

# Supplemental Materials

Molecular Biology of the Cell

Georgess et al.

## SUPPLEMENTARY DATA LEGENDS

**Figure S1. Expression ratios of the 115 genes highly expressed in OCs and not expressed in Mo, DCs and DC-MGCs as quantified by GeneChip® microarray.** The value of each gene corresponds to the  $\log_{10}$  of the ratio resulting from dividing the average expression in OCs by the average expression in Mo, DCs and DC-MGCs described in Fig. 2A. Red arrows indicate genes already known for regulating of OC functions.

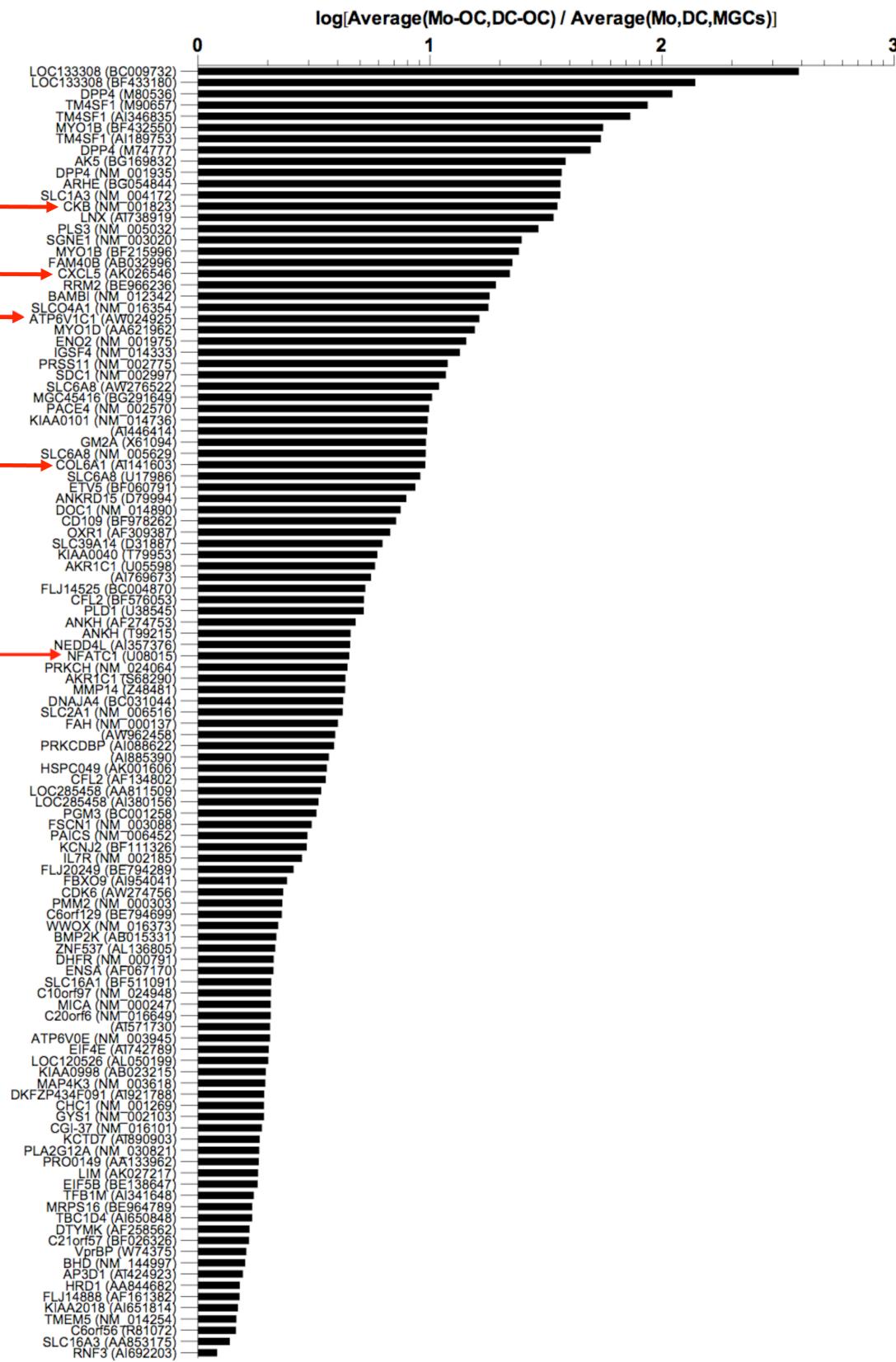
**Figure S2. Expression values (arbitrary units) of the 115 genes highly expressed in OCs.** (Mo-OCs in green; DC-OCs in violet) and not expressed (i.e. below average gene expression within each cell type) in Mo (blue), DCs (red) as quantified by GeneChip® microarray. Stacked bars depict the mean values of expression of a given gene per cell type.

