SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Effect of PLD1 or PLD2 siRNA on hyperoxia-induced ROS generation. (A) HPAECs were transfected with scrambled, PLD1, or PLD2 siRNA (50 nM, 72 h) and total RNA was isolated, and Real-time PCR was performed as described under "Experimental Procedures." Data are from three independent experiments and expressed as relative gene expression normalized to 18 S RNA. *, $p < 0.05$ compared with scrambled siRNA transfected cells. (**B**) Cell lysates from scrambled, PLD1, or PLD2 siRNA transfected HPAECs were subjected to 10% SDS-PAGE and Western blotted with anti-PLD1, -PLD2, or -actin antibodies. In parallel experiments, HPAECs were transfected with scrambled, PLD1, or PLD2 siRNA as described above, and formation of total ROS was quantified as described under "Experimental Procedures." Values for ROS production are mean $\pm$ S.D. from three independent experiments. * $p < 0.05$ compared to scrambled siRNA cells/normoxia; ** $p < 0.01$ compared to scrambled siRNA cells/ hyperoxia.

Supplemental Figure 2: Hyperoxia activates PLD in HPAECs. (A) HPAECs were labeled with [^{32}P] orthophosphate and then exposed to hyperoxia (1, 2 and 3 h) in the presence of 1-butanol (0.05% v/v) and [^{32}P]PBt formed, as a result of PLD activation, was determined as described under "Experimental Procedures". * Significantly different at $p < 0.05$ compared to normoxia. **(B)** HPAECs were pretreated for 1 h with 0.05 % 1-butanol (but-1) or tertiary-butanol (tert-but), then exposed to normoxia or hyperoxia (3 h) and ROS formation was monitored as described under "Experimental Procedures". * Significantly different at $p < 0.05$ compared to normoxia; **Significantly different at $p < 0.01$ compared to hyperoxia.

Supplemental Figure 3: Role of PLD signaling in Hyperoxia-induced translocation of Rac1 to cell periphery. HPAECs grown in slide chambers were pretreated for 1 h with 0.05 % but-1 or tert-but, then exposed to normoxia or hyperoxia (3 h), washed, fixed, permeabilized, probed with anti-Rac1 antibody, and examined under immunofluorescence microscopy using an X 60 oil objective. Exposure of cells to hyperoxia resulted in redistribution of Rac1 to cell periphery; pretreatment with but-1, but not tert-but, blocked hyperoxia-mediated Rac1 redistribution. Relative redistribution of Rac1 to cell periphery (% of normoxic cells) was calculated using MetaVue software, and values are mean $\pm$ S.D. A representative image from three independent experiments is shown.

