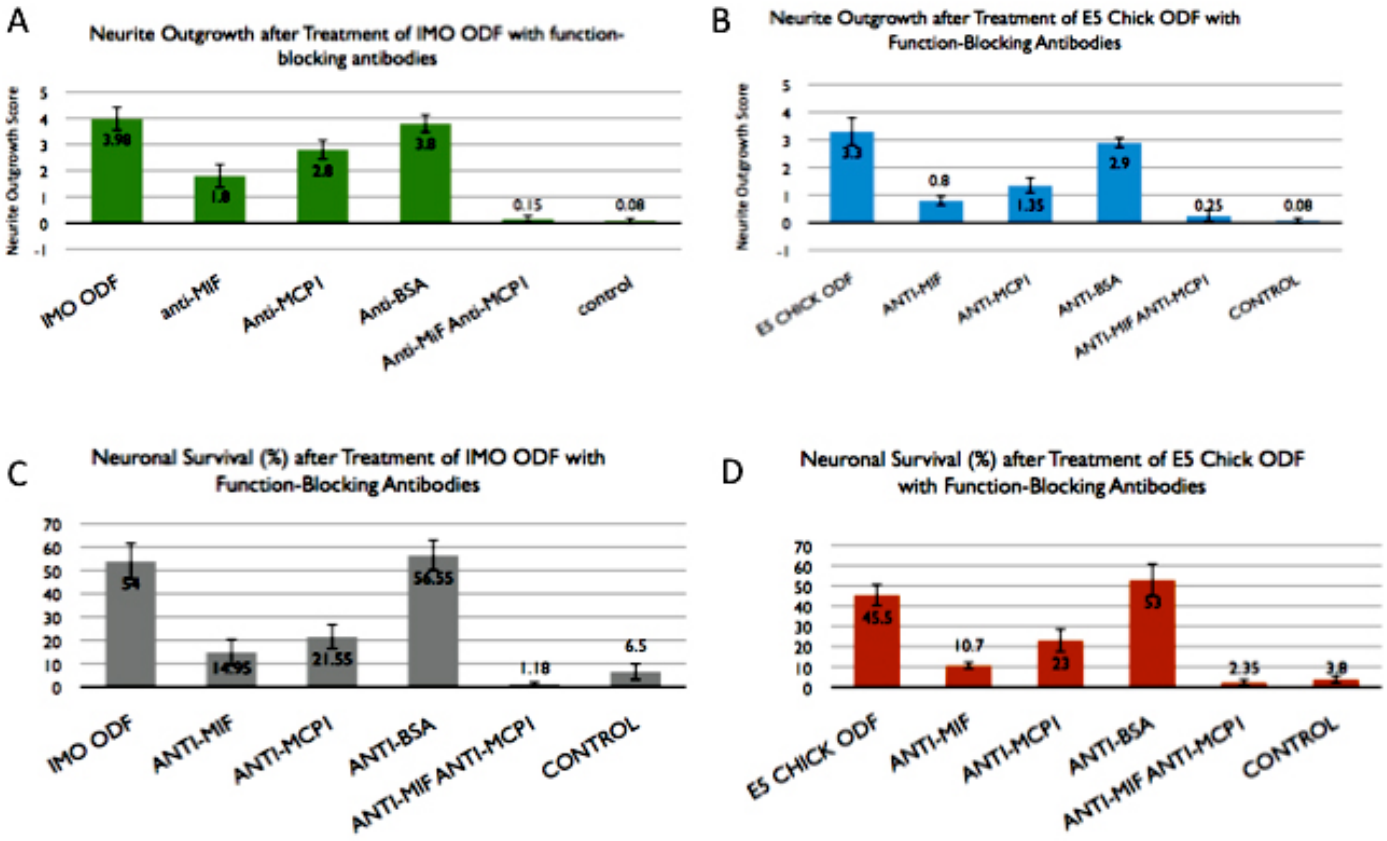

Fig. S1. Sequentially applied function-blocking antibodies to MIF and MCP1 can block the bioactivity of IMO- or chick E5 otocyst-generated ODF. (**A-D**) The MIF function-blocking antibody was added to IMO-generated and chick E5 otocyst-generated ODF to block SAG neurite outgrowth and survival. The MCP1 function-blocking antibody used in our previous work (Bianchi et al., 2005) that partially blocked ODF bioactivity in IMO- and E5 chick-generated ODF was then applied to the anti-MIF-treated CM. The bioactivity of ODF was lost with this sequential antibody treatment.

