TGF-β-induced DACT1 biomolecular condensates repress Wnt signalling to promote bone metastasis

Mark Esposito1, Cao Fang1,7, Katelyn C. Cook1,7, Nana Park1, Yong Wei1, Chiara Spadazzi2, Dan Bracha3, Ramesh T. Gunaratna1, Gary Laevsky1, Christina J. DeCoste1, Hannah Slabodkin1, Clifford P. Brangwynne3,4, Ileana M. Cristea1 and Yibin Kang1,5.6

The complexity of intracellular signalling requires both a diversity of molecular players and the sequestration of activity to unique compartments within the cell. Recent findings on the role of liquid–liquid phase separation provide a distinct mechanism for the spatial segregation of proteins to regulate signalling pathway crosstalk. Here, we discover that DACT1 is induced by TGFβ and forms protein condensates in the cytoplasm to repress Wnt signalling. These condensates do not localize to any known organelles but, rather, exist as phase-separated proteinaceous cytoplasmic bodies. The deletion of intrinsically disordered domains within the DACT1 protein eliminates its ability to both form protein condensates and suppress Wnt signalling. Isolation and mass spectrometry analysis of these particles revealed a complex of protein machinery that sequesters casein kinase 2—a Wnt pathway activator. We further demonstrate that DACT1 condensates are maintained in vivo and that DACT1 is critical to breast and prostate cancer bone metastasis.
through successive passaging in vivo\textsuperscript{12-14} (Extended Data Fig. 1a). All of the sublines established orthotopic tumours at a much greater rate compared with the parental line and acquired the ability to develop spontaneous lung metastasis (Extended Data Fig. 1b,c,e,f). However, only the SUM159-M1a (hereafter, M1a) derivative could efficiently develop spontaneous bone metastases (Extended Data Fig. 1d–f). Intracardiac injection of each subline confirmed that M1a was the most proficient at forming metastatic lesions in bone (Extended Data Fig. 1g–i). This M1a derivative has been used in our previous studies of mechanisms of osteolytic metastasis\textsuperscript{15,27,28}. Analysis using microarray followed by gene set enrichment analysis (GSEA) demonstrated that TGF-β signalling is the most enriched Hallmark signalling program in the M1a bone-metastatic derivative compared with the parental SUM159 and was highly enriched compared with the closely related but weakly bone-metastatic subline M1L1 (Fig. 1a and Supplementary Table 1). The enrichment in TGF-β signalling in highly bone-metastatic cells is corroborated by a similar analysis of the bone-metastatic variants from the MDA-MB-231 triple-negative breast cancer line\textsuperscript{12,13}, underscoring the prominent role of this pathway in driving the development of bone metastasis.

We next assessed which specific genetic elements were associated with this increase in bone-metastatic potential and enrichment in TGF-β signalling. Filtering and ranking of differentially expressed genes in highly bone-metastatic M1a cells compared with the closely related but weakly metastatic SUM159 or M1L1 cells revealed that 11 genes were upregulated by more than four-fold (Extended Data Fig. 1j,k). To determine which of these 11 enriched genes were directly regulated by TGF-β, we cross-referenced this list first to chromatin-immunoprecipitation–sequencing data of SMAD2/3-binding elements\textsuperscript{15} and then to a panel of genes induced by TGF-β at 3 h in HaCaT, HPL1, MCF10A and MDA-MB-231 cells\textsuperscript{12,13}. Only Dishevelled binding antagonist β-catenin 1 (DACT1, also known as DAPPER1 and FRDO) was present in each dataset, suggesting that it is a candidate TGF-β downstream gene with potential bone-metastasis-promoting functions. Quantitative PCR with reverse transcription (RT–qPCR) analysis verified that DACT1 was expressed at higher levels in M1a cells compared with the other derivative cell lines or the parental SUM159 cells (Extended Data Fig. 1l). The addition of recombinant TGF-β to a panel of both normal and cancer lines followed by RT–qPCR analysis at 3 h and 12 h showed that DACT1 was strongly induced by TGF-β in all of the TGF-β-responsive cell lines but not in the SMAD4-deficient cell lines (Fig. 1b). These data demonstrate that TGF-β transcriptionally induces DACT1.

DACT1 is a cytoplasmic protein that was discovered in 2002, and is a negative regulator of Wnt signalling through the stabilization of the destruction complex\textsuperscript{32}. Developmental studies have since shown that DACT1 may instead activate Wnt signalling\textsuperscript{33,34}, whereas other studies suggest that DACT1 alternately suppresses or activates Wnt depending on its concentration\textsuperscript{35}. Mechanistically, the function of DACT1 has been attributed to a diverse range of interactions, such as with p120 (ref. 36), Dishevelled\textsuperscript{37}, LEF 38, 14-3-3\textsuperscript{39} and Miz1 (ref. 40); however, considerable uncertainty remains regarding how these interactions mediate the function of DACT1 or the directionality in which they influence Wnt signalling. An exhaustive immunopurification assessment of DACT1-binding partners revealed that DACT1 indeed interacted with some of these previously described partner proteins as well as additional unknown interactions\textsuperscript{42}.

To measure whether DACT1 impacts canonical Wnt signalling, we stably transduced both normal and cancer cell lines with lentiviruses expressing either a luciferase or GFP reporter driven by upstream TCF-binding elements (12XTCF-ffLuc or 7TGC)\textsuperscript{35,41}. Validation of the Wnt reporters showed a marked increase in reporter activity after treatment with L-cell-derived Wnt3a that was lost after concurrent treatment with ICG-001, which is a specific inhibitor of canonical Wnt signalling (Extended Data Fig. 1m,n). The reporter-labelled bone-metastatic BM2 subline of MDA-MB-231\textsuperscript{12,25} or normal human pulmonary epithelial cells HPL1 were next stably transduced with DACT1 short-hairpin RNA (shRNA), scrambled control shRNA (shControl), empty vector or a human DACT1 overexpression cassette. Subsequent DACT1 knockdown (KD) or overexpression levels were then confirmed using RT–qPCR (Extended Data Fig. 2a,b). Treatment with Wnt3a for 20 h followed by assessment of luciferase activity revealed that DACT1 KD more than doubled Wnt reporter activation by Wnt3a, whereas DACT1 overexpression moderately reduced Wnt3a-dependent reporter activity (Fig. 1c). Analysis of 7TGC-GFP reporter activity on a cell-by-cell basis using flow cytometry similarly revealed that DACT1 KD increased Wnt3a-induced GFP expression, whereas DACT1 overexpression reduced the GFP signal in both BM2 and HPL1 cells (Extended Data Fig. 2c,d).

Orthogonal techniques further validated the suppression of Wnt signalling by DACT1 as DACT1 KD increased β-catenin nuclear localization in the Wnt3a-induced state (Fig. 1d and Extended Data Fig. 2e). DACT1 KD further increased total β-catenin protein in Wnt3a-treated cells (Extended Data Fig. 2f). This increase in β-catenin was not accompanied by changes in DVL2 or DVL3 levels in any of the DACT1-modified cell lines (Extended Data Fig. 2f), suggesting that DACT1 does not promote the degradation of Dishevelled to increase β-catenin destruction in this model system, in contrast to previous studies\textsuperscript{42}. Analysis of β-catenin expression in normal epithelial HPL1 cells also demonstrated that DACT1 levels were inversely correlated to β-catenin levels after Wnt3a stimulation (Extended Data Fig. 3a). Finally, RT–qPCR analysis of the Wnt downstream gene AXIN2 revealed higher induction at 24 h and 48 h in DACT1 KD cells compared with the control cells (Extended Data Fig. 3b). Collectively, these data suggest that DACT1 negatively regulates canonical Wnt signalling.

We therefore hypothesized that TGF-β induces DACT1 to negatively regulate Wnt signalling. To test this, we assessed the effects of TGF-β and LY2109761—a selective TGF-βRII/II receptor inhibitor—on Wnt signalling in the M1a-7TGC and BM2-7TGC cell lines. We reasoned that, because the M1a line shows enriched TGF-β signalling (Fig. 1a), treatment with a TGF-β inhibitor should enhance Wnt signalling by preventing TGF-β-induced DACT1 expression. Flow cytometry analysis of M1a-7TGC cells that were preincubated for 24 h with LY2109761 or TGF-β followed by 24 h of Wnt3a stimulation demonstrated that TGF-β treatment indeed suppressed the already low levels of Wnt activation, whereas LY2109761 increased Wnt activation (Fig. 1c). Testing in the BM2-7TGC line confirmed that exogenous TGF-β addition attenuated Wnt3a-mediated Wnt activation (Extended Data Fig. 3c). We next showed that these results were dependent on DACT1, as DACT1 KD in the BM2 line reduced the magnitude of TGF-β-mediated suppression of Wnt signalling compared with the control, while DACT1 overexpression enhanced this effect (Extended Data Fig. 3d,e).

DACT1 forms biomolecular condensates driven by intrinsically disordered domains. As the data showed that DACT1 does not affect Dishevelled stability, whereas it does influence β-catenin protein levels, we next sought to determine the mechanism by which DACT1 regulates Wnt signalling. Multiple molecular interactions in different cellular compartments have been ascribed to DACT1 (refs. 37,45); these include reports that DACT1 shuttles between the nucleus and cytoplasm to prevent β-catenin binding to the TCF/LEF transcription factors\textsuperscript{46}, or interacts with VPS34 to initiate the autophagosome\textsuperscript{47}.

To test the localization of DACT1, we generated tdTomato N-terminal and C-terminal fusions to DACT1 that were expressed in both M1a and BM2 cells, while tdTomato only was expressed as a control. Stable expression of either of the N-terminal or C-terminal
As well as native DACT1 protein were actively degraded as shown (Extended Data Fig. 4b). Both DACT1–tdTomato fusion proteins puncta were also observed in the HPL1 normal epithelial cell line of treatment with Wnt3a (Extended Data Fig. 4a.), while the same s.e.m. Source data are available online.

βTGF-inhibitor L Y2109761 (6 μM) for 20 h, and then luciferase activity was measured. n = 6 biological replicates per sample. Statistical analysis was performed using Student’s t-tests. The experiment was independently repeated more than three times. AU, arbitrary units.

