Neill et al., https://doi.org/10.1083/jcb.201603079

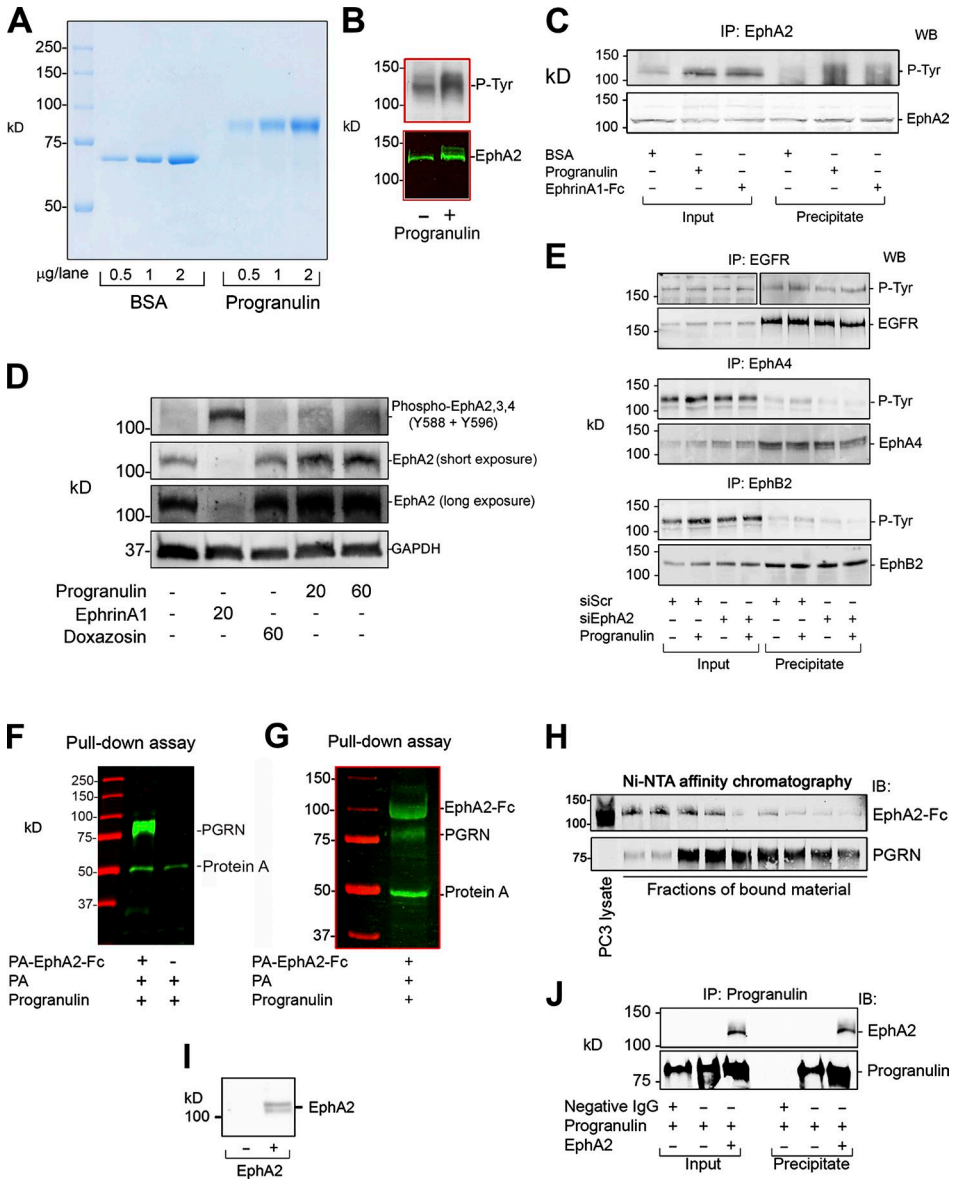

Figure S1. **Verification of human recombinant progranulin purity and binding activates EphA2 receptor.** (A) Gel stained with colloidal Coomassie blue for highly sensitive detection (as low as 5 ng) of increasing amounts of recombinant progranulin run in parallel with increasing amounts of BSA. Molecular weight markers are to the left (n = 2). (B) Immunoblotting of total cell lysates from PC3 cells after 30-min treatment with progranulin. The immunoblotting was first probed with PY20, stripped, reprobed with anti-EphA2, and detected by secondary IR800-labeled antibodies (n = 2). (C) Immunoprecipitation (IP) of EphA2 in HUVEC treated with BSA, progranulin, or EphrinA1-Fc. Membrane was first incubated with anti-phosphotyrosine antibody (PY20) for phospho-Tyr (P-Tyr) detection by chemiluminescence, stripped, and reprobed with anti-EphA2 (n = 2). (D) Representative immunoblot demonstrating EphA2 phosphorylation in PC3 cells treated with progranulin, EphrinA1-Fc, or doxazosin at the indicated time points. A long exposure of total EphA2 is provided to demonstrate the presence of total EphA2 in the EphrinA1-Fc treated condition (n = 3). (E) Immunoprecipitation of EGFR, EphA4, or EphB2. T24 cells were transiently transfected with siScramble or siEphA2 and treated with progranulin as indicated. Silencing of EphA2 was confirmed via immunoblotting before