

Supplementary Information

Rapid degradation of progressive ankylosis protein (ANKH) in craniometaphyseal dysplasia

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Supplementary Figures

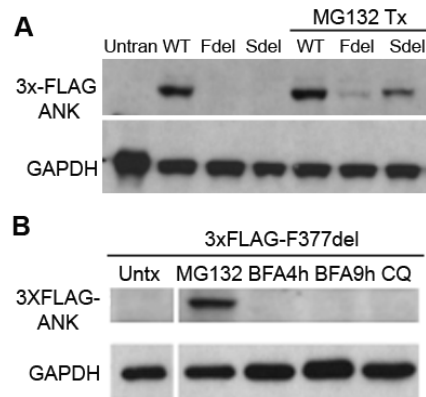


Figure S1. MG132 restores the expression of exogenous CMD mutant ANK. Immunoblotting with FLAG antibody shows that **A)** MG132 rescued both 3xFLAG-tagged F377del and S375del ANK in ROS cells; **B)** 3xFLAG-tagged F377del ANK was mostly rescued by MG132 but not lysosomal inhibitors, BFA (100 ng/ml) for 4 or 9 hours and CQ (50 μ M for 5 hours) in transfected ROS cells. GAPDH as loading control.

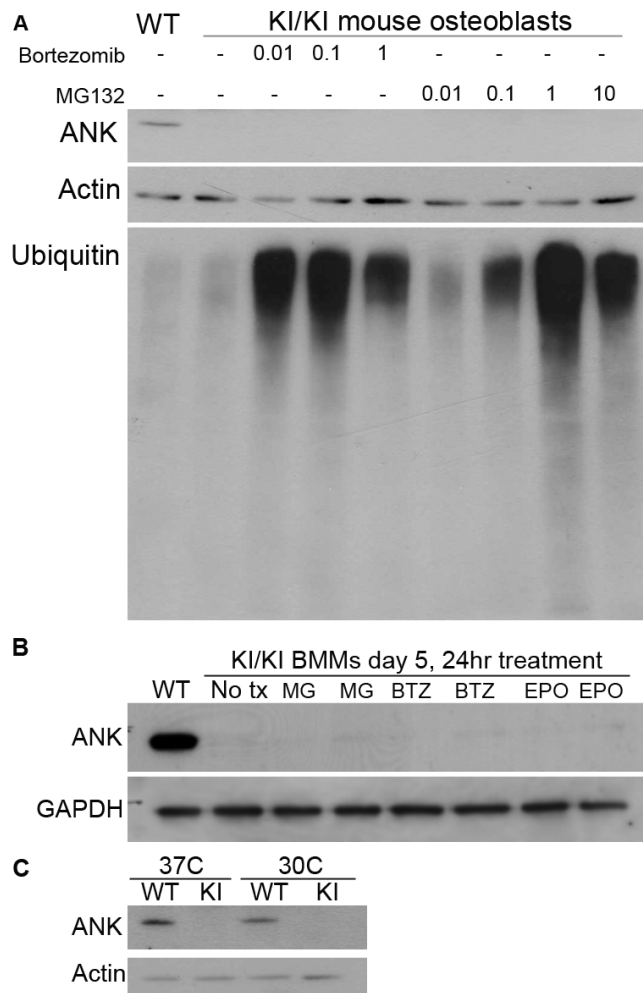


Figure S2. Inhibitors of proteasomal degradation have little effect on recovering the expression of endogenous mutant ANK: no or mild rescue of F377del ANK protein **A)** in *Ank*^{KI/KI} mouse calvarial osteoblasts incubated with increasing concentration of bortezomib or MG132. Total cell lysates were analyzed by immunoblotting for ANK (upper blot). Anti-ubiquitin blotting was used to show blockade of the 26S proteasome, with increasing amount of poly-ubiquitin-tagged proteins observed in bortezomib and MG132 treatment lanes. Actin as loading control. Inhibitor concentration in μ M; **B)** in *Ank*^{KI/KI} mouse BMM-derived osteoclasts treated with MG132, bortezomib (BTZ) or epoxomicin (EPO). Concentrations of inhibitors were MG132 10 nM (3rd lane) and 30 nM (4th lane), BTZ 1 nM (5th lane) and 5 nM (6th lane) or EPO 10 nM (7th lane) and 20 nM (8th lane); **C)** in untreated *Ank*^{KI/KI} mouse calvarial osteoblasts cultured at 30°C and 37°C for 24 hours.



