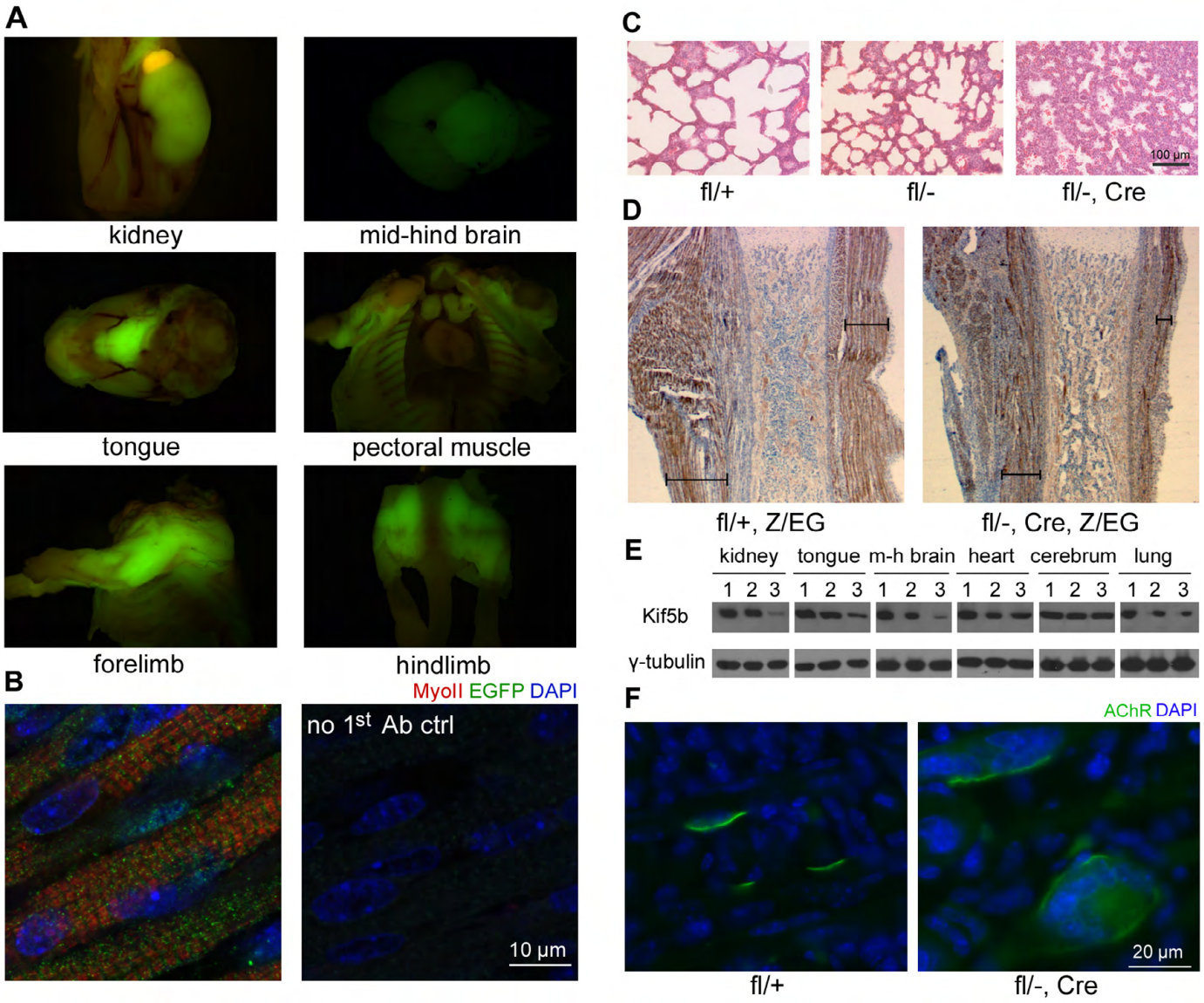

Fig. S1. Generation and characterization of *Kif5b* conditional knockout mice under regulation of *Pax2-Cre*. (**A**) Pax2-Cre-mediated gene knockout in newborn mice. *Pax2-Cre* mice were crossed with *Z/EG* reporter mice. Fluorescence signal indicating Cre activity can be detected in kidney, mid-/hindbrain, tongue, pectoral muscles and limb muscles. (**B**) Pax2-Cre positive cells gave rise to skeletal muscle cells. EGFP and myosin II were double labeled on hindlimb sections in *Pax2-Cre:Z/EG* newborn mice. The EGFP were present in the skeletal muscle cells labeled with myosin II, showing that the skeletal muscles were derivatives of Pax2-Cre expressing cells. (**C**) Lung histology of *Kif5b* conditional knockout mice. Lung sections (paraffin wax embedded) from mutants (*Kif5b*$^{fl/-}$*:Pax2-Cre*) that died soon after birth and control (*Kif5b*$^{fl/+}$ and *Kif5b*$^{+/-}$) littermates were stained with Hematoxylin and Eosin. The lung from dead mutant mice did not expand. (**D**) *Kif5b* conditional knockout mice showed reduced amount of skeletal muscle. EGFP was stained on hindlimb sections in control (*Kif5b*$^{fl/+}$*:Z/EG*) and mutant (*Kif5b*$^{fl/-}$*:Pax2-Cre:Z/EG*) newborn mice. The layer of skeletal muscle (marked by bars) was much