immunoprecipitations. In each case, the respective membranes were first reacted with the anti-phosphotyrosine antibody (4G10) for phospho-Tyr (P-Tyr) detection by chemiluminescence, stripped, and reprobed with anti-EGFR, anti-EphA4, or anti-EphB2. Note that, for EGFR only, the membrane has been split into two for differential exposure time for P-Tyr. (F) Immunoblotting of pull-down for either PA-EphA2-Fc or protein A (negative control) after incubation with IR800-labeled progranulin (100 nM; n = 1). (G) Pull-down with protein A beads where equimolar amounts of ligand and receptor were combined and allowed to interact. Protein A–Sepharose beads were then added. The immunoblotting depicts simultaneous detection of progranulin and EphA2 (n = 1). (H) Immunoblotting (IB) of EphA2 and progranulin after affinity chromatography using His_6-tagged progranulin bound to Ni-NTA beads coated with 20 µg of purified progranulin. Positive control for EphA2 is shown in lane 1 and is a PC3 whole cell lysate. Lanes 2–10 represent different fractions eluted from the column with 500 mM imidazole. (I) Verification of EphA2 from the in vitro transcription-translation reaction (n = 2). (J) Representative coimmunoprecipitation using progranulin-coated protein A magnetic beads. Protein A beads were initially coated with a polyclonal antibody against progranulin then incubated with progranulin and lysates from the coupled transcription-translation reaction (n = 2). WB, Western blot.