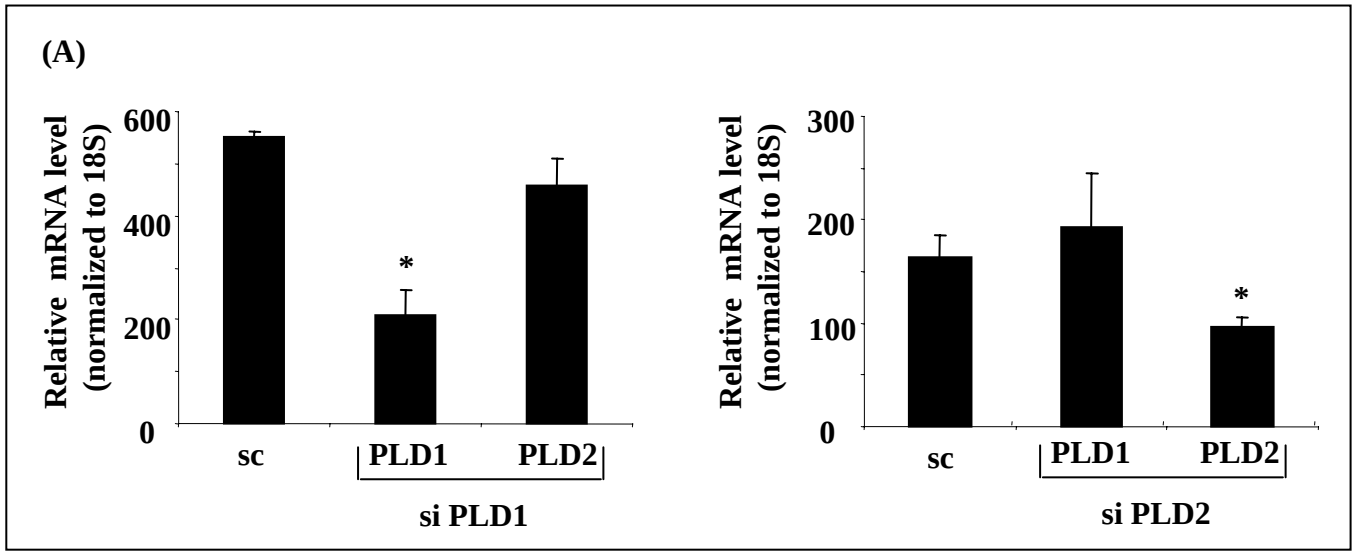
Supplemental Figure 4: Role of PLD signaling in Hyperoxia-induced translocation of IQGAP1 to cell periphery and. HPAECs grown in slide chambers were pretreated for 1 h with 0.05 % but-1 or tert-but, then exposed to normoxia or hyperoxia (3 h), washed, fixed, permeabilized, probed with anti-IQGAP1 antibody, and examined under immunofluorescence microscopy using an X 60 oil objective. Exposure of cells to hyperoxia resulted in redistribution of IQGAP1 to cell periphery; pretreatment with but-1, but not tert-but, blocked IQGAP1 redistribution. Relative redistribution of IQGAP1 to cell periphery (% of normoxic cells) was calculated using MetaVue software, and values are mean $\pm$ S.D. A representative image from three independent experiments is shown.

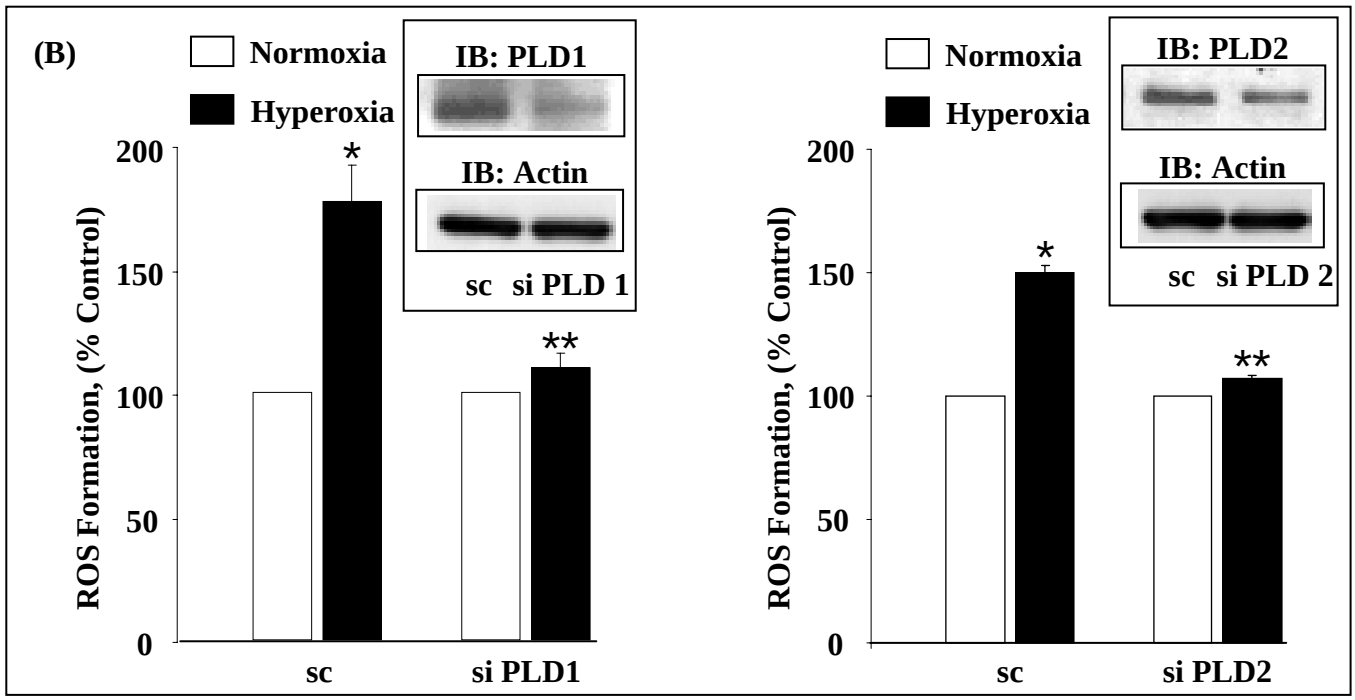
Supplemental Figure 5: Role of PLD signaling in Hyperoxia-induced translocation of cortactin to cell periphery. HPAECs grown in slide chambers were pretreated for 1 h with 0.05 % but-1 or tert-but, then exposed to normoxia or hyperoxia (3 h), washed, fixed, permeabilized, probed with anti-Cortactin antibody, and examined under immunofluorescence microscopy using an X 60 oil objective. Exposure of cells to hyperoxia resulted in redistribution of cortactin to cell periphery; pretreatment with but-1, but not tert-but, blocked cortactin redistribution. Relative redistribution of Cortactin to cell periphery (% of normoxic cells) was calculated using MetaVue software, and values are mean $\pm$ S.D. A representative image from three independent experiments is shown.