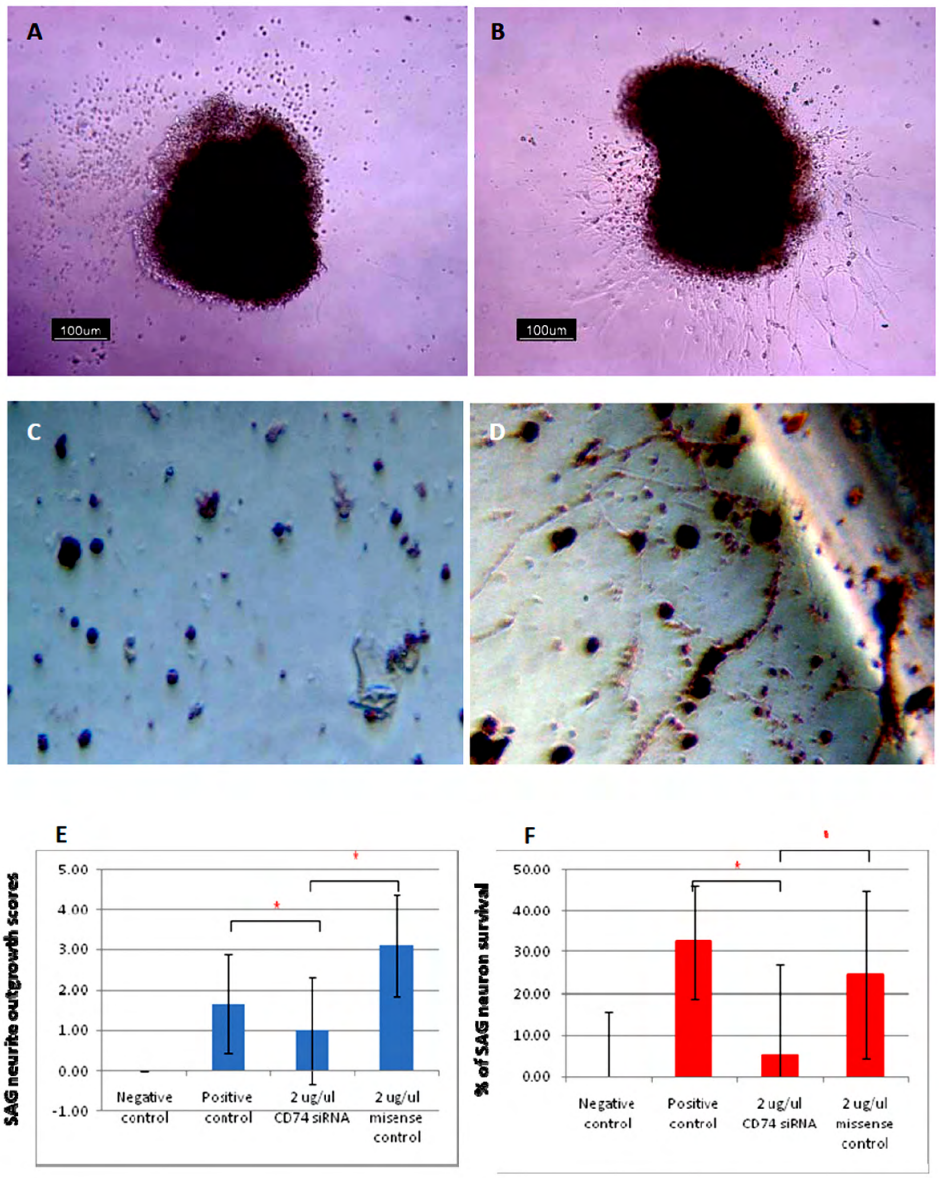

Fig. S2. Avian *Cd74* siRNA reduces SAG neurite outgrowth and survival. Neuronal bioassays using E5 chick whole SAG and dissociated SAG from the same stage embryos exposed to CM generated from E4 chick otocysts (otocyst-generated ODF) to determine the effect of blocking CD74 with siRNA. (**A**) SAG explants treated with otocyst-generated ODF in the presence of 2.0 μg/μl avian *Cd74* siRNA show little SAG neurite outgrowth. (**B**) By contrast, SAG explants treated with otocyst-generated ODF in the presence of 2.0 μg/μl missense control show SAG neurite outgrowth. (**C**,**D**) Dissociated SAG neurons treated with otocyst-generated ODF in the presence of 2.0 μg/μl avian *Cd74* siRNA demonstrate lower cell survival (C) compared with dissociated SAG neurons treated