BM2 cells with stable DACT1 were treated with control medium or Wnt3a for 24 h and 48 h. Cells were fixed and immunolabelled with anti-total-β-catenin antibodies plus Hoechst (sample images are provided in Extended Data Fig. 2e). The threshold overlap score (TOS) linear algorithm was used to assess the correlation of β-catenin nuclear colocalization in DACT1-KD cells (Extended Data Fig. 5a,b). Importantly, both or DACT1–tdTomato; notably, the DACT1 puncta were absent (Extended Data Fig. 5a, vector control) and cells expressing either wild-type DACT1 or DACT1–tdTomato; notably, the DACT1 puncta were absent in DACT1-KD cells (Extended Data Fig. 5a,b). Importantly, both fusion proteins in either cell line revealed a unique punctate localization of tdTomato-positive DACT1 bodies (1–5 bodies per M1a cell and 1–20 bodies per BM2 cell), each of which ranged in size from 200 nm to 2 μm. This result was not observed in tdTomato-only cells (Fig. 2a). The localization of these bodies was not affected by 12 h of treatment with Wnt3a (Extended Data Fig. 4a.), while the same puncta were also observed in the HPL1 normal epithelial cell line (Extended Data Fig. 4b). Both DACT1–tdTomato fusion proteins as well as native DACT1 protein were actively degraded as shown by cycloheximide pulse–chase analysis (Extended Data Fig. 4c). This degradation was mediated by the ubiquitin proteasome system and not by the lysosomal system, as demonstrated by treatment with either MG-132 or bafilomycin A, which are inhibitors of the respective protein degradation pathways (Extended Data Fig. 4d).

Staining for a panel of organelle markers revealed that these DACT1-containing organelle-like structures did not colocalize with mitochondria, p-bodies, endosomes, lysosomes, autophagosomes, the endoplasmic reticulum or the Golgi apparatus (Extended Data Fig. 4e). To verify that DACT1 bodies are not caused by fusion to tdTomato, we stained for endogenous DACT1 protein and found that the same structures were formed in native cells (Extended Data Fig. 5a, vector control) and cells expressing either wild-type DACT1 or DACT1–tdTomato; notably, the DACT1 puncta were absent in DACT1-KD cells (Extended Data Fig. 5a,b). Importantly, both

| VOL 23 | MARCH 2021 | 257–267 | www.nature.com/naturecellbiology |
the N-terminal and C-terminal DACT1–tdTomato fusion proteins could efficiently repress Wnt reporter activation compared with tdTomato alone (Fig. 2b), indicating that both fusion proteins preserved the Wnt-suppressive function of DACT1.

Given that these DACT1 bodies did not colocalize with other organelles (including autophagosomes), they did not show nuclear localization and they exhibited Wnt suppressive function, we next sought to understand what these structures were. SoRa super-resolution imaging of HEK293T cells transfected with DACT1–tdTomato revealed an assortment of structures that were mostly spherical, yet some of which were tubular or hollow (Supplementary Video 1). Further analysis using holotomographic-fluorescence imaging colocalized tdTomato signal with cell-wide measurement of the refractive index (RI). Analysis of these images in both M1a and BM2 cells revealed perfect colocalization of the tdTomato signal with distinct regions of locally increased RI (Fig. 2c and Extended Data Fig. 5c). Computational sectioning of the images revealed red fluorescent areas mapped to RIs between 1.36–1.38, a value that is normally observed for condensed protein assemblies46 (Fig. 2d).

Intracellular protein condensates provide a distinct mechanism for spatially restricted zones of signalling as compared to lipid membrane segregation47,48. Furthermore, components of the Wnt signalling pathway, including AXIN1 and APC, have recently been shown to associate together in phase-separated condensates that are critical for the activity of the β-catenin destruction complex49. Cotransfection of AXIN1–GFP and DACT1–tdTomato into HEK293T cells revealed that both proteins formed condensates; however, these formed into distinct structures (Fig. 2e). Live imaging studies of DACT1–tdTomato fusion proteins in cells showed both Brownian and directed movement of these bodies with fusion events occurring between them (Supplementary Video 2). Fluorescence recovery after photobleaching (FRAP) further supported that these were dynamic assemblies; partial recovery of fluorescence (Fig. 2f and Supplementary Videos 3 and 4) showed that both immobile and mobile populations are present, similar to other condensates50,51.

Liquid–liquid phase separation of proteins is driven in many cases by intrinsically disordered domains (IDRs)52. Prediction of DACT1 IDRs using D 2P2 identified ten disordered regions that we deleted from the DACT1–tdTomato sequence in addition to the previously reported PDZ, coiled-coil, nuclear export and nuclear localization domains of DACT1 (Extended Data Fig. 6a and Supplementary Table 2). Stable generation of each DACT1–tdTomato variant was confirmed using western blot analysis (Fig. 3a, b). These data indicate that the rescue of the phase separation of DACT1 protein segments outside the DR8 region is at least partially sufficient to support the Wnt-repressive functions of DACT1.

DACT1 biomolecular condensates contain RNA-binding, translational and signalling protein components. The data presented here show that phase separation is the central characteristic that drives DACT1 function in both cancerous and normal cells. However, this is not mutually exclusive of its other reported roles, as it may sequester important Wnt pathway components to modify Wnt signalling. Indeed, immunopurification studies have

![Fig. 2](https://example.com/fig2)

**Fig. 2 | Formation of DACT1-associated biomolecular condensates.** a, Lentiviruses expressing tdTomato (TdT) or DACT1–tdTomato C-terminal and N-terminal fusion proteins were stably transduced into M1a and BM2 cells followed by confocal z-stacking of fixed cells counterstained with Hoechst. Images are presented as a three-dimensional rendering of individual z-stacks. b, 12TFC–fLuc Wnt-reporter–expressing BM2 cells were genetically modified to express the indicated DACT1 KD, control or overexpression construct. The cell lines were treated with control medium, Wnt3a or Wnt3a plus ICG-001 (25 μM) for 20 h, and Wnt activation was quantified on the basis of luciferase activity. n = 4 biological replicates; the experiment was independently repeated three times. Statistical analysis was performed using Student’s t-tests. c, Holotomographic images coupled to epifluorescence obtained using the Tomocube imaging system shows RI as greyscale and fluorescence in red pseudocolour. The areas highlighted by dotted squares at the top are magnified at the bottom. Scale bars, 5 μm (top) and 2.5 μm (bottom). d, Quantification of RI in regions of red fluorescence in n = 22 (BM2) and n = 14 (M1a) distinct cells imaged across three independent experiments. The image sectioning analysis is demonstrated in Extended Data Fig. 5c. e, Confocal z stack of Axin1–GFP and DACT1–tdTomato transfecteds in HEK293T cells. f, FRAP analysis of DACT1–tdTomato biomolecular condensate in BM2 cells. Data are mean ± s.e.m. Source data are available online.
demonstrated that DACT1 binds to a variety of proteins, including itself, Dishevelled, β-catenin, VPS34, DACT2 and DACT3 (ref. 42). Meanwhile, previous characterization of cytoplasmic condensates has focused on p-bodies and stress granules, which are hybrid protein–RNA condensates46. We therefore tested whether these DACT1 particles contain CTNNB1 (encoding β-catenin) mRNA to prevent
translational, as our data showed that DACT1 levels are inversely correlated with β-catenin protein levels in the Wnt-activated state (Extended Data Fig. 2f). Fluorescence in situ hybridization analysis revealed that the DACT1 condensates did not bind to CTNNB1 mRNA (Extended Data Fig. 6d).

To investigate the protein contents of these DACT1 condensates, we developed a method to separate them intact from cells. We found that multiple methods of conventional lysis as well as previous methods used to isolate p-bodies led to the formation of non-specific aggregates in DACT1–tdTomato and DACT1ΔDR7–tdTomato mutants. Rather, we found that low amplitude sonication followed by the addition of 1% Triton X-100, 0.5% sodium deoxycholate, RNase inhibitors and Hoechst 33342 yielded excellent separation of DACT1–tdTomato+Hoechst particles in control or Wnt3a-stimulated cells, but did not identify particles in the tdTomato or DACT1ΔDR7–tdTomato mutant cells (Fig. 4a).

We next used fluorescence-activated particle sorting to isolate these particles and analysed their composition using liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS), quantifying the DACT1-particulate proteome under both the

![Figure 3](image_url)

**Fig. 3** | DACT1 intrinsically disordered domains drive phase separation and Wnt suppression. a, Western blot analysis of DACT1 mutants fused to tdTomato at the C-terminal region stably expressed in the BM2-7×TCF-GFP cell line. Deleted region 7 includes the antibody epitope recognition site and therefore ΔDR7 cannot be detected by western blot. Representative of three independent replicates. b, Confocal imaging of DACT1–tdTomato mutants stably expressed in the M1a cell line. Images are representative of more than three independent experiments across both the BM2 and M1a cell lines. Scale bars, 5 µm. The images marked with an asterisk have a lowered lookup table threshold to adequately visualize signal. C-coil, coiled coil.

c, Wnt activation analysis of DACT1–tdTomato mutants expressed in the BM2-7×TCF-GFP cell line treated with Wnt3a for 24 h. n = 4 biological replicates pooled from 4 independent experiments. The Student's t-test analysis compares to the tdTomato-only cells as the upper bound and the DACT1 wild-type as the lower bound. Wild type (W) indicates that Wnt repression was statistically non-significant compared to unmodified DACT1–tdTomato, while loss (L) indicates non-significance compared to the tdTomato-only control. Intermediate (I) denotes significance compared with both controls. The asterisks indicate statistical significance; *P < 0.05, **P < 0.01, ***P < 0.005; n.s., not significant; exact P values are provided as source data. Data are mean ± s.e.m. d, DACT1–tdTomato or DACT1ΔDR7–tdTomato constructs were stably expressed in BM2-7×TCF-GFP cells and were treated with Wnt3a for 24 h followed by live-cell confocal imaging. Images shown are representative of more than three independent experiments. Scale bars, 50 µm.

e, Confocal imaging of DACT1–tdTomato C-terminal fusion proteins in M1a cells with disordered region 8 deleted (ΔDR8) compared with those same constructs with wild-type Fus or Fus27×Y>S mutants. Scale bars, 10 µm.

f, Constructs from e were introduced into BM2-7×TCF-GFP Wnt reporter cells and the Wnt activation assay was performed. n = 3 biological replicates, independently repeated 2 times. Statistical analysis was performed using Student’s t-tests. Source data are available online.
control and Wnt3a-stimulated conditions (Extended Data Fig. 7a). After stringent data quality filtering and analysis, we found that DACT1 was the most abundant protein in the isolated particles, which contained approximately 600 other proteins (Supplementary Table 3). Additional analysis of the MS data uncovered a striking enrichment in proteins that are involved in RNA processing, ribosome assembly, translational control and protein folding (Extended Data Fig. 7b,c and Supplementary Table 4). This included many members of the spliceosome (for example, SNRP1, GEMIN7, TRA2A/B), ribosome assembly factors (such as RPF2, PES1, NIP7), the large and small ribosomal subunits, translation initiation factors (for example, EIF6) and protein-folding chaperones (such as HSPA1a, BAG2) among others. The protein proteome also contained proteins that control cellular organization and trafficking, such as actin-nucleating and actin-regulating proteins (for example, CAPs, ARPC1b), supporting the dynamic behaviour of DACT1 particles in cells.

