Endoglin Expression on Cancer-Associated Fibroblasts Regulates Invasion and Stimulates Colorectal Cancer Metastasis

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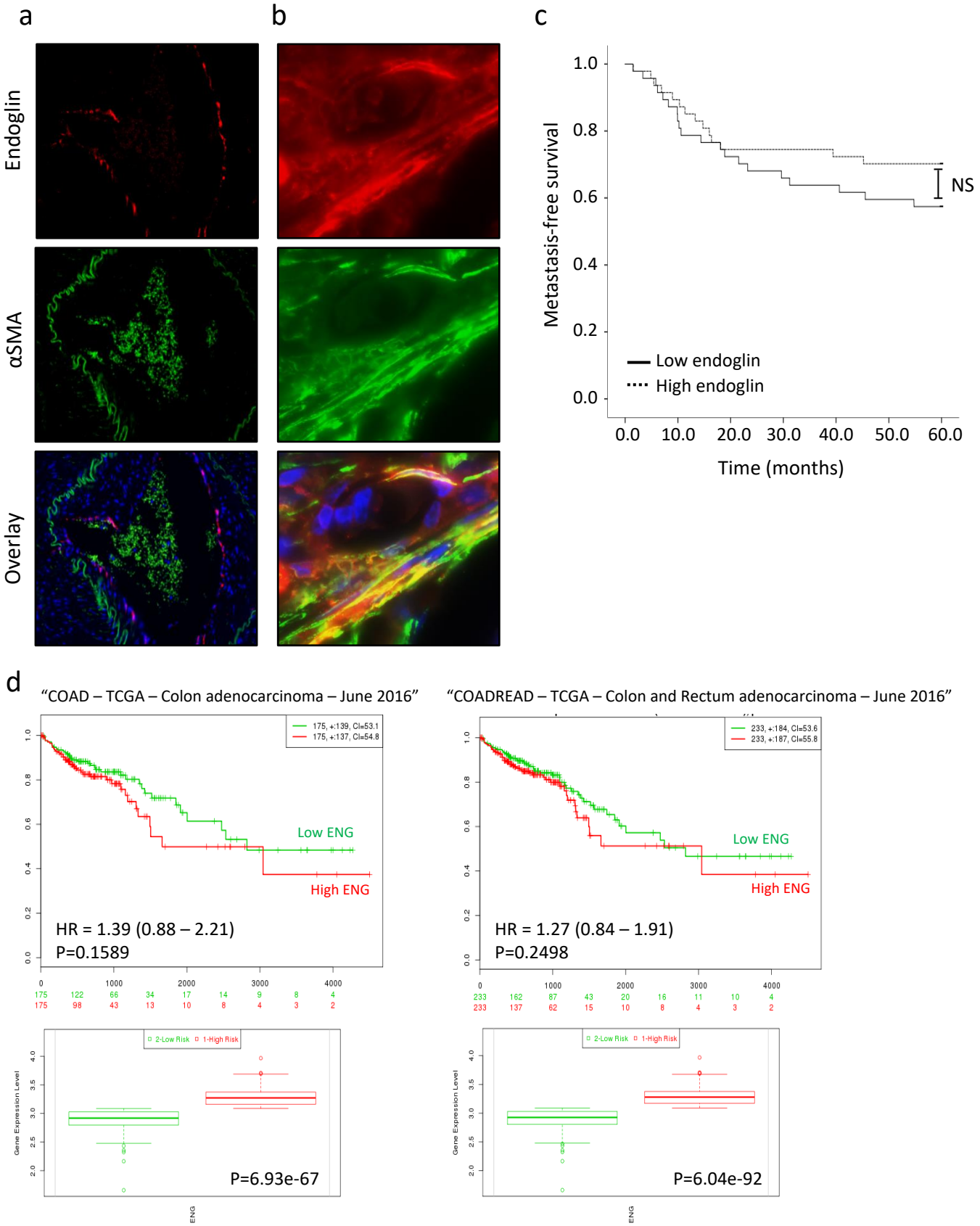
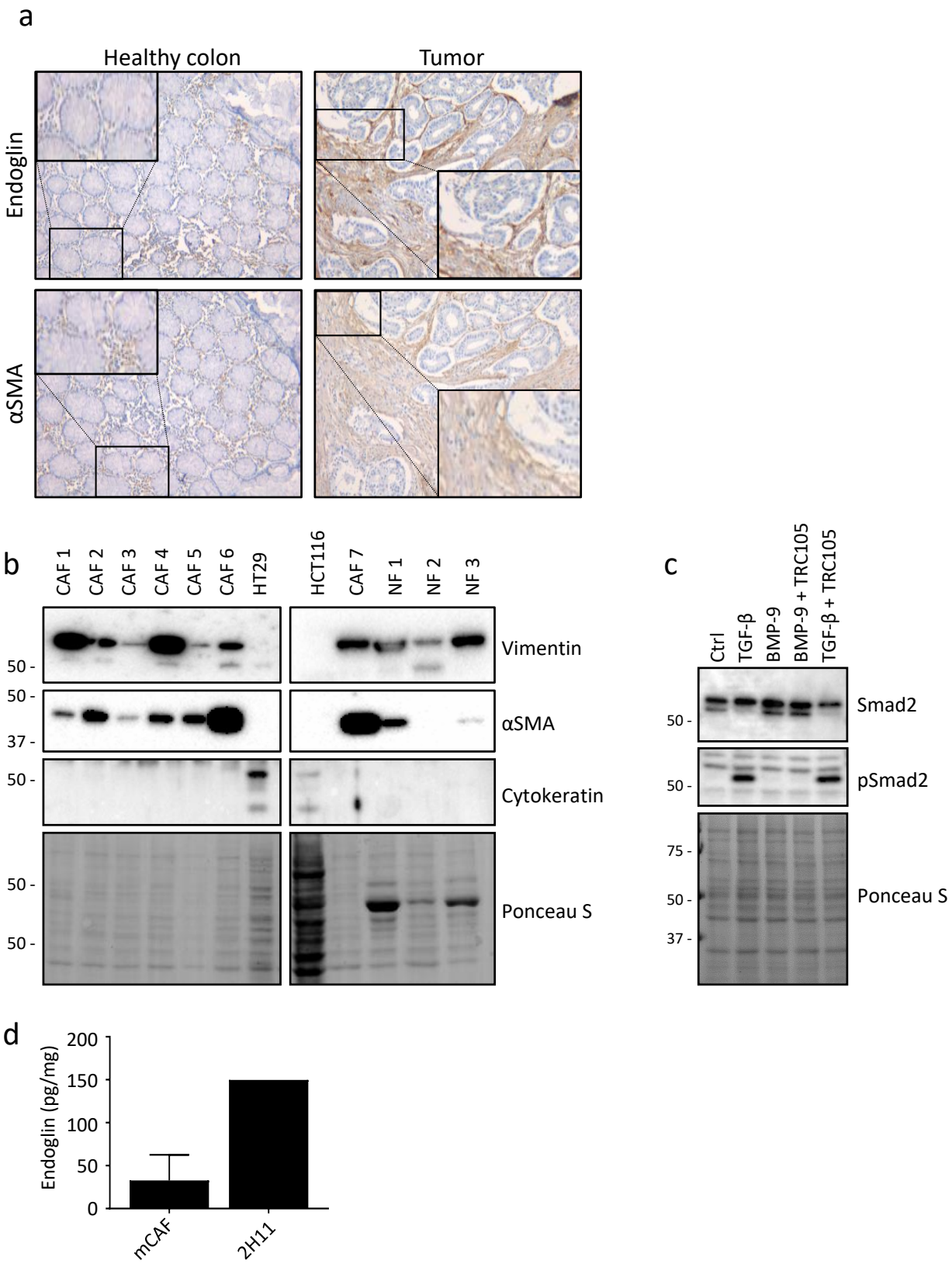