with otocyst-generated ODF in the presence of 2.0 μg/μl missense control (D). (**E**,**F**) Statistical analysis demonstrates that 2 μg/μl siRNA significantly reduced both SAG neurite outgrowth (E) and survival (F) compared with its missense control ($P<0.05$). Serum- and γ-interferon-free basal medium is used as negative control and otocyst-generated ODF is used as positive control. $n=9$, SAG neurite outgrowth; $n=12$, dissociated SAG neuronal survival.

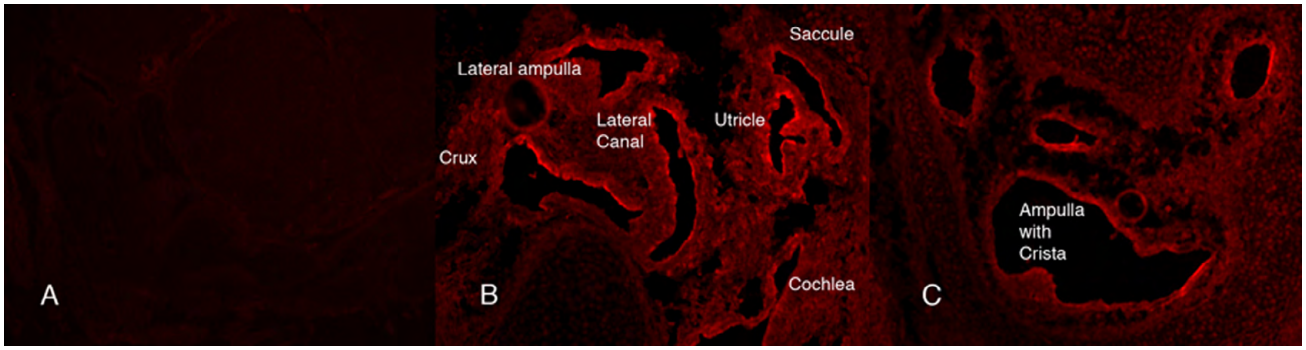

Fig. S3. MIF protein expression in the E15.5 mouse inner ear. (**A**) Control of secondary antibody alone. No background labeling is seen. (**B**,**C**) MIF expression is detected in the developing inner ear, including the cochlea, utricle, saccule, lateral canal, lateral ampulla and the crux. Magnification: 20×.

