

Supplemental Figure 1. Semaphorin3A and Neuropilin1 expression by OCLs and OBs from WT and MVNP mice. OCLs were derived from CD11b⁺ cells as described in Methods. OBs were isolated as described previously (26). Cell lysates were analyzed for Semaphorin3A and Neuropilin1. Results are representative of 2 biologic replicates.

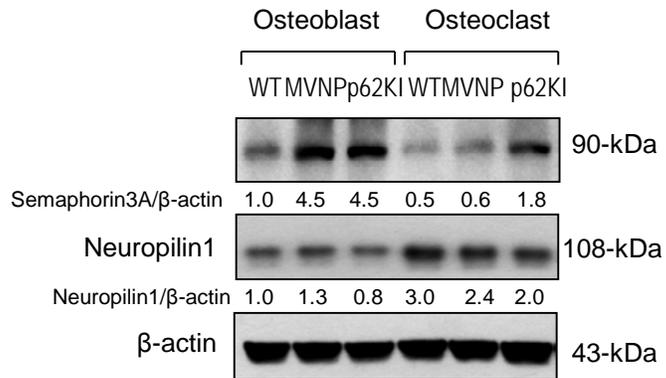
Supplemental Figure 2. Expression of MVNP by OCLs formed in cultures of peripheral blood mononuclear cells (PBMC). OCLs formed in peripheral blood cultures of OCL precursors from PD patients harboring p62^{P392L} or normal donors were tested for MVNP expression as previously described (13). A faint non-specific band is present in the normals and patient 3. MVNP expression in patients 1 and 2 was previously confirmed by sequencing (13). Results are from a single experiment and are derived from the same gel shown in Figure 2.

Supplemental Figure 3. Effects of IGF1 on PI3K and Akt activity in OBs and OCLs. (A) OCLs formed by OCL precursors of PD patients prepared as described in Methods were treated with varying concentrations of the PI3K inhibitor (LY294002) for 48 hours, the lysates prepared and ephrinB2 protein expression levels determined. Results are representative of 3 technical replicates. (B) OBs from MVNP or WT mice prepared as previously described (26), were treated with IGF1(10 ng/ml) for the time periods indicated, the lysates analyzed for Akt and Erk activation using anti-phospho-Akt (Ser473) and anti-Erk (Thr202/Tyr204) antibodies. Results are representative of 3 biologic replicates.

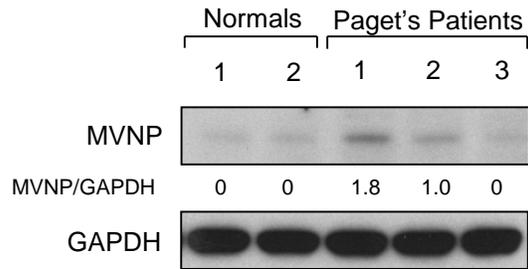
Supplemental Figure 4. OBs co-cultured with OCLs and OBs from WT or MVNP mice. OCLs formed in cultures of MVNP or WT OCL precursors were scraped with a rubber policeman, replated and cultured with 50 ng/ml RANKL overnight. OBs (1×10^5 /well) were then plated on top of the OCLs the next day, and the cells co-cultured for 72 hours. The cells were stained using a TRACP & ALP double-stain kit (Takara Bio.). OCLs stain pink, OBs stain violet (x80).

Supplemental Figure 5. IGF1 and IL-6 expression in co-cultures of mature OCLs and OBs from WT or MVNP mice. Co-cultures of OBs and OCLs from WT and MVNP mice were treated with EphB4/Fc (5 μ g/ml) using the same culture conditions as in Figure 3C. The cells were co-cultured for 72 hours and the cell lysates then tested for IGF-1 and IL-6 expression by Western blot. The relative ratios of IGF-1/ β -actin and IL-6/ β -actin were measured by ImageJ. The data are the mean of 2 independent experiments.

