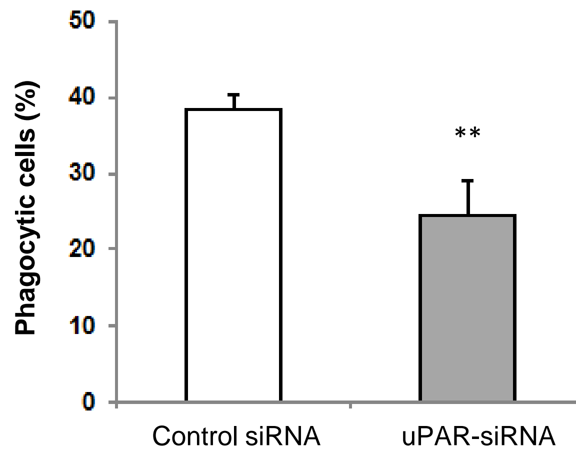
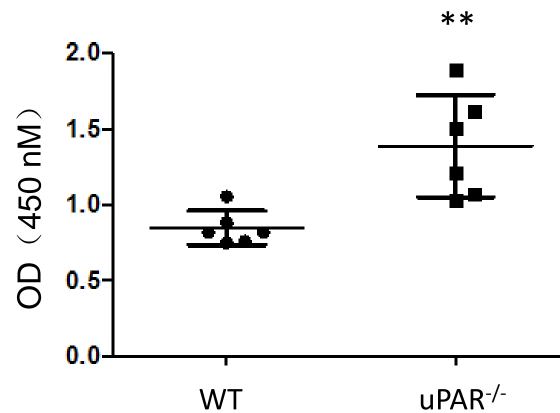
Supplemental Figure 1



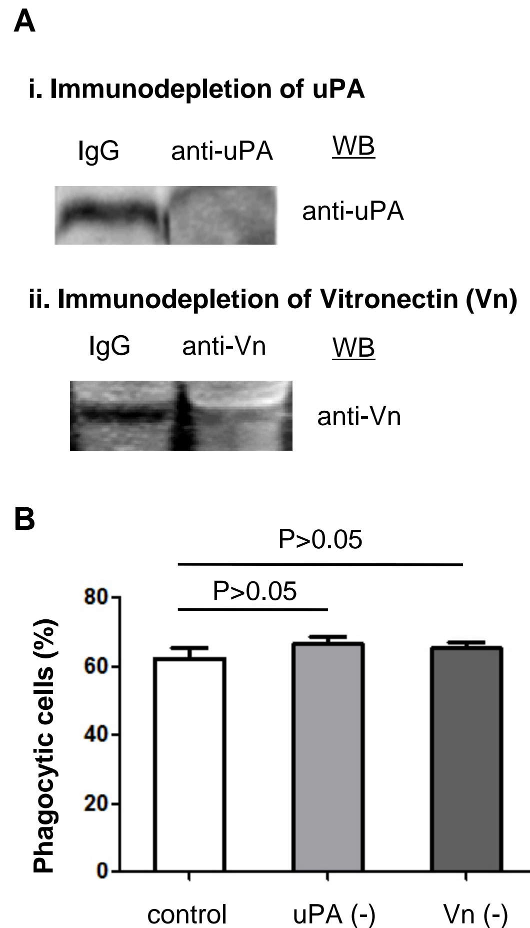
Supplemental Figure 1. Small interfering RNA (siRNA)-mediated downregulation of uPAR in human monocyte cell line THP-1 cells inhibits internalization of apoptotic cells. FITC-labeled siRNA oligonucleotides (stealth RNAi) were purchased from Invitrogen. The sequence of human uPAR cDNA (5'-GGTGAAGAAGGGCGTCCAA-3') was selected as the targeting region, and a nonsilencing siRNA, 5′-AACCTGCGGGAAGAAGTGG-3', was used as a control. The siRNA oligo was introduced into THP-1 cells purchased from the American Type Culture Collection (ATCC) by electroporation as previously described (Cell Death Differ. 2013;20:1230-1240 and J Biol Chem 2008; 283:5296-5305). Internalization of apoptotic cells by transfected THP-1 cells was performed as described in the legend for Figure 4. **, $p<0.01$.

