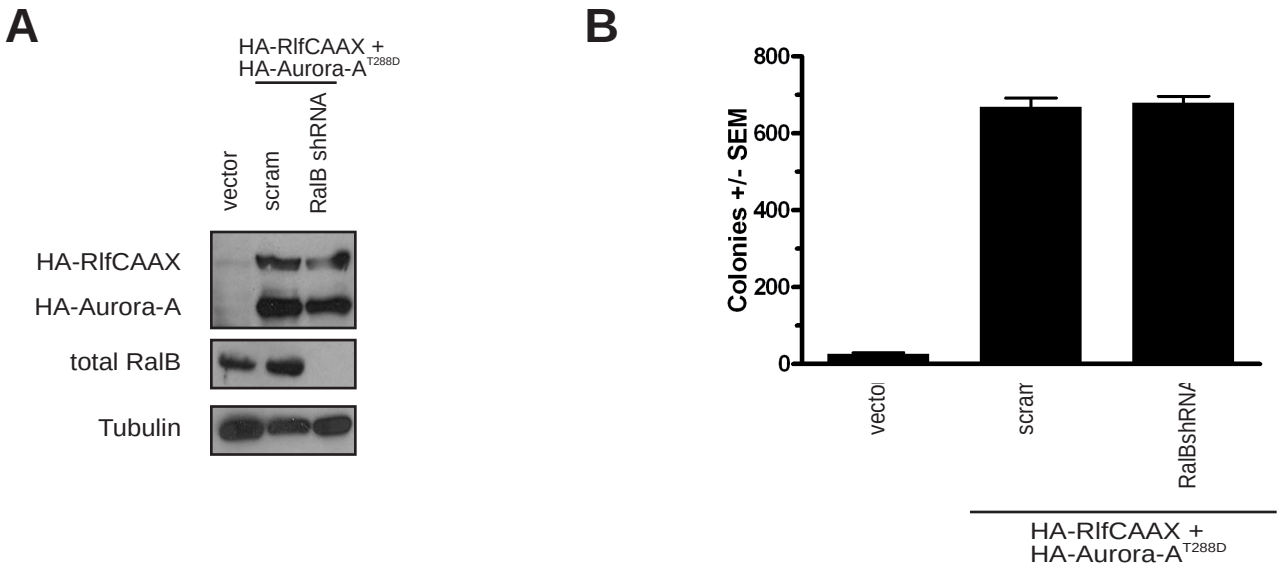
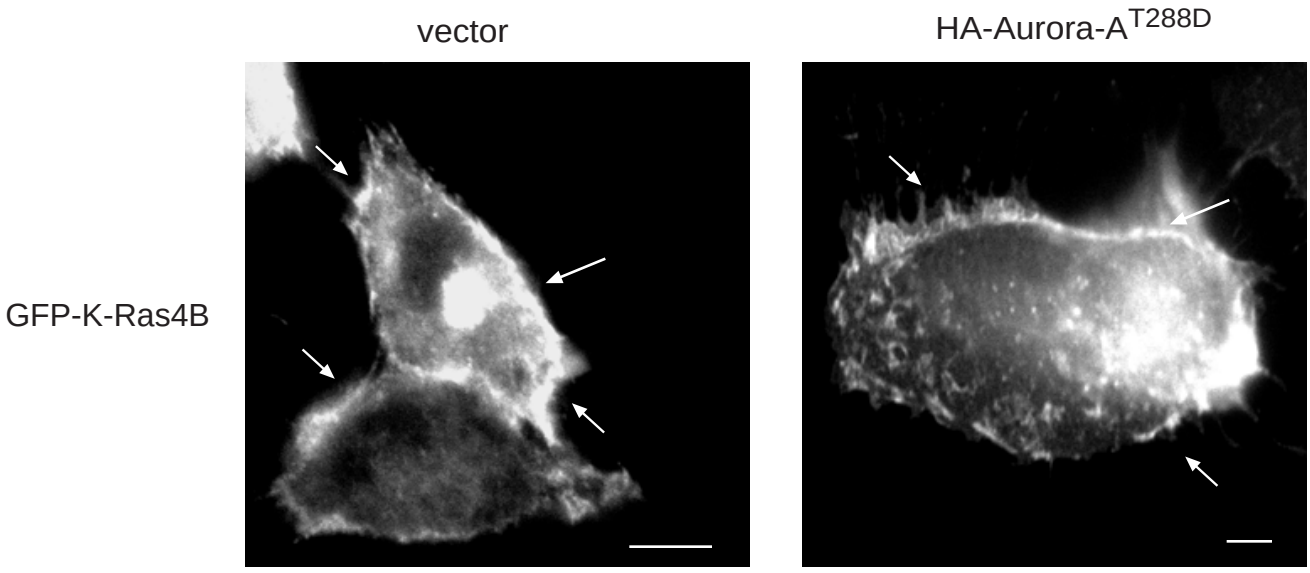


