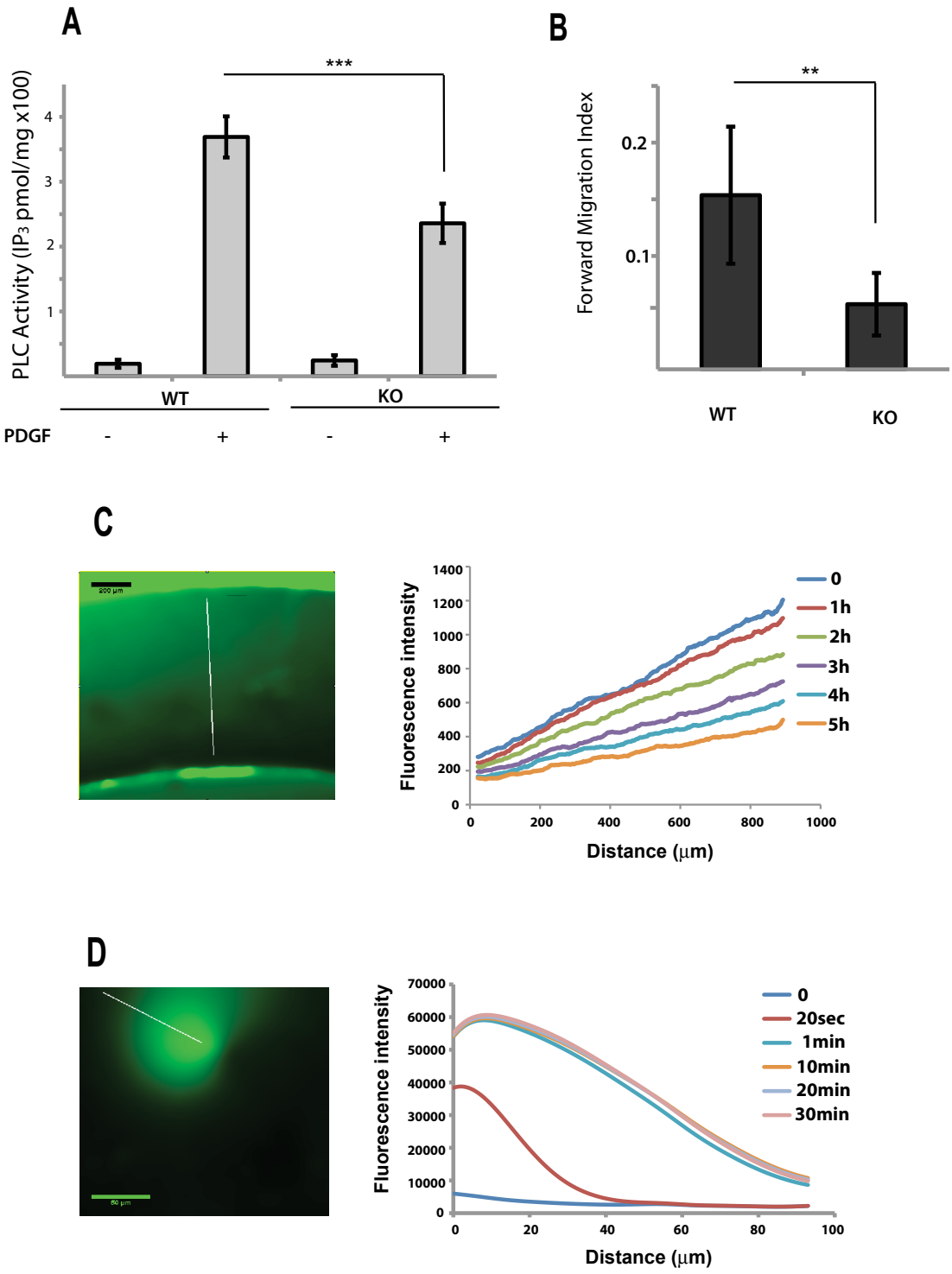

Fig. S1. Acute stimulation and chemotaxis of MEFs triggered by PDGF-BB. A) PLC responses in WT and PLCε KO MEFs. Inositol-phosphate analysis was performed on four different preparations of immortalized MEF populations (n=4); the data are from two independent experiments performed in duplicate, presented as means +/- SD.***, $P < 0.001$, ANOVA. B) Forward migration of WT and PLCε KO MEFs. Forward Migration Index was calculated for four different preparations of immortalized MEF populations (n=4) in five independent experiments. The comparison was performed using Dunn chambers. Data are shown as means +/- SD. ** $P < 0.01$, ANOVA. C) Analysis of gradient in the Dunn chamber. To quantify the gradient in the Dunn chamber Alexa-647 dye conjugated to 10,000MW dextran (similar molecular weight to PDGF-BB) was loaded in the outer annulus of the chamber and allowed to settle for 30 minutes. The fluorescence was monitored at 10 minute intervals over five hours. The fluorescence profile across the chamber (left, white line) is shown (right) at hour intervals. The gradient appears relatively constant across the chamber and as expected slowly reduces in magnitude over the time course as the PDGF-BB concentration in the two annuli begins to equilibrate. D) Analysis of gradient released by micropipette. Alexa-647-Dextran was loaded into a micropipette. The micropipette was positioned just above a coverslip and 420 hPa pressure applied. The fluorescence in the plane of the cell was monitored at 20 second intervals over 30 minutes. The fluorescence profile near the needle (left, white line) is shown. A stable gradient is established after one minute and appears stable over 30 minutes.