Supplemental Figure 6: Role of PLD in Hyperoxia-induced translocation of p47^{phox} to cell periphery. HPAECs grown in slide chambers were pretreated for 1 h with 0.05 % but-1 or tert-but, then exposed to normoxia or hyperoxia (3 h), washed, fixed, permeabilized, probed with anti-p47^{phox} antibody, and examined by immunofluorescence microscopy using a X 60 oil objective. Exposure of cells to hyperoxia resulted in redistribution of p47^{phox} to cell periphery; pretreatment with but-1, but not tert-but, blocked p47^{phox} redistribution. Relative redistribution of p47^{phox} to cell periphery (% of normoxic cells) was calculated using MetaVue software, and values are mean $\pm$ S.D. A representative image from three independent experiments is shown.

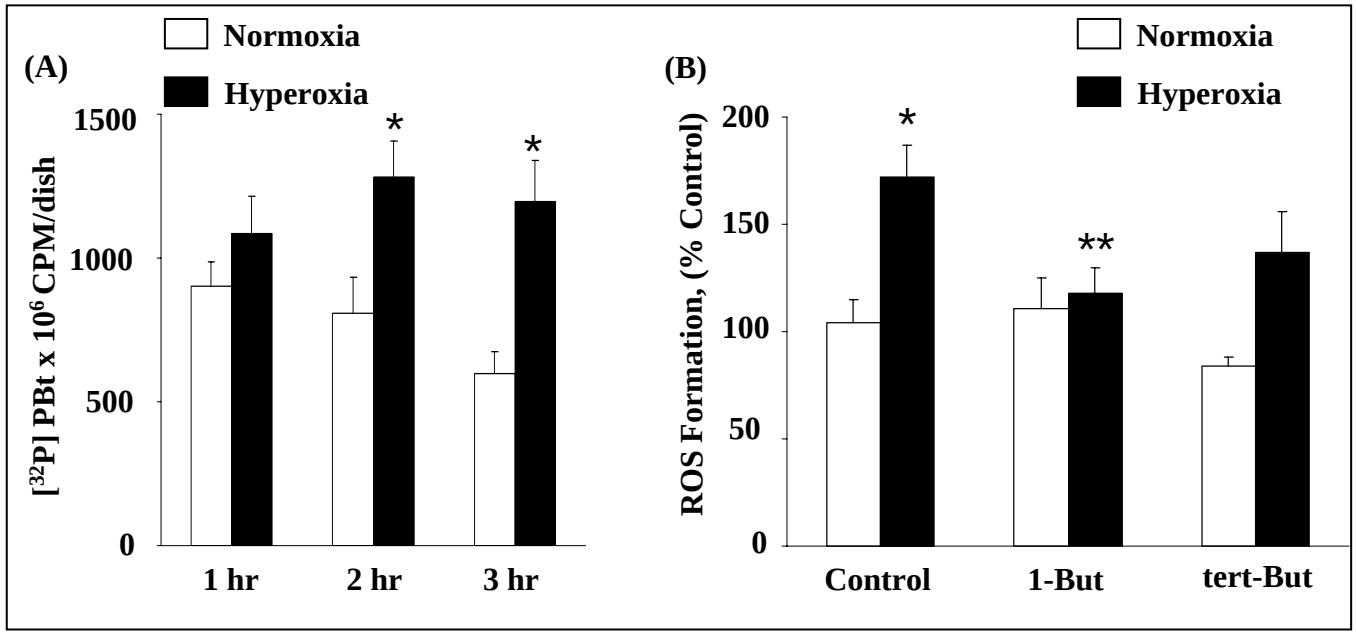
Supplemental Figure 7: Silencing IQGAP1 and Rac1 with siRNA dose not affect expression of other target proteins. HPAECs were transfected with scsiRNA, IQGAP1 siRNA or Rac1 siRNA, and total cell lysates were separated by 4-20 % SDS-PAGE and Western blotted with antibodies as

indicated. Transfection HPAECs with siRNA IQGAP1 or siRNA Rac1 reduced protein expression of IQGAP1 or Rac1, respectively without altering the expression of cortactin, Rac1 or actin. Shown is a representative blot from three independent experiments.

