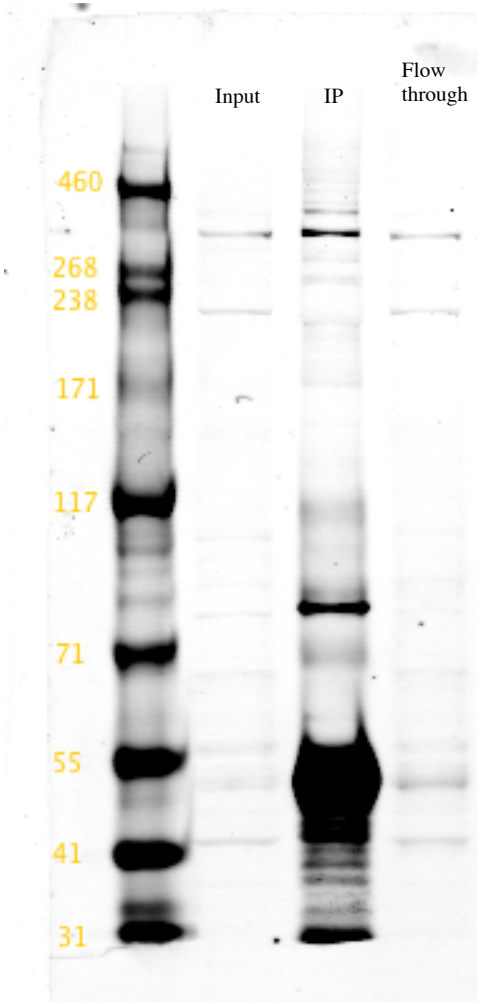

Immunoprecipitation

Whole E13.5 mouse embryos were lysed in lysis buffer consisting of 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1mM EDTA, 1%v/v Triton X-100, protease inhibitor cocktail tablet (Roche, Indianapolis, IN) by using 1 ml glass tissue grinder (Wheaton, Millville, NJ). The clear lysate was incubated with anti-Wdfy3 antibody (Novus, Littleton, CO) overnight and was then immunoprecipitated by dynabeads protein A immunoprecipitation kit (life technologies, Carlsbad, CA).

Western analysis

Total Protein lysates, immunoprecipitated samples and flow through samples obtained from whole E13.5 mouse embryos were run on a Nu-Page 3-8% Tris-Acetate gel (Invitrogen, Carlsbad, CA). Proteins were transferred to PVDF membranes and blocked in Odyssey blocking buffer (Li-Cor Biosciences, Lincoln, NE). Membranes were incubated with anti-Wdfy3 primary antibody (1:1000; Novus, Littleton, CO) diluted in Odyssey blocking buffer containing 0.1% Tween-20, overnight at 4°C. After washing, we incubated with Li-Cor IR secondary antibody (1: 5,000; Li-Cor Biosciences, Lincoln, NE) in Odyssey blocking buffer containing 0.1% Tween-20 and 0.02% SDS, washed, and imaged on the Li-Cor Odyssey IR scanner.