Supplementary Figure legends

Supplementary Figure 1. Related to Fig. 1. The loss of the C-terminal region of CdGAP has no effect on mouse mammary gland development. A, Schematic illustration of the generation of CdGAP *(P668T P670X) knock-in (ki)* mice using a CRISPR/Cas9 strategy. **B,** Representative images of whole mount inguinal mammary glands at 9-, 12- and 15-weeks of $CdGAP^{wt/wt}$ and $CdGAP^{ki/ki}$ mice (scale bar, 1 mm). Quantification of ductal branching (n=5 for each genotype, ns, not significant). **C,** H&E staining of tumors from $Neu^{+}CdGAP^{wt/wt}$ and $Neu^{+}CdGAP^{ki/ki}$ mice. scale bar, 100µm. **D-F,** Representative images of immunohistochemistry (IHC) staining and quantification of proliferation marker (Ki67) **(D)**, angiogenic marker (CD31) **(E)**, and apoptotic marker (Cleaved-Caspase 3) **(F)** on tumors from $Neu^{+}CdGAP^{wt/wt}$ and $Neu^{+}CdGAP^{ki/ki}$ mice (n=4-8 tumors, scale bar, 100µm; ns, not significant).

Supplementary Figure 2. Related to Fig. 2. CdGAP genomic alterations in various molecular subtypes of breast cancer. Distribution of genetic alterations in *CdGAP (ARHGAP31)* across breast cancer molecular subtypes from the breast cancer METABRIC dataset, classified according to: **A,** 3-Gene classifier subtype (n=1667 patients, HER2+ (183), ER-/HER2- (279), ER+/HER2- low proliferation (609), ER+/HER2- high proliferation (596)). **B,** Stacked bar chart expressing the percentage of samples with positive progesterone receptor (PR) expression in altered and unaltered *ARHGAP31* groups (n=1866 patients). **C,** Pam50 + Claudin low subtype (n=1866 patients, Claudin Low (182), Normal (135), Not categorized (NC) (6), Luminal B (454), Luminal A (673), HER2+ (218), Basal (198). **D,** Graphic representation of the distribution of the type of genetic alterations in *ARHGAP31* from **(A)** and **(C). E,** Stacked bar chart expressing the percentage of samples with histological grades 1-3 in altered and unaltered ARHGAP31 groups (n=1866).

Supplementary Figure 3. Related to Figure 2. The CdGAP C-terminus regulates cell proliferation and morphology in TNBC cells. A, Quantification of active Rac1 GTP-bound levels by G-LISA assay in lysates from the indicated MDA-MB-231 cell lines. **B and C**, Cell growth was assessed by trypan blue exclusion assays after overnight serum starvation followed by a 72h serum stimulation in **(B)** MDA-MB-231 and **(C)** HS578T KO and KI cell lines compared to control (Ctl). **D-F,** Representative immunofluorescence images of control (Ctl), CdGAP KO and

KI HS578T cell lines **(D)** cultured on fibronectin coated coverslips and analyzed by confocal microscopy. F-actin and nuclei staining was done with phalloidin-TRITC and DAPI, respectively. Scale bars,100 μm (top images) and 50 μm (bottom images). Quantification of the **(E)** cell area, and **(F)** length from **(D).** Data are presented as mean ± SEM (*p < 0.05, ** p < 0.01, ***p < 0.001, ****p < 0.0001, ns, not significant).

Supplementary Figure 4. Related to Figure 3. The CdGAP C-terminal region promotes cell migration and invasion in TNBC cells. A-D, Representative images of MDA-MB-231 **(A)** and HS578T **(C)** cell lines at 0 h and 15 h, with the initial wound marked in yellow and the closed area shaded in purple. Table shows the multiple unpaired t test results for the individual time points comparing control (Ctl) with CdGAP KO and KI mutant cell lines in **(B)** MDA-MB-231 cells and **(D)** HS578T cells. **E and F,** Migration (top images) and invasion (lower images) transwell assay representative images for **(E)** MDA-MB-231 cells and **(F)** HS578T cells. Scale bar, 500 μm.