We also observed that Wnt3a changed the abundance of the identified proteins between the control and Wnt3a conditions rather than composition (Fig. 4b). Across independent isolations, the control and Wnt3a conditions exhibited nearly complete consistency in terms of detected proteins (Fig. 4b and Supplementary Table 3). Instead, 26 proteins had more than twofold increases in the Wnt-treated condition and 11 proteins were decreased by more than twofold (Fig. 4b). These data suggest that Wnt3a stimulation does not change the proteins found within particles but, rather, alters the relative abundance of these proteins, perhaps reflecting the biological processes such as translational initiation or RNA binding that may potentially be involved in these DACT1-dependent condensates (Extended Data Fig. 7c). The majority of phase-separated condensates have been characterized as protein–RNA hybrids that are contained in p-bodies and stress granules; importantly, MS analysis did not identify either Fus or DCP1a (Supplementary Table 3), which are integral components of stress granules and p-bodies.

A key enriched candidate within these data was CK2, with all subunits, including α, α′ and β, detected at high levels of enrichment (Fig. 4b and Supplementary Table 3). CK2 is a well-known positive regulator of Wnt signalling and has also been detected as a DACT1-binding partner by high-throughput yeast two-hybrid screens. Indeed, immunostaining showed that CK2 colocalizes to DACT1–tdTomato bodies (Fig. 4c), confirming that our isolation method identified bona fide DACT1 binding to partners in cells. Treatment of BM2–TGC lines with silmitasertib, a specific CK2 inhibitor, confirmed that CK2 inhibition decreases Wnt activity (Fig. 4d). As expected, DACT1-overexpressing cells had lower basal Wnt activity, and were less sensitive to silmitasertib-induced Wnt suppression compared with the vector control cells (Fig. 4d,e).

These data suggest that DACT1 reduces the available pool of CK2 that is necessary to sustain maximal Wnt signalling, potentially through sequestration of CK2 into these biomolecular condensates (Fig. 4e). To test this hypothesis, we tethered CSKN2A1 (CK2α subunit) to GFP and expressed it by itself or in tandem with DACT1–tdTomato in both M1a and BM2 cells. Confocal imaging revealed that CK2α was diffusely localized to both the nucleus and cytoplasm when expressed by itself, whereas coexpression with DACT1–tdTomato resulted in exclusive sequestration of the cytoplasmic pool to the condensates, while the nuclear pool remained diffusely localized (Fig. 5a, Extended Data Fig. 8a and Supplementary Video 5). Further testing of this finding using particle flow cytometry revealed that CK2α–GFP was found in all of the DACT1–tdTomato particles (Fig. 5b and Extended Data Fig. 8b). These data demonstrated that DACT1 is dominant over cytoplasmic CK2 by sequestering CK2 to DACT1-generated phase separations. Importantly, DACT1-overexpressing cells were still partially sensitive to silmitasertib, an observation that may be explained by the presence of CK2 localized to the nucleus that was not accessible to sequestration within the DACT1 particles (Fig. 4c–e).

**Fig. 4 | Isolation and characterization of DACT1 biomolecular condensates.** a. BM2 cells stably expressing hDACT1–tdTomato fusion proteins were treated with control or Wnt3a medium for 24 h. Cells were sonicated in phosphate-buffered saline (PBS) followed by addition of 1% Triton X-100, 0.5% sodium deoxycholate and Hoechst (10 μg ml−1). Particulate suspensions were analysed and sorted using a modified fluorescence-activated cell sorting (FACS) protocol using tdTomato only and DACT1ΔCDB–tdTomato mutant particulate suspensions as negative gating controls. The experiment was analysed using the BD LSRII more than three times and particles were sorted using the BD FACSaria Fusion system and analysed using mass spectrometry. n = 4 independent isolations with similar results. b. Proteins enriched in the Wnt3a-treated versus control-treated conditions. Two proteins (NOP14 and IGHG1) were detected in the control condition only and not in the Wnt3a condition. n = 3 independent isolations and MS analyses per condition. c. Confocal imaging of indirect immunofluorescence staining for CK2 in BM2–hDACT1–tdTomato cells. Colocalization of DACT1–tdTomato with cytoplasmic CK2 is highlighted in the magnified images in the area indicated by a dotted line box. Scale bars, 20 μm (left) and 2 μm (right). Images are representative of two independent experiments. d. BM2–TGC cells expressing either vector or DACT1 were treated with Wnt3a with or without silmitasertib at 5 μM for 24 h. e. Median fluorescence intensities (MFI) from c of the Wnt3a treated condition (n = 3 biological replicates) were averaged and subtracted from the individual median fluorescence intensity values for silmitasertib-treated cells (n = 3 biological replicates). Statistical analysis was performed using a Student’s t-test. The experiment is representative of three independent replicates. Data are mean ± s.e.m. Source data are available online.
showed that the TGF-β signalling and the EMT gene sets were strongly enriched among genes that are correlated with DACT1 in the METABRIC clinical dataset\(^5\), suggesting that DACT1 and its coregulated genes are influenced by TGF-β signalling in human breast cancer (Extended Data Fig. 9l,m). To further assess the clinical relevance of DACT1, we analysed the EMC-MSK breast cancer clinical dataset with annotations for organ-specific metastatic relapse\(^5\). The results indicated that higher DACT1 expression is associated with a higher risk of developing bone metastasis, but is not linked to metastatic relapse to other organ sites, such as the lung, liver and brain (Fig. 6g and Supplementary Table 5).

**Discussion**

Here we described the functional mechanism of DACT1 in the suppression of Wnt signalling through the generation of phase-separated biomolecular condensates. Previous studies of the function of DACT1 function have not agreed on the directionality or the mechanism, yet have identified multiple binding partners, such as Dishevelled, β-catenin, VPS34 (ref. \(^4\)) and even itself, suggesting that a unified understanding of DACT1 was lacking\(^4\). Here we show that DACT1 serves as a scaffold to generate phase-separated condensates and, by leveraging the ability of phase-separated granules to persist in cell-free systems\(^5\), we developed a robust method to...
reveal >600 protein constituents in these DACT1 condensates. A few of these constituents have been described by previous DACT1 binding screens, including HDAC1 (ref. 38) or CK2 (ref. 55). Given the complexity of this DACT1-associated proteome, it is improbable that Wnt suppression is the sole function of DACT1.

The identification of protein regulatory, RNA-binding and translational machinery in these condensates is paralleled by previous descriptions of phase separation in which RNA–protein hybridization is the driving force behind the formation of these organelle-like structures31. Whereas our MS data identified numerous RNA-binding proteins that were previously identified as putative components of other phase-separated systems31, we did not identify the essential stress-granule or p-body proteins Fus or Dcp1a. In combination with immunostaining showing that these DACT1 particles do not colocalize with major organelles, p-bodies (Extended Data Fig. 4e) or AXIN1-driven condensates (Fig. 2e), the data here support that these DACT1 particles have an organelle-like structure that functions as a central node to integrate TGF-β and Wnt signalling.

The field of phase separation and regulation of biomolecular condensates has received considerable attention as a distinct biophysical mechanism that underlies spatial regulation21,31,58. This is particularly important to the interplay of Wnt and TGF-β signalling, as few nodes of crosstalk have emerged between these pathways. The data here indicate that DACT1 condensates prevent Wnt signalling activation by interfering with CK2 activity; however, this is probably only one of its roles. Highlighting this, nonsense mutations in DACT1 Trp 419, located in DR7, cause an autosomal dominant disorder with features that overlap Townes–Brock syndrome59.