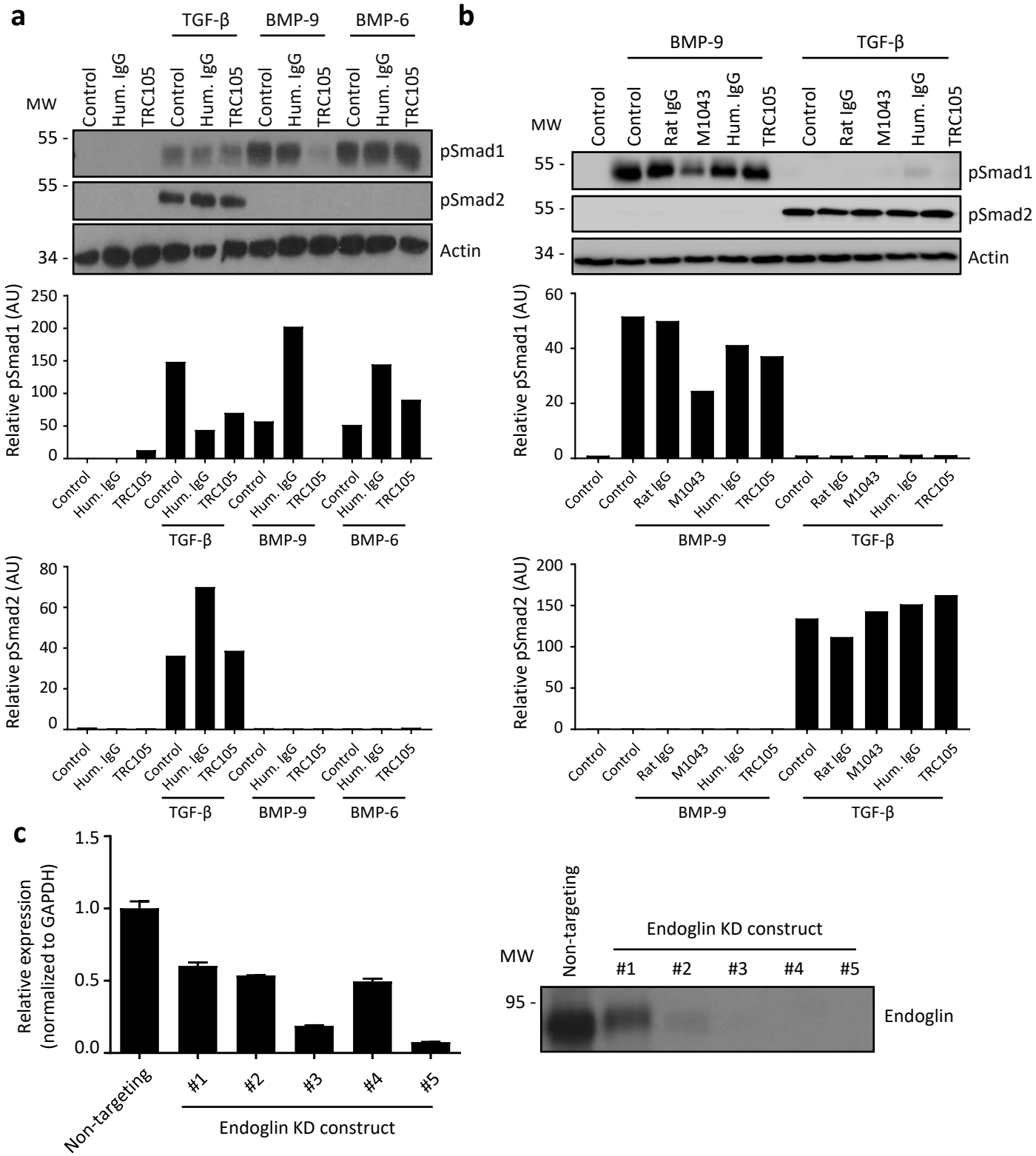


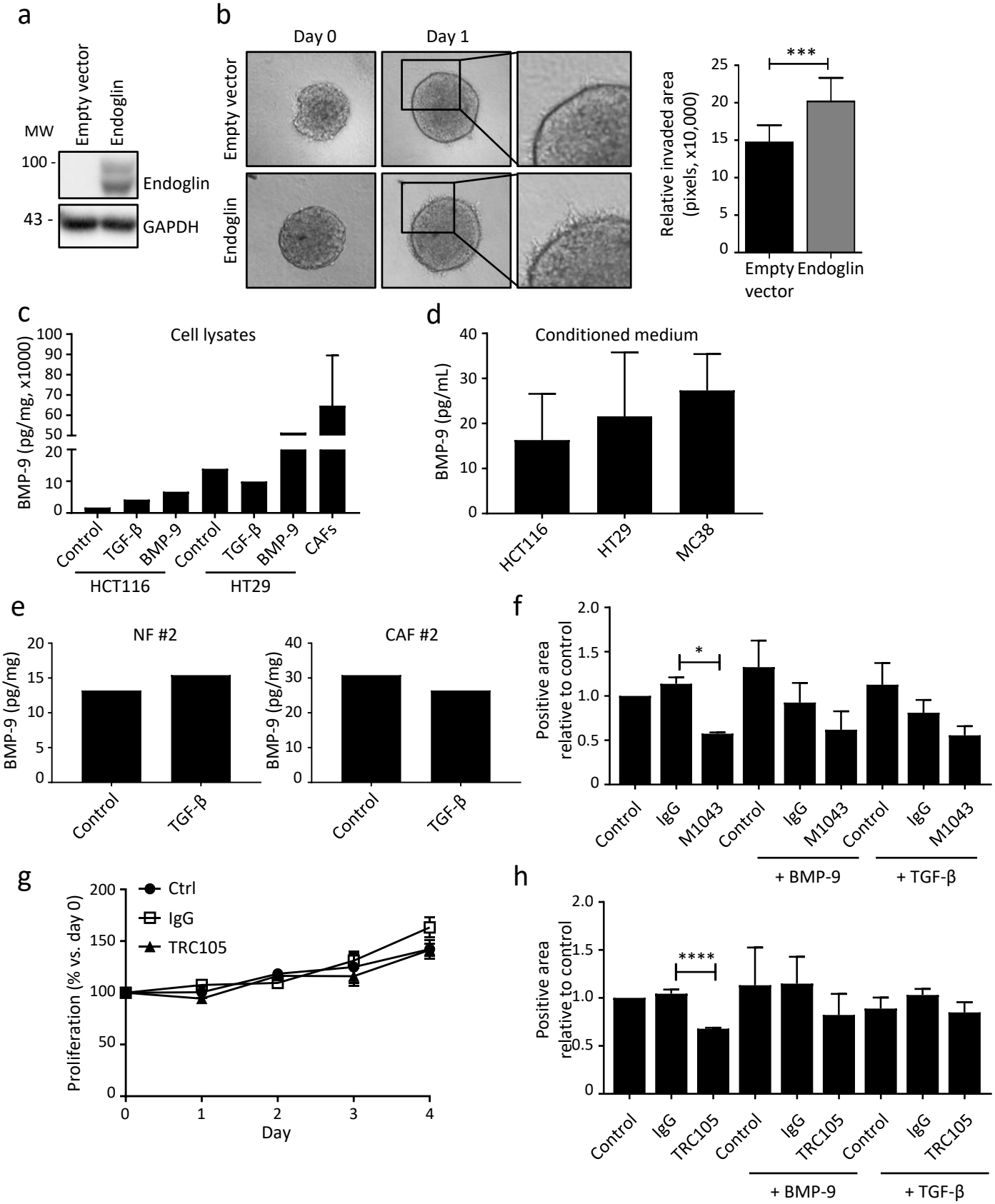
Supplementary figure S1 A. Endoglin (red) is specifically expressed by angiogenic endothelial cells within the endothelial layer of a large vessel. αSMA expression (green) on smooth muscle cell layer surrounding the vessel. Nuclei are stained blue using DAPI. B. High magnification of endoglin-expressing CAFs (yellow). C. Endoglin expression on CAFs at the invasive borders was related to distant metastasis-free survival. No correlation between the two variables was found. N=94 patients. D. SurvExpress analysis of TCGA databases "COAD – TCGA – Colon adenocarcinoma – June 2016" and "COADREAD – TCGA – Colon and Rectum adenocarcinoma – June 2016" for endoglin expression in CRC, patient survival and risk classification.