Figure S3. BFA inhibits lysosomal degradation more efficiently than chloroquine in mouse BMMs.

Total cell lysates from *Ank*^{+/+} and *Ank*^{KI/KI} BMMs were analyzed by immunoblotting with ANK and LC3B antibodies. Efficiency of blocking autophagic flux was shown by the accumulation of LC3B-II. BFA1: bafilomycin a1 100 ng/ml; BFA2: bafilomycin a1 200 ng/ml; CQ1: chloroquine 25 μ M; CQ2: chloroquine 50 μ M. GAPDH as loading control.

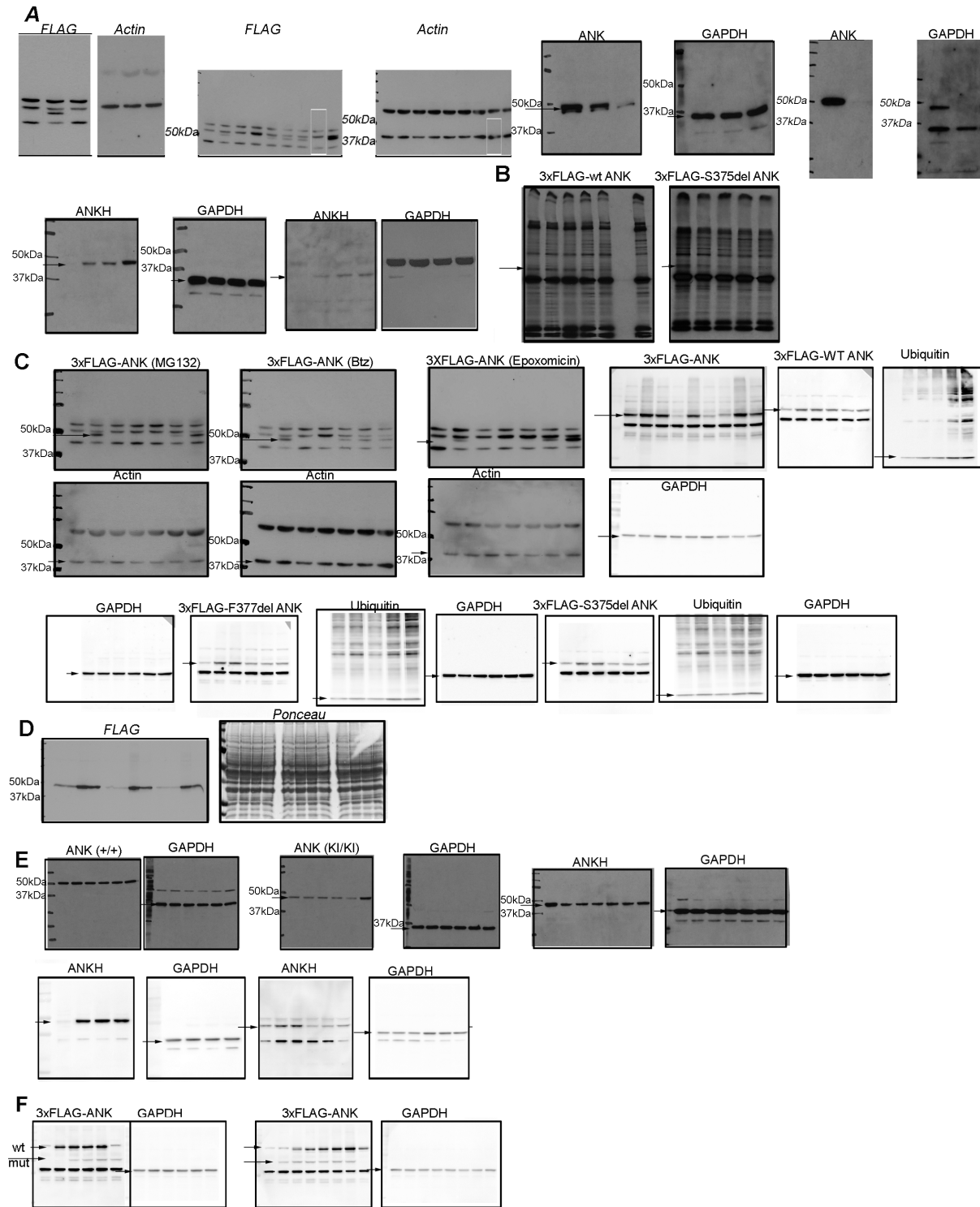


Figure S4. Images of full-length immunoblots. **A)** Immunoblots shown in Figure 1; **B)** Immunoblots shown in Figure 3; **C)** Immunoblots shown in Figure 4; **D)** Immunoblots shown in Figure 5; **E)**

Immunoblots shown in Figure 6; **F)** Immunoblots shown in Figure 8. Arrows indicate specific signal in each blot.