Supplemental Figure 1

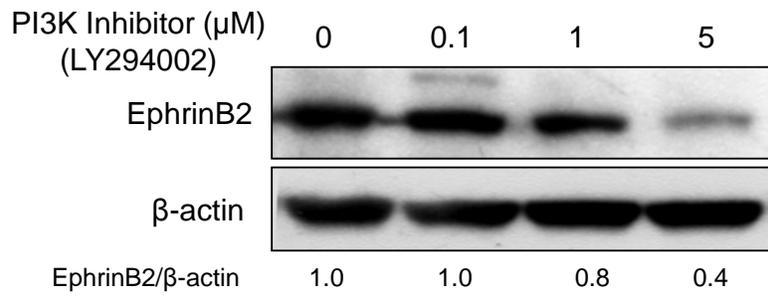


Supplemental Figure 2

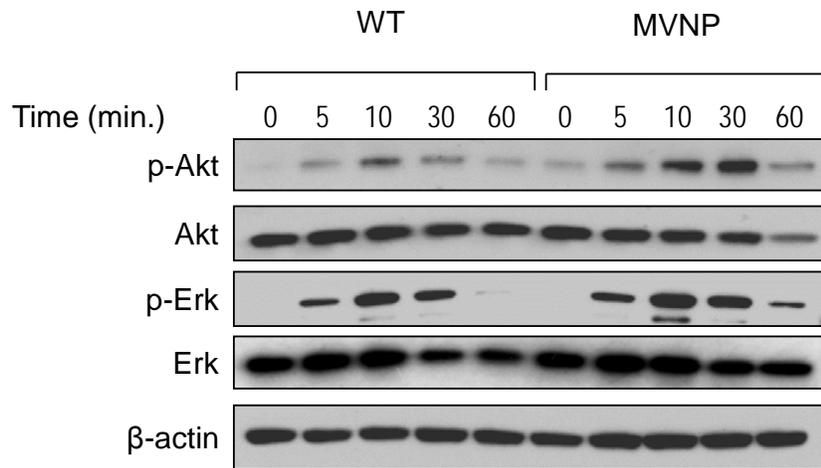


Supplemental Figure 3

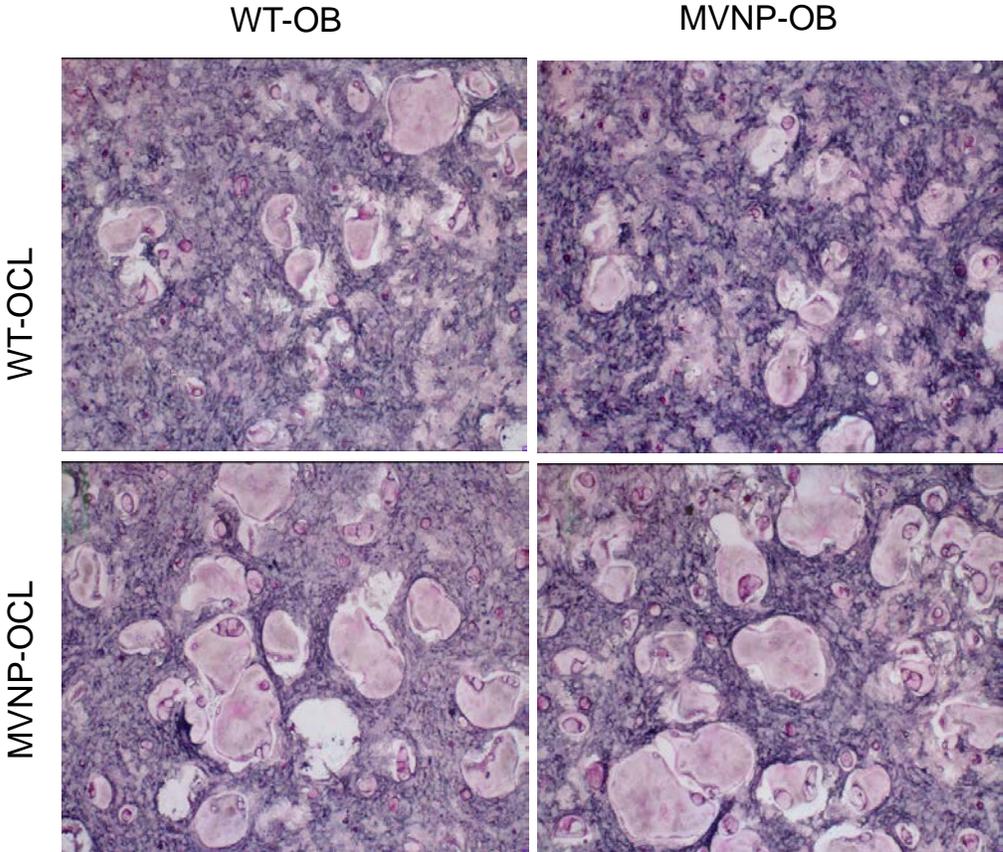
A



B



Supplemental Figure 4



Supplement Figure 5

