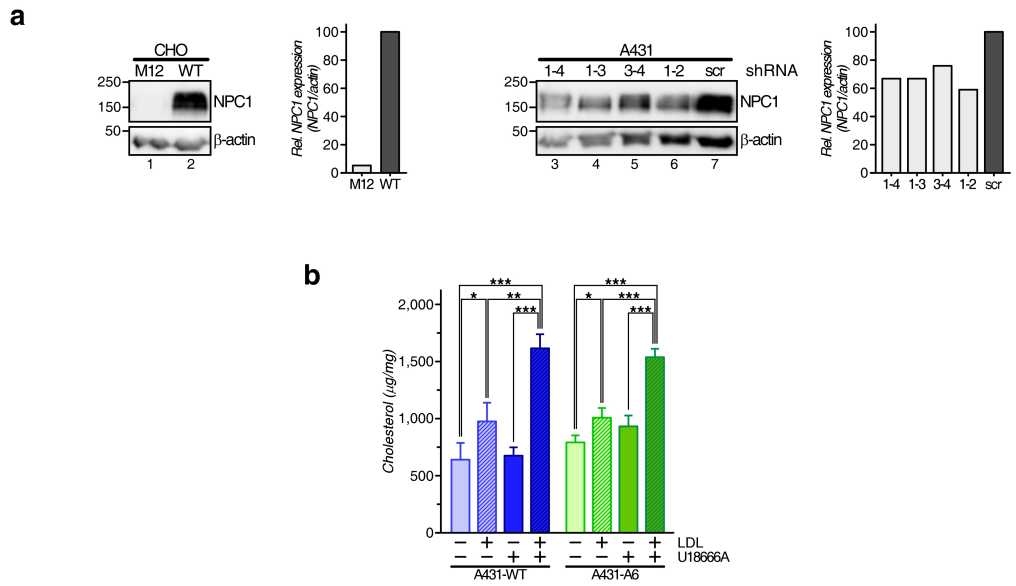
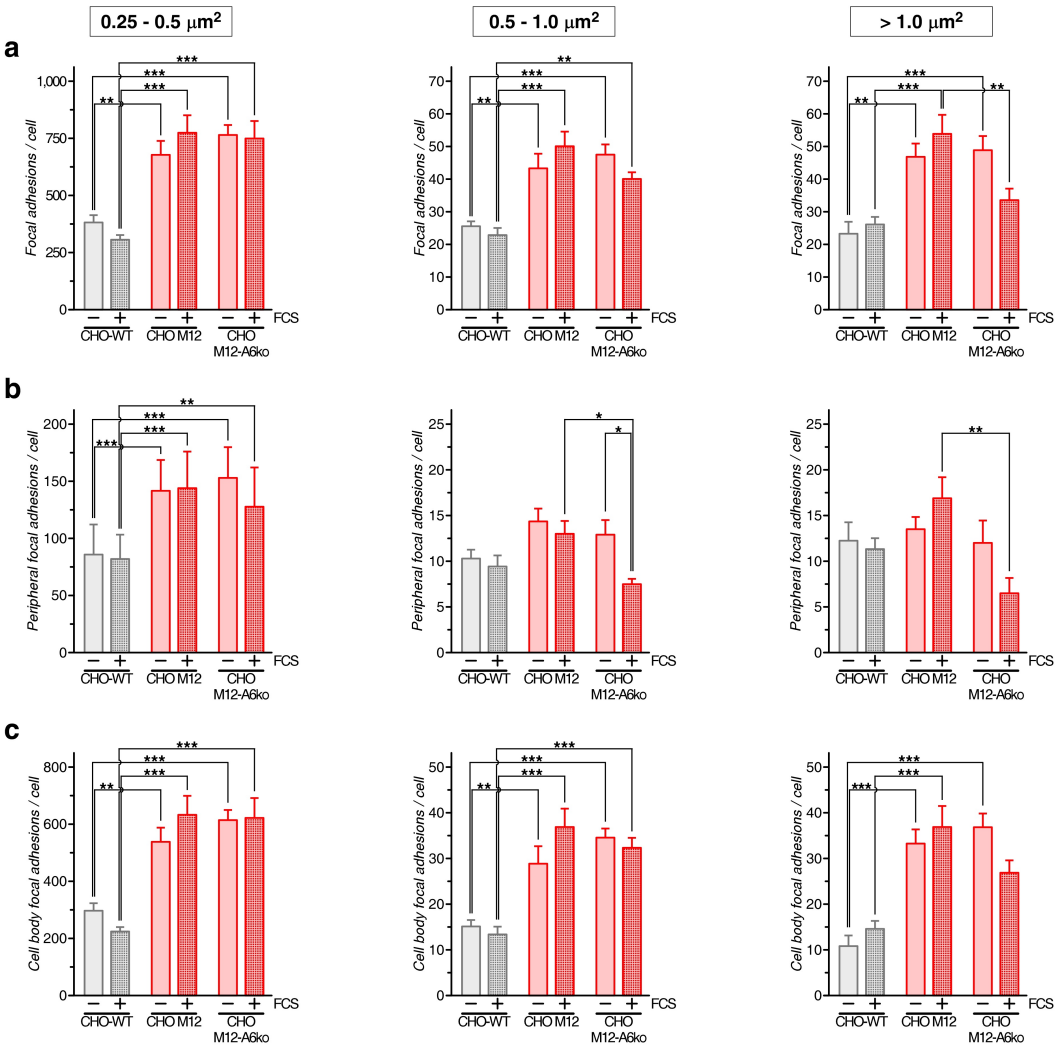
Annexin A6 and NPC1 regulate LDL-inducible cell migration and distribution of focal adhesions

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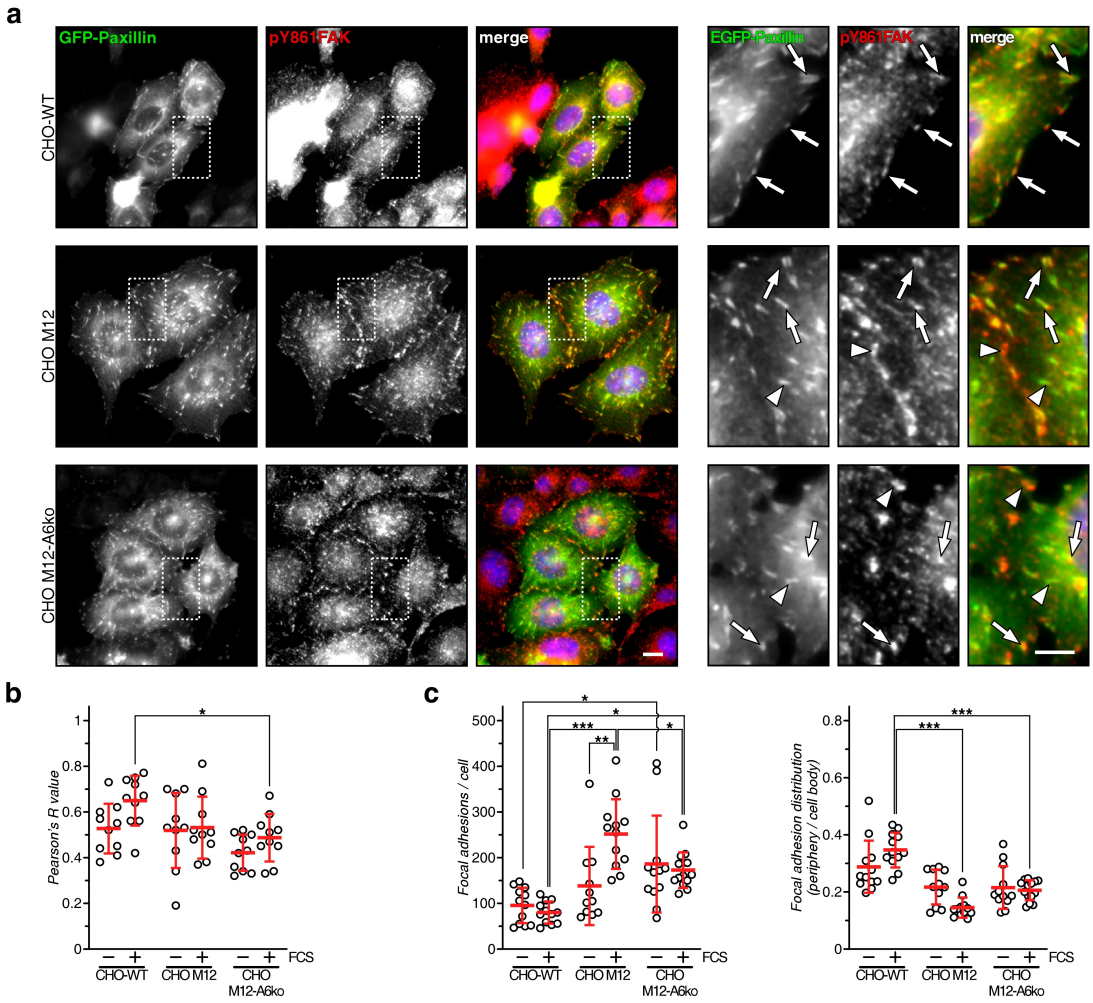
Supplementary information



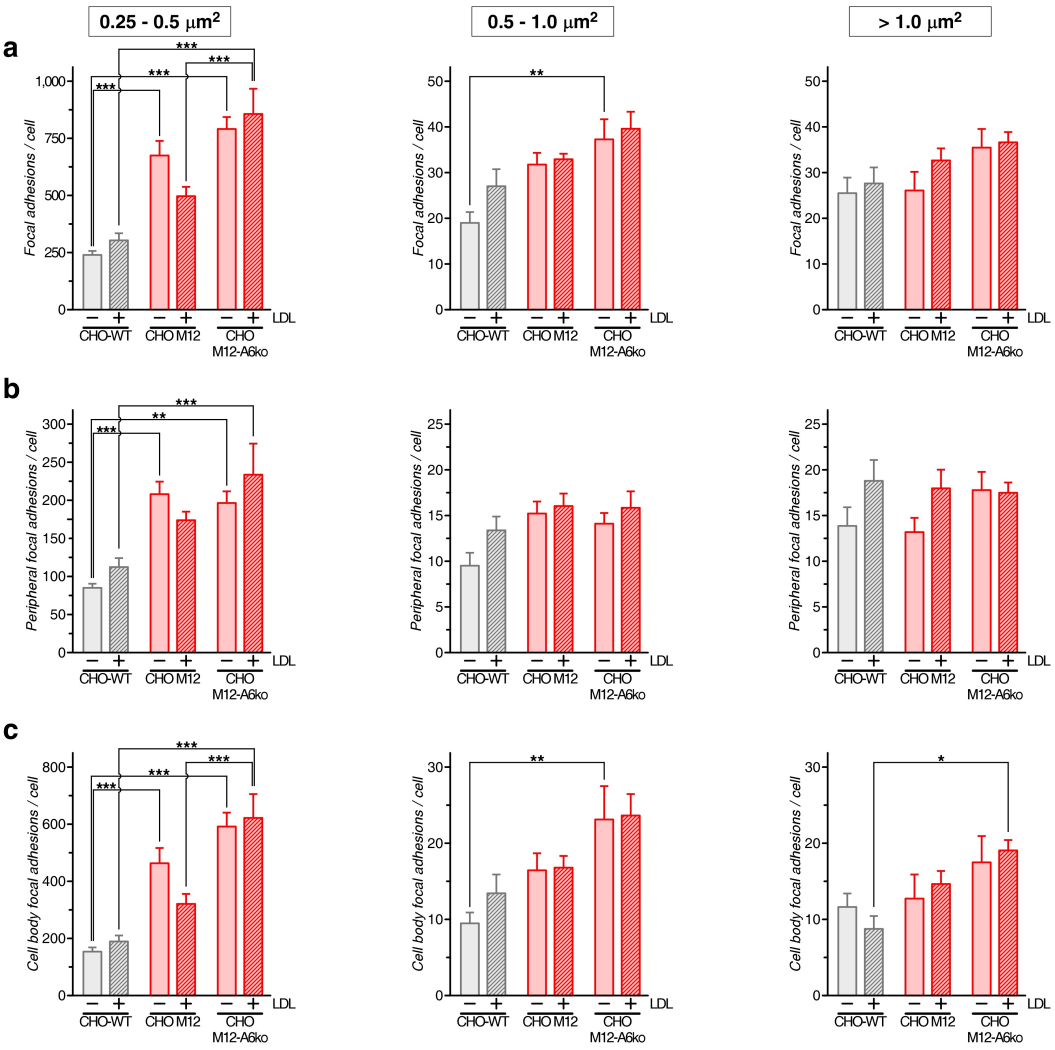
Supplementary Figure 1: (a) Cell lysates from CHO wildtype (WT), CHO NPC1 mutant (M12) (lane 1-2), and A431-WT stably expressing different combinations of four shRNAs targeting NPC1 (1-4, 1-3, 3-4, 1-2) (lane 3-6) or scrambled (scr) shRNA (lane 7) were analyzed for NPC1 and ß-actin by western blotting as indicated. Relative NPC1 expression (NPC1/ß-actin) was calculated. The A431-WT cell line expressing shRNAs 1-4 (lane 3) was used for further studies. **(b)** Cholesterol levels in A431-WT and A431-A6 cells incubated ± LDL (50 µg/ml) for 24 h in the presence or absence of the pharmacological NPC1 inhibitor U18666A. A431-WT and A431-A6 cells were seeded in 6-well plates and grown in media supplemented with 10% LPDS for 48 h before addition of LDL (50 µg/ml), U18666A (4 µg/ml), or both for additional 24 h. Cells were collected, lipids were extracted, and the amount of cholesterol (µg/mg) was determined and normalized to total cellular protein as described in Methods. The mean ± SEM from three independent experiments with triplicate samples is given. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; two-way ANOVA with Tukey's post-hoc test.