Supplemental Figure 1. Aurora-A potentiation of RalGEF-transformation does not require RalB

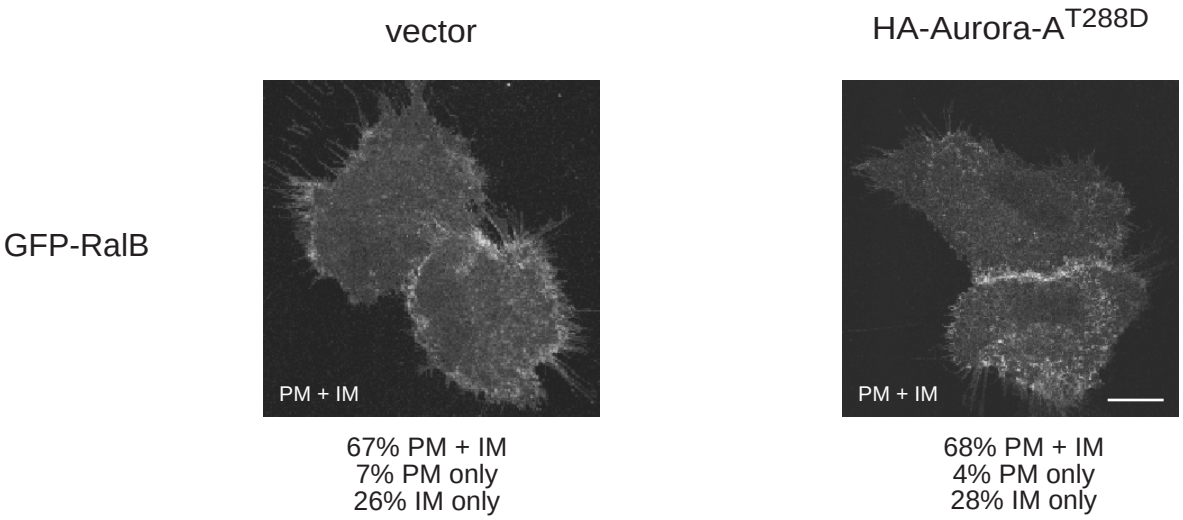
(A) Appropriate expression, as detected by immunoblot, of HA-Aurora-A^{T288D}, HA-Rlf-CAAX, knock down of endogenous RalB in HEK-TtH cells stably infected with retroviruses encoding no transgene (vector) or the indicated transgenes in the presence of shRNA specific to RalB or a scrambled version (scram) of this sequence. Actin serves as a loading control.

(B) Anchorage-independent growth in soft agar of aforementioned polyclonal HEK-TtH cells infected with retroviruses encoding the indicated shRNAs and transgenes, expressed as average colonies ± SEM from six plates (two independent experiments conducted in triplicate).