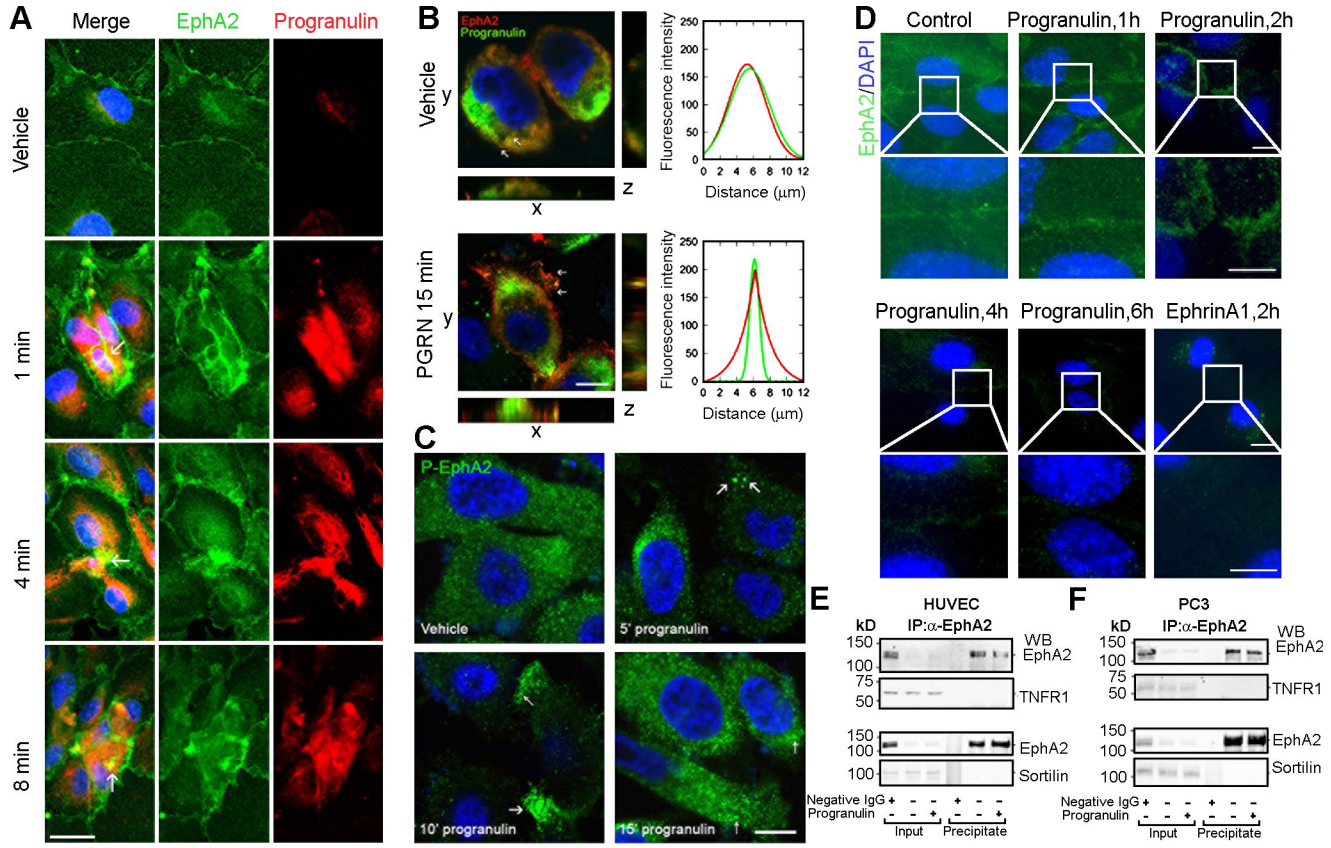

Figure S2. **Exogenous progranulin localizes with EphA2 and stimulates EphA2 phosphorylation, clustering, and internalization, but does not promote interactions with TNFR1 or sortilin.** (A) Representative confocal images after treatment with progranulin at the indicated time points. (B) Representative confocal images after 15-min treatment with progranulin. (C) Representative images denoting phosphorylated and clustered EphA2 (white arrows) at the indicated time points. (D) Representative images and corresponding insets demonstrating EphA2 internalization after progranulin at the indicated time points in HUVECs. EphrinA1-Fc was used as a positive control. Bars, ~10 µm. (E and F) Representative immunoblots depicting immunoprecipitation (IP) of EphA2 and subsequent immunoblotting for either TNFR1 or sortilin in HUVECs (E) or PC3 cells (F) in the presence of progranulin. WB, Western blot.

