Supplemental material

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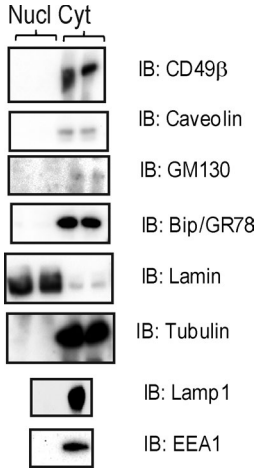

Figure S1. **Control of the purity of nuclear and cytoplasmic fractions.** The purity of nuclear (first and second lanes, denoted "Nucl") and cytoplasmic fractions (third and fourth lanes, denoted "Cyt") was determined by immunoblotting for CD49β (plasma membrane marker), caveolin (lipid raft marker), GM130 (Golgi marker), Bip/GR78 (ER marker), lamin A/C (nuclear marker), α-tubulin (cytoplasmic marker), lamp-1 (lysosomal marker), or EEA1 (early endosomes marker). IB, immunoblotting.

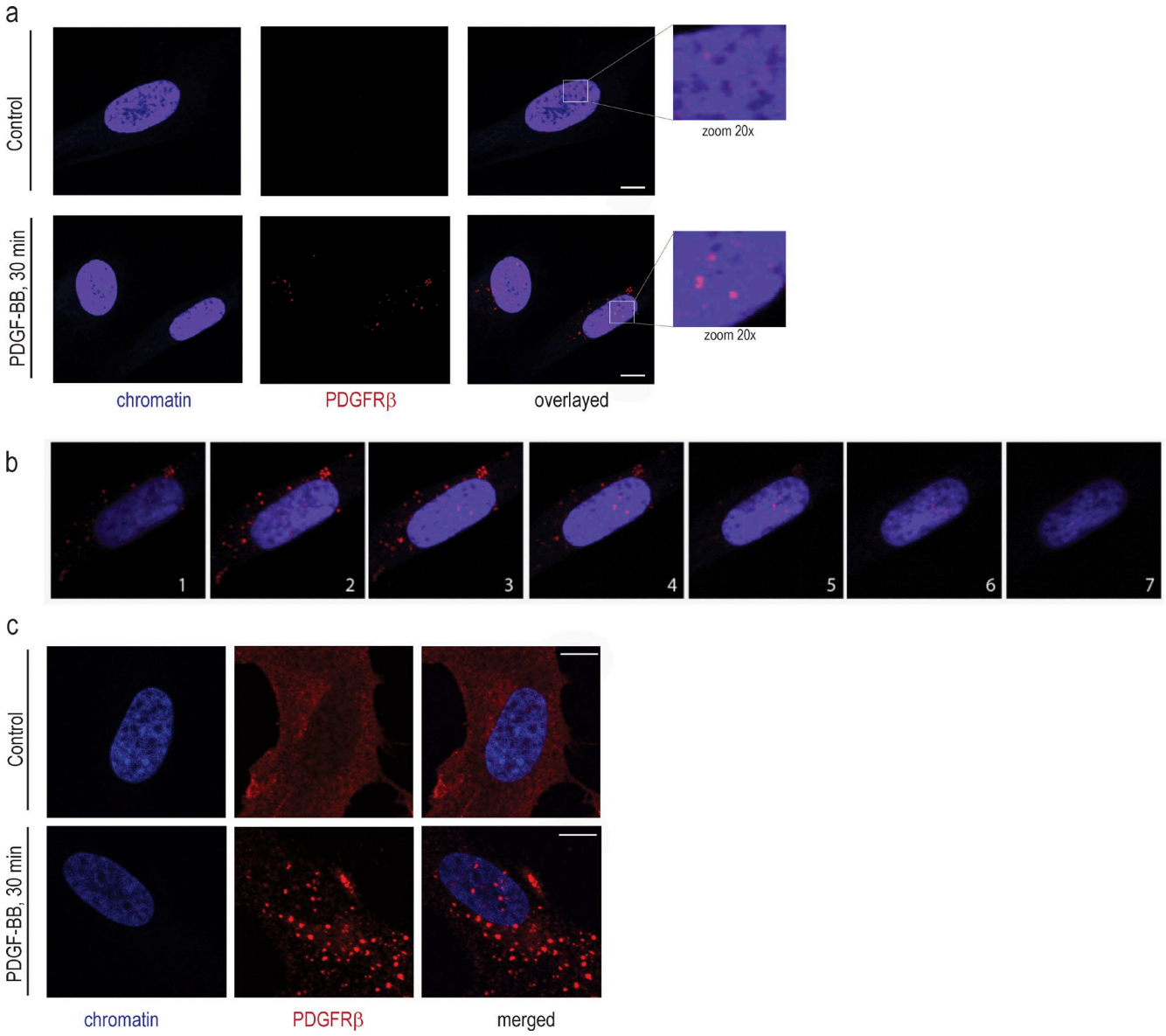

Figure S2. **Validation of nuclear localization of PDGFRβ. (a)** Immunofluorescence staining for PDGFRβ in the nucleus and cytoplasm. BJhTERT fibroblasts were stimulated (bottom) or not (top) with PDGF-BB and immunostained. PDGFRβ was stained with the rabbit intra-PDGFRβ antibody (red); chromatin was stained with DAPI (blue), and images were taken with the focus on the nucleus. After 30 min of PDGF-BB stimulation, dot-like clustering of PDGFRβ was observed in the cytoplasm, as well as throughout the nucleus (magnified images, shown on right). Bars, 10 µm. **(b)** Z-stack images through the nucleus were taken of the cell, shown on the bottom of panel. Dot-like PDGFRβ staining in the nuclear area was most clearly seen when the objective was focused in the middle of the nucleus on levels 3–5, but not in images 1, 2, or 7 with a focus above and below the nucleus, respectively. **(c)** BJhTERT fibroblasts were stimulated with PDGF-BB (bottom) or not stimulated (top). PDGFRβ was stained with a goat extra-PDGFRβ antibody (red), and chromatin was stained with DAPI (blue). Images were analyzed by laser-scanning confocal microscopy. After 30 min of PDGF-BB stimulation, dot-like clustering of PDGFRβ was observed in the cytoplasm, as well as throughout the nucleus. Bars, 10 µm.

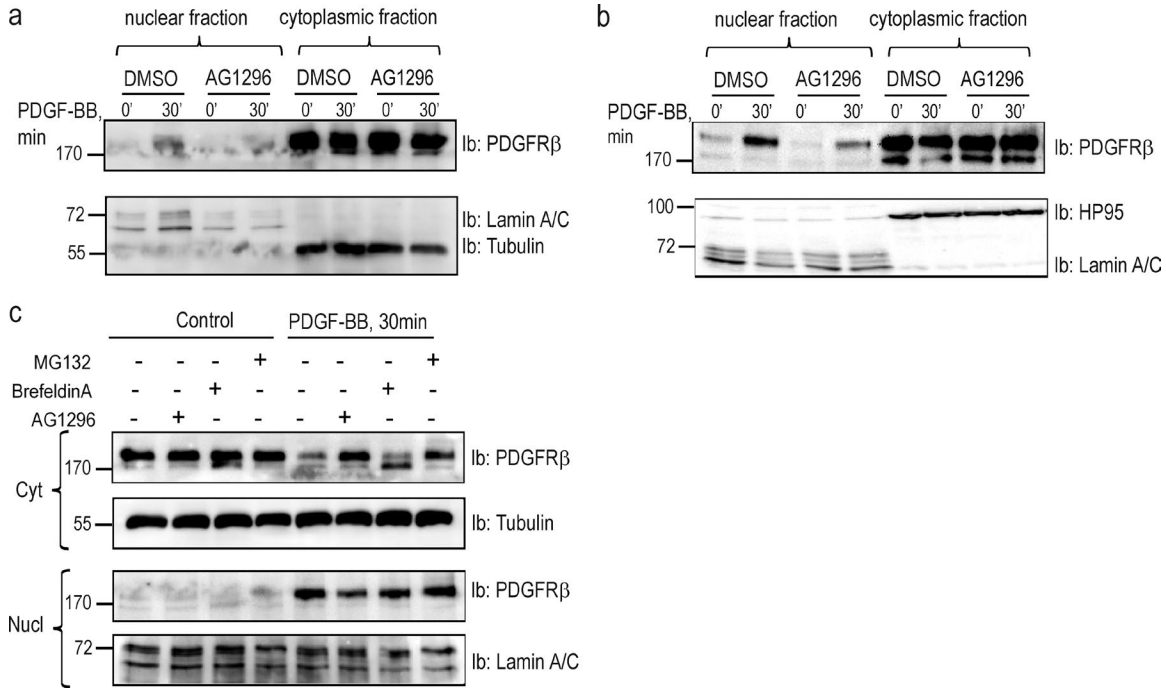

Figure S3. **Nuclear translocation of PDGFR occurs in both normal and cancer cell lines. (a)** Primary fibroblasts AG1523 were pretreated with the PDGFRβ tyrosine kinase inhibitor AG1296 (10 µM) for 1 h or with vehicle (1 µl DMSO/1 ml media). Cells were then stimulated with PDGF-BB, whereafter nuclear and cytoplasmic fractions were immunoblotted for total PDGFRβ or nuclear marker lamin C and cytoplasmic marker α-tubulin. **(b)** Glioblastoma U105MG cells were pretreated with 10 µM of AG1296 for 1 h or DMSO (as described above). Total PDGFRβ level was determined by immunoblotting (top); the purity of nuclear and cytoplasmic fractions was confirmed by blotting for the lamin A/C and cytoplasmic marker HP95 (Alix; bottom). **(c)** Osteosarcoma U2OS cells were pretreated with 25 µM MG132 for 3 h, 500 ng/ml brefeldin A for 90 min, or 10 µM AG1296 for 1 h. Cells were left unstimulated (first four lanes) or stimulated with 20 ng/ml PDGF-BB (last four lanes). Total PDGFRβ level was determined in the cytoplasmic (top) and nuclear (third panel) fractions; the purity of fractions was confirmed with immunoblotting for the cytoplasmic marker α-tubulin (second panel) or the nuclear marker lamin C (bottom). Molecular mass was measured in kilodaltons. Ib, immunoblotting.