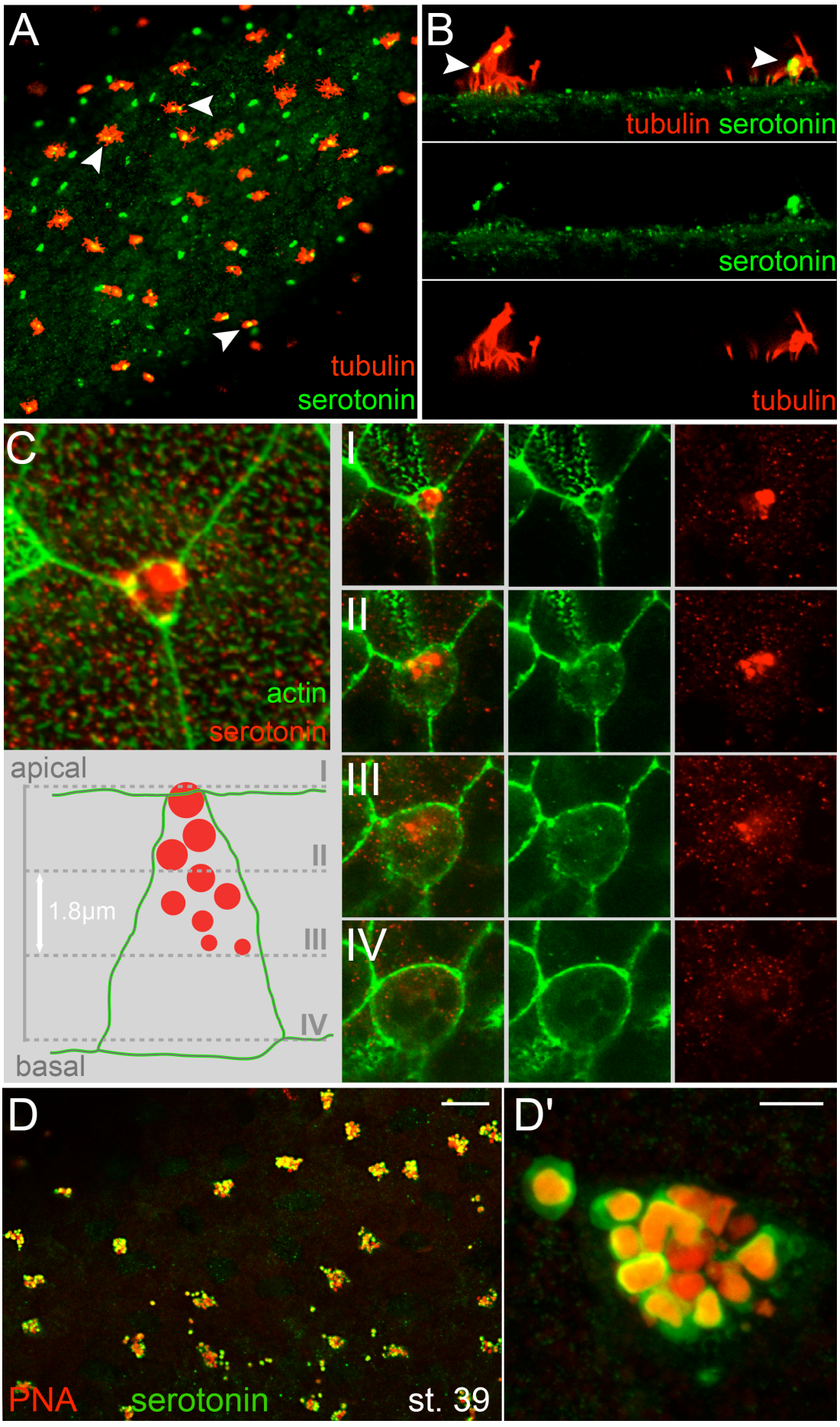

A
tubulin
serotonin
B
tubulin serotonin
serotonin
tubulin
C
actin
serotonin
apical
basal
1.8µm
I
II
III
IV
I
II
III
IV
D
PNA
serotonin
st. 39
D'

Fig. S1. Cilia association of serotonin-containing vesicles and vesicle maturation.