thinner in mutant than in control mice. (**E**) Kif5b expression level in 16.5 dpc embryos. Different tissues were taken from *Kif5b*$^{fl/+}$ (lane 1), *Kif5b*$^{fl/-}$ (lane 2) and *Kif5b*$^{fl/-}$*:Pax2-Cre* (lane 3) embryos. Kif5b levels were decreased in kidney, tongue and mid-/hindbrain specifically, but not in the heart, cerebrum and lung. (**F**) AChR expression in newborn mice. Tongue muscles from wild-type control (*Kif5b*$^{fl/+}$, left panel) and mutant (*Kif5b*$^{fl/-}$*:Pax2-Cre*, right panel) were stained with α-bungarotoxin conjugated with Alexa Fluor 488 (green) and DAPI (blue). AChR can be transported to mutant cell membranes normally.

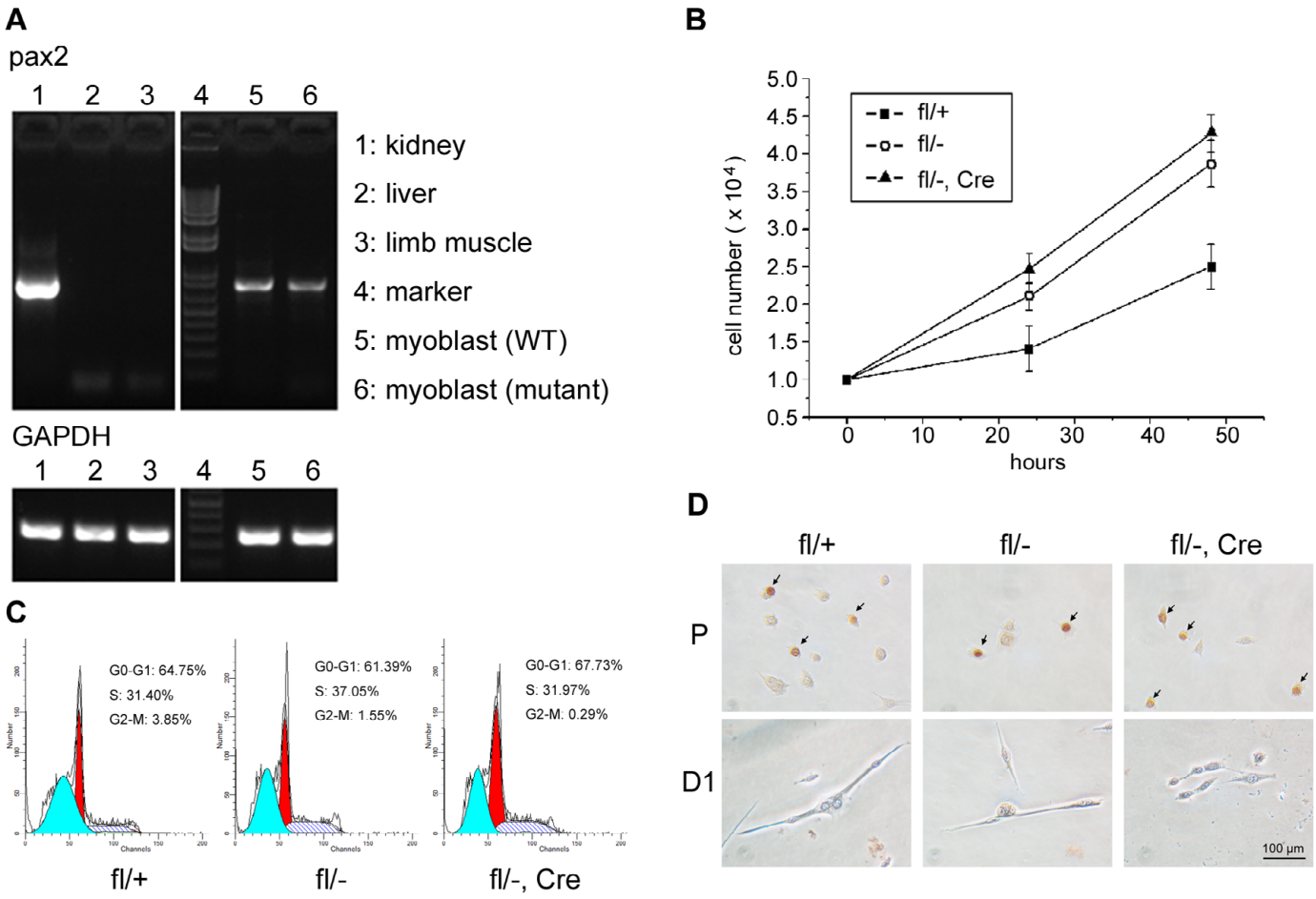

Fig. S2. Kif5b-deficient myoblast cells have normal cell cycle distribution. (**A**) Pax2 expression in myoblast cells. Endogenous Pax2 expression was examined by RT-PCR. Kidney, liver and skeletal muscle was from 7-day-old mouse. Proliferating wild-type or mutant myoblast cells were used in RT-PCR as indicated. GAPDH was used as an internal control. (**B**) Growth rate of myoblast cells. Proliferating myoblast cells from wild type (*Kif5b*$^{fl/+}$, square), heterozygous (*Kif5b*$^{fl/-}$, circle) and mutant (*Kif5b*$^{fl/-}$*:Pax2-Cre*, triangle) mice were seeded at 10,000 cells per 3.5 cm