Table S1. Summary of previous and current findings and interpretations concerning the role of cilia in otolith formation in the zebrafish ear

Topic of controversy	**Previous data and interpretations**	**New information and resolution (where applicable)**
Classes of cilia in the zebrafish ear	Two classes: short cilia throughout the ear and longer tether cilia at the poles (Riley et al., 1997; Yu et al., 2011). Two classes (motile cilia and immotile tether cilia) present at the poles (Wu et al., 2011). The rest of the ear was not examined in this study.	We describe at least three, possibly four, classes of cilia in the zebrafish otic vesicle, based on length, otolith-binding properties and motility: tether cilia, polar motile cilia, non-polar immotile cilia and non-polar motile cilia. We provide high-resolution video imaging for all classes. We have confirmed that the polar cluster contains two different classes using the *Tg(pou4f3:GFP)* line.
Motility of tether cilia	1. Tether cilia are immotile, but may be passively displaced (Riley et al., 1997; Wu et al., 2011; Yu et al., 2011). 2. Tether cilia are motile (Colantonio et al., 2009).	We confirm that tether cilia are immotile and can be passively displaced by movements of nearby motile cilia. We also show that motile cilia occasionally bind otolithic material, but do not tether the main otolith mass.
Motility of other cilia in the ear	1. Cilia lining the otic vesicle are all motile (Riley et al., 1997; Yu et al., 2011), reflecting the early pattern of *foxj1b* expression (Yu et al., 2011). 2. Cilia lining the otic vesicle are all immotile (Colantonio et al., 2009); cited in Wu et al. (Wu et al., 2011).	Each otic vesicle contains about 200 cilia at early stages, of which only 2-8% are motile. Most motile cilia are at the poles, close to the otolith tethering sites. Non-polar cilia in the ear are largely immotile, but occasional motile cilia are present. These are only found on the medial wall of the otic vesicle.
Role of cilia in otolith tethering	Cilia play an important role (all previous papers), but none has tested this by examining a mutant lacking cilia altogether. [The *igu* mutant was reported to lack cilia (Yu et al., 2011).]	Tether cilia provide a tethering site in wild-type embryos, but for the first time we show that otoliths can still nucleate and are tethered in the complete absence of cilia (*MZovl* mutant). Although the *igu* mutant has reduced numbers of cilia, tether cilia are often present (this work) (Huang and Schier, 2009).
Role of ciliary motility in otolith tethering	1. Ciliary motility throughout the ear agitates precursor particles, ensuring their even distribution and preventing their premature aggregation (Riley et al., 1997; Yu et al., 2011). 2. Motile tether cilia create vortices that attract otolith precursor particles (Colantonio et al., 2009). 3. Motility of polar cilia near the otoliths creates an advection zone and controls otolith shape; elsewhere in the ear, otolith precursor particle movement is diffusion-dominated (Wu et al., 2011).	Ciliary motility contributes to otolith formation by increasing the accuracy and robustness of the tethering process, but does not have a major role: if ciliary motility is lacking, otolith defects are not fully penetrant. Differences in number of motile cilia (between different wild-type strains) do not appear to affect otolith formation. In the absence of hair cells, the presence or absence of cilia does not have a significant effect on the otolith phenotype.
Role of the first hair cells (tether cells) in otolith tethering	Early stages (<24 hpf) not previously addressed; knockdown of *atoh1b* results in a single untethered otolith at 24 hpf (Millimaki et al., 2007). It has been proposed that the hair cells produce an otolith precursor binding factor (Riley et al., 1997).	We show that tether cells are essential for otolith tethering <24 hpf: in their absence, otolith formation is delayed and disrupted in a major way. In the complete absence of cilia, hair cells provide a tethering site for the otoliths at the poles of the otic vesicle. If more hair cells are present, otoliths are tethered over a wider area.
Role of embryonic motility in otolith formation	Not involved (Riley et al., 1997) – but this was not based on an extensive study. It is well known that ciliary mutants have a curved body axis and motility defects.	Embryonic motility has a minor role in normal otolith formation: if movement is restricted in wild-type embryos, there is an increased frequency of otolith defects; if movement is enforced in mutants, it can partially rescue

their otolith defects.