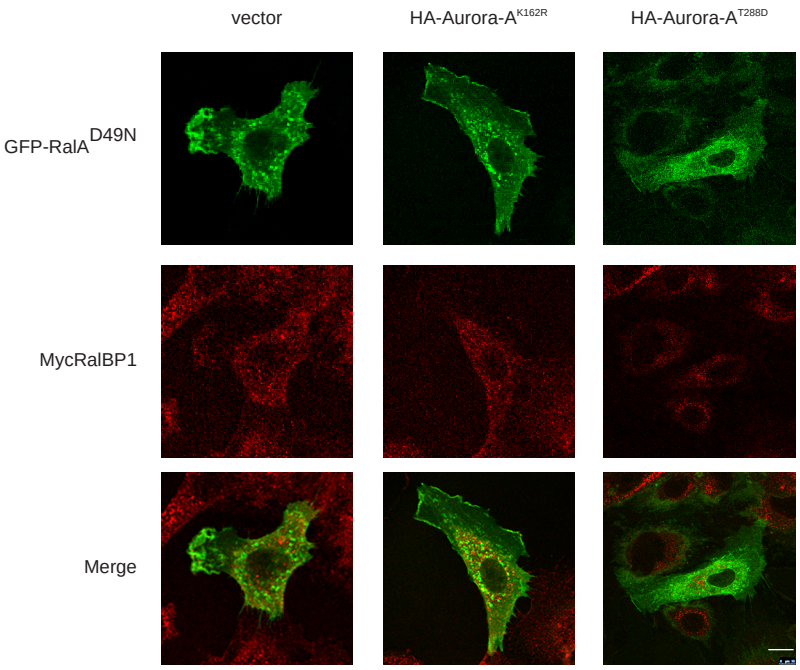
Supplemental Figure 2. K-Ras localization is unchanged upon expression of Aurora-A^{T288D}

Distribution of GFP-K-Ras4B in HEK-TtH cells stably expressing vector or HA-Aurora-A^{T288D}. arrows: plasma membrane localization. scale bar: 20μm.

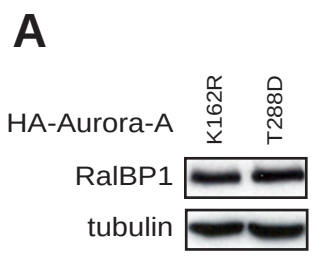


Supplemental Figure 3. RalB localization is unchanged upon expression of Aurora-A^{T288D}

