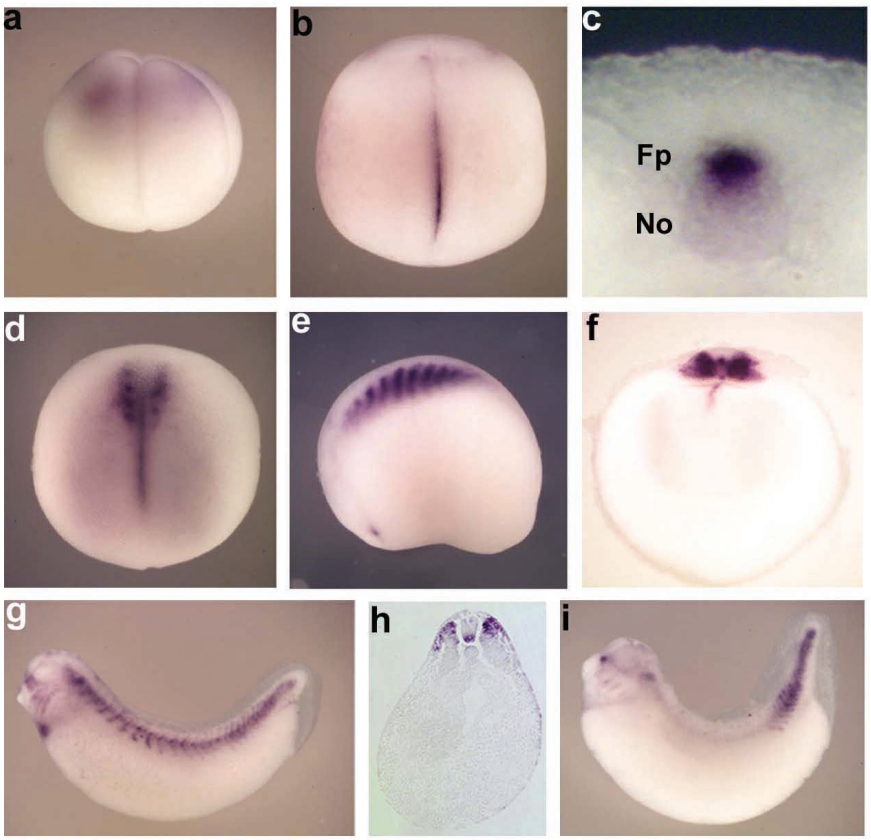

Figure S1 **Expression pattern of *CTGF* in *Xenopus* embryogenesis detected by *in situ* hybridization**. **a**, lateral view of four-cell stage embryo; **b,** dorsal view of early neural plate stage; **c**, close up of section at neural plate stage, note staining in floor plate (Fp) and notochord (No); **d**, **e**, dorsal and lateral view at the late neurula stage showing expression in somites; **f**, cross-section of a late neurula**; g, i**, lateral view of tailbud stage embryos; **h**, section through early tailbud stage. The sequence of *Xenopus* CTGF had been presented in the Genbank database (accession # U43523), but its expression during development had been not described. Our *in situ* hybridization analysis revealed the presence of maternal *CTGF* transcripts in the animal region of four-cell stage embryos and very little expression during gastrulation (panel **a** and data not shown). At early neurula stage zygotic transcripts were detected in the dorsal midline, mainly in the floor plate, and at lower levels in the underlying notochord (**b-c**). *CTGF* was also expressed throughout the developing somites at the late neurula stage and later became restricted to the dorsal aspect of the somite (**f-i**). At tailbud stage additional expression domains were seen in the developing heart, nasal placode and branchial arches (**g, i**). Thus, CTGF is expressed at various sites during early *Xenopus* development, including dorsal tissues known to be involved in embryonic patterning.