dish. The cells number was counted 24 or 48 hours after seeding to compare the cell growth rate. Data were derived from three independent experiments. (**C**) Analysis of cell cycle distribution by flow cytometry. Proliferating myoblast cells from wild type (*Kif5b*$^{fl/+}$, left panel), heterozygous (*Kif5b*$^{fl/-}$, middle panel) and mutant (*Kif5b*$^{fl/-}$*:Pax2-Cre*, right panel) mice were seeded at 10,000 cells per 3.5 cm dish. After 48 hours, the cells were collected, stained with propidium iodide, and subjected to flow cytometry to monitor the DNA contents (blue, apoptosis; red, G1 peak; shadow, S phase). (**D**) Mutant myoblast cells could exit the proliferation cycle upon differentiation. Wild-type (*Kif5b*$^{fl/+}$, left panel), heterozygous (*Kif5b*$^{fl/-}$, middle panel) and mutant (*Kif5b*$^{fl/-}$*:Pax2-Cre*, right panel) myoblast cells were cultured under proliferation conditions (P, upper panels) or induced to be differentiated for 1 day (D1, lower panels). The cells were labeled with BrdU for 30 minutes before collection to monitor the nucleotide incorporation. Under proliferating conditions, some of the control and mutant cells were labeled with BrdU (brown and arrows). One day after differentiation, no BrdU labeling was found in either control or mutant cells.