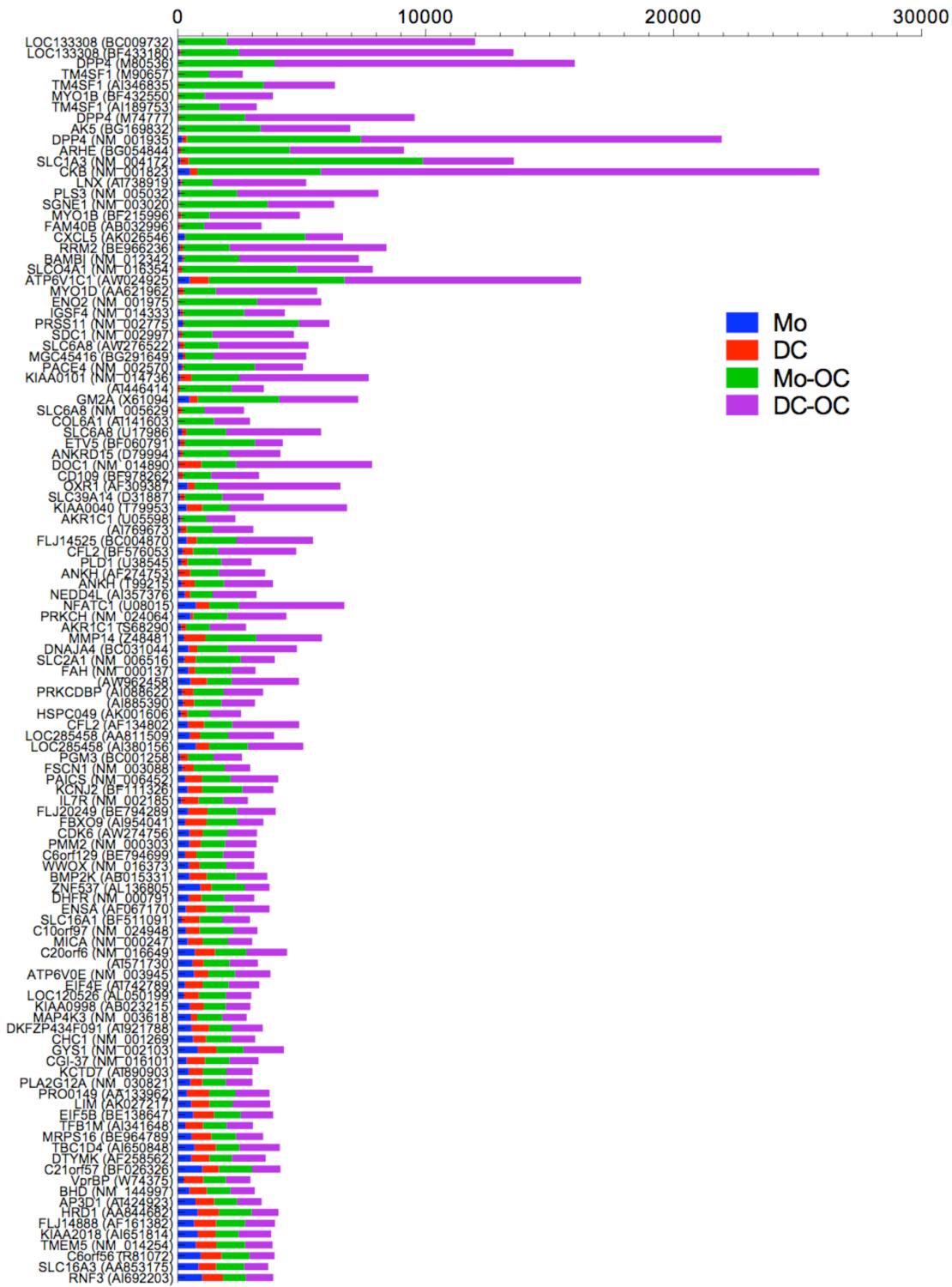
**Figure S3. RhoE<sup>gt/gt</sup> OCs have normal cell spreading and surface expression of integrin subunits  $\alpha V$  and  $\beta 3$ .** (A) Surface area ( $\mu\text{m}^2$ ) of F-actin-stained BM-OC micrographs measured manually using ImageJ software. The whisker-box plot depicts minimum and maximum (whiskers), 25% and 75% quartiles (lower and upper limits of boxes) and median (midline of boxes) of surface areas. Because, in culture, OCs exhibiting clusters, rings and SZ-like are heterogeneous in size, the values were plotted in a  $\log_{10}$  scale. (B) Flow cytometry analysis of the surface expression of  $\alpha V$  (left panels) and  $\beta 3$  (right panels) integrin subunits in RhoE<sup>+/+</sup> (green) and on RhoE<sup>gt/gt</sup> (red) BM-OCs. Isotype control staining is shown in dark purple.