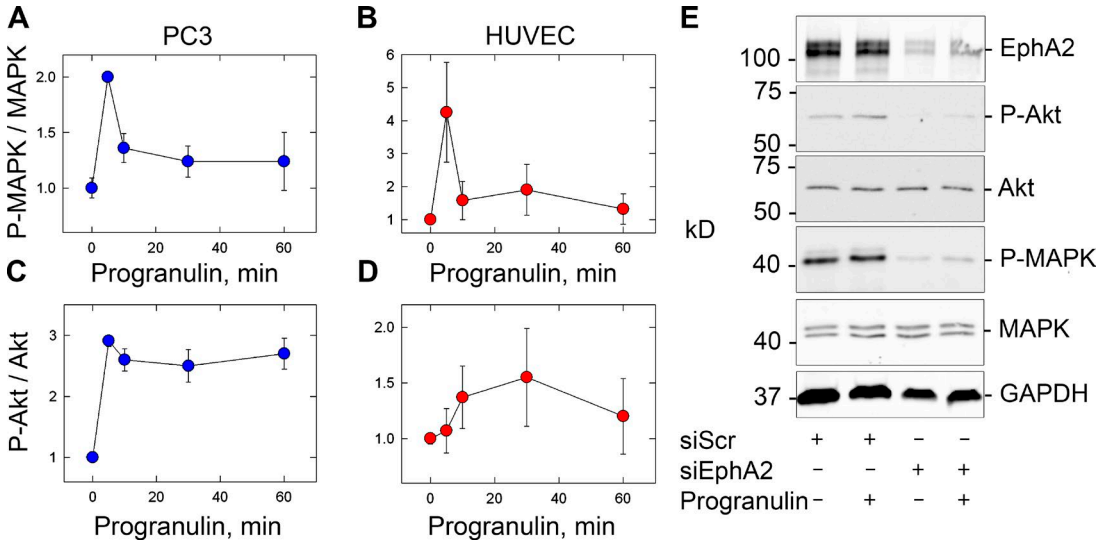

Figure S3. **Phosphorylation of MAPK and Akt requires EphA2.** (A and B) Quantification of phosphorylated MAPK in PC3 cells (A) or HUVECs (B). (C and D) Similar quantification of phosphorylated Akt in PC3 (C) or HUVECs (D). Progranulin (50 nM) was administered at the indicated time points in quiescent cells. (E) Representative immunoblots depicting EphA2 silencing in the absence or presence of progranulin and ensuing effects on Akt and MAPK phosphorylation in T24 cells. Data represent at least three independent experiments and are depicted as fold change ± SEM.