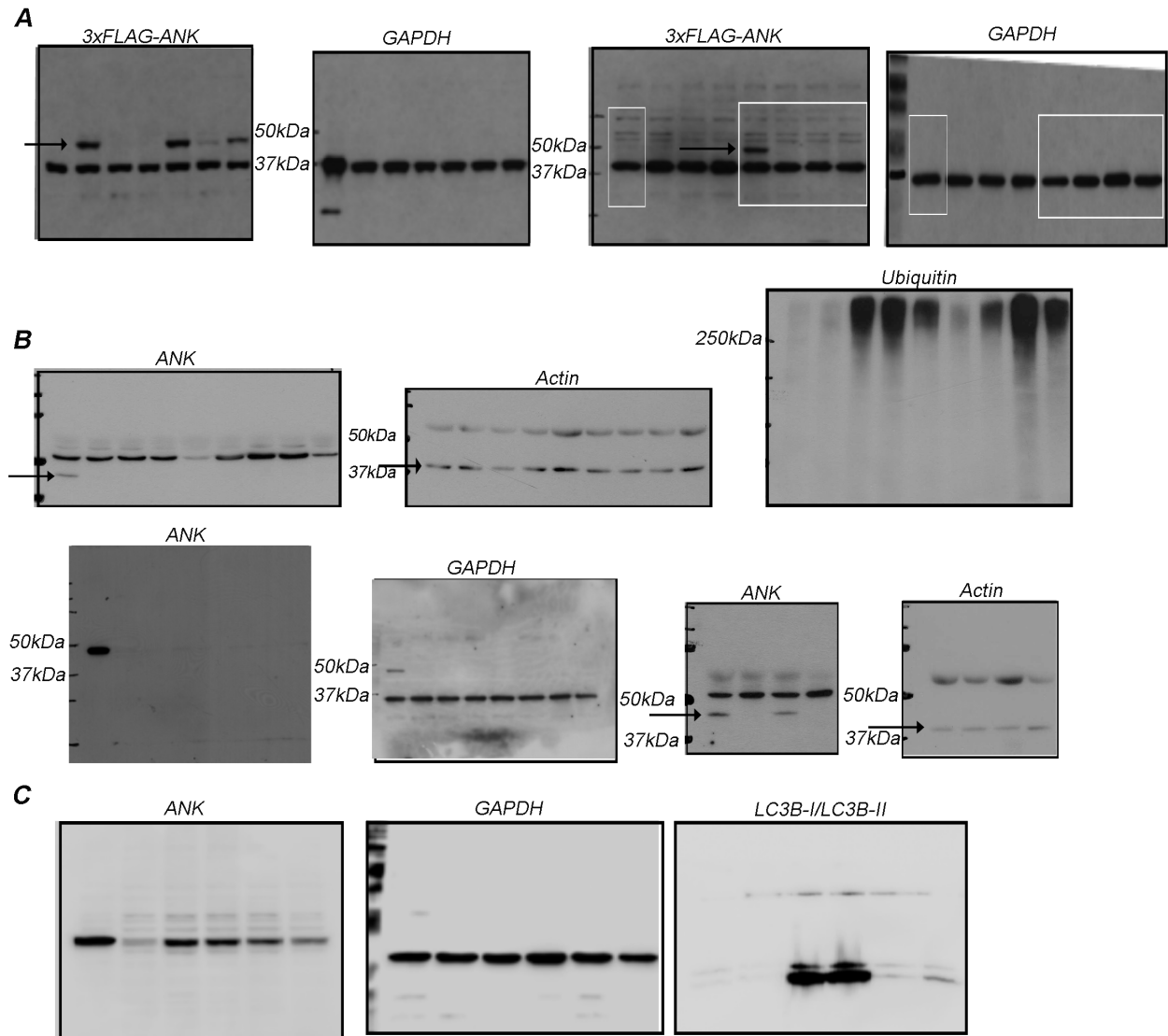


Figure S5. Images of full-length immunoblots. A) Immunoblots shown in Figure S1; B) Immunoblots shown in Figure S2; C) Immunoblots shown in Figure S3. Arrows indicate specific signal in each blot.