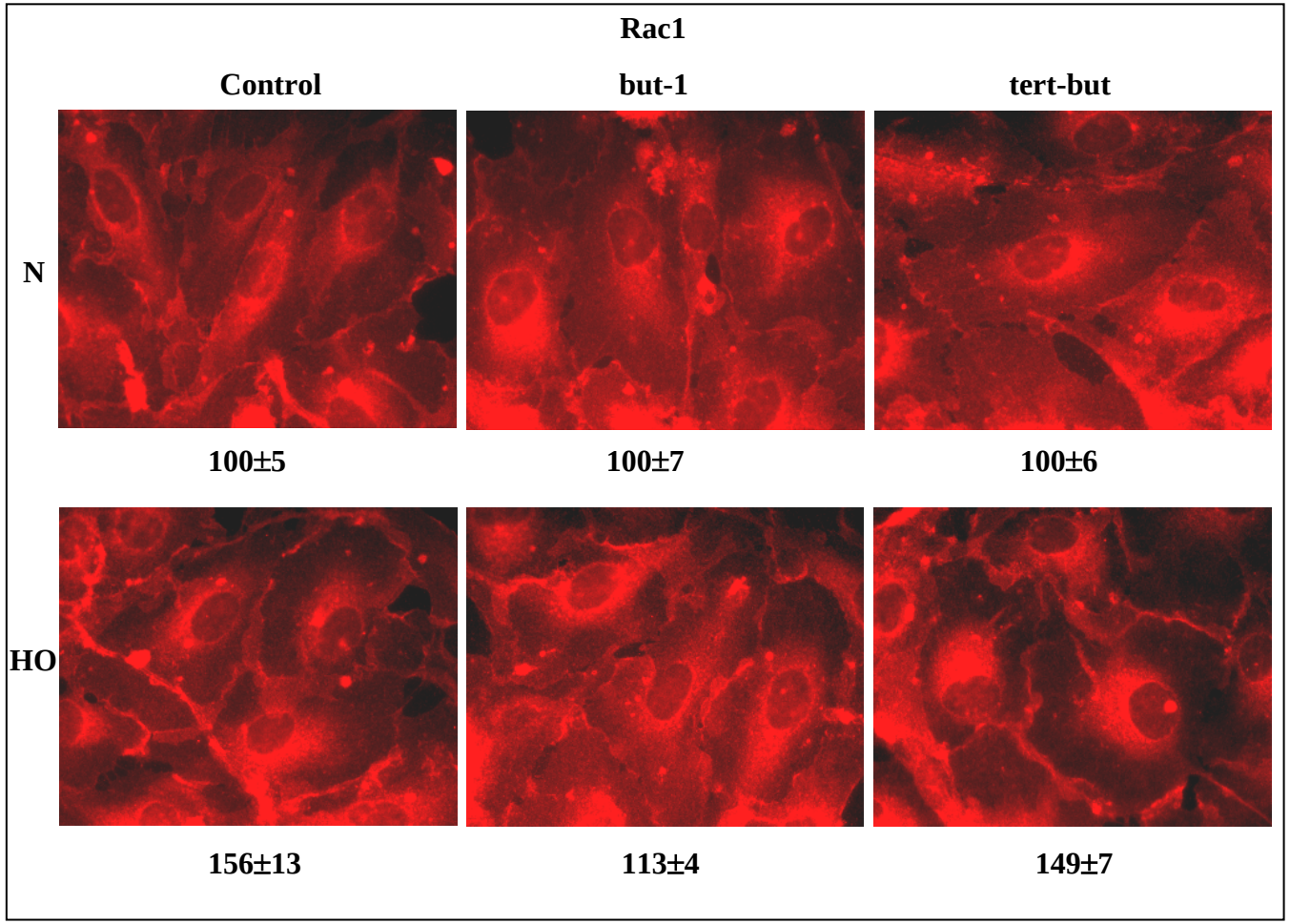




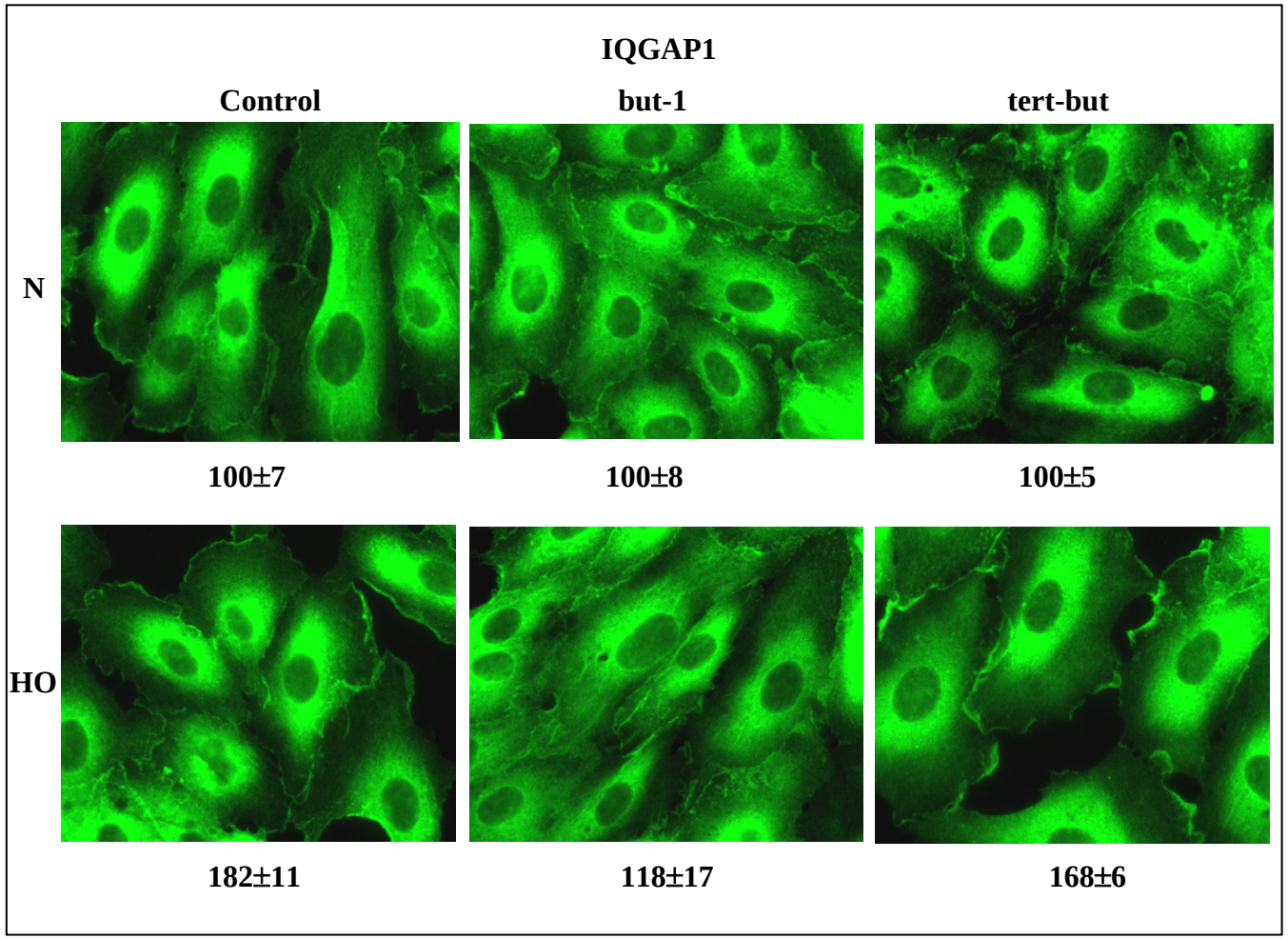
