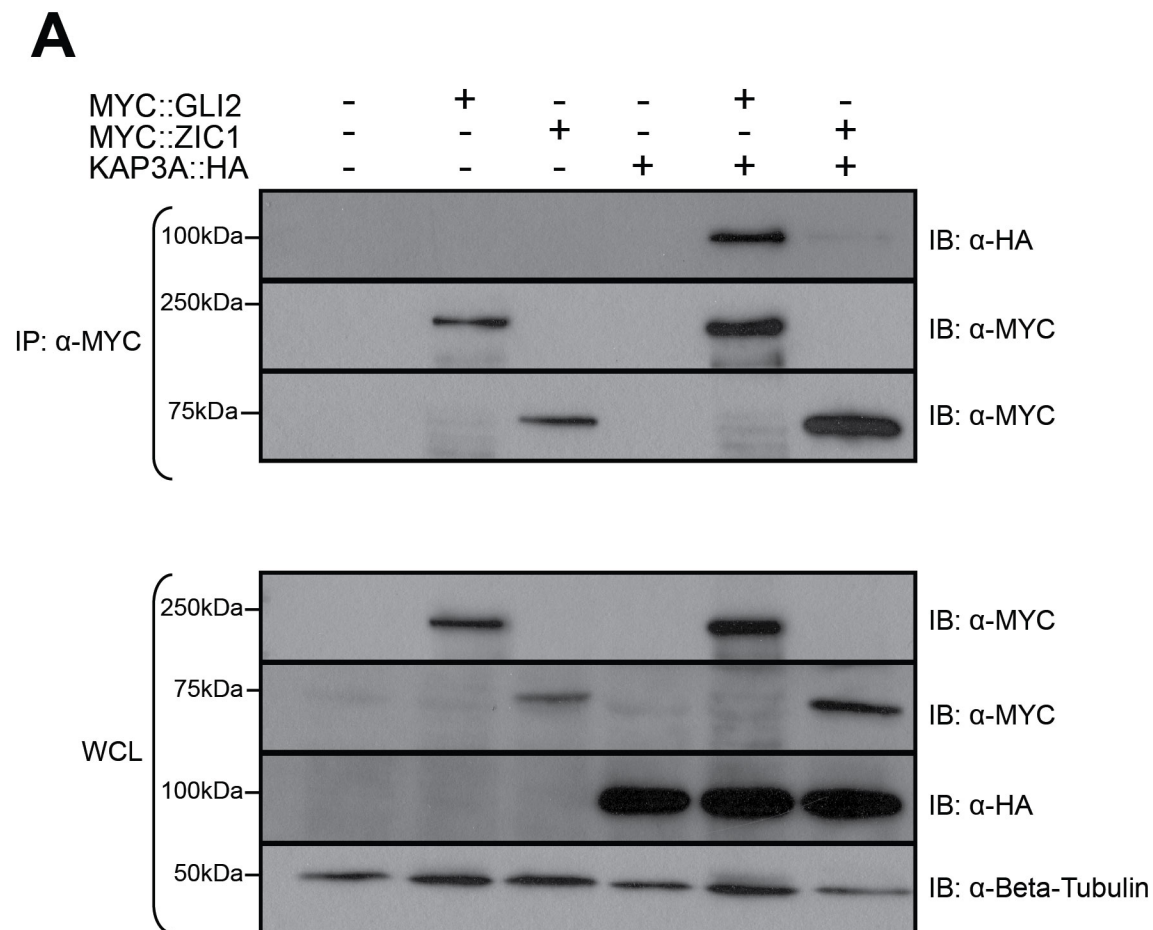**Figure S1. KAP3A co-localizes with mammalian GLI proteins.**

(A) Antibody detection of endogenous GLI3 in NIH/3T3 cells (top row; green) or *Gli2⁻/⁻;Gli3⁻/⁻* MEFs (bottom row; green) expressing HA-tagged KAP3A (KAP3::HA). Primary cilia are identified using antibodies directed against ARL13B (ARL13B; grey). DAPI denotes nuclei (blue). Insets represent high magnification images of primary cilia. Arrows indicate localization of GLI3 in primary cilia. Scale bar, 10µm. (B) Antibody detection of MYC::GLI2 (green) and KAP3A::HA (red) in NIH/3T3 cells. DAPI (blue) identifies nuclei. Scale bar, 10µm.