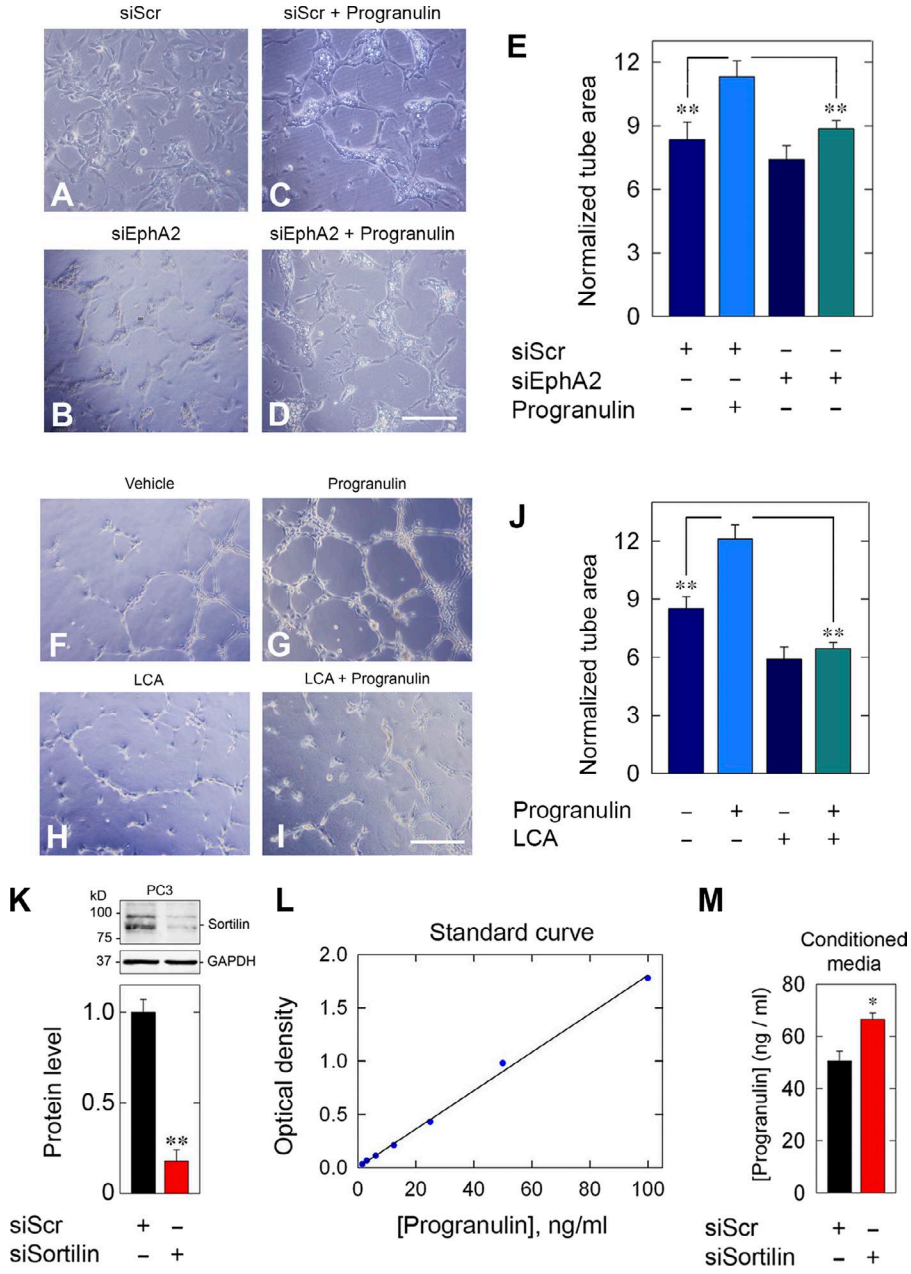

Figure S4. **EphA2 is required for capillary morphogenesis on collagen and Matrigel.** (A–D) Representative bright-field images of HUVECs embedded on collagen type I, transfected with siScr or siEphA2, and challenged with progranulin. (E) Quantification of tubular surface area of the collagen type I capillary morphogenesis assay. The accompanying quantification represents mean tube surface area ± SEM from two experiments using at least five individual fields/group. (F–I) Representative bright-field images of HUVECs on Matrigel after treatment with LCA alone or in combination with progranulin (3 h). (J) Quantification of tubular surface area of the Matrigel morphogenesis assay ± SEM from two experiments using at least 10 individual fields/group. (K) Verification of sortilin depletion. (L) Standard curve of progranulin plotted against the corresponding OD. (M) Quantification of progranulin levels found in conditioned media of PC3 cells after RNAi. Data are representative of three independent trials run in duplicate. *, $P < 0.05$; **, $P < 0.01$. Statistics calculated in E and J were analyzed via one-way ANOVA (**, $P < 0.01$).