Supplemental Fig. 1 A, B



Supplemental Fig. 2 A, B



Supplemental Fig. 3



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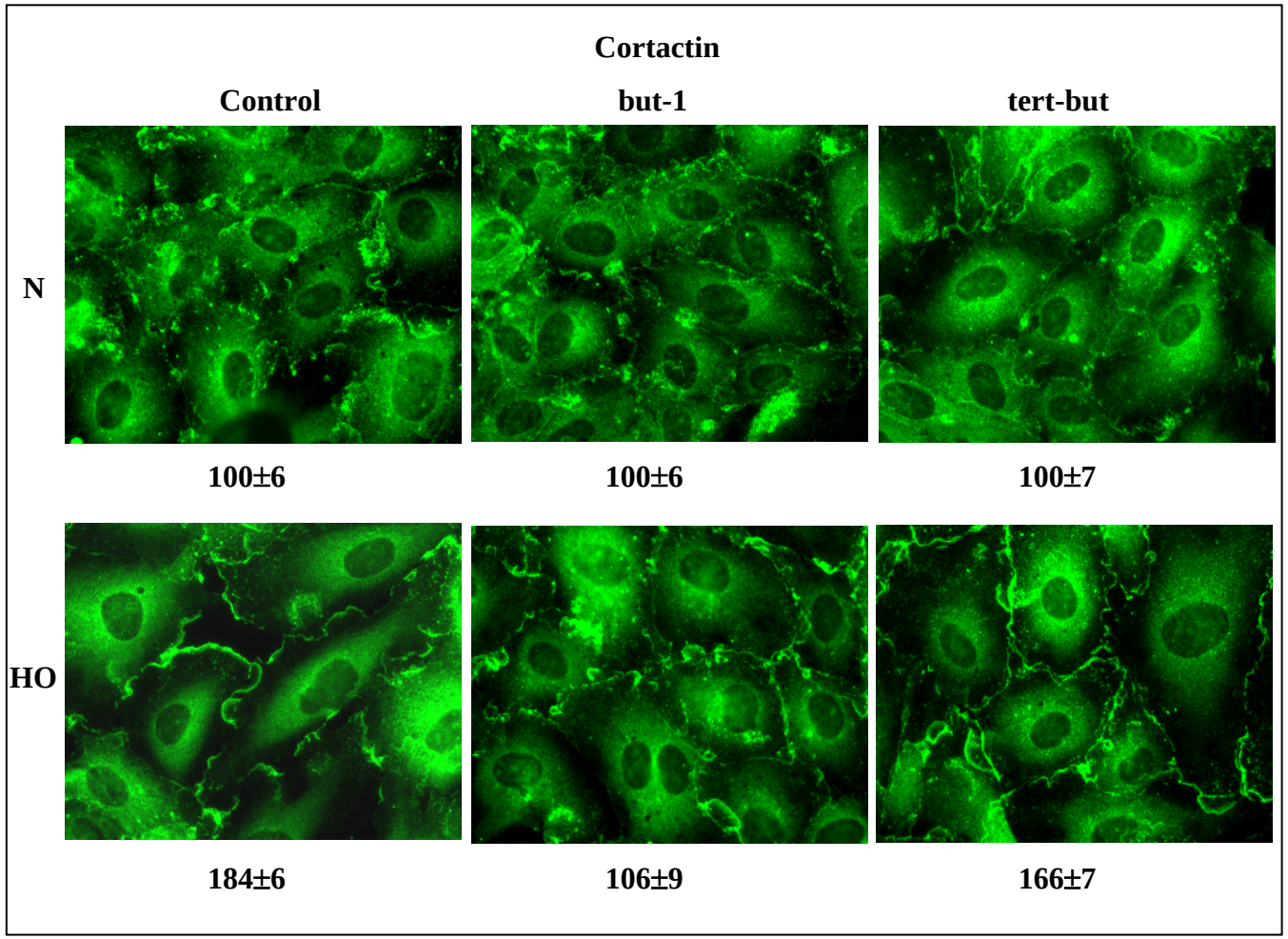