A

MYC::GLI2	-	+	-	-	+	-
MYC::ZIC1	-	-	+	-	-	+
KAP3A::HA	-	-	-	+	+	+

IP: α-MYC

100kDa—   IB: α-HA

250kDa—   IB: α-MYC

75kDa—   IB: α-MYC

WCL

250kDa—   IB: α-MYC

75kDa—   IB: α-MYC

100kDa—   IB: α-HA

50kDa—   IB: α-Beta-Tubulin

Figure S2. KAP3A does not interact with ZIC1.

(A) Immunoprecipitation of MYC-tagged GLI2 (MYC::GLI2) or MYC-tagged ZIC1 (MYC::ZIC1) from COS-7 cells expressing HA-tagged KAP3A (KAP3A::HA). Immunoprecipitates (IP) and whole cell lysates (WCL) were subjected to SDS-PAGE and western blot analysis (IB) using antibodies directed against MYC (α-MYC) and HA (α-HA). Antibody detection of Beta-Tubulin (α-Beta-Tubulin) was used to confirm equal loading across lanes. The molecular weights (in kDa) of protein standards are indicated at the left of each blot.
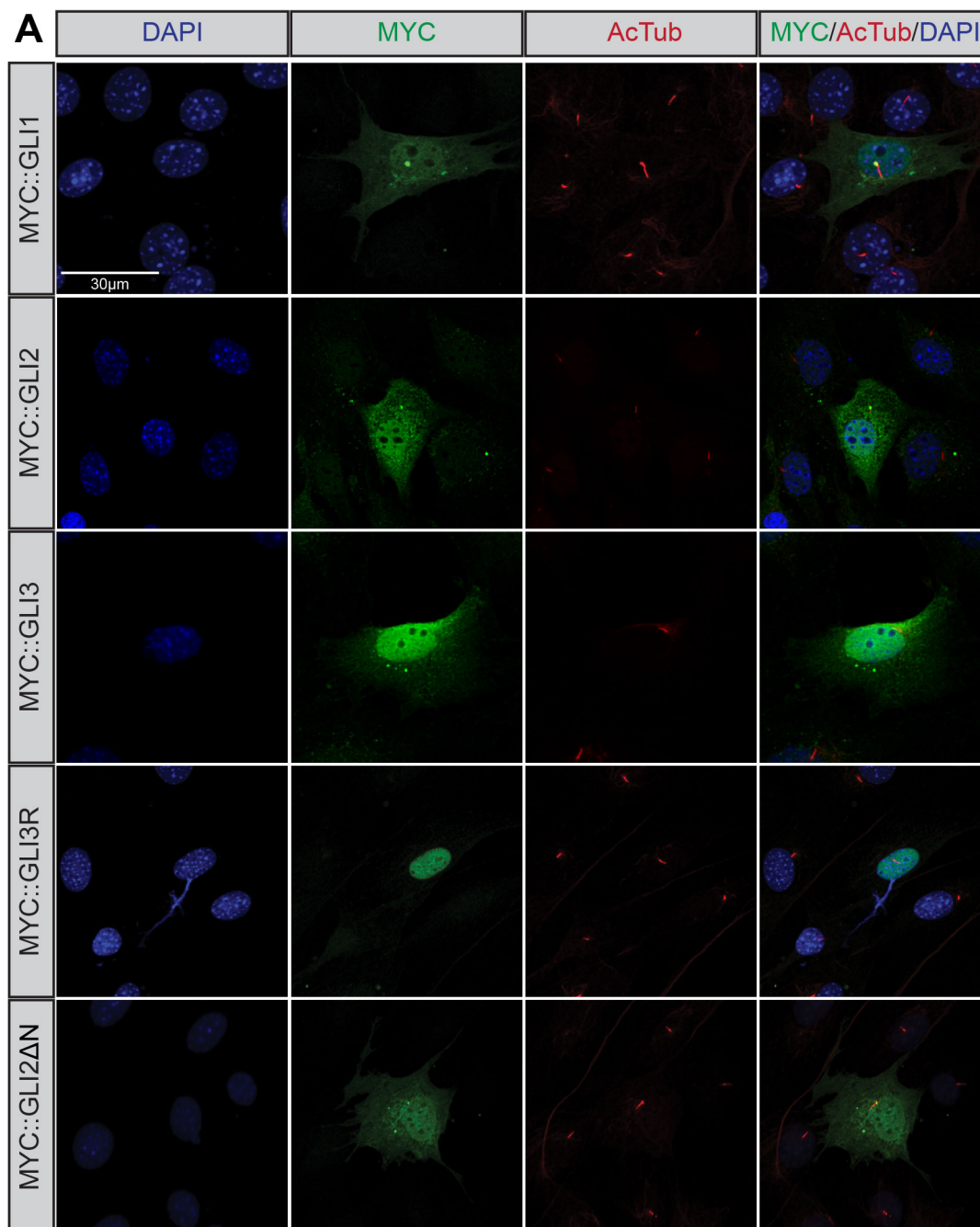