These data, when combined with previous studies of bone metastasis, suggest a model wherein initial binding of bone-metastatic cells to the sinusoidal vasculature temporarily induces Wnt signalling that is needed for the initial disseminated phase18. DACT1 is
then induced by TGF-β, which is abundant in the bone matrix, to suppress Wnt signalling in the dormancy stage\textsuperscript{15}. Further metastatic outgrowth in the bone then stimulates osteoclastogenesis, which further increases the local TGF-β concentration\textsuperscript{9}, leading to DACT1 induction and subsequent Wnt suppression that has been shown to be important to the development of osteolytic macrometastases\textsuperscript{16}. Despite strong evidence for this model from multiple distinct studies, future studies should assess the temporal contribution of DACT1 to the progression of bone metastasis. This study opens the door to future investigations into the biogenesis and function of the DACT1 particles across cellular biology in normal development and disease.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-021-00641-w.
Received: 16 June 2020; Accepted: 26 January 2021; Published online: 9 March 2021

References
1. Attisano, L. & Labbe, E. TGF-β signalling. J. Biol. Chem. 280, 2498–2502 (2005).
2. Glass, D. A. 2nd et al. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. Dev. Cell 8, 751–764 (2005).
3. Retting, K. N., Song, B., Yoon, B. S. & Lyons, K. M. BMP canonical Smad signaling through Smad1 and Smad5 is required for endochondral bone formation. Development 136, 1093–1104 (2009).
4. Takaku, K. et al. Gastric and duodenal polypos in Smad4 (Dpc4) knockout mice. Cancer Res. 59, 6113–6117 (1999).
5. Fujita, K. & Itoh. Attenuation of WNT signaling by DKK-1 and -2 regulates BMP-induced osteoblast differentiation and expression of OPG, RANKL, and M-CSF. Mol. Cell. 6, 71 (2000).
6. Winkler, D. G. et al. Sclerostin inhibition of Wnt-3a-induced CH110T1/2 cell differentiation is indirect and mediated by bone morphogenetic proteins. J. Biol. Chem. 280, 2498–2502 (2005).
7. Inkinson, C. A. et al. TGF-β1 and WISP-1/CCN4 can regulate each other’s activity to cooperatively control osteoblast function. J. Cell. Biochem. 104, 1865–1878 (2008).
8. Bragado, P. et al. TGF-β2 dictates disseminated tumour cell fate in target organs through TGF-βRII and p38α/β signalling. Nat. Cell. Biol. 15, 1351–1361 (2013).
9. Korpal, M. et al. Imaging transforming growth factor-β signaling dynamics and therapeutic response in breast cancer bone metastasis. Nat. Med. 15, 960–966 (2009).
10. Weilbaecher, K. N., Guise, T. A. & McCauley, L. K. Cancer to bone: a fatal attraction. Nat. Rev. Cancer 11, 411–425 (2011).
11. Esposito, M., Guise, T. & Kang, Y. The biology of bone metastasis. Cold Spring Harb. Perspect. Med. 8, a031252 (2018).
12. Xu, J. et al. 14-3-3 protein is associated with bone metastatic progression in prostate cancer. Cancer Sci. 109, 2024–2028 (1987).
13. Gao, X. et al. Dapper1 is a nucleocytoplasmic shuttling protein that negatively modulates Wnt signaling in the nucleus. J. Biol. Chem. 283, 35679–35688 (2008).
14. Chen, H. et al. Protein kinase A-mediated 14-3-3 association impedes human Dapper1 to promote disseminated degradation. J. Biol. Chem. 286, 14870–14880 (2011).
15. Ma, B. et al. Dapper1 promotes autophagy by enhancing the interaction with the Vps34-Ag14L complex formation. Cell Res. 24, 912–924 (2014).
16. Huang, Y., Wang, P., Chen, H., Ding, Y. & Chen, Y. G. Myc-interacting zinc-finger protein 1 positively regulates Wnt signaling by protecting Dishevelled from Dapper1-mediated degradation. Biochem. J. 466, 499–509 (2015).
17. Kiwirae, S., Yang, X. Y. & Cheyette, B. N. All Dact (Dapper/Frodo) scaffold proteins dimerize and exhibit conserved interactions with Vangl, Dvl, and serine/threonine kinases. BMC Biol. 12, 33 (2011).
18. Fuerer, C. & Nusse, R. Lentinular vectors to probe and manipulate the Wnt signaling pathway. PLoS ONE 5, e9370 (2010).
19. Biechele, T. L., Adams, A. M. & Moon, R. T. Transcription-based reporters of Wnt/beta-catenin signaling. Cold Spring Harb. Protoc. 2009, pdb.prot5223 (2009).
20. Ma, B. et al. The Wnt signaling antagonist Dapper1 accelerates Dishevelled2 degradation via promoting its ubiquitination and aggregate-induced autophagy. J. Biol. Chem. 290, 12346–12354 (2015).
21. Voros, J. The density and refractive index of adsorbing protein layers. Biophys. J. 87, 553–561 (2004).
22. Banani, S. F., Lee, H. O., Hyman, A. A. & Rosen, M. K. Biomolecular condensates: organizers of cellular physiology. Nat. Rev. Mol. Cell. Biol. 18, 285–298 (2017).
23. Weber, S. C. & Brangwynne, C. P. Getting RNA and protein in phase. Cell 149, 1188–1191 (2012).
24. Schafer, K. N. & Peifer, M. Wnt/beta-catenin signaling regulation and a role for biomolecular condensates. Dev. Cell. 48, 429–444 (2019).
25. Feric, M. et al. Coexisting liquid phases underlie nuclear subcompartments. Cell 165, 1686–1697 (2016).
26. Woodruff, J. B., Hyman, A. A. & Boke, E. Organization and function of non-dynamic biomolecular condensates. Trends. Biochem. Sci. 43, 81–94 (2018).
27. Bracha, D. et al. Mapping local and global liquid phase behavior in living cells using photo-oligomerizable seeds. J. Cell Sci. 129, 3500–3507 (2016).
28. Webb, D. et al. B浦b-body purification reveals the condensation of repressed mRNA. Mol. Cell. 68, 144–157 (2017).
29. Gao, X. & Yang, W. H. Casein kinase 2 is activated and essential for Wnt/beta-catenin signaling. J. Biol. Chem. 281, 18394–18400 (2006).
30. Vinayagam, A. et al. A directed protein interaction network for investigating intracellular signal transduction. Sci. Signal. 4, ra8 (2011).
31. Curtis, C. et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486, 346–352 (2012).
32. Bos, P. D. et al. Genes that mediate breast cancer metastasis to the brain. Nature 459, 1005–1009 (2009).
33. Bracha, D. et al. Mapping local and global liquid phase behavior in living cells using photo-oligomerizable seeds. Cell 175, 1467–1480 (2019).
34. Hubschtemann, A. et al. B浦b-body purification reveals the condensation of repressed mRNA. Nature 486, 346–352 (2012).
35. Woodruff, J. B., Hyman, A. A. & Boke, E. Organization and function of non-dynamic biomolecular condensates. Trends. Biochem. Sci. 43, 81–94 (2018).
36. Bracha, D. et al. Mapping local and global liquid phase behavior in living cells using photo-oligomerizable seeds. Cell 175, 1467–1480 (2019).
37. Webb, B. D. et al. Heterozygous pathogenic variant in DACT1 causes an autosomal-dominant syndrome with features overlapping Townes-Brocks syndrome. Hum. Mutat. 38, 373–377 (2017).

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature Limited 2021
Methods

Cell lines, cell culture and in vivo selection. MDA-MB-231 and its subline BM2, HER293T, HaCaT, MDA-MB-468 and SW480 cells were cultured in DMEM medium supplemented with 10% FBS and penicillin–streptomycin. DU415 cells and their derivatives were cultured in RPMI medium supplemented with 10% FBS and penicillin–streptomycin. The SUM159 cell line, its derivative sublines and HPL1 were grown in F12 medium supplemented with 10% FBS, 10 μg/ml insulin and 20 ng/ml EGF as well as penicillin–streptomycin. None of the cell lines used here appear in the database of commonly misidentified cell lines (ICLAC). All of the cell lines were validated with short tandem repeat (STR) analysis and compared to NCBI repository data where available (HPL1 does not have an STR registry entry). All of the cell lines were confirmed to be mycoplasma negative by monthly PCR analysis. Key reagents used in cell culture were as follows: LY2109761 (Cayman Chem), TGF-β protein (R&D systems), bafilomycin A1 (Tocris), MG-132 (MedChemExpress), siutisetib (MedChemExpress), cycloheximide (Sigma-Aldrich) and ICG-001 (Selleck Chemicals). Wnt3a or control medium was obtained from L-cell conditioned medium as described previously.

To screen for acceptable models for in vivo selection, the human breast cancer cell lines SUM159 (ER−), HS578T (ER−), T47D (ER+) and ZR-75-1 (ER+) were labelled with GFP/luciferase reporter (TR construct) and were injected into the mammary fat pad, tail vein or left ventricle of female nude/nu athymic mice (>5) to form primary tumours, lung metastases or bone metastases, respectively. These were followed for 200d, at which point, only cells from the SUM159 cell line showed a low proficiency for primary tumour growth (20%), whereas no tumours or metastatic lesions were detected from the other cell lines. A primary tumour from the SUM159 injection was dissociated, cultured (P1) and retrovirally transduced either the mammary fat pad or tail vein. Outgrowths from both injections were isolated, cultured (P2, M1a and M1b) and M1a cells were reinjected through the tail vein yielding a lung nodule that was isolated and cultured (M1L1). The original identity of each line in the cell series was confirmed by STR profiling and GFP/luciferase expression.

Mouse models and xenografts. All of the procedures involving mice and experimental protocols were approved by the University Institutional Animal Care and Use Committee (IACUC). The study is compliant with all relevant ethical regulations regarding animal research. The animal facilities were maintained at 20–22 °C under 14 h–10 h light–dark cycles at 40–70% relative humidity. All experimental protocols were approved by the University Institutional Animal Care and Use Committee. Mouse models and xenografts. To screen for acceptable models for in vivo selection, the human breast cancer cell lines SUM159 (ER−), HS578T (ER−), T47D (ER+) and ZR-75-1 (ER+) were labelled with GFP/luciferase reporter (TR construct) and were injected into the mammary fat pad, tail vein or left ventricle of female nude/nu athymic mice (>5) to form primary tumours, lung metastases or bone metastases, respectively. These were followed for 200d, at which point, only cells from the SUM159 cell line showed a low proficiency for primary tumour growth (20%), whereas no tumours or metastatic lesions were detected from the other cell lines. A primary tumour from the SUM159 injection was dissociated, cultured (P1) and retrovirally transduced either the mammary fat pad or tail vein. Outgrowths from both injections were isolated, cultured (P2, M1a and M1b) and M1a cells were reinjected through the tail vein yielding a lung nodule that was isolated and cultured (M1L1). The original identity of each line in the cell series was confirmed by STR profiling and GFP/luciferase expression.

Cloning, viral production and transduction. All KD and overexpression genetics were performed using lentiviral transduction as described previously. Kds were performed using lentiviral shRNA vectors purchased from Sigma-Aldrich (Supplementary Table 6), and DACT1-deletion and Fus-hybrid mutants are included in Supplementary Table 2 and were subcloned into the pLXe-MCS lentiviral system (Thermo Fisher Scientific). Wnt reporters (7TGC, 7TGP and 1×TGF-βLLUC/pBARL-hyg) were gifts from R. Nusse and R. Moon. All viral transduction and selection was performed on a cell-population-wide basis.