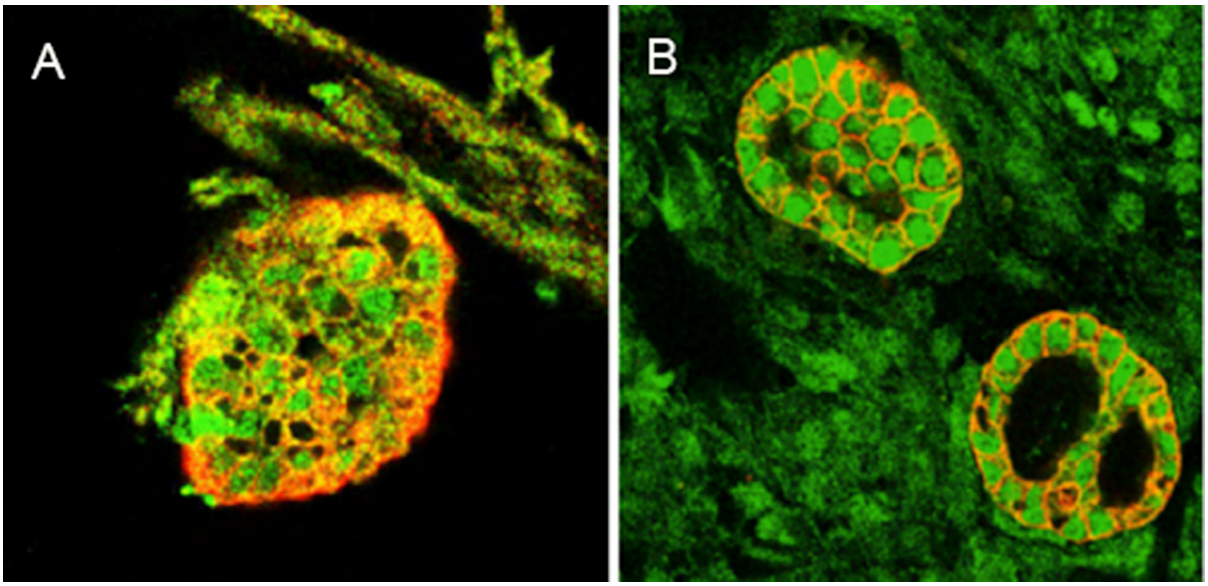

Fig. S4. Re-aggregated embryonic inner ear cells show that MIF is localized to supporting cells. (**A**) Double-labeled fluorescent immunocytochemistry shows myosin VIIa-positive hair cells primarily in the central region of the domes (green), whereas MIF (red) is detected in the surrounding (and underlying) supporting cell layer (20×). (**B**) Supporting cell external membranes are labeled with cytokeratin (red) in the underlying layer of the domes, while MIF expression (green) is detected in the cytoplasmic region of these same cells (20×). The overlying hair cells (above the focal plane) are not immunoreactive for either cytokeratin or MIF.

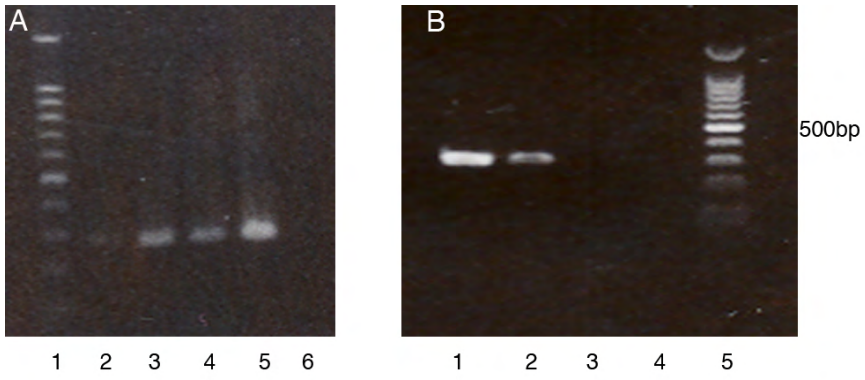

Fig. S5. Expression of *Cd74* mRNA in embryonic and adult inner ear. (**A**) Expression of *Cd74* mRNA is present in the E14 and adult WT (Balb/c) mouse SAG and otocyst. Lane 1, 100 bp ladder (NEB); lane 2, E14 SAG; lane 3, E14 embryonic ear; lane 4, adult ampulla; lane 5, adult inner ear; lane 6, blank. (**B**) Expression of *Cd74* in the adult SG (lane 1) and the sensory epithelium (lane 2). Lane 3, water control; lane 4, blank; lane 5, 100 bp DNA ladder. CD74 is also expressed at the protein level in both the embryonic SAG and postnatal spiral ganglion, as detected by western blotting (not shown).

Table S1. Summary data for the function-blocking antibody experiments

	Control	ODF	MIF	MIF+αMIF	MIF+αBSA	ODF+αMIF	ODF+αBSA
Control		NC/ $P<10^{-5}$	NC/ $P<10^{-5}$	NC/NS	NC/$P<10^{-5}$	NC/$P<10^{-2}$	NC/$P<10^{-5}$
ODF			NS/ $P<10^{-2}$	NC/NC	NC/NC	$P<10^{-4}$/ $P<10^{-3}$	NS/NS
MIF				$P<10^{-3}$/ $P<10^{-3}$	NS/NS	NC/NC	NC/NC

Function-blocking antibody effects on neurite outgrowth of whole statoacoustic ganglia (red) or on dissociated statoacoustic ganglion neuron survival (blue), showing statistical comparisons among conditions. NS, not significant; NC, no comparison was performed.