An antisense morpholino oligonucleotides directed against the immediate 5' leader region of CTGF mRNA (sequence: GTACAGCAGCAGATTAGTTCTCTTC) was microinjected into 2-cell *Xenopus* embryos. No specific morphological phenotypes were observed at 8, 10 or 80 ng of oligonucleotides per embryo (n=34, 33 and 19, respectively). The CTGF gene has been inactivated in the mouse by S. Ivkovic and K. M. Lyons (UCLA) and although the mice die perinatally, the overall body patterning is not affected by the mutation (personal communication). Thus, even if the morpholino caused phenotypes similar to those seen in mouse, we would not have expected to observe them by examination of *Xenopus* embryos at the tailbud tadpole stage.

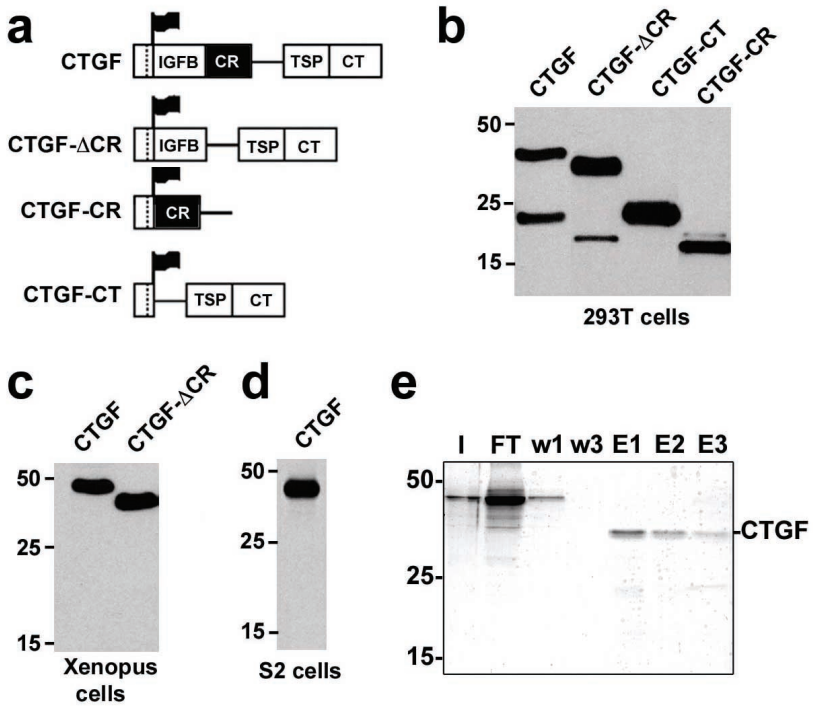

Figure S2 **CTGF constructs and expression of CTGF proteins in human 293T cells, *Xenopus* embryos and *Drosophila* S2 cells**. **a**, Schematic representation of full-length Flag epitope-tagged CTGF containing its four structural modules: the IGF binding domain (IGFB), the CR/vwc domain, the Thrombospondin-1 domain and the C-terminal cystine knot (CT). Mutants lacking the CR domain (CTGF-ΔCR and CTGF-CT), or consisting of the CR domain only (CTGF-CR) are shown. After cleavage of the signal peptide (dashed line), proteins retain twenty amino acids of the N-terminal part of Chordin (N-chd) followed by a Flag epitope sequence. The Chordin epitope tag allowed us to perform immunoprecipitation assays using an anti-N-chd polyclonal antibody[1]. **b**, Western blot using anti-Flag antibody of the proteins represented in the previous panel. Proteins were produced by transient transfection in 293T cells. Note that full-length CTGF and CTGF-ΔCR are partially cleaved and N-terminal fragments of 20 kDa and 18 kDa are generated, respectively. In other experiments the cleavage products were more prevalent than the unprocessed proteins. **c**, Expression of CTGF and CTGF-ΔCR in *Xenopus* embryonic cells. After injection of 100 pg *CTGF* or *CTGF-ΔCR* mRNA into each