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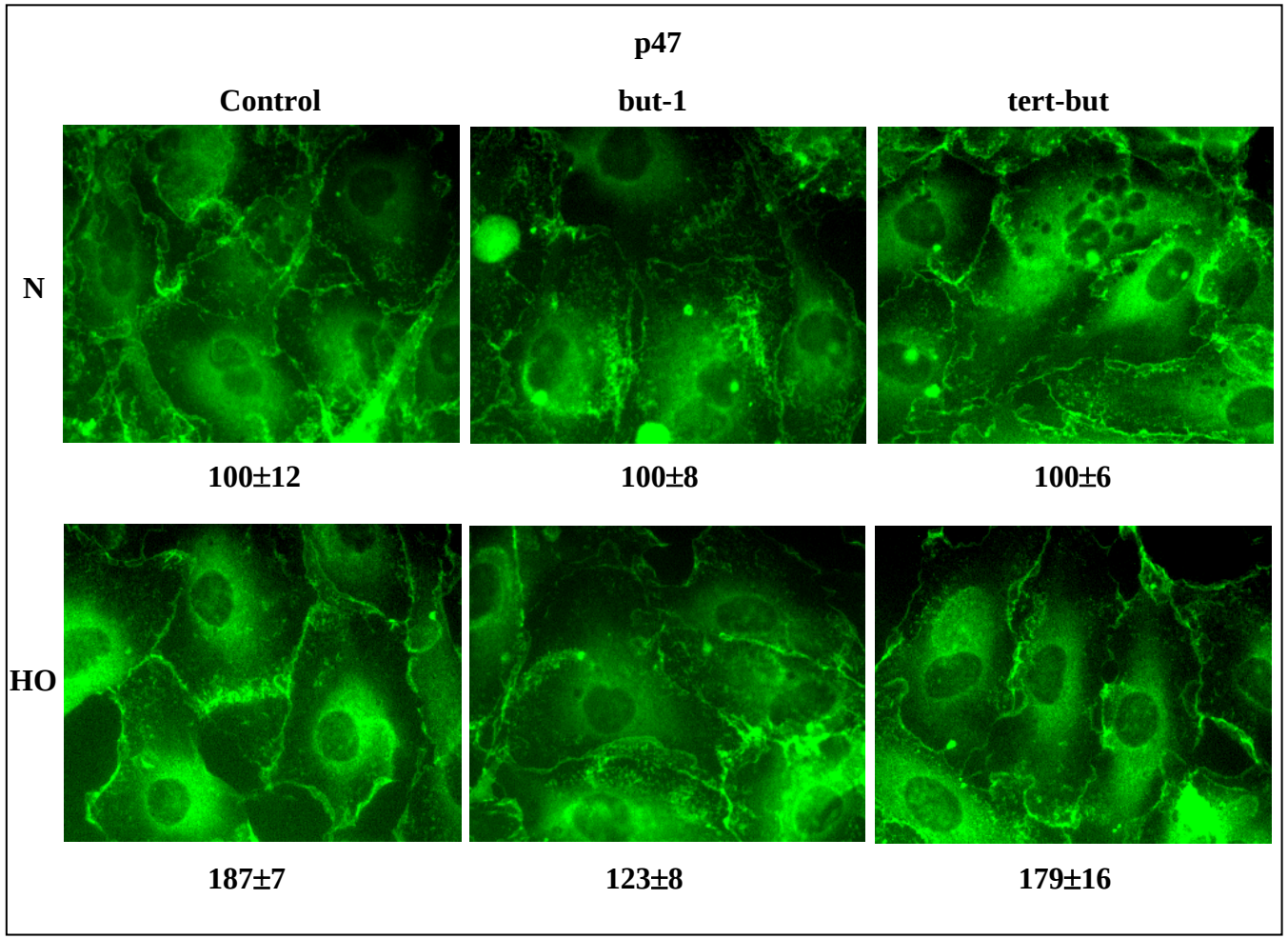