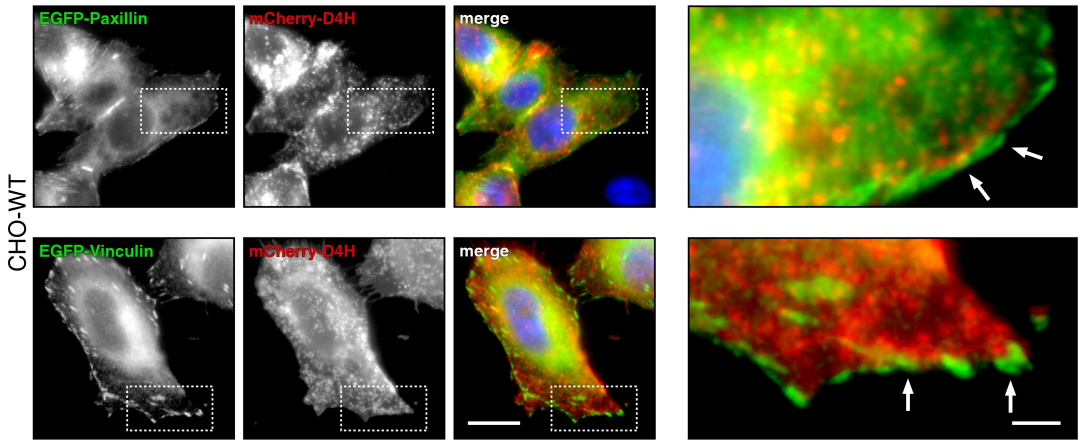
Supplementary Figure 2: Distribution and size of focal adhesions in CHO-WT, M12 and M12-A6ko cells. **(a-b)** CHO-WT, CHO M12 and CHO M12-A6ko cells were starved overnight,-serum-activated with 20% FCS for 45 min as indicated (-, +), fixed and stained for phosphorylated FAK (pY861FAK; see Fig. 5c-d). The number of small (0.25 - 0.5 μm^2), medium (0.5 – 1 μm^2) and large (> 1 μm^2) focal adhesions per cell (total), at the cell edge and throughout the cell body was quantified**.** 20-27 cells per condition and cell line were counted. The mean ± SEM is given. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, two-way ANOVA with Tukey's post-hoc test.



Supplementary Figure 3: Distribution of FA marker paxillin in CHO-WT, CHO M12 and CHO M12-A6ko cells. **(a)** CHO-WT, CHO M12 and CHO M12-A6ko cells ectopically expressing EGFP-paxillin were starved overnight, serum-stimulated (20% FCS) for 45 min, fixed and stained for phosphorylated FAK (pY861FAK, red) as indicated. The merged images and enlarged regions of interest are shown. Arrows indicate colocalization of EGFP-paxillin and pY861FAK. Arrowheads point at EGFP-paxillin or pY861FAK that do not colocalize. Bar is 10 µm and 2 µm for enlarged insets. **(b)** Colocalization of EGFR-paxillin and pY891FAK was quantified (Pearson's R value; 20-30 cells per condition and cell line were quantified. **(c)** Focal adhesions (>0.25 µm^2) per cell (left panel) and the ratio of focal adhesions at the cell edge vs. cell body (right panel) was quantified**.** 7-13 cells per condition and cell line were counted. The mean ± SD is given. * p<0.05, ** p <0.01, *** p <0.001; two-way ANOVA with Tukey's post-hoc test.



Supplementary Figure 4: Distribution and size of focal adhesions in LDL-loaded CHO-WT, M12 and M12-A6ko cells. **(a-b)** CHO-WT, CHO M12 and CHO M12-A6ko cells were grown in 10% LPDS-containing media for 2 days and then treated ± LDL (50 μg/ml) for 4 h. Cells were fixed and immunolabeled with anti-pY861FAK. The number of small (0.25 - 0.5 μm^2), medium (0.5 – 1 μm^2) and large (> 1 μm^2) focal adhesions per cell (total), at the cell edge and throughout the cell body was quantified in control and LDL-loaded cells as indicated (-, +). 16-21 cells per condition and cell line were counted. The mean ± SEM is given. * $p<0.05$, ** $p<0.01$, *** $p < 0.001$; two-way ANOVA with Tukey's post-hoc test.



Supplementary Figure 5: Association of cholesterol with FA structures in CHO-WT cells. **(a-b)** CHO-WT cells ectopically expressing the cholesterol biosensor mCherry-D4H (red) together with EGFP-tagged paxillin (green, upper panel) or vinculin (green, lower panel) were starved overnight, serum-stimulated (20% FCS) for 45 min, fixed and analyzed by microscopy as described in Methods. The merged images are shown. Arrows point at paxillin- or vinculin-containing FA structures at cell edges with D4H in the vicinity. Bar is 10 µm and 2 µm for enlarged inset.