Flow cytometry and fluorescence-activated particle sorting. For Wnt activation transduction and selection was performed on a cell-population-wide basis. The fluorescence excitation and detection parameters used were as follows: DAPI, 355 nm (40 mW) excitation, 450/50 nm bandpass filter; GFP, 488 nm (100 mW) excitation, 525/50 emission; tdTomato, 561 nm (40 mW) excitation, 610/20 emission. Gating and analysis were performed using FlowJo v.X. and FlowJo v.X.

cPCR, western blot and proliferation assay. RT-qPCR was performed using the SYBR green mastermix protocol on cDNA generated using the SuperScript III cDNA synthesis kit. The primer pairs are included in Supplementary Table 6. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qPCR was run according to the manufacturer’s recommendations. PCR conditions were 10 min at 95 °C, then 40 cycles of 15 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C. Sensitivity analysis was performed on a 10-fold dilution series. The final data were calculated using the comparative Ct method and normalized to GAPDH expression. The geometric mean fold change was calculated using REST software. For analysis of protein levels, Western blotting was used.

Nuclear localization of β-catenin. Captured images from control or Wnt-stimulated BM2 cells with the indicated genetic modifications were processed in Fiji software using a customized macro script. The script splits the 24-bit composite images into blue (DAPI), red (β-catenin) and blue channels in the ECoLocolization plugin for colocalization measurements. Images were aligned using the default settings and nuclei were

qPCR, western blot and proliferation assay. RT-qPCR was performed using the SYBR green mastermix protocol on cDNA generated using the SuperScript III cDNA synthesis kit. The primer pairs are included in Supplementary Table 6. cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qPCR was run according to the manufacturer’s recommendations. PCR conditions were 10 min at 95 °C, then 40 cycles of 15 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C. Sensitivity analysis was performed on a 10-fold dilution series. The final data were calculated using the comparative Ct method and normalized to GAPDH expression. The geometric mean fold change was calculated using REST software. For analysis of protein levels, Western blotting was used.

Quantitative image analysis. 3D RI tomograms and the correlative 3D fluorescence signals with red pseudocolour were acquired using confocal microscopy (TomoStudio, TomoCube) and the ObjectQuest plugin (TomoVision). The visualization of 3D RI maps and its correlative 3D fluorescence signal with red pseudocolour was performed using commercial software (AutoQuant X3, Media Cybernetics). The visualization of 3D RI maps and its correlative 3D fluorescence signal with red pseudocolour was performed using commercial software (TomoStudio, TomoCube). Detailed information on the principle of optical diffraction tomography, a reconstruction MATLAB code and a regularization algorithm to address the issue caused by the uncollected side-scattering signals can be found elsewhere. For 3D QPI and fluorescence imaging, BM2 and M1a cells expressing the DACT1-tdTomato fusion protein were subcultured into a microscopic dish (TomoDish, TomoCube; thickness, 1.3H; 50-mm-diameter glass bottom).

Quantitative image analysis. 3D RI tomograms and the correlative 3D fluorescence images were transformed into a same-coordinate system. After reducing noise using Gaussian filtering, a 3D mask in a RI tomogram corresponding to the 3D fluorescence signal was generated by thresholding. Median filtering was performed to further reduce the remaining noise. Blob detection was performed under the following conditions:

```
params = cv2.SimpleBlobDetector_Params()
params.minArea = 1
params.minCircularity = 0.1
params.minConvexity = 0.1
params.minArea = 50
```

Each detected particle was segmented using RI thresholds that were determined using Otus’s method and marker-controlled watershed segmentation. The volume of protein condensation was calculated by considering the physical size of individual voxels. Dry mass was calculated with 0.190 ml g⁻¹ as the RI increment (dn/dc) (refractive index increment)²⁶.

Nuclear localization of β-catenin. Captured images from control or Wnt-stimulated BM2 cells with the indicated genetic modifications were processed in Fiji software using a customized macro script. The script splits the 24-bit composite images into blue (DAPI), red (β-catenin) and blue channels in the ECoLocolization plugin for colocalization measurements. Images were aligned using the default settings and nuclei were
segmented using the prewatershed filter area (2500-Infinity). Threshold overlap score linearly rescaled was used as the metric for quantifying β-catenin nuclear localization and Coates’ algorithm to select thresholds. In total, 913 cells were analysed in 4 biological replicates under each treatment group. The box plots show the median value (horizontal line) with the upper and lower quartiles (box limits), and the whiskers represent 1.5x the interquartile range. Colocalization measurements for each cell generated by the EzColocalization plugin were aggregated and processed for visualization in R. Paired two-sided Student’s t-tests were performed to compare treatment groups statistically.

SoRa super-resolution and confocal imaging. Nikon SoRa super-resolution imaging was performed on HEK293T cells transfected with DACT1–tdTomato C-terminal fusions in the pLEX vector that were fixed with 10% formalin, stained with Hoechst 33342 and mounted in ProLong Gold. Ex vivo imaging was performed on bone samples containing metastatic BM2-DACT1–tdTomato cells that were removed and fixed for 12 h with 10% buffered formalin at 4°C. Tissues were then equilibrated in 15% and 30% sucrose for 3 h each at room temperature before embedding in SCEM (Section Lab) and cryo-sectioning using specialized adhesive film (Section Lab). Confocal imaging was performed using the Nikon A1-R system. Laser power, gain and lookup tables were kept at the same levels within the same imaging session, with the exception of DACT1–tdTomato mutant analysis, which required case-by-case modification of the laser power to best visualize fluorescence. Data with variable laser power, gain or LUTs are annotated in figure legends. Live imaging of BM2–TcG-DAC1–tdTomato mutants was performed with epifluorescence in ibidi tissue-culture-treated plastic-bottom dishes. FRAP analysis was performed on more than three concentrations per M14 and BM2 cell line using previously reported parameters.

MS analysis. MS-based quantification of the DACT1 particle proteome. All MS data presented here are the culmination of three separate proteomic analyses from three separate biological replicates of FACS-sorted DACT1-particulates, with control and WNT-treated pairs. FACS-purified particles, suspended in PBS, were brought up to 5% SDS in solution to solubilize the proteins, then centrifuged, digested with trypsin and desalted using S-Trap spin columns (Protifi). Peptides were eluted as a single fraction and analysed by LC–MS/MS using a Dionex Ultimate rNLSC coupled to a Thermo Fisher Scientific Q-Exactive HF mass spectrometer, running data dependent acquisition (DDA). For LC, peptides were separated over a 60 min gradient (5–35% of 0.1% formic acid in 97% acetonitrile) in a 45 cm column packed in-house with 1.9 mm ReproSil-Pur C18-AQ (Dr. Maisch) at 60°C. For the MS1 scan, the scan range was set to 350–1,800 m/z with an automatic gain control (AGC) of 5 × 106, with a maximum injection time of 30 ms and a resolution of 120,000. The top 10 ions were processed for collision-induced fragmentation for MS2 analysis at a resolution of 15,000, AGC of 1 × 106 and a maximum injection time of 150 ms. The isolation window was set to 1.2 m/z and normalized collision energy to 28. Tandem MS spectra were exported and analysed using Proteome Discoverer v.2.4 (Thermo Fisher Scientific, OPTON-20141).

The Proteome Discoverer v.2.4 processing workflow used the SEQUEST-HT algorithm to match identified peptides against a FASTA file containing human protein sequences and common contaminants (Uniprot), generating peptide spectrum matches and determining the false-discovery rate (FDR). The Sequest analysis was performed using settings for tryptic peptides with a maximum of 2 missed cleavages, a minimum length of 6 amino acids, and precursor and fragment mass tolerances of 0.05 and 0.02 Da respectively. Contaminant allowances for carbamidomethylation, dynamic oxidation of methionine and dynamic loss of methionine with N-terminal acetylation. Matched spectra were validated by Percolator, and only peptides with the highest confidence were retained for further analysis.

Using the KEGG Mapper and Precursor Ions Quantifier nodes in Proteome Discoverer, a consensus file of matched peptides was created. Peptides were further filtered by requiring a minimum of 5 peptide spectral matches per scan and an FDR of 1%. Missing values from peptides that were not selected for MS2 fragmentation in each replicate were identified by mapping label-free MS1 signals across samples and quantifying the maximum peak intensity for each peptide. Therefore, a minimum of 5 unique peptides for every unique peptide that contained quality-filtering. To be retained for further analysis, protein identification required at least two unique peptides that had to be found with high confidence in at least two (out of the three) replicates. For each condition (control versus Wnt-treated), all label-free quantitative data were normalized to the abundance of DACT1, as the FACS-based sorting was dependent on DACT1-positive selection. The label-free quantification was grouped across the three biological replicates to generate an average protein abundance value per condition.

**RNA fluorescence probe design and in situ hybridization.** To visualize human CTNNB1 mRNA molecules directly, we used a RNA FISH protocol described previously with minor modifications. Human CTNNB1 (Biocode technologies; VSMF-2063-5) probes were tagged with Quasar 670 to avoid spectral overlap with tdTomato. BM2 cells were cultured on circular coverslips until 50–70% confluency. Cells were washed in 1x PBS and fixed in 4% paraformaldehyde in 1x PBS for 10 min at room temperature. After fixation, cells were washed twice with 1x PBS and then permeabilized in 70% ethanol at 4°C overnight. Permeabilized cells were incubated in 500 nM CTNNB1 probes in hybridization buffer for 4 h at 37°C in a humidified chamber. Cells were washed with FISH wash buffer for 30 min at 37°C before mounting on a glass slide using ProLong Glass Antifade Mountant with NuclBlue Stain (Thermo Fisher Scientific, P36981). Cells were imaged on a Nikon A1R fluorescence microscope at x100 magnification in the 405 nm, 368 nm and 647 nm excitation channels to image NuclBlue, tdTomato and CTNNB1 RNA.