blastomere of the four-cell stage *Xenopus*, animal halves were dissected at stage 9 and cells cultured overnight in calcium-magnesium-free media[2]. Supernatant was collected, centrifuged and western blot using anti-Flag antibody performed. Note that the proteins produced in *Xenopus* were not cleaved. **d**, **e**, Large scale production and purification of full-length CTGF. Expression of Flag-CTGF in *Drosophila* S2 cells was performed as described[3]. Briefly, *Flag-CTGF* was sub-cloned into pUAST vector. To establish a cell line 5 βg of pUAST-CTGF were co-transfected with 1 βg of pCoHygro (Invitrogen, for hygromycin resistance) and 5 βg of pGal/pRmHa3, which contains the yeast Gal-4 protein under the control of the methalothioneine promoter. FuGENE (Roche) was used as transfection reagent and cells were selected with 250 βg/ml of Hygromycin for 60 days. For protein secretion, 500 μM of $CuSO_4$ was added to induce Gal4, which in turn induced CTGF expression via its UAS promoter. Note that the flag-CTGF produced by S2 cells is full-length. **e**, Flag-CTGF in S2 cell secreted media was purified by anti-Flag affinity gel (Sigma) column and eluted with Flag peptide (Sigma). Fractions were analyzed by SDS-PAGE stained with Coomassie blue. The gel was loaded with aliquots of the input in the column (I), of the flow through (FT), of the first and third washes (W1, W3) and of the three elutions (E1, E2 and E3). This method yielded approximately 100 βg of pure CTGF per 30 ml of tissue culture medium. We found that the use of full-length affinity-purified CTGF was essential for the crosslinking, binding analysis and cell culture results shown here. The use of *Drosophila* S2 cells was crucial as human 293T cells secrete large amounts of proteolytically processed CTGF. It is not known whether this proteolytic cleavage, which occurs close to the center of the protein, has a biological role on CTGF function *in vivo*.

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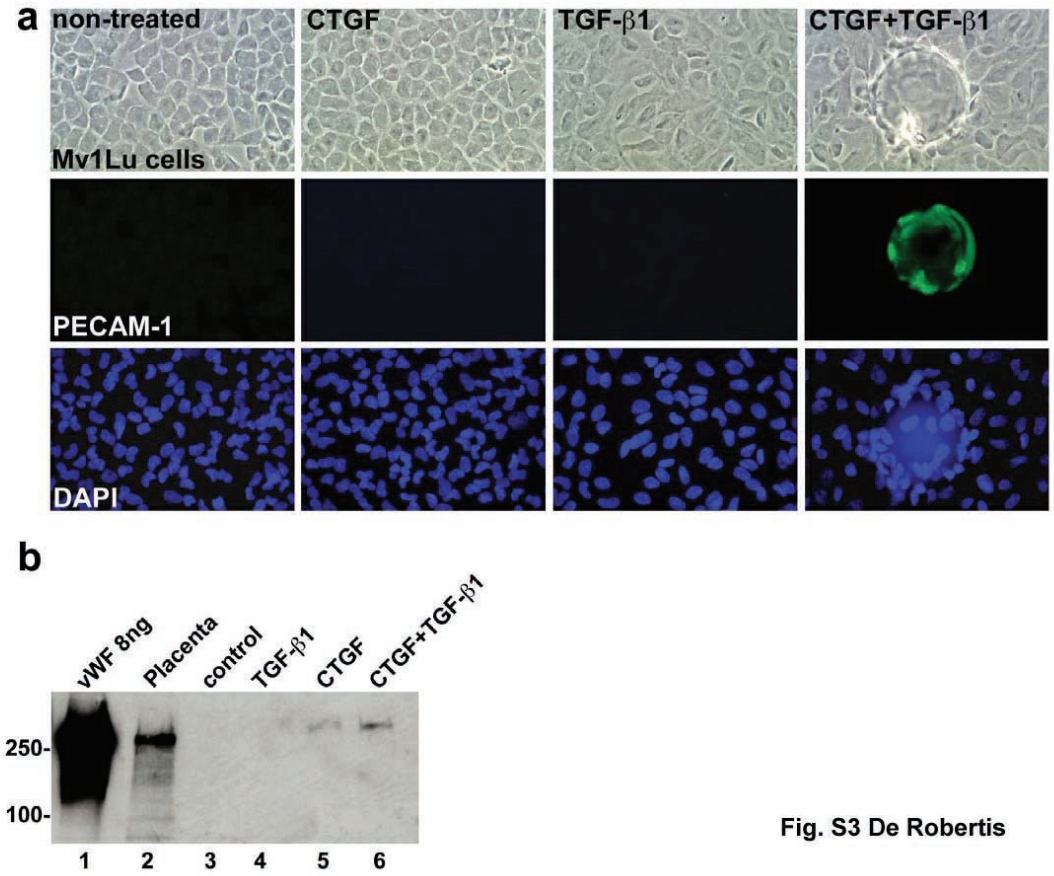