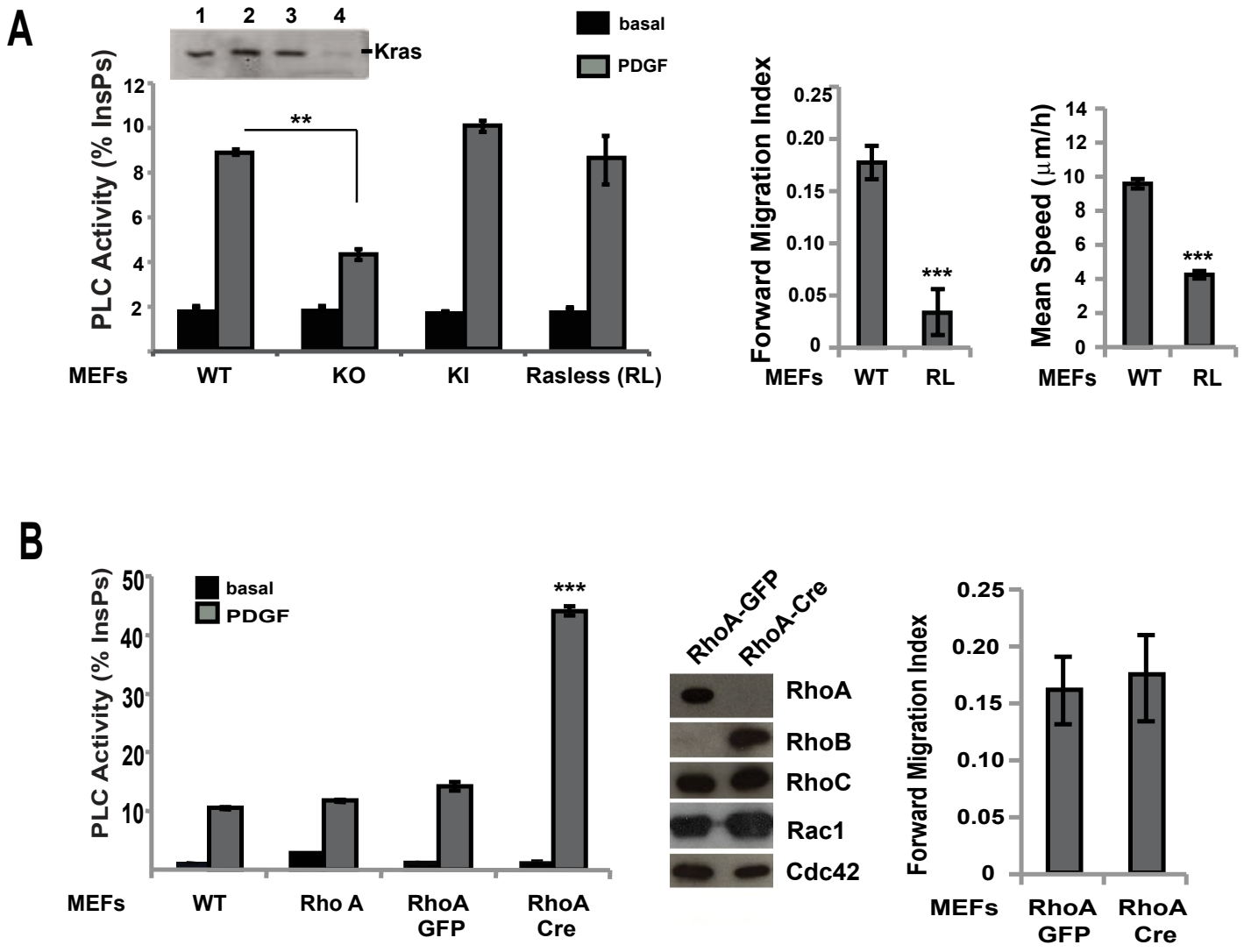

Fig. S2. A) PLC activity was determined in the WT (WT), PLCε KO (KO) and MEFs expressing PLCε RAm variant (KI) and in MEFs deficient in H- and N- Ras with further depletion of *KRas flox/flox* following infection with Ad-Cre (Rasless, RL). Immortalized cell populations were used. The PLC activity was measured in non-stimulated cells (basal) and cells stimulated by PDGF-BB (PDGF); ***P*<, t-test. Comparable PLC activity in WT and KI MEFs in this experiment is consistent with data from three independent preparations of immortalized cell populations (n=3, P<0.001, ANOVA). Expression of KRas analyzed by Western blotting (left panel). Forward Migration Index and Mean Speed of the WT (WT) and Rasless (RL) MEFs were calculated using Dunn chamber. *** *P*<0.001, ANOVA. B) PLC activity was measured in *Rhoa flox/flox* population of MEFs following immortalization (RhoA) and subsequent expression of GFP alone (RhoA GFP) or Cre (RhoA Cre). *** *P*<0.001, t-test. Expression of indicated small GTPases was analyzed by Western blotting (left panel). Forward Migration Index of RhoA GFP and RhoA Cre MEFs was calculated using Dunn chamber (right panel).