**Microarray analysis.** RNA for microarray analysis was isolated from cells using the RNeasy Minikit (Qiagen) according to the manufacturer’s instructions. RNA isolated from SUM159 cells was analysed using the Agilent human GE 8x60k two-colour microarray system (Agilent G4858A-039494). In brief, the RNA samples and a universal human reference RNA (Agilent) were labelled with CTP-c55 and CTP-c53 using the Agilent Quick Amp Labeling Kit. Labelled samples were mixed equally and hybridized to the array. The array was then scanned with the G2505C scanner (Agilent). Data were deconvoluted and analysed using Genespring v.13 (Agilent). In brief, array controls, flagged values and expression values falling below the median value were removed. Multiple values for any given gene were collapsed into the single highest expression value. Data were extracted as the log2-transformed ratio of Cy5/Cy3.

**GSEA.** log2-transformed data were subtracted and rank-ordered for each cell line. Data were analysed using GSEA v.4.0. Only datasets with nominal P ≤ 0.05 were considered in the analysis.

**Statistics and reproducibility.** All statistical comparisons were conducted using Stata v.13 (Mann–Whitney U-tests, repeated measures analysis of variance, regression and single-variable Cox’s Proportional Hazards). Microsoft Excel 2017 (t-tests) or BioMap Institute GSEA v.4.0 (preranked GSEA analysis). The results are reported as mean± S.E.M. for bar graphs with accompanying individual data points for n < 6. For all of the animal experiments, animals were excluded only if they died or had to be euthanized according to the predefined criteria listed in the IACUC protocol. Two-sided Mann–Whitney U-tests were used for bone metastasis as these data do not exhibit a normal distribution. Wilcoxon rank-sum and lesion analysis assumed each hindlimb as an independent event. Two-sided, unpaired Student’s t-tests were used for all qPCR, flow cytometry and other normal data. All statistical comparisons are non-significant at an alpha level of 0.05 if not marked by a specific P value or an asterisk, and all statistical comparisons were performed two-sided. Patient expression values in the EMC-MSK datasets were stratified by median value. Survival data for patient datasets were analysed using a single-variable Cox’s proportional Hazards model to generate hazard ratios and their associated P values. All of the in vivo experiments were repeated according to the respective figure legend. All other experiments were repeated according to their corresponding figure legend at a minimum of two independent repeats unless otherwise noted. Each experiment was repeated independently with similar results. Source data and replicates are included in the Supplementary Information. All unprocessed western blot images are included as source data. A list of the key characteristics of the cell lines used in this study is provided in Supplementary Table 8. All protocols, cell lines and reagents are available from the corresponding author on reasonable request.

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

Proteomic data have been deposited at ProteomeXchange Consortium through the PRIDE partner repository under the dataset identifier PXD019271; Microarray data are available in the Supplementary Table 8 of the manuscript using PantherDB (http://www.pantherdb.org/) and Cytoscape (https://cytoscape.org/). The microarray data generated in this study are available at the NCBI Gene Expression Omnibus (GEO) with the accession number GSE151198. The human
breast cancer data from the EMC-MSK dataset are available in Bos et al. and source data from this resource supporting Fig. 6g and Supplementary Table 5 are available in the source data for Fig. 6. The data from the METABRIC dataset are available in Curtis et al. as well as the source data for Extended Data Fig. 9. Source data are provided with this paper. All other data and image files are available from the corresponding author on reasonable request.

**Code availability**

Customized macro and R scripts used for colocalization analysis are available at GitHub (https://github.com/rgunaratna/Colocalization_Analysis).

**References**

60. Kim, K. et al. High-resolution three-dimensional imaging of red blood cells parasitized by *Plasmodium falciparum* and in situ hemozoin crystals using optical diffraction tomography. *J. Biomed. Opt.* **19**, 011005 (2014).
61. Popescu, G. et al. Optical imaging of cell mass and growth dynamics. *Am. J. Physiol. Cell Physiol.* **295**, C538–C544 (2008).
62. Sheng, H., Stauffer, W. & Lim, H. N. Systematic and general method for quantifying localization in microscopy images. *Biol. Open* **5**, 1882–1893 (2016).
63. Mellacheruvu, D. et al. The CRAPome: a contaminant repository for affinity purification-mass spectrometry data. *Nat. Methods* **10**, 730–736 (2013).
64. Raj, A., van den Bogaard, P., Rifkin, S. A., van Oudenaarden, A. & Tyagi, S. Imaging individual mRNA molecules using multiple singly labeled probes. *Nat. Methods* **5**, 877–879 (2008).

**Acknowledgements**

We thank C. DeCoste and K. Rittenbach at the Molecular Biology Flow Cytometry Resource Facility of Princeton University for flow cytometry assays and G. Laervy at the Confocal Imaging Facility of Princeton University (a Nikon Center of Excellence) for assistance with imaging; H. Cho and D. Kim for performing Tomocube image sectioning and RI analysis; and Y. Chen (Tsinghua University), B. Cheyette (UCSF), R. Moon (University of Washington) and R. Nusse (Stanford) for providing reagents. This work was supported by fellowships from the NIH (F31CA192461 to M.E. and F31A1147637 to K.C.C.), from NICCCR (DCHS19PPC029 to R.T.G.), and grants from the Brewster Foundation, NIH (R01CA212410) and the US Department of Defense (BC123187) to Y.K., and from the NIH (R01GM114141 to L.M.C. and T32GM007388 to K.C.C.).

**Author contributions**

M.E. conceived the project and cowrote the manuscript. M.E., C.F. and N.P. designed and performed flow cytometry, xenograft, genetic, RT–qPCR, confocal and bioinformatics experiments, and analysed data with assistance from Y.W., C.S., H.S. and R.T.G.; K.C.C. and L.M.C. designed and performed MS. C.J.D. assisted with particle isolation and G.L. assisted with microscopy. D.B. and C.P.B. performed and assisted with FRAP and FUS experiments and provided expert advice. Y.K. supervised the project, cowrote the manuscript, and provided experimental advice and critical guidance.

**Competing interests**

M.E. holds equity interest in KayoThera. Y.K. holds equity interest in KayoThera and Firebrand Therapeutics.

**Additional information**

Extended data is available for this paper at https://doi.org/10.1038/s41556-021-00641-w. Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41556-021-00641-w.

**Correspondence and requests for materials** should be addressed to Y.K.

**Peer review information**: *Nature Cell Biology* thanks the anonymous reviewers for their contribution to the peer review of this work. Peer reviewer reports are available.

