inhibitor on miR-494-3p expression in C2C12 cells. (A) C2C12 cells were transfected with miRNA mimic negative control (NC) or miR-494-3p mimic (MI). The expression level of *miR-494-3p* in C2C12 cells was determined using reverse transcription-quantiative PCR (n=3). The results were normalized to U6 snRNA. $^{**}P<0.01$. (B) C2C12 cells were transfected with miRNA inhibitor negative control (NC) or miR-494-3p inhibitor (IN). The expression level of *miR-494-3p* in C2C12 cells was determined using reverse transcription-quantiative PCR (n=3). The results were normalized to U6 snRNA. $^{**}P<0.01$.