Fig. S1. Whole transcriptome RNA-seq profile of *Xenopus* embryos after inhibition of NO. Volcano plot of results from DESeq2. Highlighted dots represent significant transcripts (padj < 0.1) with changes in gene expression greater than 2 times (log₂ FC > 1 or < 1). (A) PCA plot of normalized gene counts using rlog transformation (B). RT-qPCR validation of RNA-seq results of a sub-set of candidate targets (C). Heatmap of 50 transcripts with the highest changes in expression after inhibition of NO production (D). Hierarchical clustering was performed between individual experiments and transcripts. The color key indicates the log₂ value of normalized counts subtracted by their mean per transcript. Abbreviations FC: Fold-change, padj: adjusted p-value
Fig. S2. Collagens changes at protein level
Ctrl MO and combination of eNOS and nNOS MO (enNOS MO 2:1) were injected at stage 1. Embryos were fixed in 4% PFA at stage 26 and the sections of embryos were stained by Masson Trichrome staining (A). Epidermis layer was compared and amount of collagens was assessed in control (n = 9) and affected embryos (n = 8). Quantification of amount of collagens (B) Error bars indicate + s.d. and the P-value was < 0.03. At least 5 embryos were used for each condition.
Fig. S4. Inhibition of NO causes structural defects in MCCs
Embryos were injected by ctrl MO 17 ng (A) and nNOS MO 17 ng (B) at stage 1 and fixed in 4% PFA at stage 35. MCCs were stained by antibody against anti-alpha tubulin and imaged (apical surface of the embryonic epidermis) by confocal microscopy (magnification 40x, scale bar = 20 µm). At least 5 embryos were used for each condition.

Fig. S3. Changes at otogelin in embryos with inhibited NO production
Ctrl MO 34 ng (A), nNOS MO 17 ng (B) and eNOS MO 34 ng (C) were injected at stage 1 and fixed in 4% PFA at stage 26. Goblet cells were marked by otogelin (A-C) and imaged by macroscope (magnification 11.25x, scale bar = 50 µm). At least 5 embryos were used for each condition.
Fig. S5. Inhibition of NO causes accumulation of basal bodies in central region of MCCs. Ctrl MO (A) and nNOS MO (B) were injected into oocyte and fixed in 4% PFA at stage 26. Basal bodies were labeled by gamma tubulin and membranes by antibody against phalloidin and imaged (apical surface of the embryonic epidermis) by confocal microscopy (magnification 63x, scale bar = 20 µm) (A, B). Accumulation of basal bodies in central region of MCCs appears in 50% of embryos with strong phenotype. At least 5 embryos were used for each condition.

Fig. S6. Gene expression changes in embryonic epidermis with inhibited NO production relative to control (100%). Ctrl MO, nNOS MO and eNOS MO were injected into oocyte and the epidermis samples were manually collected at stage 26. Markers for MCCs (tuba1a, foxJ1a), SSCs (foxa1a) and ionocytes (foxi1e, atp6v1a) were decreased in embryos with inhibited NO production. Marker for goblet cells (itln2) was increased.