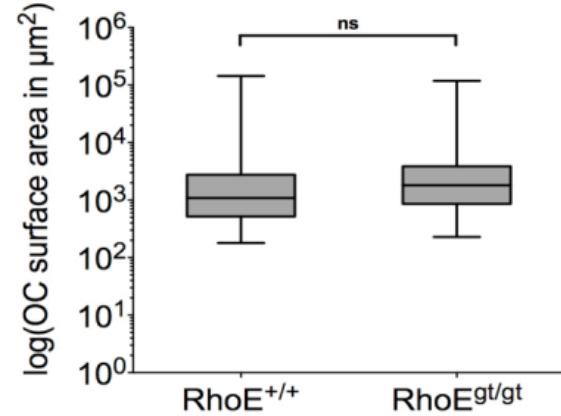
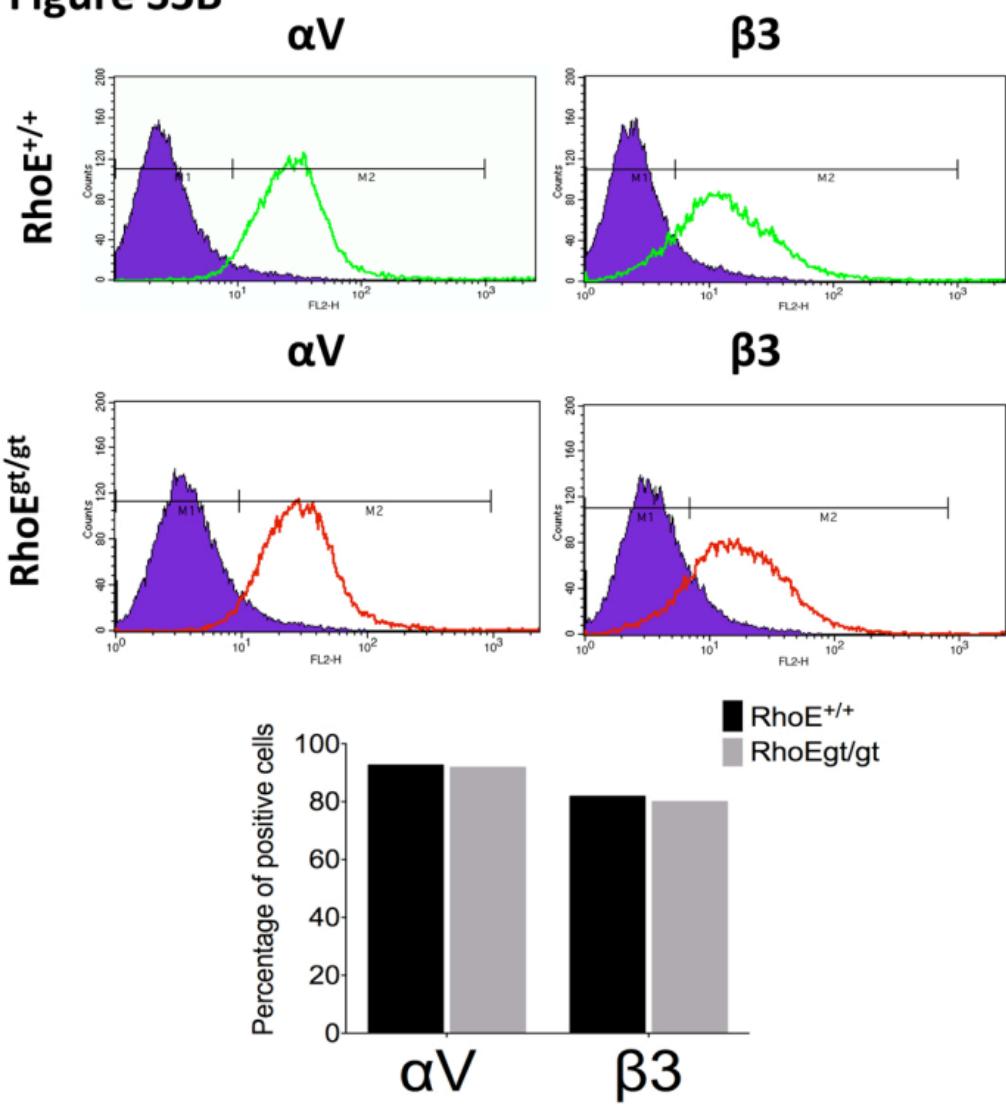
**Figure S4. Protein expression levels of essential podosome components are unchanged in RhoE<sup>gt/gt</sup> compared to RhoE<sup>+/+</sup> OCs.** Immunoblotting of cortactin, paxillin, and vinculin of total cell lysates from day-5 BM-OCs.