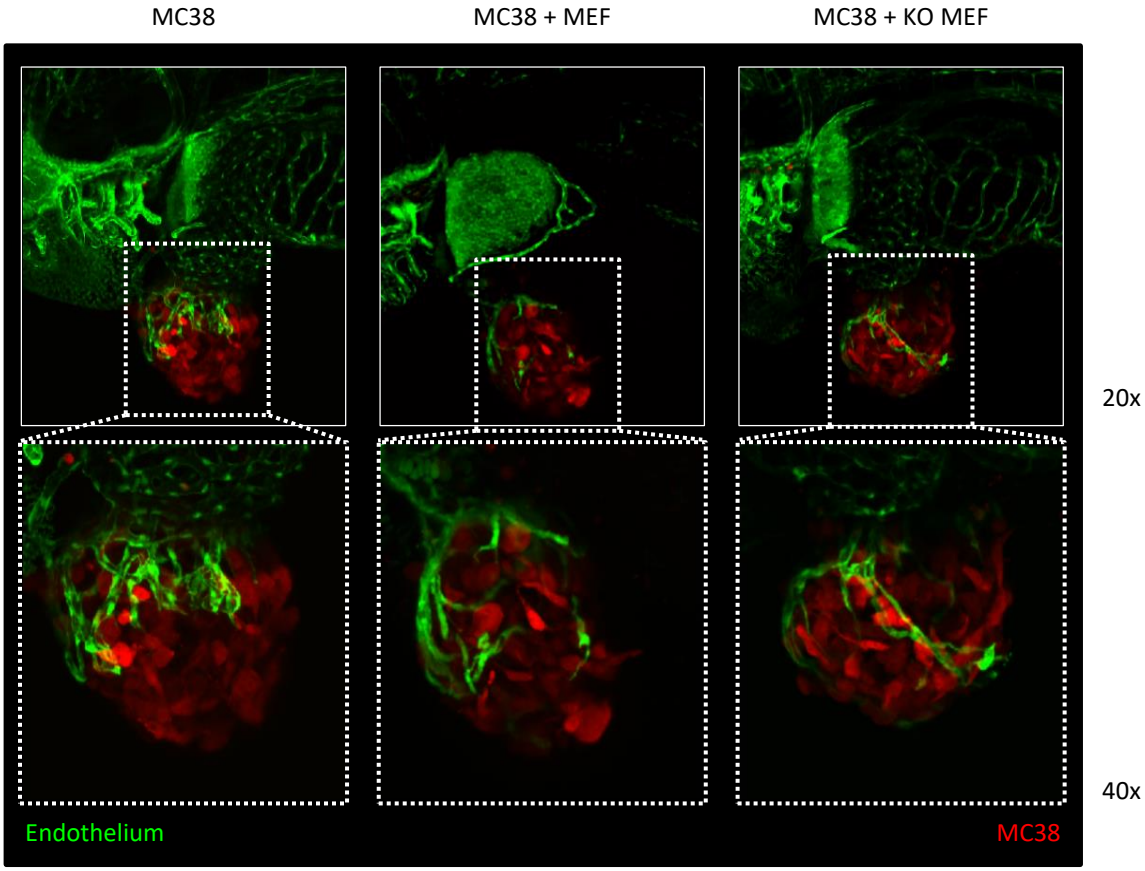
Supplementary figure S2 A. Healthy and tumor tissues from the same patient were stained for endoglin and αSMA to assess endoglin expression on (cancer-associated) fibroblasts. αSMA-positive fibroblasts in healthy colonic tissue did not show endoglin expression. Pictures are representative for normal tissue samples. Tissue from patient #4, characterized in supplementary table S3. B .Western blot analyses showing protein expression of fibroblast markers vimentin and αSMA, and absence of cytokeratin and CD31. C. Induction of Smad2 phosphorylation after ligand stimulation does not affect total Smad2 protein levels in CAFs, as shown by western blot. D. Endoglin protein expression in mouse CAFs (N=3) and mouse endothelial cells (2H11), as determined by ELISA. Mean + SD.



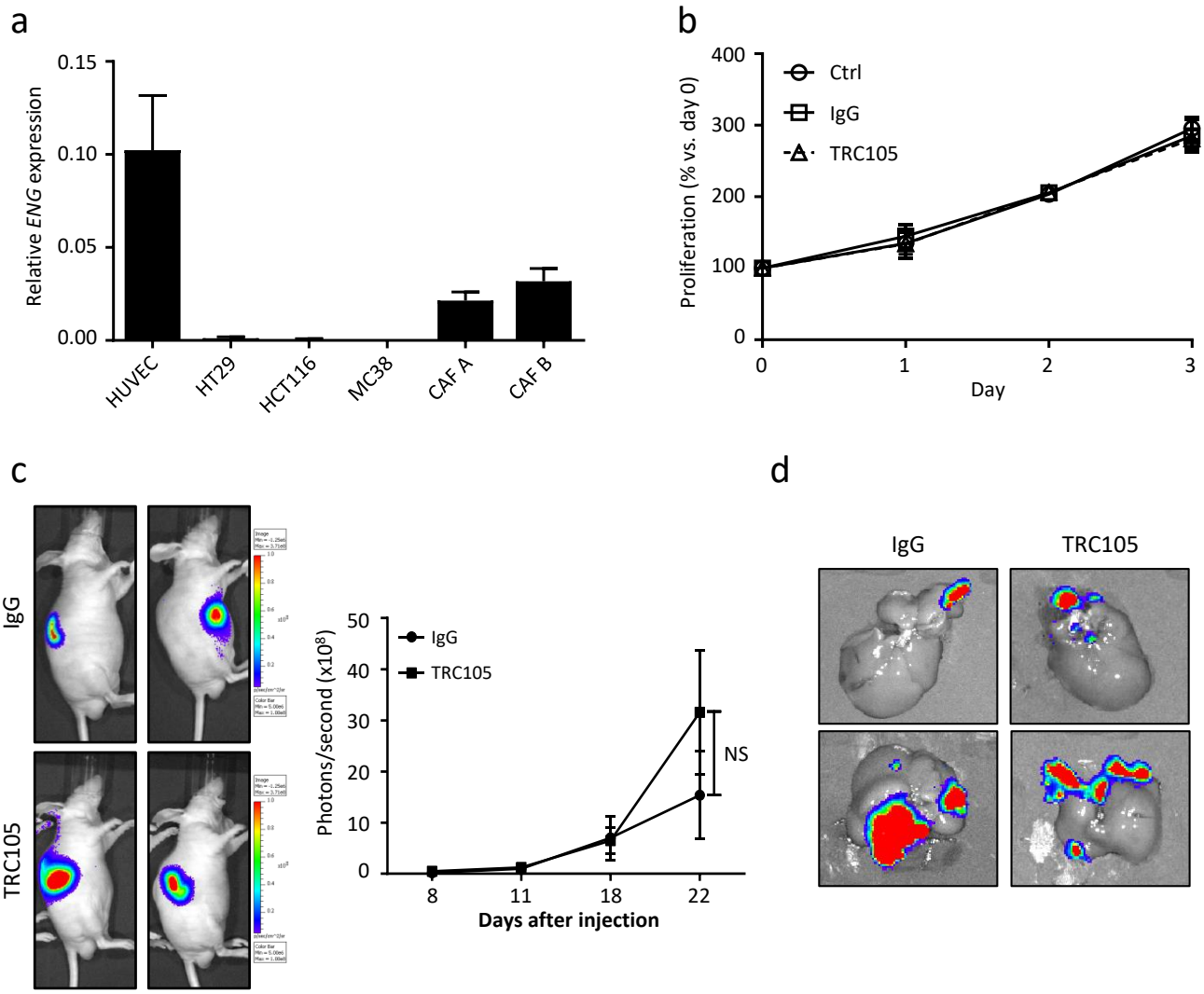
Supplementary figure S3 Quantification of Smad1 and Smad2 phosphorylation shown in figure 3D (a) and 3E (b). C. Endoglin knock down (KD) in human ECRF cells. Short hairpin RNA constructs were introduced in human endothelial cells by lentiviral transduction. All constructs reduced endoglin expression at mRNA level with at least 40% (left panel), which was reflected at the protein level (right panel).



Supplementary figure S4 HEK293T cells were transfected to express endoglin (a) and left to form spheroids. B. Invasion of collagen-I matrix after 24 hours was significantly increased in endoglin-expressing HEK293T. C. Expression