Supplemental Figure 2



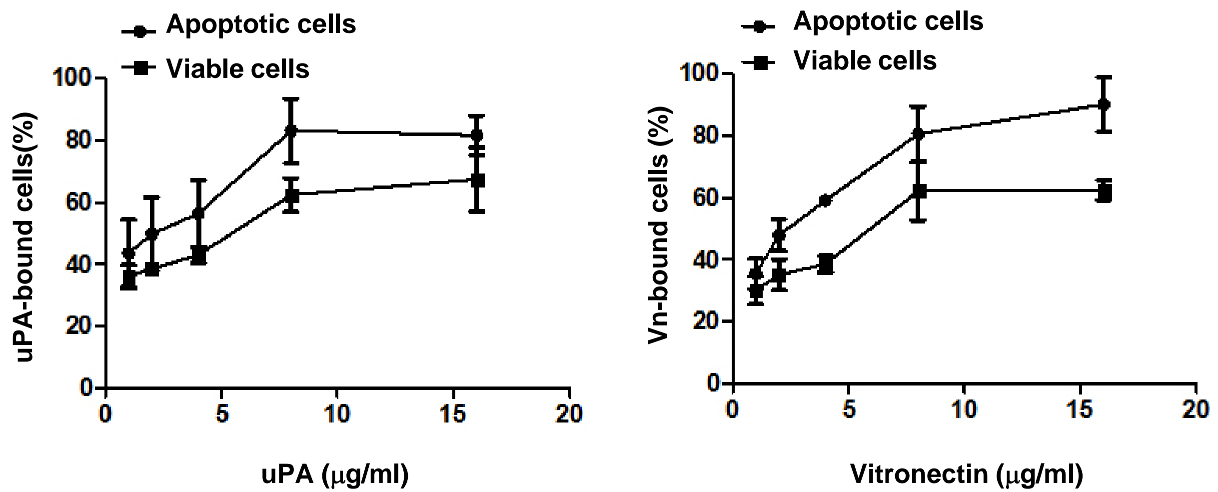
Supplemental Figure 2. Measurement of serum immunoglobulins in WT and uPAR-deficient mice. Measurement of autoantibody in plasma was performed as previously described (J Exp Med. 1998;188: 387−392, J Exp Med. 2004;200:459−467 and Blood 1995; 86:3083-3089). Briefly, after the 96-well ELISA plates were coated with 50 μg/ml PS, they were dried under nitrogen and blocked with 10% fetal calf serum (FCS). At the end of experiments described in the legend for Figure 3A, plasma was collected from WT and uPAR-deficient mice. Mouse serum was diluted 50 times in PBS and added to the 96-well ELISA plates, followed by incubation at 37℃ for 1 hour. The mouse antibodies bound to the plate were detected using HRP-conjugated goat anti−mouse immunoglobulins in PBS containing 3%FCS. The peroxidase activity was detected using TMB as a substrate, and the color reaction was read at 450 nM in a SpectraMax M5 Microplate Reader (Molecular Devices). **, $p<0.01$

Supplemental Figure 3



Supplemental Figure 3. Immunodepletion of Vitronectin or uPA does not affect internalization of apoptotic cells. A. As previously described (Investigative Ophthalmology & Visual Science 1992;33:2687-92, J Neurosurgery 2007;107:578-585), human normal serum were incubated overnight at 4C with anti-uPA antibody (R&D Systems, MAB1310), anti-vitronectin antibody (Cedarlane CL20170A) or nonimmune IgG, which was conjugated to Portein A/G Sepharose beads (Santa Cruz Biotechnology, Inc), respectively. This procedure was repeated seven more times for anti-vitronectin antibody. Immunodepletion of uPA (i) and Vitronectin (Vn, ii) was analyzed by immunoblotting. **B**. The uPAR-293 cells were incubated with apoptotic cells in serum that was immunodepleted using IgG (control), anti-uPA [uPA(-)] or anti-Vn [Vn(-)] antibodies, respectively. After treatment with TB, the internalization of apoptotic cells was evaluated by flow cytometry as described in the legend for Figure 4.

Supplemental Figure 4



Supplemental Figure 4. Vitronectin and uPA bind to both viable cells and apoptotic cells. As described in the legend for Figure 5(D), mixed apoptotic cells and viable cells were incubated with biotinylated-Vitronectin or -uPA at the indicated concentrations. After washing, the cells were labeled with PE-avidin and non-saturated amounts of FITC-annexin V. The intensity of PE fluorescence on apoptotic cells (FITC-AxV-positive) and viable cells (FITC-AxV-negative) was analyzed by flow cytometry. Shown were the quantification of the percentage of PE-positive cells, representing uPA-bound cells (%) and Vitronectin-bound cells (%).