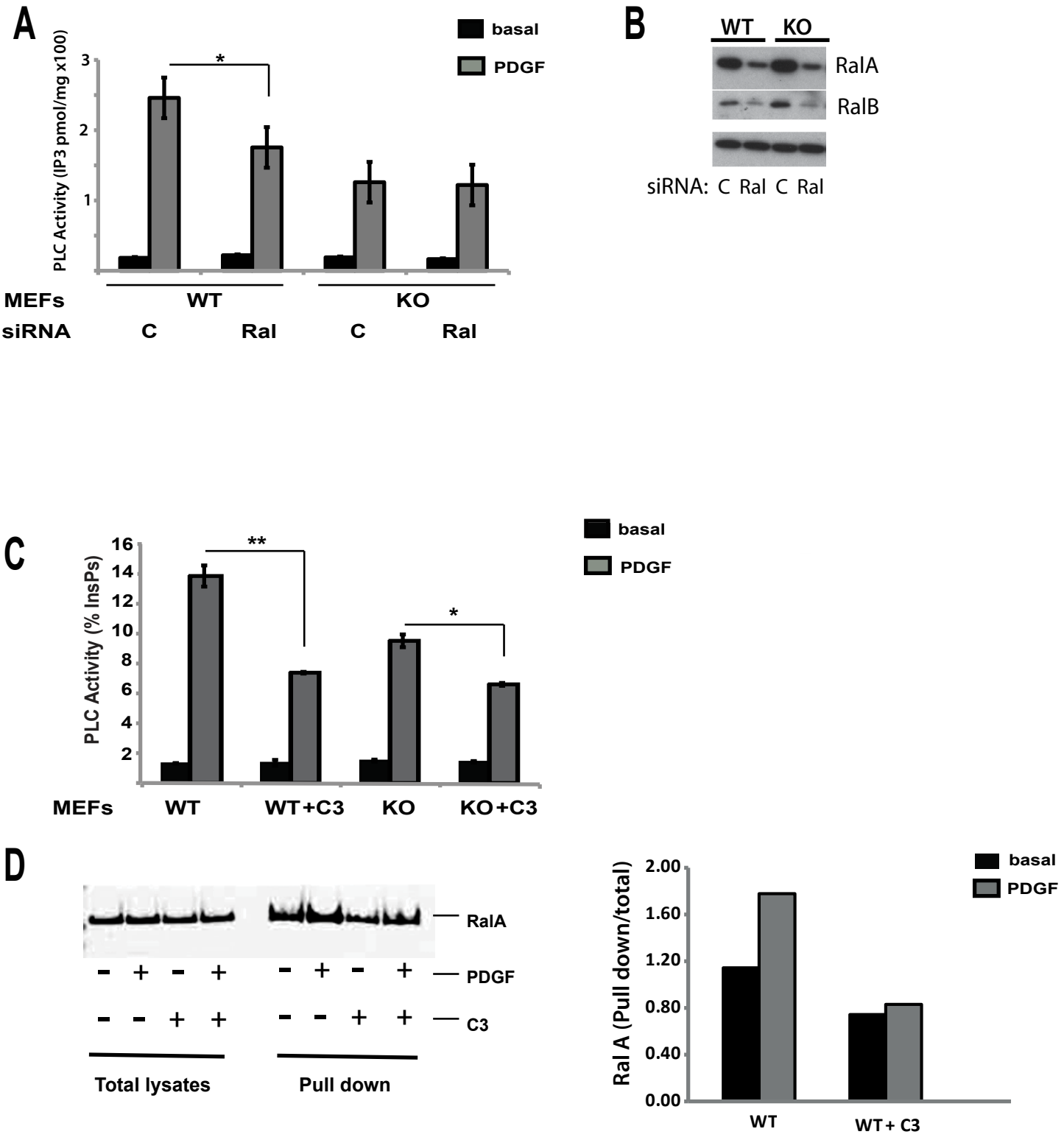

Fig. S3. A) Basel and PDGF-BB stimulated PLC activity in immortalized populations of WT and PLCε KO MEF treated with either 200 nM of non-targeting si RNA (control, C) or siRNAs specifically targeting both, RalA and RalB (Ral). B) Expression of RalA and RalB in cells described in A was analyzed by Western blotting. C) Basal and PDGF-BB stimulated PLC activity in the immortalized populations of WT and PLCε KO MEFs; control cells and cells pretreated with *C. botulinum* C3 exoenzyme (+C3) were analyzed (left panel). D) Analysis of total and GTP-bound RalA in WT MEFs before and after treatment with C3 exoenzyme and/or PDGF-BB as indicated. The Western blotting was performed using total cell lysates and pull-downs obtained by GST-RalBP-1-RBD (left), followed by quantification (right). Differences indicated in the graphs correspond to **P*<0.05; ***P*<0.01, t-test.