**Figure S5. Mypt1 phosphorylation is not Rock-dependent in OCs.** OCs were serum-starved for 2h then serum-induced for 2h with or without 30  $\mu\text{M}$  Y-27632. Rock-I inhibition resulted in decreased phosphorylation of Cofilin on Serine 3 (normalized over total Cofilin) and did not affect Mypt1 phosphorylation on Threonine 696 (normalized over total Mypt1).

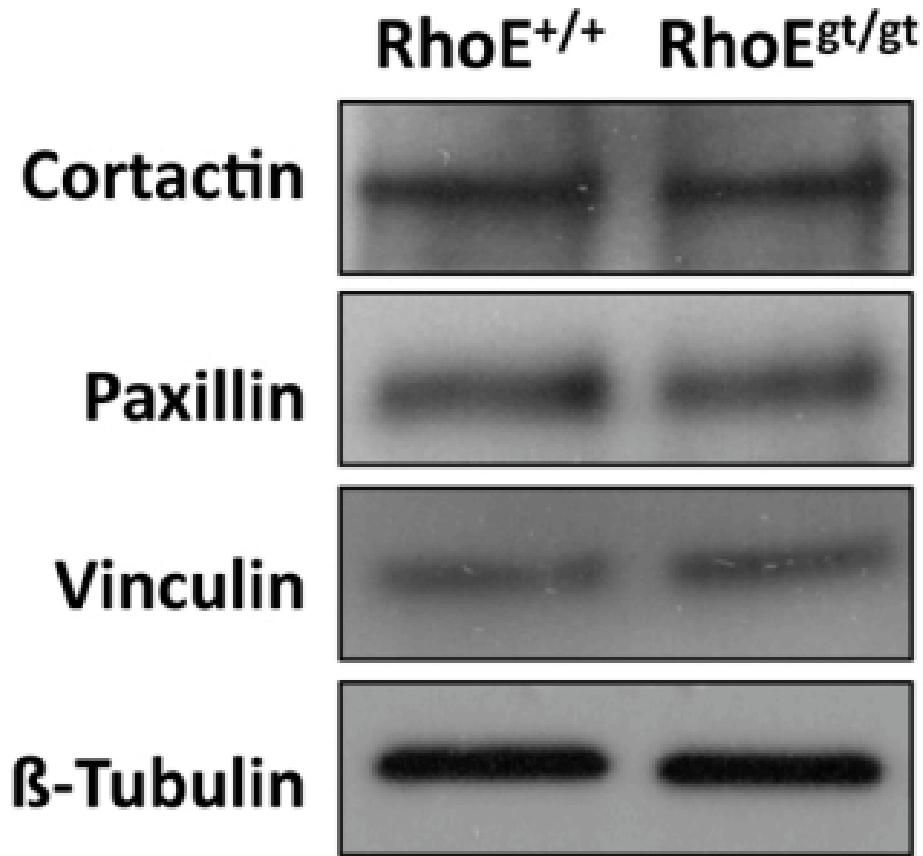
## Figure S1



**Figure S2****Mean expression values (a.u.)**

**Figure S3A****Figure S3B**

# Figure S4



**Figure S5**

**RhoE<sup>+/+</sup>**

