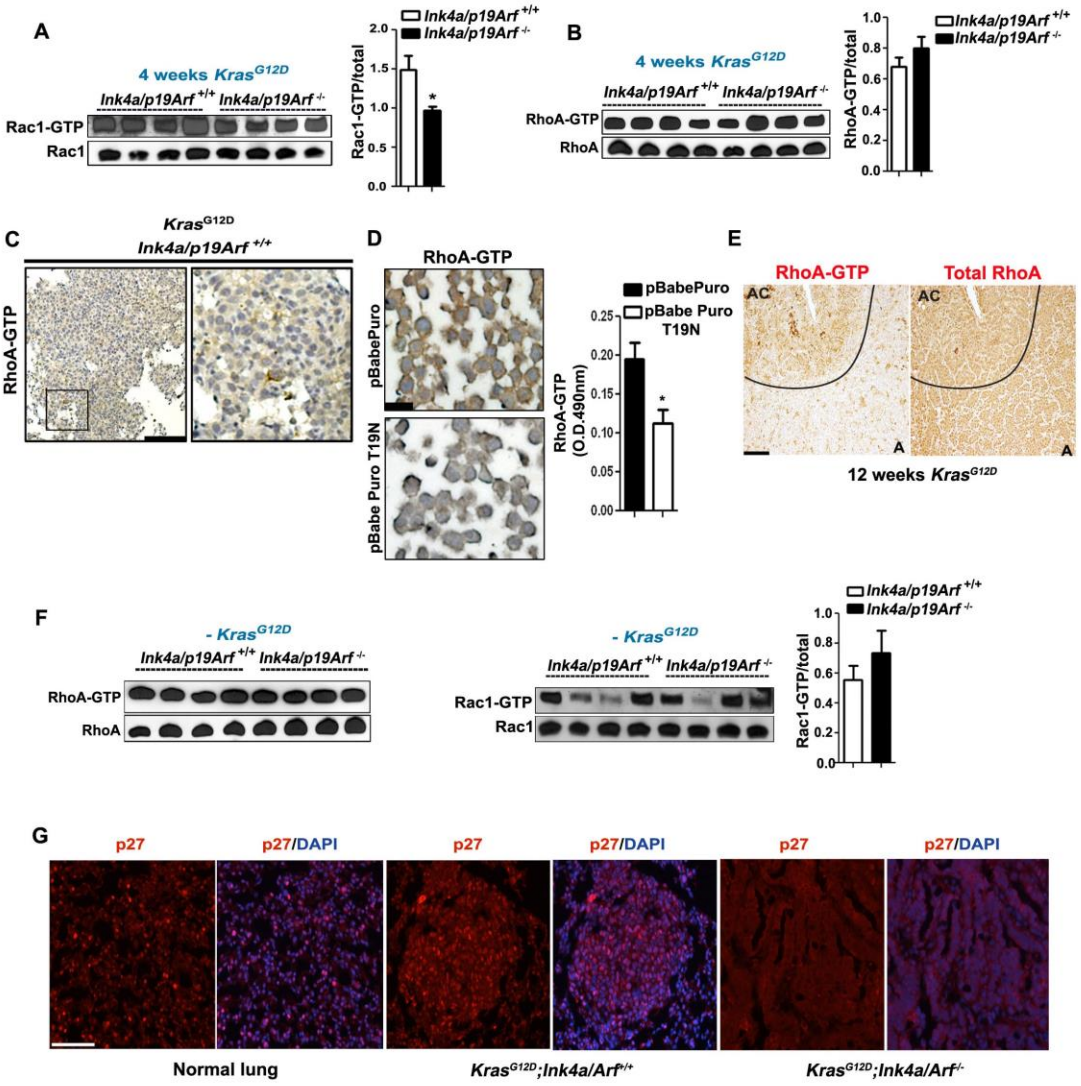
Supplementary Figure 2 Konstantinidou G. et al.



Supplementary Figure S2. RHOA deregulation in lung tumors. (A-B) Rac1-GTP or RhoA-GTP assessed in micro-dissected lung tumors by GST-PAK1 and GST-RBD pulldown respectively followed by immunoblot. Bar graphs provide a quantification of Rac1-GTP or RhoA-GTP. Each lane represents a lysate from a single mouse. **P*<0.05. **(C)** RhoA-GTP IHC staining of a lung section from *Kras*^{G12D}*;Ink4a/Arf* $^{+/+}$ mice after 12

weeks of $Kras^{G12D}$ induction. Inset indicates area selected for the higher magnification image shown in panel on the right; **S**cale bar: 100 μm. **(D)** RhoA-GTP antibody validation for IHC. Left panels: Representative images of RhoA-GTP IHC on mouse cells (LKR13) previously transduced as indicated, formalin-fixed and embedded in paraffin. The paraffin sections were mounted on the same slide in order to treat the samples in the same manner during IHC procedures. T19N is the dominant negative mutant of RhoA. Note that the pBabePuroT19N mutant clearly shows decreased RhoA-GTP staining compared to pBabePuro alone. Scale bar: 10 μm. Right panel: RhoA-GTP levels of LKR13 cells (before paraffin embedding) transduced as indicated. **(E)** RhoA-GTP and total RhoA IHC staining of lung tumors from *$Kras^{G12D}$;Ink4a/Arf $^{-/-}$* mice after 12 weeks of $Kras^{G12D}$ induction. **A**: adenomas. **AC**: adenocarcinomas; scale bar: 100 μm. The IHC clearly shows that RhoA-GTP is present only in the adenocarcinomas while total RhoA is present in both. **(F)** Rac1-GTP or RhoA-GTP assessed in healthy lung tissue from *Ink4a/Arf $^{+/+}$* and *Ink4a/Arf $^{-/-}$* mice by GST-PAK1 and GST-RBD pulldown respectively followed by immunoblot. Bar graphs provide a quantification of Rac1-GTP. Each lane represents a lysate from a single mouse. **(G)** p27/Kip1 IHC staining of normal lung tissue and of the indicated genotypes lung sections. Scale bar: 40 μm.