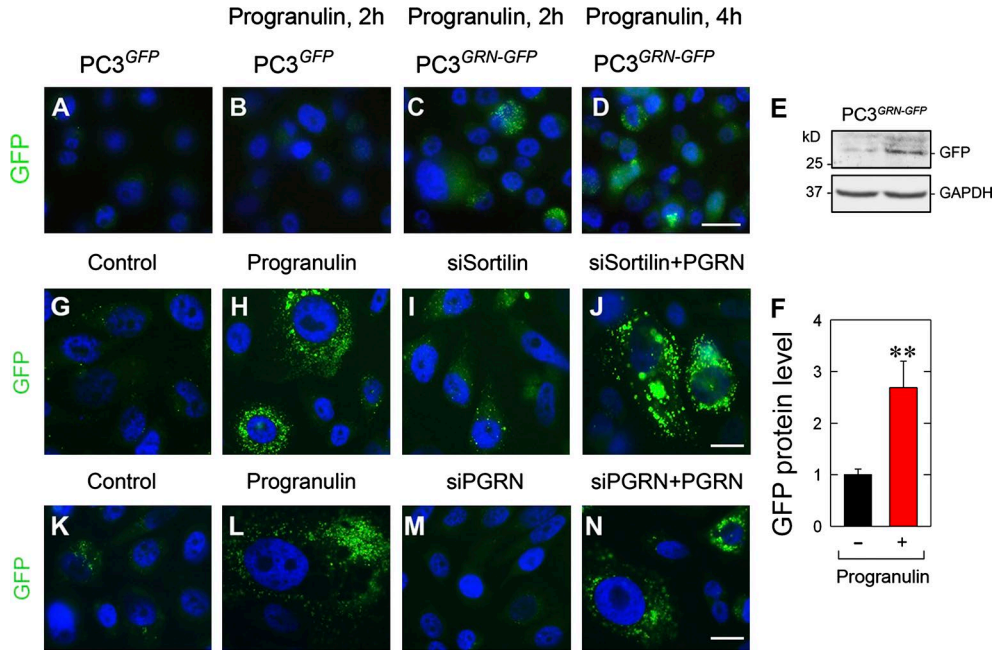

Figure S5. **Exogenous progranulin drives *GRN*-GFP activity downstream of EphA2.** (A–D) Fluorescence images of GFP after progranulin stimulation of PC3^{GFP} or PC3$^{GRN\text{-}GFP}$ cells. Note that images in A and B were derived from the negative control cells, PC3^{GFP}, after progranulin treatment as indicated, whereas images in C and D were captured from the active reporter line, PC3$^{GRN\text{-}GFP}$. (E and F) Representative immunoblotting and accompanying quantification of PC3$^{GRN\text{-}GFP}$ challenged with progranulin and probed with anti-GFP antibodies. (G–J) Images of GFP after transient transfection of siScr (G and H) or targeting siSortilin (I and J) in the absence or presence of progranulin (H and J). (K–N) Fluorescence imaging of GFP after siScr transfection (K and L) or siGRN (M and N), with or without progranulin (L and N). Data are mean fluorescence intensity. All images were taken with the same exposure, gain, and intensity. Bars, 10 µm. Data in F represent three independent experiments. **, $P < 0.01$.

Table S1. **Targeting siRNA sequences**

Targeting siRNA	Product number	Sense	Antisense
EphA2	sc-29304	sc-29304A, 5′-GGAAGUACGAGGUCACUUAtt-3′	sc-29304A, 5′-UAAGUGACCUCGUACUUCCtt-3′
		sc-29304B, 5′-GACGUUUACUUCUCCAAGUtt-3′	sc-29304B, 5′-ACUUGGAGAAGUAAACGUCtt-3′
		sc-29304C, 5′-CAACCACGAGGUGAUGAAAtt-3′	sc-29304C, 5′-UUUCAUCACCUCGUGGUUGtt-3′
Progranulin	sc-39261	sc-39261A, 5′-GAGAGUGAUUUGAGUAGAAtt-3′	sc-39261A, 5′-UUCUACUCAAAUCACUCUCtt-3′
		sc-39261B, 5′-GCUUCCAAAGAUCAGGUAAtt-3′	sc-39261B, 5′-UUACCUGAUCUUUGGAAGCtt-3′
		sc-39261C, 5′-GGACAGUACUGAAGACUCUtt-3′	sc-39261C, 5′-AGAGUCUUCAGUACUGUCCtt-3′
Sortilin	sc-42119	sc-42119A, 5′-GGAAGAAUCUUCAGAUCAUtt-3′	sc-42119A, 5′-AUGAUCUGAAGAUUCUUCCtt-3′
		sc-42119B, 5′-CAGGAACAGUUCUAUUCUAtt-3′	sc-42119B, 5′-UAGAAUAGAACUGUUCCUGtt-3′
		sc-42119C, 5′-GCAUCAUUGUGGCCAUUGAtt-3′	sc-42119C, 5′-UCAAUGGCCACAAUGAUGCtt-3′