**Reprints and permissions information** is available at www.nature.com/reprints.
Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | Characterization of the metastatic variants of SUM159 breast cancer cell line identifies DACT1 as a highly expressed gene in the bone metastatic M1a subline. a, Schematic summary of the establishment of a series of isogenic sublines with different primary tumor and metastasis potential from the parental SUM159 triple negative breast cancer cell line. Parental SUM159 cells were stably labeled with a retroviral triple reporter (TR) expressing GFP, thymidine kinase, and firefly luciferase (F-luc) and injected into the mammary fat pad of nude mice. A primary tumor was isolated, cultured, and re-injected by either tail-vein or mammary fat pad injection. Successful outgrowths were then isolated and cultured. b, Isolated cell lines were injected orthotopically into NSG mice and monitored for primary tumor growth. n = 5 mice/group. Student’s t-test. Representative of 2 independent experiments. c, d, e, Ex vivo imaging of bones and lungs was conducted once primary tumors reached mean diameter >1 cm. Values for lung (c) and bone (d) metastasis were thresholded to 0 at values below 10⁴ and 10⁵ photon/sec, respectively, to remove background noise. Individual hindlimbs were treated as independent data points. Rates of successful outgrowth were enumerated per group (e). f, Representative ex vivo bioluminescence images of spontaneous metastasis to lung and bone from each derived subline from (c–e). g, The development of bone metastasis after intracardiac injection of each derivative was monitored by bioluminescent imaging and was compared to SUM159-TR. Mann-Whitney U test. n = 6 mice/group. h, i, Representative bioluminescent (h) and X-ray images (i) at day 0 and day 25 of mice from (g). j, Gene expression values from microarray analysis were used to generate a list of genes up-regulated >4-fold in M1a compared to either SUM159PT-TR or M1L1. k, Heatmap representation of the expression levels of the 11 differentially expressed genes from (j). l, RT–qPCR analysis of DACT1 mRNA levels normalized to Gapdh in the indicated SUM159 sublines. n = 4 technical repeats, representative data from 2 independent experiments. m, n) Flow cytometry measurement of 7x-TCF–GFP Wnt reporter activity in BM2 (m) or HPL1 (n) cells with Wnt3a and Wnt inhibitor ICG-001 (25 μM) treatment. n = 3 biological replicates. Student’s t-test. Experiment independently repeated 3 times. Data represents mean ± SEM. * p < 0.05, ** p < 0.01, *** p < 0.005 in g with exact p values in Source Data. Numerical source data for b–e, g, l–n are provided.
Extended Data Fig. 2 | DACT1 represses Wnt signaling. **a, b,** qPCR analysis of DACT1 mRNA levels normalized to Gapdh in BM2-TGC (**a**) and HPL1-TGC (**b**) cells stably transduced with DACT1-targeting shRNA (KD#1 and KD#2) or overexpression constructs. n = 4 technical repeats, Student’s t-test. Data represents mean ± SEM. Student’s t-test. **c,** Flow cytometry assessment of 7x-TCF-GFP Wnt reporter activity in BM2 (**c**) or HPL1 (**d**) cells with DACT1 knockdown or overexpression; n = 3 (**c**) and n = 4 (**d**) biological replicates. Student’s t-test. Experiments were independently repeated >3 times (**c**) and 2 times (**d**). **e,** Immunofluorescence of total β-catenin in BM2 cells with DACT1 knockdown or overexpression with Wnt3a stimulation for 24 hours. Scale bars represent 50 μm. Representative of 2 independent replicates. **f,** Western blot of indicated proteins in BM2 cells with indicated DACT1-related constructs with Wnt3a or control media stimulation for 24 hours. Representative of 2 independent replicates. Numerical source data for **a–d** and uncropped blots for **f** are provided.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | TGF-β-induced DACT1 suppresses Wnt signaling. **a**, Indirect immunofluorescence of β-catenin in the indicated DACT1-modified HPL1 cells with Wnt3a or control media for 24 h. Mean pixel in intensities for Wnt3a-treated cells were measured at 9.041 (KD#1), 5.047 (KD#2), 1.562 (Vector), 1.356 (DACT1). Scale bars represent 10 μm. Representative of 2 independent replicates. **b**, qPCR analysis of Axin2 mRNA in BM2 cells with stable DACT1 KD or control after treatment with Wnt3a for 24 or 48 h. mRNA levels were normalized with Gapdh level and then Axin2 levels were normalized to the respective control condition. n = 3 technical replicates, Student’s t-test. Representative of 2 independent experiments. **c**, 7x-TCF-GFP Wnt reporter expressing BM2 cells were pre-treated for 24 hours with TGF-β and/or the TGF-β inhibitor LY2109761 followed by stimulation with Wnt3a and flow cytometry assessment of Wnt activation. n = 4 biological replicates. Student’s t-test. **d**, BM2-TGC cells with the indicated modification of DACT1 were treated with Wnt3a or Wnt3a + TGF-β (3 h or 24 h pre-treatment) were quantified by flow cytometry. n = 4 biological replicates. Student’s t-test. **f**, Gating strategy for flow cytometry quantification of 7TGC Wnt reporter activity. GFP+ counts from GFP by mCherry panel corresponds to data in Figs. 1e, 3c, 3f, 4d, 4e, Extended Figs. 1m, n, 2c, d, 3c-e. Numerical source data for b-e are provided.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | DACT1 bodies are proteasomally degraded and do not co-localize with known organelles. **a**, DACT1-TdTomato fusion protein expression in M1a cells with or without 24 h Wnt3a treatment. Scale bar represents 10 μm. Representative of n > 5 independent replicates. **b**, DACT1-TdTomato c-terminal fusions imaged in HPL1 cells. Scale bar represents 5 μm. Representative of n > 5 independent replicates. **c–d**, Western blot analysis of DACT1 wild-type and DACT1-TdTomato fusion protein expression in both M1a and BM2 cells after treatment with cycloheximide (50 μg/mL) at the indicated times (**c**) or either Bafilomycin A (10 nM) or MG-132 (20 μM) for 12 h (**d**). Images (**c, d**) representative of 3 independent experiments. * indicates expected molecular weight of fusion proteins. **e**, Representative images of DACT1-TdTomato fusions and the indicated organelle-associated proteins imaged by confocal sectioning. Scale bars represent 4 μm. Representative of 3 independent replicates. Uncropped blots for **c, d**, are provided.
Extended Data Fig. 5 | DACT1 particles show biomolecular condensation properties. a, b, DACT1 knockdown, control, overexpressing (a) or TdTomato c-terminal fusion cells (b) were probed with anti-DACT1 antibody followed by confocal imaging. Scale bars represent 5 μm. Images representative of 4 independent replicates. c, Holotomographic/epifluorescent particle sectioning analysis used to map refractive indices to 3D coordinates of TdTomato fluorescence.
Extended Data Fig. 6 | Deletion and hybrid mutant analysis of DACT1 protein localization. a, Schematic of deletions made in the DACT1 amino acid sequence with predicted intrinsically disordered domains in black background and previously reported sequence motifs labeled in multiple colors. b, c, Confocal imaging of BM2 (c) and M1a (d) cells expressing DR7-TdTomato or DR8-TdTomato fusions labeled with Hoechst. Scale bars represent 10 μm. Representative of >3 independent replicates. d, Fluorescent in situ hybridization of RNA probes for Ctnnb1 in BM2-hDACT1-TdTomato expressing cells. Scale bars represent 20 μm. Representative of 3 independent replicates.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | Mass spectrometry analysis of DACT1 biomolecular condensates. a, Isolation and analysis workflow for the characterization of DACT1-TdTomato particles. b, Proteins identified by mass spectometry were analyzed by gene ontology using the Panther DB overrepresentation test with associated enrichment values and p-values. c, High stringency STRING analysis of identified proteins shows enriched protein interaction networks as related to biological processes. Protein background color correlates to enrichment ratio observed in Wnt3a-treated as compared to control-treated particles.
Extended Data Fig. 8 | DACT1 sequesters cytoplasmic CK2 into condensates. a, BM2 cells stably expressing CK2-GFP fusions alone or in combination with DACT1-TdTomato fusions were labeled with Hoechst and imaged via confocal microscopy. Scale bars represent 10 μm. Image representative of >5 independent replicates. b, M1a cells expressing constructs from (a) were subjected to the phase separation particle isolation protocol with the addition of GFP measurement. Experiment independently repeated 2 times.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | DACT1 is essential for bone metastasis in models of breast and prostate cancer. 

a, qRT-PCR analysis of DACT1 mRNA levels normalized to Gapdh in the M1a cells stably transduced with DACT1-targeting shRNA or control shRNA. n = 4 technical repeats, representative data from 2 independent mRNA isolations and qRT-PCR experiments. Student’s t-test. 

b, Proliferation rate over 24 hours was measured by WST-8 MTT assay and normalized to Control shRNA. n = 4 biological replicates, Student’s t-test. 

c, qRT-PCR analysis of DACT1 mRNA levels normalized to Gapdh in the BM2 cells stably transduced with DACT1-targeting shRNA or control shRNA. n = 4 technical repeats, representative data from 2 independent mRNA isolations and qRT-PCR experiments. Student’s t-test. 

Extended Data Fig. 9 | DACT1 is essential for bone metastasis in models of breast and prostate cancer. 

d, BLI quantification of hind limb bone metastasis burden in mice injected with BM2 cells stably transduced with DACT1-targeting shRNA or control shRNA. Per-mouse signal was normalized to photon flux of the same mouse measured on Day 0 post-injection. n = 9 mice per group. Mann-Whitney U test. 

e, Representative bioluminescent and X-ray images from (d). 

f, g, The number (f) and area (g) of overt metastatic bone lesions were quantified per hindlimb per group with ImageJ. Mann-Whitney test. n = 18 (KD#1) and 14 (shCTL) hindlimbs per group. 

h, BLI quantification of hind limb bone metastasis burden in mice injected with DU145-ob2b cells stably transduced with DACT1-targeting shRNA or control shRNA. Per-mouse signal was normalized to photon flux of the same mouse measured on Day 0 post-injection. n = 9 mice per group. Mann-Whitney U test. 

i, qRT-PCR analysis of DACT1 mRNA levels normalized to Gapdh in the DU145-ob2b cells stably transduced with DACT1-targeting shRNA or control shRNA. n = 4 technical repeats. Student’s t-test. 

j, M1a cells stably expressing each DACT1 construct fused to TdTomato or TdTomato alone were inoculated via intracardiac injection and followed by bioluminescence. n = 9 mice per group. Experiment performed once. 

k, Representative images from (j). 

l, Correlation of DACT1 expression values to indicated GOIs from the METABRIC dataset. Pearson correlation coefficients for each gene correlated to DACT1 were ranked and GSEA analysis was performed to test the enrichment of the indicated gene sets in the Hallmark data set. Statistics by GSEA software. Data represents mean ± SEM. Student’s t-test. Numerical source data for a–d, f–j, m, are provided.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted

Give P values as exact values whenever suitable.

For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

All statistical comparisons were conducted with Stata v13 (Mann-Whitney U, repeated measures ANOVA, and single variable Cox’s Proportional Hazards), Microsoft Excel 2017 (t-test), the ImageJ EzCoLoc plugin (TOS linear measure) or Broad Institute GSEA 4.0 software (Pre-ranked GSEA analysis). Flow cytometry was collected and analyzed with FACSDiVa version 8.0.2 and FlowJo version X software packages. Tomocube data was analyzed with accompanying HT-2H system and TomoStudio. Deconvolution of a reconstructed 3-D fluorescence images was performed by using a commercial software (AutoQuant X3, Media Cybernetics). Genespring v13 software was used to deconvolute microarray data. All software used for collection is listed in the Methods. Code is available at https://github.com/rgunaratna/Colocalization_Analysis.

Data analysis

In addition to software above, Proteome Discoverer 2.4 was used with the Sequest HT algorithm, as well as PAntherDB, Cytoscape 3.0 and CRAPome to analyze mass spectrometry data.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier.
PXD019271; mass spectrometry data is further available in Supplementary Table 3. Mass spectrometry data was analyzed using PantherDB (http://www.pantherdb.org/) and Cytoscape (https://cytoscape.org/). The microarray data generated in this study are available at the NCBI Gene Expression Omnibus (GEO) with the accession number GSE151198. The human breast cancer data from the EMC-MSK dataset is available in Bos et al., 2009 and source data from this resource supporting Figures 6g and Supplementary Table 5 is available in the source data for Figure 6. The data from the METABRIC dataset is available in Curtis et al., 2012 as well as the source data for Extended Data Figure 9. All other data and image files are available from the author on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- [ ] Life sciences
- [ ] Behavioural & social sciences
- [ ] Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

### Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No sample size pre-calculation was performed as all sizes meet or exceed standards in this field. Sample size for in vitro experiments were conducted at n =/> 3 according to historical experience with data distribution in experiments such as Wnt activation and qPCR. This sample size typically yields standard error <25% of the mean value. In vivo and colocalization experiments require n=5 in order to robustly analyze this more variable data. All sample sizes meet or exceed standards in the field. |
|---|---|
| Data exclusions | No data was excluded with the exception of mouse studies. For all animal experiments, animals were only excluded if they died or had to be sacrificed according to the pre-defined criteria listed in the IACUC protocol (such as infections or moribundity due to fighting). |
| Replication | All in vivo experiments were highly reproducible and were independently repeated at least 3 times with the exception of prostate cancer bone metastasis and Dact1 mutant bone metastasis experiments which were repeated only once. All microscopy, flow cytometry, mass spectrometry, holotomography, FRAP, FISH experiments were independently repeated at least 2 times with certain critical Flow cytometry experiments (Wnt activation with Dact1 modification, particle isolation) repeated up to 6 times with identical trends. Each experiment was repeated independently with similar results. |
| Randomization | Mice were randomized from each litter into separate experimental groups prior to injection. There was no allocation of test subjects for any other experiments, thus randomization is not relevant beyond animal experiments in this study. |
| Blinding | Single blinding was performed for x-ray analysis to prevent subjective bias in scoring. Otherwise, all data analyses were performed by unbiased software programs/algorithms (e.g. Mass spec analysis, qPCR quantification, etc.) and human quantification/blinding was therefore not relevant. |

### Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Study description | Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study). |
|---|---|
| Research sample | State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source. |
| Sampling strategy | Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed. |
| Data collection | Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection. |
| Timing | Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort. |
| Data exclusions | If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established. |
| Non-participation | State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation. |
## Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Study description | Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates. |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Research sample    | Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source. |
| Sampling strategy  | Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. |
| Data collection    | Describe the data collection procedure, including who recorded the data and how. |
| Timing and spatial scale | Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken. |
| Data exclusions    | If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established. |
| Reproducibility    | Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful. |
| Randomization      | Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why. |
| Blinding           | Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study. |

Did the study involve field work?   Yes  No

### Field work, collection and transport

| Field conditions | Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall). |
|------------------|----------------------------------------------------------------------------------------------------------------------------------|
| Location         | State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth). |
| Access & import/export | Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information). |
| Disturbance      | Describe any disturbance caused by the study and how it was minimized. |

### Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

#### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology and archaeology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |
| ☑   | Dual use research of concern |

#### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |
Antibodies

| Antibodies used | Supplier | Catalog # | Type | Applications | Dilutions |
|-----------------|----------|-----------|------|--------------|----------|
| Dact1           | Abcam    | ab1260    | polyclonal | Western blot | 1:1000   |
| Dvl2            | Cell Signaling Technology | 3216   | polyclonal | Western blot | 1:1000   |
| Dvl3            | Santa Cruz | sc-8027 | 4D3  | Western blot | 1:100    |
| b-catenin (total) | Cell Signaling Technology | 8480   | D10A8 | Western blot | 1:1000   |
| b-catenin (non-phosphorylated) | Cell Signaling Technology | 8814   | D13A1 | Western blot | 1:1000   |
| Aif             | Cell Signaling Technology | 5318   | D39D2 | Immunofluorescence | 1:500 |
| Eea1            | Cell Signaling Technology | 3288   | C45B10 | Immunofluorescence | 1:500 |
| Lamp1           | Cell Signaling Technology | 9091   | D2011 | Immunofluorescence | 1:500 |
| Pdi             | Cell Signaling Technology | 3501   | C81H6 | Immunofluorescence | 1:500 |
| Rcas1           | Cell Signaling Technology | 1229   | D286N | Immunofluorescence | 1:500 |
| Dcp1a           | Abcam    | ab47811   | polyclonal | Immunofluorescence | 1:500 |
| CKBa            | Millipore-Sigma | 05-1431 | C46D9 | Immunofluorescence | 1:500 |

Validation

Dact1 antibody was validated using knockdown and overexpression cell lines as well as colocalization with TdTomato signal in fusion stains. Dact1 primary antibody was validated by the manufacturer using recombinant protein and native proteins for both mouse and human Dact1, and validated the antibody for western blot and immunofluorescence. This antibody is furthermore independently reviewed and cited by several publications on Dact1. All other antibodies are commonly used across the literature and have been validated by the manufacturer for the corresponding applications used here. Aif, Eea1, Lamp1, Pdi, Rcas1, LC3B are provided as an organelle staining kit by CST with extensive validation for immunofluorescence against human cells. Dcp1a, CK2a, Dvl2, Dvl3 and Bactin are all validated for human use in western blot and/or immunofluorescence both by manufacturer and independent reviews. All antibodies used here have been cited in more than 5 peer-reviewed articles per antibody.

Eukaryotic cell lines

Policy information about cell lines

| Cell line source(s) | Details |
|---------------------|---------|
| All cell lines were ordered from ATCC except Sum159 which was a gift from the source at University of Michigan and HPL1 which was a gift from the Massague lab. |

Authentication

All cell lines were validated by STR profiling with >90% match.

Mycoplasma contamination

All cell lines were validated mycoplasma-free by monthly PCR testing, or upon LN2 storing of cell aliquots.

Commonly misidentified lines (See ICLAC register)

No ICLAC cell lines were used

Palaeontology and Archaeology

Specimen provenance

Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).

Specimen deposition

Indicate where the specimens have been deposited to permit free access by other researchers.

Dating methods

If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.

Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not. Note that full information on the approval of the study protocol must also be provided in the manuscript.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

All xenograft experiments were conducted on 8-12 week-old female mice (athymic Nu/Nu, NOD/SCID Gamma) except in prostate cancer xenografts, which were performed on male mice of the same age. All mice were originally ordered from the Jackson Laboratory and breeding was conducted in an SPF barrier facility. Animal facilities were maintained at 20-22C with 14 h:10 h light:dark cycles at 40-70% relative humidity. No statistical method was used to predetermine the number of animals needed.

Wild animals

none
Field-collected samples | none
Ethics oversight | All procedures involving mice and experimental protocols were approved by the University Institutional Animal Care and Use Committee (IACUC). The study is compliant with all relevant ethical regulations regarding animal research.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

**Human research participants**

| Policy information about | studies involving human research participants |
|--------------------------|--------------------------------------------|
| Population characteristics | Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above." |
| Recruitment | Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results. |
| Ethics oversight | Identify the organization(s) that approved the study protocol. |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

**Clinical data**

| Policy information about | clinical studies |
|--------------------------|------------------|
| All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions. |
| Clinical trial registration | Provide the trial registration number from ClinicalTrials.gov or an equivalent agency. |
| Study protocol | Note where the full trial protocol can be accessed OR if not available, explain why. |
| Data collection | Describe the settings and locales of data collection, noting the time periods of recruitment and data collection. |
| Outcomes | Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures. |

**Dual use research of concern**

| Policy information about | dual use research of concern |
|--------------------------|------------------------------|
| Hazards | Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to: |
| No | Yes |
| | Public health |
| | National security |
| | Crops and/or livestock |
| | Ecosystems |
| | Any other significant area |

**Experiments of concern**

| Policy information about | dual use research of concern |
|--------------------------|------------------------------|
| Does the work involve any of these experiments of concern: |
| No | Yes |
| | Demonstrate how to render a vaccine ineffective |
| | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| | Enhance the virulence of a pathogen or render a nonpathogen virulent |
| | Increase transmissibility of a pathogen |
| | Alter the host range of a pathogen |
| | Enable evasion of diagnostic/detection modalities |
| | Enable the weaponization of a biological agent or toxin |
| | Any other potentially harmful combination of experiments and agents |
ChIP-seq

Data deposition
- Confirm that both raw and final processed data have been deposited in a public database such as GEO.
- Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links
For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

Files in database submission
Provide a list of all files available in the database submission.

Genome browser session
Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

Methodology

Replicates
Describe the experimental replicates, specifying number, type and replicate agreement.

Sequencing depth
Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.

Antibodies
Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.

Peak calling parameters
Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.

Data quality
Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.

Software
Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.

Flow Cytometry

Plots
Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
For Wnt activation experiments, Wnt3a or control media was isolated from L Cells and added to cell cultures as described. Following 20h of Wnt3a stimulation, cells were trypsinized, washed with PBS, resuspended in PBS with DAPI (1 μg/mL), and analyzed with the BD Biosciences LSRII flow cytometer. For particle sorting, cells were treated with control or Wnt3a media as above, then trypsinized, washed 2x with PBS and resuspended in PBS at 5 million cells per mL. Superase RNAse inhibitor (Thermo Fisher) was added to 250 U/mL. Cells were sonicated on ice at 10% amplitude using a Sonic Dismembrator 500 (Fisher) using 5 cycles of 2 sec on, 7 sec off. Excessive sonication led to decay of particle integrity. Following sonication, samples were brought first to 1% Triton X100 and then to 0.5% sodium deoxycholate from 10% stocks of each solution in ddH2O. Hoechst 33342 was added to stain nuclear DNA aggregates to 20 μg/mL. Particles were sorted using a FACSAria Fusion cell sorter (BD Biosciences) equipped with a 70 micron nozzle and run at 70psi.

Instrument
FACSAria Fusion cell sorter (BD Biosciences) and BD Biosciences LSRII flow cytometer

Software
Gating and analysis were performed with the FACSDiVa version 8.0.2 and FlowJo version X software packages.

Cell population abundance
Only fluorescent particles were sorted, and the statistics are described in Fig 4a.

Gating strategy
Cells were selected with FSC/SSC gates such that 95% of all events were within the range and only events in the lowest ~1/16 of the FSC/SSC grid were excluded. Live cells were gated using DAPI as a membrane exclusion agent using non-DAPI stained samples as a gating control. All other populations were brought to a baseline of ~100-500 MFI for corresponding negative controls. A gating strategy was provided with the final publication.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.
### Experimental resonance imaging

#### Design type

Indicate task or resting state; event-related or block design.

#### Design specifications

Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.

#### Behavioral performance measures

State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).

#### Acquisition

**Imaging type(s)**

Specify: functional, structural, diffusion, perfusion.

**Field strength**

Specify in Tesla

**Sequence & imaging parameters**

Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.

**Area of acquisition**

State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.

**Diffusion MRI**

- [ ] Used
- [ ] Not used

#### Preprocessing

**Preprocessing software**

Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).

**Normalization**

If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.

**Normalization template**

Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.

**Noise and artifact removal**

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

**Volume censoring**

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

#### Statistical modeling & inference

**Model type and settings**

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

**Effect(s) tested**

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

**Specify type of analysis:**

- [ ] Whole brain
- [ ] ROI-based
- [ ] Both

**Statistical type for inference**

(See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

**Correction**

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

#### Models & analysis

**n/a**

- [ ] Involved in the study
- [ ] Functional and/or effective connectivity
- [ ] Graph analysis
- [ ] Multivariate modeling or predictive analysis

**Functional and/or effective connectivity**

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

**Graph analysis**

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).
Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.