Supplementary Figure 5. Related to Fig. 3. The CdGAP C-terminus is required to promote lung metastasis in TNBC. Representative images of the H&E lung sections from Fig. 3K. Scale bars, 900 μm (left and right).

Supplementary Figure 6. Related to Figure 4. CdGAP interacts with LIMD1 and both proteins co-regulate cell migration. A, Volcano plot of differential protein interactors between HEK293 cells expressing GFP-CdGAP and GFP-empty vector previously reported in (7) showing LIMD1 as one of the top interactors. Green dots represent proteins with an absolute fold change >1 (log2FC=1) and P < 0.05. **B,** Representative immunoblots showing the expression of CdGAP (250 kDa), and LIMD1 (95 kDa) in MDA-MB-231 cell lines. **C,** Representative images of the indicated MDA-MB-231 cell lines at time 0 h and 15 h of a wound healing assay. Yellow line delineates initial wound at time 0 h and purple shading traces wound closure. **D,** Multiple unpaired t test results for the individual time points comparing control (Ctl) with KO cell lines.

Supplementary Figure 7. Related to Figure 4. CdGAP and its C-terminal interactor LIMD1 co-localize at focal adhesions and regulate cell morphology and focal adhesion dynamics. A, MDA-MB-231 cell lines cultured on fibronectin coated coverslips and analyzed by confocal

microscopy. Representative images for vinculin (green), F-actin (red), and nuclei staining (blue). Scale bar, 30 μm. **B and C**, CdGAP KO1 MDA-MB-231 cells transfected with GFP-empty vector (GFP-EV) or with GFP-CdGAP were cultured on fibronectin coated coverslips and analyzed by confocal microscopy. **(B)** Representative images for GFP (green), vinculin (red), and nuclei staining (blue). Scale bar, 50 μm. **(C)** Representative images for GFP (green), LIMD1 (red), and nuclei staining (blue). Scale bar, 50 μm.

Supplementary Figure 8. Related to Figure 5. The loss of CdGAP and its C-terminus display a similar transcriptomic profile in MDA-MB-231 cells. A and B, Significantly enriched and depleted hallmark pathways in CdGAP KI1 **(A)** and KO1 **(B)** vs control MDA-MB-231 cells identified via Gene Set Enrichment Analysis (GSEA) ($p < 0.05$). Stars denote hallmarks associated with immune response (pink), intracellular signaling pathways (beige), metabolism (green), cell cycle regulation (blue) and cell polarity/migration (red). **C,** Dot plot showing the results of gene ontology biological pathway analyses performed for differentially expressed (up or down regulated) genes between control and CdGAP KI1 MDA-MB-231 cells. **D,** Quantification of MMP14 and MMP16 mRNA levels in CdGAP KI cell lines. Data are presented as mean ± SEM (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). **E,** Dot plot showing the results of gene ontology biological pathway analyses performed for differentially expressed (up or down regulated) genes between control and CdGAP KO1 MDA-MB-231 cells. **F,** Heatmap portraying the 18 leading-edge genes contributing to the enrichment of a WNT signaling pathway signature performed for differentially expressed (up or down regulated) genes between control and CdGAP KO1 MDA-MB-231 cells.

Supplementary Figure 9. Related to Figure 6. The interaction of LIMD1 with the C-terminus of CdGAP contributes to limit the activation of the WNT pathway. A, Representative immunoblots of HEK293 cells transfected with GFP-empty vector (EV), GFP-CdGAP wild type (WT) or with GFP-CdGAP-R1172A treated with 5% conditioned media (CM) L-WRN. **B,** WNT transcriptional luciferase assay from **(A).** Data are presented as mean ± SEM (**$p < 0.01$, **** $p < 0.0001$, ns, not significant). **C,** Representative immunoblots of MDA-MB-231 cells transfected with GFP-empty vector (EV), GFP-CdGAP wild type (WT) or with GFP-CdGAP-R1172A treated with 5% conditioned media (CM) L-WRN.