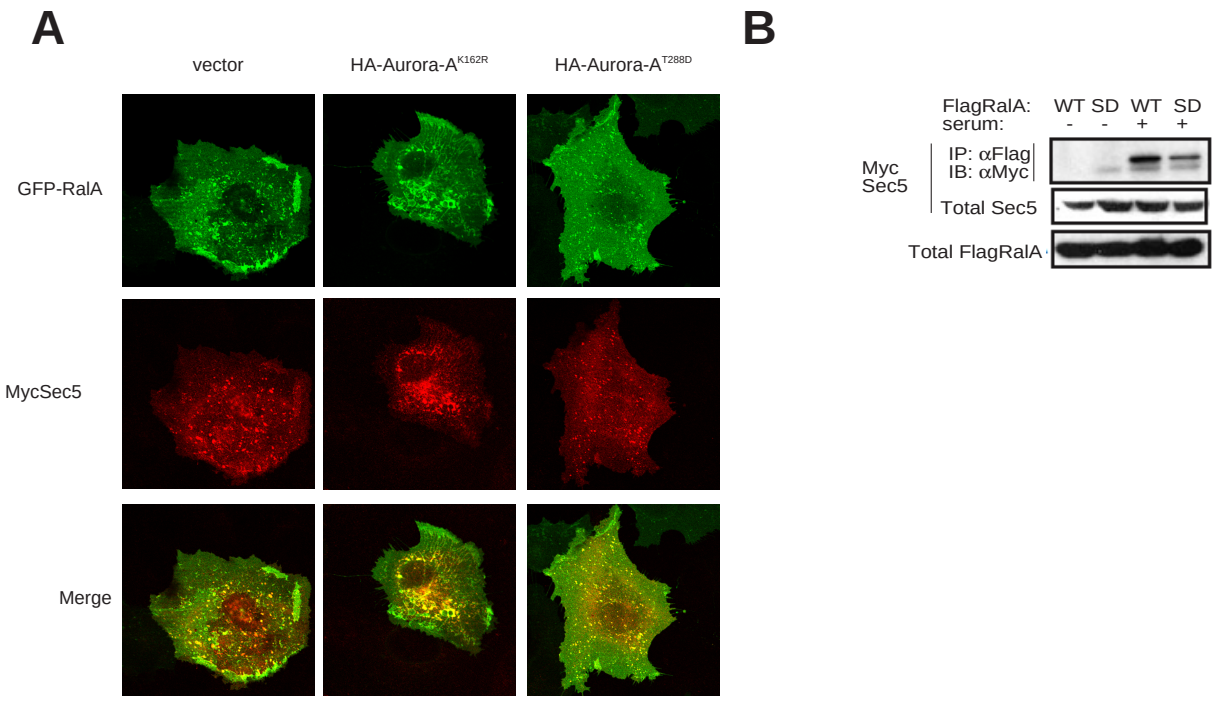
Distribution of GFP-RalB in HEK-TtH cells stably expressing vector or HA-Aurora-A^{T288D}. GFP-RalB locazation to plasma membrane + internal membrane (PM+IM), plasma membrane only (PM), or internal membrane only (IM) was quantitated in 50 cells in two independent experiments for each condition. Representative images with the primary location are displayed. arrows: plasma membrane localization. scale bar: 20μm.



Supplemental Figure 4. Aurora-A expression does not alter RalBP1 localization in prescence of effector domain mutant of RalA^{D49N}
Distribution of GFP-RalA^{D49N} and Myc-tagged RalBP1 (MycRalBP1) visualized by immunofluorescence using an α-Myc antibody in HEK-TtH cells stably expressing either vector control, kinase-inactive HA-Aurora-A^{K162R} or kinase-active HA-Aurora-A^{T288D}. scale bar: 20μm.

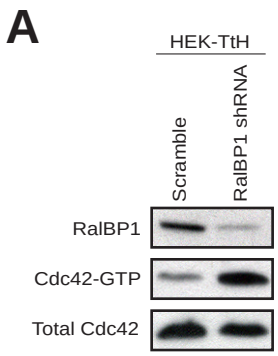


Supplemental Figure 5. Expression of activated Aurora A does not change the levels of RalBP1. (A) RalBP1 and tubulin levels in HEK-TtH cells stably expressing either HA-Aurora A^{K162} or HA-Aurora A^{T288D}.

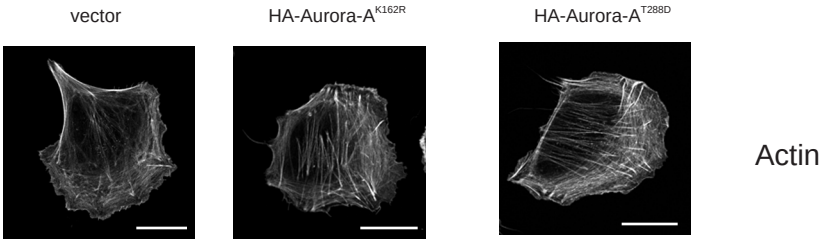


Supplemental Figure 6. Aurora-A expression does not alter Sec5 localization or association with RalA
(A) Distribution of GFP-RalA and Myc-tagged Sec5 (MycSec5) visualized by immunofluorescence using an α-Myc antibody in HEK-TtH cells stably expressing either vector control, kinase-inactive HA-Aurora-A^{K162R} or kinase-active HA-Aurora-A^{T288D}. scale bar: 20μm.