(A, B) Co-staining of serotonin (green) and cilia (red; anti acetylated- -tubulin) at stage 32 reveals occasional co-localization in the epidermis (white arrowheads). (**B**) Higher magnification of two MCCs in side view. Note the vesicular nature of serotonin staining in all cases. **(C)** Vesicle maturation in SSCs. Z-stack analysis of a single SSC stained for serotonin (red) and actin (phalloidin, green). Apical to basal horizontal optical sections (I-V) were used to reconstruct an orthogonal hypothetical model of vesicle maturation (lower panel in C). Vesicles localized basally were small (0.2 μm diameter), with a continuous increase in diameter to a maximum of 1 μm towards the apical pole of the cell. **(D, D')** Vesicular co-localization of serotonin (green) and peanut agglutinin (PNA; red) in the epidermis of *Xenopus laevis* tadpoles at stage 39. Scale bars in (D) and (D') represent 50 and 5μm, respectively.

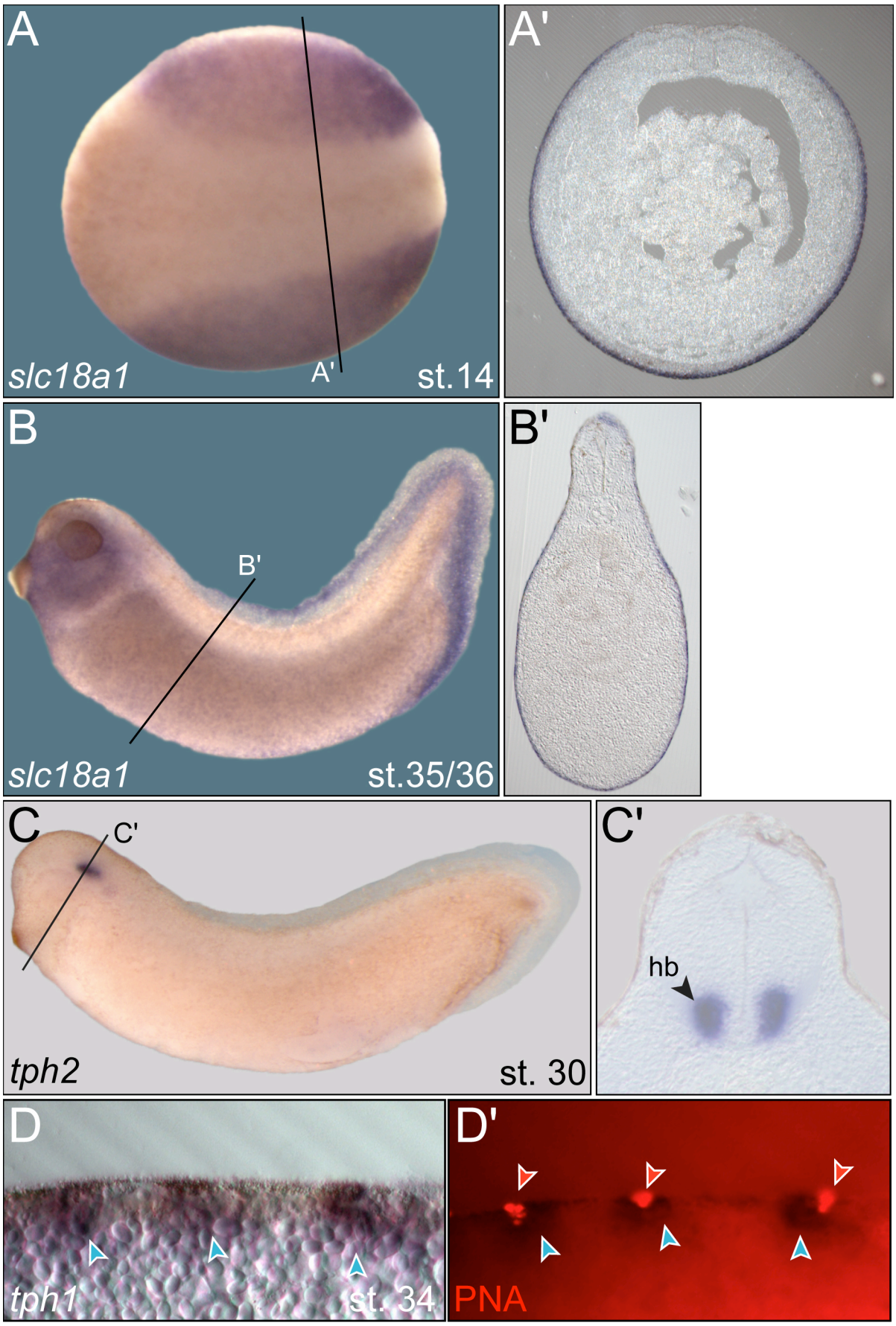


Fig. S2. Embryonic expression of serotonin pathway components.