Figure S3. Subcellular localization of MYC-tagged GLI constructs in NIH/3T3 cells.
(A) Localization of MYC::GLI1, MYC::GLI2, MYC::GLI3, MYC::GLI3R, and MYC::GLI2ΔN
in NIH/3T3 cells (green). Primary cilia are identified using antibodies directed against acetylated
tubulin (AcTub; grey). DAPI denotes nuclei (blue). (B) Summary of subcellular localization of
MYC-tagged GLI constructs (N=20 cells). Scale bar, 30μm.
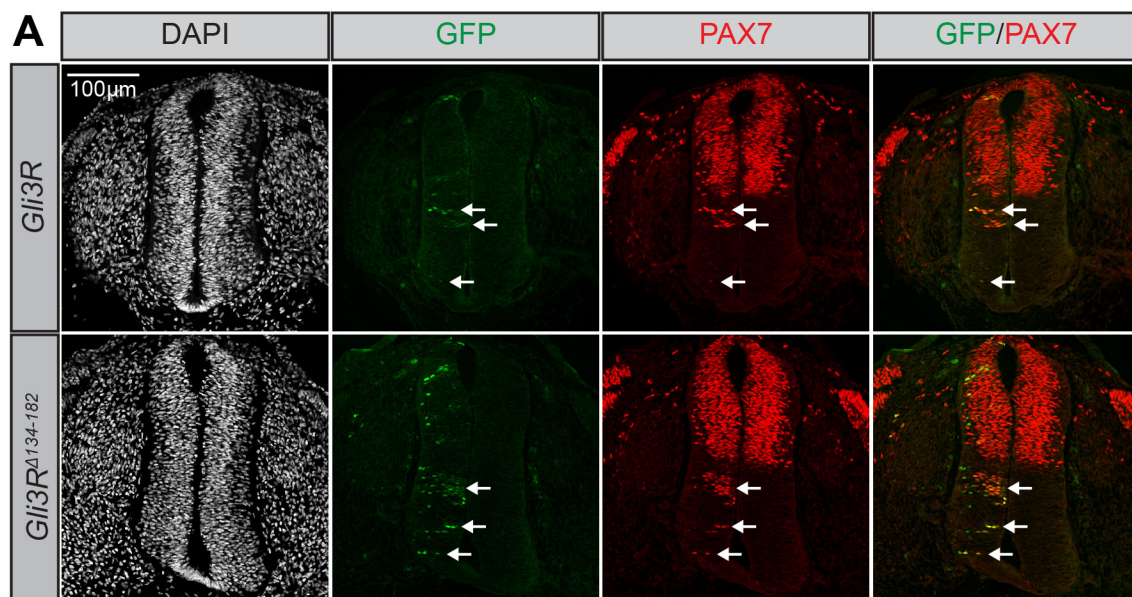
Figure S4. KAP3 binding does not alter GLI3R activity in vivo.
(A) Transverse sections of Hamburger-Hamilton stage 21-22 chicken neural tubes electroporated with Gli3R (top row) or Gli3$^{\Delta134\text{-}182}$ (bottom row) stained with antibodies raised against PAX7 (red). GFP expressing cells (green) denote electroporated cells. DAPI marks nuclei. Arrows indicate ectopic PAX7 expression. Scale bar, 100µm.