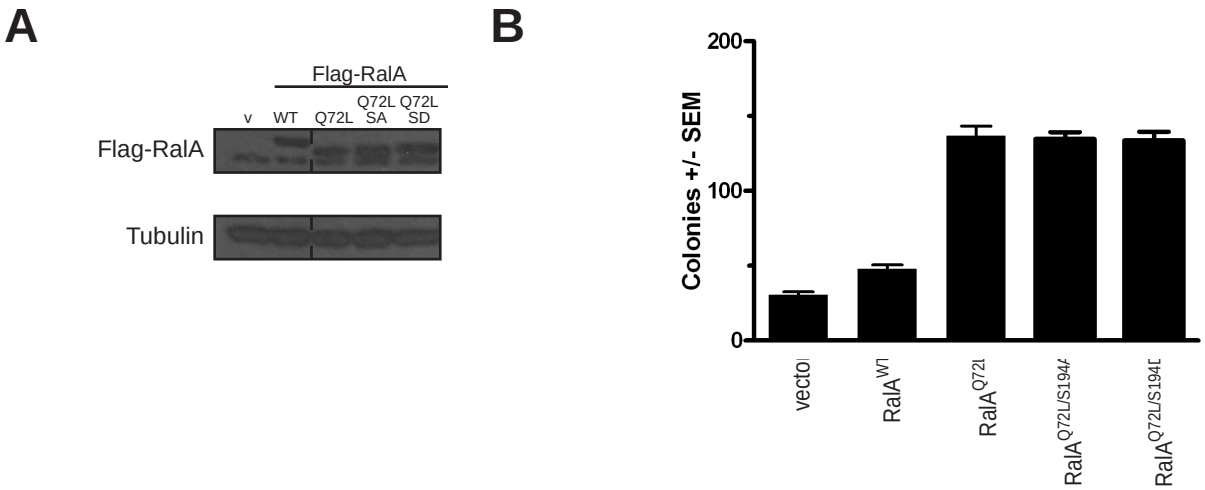
(B) Aurora-A does not alter association of RalA with Sec5. Immunoprecipitation (IP) of Flag-tagged wild-type (WT) or phosphomimetic S194D (SD) mutant version of RalA followed by immunoblot (IB) to detect Flag-tagged immunoprecipitated RalA protein or the presence or absence of co-immunoprecipitated Myc-tagged Sec5 in the presence or absence of serum to activate the RalA protein. Total Sec5 and FlagRalA serve as loading controls.



Supplemental Figure 7. Knockdown of RalBP1 activates Cdc42.
(A) GTP-Cdc42 levels in HEK-TtH cells stably expressing shRNA against either vector control or RalBP1.



Supplemental Figure 8. Aurora-A expression does not alter actin cytoskeleton organization in HEK-TtH cells
Actin organization visualized by immunofluorescence using TxRed-Phalloidin in HEK-TtH cells stably expressing either vector control, kinase-inactive HA-Aurora-A^{K162R} or kinase-active HA-Aurora-A^{T288D}. scale bar: 25μm.



Supplemental Figure 9. Mutation at S194 of RalA did not alter transformation of constitutively active RalA^{Q72L}

(A) Appropriate expression, as detected by immunoblot of HEKTtH cells stably infected with retroviruses endcoding empty vector (v), RalA^{WT} (WT), RalA^{Q72L} (Q72L), RalA$^{Q72L/S194A}$ (Q72LSA), or RalA$^{Q72L/S194D}$ (Q72LSD). Actin serves as a loading control.

(B) Anchorage-independent growth in soft agar of aforementioned polyclonal HEK-TtH cells infected with retroviruses encoding the indicated transgenes, expressed as average number of colonies ± SEM from six plates (two independent experiments conducted in triplicate)..