of BMP-9 in CRC cell and CAF lysates as determined by ELISA. D. Secretion of BMP-9 into the medium by CRC cells, assessed using ELISA. E. BMP-9 concentrations in NF and CAF lysates after TGF-β stimulation, assessed using ELISA. Representative graphs for five CAFs tested, NF/CAF# corresponds with table S4. F.M1043 significantly inhibited basal mouse CAF invasion. BMP-9- and TGF-β-induced invasion were inhibited, although not statistically significant. G. Proliferation of or human CAFs was not affected by neutralizing endoglin, as determined by MTS proliferation. H. Basal human CAF invasion was decreased by TRC105. BMP-9 and TGF-β marginally increased human CAF invasion, therefore effects of TRC105 were limited. *P≤.05, ****P≤.0001.



Supplementary figure S5 Primary tumor formation (MC38 cells in red) and angiogenesis (green) did not differ between MC38 alone, MC38 co-injected with control MEFs or MC38 co-injected with endoglin KO MEFs.



Supplementary figure S6 HT29 cells do not express endoglin (a) and TRC105 treatment did not affect proliferation *in* vitro, as determined by MTS (b). C. Mice were injected with HT29 cells and two days after injection treatment with IgG or TRC105 started. Treatment did not affect metastatic spread to the liver in the absence of human CAFs as quantified by *in vivo* luminescent imaging. D. *Ex vivo* bioluminescent imaging of mouse livers did not show differences between the two treatments when tumor cells were injected alone. N=7/8 mice/group.