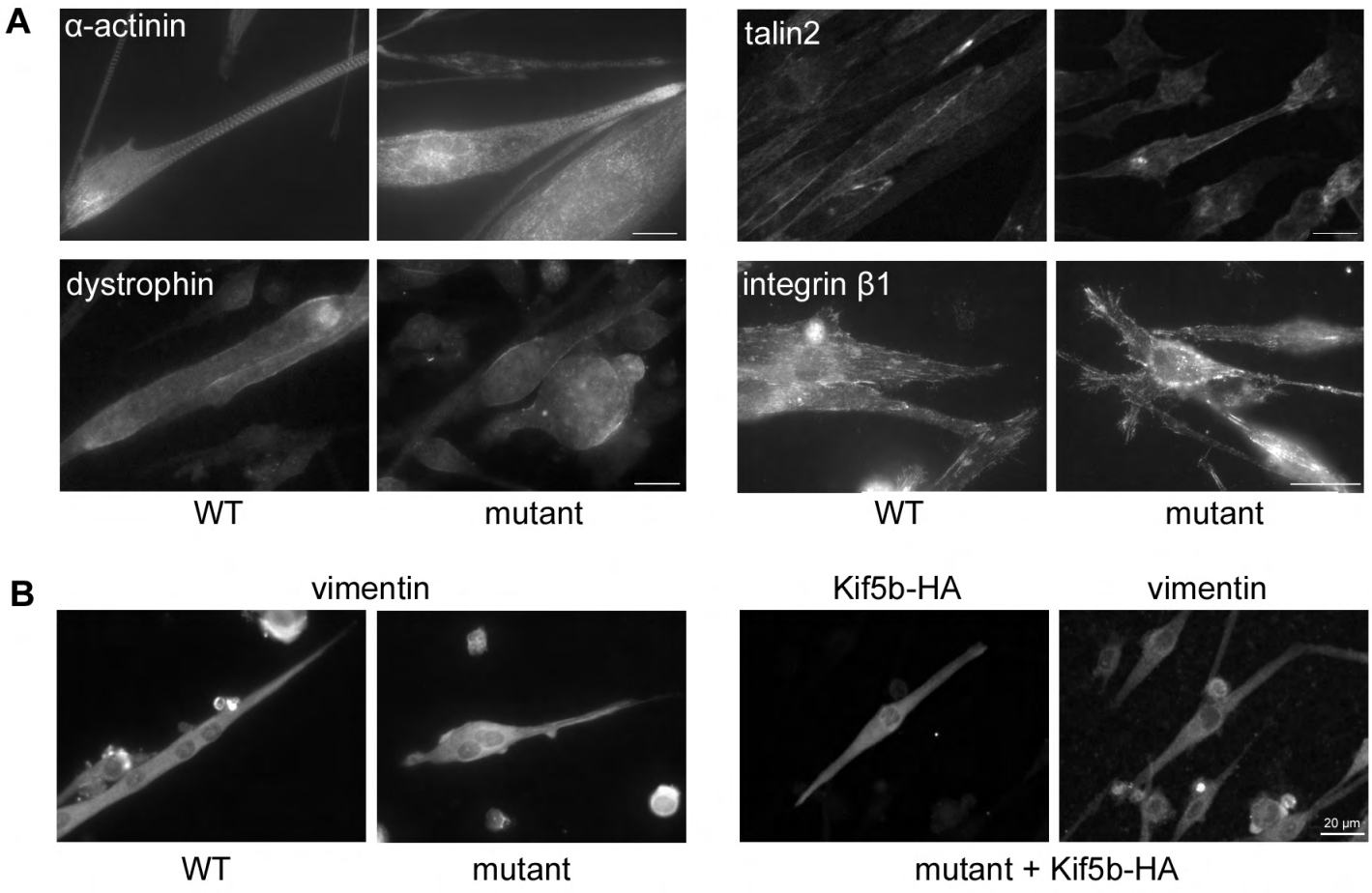

Fig. S3. Localization of marker proteins in differentiating myoblasts. (**A**) Wild-type or mutant myoblast cells differentiated for 3 days were stained with different marker proteins as indicated. No significant difference was observed between wild-type and mutant cells. Scale bar: 20 μm. (**B**) Vimentin distribution is not dependent on Kif5b. Left panels: wild type or mutant myoblast cells differentiated for 3 days were stained with anti-vimentin. Right panels: mutant cells were transfected with Kif5b-HA expression vector upon differentiation, and then double labeled with anti-HA and anti-vimentin as indicated. No significant difference in vimentin distribution was observed between wild type and mutant, or transfected and untransfected mutant cells.