(A, B) Epidermal expression of monoamine transporter *slc18a1* mRNA in stage 14 neurula (A) and stage 35/36 tadpole (B) embryos. Transverse sections (A', B') reveal uniform superficial staining in the epidermis. Note lack of expression in the neural plate at stage 14. **(C)** *tph2* expression was restricted to the hindbrain at stage 30. (C') Transverse histological section. Embryos are shown in lateral views except for panel (A), which shows a dorsal perspective. **(D, D')** *tph1* mRNA expressing SCCs (blue arrowheads) secrete PNA positive vesicles (red arrowheads). (D) Close-up bright field image of a transverse section of a *tph1* stained tadpole at stage 36 displaying three SSCs. (D') Fluorescent PNA signals (red) co-localize with *tph1* mRNA.

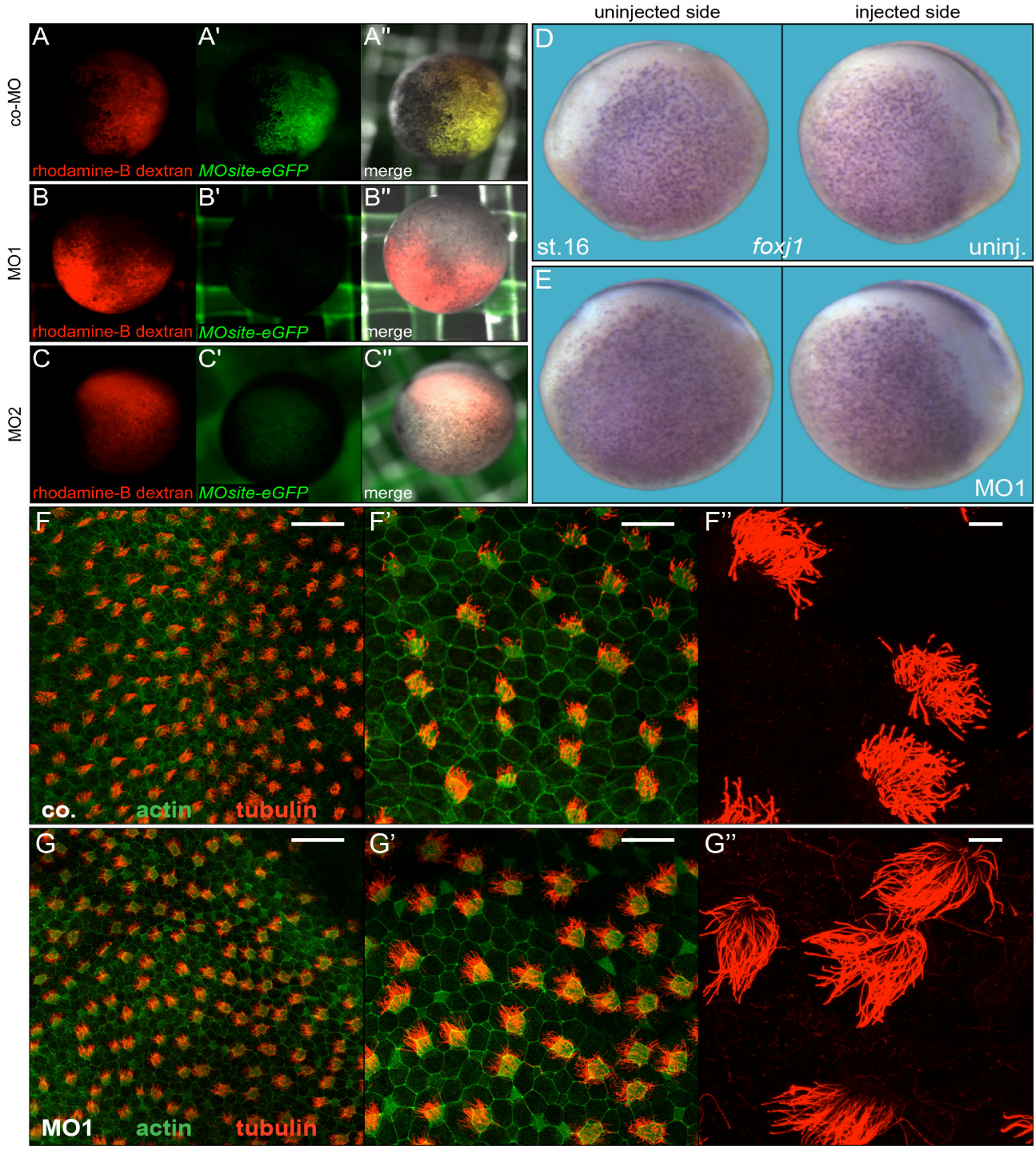


Fig. S3. MO-specificity, *foxj1* expression and ciliogenesis in *Htr3* morphants.