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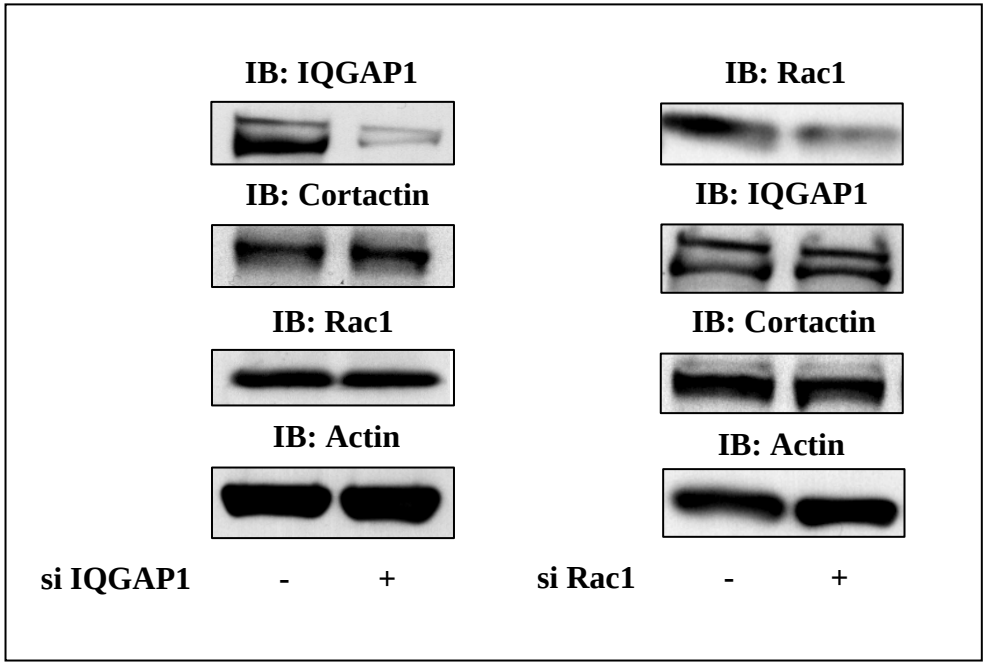

Supplemental Fig. 7