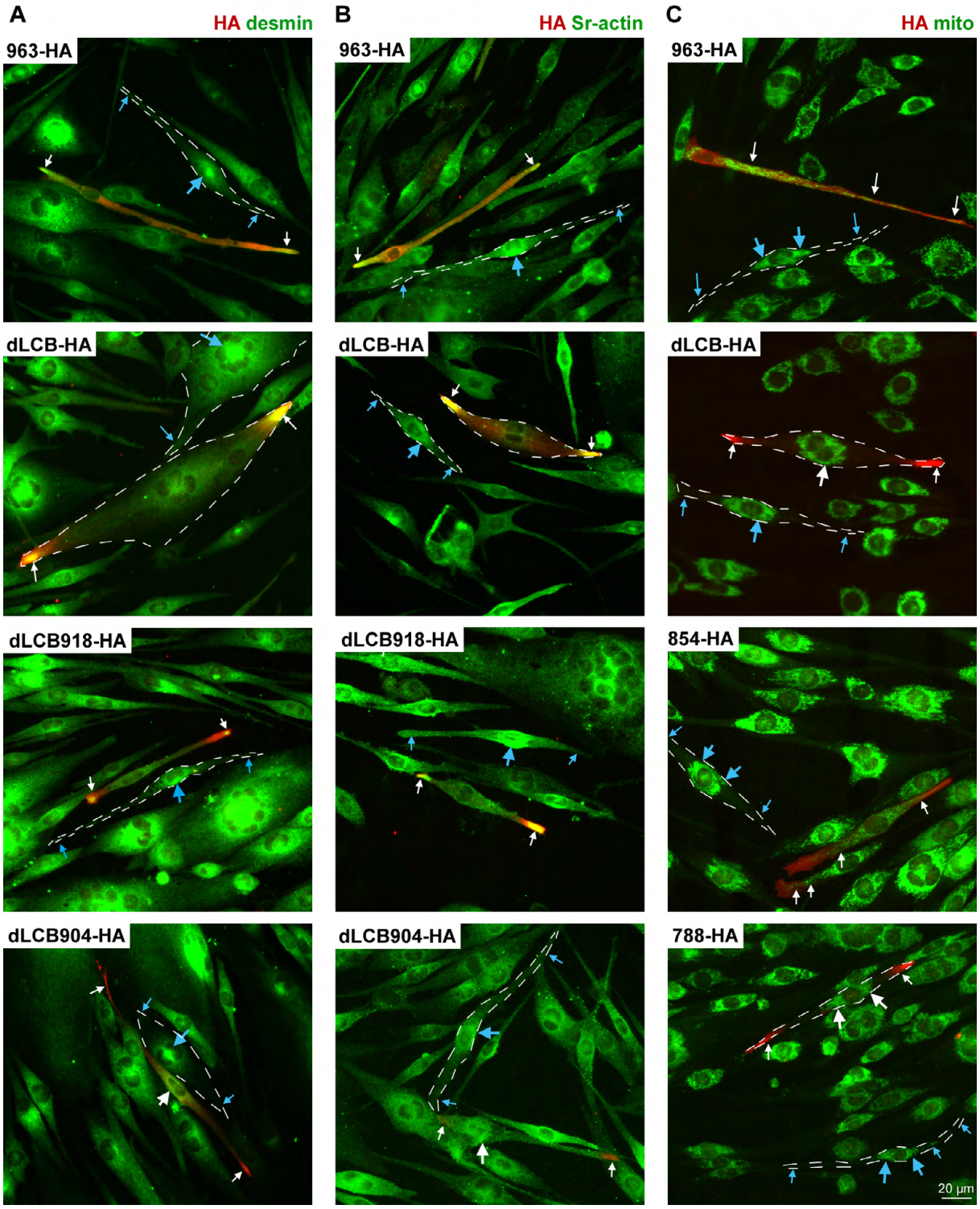

Fig. S4. Kif5b constructs in transportation of desmin, α-sarcomeric actin and mitochondria. (**A**,**B**) Rescue experiments of desmin and α-sarcomeric actin using the constructs 963-HA, dLCB-HA, dLCB918-HA and dLCB904-HA as indicated. 963-HA, dLCB-HA and dLCB918-HA could restore the tip localization of desmin, whereas dLCB904-HA could not. (**C**) Rescue experiments of mitochondria using the constructs 963-HA, dLCB-HA, 854-HA (1-854 amino acids) and 788-HA (1-788 amino acids) as indicated. 963-HA and 854-HA could promote mitochondria dispersion into the myotube shafts whereas dLCB-HA and 788-HA could not. Big and small blue arrows indicate the areas near the nuclei and the tip regions, respectively, in untransfected cells. Big and small white arrows indicate the areas near the nuclei and the tip regions, respectively, in transfected cells. Broken lines show cell boundaries.