(A-C) MO specificity. MO1 and MO2 specificity was tested using an eGFP-reporter construct. A 60 bp fragment of *Htr3* including the translational start site as well as bindings sites for both MO1 and MO2 (cf. Fig. 1C) was cloned in frame with eGFP (cf. Gessert et al., 2010). Injection into animal blastomeres of 4-cell embryos were performed using rhodamine-B dextran as lineage tracer. (**A**) Co-injection of co-MO did not affect green fluorescence of reporter construct in targeted

regions at stage 9. (**B**, **C**) Absence of green fluorescence upon co-injection of MO1 (B) or MO2 (C) demonstrated MO-specificity.

(D, E) Unaltered *foxj1* mRNA expression in the frog epidermis of *Htr3* morphant. Embryos shown in lateral view, anterior to the left. **(F, G)** Wildtype and morphant epidermis at stage 34 stained for cilia (red) and actin (green) using anti-acetylated-α-tubulin antibodies and phalloidin. MCC number and ciliation were unaffected in morphants (F', G', F'', G''). Increasing magnifications. Scale bars represent 100µm (F, G), 50µm (F', G') and 10µm (F'', G'').

Movie S1. Inhibition of serotonin synthesis reduces velocity of cilia driven mucus flow.

Time-lapse movies (10 seconds at 28.5 fps), showing transport of fluorescent beads at the epidermis of stage 32 tadpole embryos, which were either untreated (control) or incubated with 100µM TPH inhibitor PCPA alone or in combination with 1mM serotonin. Mean and relative (in % of control) velocity of bead movements are indicated.

Movie S2. Low mucus flow velocity in *Htr3* morphant embryos.

Time-lapse movies (10 seconds at 28.5 fps) show transport of fluorescent beads at the epidermis of stage 32 tadpoles. Specimens were untreated (control), injected with MO1 alone, with MO1 and a mutated *Htr3* mRNA (rescue), or with MO2 alone. Mean and relative (% of control) velocity of bead movements are indicated.

Movie S3. Serotonin signaling controls ciliary motility in the tadpole epidermis.

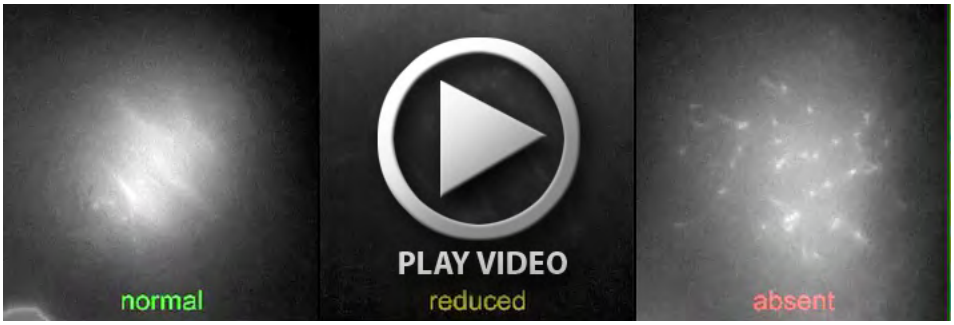
Time-lapse movies (1.7 sec; 0.22 x real time), displaying ciliary motility on wildtype MCCs (left; control), and reduced motility (middle) or virtually immotile cilia (right) in *Htr3* morphants (cf. Fig. 4D). Cilia were visualized by co-injection of 800 pg mRFP mRNA.

Supplemental reference:

Gessert, S., Bugner, V., Tecza, A., Pinker, M. and Kühl, M. (2010). FMR1/FXR1 and the miRNA pathway are required for eye and neural crest development. *Dev Biol* **341**, 222–235.

Movie 1.

Movie 2.



Movie 3.