Fig. S3 De Robertis

Figure S3 **Synergy between TGF-β1 and CTGF: Induction of striking morphological changes in Mv1Lu cells**. The combination of TGF-β1 (0.2 nM) and CTGF (3 nM) induced spherical fluid-filled aggregates of Mv1Lu cells after 12 hours of culture (panel **a**). Previous studies have implicated TGF-β1 and CTGF in angiogenesis[1-4]. Immunocytochemistry was performed with the cell surface antigen[5-6] CD31, also known as platelet-endothelial cell adhesion molecule or PECAM-1. As shown in panel **a**, it was found that, indeed, the spherical aggregates were PECAM-1 positive. Spherical aggregates of PECAM-1-positive cells were not observed in Mv1Lu cells treated with TGF-β1 or CTGF alone. Interestingly, in the pluripotent mouse embryonal carcinoma P19 cell line, addition of CTGF induced PECAM-1 positive cell aggregates without requiring the addition of TGF-β1 (Fig. 5f). The mouse P19 cell structures were positive for PECAM-1 and for von Willebrand Factor, another endothelial marker (Fig. 5f). Panel **b** shows that increased amounts of vWF protein were detected in Mv1Lu cells treated with CTGF and TGF-β1 by Western blot (lane 6); polyclonal anti-vWF (Dako Co) was used at 1:200, and purified vWF and mouse placental proteins were used as positive controls. Spherical structures are induced by CTGF alone in P19 cells (see Fig. 5f of main text) presumably because they express endogenous TGF-β1 signals, since P19 cells have higher basal luciferase levels than Mv1Lu cells when transfected with a TGF-β-inducible reporter (data not shown). Mv1Lu cells have been very useful in TGF-β research because they have an intact TGF-β signal transduction pathway[7] yet express low levels of endogenous TGF-β signals, which strongly inhibit their proliferation[8]. Further experiments will be required to determine whether Mv1Lu cells, which were established from fetal mink lung tissue, are of endothelial origin. If the aggregates induced in Mv1Lu cells treated with CTGF and TGF-β1 eventually are proven to form blood vessel-like spherical structures, they will provide a valuable system for the molecular dissection of the signals involved in angiogenesis. The CTGF gene has been inactivated by homologous recombination in the mouse and defects in angiogenesis are apparent in regions where CTGF and TGF-β are normally co-expressed, identifying CTGF as an essential mediator of angiogenesis *in vivo* (S. Ivkovic and K. M. Lyons personal communication). In gain-of-function experiments, CTGF ectopically expressed in chicken chorioallantoic membranes or in the rat cornea has been shown to promote angiogenesis and neovascularization[3,4]. Even if it is eventually demonstrated that these remarkable structures are not of vascular nature, the formation of these cell aggregates may still provide an interesting *in vitro* system for the study of mesenchymal to epithelial transformation under the control of extracellular signals.

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