Supplementary Figure 4. Assessment of the specificity of LNA-modified probes using perfectly matched and mismatched 14-mer and 22-mer LNA-modified probes for miR-124a and miR-206. In contrast to the experiment shown in Figure 1b, where the effect of central mismatches (MM) was assessed, now the effect of mismatches at the 3’ or 5’ end of the probe was tested (see Supplementary Table 1 for probe sequences). In the case of miR-206 mismatches incorporated at the 3’ or 5’ end of the probe were sufficient for adequate discrimination, although some staining was observed in the head musculature for the 14-mer MM2 probe. For miR-124a single mismatches at the 3’ or 5’ end of the probe did not result in loss of staining. As shown in figure 1b, two central mismatches in the 22-mer miR-124a probe were required for discrimination. However, a single central mismatch in the shortened 14-mer probe for miR-124a is sufficient to prevent hybridization (see Fig. 1a). The 5’ end mismatch in the 14-mer MM2 probe for miR-124a was also sufficient for adequate discrimination, whereas a clear in situ signal was observed with the 14-mer MM11 probe (3’ end mismatch) under our hybridization conditions.